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Comparative evaluation of liquid–liquid extraction, solid-phase extraction and solid-phase microextraction for the gas chromatography–mass spectrometry determination of multiclass priority organic contaminants in wastewater

José Robles-Molina, Bienvenida Gilbert-López, Juan F. García-Reyes, Antonio Molina-Díaz *

Analytical Chemistry Research Group (FQM-323), Department of Physical and Analytical Chemistry, Campus Las Lagunillas, Edif. B3, University of Jaén, 23071 Jaén, Spain

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ABSTRACT

The European Water Framework Directive (WFD) 2000/60/EC establishes guidelines to control the pollution of surface water by sorting out a list of priority substances that involves a significant risk to or via the aquatic systems. In this article, the analytical performance of three different sample preparation methodologies for the GC–MS/MS determination of multiclass organic contaminants—including priority comprounds from the WFD—in wastewater samples using gas chromatography–mass spectrometry was evaluated. The methodologies tested were: (a) liquid–liquid extraction (LLE) with n-hexane; (b) solid-phase extraction (SPE) with C_{18} cartridges and elution with ethyl acetate:dichloromethane (1:1 (v/v)), and (c) headspace solid-phase microextraction (HS-SPME) using two different fibers: polyacrylate and polydimethylsiloxane/carboxen/divinilbenzene. Identification and confirmation of the selected 57 compounds included in the study (comprising polycyclic aromatic hydrocarbons (PAHs), pesticides and other contaminants) were accomplished using gas chromatography tandem mass spectrometry (GC–MS/MS) with a triple quadrupole instrument operated in the multiple reaction monitoring (MRM) mode. Three MS/MS transitions were selected for unambiguous confirmation of the target chemicals. The different advantages and pitfalls of each method were discussed. In the case of both LLE and SPE procedures, the method was validated at two different concentration levels (15 and 150 $ng\,L^{-1}$) obtaining recovery rates in the range 70–120% for most of the target compounds. In terms of analyte coverage, results with HS-SPME were not satisfactory, since 14 of the compounds tested were not properly recovered and the overall performance was worse than the other two methods tested. LLE, SPE and HS-SPME (using polyacrylate fiber) procedures also showed good linearity and precision. Using any of the three methodologies tested, limits of quantitation obtained for most of the detected compounds were in the low nanogram per liter range.

1. Introduction

WFD [1] includes a list of priority compounds to be monitored in surface water to whom Environmental Quality Standards (EQS) are set to control their concentration levels [2]. Several analytical methodologies have been reported in the literature for the analysis of priority organic contaminants compounds in natural water and wastewater [3]. The developed methods are generally based on gas chromatography–mass spectrometry (GC–MS) rather than liquid chromatography–mass spectrometry (LC–MS), because of the physicochemical properties particularly due to their low polarity and solubility in water [4].

Despite current trends in sample handling focus on the development of faster, safer, and more environmentally friendly extraction techniques, both (LLE) and solid-phase extraction (SPE) yet are useful and widely accepted techniques for the exhaustive extraction of organic contaminants from water matrices [5]. Traditional liquid–liquid extraction does not fulfill the current requirements of green analytical chemistry. However, it allows the application of the procedure to the raw wastewater sample without filtration, thus offering the possibility to extract organic contaminants that tend to accumulate in the suspended solids [6,7]. SPE is an alternative less time-consuming and more environmentally friendly. Hence, it has been widely used for the determination of organic pollutants in natural waters [8–13].

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[*] Corresponding author. Tel.: +34 953212147; fax: +34 953 212940.
E-mail address: amolina@ujaen.es (A. Molina-Díaz).

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The need to filter sample prior to loading the sample on the SPE cartridge, makes this technique less suitable for the thorough extraction of contaminants from suspended solids.

As an alternative to these two widely accepted sample treatment methodologies, solid-phase microextraction (SPME) is also an established technique for volatile compounds and many applications in environmental analysis have been reported, since it is rapid, easily automated, and solvent-free [14–18]. Further advanced microextraction techniques described in the literature for trace analysis of organic contaminants in water [19] include stir-bar sorptive extraction [20–23], single-drop microextraction [24–26], dispersive liquid–liquid microextraction [27,28] and membrane assisted microextraction [29,30]. However, most of the studies so far are usually devoted to (surface) water samples. Only a little percentage of the abundant literature deals with complex wastewater samples such as untreated influents [31]. The inherent complexity of this matrix may originate several problems. For instance, SPME must be accomplished in headspace mode instead of by direct immersion of the SPME fiber. In addition, most of the methods developed are usually focused on a selected class of species rather than comprising a vast array of chemicals with different physicochemical properties, which is the preferred situation, so that all the species that are usually included in by water quality regulations could eventually be covered by a single method. For these demanding analytical tasks, LLE, SPE and HS-SPME are yet well established and reliable sample treatment techniques. Finally, wastewater matrices usually contained insoluble particles that retain part of the targeted contaminants, particularly those apolar species with high K_{ow} values. Depending upon the type of technique used, the analytes retained in the suspended particles may produce underestimation errors on the total amount of contaminants.

In this article, the analytical performance of three established sample preparation methodologies for the GC–MS/MS determination of multiclass organic contaminants in wastewater samples using gsa chromatography–mass spectrometry is evaluated. A total of 57 multiclass organic pollutants (44 pesticides and 13 PAHs), including priority substances from the WFD were examined. The methodologies tested were: (a) LLE with n-hexane; (b) SPE with C_{18} cartridges and elution with ethyl acetate:dichloromethane (1:1 (v/v)), and (c) HS-SPME using two different fibers: polyacrylate (PA) and polydimethylsiloxane/carboxen/divinilbenzene (PDMS/CAR/DVB). Identification and confirmation of the selected 57 compounds were accomplished using gas chromatography tandem mass spectrometry with a triple quadrupole instrument operated in the multiple reaction monitoring mode.

2. Experimental

2.1. Chemical and reagents

The selected multiclass organic contaminants included in this work are shown in Table 1, being most of them regulated by WFD (Directive 2000/60/CE). All standards were purchased from Sigma-Aldrich (Steinheim, Germany), except procymidone, which was obtained from Dr. Ehrenstofer (Augsburg, Germany). Ethyl acetate, dichloromethane (DCM) and n-hexane were obtained from Riedel-de-Häen (Seelze, Germany). Anhydrous sodium sulfate and sodium chlorine were from J.T. Baker (Deventer, Netherlands). MeOH HPLC grade was from Merck (Darmstadt, Germany) and sulfuric acid was provided by Panreac (Castellar del Vallès, España). Individual stock standard solutions of the target compounds were prepared at a concentration level of 1000 mg L^{-1}. These solutions were stored in a freezer at −20 °C. The working standard solution of multi-compounds was prepared by appropriate dilution of the stock solutions with n-hexane at a concentration level of 10 mg L^{-1}. SPME fibers (85 μm PA and 50/30 μm DVB/CAR/PDMS), 20 mL glass flat-bottomed vials (22.7 mm OD × 75 mm) as well as magnetic PTFE-silicone seals (3.0 mm i.d.) were purchased from Supelco (Madrid, Spain). PTFE-encapsulated magnetic stirring bars (6 mm × 12 mm) and SPE Bond Elut C_{18} cartridges (500 mg, 6 mL) were purchased from Varian Inc. (Walnut Creek, California, USA).

2.2. Sample treatment methodologies evaluated

The sample used for the study corresponds to the effluent of an urban wastewater sample treatment plant from the south-east of Spain with appropriate spiked amounts of analytes.

2.2.1. LLE procedure for the isolation and preconcentration of organic pollutants

The tested procedure was adapted from a previous work [32]. Samples without filtration were acidified with H_2SO_4 1 M up to pH=3 were reached. Then 250 mg of NaCl were added to an aliquot of 200 mL of wastewater sample, which was subsequently loaded in a 250-mL separatory funnel to undergo a three-step LLE. First, 25 ml of n-hexane were added and the mixture was vigorously shaken for 3 min being then the organic phase (upper) separated from the aqueous one. Then, this extraction step was repeated two times more. The three organic phases were combined and water traces were removed by adding anhydrous sodium sulfate. The extract was then carefully evaporated up to near dryness using a vacuum rotary evaporator (Büchi Rotavapor R200) equipped with a heating bath (Büchi B-490) operating at 34 °C and a vacuum controller (Büchi V800). Finally, the residue was redissolved in 1 mL of n-hexane, obtaining a preconcentration factor of 200:1.

2.2.2. SPE procedure

The selected procedure was adapted from Pitarch et al. [33] and consisted of passing 200 mL of filtered wastewater sample through the C_{18} SPE cartridge previously conditioned by passing 6 mL methanol, 6 mL ethyl acetate:DCM (50:50), 6 mL methanol and 6 mL ultrapure water, avoiding dryness. After loading the sample, cartridges were washed with 3 mL of ultrapure water. The cartridge was dried by passing air, in vacuum for at least 15 min, and then the elution was performed by passing 5 mL of the mixture ethyl acetate:DCM (50:50). The extract collected was evaporated under a gentle nitrogen stream using a Turbo Vap LV from Zymark (Hopkinton, MA), with a water bath temperature of 27 °C and a N_2 pressure of 5 psi. Finally, the residue was redissolved in 1 mL of n-hexane, obtaining a preconcentration factor of 200:1.

2.2.3. Automated HS-SPME procedure

The first step of the analysis consisted of the introduction of 10 mL of unfiltered wastewater sample in a 20-mL HS glass vial with a PTFE-encapsulated magnetic stirring bar. Then, 1 mL of MeOH (10% volume) and 1 g of NaCl were added and the vial was immediately sealed with a PTFE-silicone septum. It was placed in the CombiPAL heated module for heating with mechanical stirring (600 rpm) for 50 min at 80 °C in order to assess the equilibration between gas-phase and sample. During this incubation time, the PA fiber (85 μm thickness) was exposed to the headspace of the vial. After the exposition to the analytes, the fiber was automatically inserted in the injection port of the GC–MS system for 10 min (at a desorption temperature of 260 °C). During GC–MS/MS analysis, specific MRM transitions were recorded for each target compound.

Table 1
Optimized MS/MS transitions of the target compounds, including retention time, dwell time and collision energy.

t_R (min)	Window (min)	Compounds	Precursor ion (m/z)	Product ion (m/z)	Type of transition (Q, q_1, q_2)	Dwell time (s)	Collision energy (eV)
7.0	6.5–9.2	1,3,5-TCB	181.8	146.8	Q	0.042	20
			181.8	108.8	q_1	0.042	30
			181.8	110.9	q_2	0.042	25
7.8		1,2,4-TCB	181.8	108.8	Q	0.042	30
			181.8	146.8	q_1	0.042	20
			181.8	110.9	q_2	0.042	25
8.3		Hexachlorobutadiene	224.9	189.8	Q	0.042	20
			224.9	154.9	q_1	0.042	25
			224.9	152.7	q_2	0.042	45
8.4		1,2,3-TCB	181.8	108.8	Q	0.042	30
			181.8	146.8	q_1	0.042	20
			181.8	110.9	q_2	0.042	25
8.6		Isoproturon	161.1	146.1	Q	0.042	10
			161.1	128.0	q_1	0.042	20
			161.1	91.0	q_2	0.042	30
8.7		Chlorlotoluron	167.0	132.0	Q	0.042	10
			167.0	77.0	q_1	0.042	30
			167.0	104.0	q_2	0.042	20
9.8	9.2–10.5	Diuron	186.9	123.9	Q	0.167	20
			186.9	158.8	q_1	0.167	10
			186.9	96.8	q_2	0.167	35
11.9	10.5–12.2	Acenaphthylene	152.0	152.1	Q	0.100	10
			152.0	151.1	q_1	0.100	15
12.6	12.2–14.0	Pentachlorobenzene	249.8	214.8	Q	0.100	20
			249.8	142.0	q_1	0.100	40
			249.8	107.8	q_2	0.100	50
13.7		Fluorene	166.0	165.1	Q	0.100	10
			166.0	166.0	q_1	0.100	10
14.6	14.0–15.1	Trifluralin	306.1	264.0	Q	0.083	10
			306.1	206.0	q_1	0.083	15
			306.1	159.8	q_2	0.083	25
14.7		Atrazine desethyl	172.0	105.0	Q	0.083	10
			187.0	172.1	q_1	0.083	10
			172.0	94.0	q_2	0.083	15
15.5	15.1–16.0	α-HCH	218.9	182.8	Q	0.083	10
			218.9	146.9	q_1	0.083	25
			218.9	108.8	q_2	0.083	35
15.6		Hexachlorobenzene	283.8	248.7	Q	0.083	20
			283.8	141.8	q_1	0.083	50
			283.8	178.7	q_2	0.083	50
16.3	16.0–17.4	Simazine	201.0	173.0	Q	0.033	10
			201.0	186.1	q_1	0.033	10
			201.0	138.0	q_2	0.033	15
16.5		Atrazine	215.1	57.9	Q	0.033	10
			215.1	200.1	q_1	0.033	10
			215.1	138.1	q_2	0.033	15
16.6		Propazine	229.0	58.0	Q	0.033	15
			229.0	187.1	q_1	0.033	10
			229.0	214.1	q_2	0.033	10
16.8		β-HCH	218.9	182.8	Q	0.033	10
			218.9	144.8	q_1	0.033	25
			218.9	108.9	q_2	0.033	35
16.9		γ-HCH	218.9	182.8	Q	0.033	10
			218.9	144.8	q_1	0.033	25
			218.9	108.9	q_2	0.033	35
17.1		Terbuthylazine	214.1	132.0	Q	0.033	10
			214.1	136.0	q_1	0.033	10
			214.1	119.0	q_2	0.033	10
17.3		Diazinon	304.0	179.1	Q	0.033	15
			179.0	137.0	q_1	0.033	15
			179.0	122.0	q_2	0.033	25
17.7	17.4–18.2	Phenantrene	178.0	178.1	Q	0.250	10
			178.0	152.1	q_1	0.250	15
17.9		Anthracene	178.0	178.1	Q	0.250	10
			178.0	152.1	q_1	0.250	15
18.6	18.2–19.5	δ-HCH	218.9	182.8	Q	0.167	10
			218.9	144.8	q_1	0.167	25
			218.9	108.9	q_2	0.167	35
20.1	19.5–20.8	Alachlor	188.1	160.0	Q	0.050	10
			188.1	131.0	q_1	0.050	20
			188.1	132.0	q_2	0.050	15
20.1		Parathion methyl	263.0	109.11	Q	0.050	10
			263.0	127.1	q_1	0.050	10
			263.0	246.0	q_2	0.050	5

Table 1 (*continued*)

t_R (min)	Window (min)	Compounds	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)	Type of transition (Q, q_1, q_2)	Dwell time (s)	Collision energy (eV)
20.4		Heptachlor	272.0	237.0	Q	0.050	15
			272.0	272.1	q_1	0.050	5
			272.0	143.0	q_2	0.050	35
20.5		Ametryn	227.0	185.1	Q	0.050	10
			227.0	212.1	q_1	0.050	10
			227.0	58.0	q_2	0.050	10
21.2	20.8–21.5	Terbutryn	241.1	185.1	Q	0.167	10
			241.1	170.0	q_1	0.167	15
			241.1	111.2	q_2	0.167	25
21.9	21.5–22.7	Chlorpyrifos ethyl	314.0	258.0	Q	0.056	15
			314.0	286.0	q_1	0.056	10
			314.0	314.0	q_2	0.056	5
21.9		Aldrin	262.8	192.7	Q	0.056	30
			262.8	190.8	q_1	0.056	35
			262.8	228.0	q_2	0.056	20
22.3		Parathion ethyl	291.0	109.0	Q	0.056	10
			291.0	81.0	q_1	0.056	30
			291.0	142.0	q_2	0.056	5
23.1	22.7–24.5	Isodrin	262.8	192.7	Q	0.056	30
			262.8	190.8	q_1	0.056	30
			262.8	228.0	q_2	0.056	20
23.3		Chlorfenvinphos A	323.0	267.0	Q	0.056	10
			323.0	295.0	q_1	0.056	10
			323.0	159.0	q_2	0.056	30
23.7		Chlorfenvinphos B	323.0	267.0	Q	0.056	10
			323.0	295.0	q_1	0.056	10
			323.0	159.0	q_2	0.056	30
24.1		Procymidone	283.0	95.5	Q	0.056	10
			283.0	67.0	q_1	0.056	20
			283.0	255.1	q_2	0.056	10
25.0	24.5–26.4	Pyrene	202.1	202.0	Q	0.037	10
			202.1	201.2	q_1	0.037	10
25.0		α-endosulfan	338.8	160.1	Q	0.037	15
			338.8	194.9	q_1	0.037	10
			338.8	230.9	q_2	0.037	10
25.9		4,4′-DDE	318.0	246.0	Q	0.037	15
			318.0	248.0	q_1	0.037	10
			318.0	283.0	q_2	0.037	10
26.0		Dieldrin	262.8	192.7	Q	0.037	30
			262.8	190.8	q_1	0.037	35
			262.8	227.9	q_2	0.037	15
26.1		Oxyfluorfen	361.0	300.0	Q	0.037	10
			361.0	317.0	q_1	0.037	10
			361.0	252.0	q_2	0.037	15
26.8	26.4–28.8	Endrin	262.8	192.7	Q	0.033	35
			262.8	190.8	q_1	0.033	35
			262.8	228.0	q_2	0.033	20
27.2		β-endosulfan	338.8	266.7	Q	0.033	10
			338.8	160.0	q_1	0.033	15
			338.8	230.8	q_2	0.033	10
27.3		Ethion	231.0	129.0	Q	0.033	25
			231.0	157.0	q_1	0.033	15
			231.0	185.0	q_2	0.033	10
28.5		Endosulfan sulfate	386.8	252.9	Q	0.033	10
			386.8	288.9	q_1	0.033	10
			386.8	205.7	q_2	0.033	35
28.6		4,4′-DDT	235.0	164.9	Q	0.033	25
			235.0	200.0	q_1	0.033	10
			235.0	199.0	q_2	0.033	15
30.0	28.8–30.7	Iprodione	314.0	245.0	Q	0.062	10
			314.0	271.0	q_1	0.062	10
			314.0	55.9	q_2	0.062	20
30.3		Benzo(a)anthracene	228.0	228.0	Q	0.062	10
			228.0	226.0	q_1	0.062	25
30.5		Chrysene	228.0	228.0	Q	0.062	10
			228.0	226.0	q_1	0.062	25
30.5		Metoxychlor	227.0	169.0	Q	0.062	25
			227.0	212.1	q_1	0.062	10
			227.0	184.0	q_2	0.062	15
34.7	30.7–37.5	Benzo(b)fluoranthene	252.0	252.0	Q	0.250	10
			252.0	250.0	q_1	0.250	30
34.8		Benzo(k)fluoranthene	252.0	252.0	Q	0.250	10
			252.0	252.0	q_1	0.250	30
36.0		Benzo(a)pyrene	252.0	252.0	Q	0.250	10
			252.0	252.0	q_1	0.250	30

Table 1 (*continued*)

t_R (min)	Window (min)	Compounds	Precursor ion (*m/z*)	Product ion (*m/z*)	Type of transition (Q, q_1, q_2)	Dwell time (s)	Collision energy (eV)
38.4	37.5–39.5	Deltamethrin	252.9	93.0	Q	0.167	20
			252.9	174.0	q_1	0.167	10
			252.9	90.9	q_2	0.167	25
40.4	39.5–43.3	Indene(1,2,3-cd)pyrene	276.0	276.0	Q	0.125	10
			276.0	274.0	q_1	0.125	40
40.5		Dibenzo(a,h)anthracene	278.0	278.0	Q	0.125	10
			278.0	250.0	q_1	0.125	45
41.3		Benzo(g,h,i)perylene	276.0	276.0	Q	0.125	10
			276.0	274.0	q_1	0.125	40

2.3. GC–MS/MS conditions

2.3.1. Gas chromatography

Determination was performed using a CP-3800 gas chromatograph (Varian Inc. Walnut Creek, California, USA) equipped with electronic flow control (EFC) and a 1079 universal capillary injector that allows programmed temperature injection (a PTV injection port). The gas chromatograph was also equipped with an autosampler (CombiPAL autosampler, CTC Analytics) with capacity for 98 2-mL vials and a robotic arm. The injector temperature was held at 280 °C for 2 min during injection, then programmed at 40 °C min^{-1} to 325 °C, which was held for 10 min. Separations were performed on a Varian FactorFour Capillary Column VF-5ms analytical column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). Helium (99.9999%) was used as a carrier gas at a flow rate of 1.0 mL min^{-1}. The oven temperature was programmed as follows: 70 °C (held 2 min); 10 °C min^{-1} to 180 °C (5 min); 6.0 °C min^{-1} to 260 °C; and 4 °C min^{-1} to 300 °C (2 min). Sample extracts obtained after LLE or SPE sample treatments were injected (4 μL) using a Frit gooseneck liner (Restek, Bellefonte, USA).

2.3.2. Gas chromatography conditions for automated HS-SPME analysis

The gas chromatograph described in Section 2.3.1 was also equipped with an autosampler (CombiPAL autosampler, CTC Analytics) with capacity for 32 20-mL headspace vials composed of an oven for sample heating/headspace generation and a robotic arm where the headspace syringe was placed. For the fiber injection a split open deactivated insert liner of 5 mm OD × 54 mm × 3.4 mm ID (Varian Inc., Walnut Creek, California, USA) was placed inside the GC injection port. The column oven temperature program is described in Section 2.3.1.

2.3.3. Mass spectrometry

The GC was interfaced with a model 300-MS triple quadrupole mass spectrometer (Varian Inc. Walnut Creek, California, USA) operating in an electron ionization mode (EI, 70 eV). A filament current of 50 μA and a multiplier voltage of 1300 V were used in MS/MS mode. The temperatures of the transfer line, ion source and manifold were set at 280 °C, 250 °C and 40 °C, respectively. A filament multiplier delay of 6.4 min was fixed in order to prevent instrument damages. The mass spectrometer was calibrated as needed with perfluorotributylamine (PFTBA). For the MS/MS experiments Ar was used as a collision gas and the collision cell pressure was set at 1.80 mTorr. Multiple reaction monitoring (MRM) conditions were experimentally developed for each individual pesticide on the instrument used in this work. Precursor and product ions, collision energies and other parameters used are shown in Table 1. For instrument control, data acquisition and evaluation Varian MS Workstation, version 6.9 was used.

2.4. Optimization of MS/MS method

To set the MRM method, precursor ions, product ions, and collision energy were studied for each individual analyte. The most intense transition was used as quantifier (Q), while the other transition(s) was used as qualifier (q) peak(s) for the confirmatory analysis. Precursor and product ions, collision energies and other parameters used in the developed GC–MS/MS method are shown in Table 1. Three MRM transitions were used for pesticides, while for PAHs only two transitions were selected due to their difficulty to yield useful fragmentation. For atrazine desethyl and diazinon the selected precursor ions for quantifier and qualifier transitions were different.

To confirm peak identity in samples, retention time and the Q/q ratio criterion were used, defined as the ratio between the intensity of the quantification transition (Q) and the confirmation transitions (q_1 and q_2). Firstly, the theoretical averages Q/q_1 and Q/q_2 for each compound (only one ration available for PAHs) were calculated as the mean value obtained from the chromatographic analysis of standard solutions. The identity of a peak was confirmed by comparison of the experimental ratio in the sample with the theoretical ratio of the reference standard, considering the percentage of variability (tolerance) established in the Decision 2002/657/EC [34], based on ion-ratio statistics for the transitions monitored. As an example, Fig. 1 shows the identification of hexachlorobenzene in a wastewater sample spiked at a concentration level of 50 ng L^{-1} for the different sample treatment methods tested.

3. Results and discussion

3.1. Evaluation of the selected extraction procedures

The three sample treatment methodologies tested were: (a) LLE with n-hexane; (b) SPE with C_{18} cartridges and elution with ethyl acetate:dichloromethane (1:1 (v/v)), and (c) HS-SPME using two different fibers: PA and (DVB/CAR/PDMS). The comparative study is summarized in Table 2.

3.1.1. Method scope

The main goal of the proposed study was to evaluate which of these three methodologies was more convenient for the large-scale testing of multiclass organic contaminants in wastewater samples. First, it should be noted that wastewater influents are usually composed by two phases: aqueous phase and suspended solids. A relevant aspect to consider in an extraction method is the ability to extract contaminants from both the whole water, not only the dissolved fraction but also the suspended solids fraction. However, most of the analytical methods described in the literature for the analysis of organic contaminants in wastewater rely on the examination of the aqueous phase obtained after sample

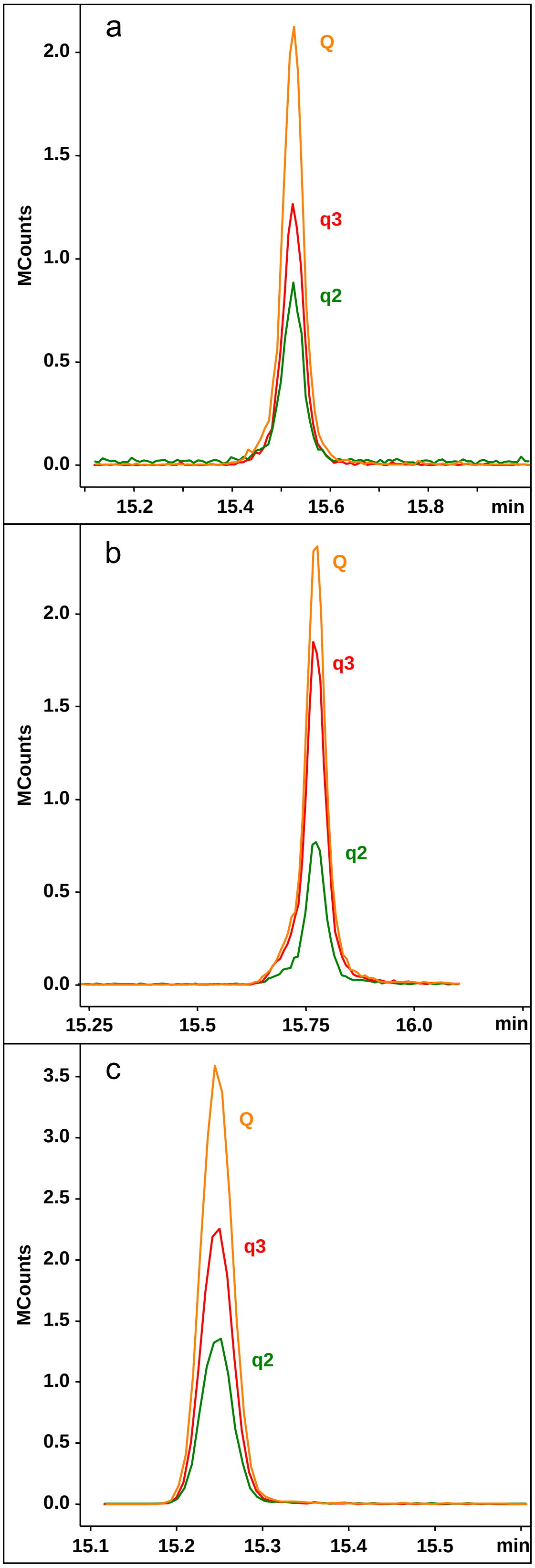

Fig. 1. Identification of hexachlorobenzene by GC–MS/MS in a wastewater sample spiked at a concentration level of 50 ng L^{-1} (a) after LLE with n-hexane; (b) after the application of the proposed SPE procedure; and (**c**) HS-SPME analysis.

filtration, disregarding thus the solid particles retained on the filters [35]. Depending on the physicochemical properties of the analytes (water solubility, octanol/water partition coefficient (log $K_{o/w}$)), each analyte exhibits a specific partitioning behavior between the two phases. This fact made clear the relevance of the analysis of both phases in wastewater samples. Otherwise, an important contribution of selected contaminants may be underestimated. According to the current regulations (EQS) [2], solid suspended particles should be considered since the whole water (dissolved + suspended solids bound fraction) must be included in the analyses.

In this sense, while LLE with solvent (hexane) permits the thorough extraction of the entire water sample (including solid suspended material), SPE usually requires a filtration prior to sample load in order to prevent clogging of cartridge, this fact being particularly important for high preconcentration factors or with complex matrices. Thus, part of the contaminants present in wastewater may be underestimated. In the case of HS-SPME, due to the complexity of the matrix and because of the suspended particulate solids, the method of choice must be HS-SPME instead of direct immersion SPME. This means that the entire unfiltered sample is subjected to analyses. In contrast, the use of HS-SPME probably along with the low volatility and behavior according to Henry's law of selected analytes offers severe drawback in terms of analyte coverage (14 of the studied compounds could not be recovered and detected). Given these data, HS-SPME was found as less versatile or at least not suited to cover such a wide variety of chemicals.

From the point of view of analyte coverage, both LLE and SPE are definitely more versatile than HS-SPME. Either with the choice of an apolar solvent (such as hexane) or a combination of two solvents to tune the extraction towards more polar compounds are effective and straightforward approaches. On the other hand, SPE with C_{18} and a careful selection of eluting solvents enabled similar recovery rates that LLE, but with the clear advantages of higher automation and lower solvent consumption. The main drawback associated with SPE is the limitation related to solid particles and the need of filtering the sample, which makes that part of the contaminants present in wastewater may be underestimated.

With regards to HS-SPME method, the choice of fiber was done considering that different chemical classes should be analyzed within a run. Under optimized values for HS-SPME variables (sample volume, heating temperature, incubation time, magnetic stirring rate, desorption time and the addition of modifiers were studied), higher signals were obtained in most cases using PA fiber. In addition, PAHs and organochlorine pesticides were strongly retained in the DVB/CAR/PDMS fiber, causing carry-over/memory effects, cross-contamination and reducing the lifetime of the fiber. Therefore, PA fiber was selected as optimum for the target analysis of this application. The main advantages of HS-SPME are the low consumption of organic solvents (only 1 mL of MeOH used as additive in each sample) and the low required volume of sample (10 mL instead of 200 mL for either LLE or SPE). The main disadvantage compared to the other studied methodologies is the limited number of species detected. Fourteen compounds were not conveniently detected: alachlor, ametryn, atrazine, atrazine desethyl, chlorotoluron, deltamethrin, endosulfan sulfate, heptachlor, iprodione, isoproturon, parathion methyl, propazine, simazine, and terbutryn. In contrast, all target compounds were effectively extracted using either LLE or SPE, and consequently detected by GC–MS/MS.

3.1.2. *Extraction efficiency: recovery rates*

To evaluate the effectiveness of the extraction using the studied LLE and SPE extraction procedures, recovery studies ($n = 6$) were carried out after spiking wastewater samples at two concentration levels, 15 and 150 ng L^{-1}. Results are detailed in Table 2, showing that both LLE and SPE in general showed similar results. At both concentration levels tested, about 60% of the studied analytes

Table 2
Comparative study of sample treatment methodologies for the determination of 57 organic pollutants by GC–MS/MS.

	LLE	SPE	HS-SPME (PA fiber results)
Number of measurable compounds	57	57	43
Treatment+analysis time per sample (min)	≃ 75	≃ 55	≃ 60[a]
Suspended particulate matter subject to analysis?	Yes	Not (unless processing unfiltered wastewater samples)	Yes (although only for relatively volatile species)
Solvents, consumed per sample	76 mL n-hexane	12 mL MeOH; 14 mL EtAc+DCM (1:1)	1 mL MeOH
LOQs (ng L^{-1})	0.03–5.10	0.03–5.00	0.10–150.00
Accuracy (recovery rates) (%)	70–110	70–120	73–119
Precision (RSD %)	3–15	1–10 in most cases	7–20
Linearity (R^2)	1–500 ng L^{-1} (at least 0.999 for most compounds)	1–500 ng L^{-1} (at least 0.999 for most compounds)	5–50 ng L^{-1} (at least 0.991 for most compounds)

EtAc: ethyl acetate and DCM: dichloromethane.

[a] Overlapped mode: injection of sample "*n*"+time incubation of sample "*n*+1".

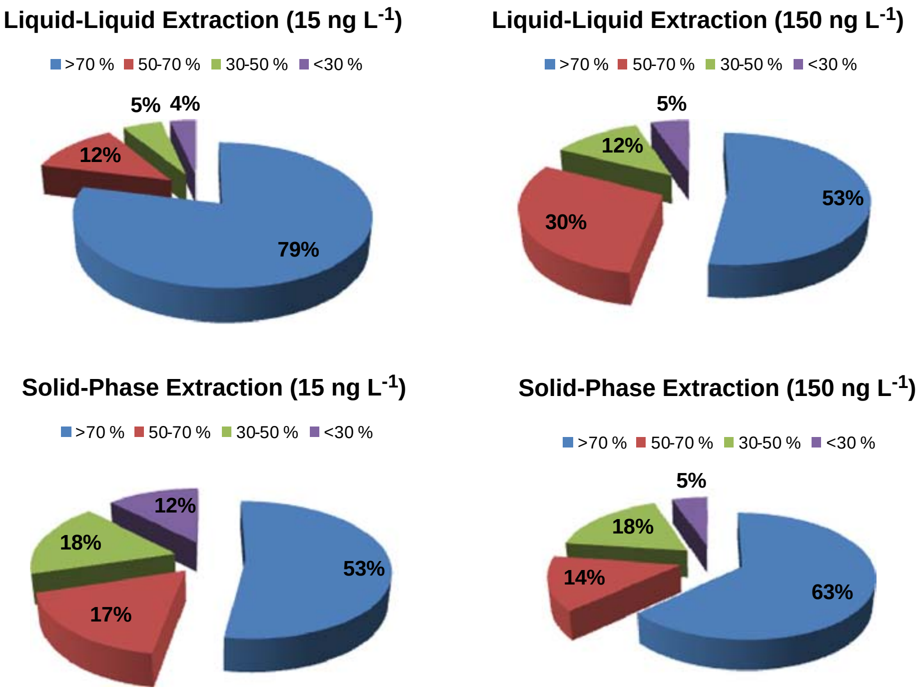

Fig. 2. Comparison between recovery studies obtained by LLE and SPE methods at both 15 and 150 ng/L spiking levels.

showed recoveries in the range 70–120%, and over 85% of the studied analytes showed recoveries at least of 50% (see Fig. 2). However, if the discussion is focused on the results by chemical class (see Table 3), it is possible to notice that a proper extraction process choice make the difference.

It is clear that SPE is the extraction process of choice for the triazine group, it showed recoveries above 90% for all compounds in both fortification levels. In contrast, four out of seven triazines gave recovery values below 60% when LLE process was applied. The extraction of these compounds is not affected by the filtration of the sample, but by the more polar solvents employed in SPE process. Similar behavior showed the phenylurea pesticides, with poor recoveries in all cases when LLE process is used. Regarding PAH compounds, at the lowest fortification level, LLE process presented in all cases better results than SPE. While recoveries values are very similar (slightly better for LLE) when comparing results at the highest fortification level. This behavior suggests that part of the PAHs is bonded to the suspended solid and this fact is of more importance at low concentration levels. Similar conclusion can be achieved for OCP, with better results for LLE at the lowest fortification level for most compounds, with recovery values below 70% for almost all of them. While slight differences are observed at the highest fortification level among both extraction processes. In the case of OPP good recovery rates (above 70% in most cases) are obtained with both extraction processes.

In the case of HS-SPME, the study was performed at 25 ng L^{-1} ($n=3$). In this case, mean recoveries ranging between 73% and 119% were obtained for the (43) detected analytes. However, it should be emphasized that in this type of microextraxtion techniques only a fraction of the analytes contained in the sample is recovered. SPME is a non-exhaustive extraction technique unlike SPE or LLE. For this reason, relative recoveries (using spiked-water standards as reference) were used instead for HS-SPME, since absolute recoveries are more appropriately used for exhaustive extraction techniques.

3.1.3. Automation, solvent consumption and waste generation

As included in Table 2, from the point of view of solvent consumption and waste generation, HS-SPME offers clear advantages against both SPE and LLE as being the more environmentally friendly approach [36], using only 1 mL of MeOH as chemical

Table 3
Recovery studies of LLE ($n=6$), SPE ($n=6$) and HS-SPME ($n=3$) methods.

Compounds	Chem. class[b]	LLE				SPE				HS-SPME	
		Fortification levels (ng L^{-1}) and RSD (%)				Fortification levels (ng L^{-1}) and RSD (%)				Fortification level (ng L^{-1}) and RSD (%)	
		15	RSD (%)	150	RSD (%)	15	RSD (%)	150	RSD (%)	25	RSD (%)
Ametryn	TrzP	47.2	19.4	33.5	11.6	90	13.3	93	4.9	–	–
Atrazine	TrzP	74.5	6.1	53.9	9.7	108	3.9	100	8.2	–	–
Atrazine desethyl	TrzP	52.1	17	44.4	19.6	95	0.3	117	5.8	–	–
Propazine	TrzP	120.1	3	82.1	7.3	94	6	101	5.8	–	–
Simazine	TrzP	–	–	25.2	15.4	–	–	94	9.9	–	–
Terbuthylazine	TrzP	139	3.8	88.8	9.2	93	9	103	8.6	86.25[a]	4.32
Terbutryn	TrzP	104.2	5.1	89.1	15.4	97	14.3	94	5.6	–	–
Chlorotoluron	PhUP	54.9	7.3	20	17.5	98	7.5	120	3.6	–	–
Diuron	PhUP	54	12.6	17.5	21.1	85	13.1	130	5.1	83.06	14.89
Isoproturon	PhUP	42	8.5	29.8	13.8	97	6.9	118	2.7	–	–
Deltamethrin	PEP	–	–	32.1	22.4	50	14.1	54	4.8	–	–
Chlorfenvinphos A	OPP	154.3	8.7	68.8	8.4	99	7	118	11.7	79.75	12.26
Chlorfenvinphos B	OPP	100.1	5.7	96.6	9.4	98	6	107	2.7	73.32	2.08
Chloropyrifos ethyl	OPP	104.6	16.4	73.8	18.1	73	11.3	74	9	118.68	6.72
Diazinon	OPP	112	5.2	90.5	10.4	89	12.7	88	9.2	123.82	5.38
Ethion	OPP	74.5	4.8	66.6	22.6	70	10	77	8.3	100.84	3.09
Parathion ethyl	OPP	112.4	5.1	100.9	8.4	112	9.8	100	2.6	89.04	12.55
Parathion methyl	OPP	101.7	3.7	102.4	10.1	79	15.3	97	5.7	–	–
Aldrin	OCP	102.1	7.3	62.1	17.8	37	14.4	30	14.3	102.57	9.42
4,4′-DDE	OCP	103.7	6.9	69.4	19.2	36	6.3	47	11.6	105.81	7.8
4,4′-DDT	OCP	93.9	9.3	69.4	18.5	48	5.9	68	7.2	102.86	6.89
Dieldrin	OCP	88.8	8.2	76.3	7.1	48	7.9	70	6.1	100.93	4.76
α-Endosulfan	OCP	175.1	7.5	85.6	8.7	–	–	74	7.2	98.71	6.94
β-Endosulfan	OCP	119.8	8.8	75.6	9.9	89	6.2	82	21.5	91.35	7.5
Endosulfan sulfate	OCP	111.3	6	69.2	18.2	110	9.8	96	6.6	–	–
Endrin	OCP	98.9	13.7	81.1	11.6	75	10	82	8.3	97.38	8.83
Heptachlor	OCP	115	5.7	67.1	11.6	49	15	37	6.3	–	–
Hexachloro-1,3-butadiene	OCP	52	15.3	31.6	19	16	6.3	18	14	104.07	1.39
Hexachlorobenzene	OCP	96.5	3.1	73.9	12.4	40	9.4	40	13.7	107.98	4.55
α-HCH	OCP	107	5.8	93	5.2	71	7	81	10.4	101.19	1.74
β-HCH	OCP	164.1	7.8	112.7	14.8	99	7.4	120	8.1	99.75	8.56
γ-HCH	OCP	133.8	3.7	93.7	13	90	8.7	91	9.9	95.53	13.92
δ-HCH	OCP	94.9	10.8	101.2	12.3	80	1.8	91	8.6	93.25[a]	8.65
Isodrin	OCP	103.9	5.2	73	12.5	38	11.4	39	8.8	101.87	7.13
Metoxychlor	OCP	94.2	1.4	78.9	17.6	74	7	89	6.1	100.04	5.28
Pentachlorobenzene	OCP	105.4	7.1	72.9	9.2	30	1.6	36	10.8	106.46	2.08
1,2,3-TCB	OCP	76.1	10.9	52.5	9.9	27	10	33	16.3	102.34	4.34
1,2,4-TCB	OCP	88.2	9.9	45.8	9.5	30	10.2	27	10.9	104.27	3.55
1,3,5-TCB	OCP	77.3	8.1	43	15.6	26	6.4	20	15.5	116.29	4.17
Oxyfluorfen	NPhEP	142.3	7.4	90.1	18	111	3.1	88	9.6	82.35	8.13
Trifluralin	DnaP	102.6	4.6	82.7	1	57	6.6	57	7.7	101.39	8.38
Iprodione	DcDP	51.7	13.9	56.5	7.7	100	13.6	120	6.1	–	–
Procymidone	DcDP	125.4	5	84.4	9.1	76	8.5	93	5.7	95.62	5.36
Alachlor	ChaP	136.6	2.7	92.7	12.2	82	8	96	9.7	–	–
Acenaphtylene	PAH	102	4.1	88.2	6.7	39	8.5	50	9.5	103.52	0.18
Anthracene	PAH	140.7	3.7	84.3	7.9	63	7.8	70	6.1	101.07	5.91
Benzo(a)anthracene	PAH	85.3	7.4	62.2	16.2	64	6.3	77	5.5	105.1	1.29
Benzo(a)pyrene	PAH	71.3	7.4	57.6	15.4	59	6.2	53	4.1	107.23	5.89
Benzo(b)fluoranthene	PAH	61.6	7.4	64.4	20.4	58	11	66	5.5	93.34	7.38
Benzo(ghi)perylene	PAH	132.4	1.6	52.1	6.8	54	15.1	49	4.5	100.89	6.68
Benzo(k)fluoranthene	PAH	63	7.1	63.7	14.6	45	14.5	63	5.3	98.49	2.3
Chrysene	PAH	98.9	6.1	86.5	13.5	60	10.1	75	4.6	105.31	0.98
Dibenzo(a,h)anthracene	PAH	76.8	4.1	54.5	11.9	50	18.1	48	4.1	97.51	7.94
Fluorene	PAH	123.4	3	79.4	12.8	48	12.8	57	9.1	106.07	0.97
Indene(1,2,3-cd)pyrene	PAH	75.7	4.8	54	11.6	63	3.6	46	2.8	105.07	18.09
Phenanthrene	PAH	108.1	5.8	74.9	14.1	70	6.5	71	10.3	111.32	7.19
Pyrene	PAH	43	20.2	70.9	13.4	73	11.2	75	8.9	104.21	4.89

[a] Terbuthylazine and δ-HCH were spiked at a concentration level of 150 ng L^{-1} ($n=3$), instead of 25 ng L^{-1}.

[b] Description of the acronyms: TrzP—Triazines pesticides, PhuP—Phenylureas pesticides, PEP—Pyrethroid ester pesticides, OPP—Organophosphate pesticides, OCP—Organochlorine pesticides, NPhEP—Nitrophenyl ether pesticides, DnaP—Dinitroanilyne pesticides, DnaP—Dinitroaniline pesticides, DcDP—Dichlorophenyl dicarboximides pesticides, ChAP—Chloroacetanilide pesticides, and PAH—Polycyclic aromatic hydrocarbon.

modifier. The solvent consumption is negligible compared to SPE and especially to LLE. In the two latter techniques, the selected sample volume was 200 mL. Considering a preconcentration factor of 200:1, the final volume of the extract is 1 mL. Working with lower volumes (e.g. 0.5 mL with 100 mL of loaded sample) was discarded because some of the matrices obtained were so complex that sometimes emulsions were obtained and a dedicated filtration of the final extracts through membrane filters was necessary. The use of SPE involves 34% reduction in organic solvent consumption compared to LLE, and is less time-consuming than

Table 4
Limits of quantitation for the determination of 57 organic pollutants by GC–MS/MS using the three studied sample treatment methodologies.

Compound	LOQ (ng L^{-1})		
	LLE	SPE	HS-SPME
Acenaphtylene	0.2	0.3	5.0
Alachlor	2.7	2.6	–
Aldrin	0.3	0.2	5.0
Ametryn	2.7	2.7	–
Anthracene	1.0	1.0	24.8
Atrazine desethyl	1.2	1.4	–
Atrazine	4.5	2.5	–
Benzo(a)anthracene	0.7	0.2	24.8
Benzo(a)pyrene	0.2	0.4	24.8
Benzo(b)fluoranthene	0.6	0.3	24.8
Benzo(ghi)perylene	0.4	0.3	5.0
Benzo(k)fluoranthene	0.3	0.2	24.8
Chlorfenvinfos A	2.2	2.4	2.6
Chlorfenvinfos B	3.2	2.7	14.9
Chloropyrifos ethyl	0.9	0.8	0.7
Chlorotoluron	1.3	2.6	–
Chrysene	1.6	0.2	24.8
Deltamethrin	2.8	4.1	–
Diazinon	0.03	0.03	0.1
Dibenzo(a,h)anthracene	3.4	0.5	5.0
4,4′-DDE	1.1	0.3	24.8
4,4′-DDT	0.6	0.6	5.0
Dieldrin	0.4	5.0	5.0
Diuron	0.3	0.3	24.8
α-Endosulfan	0.9	99.0	24.8
β-Endosulfan	0.3	9.9	24.8
Endosulfan sulfate	0.8	0.2	–
Endrin	5.0	4.1	5.0
Ethion	5.0	0.6	1.0
Fluorene	0.2	0.2	5.0
Heptachlor	0.03	0.03	–
Hexachloro-1,3-butadiene	0.3	0.7	1.0
Hexachlorobenzene	0.2	0.4	5.0
α-HCH	0.3	0.3	1.0
β-HCH	0.5	0.8	5.0
γ-HCH	0.4	0.6	5.0
δ-HCH	0.4	0.6	148.5
Indene(1,2,3-cd)pyrene	0.4	0.3	5.0
Iprodione	0.2	1.5	–
Isodrin	2.0	2.7	24.8
Isoproturon	2.2	2.7	–
Metoxychlor	0.2	0.6	5.0
Oxyfluorfen	0.4	0.0	0.7
Parathion ethyl	2.7	3.1	24.8
Parathion methyl	2.5	2.5	–
Pentachlorobenzene	0.03	0.1	5.0
Phenanthrene	0.9	0.7	24.8
Procymidone	3.0	0.4	24.8
Propazine	3.2	2.1	–
Pyrene	0.4	0.6	24.8
Simazine	5.1	5.1	–
Terbuthylazine	2.0	1.1	148.5
Terbutryn	1.4	1.4	–
1,2,3-TCB	1.7	1.9	5.0
1,2,4-TCB	2.2	2.7	5.0
1,3,5, TCB	1.8	2.7	5.0
Trifluralin	0.2	0.2	0.7

both LLE and HS-SPME. Finally, the sensitivity offered by both LLE and SPE is pretty similar (36 analytes showed LOQs below 1 ng L^{-1} in the case of SPE, while 32 analytes showed LOQs below 1 ng L^{-1} in the case of LLE).

3.2. Analytical performance of GC–MS/MS methods

The linearity of the calibration curves was studied using matrix-matched standard solutions containing the target compounds at six concentration levels in the range from 1 to 500 ng L^{-1}. The response was found to be linear with a regression coefficient (R^2) higher than 0.999 in the range tested for most of studied organic pollutants when either LLE or SPE procedures were used. In the case of HS-SPME, results were not as satisfactory with calibration curves only linear in the range 5–50 ng L^{-1} for most of studied compounds, although several analytes were linear up to 150 ng L^{-1}, obtaining values of at least 0.991 for regression coefficients.

Limit of quantitation (LOQ) was estimated as the analyte concentration that produces a peak signal of 10 times the background noise from the chromatogram at the lowest calibration level as low as it was possible using the q_2 transition. The proposed method showed LOQs in the low ng L^{-1} range, although the results were better for both SPE and LLE when compared with HS-SPME. Results are detailed in Table 4. In terms of sensitivity, LLE is similar to SPE, obtaining LOQs in the range 0.03–5.00 ng L^{-1} for both methodologies. However, 14 compounds were not effectively detected by HS-SPME. The main reasons are their low volatility and affinity towards the selected SPME fiber. For the rest of compounds detected, HS-SPME offered LOQs in the range 0.1–25 ng L^{-1}, except for terbuthylazine and δ-HCH, which showed LOQ of 150 ng L^{-1}.

A precision study was carried out by calculating the relative standard deviation (RSD) from the analysis of six replicates of fortified wastewater at 15 and 150 ng L^{-1} concentration levels for LLE and SPE. Nevertheless, in HS-SPME, 25 ng L^{-1} (three replicates) was the selected level for HS-SPME because all extractable compounds, except terbuthylazine and δ-HCH, could be measured at this level. No higher concentration was chosen for this method, due to the lack of linearity for most compounds beyond 150 ng L^{-1}. Relative standard deviation values were in most cases in the range 3–15% when LLE was used, while using SPE procedure RSD (%) was ranging 1–10% and values between 7 and 15% were found when HS-SPME methodology was applied (Table 3).

4. Conclusions

In the present work, three sample treatment methodologies have been evaluated and compared for analysis by GC–MS/MS of organic pollutants included in the list of priority substances of WFD. LLE with n-hexane and SPE using with C_{18} cartridges and ethyl acetate:dichloromethane (1:1 (v/v)) as eluent showed recoveries in the range 70–120% for the majority of the studied compounds. Linearity and precision were evaluated, obtaining satisfactory results for all the three sample treatment methodologies tested. The limits of quantitation obtained for most of the target compounds were in the low nanogram per litre range, using any of the three procedures tested. LLE and SPE were revealed as the most sensitive methods, but SPE is less time consuming and more environmentally friendly than LLE in terms of solvent consumption, although species that may be retained in suspended solids would be out of the scope of the method. Nevertheless, it has been demonstrated that depending on the chemical class, the treatment sample process of choice would be either SPE or LLE.

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