Journal of Chromatography A, 1217 (2010) 1476–1484

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Mixed-mode solid-phase extraction followed by liquid chromatography–tandem mass spectrometry for the determination of tri- and di-substituted organophosphorus species in water samples

M. García-López, I. Rodríguez*, R. Cela

Departamento de Química Analítica, Nutrición y Bromatología, Instituto de Investigación y Análisis Alimentarios, Universidad de Santiago de Compostela, Santiago de Compostela 15782, Spain

ARTICLE INFO

Article history:
Received 19 October 2009
Received in revised form
15 December 2009
Accepted 23 December 2009
Available online 4 January 2010

Keywords:
Organophosphorus compounds
Flame retardants
Wastewater
Mixed-mode solid-phase extraction
LC–MS

ABSTRACT

A procedure for the determination of three phosphoric acid diesters, eight triesters and triphenylphosphine oxide (TPPO) in water samples is presented. Analytes were simultaneously concentrated using a mixed-mode (reversed-phase and anionic-exchange) solid-phase extraction (SPE) sorbent and then sequentially eluted with methanol (triesters and TPPO) followed by a 20 mM tetrabutylammonium hydrogen sulphate (TBAHS) methanolic solution, case of diesters. After that, they were determined, in two different runs, by liquid chromatography–electrospray ionization–tandem mass spectrometry (LC–ESI–MS/MS), operating the ESI source in the positive (triesters and TPPO) and negative (diesters) ionization modes. The efficiency of the extraction step varied between 70 and 105%, except in the case of tris(2-ethylhexyl) phosphate (TEHP), and it was barely affected by the type of water sample. Moreover, low signal suppression effects were noticed in the ESI ionization of extracts obtained from different environmental water samples. As a result, the standard addition methodology was only required for the accurate quantification of tri-substituted organophosphorus (OPs) species in wastewater samples. Limits of quantification of the optimized method ranged from 0.2 to 10 ng L^{-1}, depending on the sample matrix and the considered compound. The analysis of river and wastewater samples confirmed the occurrence of several tri- and di-substituted OPs in the aquatic environment, with the highest concentrations corresponding to tris(butoxyethyl) phosphate (TBEP) and tris(chloropropyl) phosphate (TCPP).

1. Introduction

Tri-substituted organophosphorus (OPs) compounds are employed as plasticizers and flame retardant additives in textiles, wallpapers, varnishes and polymeric materials. In most cases, these species are not chemically bonded to the host materials; therefore, they can be easily emitted to the surrounding areas [1]. As a result, several OPs have been found in different environmental matrices [2], such as air [3], dust [4] and wastewater [5]. The presence of tri-substituted OPs in the latter compartment is a matter of concern because of the high mobility and poor removal rates of the most polar species, particularly tris(2-chloroethyl) phosphate (TCEP) and tris(2-chloroisopropyl) phosphate (TCPP), which pass through conventional urban wastewater treatment plants without undergoing significant removal rates [5,6].

Phosphoric acid diesters, particularly dibutyl phosphate (DBP) and diethylhexyl phosphate (DEHP), are often employed as extractants of radioactive elements [7,8]. Apart of being intentionally produced, diesters are also formed as biodegradation intermediates and metabolites [9,10] of triesters. In addition, DBP and monobutyl phosphate (MBP) might be the decomposition products of tributyl phosphate (TBP) when used as extractant in the nuclear industry [11].

During last years, several studies have addressed the determination of OPs triesters in water samples using different sample preparation approaches and mainly gas chromatography (GC) as separation technique. GC combined with nitrogen-phosphorus detection (NPD) [12,13], mass spectrometry using electron impact (EI-MS) [14] or positive chemical ionization (PCI-MS) [15], atomic emission detection (AED) [16] or inductively coupled plasma (ICP)-MS [17,18] have been used for tri-substituted OPs determination. However, none of these techniques alone meets the requirements of high selectivity, sensitivity and affordable cost. On the other hand, triesters are also amenable to liquid chromatography (LC) determination [19,20]; multiple reaction monitoring (MRM) tandem mass spectrometry (MS/MS) detection combines low limits of quantification (LOQs) and a high degree of selectivity. In addition, LC–MS/MS allows the analysis of polar molecules such as phosphoric acid diesters [21] that can only be subjected to GC analysis if derivatized [22], with all the disadvantages that entail. Up to this

* Corresponding author. Tel.: +34 981 563100x14387; fax: +34 981 595012.
E-mail address: isaac.rodriguez@usc.es (I. Rodríguez).

0021-9673/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2009.12.067

date, only a few works have dealt with the analysis of organophosphate diesters and, bearing in mind the above information, LC–MS has been the preferred choice [23–26].

Triesters extraction from water samples has been accomplished with liquid–liquid extraction (LLE) [27] or solid-phase extraction (SPE) [20] but also with microextraction approaches such as solid-phase microextraction (SPME) [28], dispersive liquid–liquid microextraction (DLLME) [29] or microporous membrane liquid–liquid extraction (MMLLE) [30]. Nevertheless, in the few papers reporting diesters extraction from water [25,26] or urine [22,24] samples, SPE has been the selected technique. Quintana et al. determined phosphoric acid diesters in wastewater samples utilizing ion-pair solid-phase extraction (IP-SPE) with tributylamine (TrBA) through Lichrolut RP18 cartridges [25]. Möller et al. [23,24] developed a molecularly imprinted solid-phase extraction (MISPE) method for extracting diphenyl phosphate (DPP) from urine samples and compared its performance with several commercially available SPE sorbents, showing that Oasis MAX cartridges were also suitable for DPP extraction, providing higher recoveries than the molecularly imprinted polymer (MIP) but lower selectivity. Finally, Shindler et al. [22] determined some diesters in urine samples by means of SPE, with Isolute Env+, followed by a derivatization step and a further clean-up prior to the determination by GC–MS/MS.

To the best of our knowledge, up to the moment, there is only one work where some triesters and diesters have been simultaneously extracted [26]. They were included in a multi-residue method for the determination of emerging organic pollutants in water samples using SPE with Oasis HLB. After elution of the SPE cartridge with methanol, OPs were determined in two different runs considering reversed-phase (triesters) and ion-pairing (diesters) LC separation mechanisms. Two different LC columns using different modifiers in the mobile phase were also employed [26]. Unfortunately, in the above work, a detailed evaluation of potential matrix effects during electrospray ionisation (ESI) of tri- and di-substituted OPs is missed.

The aim of the present investigation was to develop an improved methodology for the determination of tri- and di-substituted OPs in water samples using SPE and LC–ESI–MS/MS as extraction and determination techniques, respectively. Efforts were mainly focussed on (1) increasing the selectivity of the extraction step, which resulted in lower signal suppression effects during ESI and (2) developing common LC separation conditions for both families of OPs, allowing the use of the same column for their determination. The effects of different factors on the efficiency of both steps are thoroughly evaluated and the performance of the method evaluated using environmental water samples with different complexities.

2. Experimental

2.1. Reagents, standards and material

HPLC-grade methanol and ammonia, 25% aqueous solution, were purchased from Merck (Darmstadt, Germany). Triethylamine (TEA) and tetrabutylammonium hydrogen sulphate (TBAHS) were provided by Aldrich (Steinheim, Germany), whereas acetic and formic acid were supplied by Riedel-de Haën (Seelze, Germany). Ultrapure water was obtained from a Milli-Q (Millipore, Bedford, MA, USA) system. Tripropyl phosphate (TPrP), TBP, triisobutyl phosphate (TiBP), TCEP, tris(1,3-dichloroisopropyl) phosphate (TDCP), tris(butoxyethyl) phosphate (TBEP), triphenyl phosphate (TPP), tris(2-ethylhexyl) phosphate (TEHP), triphenyl phosphine oxide (TPPO), DPP and DEHP were acquired from Aldrich (Milwaukee, WI, USA). TCPP, as a technical mixture of isomers, was provided by Dr. Ehrenstorfer (Augsburg, Germany) and DBP was obtained from Fluka (Steinheim, Germany). Chemical structures and some relevant properties of the above compounds have been compiled in a recent review [2]. Individual standards and mixtures of tri- and di-substituted OPs were prepared in methanol and in 20 mM TBAHS in methanol, respectively.

Oasis HLB (60 mg) and MAX (60 and 150 mg) SPE cartridges were obtained from Waters (Milford, MA, USA).

2.2. Samples

Ultrapure water, river water and urban wastewater, obtained from a sewage plant equipped with primary and secondary (activated sludge) treatment units, were employed throughout this study. Samples, except ultrapure water, were passed through a combination of glass fiber pre-filters and 0.45 μm pore size nitrocellulose filters (Millipore), both 47 mm in diameter, and concentrated immediately after arriving to the laboratory.

2.3. Solid-phase extraction

2.3.1. Breakthrough and elution studies

Optimization of SPE conditions was performed with aliquots (100–500 mL) of different environmental water samples, fortified with target compounds and passed through the considered SPE sorbent (ca. 10 mL min^{-1}). Breakthrough volumes were evaluated by passing the spiked samples through two cartridges connected in series. After the extraction step, they were disconnected and processed independently. Elution volumes were determined by collecting consecutive 1 mL fractions of the SPE cartridge eluate.

2.3.2. Sample extraction conditions

Under final conditions, 500 mL of river water, or 100 mL in the case of wastewater, were concentrated using the mixed-mode Oasis MAX sorbent (150 mg), previously conditioned with a 20 mM methanolic solution of TBAHS, methanol and water, 5 mL each. After finishing the extraction step, the SPE cartridge was rinsed with 10 mL of ultrapure water and then dried under nitrogen stream. Analytes were eluted in two different fractions using 2 mL of methanol (triesters and TPPO) followed by 3 mL of a 20 mM TBAHS methanolic solution (diesters). Finally, extracts were concentrated with a gentle stream of nitrogen and made up to 1 mL using methanol or 20 mM TBAHS in methanol for triesters and diesters, respectively.

2.4. Liquid chromatography–mass spectrometry

Compounds were determined by LC–MS/MS using a Varian (Walnut Creek, CA, USA) system. The LC instrument comprised two ProStar 210 high-pressure mixing pumps (Varian), a vacuum membrane degasser (Metachem Technologies, Bath, UK) and a ProStar 410 module (Varian) consisting of an autosampler and a thermostated compartment for the LC column. The LC system was interfaced to a triple-quadrupole 1200 L mass spectrometer fitted with a ESI source (Varian).

Compounds were separated using a Luna C18 column (100 mm × 2 mm; 3 μm), acquired from Phenomenex (Torrance, CA, USA), and connected to a C18 (4 mm × 2 mm) guard cartridge from the same supplier. Five millimolar ammonium acetate in ultrapure (Milli-Q) water (A) and in methanol (B) were used as mobile phases for the separation of both groups (tri- and di-substituted) of OPs, which were determined in two different runs operating the ESI source in the negative (di-) and positive (tri-substituted species) ionization modes. For the former group of compounds the optimized gradient was: 0–2 min, 50% B; 17–22 min, 100% B; 23–27 min, 50% B. Regarding triesters and TPPO,

the composition of the mobile phase was varied accordingly to the following program: 0–2 min, 50% B; 16–30 min, 100% B; 32–35 min, 50% B. In both cases, the mobile phase flow was set at 0.2 mL min^{-1} and the column maintained at 30 °C. The injection volume for standards and sample extracts was 10 μL, filling the remaining free space in the loop (total volume 100 μL) with ultrapure water. This injection mode avoids broadening of the injection band without dilution of methanolic extracts, from SPE cartridges, previously to their injection in the LC system.

Nitrogen (99.999%), used as nebulising (50 psi) and drying gas (200 °C, 20 psi) in the ESI source, was provided by a high purity generator (Domnick Hunter, Durham, UK). The temperature of the ESI housing was maintained at 50 °C and the voltage of the ESI needle fixed at 5000 V and 4500 V in the positive and negative ionization modes, respectively. Argon (99.999%) was employed as collision gas (2.9×10^{-6} psi) for MS/MS measurements.

Search for the most intense MS/MS transitions was performed by infusion of individual standards of each OPs (ca. 2 μg mL^{-1} in methanol:water, 1:1) at a constant flow of 20 μL min^{-1}, in the positive and negative ionization modes for tri- and diesters, respectively. Firstly, the intensity of the signal for each parent ion, corresponding to protonated ($[M+1]^+$) or de-protonated ($[M-1]^-$) species, was optimized changing the capillary voltage. After that, they were subjected to collision induced dissociation (CID), optimizing the collision energy to maximize the responses of product ions. Two transitions were recorded per compound: the most intense one was used to quantify the response of each species and the other transition was employed for qualifying purposes.

2.5. Yields of SPE, matrix effects and overall recoveries

The yield of the SPE process was assessed by comparison of signals attained for samples fortified before and after extraction. In this way, the obtained responses remained unaffected by potential changes in the efficiency of the ESI ionization, depending on the complexity of SPE extracts. On the other hand, potential matrix effects related to changes in the efficiency of the ESI ionization were evaluated through a series of experiments as reported in other works [31,32]. In brief, responses (peak areas) obtained for extracts from real water samples, spiked after the SPE extraction step, were compared with those measured for a standard with the same concentration, prepared in methanol (triesters and TPPO) or in 20 mM TBAHS methanolic solution (diesters). Non-spiked aliquots of same samples were also processed in order to compensate for the presence of target species in environmental matrices.

Overall recoveries for the whole method, accounting for the efficiency of the SPE extraction and matrix effects, were evaluated using external calibration as quantification technique. The difference between peak areas obtained for spiked (before extraction) and un-spiked samples were compared with those measured for standards.

2.6. Method performance

Figures of merit corresponding to the LC–MS/MS determination step and the proposed procedure (SPE followed by LC–MS/MS) were estimated with pure standards and environmental water samples fortified at different concentration levels. The linearity in the response of the LC–MS/MS system was investigated by injection of standard mixtures at eight different concentration levels from 2 to 1000 ng mL^{-1}. Instrumental limits of quantification (LOQs) were calculated as the concentration of each species giving a signal 10 times the standard deviation of the background noise in the LC–MS/MS chromatogram. LOQs of the proposed procedure were evaluated taking into account (1) the instrumental LOQs, (2) the enrichment factor provided by the SPE procedure for each matrix (500 and 100 mL for river and wastewater, respectively) and (3) the presence of compounds in procedural blanks corresponding to the concentration of 500 mL aliquots of ultrapure water.

3. Results and discussion

3.1. Optimization of LC–ESI–MS/MS conditions

Table 1 summarizes the ionization modes (positive or negative), capillary voltages, collision energies and the two most intense transitions for each species with their relative intensities. In the case of alkylated OPs, reported transitions corresponded to the replacement of alkylated moieties by hydrogen, or their elimination as neutral fragments, rendering as later products the $[H_4PO_4]^+$ or $[PO_3]^-$ ions, for tri- and di-substituted species, respectively. The most intense transitions in the MS/MS spectra of TPPO also reflected the elimination of an aromatic ring (279 > 201) or its replacement by a hydroxyl substituent (279 > 219). The $[M-1]^-$ parent ion corresponding to DPP underwent the loss of a phenolic group, as a neutral fragment (249 > 155) or as a negatively charged phenolate (249 > 93). Finally, TPP presented a more complex fragmentation pathway (Fig. 1), with rearrangements between phenolic moieties (327 > 168), followed by a further removal of oxygen (168 > 152). Another mechanism consisted of the replacement of a phenyl group by hydrogen (327 > 251) followed by two successive losses of water, Fig. 1.

On the basis of previous works [19,20,25,33] two mobile-phase additives, ammonium acetate (0–10 mM) and formic acid (0–0.1%), were considered in order to improve compounds separation and

Table 1
Optimized ESI–MS/MS parameters for the MRM determination of target compounds.

Compound	Capillary voltage (V)	MRM transitions (m/z)	Collision energy (eV)	Relative signal intensity (%)
ESI+				
TCEP	56	285 > 99	17	100
		285 > 223	7	91
TPPO	96	279 > 201	17.5	100
		279 > 219	14.5	6
TPrP	30	225 > 99	13.5	100
		225 > 141	7	44
TCPP	40	327 > 99	15	100
		329 > 99	15.5	98
TPP	96	327 > 152	23.5	100
		327 > 215	19	80
TDCP	72	431 > 99	15	100
		433 > 99	15.5	79
TiBP	30	267 > 99	11.5	100
		267 > 155	5.5	37
TBP	30	267 > 99	11.5	100
		267 > 155	5.5	38
TBEP	48	399 > 199	9	100
		399 > 299	7.5	91
TEHP	40	435 > 99	8	100
		435 > 323	5	19
ESI−				
DPP	−76	249 > 93	26	100
		249 > 155	20.5	12
DBP	−56	209 > 79	20	100
		209 > 153	13	58
DEHP	−30	321 > 79	25	100
		321 > 209	20	36

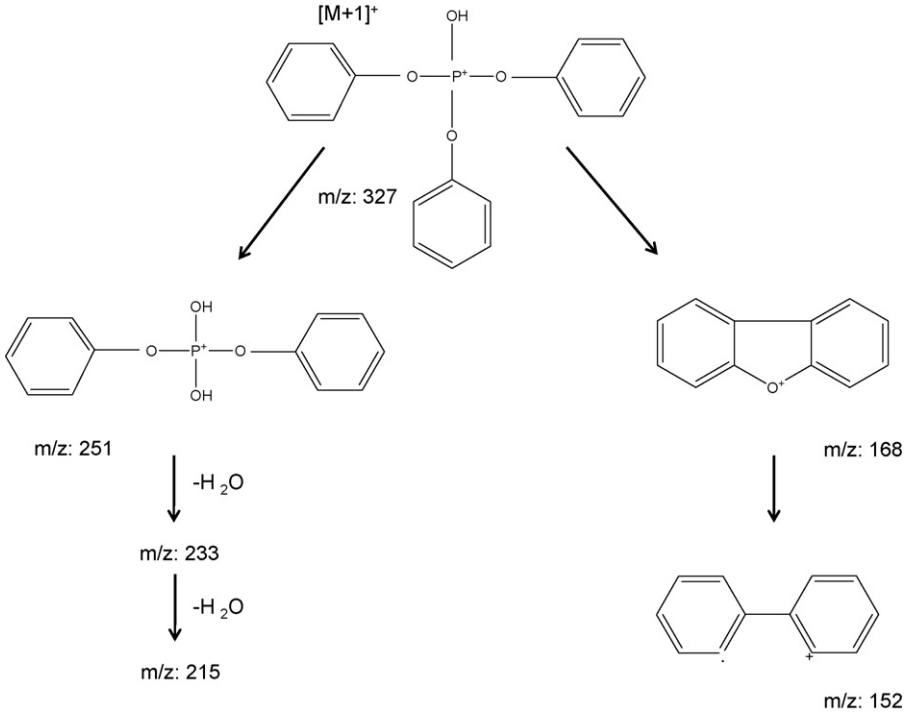

Fig. 1. Proposed MS/MS fragmentation pathways for TPP in the positive ionization mode.

the sensitivity of the method. The evaluation of their influence was performed by injection of a 0.5 μg mL^{-1} standard of each group of compounds (di- and tri-substituted ones). OPs diesters are acidic species with estimated pK_a values under 2 units [2]; moreover, two of the three species considered in this study (DBP and DPP) show log *K*ow values under 3 units, consequently they are expected to be poorly retained in reversed-phase LC column. In fact, in previous works tri-n-butylamine was added to the mobile phase as ion-pairing reagent to increase their affinity by the stationary phase of the column [25,26]. However, accordingly to our experiments, the three compounds were retained by the stationary phase of the LC column using just methanol and water, without any modifier, as mobile phases. Anyhow, broad peaks were noticed for all species. Addition of formic acid, at a 0.1% concentration, to the mobile phase (pH 2.7 units) led a slight reduction in the peak widths, without modifying the retention times of target species. Ammonium acetate produced a further reduction in peak widths of OP diesters. Moreover, it also shortened their retention times, Fig. 2 (chromatograms A and B). This latter effect is explained by the higher ionic strength of the mobile phase, which increases the solubility of the anionic diesters. Considering a 5 mM concentration of ammonium acetate, similar retention times were noticed for standards in methanol and those prepared in a 20 mM TBAHS methanolic solution, Fig. 2 (chromatograms B and C). As further described in this study, this salt was necessary to elute these species from the mixed-mode SPE sorbent.

As regards the tri-substituted OPs, none of the above modifiers showed a significant effect on the performance of their LC separation; therefore, ammonium acetate, the same modifier as for diesters, was selected as additive in the mobile phase. In general, increasing the amount of this salt (from 1 to 10 mM) caused a reduction in the efficiency of their ESI ionization, Fig. 3. Nevertheless, a 5 mM concentration was chosen in order to operate the LC column under similar conditions to those reported for diesters. Fig. 4 depicts the chromatograms corresponding to a standard of the ten tri-substituted OPs (100 ng mL^{-1} per compound), acquired under conditions reported in the experimental section. Although two pairs of compounds (TPrP-TCPP and TPP-TDCP) co-eluted, they can be quantified individually using their characteristic MRM transitions. On the other hand, a baseline separation was achieved between the isomeric tri-butyl phosphates, Fig. 4.

Table 2 summarizes the performance of LC–ESI–MS/MS system for target species using the above optimized conditions. The plot of peak areas versus the concentration of each compound fitted a linear trend with correlation coefficients (R^2) ranging from 0.991 to 1.000. Intra- and inter-day precision was evaluated at two concentration levels (25 and 100 ng mL^{-1}). The relative standard deviations (RSDs), obtained under reproducibility conditions, stayed below 9 and 8% for di- and tri-substituted compounds, respectively. Instrumental LOQs varied between 0.3

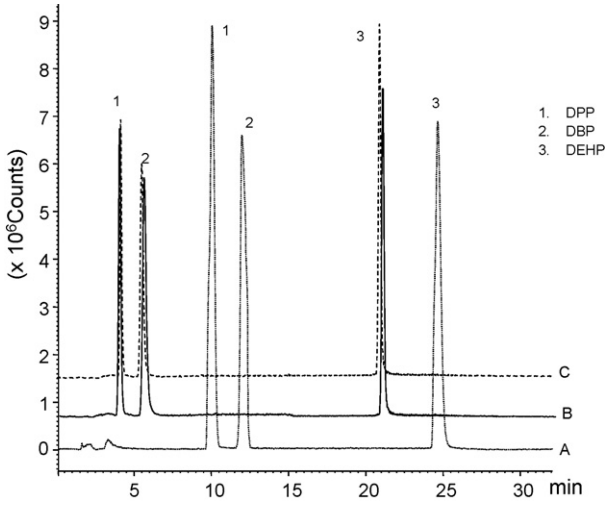

Fig. 2. Total ionic current (TIC) chromatograms for a standard of diesters (0.5 μg mL^{-1}, in methanol) using the gradient reported in the experimental section and different concentrations of ammonium acetate (NH_4AcO) as mobile phase modifier. (A) 1 mM (pH 5.5). (B) 5 mM (pH 5.3). (C) 5 mM, standard in a 20 mM methanolic solution of TBAHS.

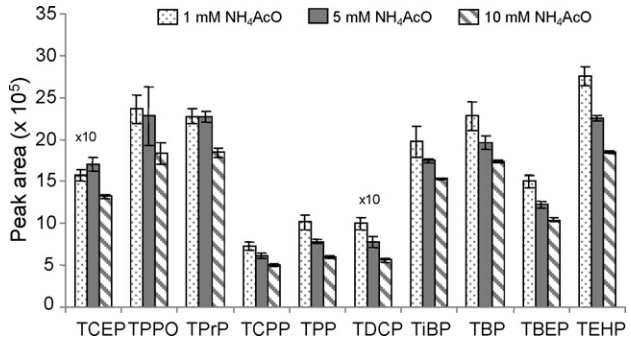

Fig. 3. Comparison of responses (average values with their standard deviations) obtained for a standard of tri-substituted OPs (0.5 μg mL^{-1}) as function of the concentration of ammonium acetate added to the mobile phase, $n = 3$ replicates.

and 1.0 ng mL^{-1} in the case of diesters, and they ranged from 0.1 to 1 ng mL^{-1} for the triesters. Overall, the above values are slightly lower than those reported by Martínez-Carballo et al. [33] for OPs triesters.

3.2. Optimization of solid-phase extraction conditions

The earlier series of experiments aiming to evaluate the performance of the SPE step were performed using aliquots of ultrapure water (from 50 to 500 mL), spiked with target compounds at 10 ng mL^{-1}. Breakthrough studies were carried out without acidification of the samples in order to limit the co-extraction of humic acids during analysis of environmental samples. Using the Oasis HLB sorbent (60 mg cartridges), the tri-substituted OPs and DEHP were retained quantitatively in the first cartridge; however, DBP and DPP showed a lower affinity by this reversed-phase polymer, appearing in the extract from the second cartridge, even when the sample intake was limited to 50 mL. On the other hand, considering the same mass of MAX sorbent, breakthrough volumes over 500 mL were achieved for all OPs, when dealing with aliquots of ultrapure water. The same behavior was observed for river water; however, when experiments were repeated using 250 mL of raw wastewater, around 10% of triesters passed to the second cartridge, whereas diesters remained quantitatively in the first one. Breakthrough studies were repeated with larger cartridges, containing 150 mg of the MAX sorbent. In this case, up to 250 mL of raw wastewater and 500 mL of river water could be concentrated without noticeable breakthrough problems for any compound.

HLB and MAX sorbents also showed different features as concerns the selectivity of the elution step. With the first polymer, all OPs were recovered with a small volume of methanol (2 mL in case of 60 mg cartridges). The same volume of methanol sufficed to elute the tri-substituted OPs from the mixed-mode sorbent; however, the diesters remained attached to the sorbent by ionic interactions. In order to favor the desorption of these latter compounds, methanol modified with formic acid (2%), TEA (20 mM), ammonium acetate (20 mM) and TBAHS (20 mM) were tested. The two latter salts resulted effective to break the electrostatic interactions between the anionic diesters and the positively charged groups in the sorbent. Using TBAHS, the three diesters were recovered with the first three fractions (1 mL each) of eluent. In the case of ammonium acetate, a considerable amount of DPP was still noticed in the 4th fraction; thus, TBAHS was selected as modifier. Taking above considerations into account water samples were concentrated using the 150 mg MAX cartridges. After drying the sorbent with a gentle stream of nitrogen, the triesters and TPPO were first eluted with 2 mL of methanol and then the diesters were recovered

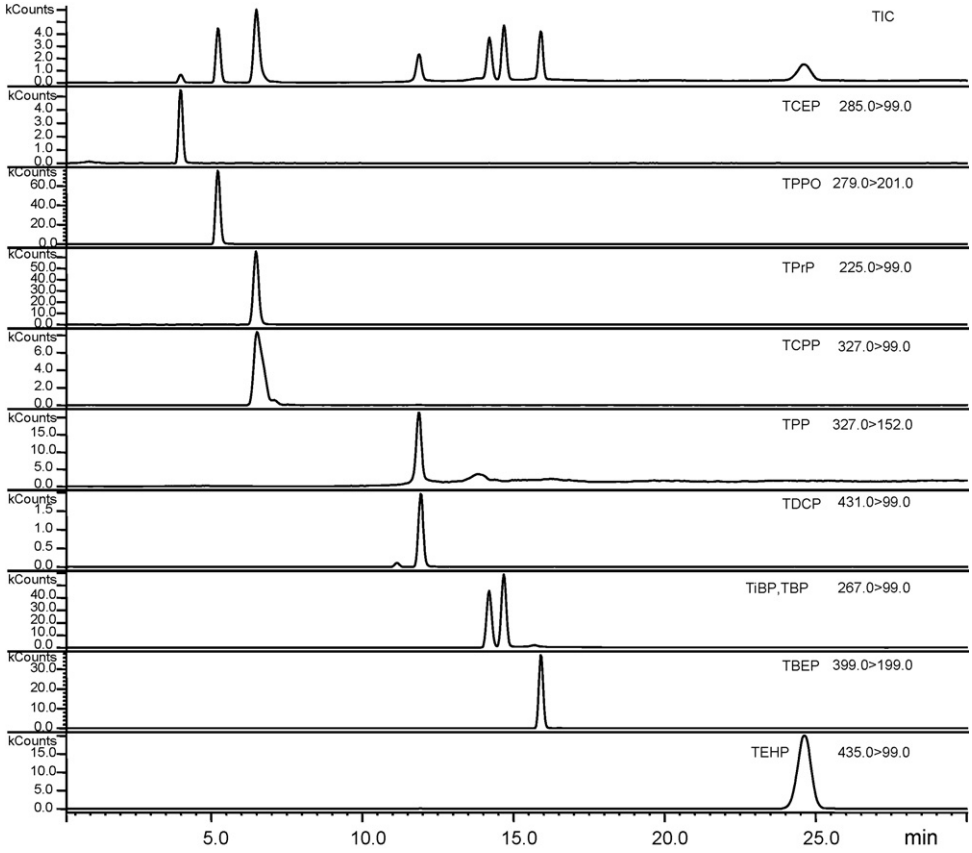

Fig. 4. TIC and MRM chromatograms for a standard of tri-substituted OPs (0.1 μg mL^{-1}) under optimized LC–ESI–MS/MS conditions.

Table 2
Linearity, inter- and intra-day precision and instrumental limits of quantification (LOQs) of the LC–ESI–MS/MS system.

Compound	Retention time (min)	Linearity, R^2 (2–1000 ng mL^{-1})	Precision (RSD%)			LOQs (ng mL^{-1})
			Intra-day (n = 5 replicates)		[a]Inter-day (n = 15 replicates)	
			25 ng mL^{-1}	100 ng mL^{-1}	100 ng mL^{-1}	
TCEP	4.13	0.996	7	2	8	0.4
TPPO	5.48	0.993	3	3	6	0.1
TPrP	6.93	0.992	2	2	6	0.1
TCPP	7.06	0.993	3	2	7	0.5
TPP	12.07	0.995	6	3	8	1.0
TDCP	12.14	0.998	7	4	8	0.3
TiBP	14.38	0.992	3	2	4	0.1
TBP	14.86	0.991	4	2	4	0.1
TBEP	16.08	0.993	4	3	4	0.1
TEHP	25.48	0.997	7	3	4	1.0
DPP	4.76	1.000	7	6	5	1.0
DBP	6.61	0.999	6	3	4	0.6
DEHP	21.74	1.000	3	5	9	0.3

[a] Injections were performed on 3 consecutive days.

with 3 mL of a 20 mM TBAHS solution in methanol. Both fractions were evaporated to 1 mL, and injected directly in the LC–MS/MS system.

Table 3 summarizes the efficiency of the SPE extraction for river (500 mL) and wastewater (100 mL) samples, spiked at two different concentrations. In general, the SPE process rendered recoveries from 70 to 105% for all compounds in the three tested matrices. The only exception was TEHP, which is a highly lipophilic compound (log *K*ow 9.49) with a strong trend to remain attached to glass material and PTFE pipes connecting the water sample with the SPE cartridge, which led to unsatisfactory recoveries. This problem can be overcome by rinsing these connections with methanol, which is further mixed with the fraction eluted from the SPE cartridge. The obvious drawback of the above strategy is that the volume of the final extract increases significantly, up to 20–30 mL [26]. Considering that, to the best of our knowledge, the occurrence of TEHP in environmental water samples has never been reported, it was preferred to exclude it from the list of target OPs than to raise the consumption of methanol in the SPE process. Globally, the above extraction efficiencies are similar to those reported for OP triesters in wastewater using the OASIS HLB sorbent [20] and diesters concentrated on C18, after formation of the corresponding ion-pairs in the aqueous sample [25]. The major advantage of the methodology optimized in this work is that both families of OPs are concentrated simultaneously and then fractionated during elution of SPE cartridges.

Table 3
Percentages of recovery (mean values with their standard deviations, SD) of the SPE step with MAX 150 mg cartridges for river (500 mL) and wastewater samples (100 mL), spiked at 1 and 5 ng mL^{-1}, respectively.

	Recovery % (SD)		
	River water	Treated wastewater	Raw wastewater
TCEP	84 (2)	82 (3)	70 (4)
TPPO	94 (2)	104 (2)	99 (3)
TPrP	87 (3)	97 (2)	92 (2)
TCPP	91 (7)	88 (2)	87 (2)
TPP	89 (5)	93 (3)	86 (2)
TDCP	86 (7)	89 (3)	80 (5)
TiBP	82 (2)	98 (3)	95 (3)
TBP	84 (2)	99 (2)	93 (2)
TBEP	75 (3)	94 (1)	96 (2)
TEHP	26 (8)	27 (2)	16 (4)
DPP	101 (5)	98 (2)	91 (4)
DBP	105 (6)	97 (2)	78 (5)
DEHP	94 (3)	91 (10)	82 (5)

3.3. Matrix effects and other figures of merit

A well-known shortcoming of LC–MS based methods, particularly when ESI sources are used, is that the yield of the ionization process might change significantly between standards and sample extracts. Thus, the presence of salts and other organic species in the extracts from SPE cartridges may produce signal enhancement or suppression effects. In previous works, signal attenuation effects up to 50 and 70% have been reported for the determination of tri- and di-substituted OPs, respectively, in raw wastewater samples concentrated 100 times over reversed-phase sorbents [20,25]. On one hand, such strong matrix effects increase the LOQs of the method; on the other one, the time-consuming standard addition method needs to be used for an accurate evaluation of analytes' levels in environmental water samples. Fig. 5 shows the responses obtained for spiked extracts from river and wastewater (concentrated 500 and 100 times, respectively) after normalization to a standard of the same concentration (0.5 μg mL^{-1}). Maximum signal suppression effects, around 35%, were noticed for a few triesters (e.g. TCEP), in the most complex of the investigated matrices: raw wastewater. For diesters, matrix effects were even less important, with a maximum of signal suppression around 20%. On the basis of these data, it appears that mixed-mode SPE cartridges provided more selective extractions than reversed-phase ones used in previous works [20,25].

The overall recoveries of the whole analytical procedure for tri-substituted OPs ranged from 47 to 109%, with standard deviations below 10%, Table 4. The lowest value corresponded to TCEP, the species showing the shorter retention time and the highest signal suppression effects during ESI ionisation (Fig. 5). Although for the rest of tri-substituted compounds the global efficiency of the sample preparation methodology remained over 65%, the standard addition method is recommended to assess their concentration in sewage water samples, whereas external calibration can be used when dealing with river water. For the three diesters involved in this study, global recoveries over 70%, with standard deviation values from 2 to 9%, were attained, Table 4. Consequently, external calibration was used to quantify their levels in river and wastewater samples.

Procedural blanks often showed traces of TiBP, TBP and TBEP (ca. 2–3 ng L^{-1}). Probably, these compounds arise from polymeric materials used in the purification water system. Moreover, the presence of TBEP in the blanks could be related with the high levels of this species in dust from indoor environments [4], and/or being due to the presence of this plasticizer in septa used to close the autosampler vials [28] or any other plastic connector used in the

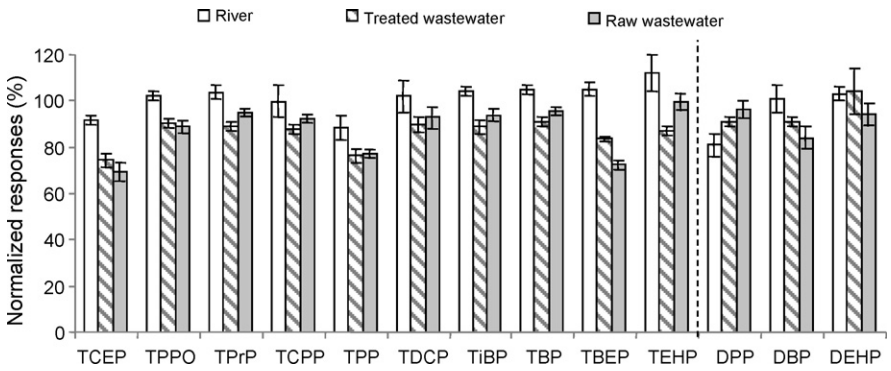

Fig. 5. Matrix effects evaluation. The differences between responses obtained for spiked and non-spiked extracts from river and wastewater samples (concentrated 500 and 100-folds, respectively) were normalized to those measured for a standard with the same concentration (0.5 μg mL^{-1}). Mean values with their standard deviations, $n = 3$ replicates.

Table 4
Overall recoveries (determined with external calibration) and estimated LOQs of the proposed method for river and wastewater samples.

	% Recovery with standard deviation, $n = 3$ replicates				LOQs (ng L^{-1})	
	[a]River water	[b]River water	[c]Treated wastewater	[c]Raw wastewater	River	Wastewater
TCEP	108 ± 6	77 ± 5	60 ± 8	47 ± 6	0.8	7
TPPO	89 ± 5	96 ± 3	94 ± 2	88 ± 3	0.2	1
TPrP	85 ± 1	90 ± 3	86 ± 2	88 ± 5	0.2	1
TCPP	86 ± 1	90 ± 2	86 ± 4	79 ± 8	1	5
TPP	85 ± 7	77 ± 6	70 ± 6	65 ± 8	2	10
TDCP	109 ± 8	88 ± 5	80 ± 7	74 ± 7	0.6	3
TiBP	85 ± 2	85 ± 6	87 ± 4	89 ± 5	10	10
TBP	88 ± 3	88 ± 5	90 ± 3	89 ± 4	10	10
TBEP	96 ± 3	78 ± 9	76 ± 7	67 ± 4	10	10
DPP	77 ± 8	86 ± 2	77 ± 4	83 ± 4	2	10
DBP	79 ± 5	73 ± 5	73 ± 9	70 ± 5	1	5
DEHP	79 ± 3	75 ± 7	90 ± 1	77 ± 6	0.7	4

[a] Addition level 0.1 ng mL^{-1}.
[b] Addition level 1 ng mL^{-1}.
[c] Additon level 5 ng mL^{-1}.

SPE process. Obviously, procedural blanks have to be run periodically in order to avoid the report of false positives during analysis of environmental water samples.

LOQs from 0.2 to 2 ng L^{-1} and ranging from 1 to 7 ng L^{-1} were achieved for those compounds not affected by contamination problems in river water and wastewater, respectively, Table 4. In the case of TiBP, TBP and TBEP a common LOQ of 10 ng L^{-1} was estimated on the basis of the above discussion related with procedural blanks.

3.4. Real samples analysis

The optimized methodology was applied to determine the levels of target species in grab samples corresponding to sewage and river water. The obtained concentrations are summarized in Table 5. Codes 1–3 correspond to pairs of samples simultaneously collected in the inlet and outlet of the same sewage plant, equipped with primary and activated sludge treatments, which receives the discharges from a 100,000-inhabitants city. The highest concentra-

Table 5
Concentrations of OPs (ng L^{-1}, with their standard deviations) in environmental water samples, $n = 3$ replicates.

Code	1	1	2	2	3	3	4	5	6	7
Sampling date	09/7/2009	09/7/2009	27/7/2009	27/7/2009	11/9/2009	11/9/2009	11/9/2009	27/7/2009	27/7/2009	27/7/2009
Type	Raw wastewater	Treated wastewater	Raw wastewater	Treated wastewater	Raw wastewater	Treated wastewater	River	River	River	River
TCEP	nd	61 ± 3	nd	nd	70 ± 10	210 ± 30	85 ± 5	nd	nd	nd
TPPO	17.2 ± 0.8	22.1 ± 0.4	5.6 ± 0.2	6.1 ± 0.5	13 ± 2	21 ± 2	13 ± 1	2.0 ± 0.2	2.8 ± 0.3	nd
TPrP	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
TCPP	290 ± 10	510 ± 30	401 ± 15	303 ± 24	540 ± 30	680 ± 50	430 ± 30	24 ± 3	64 ± 8	28 ± 3
TPP	nd	nd	nd	nd	nd	nd	nd	35 ± 4	18 ± 3	nd
TDCP	68 ± 2	103 ± 4	nd	nd	100 ± 10	140 ± 10	70 ± 10	nd	nd	nd
TiBP	188 ± 14	206 ± 9	39 ± 3	60 ± 5	115 ± 5	960 ± 60	89 ± 6	10 ± 1	11 ± 1	12 ± 2
TBP	82 ± 4	82 ± 3	nq	16 ± 1	47 ± 2	230 ± 20	50 ± 3	nq	nq	nq
TBEP	2200 ± 200	1300 ± 80	680 ± 50	1050 ± 20	3100 ± 100	3400 ± 200	2700 ± 400	nq	10 ± 1	34 ± 2
DPP	73 ± 2	137 ± 2	77 ± 4	97 ± 4	50 ± 20	105 ± 5	70 ± 2	7 ± 1	nd	9.6 ± 0.2
DBP	133 ± 6	137 ± 8	74 ± 7	84 ± 7	102 ± 7	82 ± 2	65 ± 6	8.0 ± 0.6	30 ± 1	8.6 ± 0.4
DEHP	200 ± 10	47 ± 8	120 ± 10	150 ± 20	201 ± 30	140 ± 20	92 ± 9	nq	14 ± 1	16.9 ± 0.4

nd, not detected; nq, under limit of quantification.

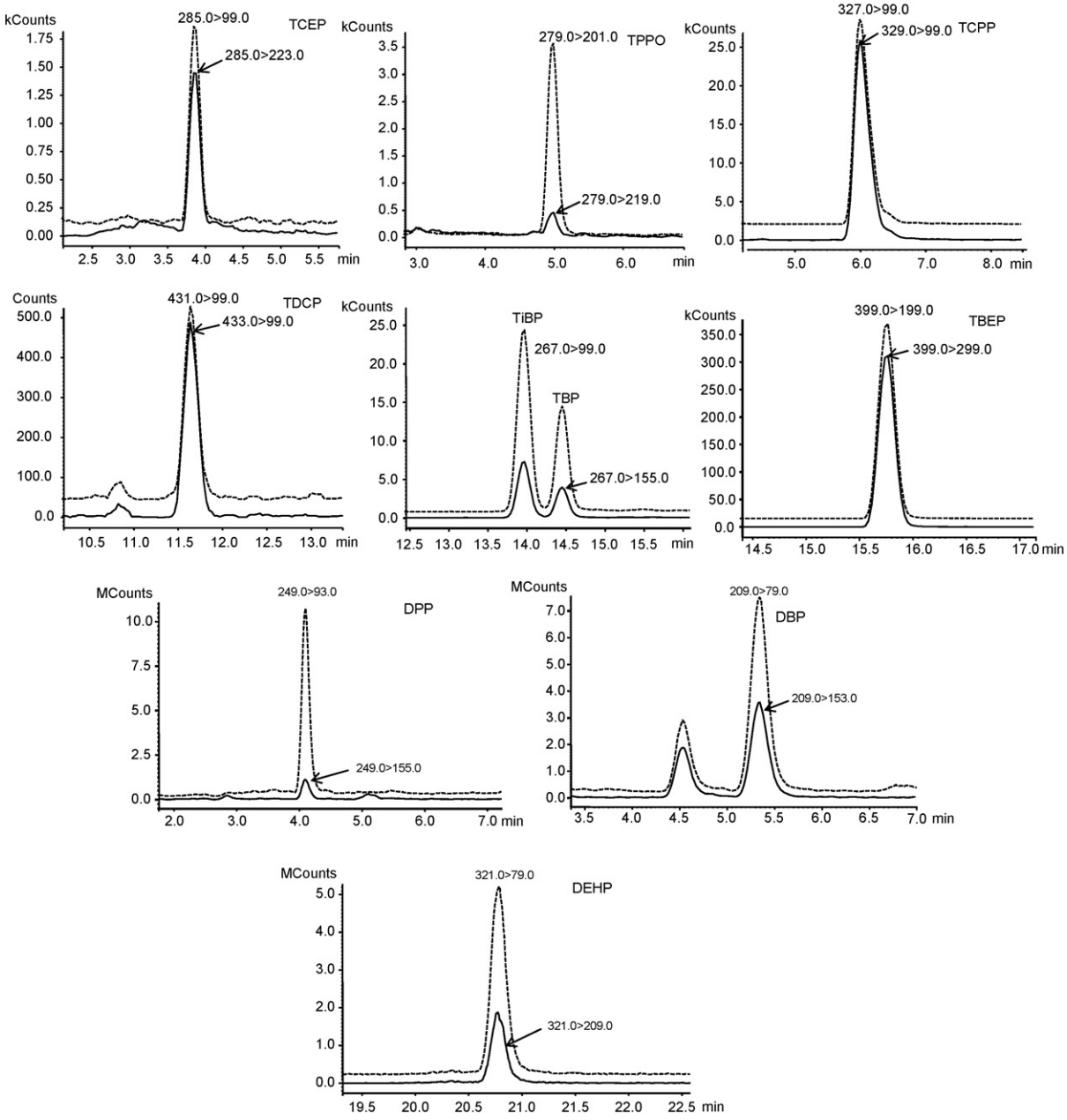

Fig. 6. Quantification (dotted line) and confirmation (solid line) MRM traces for species detected in a non-spiked river water sample (code 4, Table 5).

tions found in these samples corresponded to TBEP, followed by TCPP, with levels ranging from 300 to more than 3000 ng L^{-1}. On the other hand, TPP and TPrP stayed below the detection limit of the method. As regards diesters, their concentrations in wastewater samples varied between 50 and 200 ng L^{-1}. The presence of significant concentrations of several tri- and di-substituted OPs in the effluent of the plant confirms the contribution of urban wastewater to their introduction in the aquatic environment. Sample code 4 was collected in the river which receives the effluent of the above referred sewage plant, ca. 5 km downstream. As for the wastewater samples, TBEP and TCPP were the species showing the highest concentrations in this matrix, which indicates a certain mobility of both OPs in the aquatic environment. Chromatographic signals of compounds detected in this sample are shown in Fig. 6. According to the pioneer work of Quintana et al. [25], the peak showing the same transitions as DBP and a shorter retention time may correspond to the diester of TiBP; however, this assumption could not be confirmed due to the lack of commercially available standards.

Samples 5–7 correspond to three different rivers without known discharges of urban wastewater; however, the first two ones flow through highly industrialized areas. In these samples the concentrations of OPs were significantly lower than in the rest of specimens, being TCPP the only compound which surpassed the 50 ng L^{-1} level. Globally, data on Table 5 confirm the ubiquity of TCPP, TBEP, TBP, TiBP and TDCP in river and wastewater samples.

4. Conclusions

Mixed-mode Oasis MAX cartridges are an interesting alternative to reversed-phase sorbents for the simultaneous extraction of nine tri-substituted OPs and three diesters from environmental water samples. Analytes can be recovered in an extension higher than 70%, without the need of adjusting the pH of the samples and without

addition of ion-pairing agents. Tri- and di-substituted compounds are fractionated during the elution step and further determined by LC–MS/MS in two consecutive injections, operating the ESI source in the positive and negative modes, respectively. In comparison with previous works, devoted to the determination of either tri-esters or diesters, less signal attenuation was observed during ESI ionization, which points to a higher selectivity in sample preparation process. In fact, the three diesters involved in this study can be quantified using external calibration even in raw wastewater samples. To the best of our knowledge, this is the first time that tri- and di-substituted OPs have been determined by LC–MS/MS, using the same chromatographic column as well as the same additive in the mobile phase.

Acknowledgements

M. G.-L. thanks the Spanish Ministry of Science and Innovation for her FPU contract. Financial support from regional, national and FEDER funds (projects DGICT CTQ2009-08377 and PGIDIT06PXIB237039PR) is also acknowledged.

References

[1] A. Marklund, B. Andersson, P. Haglund, Chemosphere 53 (2003) 1137.
[2] T. Reemtsma, J.B. Quintana, R. Rodil, M. Garcia-Lopez, I. Rodriguez, Trends Anal. Chem. 27 (2008) 727.
[3] A. Marklund, B. Andersson, P. Haglund, J. Environ. Monit. 7 (2005) 814.
[4] M. García, I. Rodríguez, R. Cela, Anal. Chim. Acta 590 (2007) 17.
[5] A. Marklund, B. Andersson, P. Haglund, Environ. Sci. Technol. 39 (2005) 7423.
[6] J. Meyer, K. Bester, J. Environ. Monit. 6 (2004) 599.
[7] E.H. Lee, H.B. Yand, K.W. Kim, J.G. Lim, J.H. Yoo, J. Ind. Eng. Chem. 3 (1997) 39.
[8] S.A. Rodionov, A.N. Viznyi, V.M. Esimantovskii, B.Y. Zilberman, Radiochemistry 50 (2008) 626.
[9] T. Suzuki, K. Sasaki, M. Takeda, M. Uchiyama, J. Agric. Food Chem. 32 (2002) 603.
[10] K. Sasaki, T. Suzuki, M. Takeda, M. Uchiyama, Bull. Environ. Contam. Toxicol. 33 (1984) 281.
[11] A. Dodi, G. Verda, J. Chromatogr. A 920 (2001) 275.
[12] M. García, I. Rodríguez, R. Cela, J. Chromatogr. A 1152 (2007) 280.
[13] O.B. Jonsson, U.L. Nilsson, J. Sep. Sci. 26 (2003) 886.
[14] E. Fries, W. Puttmann, J. Environ. Monit. 3 (2001) 621.
[15] J.B. Quintana, R. Rodil, P. Lopez-Mahia, S. Muniategui-Lorenzo, D. Prada-Rodriguez, Anal. Bioanal. Chem. 388 (2007) 1283.
[16] H. Carlsson, U. Nilsson, G. Becker, C. Ostman, Environ. Sci. Technol. 31 (1997) 2931.
[17] M. Shah, J. Meija, B. Cabovska, J.A. Caruso, J. Chromatogr. A 1103 (2006) 329.
[18] M. García-López, I. Rodríguez, R. Cela, K.K. Kroening, J.A. Caruso, Talanta 79 (2009) 824.
[19] A. Bacaloni, C. Cavaliere, P. Foglia, M. Nazzari, R. Samperi, A. Laganà, Rapid Comm. Mass Spectrom. 21 (2007) 1123.
[20] R. Rodil, J.B. Quintana, T. Reemtsma, Anal. Chem. 77 (2005) 3083.
[21] J.B. Quintana, R. Rodil, T. Reemtsma, M. García-López, I. Rodríguez, Trends Anal. Chem. 27 (2008) 904.
[22] B.K. Schindler, K. Förster, J. Angerer, J. Chromatogr. B 877 (2009) 375.
[23] K. Möeller, U. Nilsson, C. Crescenzi, J. Chromatogr. A 938 (2001) 121.
[24] K. Möller, C. Crescenzi, U. Nilsson, Anal. Bioanal. Chem. 378 (2004) 197.
[25] J.B. Quintana, R. Rodil, T. Reemtsma, Anal. Chem. 78 (2006) 1644.
[26] R. Rodil, J.B. Quintana, P. López-Mahía, S. Muniategui-Lorenzo, D. Prada-Rodríguez, J. Chromatogr. A 1216 (2009) 2958.
[27] J. Andresen, K. Bester, Water Res. 40 (2006) 621.
[28] I. Rodríguez, F. Calvo, J.B. Quintana, E. Rubí, R. Rodil, R. Cela, J. Chromatogr. A 1108 (2006) 158.
[29] M. García-López, I. Rodríguez, R. Cela, J. Chromatogr. A 1166 (2007) 9.
[30] M. García-López, I. Rodríguez, R. Cela, Anal. Chim. Acta 625 (2008) 145.
[31] I. González-Mariño, J.B. Quintana, I. Rodríguez, R. Cela, Rapid Commun. Mass Spectrom. 23 (2009) 1756.
[32] J.B. Quintana, T. Reemtsma, Rapid Commun. Mass Spectrom. 18 (2004) 765.
[33] E. Martínez-Carballo, C. González-Barreiro, A. Sitka, S. Scharf, O. Gans, Sci. Total Environ. 388 (2007) 290.