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Article

Determination of Endocrine Disrupters and Pharmaceuticals in Sewage Samples by Tandem Solid Phase Clean up/Extraction and High Performance Liquid Chromatography-Negative and Positive Electrospray High-Resolution Mass Spectrometry

Fernanda B. Queiroz,[a] Júlio C. Silva,[a] Sérgio F. Aquino,[b] Maurício X. Coutrim[b] and Robson J. C. F. Afonso[b]*

[a]Programa de Pós-Graduação em Engenharia Ambiental and [b]Departamento de Química, Instituto de Ciências Exatas e Biológicas (ICEB), Universidade Federal de Ouro Preto, Campus Universitário S/N, Morro do Cruzeiro, 35400-000 Ouro Preto-MG, Brazil

Foi desenvolvido e validado um método para determinação de três perturbadores endócrinos (estradiol, etinilestradiol e bisfenol A) e cinco produtos farmacêuticos (sulfametoxazol, trimetoprima, diclofenaco, bezafibrato e miconazol) em amostras de esgoto bruto, utilizando extração em fase sólida (SPE) e cromatografia líquida à espectrometria de massas em alta resolução (HPLC-HRMS) com ionização por *eletrospray,* nos modos positivo e negativo. Foi utilizado na extração em fase sólida um cartucho de troca iônica forte (Strata SAX) e um cartucho contendo divinilbenzeno-pirrolidona, para reduzir os níveis dos alquilbenzeno-sulfonados de cadeia linear (LAS) e para concentrar os analitos de interesse das amostras de esgoto. A influência do efeito matriz na eficiência da ionização, a recuperação da EFS e a sensibilidade do método foi identificada e quantificada. O método foi aplicado com sucesso na determinação dos analitos em amostras de esgoto coletadas na entrada da estação de tratamento de efluentes (ETE) do Arrudas em Belo Horizonte, MG, Brasil.

A new method for the determination of three endocrine disrupters (estradiol, ethinyl estradiol, and bisphenol A) and five pharmaceuticals (sulfamethoxazole, trimethoprim, diclofenac, bezafibrate and miconazole) in raw sewage samples using tandem solid phase extraction (SPE) sorbents and high-performance liquid chromatography-negative and positive electrospray high-resolution mass spectrometry (HPLC-HRMS) was developed and validated. The SPE procedure used both a strong ion exchange sorbent (SAX) and a modified divinylbenzene-pyrrolidone SPE sorbent to reduce the levels of linear alkylbenzene sulfonate (LAS) and to concentrate the analytes of interest from the sewage samples. The influence of matrix composition on the ionisation efficiency, the SPE recoveries, and the sensitivity of the method was identified and quantified. The method was successfully applied to the determination of analytes in raw sewage samples collected from the entrance of the Arrudas Sewage Treatment Plant, Belo Horizonte, Brazil.

Keywords: pharmaceuticals, endocrine disrupters, high performance liquid chromatography tandem mass spectrometry, solid phase extraction, sewage

Introduction

In recent years, there have been numerous reports of a variety of organic compounds being detected throughout the world at low concentrations (ng L^{-1}) in samples of surface water, wastewater, groundwater, and even drinking water.[1-7] These compounds, commonly referred to as emerging contaminants, are widely used by humans and include drugs from different classes, such as painkillers, antibiotics, anti-inflammatory, lipid regulators, and synthetic hormones, as well as substances used as sunscreens, personal hygiene products, plasticisers and detergents. These compounds appear as a new class of organic pollutants of environmental concern because of their high potential for impacting the environment and human health.[8] Most of them are recalcitrant, polar compounds and, as a result, are highly mobile in aquatic environments.[9] Some compounds are endocrine disruptors in humans and animals[10] and have attracted the attention of both the public and the scientific community because of their potential carcinogenic and

*e-mail: sergio@iceb.ufop.br

estrogenic properties. The effects of these compounds are cumulative and may only appear in later generations.[11]

The main route of entry of these compounds into the environment is the discharge of domestic sewage, raw or treated, into watercourses. These compounds can also be introduced into the environment through the discharge of effluents of pharmaceutical industries, rural wastes and by the improper disposal of unused pharmaceutical products.[12] Therefore, it is desirable to determine the concentrations of these compounds at different stages of treatment in sewage treatment plants to evaluate their removal efficiency.

Domestic sewage is a very complex matrix, and it is challenging to work with from an analytical point of view. It can contain a variety of inorganic elements and organic compounds, such as humic and fulvic acids, proteins, lipids, and detergents, all at higher concentration when compared with the analytes of interest. For example, the most widely used anionic surfactants, linear alkylbenzene sulfonates (LAS), and their degradation products, sulphonocarboxylic acids, are found in sewage at concentrations as high as 10 mg L^{-1}. Because of their abundance and surfactant activity, LAS are significant analytical components that can interfere at the analytes recoveries during the sample preparation and on signal responses at equipment detectors.[13]

Generally, analytical procedures for determining emerging contaminants in sewage samples use solid phase extraction (SPE) for the concentration and clean-up of sample extracts. These procedures must focus on the compounds of interest and on achieving detectable levels by the instruments being used.[14] Due to its high specificity and sensitivity, high performance liquid chromatography coupled to mass spectrometry (HPLC-MS) has been used for the analysis of emerging contaminants in environmental water samples. The most commonly used ionisation sources are electrospray (ESI), used mainly for polar compounds, and atmospheric pressure chemical ionisation (APCI), used for low and medium polarity compounds.[15,16] A problem commonly encountered while using ESI and APCI for the analysis of environmental samples is the occurrence of the "matrix effect", in which the matrix composition alters the ionisation efficiency and either reduces or intensifies the ion signals of the analytes.[17] According to Taylor,[18] the matrix effect is the "Achilles' heel" of the HPLC-MS technique. Despite having been disregarded in many studies, it is extremely critical to evaluate the influence of matrix composition on the HPLC-MS responses of analytes.

Few references exist that have evaluated the matrix effect in the analysis of emerging contaminants in sewage samples, probably a result of the use of expensive isotope dilution methods. An evaluation of the suppression effect was performed by Chiu[19] with estrogenic compounds. Jahnke[20] and Koh[21] also studied the suppression effects in alkylphenols.

Possible solutions for the problem of variable analyte signal from matrix components include modifications to the mass spectrometer operation conditions, changes to the chromatographic conditions and, for very complex samples, improvements in the sample preparation steps for purification or clean-up of extracts. This work presents a novel procedure for the analysis of three endocrine disrupters and five pharmaceutical compounds. The procedure uses a strong ion exchange followed by a modified divinylbenzene-pyrrolidone SPE sorbent to reduce the levels of LAS (clean-up) and to concentrate analytes from sewage samples.

Experimental

Chemicals and materials

All solvents used were HPLC grade and were acquired from J. T. Baker, with the exception of ethyl acetate, which was acquired from Mallinckrodt. High purity water (HQ) was obtained from a purification system with activated carbon cartridges and ion exchange resin (TKA Wasseraufbereitungssysteme, Germany).

All glassware was initially washed with Extram® (2.5%), rinsed thoroughly with HQ water and placed in nitric acid (10%) in an ultrasonic bath for at least an hour. Then, the glassware was rinsed with HQ water to completely remove all nitric acid. Finally, the non-volumetric glassware was dried in an oven at 60 °C. Deactivated autosampler vials were purchased from Shimadzu, Japan.

Reference standards (> 98% purity) were purchased from Sigma-Aldrich, USA (17β-estradiol, 98%, CAS 50-28-2; 17α-etinilestradiol, 98%, CAS 257-63-6; and bisphenol A, 98%, CAS 80-05-7), from USP, USA (sulphamethoxazole, > 99%, CAS 723-46-6 and trimetroprime, > 99%, CAS 738-70-5) and from Pharma Nostra, Brazil (sodium diclofenac, 99.9%, CAS 5207-79-6; bezafibrate, 99.9%, CAS 41859-67-0; and miconazole nitrate, 98%, CAS 2832-87-7). The standards were used for analytical curves. Additionally, they were spiked into the samples before extraction for the recovery tests and into sample extracts for matrix effect corrections.

Stock solutions of the analytes (0.5 to 1 g L^{-1}) were prepared in methanol and stored in the freezer at −18 °C. Working solutions were prepared fresh daily by diluting the stock solution with methanol prior to analysis with adjustable micropipettes. LAS solutions were prepared in HQ water at appropriate concentration just before use. For method development, method validation and sewage sample analysis, samples were collected at the Center for Research

and Training on Sanitation (CePTS) of UFMG/COPASA, which is contiguous with the sewage treatment plant (STP) of Ribeirão Arrudas basin and is located in Belo Horizonte, MG, Brazil. A total of 12 samples of raw sewage were collected over a period of three months (June to August, 2010). Sewage sampling was carried out over 24 h by means of a collector device which kept the samples refrigerated with crushed ice. After the 24 hours of collection, 1 L of the cooled grabbed samples were then taken to amber bottles with polypropylene caps and preserved with the addition of 10 mL of HPLC grade methanol. The 1 L samples were then vacuum filtered through 8 µm cellulose and 0.7 µm fibreglass filters (Whatman, UK) to remove suspended solids prior to SPE extraction.

Solid phase extraction

For the SPE method development, 6 mL cartridges containing 500 mg of Strata SAX (quaternary amine strong ion exchange) and Strata X (modified divinylbenzene-pyrrolidone), all from Phenomenex, USA, were used. All SPE extractions and elutions were carried out manually on a vacuum manifold from Phenomenex, at a liquid constant flow rate of 5 mL min^{-1}. After air-drying, the SPE cartridges were wrapped in aluminium foil and stored in a freezer until elution.

Several preliminary experiments focusing on the removal of LAS from sewage samples extracts were carried out varying the type of sorbent, pH of the sample, elution solvents and conditions.

The efficiency of the Strata SAX® cartridge in the removal of LAS was qualitatively evaluated by comparing the LAS $[M\text{-}H]^-$ ion peak intensities in the extracts from a synthetic sample produced by two SPE procedures: one using only the Strata X cartridge and the other using Strata SAX followed by Strata X. The results showed that the SPE procedure proposed by US EPA Method 1694 (2007),[22] which uses HLB® (Hydrophilic-Lipophilic Balance reversed-phase sorbent) alone, and other methods that use C18 SPE yielded sewage extracts with significant amounts of LAS, as it will be seen in the Results and Discussion section. Therefore, the following experiment was devised to verify whether LAS remained after the pretreatment stages. First, two synthetic samples containing 100 µg L^{-1} of the analytes in HQ water were produced and LAS were added in one of them until a concentration of 10 mg L^{-1}. Then 100 mL of the synthetic sample containing LAS was extracted using the extraction procedure described in the US EPA Method 1694. For this, 100 mL of both synthetic samples, with and without LAS, were first passed through Strata SAX® cartridges, and then the entire eluted volumes were acidified with drops of concentrate aqueous HCl (10 mol L^{-1}) to pH 2, treated with 50 mg of EDTA and then passed through Strata X®. The conditioning of the Strata SAX® cartridges was done with 10 mL of methanol and 10 mL of water. The conditioning of the Strata X was done, at the same conditions of US EPA Method 1694, with 10 mL of methanol, 10 mL of water and 6 mL of acidified water at pH 2. The Strata X cartridge was washed with 10 mL of water, and the extracts were eluted with 6 mL of methanol and 3 mL of a mixture of acetone and methanol (1:1), as suggested by US EPA Method 1694. Elution from the Strata SAX® cartridges was accomplished with 10 mL of ethyl acetate. The extracts were separately collected into a 15 mL amber flask, evaporated to dryness with a gentle flow of nitrogen and reconstituted in 0.3 mL of methanol plus 0.1 mL of 1% formic acid in methanol.

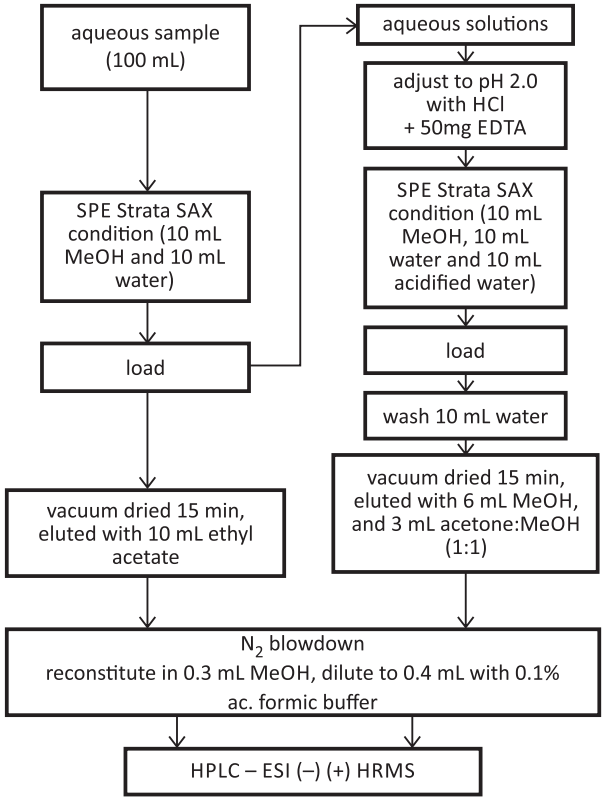

Figure 1. Flowchart of the procedures for pretreatment and extraction of analytes from the samples.

The extracts were then transferred to deactivate vials, acquired from Shimadzu, Japan, and capped with silicon/PTFE septa. All extracts were treated and analysed separately, and Figure 1 presents the flowchart of the procedures used for samples pre-treatment and extraction of the target analytes. The same serial SPE procedure was used for the method validation and to establish the recoveries of raw sewage with and without standards spiking.

High performance liquid chromatography-high resolution mass spectrometry

Liquid chromatography (LC) analyses were carried out on a Shimadzu Prominence system equipped with a high-pressure binary solvent delivery system (LC-20AD) and a SIL 20AC autosampler. The injection volume was 5 µL, and the chromatographic separation was performed on two sequential Shimadzu Shimpack VP ODS columns (3.0 µm × 150 × 2.0 mm) maintained in a GBC oven at 45 °C. The mobile phase flow rate was 0.2 mL min^{-1}, and the following five different mobile phase compositions, as described in the literature, were assessed to obtain the best signal response for the majority of the analytes: (*i*) 5 mmol L^{-1} oxalic acid in water and methanol/acetonitrile (1:1); (*ii*) 0.1% ammonium acetate and acetic acid in water and methanol/acetonitrile (1:1); (*iii*) water and methanol/acetonitrile (1:1); (*iv*) water and methanol; (*v*) 3 mmol L^{-1} NH_4OH in water and methanol.

Chromatographic separation was performed using a gradient method according to the following program: 40% B to 80% B in 6 min; hold at 80% B for 4 min; 80% to 100% B in 10 min; hold at 100% B for 8 min; reduce to 40% B in 0.5 min and hold for another 7 min. The total run time was 35 min.

Table 1. Mass spectrometry time segments for selected ion monitoring

Segment	time / min	Analyte	Selected ions monitored / (*m/z*)
1	0.0-4.0	Sulphamethoxazole	254.0594 $(M+H)^+$
2	4.0-10.5	Trimethoprim	291.1452 $(M+H)^+$
		Bezafibrate	360.1008 $(M-H)^-$
		Diclofenac	294.0091 $(M-H)^-$
3	10.5-14.5	Bisphenol A	227.1070 $(M-H)^-$
		Estradiol	271.1704 $(M-H)^-$
		Ethinyl estradiol	295.1704 $(M-H)^-$
4	14.5-18.0	SCAN	–
5	18.0-25.0	Miconazole	416.9913 $(M+H)^+$
6	25.0-35.0	Full scan	–

Mass spectrometry detection was performed using a Shimadzu LC-ESI-IT-TOF MS instrument working at high resolution and high mass accuracy (< 5 ppm) under the following conditions: ESI ionisation at +4.5 kV and −3.5 kV (positive and negative mode, respectively), nebuliser gas at 1.5 L min^{-1}, curved desorption line (CDL) interface at 200 °C, drying gas at 100 KPa, and octapole ion accumulation time of 100 ms. Full scan mass spectra from *m/z* 100 to 500 were acquired with a scan time of 0.1 s. The equipment allows segmentation in time for selected ion monitoring (SIM), which was divided into 6 segments as shown in Table 1. All mass spectra were obtained with a resolution of at least 10,000 full width at half maximum (FWHM) at *m/z* 500.

The matrix effect on the ion signals of the analytes in the ESI source was estimated for each compound in each extract produced as a percentage variation in signal intensity in the sample matrix versus the intensity observed with pure solvent using equation 1, as described by Vieno.[23] The spiked amount was such that the added concentration in the final extract for each analyte was 30 ng mL^{-1}. This procedure was applied to all SPE extracts produced.

$$\% \text{ variation of the signal} = (A_{standard} - (A_{spiked} - A_{analyte})/A_{spiked}) \times 100 \quad (1)$$

where: $A_{standard}$ = peak area of the analyte in pure solvent standard solution; A_{spiked} = peak area of analyte in the spiked extract; $A_{analyte}$ = peak area of analyte in the extract, without spike.

Since extraction/concentration recoveries should be estimated to accurately determine the analytes concentrations, the following procedure was adopt throughout this work: *i*) at least two sample aliquots were produced; *ii*) one of them was extracted without spiking (blank); *iii*) the other was spiked with a known concentration of analytes and extracted at same conditions as without spiking; *iv*) after elution, the extracts produced were divided in two fractions; *v*) to one of them it was added a known amount of analytes. The extraction/concentration recoveries were established by knowing the chromatographic peak areas of analytes in all extracts and taking into account the extract volumes and the external solvent analytical curves. Using equation 2, the corrected extraction areas could be calculated. The corrected extracted concentrations were established, based on corrected peak areas and pure solvent external analytical curves. The % recoveries were established dividing the extract concentration found by the expected spiked concentration, times 100, equation 3.

$$\text{Corrected extraction area} = (A_{sample\ spiked} - A_{smaple\ blank})/\% \text{ matrix variation of the signal} \quad (2)$$

where: $A_{sample\ spiked}$ = peak area of the analyte in the extract of spiked sample; $A_{sample\ blank}$ = peak area of analyte in the extract of sample, without spike; % matrix variation of the signal as described on equation 1.

$$\% \text{ recoveries} = (\text{extracted concentration found/expected spiked concentration}) \times 100 \quad (3)$$

Quantification and method validation parameters

The quantification of analytes was performed using high-resolution SIM areas and external analytical curves. The choice of external analytical curves and signal correction due to the matrix effect was a result of both the considerable cost of isotope-labelled compounds and the difficulty of purchasing them in Brazil.

The validation parameters used in this study were selectivity, precision, accuracy, analytical curve adjustment/linearity and range and detection and quantitation limits (LOD and LOQ) for both the instrument and method. All instrumental validation parameters were determined with known concentrations of standards.

The selectivity was ensured by liquid chromatography retention times and selected ion chromatograms of protonated and deprotonated species at high resolution. Therefore, a highly selective method was achieved because only the ions of interest in a very restricted range (± 20 mg L^{-1}) were monitored and detected by the mass analyser.

External standardisation, by means of seven injections of replicate standards at concentrations of 5, 10, 30, 50, 100, 130, 200 and 250 μg.L^{-1} in methanol was used to determine the analytical curve adjustment/linearity, which was plotted by quadratic regression. The curve adjustments/linearity were evaluated on the basis of normal distribution graphs of the residues, and the accepted correlation coefficients were above 0.97 for miconazole and 0.99 for the other analytes.

Equipment LODs and LOQs were determined using a signal-to-noise approach from successive dilutions of analytical standards. The LOD and LOQ corresponded to the concentrations that gave an estimated signal-to-noise ratio of 3:1 and 10:1, respectively. For confirmation purposes, the method quantitation limits (MQLs) were calculated using the concentration factors for the sample extracts, the recoveries and the matrix effects on the analyte ion signals.

The precision of the equipment was assessed in terms of repeatability through the calculation of the coefficient of variation (CV) for seven replicates of standards prepared in methanol. The precision of the method was also evaluated in terms of the repeatability of recovery tests using three sewage samples spiked to a final concentration of 100 ng mL^{-1}.

The accuracy was estimated by the recovery tests because the recovery indicates the amount of a given analyte recovered in the procedure in relation to the actual quantity originally present in the sample. Because other matrix components may interfere with the extraction, separation, detection and quantitation of the analytes, the effects of matrix components were considered in the recovery tests of sewage samples. The matrix effects on the analyte ion signals were corrected by the areas of the spiked analytes in the final extracts. This procedure also ensured method accuracy.

Results and Discussion

Five pharmaceuticals and three endocrine disrupters were the subject of this research (Table 1). The choice of these compounds was mainly based on data of therapeutic classes found in the environment, which was compiled by Santos[24] from 134 articles published between 1997 and 2009, and also on the occurrence of such compounds in surface waters in the state of Minas Gerais, Brazil.[25,26]

Solid phase extraction/clean-up and signal suppression

Preliminary extraction tests with C18 and Strata-X® (similar to the Oasis HLB® used in US EPA-1694) showed considerable amounts of LAS in sewage sample extracts, which, in some cases, completely suppressed the analyte ion signals. SPE experiments were carried out to evaluate the

Table 2. Chosen analytes and their physicochemical properties[27-31]

Compound	Molecular formula	Molecular weight / (g mol^{-1})	pKa	Log K_{ow}	Water solubility / (mg L^{-1})
Endocrine disrupters					
Bisphenol A (BPA)	$C_{15}H_{16}O_2$	228.29	10.2	3.32	120
17β-estradiol (E2)	$C_{18}H_{24}O_2$	272.38	10.4	3.94	13
17α-ethinyl estradiol (EE2)	$C_{20}H_{24}O_2$	296.40	10.4	4.8	4.83
Pharmaceutical					
Sulfamethoxazole (SMZ)	$C_{10}H_{11}N_3O_3S$	253.28	5.8	0.9-2.5	610
Miconazole (MCZ)	$C_{18}H_{15}Cl_4N_3O$	479,14	6.7	6.1	0.01
Sodium diclofenac (DCF)	$C_{14}H_{10}Cl_2NNaO_2$	318.13	4.2	4.2 -4.5	2.4
Bezafibrate(BZF)	$C_{19}H_{20}ClNO_4$	361.82	3.3	4.3	0.355
Trimethoprim (TMP)	$C_{14}H_{18}N_4O_3$	290.32	7.1	0.8-1.4	0.4

efficiency of the strong ion exchange Strata SAX® sorbent with regard to the removal of interfering LAS, thereby minimising the contribution to matrix effects.

The efficiency of the Strata SAX® cartridge in the removal of LAS was qualitatively evaluated by comparing the LAS $[M\text{-}H]^-$ ion peak intensities in the extracts from a synthetic sample produced by two SPE procedures, one using only the Strata X cartridge and the other using Strata SAX followed by Strata X. Figure 2 shows the two extracted ion chromatograms of LAS $[M\text{-}H]^-$ ions from varying chain lengths (C10-C13 at *m/z* 297.1530, 311.1686, 325.1843 and 339.1999) in the extracts obtained from 100 mL of a synthetic sample containing 10 mg L^{-1} of LAS. In ion chromatogram (Figure 2a), obtained from Strata X extraction only, there was a significant residual concentration of LAS with large, saturated ion peaks. In the chromatogram in Figure 2b, obtained by passing the sample in tandem through Strata SAX and Strata X, there was a significant reduction in the intensity of the LAS $[M\text{-}H]^-$ ions. The chromatogram in Figure 2b clearly showed a significant removal of LAS by using Strata SAX® sorbent before Strata X®, demonstrating that a significant fraction of LAS was retained in the first Strata SAX® cartridge.

The use of the SAX sorbent has also previously been used by Prado[32] to clean up activated sludge samples for the analysis of tetracyclines by HPLC and UV/Vis detection. However, the article did not describe what contaminants were retained by the SAX sorbent.

Another experiment was performed to evaluate the influence of the Strata SAX on the removal of analytes in the presence of LAS. The elution of analytes from the Strata SAX sorbent by ethyl acetate was also evaluated in the same experiment. In accordance to the described experimental section, the efficiencies of both extraction procedures and the recoveries of the samples generated by the two sorbents are presented in Table 3. The data were obtained from analytical curves, and the matrix effects were corrected

As shown in Table 3, when using just the Strata X sorbent, only four of the nine analysed compounds yielded an analytical signal. These compounds achieved recoveries that ranged from 12 to 87% when LAS concentrations were 10 mg L^{-1}, which is typical in Brazilian sewage.

Table 3 shows that the recovery of sulphamethoxazole was low, especially when only Strata X was used. Other references have also described low recoveries of sulfamethoxazole. Kasprzyk-Hordern[30] reported recoveries from sewage samples of 32-39% for sulfamethoxazole using MCX® cartridges. Vanderford[33] reported recoveries of 13-35% using Oasis HLB® cartridges in surface water samples. Sacher *et al.* (2001) also reported recoveries of 21-23% using Isolute ENV+® cartridges, as reviewed by Kasprzyk-Hordern.[34] In contrast, Castiglioni[35] reported a

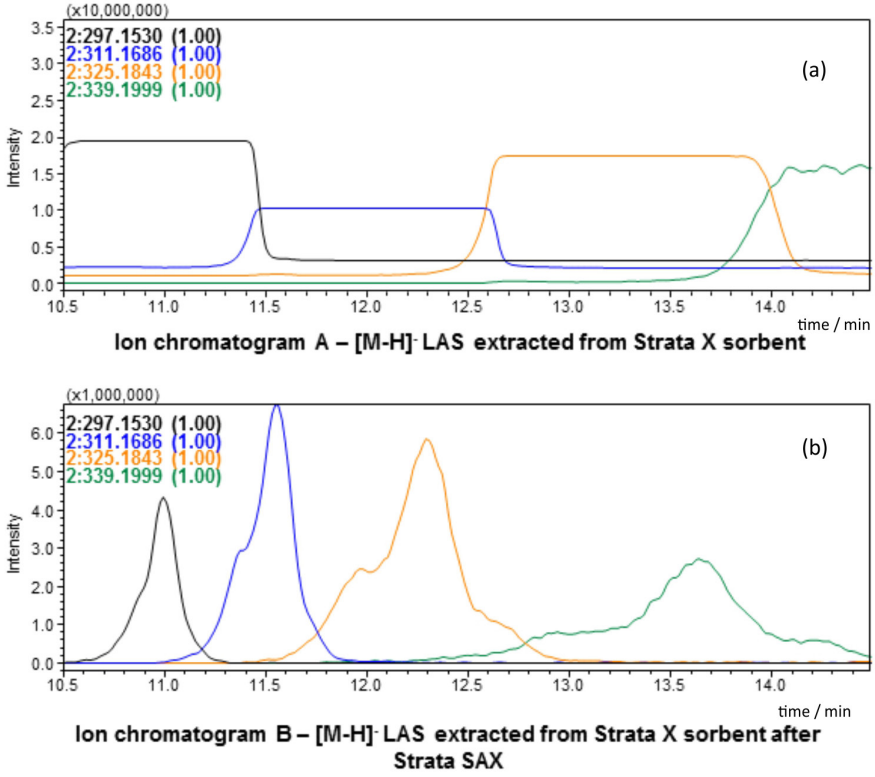

Figure 2. Extracted ion chromatograms of LAS $[M\text{-}H]^-$ species from: (a) $[M\text{-}H]^-$ LAS extracted from Strata X and (b) $[M\text{-}H]^-$ LAS extracted from Strata X sorbent after Strata SAX.

recovery of 60% for sulfamethoxazole in sewage samples using MCX®.

Diclofenac, estradiol, ethinyl estradiol and bisphenol A did not yield analytical signals when synthetic samples containing LAS were extracted using only the Strata X® cartridge. However, after serial extractions with Strata SAX® and Strata X®, such compounds were detected and quantified. The lack of any signal for diclofenac, bisphenol A, estradiol and ethinyl estradiol in the extracts obtained from just the Strata cartridge X® could be explained by the competition in the extraction procedure or by the suppression of the analyte signals due to the presence of large amounts of LAS, thus influencing the ionisation efficiency.

In the evaluated extraction procedure, it is interesting to note that when using two cartridges sequentially (Strata SAX® and Strata X®), where the solutions were first passed through the SAX ion exchange cartridge and subsequently through the Strata X®, there was a significant retention of all analytes in the first ion exchange cartridge. The strong ion exchange sorbent retained all eight tested analytes (Table 1) in the presence and absence of LAS. No reference to the use of this type of ion exchange phase with quaternary amines, for the concentration of these compounds in sewage samples, could be found in the literature.

Yet, for both analyte solutions with and without LAS, the recoveries obtained using the Strata SAX® cartridge were above 50% for six of the eight analysed compounds (diclofenac, bezafibrate, bisphenol A, estradiol, ethinyl estradiol, and miconazole). The poor recoveries for sulfamethoxazole at 26 and 36% with Strata SAX® are compatible with other studies using C18 sorbent.[36] As far as trimethoprim is concerned, Table 3 shows that this compound was not efficiently extracted by the SAX cartridge in the absence of LAS.

As shown in Table 3, trimethoprim showed low affinity for the ion exchange phase in the absence of LAS, with a recovery below 3% and a high coefficient of variation. However, there was a significant retention (36%) of trimethoprim on the Strata SAX® cartridge for solutions containing LAS. In addition, the recoveries for trimethoprim were always around 50% in all extractions using the Strata X® cartridge, and this is probably due to their similar physico-chemical and structural characteristics. Although still not conclusive, a possible explanation for the retention of the trimethoprim on the SAX cartridge in the presence of LAS is that trimethoprim can interact with the linear chains of LAS that are ionically bonded to the quaternary amines in this type of sorbent.

Table 3 also shows that the overall recovery for compounds trimethoprim, bisphenol A, estradiol and ethinyl estradiol after passing the solutions containing LAS through the two cartridges were above 80%. An excessive recovery of bisphenol A was observed in the extracts obtained from the solution in the absence of LAS, and this is rather odd considering that these results were corrected for matrix effects, as it will be seen latter.

The recoveries for the analytes of interest from real sewage samples using the Strata SAX® and Strata X® cartridges in tandem were evaluated and are presented in Table 4. SPE was performed by passing 100 mL of sewage samples through the sorbents. Three samples were spiked to obtain an added final concentration of 100 μg L^{-1} each of trimethoprim, diclofenac, bezafibrate, estradiol, ethinyl estradiol, and bisphenol A. The same procedure was applied to three non-spiked samples, which was necessary because the sewage samples are not free of the target analytes. For sulfamethoxazol and miconazol, due to technical problems, the recovery index could not be assessed as described before. For these two compounds, the recoveries were obtained using a single extract obtained combining the eluates obtained from Strata X and Strata SAX. The recovery indexes for sulfamethoxazol and miconazol were 39.6 ± 2.3 and 33.6 ± 2.1%.

As was observed in the synthetic samples, the first ion exchange cartridge retained all of the analytes. The recovery

Table 3. Percentage of recoveries (± coefficient of variation) of the analytes obtained from two different SPE extraction procedures

Compound	Synthetic solution with LAS (10 mg L^{-1}) fortified with analyte (100 μg L^{-1})				Aqueous solution with analyte (100 μg L^{-1})		
	Strata X only	Strata SAX followed by Strata X			Strata SAX followed by Strata X		
		Strata SAX	Strata X	Σ	Strata SAX	Strata X	Σ
Sulfamethoxazole	12.2 ± 10.5	26.1 ± 8.5	–	26.1 ± 8.5	35.9 ± 6.7	–	35.9 ± 6.7
Trimethoprim	56.6 ± 3.6	36.3 ± 7.5	53.2 ± 4.3	89.5 ± 9.4	2.46 ± 7.3	51.8 ± 9.8	54.3 ± 7.4
Diclofenac	–	54.3 ± 8.6	–	54.3 ± 8.6	60.8 ± 2.7	–	60.8 ± 2.7
Bezafibrate	86.3 ± 3.7	53.4 ± 5.7	–	53.4 ± 5.7	53.4 ± 10.2	–	53.4 ± 10.2
Bisphenol A	–	57.1 ± 4.9	45.4 ± 2.7	102.5 ± 6.7	118.5 ± 4.7	27.2 ± 6.4	145.7 ± 8.1
Estradiol	–	73.8 ± 2.3	6.9 ± 3.2	80.7 ± 4.5	89.4 ± 1.5	3.7 ± 7.5	93.1 ± 5.6
Ethinyl estradiol	–	85.7 ± 4.4	6.1 ± 4.3	91.8 ± 7.7	84.3 ± 1.3	4.2 ± 2.4	88.5 ± 3.9
Miconazole	39.3 ± 0.4	67.1 ± 6.7	–	67.1 ± 6.7	81.5 ± 8.3	–	81.5 ± 8.3

for the Strata SAX® cartridge varied from 17 to 35%, whereas overall recoveries from tandem SPE varied from 30 to 63%. However, in contrast to what was observed with the synthetic samples, diclofenac and bezafibrate showed better recoveries by the Strata X® cartridge. In addition, total recoveries of bisphenol A were different from that observed in the synthetic samples. In the real samples, bisphenol A was better retained on the Strata X® cartridge. For the other compounds, i.e., estradiol, ethinyl estradiol and trimethoprim, the recoveries were lower but proportionally similar to what was observed with the synthetic samples containing LAS. The total recoveries obtained for all analytes were lower than those observed in synthetic samples, but this was expected due to the fact that the sewage matrix was much more complex than the synthetic samples. In addition to the LAS surfactant, sewage also contains proteins, amino acids, oils, fats, enzymes, organic acids and a myriad of other compounds that may decrease the efficiency of sorption on SPE cartridges.[37]

Table 4. Recoveries and variation coefficients for spiked analytes (100 ng L^{-1}) in sewage samples

Compound	Recoveries and CV/ (%)	
	Strata SAX cartridge	Strata X cartridge
Trimethoprim	29.2 ± 1.9	38.5 ± 5.5
Diclofenac	26.7 ± 7.9	19.9 ± 6.8
Bezafibrate	17.3 ± 2.6	38.3 ± 6.1
Estradiol	35.4 ± 5.5	8.0 ± 6.5
Ethinyl estradiol	23.2 ± 9.5	6.6 ± 8.5
Bisphenol A	24.9 ± 9.0	37.6 ± 5.5

Despite the low recovery rates of the analytes, the coefficients of variation were less than 10%. This was regarded as satisfactory, considering that a manual SPE procedure and a vacuum manifold were used in the extraction process. The protocol for the extraction, concentration and clean-up described above was validated and used for the analyses of sewage samples from BeloHorizonte.

Liquid chromatography and mass spectrometry

Mobile phase additives are generally used to improve chromatography efficiency; however, high concentrations of mobile phase additives, despite good separation of analytes, may reduce the sensitivity of ESI-MS detection. To reduce suppression effects, the concentrations of mobile phase additives were kept to a minimum. As detailed in the Experimental section, five different mobile phases were tested. Mobile phase v (3 mmol L^{-1} NH_4OH in water and methanol) yielded the best responses (higher chromatographic peak areas) for the majority of the deprotonated analytes $[M-H]^-$ in negative mode (17β-estradiol (E2), 17α-ethinyl estradiol (EE2), bisphenol A (BPA), diclofenac (DCF) and bezafibrate (BZF)) when compared with the other tested mobile phases. Despite the observation that responses of positive ions (miconazole (MCZ), sulphamethoxazole (SMZ) and trimethoprim (TMP)) were somewhat smaller than those obtained using phase iv (containing only water and methanol), the mobile phase of water and methanol basified with 3 mmol L^{-1} of NH_4OH was chosen for analysis of such compounds. No significant retention time differences were observed upon the addition of 3 mmol L^{-1} of NH_4OH when compared to pure water and methanol mobile phases, even for the poorly retained sulphamethoxazole.

Chromatograms and high-resolution mass spectra of standards at 50 ng mL^{-1} are presented in Figure 3, whereas Figure 4 presents extracted ion chromatograms of three analytes from sewage samples. The utilisation of two columns was chosen because the autosampler and PEEK lines were not capable of dealing with high back pressure above 25 MPa, which was produced by small particle size columns (< 2.5 μm) at a flow rate of 0.2 mL min^{-1}. The oven temperature at 45 °C was also used to reduce column back pressure.

As it can be seen from Figures 3 and 4, the ion chromatograms at high resolution produced very low noise for both $[M+H]^+$ and $[M-H]^-$ ions, even for real sewage sample extracts. The retention times of selected ions were also important criteria for confirmation of the target compounds in the real sample extracts because at high mass resolution, the retention time guarantees selectivity for the analytical method. In general, the compounds in the positive ionisation mode showed higher sensitivity, although the degree of ionisation of each analyte varied significantly depending on the functional groups present in the molecule. The sensitivity for each compound was also estimated by the angular coefficients from the analytical curves. The highest responses were observed for positive ions according to the following order: miconazole > trimethoprim > sulfamethoxazole. The lowest responses were observed for the negative ions of diclofenac and bezafibrate. The estrogenic compounds 17β-estradiol, 17α-ethinyl estradiol and the xenoestrogen bisphenol A gave similar responses, which was expected from their functional and structural similarities.

Analytical curve adjustments/working range

External standardisation was employed for the quantification of the analytes during all development stages

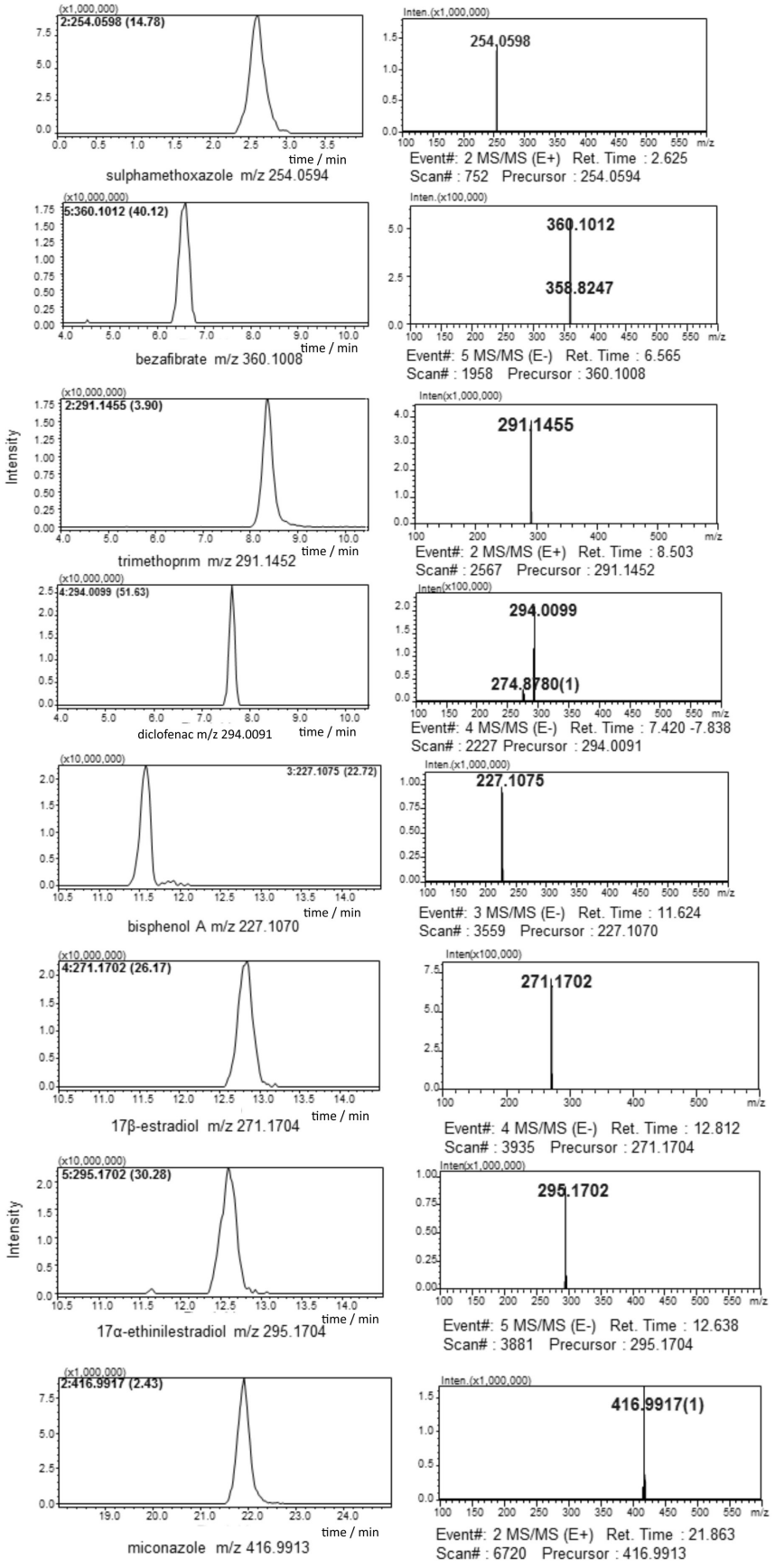

Figure 3. Single ion chromatograms and high-resolution mass spectra of analyte standards at 50 ng mL^{-1}.

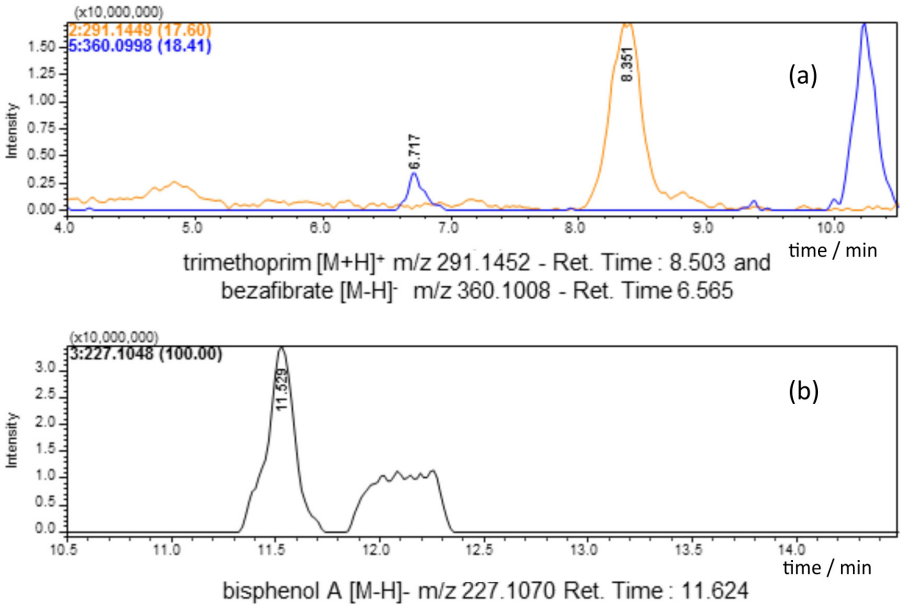

Figure 4. Extracted ion chromatograms from sewage sample extracts: (a) trimethoprim $[M+H]^+$ *m/z* 291.1452, retention time: 8.503 and bezafibrate $[M - H]^-$ *m/z* 360.1008, retention time: 6.565 of trimethoprim and (b) bisphenol A $[M-H]^-$ *m/z* 227.1070, retention time: 11.624.

and for analysis of sewage sample extracts. The analytical curve adjustments were based on quadratic regressions, and all analytical curves were constructed before analysing the sample extracts. For equipment verification, one analytical standard was re-injected every ten sample runs. For validation purposes, the analytical curve was prepared from seven injections of replicate standards over 2 days. The curve adjustments were evaluated based on the normal distribution graphs of the residues, and the correlation coefficients obtained from the analytical curves were 0.97 for miconazole (with 6 points) and 0.99 for all the other compounds (with 8 points). The obtained area values showed minor variations in the responses of the seven replicate analyses at all concentration levels. The highest relative standard deviation found was 13.6% for diclofenac at a concentration of 50 ng mL^{-1}. The working ranges for the analytical curves were from 5 to 250 ng mL^{-1} for estradiol, ethinyl estradiol, bisphenol A, sulfamethoxazole and trimethoprim; from 5 to 100 ng mL^{-1} for miconazole and from 10 to 250 ng mL^{-1} for bezafibrate and diclofenac. The quadratic equation ($f(x) = ax^2 + bx + c$) terms and correlation coefficients (r) are presented in Table 5.

Detection and quantitation limits

Table 5 presents the LOD and LOQ values for the method, which were determined by the signal-to-noise ratios. The method LOD and LOQ values were calculated considering a concentration factor of 250 times, the recovery and the matrix effect, which were all obtained from spiked standard into real sewage samples and extracts. As the matrix effects varied from one sample to another, the calculated values are described within a particular range of variation.

In this work, the limits of detection and quantification obtained for some compounds were lower than the values reported in previous studies. In a study conducted by Carballa[38] using LC-MS/MS and ESI as a source of ionisation, the method limits of detection (MLOD) and quantification (MLOQ) obtained for sulfamethoxazole were 6.7 and 20 ng L^{-1}, respectively. In another study by Kasprzyk-Hordern[30] using ultra performance liquid chromatography (UPLC)/MS and ESI, the MLOQ for bezafibrate was 94 ng L^{-1}. In that same study, the limits of quantification for trimethoprim and diclofenac were 3 ng L^{-1} and 17 ng L^{-1}, respectively, and these values are close to the values found in this work. The literature reports limits of quantification for bisphenol A that range from 10 to 63 ng L^{-1} in sewage samples[30,39] whereas the work performed by Vega Morales[27] using ESI and LC-MS/MS determined a detection limit for bisphenol A of 5.5 ng L^{-1}. In the same work, the detection limits for estradiol and ethinyl estradiol were 3.3 and 2.8 ng L^{-1}, respectively. In another study, Huang[29] reported an MLOQ of 2 ng L^{-1} for miconazole when using UPLC/MS and ESI.

Precision and accuracy

Method precision can be evaluated by reproducibility or repeatability. In this study, repeatability tests were used to verify the preparation of standards and equipment precision. The standards were injected by the same analyst, on the same instrument, under the same conditions of analysis,

Table 5. Quadratic equation coefficients for the analytical curves of the analysed compounds, their correlation coefficients (r) and the calculated method LOD and LOQ

Equation coefficient	SMZ	TMP	MCZ	DCF
A	−415.8	−2,278.1	−6,024.8	−41.6
B	271,625.8	1,449.9	1,202.6	41,668.8
C	981,172.9	6,361.3	5,109.9	−94,684.3
R	0.99	0.99	0.97	0.99
MLOD / (ng L^{-1})	1.5-2.2	1.4-3.3	2.6-4.1	5.0-8.8
MLOQ / (ng L^{-1})	5.1-7.4	3.3-4.7	8.7-13.8	16.5-29.3
Equation	BZF	E2	EE2	BPA
A	−61.7	−186.7	−146.8	−243,0
B	87,380.4	318,443.1	245,123.1	267,592.7
C	−23,384.9	−78,963.9	−85,325.0	1,895.7
R	0.99	0.99	0.99	0.99
MLOD / (ng L^{-1})	3.4-5.1	9.3	12.4	1.2-2.1
MLOQ / (ng L^{-1})	11.3-17.1	31.0	41.3	4.0-7.1

SMZ: sulfamethoxazole; TMP: trimethoprim; MCZ: miconazole; DCF: diclofenac; BZF: bezafibrate; E2: estradiol; EE2: ethinyl estradiol; BPA: bisphenol A.

at the same location, and during a short time interval. Table 6 presents the obtained mean areas and the relative coefficients of variation for the seven replicated injections of standard solutions at three concentration levels, 10, 50 and 100 ng L^{-1}. The CV values were below 13.6% for all three levels, indicating that the method was precise.

The methodology accuracy was evaluated through recovery tests of spiked sewage samples. The sewage samples were spiked with sulfamethoxazole, trimethoprim, diclofenac, bezafibrate, estradiol, ethinyl estradiol, bisphenol A and miconazole, each at final concentrations of 100 ng L^{-1}. The samples were extracted as described before, and the recoveries and their coefficients of variation in both sorbents are presented in Table 4. For sulfamethoxazole and miconazole, recovery was obtained through a combined extract obtained from Strata SAX® and Strata X® cartridges. The recoveries of sulfamethoxazole and miconazole were 39.6 ± 2.3 and 33.6 ± 2, respectively.

Because the matrix effect was corrected by the response of a known amount of spiked standard in the final extracts, and the systematic errors of the SPE recoveries were corrected by acceptable coefficients of variation, the method accuracy was assured.

Evaluation of matrix effects on response of analytes in sewage sample extracts

In complex matrices like sewage, the ion intensities of the target analytes may suffer interference from other substances simultaneously eluting from the column. Such interferences may occur at the point of ionisation

Table 6. Mean areas obtained and their relative coefficient of variation for the seven replicated injections of standards

Compound	Spiked concentration / (ng L^{-1})	Mean area (n = 7)	CV / %
Sulfamethoxazole	10	3,362,895	12.6
	50	14,470,801	9.97
	100	24,345,232	9.13
Trimethoprim	10	23,048,641	7.0
	50	71,415,297	4.2
	100	128,044,825	8.1
Miconazole	10	17,865,560	3.3
	50	50,085,023	3.4
	100	67,833,722	5.8
Diclofenac	10	260,382	12.6
	50	1,888,773	13.6
	100	3,605,733	5.6
Bezafibrate	10	833,683	12.1
	50	4,066,343	10.5
	100	8,229,485	5.1
Estradiol	10	3,285,540	8.6
	50	15,460,020	3.6
	100	29,852,603	2.0
Ethinyl estradiol	10	2,527,342	5.1
	50	11,648,516	7.3
	100	22,610,990	2.1
Bisphenol A	10	4,494,955	5.9
	50	15,611,910	2.8
	100	26,245,075	3.6

due to competition during the ionisation process, thereby enhancing or suppressing the analyte signal[26] which has also been observed by other researchers.[23,40-44] For charged analytes, such as LAS, it is likely there is no charge competition at ESI. However, LAS can suppress other analyte responses due to their tensoactive properties that bring them to droplet surface, where the liquid phase charge transfer reaction should occur on ESI. In an attempt to minimise this effect, necessary corrections to the signal areas were made for all samples. This procedure was performed by adding a known amount of standard to all final SPE extracts, for a final concentration of 30 ng mL^{-1}. Thus, the percentage of matrix effect was calculated according to equation 1 and corrected for each compound in all samples. Because the samples were different, the intensity of the matrix effect underwent variation from one sample to another. For this reason, the corrections were made in all samples. Table 7 shows the percentage of variation on the signal in all sample extracts analysed (n = 12) from each sorbent. Because estradiol and ethinyl estradiol were rarely found at quantitation levels in the sewage samples, their concentrations were not calculated.

Table 7. Matrix effects on signal responses for the analytes in extracts of 12 sewage samples analysed

Compound	Matrix effect / %	
	Strata SAX	Strata X
Sulfamethoxazole	–27.7-49.5	–
Trimethoprim	42.2-69.5	68.3-82.7
Diclofenac	–9.4-79.4	18.6-86.5
Bezafibrate	–27.3-42.2	30.4-58.5
Bisphenol A	68.6-81.5	–55.2-46.2
Miconazole	60.5-93.8	49.4-65.8

Negative values = signal enhancement. Positive values = signal suppression

Because several sewage samples were analysed, a great variation in the matrix effects was expected and subsequently observed. For some analytes, enhancement and suppression of the analytical signal were observed under the same chromatographic conditions in different sewage samples. This is an indication that matrix effects in sewage samples are far more complex than we expected and that have been reported by others authors. The correlation of matrix effect with sample composition and analyte concentration is currently being studied but is not yet established.

Environmental application

After the methodology development, this approach was used to evaluate the occurrence of sulfamethoxazole, trimethoprim, diclofenac, bezafibrate, bisphenol A, estradiol, ethinyl estradiol and miconazole in raw sewage samples generated at the metropolitan area of Belo Horizonte. Samples were also analysed to assess the removal efficiency of these compounds in simplified sewage treatment systems.[45] Figure 5 shows the variations of the concentrations of the antibiotic trimethoprim and sulfamethoxazole, the antilipemic bezafibrate, the anti-inflammatory diclofenac, the antifungal miconazole and the xenoestrogen bisphenol A in raw sewage.

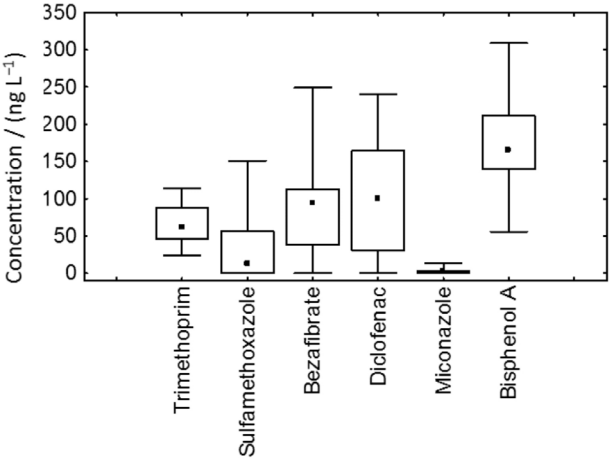

Figure 5. Variations of the concentrations of target analytes in raw sewage samples collected at the entrance of the Arrudas sewage treatment plant (STP).

Bisphenol A and the antibiotic trimethoprim were found in all analysed samples. Nonylphenols were also found in all samples but could not be quantified. The estrogens ethinyl estradiol (EE2) and estradiol (E2) were rarely detected, but when detected, their concentrations were below the MLOQs (12.4 and 9.3 ng L^{-1}, respectively). The fact that the natural (estradiol) and synthetic (ethinyl estradiol) hormones were not frequently detected may be explained by their conversion into estriol and estrone, which were not evaluated. According to Ribeiro[46] and Cajthaml,[47] the hormone estrone (E1) is a by-product of the degradation of E2, and the biodegradation of EE2 involves the formation of conjugated by-products or hydroxylated analogues (EE-OH). Studies carried out by Ternes[48] showed that E1 was found at levels as high as 70 ng L^{-1} and on average of 9 ng L^{-1}, whereas EE2 was found at a maximum of 15 ng L^{-1} and on average of 1 ng L^{-1}. Plosz[49] also found that the concentrations of E2 and EE2 were below the detection limits in all samples of raw sewage from STPs in Oslo, Norway. Desbrow[50] reported concentrations of E2 in domestic sewage that ranged from 1 to 50 ng L^{-1}, and Kim[3] and Tambosi[51] also found low concentrations of EE2 (1.3 ng L^{-1}) and E2 (< 1.0 ng L^{-1}) in samples of raw sewage.

In addition, the same work showed that the concentration of estrone was higher, ranging from 2.2 to 36 ng L^{-1}. Other research groups[52-54] found that the hormones estradiol and ethinyl estradiol were only occasionally detected in samples of raw sewage.

The presence of E2 and EE2 in waterbodies predominantly originates from the discharge of sewage (raw or treated). The data obtained in this study are consistent with results from Moreira[25,26] that reported the detection of these hormones in a few water samples that were collected from surface waters located in the region of this study.

The antibiotic sulfamethoxazole was found in 92% of the samples, and trimethoprim was found in 100% of the samples. The median concentration found of sulfamethoxazole and trimethoprim was 13 and 61 ng L^{-1}, respectively. Zucato[1] found sulfamethoxazole in raw sewage from Italy in the concentration of 246 ng L^{-1}. However, in Spain, the concentration found for sulfamethoxazole by Carballa[42] was 580 ng L^{-1}. In addition, Roberts and Thomas[4] detected trimethoprim in raw sewage from the United Kingdom in concentrations that ranged from 213 to 300 ng L^{-1}.

The anti-inflammatory diclofenac was also detected in 92% of the samples at a median concentration of 100 ng L^{-1}. This value is within the range reported by Kim[3] that found 8.8 to 127 ng L^{-1} and by Kasprzy-Hordern[30] that reported 70 ng L^{-1}. Nevertheless, the diclofenac median concentration found here is well below the values of 250-5,450 ng L^{-1} and 1,200-1,400 ng L^{-1} reported by Andreozzi[55] and Gebhardt and Schroder[9], respectively.

In this study, the antilipemic bezafibrate was detected in 92% of samples at a median concentration of 95 ng L^{-1}. This value is consistent with the study by Rosal[56] who reported concentrations from 48 to 361 ng L^{-1} (mean = 141 ng L^{-1}) in the raw sewage that feeds the STP of Alcalá de Henares in Madrid, Spain. It is also consistent with the values of 168 ng L^{-1} reported by Nodler[28] in treated sewage from Germany. However, another study, also conducted in Spain (Catalonia), reported concentrations of bezafibrate from three STPs ranging from 400 to 1,400 ng L^{-1}.[57] Kasprzy-Hordern[30] also reported a higher concentration of bezafibrate in sewage samples from the United Kingdom of 971 ng L^{-1}.

The antifungal agent miconazole was rarely detected or quantified in raw sewage samples analysed in this study. Only 16.7% of the samples were above the MLOQ, which was estimated to be 3 ng L^{-1}. When found, miconazole was always at low concentrations, and the highest concentration was 13.9 ng L^{-1}. Lindberg[58] conducted a study in Swedish STPs to assess the concentration of six antifungals, among them miconazole. In all samples, miconazole was below the LOQ of 100 ng L^{-1}, which is consistent with the present study, despite the high LOQ obtained by Lindberg.[58] Huang[29] also reported low concentrations (3 ± 1 ng L^{-1}) of miconazole in samples of raw sewage from China, while Roberts and Bersuder,[59] analysing domestic effluents in the United Kingdom, found concentrations of 9 ng L^{-1}. It should be noted that miconazole is a topical medication used at low concentrations.

The median concentration of bisphenol A was 165 ng L^{-1}. In a broad study conducted by Tan,[60] bisphenol A was found in the raw sewage from five STPs in Australia at concentrations ranging from 104 to 2,847 ng L^{-1}. Additionally, in a study from Italy,[61] bisphenol A was found in similar concentrations determined in this work, from 62 to 160 ng L^{-1}. However, other studies have reported higher concentrations of bisphenol A. For instance, Clara[62] found concentrations of bisphenol A that ranged from 720 to 2,376 ng L^{-1} in raw sewage from various STPs. According to Sodré,[63] the average concentration of bisphenol A found in sewage generated by the city of Campinas, in the state of São Paulo, Brazil, was 8,600 ng L^{-1}. This value is well above the concentration found in sewage generated by the metropolitan region of Belo Horizonte, in the state of Minas Gerais.

Conclusions

This paper presents data on the development and validation of a novel solid phase extraction strategy for environmental monitoring in sewage samples of three endocrine disrupters and five pharmaceuticals (antibiotics, anti-inflammatory, and lipid-regulating) in the low nanogram per litre range. The method involves a tandem solid phase extraction, using a strong anion exchange sorbent (Strata SAX) and a modified divinylbenzene-pyrrolidone polymeric sorbent (Strata X), followed by subsequent high performance liquid chromatography, negative and positive electrospray ionisation high resolution mass spectrometry analysis.

The strong anion exchange sorbent was capable of significantly removing linear alkylbenzene sulphonate (LAS) that is present in high concentrations in sewage samples (mg L^{-1}). It was also capable of retaining the analytes that were spiked into the sewage samples. The influences of mobile phase composition, matrix effects and SPE recovery on the sensitivity of the method were evaluated. Matrix effects and low SPE recovery, both resulting from the presence of matrix interferences, were found to be the main factors affecting the sensitivity of the method. Nevertheless, the MLOD and MLOQ were similar to other reported methods for sewage samples that used

surrogate/internal standards in the sample to compensate for losses of compounds during sample preparation and ion signal suppression. Re-analysis of the spiked extracts with known amounts of analyte is machine time-consuming, but it proved to be effective for correction of the matrix effects. Great variation was observed with the matrix effects, and both enhancement and suppression of the analytes signal was observed at identical chromatographic conditions in different sewage samples. The method developed in this study was efficient for determining the compounds of interest with regard to the validation parameters (precision, specificity, accuracy (recovery), limits of detection and quantification and linearity) and was successfully applied to the determination of selected pharmaceuticals and endocrine disrupting compounds in sewage samples.

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