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Développement de méthodes analytiques et devenir environnemental de multiples classes de contaminants pharmaceutiques

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Cette thèse intitulée

Développement de méthodes analytiques et devenir environnemental de multiples classes de contaminants pharmaceutiques

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Résumé

La consommation des composés pharmaceutiques est en constante croissance à travers le monde et leur utilisation peut entraîner une accumulation dans l'environnement d'où leur désignation à titre de contaminants émergents. De nombreux groupes de recherche se penchent donc sur les questions de leurs effets sur des organismes vivants, sur la santé de certains écosystèmes ou encore sur l'efficacité de différents types de traitement des eaux usées. Or, peu de données sont disponibles quant aux concentrations présentes dans différentes matrices telles que les eaux usées ou l'eau de surface dans lesquelles elles sont déversées ou encore l'impact que peuvent avoir les hôpitaux sur l'occurrence des composés pharmaceutiques. La recherche dans ce domaine peut s'avérer un outil clé dans l'établissement de normes et de limites pour différents contaminants puisque celles-ci sont encore marginales dans la plupart des pays du monde, surtout au niveau des médicaments. Dans le cadre de cette thèse, différentes méthodes analytiques ont été développées pour faire l'analyse de classes ciblées de médicaments par chromatographie liquide couplée à la spectrométrie de masse afin de quantifier ceux-ci dans diverses matrices environnementales.

Un des objectifs du présent ouvrage est donc de détailler les travaux de développement et de validation de méthodes analytiques robustes. Une première méthode d'extraction en phase solide en ligne avec la chromatographie liquide ultra-haute performance couplée à la spectrométrie de masse en tandem (SPE en ligne UPLC-MS/MS) a été développée afin de faire l'analyse de huit différentes classes de composés pharmaceutiques susceptibles d'être retrouvés dans les eaux usées. Afin de déterminer la distribution et l'adsorption des composés ciblés sur la phase solide de différentes matrices, une méthode d'extraction et de purification a également été optimisée de telle sorte que les extraits de matière particulaire et de sédiments soient compatibles avec cette méthode analytique. Ces méthodes ont été appliquées sur de nombreuses eaux usées d'hôpitaux et d'usine de traitement des eaux au Québec (Canada).

Les produits de chimiothérapies présentent un défi analytique supplémentaire puisqu'ils sont polaires comparativement à la plupart des autres classes de composés pharmaceutiques. Il y a donc peu d'études sur la présence et le comportement de ces contaminants bien que ceux-ci présentent un risque écotoxicologique potentiellement important. Un deuxième objectif de cette thèse a donc été de comparer différentes alternatives pour la préconcentration et la séparation chromatographique, notamment la chromatographie à interaction hydrophile (HILIC). Celle-ci a mené à la validation de deux méthodes analytiques dont la sensibilité est de l'ordre des ng/L pour ces composés dans des eaux usées brutes. Ces méthodes de SPE en ligne UPLC-MS/MS ont

été appliquées à de nombreux échantillons d'effluents d'hôpitaux ainsi qu'à des usines de traitement des eaux usées au Québec (Canada).

D'autre part, la méthode SPE en ligne UPLC-MS/MS pour l'analyse des multiples classes de composés pharmaceutiques a été validée pour l'eau de surface enfin d'atteindre un dernier objectif de cet ouvrage qui consiste à évaluer différentes sources de contamination de cette matrice à proximité de zones densément peuplées. Durant de larges campagnes d'échantillonnage sur le fleuve Saint-Laurent, échelonnées sur une période de cinq ans et ayant couvert une zone géographique de près de 700 km entre le Lac Ontario et l'estuaire, plus de 400 échantillons ont été prélevés et analysés. Parmi ceux-ci figurent des échantillons provenant de 56 rivières tributaires au fleuve prélevés afin de déterminer l'impact de celles-ci en termes de pollution en composés pharmaceutiques et de les comparer avec des points de rejet d'eau usée de villes telles que Montréal ou Québec.

Enfin, ces mêmes méthodes ont été appliquées lors de projets en collaboration avec des chercheurs se penchant sur les effets écotoxicologiques des composés pharmaceutiques sur des organismes aquatiques dans des matrices d'eau de surface ainsi que pour de développement de technologies alternatives pour le traitement des eaux usées.

Mots clés :

SPE en ligne, UPLC-MS/MS, pharmaceutiques, hôpitaux, eau usée, eau de surface

Abstract

A constant increase in pharmaceutical compounds worldwide can accentuate problems related to these pollutants of emerging concern in environmental matrices. Therefore, many researchers are studying their effects on living organisms, the health of impacted ecosystems, and the efficiency of different types of wastewater treatment systems. However, there is a lack of available data on the occurrence and concentrations of pharmaceuticals in different matrices, such as wastewaters or receiving surface waters, and the relative importance of hospital effluents on the load of these contaminants. Research in this field can be a key tool on different drugs since only a few countries have implemented norms and guidance, especially for medications. As part of this thesis, different analytical methods have been developed for the analysis of selected drugs, and liquid chromatography coupled with mass spectrometry was used to quantify multi-class pharmaceuticals occurring in various environmental matrices.

One objective of this manuscript is to develop and validate robust analytical methods. A first extraction method on-line with liquid chromatography coupled to tandem mass spectrometry (on-line SPE UPLC-MS/MS) was developed to analyze eight different pharmaceutical classes susceptible to be found in wastewaters. With the aim of evaluating the partitioning and adsorption of target compounds onto a solid phase, an extraction and purification method was optimized to ensure that particulate matter and sediment extracts were compatible with the analytical method. These optimized methods were then applied to several hospital effluents and wastewater treatment plants samples in the province of Quebec (Canada).

Chemotherapy agents present an additional analytical challenge since these drugs are relatively polar compared with most of other pharmaceutical classes. This explains the low number of publications relating to their occurrence and behaviour even though they present a high ecotoxicological hazard. A second objective of this thesis was to compare different potential alternatives for enrichment and chromatographic separation of these pollutants, notably the use of hydrophilic interaction chromatography (HILIC). This work led to the validation of two analytical methods for which the sensitivity reached ng/L levels for these compounds in raw sewage. These methods were successfully applied to samples from numerous hospital effluents and wastewater treatment plants from the province of Quebec (Canada).

Also, a multi-class pharmaceutical method was validated for surface water in order to achieve the last objective of this thesis which consisted in the determination of the different contamination sources of this matrix near densely populated areas. During major sampling campaigns on the

Saint-Lawrence River, spanning over five years and covering a geographical area of approx. 700 km between Lake Ontario and the estuary, a total of more than 400 samples were collected and analyzed. Among those, 56 different rivers tributary to the Saint-Lawrence were sampled to establish the impact they may have on pharmaceutical pollution. Data were compared with wastewater rejection points from cities, such as Montreal and Quebec.

Finally, these methods were applied in the context of collaboration projects with researchers focusing on measuring ecotoxicological effects of pharmaceutical compounds on living organisms in surface water matrices and on the development of alternative wastewater treatment technologies.

Keywords :

On-line SPE, UPLC-MS/MS, pharmaceuticals, hospitals, wastewater, surface water

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2OH-CBZ	10,11-Dihydroxy-10,11-dihydrocarbamazépine
5-FU	5-Fluorouracile
a. u.	Unité arbitraire (*arbitrary unit*)
ACE	Acébutolol
ACN	Acétonitrile
AmAc	Acétate d'ammonium (*ammonium acetate*)
AmFo	Formate d'ammonium (*ammonium formate*)
AMOX	Amoxicilline
APCI	Ionisation chimique à pression atmosphérique (*atmospheric pressure chemical ionization*)
AA-QS	Standard de qualité basé sur la concentration moyenne annuelle (*annual average concentration based quality standard*)
AS	Ratio d'asymétrie
ASE	Extraction par solvant accéléré (*accelerated solvent extraction*)
C18	Octadécyle
CAF	Caféine
CAP	Capécitabine
CBZ	Carbamazépine
CCME	Conseil canadien des ministres de l'environnement (*Canadian council of Ministers of the Environment*)
CCV	Vérification continue de l'étalonnage (*continued calibration verification*)
CE	Électrophorèse capillaire (*capillary electrophoresis*)
CEAEQ	Centre d'expertise en analyse environnementale du Québec
CLA	Clarithromycine
CT	Transition de confirmation (*confirmation transition*)
CWM	Masse d'eau centrale (*central water mass*)
CWQG	*Canadian Water Quality Guideline*
CYC	Cyclophosphamide
CYT	Cytarabine
D	Désirabilité de Derringer
DCF	Diclofénac
DEA	Diethylamine
dFdU	2',2'-Difluorodeoxyuridine
DVB	Divinylbenzène
DVEN	Desvenlafaxine
E1	Estrone
E2	Estradiol
E3	Estriol
EE2	Éthinylestradiol
EI	Ionisation électronique (*electron ionization*)
EQS	Standards de qualité environnementale (*environment quality standards*)
ESI	Ionisation par électronébulisation (*electrospray ionization*)
ESRD	*Alberta Environment & Sustainable Ressource Development*
ETZ	Zone de transition estuarienne (*estuarine transition zone*)

FA	Acide formique (*formic acid*)
FDA	Agence fédérale américaine des produits alimentaires et médicamenteux (*Food and Drug Administration*)
FLU	Fluoxétine
FT-ICR	Spectrométrie de masse par résonance cyclonique ionique (*Fourier-transform ion cyclotron resonance*)
FW	Eeau douce (*freshwater*)
GC	Chromatographie gazeuse (*gas chromatography*)
GEM	Gemcitabine
GFF	Filtre en fibre de verre (*glass fiber filter*)
GL	Grands Lacs (Great Lakes)
GW	Eau souterraine (*ground water*)
GWF	Empreinte des eaux grises (*grey water footprint*)
HA	Hexylamine
HILIC	Chromatographie à interaction hydrophile (*hydrophilic interaction chromatography*)
HLB	Chromatographie d'équilibre hydrophile-lipophile (*hydrophilic-lipophilic balance*)
HMOR	Hydromorphone
HPLC	Chromatographie liquide haute performance (*high performance liquid chromatography*)
HRMS	Spectrométrie de masse à haute résolution (*high resolution mass spectrometry*)
IBU	Ibuprofène
IFO	Ifosfamide
IS	Étalon interne (*internal standard*)
K_d	Coefficient de distribution
kg	Kilogramme
K_{ow} ou log P	Coefficient de partage octanol/eau
L	Litre
LC	Chromatographie liquide (*liquid chromatography*)
LD_{50}	Dose létale médiane
LEVO	Lévonorgestrel
LOD	Limite de détection (*limit of detection*)
LOEC	Plus petite concentration induisant un effet observable (*lowest-observed effect concentration*)
LOQ	Limite de quantification (*limit of quantification*)
m/z	Ratio masse sur charge
m^2	Mètre carré
m^3	Mètre cube
MAC-QS	Standard de qualité basé sur la concentration maximale admissible (*maximum admissible concentration based quality standard*)
MAE	Extraction assistée par micro-ondes (*microwave assissted extraction*)
MALDI	Désorption-ionisation laser assistée par matrice (*matrix assisted laser desorption ionization*)

ME	Effet de matrice (*matrix effect*)
MELCCFP	Ministère de l'Environnement, de la Lutte contre les changements climatiques, de la Faune et des Parcs
MeOH	Méthanol
MPROG	Médroxyprogestérone
MRM	Multiples fragmentations sélectives (*multiple reaction monitoring*)
MRNF	Ministère des Ressources naturelles et des Forêts
MS	Spectrométrie de masse (*mass spectrometry*)
MS/MS	Spectrométrie de masse en tandem (*tandem mass spectrometry*)
MTX	Méthotrexate
n. a.	Non applicable
n. d.	Non détecté
NOEC	Concentration sans effet obervable (*no-observed effect concentration*)
NOR	Noréthindrone
NORF	Norfluoxétine
NO-SMX	4-Nitrososulfaméthoxazole
NPLC	Chromatographie liquide en phase normale (*normal phase liquid chromatography*)
OH-DCF	4-Hydroxydiclofénac
OH-IBU	2-Hydroxyibuprofène
OH-MTX	Hydroxyméthotrexate
OR	Rivière des Outaouais (Ottawa River)
PFP	Pentafluorophényle
PLE	Extraction par liquide pressurisé (*pressurized liquid extraction*)
PNEC	Concentration prédite sans effet (*predicted no-effect concentration*)
ppb	Partie par milliard (*part per billion*)
ppm	Partie par million
ppq	Partie par billiard (*part per quadrillion*)
ppt	Partie par billion (*part per trillion*)
PROG	Progestérone
QA	Assurance qualité (*quality assurance*)
QC	Contrôle de qualité (*quality control*)
QqQ	Triple quadripôle
$QS_{biota\ sec\ pois}$	Standard de qualité pour la prévention de l'intoxication indirecte des prédateurs supérieurs dans la chaîne trophique (*prevention of secondary poisoning of top predator based quality standard*)
$QS_{fw\ eco}$	Standard de qualité pour la protection des écosystèmes d'eau douce (*freshwater ecosystems protection based quality standard*)
QT	Transition de quantification (*quantification transition*)
R	Résolution
RF lens	Lentille à radio fréquence (*radio frequency lens*)
RPLC	Chromatographie liquide en phase inverse (*reversed phase liquid chromatography*)
RPM	Tour par minute (*revolution per minute*)
RQ	Quotient de risque (*risk quotient*)
RQM	Réseau Québec Maritime

RSD	Écart-type relatif (*relative standard deviation*)
R/V	Navire de recherche (*research vessel*)
S/N	Ratio signal sur bruit (*signal-to-noise ratio*)
SCHEER	*Scientific Committee on Health, Environmental and Emerging Risks*
SED	Sédiments
SI	Information complémentaire (*supporting information*)
SIM	Surveillance d'ion unique (*single ion monitoring*)
SL	Boues d'épuration (*sludge*)
SMX	Sulfaméthoxazole
SPE	Extraction sur phase solide (*solid phase extraction*)
SPM	Particules en suspension (*suspended particulate matter*)
SRM	Suivi de réaction ciblée (*selected reaction monitoring*)
ST	Tributaires sud (*south tributaries*)
STP	Station d'épuration (*sewage treatment plant*)
SW	Eau de surface (*surface water*)
TESTO	Testostérone
TOF	Analyseur à temps de vol (*time-of-flight mass spectrometry*)
TW	Eau du robinet (*tap water*)
UAE	Extraction assistée ultrasons (*ultrasound assissted extraction*)
UPLC	Chromatographie liquide ultra-haute performance (*ultra performance liquid chromatography*)
US EPA	Agence de protection de l'environnement des États-Unis (*U. S. Environmental Protection Agency*)
UV-vis	Spectroscopie ultraviolet-visible
VEN	Venlafaxine
WFD	*Water Framework Directive*
WW	Eau usée (*wastewater*)
WWTP	Usine d'épuration des eaux usées (*wastewater treatment plant*)
σ	Écart-type
$\sum$	Somme
Φ	Fraction massique associée à la phase particulaire
ω	Fraction massique associée à la phase dissoute
$\bar{x}$	Moyenne

“We demand rigidly defined areas of doubt and uncertainty!”

- Douglas Adam

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Je tiens à remercier en premier mes parents qui m'ont toujours encouragé à faire ce que je voulais dans la vie, tout en insistant pour que je fasse bien les choses et jusqu'au bout. On peut dire qu'en quelque sorte le Ph. D. c'est le bout de quelque chose! Je veux aussi remercier Annie ma Choumpie qui m'a soutenu et enduré durant toute la période de mes études graduées. Avec ton support, on aura réussi, en l'espace d'une année, à se bâtir un chez nous, à me trouver une job et à finaliser ma thèse, ce qui n'est pas rien! Avec Pitouak et Pitouette on te câline très fort!

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1 Introduction

1.1 Historique de la problématique des composés pharmaceutiques

Les composés pharmaceutiques permettent le contrôle de certaines maladies, d'augmenter l'espérance de vie ou de diminuer les risques de problèmes de santé chroniques, tels que le diabète ou les maladies cardiovasculaires. En plus de traitements contre des maladies, certains médicaments sont utiles dans la vie quotidienne comme les antihistaminiques contre les allergies, les pilules contraceptives ou encore des analgésiques communs tels que l'acétaminophène (Tylenol) ou l'ibuprofène (Advil). Lorsque ces composés sont développés, il est essentiel de démontrer l'efficacité et l'innocuité de ces médicaments pour les personnes qui les consomment. Toutefois, ces composés peuvent se retrouver de manière intacte ou sous forme métabolisée dans l'environnement. Les études sur les effets que ces contaminants peuvent engendrer sont de plus en plus nombreuses ces dernières années [1–5]. Des groupes de recherche ont commencé à s'y intéresser dans les dernières décennies et l'avancement technologique et les développements de méthodes analytiques actuels permettent des études sur le comportement et les effets des composés pharmaceutiques dans l'environnement [6]. D'ailleurs, plusieurs organismes gouvernementaux, notamment le Ministère de l'Environnement, de la Lutte contre les changements climatiques, de la Faune et des Parcs (MELCCFP), ont classé les composés pharmaceutiques parmi les contaminants d'intérêts émergents et les substances toxiques qui doivent être étudiés et suivis dans différentes matrices environnementales [7]. Un nombre significatif de données sur ces polluants doit être recueilli, tant quant à leur concentration et leur occurrence qu'en lien avec l'efficacité des systèmes de traitement des eaux usées et de l'eau potables. Ces données permettent également de faire l'évaluation des effets écotoxicologiques de ces contaminants sur la faune, la flore ou sur les êtres humains [8]. Enfin, des campagnes d'échantillonnages répétées permettent de dresser des tendances de la pollution à travers le temps et de déterminer les possibles mesures à mettre en place afin d'éviter les différentes problématiques en découlant.

1.2 Consommation des composés pharmaceutiques dans le monde

Avec la croissance démographique mondiale, la production et la consommation de médicaments sont en constante augmentation et par le fait même, les enjeux environnementaux que peuvent engendrer les composés pharmaceutiques sont le sujet d'études diverses. Des centaines de tonnes de ces composés sont vendues chaque année et une partie non négligeable

de ceux-ci et de leurs métabolites peuvent se retrouver dans l'environnement via différentes sources de contamination [9]. Les pays d'Amérique du Nord comptent parmi les plus grands producteurs et consommateurs de composés pharmaceutiques avec des dépenses pouvant aller jusqu'à 18 milliards de dollars par an pour l'ensemble des citoyens canadiens et plus de la moitié de ceux-ci consommant un médicament sous prescription chaque mois [10,11]. Avec des milliers de produits en vente et des centaines de milliers en cours de développement, le marché pharmaceutique nord-américain en constante expansion se chiffre à des centaines de milliards de dollars par année et seulement aux États-Unis; les 200 composés les plus répandus sont prescrits plus de deux milliards de fois [10]. Lorsque l'on considère les analgésiques, ce sont des pays d'Europe tels que la France et la Suède qui sont les plus grands consommateurs. Certains composés tels que l'acétaminophène ou l'ibuprofène sont consommés respectivement à plus de 3000 tonnes et 200 tonnes par année [12,13]. Un bon exemple pour illustrer l'augmentation de la demande de certains médicaments est celui des psychostimulants servant aux troubles d'attention et d'hyperactivité puisqu'ils connaissent une croissance de consommation moyenne de 12% par année dans différents pays tels que le Canada [14]. Les problèmes de plus en plus connus de résistance bactérienne en lien avec les antibiotiques ont encouragé de nombreuses études sur cette classe de médicaments qui connaissent une augmentation de consommation de l'ordre de 35 % à l'échelle mondiale et jusqu'à 90% pour certains composés dans des pays en voie de développement [15–17]. Or des études démontrent que la présence et les concentrations de ces contaminants dans l'environnement peuvent être reliées à leur consommation [18,19].

1.3 Sources de contamination des composés pharmaceutiques

La consommation des composés pharmaceutiques étant répandue à l'ensemble de la population et non pas à un secteur d'activité spécifique, tel que pourrait être le cas de contaminants comme les pesticides, fait en sorte que les sources de contaminations sont multiples. Les usines de production et de recherche sur les médicaments sont sujettes à des réglementations de certaines municipalités qui dictent la gestion des déchets et des rejets pour limiter les quantités de contaminants pharmaceutiques. En absence de ces restrictions, des concentrations importantes sont observées dans des réseaux d'égouts avoisinant des industries pharmaceutiques et ceux-ci sont alors mélangés aux eaux usées municipales [20].

Les hôpitaux et les établissements de soins de santés sont aussi une source importante de rejets de médicaments. Certains traitements spécifiques y sont administrés et des contaminants sont donc susceptibles d'être retrouvés en concentrations élevées dans leurs eaux usées [21,22].

C’est notamment le cas pour les agents de chimiothérapie qui, malgré qu’il y ait de plus en plus de prescriptions pour leur consommation en dehors de l’hôpital, sont souvent administrés par intraveineuse au sein de ces établissements [23]. Les concentrations de certains autres médicaments comme les analgésiques ou les antibiotiques sont soupçonnés d’y être rejetés à de hauts niveaux puisqu’une forte proportion des gens dans ces établissements les consomment [21].

Des campagnes de sensibilisation sont effectuées au Canada afin d’informer les gens sur les bonnes manières de disposer des médicaments. Ainsi, on tente de limiter les quantités de ces contaminants qui peuvent se retrouver dans des sites d’enfouissement. On veut également éviter que des comprimés soient jetés aux toilettes puisque ceux-ci se retrouveraient mélangés aux eaux usées municipales [24]. Or, lorsque des médicaments sont consommés, ceux-ci peuvent être excrétés de manière intacte ou sous forme de métabolites divers via l’urine et les excréments et une quantité non négligeable de composés pharmaceutiques se retrouvent tout de même dans les réseaux d’égout [25].

Les usines de traitement qui reçoivent les eaux usées municipales ne sont généralement pas conçues pour éliminer les médicaments et leurs métabolites [26–28]. La Figure 1-1 illustre que l’enlèvement des contaminants pharmaceutiques dépend grandement du type de traitement employé. En effet, un traitement primaire, qui consiste en un traitement mécanique tel qu’une décantation, a une efficacité très limitée comparativement à d’autres technologies plus poussées telles que celle des bioréacteurs à membrane ou encore l’électro-oxydation avancée. L’efficacité de ces différentes approches de traitement dépendant des propriétés physico-chimiques des contaminants, certaines classes pharmaceutiques telles que les analgésiques subissent un abattement plus important lors de leur séjour dans les usines de traitement des eaux usées [28,29].

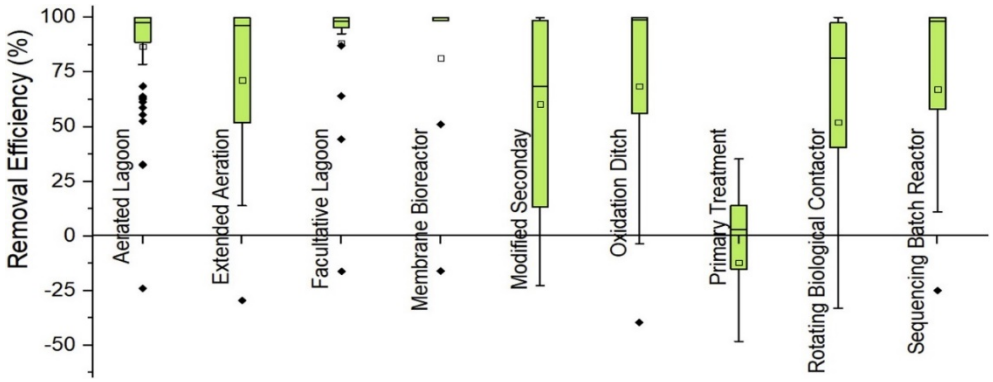


Figure 1-1. Efficacité de l'élimination de composés pharmaceutiques par différentes usines de traitement des eaux usées au Canada (figure tirée de Greenham et al, 2019).

Au Canada, leurs rejets sont réglementés, mais principalement pour contrôler la matière organique et les phosphates alors qu'il n'y a pas vraiment de contrôle en termes de composés pharmaceutiques. Il y a donc des concentrations importantes de ces contaminants qui sont rejetées dans l'eau de surface, notamment dans des cours d'eau à proximité de villes densément peuplées autour des Grands Lacs ou le long du Fleuve Saint-Laurent [23,30]. Différents projets sont en cours de développement afin de modifier les systèmes d'épuration actuels ou de proposer de nouvelles alternatives pour remédier à ce problème. Certains groupes de recherche s'intéressent à la possibilité de cibler certaines sources spécifiques de composés pharmaceutiques telles que les hôpitaux ou les usines qui les produisent.

Une autre source non négligeable de certains composés pharmaceutiques est l'industrie vétérinaire qui administre de grandes quantités d'hormones et d'antibiotiques pour les élevages de bétail, de volaille ainsi que pour des productions en aquaculture. Une contamination peut donc avoir lieu via les eaux usées, mais également par un lessivage du sol ou un ruissèlement des contaminants dans les cours d'eau avoisinants les sites d'utilisation [31–34].

La Figure 1-2 illustre bien les différentes voies majeures de contamination des pharmaceutiques entre leur production, leur consommation et leur utilisation jusqu'à leur dispersion dans l'environnement via les eaux de surfaces et souterraines. Certaines études se sont intéressées à la quantification de ces contaminants dans diverses matrices environnementales au Canada [28–30,35–40]. Or, plus de données sont nécessaires pour étudier les effets de ces contaminants sur des organismes près de points de rejet d'eaux usées ou sur la santé d'écosystèmes complexes à proximité de ceux-ci tel que dans les Grands Lacs et le Fleuve Saint-Laurent [23]. Plus de données sur les sources de ces contaminants et leur traitement peuvent également apporter des arguments pour l'amélioration des technologies actuelles ou encore sur l'établissement de normes plus sévères pour des établissements qui contribuent plus lourdement à la pollution des produits pharmaceutiques.

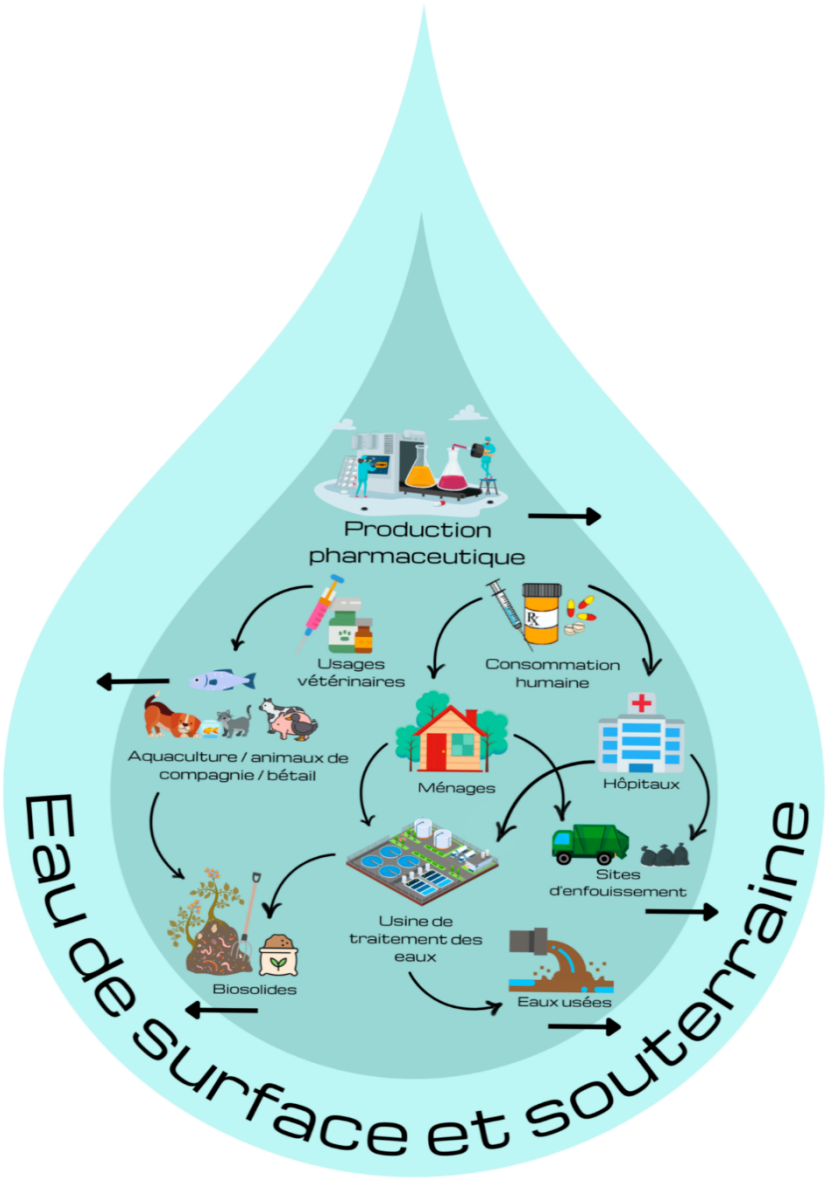


Figure 1-2. Principales voies de contamination environnementale des composés pharmaceutiques de la production jusqu'au rejet dans l'eau de surface et souterraine (figure adaptée de OECD, 2019).

D'autre part, les voies de pollution de l'environnement dépendent de l'utilisation de ces composés et donc de la classe pharmaceutique à laquelle ils appartiennent. Ceci aura également un effet sur le transport, la persistance ainsi que sur la toxicité des contaminants sur les différents milieux affectés. Il importe donc de cibler des composés de différentes classes pharmaceutiques lors d'études sur leur pollution et leurs impacts environnementaux.

1.4 Classes de composés pharmaceutiques et effets écotoxicologiques

Les très nombreux composés pharmaceutiques actuellement disponibles ou en cours de développement peuvent être catégorisés de différentes manières, soit par famille de structure chimique, par mode d'action, par effet physiologique ou encore par classe thérapeutique. Cette dernière classification est utile pour déterminer l'usage médical des médicaments pour traiter différentes pathologies et elle contient 40 différents sous-groupes selon les critères de la FDA [41]. Des médicaments de certains de ces groupes ont été sélectionnés parmi les composés les plus consommés afin de représenter leur classe thérapeutique pour avoir une vue d'ensemble de la contamination environnementale par les composés pharmaceutiques dans les matrices ciblées.

1.4.1 Analgésiques

Les analgésiques, qui sont utilisés pour traiter la douleur, comprennent des composés en vente libre tel que ceux pour traiter les maux de tête (ex : Advil, Tylenol, Aspirine) ou des composés plus puissants tels que des opioïdes (ex : Fentanyl, Codéine, Dilaudid). La Figure 1-4 présente certains des analgésiques ciblés par les méthodes présentées dans cette thèse.

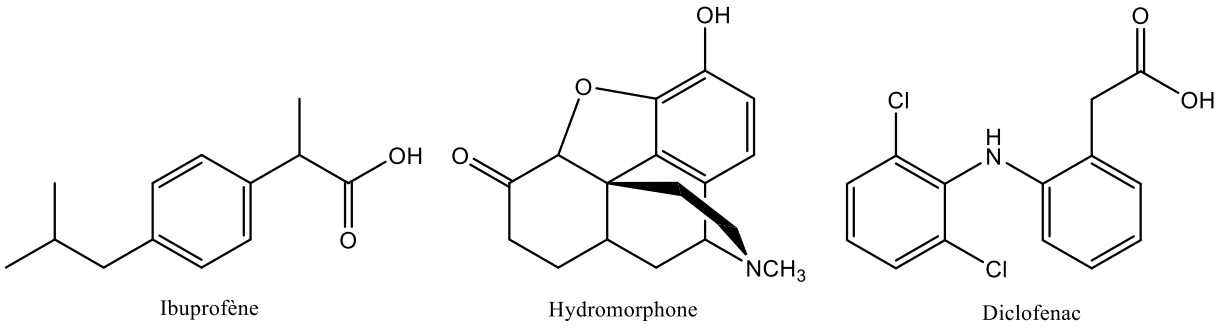


Figure 1-3. Structure de molécules ciblées pour les analgésiques.

Des publications ont soulevé des problèmes liés à une exposition à de fortes concentrations d'analgésiques telles que celles pouvant être retrouvées près de certains effluents municipaux. Parmi ces effets, on observe une baisse de fécondité de différents organismes dans la chaîne trophique [40,42]. Une exposition chronique à ce type de contaminants a aussi mené à des changements comportementaux et à une hausse de mortalité de certaines espèces de poissons et crustacés [43–45].

1.4.2 Antibiotiques

Les antibiotiques forment une famille de composés utilisés pour traiter ou prévenir des infections bactériennes qui peuvent être regroupés en sept grandes familles selon leur mécanisme d'action et les microbes qu'ils ciblent: les pénicillines (ex. amoxicilline, Figure 1-5), les fluoroquinolones, les tétracyclines, les macrolides (ex. clarithromycine, Figure 1-5), les céphalosporines, les aminoglycosides et les sulfonamides (ex. sulfaméthoxazole, Figure 1-5).

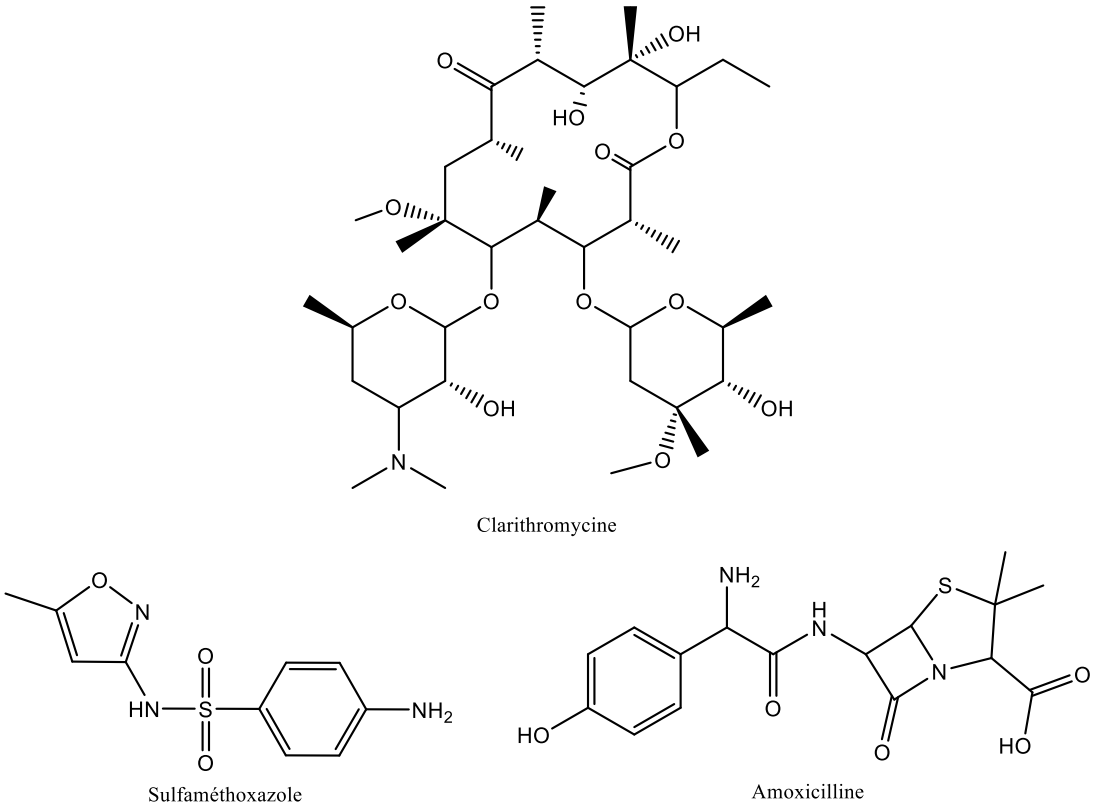


Figure 1-4. Structures de certaines molécules d'antibiotiques ciblées.

Certains antibiotiques sont efficaces seulement contre un nombre limité d'infections alors que certains autres, dits à spectre large, peuvent servir à traiter de nombreuses maladies. Or quand ces derniers se retrouvent dans l'environnement, ils sont plus susceptibles d'être à l'origine de résistance bactérienne. Malgré qu'une majeure partie des antibiotiques dans l'environnement proviennent de l'aquaculture ou de source vétérinaire en lien avec la production de viande, des quantités importantes de ces composés sont consommées par les humains. Les quantités résiduelles de ces contaminants dans l'environnement accentuent le phénomène de résistance bactérienne existant dans l'organisme traité avec un antibiotique qui n'élimine pas certaines souches bactériennes qui possèdent des gènes de résistance à celui-ci. Cette résistance peut se trouver chez des microorganismes pathogènes, mais peut aussi être transférée par différents mécanismes à d'autres bactéries. Ainsi, certaines maladies seront potentiellement très

difficilement combattues par les traitements actuellement disponibles [46,47]. Certaines études suggèrent que les antibiotiques à des concentrations relativement faibles dans l'environnement peuvent avoir des effets toxiques pour certains organismes aquatiques [42,48].

1.4.3 Chimiothérapie

Les agents de chimiothérapie sont utilisés dans le traitement de différents cancers et sont principalement divisés selon leur mécanisme d'action soit les antinéoplasiques qui stoppent la progression des masses cancéreuses ou les cytostatiques qui inhibent la division cellulaire. Certains d'entre eux sont utilisés dans le traitement d'autres pathologies tel que le méthotrexate (Figure 1-6) consommé pour diminuer la réponse immunitaire et aider à gérer l'arthrite rhumatoïde et psoriasique [49].

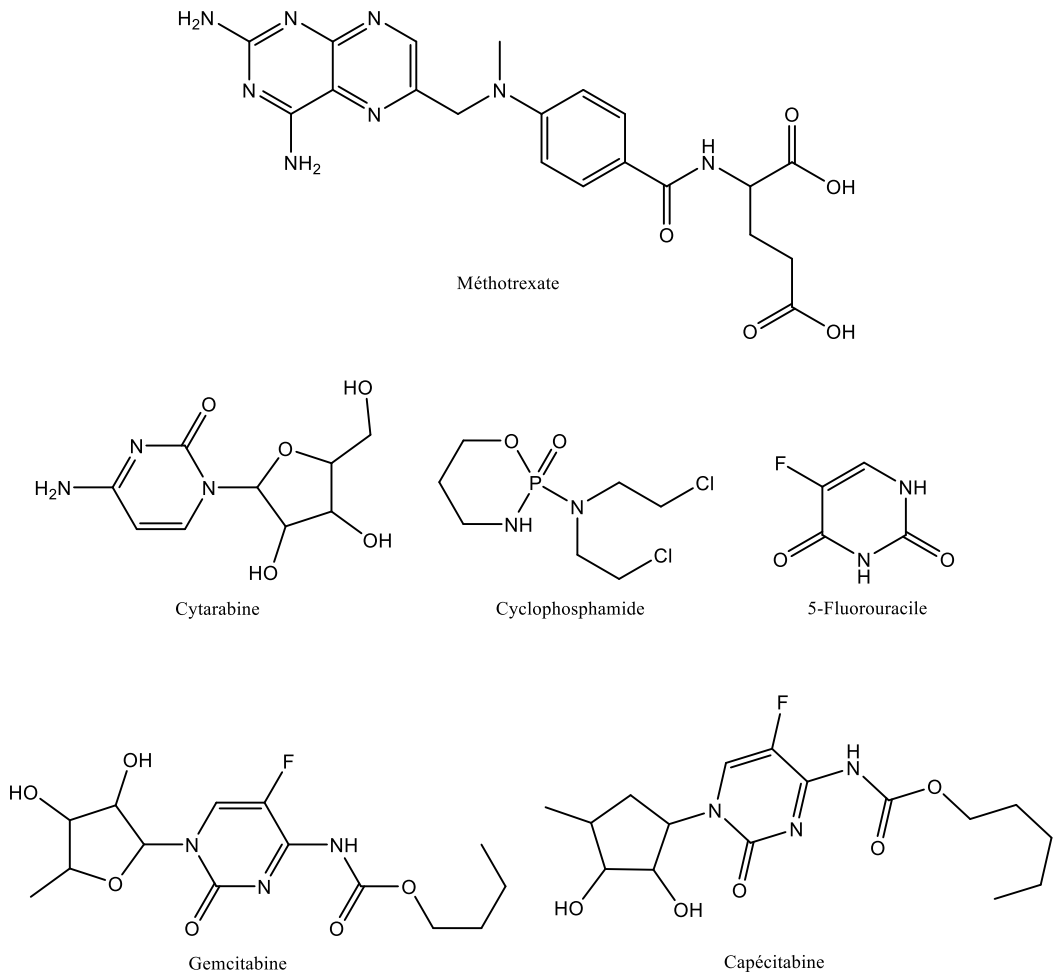


Figure 1-5. Structure de molécules ciblées pour les agents de chimiothérapie.

Leur mécanisme d'action est directement lié à leur potentiel de dangerosité lorsque consommé sans fins thérapeutiques. Or, ces contaminants peuvent se retrouver dans l'environnement et causer des effets toxiques sur la faune et la flore à proximité d'effluents municipaux [50]. Des problèmes de fertilité et le développement de cancer seraient potentiellement causés par des expositions à de faibles doses de produits de chimiothérapie chez des mammifères et chez l'humain [51].

1.4.4 Hormones

Les hormones, tant naturelles que synthétiques, ont des applications pharmaceutiques multiples. En plus d'une utilisation très répandue des hormones pour la contraception, celles-ci sont utilisées en tant qu'anti-inflammatoire, comme thérapie de remplacement hormonal, pour traiter un déséquilibre menstruel ou durant la ménopause. Elles sont aussi utilisées pour augmenter les performances physiques comme dans le cas de certains agents de dopage dans les sports de compétition ou parfois dans le traitement de certains cancers [52]. Des exemples de composés utilisés à ces fins sont présentés à la Figure 1-7.

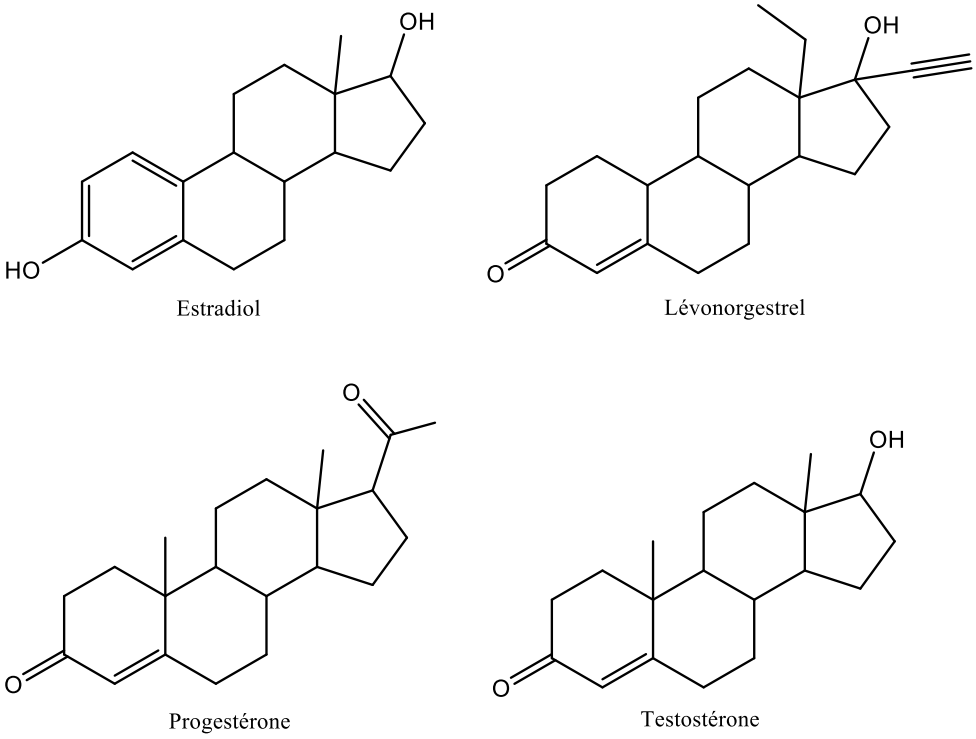
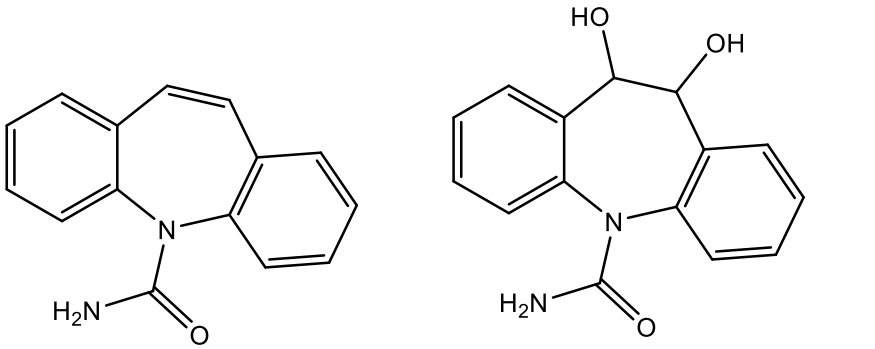


Figure 1-6. Structure de molécules ciblées pour les hormones.

Ces contaminants sont en partie de source naturelle puisque certaines hormones sont sécrétées chez l'humain et certains animaux sans médication. Toutefois, sans l'apport en hormones des rejets d'eaux usées ou de déchets de l'industrie vétérinaire, ces composés n'atteignent normalement pas des seuils de pollution pour les milieux aquatiques [53]. Ces derniers sont toutefois à des niveaux de concentration très faibles et sont reconnus comme perturbateurs endocriniens [43,54]. Lors d'études de l'exposition aux hormones chez des organismes aquatiques, des effets sont observés au niveau de plusieurs organes tels que le foie, le cerveau ou les reins, mais un des effets les plus notables est au niveau des gonades [55]. On observe une baisse drastique de la fertilité ou de la mobilité, une féminisation des spécimens mâles et ultimement un déclin sévère de la population pour la faune exposée [56]. Des effets de perturbation endocrinienne peuvent potentiellement affecter les humains lorsque des quantités résiduelles d'hormones sont consommées, par exemple via l'eau potable [57].

1.4.5 Anticonvulsivants

Les anticonvulsivants sont des composés psychoactifs utilisés dans les traitements de l'épilepsie. Certains d'entre eux sont également utilisés pour traiter les troubles d'humeur tels que la bipolarité ou atténuer les symptômes de certains cancers du cerveau [58]. Les structures du médicament antiépileptique le plus consommé au Canada ainsi que son principal métabolite sont présentées à la Figure 1-8.



Carbamazépine 10,11-Dihydroxy-10,11-dihydrocarbamazepine

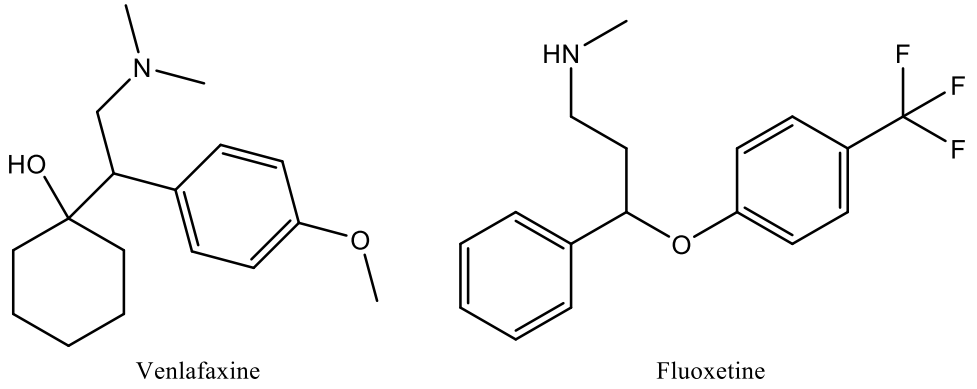
Figure 1-7. Structure de molécules ciblées pour les anticonvulsivants.

Ces composés pharmaceutiques sont parmi les plus retrouvés dans l'environnement et souvent cités comme contaminants prioritaires pour l'étude de ces effets écotoxicologiques [54]. Malgré que ce ne sont pas les molécules présentant la plus grande toxicité, les hauts niveaux de

contamination et leur résistance à la dégradation contribuent à causer des cas de toxicité aigüe pour certains organismes vivants dans ces milieux [9]. Une atteinte au niveau de certains organes tels que le foie et les reins chez des poissons ou encore une diminution en biodiversité de certaines algues sont des exemples observés d'effets néfastes sur la faune et la flore de milieux naturels contaminés par les anticonvulsivants [59,60].

1.4.6 Antidépresseurs

Les antidépresseurs sont principalement utilisés pour traiter la dépression et les troubles d'anxiété, mais également pour contrôler des troubles alimentaires tels que la boulimie ou atténuer certaines douleurs chroniques [61,62]. Deux de ces composés fréquemment retrouvés dans l'environnement sont présentés à la Figure 1-9.



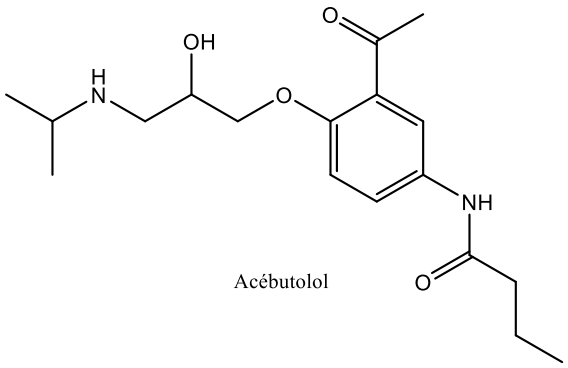
Venlafaxine Fluoxetine

Figure 1-8. Structure de molécules ciblées pour les antidépresseurs.

Cette classe de composés pharmaceutiques est particulièrement nocive pour la faune et présente un facteur d'impact parmi les plus importants en dépit de leurs faibles concentrations dans l'environnement [54,63]. Cet impact dépend des organismes qui sont touchés, mais va de la perte de mobilité et de comportement de fuite face à des prédateurs jusqu'à une toxicité sévère en passant par des effets délétères sur le développement de certaines espèces d'invertébrés, d'amphibiens et de poissons [64].

1.4.7 Bêtabloquants

Les bêtabloquants sont compris parmi les traitements contre les troubles circulatoires ou cardiaques. Ils servent à traiter l'hypertension ou à réduire les battements cardiaques. L'acébutolol (Figure 1-10) est un représentant de cette classe pharmaceutique qui est fortement utilisé au Canada.



Acébutolol

Figure 1-9. Structure de molécules ciblées pour les bêtabloquants.

Ces médicaments ne sont pas reconnus pour être particulièrement problématiques puisqu'ils sont généralement bien gérés par les usines de traitement des eaux et qu'ils ne possèdent pas un grand potentiel de toxicité [65,66]. Toutefois, certaines études soulèvent que les modèles généralement utilisés pour mesurer ce potentiel tiennent rarement compte d'une exposition répétée à long terme et que certains bêtabloquants ont des effets chroniques sur des crustacés et poissons à proximité de points de rejet d'eaux usées [67,68].

1.4.8 Caféine

La caféine (Figure 1-11), qui n'est pas en soi un composé pharmaceutique, est un stimulant souvent utilisé comme marqueur de contamination anthropique. Le café étant fortement consommé à travers le monde, cette molécule est retrouvée presque systématiquement dans les eaux usées et parfois dans des milieux très isolés tels que des lacs montagneux et peut servir à déterminer le niveau de contamination de sites à proximité de points de rejet ou d'activité humaine ou encore à prédire la concentration de coliformes fécaux de l'eau de surface [69–73]. Ce composé est donc fréquemment ajouté à la liste des composés cibles de méthodes analytiques pour l'analyse de contaminants organiques dans des matrices environnementales [54,74]. Une exposition chronique à la caféine peut être cause de troubles de la croissance ou de mortalité de

certains organismes aquatiques ou de grenouilles et pourrait même contribuer au phénomène du blanchissement des récifs coralliens [72].

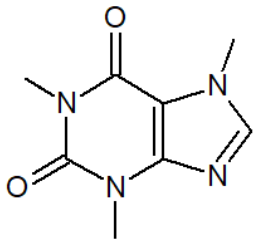


Figure 1-10. Structure de la caféine.

Vu les grandes quantités consommées de ces composés pharmaceutiques, ceux-ci sont susceptibles d'être retrouvés en fortes concentrations dans différentes matrices environnementales où ils auront de potentiels effets écotoxicologiques. L'impact qu'un contaminant peut avoir sur des organismes dans un milieu environnemental dépend de plusieurs facteurs tels que ses propriétés physicochimiques, son mécanisme d'action, sa concentration, sa persistance ou dégradabilité, sa biodisponibilité, bioaccumulation et bioamplification ou encore sa toxicité [75–78]. Par exemple, un contaminant pourrait avoir un impact important à long terme s'il est très stable alors qu'un contaminant se dégradant rapidement, mais émis de manière continue, pourrait être considéré comme pseudo-persistant et avoir des effets sur la faune et la flore. Il est impératif de prendre en compte ces phénomènes lorsque vient le temps de déterminer les effets des composés pharmaceutiques dans des matrices environnementales [79].

1.5 Toxicité des contaminants pharmaceutiques

Pour comparer la toxicité de différents contaminants, des valeurs de concentrations prévisibles sans effet sur le milieu (PNEC) sont déterminées en appliquant un facteur de précaution à la valeur de concentration sans effet observable (NOEC) sur la croissance ou la reproduction ou à des valeurs de doses létales (LD_{50}) [80–82]. Ces valeurs peuvent être déterminées à la suite de tests d'expositions en laboratoire des substances individuelles à différents niveaux de concentrations et pour divers organismes. Or, pour les composés pharmaceutiques, il existe une lacune dans la collecte de données de toxicité pouvant permettre d'obtenir des valeurs précises de PNEC [80–83]. Ce manque est partiellement comblé par l'utilisation de modèles prédictifs qui permettent d'estimer des valeurs de PNEC en l'absence de données empiriques. La détermination des composés cibles prioritaires pour améliorer la qualité des milieux environnementaux suite à l'activité humaine demeure toute de même un défi.

Certaines études suggèrent tout de même des valeurs estimées de PNEC en se basant sur les quelques données disponibles pour différents organismes et en appliquant un facteur d'incertitude [84,85]. Le Tableau 1-1 contient des exemples de valeurs de PNEC pouvant éventuellement servir à une évaluation du potentiel de risque d'échantillons contaminés par les composés pharmaceutiques. Toutefois, une approche plus complète nécessiterait l'évaluation d'autres paramètres environnementaux tels que la persistance, la mobilité ou encore la bioaccessibilité de ces contaminants.

Tableau 1-1. Exemple de données de PNEC basées sur la toxicité pour des organismes d'eau douce [86–99].

Composé	**Valeurs de PNEC (ng/L)**
10,11-Dihydroxy-10,11-dihydrocarbamazépine	1910
2-Hydroxyibuprofène	7880
4-Hydroxydiclofénac	220
4-Nitrososulfaméthoxazole	3810
Acébutolol	2930 ; 10100
Amoxicilline	78 ; 37
Caféine	1200 ; 320 ; 87000
Carbamazépine	2000 ; 420 ; 10 ; 250
Clarithromycine	120 ; 20
O-Desmethylvenlafaxine	7110
Diclofénac	50 ; 20 ; 1 ; 5
Estradiol (E2)	0.3 ; 2 ; 0.4
Estriol (E3)	60 ; 51
Estrone (E1)	3.6 ; 20
Éthinylestradiol (EE2)	0.037
Fluoxétine	100 ; 3 ; 50
Hydromorphone	3640
Ibuprofène	11 ; 10
Lévonorgestrel	0.01
Médroxyprogestérone	6720
Méthotrexate	69
Noréthindrone	4520
Norfluoxétine	1700
Progestérone	1000 ; 19
Sulfaméthoxazole	600 ; 30 ; 2400 ; 18 ; 27
Testostérone	4370
Venlafaxine	880 ; 92

Bien que certaines valeurs de PNEC soient relativement élevées et que le potentiel de risque des composés individuels soit faible comparativement aux autres contaminants émergents tels que les pesticides, certaines études suggèrent que ces valeurs devraient être revues fortement à la baisse en considérant leur mode d'action [81–83,100]. Ces valeurs sont aussi généralement plus faibles dans le cas d'organismes inférieurs dans la chaîne trophique. Tel est le cas par exemple de l'hormone éthynylestradiol, de l'antidépresseur venlafaxine ou des analgésiques ibuprofène et diclofénac pour lesquelles des valeurs jusqu'à 1000x plus faibles sont parfois mentionnées dans la littérature [101,102]. Ces valeurs, prenant parfois compte d'une exposition chronique, négligent l'effet synergique potentiel de ces substances dans différents milieux exposés à des sources variables de contamination [81,82]. Or pour arriver à des valeurs de PNEC plus justes et déterminer le potentiel de ces contaminants émergents, des données de concentrations doivent être recueillies en plus grand nombre, et ce pour différentes matrices et pour différents organismes cibles.

1.6 Devenir environnemental des composés pharmaceutiques

Les gammes de concentrations de ces différentes classes de composés pharmaceutiques dans l'environnement dépendent de la nature de la matrice ciblée, de la stabilité des molécules, mais surtout de la consommation de celles-ci. Les quantités maximales mesurées varient donc grandement entre différents pays n'ayant pas les mêmes tendances de consommation ou les mêmes politiques de gestion des eaux usées et des déchets. Le Tableau 1-2 présente certaines valeurs rapportées dans la littérature à travers le monde et au Canada.

Tel que mentionné précédemment, il n'est pas étonnant de retrouver de la caféine dans les eaux usées. Or, des concentrations très élevées sont parfois mesurées telles que l'exemple de près de 4 mg/L dans un affluent de Singapour, indiquant un très haut niveau de contamination des eaux usées de cette région [103]. Cette donnée n'est pas un cas isolé puisque des dizaines de µg/L de certains médicaments sont retrouvés dans des eaux usées de divers pays, comme l'ibuprofène (52 µg/L en Australie), l'amoxicilline (21 µg/L au Vietnam) ou encore l'aténolol (310 µg/L aux États-Unis) [104–107]. Certains composés sont heureusement présents en moindre quantité, comme le cas des hormones ou de certains agents de chimiothérapie qui possèdent généralement un potentiel de toxicité plus élevée comparativement à d'autres médicaments tels que les anticonvulsivants ou les antidépresseurs souvent retrouvés à des centaines ou des milliers de ng/L [108–118].

Tableau 1-2. Exemple de concentrations de divers composés pharmaceutiques dans l'eau usée à travers le monde.

Classe Pharmaceutique	Composé	Concentration (ng/L)	Pays	Référence
Analgésiques	Diclofénac	400	Australie	Ahmed et al. 2021
	Ibuprofène	52 000		
Antibiotiques	Amoxicilline	21 000	Vietnam	Anh et al. 2021
	Sulfaméthoxazole	6 400		
	Clarithromycine	640	Singapour	
Chimiothérapie	5-Fluorouracile	280	Slovénie	Ljoncheva et al. 2020
	Méthotrexate	4700	Espagne	Castelano-Hinojosa et al. 2023
	Ifosfamide	86 000		
	Gemcitabine	61		
Hormones	Testostérone	390	Émirats Arabes Unis	Almazrouei et al. 2023
	Éthinylestradiol	31	Chine	Tang et al. 2021
Anticonvulsivants	Carbamazépine	260 000	États-Unis	Feijoo et al. 2023
Antidépresseurs	Venlafaxine	1 700	Chine	Wang et al. 2023
	Fluoxétine	61		
Bêtabloquants	Acébutolol	250	Suède	Yi et al. 2020
	Aténolol	310 000	États-Unis	
Stimulant	Caféine	3 600 000	Singapour	Li et al. 2020

Les eaux de surface qui reçoivent les eaux usées atteignent parfois des niveaux de contamination dans les mêmes ordres de grandeur que dans les effluents des usines de traitement des eaux, surtout lorsque les traitements appliqués ne sont pas adaptés à leur enlèvement. Par exemple, des hormones ont été quantifiées à des concentrations de quelques dizaines de ng/L dans des rivières en Asie [111,112]. Toutefois, les concentrations mesurées sont généralement plus faibles dans l'eau de surface en raison de différents facteurs tels que la photodégradation ou parfois un important facteur de dilution. Or, c'est surtout dans ces milieux que des effets délétères pourront être observés sur la faune et la flore.

Ainsi, tel qu'illustré par les exemples de valeurs présentées dans le Tableau 1-3, des quantités importantes des mêmes composés que dans les effluents sont également rapportées à proximité de points de rejet. Tel est le cas pour la clarithromycine (1.2 µg/L au Japon), la carbamazépine (12 µg/L en Italie), l'aténolol (11 µg/L en Espagne) ou la caféine (12 µg/L au Brésil) [54,103,105,106,114]. Les concentrations d'autres composés sont significativement plus faibles dans l'eau de surface comme dans le cas des hormones ou de la plupart des produits de chimiothérapie qui sont détectés de manière plus sporadique [112,116,117,119]. Toutefois, ces

composés sont de plus en plus utilisés et il y a un manque flagrant de données sur leur occurrence et celle de leurs différents métabolites qui présentent souvent des mécanismes d'action similaire à leur molécule mère [109,120]. Enfin, certains médicaments sont présents à des concentrations inférieures à ce qu'on retrouve dans les eaux usées, mais sont détectés de manière presque systématique dans des échantillons d'eau de surface à proximité de zones densément peuplées [54,110,121]. Des analgésiques tels que l'ibuprofène ou des anticonvulsivants tels que la carbamazépine ont donc servi dans de nombreuses études comme marqueur de la contamination par les produits pharmaceutiques dans des matrices environnementales [104,113].

Tableau 1-3. Exemple de concentrations de divers composés pharmaceutiques dans l'eau de surface à travers le monde.

Classe Pharmaceutique	Composé	Concentration (ng/L)	Pays	Référence
Analgésiques	Diclofénac	72 000	Pakistan	Waleng et al. 2022
	Ibuprofène	85 000	Afrique du Sud	
Antibiotiques	Sulfaméthoxazole	575	Thaïlande	Anh et al. 2021
	Clarithromycine	1 200	Japon	
Chimiothérapie	Méthotrexate	6.3		
	Capécitabine	20	Espagne	Hinojosa et al. 2023
	Cyclophosphamide	500		
	5-Fluorouracile	160	Taiwan	Ljoncheva et al. 2020
Hormones	Testostérone	480	Chine	Yazdan et al. 2022
	Éthinylestradiol	28	Vietnam	Tang et al. 2021
Anticonvulsivants	Carbamazépine	12 000	Italie	Adeleye et al. 2022
Antidépresseurs	Venlafaxine	240	États-Unis	Wang et al. 2023
	Fluoxétine	100	Royaume-Uni	Adeleye et al. 2022
Bêtabloquants	Acébutolol	8.0	Finlande	Yi et al. 2020
	Aténolol	11 000	Espagne	
Stimulant	Caféine	12 000	Brésil	Li et al. 2020

Au Canada, quelques études ont aussi documenté de manière sporadique la présence de ces contaminants, tant dans les eaux usées que dans l'eau de surface. Le Tableau 1-4 présente des exemples de concentrations de produits pharmaceutiques quantifiés dans ces matrices. Les classes pharmaceutiques les plus fréquemment détectées dans d'autres pays, ainsi que les composés rapportés à des concentrations élevées sont aussi détectés au Canada. Tel est le cas des analgésiques, des antibiotiques, des anticonvulsivants ainsi que des antidépresseurs [35,36,38,39,114,122–127]. Les composés généralement présents à plus faible concentration, tels que les produits de chimiothérapie, les hormones et les bêtabloquants, le sont également

dans les matrices environnementales du Canada [36,38,39,120,128–130]. Globalement, les concentrations sont moindres que dans certains pays avec une densité de population nettement plus élevée ou pour lesquels il peut y avoir des manques au niveau du traitement des eaux et des politiques environnementales. D'autre part, malgré que la contamination dans l'eau de surface soit moindre que dans l'eau usée, la somme des concentrations en composés pharmaceutiques peut tout de même représenter un problème, d'autant plus que ceux-ci sont rejetés quotidiennement via les effluents municipaux puisque les usines de traitement des eaux ne sont que partiellement efficaces à leur enlèvement.

Tableau 1-4. Exemple de concentrations de divers composés pharmaceutiques dans les eaux usées et l'eau de surface du Canada.

Matrice	Classe Pharmaceutique	Composé	Concentration (ng/L)	Référence
Eau usée	**Analgésiques**	Diclofénac	28 000	Metcalfe et al. 2004
		Ibuprofène	25 000	
	Antibiotiques	Sulfaméthoxazole	3 300	Koné et al. 2013
		Clarithromycine	540	Metcalfe et al. 2004
	Chimiothérapie	Méthotrexate	59	Garcia-Ac et al. 2009
		Cyclophosphamide	5	Metcalfe et al. 2004
	Hormones	Testostérone	59	Goeury et al. 2019
		Éthinylestradiol	20	Metcalfe et al. 2004
	Anticonvulsivants	Carbamazépine	3 300	Koné et al. 2013
	Antidépresseurs	Venlafaxine	3 000	Melchor-Martinez et al. 2021
		Fluoxétine	510	Fong et al. 2014
	Bêtabloquants	Acébutolol	660	Koné et al. 2013
		Aténolol	1 700	
	Stimulant	Caféine	680	Metcalfe et al. 2004
Eau de surface	**Analgésiques**	Diclofénac	190	Metcalfe et al. 2004
		Ibuprofène	1 200	Marcogliese et al. 2015
	Antibiotiques	Sulfaméthoxazole	290	Koné et al. 2013
		Clarithromycine	260	Segura et al. 2007
	Chimiothérapie	Méthotrexate	53	Poirier Larabie et al. 2022
		Cyclophosphamide	5	Metcalfe et al. 2004
	Hormones	Testostérone	10	Berryman et al. 2014
		Estradiol	11	
	Anticonvulsivants	Carbamazépine	700	Koné et al. 2013
	Antidépresseurs	Venlafaxine	900	
		Fluoxétine	140	
	Bêtabloquants	Propanolol	50	Brun et al. 2009
	Stimulant	Caféine	950	Berryman et al. 2014

Enfin pour bien évaluer l'ampleur de la contamination par les produits pharmaceutiques, il est important de ne pas omettre la phase particulaire dans les eaux usées ou les sédiments des rivières qui reçoivent des effluents. En effet, certains composés auront tendance à s'adsorber à aux particules, les rendant plus persistants et pouvant les rendre plus biodisponibles pour les organismes en contact avec les sédiments [131–133]. Il y a donc un intérêt croissant à développer des méthodes d'extraction et de purification afin d'évaluer la teneur de ces contaminants dans ces matrices. Avec une forte teneur dans des eaux de surface, il n'est pas étonnant que des concentrations élevées d'antibiotiques soient quantifiées dans des sédiments de rivières polluées. C'est le cas de la norfloxacine et de l'ofloxacine qui atteignent respectivement 5 800 et 1600 ng/g de sédiments (poids sec) provenant de rivières en Chine. Certains analgésiques, dont l'usage est très répandu, sont également retrouvés dans grand nombre d'échantillons de sédiments avec des concentrations pouvant atteindre plusieurs dizaines de ng/g de sédiments (poids sec). De surcroît, certains composés moins fréquemment détectés dans des eaux de surface sont retrouvés dans la phase particulaire. Par exemple, des produits de chimiothérapie au Japon (entre 0.11 et 0.40 ng/g), des œstrogènes en Chine (entre 1 et 52 ng/g) ou des antidépresseurs en Allemagne (jusqu'à 15 ng/g) qui malgré de plus faibles concentrations sont reconnus pour avoir un plus grand potentiel écotoxicologique [134–136]. Une fois stabilisés à la surface de particules, certains contaminants pourront être transportés sur de grandes distances et ainsi contaminer des milieux naturels plus éloignés. Ainsi, des contaminants pharmaceutiques sont présents dans les sédiments des fonds marins, dans des régions forestières ou montagneuses et également dans les zones polaires de l'Arctique et de l'Antarctique. Ces polluants ont également été retrouvés dans des particules atmosphériques, ce qui augmente davantage leur distribution [137–142]. D'autre part, bien que des traitements plus poussés soient employés pour le traitement de l'eau destinée à la consommation, de nombreux médicaments sont tout de même retrouvés dans cette matrice. L'eau potable peut donc être une voie d'exposition directe et chronique à différents polluants pour l'humain [143–146].

La plupart des études se concentrant sur une classe spécifique de composés pharmaceutiques et l'impossibilité méthodologique de quantifier l'ensemble des médicaments dans une matrice environnementale font en sorte que la charge de médicaments est souvent sous-estimée. Il y a donc un intérêt à développer des méthodes multiclasses avec des composés marqueurs pour chaque classe pharmaceutique afin de brosser un portrait plus juste de la contamination dans les matrices ciblées. Toutefois, ses méthodes doivent faire un compromis en terme de performances analytiques afin de parvenir à une quantification de composés possédant des propriétés physico-chimiques très diverses [147–151]. Un autre facteur qui contribue à la

sous-estimation de la charge totale de la contamination par les composés pharmaceutiques est le nombre limité de publications s'intéressant aux produits métaboliques des médicaments. Pourtant, ceux-ci sont généralement la forme principale retrouvée dans les déjections humaines et ont donc été quantifiés à des concentrations plus importantes dans des eaux usées [152–154]. Plusieurs réactions de dégradation peuvent survenir selon la nature du médicament, comme des réactions d'oxydoréduction, l'hydrolyse ou l'hydratation, l'isomérisation ou encore la conjugaison [155,156]. Les dérivés ainsi formés peuvent parfois prendre la forme de composés actifs et avoir des effets semblables à leur molécule mère, mais les impacts sur des organismes non ciblés sont peu documentés. La métabolisation permet une élimination plus efficace des pharmaceutiques résiduels dans l'organisme, mais peut, par le fait même, rendre ces contaminants plus solubles dans des matrices environnementales. En plus de leur conférer une plus grande mobilité, ceux-ci sont donc parfois plus stables à la dégradation, moins bien pris en charge par les usines de traitement des eaux usées et certains sont même retransformés en leur molécule mère par des microorganismes (i.e, plusieurs molécules conjuguées peuvent être relativement facilement déconjuguée dans l'environnement) [25,157,158].

Afin de prévenir les impacts écotoxicologiques, il est primordial de limiter les quantités de médicaments susceptibles de se retrouver dans l'environnement. Or, c'est un défi de grande taille vu l'impossibilité de stopper l'utilisation massive des composés pharmaceutiques. À défaut de pouvoir moderniser l'ensemble des usines de traitement des eaux usées afin d'assurer une meilleure gestion des composés pharmaceutiques, des campagnes de sensibilisation et certaines politiques sont mises en place pour tenter de réduire la contamination environnementale.

1.7 Politiques et gestion des composés pharmaceutiques

La gestion des déchets de production de médicaments, de l'élimination de leur surplus dans la population ou encore sur les technologies de traitement des eaux et de leur efficacité sont très variables dans le monde. Il est démontré que les politiques pour contrôler la consommation et limiter la pollution des composés pharmaceutiques peuvent avoir un impact majeur sur les concentrations retrouvées dans des matrices telles que l'eau de surface et les effets écotoxicologiques qui en découlent [9]. Malgré l'efficacité incomplète des usines de traitement à dégrader ces molécules, cette option est tout de même préférable au rejet direct d'effluents non traités tel que c'est encore le cas dans plusieurs pays [159].

Les centres de production de médicaments ont majoritairement des protocoles pour éviter de rejeter des produits chimiques dangereux, mais la plupart des municipalités n'effectuent pas des tests pour déterminer les concentrations en pharmaceutiques qui sont rejetées via les eaux usées. Aux États-Unis, cet enjeu a été récemment adressé en exigeant que les institutions susceptibles de générer une grande contamination par des produits pharmaceutiques développent un système de récupération et de destruction de substances contrôlées afin d'éviter que celles-ci ne se retrouvent dans les eaux usées ou les sites d'enfouissement. Or, cette politique est difficilement applicable aux rejets dus à la consommation par les patients dans les hôpitaux ou encore aux médicaments qui sont disposés par la population en général [160]. En Europe, certains pays adoptent des règlementations plus strictes afin de limiter la pollution par les composés pharmaceutiques, notamment avec l'approche du principe de précaution. Toutefois, peu de produits pharmaceutiques ont été désignés comme substances prioritaires par manque de données et il s'avère difficile de s'assurer que ces dernières ne se retrouvent jamais dans des matrices environnementales [161,162]. Au Canada, les composés pharmaceutiques ne sont typiquement pas inclus dans la liste des substances prioritaires bien que les effets de certains médicaments, tels que les hormones, soient étudiés pour établir des recommandations sur des seuils à ne pas dépasser [38,163]. Avec l'élaboration de méthodes robustes et sensibles, plus de données pourraient être récoltées lors d'études sur les comportements de ces contaminants dans différents effluents ainsi que sur leurs effets sur la faune et la flore. Celles-ci seraient un outil considérable pour l'établissement de critères plus sévères quant à la qualité de différentes matrices en termes de composés pharmaceutiques.

2 Développement de méthodes analytiques pour l'analyse des composés pharmaceutiques

2.1 Défi analytique

Les composés pharmaceutiques présentent un défi pour le développement de méthodes analytiques en raison de leurs propriétés physicochimiques très variées selon leurs classes thérapeutiques ou de leurs mécanismes d'action. De plus, leur présence dans différentes matrices environnementales fait en sorte que ces méthodes doivent être à la fois robustes et versatiles. Certains médicaments, notamment les composés de chimiothérapie, sont très polaires et peuvent donc s'avérer difficiles à extraire et préconcentrer à partir d'échantillons aqueux. De plus, les échantillons environnementaux sont des matrices complexes qui peuvent être très chargées tel que dans le cas des eaux usées requérant l'utilisation de méthodes de purification permettant de s'affranchir d'une majorité d'interférents affectant les analyses [164]. Ce défi est d'autant plus grand pour des échantillons solides pour lesquels des étapes d'extraction doivent être minutieusement optimisées en amont de la purification afin de procéder aux analyses de contaminants.

Parmi les techniques les plus fréquemment utilisées dans les dernières années, il y a une emphase particulière sur la chromatographique liquide ou gazeuse (LC ou GC) couplée à la spectrométrie de masse (MS). Avant ces analyses, les échantillons doivent être préparés à l'aide de techniques de séparation telles que l'extraction assistée par micro-onde (MAE) ou par ultrasons (UAE), l'extraction accélérée par solvant (ASE) ou sous-pression (PLE) et l'extraction sur phase solide (SPE). Ces techniques sont choisies pour leur versatilité et leur simplicité résultant en des analyses robustes et reproductibles pour des contaminants organiques aux propriétés physicochimiques diverses dans des matrices contaminées ou complexes telles que les eaux usées ou des échantillons biologiques [165].

2.2 Extraction assistée par ultrasons

La technique utilisée pour l'analyse des composés pharmaceutiques ciblés dans les échantillons solides (matière en suspension, sédiments, boue de traitement, tissus biologiques) est l'extraction assistée par ultrasons. Elle consiste en l'immersion des échantillons préalablement homogénéisés dans un solvant organique exposé à des ultrasons qui par la formation de bulles de vapeurs favorisent la pénétration dans la matrice et par le fait même accélèrent l'extraction. L'utilisation d'un bain à ultrasons (Figure 2-1) permet de procéder à l'extraction d'un grand nombre d'échantillons simultanément et est une option abordable qui a démontré des recouvrements satisfaisants pour diverses classes de contaminants organiques dans des matrices solides complexes [166–169].

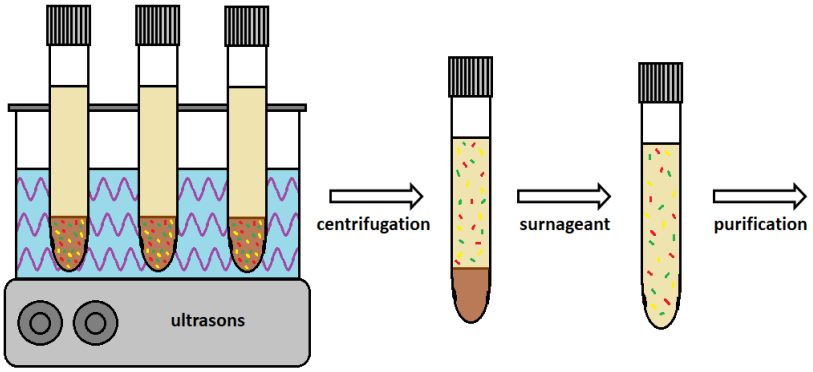


Figure 2-1. Protocole général pour l'extraction assistée par ultrasons.

Afin de maximiser l'efficacité de cette technique pour des analytes aux propriétés physico-chimiques diverses, plusieurs paramètres doivent être optimisés. Parmi ceux-ci, on compte la nature du solvant, y compris son pH, sa température et son volume, le nombre de cycles ainsi que le temps de sonication [170–173]. Une étape de lyophilisation, un procédé qui consiste à déshydrater une substance congelée par sublimation, est souvent utilisée en amont des étapes d'extractions enfin d'extraire l'eau sans dénaturer la structure et les propriétés physicochimiques de l'échantillon tout en augmentant sa perméabilité au solvant d'extraction. Cette technique de préparation d'échantillons est particulièrement utile pour des matrices complexes telles que les tissus biologiques et permet une meilleure homogénéité, précision et améliore le rendement de l'extraction subséquente [174–177]. Enfin, une mesure du taux de récupération permet de valider qu'un maximum d'analytes puisse être extrait et que les différentes étapes d'extraction ne mènent pas à des pertes de certains composés ciblés. Le Tableau 2-1 présente différentes procédures d'extraction assistées par ultrasons qui ont été précédemment appliquées pour l'analyse de composés pharmaceutiques dans diverses matrices.

Tableau 2-1. Exemples de méthodes d'extraction assistée par ultrasons pour des contaminants pharmaceutiques dans des matrices environnementales.

Analytes	Matrice ciblée	Prétaitement des échantillons	Solvant	Sonication	Référence
Pharmaceutiques, parabènes et métabolites	Boues d'épuration	Lyophilisation et homogénéisation	MeOH + 0.5 % FA	3 cycles de 15 min	Malvar et al. 2020
Pharmaceutiques et hormones	Sol agricole	Séchage et homogénéisation	Acétate d'éthyle + 2% FA	3 cycles de 15 min	Kumirska et al. 2019
Antibiotiques et analgésiques	Muscle et plasma de poisson	Broyage et homogénéisation	Tampon phosphate : ACN + 0.2% FA (V/V = 1:3)	3 cycles de 10 min	Liu et al. 2018
Analgésiques et antiépileptiques	Sol et sédiments	Séchage et tamisage	1. Eau, 2. MeOH/ACN (1/1)	1. 15 min, 2. 20 min	Hlengwa et al. 2020
Pharmaceutiques et caféine	Sédiments	Séchage et homogénéisation	1. MeOH, 2. acétone	1. 2 cycles 5 min, 2. 2 cycles 5 min	Ohoro et al. 2021

En plus de pouvoir être appliquée à différentes matrices environnementales telles que des sédiments ou des sols agricoles, l'extraction assistée par ultrasons permet d'extraire des quantités traces de contaminants dans celles-ci. Ainsi, les limites de détections peuvent atteindre des niveaux de l'ordre du ng/g ou du pg/g dépendamment la complexité et la nature des échantillons [172,178,179]. D'autre part, il a été démontrer que cette technique est aussi applicable pour la quantification de concentrations élevées en pharmaceutiques dans des boues d'épuration [173]. Pour ce faire, il faut impérativement inclure une étape de purification des extraits avant leur injection sur des systèmes analytiques.

2.3 Extraction sur phase solide

Parmi les techniques de purification les plus utilisées, l'extraction sur phase solide (SPE) a été choisie pour sa simplicité d'exécution et un large choix de phases solides permettant de réduire les impuretés et interférents présents dans les matrices environnementales. Cette technique a aussi pour avantage d'utiliser de faibles volumes de solvants tout en permettant d'atteindre des recouvrements supérieurs aux techniques telles que l'extraction liquide-liquide. La SPE est également un outil très efficace pour la préconcentration d'analytes présents à l'état de trace dans les matrices environnementales [180].

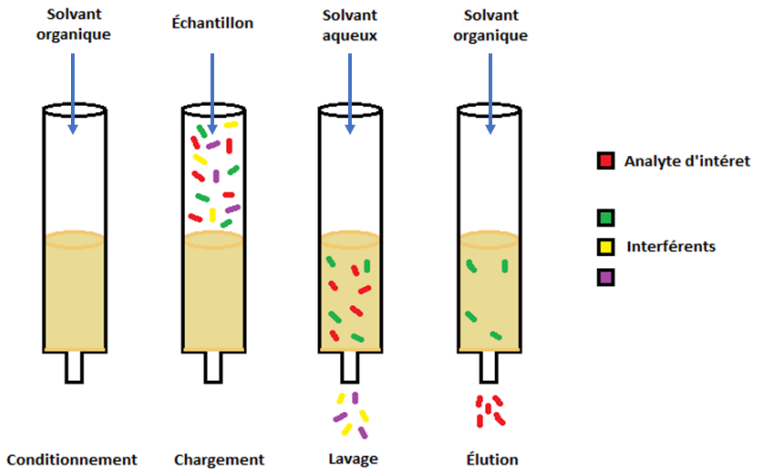


Figure 2-2. Protocole général de l'extraction en phase solide (SPE).

Cette technique repose sur l'affinité des analytes avec une phase stationnaire empaquetée dans une cartouche (Figure 2-2). Lors du passage d'un échantillon par la cartouche, les composés seront adsorbés à la surface de la phase stationnaire, puis une étape de lavage avec un solvant de faible force éluotropique permet de s'affranchir de certains interférents et une grande partie de la matrice qui ne possèdent pas une bonne affinité avec la phase stationnaire. Ensuite, les analytes sont élués avec un solvant ayant une force éluotropique plus grande et dans lequel les analytes d'intérêt sont solubles. Ce dernier, souvent un solvant organique, peut alors être évaporé afin que l'extrait puisse être reconstitué dans un solvant qui sera compatible avec le système analytique employé pour l'analyse des échantillons. Pour les cartouches chromatographiques en mode normal et inverse la force éluotropique dépendra de la polarité des solvants alors que des cartouches d'échange ionique utiliseront des phases mobiles dont le pH est contrôlé pour obtenir la forme neutre ou ionisée des composés d'intérêt pour permettre d'abord leur rétention lors de l'étape de charge et de lavage, puis leur percolation lors de l'élution [181–183].

Dans le cadre des travaux présentés dans cette thèse, différentes cartouches possédant une phase stationnaire contenant des groupements amphiphiles, ont été testées afin de déterminer leur potentiel pour la purification de multiples classes de composés pharmaceutiques dans les matrices environnementales ciblées. Leur efficacité a été comparée avec des cartouches de type C18 afin de déterminer si elles s'avèrent plus aptes à l'enrichissement simultané de molécules présentant une variabilité de polarité et de constante d'acidité (pKa) [184–186]. Le Tableau 2-2 présente différentes applications de ce type de cartouche pour la préconcentration et la purification de composés pharmaceutiques dans des échantillons environnementaux.

Tableau 2-2. Exemples de procédures SPE hors ligne pour la préconcentration et la purification de contaminants pharmaceutiques dans des matrices environnementales.

Analytes	Matrice ciblée	Volume/masse d'échantillon	Type de cartouche	Référence
Pharmaceutiques et drogues	Eau potable	200 mL	Polymérique en mode mixte (Strata-X-Drug B)	Peng et al. 2019
Pharmaceutiques et produits d'hygiène personnelle	Eau de surface, eau souterraine et eau potable	500 mL	Échange de cation en mode mixte (Oasis MXC)	Ebele et al. 2020
Pharmaceutiques et caféine	Eau usée	250 mL	Balance hydrophilique-lipophilique (Oasis HLB)	Al-Qaim et al. 2018
Pharmaceutiques et hormones	Boues d'épuration et sols	500 mg	Balance hydrophilique-lipophilique (Oasis HLB) en mode purification de l'extrait	Silva et al. 2021
Pharmaceutiques	Boues d'épuration	300 mg	Balance hydrophilique-lipophilique (Oasis HLB) en mode purification de l'extrait	Pérez-Lemus et al. 2022
Antibiotiques	Viande et œufs	5000 mg	Balance hydrophilique-lipophilique (PRiME HLB) en mode purification de l'extrait	Lu et al. 2019
Antibiotiques, hormones et sédatifs	Viande	2500 mg	Balance hydrophilique-lipophilique (PRiME HLB) en mode purification de l'extrait	Zhang et al. 2018

De telles cartouches sont donc utilisées en mode de préconcentration classique, mais également en mode de purification par percolation où un extrait en phase organique est passé à travers cette dernière afin d'éliminer rapidement une partie des interférents. Ce mode est particulièrement intéressant lorsqu'utilisé à la suite de l'extraction d'analytes dans des échantillons solides fortement contaminés ne nécessitant pas une forte préconcentration, mais pouvant présenter une matrice complexe fortement chargée [187–190].

La SPE peut également être directement couplée avec un système de chromatographie liquide afin d'automatiser l'extraction. Ceci permet entre autres de minimiser les manipulations requises, augmentant la répétabilité d'une méthode, ainsi que de diminuer le temps d'analyse et la quantité de solvant par échantillon. Une colonne chromatographique peut être conditionnée simultanément au chargement de l'échantillon sur la colonne SPE qui sera rincée avant le couplage. Les composés qui seront ensuite élués de la cartouche pourront ainsi être directement séparés lors de leur transfert sur la colonne chromatographique (Figure 2-3). Enfin, la cartouche SPE peut être conditionnée pendant la durée du gradient chromatographique pour recevoir le prochain échantillon [191,192]. Cette technique s'avère particulièrement utile dans le cadre d'études environnementales nécessitant l'analyse d'un grand nombre d'échantillons.

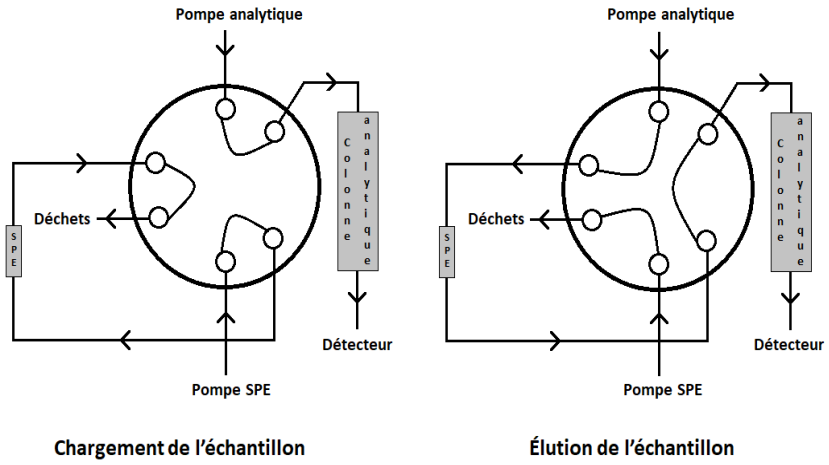


Figure 2-3. Schéma simplifié du fonctionnement de la SPE en ligne.

Plusieurs paramètres tels que la vitesse de charge, les conditions de lavage, le volume de l'échantillon ou la nature des phases mobiles doivent être optimisés afin d'obtenir une méthode avec des recouvrements satisfaisants pour l'ensemble des analytes ciblés, surtout dans le cas des composés pharmaceutiques appartenant à diverses classes de structures moléculaires. Par contre, il existe certaines limitations pour ce type de prétraitement d'échantillons comparativement à la SPE classique, tel qu'un moindre choix de phases stationnaires, des volumes restreints

d'échantillons ou encore l'ajout de pression supplémentaire sur le système de pompes chromatographiques. Aussi, la nature des solvants, l'utilisation de tampons ou de sels ou encore le pH des phases mobiles doivent être compatibles avec les conditions chromatographiques ainsi qu'avec le détecteur employé à la suite de la SPE en ligne. Cette technique gagnant en popularité, de plus en plus de méthodes y font appel lors de l'analyse d'échantillons environnementaux (Tableau 2-3).

Tableau 2-3. Exemples de procédures SPE en ligne pour la préconcentration et la purification de contaminants pharmaceutiques dans des matrices environnementales.

Analytes	Matrice ciblée	Volume d'échantillon	Type de cartouche	Référence
Pharmaceutiques et hormones	Eau de surface	2.5 mL	Balance hydrophilique-lipophilique (Oasis HLB)	Camilleri et al. 2015
Pharmaceutiques	Eau de surface, eau potable et eau souterraine	5 mL	Balance hydrophilique-lipophilique (Oasis HLB)	Liang et al. 2019
Pharmaceutiques, PFAS et retardateurs de flamme	Eau potable	1.8 mL	Phase inverse polymérique (PLRP-s)	Zhong et al. 2019
Pharmaceutiques	Eau usée	50 µL	Balance hydrophilique-lipophilique (Oasis HLB)	Marasco Junior et al. 2021
Chimiothérapie et antidépresseurs	Eau usée	0.9 mL	C18 (Hypersil GOLD aQ)	Belay et al. 2022

L'utilisation de méthodes SPE en ligne est donc tout indiquée pour l'analyse de contaminants organiques tels que les produits pharmaceutiques dans des matrices environnementales comme les eaux usées ou l'eau de surface. Les exemples ci-dessus démontrent que des petits volumes d'échantillons sont requis lors de l'application de telles méthodes, ce qui facilite l'échantillonnage et l'entreposage des échantillons. Enfin, suite aux étapes de préconcentration et de purification des échantillons, leurs différents constituants doivent être séparés afin d'améliorer les performances d'analyse.

2.4 Chromatographie liquide

La chromatographie liquide (LC) est une technique de séparation fréquemment utilisée pour l'analyse de composés pharmaceutiques dans des matrices environnementales. Sa versatilité et sa robustesse en ont fait un choix indéniable vis-à-vis d'autres techniques telles que la chromatographie gazeuse (GC) ou l'électrophorèse capillaire (CE). Ces techniques peuvent présenter des avantages, mais aussi des limitations, justifiant l'utilisation plus répandue de la LC-MS pour l'analyse de ces contaminants dans l'environnement. Par exemple, l'emploi de la chromatographie gazeuse permet d'atteindre de faibles limites de détections et le couplage avec l'impact électronique est moins sensible aux effets de matrice. Toutefois, cette technique est limitée à l'analyse de composés à la fois volatils et thermostables, ce qui requiert souvent une étape de dérivatisation pour l'analyse de composés pharmaceutiques [147,193–196]. L'électrophorèse capillaire est, quant à elle, utilisée en raison de sa grande résolution lors de la séparation des échantillons, mais celle-ci ne permet pas d'atteindre des limites de détection aussi poussées étant donné qu'un volume limité d'échantillon d'une matrice complexe peut être injecté. Tout comme la GC, cette technique requiert généralement un prétraitement d'échantillon plus laborieux que la chromatographie liquide [147,197,198]. La LC est donc particulièrement avantageuse lors de l'analyse de contaminants organiques, spécialement pour les analytes polaires, dans des matrices très chargées telles que les eaux usées, les extraits de sédiments ou les échantillons biologiques.

Afin d'obtenir un grand pouvoir de séparation avec des méthodes de courtes durées, des particules plus fines (de taille <2 µm) sont utilisées avec un système de pompes à haute pression. L'augmentation de résolution obtenue avec cette technique, la chromatographie liquide à ultra-haute performance (UPLC), permet l'obtention de pics plus fins et de plus grande intensité, ce qui permet l'optimisation de méthodes très sensibles pour un nombre important de composés par injection.

Les matrices environnementales étant souvent en milieux aqueux, la chromatographie en phase inverse (RPLC) est généralement un premier choix judicieux pour l'analyse de contaminants organiques tels que les composés pharmaceutiques. La phase stationnaire la plus fréquemment utilisée fait appel à des chaînes carbonées octadécyles (C18) greffées à la surface de silice qui permettra la séparation des constituants d'un échantillon en fonction de leur polarité (Figure 2-4).

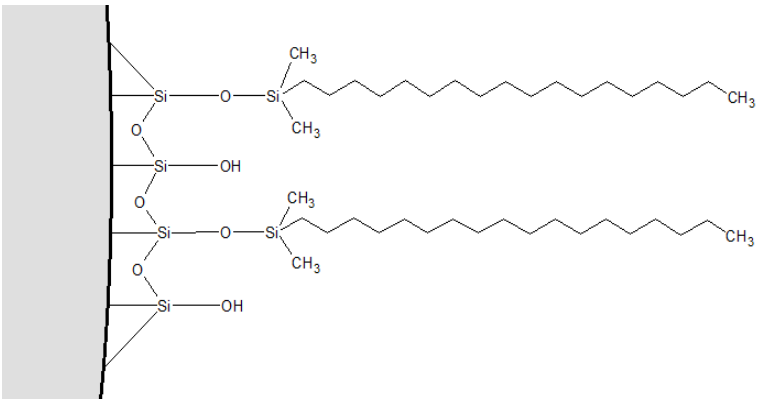


Figure 2-5. Schéma de phase stationnaire en phase inverse de type C18.

Certains polluants de matrices environnementales sont difficilement analysables par RPLC en raison de leur polarité qui diminue grandement leur interaction avec les phases stationnaires classiques. Alors que certaines techniques de chromatographie en phase normale ou de dérivatisation des analytes sont parfois employées, ces options sont parfois difficilement applicables à des échantillons aqueux et à des matrices environnementales complexes [199–201]. La chromatographie à interaction hydrophile (HILIC) est une alternative pouvant résoudre ce problème en combinant certains avantages de la chromatographie en phase normale, en phase inverse ainsi que la chromatographie ionique [202]. Celles-ci permet donc la séparation d'analytes ayant des valeurs de log *P* négatives, comme c'est le cas par exemple de certains composés pharmaceutiques dont notamment les produits de chimiothérapie [203,204].

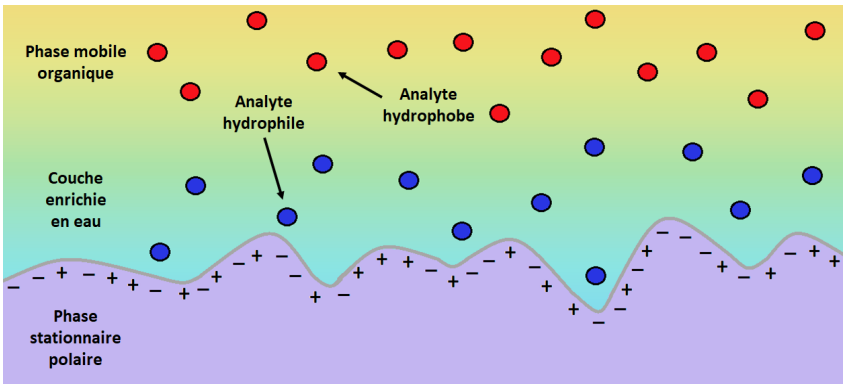


Figure 2-4. Schématisation du principe de séparation HILIC.

La séparation HILIC repose sur la formation d'une couche enrichie d'eau à la surface de la phase stationnaire de la colonne chromatographique (Figure 2-5). Les analytes polaires auront donc une plus forte affinité avec la phase stationnaire, qui est composée de groupements polaires greffés sur des supports de silice de même type qu'en chromatographie en phase normale [205]. Les analytes peuvent être injectés en phase organique, ce qui s'avère utile pour l'injection suivant une extraction dans un solvant tel que dans le cas de la SPE, ou suite à l'extraction d'échantillons

solides. L'utilisation d'un solvant miscible dans l'eau permet enfin de varier la proportion de la phase aqueuse afin d'éluer les analytes. L'ajout de certains additifs tels que des sels et des tampons est souvent nécessaire pour ce type de chromatographie afin de stabiliser le pH de la phase mobile ou de conserver la forme ionisée de certains analytes. Cette technique a l'avantage d'être compatible avec la plupart des systèmes analytiques tels que l'ionisation par électronébulisation couplée à la spectrométrie de masse. Par contre, la séparation HILIC nécessite parfois des méthodes plus longues puisque les colonnes de ce type requièrent un conditionnement afin de former et de stabiliser la couche enrichie en eau sans laquelle il n'y a que très peu de séparation [202,205,206].

2.5 Spectrométrie de masse

La spectrométrie de masse est un outil puissant pour l'analyse de contaminants à l'état de trace dans des échantillons complexes tel que dans des matrices environnementales. Grâce à sa spécificité, elle permet d'atteindre des limites de détections de l'ordre des parties par billiard (ppq) qui peuvent s'avérer nécessaires lors de l'analyse des effets écotoxicoloques de certaines substances sur des organismes. De plus, cette technique est applicable à un large éventail de molécules tant en termes de masse molaire qu'en terme de polarité, et sans certaines limitations spécifiques à d'autres techniques telles que la présence de groupement chromophore/fluorophore. Cette technique permet également de faire l'élucidation de structure de molécules ou encore l'analyse de composés non ciblés dans matrices biologiques ou environnementales [207,208]. L'analyse par spectrométrie de masse s'effectue en deux temps :

- Les analytes sont d'abord chargés dans une source d'ionisation. Les techniques d'ionisation les plus utilisées sont l'impact électronique (EI), l'ionisation chimique à pression atmosphérique (APCI), la désorption-ionisation assistée laser assistée par matrice (MALDI) et l'électronébulisation (ESI).
- Ensuite, les ions seront analysés selon leur rapport masse sur charge (m/z). Les analyseurs les plus utilisés sont le temps de vol (TOF), l'orbitrap, la résonance cyclotronique (FT-ICR) et le quadripôle.

Le choix de la source d'ionisation et du type d'analyseur est dicté en fonction du besoin de la méthode ou de la nature des analytes. Dans le cadre de cette thèse, les méthodes employées font appel à l'électronébulisation couplée avec un spectromètre de masse de type triple quadripôle.

2.5.1 L'ionisation par électronébulisation (ESI)

Les propriétés physicochimiques des analytes pouvant influer sur l'efficacité de leur ionisation, il est important d'utiliser une source d'ionisation ayant un bon rendement à la suite d'un couplage avec la chromatographie liquide. Il faut par exemple sélectionner une source d'ionisation qui tienne compte de la gamme de masses molaires et de polarités de nos composés ainsi que de leur stabilité thermique. Les différentes classes de composés pharmaceutiques ayant des propriétés très variées, la source d'ionisation par électronébulisation (ESI) a été sélectionnée. Cette technique est dite douce puisqu'elle permet de générer des ions relativement stables et de contrôler la fragmentation des analytes lors du processus d'ionisation. En plus d'être versatile en termes de polarité des composés permettant l'analyse de composés relativement apolaires à très polaires, c'est la source d'ionisation ayant le meilleur rendement pour l'analyse de molécules de haut poids moléculaire, mais pouvant également ioniser des petits analytes (Figure 2-6) [209]. De plus, elle permet d'obtenir des ions multiplement chargés, ce qui s'avère utile pour l'analyse de molécules de grande masse molaire telle que des protéines ou des chaînes peptidiques.

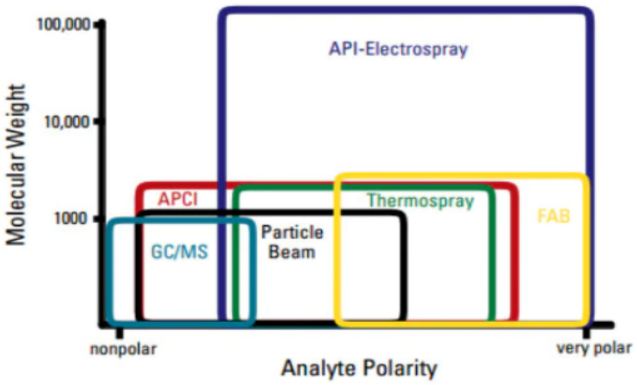


Figure 2-6. Comparaison des gammes de masse et de polarité d'analytes compatibles avec différentes sources d'ionisation (figure tirée de Agilent-Technologies, 1998).

Le principe la technique ESI repose sur l'application d'un potentiel électrique menant à la formation de gouttelettes chargées du solvant provenant de la chromatographie liquide. Cette ionisation se fait à pression atmosphérique avec l'application de gaz inerte et de chaleur afin de favoriser l'évaporation du solvant afin de réduire la taille de gouttelettes chargées. Les analytes dissous se trouveront sous forme chargée et libre après que les forces de répulsions électrostatiques auront dépassées la limite de Rayleigh, dictée par la tension de surface des gouttelettes de solvant, résultant en à la fission de Coulomb (Figure 2-7). Pendant ce processus, les molécules chargées se déplacent sous l'effet de la différence de potentiel entre le capillaire et la sortie de la source d'ionisation menant à l'analyseur [210,211].

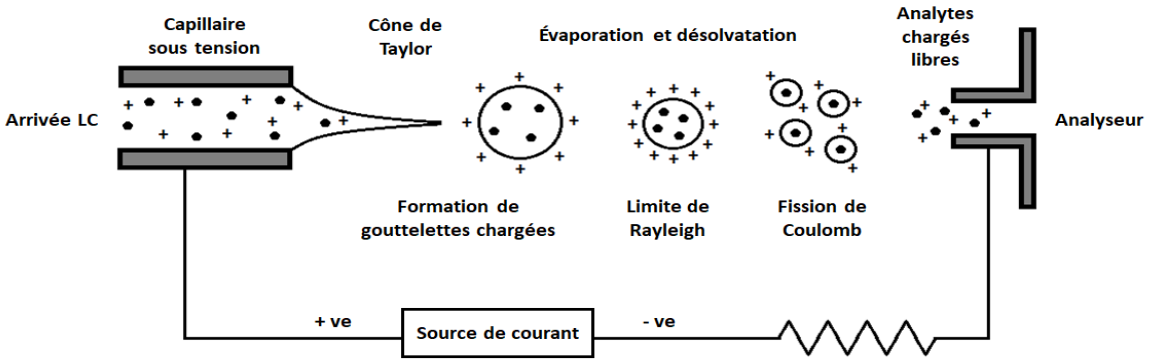


Figure 2-7. Principe général de l'ionisation positive dans une source ESI.

De manière générale, des paramètres tels que le potentiel appliqué ou la nature du solvant utilisé en combinaison avec certains additifs doivent être optimisés afin d'obtenir une ionisation favorable pour l'ensemble des analytes d'une même méthode et pour contrôler le mode d'ionisation appliqué (charge positive ou négative). Le débit des gaz et la température de la source sont également des éléments pouvant affecter l'intensité du signal observé lors de l'analyse des différentes molécules cibles.

2.5.2 Analyseur triple quadripôle

Certains analyseurs en spectrométrie de masse sont dits à haute résolution (R> 10 000) puisqu'ils peuvent faire l'analyse d'ions avec une précision sur des signaux m/z avec plusieurs décimales[212,213]. Ces analyseurs permettent de différencier deux composés de même masse nominale ayant des différences de masse exacte de l'ordre du ppm. Ceux-ci peuvent donc s'avérer incontournables dans la détermination de la composition de certains mélanges complexes ou encore pour effectuer la recherche de composés non ciblés dans des matrices chargées [214,215]. Or, pour l'élucidation de structure moléculaire ou encore la quantification spécifique d'analytes au sein d'une matrice complexe, des analyseurs de spectrométrie de masse en tandem (MS/MS), pouvant effectuer l'analyse de masse d'ions précurseurs et de leurs fragments, sont privilégiés. En effet, ces types d'analyseurs sont reconnus pour offrir une grande sensibilité et spécificité, ce qui permet de s'affranchir d'interférences isobariques pour des molécules ayant une même masse molaire [216]. Des analyseurs de masse en tandem ne nécessitent donc pas une résolution aussi élevée lorsqu'ils sont utilisés dans des méthodes pour l'analyse de composés ciblés. Tel est le cas des méthodes décrites dans cette thèse qui font appel à un analyseur de type triple quadripôle qui possède généralement une résolution de l'ordre de 1000 [217,218].

Un quadripôle consiste en deux paires d'électrodes cylindriques parallèles, l'une avec un potentiel électrique positive et l'autre négative (Figure 2-8). Avec l'application d'un courant continu fixe et en variant l'intensité d'un courant alternatif sur ces électrodes, il est possible de filtrer les analytes chargés provenant de la source d'ionisation puisque seulement les ions de masse sur charge (m/z) spécifique auront une trajectoire stable dans le quadripôle. Ainsi, seules les molécules d'intérêt seront transférées vers le détecteur alors que les ions avec des trajectoires instables percuteront l'une des électrodes et ne seront pas transmises. La résolution et la gamme de m/z pouvant être analysées par un quadripôle sont dictées par la gamme de champ électrique et les dimensions des électrodes [219,220].

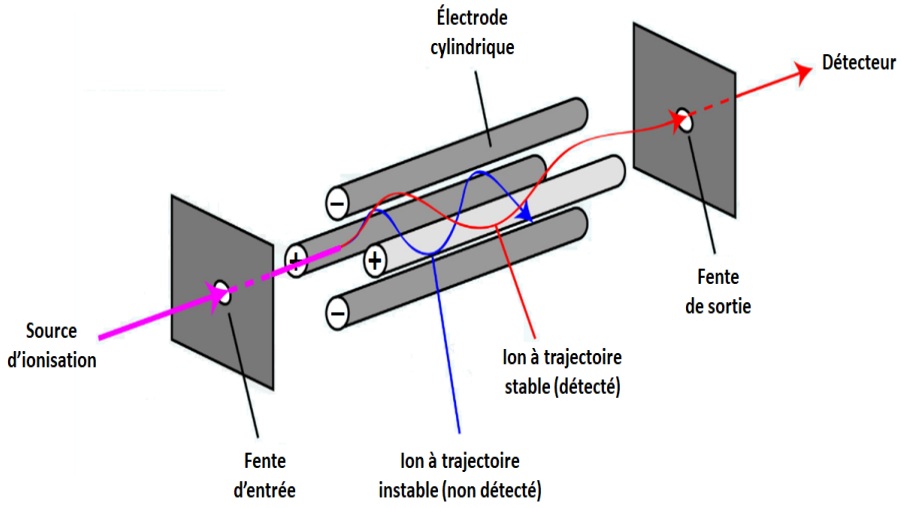


Figure 2-8. Schéma du fonctionnement d'un quadripôle linéaire (adaptée de Gates et al. 2014).

Le triple quadripôle (QqQ) est l'un des analyseurs les plus utilisés en spectrométrie de masse pour la quantification des contaminants émergents. Suite à l'introduction d'analytes chargés à la sortie de la source d'ionisation, ceux-ci sont guidés vers un premier quadripôle (Q) qui filtrera les différents ions précurseurs sélectionnés selon la méthode. Par la suite, ceux-ci peuvent être fragmentés dans la cellule de collision (q) et les ions résultants pourront être davantage filtrés lors du passage dans un deuxième filtre de masse (Q) avant d'atteindre un détecteur qui amplifiera le signal des différents ions (Figure 2-9). Le multiplicateur d'électron, l'un détecteur parmi les plus utilisés, repose sur l'amplification du signal des ions par une cascade d'électrons secondaires générés par des dynodes disposées en série [221,222].

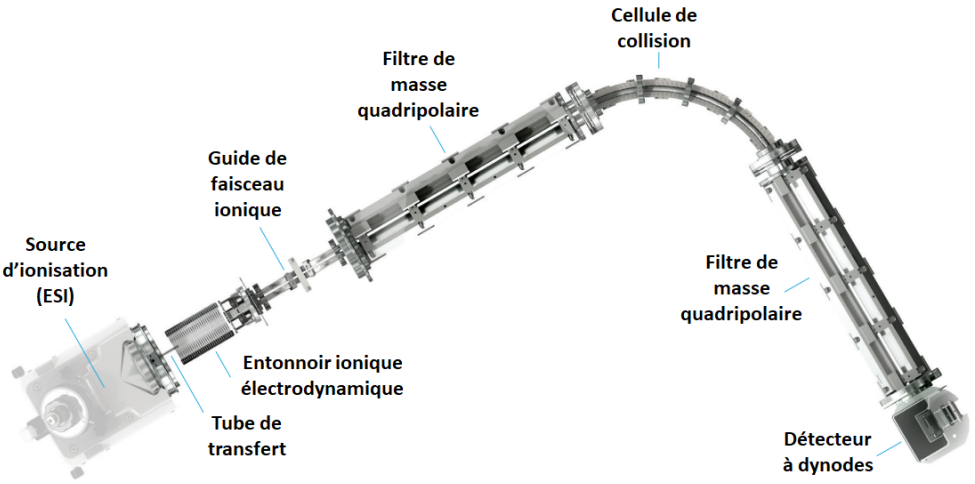


Figure 2-10. Schéma du triple quadripôle d'un appareil TSQ Quantiva de Thermo Scientific (adaptée de Thermo Fisher Scientific. 2016).

Le triple quadripôle peut être utilisé en plusieurs modes dépendamment du besoin de la méthode analytique. En ne filtrant pas les ions et en n'effectuant pas de fragmentation, on peut analyser l'ensemble des molécules ionisables dans un échantillon (mode balayage complet), or la capacité de sélectivité du triple quadripôle n'est pas utilisée. Il est également possible de sélectionner spécifiquement des ions précurseurs (balayage sélectif), mais la sélectivité est accrue lorsque des fragments sont générés dans la cellule de collision. Ainsi, il est possible de faire l'analyse de l'ensemble des ions qui subissent une perte de masse spécifique (perte de neutre), des ions parents qui possèdent un même fragment (mode des précurseurs) ou encore de mesurer le signal de l'ensemble des fragments d'un ion précurseur (balayage d'ions fragments). Enfin, le mode à multiples fragmentations sélectives (MRM) permet de filtrer des fragments spécifiques résultants d'un ou plusieurs ions précurseurs (Figure 2-10) [219,220,223].

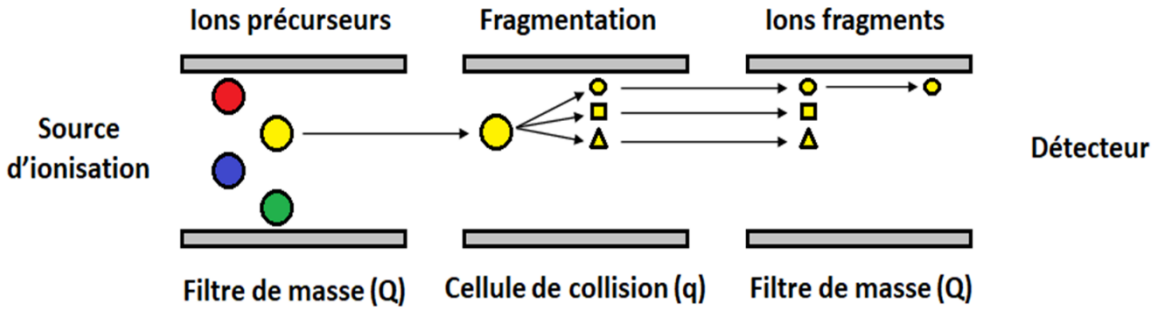


Figure 2-9. Mode de multiples fragmentations sélectives (MRM) du triple quadripôle.

La MRM permet ainsi de développer des méthodes avec un nombre important de composés ciblés, la séquence de filtration des ions précurseurs et de leurs fragments spécifiques se faisant de manière consécutive, et ce cycle d'acquisition étant répété à une grande fréquence afin de maximiser l'intensité du signal mesuré [224–227]. Ce mode est privilégié lors de la quantification dans des échantillons complexes puisqu'il est hautement spécifique et est donc celui utilisé dans l'ensemble des méthodes décrites dans cette thèse. C'est également le mode le plus fréquemment employé dans les études de quantification de contaminants environnementaux faisant appel au spectromètre de masse de type triple quadripôle [228,229].

Tableau 2-4. Exemples de méthodes analytiques faisant appel à la spectrométrie de masse pour la quantification de composés pharmaceutiques dans l'environnement.

Matrice ciblée	Nombre de composés ciblés	Prétraitement des échantillons	Séparation	Analyseur	Référence
Eau usée	32	Extraction SPE	LC	Triple quadripôle	Mostafa et al. 2023
	64	Extraction SPE	LC	Triple quadripôle	Konda et al. 2022
	35	Extraction SPE	LC	Orbitrap	Kosma et al. 2020
	22	Dérivatisation sur fibre SPME	GC	Triple quadripôle	Lopez-Serna et al. 2018
Eau de surface	26	Extraction SPE	LC	Orbitrap	Rendedula et al. 2020
	15	Extraction SPE	LC	Triple quadripôle	Yuan et al. 2020
	168	Extraction SPE	LC	Triple quadripôle	Zhang et al. 2020
	81	Extraction liquide-liquide	GC	Triple quadripôle	Skrbic et al. 2018

Ainsi, il est possible de retrouver des méthodes avec plusieurs dizaines, voire plus d'une centaine de composés pharmaceutiques, tant dans l'eau usée que dans l'eau de surface [150,230]. Or, ces méthodes font généralement la préconcentration de composés acides, basiques et neutres sur différentes cartouches SPE et des injections consécutives en mode d'ionisation positive et négative sont parfois requises afin d'inclure autant de composés ciblés [151,231]. Certaines études présentent des méthodes GC-MS, faisant souvent appel au triple quadripôle, mais tel que discuté auparavant, celles-ci se limitent à des composés compatibles avec cette technique de séparation ou nécessitant une dérivatisation [195,196]. Enfin, certaines méthodes font plutôt appel à des appareils à haute résolution, tel que l'orbitrap, ce qui, comme mentionné précédemment, permet de faire l'analyse de composés non ciblés étant donné une meilleure exactitude sur le ratio m/z mesuré [232,233]. Or, cette approche ne permet pas une quantification absolue de ceux-ci sans l'ajout de standards afin de faire l'étalonnage.

2.6 Validation de méthode

Une des étapes cruciales dans le développement d'une méthode analytique est la validation qui permet de confirmer un certain niveau de rigueur de celle-ci. Plusieurs paramètres sont donc mesurés afin de connaître entre autres les limitations de cette méthode, mais également sa justesse ainsi que sa précision. Il existe plusieurs critères de validation qui dépendent du type d'analytes et de la nature des échantillons, mais principalement de la technique analytique concernée dans la méthode. Par exemple, pour l'analyse de contaminants émergents tels que les composés pharmaceutiques dans des échantillons environnementaux, des organismes gouvernementaux tels que le Centre d'expertise en analyse environnementale du Québec du ministère de l'Environnement et de la Lutte contre les changements climatiques, de la Faune et des Parcs (CEAEQ, MELCCFP) au Canada ou de l'Agence de protection de l'environnement des États-Unis (US EPA), donnent des balises pour les critères d'acceptation d'une méthode d'analyse. Les critères utilisés dans le cadre de cette thèse sont ceux indiqués par US EPA quant au développement de méthodes analytiques pour la quantification des composés pharmaceutiques et des hormones dans des matrices telles que l'eau potable, l'eau usée, les sédiments ou les biosolides [234,235].

Afin de déterminer la concentration des analytes dans des échantillons inconnus, une solution standard contenants les analytes ciblés sera diluée afin de préparer une courbe d'étalonnage. La linéarité de cette courbe est mesurée grâce au coefficient de détermination (R^2) qui permet de confirmer qu'il y a une corrélation linéaire entre la concentration injectée et le signal mesuré. La valeur de ce coefficient doit être supérieure à 0.99 pour une courbe d'étalonnage dans une matrice environnementale. Le domaine de concentrations pour lesquelles une méthode est applicable se trouvera donc entre la limite de quantification (LOQ) et la concentration supérieure limite du domaine de linéarité, puisqu'au-delà de cette dernière, la sensibilité de la méthode n'est plus constante.

Les limites de détection (LOD) et de quantification (LOQ) sont respectivement la plus faible concentration détectable qui soit distinguable du bruit et la plus faible concentration pouvant être quantifiée avec une fiabilité suffisante. Ces valeurs sont déterminées en prenant en compte l'ensemble des étapes de préparation et d'analyse d'un échantillon et en comparant le signal obtenu d'une faible concentration dans la matrice d'intérêt avec le signal du bruit. Ces paramètres sont définis comme étant respectivement la concentration correspondant à un ratio signal/bruit de 3 pour 1 (LOD) et de 10 pour 1 (LOQ) dans la matrice ciblée. Ces limites peuvent également être déterminées à partir de mesures répétées sur un blanc (n=20) pour obtenir une moyenne et un

écart-type [236–238]. Enfin, ils peuvent être déterminées à l'aide de l'écart-type (σ) d'un étalon à faible concentration analysé en *replica* (n = 10) selon les formules suivantes [239] :

$$LOD = 3\,x\,\sigma \qquad \mathbf{Eq.\,2-1}$$

$$LOQ = 10\,x\,\sigma \qquad \mathbf{Eq.\,2-2}$$

Enfin, si les échantillons sont dans un milieu complexe, tels que des échantillons biologiques ou environnementaux, des calculs d'effets matrices peuvent s'avérer utiles afin de déterminer l'impact de l'ensemble des constituants sur la sensibilité de la méthode. En comparant la pente d'une courbe d'étalonnage exempte d'interférant (directement dans le solvant, B) avec celle préparée dans la matrice (A), l'effet de matrice (ME) est calculé. Une diminution de la pente dans la matrice peut être attribuée par exemple à une suppression de l'ionisation d'un analyte alors qu'une augmentation indique que la matrice affecte positivement le signal de cet analyte. Les valeurs d'effet matrice doivent être entre -30 et 30%. L'évaluation de l'effet de matrice résiduelle entre des échantillons d'une même nature (eau usée, urine, sédiments) permet de mesurer la variabilité de l'étalonnage entre ceux-ci, ce qui permet de déterminer si des solutions étalons appariées à la matrice sont suffisantes ou si la technique des ajouts dosés doit être utilisée pour chacun des échantillons, ce qui peut s'avérer laborieux en termes de manipulations. Les effets de matrice peuvent être calculés selon la formule suivante :

$$ME\ (\%) = \left(\frac{A}{B}\,x\,100\%\right) - 100\% \qquad \mathbf{Eq.\,2-3}$$

En plus des paramètres de calibration, il est nécessaire pour la validation d'une méthode de déterminer la robustesse de la quantification des échantillons. Pour ce faire, il est possible de calculer le taux de récupération de la méthode ainsi que la précision et l'exactitude sur les valeurs de concentration obtenues. Le pourcentage de récupération permet de déterminer la perte d'analytes ou l'impact d'interférents sur l'analyse de ceux-ci lorsqu'une solution dopée est soumise au protocole de préparation et d'analyse dans la matrice. Ainsi on peut connaître, par exemple, l'efficacité d'une étape d'extraction, de préconcentration ou de dérivatisation appliquée à des échantillons. L'exactitude permet de déterminer la justesse entre une concentration attendue et la valeur obtenue par la quantification alors que la précision permet de déterminer l'étroitesse de la variation des concentrations obtenues pour différentes analyses d'une même concentration. Les valeurs de récupération et d'exactitude doivent se trouver entre 70 et 130% lors d'analyse de contaminants dans des matrices environnementales. Il est possible de calculer la réplicabilité et la répétabilité qui donnent respectivement une valeur de précision d'injections

successives d'un même échantillon sur le même appareil dans une même journée et du même échantillon sur le même appareil à des journées différentes. La reproductibilité, quant à elle, donne une valeur de précision sur une quantification effectuée par des opérateurs différents dans des laboratoires différents qui appliquent le même protocole expérimental et la même méthode à un échantillon commun. Ces valeurs peuvent être exprimées sous forme d'intervalle de confiance ou d'écart-type relatif (RSD) qui doit être au plus de 30% pour une quantification dans des échantillons environnementaux. Ces différents paramètres peuvent être calculés selon les équations 2-4, 2-5 et 2-6 pour lesquelles C*f* est la concentration fortifiée, C*i* est la concentration non fortifiée, C*a* est la concentration ajoutée, C*m* est la concentration mesurée et $\bar{x}$ est la moyenne:

$$Taux\ de\ récupération\ (\%) = \frac{Cf - Ci}{Ca}\ x\ 100\% \qquad \mathbf{Eq.\ 2-4}$$

$$Exactitude\ (\%) = 100\% - \left(\frac{Cm - Ca}{Ca}\ x\ 100\%\right) \qquad \mathbf{Eq.\ 2-5}$$

$$Précision\ (\%) = RSD = \frac{\sigma}{\bar{x}} \qquad \mathbf{Eq.\ 2-6}$$

L'utilisation d'étalons internes marqués avec des isotopes stables permet de faire la quantification par étalonnage interne, ce qui permet de s'affranchir d'une grande part des interférences au niveau de la préparation des échantillons et de l'analyse. Le taux de récupération ne tient pas compte de cette correction, mais la précision et l'exactitude s'en trouvent améliorés. L'impact de la matrice affectant également les étalons internes et les standards, l'effet de matrice est également fortement amoindri. L'utilisation de ces étalons internes est souvent associée avec les méthodes par spectrométrie de masse qui permettent de les différencier de leur analogue non marqué puisque le rapport m/z est changé par la masse des isotopes, les plus souvent utilisés étant les isotopes stables du carbone (^{13}C), de l'azote (^{15}N) et le deutérium (^{2}H). D'autres types d'étalon interne peuvent également être utilisés, par exemple avec des méthodes ne faisant pas appel à la spectrométrie de masse, or le choix d'un étalon interne repose sur le fait que celui-ci doit être absent des échantillons et avoir des propriétés physicochimiques similaires aux analytes d'intérêts. Les molécules marquées ne pouvant se trouver dans des échantillons qu'après un ajout volontaire, et leur structure étant identique à leur analogue non marqué, ils peuvent donc corriger de manière optimale la réponse d'un analyte puisqu'ils subiront exactement les mêmes conditions et interférences au cours d'une analyse.

3 Échantillonnage pour l'application de méthode

La collecte d'échantillon est une étape cruciale pour l'application de méthode à des échantillons réels et elle demande une attention particulière. En plus du choix du matériel qui doit minimiser la contamination et l'altération des échantillons, plusieurs protocoles d'échantillonnage existent pour répondre à différentes situations de terrain. Par exemple, lors de l'échantillonnage d'un plan d'eau ou d'un sol contaminé, il faut non seulement considérer les points d'échantillonnage, mais également la profondeur à laquelle sera effectué le prélèvement. Une forte variation temporelle, comme à un point de rejet d'eau usée, peut favoriser l'utilisation d'échantillonneurs automatiques pouvant récolter des aliquotes à des temps donnés ou faire des échantillons composites pour avoir une idée des concentrations globales d'un contaminant. Un retour régulier à un même site pourrait s'avérer intéressant pour obtenir des informations sur les tendances saisonnières ou l'impact de technique de remédiation sur des contaminants.

En plus d'une bonne planification des sites, il est important d'évaluer la contamination potentielle des échantillons par le protocole de prélèvement. C'est pourquoi des blancs protocoles ainsi que des blancs terrains et de transport sont inclus dans la plupart des campagnes d'échantillonnage. En raison de l'imprécision qu'il peut y avoir de la manière dont sont prélevés les échantillons, il est préférable qu'un même opérateur collecte l'ensemble des échantillons et l'ajout de certains *replica* d'échantillon est utile pour tenir compte de cette variation. Lors de l'analyse instrumentale, plusieurs aliquotes d'un même échantillon seront injectés afin de s'assurer de l'homogénéité de celui-ci et une analyse successive d'un même échantillon peut être effectuée lorsque les échantillons sont conservés sur une longue période afin de confirmer que la dégradation des analytes est minimale.

Pour l'évaluation de la distribution des contaminants émergents dans le fleuve Saint-Laurent et ses tributaires, de grandes campagnes d'échantillonnage ont été mises en œuvre afin de couvrir une grande zone géographique. Avec approximativement 80% de la population de la province de Québec qui réside sur ses rives, ce fleuve qui débouche dans l'océan Atlantique représente l'une des sources d'eau potable les plus importantes au monde en considérant l'apport des Grands Lacs qui s'y jettent. Le fleuve est aussi grandement utilisé comme couloir de transport de marchandises et est fortement exposé à des activités industrielles et agricoles. Les rejets des eaux usées de villes densément peuplées impactent également l'environnement du fleuve et des rivières qui l'alimentent.

Alors que ses tributaires sont majoritairement accessibles en voiture, l'échantillonnage sur le fleuve requiert l'utilisation d'embarcation et de matériel spécialisé afin de garantir l'intégrité des échantillons. Pour ce faire, le navire de recherche Lampsilis (Figure 3-1) de l'Université du Québec à Trois-Rivières a été nolisé durant plusieurs étés consécutifs (depuis 2017). Celui-ci a été spécialement équipé afin de pouvoir faire des prélèvements d'échantillons dans des zones peu profondes du fleuve et des espaces de laboratoire y sont aménagés pour que des manipulations simples puissent être effectuées pour des analyses *in situ* ou pour la conservation des échantillons. Des dispositifs spécialisés pour la pêche et la collecte d'échantillons de sédiments, de plancton et de benthos sont également disponibles pour des campagnes d'échantillonnages ciblées pour ce type d'études. Le trajet le plus étendu ayant couvert du lac Ontario jusqu'à l'estuaire, différentes zones ont été échantillonnées au cours des différentes années en passant par des sites récurrents à chaque campagne dans la région de Montréal ou l'impact anthropique et le rejet des composés pharmaceutiques était soupçonnés d'être parmi les plus importants.

Figure 3-1. Navire scientifique Lampsilis de l'UQTR.

Afin de récolter les échantillons d'eau de surface à une profondeur constante et en évitant une contamination par des rejets de navires, une bouteille de Niskin (de type Go-Flo, Figure 3-2) installée sur un treuil est submergée pour prélever l'eau des sites à 1m de la surface. Des blancs d'échantillonnage ont été effectués afin de vérifier que le dispositif et le protocole ne sont pas source de contamination.

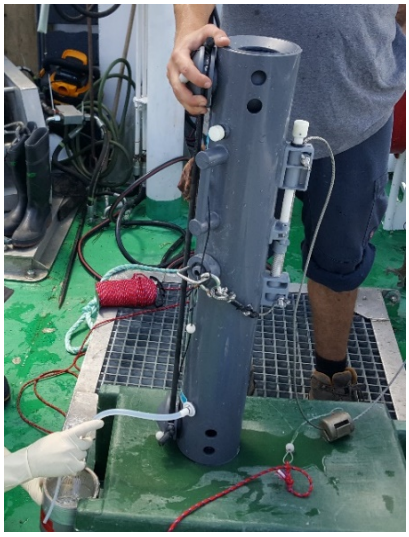


Figure 3-2. Bouteille d'échantillonnage Go-Flo.

Des transects du fleuve ont également été effectués puisque sur une majeure partie de celui-ci différentes masses d'eau distinctes subissant peu de mélange le composent. Par exemple, dans le lac Saint-Pierre, des images satellites montre des couloirs de masse d'eau aux couleurs distinctes qui peuvent être associées à l'apport des Grands Lacs et de tributaires du sud, à la Voie maritime au centre et à la contribution de la rivière des Outaouais et des tributaires du nord (Figure 3-3) [240,241].

Figure 3-3. Masses d'eau du lac Saint-Pierre (Biophare, 2009).

4 Objectifs et structure de la thèse

Un premier objectif de cette thèse était de déterminer le niveau de contamination d'effluents d'hôpitaux en termes de composés pharmaceutiques afin de le comparer avec celui des usines de traitements des eaux usées. Ces dernières n'étant que partiellement efficaces pour décontaminer les eaux avant leur rejet et les hôpitaux étant soupçonnées d'être une source importante de ces contaminants, leur contribution aux réseaux d'égouts municipaux pourrait être limitée par des traitements à la source. Or pour justifier de telles mesures et tenter de diminuer la pollution gérée par les stations d'épurations, il existe un manque important de données quant à la nature des composés pharmaceutiques et leur concentration dans ces différentes matrices d'eaux usées. Une des hypothèses principales de cette thèse était que les effluents d'hôpitaux contiendraient des concentrations plus importantes en contaminants pharmaceutiques, notamment pour certaines classes telles que les produits de chimiothérapie.

Afin de répondre à ces questionnements, une première méthode analytique a été développée par SPE en ligne couplée avec la UPLC-MS/MS pour l'analyse de composés pharmaceutiques multiclasses. Plusieurs paramètres ont été soigneusement optimisés, tels que le choix des colonnes et conditions de préconcentration et séparation chromatographiques, le volume d'échantillon, le choix de filtre pour la préparation des échantillons ou encore les paramètres pour l'extraction et la purification des échantillons en phase solide. Cette méthode a été validée pour la phase dissoute et la phase particulaire d'eaux usées. Elle a ensuite été appliquée à des échantillons d'effluents d'usine d'épuration et d'hôpitaux du Québec. Une comparaison entre les concentrations mesurées dans la phase dissoute et dans la matière en suspension a permis de déterminer la distribution et le partitionnement de certains contaminants selon la nature de la matrice étudiée.

Lors du développement de méthode, les composés de chimiothérapie étant trop polaires pour la préconcentration et la séparation sur des phases stationnaires classiques en chromatographie en phase inverse, une attention particulière leur a été portée. Une cartouche SPE a été sélectionnée pour l'adsorption de contaminants organiques polaires afin de procéder à la préconcentration en ligne. Celle-ci a été couplée avec une sélection de colonnes chromatographiques qui ont été comparées afin de déterminer une combinaison pouvant mener aux meilleures conditions chromatographiques en termes de séparation, de sensibilité et de forme de pics. Une première méthode, utilisant une colonne de type C18 modifiée, a été développée afin de pouvoir faire l'analyse de différentes familles de cytostatiques susceptibles d'être retrouvées en forte concentration dans des eaux usées. Une optimisation rigoureuse des

différents paramètres chromatographiques a menée à deux méthodes en ligne sensibles, robustes et efficaces pouvant se comparer à des méthodes plus complexes exigeant parfois des dérivations. Suite à leur validation, elles ont été appliquées à des effluents d'hôpitaux ainsi que des eaux usées d'usines d'épurations afin de vérifier l'hypothèse selon laquelle il y aurait des différences dans les profils et les niveaux de contamination de ces matrices.

Enfin, sachant que la plupart des technologies de traitement d'eaux usées n'ont pas été conçues pour dégrader les composés pharmaceutiques, des quantités importantes de ceux-ci peuvent donc se retrouver dans les eaux de surface à proximité des points de rejet. La participation à une large campagne d'échantillonnage sur le fleuve Saint-Laurent a rendu possible l'étude de l'impact de points de rejet d'eaux usées à proximité de régions densément peuplées. Une des hypothèses de cette thèse était qu'un impact important pourrait être mesuré à proximité du point de rejet de la ville de Montréal et que celui-ci pourrait être perçu sur une grande distance. Un échantillonnage annuel sur une période de cinq ans a été maintenu pour étudier d'éventuelles tendances temporelles. Des échantillons de sédiments et de l'eau près du lit du fleuve ont été obtenus grâce à la collaboration avec une plongeuse professionnelle pour déterminer si les tendances de concentration des composés pharmaceutiques s'étendent à différentes profondeurs. Également, plus de 50 tributaires se jetant dans le fleuve ont également été échantillonnés afin de déterminer si certains d'entre eux peuvent contribuer de manière importante à la pollution des différentes masses d'eaux qui le compose par ce type de contaminants émergents. Une autre hypothèse de cette thèse étant que les rivières s'écoulant dans des zones densément peuplées seraient plus impactées d'un point de vue de pollution pharmaceutique.

4.1 Articles composant la thèse

Les chapitres 5 à 7 présentent les articles qui composent cette thèse et qui répondent aux objectifs mentionnés précédemment.

Le **Chapitre 5** correspond au développement et la validation d'une méthode d'analyse par SPE en ligne couplée avec la UPLC-MS/MS pour les composés pharmaceutiques multiclasses dans les eaux usées. Les résultats sont détaillés dans la publication suivante:

Vaudreuil, M.-A., Vo Duy, S., Munoz, G., Sauvé, S. Pharmaceutical pollution of hospital effluents and municipal wastewaters of Eastern Canada. *STOTEN,* 846, 2022

Le **Chapitre 6** correspond au développement et la validation des méthodes d'analyse spécifiques aux produits de chimiothérapie et à l'application aux eaux usées. Les résultats sont détaillés dans la publication suivante :

Vaudreuil, M.-A., Vo Duy, S., Munoz, G., Furtos, A., Sauvé, S. A framework for the analysis of polar anticancer drugs in wastewater: On-line extraction coupled to HILIC or reversed phase LC-MS/MS. Talanta, 220, 1-12, 2020

Le **Chapitre 7** correspond aux analyses d'échantillons d'eau de surface du fleuve Saint-Laurent et de ses tributaires entre 2017 et 2021. Les résultats sont compilés dans une publication qui a été soumise à un périodique scientifique en date de dépôt de cette thèse.

Vaudreuil, M-A., Munoz, G., Vo Duy, S., Sauvé, S. Tracking down pharmaceutical pollution in surface waters of the St. Lawrence River and its major tributaries. Submitted to *STOTEN*, 2023

4.2 Autres travaux :

Les méthodes détaillées dans cette thèse ont aussi été utilisées dans d'autres applications dans le cadre de collaborations avec d'autres groupes de recherche. Par exemple, les composés pharmaceutiques dans des échantillons d'eaux usées ont été quantifiés afin de développer et d'optimiser des prototypes de traitement des eaux usées d'hôpitaux à la source, ce qui pourrait éventuellement être appliqué à large échelle afin de diminuer la charge en médicaments se rendant jusqu'aux stations d'épuration. Des analyses d'eau de surface et l'extraction et la purification d'échantillons de moules exposées à des effluents ont été utiles à une étude sur les effets écotoxicologiques des rejets d'eaux usées sur des organismes aquatiques. Ces contributions seront succinctement énoncées dans le **Chapitre 8** de cette thèse.

5 Pollution par les composés pharmaceutiques dans des effluents hospitaliers et des eaux usées municipales de l'Est du Canada

Article publié dans *Science of The Total Environment* (2022) 846, 1-14.

"Pharmaceutical pollution of hospital effluents and municipal wastewaters of Eastern Canada"

Auteurs: Marc-Antoine Vaudreuil, Sung Vo Duy, Gabriel Munoz, Sébastien Sauvé

Description : Cet article décrit l'optimisation, la validation et l'application de méthodes faisant appel à la SPE couplée à la LC-MS/MS pour l'analyse de multiples classes de composés pharmaceutiques dans des échantillons d'eaux usées municipales et d'hôpitaux ainsi que dans la phase particulaire.

Contribution : J'ai effectué la conception du projet, le développement de méthodes, la collecte et la préparation des échantillons, le traitement de données, ainsi que la rédaction de l'article.

Coauteurs : Sung Vo Duy et Gabriel Munoz m'ont aidé à la conception du projet ainsi qu'à la révision de l'article.

Directeur : Sébastien Sauvé m'a aidé à la conception du projet ainsi qu'à la révision de l'article.

ABSTRACT

Quantification of drugs residues in wastewaters of different sources could help better understand contamination pathways, eventually leading to effluent regulation. However, limited data are available for hospital-derived wastewaters. Here, an analytical method based on automated on-line solid-phase extraction liquid chromatography tandem mass spectrometry (on-line SPE – UPLC-MS/MS) was developed for the quantification of multi-class pharmaceuticals in wastewaters. Filtrate phase and suspended solids (SPM) were both considered to evaluate the distribution of targeted analytes. Experimental design optimization involved testing different chromatographic columns, on-line SPE columns, and loading conditions for the filtrate phase, and different organic solvents and cleanup strategies for suspended solids. The selected methods were validated with suitable limits of detection, recovery, accuracy, and precision. A total of 30 hospital effluents and 6 wastewater treatment plants were sampled to evaluate concentrations in real field-collected samples. Certain pharmaceuticals were quantified at high levels such as caffeine at 670,000 ng/L in hospital wastewaters and hydroxyibuprofen at 49,000 ng/L in WWTP influents. SPM samples also had high contaminant concentrations such as ibuprofen at 31,000 ng/g in hospital effluents, fluoxetine at 529 ng/g in WWTP influents or clarithromycin at 295 ng/g in WWTP effluents. Distribution coefficients (K_d) and particle-associated fractions (Φ) indicate that pharmaceuticals tend to have better affinity to suspended solids in hospital wastewater than in municipal wastewaters. The results also bring arguments for at source treatment of these specific effluents before their introduction into urban wastewater systems.

Keywords

Pharmaceuticals; On-line SPE LC-MS/MS validation; Hospital effluents; Municipal wastewater; Suspended solids; Field distribution coefficients.

5.1 INTRODUCTION

With the continued increase in the consumption of pharmaceuticals, their active ingredients may occur more frequently in the environment and at higher concentrations [9,242,243]. The main contamination source is via the urban wastewaters that are discharged to surface water after treatment. Wastewater treatment plant technologies were not originally designed to remove these contaminants. Many pharmaceuticals are thus only partially degraded and the treatment effect is even null for some compounds, leading to drug discharges that can reach concentrations as high as hundreds of µg/L [89,244]. Another important source of contamination are hospital facilities (drugs for human use) and agriculture and aquaculture (prophylactic and therapeutic veterinary drugs) [32,245].

Because of the potential ecotoxicological effects on aquatic organisms exposed to these pollutants, pharmaceuticals and personal care products have been recognized as emerging contaminants [25,246]. Hormones used primarily for contraception are endocrine disruptor chemicals that can induce feminization in aquatic species and exert reprotoxic effects on wildlife exposed to concentrations as low as the part-per-trillion (ng/L) [56,247]. Reproduction, growth and prey/predator behaviors were also affected during exposures to antidepressants at environmentally relevant concentrations, including for larva and adult fish specimens [248–250]. Analgesics are among the most prevalent pharmaceutical contaminants and can present a high toxicity for crustaceans [251,252]. How mixtures of these contaminants can affect organisms also raises concerns, due to the co-occurrence of numerous pharmaceuticals in impacted environments and their possible synergistic effects [253,254].

To address these problematics, there is a constant need for analytical method improvements to enable ultra-trace quantification of pharmaceuticals in complex natural matrices. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is the typical technique of choice for this purpose [255,256]. The specificity, sensitivity and robustness of MS detectors is a great advantage compared with other techniques such as UV/vis or electrochemical sensors [257–259]. Sample pre-treatments are also frequently used to pre-concentrate samples and reduce sample matrix complexity by eliminating potential interferents. Techniques such as solid-phase extraction (SPE) are commonly used in the field of environmental analysis because of the ease of use for aqueous samples and the wide range of adsorbents that can be functionalized to extract specific pollutants according to their charge and polarity [260–262]. For instance, several recent studies used off-line SPE cartridges (Waters Oasis HLB 200-1000 mg) of 150-500 mL wastewater samples (domestic, hospital, or municipal sources) for the pre-

concentration of pharmaceutically active compounds prior LC-MS/MS analysis [263–265]. Though limits of detection (LODs) in the typical range of 1-50 ng/L can be achieved, the high pre-concentration factor often goes on par with substantial matrix effects [263,264]. Multi-step off-line SPE workflows also present an obvious limitation considering the wide concentration range of pharmaceuticals in wastewaters: several sample preparations might be required (involving different pre-concentration factors) to remain within linear instrument working range for all target analytes.

Contrastingly, automation of SPE methods is trending because it can significantly reduce sample handling and increase method throughput. Wastewater is a relevant test matrix for automated on-line SPE LC-MS methods, known to reach LODs in the order of ng/L [266,267]. Previously published methods had to overcome some limitations of on-line methods such as higher LODs, matrix effects, and method ruggedness during consecutive injections of a large number of wastewater samples [268–270]. Standardized methods have been frequently published for the analysis of pharmaceuticals in samples such as surface and drinking water while fewer methods are available for highly complex matrices such as urban wastewater or hospital effluents [234,271]. Standardized methods are also often based on off-line SPE, and modifications to the extraction procedure require analysts to meet performance requirements as part of the initial demonstration of capability. Achieving analysis of multi-class compounds within a single workflow remains a challenge for wastewaters [272].

In the present study, we aimed to develop a fast and robust on-line SPE LC-MS/MS analytical method for the quantification of 8 different classes of pharmaceuticals (analgesics, antibiotics, chemotherapy agents, steroid hormones, anticonvulsants, antidepressants, stimulants, and beta blockers) in wastewater matrixes, including hospital effluents and influents/effluents of municipal WWTPs. The selection of targeted analytes was based on a list of consumed and prescribed compounds in a hospital facility and are also among the major pollutants and metabolites often found in wastewater matrices. The final method was selected upon multi-criteria optimization including choice of the chromatographic column and SPE sorbent material, sample pre-treatment, and mobile phase variations. In addition, an extraction method was developed to quantify analytes adsorbed onto suspended solids (i.e., suspended particulate matter (SPM) of the wastewater). The proposed methods were validated and performance endpoints such as method recoveries, precisions, accuracies, linearity, limits of detections and matrix effects are discussed. The methods were applied to wastewater from hospitals and municipal WWTPs. Field-based suspended solid–dissolved phase distribution coefficients (K_d)

and particle-associated fractions (Φ) are presented. To our knowledge, this is one of the first studies to document the dissolved-solid distribution of pharmaceuticals in hospital and urban effluents.

5.2 EXPERIMENTAL

5.2.1 Standards and reagents

A list of the 28 targeted compounds grouped by pharmaceutical classes is presented in **Table 5-1**. Native standards for all selected analytes were purchased from Sigma Aldrich (Saint-Louis, MO, U.S.A.) with a purity of ≥97%. Isotope-labeled internal standards (IS) were purchased from Santa Cruz Biotechnology (Dallas, TX, U.S.A.). Stock solutions were individually prepared by dissolving an exact mass (25 mg) of standards in 25 mL of methanol and kept at a temperature of -20°C. A mix containing all compounds at 1 µg/L was prepared by dilution from the previous individual 1 mg/mL and kept at the same temperature. Further details on compound structures and physicochemical properties are available in SI (**Table 5-6**). Information on solvents, reagents, and filters, and other materials is available in SI (**Text 5-1**).

Table 5-1. List of targeted analytes, arranged by pharmaceutical class.

Pharmaceutical Class	Compound Name	Abbreviation
Analgesics	Ibuprofen	IBU
	2-Hydroxyibuprofen	OH-IBU
	Hydromorphone	HMOR
	Diclofenac	DCF
	4-Hydroxydiclofenac	OH-DCF
Antibiotics	Sulfamethoxazole	SMX
	4-Nitrososulfamethoxazole	NO-SMX
	Amoxicilline	AMOX
	Clarithromycine	CLA
Chemotherapeutics	Methotrexate	MTX
	7-Hydroxymethotrexate	OH-MTX
Hormones	Estradiol	E2
	Estrone	E1
	Estriol	E3
	Ethinylestradiol	EE2
	Levonorgestrel	LEVO
	Norethindrone	NOR
	Progesterone	PROG
	Medroxyprogesterone	MPROG
	Testosterone	TESTO
Anticonvulsants	Carbamazepine	CBZ
	10,11-Dihydroxy-10,11-dihydrocarbamazepine	2OH-CBZ
Antidepressants	Venlafaxine	VEN
	Desvenlafaxine	DVEN
	Fluoxetine	FLU
	Norfluoxetine	NORF
Stimulants	Caffein	CAF
Beta Blockers	Acebutolol	ACE

5.2.2 Sample collections

For water samples collections, 1L amber glass bottles were previously cleaned in the laboratory with pure solvents (MeOH and H_2O HPLC). Sodium omadine salt was added (70 g/L) for its biocide effect to minimize bacterial biodegradation of pharmaceuticals during storage time [130,273]. When applicable, a pump system was used to sample a larger volume (10 L at approximately 1 L/min) that was then homogenized by agitation and bottles were filled. All bottles were transported to the university in a cooler with ice until filtration and storage at -20°C prior analysis. Acidification of samples could help stabilizing some environmental contaminants for long storage time, but lower pHs were reported to have a negative effect for some pharmaceuticals [274,275]. For this reason, the samples were not acidified and kept at their original pH.

Overall, 30 hospital effluents, 6 municipal WWTP influents (before treatment), and 6 municipal effluents (after treatment) were collected in Canada. Additional information on the WWTP sampling sites, including treatment type and average flow rates, can be found in SI (**Table 5-7**).

5.2.3 Sample preparation

Water sample filtration was done using glass fiber membranes (0.3 µm, 25 mm, Sterlitech) to separate the dissolved phase from suspended solids. The filtrate was collected in a clean glass flask and an exact volume (5 mL) was transferred to an amber glass vial and amended with internal standards (2000 ng/L) prior analysis by the on-line SPE – UPLC-MS/MS method.

Particulate matter samples (filters) were extracted twice with 5 mL MeOH/ACN (75/25: v/v) + 0.1% FA. Each extraction cycle involved brief vortexing (10 s), sonication (20 min), and centrifugation (5000 rpm, 5 min). The supernatants were combined and evaporated to 1 mL in a heated bath (40 °C) under a nitrogen stream and reconstituted in 100 mL of HPLC water for SPE purification. The extract was loaded on a Strata-X polymeric cartridge (Phenomenex, 200 mg/6 mL) previously conditioned with MeOH (2 x 2 mL) and water (2 x 2 mL). The cartridge was rinsed with water (2 x 3 mL) and dried under vacuum (30 min) before elution with MeOH (2 x 3 mL). The purified extract was brought to dryness with a nitrogen flow and mild heating (40 °C), reconstituted in 200 µL of H_2O/MeOH (90/10), and aliquoted in a 250-uL injection vial. Analysis proceeded using the small injection off-line method (50 µL injection volume, see also SI **Table 5-8**).

5.2.4 Instrumental analysis

A dual pump system was used for both pre-concentration (Accela 600 pump) and chromatographic separation (Accela 1250 pump). In the case of the dissolved phase samples, an exact volume of sample (2 mL) was withdrawn and injected with a 2500 µL automated syringe maneuvered by an HTC Thermopal autosampler system (CTC Analytic AG, Zwingen, Switzerland). A switching valve system was used for the on-line pre-concentration methods [33]. The sample enrichment and separation were executed using a C18 material compacted in different LC column formats: Hypersil Gold aQ (20 x 2.1 mm; 1.9 µm) and Hypersil Gold (50 x 2.1 mm; 1.9 µm), respectively as the on-line SPE and UPLC chromatographic columns. The chromatographic column was kept at 40°C. For the sample pre-concentration, injection volume, flow rate and washing volume were conjointly optimised. Final conditions were as follows: a 2-mL sample volume was loaded at a flow rate of 2500 µL/min with an additional 2 mL of aqueous solvent passing through the SPE column for interferent reduction (washing step). An 8-min chromatographic gradient followed the enrichment step and was found sufficient for analyte separation. The detailed chromatographic gradient is presented in SI (**Table 5-9**).

A TSQ Quantiva triple quadrupole mass spectrometer analyser (Thermo Scientific, Waltham, MA, U.S.A.) was used with an electrospray ionization source (ESI) for analyte detection and quantification. Ionization parameters have been optimized during analyte infusion steps and were as follows: spray voltage set to -3000V and +3000V (negative and positive mode, respectively), sheath gas (50 a.u.), auxiliary gas (10 a.u.), sweep gas (0 a.u.), ion transfer tube temperature (350 °C), and vaporizer temperature (400 °C).[1] Selected *m/z* ions (precursor and fragments) and corresponding polarity, collision energy and RF lens information can be found in SI (**Table 5-10**). Acquisition was performed using the two must abundant MS/MS fragments in selected reaction monitoring mode (SRM) as quantification (QT) and confirmation (CT) transitions.

5.2.5 Method optimization

5.2.5.1 On-line SPE method (dissolved phase)

The chromatographic gradient and flow rates have been optimized to have a sufficient retention of the most polar targeted analytes simultaneously with a sufficient separation of all selected compounds in an overall minimum method time. Three chromatographic columns were tested considering the resulting compounds retention, peak shapes, and signal intensities. Details

[1] Negative and positive ions are analyzed in a single run using polarity switching.

on the tested chromatographic columns are presented in SI (**Table 5-11**). Different mobile phase variations have also been tested with either organic solvent nature (methanol (MeOH) or acetonitrile (ACN)), pH adjustment (with formic acid (FA) or ammonium hydroxide (NH_4OH)) or ammonium fluoride (NH_4F) addition.

Different on-line column materials have been tested for sample pre-concentration. The selection of the more suitable sorbent for pharmaceutical compounds was based on the absolute signal intensity and corresponding variation (with n = 3 for the different conditions). The physical and analytical parameters of the tested on-line SPE columns are described in SI (**Table 5-12**). As for the chromatographic mobile phase, different organic eluents were tested (MeOH / ACN), while acidic addition (from 0.1 to 1%) was also tested in both the aqueous and organic phases. The best solvent combination led to the final optimization of sample loading steps.

To achieve optimal SPE enrichment, a Derringer's desirability approach was used [276]. A wastewater sample spiked at 500 ng/L with the target pharmaceuticals was used for these tests. Different injection volumes were tested using corresponding loops (1, 2 and 5 mL) for which a washing volume (between 0 and 3 mL) was previously optimised. For each volume, different flow rates (between 0.5 and 3 mL/min) were tested, and the following parameters were accounted to discriminate the best combination: precision (relative standard deviation, RSD) (d_1), the absolute signal intensity (to favor LOD) (d_2), and signal intensity normalized to injection volume (to favor SPE recoveries) (d_3). For each injection volume and loading flow rate combination, the overall desirability (D) was calculated with a Derringer's function (as follows). The highest D value across conditions led to the choice of final settings. Additional details on calculation of d_i criteria can be found in SI (**Text 5-2**).

$$D = \left(\prod_{i=1}^{n} d_i \right)^{1/n} \qquad \textbf{Eq. 5 – 1}$$

5.2.5.2 Suspended particulate matter

An extraction method was also optimized for suspended solids. Variations of extraction solvent nature (mixtures of MeOH/acetone or MeOH/ACN), ratios of MeOH, and solvent pH were considered. SPE pre-concentration of aqueous-diluted extracts was compared to pass-through SPE cleanup of organic extracts, and different parameters were optimized to achieve the best possible recoveries. This involved testing different SPE cartridges, including HyperSep C18 and HyperSep Retain PEP (Thermo Fisher Scientific, Waltham, MA, USA), Oasis HLB (Waters, Milford, MA, USA), Strata-X (Phenomenex, Torrance, CA, USA) and Isolute ENV+ (Biotage,

Uppsala, Sweden) (see also SI **Table 5-13**). pH adjustment of the samples and SPE conditioning and elution solvents were also tested.

5.2.6 Choice of the filter type

Hospital effluents and influents/effluents of municipal wastewaters being complex matrices, they may contain high loads of suspended solids that needs to be removed prior to HPLC injection. The selection of a suitable filter material was based on recovery assessment to reduce filtration biases (i.e., avoid artefactual decrease of the analyte dissolved concentration through unwanted sorption onto the filter material). Recoveries were obtained for the different tested membranes, namely, glass fiber (0.3 µm, 25mm), nylon (0.22 µm, 25mm), cellulose acetate (0.2 µm, 25mm), polycarbonate (0.2 µm, 25mm), polyester (0.2 µm, 25mm), and polypropylene (0.2 µm, 30mm). Ease of filtration was another parameter considered, since wastewater samples could rapidly clog the filter membrane and limit the filtered volume.

5.2.7 Method validation and quality control

A mix of all targeted analyte standards was used for calibration curves in corresponding matrix-matched matrices, allowing comparison of retention times and QT/CT ratios. Integration of detected peaks was performed using the Xcalibur 4.3 software from Thermo Fisher Scientific (Waltham, MA, U.S.A.).

During each sampling campaign, field blanks were included by filling bottles with HPLC water. These blanks were injected during analysis sequences to ensure there is no system/protocol contaminations. No signal was detected for the selected pharmaceutical compounds. HPLC water injection blanks were also run following QC spikes to confirm there was no carryover.

The methods were validated in matrix-matched samples, including a real wastewater matrix (obtained from an anonymous wastewater treatment plant located in Quebec, Canada) and suspended solids (sediment matrix used as a surrogate for solids). These matrices were used for the determination of overall method recoveries, precisions, and accuracies at two levels for wastewater (QC1 = 100 ng/L and QC2 = 20 000 ng/L) and solids (QC1 = 0.20 ng/g for SMX, CLA, E2, E1, PROG, MPROG, TESTO, CBZ, 2OH-CBZ, VEN, CAF and ACE, 2 ng/g for IBU, DCF, E3,

EE2, LEVO, NOR and DVEN, 10 ng/g for HMOR, OH-DCF, MTX and FLU and 40 ng/g for OH-IBU and QC2 = 250 ng/g).

For the dissolved phase, absolute recoveries were calculated as the ratio between absolute peak areas of the mix of analytes enriched via on-line SPE LC-MS/MS (2 mL) and of an equivalent amount analysed by small volume on-line SPE LC-MS/MS (50 µL). Absolute recoveries were also determined for the solid matrix, by comparing the analyte absolute area in samples spiked before extraction *vs.* reference samples spiked after performing all preparation steps.

Whole-method accuracies were determined by spiking target analytes (at the two QC levels previously mentioned) as well as internal standards to wastewater and solid matrix. Measured concentrations (subtracted by the matrix blank) were compared to theoretically spiked concentrations to derive accuracy percentage, with 100% reflecting a perfect match between the two values. Relative standard deviations (RSDs) of accuracy replicates prepared during the same workday (n = 5) correspond to the intraday precision; RSDs of the same process performed on three workdays (overall n = 15) correspond to the inter-day precision. Compliance with acceptance criteria was verified using US EPA guidelines for trace organic pollutants in real environmental samples, i.e., accuracy range of 70-130% and RSD <20-30% [277].

These matrices also were used for determination of the linear ranges and determination coefficients (R^2). Linear regressions were fitted after the analysis of 17 calibration levels in matrix-matched wastewater: unspiked, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, 100, 500, 1000, 5000, 10 000, 25 000, 50 000 and 75 000 ng/L. These large concentration ranges are evaluated to reflect the array of possible contaminant concentrations in real environmental samples. A similar process was followed for solids, with a 14-point based matrix-matched calibration curve. In the event of samples exceeding the tested linear range of each matrix, a sample dilution was performed to lower the measured concentration within linear working range, and the exact values were derived considering the dilution factor.

Limits of detection (LOD) and limits of quantification (LOQ) were derived from signal to noise ratios (LOD: S/N>3, LOQ: S/N>10) using the lower end levels of the calibration curves (when the compounds were absent in the matrix blank) or using field samples with low levels of analytes.

Matrix effects were calculated from the comparison of calibration curve slopes of real sample matrix to neat solvent (HPLC water) [215,278,279].

5.3 RESULTS AND DISCUSSION

5.3.1 Optimisation of the filtration step

Filtration is a key step for achieving a robust on-line SPE method that can sustain a long injection sequence. This step was optimised to ensure that there was no significant analyte loss, avoiding false negative results. Recoveries were compared for different filtration membrane materials (**Figure 5-1** and SI **Table 5-14**). The teflon filters could not be efficiently used with samples such as wastewater because of the fast membrane clogging and impractical pressure needed for use in capsulated syringe filters. While polypropylene showed low analyte recovery overall (mean = 23%), nylon filter presented some recoveries exceeding a reasonable value (DCF = 128%, CLA = 154% and FLU = 139%) or with low recoveries (e.g., E2, E1, E3 = 0%, NORF = 0.46%, OH-DCF = 1.4% and EE2 = 6%); these options were thus discarded. Polyester and cellulose filters were found to be efficient for some of the analytes (means = 69% and 41%, respectively), but unacceptable losses was still observed for some compounds (e.g., recoveries of DCF, CLA, FLU and several hormones below 10%). The polycarbonate membrane was not suitable for FLU (1.3%) and DCF (1.1%), but showed satisfactory recoveries for the remaining pharmaceuticals (ranging from 66 to 129%, mean = 91%). Only the glass fiber membrane showed satisfactory recoveries for all targeted analytes with values ranging from 70 to 107% without exceptions (overall mean = 84%). A previous study by Miossec et al. showed that glass fiber filters were adequate for the vast majority of pharmaceutical classes [280] and is a reccurent choice in literature [275]. Glass fiber filters were thus used to prepare liquid samples (retrieve the dissolved phase of wastewater) and for the collection of suspended particulate matter (SPM).

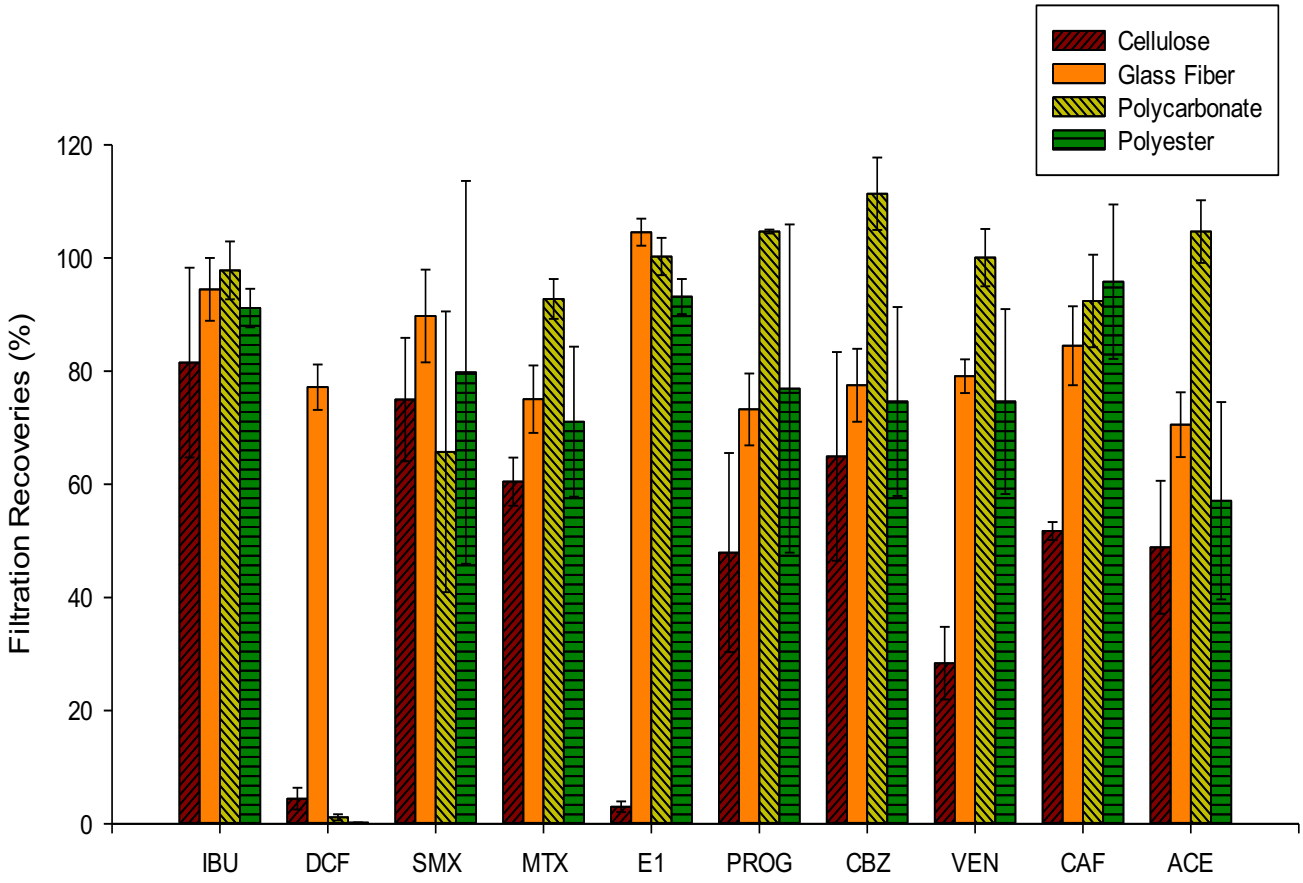


Figure 5-1. Selection of the filter membrane for sample pre-treatment and suspended particulate matter collection, illustrated for selected pharmaceuticals (see also SI **Table 5-14**).

5.3.2 Optimization of UPLC parameters

Designing a method capable of efficiently retaining a wide range of pharmaceutical compounds from highly polar (ex: MTX, Log *P* = -1.9) to apolar (ex: DCF, Log *P* = 4.5) first required optimization of the chromatographic parameters. Off-line injection on a Hypersil GOLD C18 column showed suitable peak separation between the different analytes; retention time windows were used to ensure enough points per peak for each MS/MS transition. Other columns options were tested (SI **Table 5-11**) but showed no performance improvement in terms of separation and signal intensities. For the HYPERCARB column, ion-pairing agents such as hexylamine and diethylamine were used to enhance retention of highly polar compounds, but these conditions did not meet satisfactory separation for most targeted analytes. The chromatographic gradient was programmed to yield satisfactory separation of the 28 targeted compounds during a limited method time (10 min total run time). A final workflow of 500 µL/min was used, that showed symmetrical

and narrow peak shapes. Among the selected pharmaceuticals, the chemotherapy metabolite OH-MTX showed no retention on all tested columns and was thus discarded from the target analyte list; hydrophilic interaction chromatography might be better suited for this compound [276].

The pH adjustment of the aqueous mobile phase did not yield better peak separation or greater signal intensities (see **Figure 5-5** in SI). Amending the mobile phase with NH_4F was reported to improve the sensitivity of hormones [281]. EPA method 539.1 (steroid hormones and bisphenol A in drinking water) also uses NH_4F as the mobile phase modifier [235]. Our in-house method for EPA-priority endocrine disruptors [281] showed that using 1mM NH_4F had a positive impact on targeted hormones. We also found that 1mM NH_4F has a positive influence for DCF and its metabolite, and that higher concentrations did not yield further signal improvement (**Figure 5-2** and SI **Table 5-15**). These observations and the selected NH_4F concentration agree with earlier literature on emerging contaminants [282,283]. Final gradient conditions are presented in SI (**Table 5-8**).

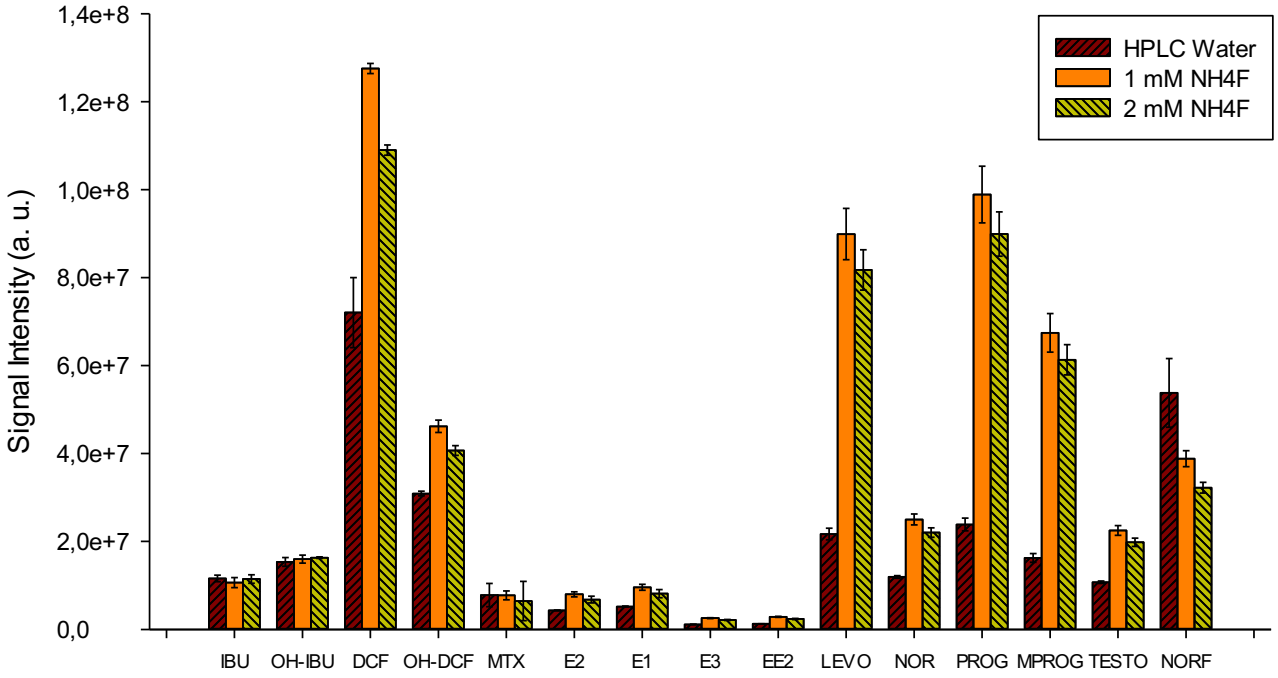


Figure 5-2. Effect of NH4F addition to the mobile phase for select analytes (see also SI **Table 5-15**).

5.3.3 Selection of the on-line SPE column

On-line SPE columns based on modified C18 were tested concurrently to a regular C18 sorbent (**Figure 5-3**), which is a widely used option for environmental organic pollutants. Among the different options was also the Isolute ENV+ from Biotage that showed improved recoveries for very polar compounds such as chemotherapy agents [276]. Modified C18 (e.g., Hypersil Gold aQ) showed a reasonable compromise and the overall best retention and was thus selected for further optimization.

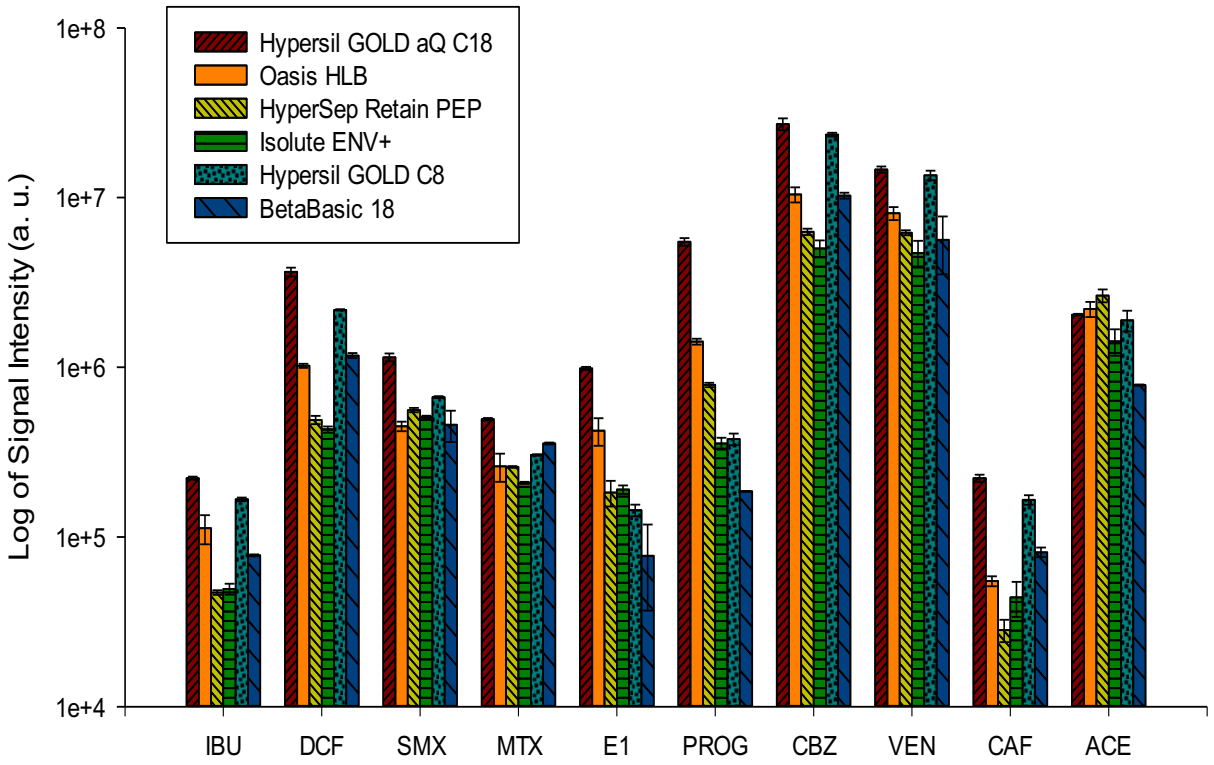


Figure 5-3. Absolute signal intensity of targeted analyte enriched with different SPE columns (see also SI **Table 5-16**).

5.3.4 Optimization of on-line SPE parameters

With the selected sorbent, the first parameters to refine were the washing volume (which should be adapted to sample injection volume) and the nature of the on-line mobile phase. For on-line SPE enrichment, MeOH was selected as the organic phase. FA addition to both mobile phases led to an increase in analyte signal by *circa* 3 times (**Figure 5-6, SI**). No acid in the mobile phases negatively affected the response of all targeted compounds, except for HMOR and MTX

for which FA slightly reduced signal intensity, and 1% FA led to losses (by *circa* 20%) for some compounds. A final concentration of 0.5% FA in each mobile phase was eventually selected, based on improvements for analytes such as NORF, CLA and FLU (signal increase by *circa* 1.5 times).

As it can been seen in **Figure 5-7** in SI, the aqueous wash volume applied after on-line SPE loading was an influential parameter, key in removing interferents that could cause signal suppression. However, it should be kept low enough to avoid analyte breakthrough, especially for the less hydrophobic analytes. As could be expected for the complex wastewater matrix, avoiding the wash step after SPE enrichment caused drastic signal reduction for most analytes (decrease ranging from 1.3 to 77 times, mean of 11 times) and complete signal extinction for three compounds (NO-SMX, AMOX and 2OH-CBZ). A wash volume higher than 2 mL had a negative effect on polar compounds such as MTX and OH-DCF, leading to significant signal loss (by 13 times). In most cases, higher volumes showed no improvement in signal intensity and were discarded to reduce method time. A 2-mL aqueous wash volume was finally selected to reduce matrix effects (SI **Figure 5-7**).

Loading volume and speed were tested using an experimental design with multi-criteria optimization (Derringer's desirability approach). Three loop volumes (1, 2 and 5 mL) and four flow rates (1, 1.5, 2, 2.5 and 3 mL/min) were tested. To achieve the best LOD, a larger volume could be preferred (leading to higher absolute signal intensity), while SPE recoveries would be favored by smaller injection volumes. The flow rate at which the samples are loaded onto the SPE column influences enrichment and chromatographic stability but has a major impact in total method length per sample. Faster sample loading could lead to signal loss for the least retained analytes, and also higher pressure on the on-line SPE column. The best condition was obtained with a 2 mL sample loaded at 2.5 mL/min, with a Derringer's desirability of D = 0.78 (**Figure 5-8**). This result is obtained with equal d_i criteria weighting on the geometric mean used to calculate the Derringer's desirability function (D) (see also SI **Text 5-2**). To reach the best possible method sensitivity, the statistical weight of d_2 (absolute signal criterion) can be increased which tends to favor a larger sample volume (see **Figure 5-9-B**, SI). As expected, the 5 mL conditions had among the lower desirability values (D = 0.52 to 0.60) and might be explained by sorbent saturation when working with highly complex matrices such as wastewater (see **Figure 5-9-C**, SI). A typical chromatogram showing final peak separation and shapes is available in SI (see **Figure 5-10**).

5.3.5 Extraction and purification of suspended solids

Solvent extraction using sonication is a simple-to-use, efficient method to extract organic contaminants from solid samples [166,284–286]. Among the tested extraction solvents, MeOH/ACN (75:25 v/v) provided the best performance overall (SI **Figure 5-11**). Acetone was also tested but led to slightly lower recoveries for some compounds (e.g., HMOR, E3, VEN or ACE). Addition of 0.5% NH_4OH was beneficial only for HMOR, while acidification (0.5% AF) led to better extraction for some compounds such as MTX and NO-SMX that are otherwise poorly recovered. Acidification also led to improved recoveries for other metabolites (OH-IBU and OH-DCF). The FA percentage was optimized, leading to the choice of the 0.1% FA condition (SI **Figure 5-12**). Acidification of solvents was also found to be beneficial in extraction methods of other targeted organic pollutants from solids [286–288]. Increasing the number of sonication cycles with this organic solvent to three (unpublished data) did not significantly increase the extraction efficiency and would be more time consuming.

The next step involved the selection of the purification mode between regular SPE (loading of the aqueous-diluted extract) or pass-through SPE cleanup (filtration of organic extract). Recoveries are presented in SI **Figure 5-13**. The cleanup option was found to be inefficient for MTX, VEN and CLA (recoveries under 10%) and lacking recovery for HMOR (47%) and ACE (36%). The SPE option was selected for further optimization.

Considering the regular SPE mode (loading of water-diluted extracts), different sorbent options were tested. C18 as well as HyperSep Retain PEP cartridges led to good recoveries (means of 68 and 67 % respectively) for the majority of pharmaceuticals but had low retention for the more polar compounds (e.g., NO-SMX, MTX, FLU or NORF). The HLB cartridge (mean recovery of 87%) was the only option yielding MTX recovery over 50%, but showed poor results for compounds such as DVEN, FLU, NORF and CLA (recoveries under 70%). These compounds were better purified using Strata-X cartridges and this option was chosen as a compromise with recoveries ranging from 71 to 122% (mean of 97%). The exception was MTX (32%), but this compound is expected to remain mostly in the aqueous phase due to its highly polar nature. Modifying solvent pH for the purification steps did not improve final recoveries (unpublished data).

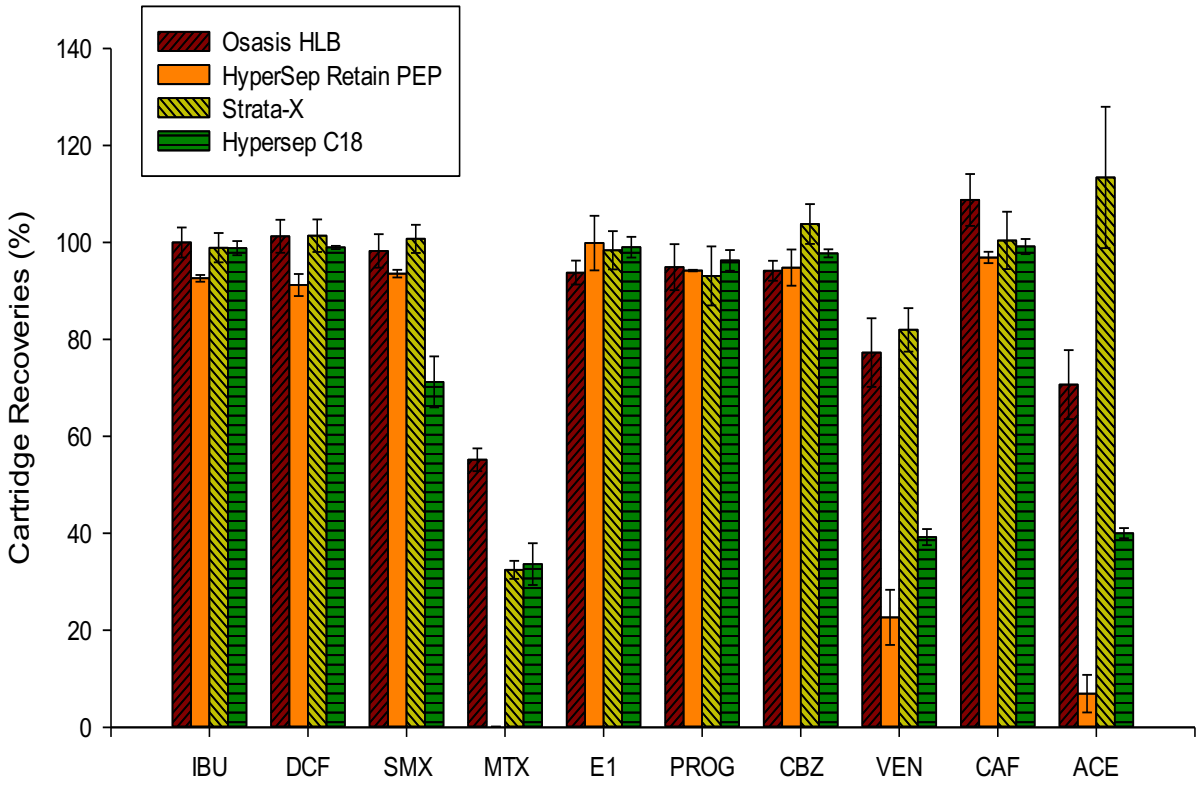


Figure 5-4. Selection of off-line SPE cartridge for the purification of extracted solid samples (see also SI **Table 5-17**).

5.3.6 Analytical method validation

To assess the method performance, calibration and validation parameters have been jointly measured. Validation was separately conducted for wastewater filtrate (dissolved phase) and solids, and the corresponding results are presented in **Tables 5-18** to **5-21**.

5.3.6.1 Wastewater (dissolved phase)

Determination coefficients met the EPA performance requirement with the minimal R^2 value of 0.9904 for CAF, except for NO-SMX for which the signal is lost. NO-SMX and AMOX were discarded from the analyte list in wastewater since only very concentrated spiked solutions led to distinctive chromatographic peaks. For example, a calculated LOD of 2000 ng/L was obtained for AMOX, which is not considered suitable in view of expected contamination ranges in wastewater. In contrast, the LODs of other compounds were in the low ng/L or sub ng/L range with values comprised between 0.1 and 20 ng/L. The higher LOD values are mainly obtained for hormones or polar metabolites such as 2OH-CBZ which are compounds with low relative signal

intensity when compared with other targeted analytes. A more specific method for these compounds could be optimized, but the obtained values are deemed sufficient for the analysis of untreated hospital wastewater or to investigate their removal by wastewater treatment plants (SI **Table 5-18**).

The low level (QC1) for wastewater was set at 100 ng/L and QC2 was set to 20 000 ng/L to evaluate performances at high concentration levels. Recoveries were between 74 and 121% at QC1 (mean = 91%) and between 81 and 122% at QC2 (mean = 99%). Accuracies values ranged from 84 to 119% at QC1 (mean = 103%) and from 91 to 118% (mean = 103%). The maximum RSD values were 9.3 % (QC1, mean 4.5%) and 9.9% (QC2, mean = 4.8%) for intraday precision and 16% (QC1, mean = 9.0%) and 15.5% (QC2, mean = 9.4%) for inter-day precision. Apart from NO-SMX, all targeted analytes met US EPA analytical performance requirements [277] (SI **Table 5-19**).

5.3.6.2 Solids

With the need of extra steps prior to LC-MS injection for extraction and purification, obtaining low LODs for solid samples may present challenges. Analyte loss could occur during the multi-step process, including extraction, cleanup, or evaporation. Such as in the case of wastewater, NO-SMX and AMOX showed poor results and could not be retrieved by the extraction protocol. This is observed as well for the antidepressant metabolite NORF that is discarded from target analytes for solid phase samples. The extraction/purification protocol led to a satisfying method sensitivity for the remaining target compounds, with LODs ranging from 0.002 ng/g (CBZ) to 10 ng/g (OH-IBU). Relatively high LODs were obtained for the more polar pharmaceuticals (e.g., MTX or OH-IBU) that have less affinity with organic solvents during the extraction and are less retained by tested SPE sorbents. Regardless, all compounds had suitable linearity over the tested range with R^2 values greater than 0.9900 (overall range of R^2 = 0.9912–0.9999) (SI **Table 5-20**).

Recoveries were determined at two spiked concentrations (QC1 = 0.20 ng/g for SMX, CLA, E2, E1, PROG, MPROG, TESTO, CBZ, 2OH-CBZ, VEN, CAF and ACE, 2 ng/g for IBU, DCF, E3, EE2, LEVO, NOR and DVEN, 10 ng/g for HMOR, OH-DCF, MTX and FLU and 40 ng/g for OH-IBU and QC2 = 250 ng/g). Some of the compounds are showing poor recoveries (HMOR = 28% and OH-DCF = 36%) at QC1. Two pharmaceuticals had very low (MTX: QC1 = 1.89%, QC2 = 35%) or null (NORF) recoveries and were discarded from the list of target analytes for solids. Apart from these compounds, the recoveries at QC1 are within an acceptable range with values between 63 and 122% (mean = 77%). All compounds had acceptable recoveries at QC2, with values ranging from 76 to 122% (mean = 101%).

Matrix spike accuracies ranged from 73 to 123% (mean = 97%) at QC1, except for HMOR (69%) and from 71 to 123% (mean 91%) at QC2. The maximum precision values are both 25% with mean of 15% for QC1 and 11% for QC2. Both accuracies and precision values are deemed sufficient for the validation of the extraction/purification protocol (SI **Table 5-21**).

5.3.7 Matrix effect evaluation

The absolute matrix effect of wastewater was measured by comparing matrix-matched calibration curve slopes to a matrix free calibration curve (HPLC water). The calculated matrix effect ranged from -54 to 96% for wastewater, indicating that the use of a matrix-free curve would not be suitable for quantitation. Compounds with a corresponding isotope labeled internal standard have typically smaller matrix effects.

To ensure a reliable calibration for every targeted pharmaceutical, a matrix-matched calibration curve approach was tested. When analyzing a series of samples, a matching matrix is used to prepare the calibration curve minimizing the possible matrix effect. To ensure this calibration option was suitable, the residual matrix effect was evaluated by comparing calibration slope of standard additions to individual samples with the slope of the curve at the same concentrations for a mix of the samples. In both cases, the values did not exceed the US EPA requirement of ± 30% for matrix effect with values ranging between -19 and 11% for wastewater. This highlights the importance of matrix-matched calibration curves to compensate intensity variation that might otherwise lead to major quantification errors if using neat solvent. Matrix-matched calibration was thus used for the analysis of field samples in the present study. Further details on matrix effect and residual matrix effect values are presented in SI (**Table 5-22**).

5.3.8 Comparison with literature and potential method limitations

Although the present study is not the first to describe methods for the analysis of pharmaceuticals in wastewater matrices (**Table 5-2**), it shows some advantage over previous publications. The use of on-line SPE coupled with LC-MS reduces the pre-treatment steps to a simple filtration. When considering previous on-line SPE workflows, the method duration includes the pre-concentration and purification steps as well as the analysis time, thus leading to a method that offers fast turnaround. Moreover, the need of derivatization for GC-MS methods demands further method optimization and requires more sample preparation when compared with LC-MS. The automatization of the on-line methods has been proven to have low variations compared to

manual SPE and usually yield better method exactitudes and precisions. The method described in this study is among the shortest, with an overall duration of 8 min per injection, allowing the analysis of many water samples per analysis sequence. Allowing the analysis of multiclass pharmaceutical in wastewater, the method performance is comparable of previous methods in terms of recoveries and LODs. These results are obtained with a relatively low sample volume (2 mL) compared with other on-line methods and much smaller compared with off-line methods. This presents an obvious advantage for sampling campaigns and sample handling.

When comparing the solid sample method, the different extraction and purification steps are comparable to previous publications (**Table 5-2**), but the injections are done with a short method as well (10 min). Recoveries are in the same range as other method with some compounds presenting slightly smaller values. In term of LODs, the present method reaches lower detectable concentrations using a smaller sample mass (0.5 g). Finally, having measured validation parameters and matrix effect in real samples matrices highlight the robustness of the described method.

Table 5-2. Analytical method comparison for pharmaceutical in environmental matrices.

Reference	# of pharmaceuticals	Targeted Matrix	Method duration	Sample volume/mass	Analytical method	Recoveries (%)	LODs
Samaras et al. [289]	10	WW, SL	24 min. + SPE + derivatization	100 mL	SPE GC-MS	91 – 117 (WW), 84 – 107 (SL)	0.3 – 3.2 ng/L (WW), 18 – 25 ng/g (SL)
Gumbi et al. [290]	8	SED	14 min. + purification & SPE + derivatization	10 g	SPE GC-MS	66 – 130	0.024 – 1.9 ng/g
US EPA [271]	12	TW	2 x 25 min. + SPE	1 L	SPE LC-MS	55 – 111	0.27 – 5 ng/L
US EPA [234]	74	WW	4 x 33 min. + SPE	1 L	SPE LC-MS	5 – 200	0.1 – 170 ng/L
		SL, SED	4 x 33 min. + extraction & SPE	1 g			0.22 – 270 ng/g
Asghar et al. [291]	29	SW	35 min. + SPE	200 mL	SPE LC-MS	50 – 106	10 – 2500 ng/L
Camilleri et al. [292]	12	SW	30 min. (on-line SPE)	2.5 mL	On-line SPE LC-MS	85 – 110	0.1 – 10
Liang et al. [293]	62	TW, SW, GW	14 min. (on-line SPE)	5 mL	On-line SPE LC-MS	81 – 120	0.005 – 2.5 ng/L
Zhong et al. [294]	58	TW	30 min. (on-line SPE)	1.8 mL	On-line SPE LC-MS	70 – 130	0.16 – 5.1 ng/L
Boulard et al. [295]	90	SPM	2 x 33 min. + extraction & purification	N. A.	PLE LC-MS	70 – 127	0.03 – 12 ng/g
Nannou et al. [132]	25	SED	11 min. + extraction & SPE	2 g	SPE LC-MS	57 – 92	0.4 – 14 ng/g
Al-Khazrajy et al. [296]	6	SED	25 min. + extraction & SPE	5 g	SPE LC-MS	30 – 125	0.03 – 3.5 ng/g
Present study	**25**	**WW**	**8 min. (on-line SPE)**	**2 mL**	**On-line SPE LC-MS**	**74 – 121**	**0.1 – 20 ng/L**
	23	**SED/SPM**	**10 min. + extraction & SPE**	**0.5 g**	**SPE LC-MS**	**63 – 122**	**0.002 – 10 ng/g**

WW: wastewater, SL: sludge, SED: sediment, TW: tap water, SW: surface water, GW: ground water, SPM: suspended particulate matter

Focusing on one group of pharmaceuticals or one environmental matrix could potentially lead to lower LODs or higher recoveries and precisions. The present method focused on some representative compounds of different pharmaceutical classes, but other targeted compounds might be a better choice depending on some specific samples like hospital in other countries where prescribed drugs may vary. The method could be applied to a larger number of compounds that are within the physicochemical properties range of the selected analytes, especially in terms of molecular mass, pK_a and polarity. Previous works by Liang et al., Zhong et al. or Boulard et al. proposed methods for a larger number of compounds but often required different LC-MS injections to analyze positively and negatively charged analytes separately, while the triple quadrupole system used for the present method can alternate between those modes in a single run [293–295]. This can be an advantage with a view to accomplish large sampling campaigns that need to be readily analyzed to avoid sample alteration during storage time. On the other hand, this also limits the number of total method analytes since switching between ionization modes restricts the scan time/events devoted to each MS/MS transition. Some compounds were found to be more sensible to sample pre-treatment steps such as extraction/purification for the solid samples, and not all initially targeted compounds could be retained in the final method due to noted QA/QC failures (e.g., MTX and NORF were excluded from the method for solids due to low recoveries). To improve overall validation parameters, additional IS could be introduced to better correct for compounds with different retention times, ionization mode and polarity. The present method demonstrates that with a limited number of IS, US EPA requirements could be met and that even with samples of different origins, the matrix effect can be compensated [277].

5.4 Method application to field samples

Different matrices were selected to assess pharmaceutical contamination from different wastewater sources in Eastern Canada. The on-line method was used for quantification in both hospital effluents and wastewater treatment plants (influent and effluent). The developed method for solids was used to treat the SPM of all wastewater samples. We hypothesized that hospital samples would present the highest concentration for specific pharmaceuticals, while compounds largely used and prescribed to the general population (such as IBU or estrogenic hormones) might reach higher levels in urban wastewater. Analysing influents prior to treatment by different facilities could provide insight into the efficiency of pharmaceutical removal.

5.4.1 Hospital wastewater

A total of 30 hospital effluents were analyzed from institutions of different capacities, part of which are general facilities, and some are more specialised clinics such as mental health departments or emergency clinics. Among the targeted analyte list, only the two hormones (EE2 and NOR) were not detected and in general this pharmaceutical group has the lowest detection frequency in the aqueous phase. This might be explained by the fact that these compounds are sensitive to degradation and because hormones are generally found at lower concentrations compared to other pharmaceutical classes [297]. Analgesics are the most frequently detected compounds with IBU being detected in 100% of the hospital samples. CAF was also present in every analysed sample and had the highest maximum concentration (670 000 ng/L) among contaminants, suggesting that it could be a good tracer of anthropic urban contamination for other matrix types such as surface or tap water. Antidepressant (VEN), antibiotics (SMX, CLA) and anticonvulsant (CBZ) were found in most samples with detection frequencies of 87% or higher (**Table 5-3**). Metabolites were less recurrently detected than their parent compounds, though in some cases the maximum concentrations surpassed those of the parent compounds such as OH-IBU *vs.* IBU, 2OH-CBZ *vs.* CBZ, or DVEN *vs.* VEN. The lowest concentrations were measured for hormones as well as for antidepressant FLU and its metabolite (NORF). The chemotherapy agent MTX was found in almost half of the samples but only at relatively small concentrations. Facilities with mental health department did not show trends in detected pharmaceuticals. For example, antidepressants were not more frequently detected in their effluents. On the other hand, the highest concentration of IBU was found in an emergency clinic effluent and MTX was slightly more concentrated in a larger institution hosting a chemotherapy department. However, the limited number of samples and institutions is not sufficient to conclude if this difference would be significative. In a future study, it would be interesting to obtain samples from retirement homes where high pharmaceutical consumption could enhance differences in contamination profile compared to hospital or urban wastewater.

In SPM samples (**Table 5-3**), the proportion of the different quantified analytes was distinctly different from the aqueous phase. Some hormones were detected more frequently denoting a better affinity or higher stability when adsorbed to solid particles in wastewater samples, also in accordance with previous studies [298,299]. In one case, EE2 concentration reached a relatively high level (810 ng/g) while it was not detected in the dissolved phase. On the contrary, some metabolites with some additional polar group to the parent compound, such as OH-IBU and OH-DCF, are much less recurrent in the SPM than in the dissolved phase. CAF is also present in all the analysed solid phase samples, along with CBZ and its metabolite 2OH-CBZ.

These anticonvulsants are known to have affinity to SPM in environmental samples [295]. Apart from EE2, the most concentrated pollutants found in the wastewater SPM were IBU (maximum of 31 000 ng/g) and its metabolite OH-IBU, DCF, DVEN, FLU and CAF. The antidepressant FLU had a much higher detection frequency in the SPM than in the filtrate samples.

Table 5-3. Pharmaceuticals detected in hospital effluent samples[2].

	Hospital Aqueous Phase		Hospital SPM	
Compound	**Detection Frequency (%)**	**Quantified Range (ng/L)**	**Detection Frequency (%)**	**Quantified Range (ng/g)**
IBU	100	28 – 47 000	83	40 – 31 000
OH-IBU	97	230 – 140 000	28	490 – 3800
HMOR	40	69 – 5500	28	9.3 – 93
DCF	90	4.0 – 2300	94	7.2 – 1200
OH-DCF	77	1.3 – 960	11	6.4 – 10
SMX	77	11 – 42 000	33	0.75 – 310
CLA	73	0.60 – 1900	22	0.50 – 580
MTX	43	5.6 – 360	n.a.	n.a.
E2	10	28 – 86	n.a.	n.a.
E1	70	2.1 – 440	72	0.11 – 23
E3	6.7	120 – 180	39	1.4 – 220
EE2	0	n. a.	5,6	810*
LEVO	6.7	15 – 19	n.a.	n.a.
NOR	0	n. a.	n.a.	n.a.
PROG	40	5.0 – 99	56	25 – 220
MPROG	3.3	55*	n.a.	n.a.
TESTO	6.7	250 –260	11	110 – 370
CBZ	87	3.8 – 4000	100	0.20 – 160
2OH-CBZ	33	7.2 – 130 000	100	0.085 – 6.6
VEN	93	1.8 – 5200	5,6	58*
DVEN	83	14 – 21 000	50	6.7 – 1700
FLU	6.7	2.1 – 9.5	44	6.8 – 2100
NORF	6.7	9.8 – 15	n.a.	n.a.
CAF	100	270 – 670 000	100	8.0 – 4900
ACE	63	1.0 – 15 000	33	0.16 – 308

* Only one concentration obtained above the LOQ.

[2] These samples consist of wastewater collected directly after their rejection from hospital facilities before mixing with municipal sewage.

Previous studies of hospital effluents reported antibiotics at *circa* 20 µg/L [300], chemotherapy agents and hormones at 0.20 and 0.10 µg/L respectively [301], and analgesics at 200 µg/L [302]. Pharmaceutical concentrations in hospital effluents may vary considerably between countries. For example, Zafar et al. reported SMX concentrations up to 16 000 µg/L with this antibiotic being the less concentrated among their targeted compound [303]. Seasonal variation might also occur and a more frequent sampling of hospital wastewater would give a better approximation of the load of the many pharmaceuticals rejected over a year [304].

5.4.2 Urban wastewater

Among the targeted analytes, 16 compounds were found in the dissolved phase of the influent and/or effluent of the six municipal wastewater treatment plants. Of these, 11 pharmaceuticals were present in all samples (see SI **Table 5-23**). Concentration profiles varies from low concentrations for compounds such as the hormone E1 (3.9 to 28 ng/L in influent and 3.8 to 11 ng/L in effluent) or the chemotherapy MTX (4.1 to 40 ng/L in influent and 3.4 to 5.2 ng/L in effluent) to high concentrations for the stimulant CAF (12 000 ng/L to 67 000 ng/L in influent and 3600 to 32 000 ng/L in effluent) or the analgesic metabolite OH-IBU (2700 to 49 000 ng/L in influent and 1800 to 9700 ng/L in effluent). Among the six wastewater treatment plants, WWTP #4 and #5 presented the highest concentrations for every analyte except for 2OH-CBZ and EE2. EE2 was quantified only in influent WWTP #1 and #4 while FLU was detected only in influent WWTP #4, and these were the only analytes not found in any sampled effluents. The high concentrations of WWTP #4 and #5 could not be related to the population served by these facilities, but these two WWTP having the highest BOD_5 compared to the average flow rate of treated water might indicate wastewaters heavily loaded with organic matter (see SI **Table 5-7**) [305,306]. The average concentrations of the beta blocker ACE were similar between influent and effluent (mean concentrations = 320 ng/L) and might indicate that this compound is not significantly affected by the treatment of the sampled facilities. Besides ACE, contaminants in effluents were generally found in smaller concentrations than in influents with some exceptions such as the antibiotic SMX (influent mean concentration = 37 ng/L, effluent mean concentration = 61 ng/L) or the anticonvulsant CBZ (influent mean concentration = 96 ng/L, effluent mean concentration = 110 ng/L). This might be explained by the fact that wastewater pharmaceutical concentrations are not stable over time and that there is a challenge of collection grab samples of corresponding influent/effluent pairs. However, considerable amounts of pharmaceutical are still present in all the six wastewaters treatment plant effluent after their

different cleaning process indicating that these facilities are only partially effective for the removal of these type of organic contaminants. Previous studies report that pharmaceutical elimination from wastewater are variable and might depend on factor such as treatment process, compound physicochemical properties or season and might range from null to total removal [307–310].

When comparing contamination of the WWTP effluent to hospital wastewater, all pharmaceuticals were found at lower concentrations except for the birth control hormone EE2 that was not detected in the sampled hospital effluents. On the contrary, HMOR (an analgesic derived from morphine) was found only in hospital effluent and can be expected to be found only in trace amounts at WWTP. However, there are some analytes found in close maximal concentrations in both matrices such as CLA, OH-IBU or VEN, but these are compounds that can be prescribed and consumed at home. Another factor that needs to be taken into consideration is the dilution factor, since hospital buildings are known to have a high water consumption for different services such as patient restrooms, kitchens or laundry [311].

Out of the targeted compounds, 11 pharmaceuticals were detected in the solid phase of the WWTP samples, with 7 of them being present in 100% of the samples (see SI **Table 5-24**). Even though most of their concentrations are in the low ng/g range, more hormones are detected in the SPM when compared to the dissolved phase. PROG was found in only 2 of the sites and in none of the effluents while TESTO was present only in WWTP#4 even after water treatment. The antidepressant FLU was detected in all samples and was detected at the highest concentration (529 ng/g) followed by CAF (452 ng/g). Highest contamination of effluent was observed for the antibiotic CLA (295 ng/g) and CAF (282 ng/g). On the lower end of contamination, 2OH-CBZ, for which the LOD is relatively low, could be detected between 0.10 and 0.82 ng/g in influents and between 0.13 and 0.79 ng/g in effluents. Average concentrations in the SPM were notably lower after the treatment process, except for DCF for which the average concentration remained similar (influent mean concentration = 40 ng/g, effluent mean concentration = 41 ng/g). Despite a smaller average SPM mass collected on the filters (see SI **Table 5-25**), the presence of targeted analytes on the solid phase following the different treatment plants indicate that the processes are insufficient to remove all particles or that a coagulation process occur in the effluent and lead to pharmaceutical adsorption. These particles can transport stabilized pharmaceutical to the environment after the rejection of wastewater to surface water via the effluents and contribute to sediments pollution [312,313].

The quantified amounts of pharmaceuticals in the dissolved phase fall in the ranges of previous monitoring of urban wastewaters, for example around 1000 ng/L for VEN in Spain [314],

300 ng/L for CBZ in the United States [309], 30 ng/L for E1 in South Africa [310], 500 ng/L for SMX in Canada [308] and 2000 ng/L for IBU in France [307]. Pharmaceutical concentrations are variable between regions and over time because of seasonal consumption patterns or degradation rates [307–309].

When trying to compare values for the SPM, there is a lack of data for many targeted analytes. Taking this phase into account might change the pharmaceutical removal evaluation by wastewater treatment processes due to contaminant adsorption and gain in stability [315,316]. A study by Lahti et al. quantified antibiotics around 400 ng/g, while 200 ng/g of the antidepressant FLU was reported by Baker et al. [317,318]. The lack of existing regulations can lead to much higher values such as up to 80 000 ng/g of antibiotics in SPM from a wastewater of Kenya [316]. A previous work by Darwano et al. reported slightly higher concentrations for the stimulant CAF (approx. 540 ng/g) and the anticonvulsant CBZ (approx. 70 ng/g) in SPM of municipal wastewaters in Canada [284]. More studies have focused on the sludges that results of different treatment plants process that can accumulate particles reaching high pollutant concentrations. For example, Bisognin et al. quantified CAF up to 35 000 ng/g, Martinez-Alcala et al. reported CBZ at 23 000 ng/g and Martin et al. measured IBU at 51 000 ng/g in sludges of municipal wastewaters in Brazil and Spain [319–321].

5.4.3 Distribution coefficients and mass balance

A limited number of publications have examined suspend solids in hospital and municipal wastewaters and previous efforts on partitioning are more focused on sludge of different wastewater treatment processes [322]. In addition to better understanding persistence and degradation behaviors, establishing the distribution coefficients is key in understanding their environmental fate [323].

Individual pharmaceutical suspended solid to wastewater distribution coefficient (K_d) were calculated for samples containing analytes in both phases. There is a challenge in K_d values comparison since this parameter is sensible to many variables such as matrix temperature, pH, particulate organic carbon, or suspended matter concentration [324,325]. For this reason, **Table 5-4** present the ranges of log (K_d) values for targeted analytes in the selected wastewater matrices and details of K_d calculation are presented in SI (**Text 5-3**). Additional data on calculated K_d such as medians, means and standard deviations are presented in SI (**Table 5-26**). When considering each matrix, the log (K_d) values ranged between 0.10 (2OH-CBZ) and 5.90 (DCF) in

hospital effluents, -1.31 (2OH-CBZ) and 3.51 (FLU) in WWTP influents and -0.90 (2OH-CBZ) and 3.55 (CLA) in WWTP effluents. Highly polar compounds such as 2OH-CBZ (log *P* = 0.13) were in the lower range of the obtained K_d values, while the opposite trends were observed for more apolar compounds such as FLU (log *P* = 4.05) or DCF (log *P* = 4.51). However, the log *P* values alone do not allow an accurate prediction of distribution coefficients like in the case of CAF (log K_d = 2.14 – 3.94, log *P* = -0.05). Similar conclusions were drawn in Petrie et al. and Aminot et al., and might indicate that factors such as electrostatic interactions can play a significant role in their partitioning behavior [325,326].

When comparing ranges of K_d values between selected matrices, it can be observed that hospital effluents tended to positively impact K_d values. For all analytes present in both the dissolved and the adsorbed phase, maximal log of K_d values were higher for hospital effluents when compared to both influents and effluents of the sampled WWTP. For some analytes, the hospital samples were the only one for which a K_d value could be calculated, and this might be due to higher contamination levels in these specific wastewaters or by a higher amount of suspended solids available for analysis when compared to WWTP samples (see SI **Table 5-25**). When comparing ranges of Log K_d values of the two WWTP matrices, the difference is not as clear for detected pharmaceuticals, with higher values obtained for either influents or effluents. This might be due to partial removal of suspended solids or incomplete removal of targeted compounds that can equilibrate with particles after the different treatment processes [326].

Table 5-4. Range of logarithm of distribution coefficient (Kd) for the selected wastewater matrices.

	Log (K_d)		
	Hospital Effluent	**WWTP Influent**	**WWTP Effluent**
IBU	1.83 - 4.73	1.36 - 2.30	1.30 - 2.16
OH-IBU	2.79 - 4.41	n.a.	n.a.
HMOR	3.29	n.a.	n.a.
DCF	2.72 - 5.90	1.62 - 3.14	2.04 - 3.22
OH-DCF	2.89 - 4.14	1.35 - 1.97	n.a.
SMX	2.12 - 2.96	n.a.	n.a.
CLA	2.91 - 5.08	1.18 - 2.87	1.67 - 3.55
MTX	4.13	n.a.	n.a.
E1	3.33 - 4.75	2.47 - 3.32	2.54 - 2.79
E3	3.85	n.a.	n.a.
TESTO	4.20	n.a.	n.a.
CBZ	3.07 - 3.98	1.70 - 2.20	1.60 - 2.20
2OH-CBZ	0.10 - 2.16	-1.31 - 0.033	-0.90 - 0.21
VEN	3.28	n.a.	n.a.
DVEN	2.69 - 4.36	n.a.	n.a.
FLU	n.a.	3.51	n.a.
CAF	2.14 - 3.94	0.76 - 1.37	0.87 - 1.70
ACE	1.05 - 4.33	1.96 - 3.19	1.58 - 2.79

Considering the mass balance between dissolved and adsorbed fractions can give further information on the relative weights of these phases and fate of organic pollutants [327,328].[3] The particle-associated fraction (Φ) was calculated (SI **Text 5-3** and **Table 5-5**). Additional data on calculated particle-associated fractions such as medians, means and standard deviations are presented in SI (**Table 5-27**). When considering individual matrices, Φ ranged between 0.0009% (2OH-CBZ) and 56% (DCF) for hospital wastewaters, 0.0001% (2OH-CBZ) and 11% (FLU) for WWTP influents and 0.0001% (2OH-CBZ) and 6.2% (DVEN) in WWTP effluents. Punctually higher values were found for DVEN (13%) and CLA (31%) among the hospital samples while no other compound than FLU exceeded 10% of analytes adsorbed to particles in the municipal WWTPs. Particle-associated fractions followed similar trends as for K_d values, with some very polar compounds such as 2OH-CBZ being the compound least associated to suspended solids, and DCF, FLU and CLA being amongst the most particle-associated analytes.

[3] The mass balance refers to the adsorbed fraction of pharmaceutical unto the particulate matter.

Analytes in the hospital effluents tend to reach higher particle-associated fractions than municipal WWTP matrices for all detectable compounds. Hospital and WWTP influents being untreated matrices with different dilution levels and WWTP effluents being sampled after treatment process are expected to be less contaminated and with partial removal of SPM. Hospital effluents are expected to be more contaminated by pharmaceuticals, both in the dissolved phase and on the suspended solids, and this raises concerns about the release of pharmaceuticals to urban wastewater systems without any specific pre-treatment steps. Such technologies are being studied and have been proven more efficient than classical municipal WWTP on some pilot installations as previously discussed by Verlicchi [323].

Table 5-5. Range of particle-associated fraction (Φ) for the selected wastewater matrices.

	Φ (%)		
	Hospital Effluent	**WWTP Influent**	**WWTP Effluent**
IBU	0.0022 - 1.8	0.022 - 0.79	0.0057 - 0.29
OH-IBU	0.037 - 2.9	n.a.	n.a.
DCF	0.033 - 56	0.013 - 0.31	0.023 - 0.50
OH-DCF	0.048 - 3,3	0.049 - 0.090	n.a.
SMX	0.024 - 0.14	n.a.	n.a.
CLA	0.0086 - 31	0.040 - 3.2	0.013 - 4.4
E1	0.090 - 7.3	0.16 - 7.2	0.049 - 0.64
E3	0.79 - 2.0	n.a.	n.a.
PROG	4.7	n.a.	n.a.
TESTO	0.58 - 5.5	n.a.	n.a.
CBZ	0.012 - 2,2	0.051 - 0.44	0.017 - 0.14
2OH-CBZ	0.0009 - 0.027	0.0001 - 0.0008	0.0001 - 0.0007
VEN	0.085 - 0.086	n.a.	n.a.
DVEN	0.031 - 13	n.a.	0.016 - 6.2
FLU	n.a.	8.9 - 11	n.a.
CAF	0.0097 - 0.51	0.0033 - 0.040	0.0056 - 0.037
ACE	0.0011 - 1.3	0.098 - 0.52	0.011 - 0.32

To compare partitioning data with previous publications, values obtained for sludges in WWTP had to be used because of the lack of available data for hospital effluents. Distribution coefficient values (log K_d) were found in similar ranges for antimicrobials (between 1.2 and 3.8) by Senta et al. and in some multiclass pharmaceutical studies with values not exceeding 4.3 [324–326,329]. Some higher values obtained in the hospital matrix, such as 5.9 for DCF, are greater than these ranges, but are still relatively close. Particle-associated fractions in WWTP effluent was found to be smaller compared to raw wastewater in a publication by Petrie et al. [326]. Calculated

ranges of Φ values often lead to conclusion of a much greater pharmaceutical mass associated with the dissolved phase. However, some compounds in raw WWTP effluents sometimes reach values as high as 50%, comparable to findings in the present study [324,326,330].

5.5 Conclusions

The present study aimed at developing a fast and robust method for the quantification of multiclass pharmaceuticals that could be applied to different matrices such as hospital effluents, urban WWTP influents/effluents, and corresponding suspended solids. A chromatographic method was developed and was coupled with an on-line SPE pre-concentration gradient for the analysis of dissolved phase samples. An extraction/purification method was also optimized for the SPM. During method development and optimization, particular focus has been put on sorbent material for sample enrichment, the choice of filter material or selection between cleanup or off-line SPE for the SPM. A relatively fast final method (8 min.) allows high throughput of samples, which is advantageous for environmental analysis. Satisfactory validation parameters (linearities, recoveries, accuracies and precisions) were obtained for the selected pharmaceuticals, falling in the expected range prescribed by US EPA requirements [277] with a few exceptions. LODs are in the range of low or sub ng/L for wastewater and in the range of low or sub ng/g for SPM. These values were comparable with previous published work for these matrices and slightly better than some other studies focusing on SPM or sediments. A matrix-matched calibration approach was deemed appropriate (residual matrix effect ≤20%) for the quantification of field samples.

The on-line SPE method was applied to different hospital wastewaters (n = 30) of Eastern Canada. Only two targeted analytes were under LODs in all samples, while 23 of the remaining pharmaceutical were quantified between 0.60 ng/L (CLA) and 670 µg/L (CAF). Evaluation of these specific wastewaters could help understand the weight of these institutions in the total load of pharmaceutical that ends up at treatment plants with some research suggesting a specific treatment to hospital effluents to lower the burden of municipal WWTP [311,331]. Additionally, 6 WWTPs were sampled, with both influents and effluents analysed. The obtained concentrations ranged between low ng/L (E1 = 3.8 ng/L) to thousands of ng/L (CAF = 67 000 ng/L). Effluents were generally less contaminated than influents, but non-negligeable concentrations of selected compounds confirms that WWTPs are only partially efficient for pharmaceutical removal. A more extensive sampling and use of composite sampling technique could help more rigorously assess the abatement efficiency of different treatment technologies. The developed extraction and

purification method was successfully used for quantification of pharmaceuticals in SPM of both hospital and urban wastewaters. This allowed calculation of distribution coefficients (K_d) and particle-associated fractions (Φ), more frequently documented in wastewater sludges in previous literature.

In follow-up studies, the developed extraction/purification method could be adapted to the analysis of wastewater treatment sludges. Analysis of these samples would be of great interest since they can contain high pollutant loads and are frequently reused as fertilizers for agricultural field application [332,333]

Acknowledgments

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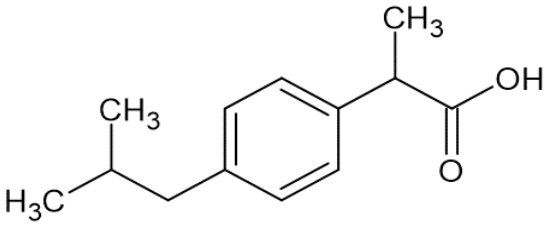
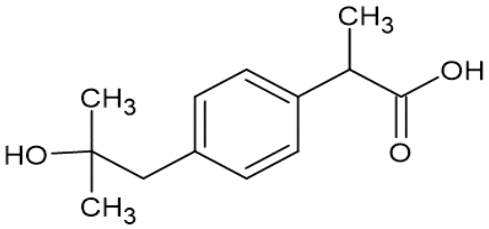
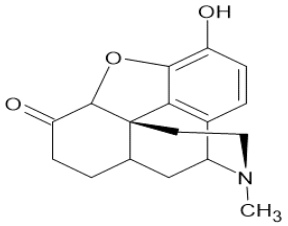
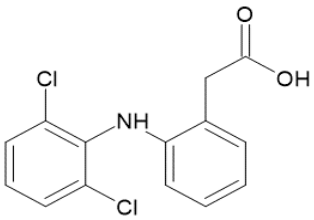
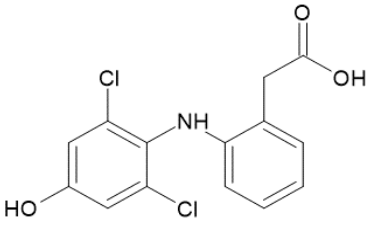
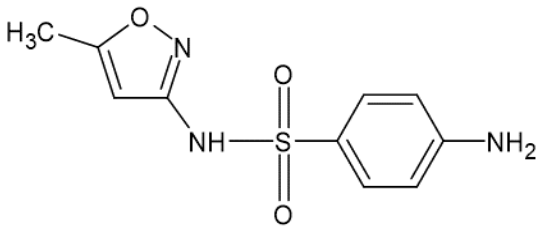
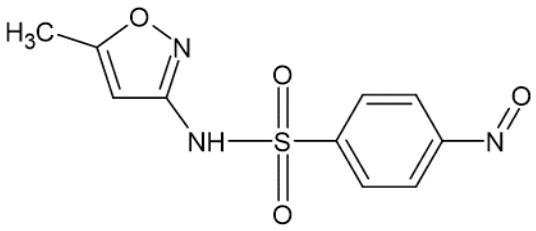
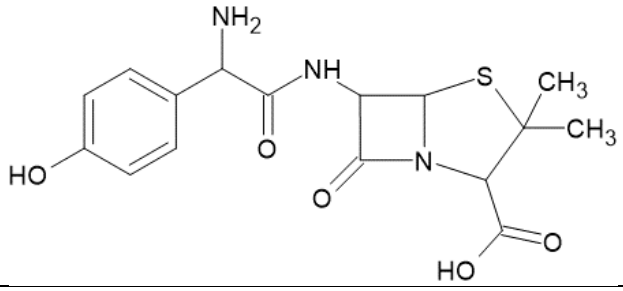
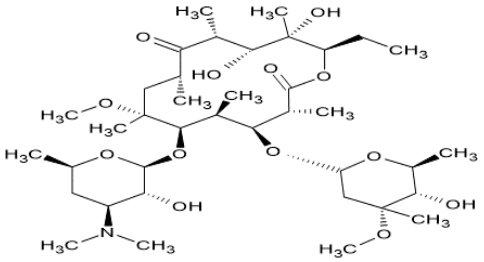
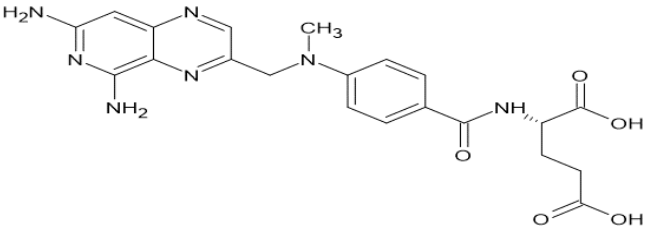
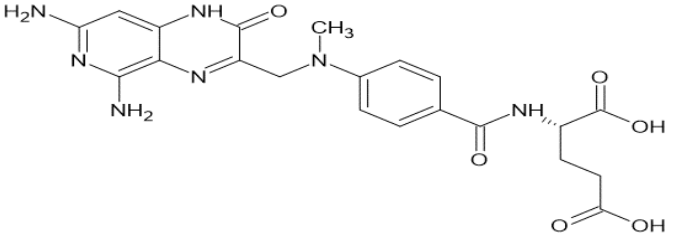
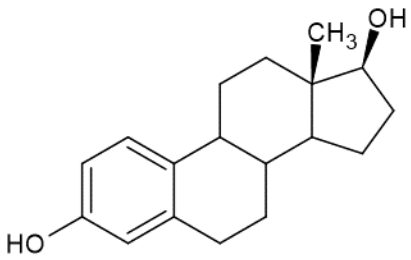
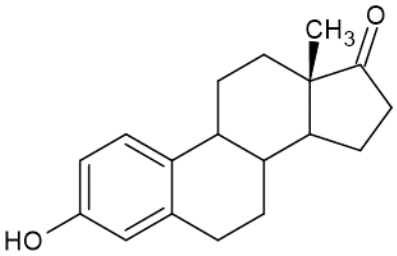
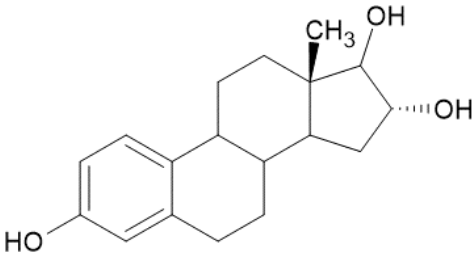
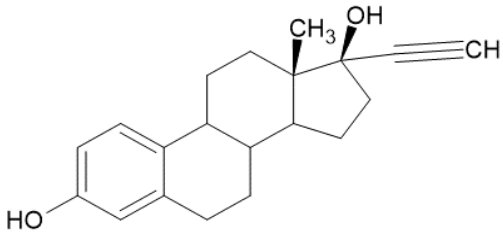
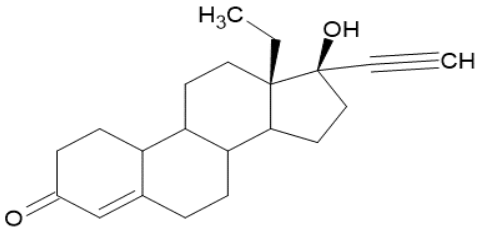
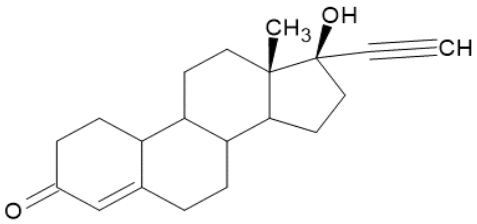
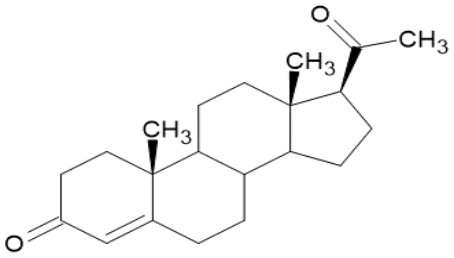
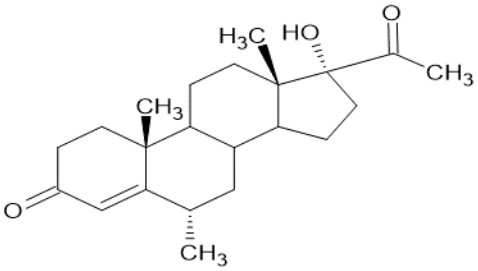
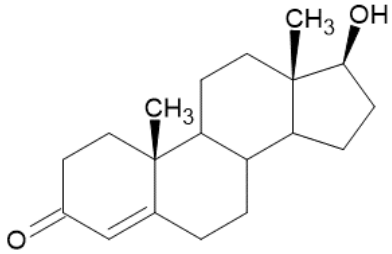
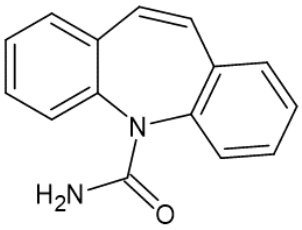
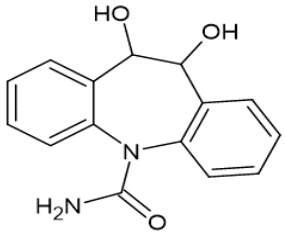
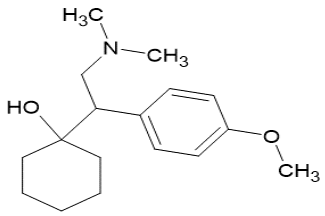
Supporting Information

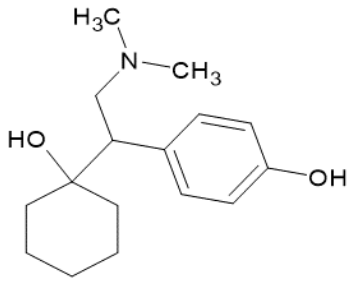
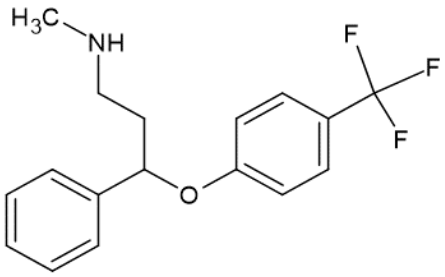
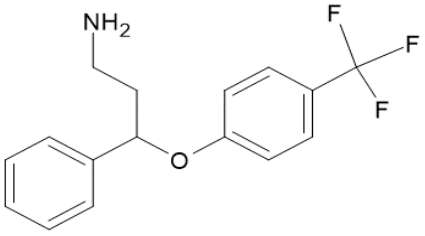
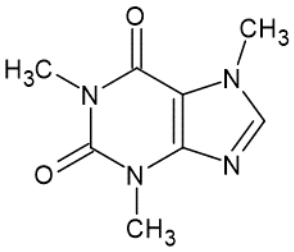
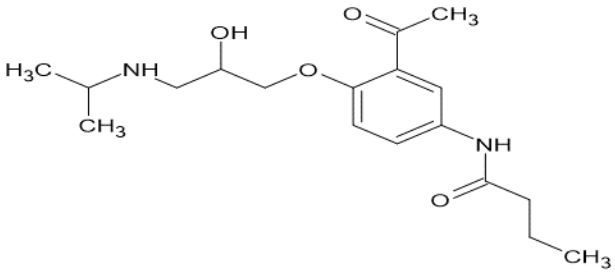
Table 5-6. Structure and properties of the targeted pharmaceuticals (data retrieved from ChemAxon Ltd).

Nom	MW g/mol	Log *P*	Structure
Ibuprofen	206.28	3.97	
IBU			
2-Hydroxyibuprofen	222.28	2.41	
OH-IBU			
Hydromorphone	285.34	1.06	
HMOR			
Diclofenac	296.16	4.51	
DCF			
4-Hydroxydiclofenac	312.15	3.70	
OH-DCF			

Sulfamethoxazole	253.28	0.89	
SMX			
4-Nitrososulfamethoxazole	267.26	0.87	
NO-SMX			
Amoxicillin	365.40	0.87	
AMOX			
Clarithromycin	747.95	3.16	
CLA			
Methotrexate	454.44	-1.85	
MTX			
7-Hydroxymethotrexate	470.44	-2.87	
OH-MTX			

Estradiol **E2**	272.38	4.01	
Estrone **E1**	270.37	3.13	
Estriol **E3**	288.38	2.45	
Ethinylestradiol **EE2**	296.41	3.67	
Levonorgestrel **LEVO**	312.45	3.48	
Norethindrone **NOR**	298.43	2.97	

Progesterone **PROG**	314.47	3.87	
Medroxyprogesterone **MPROG**	344.23	3.50	
Testosterone **TESTO**	288.42	3.32	
Carbamazepine **CBZ**	236.27	2.45	
10,11-Dihydroxy-10,11-dihydrocarbamazepine **2OH-CBZ**	270.28	0.13	
Venlafaxine **VEN**	277.4	3.20	

Desvenlafaxine	263.38	0.21	
DVEN			
Fluoxetine	309.33	4.05	
FLU			
Norfluoxetine	295.3	3.74	
NORF			
Caffeine	194.19	-0.07	
CAF			
Acebutolol	336.43	1.71	
ACE			

Text 5-1. Information on solvents, reagents, and filter providers.

HPLC grade solvents including water (H_2O), acetonitrile (ACN), methanol (MeOH), and acetone were obtained from Thermo Fisher (Whitby, ON, Canada). Formic acid (FA, purity ≥ 98%), ammonium hydroxide (NH_4OH, purity 30%), and ammonium fluoride (NH_4F, purity ≥ 98%) were purchased from Sigma Aldrich (Saint-Louis, MO, U.S.A.). Glass fiber (0.3 µm, 25mm), nylon (0.22 µm, 25mm), cellulose acetate (0.2 µm, 25mm), polycarbonate (0.2 µm, 25mm), polyester (0.2 µm, 25mm) and polypropylene (0.2 µm, 30mm) filters were from Sterlitech (Kent, WA, U.S.A.).

Table 5-7. Details on wastewater treatment plants, including population served and average flowrate.

WWTP	Population	Average influent flowrate (m^3/day)	DBO_5 (kg/day)	Treatment type
#1	48 000	37 000	2 600	settling, biofiltration, UV disinfection
#2	59 000	44 000	n. a.	settling, UV disinfection
#3	270 000	230 000	22 000	settling, UV disinfection
#4	17 000	11 000	1 400	settling
#5	70 000	43 000	4 200	settling, physico-chemical
#6	47 000	30 000	2 800	settling, biofiltration

Table 5-8. Chromatographic gradient for the off-line method at a flow rate of 500 µL min^{-1}.

Time / min.	H_2O / %	MeOH / %	H_2O + 1 mM NH_4F / %
0.00	90	5	5
1.00	90	5	5
7.00	0	95	5
8.00	0	95	5
8.10	90	5	5
10.00	90	5	5

Table 5-9. Chromatographic gradient for the on-line method at a flow rate of 500 µL min^{-1}.

Analytical pump gradient:

Time / min.	H_2O / %	MeOH / %	H_2O + 1 mM NH_4F / %
0.00	55	40	5
1.60	55	40	5
5.20	0	95	5
7.20	0	95	5
7.21	55	40	5
8.00	55	40	5

SPE charging pump gradient:

Time / min.	H_2O + 0.5 % FA	MeOH + 0.5 % FA	Flow rate (µL min^{-1})
0.00	100	0	2500
1.60	100	0	2500
1.70	0	100	2000
5.00	0	100	2000
5.01	100	0	1500
8.00	100	0	1500

Table 5-10. Optimized mass spectrometry acquisition parameters for targeted compounds and isotope-labeled internal standards.

Compound	Ionization mode	Precursor ion (m/z)	Product ion (m/z)	Collision energy (V)	RF lens (V)
IBU	Negative	205.3	161.2	10	30
			204.8	10	
IBU-d3	Negative	208.2	164.3	10	33
OH-IBU	Negative	221.2	177.0	10	30
			220.7	10	
HMOR	Positive	286.2	184.9	30	79
			156.9	43	
DCF	Positive	296.1	214.0	34	43
			215.0	19	
DCF-d4	Positive	300.0	218.0	35	44
OH-DCF	Positive	312.0	230.0	35	51
			231.0	20	
SMX	Positive	254.1	156.0	16	49
			108.1	24	
SMX-13C6	Positive	260.2	162.0	16	52
NO-SMX	Negative	266.0	122.1	24	60
			170.0	14	
AMOX	Positive	398.2	349.1	16	49
			381.1	10	
CLA	Positive	748.5	590.6	16	81
			558.3	20	
MTX	Positive	455.2	308.0	20	83
			175.0	39	
MTX-d3	Positive	458.3	311.1	20	78
OH-MTX	Negative	469.2	340.1	22	92
			451.1	19	
E2	Negative	271.0	269.1	33	99
			182.9	41	
E2-13C6	Negative	277.2	186.1	43	97
E1	Negative	269.2	145.1	41	97
			159.2	39	
E3	Negative	287.2	145.1	43	105
			171.1	39	
EE2	Negative	295.0	269.1	33	90
			267.1	27	
LEVO	Positive	313.3	245.1	18	61
			185.1	19	
NOR	Positive	299.3	231.2	18	57
			171.1	20	

PROG	Positive	315.2	97.1 109.1	24 27	55
PROG-d9	Positive	324.3	100.1	25	53
MPROG	Positive	345.2	97.1 123.1	28 27	58
TESTO	Positive	289.3	97.1 109.1	24 27	54
CBZ	Positive	237.2	194.1 192.1	20 24	58
CBZ-d10	Positive	247.3	204.1	22	60
2OH-CBZ	Positive	271.2	180.1 253.1	30 10	30
VEN	Positive	278.3	260.2 58.2	10 20	48
VEN-d6	Positive	284.4	266.1	10	45
DVEN	Positive	264.3	246.0 58.1	10 20	45
FLU	Positive	310.2	148.1 44.2	10 12	43
NORF	Positive	296.2	134.1 30.3	10 10	30
CAF	Positive	195.2	138.1 110.1	20 23	58
CAF-13C3	Positive	198.2	140.1	20	61
ACE	Positive	337.3	319.1 116.0	15 22	72

Table 5-11. Characteristics of the screened analytical columns.

Chromatographic column type	Sorbent	Dimensions & particle size	Pore size (Å)	pH range
Hypersil GOLD C18 [a]	C18	50 x 2.1 mm; 1.9 µm	175	1 - 11
BetaBasic 18 [a]	C18	100 x 2.1 mm; 3 µm	150	2 - 12
HYPERCARB [a]	Porous graphitic carbon	100 x 2.1 mm; 3 µm	250	0 - 14

[a] Thermo Fisher

Table 5-12. Characteristics of the screened on-line SPE columns.

SPE column type	Sorbent	Dimensions & particle size	Pore size (Å)	Surface area (m^2/g)	pH range
Hypersil GOLD C8 [a]	C8	20 mm x 2.1 mm; 5 µm	175	220	2 - 9
Hypersil GOLD aQ C18 [a]	C18	20 mm x 2.1 mm; 12 µm	175	220	2 - 9
HyperSep Retain PEP [a]	PS-DVB, urea groups	20 mm x 2.1 mm; 40 - 60 µm	90	550 - 750	0 - 14
BetaBasic 18 [a]	C18	20 mm x 2.1 mm; 3 µm	150	200	2 - 12
Oasis HLB [b]	NVP-DVB	20 mm x 2.1 mm; 5 µm	80	830	0 - 14
Isolute ENV+ [c]	Hydroxylated PS-DVB	30 mm x 2.1; 40 µm	800	1000	1 - 14

[a] Thermo Fisher; [b] Waters; [c] Biotage

Text 5-2. Calculation of the different criteria for the Derringer's desirability approach

Criterion d_1:

The d_1 criterion is defined to take into account the conditions that allow the best precision. It is calculated as the No. of targeted analytes that meet the requirement of an RSD ≤ 10%, divided by the total number of analytes.

$$\mathrm{d1} = \frac{\#\ \text{of analytes with RSD}\ \leq 10\%}{\text{total}\ \#\ \text{of analytes}} \qquad \mathbf{Eq.\ 5-2}$$

Criterion d_2:

The d_2 criterion is defined to take into account the conditions that allow the best sensitivity. It is calculated as the mean of normalized area (each targeted analyte normalized to the maximum observed value between all the different conditions).

$$d2 = average\left(\frac{analyte\ signal\ area}{(analyte\ signal\ area)max}\right) \qquad \mathbf{Eq.\ 5-3}$$

Criterion d_3:

The d_3 criterion is defined to take into account the conditions that allow the best recoveries. It is calculated as the mean of normalized area/volume ratio (each targeted analyte ratio normalized to the maximum area/volume observed value between all the different conditions).

$$d3 = average\left(\frac{\frac{analyte\ signal\ area}{injection\ volume}}{\left(\frac{analyte\ signal\ area}{injection\ volume}\right)max}\right) \qquad \mathbf{Eq.\ 5-4}$$

Each of these criteria result in a value between 0 and 1. For each combination of sample volume and loading flow rate, the overall desirability D is derived as the geometric mean of d_i values.[4]

$$D = \left(\prod_{i=1}^{n} d_i \right)^{1/n} \qquad \textbf{Eq. 5 – 1}$$

Table 5-13. Characteristics of the tested off-line SPE sorbents.

SPE cartridge type	Cartridge Volume (mL)	Sorbent mass (mg)	Particle size (µm)
HyperSep C18 [a]	3	200	40-60
HyperSep Retain PEP [a]	6	200	40-60
Oasis HLB [b]	6	200	30
Strata-X [c]	6	200	33
Isolute ENV+ [d]	6	1000	90

[a] Thermo Fisher; [b] Waters; [c] Phenomenex; [d] Biotage

[4] E.A. Bekele, C.E.P. Annaratone, M.L.A.T.M. Hertog, B.M. Nicolai, A.H. Geeraerd, Multi-response optimization of the extraction and derivatization protocol of selected polar metabolites from apple fruit tissue for GC–MS analysis, *Anal. Chim. Acta* 824 (2014) 42–56. https://doi.org/https://doi.org/10.1016/j.aca.2014.03.030.

Table 5-14. Percentage recoveries of filtration on different filter membrane material. Each compound was spiked to a final concentration of 500 ng L-1 in HPLC water sample. Error bars correspond to standard deviations (n = 3).

	Cellulose		Glass Fiber		Nylon		Polycarbonate		Polyester		Polypropylene	
	Mean	Std dev	Mean	Std dev	Mean	Std dev	Mean	Std dev	Mean	Std dev	Mean	Std dev
IBU	82	17	94	5.5	64	8.8	98	5.1	91	3.4	55	4.5
OH-IBU	18	8.5	107	7.7	29	1.2	90	49	109	0.0	53	11
HMOR	56	2.8	78	6.3	52	3.5	129	27	60	11	41	2.4
DCF	4.4	1.9	77	4.0	128	5.3	1.1	0.53	0.18	0.0	0.11	0.0
OH-DCF	62	5.1	79	6.6	1.4	0.26	87	3.6	87	6.0	120	4.4
SMX	75	11	90	8.2	37	7.0	66	25	80	34	42	14
NO-SMX	15	11	78	8.3	29	8.6	109	43	39	59	25	24
AMOX	8.1	8.6	88	6.4	13	4.9	70	30	31	12	22	19
CLA	117	34	84	7.6	154	5.0	119	0.67	0.45	0.39	0.0	0.0
MTX	60	4.2	75	6.0	13	1.0	93	3.5	71	13	36	1.3
E2	2.7	0.87	95	2.2	0.0	0.0	91	3.0	85	2.8	1.4	0.32
E1	3.0	1.0	105	2.4	0.0	0.0	100	3.3	93	3.1	1.5	0.36
E3	2.5	0.80	87	2.0	0.0	0.0	84	2.8	78	2.6	1.2	0.30
EE2	0.70	0.11	92	1.5	6.0	3.0	84	2.3	73	2.4	10	3.1
LEVO	50	19	77	6.7	31	3.5	110	0.36	81	31	0.10	0.08
NOR	19	1.9	81	7.1	46	2.2	110	4.6	87	6.1	0.50	0.18
PROG	48	18	73	6.4	29	3.3	105	0.34	77	29	0.09	0.08
MPROG	43	16	71	5.7	26	3.0	94	0.31	69	26	0.08	0.07
TESTO	18	1.8	77	6.7	44	2.1	104	4.3	82	5.8	0.50	0.17
CBZ	65	18	78	6.5	45	1.5	111	6.4	75	17	36	11
2OH-CBZ	91	6.1	107	3.0	19	1.3	104	1.7	95	6.3	79	7.6
VEN	28	6.4	79	3.0	105	4.1	100	5.1	75	16	0.21	0.12
DVEN	63	3.7	78	6.6	71	5.1	117	6.3	84	5.3	12	4.9
FLU	9.3	5.0	70	0.91	139	7.6	1.3	0.72	0.16	0.07	0.0	0.0
NORF	56	3.2	85	1.8	0.46	0.07	91	2.7	89	7.0	33	1.4
CAF	52	1.6	84	7.0	41	3.2	92	8.2	96	14	52	2.1
ACE	49	12	71	5.7	65	5.4	105	5.5	57	17	0.09	0.12

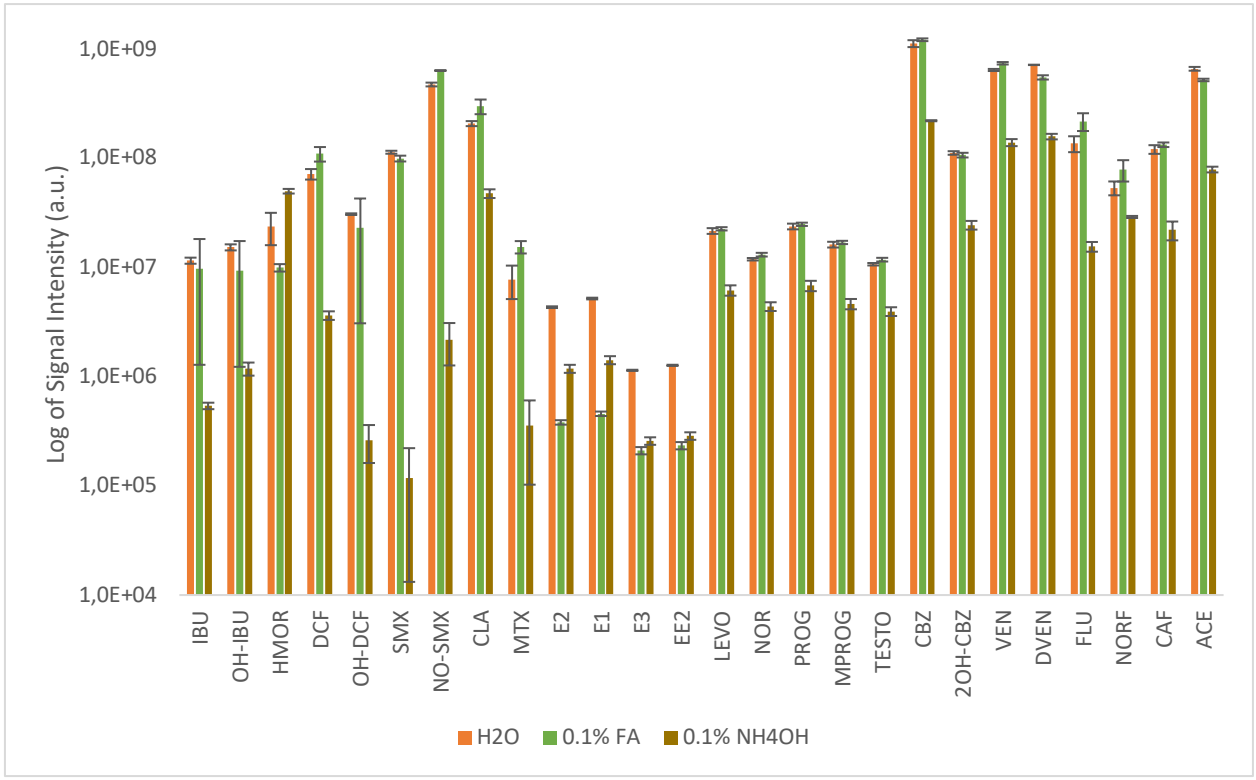


Figure 5-5. Effect of mobile phase modifier (added to the aqueous mobile phase, chromatographic gradient) on analyte signal intensity.

Table 5-15. Effect of addition of NH4F to the chromatographic mobile phase. These concentrations correspond to a 5% of total mobile phase added to the gradient for all method duration.

	H2O		1mM NH_4F		2mM NH_4F	
	Mean	**Std dev**	**Mean**	**Std dev**	**Mean**	**Std dev**
IBU	11598401	746256	10661379	1140698	11466238	975926
OH-IBU	15364453	982473	15970414	902732	16310515	171969
HMOR	23917263	7859693	97778007	12990618	103709850	30648879
DCF	72046718	7967755	127544589	1147965	108983848	1170572
OH-DCF	30898784	532904	46191737	1414199	40684843	1106363
SMX	114137948	3571237	125298691	884529	108488430	3074725
NO-SMX	476667000	19018950	413528978	5248633	403123696	1431321
AMOX	82657973	678154	26609374	1095024	20500326	2560880
CLA	208984584	11057747	250171513	6304286	281087186	18684842
MTX	7792513	2642414	7745532	1005326	6465663	4465432
E2	4341999	77698	7982015	559636	6789992	769011
E1	5210398	93237	9578418	671563	8147990	922813
E3	1145382	14129	2579002	98748	2141490	124014
EE2	1272646	15699	2865558	109720	2379434	137794
LEVO	21691551	1310411	89892243	5842035	81732614	4581669
NOR	11939508	309756	25001584	1238335	22033017	1057571
PROG	23860706	1441452	98881467	6426239	89905875	5039835
MPROG	16268663	982809	67419182	4381526	61299460	3436251
TESTO	10745557	278781	22501426	1114501	19829715	951814
CBZ	1130925031	82331764	1268690168	104340542	1207565054	185412045
2OH-CBZ	112406400	4232274	146711571	5009738	126059716	29441655
VEN	647316586	14249203	579367674	13471801	605501799	20427889
DVEN	720640918	2861118	657938183	14487954	652930749	49443569
FLU	137013511	22756290	102829364	5704526	96096961	6715930
NORF	53786773	7805004	38834858	1810939	32217491	1219282
CAF	121338368	11064264	162072490	8273793	143929203	17248128
ACE	663750737	26469773	598986012	13825213	542813122	24966783

Table 5-16. Selection of on-line SPE column for the enrichment of the targeted pharmaceuticals.

	Hypersil GOLD aQ C18		Oasis HLB		HyperSep Retain PEP		Isolute ENV+		Hypersil GOLD C8		BetaBasic 18	
	Mean	Std dev	Mean	Std dev	Mean	Std dev	Mean	Std dev	Mean	Std dev	Mean	Std dev
IBU	222179	4602	112507	22067	47079	1449	49367	3676	166880	2121	77902	1043
OH-IBU	2058928	64215	1602973	213008	829610	107506	1285604	116589	1709039	67288	986104	8584
HMOR	315302	31119	192416	10090	142941	18703	241917	15372	289447	8872	174032	7772
DCF	3646677	216283	1020703	27336	489068	27172	429540	18726	2176035	10808	1172743	39669
OH-DCF	1757614	193181	470197	27637	255513	22701	236082	27373	993366	15798	717528	74284
SMX	1145863	61086	448768	28590	559097	16834	509253	8666	666945	5002	458472	95359
NO-SMX	21009	5291	0	0	7322	2276	9883	4663	18892	2691	9331	1845
AMOX	14916	4392	0	0	4027	1274	3360	2192	13413	2598	0	0
CLA	12606347	478601	3574714	121433	4588487	15623	4751930	681800	7197128	393496	4174903	99728
MTX	494019	8314	260557	49158	258470	2704	208178	2941	303744	3092	355183	4798
E2	227817	16248	101370	15042	48677	3753	0	0	179526	0	96924	50925
E1	983708	21708	422650	78527	182567	31285	190330	10828	143621	6250	77539	40740
E3	77341	1280	13207	2419	0	0	0	0	0	0	0	0
EE2	114435	9511	47106	9916	25024	6057	22285	1857	99767	1072	51882	2372
LEVO	1607195	91276	441319	8121	257632	6332	139946	9797	1233485	5654	614139	33580
NOR	1041342	76618	247309	7424	161673	3442	113817	9499	861188	5482	418229	33174
PROG	5482436	283147	1415356	56487	786838	23125	355337	28590	377204	16500	185353	1012
MPROG	8641188	210500	1995883	43966	1194207	26317	503536	37557	688950	21675	334584	26540
TESTO	6024553	528016	1559315	54245	1029960	92770	630794	49191	79813	28390	41505	1898
CBZ	27100487	2184323	10422817	1069490	6277451	265301	5009426	577273	23496280	333169	10263433	408126
2OH-CBZ	206505	6633	73839	12255	28859	3869	62924	8731	165550	5039	86108	865
VEN	14623048	604819	8074986	716900	6208154	207331	4711264	842376	13523416	486171	5631984	2102416
DVEN	2245982	133270	2744736	206141	1813572	74847	1318630	249192	1757596	143819	925095	33377
FLU	4147164	267018	1211879	60686	1096059	44673	937766	50096	2395373	28913	1382471	142327
NORF	543207	20759	141065	12418	125935	10399	136096	12147	471505	7010	231691	1265
CAF	221794	10557	54963	3739	28221	4249	44097	10270	165316	5927	81254	5273
ACE	2039221	24060	2201420	227072	2643104	232235	1420605	251124	1889673	144934	784368	7248

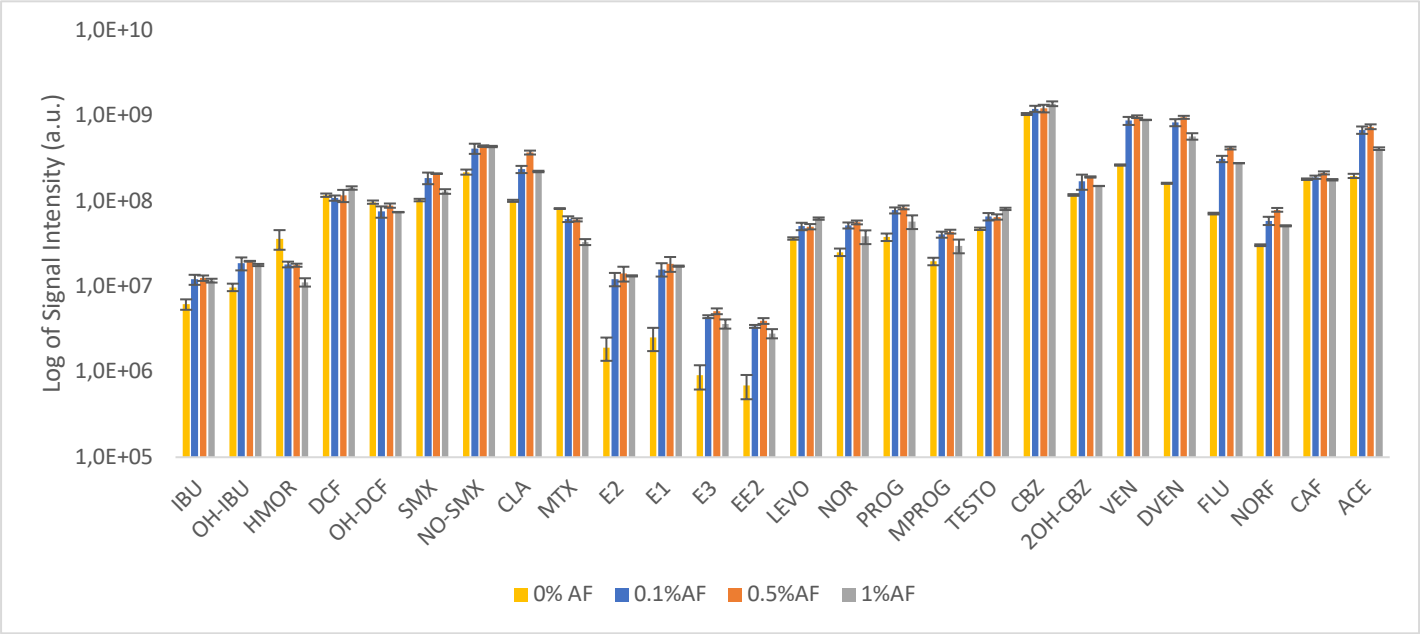


Figure 5-6. Effect of FA addition on analytes signals after on-line SPE enrichment in wastewater.

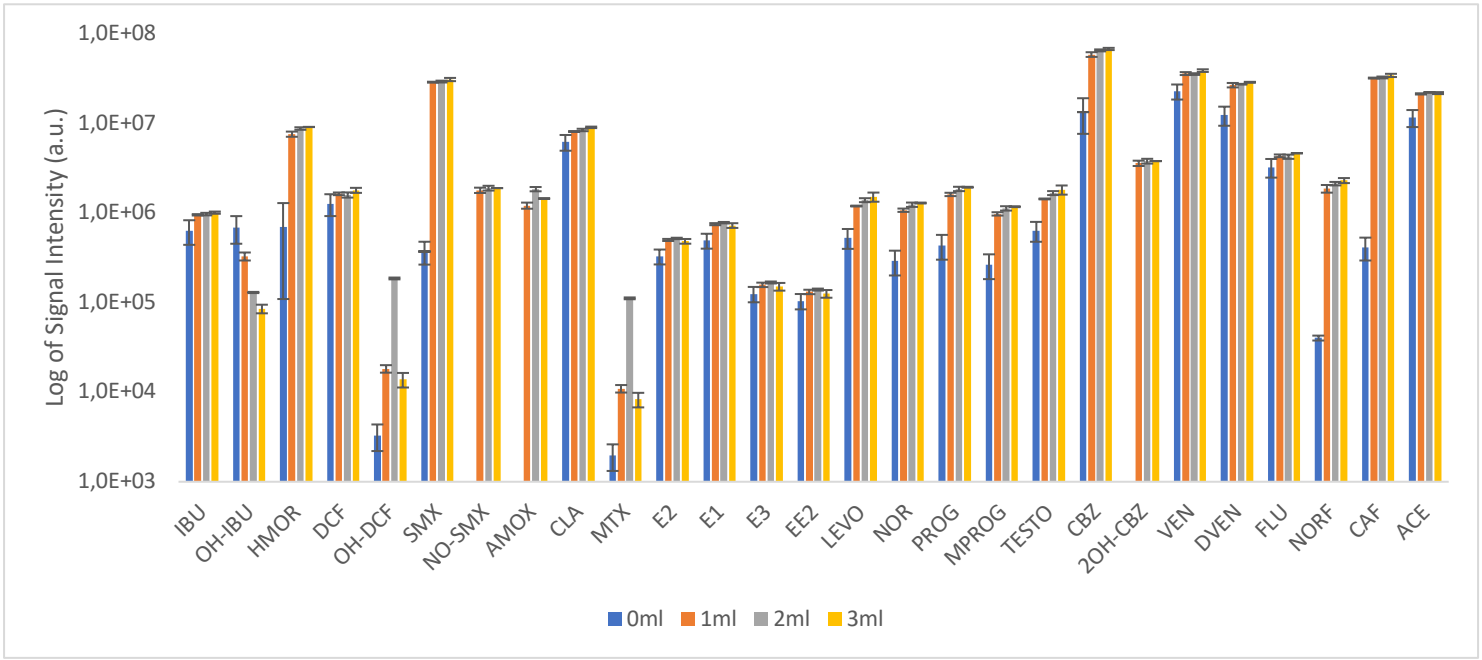


Figure 5-7. Effect of washing volume on signal intensity when loading 2 mL wastewater sample.

Figure 5-8. Selection best combination of loop volume and flow rate for on-line SPE pre-concentration of wastewater samples using a Derringer's desirability function (C1-C5 = 1 mL loop volume, flow rates of 1, 1.5, 2, 2.5 and 3 mL/min; C6-C10 = 2 mL loop volume, flow rates of 1, 1.5, 2, 2.5 and 3 mL/min) with equal d_i criteria weighting on the geometric mean. These conditions are tested with a 2 mL wash volume for each loop volume.

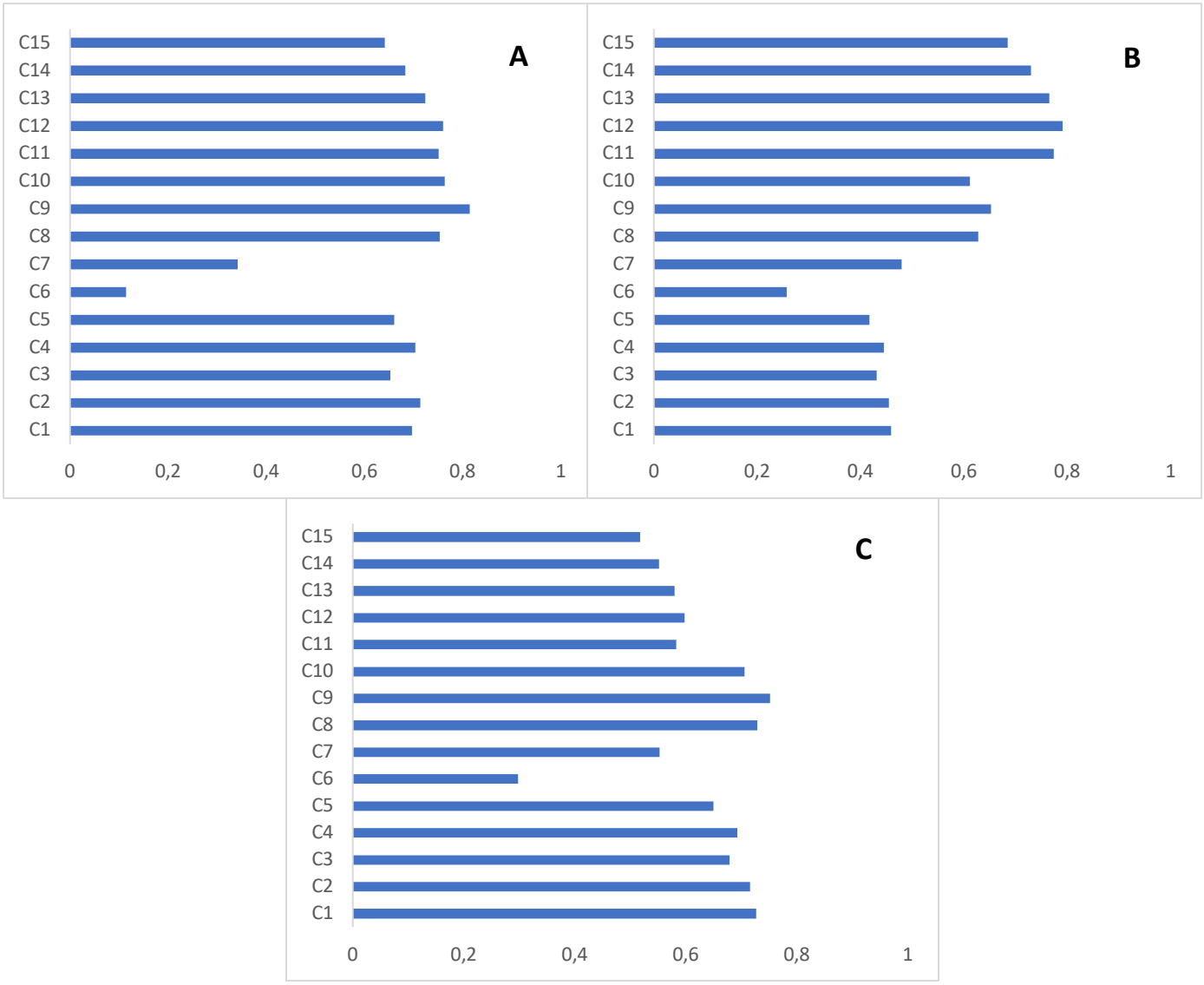


Figure 5-9. Selection best combination of loop volume and flow rate for on-line SPE preconcentration of wastewater samples using a Derringer's desirability function (C1-C5 = 1 mL loop volume, flow rates of 1, 1.5, 2, 2.5 and 3 mL/min; C6-C10 = 2 mL loop volume, flow rates of 1, 1.5, 2, 2.5 and 3 mL/min). These conditions are tested with a 2 mL wash volume for each loop volume. A. Emphasise on RSD criterion (d1) B. Emphasise on LOD criterion (d2) C. Emphasise on recovery criterion (d3).

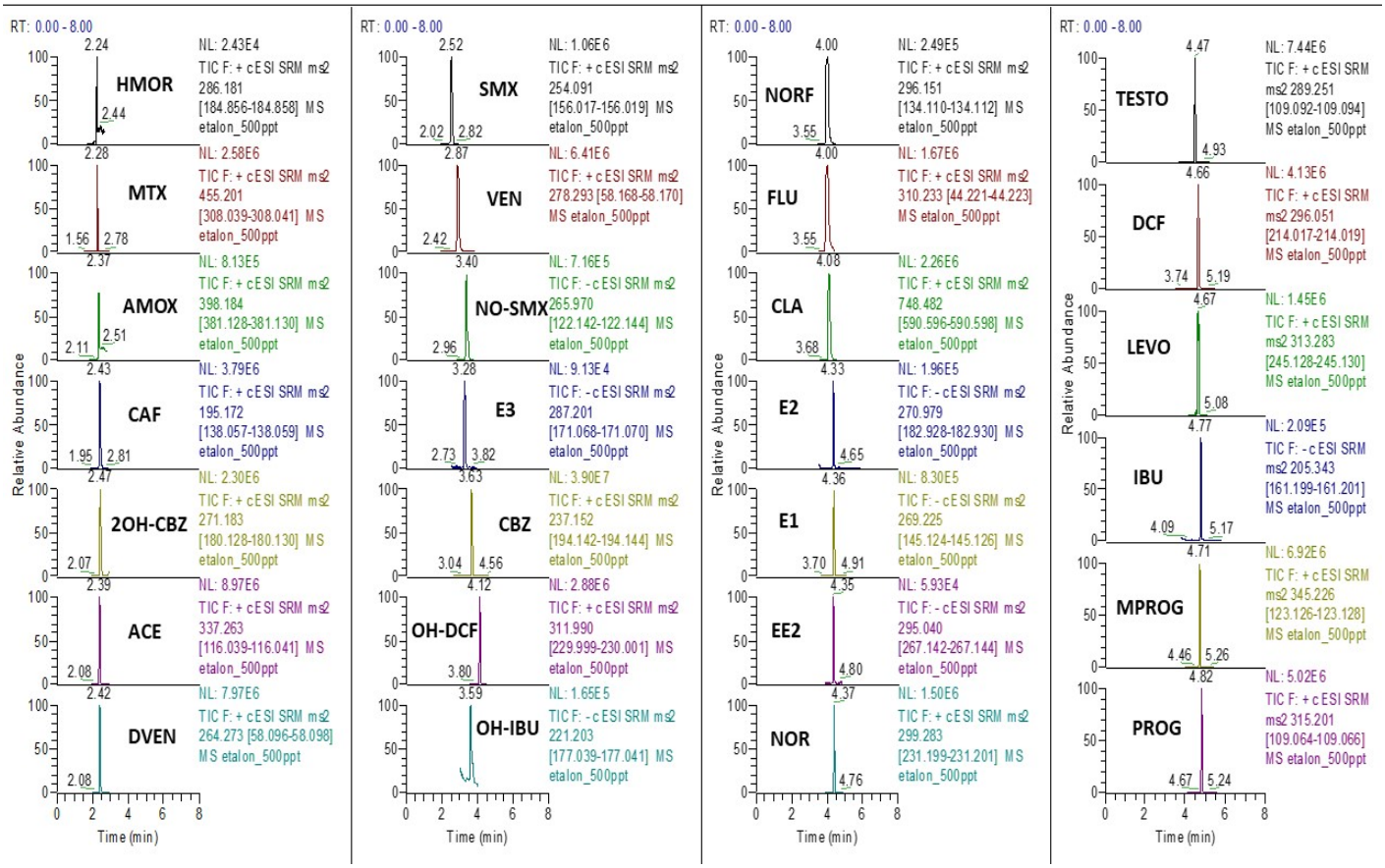


Figure 5-10. On-line SPE method final chromatographic separation of a 2 mL wastewater sample spiked at 500 ng/L.

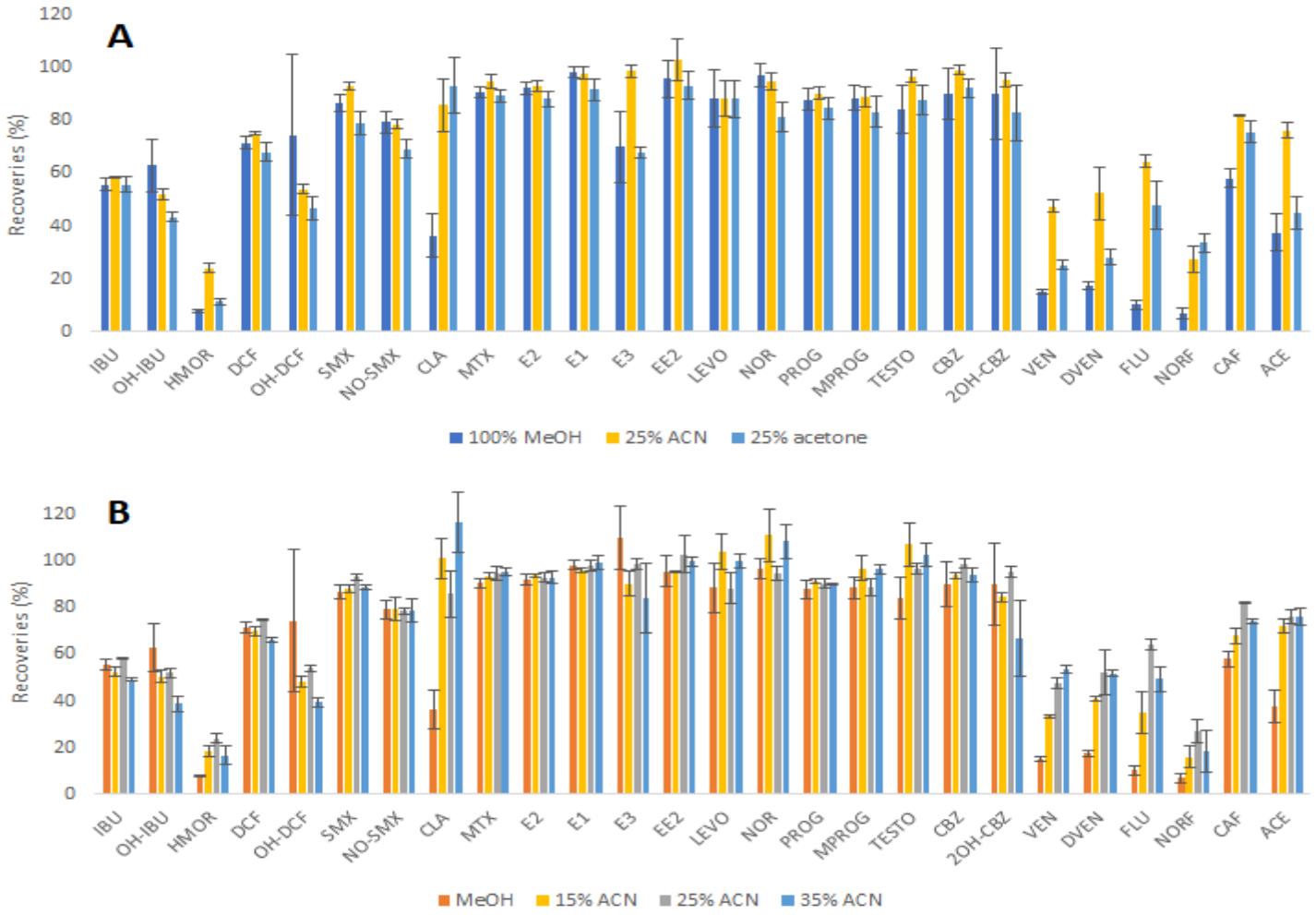


Figure 5-11. Comparison of recoveries for different solvent nature for sediment extraction (A) and optimization of the MeOH/ACN ratio for the sediment extraction (B).

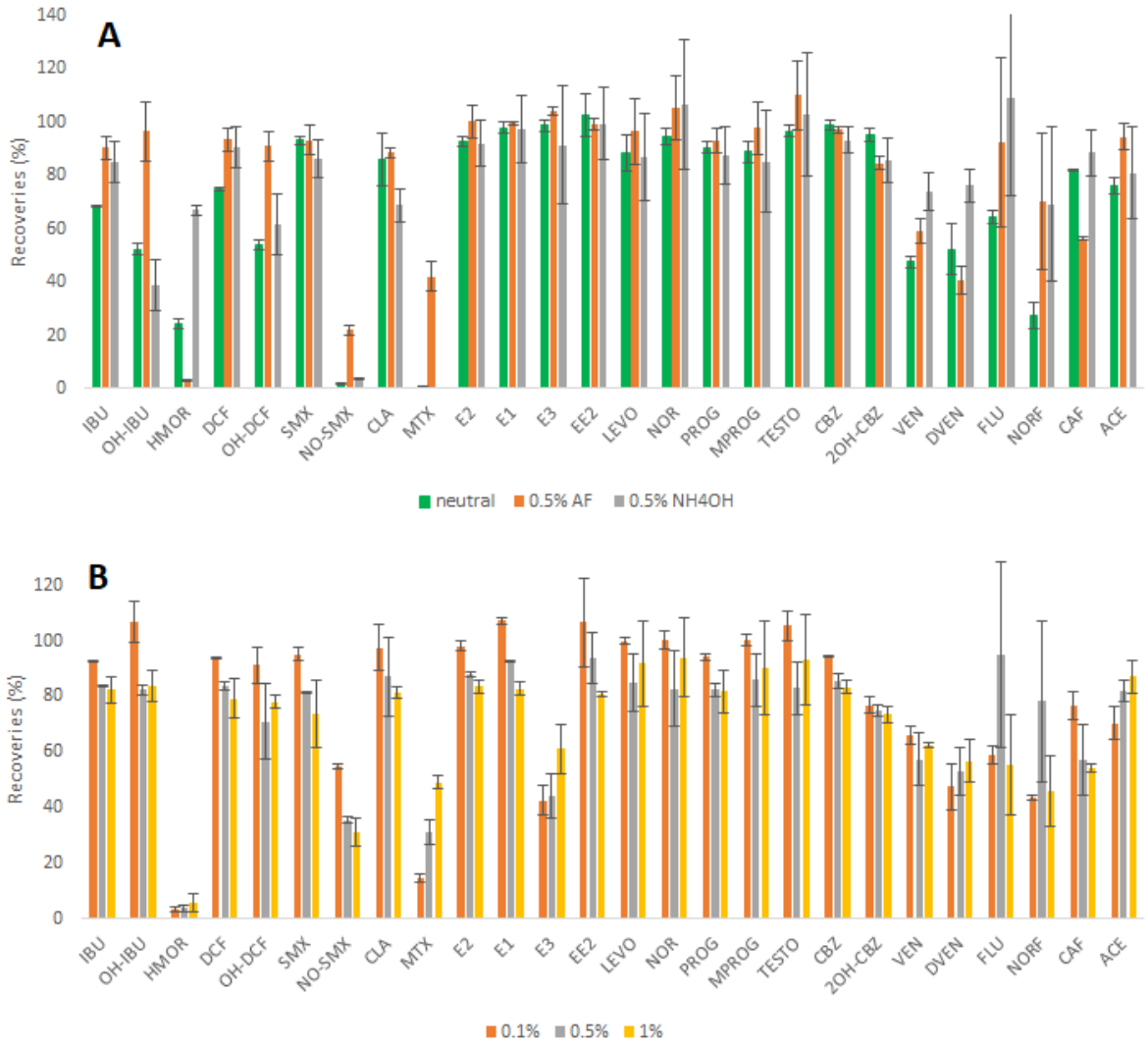


Figure 5-12. Effect of acid/base addition to organic solvent on sediment extraction recoveries (A) and optimization of the formic acid percentage in organic solvent for sediment extraction recoveries (B).

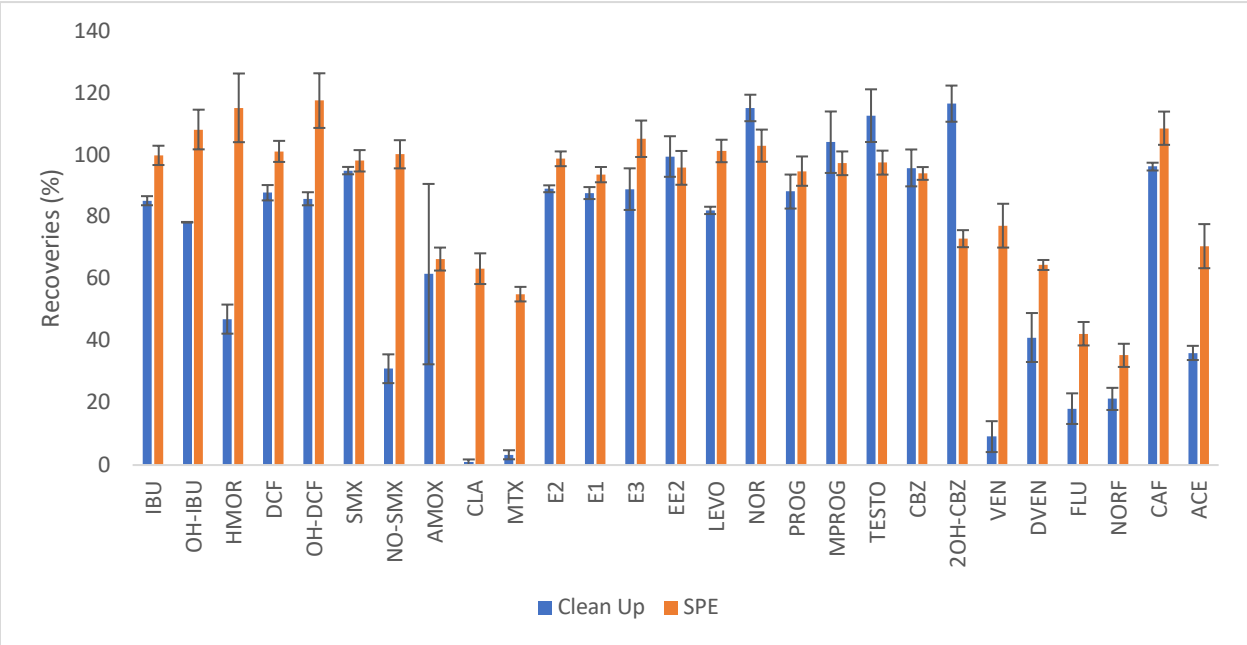


Figure 5-13. Selection of the method for purification of the solid sample extracts.

Table 5-17. Selection of off-line SPE cartridge for the purification of extracted sediments/SPM samples.

	Oasis HLB		HyperSep Retain PEP		Strata-X		HyperSep C18	
	Mean	**Std dev**	**Mean**	**Std dev**	**Mean**	**Std dev**	**Mean**	**Std dev**
IBU	100	3.1	93	0.70	99	3.0	99	1.5
OH-IBU	108	6,4	49	2.5	76	4.5	94	3.6
HMOR	115	11	79	11	115	3.1	18	0.50
DCF	101	3.4	91	2.3	101	3.4	99	0.34
OH-DCF	118	8.8	89	1.0	122	14	99	2.0
SMX	98	3.5	94	0.79	101	2.9	71	5.3
NO-SMX	59	2.9	20	0.40	71	2.2	15	1.7
AMOX	67	3.7	24	3.7	73	4.1	14	2.8
CLA	63	5.0	30	1.7	88	5.0	23	2.5
MTX	55	2.4	0.12	0.03	32	1.9	34	4.3
E2	99	2.4	93	1.4	99	3.49	96	1.3
E1	94	2.5	100	5.6	98	3.9	99	2.1
E3	105	5.9	96	5.6	97	1.2	97	1.2
EE2	96	5.5	102	9.7	102	4.3	95	3.1
LEVO	101	3.6	99	5.5	96	6.1	92	4.9
NOR	103	5.2	111	16	98	7.7	95	8.6
PROG	95	4.7	94	0.16	93	6.1	96	2.1
MPROG	97	3.8	95	6.3	96	5.4	91	6.3
TESTO	98	3.9	107	8.7	102	5.3	96	6.5
CBZ	94	2.1	95	3.7	104	4.1	98	0.83
2OH-CBZ	73	2.7	87	3.3	98	9.2	69	14
VEN	77	7.1	23	5.7	82	4.5	39	1.66
DVEN	65	1.6	27	3.8	120	11	33	0.63
FLU	42	3.8	1.6	0.17	115	9.2	9.93	0.86
NORF	35	3.7	2.7	0.95	120	11	13	10
CAF	109	5.4	97	1.2	100	5.9	99	1.6
ACE	71	7.1	6.9	3.9	113	15	40	1.1

Table 5-18. Calibration performances for wastewater.

Compound	LOD (ng/L)	Linear range (ng/L)*	Linearity (R^2)
IBU	1	3.3 – 75 000	0.9995
OH-IBU	1	3.3 – 75 000	0.9956
HMOR	5	17 – 25 000	0.9986
DCF	0.1	0.33 – 75 000	0.9998
OH-DCF	0.1	0.33 – 25 000	0.9988
SMX	0.1	0.33 – 75 000	0.9989
CLA	0.1	0.33 – 75 000	0.9954
MTX	0.5	1.7 – 25 000	0.9981
E2	10	33 – 25 000	0.9993
E1	0.5	1.7 – 25 000	0.9996
E3	10	33 – 25 000	0.9982
EE2	20	67 – 25 000	0.9993
LEVO	8	27 – 25 000	0.9993
NOR	20	67 – 25 000	0.9998
PROG	1	3.3 – 25 000	0.9993
MPROG	10	33 – 25 000	0.9998
TESTO	20	67 – 25 000	0.9994
CBZ	0.1	0.33 – 75 000	0.9995
2OH-CBZ	10	33 – 75 000	0.9981
VEN	0.1	0.33 – 75 000	0.9987
DVEN	0.5	1.7 – 25 000	0.9913
FLU	1	3.3 – 25 000	0.9985
NORF	5	17 – 25 000	0.9980
CAF	0.5	1.7 – 75 000	0.9904
ACE	0.5	1.7 – 75 000	0.9993

* The linear range reflect values between LOQ and the maximum analysed concentration that do not compromise linearity

Table 5-19. Wastewater matrix analytical validation performance, evaluated at two spike levels (QC1 = 100 ng/L and QC2 = 20 000 ng/L).

	Recovery (%)		Accuracy (%)		Intraday Precision (RSD %)		Interday Precision (RSD %)	
	QC1	QC2	QC1	QC2	QC1	QC2	QC1	QC2
IBU	82 ± 4	91 ± 2	111 ± 9	95 ± 2	1.73	1.62	2.12	3.16
OH-IBU	86 ± 5	87 ± 2	101 ± 3	113 ± 2	3.85	2.56	10.4	6.90
HMOR	76 ± 3	108 ± 5	95 ± 2	91 ± 3	7.08	8.34	10.8	15.5
DCF	82 ± 2	82 ± 4	98 ± 2	104 ± 1	4.04	1.81	3.11	5.07
OH-DCF	75 ± 1	113 ± 1	110 ± 4	105 ± 1	3.85	6.14	14.9	10.4
SMX	92 ± 1	110 ± 1	98 ± 4	118 ± 1	5.92	5.26	7.23	11.0
CLA	119 ± 9	99 ± 6	107 ± 4	99 ± 3	1.30	7.20	12.4	11.2
MTX	121 ± 5	122 ± 6	103 ± 8	96 ± 9	8.64	5.53	7.78	10.4
E2	74 ± 6	81 ± 3	96 ± 6	95 ± 7	5.87	2.02	6.50	6.95
E1	87 ± 3	89 ± 3	99 ± 4	99 ± 4	6.70	5.56	7.49	8.09
E3	74 ± 5	90 ± 4	108 ± 9	103 ± 4	9.26	4.51	11.6	8.04
EE2	76 ± 5	89 ± 2	117 ± 5	101 ± 7	6.48	4.62	8.41	5.89
LEVO	84 ± 1	90 ± 1	103 ± 4	103 ± 8	6.29	8.58	12.4	14.5
NOR	82 ± 1	92 ± 1	92 ± 3	97 ± 8	4.82	6.61	12.8	11.6
PROG	90 ± 5	101 ± 6	112 ± 3	98 ± 3	5.12	2.62	5.91	5.21
MPROG	100 ± 6	110 ± 5	119 ± 1	110 ± 6	4.80	4.90	9.23	4.91
TESTO	85 ± 1	99 ± 1	106 ± 8	115 ± 7	6.31	6.72	6.85	7.90
CBZ	86 ± 2	102 ± 1	117 ± 2	107 ± 5	1.47	1.91	1.95	4.56
2OH-CBZ	102 ± 2	100 ± 2	103 ± 3	95 ± 3	4.26	9.90	8.68	13.9
VEN	89 ± 1	102 ± 1	96 ± 6	105 ± 6	2.85	2.02	6.99	9.83
DVEN	97 ± 1	114 ± 1	102 ± 3	112 ± 2	2.80	1.33	11.1	13.0
FLU	115 ± 2	105 ± 8	89 ± 6	104 ± 2	4.19	8.11	14.4	13.8
NORF	102 ± 7	103 ± 7	84 ± 4	91 ± 2	2.53	5.91	16.0	10.7
CAF	104 ± 7	99 ± 2	116 ± 6	107 ± 8	2.08	1.52	11.1	14.1
ACE	105 ± 1	109 ± 3	96 ± 2	104 ± 2	0.73	5.24	5.09	9.25

Table 5-20. Calibration performances for the solid phase.

Compound	LOD (ng/g)	Linear range (ng/g)*	Linearity (R^2)
IBU	0.4	1.3 – 1000	0.9998
OH-IBU	10	33 – 1000	0.9912
HMOR	2	6.7 – 1000	0.9994
DCF	0.1	0.33 – 1000	0.9998
OH-DCF	0.8	2.6 – 1000	0.9954
SMX	0.02	0.067 – 1000	0.9999
CLA	0.02	0.067 – 1000	0.9995
MTX	2	6.7 – 1000	0.9992
E2	0.02	0.067 – 1000	0.9988
E1	0.02	0.067 – 1000	0.9999
E3	0.12	0.40 – 1000	0.9988
EE2	0.02	0.067 – 1000	0.9939
LEVO	0.4	1.3 – 1000	0.9999
NOR	0.4	1.3 – 1000	0.9970
PROG	0.012	0.040 – 1000	0.9988
MPROG	0.04	0.13 – 1000	0.9981
TESTO	0.04	0.13 – 1000	0.9996
CBZ	0.002	0.0067 – 1000	0.9999
2OH-CBZ	0.02	0.067 – 1000	0.9998
VEN	0.02	0.067 – 1000	0.9999
DVEN	0.2	0.67 – 1000	0.9999
FLU	0.8	2.6 – 1000	0.9999
CAF	0.02	0.067 – 1000	0.9988
ACE	0.002	0.0067 – 1000	0.9977

* The linear range reflect values between LOQ and the maximum analysed concentration that do not compromise linearity

Table 5-21. Solid phase (particulate matter) analytical validation performance, evaluated at two spike levels (QC1 = 0.20 ng/g for SMX, CLA, E2, E1, PROG, MPROG, TESTO, CBZ, 2OH-CBZ, VEN, CAF and ACE, 2 ng/g for IBU, DCF, E3, EE2, LEVO, NOR and DVEN, 10 ng/g for HMOR, OH-DCF, MTX and FLU and 40 ng/g for OH-IBU and QC2 = 250 ng/g).

	Recovery (%)		Accuracy (%)		Precision (interday) (%)	
	QC1	QC2	QC1	QC2	QC1	QC2
IBU	98 ± 2	99 ± 3	105 ± 2	123 ± 3	1.04	2.09
OH-IBU	79 ± 3	76 ± 4	70 ± 10	71 ± 6	24.9	16.8
HMOR	28.1 ± 0.4	115 ± 3	69 ± 7	85 ± 5	17.6	25.5
DCF	122 ± 4	101 ± 3	102 ± 9	74 ± 4	12.3	6.97
OH-DCF	36 ± 3	122 ± 13	72 ± 9	72 ± 4	23.7	17.2
SMX	78 ± 3	101 ± 3	113 ± 10	112 ± 5	9.72	4.09
CLA	71 ± 1	88 ± 5	94 ± 5	93 ± 5	15.6	16.2
E2	84 ± 1	99 ± 3	91 ± 2	78 ± 2	8.73	11.5
E1	78 ± 2	98 ± 4	123 ± 8	84 ± 7	7.31	10.2
E3	90 ± 4	97 ± 1	78 ± 6	76 ± 8	18.5	7.65
EE2	76.5 ± 0.2	102 ± 4	78 ± 8	81 ± 8	22.0	10.6
LEVO	71 ± 1	96 ± 6	123 ± 6	88 ± 9	15.1	13.8
NOR	72 ± 2	98 ± 8	95 ± 10	85 ± 6	22.3	12.8
PROG	78 ± 1	93 ± 6	75 ± 7	84 ± 1	16.3	16.6
MPROG	68 ± 1	96 ± 5	118 ± 2	86 ± 8	16.8	9.59
TESTO	72 ± 3	102 ± 5	112 ± 8	110 ± 11	18.4	11.5
CBZ	83 ± 3	104 ± 4	90 ± 3	108 ± 3	2.5	2.37
2OH-CBZ	73 ± 1	98 ± 9	73 ± 7	75 ± 11	24.3	10.7
VEN	78 ± 2	82 ± 4	83 ± 7	107 ± 7	24.9	3.65
DVEN	63 ± 1	119 ± 10	109 ± 11	93 ± 5	19.1	14.8
FLU	76 ± 4	114 ± 9	100 ± 6	71 ± 5	7.34	17.2
CAF	94 ± 1	100 ± 6	102 ± 6	106 ± 7	23.4	15.9
ACE	106 ± 3	113 ± 4	112 ± 5	98 ± 4	20.7	14.6

Table 5-22. Matrix effect assessment for wastewater. A matrix effect of <0% reflects net ion suppression and >0% net ion enhancement.

	Matrix effect (%)	Residual matrix effect (%)
IBU	17	-2.4
OH-IBU	25	-10
HMOR	94	-19
DCF	6.9	-2.2
OH-DCF	-16	-2.1
SMX	32	-14
NO-SMX	n.a.	n.a.
AMOX	n.a.	n.a.
CLA	2.6	6.3
MTX	-9.4	11
E2	10	-3.2
E1	8.3	-1.1
E3	-12	-1.6
EE2	36	-3.2
LEVO	14	-1.4
NOR	-5.5	-1.8
PROG	15	-1.6
MPROG	59	-1.6
TESTO	-32	-0.9
CBZ	96	-2.6
2OH-CBZ	-54	-6.2
VEN	13	-3.5
DVEN	-35	4.0
FLU	-26	9.4
NORF	25	10
CAF	15	-2.5
ACE	-35	3.0

Table 5-23. Quantified concentration (ng/L) of pharmaceuticals in the aqueous phase of the selected WWTP.

	Quantified Concentrations (ng/L)											
Compound	**Influent #1**	**Effluent #1**	**Influent #2**	**Effluent #2**	**Influent #3**	**Effluent #3**	**Influent #4**	**Effluent #4**	**Influent #5**	**Effluent #5**	**Influent #6**	**Effluent #6**
IBU	1182 ± 2	722 ± 10	1807 ± 22	726 ± 11	953 ± 9	656 ± 13	1491 ± 69	45.7 ± 0.3	3068 ± 54	2087 ± 8	2356 ± 20	1388 ± 40
OH-IBU	5240 ± 97	2310 ± 108	2833 ± 13	2357 ± 47	2743 ± 5	1822 ± 93	49387 ± 865	9701 ± 235	34942 ± 546	4941 ± 65	6790 ± 24	3769 ± 35
DCF	52.3 ± 0.5	50.7 ± 1.8	100 ± 2	46.5 ± 1.2	77.3 ± 0.9	53.1 ± 1.5	609 ± 9	259 ± 2	424 ± 16	233 ± 1	132 ± 3	35.3 ± 0.04
OH-DCF	14.3 ± 0.2	9.31 ± 0.16	17.6 ± 0.2	7.41 ± 0.68	16.7 ± 0.3	10.0 ± 0.1	58.6 ± 1.7	9.19 ± 0.20	58.4 ± 2.4	25.2 ± 0.4	12.8 ± 0.4	8.28 ± 0.29
SMX	25.8 ± 0.1	33.5 ± 0.9	37.0 ± 5.7	15.5 ± 0.4	21.8 ± 0.9	16.8 ± 0.7	15.8 ± 1.1	147 ± 4	91.7 ± 0.3	81.2 ± 3.7	28.8 ± 1.0	73.7 ± 0.5
CLA	1210 ± 9	77.9 ± 0.8	374 ± 5	96.1 ± 0.02	382 ± 3	84.0 ± 2.0	8671 ± 49	279 ± 4	<LOD	1288 ± 21	229 ± 5	184 ± 1
MTX	<LOQ	<LOQ	4.65 ± 0.36	3.42 ± 0.05	5.90 ± 0.09	<LOQ	40.1 ± 1.1	<LOD	9.29 ± 0.03	5.16 ± 0.48	4.12 ± 0.36	<LOQ
E1	<LOQ	<LOQ	6.96 ± 0.43	<LOQ	3.85 ± 0.18	3.83 ± 0.50	28.1 ± 0.5	9.75 ± 0.11	8.00 ± 0.19	10.6 ± 0.4	8.96 ± 0.06	<LOD
EE2	319 ± 2	<LOD	<LOD	<LOD	<LOD	<LOD	79.3 ± 0.8	<LOD	<LOD	<LOD	<LOD	<LOD
CBZ	59.2 ± 0.8	57.5 ± 1.5	54.2 ± 0.6	54.6 ± 2.3	79.5 ± 1.9	87.9 ± 1.3	164 ± 4	258 ± 8	127 ± 8	126 ± 1	91.1 ± 1.7	64.3 ± 2.4
2OH-CBZ	726 ± 28	497 ± 16	593 ± 11	342 ± 8	1135 ± 15	483 ± 26	1572 ± 8	1344 ± 37	2041 ± 22	768 ± 3	2640 ± 42	430 ± 2
VEN	168 ± 5	114 ± 2	215 ± 0.2	158 ± 1	181 ± 1	172 ± 7	1129 ± 5	781 ± 5	563 ± 5	387 ± 8	349 ± 4	217 ± 0.3
DVEN	1115 ± 19	256 ± 0.2	417 ± 1	278 ± 4	447 ± 3	186 ± 5	5313 ± 15	2848 ± 6	2288 ± 23	1106 ± 15	793 ± 4	538 ± 3
FLU	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOD	24.3 ± 0.6	<LOQ	<LOD	<LOD	<LOD	<LOD
CAF	22636 ± 318	3609 ± 9	18890 ± 45	8085 ± 30	12277 ± 10	5572 ± 49	66560 ± 256	5130 ± 23	34396 ± 151	31942 ± 75	19213 ± 106	8231 ± 23
ACE	70.3 ± 0.9	58.9 ± 1.4	44.8 ± 1.3	46.7 ± 0.5	91.6 ± 2.7	97.0 ± 2.0	347 ± 0.3	1039 ± 1	1288 ± 18	643 ± 24	61.6 ± 0.6	48.7 ± 0.3

Table 5-24. Quantified concentration (ng/g) of pharmaceuticals in the SPM of the selected WWTP.

	Quantified Concentrations (ng/g)											
Compound	**Influent #1**	**Effluent #1**	**Influent #2**	**Effluent #2**	**Influent #3**	**Effluent #3**	**Influent #4**	**Effluent #4**	**Influent #5**	**Effluent #5**	**Influent #6**	**Effluent #6**
IBU	152 ± 20	105 ± 7	126 ± 1	95.1 ± 7.2	21.8 ± 3.0	25.8 ± 1.5	301 ± 43	<LOD	97.8 ± 1.6	96.5 ± 6.6	252 ± 20	27.6 ± 3.9
DCF	72.0 ± 9.3	63.7 ± 3.9	18.5 ± 1.6	25.3 ± 2.6	82.1 ± 6.6	88.9 ± 9.2	27.2 ± 0.1	28.6 ± 0.7	31.8 ± 4.2	28.1 ± 0.8	5.48 ± 0.66	9.41 ± 0.72
CLA	104 ± 7	25.9 ± 3.6	18.8 ± 2.1	<LOQ	283 ± 27	295 ± 31	131 ± 13	13.1 ± 1.1	56.2 ± 6.7	75.9 ± 6.3	28.4 ± 3.2	34.7 ± 4.8
E1	8.67 ± 0.63	1.37 ± 0.10	14.4 ± 1.9	6.64 ± 0.39	2.84 ± 0.40	2.34 ± 0.22	8.31 ± 0.58	3.37 ± 0.21	13.7 ± 2.0	5.64 ± 0.40	9.15 ± 0.90	8.08 ± 1.13
PROG	21.7 ± 2.1	<LOD	<LOD	<LOD	<LOD	<LOD	98.3 ± 6.0	<LOD	<LOD	<LOD	<LOD	<LOD
TESTO	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	48.8 ± 3.6	44.2 ± 2.7	<LOD	<LOD	<LOD	<LOD
CBZ	6.10 ± 0.75	4.20 ± 0.32	8.53 ± 0.68	8.60 ± 0.88	3.99 ± 0.52	5.74 ± 0.44	10.2 ± 1.1	10.2 ± 0.2	12.2 ± 1.1	7.75 ± 0.53	10.4 ± 0.9	5.48 ± 0.11
2OH-CBZ	0.784 ± 0.011	0.698 ± 0.005	0.313 ± 0.028	0.339 ± 0.050	0.824 ± 0.095	0.789 ± 0.004	<LOD	0.171 ± 0.017	0.101 ± 0.004	0.133 ± 0.016	1.06 ± 0.11	0.215 ± 0.022
FLU	74.2 ± 4.3	37.7 ± 0.9	51.8 ± 5.9	<LOQ	529 ± 32	188 ± 8	79.1 ± 7.3	15.6 ± 2.1	74.0 ± 1.4	58.1 ± 8.5	257 ± 29	160 ± 11
CAF	198 ± 15	89.0 ± 7.0	193 ± 14	121 ± 15	208 ± 22	282 ± 5	383 ± 28	203 ± 16	261 ± 26	238 ± 23	452 ± 61	162 ± 21
ACE	6.44 ± 0.61	8.48 ± 0.34	68.7 ± 0.9	28.7 ± 3.9	13.3 ± 1.4	16.2 ± 1.8	39.6 ± 2.6	39.1 ± 3.3	130 ± 13	76.9 ± 5.7	11.6 ± 0.2	9.83 ± 0.62

Table 5-25. Mass to volume ratio of filters of the different wastewater samples.

	Mass/Volume of filters (g/L)	
Matrix	**Mean**	**Median**
Hospital effluent	0.104	0.0760
WWTP influent	0.0299	0.0280
WWTP effluent	0.0044	0.0033

Text 5-3. Distribution coefficients and mass balance in water.

$$Kd\ \left(\frac{L}{kg}\right) = \frac{\left[solid\ phase\ \left(\frac{ng}{kg}\right)\right]}{\left[dissolved\ phase\ \left(\frac{ng}{L}\right)\right]} \qquad \mathbf{Eq.\ 5-5}$$

Calculation of individual analyte mass balance in total water:

$$\phi\ (\%) = 100\% * \frac{a}{a+b} \qquad \mathbf{Eq.\ 5-6}$$

$$a = analyte\ concentration\ in\ the\ solid\ phase\ \left(\frac{ng}{g}\right) * sample\ mass\ (g) * \frac{1000\ (mL)}{filtrate\ V\ (mL)}$$

$$b = analyte\ concencentration\ in\ the\ dissolved\ phase\ \left(\frac{ng}{L}\right) * 1L$$

$$\omega\ (\%) = 100 - \varphi\ (\%) \qquad \mathbf{Eq.\ 5-7}$$

Table 5-26. Calculated medians, means and standard deviations of logarithm of distribution coefficient (Kd) for the selected wastewater matrices.

	Log (K_d)								
	Hospital Effluent			WWTP Influent			WWTP Effluent		
	Median	Mean	Standard deviation	Median	Mean	Standard deviation	Median	Mean	Standard deviation
IBU	3.23	3.72	4.13	1.95	1.97	1.83	1.66	1.88	1.76
OH-IBU	3.73	3.95	3.99	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
HMOR	3.29	3.29	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
DCF	3.79	5.00	5.33	2.11	2.67	2.77	2.61	2.82	2.82
OH-DCF	3.86	3.86	3.97	1.76	1.76	1.70	n.a.	n.a.	n.a.
SMX	2.60	2.66	2.46	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CLA	4.78	4.78	4.93	1.93	2.31	2.48	2.27	2.92	3.18
MTX	4.13	4.13	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
E1	3.93	4.31	4.35	3.01	3.07	2.86	2.73	2.70	2.13
E3	3.85	3.85	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
TESTO	4.20	4.20	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CBZ	3.51	3.59	3.38	2.00	1.99	1.58	1.84	1.90	1.61
2OH-CBZ	0.63	1.48	1.70	-0.28	-0.25	-0.42	-0.13	-0.09	-0.20
VEN	3.28	3.28	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
DVEN	3.41	3.74	3.87	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
FLU	n.a.	n.a.	n.a.	3.51	3.51	n.a.	n.a.	n.a.	n.a.
CAF	3.07	3.20	3.27	0.98	1.08	0.83	1.35	1.42	1.21
ACE	3.59	3.86	4.00	2.11	2.56	2.76	2.19	2.33	2.31

Table 5-27. Calculated medians, means and standard deviations of particle-associated fraction (Φ) for the selected wastewater matrices.

	Φ (%)								
	Hospital Effluent			WWTP Influent			WWTP Effluent		
	Median	Mean	Standard deviation	Median	Mean	Standard deviation	Median	Mean	Standard deviation
IBU	0.19	0.33	0.42	0.090	0.18	0.23	0.014	0.075	0.099
OH-IBU	0.33	0.88	1.0	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
DCF	0.67	7.2	12	0.073	0.099	0.095	0.17	0.20	0.17
OH-DCF	1.1	1.4	1.6	0.059	0.064	0.019	n.a.	n.a.	n.a.
SMX	0.052	0.063	0.037	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CLA	1.2	8.4	15	0.12	0.65	1.1	0.35	0.80	1.3
E1	1.4	1.9	1.8	1.6	2.2	2.1	0.35	0.32	0.22
E3	1.4	1.4	0.86	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
PROG	4.7	4.7	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
TESTO	3.0	3.0	3.5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CBZ	0.34	0.59	0.58	0.18	0.19	0.12	0.033	0.051	0.039
2OH-CBZ	0.0012	0.0072	0.010	0.00024	0.00031	0.00021	0.00014	0.00026	0.00021
VEN	0.086	0.086	0.0012	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
DVEN	0.15	1.15	3.3	n.a.	n.a.	n.a.	0.042	1.4	2.7
FLU	n.a.	n.a.	n.a.	10	10	1.8	n.a.	n.a.	n.a.
CAF	0.12	0.15	0.13	0.016	0.017	0.012	0.011	0.016	0.010
ACE	0.13	0.38	0.51	0.45	0.36	0.17	0.058	0.10	0.099

6 Une approche d'analyse des médicaments anticancéreux polaires dans l'eau usée : l'extraction en ligne avec la chromatographie HILIC ou la chromatographie en phase inverse couplées avec la spectrométrie de masse en tandem

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"A framework for the analysis of polar anticancer drugs in wastewater: On-line extraction coupled to HILIC or reverse phase LC-MS/MS"

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Description : Cet article décrit l'optimisation, la validation et l'application de méthodes faisant appel à la SPE couplée à la LC-MS/MS pour l'analyse de composés de chimiothérapie dans des échantillons d'eaux usées municipales et d'hôpitaux. L'article présente deux différentes approches, l'une faisant appel à la chromatographie en phase inverse et l'autre à la chromatographie HILIC suite à la détermination des conditions chromatographiques les plus prometteuses pour l'analyse de ces composés peu étudiés en raison de leur polarité.

Contribution : J'ai effectué la conception du projet, le développement de méthodes, la collecte et la préparation des échantillons, le traitement de données, ainsi que la rédaction de l'article.

Coauteurs : Sung Vo Duy, Gabriel Munoz et Alexandra Furtos m'ont aidé à la conception du projet ainsi qu'à la révision de l'article.

Directeur : Sébastien Sauvé m'a aidé à la conception du projet ainsi qu'à la révision de l'article.

ABSTRACT

The consumption of chemotherapy agents is increasing due to common cancer incidence and aging of population. Residues of anticancer drugs may thus be increasingly found in hospital and municipal wastewaters. Quantification of these highly polar micropollutants remains challenging due to poor chromatographic retention on typical reversed phases. This study investigated different solid-phase extraction (SPE) materials for automated on-line preconcentration of complex matrices (hospital and municipal wastewaters) and various chromatographic column options. A hyper crosslinked hydroxylated polystyrene-divinylbenzene copolymer SPE sorbent coupled on-line with hydrophilic interaction liquid chromatography tandem mass spectrometry (HILIC-MS/MS) yielded suitable limits of detection (LOD: 1-2 ng L^{-1}) for 5-fluorouracil (5-FU) and 2',2'-difluorodeoxyuridine (dFdU). Optimization of chromatographic conditions lead to a single LC-MS/MS method for the analysis of other cytostatic drugs including cytarabine (CYT), gemcitabine (GEM), methotrexate (MTX), ifosfamide (IFO), cyclophosphamide (CYC) and capecitabine (CAP). The filter membrane for sample pre-treatment, HPLC mobile phase additives, and on-line SPE loading parameters were also investigated. The methods were validated in wastewater matrix with suitable determination coefficients (R^2 range: 0.9982–0.9999), LODs (0.5–5 ng L^{-1}), accuracy (78–111%), intraday precision (2.6–12%), and interday precision (2.1–13%). The occurrence of cytostatic drugs was examined in field-collected water samples from hospital effluents and municipal wastewater treatment plants (WWTP) in Canada. CAP (3.7-64 ng L^{-1}), dFdU (6.1–300 ng L^{-1}), and MTX (1.8–68 ng L^{-1}) were frequently detected across both matrix types, while IFO was detected in hospital wastewater (23–140 ng L^{-1}) but not in municipal WWTPs.

Keywords

Anticancer drugs; Hospital effluent; Municipal wastewater; Hydrophilic interaction liquid chromatography (HILIC); LC-MS/MS, On-line SPE.

6.1 Introduction

The advent of sensitive instrumental methods for the analysis of polar molecules not easily amenable to gas chromatography has paved the way for the discovery of contaminants of emerging concern in biological, food, and environmental samples. Reversed-phase liquid chromatography (RPLC) coupled to tandem mass spectrometry (MS/MS) has gained considerable popularity and may now be considered the "gold standard" to analyze moderately polar compounds [334–336]. RPLC retention involves analyte partitioning between a rather polar mobile phase and a non-polar stationary phase (e.g., C18-based silica). Despite the versatility of RPLC, the technique may show limited applicability for highly polar analytes (Log K_{OW} < 0). Little or no retention of such analytes is typically observed along with limited MS sensitivity. This is due to lower ionization efficiency under the highly aqueous composition of the mobile phase at the start of the chromatographic gradient [337,338]. Another possible caveat relates to the increased risks of matrix effects near the chromatographic dead time, due to the simultaneous elution of non-retained polar matrix interferences [339].

Alternative LC methods include the use of ion exchange sorbents—depending on the analyte pKa—, carbon-based sorbents, and normal phase liquid chromatography (NPLC) [340–343]. The latter techniques present, however, their own shortcomings. In practice, the implementation of carbon-based LC for large samples series may be compounded by the equilibration time required between consecutive injections; additionally, elution from the sorbent may be difficult to achieve without ion-pairing reagents [344]. Ion-exchange chromatography requires the analytes to be in their ionized form when injected, which may not be easily attainable when analytes of different properties (pKa) are present. Retention of polar analytes using non-aqueous NPLC involves adsorption onto a polar stationary phase from a highly apolar mobile phase (e.g., hexane) prior to elution using a slightly more polar mobile phase composition. Highly hydrophilic compounds may be excessively retained using the latter setup and could also display poor solubility in the mobile phase [339]. This can be addressed by using a modified normal phase sorbent compatible with the introduction of water into the mobile phase composition, a technique known as Hydrophilic Interaction Chromatography (HILIC) [204,338,339].

Hitherto, HILIC has been demonstrated as a powerful complementary technique to RPLC for the retention and separation of highly polar analytes [206,345–347]. To achieve a suitable method detection limit for water samples, a pre-concentration step is typically performed off-line by loading 50-250 mL of aqueous sample into solid phase extraction (SPE) cartridges suited for this purpose (e.g., Oasis HLB, Isolute ENV$^+$, or Hypersep Retain PEP). Elution of these cartridges

is typically performed with a high organic solvent content (e.g., ACN:Water 90:10 v/v), suitable for subsequent injection to HILIC-MS/MS. Alternatively, a pre-concentration method coupled on-line to HILIC-MS/MS would be advantageous because of the minimal pre-treatment steps required and fast turnaround in the analysis of large sample batches. To date, the coupling of on-line pre-concentration with HILIC has been the subject of a limited number of studies, mainly in the field of bioanalytical applications and involving relatively small injection volumes. Some examples include the analysis of folate catabolites in human biofluids [348], neurotransmitters and neuropeptides in whole blood [349], and saxitoxin in human urine [350]. For aqueous environmental samples, a previous method demonstrated the feasibility of on-line SPE coupling with HILIC-MS for analysis of polar pharmaceuticals in slightly larger sample volumes [351]. The targeted analytes were not as polar as the chemotherapy agents targeted in the present study, and the applicability of on-line SPE HILIC-MS for such small polar compounds remained to be validated.

The challenge of analyzing highly polar cytostatic anticancer drugs in complex water matrixes provides an appropriate case-study to explore the knowledge gap. Antimetabolites and cross-linking agents—the specific classes of chemotherapeutic agents targeted in this study—are among the most widely consumed anticancer drugs in the European Union and North American countries [109,352]. A variable yet significant fraction of cytostatic drugs is typically excreted by patients in hospital wards or homes [353,354]. Their surveillance in environmental waterways appears essential to better document their ecotoxicological risks and adjust treatment countermeasures [109,355]. Alkylating agents such as cyclophosphamide and ifosfamide have been reported to occur in the range of 2–10,000 ng L^{-1} in hospital effluents and wastewater treatment plant (WWTP) influents [109,356]. Among the antimetabolites surveyed in hospital effluents, 5-fluorouracil and methotrexate have been recorded at the highest concentrations (<2-120,000 and <2–4,700 ng L^{-1}, respectively) [357]. These compounds typically occur at much lower levels in WWTP effluents and surface waters, because of dilution effects and transformation processes including microbial degradation and photolysis [358]. Negreira et al. (2013) [359] and Rabii et al. (2014) [352] previously introduced an on-line SPE – LC-MS/MS workflow for selected cytostatics, coupled either to a reversed phase C18 or pentafluorophenyl (PFP) column. However, the method could not be expanded to the analysis of some highly challenging analytes such as 5-fluorouracil [352,359]. In order to help refining the prioritization of anticancer drug contaminants with regard to their occurrence levels, a fast and multi-residue method for the analysis of antimetabolites and alkylating agents in water would be useful.

The objective of the present study was to provide a framework for the determination of challenging small polar anticancer drugs in hospital effluents and municipal wastewater, involving on-line pre-concentration – LC-MS/MS. The rationales for analyte selection relied on consumption patterns, occurrence data in real samples, and results from previous categorization approaches [109,357]. A target list of 5 antimetabolites was established, including pyrimidine-based antimetabolites (5-fluorouracil, capecitabine, cytarabine, gemcitabine), and one folic acid analogue (methotrexate). A metabolite of gemcitabine (2',2'-difluorodeoxyuridine) was also included. Cross-linking agents of the nitrogen mustard class (cyclophosphamide and ifosfamide) were the two additional analytes selected, due to their widespread use. A table containing the list of selected analytes with their respective structure and physicochemical properties (molecular weight, pKa and log *P*) is presented in Supporting Information (SI, **Table 6-9**). In the initial screening step, their retention and separation were investigated on various HILIC and polar-modified RPLC analytical columns, in combination with HLB-type (hydrophilic-lipophilic balance) on-line SPE sorbents from different brands. The selected on-line SPE – HILIC-MS/MS and on-line SPE – modified RPLC-MS/MS methods were validated to determine recoveries, limits of detection (LODs), limits of quantification (LOQs), linearity range, accuracy, and precision. A detailed assessment of matrix effects was also conducted to verify the method robustness and delineate a suitable quantification procedure. The results of the present screening study provide a framework regarding column selection, operating conditions, and quantification strategies of therapeutic drugs in complex water samples. We also demonstrate the applicability of the newly developed methods to analyze polar anticancer drugs in hospital and urban wastewaters collected in eastern Canada.

6.2 Materials and methods

6.2.1 Chemicals and standards

Native standards including cytarabine (CYT, purity ≥97%), 5-fluorouracil (5-FU, purity ≥ 99%), gemcitabine (GEM, purity ≥ 98%), methotrexate (MTX, purity ≥97%), ifosphamide (IFO, purity ≥ 98%), cyclophosphamide (CYC, purity ≥ 97%), and capecitabine (CAP, purity ≥ 99%) were purchased from Sigma Aldrich (Saint-Louis, MO, U.S.A.), while 2',2'-difluorodeoxyuridine (dFdU, purity ≥ 97%) was obtained from Santa Cruz Biotechnology (Dallas, TX, U.S.A.). Isotope-labeled internal standards (IS) including 5-fluorouracil-$^{13}C,^{15}N_2$ (5-FU-1C2N, purity ≥97%), cyclophosphamide-d4 (CYC-d4, purity ≥ 98%), gemcitabine-$^{13}C,^{15}N_2$ (GEM-1C2N, purity ≥ 98%), ifosfamide-d4 (IFO-d4, purity ≥97%), 5-bromouracile (5-BU,

purity ≥ 98%), and methotrexate-d3 (MTX-d3, purity ≥97%) were purchased from Santa Cruz Biotechnology (Dallas, TX, U.S.A.).

HPLC grade solvents including water (H_2O), acetonitrile (ACN), and methanol (MeOH) were obtained from Thermo Fisher Scientific (Waltham, MA, U.S.A.). Formic acid (FA, purity ≥ 98%), ammonium acetate (AmAc, purity ≥ 98%), ammonium formate (AmFo, purity ≥ 99%), diethylamine (DEA, purity ≥ 99.5%) and hexylamine (HA, purity ≥ 99%) were purchased from Sigma Aldrich (Saint-Louis, MO, U.S.A.). Glass fiber (0.22 µm, 13mm) filters were purchased from Adventec, Cole Parmer (Vernon Hills, IL, U.S.A.), while nylon (0.22 µm, 25mm), polypropylene (0.2 µm, 30mm), polycarbonate (0.2 µm, 25mm), polyester (0.2 µm, 25mm) and cellulose acetate (0.2 µm, 25mm) filters were from Sterlitech (Kent, WA, U.S.A.) and GHP (0.2 µm, 25mm) from Acrodisc, Waters (Milford, MA, U.S.A.).

6.2.2 Sample collections

Water samples were collected in 1L amber glass bottles previously cleaned in the lab with MeOH and HPLC water. At each field sampling site, a pump system was used to sample 10 L at approximately 1 L min^{-1} wastewater in a clean 20 L bucket and this grab sample was homogenized by agitation. Then the bottles were rinsed three times with the site wastewater/hospital wastewater, filled, capped, and stored in a cooler with ice. Upon arrival at the laboratory facilities, the samples were stored at -20°C [360].

Hospitals (n=11) from Quebec province (Canada) were sampled in the 2018 autumn season with the assistance of the environmental service and industrial waste control division of the city. Affluent and effluent samples from several wastewater treatment plants (n = 6, Quebec province, Canada) were sampled in the 2019 winter season. Additional details on sampling sites, including number of hospital beds and average wastewater volume processed through treatment plants, are presented in Supporting Information (**Tables 6-10** and **6-11**). Field blanks were generated within the different sampling campaigns using 1L amber glass bottles filled on site with HPLC water prior filtration, preparation, and analysis in the same LC-MS sequence as other samples.

6.2.3 Sample preparation and instrumental analysis

Waters samples were passed through cellulose acetate filters (0.2 µm, 25mm) to remove suspended particles and the filtrate was retrieved in a glass flask. An exact volume of this filtrate was transferred to an amber glass vial and spiked with isotope-labelled internal standards for a final concentration of 200 ng L^{-1} each. The samples were then analyzed by two sets of on-line SPE UPLC-MS/MS methods.

The two-pump system used for on-line pre-concentration and chromatographic separation consisted of an Accela 600 pump and an Accela 1250 pump (Thermo Fisher, San Jose, CA, U.S.A.). Depending on the method, samples were injected onto a 1 mL or 2 mL loop by an HTC Thermopal autosampler (CTC Analytic AG, Zwingen, Switzerland). The valve switching process was performed using a set of two distinct six-position valves and one ten-position valve (VICI Valco Instruments Co., Houston, TX, U.S.A.). A diagram of the system was presented elsewhere in a recent study [361].

Two different methods were retained for the chromatographic separation of the analytes of interest: 5-FU and dFdU were analyzed using on-line SPE – HILIC-MS/MS (method #1) while the other targeted compounds were analyzed by on-line SPE coupled to RPLC-MS/MS (method #2). Both methods shared the same on-line SPE enrichment cartridge: Isolute ENV+ SPE column (30 x 2.1 mm, 40 µm) (Biotage, Uppsala, Sweden). In method #1, 1 mL of sample was loaded at a flow rate of 1000 µL min^{-1}. In method #2, 2 mL of sample were loaded at 2500 µL min^{-1}. After the sample transfer from the injection loop to the on-line SPE column, in both methods, the aqueous mobile phase was left to flow for an additional 1 mL to allow matrix removal (wash step). The sample was then backflushed to the LC chromatographic column for analyte separation. Method #1 used a Hypersil GOLD PEI HILIC column (100 x 2.1 mm; 1.9 µm particle size) from Thermo Scientific (Waltham, MA, U.S.A.), thermostated at 30°C. Method #2 used a Luna Omega Polar C18 column (100 x 2.1 mm; 1.6 µm) from Phenomenex (Torrance, CA, U.S.A.), thermostated at 40°C; note that a Hypersil GOLD aQ column (100 x 2.1 mm; 1.9 µm) from Thermo Scientific (Waltham, MA, U.S.A.) would provide equivalent performance. The full details of each chromatographic method, including flow rate, mobile phases, and gradient elution programs, are provided in the SI (**Tables 6-12** and **6-13**).

A TSQ Quantiva triple quadrupole mass spectrometer (Thermo Scientific, Waltham, MA, U.S.A.) equipped with a heated electrospray ionization source (heated-ESI) was used for compound detection. Optimized ionization parameters were as follows: spray voltage was set at -3000 V in negative ionisation mode and at +4000 V in positive mode; sheath gas was set at

50 a.u., auxiliary gas at 10 a.u., sweep gas at 0 a.u., ion transfer tube temperature at 350°C, and vaporizer temperature at 400°C. Acquisition was performed using selected reaction monitoring mode (SRM), with the two must abundant MS/MS fragment ions as quantification and confirmation transitions. Further details on compound-dependent MS parameters including acquisition window time, polarity, precursor and product ion (*m/z*), collision energy, and RF lens are provided in SI (**Table 6-14**).

6.2.4 Method optimization and experimental designs

Diverse on-line SPE columns were first investigated using water samples spiked at 100 ng L^{-1} with the targeted cytostatics. The analytical column used for these tests was a Luna Omega Polar C18 (modified RPLC). Despite the fact that retention was not optimal yet for all targeted analytes, a sufficient separation was obtained and signal area could be integrated in order to select the best SPE sorbent. The characteristics of the 5 tested on-line SPE columns are listed in **Table 6-1**. The selection of a suitable on-line SPE sorbent was based on signal intensity and precision (n = 3 per condition).

Table 6-1. Characteristics of the different on-line SPE columns tested at the screening step.

SPE cartridge type	Sorbent	Dimensions & particle size	Pore size (Å)	Surface area (m^2/g)	pH range
Hypersil GOLD C8[a]	C8	20 mm × 2.1 mm ; 5 µm	175	220	2 – 9
Isolute ENV+[b]	Hydroxylated PS-DVB	30 mm × 2.1 mm ; 40 µm	800	1000	1 – 14
Oasis HLB[c]	NVP-DVB	20 mm × 2.1 mm ; 5 µm	80	830	0 – 14
Hypersep Retain PEP[a]	PS-DVB, urea groups	20 mm × 2.1 mm ; 40-60 µm	90	550-750	0 – 14
BetaBasic C18[a]	C18	20 mm × 2.1 mm ; 3 µm	150	200	2 – 12

a. Thermo Fisher, b. Biotage, c. Waters

Following the selection of the on-line SPE column (Isolute ENV+), a wide screening of potential analytical columns was conducted covering different sorbent types and brands (**Table 6-2**). The need to retain the polar anticancer drugs led to focus onto modified C18 and HILIC type column materials [347,352,356,360,362]. The tested columns included 8 HILIC type columns (Hypersil GOLD Silica, Hypersil GOLD PEI HILIC, Accucore-Amide-HILIC, Syncronis HILIC, Kinetex HILIC, Luna HILIC, ACQUITY UPC^2 Torus Diol and ACQUITY UPLC BEH Amide), one rather polar column (Luna CN), 2 modified reverse phase columns (Luna Omega Polar C18, Hypersil GOLD aQ), and 1 porous graphitic carbon column (PGC: Hypercarb). Each column was equipped with a precolumn filter to reduce the risk of clogging, while the rise in pressure during

the LC-MS/MS sequence can be limited by changing the prefilter after approximately twenty consecutive sample injections [363].

Table 6-2. Summary of the different chromatographic columns tested in HILIC and/or RPLC mode at the screening step.

Name	Sorbent	Dimensions	Pore size (Å)	Mode	pH range
Hypersil GOLD Silica[a]	Unbonded silica	100 x 2.1 mm ; 1.9 µm	175	HILIC	2 - 8
Hypersil GOLD PEI HILIC[a]	Polyethyleneimine	100 x 2.1 mm ; 1.9 µm	175	HILIC	2 - 8
Accucore-Amide-HILIC[a]	Amide	100 x 2.1 mm ; 2.6 µm	150	HILIC	2 - 9
Syncronis HILIC[a]	Zwitterionic	100 x 2.1 mm ; 1.7 µm	100	HILIC	2 - 8
Kinetex HILIC[b]	Unbonded silica	100 x 4.6 mm ; 2.6 µm	100	HILIC	2 - 7.5
Luna HILIC[b]	Cross-linked diol	150 x 4.6 mm ; 3.0 µm	200	HILIC	1.5 - 8
ACQUITY UPC^2 Torus Diol[c]	High density diol	100 x 2.1 mm ; 1.9 µm	130	HILIC	2 - 8
ACQUITY UPLC BEH Amide[c]	Trifunctional Amide	150 x 3.0 mm ; 1.7 µm	130	HILIC	2 - 12
Luna CN[b]	Nitrile groups	100 x 4.6 mm ; 3.0 µm	100	HILIC/RPLC	1.5 - 7
Luna Omega Polar C18[b]	100% aqueous stable C18	100 x 2.1 mm ; 1.6 µm	100	RPLC	1.5 - 8.5
Hypersil GOLD aQ[a]	Polar endcapped C18	100 x 2.1 mm ; 1.9 µm	175	RPLC	2 - 9
HYPERCARB[a]	Porous graphitic carbon	100 x 2.1 mm ; 3 µm	250	RPLC	0 - 14

[a]. Thermo Fisher, [b]. Phenomenex, [c]. Waters

To evaluate the performance of the different chromatographic conditions, the following analytical parameters were considered: retention times of the targeted compounds, signal intensity, chromatographic peak shape, and separation performance of the two isomeric compounds (CYC and IFO). Assessment of these parameters led to a score for each targeted analyte using the selected columns under different chromatographic conditions. More details about the score calculation is presented in Supporting Information. Mobile phases were screened with variations of organic mobile phase (MeOH *vs.* ACN), pH modifications of the aqueous mobile phase (e.g., 0.1% AF *vs.* NH_4OH addition up to 0.1%), buffer amendment (ammonium acetate or ammonium formate, tested at 5mM) in one or both phases, and variations in the gradient elution program. While slightly basic pH conditions were tested for columns that can withstand such conditions, the tested values did not exceed the upper limit of pH to preserve column lifetime. For the HYPERCARB, ion-pairing agents (hexylamine (HA) and diethylamine (DEA)) were also tested to improve the elution and separation of targeted analytes [364,365]. Different column temperatures (30 to 50 °C) and mobile phase flow rates (ranging from 300 to 500 µL min^{-1}) were

tested to reduce the method total run time without affecting chromatographic efficiency. For the HILIC type columns, known to require longer equilibration times, different durations of isocratic flow at the end of the gradient program were investigated. The shorter time that still allowed to maintain reproducible retention times and peak shapes between consecutive runs was selected.

Further optimization of the on-line SPE loading step was then performed. Acid additives (between 0.1 and 1%) were tested in the loading mobile phase. In view of the complexity of targeted wastewater matrix, a wash step following the on-line SPE loading was also investigated. The wash mobile phase, wash flow rate, and wash volume were thus optimised. The wash volume was tested at discrete levels (no wash applied *vs.* wash volumes between 0.5 and 3 mL).

Optimization of the sample volume injected (using different loop volumes) and on-line loading speed was conducted using an experimental design, since these parameters can be interdependent [366]. The sample loading volume was tested between 1 – 5 mL and loading speed between 250–3000 µL min^{-1}. A Derringer's desirability approach was then used to discriminate the best combination of parameters, with three d_i criteria: minimal relative standard deviation (RSD) of replicate injections (d_1), absolute signal intensity (d_2) and signal response normalized to injection volume (d_3) (to attain maximal recoveries). The calculation method of the d_i criteria was based on a previous study [366]. More details about the calculation of each criterion is presented in Supporting Information. For each combination of sample volume and loading flow rate, the overall desirability D was then derived as the geometric mean of d_i values [367].

$$D = \left(\prod_{i=1}^{n} d_i \right)^{1/n} \qquad \textbf{Eq. 6 – 1}$$

6.2.5 Method validation and quality control

Identification of the anti-cancer drugs in field samples was based on matching retention times with certified standards, the detection of both quantification (QT) and confirmation (CT) MS/MS transitions, and QT/CT ratios compared to the average in calibration curve standards. Xcalibur 3.0 software from Thermo Fisher Scientific (Waltham, MA, U.S.A.) was used for peak integration.

No detectable analyte levels were reported in field blanks generated within the various sampling campaigns. In each analysis sequence, blanks consisting of HPLC water aliquots were also injected regularly to ensure the absence of carryover artifacts. The absence of observable

carryover was verified by injecting a blank sample (HPLC water) immediately after a 100 ng L^{-1} calibration level.

The selection of a suitable filtration membrane for suspended particle removal proceeded from the comparison of the recovery of a spiked solution percolated through different filter types, namely, glass fiber (0.22 µm, 13mm), nylon (0.22 µm, 25mm), polypropylene (0.2 µm, 30mm), polycarbonate (0.2 µm, 25mm), polyester (0.2 µm, 25mm), cellulose acetate (0.2 µm, 25mm) and GHP (0.2 µm, 25mm).

Analytical method validation was conducted using untreated wastewater obtained from a wastewater treatment plant in the Quebec province area. The linearity range and determination coefficient (R^2) were determined from linear regressions on up to 11 calibration levels (native analyte concentrations: unspiked, 0.1, 0.5, 1, 5, 10, 50, 100, 500, 1000, 5000 and 10000 ng L^{-1}).

The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on a signal to noise ratio greater than three (S/N>3) and greater than 10 (S/N>10) respectively, using low-end calibration curve levels in wastewater matrix. If one of the analyte was found in the non-spiked matrix blank, the LOD was derived from the S/N observed in a weakly contaminated field sample.

The recovery of the on-line SPE step was determined as the absolute peak area of analytes submitted to on-line SPE–LC-MS/MS (1 mL injected for method #1 and 2 mL injected for method #2), compared to the absolute peak area of an equivalent amount submitted to small volume on-line (100 µL) LC-MS/MS [366]. Four spike levels were tested for each set up and each condition was analyzed in triplicate.[5]

Accuracy and precision were evaluated at both low (QC_1 = 5 to 15 ng L^{-1}) and high levels (QC_4 = 2500 ng L^{-1}) with intermediary levels (QC_2 = 25 ng L^{-1} and QC_3 = 200 ng L^{-1}). Precision was assessed by injecting replicate spiked samples on a first day (intraday variation, n = 5) and then on two additional days (interday variation, n = 15). Precision is presented as relative standard deviation (RSD, %). The low QC level was set at a concentration that exceeds the LOQ, while the high level was set to be in the same order of magnitude as the maximum concentration quantified in contaminated field sample within the linear range.

[5] The internal standards recoveries were verified to meet the US EPA requirement (values between 70 and 130%) [524].

The matrix effect was derived by comparing the slope in wastewater matrix to that in a matrix-free (HPLC water) calibration curve. In the absence of matching isotope-labeled internal standards for every targeted compound, the best association of IS with each anti-cancer drug has been confirmed by a selection based on ionization mode (positive/negative), retention time, and efficiency at correcting method variations.

6.3 Results and discussion

6.3.1 3.1. Selection of the on-line SPE column

In the initial step of method development, on-line SPE columns were screened for their potential to retain small polar pharmaceuticals. Poor retention and inadequate preconcentration was expected on typical hydrophobic phases, especially for analytes with negative log *P* values. Therefore, classical C18 sorbents were not included in this screening step. A C8 material was tested due to its less hydrophobic alkyl chains, but this option did not show satisfactory retention for the most polar analytes and was therefore discarded. Similar issues were noted with a BetaBasic column which exhibited low recoveries (<10%) for 5-FU. These two SPE columns having much less sorbent surface area when compared with the other options can be a possible explanation for lack of retention ability [368]. Kovalova et al. (2009) [347] previously introduced Isolute ENV+ cartridges (off-line) to retain more effectively 5-FU, a major chemotherapy agent yet infrequently monitored due to the lack of affinity with more commonly used cartridges. This material, along with the Oasis HLB and the Hypersep Retain PEP, yielded similar performance for a number of slightly hydrophobic chemotherapy agents such as GEM, dFdU, MTX, IFO, CYC and CAP (**Figure 6-1**).

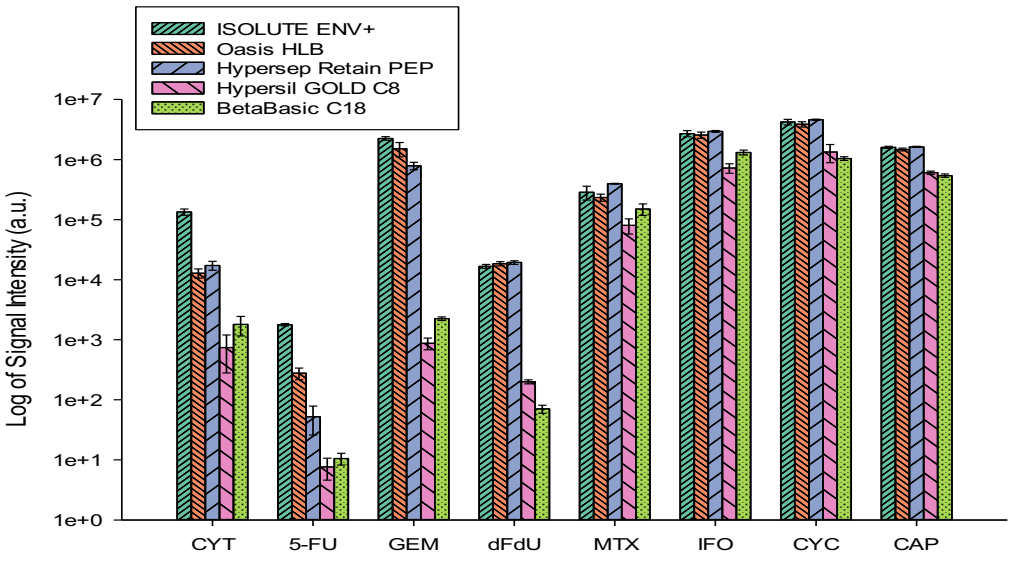

Figure 6-1. Comparative assessment of different on-line SPE columns (signal intensity shown in log scale). Each condition was tested at 100 ng L^{-1}. Error bars correspond to standard deviations (n = 3).

Significant differences were, however, noted for the most hydrophilic compounds. The Isolute ENV+ sorbent from Biotage produced the best results for 5-FU as well as for CYT (**Figure 6-1**), suggesting promising potential for further optimisation (Sections 6.3.2 and 6.3.3). The signal improvement observed in **Figure 6-1** reflects a better enrichment on the Biotage on-line SPE column, presumably due to the arrangement of the copolymer (hyper crosslinked hydroxylated polystyrene-divinylbenzene copolymer) compared to the other sorbent types. When looking at dimensions of the different SPE columns, it can be observed that the selected one (Isolute ENV+) is slightly longer (**Table 6-1**). This difference is not considered to have a major impact on selecting the best material since previous studies looking at the different options for off-line cartridges with the same sorbent mass led to similar conclusions for cytostatics [362].

6.3.2 Screening of HPLC chromatographic conditions

The next step of method development aimed at achieving suitable chromatographic separation. Some chromatographic options had to be discarded due to the lack of retention for the target compounds or difficulty to hyphenate the analytical column with the on-line SPE for complex wastewater samples. Prior studies on LC-MS/MS analysis of polar compounds such as nucleotides by Crauste and al. (2009) [365] showed the potential of porous graphitic carbon columns for analytes with structural similarities to some of the targeted cytostatics. For this reason, a porous graphitic carbon column was tested under different chromatographic conditions and with

some ion pairing agents as additives. Even with the use of DEA or HA, that are used to enhance chromatographic performance (separation and peak shape) [364], the PGC column did not show sufficient potential. The other columns were tested under the chromatographic mode mentioned in **Table 6-3**. The Luna CN was the only column tested in both modes. However, HILIC separations were unsatisfactory and only the RPLC results were retained for comparison purpose. For each combination, the evaluation proceeded considering the three following parameters: retention time, peak shape (width and symmetry), and intensity. Scoring of these parameters rates from poor (1), intermediate (2) to good (3) performance, with some results that lead to unacceptable peak shape and were therefore rejected (0). Scores are calculated as the sum of criteria corresponding to each of the three parameters that have values ranging from 0 to 1. More details about the calculation of each criterion are presented in Supporting Information. This analysis allows to delineate the most promising column/mobile phase combinations to be further submitted to optimization (**Table 6-3**).

Table 6-3. Comparison of the most promising chromatographic conditions and columns.[6]

Column	Condition	Column performance (retention, peak shape, intensity)							
		CYT	5-FU	GEM	dFdU	MTX	IFO	CYC	CAP
Hypersil GOLD Silica	A	1.6	1.1	0.9	1.2	1.2	1.1	1.1	1.1
	B	1.8	1.2	1.6	1.3	2.6	1.3	1.4	1.4
	C	1.4	1.3	2.1	1.8	1.0	1.4	1.5	2.1
	D	0.0	0.1	1.6	1.2	1.0	1.1	1.1	1.1
Hypersil GOLD PEI HILIC	A	0.8	1.8	0.0	1.2	0.0	1.0	1.0	1.1
	B	0.7	1.5	0.0	1.8	2.0	1.2	1.3	1.2
	C	1.2	2.2	0.0	1.7	0.0	1.2	1.3	1.2
	D	**0.6**	**2.9**	**0.1**	**3.0**	**1.0**	**1.0**	**1.0**	**1.1**
Accucore-Amide-HILIC	A	1.9	0.5	1.1	0.4	1.1	0.4	0.4	0.4
	B	1.9	1.0	0.6	0.0	1.6	1.1	1.1	1.1
	C	0.0	1.0	0.1	1.0	1.0	0.9	0.9	1.0
	D	0.0	0.0	0.1	0.2	0.9	0.7	0.7	0.4
Syncronis HILIC	A	0.0	1.1	0.7	1.2	1.6	1.0	1.0	1.1
	B	0.0	1.1	0.0	1.1	1.5	1.0	1.0	1.1
	C	0.0	0.0	0.0	1.2	1.0	1.1	1.1	1.1
	D	0.0	0.0	0.0	0.8	1.5	1.0	1.0	1.0
Kinetex HILIC	A	0.0	0.0	0.0	1.2	1.4	0.8	0.7	0.7
	B	0.0	0.3	2.0	0.4	0.0	0.3	0.4	0.3
	C	0.0	0.0	0.3	0.6	1.1	0.6	0.7	0.7
	D	0.0	0.6	0.0	0.8	0.3	0.9	0.9	0.6
Luna HILIC	A	0.0	0.0	1.7	0.0	1.3	0.5	0.6	0.7
	B	2.0	0.0	1.9	0.0	1.7	0.0	0.0	0.0
	C	0.0	0.0	0.0	0.0	2.2	0.0	0.0	0.0
	D	0.0	0.0	0.0	0.0	1.6	0.5	0.6	0.6
ACQUITY UPC² Torus Diol	A	1.3	0.0	1.0	0.0	0.0	0.9	0.9	1.0
	B	0.0	0.0	1.6	0.0	1.4	0.0	0.0	0.0
	C	1.1	0.0	0.8	0.0	1.3	0.7	0.9	1.0
	D	1.3	0.0	0.5	0.0	1.2	0.8	0.8	0.9
ACQUITY UPLC BEH Amide	A	1.3	0.0	0.7	0.0	0.0	1.1	1.1	1.1
	B	0.6	1.2	0.0	1.4	0.0	1.2	1.3	1.3
	C	0.0	1.2	0.9	1.6	0.0	1.3	1.4	1.6
	D	0.8	1.0	1.0	1.1	1.5	1.1	1.1	1.1
Luna CN	A	1.7	0.0	1.5	0.0	1.0	0.7	0.7	0.7
	B	1.8	0.6	1.8	0.7	1.6	0.8	0.9	0.9
	C	0.0	0.0	0.0	0.7	0.0	1.3	1.4	0.9
	D	0.0	0.0	0.7	0.0	1.2	0.8	0.8	0.0
Luna Omega Polar C18	A	1.6	1.4	1.4	2.8	1.4	2.7	2.8	2.7
	B	1.3	1.5	1.4	2.0	1.3	2.6	2.7	2.7
	C	**2.0**	**2.0**	**1.9**	**2.3**	**2.6**	**3.0**	**3.0**	**2.8**
	D	1.7	1.4	1.5	2.0	1.5	2.4	1.9	1.9
Hypersil GOLD aQ	A	1.6	1.4	1.4	2.8	1.4	2.7	2.7	2.6
	B	1.3	1.5	1.4	2.1	1.2	2.5	2.8	2.7
	C	**2.0**	**2.0**	**1.9**	**2.3**	**2.5**	**3.0**	**3.0**	**2.8**
	D	1.7	1.4	1.5	2.0	1.5	2.4	1.9	1.9

Conditions : A. H2O/ACN; B. H2O + 0.1%AF/ACN; C. H2O + 5mM AmFo/ACN; D. H2O + 5mM AmAc/ACN. Each condition has been applied several times (n=5) to obtain values for score calculation. A first analytical gradient method was used to compare all normal and HILIC type columns and a second one was used for reverse phase type columns (See Supporting Information **Tables 6-12** and **6-13**).

[6] Bold characters denote the most optimal chromatographic conditions for chemotherapy separation following the preconcentration on a ISOLUE ENV+ SPE column.

Only few conditions led to acceptable chromatographic performance for 5-FU (**Table 6-3**). This compound being a major chemotherapy agent in terms of consumption and potential release via wastewaters, finding a specific method appears essential. The Isolute ENV+ on-line column coupled with Hypersil GOLD PEI HILIC analytical column provided a fast and robust analytical method. Addition of AmAc contributed to improving 5-FU retention and signal response. Low concentrations of AmAc (0.1-0.5 mM) yielded marginal signal improvements compared to non-amended mobile phase, while the effect was more pronounced at intermediate (1 mM) tested range (+19%) along with improved precision. Higher concentrations led to a loss of 5-FU signal in addition to a gradual pressure increase affecting retention time reproducibility and limiting the number of samples that could be analyzed in a single sequence. Similar trends were noted for dFdU signal response. Taking these limitations into account, the most advantageous buffer concentration was determined to be 1 mM (SI **Figure 6-6**). Stability tests were also conducted to ensure that consecutive injections on the HILIC type column presented reproducible retention times. A 9-min stabilization plateau (10/90 H_2O + 1mM AmAc/ACN) was deemed sufficient to maintain steady retention times across consecutive injections. Shorter equilibration times yielded retention time variations across injections (SI **Figure 6-7**). This could lead to problematic peak identification, especially in a complex matrix such as wastewater. Longer equilibration times have also been tested but methods of more than 20 minutes (25 and 30 minutes) showed no further improvements.

The same approach led to the choice of a method that can be suitable for the analysis of other cytostatic drugs. The Luna Omega Polar C18 and Hypersil GOLD aQ, both running under reverse phase conditions, presented the best chromatographic performances overall (**Table 6-3**). Adding AmFo buffer resulted in improved peak shapes and intensities and permitted the chromatographic resolution of IFO and CYC (isomers). Further optimization of the mobile phase additives in the RPLC set-up was performed. The use of buffer in the mobile phase can help achieve better separation, increased sensitivity, and reduced variations in matrix effects between samples. However, the addition of non-volatile buffers at high concentration can lead to a decrease in performance due to increased background signal, signal suppression of target analyte, and spray instability [369,370]. For the RPLC method (using either Luna Omega Polar C18 or Hypersil GOLD aQ columns), the addition of AmFo led to better chromatographic performances at a concentration of 10 mM (**Figure 6-2**). Addition of MeOH to the mobile phase could also lead to better chromatographic performances. A mix of ACN and MeOH (50/50) use as solvent for the organic mobile phase showed better results when compared with MeOH or ACN alone.

Other conditions presented in **Table 6-3** may show better results for specific compound types (but not for all compounds) and could be further explored when focusing on a specific cytostatic. For instance, enhanced analysis of CYT could be performed under different conditions, e.g., by using formic acid additive combined with different columns (Hypersil GOLD Silica, Accucore-Amide-HILIC, Luna HILIC, Luna CN) or without any additives with the Accucore-Amide-HILIC column (**Table 6-3**). This last condition was chosen for comparison purposes in the method development experiment. However, HILIC chromatography, especially with complex matrices, tends to be less reproducible without buffer addition to the mobile phase [206,371] and therefore could be unsuitable for this application. Another compound for which better results could be achieved under acidic mobile phase conditions is GEM with different columns (Kinetex HILIC, Luna HILIC). Addition of volatile acid in elution solvents, such as formic acid, generally favors the ionization of positively charged analytes in ESI [370]. For a majority of the selected columns, it was not possible to test basic pH conditions without threatening sorbent integrity. The Hypercarb and ACQUITY UPLC BEH Amide columns, that support pH value up to 10, were tested under these conditions, but there was no significant improvement in terms of signal nor analyte separation.

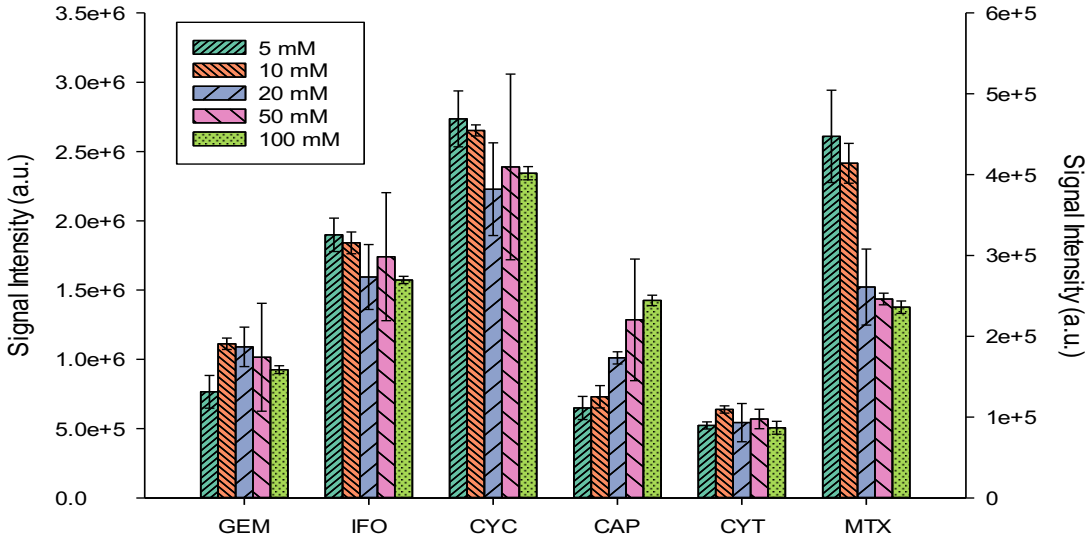

Figure 6-2. Effect of AmFo additive concentration on the cytostatics signal on a Luna Omega Polar C18 column. Each condition was tested at 100 ng L^{-1}. Error bars correspond to standard deviations (n = 5). CYT and MTX are related to the right axis.

Additional HPLC method parameters were optimized for the HILIC and RPLC selected methods. The starting ratio (aqueous phase/organic phase), elution gradient, flow rate, and column temperature were investigated. The selected chromatographic gradient conditions are presented in the SI (**Tables 6-12** and **6-13**).

Flow rate and column temperature were chosen to reduce method time and favor gaussian peak shapes without affecting retention time or back-pressure increase. A lower column temperature was needed for the HILIC columns in order to preserve analytes' affinity with the stationary phase since increasing temperature can affect both their kinetics and thermodynamics [372,373]. The HILIC column oven was set at 30°C to prevent artifacts from potentially variable room temperature and to extend the column working lifetime [372].

6.3.3 Optimization of on-line SPE loading conditions

On-line SPE loading parameters with the Isolute ENV+ on-line SPE column were subjected to further optimization. Acidic amendment of the SPE mobile phases was investigated. While the SPE enrichment step showed best results under neutral conditions (using HPLC water without additives), the elution and washing steps were more effective under acidic conditions (0.1% formic acid). Higher FA percentages (0.5 and 1%) in the wash phase did not yield further improvement. Another critical parameter was the on-line wash volume, which was assessed for different sample loading volumes (1, 2 and 5 mL of wastewater). For each loading volume, the wash volumes (with HPLC water) were tested at discrete levels between 0 and 3 mL, applied before the back-flush elution. An on-line wash volume of 1 mL reduced the matrix effect and yielded better signals than conditions involving low or no wash for such complex samples (wastewater). Selecting the lowest possible wash volume allows to expedite the loading/wash process thus reducing overall method duration and limiting the risk of breakthrough losses (**Figure 6-3**). For more easily retained compounds, only a small difference could be observed between a wash volume of 1 and 2 mL, but slightly more stable signals were obtained for the triplicate injection of the smaller volume (SI **Figure 6-8**). The result of 1 mL was obtained for both methods and for the three different loop volumes (data not shown). Only the results for the selected loop volume corresponding to each method are presented.

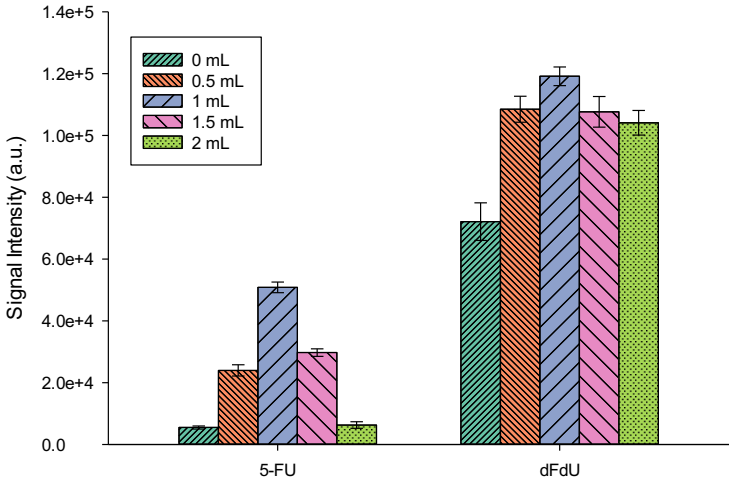

Figure 6-3. Influence of wash volume on analyte response, illustrated for 5-FU and dFdU on Isolute ENV+ when loading 1 mL of sample. Each condition was tested at 100 ng L^{-1}. Error bars correspond to standard deviations (n = 3).

Other on-line SPE conditions were jointly optimized by creating an experimental design, with methods that vary in terms of sample volume (using different loops) and loading flow rate. To refine the best combination of conditions for a maximum number of analytes, Derringer's desirability functions were constructed based on three d_i criteria (Section 2.4). The weight of each parameter can be augmented in order to achieve better recoveries (relative signal), better LODs (signal intensity that will tend to favor larger loading volumes), or better stability and precision (RSD) [366]. These tests were conducted for the HILIC and RPLC methods separately. Details on calculated d_i values are provided in supporting information (see **Figures** 6-9 and 6-10), and **Figure 6-4** and **Figure 6-5** presents the overall Desirability across tested conditions.

In the method focusing on 5-FU, the difficulty of pre-concentration drives toward focusing on the relative signal (d_3). This favors conditions with smaller loop volumes and a relatively slow loading speed to improve SPE recovery, leading to the selection of a 1-mL sample volume loaded at 1000 µL min^{-1} (**Figure 6-4**). Inconclusive results were obtained for the 5-mL loop with low recoveries and problematic pressure increase after a few injections due to the complex matrix. For the method focusing on other chemotherapy agents, the emphasis was on signal intensity to promote better LODs (d_2). On the Luna Omega Polar C18, the selected on-line SPE parameters were a 2-mL loop volume and a flow rate of 2500 µL min^{-1} (**Figure 6-5**). The use of a 5-mL loop for this method is possible but might lead to lower analyte recoveries and higher risks of matrix effects. The null D values that can be observed for some methods in **Figures** 6-4 and 6-5 reflect

poor reproducibility leading to unsuccessful compliance with the RSD criterion (d_1). Overall, a slower flow rate for sample enrichment results in less precise injections, but the highest flow rate tested for each loop volume did not let enough contact time between sorbent and analytes leading to a higher RSD. Thus, higher flow rates were not subjected to further testing. As expected, a higher volume of sample injected resulted in higher intensities, but working with very polar analytes also means difficult retention and lower recoveries. This can be observed for both methods when comparing d_2 and d_3 criteria and is more pronounced for the 5 mL conditions (see **Figures** 6-9 and 6-10).

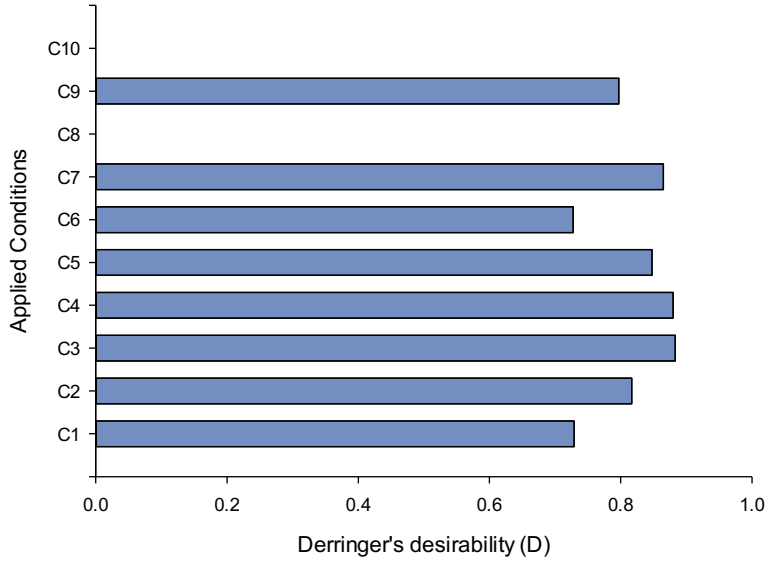

Figure 6-4. Optimization of the loading parameters for the on-line enrichment of 5-FU and dFdU using an experimental design and Derringer's desirability functions (C1-C5 = 1-mL injection volume, flow rates investigated at 0.25, 0.5, 1, 1.5, and 2 mL min^{-1}; C6-C10 = 2-mL injection volume, flow rates investigated at 0.25, 0.5, 1, 1.5, and 2 mL min^{-1}). These conditions were tested using a wash volume of 1 mL and chromatographic separation on a Hypersil GOLD PEI HILIC column.

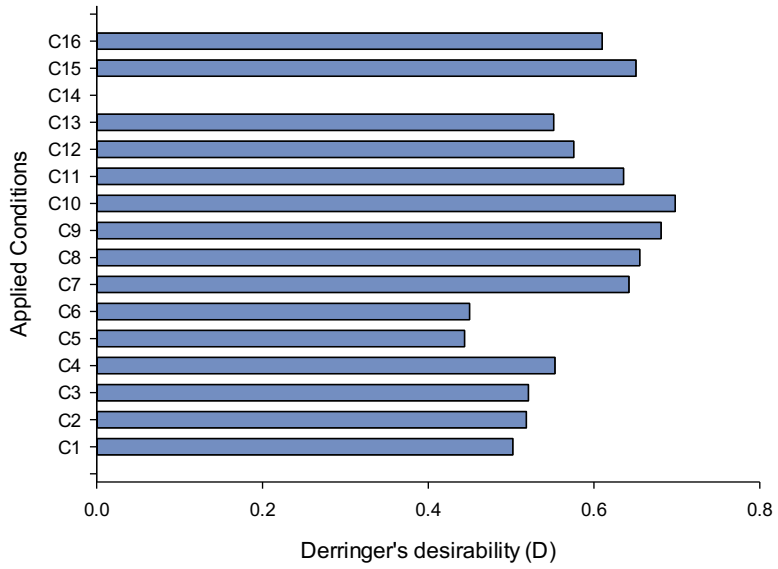

Figure 6-5. Optimization of the loading parameters for the on-line enrichment of CYT, GEM, MTX, IFO, CYC and CAP under experimental design to evaluate Derringer's desirability (C1-C6 = 1-mL injection volume, flow rates investigated at 0.5, 1, 1.5, 2, 2.5, 3 mL min^{-1}; C_7-C_{11} = 2-mL injection volume, flow rates investigated at 1, 1.5, 2, 2.5, 3 mL min^{-1}; C_{12}-C_{16} = 3-mL injection volume, flow rates investigated at 1, 1.5, 2, 2.5, 3 mL min^{-1}). These conditions were tested with a wash volume of 1 mL and chromatographic separation on a Luna Omega Polar C18 column.

6.3.4 Optimization of the filtration step

The choice of filtration membrane used as a pre-treatment step prior on-line SPE – UHPLC-MS/MS should be made upon appropriate testing to avoid biased-low water concentrations or false negatives due to adsorption artifacts. **Figure 5** presents the recoveries of the targeted compounds percolated through the different membranes. The polyester filter did not allow the filtration of a large enough volume to be suitable for the application to wastewater because of rapid filter clogging and demanding high pressure in practice. Other membranes showed suitable recoveries for most compounds except for nylon filters (ranging between 40 to 100%). Filtration recoveries ranged between 73 to 100% for cellulose, 62 to 95% for glass fiber, 60 to 90% for GHP, 77 to 96% for polycarbonate and 68 to 98% for polypropylene. As shown in **Figure 6-11** (SI), the cellulose membrane was the one with the highest mean recoveries and smaller variation compared with other filters of similar recovery performance. Cellulose filters were finally retained for their filtration ease and superior recovery and precision.

6.3.5 Analytical method validation

Analytical validation was conducted to verify standard performance requirements for quantification. The recoveries of the targeted analytes on the on-line SPE sorbent are shown in **Table 4**. Recoveries ranges were between 92 and 108% at QC_1 (mean: 101%), 87 and 119% at QC_2 (mean: 109%), 92 and 114% at QC_3 (mean: 105%) and 79 and 124% at QC_4 (mean: 97%).

Accuracies ranged from 81 to 113% at QC_1 (mean: 102%), 80 to 111% at QC_2 (mean: 92%), 89 to 104% at QC_3 (mean: 96%) and 78 to 106% at QC_4 (mean: 92%) (**Table 6-4**). The accuracy of dFdU at high level is more affected when comparing with the other analytes. The slightly biased-low dFdU accuracy (78%) at 2500 ng L^{-1} may be related to the lower recovery at high concentration level, which is not fully compensated by internal standardization. Apart from this lower value, all accuracies are compliant with US EPA guidelines (70-130%) for trace organic pollutants in wastewater [277]. The maximum RSD values for intraday precision were 6.3% at QC_1 (mean: 5.0%), 12% at QC_2 (mean: 5.6%), 9.6% at QC_3 (mean: 5.2%) and 11% at QC_4 (mean: 5.1%). For interday precision, maximum RSD values were 8.6% at QC_1 (mean: 6.1%), 13% at QC_2 (mean: 7.2%), 12% at QC_3 (mean: 6.9%) and 12% at QC_4 (mean: 6.3%). The mean values of intraday precision is slightly higher when compared to intraday precision, but these values are within the European Commission and US EPA requirements that recommend RSDs not exceeding 20% to 30% [277,374].

Table 6-4. Analytical validation parameters, including recovery, accuracy, and precision (intraday and interday) tested at four spike levels (QC1 = 5 ng L^{-1} for MTX and CYC, 10 ng L^{-1} for CAP, IFO and dFdU and 15 ng L^{-1} for 5-FU, QC2 = 25 ng L^{-1}, QC3 = 200 ng L^{-1} and QC4 = 2500 ng L^{-1}).

		CYT	5-FU	GEM	dFdU	MTX	IFO	CYC	CAP
Recovery (%)	QC_1	N. A.	99 ± 7	N. A.	97 ± 5	92 ± 7	108 ± 2	108 ± 2	102 ± 1
	QC_2	87 ± 5	111 ± 4	119 ± 6	115 ± 4	102 ± 2	118 ± 3	107 ± 9	112 ± 5
	QC_3	105 ± 5	108 ± 4	106 ± 6	105 ± 5	92 ± 3	114 ± 4	107 ± 6	103 ± 9
	QC_4	124 ± 4	84 ± 7	116 ± 8	79 ± 6	82 ± 6	95 ± 4	81 ± 2	113 ± 5
Accuracy (%)	QC_1	N. A.	110 ± 8	N. A.	105 ± 9	81 ± 8	100 ± 8	113 ± 9	101 ± 4
	QC_2	95 ± 9	93 ± 3	80 ± 2	102 ± 8	84 ± 2	111 ± 5	88 ± 2	83 ± 12
	QC_3	97 ± 8	96 ± 9	98 ± 4	94 ± 7	96 ± 5	89 ± 4	91 ± 5	104 ± 8
	QC_4	93 ± 2	90 ± 10	102 ± 3	78 ± 5	106 ± 3	86 ± 2	97 ± 3	81 ± 7
Intraday Precision (%)	QC_1	N. A.	4.5	N. A.	3.8	6.3	8.2	4.4	2.6
	QC_2	9.0	2.8	3.1	7.6	3.0	4.8	2.8	12
	QC_3	9.6	2.4	3.0	5.4	7.2	7.4	4.6	2.2
	QC_4	11	2.6	3.0	6.0	3.3	2.6	3.1	8.9
Interday Precision (%)	QC_1	N. A.	4.6	N. A.	8.5	4.9	7.1	7.4	4.0
	QC_2	12	6.4	4.3	12	3.8	3.9	2.1	13
	QC_3	12	9.2	4.0	7.0	5.0	4.7	5.6	8.0
	QC_4	12	7.9	2.7	5.7	7.3	4.9	2.6	7.0

* N.A: QC1 results for CYT and GEM are not available because their LOQ is already close to QC2 level.

The LOD performance was in the range of 0.5–5 ng L^{-1} (**Table 6-5**). Calibration curves were produced with determination coefficients (R^2) in the range of 0.9982–0.9999, compliant with EPA acceptance requirements (R^2 >0.99) [375]. For some of the targeted compounds, signals deviated from linearity or plateaued at concentrations higher than 5000 ng L^{-1}. No concentrations exceeding 10000 ng L^{-1} have been injected since this is above the worst-case expected concentration for Eastern Canada, even for contaminated samples such as hospital wastewater. However, for samples that may have concentrations above the highest calibration curve level, a dilution step can be performed. The developed on-line methods still allow the quantification over a 3 order of magnitude concentration range.

Table 6-5. Method performance for the targeted chemotherapy agents using the developed on-line SPE – UPLC-MS/MS methods.

Compound	LOD (ng L^{-1})	Linear range* (ng L^{-1})	Linearity (R^2)
CYT	5	16 - 10,000	0.9982
5-FU	2	6.6 - 10,000	0.9997
GEM	5	16 - 10,000	0.9997
dFdU	1	3.3 - 10,000	0.9994
MTX	0.5	1.6 - 10,000	0.9999
IFO	1	3.3 - 5,000	0.9999
CYC	0.5	1.6 - 10,000	0.9996
CAP	1	3.3 - 5,000	0.9981

* The linear range is comprised between LOQ values and the maximum injected concentration that maintained suitable linearity.

6.3.6 Assessment of total matrix effects

Comparing calibration curves constructed in matrix-free water (HPLC water) to those in wastewater allows the determination of the global matrix effect coming from the SPE step, chromatography and MS detection. There is only limited effect of the hospital wastewater matrix on the calibration curves, implying that the use of internal standardization is efficient. For instance, slight matrix suppression was observed for IFO (-1.2%) and moderate matrix enhancement for CYT (+12%) when comparing to the matrix-free calibration curve (see SI **Table 6-15**). A noteworthy exception was CAP, with deviations exceeding the acceptance threshold set at ±30%. Similar results were observed when assessing matrix effects in urban wastewaters (SI **Table 6-16**). The matrix effect values are within the anticipated range for such complex aqueous matrices according to previous studies [376]. For quantifying CAP alone, performing standard additions may yield improved accuracy. However, this approach is not suitable when a large number of samples needs to be analyzed.

The residual matrix effect was generally low for targeted compounds with matched internal standards, and more pronounced for those without matched internal standards (e.g., CAP, CYT, dFdU). This may be addressed in the future by acquiring their matched isotope labeled internal standards. This would compensate for variations and reduce the discrepancy between matrix-free and wastewater curve to the level of other compounds with a mean matrix effect generally within ±5%. This may also endorse the use of matrix-free calibration curve for routine quantification, provided that all compounds are in the acceptable matrix effect range of ±30% according to US

EPA requirements. In the present study, calibration curves were constructed in matrix-matched wastewater for improved accuracy in the quantification of CAP, CYT, and dFdU in field samples.

6.3.7 Comparison with the literature and potential limitations

Considering previous studies on cytostatic drugs, some parameters can be highlighted to show the advantage of using on-line SPE methods (**Table 6-6**). The total method time (preparation + instrumental analysis) is greatly improved by automation. Sample volume needed for on-line pre-concentration are also reduced while yielding the same ranges of LODs. Few studies achieved suitable recoveries for the list of targeted compounds, and the analysis of 5-FU was often problematic. For instance, one study achieved suitable LODs involving on-line SPE injections of a 5-mL sample, but low enrichment recoveries were noted for GEM [359]. Some gas chromatography methods have been developed and are achieving very low LODs like 0.5 ng L^{-1} for 5-FU using off-line SPE with large sample volume enrichment (100 mL), but with lower analyte recovery (53%) (Kosjek et al. 2013). A derivatization is required so that cytostatics are amenable to GC-MS analysis; this results in a multi-step process (off-line SPE + derivatization) resource-intensive to implement in practice. Comparing the method performance of the present study with literature is not always straightforward, since analytical validation is sometimes performed in matrix-free medium (HPLC water) instead of a real matrix. However, most of the methods included at least testing of the matrix effect by spiking real wastewater or surface water samples. The previous methods highlighted the possibility of matrix effects including signal suppression for IFO and CYC (-40%) or signal enhancement for CAP (+46%) [359,377]. The method reported herein presented similar performance in terms of LODs, but generally better recoveries and lower matrix effects (highlighting the importance of a suitable wash step for matrix removal).

Table 6-6. Analytical method comparison for chemotherapy agents.

Analytes[1]	Matrix[2]	Method duration	Sample volume	Chromatographic column	SPE cartridge	Recoveries (%)	LOD (ng L^{-1})	Accuracy (%)	Precision (%)	Reference
CYC, IFO	SW, WWTP	12 min + off-line SPE	1 L	Xterra RP18	Bio-Beads SM-2	74 - 93	0.2 - 2	N. A.	N. A.	[378]
5-FU, GEM, dFdU	HWW	30 min + off-line SPE	50 mL	ZIC HILIC PEEK	Isolute ENV+	40 - 79	0.3 - 3	54 - 118	5.6 - 22	[347]
CYC, IFO, MTX	WWTP, HWW	5.5 min + off-line SPE	50 mL	Acquity HSS T3	Oasis HLB	73 - 82	1.1 - 1.8	82 - 121	8.4 - 10.3	[360]
IFO, CYC, MET, CAP, GEM	GW, SW, WWTP	40 min with on-line SPE	5 mL	Purospher STAR RP-18e	Oasis HLB, PLRP-s	0.13 - 119	0.1 - 0.3	81 - 116	2.3 - 7	[359]
CYC, IFO, CYT, GEM, CAP	GW, SW, WWTP, HWW	50 min + off-line SPE	100 mL	Luna C18	Oasis HLB	6 - 92	4.4 - 262	N. A.	10 - 21	[379]
CYC, IFO, MTX	WWTP	11 min with on-line SPE	1 mL	Hypersil GOLD PFP	Hypersil GOLD PFP	73 - 85	4 - 12	N. A.	9 - 17	[352]
CYC, CAP, 5-FU	SW, WWTP	27 min + off-line SPE	100 mL	Xbridge amide	Oasis WAX	31 - 83	2 - 46	N. A.	2 - 13	[377]
5-FU	HWW	26 min + off-line SPE + derivatization	100 mL	Factor Four 5-ms (GC method)	Isolute ENV+	95 - 101	12	90 - 130	5	[380]
CYC	SW, WWTP	22 min + off-line SPE + derivatization	500 mL	HP-5MS (GC method)	Oasis HLB	55 - 110	10	N. A.	<15	[381]
5-FU, CAP	SW, WWTP, HWW	17 min + off-line SPE + derivatization	100 mL	DB-5 MS (GC method)	Isolute ENV+	53	0.5	90	14	[108]
5-FU, dFdU	**WWTP, HWW**	**20 min with on-line SPE**	**1 mL**	**Hypersil GOLD PEI HILIC**	**Isolute ENV+**	**111 - 115**	**1 - 2**	**93 - 102**	**6.4 - 12**	**Present study**
CYT, GEM, MTX, IFO, CYC, CAP	**WWTP, HWW**	**10 min with on-line SPE**	**2 mL**	**Hypersil GOLD aQ or Luna Omega Polar C18**	**Isolute ENV+**	**87 - 119**	**0.5 - 5**	**80 - 111**	**2.1 - 13**	**Present study**

1. Only the common analytes to the present study are shown to simplify the table
2. GW = ground water, SW = surface water, WWTP = wastewater treatment plant, HWW = hospital wastewater

The HILIC analytical method designed for dFdU and 5-FU in the present study, required a rather long conditioning time in order to ensure reproducible separations. While the on-line method reduced sample pretreatment, the need for consistent retention times thus decreased sample throughput. The working pressure of the column might rapidly build up when injecting heavily charged samples so an efficient filtration was needed prior to injection; filtration through cellulose membrane allowed efficient removal of particles >0.2 µm without significant analyte adsorption. Analysis of even more polar compounds, such as some cytostatics metabolites (e.g., hydroxymethotrexate or fluoro-beta-alanine), could be of interest but may require re-evaluation of the preconcentration method. These compounds can provide insights on the consumption and metabolism of the parent molecule [382,383], and can also present a hazardous potential [384,385].

6.3.8 Method application to hospital effluents and municipal wastewaters

The selected analytical methods were applied to a set of hospital wastewaters and urban wastewaters (influents and effluents) collected in eastern Canada. The two matrices were expected to contain numerous pharmaceutical compounds at different concentration ranges [360,378,386]. Some chemotherapy agents were anticipated to occur at higher concentrations at the hospital reject point. The hospital wastewaters that are not treated at the reject point eventually end up in wastewater treatment plants and combine with urban wastewaters. Those can also contain some anticancer drugs increasingly consumed at home [387,388]. Analysing both affluent and effluent wastewater can help estimate the removal efficiency by different types of WWTPs. Knowing the amount of these compounds that are still present at the exit of treatment also provides an idea of the amount of anticancer drugs that are released to the environment.

The anticancer drugs were screened in the wastewater of 11 hospital institutions (**Table 6-7**), some with and some without cancerology departments. For instance, hospitals #10 and #11 are institutions where no chemotherapy treatments are given. Theoretically, there may still be traces of cytostatics since a patient could receive a prescription from another hospital but being treated in this one for another purpose. However, none of the targeted compounds were present at detectable levels in wastewaters from hospitals #10 and #11.

Table 6-7. Concentrations (ng L-1) of the targeted cytostatic agents in hospital wastewater effluents.

Hospital	CYT	5-FU	GEM	dFdU	MTX	IFO	CYC	CAP
#1	nd	nd	<LOQ	48.8 ± 6.8	68.4 ± 3.5	22.7 ± 0.5	2.17 ± 0.08	4.42 ± 0.60
#2	nd	nd	nd	nd	nd	144 ± 9	nd	6.13 ± 0.32
#3	nd	nd	31.4 ± 0.1	38.1 ± 3.0	42.4 ± 2.0	nd	nd	nd
#4	nd	nd	nd	41.8 ± 2.6	6.06 ± 0.18	nd	nd	nd
#5	nd	nd	21.9 ± 0.2	<LOQ	nd	nd	nd	3.71 ± 0.21
#6	nd	nd	25.5 ± 1.7	16.7 ± 2.5	8.32 ± 0.62	nd	1.90 ± 0.08	nd
#7	nd	nd	17.3 ± 0.6	nd	5.42 ± 0.76	nd	nd	nd
#8	nd	nd	nd	nd	nd	nd	nd	nd
#9	nd	nd	<LOQ	nd	nd	nd	nd	nd
#10	nd	nd	nd	nd	nd	nd	nd	nd
#11	nd	nd	nd	nd	nd	nd	nd	nd

Hospital wastewaters showed detectable levels of GEM (17-31 ng L^{-1}), dFdU (17-49 ng L^{-1}), MTX (5.4-68 ng L^{-1}), IFO (23-140 ng L^{-1}), CYC (1.9-2.2 ng L^{-1}), and CAP (3.7-6.1 ng L^{-1}). Interestingly, some analytes absent in WWTP samples were reported in hospital effluents. For instance, GEM was found in 5/11 hospitals and IFO in 2/11 hospitals, while these compounds were never detected in municipal WWTPs. Conversely, CAP was systematically found in WWTP influents, but rarely detected in hospital effluents (3 out of 11 samples). CAP is an orally administered prodrug of 5-FU for use at home, which could explain its detection in urban wastewaters [389].

In WWTP influent samples (**Table 6-8**), the most recurrently detected compounds were CAP (detection in 6 out of 6 WWTPs), CYT (6/6), MTX (5/6), and dFdU (5/6). Cyclophosphamide was detected in 3/6 WWTP influents while its related isomer IFO, which is less commonly prescribed, was not detected in any urban WWTP sample. MTX (used for arthritis treatment) and CAP (orally-administered form of 5-FU) are among the cytostatic agents most consumed at homes, which supports their detection in urban WWTP influents. Concentrations ranges observed for WWTP influents in the present study (positive samples only) were 74.4–924 ng L^{-1} for CYT, 9.2–300 ng L^{-1} for dFdU, 4.3–27 ng L^{-1} for MTX, 8.6–118 ng L^{-1} for CYC, and 4.2–64 ng L^{-1} for CAP. These values fall within the range of concentrations reported in previous literature, for example 17–60 ng L^{-1} for MTX [352], around 25 ng L^{-1} for CYC [360] and 15 ng L^{-1} for CAP [379]. In the case of CYT, a high concentration (>900 ng L^{-1}) was quantified for WWTP influent #3 but the rest of measured concentrations were in the range of previously reported contamination [390].

The compounds were also detected in WWTP effluents in the present study, implying that removal from the sampled sites is not totally effective for these selected polar anticancer drugs (**Table 6-8**).

Table 6-8. Concentrations (ng L-1) of the targeted cytostatic agents in influents and effluents from six municipal WWTPs in southern Quebec (Canada).

Sample	CYT	5-FU	GEM	dFdU	MTX	IFO	CYC	CAP
Influent #1	329 ± 6	nd	nd	130 ± 11	4.34 ± 0.33	<LOD	<LOD	4.18 ± 0.41
Effluent #1	349 ± 5	nd	nd	170 ± 14	1.84 ± 0.03	nd	<LOQ	52.2 ± 0.6
Influent #2	108 ± 4	nd	nd	300 ± 2	5.16 ± 0.49	nd	<LOD	26.9 ± 2.7
Effluent #2	145 ± 12	nd	nd	150 ± 9	6.59 ± 0.17	nd	<LOD	8.62 ± 0.76
Influent #3	924 ± 65	nd	nd	24.2 ± 1.3	7.08 ± 0.26	nd	8.58 ± 0.13	21.3 ± 1.0
Effluent #3	217 ± 21	nd	nd	6.07 ± 0.21	5.57 ± 0.10	nd	1.68 ± 0.12	33.7 ± 1.7
Influent #4	74.4 ± 0.9	nd	nd	nd	27.3 ± 2.8	nd	118 ± 3	64.4 ± 0.2
Effluent #4	54.8 ± 4.3	nd	nd	nd	nd	nd	5.15 ± 0.32	9.48 ± 0.76
Influent #5	101 ± 8	nd	nd	16.8 ± 1.1	7.63 ± 0.24	nd	nd	7.57 ± 0.77
Effluent #5	176 ± 10	nd	nd	24.7 ± 2.5	25.0 ± 0.5	nd	18.2 ± 0.4	15.8 ± 1.3
Influent #6	145 ± 13	nd	nd	9.15 ± 0.89	16.5 ± 0.8	nd	18.4 ± 0.0	41.0 ± 0.7
Effluent #6	114 ± 11	nd	nd	11.8 ± 0.9	1.80 ± 0.16	nd	13.8 ± 0.3	20.6 ± 0.5

When comparing ranges of concentrations with previously reported data in the literature, only GEM is found in comparable amounts in hospital wastewaters. The other compounds are found in the same minimal concentration ranges, but some articles measured heavily contaminated samples that can reach 100-fold concentrations or even higher. For example, IFO and CYC were reported as the prevalent cytostatics in hospital effluents from Catalonia (Spain) at concentrations of 86200 and 4720 ng L^{-1}, respectively [362,379]. However, 5-FU was not detected in any of the hospital wastewater from the present study. Despite the fact that this is one of the most consumed chemotherapy agents worldwide, consumption and prescription data for these pharmaceuticals were not available for the sites sampled and it could not be certain that it was administered in these hospitals. The absence of this compound could also be attributed to degradation or high metabolism rate to fluoro-beta-alanine [357,358,382]. Considering WWTPs, both influent and effluent quantifications are leading to concentration in the same range of literature reports, with the exception of CYC that was measured at up to 13000 ng L^{-1} in influent wastewater samples [379].

6.4 Conclusions

The present study aimed at evaluating a combination of on-line SPE – LC-MS methods to analyze some of the most consumed small polar cytostatics. The results of the different chromatographic column and analyte combinations provided a framework for future applications. 5-FU and dFdU were analyzed by HILIC-MS/MS while CYT, GEM, MTX, IFO, CYC, CAP were analyzed using RPLC-MS/MS (Luna Omega Polar C18 and Hypersil GOLD aQ). Both methods employed the same on-line SPE sorbent (hyper crosslinked hydroxylated polystyrene-divinylbenzene copolymer). The LOD performance was in the low ng L^{-1} range, which is comparable to previous studies with the further advantage of a short total analysis time and improved recovery. Matrix effects were generally moderate (≤12%) except for CAP (-46.7%). Quantitation with matrix-matched calibration resulted in suitable accuracy (78-111%) and precision (<13%). Depending on the list of analytes targeted in future studies, HPLC column options could be further tailored to maximize sensitivity for specific analytes. If focusing on cyclophosphamide and ifosfamide isomers only, Hypersil GOLD aQ would appear as the best option, while if the interest is on gemcitabine and methotrexate, the Hypersil GOLD Silica column could be selected.

When applying the methods to real wastewater, 6 out of the 8 targeted compounds were detected in hospital wastewater samples while 4 compounds were also detected in WWTP samples. The concentrations of some cytostatics ranged between low ng L^{-1} to hundreds of ng L^{-1}. Since conventional wastewater treatment plants are not efficient in terms of pharmaceuticals removal, these compounds are eventually discharged to receiving surface waters [391–393]. Some of the antineoplastics, such as 5-FU, can present adverse effects on aquatic species [394–397]. The occurrence of these compounds in Canadian surface waters is currently unknown. With small protocol adjustments, the developed analytical methods could be expanded to additional matrix types, including environmental surface waters with potentially larger injection loops.

Acknowledgments

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Supporting Information

Table 6-9. Structure and properties of the targeted anticancer drugs (data retrieved from ChemAxon Ltd).

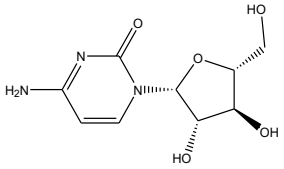
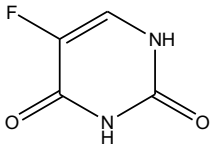
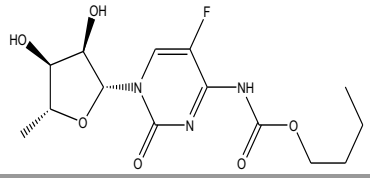
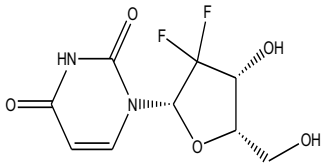
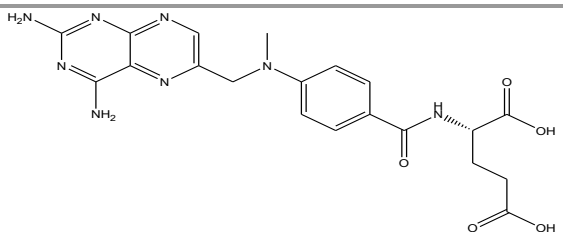
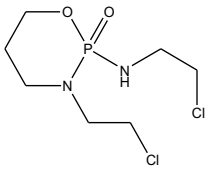
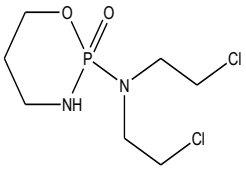
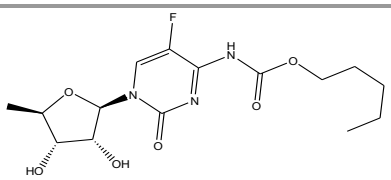
Compound	MW ($g\ mol^{-1}$)	pK_a	LogP	Structure
Cytarabine (CYT)	243.219	4.19, 12.55	-2.80	
5-Fluorouracile (5-FU)	130.078	7.18	-0.66	
Gemcitabine (GEM)	263.201	3.65, 11.52	-1.47	
2',2'-Difluorodeoxyuridine (dFdU)	264.185	9.89	-1.08	
Methotrexate (MTX)	454.447	2.80, 3.25	-0.24	
Ifosfamide (IFO)	261.08	14.64	0.10	
Cyclophosphamide (CYC)	261.08	13.48	0.10	
Capecitabine (CAP)	359.354	0.07, 8.63	0.77	

Table 6-10. Further details on hospital facilities, including approximate number of beds and whether presence of chemotherapy treatment administered.

Hospital Sample	Approx. No. Of beds	Chemotherapy treatment administered
#1	1400	Yes
#2	1300	Yes
#3	200	Yes
#4	300	Yes
#5	100	Yes
#6	500	Yes
#7	400	Yes
#8	600	Yes
#9	300	Yes
#10	N/A	No
#11	N/A	No

Table 6-11. Further details on wastewater treatment plants, including population served and average flowrate.

WWTP	Population	Average flowrate (m^3/day)
#1	40000	38000
#2	59000	44000
#3	280000	240000
#4	49000	36000
#5	65000	26000
#6	41000	30000

Table 6-12. Chromatographic gradient for the PEI column method (HILIC mode) at a flow rate of 500 µL min^{-1}.

Time / min.	H_2O + 1 mM AmAc / %	ACN / %
0	10	90
4	10	90
8	60	40
11	10	90
20	10	90

Table 6-13. Chromatographic gradient for the Luna column method (RPLC mode) at a flow rate of 500 µL min^{-1}.

Time / min.	H_2O + 10 mM AmFo / %	MeOH/ACN (50/50) / %
0	90	10
1.2	90	10
7.2	0	100
7.7	0	100
7.8	90	10
10	90	10

Table 6-14. Optimized mass spectrometer parameters for selected compounds and isotope-labeled internal standards.

Compound	Polarity	Precursor ions (m/z)	Product ions (m/z)	Collision Energy (V)	RF lens (V)
CYT	Positive	244.2	112.1	15	39
			95.1	39	
5-FU	Negative	129.2	42.2	20	49
			86.1	20	
5-FU-1C2N	Negative	132.3	44.2	19	48
5-BU	Negative	190.9	80.9	50	60
GEM	Positive	264.1	112.2	18	64
			95.0	38	
GEM-1C2N	Positive	267.2	115.1	18	58
dFdU	Negative	263.1	220.1	10	55
			111.2	10	
MTX	Positive	455.2	308.0	20	83
			175.0	39	
MTX-d3	Positive	458.3	311.1	20	78
IFO	Positive	261.1	154.1	23	67
			182.1	18	
IFO-d4	Positive	265.3	156.1	22	64
CYC	Positive	261.1	140.1	22	65
			142.1	21	
CYC-d4	Positive	265.2	140.0	22	35
CAP	Positive	360.2	244.1	11	46
			174.0	21	

Calculation of the different criteria for the overall appreciation score (Table 6-3):

Criterion for intensity (I):

This criterion is defined to take into account the conditions that allow high peak intensity (I). It is calculated as the ratio of the signal for each tested condition to the highest signal of all conditions. This value is calculated separately for each targeted analyte.

$$I = \frac{analyte\ signal\ under\ condition\ x}{best\ signal\ obtain\ of\ all\ conditions} \qquad \mathbf{Eq.\ 6-2}$$

Criterion for retention time (t_R):

This criterion is defined to take into account the conditions that allow efficient retention of targeted compounds (t_R). It is calculated as the ratio of the capacity factor (K') for each tested condition to the highest capacity factor of all conditions. This value is calculated separately for each targeted analyte.

$$\mathrm{t(R)} = \frac{\mathrm{K'}\ of\ analyte\ under\ condition\ x}{\mathrm{best\ K'\ obtain\ of\ all\ conditions}}\ with\ K' = \frac{t(analyte) - t(0)}{t(0)} \qquad \mathbf{Eq.\ 6-3}$$

Criterion for asymmetry ratio (AS):

This criterion is defined to take into account the conditions which leads to symmetrical peak. It is calculated as the asymmetry ratio (AS) for each tested condition. This value is calculated separately for each targeted analyte.

$$\mathrm{AS} = \frac{\mathrm{a}}{\mathrm{b}}\ with\ a < b \qquad \mathbf{Eq.\ 6-4}$$

In this formula, a and b are the distance from peak center (highest point) measured at 10% of maximum peak height and a is defined as the smallest distance so the criterion value is ranging from 0 to 1.

The different conditions have been applied several times (n=5) to obtain values (signal intensity, retention time, peak shape) for individual criterion. Each of these criteria results in a value between 0 and 1 and are added to obtain overall appreciation score:

$$Score = I + t(R) + AS \qquad \mathbf{Eq.\,6-5}$$

Calculation of the different criteria for the Derringer's desirability approach:

Criterion d_1:

The d_1 criterion is defined to take into account the conditions that allow the best precision. It is calculated as the No. of targeted analytes that meet the requirement of a RSD ≤ 10%, divided by the total number of analytes.

$$\mathrm{d1} = \frac{\#\text{ of analytes with RSD } \leq 10\%}{\text{total \# of analytes}} \qquad \mathbf{Eq.\,6-6}$$

Criterion d_2:

The d_2 criterion is defined to take into account the conditions that allow the best sensitivity. It is calculated as the mean of normalized area (each targeted analyte normalized to the maximum observed value between all the different conditions).

$$d2 = average\left(\frac{analyte\ signal\ area}{(analyte\ signal\ area)max}\right) \qquad \mathbf{Eq.\,6-7}$$

Criterion d_3:

The d_3 criterion is defined to take into account the conditions that allow the best recoveries. It is calculated as the mean of normalized area/volume ratio (each targeted analyte ratio normalized to the maximum area/volume observed value between all the different conditions).

$$d3 = average\left(\frac{\frac{analyte\ signal\ area}{injection\ volume}}{\left(\frac{analyte\ signal\ area}{injection\ volume}\right)max}\right) \qquad \mathbf{Eq.\,6-8}$$

Each of these criteria result in a value between 0 and 1. For each combination of sample volume and loading flow rate, the overall desirability D is derived as the geometric mean of d_i values[7].

$$D = \left(\prod_{i=1}^{n} d_i \right)^{1/n} \qquad \textbf{Eq. 6 – 1}$$

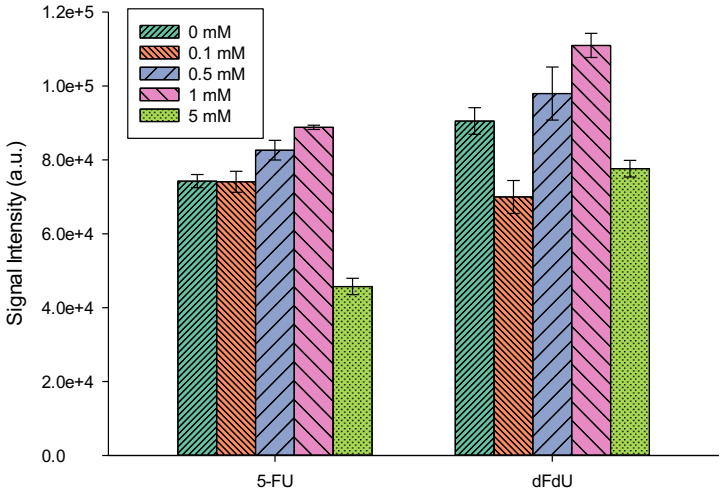


Figure 6-6. Effect of AmAC additive concentration on 5-FU and dFdU signal on a Hypersil GOLD PEI HILIC column. Each condition was tested at 100 ng L^{-1}. Error bars correspond to standard deviations (n = 5).

[7] E.A. Bekele, C.E.P. Annaratone, M.L.A.T.M. Hertog, B.M. Nicolai, A.H. Geeraerd, Multi-response optimization of the extraction and derivatization protocol of selected polar metabolites from apple fruit tissue for GC–MS analysis, Anal. Chim. Acta. 824 (2014) 42–56. https://doi.org/https://doi.org/10.1016/j.aca.2014.03.030.

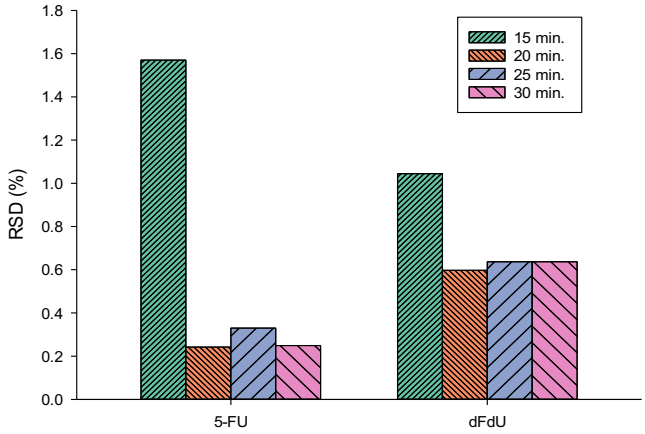


Figure 6-7. Stability of the HILIC method calculated as RSD (n = 5) of retention time at different total method times (15, 20, 25 and 30 min.), for 5-FU and dFdU, on a Hypersil GOLD PEI HILIC column. Each condition was tested at 100 ng L^{-1}.

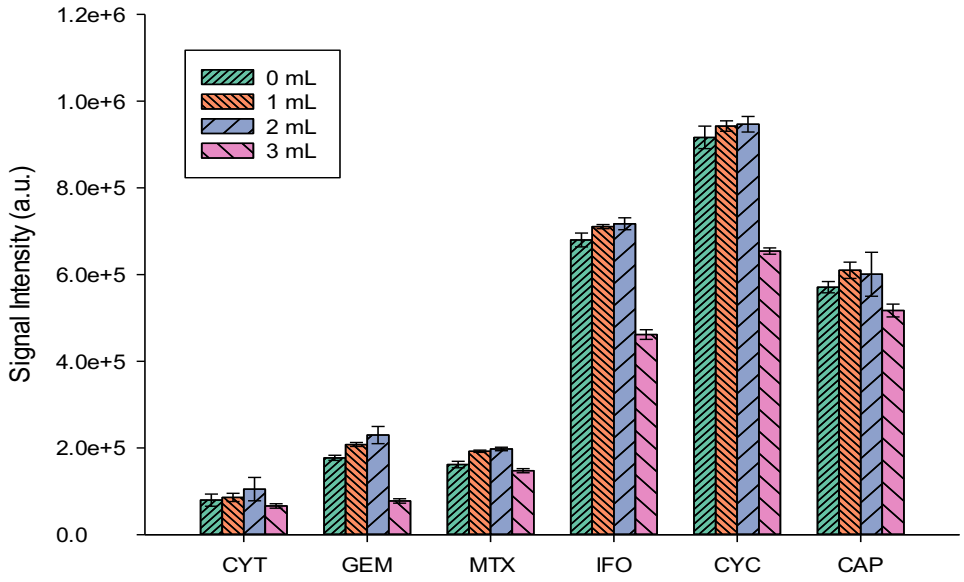


Figure 6-8. Influence of wash volume on analyte response, illustrated for CYT, GEM, MTX, IFO, CYC, and CAP on Isolute ENV+ when loading with 2 mL of sample. Each condition was tested at 100 ng L^{-1}. Error bars correspond to standard deviations (n = 3).

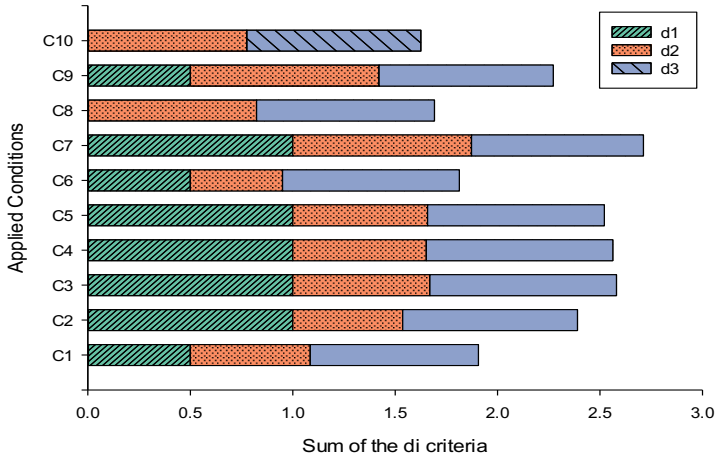


Figure 6-9. Visual representation of the di criteria used for calculation of overall desirability (D) for the on-line enrichment of 5-FU and dFdU using an experimental design and Derringer's desirability functions (C1-C5 = 1-mL injection volume, flow rates investigated at 0.25, 0.5, 1, 1.5, and 2 mL min^{-1}; C_6-C_{10} = 2-mL injection volume, flow rates investigated at 0.25, 0.5, 1, 1.5, and 2 mL min^{-1}). These conditions were tested using a wash volume of 1 mL and chromatographic separation on a Hypersil GOLD PEI HILIC column.

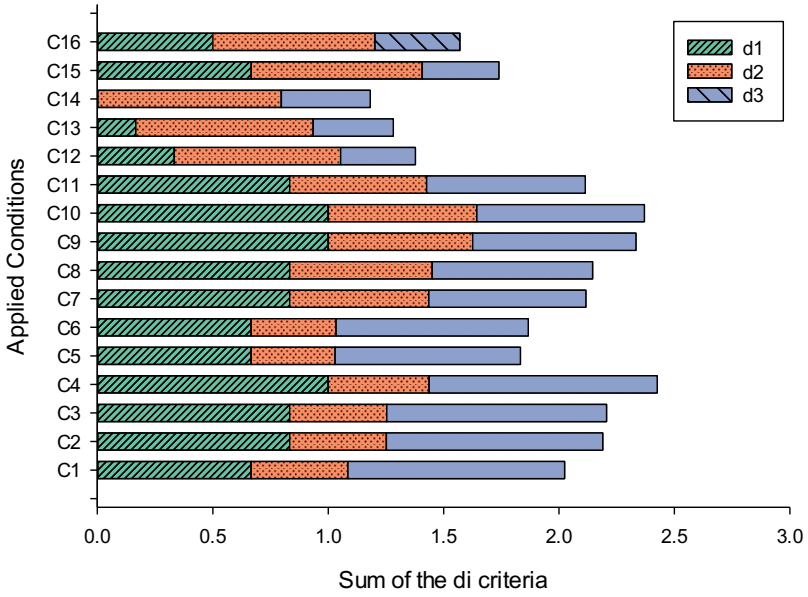


Figure 6-10. Visual representation of the di criteria used for calculation of overall desirability (D) for the on-line enrichment of CYT, GEM, MTX, IFO, CYC and CAP under experimental design to evaluate Derringer's desirability (C1-C6 = 1-mL injection volume, flow rates investigated at 0.5, 1, 1.5, 2, 2.5, 3 mL min^{-1}; C_7-C_{11} = 2-mL injection volume, flow rates investigated at 1, 1.5, 2, 2.5, 3 mL min^{-1}; C_{12}-C_{16} = 3-mL injection volume, flow rates investigated at 1, 1.5, 2, 2.5, 3 mL min^{-1}). These conditions were tested with a wash volume of 1 mL and chromatographic separation on a Luna Omega Polar C18 column.

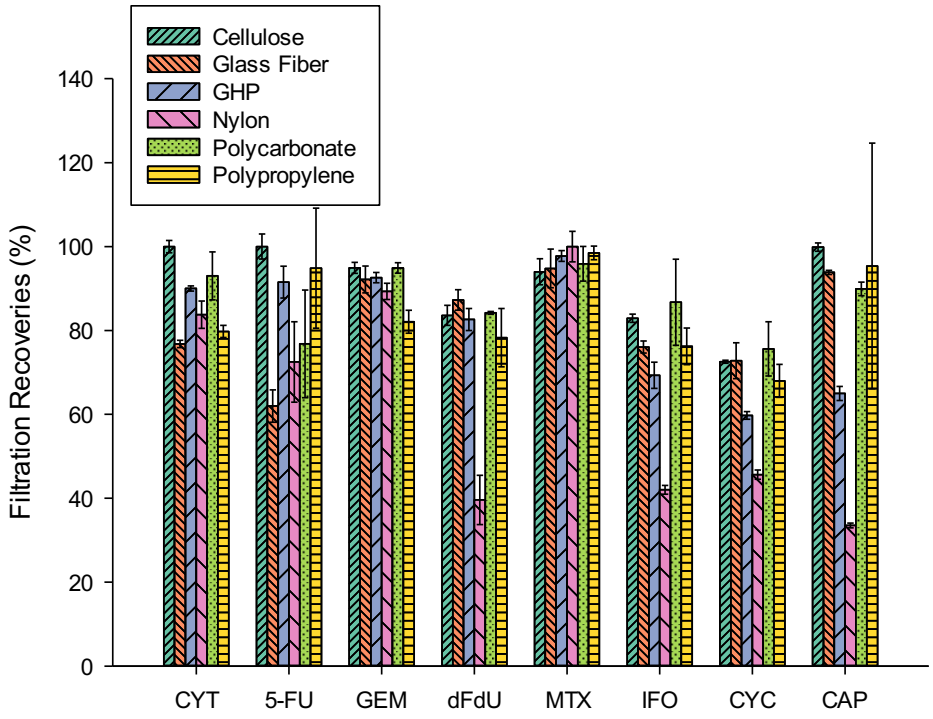


Figure 6-11. Percentage recoveries of filtration on different filter membrane material. Each compound was spiked to a final concentration of 100 ng L^{-1} in HPLC water sample. Error bars correspond to standard deviations (n = 3).

Table 6-15. Hospital wastewater matrix effect on the targeted compounds.

Compound	CYT	5-FU	GEM	dFdU	MTX	IFO	CYC	CAP
Matrix Effect (%)	+12.2	+5.83	-6.05	-14.9	+3.96	-1.18	+2.13	-46.7

Table 6-16. Matrix effect (Influent vs Effluent wastewater from different treatment plants).

Compound	CYT	5-FU	GEM	dFdU	MTX	IFO	CYC	CAP
Matrix Effect (%)	+11.4	+3.49	+0.35	-14.7	+4.97	+3.33	+9.73	+17.4

7 Suivi de la pollution pharmaceutique dans les eaux de surface du fleuve Saint-Laurent et de ses principaux affluents

Article soumis dans *Science of the Total Environment* (août 2023).

“Tracking down pharmaceutical pollution in surface waters of the St. Lawrence River and its major tributaries”

Auteurs: Marc-Antoine Vaudreuil, Gabriel Munoz, Sung Vo Duy, Sébastien Sauvé

Description : Cet article décrit l’application de méthodes validées faisant appel à la SPE couplée à la LC-MS/MS pour l’analyse de multiples classes de composés pharmaceutiques dans des échantillons d’eaux de surface et de sédiments. De larges campagnes d’échantillonnage sur le fleuve Saint-Laurent entre le lac Ontario et Cacouna ont permis de déterminer les sources principales de contamination pharmaceutique et leur impact dans cette importante rivière ainsi que dans plus de 50 de ses tributaires sur une période de cinq ans.

Contribution : J’ai effectué la conception du projet, le développement de méthodes, la collecte et la préparation des échantillons, le traitement de données, ainsi que la rédaction de l’article.

Coauteurs : Gabriel Munoz et Sung Vo Duy m’ont aidé à la conception du projet ainsi qu’à la révision de l’article.

Directeur : Sébastien Sauvé m’a aidé à la conception du projet ainsi qu’à la révision de l’article.

ABSTRACT

A reconnaissance survey was undertaken to evaluate the occurrence and risks of 27 pharmaceuticals and metabolites in the St. Lawrence watershed. Surface water samples were collected over a five-year period (2017-2021) along a 700-km reach of the St. Lawrence River as well as 55 tributary rivers (overall N = 406 samples). Additionally, depth water samples and sediments were collected near a major wastewater effluent. Caffeine, diclofenac, and venlafaxine were the most recurrent substances (detection rates >80%), and extremely high levels were found near a municipal effluent (e.g., ibuprofen (860 ng/L), hydroxyibuprofen (1800 ng/L) and caffeine (7200 ng/L)). Geographical mapping and statistical analyses indicated that the St. Lawrence River water mass after the Montreal City effluent was significantly more contaminated than the other water masses, and that contamination could extend up to 70 km further downstream. This phenomenon was repeatedly observed over the five years of sampling, confirming that this is not a random trend. A slight increase in contamination was also observed near Quebec City, but concentrations rapidly declined in the estuarine transition zone. Tributaries with the highest pharmaceutical levels (ΣPharmas ~ 400-900 ng/L) included the Mascouche, Saint-Regis, and Bertrand rivers, all located in the densely populated Greater Montreal area. When flowrate was factored in, the top five tributaries in terms of mass load (ΣPharmas ~ 200-2000 kg/year) were the Des Prairies, Saint-François, Richelieu, Ottawa, and Yamaska rivers. All samples met the Canadian Water Quality Guideline for carbamazepine. Despite the large dilution effect of the St. Lawrence River, a risk quotient approach based on freshwater PNEC values suggested that four compounds (caffeine, carbamazepine, diclofenac, and ibuprofen) could present intermediate to high risks for aquatic organisms in terms of chronic exposure.

Keywords

Pharmaceutical pollution; Canada; Municipal effluents; St. Lawrence River and tributaries; Mass loads; Risk quotients.

7.1 Introduction

As the world's population grows and healthcare systems improve, the consumption of pharmaceuticals continues to rise [398,399]. The use of pharmaceutical compounds results in the release of unaltered drugs and metabolites to urban wastewater via excretion or inappropriate disposal of drugs [400,401]. These compounds are then partially eliminated by wastewater treatment plants, but their removal varies depending on the type of treatment [25,161]. Classical wastewater treatment technologies were not developed to remove these specific organic pollutants and elimination efficiency is sometimes low or null for pharmaceuticals, resulting in the contamination of receiving surface waters [307,402]. Contamination of water resources by pharmaceuticals is of concern because of the antimicrobial resistance issue. Pharmaceuticals and hormones can also lead to adverse effects on aquatic organisms, even at trace levels. Effects depend on different factors such as chronic or acute toxicity, concentration, bioavailability, and persistence [403,404]. Traces of pharmaceuticals have the potential to severely disrupt ecosystems by lowering the life expectancy of plankton, thereby affecting the food chain, or by causing the collapse of fish populations after acute endocrine disruption or induced feminization [405–407]. Higher trophic level organisms are also at risk, as illustrated by the collapse of Asian vulture populations after exposure to diclofenac [408].

Pharmaceutical consumption in Canada has been increasing by over 35% in the last decade, with approximately 13% associated with hospital needs. Drug manufacturing is a lucrative production sector with nearly 30 billion dollars in sales in Canada for the year 2019 [409]. Despite the vast amount of pharmaceuticals consumed each year, there are very few policies to limit the various pathways through which these compounds contribute to environmental pollution. Although governmental agencies emit regulations on the amounts of different classes of pollutants (e.g., biological and chemical pollutants) that can be released via wastewater, no guideline is currently available for pharmaceuticals and many other classes of emerging contaminants. To protect freshwater aquatic life, the Canadian Council of Ministers of the Environment (CCME) has established a Canadian Water Quality Guideline for the anticonvulsant carbamazepine at 10 µg/L in Canadian surface waters; most other pharmaceuticals do not have available Canadian or provincial guidelines [38,410,411]. As part of the Water Framework Directive, the European Union has established environmental quality standards for several priority substances, including a few pharmaceuticals (e.g., carbamazepine, diclofenac, ibuprofen, and steroid hormones) [412]. For many pharmaceuticals, the lack of sufficient environmental monitoring data and ecotoxicity studies impedes the derivation of guidelines for aquatic ecosystems.

Monitoring studies of pharmaceuticals have mainly addressed their occurrence in wastewater influents/effluents and small- and medium-scale rivers impacted by municipal effluents, with relatively fewer studies for large-scale rivers and lakes [262,413]. Because some of the world's largest freshwater hydrosystems are located in densely urbanized areas, the dilution effect may not mitigate the risk posed by pharmaceutically-active compounds. For instance, Blair et al. (2013) reported intermediate to high ecological risks for 14 pharmaceuticals downgradient a municipal effluent discharging into one of the Laurentian Great Lakes (Lake Michigan, WI, USA). Loos et al. (2017) reported diclofenac concentrations of ~10 ng/L in surface waters collected as part of an international survey along the Danube River (European Union), values that would be higher than the proposed E.U. quality standard to prevent the secondary poisoning of top predators.

Previous studies conducted in the St. Lawrence River (Canada), one of the largest rivers in North America, also suggested variable water quality along its course. According to a longitudinal survey led in 2012-2014 by Québec's Ministry of the Environment, the water quality (based on a bacteriological/physicochemical index) was good upstream from Montreal, but deteriorated downstream due to fecal contamination from several WWTPs [414]. In particular, discharges of wastewaters from the Communauté Métropolitaine de Montréal represent a combined input of ~3-5 million m3 per day – most of which originates from undisinfected wastewaters from the Montreal WWTP [37,415]. The release of such a large volume of untreated wastewater into the river has been linked with ecotoxicological disturbances [37]. Fishes (Northern pike E. lucius) collected near Ilet Vert (4 km downstream from the Montreal effluent outflow) had increased vitellogenin levels, while mussels (E. complanata) caged 7 km downstream from the municipal effluent outflow had the proportion of females increase from 41 to 67% after one year of field exposure [37,416,417]. Limited information is available as regards the longitudinal distribution of endocrine-disrupting chemicals, such as some pharmaceutical contaminants, along the St. Lawrence River and its tributary rivers, representing an important knowledge gap to bridge.

Here, we aimed to investigate the contamination sources of pharmaceuticals in the St. Lawrence River and more than 50 of its tributary rivers, for the first time. Five sampling campaigns were performed between 2017 and 2021 to document longitudinal variations along a 700-km gradient of the St. Lawrence River and estuary, with multiple transects to evaluate cross-sectional variations in the distinct water masses that compose this complex hydrosystem. We also analyzed depth water and sediment samples near the rejection point of a major wastewater treatment plant upstream and downstream the Montreal Island to refine spatial trends. Statistical analyses were

applied to evaluate interannual differences, relative levels of contamination between distinct water masses, and identify clusters of sites having similar contamination patterns. A preliminary risk assessment was also performed by comparing measured concentrations to available quality guidelines and predicted no effect concentrations. This work provides a valuable database to fill the knowledge gap on pharmaceutical pollution in the St. Lawrence River. The data could also be of great interest for the future elaboration of quality criteria and the implementation of improved wastewater treatment technologies.

7.2 Materials and methods

7.2.1 Standards and reagents

Table 7-1 lists the 27 targeted analytes and their corresponding pharmaceutical classes. The selection of targeted analytes was based on lists of consumed pharmaceuticals (with or without prescription) in Canada and Quebec [418] and available monitoring data on pharmaceuticals and metabolites found in municipal effluents and receiving surface waters in Eastern Canada [7,126,419,420]. For instance, amoxicillin, carbamazepine, fluoxetine, ibuprofen, methotrexate, steroid hormones (estradiol, levonorgestrel, medroxyprogesterone), and sulfamethoxazole are all on the list of essential drugs sold in Canada, with combined national sales of CAD $600 million in 2015 [418], while hydromorphone is within the top ten prescription drugs in Québec [421]. We note that several of the target pharmaceuticals are also on the watch list of the EU for surface water monitoring. In addition, caffeine was enlisted as a marker of anthropic pollution; high concentrations in surface water might reflect combined sewer overflows or direct discharge of municipal effluents to the environment [422,423]. Details on the physicochemical properties of selected drugs can be found in the Supporting Information (**Table 7-4**) [424]. Native analyte standards were all purchased from Sigma-Aldrich while nine isotope-labelled internal standards were bought from Santa Cruz Biotechnology (purity >97%). Native analyte standards and internal standards were kept in the freezer (-20°C) along with mix solutions. Dilutions of mix solutions used for the preparation of calibration curves and quality control solutions (QC) were freshly prepared before individual runs to ensure standard stability over time. Solvents such as water and MeOH were all HPLC grade and were purchased from Thermo Fisher Scientific, while formic acid (FA, purity ≥ 98 %) and ammonium fluoride (NH4F, purity ≥ 98 %) were purchased from Sigma-Aldrich.

Table 7-1. Targeted analytes presented by pharmaceutical class.

Pharmaceutical Class	Compound Name	Abbreviation
Analgesic	Ibuprofen	IBU
	2-Hydroxyibuprofen	OH-IBU
	Hydromorphone	HMOR
	Diclofenac	DCF
	4-Hydroxydiclofenac	OH-DCF
Antibiotic	Sulfamethoxazole	SMX
	4-Nitrososulfamethoxazole	NO-SMX
	Amoxicillin	AMOX
	Clarithromycin	CLA
Chemotherapy	Methotrexate	MTX
Hormone	Estradiol	E2
	Estrone	E1
	Estriol	E3
	Ethinylestradiol	EE2
	Levonorgestrel	LEVO
	Norethindrone	NOR
	Progesterone	PROG
	Medroxyprogesterone	MPROG
	Testosterone	TESTO
Anticonvulsant	Carbamazepine	CBZ
	10,11-Dihydroxy-10,11-dihydrocarbamazepine	2OH-CBZ
Antidepressant	Venlafaxine	VEN
	O-Desmethylvenlafaxine	DVEN
	Fluoxetine	FLU
	Norfluoxetine	NORF
Stimulant	Caffeine	CAF
Beta Blocker	Acebutolol	ACE

7.2.2 Sample collections

Surface water samples of the St. Lawrence River were sampled with a 20 L Go-Flo sampler from General Oceanics (Miami, FL, U.S.A.) at a 1 m depth and transferred to 1 L amber glass bottles, during onboard cruises on the Lampsilis Research Vessel (R/V). Bottom water samples of the St. Lawrence River were sampled by a professional scuba diver at a depth of approx. 10 m. The surface sample bottles contained sodium omadine salt to ensure pharmaceutical stability and minimal degradation during transport and storage [130,425]. In the case of the depth samples, the omadine salt was added directly upon arrival at the laboratory. Some sediment samples were collected from the riverbed at the same time as the depth water samples by the scuba diver by filling a 50 mL amber glass vial directly under the corresponding water sample. The excess water was removed, and the samples were brought back to the lab and frozen prior to freeze-drying. During the long uninterrupted sampling campaign (several consecutive days) onboard the Lampsilis R/V, the surface water samples were kept in a refrigerator until shipment to the laboratory.

To better assess the pharmaceutical contamination in the St. Lawrence River, a transect approach was used for sampling the different water masses that flow in parallel with limited mixing. A graphical representation of the St. Lawrence River sampling sites is shown in **Figure 7-1**. The water masses present an array of variations in terms of dissolved organic matter, total nitrogen and nitrogen species, phytoplankton communities, or suspended particulate matter concentrations. There are also distinctive colors and water flow between these different water masses that have a potential impact on water quality and contaminant dispersion along the St. Lawrence River [240,426].

The Lampsilis R/V made cross-sections at the pre-set sampling points along the freshwater section of the river to evaluate transversal and longitudinal spatial variations. With a focus on recurring sites around the Montreal effluent rejection point, a large geographic portion of the river was sampled between its origin in the Great Lakes (Lake Ontario) to the Estuarine Transition Zone (ETZ) after Quebec City. This large-scale sampling was performed five times between the summers of 2017 and 2021. Sites were classified among six different water masses, listed as follows: The Great Lakes (GL) water mass that is upstream from the Montreal effluent and surrounded by the Ottawa River (OR) water mass on the north shore and the South Tributaries (ST) water mass on the south shore. The Central Water Mass (CWM) is the continuity of the GL water mass that is suspected to be heavily impacted by the presence of the rejection point of Montreal's effluents. The CWM flows alongside the ST and OR water masses until they are substantially mixed after the Lake St. Pierre area and form a mostly homogenous water mass named the Mixed Water Mass (MWM) in the present study. A final distinct water mass is reached in the ETZ past Quebec City where freshwater starts to mix with brackish water with marked changes in terms of salinity, temperature, turbidity, and nutrient concentrations [427]. Sampling of this region was performed against the tidal direction to avoid sampling the body of water moving in the same direction as the sampling vessel. Sampling was not conducted in the maritime estuary because marine water might exert a different matrix effect and the impact on method performance was not evaluated for this type of samples. A previous study investigated emerging contaminants such as pesticides, pharmaceuticals, and perfluoroalkyl substances in the maritime estuary and Gulf of St. Lawrence [419].

In addition to the St. Lawrence River samples, some of its major tributaries in terms of waterflow and some potentially polluted urban streams were sampled to assess additional sources of pharmaceutical pollution. These destinations could be reached by car and each river was

sampled from a bridge or location near the river mouth. These grab samples were transported in a cooler with ice before filtration and storage in the lab prior to analysis.

A total of 261 (78 sites) St. Lawrence River surface water samples and 145 (55 sites) tributary surface water samples were collected from the field sampling efforts (overall N = 406 surface water samples). Additionally, 15 depth water and sediments samples were collected. A list of the St. Lawrence River and tributary rivers sampling sites with coordinates, average river flow and corresponding sampling years is available in the Supporting Information (SI, **Tables 7-5 and 7-6**).

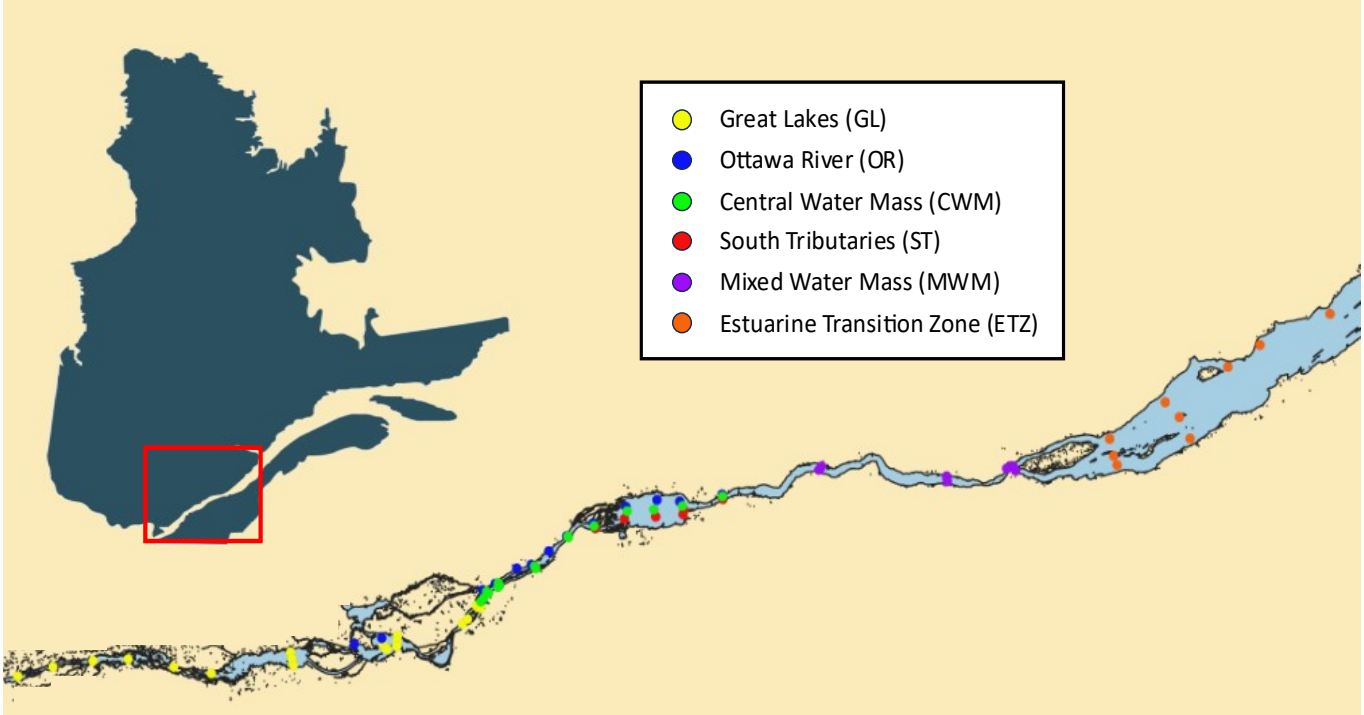


Figure 7-1. Spatial distribution of Lampsilis surveys sampling sites on the St. Lawrence River in Quebec, Canada.

7.2.3 Sample preparation and chemical analysis

The only pretreatment steps prior to the analysis of water samples are sample filtration and the addition of isotope-labelled internal standards. The selection of an appropriate filter material was based on a screening of different filtration membranes [424]. Under the retained conditions, filtration was done by percolation through a glass fiber membrane (0.3 µm, 25 mm, Sterlitech). An exact volume of 5 mL of the filtrate was transferred to an amber glass vial and spiked with internal standards (1 µg/L) prior to injection of 2 mL onto an on-line solid phase extraction column coupled

to ultra-high-performance liquid chromatography tandem mass spectrometry (on-line SPE – UHPLC-MS/MS).

Analysis of suspended solids was based on an optimized protocol [424]. Filters were extracted by two ultrasonic extraction cycles (20 min each) in 5 mL MeOH/ACN (3/1 v/v) + 0.1% FA. After centrifugation (5000 RPM, 5 min) and N_2 concentration, the water-diluted extracts were purified by off-line SPE on a Strata-X cartridge (Phenomenex, 200 mg, 6 mL). The cartridges were rinsed with water (2 x 3 mL) and dried under vacuum (30 min) after sample loading. Elution was done with MeOH (2 x 3 mL), and the purified extract was evaporated to dryness (N_2, 40 °C) and reconstituted in 200 µL of water/MeOH (90/10) prior UHPLC-MS/MS analysis (50 µL injection volume).

Details on instrumental methods for water and solid samples are provided in SI (**Tables 7-7 and 7-8**). On-line SPE preconcentration was done on a Hypersil Gold aQ column (20 x 2.1 mm, 12 µm, Thermo Scientific), and chromatographic separation with a Hypersil Gold column (50 x 2.1 mm, 1.9 µm, Thermo Scientific). Analyses were performed on a TSQ Quantiva triple quadrupole mass spectrometer system (Thermo Scientific) in selected reaction monitoring mode (SRM) with electrospray ionization (ESI). Specific details about compound-dependent MS/MS acquisition parameters and ionization source parameters are provided in the Supporting Information (**Table 7-9**) [424].

7.2.4 Quality assurance and quality control (QA/QC)

A mix of all the targeted analytes was used to freshly prepare calibration curves for each analytical run. To assess calibration ranges and determination coefficients (R^2), calibration curves were generated in matrix-matched surface water: a composite matrix made of aliquots of different freshwater river water samples was spiked with increasing levels of native pharmaceuticals (tested concentration levels: unspiked, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50, 100, 500, 1000, 5000 and 10 000 ng/L), while surrogate isotope-labelled internal standards were kept constant. This calibration curve was used to derive pharmaceutical concentrations in field samples (also added with internal standards).

As part of a separate experiment, we checked whether the composite matrix-matched curve was efficient at compensating matrix effects for different water samples. For this test, we ran standard addition calibration curves (constructed in several individual surface water samples) and compared the slopes to that of the composite curve to derive the *residual* matrix effects. For

information purposes, we also evaluated the *absolute* matrix effects by comparing the sensitivity (absolute response) of the calibration curve in the surface water matrix to the one in neat solvent.

After running the initial calibration, quality control solutions (QC) of spiked analytes to HPLC water were also included in each run to monitor the instrumental accuracy during long LC-MS/MS batch sequences (continued calibration verification (CCV, 500 ng/L) samples, analyzed after every 10 injections); the accuracy of CCV samples was required to fall within 70-130%. Before each run and at the start and end of the calibration curve, injection blanks (HPLC water) were also injected to confirm that there was no carryover.

Limits of detection (LOD) and limits of quantification (LOQ) were derived by injecting a low-concentration mix solution and determining signal to noise ratios (S/N) of 3 and 10 respectively.

Analytical validation also included the evaluation of method recoveries, accuracy, and precision in matrix-matched surface water fortified at two levels with the target pharmaceuticals (QC_1 = 20 ng/L and QC_2 = 5000 ng/L). Intraday precision and interday precision were calculated as the relative standard deviation (RSD) of replicate analyses performed on the same day (n = 5) and on three distinct days (n = 15), respectively. Method performance is presented in SI (**Tables 7-10 and 7-11**) and was found to reach US EPA methods performance criteria [277].

Different types of blanks were included during each sampling campaign to evaluate potential contamination occurring at different sampling steps. Field/transport blanks were obtained by filling a bottle with HPLC water onsite during a sampling event, while field sampler blanks were obtained by filling the Go-Flo sampling kit with HPLC water and transferring it to a container bottle while onboard the *Lampsilis* R/V. No pharmaceutical compounds were detected in such blanks.

Some duplicate bottles of field surface water samples were collected throughout sampling campaigns to measure variation that may occur during the sampling of two consecutive Go-Flo bottles at the same site. During the analytical run, some of the samples were also prepared and analyzed in triplicate to evaluate the analytical method precision for unspiked field samples.

7.2.5 Statistical analyses and GIS

The R software version 4.2.2 was used to conduct statistical analyses [428]. Statistical significance was set at a p-value of <0.05. Paired Wilcoxon's signed rank tests were used to test for differences between subsurface and bottom-water concentrations of pharmaceuticals (paired surface/depth data by geographical location). Interannual differences were tested considering the

sum of detected pharmaceuticals between years, also using paired Wilcoxon's signed rank tests. Differences between water masses within the St. Lawrence River were tested by comparing the overall means between groups (CWM, n = 73; ETZ, n = 17; GL, n = 55; MWM, n = 35; OR, n = 52; ST, n = 29), using a Kruskal-Wallis rank sum test; a pairwise comparison between groups was then performed using Wilcoxon rank sum tests. Boxplots were plotted using the ggplot2 and ggpubr R packages, while hierarchical clustering (cluster dendrogram per year) was performed using the ade4 R package. Quantum GIS (version 3.26.2 "Buenos Aires") was used as a geographic information system and base maps were retrieved from the *Ministère des Ressources naturelles et des Forêts* (MRNF) geographic database [429].

7.2.6 Risk quotient approach

Ecological risks to freshwater aquatic organisms were evaluated using a risk quotient (RQ) approach [413]. Predicted no effect concentrations (PNECs) of pharmaceuticals were found using the NORMAN Ecotoxicology Database (lowest PNECs based on experimental ecotoxicity data or QSAR predictions in case of insufficient empirical endpoints [https://www.norman-network.com/nds/ecotox/lowestPnecsIndex.php], [86]) and available literature data [87–92,94–99]. The RQ was then derived as the ratio between the measured environmental concentration (MEC) and the lowest PNEC (SI **Table 7-12**). Numerical RQ values can be interpreted as low risk (RQ <0.1), medium risk (0.1≤ RQ <1) and high risk (RQ ≥1) [413].[8]

7.3 Method performance and analytical validation in surface water matrix

7.3.1 Linearity and detection limits

The surface water being a relatively clean matrix, calibration curves are expected to be highly linear with the use of the isotope-labelled internal standards. Determination coefficients (R^2) of the targeted pharmaceutical were between 0.9937 and 1.0000, with no compounds under the 0.9900 performance objective (SI **Table 7-10**). Suitable linearity was observed up to 10 µg/L except for HMOR showing a signal saturation or loss of linearity at concentrations higher than 5 µg/L. This range was selected to reflect the concentrations that can be found in contaminated surface water

[8] The choice of RQ thresholds was based on previous pharmaceutical pollutants risk assessment studies [89,413,525–527].

close to wastewater rejection points. LODs were in the low to sub ng/L range with values between 0.05 and 5 ng/L and are deemed sufficient for river samples of regions with relatively low anthropogenic impacts [138,430–432].

7.3.2 Accuracy and precision of matrix spikes

Since this work proposes method application to different surface water samples, this matrix was submitted for validation to evaluate the meeting of performance requirements. Two spike levels were evaluated since pharmaceuticals may be found in a broad range of concentrations in real samples. The low spike level (QC1) was evaluated at 20 ng/L, while QC2 was set at 5000 ng/L since some compounds can be found in the µg/L range in surface waters near wastewater reject points. Recoveries all fell between 70 and 89 % at QC1 (mean = 78 %) and between 70 and 108 % at QC2 (mean = 88 %), except for NO-SMX (QC1 = 63 % and QC2 = 65 %) (SI **Table 7-11**).

Accuracy values ranged from 71 to 125 % at QC1 (mean = 102%) and from 92 to 116 % at QC2 (mean = 101%). The maximum RSD values were 8.8 % (QC1, mean 4.4 %) and 9.3 % (QC2, mean = 3.0 %) for intraday precision and 20 % (QC1, mean 8.6 %) and 15 % (QC2, mean = 7.0%) for interday precision. Apart from NO-SMX, all targeted analytes met US EPA precision requirements for surface water.

7.3.3 Matrix effects and quantification procedure

When comparing the absolute sensitivity in pure solvent (HPLC water) and in surface water matrix (see **Table 7-13** in SI), significant absolute matrix effects were observed ranging from signal suppression (-87%) to signal enhancement (+60%) with an overall mean absolute deviation of 21%. This effect was largely reduced by the matrix-matched approach with residual matrix effects ranging from -13% to +20% (mean in absolute terms = 5.3%), which is within the expected thresholds of ±20% or ±30% [277,279]. Even better correction could be achieved in the future by using additional isotope-labelled internal standards for those compounds that are more liable to matrix effects. The matrix effect assessment confirmed that a matrix-matched calibration in surface water matrix (with isotope-labelled internal standard correction) was sufficient to compensate matrix effects.

7.4 Reconnaissance survey of pharmaceuticals on the St. Lawrence watershed

7.4.1 Overall detection frequencies

Of 27 target pharmaceuticals, 16 were detected at least once in surface water samples from the Lampsilis and tributary survey (overall N = 406). Nearly all samples (99.3%) were positive with at least one pharmaceutical compound being detected (up to 14 compounds detected simultaneously within a single sample). Compounds of high detection frequencies and maximum concentrations above 50 ng/L included caffeine (detected in 347/406 samples, or a detection rate of 85%; max = 7200 ng/L), venlafaxine (81%; max = 97 ng/L), diclofenac (78%; max = 58 ng/L), carbamazepine (72%; max = 233 ng/L), hydroxyibuprofen (57%; max = 2700 ng/L), ibuprofen (66%; max = 860 ng/L), and O-desmethylvenlafaxine (58%; 430 ng/L). Other studies reported high detection frequencies of these pharmaceuticals in impacted rivers, such as in the UK and Japan [433,434]. The same drugs could be measured at much higher concentrations in Nigeria and some South American countries where environmental pollution was attributed to fast urbanization combined with a lack of efficient wastewater treatment and environmental policies [55,435].

Table 7-2. Occurrence of targeted pharmaceuticals, including detection frequencies (% of data above the LOD) and concentration ranges (ng/L, min-max of detected data) in surface water samples of the St. Lawrence River and tributary rivers. Individual analyte concentrations per site and sampling year are provided in SI **Tables 7-16** to **7-24.**

	St. Lawrence River			Tributary rivers		
	Detection frequency (%)	Concentration range (ng/L)	Mean of quantified concentrations (ng/L)	Detection frequency (%)	Concentration range (ng/L)	Mean of quantified concentrations (ng/L)
IBU	66	3.6 – 860	27	65	3.3 - 84	14
OH-IBU	39	3.6 – 1 800	290	-	-	-
HMOR	8	17 – 110	53	1	32	32
DCF	84	0.16 – 58	3.0	69	0.24 - 21	3.0
OH-DCF	43	0.33 – 10	1.0	25	0.33 - 2,1	1.0
SMX	> 1	27	27	3	13 - 350	130
CLA	24	0.13 – 25	1.6	44	0.34 – 7.8	1.1
MTX	12	0.39 – 2.6	1.3	1	4.2	4.2
E2	-	-	-	1	<LOQ	<LOQ
E1	15	0.36 – 9.7	1.9	4	3.2 – 6.5	5.0
CBZ	72	0.84 – 85	4.5	72	1.0 - 230	13
2OH-CBZ	17	4.1 – 483	49	33	31 - 450	110
VEN	85	1.2 – 97	5.5	75	1.7 - 62	12
DVEN	57	3.3 – 270	11	60	3.5 - 430	37
FLU	2	<LOQ	<LOQ	1	<LOQ	<LOQ
CAF	93	12 – 7 200	210	73	3.7 - 720	110
ACE	78	0.34 – 15	1.4	75	0.35 - 14	1.8

A few compounds were marginally detected in sediments (i.e., CAF, CBZ, E2, E1, and PROG), but their concentrations remained below the method LOQ. Thus, the remainder of this section will be focused on trends in river water. A larger number of sediments collected in different sections of the river with lesser current might have led to finding samples with higher organic content, which have a higher potential for organic pollutant adsorption [131,312].

7.4.2 Pharmaceutical contamination in the St. Lawrence River

The St. Lawrence River originates from the Great Lakes and is fed by different tributaries along its course sometimes creating distinct water masses that flow alongside each other with minimal lateral mixing [426,436,437]. For this reason, samples were taken near south/north shores and at the center of the river. With the central water mass being mostly composed of blue-green waters

originating from the Great Lakes and the influence of Montreal WWTP outfall (also within the central water mass), this portion of the river was expected to be the most contaminated [240,436]. To verify this assumption and evaluate the possible influence of other major conurbations along the St. Lawrence River, cross-sectional trends were evaluated along a 700-km longitudinal gradient including the entire fluvial section (from Kingston to Québec City) and the estuarine transition zone (Orléans Island to Cacouna).

Several pharmaceuticals were ubiquitously detected along the St. Lawrence River (**Table 7-2**). For instance, DCF, VEN, and CAF had detection rates greater than 80%. Despite the high dilution imparted by the St. Lawrence (mean river discharge of 8000 m^3/s at Sorel-Tracy in the summer), a few compounds occasionally surpassed µg/L levels (i.e., CAF and OH-IBU). Previous studies have reported even higher concentrations of analgesics and CAF in surface water near rejection points of WWTPs in heavily populated areas of Europe, Asia, or America [103,438–443]. Metabolites were typically found in fewer samples but most of them were quantified at higher concentrations than their parent molecule, consistent with previous studies [153,158].

7.4.2.1 Spatial variations within the St. Lawrence

A Kruskal-Wallis rank sum test indicated significant differences between water masses in terms of summed pharmaceutical (ΣPharmas) levels (p-value = 5.2e-8). Based on Wilcoxon's rank sum tests (SI **Table 7-14**), samples collected within the CWM (n = 73) had the highest ΣPharmas (mean 771 ng/L, median 247 ng/L, maximum 10 600 ng/L) (see **Figure 7-2**). CWM samples were also statistically higher than all other water masses except for MWM samples (**Figure 7-2**). We also concluded that MWM (mean 263 ng/L) > ST (118 ng/L) (p-value = 0.011), MWM (263 ng/L) > GL (152 ng/L) (p-value = 0.0023) and ST ~ GL (p-value = 0.35).

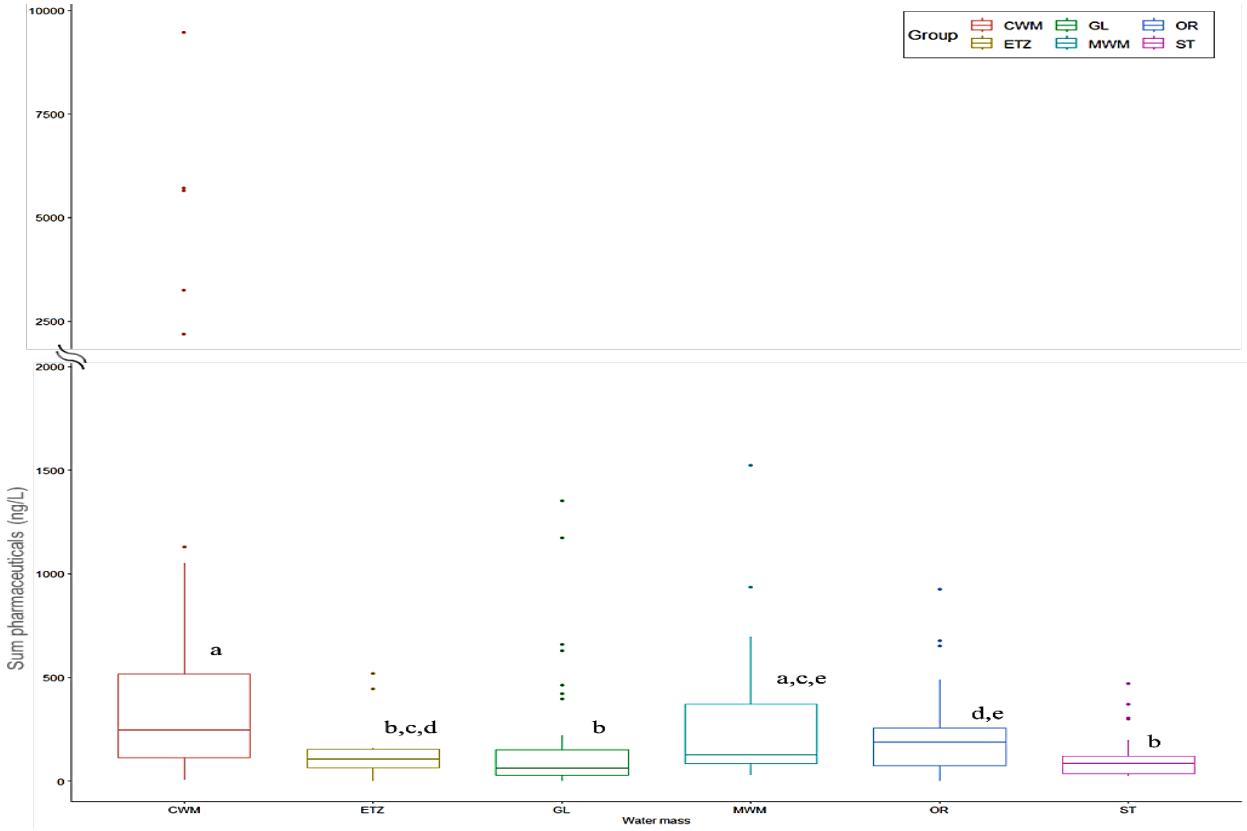


Figure 7-2. Boxplot showing the data distribution of summed pharmaceuticals (ΣPharmas, ng/L) between the different water masses (CWM: Central Water Mass; GL: Great Lakes (upstream Montreal); OR: North Shore / Ottawa River; ETZ: Estuarine Transition Zone; MWM: Mixed Water Mass; ST: South Shore). Note that a vertical axis scale break is applied at 2000 ng/L for better visualization. Statistical differences ($p<0.05$) between groups (water masses) are noted in letters (a,b,c,d,e).

A mapping of the pharmaceuticals in the St. Lawrence River is presented in **Figure 7-3**. The first fluvial section ("international section" of the St. Lawrence (Canada/USA)) was characterized by low pharmaceutical levels. For instance, no contamination peak occurred at ONT1 and ONT2 sites (near the source of the St. Lawrence), despite the presence of distant effluents in some major cities around Lake Ontario such as Toronto (population of 2.6 million people) and further upstream (other Laurentian Great Lakes). This may be explained by the major dilution factor from Lake Ontario (1 600 km^3) and water residence time (allowing natural attenuation of less persistent pharmaceuticals) prior to entering the St. Lawrence. In the international fluvial section (from Lake Ontario to Lake St. Francis, upstream from Lake St-Louis), samples had a few detectable pharmaceutical compounds (mostly IBU, VEN, and CAF) present at trace levels; in many sites, targeted analytes were in fact below LODs, also reflecting the lack of major conurbations along the shores of the river.

The ST samples showed levels of pharmaceuticals comparable to the GL water mass, while the OR samples were slightly more polluted. The latter water mass originates from the Outaouais (Ottawa) river and mixes with the Mille-Iles River that runs along Laval Island (population over 400 000) where it is heavily impacted by municipal effluents and combined sewer overflows from Laval and other municipalities in the north shore [444–447].

Downstream Lake St. Louis, an increase in pharmaceutical concentrations can be observed within the GL water mass just before it reaches the CWM due to the presence of effluents of several densely populated cities of the south shore of the St. Lawrence River (e.g., Longueuil WWTP).

The Montreal effluent rejection point appears as a major source of pharmaceuticals to the St. Lawrence, with summed pharmaceuticals (ΣPharmas) increasing sharply immediately after Ile aux Vaches (TCM sites) (**Figure 7-3a**). This was expected considering the fact that two million people live in the Montreal Island leading to approximately 900 billion liters processed by the city's wastewater treatment plant each year [448,449], and that the current wastewater treatment technologies only partially remove pharmaceuticals. The highest contamination was observed for the sites closest to the effluent rejection point and the next two sites, immediately downstream and located in the same water mass (CWM). The impact of the WWTP is more clearly marked for classes of pharmaceuticals found in greater concentrations such as analgesics, stimulant (CAF), and anticonvulsants.

Samples collected along the effluent plume still had relatively high pharmaceutical levels until 70 km downstream, where concentrations had receded to near pre-effluent levels. A secondary contamination peak was found in the MWM water mass at Quebec City (**Figure 7-3b**). Quebec City's wastewater treatment plant serves a much smaller population and manages a volume almost 10 fold smaller than that of Montreal City (slightly under 100 billion liters per year) [450]. Pharmaceutical levels then decreased rapidly when entering the ETZ water mass. Past the Orléans Island, there is an increase in salinity and turbidity due to sediment resuspension (maximum turbidity zone) and greater dilution effects related to tides that can explain the sharp decrease in pharmaceutical concentrations [451,452].

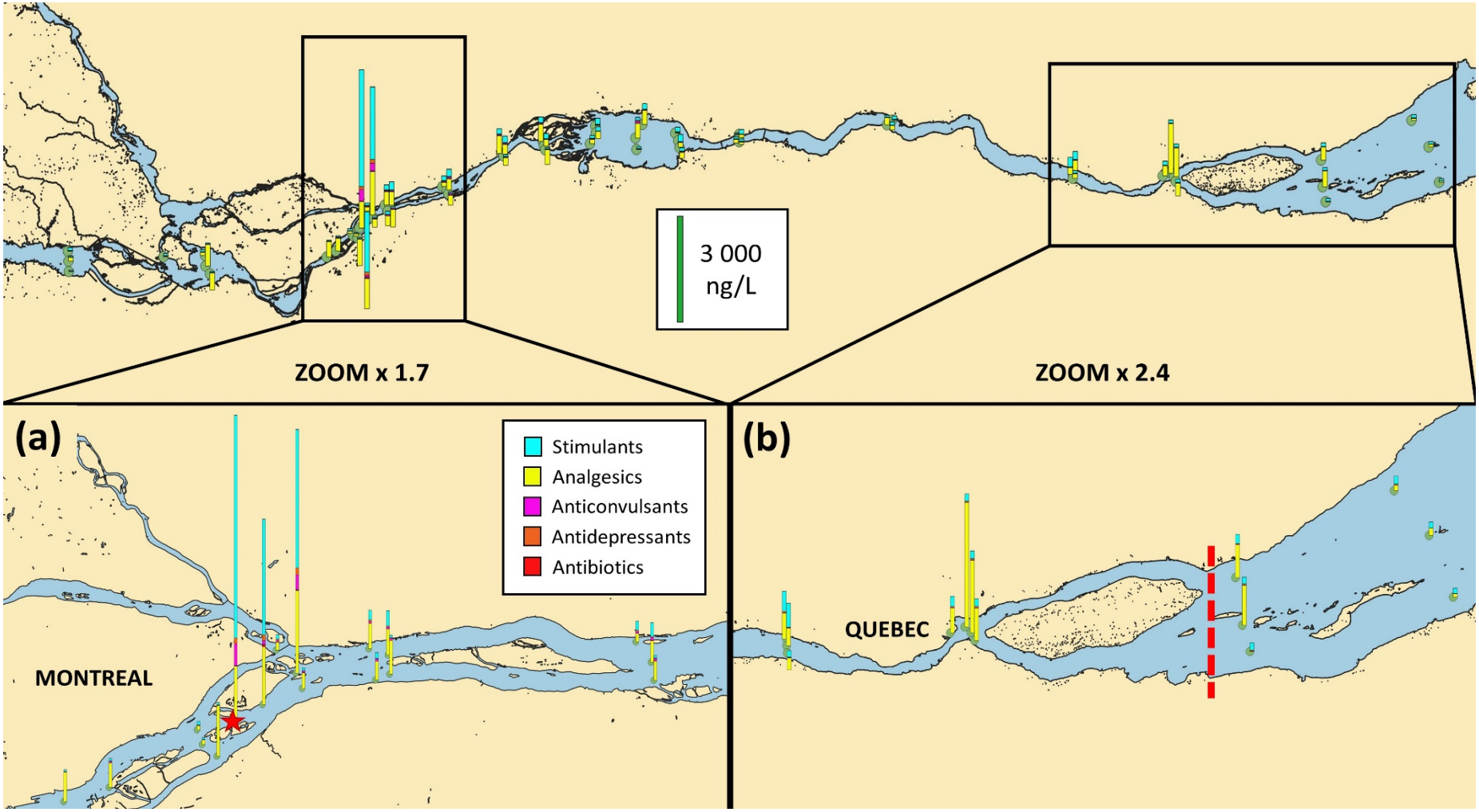


Figure 7-3. Spatial distribution of pharmaceutical contaminants in the St. Lawrence River with a zoom-in on the denser populated regions of Montreal City (**a**) and Quebec City (**b**). The location of the Montreal effluent reject point is marked by a red star (**a**). The red dotted line indicates the limit of the ETZ water mass where freshwater starts to mix with brackish water (**b**).

7.4.2.2 Inter-annual variations

The spatial distribution patterns discussed above were consistently observed during each of the St. Lawrence wide sampling campaigns (*Lampsilis* surveys of 2017, 2018, 2019, 2020, and 2021). Hierarchical classification resulted in overall similar dendrograms (see **Figure 7-6**), with samples TCM 1-5, TCM 2, and/or TCM 2-5 being singled out due to extremely high concentrations, reflecting the proximity to Montreal's WWTP effluent release point. Some recurring clusters indicate proximity or continuity of concentrations of samples collected in the same water mass, which reinforces the hypothesis that there is only limited mixing of the water masses that flow alongside each other. Hence, the following samples repeatedly grouped together: REP1, CON1, TRA1 SORAM1, SORAV1, LSPM1 and LSPA1 (2018, 2019 and 2021; northern water mass); REP2, CON2, SORAM2, SORAV2, LSPM2, LSPA2 and STM2 (2017, 2018 and 2019; central water mass); or SORAM3, SORAV3, LSPM3, LSPA3 and STM3 (2019 and 2020; southern water mass). Other clusters indicate similar levels of pharmaceuticals in samples close to contamination points such as TCM2-5, REP2, and CON2 (2018; same water mass and downstream from Montreal WWTP reject point), TCL1, BOU3, TCM1, and TCM3 (2020) or the QEC sites (near Quebec City; 2019 to 2021).

Though pharmaceutical profiles were often similar, concentration levels somewhat varied between sampling years. Wilcoxon's signed rank test conducted between years (paired per sampling location, with 28 common sampling points from 2017 to 2021), indicated significantly higher ΣPharmas concentrations for 2020 (median of 370 ng/L) vs. 2017 (57 ng/L, p-value = <0.0001), 2018 (200 ng/L, p-value = 0.03), 2019 (141 ng/L, p-value = 0.0007), or 2021 (178 ng/L, p-value = 0.005). The ΣPharmas was also significantly lower in 2017 than in other years (p-value range = <0.0001-0.002). No significant differences were found between 2018/2019, 2018/2021 or 2019/2021 (p-value range = 0.29-0.76). The higher ΣPharmas concentrations in 2020 are mainly due to OH-IBU, the main metabolite of IBU, for which the mean value is much higher than the other sampling years. Although some metabolites are less stable in environmental matrices, this compound is known to be more resilient to conventional treatment plants and with a higher solubility and mobility, is frequently reported in higher concentrations and at more distant sites from wastewater treatment plants [453–456]. These fluctuations might be attributed to annual changes in physicochemical characteristics of the receiving surface water such as the pH, organic matter content, or temperature that are known to affect polar metabolites in environmental matrices [158,456,457]. When comparing the ΣPharmas of the year 2020 without OH-IBU, the median concentrations are much closer to that of the years 2018, 2019, and 2021.

It should also be nuanced that not all relevant pharmaceuticals could be included for study. Additionally, the sampling design did not include any covid-19 related drugs (since the surveys started before the pandemic), which could have revealed different trends. For instance, the Covid-19 pandemic has been linked with changes in terms of some pharmaceutical classes such as antibiotics and analgesics found in wastewater [83,458–463]. The inclusion of specific compounds such as ritonavir, chloroquine, or ivermectin, used to treat Covid-19, might have allowed to confirm whether this was the case [464–466]. The higher concentrations of OH-IBU observed in 2020 might still be associated with increased usage of over-the-counter cold medications during the the pandemic. Finally, attempts to compare water level and rainfall with contamination levels did not yield conclusive results, and the lack of available data on the different effluents treated volumes and flows would prevent a more in-depth evaluation.

7.4.2.3 Depth effect

Along with the 2018 St. Lawrence sampling campaign, a distinct campaign was done to evaluate wastewater discharge near the river bed (approx. 10 m depth) in the Montreal Island area. **Figure 7-4** represents the ratio of quantified pharmaceuticals normalized to the highest concentration for each compound. This representation was used to superimpose contamination peaks of different compounds found in a broad array of concentrations.

Of the targeted compounds, eight analytes were detected both at the bottom and the surface of the river and in many cases in all of the samples with concentrations ranging from sub ng/L (for example, VEN = 0.42 ng/L and CBZ = 0.40 ng/L) to hundreds of ng/L (for example, CAF = 190 ng/L and OH-IBU = 310 ng/L). The increase of concentrations prior to the effluent rejection point might be attributable to other municipal effluents of the suburbs of the Montreal region that have a lesser impact but are contributing to the total pharmaceutical pollution of the St. Lawrence River such as the TCL1 site near Longueuil City or from the Mille-Iles River that is impacted by wastewater effluents and combined sewer overflows [446].

For water sampled at the near-surface of the river (**Figure 7-4a**), a rise in the concentration of every analyte can be observed in the region of the rejection point of the wastewater treatment plant of Montreal City and is peaking over a short distance (approx. 15 km). Concentrations then decrease over a longer distance (approx. 70 km) before reaching concentrations similar to those obtained for samples upstream from the effluent. For depth samples (**Figure 7-4b**), a similar trend can be observed, wherein pharmaceutical concentrations increase after the wastewater rejection to the river. However, maximum concentrations are observed for the sample located five km apart from the one nearest to the effluent, which might indicate that the sampling sites might have been

located slightly distant from the wastewater plume or in a region where the effluent is not well homogenized with river water. For safety reasons, the depth water was sampled on the northern portion of the river whereas the effluents exit pipe is located in the St. Lawrence Seaway where there is important maritime traffic and high water current. Surface water was sampled in the St. Lawrence Seaway and a much more intense demarcation can be observed when meeting the Montreal effluent rejection location (**Figure 7-7**).

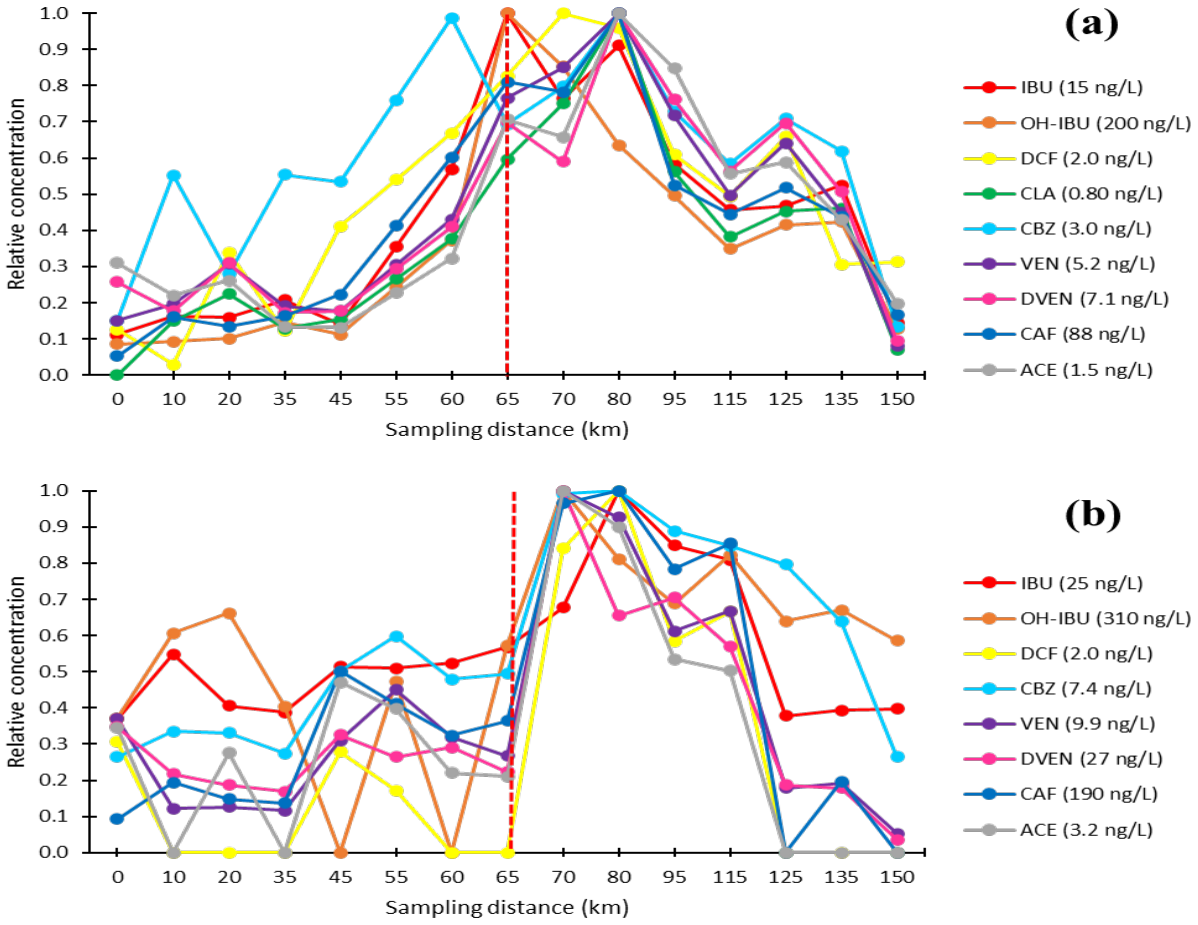


Figure 7-4. Ratio of detected pharmaceuticals normalized to the maximum quantified concentration (the maximum amount is indicated aside each compound name) of (a) surface water (approx. 1 m depth) and (b) riverbed water (approx. 10 m depth). The dotted vertical line represents the position of the wastewater rejection point in the river.Individual analyte concentrations per site are provided in SI **Tables 7-25** and **7-26**.

When comparing subsurface vs. bottom water samples, no statistical differences were found for acebutolol (p-value = 0.093), while diclofenac (p-value = 0.0011) was relatively higher in subsurface water; however, these two compounds were often non-detect and present at sub-ng/L concentrations. Wilcoxon's signed rank tests (paired by sampling site, n = 15 pairs) yielded statistically higher concentrations of bottom-water samples for high-concentration

pharmaceuticals such as carbamazepine (p-value = 6.1e-5), venlafaxine (0.041), O-desmethylvenlafaxine (0.0011) and caffeine (0.018). However, the difference between bottom-water and subsurface water samples remained relatively limited (e.g., average bottom-to-subsurface ratios of 2.1 for IBU, 1.9 for OH-IBU, and 2.6 for CBZ). Higher concentrations of major detected pharmaceuticals in bottom-water could reflect higher persistence due to slower photodegradation (limited sunlight penetration) and the fact that municipal wastewater effluents can be released near the riverbed with limited vertical mixing in the investigated area [467,468].

7.4.3 Tributary samples

Apart from the contribution originating from the Laurentian Great Lakes, many rivers are tributaries to the St. Lawrence River along its course and might act as potential sources of pharmaceutical pollution. Some of them flow through densely populated areas while others are more impacted by agricultural activities, which could influence the type and concentrations of pollutants.

In terms of detection frequencies (**Table 7-2**), CBZ, VEN, CAF et ACE were present in most of the tributary samples with occurrences >70%. The pharmaceuticals found at the greatest concentrations, i.e., CAF, CBZ, and SMX (max levels of hundreds of ng/L), still remained at lower values than maximum observed levels in the St. Lawrence River. However, the antibiotics SMX and CLA were more frequently detected in tributary rivers than in the St. Lawrence, which might reflect their veterinary usage in tributaries located in agricultural areas [469–471]. Some impacted tributaries also showed relatively high levels of the metabolites 2OH-CBZ and DVEN, in the same order of magnitude as in the most polluted St. Lawrence River samples. These metabolites were reported at similar concentration ranges in urban rivers from the USA [472].

Depending on the tributary, the ΣPharmas varied from low ng/L to sub-µg/L level (**Figure 7-5a**). In a few cases, all target pharmaceuticals remained undetected, such as in the Ogdensburg River (2019) and the Lac Saint-Jean (2021). Overall, the most polluted rivers in terms of concentrations are by far the Mascouche River (940 ng/L) followed by the Saint-Regis (480 ng/L), Ruisseau Bertrand (420 ng/L), Saint-Jacques (360 ng/L), and Richelieu (340 ng/L) rivers. These rivers are affected by anthropic activities and wastewater discharge and are all flowing in densely populated areas on both the south and north suburbs of the Greater Montreal Area, except for Ruisseau Bertrand, a small stream located within Montreal Island. Previous studies have suggested bacterial contamination issues due to industrial and residential wastewater discharges

into this watercourse [473]. The tributaries in less populated urban areas as well as the rivers flowing through agricultural or forest environments showed overall low concentrations of pharmaceuticals.

The number of pharmaceuticals detected in tributaries varied somewhat between sampling years. For example, although they are not usually heavily contaminated for other years, no analyte was found in the Batiscan and Sainte-Anne rivers in 2018 or the Cap Rouge River in 2021. The rivers with the most detected analytes also changed over the years, e.g., Yamachiche (10 detected analytes in 2018), Bécancour (11 in 2019), Du Sud (10 in 2020) or Saint-Charles and Yamaska (8 in 2021). Other tributary samples contained among the maximum detected analytes for recurrent years such as Mille-Iles (2018 to 2021), Mascouche (2018/2019/2021), Saint-Jacques (2018/2019), Saint-Regis (2018/2020), Du Loup (2019/2021) or Richelieu (2019/2020). These results generally coincide with rivers that were among the most polluted in terms of concentrations or mass loads. Among recurring tributaries that were sampled each year (n = 24), a higher ΣPharmas concentrations was quantified in 2019 (median of 210 ng/L) followed by 2021 (130 ng/L), while 2018 (56 ng/L) and 2020 (76 ng/L) had comparable contamination levels.

To better evaluate the potential contribution of individual tributary rivers to pharmaceutical pollution in the St. Lawrence River environment, the daily load of the sums of pharmaceuticals was calculated using the seasonal average flow of each river (data compiled in **Table 7-6**). The tributaries with the highest load of pharmaceuticals are by far the Des Prairies River (5200 g/day), followed by the Saint-François (1100 g/day), Richelieu (850 g/day), Outaouais (790 g/day), Yamaska (590 g/day), and Mille-Iles (570 g/day) rivers (**Figure 7-5b**). Again, these tributaries receive effluent outfalls and are flowing through areas impacted by anthropic activities; their flowrate is also within the highest among the tributaries to the St. Lawrence. Interestingly, the Des Prairies River was not among the rivers with the highest pharmaceutical concentrations, but the fact that it flows along the island of Montreal Island and has a high flowrate makes it a major source in terms of mass balance. The same comment applies to the Outaouais River, while the Richelieu and the Mille-Iles rivers are present at the higher end both in terms of total concentrations and daily loads of pharmaceuticals. Ruisseau Bertrand and Ruisseau Bouchard (two streams within the Montreal Island) can be considered low sources of contaminants despite their high concentrations since their water flow is quite limited [473]. Overall, the calculated pharmaceutical mass loads are comparable to previously published values of rivers impacted by wastewater effluents [474].

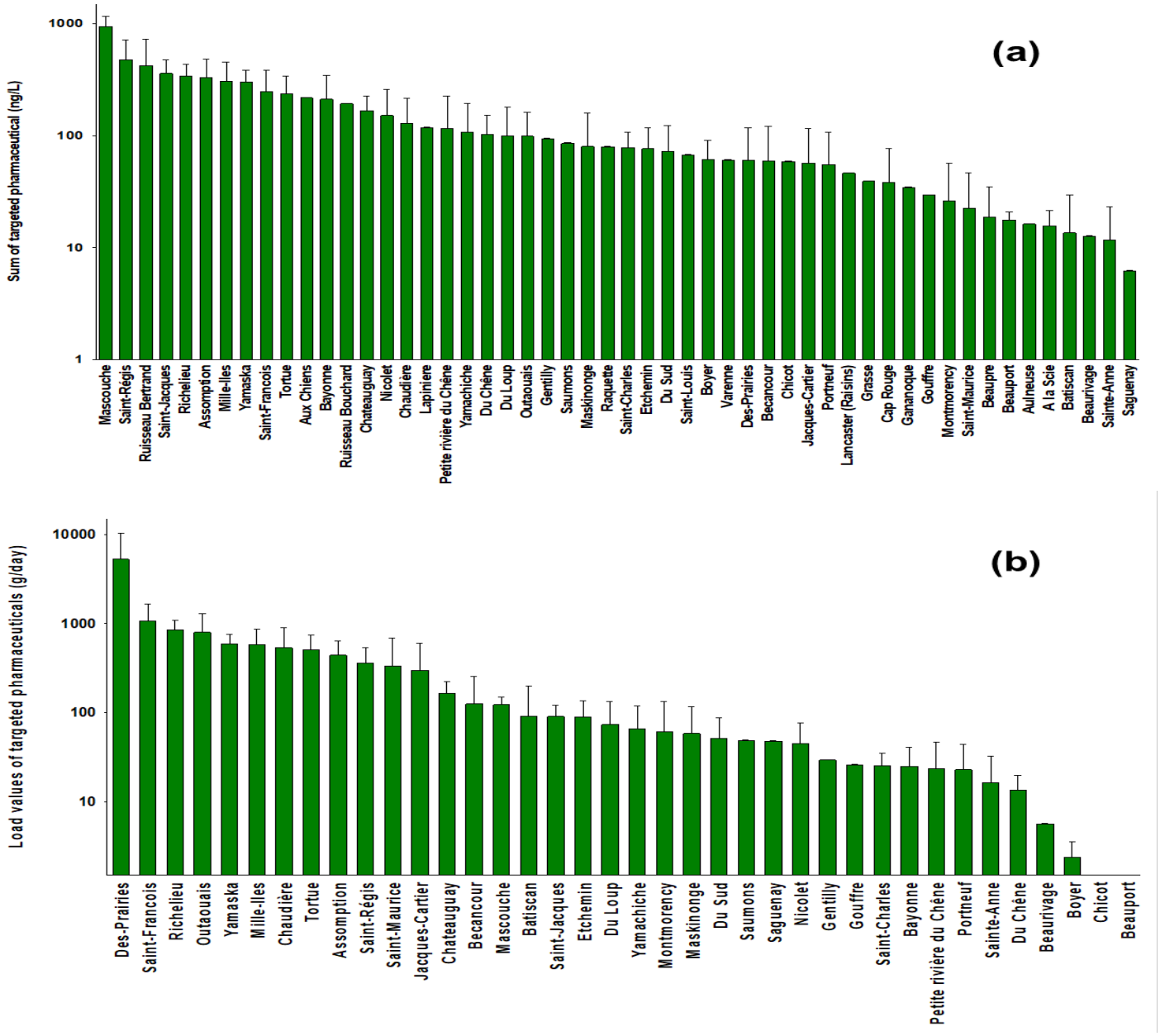


Figure 7-5. Overview of the most polluted tributary rivers sorted by **(a)** average summed concentration (ng/L) and **(b)** daily load (g/day) (when flowrate information was available) of the targeted pharmaceuticals.

7.5 Compliance with guidelines and ecological risk assessment

7.5.1 Compliance with Canadian guidelines

No Canadian guidelines are yet available for most pharmaceutical compounds. However, the Canadian Council of Ministers of the Environment (CCME) has developed a Canadian Water Quality Guideline (CWQG) for carbamazepine in freshwater environmental waters [410], set at 10 000 ng/L (long-term exposure of aquatic life). The value was derived as a type B2 (deterministic) guideline, from the most sensitive Lowest-Observed Effect Concentration (LOEC) found among available long-term exposure studies and applying a safety factor of 10 times [410]. The maximum CBZ concentration from our large-scale survey of surface waters from the St. Lawrence watershed is 233 ng/L, or about 40 times lower than the CWQG.

7.5.2 Comparison with European Union EQS

As part of the European Union Water Framework Directive (WFD), environment quality standards (EQS) have been developed for priority pollutants, including an annual average concentration (AA-QS) for long-term exposure (based on chronic toxicity data), and a maximum admissible concentration (MAC-QS) to protect aquatic organisms from short-term contamination peaks (acute toxicity). Guidelines to prevent the secondary poisoning of top predators ($QS_{biota\ sec\ pois}$) are also sometimes available. Although European Union EQS would not apply to Canadian surface waters, we used the available freshwater values for information purposes, including current and draft guidelines (see also SI **Table 7-15**).

Carbamazepine. The current EU environmental quality standard for CBZ in freshwater surface water is 2000 ng/L as annual average for the protection of freshwater ecosystems (AA-$QS_{fw\ eco}$). The European Commission Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) recently proposed a draft alternative AA-$QS_{fw\ eco}$ of 2500 ng/L, and a draft maximum admissible concentration (MAC-QS_{fw}) of 1 600 000 ng/L [475]. The measured CBZ concentrations in our study (max of 233 ng/L) are lower than these quality standards.

Clarithromycin. SCHEER recently endorsed the draft AA-$QS_{fw\ eco}$ and MAC-QS_{fw} for CLA, both at 130 ng/L [476]. The maximum measured concentration of CLA in our dataset (25 ng/L) is about 5 times lower than the draft AA-$QS_{fw\ eco}$ and MAC-QS_{fw}.

Diclofenac. The current AA-$QS_{fw\ eco}$ for diclofenac is set at 100 ng/L; using this guideline, all the surface water samples from our survey would be compliant (maximum DCF of 58 ng/L).

However, SCHEER recently supported a draft revised AA-$QS_{fw\ eco}$ of 40 ng/L; using this new guideline, 3 samples (all belonging to the TCM sample series) would be above the 40 ng/L threshold [477].

Due to the deleterious effects of DCF on Asian vulture populations, the EQS dossier also proposed a draft $QS_{biota\ sec\ pois}$ of 1.2 µg kg^{-1} (DCF concentration in filter-feeding bivalves) to protect avian wildlife; based on bioaccumulation factors this was translated to a $QS_{biota\ sec\ pois}$ of 5.4 ng/L in freshwater [408,477]. While most samples from our survey would still be compliant with this more stringent guideline, 30/406 samples (or 7.4%) would be above the $QS_{biota\ sec\ pois}$ of 5.4 ng/L.

Ibuprofen. A draft AA-$QS_{fw\ eco}$ of 140 ng/L is being proposed for ibuprofen (SCHEER 2023), while no MAC-QS_{fw} is available [478]. The vast majority of the surface water samples from our survey (99%) are lower than the proposed AA-$QS_{fw\ eco}$. Only 4 exceedances were reported: TCM2_2018 (860 ng/L), TCM2-5_2019 (820 ng/L), TCM1-5_2020 (360 ng/L), and TCM1-5_2021 (190 ng/L). These samples were all collected in the St. Lawrence River within the effluent plume downstream the Montreal WWTP outflow. Due to the scope of IBU concentrations found considering all samples from the TCM1-5/2/2-5 series (average from the five-year sampling: 210 ng/L; median: 120 ng/L), it is likely that the annual average AA-$QS_{fw\ eco}$ of 140 ng/L could be exceeded for this area of the St. Lawrence River.

Steroid hormones. The current AA-$QS_{fw\ eco}$ for EE2 is 0.035 ng/L and a draft alternative AA-$QS_{fw\ eco}$ of 0.017 ng EE2/L was recently proposed by SCHEER [93]. The current AA-$QS_{fw\ eco}$ of E2 is 0.4 ng/L, with a draft alternative AA-$QS_{fw\ eco}$ of 0.18 ng/L [93]. Comparison with these guidelines was not possible due to our relatively high method detection limits for EE2 and E2 (LDM of 5 ng/L and 1 ng/L, respectively).

Sulfamethoxazole. The current AA-$QS_{fw\ eco}$ for SMX is 600 ng/L, while the current MAC-QS_{fw} is set at 2700 ng/L [479]. The measured SMX concentrations in our study (max of 351 ng/L) are lower than these quality standards.

7.5.3 Risk quotients

Risk quotients were calculated following the approach described in Section 2.6 (see also **Table 7-12** for a compilation of freshwater PNEC data) and associated summary statistics are provided in **Table 7-3**. Samples collected in the ETZ were not considered in these assessments since PNEC data for aquatic organisms living in brackish water regions are generally not available; for

instance, the Norman Ecotoxicological Database only reports freshwater and marine PNECs. A majority of the tested samples presented intermediate to high risks for CBZ (66% of samples with RQ ≥0.1), IBU (63%), and DCF (80%). The TCM samples series often presented the maximum RQ values of the dataset (e.g., a maximum RQ of 86 for IBU, 58 for DCF or 22 for CAF at site TCM2). Example sites presenting multiple exceedances to the RQ >1 threshold across years were the TCM series of the St. Lawrence River (immediate downstream area of Montreal WWTP outfall: CAF, CBZ, DCF, IBU) and urban tributaries including the Mascouche River (DCF, IBU) and the St. Regis River (DCF, IBU).

Aquatic organisms living near these sampling sites might be subject to deleterious effects on their growth and reproduction and the exceeding of some EQS might suggest possible toxicity for the most sensible organisms [480–482]. In addition, municipal wastewater effluents may bring other trace organic chemicals (e.g., nonylphenol and associated ethoxylates (NPEO), polybrominated diphenyl ethers), metals (e.g., Cd and Cu) and pathogens that can contribute to ecotoxicological effects [37].

Table 7-3. Summary statistics of risk quotients (RQ, derived from PNEC values) of pharmaceuticals in freshwater surface water samples from the present survey (overall N = 389; samples from the ETZ excluded), including maximum, mean, median, % belonging to each class of risk, and sites corresponding to Max RQs.

	Max RQ	Mean RQ	Median RQ	% of samples with RQ <0.1	% of samples with 0.1< RQ <1	% of samples with RQ >1	Sites corresponding to Max RQs
IBU	85.8	1.45	0.54	38	35	28	TCM2
OH-IBU	0.34	0.029	0.0069	94	5.9	0	Ruisseau Bertrand
HMOR	0.03	0.0005	0	100	0	0	IRQ1-5
DCF	57.9	2.32	1.2	21	21	59	TCM2
OH-DCF	0.05	0.0012	0	100	0	0	TCM2-5
SMX	19.5	0.059	0	99	0.26	0.77	Mascouche River
CLA	1.25	0.021	0	95	4.4	0.26	TCM2
MTX	0.06	0.0010	0	100	0	0	Yamachiche River
E1	2.70	0.047	0	93	6.2	1.3	TCM2-5
CBZ	23.3	0.56	0.27	34	56	9.5	St. Regis River
2OH-CBZ	0.25	0.0092	0.000	98	2.3	0	TCM2
VEN	1.05	0.060	0.029	87	13	0.26	TCM2-5
DVEN	0.06	0.0018	0.0006	100	0	0	Mascouche River
FLU	0	0	0	100	0	0	-
CAF	22.4	0.47	0.17	31	64	4.4	TCM2
ACE	0.005	0.0003	0.0002	100	0	0	TCM2-5

Most compounds had average RQs <0.1, falling within the low/minimal risk category, though relatively high RQs were punctually observed at some sites (e.g., E1: an overall mean RQ of 0.045 but a maximum of 2.7 at site TCM2-5). The average RQs of two compounds presented a higher risk with average RQs above unity, IBU (mean RQ = 1.4) and DCF (mean RQ = 2.2), due to their low PNEC values set at 10 and 1 ng/L, respectively. Overall, the average RQ values are close to previously reported data in some urban rivers of countries such as China or Spain and are relatively small when compared to some extreme values calculated in the Middle East and North Africa where there are limited environmental legislations [483–486].

7.6 Conclusions

The present study provides the first large-scale characterization of pharmaceuticals in the St. Lawrence watershed. The extensive five-year sampling campaigns, covering over 700 km of the St. Lawrence River and more than 50 tributaries, provided precious information on spatial and temporal trends of these emerging pollutants. Targeted pharmaceuticals such as carbamazepine, diclofenac, and venlafaxine were found in most samples with detection rates >80%. Specific compounds showed maximum concentrations in the hundred ng/L range (e.g., dihydroxy-dihydrocarbamazepine, ibuprofen) and some even surpassed µg/L levels (e.g., caffeine, hydroxyibuprofen).

In the upstream section of the St. Lawrence River, pharmaceuticals had typically low (yet constant) concentrations, reflecting the high dilution effect from Lake Ontario and gradual attenuation prior to entering the St. Lawrence. Concentrations gradually increased as the river crossed the densely populated suburbs of Montreal, culminating in a sharp contamination peak right after the outflow of Montreal's wastewater treatment plant. Concentrations remained relatively high within the effluent plume until 70 km downstream where they had receded to pre-effluent levels. A secondary contamination peak was noted around Quebec City, but concentrations rapidly declined afterwards when reaching the estuarine transition zone.

Tributary rivers were characterized by a wide span of pharmaceutical concentrations, ranging from below detection limits (e.g., Lac Saint-Jean / Saguenay River) to hundreds of ng/L (e.g., Mascouche River). Contamination profiles were often similar to that of the St. Lawrence River, with some exceptions: the antibiotics clarithromycin and sulfamethoxazole, used for livestock, were found at greater occurrence in tributaries.

All surface water samples were compliant with the Canadian Water Quality Guideline available for carbamazepine, and most samples would also meet European Union quality standards available for carbamazepine, clarithromycin, diclofenac, ibuprofen, and sulfamethoxazole. A preliminary risk assessment was performed by comparing measured concentrations to PNEC values. Despite the large dilution effect, average risk quotients >1 were observed for diclofenac and ibuprofen, with even greater values for the sample series in the immediate downstream section of Montreal wastewater effluents (risks quotients occasionally >50). The latter sample series would also likely exceed the European Union annual average quality standard for ibuprofen. The high dilution from the St. Lawrence River hence does not appear to be sufficient to mitigate the impact of the Montreal effluent (primary treatment), further supported by the observation of endocrine disruption effects and feminization in aquatic organisms collected in the effluent plume downstream Montreal [37,487]. Apart from encouraging efforts by industries and the general population to correctly dispose of pharmaceutical wastes, the implementation of advanced wastewater treatments reducing chemical loads could help improve water quality. Our study provides valuable baseline data of pharmaceuticals prior to the implementation of such treatments and could be repeated in the future to measure the effect of advanced treatments and the implementation of environmental guidelines. Future sampling campaigns could consider adding seasonal sampling at some impacted sites to assess variations in terms of pharmaceutical classes and their concentrations, since the nature of consumed drugs may change during the year.

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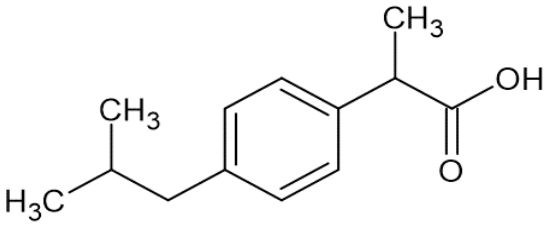
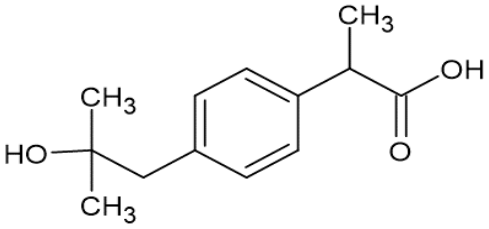
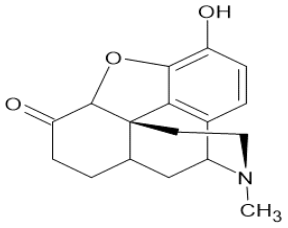
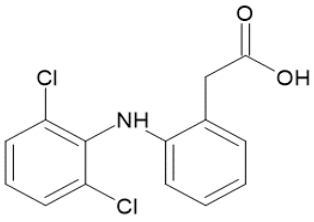
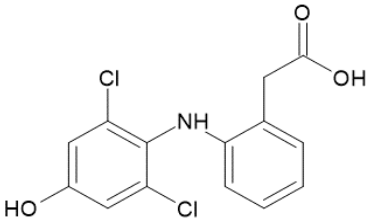
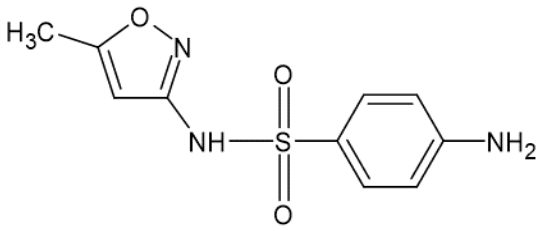
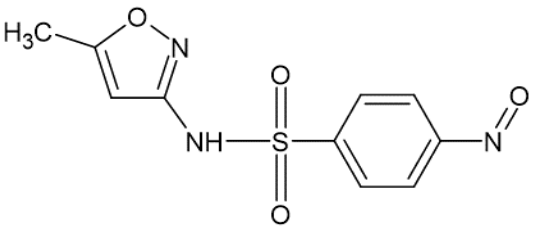
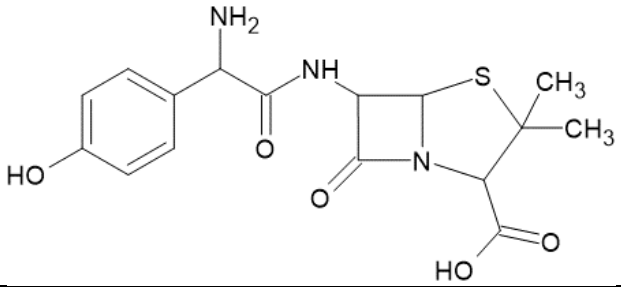
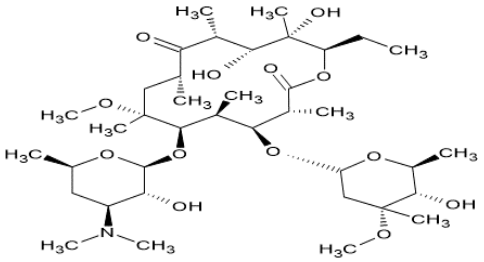
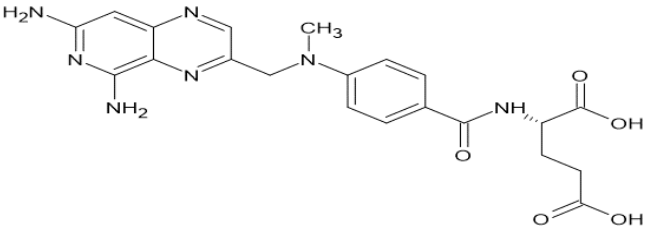
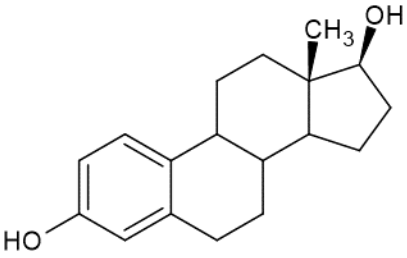
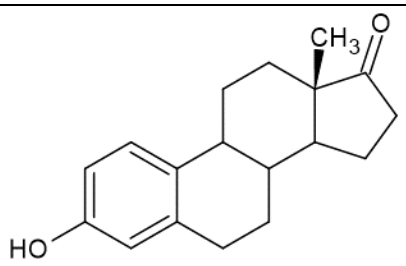
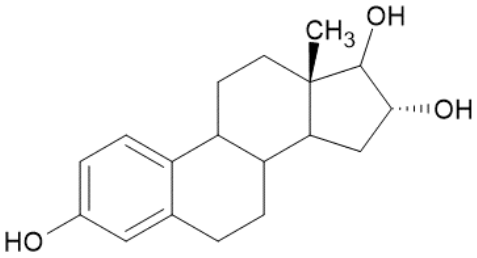
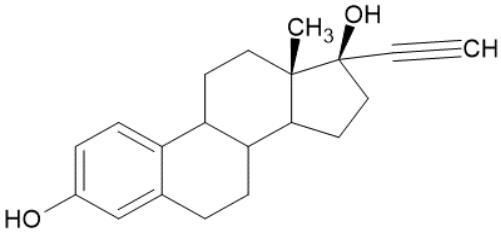
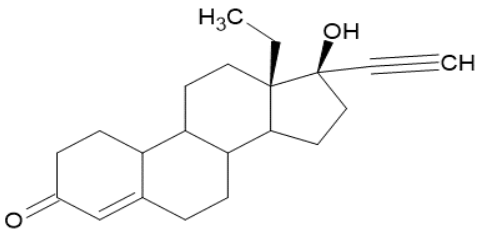
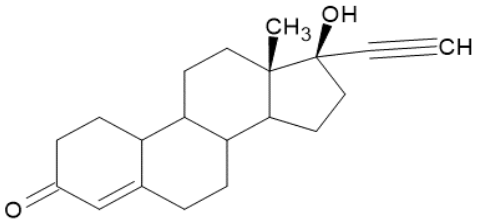
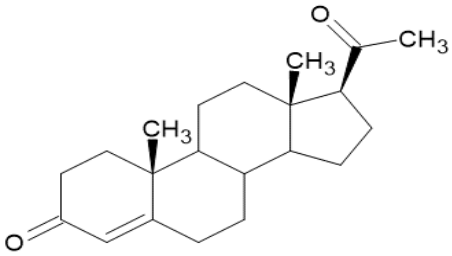
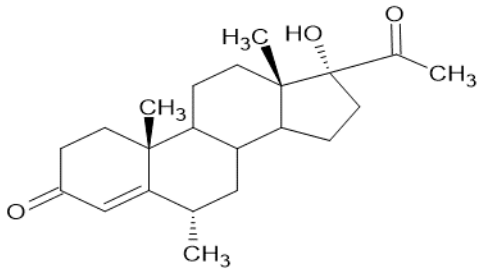
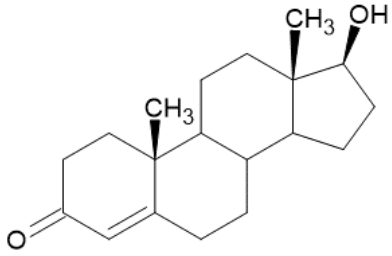
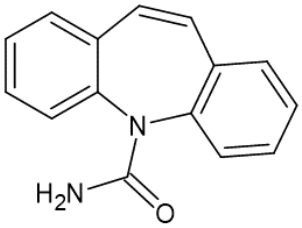
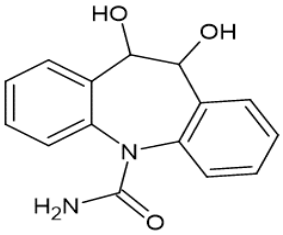
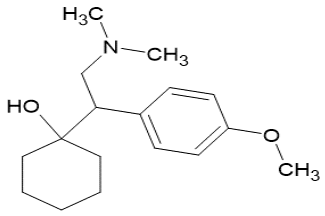
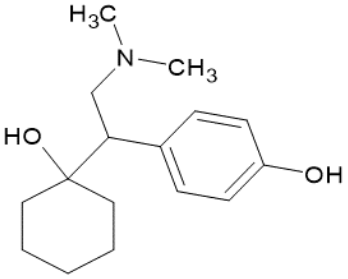
Supporting Information

Table 7-4. Structure and properties of the targeted pharmaceuticals (data retrieved from ChemAxon Ltd).

Compound name and acronym	MW g/mol	Log *P*	Structure
Ibuprofen	206.28	3.97	
IBU			
2-Hydroxyibuprofen	222.28	2.41	
OH-IBU			
Hydromorphone	285.34	1.06	
HMOR			
Diclofenac	296.16	4.51	
DCF			
4-Hydroxydiclofenac	312.15	3.70	
OH-DCF			

Sulfamethoxazole	253.28	0.89	
SMX			
4-Nitrososulfamethoxazole	267.26	0.87	
NO-SMX			
Amoxicillin	365.40	0.87	
AMOX			
Clarithromycin	747.95	3.16	
CLA			
Methotrexate	454.44	-1.85	
MTX			
Estradiol	272.38	4.01	
E2			

Estrone	270.37	3.13	
E1			
Estriol	288.38	2.45	
E3			
Ethinylestradiol	296.41	3.67	
EE2			
Levonorgestrel	312.45	3.48	
LEVO			
Norethindrone	298.43	2.97	
NOR			
Progesterone	314.47	3.87	
PROG			

Medroxyprogesterone **MPROG**	344.23	3.50	
Testosterone **TESTO**	288.42	3.32	
Carbamazepine **CBZ**	236.27	2.45	
10,11-Dihydroxy-10,11-dihydrocarbamazepine **2OH-CBZ**	270.28	0.13	
Venlafaxine **VEN**	277.4	3.20	
Desvenlafaxine **DVEN**	263.38	0.21	

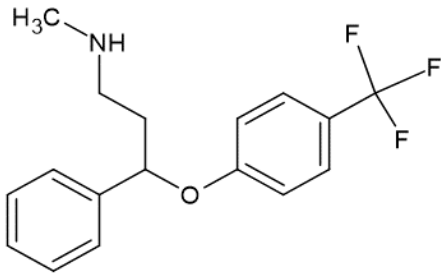
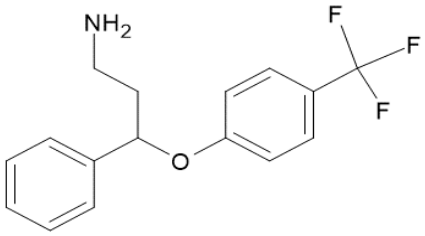
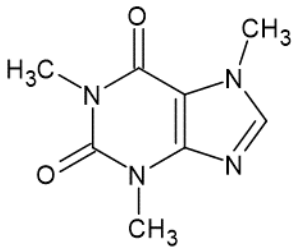
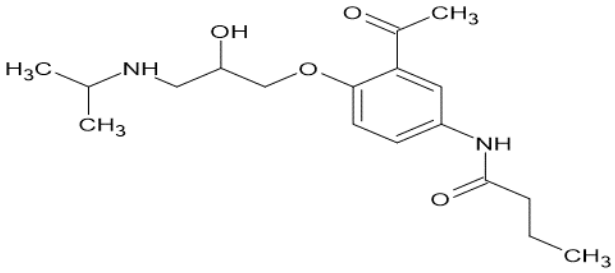
Fluoxetine **FLU**	309.33	4.05	
Norfluoxetine **NORF**	295.3	3.74	
Caffeine **CAF**	194.19	-0.07	
Acebutolol **ACE**	336.43	1.71	

Table 7-5. GPS coordinates of the Saint-Lawrence River sites, sampling years, and associated water mass (CWM: Central Water Mass; ETZ: Estuarine Transition Zone; GL: Great Lakes; MWM: -Mixed Water Mass; OR: Ottawa River; ST: South Tributaries).

				Sampling years				
Site code	**Water mass**	**Coordinates**		**2017**	**2018**	**2019**	**2020**	**2021**
BOU1	GL	45°38.810'N	73°28.592'O	x	x	x	x	x
BOU2	GL	45°38.651'N	73°27.974'O	x	x	x	x	x
BOU3	GL	45°38.787'N	73°27.156'O	x	x		x	x
BOU4	GL	45°34.145'N	73°30.392'O	x		x	x	x
CAC1	ETZ	47° 58.498'N	69° 42.990'O					x
CHA1	GL	45°26'12.98"N	73°47'14.06"O		x			
CHA2	GL	45°25'9.86"N	73°46'39.62"O		x			
CHA3	GL	45°24'11.12"N	73°46'8.16"O		x			
CON1	OR	45° 50.743'N	73° 18.604'O	x	x	x	x	x
CON1-5	OR	45°55'21.35"N	73°14'32.38"O					x
CON2	CWM	45° 51.280 N	73° 16.810 O	x	x	x	x	x
CON3	CWM	45° 51.000 N	73° 16.150 O	x	x	x	x	x
IRQ1	GL	44°34.867'N	75°40.775'O		x			x
IRQ1-5	GL	44°44'09.6"N	75°27'23.3"O		x			x
IRQ2	GL	44°49.185'N	75°18.931'O		x			x
LSF1	GL	45°13'34.68"N	74°15'5.69"O	x				x
LSF2	GL	45°12'32.51"N	74°13'56.21"O	x	x			x
LSF3	GL	45°11'25.73"N	74°12'45.47"O	x				x
LSF3-5	OR	45°20'50.40"N	73°58'17.47"O					x
LSF4	GL	45° 2'30.42"N	74°34'30.77"O		x			
LSL1	OR	45° 24.394'N	73° 51.319'O	x	x	x	x	x
LSL2	GL	45°23.263'N	73°50.151'O	x		x	x	x
LSL3	GL	45°22.780'N	73°48.563'O		x	x	x	x
LSPA1	OR	46° 15.980 N	72° 43.002 O	x	x	x	x	x
LSPA2	CWM	46° 15.360 N	72° 41.700 O	x	x	x	x	x
LSPA3	ST	46° 14.710 N	72° 40.730 O	x	x	x	x	x
LSPA4	ST	46°13'52.35"N	72°40'31.83"O					x
LSPM1	OR	46° 14.310 N	72° 49.695 O		x	x	x	x
LSPM2	CWM	46° 12.240 N	72° 49.470 O		x	x	x	x
LSPM3	ST	46° 11.050 N	72° 48.080 O		x	x	x	x
MASS1	ETZ	47° 15.050 N	70° 32.720 O				x	x
MASS2	ETZ	47° 13.350 N	70° 26.870 O				x	
MASS3	ETZ	47° 10.130 N	70° 21.350 O				x	
ONT1	GL	44°11.646'N	76°34.673'O		x			x
ONT2	GL	44°14.261'N	76°23.901'O		x			x
ORL1	ETZ	47° 03.620 N	70° 45.080 O			x	x	x
ORL2	ETZ	47° 00.720 N	70° 42.060 O			x	x	x
ORL3	ETZ	46° 59.260 N	70° 39.980 O			x	x	x
QEC1	MWM	46° 50.550 N	71° 10.830 O			x	x	x
QEC2	MWM	46° 50.390 N	71° 09.920 O			x	x	x
QEC3	MWM	46° 50.010 N	71° 09.150 O			x	x	x
QEC4	MWM	46° 49.633 N	71° 12.034 O			x		x
REP1	OR	45° 44.595 N	73° 25.899 O	x	x	x	x	x
REP1-5	OR	45°49'19.56"N	73°21'35.86"O		x			

REP2	CWM	45° 44.950 N	73° 25.113 O	x	x	x	x	x
REP3	CWM	45° 44.623 N	73° 24.460 O			x	x	x
REP4	CWM	45°44'9.72"N	73°24'42.60"O	x	x		x	x
SAD1	MWM	46° 43.240 N	71° 28.700 O			x	x	x
SAD2	MWM	46° 42.790 N	71° 28.330 O			x	x	x
SAD3	MWM	46° 42.342 N	71° 27.906 O			x	x	x
SORAM1	OR	46° 04.420 N	73° 05.320 O	x	x	x	x	x
SORAM2	CWM	46° 03.990 N	73° 04.620 O	x	x	x	x	x
SORAM3	ST	46° 03.670 N	73° 04.000 O	x	x	x	x	x
SORAV1	OR	46° 10.350 N	72° 57.760 O	x	x	x	x	x
SORAV2	CWM	46° 09.640 N	72° 56.950 O	x	x	x	x	x
SORAV3	ST	46° 07.950 N	72° 56.810 O	x	x	x	x	x
STA1	MWM	46° 34.696 N	72° 06.154 O	x	x	x	x	x
STA2	MWM	46° 34.207 N	72° 06.252 O	x	x	x	x	x
STA3	MWM	46° 33.760 N	72° 06.562 O	x	x	x	x	x
STAN1	ETZ	47° 44.925'N	69° 53.265'O					x
STIR1	ETZ	47° 33.489'N	70° 10.827'O					x
STJO1	ETZ	47° 26.740 N	70° 18.010 O					x
STL1	GL	44°53.989'N	75°8.708'O		x			x
STL1-5	GL	44°57'31.9"N	74°59'16.3"O					x
STL2	GL	45°0.301'N	74°45.711'O		x			x
STM1	OR	46° 20.950 N	72° 31.670 O	x	x	x	x	x
STM2	CWM	46° 20.600 N	72° 31.330 O	x	x	x	x	x
STM3	ST	46° 20.280 N	72° 31.210 O	x	x	x	x	x
STM4	ST	46° 20.123'N	72° 30.893'O	x	x		x	x
TCL1	GL	45° 35.532'N	73° 29.462'O			x	x	x
TCM1	OR	45°42.288'N	73°28.687'O	x	x	x	x	x
TCM1-5	CWM	45° 39.970'N	73° 27.853'O		x	x	x	x
TCM2	CWM	45°42.329'N	73°27.361'O	x	x	x	x	x
TCM2-5	CWM	45° 40.906'N	73° 27.391'O	x	x	x	x	x
TCM3	CWM	45°42.190'N	73°26.682'O		x	x	x	x
TRA1	OR	45° 59.710 N	73° 11.230 O	x	x	x	x	x
TRA2	CWM	45° 59.757'N	73° 10.883'O	x	x	x	x	x
TRA3	CWM	45° 59.630 N	73° 10.600 O		x	x	x	x

Table 7-6. GPS coordinates of the sampled Saint-Lawrence tributaries.

Site name	Average river flow (June-July) m^3/s	Coordinates		Sampled years			
				2018	2019	2020	2021
A la Scie		46°46'3.16"N	71°13'1.94"O			x	x
Assomption	12	45°43'4.41"N	73°28'54.77"O	x	x	x	x
Aulneuse		46°42'21.15"N	71°22'51.54"O			x	
Aux Chiens		45°39'15.27"N	73°46'53.47"O			x	
Batiscan	96	46°31'13.82"N	72°14'47.59"O	x	x	x	x
Bayonne	1	46° 5'28.53"N	73°10'11.62"O	x	x	x	x
Beauport		46°51'5.53"N	71°11'59.04"O			x	x
Beaupre		47° 2'54.77"N	70°52'59.35"O		x	x	x
Beaurivage		46°39'4.40"N	71°18'2.93"O			x	
Becancour	59	46°21'9.62"N	72°26'19.03"O	x	x	x	x
Boyer	0.22	46°53'3.39"N	70°51'32.83"O	x	x	x	x
Cap Rouge		46°45'2.48"N	71°20'54.20"O			x	x
Chateauguay	114	45°21'39.89"N	73°44'51.15"O	x	x	x	x
Chaudière		46°42'52.14"N	71°16'51.59"O	x	x	x	x
Chicot		45°34'54.89"N	73°51'23.40"O			x	
Coulee Grou		45°41'53.91"N	73°30'13.28"O				x
Des-Prairies	907	45°31'59.94"N	73°43'40.29"O	x	x	x	x
Petite rivière du Chêne		46°34'21.84"N	71°59'35.37"O		x	x	
Du Chêne		45°33'25.01"N	73°53'24.88"O	x	x	x	x
Du Loup	26	47°50'42.93"N	69°32'17.01"O	x	x	x	x
Du Sud		46°59'3.15"N	70°32'54.99"O		x	x	x
Etchemin		46°45'35.37"N	71°13'42.60"O		x	x	x
Gananoque		44°19'37.72"N	76° 9'52.21"O		x		
Gentilly		46°22'57.24"N	72°20'10.27"O		x		
Gouffre		47°26'29.74"N	70°30'17.84"O				x
Grasse		44°57'33.27"N	74°49'49.31"O		x		
Jacques-Cartier	190	46°40'42.85"N	71°45'6.37"O	x	x	x	x
Lac St-Jean		48°35'52.40"N	71°48'35.02"O				x
Lapiniere		45°37'27.69"N	73°38'23.65"O			x	
Lancaster (Raisins)		45° 7'41.38"N	74°29'38.11"O		x		
Mascouche	1.5	45°41'44.64"N	73°35'36.63"O	x	x	x	x
Maskinonge	18	46°10'55.68"N	73° 2'0.71"O	x	x	x	x
Mille-Iles	286	45°36'50.58"N	73°47'39.09"O	x	x	x	x
Montmorency	25	46°53'10.93"N	71° 8'41.57"O		x	x	x
Nicolet	33	46°13'31.07"N	72°37'22.68"O	x	x	x	x
Ogdensburg		44°41'24.50"N	75°29'37.50"O		x		
Outaouais	1950	45°27'29.46"N	74° 5'40.14"O	x	x	x	x
Portneuf		48°38'26.17"N	69° 5'36.01"O			x	x
Raquette		44°58'42.71"N	74°43'1.52"O		x		
Richelieu	330	46° 2'30.27"N	73° 7'3.88"O	x	x	x	x
Ruisseau Bertrand		45°30'41.89"N	73°45'19.65"O			x	x
Ruisseau Bouchard		45°26'26.72"N	73°43'35.10"O				x
Sainte-Anne		46°34'16.79"N	72°12'22.43"O	x	x	x	x
Saint-Charles		46°49'8.91"N	71°13'24.74"O	x	x	x	x
Saint-François	100	46° 4'3.12"N	72°48'50.06"O	x	x	x	x
Saint-Jacques		45°25'49.81"N	73°28'45.69"O	x	x	x	x
Saint-Maurice	300	46°21'38.37"N	72°31'54.00"O		x	x	x
Saint-Louis		45°18'51.52"N	73°52'47.80"O		x		

Saint-Régis	8.7	44°58'23.27"N	74°39'55.74"O	x	x	x	
Saguenay	1750	48° 8'17.13"N	69°43'37.98"O				x
Saumons		45°39'3.04"N	74°54'6.91"O		x		
Tortue	25	45°24'3.30"N	73°32'9.48"O	x	x	x	x
Varennes		45°40'16.16"N	73°26'8.91"O		x		
Yamachiche	7	46°15'57.60"N	72°48'23.92"O	x	x	x	x
Yamaska	83	46° 0'18.85"N	72°54'34.44"O	x	x	x	x

Table 7-7. Chromatographic gradient for the off-line method at a flow rate of 500 µL min^{-1}.

Time / min.	H_2O / %	MeOH / %	H_2O + 1 mM NH_4F / %
0.00	90	5	5
1.00	90	5	5
7.00	0	95	5
8.00	0	95	5
8.10	90	5	5
10.00	90	5	5

Table 7-8. Chromatographic gradient for the on-line method at a flow rate of 500 µL min^{-1}.

Analytical pump gradient:

Time / min.	H_2O / %	MeOH / %	H_2O + 1 mM NH_4F / %
0.00	55	40	5
1.60	55	40	5
5.20	0	95	5
7.20	0	95	5
7.21	55	40	5
8.00	55	40	5

SPE loading pump gradient:

Time / min.	H_2O + 0.5 % FA	MeOH + 0.5 % FA	Flow rate (µL min^{-1})
0.00	100	0	2500
1.60	100	0	2500
1.70	0	100	2000
5.00	0	100	2000
5.01	100	0	1500
8.00	100	0	1500

Table 7-9. Optimized mass spectrometry acquisition parameters for targeted compounds and isotope-labeled internal standards.

Compound	Ionization mode[9]	Precursor ion (m/z)	Product ion (m/z)	Collision energy (V)	RF lens (V)	Isotope-labelled IS used
IBU	Negative	205.3	161.2	10	30	IBU-d3
			204.8	10		
IBU-d3	Negative	208.2	164.3	10	33	n.a.
OH-IBU	Negative	221.2	177.0	10	30	IBU-d3
			220.7	10		
HMOR	Positive	286.2	184.9	30	79	DCF-d4
			156.9	43		
DCF	Positive	296.1	214.0	34	43	DCF-d4
			215.0	19		
DCF-d4	Positive	300.0	218.0	35	44	n.a.
OH-DCF	Positive	312.0	230.0	35	51	DCF-d4
			231.0	20		
SMX	Positive	254.1	156.0	16	49	SMX-13C6
			108.1	24		
SMX-13C6	Positive	260.2	162.0	16	52	n.a.
NO-SMX	Negative	266.0	122.1	24	60	IBU-d3
			170.0	14		
AMOX	Positive	398.2	349.1	16	49	SMX-13C6
			381.1	10		
CLA	Positive	748.5	590.6	16	81	VEN-d6
			558.3	20		
MTX	Positive	455.2	308.0	20	83	MTX-d3
			175.0	39		
MTX-d3	Positive	458.3	311.1	20	78	n.a.
E2	Negative	271.0	269.1	33	99	E2-13C6
			182.9	41		
E2-13C6	Negative	277.2	186.1	43	97	n.a.
E1	Negative	269.2	145.1	41	97	E2-13C6
			159.2	39		
E3	Negative	287.2	145.1	43	105	E2-13C6
			171.1	39		
EE2	Negative	295.0	269.1	33	90	E2-13C6
			267.1	27		
LEVO	Positive	313.3	245.1	18	61	PROG-d9
			185.1	19		
NOR	Positive	299.3	231.2	18	57	PROG-d9

[9] Negative and positive ions are analyzed in a single run using polarity switching.

			171.1	20		
PROG	Positive	315.2	97.1	24	55	PROG-d9
			109.1	27		
PROG-d9	Positive	324.3	100.1	25	53	n.a.
MPROG	Positive	345.2	97.1	28	58	PROG-d9
			123.1	27		
TESTO	Positive	289.3	97.1	24	54	PROG-d9
			109.1	27		
CBZ	Positive	237.2	194.1	20	58	CBZ-d10
			192.1	24		
CBZ-d10	Positive	247.3	204.1	22	60	n.a.
2OH-CBZ	Positive	271.2	180.1	30	30	CBZ-d10
			253.1	10		
VEN	Positive	278.3	260.2	10	48	VEN-d6
			58.2	20		
VEN-d6	Positive	284.4	266.1	10	45	n.a.
DVEN	Positive	264.3	246.0	10	45	VEN-d6
			58.1	20		
FLU	Positive	310.2	148.1	10	43	VEN-d6
			44.2	12		
NORF	Positive	296.2	134.1	10	30	VEN-d6
			30.3	10		
CAF	Positive	195.2	138.1	20	58	CAF-13C3
			110.1	23		
CAF-13C3	Positive	198.2	140.1	20	61	n.a.
ACE	Positive	337.3	319.1	15	72	CAF-13C3
			116.0	22		

Table 7-10. Calibration performance in surface water matrix.

Compound	LOD (ng/L)	Linear range (ng/L)*	Linearity (R^2)
IBU	1	3.3 – 10 000	0.9999
OH-IBU	1	3.3 – 10 000	0.9992
HMOR	2	6.6 – 5000	0.9993
DCF	0.05	0.16 – 10 000	0.9999
OH-DCF	0.1	0.33 – 10 000	0.9999
SMX	0.1	0.33 – 10 000	0.9998
NO-SMX	5	16 – 10 000	0.9970
AMOX	5	16 – 10 000	0.9937
CLA	0.1	0.33 – 10 000	1.0000
MTX	0.1	0.33 – 10 000	0.9999
E2	1	3.3 – 10 000	0.9999
E1	0.1	0.33 – 10 000	0.9999
E3	5	16 – 10 000	0.9994
EE2	5	16 – 10 000	0.9998
LEVO	5	16 – 10 000	0.9999
NOR	0.5	1.6 – 10 000	0.9996
PROG	1	3.3 – 10 000	1.0000
MPROG	1	3.3 – 10 000	0.9996
TESTO	2	6.6 – 10 000	0.9999
CBZ	0.05	0.16 – 10 000	0.9996
2OH-CBZ	1	3.3 – 10 000	0.9989
VEN	0.05	0.16 – 10 000	1.0000
DVEN	1	3.3 – 10 000	0.9995
FLU	1	3.3 – 10 000	0.9996
NORF	5	16 – 10 000	0.9990
CAF	1	3.3 – 10 000	0.9998
ACE	0.1	0.33 – 10 000	0.9998

* The linear range reflects values between LOQ and the upper calibration curve level.

Table 7-11. Analytical validation performed in surface water matrix at two spike levels (QC1 = 20 ng/L and QC2 = 5000 ng/L), including recovery %, accuracy %, intraday precision (RSD%), and interday precision (RSD%).

	Recovery (%)		Accuracy (%)		Intraday Precision (RSD%)		Interday Precision (RSD%)	
	QC1	QC2	QC1	QC2	QC1	QC2	QC1	QC2
IBU	87 ± 5	100 ± 5	98 ± 7	96 ± 3	7.51	2.81	10.5	2.15
OH-IBU	86 ± 8	104 ± 1	104 ± 5	105 ± 4	4.62	4.92	6.61	5.90
HMOR	73 ± 2	75 ± 6	117 ± 5	99 ± 6	8.82	9.31	19.5	14.2
DCF	72 ± 1	79 ± 1	108 ± 1	101 ± 2	1.19	2.37	6.07	2.25
OH-DCF	74 ± 1	84 ± 1	100 ± 3	96 ± 3	3.18	2.84	5.84	3.37
SMX	71 ± 2	87 ± 1	104 ± 9	101 ± 5	2.03	1.22	5.13	9.46
NO-SMX	63 ± 6	65 ± 1	71 ± 3	114 ± 8	2.31	3.73	8.33	7.89
AMOX	80 ± 5	88 ± 1	117 ± 7	102 ± 3	6.43	3.50	13.1	7.28
CLA	83 ± 2	100 ± 2	98 ± 5	98 ± 2	6.38	2.04	8.80	14.6
MTX	89 ± 5	108 ± 2	108 ± 6	102 ± 6	4.90	3.79	18.3	13.3
E2	80 ± 5	103 ± 1	93 ± 5	96 ± 2	5.59	0.90	9.70	3.08
E1	89 ± 4	88 ± 1	99 ± 3	96 ± 2	4.75	2.99	7.93	3.44
E3	79 ± 8	81 ± 1	113 ± 4	92 ± 1	3.37	1.21	8.94	8.60
EE2	82 ± 4	99 ± 1	99 ± 5	106 ± 5	5.29	2.56	10.9	2.96
LEVO	85 ± 1	86 ± 1	118 ± 2	100 ± 3	1.01	1.52	3.56	4.98
NOR	79 ± 2	85 ± 1	101 ± 5	112 ± 3	4.71	2.95	6.74	4.39
PROG	77 ± 1	73 ± 1	104 ± 3	104 ± 1	2.59	0.41	5.55	1.44
MPROG	76 ± 2	83 ± 1	101 ± 4	97 ± 3	3.67	1.37	7.69	7.44
TESTO	75 ± 1	78 ± 1	105 ± 3	104 ± 1	2.81	0.38	5.33	3.07
CBZ	77 ± 1	83 ± 1	107 ± 5	106 ± 2	2.52	2.25	5.18	6.33
2OH-CBZ	70 ± 1	70 ± 1	125 ± 8	107 ± 4	6.69	1.97	11.9	14.4
VEN	76 ± 5	89 ± 1	111 ± 4	96 ± 2	3.17	1.93	4.65	1.72
DVEN	73 ± 2	98 ± 1	80 ± 4	98 ± 4	4.09	7.80	8.85	8.41
FLU	73 ± 2	87 ± 2	81 ± 4	93 ± 8	2.12	3.55	5.31	13.1
NORF	83 ± 1	90 ± 2	81 ± 7	86 ± 4	7.40	5.50	10.4	4.02
CAF	74 ± 2	85 ± 1	103 ± 5	96 ± 7	4.50	2.99	6.21	7.44
ACE	73 ± 2	81 ± 1	106 ± 6	116 ± 4	6.61	3.63	12.5	13.8

Table 7-12. Literature PNEC data (ng/L) for pharmaceuticals (freshwater aquatic organisms) and retained PNEC for risk assessment of surface waters in the present study.

Compound	PNEC data in literature (ng/L)	Retained PNEC (ng/L)
10,11-Dihydroxy-10,11-dihydrocarbamazepine	1910[a]	1910
2-Hydroxyibuprofen	7880[a]	7880
4-Hydroxydiclofenac	220[a]	220
4-Nitrososulfamethoxazole	3810[a]	3810
Acebutolol	2930[a] ; 10100[b]	2930
Amoxicillin	78[a] ; 37[c]	37
Caffeine	1200[a] ; 320[c] ; 87000[d]	320
Carbamazepine	2000[a] ; 420[b,g] ; 10[c] ; 250[e,f]	10
Clarithromycin	120[a] ; 20[c]	20
Desvenlafaxine	7110[a]	7110
Diclofenac	50[a] ; 20[b,h] ; 1[c] ; 5[f,i]	1
Estradiol (E2)	0.3[a] ; 2[c] ; 0,4[j]	0.3
Estriol (E3)	60[a] ; 51[k]	51
Estrone (E1)	3.6[a,j] ; 20[c]	3.6
Ethinylestradiol (EE2)	0.037[a]	N/A (<LOD)
Fluoxetine	100[a] ; 3[c] ; 50[l]	3
Hydromorphone	3640[a]	3640
Ibuprofen	11[a] ; 10[c]	10
Levonorgestrel	0.01[a]	N/A (<LOD)
Medroxyprogesterone	6720[a]	3720
Methotrexate	69[a]	69
Norethindrone	4520[a]	4520
Norfluoxetine	1700[a]	1700
Progesterone	1000[a] ; 19[c]	19
Sulfamethoxazole	600[a] ; 30[b,n] ; 2400[c] ; 18[f,m] ; 27[l]	18
Testosterone	4370[a]	4370
Venlafaxine	880[a] ; 92[c]	92

[a] Norman Ecotoxicology Database; [b] Gosset et al. 2021; [c] Zhou et al. 2019; [d] ECHA; [e] Ferrari et al. 2004; [f] Vergeynst et al. 2015; [g] Helwig et al. 2016; [h] Orias and Perrodin 2013; [i] Hoeger et al. 2005; [j] Čelić et al. 2020; [k] Nie et al. 2015; [l] Verlicchi et al. 2012; [m] Grung et al. 2008; [n] Rivera-Jaimes et al. 2018.

Table 7-13. Matrix effect assessment in surface water, including absolute* and residual** matrix effects.

	Absolute * matrix effect (%)	Residual ** matrix effect (%)
IBU	2.6	-2.3
OH-IBU	50	-4.6
HMOR	23	19
DCF	3.0	-1.2
OH-DCF	-18	5.6
SMX	7.9	-3.3
NO-SMX	-19	13
AMOX	52	3
CLA	53	-7.1
MTX	2.4	-5.2
E2	1.9	-2.5
E1	5.4	-1.2
E3	-21	6.8
EE2	19	-3.7
LEVO	-2.6	-3.8
NOR	-7.1	-1.5
PROG	7.6	-3.6
MPROG	1.1	-1.3
TESTO	-36	-4.2
CBZ	60	-13
2OH-CBZ	-87	5.4
VEN	18	-3.8
DVEN	-2.0	0.42
FLU	-20	-4.5
NORF	-18	20
CAF	12	-2.6
ACE	-4.0	-1.2

*Absolute matrix effects were evaluated by comparing the sensitivity (absolute response) of the calibration curve in the surface water matrix to the one in pure solvent. **Residual matrix effects were calculated by comparing the slopes of the standard addition curve in individual surface water samples to the composite matrix used for quantification.

Table 7-14. Wilcoxon's rank sum tests showing statistical differences between water masses (p-values in bold characters indicate statistical significance (p<0.05)).

	CWM	ETZ	GL	MWM	OR
ETZ	**0.01180**	-	-	-	-
GL	**2e-06**	0.23106	-	-	-
MWM	0.11371	0.23106	**0.00225**	-	-
OR	**0.02702**	0.11371	**0.00335**	0.98964	-
ST	**0.00019**	0.41480	0.35464	**0.01180**	**0.02702**

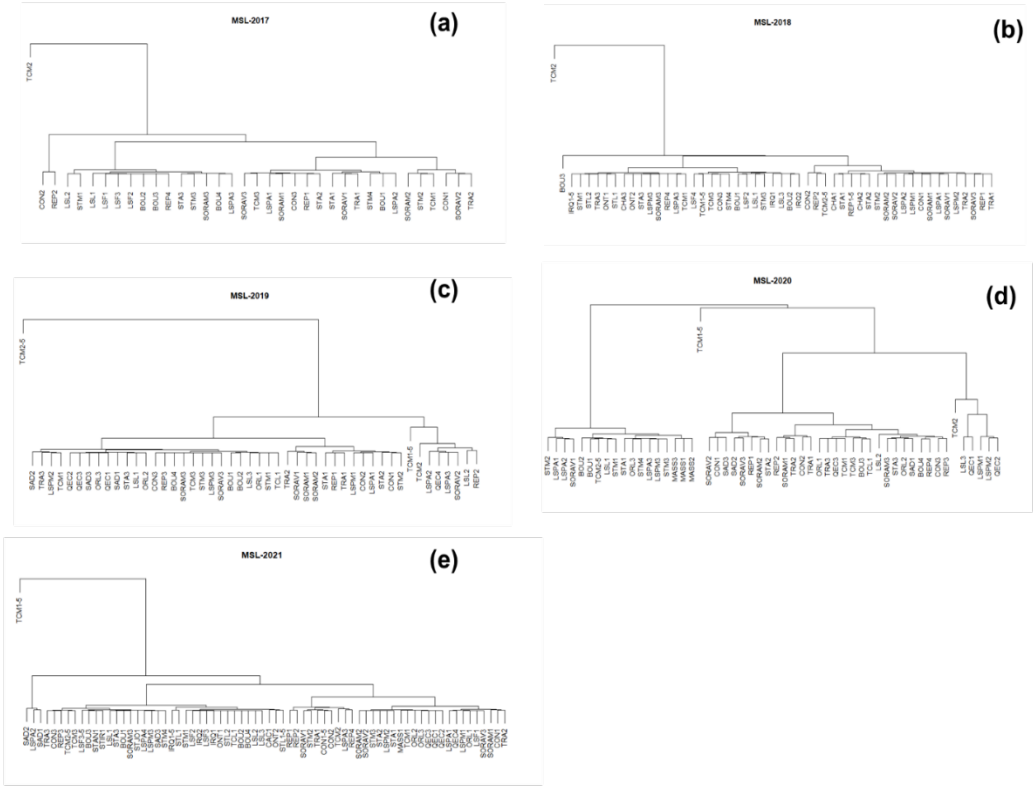


Figure 7-6. Cluster dendrograms (hierarchical cluster analysis, Euclidean distance, Ward's method) generated based on pharmaceutical data generated for St. Lawrence in 2017(a), 2018 (b), 2019 (c), 2020 (d), and 2021 (e).

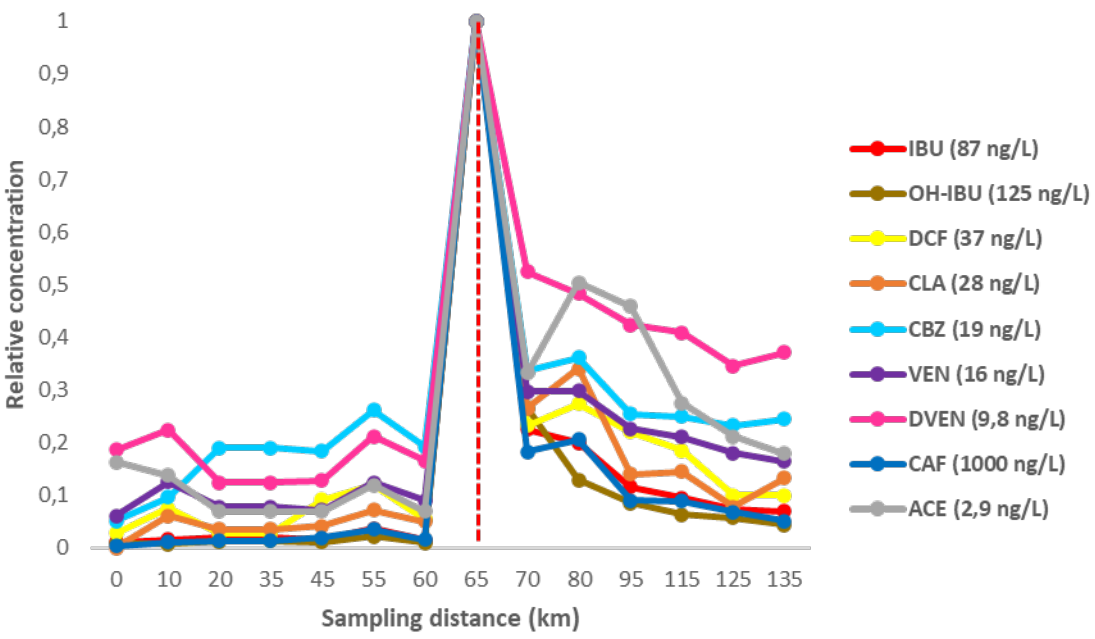


Figure 7-7. Ratio of detected pharmaceuticals normalized to the maximum quantified concentration (amount aside compound name) of surface water (approx. 1 m depth). The dotted vertical line represents the position of the wastewater rejection point in the river.

Table 7-15. Environmental guidelines for pharmaceuticals in freshwater surface waters (ng/L), published or under review by the European Union (EU), Canada (https://ccme.ca/en/summary-table) and British Columbia (BC, Canada) (https://bcgov-env.shinyapps.io/bc_wqg/).

Compound	EU annual average QS (ng/L)	EU maximum admissible concentration QS (ng/L)	Canadian or BC guideline (ng/L)
Diclofenac	100[a]; 40[b]; 5.4[c]	250 000[d]	
Carbamazepine	2000[e]; 2500[f]	1 600 000[g]	10 000[h]
Sulfamethoxazole	600[i]	2700[j]	
EE2	0.035[k]; 0.017[l]		0.5[m]; 0.75[n]
E2	0.4[o]; 0.18[p]		
Clarithromycin	130[q]	130[r]	
Ibuprofen	140[s]		

[a] Current European Union environmental quality standard (EQS) for diclofenac in freshwater surface water (aquatic biota), annual average concentration (AA-$QS_{fw\ eco}$) (Diclofenac Dossier 2011); [b] Draft European Union EQS for diclofenac in freshwater surface water (aquatic biota), annual average concentration (AA-$QS_{fw\ eco}$) (SCHEER 2022); [c] Draft European Union QS for diclofenac in freshwater surface water to prevent the secondary poisoning of birds ($QS_{biota\ sec\ pois}$) (SCHEER 2022); [d] Draft maximum admissible concentration of diclofenac in freshwater surface water (MAC-QS_{fw}) (SCHEER 2022); [e] Current European Union environmental quality standard (EQS) for carbamazepine in freshwater surface water (aquatic biota), annual average concentration (AA-$QS_{fw\ eco}$); [f] Draft alternative AA-$QS_{fw\ eco}$ proposed by SCHEER for carbamazepine (SCHEER 2022); [g] Draft MAC-QS_{fw} proposed by SCHEER for carbamazepine (SCHEER 2022); [h] CCME 2018, Long-term exposure of aquatic biota, freshwater Canadian Water Quality Guideline (CWQG); [i] Current European Union environmental quality standard (EQS) for sulfamethoxazole in freshwater surface water (aquatic biota), annual average concentration (AA-$QS_{fw\ eco}$); [j] Current European Union environmental quality standard (EQS) for sulfamethoxazole in freshwater surface water (aquatic biota), maximum admissible concentration (MAC-QS_{fw}); [k] Current European Union environmental quality standard (EQS) for EE2 in freshwater surface water (aquatic biota), annual average concentration (AA-$QS_{fw\ eco}$) (EE2 Dossier 2011); [l] Draft alternative AA-$QS_{fw\ eco}$ proposed by SCHEER for EE2 (SCHEER 2022); [m] British Columbia Ministry of Environment and Climate Change Strategy. Long-term chronic guideline for aquatic biota; [n] British Columbia Ministry of Environment and Climate Change Strategy. Short-term acute guideline for aquatic biota; [o] Current European Union environmental quality standard (EQS) for E2 in freshwater surface water (aquatic biota), annual average concentration (AA-$QS_{fw\ eco}$) (E2 Dossier 2011); [p] Draft alternative AA-$QS_{fw\ eco}$ proposed by SCHEER for E2 (SCHEER 2022); [q] Draft European Union EQS for clarithromycin in freshwater surface water (aquatic biota), annual average concentration (AA-$QS_{fw\ eco}$) (SCHEER 2022); [r] Draft maximum admissible concentration of clarithromycin in freshwater surface water (MAC-QS_{fw}) (SCHEER 2022); [s] Draft European Union EQS for ibuprofen in freshwater surface water (aquatic biota), annual average concentration (AA-$QS_{fw\ eco}$) (SCHEER 2022).

Table 7-16. St. Lawrence River data (2017).

		Concentrations of detected pharmaceuticals (ng/L)							
Water Mass	Site	IBU	DCF	CLA	CBZ	VEN	DVEN	CAF	ACE
GL	BOU1	7,12	1,33	<LOD	2,97	2,25	<LOD	52,7	0,49
GL	BOU2	<LOD	0,49	<LOD	<LOD	<LOD	<LOD	16,1	<LOD
GL	BOU3	<LOD	0,29	<LOD	<LOQ	<LOD	<LOD	16,2	<LOD
GL	BOU4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	19,5	<LOD
OR	CON1	11,4	1,91	0,80	3,00	5,22	7,08	87,6	1,51
CWM	CON2	24,2	2,44	1,00	3,16	3,82	4,74	210	1,44
CWM	CON3	3,77	0,93	<LOD	<LOQ	<LOQ	<LOD	34,2	<LOD
GL	LSF1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	14,2	<LOD
GL	LSF2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	17,0	<LOD
GL	LSF3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	15,2	<LOD
OR	LSL1	<LOD	<LOD	<LOD	<LOD	1,61	<LOD	11,8	0,39
GL	LSL2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
OR	LSPA1	6,57	0,61	0,37	1,86	2,35	3,59	38,3	0,65
CWM	LSPA2	8,52	0,88	0,39	2,14	2,11	3,64	52,8	0,51
ST	LSPA3	<LOD	0,47	<LOD	<LOQ	1,50	<LOD	21,5	<LOD
OR	REP1	4,57	0,99	<LOD	<LOD	2,44	<LOD	38,6	0,69
CWM	REP2	27,4	2,07	0,78	2,93	3,81	5,14	186	0,95
CWM	REP4	<LOD	0,29	<LOD	<LOD	<LOD	<LOD	16,3	<LOD
OR	SORAM1	5,72	0,98	<LOD	<LOQ	2,59	3,99	39,0	0,84
CWM	SORAM2	9,04	0,89	<LOD	2,03	2,31	3,39	69,4	0,61
ST	SORAM3	3,74	0,34	<LOD	<LOD	<LOD	<LOD	23,1	<LOD
OR	SORAV1	5,87	1,31	0,36	2,13	3,34	4,93	45,3	0,89
CWM	SORAV2	11,5	1,65	0,43	2,19	2,70	4,01	90,0	0,79
ST	SORAV3	<LOD	0,66	<LOD	<LOQ	<LOD	<LOD	39,2	<LOD
MWM	STA1	7,49	0,64	0,38	<LOQ	2,18	3,71	44,3	0,66
MWM	STA2	5,96	0,68	<LOD	1,92	1,80	<LOD	38,1	0,59
MWM	STA3	<LOD	0,30	<LOD	2,10	2,13	3,35	19,2	0,78
OR	STM1	<LOD	1,22	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
CWM	STM2	10,3	1,49	0,36	2,58	2,65	5,78	71,2	1,55
ST	STM3	<LOD	0,43	<LOD	2,34	2,43	3,73	20,8	0,96
ST	STM4	10,6	3,18	0,45	2,22	2,63	3,98	58,1	0,68
OR	TCM1	12,5	1,64	0,48	2,08	4,00	4,94	70,9	1,06
CWM	TCM2	119	9,29	3,05	9,20	13,3	10,4	962	2,97
CWM	TCM3	3,60	0,79	<LOD	<LOQ	<LOQ	<LOD	44,0	<LOD
OR	TRA1	7,31	1,21	0,45	2,19	3,75	5,39	46,0	1,28
CWM	TRA2	14,1	1,96	0,41	2,23	2,90	4,16	92,1	1,31

Table 7-17. St. Lawrence River data (2018).

		Concentrations of detected pharmaceuticals (ng/L)										
Water Mass	**Site**	**IBU**	**OH-IBU**	**DCF**	**CLA**	**E1**	**CBZ**	**2OH-CBZ**	**VEN**	**DVEN**	**CAF**	**ACE**
GL	**BOU1**	<LOD	84,1	<LOD	<LOD	<LOD	2,46	<LOD	1,83	7,45	63,9	0,36
GL	**BOU2**	<LOD	123	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4,55	26,9	<LOD
GL	**BOU3**	<LOD	1142	<LOD	<LOD	<LOD	2,30	<LOD	<LOQ	6,52	23,6	<LOD
GL	**CHA1**	<LOD	<LOD	0,36	<LOD	<LOD	2,61	<LOD	2,39	<LOD	88,9	0,42
GL	**CHA2**	5,05	36,9	1,44	<LOD	<LOD	2,78	<LOD	2,60	7,63	99,6	0,36
GL	**CHA3**	<LOD	7,98	0,63	<LOD	<LOD	2,81	<LOD	2,24	5,16	37,5	0,69
OR	**CON1**	21,6	<LOD	3,53	0,46	<LOD	3,62	13,5	4,75	5,88	186	1,14
CWM	**CON2**	15,1	46,0	2,21	0,42	<LOD	4,22	<LOD	4,91	16,3	292	0,90
CWM	**CON3**	<LOD	70,7	<LOD	<LOD	<LOD	<LOQ	<LOD	1,37	<LOD	22,3	<LOD
GL	**IRQ1**	<LOD	153	0,56	<LOD	<LOD	<LOD	<LOD	<LOD	8,31	59,5	<LOD
GL	**IRQ1-5**	<LOD	8,01	<LOD	<LOD	<LOD	<LOD	<LOD	1,43	3,59	<LOD	<LOD
GL	**IRQ2**	<LOD	112	<LOQ	<LOD	<LOD	<LOD	<LOD	1,66	5,83	33,0	<LOD
GL	**LSF2**	<LOD	94,4	3,00	<LOD	<LOD	<LOQ	<LOD	<LOD	5,09	53,7	<LOD
GL	**LSF4**	<LOD	64,4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4,47	<LOD	<LOD
OR	**LSL1**	<LOD	83,2	2,20	<LOD	<LOD	2,67	<LOD	2,01	6,99	90,0	<LOD
GL	**LSL3**	<LOD	111	0,79	<LOD	<LOD	<LOD	<LOD	<LOD	4,48	<LOD	<LOD
OR	**LSPA1**	22,3	6,91	<LOQ	<LOD	<LOD	3,35	4,8	3,58	12,1	167	0,75
CWM	**LSPA2**	6,66	23,8	3,24	<LOD	<LOD	3,08	7,8	3,11	7,14	205	0,50
ST	**LSPA3**	<LOD	15,6	<LOD	<LOD	<LOD	4,75	18,0	3,89	7,76	34,8	1,34
OR	**LSPM1**	18,7	39,8	2,26	<LOQ	<LOD	3,47	8,8	3,53	10,1	206	0,58
CWM	**LSPM2**	15,6	<LOD	1,36	<LOD	<LOD	2,36	57,4	2,91	35,2	120	<LOD
ST	**LSPM3**	<LOD	<LOD	0,37	<LOD	<LOD	<LOD	10,8	2,75	<LOD	36,1	<LOQ
GL	**ONT1**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	35,8	<LOD	7,87	<LOD	<LOD
GL	**ONT2**	<LOD	<LOD	2,88	<LOD	<LOD	<LOD	<LOD	1,37	9,26	33,7	<LOD
OR	**REP1**	22,2	<LOD	1,19	0,43	<LOD	3,42	8,6	6,19	16,7	143	0,71
OR	**REP1-5**	18,1	36,9	0,23	<LOD	<LOD	2,56	<LOD	3,07	9,87	73,5	<LOD
CWM	**REP2**	38,1	27,6	4,47	0,68	2,00	4,51	13,8	5,52	17,6	413	0,92
CWM	**REP4**	<LOD	43,3	<LOD	<LOD	<LOD	<LOD	<LOD	1,45	5,31	24,4	<LOD
OR	**SORAM1**	16,0	<LOD	1,71	<LOD	<LOD	3,37	23,9	5,07	6,87	194	0,76
CWM	**SORAM2**	11,4	54,3	1,63	<LOD	<LOD	3,22	<LOD	3,19	12,8	173	0,64
ST	**SORAM3**	<LOD	<LOD	0,33	<LOD	<LOD	<LOD	5,7	1,37	<LOD	21,1	<LOQ
OR	**SORAV1**	14,3	8,90	1,71	0,51	<LOD	3,90	<LOD	4,62	21,8	176	0,68
CWM	**SORAV2**	10,6	64,0	4,55	<LOD	<LOD	2,82	<LOD	2,13	9,57	165	0,39
ST	**SORAV3**	10,3	<LOD	0,86	<LOD	<LOD	2,57	17,4	2,78	4,34	135	0,36
MWM	**STA1**	5,60	<LOD	0,41	<LOQ	<LOD	2,63	<LOD	2,33	9,66	83,0	0,45
MWM	**STA2**	5,74	20,0	1,64	<LOD	<LOD	2,74	<LOD	2,65	9,44	105	0,49
MWM	**STA3**	<LOD	<LOD	2,04	<LOD	<LOD	2,45	<LOQ	1,98	4,10	38,5	<LOQ
GL	**STL1**	<LOD	31,7	0,66	<LOD	<LOD	<LOD	24,2	<LOD	5,32	<LOD	<LOD
GL	**STL2**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	11,7	1,91	7,56	<LOD	<LOD
OR	**STM1**	<LOD	12,1	1,76	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
CWM	**STM2**	5,11	73,2	0,16	<LOD	<LOD	2,94	<LOD	3,16	10,3	145	0,57
ST	**STM3**	4,09	104	0,69	<LOD	<LOD	2,39	<LOD	1,60	8,54	76,3	0,55
ST	**STM4**	<LOD	64,9	<LOD	<LOD	<LOD	2,68	11,3	2,31	3,33	34,9	0,51
OR	**TCM1**	<LOD	18,7	0,73	<LOQ	<LOD	2,58	<LOD	3,67	20,9	29,2	0,50
CWM	**TCM1-5**	<LOD	55,7	12,2	<LOD	<LOD	<LOD	<LOD	3,10	4,13	<LOQ	<LOD
CWM	**TCM2**	858	1583	57,9	25,1	8,38	62,8	482,7	87,5	267	7175	11,7
CWM	**TCM2-5**	52,4	60,1	5,19	0,71	1,71	5,57	16,7	5,52	20,2	438	0,76
CWM	**TCM3**	15,4	81,2	<LOD	<LOD	<LOD	<LOD	<LOD	1,59	5,18	38,7	<LOQ
OR	**TRA1**	16,4	<LOD	1,44	<LOD	<LOD	4,08	12,4	5,17	13,7	156	1,00
CWM	**TRA2**	12,5	27,7	0,65	<LOD	<LOD	3,00	12,1	2,79	10,3	151	0,68
CWM	**TRA3**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	4,1	1,29	<LOD	<LOD	<LOD

Table 7-18. St. Lawrence River data (2019).

		Concentrations of detected pharmaceuticals (ng/L)												
Water Mass	**Site**	**IBU**	**OH-IBU**	**DCF**	**OH-DCF**	**SMX**	**CLA**	**E1**	**CBZ**	**VEN**	**DVEN**	**FLU**	**CAF**	**ACE**
GL	**BOU1**	<LOD	17,7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ
GL	**BOU2**	<LOD	23,0	<LOD	1,04	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ
GL	**BOU4**	<LOD	<LOD	<LOQ	<LOQ	<LOD	0,50	<LOD	<LOD	<LOD	<LOD	<LOQ	24,0	<LOD
OR	**CON1**	6,74	<LOD	1,11	<LOD	<LOD	0,57	<LOD	4,61	5,27	10,8	<LOD	86,5	1,61
CWM	**CON2**	8,41	<LOD	0,96	<LOQ	<LOD	0,35	0,82	5,22	3,47	4,87	<LOD	114	1,76
CWM	**CON3**	<LOD	<LOD	0,51	<LOD	<LOD	<LOD	0,47	<LOD	1,33	<LOD	<LOD	19,6	1,30
OR	**LSL1**	<LOD	<LOD	0,91	<LOQ	<LOD	0,62	<LOQ	<LOD	1,36	<LOD	<LOD	45,8	<LOQ
GL	**LSL2**	<LOD	626	1,14	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	30,9	1,30
GL	**LSL3**	<LOQ	7,72	<LOD	<LOQ	<LOD	<LOD	0,44	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ
OR	**LSPA1**	9,40	<LOD	0,90	<LOQ	<LOD	0,53	<LOD	3,91	3,50	6,16	<LOD	116	1,30
CWM	**LSPA2**	<LOQ	228	0,51	<LOQ	<LOD	<LOD	<LOQ	3,11	2,05	<LOD	<LOD	43,8	1,34
ST	**LSPA3**	<LOD	323	0,69	<LOQ	<LOD	<LOD	<LOD	<LOD	1,77	<LOD	<LOD	46,4	<LOQ
OR	**LSPM1**	10,0	<LOD	1,25	<LOD	<LOD	0,55	0,51	4,60	5,42	8,40	<LOD	128	1,71
CWM	**LSPM2**	3,70	55,7	0,38	<LOD	<LOD	<LOD	<LOQ	<LOQ	1,99	<LOD	<LOD	38,1	1,45
ST	**LSPM3**	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	1,77	<LOD	<LOD	26,4	<LOD
ETZ	**ORL1**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3,44	2,10	<LOQ	<LOD	<LOQ	<LOD
ETZ	**ORL2**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3,51	2,23	<LOQ	<LOD	44,9	<LOD
ETZ	**ORL3**	<LOD	<LOD	<LOD	<LOD	<LOD	0,41	<LOD	3,56	2,58	<LOQ	<LOD	58,1	<LOD
MWM	**QEC1**	<LOQ	<LOD	0,95	<LOQ	<LOD	<LOD	<LOD	3,40	2,69	3,98	<LOD	60,7	1,35
MWM	**QEC2**	4,08	<LOD	0,59	<LOQ	<LOD	<LOD	<LOD	3,54	3,22	4,79	<LOD	69,6	1,36
MWM	**QEC3**	<LOQ	<LOD	1,11	<LOQ	<LOD	<LOD	<LOD	3,27	2,58	<LOD	<LOD	65,8	<LOQ
MWM	**QEC4**	<LOQ	286	0,43	<LOQ	<LOD	<LOD	<LOD	3,45	3,12	3,49	<LOD	67,9	1,54
OR	**REP1**	14,2	<LOD	2,71	<LOD	<LOD	0,43	<LOD	5,24	6,52	13,5	<LOD	165	1,54
CWM	**REP2**	11,9	441	1,71	<LOQ	<LOD	0,59	0,92	4,61	3,41	4,23	<LOD	158	1,45
CWM	**REP3**	<LOD	<LOD	0,45	<LOD	<LOD	<LOD	<LOD	<LOD	1,18	<LOD	<LOD	19,3	<LOQ
MWM	**SAD1**	<LOQ	28,6	0,24	<LOQ	<LOD	<LOD	<LOD	<LOQ	2,37	3,77	<LOD	47,5	1,37
MWM	**SAD2**	<LOQ	111	0,37	<LOQ	<LOD	0,62	<LOD	3,00	2,37	3,37	<LOD	61,8	1,40
MWM	**SAD3**	<LOQ	<LOD	0,31	<LOD	<LOD	0,34	<LOD	3,11	2,24	3,81	<LOD	53,1	1,32
OR	**SORAM1**	23,4	<LOD	2,39	<LOQ	<LOD	0,61	0,36	5,88	5,13	10,7	<LOD	253	1,89
CWM	**SORAM2**	21,6	<LOD	2,08	<LOD	<LOD	0,51	0,53	5,53	4,37	8,48	<LOD	269	1,92
ST	**SORAM3**	<LOD	<LOD	0,42	<LOD	<LOD	<LOD	0,41	<LOQ	1,55	<LOD	<LOD	20,9	<LOQ
OR	**SORAV1**	18,4	<LOD	1,94	<LOQ	<LOD	1,12	0,81	6,74	5,69	10,7	<LOQ	217	1,87
CWM	**SORAV2**	7,14	332	0,71	1,04	<LOD	<LOD	0,45	4,18	3,23	4,62	<LOD	81,0	1,56
ST	**SORAV3**	<LOQ	3,63	0,35	<LOQ	<LOD	<LOD	<LOQ	<LOD	1,48	<LOD	<LOD	24,7	1,40
MWM	**STA1**	<LOD	<LOD	1,23	<LOQ	<LOD	0,60	0,86	3,45	3,23	13,1	<LOQ	152	2,99
MWM	**STA2**	6,80	27,5	0,58	<LOQ	<LOD	<LOD	<LOQ	3,51	2,87	5,03	<LOD	96,8	1,45
MWM	**STA3**	<LOD	6,83	0,90	<LOD	<LOD	<LOD	<LOD	<LOQ	1,98	<LOD	<LOD	41,7	<LOQ
OR	**STM1**	<LOD	<LOD	0,26	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1,30
CWM	**STM2**	4,62	<LOD	1,24	1,01	<LOD	<LOD	<LOQ	3,66	2,73	5,09	<LOD	89,2	1,47
ST	**STM3**	3,67	<LOD	0,33	<LOQ	<LOD	<LOD	<LOD	3,01	1,85	<LOD	<LOD	28,5	<LOQ
GL	**TCL1**	<LOD	<LOD	0,59	<LOQ	<LOD	0,52	<LOQ	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD
OR	**TCM1**	<LOQ	69,7	1,61	<LOQ	<LOD	<LOQ	<LOD	3,28	4,22	11,3	<LOD	44,3	1,41
CWM	**TCM1-5**	123	358	5,52	<LOQ	<LOD	1,94	2,21	12,5	14,6	22,5	<LOD	1650	2,66
CWM	**TCM2**	52,2	318	4,92	1,19	<LOD	0,94	2,37	7,97	7,61	13,9	<LOD	640	2,76
CWM	**TCM2-5**	817	1807	46,0	10,2	27,1	16,0	4,76	84,9	96,7	154	<LOQ	6396	15,1
CWM	**TCM3**	<LOQ	<LOD	0,25	<LOD	<LOD	0,51	<LOD	<LOD	1,28	<LOD	<LOQ	22,6	<LOD
OR	**TRA1**	17,1	<LOD	1,95	<LOQ	<LOD	0,50	<LOQ	5,54	5,87	14,3	<LOD	162	1,98
CWM	**TRA2**	31,7	<LOD	2,81	<LOQ	<LOD	0,66	1,13	7,34	6,33	11,3	<LOD	344	2,00
CWM	**TRA3**	<LOD	77,5	0,46	<LOQ	<LOD	0,13	<LOD	<LOD	1,17	<LOD	<LOD	<LOD	<LOQ

Table 7-19. St. Lawrence River data (2020).

		Concentrations of detected pharmaceuticals (ng/L)											
Water Mass	Site	IBU	OH-IBU	DCF	OH-DCF	CLA	MTX	E1	CBZ	2OH-CBZ	VEN	CAF	ACE
GL	BOU1	5,83	<LOD	1,28	1,12	<LOD	<LOD	<LOD	<LOD	<LOD	2,38	20,1	<LOQ
GL	BOU2	6,79	31	0,91	1,22	<LOD	<LOD	<LOD	<LOD	<LOD	2,24	20,3	<LOD
GL	BOU3	5,32	366	1,24	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	22,7	1,46
GL	BOU4	4,30	436	<LOD	1,05	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	19,0	2,04
OR	CON1	14,0	108	2,59	<LOD	<LOD	<LOD	<LOD	4,47	<LOD	6,47	144	1,58
CWM	CON2	16,6	560	2,49	<LOQ	<LOD	1,40	<LOD	4,17	<LOD	4,45	226	2,36
CWM	CON3	5,29	492	1,13	<LOQ	<LOD	1,07	<LOD	<LOD	<LOD	2,15	56,7	1,75
OR	LSL1	4,74	<LOD	1,95	1,27	<LOD	<LOD	<LOD	3,28	<LOD	6,02	<LOD	<LOD
GL	LSL2	5,44	595	9,38	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	19,5	<LOD
GL	LSL3	<LOD	1332	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	19,1	1,75
OR	LSPA1	11,8	<LOD	1,59	1,18	<LOD	<LOD	<LOD	3,71	<LOD	4,40	174	<LOQ
CWM	LSPA2	14,4	<LOD	1,21	<LOQ	<LOD	<LOD	<LOD	3,48	<LOD	5,01	189	<LOQ
ST	LSPA3	6,92	<LOD	1,12	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	2,70	65,9	1,72
OR	LSPM1	15,0	739	1,86	1,08	<LOD	<LOD	<LOD	3,99	<LOD	3,70	159	1,44
CWM	LSPM2	5,89	781	0,87	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	2,43	59,5	1,53
ST	LSPM3	8,80	<LOQ	0,60	<LOD	<LOD	<LOD	<LOD	3,03	<LOD	3,24	68,2	1,92
ETZ	MASS1	<LOD	64	<LOD	<LOQ	<LOD	<LOD	<LOD	2,83	<LOD	<LOQ	56,2	1,82
ETZ	MASS2	<LOD	83	<LOD	1,25	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOQ	68,3	1,89
ETZ	MASS3	<LOD	37	<LOD	1,15	<LOD	<LOD	<LOD	3,24	<LOD	<LOQ	60,9	2,05
ETZ	ORL1	<LOD	376	<LOD	<LOQ	<LOD	<LOD	<LOD	3,07	<LOD	<LOQ	63,2	2,12
ETZ	ORL2	<LOD	433	1,04	<LOD	<LOD	<LOD	<LOD	3,51	<LOD	2,88	76,9	2,10
ETZ	ORL3	<LOD	<LOD	1,07	<LOD	<LOD	<LOD	<LOD	3,29	<LOD	3,91	78,3	1,90
MWM	QEC1	8,03	1417	1,10	<LOD	<LOD	<LOD	<LOD	3,14	<LOD	2,98	89,0	1,70
MWM	QEC2	5,98	823	1,04	<LOQ	<LOD	2,19	<LOD	3,35	<LOD	3,41	95,9	1,75
MWM	QEC3	7,70	362	1,19	<LOD	<LOD	1,22	<LOD	2,92	<LOD	3,46	86,3	1,91
OR	REP1	23,7	254	3,17	0,99	0,45	1,50	<LOD	5,30	<LOD	10,4	188	<LOQ
CWM	REP2	20,2	392	2,39	<LOQ	<LOD	<LOD	<LOD	3,45	<LOD	4,62	224	2,86
CWM	REP3	5,31	496	1,84	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2,39	22,9	1,68
CWM	REP4	6,63	446	1,20	1,03	<LOD	<LOD	<LOD	<LOQ	<LOD	2,04	32,7	1,91
MWM	SAD1	<LOD	457	1,00	1,08	<LOD	<LOD	<LOQ	2,86	<LOD	2,51	88,4	<LOD
MWM	SAD2	6,61	270	1,18	<LOD	<LOD	<LOD	<LOD	2,92	<LOD	3,10	91,7	<LOQ
MWM	SAD3	5,97	131	0,93	<LOQ	<LOD	<LOD	<LOD	2,74	<LOD	3,07	83,0	<LOD
OR	SORAM1	17,1	435	2,45	<LOD	<LOD	0,39	<LOD	3,72	<LOD	5,57	186	1,87
CWM	SORAM2	16,6	295	1,46	0,97	<LOD	<LOD	<LOD	2,81	<LOD	2,31	129	<LOQ
ST	SORAM3	<LOD	415	1,02	1,33	<LOD	<LOD	<LOD	<LOQ	<LOD	2,30	52,2	<LOQ
OR	SORAV1	16,6	<LOD	1,95	1,04	<LOD	<LOD	<LOD	3,92	<LOD	5,89	202	2,10
CWM	SORAV2	8,53	189	1,76	<LOQ	<LOD	<LOD	<LOD	3,35	<LOD	3,06	133	1,52
ST	SORAV3	5,77	239	0,99	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	51,6	1,66
MWM	STA1	8,31	<LOD	1,10	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	2,98	90,7	<LOD
MWM	STA2	9,46	303	0,97	<LOD	<LOD	<LOD	<LOD	3,00	<LOD	2,93	118	1,59
MWM	STA3	4,38	405	0,62	<LOD	<LOD	<LOD	<LOD	3,18	<LOD	2,38	75,9	1,73
OR	STM1	<LOD	<LOD	0,86	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
CWM	STM2	12,8	<LOD	1,24	<LOD	<LOD	<LOD	<LOD	2,63	<LOD	4,01	130	1,94
ST	STM3	5,81	<LOD	0,88	<LOD	<LOD	<LOD	<LOD	2,76	<LOD	2,45	70,3	1,69
ST	STM4	<LOD	<LOD	0,60	<LOD	<LOD	<LOD	<LOD	3,43	<LOD	2,33	73,9	1,73
GL	TCL1	4,29	363	0,87	<LOD	0,69	0,98	<LOD	<LOD	20,3	<LOQ	28,4	2,56
OR	TCM1	7,49	339	1,86	<LOD	0,38	0,84	<LOD	3,35	18,6	3,79	77,4	<LOD
CWM	TCM1-5	362	1211	30,6	6,77	4,82	2,59	2,94	24,6	253	51,6	3755	3,87
CWM	TCM2	126	1668	11,1	1,75	2,12	1,89	<LOQ	11,6	97,1	21,1	1305	4,58
CWM	TCM2-5	5,19	<LOD	1,08	1,23	0,83	2,30	1,14	<LOD	33,5	2,34	15,7	2,22
CWM	TCM3	8,12	332	1,16	1,78	0,61	1,33	<LOD	<LOQ	25,1	2,40	24,0	2,24
OR	TRA1	13,3	495	2,58	1,01	<LOD	1,24	<LOD	4,56	<LOD	8,15	150	1,66
CWM	TRA2	19,5	455	2,04	<LOQ	<LOD	<LOD	<LOD	3,77	<LOD	4,86	215	1,89
CWM	TRA3	5,55	356	0,98	0,96	<LOD	1,21	<LOD	2,77	<LOD	2,45	57,7	1,66

Table 7-20. St. Lawrence River data (2021).

		Concentrations of detected pharmaceuticals (ng/L)													
Water Mass	**Site**	**IBU**	**OH-IBU**	**HMOR**	**DCF**	**OH-DCF**	**CLA**	**MTX**	**E1**	**CBZ**	**2OH-CBZ**	**VEN**	**DVEN**	**CAF**	**ACE**
GL	**BOU1**	10,9	<LOD	<LOD	1,90	0,41	<LOD	<LOD	<LOD	1,59	<LOD	2,87	6,17	76,0	0,47
GL	**BOU2**	6,99	<LOD	<LOD	0,98	0,55	<LOD	<LOD	<LOD	1,61	<LOD	2,29	4,25	41,2	0,44
GL	**BOU3**	6,17	<LOD	<LOD	3,12	0,43	<LOD	<LOD	<LOD	1,58	<LOD	2,68	4,61	61,0	0,46
GL	**BOU4**	6,57	<LOD	<LOD	<LOD	0,47	0,53	<LOD	<LOD	1,70	<LOD	1,93	4,34	40,3	<LOQ
ETZ	**CAC1**	<LOD	<LOD	<LOQ	<LOD	0,53	<LOD	<LOD	<LOD	1,01	<LOD	<LOD	<LOD	<LOD	<LOD
OR	**CON1**	23,9	<LOD	<LOD	2,90	0,36	<LOD	<LOQ	<LOD	5,12	37,9	8,69	15,0	167	0,75
OR	**CON1-5**	20,0	<LOD	<LOD	2,13	<LOD	<LOD	<LOQ	<LOD	5,09	40,5	8,23	14,4	208	0,83
CWM	**CON2**	23,9	<LOD	<LOD	2,43	0,35	<LOD	<LOQ	1,23	3,96	41,9	5,14	10,1	195	0,53
CWM	**CON3**	9,59	<LOD	<LOQ	1,71	0,56	<LOD	<LOQ	<LOD	3,10	36,7	2,33	4,41	64,4	<LOQ
GL	**IRQ1**	5,94	<LOD	<LOD	6,69	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1,91	<LOD	49,6	0,38
GL	**IRQ1-5**	4,88	<LOD	93,7	5,69	0,45	<LOD	<LOD	<LOD	<LOD	<LOD	2,12	<LOD	43,0	<LOQ
GL	**IRQ2**	6,13	<LOD	<LOD	7,85	0,55	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3,82	38,7	0,34
GL	**LSF1**	7,99	<LOD	<LOD	8,40	0,60	<LOD	<LOD	<LOD	<LOD	<LOD	2,19	3,76	153	<LOQ
GL	**LSF2**	6,40	<LOD	<LOD	7,56	0,62	<LOD	<LOD	<LOD	<LOD	<LOD	2,19	<LOD	35,4	<LOQ
GL	**LSF3**	6,91	<LOD	<LOD	9,42	0,41	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	39,6	<LOQ
OR	**LSF3-5**	8,67	<LOD	<LOD	9,27	0,49	<LOD	<LOD	<LOD	<LOD	<LOD	8,15	12,6	55,9	0,72
OR	**LSL1**	10,3	<LOD	<LOD	4,42	0,36	<LOD	<LOD	<LOD	<LOD	<LOD	5,93	8,74	74,1	0,54
GL	**LSL2**	6,93	<LOD	<LOD	3,09	0,48	<LOD	<LOD	<LOD	<LOD	<LOD	2,03	3,60	42,0	0,39
GL	**LSL3**	7,63	<LOD	<LOD	6,44	0,54	<LOD	<LOD	<LOD	<LOD	<LOD	1,91	3,34	41,8	<LOQ
OR	**LSPA1**	<LOD	<LOD	<LOD	1,88	0,41	<LOD	<LOD	<LOD	2,56	<LOD	8,07	8,99	160	<LOQ
CWM	**LSPA2**	<LOD	<LOD	<LOD	1,58	<LOD	2,31	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	542	<LOD
ST	**LSPA3**	<LOD	<LOD	<LOD	2,78	<LOD	2,60	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	300	<LOD
ST	**LSPA4**	<LOD	<LOD	<LOD	1,48	<LOD	0,45	0,50	<LOD	<LOD	<LOD	6,63	3,72	72,8	<LOQ
OR	**LSPM1**	15,0	<LOD	<LOD	1,86	<LOD	<LOD	<LOD	<LOD	2,55	<LOD	5,57	10,6	138	<LOQ
CWM	**LSPM2**	8,63	<LOD	<LOD	1,57	<LOD	<LOD	<LOD	<LOD	2,04	<LOD	3,47	6,37	101	<LOD
ST	**LSPM3**	<LOD	<LOD	<LOD	1,15	<LOD	<LOD	<LOD	<LOD	1,93	<LOD	3,22	5,73	71,2	<LOQ
ETZ	**MASS1**	<LOD	<LOD	<LOD	0,98	<LOD	<LOD	<LOD	<LOD	1,84	<LOD	4,64	6,06	124	0,54
GL	**ONT1**	6,31	<LOD	<LOD	<LOD	0,55	0,60	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	45,4	<LOQ
GL	**ONT2**	6,67	<LOD	51,8	<LOD	0,41	<LOD	<LOD	<LOD	<LOD	<LOD	1,81	<LOD	43,3	<LOQ
ETZ	**ORL1**	<LOD	<LOD	<LOD	0,85	0,44	<LOD	<LOD	<LOD	1,51	<LOD	5,55	6,58	144	0,47
ETZ	**ORL2**	<LOD	<LOD	22,6	1,21	0,41	<LOD	<LOD	<LOD	1,88	<LOD	13,5	6,97	106	0,57
ETZ	**ORL3**	<LOD	<LOD	<LOQ	1,69	0,38	<LOD	<LOD	<LOD	2,21	<LOD	8,13	7,07	107	0,34
MWM	**QEC1**	<LOD	<LOD	<LOD	0,96	<LOD	<LOD	<LOD	<LOD	1,87	<LOD	12,1	7,82	98,6	0,65
MWM	**QEC2**	<LOD	<LOD	<LOD	1,02	0,36	<LOD	<LOD	<LOD	2,16	<LOD	17,5	8,44	95,4	0,69
MWM	**QEC3**	<LOD	<LOD	<LOQ	1,14	<LOD	<LOD	<LOD	<LOD	1,92	<LOD	17,5	9,28	105	0,51
MWM	**QEC4**	<LOD	<LOD	<LOD	1,24	<LOD	<LOD	<LOD	<LOD	2,12	<LOD	13,0	8,39	161	0,46
OR	**REP1**	20,2	<LOD	108	4,67	0,69	<LOD	<LOQ	<LOD	4,41	37,2	9,81	14,7	186	0,95
CWM	**REP2**	21,7	<LOD	74,0	4,82	0,73	<LOD	<LOQ	1,04	3,82	40,4	4,76	8,57	174	0,44
CWM	**REP3**	11,6	<LOD	<LOD	1,52	0,60	<LOD	<LOQ	<LOD	2,87	31,7	2,83	5,05	66,3	<LOQ
CWM	**REP4**	9,44	<LOD	26,0	1,62	0,47	<LOD	<LOQ	<LOD	2,95	33,5	2,42	4,30	279	<LOD
MWM	**SAD1**	10,5	<LOD	39,2	3,71	<LOD	<LOD	<LOD	<LOD	1,63	<LOD	36,8	5,72	547	0,57
MWM	**SAD2**	9,61	<LOD	<LOD	3,52	<LOD	<LOD	<LOD	<LOD	1,57	<LOD	12,6	4,80	664	0,36
MWM	**SAD3**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1,78	<LOD	5,23	6,24	77,5	0,38
OR	**SORAM1**	18,6	<LOD	37,4	5,10	0,36	<LOD	<LOD	<LOD	4,47	<LOD	6,06	11,1	135	0,42
CWM	**SORAM2**	14,5	<LOD	<LOD	1,51	0,37	<LOD	<LOD	<LOD	3,86	<LOD	4,58	9,11	103	0,39
ST	**SORAM3**	9,25	<LOD	<LOD	1,97	0,43	<LOD	<LOD	<LOD	3,17	<LOD	3,17	5,59	75,7	<LOD
OR	**SORAV1**	21,7	<LOD	<LOD	5,02	0,49	<LOD	<LOD	<LOD	4,66	<LOD	6,33	12,9	205	<LOQ
CWM	**SORAV2**	11,8	<LOD	<LOD	1,98	0,42	<LOD	<LOD	<LOD	3,33	<LOD	3,74	7,91	102	<LOQ
ST	**SORAV3**	11,5	<LOD	<LOD	1,52	<LOD	<LOD	<LOD	<LOD	3,32	<LOD	3,40	5,69	148	<LOD
MWM	**STA1**	9,68	<LOD	<LOD	2,08	<LOD	<LOD	<LOD	<LOD	1,75	<LOD	4,78	6,39	100	0,41
MWM	**STA2**	9,80	<LOD	<LOD	<LOD	0,39	<LOD	<LOD	<LOD	1,49	<LOD	5,14	6,31	102	0,36
MWM	**STA3**	8,78	<LOD	<LOQ	1,03	0,46	<LOD	<LOD	<LOD	1,54	<LOD	5,07	5,61	80,2	0,36
ETZ	**STAN1**	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,92	<LOD	<LOD	<LOQ	59,6	<LOD

ETZ	**STIR1**	<LOD	<LOD	<LOD	<LOD	0,43	<LOD	<LOD	<LOD	0,84	<LOD	<LOD	<LOD	63,3	<LOD
ETZ	**STJO1**	<LOD	<LOD	<LOD	<LOD	0,43	<LOD	<LOD	<LOD	1,33	<LOD	<LOQ	<LOQ	87,3	<LOQ
GL	**STL1**	6,03	<LOD	74,5	6,11	0,68	<LOD	<LOD	<LOD	<LOD	<LOD	1,90	<LOD	37,5	<LOQ
GL	**STL1-5**	5,05	<LOD	21,5	1,13	0,69	<LOD	<LOD	<LOD	<LOD	<LOD	1,86	3,33	36,6	0,35
GL	**STL2**	5,59	<LOD	<LOD	1,10	0,51	<LOD	<LOD	<LOD	<LOD	<LOD	1,78	3,38	36,2	<LOQ
OR	**STM1**	<LOD	<LOD	<LOD	0,68	<LOD	<LOQ	0,92	<LOD	<LOD	<LOD	2,12	<LOD	27,8	0,41
CWM	**STM2**	12,8	<LOD	<LOD	1,60	<LOD	<LOD	<LOD	<LOD	2,18	<LOD	6,40	6,83	217	<LOQ
ST	**STM3**	8,32	<LOD	<LOD	0,96	<LOD	<LOD	<LOD	<LOD	1,79	<LOD	5,64	6,39	95,5	<LOQ
ST	**STM4**	4,98	<LOD	<LOD	0,66	<LOD	<LOD	<LOD	<LOD	1,89	<LOD	6,60	5,95	74,9	<LOQ
GL	**TCL1**	6,13	<LOD	<LOD	0,75	0,57	<LOD	<LOD	<LOD	1,49	<LOD	2,09	3,86	36,6	0,42
OR	**TCM1**	17,2	4,16	<LOD	3,49	0,35	<LOD	<LOQ	0,97	4,00	21,8	7,05	11,8	118	0,63
CWM	**TCM1-5**	188	123	75,0	44,7	3,48	5,53	<LOQ	1,85	42,7	357	71,1	123	4615	6,59
CWM	**TCM2**	64,6	11,5	82,5	3,45	0,39	<LOD	<LOQ	1,28	3,01	38,8	7,54	13,3	289	0,79
CWM	**TCM2-5**	12,7	7,46	<LOQ	1,50	0,33	<LOD	<LOQ	9,73	2,01	21,7	4,32	5,98	75,7	<LOQ
CWM	**TCM3**	8,09	<LOQ	16,7	1,38	0,35	<LOD	<LOQ	0,94	1,84	21,1	3,14	5,33	69,2	0,35
OR	**TRA1**	25,9	<LOD	<LOD	1,60	<LOD	<LOD	<LOQ	<LOD	5,37	31,7	8,50	13,5	232	0,59
CWM	**TRA2**	17,7	<LOD	25,1	3,44	0,38	<LOD	<LOQ	<LOD	3,78	42,5	4,54	9,10	133	0,45
CWM	**TRA3**	7,44	<LOD	<LOD	0,91	<LOD	<LOD	<LOQ	<LOD	3,03	29,2	2,65	4,58	53,1	<LOQ

Table 7-21. Tributary rivers data (2018).

	Concentrations of detected pharmaceuticals (ng/L)												
	IBU	**OH-IBU**	**DCF**	**OH-DCF**	**CLA**	**MTX**	**CBZ**	**2OH-CBZ**	**VEN**	**DVEN**	**FLU**	**CAF**	**ACE**
Assomption	<LOD	269	2,29	<LOD	1,20	<LOD	20,0	37,4	27,9	79,5	<LOD	54,0	4,81
Batiscan	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Bayonne	<LOD	215	1,54	<LOD	0,34	<LOD	11,5	<LOQ	11,1	37,6	<LOD	194	4,36
Becancour	<LOD	290	<LOD	<LOD	<LOD	<LOD	6,28	<LOD	<LOQ	<LOD	<LOQ	17,0	1,30
Boyer	<LOD	294	0,24	<LOD	<LOD	<LOD	8,02	<LOQ	<LOD	<LOD	<LOD	38,8	<LOQ
Chateauguay	3,94	348	0,84	<LOD	0,79	<LOD	11,6	<LOQ	12,0	27,3	<LOD	<LOD	1,87
Chaudière	<LOD	237	0,98	<LOD	1,04	<LOD	11,9	<LOQ	13,1	27,1	<LOD	12,3	1,35
Des-Prairies	<LOD	211	1,60	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	13,1	<LOD	12,9	<LOQ
Du Chêne	<LOD	<LOD	2,09	<LOD	<LOD	<LOD	3,84	<LOD	<LOQ	18,7	<LOD	3,69	<LOD
Du Loup	<LOD	217	1,05	<LOD	<LOD	<LOD	7,09	<LOD	7,24	29,1	<LOD	<LOD	2,16
Jacques-Cartier	<LOD	247	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	7,03	<LOD
Mascouche	50,3	541	18,8	<LOD	3,16	<LOD	34,9	86,3	60,9	425	<LOD	116	5,89
Maskinonge	<LOD	216	1,21	<LOD	<LOD	<LOD	4,98	<LOD	8,17	10,2	<LOD	<LOD	<LOD
Mille-Iles	25,4	214	4,77	0,44	1,24	<LOD	4,03	<LOD	9,77	34,3	<LOD	144	0,94
Nicolet	<LOD	267	<LOD	<LOD	0,49	<LOD	18,9	34,4	8,70	<LOD	<LOD	<LOD	2,52
Outaouais	<LOD	<LOD	1,16	<LOD	<LOD	<LOD	<LOD	<LOD	3,48	<LOD	<LOD	43,2	<LOQ
Richelieu	19,0	394	1,37	<LOD	0,65	<LOD	6,22	<LOD	5,27	<LOD	<LOD	223	2,33
Saite-Anne	<LOD	280	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Saint-Charles	<LOD	197	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	23,2	<LOD
Saint-François	<LOD	<LOD	0,86	<LOD	0,74	<LOD	9,83	<LOQ	18,5	18,9	<LOD	<LOD	4,50
Saint-Jacques	83,8	475	5,74	<LOD	3,59	<LOD	10,4	34,3	24,5	182	<LOD	206	0,85
Saint-Régis	33,7	436	1,25	<LOD	1,90	<LOD	202	204	33,2	135	<LOD	127	4,86
Tortue	58,3	515	9,43	<LOD	0,70	<LOD	12,5	51,7	23,5	121	<LOD	154	<LOQ
Yamachiche	45,5	525	7,37	<LOD	0,39	4,19	4,13	<LOQ	6,50	41,5	<LOD	168	3,58
Yamaska	<LOD	209	5,73	<LOD	0,46	<LOD	35,3	65,4	21,5	18,8	<LOD	110	3,65

Table 7-22. Tributary rivers data (2019).

	Concentrations of detected pharmaceuticals (ng/L)											
	IBU	**OH-IBU**	**DCF**	**OH-DCF**	**CLA**	**E1**	**CBZ**	**2OH-CBZ**	**VEN**	**DVEN**	**CAF**	**ACE**
Assomption	9,06	656	7,59	<LOD	2,65	<LOD	14,1	257	20,1	104	94,1	1,49
Batiscan	4,43	356	<LOD	1,42	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	1,96
Bayonne	16,4	956	4,52	1,68	2,17	<LOD	8,83	175	14,3	78,5	131	<LOD
Beaupre	5,39	493	<LOD	1,64	0,60	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ
Becancour	4,64	1144	2,15	1,60	1,12	<LOQ	5,22	103	5,40	19,1	39,2	2,08
Boyer	7,10	232	2,45	<LOD	1,98	<LOQ	4,64	59,3	4,76	18,5	<LOD	1,41
Chateauguay	7,67	546	2,50	<LOD	0,58	<LOD	3,98	51,6	4,90	14,2	69,8	<LOD
Chaudière	4,71	<LOD	3,87	<LOD	0,83	<LOD	10,2	147	10,4	46,9	64,6	1,46
Des-Prairies	4,58	<LOD	3,61	<LOD	<LOD	<LOD	5,22	79,1	8,35	30,0	44,5	<LOD
Petite rivière du Chêne	5,00	522	2,96	<LOD	<LOD	<LOD	7,18	143	<LOQ	22,8	45,0	<LOD
Du Chêne	11,4	298	3,32	1,61	1,97	<LOD	4,80	<LOQ	7,17	26,5	69,8	<LOD
Du Loup	4,93	354	3,08	1,81	0,59	<LOD	6,93	135	11,1	58,6	38,1	1,91
Du Sud	5,69	550	2,27	1,46	<LOD	<LOD	4,74	43,4	<LOQ	<LOD	<LOD	2,76
Etchemin	4,84	1052	2,87	<LOD	2,12	<LOD	5,09	67,3	7,42	32,4	<LOQ	1,43
Gananoque	<LOQ	265	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	34,6	<LOD
Gentilly	4,54	464	2,51	<LOD	1,31	<LOD	<LOD	<LOD	<LOQ	<LOD	84,2	1,61
Grasse	4,37	952	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	33,4	1,49
Jacques-Cartier	<LOD	747	3,65	2,11	0,74	<LOD	4,49	82,0	8,98	31,9	39,2	1,75
Lancaster	5,69	374	1,93	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	38,8	<LOD
Mascouche	12,3	985	10,9	1,71	3,98	<LOD	30,6	451	49,3	258	92,0	2,46
Maskinonge	<LOD	<LOD	4,24	1,45	0,50	<LOD	8,12	157	10,2	13,7	40,7	2,54
Mille-Iles	35,6	236	6,94	1,67	7,80	<LOD	9,29	165	19,7	86,1	271	1,48
Montmorency	3,86	529	<LOD	1,58	0,39	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ
Nicolet	7,70	258	4,87	1,66	2,87	<LOD	13,1	212	21,8	44,3	31,3	5,81
Ogdensburg	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Outaouais	4,11	703	3,71	<LOD	0,88	<LOD	4,91	73,3	8,13	34,3	<LOQ	<LOQ
Raquette	9,93	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	69,6	<LOQ
Richelieu	27,1	<LOD	4,27	1,65	2,37	<LOD	6,86	131	13,1	64,2	243	2,02
Saite-Anne	4,53	983	<LOD	1,63	0,65	<LOD	<LOD	<LOD	<LOQ	8,82	<LOD	2,42
Saint-Charles	12,1	409	2,01	1,51	0,71	<LOD	<LOD	<LOQ	<LOD	19,2	74,2	<LOQ
Saint-François	20,1	655	4,48	1,56	1,61	<LOD	7,26	125	12,7	55,8	182	2,43
Saint-Jacques	21,1	756	4,11	<LOD	0,45	3,18	5,84	63,2	7,36	27,6	130	2,35
Saint-Maurice	<LOD	861	<LOD	1,63	<LOD	<LOD	<LOD	<LOD	<LOD	6,88	<LOQ	<LOQ
Saint-Louis	6,39	<LOD	1,96	<LOD	<LOD	<LOD	3,39	<LOD	<LOQ	10,7	44,6	<LOD
Saint-Régis	<LOD	2295	21,0	<LOD	<LOD	<LOD	46,8	<LOD	<LOD	<LOD	55,2	<LOD
Saumons	5,66	713	<LOD	<LOD	<LOD	<LOD	<LOD	46,9	<LOQ	<LOD	32,9	<LOD
Tortue	13,6	244	3,38	<LOD	0,53	<LOD	<LOD	<LOD	4,46	14,6	49,3	<LOQ
Varenne	10,1	273	2,73	<LOD	<LOD	<LOD	3,84	<LOD	4,18	<LOD	37,5	2,26
Yamachiche	4,82	240	2,04	<LOD	<LOD	<LOD	4,28	68,9	<LOD	<LOD	<LOQ	1,41
Yamaska	7,00	673	9,11	<LOD	1,21	<LOD	14,6	271	26,8	89,0	47,4	2,59

Table 7-23. Tributary rivers data (2020).

	Concentrations of detected pharmaceuticals (ng/L)													
	IBU	**OH-IBU**	**HMOR**	**DCF**	**OH-DCF**	**SMX**	**CLA**	**E2**	**E1**	**CBZ**	**VEN**	**DVEN**	**CAF**	**ACE**
A la Scie	8,20	249	<LOD	1,24	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,46
Assomption	15,4	1299	<LOD	2,05	<LOD	<LOD	<LOD	<LOD	<LOD	14,4	14,5	<LOD	90,6	0,73
Aulneuse	<LOD	<LOD	<LOD	2,27	<LOD	<LOD	<LOD	<LOD	<LOD	6,19	6,66	<LOD	<LOD	1,13
Aux Chiens	25,3	317	<LOD	5,20	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	10,8	<LOD	177	<LOD
Batiscan	<LOD	358	<LOD	1,15	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	44,4	0,40
Bayonne	9,30	355	<LOD	2,39	0,43	<LOD	<LOD	<LOD	5,33	8,72	13,7	<LOD	<LOQ	1,05
Beauport	<LOQ	360	<LOD	2,69	<LOD	<LOD	0,38	<LOD	<LOD	<LOD	<LOD	11,7	<LOQ	<LOQ
Beaupre	<LOQ	586	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	42,2	0,42
Beaurivage	7,66	212	<LOD	<LOD	<LOD	<LOD	0,49	<LOD	<LOD	3,79	<LOQ	<LOD	<LOD	0,76
Becancour	5,10	344	<LOD	<LOD	<LOD	<LOD	0,38	<LOD	<LOD	4,16	4,57	<LOD	<LOD	0,74
Boyer	7,07	357	<LOD	<LOD	<LOD	<LOD	0,51	<LOD	<LOD	8,82	<LOD	4,64	53,5	7,06
Cap Rouge	<LOQ	283	<LOD	1,56	<LOD	<LOD	0,41	<LOD	<LOD	<LOD	<LOQ	<LOQ	74,6	<LOQ
Chateauguay	10,9	214	<LOD	2,23	0,61	<LOQ	1,18	<LOD	<LOD	9,64	11,2	<LOD	198	1,03
Chaudière	<LOD	1087	<LOD	1,29	<LOD	<LOD	0,53	<LOD	<LOQ	4,66	5,04	6,40	<LOD	0,81
Chicots	6,61	217	<LOD	<LOD	0,75	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	51,3	<LOD
Des-Prairies	<LOQ	<LOD	<LOD	1,80	<LOD	<LOD	0,47	<LOD	<LOD	2,58	3,71	7,92	<LOD	0,46
Petite rivière du Chêne	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	3,45	<LOQ	<LOD	<LOD	0,89
Du Chêne	9,72	258	<LOD	1,07	<LOD	<LOD	0,55	<LOD	<LOD	4,90	<LOQ	<LOD	62,6	<LOD
Du Loup	4,00	695	<LOD	<LOD	0,34	<LOD	<LOD	<LOD	<LOD	6,97	5,59	<LOD	<LOD	1,02
Du Sud	4,12	588	31,7	1,08	<LOD	<LOQ	1,04	<LOD	<LOD	6,17	<LOQ	5,75	98,6	0,42
Etchemin	<LOD	<LOD	<LOD	1,49	<LOD	<LOD	0,76	<LOD	<LOD	5,57	7,02	<LOD	<LOD	0,57
Jacques-Cartier	<LOD	252	<LOD	<LOD	0,96	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOQ	<LOQ	<LOQ	0,83
Lapiniere	48,5	1700	<LOD	1,30	0,41	<LOD	<LOD	<LOD	6,47	14,6	9,68	<LOD	36	1,60
Mascouche	42,5	543	<LOD	6,09	<LOD	351	1,32	<LOD	<LOD	68,2	38,2	<LOD	170	2,28
Maskinonge	<LOQ	922	<LOD	1,51	<LOD	<LOD	0,36	<LOD	<LOD	3,79	5,35	<LOD	<LOD	0,58
Mille-Iles	18,3	447	<LOD	2,22	<LOD	<LOD	0,47	<LOD	<LOQ	4,71	7,19	17,1	156	0,82
Montmorency	<LOQ	504	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	0,47
Nicolet	<LOQ	828	<LOD	1,29	<LOD	<LOD	0,39	<LOD	<LOD	3,86	6,50	7,95	<LOD	0,84
Outaouais	<LOQ	230	<LOD	2,07	0,34	<LOD	0,50	<LOD	<LOD	2,65	4,45	<LOD	183	<LOD
Portneuf	<LOD	344	<LOD	2,60	<LOD	<LOD	0,69	<LOD	<LOD	3,39	8,62	15,8	75,5	0,54
Richelieu	15,7	296	<LOD	1,75	0,38	<LOD	0,39	<LOD	<LOD	6,47	8,10	13,4	193	0,97
Ruisseau Bertrand	11,5	429	<LOD	2,66	0,39	12,6	0,37	<LOD	<LOD	<LOD	<LOD	<LOD	87,1	<LOQ
Saite-Anne	<LOD	266	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOQ	0,48
Saint-Charles	3,70	380	<LOD	<LOD	<LOD	<LOD	0,62	<LOD	<LOD	1,95	<LOQ	5,89	60,6	0,47
Saint-François	6,07	563	<LOD	1,59	<LOD	<LOD	0,67	<LOD	<LOD	18,7	26,3	24,3	88,9	4,11
Saint-Jacques	22,6	1719	<LOD	1,56	<LOD	<LOD	1,45	<LOD	<LOD	11,5	9,05	<LOD	173	0,58
Saint-Maurice	<LOQ	328	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	58,7	<LOQ
Saint-Régis	74,5	306	<LOD	1,90	0,51	21,5	3,16	<LOD	<LOD	233	44,5	<LOD	175	14,3
Tortue	14,3	389	<LOD	4,25	<LOD	<LOD	0,62	<LOD	<LOD	5,74	4,32	<LOD	147	<LOD
Yamachiche	4,45	423	<LOD	<LOD	0,37	<LOD	0,39	<LOD	<LOD	3,37	<LOQ	<LOD	42,9	<LOQ
Yamaska	5,93	247	<LOD	2,72	<LOD	<LOD	0,48	<LOD	<LOD	18,2	16,9	15,4	124	1,26

Table 7-24. Tributary rivers data (2021).

	Concentrations of detected pharmaceuticals (ng/L)									
	IBU	**OH-IBU**	**DCF**	**OH-DCF**	**CBZ**	**2OH-CBZ**	**VEN**	**DVEN**	**CAF**	**ACE**
A la Scie	13,7	318	1,16	<LOD	1,02	<LOD	<LOD	5,52	<LOD	<LOD
Assomption	<LOD	237	1,82	<LOD	22,8	78,3	10,5	27,8	307	0,88
Batiscan	<LOD	562	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,43
Bayonne	<LOD	237	2,90	<LOD	9,59	36,5	6,59	38,0	18,1	0,60
Beauport	4,42	452	<LOD	<LOD	3,00	<LOD	<LOQ	4,33	8,64	0,35
Beaupre	5,00	287	0,52	0,61	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ
Becancour	3,34	269	<LOD	0,44	5,31	<LOQ	<LOQ	<LOD	5,42	0,51
Boyer	4,52	263	1,15	<LOD	2,68	<LOD	<LOQ	6,38	<LOD	<LOQ
Cap Rouge	<LOD	309	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Chateauguay	<LOD	523	2,32	<LOD	4,21	31,0	4,11	22,2	154	0,38
Chaudière	<LOD	274	2,02	<LOD	13,0	59,3	4,81	20,0	42,1	<LOQ
Coulee Grou	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0,46
Des-Prairies	<LOD	281	1,63	<LOD	1,11	<LOD	<LOQ	6,92	12,6	<LOQ
Du Chêne	20,8	443	3,13	<LOD	7,56	<LOD	4,31	19,9	121	<LOD
Du Loup	3,98	435	1,41	0,47	3,55	34,3	5,25	20,6	<LOD	1,49
Du Sud	4,35	272	<LOD	0,48	1,49	<LOD	<LOD	<LOD	<LOD	<LOD
Etchemin	<LOD	235	<LOD	<LOD	12,2	42,2	7,32	15,40	12,4	0,71
Gouffre	6,12	348	3,02	<LOD	4,04	<LOQ	<LOQ	15,70	<LOD	0,47
Jacques-Cartier	<LOD	332	<LOD	0,35	1,17	<LOD	<LOD	<LOD	42,1	<LOD
Lac St-Jean	<LOD	336	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Mascouche	78,3	647	15,0	<LOD	73,6	306	61,9	227	610	3,49
Maskinonge	<LOD	325	1,28	<LOD	4,22	<LOQ	4,86	7,32	28,6	<LOD
Mille-Iles	10,0	423	2,36	0,72	1,94	<LOD	<LOQ	12,0	155	0,36
Montmorency	<LOD	263	<LOD	<LOD	1,31	<LOD	<LOD	<LOD	71,0	<LOQ
Nicolet	<LOD	269	<LOD	<LOD	26,5	104	<LOQ	20,1	22,9	0,67
Outaouais	5,82	<LOD	1,67	<LOD	2,74	<LOD	4,04	10,5	<LOD	0,35
Portneuf	<LOQ	398	<LOD	<LOD	<LOD	<LOD	<LOQ	3,58	<LOD	<LOQ
Richelieu	22,1	329	<LOD	<LOD	3,47	<LOD	<LOQ	7,23	333	0,41
Ruisseau Bertrand	<LOD	2687	1,17	<LOD	<LOD	<LOD	<LOQ	7,92	719	<LOD
Ruisseau Bouchard	<LOD	383	<LOD	<LOD	<LOD	<LOD	<LOD	3,48	190	<LOD
Saite-Anne	8,66	312	1,00	<LOD	1,48	<LOD	<LOQ	3,91	13,2	<LOD
Saint-Charles	8,82	335	1,17	0,80	1,99	<LOD	<LOQ	10,6	82,1	0,72
Saint-François	<LOD	240	<LOD	<LOD	17,4	68,9	10,7	9,15	251	1,73
Saint-Jacques	<LOD	322	1,97	<LOD	27,3	86,0	9,37	87,7	194	<LOD
Saint-Maurice	<LOD	433	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	<LOD
Saguenay	4,33	388	<LOD	<LOD	1,50	<LOD	<LOD	<LOD	<LOD	0,38
Tortue	<LOD	209	2,81	<LOD	8,94	59,5	7,86	42,5	128	0,40
Yamachiche	<LOD	274	<LOD	0,33	5,27	<LOD	<LOD	<LOD	12,8	<LOD
Yamaska	<LOD	245	1,12	0,86	56,7	163	22,3	17,0	27,8	0,83

Table 7-25. Scuba diving data (sub-surface samples, approx. 1m).

	Position (km)	IBU	OH-IBU	DCF	CLA	CBZ	VEN	DVEN	CAF	ACE
	0	3,40	16,9	0,25	<LOD	0,45	0,79	<LOQ	4,55	0,47
	10	4,02	18,2	<LOQ	<LOQ	1,66	1,02	<LOQ	14,0	0,33
	20	3,99	19,9	0,68	<LOQ	0,84	1,61	<LOQ	11,8	0,39
	35	4,61	28,7	0,24	<LOQ	1,66	1,00	<LOQ	14,2	<LOQ
	45	3,76	22,0	0,82	<LOQ	1,60	0,93	<LOQ	19,5	<LOQ
	55	6,44	47,8	1,07	<LOQ	2,29	1,59	<LOQ	36,1	0,34
	60	9,12	73,6	1,33	0,35	2,97	2,25	<LOQ	52,7	0,49
Effluent MTL	**65**	14,52	197,7	1,64	0,48	2,08	4,00	4,94	70,9	1,06
	70	11,57	168,6	1,99	0,60	2,40	4,44	4,18	68,6	0,99
	80	13,40	125,6	1,91	0,80	3,00	5,22	7,08	87,6	1,51
	95	9,31	98,0	1,21	0,45	2,19	3,75	5,39	46,0	1,28
	115	7,72	68,9	0,98	0,34	1,75	2,59	3,99	39,0	0,84
	125	7,87	82,2	1,31	0,36	2,13	3,34	4,93	45,3	0,89
	135	8,57	83,7	0,61	0,37	1,86	2,35	3,59	38,3	0,65
	150	3,82	25,2	0,62	<LOD	0,40	0,42	<LOD	14,6	<LOQ

Table 7-26. Scuba diving data (depth samples, approx. 10m).

	Position (km)	IBU	OH-IBU	DCF	CBZ	VEN	DVEN	CAF	ACE
	0	9,10	25,6	0,63	1,95	3,65	9,22	17,4	1,10
	10	13,5	98,8	<LOD	2,47	1,20	5,78	36,1	<LOD
	20	10,0	116	<LOD	2,44	1,25	4,97	27,7	0,88
	35	9,54	36,0	<LOD	2,02	1,15	4,48	25,5	<LOD
	45	12,6	<LOD	0,57	3,70	3,06	8,63	93,3	1,51
	55	12,6	57,1	0,35	4,41	4,44	7,01	76,7	1,27
	60	12,9	<LOD	<LOD	3,54	3,15	7,71	60,3	0,70
Effluent MTL	**65**	14,0	88,2	<LOD	3,65	2,64	5,93	67,9	0,68
	70	16,7	221	1,72	7,32	9,87	26,5	180	3,20
	80	24,6	162	2,05	7,38	9,14	17,4	186	2,88
	95	20,9	124	1,20	6,56	6,04	18,7	146	1,71
	115	19,9	166	1,36	6,25	6,59	15,1	159	1,61
	125	9,30	109	<LOD	5,87	1,77	4,94	<LOD	<LOD
	135	9,69	119	<LOD	4,71	1,89	4,74	36,5	<LOD
	150	9,79	92,3	<LOD	1,95	0,52	<LOD	<LOD	<LOD

8 Contributions à d'autres articles

Les méthodes analytiques développées dans le cadre de cette thèse étant versatiles et leur validation ayant démontrée leur robustesse ainsi que la possibilité d'atteindre des limites de détection à des niveaux traces, celles-ci ont pu être utilisées dans le cadre de travaux en collaboration avec divers groupes de recherche. Les diverses publications issues de ceux-ci sont présentées dans le Tableau 8-1.

Afin de diminuer la charge en composés pharmaceutiques provenant d'effluents hospitaliers, certains groupes de recherche testent des technologiques alternatives pour l'enlèvement de ces polluants à la source. Or, pour confirmer l'efficacité de ces prototypes, il importe de quantifier les concentrations des composés pharmaceutiques avant et après le traitement des eaux usées. Ainsi, les méthodes d'extractions par ultrasons ainsi que la SPE en ligne avec l'UPLC-MS/MS ont été appliquées pour optimiser différents paramètres dans des procédés d'électro-oxydation et dans des bioréacteurs à membrane pour maximiser l'enlèvement des composés pharmaceutiques [488–494]. Il a également été intéressant de démontrer la présence de hautes concentrations de certains contaminants pharmaceutiques dans les boues de traitement d'eaux usées puisque celles-ci sont parfois revalorisées par leur utilisation comme engrais dans des sols agricoles.

Ces méthodes ont également été utilisées dans le cadre d'études sur les effets écotoxicologiques de rejets hospitaliers ou d'effluents municipaux sur des organismes aquatiques tels que des larves de poissons, des daphnies ou encore des moules (certains travaux sont toujours en cours) [487]. La quantification des composés pharmaceutiques dans les milieux d'expositions et dans les organismes eux-mêmes permet de lier les divers contaminants et leurs concentrations à des effets sur des biomarqueurs ou encore de déterminer si ceux-ci ont un effet synergique dans des matrices contaminées telles que des eaux usées.

Tableau 8-1. Articles résultant de collaborations réalisées grâce aux méthodes analytiques décrites dans cette thèse.

Collaborateurs	Publications
Technologie alternative de traitement des eaux usées (INRS)	**1.** Y. Ouarda, B. Tiwari, A. Azaïs, M.A. Vaudreuil, S.D. Ndiaye, P. Drogui, R.D. Tyagi, S. Sauvé, M. Desrosiers, G. Buelna, R. Dubé, Synthetic hospital wastewater treatment by coupling submerged membrane bioreactor and electrochemical advanced oxidation process: Kinetic study and toxicity assessment, Chemosphere. 193 (2018) 160–169. https://doi.org/10.1016/j.chemosphere.2017.11.010.
	2. Y. Ouarda, F. Bouchard, A. Azaïs, M.A. Vaudreuil, P. Drogui, R. Dayal Tyagi, S. Sauvé, G. Buelna, R. Dubé, Electrochemical treatment of real hospital wastewaters and monitoring of pharmaceutical residues by using surrogate models, J Environ Chem Eng. 7 (2019) 1–9. https://doi.org/10.1016/j.jece.2019.103332.
	3. B. Tiwari, B. Sellamuthu, S. Piché-Choquette, P. Drogui, R.D. Tyagi, M.A. Vaudreuil, S. Sauvé, G. Buelna, R. Dubé, The bacterial community structure of submerged membrane bioreactor treating synthetic hospital wastewater, Bioresour Technol. 286 (2019) 1–9. https://doi.org/10.1016/j.biortech.2019.121362.
	4. B. Tiwari, B. Sellamuthu, S. Piché-Choquette, P. Drogui, R.D. Tyagi, G. Buelna, M.A. Vaudreuil, S. Sauvé, R. Dube, R.Y. Surampalli, Compositional Microbial-Community Shift of Submerged Membrane Bioreactor Treating Hospital Wastewater at Varying Temperatures, Journal of Environmental Engineering. 147 (2021) 1–10. https://doi.org/10.1061/(asce)ee.1943-7870.0001842.
	5. B. Tiwari, B. Sellamuthu, S. Piché-Choquette, P. Drogui, R.D. Tyagi, M.A. Vaudreuil, S. Sauvé, G. Buelna, R. Dubé, Acclimatization of microbial community of submerged membrane bioreactor treating hospital wastewater, Bioresour Technol. 319 (2021) 1–8. https://doi.org/10.1016/j.biortech.2020.124223.
	6. B. Tiwari, Y. Ouarda, P. Drogui, R.D. Tyagi, M.A. Vaudreuil, S. Sauvé, G. Buelna, R. Dubé, Fate of Pharmaceuticals in a Submerged Membrane Bioreactor Treating Hospital Wastewater, Frontiers in Water. 3 (2021) 1–8. https://doi.org/10.3389/frwa.2021.730479.
	7. B. Tiwari, B. Sellamuthu, S. Piché-Choquette, P. Drogui, R.D. Tyagi, M.A. Vaudreuil, S. Sauvé, G. Buelna, R. Dubé, Dynamics of bacterial community at varying sludge retention time within membrane bioreactor treating synthetic hospital wastewater, Systems Microbiology and Biomanufacturing. 1 (2021) 471–482. https://doi.org/10.1007/s43393-021-00034-y.
Écotoxicologie (ECCC)	**8.** C. André, M.-A. Vaudreuil, S. Vo Duy, S. Sauvé, F. Gagné, Long-term and comparative impacts of combined sewers and municipal effluents to freshwater mussels, 2020. https://doi.org/10.25431/1824-307X/isj.v0i0.75-89

Conclusion

Les composés pharmaceutiques étant essentiels et leur consommation étant en constante croissance avec l'augmentation de la population, ils sont susceptibles de se retrouver à des niveaux de plus en plus élevés dans l'environnement [9]. Ainsi, depuis plusieurs années, la pollution de différents milieux est le sujet d'étude, mais l'éventail des problématiques environnementales ou des impacts écotoxicologiques de ces contaminants sur la faune et la flore ne sont que partiellement documentés. Ces composés ont un impact environnemental important à proximité des zones urbanisées car les usines de traitement des eaux usées n'ont pas été conçues pour traiter ce type de molécules [26,27]. D'autre part, les milieux hospitaliers sont susceptibles d'être des sources de rejets des produits pharmaceutiques soulevant l'idée de développer des technologies spécifiques pour le traitement à la source de ces établissements pour alléger les apports aux stations d'épurations municipales [21,22]. Or, pour supporter ces initiatives, il est important de collecter des données sur la diversité et les niveaux de concentration des différentes classes de composés pharmaceutiques qui sont retrouvés dans les rejets hospitaliers et les effluents municipaux. Les différents chapitres qui composent cette thèse ont pour objectif de tenter de répondre à ces questionnements.

Le **Chapitre 5** décrit le développement d'une méthode rapide et robuste pour l'analyse de 28 composés de diverses classes pharmaceutiques par SPE en ligne avec la chromatographie liquide ultra-haute performance couplée à la spectrométrie de masse en tandem (UPLC-MS/MS). Une étape d'extraction et de purification a également été optimisée afin de quantifier les composés ciblés dans la portion solide des échantillons. En effet, une quantité non négligeable de composés pharmaceutiques peut s'adsorber à la surface des particules dans les eaux usées et la quantification seule de la phase dissoute ne donne pas l'image globale de la contamination. La validation de cette méthode a permis de démontrer qu'elle respecte les critères de l'US EPA en termes de précision et d'exactitude, mais également qu'une approche de calibration dans une matrice mixte d'eau usée est suffisante pour contrer les effets de matrice. Ainsi, des limites de détection de l'ordre du ng/L sont obtenues, ce qui est nettement suffisant pour des échantillons contaminés tels que les eaux usées. Les performances sont équivalentes à celles d'autres méthodes publiées, mais avec un temps total d'analyse plus court et un volume restreint d'échantillon. Celle-ci a été appliquée à un total de 30 effluents hospitaliers ainsi qu'aux affluents et effluents de six différentes usines d'épuration. Ces matrices contenaient de 18 à 25 des composés ciblés avec des concentrations en analgésiques, en antibiotiques et en caféine atteignant des centaines de µg/L. De manière générale, des concentrations plus élevées sont

retrouvées dans les effluents hospitaliers comparativement aux eaux usées municipales, à l'exception d'hormones de contraception largement utilisées par la population. Ceci confirme l'hypothèse de départ selon laquelle les eaux usées d'hôpitaux peuvent être plus polluées par des contaminants pharmaceutiques. Aussi, les concentrations mesurées à la suite du traitement des eaux sont inférieures, mais des quantités importantes de produits pharmaceutiques sont mesurées dans les effluents, ce qui confirme que celles-ci ne traitent que partiellement ce type de contaminants qui seront inévitablement rejetés dans l'environnement via des eaux de surface. Même si les concentrations des composés pharmaceutiques varient entre différents pays, les conclusions de cette étude abondent dans le sens d'autres publications s'intéressant à la quantification de ces contaminants dans des effluents municipaux et d'hôpitaux.

Au cours du développement de la méthode décrite au **Chapitre 5**, une limitation a été rencontrée en lien avec l'analyse des produits de chimiothérapie. Ceux-ci étant majoritairement de molécules de faible masse et très polaires, leur préconcentration et leur rétention, par la méthode avec des phases stationnaires de type C18 conventionnelles, sont insuffisantes pour les analyser à des niveaux traces ou dans des matrices très chargées telles que des eaux usées. Le **Chapitre 6** décrit donc les travaux visant à choisir une approche alternative pour l'analyse ciblée de ces composés pharmaceutiques dans des effluents hospitaliers et municipaux. Différentes options prometteuses pour la préconcentration (cartouches SPE) ainsi que diverses colonnes chromatographiques susceptibles d'offrir une bonne rétention pour les analytes ciblés ont été combinées afin d'établir les conditions optimales pour leur analyse. Une approche novatrice couplant la SPE en ligne avec la chromatographie HILIC-MS/MS a été optimisée pour la quantification du fluorouracile (5-FU) et de son métabolite dans des échantillons aqueux. Ce composé de chimiothérapie est parmi les plus utilisés au Canada, or peu de données sont disponibles quant à sa présence et sa concentration dans des matrices environnementales en raison de la difficulté que représente son analyse. C'est également le cas pour les six autres produits cytostatiques ciblés pour lesquels une méthode utilisant la même cartouche SPE (Isolute ENV+, Biotage), mais couplée avec une colonne chromatographique à base de chaînes C18 modifiées a été optimisée. Ces méthodes ont été validées et sont conformes aux critères de l'US EPA. Aussi, des limites de détection de l'ordre du ng/L sont obtenues et sont jugées suffisantes pour l'analyse à des niveaux traces dans des eaux usées. Les performances de ces méthodes sont comparables à celles décrites dans d'autres études avec toutefois de meilleurs recouvrements pour certains analytes tels que 5-FU et GEM et avec un temps total d'analyse plus court. Ainsi, six des huit analytes ont été retrouvés dans les échantillons d'eaux usées d'hôpitaux (n = 11) alors que seulement quatre sont détectés dans des effluents municipaux (n = 6), mais à

des concentrations parfois plus importantes allant des faibles ng/L à quelques centaines de ng/L. Encore une fois, les concentrations dans les effluents des usines d'épuration suggèrent que celles-ci ne sont pas adaptées à l'enlèvement de cette classe de composés pharmaceutiques qui se retrouveront dans des eaux de surface à proximité de régions densément peuplées. La contamination mesurée dans les effluents d'hôpitaux échantillonnés est nettement moins élevée que celle rapportée auparavant dans d'autres pays, notamment pour IFO et CYC. Les eaux usées municipales, quant à elles, contenaient des concentrations comparables aux valeurs de précédentes études.

Ainsi, en raison de l'importante consommation de composés pharmaceutiques et l'inefficacité des stations d'épuration à les traiter, ces contaminants sont retrouvés dans l'environnement à proximité de zones urbaines, mais également dans des régions éloignées de l'activité humaine telles que sur des sommets montagneux, dans eaux océaniques ou encore aux pôles [137–142]. Certains groupes de recherche ont quantifié des groupes spécifiques de composés pharmaceutiques dans des eaux de surface, or le nombre de données sur la contamination environnementale au Canada est limité malgré que le Canada figure parmi les plus grands producteurs et consommateurs de médicaments [10,11,23]. Il importe de déterminer les sources de contamination ainsi que d'étudier la dispersion des composés pharmaceutiques dans l'environnement via l'eau de surface afin d'avoir une vue d'ensemble de la problématique. Le **Chapitre 7** compile les données récoltées dans le cadre d'une large campagne d'échantillonnage sur le fleuve Saint-Laurent échelonnée sur cinq ans. Dans l'ensemble, plus de 400 échantillons pris sur une distance d'environ 700 km ainsi que plus de 50 rivières tributaires au fleuve ont été analysés afin de déterminer la présence et les concentrations des différents composés pharmaceutiques ciblés. Pour ce faire, la méthode présentée au **Chapitre 5** a été validée pour l'eau de surface en respectant les critères de l'US EPA. Une méthode SPE en ligne couplée avec l'UPLC-MS/MS s'est avérée fort utile pour l'analyse d'un nombre si important d'échantillons et la méthode d'extraction/purification a pu être appliquée à des échantillons de sédiments prélevés à proximité d'un point de rejets d'eau usée. Des échantillons d'eau en profondeur ont également été obtenus lors d'une plongée sous-marine permettant ainsi de déterminer qu'un profil similaire de contamination est observé pour des échantillons prélevés en surface et que cette dernière approche, beaucoup plus simple d'un point de vue technique, est suffisante pour déterminer l'emplacement d'une source de contamination par les composés pharmaceutiques. Certains médicaments tels que CBZ, DCF et VEN sont retrouvés dans une vaste majorité des échantillons (fréquence de détection > 80%) et certains sont retrouvés à des concentrations importantes telles que 2OH-CBZ à des centaines de ng/L ou encore OH-IBU atteignant le niveau des µg/L. Alors

que certains sites tels que ceux près du lac Ontario ou de l'estuaire du fleuve sont peu contaminés en raison d'une forte dilution, des pics de contamination sont observés à l'approche des effluents de la ville de Québec et de Montréal. Ceci corrobore l'hypothèse selon laquelle un impact important pourrait être mesuré à proximité du point de rejet des eaux usées de Montréal et que celui-ci aurait une répercussion sur une grande distance. Les sites près de ce dernier présentent d'ailleurs un haut facteur de risque en raison de fortes concentrations d'analgésiques qui dépassent les seuils de recommandation de l'Union européenne concernant les contaminants pharmaceutiques. Des conclusions similaires ont été obtenues lors d'études sur des effluents de métropoles à travers le monde. Des analyses statistiques ont également permis de confirmer qu'il y a peu de mélange entre les masses d'eau qui composent le fleuve et que les contaminants rejetés à l'effluent de Montréal sont transportés avec un impact ressenti sur plus de 70 km, et ce durant les cinq années d'échantillonnage. Enfin, des profils similaires de contaminants pharmaceutiques ont pu être observés dans les tributaires échantillonnés, mais à des concentrations moindres, avec l'exception de certains antibiotiques qui sont retrouvés plus fréquemment que dans l'eau du fleuve. Les rivières les plus contaminées étant celles s'écoulant dans des zones densément peuplées dans la grande région de Montréal, certaines sont jugées comme étant des sources non négligeables de contamination pharmaceutique lorsque l'on prend en compte la charge massique de ceux-ci, calculés en utilisant les débits moyens des rivières ciblées. Cette tendance vérifie donc l'hypothèse de l'impact de l'intensité de l'activité humaine sur les niveaux de contaminations des composés pharmaceutiques dans des rivières. Il est également important de noter que les niveaux de concentration mesurés ainsi que les profils de contamination pharmaceutique sont comparables aux données qui ont été sporadiquement rapportées dans des études précédentes à travers le Canada.

En somme, cette thèse présente des méthodes rendant possible le suivi des contaminants pharmaceutiques multi-classes de leur source dans les eaux usées jusqu'à leur rejet dans l'environnement à des niveaux traces dans les eaux de surface. Celles-ci pourraient s'avérer utiles pour des suivis plus rigoureux de la pollution des composés pharmaceutiques, ce qui pourrait permettre de mieux comprendre leur impact sur la faune et la flore. Celles-ci pourraient également être mises à profit lors de l'établissement de nouvelles technologies pour le traitement des eaux usées qui devront probablement être mise en place dans le futur afin d'éviter le déclin de populations animale et végétale ou que le problème n'est un impact trop considérable d'un point de vue de santé publique [495–498].

Perspectives

Bien que les différentes matrices ciblées dans cette thèse soient peu susceptibles d'impacter directement la santé humaine, il est important de noter que la vaste présence des composés pharmaceutiques dans l'environnement fait en sorte qu'ils sont à même de se retrouver dans certains de nos produits de consommation. Par exemple, de nombreuses villes au Québec puisent leur eau potable dans le fleuve, qui comme discuté dans le **Chapitre 7** contient divers médicaments. Bien que des traitements soient appliqués avant la consommation, certaines études ont démontré que des composés pharmaceutiques peuvent se retrouver dans cette matrice [144–146,499]. Les faibles concentrations de ces composés nécessitent le développement de méthodes de préconcentration et d'analyse avancées. Or, il serait possible d'augmenter le volume de préconcentration utilisée lors de l'étape de SPE en ligne de la méthode décrite au **Chapitre 5** afin d'atteindre de telles limites de détection. Ainsi, des échantillons d'eau potable pourraient être échantillonnés au Canada afin de combler un manque de données quant à la présence et aux concentrations des contaminants pharmaceutiques dans cette matrice. Il aurait également été intéressant d'évaluer l'efficacité de certains dispositifs de filtration de l'eau du robinet tels que les pichets de type *Brita* qui utilisent le charbon activé et dont l'efficacité a été démontrée pour capter des métaux et certains contaminants organiques [500,501].

D'autre part, outre la consommation directe d'antibiotiques lors d'une infection bactérienne, ces médicaments peuvent se retrouver dans notre nourriture à la suite de l'exposition de certains fruits et légumes à des fertilisants contenant des déjections animales. En effet, des quantités importantes de ces composés, ainsi que certaines hormones, sont utilisées lors de l'élevage de bétail. Certains sols agricoles sont également irrigués avec des eaux usées et certaines boues issues d'usines d'épuration sont également épandues afin de servir d'engrais, or ces matrices contiennent fréquemment des quantités importantes de composés pharmaceutiques [502–504]. Ainsi, il aurait été intéressant d'inclure certains composés pharmaceutiques spécifiques aux usages vétérinaires afin de déterminer leurs niveaux de concentration dans les matrices ciblées. Par exemple, lors de l'analyse des échantillons du fleuve et de ses tributaires détaillée au **Chapitre 7**, il aurait été possible de distinguer des sources de contamination de composés pharmaceutiques pour usage vétérinaire qui aurait probablement différées de celles trouvées à proximité de zones densément peuplées.

En plus de déterminer l'impact de certains secteurs d'activité sur la contamination dans les effluents et l'eau de surface, des échantillonnages à différentes périodes de l'année auraient pu être effectués afin de voir s'il en ressort des tendances saisonnières. Puisque la consommation

de certains composés pharmaceutiques varie selon les saisons, il aurait pu y avoir des fluctuations en termes de profils des classes pharmaceutiques et de leur concentration dans les eaux usées et les milieux naturels [505–507]. De surcroît, l'ajout de médicaments spécifiques pour le traitement de la grippe ou d'autres infections saisonnières aurait pu mener à des tendances indiquant les périodes de l'année où une portion importante de la population est affectée. Il aurait alors été intéressant de cibler des composés tels que le ritonavir, la chloroquine ou l'ivermectine dont l'utilisation a été exacerbée en raison de la pandémie de Covid-19 [464–466]. En effet, en fonction des fluctuations des nombres de cas ou de certaines directives des instances gouvernementales, des études ont pu établir des tendances en termes de la contamination par certaines classes pharmaceutiques telles que les antibiotiques ou les analgésiques dans des échantillons de stations d'épuration [458–461].

Pour répondre à des telles questions quant à l'impact de secteurs d'activité ou de variations temporelles, il aurait été possible de faire appel à de traitements de données multivariés plus poussés. Ceux-ci auraient aussi pu être employés afin de tirer davantage de conclusions des données récoltées dans le cadre des différents chapitres présentés dans cette thèse. Par exemple, il serait peut-être possible de déterminer si le type de traitement employé par les usines d'épuration échantillonnées a un impact sur les profils de pharmaceutiques retrouvés dans les affluents comparés aux effluents ou encore si les différentes masses d'eaux du fleuve sont contaminées par des profils distincts de classes pharmaceutiques.

D'autre part, l'accessibilité aux points d'échantillonnage des eaux usées étant complexe, le nombre de ces échantillons s'en trouve souvent limité. Il est ainsi plus difficile de déterminer des tendances dans cette matrice. Or, pour déterminer l'efficacité des usines de traitement des eaux usées, il serait nécessaire d'avoir un plus grand nombre d'échantillons afin de pallier le manque de données à ce sujet. Il importe également de considérer le temps de résidence des eaux usées lorsque l'on compare les affluents aux effluents. Pour pallier ces difficultés techniques, il existe des alternatives en termes d'échantillonnage, notamment par l'usage d'échantillonneurs passifs qui peuvent préconcentrer certains contaminants ou des échantillonneurs automatiques qui offrent l'option d'obtenir des échantillons à des laps de temps contrôlés ou encore de faire des échantillons composites qui seront plus représentatifs de la contamination moyenne sur une période de temps donnée (journalière, hebdomadaire, mensuelle, etc.) [508–510].

Une telle approche pourrait être mise en pratique lors de l'instauration de la nouvelle usine d'épuration de la ville de Montréal qui utilisera un procédé d'ozonation qui pourrait être prometteur pour le traitement de contaminants organiques tels que les composés pharmaceutiques. Une

campagne d'échantillonnage comme celle présentée au **Chapitre 7** pourrait alors être réalisée de nouveau afin de déterminer si l'impact du point de rejet des effluents de Montréal génère un pic de contamination dans les eaux de surface. Les concentrations en pharmaceutiques et le facteur de risque pour la vie aquatique pourraient alors être comparés aux données récoltées de 2017 à 2021 afin de déterminer si ce nouveau procédé permet d'améliorer la qualité de l'eau. Il serait toutefois important d'inclure des produits de dégradation des composés pharmaceutiques en raison de la tendance reconnue de l'ozonation à générer ce type de sous-produits [511–513].

Enfin, pour brosser un portrait plus complet de la charge totale en produits pharmaceutiques dans les matrices environnementales ciblées, il serait important de mettre au point des méthodes de détection de composés non ciblés. Par exemple, l'utilisation d'un spectromètre de masse à haute résolution (HRMS) permettrait de déterminer la masse exacte de certains contaminants présents en forte concentration et de procéder à l'élucidation de leur structure à l'aide d'analyses MS/MS. L'utilisation de bases de données ou de modèles prédictifs pourrait s'avérer des outils fort utiles pour la détermination de métabolites et de produits de dégradation pour lesquels il n'existe pas de standards commercialement disponibles et qui ne sont donc pas inclus dans les méthodes analytiques pour le moment. Les composés les plus importants ou présentant un potentiel écotoxicologique pourraient alors être ajoutés aux méthodes quantitatives développées pour faire des suivis réguliers de la pollution de milieux environnementaux par les composés pharmaceutiques. Conjointement aux études quantitatives, la toxicité des différents échantillons d'eaux usées et de surface aurait pu être déterminée par une exposition à différents organismes tels que des daphnies, des moules ou des alevins [514–519]. En outre, la persistance et la bioaccumulation des composés individuels auraient également pu être évaluées lors d'essais d'exposition, ce qui aurait permis de faire une analyse plus exhaustive du niveau de risque environnemental dans les milieux échantillonnés [520–523].

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