Comparison of the performances of Stir Bar Sorptive Extraction (SBSE) coupled to GC-MS-MS and UHPLC-MS-MS for the analysis of pesticides in freshwaters

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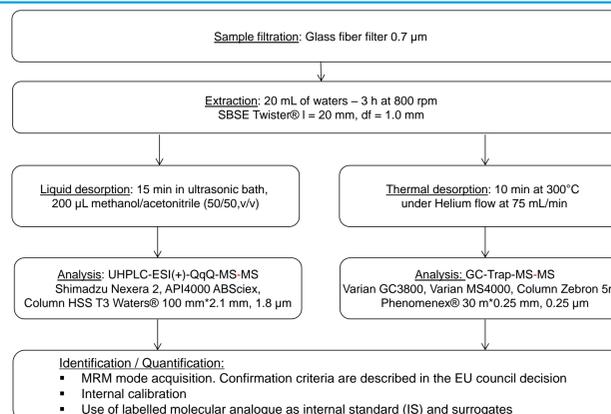
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INTRODUCTION

Monitoring of organic pesticides in freshwaters requires developing robust methods that usually involve an extraction step followed by gas or liquid chromatography. Stir Bar Sorptive Extraction (SBSE) is an innovative solvent free sample preparation technique for moderately hydrophobic to hydrophobic organic compounds in aqueous samples [1]. This fast extraction technique can be followed by two possible desorption modes: liquid desorption (LD) for liquid chromatography or thermal desorption (TD) for gas chromatography. The aim of this work was to validate SBSE-TD-GC-MS-MS and SBSE-LD-UHPLC-MS-MS methods for the determination of several pesticides in surface waters and to compare their performances.

MATERIAL AND METHOD

Method synoptic [2], [3]

Molecule	Abbreviation	UHPLC-MS-MS	GC-MS-MS	Use	Log K_{OW}
Azoxystrobine	AZS	X		F	2.5
Dimethomorph	DMM	X		F	2.7
Procymidone	PCM	X	X	F	3.3
Spiroxamine	SPX	X		F	2.9
Tebuconazole	TBZ	X		F	3.7
Acetochlor	ATC	X	X	H	3.0
Atrazine	ATZ	X		H	2.7
Chlortoluron	CTU	X		H	2.5
Diflufenican	DFF	X	X	H	4.2
Diuron	DIU	X		H	2.7
Flumioxazine	FMX	X		H	2.6
Isoproturon	IPU	X		H	2.9
Metolachlor	MTC	X	X	H	3.1
Norflurazon	NFZ	X		H	2.5
Simazine	SMZ	X		H	2.3
Dichloraniline	DCA	X	X	metab H	2.7
3-(3,4-dichlorophenyl)-1methylurea	DCPMU	X		metab H	2.5
Chlorfenvinphos	CFV	X	X	I	3.8
Chlorpyrifos Ethyl	CPE	X	X	I	4.7
Chlorpyrifos Methyl	CPM	X	X	I	4.0
Fenitrothion	FNT	X	X	I	3.3

F: Fungicide, H: Herbicide, I: Insecticide
Log Kow: Octanol-water partition coefficient

Sample filtration: Glass fiber filter 0.7 µm

Extraction: 20 mL of waters – 3 h at 800 rpm
SBSE Twister® l = 20 mm, df = 1.0 mm

Liquid desorption: 15 min in ultrasonic bath, 200 µL methanol/acetonitrile (50/50,v/v)

Thermal desorption: 10 min at 300°C under Helium flow at 75 mL/min

Analysis: UHPLC-ESI(+)-QqQ-MS-MS
Shimadzu Nexera 2, API4000 ABSciex, Column HSS T3 Waters® 100 mm*2.1 mm, 1.8 µm

Analysis: GC-Trap-MS-MS
Varian GC3800, Varian MS4000, Column Zebron 5ms Phenomenex® 30 m*0.25 mm, 0.25 µm

Identification / Quantification:
- MRM mode acquisition. Confirmation criteria are described in the EU council decision
- Internal calibration
- Use of labelled molecular analogue as internal standard (IS) and surrogates

Method performances [4], [5]

Linearity range

Experimental:
5 calibration regression curves from the extraction of standards at 6 levels.

2 evaluation criteria:
Fisher test with α = 0.01.
Comparison of the deviations between theoretical and calculated standard concentration values to a maximal acceptable deviation (MAD: from 10% to 60%).

Limits of quantification (LOQ)

Experimental:
Extraction of 5 duplicates of water spiked at the LOQ_{th} (estimated for S/N = k*10).
Experimental LOQ and its standard deviation (s_{LOQ}) were determined.

Evaluation criterion:
LOQ - 2 s_{LOQ} > LOQ_{th} - 60% LOQ_{th}
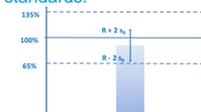LOQ + 2 s_{LOQ} < LOQ_{th} + 60% LOQ_{th}
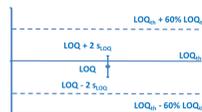
Accuracy and repeatability

Experimental:
Extraction of 5 duplicates of water spiked at 2 levels: 20% and 80% of the highest concentration level of the calibration range. Recoveries (R) were determined with calibration curve from extracted standards.

Evaluation criterion:
R - 2 s_R > 65%
R + 2 s_R < 135%

Analytical uncertainties (U)

Experimental:
Extraction of 10 duplicates of 3 natural waters spiked at 3 levels: LQ, 20% and 80% of the highest concentration level of the calibration range.

Determination of uncertainties:
For each concentration level, the average concentration of the samples x and the reproducibility errors s_{repro} were determined. Then U was calculated as follows, with k = 2:
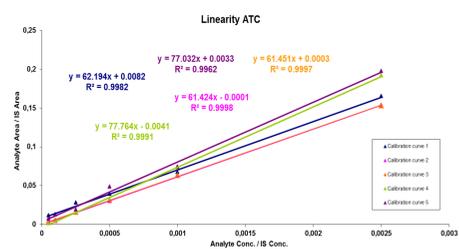
$$U\ (\%) = (k \times s_{repro} \times 100) / x$$

RESULTS AND DISCUSSION

Linearity range

For all compounds, the linearity was validated with a Fisher test or MAD. The ranges for GC method are forty times larger: LOQ to 2000LOQ for GC method and LOQ to 50LOQ for UHPLC method.

Molecule	Linearity range (µg/L) UHPLC-MS-MS	R^2	GC-MS-MS	R^2
AZS	0.02 - 1	0.9995	-	-
DMM	0.1 - 5	0.9996	-	-
PCM	0.2 - 10	0.9993	0.005 - 10	0.9992
SPX	0.02 - 1	0.9985	-	-
TBZ	0.1 - 5	0.9995	-	-
ATC	0.05 - 2.5	0.9993	0.005 - 10	0.9986
ATZ	0.005 - 0.25	0.9993	-	-
CTU	0.05 - 2.5	0.9994	-	-
DFF	0.2 - 10	0.9991	0.05 - 100	0.9981
DIU	1 - 50	0.9992	-	-
FMX	0.2 - 10	0.9991	-	-
IPU	0.1 - 5	0.9994	-	-
MTC	0.005 - 0.25	0.9992	0.005 - 10	0.9992
NFZ	0.2 - 10	0.9991	-	-
SMZ	0.05 - 2.5	0.9995	-	-
DCA	0.05 - 2.5	0.9984	0.1 - 200	0.9982
DCPMU	1 - 50	0.9992	-	-
CFV	0.1 - 5	0.9993	0.025 - 50	0.9972
CPE	0.05 - 2.5	0.9993	0.025 - 50	0.9989
CPM	0.05 - 2.5	0.9992	0.0025 - 5	0.9989
FNT	0.5 - 25	0.9991	0.005 - 10	0.9985

Linearity ATC

$y = 77.032x + 0.0033$ $R^2 = 0.9962$
$y = 91.451x + 0.0003$ $R^2 = 0.9997$
$y = 62.194x + 0.0082$ $R^2 = 0.9982$
$y = 61.424x - 0.0001$ $R^2 = 0.9998$
$y = 77.764x - 0.0041$ $R^2 = 0.9991$

Limits of quantification (LOQ)

For the UHPLC method, LOQ are between 5 and 1000 ng/L with recoveries higher than 83% and relative standard deviation (RSD) below 18% (n = 10).
For the GC method, LOQ are between 2.5 and 100 ng/L with recoveries higher than 93% and RSD below 19% (n = 10).
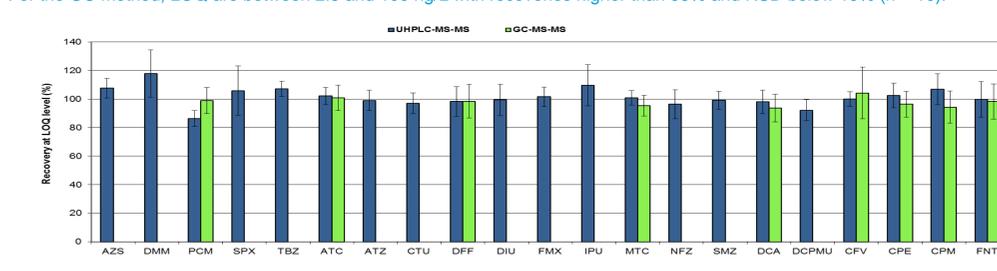
Molecule	LOQ (ng/L) UHPLC-MS-MS	GC-MS-MS
AZS	20	-
DMM	100	-
PCM	200	5
SPX	20	-
TBZ	100	-
ATC	50	5
ATZ	5	-
CTU	50	-
DFF	200	50
DIU	1000	-
FMX	200	-
IPU	100	-
MTC	5	5
NFZ	200	-
SMZ	50	-
DCA	100	100
DCPMU	1000	-
CFV	100	2.5
CPE	50	25
CPM	50	2.5
FNT	500	5

Recovery at LOQ level (%) — ■ UHPLC-MS-MS ■ GC-MS-MS (AZS, DMM, PCM, SPX, TBZ, ATC, ATZ, CTU, DFF, DIU, FMX, IPU, MTC, NFZ, SMZ, DCA, DCPMU, CFV, CPE, CPM, FNT)

Accuracy and repeatability

Accuracy and repeatability were validated for both methods.

UHPLC method obtains recoveries higher than 94% with RSD below 13% (n = 10 for each level).

GC method reaches recoveries higher than 90% with RSD below 11% (n = 10 for each level).

Recoveries at 2 spiking levels for each method
■ UHPLC 20% ■ UHPLC 80% ■ GC 20% ■ GC 80%
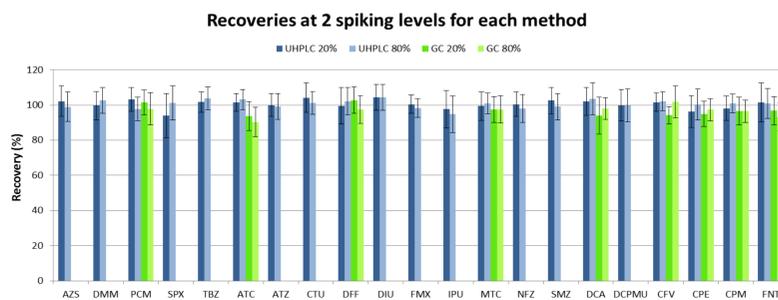
Analytical uncertainties

Expanded uncertainties for the UHPLC method varied from 9% to 28% depending on the compound and the concentration level, except for SPX at LOQ level.

Similarly, the expanded uncertainties for the GC method varied from 9% to 25% depending on the compound and the concentration level.

Molecule	Uncertainties % UHPLC LOQ	UHPLC 20%	UHPLC 80%	GC LOQ	GC 20%	GC 80%
AZS	16.1	17.1	17.4	-	-	-
DMM	18.7	16.5	14.6	-	-	-
PCM	16.8	13.4	14.2	18.6	12.9	15.3
SPX	38.7	26.9	19.4	-	-	-
TBZ	14.6	11.4	12.7	-	-	-
ATC	12.5	9.9	11.3	16.9	16.7	20.4
ATZ	13.1	12.9	15.1	-	-	-
CTU	12.8	16.4	12.9	-	-	-
DFF	25.0	21.5	15.5	25.0	14.3	16.6
DIU	23.1	14.3	14.4	-	-	-
FMX	14.8	10.6	10.8	-	-	-
IPU	21.0	22.2	22.2	-	-	-
MTC	11.7	16.2	12.1	14.7	14.0	12.6
NFZ	20.7	14.0	16.4	-	-	-
SMZ	11.3	14.7	15.1	-	-	-
DCA	16.5	15.6	17.8	18.8	20.2	12.2
DCPMU	19.3	18.3	19.0	-	-	-
CFV	9.0	10.3	11.6	20.3	12.8	14.4
CPE	16.7	18.8	17.9	18.7	15.5	9.5
CPM	22.4	14.5	10.8	20.8	14.5	11.5
FNT	28.1	21.9	17.1	23.0	14.4	16.1

METHODS COMPARISON

SBSE-LD-LC-MS-MS method:
- ☺ 21 molecules analyzed
- ☺ Larger polarity range
- ☺ > 80% recoveries
- ☹ Less sensitive method
- ☹ Non automated method

SBSE-TD-GC-MS-MS method:
- ☺ More sensitive method
- ☺ Larger linearity range
- ☺ > 90% recoveries
- ☺ Easy and automated method
- ☹ 9 molecules analyzed

CONCLUSIONS

Both methods were validated for the accurate and robust determination of ultra traces of pesticides in freshwaters. SBSE-LD-UHPLC-MS/MS targets a larger number of pesticides whereas SBSE-TD-GC-MS/MS reaches lower LOQ. Additionally, the automated online thermal desorption system allows faster and easier sample treatment.

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[4] AFNOR, T90-210 Qualité de l'eau : Protocole d'évaluation initiale des performances d'une méthode dans un laboratoire, 2009, p. 43.
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