

Université de Montréal

**Développement de techniques analytiques pour la
détermination des agents anti-infectieux dans les eaux
environnementales**

par

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dans les eaux environnementales

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Résumé

Les agents anti-infectieux sont utilisés pour traiter ou prévenir les infections chez les humains, les animaux, les insectes et les plantes. L'apparition de traces de ces substances dans les eaux usées, les eaux naturelles et même l'eau potable dans plusieurs pays du monde soulève l'inquiétude de la communauté scientifique surtout à cause de leur activité biologique.

Le but de ces travaux de recherche a été d'étudier la présence d'anti-infectieux dans les eaux environnementales contaminées (*c.-à-d.* eaux usées, eaux naturelles et eau potable) ainsi que de développer de nouvelles méthodes analytiques capables de quantifier et confirmer leur présence dans ces matrices.

Une méta-analyse sur l'occurrence des anti-infectieux dans les eaux environnementales contaminées a démontré qu'au moins 68 composés et 10 de leurs produits de transformation ont été quantifiés à ce jour. Les concentrations environnementales varient entre 10^{-1} et 10^9 ng L⁻¹, selon le composé, la matrice et la source de contamination. D'après cette étude, les effets nuisibles des anti-infectieux sur le biote aquatique sont possibles et ces substances peuvent aussi avoir un effet indirect sur la santé humaine à cause de sa possible contribution à la dissémination de la résistance aux anti-infectieux chez les bactéries.

Les premiers tests préliminaires de développement d'une méthode de détermination des anti-infectieux dans les eaux usées ont montré les difficultés à surmonter lors de l'extraction sur phase solide (SPE) ainsi que l'importance de la sélectivité du détecteur.

On a décrit une nouvelle méthode de quantification des anti-infectieux utilisant la SPE en tandem dans le mode découplé et la chromatographie liquide couplée à la spectrométrie de masse en tandem (LC-MS/MS). Les six anti-infectieux ciblés (sulfaméthoxazole, triméthoprime, ciprofloxacine, levofloxacine, clarithromycine et azithromycine) ont été quantifiés à des concentrations entre 39 et 276 ng L⁻¹ dans les échantillons d'affluent et d'effluent provenant d'une station d'épuration appliquant un traitement primaire et physico-

chimique. Les concentrations retrouvées dans les effluents indiquent que la masse moyenne totale de ces substances, déversées hebdomadairement dans le fleuve St. Laurent, était de ~ 2 kg.

En vue de réduire le temps total d'analyse et simplifier les manipulations, on a travaillé sur une nouvelle méthode de SPE couplée-LC-MS/MS. Cette méthode a utilisé une technique de permutation de colonnes pour préconcentrer 1.00 mL d'échantillon dans une colonne de SPE couplée. La performance analytique de la méthode a permis la quantification des six anti-infectieux dans les eaux usées municipales et les limites de détection étaient du même ordre de grandeur ($13\text{-}60 \text{ ng L}^{-1}$) que les méthodes basées sur la SPE manuelle.

Ensuite, l'application des colonnes de SPE couplée de chromatographie à débit turbulent pour la préconcentration de six anti-infectieux dans les eaux usées a été explorée pour diminuer les effets de matrice. Les résultats obtenus ont indiqué que ces colonnes sont une solution de réchange intéressante aux colonnes de SPE couplée traditionnelles.

Finalement, en vue de permettre l'analyse des anti-infectieux dans les eaux de surface et l'eau potable, une méthode SPE couplée-LC-MS/MS utilisant des injections de grand volume (10 mL) a été développée. Le volume de fuite de plusieurs colonnes de SPE couplée a été estimé et la colonne ayant la meilleure rétention a été choisie. Les limites de détection et de confirmation de la méthode ont été entre 1 à 6 ng L^{-1} . L'analyse des échantillons réels a démontré que la concentration des trois anti-infectieux ciblés (sulfaméthoxazole, triméthoprime et clarithromycine) était au dessous de la limite de détection de la méthode. La mesure des masses exactes par spectrométrie de masse à temps d'envol et les spectres des ions produits utilisant une pente d'énergie de collision inverse dans un spectromètre de masse à triple quadripôle ont été explorés comme des méthodes de confirmation possibles.

Mots-clés : anti-infectieux, antibiotiques, pharmaceutiques, analyse de traces, eaux usées, eaux de surface, eau potable, extraction sur phase solide, chromatographie liquide, spectrométrie de masse en tandem, spectrométrie de masse à temps d'envol.

Abstract

Anti-infectives are used to treat or prevent infections in humans, animals, insects and plants. The occurrence of traces of these substances in wastewaters, natural waters and even drinking water has caused concern among the scientific community especially because of their biological activity.

The goal of this research was to study the occurrence of anti-infectives in contaminated environmental waters (wastewaters, natural waters, and drinking water) and to develop new analytical methods able to quantify and confirm their presence in these matrices.

A meta-analysis on the occurrence of anti-infectives in contaminated environmental waters demonstrated that at least 68 parent compounds and 10 transformation products have been quantified to date. Environmental concentrations vary between 10^{-1} and 10^{-9} ng L⁻¹ depending on the compound, the matrix and the source of contamination. According to this study, detrimental effects of anti-infectives on aquatic biota are possible and these substances could also affect indirectly human health because of their possible contribution to the dissemination of antibiotic resistance in bacteria.

Preliminary tests on the development of a method of determination of anti-infectives in wastewaters showed the main difficulties to overcome during solid-phase extraction (SPE) as well as the importance of the detector selectivity.

A novel method of determination of anti-infectives was described using off-line tandem SPE and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Six target anti-infectives (sulfamethoxazole, trimethoprim, ciprofloxacin, levofloxacin, clarithromycin and azithromycin) were quantitated at concentrations between 39 and 276 ng L⁻¹ in samples of influent and effluent collected from a primary and physico-chemical wastewater treatment plant. Reported effluent concentrations indicate that the mean mass of these substances discharged daily in the St. Lawrence River was ~ 2 kg.

In order to reduce total analysis time and simplify sample preparation, a new on-line SPE-LC-MS/MS was presented. This method used a column-switching technique to preconcentrate 1.00 mL of sample in an on-line SPE column. Method analytical performance allowed the quantitation of six anti-infectives in municipal wastewaters and limits of detection were of the same magnitude (13-60 ng L⁻¹) than methods based in off-line SPE.

Next, the application of turbulent flow chromatography on-line SPE columns for the preconcentration of six anti-infectives in wastewaters was explored. Results showed that these columns are an interesting alternative to traditional on-line SPE columns.

Finally, in order to allow analysis of anti-infectives in surface and drinking water, we developed an on-line SPE-LC-MS/MS method using large-volume injections (10 mL). Breakthrough volumes of several on-line SPE columns were estimated and the column having the best retention, Strata-X, was chosen. Method detection and confirmation limits were between 1 and 6 ng L⁻¹. Analysis of real samples indicated that the concentration of the three target anti-infectives (sulfamethoxazole, trimethoprim and clarithromycin) was lower than the method detection limits. Accurate mass measurement by time-of-flight mass spectrometry and product ion spectra obtained by a reversed-energy ramp in a triple quadrupole mass spectrometer were explored as alternative confirmation methods.

Keywords: anti-infectives, antibiotics, pharmaceuticals, trace analysis, wastewaters, surface waters, drinking water, solid-phase extraction, liquid chromatography, tandem mass spectrometry, time-of-flight mass spectrometry.

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Liste d'abréviations, sigles et acronymes

Les mots dans la langue anglaise sont indiqués en italique. Certaines termes n'apparaissent que dans une langue parce qu'elles n'ont pas été utilisées dans l'autre langue.

| | |
|------------------|--|
| %ME | <i>Matrix effects percentage</i> |
| ACN | Acétonitrile; <i>acetonitrile</i> |
| ANOVA | Analyse de la variance; <i>analysis of variance</i> |
| A _s | <i>Peak asymmetry factor</i> |
| AS | Ajout de sel |
| ATZ | <i>Atrazine</i> |
| AZI | Azithromycine; <i>azithromycin</i> |
| BOD ₅ | <i>Biological oxygen demand within a five-day period</i> |
| CAF | <i>Caffeine</i> |
| CBZ | <i>Carbamazepine</i> |
| cc-DI | <i>Calibarion curve in deionized water</i> |
| cc-dWW | <i>Calibarion curve in diluted wastewater</i> |
| CE | Énergie de collision; <i>collision energy</i> |
| CIP | Ciprofloxacin; <i>ciprofloxacin</i> |
| CLA | Clarithromycine; <i>clarithromycin</i> |
| CLF | <i>Chloroform</i> |
| COD | Demande chimique en oxygène; <i>chemical oxygen demand</i> |
| CYA | <i>Cyanazine</i> |
| CYC | <i>Cyclophosphamide</i> |
| DEA | <i>Deethylatrazine</i> |
| DHC | <i>10;11-dihydrocarbamazepine</i> |
| DI | <i>Deionized water</i> |
| DIA | <i>Diaveridine</i> |
| DIA | <i>Deisopropylatrazine</i> |
| DOC | <i>Dissolved organic carbon</i> |
| DOM | <i>Disssolved organic matter</i> |
| DWTP | <i>Drinking water treatment plant</i> |

| | |
|----------------------|---|
| dWW | <i>Diluted wastewater</i> |
| EC01 | Concentration effective à 1%; <i>1% Effective concentration</i> |
| EC ₅₀ | Concentration effective à 50%; <i>50% effective concentration</i> |
| ÉI | Étalon interne |
| ENA | <i>Enalapril</i> |
| ESI+ | <i>Positive mode electrospray ionization</i> |
| F | Filtration |
| F.A. | Acide formique; <i>formic acid</i> |
| FAB-MS (BE) | Bombardement par atomes rapides et spectrométrie de masse avec analyseur à double focalisation |
| FD-MS(BE) | Désorption à champ élevé et spectrométrie de masse avec analyseur à double focalisation |
| FWHM | <i>Full width at half maximum</i> |
| GC-MS | Chromatographie gazeuse couplée à la spectrométrie de masse; <i>gas chromatography-mass spectrometry</i> |
| I.D. | <i>Internal diameter</i> |
| ICH | <i>International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use</i> |
| i-PrOH | <i>Isopropanol</i> |
| IS | <i>Internal standard</i> |
| JOS | <i>Josamycin</i> |
| K _{d,solid} | <i>Sorption coefficient to soil solids</i> |
| LC ₅₀ | <i>50% lethal concentration</i> |
| LC-MS | Chromatographie liquide couplée à la spectrométrie de masse; <i>liquid chromatography-mass spectrometry</i> |
| LC-MS/MS | Chromatographie liquide couplée à la spectrométrie de masse en tandem; <i>liquid chromatography-tandem mass spectrometry</i> |
| LC-TOFMS | Chromatographie liquide couplée à la spectrométrie de masse de temps d'envol; <i>liquid chromatography-time of flight mass spectrometry</i> |
| LDR | <i>Linear dynamic range</i> |
| LOC | <i>Limit of confirmation</i> |

| | |
|-------------------------|---|
| LOD | Limite de détection; <i>limit of detection</i> |
| LOEC | Plus basse concentration observée ayant un effet; <i>lowest observed effective concentration</i> |
| $\log D_{ow}$ | <i>Octanol-water distribution constant</i> |
| $\log k'_w$ | <i>Logarithm of the capacity factor of the analyte when the mobile phase is 100% aqueous</i> |
| $\log K_{ow}$ | Logarithme de la constante de partition octanol-eau <i>Logarithm of the octanol-water partition coefficient</i> |
| LOM | <i>Lomefloxacin</i> |
| LOQ | Limite de quantification; <i>limit of quantification</i> |
| LYO | Lyophilisation |
| m/z | <i>mass-to-charge ratio</i> |
| MCL | <i>Method confirmation limit</i> |
| MDL | <i>Method detection limit</i> |
| MeOH | Méthanol; <i>methanol</i> |
| meq g^{-1} | <i>milliequivalents per gram</i> |
| MIC | <i>Minimum inhibitory concentration</i> |
| MTX | <i>Methotrexate</i> |
| OWC | <i>Organic wastewater contaminant</i> |
| pABA | Acide <i>para</i> -amino-benzoïque |
| PEEK | <i>Polyetheretherketone</i> |
| PPCPs | <i>Pharmaceuticals and personal care products</i> |
| PYR | <i>Pyrimethamine</i> |
| QL | Quinolone; <i>quinolone</i> |
| r^2 | <i>Determination coefficient</i> |
| RP | Polarité de phases inversée; <i>reversed phase</i> |
| RPLC-ESI-MS/MS (IT) | Chromatographie liquide à polarité de phases inversée couplé à l'ionisation par électro-nébulisation et à la spectrométrie de masse en tandem avec une trappe ionique |
| RPLC-ESI-MS/MS (QqQ) | Chromatographie liquide à polarité de phases inversée couplé à l'ionisation par électro-nébulisation et à la spectrométrie de masse en tandem avec un triple quadripôle |

| | |
|-----------|--|
| RPLC-FLD | Chromatographie liquide à polarité des phases inversée avec détection par fluorescence |
| RPLC-UV | Chromatographie liquide à polarité des phases inversée avec détection par absorption UV |
| RSD, %RSD | <i>Relative standard deviation</i> |
| S/N | Rapport signal sur bruit; <i>signal-to-noise ratio</i> |
| STEP | Station d'épuration d'eau usée |
| SEVM | Station d'épuration des eaux usées de la Ville de Montréal |
| SIM | <i>Simazine</i> |
| SMX | <i>Sulfamethoxazole</i> |
| SOS | Chromotest SOS pour détecter substances capable d'endommager le ADN; <i>SOS Chromotest bacterial test for detecting DNA-damaging agents)</i> |
| SPE | Extraction sur phase solide; <i>solid-phase extraction</i> |
| SRM | Suivi des réactions de fragmentation caractéristiques; <i>Selected reaction monitoring</i> |
| SS | <i>Suspended solids</i> |
| STD | <i>Standard deviation</i> |
| TCC | <i>Triclocarban</i> |
| TFC | <i>Turbulent flow chromatography</i> |
| TFU | Ultrafiltration par flux tangentiel |
| THF | Tétrahydrofolate |
| TIC | <i>Total ion current</i> |
| TL | <i>Tube lens</i> |
| t_R | <i>Retention time</i> |
| TRI | Triméthoprim |
| u | <i>Unified atomic mass unit</i> |
| UV-Vis | <i>Ultraviolet-visible</i> |
| V'_b | <i>Estimated breakthrough volume</i> |
| V_m | <i>Void volume</i> |
| WW | <i>Wastewater</i> |

| | |
|----------------|---|
| WWTP | <i>Wastewater treatment plant</i> |
| XIC | <i>Extracted ion current</i> |
| λ_{em} | Longueur d'onde d'émission <i>Emission wavelength</i> |
| λ_{ex} | Longueur d'onde d'excitation <i>Excitation wavelength</i> |

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*Au Créateur, à ma famille et
à l'amour de ma vie, Paola*

Chapitre 1 : Introduction générale

1.1. Les anti-infectieux

1.1.1. Définition

Les anti-infectieux, aussi connus sous le nom d'antibiotiques, antimicrobiens ou antibactériens, sont des substances capables d'inhiber en petites doses la croissance ou la survie des microbes sans affecter l'hôte. Ces substances montrent alors une toxicité sélective puisqu'elles empêchent le déroulement de certains processus vitaux des micro-organismes sans être nocives pour les cellules des macro-organismes (Korolkovas, 1976; Mitscher, 1995; Walsh, 2003). Cette toxicité sélective démarque les anti-infectieux d'autres substances capables d'éliminer les micro-organismes tels que les antiseptiques ou les désinfectants. Les anti-infectieux peuvent être d'origine complètement naturelle comme c'est le cas de la benzylepénicilline, produite par le champignon *Penicillium notatum*; semi-synthétiques, comme l'azithromycine, dérivée de l'érythromycine (produite par l'actinomycète *Saccharopolyspora erythraea*) ou complètement synthétiques comme la sulfaméthoxazole (Radetsky, 1996; Katz et Ashley, 2005).

De façon plus spécifique, le terme antibiotique est utilisé pour se référer aux dérivés produits par des micro-organismes ainsi qu'aux substances créées de façon semi-synthétique ayant des propriétés anti-infectieuses. Les substances ayant ces mêmes propriétés, mais obtenues par synthèse totale sont plutôt des antibactériens ou antimicrobiens et non des antibiotiques (Mitscher, 1995; Bryskier, 2005d). Afin de respecter la nomenclature scientifique, le terme anti-infectieux est alors préféré et sera utilisé tout au long de ce texte.

1.1.2. Bref aperçu historique

L'utilisation des anti-infectieux n'est pas moderne. Des études réalisées sur « l'Homme des glaces », trouvé dans les Alpes de l'Ötztal en 1991, ont démontré qu'il y a plus de 5000 ans, les huiles du champignon *Piptoporus betulinus* étaient utilisées comme des anti-infectieux pour lutter contre les parasites intestinaux (Capasso, 1998). Nos ancêtres connaissaient les propriétés thérapeutiques de nombreuses substances naturelles (Cowan,

1999) et avant le XVI^e siècle, les Amérindiens du Brésil utilisaient de l'extrait du rhizome de l'ipéca (*Psychotria ipecacuanha*) pour traiter la dysenterie amibienne (Alves Garcia *et al.*, 2005). Cependant, ces remèdes sont restés peu connus ou rarement accessibles et avant le XX^e siècle les infections bactériennes étaient une des causes majeures de mortalité (Bryskier, 2005d). Durant la Guerre civile américaine, 50 % des soldats blessés sont morts à cause des infections (Le Couteur et Burreson, 2003). À cette époque des infections comme la syphilis étaient traitées en respirant les vapeurs du mercure dans un four chauffé. Évidemment, si les patients survivaient à ce type de traitement, l'empoisonnement au mercure était la cause de mortalité par la suite (Le Couteur et Burreson, 2003).

Cette situation a changé grâce au développement de la chimiothérapie vers le début du XX^e siècle. Selon le chimiste allemand Paul Ehrlich, il était possible de trouver une substance capable d'agir comme une sorte de « balle magique » *c.-à-d.*, une substance ayant la capacité d'éliminer les micro-organismes pathogènes sans être toxique pour l'hôte. Pour arriver à cette fin, il a appliqué la chimie à la bactériologie en étudiant de façon systématique l'effet des colorants à base d'aniline sur les bactéries (Petri, 2007). Ses travaux ont abouti à la découverte en 1909 du salvarsan, un colorant à base d'arsenic capable de traiter la syphilis (Levy, 1992b). Quoique le salvarsan ne fût pas une « balle magique », cette drogue était aussi toxique pour les patients; son approche a inspiré d'autres chimistes. C'est au début des années 1930 que les travaux de Gerhard Domagk ont mené à la découverte des premiers anti-infectieux, les sulfonamides (Levy, 1992b; Radetsky, 1996).

Cette découverte a commencé une « révolution thérapeutique » et depuis, des milliers d'anti-infectieux ont été découverts ou synthétisés (Greenwood, 2003a). Les anti-infectieux ont été reconnus comme « un trésor médical, peut-être la découverte thérapeutique la plus importante de l'histoire de la médecine » (Levy, 1992c). En effet, l'introduction de la benzylpénicilline et de la streptomycine a réduit la mortalité de 30 % en 1947 comparé à celle de 1938 et aussi a contribué à augmenter l'espérance de vie qui est passée de 47 ans en 1900 à plus de 70 ans en 1960 (Bunker, 2001). La popularité des anti-infectieux a vite grandi et durant les années 1970, et durant cette période ces substances étaient la classe de drogues la plus prescrite dans les É.-U. (Korolkovas, 1976). En 1997, le marché mondial des anti-infectieux s'élevait à 17 milliards de dollars américains (Carbon et Bax, 1998). Il

est estimé qu'entre 100 000 et 200 000 tonnes des ces substances sont consommées annuellement dans le monde entier (Wise, 2002).

1.2. Classification, mode d'action et utilisation des anti-infectieux

Les anti-infectieux sont souvent classifiés selon leur structure moléculaire. Nous décrirons brièvement les classes ou familles d'anti-infectieux les plus utilisées selon l'ordre chronologique de leur découverte. Le **Tableau 1-1** résume l'information de cette section. Le développement des nouvelles substances suit souvent le même patron : découverte d'une molécule piste, servant de structure de base, et ensuite synthèse des nouveaux dérivés par l'ajout ou substitution des groupements fonctionnels à la molécule de base pour augmenter l'activité anti-infectieuse de cette dernière.

La recherche et développement des nouveaux anti-infectieux ont été encouragés par le besoin de nouvelles substances avec spectres d'activité élargis ainsi que l'apparition de micro-organismes résistants aux anti-infectieux. En effet, la prolifération de la résistance a diminué l'efficacité des premières substances découvertes, en les rendant presque inutiles (Bryskier, 2005d).

1.2.1. Sulfonamides

Les sulfonamides ont été les premiers anti-infectieux ayant un spectre d'activité élargie (Veyssier et Bryskier, 2005). La première sulfonamide introduite dans le marché a été le colorant Prontosil (sulfamidochrysoïdine) au milieu des années 1930. La découverte de ce composé est due au scientifique allemand Gerhard Domagk. Celui-ci avait observé une propriété intéressante du Prontosil : le composé montrait l'activité antibactérienne *in vivo*, cependant aucune activité n'a été observée *in vitro* (Le Couteur et Burreson, 2003). Par la suite, il a été découvert que le colorant n'était pas le composé actif, mais plutôt la sulfanilamide, un produit de sa métabolisation par l'organisme. Entre 1935 et 1946 plus de 5 000 dérivés de la sulfanilamide ont été synthétisés. Grâce à la recherche sur la sulfanilamide, il a été découvert que la substitution d'un hydrogène sur le groupement

aminosulfonyle par certains groupements fonctionnels augmentaient son activité antimicrobienne (Veyssier et Bryskier, 2005). La **Figure 1-1** montre les sulfonamides les plus mentionnées dans la littérature environnementale.

1.2.1.a. Mode d'action

L'activité antibactérienne des sulfonamides découle de leur capacité à interférer avec la biosynthèse du tétrahydrofolate (THF), une coenzyme essentielle à la synthèse des acides aminés et des acides nucléiques chez les bactéries. Les sulfonamides bloquent la synthèse d'un précurseur du THF, la dihydroperoate, et sont en compétition avec l'acide *para*-amino-benzoïque (pABA) pour le site actif de l'enzyme dihydroptéroate synthétase (Walsh, 2003).

1.2.1.b. Utilisation

Les sulfonamides sont des agents bactériostatiques, c'est-à-dire, ils empêchent la multiplication cellulaire sans tuer complètement les bactéries. Elles sont utilisées pour le traitement des infections urinaires et respiratoires. Ces anti-infectieux ont un spectre élargi d'activité, et sont alors capables d'arrêter la prolifération des bactéries Gram positif et Gram négatif. Les bactéries Gram-positif possèdent une paroi cellulaire externe de peptidoglycane et celles Gram négatif, une paroi cellulaire externe constituée de peptidoglycane et de lipides. Des exemples des bactéries Gram-négatif susceptibles aux sulfonamides sont *Haemophilus influenzae* (causant la méningite purulente) et *Escherichia Coli* (responsable de la majorité des infections urinaires) Des exemples des bactéries Gram positif susceptibles aux sulfonamides sont *Staphylococcus aureus* (causant entre autres, l'ostéomyélite), *Streptococcus pyogenes* (causant l'otite) et *Streptococcus pneumoniae* (responsable de la pneumonie bactérienne) (Avril, 1988).

Cependant à cause de l'apparition de souches résistantes aux sulfonamides depuis le début des années 1940, elles sont rarement administrées seules (Mitscher, 1995; Greenwood, 2003b). Vers la fin des années 1960, les sulfonamides ont commencé à

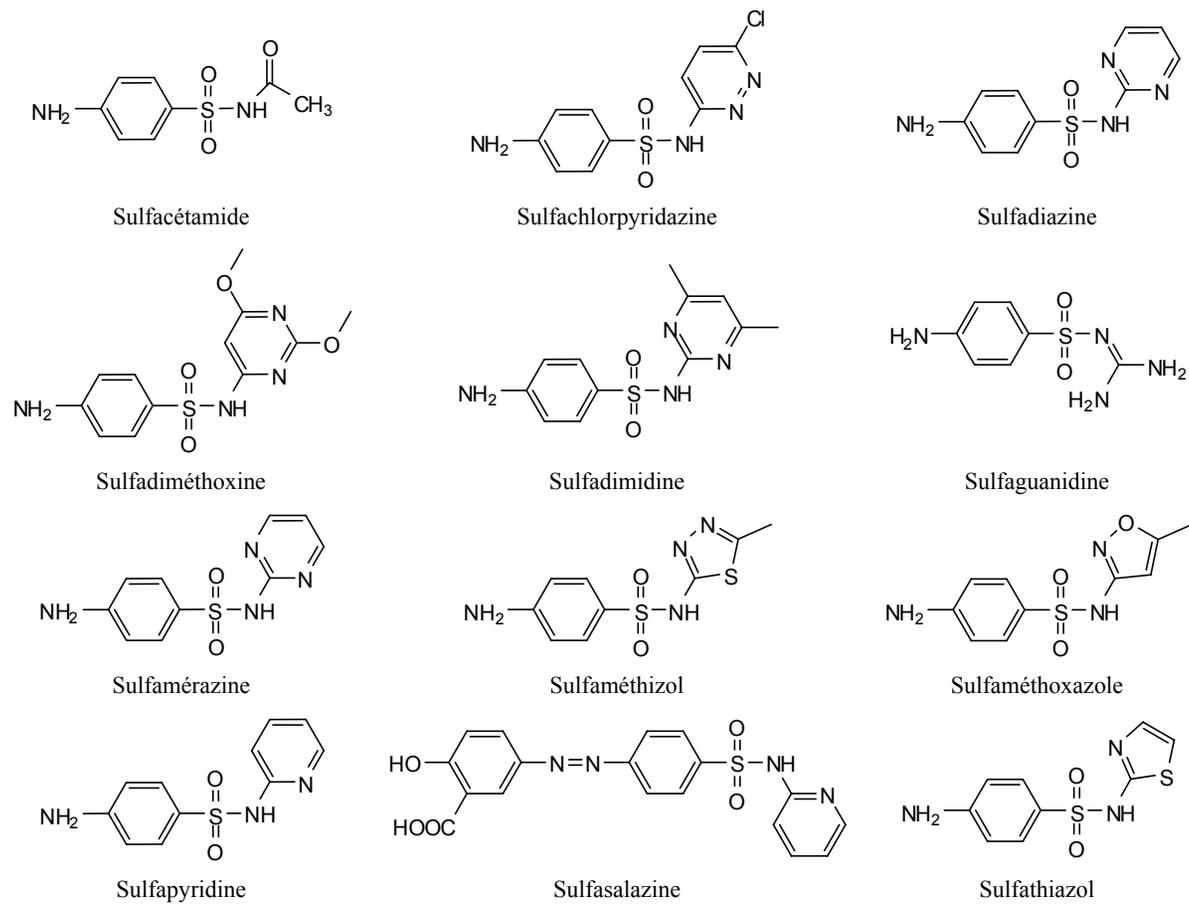


Figure 1-1. Structures des sulfonamides les plus mentionnées dans la littérature environnementale.

être administrées en combinaison avec un inhibiteur de l'hydrofolate réductase tel que le triméthoprim (Veysier et Bryskier, 2005).

1.2.2. β -lactamines

Les β -lactamines sont un groupe très large d'anti-infectieux comportant plusieurs sous-classes telles que les pénicillines, les céphalosporines, les carbapénèmes et les monobactames, parmi d'autres. Cette section focalisera seulement sur les pénicillines et les céphalosporines, les deux classes de β -lactamines les plus utilisées actuellement. Ces deux classes se différencient par le cycle attaché à l'anneau azetidone (anneau β -lactamine) : pour les pénicillines un pentacycle (thiazolidine) et pour les céphalosporines un hexacycle insaturé (dihydrothiazine) (Bryskier, 2005b). Les β -lactamines les plus mentionnées dans la littérature environnementale apparaissent la **Figure 1-2**.

Les propriétés antibactériennes de la pénicilline ont été découvertes vers la fin des années 1920 par Alexander Flemming, lorsqu'il a observé qu'une culture de *Staphylococcus aureus* était inhibée par la moisissure *Penicillium notatum* (Radetsky, 1996). Sa découverte a été vite oubliée et c'est en 1940 que Florey et Chain ont réussi à isoler la pénicilline, un mélange de quatre β -lactamines, dont la benzylepénicilline a été sélectionnée pour développement (Bush, 2003). Cette substance a servi de modèle pour la conception des pénicillines semi-synthétiques.

Les céphalosporines, ont été découvertes au milieu des années 1940 par Giuseppe Brotzu qui a observé qu'un filtrat de culture de *Cephalosporium acremonium*, (maintenant appelé *Acremonium crysogenum*), montrait de l'activité antibactérienne. La première céphalosporine, céphalosporine C a été isolée par l'équipe de Abraham et Newton de l'Université d'Oxford (Bryskier et Lebel, 2005). La modification synthétique de cette molécule a donné lieu à de nombreux dérivés qui se trouvent parmi les anti-infectieux les plus utilisés. En effet, actuellement les céphalosporines, avec les pénicillines et les autres β -lactamines, représentent parmi 50 et 70% de la consommation humaine des anti-infectieux dans nombreux pays (Kummerer, 2009).

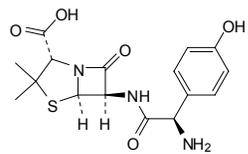
1.2.2.a. Mode d'action

Les β -lactamines, agissent sur la synthèse du peptidoglycane, un composant essentiel de la paroi cellulaire des procaryotes. L'activité antibactérienne des β -lactamines découle de leur capacité à inhiber la transpeptidase, une enzyme responsable de catalyser la transpeptidation, une des étapes nécessaires à la réticulation du peptidoglycane (Walsh, 2003).

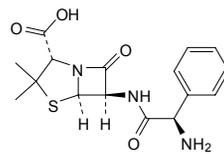
1.2.2.b. Utilisation

Les pénicillines sont des agents bactéricides, *c.-à-d.*, les doses prescrites sont létales pour les bactéries. Elles sont utilisées pour le traitement des nombreuses infections causées par des streptocoques, des staphylocoques et d'autres bactéries (Bush, 2003). Le spectre d'activité des pénicillines incluent les coccus Gram négatif *Neisseria gonorrhoeae* (causant la blennorragie) et *Neisseria meningitidis* (responsable de la méningite cérébro-spinale) ainsi que les bacilles Gram-positif *Listeria monocytogenes* (responsable de la listériose), *Corynebacterium diphtheriae* (causant l'angine diphtérique), *Bacillus anthracis* (responsable de la maladie du Charbon). (Avril *et al.*, 2000).

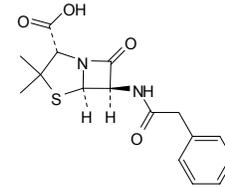
Les céphalosporines sont des aussi bactéricides et elles sont souvent utilisées pour le traitement des infections des voies respiratoires supérieures, urinaires, des tissus mous et d'autres infections lorsque les patients sont allergiques aux pénicillines. Elles sont actives contre les bactéries Gram positif telles que *Enterococcus faecalis* (impliquée dans l'endocardite aiguë) et *Staphylococcus epidermidis* (impliquée dans la péritonite) et contre des bactéries Gram négatif telles que *N. gonorrhoeae*, *H. influenzae*, et *Klebsiella rhinoscleromatis* (causant le rhinosclérome) parmi d'autres (Avril *et al.*, 2000; Greenwood, 2003c).



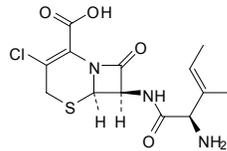
Amoxicilline



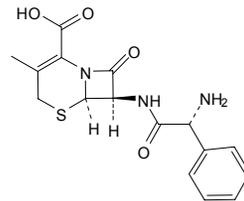
Ampicilline



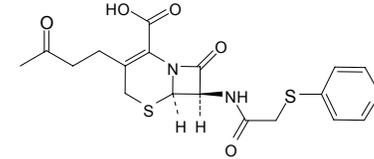
Benzylepénicilline



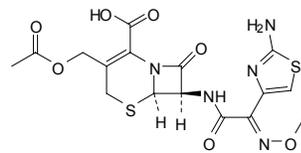
Céfáclor



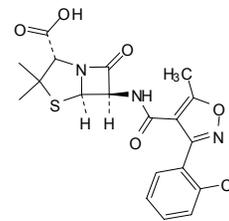
Céfalexine



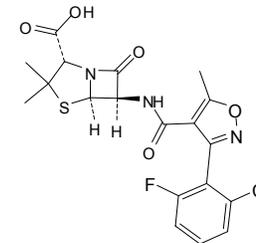
Céfapirine



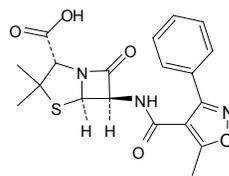
Céfotaxime



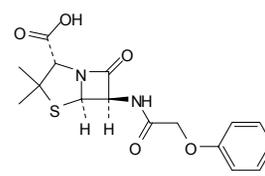
Cloxacilline



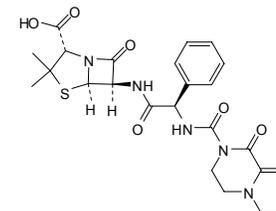
Flucloxacilline



Oxacilline



Phenoxyéthylpénicilline



Pipéracilline

Figure 1-2. Structures des β -lactamines les plus mentionnées dans la littérature environnementale.

1.2.3. Tétracyclines

La recherche des nouveaux anti-infectieux ayant des spectres d'activité élargis a amené à la découverte des tétracyclines. La première tétracycline, chlortétracycline, a été isolée au milieu des années 1940 à partir d'une culture de *Streptomyces aureofaciens* par Benjamin Duggar et ensuite utilisée de façon thérapeutique vers la fin des années 1940 (Bryskier, 2005a). Cette molécule a servi de modèle pour le développement de la classe des tétracyclines (**Figure 1-3**). En 1955, plus de 8 000 papiers ont été publiés en décrivant l'application de chlortétracycline pour des infections aiguës et chroniques (Levy, 1992b).

1.2.3.a. Mode d'action

L'activité antibactérienne des tétracyclines est basée sur l'inhibition de la synthèse des protéines (Chopra et Roberts, 2001). Pour induire leur effet anti-infectieux, les tétracyclines doivent traverser les membranes cellulaires pour se rendre aux ribosomes. Sur ces organites, elles empêchent l'association de l'ARN de transfert avec le site A des sous-unités 30S du ribosome (Walsh, 2003). La fixation particulière des tétracyclines sur ce site des ribosomes évite que les acides aminés soient assemblés dans des chaînes peptidiques pour former des protéines (Bryskier, 2005a).

1.2.3.b. Utilisation

Au milieu agricole, les tétracyclines sont parmi les anti-infectieux les plus utilisées (Sarmah *et al.*, 2006). Les tétracyclines sont des anti-infectieux bactériostatiques à spectre élargi, cependant, leur utilisation a diminué à cause de l'apparition des souches résistantes, et elles ont été substituées à des agents plus efficaces (Chopra et Roberts, 2001; Chopra, 2003). Les tétracyclines sont encore utilisées lors du traitement multi-drogues de la gastrite et des ulcères peptidiques causées par *Helicobacter pylori*, le cholera, l'acné et certaines infections génitales. Elles sont actives contre les bactéries Gram positif tels que *S. pyogenes*, et celles Gram négatif telles que *N. gonorrhoeae* et *H. influenzae*.

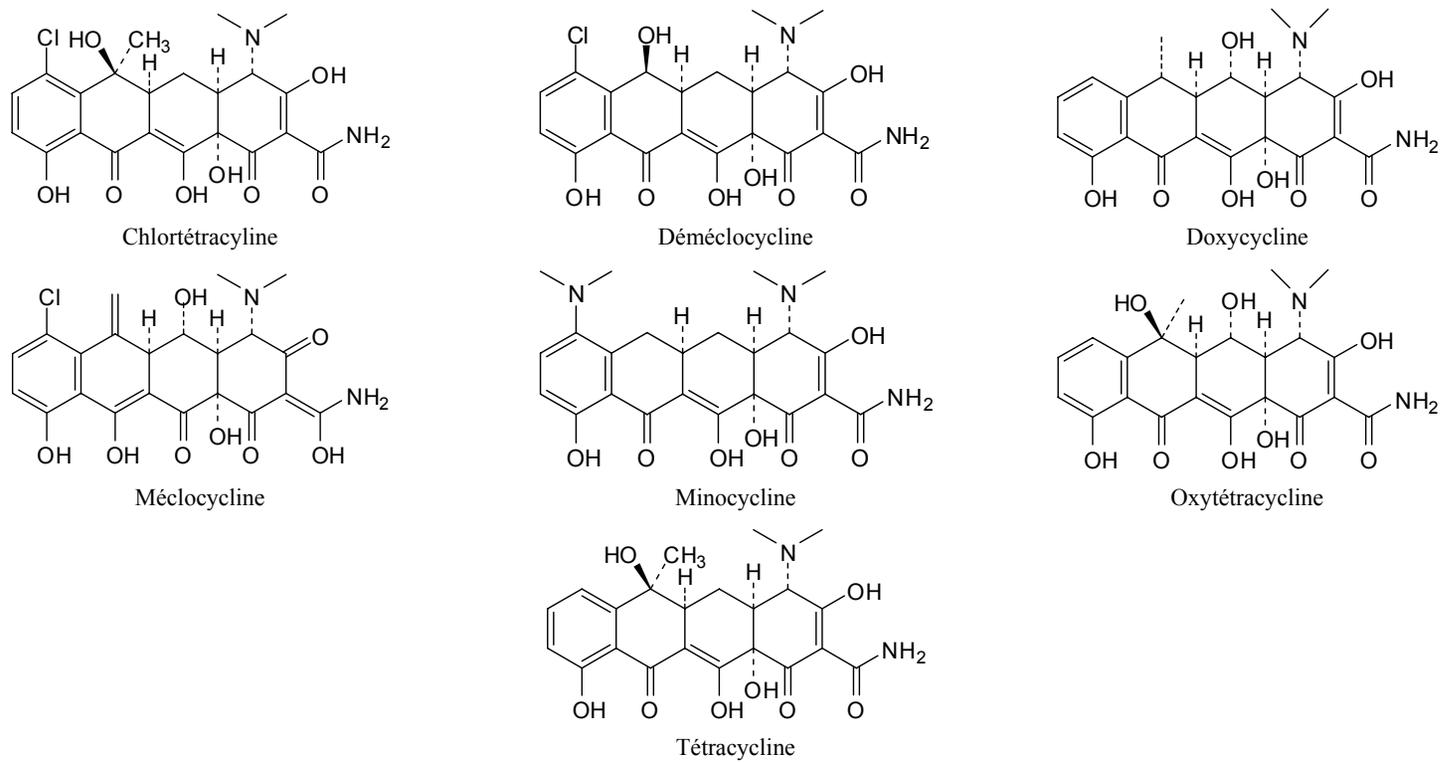


Figure 1-3. Structures des tétracyclines les plus mentionnées dans la littérature environnementale.

1.2.4. Macrolides

Vers la fin des années 1940, le développement des anti-infectieux semblait être complet, les substances découvertes à l'époque étaient capables de guérir la plupart des infections causées par une multitude de micro-organismes. Cependant, il a été observé que l'efficacité de certains anti-infectieux diminuait avec le temps, et que certaines souches des bactéries subtiles aux anti-infectieux devenaient résistantes. Alors, la recherche de nouvelles substances anti-infectieuses a continué dans les années 1950 (Bryskier et Bergogne-Bérézin, 2005). Le premier macrolide introduit pour utilisation clinique, l'érythromycine, a été découvert par les chercheurs de la société Eli Lilly vers le début des années 1950 et a servi de piste pour le développement de la classe des macrolides (Bryskier et Bergogne-Bérézin, 2005). Le terme « macrolide » est une abréviation de « antibiotiques à macrolactone hétéroside », elles sont alors formées par un hétérocycle oxygéné lié à un ou plus résidus de désoxyoses (**Figure 1-4**) (Katz et Ashley, 2005).

1.2.4.a. Mode d'action

L'activité antibactérienne des macrolides est basée sur un mécanisme semblable à celui des tétracyclines. Les macrolides inhibent la synthèse des protéines bactériennes en se liant à la sous-unité 50 du site P des ribosomes, causant ainsi l'expulsion prématurée de l'ARN de transfert des ribosomes (Katz et Ashley, 2005).

1.2.4.b. Utilisation

Les macrolides sont utilisés pour traiter les infections urogénitales, des voies respiratoires supérieures et inférieures, de la peau, et des tissus mous, parmi d'autres (Bryskier et Butzler, 2003). Le spectre d'activité des macrolides inclut les bactéries Gram positif, les cocci et bacilles Gram négatif, les bactéries anaérobies et les spirochètes entre autres. Elles montrent aussi l'activité contre des nombreux pathogènes intracellulaires tels que *Chlamydia trachomatis* (responsable du trachome), *Chlamydomphila pneumoniae*

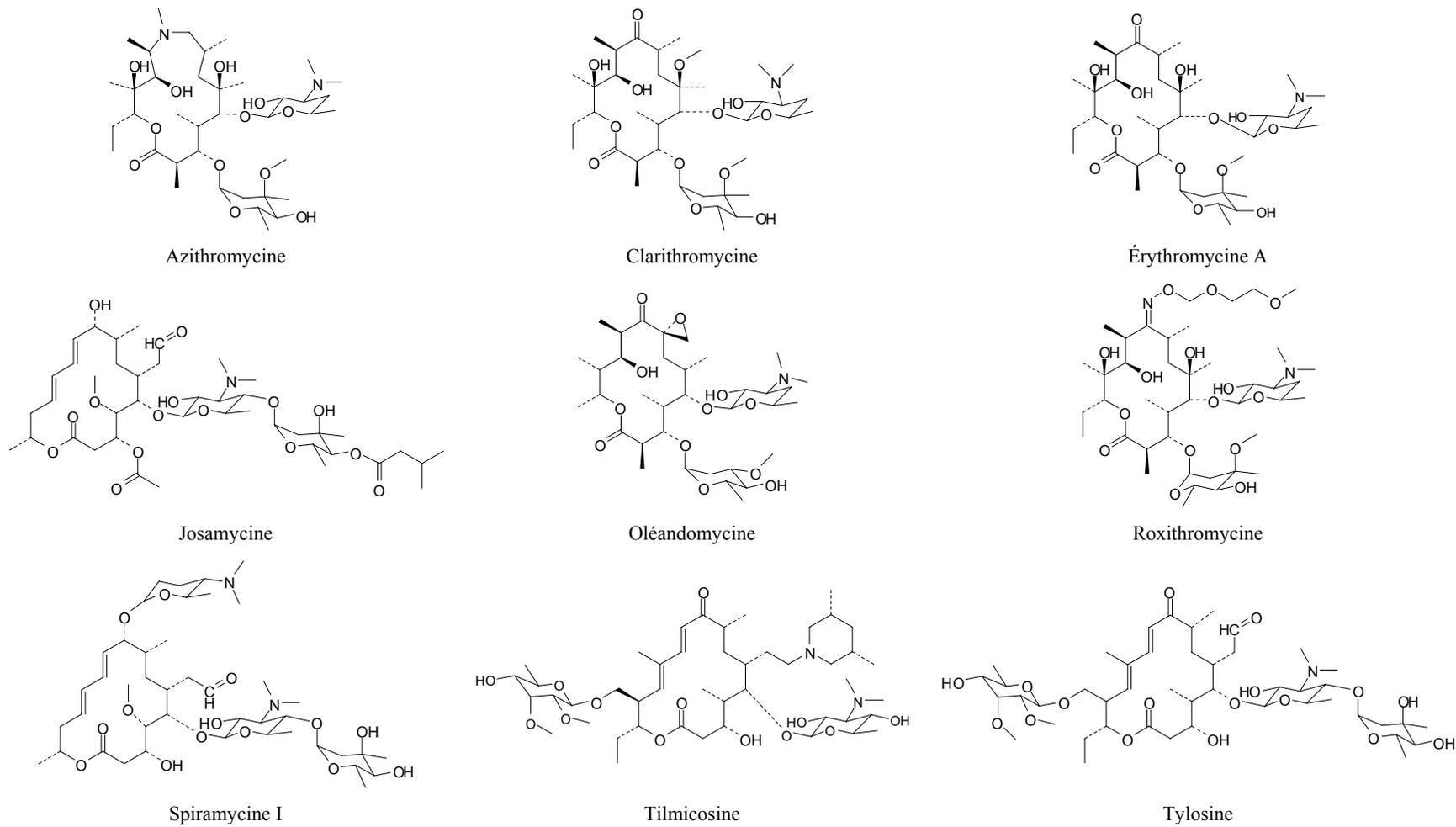


Figure 1-4. Structures des macrolides les plus mentionnés dans la littérature environnementale.

(provoquant aussi la pneumonie), *Legionella pneumophila* (causant la maladie des légionnaires), etc. (Avril *et al.*, 2000).

1.2.5. Quinolones

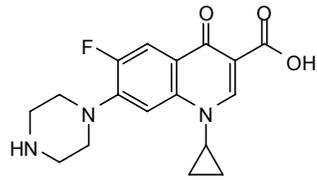
Leshner et ses collaborateurs ont observé lors de la fabrication d'un agent contre le paludisme, que la chloroquine, une impureté du processus avait des propriétés antibactériennes. La modification chimique de cette impureté a abouti dans la découverte, au début des années 1960, de l'acide nalidixique, la molécule piste des quinolones (**Figure 1-6**) (Bryskier, 2005c; Mitscher, 2005). Les fluoroquinolones, un sous groupe des quinolones, ont été introduites plus tard lors de la découverte de la norfloxacin, un dérivé ayant un atome de fluor à la position 6 et un groupement piperazinyle à la position 7 étant 10 à 100 plus active que l'acide nalidixique (Bryskier, 2005c).

1.2.5.a. Mode d'action

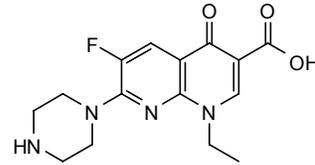
Le mécanisme de fonctionnement des quinolones consiste en leur capacité à traverser la paroi cellulaire des bactéries et inhiber la synthèse de l'ADN (Bryskier, 2005c). L'inhibition de la synthèse de l'ADN a lieu à la suite de la formation d'un complexe ternaire composé de la quinolone, l'ADN et une enzyme topoisomérase. Ce complexe perturbe les fonctions de base de l'ADN telles que la transcription, la réplication et la réparation, résultant dans la mort cellulaire (Mitscher, 2005).

1.2.5.b. Utilisation

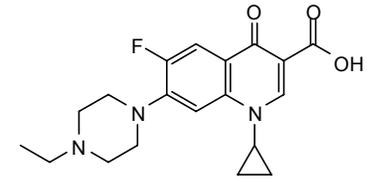
Les quinolones sont souvent utilisées pour le traitement de différents types d'infections: gastro-intestinales, gynécologiques, urinaires, des voies respiratoires supérieures et inférieures, de la peau, des os et des tissus mous. Aussi elles sont utilisées



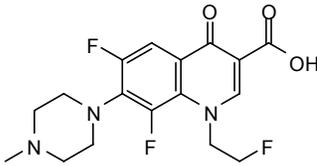
Ciprofloxacin



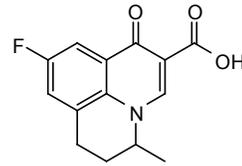
Énoxacin



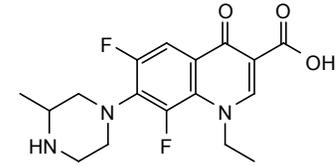
Enrofloxacin



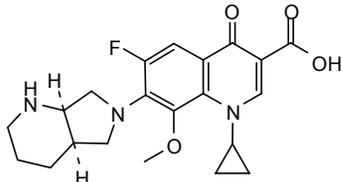
Fléroxacin



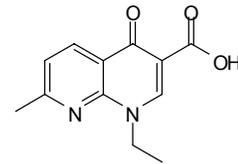
Fluméquine



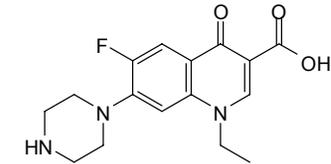
Loméfloxacine



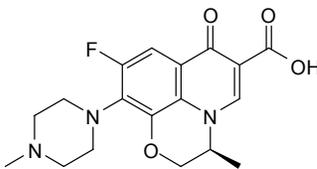
Moxifloxacine



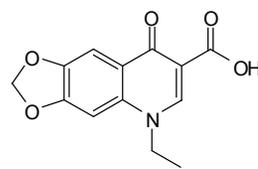
Acide nalidixique



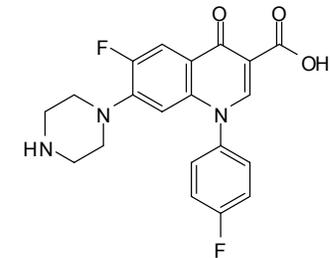
Norfloxacine



Lévofloxacine



Acide oxolinique



Sarafloxacine

Figure 1-5. Structures des quinolones les plus mentionnées dans la littérature environnementale.

pour le traitement de maladies transmissibles sexuellement et la prostatite (Mitscher, 2005).

Les quinolones montrent une activité bactéricide et sont efficaces contre les cocci Gram positif tels que *S. pyogenes*, *Streptococcus agalactiae* (pouvant causer des infections précédant ou suivant l'accouchement) et *E. faecalis*; les bacilles Gram-positif *C. diphtheriae*, *L. monocytogenes* et les entérobactéries *E. coli* et *Klebsiella pneumoniae* (impliquée dans la pneumonie). Les quinolones aussi sont efficaces contre les bacilles Gram négatif tels que *Pseudomonas stutzeri* et *Pseudomonas putida*, deux bactéries pouvant contaminer les solutions antiseptiques dans les milieux hospitaliers (Avril *et al.*, 2000; Mitscher, 2005).

1.2.6. Autres anti-infectieux utilisés fréquemment

La **Figure 1-6** montre les structures d'autres anti-infectieux employés couramment. Certains de ces composés tels que l'amprolium et le chloramphénicol sont rarement utilisés sur les humains, ils sont plutôt administrés aux animaux d'élevage (Boxall, 2004).

1.2.6.a. Lincosamides

Lincomycine, le composé piste des lincosamides, a été isolée en 1962 à partir d'une culture de *Streptomyces lincolnensis*. Le développement des composés semi-synthétiques avec des propriétés améliorées n'a pas trouvé beaucoup de succès à l'exception de la clindamycine (Greenwood, 2003b).

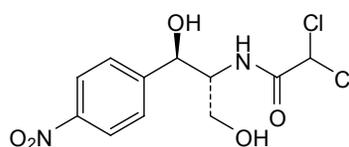
Les lincosamides agissent en inhibant la synthèse des protéines bactériennes de façon similaire aux macrolides (Mukhtar, 2005). Les lincosamides sont administrées pour le traitement des infections chez les patients allergiques aux pénicillines. Ces anti-infectieux sont aussi actifs contre les protozoaires et les mycoplasmes (Greenwood, 2003b).

1.2.6.b. Triméthoprime

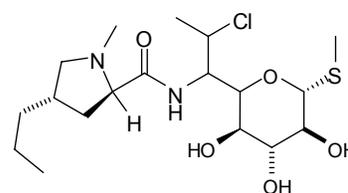
Le triméthoprime appartient à la classe des diaminopyrimidines. Le triméthoprime agit en interrompant le cycle de la biosynthèse des folates (comme les sulfonamides), en empêchant la conversion de la coenzyme dihydrofolate à THF par inhibition sélective de l'enzyme dihydrofolate réductase (Walsh, 2003). À cause de l'effet synergétique sur l'activité antibactérienne qui montrent les sulfonamides combinées avec triméthoprime (Veysier et Bryskier, 2005), ce dernier est habituellement prescrit sous forme d'une mixture avec sulfaméthoxazole. Le triméthoprime seul est utilisé pour le traitement des infections urinaires (Then, 2003).



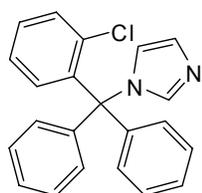
Amprolium



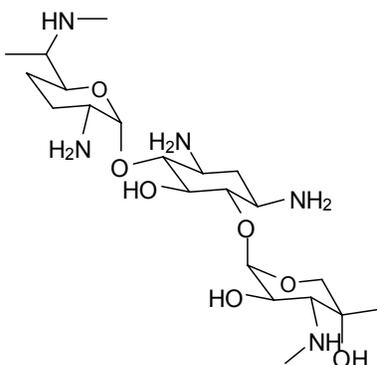
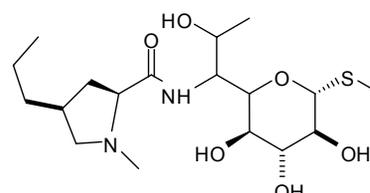
Chloramphénicol



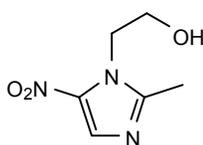
Clindamycine



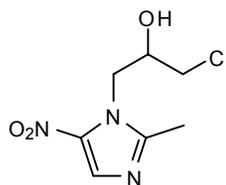
Clotrimazole

Gentamicine C₁

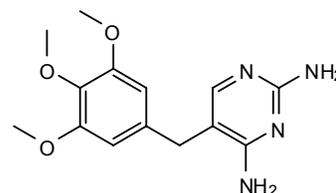
Lincomycine



Métronidazole



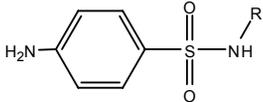
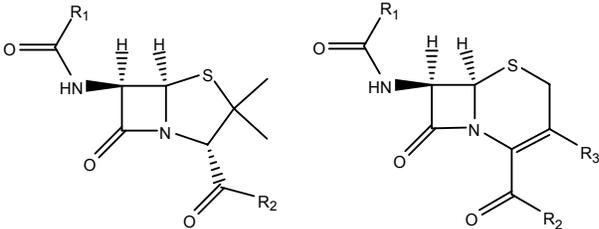
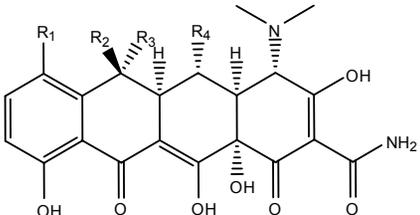
Ornidazole



Triméthoprime

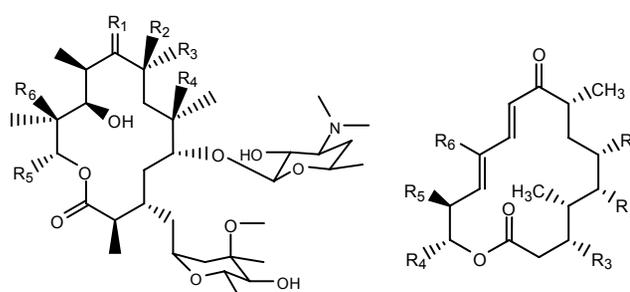
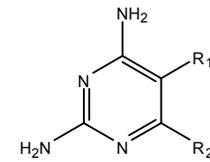
Figure 1-6. Structures d'autres anti-infectieux souvent mentionnées dans la littérature environnementale.

Tableau 1-1. Structures communes et mode d'action des principales classes d'anti-infectieux.

| Classe | Structure commune ^a | Cible d'inhibition microbienne | Composés les plus prescrits au Québec ^b |
|---------------|--|------------------------------------|--|
| Sulfonamides |  | Métabolisme de l'acide folique | Sulfaméthoxazole (13) Sulfasalazine (26) Sulfisoxazole (46) Sulfadiazine (71) |
| β-lactamines |  | Biosynthèse de la paroi cellulaire | Amoxicilline d (1) Phenoxyméthylpénicilline (5) Cefprozil (7) Céfalexine (15) |
| Tétracyclines |  | Biosynthèse des protéines | Minocycline (11) Doxycycline (23) Tétracycline (27) Déméclocycline (63) |

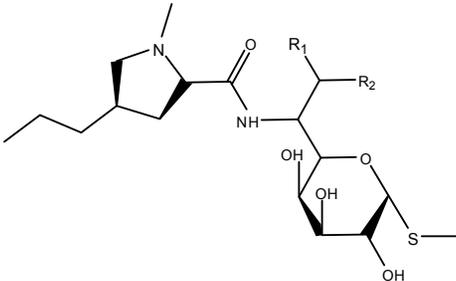
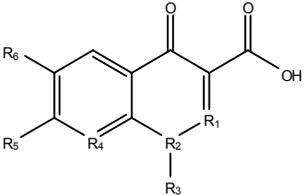
^a Les « R » indiquent les endroits où des groupements caractéristiques à chaque substance peuvent se trouver, ceci est applicable à la plupart des anti-infectieux. ^b Rang d'après le nombre de prescriptions vendues au Québec en 2006, selon IMS Health Canada (IMS Health Canada, 2007).

Tableau 1-1. Structures communes et mode d'action des principales classes d'anti-infectieux (suite).

| Classe | Structure commune ^a | Cible d'inhibition microbienne | Composés les plus prescrits au Québec ^b |
|--------------------|---|--------------------------------|--|
| Macrolides |  <p>Anneaux à 14 membres Anneaux à 16 membres</p> | Biosynthèse des protéines | Clarithromycine (3) Azithromycine (4) |
| Diaminopyrimidines |  | Métabolisme de l'acide folique | Triméthoprime (8) |

^a Les « R » indiquent les endroits où des groupements caractéristiques à chaque substance peuvent se trouver, ceci est applicable à la plupart des anti-infectieux. ^b Rang d'après le nombre de prescriptions vendues au Québec en 2006, selon IMS Health Canada (IMS Health Canada, 2007).

Tableau 1-1. Structures communes et mode d'action des principales classes d'anti-infectieux (suite).

| Classe | Structure commune ^a | Cible d'inhibition microbienne | Composés les plus prescrits au Québec ^b |
|--------------|---|------------------------------------|---|
| Lincosamides |  | Biosynthèse de protéines | Clindamycine (14) Lincomycine (86) |
| Quinolones |  | Réplication et réparation de l'ADN | Ciprofloxacine (2) Moxifloxacine (9) Lévofloxacine (21) |

^a Les « R » indiquent les endroits où des groupements caractéristiques à chaque substance peuvent se trouver, ceci est applicable à la plupart des anti-infectieux. ^b Rang d'après le nombre de prescriptions vendues au Québec en 2006, selon IMS Health Canada (IMS Health Canada, 2007).

1.3. Les anti-infectieux comme contaminants d'intérêt émergent

1.3.1. Les contaminants « émergents »

Les contaminants « émergents » sont un ensemble hétérogène de substances dont leur présence dans l'environnement n'avait pas été décelée auparavant. L'étude et surveillance de ces contaminants est relativement récent, vers le début des années 1990 (Erickson, 2002; Snow *et al.*, 2007). Cet ensemble comprend des composés tels que les surfactants, les pharmaceutiques, les hormones, les écrans solaires, les retardateurs des flammes et les toxines algales, parmi d'autres. Le terme « émergent » sert alors à les distinguer des substances dénommées comme des « polluants prioritaires » tels que les pesticides, les métaux traces ou les hydrocarbures aromatiques polycycliques dont leurs concentrations dans l'environnement sont réglementées et leurs effets sur l'environnement sont étudiés depuis des années (CCME, 2005; CCME, 2007). D'après Daughton (Daughton, 2004b), la dénomination « émergent » est impropre, puisqu'il suggère que l'occurrence de ces contaminants dans l'environnement est récente.

L'acide clofibrique, un contaminant qu'on peut classer comme « émergent » a été détecté dans l'environnement pour la première fois en 1976 (Garrison *et al.*, 1976). Les anti-infectieux ont été identifiés pour la première fois dans les eaux de surface du Royaume-Uni par Watts et son équipe en 1983 (Watts *et al.*, 1983).

Nos connaissances actuelles suggèrent que la présence de contaminants « émergents » dans l'environnement date du temps qu'ils ont été introduits pour la première fois dans le marché (Erickson, 2002; Daughton, 2004a). Dans les cas des anti-infectieux ceci suggère que l'environnement aquatique a été exposé durant plus de 70 ans à ces substances. Alors, le terme « émergent » se réfère plutôt à l'intérêt récent tant de la communauté scientifique comme celle du public général pour l'occurrence et le devenir environnemental de ces substances. Cet intérêt est naissant non pas à cause de l'inadvertance de la communauté scientifique, mais plutôt à cause de son incapacité à détecter leur présence (Daughton, 2004a).

Les études sur l'occurrence des anti-infectieux dans l'environnement ont été inhibées par la disponibilité des techniques d'analyse assez sensibles et particulières telles que la chromatographie liquide couplée à la spectrométrie de masse (LC-MS) (Daughton et Ternes, 1999; Ternes *et al.*, 2004b). Le développement de la chimie analytique dans les derniers 20 ans a permis la détection des substances qu'on croyait inexistantes dans l'environnement. Durant cette période, les limites instrumentales de détection de la LC-MS ont été abaissées d'un facteur de $\sim 10^4$ (Barceló et Petrovic, 2007). Pour ces raisons, le terme « contaminants d'intérêt émergent » est préféré et sera utilisé désormais dans ce document.

C'est vers la fin des années 1990 et le début de 2000 que des méthodes sensibles d'analyse de traces ont été publiées (Hirsch *et al.*, 1998; Golet *et al.*, 2001; Lindsey *et al.*, 2001). Une discussion plus approfondie du développement des méthodes d'analyse des anti-infectieux dans les eaux environnementales sera présentée dans la section **1.4**.

1.3.2. Sources et transport

Les sources des anti-infectieux dans l'environnement aquatique sont très diverses (**Figure 1-7**). Cependant, il a été reconnu que les sources principales de ces substances dans l'environnement sont les excréments des personnes ou des animaux sous traitement (Hirsch *et al.*, 1999; Golet *et al.*, 2002). Lorsqu'un individu consomme un anti-infectieux, une partie de cette substance (30-90%) est éliminée par les voies d'excrétion (urine, fèces, etc.) sans être métabolisée, *c.-à-d.*, transformée par l'organisme (Cooper *et al.*, 2008) (**Tableau 1-2**). Une partie importante de la dose ingérée peut alors se retrouver dans les eaux usées domestiques ou des hôpitaux (dans les cas de la consommation humaine), ou dans les eaux usées agricoles et le fumier (consommation animale). Par conséquent, les deux voies de contamination majeures sont la voie urbaine et la voie agricole.

De façon générale, ces deux voies se différencient principalement par la manière dont les eaux contaminées sont rejetées dans l'environnement. D'après les connaissances actuelles, la présence naturelle des anti-infectieux dans l'environnement, due à la

production des antibiotiques par les bactéries du sol peut être négligée. Leur contribution dans les eaux de surface doit être davantage inférieure étant donné la faible densité des bactéries dans ces matrices par rapport aux sols (Kummerer, 2009).

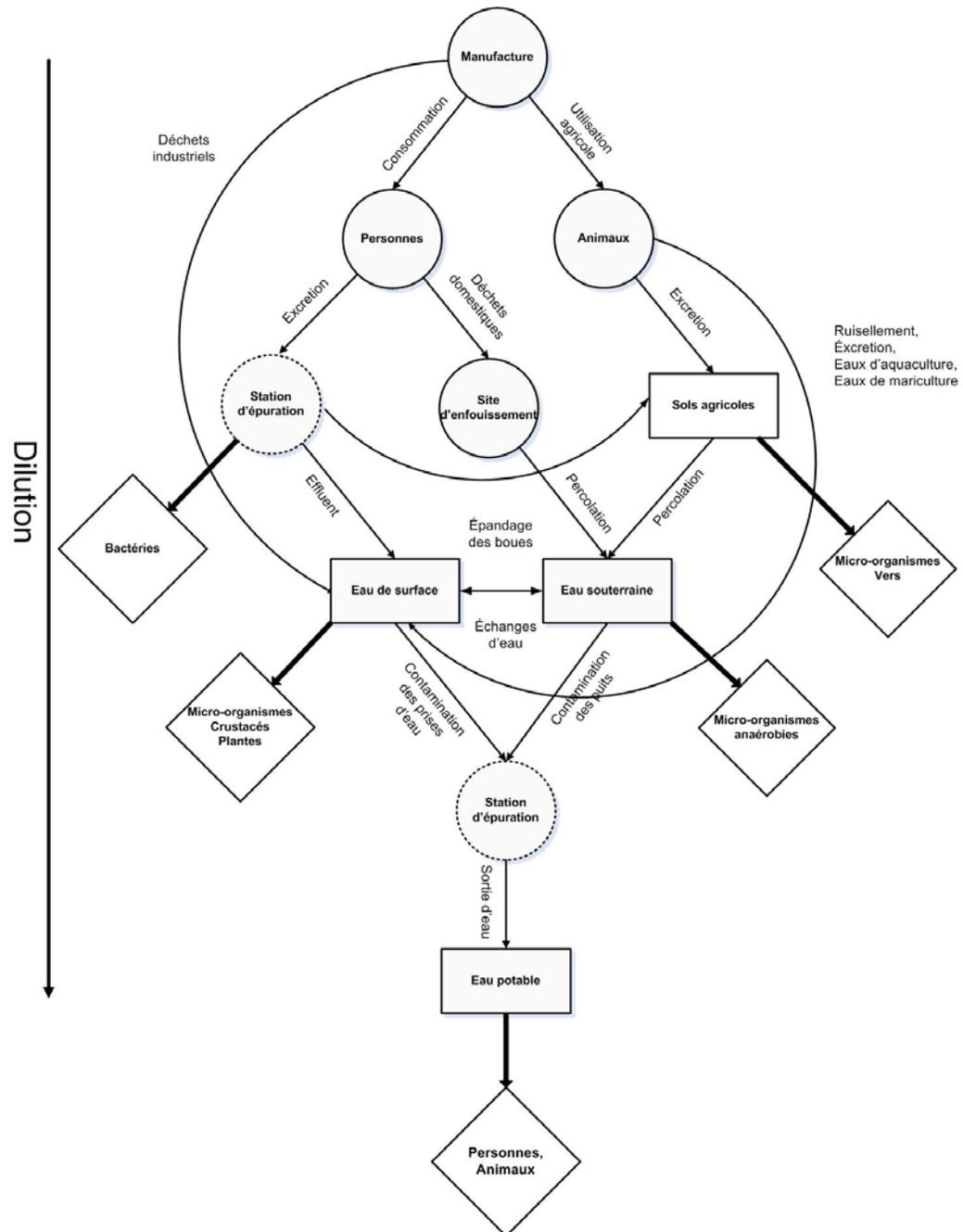


Figure 1-7. Devenir environnemental des anti-infectieux. Les cercles indiquent les sources; les rectangles, les milieux récepteurs et les losanges, les cibles. Les cercles pointillés indiquent que ces sources ne sont pas parfois présentes et par conséquent le transport se fait directement.

Tableau 1-2. Propriétés des anti-infectieux ayant un effet sur leur occurrence dans les eaux environnementales.

| Anti-infectieux | Taux d'élimination ^a (%) | Référence | Dégradation (%) | Référence |
|------------------------|--|--|---------------------------------------|---|
| Amoxicilline | 44 - 79 | (Bryskier, 2005b) | 5 ^b , 13 - 63 ^c | (Alexy <i>et al.</i> , 2004; Gartiser <i>et al.</i> , 2007a) |
| Azithromycine | 6 - 10 | (Warnock, 2003; Veyssier et Bryskier, 2005) | ND | |
| Céfuroxime | 30 - 32 | (Bryskier et Lebel, 2005) | (1) ^b , 10 ^c | (Alexy <i>et al.</i> , 2004; Gartiser <i>et al.</i> , 2007a) |
| Ciprofloxacine | 29 - 33 | (Veyssier et Bryskier, 2005) | 0 ^b | (Kümmerer <i>et al.</i> , 2000) |
| Clarithromycine | 20 - 40 | (Bryskier et Butzler, 2003) | 0 ^b | (Alexy <i>et al.</i> , 2004) |
| Clindamycine | 13 | (Greenwood, 2003b) | 3 ^b , (4) ^c | (Alexy <i>et al.</i> , 2004; Gartiser <i>et al.</i> , 2007a) |
| Lévofloxacine | 69.6 - 80.8 | (Veyssier et Bryskier, 2005) | 0 ^b , (19) ^c | (Kümmerer <i>et al.</i> , 2000; Gartiser <i>et al.</i> , 2007a) |
| Minocycline | 4 - 10 | (Chopra, 2003; Bryskier, 2005a) | 4 ^b | (Alexy <i>et al.</i> , 2004) |
| Sulfaméthoxazole | 10 - 30 | (Greenwood, 2003b; Veyssier et Bryskier, 2005) | 0 ^b , (10) ^c | (Al-Ahmad <i>et al.</i> , 1999; Gartiser <i>et al.</i> , 2007a) |
| Triméthoprime | 33.1 - 90 | (Then, 2003; Veyssier et Bryskier, 2005) | (11) ^c | (Junker <i>et al.</i> , 2006; Gartiser <i>et al.</i> , 2007a) |

ND : Non disponible ^a Élimination rénale pour une dose orale. ^b Test de la bouteille fermée (Organisation for Economic Co-Operation and Development, 1992), ^c Test de biodégradabilité ultime combiné. Les valeurs négatives (entre parenthèses) indiquent possiblement des effets d'inhibition de la population des micro-organismes utilisé dans le test.

1.3.2.a. La voie urbaine

Dans la voie urbaine, les eaux contenant les anti-infectieux excrétés sont généralement conduites par les égouts vers les stations d'épuration des eaux usées (STEP). Historiquement, les traitements utilisés dans les STEP ont été implantés pour diminuer les solides en suspension, la demande biologique en oxygène, le phosphore total ainsi que l'ammoniaque (Ternes *et al.*, 2004b). Cependant, les STEP n'ont pas été conçues pour éliminer les anti-infectieux ou d'autres contaminants d'intérêt émergent et par conséquent, ceux-ci sont déversés en presque totalité ou partiellement dans les cours d'eau. Il s'agit alors d'une source ponctuelle de contamination, puisque les anti-infectieux sont rejetés dans un endroit précis.

Les hôpitaux sont considérés comme une des sources majeures d'anti-infectieux dans la voie urbaine (Gómez *et al.*, 2006), cependant des études récentes (Kummerer, 2009) indiquent que l'impact des hôpitaux n'est pas aussi élevé qu'attendu puisque l'usage communautaire représente plus de deux tiers de la consommation totale dans plusieurs pays (70% dans le Royaume-Uni, 75% dans les États-Unis et 75% en Allemagne) et les concentrations reportées sont souvent similaires ou inférieures à celles que les concentrations trouvées dans les STEP et les maisons de retraite.

Dans les STEP les anti-infectieux peuvent être partiellement éliminés des eaux usées, cependant ils peuvent se retrouver dans les boues d'épuration (Golet *et al.*, 2003). L'épandage de ces boues sur les sols agricoles donne lieu à une autre voie de transport des anti-infectieux dans l'environnement. Les anti-infectieux qui n'ont pas été éliminés par les STEP se rendent aux eaux de surface où leur dilution dépend du débit du cours d'eau. La présence d'anti-infectieux dans l'eau potable résulte de la contamination des prises d'eau et illustre la mobilité de ces composés dans l'environnement aquatique.

1.3.2.b. La voie agricole

Il est peu surprenant que les zones agricoles soient des sources importantes des anti-infectieux dans l'environnement étant donné le volume de ces substances utilisées pour

l'élevage d'animaux. En l'Union européenne et en Suisse plus de 4 000 tonnes d'anti-infectieux vétérinaires ont été utilisées en 1999 et aux États-Unis plus de 11 000 tonnes en 2001 (Kemper, 2008; Kummerer, 2009). Dans la voie agricole, les anti-infectieux utilisés à des fins thérapeutiques, prophylactiques ou pour l'augmentation de la croissance chez les animaux d'élevage sont excrétés sous leur forme active à des taux pouvant aller jusqu'à 95% de la dose administrée (Sarmah *et al.*, 2006). Dans les fermes, les excréments des animaux sont souvent emmagasinés dans des lagunes de rejet ou d'autres structures d'entreposage de fumier et ensuite appliquées comme fertilisants dans les sols agricoles (Boxall, 2004). Les anti-infectieux peuvent alors se rendre des sols aux eaux de surface par ruissellement et écoulement dans les eaux souterraines par percolation (Lee *et al.*, 2007b). Les anti-infectieux sont aussi utilisés pour le contrôle des infections chez les plantes et les abeilles (Kummerer, 2009). Cependant, la contribution de cette voie de contamination n'a pas été encore documentée.

Dans les aquacultures et les maricultures, les anti-infectieux sont administrés aux animaux d'élevage dans la nourriture. Ces substances sont alors introduites directement dans l'environnement aquatique par la nourriture non consommée ou par les excréments des animaux. Les anti-infectieux libérés peuvent aussi se déposer dans les sédiments et être ensuite relargués dans l'eau (Cabello, 2006). Le transport des anti-infectieux vers les milieux récepteurs se fait d'une façon plus diffuse et pour cette raison les zones agricoles sont considérées comme des sources de contamination non-ponctuelles (Halling-Sørensen *et al.*, 1998; Daughton et Ternes, 1999; Ternes et Joss, 2006).

1.3.2.c. Sites d'enfouissement

Les anti-infectieux peuvent aussi se rendre aux eaux souterraines par l'écoulement à partir des anti-infectieux présents dans les rejets domestiques solides dans les sites d'enfouissement. Cette voie de transport, quoique peu enquêtée et considérée comme une voie mineure (Boxall, 2004), ne doit pas être négligée, surtout lorsqu'on considère qu'une proportion significative de la population des États-Unis (54%) et du Royaume-Uni (71%) jette les médicaments inutilisés à la poubelle (Kuspis et Krenzelok, 1996; Bound et Voulvoulis, 2005).

1.3.3. Devenir des anti-infectieux

Le devenir environnemental des anti-infectieux dépend évidemment des propriétés physico-chimiques de chaque substance ainsi que les conditions du milieu récepteur (Boxall *et al.*, 2004; Peschka *et al.*, 2007). Le transport des anti-infectieux dans la phase aqueuse est affecté par d'autres facteurs que leur hydrophobicité; parmi les plus importants se retrouvent l'échange cationique, la formation de ponts H et la formation de complexes. Pour ces raisons leur comportement dans l'environnement ne peut pas être prédit facilement par la constante de partition octanol-eau ($\log K_{ow}$) (Tolls, 2001).

Le transport des anti-infectieux dans l'environnement aquatique amène sans doute à la diminution de leur concentration dès la source jusqu'aux milieux récepteurs (**Figure 1-7**, **Figure 1-8**). Cette diminution est causée par trois facteurs principaux durant leur cycle de vie : sorption, dilution et dégradation.

Une fois dans les eaux de rejet domestiques, les anti-infectieux subissent généralement une dilution par le mélange avec les eaux ne contenant pas ces substances (en moyenne, 35% de l'utilisation journalière d'eau par personne est réservée aux W.-C.) (Ville de Montréal, 2002b).

Ce phénomène de dilution est essentiel lorsque ces substances sont rejetées par les STEP puisque les rivières ayant les plus faibles débits par rapport aux ceux des STEP auront un plus petit pouvoir de dilution et par conséquent les concentrations des anti-infectieux seront supérieures.

Certains anti-infectieux tels que les quinolones et les tétracyclines ont la capacité de se sorber à des particules. Ces substances sont capables de former des complexes avec les cations divalents et trivalents tels que Al^{3+} , Fe^{3+} , Mg^{2+} et Ca^{2+} (Tolls, 2001) et de s'associer avec la matière organique. Ces interactions sont de nature électrostatique (ponts hydrogène, interactions ioniques), hydrophobe, ou covalente (liens de coordination) (Lee *et al.*, 2007b).

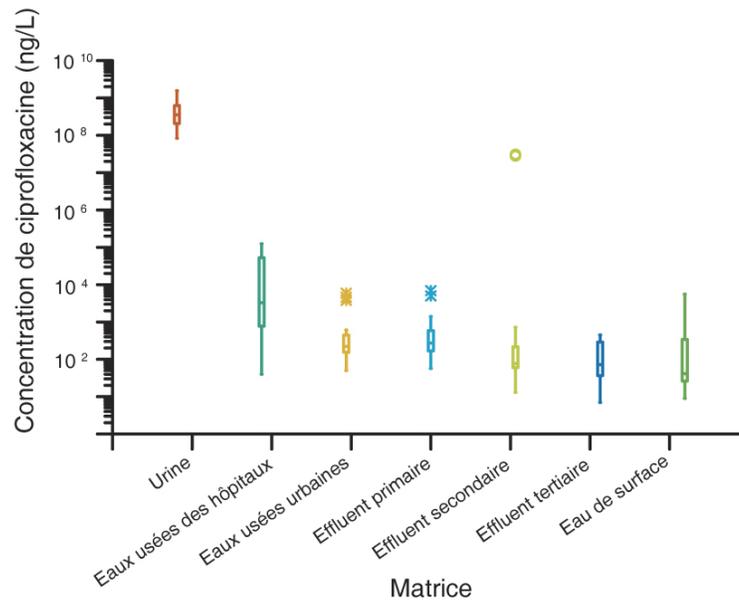


Figure 1-8. Diminution de la concentration de ciprofloxacine dans l'environnement suite à leur excrétion. Source : publications sélectionnées reportant la présence de ciprofloxacine dans l'environnement.

Ces interactions peuvent être affectées par le pH et la force ionique étant donné le caractère ionisables des anti-infectieux (Gu et Karthikeyan, 2005b; Gu et Karthikeyan, 2005a). La capacité des substances à se sorber à des solides est quantifiée par le coefficient de sorption $K_{d, \text{solid}}$. Ce coefficient exprime le ratio entre la concentration d'un composé dans le sorbant et la concentration dans la phase aqueuse à l'équilibre et indique le degré de mobilité d'une substance dans l'environnement (Tolls, 2001). Les $K_{d, \text{solid}}$ des anti-infectieux ($0.2\text{-}6000 \text{ L kg}^{-1}$) démontrent que leur mobilité est très variable. Les anti-infectieux peuvent aussi s'associer avec la matière organique dissoute (DOM). Contrairement à la sorption sur des solides, l'association à la DOM peut augmenter la mobilité des substances. (Tolls, 2001; Lee *et al.*, 2007b).

La dégradation des anti-infectieux peut avoir lieu tout au long de leur trajet vers les milieux récepteurs et aussi une fois rendus dans les eaux naturelles. La nature des phénomènes de dégradation naturelle peut être abiotique ou biotique. Les principaux mécanismes de dégradation abiotique des anti-infectieux sont la photolyse, l'hydrolyse et l'oxydoréduction.

La photodégradation est un des principaux mécanismes de réduction des concentrations des anti-infectieux susceptibles à la lumière (quinolones, tétracyclines) dans les eaux de surface (Boreen *et al.*, 2004). La réactivité de ces substances à la lumière est due à leurs structures moléculaires contenant des groupements fonctionnels capables d'absorber les longueurs d'onde de la lumière naturelle (350-800 nm) ou de réagir avec des espèces réactives (O_2 singulet et OH^*) présentes dans les milieux aquatiques (Boreen *et al.*, 2003).

En ce qui concerne l'oxydoréduction, les études récentes (Zhang et Huang, 2005) ont démontré que les quinolones sont oxydées rapidement par les oxydes de Mn (IV). Ces processus réactifs ont plus d'importance dans les milieux terrestres, étant donné la présence commune des oxydes de manganèse dans les sols et sédiments, mais pourraient contribuer à la diminution des concentrations de ces anti-infectieux dans les zones agricoles.

La dégradation par hydrolyse est un des principaux phénomènes expliquant les basses concentrations des β -lactamines dans les eaux environnementales (Andreozzi *et al.*, 2004). Tel que mentionné antérieurement, l'anneau β -lactamine (anneau azetidione) est clivé en milieu aqueux résultant en la formation d'un groupement carboxylique et une amine secondaire (**Figure 1-9**). Certaines tétracyclines semblent aussi être affectées par ce type de dégradation (Halling-Sørensen, 2000). Cependant, d'autres classes d'anti-infectieux tel que les quinolones et les sulfonamides semblent être résistantes à l'hydrolyse (Kummerer, 2009).

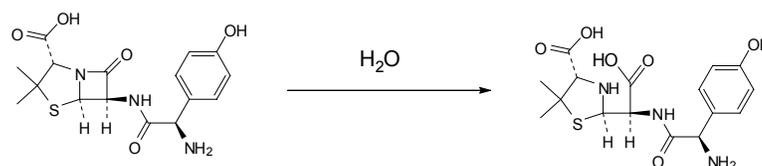


Figure 1-9. L'hydrolyse de l'amoxicilline. D'après (Längin *et al.*, 2009)

La biodégradabilité des contaminants organiques est mesurée par des tests standardisés. Par exemple, le test de la bouteille fermée détermine si un composé sera rapidement biodégradé dans l'environnement aquatique sous des conditions d'aérobies. Ce test mesure la quantité d'oxygène consommée par une population microbienne durant la biodégradation de la substance testée (Organisation for Economic Co-Operation and Development, 1992). Le test de biodégradabilité ultime combiné développé par Gartiser (Gartiser *et al.*, 2007a) détermine

si une substance sera totalement utilisée par les micro-organismes pour former du $\text{CO}_{2(g)}$, de l'eau, des sels et de la biomasse. Ce test mesure la production de $\text{CO}_{2(g)}$ et l'élimination du carbone organique dissout par sorption. Ces tests ont démontré que la plupart d'anti-infectieux ne sont pas facilement biodégradables (Alexy *et al.*, 2004; Gartiser *et al.*, 2007a). Toutefois, la capacité à résister à la biodégradation pour de très longues périodes (> 28 jours) n'est pas aussi critique pour les anti-infectieux comme on le discutera à la section **1.3.4**. La transformation biotique des substances résulte généralement dans des produits de dégradation plus polaires et hydrophiles que le composé parent (Boxall *et al.*, 2004).

Les sols agricoles peuvent agir comme des réservoirs environnementaux des anti-infectieux, puisque certaines substances peuvent se lier aux particules du sol en s'adsorbant sur les argiles et la matière organique naturelle (Rooklidge, 2004; Kumar *et al.*, 2005). Il est important de remarquer l'importance des interactions avec la phase particulaire. Certains anti-infectieux ont la capacité de s'associer aux particules en suspension ce qui modifie leur transport ainsi que leur devenir environnemental. Cependant, la phase particulaire n'est pas l'objet de cette thèse.

Tous les phénomènes contribuant à la réduction des concentrations des molécules parents des anti-infectieux dans l'environnement diminuent aussi leur activité anti-infectieuse. Par exemple, des études ont démontré que l'adsorption des anti-infectieux aux sols diminue leur effet inhibitoire sur les bactéries (Koschorreck *et al.*, 2002). Les concentrations des produits de dégradation ont une importance environnementale seulement si ceux-ci possèdent une activité biologique.

1.3.3.a. Devenir dans les STEP

L'efficacité des STEP à éliminer les anti-infectieux présents dans les affluents municipaux dépend évidemment du type de traitement appliqué, mais aussi de la conception de la STEP et des propriétés physico-chimiques des composés (Gulkowska *et al.*, 2008). L'effet du type de traitement est illustré par les résultats obtenus par Göbel (Göbel *et al.*, 2005b) lors de mesures de l'occurrence de triméthoprimine dans deux STEP en Suisse. La concentration médiane de l'affluent était de 290 ng L^{-1} , après traitement primaire (dégrillage,

dessablage aéré et clarification) la concentration médiane a diminué à 230 ng L⁻¹ (réduction de 21%). Suite au traitement secondaire (boues activées et bioréacteur) la concentration médiane de triméthoprime est passée à 200 ng L⁻¹ (réduction de 31%). Finalement après traitement tertiaire (filtration sur lit de sable), la concentration dans l'effluent était de 70 ng L⁻¹ (réduction de 76%). Aussi, la conception de la STEP est prépondérante puisque deux traitements similaires peuvent avoir des efficacités distinctes comme le démontre le cas des STEP de Tai Po Tai Po et Shatin en Chine. Les deux STEP appliquent un traitement secondaire (boues activées), cependant la STEP de Shatin a été capable d'enlever 58% plus de norfloxacin que la STEP de Tai Po (Gulkowska *et al.*, 2008). D'après les auteurs cette différence pourrait être expliquée par les différences dans la capacité de traitement. De plus, l'efficacité d'enlèvement dépend de la nature du composé comme est illustré par l'étude faite par Batt (Batt *et al.*, 2007) dans la STEP de East Aurora aux États-Unis. Cette STEP applique un traitement tertiaire (filtration sur lit de sable et chloration) et est capable de réduire 97% de la concentration initiale de triméthoprime, pendant que pour ciprofloxacine l'élimination a été de 67%.

La sorption à des micro-organismes ou à des solides en suspensions est un processus clé pour l'élimination des contaminants dans les STEP. Celle-ci résulte des interactions hydrophobes (Ternes *et al.*, 2004b). Par conséquent, des facteurs importants dans l'enlèvement des anti-infectieux sont la composition des boues activées, ainsi que le pH de l'eau usée qui peut avoir un effet sur la ou les formes prédominantes des composés ionisables. Aussi les conditions d'oxydoréduction sont importantes pour l'élimination des anti-infectieux parce que les pharmaceutiques peuvent être plus facilement dégradables dépendamment de la disponibilité de O₂ et NO₃⁻ (Ternes *et al.*, 2004b). La biodégradation partielle et la minéralisation des anti-infectieux dans les STEP sont possibles parce que les bactéries peuvent co-métaboliser ces substances (c.-à-d. les modifier ou les dégrader à l'aide d'un substrat) ou les utiliser directement comme une source de carbone et d'énergie pour se multiplier (Ternes *et al.*, 2004b). Par conséquent, l'âge des boues a un effet l'efficacité de l'enlèvement parce que la diversité microbienne est augmentée par les temps de rétention des solides.

1.3.4. Occurrence des anti-infectieux dans les eaux environnementales

La recherche faite sur l'occurrence des anti-infectieux depuis 25 ans a démontré la présence de ces substances dans les eaux usées, les eaux de surface, les eaux souterraines et l'eau potable dans plusieurs pays (Zuccato *et al.*, 2000; Kolpin *et al.*, 2002; Göbel *et al.*, 2005b). Tel que mentionné antérieurement, la présence de ces composés dans les eaux environnementales est due à leur utilisation courante ainsi qu'à certains de leurs propriétés (mobilité, non-biodégradabilité) qui permettent leur transport et leur dissémination en quantités suffisantes pour qu'ils soient détectés dans l'environnement aquatique. Les anti-infectieux et d'autres contaminants à intérêt émergent sont souvent considérés comme des contaminants pseudo-persistants (Khetan et Collins, 2007). Les contaminants (ou polluants) persistants sont ceux qui une fois rejetés, ont la capacité de résister à la dégradation et perdurer dans l'environnement (vanLoon et Duffy, 2000). Cependant, les anti-infectieux vont aussi se retrouver continuellement dans l'environnement (sans être pourtant toujours résistants à la dégradation) par l'effet de leur déversement continu.

Le **Tableau 1-3** montre des données sur la présence d'anti-infectieux dans les eaux contaminées publiées dans 143 articles scientifiques publiées entre 1984 et 2008. Ces valeurs indiquent que la concentration médiane de la plupart des anti-infectieux dans les eaux usées sont de l'ordre de 100 à 500 ng L⁻¹, dans les eaux naturelles de 10 à 200 ng L⁻¹ et dans l'eau potable autour de 1 ng L⁻¹.

Tableau 1-3. Présence des principales classes d'anti-infectieux dans les eaux environnementales contaminées.

| Classe | Eaux usées | | Eaux naturelles | | Eau potable | |
|--------------------|----------------------------------|------------------------------|----------------------------------|------------------------------|----------------------------------|------------------------------|
| | Médiane (ng L ⁻¹) | 95% (ng L ⁻¹) | Médiane (ng L ⁻¹) | 95% (ng L ⁻¹) | Médiane (ng L ⁻¹) | 95% (ng L ⁻¹) |
| Sulfonamides | 330 | 31000 | 120 | 472000 | 0.4 | 0.5 |
| β-Lactamines | 300 | 5360 | 11 | 350 | N.D. | N.D. |
| Tétracyclines | 530 | 6095000 | 192 | 623550 | N.D. | N.D. |
| Macrolides | 110 | 1000 | 11 | 197 | 3 | 5 |
| Diaminopyrimidines | 270 | 5000 | 18 | 510 | N.D. | N.D. |
| Lincosamides | 60 | 190500 | 18 | 1020 | N.D. | N.D. |
| Quinolones | 205 | 41922 | 27 | 640 | 2 | 4 |

N.D. : Non disponible.

1.3.5. Cibles et effets des anti-infectieux dans les eaux environnementales

À ce jour, une des principales inquiétudes de la communauté scientifique par rapport à la présence d'anti-infectieux dans l'environnement aquatique est leur contribution potentielle à la prolifération de la résistance aux anti-infectieux chez les bactéries. Plus récemment, de nouvelles études ont focalisé sur les espèces aquatiques non ciblées par l'activité biologique des anti-infectieux. D'après une étude récente d'analyse de risque (Cooper *et al.*, 2008), les anti-infectieux se retrouvent parmi les pharmaceutiques posant un plus grand risque pour l'environnement. L'investigation scientifique a donné peu d'attention aux effets directs sur la santé humaine (la résistance chez les bactéries est considérée un effet indirect) étant donné les faibles concentrations de ces composés dans l'eau potable (**Tableau 1-3**).

1.3.5.a. Prolifération de la résistance aux anti-infectieux chez les bactéries

La résistance aux anti-infectieux est définie comme la capacité d'un micro-organisme à désactiver l'effet toxique des anti-infectieux par l'acquisition des mécanismes de protection (Suling et O'Leary, 1975; Levy, 1992a). La résistance aux anti-infectieux est un phénomène d'adaptation. Lorsqu'une pression évolutive est exercée sur une population de bactéries (par exemple, la présence continue des anti-infectieux dans un milieu), la prolifération de la résistance est accrue. Ceci s'explique par le fait que seulement les espèces ayant la capacité de neutraliser les anti-infectieux vont survivre et par conséquent ils passeront cette capacité à leur progéniture.

Comme il a été vu à la section 1.2., les anti-infectieux ont été désignés pour inhiber spécifiquement la croissance ou la survie des bactéries. Évidemment, ces « balles magiques » doivent exercer leur fonction à l'intérieur de l'organisme du patient, mais une fois rejetés dans les eaux environnementales, les anti-infectieux (excrétés sous leur forme parent) conservent leur activité biologique et peuvent avoir un effet sur les populations des bactéries présentes dans ces milieux. Ce sujet est encore très controversé puisqu'il est argumenté que les concentrations environnementales des anti-infectieux ne sont pas assez élevées pour avoir un effet sur bactéries (Summers, 2002), cependant plusieurs études ont observé une incidence sur

la prolifération de la résistance chez les bactéries dans les eaux usées contaminées par les anti-infectieux (Kümmerer, 2004).

1.3.5.b. Effets sur les espèces aquatiques

Des études réalisées sur des espèces aquatiques ont démontré la toxicité des anti-infectieux à des concentrations environnementales **Tableau 1-4**. Ces études indiquent que certaines espèces aquatiques telles que les cyanobactéries, les algues et les diatomées sont particulièrement sensibles à la présence d'anti-infectieux dans l'environnement aquatique.

La toxicité d'une substance est quantifiée par l'utilisation de seuils limites, tels que la concentration effective à 50% (EC_{50}) ou la plus petite concentration ayant un effet observable (LOEC). La EC_{50} représente la concentration d'une substance ayant un effet donné sur 50% des individus de l'espèce testée lors d'un temps exposition fini. Plus spécifiquement, la toxicité aiguë est mesurée par la concentration (ou dose) létale à 50% (LC_{50}) qui est en effet une EC_{50} où l'effet étudié est la mortalité. Ces tests sont réalisés pour une courte période de temps tandis que les tests de toxicité chronique étudient d'autres effets sur l'espèce (croissance de la population, reproduction) et généralement pour une période de temps plus élevée (Cunningham *et al.*, 2006).

Les études indiquent que les anti-infectieux démontrent une toxicité aiguë limitée envers les organismes non cibles (Isidori *et al.*, 2005), cependant leur toxicité chronique est plus importante. Par exemple, la toxicité aiguë d'ofloxacine pour le rotifère *Branchionus calyciflorus* est relativement élevée ($LC_{50}=29\ 880\ 000\ \text{ng L}^{-1}$) tandis que la toxicité chronique (inhibition de la croissance de la population) de la même substance pour la même espèce ($EC_{50}=530\ 000\ \text{ng L}^{-1}$) est plus basse et plus proche des concentrations environnementales

(Isidori *et al.*, 2005). Une autre étude réalisée avec des communautés des algues d'eau douce (Wilson *et al.*, 2003) a démontré la capacité du ciprofloxacine à modifier leur structure et leurs fonctions à des concentrations aussi basses que $12\ \text{ng L}^{-1}$.

Tableau 1-4. Concentrations effectives des anti-infectieux les plus basses reportées dans la littérature.

| Anti-infectieux | Espèce | Effet | Temps d'exposition | Point de toxicité | Concentration (ng L ⁻¹) | Référence |
|-------------------|-----------------|----------------------------------|--------------------|-------------------|-------------------------------------|---|
| Ciprofloxacin | Algue diatomée | Réduction de la biomasse | 13 d | LOEC | 12 | (Wilson <i>et al.</i> , 2003) |
| Ciprofloxacin | Diatomée | Biomasse | 13 d | LOEC | 120 | (Wilson <i>et al.</i> , 2003) |
| Ciprofloxacin | Bactérie | Genotoxicité | 2 h | SOS | 200 | (Kümmerer <i>et al.</i> , 2000) |
| Ofloxacin | Bactérie | Inhibition de la bioluminescence | 24 h | EC01 | 940 | (Backhaus <i>et al.</i> , 2000) |
| Ofloxacin | Bactérie | Inhibition de la croissance | 2 h | SOS | 1000 | (Kümmerer <i>et al.</i> , 2000) |
| Loméfloxacine | Bactérie | Inhibition de la bioluminescence | 24 h | EC01 | 1610 | (Backhaus <i>et al.</i> , 2000) |
| Clarithromycine | Algue | Inhibition de la croissance | 72 h | EC50 | 2000 | (Isidori <i>et al.</i> , 2005) |
| Sulfaméthoxazole | Bactérie | Inhibition de la population | NA | EC50 | 2000 | (Al-Ahmad <i>et al.</i> , 1999) |
| Ciprofloxacin | Bactérie | Inhibition de la population | NA | MIC50 | 2000 | (Al-Ahmad <i>et al.</i> , 1999) |
| Spiramycine | Cyanobactérie | Inhibition de la croissance | 7d | EC50 | 5000 | (Halling-Sørensen <i>et al.</i> , 2000) |
| Sulfaméthoxazole | Bactérie | Inhibition de la population | NA | MIC50 | 2000 | (Al-Ahmad <i>et al.</i> , 1999) |
| Benzylpénicilline | Bactérie | Inhibition de la population | NA | MIC50 | 4000 | (Al-Ahmad <i>et al.</i> , 1999) |
| Ciprofloxacin | Cyanobactérie | Inhibition de la croissance | 4-6 h | EC50 | 5000 | (Halling-Sørensen <i>et al.</i> , 2000) |
| Ciprofloxacin | Bactérie | Genotoxicité | 30 min | LOEC | 5000 | (Hartmann <i>et al.</i> , 1998) |
| Spiramycine | Cyanobactérie | Inhibition de la croissance | 7d | EC50 | 5000 | (Halling-Sørensen <i>et al.</i> , 2000) |
| Clarithromycine | Crustacé | Inhibition de la reproduction | 21 d | EC50 | 6300 | (Yamashita <i>et al.</i> , 2006) |
| Clarithromycine | Algue | Inhibition de la croissance | 96 h | EC50 | 6300 | (Yamashita <i>et al.</i> , 2006) |
| Sulfaméthoxazole | Plant aquatique | pABA total | 7 d | LOEC | 9000 | (Brain <i>et al.</i> , 2008) |

LOEC : Plus basse concentration observée ayant un effet. SOS : Chromotest SOS pour détecter les substances capables d'endommager l'AND; EC01 : Concentration effective à 1%; EC50 : Concentration effective à 50%; MIC50 : Concentration inhibitoire minimale à 50.

Il est aussi notable de remarquer qu'il existe un certain décalage entre le développement de la chimie analytique et celui de l'écotoxicologie dans les dernières années. L'avancement qui a eu lieu dans chimie analytique et qui a permis la détection des anti-infectieux dans les eaux environnementales n'a pas eu de contrepartie dans l'écotoxicologie et maintenant nous sommes capables de détecter des concentrations dont les effets sur le biote sont encore inconnus. L'étude de nouveaux seuils de toxicité tels que l'inhibition des réactions métaboliques comme celui du pABA chez les plantes aquatiques (Brain *et al.*, 2008) est un exemple des effets ignorés qui ne pouvaient pas être détectés par les méthodes conventionnelles.

1.3.5.c. Effets directs sur la santé humaine par la consommation de l'eau potable

Il est difficile d'étudier les effets des anti-infectieux sur la santé humaine à cause de faibles concentrations (0.1-5 ng L⁻¹) trouvées dans l'eau potable. Comparées aux doses recommandées pour les adultes (Conseil du médicament, 2005), ces concentrations sont entre 2×10^7 et 1×10^{10} fois plus faibles. Même si l'on détermine la quantité totale d'un anti-infectieux ingérée durant une période de 70 ans (en supposant une consommation journalière de 2 L d'eau potable contaminée avec 2 ng L⁻¹ d'ofloxacine) celle-ci est ~ 2500 fois inférieure à la dose journalière recommandée pour un patient.

Cependant, des études récentes ont démontré que les effets sur la santé humaine sont possibles surtout pour les fœtus et les enfants (Pomati *et al.*, 2008), lorsqu'on considère l'effet combiné des mélanges des pharmaceutiques. Yargeau et ses collaborateurs ont démontré que l'exposition des cellules humaines à des produits de dégradation de l'ozonation de sulfaméthoxazole peut causer des changements de morphologie, indiquant ainsi la possibilité des effets sur la santé humaine par l'ingestion d'eau potable contaminée (Yargeau *et al.*, 2008). Ternak (Ternak, 2005) a aussi proposé une hypothèse d'après laquelle les traces des anti-infectieux dans la nourriture et l'eau potable pourraient agir comme des promoteurs de la croissance et de l'obésité chez les humains, de façon similaire comme il est observé chez les animaux.

1.3.6. Solutions proposées

D'après le principe de précaution, l'incertitude scientifique ne doit pas être utilisée pour empêcher les solutions à des menaces environnementales (Tickner et Geiser, 2004). Pour cette raison, de nombreuses mesures pour éviter la présence d'anti-infectieux ou de façon plus générale des contaminants d'intérêt émergent dans l'environnement ont été proposées à ce jour. Les deux approches majeures pour les milieux urbains sont le contrôle à la source et l'amélioration du traitement des eaux usées (Larsen *et al.*, 2004; Ternes *et al.*, 2004a; Ternes *et al.*, 2004b).

Les solutions de contrôle à la source cherchent à réduire la charge de contaminants émergents provenant des eaux résidentielles et des hôpitaux avant qu'ils atteignent les systèmes d'égouts publics. Ceci peut être réalisé au niveau de la consommation au moyen de l'étiquetage environnemental, c'est-à-dire en informant les patients et médecins sur les substances plus nuisibles à l'environnement et les précautions à prendre. Un autre type de contrôle à la source est réalisable au niveau du traitement des rejets résidentiels au moyen de la séparation de l'urine (étant donné qu'une grande partie des pharmaceutiques est excrétée par voie rénale) et le prétraitement des eaux usées hospitalières (Larsen *et al.*, 2004; Ternes *et al.*, 2004b).

L'amélioration du traitement des eaux usées cherche à augmenter l'efficacité de l'élimination des anti-infectieux dans les STEP en optimisant la technologie déjà en place ou en installant des technologies de traitement plus avancées, par exemple l'ozonation, les processus d'oxydation avancés, la filtration par membrane et le carbone activé (Ternes *et al.*, 2004a; Ternes et Joss, 2006).

Dans le cas des anti-infectieux d'origine agricole, de meilleures pratiques d'exploitation ont été proposées telles que l'application des techniques de contrôle de l'érosion pour réduire le lessivage (Davis *et al.*, 2006), l'augmentation du temps de maturation du fumier pour rehausser la dégradation (De Liguoro *et al.*, 2003) et l'utilisation des filtres industriels à base de polyester pour réduire les décharges provenant des aquacultures (Smith *et al.*, 1994).

Toutes ces mesures devraient contribuer à réduire les apports urbains et agricoles des anti-infectieux dans l'environnement.

1.4. Méthodes d'analyse des anti-infectieux dans les eaux environnementales

1.4.1. Premières études

Les premières études d'identification des contaminants organiques dans les eaux environnementales ont focalisé sur les composés volatils, étant donné la disponibilité de la chromatographie gazeuse couplée à la spectrométrie de masse (GC-MS) ainsi que des bases de données standardisées (Crathorne *et al.*, 1984). Cependant, la fraction volatile ne représente que 20% du carbone organique total et l'identification de la fraction organique non volatile (d'origine non humique) est restée difficile à étudier avant l'apparition des sources d'ionisation « douces » permettant l'analyse des substances plutôt polaires (Crathorne *et al.*, 1984).

Le premier rapport connu de la présence d'anti-infectieux dans l'environnement aquatique date de 1983 (Watts *et al.*, 1983). Dans cette étude, les auteurs ont utilisé l'extraction sur phase solide (SPE) avec une résine à base de copolymère de styrène-divinylbenzène ou la lyophilisation comme techniques de préparation des échantillons (Crathorne *et al.*, 1982). Pour la séparation des extraits, la technique utilisée a été la chromatographie liquide à polarité des phases inversée avec détection par absorption UV (RPLC-UV). Finalement, les extraits SPE et les fractions RPLC-UV ont été analysés par désorption à champ élevé et spectrométrie de masse avec un analyseur à double focalisation [(FD-MS(BE))]. Des études postérieures par la même équipe (Crathorne *et al.*, 1984) ont appliqué une autre technique d'ionisation « douce » couplée à spectrométrie de masse, le bombardement par atomes rapides [(FAB-MS(BE))]. Quoique les auteurs n'aient pas fait une étude quantitative, la concentration d'érythromycine et de tétracycline a été estimée dans l'ordre du microgramme par litre dans l'eau de surface d'une rivière anglaise. Ces

études ont démontré que la fraction organique non volatile présente dans les eaux environnementales peut contenir une quantité élevée de traces des composés organiques dont la plupart sont d'origine anthropique et qui n'avaient pas été rapportés auparavant.

Des études suivantes ont démontré la présence d'anti-infectieux dans divers types d'eaux environnementales, cependant ces méthodes étaient incapables d'analyser les anti-infectieux à de faibles concentrations ($< 1 \mu\text{g L}^{-1}$). Ces études ont utilisé la RPLC-UV pour étudier l'oxytétracycline dans les eaux de rejet d'une aquaculture (Smith *et al.*, 1994); cinq sulfonamides (sulfadiazine, sulfadimidine, sulfaguanidine, sulfaméthizol et sulfonilamide) dans les eaux souterraines proches à un site d'enfouissement de déchets domestiques et industriels (Holm *et al.*, 1995), la fluméquine dans les eaux de mariculture (Migliore *et al.*, 1996) et sept sulfonamides (sulfacétamide, sulfadiazine, sulfadimidine, sulfamérazine, sulfaméthoxazole, sulfamonométhoxine, sulfathiazole) dans les eaux usées d'une ferme d'élevage des porcs (Jen *et al.*, 1998). Les limites de quantification étaient entre 1 à $49.5 \mu\text{g L}^{-1}$, soient des valeurs nettement trop élevées pour pouvoir réaliser des évaluations environnementales.

1.4.2. Revue de principales méthodes d'analyse des traces d'anti-infectieux de 1998 à 2005

Dans cette section, on résume les méthodes d'analyse d'anti-infectieux les plus remarquables publiées entre 1998 et 2005. Cette revue s'arrête en 2005 pour décrire l'état du développement analytique, lors du début des travaux préliminaires. Le **Tableau 1-5** résume la performance de ces méthodes

La première méthode capable de quantifier les traces d'anti-infectieux à des concentrations inférieures à $1 \mu\text{g L}^{-1}$ dans les eaux environnementales a été introduite par Hartmann et son équipe (Hartmann *et al.*, 1998). Ils ont détecté le ciprofloxacine dans les eaux usées d'un hôpital en utilisant la chromatographie liquide à polarité des phases inversée avec détection par fluorescence (RPLC-FLD). La limite de quantification de leur méthode était de 500 ng L^{-1} .

Cette même année, Hirsch et ses collaborateurs ont publié la première méthode utilisant la chromatographie liquide à polarité de phases inversée couplée à l'ionisation par électro-nébuliseur et spectrométrie de masse en tandem avec un triple quadripôle [RPLC-ESI-MS/MS(QqQ)] (Hirsch *et al.*, 1998). Les auteurs ont utilisé deux méthodes de préconcentration différentes : la lyophilisation et la SPE. Les résultats ont démontré que la lyophilisation était la technique de choix pour l'analyse routinière d'un grand nombre d'échantillons, cependant le développement analytique ultérieur s'est focalisé sur la SPE. Cette méthode était capable de quantifier 18 anti-infectieux dans l'eau de surface avec des limites de quantification (LOQ) de 50 ng L⁻¹ pour les quatre tétracyclines étudiées et de 20 ng L⁻¹ pour le reste des analytes. Les taux de recouvrement dans l'eau de surface étaient entre 45 et 137 %.

En 2001, le premier article portant sur l'analyse de traces quinolones dans les eaux usées municipales a été publié (Golet *et al.*, 2001). Dans cet article les auteurs ont démontré que l'analyse de traces d'anti-infectieux dans les eaux usées municipales pouvait être faite en préconcentrant l'échantillon par SPE et ensuite l'analyse par RPLC-FLD. Les auteurs ont utilisé de disques d'extraction mixtes (c.-à-d. phases solides ayant une chimie de type polarité des phases inversée et aussi échangeur de cations) et ont obtenu des limites de quantification de 45-150 ng L⁻¹ dans les effluents primaires et de 15-45 ng L⁻¹ dans les effluents tertiaires pour neuf fluoroquinolones. Les taux de recouvrement dans l'effluent primaire étaient entre 81 et 96%.

En 2003, Calamari et ses collaborateurs (Calamari *et al.*, 2003) ont introduit de nouvelles approches d'extraction pour l'analyse d'anti-infectieux et d'autres contaminants émergents dans les eaux de surface. Les auteurs ont choisi 25 pharmaceutiques pour leur étude et ensuite ils les ont distribués en trois groupes d'après les conditions d'extraction. Lincomycine, oxytétracycline, ciprofloxacine, tilmicosine et oléandomycine ont été extraits avec des cartouches mixtes à pH acide. Pour extraire spiramycine, érythromycine, tylosine et clarithromycine, une cartouche de styrène-divinylbenzène a été utilisée et le pH a été fixé à 7. Finalement pour amoxicilline, l'échantillon a été rendu basique et chargé dans une cartouche C₁₈. La modification du pH de l'échantillon favorise la rétention puisque ces anti-infectieux sont des composés ionisables et selon la phase stationnaire utilisée, la forme neutre ou la forme ionique vont montrer une meilleure affinité envers celle-ci (Simpson et

Simpson, 2000). Les limites de quantification déterminées par les auteurs étaient parmi les plus bas publiées à l'époque : 0.02 à 0.29 ng L⁻¹ et les taux de recouvrement entre 26 et 135%.

Les méthodes publiées postérieurement en 2004 (Göbel *et al.*, 2004; Miao *et al.*, 2004; Renew et Huang, 2004) et 2005 (Batt et Aga, 2005) cherchaient à développer des méthodes de SPE capables d'analyser plusieurs familles d'anti-infectieux pour diminuer le temps d'extraction et préconcentration des échantillons. Renew et ses collaborateurs (Renew et Huang, 2004) ont proposé une méthode utilisant deux cartouches SPE en tandem (cartouche échangeur d'anions et cartouche à polarité des phases inversée). La cartouche échangeur d'anions était utilisée pour retenir les interférences présentes dans la matrice. Cette méthode permettait l'analyse de traces des quinolones, des sulfonamides et du triméthoprime (LOQ : 30-90 ng L⁻¹ dans les effluents secondaires) mais les taux de recouvrement pour certaines sulfonamides étaient très faibles (37-57%).

La méthode proposée par Miao et ses collaborateurs (Miao *et al.*, 2004) montrait une amélioration des taux de recouvrement (74-99%) par rapport à la méthode de Renew *et al.* et des LOQ assez basses pour l'analyse de traces dans les effluents finaux des STEP (1-8 ng L⁻¹). Les auteurs ont sélectionné un groupe de 31 anti-infectieux comportant des sulfonamides, des macrolides, des quinolaxine-dioxydes, des quinolones et des tétracyclines. Deux méthodes à base de SPE ont été proposées: la première pour l'extraction des macrolides (à pH 6.0) et la deuxième (à pH 3.0 et avec ajout de Na₂EDTA) pour le reste des anti-infectieux.

La méthode développée par Göbel (Göbel *et al.*, 2004) déterminait un nombre plus restreint de composés que celle de Miao, cependant cette méthode a été validée pour l'analyse des matrices plus difficiles telles que les effluents tertiaires, secondaires et primaires. Les travaux de Göbel ont démontré les difficultés rencontrées lors de l'analyse des effluents primaires et l'impact de la matrice sur les LOQ. Par exemple pour la sulfathiazole la LOQ dans l'effluent tertiaire était de 16 ng L⁻¹ tandis que pour l'effluent primaire cette dernière était de 214 ng L⁻¹. Cette méthode a été aussi une des premières à utiliser deux transitions de suivi des réactions de fragmentation caractéristiques (SRM) pour confirmer la présence d'anti-infectieux.

Tableau 1-5. Méthodes sélectionnées d'analyse des anti-infectieux dans les eaux environnementales.

| Prétraitement | Extraction | Séparation et détection | Recouvrement (%) | LOQ (ng L ⁻¹) | Référence |
|---|--|--------------------------|--|---|-------------------------------|
| F : 0.45 µm fibre de verre AS : 1 g L ⁻¹ Na ₂ EDTA | LYO: -30°C / EtOH F: 0.45 µm PTFE FP : 100 | RPLC-ESI(+/-)-MS/MS(QqQ) | <i>Eau de surface (n=3)</i> β-lactamines : 106-137 Chloramphénicol: 98 Macrolides: 79-120 Sulfonamides: 40-88 Tétracyclines: 45-68 Triméthoprim: 60-84 | <i>Eau de surface</i> β-lactamines : 20 Chloramphénicol:20 Macrolides: 20 Sulfonamides: 50 Tétracyclines: 50 Triméthoprim: 20 | (Hirsch <i>et al.</i> , 1998) |
| F: 0.45 µm nitrate de cellulose pH = 3.0 | SPE: Disques MPC FP: 16.6 - 50 | RPLC-FLD | <i>Effluent primaire (n=6)</i> Quinolones: 81±9.9%-100±5.0% <i>Effluent tertiaire (n=6)</i> Quinolones: 72-97 | <i>Effluent primaire</i> Quinolones: 45-150 <i>Effluent tertiaire</i> Quinolones:15-45 | (Golet <i>et al.</i> , 2001) |

AS: Ajout de sel; F: Filtration, FP: Factor de préconcentration; LYO: Lyophilisation; pH: ajustement du pH de l'échantillon; RPLC-ESI(+/-)-MS/MS(QqQ) : Chromatographie liquide à polarité de phases inversée couplée à l'ionisation par électronébuliseur (mode positif ou négatif) et à la spectrométrie de masse en tandem avec un triple quadripôle; RPLC-FLD: Chromatographie liquide à polarité des phases inversée avec détection par fluorescence, SPE: Extraction sur phase solide.

Tableau 1.5. Méthodes sélectionnées d'analyse des anti-infectieux dans les eaux environnementales (suite).

| Prétraitement | Extraction | Séparation et détection | Recouvrement (%) | LOQ (ng L ⁻¹) | Référence |
|---|--------------------------------|-------------------------|---|-----------------------------------|------------------------------|
| F: 0.45µm nitrate de cellulose FD: 4 AS: 4-5 g L ⁻¹ NaCl pH = 4.0 | SPE: Oasis HLB FP: 400- 500 | RPLC-ESI(+)-MS/MS(QqQ) | <i>Effluent primaire (n=4)</i> | <i>Effluent primaire</i> | (Göbel <i>et al.</i> , 2004) |
| | | | Macrolides: 83-100 | Macrolides: 3-19 | |
| | | | Sulfonamides: 91-108 | Sulfonamides: 42-214 | |
| | | | Triméthopriame: 47 | Triméthopriame: 21 | |
| | | | <i>Effluent secondaire (n=4)</i> | <i>Effluent secondaire</i> | |
| | | | Macrolides: 81 | Macrolides: 1-5 | |
| | | | Sulfonamides: 95-105 | Sulfonamides: 9-23 | |
| | | | Triméthopriame: 30 | Triméthopriame: 6 | |
| | | | <i>Effluent tertiaire (n=4)</i> | <i>Effluent tertiaire</i> | |
| | | | Macrolides: 78-124 | Macrolides: 1-6 | |
| | | | Sulfonamides: 93-106 | Sulfonamides: 7-22 | |
| | | | Triméthopriame: 35 | Triméthopriame: 4 | |
| F: 1.0 µm fibre de verre pH = 3.0-6.0 AS: 500 mg L ⁻¹ | SPE: Oasis HLB FP: 1000 | RPLC-ESI(+)-MS/MS(QqQ) | <i>Effluent tertiaire</i> | <i>Effluent tertiaire</i> | (Miao <i>et al.</i> , 2004) |
| | | | Quinolones: 88-96 | Quinolones: 3-27 | |
| | | | Macrolides: 73-87 | Macrolides: 3 | |
| | | | Sulfonamides: 72-90 | Sulfonamides: 3-17 | |
| | | | Tétracyclines: 79-99 | Tétracyclines: 7-20 | |

AS: Ajout de sel; F: Filtration, FD : Facteur de dilution; FP: Factor de préconcentration; pH: ajustement du pH de l'échantillon; RPLC-ESI(+/-)-MS/MS(QqQ) : Chromatographie liquide à polarité de phases inversée couplée à l'ionisation par électronébuliseur (mode positif) et à la spectrométrie de masse en tandem avec un triple quadripôle; SPE: Extraction sur phase solide;

Tableau 1.5. Méthodes sélectionnées d'analyse des anti-infectieux dans les eaux environnementales (suite).

| Prétraitement | Extraction | Séparation et détection | Recouvrement (%) | LOQ (ng L ⁻¹) | Référence |
|---|---------------------------|-------------------------|--|---|---------------------|
| AS: 100 mg L ⁻¹ Na ₂ EDTA pH = 2.8-3.0 | SPE: Oasis HLB FP: 500 | RPLC-ESI(+)-MS/MS(IT) | <p><i>Eau de surface</i></p> <p>Quinolones: 104-106 Macrolides: 74-112 Sulfonamides: 99-108 Tétracyclines: 106-127 Triméthoprième: 88</p> <p><i>Effluent</i></p> <p>Quinolones: 91-92 Macrolides: 82-85 Sulfonamides: 99-107 Tétracyclines: 111-126 Triméthoprième: 94</p> | <p><i>Eau déionisée</i></p> <p>Quinolones: 100-120 Macrolides: 120-650 Sulfonamides: 170-270 Tétracyclines: 200 Triméthoprième: 310</p> | (Batt et Aga, 2005) |

AS: Ajout de sel; F: Filtration, FP: Factor de préconcentration; pH: ajustement du pH de l'échantillon; RPLC-ESI(+/-)-MS/MS(IT) : Chromatographie liquide à polarité de phases inversée couplée à l'ionisation par électronébuliseur (mode positif) et à la spectrométrie de masse en tandem avec un e trappe ionique; SPE: Extraction sur phase solide.

Finalement, les travaux de Batt et ses collaborateurs (Batt et Aga, 2005) sur le développement d'une méthode analytique capable d'analyser différentes familles d'anti-infectieux dans les eaux usées et naturelles ont démontré quelles étaient les meilleures conditions pour obtenir des taux de recouvrement optimaux (**Tableau 1-5**) en utilisant des cartouches polymériques à polarité des phases inversées.

Tous ces travaux ont permis d'établir un point de départ pour le développement d'une nouvelle méthode analytique mieux adaptée à l'analyse des affluents et des effluents primaires pour cette classe de contaminants.

1.5. Perspectives de la recherche

En vue de l'état de la recherche et des connaissances sur la présence d'anti-infectieux dans l'environnement aquatique, il a été décidé à développer de nouvelles approches analytiques pour améliorer les méthodes déjà existantes. Aussi on a entrepris une revue des concentrations environnementales reportées dans la littérature scientifique pour mettre en perspective leur importance environnementale par rapport aux effets sur le biote.

Premièrement, une méta-analyse des concentrations des anti-infectieux reportés dans la littérature était encore manquante. Ce recensement et l'analyse de ces données sont importants puisqu'ils donnent une vue globale de la situation quant à la contamination des anti-infectieux dans l'environnement et les risques pour les êtres humains et le biote aquatique.

Deuxièmement, au début du projet il y avait peu de méthodes validées ou capables d'analyser les anti-infectieux dans les affluents et les effluents provenant des usines d'épuration primaire et physico-chimique, comme ceux de la station d'épuration de la Ville de Montréal (SEVM). Le but de ce traitement est de diminuer la charge des effluents en phosphore total ($< 0.5 \text{ mg L}^{-1}$) et en matières en suspension ($< 30 \text{ mg L}^{-1}$) (Communauté Urbaine de Montréal, 1999a; Ville de Montréal, 2001). Par conséquent, l'effluent montréalais contient une quantité considérable de solides en suspension ainsi qu'une demande chimique en oxygène élevée (Segura *et al.*, 2007b). De plus, les eaux usées

montréalaises sont composées des rejets domestiques et industriels ainsi que des matériaux organiques et inorganiques provenant des rejets pluviaux et de la fonte des neiges. Les eaux usées de Montréal sont alors une matrice complexe et variable, posant un défi analytique important. Une méthode capable de déterminer les analytes à des concentrations l'ordre du nanogramme par litre dans une matrice si chargée en matière organique doit alors être très sensible et spécifique.

Troisièmement, il a été décidé à s'attarder sur le problème de la préconcentration des échantillons. Comme le démontre le **Tableau 1-5**, les méthodes développées se basent principalement sur l'extraction sur phase solide (SPE) manuelle, c'est-à-dire l'étape de préconcentration de l'échantillon se réalise séparément de la séparation et détection. Cette technique exige beaucoup de temps et dépend de plusieurs variables expérimentales telles que le débit de charge, le temps de séchage, l'évaporation à sec, etc. qui ont un effet sur la précision. Par conséquent, la préconcentration automatisée des échantillons a été explorée pour améliorer la vitesse de la méthode ainsi que la qualité des résultats. Cette nouvelle approche pose aussi de nouveaux défis analytiques comme celui de la robustesse de la méthode lors de l'analyse de nombreux échantillons des eaux usées ou celui de la limite de détection, puisque les méthodes automatisées utilisent souvent des volumes d'échantillon relativement faibles (~1 mL) et par conséquent, leur facteur de préconcentration est faible. Toutes ces limitations doivent être surmontées pour pouvoir déterminer les anti-infectieux dans les eaux usées, les eaux de surface et l'eau potable d'une manière efficace.

1.6. Structure de la thèse

Les chapitres de la thèse sont organisés de façon à montrer la progression du développement analytique.

Le **Chapitre 2**, une revue et méta-analyse, introduit la problématique environnementale d'une façon plus détaillée et fait un sommaire des concentrations environnementales rapportées dans la littérature. Ce chapitre étudie aussi la signifiante de ces concentrations par rapport aux concentrations ayant un effet sur le biote aquatique.

Le **Chapitre 3**, chapitre sous la forme traditionnelle, décrit les travaux préliminaires réalisés au début du projet et explique les défis les plus importants à surmonter pour détecter les anti-infectieux dans les eaux usées, surtout celles provenant des affluents et des effluents d'une usine de traitement primaire et physico-chimique.

Le **Chapitre 4**, article publié dans *Journal of Environmental Monitoring*, se sert de la connaissance acquise durant les travaux préliminaires pour exposer le développement et validation d'une nouvelle méthode analytique capable de détecter six anti-infectieux dans les eaux usées d'une STEP primaire et physico-chimique. Cette méthode a utilisé la SPE manuelle en tandem pour la préconcentration et la LC-MS/MS pour l'analyse.

Le **Chapitre 5**, article publié dans *Analytica Chimica Acta*, vise à améliorer la méthode précédente en la transférant dans le mode SPE couplée ou automatique. Cette nouvelle méthode diminue le temps de préparation des échantillons et simplifie l'étape de préconcentration lors de l'analyse des anti-infectieux dans les eaux usées.

Le **Chapitre 6**, article publié dans *Chromatographia*, explore l'utilisation des colonnes à débit turbulent pour diminuer les effets de matrice observés lors du développement de la méthode SPE couplée-LC-MS/MS du chapitre précédent.

Le **Chapitre 7**, une conclusion générale, résume les résultats obtenus et met en perspective la recherche effectuée.

Le **Chapitre 8** (*en annexe*), article publié dans *Journal of Chromatography A*, développe une nouvelle méthode SPE couplée-LC-MS/MS pour l'analyse des anti-infectieux dans l'eau de surface et l'eau potable. Cette méthode utilise des injections de 10 mL au lieu des injections de 1 mL (comme les méthodes SPE couplée précédentes) pour améliorer les limites de détection.

Chapitre 2 : Méta-analyse et revue de la présence d’anti-infectieux dans les eaux usées, les eaux naturelles et l’eau potable contaminées

Review of the Occurrence of Anti-infectives in Contaminated Wastewaters and Natural and Drinking Waters

Segura P. A., François M., Gagnon C. et Sauvé S. 2009.

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Note sur ma contribution

Ma participation aux travaux de recherche: J’ai compilé l’information et j’ai aussi réalisé la majorité de l’analyse et de l’interprétation de données.

Rédaction : J’ai rédigé l’article en m’appuyant sur les commentaires de Dr. Gagnon et du Prof. Sauvé, mes directeurs de thèse.

Collaboration des co-auteurs: M. François m’a assisté avec l’analyse statistique et la correction de l’article.

Abstract

Objective. Anti-infectives are constantly discharged at trace levels in natural waters near urban centers and agricultural areas. They represent a cause for concern because of their potential contribution to the spread of anti-infective resistance in bacteria and other effects on aquatic biota. We compiled data on the occurrence of anti-infectives published in the last 24 years in environmental water matrices. The collected information was then compared with the available ecotoxicologic values to evaluate potential environmental concerns.

Data Sources. We used Web of Science and Google Scholar to search for articles published in peer-reviewed journals written in the English language since 1984.

Data Extraction. Information on compound concentrations in wastewaters and natural and drinking waters, the source of contamination, country of provenance of the samples, year of publication, limits of quantification and method of analysis was extracted.

Data Synthesis. From the 126 different substances analyzed in environmental waters, 68 different parent compounds and 10 degradation products or metabolites have been quantified so far. Environmental concentrations vary from about 10^{-1} to 10^9 ng L⁻¹ depending on the compound, the matrix and the source of contamination.

Conclusions. Detrimental effects of anti-infectives on aquatic microbiota are possible with the constant exposure of sensitive species. Indirect impact on human health cannot be ruled out when considering the potential contribution of high anti-infective concentrations to the spreading of anti-infective resistance in bacteria.

2.1. Introduction

Anti-infectives are substances that in small doses can inhibit the growth or the survival of micro-organisms without affecting the host (Korolkovas, 1976; Foye *et al.*, 1995). They comprise several classes of biologically-active compounds of natural or semi-natural origin such as antibiotics (e.g., β -lactams, tetracyclines) or from synthetic sources such as antimicrobials (e.g., sulfonamides, quinolones) and some antifungals (e.g., azoles). These substances are used to treat infections or to prevent them in humans, animals and food-producing insects and plants. In some food animals, subtherapeutic amounts of anti-infectives are also used as feed additives to reduce their susceptibility to stress-related diseases and to enhance growth (Kumar *et al.*, 2005). Anti-infectives have been recognized as the most significant therapeutic breakthrough in the history of medicine (Levy, 1992b), and estimates indicate that between 100 and 200×10^6 kg of these compounds are consumed annually worldwide (Wise, 2002).

The first report on the appearance of anti-infectives in the environment was published in 1983 (Watts *et al.*, 1983). Further studies were limited by the availability of sufficiently sensitive techniques, and it was not until the late 1990's and early 2000 that sensitive methods of trace analysis were first introduced (Hirsch *et al.*, 1998; Golet *et al.*, 2001; Lindsey *et al.*, 2001). The occurrence of these compounds in the environment has raised concerns about their potential role in the dissemination of anti-infective resistance in bacteria and the impact of their biological activity on the health of aquatic ecosystems (Daughton et Ternes, 1999).

To date, no comprehensive review on the occurrence of anti-infectives in the environment has been published. Therefore, the main goal of this work is 3-fold: *i*) to summarize published information on the environmental concentrations of anti-infectives in water matrices (wastewaters, natural and drinking waters) in the last 24 years, *ii*) to summarize the present body of knowledge on the presence of anti-infectives in the aquatic environment *iii*) to estimate possible effects of anti-infectives in the environment by comparing environmental concentrations to environmental effective concentrations.

Fate and occurrence in soils or sediments will not be discussed, as other papers have already commented on this subject (Tolls, 2001; Thiele-Bruhn, 2003). Analytical methods (Ternes, 2001; Díaz-Cruz et Barceló, 2005; Fatta *et al.*, 2007), the fate in wastewater treatment plants (Petrović *et al.*, 2003; Jones *et al.*, 2005), and ecotoxicity (Jones *et al.*, 2004; Crane *et al.*, 2006; Jjemba, 2006) are also among the subjects that will not be discussed in detail given the excellent earlier published reviews.

2.1.1. Sources and transport

Several sources of anti-infectives in the environment have been identified, such as manufacturing wastes (Babić *et al.*, 2007; Larsson *et al.*, 2007), improper disposal of unused medication (Bound et Voulvoulis, 2005) and landfill leachates (Holm *et al.*, 1995). However it has been recognized that the excretions of people or animals under treatment are the foremost source of anti-infectives in the aquatic environment (Hirsch *et al.*, 1999). Hence, anti-infectives reach the aquatic environment through two main routes: urban and agricultural.

In the urban route, the anti-infectives excreted [for some compounds as much as 90% in the parent form (Jjemba, 2006)], washed-off (in the case of topical formulations) or discarded by people in households, hospitals or industries, will end up in sewage. Once in wastewater, anti-infectives are discharged directly to surface waters or transported by sewers to wastewater treatment plants (WWTPs). During this process, the anti-infectives loads in sewage may be diluted by the mixing with used water containing none of these substances (Alexy, 2004). Anti-infectives may also reach the aquatic environment directly because of leaking sewers and sewer overflows (Sedlak *et al.*, 2004). Compounds arriving at WWTPs may be eliminated from wastewater, depending mainly on their capacity to associate with particulate matter (which influences their removal by physico-chemical or biological treatments) and their susceptibility to biological transformation (which certainly affects their elimination by biological treatment) (Ternes et Joss, 2006). Partial biodegradation and mineralization of anti-infectives in WWTPs is possible, as bacteria may co-metabolize these substances or use them as a source of carbon and energy to grow (Ternes *et al.*, 2004b). Substances having a lower affinity for solids and higher resistance to

biotransformation, will be subsequently discharged into streams (Roberts et Thomas, 2006). Substances sorbed to sludge during treatment in WWTPs, can also reach the environment by the application of sewage sludge in agricultural fields or by leaching in landfills. For these reasons, WWTPs are the main entry point of urban anti-infectives in the aquatic environment (Ternes *et al.*, 2004b; Glassmeyer *et al.*, 2008)

In the agricultural route, anti-infectives present in animal excreta may reach the aquatic environment by drainage and runoff to surface water and by percolation to groundwater. Studies have shown that compounds may be transported by the aqueous phase or bound to particulates in suspension (Kay *et al.*, 2004; Kay *et al.*, 2005), and this pathway is enhanced mainly because of land application of manure (Alexy, 2004; Kumar *et al.*, 2005). Substances retained and progressively accumulated in soils can be gradually released into the aqueous phase; agricultural soils may therefore act as environmental reservoirs for anti-infectives (Rooklidge, 2004; Lee *et al.*, 2007b). These substances can also reach natural waters directly by leaking from manure storage structures or constructed lagoons (Meyer, 2004) or through dust (Hamscher *et al.*, 2003). Compounds used in aquaculture are often released directly into surface waters by leaching from food pellets, fish feces or pond sediments (Cabello, 2006; Lee *et al.*, 2007b). Anti-infectives sprayed on fruit plants may reach the aquatic environment; however, this pathway has not yet been documented. Therefore, agricultural activities may be considered among the main non-point sources of anti-infectives in the aquatic environment.

2.1.2. Fate and effects

Once in the aquatic environment, anti-infectives are affected by different abiotic and biotic processes influencing their bioavailability and their subsequent impact on aquatic biota. The relative importance of these processes on the fate of anti-infectives is dictated by their physico-chemical properties as well as by the conditions of the medium in which they are present (Peschka *et al.*, 2007). Biodegradation and non-biological processes such as sorption, photolysis and hydrolysis may reduce anti-infective loads in the environment and result in partial degradation or mineralization of these compounds (Halling-Sørensen *et al.*, 1998; Alexy, 2004). Compounds having a lower susceptibility to these processes may be

persistent, in which case their environmental concentrations are reduced mainly through dilution in the aquatic environment. However, anti-infectives do not need to be very persistent in the environment to be able to have an effect. Contrary to other organic pollutants such as pesticides (Comoretto et Chiron, 2005), anti-infectives are constantly released in the environment; therefore substances degrading in a few days can be considered persistent with respect to natural waters at the point of discharge where releases are continuous (Sedlak *et al.*, 2004).

2.1.2.a. Anti-infective resistance

To date, the main interest for the study of anti-infectives in the environment has focused on their potential role on the spread of anti-infective resistance, since these substances are specifically designed and used with the purpose of inhibiting microbes. Anti-infective resistance is defined as the ability of a population of microorganism cells to neutralize the toxic effect of an anti-infective (Suling et O'Leary, 1975). When a bacterium develops resistance to a particular anti-infective mode of action, either by the acquisition of genes via mobile agents (e.g., plasmids and transposons) or by means of mutations, it gains an evolutionary advantage over its non-resistant congeners when the host is under anti-infective treatment (Levy et Marshall, 2004). However, this advantage ends once therapy on the patient is over because the recolonization of the medium by non-resistant bacteria will not be impeded and, as a consequence, resistant strains become diluted (Levy et Marshall, 2004).

So is it possible that anti-infective occurrence in environmental waters promotes resistance? Several authors have argued that if concentrations are higher than the minimum inhibitory concentrations (MICs) of some pathogenic bacteria, a selective pressure would be exerted and, as a result, the dissemination of anti-infective resistance could be enhanced (Kümmerer et Henninger, 2003). It has also been proven that even sub-inhibitory concentrations (e.g., one-quarter of the MIC) of several anti-infectives are able to have an effect, [e.g., as shown for the modulation of the expression of toxin-encoding genes in *Staphylococcus aureus* (Ohlsen *et al.*, 1998)]. Transfer of genetic elements among bacteria has been observed under natural conditions in WWTPs (Marcinek *et al.*, 1998), and

selection of resistant bacteria has been documented in sewers receiving wastewaters from pharmaceutical plants (Guardabassi *et al.*, 1998). Agricultural activities may also contribute to the transfer of resistance genes from wastewater bacteria to indigenous soil microbiota (Chee-Sanford *et al.*, 2001). However, the extent of the impact of the occurrence of anti-infectives in the aquatic environment on the dissemination of resistance in bacteria is still a subject of debate (Summers, 2002; Ohlsen *et al.*, 2003) and present knowledge indicates that its impact is still questionable (Kümmerer, 2004).

2.1.2.b. *Ecosystem health*

Anti-infectives are biologically-active substances; therefore they pose a potential threat to aquatic biota. Recently, numerous studies have reported the acute and chronic toxic effects of anti-infectives on non-target organisms such as diatoms (Wilson *et al.*, 2003), algae (Halling-Sørensen, 2000; Ferrari *et al.*, 2004) crustaceans (Ferrari *et al.*, 2004; Flaherty et Dodson, 2005; Isidori *et al.*, 2005), rotifers (Ferrari *et al.*, 2004; Isidori *et al.*, 2005), cnidarians (Quinn *et al.*, 2008) and amphipods (Maul *et al.*, 2006). These reports indicate that effective concentrations of most studied aquatic species are 2-5 orders of magnitude higher than those generally found in contaminated natural waters. Nevertheless, sensitive species, such as diatoms, green algae, cyanobacteria and some pathogenic bacteria (Al-Ahmad *et al.*, 1999; Kümmerer *et al.*, 2000; Wilson *et al.*, 2003) are affected by concentrations lower than 2000 ng L⁻¹. We must also consider that most of these studies target effects using a single species and single compounds. Surface waters near WWTP outfalls contain a myriad of organic and inorganic compounds that can interact as toxic mixtures. In comparison to short-term exposures in the laboratory, chronic exposures are likely to result in observable effects at lower thresholds. Other changes in the environment, indirect effects or more subtle effects that may affect species such as food selection behavior (Daughton et Ternes, 1999; Hahn et Schulz, 2007) or the fate of other organic pollutants such as pesticides should also be contemplated (Boxall *et al.*, 2003).

2.2. Methods

2.2.1. Selection and classification of literature data

Because an enormous amount of data has been published over the last 24 years on the occurrence of anti-infectives in the environment, we decided to set the following criteria to select and assure the quality of the compiled values. Only data reported in peer-reviewed journals written in the English language were considered for compilation. Only papers indicating or citing the limit of quantification (LOQ) of their determination method were selected. Therefore, only values higher than or equal to the reported LOQ were considered. When the authors reported only the limit of detection (LOD), the LOQ was evaluated by multiplying the LOD by a conversion factor x according to the definition of the LOD used by the authors. For example, when the authors defined the LOD as the concentration giving a signal-to-noise ratio (S/N) of 3, the LOQ was calculated by multiplying the LOD by a factor $x = 3.3$, as the LOQ is equivalent to S/N=10. In addition, when the same substance was analyzed by more than one method in the same study, the concentration reported using the most sensitive method (lower LOQ) was used. When the authors did not report any concentrations in real samples, their LOQ values were selected for compilation. Only concentrations in which the type (e.g., raw sewage, surface water) and the country of provenance of the sample were clearly indicated were used. Only data reported as numeric values were used. Data reported in figures were not considered because of the uncertainty of their interpretation. Only natural concentrations were reported; values in natural waters with experimental amendment of manure or sediments were not considered. Urban effluents were classified in three categories according to the treatment applied: primary (physical or mechanical), secondary (biological), and tertiary (advanced, such as disinfection by chlorination or ultraviolet radiation) (vanLoon et Duffy, 2000). Waters found in agricultural matrices such as runoff, lagoons, and field streams were classified as wastewaters. Ecotoxicologic data such as lowest-observed effective concentrations (LOEC) and 50% effective concentration (EC_{50}) of sulfamethoxazole and ofloxacin for several types of bacteria and aquatic species were gathered from the peer-reviewed literature. We

included as many different species as possible to illustrate the distribution of effective concentrations on aquatic biota of these two anti-infectives (**Tableau 2-3**, **Tableau 2-4**).

2.2.2. Statistical analysis

The distributions of anti-infectives concentrations in the different matrices are described by their arithmetic mean, median, 75th and 95th percentiles and maximal concentrations. We mined the data looking for expected trends or for possible relations with factors such as the geographic region, anti-infective class and the treatment process, if any. The matrix of selected data cannot be processed entirely through statistical analyses because of the heterogeneity of the studied compounds between matrices and countries. However, focusing on urban wastewater and on three data-rich classes (macrolides, quinolones, sulfonamides) and one compound (trimethoprim), we compared their concentrations in raw and treated wastewaters from three different regions (East Asia, Europe, and North America). An analysis of variance with the general linear model procedures of SPSS (version 16.0, SPSS Inc., Chicago, IL, USA) was used for that purpose. Density histograms and normal density functions of sulfamethoxazole, ofloxacin and their LOEC and EC₅₀ were calculated by Systat (version 11.0, Systat Software Inc., Chicago, IL, USA).

2.3. Results and discussion

2.3.1. Occurrence of anti-infectives in the environment

A bibliographic research of the scientific literature found 159 papers published between 1984 and mid-2008 reporting analyses of anti-infectives in wastewaters, surface waters or drinking waters. The attention that the occurrence of anti-infectives in the aquatic environment has generated in the recent years is reflected by the number of papers published each 5-year period since 1984: 2 (1984-1988), 0 (1989-1993), 6 (1994-1998), 27 (1999-2003) and 124 (2004-2008). Most of the studies we found reported concentrations of

these compounds in environmental waters coming from countries defined as having high incomes (World Bank, 2008); in fact, only 28 papers of 159 originally found (18%) analyzed waters from low to middle-income countries.

Applying the selection criteria enumerated in the Methods section under “Selection and classification of literature data”, 16 papers were eliminated. A database was built containing over 2200 values of concentrations and 2500 LOQ values of anti-infectives in drinking, natural, and wastewaters reported in the 143 selected publications. A summary of the detection instruments showed that 75% of the reported values come from methods using tandem mass spectrometry, 14% from single mass spectrometry, and 11% from molecular spectroscopy (fluorescence or ultraviolet). By compiling the data obtained from different analytical methods and sources we tried to offset a bias in our results caused by the publications reporting more values. In total, from the 126 different substances (parent compounds, degradation products, or metabolites) for which at least one method of determination exists, only 68 different parent compounds and 10 degradation products or metabolites have been quantified so far in environmental waters. **Tableau 2-1** shows occurrence data sets organized by anti-infective class and matrix. For more detailed information, see Matériel supplémentaire (**Tableau 2-2**).

Several classes of anti-infectives have been less frequently reported in spite of the numerous studies that have tried to determine them. One example is the β -lactams, for which only 12 of 24 different compounds have been determined so far in environmental waters. As mentioned earlier, this is due to their high reactivity in aquatic media. Also, among the most important classes of anti-infectives that have not yet been determined are the quinoxaline dioxides (carbadox, olaquinox), which at least 13 different studies have been unable to quantitate.

The study of metabolites is important for the accurate determination of anti-infectives, as some conjugated metabolites, such as glucuronide, sulfate, and N-acetyl, can be deconjugated in wastewaters by bacterial enzymes, thus increasing the concentration of the parent drug (Jones *et al.*, 2005). Few studies have looked into this problem, and only N^4 -acetylsulfamethoxazole, a metabolite of the sulfonamide sulfamethoxazole, has been quantitated to date (Hilton et Thomas, 2003; Ashton *et al.*, 2004; Göbel *et al.*, 2004; Göbel

et al., 2005a; Göbel *et al.*, 2007). As for degradation products, their occurrence is less significant in the environment if they are not as biologically active as the parent drug. This is the case of anhydro-erythromycin (erythromycin-H₂O), the degradation product of the macrolide erythromycin, which has been widely quantitated in the literature. Other degradation products such as those of the β -lactams or the tetracyclines have been only sparsely documented (Mackie *et al.*, 2006; Li *et al.*, 2008b).

An important aspect of our study, that we must emphasize, is that the selected occurrence data are not representative of the water matrices or the global state of water contamination with respect to anti-infectives. Published values are biased because analyzed samples are often collected in sites where contamination is suspected. In addition, information on frequency of detection is not always available, which also overestimates the occurrence of anti-infectives. Therefore, it must be kept in mind that throughout our study, our results and conclusions apply mostly to contaminated waters.

2.3.2. Drinking water

Occurrence of anti-infectives in drinking water is the least reported so far. Only about 2% (3 of 143 selected papers) indicate quantitative values in drinking water, even though more than eight different studies have tried to measure them. This can be explained by the low limits of quantification necessary to achieve their determination in drinking water which often must be $< 1 \text{ ng L}^{-1}$. Anti-infectives concentrations in contaminated tap water range from 0.3 to 5 ng L^{-1} , with a median concentration of 2 ng L^{-1} . We found only one study attempting to measure concentration of degradation products or metabolites. Anti-infectives reach drinking water, albeit in very low amounts, because they are able to persist in natural water sources and resist purification processes in drinking water treatment plants (DWTPs). However, anti-infectives seem to be more affected by purification processes than other more frequently reported organic wastewater contaminants (OWCs). In a study on the fate of 106 OWCs (including 25 anti-infectives) in a conventional DWTP using several physico-chemical processes in sequence, from the 42 OWCs detected above their reporting limit in stream and raw water samples, only five were anti-infectives. In finished waters, only 17 OWCs were detected and none of them were anti-infectives (Stackelberg *et al.*,

2004). A study on the effectiveness of several treatment processes used in DWTPs showed that activated carbon sorption, reverse osmosis and oxidation (chlorination or ozonation) were among the most efficient treatments to remove anti-infectives from source water (Adams *et al.*, 2002).

2.3.3. Natural waters

Occurrence of anti-infectives has been well documented in both groundwater and surface waters (44% of selected papers). Rivers, creeks, lakes, estuaries, basins, sea waters and wells have been reported to be contaminated by several of these compounds. Values found in the literature show up to eight orders of magnitude of variation and concentrations often decrease as the distance from the source (WWTP outfalls, landfills, etc.) increases. The cause of the attenuation of anti-infectives in surface waters cannot be easily interpreted because different attenuation mechanisms may operate simultaneously (Sedlak *et al.*, 2004). Published data in the literature do not indicate the present state of the global anti-infective contamination of surface waters and this may appear overestimated by the choice of sampling points, often near WWTP outfalls or agricultural areas. For example, a study of 139 streams in the United States showed that only 2 of the 23 targeted anti-infectives were detected in more than 20 % of the samples collected in zones susceptible of contamination (Kolpin *et al.*, 2002). These observations were later confirmed by Focazio *et al.* (Focazio *et al.*, 2008), who showed that only 6 anti-infectives, from the 25 initially targeted, were found in less than 35% of the 74 untreated drinking water sources across the United States.

2.3.3.a. Surface waters

As the receptors of most WWTPs final effluents, outfalls in streams and the nearby downstream zones act as collectors of all the substances that were not removed by the treatment process. Our database showed that a total of 52 of 143 papers (36%) report concentrations of anti-infectives in surface waters. Median concentration of these drugs in contaminated surface waters was 30 ng L⁻¹ and values varied between 0.07 and 712 000 ng L⁻¹. As for metabolites and degradation products, their median concentration was 548 ng L⁻¹, and reported amounts ranged from 2 to 10 540 000 ngL⁻¹. Detection of anti-

infectives in surface waters upstream of WWTPs outfalls, indicate the persistence and mobility of these compounds, which may be discharged by point or non-point sources. The importance of these sources on the anti-infective occurrence in downstream surface water depends clearly on the season and hydrology of the region. Kolpin *et al.* (Kolpin *et al.*, 2004) demonstrated that stream flow conditions significantly affect occurrence of organic wastewater contaminants, as higher flows increase the dilution factor of WWTPs effluents. Also, the contribution of WWTPs effluents to the total flow of rivers is determinant. Rivers in which the majority of the flow is composed of WWTP effluent will have a lower diluting power (some rivers are composed of up to 75-80% of WWTP discharges) and anti-infective concentrations downstream will be approximately constant depending on other attenuation processes such as photolysis or sorption (Hirsch *et al.*, 1999; Bendz *et al.*, 2005).

2.3.3.b. Groundwaters

Ground waters are affected by a variety of sources, with landfills, septic systems and agricultural fields representing the most significant potential sources of anti-infective contamination. A recent national reconnaissance study of 65 OWCs in groundwaters in the United States (Barnes *et al.*, 2008) detected only 3 anti-infectives of the 21 targeted. Sulfamethoxazole was the most frequently detected (23.4%), and its maximum concentration was 1,110 ng L⁻¹. However, the detected anti-infectives represented less than 5% of the total OWCs concentration. According to our database, 10 % (13 of 143 sampled papers) report concentrations of anti-infectives in ground waters in the range of 0.2 to 1400 ng L⁻¹, and a median concentration of 71 ng L⁻¹. Only one paper, a study on the occurrence of sulfonamides in ground waters near a pharmaceutical waste landfill in Denmark (Holm *et al.*, 1995), reported higher amounts, with a median concentration of 190 000 ng L⁻¹ and a maximum concentration as high as 1 600 000 ng L⁻¹. Landfills containing WWTPs biosolids or discarded anti-infectives contaminate ground waters because leachate plumes may reach nearby aquifers. In addition, as opposed to surface waters, anaerobic or suboxic conditions are often observed in ground waters and may prevent or slow down the degradation rates of some anti-infectives (Verstraeten *et al.*, 2005). Studies on the disposal of pharmaceuticals in the United States and the United Kingdom (Kuspis et Krenzelok, 1996; Bound et Voulvoulis, 2005) showed that a significant proportion of people (54 % in

the United States, 71 % in the United Kingdom) disposed of unused medication in the trash. Hence, the role of landfills in the contamination of groundwaters should be reassessed as disposal of anti-infectives is usually considered only a minor source of contamination (Boxall, 2004).

2.3.4. Wastewaters

Wastewaters produced by urban centers as well as by agricultural activities were the most studied matrices in the selected literature (62%). Reported concentrations in this matrix are obviously the highest but they vary by up to 10 orders of magnitude. This huge variability is mainly a consequence of the diverse origin of the wastewaters which may come from industries, hospitals, municipal WWTPs, farm lagoons, field runoff, etc. Also, anti-infective concentrations are affected by the different treatment process applied to wastewaters, which in some cases are non existent (as is the case of direct discharges of urban or agricultural origin) and in others very advanced such as tertiary wastewater treatment systems that include reverse osmosis and micro and nano-filtration as well as ozonation.

2.3.4.a. Industrial sewage

Manufacture of anti-infectives often generates highly contaminated sewage. For example, biosynthetic fabrication of tetracyclines produces wastes having high chemical oxygen demand (COD) loads and treatment of these waters is both difficult and expensive (Li *et al.*, 2004). Consequently, anti-infectives concentrations in these manufacturing wastes are worryingly high, as is the case of wastewaters from oxytetracycline production facilities reaching values as high as 920 000 000 ng L⁻¹ (Li *et al.*, 2008a) which are several times higher than the EC₅₀ for some aquatic species such as *Microcystis aeruginosa* (EC₅₀=20 700 ng L⁻¹), or *Rhodomonas salina* (EC₅₀=160 000 ng L⁻¹) (Holten-Lützhøft *et al.*, 1999). Other compounds, such as the quinolones, have also been reported in effluents from drug manufactures (Larsson *et al.*, 2007) with concentrations of ciprofloxacin up to 30 000 000 ng L⁻¹ which are well above EC₅₀ values for several aquatic species as well

(Larsson *et al.*, 2007). The mixing of industrial wastes with human sewage creates further concerns, as it generates an ideal environment for the spreading of anti-infective resistance in bacteria (Larsson *et al.*, 2007). The β -lactams and their metabolites have been also reported in manufacturing plant effluents, with a concentration of benzylpenicillin (153 000 ng L⁻¹) comparable to published MICs (Li *et al.*, 2008b).

According to our bibliographic research, about 5 % (7 of 143 of the sampled papers) report the presence of anti-infectives in industrial wastewaters and concentrations range from 4900 to 920 000 000 ng L⁻¹, with a median concentration of 300 000 ng L⁻¹. The extent of the contribution coming from manufacturing plants to the overall occurrence of anti-infectives in the environment cannot be evaluated at present, because published data are still scarce. (These reports were limited to only three countries: China, India and Croatia). Industrial discharges in the environment in high-income countries like the United States are controlled by current Good Manufacturing Practices and emissions regulations (Velagaleti *et al.*, 2002) therefore, the impact of drug manufacturers should be limited only to countries with more flexible legislation (or lacking the resources to enforce them). In 1999, low- to middle-income countries accounted for only 7.1% (by value) of the world pharmaceutical production; nevertheless, at least 10 low- to middle-income countries produce active ingredients, with China and India leading this group (World Health Organization, 2004). In countries with less strict regulations, anti-infective production facilities may be among the most important sources of these substances in their nearby aquatic environment (Larsson *et al.*, 2007).

2.3.4.b. Hospital sewage

Hospitals are considered one of the most important sources of anti-infectives in the aquatic environment (Gómez *et al.*, 2006). However, less than 8% of the selected papers (12 of 143 sampled papers) report the occurrence of these compounds in hospital sewage. Concentrations of anti-infectives in contaminated hospital waters range from 10 -124,500 ng L⁻¹, with a median value of 2,100 ng L⁻¹. The maximum concentration of ciprofloxacin found in hospital effluents (124,500 ng L⁻¹) (Hartmann *et al.*, 1988) is considerably higher than the lowest effect concentration of ciprofloxacin for genotoxicity (LOEC= 200 ng L⁻¹)

or the EC_{50} of some pathogens ($EC_{50}= 2000 \text{ ng L}^{-1}$) (Kümmerer *et al.*, 2000). Quinolones, especially ciprofloxacin, were the main cause of the DNA-damaging effects detected in wastewater samples from hospitals (Hartmann *et al.*, 1988). Additionally, compared with antineoplastic drugs, quinolones have a greater potential to cause DNA damage (Hartmann, 1999). These findings support the concern for the potential impact of anti-infectives residues on the spread of bacterial resistance, although according to other studies, the concentrations of anti-infectives found in hospital wastewaters are below the concentrations known to promote resistance (Ohlsen *et al.*, 2003; Jarnheimer *et al.*, 2004).

Although detection frequencies and concentrations are generally higher in hospital sewage than in municipal wastewaters, it has also been reported that anti-infective concentrations in hospital sewage are similar or lower than the concentrations found in municipal WWTPs influents (Karthikeyan et Meyer, 2006) or retirement homes (Brown *et al.*, 2006). Therefore, the impact of hospitals may depend on the communities, the season, and water use, but results tend to indicate that wherever large groups of individuals under medication cohabit in the same location, significant concentrations of anti-infectives will be found in sewage.

2.3.4.c. Agricultural and aquacultural wastewaters

Papers reporting the presence of anti-infective in agricultural waters coming from hog, fish and shrimp breeding are among the first reports published in the anti-infectives in the environment literature (Smith *et al.*, 1994; Migliore *et al.*, 1996). These studies reflect early concerns on the intense use of these compounds in farming and their fate. About 7% of the selected papers (10 of 143) report values in agricultural wastewaters. Concentrations of anti-infectives in lagoons or aquaculture ponds range between 1 and 13 000 000 ng L^{-1} , with a median concentration of 22,930 ng L^{-1} . Residues of these drugs in aquaculture waters may reach high values because they are used as feed additives; they may leach from the food pellets and are also excreted by the animals. Sediments may also accumulate anti-infectives, which may be released later in the water. Estimates indicate that 70 to 80% of drugs used as feed in aquaculture may reach the environment (Migliore *et al.*, 1996; Holten-Lützhøft *et al.*, 1999).

Occurrence of anti-infectives in field tiles, field streams and runoff show much lower concentrations (2 to 4000 ng L⁻¹). Difference in concentrations for anti-infectives in these matrices may be due to several factors such as the dose used, resistance to biodegradation, and mobility, as well as soil characteristics. Because the mobility of anti-infectives is affected by their affinity to particulate matter, their occurrence in overland flow seems to be more important for rather hydrophilic compounds such as the sulfonamides or trimethoprim than more hydrophobic or binding-capable compounds such as the tetracyclines or the macrolides (Kay *et al.*, 2004). However, compounds having high sorption coefficients are still able to be transported and reach environmental waters (Kay *et al.*, 2004). Sorption of more hydrophilic compounds (e.g. sulfonamides) onto soils may increase with time, thus reducing their release into environmental waters (Stoob *et al.*, 2007). The influence of other transport process such as binding to dissolved organic matter remains unclear (Lee *et al.*, 2007b).

2.3.4.d. Urban wastewaters

Because of the preponderant role of WWTPs on the anti-infective contamination of surface waters, urban wastewaters have been extensively studied in the past 10 years. In fact 51% of the sampled papers reported concentrations in urban wastewaters. In our database, occurrence of anti-infectives in urban raw sewage range from 3 to 10,570 ng L⁻¹, with a median concentration of 300 ng L⁻¹. In treated effluents, concentrations vary between 1 and 29 000 ng L⁻¹ and a median of 136 ng L⁻¹. Analyses in raw sewage and WWTP effluents applying different types of processes have demonstrated the failure of the commonly used wastewater treatment technologies to completely remove anti-infectives present in wastewaters.

As our bibliographic research of urban wastewaters provides enough data for three classes of substances [macrolides (including anhydro-erythromycin, the degradation product of erythromycin), quinolones and sulfonamides] and one compound (trimethoprim), four levels of treatment (none, primary, secondary and tertiary) in three geographic areas: East Asia (China, Japan, and South Korea), Europe (Austria, Croatia, Denmark, France, Finland, Germany, Greece, Italy, Norway, Sweden, Spain, Switzerland,

and the United Kingdom) and North America (Canada, Mexico, and the United States), it was possible to investigate the influence of these factors on anti-infective concentrations. Analysis of variance with Dunnett's T3 post-hoc tests showed significantly higher ($p < 0.05$) concentrations in raw wastewaters compared with primary, secondary and tertiary effluents. Analysis of variance also showed that the concentrations measured in North America and East Asia were significantly higher than those in Europe. Furthermore, resulting concentrations of sulfonamides and trimethoprim are significantly higher than those of macrolides and quinolones. These results confirm previous observations about the removal of anti-infectives in WWTPs (Ternes *et al.*, 2004b; Göbel *et al.*, 2005b; Batt *et al.*, 2007; Gulkowska *et al.*, 2008). Removal efficiency is dependent of many factors, the most important being the type of treatment (primary, secondary, etc.), the WWTP design (hydraulic and solid retention times, sludge age, etc) and the physico-chemical properties of each compound (e.g. pKa and log D_{ow}).

2.4. Ecological significance of ambient concentrations of anti-infectives

2.4.1. The case of sulfamethoxazole and ofloxacin

Sulfamethoxazole and ofloxacin were chosen for further insight into the significance of the occurrence data reported in the literature from an ecotoxicologic standpoint. **Figure 2-1** shows the histogram and the normal density function of sulfamethoxazole for natural and wastewaters and the LOEC and EC_{50} for different species (**Tableau 2-3**). As can be observed, the distribution of sulfamethoxazole concentration in natural waters clusters around 10^2 ng L⁻¹, and for wastewaters this value is about an order of magnitude superior. This difference coincides well with fate data, showing a rather weak affinity of sulfamethoxazole for solids, which enhances its transport in the aqueous phase. Ecotoxicologic values show a much wider distribution, with a density function maximum around 10^6 ng L⁻¹ for LOEC and 10^7 ng L⁻¹ for EC_{50} . In some cases, these density curves lie over common ranges, and we interpreted this overlapping as an increased risk for aquatic species. We observed that less than 1% of LOEC values and less than 0.1% of the EC_{50} values were lower than the highest 10% of the concentrations of sulfamethoxazole in

Tableau 2-1. Occurrence in nanograms-per-liter of anti-infectives in contaminated wastewaters, and natural and drinking waters organized by class and matrix.

| Anti-infective class | Matrix: Wastewaters | | | | | | | | |
|--|---------------------|----------------|----------|---------|----------|-----------|-----------|---------------------------------|----------------------------------|
| | N ^a | P ^b | Mean | Median | 75% | 95% | Max | LOQ _{low} ^c | LOQ _{high} ^d |
| <i>As parent compounds</i> | | | | | | | | | |
| Azoles | 17 | 6 | 5987 | 26 | 50 | 61920 | 90200 | 5 | 112 |
| β-Lactams | 42 | 15 | 4633 | 300 | 1200 | 5360 | 153000 | 1 | 100000 |
| Quinolaxine-Dioxide | 0 | 5 | | | | | | 5 | 100 |
| Lincosamides | 33 | 10 | 18715 | 60 | 2125 | 190500 | 240000 | 0.3 | 100 |
| Macrolides | 217 | 43 | 352 | 110 | 271 | 1000 | 27000 | 0.2 | 1155 |
| Poliether ionophores | 13 | 2 | 29 | 11 | 26 | 167 | 190 | 1 | 3 |
| Quinolones | 420 | 51 | 152247 | 205 | 570 | 41922 | 31000000 | 1 | 20600 |
| Sulfonamides | 289 | 57 | 11972 | 330 | 800 | 31000 | 1158680 | 1 | 300000 |
| Tetracyclines | 161 | 32 | 11642200 | 530 | 7250 | 6095000 | 920000000 | 1 | 700000 |
| Trimethoprim ^e | 210 | 52 | 1351 | 270 | 795 | 5000 | 55200 | 1 | 150000 |
| Other | 17 | 11 | 750 | 39 | 1115 | 4101 | 5000 | 3 | 667 |
| <i>As metabolites / degradation products</i> | | | | | | | | | |
| β-Lactams | 10 | 1 | 55794600 | 7630000 | 44500000 | 389000000 | 389000000 | 410 | 1360 |
| Macrolides | 52 | 16 | 2848 | 450 | 1414 | 5650 | 83000 | 1 | 500 |
| Sulfonamides | 13 | 5 | 779 | 570 | 1200 | 2230 | 2235 | 20 | 212 |
| Tetracyclines | 6 | 1 | 5092167 | 1445000 | 9420000 | 18100000 | 18100000 | 600 | 1300 |

^a Number of values reported being > LOQ for each class. ^b Number of papers reporting analysis in each matrix for each class. ^c Lowest LOQ reported. ^d Highest LOQ reported. ^e Dihydrofolate reductase inhibitor. NA: Not available.

Tableau 2–1. Occurrence in nanograms-per-liter of anti-infectives in contaminated wastewaters, and natural and drinking waters organized by class and matrix (suite).

| | N ^a | P ^b | Mean | Median | Matrix: Natural waters | | Max | LOQ _{low} ^c | LOQ _{high} ^d |
|--|----------------|----------------|---------|---------|------------------------|----------|----------|---------------------------------|----------------------------------|
| | | | | | 75% | 95% | | | |
| <i>As parent compounds</i> | | | | | | | | | |
| Azoles | 39 | 11 | 18 | 18 | 23 | 44 | 58 | 1 | 370 |
| β-Lactams | 6 | 16 | 73 | 11 | 48 | 350 | 350 | 2 | 24000 |
| Quinolaxine-Dioxide | 0 | 9 | | | | | | 35 | 1400 |
| Lincosamides | 46 | 21 | 147 | 18 | 100 | 1020 | 1400 | 0.04 | 198 |
| Macrolides | 128 | 38 | 58 | 11 | 46 | 197 | 1022 | 0.02 | 1155 |
| Poliether ionophores | 4 | 4 | 312 | 35 | 606 | 1172 | 1172 | 0.1 | 380 |
| Quinolones | 78 | 31 | 199 | 27 | 108 | 640 | 5600 | 0.3 | 7000 |
| Sulfonamides | 234 | 60 | 66531 | 120 | 700 | 472000 | 1600000 | 0.2 | 33000 |
| Tetracyclines | 47 | 33 | 97369 | 192 | 658 | 623550 | 712000 | 0.07 | 1650 |
| Trimethoprim ^f | 90 | 24 | 94 | 18 | 42 | 510 | 3000 | 0.2 | 4000 |
| Other | 5 | 21 | 136 | 127 | 207 | 266 | 266 | 2 | 2000 |
| <i>As metabolites / degradation products</i> | | | | | | | | | |
| β-Lactams | 4 | 1 | 4719500 | 4037500 | 8840000 | 10540000 | 10540000 | | |
| Macrolides | 51 | 24 | 184 | 40 | 146 | 1186 | 1700 | | |
| Sulfonamides | 6 | 6 | 86 | 14 | 239 | 240 | 240 | | |
| Tetracyclines | 27 | 25 | 12367 | 11100 | 14750 | 32840 | 34200 | | |

^a Number of values reported being > LOQ for each class. ^b Number of papers reporting analysis in each matrix for each class. ^c Lowest LOQ reported. ^d Highest LOQ reported. ^f Dihydrofolate reductase inhibitor. NA: Not available.

Tableau 2–1. Occurrence in nanograms-per-liter of anti-infectives in contaminated wastewaters, and natural and drinking waters organized by class and matrix (continuation).

| | N ^a | P ^b | Matrix : Drinking waters | | | | | | |
|--|----------------|----------------|--------------------------|--------|-----|-----|-----|---------------------------------|----------------------------------|
| | | | Mean | Median | 75% | 95% | Max | LOQ _{low} ^c | LOQ _{high} ^d |
| <i>As parent compounds</i> | | | | | | | | | |
| Azoles | 0 | 1 | | | | | | 370 | 370 |
| β-Lactams | 0 | 0 | | | | | | N.A. | N.A. |
| Quinolaxine-Dioxide | 0 | 1 | | | | | | 100 | 100 |
| Lincosamides | 0 | 2 | | | | | | 0.07 | 5 |
| Macrolides | 4 | 2 | 3 | 3 | 5 | 5 | 5 | 0.07 | 220 |
| Poliether ionophores | 0 | 0 | | | | | | N.A. | N.A. |
| Quinolones | 5 | 2 | 2 | 2 | 3 | 4 | 4 | 0.3 | 10 |
| Sulfonamides | 2 | 5 | 0.4 | 0.4 | 0.5 | 0.5 | 0.5 | 0.2 | 1155 |
| Tetracyclines | 0 | 1 | | | | | | 3 | 12 |
| Trimethoprim ^e | 0 | 4 | | | | | | 0.5 | 250 |
| Other | 0 | 1 | | | | | | 5 | 5 |
| <i>As metabolites / degradation products</i> | | | | | | | | | |
| β-Lactams | 0 | 0 | | | | | | N.A. | N.A. |
| Macrolides | 0 | 1 | | | | | | 10 | 10 |
| Sulfonamides | 0 | 0 | | | | | | N.A. | N.A. |
| Tetracyclines | 0 | 0 | | | | | | N.A. | N.A. |

^a Number of values reported being > LOQ for each class. ^b Number of papers reporting analysis in each matrix for each class. ^c Lowest LOQ reported. ^d Highest LOQ reported. ^e Dihydrofolate reductase inhibitor. NA: Not available.

natural water. When looking at the density curves of effective concentrations versus occurrence of sulfamethoxazole in wastewaters, we can see that overlapping between them is slightly more important. About 3% of LOEC and less than 1% of EC₅₀ values were lower than the highest 10% of the concentrations of sulfamethoxazole in wastewaters. The presence of this overlapping region suggests that the observed concentrations of sulfamethoxazole in natural waters are not high enough to affect most studied aquatic species but that concentrations in wastewaters could have an impact on the most sensitive species such as bacteria.

In the case of ofloxacin (**Figure 2-2**) the distribution of its concentration shows that occurrence of this quinolone in natural waters centers around 10² ng L⁻¹, whereas in wastewaters, this value is between 1 and 2 orders of magnitude higher. Compared with sulfamethoxazole, this difference is more pronounced which is not surprising given the higher affinity of ofloxacin for solids and its subsequent better elimination by wastewater treatments. As with sulfamethoxazole, ecotoxicologic values (**Tableau 2-4**) show a wide distribution, having a maximum close to 10⁵ ng L⁻¹ for LOEC and 10⁶ ng L⁻¹ for EC₅₀. Less than 1 % of LOEC values and less than 0.1 % of the EC₅₀ density curves were lower than the highest 10 % of concentrations of ofloxacin in natural water. Also, the density curve overlapping of wastewaters and effective concentrations is much more important than in the case of sulfamethoxazole, with around 8 % of LOEC values and less than 2 % of the EC₅₀ values being lower than the highest 10 % of concentrations of ofloxacin in wastewaters. Therefore, ofloxacin seems to present a higher ecotoxicological risk than sulfamethoxazole, and detrimental effects on wastewater bacteria are more likely to occur than in aquatic biota.

Thus, what is the ecotoxicologic significance of the occurrence of anti-infectives in environmental waters? Assuming that our sampled data are representative of ambient anti-infective concentrations in most contaminated environmental waters, it can be argued that even a weak overlapping between concentrations values corresponding to environmental waters and ecotoxicological data could have detrimental effects on the most sensitive species such as bacteria or algae. In the case of surface waters, because anti-infectives are constantly being released into the environment, microbiota are constantly exposed to these compounds. These harmful effects should be more important in small streams affected by

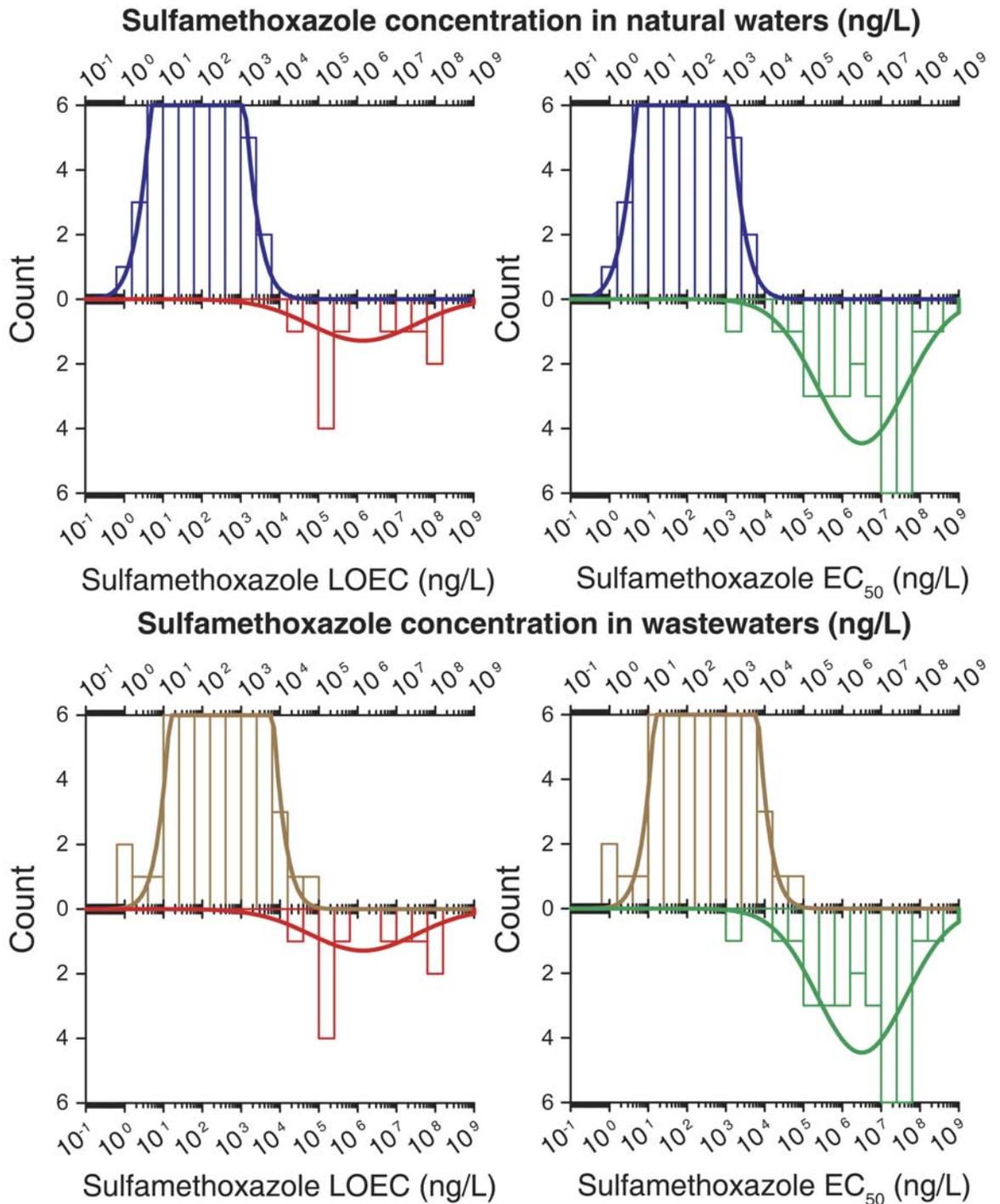


Figure 2-1. Density histogram (bars) and density function (line) of ofloxacin occurrence in natural waters (A) and wastewaters (B) compared with density histogram and density function of LOEC (left) and EC₅₀ (right) values for several aquatic species exposed to ofloxacin.

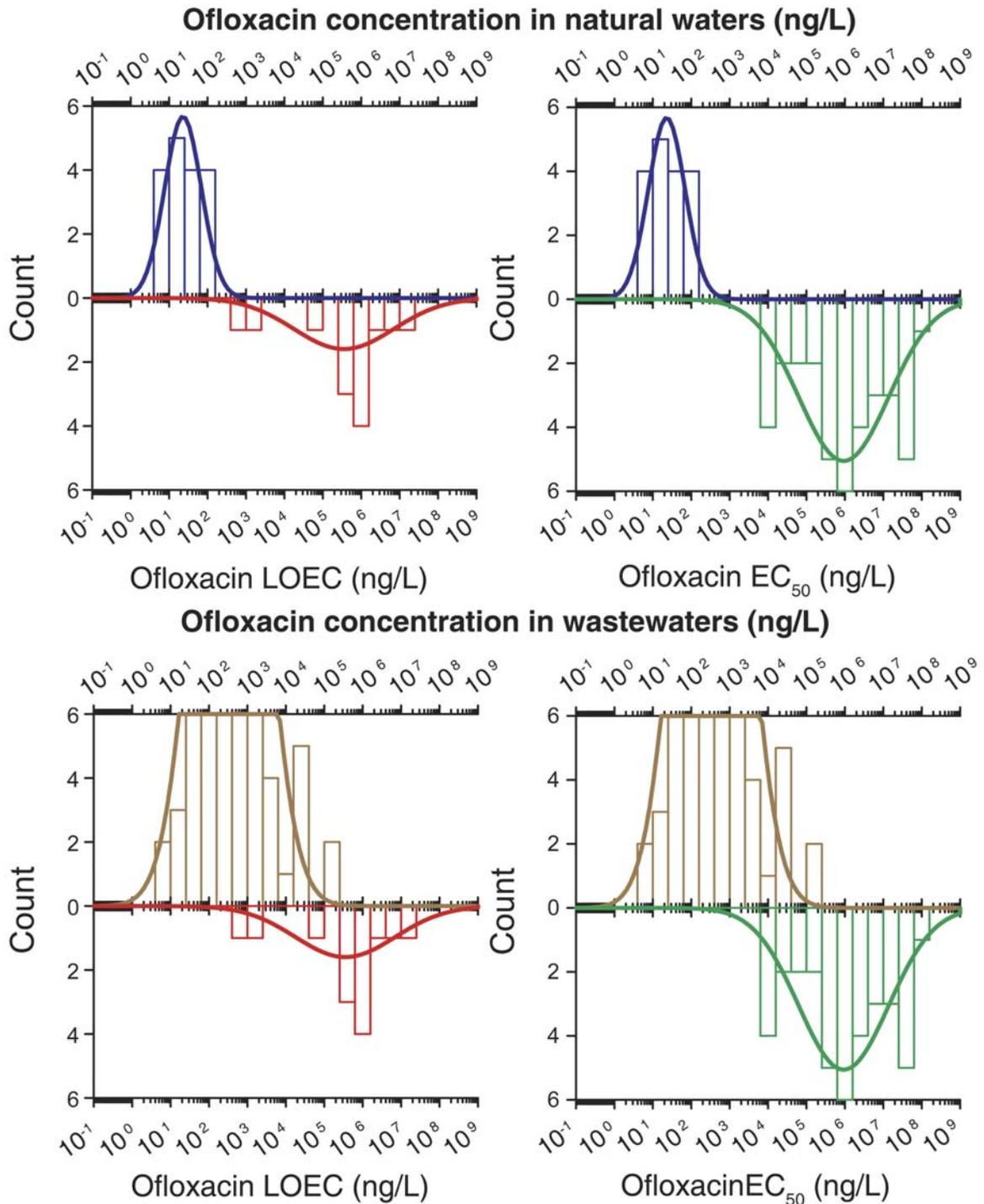


Figure 2-2. Density histogram (bars) and density function (line) of sulfamethoxazole occurrence in natural waters and wastewaters compared with density histogram and density function of LOEC (left) and EC₅₀ (right) values for several aquatic species exposed to sulfamethoxazole.

urban or agricultural discharges, because of their reduced dilution capacity. With regard to wastewaters, even if our results show that high concentrations ($> 10\,000\text{ ngL}^{-1}$) of anti-infectives in these waters are more the exception than the rule, the existence of a few locations where these concentrations can be reached are enough to contribute to the global spreading of anti-infective resistance (Okeke et Edelman, 2001). Given that large populations of bacteria are being exposed to a selective pressure, environmental waters and especially wastewaters become ideal settings for the assembly and exchange of mobile genetic agents encoding for resistance in bacteria (O'Brien, 2002).

2.4.2. Additional factors to be considered

2.4.2.a. Mixture effects

Mixture effects are expected in environmental waters because many other organic and inorganic contaminants are discharged in conjunction with different anti-infectives. According to a recent study using predicted environmental concentrations, when strong synergistic effects are present between anti-infectives and other pharmaceuticals occurring in wastewater, an impact on resistance in bacteria is possible but not in fungi (Kostich et Lazorchak, 2008). Certain substances that may be present in environmental waters have a synergistic effect on some anti-infectives. For example the MIC of ampicillin for *Pseudomonas aureginosa* is $1,500\text{ mg L}^{-1}$, but in combination with 500 mg L^{-1} of EDTA, the MIC was reduced to 22 mg L^{-1} (Lambert *et al.*, 2004). Surfactants have also been reported as potentiators (agents capable to enhance the activity of a substance) of chlortetracycline and benzylpenicillin in certain bacterial strains (Suling et O'Leary, 1975). In contrast, antagonistic interactions between anti-infectives and other pharmaceuticals on *Escherichia coli* and human ovarian carcinoma cells have been observed *in vitro* with environmentally realistic concentrations (Pomati *et al.*, 2008). Thus, more information is necessary to better predict the effect of chronic exposure to complex mixtures such as surface waters near WWTP outfalls.

2.4.2.b. Occurrence of anti-infectives in low to middle-income countries

Our bibliographic research showed an important gap in the present knowledge of anti-infective contamination on a global scale with regard to low- to middle-income countries. From the total 159 papers identified, 84% analyzed environmental waters in high-income countries; the remaining 16% are from four Asian countries (China, India, Malaysia and Vietnam), two European countries (Croatia and Poland) and one Latin American country (Mexico). We did not find any data in the peer-reviewed literature concerning the environmental occurrence of anti-infectives in Africa or in other Asian or Latin American countries. In 1999, low- and middle-income countries consumed less than 10 % (by value) of the world's medicines (World Health Organization, 2004). Nevertheless, the high rates of over-the-counter self-medication (Kunin, 1993; Kamat et Nichter, 1998) and wide availability of inexpensive anti-infectives combined with a lower access to public sewage networks could result in environmental waters containing significantly higher amounts of anti-infectives in these countries than in high-income countries. Therefore, more research focusing on the occurrence of these substances in low and middle-income countries is necessary to properly evaluate the state and impact of global contamination of waters. In addition, discharge from anti-infective manufacturing in low- to middle-income countries needs to be studied, as their input could actually be the most important point source in the local environment of some regions (Larsson *et al.*, 2007). If anti-infective occurrence in environmental waters does promote resistance in bacteria, the contamination caused by anti-infectives should be approached from a global perspective, as people and products affected by these waters can contribute to the spread of anti-infective resistance to other parts of the world (Okeke et Edelman, 2001; O'Brien, 2002).

2.5. Conclusion

Anti-infectives, the miracle drugs of the 20th century, have become environmental contaminants of emerging concerns in 21st century. Research has shown these compounds are persistent and mobile enough to be transported from landfills, agricultural fields, and urban centers to natural waters. To gain a better insight of the global contamination caused by anti-infectives in environmental waters, we created a database with more than 2200

concentration values of 68 parent drugs and 10 metabolites or degradation products reported in 143 peer-reviewed papers. Statistical analysis of concentrations of three classes of anti-infectives (macrolides, quinolones and sulfonamides) and trimethoprim in urban wastewaters in three geographic areas (East Asia, Europe and North America) confirmed significantly higher concentrations in raw wastewaters compared with treated wastewaters. Also, concentrations measured in Europe were significantly lower than those in North America and East Asia.

Furthermore, resulting concentrations of sulfonamides and trimethoprim are significantly higher than those of macrolides and quinolones. These results confirm previous observations about the factors influencing the removal of anti-infectives. Comparison between sulfamethoxazole and ofloxacin occurrence in natural and wastewater and their effective concentrations in aquatic biota showed that there is a weak overlapping of the distribution curves and that only highly contaminated waters could affect the most sensitive species. However, potential effects on aquatic microbiota can not be ruled out for the following reasons:

- Effects of chronic exposure of sensitive organisms such as bacteria or algae to sub-inhibitory concentrations of anti-infectives over long periods of time are still unknown.
- Even if highly contaminated wastewaters are rather rare with respect to anti-infectives, heavily impacted industrial or agricultural wastewaters could become a non-negligible environmental reservoir of anti-infective resistant bacteria, given that they have all the necessary elements of an ideal setting for the assembly and exchange of mobile genetic agents encoding for resistance
- Current knowledge on the global occurrence of anti-infectives in environmental waters is far from complete. More research is necessary, especially for low to middle-income countries, which may be more impacted by anti-infective contamination than high-income countries because of less extended public sewage infrastructures, higher rates of self-prescription and often less-strict industrial emissions legislations.
- The effects of cumulative and synergistic effects of anti-infectives in complex mixtures such as wastewaters are yet to be unraveled.

The current tendency towards larger and more densely populated production facilities such as concentrated animal feeding operations (CAFOs) suggests that occurrence of anti-infective in agricultural wastewaters may increase in the near future (Lee *et al.*, 2007b). Also, water-saving policies in urban settings would result in a reduction of wastewater volumes and consequently, in the increase of anti-infective levels because of lower dilution (Kümmerer, 2004). Many measures to avoid the presence of pharmaceuticals in the environment have been proposed so far. Two main approaches in urban settings can be distinguished: source control and improvement of wastewater technologies. Source control solutions look for the reduction of pharmaceutical inputs before they reach public sewer systems, at the consumer level (e.g., environmental labeling to inform patients and physicians) or at the waste management level [e.g., urine separation (Larsen *et al.*, 2004)], as well as pretreatment of hospital sewage (Ternes *et al.*, 2004b). Improvement of sewage treatment processes to increase removal efficacy of WWTPs includes optimization of current technology and the implementation of more advanced treatment techniques such as ozonation, advanced oxidation processes, membrane filtration, and activated carbon (Ternes *et al.*, 2003; Ternes et Joss, 2006). With regard to anti-infectives of agricultural origin, better farming practices have been proposed such as erosion control to reduce runoff (Davis *et al.*, 2006), increasing the maturation time of manure before application to enhance degradation (De Liguoro *et al.*, 2003) and the use of filters to reduce discharges by aquaculture operations (Smith *et al.*, 1994). All of these measures should contribute to the reduction of urban and agricultural inputs of anti-infectives in the aquatic environment.

Supplementary material

Tableau 2-2. Occurrence of anti-infectives in nanograms-per-liter in contaminated wastewaters, and natural and drinking waters.

| Compound | Matrix | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|--------------------------------------|----------------|-------|--------|-------|--------|--------|---------------------------------|----------------------------------|----------------|----------------|--------|-----|-----|-----|---------------------------------|----------------------------------|----------------|------|--------|-----|-----|-----|---------------------------------|----------------------------------|------|------|------|------|------|
| | Wastewaters | | | | | | | | | Natural waters | | | | | | Drinking water | | | | | | | | | | | | | |
| | N ^a | Mean | Median | 75% | 95% | Max | LOQ _{low} ^b | LOQ _{high} ^c | N ^a | Mean | Median | 75% | 95% | Max | LOQ _{low} ^b | LOQ _{high} ^c | N ^a | Mean | Median | 75% | 95% | Max | LOQ _{low} ^b | LOQ _{high} ^c | | | | | |
| Azoles | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Clotrimazole | 13 | 21 | 23 | 28 | 33 | 33 | 5 | 5 | 35 | 15 | 17 | 22 | 30 | 34 | 1 | 370 | 0 | | | | | | | 370 | 370 | | | | |
| Metronidazole | 4 | 25375 | 5600 | 49800 | 90200 | 90200 | 26 | 112 | 0 | | | | | | 2 | 9 | 0 | | | | | | | N.A. | N.A. | | | | |
| Miconazole | 0 | | | | | | 18 | 18 | 0 | | | | | | 18 | 18 | 0 | | | | | | | | N.A. | N.A. | | | |
| Ornidazole | 0 | | | | | | N.A. | N.A. | 4 | 36 | 38 | 56 | 58 | 58 | 10 | 10 | 0 | | | | | | | | N.A. | N.A. | | | |
| Ronidazole | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 11 | 11 | 0 | | | | | | | | N.A. | N.A. | | | |
| Tiabendazole | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 11 | 11 | 0 | | | | | | | | N.A. | N.A. | | | |
| β-Lactams | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Amoxicillin | 13 | 99 | 25 | 200 | 279 | 280 | 1.8 | 74 | 0 | | | | | | 5 | 20 | 0 | | | | | | | | N.A. | N.A. | | | |
| Ampicillin | 0 | | | | | | 13 | 66 | 1 | 11 | 11 | NC | NC | 11 | 5 | 10 | 0 | | | | | | | | | N.A. | N.A. | | |
| Benzylpenicillin ^d | 5 | 32152 | 1680 | 42150 | 153000 | 153000 | 4 | 100000 | 1 | 350 | 350 | NC | NC | 350 | 2 | 33 | 0 | | | | | | | | | N.A. | N.A. | | |
| Cefaclor | 4 | 740 | 740 | 890 | 980 | 980 | 59 | 59 | 0 | | | | | | 16000 | 16000 | 0 | | | | | | | | | N.A. | N.A. | | |
| Cefadroxil | 0 | | | | | | 77 | 84 | 0 | | | | | | 24000 | 24000 | 0 | | | | | | | | | | N.A. | N.A. | |
| Cefalexin | 13 | 2143 | 1800 | 3750 | 5450 | 5600 | 26 | 37 | 0 | | | | | | N.A. | N.A. | 0 | | | | | | | | | N.A. | N.A. | | |
| Cefapirin | 0 | | | | | | 15 | 18 | 1 | 9 | 9 | NC | NC | 9 | 8 | 8 | 0 | | | | | | | | | | N.A. | N.A. | |
| Cefazolin | 0 | | | | | | 11 | 14 | 0 | | | | | | N.A. | N.A. | 0 | | | | | | | | | | N.A. | N.A. | |
| Cefdinir | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 18000 | 18000 | 0 | | | | | | | | | | N.A. | N.A. | |
| Cefotaxime | 3 | 386 | 34 | 834 | 1100 | 1100 | 12 | 15 | 0 | | | | | | N.A. | N.A. | 0 | | | | | | | | | | N.A. | N.A. | |
| Cefprozil | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 12000 | 12000 | 0 | | | | | | | | | | | N.A. | N.A. |
| Ceftazidime | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 10 | 10 | 0 | | | | | | | | | | | N.A. | N.A. |
| Ceftiofur | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 8700 | 8700 | 0 | | | | | | | | | | | N.A. | N.A. |
| Ceftriaxone | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 4 | 4 | 0 | | | | | | | | | | | N.A. | N.A. |
| Cefuroxime | 0 | | | | | | 413 | 413 | 0 | | | | | | 19200 | 19200 | 0 | | | | | | | | | | | N.A. | N.A. |
| Cloxacillin | 2 | 168 | 168 | 320 | 320 | 320 | 1 | 20 | 0 | | | | | | 5 | 20 | 0 | | | | | | | | | | N.A. | N.A. | |
| Dicloxacillin | 0 | | | | | | 20 | 25 | 0 | | | | | | 5 | 25 | 0 | | | | | | | | | | | N.A. | N.A. |
| Flucloxacillin | 0 | | | | | | N.A. | N.A. | 1 | 7 | 7 | NC | NC | 7 | 5 | 5 | 0 | | | | | | | | | | | N.A. | N.A. |
| Meticillin | 0 | | | | | | 20 | 25 | 0 | | | | | | 5 | 50 | 0 | | | | | | | | | | | N.A. | N.A. |
| Mezlocillin | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 5 | 5 | 0 | | | | | | | | | | | N.A. | N.A. |
| Nafcillin | 0 | | | | | | 20 | 25 | 0 | | | | | | 15 | 25 | 0 | | | | | | | | | | | N.A. | N.A. |
| Oxacillin | 0 | | | | | | 8 | 25 | 1 | 10 | 10 | NC | NC | 10 | 5 | 25 | 0 | | | | | | | | | | | N.A. | N.A. |
| Phenoxymethylpenicillin ^e | 2 | 120 | 120 | 160 | 160 | 160 | 5 | 82 | 0 | | | | | | 2 | 33 | 0 | | | | | | | | | | | N.A. | N.A. |
| Piperacillin | 0 | | | | | | N.A. | N.A. | 1 | 48 | 48 | NC | NC | 48 | 2 | 5 | 0 | | | | | | | | | | | N.A. | N.A. |

| Compound | Matrix | | | | | | | | | | | | | | | | | | | | | | | | |
|---|----------------|-----------|-----------|-----------|-----------|-----------|---------------------------------|----------------------------------|----------------|---------|---------|----------|----------|----------|---------------------------------|----------------------------------|----------------|------|--------|-----|-----|-----|---------------------------------|----------------------------------|------|
| | Wastewaters | | | | | | | Natural waters | | | | | | | Drinking water | | | | | | | | | | |
| | N ^a | Mean | Median | 75% | 95% | Max | LOQ _{low} ^b | LOQ _{high} ^c | N ^a | Mean | Median | 75% | 95% | Max | LOQ _{low} ^b | LOQ _{high} ^c | N ^a | Mean | Median | 75% | 95% | Max | LOQ _{low} ^b | LOQ _{high} ^c | |
| <i>metabolites / degradation products</i> | | | | | | | | | | | | | | | | | | | | | | | | | |
| Iso-penicillic acid | 2 | 598000 | 598000 | 1050000 | 1050000 | 1050000 | 960 | 960 | 2 | 599000 | 599000 | 935000 | 935000 | 935000 | 960 | 960 | 0 | | | | | | | N.A. | N.A. |
| Penicilloaldehyde | 2 | 41035000 | 41035000 | 75300000 | 75300000 | 75300000 | 1360 | 1360 | 0 | | | | | | 1360 | 1360 | 0 | | | | | | | N.A. | N.A. |
| Penicilloic acid | 2 | 6095000 | 6095000 | 8490000 | 8490000 | 8490000 | 640 | 640 | 2 | 8840000 | 8840000 | 10540000 | 10540000 | 10540000 | 640 | 640 | 0 | | | | | | | N.A. | N.A. |
| Penillic acid | 2 | 14495000 | 14495000 | 23500000 | 23500000 | 23500000 | 410 | 410 | 0 | | | | | | 410 | 410 | 0 | | | | | | | N.A. | N.A. |
| Penilloic acid | 2 | 216750000 | 216750000 | 389000000 | 389000000 | 389000000 | 1050 | 1050 | 0 | | | | | | 1050 | 1050 | 0 | | | | | | | N.A. | N.A. |
| Quinoxaline-dioxides | | | | | | | | | | | | | | | | | | | | | | | | | |
| Carbadox | 0 | | | | | | 5 | 100 | 0 | | | | | | 35 | 1400 | 0 | | | | | | | 100 | 100 |
| Olaquinox | 0 | | | | | | 6 | 6 | 0 | | | | | | N.A. | N.A. | 0 | | | | | | | N.A. | N.A. |
| Lincosamides | | | | | | | | | | | | | | | | | | | | | | | | | |
| Clindamycin | 5 | 5 | 5 | 5 | 5 | 5 | 3 | 100 | 20 | 116 | 6 | 29 | 1050 | 1100 | 0.1 | 198 | 0 | | | | | | | N.A. | N.A. |
| Lincomycin | 28 | 22056 | 190 | 2500 | 213000 | 240000 | 0.3 | 50 | 26 | 171 | 33 | 249 | 864 | 1400 | 0.04 | 50 | 0 | | | | | | | 0.07 | 5 |
| Macrolides | | | | | | | | | | | | | | | | | | | | | | | | | |
| Azithromycin | 28 | 177 | 141 | 278 | 405 | 450 | 0.8 | 231 | 12 | 40 | 17 | 40 | 173 | 175 | 0.5 | 100 | 0 | | | | | | | N.A. | N.A. |
| Clarithromycin | 60 | 334 | 215 | 420 | 1245 | 2000 | 0.2 | 500 | 20 | 44 | 14 | 43 | 225 | 260 | 0.02 | 30 | 0 | | | | | | | N.A. | N.A. |
| Erythromycin | 60 | 704 | 149 | 280 | 1200 | 27000 | 0.4 | 500 | 45 | 85 | 17 | 45 | 715 | 1022 | 0.1 | 220 | 1 | 5 | 5 | NC | NC | 5 | 0.1 | 220 | |
| Josamycin | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 0.07 | 0.07 | 0 | | | | | | | N.A. | N.A. |
| Oleandomycin | 2 | 18 | 18 | 30 | 30 | 30 | 0.3 | 3 | 9 | 1 | 0.4 | 3 | 5 | 5 | 0.04 | 8 | 0 | | | | | | | 0.07 | 0.07 |
| Roxithromycin | 48 | 143 | 37 | 110 | 829 | 1000 | 0.3 | 650 | 31 | 59 | 30 | 65 | 190 | 560 | 0.04 | 650 | 1 | 1 | 1 | NC | NC | 1 | 1 | 1 | |
| Spiramycin | 9 | 69 | 75 | 101 | 161 | 161 | 1 | 70 | 8 | 42 | 44 | 71 | 74 | 74 | 0.05 | 35 | 0 | | | | | | | 2 | 2 |
| Tilmicosin | 0 | | | | | | 0.7 | 0.7 | 1 | 0.4 | 0.4 | NC | NC | 0.4 | 0.1 | 2 | 0 | | | | | | | 2 | 2 |
| Tylosin | 10 | 168 | 58 | 65 | 1150 | 1150 | 0.8 | 1155 | 12 | 55 | 26 | 70 | 265 | 280 | 0.04 | 1155 | 2 | 3 | 3 | 4 | 4 | 4 | 0.8 | 1 | |
| <i>metabolites / degradation products</i> | | | | | | | | | | | | | | | | | | | | | | | | | |
| Erythromycin-H ₂ O | 52 | 2848 | 450 | 1414 | 5650 | 83000 | 1 | 500 | 51 | 184 | 40 | 146 | 1186 | 1700 | 0.3 | 250 | 0 | | | | | | | 10 | 10 |
| Polyether ionophores | | | | | | | | | | | | | | | | | | | | | | | | | |
| Monensin A | 12 | 29 | 10.5 | 27 | 175 | 190 | 1 | 3 | 3 | 403 | 30 | 887 | 1172 | 1172 | 0.1 | 380 | 0 | | | | | | | N.A. | N.A. |
| Monensin B | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 50 | 50 | 0 | | | | | | | N.A. | N.A. |
| Narasin A | 0 | | | | | | N.A. | N.A. | 1 | 40 | 40 | NC | NC | 40 | 40 | 40 | 0 | | | | | | | N.A. | N.A. |
| Salinomycin | 1 | 25 | 25 | NC | NC | 25 | 3 | 3 | 0 | | | | | | 40 | 40 | 0 | | | | | | | N.A. | N.A. |
| Quinolones | | | | | | | | | | | | | | | | | | | | | | | | | |
| Cinoxacin | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 33 | 33 | 0 | | | | | | | N.A. | N.A. |
| Ciprofloxacin | 156 | 382207 | 251 | 595 | 35630 | 31000000 | 1 | 3630 | 22 | 470 | 180 | 360 | 2822 | 5600 | 0.3 | 2000 | 0 | | | | | | | 1 | 3 |
| Clinafloxacin | 0 | | | | | | 5 | 5 | 0 | | | | | | N.A. | N.A. | 0 | | | | | | | N.A. | N.A. |
| Danofloxacin | 0 | | | | | | 15 | 1650 | 0 | | | | | | 5 | 40 | 0 | | | | | | | N.A. | N.A. |
| Difloxacin | 0 | | | | | | 30 | 130 | 0 | | | | | | 9 | 130 | 0 | | | | | | | N.A. | N.A. |
| Enoxacin | 2 | 225000 | 225000 | 300000 | 300000 | 300000 | 36 | 3630 | 1 | 11 | 11 | NC | NC | 11 | 10 | 43 | 0 | | | | | | | N.A. | N.A. |
| Enrofloxacin | 14 | 126149 | 60 | 23700 | 876000 | 900000 | 3 | 20600 | 2 | 10 | 10 | NC | NC | 10 | 3 | 200 | 0 | | | | | | | 5 | 5 |

| Compound | Matrix | | | | | | | | | | | | | | | | | | | | | | | | |
|---------------------------------|----------------|---------|---------|---------|---------|---------|---------------------------------|----------------------------------|----------------|--------|--------|--------|---------|---------|---------------------------------|----------------------------------|----------------|------|--------|-----|-----|-----|---------------------------------|----------------------------------|------|
| | Wastewaters | | | | | | | Natural waters | | | | | | | Drinking water | | | | | | | | | | |
| | N ^a | Mean | Median | 75% | 95% | Max | LOQ _{low} ^b | LOQ _{high} ^c | N ^a | Mean | Median | 75% | 95% | Max | LOQ _{low} ^b | LOQ _{high} ^c | N ^a | Mean | Median | 75% | 95% | Max | LOQ _{low} ^b | LOQ _{high} ^c | |
| Fleroxacin | 2 | 20 | 20 | 20 | 20 | 20 | 1 | 90 | 0 | | | | | | 1 | 9 | 0 | | | | | | | N.A. | N.A. |
| Flumequine | 11 | 18779 | 14880 | 24138 | 49586 | 49820 | 5 | 2310 | 10 | 20 | 16 | 29 | 32 | 32 | 2 | 43 | 2 | 2 | 2 | 3 | 3 | 3 | 2 | 2 | |
| Gatifloxacin | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 7000 | 7000 | 0 | | | | | | | N.A. | N.A. |
| Lomefloxacin | 2 | 225000 | 225000 | 300000 | 300000 | 300000 | 2 | 3300 | 0 | | | | | | 2 | 6000 | 0 | | | | | | | N.A. | N.A. |
| Moxifloxacin | 2 | 1273 | 1273 | 2175 | 2175 | 2175 | 0.6 | 0.6 | 1 | 17 | 17 | NC | NC | 17 | 0.6 | 2 | 0 | | | | | | | N.A. | N.A. |
| Nalidixic acid | 4 | 94 | 65 | 138 | 200 | 200 | 3 | 4620 | 0 | | | | | | 10 | 66 | 0 | | | | | | | N.A. | N.A. |
| Norfloxacin | 115 | 7911 | 76 | 253 | 7150 | 420000 | 3 | 8000 | 21 | 58 | 25 | 95 | 203 | 251 | 2 | 2700 | 0 | | | | | | | 4 | 6 |
| Ofloxacin/Levofloxacin | 109 | 4807 | 288 | 739 | 25775 | 160000 | 1 | 8000 | 17 | 39 | 20 | 62 | 109 | 109 | 1 | 4400 | 2 | 1 | 1 | 2 | 2 | 2 | 0.3 | 0.3 | |
| Oxolinic acid | 4 | 1207500 | 1160000 | 2405000 | 2500000 | 2500000 | 5 | 2640 | 2 | 16 | 16 | 19 | 19 | 19 | 4 | 33 | 1 | 4 | 4 | NC | NC | 4 | 4 | 4 | |
| Pipemidic acid | 0 | | | | | | 7 | 155 | 0 | | | | | | 10 | 155 | 0 | | | | | | | 10 | 10 |
| Sarafloxacin | 3 | 3333 | 3000 | 3750 | 4000 | 4000 | 5 | 220 | 2 | 1505 | 1505 | 3000 | 3000 | 3000 | 2 | 220 | 0 | | | | | | | 2 | 2 |
| Sparfloxacin | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 5700 | 5700 | 0 | | | | | | | N.A. | N.A. |
| Tosufloxacin | 0 | | | | | | 245 | 245 | 0 | | | | | | 245 | 245 | 0 | | | | | | | N.A. | N.A. |
| Sulfonamides | | | | | | | | | | | | | | | | | | | | | | | | | |
| Sulfacetamide | 3 | 77 | 64 | 129 | 151 | 151 | 1 | 13200 | 0 | | | | | | 0.5 | 9600 | 0 | | | | | | | 1 | 1 |
| Sulfabenzamide | 0 | | | | | | 1 | 1 | 0 | | | | | | | | 0 | | | | | | | N.A. | N.A. |
| Sulfachlorpyridazine | 3 | 2697 | 1560 | 5288 | 6530 | 6530 | 1 | 165 | 5 | 17 | 20 | 30 | 30 | 30 | 0.5 | 825 | 0 | | | | | | | 7 | 7 |
| Sulfadiazine | 10 | 30966 | 4640 | 76000 | 111400 | 111400 | 1 | 100000 | 13 | 310825 | 170000 | 480000 | 1094000 | 1160000 | 0.3 | 33000 | 0 | | | | | | | 0.3 | 1155 |
| Sulfadimethoxine | 14 | 2206 | 2500 | 2500 | 10284 | 12230 | 1 | 2800 | 10 | 1553 | 53 | 60 | 15000 | 15000 | 0.2 | 2800 | 0 | | | | | | | 4 | 4 |
| Sulfadimidine ^f | 31 | 45154 | 363 | 3633 | 369000 | 400000 | 1 | 49500 | 35 | 114193 | 215 | 87500 | 835000 | 900000 | 0.3 | 33000 | 0 | | | | | | | 0.8 | 495 |
| Sulfadoxine | 0 | | | | | | 1 | 1 | 0 | | | | | | 1 | 1 | 0 | | | | | | | N.A. | N.A. |
| Sulfaguandine | 1 | 211100 | 211100 | NC | NC | 211100 | 5 | 300000 | 10 | 473000 | 270000 | 540000 | 1600000 | 1600000 | 33000 | 33000 | 0 | | | | | | | N.A. | N.A. |
| Sulfamerazine | 4 | 20505 | 2490 | 40225 | 77000 | 77000 | 1 | 1700 | 3 | 87 | 60 | 158 | 190 | 190 | 0.3 | 1700 | 0 | | | | | | | 6 | 6 |
| Sulfamethizole ^e | 0 | | | | | | 1 | 600 | 11 | 136387 | 70000 | 205000 | 329000 | 330000 | 1 | 33000 | 0 | | | | | | | 5 | 5 |
| Sulfamethoxazole | 177 | 1301 | 352 | 643 | 4167 | 69000 | 1 | 2500 | 115 | 285 | 72 | 298 | 1080 | 6000 | 0.3 | 14000 | 2 | 0.4 | 0.4 | 1 | 1 | 1 | 0.2 | 495 | |
| Sulfamethoxypridazine | 2 | 5 | 5 | 9 | 9 | 9 | 1 | 2800 | 0 | | | | | | 1 | 2800 | 0 | | | | | | | N.A. | N.A. |
| Sulfametoxydiazine ^h | 1 | 4 | 4 | NC | NC | 4 | 1 | 6 | 0 | | | | | | 1 | 1 | 0 | | | | | | | N.A. | N.A. |
| Sulfamonomethoxine | 2 | 4110 | 4110 | 5110 | 5110 | 5110 | 1 | 49500 | 0 | | | | | | 1 | 110 | 0 | | | | | | | N.A. | N.A. |
| Sulfamoxole | 0 | | | | | | 1 | 1 | 0 | | | | | | N.A. | N.A. | 0 | | | | | | | N.A. | N.A. |
| Sulfanilamide | 0 | | | | | | 5 | 10200 | 9 | 138889 | 140000 | 180000 | 300000 | 300000 | 10200 | 33000 | 0 | | | | | | | N.A. | N.A. |
| Sulfapyridine | 17 | 123 | 85 | 158 | 308 | 350 | 1 | 528 | 8 | 19 | 15 | 30 | 39 | 39 | 1 | 528 | 0 | | | | | | | 0.5 | 528 |
| Sulfaquinoxaline | 0 | | | | | | 1 | 2200 | 0 | | | | | | 2200 | 2200 | 0 | | | | | | | N.A. | N.A. |
| Sulfasalazine | 13 | 272 | 290 | 400 | 437 | 438 | 10 | 64 | 11 | 158 | 120 | 155 | 757 | 780 | 5 | 5 | 0 | | | | | | | N.A. | N.A. |
| Sulfathiazole | 7 | 167683 | 180 | 8995 | 1158680 | 1158680 | 3 | 33000 | 4 | 14 | 13 | 23 | 30 | 30 | 1 | 3600 | 0 | | | | | | | 8 | 858 |
| Sulfisomidine | 0 | | | | | | 3 | 8800 | 0 | | | | | | 8800 | 8800 | 0 | | | | | | | N.A. | N.A. |

| Compound | Matrix | | | | | | | | | | | | | | | | | | | | | | | |
|-------------------------------|---|----------|---------|----------|----------|-----------|---------------------------------|----------------------------------|----------------|--------|--------|--------|--------|--------|---------------------------------|----------------------------------|----------------|------|--------|-----|-----|-----|---------------------------------|----------------------------------|
| | Wastewaters | | | | | | | Natural waters | | | | | | | Drinking water | | | | | | | | | |
| | N ^a | Mean | Median | 75% | 95% | Max | LOQ _{low} ^b | LOQ _{high} ^c | N ^a | Mean | Median | 75% | 95% | Max | LOQ _{low} ^b | LOQ _{high} ^c | N ^a | Mean | Median | 75% | 95% | Max | LOQ _{low} ^b | LOQ _{high} ^c |
| Sulfisoxazole | 4 | 22 | 21 | 28 | 34 | 34 | 1 | 3600 | 0 | | | | | | 0.4 | 3600 | 0 | | | | | | 0.4 | 0.4 |
| | <i>metabolites / degradation products</i> | | | | | | | | | | | | | | | | | | | | | | | |
| Acetyl-Sulfadiazine | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 17 | 17 | 0 | | | | | | N.A. | N.A. |
| Acetyl-Sulfadimethoxine | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 17 | 17 | 0 | | | | | | N.A. | N.A. |
| Acetyl-Sulfamethazine | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 17 | 17 | 0 | | | | | | N.A. | N.A. |
| Acetyl-Sulfamethoxazole | 13 | 779 | 570 | 1200 | 2230 | 2235 | 20 | 212 | 6 | 86 | 14 | 239 | 240 | 240 | 2 | 50 | 0 | | | | | | N.A. | N.A. |
| Acetyl-Sulfathiazole | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 17 | 17 | 0 | | | | | | N.A. | N.A. |
| | Tetracyclines | | | | | | | | | | | | | | | | | | | | | | | |
| Chlortetracycline | 24 | 703804 | 80450 | 230000 | 4300000 | 12000000 | 4.0 | 500 | 8 | 225 | 171 | 315 | 690 | 690 | 7 | 1650 | 0 | | | | | | 25 | 25 |
| Demeclocycline | 7 | 1215 | 1140 | 2310 | 3150 | 3150 | 10 | 165 | 4 | 208 | 185 | 380 | 440 | 440 | 12 | 165 | 0 | | | | | | 12 | 12 |
| Doxycycline | 33 | 531 | 83 | 353 | 2440 | 6700 | 2 | 165 | 4 | 34 | 30 | 62 | 73 | 73 | 1 | 165 | 0 | | | | | | 4 | 4 |
| Meclocycline | 4 | 530 | 435 | 785 | 1070 | 1070 | 23 | 90 | 2 | 55 | 55 | 100 | 100 | 100 | 5 | 500 | 0 | | | | | | N.A. | N.A. |
| Minocycline | 2 | 4640 | 4640 | 8900 | 8900 | 8900 | 10 | 165 | 0 | | | | | | 5 | 165 | 0 | | | | | | 5 | 5 |
| Oxytetracycline | 29 | 34693400 | 1100000 | 2750000 | 64525000 | 920000000 | 1 | 100000 | 19 | 240611 | 1340 | 513250 | 701650 | 712000 | 0.07 | 1155 | 0 | | | | | | 4 | 4 |
| Tetracycline | 62 | 13731500 | 370 | 850 | 11180 | 850000000 | 2 | 700000 | 10 | 186 | 110 | 300 | 560 | 560 | 3 | 250 | 0 | | | | | | 3 | 3 |
| | <i>metabolites / degradation products</i> | | | | | | | | | | | | | | | | | | | | | | | |
| α-apo-oxytetracycline | 2 | 4765000 | 4765000 | 9420000 | 9420000 | 9420000 | 1300 | 1300 | 9 | 8986 | 9570 | 11275 | 12900 | 12900 | 1300 | 1300 | 0 | | | | | | N.A. | N.A. |
| β-apo-oxytetracycline | 2 | 1061500 | 1061500 | 2090000 | 2090000 | 2090000 | 600 | 600 | 9 | 6339 | 4740 | 8915 | 15100 | 15100 | 600 | 600 | 0 | | | | | | N.A. | N.A. |
| Doxycycline-H ₂ O | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 100 | 100 | 0 | | | | | | N.A. | N.A. |
| Epi-oxytetracycline | 2 | 9450000 | 9450000 | 18100000 | 18100000 | 18100000 | 1200 | 1200 | 9 | 21778 | 22800 | 28925 | 34200 | 34200 | 1200 | 1200 | 0 | | | | | | N.A. | N.A. |
| Iso-chlortetracycline | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 100 | 100 | 0 | | | | | | N.A. | N.A. |
| Iso-epi-chlortetracycline | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 100 | 100 | 0 | | | | | | N.A. | N.A. |
| Tetracycline-H ₂ O | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 10 | 10 | 0 | | | | | | N.A. | N.A. |
| | Other | | | | | | | | | | | | | | | | | | | | | | | |
| Amprolium | 9 | 59 | 24 | 53 | 288 | 288 | 8 | 8 | 0 | | | | | | N.A. | N.A. | 0 | | | | | | N.A. | N.A. |
| Bacitracin | 0 | | | | | | 40 | 40 | 0 | | | | | | N.A. | N.A. | 0 | | | | | | N.A. | N.A. |
| Chloramphenicol | 6 | 1038 | 1010 | 1730 | 2430 | 2430 | 5 | 667 | 5 | 136 | 127 | 207 | 266 | 266 | 2 | 80 | 0 | | | | | | 5 | 5 |
| Dapsone | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 7 | 7 | 0 | | | | | | N.A. | N.A. |
| Furazolidone | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 4 | 4 | 0 | | | | | | N.A. | N.A. |
| Gentamicin | 2 | 3000 | 3000 | 5000 | 5000 | 5000 | 200 | 200 | 0 | | | | | | N.A. | N.A. | 0 | | | | | | N.A. | N.A. |
| Novobiocin | 0 | | | | | | 3 | 3 | 0 | | | | | | 3 | 3 | 0 | | | | | | N.A. | N.A. |
| Ormetoprim | 0 | | | | | | 5 | 5 | 0 | | | | | | 5 | 5 | 0 | | | | | | N.A. | N.A. |
| Roxarsone | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 2000 | 2000 | 0 | | | | | | N.A. | N.A. |
| Trimethoprim | 210 | 1351 | 270 | 795 | 5000 | 55200 | 0.9 | 150000 | 90 | 94 | 18 | 42 | 510 | 3000 | 0.2 | 4000 | 0 | | | | | | 0.5 | 250 |
| Vancomycin | 0 | | | | | | N.A. | N.A. | 0 | | | | | | 30.0 | 30 | 0 | | | | | | N.A. | N.A. |

| Compound | Matrix | | | | | | | | | | | | | | | | | | | | | | | | | |
|---------------|----------------|------|--------|-----|-----|-----|---------------------------------|----------------------------------|----------------|----------------|--------|-----|-----|-----|---------------------------------|----------------------------------|----------------|------|--------|-----|-----|-----|---------------------------------|----------------------------------|------|------|
| | Wastewaters | | | | | | | | | Natural waters | | | | | | Drinking water | | | | | | | | | | |
| | N ^a | Mean | Median | 75% | 95% | Max | LOQ _{low} ^b | LOQ _{high} ^c | N ^a | Mean | Median | 75% | 95% | Max | LOQ _{low} ^b | LOQ _{high} ^c | N ^a | Mean | Median | 75% | 95% | Max | LOQ _{low} ^b | LOQ _{high} ^c | | |
| Virginiamycin | 0 | | | | | | 5 | 12 | 0 | | | | | | 5 | 170 | 0 | | | | | | | | N.A. | N.A. |

^a Number of values reported being > LOQ for each compound. ^b Lowest LOQ reported. ^c Highest LOQ reported. ^d Also known as penicillin G. ^e Also known as penicillin V. ^f Also known as sulfamethazine. ^g Also known as sulfamethiazole. ^h Also known as sulfameter. NA : not available. NC : Not calculated.

Tableau 2-3. Effective concentrations of sulfamethoxazole on several aquatic species

| Category | Name | Test type | Effect | Author and publication year | Value (ng L ⁻¹) |
|------------------|--------------------------------|-------------------|----------------------------|---------------------------------|--------------------------------|
| LOEC | <i>L. gibba</i> | LOEC | Frond number | (Brain <i>et al.</i> , 2004) | 30000 |
| LOEC | <i>L. gibba</i> | LOEC | Chlorophyll a | (Brain <i>et al.</i> , 2004) | 100000 |
| LOEC | <i>L. gibba</i> | LOEC | Chlorophyll b | (Brain <i>et al.</i> , 2004) | 100000 |
| LOEC | <i>L. gibba</i> | LOEC | Wet weight | (Brain <i>et al.</i> , 2004) | 100000 |
| LOEC | <i>L. gibba</i> | LOEC | Carotenoids | (Brain <i>et al.</i> , 2004) | 300000 |
| LOEC | <i>S. typhimurium TA98</i> | AMES | Mutagenicity | (Isidori <i>et al.</i> , 2005) | 6250000 |
| LOEC | <i>S. typhimurium TA100</i> | AMES | Mutagenicity | (Isidori <i>et al.</i> , 2005) | 50000000 |
| LOEC | <i>S. typhimurium TA98</i> | AMES | Mutagenicity | (Isidori <i>et al.</i> , 2005) | 100000000 |
| LOEC | <i>S. typhimurium TA100</i> | AMES | Mutagenicity | (Isidori <i>et al.</i> , 2005) | 100000000 |
| LOEC | <i>H. attenuata</i> | LOEC | Morphology | (Quinn <i>et al.</i> , 2008) | 10000000 |
| LOEC | <i>B. stearrowthermophilus</i> | LOEC | Color change | (Smith <i>et al.</i> , 2007) | 132500 |
| EC ₅₀ | Pathogens | MIC ₅₀ | Population Inhibition | (Al-Ahmad <i>et al.</i> , 1999) | 2000 |
| EC ₅₀ | <i>Ps. Putida</i> | IC ₅₀ | Growth inhibitory activity | (Al-Ahmad <i>et al.</i> , 1999) | 256000 |
| EC ₅₀ | Pathogens | MIC ₅₀ | Population Inhibition | (Al-Ahmad <i>et al.</i> , 1999) | 256000 |
| EC ₅₀ | <i>C. vulgaris</i> | EC ₅₀ | Growth inhibition | (Baran <i>et al.</i> , 2006) | 15689230 |
| EC ₅₀ | <i>P. subcapitata</i> | EC ₅₀ | Growth inhibition | (Blaise <i>et al.</i> , 2006) | 13400000 |
| EC ₅₀ | <i>H. attenuata</i> | EC ₅₀ | Morphology perturbation | (Blaise <i>et al.</i> , 2006) | 19300000 |
| EC ₅₀ | <i>V. fischeri</i> | EC ₅₀ | Luminescence inhibition | (Blaise <i>et al.</i> , 2006) | 65300000 |
| EC ₅₀ | <i>L. gibba</i> | EC ₅₀ | Wet weight | (Brain <i>et al.</i> , 2004) | 81000 |
| EC ₅₀ | <i>L. gibba</i> | EC ₅₀ | Frond number | (Brain <i>et al.</i> , 2004) | 249000 |
| EC ₅₀ | <i>L. gibba</i> | EC ₅₀ | Chlorophyll b | (Brain <i>et al.</i> , 2004) | 682000 |
| EC ₅₀ | <i>L. gibba</i> | EC ₅₀ | Chlorophyll a | (Brain <i>et al.</i> , 2004) | 985000 |
| EC ₅₀ | <i>L. gibba</i> | EC ₅₀ | Carotenoids | (Brain <i>et al.</i> , 2004) | 4983000 |
| EC ₅₀ | <i>S. capricornutum</i> | EC ₅₀ | Growth inhibitory activity | (Eguchi <i>et al.</i> , 2004) | 1530000 |
| EC ₅₀ | <i>S. leopolensis</i> | EC ₅₀ | Growth | (Ferrari <i>et al.</i> , 2004) | 26800 |
| EC ₅₀ | <i>P. subcapitata</i> | EC ₅₀ | Growth | (Ferrari <i>et al.</i> , 2004) | 146000 |
| EC ₅₀ | <i>C. meneghiniana</i> | EC ₅₀ | Growth | (Ferrari <i>et al.</i> , 2004) | 2400000 |
| EC ₅₀ | <i>C. dubia</i> | EC ₅₀ | Chronic toxicity | (Isidori <i>et al.</i> , 2005) | 210000 |
| EC ₅₀ | <i>P. subcapitata</i> | EC ₅₀ | Chronic toxicity | (Isidori <i>et al.</i> , 2005) | 520000 |
| EC ₅₀ | <i>B. calyciflorus</i> | EC ₅₀ | Chronic toxicity | (Isidori <i>et al.</i> , 2005) | 9630000 |
| EC ₅₀ | <i>C. dubia</i> | EC ₅₀ | Acute toxicity | (Isidori <i>et al.</i> , 2005) | 15510000 |
| EC ₅₀ | <i>V. fischeri</i> | EC ₅₀ | Acute toxicity | (Isidori <i>et al.</i> , 2005) | 23300000 |
| EC ₅₀ | <i>D. magna</i> | EC ₅₀ | Acute toxicity | (Isidori <i>et al.</i> , 2005) | 25200000 |
| EC ₅₀ | <i>B. calyciflorus</i> | EC ₅₀ | Acute toxicity | (Isidori <i>et al.</i> , 2005) | 26270000 |
| EC ₅₀ | <i>T. platyurus</i> | EC ₅₀ | Acute toxicity | (Isidori <i>et al.</i> , 2005) | 35360000 |
| EC ₅₀ | <i>D. magna</i> | EC ₅₀ | Acute toxicity | (Jung <i>et al.</i> , 2008) | 177600000 |
| EC ₅₀ | <i>E. coli DH5a</i> | EC ₅₀ | Growth inhibition | (Wammer <i>et al.</i> , 2006) | 31125408 |
| EC ₅₀ | <i>B. fragilis</i> | MIC ₅₀ | Growth inhibition | (Wüst et Wilkins, 1978) | 2400000 |
| EC ₅₀ | Gram-positive bacteria | MIC ₅₀ | Growth inhibition | (Wüst et Wilkins, 1978) | 4000000 |
| EC ₅₀ | Fusobacterium | MIC ₅₀ | Growth inhibition | (Wüst et Wilkins, 1978) | 11000000 |
| EC ₅₀ | Peptococcus/Peptostreptococcus | MIC ₅₀ | Growth inhibition | (Wüst et Wilkins, 1978) | 32000000 |
| EC ₅₀ | <i>Clostridium</i> | MIC ₅₀ | Growth inhibition | (Wüst et Wilkins, 1978) | 32000000 |
| EC ₅₀ | <i>Bacteroides</i> | MIC ₅₀ | Growth inhibition | (Wüst et Wilkins, 1978) | 53000000 |

Tableau 2-4. Effective concentrations of ofloxacin/levofloxacin on several aquatic species

| Category | Name | Test type | Effect | Author and publication year | Value (ng L ⁻¹) |
|------------------|----------------------------|-------------------|----------------------------|----------------------------------|-----------------------------|
| LOEC | <i>V. fischeri</i> | EC ₀₁ | Bioluminescence inhibition | (Backhaus <i>et al.</i> , 2000) | 940 |
| LOEC | <i>L. gibba</i> | LOEC | Wet weight | (Brain <i>et al.</i> , 2004) | 300000 |
| LOEC | <i>L. gibba</i> | LOEC | Carotenoids | (Brain <i>et al.</i> , 2004) | 1000000 |
| LOEC | <i>L. gibba</i> | LOEC | Chlorophyll a | (Brain <i>et al.</i> , 2004) | 1000000 |
| LOEC | <i>L. gibba</i> | LOEC | Chlorophyll b | (Brain <i>et al.</i> , 2004) | 1000000 |
| LOEC | <i>L. gibba</i> | LOEC | Fronde number | (Brain <i>et al.</i> , 2004) | 1000000 |
| LOEC | <i>S. typhimurium TA98</i> | AMES | Mutagenicity | (Isidori <i>et al.</i> , 2005) | 312500 |
| LOEC | <i>S. typhimurium TA98</i> | AMES | Mutagenicity | (Isidori <i>et al.</i> , 2005) | 2500000 |
| LOEC | <i>E. coli PQ37</i> | SOS | Genotoxicity | (Isidori <i>et al.</i> , 2005) | 6250000 |
| LOEC | <i>E. coli PQ37</i> | SOS | Genotoxicity | (Isidori <i>et al.</i> , 2005) | 25000000 |
| LOEC | <i>E. coli PQ37</i> | SOS | Genotoxicity | (Kümmerer <i>et al.</i> , 2000) | 1000 |
| LOEC | <i>D. magna</i> | LOEC | Reproduction inhibition | (Yamashita <i>et al.</i> , 2006) | 63000 |
| LOEC | <i>P. subcapitata</i> | LOEC | Growth inhibition | (Yamashita <i>et al.</i> , 2006) | 630000 |
| EC ₅₀ | <i>V. fischeri</i> | EC ₅₀ | Bioluminescence inhibition | (Backhaus <i>et al.</i> , 2000) | 13590000 |
| EC ₅₀ | <i>L. gibba</i> | EC ₅₀ | Wet weight | (Brain <i>et al.</i> , 2004) | 532000 |
| EC ₅₀ | <i>L. gibba</i> | EC ₅₀ | Fronde number | (Brain <i>et al.</i> , 2004) | 653000 |
| EC ₅₀ | <i>L. gibba</i> | EC ₅₀ | Chlorophyll b | (Brain <i>et al.</i> , 2004) | 840000 |
| EC ₅₀ | <i>L. gibba</i> | EC ₅₀ | Chlorophyll a | (Brain <i>et al.</i> , 2004) | 989000 |
| EC ₅₀ | <i>L. gibba</i> | EC ₅₀ | Carotenoids | (Brain <i>et al.</i> , 2004) | 1374000 |
| EC ₅₀ | <i>Ps. putida</i> | EC ₅₀ | Growth | (Ferrari <i>et al.</i> , 2004) | 10000 |
| EC ₅₀ | <i>S. Leopoldensis</i> | EC ₅₀ | Growth | (Ferrari <i>et al.</i> , 2004) | 16000 |
| EC ₅₀ | <i>C. meneghiniana</i> | EC ₅₀ | Growth | (Ferrari <i>et al.</i> , 2004) | 90600 |
| EC ₅₀ | <i>P. subcapitata</i> | EC ₅₀ | Growth | (Ferrari <i>et al.</i> , 2004) | 4740000 |
| EC ₅₀ | <i>C. dubia</i> | EC ₅₀ | Mortality | (Ferrari <i>et al.</i> , 2004) | 26700000 |
| EC ₅₀ | <i>D. magna</i> | EC ₅₀ | Mortality | (Ferrari <i>et al.</i> , 2004) | 76580000 |
| EC ₅₀ | <i>E. coli</i> | MIC ₅₀ | Growth inhibition | (Fu <i>et al.</i> , 1992) | 120000 |
| EC ₅₀ | <i>S. aureus</i> | MIC ₅₀ | Growth inhibition | (Fu <i>et al.</i> , 1992) | 500000 |
| EC ₅₀ | <i>S. pneumonia</i> | MIC ₅₀ | Growth inhibition | (Fu <i>et al.</i> , 1992) | 2000000 |
| EC ₅₀ | <i>E. faecalis</i> | MIC ₅₀ | Growth inhibition | (Fu <i>et al.</i> , 1992) | 2000000 |
| EC ₅₀ | Peptostreptococcus | MIC ₅₀ | Growth inhibition | (Fu <i>et al.</i> , 1992) | 2000000 |
| EC ₅₀ | <i>B. fragilis</i> | MIC ₅₀ | Growth inhibition | (Fu <i>et al.</i> , 1992) | 4000000 |

| Category | Name | Test type | Effect | Author and publication year | Value (ng L ⁻¹) |
|------------------|------------------------|-------------------|-----------------------------|----------------------------------|-----------------------------|
| EC ₅₀ | <i>Bacteria</i> | EC ₅₀ | Anaerobic inhibition | (Gartiser <i>et al.</i> , 2007b) | 37400000 |
| EC ₅₀ | <i>B. calyciflorus</i> | EC ₅₀ | Population growth inhibiton | (Isidori <i>et al.</i> , 2005) | 530000 |
| EC ₅₀ | <i>P. subcapitata</i> | EC ₅₀ | Growth inhibition | (Isidori <i>et al.</i> , 2005) | 1440000 |
| EC ₅₀ | <i>C. dubia</i> | EC ₅₀ | Population growth inhibiton | (Isidori <i>et al.</i> , 2005) | 3130000 |
| EC ₅₀ | <i>C. dubia</i> | EC ₅₀ | Acute toxicity | (Isidori <i>et al.</i> , 2005) | 17410000 |
| EC ₅₀ | <i>B. calyciflorus</i> | EC ₅₀ | Acute toxicity | (Isidori <i>et al.</i> , 2005) | 29880000 |
| EC ₅₀ | <i>D. magna</i> | EC ₅₀ | Acute toxicity | (Isidori <i>et al.</i> , 2005) | 31750000 |
| EC ₅₀ | <i>T. platyurus</i> | EC ₅₀ | Acute toxicity | (Isidori <i>et al.</i> , 2005) | 33980000 |
| EC ₅₀ | Pathogens | MIC ₅₀ | Population Inhibition | (Kümmerer <i>et al.</i> , 2000) | 7500 |
| EC ₅₀ | <i>Ps. putida</i> | EC ₅₀ | Growth inhibitory activity | (Kümmerer <i>et al.</i> , 2000) | 10000 |
| EC ₅₀ | <i>S. aureus</i> | EC ₅₀ | Growth probability | (Nix <i>et al.</i> , 1997) | 320000 |
| EC ₅₀ | <i>M. aeruginosa</i> | EC ₅₀ | Growth inhibition | (Robinson <i>et al.</i> , 2005) | 7900 |
| EC ₅₀ | <i>M. aeruginosa</i> | EC ₅₀ | Growth inhibition | (Robinson <i>et al.</i> , 2005) | 21000 |
| EC ₅₀ | <i>P. subcapitata</i> | EC ₅₀ | Growth inhibition | (Robinson <i>et al.</i> , 2005) | 7400000 |
| EC ₅₀ | <i>P. subcapitata</i> | EC ₅₀ | Growth inhibition | (Robinson <i>et al.</i> , 2005) | 12100000 |
| EC ₅₀ | <i>L. minor</i> | EC ₅₀ | Reproduction inhibition | (Robinson <i>et al.</i> , 2005) | 51000 |
| EC ₅₀ | <i>D. magna</i> | EC ₅₀ | Reproduction inhibition | (Yamashita <i>et al.</i> , 2006) | 340000 |
| EC ₅₀ | <i>P. subcapitata</i> | EC ₅₀ | Growth inhibition | (Yamashita <i>et al.</i> , 2006) | 1200000 |

**Chapitre 3 : Tests d'extraction sur phase solide de trois
quinolones et analyse par chromatographie liquide
couplée à différents détecteurs**

3.1. Introduction

La présence d'anti-infectieux à l'état de traces dans les eaux usées et de surface a éveillé depuis quelques années l'intérêt de la communauté scientifique (Daughton et Ternes, 1999; Hirsch *et al.*, 1999). Comme il a été mentionné dans le chapitre antérieur, les principales raisons de cet intérêt sont que malgré les faibles concentrations dont ces substances se trouvent dans l'environnement (Hirsch *et al.*, 1998; Kolpin *et al.*, 2002), celles-ci peuvent contribuer à la propagation de la résistance aux anti-infectieux chez les bactéries (Kümmerer et Henninger, 2003) ainsi qu'avoir un effet sur les organismes aquatiques sensibles, tels que les algues (Wilson *et al.*, 2003). La situation du Québec à ce sujet étant peu connue, il a été décidé de développer une méthode de détermination capable de quantifier les traces de ces substances dans l'effluent de la station d'épuration de la Ville de Montréal (SEVM).

Nous avons identifié deux défis analytiques qu'il a fallu surmonter pour arriver à déterminer les analytes dans les matrices échantillonnées : *i) Facteur de dilution*. La ville de Montréal est parmi les plus grandes productrices des eaux usées au monde (cinq fois plus que Paris). La SEVM de Montréal traite entre 2.5 et 7.6 Mm³ jour⁻¹ des eaux usées (Ville de Montréal, 2001). Pour les échantillons d'eau de surface, le défi est encore plus grand puisque le St.-Laurent est le fleuve avec le troisième plus haut débit de l'Amérique du Nord avec un débit moyen de 846.72 Mm³ jour⁻¹ dans la région de Montréal (Encyclopædia Britannica Online, 2008; L'Encyclopédie canadienne, 2008), ce qui lui donne une grande capacité de dilution des eaux de rejets municipaux. *ii) Matrice complexe et variable*. Les eaux usées montréalaises sont composées des rejets domestiques et industriels ainsi que des matériaux organiques et inorganiques provenant des rejets pluviaux et de la fonte des neiges. De plus, la SEVM n'applique qu'un traitement primaire et physico-chimique aux eaux rejetées. Par conséquent, l'effluent montréalais contient une quantité considérable de solides en suspension ainsi qu'une demande chimique en oxygène élevé. La méthode développée doit alors être très sensible et sélective pour arriver à déterminer les analytes à des concentrations l'ordre du nanogramme par litre dans une matrice très chargée en matière organique où des effets de matrice sont attendues.

Compte tenu de la faible teneur des anti-infectieux dans les eaux, il est nécessaire d'utiliser une technique d'extraction, de purification et de préconcentration permettant l'élimination de la majeure partie des interférences trouvées dans la matrice et la rétention de la plus grande quantité des analytes d'intérêt. La technique d'extraction moderne la plus utilisée à cette fin est l'extraction sur phase solide (SPE) (Ternes, 2001; Díaz-Cruz et Barceló, 2006).

Dans notre recherche bibliographique, nous avons remarqué l'utilisation fréquente, pour l'analyse des anti-infectieux dans les eaux, des cartouches à polarité de phases inversée telle que la cartouche polymérique Oasis HLB (Göbel *et al.*, 2004; Miao *et al.*, 2004; Renew et Huang, 2004). Un autre type de cartouche utilisée est celle avec une phase mixte d'échange cationique (Golet *et al.*, 2001; Calamari *et al.*, 2003). Ensuite, nous avons exploré trois techniques analytiques différentes: la chromatographie liquide (LC) couplée à un détecteur à fluorescence (LC-FLD), LC couplée à un spectromètre de masse à temps d'envol [LC-TOFMS] et LC couplée à la spectrométrie de masse en tandem avec un triple quadripôle [LC-MS/MS(QqQ)]. Finalement, nous avons aussi obtenu de l'information sur consommation des anti-infectieux au Québec avec l'aide de la société IMS Health Canada pour confirmer les résultats obtenus.

Nous avons ciblé trois anti-infectieux de la famille de quinolones (QLs): ciprofloxacin, norfloxacin et enrofloxacin (**Figure 3-1**) qui ont été détectées dans les eaux de nombreux pays européens (Golet *et al.*, 2001; Andreozzi *et al.*, 2003; Christian *et al.*, 2003) et aux États-Unis (Campagnolo *et al.*, 2002; Kolpin *et al.*, 2002) pour entreprendre nos tests préliminaires de développement analytique.

Le but de ces premiers travaux préliminaires a été de tester différentes cartouches de SPE pour l'analyse de traces d'anti-infectieux ainsi que d'explorer l'utilisation de différents détecteurs couplés à la LC. Aussi nos recherches sur la consommation de ces substances au Québec ont permis de cibler les anti-infectieux les plus couramment utilisés et qui sont probablement ceux ayant les concentrations les plus élevées.

3.2. Partie expérimentale

3.2.1. Matériel

Tous les solvants et les réactifs utilisés sont de grade LC ou supérieur. Le méthanol (MeOH) et l'hydroxyde de sodium 28-30% (NH₄OH) ont été fournis par Caledon Laboratories (Georgetown, ON), l'acétonitrile (ACN) par Anachemia (Montréal, QC), l'acide chlorhydrique 36.5-38% (HCl) par Produits Chimiques Américains (Montréal, QC) et l'acide ortho-phosphorique (*o*-H₃PO₄), par EMD Chemicals (Gibbstown, NJ). Les quinolones norfloxacin (NOR) et enrofloxacin (ENR) ont été fournies par Sigma-Aldrich (Oakville, ON). Ciprofloxacin (CIP) ainsi que le standard interne utilisé, énoxacin ont été fournis par MP Biomedicals (Irvine, CA).

Nous avons évalué les performances de trois cartouches différentes disponibles commercialement : Oasis HLB Waters de 6 mL et 200 mg de masse de sorbent (Milford, MA), Strata-X 5 mL, 200 mg et Strata-XC de 5 mL, 200 mg de Phenomenex (Torrence, CA). Les propriétés des cartouches apparaissent sur le **Tableau 3-1**.

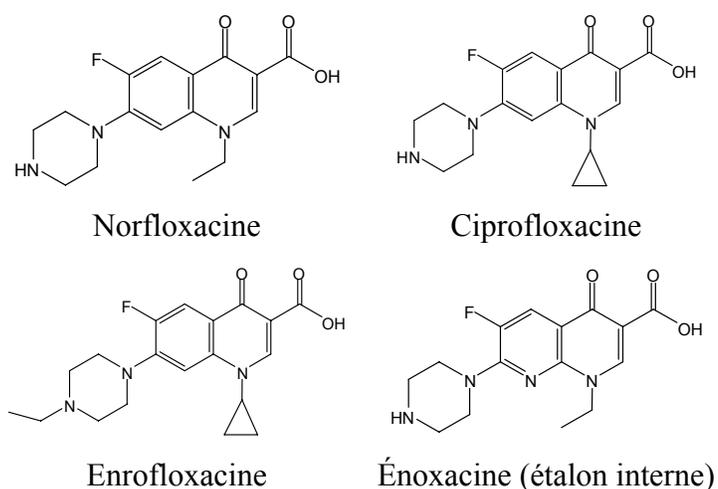


Figure 3-1. Quinolones ciblées dans cette étude.

3.2.2. Instrumentation

Les mesures d'absorption UV-Vis ($\lambda = 254-400$ nm) ont été réalisées dans un spectrophotomètre Cary-100 Bio UV-Visible de Varian (Mississauga, ON). Ces mesures ont été réalisées pour évaluer l'efficacité de l'ultrafiltration par débit tangentiel (TFU). À fin de préconcentrer les échantillons par SPE, deux collecteurs à vide différents ont été utilisés: le VacElut SPS 24 de Varian pour charger les analytes dans la cartouche de SPE et le IST VacMaster-10 d'Argonaut (Redwood City, CA) pour faire le lavage des cartouches et la désorption des analytes.

La séparation et détection des analytes par LC-FLD a été effectuée dans un système muni d'une pompe P4000, d'un auto-échantillonneur AS400 et d'un détecteur FL300 de fabriqués par Thermo Electron. L'analyse par LC-TOFMS a été faite avec un système de LC HP 1000 Series couplé à un spectromètre à temps d'envol 6210 d'Agilent (Santa Clara, CA). L'analyse par LC-MS/MS(QqQ) a été réalisée avec un système de LC Surveyor couplé à un spectromètre de masse à triple quadripôle TSQ Quantum Ultra AM de Thermo Electron.

3.2.3. Préparation des solutions

Les solutions mères des trois quinolones (QLs) et de l'étalon interne (ÉI) de 400 mg L⁻¹ ont été préparées avec une solution de 0.2% HCl dans 50% MeOH-50% H₂O (v/v).

Ces solutions ont été conservées à -20 °C durant au plus 3 mois. À partir de ces dernières, des solutions mixtes de travail de 400 µg L⁻¹ dans *o*-H₃PO₄ 0.027% (pH = 2.6) ont été faites pour doper les échantillons d'eau désionisée et d'eaux usées.

Tableau 3-1. Caractéristiques de cartouches SPE utilisées.

| Cartouche | Type | Matériel | Aire de surface spécifique (m ² g ⁻¹) | Diamètre moyen de pores (nm) | Diamètre moyen de particules (µm) |
|-----------|--|---|--|------------------------------|-----------------------------------|
| Oasis HLB | Polarité des phases inversée | Poly(styrène-divinylebenzène-co- <i>N</i> -vinylpyrrolidone) | 727-889 ^a , 831 ^b | 0.118-0.144 ^b | 25.0-35.0 ^b |
| Strata-X | Polarité des phases inversée | Polymère de styrène-divinylebenzène modifié à la surface | 660 ^a , 800 ^b | 8.1-9.1 ^b | 28-34 ^b |
| Strata-XC | Polarité des phases inversée et échangeur cationique | Polymère de styrène-divinylebenzène greffé de l'acide benzènesulfonique | 705-825 ^b | 7.5-9.1 ^b | 28-34 ^b |

^a Valeur expérimentale déterminée par l'isotherme d'adsorption/désorption de l'azote. Source : (Fontanals *et al.*, 2004); ^b Valeur donnée par le manufacturier

3.2.4. Échantillonnage et préparation des échantillons d'eaux usées

La station d'épuration des eaux usées la Ville de Montréal (SEVM) est la plus grande station de traitement d'eaux usées des Amériques utilisant un processus primaire et physico-chimique (Ville de Montréal, 2002a). Ce dernier consiste en l'élimination mécanique des débris et la précipitation des matières en suspension et des phosphates par l'ajout de chlorure ferrique (FeCl_3) ou de l'alun (sulfate d'aluminium et de potassium dodecahydraté, $[\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}]$) et d'un polymère anionique (Communauté Urbaine de Montréal, 1999a). La SEVM collecte toutes les eaux usées produites sur le territoire des îles de Montréal, Bizard et des Sœurs (Communauté Urbaine de Montréal, 1999b). En 2006, le débit des eaux usées traitées par la station était autour de $2\,786\,000\text{ m}^3\text{ d}^{-1}$, ce qui représente approximativement 43 % des eaux usées produites au Québec (Ministère des Affaires municipales et des Régions du Québec, 2006). Une fois traitées, les eaux sont rejetées au fleuve St-Laurent en face de l'île aux Vaches.

L'échantillonnage d'eaux usées de la SEVM a été réalisé le 11 avril 2005. L'échantillon était un composé à débit proportionnel de 24 h de l'effluent. L'échantillon d'eaux usées a été transporté dans un contenant en acier inoxydable et ensuite filtré avec des filtres de fibre de verre TCLP manufacturés par Pall Corporation (Ann Arbor, MI) et ayant un diamètre de pores moyen de $0.7\ \mu\text{m}$.

L'échantillon d'eau a été ensuite filtré avec un système d'ultrafiltration par débit tangentiel (TFU), constitué d'une pompe péristaltique et une cartouche Prep/Scale-TFF-2 PLAC manufacturée par Millipore (Bedford, MA). Cette membrane de cellulose régénérée ayant une aire de membrane de 0.23 m^2 est capable de ségréger les particules de taille inférieure à 1000 u. Il est important d'éliminer ces substances puisqu'elles sont co-extraites et peuvent interférer avec l'analyse. Les eaux usées ont été ensuite acidifiées à $\text{pH} = 2.6$ avec $o\text{-H}_3\text{PO}_4$ pour empêcher la biodégradation microbienne. L'effet de l'ultrafiltration sur la coloration des eaux a été étudié en mesurant l'absorption UV-Vis (225-700 nm).

3.2.5. SPE

Les cartouches SPE ont été d'abord conditionnées avec MeOH 100% puis avec 0.023% *o*-H₃PO₄ dans H₂O (v/v). Un aliquote 500 µL d'une solution de NaCl (0.1 M) a été ensuite ajouté à un volume d'échantillon [100 mL pour LC-FLD, 500 mL pour LC-MSTOF et LC-MS/MS(QqQ)]. Le volume total est alors aspiré dans la cartouche à l'aide du collecteur à vide. Une fois le volume complet est passé par la cartouche, les cartouches SPE sont traitées différemment.

Les cartouches polymériques à polarité des phases inversée (Oasis HLB et Strata-X) ont été lavées avec 1 × 1 mL d'une solution de 5% MeOH-95% H₂O (v/v) pour enlever une partie des impuretés retenues par la cartouche, tandis que la cartouche de phase mixte (Strata-XC) a été rincée avec 1 × 1 mL d'une solution de 100% MeOH, et après avec 1 × 1 mL de H₃PO₄ 0.1% dans H₂O. Pour les polymériques, la désorption de l'échantillon s'est réalisée avec 2 × 2 mL à un débit d'à peu près 250 µL s⁻¹. Pour la cartouche de phase mixte, la désorption se réalise avec 2 × 2 mL d'une solution 5% NH₄OH-95% MeOH (v/v) au même débit des cartouches polymériques à polarité des phases inversée.

Après la désorption, l'échantillon est recueilli dans une éprouvette de verre et évaporé à sec avec un flux léger d'azote sur une plaque chauffée à 40°C. Pour les analyses par LC-FLD, l'échantillon est ensuite reconstitué à 1 mL avec une solution de 13% MeOH-87% H₃PO₄ 0.023% dans H₂O (v/v). Pour les analyses par LC-MSTOF et LC-MS/MS(QqQ) la reconstitution se fait à 1 mL avec une solution de 20% MeOH dans H₂O (v/v) contenant l'ÉI.

3.2.6. LC-FLD

Pour réaliser la séparation chromatographique par LC-FLD, la colonne utilisée est une Discovery RP-Amide C₁₆ de dimensions 250 × 3 mm et ayant une taille de particules de 5µm, a été fournie par Supelco (Bellefonte, PA). La colonne analytique a été protégée par une précolonne de 3 mm de la même nature et manufacturier.

La séparation des analytes s'est faite en utilisant une méthode basée sur celle de Golet (Golet *et al.*, 2001). Le gradient binaire était constitué de *o*-H₃PO₄ 0.023% (A) et ACN (B). Le gradient d'élution a été le suivant (% B) : 5% à 0 min, 7% à 17 min, 7% à 23 min, 85 % à 23 min, 85% à 27 min, 5% à 31 min, 5% à 41 min. En ce qui concerne la détection, la longueur d'onde d'excitation (λ_{ex}) était de 278 nm et la longueur d'onde d'émission (λ_{em}) de 444 nm. Le débit de la phase mobile était de 0.7 mL min⁻¹ et le volume d'injection de 20 μ L.

3.2.7. LC-TOFMS et LC-MS/MS(QqQ)

La méthode LC utilisée est basée sur celle de Miao (Miao *et al.*, 2004). Pour la LC-TOFMS la colonne utilisée est une Gemini C₁₈ de 150 \times 4.6 mm et 5 μ m, manufacturée par Phenomenex. Enfin, la colonne utilisée en LC-MS/MS(QqQ) était une C₁₈ Hypersil Gold de 100 \times 2.1 mm et 3 μ m, fournie par Thermo Electron (Waltham, MA). Le solvant A était l'acide formique 0.1% (v/v) dans l'eau et le solvant B était l'acétonitrile. Le gradient d'élution a été le suivant (% B) : 12% à 0 min, 55% à 8 min, 100% à 10 min, 100 % à 15 min, 12 % à 16 min et 12 % à 22 min. Pour la LC-TOFMS le débit de la phase mobile était de 500 μ L min⁻¹ et le volume d'injection 20 μ L, tandis que pour la LC-MS/MS le débit de la phase mobile était de 100 μ L min⁻¹ et le volume d'injection 2 μ L.

Les analytes ont été ionisés par électronébulisation dans le mode positif (ESI+) pour les analyses par spectrométrie de masse (TOF et QqQ). Pour la LC-TOFMS les paramètres de la source ont été les suivants : voltage du capillaire (3200 V), nébuliseur (15 psig), gaz de séchage (7.0 L min⁻¹) et température du gaz (300 °C). La détection par TOF s'est faite en balayant de 100 *m/z* à 1000 *m/z*. Pour la LC-MS/MS(QqQ) les paramètres de la source ont été les suivants : voltage de l'électronébuliseur (3000 V), gaz de nébulisation (15 unités arbitraires), gaz auxiliaire (0 unités arbitraires), température du tube de transfert d'ions (310 °C), voltage du skimmer (8 V). La détection par QqQ s'est faite dans le mode de suivi des réactions de fragmentation caractéristiques (SRM) avec l'argon comme gaz de collision à une pression de 1.2 mTorr. Les transitions SRM surveillées ont été les suivantes : NOR (ion précurseur 320.2 *m/z* \rightarrow ion produit 233.1 *m/z*, électro-lentille de transfert (TL) : 90 V,

énergie de collision (CE) : 25, CIP (332.2 m/z → 231.2 m/z , TL : 93 V, CE : 36 V) et ENR (360.2 m/z → 316.2 m/z , TL : 90 V, CE : 20 V).

3.2.8. Information sur la consommation des anti-infectieux au Québec

L'information sur la consommation des anti-infectieux au Québec a été fournie par la compagnie IMS Health Canada. Cette information est recueillie mensuellement par le système informatisé CompuScript qui trace entre autres le nombre et les composé actifs des prescriptions délivrées dans les pharmacies au Canada (IMS Health Canada, 2008).

3.2.9. Validation de la méthode

Le recouvrement de l'extraction a été mesuré en dopant l'eau déionisée à une concentration de 200 ng L⁻¹ en NOR, CIP et ENR et en comparant ensuite avec des étalons non-extraits de concentration équivalente selon le facteur de préconcentration [100 pour la LC-FLD et 500 pour LC-MSTOF et LC-MS/MS(QqQ)] selon l'équation suivante :

$$\text{Recouvrement (\%)} = \frac{\left(\frac{H_{QL}}{H_{\acute{E}I}} \right)_{\text{Extrait SPE}}}{\left(\frac{H_{QL}}{H_{\acute{E}I}} \right)_{\text{Non-extrait}}} \times 100\% \quad (\text{Équation 3-1})$$

Où H_{QL} est la réponse du détecteur pour les quinolones, déterminée en mesurant la hauteur du pic, $H_{\acute{E}I}$ est la hauteur du pic de l'étalon interne. Les étalons non-extraits correspondent à une concentration équivalente (*i.e.* concentration rehaussée en fonction du facteur de préconcentration), pour un recouvrement de 100%. Ensuite le recouvrement est déterminé dans l'eau usée. Comme les analytes sont potentiellement présents dans la matrice, le signal d'un blanc de matrice est soustrait à celle de l'extrait SPE.

L'étalonnage par ajouts dosés a été utilisé pour déterminer la concentration des analytes dans l'eau usée. Cette dernière a été dopée avec les analytes pour des

concentrations finales de 40, 200, 1000, et 2000 $\mu\text{g L}^{-1}$ et en analysant deux fois chaque échantillon dopé. Le dopage s'est réaslisé après la SPE.

L'analyse statistique des résultats a été effectuée avec le logiciel SPSS 13.0 créée par SPSS Inc. (Chicago, IL). L'homogénéité de la variance des résultats a été déterminée par le test de Levene. Les différences entre les recouvrements des cartouches SPE ont été évaluées par un test d'analyse de la variance (ANOVA) et le test posthoc Dunnett's T3. Pour la calibration, si la variance n'est pas homogène, une régression linéaire de moindres carrées pondérée a été effectuée au lieu d'une régression linéaire de moindres carrés ordinaire.

3.3. Résultats et discussion

3.3.1. SPE

Àfin d'évaluer l'efficacité des trois cartouches testées lors de l'extraction des QLs, nous avons procédé à l'application du test statistiques ANOVA pour déterminer si les rendements obtenus montrent des différences significatives (**Tableau 3-2**). Le niveau de signifiante pour les recouvrements du NOR était de $p = 0.0001$, pour CIP $p = 0.0005$ et pour ENR $p = 0.3$. Ces résultats démontrent qu'ils existent des différences qui ne sont pas dues à la chance ($p < 0.05$) entre les rendements des trois cartouches pour NOR et CIP. En ce qui concerne ENR, le recouvrement des cartouches Oasis HLB, Strata-X et Strata-XC n'est pas significativement différent ($p > 0.05$).

Les tests post-hoc déterminent quelles moyennes sont significativement différentes. Le test de Levene a montré que les variances n'étaient homogènes, par conséquent, le test post-hoc Dunnett's T3 a été employé pour réaliser les comparaisons multiples pour chaque cartouche. Ce test a été choisi parce qu'il n'assume pas l'homogénéité des variances. Le **Tableau 3-2** affiche les résultats obtenus. Les résultats des tests post-hoc montrent que les recouvrements de NOR et CIP avec les trois cartouches sont significativement différents.

Cependant à des fins pratiques l'extraction des QLs peut être faite avec les trois cartouches puisque le recouvrement des extractions est excellent (> 88 %).

Tableau 3-2. Comparaison des taux de recouvrement des trois QLs dans l'eau déionisée obtenus avec les trois cartouches de SPE ($n=3$).

| QL | Oasis HLB ^a (% Rec.) | Strata-X ^a (% Rec.) | Strata-XC ^a (% Rec.) |
|-----|------------------------------------|-----------------------------------|------------------------------------|
| NOR | 88 ± 1 ^A | 113 ± 5 ^B | 91 ± 1 ^C |
| CIP | 89 ± 1 ^A | 116 ± 7 ^B | 95 ± 2 ^C |
| ENR | 140 ± 7 ^A | 151 ± 13 ^A | 140 ± 7 ^A |

^a Les valeurs suivies de mêmes lettres ne sont pas statistiquement différents au niveau de signifiante $p = 0.05$ selon le test post-hoc Dunnetts's T3.

Il est difficile à expliquer le recouvrement élevé de ENR. D'après l'équation (1), pour avoir des valeurs de recouvrement > 100 % le numérateur doit être plus grand que le dénominateur, *i.e.* le signal de ENR extrait est plus élevé que celui de l'étalon. Une façon dont ceci aurait pu se produire, est la possibilité que l'étalon s'est dégradé avant l'analyse. Cependant une dégradation de l'ordre de 30 % de la concentration initiale semble peu probable en quelques heures. Pour empêcher toute possible dégradation, les solutions ont été désormais entreposés à 4°C jusqu'à son utilisation. Une explication plus probable est celle de la contamination de la verrerie utilisée pour l'extraction, augmentant ainsi la concentration dans la solution dopée (solution pour extraction SPE). Pour empêcher la contamination de la verrerie, celle-ci a été trempée dans le savon acidifié et lavé avec acétone, dicholorométhane et MeOH avant son utilisation.

Un excellent recouvrement dans l'eau déionisée n'assure pas un bon recouvrement dans la matrice, spécialement dans une matrice aussi complexe que les effluents de la SEVM. Alors nous avons procédé à la détermination du taux de recouvrement de l'extraction dans la matrice. Compte tenu que la cartouche Strata-XC offre une meilleure sélectivité envers les QLs et que le lavage « agressif » avec 100% MeOH permet d'éliminer

une grande partie des impuretés présentes dans la matrice, il a été décidé de débiter nos travaux avec cette cartouche.

Les résultats obtenus (**Error! Reference source not found.**) démontrent que la matrice exerce une influence déterminante sur le pourcentage de recouvrement de l'extraction. Dans la matrice, malgré l'ultrafiltration, la récupération du NOR est passée de $91 \pm 1 \%$ à $7 \pm 3 \%$ et celle du CIP de 95 ± 2 à $2 \pm 4 \%$. Quant à ENR, le recouvrement était négatif, c'est-à-dire que le signal dans la matrice (corrigée par la présence probable de ENR) était plus fort que dans l'eau dopée. Cette forte diminution dans le pourcentage de récupération peut être expliquée par la saturation des sites d'échange cationique sur la cartouche qui a une capacité d'échange ionique de $0.9-1.2 \text{ meq g}^{-1}$. Si la matrice contient une concentration élevée en cations monovalents (Na^+ , K^+) ou encore divalents (Mg^{2+} , Ca^{2+}) la capacité de l'adsorbant à retenir les QLs chargées peut se voir grandement affectée (Hennion, 1999). Cette situation est fort probable durant les mois d'hiver, lorsque les eaux ont de fortes concentrations en sel à cause de l'épandage de ces dernières sur les rues à déglacer. D'après les mesures réalisées par les laboratoires de la SEVM les eaux usées peuvent contenir $30-50 \text{ mg L}^{-1}$ de Ca et $8-10 \text{ mg L}^{-1}$ de Mg pendant les mois d'hiver (Tremblay, 2006).

Tableau 3-3. Pourcentage de recouvrement de QLs dans la matrice ultrafiltrée avec la cartouche Strata-XC ($n=3$).

| QL | Concentration dopée (ng L^{-1}) | Recouvrement (%) |
|-----|---|---------------------|
| NOR | 200.24 | 7 ± 3 |
| CIP | 200.01 | 2 ± 4 |
| ENR | 199.91 | -4 ± 7 |

Pour cette raison, les travaux préliminaires ultérieurs ont utilisé la cartouche à polarité de phases inversée Strata-X, puisque ce type de cartouche est moins affectée par les concentrations en sel élevées.

3.3.2. LC-FLD

L'application d'une méthode LC-FLD aux effluents de la SEVM s'est avérée difficile à cause de la nature particulière de l'effluent montréalais, riche en déchets industriels et en substances organiques et inorganiques ayant la capacité d'interférer la détection par fluorescence. Comme le montre la **Figure 3-2**, le chromatogramme des étalons dans l'eau désionisée (**a**), les QLs montrent des pics bien définis et résolus à la base, cependant lorsque la matrice des eaux usées dopées avec la même concentration équivalente est préconcentrée par SPE, le chromatogramme obtenu (**b**) montre que les pics ne sont plus résolus à la base et leur détermination est difficile.

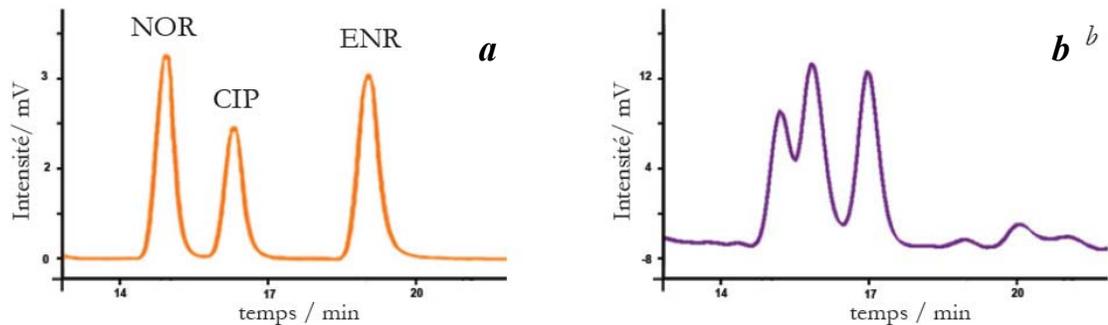


Figure 3-2. Chromatogrammes LC-FLD. ^a Solution contenant les étalons à une concentration équivalente à 200 ng L⁻¹ dans l'eau désionisée ^b Effluent dopé avec 200 ngL⁻¹ et préconcentré avec la cartouche Strata-X.

Ces résultats montrent que l'analyse par LC-FLD de la matrice de l'effluent de la SEVM se voit affectée par la présence de substances qui co-éluent avec les QLs et ayant des λ_{ex} et λ_{em} proches des analytes.

3.3.3. Ultrafiltration

L'ultrafiltration par flux tangentiel (TFU) consiste en l'application d'un débit d'échantillon tangentiel à la surface d'une membrane perméable aux substances ayant une masse moléculaire donnée (dans ce cas-ci une membrane de cellulose ségrégeant les masses < 1000 u). La rétention ou le rejet des composés se fait alors en fonction de la masse et de

la forme moléculaire et elle est influencée par plusieurs facteurs tels que le pH, la force ionique et la concentration (Guéguen *et al.*, 2002). Le débit est appliqué en continu de manière à ce que l'échantillon entre en contact avec la membrane plusieurs fois. Ceci donne lieu au fractionnement de l'échantillon en deux parties : le rétenat et le perméat. Le rétenat (partie de l'échantillon qui ne traverse pas la membrane) est enrichie, dans notre cas, majoritairement des molécules ayant une masse > 1000 u. Le perméat qui est partie de l'échantillon qui réussit à traverser la membrane, est contient majoritairement des molécules ayant une masse < 1000 u.

Nous avons ajouté une étape de TFU avant l'extraction parce celle-ci est une technique couramment utilisée dans l'analyse environnementale (Guéguen *et al.*, 2002; Morrison et Benoit, 2004). Par exemple, elle a été appliquée à l'analyse des eaux usées pour séparer les colloïdes de haute masse moléculaire des substances de basse masse moléculaire dans la fraction dissoute (*i.e.* $< 0.45 \mu\text{m}$) (Gagnon *et al.*, 2006). Nous avons utilisé la TFU pour nettoyer l'échantillon d'eau usée et éliminer une partie des interférences de haute masse moléculaire présentes dans la matrice avant la SPE.

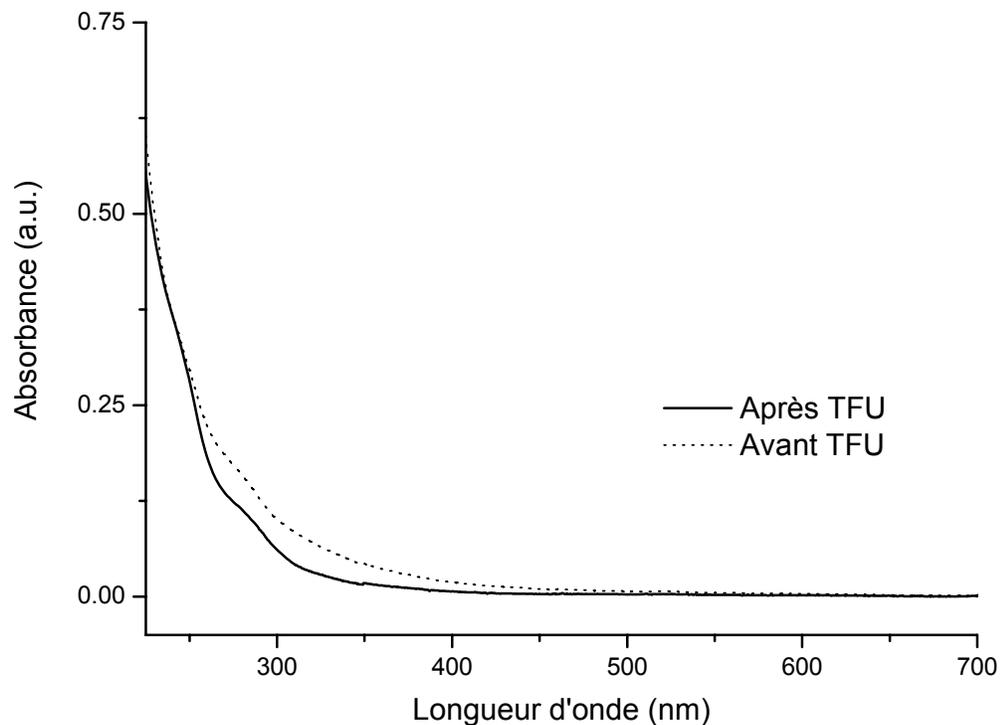


Figure 3-3. Absorption UV-Vis de l'échantillon d'effluent de la SEVM avant ultrafiltration (ligne pointillée) et après ultrafiltration (ligne pleine).

L'inspection visuelle de l'échantillon d'effluent après TFU montrait une différence au niveau de la coloration. La **Error! Reference source not found.** illustre la réduction de l'absorption UV-Vis dans l'échantillon du perméat (*c.-à-d.* l'échantillon après TFU) comparée à celle de l'effluent. Une diminution significative de l'absorbance est observée entre la région de 250 à 400 nm. D'après Renew (Renew et Huang, 2004) l'absorption UV à 254 nm est corrélée positivement avec le contenu en carbone organique de l'eau. D'après cette expérience l'absorption des eaux usées après TFU est passée de 0.265 à 0.239 u.a.

Nos données indiquent alors que seulement une partie du carbone organique dans l'échantillon possède une masse moléculaire > 1000 u. Ces résultats sont confirmés par Kloepfer (Kloepfer *et al.*, 2005) qui a calculé que plus du 70 % du carbon organique dans les eaux usées était constitué par des substances ayant des masses moléculaires < 1000 u. Par conséquent, une technique analytique plus sélective et moins affectée par les interférences présentes dans la matrice que la LC-FLD est indispensable.

3.3.4. LC-MS

3.3.4.a. LC-MSTOF

Nous avons alors procédé à l'application de la technique de choix pour l'analyse des anti-infectieux au niveau de trace dans les effluents urbains : la chromatographie liquide couplée à la spectrométrie de masse. Le même échantillon a été analysé avec un LC-TOFMS et un LC-MS/MS(QqQ).

L'utilisation de la spectrométrie de masse diminue considérablement les interférences de la matrice et offre un mode de détection plus sélectif que celui de la fluorescence, puisque la détection se fait en fonction du rapport masse sur charge (m/z) plutôt que par l'excitation et l'émission à des longueurs d'onde spécifiques. Les paramètres de l'analyse LC-MSTOF sont présentés au **Tableau 3-4**. Les droites de calibration par ajouts dosés ont été linéaires ($r^2 > 0.99$ pour NOR et CIP, 0.97 pour ENR). Cependant pour LC-MSTOF, les

limites de détection (36-40 ng L⁻¹) ne sont pas assez basses pour détecter la présence de QLs ciblées.

Tableau 3-4. Paramètres de la méthode de détermination par ajouts dosés des QLs dans les eaux usées de la SEVM utilisant la SPE suivie de la LC-TOFMS.

| QLs | Équation de la droite ^a | r^2 | Conc. dans l'effluent (ng L ⁻¹) | LOD (ng L ⁻¹) |
|-----|------------------------------------|--------|--|------------------------------|
| NOR | $y = -5720 + 366x$ | 0.9950 | < LOD | 69 |
| CIP | $y = 11719 + 391x$ | 0.9968 | < LOD | 40 |
| ENR | $y = -2065 + 329x$ | 0.9756 | < LOD | 36 |

^a La pondération w pour NOR et CIP a été de $w = y^{-0.5}$ et celle de ENR $w = y^{-1}$. Les unités de la pente sont en nanogramme par litre.

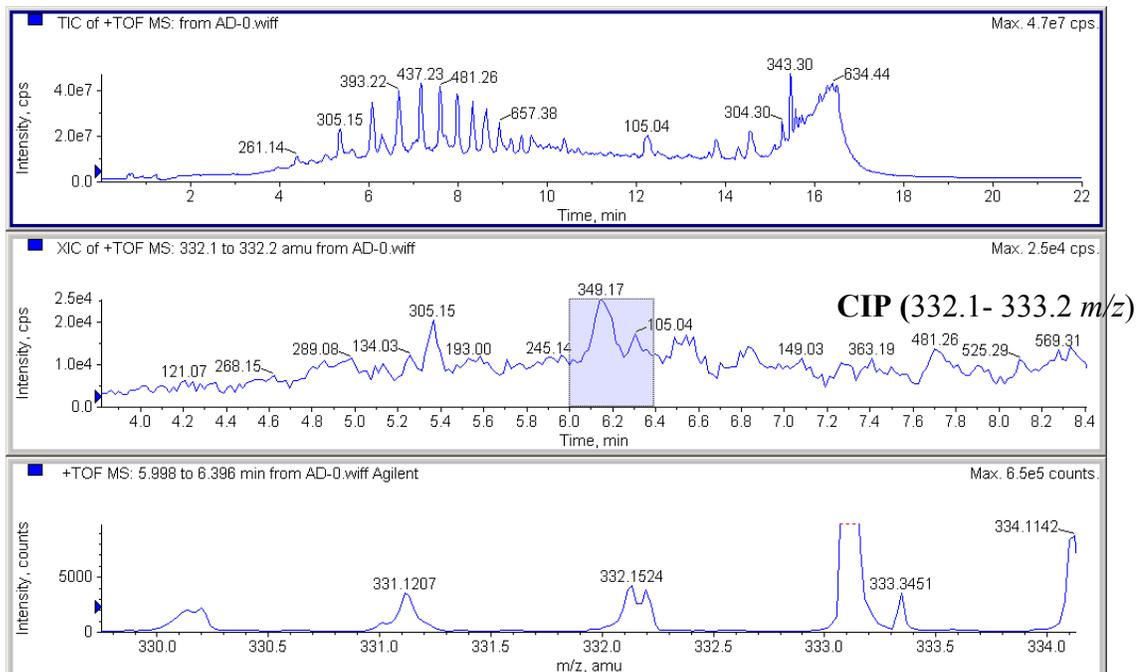


Figure 3-4. Résultats de l'analyse par LC-TOFMS de CIP dans un échantillon non-dopé de l'effluent de la SEVM: Chromatogramme de courant total (TIC, image supérieure), chromatogramme de courant extrait (XIC, image du milieu) et le spectre de masse de l'ion 332 m/z (TOFMS, image inférieure).

La **Figure 3-4** montre les résultats de l'analyse LC-TOFMS de la matrice. Le chromatogramme de courant total (TIC) montre le signal généré par les ions arrivant au détecteur et ayant un rapport m/z entre 100 et 1000 m/z . Le chromatogramme de courant extraite (XIC) pour l'ion 332 m/z que le signal de CIP dans la matrice non-dopée est faible et n'est pas plus que 3 fois supérieur à la ligne de base. Alors pour quantifier les analyses de traces de QLs dans l'effluent de la SEVM, un détecteur de spectrométrie de masse encore plus sensible et la TOFMS est nécessaire.

3.3.4.b. LC-MS/MS(QqQ)

La spectrométrie de masse en tandem (MS/MS) offre une sélectivité accrue sans avoir un effet sur la sensibilité (le bruit et le signal diminuent en fonction des étapes d'analyse par spectrométrie de masse tandis que le rapport signal sur bruit (S/N) augmente) (Boyd, 2006). Alors, la MS/MS(QqQ) a été ensuite explorée.

Les résultats des analyses ont démontré que la LC-MS/MS(QqQ) était la technique d'analyse la plus appropriée pour la détection et quantification de traces d'anti-infectieux dans les effluents des stations d'épuration. L'utilisation de cette technique apporte une indéniable sélectivité par son mode SRM tout en éliminant certains effets d'interférence. Les droites des ajouts dosés ont été linéaires ($r^2 > 0.98$) et les limites de détection assez bas (2-7 ng L⁻¹) pour quantifier les trois QLs dans l'effluent de la SEVM (**Tableau 3-5**.)

Nos résultats indiquent que les trois QLs étudiées, ont été détectés et quantifiés dans l'effluent de la SEVM (**Figure 3-5**). Néanmoins, sa concentration dans l'effluent montréalais s'avère plus basse que les autres concentrations reportées par Miao (Miao *et al.*, 2004) qui a détecté CIP (118-400 ng L⁻¹) et NOR (50-112 ng L⁻¹) dans les effluents d'autres villes canadiennes. Toutefois, le débit moyen de la SEVM est très élevé (2 786 000 m³ d⁻¹ en 2006) et la charge environnementale est donc très significative. La masse totale de trois QLs déversé sur le fleuve dépend lors de la consommation journalière, ainsi que de la résistance à la dégradation.

Tableau 3-5. Paramètres de la méthode de détermination par ajouts dosés des QLs dans les eaux usées de la SEVM utilisant la SPE suivie de la LC-MS/MS(QqQ).

| QLs | Équation de la droite ^a | r^2 | Conc. dans l'effluent (ng L ⁻¹) | LOD (ng L ⁻¹) |
|-----|------------------------------------|--------|--|------------------------------|
| NOR | $y = 2766 + 217x$ | 0.9811 | 13 ± 1 | 4 |
| CIP | $y = 13443 + 260x$ | 0.9925 | 52 ± 3 | 7 |
| ENR | $y = 4542 + 574x$ | 0.9886 | 8 ± 3 | 2 |

^a La pondération w pour NOR et CIP a été de $w = y^{-0.5}$ et celle de ENR $w = y^{-1}$. La pondération augmente la précision de la régression en donnant plus d'importance aux points les plus précis de la courbe (ceux qui varient le plus).

Il est important de souligner que les résultats obtenus doivent être interprétés avec soin. Comme il peut être observé à la **Figure 3-5**, certaines interférences peuvent être observées dans les chromatogrammes même dans le mode SRM. Il est possible que des substances dans l'effluent de la SEVM puissent avoir une transition SRM semblable à celle des QLs, faussant aussi les résultats obtenus. Pour éliminer ces « faux positifs » une deuxième transition SRM devrait être utilisée (Hernández *et al.*, 2004).

3.3.5. Information sur la consommation d'anti-infectieux au Québec

Les données sur le nombre de prescriptions d'anti-infectieux délivrées dans les pharmacies de Québec dans la période de 2004-2006 ont été obtenues de la compagnie IMS Health Canada.

La **Figure 3-6** montre la variation du nombre des prescriptions des 25 anti-infectieux les plus populaires de 2006 dans la période de 2004 à 2006. Dans cette période le nombre totale de prescriptions d'anti-infectieux ont été proches de 5 millions. Il est important de mentionner que l'information obtenu montre seulement le nombre de prescriptions et non la quantité ou la masse totale des anti-infectieux vendus. Aussi l'utilisation des anti-infectieux au milieu hospitalier n'est pas incluse dans ces données.

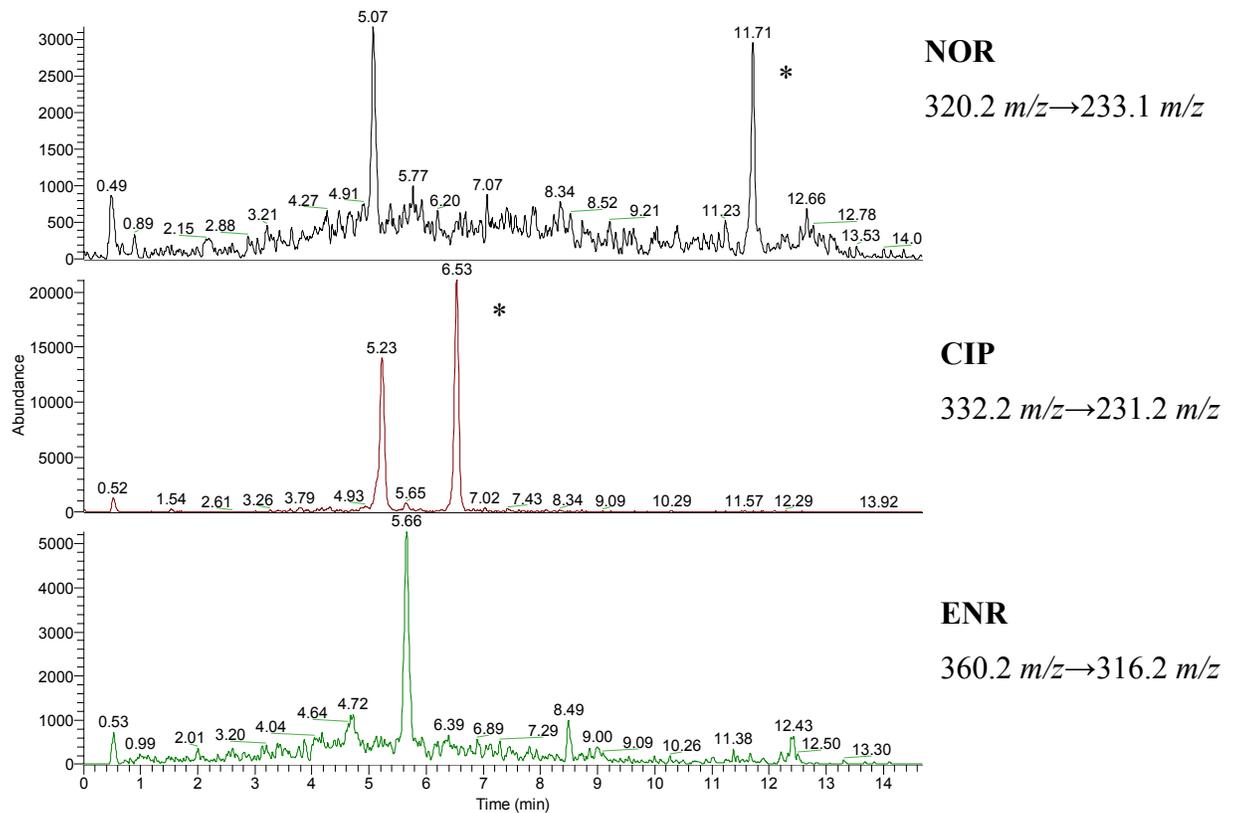


Figure 3-5. Chromatogramme LC-MS/MS en mode suivi des réactions de fragmentation caractéristiques (SRM) d'un extrait d'effluent de la SEVM. Les pics ayant un asterisk sont des interférences.

Les données de IMS Health Canada montrent que parmi les 25 anti-infectieux les plus prescrits au Québec, on retrouve des substances appartenant à différentes familles : β -lactames (amoxicilline, phénoxyméthylpénicilline, cefprozil, céfalexine, etc.), quinolones (ciprofloxacine, moxifloxacine, lévofloxacine, gatifloxacine), macrolides (azithromycine, clarithromycine), azoles (fluconazole) sulfonamides (sulfaméthoxazole) et les tétracyclines (minocycline, doxycycline) parmi d'autres. Alors une méthode capable d'extraire des composés appartenant à différentes familles devra être développée pour quantifier les substances se trouvant à plus haute concentration et par conséquent posant le plus des risques pour l'environnement.

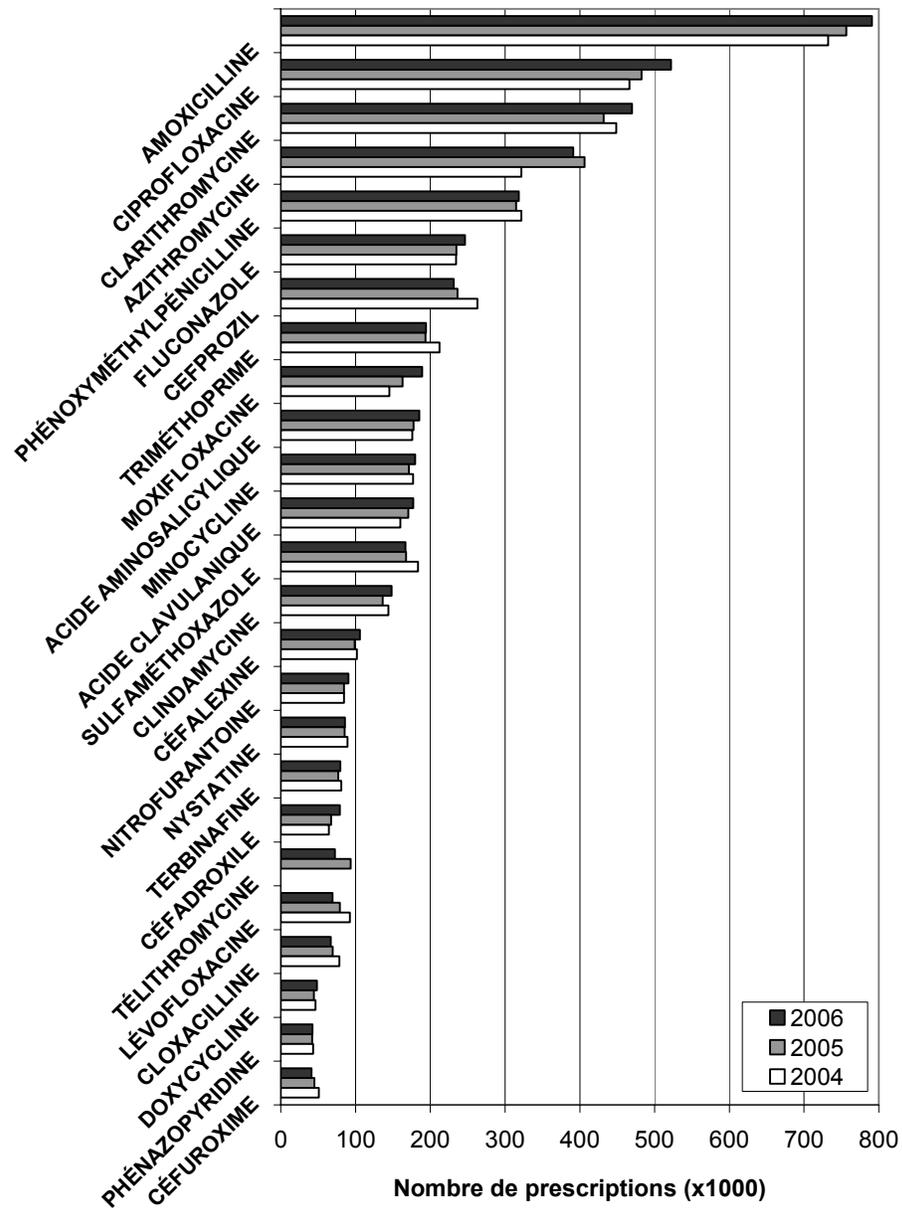


Figure 3-6. Prescriptions au Québec entre 2004 et 2006 des 25 anti-infectieux les plus populaires. Les données de 2004 ont été calculées à partir des données de croissance de 2005. Source : (IMS Health Canada, 2007).

Un autre point important est celui de la persistance dans l'environnement. Il ne suffit pas de connaître quels anti-infectieux sont les plus consommés pour déterminer lesquels se trouveront dans l'environnement. La capacité d'une substance à résister la dégradation de nature abiotique (hydrolyse, photolyse) ou biotique (bactéries) détermine son devenir

environnemental après avoir été introduit dans l'environnement. L'exemple des β -lactames illustre ce fait: malgré leur consommation élevée, ils ont été peu souvent détectés (Andreozzi *et al.*, 2004; Costanzo *et al.*, 2005; Brown *et al.*, 2006). Ceci est expliqué par l'instabilité de leur anneau β -lactame à 4 membres, qui est susceptible à l'hydrolyse (Hirsch *et al.*, 1999).

Étant donné que les valeurs expérimentales sur la persistance des anti-infectieux dans l'environnement sont rares dans la littérature scientifique, il faut alors évaluer quelles substances, parmi celles qui ont été reportées dans plusieurs études d'occurrence d'anti-infectieux réalisées aux Etats-Unis et en Europe (Hirsch *et al.*, 1999; Lindsey *et al.*, 2001; Kolpin *et al.*, 2002; Göbel *et al.*, 2004), se trouvent parmi les plus utilisées au Québec. Ceci permettra de faire une sélection qui tient compte à la fois de la consommation locale et de leur persistance. Cette approche est clairement imparfaite, puisque certaines conditions du milieu récepteur (par exemple : traitements d'épuration avancés, facteur de dilution) peuvent fausser cette présomption. Cependant cette manière de procéder nous a permis de mieux cibler le développement analytique.

3.4. Conclusion

L'information sur la présence de traces des anti-infectieux dans les effluents urbains de Québec est rare. Un développement méthodologique a été entrepris afin d'être en mesure de déterminer la présence de trois QLs dans la matrice complexe de l'effluent de la SEVM. Les premiers tests réalisés avec les cartouches SPE indiquent que plusieurs difficultés sont à prévoir lors de l'extraction de traces d'anti-infectieux des eaux usées : présence d'interférences, faibles recouvrements, besoin de facteurs de préconcentration élevés (> 500).

L'utilisation des trois détecteurs différents couplés à la LC [FLD, TOFMS et MS/MS(QqQ)] a démontré que la technique analytique la plus convenable à l'analyse de traces anti-infectieux dans les effluents des stations d'épuration est sans doute la LC-MS/MS(QqQ).

Une méthode multi-classes capable d'extraire, quantifier et confirmer les traces d'anti-infectieux dans les eaux usées non traitées et celles subissant seulement un traitement primaire et physico-chimique est encore manquante. L'information sur la consommation des anti-infectieux au Québec ainsi que l'information sur l'occurrence trouvée dans la littérature permettra de mieux guider la sélection des anti-infectieux à cibler pour les futurs analyses.

Chapitre 4 : Détermination de six anti-infectieux dans les eaux usées en utilisant l'extraction sur phase solide en tandem et la chromatographie liquide couplée à la spectrométrie de masse en tandem

Determination of six anti-infectives in wastewater using tandem solid-phase extraction and liquid chromatography-tandem mass spectrometry

Segura P. A., Garcia-Ac A., Lajeunesse A., Ghosh D., Gagnon C. et Sauvé S.
J. Environ. Monit., 2007, 9: 307-313.

Note sur ma contribution

Ma participation aux travaux de recherche: J'ai réalisé les manipulations, l'analyse et l'interprétation des résultats.

Rédaction : J'ai rédigé l'article en m'appuyant sur les commentaires de Dr. Gagnon et du Prof. Sauvé, mes directeurs de thèse.

Collaboration des co-auteurs: A. Garcia-Ac et A. Lajeunesse m'ont assisté avec la préparation des échantillons. D. Ghosh a participé à la révision du manuscrit.

Abstract

A rugged and specific method based on tandem solid-phase extraction and liquid chromatography-tandem mass spectrometry for the determination of anti-infectives in raw sewage and wastewater plant effluents was developed. Analyte recoveries from spiked effluents ranged from 68 to 104%. Two specific selected reaction monitoring transitions and their peak area ratio were used to avoid false positives and confirm the presence of the targeted substances. Detection limits allowed low nanogram-per-liter detection (0.3-22 ng L⁻¹). The method was successfully applied to real samples from the Montréal wastewater treatment plant. All the studied anti-infectives were found in the wastewater samples in concentrations ranging from 39 to 276 ng L⁻¹. Mean flows of anti-infectives were estimated from effluent concentrations and it was found that large amounts (> 118 g day⁻¹ up to 830 g day⁻¹) are discharged in the receiving waters of the St. Lawrence River.

4.1. Introduction

“Anti-infectives” is a general term that refers to several classes of biologically active compounds used to treat or to prevent infections and includes therapeutic agents of synthetic origin such as the antimicrobials (e.g. sulfonamides, fluoroquinolones) or derived from natural or semi-natural sources such as the antibiotics (e.g. macrolides, tetracyclines). The widespread use of anti-infectives in urban centers as well as their resistance to biodegradation or elimination in wastewater treatment plants (WWTP) has led to their appearance in WWTP effluents and surface waters (Hirsch *et al.*, 1999; Kolpin *et al.*, 2002; Metcalfe *et al.*, 2003a). In the last few years, there has been a growing concern about the environmental fate and the possible effects of these agents on the aquatic environment (Wilson *et al.*, 2003; Richards *et al.*, 2004; Wilson *et al.*, 2004).

The first report on the occurrence of anti-infective traces in the aquatic environment was published as early as 1983 (Watts *et al.*, 1983). Later studies (Richardson et Bowron, 1985; Golet *et al.*, 2002) acknowledged that the source of pharmaceuticals in the environment was mainly the excreta of individuals taking medication at home, in hospitals or clinics and transported *via* wastewaters into receiving surface waters. Hence it is important to know the amounts of these substances released in the aquatic environment to be able to properly evaluate the risks, the effects and the potential impacts of these products.

According to IMS Health Canada (IMS Health Canada, 2005), anti-infectives were the 4th most prescribed therapeutic class in 2004 in Canada and almost 5 millions such prescriptions were dispensed in Québec (**Figure 4-1**). Since Montréal is the most populous city of the province, significant amounts of anti-infectives in the city’s sewage can be expected. However, because Montréal’s wastewaters are processed by one of the largest primary treatment plants of North America (average flow $\sim 2\,500\,000\text{ m}^3\text{ day}^{-1}$) the effluent wastewaters are made of a quite complex matrix (**Tableau 4-1**) and a selective extraction

method is necessary to properly determine the concentrations of anti-infectives released to the St-Lawrence River.

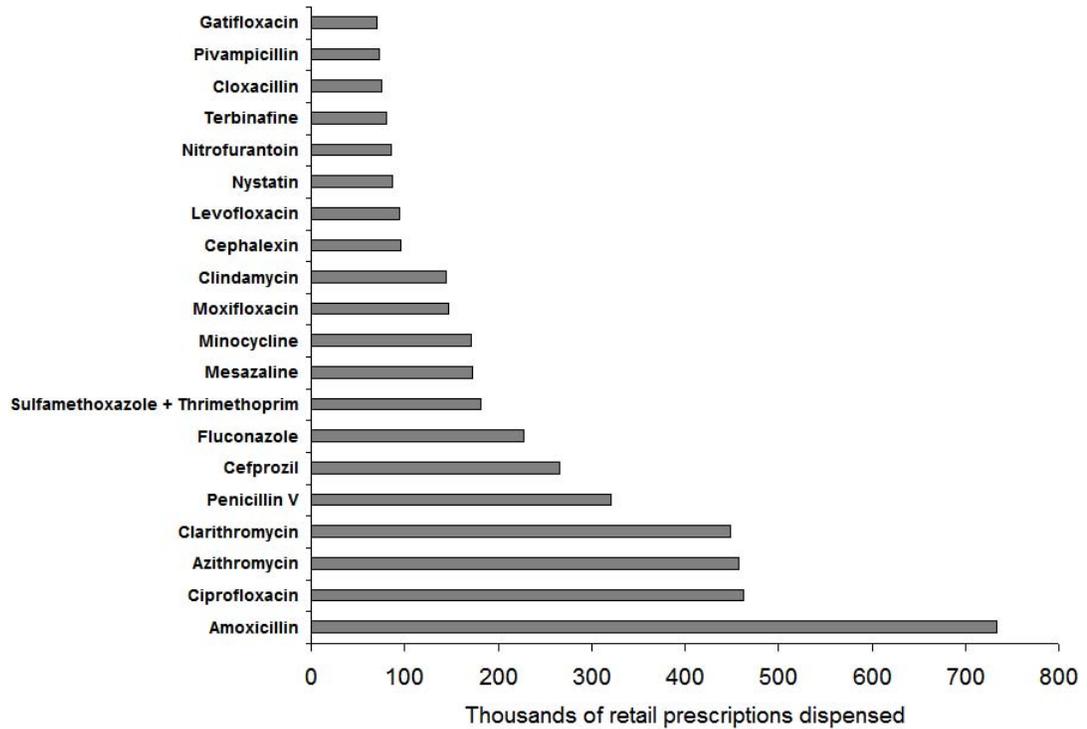


Figure 4-1. Most prescribed anti-infectives in Québec in 2004.

Presently, there are numerous solid-phase extraction (SPE) and liquid chromatography-mass spectrometry methods published in the literature for the determination of anti-infectives in environmental matrices, but few are able to successfully extract and quantify those compounds in more complex waters. To the authors' knowledge, only a handful of published methods show SPE recoveries in raw sewage (Lindberg *et al.*, 2004; Vieno *et al.*, 2006) or primary effluents (Golet *et al.*, 2001; Göbel *et al.*, 2004). Göbel *et al.* (Göbel *et al.*, 2004) obtained high recoveries (> 80 %) of various sulfonamide and macrolide anti-infectives but lower recoveries on trimethoprim (47 % \pm 2.8 %) on primary-treated effluents. Golet *et al.* (Golet *et al.*, 2001) reported high recoveries (> 81%) on different quinolones and fluoroquinolones. However the later method is based on fluorescence detection and proved to be unsuitable for Montréal's wastewaters which contain important

inputs of industrial wastes that seem to interfere with fluorescence detection. When analyzing the Montréal wastewater matrix, Segura *et al.* (Segura *et al.*, 2005) observed the presence of intense peaks eluting near the retention times of the analytes, making the quantification of norfloxacin, ciprofloxacin and enrofloxacin impossible by fluorescence. The authors explained the presence of those peaks by the possible presence of organic substances, not found in the Swiss effluent of the original work (Golet *et al.*, 2001), having excitation and emission wavelengths near those of the fluoroquinolones ($\lambda_{\text{ex}}=278$ nm, $\lambda_{\text{em}}=445$ nm). Lindberg *et al.* (Lindberg *et al.*, 2004) reported good recoveries of sulfamethoxazole (77 % \pm 5) and trimethoprim (87 % \pm 9) but weaker recoveries on ciprofloxacin (61 % \pm 5) in fortified hospital sewage samples and recently, Vieno *et al.* (Vieno *et al.*, 2006) obtained low absolute recoveries of ciprofloxacin (32 % \pm 24) in WWTP influents. To properly determine the occurrence of anti-infectives in raw sewage and primary-treated effluents, such as those of the city of Montréal, it is therefore necessary to develop a new rugged and specific method capable to quantify target analytes in spite of high dissolved organic carbon (DOC) concentrations.

Tableau 4-1. Physico-chemical parameters of raw and treated sewage of the city of Montréal.

| Property | North Influent | South Influent | Effluent |
|---|----------------|----------------|-----------------------------------|
| pH ^a | 7.5 | 7.4 | 7.5 |
| DOC (mg L ⁻¹) ^a | | | 39-44(Sabik <i>et al.</i> , 2004) |
| COD (mg L ⁻¹) ^a | 102 | 161 | 73 |
| BOD ₅ (mg L ⁻¹) ^a | 42 | 72 | 31 |
| SS (mg L ⁻¹) ^a | 89 | 110 | 17 |

^a Average values for the month of May of the physico-chemical properties of the month of May. DOC: dissolved organic carbon; COD: chemical oxygen demand, BOD₅: biological oxygen demand within a 5 day period; SS: Suspended solids.

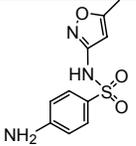
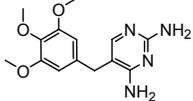
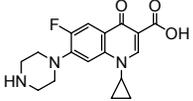
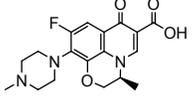
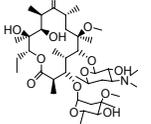
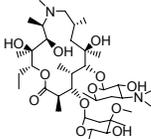
The main goals of the present study are: *i*) to develop a new SPE method optimized for the extraction of six highly prescribed (**Tableau 4-2**) anti-infectives from WWTP influents and primary effluents, *ii*) to determine the amounts of these compounds in raw sewage and WWTP primary effluents using liquid chromatography-tandem mass spectrometry (LC-MS/MS), *iii*) to confirm the presence of the detected compounds using a second specific selected reaction monitoring (SRM) transition and the area ratio of the two transitions.

4.2. Experimental

4.2.1. Materials and instruments

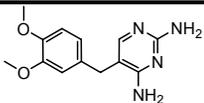
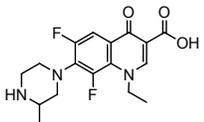
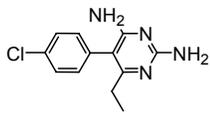
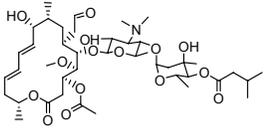
Pyrimethamine (PYR), sulfamethoxazole (SMX), Diaveridine (DIA), trimethoprim (TRI), ciprofloxacin (CIP), lomefloxacin (LOM), levofloxacin (LEV), azithromycin (AZI) josamycin (JOS), were purchased from Sigma-Aldrich Canada (Oakville, ON). Clarithromycin (CLA), was kindly provided by Abbot Canada (Montréal, QC). Disodium ethylenediaminetetraacetate (Na₂H₂EDTA) and formic acid 95% were purchased from Sigma-Aldrich Canada, sodium hydroxide 99% was obtained from Fischer Scientific Canada (Ottawa, ON) and ammonia 25% was purchased from BDH Chemicals (Toronto, ON). Solvents used for mobile phase preparation such as water, methanol (MeOH) and acetonitrile (ACN) were LC/MS grade, solvents used for cartridge elution such as MeOH, ACN and isopropanol (*i*-PrOH) were Optima grade and they were all obtained from Fisher Scientific Canada. Glass fiber pre-filters (1.2- μ m pore-size) and MF membranes (0.45- μ m pore diameter) were manufactured by Millipore (Billerica, MA). SPE cartridges Strata-X (surface-modified styrene divinylbenzene polymer) 200 mg/6 mL and Strata- X-C (benzenesulfonic acid group bonded on a polymeric surface) 200 mg/3 mL were purchased from Phenomenex (Torrance, CA). Liquid chromatography was carried out using a BetaBasic C₁₈ column (50 x 2.1 mm, 3- μ m diameter particle size) manufactured by Thermo Scientific (Waltham, MA).

Tableau 4-2. Physico-chemical properties and molecular structures of the studied anti-infectives.

| Anti-infective (Acronym) | Structure | MW (g mol ⁻¹) | pKa ^a | log Dow ^b |
|-----------------------------|---|------------------------------|--|---|
| Sulfamethoxazole (SMX) |  | 253.2776 | 1.7 - 1.85 (Szczepaniak et Szymański, 2000; Qiang et Adams, 2004) 5.60 - 5.70 (Vree et Hekster, 1987; Qiang et Adams, 2004) | 0.88 (Chemical Abstracts Service, 2006) |
| Trimethoprim (TRI) |  | 290.3177 | 1.32 - 3.23 (Cao et Cross, 1995; Qiang et Adams, 2004) 6.6 - 7.34 (Martindale, 1999; Kan et Petz, 2000) | -0.170 (Chemical Abstracts Service, 2006) |
| Ciprofloxacin (CIP) |  | 331.3415 | 5.90 - 6.09 (Ross et Riley, 1992; Drakopoulos et Ioannou, 1997) 8.74 - 8.89 (Ross et Riley, 1992; Drakopoulos et Ioannou, 1997) | -1.78 (Chemical Abstracts Service, 2006) |
| Levofloxacin (LEV) |  | 361.3675 | 5.97 - 6.10 (Drakopoulos et Ioannou, 1997; Park <i>et al.</i> , 2002) 8.22 - 8.28 (Ross et Riley, 1992; Drakopoulos et Ioannou, 1997) | -1.4 (Chemical Abstracts Service, 2006) |
| Clarithromycin (CLA) |  | 747.9534 | 8.3 - 8.99 (Bryskier <i>et al.</i> , 1993; McFarland <i>et al.</i> , 1997) | 0.06 (Chemical Abstracts Service, 2006) |
| Azithromycin (AZI) |  | 748.9845 | 8.1-8.74 (Bryskier <i>et al.</i> , 1993; McFarland <i>et al.</i> , 1997) 8.8-9.45 (Bryskier <i>et al.</i> , 1993; McFarland <i>et al.</i> , 1997) | -0.77 (Chemical Abstracts Service, 2006) |

^a Lowest and highest values reported in the literature. ^b Estimated values.

Tableau 4-2. Physico-chemical properties and molecular structures of the studied anti-infectives (suite).

| Internal standard (Acronym) | Structure | For compounds | Surrogate ^a |
|--------------------------------|--|---------------|---|
| Diaveridine (DIA) |  | TRI, SMX | |
| Lomefloxacin (LOM) |  | CIP, LEV |  |
| Josamycin (JOS) |  | CLA, AZI | Pyrimethamine (PYR) |

^a For all compounds.

SPE work was done on a 12-position vacuum manifold from Phenomenex. SPE eluates were evaporated using a 9-port Reacti-vap unit from Pierce (Rockford, IL). LC-MS/MS analyses were performed on a Thermo Scientific HPLC Surveyor System coupled to a TSQ Quantum Ultra triple quadrupole by Thermo Scientific.

4.2.2. Solutions

Stock solutions of 200 mg L^{-1} were prepared in MeOH except for the fluoroquinolones (CIP, LOM and LEV) which were dissolved in 5% NaOH 0.1 M/ 95% MeOH. All stock solutions were conserved at -15°C and used for no more than 3 months. ESI source optimization solutions of 1 mg L^{-1} each compound were prepared in 0.1 % formic acid in 50% H_2O / 25% MeOH /25% ACN. Mixed working solutions containing $500 \text{ } \mu\text{g L}^{-1}$ of the anti-infectives or $500 \text{ } \mu\text{g L}^{-1}$ of the internal standards were prepared fresh daily in 0.1% formic acid in 90% H_2O / 5% MeOH/ 5% ACN.

4.2.3. Sample collection and preparation

4.2.3.a. Recovery tests

The Montréal WWTP processes all of the wastewaters from the Montréal and Bizard islands, serving a population of over 1.8 million people. The primary treatment consists in a preliminary process (screening and grit removal) and a physico-chemical treatment (coagulation and sedimentation of suspended solids).

A series of 24-h composite samples of the primary effluent were collected in amber bottles at the WWTP. The bottles were placed in a cooler at 4°C and then transported to the laboratory. They were immediately filtered through $1.2\text{-}\mu\text{m}$ pore-size fiber glass filters and then $0.45\text{-}\mu\text{m}$ pore-size mixed cellulose ester membranes. Sub samples of 250 mL of the filtered wastewater were then transferred to volumetric glass flasks. To test the recovery, they were combined with a $250\text{-}\mu\text{L}$ aliquot of the $500 \text{ } \mu\text{g L}^{-1}$ anti-infective mixed working

solution. Sample pH was adjusted to 3 using 12.5 mmol of formic acid and NaOH 1.0 M. As a sample additive, 1 mL of a 5% Na₂EDTA (w/v) solution was added to increase the recovery of the macrolide antibiotics (Hirsch *et al.*, 1998).

Three different methods were tested. For method I and II, a Strata-X cartridge was used and for method III a Strata-X in tandem with the Strata-X-C cartridge (Strata-X in top of Strata-X-C) was used. For all methods tested, the cartridges were conditioned using 2 x 2.5 mL MeOH and then 2 x 2.5 mL of deionized water containing 50 mM of formic acid and 200 mg L⁻¹ Na₂EDTA adjusted to pH=3 with NaOH. Samples were introduced in the cartridges at a flow rate of 2-3 mL min⁻¹ by negative pressure using Teflon tubes and a vacuum manifold connected to a water trap and a pump. After the cartridges were loaded with the sample, they were dried for 15 min with N_{2(g)} at maximum pressure (20 in Hg). After drying, the cartridges were eluted in three different ways at a flow rate of 2.5 mLmin⁻¹: 2 x 2.5 mL MeOH:ACN 1:1 (method I), 2 x 2.5 mL MeOH:ACN:*i*-PrOH 5:5:2 (method II) and 2 x 2.5 mL MeOH:ACN 1:1 for the Strata-X cartridge and 2 x 2.5 mL 5% NH₃/MeOH:ACN 1:1 for the Strata-XC cartridge (method III). The elution solvent was allowed to soak the cartridge for 5 min before each elution.

Eluates were recovered in conical-bottom centrifuge tubes. For method III, the eluates were recovered from both cartridges and were collected on the same conical-bottom centrifuge tube. Eluates were then evaporated to dryness with a gentle stream of N_{2(g)} at 30°C and then reconstituted to 250 µL with 0.1% formic acid in 90% H₂O/ 5% MeOH/ 5% ACN solution.

4.2.4. LC-MS/MS conditions

For LC, the column temperature was set to 30°C. Solvent A was 0.1 % formic acid/H₂O (pH≈2.9) and solvent B was 0.1% formic acid/MeOH:ACN 1:1. The injection volume was 20 µL using the full loop mode. Mobile phase flow rate was set to 200 µL min⁻¹. The following gradient elution program (mobile phase B) was used: 0 min 10 %, 2 min 10 %, 8 min 20%, 10 min 20%, 16 min 60%, 20 min 95%, 25 min 95%, 30 min 10%, 35 min 10%.

For MS/MS, ionization was performed using electrospray in the positive mode (ESI+). Ion source parameters were optimized for each compound using the Quantum Tune application. ESI optimization solutions were infused with syringe pump of the TSQ Quantum Ultra and mixed using a tee with the LC column flow (200 $\mu\text{L min}^{-1}$) before being introduced into the ESI source. Quantum Tune was programmed to look for the most intense SRM transitions for each compound. Non-specific SRM transitions (*i.e.* showing neutral losses of H₂O or CO₂) were not used because many molecules could have similar fragment losses and therefore are not specific to the analyte parent ion. For example, fragmentation of CIP yielded three major product ions: m/z 231, m/z 314 and m/z 288. While m/z 314 was more intense than m/z 288, the later was not used as it represented a loss of water. Only the two more intense and specific SRM transitions were re-optimized to get the best possible source and ion focusing values.

Tableau 4-3. SRM transitions used for quantification (SRM #1) and confirmation (SRM #2).

| Compound | SMR #1 (m/z) | CE (V) | SRM #2 (m/z) | CE (V) | Tube Lens |
|----------|---------------------|-----------|---------------------|-----------|-----------|
| PYR | 249.10 → 177.07 | 40 | | | 86 |
| SMX | 254.08 → 92.11 | 36 | 254.08 → 108.10 | 37 | 70 |
| DIA | 261.15 → 123.11 | 34 | | | 86 |
| TRI | 291.16 → 123.10 | 33 | 291.16 → 230.17 | 34 | 91 |
| CIP | 332.16 → 288.15 | 27 | 332.16 → 231.07 | 49 | 82 |
| LOM | 352.17 → 265.13 | 34 | | | 89 |
| LEV | 362.17 → 261.12 | 35 | 362.17 → 221.05 | 43 | 92 |
| CLA | 748.55 → 590.36 | 19 | 748.55 → 115.99 | 35 | 96 |
| AZI | 375.33 → 82.96 | 25 | 749.54 → 158.04 | 38 | 74/112 |
| JOS | 828.53 → 108.87 | 46 | 828.53 → 173.96 | 47 | 126 |

Common source parameters for each compound were averaged to get overall optimal signal intensity and stability and set to the following values: spray voltage, 3500 V; ion transfer capillary temperature, 350 °C; sheath gas pressure, 21 mTorr; auxiliary gas

pressure, 4 mTorr; collision gas pressure, 1.5 mTorr and source CID, -12 V. Tube lens and collision energies (CE) are compound-specific and appear in **Tableau 4-3**.

4.2.5. Anti-infective determination in WWTP influents and primary effluents

The wastewater interception network of the city of Montréal is divided in two main sectors: north and south. The northern interceptor collects sewage coming from the west and north side of the city while the southern interceptor collects sewage and rainwater from the south side of the city. We collected 24-h composite samples from the WWTP influent (North and South interceptors) and effluent on May 16th 2006. The mean flow for this date was $35.3 \text{ m}^3 \text{ s}^{-1}$. Matrix characteristics are shown in **Tableau 4-1**. The samples were prepared exactly as above, and they were extracted using method III. To verify the extraction recovery, prior to the extraction, 250 μL of the surrogate standard (PYR) was spiked to get a final concentration of 500 ng L^{-1} . After evaporation, dried residues were reconstituted to 250 μL with 0.1% formic acid in 90% H_2O / 5% MeOH/ 5% ACN solution containing $500 \mu\text{g L}^{-1}$ of the internal standards (DIA, LOM and JOS).

4.2.6. Quantification and data analysis

Chromatogram peaks were integrated using the ICIS algorithm of Xcalibur 1.2 by Thermo Scientific. Signal-to-noise (S/N) ratios were determined with the manual noise region option in Xcalibur. This method was preferred to the default peak to peak method as it was observed that the later overestimated S/N ratios. Analyte recoveries were compared using a one-way ANOVA test and significant differences ($p < 0.05$) were elucidated with Tukey's b post-hoc test using the statistical software SPSS 13 by SPSS Inc. (Chicago, IL).

Calibration standards used for quantification were prepared by diluting the primary effluent matrices with deionized water by a factor of 10 and then preparing the samples using method III. Five concentration levels were used: 0, 25, 50, 250 and $1000 \mu\text{g L}^{-1}$ and the internal standards and the surrogate were added at a constant concentration of $500 \mu\text{g L}^{-1}$. Calibration curve standards were injected twice during the analysis sequence.

The zero level was subtracted from all levels to correct for the anti-infectives already present in the diluted matrix. Internal standards were chosen because of similar structure and physico-chemical properties. These substances are not marketed in Canada the probability of their presence in the matrix is very low. Their absence in the sample matrices was confirmed by LC-MS/MS analysis of non-fortified samples.

4.2.7. Method validation

To avoid false blanks, we used a second SRM transition as well as the area ratio of the two transitions to confirm the presence of the identified anti-infectives. According to the identification point system proposed by the European Commission (Commission of the European Communities, 2002), by using one precursor ion and two product ions, each compound earns four identification points (IP), which then fulfils the requirements for identification and confirmation of environmental contaminants (Petrović et Barceló, 2006). In order to qualify for the IPs required for confirmation, ion ratios must agree within specified tolerances (from $\pm 20\%$ for relative ion intensities $> 50\%$ to $\pm 50\%$ for relative ion intensities $\leq 10\%$).

Limits of detection (LOD) were determined using the wastewater effluent samples since field blanks are not available. LODs were estimated by averaging the measured S/N ratio of the analyte peak in the samples ($n=3$) and then downscaling to calculate the concentration of the target analyte able to generate a S/N ratio equal to 3. Linear dynamic ranges (LDR) were estimated using calibration curves prepared with the diluted effluent matrix.

4.3. Results and discussion

4.3.1. Recovery Tests

The Strata-X cartridge is a reversed-phase sorbent made of a surface modified styrene divinylbenzene polymer. It is designed to enhance retention on polar and aromatic analytes by H-bonding and π - π interactions. The Strata-X-C sorbent has a benzenesulfonic acid group uniformly bonded on a polymeric surface and therefore has cation-exchange properties. This type of cartridges have been used previously in trace analysis of anti-infectives (Calamari *et al.*, 2003) but it shows lower recoveries on the less polar compounds. Recovery tests results are shown in **Tableau 4-4**. For method I, analyte recoveries of SMX ($50\% \pm 3$), CIP ($44\% \pm 1$) and LEV ($78.7\% \pm 0.2$) were lower than those of TRI ($90\% \pm 2$), AZI ($88\% \pm 6$) and CLA ($88\% \pm 6$). Elution of the cartridges with a solvent mix containing 16% *i*-PrOH (method II) provided no significant differences compared to method I.

The tandem SPE approach (method III) significantly improved the recovery of SMX ($68\% \pm 5$) TRI ($104\% \pm 4$), CIP ($76\% \pm 5$) and LEV ($97\% \pm 5$) compared to the single cartridge methods (I and II) and was therefore applied to all subsequent analyses. Extraction recoveries of CLA ($100\% \pm 2$) and AZI ($92\% \pm 4$) with the tandem SPE method were not significantly different to method I and II. Tandem SPE methods have been used to eliminate interferences (Christian *et al.*, 2003; Renew et Huang, 2004) but to the authors' knowledge, they have never been applied to more complex water matrices such as raw sewage or primary effluents. The combination of reversed-phase and ion exchange surface chemistry proved to be an effective way to simultaneously extract from wastewaters various anti-infectives having different chemical properties such as pK_a and D_{ow} .

The improvement on the recovery of compounds such as the fluoroquinolone antimicrobials CIP and LEV can be explained by their ionic nature. Batt *et al.* (Batt et Aga, 2005) reported that the mechanism for the interaction of the fluoroquinolones with the solid phase in the Oasis HLB cartridges (poly[divinylbenzene-co-N-vinylpyrrolidone]) is based on electrostatic rather than hydrophobic interactions. At pH=3, both CIP and LEV are almost completely in their protonated form which strongly interacts with the ion exchanger benzenesulfonic acid group of the Strata-X-C cartridge, therefore increasing overall retention. Interestingly, SMX recovery is also improved using the tandem SPE method. At pH=3, SMX is mostly in its neutral form ($pK_a=1.7-1.85$) and thus its retention can not be improved by ion exchange. However SMX has a benzene ring that can interact *via* London

π - π interactions with the polymer backbone of the Strata-XC cartridge, which yields a higher retention. The recovery of the macrolide antibiotics AZI and CLA was very high on all three methods (> 85%) and no significant differences were observed among them, which suggests that the main retention mechanism of these compounds is by Van der Waals forces on the Strata-X cartridge.

Tableau 4-4. Mean recoveries obtained with the three methods tested. The anti-infectives were spiked in the primary effluent at a 500 ng L⁻¹ level ($n=2$).

| Compound | Recovery (%) \pm STD | | | | | |
|----------|------------------------|------------------------|-----------|------------------------|------------|----------------------|
| | Method I | | Method II | | Method III | |
| SMX | 50 | \pm 3 ^a | 38 | \pm 3 ^a | 68 | \pm 5 ^b |
| TRI | 90 | \pm 2 ^a | 89 | \pm 3 ^a | 104 | \pm 4 ^b |
| CIP | 44 | \pm 1 ^a | 50 | \pm 3 ^a | 76 | \pm 5 ^b |
| LEV | 78.7 | \pm 0.2 ^a | 74.1 | \pm 0.8 ^a | 97 | \pm 5 ^b |
| CLA | 96 | \pm 6 ^a | 109 | \pm 3 ^a | 100 | \pm 2 ^a |
| AZI | 88 | \pm 6 ^a | 93.6 | \pm 0.1 ^a | 92 | \pm 4 ^a |

Results followed by the same letters in the same row are not significantly different ($p < 0.05$).

4.3.2. Method validation

Tandem mass spectrometry in the SRM mode proved to be highly specific. When two specific SRM transitions are employed, the possibility of false positives is reduced as some matrix interferences co-extracted with the analytes could have the same SRM transition (**Figure 4-2**). Sample SRM transition area ratios were reproducible (RSD <10%) and differences with SRM transition area ratios of spiked standards in the matrix were not higher than 19 %, except for AZI (64%). Instrument response was linear ($r^2 \geq 0.99$) in the dynamic range (25-1000 ng L⁻¹) in spite of the presence of high concentrations of organic as well as inorganic interferences in the matrix. Limits of detection ranged from 0.3 to 22 ng L⁻¹ (**Tableau 4-5**). Method uncertainty was in most cases < 8% RSD, except for CLA and AZI in the South influent.

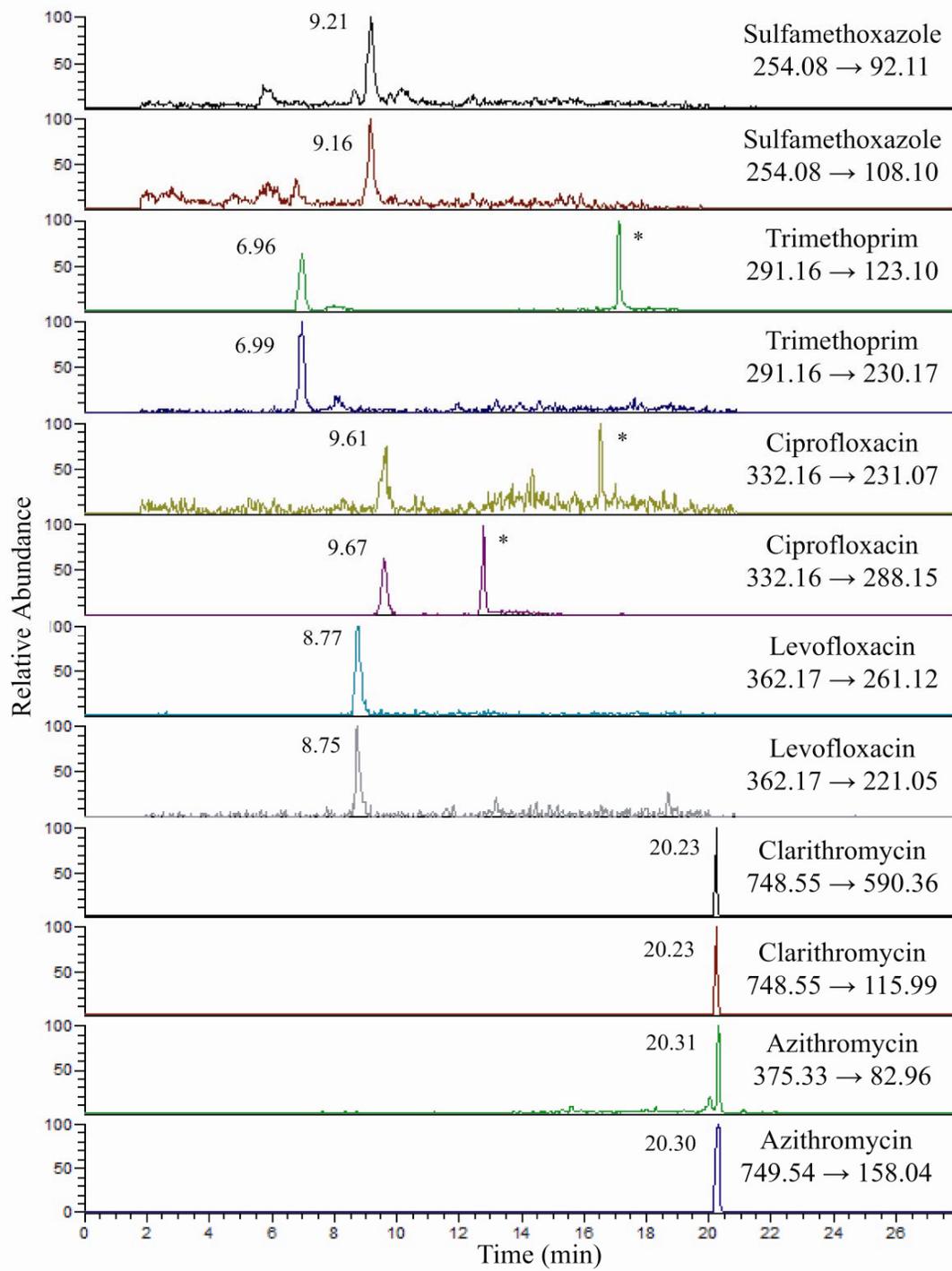


Figure 4-2. Chromatograms showing two SRM transitions of the studied compounds in treated wastewater. Peaks due to interferences are marked by asterisks (*). The presence of the studied anti-infectives was effectively and unambiguously confirmed by their two specific SRM transitions as well as their area ratio.

4.3.3. Anti-infective determination in WWTP influents and primary effluents

Results of the analysis of real samples (**Figure 4-3**) showed that all targeted anti-infectives were found in the Montréal WWTP influents and effluents. The anti-infective found at the highest concentration was CLA in the North influent ($263 \pm 7 \text{ ng L}^{-1}$) and LEV was found at the lowest concentration in the treated effluent ($39 \pm 1 \text{ ng L}^{-1}$). Concentrations of SMX and TRI in the effluent are similar to previously published data of samples collected at the same WWTP (Gagné *et al.*, 2006). The reported levels for all anti-infectives are lower or in the same order of magnitude of other Canadian (Metcalf *et al.*, 2003b; Miao *et al.*, 2004), American (Yang *et al.*, 2003; Renew *et al.*, 2004) or European cities (Hartig *et al.*, 1999; McArdell *et al.*; Petrović *et al.*, 2003; Göbel *et al.*, 2004).

Tableau 4-5. Analytical method parameters

| Compound | r^2 matrix ^a | Limit of detection ^b (ng L ⁻¹) | Standard SRM ratio \pm SD ^c | Sample SRM ratio \pm SD ^d | SRM ratio difference ^e (%) |
|----------|---------------------------|--|--|--|--|
| SMX | 0.9995 | 22 | 1.53 ± 0.03 | 1.6 ± 0.2 | -2.6 |
| TRI | 0.9998 | 7 | 4.2 ± 0.1 | 4.39 ± 0.07 | -3.3 |
| CIP | 0.9996 | 21 | 5.5 ± 0.8 | 6.59 ± 0.05 | -18.9 |
| LEV | 0.9996 | 4 | 3.65 ± 0.07 | 3.83 ± 0.06 | -5.0 |
| CLA | 0.9997 | 0.3 | 1.67 ± 0.04 | 1.59 ± 0.09 | 4.3 |
| AZI | 0.9900 | 12 | 1.2 ± 0.1 | 0.44 ± 0.1 | 64 |

^a Determination coefficient of the calibration curve made using the WWTP effluent diluted by a factor of 10; ^b Calculated from the effluent data based on a signal-to-noise ratio=3; ^c Standards spiked WWTP effluent diluted by a factor of 10, $n=4$; ^d WWTP effluent, $n=3$; ^e Percentage difference between the standard and sample SRM ratio.

Estimated mean flows of the studied anti-infectives in the St. Lawrence River (SMX 340 ± 30 , TRI 310 ± 20 , CIP 320 ± 10 , LEV 118 ± 2 , CLA 830 ± 60 and AZI $310 \pm 20 \text{ g day}^{-1}$) show that in spite of the sub-microgram per liter concentrations of anti-infectives found in the Montréal WWTP effluent, large amounts of those products are discharged into

the receiving waters of the St. Lawrence River. Compared to the mass flow of CIP discharged by Klotten-Opfikon tertiary WWTP in the Glatt River in Switzerland (1.3 ± 0.6 g day⁻¹), the mean mass flow of CIP coming from the Montréal WWTP is more than 200 times higher. More information is necessary to evaluate the ecotoxicological consequences arising from the large amounts of these biologically active compounds being continuously released into the St. Lawrence River.

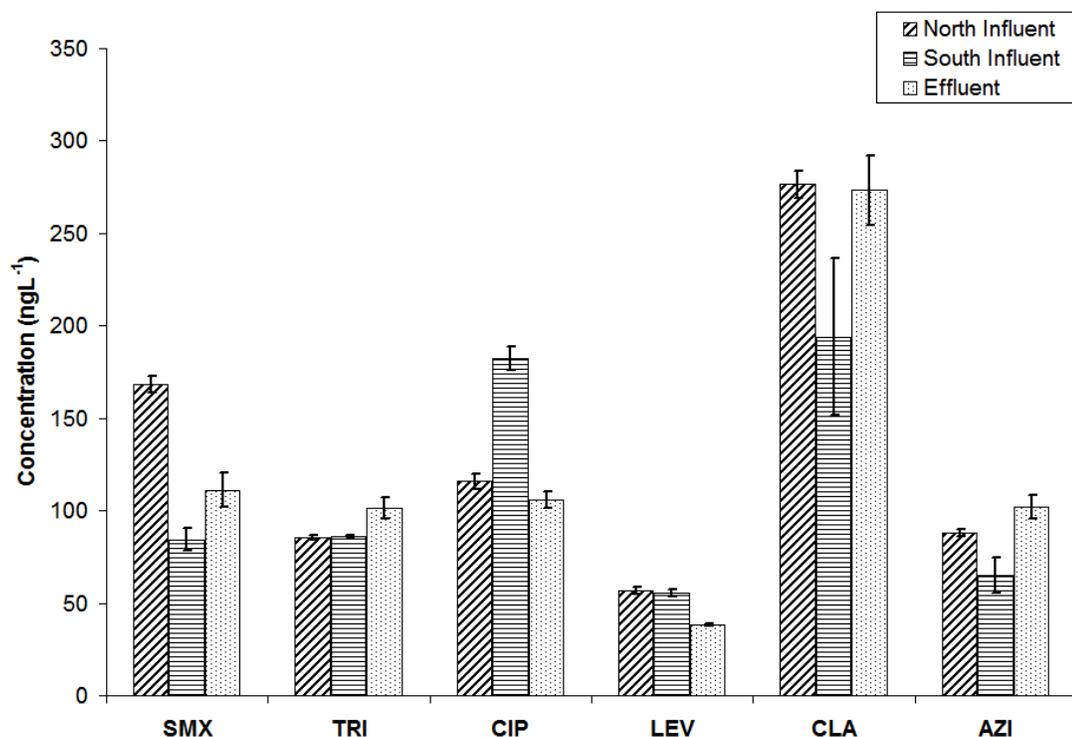


Figure 4-3. Occurrence of the targeted anti-infectives in the North influent, South influent and primary effluent of the Montréal WWTP.

4.4. Conclusion

The developed tandem SPE LC-MS/MS method proved to be a rugged and specific method for the extraction and quantification of anti-infectives in wastewaters containing high concentrations of DOC. The tandem SPE method improved anti-infectives recoveries compared to single cartridge methods. Recoveries using tandem SPE cartridges were higher than 75%, except for SMX which was 68%. Detection limits ranged from 0.3 to 22 ng L⁻¹

and instrument response was linear ($r^2 \geq 0.99$) in the dynamic range (25-1000 ng L⁻¹). The method was successfully applied to real samples from the Montréal wastewater treatment plant. The use of two specific SRM transitions and their area ratio proved to be a reliable and effective way to reduce false positives and confirm the presence of the targeted substances. All the studied anti-infectives were found in the wastewater samples in concentrations ranging from 39 to 276 ng L⁻¹. Mean flows of anti-infectives were estimated from effluent concentrations and it was found that, despite the low concentrations of these biological active compounds in the treated sewage, given the significant water flow, large amounts are actually discharged in the receiving waters of the St. Lawrence River. More studies are necessary to assay the potential risk and effects of anti-infectives on aquatic biota.

4.5. Acknowledgements

We thank the Environment Canada-Conservation Service Research Fund, the National Research and Engineering Research Council of Canada and the Canadian Foundation for Innovation for their financial support. We are also grateful for the help of Luc Tremblay and Christine Yelle at the Montréal WWTP.

Chapitre 5 : Méthode complètement automatisée de préconcentration et chromatographie liquide couplée à la spectrométrie de masse en tandem pour l'analyse des anti-infectieux dans les eaux usées

A fully automated on-line preconcentration and liquid chromatography-tandem mass spectrometry method for the analysis of anti-infectives in wastewaters

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Anal. Chim. Acta, 2007, **604**, 147-157.

Note sur ma contribution

Ma participation aux travaux de recherche: J'ai réalisé les expériences, l'analyse des résultats et leur interprétation.

Rédaction : J'ai rédigé l'article en m'appuyant sur les commentaires de Dr. Gagnon et du Prof. Sauvé, mes directeurs de thèse.

Abstract

We developed and validated a novel on-line preconcentration liquid chromatography tandem mass spectrometry method for the determination of anti-infectives in wastewaters. The presented method preconcentrates 1 mL of sample in a load column using a switching-valve technique. The method was optimized with respect to sample load flow rate, volume of the load column wash and organic solvent content of the load column wash. The sample is cleaned using a 30 % organic solvent washing step and then gradually eluted to an analytical column for separation. To compensate for matrix effects, quantitation was performed using standard additions. Confirmation of the presence of the detected compounds was done using a second selective reaction monitoring transition. Method intra-day precision was less than 9% and inter-day precision %RSD varied between 2.5 and 23%. Limits of detection for the selected anti-infective compounds ranged from 13 to 61 ng L⁻¹. All the target anti-infectives were found in the city of Montreal WWTP effluent in concentrations ranging from 71 to 289 ng L⁻¹. This automated method eases the rapid quantitation of those trace contaminants using small sample volumes.

5.1. Introduction

The anti-infectives are a class of therapeutic pharmaceuticals comprising several families of biologically active compounds used to treat or to prevent infections and includes substances of synthetic origin such as the antimicrobials (e.g. sulfonamides, fluoroquinolones) or derived from natural or semi-natural sources such as the antibiotics (e.g. macrolides, tetracyclines).

The occurrence of anti-infectives in surface waters has generated a lot of concern in the scientific community in past years because of the possible effects of these compounds on the aquatic environment (Wilson *et al.*, 2003; Richards *et al.*, 2004) as well as their potential contribution to increasing bacterial resistance to anti-infectives (Kümmerer et Henninger, 2003). The first record on the occurrence of anti-infectives in the environment was published by Watts *et al.* in the United Kingdom. The authors reported the presence of erythromycin and tetracycline at the microgram-per-liter level in river water (Watts *et al.*, 1983).

Since wastewater treatment plants (WWTP) have been identified as the main entry point of anti-infectives in the environment (Hirsch *et al.*, 1999; Ternes et Joss), the development of effective analytical methods is essential for the determination of the amounts of these contaminants that enter the environment as well as the efficiency of WWTP to remove them. Numerous methods for the determination of anti-infectives in sewage and surface waters have been published in the scientific literature (Díaz-Cruz et Barceló, 2006; Gros *et al.*). However, most of these methods are based on off-line solid-phase extraction (off-line SPE), *i.e.* extraction and preconcentration steps are done separately from the chromatographic separation and quantitation. Although some on-line preconcentration methods exist for the analysis of pesticides (Hernández *et al.*, 2001; Sancho *et al.*, 2004), herbicides (Castro *et al.*, 2000), drug residues in animal tissue (Tang *et al.*, 2006) or estrogens in natural waters and waterworks (Rodríguez-Mozaz *et al.*, 2004; Salvador *et al.*), only a few on-line methods for the determination of anti-infectives in environmental matrices have been published up to now. Stoob *et al.* (Stoob *et al.*, 2005) analyzed various sulfonamide antimicrobials and their metabolites in surface waters and

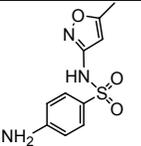
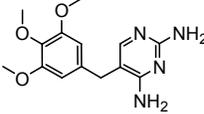
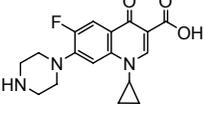
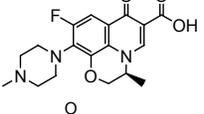
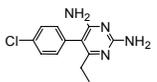
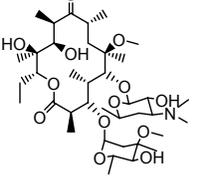
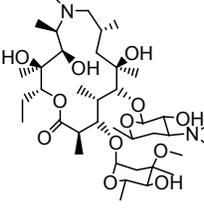
obtained limits of detection (LOD) as low as 1 ng L^{-1} using 18 mL sample injections employing on-line solid-phase extraction-liquid chromatography-tandem mass spectrometry (SPE-LC-MS/MS). Pozo *et al.* (Pozo *et al.*, 2006) detected quinolones and β -lactams in ground and surface waters using only 9.8 mL of sample and obtained LOD ranging from 0.4 to 4.4 ng L^{-1} using SPE/LC-MS/MS. Recently Choi *et al.* (Choi *et al.*, 2007) used on-line solid-phase extraction-liquid chromatography-mass spectrometry to quantify several sulfonamides and tetracyclines in sewage and agricultural wastewaters utilizing 10 mL of sample and reported a LOD of 30 ng L^{-1} .

The development of SPE on-line methods is an important step for the study of anti-infectives in the environment because they have clear advantages compared to SPE off-line methods such as reduced sample manipulation and time, smaller sample volume requirements, better intra and inter-day reproducibility, higher sample throughputs as well as an increase on the number of replicas that can be analyzed in a single day, all of which further improves the quality of the analytical data reported.

However, the development of SPE on-line methods presents several difficulties such as purchase of specialized equipment, hardware modifications and staff training. In addition, the transfer of previous off-line methods to on-line mode is not straightforward: incompatibility between SPE sorbents and analytical columns, mobile phase pH incompatibility and peak broadening, among other problems, are often observed (Oliferova *et al.*, 2006).

In this paper, we report the development of a simple and economic on-line preconcentration method for the analysis of six selected anti-infectives (**Tableau 5-1**). These compounds account for more than a third of all the annual anti-infectives retail prescriptions dispensed by community drugstores in Québec, which were close to 5 million in 2006 (IMS Health Canada, 2007). Our method uses standard liquid chromatography-tandem mass spectrometry equipment with minor modifications that allow quantitation and confirmation down to the low nanogram-per-liter level of six target analytes in complex matrices such as sewage.

Tableau 5-1. Structures and physico-chemical properties of the target compounds.

| Anti-infective (Acronym) | Structure | MW (g mol ⁻¹) | pK _a ^a | log K _{ow} ^b | Internal standard |
|-----------------------------|---|------------------------------|--|--|---|
| Sulfamethoxazole (SMX) |  | 253.2776 | 1.7 - 1.85 (Szczepaniak et Szymański, 2000; Qiang et Adams, 2004) 5.60 - 5.70 (Vree et Hekster, 1987; Qiang et Adams, 2004) | 0.91 (Szczepaniak et Szymański, 2000) | |
| Trimethoprim (TRI) |  | 290.3177 | 1.32 - 3.23 (Cao et Cross, 1995; Qiang et Adams, 2004) 6.6 - 7.34 (Martindale, 1999; Kan et Petz, 2000) | 0.91 - 1.364 (Boxall <i>et al.</i> , 2006; Wishart <i>et al.</i> , 2006) | |
| Ciprofloxacin (CIP) |  | 331.3415 | 5.90 - 6.09 (Ross et Riley, 1992; Drakopoulos et Ioannou, 1997) 8.74 - 8.89 (Ross et Riley, 1992; Drakopoulos et Ioannou, 1997) | (0.94) - 0.4 (Ross <i>et al.</i> , 1992; Tolls, 2001) | |
| Levofloxacin (LEV) |  | 361.3675 | 5.97 - 6.10 (Drakopoulos et Ioannou, 1997; Park <i>et al.</i> , 2002) 8.22 - 8.28 (Ross et Riley, 1992; Drakopoulos et Ioannou, 1997) | (0.28) - 0.35 (Ross <i>et al.</i> , 1992; Takács-Novák <i>et al.</i> , 1992) |  Pyrimethamine (PYR) |
| Clarithromycin (CLA) |  | 747.9534 | 8.3 - 8.99 (Bryskier <i>et al.</i> , 1993; McFarland <i>et al.</i> , 1997) | 3.16 (McFarland <i>et al.</i> , 1997) | |
| Azithromycin (AZI) |  | 748.9845 | 8.1 - 8.74 (Bryskier <i>et al.</i> , 1993; McFarland <i>et al.</i> , 1997) 8.8 - 9.45 (Bryskier <i>et al.</i> , 1993; McFarland <i>et al.</i> , 1997) | 4.02 (McFarland <i>et al.</i> , 1997) | |

^aLowest and highest values reported in the literature. ^bParentheses indicate negative values.

5.2. Experimental

5.2.1. Reagents and material

Pyrimethamine (PYR), sulfamethoxazole (SMX), trimethoprim (TRI), ciprofloxacin (CIP), levofloxacin (LEV), azithromycin (AZI) and formic acid 98% pure (F.A.), were purchased from Sigma-Aldrich Canada (Oakville, ON). Clarithromycin (CLA) was kindly provided by Abbot Canada (Montréal, QC). PYR was used as internal standard because it was not present in the wastewater samples, showed good stability in solution and it also gives a strong signal with electrospray ionization on the positive mode (ESI+). Solvents used for mobile phase preparation such as water, methanol (MeOH) and acetonitrile (ACN) were LC/MS grade and were obtained from J.T. Baker (Phillipsburg, NJ). Glass fiber pre-filters (1.2- μm pore-size) and mixed cellulose ester membranes (0.45- μm pore diameter) were manufactured by Millipore (Billerica, MA). Deionized water (DI water) was generated by a Millipore Milli-Q RG water purification system.

5.2.1.a. Solutions

Stock solutions of 200 mg L^{-1} were prepared in MeOH except for the fluoroquinolones (CIP and LEV) which were dissolved in 5% NaOH 0.1 M-95% MeOH. All stock solutions were conserved at -15°C and used for no more than 3 months. ESI+ source optimization solutions of 1 mg L^{-1} of each compound were prepared in 0.1% F.A. in 50% H_2O -25% MeOH-25% ACN. Mixed working solutions containing $100 \mu\text{g L}^{-1}$ of the anti-infectives or $100 \mu\text{g L}^{-1}$ of the internal standard (PYR) were prepared weekly in ACN and kept at -15°C .

5.2.1.b. Instrumentation

Sample preconcentration and separation was performed using the EQuan system developed by Thermo Fisher Scientific (Waltham, MA). The EQuan system is based on a column-switching technique. It consists in an upgrade kit for the Surveyor HPLC system,

allowing the injection and preconcentration of up to 1.8 mL of sample using a high-flow pump, a low-flow pump, a load column and an analytical column (**Figure 5-1**).

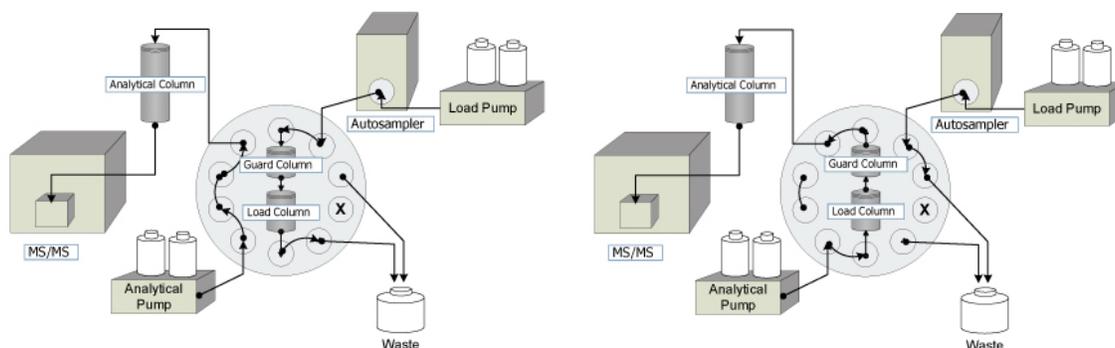


Figure 5-1. *Left:* Divert valve in the “load” position. *Right:* Divert valve in the “inject” position Experimental setup of the on-line preconcentration LC-MS/MS system. Arrows indicate flow direction. To make the diagram easier to understand, the analytical column was drawn outside the autosampler but in reality it is placed inside it for temperature control.

Sample delivery in the EQUAN system is carried out with the Surveyor Autosampler Plus. This autosampler was adapted to allow one-milliliter injections. Modifications to the autosampler include the installation of a 2.5-mL injection syringe, a sample transfer tube extension and a 2.5-mL PEEK loop made by Upchurch Scientific Inc. (Oak Harbor, WA). A high-flow Surveyor LC Pump Plus was used for sample loading (load pump) and a low-flow Surveyor MS pump was used for liquid chromatography (analytical pump). Column-switching was performed using a Rheodyne 9750E 10-port valve manufactured by Rheodyne LLC (Rohnert Park, CA) but a standard 6-port valve can be used as well. Sample loading and preconcentration was performed on a Hypersil Gold C₁₈ (20 x 2.1 mm, 12 μm particle-diameter, 175 Å pore-size) manufactured by Thermo Fisher Scientific (Waltham, MA). A Hypersil Gold Guard column C₁₈ (10 x 2.1 mm, 3 μm) was used to protect the load column. Liquid chromatography was carried out using a Hypersil Gold column C₁₈ (50 x 2.1 mm, 3-μm, 175 Å). Sample ionization was done by ESI⁺ with the Ion Max API Source and tandem mass spectrometry was performed on a TSQ Quantum Ultra AM triple quadrupole (Thermo Fisher Scientific).

5.2.2. Sample collection and preparation

The Montréal WWTP is the largest primary physico-chemical treatment plant of the Americas treating each day, on average, 2 500 000 m³ of water (Ville de Montréal, 2002a). Its treatment process consists in a mechanical removal of large solid matter and grit, as well elimination of suspended solid matter and phosphorus by flocculation (Communauté Urbaine de Montréal, 1999a).

A series of flow-proportional 24-h composite samples of the Montréal WWTP effluent were collected in amber bottles on May 28th 2007. The bottles were placed in a cooler at 4 °C and then transported to the laboratory. They were immediately filtered through 1.2- μ m pore-size fiber glass filters and then 0.45- μ m pore-size mixed cellulose ester membranes. The wastewater samples were transferred to 25-mL volumetric flasks and pH was adjusted to ~3 with 250 μ L of F.A. To avoid C₁₈ chain collapse during sample loading, 750 μ L of MeOH : ACN (1:1) was also added to the sample. For internal calibration, a 120- μ L aliquot of the 100 μ g L⁻¹ internal standard (PYR) working solution was added. Sub samples of 1.8 mL were then transferred to amber glass vials for on-line preconcentration followed by LC-MS/MS analysis.

5.2.3. On-line preconcentration

On-line sample preconcentration was optimized by a series of tests to study the behavior of the system to variations of key parameters such as sample load flow rate, volume of the load column wash and organic solvent content of the load column wash. These assays were used to find the optimal parameters for maximum performance of the system. Sample preconcentration in the EQuan system is illustrated in **Figure 5-1**. Vials containing the samples were placed in the autosampler tray kept at 4°C. The divert valve was at the “load” position and the autosampler programmed to draw 1.00 mL of the sample from the vial and inject it in the 2-mL injection loop using the partial loop mode.

Tableau 5-2. Mobile phase program for the load and analytical pumps.

| Load pump ^a | | | Analytical pump | | |
|------------------------|---------------------------------|------------------------|-----------------|---------------------------------|------------------------|
| Time (min) | Flow (mL min ⁻¹) | % B in mobile phase | Time (min) | Flow (μL min ⁻¹) | % B in mobile phase |
| 0.00 | 1.50 | 3.0 | 0.00 | 200.0 | 10 |
| 0.75 | 1.50 | 3.0 | 4.00 | 200.0 | 10 |
| 0.76 | 1.50 | 30.0 | 10.00 | 200.0 | 20 |
| 2.50 | 1.50 | 30.0 | 12.00 | 200.0 | 20 |
| 2.75 | 0.250 | 30.0 | 18.00 | 200.0 | 60 |
| 3.00 | 0.250 | 97.0 | 22.00 | 200.0 | 95 |
| 22.50 | 0.250 | 97.0 | 22.50 | 500.0 | 95 |
| 23.00 | 0.500 | 97.0 | 23.00 | 500.0 | 95 |
| 26.00 | 0.500 | 97.0 | 26.00 | 500.0 | 95 |
| 26.50 | 0.500 | 3.0 | 26.50 | 500.0 | 10 |
| 27.00 | 0.500 | 3.0 | 30.00 | 500.0 | 10 |
| 30.00 | 0.500 | 3.0 | | | |

^a Divert valve initial position was “Load”. The valve was actuated at 2.5 min to the “Inject” position and then at 23.0 min it was actuated again to the “Load” position.

The injected sample was then introduced in the load column with 97% of solvent A (0.1% F.A.-H₂O) and 3% of solvent B [0.1% F.A.-MeOH : ACN (1:1)] using the load pump at a flow rate of 1.5 mL min⁻¹. The column was then washed by increasing the percentage of solvent B in the mobile phase from 3% to 30%. After 2.5 min, the divert valve was switched to the “inject” position and the column is back-flushed using the analytical pump flow. The complete load pump gradient is shown in **Tableau 5-2**.

5.2.4. Liquid chromatography

Analytes retained by the load column were gradually eluted by back-flushing the load column (flow direction inside the column was reversed) with the analytical pump gradient composed of solvent A: 0.1 % F.A.-H₂O and solvent B: 0.1% F.A.-MeOH : ACN (1:1). Once the analytes were eluted from the load column, they were introduced in the analytical column where chromatographic separation takes place. The gradient elution program is

shown in **Tableau 5-2**. Column temperature was set to 30°C. Separated compounds were then directed to the ion source.

5.2.5. Electrospray-tandem mass spectrometry

Ionization of analytes was performed using ESI+. Ion source parameters were optimized for each compound using the Quantum Tune application of Xcalibur 2.0 software. ESI+ optimization solutions were infused with the syringe pump of the TSQ Quantum Ultra AM and mixed using a tee with the LC flow (200 $\mu\text{L min}^{-1}$) before being introduced into the ESI+ source. Quantum Tune was programmed to look for the most intense selected reaction monitoring (SRM) transitions for each compound. Non-specific SRM transitions (*i.e.* showing neutral losses of H₂O or CO₂) were not used for quantitation because many molecules could have similar fragment losses and those are therefore not specific to the analyte parent ion. However, for the second SRM transition, used only for confirmation, non-specific transitions were allowed. Only two SRM transitions were retained and they were re-optimized to get the best possible source and ion focusing values.

Common source parameters for each compound were averaged to get overall optimal signal intensity and stability and set to the following values: spray voltage, 4000 V; ion transfer capillary temperature, 350 °C; sheath gas pressure, 50 arbitrary units and auxiliary gas pressure, 15 arbitrary units. Skimmer offset was set to -10 V and collision gas pressure to 1.5 mTorr. Tube lens and collision energies (CE) are compound-specific and appear in **Tableau 5.1**. Mass spectrometer resolution was set to 0.7 u Full Width at Half Maximum (FWHM) and the scan time to 0.25 s.

5.2.6. Data analysis

Integration of chromatographic peaks and quantitation were performed using the LCQuan 2.5 software (Thermo Fisher). Calibration curves were built with the response ratio (area of the analyte standard divided by area of the internal standard) as a function of the

analyte concentration. Statistical analysis such as linear regressions and Dunnett's T3 post-hoc test were done with SPSS 13 software (SPSS Inc., Chicago, IL).

Tableau 5.1. SRM transitions used for quantitation (SRM #1) and confirmation (SRM #2).

| Compound | SMR #1 | | Collision | | SRM #2 | | Collision | | Tube Lens |
|----------|--------|---|------------|----|--------|---|------------|----|-----------|
| | (m/z) | | Energy (V) | | (m/z) | | Energy (V) | | |
| PYR | 249.1 | → | 177.1 | 30 | N.A | | N.A | | 93 |
| SMX | 254.1 | → | 156.0 | 13 | 254.1 | → | 108.1 | 18 | 72 |
| TRI | 291.2 | → | 230.1 | 23 | 291.2 | → | 123.1 | 35 | 92 |
| CIP | 332.1 | → | 231.0 | 32 | 332.1 | → | 288.1 | 16 | 80 |
| LEV | 362.2 | → | 261.1 | 27 | 362.2 | → | 318.1 | 33 | 92 |
| CLA | 748.5 | → | 158.0 | 28 | 748.5 | → | 590.2 | 17 | 110 |
| AZI | 749.5 | → | 591.2 | 25 | 375.4 | → | 83.1 | 17 | 121/72 |

5.2.7. Method validation

Sample carryover was evaluated by injecting a series of blanks after a high concentration standard (1000 ng L⁻¹) with the following formula:

$$\text{Carryover (\%)} = \left(\frac{A_{\text{blank}}}{A_{\text{standard}}} \right) \times 100\% \quad (\text{Équation 5-1})$$

where A_{blank} is the average ($n=3$) peak area of the analyte in the blank and A_{standard} is the average peak area of the analyte at 1000 ng L⁻¹. Three different types of calibration curves were tested for quantitation: *i*) 0.1% F.A. in 1.5% ACN-1.5 MeOH-97% DI water (v/v) (internal calibration), *ii*) WWTP effluent diluted by a factor of 10 with 0.1% F.A. in 1.5% ACN-1.5 MeOH-97% DI water (v/v) (internal calibration) and *iii*) WWTP effluent with 0.1% F.A. and 1.5 % ACN-1.5% MeOH (v/v) (standard additions). They contained the analytes at five different concentrations (0, 75, 150, 250 and 1000 ng L⁻¹) and were injected in triplicate.

Method precision (intra and inter-day) was determined using a wastewater sample spiked at the 500 ng L⁻¹ level, injected three times each during the sequence run (intra-day

precision) or three times on three different days (inter-day precision). Recoveries were evaluated in tap water, diluted WWTP effluent and in the wastewater effluent by analyzing five replicates and then applying the following equation:

$$\text{Recovery (\%)} = \left(\frac{C_{m+s} - C_m}{C_s} \right) \times 100\% \quad (\text{Équation 5-2})$$

where C_{m+s} is the measured concentration of the analyte in the spiked matrix, C_m is the original concentration of the analyte in the matrix and C_s is the concentration spiked in the matrix. Limits of detection (LOD) were determined using the standard error of the intercept and the slope of the calibration curve as proposed by The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (ICH, 2005). Matrix effects were evaluated using the following equation:

$$\text{Matrix Effects (\%)} = \left(\frac{R_{m+s} - R_m}{R_0} - 1 \right) \times 100\% \quad (\text{Équation 5-3})$$

where R_{m+s} is the response ratio of the analyte in the spiked matrix, R_m is the response ratio of the analyte in the unspiked matrix and R_0 is the response ratio of the analyte in DI water or diluted wastewater. In this equation, similar to a previously published equation to calculate signal suppression (Renew et Huang, 2004), positive values indicate signal enhancement and negative values represent signal suppression due to the matrix. Column lifetime was assessed by monitoring changes on the peak asymmetry factor (A_s) (Foley et Dorsey, 1983) and the retention time (t_R) of CLA in standards prepared in 0.1% F.A. in 1.5% ACN-1.5 MeOH-97% DI water (v/v) at the 250 ng L⁻¹ level injected at the beginning and the end of the analyses.

5.3. Results and discussion

Contrary to the majority of published on-line methods for the analysis pharmaceuticals and personal care products (PPCPs) in environmental samples, which use additional equipment for the preconcentration step, the proposed method uses standard HPLC equipment with minor modifications, which eases the transfer of validated off-line methods to the on-line mode without the need to purchase specialized equipment or specific staff

training. Also, because only one-milliliter of sample is used, analysis time is further decreased (down to ~30 min from up to ~10 h for off-line methods) and instrument maintenance is notably reduced.

5.3.1. Optimization

In order to obtain the highest sensitivity, to maximize recoveries and to eliminate as much matrix interferences as possible, we performed a series of tests to optimize the preconcentration step of our method. Three essential parameters were optimized: sample load flow rate, wash volume and wash organic solvent content.

5.3.1.a. Effect of sample load flow rate

While performing solid-phase extraction, load or elute flow rates that are too fast may not allow enough time for the analytes of interest to be bound or removed from the sorbent (Jordan). Also, very high flow rates cannot be used because of the pressure limits of the load column. Conversely, very low flow rates are impractical, because of the large dead volume of the system. Therefore, it is of critical importance to optimize the speed at which the analytes are introduced in the load column. We optimized the sample load flow rate by measuring the effect of three different flow rates on the analyte response (**Figure 5-2**).

As can be observed in **Figure 5-2**, at lower flow rates, the analyte response is greatly reduced or even completely inexistent. These results were surprising because we expected to have stronger signals for the lower flow rates. A possible explanation might be that the slower flow rates may have contributed to a deconditioning of the stationary phase, inhibiting its capacity to retain the analytes. A sample load flow rate of 1.5 mL min⁻¹ showed the best response and reproducibility and was used for all subsequent analysis as well as for the washing step. Higher flow rates were not tested to avoid system pressure increase which may affect the long term reliability of the system as column back pressure will gradually increase with time because of the accumulation of particulate matter (Snyder *et al.*, 1997).

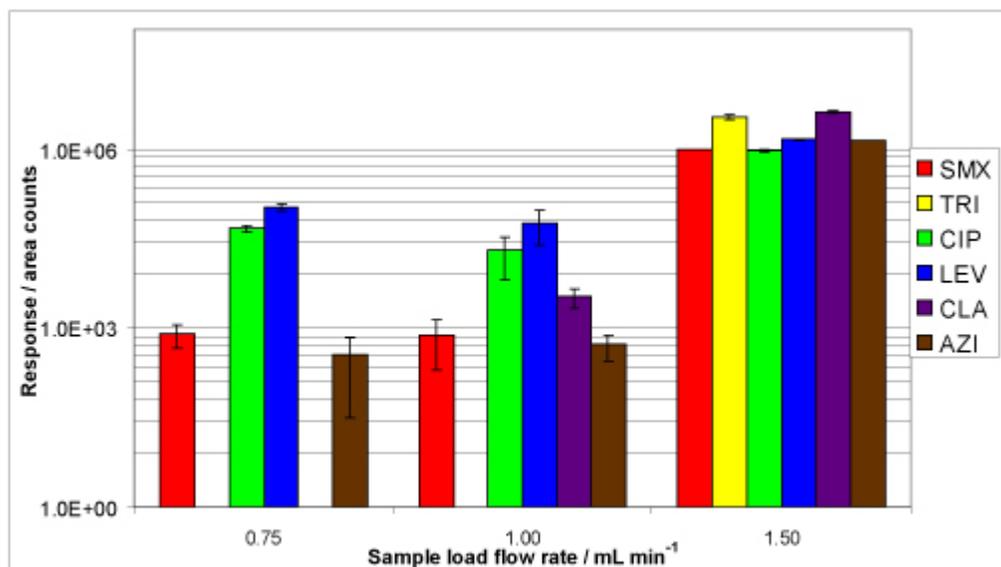


Figure 5-2. Effect of sample load flow rate on analyte response ratios. Analyte response was used instead of response ratio because sample load flow rates affected internal standard signal intensity. Length of error bars represents two standard deviations (2σ) of triplicate measurements.

5.3.1.b. Effect of wash volume

A column-switching method applied to wastewater samples must be able to eliminate as much matrix interferences as possible without affecting sensitivity. Matrix interferences are defined as the components from the sample that inhibit the ability to accurately quantitate the components of interest (Jordan, 2000). Matrix interferences can reduce column life-time and cause source fouling as well. Matrix interference elimination is usually done by washing the loading column with a higher proportion of organic solvent than the initial conditions or by switching the divert valve to prevent highly-retained compounds from entering the analytical column. However, one must be careful with this washing step, because excessive washing could lead to analyte losses. We tested the effect of four different washing volumes on the analyte response ratio, the washing were done using a 90% aqueous (0.1% F.A-H₂O) and 10% organic (0.1% F.A-MeOH : ACN (1:1)) composition (**Figure 5-3**).

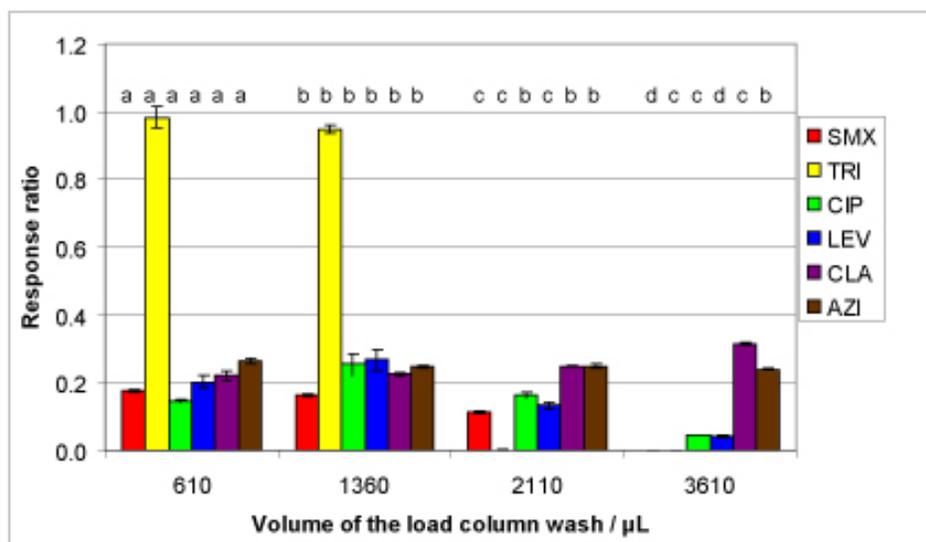


Figure 5-3. Effect of wash volume on analyte response ratios. Length of error bars represents two standard deviations (2σ) of triplicate measurements. Bars of the same anti-infective with the same letters are not significantly different ($p < 0.05$) according to Dunnet's T3 post-hoc test.

As expected, the response ratio of the first four eluting compounds (TRI, LEV, CIP and SMX) is reduced as these are washed out of the loading column. The response ratio of the last two eluting compounds remains more or less constant as higher amounts of organic are necessary to elute them from the loading column. We performed a one-way ANOVA Dunnet's T3 post-hoc test to discern significant differences ($p > 0.05$) among the observed results. This showed that there are no significant differences between the 610 and 1360 μL wash. We chose these two volumes to measure the effects of the wash organic solvent content.

5.3.1.c. Effect of wash organic solvent content

We tested the effect of four different amounts of organic solvent in the composition of the wash solvent: 10, 20, 30 and 50 %, using 610 and 1360 μL wash volumes. Assay results are shown in **Figure 5-4**.

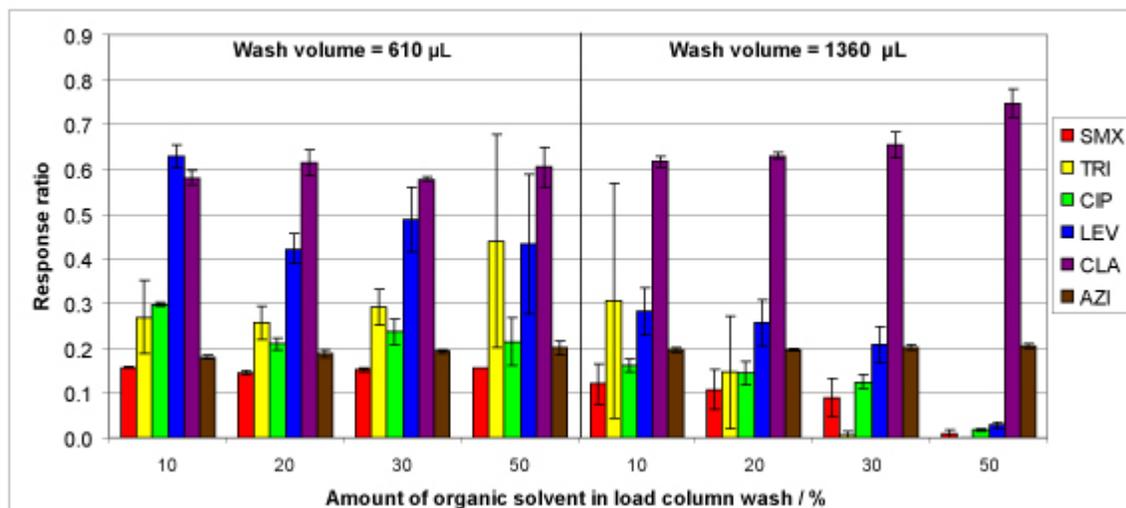


Figure 5-4. Effect of wash solvent strength on analytes response ratios. Length of error bars represents two standard deviations (2σ) of triplicate measurements.

Similarly to the effect of the wash solvent volume, response ratios of the early eluting compounds decrease as the organic solvent content of the wash is increased. This effect is more drastic for the 1360- μ L wash volume in which case we see that at 50 % content of organic solvent, most analytes are washed out of the column and only the most highly retained compounds (CLA and AZI) maintain a strong response. Results show that either a 20 or 30 % of organic solvent using a 610- μ L wash volume are seemingly optimal to obtain the highest response ratios and the lowest signal variability for all target compounds. To improve the wash benefits on decreasing potential interfering compounds, we chose to continue with the 30 % organic solvent wash and we applied it to all subsequent analyses.

5.3.2. On-line preconcentration

The sample volume that can be injected in this configuration of the EQUAN system is limited by the volume of the vials (1.8 mL). Injection volumes higher than 1 mL were not tested because of the dead volume increase when using the partial loop mode. The maximum injection volume allowed by the Surveyor Plus system in the partial loop mode is half the volume of the sample loop. Full loop injection, albeit more precise, cannot be performed by the system with 1-mL injections because it withdraws from the vial a volume largely superior to the vial volume (Thermo Electron Corporation, 2005). So in order to perform 1-mL injections, a 2-mL loop must be used. When adding a large volume loop, the

dead volume of the system is increased and this has a profound effect on the load pump program. First, the flow rate has to be high enough to introduce the sample in the loading column in a reasonable time. Second, any mobile phase composition change has to take into account the increased dead volume of the system. Therefore the mobile phase composition change necessary for washing the load column must start before the sample has finished being loaded. We programmed the load pump to change the composition of the mobile phase from 3% organic to 30% organic at 0.76 min because at a 1.5 mL min⁻¹ flow rate, it takes ~1.33 min to reach the loading column. The divert valve is then switched at 2.5 min, which allows a ~ 610- μ L wash.

5.3.3. Liquid Chromatography

We used an existing liquid chromatography method for the determination of anti-infectives in wastewaters (Segura *et al.*, 2007b) and adapted it for on-line analysis. Compared to classical small injection volumes, we observed that the on-line preconcentration analyte peaks show a major increase in peak width, however, this seems to be a natural consequence of the column switching process (Oliferova *et al.*, 2006). Original gradient table times were modified for the on-line setup by adding an extra period for sample introduction. The sample is desorbed using the same mobile phase composition as in the offline SPE method, thus maintaining the original chromatographic conditions.

5.3.4. Method validation

5.3.4.a. Carryover

Carryover is a memory effect in which sample from a previous injection is observed during the analysis of subsequent injections (Elmashni, 2004). It is often the result of an inadequate flush of the liquid chromatography injection system, and therefore, it is important to reduce it to a minimum, to avoid sample contamination. On-line preconcentration methods, because they use larger samples volumes than their classic off-

line counterparts, are then more prone to be affected by carryover. We used two different strategies to reduce carryover: *i*) use of higher volumes of rinse and flushing solvents and *ii*) actuate the valve back to the “load” position once the last compound was eluted from the chromatography column to effectuate a better wash of the load column.

Elmashni (Elmashni, 2004) recommends using a higher volume of solvent to flush the autosampler injection system. We used a volume of 2 mL for flushing and 3 mL for washing. Also, by actuating the switching valve to the “load” position after the last analyte was eluted from the analytical column, a more aggressive wash (3% of mobile phase A and 97% of mobile phase B) of the load column could be performed without desorption of retained interferences, avoiding their entry into the analytical column. Results showed that carryover was less than 0.3% after the first injection and it was reduced to less than 0.2 % after the second injection. After a third injection carryover was not further decreased; however such low peak areas do not have impact on quantitation because they are much lower than the detection limits.

5.3.4.b. Recovery

Method recoveries were initially determined using a low (100 ng L^{-1}) and high (500 ng L^{-1}) spiked concentrations to evaluate the proportion of analyte added to the matrix that the method is able to accurately quantify. **Figure 5-5** shows that recoveries for most compounds deviate from the ideal value of 100% for the two spike levels and their standard deviation increases with matrix complexity. For all analytes in the two spike levels, recoveries in tap water were found between $98 \% \pm 5$ and $124 \% \pm 9$, which shows that the column-switching system is able to quantitatively concentrate and extract nanogram-per-liter levels of anti-infectives. However, recoveries in WWTP effluent, calculated using calibration curves in DI water, varied between 80 ± 13 and 224 ± 8 . As these results show, calibration curves in DI water cannot be used for the quantitation of the target anti-infectives in wastewater because of the complexity of the matrix and its effect on the electrospray signal. According to Taylor *et al.* (Taylor, 2005) co-eluting matrix components modify the ionization efficiency of the ESI source causing either analyte signal suppression or enhancement. The exact mechanism of these matrix effects still remains unknown but is

probably due to interactions between the analyte and the matrix molecules on the electrospray micro-droplets surface during the ESI process (Kuhlmann *et al.*, 1995; King *et al.*, 2000). Renew *et al.* studied the effect of the amount of organic matter on matrix effects. They observed that signal suppression for anti-infectives such as fluoroquinolones, sulfonamides and trimethoprim showed approximately a positive linear correlation to Suwannee River organic matter concentration (Renew et Huang, 2004).

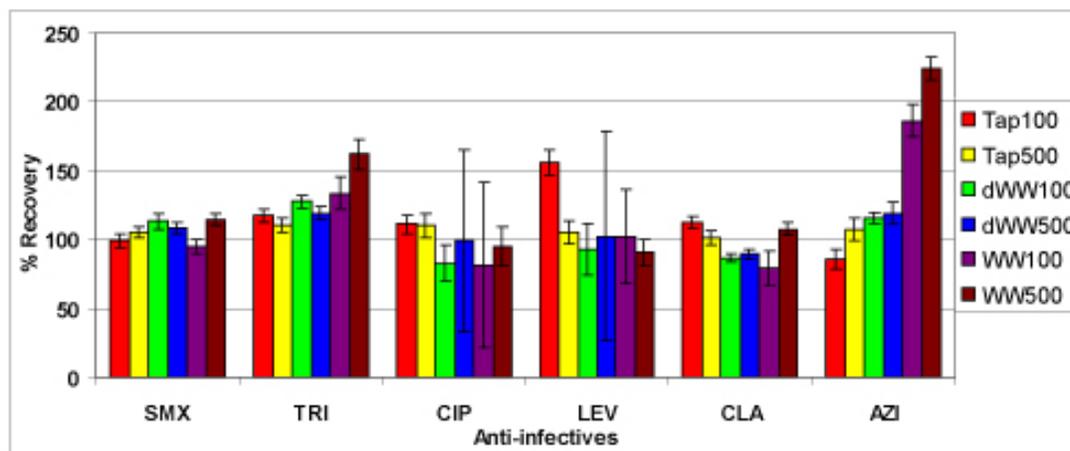


Figure 5-5. Method percentage recoveries for 100 ng L⁻¹ and 500 ng L⁻¹ spike of analyte in tap water (Tap), diluted WWTP (dWW) effluent water (by a factor of 10) and WWTP effluent water (WW). The length of error bars was determined using propagation of uncertainty rules (Harris, 1999).

Percentage matrix effect (%ME) calculated using the internal calibration curves made in DI water (**Tableau 5.2**) shows that for some compounds, signal suppression is low (*e.g.* SMX, %ME=-4%) while for other compounds, signal enhancement more that doubles the response of the analyte in wastewater compared to DI water (*e.g.* AZI, %ME=+116%). Quantitation of anti-infectives in wastewater using calibration curves in pure water or groundwater is a common practice in the literature (Göbel *et al.*, 2004; Batt et Aga, 2005; Vieno *et al.*, 2006), however in the present method, no isotopically-labeled internal standards are used to compensate for matrix effects, and therefore other calibration methods are necessary (Stüber et Reemtsma, 2004).

To account for the presence of matrix effects during the ESI+ process, it was decided to prepare a calibration curve using the diluted WWTP effluent as done in the previous work that used off-line SPE (Segura *et al.*, 2007b). As **Tableau 5.2** shows, this approach proved to be unsuccessful, and while %ME were reduced for some of the analytes (*e.g.* LEV and CLA) compared to the calibration curve in DI water, ESI+ signal is still altered, and its utilization for quantitation is unacceptable. A reason for the failure of this technique in the present on-line method may be due to an incomplete sample clean-up or to differences between the retention properties of the C₁₈ preconcentration column and the polymeric SPE cartridge used in the previous work. Consequently, interferences present in the matrix are not totally eliminated during the washing step and they co-elute with the compounds of interest, distorting the ESI+ signal.

Tableau 5.2. Matrix effects calculated using the calibration curve in DI water (cc-DI) and diluted wastewater (cc-dWW)

| Compound | cc-DI | | | | | | cc-dWW | | | | | |
|----------|---------------------|---|-----|---------------------|---|------|---------------------|---|-----|---------------------|---|------|
| | %ME100 ^a | | | %ME500 ^b | | | %ME100 ^c | | | %ME500 ^d | | |
| | (%) | | | (%) | | | (%) | | | (%) | | |
| SMX | -4.0 | ± | 0.2 | 10.2 | ± | 0.2 | -36.3 | ± | 1.9 | -1.54 | ± | 0.03 |
| TRI | 14 | ± | 2 | 43 | ± | 3 | -8.9 | ± | 0.6 | 32 | ± | 2 |
| CIP | -27 | ± | 20 | -5.2 | ± | 0.8 | -38 | ± | 24 | -13 | ± | 2 |
| LEV | -35 | ± | 12 | -25 | ± | 2 | -9 | ± | 3 | -15.0 | ± | 2.2 |
| CLA | -29 | ± | 4 | -1.67 | ± | 0.05 | -29 | ± | 4 | 14.0 | ± | 0.6 |
| AZI | 116 | ± | 7 | 151 | ± | 3 | 62 | ± | 2 | 88 | ± | 3 |

Percentage matrix effects in the WWTP effluent compared to: ^a DI water for a 100 ng L⁻¹ spike, ^b DI water for a 500 ng L⁻¹ spike, ^c Diluted wastewater for a 100 ng L⁻¹ spike and ^d Diluted wastewater for a 500 ng L⁻¹ spike.

It has been pointed out that the best strategy to correct matrix effects is the standard addition method (Reemtsma, 2001; Stüber et Reemtsma, 2004). Standard additions is an accurate and powerful method for quantification in analytical chemistry, nonetheless it has seldom been used in the environmental determination of anti-infectives (Lindsey *et al.*, 2001; Renew et Huang, 2004) because it is laborious and time-consuming (Stüber et Reemtsma, 2004; Ternes et Joss, 2006). However, when working with an on-line

preconcentration and detection system, standard additions become the method of choice for quantification.

Standard additions calibration curves (**Tableau 5-3**), show excellent determination coefficients ($r^2 > 0.99$), for most of the compounds except for TRI, CIP and LEV ($0.8674 < r^2 < 0.9555$). However, significance values of the F statistic of the ANOVA test were in all cases lower than 0.05 which suggests that the variation of the response ratio is well explained by the variation of the analyte concentration.

5.3.4.c. Precision, limits of detection and column lifetime

Limits of detection (LOD) were evaluated in the WWTP effluent based on the calibration curve, by multiplying the standard error of the y-intercept by 3.3 and dividing the result by the slope of the standard additions calibration curve (ICH, 2005). LOD for the quantification and the confirmation SRM transitions are shown in Table 5. As the presence of any analyte must be confirmed by the presence of a detectable peak by a second SRM transition, method LOD are limited by the sensitivity of the second SRM transition.

Tableau 5-3. Standard additions calibration curve parameters, limits of detection and method precision.

| Compound | Equation ^a | r^2 | LOD _{ww} | LOD _{ww} | Intra-day | Inter-day |
|----------|------------------------|--------|---|---|----------------------------------|----------------------------------|
| | | | SRM#1 ^b (ng L ⁻¹) | SRM#2 ^c (ng L ⁻¹) | precision ^d (%RSD) | precision ^e (%RSD) |
| SMX | $y = 0.065 + 0.00023x$ | 0.9950 | 12 | 13 | 1.6 | 2.5 |
| TRI | $y = 0.049 + 0.00047x$ | 0.9555 | 22 | 21 | 0.30 | 9.8 |
| CIP | $y = 0.026 + 0.00011x$ | 0.8674 | 59 | 61 | 4.8 | 21 |
| LEV | $y = 0.011 + 0.00015x$ | 0.8818 | 29 | 32 | 8.6 | 23 |
| CLA | $y = 0.231 + 0.00097x$ | 0.9981 | 19 | 17 | 3.4 | 9.8 |
| AZI | $y = 0.045 + 0.00058x$ | 0.9965 | 5 | 37 | 1.7 | 21 |

^a All regression lines were weighted with a factor $w=1/y^2$ except for CLA which had homogeneous variance across all the levels, units of x are in ng L⁻¹ ^b Limit of detection for the quantitation SRM transition ^c Limit of detection for the confirmation SRM transition ^d Relative standard deviation of wastewater samples ($n=3$)

spiked at the 500 ng L⁻¹ level the same day ^e Relative standard deviation of wastewater ($n=3$) samples spiked at the 500 ng L⁻¹ injected on three different days ($N=9$).

Method precision evaluated during the same day (intra-day) and three different days during a three-week period (inter-day) using a 500 ng L⁻¹ level standard are shown in Table 5. Intra-day method precision ranged from 0.3 to 8.6 % while for the inter-day assay it varied from 2.5 to 23%. While inter-day precision values for CIP, LEV and AZI maybe be higher than the 15 % that it is normally accepted (Rosing *et al.*, 2000) given the complexity of the matrix and the possibility of degradation of the analytes during the sequence runs, precision can thus be considered acceptable.

Tests of the lifetime of the columns showed that more than 250 samples (of which more than 100 were wastewater samples) could be analyzed with the same columns without a major degradation of the peak shape (A_s increased only by 3.3%) and change of the retention time (t_R decreased by less than 0.2 s).

5.3.4.d. Quantitation in WWTP effluents

In most on-line methods found in the literature, large sample volumes (up to 500 mL) are often used to detect PPCPs because of their low concentration in the environment (Bones *et al.*, 2006). However, our method proved to be efficient to quantitate and confirm anti-infectives down to the low nanogram-per-liter level in a complex matrix (Total Organic Carbon = 24.1 mg L⁻¹) such as wastewater using 1-mL injections. Results show that all the six studied anti-infectives were detected in the WWTP effluent samples. Their presence was unequivocally confirmed by a second SRM transition (**Figure 5-6**).

Measured concentrations ranged from 71 ± 12 ng L⁻¹ for LEV to 289 ± 7 ng L⁻¹ for SMX. Occurrence of anti-infectives in the WWTP effluent of the City of Montréal has been previously reported. (Gagné *et al.*, 2006; Segura *et al.*, 2007b) **Figure 5-7** shows the concentrations found in the literature compared to our on-line preconcentration method.

While most of the reported values show significant differences, concentrations show the same order of magnitude for samples taken at different dates. Similar low nanogram per liter concentration values of anti-infectives have been reported in most of the municipal effluents of other Canadian cities as well (Metcalf *et al.*, 2003a; Miao *et al.*, 2004; Lee *et al.*, 2007a).

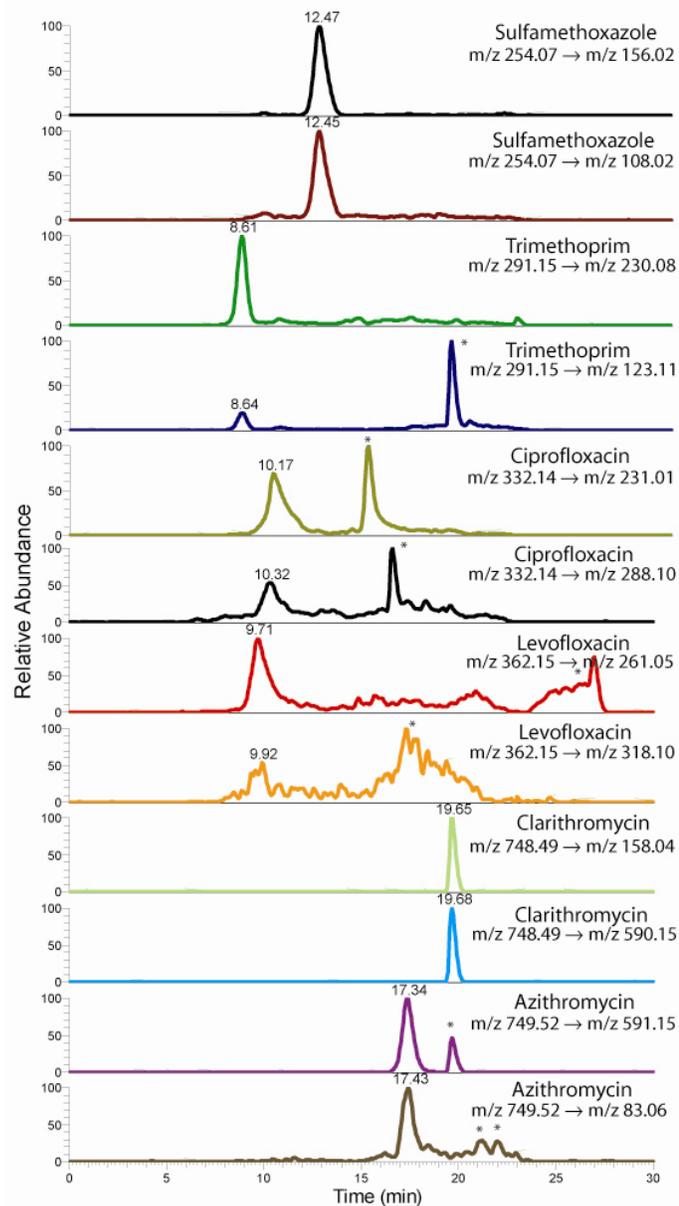


Figure 5-6. LC-MS/MS chromatogram of the WWTP effluent showing the two SRM transitions used for quantification and confirmation of the target anti-infectives. Peaks due to interferences are indicated by asterisks (*).

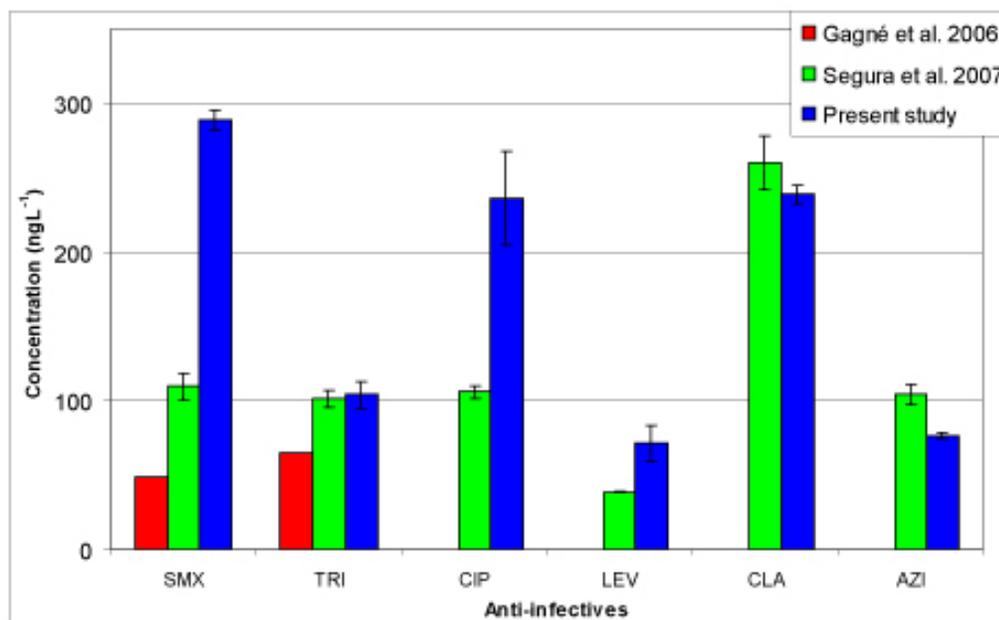


Figure 5-7. Comparison of measured concentrations of the studied anti-infectives in different effluent samples from the Montreal WWTP (Gagné *et al.*, 2006; Segura *et al.*, 2007b). Values reported by Gagné *et al.* are for SMX and TRI only and were done in instantaneous samples. For the present study, the length of error bars was estimated using propagation of uncertainty rules according to Bruce and Gill (Bruce et Gill, 1999).

5.4. Conclusion

An on-line preconcentration LC-MS/MS method for the determination of anti-infectives was developed and validated for the analysis of wastewater samples. Contrary to most published methods which use large sample volumes, we were able to quantitate six anti-infectives in wastewater using only one-milliliter injections. Our method uses a switching-valve technique for the preconcentration of analytes as well as 30 % organic solvent washing step for the elimination of matrix interferences. Samples are analyzed in 30 min runs without having to perform the tedious and time-consuming tasks required for the SPE of large volumes of water and subsequent SPE eluate evaporation.

Measurements of analyte recoveries in wastewaters showed that despite the washing step before the back-flush elution of the load column, matrix effects ($-4.0 \% \pm 0.2$ to 116%

± 7) made impossible the quantitation by internal calibration when non isotopically-labeled internal calibrant is used. Quantitation was performed using standard additions to compensate for signal alteration due to interferences present in the samples. Limits of detection of the six anti-infectives in WWTP effluents ranged from 13 to 61 ng L⁻¹. For all analytes, method intra-day precision was less than 9% while for inter-day precision %RSD ranged between 2.5 and 23%. Column tests showed that more than 100 wastewater samples could be analyzed without a major degradation of analyte peak shape and changes on retention times. All the studied anti-infectives were found in the tested municipal WWTP effluent samples and their concentrations ranged from 71 to 289 ng L⁻¹. These results were in the same order of magnitude than previously reported concentrations using off-line methods.

Although some properties of the analytical system such as breakthrough volume and load column capacity are yet to be studied in details to fully understand its range of applications as well as its limitations, our results show that the presented method can potentially be applied to the analysis of many other compounds present at low nanogram-per-liter levels in complex matrices.

5.5. Acknowledgements

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Chapitre 6 : Application des colonnes de charge de chromatographie à débit turbulent pour l'analyse des anti-infectieux dans les eaux usées

Application of turbulent flow chromatography load columns for the on-line analysis of anti-infectives in wastewaters

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Chromatographia 2009, 70: 239-245.

Note sur ma contribution

Ma participation aux travaux de recherche: J'ai réalisé les expériences, l'analyse des résultats et leur interprétation.

Rédaction : J'ai rédigé l'article en m'appuyant sur les commentaires de Dr. Gagnon et du Prof. Sauvé, mes directeurs de thèse.

Abstract

We developed an on-line preconcentration liquid chromatography-tandem mass spectrometry method for the determination of the anti-infectives sulfamethoxazole, trimethoprim, ciprofloxacin, levofloxacin, clarithromycin and azithromycin in wastewaters using a turbulent flow chromatography load column. Recoveries for the target analytes were between 86 and 141%. Limits of quantification ranged from 45 to 122 ng L⁻¹ and limits of confirmation from 37 to 142 ng L⁻¹. This study shows that turbulent flow chromatography load columns are an interesting alternative for the on-line preconcentration of wastewater samples because they can be loaded at higher flow rates and are less affected by fouling, thus decreasing analysis time and enhancing method robustness necessary for the analysis of environmental trace pollutants.

Keywords

Turbulent flow chromatography, switching-column, anti-infectives, wastewater, on-line preconcentration.

6.1. Introduction

According to the van Deemter equation, optimal column efficiency is achieved both at low linear flow rates and by using columns with small particles, thus decreasing resistance to mass transfer (Harris, 1999). However, it has been demonstrated that when the mobile phase flow rate is high enough to produce eddies inside the column, *i.e.* a turbulent flow, low plate heights can be reached as well (Oberhauser *et al.*, 2000; Edge, 2002).

Turbulent flow chromatography (TFC) columns have large particle sizes (40-60 μm), large interstitial spacing and small internal diameters (0.5-1.0 mm) to reduce both solvent consumption and high pressures caused by high (> 1.0 mL min⁻¹) flow rates. TFC columns combine partition and size exclusion chromatography to retain small molecules which eases the elimination of larger molecular weight compounds from the matrix (Thermo Scientific, 2007).

The presence of pharmaceutical compounds such as anti-infectives (antibiotics and antimicrobials) in the environment has attracted scientific and public attention in the last few years (Daughton et Ternes, 1999; Associated Press). This interest arises from their potential contribution to the spread of anti-infective resistance in bacteria and their effect on sensitive aquatic biota (Kümmerer et Henninger, 2003; Wilson *et al.*, 2003). Therefore, robust and fast methods of analysis are necessary to determine their presence in raw sewage, wastewater treatment plant (WWTP) effluents and surface waters.

Several on-line methods of analysis of anti-infectives have been published in the literature recently (Stoob *et al.*, 2005; Pozo *et al.*, 2006; Choi *et al.*, 2007; Feitosa-Felizzola *et al.*, 2007; Segura *et al.*, 2007a). Analyte recoveries for these methods are usually high (> 80 %) and the limits of quantification (LOQ) are in the low nanogram-per-liter range (1-150 ng L⁻¹). Load columns used for preconcentration are either polymer- (e.g. Oasis HLB) or silica-based (e.g. C₁₈) having particle diameters between 10 and 30 μm . Method performance of on-line methods could be further improved by using TFC columns as trapping or load columns because they are less prone to fouling and can also be used at higher flow rates without generating excessive backpressures compared to smaller particle

diameter columns. TFC has been previously used for the analysis of pharmaceuticals in plasma (Ayrton *et al.*, 1997; Brignol *et al.*, 2000; Herman, 2002; Chassaing *et al.*, 2005; Herman, 2005), however there are few published applications for environmental trace analysis (Takino *et al.*, 2003).

Our research objective is to develop and validate a TFC-based on-line preconcentration and liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the analysis of six commonly used anti-infectives frequently encountered in urban wastewaters. We tested two different TFC load columns and optimized several critical parameters such as sample load flow rate, wash volume and wash organic content. The optimized method was then validated and applied to the analysis of WWTP effluent samples.

6.2. Experimental

6.2.1. Standards, reagents and solutions

Sulfamethoxazole (SMX), trimethoprim (TRI), ciprofloxacin (CIP), levofloxacin (LEV), azithromycin (AZI) and clarithromycin (CLA), formic acid (98% pure) and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich (St-Louis, MO). Methanol (MeOH), acetonitrile (ACN) and 0.1 % formic acid in water were purchased from J. T. Baker (Phillipsburg, NJ). Stock solutions of 200 mg L⁻¹ of each compound were prepared in MeOH except for the fluoroquinolones (CIP and LEV) which were dissolved in 5% NaOH 0.1 M-95% MeOH. All stock solutions were conserved at -15°C and used for no more than 3 months. Mixed working solutions containing 100 µg L⁻¹ of the anti-infectives were prepared weekly in ACN and kept at -15 °C. Solvents used for on-line preconcentration and liquid chromatography were 0.1% formic acid in H₂O (v/v) (A) and 0.1% formic acid in 50 % methanol-50 % acetonitrile (v/v) (B).

6.2.2. Apparatus

Sample preconcentration and detection was performed using the EQUan system developed by Thermo Fisher Scientific (Waltham, MA). The EQUan system is based on a column-switching technique (Segura *et al.*, 2007a) (**Figure 6-1**). It consists in a sample delivery system (autosampler: CTC analytics HTC Pal; quaternary load pump: Thermo Scientific Surveyor LC Pump Plus), a switching-column array (six-port switching valve: Rheodyne 7750E, load column: 1) Cohesive C₁₈ XL (C₁₈ bonded silica) of dimensions 50×1 mm, particle size 50 μm and pore size 60 Å or 2) Cohesive Cyclone-P (styrene-divinylbenzene copolymer bead with polar modification) of dimensions 50×1 mm, particle size 60 μm and pore size 100 Å; quaternary analytical pump: Thermo Scientific Accela Pump; analytical column: Hypersil Gold (50×2.1) mm, particle size 3 μm and pore size 175 Å) and an ionization and detection system (source: heated-electrospray; detector: Thermo Scientific TSQ Quantum Access triple quadrupole).

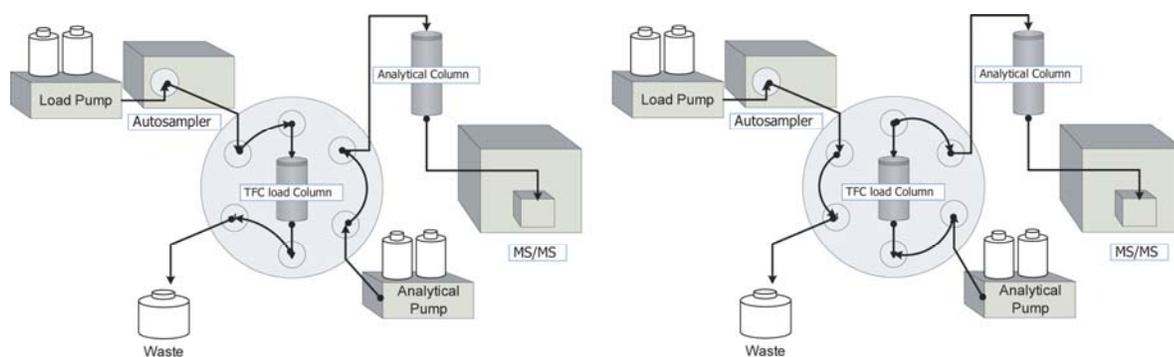


Figure 6-1. Diagram showing the EQUan system. *Left* : system in the “load” mode; *right* : system in the “inject” mode.

6.2.3. Method optimization and parameters

TFC load columns were optimized for three key parameters: 1) sample load flow rate, 2) load column wash volume and 3) amount of organic solvent in the wash. Optimization was carried out by comparing analyte peak areas obtained under different conditions after injecting 1.00 mL of a mixed solution of 500 ng L⁻¹ of the target anti-infectives in 0.1

formic acid in MeOH 3%-H₂O 97% (v/v) (Segura *et al.*, 2007a). Operating conditions for the optimal method were: 1.00 mL of sample was injected by the autosampler in a 1.00 mL loop and then the mobile phase (solvent A: 97%, solvent B: 3%) delivered by the load pump (flow rate: 3 mL min⁻¹) introduced the sample in the load column (**Figure 6-1**). The column was then washed with 420 µL (~10 column volumes). The organic content of the wash was 50% of solvent B. The binary gradient of the load pump was (%B): 0 min (3%), 0.10 min (3%), 0.11 min (50%), 0.57 min (50%), 0.82 min (95%), 20.07 min (95%), 23.32 min (95%), 23.57 min (3%) 27.07 min (3%), 28.07 (3%). The valve initially in the “load” position is actuated to the “inject” position after the load column wash is finished at 0.57 min and back to the “load” position at 20.07 min to rinse and recondition the load column separately from the analytical column. Sample desorption and separation were performed by back-flushing the load column with the binary gradient of the analytical pump (flow rate: 200 µL min⁻¹). The analytical column was rinsed and reconditioned at 20.07 min at a flow rate of 500 µL min⁻¹. The binary gradient of the analytical pump was: (% B): 0 min (10%), 2.07 (10%), 8.07 min (20%), 10.07 min (20%), 16.07 min (60%), 20.07 min (95%), 24.07 min (95%), 24.57 min (10%), 28.07 min (10%).

Positive electrospray ionization (ESI+) parameters were the following: spray voltage: 4000 V, sheath gas: 20 arbitrary units, auxiliary gas: 15 arbitrary units, ion sweep gas: 0.5 arbitrary units, ion transfer tube temperature: 350 °C. Tandem mass spectrometry detection was done on the selected reaction monitoring (SRM) transition mode. Two SRM transitions were used to avoid false positives according to the identification point system proposed by the European Union (Commission of the European Communities, 2002). For quantification, a specific SRM transition was used (SMR#1) which consisted in the precursor ion (the singly charged pseudo-molecular ion [M+H]⁺) and its specific product ion generated after collision-induced dissociation: SMX (precursor ion: 254.0 *m/z* → product ion: 156.0 *m/z*, collision energy (CE): 15 V, tube lens (TL): 82 V; TRI (291.1 *m/z*→230.0 *m/z*), CE: 24 V, TL: 98 V; CIP (332.1 *m/z*→231.0 *m/z*), CE: 35 V, TL: 96 V; LEV (362.1 *m/z*→260.8 *m/z*), CE: 26 V, TL: 90 V; CLA (748.5 *m/z*→589.9 *m/z*), CE: 18 V, TL: 110 V; AZI (749.5 *m/z*→591.2 *m/z*), CE: 28 V, TL: 116 V. Analyte confirmation was done by using a second SRM transition (SRM#2) consisting in a [M+H]⁺ precursor ion or, in the case of AZI, a doubly-charged pseudo-molecular [M+2H]²⁺ precursor ion and a product ion generated after collision-induced dissociation: SMX (254.0 *m/z*→108.2 *m/z*), CE: 23 V, TL: 82 V;

TRI (291.1 m/z →123.1 m/z), CE: 27 V, TL: 98 V; CIP (332.1 m/z →288.0 m/z), CE: 17 V, TL: 96 V; LEV (362.1 m/z →318.0 m/z), CE: 19 V, TL: 90 V; CLA (748.5 m/z →157.9 m/z), CE: 17 V, TL: 110 V; AZI (375.4 m/z →83.1 m/z), CE: 20 V, TL: 81 V.

6.2.4. Method validation and application to environmental samples

Effluent samples from the wastewater treatment plant of the city of Montréal were used in our tests. To investigate the effect of the matrix of the electrospray signal we performed a matrix effects study by measuring the analyte signal on both deionized water and wastewater as in our previous work (Segura *et al.*, 2007a). Calibration was performed by the standard additions method by analyzing triplicates 1.00 mL aliquots of unspiked and spiked wastewater samples with 100, 200, 300, 400, 500 and 600 ng L⁻¹ of the target anti-infectives. Recovery was determined using the following equation:

$$\text{Recovery (\%)} = \left(\frac{C_{2s} - C_{1s}}{D} \right) \times 100\% \quad (\text{Équation 6-1})$$

where C_{2s} and C_{1s} are the calculated concentration (*i.e.*, from the amounts spiked using the calibration curve) of the second lowest and lowest spike, respectively and D is the concentration difference between those spikes (in ng L⁻¹). Limits of detection (LOD) and quantification (LOQ) were determined by multiplying the standard error of the intercept by 3.3 and 10, respectively and then dividing by the slope of the calibration curve as proposed by The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use [ICH], 2005). The presence of the analytes was confirmed if their concentration was higher than the limit of confirmation (LOC) of SRM#2. The LOC was determined in a similar way but using a factor of 3.3 instead. System carryover was evaluated by comparing the analyte peak area in a blank and in a previously injected low concentration standard (100 ng L⁻¹) (Segura *et al.*, 2007a). Precision was determined by analyzing wastewater samples spiked with 50 ng L⁻¹ in two ways: intra-day ($n=5$, on the same day) and inter-day ($n=14$, on three different days).

6.3. Results

6.3.1. Method optimization

Optimization tests showed that the sample load flow rate had different effects on the two columns. For the C₁₈ analyte peak areas generally reached a maxima between 1.5 and 3.0 mL min⁻¹ while few statistically significant ($p < 0.05$) differences were observed for the Cyclone-P. As for the load wash volume, the best peak intensities were observed for the C₁₈ when using the smallest wash volume (420 µL); with increasing wash volumes the signal decreased. For the Cyclone-P, few differences were observed among the analyte peak intensities when using different wash volumes tested, except for LEV which showed a decreased signal after the 2000 µL wash. As LEV was one of the less intense peaks, we decided to use 1 200 µL as wash volume for this column. Finally for the amount of organic solvent used in the washing step on the C₁₈, we observed that the signal generally augmented with the amount of solvent B in the wash, and 50% of solvent B provided the best overall results. For the Cyclone-P, the best compromise was found to be 20% of solvent B in the wash, as signal diminished with higher amounts of organic solvent. To summarize, optimal conditions for the C₁₈ were: 3 mL min⁻¹ (sample load flow rate), 420 µL (wash volume) and 50% of solvent B (composition of wash). Optimal conditions for the Cyclone-P were: 5 mL min⁻¹, 1200 µL and 20% of solvent B. Comparison of both load columns using the optimal conditions showed that analyte peak areas with the C₁₈ were significantly higher ($p < 0.05$) for SMX, TRI, CIP and AZI than those obtained with the Cyclone-P. Therefore, we proceeded to validate a method using the C₁₈ load column. A chromatogram of the pure standards obtained with the optimal conditions is shown on **Figure 6-2**.

6.3.2. Method validation and application to environmental samples

Assays showed that in spite of the load column wash, matrix effects were always present either as signal enhancement (CIP: 51 % ± 3; LEV: 114 % ± 5; AZI: 24 % ± 3) or as signal suppression: (SMX: -19 % ± 1; TRI: -18.1 % ± 0.8; CLA: -8.7 % ± 0.5).

According to Taylor (Taylor, 2005) the competition for access to the ESI droplet surface and subsequent transfer to the gas phase between non-volatile matrix components and analytes results in signal suppression or signal enhancement. Signal suppression is a common phenomenon for ESI (Niessen, 1999) and signal enhancement albeit less known, has been reported in the literature by several authors (Benijts *et al.*, 2004; Cahill *et al.*, 2004; Kloepfer *et al.*, 2005). Method performance parameters appear on Table 1. Standard additions calibration curves were linear in the added concentration range (100 to 600 ng L⁻¹) and analyte recoveries were > 85 %. LOD (SRM#1) ranged from 15 to 53 ng L⁻¹, and LOQ (SRM#1) from 45 to 161 ng L⁻¹. LOC (SRM#2) varied between 37 and 142 ng L⁻¹. Method intraday precision was ≤ 7% and inter-day precision was ≤ 15%, except for TRI and AZI.

Carryover was < 0.7 % for the analyzed substances, with the exception of SMX (6%). This high value, however, did not affect its quantification as it represented a signal ten times lower than the LOQ. After more than 250 injections (of which 135 were wastewater sample injections) no major degradation of peak shape was observed (CLA peak asymmetry decreased by 25 %) and also no significant change in retention times was observed (CLA retention time decreased by < 0.08 min).

Of the six target anti-infectives, only the presence of CLA (113 ± 8 ng L⁻¹) could be quantified and confirmed. For the other compounds, they failed to be determined because their concentrations were < LOC (as in the case of CIP), < LOQ (SMX, and AZI) or as in the case of TRI and LEV, < LOD.

6.4. Discussion

Higher sample flow rates can be used with TFC columns compared to HPLC columns because bigger particle sizes result in lower column backpressures. Our results showed that optimal flow rates for the turboflow load columns were 2 to 4 times the optimal flow rate of a 12-µm particle size HPLC C₁₈ column (1.5 mL min⁻¹) (Segura *et al.*, 2007a). The capacity of TFC columns to work at higher flow rates is an important property because larger volumes of sample could be preconcentrated without increasing too much the total

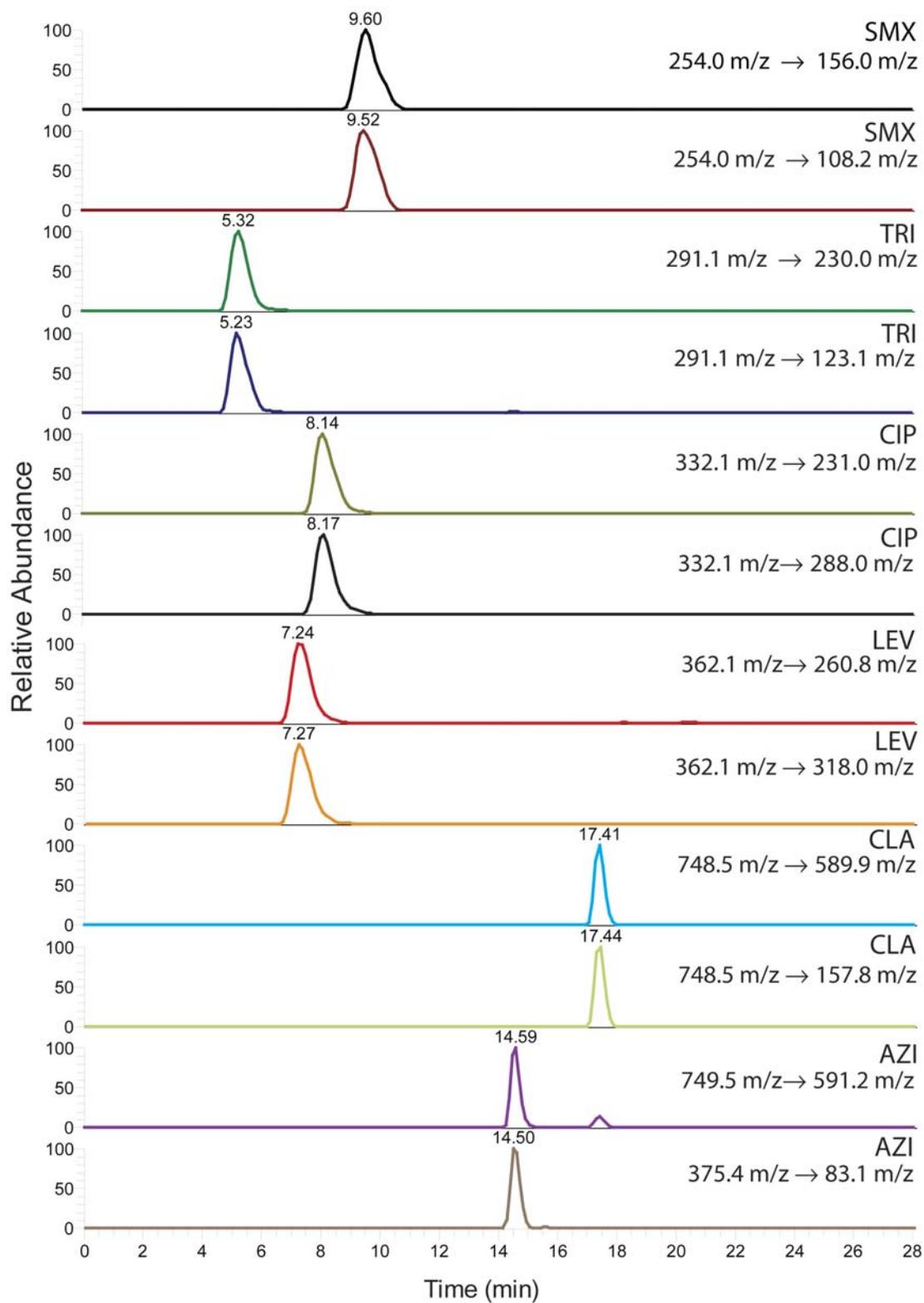


Figure 6-2. Chromatograms and SRM signals of 500 ng L⁻¹ of the pure standards spiked in 0.1 % formic acid in MeOH 3%-H₂O 97% (v/v).

analysis time. Signal intensity differences between the two columns could be explained by their chemistry. The C₁₈ is silica-based, while the Cyclone-P is a styrene-divinylbenzene copolymer with polar modification. The presence of π - π interactions in the latter could enhance the retention of SMX, TRI, CIP and AZI compared to the former. Therefore, gradients containing a higher proportion of organic solvent containing capable of π - π interactions (such as ACN) could elute more effectively those compounds from the Cyclone-P.

The influence of matrix on the detector's signal in LC-MS/MS has been extensively reported in the literature (Reemtsma, 2001; Kloepfer *et al.*, 2005; Taylor, 2005). Several authors have reported matrix effects in on-line preconcentration methods (Stoob *et al.*, 2005; Feitosa-Felizzola *et al.*, 2007; Segura *et al.*, 2007a). The use of a wash step before elution has failed to remove them (Segura *et al.*, 2007a) and it has been pointed out that the importance of this signal alteration may differ depending on the type of matrix studied (Poza *et al.*, 2006). According to our results, interfering components present in the wastewater samples and causing ESI signal alteration are: 1) retained by the load column, 2) not washed off the column during the wash step 3) co-eluted from the analytical column with the analytical gradient. As these interfering compounds reach the electrospray source in spite of the size exclusion chemistry of the TFC load columns, it can be speculated that, in our case, matrix effects are not caused by large molecular weight substances (>2000-3000 u, according to the manufacturer) but rather by small molecules having an affinity for the stationary phases similar to that of the analytes. These results are confirmed by Kloepfer *et al.* who concluded that the signal distortion observed in wastewaters is caused by matrix components with molecular masses below 1000 u, which also represented > 70% of the samples' dissolved organic carbon (Kloepfer *et al.*, 2005). Therefore in the absence of isotopically-labeled internal standards, the standard addition method is the best solution to correct matrix effects.

The column-switching system demonstrated good durability as no major changes in analyte peak asymmetry or retention time were observed after injecting more than 250 samples of which 135 were wastewater and without the need of a pre-column protecting the load or the analytical column.

Parameters of analytical performance (**Tableau 6-1**) were acceptable for the most part, except for linearity. This indicates that our method should be considered as a screening method, rather than a truly quantitative one. Low r^2 values for certain substances, especially in the case of SMX ($r^2=0.9235$) could be explained by extra-column adsorption (which also explains its high carryover). However, the proposed method is sensitive and robust enough to allow the trace analysis of anti-infectives in polluted raw sewage and WWTP effluents (Batt et Aga, 2005; Castiglioni *et al.*, 2005; Gulkowska *et al.*, 2008; Siemens *et al.*, 2008) and without being affected by matrix effects. Although the sensitivity of the method was slightly lower than those reported by other studies (Stoob *et al.*; Pozo *et al.*; Choi *et al.*; Feitosa-Felizzola *et al.*; Segura *et al.*) the robustness, rapidity and reliability of the method are much sought-after. LOQ and LOC could be improved by using larger sample volumes for the preconcentration.

Analysis of the urban wastewater effluent of the City of Montreal (**Figure 6-3**), showed that the developed on-line preconcentration and LC-MS/MS method was able to quantify and confirm the presence of CLA. As for the rest of the compounds except CIP, they could not be quantified because their calculated concentration was $< \text{LOQ}$. In the case of CIP, the concentration calculated with SRM#1 was $> \text{LOQ}$, but the concentration calculated with SRM#2 was $< \text{LOC}$, therefore its presence of the wastewater samples cannot be confirmed.

6.5. Conclusion

We developed and validated an on-line preconcentration and LC-MS/MS method of trace determination of six anti-infectives in wastewaters using a TFC load column. Recoveries ($> 85\%$), linearity ($0.9235 < r^2 < 0.9901$), precision (intra-day: $\leq 7\%$; inter-day: $\leq 35\%$), carryover ($< 6.1\%$), LOD ($15\text{-}53 \text{ ng L}^{-1}$), LOQ ($45\text{-}161 \text{ ng L}^{-1}$) and LOC ($17\text{-}142 \text{ ng L}^{-1}$) were in most cases similar to current methodology and allow trace determination in polluted urban wastewaters. Our results showed TFC load columns are an interesting alternative over traditional HPLC columns for the on-line preconcentration of environmental trace pollutants in wastewaters because they can be loaded at higher flow

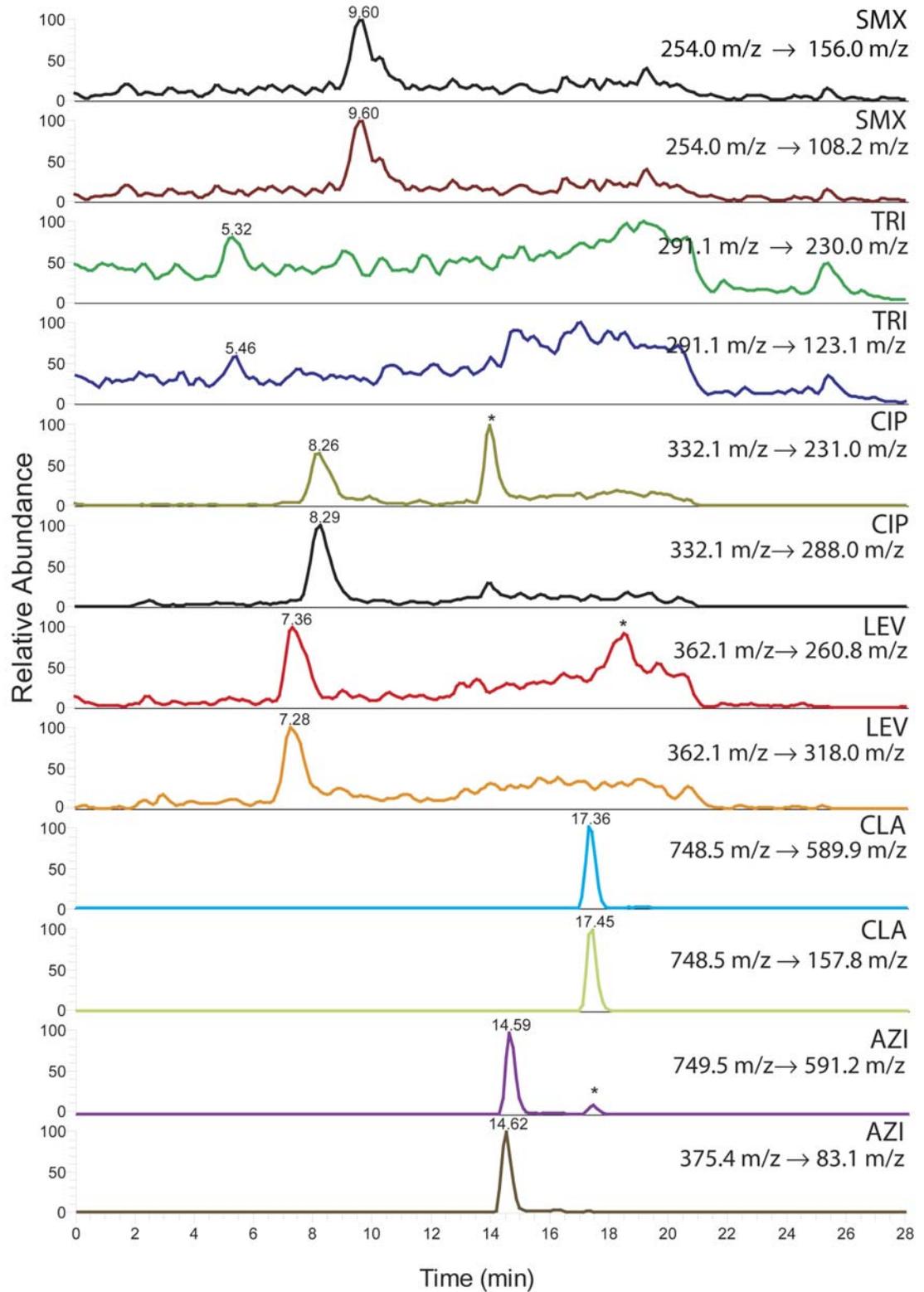


Figure 6-3. Chromatograms and SRM signals of the target analytes in the unspiked wastewater effluent of the City of Montreal.

Tableau 6-1. Method performance parameters of the on-line preconcentration and LC-MS/MS method using the C₁₈ TFC load column.

| Compound | Equation | r^2 | Recovery (%±SD) | LOD _{SRM#1} (ng L ⁻¹) | LOQ _{SRM#1} (ng L ⁻¹) | LOC _{SRM#2} (ng L ⁻¹) | Intra-day precision (%RSD) | Inter-day precision (%RSD) | Carryover (%) |
|----------|----------------------|--------|--------------------|---|---|---|----------------------------------|----------------------------------|------------------|
| SMX | $y = 82405 + 800x$ | 0.9235 | 109 ± 26 | 34 | 104 | 142 | 5.4 | 12.1 | 6.1 |
| TRI | $y = 77397 + 6238x$ | 0.9880 | 104 ± 7 | 34 | 104 | 17 | 6.6 | 16.7 | 0 |
| CIP | $y = 208925 + 2798x$ | 0.9901 | 141 ± 32 | 15 | 45 | 60 | 6.5 | 15.1 | 0.3 |
| LEV | $y = 85536 + 7682x$ | 0.9821 | 86 ± 4 | 40 | 122 | 37 | 7.2 | 7.1 | 0.7 |
| CLA | $y = 409993 + 3634x$ | 0.9686 | 102 ± 22 | 22 | 68 | 86 | 4.2 | 5.0 | 0.2 |
| AZI | $y = 410528 + 3554x$ | 0.9696 | 96 ± 20 | 53 | 161 | 53 | 3.9 | 35.0 | 0.1 |

rates and are less affected by fouling, thus decreasing analysis time and enhancing method robustness. Study of matrix effects showed that despite the size exclusion chemistry of TFC columns and the wash step performed before desorption, signal distortion caused by matrix components is still important and in the absence of the appropriate isotopically-labeled internal standards, accurate quantification by internal calibration is not possible. Therefore, quantification by the standard additions method is the best solution to compensate for matrix effects. This method could be applied to surface and drinking water analysis by using larger sample injection volumes, which will be the focus of future work.

Chapitre 7 : Conclusion

7.1. Remarques générales

Les anti-infectieux, les médicaments miraculeux du XXe siècle sont devenus des contaminants à intérêt émergent dans le XXIe siècle. Les bienfaits des anti-infectieux à l'humanité sont incontestables, cependant leur apparition dans l'environnement aquatique est un nouveau rappel de la prudence avec laquelle ils doivent être utilisés.

Une revue et méta-analyse de la littérature scientifique, reportant l'analyse des anti-infectieux dans les eaux environnementales contaminées, a résumé l'information sur la présence de ces substances dans les eaux environnementales contaminées. D'après cette étude les anti-infectieux se retrouvent dans ces eaux à des concentrations variant 11 ordres de grandeur (10^{-1} - 10^9 ng L⁻¹) (**Chapitre 2**). La contribution potentielle de ce phénomène à la dissémination de la résistance aux anti-infectieux est incertaine et reste toutefois un sujet de débat. En ce qui concerne les eaux naturelles, la présence d'anti-infectieux dépend de différents facteurs abiotiques et biotiques, mais les impacts potentiels aux écosystèmes proches des points de rejet des eaux usées ne peuvent pas être négligés, surtout lorsque l'on considère l'exposition chronique des organismes aquatiques à ces substances. Dans l'eau potable les concentrations rapportées à ce jour sont dans le bas nanogramme par litre (0.1-10 ng L⁻¹) et les effets chroniques chez les humains causés par l'ingestion de basses concentrations des mélanges contaminants organiques commencent à être étudiés (Pomati *et al.*, 2008), ainsi que la bioactivité de leurs sous-produits après traitement (Yargeau *et al.*, 2008). On a aussi souligné deux facteurs importants qui devront être étudiés dans un proche avenir : le manque d'information concernant l'occurrence des anti-infectieux dans les pays à revenu moyen et bas ainsi que l'impact des effets des mixtures. On espère que ceci attirera l'attention de la communauté scientifique, étant donné l'importance de ces facteurs.

Le développement des méthodes de détermination des anti-infectieux dans les eaux environnementales présentées dans cette thèse a alors eu comme but de trouver les approches plus efficaces et performantes pour quantifier ces substances dans les eaux usées, les eaux de surface et l'eau potable. Les essais préliminaires avec les eaux usées non traitées et celles subissant seulement un traitement primaire et physico-chimique ont démontré les difficultés liées à cette tâche : besoin de facteurs de préconcentration élevés

ainsi que la présence d'une multitude d'interférences (**Chapitre 3**). D'après les résultats obtenus, l'utilisation de l'ultrafiltration par débit tangentiel (TFU) n'est pas recommandée pour les analyses par LC-MS/MS puisque seulement une faible portion de la matière organique a une masse < 1000 u. Les tests de SPE ont indiqué que les cartouches polymériques étaient les plus appropriées pour la préconcentration des quinolones dans les effluents contenant des concentrations élevées de Mg^{2+} et Ca^{2+} . Trois outils ont été essentiels au développement analytique qui s'est poursuivi : 1) l'utilisation de la chromatographie liquide couplée à la spectrométrie de masse en tandem (LC-MS/MS) dans le mode de suivi de réaction de fragmentation caractéristique (SRM), 2) l'information sur la consommation locale ainsi et 3) les rapports sur la présence de ces substances dans les eaux de surface d'autres pays.

La première méthode développée pour l'analyse des eaux usées a utilisé l'extraction sur phase solide (SPE) dans le mode découplé ou « off-line » comme technique de préparation des échantillons pour préconcentrer les analytes et éliminer une fraction des impuretés retrouvées dans la matrice (**Chapitre 4**). Cet article a été le premier à démontrer la présence de six anti-infectieux ciblés (sulfaméthoxazole, triméthoprim, ciprofloxacine, lévofloxacine, clarithromycine et azithromycine) à des concentrations traces (39 à 276 $ng\ L^{-1}$) dans l'affluent et l'effluent de la Station d'épuration de la Ville de Montréal. Ces résultats ont indiqué que ces anti-infectieux étaient rejetés dans le fleuve St.-Laurent et que malgré leur basse concentration, des quantités importantes (~ 1 kg en total) atteignaient la zone du point de rejet chaque jour.

Les difficultés associées à l'extraction sur phase solide dans le mode découplé ont amené à travailler sur une nouvelle méthode plus performante d'analyse des anti-infectieux dans les eaux usées (**Chapitre 5**). Une méthode de préconcentration totalement automatisée a été développée, capable de quantifier et confirmer la présence de traces d'anti-infectieux (> 13-61 $ng\ L^{-1}$) dans les eaux usées traitées et non traitées. Cette nouvelle méthode utilisait des injections de 1.0 mL et la technique de permutation de colonnes pour préconcentrer les analytes dans une petite colonne de charge (ou colonne de SPE couplée) et ensuite les séparer et les quantifier par LC-MS/MS. Ces travaux ont démontré qu'il était possible de quantifier les traces d'anti-infectieux dans les eaux usées avec une méthode automatisée beaucoup plus rapide et simple et avec une performance similaire aux méthodes utilisant la

SPE manuelle. Cependant, cette méthode n'était pas assez sensible pour analyser les anti-infectieux dans les eaux de surface ou l'eau potable. Aussi, la robustesse de la méthode doit être améliorée pour permettre l'analyse d'un plus grand nombre d'échantillons. Cet article a illustré l'avenir des méthodes de SPE couplée pour l'analyse des contaminants à intérêt émergent dans les eaux usées.

Par la suite, en vue d'augmenter la robustesse des méthodes automatisées, l'utilisation des colonnes de charge à débit turbulent a été ensuite explorée (**Chapitre 6**). Ces colonnes ont des propriétés spéciales (diamètre des particules élevé, petit diamètre interne) qui les rendent très attirantes pour l'analyse environnementale. La méthode développée avec ces nouvelles colonnes a montré des performances similaires (LOD : 15-53 ng L⁻¹; recouvrement : > 85%) à celle utilisant les colonnes de charge traditionnelles, mais avec une robustesse accrue. Aucun changement majeur au niveau de l'asymétrie des pics n'a été observé après l'injection de 135 échantillons d'eaux usées et sans utiliser de colonne de garde.

Les méthodes SPE couplées quoique plus performantes que la méthode manuelle en termes de vitesse et de précision se sont avérées insuffisamment sensibles pour être appliquées à l'analyse des anti-infectieux dans les eaux de surface et encore moins dans l'eau potable où des limites de détection < 10 ng L⁻¹ sont requises. Ainsi, une façon d'améliorer les limites de détection et confirmation des méthodes de SPE couplée (**Chapitre 8, voir l'annexe**) a été recherchée. Il a été trouvé que l'utilisation des injections de grand volume (10 mL) dans une colonne de charge ayant des volumes de fuite élevés (> 16 mL) permettait d'atteindre la performance analytique désirée.

La recherche effectuée s'est surtout focalisée sur l'étape de l'extraction et préconcentration des analytes de la matrice, étape essentielle à toute analyse de traces. Le fil conducteur des travaux a été alors l'amélioration des limites de détection pour permettre la détection à des niveaux s'approchant de plus en plus au picogramme par litre et la simplification de l'étape de préparation des échantillons. L'application de la SPE dans ces diverses modes (manuelle en utilisant des cartouches, couplée en utilisant des colonnes de charge traditionnelles et couplée en utilisant des colonnes de charge à débit turbulent) a démontré les faiblesses et avantages de ces techniques. Dans son ensemble, ces travaux-ci

ont réussi à identifier les approches les plus appropriées pour quantifier les anti-infectieux dans les eaux environnementales.

D'après nos résultats, les méthodes SPE couplées représentent l'avenir pour l'analyse des anti-infectieux dans les eaux usées et de surface. Les raisons principales sont la simplification des tâches de préparation et la performance similaire (précision, limites de détection) à celles obtenues par SPE manuelle, qui est actuellement la méthode la plus répandue de préconcentration. De plus, un autre facteur très important à considérer est celui de la calibration. La SPE couplée permet de contourner le problème des effets de matrice qui modifient beaucoup le signal de l'électronébuliseur lors de l'analyse des matrices complexes, par moyen de la quantification par ajouts dosés. Cependant, en ce qui concerne l'analyse des anti-infectieux dans l'eau potable, les méthodes SPE couplées ne sont pas encore assez sensibles pour être appliquées à des programmes de suivi. Idéalement, la limite de détection des anti-infectieux dans l'eau potable devrait être autour de 50-100 pg L⁻¹. À ce jour seulement les méthodes SPE manuelles, beaucoup moins rapides et pratiques, peuvent atteindre ces limites.

L'apport des travaux de recherche à l'analyse de traces ne se limite ni aux anti-infectieux ni aux applications environnementales, évidemment. Les méthodes décrites dans cette thèse sont applicables à un grand nombre de molécules organiques, comme il a été vu au **Chapitre 8 (annexe)**. Les méthodes de SPE couplée à la LC-MS/MS que l'on a développées peuvent aussi être appliquées à la détermination de nombreuses molécules organiques dans une multitude des matrices (urine, sang, etc.). D'après nos observations, une des principales limitations de ces méthodes est la sorption des analytes d'intérêt dans la colonne de SPE couplée. Les contaminants de nature plutôt polaire sont souvent peu retenus et par conséquent, leurs volumes de fuite sont très faibles. Puisqu'à ce jour, le nombre de phases stationnaires disponibles dans le marché est assez restreint, l'applicabilité de ces méthodes dépendra du développement futur des nouvelles phases capables de mieux retenir les composés organiques de nature polaire.

7.2. Les défis de l'avenir

Quels sont les défis analytiques de l'avenir dans ce domaine? Il y a plus de 25 ans, on appelait la fraction organique dans les eaux qui était difficilement analysable « la fraction organique non volatile » (Watts *et al.*, 1982). Aujourd'hui on sait que cette fraction contient en effet une multitude de résidus.

Cette thèse a focalisé sur les méthodes de détermination des anti-infectieux dans les eaux environnementales, mais ils ne sont pas bien sûr les seuls composés bioactifs actuellement présents dans l'environnement aquatique : les composés d'origine industriels, les pesticides, les produits pharmaceutiques et de soin personnel ainsi que les métaux trace sont parmi les plus importantes classes de substances qui ont été détectées à ce jour. Cependant, la société actuelle produit et consomme une quantité énorme de composés, et certains de ceux-ci, à cause de leur volume de production élevé ou à leur résistance à la dégradation, finissent pour se rendre dans l'environnement aquatique.

Les composés qui sont étudiés actuellement dans l'environnement ne sont alors qu'une petite fraction du nombre total qui peut se retrouver dans l'environnement (Daughton, 2003). D'après le Chemical Abstracts Service, en juillet 2009 il y avait plus de 35 000 000 composés disponibles commercialement (Chemical Abstracts Service, 2009). En mars 2004, ce chiffre n'était que 7 000 000 (Daughton, 2004b). Alors, il est à prévoir une augmentation de la diversité chimique des contaminants dans les années à venir, surtout lorsque la recherche actuelle indique qu'il y a encore des structures chimiques inexplorées par la synthèse organique (Lipkus *et al.*, 2008). Malgré ceci, actuellement, moins de 150 sont régulés actuellement dans l'eau potable (Health Canada, 2008) ou de surface (CCME, 2007) au Canada.

Un des plus grands défis de l'avenir sera alors la diversité des contaminants (on focalisera ici sur ceux d'origine organique). On essaiera alors de répondre deux questions qui devront aider à guider la recherche dans ce domaine dans les années futures.

7.2.1. Question #1 : Comment peut-on identifier d'autres substances organiques d'origine anthropique qui se retrouvent dans les eaux environnementales?

L'analyse de traces en LC-MS/MS dans le mode SRM est une analyse ciblée. Cette spécificité permet en partie leur sensibilité élevée. Cependant, dans ce type d'analyse une grande partie de l'information sur la présence d'autres composés non ciblés est perdue. Une façon de répondre cette question a été essayée en développant une technique de préconcentration des injections de grand volume (**Chapitre 8, annexe**). Cette méthode, quoique ne ciblait que 14 substances, est aussi une « trappe » non sélective des composés organiques. Cette approche couplée avec de plus puissants instruments hybrides tels que le spectromètre de masse à quadripôle-temps d'envol (QqTOF) ou la trappe ionique linéaire-orbitrap (LIT-Orbitrap) est une voie de recherche prometteuse pour l'identification et détection de nouvelles substances inconnues. Ces techniques permettent la mesure des masses exactes de l'ion précurseurs et de ses produits, ce qui facilite la tâche d'identification structurale par spectrométrie de masse. De cette façon, il sera possible de réaliser des études non ciblées de dépistage environnemental dans les eaux de surface. Pour pouvoir accomplir le même type d'expérience dans l'eau potable, une combinaison de la SPE manuelle et la SPE couplée est envisageable. Ceci peut être fait en réalisant la SPE manuelle avec un volume de 1 L et ensuite faire une injection de grand volume avec l'extrait préconcentré dans un système de permutation de colonnes. Ainsi, il sera possible d'obtenir des facteurs de préconcentration aussi élevés que 100 000.

Le développement futur des ces techniques rendront possible d'identifier de nouvelles substances organiques présentes dans nos eaux, cependant il reste peu probable qu'on arrive à déterminer le nombre exact de ces substances, étant donné certaines limitations de la préconcentration. Cependant, d'un point de vue toxicologique, il en demeure qu'il est plus important de connaître combien de substances toxiques ou potentiellement toxiques se retrouvent parmi cet ensemble, ce qui nous amène nécessairement à la deuxième question.

7.2.2. Question #2 : Quelle est l'importance environnementale de ce « cocktail »?

Tout développement analytique dans ce domaine devra avoir comme but ultime celui de donner l'information nécessaire pour permettre de mieux comprendre les effets chroniques sur les êtres vivants, y compris l'être humain. D'après Daughton (Daughton et Ternes, 1999), un des plus grands dangers des pharmaceutiques et les produits de soin personnel (PPCPs) dans l'environnement aquatique est leur capacité à avoir « effets subtils », c'est-à-dire causer des petits changements qui s'accumulent avec le temps, mais qui sont si discrets que nos techniques de mesure les rendent indétectables. Aussi, peu est connu sur la toxicologie et l'écotoxicologie des faibles concentrations d'un grand nombre PPCPs et leurs effets combinés. Alors, un avancement considérable de ces domaines dans l'avenir est indispensable.

Le progrès dans l'identification de traceurs, de récepteurs et de réactions métaboliques amènera possiblement au développement de nouvelles techniques bioanalytiques capables de quantifier directement le potentiel toxique (perturbation endocrinienne, génotoxicité, etc.) d'un échantillon d'eau donnée.

Par exemple, le test biologique E-Screen (Soto *et al.*, 1995), est capable de mesurer l'affinité d'une substance avec le récepteur de l'estrogène (*c.-à-d.* l'activité oestrogénique). Ce test se base sur la prolifération des cellules cancérogènes du sein induite par l'attachement de la molécule testée au récepteur de l'estrogène (Soto *et al.*, 1995). Ce test peut être aussi couplé à la SPE pour purifier et préconcentrer les échantillons analysés (Körner *et al.*, 1999). Cependant, l'application de ce test pour le suivi environnemental est limitée puisque les résultats sont obtenus après 6 jours. Il est alors désirable de développer une nouvelle technique plus performante utilisant un biocapteur à base du récepteur de l'estrogène et capable de quantifier « l'activité oestrogénique totale » d'un échantillon d'eau. Ce même principe peut être appliqué à d'autres effets toxiques.

De cette façon, il serait possible de quantifier la capacité d'une certaine source de pollution d'avoir un effet sur le biote aquatique et aussi sur les êtres humains. Ceci est une voie holistique qui pourra mieux répondre aux défis de l'avenir.

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Annexe

Chapitre 8 : Extraction sur phase solide d'injections de grands volumes couplée à la LC-MS/MS pour la quantification et la confirmation de 14 contaminants organiques trace dans l'eau potable et l'eau de surface

On-line solid-phase extraction of large-volume injections coupled to liquid chromatography-tandem mass spectrometry for the quantitation and confirmation of 14 selected trace organic contaminants in drinking and surface water

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Note sur ma contribution

Ma participation aux travaux de recherche: J'ai réalisé les calculs et la rédaction de l'introduction, la partie expérimentale et la discussion pour la partie détermination du volume de fuite.

Rédaction : J'ai écrit une portion de l'introduction et de la partie expérimentale. J'ai écrit la section de la discussion relative au volume de fuite, à la TOFMS et à la RER et aussi tout ce qui concerne les anti-infectieux (CLA, SMX, TRI).

Collaboration des co-auteurs: A. Garcia-Ac (premier auteur) a analysé les résultats de CYC, DHC, ENA, et MTX. Elle a écrit la plupart de l'introduction, de la partie expérimentale et de la discussion. L. Viglino a participé au développement analytique, a fait l'analyse de résultats pour les composés ATZ, CBZ, CYA, DEA, DIA, SIM, et TCC. Elle a aussi révisé le manuscrit. A. Fürtös nous a assistés avec les analyses par TOFMS et a révisé le manuscrit. M. Prévost, C. Gagnon, et S. Sauvé ont guidé l'avancement des travaux et révisé le manuscrit.

Abstract

We describe the development and validation of an on-line solid-phase extraction of large-volume injections coupled to liquid chromatography–tandem mass spectrometry method for the simultaneous quantitation and confirmation of 14 selected trace organic contaminants in drinking and surface water. Selected compounds were: anti-infectives (clarithromycin, sulfamethoxazole and trimethoprim), an anticonvulsant (carbamazepine) and its transformation product 10,11-dihydrocarbamazepine, an antihypertensive (enalapril), antineoplastics (cyclophosphamide and methotrexate), herbicides (atrazine, cyanazine, and simazine) and two of their transformation products (deethylatrazine and deisopropylatrazine) and an antiseptic (triclocarban). The breakthrough volume determinations showed that out of all the investigated sorbents, the Strata-X on-line solid-phase extraction column showed the best performance. The method used a load volume of 10.0 mL and was validated using the corresponding matrices, yielding for most compounds, $R^2 > 0.99$. Extraction recoveries ranged from 60 to 109%. The intra- and inter-day precision were $< 14\%$ and $< 16\%$, respectively. The method detection limits ranged from 0.6 to 6 ng L⁻¹. Matrix effects were in general low. The performance of the on-line method was demonstrated with the analysis of real water samples. The application of alternative techniques of confirmation was also explored using accurate mass measurements on a time-of-flight mass spectrometer and the data-dependent reverse energy ramp scan on a triple quadrupole.

Introduction

Numerous studies have reported the presence of traces (nanogram to micrograms-per-liter) of organic contaminants such as pharmaceuticals and personal care products (PPCPs) and pesticides in drinking and surface waters in many countries (Ternes, 1998; Kolpin *et al.*, 2002; Comoretto et Chiron, 2005; Siemens *et al.*, 2008). Pesticides are ubiquitous in today's agriculture and given their persistence and transport they appear in the aquatic environment. PPCPs are constantly released from urban centers into surface waters via wastewater treatment plants. Major concerns about the presence of organic contaminants in the environment are endocrine disruption (e.g. pharmaceuticals, personal care products, pesticides), genotoxicity (e.g. antineoplastics) and possible contribution to the spread of antibiotic resistance in bacteria as well as their toxicity to sensitive non-target species (anti-infectives) (Daughton et Ternes, 1999; Segura *et al.*, 2009a). Therefore these substances could have a significant impact on the natural environment, especially to the aquatic ecosystems where they are being released. Human exposure to trace organic contaminants, via drinking water consumption is also of potential concern due to possible long-term effects from chronic exposure at low concentrations (Pomati *et al.*, 2008).

In this context, sensitive analytical methods capable of detecting traces in the low nanogram-per-liter range are essential for the monitoring of organic contaminants in the aquatic environment. In addition, high selectivity is required in order to avoid interference by matrix components. In order to avoid false positives, confirmation techniques using identification points have been implemented (Commission of the European Communities, 2002) or proposed (Hernández *et al.*, 2004). However, these techniques are often hindered by the low concentrations found in the environment. Analyte confirmation is usually performed using a second selective reaction monitoring transition (SRM) and ion ratios, nonetheless is not always possible to obtain a strong signal for secondary SRM transitions, which in turn limits the quantitation process as the presence of the detected compound cannot be confirmed. Environmental applications of time-of-flight mass spectrometry (TOFMS) to trace contaminants have been previously reported (Bobeldijk *et al.*, 2001; Hernández *et al.*, 2004; Ibáñez *et al.*, 2008), but its successful use for confirmatory

purposes has been hampered by low sensitivity. Recently, novel approaches such as data-dependent scans have also been described (Martínez Bueno *et al.*, 2009).

Achieving quantification of minute analyte amounts is one of the main challenges in environmental trace analysis. Usually samples must be preconcentrated before analysis with an enrichment step such as solid-phase extraction (SPE) in order to allow trace detection in relatively clean surface waters. To date, most of the published multi-residue methods for the determination of ultra traces of organic compounds in surface and drinking water use off-line SPE followed gas by chromatography mass spectrometry (GC-MS) or by liquid-chromatography tandem mass spectrometry (LC-MS/MS) (Lee *et al.*; Hao *et al.*, 2007). However, off-line SPE can be a lengthy sample preparation step, especially when large volumes of sample are preconcentrated and does not seem adequate for the analysis of numerous samples for monitoring programs.

Automation of SPE, by coupling it on-line to the LC system using a column-switching technique, is an alternative approach that is faster and more precise since the evaporation and reconstitution steps are eliminated and sample handling errors are reduced. Nowadays, a large number of on-line SPE column materials (e.g., alkyl-bonded silica, polymers, and porous graphitic carbon) are available, covering a wide range of selectivity and thus a large variety of applications (Pichon, 2000). Although automated on-line preconcentration methods have some clear advantages over off-line SPE (Jordan, 2000), they are often inadequate for surface and drinking water trace analysis due to their low preconcentration factor which results in relatively higher method detection limits (MDLs). To improve the MDLs of on-line SPE, it is possible to increase the preconcentration volume. Nevertheless, this would only be achievable if the analytes do not break through the SPE column. The potential for analyte breakthrough is significantly higher with polar compounds that are more difficult to retain on the SPE column.

The first objective of this paper is to describe a fully automated on-line SPE-LC-MS/MS setup that allows the analysis of different groups of organic contaminants at trace levels in surface and drinking waters. To date, several on-line SPE methods of trace determination of organic contaminants have been published (Rodríguez-Mozaz *et al.*,

2004; Stoob *et al.*, 2005; Pozo *et al.*, 2006; Salvador *et al.*, 2007), however they are often limited to single class of compounds or not sensitive enough for drinking water analysis (Choi *et al.*, 2007). Our goal was to improve the MDL of previously published on-line preconcentration methods (Segura *et al.*, 2007a; Viglino *et al.*, 2008; Garcia-Ac *et al.*, 2009) by loading a higher sample volume (10 mL) in the SPE column. To ensure that the analytes are well retained by the SPE column, we estimated the breakthrough volumes of 17 target compounds for several on-line SPE columns made of different sorbent materials. Target compounds were chosen because of their high use, toxicity and previous detection in wastewaters and surface waters (Segura *et al.*, 2007a; Segura *et al.*, 2007b; Viglino *et al.*, 2008; Garcia-Ac *et al.*, 2009). The column having the highest retention capacity was chosen for method development and validation using real samples. The analytical method was then applied for a field study of PPCPs and pesticides in drinking and surface waters in the region of Montreal, Canada. Our second objective is to evaluate the potential of two alternative approaches of confirmation of the presence of trace contaminants: data-dependent reversed energy ramp (RER) scan in a triple quadrupole and accurate mass measurement using time-of-flight mass spectrometry (TOFMS).

8.1. Experimental methods

8.1.1. Reagents and solutions

Standards of atrazine (ATZ), azithromycin (AZI), caffeine (CAF), carbamazepine (CBZ), ciprofloxacin (CIP), clarithromycin (CLA), cyanazine (CYA), cyclophosphamide (CYC), deethylatrazine (DEA) [transformation product of atrazine and simazine], deisopropylatrazine (DIA) [transformation product of atrazine], 10,11-dihydrocarbamazepine (DHC) [transformation product of carbamazepine], levofloxacin (LEV), methotrexate (MTX), simazine (SIM), sulfamethoxazole (SMX), triclocarban (TCC) and trimethoprim (TRI), were obtained from Sigma-Aldrich Canada (Oakville, ON, Canada). Enalapril (ENA) was kindly supplied as a formulation by a local pharmacy. The internal standard (IS) [$^{13}\text{C}_3$]-atrazine was supplied by ACP Chemical Inc. (Montreal, QC, Canada) with certified purity $\geq 99\%$. Formic acid (F.A.) 98% pure and sodium hydroxide

(NaOH) were purchased from Sigma-Aldrich Canada. LC-MS grade methanol (MeOH), acetonitrile (ACN), H₂O and 0.1% F.A. in H₂O were purchased from J. T. Baker (Phillipsburg, NJ). ACS grade chloroform (CLF) was supplied by Fisher Scientific (Mississauga, ON).

A working solution of 100 µg L⁻¹ of the IS was prepared by dissolving 100 µL of [¹³C₃]-ATZ in 1 mL of MeOH. Stock solutions of 400 mg L⁻¹ of each compound were prepared by dissolving the appropriate amount of each substance in MeOH except for CIP and LEV which were dissolved in 0.1 M NaOH /MeOH 5/95 (v/v). All stock solutions were conserved at -25°C and used for no longer than 3 months.

8.1.2. Instrumentation

Three different experimental setups were used during method development and sample analysis.

Small injection setup: For the estimation of breakthrough volumes, we used a liquid chromatography-tandem mass spectrometry (LC-MS/MS) made by Thermo Fisher Scientific (Waltham, MA). It consists of a LC Surveyor system (Surveyor autosampler and Surveyor MS quaternary pump) coupled with an electrospray ionization (ESI) source and a Quantum Ultra triple quadrupole mass spectrometer. Information on the on-line SPE columns tested is given in Table S1 (Matériel supplémentaire).

On-line SPE setup: For the analysis of drinking and surface water, the Environmental Quantification (EQuan™) system manufactured by Thermo Fisher Scientific was used. This system is based on a column-switching technique (Segura *et al.*, 2007a; Viglino *et al.*, 2008; Garcia-Ac *et al.*, 2009). It consists in a sample delivery system, a switching column array and a LC-MS/MS system. The sample delivery system consists in an HTC Pal autosampler manufactured by CTC analytics AG (Zwingen, Switzerland) and a quaternary load pump (Thermo Scientific Surveyor LC Pump Plus). The switching-column array is composed of the Rheodyne 7750E six-port switching valve made by IDEX (Oak Arbor, WA), a SPE on-line column and an analytical column. The analytical column used was a Synergi Fusion RP column (50 × 2 mm, 4 µm particle size) from Phenomenex (Torrance, CA).

LC-TOFMS setup: For accurate mass measurements, a 6210 Time-of-Flight (TOF) mass spectrometer coupled to an 1100 Series liquid chromatography system manufactured by Agilent (Santa Clara, CA) was used. The columns used were the same as described in *on-line setup* section.

8.1.3. Sample collection and preparation

8.1.3.a. Drinking water and surface samples

All water samples were collected in clean glass bottles and then immediately transported to the laboratory, stored in the dark at 4 °C and analyzed within 24 h. Drinking water samples were collected directly from residential tap water in three Montréal boroughs, and did not need any further preparation. Surface water samples were collected at three sampling sites located around the Island of Montreal: the St. Lawrence River, the Thousand Islands River and the Des Prairies River. The Thousand Island River and St. Lawrence River, two sources of drinking water for the region of Montreal, were selected due to the documented discharges of urban and agricultural wastes (Viglino *et al.*, 2008). Surface water samples were vacuum-filtered through 0.7- μm pore size fiber glass filters followed by 0.45- μm pore size cellulose ester membranes (Millipore - Billerica, MA), to remove suspended particles. A previous study showed that this filtration step did not cause analyte losses (Garcia-Ac *et al.*, 2009).

Aliquots of 100 mL of the drinking and surface water samples were transferred to volumetric glass flasks, acidified with 100 μL of F.A. and spiked with the IS for a final concentration of 200 ng L^{-1} . The samples were then transferred to 20-mL amber glass vials for on-line SPE-LC-MS/MS analysis.

8.1.3.b. Breakthrough volume estimation

The breakthrough volume is defined as the maximum sample volume that can be loaded in a sorbent without significant analyte losses. Breakthrough volume estimation

experiments were done on the small injection setup using the graphical extrapolation method (Hennion, 1999). In order to keep dead volume of the system to a minimum, we used short PEEK tubings with narrow I.D. (127 μm) to make the connections.. Tests were done by injecting 10 μL of a 100 $\mu\text{g L}^{-1}$ standard mix solution on a on-line SPE column and then eluting with different isocratic mobile phase compositions using solvent A [0.1% F.A. in H_2O (v/v)] and solvent B [0.1% F.A. in MeOH (v/v)]. Five isocratic mobile phase compositions (between 25 and 97% of B, depending on the compound) were tested in triplicate for each column. The variation of the retention time of each analyte as a function of the percentage of MeOH in the mobile phase was used to estimate the breakthrough volume. First we measured the void volume (V_m) by pycnometry (Rimmer *et al.*, 2002):

$$V_m = \left(\frac{W_{CLF} - W_{ACN}}{\rho_{CLF} - \rho_{ACN}} \right) \quad \text{(Équation 8-1)}$$

where W_{CLF} and W_{ACN} are the weight of the column filled with CLF and ACN respectively, and ρ_{CLF} and ρ_{ACN} the densities of the solvents at 20 °C. The dead time of the column (t_m) was then calculated by dividing V_m by the flow rate (200 $\mu\text{L min}^{-1}$). Next, we calculated the capacity (or retention) factor (k') of each analyte at a given mobile phase composition. We used a quadratic regression to fit the logarithm of k' as a function of the volume fraction of MeOH (ϕ) in the mobile phase (Jandera *et al.*, 1982):

$$\log k' = \log k'_w - a\phi + b\phi^2 \quad \text{(Équation 8-2)}$$

where the y-intercept of the quadratic regression, $\log k'_w$, is the logarithm of the capacity factor of the analyte when the mobile phase is 100% aqueous. This value is used to calculate the estimated breakthrough volume (V_b') according to:

$$V_b' = V_m (k'_w + 1) \quad \text{(Équation 8-3)}$$

8.2. On-line SPE and LC parameters

The HTC Pal autosampler allows the injection of large sample volumes using a 5.0-mL dispenser syringe. We used three consecutive 4-mL injections to fill the 10-mL loop of the autosampler. During method development, we observed that method precision was

improved by injecting a sample volume larger than the maximum capacity of the loop. This reduces the sample dilution effect inside the loop during the injection process. The injection of a volume of 12 mL was sufficient to minimize this effect and to obtain an acceptable precision. The syringe was filled at flow rate of $75 \mu\text{L s}^{-1}$ and the injections were performed at the same flow rate. The sample was then introduced into the on-line SPE column using a 0.1% F.A. in H_2O (v/v) solution delivered by the load pump at a flow rate of 1.5 mL min^{-1} .

After the sample was loaded, the divert valve of the column-switching array was actuated (8.5 min) and the on-line SPE column was back-flushed with the LC mobile phase [solvent A: 0.1% F.A. in H_2O (v/v), solvent B: 0.1% F.A. in MeOH (v/v) and solvent C: 0.1% F.A. in ACN (v/v)] delivered by the analytical pump (details on the load and analytical pumps program are shown in the Supplementary material, Table S2). The LC gradient allows the transfer of the retained components of the sample from the on-line SPE to the analytical column where the analyte separation takes place. All the on-line operations are fully automated for routine analysis with a total run time of ~ 30 min (26.5 min for the chromatographic run and ~ 3.5 min for sample injection). To avoid sample cross-contamination, the syringe, the injection port and the injection valve were washed with 5 mL of $\text{H}_2\text{O}/\text{MeOH}$ 50/50 (v/v) before each injection.

For LC-TOFMS, we employed a similar LC method (see Supplementary material, Table S3). In order to perform accurate mass measurements on the preconcentrated samples, a two step procedure was used. In the first step, the on-line SPE column was using the on-line setup. In the second step, both, the SPE column and the analytical columns were then removed and connected to the LC-TOFMS setup for desorption and separation.

8.2.1. Mass spectrometry parameters

Electrospray ionization (ESI) was performed on the positive mode using a spray voltage of 4.5 kV. The sheath gas (N_2) was set to 30 arbitrary units and the auxiliary gas (N_2) to 5 arbitrary units. Ion transfer capillary temperature was set to $350 \text{ }^\circ\text{C}$. The tandem mass spectrometer was operated in selected reaction monitoring (SRM) mode at unit

resolution (full width at half maximum = 0.7 u). In order to select precursor ions for each analyte, chromatograms were initially recorded in full scan mode. In all cases, the pseudo-molecular $[M+H]^+$ ion was selected. Once precursors were selected, full product ion scans were recorded, and collision energies were optimized to get the maximum intensity of the fragment ions obtained. The pressure of the collision gas (Ar) in the collision cell was set to 1.5 mTorr. The most intense SRM transition (SRM#1) was selected for quantitation and the second most intense (SRM#2) was used for confirmation. SRM transitions, collision energy and skimmer offset were compound-dependent and appear in Table S4 (Supplementary material).

The reverse energy ramp (RER) scan allows a linear increase of the collision energy as a function of the decreasing m/z values scanned on the third quadrupole. RER scan energy parameters are compound-dependent, but generally collision energy was set to a low value (5-15 V) and the energy ramp to a higher value (30-40 V). This allows the acquisition of rich full product ion spectra as abundant high and low m/z products ions are generated by the increasing collision energy (Beck *et al.*, 2006). This data-dependent scan was triggered when the SRM signal was $> 10^4$ counts.

The TOFMS acquisition range was 105-1000 m/z with a scan rate of 1.03 scans s^{-1} . ESI source gas (N_2) temperature was set to 350 °C, flow to 12 L min^{-1} and nebulizer gas pressure to 30 psi. Capillary voltage was set to 3.5 kV, fragmentor to 175 V and skimmer to 65 V.

8.3. Method validation

8.3.1.a. Drinking water

Internal calibration was used for the quantitation of drinking water samples. Calibration solutions of the target compounds were prepared in 0.1% F.A. in H_2O (v/v) by dilution of a mixed working solution (100 $\mu g L^{-1}$) at the following concentrations: 5, 10, 25, 50, 75, 100, 150, 250 $ng L^{-1}$. The IS was added to every solution for a final concentration of

200 ng L⁻¹. ¹³C₃-atrazine was chosen as IS because it gave a strong and stable signal in ESI(+), which helped to reduce method variability and to compensate for losses in the system.

Extraction recovery of target compounds in the on-line system was determined by applying a previously published method (Koal *et al.*, 2003). Briefly, analyte peak areas resulting from 4 replicate 10-mL injections of 0.1% F.A. in H₂O (v/v) spiked at 20 ng L⁻¹ level were compared to those obtained by 4 replicate 500-μL injections of spiked 0.1% F.A. in H₂O (v/v) with a concentration 20 times higher (400 ng L⁻¹). Precision was determined using spiked solutions of 0.1% F.A. in H₂O (v/v) at the 5 ng L⁻¹ level injected four times each during the sequence run (intra-day precision) and four times on three different days (inter-day precision). MDLs were calculated as the standard deviation of the calculated concentration (using SRM #1) of seven replicate measurements of a low concentration solution, multiplied by the Student's *t* value for a one-tailed test at the 99% confidence level and *n*-1 degrees of freedom (Glaser *et al.*, 1981). MDL is defined as the “minimum concentration of a substance that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero” (Glaser *et al.*, 1981). The MDLs of the SRM #2 were calculated the same way and will be called method confirmation limits (MCL) hereafter. The concentration of the solution was 5 or 10 ng L⁻¹ depending on the analyte. To ensure that the concentration used to determine the MDL and the MCL was appropriate, the ratio of the average of seven replicate measurements and the measured MDL or MCL should be higher than 4 and lower than 10 (Centre d'Expertise en Analyse Environnementale du Québec, 2002). Additionally, ion ratios (relative intensity of SRM#2 vs. SRM#1) of each compound detected in the samples were calculated and compared to ion ratios in pure standards. In order to confirm the presence of an analyte in the samples, ion ratio differences between the former and the later had to be lower than maximum tolerance levels according to European Union Commission Decision 2002/657/EC (Commission of the European Communities, 2002). Blanks subjected to the entire preparation and analysis procedure (method blanks) were used to correct for sample contamination.

8.3.1.b. Surface Water

The method of standard additions was used for quantitation of the surface water samples and also an IS ($^{13}\text{C}_3$ -atrazine) was added to all samples. Standard additions was necessary to compensate for the presence of matrix effects affecting the ESI process usually observed when analyzing environmental samples, while the IS contributed to improve method precision. Eight calibration solutions were prepared per sample: five spiked solutions (10, 25, 50, 75 and 100 ng L⁻¹) and three unspiked. The amounts spiked were chosen to represent the average analyte concentration we expected to find in the surface samples according previous studies (Segura *et al.*, 2007a; Viglino *et al.*, 2008; Garcia-Ac *et al.*, 2009). Consequently, it is important to adjust the amounts spiked on each sample depending on the sites studied. The IS (200 ng L⁻¹) and F.A. (0.1%, v/v) were added to all solutions. Extraction recovery was measured the same way as for drinking water, except that the matrix used was surface water acidified with F.A. (0.1%, v/v). Intra- and inter-day precision and MDL were determined using the same method as for drinking water but surface river water was spiked at the 25 ng L⁻¹ level. Matrix effects were determined with the same extraction processes in surface water samples and were calculated according to the following equation:

$$\text{Matrix effects (\%)} = \left(\frac{SW_S - SW_{NS}}{W} \right) \times 100\% \quad \text{(Équation 8-4)}$$

where SW_S is the analyte peak area in spiked surface water, SW_{NS} the analyte peak area in the non-spiked surface water and (W) the analyte peak area of a known amount spiked in 0.1% F.A. in H₂O (v/v). A value of 100% indicates that there is no absolute matrix effect. Signal enhancement is indicated by matrix effects values > 100% and signal suppression by values < 100% (Salvador *et al.*, 2007).

8.4. Results and discussion

8.4.1. Breakthrough volume estimation

The compounds studied in this work represent a wide range of octanol–water distribution coefficients and water solubilities. Furthermore, some of them are ionic

depending on the pH and others are neutral (for details on the physico-chemical properties of the target compounds consult (Segura *et al.*, 2007a; Viglino *et al.*, 2008; Garcia-Ac *et al.*, 2009) and references therein). Therefore, we used the graphical extrapolation method for the estimation of breakthrough volumes (V_b') in order to find the on-line SPE column best fitted for the preconcentration of large sample volumes of analytes having such different physico-chemical properties. This approach has been used previously for the determination of the breakthrough volume of various pesticides (Koal *et al.*, 2003) and chlorophenols (Werkhoven-Goewie *et al.*, 1981).

It is widely accepted that a quadratic equation can be used to describe the retention as a function of binary mobile phase mixtures observed in reversed-phase liquid chromatography (RPLC) systems (Poole et Poole, 2002). The terms a and b of equation (2) depend on the size and polarity of the solute as well as the temperature (Jandera *et al.*, 1982). However, according to Jandera and Kubát (Jandera et Kubát, 1990) the quality of the prediction is affected by the range of ϕ values chosen, and the closer these values are to 0% organic modifier, the better. In practice this is not always possible and therefore accuracy may fall within an order of magnitude. For some strongly retained compounds such as CBZ, CLA and CYC it was not possible to use $\phi < 50\%$ MeOH because of the intense peak broadening which made the identification of the peaks difficult. For these compounds, the error on the estimation of k'_w is expected to be higher than for the early eluting compounds (DIA, MTX and TRI) for which we could use ϕ as low as 25%. Also this method fails to accurately determine k'_w because of the presence of preferential sorption of the organic solvent on the apolar surface of the sorbent (Jandera et Kubát, 1990). Therefore we used the results shown on Table 1 only as a comparative estimate that allowed us to choose the best on-line SPE column. The results obtained by this method must be interpreted with caution as large errors do not imply that a given column is not appropriate for the preconcentration of large volumes. They rather reflect the limitation of this method to predict the breakthrough volumes of strongly retained compounds.

In spite of the shortcomings and errors associated with this method, it proved useful to evaluate the on-line SPE column best suited for the preconcentration of a large array of heterogeneous organic contaminants. The results in Table 1 indicate that the Strata-X column is the best candidate for the preconcentration of large sample volumes given that in

most cases, the V_b' are all higher than 10 mL, even considering the estimation errors. The large error observed for many of the studied compounds on the Strata-X also suggests that the retention in this column was stronger as the ϕ values used for the quadratic regression were larger compared to other columns. We paid particular attention to early eluting compounds in RPLC systems such as DIA, MTX and TRI. The common assumption that more polar columns such as C_8 will retain better more polar compounds could not be applied to all the target compounds, as the V_b' of the DIA, MTX and TRI in the Strata C_8 column was lower or similar than the V_b' of the Strata C_{18} or Hypersil Gold columns. Our results are similar to those obtained in a previous study of breakthrough volume of nitrogen-containing pesticides, which showed that polymeric phases show higher breakthrough volume than silica-based phases (Soriano *et al.*, 1998).

The hydrophobic and electrostatic interactions that occur between compounds and the polarizable surface of graphite on the Hypercarb column (Hennion, 1999) may explain why some compounds were not desorbed, as strong interactions are more difficult to break. Also planar molecules show increased retention because of stronger interactions with graphite surface (Thermo Electron, 2004). The V_b' volumes of DEA and DIA obtained with the Hypercarb on-line SPE column were high (580 mL \pm 14% and 1000 mL \pm 13%, respectively) which agrees with the results obtained by Barceló and Hennion (Barceló et Hennion, 1995) which found breakthrough volumes higher than 100 mL for these compounds.

The validity of the results obtained for V_b' by the graphical extrapolation method was confirmed by the extraction recovery tests on drinking water, as shown in Table S5 and S6 (Supplementary material). As expected, the extraction recovery values for most compounds were good (> 80%), except for the most polar compounds such as TRI (64 \pm 5%) and MTX (69 \pm 2%). Better recoveries could be obtained by using larger I.D. columns (3-4 mm) (Pocurull *et al.*, 1995), however on-line SPE columns of these dimensions are not always commercially available.

Tableau 8-1. Comparison of estimated breakthrough volumes (V_b') of 17 selected trace organic contaminants in the on-line SPE columns tested.

| Compound | Column | | | | | | | | | | | | | | | | | | | | |
|----------|----------------|--------------|--------|-----------------|--------------|--------|----------------|--------------|--------|------------------------|--------------|--------|---------------------------|--------------|--------|------------------|--------------|--------|----------------|--------------|--------|
| | C ₈ | | | C ₁₈ | | | Cyclone | | | Hypercarb ^a | | | HypersilGold ^d | | | HypersilGold PFP | | | Strata-X | | |
| | V_b' (mL) | error (%) | r^2 | V_b' (mL) | error (%) | r^2 | V_b' (mL) | error (%) | r^2 | V_b' (mL) | error (%) | r^2 | V_b' (mL) | error (%) | r^2 | V_b' (mL) | error (%) | r^2 | V_b' (mL) | error (%) | r^2 |
| ATZ | 300 | 7 | 0.9998 | 199 | 5 | 0.9998 | 2000 | 94 | 0.9907 | NA | NA | NA | 11 | 47 | 0.9800 | 58 | 10 | 0.9974 | 1000 | 45 | 0.9991 |
| AZI | 50 | 20 | 0.9538 | 7 | 32 | 0.9538 | 300 | 46 | 0.9538 | NA | NA | NA | 100 | 121 | 0.9538 | 700000 | 41 | 0.9538 | 800 | 25 | 0.9538 |
| CAF | 3.7 | 10 | 0.9939 | 3.0 | 9 | 0.9935 | 2.1 | 12 | 0.9872 | NA | NA | NA | 1.2 | 29 | 0.9446 | 6.9 | 8 | 0.9946 | 16 | 24 | 0.9853 |
| CBZ | 500 | 6 | 0.9998 | 400 | 46 | 0.9783 | 20000 | 324 | 0.9670 | 4 | 161 | 0.9847 | 2400 | 16 | 0.9994 | 130 | 11 | 0.9980 | 4000 | 26 | 0.9991 |
| CIP | 2.2 | 13 | 0.8432 | 1.6 | 23 | 0.8432 | 40 | 75 | 0.8432 | NA | NA | NA | 22 | 84 | 0.8432 | 57 | 6 | 0.8432 | 100 | 19 | 0.8432 |
| CLA | 260000 | 27 | 0.9997 | 360000 | 9 | 0.9997 | 3000000000 | 155 | 0.9997 | NA | NA | NA | 600000 | 21 | 0.9997 | 2000000 | 49 | 0.9997 | 30000000 | 108 | 0.9997 |
| CYA | 199 | 5 | 0.9998 | 148 | 5 | 0.9998 | 10000 | 196 | 0.9842 | 11 | 115 | 0.9884 | 10 | 48 | 0.9718 | 80 | 12 | 0.9982 | 2100 | 25 | 0.9991 |
| CYC | 40 | 9 | 0.9992 | 25 | 33 | 0.9799 | 120 | 76 | 0.9630 | 90 | 187 | 0.9512 | 51 | 7 | 0.9998 | 8.9 | 55 | 0.9998 | 3000 | 174 | 0.9651 |
| DEA | 16.3 | 4 | 0.9997 | 12.5 | 5 | 0.9995 | 10 | 21 | 0.9927 | 580 | 14 | 0.9987 | 2.4 | 33 | 0.9632 | 6.8 | 8 | 0.9965 | 90 | 51 | 0.9742 |
| DIA | 5.3 | 4 | 0.9993 | 4.3 | 6 | 0.9980 | 3.5 | 19 | 0.9843 | 1000 | 13 | 0.9988 | 1.2 | 28 | 0.9526 | 3.0 | 8 | 0.9942 | 22 | 35 | 0.9788 |
| DIH | 600 | 9 | 0.9997 | 500 | 54 | 0.9794 | 1000 | 143 | 0.9582 | 70 | 81 | 0.9953 | 1600 | 10 | 0.9998 | 80 | 9 | 0.9997 | 11000 | 41 | 0.9989 |
| ENA | 100 | 24 | 0.9958 | 300 | 15 | 0.9986 | 1000 | 81 | 0.9706 | 5000000 | 348 | 0.9542 | 1100 | 13 | 0.9997 | 900 | 12 | 0.9786 | 60000 | 247 | 0.9399 |
| LEV | 1.1 | 16 | 0.9116 | 0.9 | 25 | 0.9116 | 60 | 57 | 0.9116 | NA | NA | NA | 3 | 39 | 0.9116 | 38 | 5 | 0.9116 | 66 | 10 | 0.9116 |
| MTX | 0.49 | 17 | 0.9098 | 1.1 | 37 | 0.8571 | 2.2 | 10 | 0.9662 | NA | NA | NA | 0.6 | 29 | 0.8181 | 7.2 | 14 | 0.9953 | 30 | 66 | 0.9655 |
| SIM | 72 | 5 | 0.9998 | 65 | 4 | 0.9999 | 300 | 30 | 0.9965 | NA | NA | NA | 6 | 42 | 0.9647 | 25 | 9 | 0.9972 | 200 | 169 | 0.9505 |
| SMX | 24 | 8 | 0.9795 | 14 | 9 | 0.9795 | 220 | 30 | 0.9795 | 400 | 29 | 0.9795 | 4 | 29 | 0.9795 | 21 | 7 | 0.9795 | 1200 | 20 | 0.9795 |
| TRI | 0.53 | 10 | 0.9092 | 0.8 | 22 | 0.9092 | 18 | 25 | 0.9092 | 20 | 105 | 0.9092 | 1.8 | 32 | 0.9092 | 7.8 | 8 | 0.9092 | 29 | 9 | 0.9092 |

^a Elution of ATZ, AZI, CAF, CIP, LEV, CLA, LEV, MTX and SIM and was not possible with MeOH on the Hypercarb column.

8.4.2. Development of a method for the analysis of PPCPs and pesticides in drinking and surface waters

On the basis of the results obtained for V_b' , the chromatographic method was further optimized. A suite of preliminary experiments was performed, testing several mobile phase gradients (results not shown). The optimal separation of the 17 compounds was achieved using a ternary mobile phase composed of solvents A (0.1% F.A. in H₂O), B (0.1% F.A. in MeOH) and C (0.1% F.A. in ACN) and a Synergi Fusion RP analytical column (for details on the time program, see Supplementary material, Table S2). The use of this column is justified by the fact that peak broadening could occur if the on-line SPE column is coupled with an analytical column having a different type of retention (Soriano *et al.*, 1998; Oliferova *et al.*, 2006). Representative chromatograms of a 25 ng L⁻¹ standard mixture of the compounds analyzed are illustrated in Fig. S1 (Supplementary material).

In previous studies (Segura *et al.*, 2007a; Viglino *et al.*; Garcia-Ac *et al.*), important on-line SPE parameters such as sample loading flow rate, wash volume and organic modifier in the wash volume were optimized; therefore there was no need to further study their effects. Based on that, a sample load rate of 1.5 mL min⁻¹ was chosen because it gave optimal results in terms of system stability and run time. Higher flow rates could be used, in particular with on-line SPE columns having larger particle sizes (Segura *et al.*, 2009b). In spite of that, we observed that high flow rates sometimes affected the long-term functioning of the load pump and a higher tendency to provoke leaks. Consequently, we preferred longer run times to achieve better system stability. We did not perform any wash containing organic modifiers after the load step to avoid analyte losses (especially for the weakly retained polar compounds). Additionally, it has been shown that matrix effects can be caused by co-eluting substances (Kloepfer *et al.*, 2005; Segura *et al.*, 2009b), therefore a wash step containing organic modifier fails to eliminate these interferences, and it becomes unnecessary. Consequently only an aqueous wash was performed after the sample was loaded into the on-line SPE column to eliminate salts and other hydrophilic substances present in the samples.

8.4.3. Method validation

Validation data, determined for both drinking and surface water are presented in Tableau S5 and S6, respectively (Supplementary material). During the validation process, we observed that we could not obtain adequate results for four of the selected compounds (AZI, CAF, CIP and LEV), therefore they were not further analyzed. At this point we do not have a good explanation for this, however in the case of the two quinolones anti-infectives (CIP and LEV) the presence of metal cations in the samples could cause the formation of complexes (Gu et Karthikeyan, 2005b). Also we decided to add a new compound to the final analyte list, TCC for which no breakthrough study was performed but that had a strong retention in the Strata-X on-line SPE column.

For drinking water, linearity was excellent for all compounds with determination coefficients (R^2) ≥ 0.99 except for CYC, DHC and ENA which were > 0.98 . Method intra-day precision was between 1-7% and between 4-16% for inter-day precision. Extraction recovery achieved for all target compounds was generally good ($> 80\%$) except for CYC, ENA, MTX, SMX and TRI, which showed somewhat lower recoveries (64-72%). The MDL ranged from 0.4 to 3 ng L⁻¹ while MCL ranged from 1 to 6 ng L⁻¹.

For surface water samples, an excellent linearity ($R^2 \geq 0.99$) was achieved for most calibration curves except for CYC in the St. Lawrence River sample and ENA in the Des Prairies River which had a R^2 of 0.9550 and 0.9711, respectively. MDL ranged from 0.6 to 6 ng L⁻¹ and the MCL from 0.9 to 14 ng L⁻¹. Extraction recovery was between $60 \pm 2\%$ and $109 \pm 2\%$. Intra-day precision was $\leq 10\%$ except for TCC (14%), while inter-day precision was $\leq 12\%$ except for CYC and MTX (15% for both). During our preliminary experiments, we worked using only standard additions without an IS but method precision was poor (results not shown). Therefore we decided to add an IS to reduce signal variability to acceptable values ($<15\%$).

8.4.4. Matrix effects

A major problem for quantitative analysis using ESI is the presence of matrix effects, which are defined as the unexpected suppression or enhancement of the analyte response due to co-eluting matrix constituents (Schuhmacher *et al.*, 2003). Our matrix effects study showed that some compounds were not subjected to matrix effects (ATZ, CLA, DEA, DIA, MTX, SIM, and TRI) while others were susceptible to signal enhancement (CBZ, CYA, CYC, DHC, and ENA) or signal suppression (SMX and TCC). Strategies to reduce matrix effects such as external calibration using matrix-matched samples, isotope dilution and standard additions have been recommended (Kloepfer *et al.*, 2005). Standard additions are often considered a lengthy and labor-intensive method, but when working with on-line SPE systems, standard additions becomes an efficient means to correct for signal distortion caused by matrix interferences.

8.4.5. Improvement of limits of detection

The preconcentration of 10 mL of sample lowered MDLs by a factor of 1.7 to 20 compared to previous methods using 1 mL (Segura *et al.*, 2007a; Viglino *et al.*, 2008; Garcia-Ac *et al.*, 2009). Four compounds (ATZ, CBZ, DEA and SIM) showed lower improvement factors (< 5) than expected, while for DIA, ENA and MTX the improvement factor was much better (> 10). We attribute this compound-dependent behavior of the MDLs to the presence of co-extracted substances during the load step which may differentially affect the signal variability of each analyte. In spite of this, the MDLs were in the low ng L⁻¹ range which allows the detection of trace amounts of the selected contaminants in drinking and surface water. Also, the low MCL values achieved reduce the probability of false positives. These detection limits are of the same magnitude or lower than other on-line (Stoob *et al.*, 2005; Pozo *et al.*, 2006) or off-line SPE methods (Ternes, 2001; Vanderford *et al.*, 2003) used for the analysis of PPCPs and pesticides reporting detection limits in the 0.1-20 ng L⁻¹ range.

8.4.6. Analysis of drinking and surface water samples

To demonstrate the applicability of the developed method, drinking water and surface water samples from the region of Montreal, Canada, were analyzed. Results are summarized in Table 2. The targeted *s*-triazine herbicides and their transformation products were the most frequently detected compounds (Fig. 1 and Fig. 2). In drinking water, their concentration ranged from 2.0 ± 0.1 for CYA to 331 ± 9 ngL⁻¹ for DEA. In surface water, ATZ and DEA, were detected in the three surface water samples. The highest concentration detected in surface water was 479 ng L⁻¹ for DEA, in the St. Lawrence River. ATZ and its transformation products are among the most frequently detected organic contaminants in surface, ground and drinking water samples (Battaglin et Hay, 1996; Squillace *et al.*, 2002). This can be explained by its intense application in agricultural fields in US and Canada (Hua *et al.*, 2006; Poissant *et al.*, 2008).

The only PPCP detected in the surface water that we sampled was CBZ, with a concentration range of 6.0 ± 0.5 to 11 ± 1 ng L⁻¹. These levels were generally similar or lower to those previously reported in Canadian surface waters (Hua *et al.*, 2006; Yargeau *et al.*, 2007). Studies have shown that among the different pharmaceutical compounds detected in the environment, CBZ, an anti-convulsion drug used mainly for the treatment of epilepsy, is one of the most frequently detected compound in surface waters (Miao et Metcalfe, 2003; Benotti *et al.*, 2009). The absence of other targeted PPCPs may be influenced by the choice of sampling sites, which were not located close to wastewater plants discharges. However, the MDLs and MCLs achieved are sufficiently low to detect and confirm the presence of the targeted PPCPs in surface waters affected by effluent dispersion plumes (Focazio *et al.*, 2008).

Tableau 8-2. Analysis of drinking and surface water samples of the region of Montreal. All values are in nanograms-per-liter (ng L⁻¹).

| Compound | Drinking water | | | | | Surface water | | | | |
|----------|----------------|-----|------------|------------|------------|---------------|-----|--------------------|--------------------|----------------------------|
| | MDL | MCL | Sample A | Sample B | Sample C | MDL | MCL | St. Lawrence River | Des Prairies River | One Thousand Islands River |
| ATZ | 0.7 | 1 | 15.0 ± 0.4 | 28.0 ± 0.2 | 21.0 ± 0.3 | 0.6 | 0.9 | 48 ± 3 | 2.0 ± 0.8 | 2.0 ± 0.4 |
| CBZ | 0.7 | 3 | < MCL | 5.6 ± 0.4 | < MCL | 1 | 4 | 11 ± 1 | 6.0 ± 0.5 | 10 ± 1 |
| CLA | 2 | 2 | < MDL | < MDL | < MDL | 6 | 6 | < MDL | < MDL | < MDL |
| CYA | 0.8 | 1 | 2.0 ± 0.1 | 3.5 ± 0.1 | 2.0 ± 0.2 | 0.8 | 2 | 4.0 ± 0.5 | < MCL | < MCL |
| CYC | 1 | NA | < MDL | < MDL | < MDL | 1 | NA | < MDL | < MDL | < MDL |
| DEA | 3 | 5 | 278 ± 13 | 331 ± 9 | 317 ± 10 | 3 | 5 | 479 ± 4 | 20 ± 4 | 19 ± 1 |
| DHC | 1 | 5 | < MDL | < MDL | < MDL | 1 | NA | < MDL | < MDL | < MDL |
| DIA | 0.4 | NA | 8.9 ± 0.5 | 11.0 ± 0.4 | 8 ± 1 | 1 | 5 | 12 ± 1 | 1.0 ± 0.2 | 2.0 ± 0.2 |
| ENA | 1 | NA | < MDL | < MDL | < MDL | 1 | NA | < MDL | < MDL | < MDL |
| MTX | 1 | NA | < MDL | < MDL | < MDL | 2 | NA | < MDL | < MDL | < MDL |
| SIM | 2 | 3 | 7.0 ± 0.4 | 16 ± 3 | 13 ± 2 | 2 | 2 | 22 ± 4 | 4 ± 1 | 4 ± 1 |
| SMX | 1 | 2 | < MDL | < MDL | < MDL | 3 | 14 | < MCL | < MDL | < MDL |
| TCC | 3 | 5 | < MDL | < MDL | < MDL | 6 | 10 | < MDL | < MDL | < MDL |
| TRI | 3 | 6 | < MDL | < MDL | < MDL | 2 | 3 | < MDL | < MDL | < MDL |

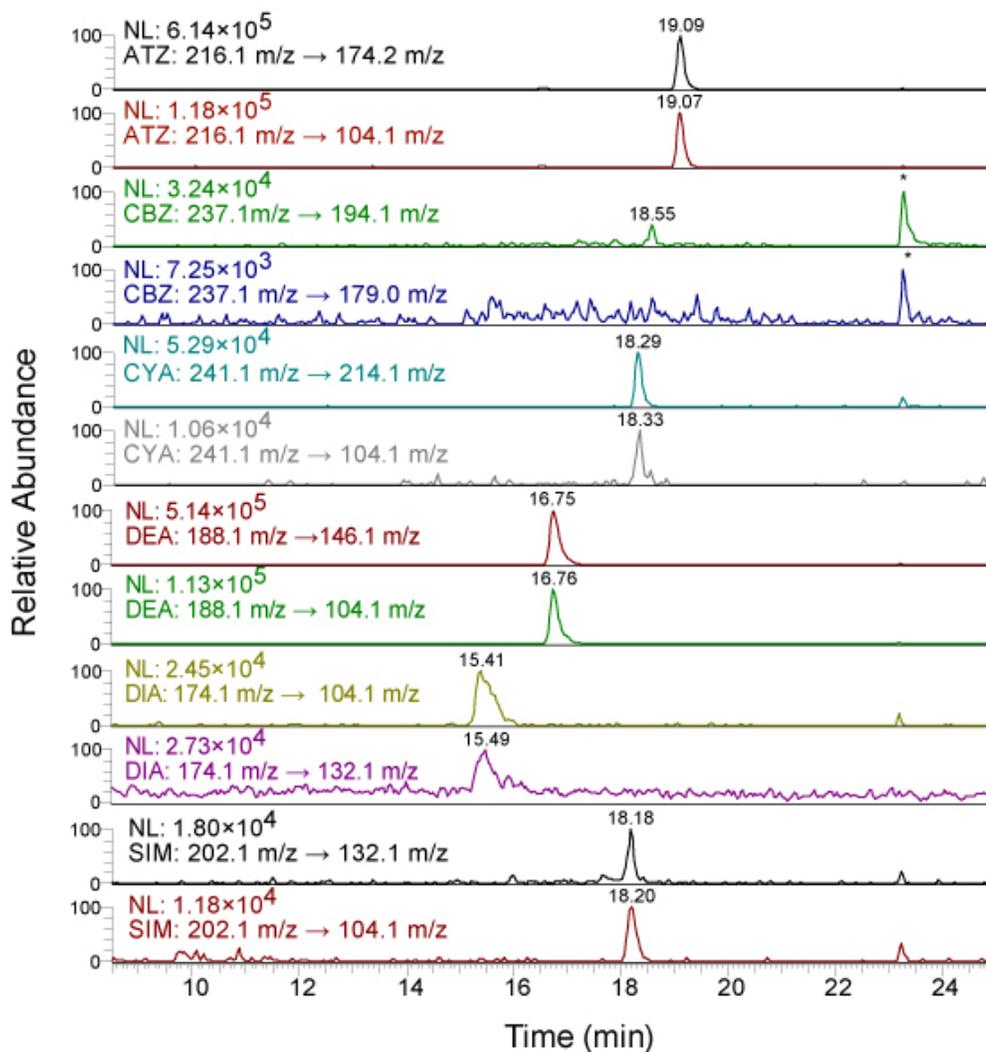


Figure 8-1. LC-MS/MS chromatograms in SRM mode of the detected compounds in drinking water sample A (not spiked). The presence of CBZ could not be confirmed because the SRM #2 signal was lower than the MCL. Peaks due to matrix components having the same SRM as the analyte are indicated by asterisks (*).

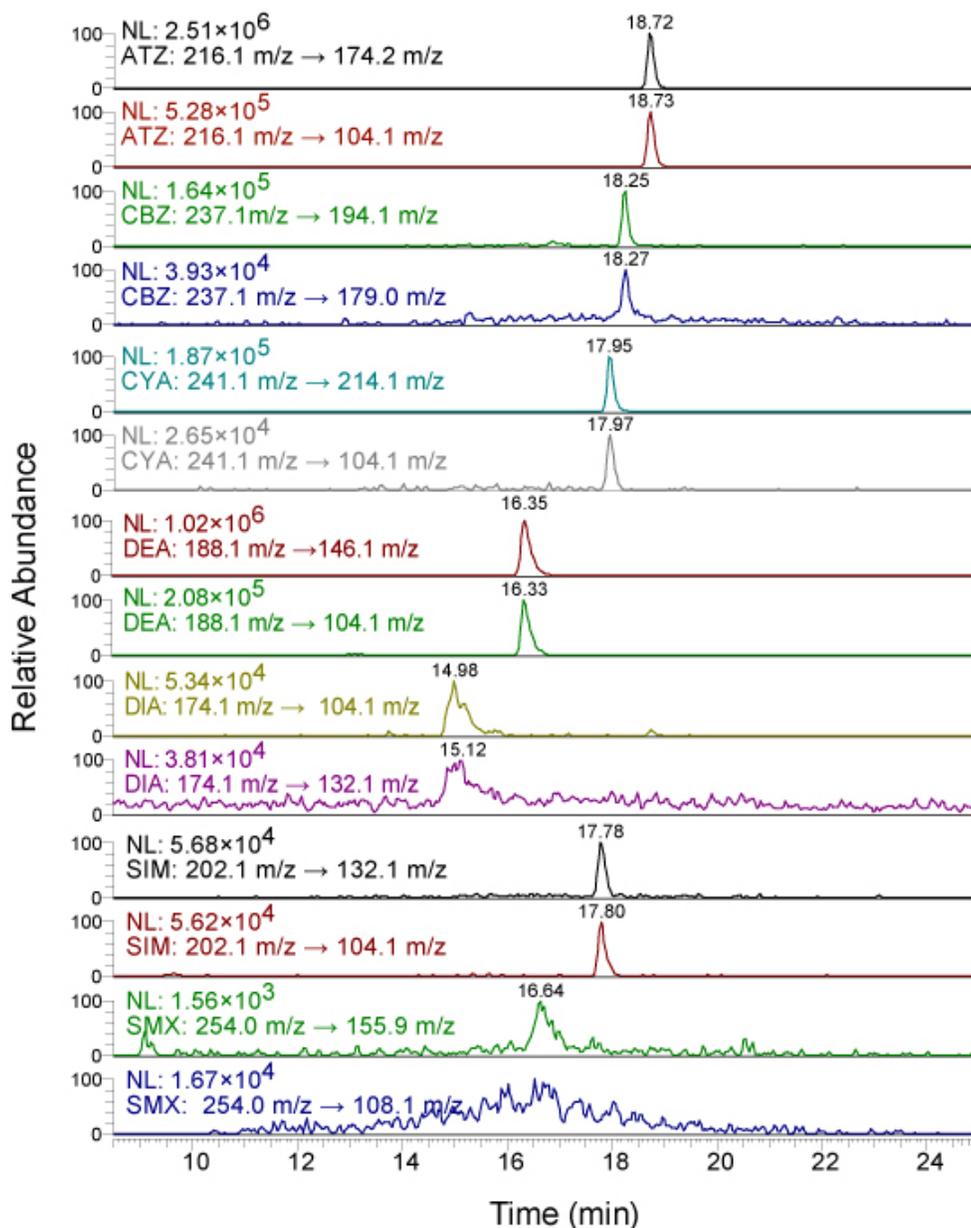


Figure 8-2. LC-MS/MS chromatograms in SRM mode of the detected compounds in the St. Lawrence River sample (not spiked). The presence of SMX could not be confirmed because the SRM #2 signal was lower than the MCL.

The only PPCP detected in the surface water that we sampled was CBZ, with a concentration NL range of 6.0 ± 0.5 to 11 ± 1 ng L⁻¹. These levels were generally similar or lower to those previously reported in Canadian surface waters (Metcalf *et al.*, 2003b; Hua *et al.*, 2006; Yargeau *et al.*, 2007). Studies have shown that among the different

pharmaceutical compounds detected in the environment, CBZ, an anti-convulsion drug used mainly for the treatment of epilepsy, is one of the most frequently detected compound in surface waters (Miao et Metcalfe, 2003; Benotti *et al.*, 2009).

The absence of other targeted PPCPs may be influenced by the choice of sampling sites, which were not located close to wastewater plants outfalls. However, the MDLs and MCLs achieved are sufficiently low to detect and confirm the presence of the targeted PPCPs in surface waters affected by effluent dispersion plumes (Kolpin *et al.*, 2002; Focazio *et al.*, 2008).

8.4.7. Alternative methods of confirmation

As seen on Table S4 (Supplementary material) an intense second SRM could not be found for 4 compounds (CYC, DHC, ENA and MTX). Thus, their confirmation is not possible with SRM#2. In order to solve this limitation, we explored the potential of liquid chromatography-time-of-flight mass spectrometry (LC-TOFMS) and the data-dependent RER scan in a triple quadrupole using the same environmental samples.

With accurate mass measurements, it is possible to increase the certainty of the presence of a compound based on its molecular formula which helps to avoid false positives. Only six of the 14 target compounds were possible to detect by LC-TOFMS (Fig. 3), while using spiked surface water at the 50 ngL⁻¹ level. The measured masses obtained for each compound were compared with the theoretical ones and errors were < 1.2 mmu. These results suggest that PPCPs and pesticides confirmation by LC-TOFMS in environmental samples using our method is rather limited, due to the low concentrations (1-10 ng L⁻¹) normally present in surface waters. To achieve a significant response, we would need to preconcentrate a much higher sample volume. Therefore, to be efficiently applied with our method, trace organic pollutants should occur at higher concentration levels, roughly 20-200 ng L⁻¹.

LC-MSTOF analysis of a non-spiked St. Lawrence River sample (Fig. S2, Supplementary material), allowed additional confirmation of the presence of ATZ and

DEA. A screening study using a homemade database of 60 common surface water contaminants with the “Find compounds by formula” feature on the Agilent MassHunter software (version B.02.00) also revealed the possible presence of *N,N*-Diethyl-*m*-toluamide (DEET) with a mass error of 0.2 mmu. The presence of this compound was further investigated using LC-MS/MS with the SRM transition 192 *m/z* → 119 *m/z* (Rodil *et al.*, 2009) and we obtained an intense peak at an equivalent retention time to LC-MSTOF (results not shown). Because a standard of this substance was not available, we cannot be certain of the presence of this compound. Nevertheless, these results show the potential of on-line preconcentration of large-volume injections coupled to LC-MS/MS and LC-TOFMS for the screening of other environmental contaminants.

In addition to accurate mass measurements for analyte confirmation, product ion mass spectra were collected using the RER scan in a triple quadrupole. These mass spectra provided additional structural information on the compounds analyzed and were used to confirm their presence by comparing them with the mass spectra of standard solutions. Fig. 4 shows the RER product ion mass spectrum of ENA collected during the analytical run for a spiked sample (100 ng L⁻¹). This figure also shows that when the signal generated by the ENA SRM transition exceeds the threshold value set in the acquisition method, a product ion scan of the precursor is triggered (Fig 4-B, C). As opposed to static product ion scan mass spectra which uses a single collision value, RER-generated product ion mass spectra shows high abundances across the whole scan range (Fig. 4-C). This results from the dynamic collision energy used, which is increased while the second quadrupole scans the lower mass range. As it can be observed, the ENA RER mass spectrum obtained using our method (Fig. 4-C) is almost identical to the one acquired by infusion of a pure standard (Fig. 4-D). This allows us to confirm the presence of ENA using the product ion spectrum. Other compounds that could be confirmed by the data-dependent RER scan were DEA and CLA (results not shown) and SIM (Fig. S3, Supplementary material) in the Des Prairies River sample spiked at the 25 ng L⁻¹ level. This figure shows that the RER technique works well in surface water at environmentally relevant concentrations. Nonetheless, the performance of this technique is hindered by the presence of co-eluting compounds having similar *m/z* ratios as the precursor ion. The presence of such species results in a “dirty” product ion mass spectra containing fragments belonging to interfering precursors.

Therefore it is essential to have an excellent chromatography to take full advantage of this technique.

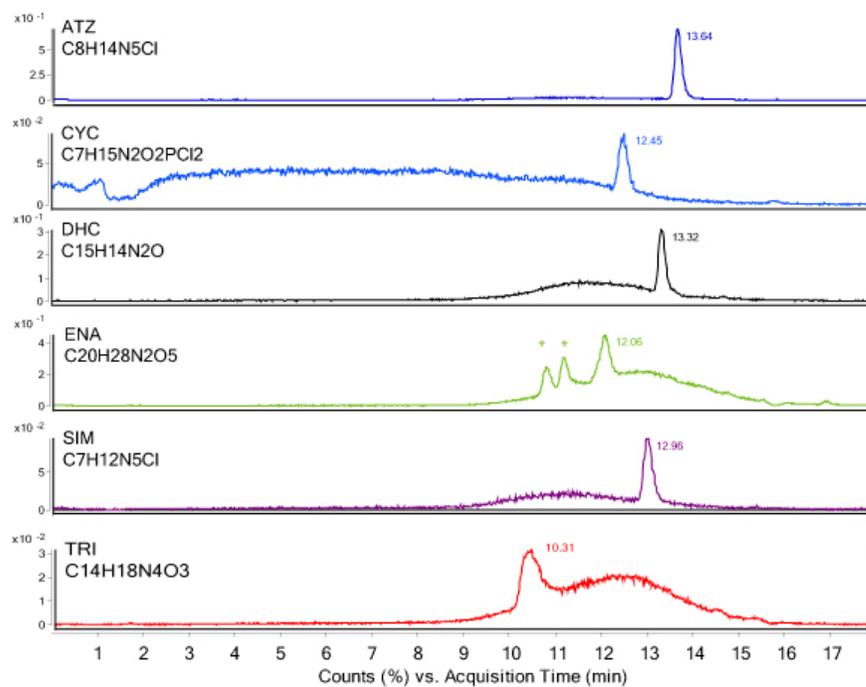


Figure 8-3. LC-MSTOF extracted ion chromatogram of the Des Prairie River sample spiked at the 50 ng L-1 level. Retention times are different to those obtained by the online SPE setup because of differences on the experimental setups. Peaks due to matrix components having accurate masses close to the analyte are indicated by asterisks (*).

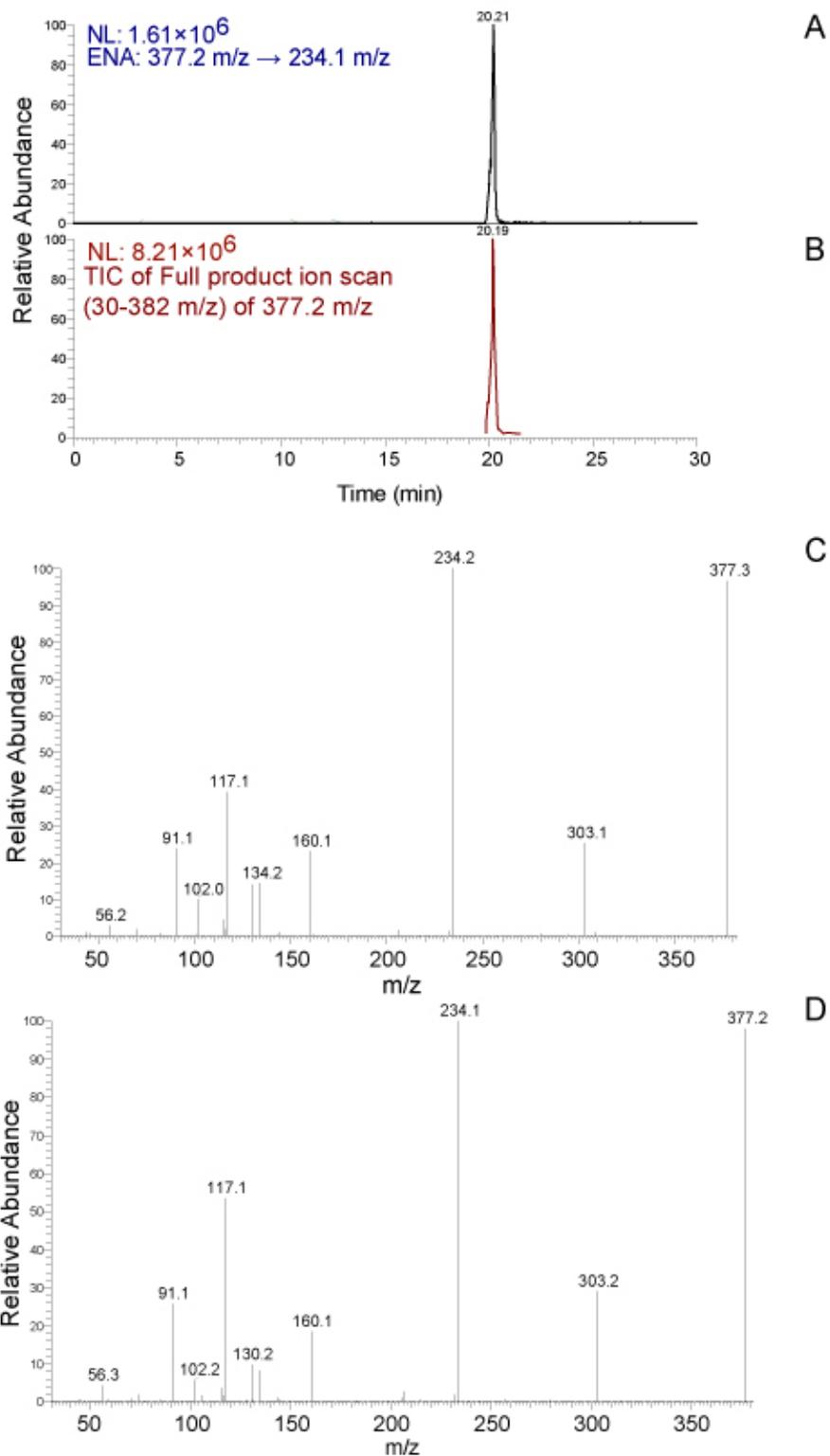


Figure 8-4. LC-MS/MS chromatograms and mass spectra of ENA. (A) SRM transition of ENA. (B) TIC of the RER scan. (C) ENA mass spectrum generated by the RER scan of a

0.1% F.A in H₂O sample spiked at the 100 ng L⁻¹ level. (D) ENA mass spectrum of a standard solution spiked at the 1 mg L⁻¹ level.

8.5. Conclusion

The multi-residue analytical method developed, based on SPE–LC–MS/MS allowed the simultaneous extraction of 14 multiple-class PPCPs and pesticides in a single extraction step, with minimal sample preparation. This method is a reliable, sensitive and robust tool that could be used for routine analysis of multiple organic trace contaminants in drinking and surface water. After breakthrough volume estimations, recoveries using the Strata-X on-line SPE column were generally > 70%. The preconcentration of 10 mL-samples allowed the improvement of the MDLs, which were in the low nanogram-per-liter range (0.6-6 ng L⁻¹) for both surface and drinking water samples. The application of LC–MS/MS operating in SRM mode, with a second SRM transition monitored for each compound provided a good means of confirmation. Other tools of confirmation such as the data-dependent RER scan and TOFMS were found to be successful in some cases but not sensitive or selective enough for confirmatory purposes during routine analysis. The presented method also showed potential for its application to screening studies.

8.6. Acknowledgements

The authors would like to thank the staff of the Industrial Chair of Drinking Water for their help with sample collection. This study was supported by the Natural Science and Engineering Research Council of Canada (NSERC), the NSERC Industrial Chair on Drinking Water of the École Polytechnique, the St. Lawrence Action Plan, the Canadian Foundation for Innovation and the CONACYT (Mexico).

Supplementary material

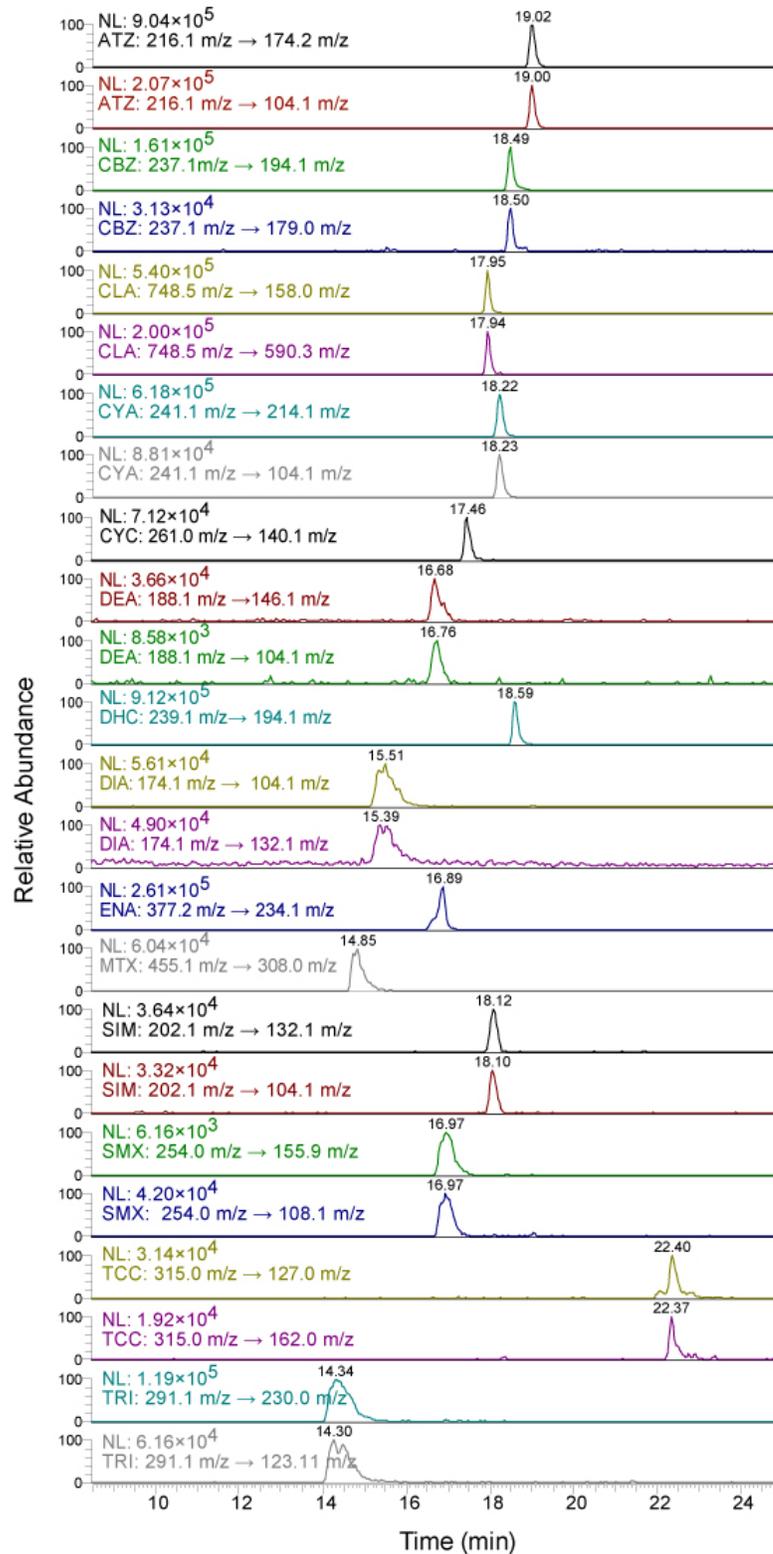


Figure 8-5. LC-MS/MS chromatograms in SRM mode of the target compounds spiked at the 25 ng L⁻¹ level in 0.1 % F.A. in H₂O.

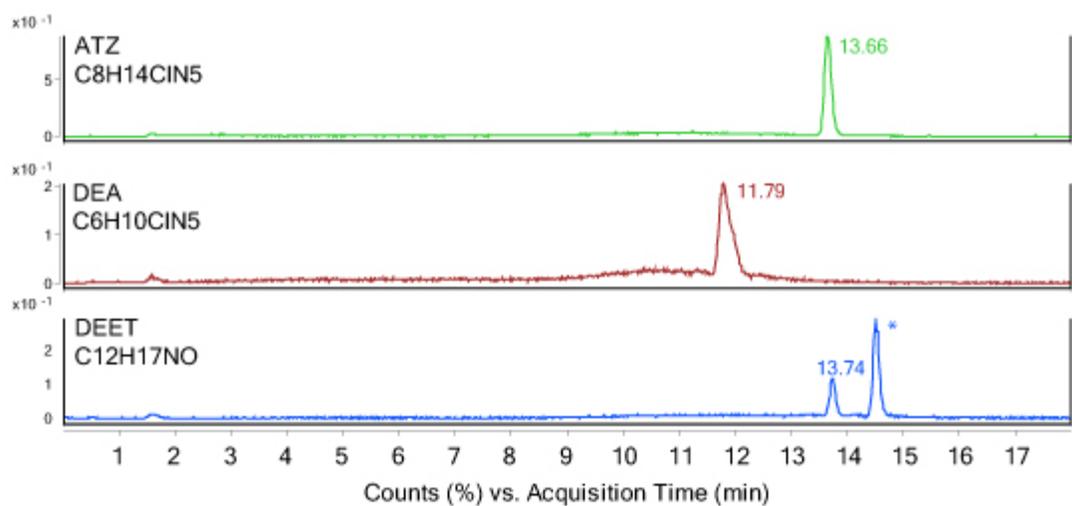


Figure 8-6. LC-TOFMS extracted ion chromatogram of the St. Lawrence River sample (not spiked). Retention times are different to those obtained by the online SPE setup because of differences in the experimental setups.

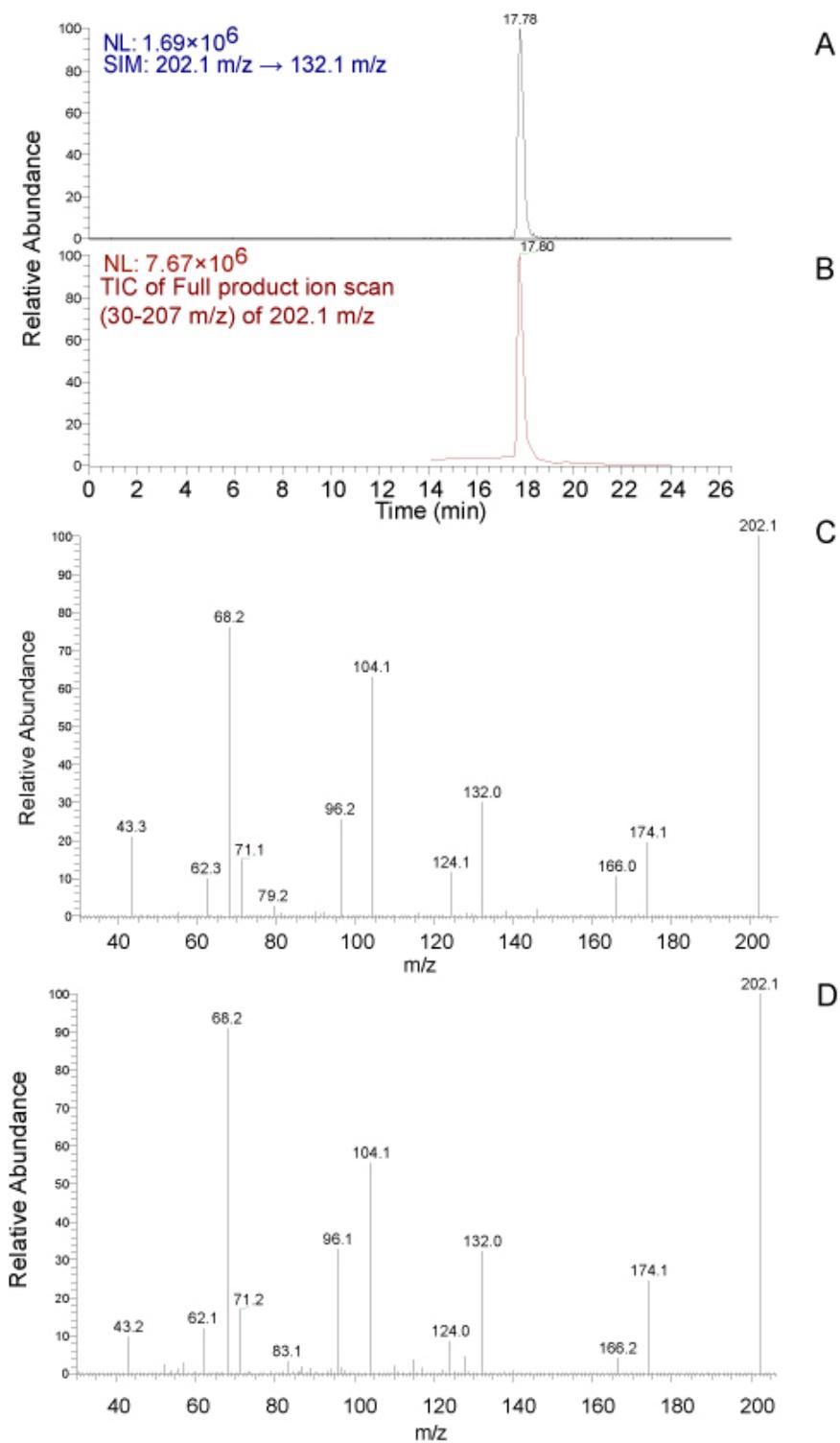


Figure 8-7. LC-MS/MS chromatograms and mass spectra of SIM. (A) SRM transition of SIM. (B) TIC of the RER scan. (C) SIM mass spectrum generated by the RER scan of a Des Prairies River sample spiked at the 25 ng L⁻¹ level. (D) ENA mass spectrum of a standard solution spiked at the 1 mg L⁻¹ level.

Tableau 8-3. Columns tested for breakthrough of seventeen selected organic contaminants.

| Column | Manufacturer | Length (mm) | I.D. (mm) | Particle size (μm) | Pore size (\AA) | Material | pH range |
|------------------------|--------------------------|------------------------|----------------------|---|--|--|-----------------|
| Strata C ₈ | Phenomenex | 20 | 2 | NA | NA | Octyl-bonded silica (endcapped) | NA |
| Strata C ₁₈ | Phenomenex | 20 | 2 | NA | NA | Octadecyl-bonded silica (endcapped) | NA |
| Cyclone | Thermo Fisher Scientific | 50 | 1 | 60 | 100 | Styrene-Divinylbenzene copolymer bead | 1-13 |
| Hypercarb | Thermo Fisher Scientific | 20 | 2.1 | 7 | 250 | Porous graphitic carbon | 0-14 |
| Hypersil Gold | Thermo Fisher Scientific | 20 | 2.1 | 12 | 175 | Alkyl-bonded silica (endcapped) | 1-11 |
| Hypersil Gold PFP | Thermo Fisher Scientific | 20 | 2.1 | 12 | 175 | Perfluorophenyl-bonded silica (endcapped) | 2-8 |
| Strata-X | Phenomenex | 20 | 2 | 25 | 85 | Styrene-Divinylbenzene copolymer surface modified) | 1-14 |

Tableau 8-4. Load and analytical pumps program.

| Time (min) | Load Pump | | Analytical Pump | | | Divert valve position |
|---------------|------------------|---|------------------|------------------|---|-----------------------|
| | Solvent A (%) | Flow rate ($\mu\text{L min}^{-1}$) | Solvent B (%) | Solvent C (%) | Flow rate ($\mu\text{L min}^{-1}$) | |
| 0.0 | 100 | 200 | 2.5 | 2.5 | 200 | Load |
| 0.5 | 100 | 1500 | ↓ | ↓ | ↓ | Elution |
| 8.5 | 100 | 1500 | ↓ | ↓ | ↓ | |
| 9.5 | 100 | 200 | ↓ | ↓ | ↓ | |
| 10.5 | ↓ | ↓ | 2.5 | 2.5 | ↓ | |
| 18.5 | ↓ | ↓ | 47.5 | 47.5 | ↓ | |
| 20.5 | ↓ | ↓ | 47.5 | 47.5 | ↓ | |
| 21.0 | ↓ | ↓ | 10 | 89 | ↓ | |
| 24.5 | ↓ | ↓ | 20 | 89 | ↓ | |
| 25.0 | ↓ | ↓ | 2.5 | 2.5 | ↓ | |
| 26.5 | 100 | 200 | 2.5 | 2.5 | 200 | Load |

Tableau 8-5. LC-TOFMS pump program.

| Time (min) | Pump | |
|-----------------------|--------------------------|--|
| | Solvent B (%) | Flow rate ($\mu\text{L min}^{-1}$) |
| 0.0 | 5 | 200 |
| 2.0 | 5 | ↓ |
| 10.0 | 95 | |
| 16.0 | 95 | |
| 16.5 | 5 | 200 |

Tableau 8-6. SRM transitions parameters.

| Compound | Skimmer offset (V) | Tube Lens (V) | SRM #1 (Quantification) | | | SRM #2 (Confirmation) | | |
|----------|-----------------------|------------------|-------------------------|------------------|-----------|-----------------------|------------------|-----------|
| | | | Precursor (m/z) | Product (m/z) | CE (V) | Precursor (m/z) | Product (m/z) | CE (V) |
| ATZ | 0 | 80 | 216.1 | 174.2 | 15 | 216.1 | 104.1 | 27 |
| CBZ | 0 | 86 | 237.1 | 194.1 | 20 | 237.1 | 179.0 | 35 |
| CLA | 5 | 123 | 748.5 | 158.0 | 28 | 748.5 | 590.3 | 17 |
| CYA | 0 | 80 | 241.1 | 214.1 | 19 | 241.1 | 104.1 | 27 |
| CYC | 0 | 97 | 261.0 | 140.1 | 24 | NA | NA | NA |
| DEA | 0 | 70 | 188.1 | 146.1 | 17 | 188.1 | 104.1 | 27 |
| DIA | 0 | 80 | 174.1 | 104.1 | 23 | 174.1 | 132.1 | 15 |
| DHC | 0 | 91 | 239.1 | 194.1 | 23 | NA | NA | NA |
| ENA | 0 | 92 | 377.2 | 234.1 | 16 | NA | NA | NA |
| MTX | 0 | 122 | 455.1 | 308.1 | 19 | NA | NA | NA |
| SIM | 0 | 78 | 202.1 | 132.1 | 22 | 202.1 | 104.1 | 27 |
| SMX | 5 | 82 | 254.1 | 155.9 | 16 | 254.1 | 108.1 | 24 |
| TCC | 0 | 86 | 315.0 | 127.0 | 35 | 315.0 | 162.0 | 19 |
| TRI | 5 | 91 | 291.1 | 230.0 | 22 | 291.1 | 123.1 | 31 |

Tableau 8-7. Method validation parameters for the analysis of drinking water.

| Compound | r^2^a | MDL (ng L⁻¹) | MCL (ng L⁻¹) | Intra-day precision^b (% RSD) | Inter-day precision^c (% RSD) | Recovery^d (%) | |
|-----------------|-------------------------------------|------------------------------------|------------------------------------|--|--|-------------------------------------|-----|
| ATZ | 0.9997 | 0.7 | 1 | 2 | 5 | 84 | ± 3 |
| CBZ | 0.9994 | 0.7 | 3 | 3 | 8 | 105 | ± 3 |
| CLA | 0.9971 | 2 | 2 | 6 | 7 | 91 | ± 3 |
| CYA | 0.9993 | 0.8 | 1 | 1 | 4 | 93 | ± 2 |
| CYC | 0.9890 | 1 | NA | 5 | 6 | 70 | ± 4 |
| DEA | 0.9991 | 3 | 5 | 2 | 11 | 92 | ± 3 |
| DIA | 0.9988 | 1 | 5 | 3 | 5 | 88 | ± 2 |
| DHC | 0.9872 | 0.4 | NA | 4 | 4 | 86 | ± 6 |
| ENA | 0.9890 | 1 | NA | 7 | 7 | 71 | ± 6 |
| MTX | 0.9914 | 1 | NA | 5 | 8 | 69 | ± 5 |
| SIM | 0.9986 | 2 | 3 | 4 | 10 | 92 | ± 7 |
| SMX | 0.9999 | 1 | 2 | 7 | 12 | 72 | ± 7 |
| TCC | 0.990 | 3 | 5 | 6 | 16 | 103 | ± 6 |
| TRI | 0.9996 | 3 | 6 | 3 | 5 | 64 | ± 5 |

^a Equation and coefficient of determination for the calibration curve using SRM#1 ^b Relative standard deviation of samples ($n=4$) spiked for a final concentration of 5 ng L⁻¹ the same day in 0.1 % F.A. in H₂O ^c Relative standard deviation of samples ($n=4$) spiked for a final concentration of 5 ng L⁻¹ on 3 different days ($N=12$) in 0.1 % F.A. in H₂O, except for CLA (2 days, $N=8$). ^d Sample spiked for a final concentration of 20 ng L⁻¹.

Tableau 8-8. Method validation parameters for the analysis of surface water

| Compound | r^2 ^a | r^2 ^b | r^2 ^c | MDL (ng L ⁻¹) | MCL (ng L ⁻¹) | Intra-day precision ^d (% RSD) | Inter-day precision ^e (% RSD) | Recovery ^f (%) | Matrix effects ^g (%) |
|----------|--------------------|--------------------|--------------------|------------------------------|------------------------------|--|--|------------------------------|------------------------------------|
| ATZ | 0.9999 | 0.9999 | 0.9999 | 0.6 | 0.9 | 2 | 4 | 101 ± 3 | 105 ± 3 |
| CBZ | 0.9999 | 0.9999 | 0.9999 | 1 | 4 | 3 | 4 | 103 ± 2 | 111 ± 3 |
| CLA | 0.9938 | 0.9939 | 0.9931 | 6 | 6 | 7 | 6 | 67 ± 3 | 104 ± 7 |
| CYA | 0.9999 | 0.9995 | 0.9994 | 0.8 | 2 | 2 | 6 | 89 ± 3 | 116 ± 4 |
| CYC | 0.9550 | 0.9977 | 0.9937 | 1 | NA | 5 | 15 | 61 ± 7 | 120 ± 5 |
| DEA | 0.9983 | 0.9980 | 0.9992 | 3 | 5 | 4 | 9 | 82 ± 1 | 96 ± 5 |
| DHC | 0.9910 | 0.9993 | 0.9958 | 1 | NA | 6 | 6 | 71 ± 2 | 133 ± 3 |
| DIA | 0.9998 | 0.9983 | 0.9991 | 1 | 5 | 2 | 6 | 109 ± 2 | 102 ± 2 |
| ENA | 0.9963 | 0.9711 | 0.9940 | 1 | NA | 4 | 7 | 60 ± 2 | 169 ± 3 |
| MTX | 0.9944 | 0.9927 | 0.9906 | 2 | NA | 9 | 15 | 67 ± 6 | 105 ± 7 |
| SIM | 0.9976 | 0.9987 | 0.9967 | 2 | 2 | 3 | 9 | 102 ± 5 | 104 ± 11 |
| SMX | 0.9989 | 0.9963 | 0.9948 | 3 | 14 | 10 | 9 | 66 ± 7 | 81 ± 9 |
| TCC | 0.9991 | 0.9947 | 0.9973 | 6 | 10 | 14 | 12 | 92 ± 5 | 80 ± 4 |
| TRI | 0.9982 | 0.9992 | 0.9990 | 2 | 3 | 6 | 9 | 63 ± 3 | 103 ± 7 |

^a Coefficient of determination of the standard additions calibration curve for the St. Lawrence River sample. ^b Des Prairies River sample ^c One Thousand Islands River sample. ^d Relative standard deviation of analytes in the Des Prairies River sample ($n=4$) spiked for a final concentration of 5 ng L⁻¹ the same day. ^e Relative standard deviation of analytes in the Des Prairies River sample ($n=4$) spiked for a final concentration of 5 ng L⁻¹ on 3 different days ($N=12$). ^f Sample spiked for a final concentration of 25 ng L⁻¹. ^g Calculated comparing signal intensities of St. Lawrence River and 0.1 % F.A. in H₂O samples spiked for a final concentration of 20 ng L⁻¹.

