Available online at www.sciencedirect.com
ScienceDirect
Talanta 73 (2007) 314–320
Talanta
www.elsevier.com/locate/talanta

Simultaneous analysis of neutral and acidic pharmaceuticals as well as related compounds by gas chromatography–tandem mass spectrometry in wastewater

M.J. Gómez [a], A. Agüera [a,*], M. Mezcua [a,b], J. Hurtado [a], F. Mocholí [a], A.R. Fernández-Alba [a]

[a] *Pesticide Residue Research Group, University of Almería, 04120 Almería, Spain*
[b] *Department of Chemical Engineering and Analytical Chemistry, University of Alcalá de Henares, Spain*

Received 5 December 2006; received in revised form 20 March 2007; accepted 23 March 2007
Available online 1 April 2007

Abstract

This work presents a new multi-residue analytical method based on solid phase extraction (SPE) with Oasis HLB sorbent, followed by gas chromatography tandem mass spectrometry (GC–MS/MS) for the simultaneous determination of a group of 10 acidic and neutral pharmaceuticals and related compounds in wastewaters. The typical derivation step was avoided, allowing the determination of acidic and neutral pollutants in a single analysis as well as providing a fast and easy method suitable for routine monitoring. Target pollutants include: anti-inflammatory drugs (ibuprofen, acetaminophen and diclofenac); an antiepileptic agent (carbamazepine); stimulants (caffeine and nicotine); an antiseptic (triclosan); a plasticizer (bisphenol A) and two of their more relevant metabolites (2,8-dichlorodibenzo-*p*-dioxin and 1,7-dimethylxanthine). Recoveries between 66 and 112% were achieved for all the target compounds (except for 2,8-dichlorodibenzo-*p*-dioxin). Good linearity was observed within the studied ranges ($R^2 > 0.993$). Acceptable intra and inter-day precision was obtained, with relative standard deviation between 2 and 18%. The application of the optimized MS/MS mode allowed method detection limits in the range of 0.2–16 ng/L, with the exception of ibuprofen (120 ng/L). Finally, the methodology was successfully applied to the analysis of hospital effluent samples. All target analytes were detected at concentrations between 1 ng/L and 83215 μg/L. Even in the absence of derivatization, all the analytes showed good peak shape, except acetaminophen, which exhibited peak tailing. However, the method proved to be repetitive and reproducible, and the peak shape did not represent a problem for the reliable quantification of this compound. For most of the analytes studied, the detection limits achieved compare well against values reported in previously published methods.

Keywords: Gas chromatography–tandem mass spectrometry; Wastewater; Hospital effluents; Pharmaceuticals; caffeine; Nicotine; Bisphenol A

1. Introduction

Nowadays, one of the most relevant topics in environmental analytical chemistry is water quality. Ever since the presence of non-regulated contaminants was pointed out as a possible cause of the quality reduction of natural water, many studies have been performed to evaluate the source, occurrence, fate and environmental effects of these pollutants [1–8]. Active pharmaceutical ingredients, surfactants, personal care products or substances with endocrine disrupting activities are among the compounds which have provoked greatest interest over the last decade. These compounds and their active metabolites are continually introduced into the aquatic environment in the form of complex mixtures via a number of routes, but primarily by both untreated and treated municipal wastewater. Contrasting with the wide-ranging information concerning the occurrence of pharmaceuticals and other emergent contaminants in effluents from conventional sewage treatment plants (STPs) [1,5], data is still lacking relating to the contribution of other different sources, such as hospitals. Hospital effluents generally reach the municipal sewer network without preliminary treatment and may represent a significant source of chemicals released into the aquatic environment. Research has also shown that conventional treatments employed in STPs are not specifically designed to remove many of these compounds efficiently [1–9] and they have been identified in the receiving water in the ng/L to μg/L range [3,4,10].

* Corresponding author. Tel.: +34 950015531; fax: +34 950015483.
E-mail address: aaguera@ual.es (A. Agüera).

0039-9140/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2007.03.053

Due to the biological activity of these emergent contaminants [11,12,13] their concentration, fate and behaviour in the environment must be understood and quantified. With this purpose in mind, accurate and sensitive analytical methods have to be developed capable of monitoring these residues at the ng/L level. To date, numerous analytical methodologies for the determination of pollutants and their metabolites in water are available in the literature. However, most of them encompass only groups of compounds containing similar polarities, similar structures or similar activities [14,15,16]. The development of multi-residue methods useful for the determination of a broad group of compounds is required in order to obtain a correct chemical evaluation of the effluents [5,17]. Nevertheless, the majority of these methods require separate and time-consuming sample pre-treatment and/or different analysis for each group of compounds. A simultaneous extraction and analysis for various compound classes generally require a compromise in the selection of experimental conditions, but result in shortened overall analysis time, cost reduction and is more suitable for routine analysis [1,18]. Öllers et al. [19] have reported a GC–MS method for the simultaneous analysis of neutral and acidic pharmaceuticals and pesticides in surface and wastewater, but two separated analyses were required, since the acidic compounds were derivatized and simultaneous derivatization of both neutral and acidic analytes, was not advisable. Verenitch et al. [3], found that caffeine was completely destroyed during methylation processes and for this reason it could not be determined along with other acidic drugs.

Solid phase extraction (SPE) is the most common sample isolation and pre-concentration technique for the target analytes and Oasis HLB® the preferred sorbent, used for this purpose [1,3,4]. Gas chromatography coupled to mass spectrometry (GC–MS or GG–MS/MS) or liquid chromatography coupled to mass spectrometry (LC–MS) or tandem mass spectrometry (LC–MS/MS) are the most widely used techniques [1,3,11,20]. Recently, the authors have applied a LC–TOF/MS technique to determine polar pharmaceuticals in hospital effluents [18], demonstrating that it can be a useful approach. LC–MS/MS has progressed over the last decades, and nowadays it is the main choice in determining ultratrace concentrations of polar pharmaceuticals in environmental samples [20,21]. However, when highly complex samples are analysed, such as wastewater, suppression of the electrospray ionization is likely to occur, especially with electrospray ionization (ESI). The published papers have reported GC–MS in selective ion monitoring (SIM) and GC–MS/MS as very suitable methods for the environmental analysis of the majority of the studied compounds, which are neutral or medium acidic [1,3,19,22]. Although, due to the poor volatility of some compounds and the presence of various polar groups in the molecule, derivatization steps aimed to produce more volatile products and improve the sensitivity of subsequent GC analysis, are applied [3,19]. Thus, the advantages of better sensitivity are sometimes largely offset by loss of sample during the additional manipulation. Most of the analytical methods proposed in the literature apply derivatization procedures before GC–MS analysis.

The group of organic compounds which are the object of this study includes the anti-inflammatory drugs ibuprofen, acetaminophen and diclofenac, the antiepileptic agent carbamazepine, the plasticizer bisphenol A, the antiseptic triclosan and its toxic metabolite 2,8-dichlorodibenzo-*p*-dioxin (2,8-DCDD) and the stimulants nicotine, caffeine and its metabolite 1,7-dimethylxanthine. This group of compounds was selected based on preliminary studies performed in a municipal STP where the selected hospital discharges the effluent. The authors have documented in a previous work [1] the presence of this group of compounds in the influent and effluent of the STP.

A single method based on SPE and direct analysis of the extract by GC–MS/MS is proposed, avoiding the tedious and critical step of derivatization. A comparison of MDLs achieved with other different published methods, for the analysis of these compounds in wastewater is discussed in this paper.

The aim of this study was to develop a sensitive analytical method for the rapid and quantitative determination of neutral and acidic organic contaminants in wastewaters. The described method was then used to analyze hospital effluent samples collected from a hospital in Almería (Spain).

2. Experimental

2.1. Chemicals and reagents

Pure standards (>98%) of acetaminophen, ibuprofen, caffeine, 1,7-dimethylxanthine, carbamazepine, diclofenac, (−)-nicotine, triclosan and bisphenol A were purchased by Sigma–Aldrich (Steinheim, Germany). 2,8-dichlorodibenzo-*p*-dioxin (DCDD) was obtained from LGC Promochem (USA). Individual stock standard solutions were prepared in ethyl acetate, or methanol in the case of carbamazepine, 1,7-dimethylxanthine, diclofenac and acetaminophen, and stored at −20 °C. Standard mixtures, at different concentrations, were prepared by appropriate dilution of the stock solutions.

Pesticide-grade ethyl acetate was obtained from Panreac (Barcelona, Spain) and HPLC-grade methanol from Merck (Darmstadt, Germany).

2.2. Sampling and sample preparation

Discrete samples were collected from the main sewer of a health-care centre with a 75 bed capacity. Amber glass bottles rinsed with ultra-pure water were used for this purpose. Once in the laboratory, the samples were vacuum filtered through a 0.7 μm glass fibre filter (Teknokroma, Barcelona, Spain) and stored at 4 °C until solid phase extraction (SPE), which was performed within 24 h in order to avoid any degradation.

A SPE procedure was applied to the samples using commercial Oasis HLB® (divinylbenzene/*N*-vinylpyrrolidone copolymer) cartridges (200 mg, 6 cm^3) from Waters (Mildford, MA, USA). An automated sample processor ASPEC XL fitted with an 817 switching valve and an external 306 LC pump from Gilson (Villiers-le-Bel, France) was used for this purpose. A conditioning step was performed with 5 ml of ethyl acetate, 5 ml of methanol and 5 ml of LC-grade water at a flow rate of 1 ml/min. After the conditioning step, sample aliquots of 100 ml (pH adjusted to 7 with H_2SO_4) were passed through the car-

tridges at a flow rate of 10 ml/min and then rinsed with 5 ml of deionized water prior to elution. After that, the cartridges were dried by nitrogen stream for approximately 10 min and finally eluted with 2× 4 ml of ethyl acetate at 1 ml/min. The extracts were evaporated until almost dry using a Turbo-Vap from Zymark (Hopkinton, Massachusetts), with the water bath at 40 °C. The samples were then reconstituted with 1 ml of ethyl acetate and transferred to an auto-sampler vial for direct analysis by GC–MS.

2.3. GC-IT-(EI) MS/MS analysis

Determination was performed using a Varian 4000-GC–MS/MS system with a 1079 PTV injector and a CP-8400 autosampler (Varian, Walnut Creek, CA, USA). Data acquisition and processing were performed using the Varian Star Workstation software 6.42 version. The system worked in internal ionization mode. A fused silica untreated capillary column (1 m × 0.25 mm i.d.) from Varian (Middelburg, The Netherlands) was used as a guard column, connected to a Varian FactorFour VF-5ms capillary column (5% diphenyl 95% dimethylsiloxane), 30 m × 0.25 mm i.d., 0.25 μm film thickness. The helium carrier gas flow was constant and set at 1 ml/min. Automatic injections (1 μL) were performed in splitless mode at 280 °C and with the split vet opening set at 1.5 min. Transfer line temperature was 290 °C and ion trap temperature was 200 °C.

The GC temperature program was 70 °C for 2.00 min, programmed to 200 °C at 30 °C/min, then programmed to 220 °C at 2 °C/min (held for 6 min) and finally programmed to 300 °C at 10 °C/min and held at this temperature for 5 min. Total run time was 31.33 min.

3. Results and discussions

This work presents an easy multi-residue analytical method, useful for the rapid and simultaneous determination of acidic and neutral pharmaceutical residues in wastewater samples. Most of the GC-based analytical methods described in the literature for the analysis of acidic pharmaceuticals apply a derivatization technique prior to injection in order to transform polar analytes into their less polar and more volatile forms. The reduction in polarity improves the chromatographic properties and/or the sensitivity of the detection.

Nevertheless, derivatization procedures are laborious and time-consuming, and present other disadvantages such as the reduction in the analytical columns lifetimes or side effects that can occur during the derivatization reactions. In addition, some derivatization processes are susceptible to reaction inversion, while preparation and use of the most commonly used derivatization reagents carries some risk because of their toxicity, carcinogenicity or danger of explosions. Besides these difficulties, the derivatization can destroy non-acidic compounds, as in the case of caffeine [3], or reduce the analysis efficiency of the neutral analytes [19], and therefore, these pharmaceuticals have to be processed separately from acid drugs. To overcome these difficulties, a rapid and simple GC–MS/MS based method, without previous derivatization of acidic drugs, is proposed. Tandem mass spectrometry is a highly selective and sensitive 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. The advantages and suitability of the method for each compound is discussed and compared with previous works.

3.1. GC-IT-(EI) MS/MS method

Fig. 1 shows the selected ion GC–MS/MS chromatogram obtained from a hospital effluent extract spiked at the 500 ng/L level. As it can be seen, a complete separation of the compounds is achieved in a total time of 31 min. The last analyte eluted at 20 min, but the analysis was maintained until reaching a final temperature of 300 °C to ensure complete elimination of the matrix components from the analytical column. Even in the absence of derivatization, all the peaks showed a good peak shape, with the exception of acetaminophen, which suffered from peak tailing. Although this is an undesirable effect in chromatographic analysis, it did not represent an obstacle for the reliable determination of this compound, as will be discussed later on. Furthermore, the sensitivity obtained was enough to achieve a detection limit according to the concentration levels present in the samples.

MS–MS parameters were individually optimized for each analyte. Spiked hospital effluent extracts, at a concentration level of 10 μg/L, were used for this purpose because the presence of a matrix had an effect upon the intensity ration that needed to be evaluated. The different optimized parameters and the precursor ion isolated, together with the main product ions obtained, and their relative intensities, are presented in Table 1. Since the molecular ion is considered as the most characteristic ion for each compound, it was selected as the precursor ion in the case of nicotine, caffeine, 1,7-dimethylxanthine, 2,8-DCDD, and triclosan. For these compounds, the molecular ion also coincided with the base peak – with the exception of nicotine, which presented a base peak at m/z 84. This low-mass ion, which resulted from the loss of the pyridyl group, was not selective enough, thus increasing the probability of matrix interference. In addition, further fragmentation of this ion did not provide structural information of interest, so the molecular ion at m/z 161, was selected. The precursor ion chosen for diclofenac was the fragment ion at m/z 277, corresponding to the loss of water $[M - H_2O]^+$. The molecular ion is not present in the *full scan* spectrum. The base peak in this case corresponded to $[M - H_2OClCO]^+$ ($m/z = 214$), but the ion at $m/z = 277$ provided more structural information, with a characteristic product ion at $m/z = 242$, corresponding with the loss of one chlorine atom. For ibuprofen, acetaminophen, carbamazepine and bisphenol A, the base peak of the EI spectrum was selected as the precursor ion in order to get higher sensitivity. For ibuprofen, this peak corresponded with the loss of carbon dioxide $[M - H - CO_2]^+$ ($m/z = 161$), typical of carboxylic acids. The ion $[M - COCH_3]^+$ ($m/z = 109$), was the base peak in the case of acetaminophen. For the neutral drug carbamazepine, the base peak was observed at $m/z = 193$ due to the loss of the $CONH_2$ group. It must be pointed out

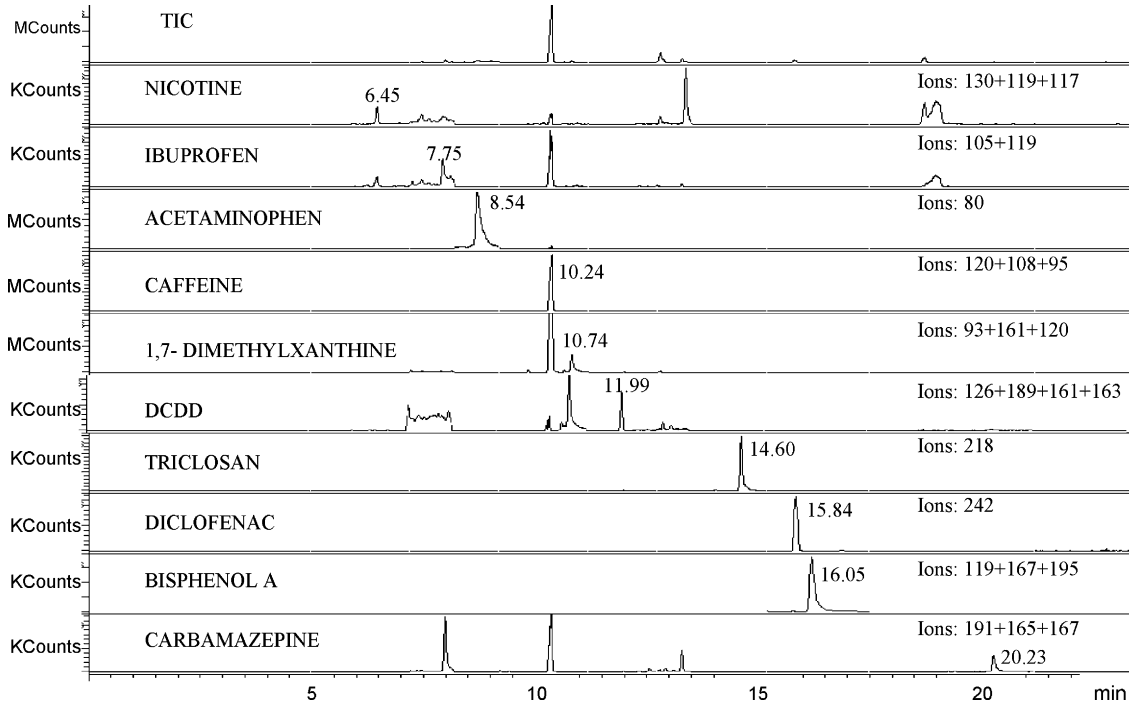

Fig. 1. Total ion (TIC) and selected ion GC–MS/MS chromatograms corresponding to a spiked wastewater sample at 0.5 (g/l level.

that typical degradation of carbamazepine to iminostilbene as a consequence of thermal degradation in the injection port of the gas-chromatograph, was observed. However, as will be discuss later on, precision studies showed an acceptable repeatability and reproducibility for the carbamazepine peak (around 10%), and the MDL (0.2 ng L^{-1}) obtained. Despite degradation, it was low enough to reach the concentration levels present in the samples. For this reason, the contribution of iminostilbene was not considered for quantification. The most intense fragment for bisphenol A was the ion at $m/z = 213$, which corresponded to the loss of a methyl group $[M - 15]^+$. In all these cases, selected precursor ions delivered daughter ions which were indicative of the structure of the analyte, considered adequate for an accurate identification.

At least three fragments were selected as qualifier ions, which are shown in Table 1. The most intense fragment ion/s in the MS/MS spectrum of each pharmaceutical, were selected for quantification purposes.

3.2. Performance of the analytical method

The analytical method was evaluated to prove its applicability to the analysis of neutral and acidic chemicals in wastewater. Recoveries, linearity range, precision and sensitivity were calculated. Validation results, determined in hospital effluent extracts, are presented in Table 2. Concentrations of the pharmaceuticals under investigation in the original hospital effluent samples have been taken into account.

Table 1
Diagnostic ions and quantification masses (in bold) used for the GC–MS/MS analysis of the studied compounds in wastewater

Compound	Rt window (min)	Rt (min)	Molecular ion (*m/z*)	Precursor ion (*m/z*)	Product ions (*m/z*) (relative abundance, %)	Storage level (*m/z*)	Excitation voltage (V)
Nicotine	5.0–7.2	6.45	162.23	161	**130** (100), **119** (90), **117** (56)	60	58
Ibuprofen	7.2–8.2	7.75	206.28	161	**105** (100), **119** (68), 91 (35)	40	40
Acetaminophen	8.2–9.2	8.54	151.17	109	**80** (100), 81 (43), 53 (10)	60	46
Caffeine	9.2–10.6	10.24	194.19	194	**120** (100), **108** (78), **95** (62)	60	53
1,7-Dimethylxanthine	10.6–11.2	10.74	180.17	180	**93** (100), **161** (68), **120** (28)	70	66
DCDD	11.2–12.25	11.99	252.88	252	**126** (100), **189** (92), **161** (79), **163** (31)	90	85
Triclosan	13.2–15.2	14.60	287.95	288	**218** (100), 146 (35), 220 (27)	100	75
Diclofenac	15.2–17.5	15.84	295.13	277	**242** (87), 243 (26), 207 (16), 214(8)	100	66
Bisphenol A	15.2–17.5	16.05	228.29	213	**119** (100), **167** (49), **195** (39)	80	69
Carbamazepine	17.5–24.0	20.23	236.27	193	**191** (100), **165** (97), **167** (35)	80	81

Retention times obtained for each compound and fragmentation conditions are also included.
Isolation window: 3.0; excitation time: 20 ms.

Table 2
Analytical parameters obtained with the GC–MS/MS method for the analysis of selected compounds in wastewater

Compound	Recovery (R.S.D., %), $n=3$	Linearity			MDL (ng/L)	MQL (ng/L)	Repeatability (R.S.D., %), $n=10$	Reproducibility (R.S.D, %), $n=10$
		Range (ng/L)	Cal. equation	R^2				
Nicotine	66 (10)	50–1000	$Y=1231x+1.8e+5$	0.9969	6	21	2	8
Ibuprofen	80 (8)	500–1000	$Y=2774x-4.7e+4$	0.9939	120	401	8	18
Acetaminophen	71 (5)	100–1000	$Y=4152x+3.2e+6$	0.9974	16	55	13	11
Caffeine	92 (5)	10–1000	$Y=6295x-3.1e+4$	0.9995	0.7[a]	2.3[a]	3	4
1,7-Dimethylxanthine	92 (7)	50–1000	$Y=1776x+9.8e+5$	0.9966	10	34	8	13
DCDD	25 (9)	10–1000	$Y=4718x+2.2e+4$	0.9998	1.5	5	2	3
Triclosan	112 (10)	10–1000	$Y=2168x+1.4e+4$	0.9998	2	8	12	12
Diclofenac	79 (3)	10–1000	$Y=873x+4014$	0.9997	1.1	4	6	7
Bisphenol A	72 (4)	10–1000	$Y=1e+4x+2.6e+5$	0.9999	0.5	2	11	14
Carbamazepine	81 (5)	1–1000	$Y=6807x+9.4e+4$	0.9993	0.2	0.7	9	10

R.S.D.: relative standard deviation; MDL: method detection limit; MQL: method quantification limit.
[a] Calculated from a pure solvent standard solution.

3.2.1. Recovery

Recovery studies were performed in triplicate by spiking wastewater samples at a concentration level of 1 μg/l, before and after extraction. Quantitative recoveries obtained for the target compounds ranged from 66 to 112%, except for 2,8-DCDD, which showed a lower recovery (25%). Most of the methods described in the literature for the extraction of polychlorinated dibenzo-*p*-dioxins in environmental samples are focused on solid samples. For the analysis of water samples, SPE with C_{18} sorbent yields better recoveries [23]. However, this hydrophobic stationary phase does not efficiently extract acidic compounds [24]. Simultaneous extraction of acidic and neutral pharmaceutical residues is better obtained by HLB cartridges at pH = 7 [1,18,20,24]. Ethyl acetate was selected as the elution solvent because it desorbs analytes efficiently over a wide range of polarities. Despite the variety of chemical classes of compounds analyzed, which makes it difficult to obtain the best extraction conditions for all of them, HLB-SPE cartridges represented a very good choice with good results in most of the cases. Although the recovery obtained for 2,8-DCDD was not enough for reliable quantitative analysis, other validation parameters such as sensitivity (MDL, 1.5 ng/L) or repeatability (R.S.D., 1.7%) were quite good, and therefore an acceptable quantitative estimation of the concentration of 2,8-DCDD in the samples could be obtained.

3.2.2. Linear dynamic range

The linearity in the response was studied using matrix-matched calibration solutions prepared by spiking hospital effluent extracts at seven concentration levels, ranging from 10 to 1000 ng/L in the samples. The linear dynamic ranges, calibration equations and correlation coefficients (r^2) obtained are shown in Table 2. Good linearity was observed in the studied range with r^2 values higher than 0.99 in all of the cases.

3.2.3. Sensitivity

Method detection limits (MDLs) and method quantification limits (MQLs) were determined from spiked wastewater samples, taking into account the concentration of the target pharmaceuticals in the original samples. Only in the case of caffeine, were MDL and MQL determined from a standard solution in ethyl acetate, due to the fact that caffeine was found at very high concentration in all the analyzed samples. Values obtained ranged from 0.2 to 16 ng/L and 0.7 to 55 ng/L, respectively, except for ibuprofen, which exhibited higher detection and quantification limits, 120 and 401 ng/L, respectively. These levels were low enough however to quantify this compound in the samples. Better detection limits, of 61 and 0.8 ng/L, have been reported [3,7] for ibuprofen after derivatization by GC–MS and GC–MS/MS, respectively, and a MDL of 31 ng/L was achieved by LC–MS/MS [20].

For acetaminophen, even though this compound did not show a very good peak shape, an acceptable MDL (16 ng/L) was reached. Higher levels have been previously reported by GC–MS (129 ng/L) [1] or LC–MS/MS (47 ng/L) [20].

The neutral drug carbamazepine, present in the samples at the lower concentration levels, achieved a MDL of 0.2 ng/L, which was lower than others previously reported by GC–MS, with and without derivatization (100 and 159 ng/L, respectively) [1,5] or. by LC–MS/MS (7 ng/L) [20], which has also been frequently used.

A detection limit of 0.5 ng/L was obtained for bisphenol A. MDLs of 0.5, 2, 27, 396 and 1 ng/L were previously reported for the analysis of bisphenol A by GC–MS/MS with and without derivatization, GC–MS with and without derivatization and LC–MS/MS, respectively [25,26,1,27,21].

Triclosan yielded a MDL of 2.5 ng/L derivatization however, considerably, improves the response of this acidic compound. MDLs of 200, 31 and 10 ng/L have been reported in previous works by GC–MS with and without derivatization and by LC–MS/MS, respectively [1,7,28].

The diclofenac detection limit was 1 ng/L. The same value was reported by GC–MS/MS analysis with derivatization [3], and MDLs of 144, 62 and 30 ng/L were achieved by GC–MS without and with derivatization and LC–MS/MS, respectively [1,7,20].

The neutral drugs caffeine and nicotine could be detected at very low concentrations (0.7 and 6 ng/L), which were, in all cases, one or two orders of magnitude lower than the MDLs reported by other authors (from 14 to 397 ng/l) [3,1,10].

It must be considered that detection limits depend on the sample nature and the preconcentration factor reached during the sample treatment. Most of the papers previously cited analyzed wastewater effluent samples, but the preconcentration factors applied were different.

Although lower MDLs could be obtained by increasing the preconcentration factor, this is not recommendable since it leads to more complex extracts, making the use of additional clean-up steps necessary.

3.2.4. Precision

In order to evaluate the precision of the proposed method, within-laboratory repeatability and reproducibility were estimated. To ensure correct quantification of the underivatized analytes, a spiked extract at 1 μg/L was analyzed 10 times in the same day and 10 times in different days. The repeatability, expressed as percent relative standard deviation (R.S.D.), varied between 2 and 13%, a little bit higher variability was achieved for the reproducibility, from 3 to 18%.

3.2.5. Identification capability of the GC–MS/MS method

Under the conditions described, the MS/MS method provided high sensitivity and selectivity. The isolation of only one ion from the rest of the matrix to obtain the product ion spectra, reduces background interferences and increases the signal-to-noise (S/N) ratio, thus improving confidence in the identification.

The confirmation criteria applied to the target analytes in the samples was based on the peak retention times and the product ion spectra. A peak retention time window within 2% and the presence of three of the most intensive and characteristic ions of the MS/MS spectrum were used to verify a correct identification. The ratio of each parent/product peak combination has to fall within 30% of the established references.

These results indicated a good performance for the developed method.

Table 3
Concentration of selected compounds in hospital effluent samples

Compounds	Sample 1 (ng/L)	Sample 2 (ng/L)	Sample 3 (ng/L)
Nicotine	428	3786	1573
Ibuprofen	3573	4572	921
Acetaminophen	1090	3130	1080
Caffeine	11266	68404	83215
1,7-Dimethylxanthine	1028	1243	638
DCDD	522	714	28
Triclosan	115	212	268
Diclofenac	96	37	510
Bisphenol A	25	82	146
Carbamazepine	3	1	8

3.3. Application of the method to real samples

To demonstrate the applicability of the optimized method, three discrete hospital effluent samples were analyzed. Results obtained are summarized in Table 3. All the target compounds were present in the three samples. Variations in concentrations between samples are explained by the use of discrete samples [20]. Maximum concentrations were detected for caffeine, with concentrations between 11266 and 83215 ng/L. Nicotine, 1,7-dimethylxanthine and the antiinflammatories

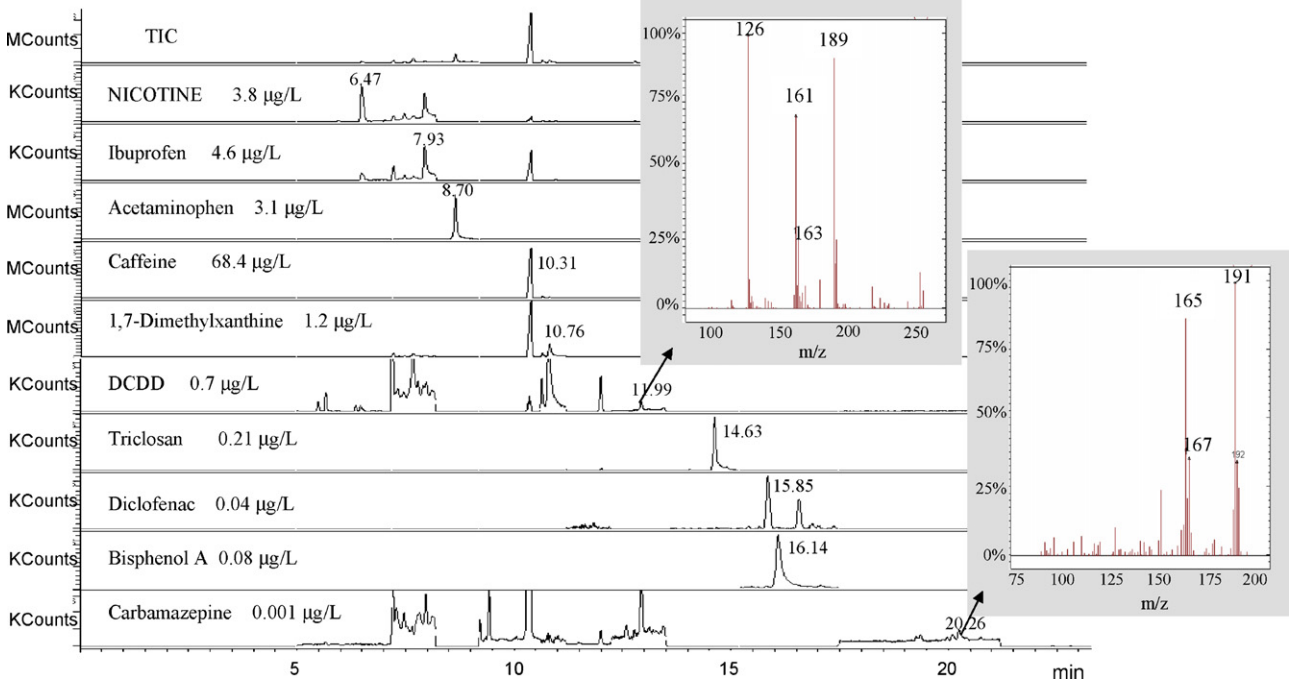

Fig. 2. Selected ion GC–MS/MS chromatogram corresponding to an hospital effluent sample where the target compounds have been identified. Spectra of two of the analytes found at lower concentration are also included.

ibuprofen and acetaminophen were found in the samples at concentrations higher than 1000 ng/L. Carbamazepine was the compound present in the samples at lower concentrations, from 1 to 8 ng/L. The rest of the compounds were detected at concentrations between 25 and 700 ng/L. Fig. 2 shows an extracted ion GC–MS/MS chromatogram of a hospital effluent extract in which all the compounds have been identified. As can be observed in the figure, the high selectivity and structural information provided by the product ions mass spectra allows a reliable identification of the target compounds in wastewater, even with the analytes found at lower concentrations. These results demonstrate the usefulness of the proposed method for this application. The LODs achieved are also low enough to determinate these analytes in urban wastewater, at the levels currently reported in the literature.

4. Conclusions

In the present work, a simple and fast multi-residue method based on SPE followed by GC–MS/MS has been developed for the simultaneous extraction and analysis of neutral and underivatized acid pharmaceuticals and related compounds in wastewater samples. It has been shown that this method represents an easy and fast analytical approach, viable for routine analysis, avoiding the inconvenience associated with the application of derivatization processes. Quantitative recoveries were obtained for all the target compounds, ranging from 66 to 112% (except for 2,8-DCDD: 25%). Linearity with $R^2 > 0.994$ and precision of the method with relative standard deviation between 2 and 18% were also acceptable. With the application of the optimized MS/MS parameters, MDLs of 0.2–16 ng/L can be achieved, except for ibuprofen (120%). For this acid pharmaceutical, derivatization prior to the analysis has been reported to give better MDLs, but this detection limit was low enough to analyze ibuprofen in hospital effluent samples. Most of detection limits achieved were lower than those obtained with previously published methods.

The higher selectivity and structural information provided by the product ions mass spectra allows reliable confirmation of the target compounds in these complex matrices.

This method was successfully applied to the analysis of the studied compounds in hospital effluents. All the compounds were detected, except the carbazepine metabolite. Even in the absence of derivatization, the proposed method showed a high sensitivity for the target compounds and all the analytes showed a good peak shape, with the exception of acetaminophen, which suffered from peak tailing, although this did not represent an impediment to the reliable quantification of this compound. The proposed method is therefore, a useful alternative to previous methods, for simultaneously analyzing neutral and acidic compounds in wastewaters.

Acknowledgements

The authors acknowledge The Spanish Ministry of Education and Science (Project No. CTM2004-06265-C03-03 and Programa Consolider Ingenio 2010 CE-CSD2006-004). M. Mezcua acknowledges the “Juan de la Cierva” research contract from The Spanish Ministry of Science and Technology and M.J. Gómez acknowledges the fellowship from The Spanish Ministry of Science and Technology.

References

[1] M.J. Gómez, M.J. Martinez, S. Lacorte, A.R. Fernández-Alba, A. Agüera, Chemosphere 66 (2007) 993.
[2] P.H. Roberts, K.V. Thomas, Sci. Total Environ. 356 (2006) 143.
[3] S.S. Verenitch, C.J. Lowe, A. Mazumder, J. Chromatogr. A 1116 (2006) 193.
[4] Z. Moldovan, Chemosphere 64 (2006) 1808.
[5] T.A. Ternes, Trends Anal. Chem. 20 (2001) 419.
[6] M.D. Hernando, M. Mezcua, A.R. Fernández-Alba, D. Barceló, Talanta 69 (2006) 334.
[7] L. Lishman, S.A. Smyth, K. Sarafin, S. Kleywegt, J. Toito, T. Peart, B. Lee, M. Servos, M. Beland, P. Seto, Sci. Total Environ. 367 (2006) 544.
[8] A. Agüera, A.R. Fernández-Alba, L. Piedra, M. Mézcua, M.J. Gómez, Anal. Chem. Acta 480 (2003) 193.
[9] N. Paxeus, Water Sci. Technol. 50 (2004) 253.
[10] J.D. Cahill, E.T. Furlong, M.R. Burkhardt, D. Kolpin, L.G. Anderson, J. Chromatogr. A (2004) 171.
[11] E. Emmanuel, G. Keck, J.-M. Blanchard, P. Vermande, Y. Perrodin, Environ. Int. 30 (2004) 891.
[12] T. Schwartz, W. Kohnen, B. Jansen, U. Obs, FEMS Microbiol. Ecol. 1470 (2002) 1.
[13] A.K. Gautam, S. Kumar, P.C. Sabumon, J. Environ. Manage., in press.
[14] T. Kosjek, E. Heath, A. Krbavcic, Environ. Int. 31 (2005) 679.
[15] K.D. Brown, J. Kulis, B. Thomson, T.H. Chapman, D.B. Mawhinney, Sci. Total Environ., in press.
[16] R. Lindberg, P. Jarnheimer, B. Olsen, M. Johansson, M. Tysklind, Chemosphere 57 (2004) 1479.
[17] F. Sacher, F. Thomas Lange, H.J. Brauch, I. Blankenhorn, J. Chromatogr. A 938 (2001) 199.
[18] M.J. Gómez, O. Malato, I. Ferrer, A. Agüera, A.R. Fernández-Alba, J. Environ. Monit. 9 (2007) 718.
[19] S. Öllers, H. Singer, P. Fassler, S.R. Müller, J. Chromatogr. A 911 (2001) 225.
[20] M.J. Gómez, M. Petrovic, A.R. Fernández-Alba, D. Barceló, J. Chromatogr. A 1114 (2006) 224.
[21] B.J. Vanderford, R.A. Pearson, D.J. Rexing, S.A. Snyder, Anal. Chem. 75 (2003) 6265.
[22] S. Morales-Muñoz, J.L. Luque-García, M.J. Ramos, A. Fernández-Alba, M.D. Luque de Castro, Anal. Chem. Acta 552 (2005) 50.
[23] J.-F. Focant, C. Pirard, E. Pauw, Talanta 63 (2004) 1101.
[24] W.-C. Lin, H.-C. Chen, W.-H. Ding, J. Chromatogr. A 1065 (2005) 279.
[25] M.J. Gómez, M. Mezcua, M.J. Martinez, A.R. Fernández-Alba, A. Agüera, Intern. J. Environ. Anal. Chem. 86 (2006) 3.
[26] R. Jeannot, H. Sabik, E. Sauvard, T. Dagnac, K. Dohrendorf, J. Chromatogr. A 974 (2002) 143.
[27] M.D. Hernando, M. Mezcua, M.J. Gómez, O. Malato, A. Agüera, A.R. Fernández-Alba, J. Chromatogr. A 1047 (2004) 129.
[28] W. Hua, E.R. Bennett, R.J. Letcher, Environ. Int. 31 (2005) 621.