Development of an LC-MS-based method to study the fate of nanoencapsulated pesticides in soils and strawberry plant

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Abstract

The increased production and use of nanopesticides will increase the likelihood of their exposure to humans and the environment. In order to properly evaluate their risk, it will be necessary to rigorously quantify their concentrations in major environmental compartments including water, soil and food. Due to major differences in the characteristics of their formulation, it is unclear whether analytical techniques that have been developed for conventional pesticides will allow quantification of the nano-forms. Therefore, it is necessary to develop and validate analytical techniques for the quantification of nanopesticides in foods and the environment. The goal of this study was to validate a method for analyzing the active ingredients of two pesticides with different physicochemical properties: azoxystrobin (AZOX, a fungicide, log K_{ow} 3.7) and bifenthrin (BFT, an insecticide, log K_{ow} 6.6) that were applied to agricultural soils, either as a conventional formulation or encapsulated in nanoparticles (either Allosperse® or porous hollow nSiO_2). Pesticide-free strawberry plants (*Fragaria* × *ananassa*) and three different agricultural soils were spiked with the active ingredients (azoxystrobin and bifenthrin), in either conventional or nano formulations. A modified QuEChERS approach was used to extract the pesticides from the strawberry plants (roots, leaves and fruits) and a solvent extraction (1:2 acetonitrile) was employed for the soils. Samples were analyzed by liquid chromatography-hybrid quadrupole time-of-flight mass spectrometry in order to determine method detection limits, recoveries, precision and matrix effects for both the “conventional” and nanoencapsulated pesticides. Results for the modified method indicated good recoveries and precision for the analysis of the nanoencapsulated pesticides from strawberries and agricultural soils, with recoveries ranging from 85-127% (AZOX) and 68-138% (BFT). The results indicated that the presence of the nanoencapsulants had significant effects on the efficiency of extraction and the quantification of

the active ingredients. The modified analytical methods were successfully used to measure strawberry and soil samples from a field experiment, providing the means to explore the fate of nanoencapsulated pesticides in food and environmental matrices.

Keywords: Nanoencapsulated pesticides; Azoxystrobin; Bifenthrin; Soil; Strawberry, Liquid chromatography–mass spectrometry.

1. Introduction

Sustainable agricultural practices, potentially implicating nanotechnology, are required to meet the demand of a rapidly increasing global population (Rodrigues et al., 2017; Hofmann et al., 2020). Nanopesticides, particles with at least one dimension in the 1-100 nm size range (Iavicoli, Leso, Beezhold & Shvedova, 2017), have been developed with the promise of a higher efficacy of the active ingredients, minimal environmental impacts and reduced undesirable consequences as compared to conventional pesticides (Rodrigues et al., 2013; Camara et al., 2019). Although nanopesticides have great potential to increase crop productivity, their potential risks have also raised concerns (Adisa et al., 2019), especially with respect to their toxicity or changes to the fate (aging, mobility, etc.) of the active ingredients in the environment (Hofmann et al., 2020; Singh et al., 2020). Since some nanopesticides have been shown to be systemic for plants (Melissa et al., 2013; Zhao et al., 2017; Zhao et al., 2018; Dong et al., 2020; Mathur & Roy, 2020), there is a need to investigate if nanoencapsulation could modify the fate of active ingredients. In previous pesticide residual experiments, nanoencapsulated pesticides were analyzed by methods developed for conventional formulations (Liang et al., 2017; Zhao et al., 2017; Zhao et al., 2018). The efficiency of those analytical methods for pesticides capsuled in nanocarriers has not been validated.

Among the most promising nanopesticides are those where the active ingredient is encapsulated within nanomaterials comprised of lipid and polymer carriers (e.g. polyacrylates), inorganic nanoparticles such as SiO_2 or carbon nanotubes (Chhipa, 2017; Kumar et al., 2019). Due to interactions of the pesticides with the nanocarriers, modifications to the solubility of the active ingredients and analytical difficulties associated with their extraction, the analytical approach required for the quantification of nanopesticides is likely to differ from the ones that have been

developed for conventional pesticides (Mohd Firdaus et al., 2018; Adisa et al., 2019). There is presently little information in the literature on the extraction and quantification of nanopesticides in plants and soils.

Azoxystrobin (AZOX, log K_{ow} 3.7) and bifenthrin (BFT, log K_{ow} 6.6) are among the active ingredients currently being incorporated into nanocarriers for commercialization for crop protection (Vive Crop Protection, 2021). AZOX is a major strobilurin fungicide, with annual global sales reaching 1.2 billion in 2014 (Cao et al., 2016). AZOX inhibits mitochondrial respiration via a blockage of the electron transfer between cytochromes *b* and *c*1, leading to an oxidative stress in the target fungus (Zhang et al., 2020). BFT is a pyrethroid insecticide, which is neurotoxic to insects by interfering with the nerve cells' ability to transfer signals (Yang & Li, 2015). Both AZOX and BFT have been applied to strawberry crops in order to increase their yield (Abrol & Anil, 2009; Pandey, Shankar & Sharma, 2012).

The extraction of pesticides from plants and soils can be challenging due to their affinity with organic matter (Harrison, Bull & Michaelides, 2013). Among the various extraction methods reported for conventional pesticide analysis in food, QuEChERS (quick, easy, cheap, effective, rugged, and safe) has emerged as a popular method (Lehotay, 2007). Nonetheless, methodologies for the simultaneous extraction and analysis of the nano-based pesticides still need development (Singh et al., 2014). Since extraction shaking time and solvent volumes are known to affect the recovery of the pesticides from fruit matrices (Jia et al., 2010), these parameters need to be optimized. Furthermore, BFT is relatively hydrophobic (log K_{ow} = 6.6), so its affinity with plastic materials may be relatively high (Guo et al., 2020), implying that the type of materials used for sample preparation may impact the recoveries of the target analytes.

The goal of this paper is to develop and validate a method for the extraction and quantification of AZOX and BFT from agricultural soils and strawberry plants (roots, leaves and fruits), for compounds that are either present in their conventional form or encapsulated with two important types of nanoparticles: polyacrylic acid nanoparticles (Allosperse®) and porous hollow nano-sized SiO_2. Precision, matrix effects and recoveries of the methods were determined. The methods were then applied to field samples for further validation.

2. Methods

2.1 Chemicals and reagents

Analytical standards of the pure compounds, azoxystrobin (AZOX) (≥98%, CAS#131860-33-8) and bifenthrin (BFT) (≥98.0%, CAS#82657-04-3) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Deuterated internal standards (D_4-azoxystrobin and D_5-bifenthin) were purchased from Toronto Research Chemicals (North York, ON, Canada). HPLC grade solvents (water, acetonitrile (ACN), and methanol), anhydrous magnesium sulphate, sodium acetate, LC/MS grade formic acid and ammonium acetate (NH_4Ac) were obtained from Fisher Chemicals (Pittsburgh, PA, USA). Primary and secondary amine (PSA) salts were purchased from Agilent (Santa Clara, CA, USA). Allosperse® is a polymeric nanoparticle, comprised of polyacrylic acid, that is used as a nanocarrier for the pesticides (AZOX, BFT). Allosperse®-AZOX and Allosperse®-BFT were prepared and supplied by Vive Crop Protection Inc. (Toronto, Canada). Porous hollow silica nanoparticles ($nSiO_2$) were synthesized as reported in an earlier study (Bueno & Ghoshal, 2020). The feasibility of loading dissolved solutes into the $nSiO_2$ were also evaluated in that study. For the experiments conducted in this study, the $nSiO_2$ was loaded with the analytical

standards to produce $nSiO_2$–AZOX and $nSiO_2$–BFT as described above for the Allosperse®. Stock solutions of the nanopesticides used for method validation were prepared in methanol.

2.2 Field samples

A controlled field experiment was carried at the Macdonald Campus of McGill University, Sainte-Anne-de-Bellevue, QC, Canada. Strawberry plants (*Fragaria* x *ananassa* Duch. "Seascape"), were cultivated under field conditions (n = 5) and exposed to seven different treatments: (i) control ("pesticide-free" soil); (ii) BFT; (iii) AZOX; (iv) Allosperse® containing BFT; (v) Allosperse® containing AZOX; (vi) $nSiO_2$ containing BFT; (vii) $nSiO_2$ containing AZOX (0.22 $mg.kg^{-1}$ of the active ingredient). Treatments with AZOX all contained 7.6 mg active ingredient / pot; treatments with BFT all contained 7.98 mg active ingredient / pot based on the US EPA guidelines (2015a; 2015b). Strawberry plants without fruit (Pépinière Lareault, Canada) were planted in the first week of June and the treatments was applied twice: 15 and 30 days after planting, following the instructions for commercial pesticides. In summary, 200 mL of the different formulations were diluted to 1 L using irrigation water, which was then used to drench on the soils of each pot (n = 5), avoiding the direct contact of the solutions with the plants. Strawberry plants and the corresponding soil samples were collected 30 days after the first exposition prior to treatment using the methodology described in **2.3** and **2.4**.

2.3 Extraction of the pesticides from the strawberry plants

Initial tests to adapt the extraction method for nanoencapsulated pesticides in plant tissues and the subsequent method validation tests were conducted on strawberry tissues from plants grown in pesticide-free soils (See section **2.2**). Fruits were homogenized in a stainless-steel blender. Leaves

and roots were freeze dried and homogenized. All field samples were stored in at -20 °C until analysis.

Pesticide extraction for the strawberry plants was adapted from a method based on the original QuEChERS approach (AOAC, 2007). The method was scaled to a smaller sample size (2 g) in order to accommodate field samples that may be available in limited amounts on some harvest days. In the present study, pesticide recovery was assessed for strawberry samples (spiked at 10 $\mu g\ kg^{-1}$ for AZOX or BFT) for several shaking times (1, 5, 15 and 30 min) and solvent volumes (2 and 4 mL). Two types of centrifuge tubes (glass and plastic) were tested for the extraction of conventional and Allosperse®-BFT, spiked at 10 $\mu g\ kg^{-1}$ and 1000 $\mu g\ kg^{-1}$ (concentration corresponds to the active ingredient). The mass-labeled standards D_4-AZOX and D_5-BFT were spiked in the strawberry plant samples at 40 and 60 $\mu g\ kg^{-1}$, respectively. For the extraction, 2 g of homogenized fruit or 0.2 g of homogenized dried leaves and roots (n = 3) were weighed in a 15 mL plastic centrifuge tube to which 4 mL of 1% acetic acid in acetonitrile, 0.8 g of magnesium sulphate and 0.2 g of sodium acetate were added. Solutions were vortexed for 15 minutes then centrifuged at 2240 × g (5 min, 20 °C). One mL of the supernatant was transferred to centrifuge tubes containing 50 mg PSA and 150 mg of $MgSO_4$. Solutions were then vortexed for 1 min, centrifuged (2240 × g, 5 min, 20 °C), and filtered through a 0.22 μm polytetrafluoroethylene (PTFE, Chrom4; Thuringen, Germany) filter into HPLC vials.

2.4 Extraction of the pesticides from the soil samples

Method validation was performed on three different types of soils collected in Quebec, Canada (Table 1), including a clay soil (relatively rich in organic matter – OM; 6.1%), a loamy sand soil (intermediate OM content; 4.7%), and a loam soil (lower OM content; 3.6%) (Table 1). Soil 1

corresponded to the soil used for the strawberry crop described in **2.2**. Soils were dried at room temperature until constant weight, sieved through a 2 mm nylon mesh, then ground to a fine powder. Prior to the extraction, soils (n = 3) were spiked with 10 μg kg^{-1} or 1000 μg kg^{-1} of the different treatments (AZOX, BFT, Allosperse®, Allosperse®-AZOX, Allosperse®-BFT, $nSiO_2$, $nSiO_2$-AZOX and $nSiO_2$–BFT) and with deuterated standards (40 ug kg^{-1} of D_4-AZOX and 60 ug kg^{-1} of D_5-BFT). Samples were then vortexed for 1 min and left to equilibrate for at least one hour prior to extraction. The extraction method was adapted from Kah et al. (2016) and consisted in shaking 1 g of dried and sieved (2 mm) soil in 2 mL of ACN for 1 hour at 20 rpm on a vertical shaker at room temperature; followed by centrifugation (1882 × g; 5 min, 20 °C) and filtration of the supernatant through 0.22 μm filters into HPLC glass vials.

Table 1. Characteristics of the three agricultural soils used for method validation.

	% sand	**% silt**	**% clay**	**Soil texture class**	**pH**	**% OM[1]**
Soil 1	30	31	38	clay	7.2	6.1
Soil 2	81	14	5	loamy sand	6.9	4.7
Soil 3	53	32	15	loam	7.2	3.6

1. OM is Organic material.

2.5 Instrumental analysis

Extracts were analyzed with an Agilent 1290 Infinity II liquid chromatograph (LC) coupled to a 6545 QTOF mass spectrometer (Agilent Technologies, Santa Clara, USA) operating in positive electrospray ionization mode. The LC separation was conducted on a Poroshell 120 phenyl hexyl column (Agilent Technologies; 2.7 μm × 3.0 mm × 100 mm) fitted with a Poroshell 120 EC-C18 (2.7 μm × 3.0 mm × 5 mm) guard column. Elution was performed in gradient mode

(0.4 mL min^{-1}) using A = water and B = Acetonitrile: Methanol (1:1), both containing 0.1% formic acid and 5 mM NH_4Ac (0 min: 70% A; 0-3 min: B increased from 30 to 100%; 3-6 min: 100% B; 6-8 min: B decreased from 100% to 30%). The injection volume was 10 μL and the column temperature was maintained at 30°C. Nitrogen was used as the drying gas (110°C, 12 L min^{-1}). Samples were run in the *All Ions MS/MS* mode. The fragmentor voltage was 110 V and MS data was acquired in the 50-750 *m/z* range. The following *m/z* were extracted from total ion chromatogram (TIC) (±10 ppm) for quantification: 404.1247 for AZOX and 440.1604 for BFT. The *m/z* of the qualifier ions were 372.0971 and 181.1009 for AZOX and BFT, respectively.

2.6 Linearity, IDLs, MDLs and MQLs

Calibration curve linearity was evaluated from the coefficient of determination (r^2) using injections of the standards prepared in acetonitrile at 1, 5, 10, 25, 50, and 100 ng mL^{-1}. Instrument detection limits (IDLs) were calculated as the amount of analyte injected that resulted in a signal-to-noise ratio (S/N) of 3, as determined from the lowest standard of the calibration curve in pure solvent (Indrayanto, 2018). Method detection limits (MDLs) were assessed as 3σ of the response obtained for procedural blanks. Method quantification limits (MQLs) were determined from 10σ of the procedural blanks.

2.7 Recoveries, matrix effects and precision

Recoveries, matrix effects and precision were assessed for conventional AZOX and BFT (AZOX and BFT spiked together), Allosperse®-AZOX, Allosperse®-BFT, $nSiO_2$–AZOX and $nSiO_2$–BFT, for all plant and soil samples. As of 2021, maximum residue limits (MRLs) for AZOX and BFT in strawberry fruits in Canada are 10 and 3 mg kg^{-1}, respectively (Government of Canada,

2016, 2018). For soils, spiking concentrations were set according to residue levels commonly reported in agricultural soils: AZOX in the range of 30 - 250 μg kg^{-1} (Silva et al., 2019); and BFT in the range of 2.28 to 112.9 μg kg^{-1} (Leyva-Morales et al., 2015). Recovery was determined by spiking the homogenized samples prior to extraction with both pesticides and their mass-labeled surrogates. For each treatment (AZOX, BFT, Allosperse®, Allosperse®-AZOX, Allosperse®-BFT, $nSiO_2$, $nSiO_2$-AZOX and $nSiO_2$–BFT), samples (n = 3) were spiked at two levels: strawberries and soils (10 μg kg^{-1} and 1000 μg kg^{-1}); leaves and roots (20 μg kg^{-1} and 1000 μg kg^{-1}). Recoveries of the pesticides were considered acceptable when in the 70-120% range (Rutkowska, Lozowicka & Kaczynski, 2018).

Matrix effects were studied by comparing the slope of a matrix-matched calibration curve with the slope of the calibration curve in pure solvent. Four different concentrations (10, 25, 50, and 100 μg kg^{-1}, n = 3) were added to each matrix in order to assess matrix effects according to:

$$\text{Matrix effect (\%)} = (1-B/A) \times 100 \quad (1)$$

where A is the average peak area obtained for a given concentration of standard in the pure solvent and B is the average peak area obtained for the sample extracts (Chambers et al., 2007). Intraday and interday precision were determined from the analysis of samples (n = 5) spiked at a level of 100 μg kg^{-1} spike for each pesticide.

2.8 Statistical analysis

Analysis of variance (one-way ANOVA, Microsoft Excel) was used to identify differences among results obtained for different pesticide formulations and different types of samples, by applying a confidence range of 95% (α = 0.05, n = 3). When differences were identified,

Tukey's test was then used to determine which pairs of means were statistically different ($p<0.05$). In the figures, error bars represent standard deviations (n = 3).

3. Results and Discussion

3.1 Instrument validation

Instrument validation was performed for the LC-MS analysis (Table 2). Good instrumental linearity was achieved ($r^2 > 0.999$) in the range of 10-1000 pg injected for AZOX and 50-1000 pg for BFT. Low IDLs for AZOX and BFT were obtained (0.3 pg and 2.2 pg). Mass measurement errors were generally below 2.5 ppm among the various formulations (Table S3 and S4). As can be seen in Figs. S2 and S3, *m/z* and retention times were similar for the target compounds when they were prepared in extracts or when they were present as pure active ingredients or encapsulated into the different nanocarriers. The relative intensities of the qualifier and quantifier ions for both AZOX and BFT in acetonitrile and samples (Table S5) were acceptable according to the SANCO/12495/2011 guideline (European Commission, 2012).

Table 2. Instrument validation for the LC-MS analysis of AZOX and BFT

Target analytes	RT (min)	Formulation	Quantifier ion (*m/z*)	Qualifier ion (*m/z*)	IDLs[a] (pg)	r^2 [b]
AZOX	3.72	$C_{22}H_{17}N_3O_5$	404.1247	372.0971	0.3	0.9997
BFT	4.97	$C_{23}H_{22}ClF_3O_2$	440.1604	181.1009	2.2	0.9981

[a]. IDLs are the instrument detection limits. [b]. r^2 is the coefficient of variation of calibration curve

3.2 Development and validation of the methods

In the initial tests, the performances of the solvent extraction methods for AZOX and BFT in soil samples were acceptable. For strawberries, initial tests conducted with the original

approach (AOAC, 2007) gave acceptable recoveries for the three forms of AZOX pesticides. On the other hand, BFT (conventional and Allosperse®) was not detectable in samples spiked at 10 μg kg^{-1} (Fig. S1). In order to increase the recovery of the BFT (conventional and Allosperse®) to acceptable levels, several conditions were tested, including the use of different tube materials (glass, plastic), variable extraction solvent volumes, and shaking times. The developed extraction method was then applied to all strawberry plant matrices (strawberry, leaves and roots).

3.2.1 Development of an extraction method for the strawberries

Initially, when using plastic centrifuge tubes, only 23 ± 32% of the conventional BFT was recovered from the spiked strawberry samples (10 μg kg^{-1}) and no signal was detected in the Allosperse®-BFT treatment. By increasing the extraction solvent volumes from 2 to 4 mL (Fig. S1), recoveries for Allosperse®-BFT increased to 61 ± 4%. For both BFT formulations, recoveries were improved further when switching to glass centrifuge tubes: 78 ± 17% for the conventional BFT and 60 ± 4% for the Allosperse®-BFT (Fig. S1). Note that when using the longer extraction times (15 min), acceptable recoveries for plastic centrifuge tubes were also obtained (80 ± 12% for conventional BFT and 98 ± 4% for Allosperse®-BFT). Considering the efficiency, cost and labor-consumption, the final conditions for the extraction combined the plastic tube, 4 mL of solvent and 15 min of shaking time. Given our initial observation that 15 minutes of shaking improved the extraction efficiency, a subsequent optimization below examined the role of shaking time (1, 5, 15 and 30 minutes). This point is important given that the two nanocarriers provide slow release of the loaded pesticides (Walker et al., 2017).

Shaking time had no perceptible influence on the extraction of AZOX, for any of the formulations and recoveries were already acceptable when using 1 min shaking (Fig. 1). Furthermore, there were no significant differences observed when comparing the extraction of the

conventional AZOX with respect to the two nanocarriers (Allosperse® and porous hollow nano-silica).

On the other hand, for BFT, recoveries were improved ($p < 0.05$) for Allosperse® and the conventional formulations of the longer shaking times (5, 15 or 30 min). For BFT, the nanoencapsulated pesticides generally had better recoveries than the conventional ones (Fig. 1). This may be linked to a faster release rate of the pesticides from those nanoparticles compared with conventional pesticides, which is controlled by many factors, including shell thickness, pore size, inner polarity and the solubility of the active ingredient (Botterhuis, Sun, Magusin, Van Santen & Sommerdijk, 2006; Yao, Shi, Jin, Li & Zhang, 2010). Pesticide encapsulation has also been shown to modify the hydrophobic partitioning of pesticides (Slattery et al., 2019). Although the basic AOAC QuEChERS method was efficient and accurate with respect to the extraction of the AZOX and the silica nanopesticides, the increased extraction times clearly improved the efficiency of the Allosperse® encapsulated and conventional pesticide formulations.

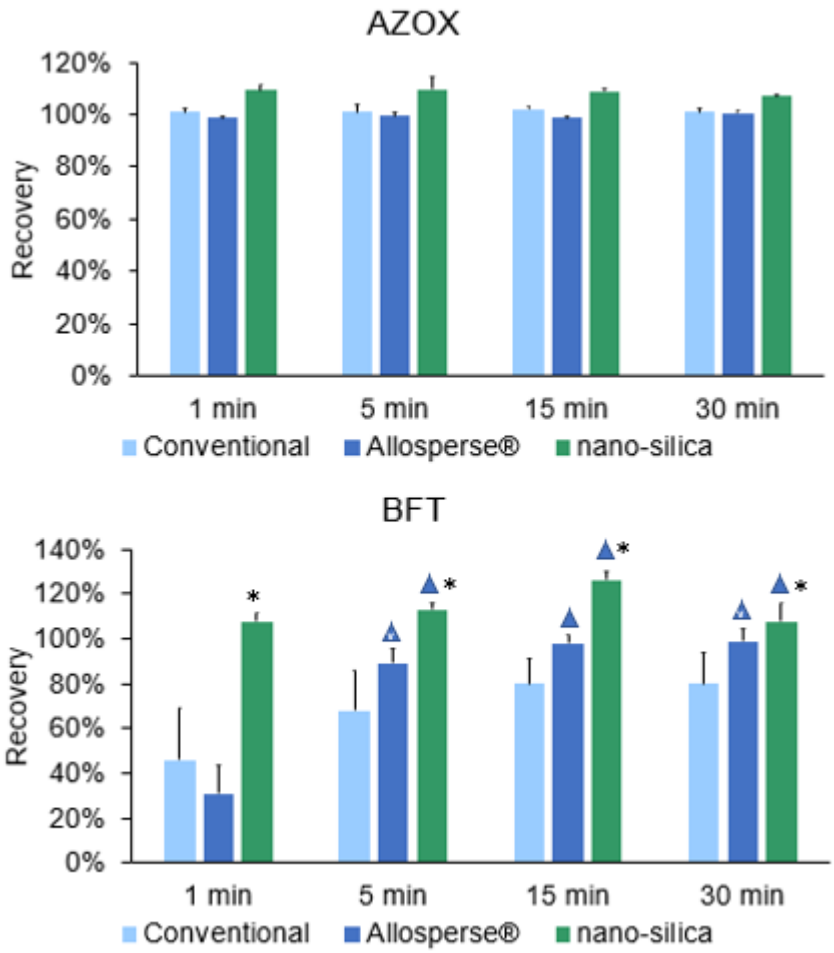

Fig. 1 –The recovery of AZOX and BFT pesticide (conventional and nanoencapsulated, 10 µg kg^{-1}) from strawberries using QuEChERS with different extraction time (extraction solvent volume: 4 mL; plastic centrifuge tubes; n = 3). Δ indicates a significantly higher recovery with respect to the 1 min extraction time for a given formulation; * indicates a significantly different recovery when compared to results for the conventional pesticides for an identical extraction time.

3.2.2 Validation of the developed extraction method for strawberry plant matrices

Recoveries, matrix effects, precisions and MDLs were assessed using the above method for both the conventional pesticide formulation and the nanopesticides (Table 3). MDLs ranged from

0.02 to 0.65 µg kg^{-1} among the various plant matrices for azoxystrobin, and from 0.03 to 0.36 µg kg^{-1} for bifenthrin. These MDLs were comparable or lower than those reported in the literature (Chauhan, Monga & Kumari, 2012; Vera et al., 2013; Bhattacharyya & Roy, 2014). For the lowest spiking level, recoveries ranged from 80 ± 12% to 125 ± 2% for the conventional formulations; from 87 ± 10% to 126 ± 6% for the Allosperse® and from 103 ± 13% to 126 ± 4% for the $nSiO_2$-based nanopesticides. Recoveries were also satisfactory when plant samples were spiked with the higher concentration of conventional pesticides (between 88 ± 6% and 111 ± 12%); Allosperse® (83 ± 4% to 138 ± 14%) and $nSiO_2$ (68 ± 3% to 118 ± 6%). There was an important improvement in the recovery of BFT from no detection to 80% (Fig. S1), when using the modified method. For strawberries spiked with 10 µg kg^{-1} (lower spiked level), recoveries (87 ± 10%-126 ± 6%) were higher than those at the higher spiked level. These lower pesticide concentrations correspond to levels that were found in the strawberries taken from the experimental field (Fig. S4), and 2–98 ug kg^{-1} of AZOX residue levels and 2-85 ug kg^{-1} of BFT residue levels reported by the U.S. Dept. of Agriculture (USDA, 2019). Furthermore, the precision (RSD%) was in the range of 1.99-16.71% (Table 3, Table S2) for both AZOX and BFT in the plant samples. This confirms the good performance of the modified method.

Note that the above recovery values were obtained after correction for matrix effects. In LC-ESI-MS, matrix effects are commonly caused by coeluting compounds, including endogenous metabolites, impurities or degradation products found in the extract (Chambers et al., 2007). These substances can promote or compete with the target analyte for the available charges in the ion source, which may either cause an increase (enhancement) or decrease (suppression) in the detector response as compared to the analyte in pure solvent. When the average matrix effect

exceeds ± 20%, the matrix is considered to have a significant effect on quantitative determinations (European Commission, 2017).

For AZOX, matrix effects were not significant (<±20%). For BFT, matrix effects were below 20% except for two observed matrix effect values linked to the $nSiO_2$ formulation: 27 ± 1% in the strawberries (fruit) and 43 ± 6% in the strawberry roots (Table 3). When comparing the conventional-BFT and the nanopesticides, several significant ($p < 0.05$) matrix effects were observed for both the Allosperse®-BFT and $nSiO_2$-BFT (Table S1). These results again demonstrate that impact of nanoencapsulation on the extraction of BFT from the strawberry samples.

Mass-labeled surrogates can be added prior to extraction to correct for matrix effects (Niessen, Manini & Andreoli, 2006). In the present study, the use of AZOX-D_4 and BFT-D_5 indeed reduced the effect of the matrix on the quantification. The combination of longer extraction times and higher solvent volumes and the use of labeled pesticides allowed us to attain the higher recoveries discussed above for the nanopesticides in the strawberries. Similar recoveries of 90.6-116.2% have been reported for the extraction of a nanoformulation of pyridalyl from tomatoes using a different QuEChERS protocol (Saini, Gopal, Kumar, Gogoi, & Srivastava, 2015).

Table 3. Recoveries (%) and matrix effect (%) of the pesticides in the conventional and nano formulations for samples of strawberry, leaves, roots and soils (n = 3).

Azoxystrobin						
Matrix	**Treatment**	**Recovery % (n = 3)**		**Matrix Effect % (n = 3)**	**Precision % (n = 5)**	**MDLs[1] µg kg^{-1} (n = 5)**
		Spiked @0.01mg kg^{-1} (wet wt.)	**Spiked @1 mg kg^{-1} (wet wt.)**			
Soil 1	**Conventional**	105 ± 3	109 ± 19	-2.3 ± 7	1.27	0.65
	Allosperse®	85 ± 2	110 ± 1	-8.5 ± 5.3		
	$nSiO_2$	108 ± 3	127 ± 1	-4.9 ± 5.6		
Soil 2	**Conventional**	120 ± 2	122 ± 1	-29 ± 6		
	Allosperse®	87 ± 4	104 ± 1	-11 ± 3		
	$nSiO_2$	117 ± 2	125 ± 1	-12 ± 1		
Soil 3	**Conventional**	126 ± 1	125 ± 1	-14 ± 1		
	Allosperse®	126 ± 4	102 ± 2	36.5 ± 5		
	$nSiO_2$	107 ± 3	122 ± 5	-12 ± 9		
Strawberry	**Conventional**	102 ± 1	97 ± 6	-19 ± 2	3.90	0.14
	Allosperse®	99 ± 1	88 ± 3	-15 ± 1		
	$nSiO_2$	109 ± 1	111 ± 5	-9 ± 3		
		Spiked @0.02 mg kg^{-1} (dry wt.)	**Spiked @1 mg kg^{-1} (dry wt.)**			
Leaves	**Conventional**	93 ± 3	91 ± 1	-3 ± 6	4.33	0.02
	Allosperse®	114 ± 26	115 ± 5	-13 ± 3		
	$nSiO_2$	108 ± 13	116 ± 5	-10 ± 2		
Roots	**Conventional**	125 ± 2	94 ± 1	-11 ± 8	1.99	0.07
	Allosperse®	115 ± 16	114 ± 8	-15 ± 8		
	$nSiO_2$	113 ± 15	118 ± 6	-13 ± 2		

Bifenthrin						
Matrix	**Treatment**	**Recovery % (n = 3)**		**Matrix Effect % (n = 3)**	**Precision % (n = 5)**	**MDLs [a] µg kg^{-1} (n = 5)**
		Spiked @0.01 mg kg^{-1} (wet wt.)	**Spiked @1 mg kg^{-1} (wet wt.)**			
Soil 1	**Conventional**	92 ± 9	104 ± 4	66 ± 3	2.36	0.36
	Allosperse®	84 ± 3	92 ± 3	57 ± 9		
	$nSiO_2$	91 ± 6	106 ± 5	33 ± 10		
Soil 2	**Conventional**	91 ± 1	93 ± 2	-80 ± 8		
	Allosperse®	78 ± 3	81 ± 2	-60 ± 8		
	$nSiO_2$	83 ± 5	95 ± 2	-72 ± 9		
Soil 3	**Conventional**	86 ± 3	98 ± 2	-51 ± 4		
	Allosperse®	71 ± 1	78 ± 5	-73 ± 15		
	$nSiO_2$	86 ± 2	101 ± 1	-71 ± 11		
Strawberry	**Conventional**	80 ± 12	88 ± 6	0.1 ± 2	16.71	0.03
	Allosperse®	98 ± 4	87 ± 4	15 ± 5		
	$nSiO_2$	126 ± 4	68 ± 3	27 ± 1		
		Spiked @0.02 mg kg^{-1} (dry wt.)	**Spiked @1 mg kg^{-1} (dry wt.)**			
Leaves	**Conventional**	107 ± 8	111 ± 12	7 ± 8	8.72	0.08
	Allosperse®	126 ± 6	138 ± 14	20 ± 7		
	$nSiO_2$	103 ± 13	98 ± 8	18 ± 5		
Roots	**Conventional**	115 ± 3	99 ± 1	20 ± 19	2.97	0.25
	Allosperse®	87 ± 10	114 ± 15	4 ± 21		
	$nSiO_2$	107 ± 9	79 ± 4	43 ± 6		

[a]. MDLs are method detection limits

3.2.3 Validation of the developed extraction method for soil

Recoveries were also assessed for three types of agricultural soils. For the lowest spiking level, recoveries ranged from 86-126% for the conventional formulations; from 71-126% for the Allosperse® and from 83-117% for the $nSiO_2$-based nanopesticides. Recoveries were also satisfactory when the soils were spiked with the higher concentration of conventional pesticides (98-122%); Allosperse® (78-110%) and $nSiO_2$ (95-127%). These recoveries were thus comparable to those reported using a QuEChERS approach for the multi-pesticide extraction of several conventional formulations in soils (range of 70 to 120%, MQL for AZOX = 0.01 mg kg^{-1}; Silva et al., 2019). Furthermore, results were similar to those obtained by an accelerated solvent extraction (dichloromethane:acetone, 50:50, v/v) and analysis by GC coupled to selective detectors (reported recoveries for conventional AZOX ranged from 78-130%, MQL = 6.432 µg kg^{-1}; BFT ranged from 71-126%, MQL = 4.779 µg kg^{-1}) (Leyva-Morales et al., 2015). Overall, the extraction procedure proposed here was appropriate for the fast quantification of the different formulations of the two different pesticides in the soils, with a MQL lower than previously reported.

Matrix effects (Table 3) were significant (>±20%) for all three of the BFT formulations, for all of the tested soils and for conventional AZOX (Soil 2) and Allosperse®-AZOX (Soil 3). Matrix effects were generally less important for the AZOX formulations as compared to the BFT formulations, although some different tendencies were observed based upon the type of soil examined (Table S1, $p < 0.05$). For Soil 1, which was the most OM rich soil, an enhancement of the signal was observed for BFT, whereas for Soil 2 and Soil 3, the signal was suppressed. For example, it was possible to observe a slightly higher recovery for BFT-SiO_2 extracted from Soil 1 when compared to the other soils (Table 3; Table S1, $p < 0.05$). Matrix effects appeared to be related to the soil, the pesticide type and to the nature of the formulation. Clearly, the addition of

the internal standards D_4-AZOX and D_5-BFT was necessary to compensate for matrix effects and to improve the precision and robustness of the analytical method (Tan and Awaiye 2013; Stachniuk and Fornal 2016; Hu et al., 2016). Overall, the recoveries (Table 3) and precision (Table S2) were consistent with an accurate, simultaneous extraction of these two pesticides in their formulations, from different soils.

3.3 Application to real samples

Chromatograms obtained for strawberries, leaves, roots and soils that were exposed to the different pesticide formulations showed clear, symmetrical peaks at 3.7 min for AZOX and 5.0 min for BFT (Fig. 2). The chromatograms for AZOX and BFT standards in ACN solvent were also shown in Fig. 2 (Panels 4, 8, 12, 16, 20, 24, 28, and 32), and were used to quantify the target compounds in sample extracts. AZOX could be detected in all treated plant matrices and in soil samples. BFT was detected in leaves, roots and soils, but not in the fruit (strawberries).

Before widespread the application of those nano herbicides, a reliable and comprehensive risk assessment will be necessary to ensure environmental safety and protect the human health (Kah et al., 2016). Because the standard guidelines for pesticide characterization in environmental and food samples have been established for conventional formulations, the adjusted analytical techniques presented here will be required in order to quantify the nanoformulations and therefore allow a reliable and comprehensive risk assessment, prior to the registration, commercialization and widespread the application of those nano pesticides (Kah et al., 2016; Li et al., 2019).

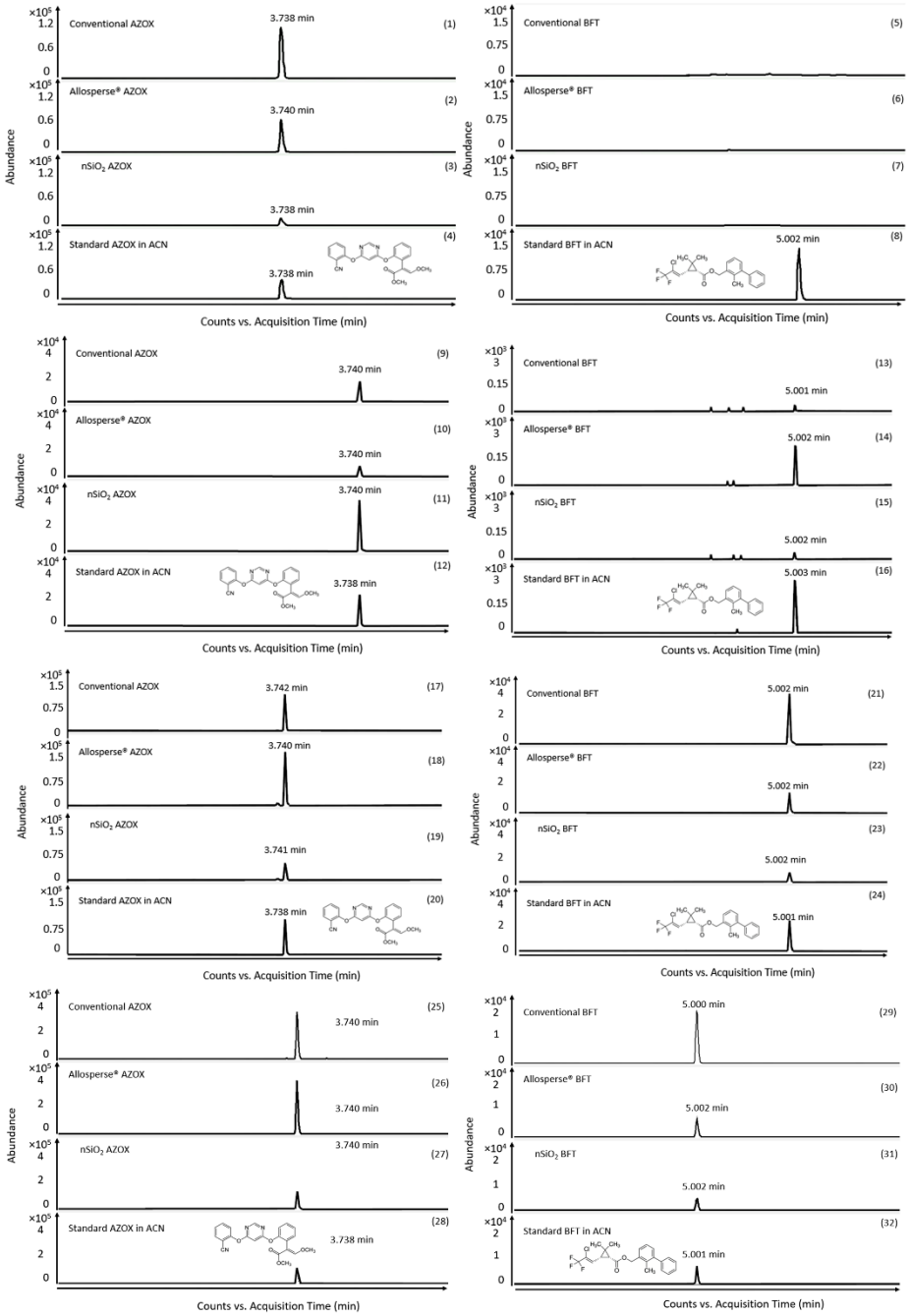

Fig. 2. Extracted ion chromatograms at m/z 404.1250 for conventional, Allosperse®, $nSiO_2$ and ACN standard - AZOX (Treated strawberry extract: Panel **1-4**; Leaf extract: Panel **9-12**; Root extract: Panel **17-20**; Soil extract: Panel **25-28**). Extracted ion chromatograms at m/z 440.1604 for conventional, Allosperse®, $nSiO_2$ and ACN standard - AZOX (Treated strawberry extract: Panel **5-8**; Leaf extract: Panel **13-16**; Root extract: Panel **21-24**; Soil extract: Panel **29-32**).

4. Conclusion

This paper described rapid and accurate analytical techniques for analyzing nano-based pesticides in strawberry plants and agricultural soils with different characteristics. For the strawberries, a QuEChERS technique was modified, followed by LC-QToF-MS. Extraction time and solvent volume were successfully optimized. For the extraction of the fungicide AZOX in strawberries, plastic extraction tubes were shown to have minimal impact on the recovery of the conventional and nano formulations of the pesticide. When extracting BFT from the fruits, the use of doubling extraction solvent and longer extraction time were shown to give improved recoveries. For 3 different agricultural soils, acceptable recovery and precision could be obtained when using the modified extraction. Given the significant matrix effects that were observed, the use of stable isotopes (AZOX-D_4 and BFT-D_5) as internal standards was necessary to properly quantify these emerging products. Because BFT and AZOX are major pesticides from different classes, the modified procedures may be useful for rapid and efficient extractions of other nanopesticides from similar samples, increasing the possibilities for research on nano enabled pesticides and facilitating a more complete understanding of the effects of the nanopesticides on these systems.

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