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Gas chromatography triple quadrupole mass spectrometry method for monitoring multiclass organic pollutants in Spanish sewage treatment plants effluents

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ABSTRACT

In Spain, although more than 50% of urban wastewaters are currently being treated, only half of them are subjected to biological treatments and only 3% undergo advanced treatment technologies. Consequently, the application of more exhaustive wastewater treatment protocols, including the use of new and improved technologies, the application of wider and integrated quality control and water reuse strategies are a priority. We have used as a reference, the European Water Framework Directive (WFD; Directive 2000/60/CE), which establishes a framework for Community action in the field of water policy, setting a list of priority compounds to be monitored in water in order to evaluate their levels. The aim of the present study is to develop and validate a multi-residue method for the analysis of 57 multi-class organic contaminants in wastewater samples using gas chromatography coupled to triple quadrupole mass spectrometry and apply it to evaluate the presence of such compounds in different wastewater treatment plants. The proposed method is based on a sample treatment using liquid–liquid extraction with n-hexane followed by identification, confirmation and quantitation with gas chromatography tandem mass spectrometry using a triple quadrupole analyzer operating in the selected reaction monitoring mode. Three MS/MS transitions were selected for unambiguous confirmation of the target chemicals. The method was validated at two different concentration levels (15 and 150 ng L^{-1}) obtaining recovery rates in the range 70–110% in most cases. The limits of quantitation obtained for most of the compounds tested were in the low nanogram per liter range (below 3 ng L^{-1} in all cases). Treated and untreated effluent wastewater samples of different origin (industrial, coastal and urban) provided by several sewage treatment plants (STPs) located throughout Spain were tested. Results so far showed that most of the samples assayed did not contain large amount of these contaminants. Hexachlorobenzene was found to be the more frequently detected contaminant in the studied samples, although at levels below 5 μg L^{-1}.

1. Introduction

Protection of aquatic environment from chemical pollution resulting from human activity is a current challenge for European governments. To cope with this issue, one of the first steps is the monitoring of sewage treatment plants (STPs) discharges. In Spain, the vast majority of urban wastewater treatment stations have been designed and operate so as to meet European Directive concerning urban wastewater treatment [1]. This regulation sets objectives for treated water quality to minimize the environmental impact on areas of discharge, but is not explicitly oriented to reuse. Nevertheless, treated wastewater is indirectly reused through natural courses. The discharged treated wastewaters (except in the case of coastal sewage treatment plants, which discharge treated wastewaters to the sea) are diluted with circulating flows and are partially reused in downstream areas for new urban uses, agriculture and industry [2]. Furthermore, secondary treatment involves biological treatment or other process designed to control the biochemical oxygen demand (BOD) and the chemical oxygen demand (COD) of the wastewater under treatment. Therefore, it is not specifically focused on the elimination of certain hazardous compounds that can impact negatively the environment, and some organic compounds are becoming ubiquitous in the environment [3,4].

In order to achieve a good chemical status of water in the EU [5], the European Water Framework Directive 2000/60/CE (WFD) [6] establishes a framework for Community action in the field of water policy, setting a list of priority compounds to be monitored in water in order to evaluate their levels. Environmental Quality Standards (EQS) have been set to control the concentration levels

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of these compounds in surface waters [7]. On the basis of these EQS, Member States have to establish monitoring programs in order to have a comprehensive overview of water status within each river basin district. Additionally, Member States have to identify their river basin specific pollutants, which will be the basis of further updates of the priority substances list. Consequently, improved and powerful analytical methodologies need to be available to accurately determine the very low concentration levels set [8].

Since WFD became into force, numerous studies have been undertaken to identify the occurrence and significance of priority substances in wastewater systems [9–14]. The most widely used approach in the last years for the analysis of priority organic contaminants in environmental samples is gas chromatography/mass spectrometry (GC–MS) with single quadrupole detector operating in selected ion monitoring (SIM) [15–17], not only by its cost effectiveness, sensitivity and selectivity but also by the physicochemical characteristics of most the target analytes. Considering the list of priority compounds, pesticides such as triazines (atrazine, simazine), phenylurea herbicides (diuron, isoproturon) and alachlor can be analyzed by either LC–MS or GC–MS, while other of them as cyclodiene pesticides (aldrin, dieldrin, endrin, isodrin), chlorfenvinphos, chlorpyrifos ethyl, 4,4′–DDT, endosulfan, hexachlorocyclohexane (HCH) and trifluralin are only GC-amenable compounds. Polycyclic aromatic hydrocarbons (PAHs) are other group of priority pollutants that cannot be properly analyzed by LC–MS using an electrospray (ESI) interface. Numerous applications in water analysis based on GC–MS have been reported for the determination of PAHs [18–21], pesticides [22–24] and multi-residue procedures for the determination of priority and persistent organic pollutants [25,26]. As an alternative to classic GC–MS, several approaches with enhanced performance has been proposed for the sensitive and selective detection of priority organic contaminants in aqueous matrices including gas chromatography tandem mass spectrometry (GC–MS/MS) with triple quadrupole mass spectrometry [27–30], gas chromatography high resolution mass spectrometry (GC–HRMS) using either time-of flight mass spectrometry (GC–TOFMS) [29,31] or magnetic sector analyzer [10], comprehensive two dimensional gas chromatography time-of-flight mass spectrometry (GC × GC-TOFMS) [32–34] and fast GC–MS [35]. These detection methods have been accompanied by different sample preparation approaches including liquid–liquid extraction [36], solid-phase extraction (SPE) [28,33], SPME [30], liquid–liquid microextraction (LLME) [32], or stir bar sorptive extraction (SBSE) [33,37,38].

In this work, we report on the development of a multi-residue method for analysis of 57 organic pollutants (including priority substances from the European Water Framework Directive) by gas chromatography coupled to tandem mass spectrometry, and its application to the monitoring of 33 wastewaters samples at the final stage of three Spanish Sample Treatment Plants (STP) (ready to be discharged into the environment). The proposed method is based on liquid–liquid extraction of the raw sample without previous filtration, since the WFD requires methods allowing complete extraction of organic contaminants from whole water samples [7] (thus avoiding losses in the suspended solids). The method was validated and its performance tested with different samples from sewage treatment plant (STP) effluents collected in different regions of Spain.

2. Experimental

2.1. Chemical and reagents

Organic pollutants investigated in this work are shown in Table 1, being several of them regulated by European Water Framework Directive (WFD; Directive 2000/60/CE). All compounds standards were purchased from Sigma-Aldrich (Steinheim, Germany), except for procymidone, which was obtained from Dr. Ehrenstorfer (Augsburg, Germany).

Ethyl acetate, n-hexane were provided from Riedel-de-Häen (Seelze, Germany), anhydrous sodium sulfate, and sodium chlorine from J.T. Baker (Deventer, Netherlands), MeOH HPLC grade from Merk (Darmstadt, Germany) and sulfuric acid concentrated provided from Panreac (Castellar del Vallès, España). Individual stock standard solutions of the target compounds were prepared at a concentration level of 1000 $\mu g\,mL^{-1}$ by dissolving them in n-hexane, ethyl acetate or mixtures of these solvents. Purity was included in the calculation of actual concentration of each standard solution. These solutions were stored in a freezer at −20 °C. The working standards mix solution was prepared by appropriate dilution of the stock solutions with n-hexane at a concentration level of 10 $\mu g\,mL^{-1}$.

2.2. Sample material

A total number of 33 wastewater samples of urban origin were collected from three different Sewage Treatment Plants, located in the North (N-STP), North-East (NE-STP) and South-East (SE-STP) of Spain. Wastewater samples were collected during several months after they have passed different stages of treatment, primary, secondary and tertiary. Effluents of the secondary and non-subsequent tertiary treatment stages were analyzed. Thirteen (4 from the SE-STP, 4 from the NE-STP and 5 from the N-STP) samples corresponded to effluents of the secondary treatment stage and twenty (4 from the SE-STP, 4 from the NE-STP and 12 from the N-STP) samples corresponded to effluents of the tertiary treatment stage. Integrated samples were collected at 1-h intervals and used as representative of 24-hour work in the STPs.

2.3. Liquid–liquid extraction procedure for the isolation and preconcentration of organic pollutants

Samples (200 mL) without filtration were acidified with H_2SO_4 1 M up to pH = 3 was 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 liquid–liquid extraction. 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 twice more. The 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) and a vacuum controller (Büchi V800). Finally, the residue was re-dissolved in 1 mL of n-hexane, obtaining thus a preconcentration factor of 200:1.

2.4. GC–MS/MS conditions

2.4.1. Gas chromatography

Determination were performed using a CP-3800 gas chromatograph (Varian Inc. Walnut Creek, California, USA) equipped with electronic flow control (EFC), a 1079 universal capillary injector and CombiPal autosampler (CTC Analytics). The injector temperature was held at 280 °C for 2.0 min during injection, then programmed at 40 °C min^{-1} to 325 °C, which was held for 10 min. A split ratio was initially set at 50:1, at 0.01 min the split vent was closed until 2 min to ensure complete sample transfer, then, the split ratio was held at 100:1 until 13 min, and finally

Table 1
GC–MS/MS MRM identification parameters of the studied compounds: retention time and optimized MS/MS transitions.

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)
7.048	6.50–9.20	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
7.834		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
8.313		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
8.372		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
8.560		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
8.723		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
9.795	9.20–10.50	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
11.909	10.50–12.20	Acenaphthylene	152.0	152.1	Q	0.100	10
			152.0	151.1	q_1	0.100	15
12.595	12.20–14.00	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
13.682		Fluorene	166.0	165.1	Q	0.100	10
			166.0	166.0	q_1	0.100	10
14.597	14.00–15.10	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
14.715		Atrazine desethyl	172.0	105.0	Q	0.083	10
			187.0	172.0	q_1	0.083	10
			172.0	94.0	q_2	0.083	15
15.528	15.10–16.00	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
15.612		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
16.340	16.00–17.40	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
16.504		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
16.633		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
16.785		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
16.949		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
17.125		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
17.312		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
17.665	17.40–18.20	Phenantrene	178.0	178.1	Q	0.250	10
			178.0	152.1	q_1	0.250	15

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)
17.960		Anthracene	178.0	178.1	Q	0.250	10
			178.0	152.1	q_1	0.250	15
18.593	18.20–19.50	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
20.093	19.50–20.80	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
20.113		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
20.357		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
20.549		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
21.236	20.80–21.50	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
21.875	21.50–22.70	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
21.917		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
22.263		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
23.140	22.70–24.50	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
23.286		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
23.705		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
24.089		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
24.975	24.50–26.40	Pyrene	202.1	202.0	Q	0.037	10
			202.1	201.2	q_1	0.037	10
24.984		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
25.860		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
26.006		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
26.152		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
26.755	26.40–28.85	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
27.198		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
27.339		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

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)
28.526		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
28.646		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
30.030	28.85–30.70	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
30.320		Benzo(a)anthracene	228.0	228.0	Q	0.062	10
			228.0	226.0	q_1	0.062	25
30.466		Chrysene	228.0	228.0	Q	0.062	10
			228.0	226.0	q_1	0.062	25
30.507		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
34.698	30.70–37.50	Benzo(b)fluoranthene	252.0	252.0	Q	0.250	10
			252.0	250.0	q_1	0.250	30
34.810		Benzo(k)fluoranthene	252.0	252.0	Q	0.250	10
			252.0	252.0	q_1	0.250	30
35.987		Benzo(a)pyrene	252.0	252.0	Q	0.250	10
			252.0	252.0	q_1	0.250	30
38.378	37.50–39.50	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
40.367	39.50–43.33	Indene(1,2,3-cd)pyrene	276.0	276.0	Q	0.125	10
			276.0	274.0	q_1	0.125	40
40.520		Dibenzo(a,h)anthracene	278.0	278.0	Q	0.125	10
			278.0	250.0	q_1	0.125	45
41.326		Benzo(g,h,i)perylene	276.0	276.0	Q	0.125	10
			276.0	274.0	q_1	0.125	40

reduced to 20:1. Sample extracts (4 μL) in n-hexane were injected using a Frit gooseneck liner (Restek, Bellefonte, USA). Separations were performed on a Varian FactorFour Capillary Column VF-5 ms analytical column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). Helium 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; 4 °C min^{-1} to 300 °C (2 min).

2.4.2. 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.

3. Results and discussions

3.1. GC–QqQ–MS/MS method development

As a general rule, the chromatographic separation is not a critical aspect in the development of a multi-residue method with triple quadrupole analyzers because of the possibility of monitoring co-eluted compounds in MRM mode. Optimization of triple quadrupole MS/MS was performed for each target compound in order to optimize the MRM method. Precursor ions, product ions, and collision energy optimization was carefully studied for each individual analyte for best response. The optimization was carried out by the analysis of 4 μL of mixtures of pesticide standard solutions in hexane, containing 1 mg L^{-1} of each analyte. After obtaining the full scan spectra of each analyte, the precursor ion with the highest m/z ratio (increase of selectivity) and relative abundance (increase in sensitivity) was selected for fragmentation purposes. The same criterion was also applied to choose the more suitable product ions. Collision energies (CEs) from 5 to 50 eV were evaluated.

Three MRM transitions were used for pesticides, while for PAHs only two transitions were selected due to their difficulty to yield useful fragmentation. Most of PAHs are quantified using the same ion as precursor and as product ion, respectively. The pairs of isomers phenanthrene–anthracene, and benzo(b)fluoranthene–benzo(k)fluoranthene, use exactly the same transitions (respectively), and both compounds in each pair elute very close one to

Table 2
Preliminary evaluation of different solvents (n-hexane and dichloromethane) for the liquid–liquid extraction (LLE) of multiclass organic contaminants (fortification level: 100 ng L^{-1}).

	LLE with n-hexane[a]		LLE with dichloromethane[b]	
	Recovery (%)	R.S.D (%) ($n=3$)	Recovery (%)	R.S.D (%) ($n=3$)
1,3,5-TCB	42.2	9.7	34.5	16.7
1,2,4-TCB	43.3	9.2	35.6	13.7
1,2,3-TCB	49.4	6.0	36.6	13.5
Hexachlorobutadiene	45.4	10.8	27.2	20.4
Pentachlorobenzene	56.0	1.6	43.2	18.0
α-HCH	75.3	2.8	59.6	19.9
β-HCH	71.6	5.0	48.2	21.1
γ-HCH	78.1	3.5	60.1	17.8
δ-HCH	67.3	5.5	46.2	22.4
Hexachlorobenzene	58.5	2.1	51.7	23.1
Alachlor	83.4	5.8	63.9	15.9

[a] Proposed method [this work].
[b] Adapted from Ref. [40]. Briefly, 200 ml water sample +500 mg NaCl, add 30 mL dichloromethane (DCM); 5 min vortex (separate organic phase). Add HCl to change aqueous phase to pH=2, add an additional aliquot of 30 mL DCM, extract and separate DCM phase; change pH of aqueous phase to 11 with NaOH, add 30 mL DCM. Combine DCM extracts and evaporate with rotavapor and finally taken up with 2-mL hexane.

the other. Therefore for the identification and quantification of PAHs is very important to achieve a good chromatographic separation. For atrazine desethyl and diazinon the selected precursor ion for quantifier and qualifier transitions was different. The most intense transition was used as quantifier (Q), while the other transition(s) were 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.

To confirm peak identity in samples, the Q/q ratio criterion was 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 average Q/q_1 and Q/q_2 for each compound (only one ratio 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 [39], based on ion-ratio statistics for the transitions monitored. Obviously, the agreement in the retention time with standards is also required for a positive confirmation, which is particularly important for the PAHs isomers whose identification is based on the same MS/MS transitions (phenanthrene–anthracene, and benzo(b) fluoranthene–benzo(k) fluoranthene).

3.2. Liquid–liquid extraction method considerations

The main difficulty to tackle with when developing a multiresidue method is the vast array of species with very different physicochemical properties that need to be extracted with a unique set of conditions. Hence, any extraction solvent choice may eventually be biased towards the extraction of a selected class of analytes. The list of priority species included in the study (using lists from current regulations [6,7]) involved non-polar species such as PAHs and organochlorine species together with relatively polar herbicides such as diuron or simazine, whose analyses are more convenient by LC–MS. The choice of the solvent is therefore difficult given this diversity of chemicals. Besides n-hexane, other solvents such as acetone, ethyl acetate or dichloromethane might be possible choices. Considering previous literature [40], a preliminary experiment was undertaken evaluating the performance of dichloromethane and n-hexane for 11 out of the more representative organic priority species. Results obtained are shown in Table 2.

From the data shown we found some problems with dichloromethane as solvent, particularly for trichlorobenzene isomers, hexachlorobenzene, pentachlorobenzene and hexachlorobutadiene. Meanwhile, the recovery rates provided by n-hexane were satisfactory in most cases -given the difficulty to find out universal conditions for all the tested species-, even for relatively polar pesticides (for GC analyses) such as triazines. Hence, the performance of n-hexane for relatively polar species was much better than the performance of dichloromethane for non-polar species. For this reason, n-hexane was selected as extraction solvent. In addition, considering aspects such as occupational safety and health and green chemistry principles, the choice of n-hexane is more convenient (or less inconvenient) than dichloromethane.

One the other hand, with regards to the sample volume used a 200-mL of wastewater were preconcentrated to a final volume of 1 mL. This involved a relative high consumption of hexane (75 mL). This sample size was selected considering the case of highly dirty samples (e.g. from untreated effluents) which were difficult to filter before GC–MS. The extracts obtained were so dirty that most of the sample extracts were lost during the filtration step, and eventually the entire extract from the extraction could be lost. Hence, a final volume of 1-mL was selected for the stuffy. Keeping this in mind, in the case of less complex samples (i.e. effluents), the procedure could be re-sized so that less organic solvent was consumed, for instance, 3 replicates with 10–15 mL with half of sample volume (e.g. 100 mL), obtaining a final volume of 0.5 mL which would enable the same preconcentration factor. This selection may vary depending on the type of samples tested.

3.3. Analytical performance

The linearity of the calibration curves was studied using matrix-matched target compounds standard solutions at seven concentrations ranging between 0.1 and 100 μg L^{-1} which corresponded to 0.5 and 500 ng L^{-1} in the wastewater samples using 7 calibration solutions prepared in n-hexane and in extract of wastewater obtained by LLE extraction procedure as any other sample. The response function in matrix-matched standards was found to be linear with a regression coefficient (r^2) higher than 0.993 in the tested range for all organic pollutants.

Limit of detection (LOD) was estimated as the analyte concentration that produces a peak signal of three times the background noise from the chromatogram at the lowest matrix-matched calibration level as low as it was possible. Limit of quantitation (LOQ) was calculated as 3.3 times the LOD. As can be seen in Table 3, LOQs were in the low range 0.03–5.00 ng L^{-1}, improving those previously reported by using the same technique for the determination of a similar mixture of compounds [28]. Taking into account that EQS for the studied compounds are in the range 2–1000 ng L^{-1}, the proposed method fulfills by far the performance criterion "LOQ less than 30% of EQS" proposed by the Chemical Monitoring Activity (CMA) working group of the European Commission [7].

It should be also emphasized the satisfactory performance achieved with GC–MS/MS for relatively polar compounds such as

Table 3
Analytical parameters of the studied compounds: equation, lineal dynamic range, regression coefficient, limits of detection and quantification.

Compounds	Equation	Linearity (r^2)	LOD (ng L^{-1})	LOQ (ng L^{-1})	LDR (ng L^{-1})
Acenaphthylene	$y=33.97x+3.18$	0.999	0.07	0.23	0.5–500
Alachlor	$y=3.04x+0.87$	0.998	0.81	2.69	2.5–500
Aldrin	$y=0.10x+0.002$	0.998	0.10	0.33	2.5–500
Ametryn	$y=1.92x+0.48$	0.997	0.83	2.78	0.5–500
Anthracene	$y=57.36x+9.84$	0.998	0.30	1.00	0.5–500
Atrazine	$y=1.21x+0.24$	0.997	1.36	4.55	2.5–500
Atrazine desethyl	$y=1.32x+0.21$	0.998	0.35	1.17	2.5–500
Benzo(a)anthracene	$y=9.51x+0.86$	0.999	0.20	0.67	0.5–500
Benzo(a)pyrene	$y=9.05x+0.39$	0.999	0.06	0.20	0.5–500
Benzo(b)fluoranthene	$y=10.33x+0.54$	0.999	0.17	0.58	2.5–500
Benzo(ghi)perylene	$y=10.27x+0.59$	0.998	0.13	0.42	0.5–500
Benzo(k)fluoranthene	$y=10.75x+0.62$	0.998	0.09	0.30	2.5–500
Chlorfenvinphos A	$y=0.13x+0.01$	0.999	0.66	2.19	2.5–500
Chlorfenvinphos B	$y=1.08x+0.07$	0.999	0.96	3.21	0.5–500
Chlorotoluron	$y=1.36x+0.0008$	0.998	0.38	1.25	2.5–500
Chlorpyrifos ethyl	$y=1.03x+0.21$	0.998	0.27	0.90	0.5–500
Chrysene	$y=10.16x+1.37$	0.999	0.50	1.67	0.5–500
Deltamethrin	$y=0.29x+0.01$	0.998	0.82	2.75	2.5–500
Diazinon	$y=1.10x+0.71$	0.998	0.01	0.03	0.5–500
Dibenzo(a,h)anthracene	$y=8.64x+0.10$	0.999	1.01	3.38	2.5–500
4,4′-DDE	$y=0.38x+0.02$	0.999	0.33	1.12	0.5–500
4,4′-DDT	$y=1.32x+0.09$	0.999	0.18	0.59	0.5–500
Dieldrin	$y=0.05x+0.001$	0.996	0.10	0.34	5–500
Diuron	$y=0.94x+0.57$	0.998	0.10	0.33	0.5–500
α-Endosulfan	$y=0.07x+0.01$	0.999	0.27	0.89	5–500
β-Endosulfan	$y=0.02x+0.006$	0.998	0.09	0.29	50–500
Endosulfan sulfate	$y=0.04x+0.002$	0.998	0.23	0.78	3–500
Endrin	$y=0.06x+0.006$	0.998	1.50	5.00	2.5–500
Ethion	$y=0.80x+0.01$	0.999	1.50	5.00	0.5–500
Fluorene	$y=30.39x+3.05$	0.999	0.05	0.17	0.5–500
Heptachlor	$y=2.41x+0.31$	0.998	0.01	0.03	0.5–500
Hexachlorobenzene	$y=1.18x+0.07$	0.999	0.07	0.24	0.5–500
Hexachloro-1,3-butadiene	$y=1.41x+0.10$	0.999	0.09	0.29	0.5–500
α-HCH	$y=1.00x+0.08$	0.999	0.09	0.28	0.5–500
β-HCH	$y=1.59x+0.13$	0.999	0.16	0.55	2.5–500
δ-HCH	$y=1.02x+0.09$	0.999	0.11	0.37	0.5–500
γ-HCH	$y=0.71x+0.32$	0.997	0.14	0.46	0.5–500
Indene(1,2,3-cd)pyrene	$y=8.73x+0.10$	0.998	0.11	0.36	2–500
Iprodione	$y=0.04x+0.01$	0.998	0.07	0.25	3.5–500
Isodrin	$y=0.03x+0.002$	0.998	0.60	1.98	2.5–500
Isoproturon	$y=0.71x+0.11$	0.993	0.67	2.23	2.5–500
Metoxychlor	$y=0.82x+0.10$	0.998	0.07	0.23	0.5–500
Oxyfluorfen	$y=0.09x+0.005$	0.999	0.11	0.37	0.5–500
Parathion ethyl	$y=0.80x+0.04$	0.999	0.82	2.72	2.5–500
Parathion methyl	$y=1.06x+0.04$	0.998	0.75	2.50	2.5–500
Pentachlorobenzene	$y=1.00x+0.09$	0.999	0.01	0.03	0.5–500
Phenanthrene	$y=58.71x+16.16$	0.998	0.28	0.94	0.5–500
Procymidone	$y=0.39x+0.02$	0.998	0.90	3.01	0.5–500
Propazine	$y=1.26x+0.19$	0.998	0.95	3.16	2.5–500
Pyrene	$y=10.10x+6.78$	0.997	0.13	0.45	0.5–500
Simazine	$y=0.94x+0.22$	0.999	1.53	5.10	0.5–500
Terbutryn	$y=2.42x+0.83$	0.997	0.42	1.41	0.5–500
Terbuthylazine	$y=1.33x+0.77$	0.999	0.60	2.00	0.5–500
1,2,3-TCB	$y=0.94x+0.02$	0.999	0.51	1.70	0.5–500
1,2,4-TCB	$y=0.98x+0.05$	0.999	0.67	2.23	0.5–500
1,3,5-TCB	$y=1.03x+0.05$	0.999	0.53	1.77	0.5–500
Trifluralin	$y=3.05x+0.29$	0.999	0.04	0.15	0.5–500

diuron, isoproturon, chlortoluron, or desethyl atrazine, whose analyses fit better with LC-MS. The idea of the proposed GC–MS/MS method was to explore the potential use of the selected technique for a suite of representative multiclass compounds. For a comprehensive evaluation of the total amount of priority and emerging contaminants, the combination of GC–MS and LC–MS is mandatory. However, we focused our efforts in the highest number of compounds included in EU regulations [6,7] -amongst them atrazine diuron isoproturon and simazine- whose analysis could be also suitable by GC–MS. For these relatively polar species, LC-MS may eventually be more convenient, but however those more apolar species such as PAHs or organochlorinated species must be tested with GC–MS. Hence, in this case dealing with priority substances targeted in this work, if only one technique should be selected, GC–MS should be the choice.

The accuracy and precision of the method was estimated by means of recovery experiments, analyzing wastewater sample fortified ($n=7$) at two concentration levels, 15 ng L^{-1} and 150 ng L^{-1}. The results are detailed in Table 4, showing recoveries in the range 70–120% for about 60% of the studied analytes (with some differences between concentration levels). Particularly outstanding is the fact that all studied PAHs showed good recoveries, taking into account that these chemicals tend to accumulate in the suspended solids.

Table 4
Average recovery (%) and R.S.D. (in parenthesis) after the application of the GC-(EI)MS/MS procedure to wastewater sample fortified ($n=7$) at two concentration levels, 15 and 150 ng L^{-1}.

Compounds	Fortification levels and relative standard deviation			
	15 ng L^{-1}	R.S.D. (%)	150 ng L^{-1}	R.S.D. (%)
Acenaphthylene	102	4	88	7
Alachlor	137	3	93	12
Aldrin	102	7	62	18
Ametryn	47	19	33	12
Anthracene	141	4	84	8
Atrazine	74	6	54	10
Atrazine desethyl	52	17	44	20
Benzo(a)anthracene	85	7	62	16
Benzo(a)pyrene	71	7	57	15
Benzo(b)fluoranthene	62	7	64	20
Benzo(ghi)perylene	132	2	52	7
Benzo(k)fluoranthene	63	7	64	15
Chlorfenvinphos A	154	9	69	8
Chlorfenvinphos B	100	6	97	9
Chlorotoluron	55	7	20	17
Chlorpyrifos ethyl	105	16	74	18
Chrysene	99	6	86	13
Deltamethrin	–	–	32	22
Diazinon	112	5	90	10
Dibenzo(a,h)anthracene	77	4	54	12
4,4′-DDE	104	7	69	19
4,4′-DDT	94	9	69	18
Dieldrin	89	8	76	7
Diuron	54	13	17	21
α-Endosulfan	175	7	86	9
β-Endosulfan	120	9	75	10
Endosulfan sulfate	111	6	69	18
Endrin	99	14	81	12
Ethion	74	5	67	22
Fluorene	123	3	79	13
Heptachlor	115	6	67	12
Hexachlorobenzene	96	3	74	12
Hexachloro-1,3-butadiene	52	15	32	19
α-HCH	107	6	93	5
β-HCH	164	8	113	15
δ-HCH	95	11	101	12
γ-HCH	134	4	94	13
Indene(1,2,3-cd)pyrene	76	5	54	12
Iprodione	52	14	56	8
Isodrin	104	5	73	12
Isoproturon	42	8	30	14
Metoxychlor	94	1	79	18
Oxyfluorfen	142	7	90	18
Parathion ethyl	112	5	101	8
Parathion methyl	102	4	102	10
Pentachlorobenzene	105	7	73	9
Phenanthrene	108	6	75	14
Procymidone	125	5	84	9
Propazine	120	3	82	7
Pyrene	43	20	71	13
Simazine	–	–	25	15
Terbuthylazine	139	4	89	9
Terbutryn	104	5	89	15
1,2,3, TCB	76	11	52	10
1,2,4, TCB	88	10	46	9
1,3,5, TCB	77	8	43	16
Trifluralin	103	5	83	1

3.4. Application to analysis of real (STP) samples

The optimized MRM procedure was applied to the analysis of 33 wastewater samples of urban origin, collected as previously described (see Section 2.2) at different months from 3 STP located in the North (N-STP), North-East (NE-STP) and South-East (SE-STP) of Spain (see Fig. 1). Results are summarized in Table 5.

Fourteen of the studied compounds were found in more than 50% of the analyzed samples. Amongst them, the sum of hexachlorocyclohexane (HCH) isomers and the sum of benzo(ghi)perylene and indeno(1,2,3-cd)pyrene are identified as priority hazardous substances and environmental quality standards (EQS) have been established in the European Union regarding the concentration of these hazardous contaminants in surface waters [7]. Besides, priority hazardous compounds, EQS have also been set for pesticides as chlorpyrifos ethyl and diuron, frequently detected in the present survey. Chlorpyrifos ethyl was found in all analyzed samples, and except in the case of effluents of the secondary treatment stage of the SE-STP, the average concentration level of this compound was below its EU annual EQS, established at 30 ng L^{-1} for inland surface waters [7]. With regards to effluents of the secondary treatment stage of the SE-STP, samples collected from March to May contained concentrations of chlorpyrifos ethyl between 1.6 and 5.6 times higher than the maximum allowable concentration for this compound, established at 100 ng L^{-1} [7]. Therefore, the necessity of tertiary treatment to keep the contaminants at low levels is pointed out. Similarly, benzo(ghi)perylene and indeno(1,2,3-cd)pyrene, identified as priority hazardous compounds, were frequently detected at concentration levels below the 2 ng L^{-1} EU EQS, except in the case of effluents of the secondary treatment stage of the N-STP, enforcing the necessity of tertiary treatment to keep the contaminants at low levels. As an example, Fig. 2 shows the positive findings of chlorpyrifos ethyl and pyrene in a wastewater effluent of the secondary treatment of the NE-STP.

Lindane (γ–HCH isomer) was detected in the 97% of the analyzed effluent samples and diuron was found in the 82% of the studied samples. The average value of the sum of hexachlorocyclohexane isomers individual concentrations and the average concentration level of diuron were below their corresponding EU environmental quality standards, set at 20 ng L^{-1} and 200 ng L^{-1}, respectively. Hexachlorobenzene was detected in the 35% of the studied samples, corresponding mainly to the N-STP, while it was detected in the 98% of the samples in a previous study [10] comprising samples from 5 STPs. Interestingly, fluorene, phenanthrene and pyrene were frequently detected and several samples presented concentration levels of these compounds higher than 40 ng L^{-1}, even higher than 100 ng L^{-1}, but no EQS have been set for these compounds.

Twenty six compounds were not detected in the studied samples. In this group are included priority compounds as anthracene (the only one not detected amongst the studied PAHs), cyclodiene pesticides, endosulfan, simazine, atrazine, or δ-HCH.

4. Concluding remarks

In this study, an analytical method based on liquid–liquid extraction followed by GC–MS/MS has been developed for the detection of 57 priority organic contaminants in wastewater samples. The proposed LLE method, which comprises the analysis of both liquid phase and suspended solids of wastewater samples—as required by the European WFD—, used n-hexane as it was found the more appropriate solvent for the extraction of such variety of species. This LLE step was followed by determination with the proposed GC–MS/MS method, which was found to provide the excellent selectivity and sensitivity attained, enabling the unambiguous identification of the target species by the determination of three MRM transitions (when available). It should be also emphasized the satisfactory performance achieved with GC–MS/MS for relatively polar compounds such as diuron, isoproturon, chlortoluron, or desethyl atrazine, whose analyses fit

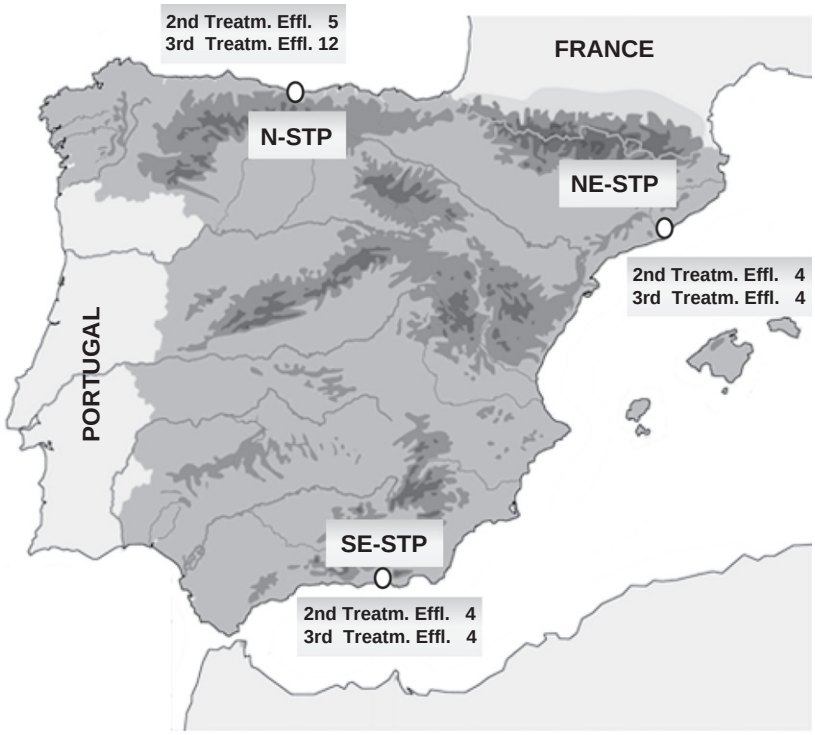

Fig. 1. Geographical location of the monitored sewage treatment plants, including the number of effluents of the secondary and tertiary treatments collected, respectively.

Table 5
Results from the analyses of wastewater samples. Percentage of positive samples and concentration ranges of the most frequently detected analytes.

Compound	Secondary treatment STP effluents (13 samples)			Tertiary treatment STP effluents (20 samples)			Priority hazardous substances regulation [7]	
	% positive samples	Concentration range (ng L^{-1})	Average concentration (ng L^{-1})	% positive samples	Concentration range (ng L^{-1})	Average oncentration (ng L^{-1})	AA-EQS[a](ng L^{-1})	MAC-EQS[b](ng L^{-1})
Acenaphthylene	76.9	1.0–89.1	13.1	70.0	1.1–7.9	2.7	–	–
Benzo[a]anthracene	76.9	0.3–15.1	5.7	45.0	0.3–2.7	1.1	–	–
Benzo[ghi]perylene	84.6	0.3–6.9	2.4	55.0	0.2–2.9	1.0	Σ=2	Σ=2
Indeno[1–3]–cd pyrene	38.5	0.4–8.0	2.6	25.0	0.4–2.2	1.1		
Chlorpyrifos ethyl	100.0	4.7–590.0	87.2	100.0	0.6–50.3	11.9	30	100
Diazinon	61.5	1.5–92.6	27.9	70.0	1.1–43.4	9.0	–	–
Diuron	92.3	0.2–68.5	15.6	80.0	1.1–46.1	11.1	200	1800
Fluorene	92.3	1.2–80.7	21.0	90.0	0.7–17.6	4.5	–	–
Hexachlorobenzene	23.1	0.4–1.8	0.9	40.0	0.2–3.3	1.2	10	50
α-HCH	23.1	0.2–0.8	0.5	35.0	0.1–0.7	0.4	Σ=20	Σ=40
β-HCH	15.4	0.8–1.3	1.1	10.0	0.4–1.4	0.9		
γ-HCH	92.3	1.8–29.1	7.4	100.0	0.8–37.3	7.1		
Pentachlorobenzene	0.0	–	–	0.0	–	–	7	–
Phenanthrene	92.3	2.6–141.0	35.0	80.0	1.4–41.0	11.1	–	–
Procymidone	46.2	1.2–27.5	7.9	65.0	0.8–15.4	6.6	–	–
Pyrene	100.0	4.9–73.9	27.2	75.0	0.2–121.4	23.2	–	–
Terbutryn	30.8	4.4–46.8	20.1	85.0	0.6–19.0	10.0	–	–

[a] AA-EQS: Environmental Quality Standard established for the annual average concentration of the corresponding contaminant in inland surface waters. Directive 2008/105/EC.
[b] MAC-EQS: Environmental Quality Standard established for the maximum allowable concentration of the corresponding contaminant in inland surface waters. Directive 2008/105/EC.

better with LC–MS. In contrast, for PAHs only two transitions were selected due to their difficulty to yield useful fragmentation. Most of PAHs were quantified using, respectively, the same ion as precursor and as product ion. In particular, the identification of isomers phenanthrene–anthracene, and benzo(b)fluoranthene–benzo(k)fluoranthene, which used the same MS/MS transitions, relied on the chromatographic separation. Finally, the results obtained from the monitoring study of these organic contaminants in wastewater samples as provided by the developed procedure, demonstrated their ubiquitous presence which emphasizes the need to control such group of contaminants. Thus, it has been demonstrated that the proposed GC–MS/MS method for multiclass organic pollutants is useful in order to know concentration levels of pollutants in waste water sewage treatment plant effluents, and accordingly to proceed with appropriate tertiary treatments when necessary.

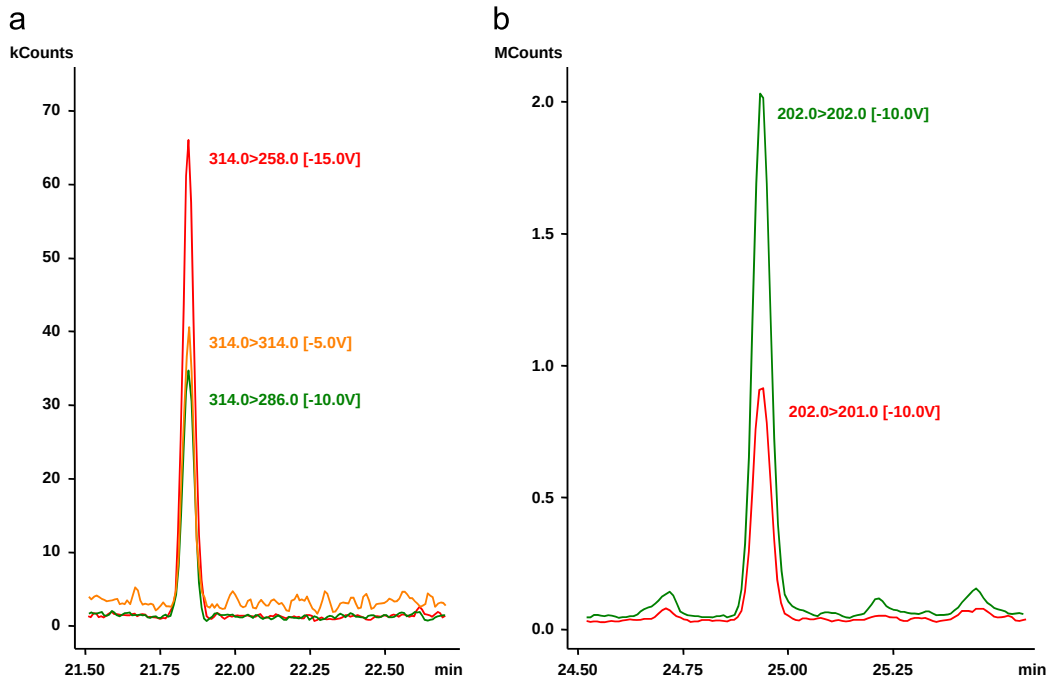

Fig. 2. Positive findings of (a) chlorpyrifos ethyl (6 ng L^{-1}) and (b) pyrene (12.4 ng L^{-1}) in an effluent wastewater sample corresponding to the secondary treatment stage of the STP located in the North-East of Spain.

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