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Comparative study of analytical methods involving gas chromatography–mass spectrometry after derivatization and gas chromatography–tandem mass spectrometry for the determination of selected endocrine disrupting compounds in wastewaters☆

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Abstract

Two GC–MS methods, based on the application of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide-derivatization–GC–MS (selected-ion monitoring) and GC–MS–MS without derivatization, respectively, were optimised and applied to the determination of a group of five selected endocrine disrupting compounds (EDCs) in wastewaters. Both methods included solid-phase extraction with Oasis HLB cartridges allowing an enrichment factor for wastewater samples of 100-fold. The investigated EDCs were estrone, 17β-estradiol, 17α-ethynylestradiol, 4-*tert*-octylphenol and bisphenol A. Results obtained from the validation studies yielded comparable results in both cases. Recoveries in spiked wastewaters at 50 ng/l were higher than 90% for all the compounds, except for 4-*tert*-octylphenol (75%). Repeatability and reproducibility were adequate, varying from 1.6 to 14%, except for estrone which reproducibility was 28% when the derivatization–GC–MS method was applied. Limits of detection calculated ranged from 2.5 to 27.5 ng/l with differences between both methods from 1.1 (estrone) to 10.4 (bisphenol A) times. Both methods were successfully applied to the analysis of the target compounds in sewage treatment plant influents and effluents. Traces of bisphenol A, 4-*tert*-octylphenol, estrone and 17β-estradiol were detected at concentration levels ranging from 13.3 to 1105.2 ng/l.

Keywords: Water analysis; Endocrine disruptors; Estrogens

1. Introduction

Increasingly, evidence that endocrine disrupting compounds (EDCs) can have harmful effects on the aquatic organisms has emerged. Some of the compounds with highest estrogenic capacity include both natural (e.g. 17β-estradiol, estrone) and synthetic estrogens (17α-ethynylestradiol). Apart from these, chemicals from household or industrial processes, such as bisphenol A, or alkylphenol polyethoxylates ($APEO_n$, e.g. 4-nonylphenol or 4-*tert*-octylphenol) can exert endocrine disruption by different mechanisms by mimicking or antagonising the effects of hormones, by altering the synthesis and metabolism of hormones, and by modifying hormone receptor levels [1].

The EDCs may be released directly or indirectly to the aquatic environment. Wastewater treatment plants appear to be one of the major sources of pollution because these compounds are not totally removed or degraded by biological treatments. They have been detected in wastewaters and surface waters at concentration levels of ng/l [2,3]. However, the exposition of aquatic organisms, even at these very low concentration levels can induce estrogenic responses. Reported

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studies have observed the vitellogenin production (feminisation processes) in male fish exposed to low ng/l levels of EDCs [4,5].

APEOn [nonylphenol, octylphenol and alkylphenols (4-*p*-nonylphenol, 4-*p*-*tert*-octylphenol] have been recently included as priority substances in the field of water policy and octylphenols will be subject to a review for identification as possible "priority hazardous substance" (Decision No. 2455/2001/EC) [6].

Different analytical methods have been developed for analysing EDCs from wastewater samples. The most common are liquid or gas chromatography coupled with mass spectrometry (LC–MS or GC–MS). LC–MS enables the determination of APEOn using electrospray ionization (ESI) in both positive and negative mode at μg/l level [7]. Few papers reporting extremely high sensitivity (<0.1–5.0 ng/l) have been published using LC–MS with ESI or atmospheric pressure chemical ionization (APCI) detection [8], or LC–tandem MS [7,9,10]. However, important signal suppression effects are frequently observed when LC–atmospheric pressure ionization (API) MS is applied [11]. Low concentrations (ng/l) of EDCs are generally determined by GC–MS [7,12–17]. In our knowledge, all the analytical methods proposed in the literature apply derivatization procedures before GC–MS analysis. Different reagents have been used to derivatize EDCs, including pentafluorobenzyl (PFBr), *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) or *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA) that lead to the formation of TMS and TBS derivatives. These are often chosen because they are stable during approximately 30 min and allow improving the sensitivity [18–21]. However, derivatization processes can make the sample preparation laborious and time consuming, and can increase the possibility of contamination as consequence of undesirable reactions with the matrix.

This paper proposes avoiding this tedious and critical step performing SPE and direct analysis of the extracts by GC–MS–MS. This method is compared with a well-established method that uses BSTFA as derivatizating agent by using the same fortified samples. So, a comparison between the two methods has been performed. The obtaining of similar results in both cases will allow demonstrate that the elimination of the derivatization step is feasible.

2. Experimental

2.1. Chemical and reagents

Standards of estrogens: estrone, 17β-estradiol and 17α-ethynylestradiol were obtained from Sigma (Oakville, Canada). Standards of APEOn: 4-*tert*-octylphenol and bisphenol A, were supplied from Aldrich (L'Isle d'Abeau, France). [$^2H_2$]17β-estradiol (17β-estradiol-d_2) and [$^2H_{16}$] bisphenol A (bisphenol A-d_6) (from Sigma and Aldrich, respectively) were used as internal standards to perform quantification of the analytes in the wastewater samples when the derivatization GC–MS method was applied. BSTFA was obtained from Sigma. All the standards were of analytical grade (>90%). Stock solutions of the standards were prepared in methanol and stored at −20 °C.

Solvents including methanol and ethyl acetate were from Panreac (Barcelona, Spain) and Oasis HLB SPE cartridges (200 mg, 6 ml) were from Waters (Milford, MA, USA).

2.2. Sample collection and preparation

Influent and effluent wastewaters samples were collected from three municipal WWTPs located in the south of UK (East of Sussex) and in the southeast of Spain (Almeria). All these WWTPs apply conventional treatments based on a preliminary clarification followed by an activated sludge biological treatment and finally, as end point, clarification. Samples were collected in amber glass bottles pre-rinsed with ultrapure water. After collection, samples were filtered through a 0.7 μm glass fibber filter (Teknokroma, Barcelona, Spain) to remove particles that may difficult the extraction procedure. pH of the samples was adjusted at 8, and sodium azide was added to avoid changes in the sample composition by degradation processes. Samples were stored in the dark at 4 °C until SPE extraction was performed, before 48 h of their reception in all the cases.

For EDCs, determination is at the trace level (ng/l) and requires concentration of the samples to reach these levels. Prior the extraction, 100 ml volumes of the wastewater samples were spiked with a mixture of the internal standards at 100 ng/l. SPE was carried out with the following scheme: (a) conditioning step, by the sequential addition of 5 ml of AcEt, 5 ml of methanol and 5 ml of Milli-Q water at a flow rate of 1 ml/min; (b) loading step, by passing 100 ml of the wastewater sample through the cartridge at a flow of 5 ml/min; (c) washing step, the cartridge is rinsed with 5 ml methanol–water (5:95) and dried by vacuum pressure during approximately 15 min; and (d) final elution is performed with 2 × 4 ml of EtAc, at a flow of 1 ml/min.

After elution, the extracts were evaporated by a gentle nitrogen stream until a final volume of 500 μl and directly analysed by GC–MS–MS or transferred into reaction vials for derivatization GC–MS analysis.

2.3. BSTFA derivatization process and GC–MS analysis

Both, standard solutions and wastewater extracts were derivatized in a graduated reaction vial by addition of BSTFA as silylation agent. The derivatization process was performed by evaporating 500 μl of standard solution or extract to dryness at 30 °C under a gentle nitrogen stream. Aliquots (50 μl) of BSTFA and pyridine were added into each reaction vial. After that, the vials were closed and placed in a heater at 65 °C for 25 min. Once the derivatization was completed, 1 μl of the reaction mixture was injected into the GC–MS system before 30 min to avoid the reaction inversion.

Table 1
Quantitation (in bold) and diagnostic ions used for the GC–MS and GC–MS–MS analysis of EDCs in wastewater and MS–MS fragmentation conditions

Compound	M_r	Derivatization GC–MS	GC–MS–MS[a]		
		SIM ions (RA, %)	Precursor (RA, %)	Main product ions (RA, %)	Fragmentation voltage (V)
4-*tert*-Octylphenol	206	**207** (100), 208 (17)	**135** (17)	**107** (100)	0.8
Bisphenol A	228	**357** (100), 358 (30), 372 (12)	213 (20)	**198** (100), 119 (98), **165** (95)	1.2
Estrone	270	**342** (100), 257 (82), 244 (35)	270 (20)	**185** (100), 157 (54), **170** (40)	0.95
17β-Estradiol	272	285 (100), **416** (60), 326 (32)	272 (29)	**213** (100), **188** (85), 186 (75)	0.9
17α-Ethynylestradiol	296	**425** (100), 285 (95), 232 (55)	213 (15)	**157** (100), 128 (38), **133** (40)	1.0

[a] Isolation time, 16 ms; isolation window, 2.

GC–MS analyses were performed on a Trace 2000 gas chromatograph (Thermoquest CE Instrument, Austin, TX, USA) interfaced to a GCQ ion trap mass spectrometer (Finnigan, Austin, TX, USA). Analytes were separated in a crosslinked 5% diphenyl–95% dimethylsiloxane (HP-5 MS, Hewlett-Packard, Palo Alto, CA, USA) capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). A 2.5 m × 0.25 mm i.d. uncoated retention gap (Hewlett-Packard) was coupled to the front of the analytical column via a press fit connector. The temperature program was 1 min at 100 °C, 10 °C/min to 200 °C and then 3 °C/min to 275 °C (1 min). Helium was used as the gas carrier at a constant flow of 1 ml/min. Data acquisition was performed in electron impact ionisation (EI) and in selected-ion monitoring (SIM) mode. Identification and quantitation ions are showed in Table 1. The transfer line was set at 275 °C and the source at 250 °C.

2.4. GC–MS–MS analysis

GC–MS–MS analyses were performed with the same system and chromatographic conditions described above. For MS–MS operation, typical ion trap mass spectrometer conditions were optimised at the following values: electron multiplier at 1425 V, trap offset at 10 V, lens 1 at 25 V, lens 3 at 18 V and gate lens at −83 V. The external ion source worked in EI mode at a temperature of 200 °C. Source pressure was optimised at 30 mTorr (1 Torr = 133.322 Pa) MS–MS conditions such as isolation (wideband application, isolation time) and fragmentation (resonance excitation voltage, fragmentation voltage) were optimised for each analyte and the results are shown in Table 1. The product ion mass spectra resulting from fragmentation were scanned from *m*/*z* 60 to two masses over the mass of the precursor ion selected. Precursor and product ions for identification and quantitation are showed in Table 1.

2.5. Validation studies

All the validation studies were performed by using wastewater samples taken from WWTP effluents. The samples were previously analysed and presence of the target compounds considered. The linearity in the response was studied by using matrix-matched calibration solutions prepared by spiking sewage SPE extracts at six concentration levels, ranging from the analytes, limits of detection (LODs) to 500 ng/l, except for 4-*tert*-octylphenol that was up to 2000 ng/l. Integrated peak area data of the selected quantification masses (see Table 1) were used to construct the curves. Precision of the chromatographic method, determined as relative standard deviation (R.S.D.), was obtained from the repeated injection (three times) of a spiked extract, at the 50 ng/l level, during the same day (repeatability) and in different days (reproducibility). The LODs were determined experimentally from the injection of spiked wastewaters and calculated using a signal-to-noise ratio of 3. The recovery studies were carried out (four replicates) by spiking 100 ml volumes of wastewater samples with the analytes, at the concentration level of 50 ng/l. This concentration level was selected because it is representative of the concentrations usually founded in wastewaters.

3. Results and discussion

3.1. Derivatization GC–MS method

Trimethylsilyl derivatives of the target EDCs were obtained using BSTFA as silylation reagent. This reagent was selected because of its fast reactivity with compounds containing hydroxyl groups, its high volatility resulting in non-coelution of early eluting peaks, and low thermal degradation and good solubility in common organic solvents of the derivatized compounds. Derivatized samples presented an improved separation of the analytes under GC–MS analysis, because of their higher volatility and lower interaction with the stationary phase. The use of the SIM mode during the GC–MS analysis also contributed to an improvement in the selectivity and sensitivity. GC–MS (SIM) chromatograms corresponding to derivatizated and non derivatizated spiked wastewater samples at concentrations of 60 ng/l for 4-*tert*-octylphenol, estrone and 17β-estradiol and 800 ng/l for bisphenol A, are showed in Fig. 1. An increment in the signal to noise ratio of the target compounds peaks is clearly observed in the derivatizated sample. Another feature of the application of derivatization reactions is that trimethylsilyl derivatives produce ions with higher *m*/*z* in the GC–MS system in contrast to those obtained from underivatizated compounds. Table 1 shows the most abundant

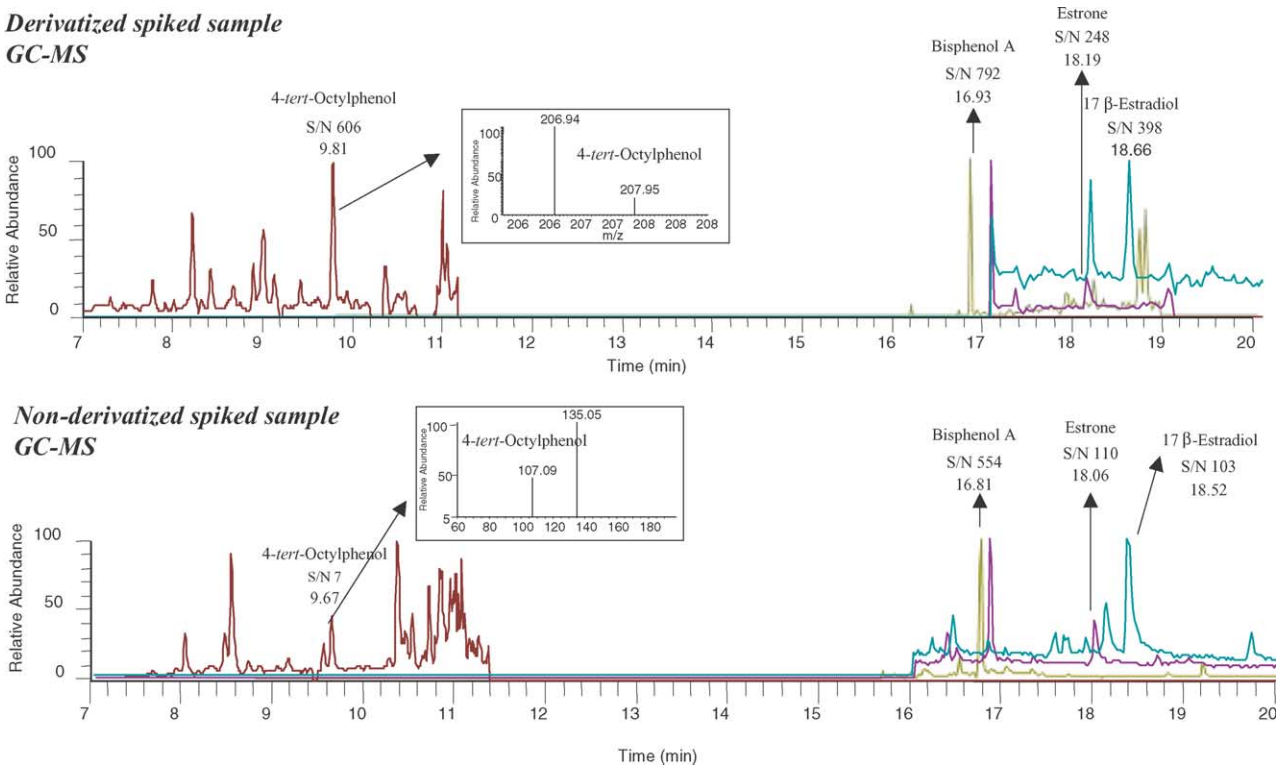

Fig. 1. GC–MS (SIM) chromatograms corresponding to a derivatized and non derivatized spiked wastewater sample at 60 ng/l level for 4-*tert*-octylphenol, estrone, 17β-estradiol, 17α-ethynylestradiol, and 80 ng/l level for bisphenol A.

fragments, and their relative abundances (RAs), selected in the SIM program. As an example, the mass spectra of 4-*tert*-octylphenol in derivatizated and non-derivatizated spiked wastewater samples are also presented in Fig. 1. The EI *full scan* mass spectrum of 4-*tert*-octylphenol showed two significant ion fragments: the base peak at *m*/*z* 135, corresponding to $[HO–C_6H_4–C(CH_3)_2]^+$, and a fragment at *m*/*z* 107 (abundance 50%) assigned to the loss of the methyl groups, $[HO–C_6H_4–CH_2]^+$. The silylated 4-*tert*-octylphenol showed an only major fragment at the higher *m*/*z* 207. This ion correspond to $[(CH_3)_3Si–O–C_6H_4–C(CH_3)_2]^+$ and it was selected as quantification ion. The same happened with the rest of compound that showed base peaks (100% abundance) at *m*/*z* > 300 that were selected as quantification ions. Only in the case of 17β-estradiol the major ion at *m*/*z* 285 was substituted for the molecular ion at *m*/*z* 416 (60% abundance) because of its higher selectivity. The selection of high mass fragments as quantification ions is of great interest, especially when complex matrices are going to be analysed, because of the lower probability of presence of interferences. The ion at *m*/*z* 425 belonging to 17α-ethynylestradiol was selected as quantification ion. This ion can be also produced by humic acids, usually presents in natural waters. So, although presence of humic matters in wastewater is not relevant, their absence must be checked in order to avoid quantification errors. Another alternative can be selecting the ion at *m*/*z* 285. Presence of humic acids was not detected in the samples analysed.

Quantification of the target compounds in the samples was performed by internal standard calibration. Generally, various internal standards have to be used according to the chemical structure and retention time of the analytes. In this work, two internal standard were selected, bisphenol A-d_{16} for 4-*tert*-octylphenol and bisphenol A, and 17β-estradiol-d_2 for the selected natural and synthetic estrogens.

With respect to the identification capability of the method, mass spectra of the derivatizated compounds showed, in most of the cases, enough fragmentation to an accurate identification of the target compounds in the samples. Presence of three diagnostic ions in the SIM mode at their correct relative abundances was imposed as identification criterion. In the case of 4-*tert*-octylphenol, however, only one significant mass was obtained for identification purposes so limiting the confirmation of this compound in real wastewater samples.

Concerning the derivatization technique, complete derivatization of all the aliphatic and aromatic hydroxyl groups present in the molecules of the studied EDCs was achieved. Efficiency of the silylation reaction was studied by the derivatization and analysis of spiked SPE extracts. Results obtained showed that the process was homogeneous for the selected analytes, with R.S.D.s ranging from 5.4 to 11.2%, considering both inter and intra-day precision (see Table 2). Only in the case of the estrone, the reproducibility of the method could be affected because the efficiency was variable (28%).

Table 2
Validation studies of the analytical methods in matrix matched standards

Compound	LOD (ng/l)		Repeatability (R.S.D., %)		Reproducibility (R.S.D., %)		Linearity R^2	
	Derivatization GC–MS (SIM)	GC–MS–MS	Derivatization GC–MS (SIM)	GC–MS–MS	Derivatization GC–MS (SIM)	GC–MS–MS	Derivatization GC–MS (SIM)	GC–MS–MS
4-*tert*-Octylphenol	13.0	20.0	5.4	6.4	10.6	14	0.997	0.995
Bisphenol A	26.5	2.5	8.0	1.6	6.4	10	0.999	0.991
Estrone	8.5	7.5	6.6	9.5	28.0	13	0.995	0.997
17β-Estradiol	17.0	27.5	7.6	10.2	6.0	9	0.999	0.997
17α-Ethynylestradiol	4.0	17.5	5.4	8.5	11.2	10	0.995	0.999

R.S.D., relative standard deviation.

3.2. GC–MS–MS method

In addition to the derivatization GC–MS method and with the aim of reduce labour and time consuming during the sample preparation step, a GC–MS–MS based method without previous derivatization was developed. Tandem mass spectrometry is a highly selective technique that provides very good results in the analysis of trace compounds in complex matrices, so representing a very good choice in the analysis of wastewater samples.

MS–MS parameters were optimised individually for each analyte by using spiked SPE extracts of wastewater samples, at a concentration level of 10 μg/l. Information about the precursor ion isolated, resonance excitation voltage applied and the main product ions obtained with their relative intensities is included in Table 1. The most intense fragment ion on the EI spectrum of the target compounds was selected as precursor ion in all the cases in order to get maximal sensitivity. The base peak corresponded with the molecular ion in the case of estrone and 17β-estradiol. For bisphenol A and 17α-ethynylestradiol the *m*/*z* 213 was the major ion corresponding to the loss of a methyl group $[M\text{-}15]^+$ and part of the aliphatic chain $[M\text{-}83]^+$, respectively. In both cases these ions deliver daughter ions, which were indicative of the structure of the analyte, being considered adequate for an accurate identification. The isolation time was set at 16 ms mass and the isolation window was fixed at 2 in all the cases, in order to get good sensitivity and selectivity.

The resonance excitation voltage applied to the fragmentation of the parent ion was adjusted in order to avoid its complete disappearance. So, the parent ion was present in the MS–MS spectrum of each compound with a relative abundance of around 20%. In these conditions, enough fragmentation was observed in the spectra, where at least three fragments were present (see Table 1).

As it is generally accepted, in addition to the chromatographic retention time match, the match of *m*/*z* values and signal intensity ratios (within 30%) of one or two transitions (parent-to-product ions) between an unknown peak and that due to the correspondent matrix matched standard were considered as accurate identification criteria. Comparing with the derivatization GC–MS method, a more reliable identification of 4-*tert*-octylphenol could be obtained, considering the presence of the parent ion and one transition, as a consequence of the higher selectivity of the GC–MS–MS technique. This selectivity also contributed to the diminution of matrix interferences in the chromatogram, clearly patent in Fig. 2.

For routine analysis, the GC–MS–MS method has many other advantages over the derivatization GC–MS method. For example, GC–MS–MS method does not require the use of internal standards for an accurate quantification and does not require a laborious and time consuming sample preparation, such as the derivatization technique. Furthermore, the derivatization reaction has an important limitation because derivatives can to end up as underivative analytes. This can occurs after approximately 30 min, forcing to do the GC analysis

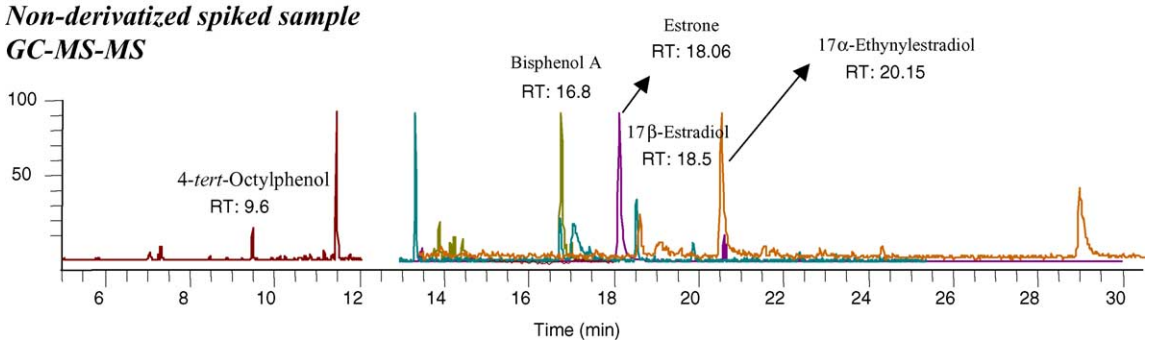

Fig. 2. GC–MS–MS chromatogram corresponding to a spiked wastewater sample at 30 ng/l level for 4-*tert*-octylphenol, estrone, 17β-estradiol, 17α-ethynylestradiol, and 80 ng/l level for bisphenol A.

Table 3
Analysis of wastewater samples (concentration in ng/l)

Wastewater samples	4-*tert*-Octylphenol		Bisphenol A		Estrone		17β-Estradiol	
	GC–MS–MS	GC–MS (derivatization)	GC–MS–MS	GC–MS (derivatization)	GC–MS–MS	GC–MS (derivatization)	GC–MS–MS	GC–MS (derivatization)
Influent WW1	39.6	37.5	1105.2[a]	1270.9[a]	86.9	94.7	49.4	55.0
Influent WW2	43.2	48.4	890.9[a]	916.8[a]	62.9	70.2	83.7	77.1
Influent WW3	43.5	47.1	884.7[a]	982.0[a]	60.4	67.8	93.3	101.3
Effluent WW1	–	18.5	19.2	–	–	–	–	–
Effluent WW2	–	16.7	13.3	–	–	–	–	–

[a] Recoveries at this concentration level have not been evaluated.

before this time and limiting the use of automatic analysis sequences.

3.3. Performance of the analytical methods

The analytical performance of both methods was evaluated estimating the linearity, accuracy, precision, and sensitivity (Table 2).

Linearity of the calibration curve was tested for both methods. Using both MS (SIM) and MS–MS detection, correlation coefficients obtained were higher than 0.991, indicating the concordance of the responses with the linear model for each compound.

Quantitative recoveries were obtained for all the compounds at the concentration level studied, varying from 90 to 99% in all the cases, except for 4-*tert*-octylphenol, which yield a lower recovery (75%). R.S.D.s were ≤10% in all the cases.

Precision of the methods has been commented above. It was determined in terms of reproducibility and repeatability, as R.S.D.s, inter- and intra-day, respectively. Values obtained ranged from 1.6 to 10.2% (repeatability) and 6.4 to 14% (reproducibility), with the exception of estrone, already commented.

LODs were very similar for both methods, ranging from 2.5 to 27.5 ng/l Only in the case of bisphenol A higher differences were obtained, being the GC–MS–MS method ten times more sensitive than the MS (SIM) method.

3.4. Analysis of wastewater samples

Both methods were applied to the analysis of the studied compounds in influent and effluent wastewaters from different European WWTPs located in the UK (East of Sussex) and in Spain (Almeria). A total of twenty wastewater samples were analysed and five of them yielded positive results for the EDCs selected in this study. 4-*tert*-Octylphenol and bisphenol A were the analytes more frequently founded in both influents and effluents. Presence of traces of these compounds in the sewage effluents indicates that they escape elimination in WWTPs because they are not clearly designed to remove this type of compounds. The higher concentrations were detected for bisphenol A, that reached concentrations up to 1105 ng/l in the influent. As the concentration of this compound in the samples exceeded the concentration range of the proposed methods, the samples were diluted to perform an adequate quantification.

Lower concentrations of this compound in the effluent samples could be only detected by GC–MS–MS. Estrone and 17β-estradiol were founded only in the influent of the plants at concentrations that ranged from 49 to 93 ng/l. They were not detected in the effluents. These preliminary results, listed in Table 3, show the applicability of both methods to the analysis of EDCs in wastewaters, demonstrating that the elimination of the derivatization step is feasible

4. Conclusions

Results obtained from the comparison of the two methods described have proved that both methods are applicable to the analysis of the six EDCs studied in wastewater samples. Quantitative recoveries were obtained in all the cases and linearity ($R^2 > 0.991$), precision (R.S.D. < 28%) and limits of detection (4.0–27.5 ng/l) yielded very similar good results for both methods. However in their application to routine analysis the use of the GC–MS–MS method represent the easiest and fast analytical approach avoiding the inconvenient associated with the application of derivatization processes. In addition, the higher selectivity and structural information provided by the product ions mass spectra allows a more reliable confirmation of the target compounds in the samples. The study performed in real samples from sewage treatment plants has evidenced the presence of traces of bisphenol A, 4-*tert*-octylphenol, estrone and 17β-estradiol in the WWTP influents at concentration levels that ranged from 39.6 to 1105.2 ng/l. In the effluents, only 4-*tert*-octylphenol and bisphenol A have been detected at

concentrations between 13.3 and 19.2 ng/l, so evidencing their entry in the environment.

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References

[1] M.H. Deplegde, Z. Billinghurst, Mar. Pollut. Bull. 39 (1999) 32.
[2] T. Ternes, P. Kreckel, J. Mueller, Sci. Total Environ. 225 (1999) 91.
[3] M. Solé, M. Lopez de Alda, M. Castillo, C. Porte, K. Ladegaard-Pedersen, D. Barcelo, Environ. Sci. Technol. 34 (2000) 5076.
[4] E. Routledge, D. Sheahan, C. Desbrow, G.C. Brighty, M. Waldock, J.P. Sumpter, Environ. Sci. Technol. 32 (1998) 1550.
[5] G. Panter, R. Thompson, J. Sumpter, Environ. Sci. Technol. 34 (2000) 2756.
[6] Decision No. 2455/2001/EC of the European Parliament and of the Council of 20 November establishing the list of priority substances in the field of water policy and amending Directive 2000/60/EC, Offi. J. Eur. Commun. 15 December 2001.
[7] R. Jeannot, H. Sabik, E. Sauvard, T. Dagnac, K. Dohrendorf, J. Chromatogr. A 974 (2002) 143.
[8] M.J. Lopez de Alda, D. Barceló, J. Chromatogr. A 892 (2000) 391.
[9] V. Ingrand, G. Herry, J. Beausse, M.-R. De Roubin, J. Chromatogr. A 1020 (2003) 99.
[10] C. Baronti, R. Curini, G. D'Ascenzo, A.Di. Corcia, A. Gentili, R. Samperi, Environ. Sci. Technol. 34 (2000) 5059.
[11] Internal communication.
[12] H.G.J. Mol, S. Sunarto, O.M. Steijger, J. Chromatogr. A 879 (2000) 97.
[13] I. Rodriguez, J.B. Quintana, J. Carpinteiro, A.M. Carro, R.A. Lorenzo, R. Cela, J. Chromatogr. A 985 (2003) 265.
[14] C. Kelly, J. Chromatogr. A 872 (2000) 309.
[15] M.I.H. Helaleh, Y. Takabayashi, S. Fujii, T. Korenanga, Anal. Chim. Acta 428 (2001) 227.
[16] W.-H. Ding, C.-C. Chiang, Rapid Commun. Mass. Spectrom. 17 (2003) 56.
[17] T. Jones-Lepp, C.L. Gerlach, E.J. Cooter, Trends Anal. Chem. 19 (2000) 286.
[18] K. Schoene, H.-J. Bruckert, J. Steinhanses, A. Konig, Fresenius J. Anal. Chem. 348 (1994) 364.
[19] A. Shareef, C.J. Parnis, M.J. Angove, J.D. Wells, B.B. Johnson, J. Chromatogr. A 1026 (2004) 295.
[20] S. Nakamura, T. Hwee Sian, S. Daishima, J. Chromatogr. A 919 (2001) 275.
[21] D.D. Fine, G.P. Breidenbach, T.L. Price, S.R. Hutchins, J. Chromatogr. A 1017 (2003) 167.