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Determination of selected human pharmaceutical compounds in effluent and surface water samples by high-performance liquid chromatography–electrospray tandem mass spectrometry

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Abstract

A simple method is presented for the analysis of 13 pharmaceutical and pharmaceutical metabolite compounds in sewage effluents and surface waters. The pharmaceutical compounds were extracted using a generic solid-phase extraction (SPE) procedure using Phenomenex Strata X as a stationary phase. Extracts were quantitatively analysed by four separate reversed-phase high-performance liquid chromatography–electrospray tandem mass spectrometry (HPLC–ESI-MS/MS) techniques and quantified by comparison with an internal standard ([^{13}C]-phenacetin). Recoveries and limits of detection (LOD) for sulfamethoxazole (120%, 50 ng l^{-1}), acetyl-sulfamethoxazole (56%, 50 ng l^{-1}), trimethoprim (123%, 10 ng l^{-1}), erythromycin (73%, 10 ng l^{-1}), paracetamol (75%, 50 ng l^{-1}), ibuprofen (117%, 20 ng l^{-1}), clofibric acid (83%, 50 ng l^{-1}), mefenamic acid (24%, 50 ng l^{-1}), diclofenac (62%, 20 ng l^{-1}), propranolol (45%, 10 ng l^{-1}), dextropropoxyphene (63%, 20 ng l^{-1}) and tamoxifen (42%, 10 ng l^{-1}) were all acceptable. The recovery of lofepramine (4%) was too low to be of use in a monitoring programme. Application of the method to samples collected from UK sewage effluents and surface waters showed detectable concentrations of mefenamic acid, diclofenac, propranolol, erythromycin, trimethoprim and acetyl-sulfamethoxazole in both matrices. Ibuprofen and dextropropoxyphene were detected in sewage effluents alone. All other pharmaceutical compounds were below the methods limits of detection.

Keywords: Water analysis; Environmental analysis; Drugs

1. Introduction

Increased attention is currently being paid to pharmaceutical substances as a class of environmental contaminants [1,2]. Pharmaceutical substances are used extensively in human and veterinary medicine and can enter the aquatic environment following manufacture, application or ingestion/excretion [3]. The majority of human pharmaceutical compounds enter aquatic systems after ingestion and subsequent excretion in the form of the non-metabolised parent compounds or as metabolites via the sewage treatment network. Inputs of pharmaceutical substances into aquatic systems via this route have led to their occurrence being reported in sewage treatment work (STW) effluent, river and marine surface water, ground water and exceptionally in drinking water [4–12]. Much of this work has

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been conducted in continental Europe and the USA with very little or no recent work being performed in the UK. The absence of occurrence data for the UK is significant since use patterns and volumes differ from country to country suggesting that occurrence data obtained in Europe may not be applicable to the UK. Occurrence data are therefore required for human pharmaceuticals in aquatic systems in the UK. In order to address this gap in our knowledge the UK Environment Agency (EA) commissioned a study to conduct targeted monitoring of pharmaceutical compounds in STW effluents and receiving waters [13].

Approximately 3000 different human pharmaceutical substances are registered for use in the UK [14]. Prior to conducting a targeted monitoring study it was necessary to rank substances on their relative risk, enabling those substances with the greatest potential to pose a risk to the aquatic environment to be identified. The UK Environment Agency conducted such a ranking procedure, and identified 13 compounds for priority investigation [13] (Fig. 1).

Methods have been reported for 9 of the 13 compounds targeted [8,15], although the only methods reported for paracetamol in environmental samples had a very low recovery (0%; [15]) or a high limit of detection (LOD) (500 ng l^{-1}; [5]) and are therefore unsuitable for use in monitoring studies. As far as we are aware no published methods are available for the environmental analysis of dextropropoxyphene, lofepramine, tamoxifen and the sulfamethoxazole metabolite, acetyl-sulfamethoxazole.

In this paper, we report the development of a suite of methods for the analysis of the targeted pharmaceutical compounds and pharmaceutical metabolites (Fig. 1) in surface water and effluent samples using a generic solid-phase extraction (SPE) and preconcentration procedure, followed by liquid chromatography–electrospray tandem mass spectrometry (LC–ESI-MS/MS) analysis.

2. Experimental

2.1. Materials

Methanol and water (HPLC-grade) were obtained from Rathburns (Walkerburn, UK), hydrochloric acid from BDH (Poole, UK) and dichlorodimethylsilane, ammonium acetate and formic acid from Aldrich (Gillingham, UK). [^{13}C]-phenacetin [phenacetin-(ethoxy)-1-^{13}C] was obtained from Cambridge Isotope Labs. (Andover, MA, USA). Erythromycin, paracetamol (4-acetamidophenol), clofibric acid [2-(4-chlorophenoxy)-2-methylpropionic acid], ibuprofen [s(+)-2-(4-isobutylphenyl)propionic acid], mefenamic acid {2-[(2,3-dimethylphenyl)amino]benzoic acid}, diclofenac {[2-(2,6-dichloropheny)amino]benzeneacetic acid}, propranolol (propranolol hydrochloride), and tamoxifen {[*z*]-1-(*p*-dimethylaminoethoxyphenyl)-1,2-diphenyl-1-butene} were all obtained from Aldrich. Lofepramine (lofepramine hydrochloride), dextropropoxyphene (dextropropoxyphene hydrochloride) and trimethoprim were all supplied by the British Pharmacopoeia Commission Laboratory (Stanmore, UK), whilst sulfamethoxazole, and acetyl-sulfamethoxazole [4-acetylamino-*N*(5-methyl-3-isoxazolyl)benzenesulfonamide] were obtained from RdH (Germany) and Micromol (Germany), respectively. All compounds were of a purity >95%.

2.2. Methods

All glassware was silanised by rinsing with 10% (v/v) dimethyldichlorosilane in dichloromethane (DCM), followed by DCM (2×), and methanol (2×).

2.2.1. Quantification and preparation of standards and spiked samples

External calibration over a range of 10–5000 ng l^{-1} was used alongside a ^{13}C-labelled internal standard ([^{13}C]-phenacetin). Calibration standards were prepared at concentrations of 0.01, 0.05, 0.1, 0.45, 1.0, 5.0, 10.0 and 45 μg l^{-1} in a 50:50 mix of methanol:40 mM ammonium acetate at pH 5.5.

Spiked tapwater samples (100 ml) were prepared by the addition of an accurate amount of multi-compound standard for recovery determination (ca. 1 μg in each 100 ml spike sample). Tapwater (100 ml) was also used for blanks. For quantification an internal standard solution (990 μg l^{-1} solution of [^{13}C]-phenacetin in 50:50 methanol:40 mM ammonium acetate at pH 5.5) was added to all samples, spikes and blanks giving a concentration of 990 ng 100 ml^{-1} (990 ng l^{-1} for samples).

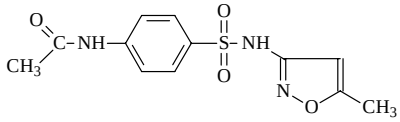
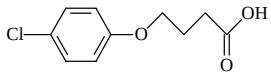
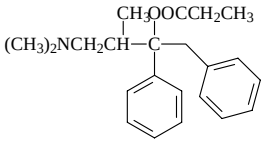
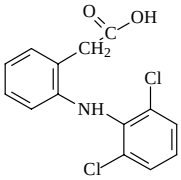
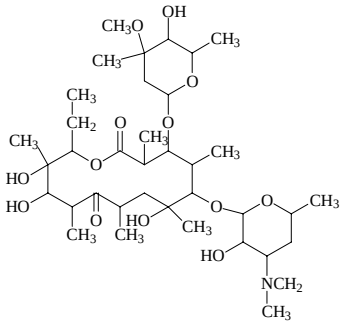
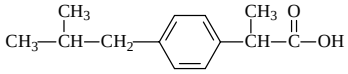
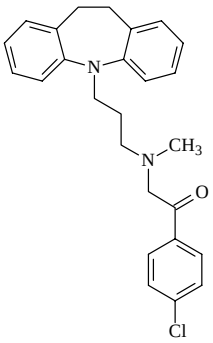
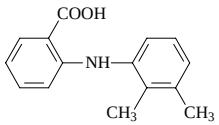
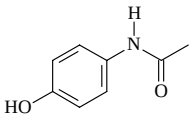
Pharmaceutical	Structure
Acetyl-sulfamethoxazole	
Clofibric Acid	
Dextropropoxyphene	
Diclofenac	
Erythromycin	
Ibuprofen	
Lofepramine	

Fig. 1. Structure of pharmaceutical compounds selected for targeted monitoring.

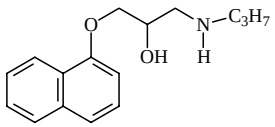
Mefenamic acid



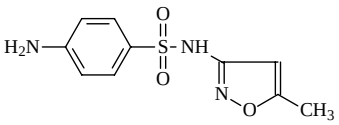
Paracetamol



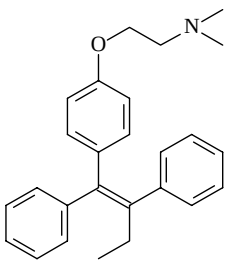
Propranolol



Sulfamethoxazole



Tamoxifen



Trimethoprim

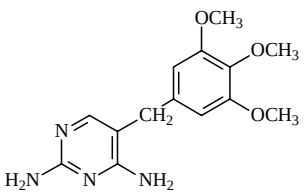


Fig. 1. (*Continued*).

2.2.2. Sample collection

Effluent and surface water samples (2.7 l) were collected using two similar procedures due to the different types of sampling location. Direct sampling in a silanised, clean amber glass Winchester using a weighted stainless steel water sampler was used for surface waters and most of the effluents. Where it was not possible to use this approach, samples were first collected using a stainless steel bucket and then transferred into a silanised, clean amber glass Winchester. All samples were extracted by SPE within 48 h of collection.

2.2.3. Sample extraction

Water samples (1 l) were passed through a glass fibre filter (GFC, 0.45 μm) and the pH adjusted to 3 by the addition of concentrated HCl. [^{13}C]-phenacetin in methanol (1 ml) was then added, the sample agitated

and then transferred to a separate clean, silanised amber Winchester before being passed through a Strata X 6 ml SPE column (200 mg; Phenomenex, UK). Prior to extraction the SPE columns had been solvated with methanol (3× 2 ml) water (3× 2 ml) and water adjusted to pH 3 (3× 2 ml). Without allowing the column to go dry, the sample was introduced directly to the column, by means of a PTFE tube, at a flow rate of approximately 10 ml min^{-1}. Following extraction, the SPE columns were dried by vacuum aspiration for 30 min, wrapped in aluminium foil, and stored in a self-seal plastic bag at −30 °C until eluted.

Columns were allowed to defrost before elution of the analytes. The analytes were eluted with methanol (3× 2 ml). The column was soaked in methanol for 5 min before the methanol was allowed to run through the column at a flow rate of approximately 5 ml min^{-1}. Following elution, the sample was reduced in volume to ∼100 μl under a nitrogen stream (TurboVap, Zymark, USA; 40 °C). The samples were then transferred into an analysis vial, made up to 1 ml with 50:50 methanol:40 mM ammonium acetate (pH 5.5), and stored at −20 °C until analysed.

2.3. Analysis

2.3.1. HPLC

HPLC was carried out on a Thermo-Finnigan Surveyor system (Thermo Finnigan, Hemel Hempstead, UK). The analytes were separated on a 250 mm × 2 mm × 5 μm C_{18} Luna (2) column (Phenomenex, Macclesfield, UK) using a mobile phase of water, methanol and 40 mM ammonium acetate in water, adjusted to pH 5.5 by the addition of formic acid. In order to get good separation of each analyte four separate solvent gradients were used at a flow rate of 200 μl min^{-1} (solvent gradients are shown in Tables 1–4). The antibiotics erythromycin, trimethoprim sulfamethoxazole and acetyl-sulfamethoxazole were analysed using one procedure. Mefenamic acid, lofepramine, propranolol, dextropropoxyphene, diclofenac and tamoxifen were analysed using another. Clofibric acid and ibuprofen were analysed on a separate gradient, as was paracetamol. An injection volume of 20 μl and a post-run equilibrium time of 3 min was used for all samples. Approximate retention times are shown in Table 7.

Table 1
HPLC solvent gradient for the separation of erythromycin, sulfamethoxazole, acetyl-sulfamethoxazole and trimethoprim

Time	Solvent A	Solvent B	Solvent C
0	10	15	75
2	10	15	75
15	10	90	0
20	10	90	0
25	10	15	75

Solvent A: 40 mM ammonium acetate adjusted to pH 5.5 with formic acid; solvent B: methanol; solvent C: water.

Table 2
HPLC solvent gradient for the separation of mefenamic acid, lofepramine, propranolol, dextropropoxyphene, diclofenac and tamoxifen

Time	Solvent A	Solvent B	Solvent C
0	10	15	75
3	10	15	75
10	10	90	0
20	0	100	0
25	0	100	0
26	10	15	75
30	10	15	75

Solvent A: 40 mM ammonium acetate adjusted to pH 5.5 with formic acid; solvent B: methanol; solvent C: water.

Table 3
HPLC solvent gradient for the separation of clofibric acid and ibuprofen

Time	Solvent A	Solvent B	Solvent C
0	0	15	85
3	0	15	85
10	0	100	0
20	0	100	0
25	0	15	85

Solvent A: 40 mM ammonium acetate adjusted to pH 5.5 with formic acid; solvent B: methanol; solvent C: water.

Table 4
HPLC solvent gradient for the separation of paracetamol

Time	Solvent A	Solvent B	Solvent C
0	0	10	90
5	0	10	90
25	0	100	0
30	0	10	90

Solvent A: 40 mM ammonium acetate adjusted to pH 5.5 with formic acid; solvent B: methanol; solvent C: water.

2.3.2. Mass spectrometry

Mass spectra were obtained on a Thermo Finnigan LCQ Advantage mass spectrometer. The mass spectrometer was set-up for electrospray ionisation and the probe temperature set to 220 °C. The flow from the HPLC was passed through a 1:1 split with 100 μl min^{-1} of eluent introduced into the MS interface.

Full scan acquisitions were made over specific mass ranges for individual compounds to determine the optimum mode of ionisation. Following preliminary evaluation of the most sensitive ionisation mode for each analyte four detection methods were developed. Erythromycin, trimethoprim, sulfamethoxazole and acetyl-sulfamethoxazole were detected using tandem mass spectrometry in the positive ionisation mode with consecutive reaction monitoring (CRM). Mefenamic acid, diclofenac, propranolol, dextropropoxyphene, lofepramine and tamoxifen were detected by tandem mass spectrometry using positive ionisation CRM. A negative ionisation method was used to detect clofibric acid and ibuprofen. Single ion monitoring (SIM) was used for ibuprofen, whist tandem mass spectrometry CRM was used to detect clofibric acid. Paracetamol was detected using positive ionisation SIM. The mass/charge ratios shown in Table 6 were set for the detection of each compound. Tuning was performed on the ion displaying the weakest signal for each group of analytes. Paracetamol was used for tuning the paracetamol and the positive ionisation methods, whilst erythromycin was used to tune the antibiotic method. Clofibric acid was used to tune the negative ionisation method.

For all methods the sheath gas flow was 10 (arbitrary units), auxiliary flow was 4 (arbitrary units) and capillary temperature was set at 220 °C. Other variable parameters are shown in Table 5.

3. Results and discussion

3.1. General

Previously published methods for the analysis of pharmaceuticals in surface waters and effluents commonly use gas or liquid chromatography coupled to mass spectrometry (GC–MS or LC–MS) following extraction and pre-concentration using SPE [8,10–12,15–17]. Many of the compounds selected for this study are polar in nature and would require derivatisation prior to analysis by GC. Derivatisation of both acidic and basic compounds in the same process is difficult and time consuming, therefore LC–MS was chosen for this study. ESI-MS based methods have been commonly reported as suitable for pharmaceutical compounds. ESI is known to provide lower limits of detection than atmospheric pressure chemical ionisation (APCI) for the detection of clofibric acid, diclofenac, ibuprofen, mefenamic acid and paracetamol [15] and an initial assessment of APCI and ESI in this study showed ESI to be the most sensitive ionisation technique.

A multi-residue extraction and pre-concentration SPE step was developed in order to keep the number of samples collected, analysis times and costs to a minimum.

3.2. Selection of ionisation mode

Preliminary evaluation of the most sensitive ionisation mode was carried out for the selected pharmaceuticals, by direct injection of a 1 μg ml^{-1} standard. Good ionisation of erythromycin, sulfamethoxazole, acetyl-sulfamethoxazole, trimethoprim, lofepramine, dextropropoxyphene, tamoxifen, mefenamic acid, paracetamol, diclofenac and propranolol was observed in the positive ESI mode producing an intense

Table 5
Mass spectrometer parameters used

MS method reference	Method	Current (μA)	Voltage (kV)	Capillary voltage (V)
1	Positive ionisation	2.0	5.0	31.0
2	Negative ionisation	6.3	4.2	−32.7
3	Antibiotics	2.0	5.0	2.8
4	Paracetamol	2.0	5.0	31.0

protonated molecule $[M + H]^+$ for each compound (Table 6). Poor ionisation of clofibric acid and ibuprofen was observed in the positive mode whilst an intense de-protonised molecule $[M - H]^-$ was observed for both compounds in the negative ESI mode. For all compounds except paracetamol and ibuprofen consecutive reaction monitoring (CRM) of the precursor molecule produced a single intense primary product ion (Table 6). In complex matrices such as STW effluent greater selectivity is desirable, tandem mass spectrometry (MS/MS) was therefore used to detect all compounds bar ibuprofen and paracetamol, which were detected by MS in single ion monitoring (SIM) mode. For both MS and tandem MS, detection in full scan was not as sensitive as detection in SIM and CRM modes, respectively.

Using positive and negative ESI in CRM and SIM mode, the calibration data obtained for the standards in methanol are shown in Table 7. The system was linear in all cases, with linear ranges for each ion identified by injecting known concentrations of standards (10–5000 ng l^{-1}) directly into the mass spectrometer. Limits of detection (LODs) were calculated as the lowest identifiable standard concentration using a signal to noise ratio of 10, and are shown in Table 7.

Quantification of real samples was achieved by comparison with the internal standard [^{13}C]-phenacetin. [^{13}C]-phenacetin displays good ionisation in the positive ESI mode, but poor ionisation in the negative ESI mode. Therefore, the peak values in the previous positive ionisation run for [^{13}C]-phenacetin were used for the quantification of ibuprofen and clofibric acid. Whilst not ideal, this did not affect repeatability to a great extent, relative standard deviations (R.S.D.s) being 22 and 7%, respectively.

3.3. Extraction

Initial extraction protocols were based on published methods [8,15]. Attempts were made to incorporate into these methods those compounds that had not previously been included. Satisfactory performance data could not be obtained by replicating the published methods and a number of stationary phases were initially evaluated for the extraction of the selected pharmaceutical compounds. These were Isolute ENV+, Isolute C_{18} and Isolute C_8, Oasis HLB and Oasis MCX, Varian Bond Elut C_{18} and Phenomenex Strata X. Varian Bond Elut C_{18} and Phenomenex Strata X were identified as being the most effective and selected for further investigation. Performance data were generated for both cartridges and Strata X was shown to be the better phase for extracting the majority of the selected compounds (Table 7). All glassware was silanised in order to minimise the surface adsorption of analytes and the sample pH was adjusted to 3 in order to achieve reproducible and high analyte recoveries. Since Hirsch et al. [8] reported lower recoveries in Milli-Q water, which has little or no salt content, the effect of adding NaCl to the tap-water spike sample was investigated. No significant difference in the recoveries was obtained, and so NaCl was not added to further samples.

Given the different physico-chemical properties of the compounds selected the recoveries using the Strata X SPE columns are good (typically >60%) and reproducible R.S.D.s $<$ 10%) (Table 7). Paracetamol, which has been found to breakthrough certain SPE columns [15] was also reproducibly extracted with good recoveries (75%, R.S.D. $=$ 6.9%). Of the compounds with recoveries less than 60%, acetyl-sulfamethoxazole, propranolol and tamoxifen were all sufficiently high and reproducible to be used for environmental monitoring. Lofepramine could not be efficiently extracted, and does not appear to be compatible with this method. Additional work will be carried out to establish a method of analysis for lofepramine. The recovery of mefenamic acid (24%; R.S.D. $=$ 7.9%) was also inadequate, again additional work will be done to attempt to improve the recovery, however the current method will be tested using real samples to assess it's suitability until a better method for mefenamic acid can be developed.

3.4. HPLC

Chromatograms typical of spiked tapwater and effluent, obtained using HPLC–ESI-MS/MS using both CRM are shown in Figs. 2 and 3. Each compound is resolved to baseline with good peak shape.

Four solvent gradients were used (Tables 1–4). The negative ionisation of clofibric acid and ibuprofen required that a separate gradient was used since the ammonium acetate buffer, used in some of the gradients to stabilise retention times, was omitted since it suppressed the signal from clofibric acid and ibuprofen.

Table 6
Precursor/SIM mass, product ion mass, LC gradients and MS procedures used for the selected pharmaceuticals analysed

Compound	Therapeutic class	Chemical Abstracts Service (CAS) no.	Gradient programme (see table indicated)	MS procedure (see Table 5)	Precursor mass (SIM)	Product ion 1 (MS/MS)
Acetyl-sulfamethoxazole	Antibiotic metabolite		Table 1	3	296.1 $[M + H]^+$	236.2
Clofibric acid	Lipid regulator metabolite	882-09-7	Table 3	2	213.1 $[M - H]^-$	127.1 $[M - (CH_2)_3COOH]^-$
Dextropropoxyphene	Analgesic	469-62-5	Table 2	1	340.1 $[M + H]^+$	266.2 $[M - CH_3CH_2CO_2]^+$
Diclofenac	Anti-inflammatory	15307-86-5	Table 2	1	296.1 $[M + H]^+$	277.9 $[M + H - H_2O]^+$
Erythromycin	Antibiotic	114-07-8	Table 1	3	734.7 $[M + H]^+$	576.3 $[M + H - C_8H_{15}O_3]^+$
Ibuprofen	Analgesic	15687-27-1	Table 3	2	205.2 $[M - H]^-$	–
Lofepramine	Anti-depressant	23047-25-8	Table 2	1	419.2 $[M + H]^+$	224.1 $[M - C_{14}H_{12}N]^+$
Mefenamic acid	Anti-inflammatory	61-68-7	Table 2	1	242.2 $[M + H]^+$	224.2 $[M + H - H_2O]^+$
Paracetamol	Analgesic	103-90-2	Table 4	4	152.2 $[M + H]^+$	–
Propranolol	Anti-hypertensive	525-66-6	Table 2	1	260.2 $[M + H]^+$	183.1 $[M - H_2O - C_3H_7NH]^+$
Sulfamethoxazole	Antibiotic	723-46-6	Table 1	3	254.2 $[M + H]^+$	188.1
Tamoxifen	Anti-cancer	10540-29-1	Table 2	1	372.3 $[M + H]^+$	327.1 $[M - (CH_3)_2N]^+$
Trimethoprim	Antibiotic	738-70-5	Table 1	3	292.6 $[M + H]^+$	230.2 $[M - 2CH_3O]^+$

Table 7
Performance data for selected pharmaceuticals including recoveries for Varian Bond Elut C_{18} and Phenomenex Strata X SPE columns, with LOD for Phenomenex Strata X, calibration data for ESI-MS/MS detection, peak retention time and ion suppression ranges for relevant matrices

Compound	Calibration equation	Linear range ($\mu g\ l^{-1}$)	Correlation coefficient	Approximate retention time (min)	Ion suppression range surface water (min)	Ion suppression range effluent (min)	SPE column recoveries[a]		
							Varian R.S.D. (%)	Strata X R.S.D. (%)	LOD[b] ($ng\ l^{-1}$)
Acetyl-sulfamethoxazole	$y = 2 \times 10^6x - 1 \times 10^6$	0.05–45	0.9989	4.3	21–22	3.0–3.6	56 (4.8)	56 (5.4)	50
Clofibric acid	$y = 5 \times 10^5x + 5 \times 10^5$	0.05–10	0.9690	13.8	2.7–4.0	2.5–3.2	62 (10)	83 (7.0)	50
Dextropropoxyphene	$y = 3 \times 10^5x + 6 \times 10^5$	0.02–45	0.9843	16.4	28–29		43 (25)	63 (3.9)	20
Diclofenac	$y = 2 \times 10^6x + 1 \times 10^5$	0.02–45	0.9998	16.6	28–29		44 (9.7)	62 (20)	20
Erythromycin	$y = 5 \times 10^7x + 9 \times 10^6$	0.01–5	0.9942	19.4	1.0–5.5		0.92 (16)	73 (30)	10
Ibuprofen	$y = 4 \times 10^6x + 1 \times 10^6$	0.02–10	0.9784	15.5	3.1–4.2	3.2–3.9	108 (5.5)	117 (22)	20
Lofepramine	$y = 2 \times 10^7x + 1 \times 10^6$	0.01–10	0.9989	23.8			0.13 (54)	4.2 (35)	10
Mefenamic acid	$y = 5 \times 10^7x - 2 \times 10^5$	0.05–10	0.9988	17.8	28–29		19 (14)	24 (7.9)	50
Paracetamol	$y = 6 \times 10^6x + 3 \times 10^6$	0.05–45	0.9989	14.8		3.1–4.8	61 (6.8)	75 (6.9)	50
Propranolol	$y = 3 \times 10^7x + 2 \times 10^6$	0.01–1	0.9947	15.3			41 (4.2)	45 (5.6)	10
Sulfamethoxazole	$y = 5 \times 10^6x - 3 \times 10^6$	0.05–45	0.9966	4.0	21–22		43 (26)	120 (16)	50
Tamoxifen	$y = 3 \times 10^6x + 4 \times 10^5$	0.01–5	0.9941	20.5			17 (37)	42 (40)	10
Trimethoprim	$y = 1 \times 10^7x + 2 \times 10^7$	0.01–45	0.9943	14.5	21–22	3.0–3.6	39 (9.3)	123 (2.5)	10

[a] Calculated using: recovery $= 100(X_S - X_U)/K$, where X_S = concentration measured in spiked sample, X_U = concentration measured in unspiked sample and K = known value of the spike in the sample; $n = 3$; 100 ng spiked into each sample.

[b] Limit of detection (LOD) calculated using a signal to noise ratio of 10.

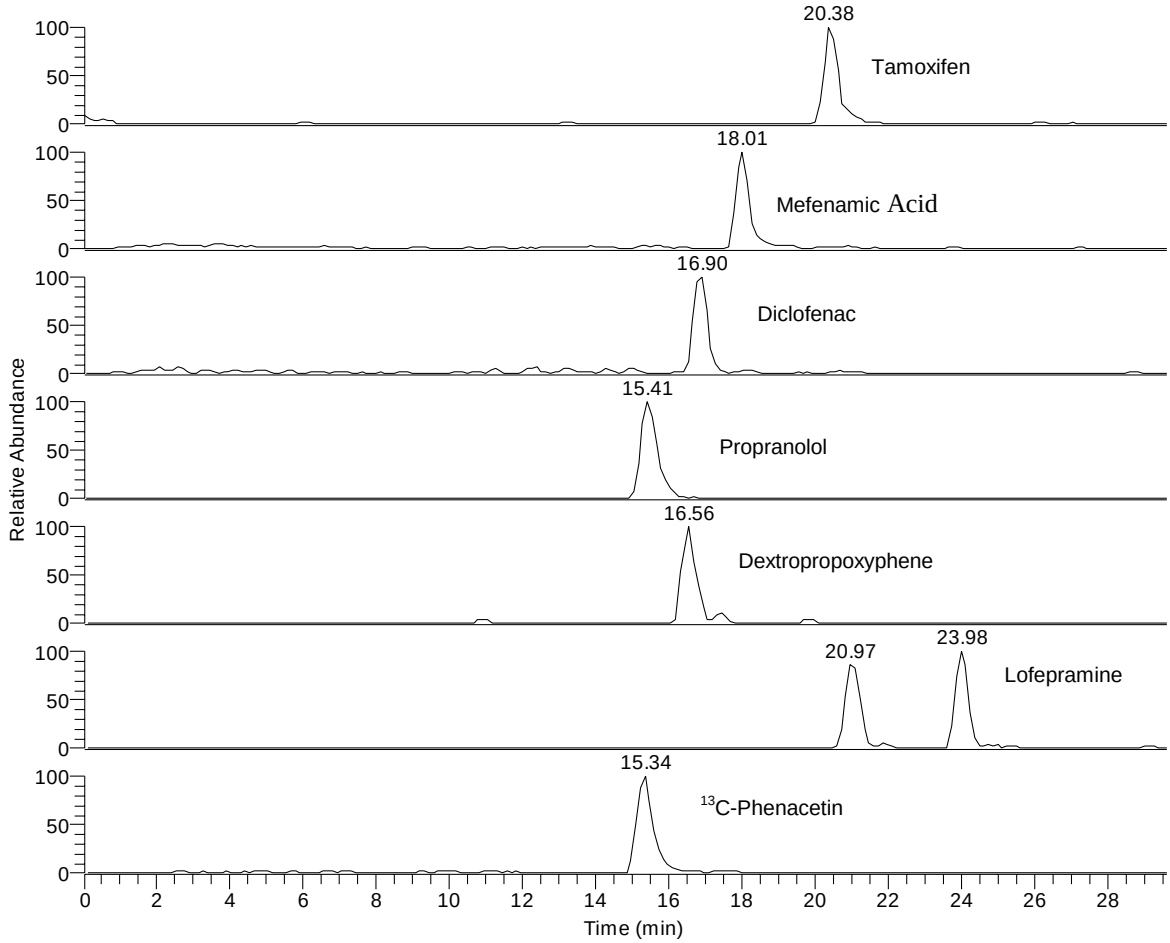


Fig. 2. Chromatograms showing the analysis of selected pharmaceutical standards spiked into tap-water and extracted by SPE. Retention times (min) from top: tamoxifen (20.4), mefenamic acid (18), diclofenac (16.9), propranolol (15.4), dextropropoxyphene (16.6), lofepramine (24.0) and [^{13}C]-phenacetin (15.3).

The same effect was found for positively ionised paracetamol and so an additional gradient was set up for paracetamol alone. The omission of ammonium acetate was done knowing that the absence of a buffer would also result in less stable peak retention times. However, variation of these retention times was not so great that peak identification was significantly hindered. Ammonium acetate was used as a buffer in the other gradients since it is known to have a low suppressing effect on the signal [15]. In the case of the positive ionisation gradient, >90% methanol was required to elute some of the compounds from the column, hence the gradient was set-up to increase to 100% methanol. This resulted in a decrease in the amount of buffer used in the positive ionisation gradient. In the majority of cases this decrease in ammonium acetate would have a minimal effect since the majority of the compounds are eluted between 15 and 18 min. However, the addition of a fourth solvent to ensure that ammonium acetate concentration remains constant will be investigated in future as this may improve the recoveries of tamoxifen and lofepramine.

Areas of ion suppression by the matrices were identified by injecting a blank sample matrix into a stream of analyte, which caused an elevated baseline. Any suppression caused by the matrix was then observed

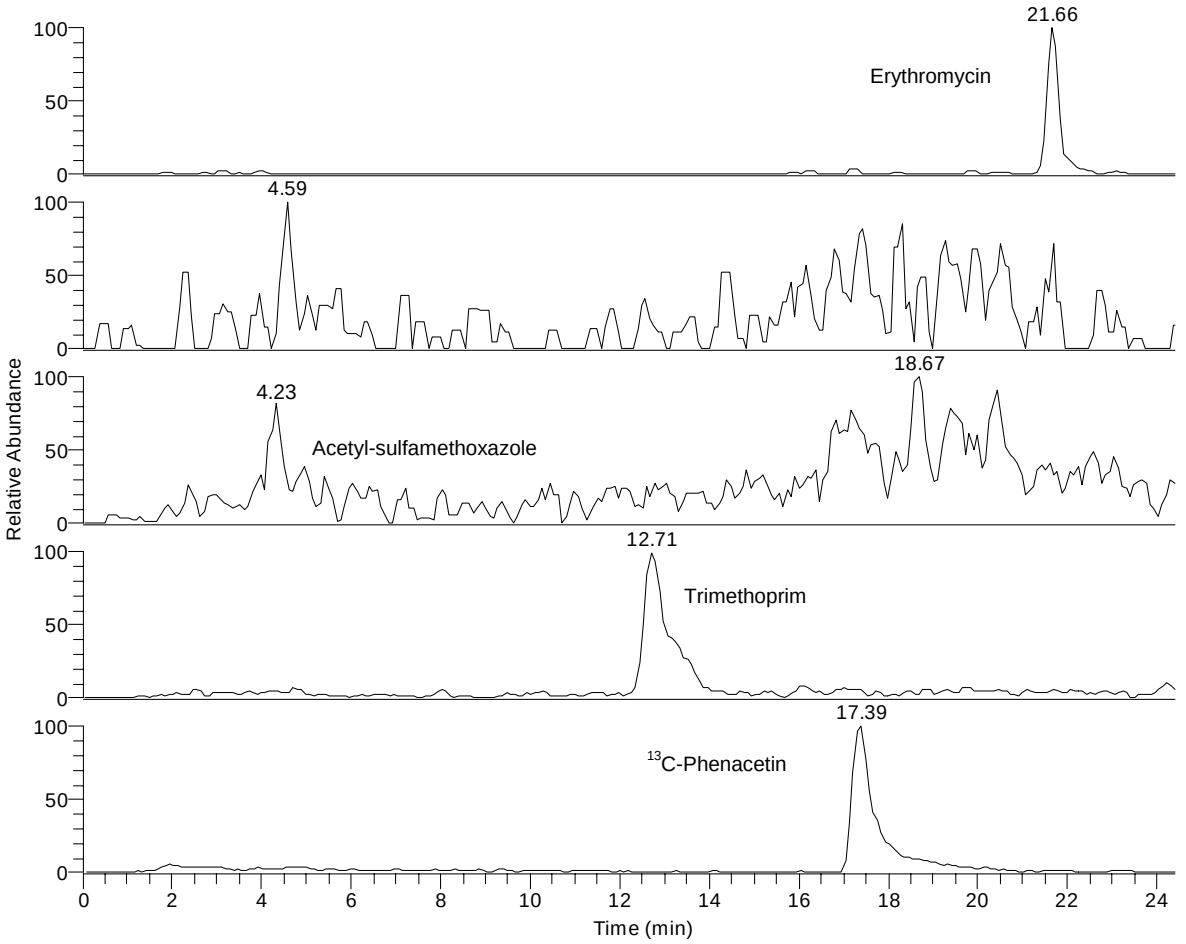


Fig. 3. Chromatograms showing the analysis of target antibiotic compounds in sewage effluent. Retention times (min) from top: erythromycin (21.7), sulfamethoxazole (not detected), acetyl-sulfamethoxazole (4.2), trimethoprim (12.7), and [^{13}C]-phenacetin (17.4).

as a drop in that baseline [18]. This was performed for the sewage effluent and freshwater matrices. Areas of suppression are shown in Table 7. Only the suppression of acetyl-sulfamethoxazole caused by the effluent matrix was a cause for concern. However, sample peaks occurred around 4 min, approximately 0.5 min after the identified area of suppression, and therefore would not usually be expected to affect sample quantification.

3.5. Application

As an application the occurrence of selected pharmaceutical compounds in three sewage effluent samples and an upstream and downstream sample, were investigated. Concentrations are shown in Table 8. Mefenamic acid, diclofenac, propranolol, erythromycin, trimethoprim and acetyl-sulfamethoxazole were all detected in surface waters downstream of the discharge and effluent samples. Ibuprofen was detected in the highest concentrations in the discharge being in the $\mu g\,l^{-1}$ range for all three effluent samples (maximum $3.8\,\mu g\,l^{-1}$) though surprisingly was not detected downstream of the site. However, additional downstream samples analysed using this method showed detectable concentrations of ibuprofen present in surface water samples downstream of sewage discharges (see [13]). Dextropropoxyphene

Table 8
Concentration ($ng\,l^{-1}$) of selected pharmaceuticals in effluents and surface waters upstream and downstream of the STW discharge

	Paracetamol	Mefenamic acid	Diclofenac	Propranolol	Dextropropoxyphene	Lofepramine	Tamoxifen	Clofibric Acid	Ibuprofen	Erythromycin	Sulfamethoxazole	Trimethoprim	Acetyl-sulfamethoxazole
Effluent 1	<50	1100	460	180	230	<10	<10	<50	1800	180	<50	270	2200
Effluent 2	<50	800	350	160	110	<10	<10	<50	1700	130	<50	83	690
Effluent 3	<50	720	410	130	260	<10	<10	<50	3800	180	<50	140	1200
Upstream	<50	<50	<20	<10	<20	<10	<10	<50	<20	57	<50	<10	<50
Downstream	<50	65	91	37	<20	<10	<10	<50	<20	1000	<50	39	240

was also detected in effluent samples only. Concentrations of this magnitude would be expected for ibuprofen since it has previously been detected at similar concentrations, is used in large amounts in the UK, undergoes a low degree of human metabolism, and has also been reported as relatively persistent in aquatic systems [5,6,10–12,14,19].

These data show that the methods are suitable for environmental monitoring. The methods will be used to establish the occurrence of selected human pharmaceutical compounds in UK STW effluents and surface waters.

4. Conclusions

A simple procedure has been developed that allows the simultaneous extraction and pre-concentration of 13 pharmaceutical compounds. The analytes were then quantified using four separate HPLC–MS/MS or HPLC–MS techniques, which provided good sensitivity and selectivity. The method has been shown to provide good reproducible recoveries and low limits of detection that allow the accurate quantification of the pharmaceutical compounds in environmental samples. The method should be a valuable tool in the detection and determination of the selected pharmaceuticals in surface waters and sewage effluents.

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