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Fast ultrahigh performance liquid chromatographic method for the simultaneous determination of 25 emerging contaminants in surface water and wastewater samples using superficially porous sub-3 μm particles as an alternative to fully porous sub-2 μm particles

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ABSTRACT

The development of fused-core silica stationary phases was considered a breakthrough in column manufacturing. In this study, a fast LC–UV method was developed and validated for the simultaneous determination of 25 emerging contaminants in surface water and wastewater. The selected analytes belonged to various classes such as veterinary antibiotics, central nervous system stimulants, non-steroidal anti-inflammatory drugs, steroids and preservatives. The chromatographic separation was optimized in order to achieve suitable retention times and good resolution for all analytes in a single run using solvent gradient and flow rate gradient. All analytes eluted within 10 min on a Kinetex® C18 column packed with fused core particles. Sample preparation was executed with solid-phase extraction on Oasis HLB cartridges. The method was validated by assessing linearity, selectivity, accuracy, precision, limits of detection and limits of quantification. Good recoveries were obtained for all analytes ranging from 67.5% to 97.0% with standard deviations not higher than 5.7%, except for acetaminophen, sulphanilamide and acetyl salicylic acid, for which lower recoveries were obtained. The detection limits ranged from 1.5 to 15 μg L^{-1}, while limits of quantification were in the range from 5 to 50 μg L^{-1}. The short analysis time achieved by this method allowed analysis of a large number of samples in a short time, minimizing organic solvent consumption and lowering LC solvents cost. This fast method offers benefits both environmentally and economically.

1. Introduction

High performance liquid chromatography (HPLC) is one of the most popular techniques for qualitative and quantitative analysis of organic compounds. The performance of this technique is highly related to the properties of the stationary phase used [1]. Reducing the analysis time, increasing the selectivity and enhancing the efficiency at the same time are the main criteria in method development for HPLC [2]. High throughput analysis with high resolution is highly desirable for environmental, pharmaceutical and food analyses [3]. The reduction in run time using fast analysis results in the reduction in the overall solvent consumption making the LC analysis greener and lowering costs; these improvements are highly needed in commercial laboratories.

Various analytical strategies have been proposed for fast chromatographic separations based on several approaches. The first one is increasing the mobile phase linear velocity by increasing the pressure or reducing the solvent viscosity. Reduction in mobile phase viscosity can be achieved by using elevated temperature [4]. The second approach aiming at speeding up the analysis without the need for high inlet pressure is to increase the column permeability, which can be achieved by using monolithic columns. These columns are characterized by decreased mass transfer effects compared to conventional fully porous particles [5,6], high permeability and small skeleton size allowing high flow rates to be used with low backpressure on conventional LC systems [7]. However, these columns have poor efficiencies as discussed by Gritti and Guiochon [8].

Another alternative to reducing the analysis time without sacrificing the efficiency is to reduce the column length simultaneously with the reduction of the plate height. This could be done by using small particles, such as sub-2 μm packing; however, very high pressure is needed due to the reduction in the column permeability. Columns packed with fully porous sub-2 μm particles provide faster and more efficient separations compared to conventional 3–5 μm particles [9,10]. Small particle size packing

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Table 1
Characteristics of the studied analytes.

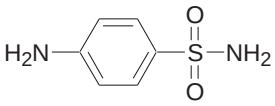
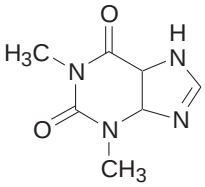
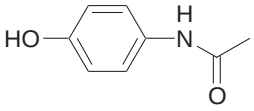
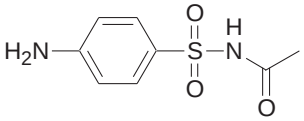
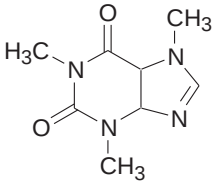
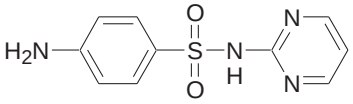
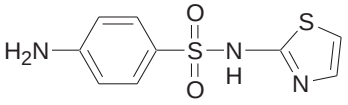
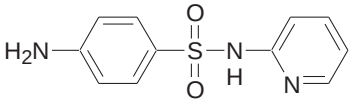
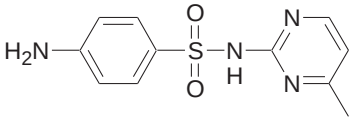
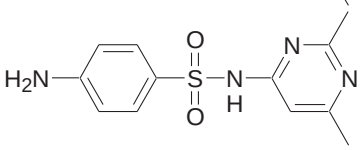
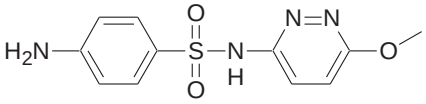
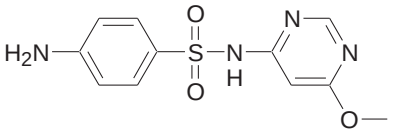
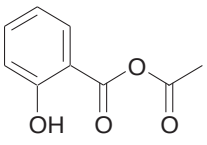
Analyte	Abb	Chemical structures	pKa	λ	Therapeutic use
Sulfanilamide	SNM		10.4	272	Bacteriostatic
Theophylline	THP		8.8	272	CNS stimulant
Acetaminophen	APH		9.5	247	Analgesic
Sulfacetamide	SAM		5.4	272	Bacteriostatic
Caffeine	CFN		14.06	272	CNS stimulant
Sulfadiazine	SDZ		6.5	272	Bacteriostatic
Sulfathiazole	STZ		7.2	272	Bacteriostatic
Sulfapyridine	SPD		8.4	272	Bacteriostatic
Sulfamerazine	SRZ		7.1	272	Bacteriostatic
Sulfamethazine	SMZ		7.4	272	Bacteriostatic
Sulfamethoxypyridazine	SPZ		7.2	272	Bacteriostatic
Sulfamonomethoxine	SMX		6.0	272	Bacteriostatic
Acetyl salicylic acid	ASA		2.97	230	Anti-inflammatory

Table 1 (*continued*)

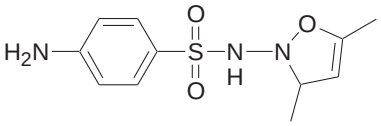
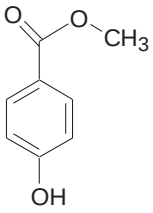
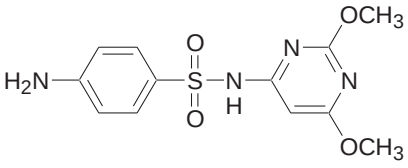
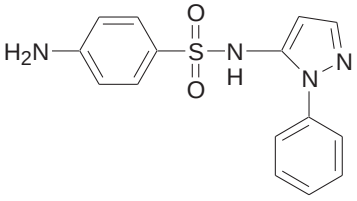
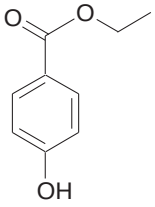
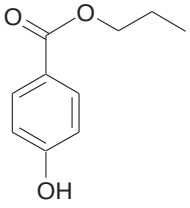
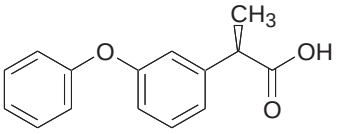
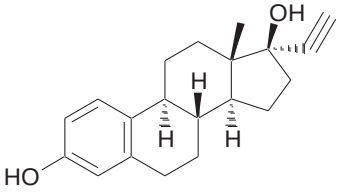
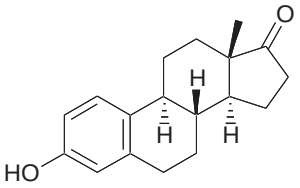
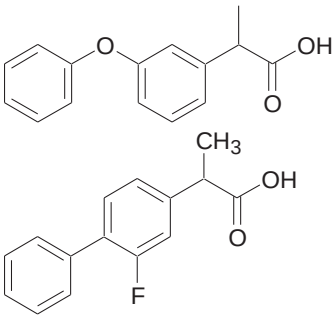
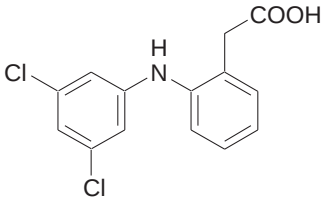
Analyte	Abb	Chemical structures	pKa	λ	Therapeutic use
Sulfamethoxazole	SXZ		5.7	272	Bacteriostatic
Methylparaben	MPN		8.5	254	Preservative & antifungal
Sulfadimethoxine	SDM		6.1	272	Bacteriostatic
Sulfaphenazole	SPZ		6.5	272	Bacteriostatic
Ethylparaben	EPB		8.4	254	Preservative & antifungal
Propylparaben	PPN		8.4	254	Preservative
Ketoprofen	KFN		5.9	254	Anti-inflammatory
17 α-ethinyl estradiol	ESD		10.0	280	Steroid
Estrone	ETN		10.4	280	Steroid
Fenoprofen	FEN		4.5	272	Anti-inflammatory

Table 1 (*continued*)

Analyte	Abb	Chemical structures	pKa	λ	Therapeutic use
Flurbiprofen	FLP		4.2	247	Anti-inflammatory
Diclofenac	DCF		4.0	272	Anti-inflammatory

can reduce the analysis time according to the equation $u = v\ D_m/d_p$ [11], where, v is the reduced mobile phase velocity, u is the optimal velocity and D_m is the diffusion coefficient of the solute in the mobile phase. These particles give flatter van Deemter curves allowing higher flow rates to be used while maintaining high efficiencies. However, these particles are not compatible with conventional LC systems capable of a maximum of 400 bar pressure. Ultra high-pressure systems (UHPLC) providing pressures up to 1000 bar typically have to be used with small packing particles to overcome the high pressure drop [12,13].

Recently, superficially porous particles were introduced to reduce the analysis time and enhance the efficiency with improved column permeability compared to sub-2 μm particles [14,15]. Columns packed with these particles show significant progress in column performance compared to sub-2 μm particles. Fused-core Kinetex particles are comprised of 1.9 μm diameter solid silica core surrounded by 0.35 μm thick porous silica shell [16]. The small diffusion path (0.35 μm) significantly reduces peak broadening, thus enhancing the efficiency compared to fully porous particles. The thin porous shell on fused-core particles allows solutes to diffuse more quickly in and out of the porous structure for interaction with the stationary phase resulting in improved mass transfer and allowing higher flow rates to be used without significant loss in efficiency [17]. The most important advantage from the economical point of view is that fused-core particles produce lower back pressure compared to fully porous smaller particles, allowing the use of these particles with conventional HPLC systems. In addition, columns packed with fused-core particles are less susceptible to the plugging problems that are sometimes evident with sub-2 μm columns, especially for pharmaceutical samples with complex matrices [7].

In recent years, the continual discharge of pharmaceuticals and personal care products to the aquatic environment has become an issue of great concern [18]. These substances enter the aquatic environment from households, hospitals and industrial units. Most of them are not completely eliminated in wastewater treatment plants because of their polarity and high stability [19]. These compounds could be detected in the aquatic environment at concentrations ranging from ng L^{-1} to μg L^{-1} [19,20]. Continual release of pharmaceuticals into the environment might lead to adverse effects on humans and wildlife, including disruption of the endocrine system, inhibition of primary productivity and resistance in some bacterial strains. Other adverse effects of the occurrence of pharmaceuticals in the aquatic environment are descried elsewhere [21].

Consequently, monitoring of the occurrence of pharmaceuticals in the aquatic environment is of great importance and has been the focus of an increasing number of recent studies. The most important groups of pharmaceuticals which are detected in the aquatic environment based on recent reviews [22,23] include non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics, central nervous system (CNS) stimulants, estrogens and lipid regulators [22].

Several chromatographic methods have been developed for the analysis of pharmaceuticals in environmental water samples using LC–MS [24–27], LC–MS–MS [28–33] and LC–UV [18,34–36]. Analytical methods based on mass spectrometric detection, such as GC–MS and LC–MS are favored by many researchers because they offer advantages such as high sensitivity and ability to provide compound confirmation. However, GC–MS requires analyte derivatization before the analysis of many pharmaceuticals to increase their volatility. The derivatization process is time consuming, especially when a large number of samples have to be analyzed.

Due to the polarity of most pharmaceuticals, HPLC remains the technique of choice for the analysis of pharmaceuticals in most commercial and research laboratories. While LC–MS is the preferred combination, it is not widely available in many laboratories due to its high cost.

Recently, the development of fast methods becomes more feasible using techniques such as UPLC which is one of the most suitable analytical tools for the determination of contaminants in environmental samples. UPLC provides great resolution, increased sensitivity and high speed of analysis. Although the use of fused-core particle columns is a promising approach for achieving high speed and high resolution analyses, its application in environmental analysis is very limited. Only a few applications of fused-core columns for the determination of pharmaceuticals in the environmental samples have been reported [37,38].

The objective of this work was to illustrate the advantages of using columns packed with fused core particles as an alternative to fully porous sub-2 μm particles in the analysis of environmental

pollutants for achieving fast HPLC separations. In this study, a fast method using fused-core C18 silica particle column was developed and validated to determine 25 emerging contaminants from different classes in river, lake and wastewater samples in the shortest possible time (10 min) in a single run. Sample preconcentration was performed using solid-phase extraction (SPE) with Oasis HLB cartridges. The short analysis time achieved by this method allowed the analysis of a large number of samples in a short time, which can save expensive HPLC solvents and reduce labour costs.

2. Experimental

2.1. Materials and reagents

HPLC grade acetonitrile and acetic acid were purchased from Sigma-Aldrich. Ultrapure water used in the analysis was purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA). All solvents were filtered using 0.45 μm × 47 mm nylon membrane filters (Supelco, Bellefonte, PA, USA) and degassed by sonication for 20 min using a sonication bath (Crest Ultrasonics, USA, Model 275 D) before use. The studied analytes were sulphanilamide, sulfacetamide, sulfadiazine, sulfathiazole, sulfapyridine, sulfamerazine, sulfamethazine, sulfamethoxypyridazine, sulfamonomethoxine, sulfamethoxazole, sulfadimethoxine, sulfaphenazole acetaminophen, acetyl salicylic acid, ketoprofen, fenoprofen, flurbiprofen, diclofenac, theophylline, caffeine, methylparaben, ethylparaben, propylparaben, 17 α-ethinyl estradiol and estrone. The chemical structures and pKa values of the analytes studied are given in Table 1. All standards were purchased from Sigma-Aldrich with purity greater than 98%. All solutions were stored in the dark at 4 °C. Stock solutions of the analytes were prepared by dissolving 10 mg of each standard in 10 mL methanol. The working standard solutions were prepared from the stock solutions by serial dilutions. The standards were distributed into five concentrations ranging from 5 μg L^{-1} to 1000 μg L^{-1}.

2.2. Instrumentation and chromatographic conditions

All separations were performed using an Agilent model 1200 HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with an autosampler, a thermostated column compartment, a binary pump and a UV diode array detector. The maximum backpressure of the system was 600 bar. The injection volume was 5 μL. Agilent Chemstation Software was used for instrument control and data acquisition. The columns used in this study were fused-core Kinetex® C18 particle columns of 4.6 mm ID, 150 mm length and 2.6 μm O.D. particles (Phenomenex, USA) and Zorbax Stable Bond C18 columns (Agilent Technologies, Waldbronn, Germany) of 4.6 mm ID, 150 mm length and 1.8 μm O.D. particles. A 4.6 mm I.D. in-line filter (0.2 μm) (Agilent Technologies, Waldbronn, Germany) was used to protect the analytical columns. Analytes were separated by gradient elution at 30 °C using a mobile phase consisting of acetonitrile (solvent B) and ultra-pure water containing 0.5% acetic acid (solvent A). The gradient used for the analysis was 0 min, 15% acetonitrile and flow rate 1 mL min^{-1}; 3 min, 15% acetonitrile and flow rate 1.3 mL min^{-1} then increasing to 82% acetonitrile at 10 min with flow rate 1.3 mL min^{-1}. The equilibrium time was set to 5 min.

For higher sensitivity, each analyte was detected using the diode array detector (DAD) at its wavelength of maximum absorption. Table 1 shows the wavelengths used for the detection of each analyte.

2.3. Sample preparation

Solid phase extraction (SPE) was used for sample preconcentration and clean-up using Visiprep solid phase extraction vacuum manifold system purchased from Supelco (Bellefonte, PA, USA). Two SPE cartridges packed with polymeric sorbents were investigated: Oasis HLB cartridges with 60 mg packing material of the hydrophilic–lipophilic balance (HLB) type and 3 mL reservoir purchased from Waters (Milford, MA, USA) and SampliQ OPT polymer, 60 mg packing material and 3 mL reservoir donated by Agilent Technologies (Waldbronn, Germany). Analytes were extracted from spiked deionized water. Before applying the spiked water samples, the cartridges were conditioned with 3 mL methanol followed by 3 mL deionized water. A sample volume of 100 mL was applied to the cartridge and the flow was kept at no greater than 4 mL min^{-1}. The sorbent was never allowed to dry during either the conditioning period or sample loading procedures. After the vacuum drying period of the sorbent, the loaded cartridges were eluted with 5 mL of ammoniated methanol (ammonia: methanol, 1:19 v/v). Following the elution, the filtrates were evaporated to dryness under nitrogen stream. The dried extract was dissolved in l mL of the mobile phase to obtain a 100-fold pre-concentration. Non-spiked water samples were also extracted in all experiments, using the same procedure, in order to detect any possible contribution of the water matrix to analyte signals. For extraction of real water samples, 500 ml of river and lake water were extracted while 150 ml of wastewater sample was used in order to avoid blocking of the SPE sorbents. The extracted samples were evaporated and dissolved in 0.5 ml of the mobile phase to increase the sensitivity. Finally, 5 μL of the extract was injected into the LC system.

2.4. Sample collection

Water samples were collected from a small river, a lake and a municipal wastewater treatment plant in Southern Ontario, Canada. Raw influent wastewater samples were used in this study and sewage water was pumped directly from the sewage pipe (4 m below the surface) straight into the sampling bottles. All samples were collected in pre-washed amber glass bottles with Teflon-lined caps. Prior to sampling, the pump was allowed to run for a few minutes to ensure that a representative sample was collected. Bottles were completely filled with the sample, wrapped in hermetic plastic bags and transported to the laboratory on ice. The samples were filtered to eliminate suspended matter through 0.45 μm cellulose acetate filters. The samples were stored at 4 °C until the extraction, which was performed within 24 h in order to avoid degradation of the analytes.

3. Results and discussion

3.1. HPLC method

The main goals when developing an LC method are to achieve sufficient resolution for all analytes, short analysis time and high sensitivity. Many chromatographic factors can be adjusted to give the desired response including column dimensions, stationary phase, mobile phase composition and flow rate.

In this study, 4.6 mm I.D. columns were selected for the analysis to maintain high efficiency. Theses standard columns are still the most popular. Narrower diameter columns offer advantages such as solvent savings and improved detection limits, yet a loss in efficiency is often observed due to extra-column effects [39,40] and the difficulty of packing of sub-2 μm particles into small diameter columns [41]. 150 mm long columns were used in this study to maximize the efficiency. Columns packed with 2.6 μm O.D. superficially porous C18 silica particles and columns of the same length and internal diameter packed with sub-2 μm fully porous C18 silica particles were used to perform the analysis.

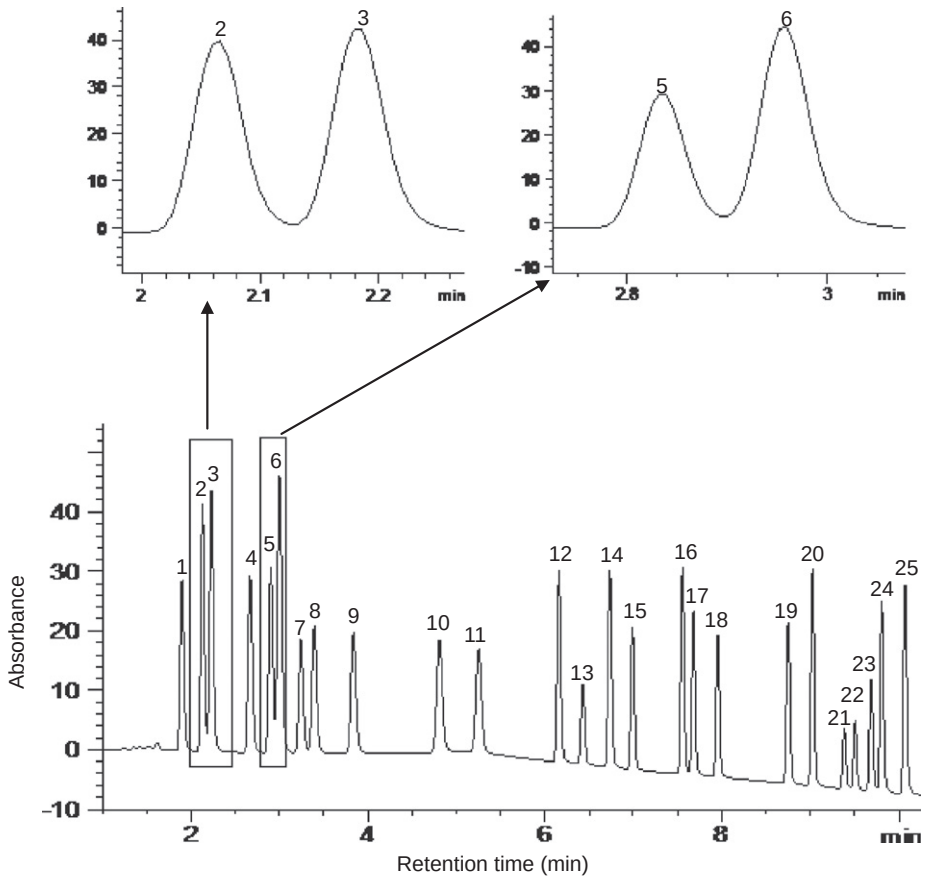


Fig. 1. Chromatogram of the studied analytes separated on the column packed with fused-core particles. Peak identification: 1-sulphanilamide, 2-theophylline, 3-acetaminophen, 4-sulfacetamide, 5-caffeine, 6-sulfadiazine, 7-sulfathiazole, 8-sulfapyridine, 9-sulfamerazine, 10-sulfamethazine, 11-sulfamethoxypyridazine, 12-sulfamonomethoxine, 13-acetyl salicylic acid, 14-sulfamethoxazole, 15-methylparaben, 16- sulfadimethoxine, 17-sulfaphenazole, 18-ethylparaben, 19-propylparaben, 20-ketoprofen, 21-17 α-ethinyl estradiol, 22-estrone, 23-fenoprofen, 24-flurbiprofen and 25-diclofenac. The inset shows baseline separation of peaks 2, 3 and 5, 6.

The mobile phase used in the study was not buffered. Acetic acid (0.5%) was added to the mobile phase to enhance peak resolution. An investigation to select the adequate composition of the mobile phase was performed and different mobile phase compositions were tested to obtain the best separation of the studied analytes in the shortest possible time without sacrificing peak shape. Solvent gradient and flow rate gradient were used to obtain the best separation for the studied analytes. The gradient elution program described in Section 2.2 was the best option in terms of analysis time and peak shape. Under these conditions, all analytes were fully separated on the fused-core particle column in the shortest possible time (10 min), as shown in Fig. 1.

After the optimization of the separation conditions on the column packed with fused core particles, the same gradient was applied to the column packed with fully porous sub-2 μm particles for comparison. The chromatogram obtained is presented in Fig. 2.

A comparison of the chromatograms obtained with columns packed with fused-core particles (Fig. 1) and fully porous sub-2 μm particles (Fig. 2) under the same conditions indicated a change in selectivity manifesting itself through the change in the elution order between some analytes such as caffeine (peak 5) and sulfadiazine (peak 6), as well as acetyl salicylic acid (peak 13) and sulfamethaxazole (peak 14). The change in selectivity could be due to differences in chemistry of both stationary phases.

While all analytes were fully resolved on the column packed with fused-core particles (resolution between 2.09 and 18.06, above the critical value of 1.5 in all cases), coelutions of many analytes were observed on the column packed with fully porous particles. For example, the critical pair of theophylline and acetaminophen (peaks 2 and 3), which were fully separated on the column packed with fused-core particles with a resolution of 2.25, coeluted completely on the column packed with fully porous sub-2 μm particles. Also, partial coelutions were observed between caffeine and sulfathiazole, as well as acetyl salicylic acid and methyl paraben with this column. While all analytes could be fully separated on the column packed with fused-core particles in only 10 min, the analysis time on the column packed with fully porous

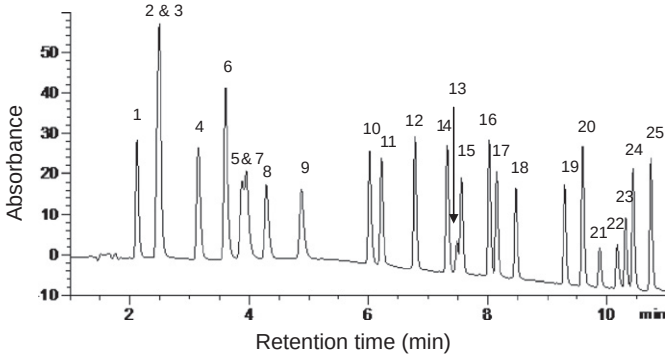


Fig. 2. Chromatogram of the studied analytes separated on the column packed with fully porous sub-2 μm particles. Peak identification: 1-sulphanilamide, 2 and 3-theophylline and acetaminophen, 4-sulfacetamide, 5-caffeine, 6-sulfadiazine, 7-sulfathiazole, 8-sulfapyridine, 9-sulfamerazine, 10-sulfamethazine, 11-sulfamethoxypyridazine, 12-sulfamonomethoxine, 13-acetyl salicylic acid, 14-sulfamethoxazole, 15-methylparaben, 16-sulfadimethoxine, 17-sulfaphenazole, 18-ethylparaben, 19-propylparaben, 20-ketoprofen, 21-17 α-ethinyl estradiol, 22-estrone, 23-fenoprofen, 24-flurbiprofen and 25-diclofenac.

sub-2 μm particles was longer than 10 min without complete separation of all analytes under the same conditions. Complete separation of the analytes on the column packed with fully porous sub-2 μm particles would require further optimization at a cost of a longer analysis time. The system pressure observed when the separation was performed on the column packed with fused-core particles was 355 bar compared to 520 bar when the column packed with fully porous sub-2 μm particles was used under the same mobile phase composition and flow rate conditions. The low back pressure obtained for the column packed with fused-core particles is advantageous when conventional HPLC systems (max. 400 bar) are used, while ultrahigh pressure instrumentation ($\geq$600 bar) is needed when the separation has to be performed on columns packed with fully porous sub-2 μm particles.

Based on this comparison, the column packed with fused-core particles was chosen for the analysis, as it allowed reducing the analysis times at relatively low back pressures compared to fully porous sub-2 μm particles. The column packed with fused-core particles was then used for the determination of the 25 emerging contaminants in real water samples. The chromatographic parameters (retention time, capacity factor, resolution and selectivity) of the analytes on the column packed with fused-core particles are listed in Table 2.

3.2. SPE optimization

To meet the objectives for the monitoring of the analytes studied in surface water and wastewater, a preliminary preconcentration step is required to reach the sensitivity necessary to detect the low concentrations normally present in these samples (in the range of ng L^{-1} to μg L^{-1}). SPE is considered the technique of choice for analyte enrichment since it provides clean extracts, high selectivity, high recoveries for polar compounds and is easy to automate.

The choice of sorbents is critical for efficient extraction of contaminants from water matrices. Several types of sorbents have been reported for isolation and enrichment of pharmaceuticals. In this study, two polymeric sorbents, Oasis HLB and SampliQ OPT, were evaluated to quantitatively extract the investigated analytes and to eliminate the influence of matrix components. Polymeric sorbents were selected in this study because of their higher adsorption capacity than C18 sorbents for polar analytes and a broader pH stability range [42]. These sorbents are composed of a variety of hyper-cross-linked polystyrene-divinylbenzene polymers with different degrees of linkage, porosity and surface area. Oasis HLB is a polymer with lipophilic divinylbenzene and hydrophilic N-vinylpyrrolidine groups. SampliQ OPT is an amide-modified divinylbenzene polymer which also combines hydrophilic and lipophilic characteristics. The performance of the selected sorbents was evaluated using the extraction conditions described in Section 2.4.

From the comparison of the two tested SPE cartridges, higher average recovery was achieved with Oasis HLB. With this sorbent, the recovery ranged from 67.5% to 97% for all analytes except SND, APH and ASA, which were characterized by low recoveries (33.9%, 32.2% and 15% respectively). Compared to Oasis HLB, SampliQ OPT cartridge revealed higher recovery for SND, APH, SMZ, ASA and SDM (54.8%, 48%, 100%, 24.4% and 98% respectively). On the other hand, SampliQ OPT produced very low recoveries for many analytes such as THP, CFN, SDZ, STZ and FPB (12.3%, 11.7%, 35.5%, 35% and 31% respectively). Fig. 3 shows the comparison of the recoveries of the analytes studied obtained with both sorbents. Based on this comparison, Oasis HLB was chosen as the optimal sorbent, as it produced higher recoveries on average. Oasis HLB cartridges were preferred for the extraction of contaminants in water samples because of their improved wetting characteristics leading to better mass transfer, higher retention capabilities for extracting acidic analytes from water samples without acidification and the ability to extract a large number of compounds simultaneously without the need for sample pH adjustment [11]. Performing the extraction without pH adjustment could simplify sample handling, especially for large volume samples.

Table 2
Chromatographic parameters for the analytes separated on the Kinetex® C18 column packed with 2.6 μm fused-core particles.

Analyte	$t_R \pm$ R.S.D	k'	R_s	α
Sulfanilamide	1.88 ± 1.02	0.36	4.48	1.43
Theophylline	2.09 ± 2.03	0.51	2.25	1.15
Acetaminophen	2.20 ± 1.38	0.59	8.58	1.54
Sulfacetamide	2.64 ± 0.96	0.91	4.23	1.18
Caffeine	2.87 ± 1.53	1.07	2.09	1.08
Sulfadiazine	2.98 ± 0.97	1.15	4.21	1.15
Sulfathiazole	3.21 ± 1.15	1.32	2.73	1.08
Sulfapyridine	3.36 ± 0.99	1.43	7.62	1.22
Sulfamerazine	3.81 ± 0.82	1.75	15.01	1.40
Sulfamethazine	4.78 ± 0.71	2.46	6.32	1.13
Sulfamethoxypyridazine	5.22 ± 0.75	2.77	14.45	1.24
Sulfamonomethoxine	6.14 ± 0.23	3.44	5.24	1.06
Acetyl salicylic acid	6.42 ± 0.18	3.64	6.62	1.06
Sulfamethoxazole	6.72 ± 0.13	3.86	5.62	1.05
Methylparaben	6.98 ± 0.11	4.05	12.81	1.10
Sulfadimethoxine	7.55 ± 0.07	4.46	2.84	1.02
Sulfaphenazole	7.67 ± 0.06	4.55	6.27	1.04
Ethylparaben	7.95 ± 0.06	4.75	18.06	1.12
Propylparaben	8.74 ± 0.04	5.32	6.26	1.04
Ketoprofen	9.02 ± 0.04	5.52	8.13	1.05
17 α-ethinyl estradiol	9.38 ± 0.04	5.78	2.75	1.02
Estrone	9.50 ± 0.03	5.87	4.02	1.02
Fenoprofen	9.68 ± 0.04	6.00	2.68	1.01
Flurbiprofen	9.80 ± 0.03	6.09	5.87	1.03
Diclofenac	10.07 ± 0.03	6.28		

t_R: retention time; R.S.D: relative standard deviation; k': capacity factor; R_s: resolution; α: selectivity.

3.3. Validation of the method

The SPE–HPLC method was validated based on parameters such as linearity, precision, detection and quantification limits, selectivity and accuracy.

3.3.1. Linearity

The linearity of the method was evaluated using deionized water spiked with the analytes in the concentration range from 5 μg L^{-1} to 1000 μg L^{-1}. Calibration curves were prepared for each compound by plotting the peak area versus concentration. The characteristic parameters of the regression equations for the analytes studied are given in Table 3.

3.3.2. Precision

Precision was validated based on the evaluation of intra- and inter-day repeatability of the method. Intra-day and inter-day repeatability were determined by analyzing three replicates of deionized water samples spiked with the analytes at two concentration levels. Satisfactory results were achieved for all analytes. The intra-day repeatability RSDs ranged from 1.1% to 5.4%, and the inter-day repeatability ranged from 1.4% to 5.4% except for acetyl salicylic acid (which had very low recovery). The results of the intra-day and inter-day repeatability experiments expressed as relative standard deviations are summarized in Table 4.

3.3.3. Detection and quantitation limits

The limits of detection (LOD) and quantification (LOQ) were determined according to International Conference on Harmonization

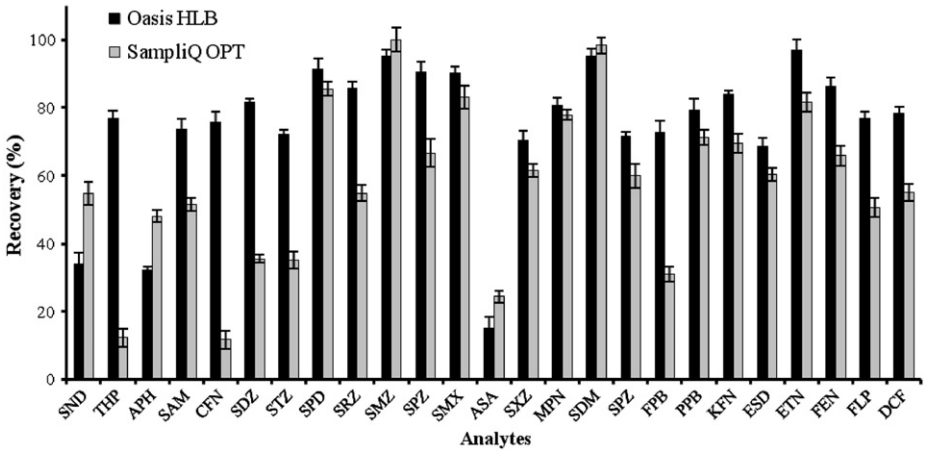


Fig. 3. Recovery of the studied analytes on Oasis HLB and SampliQ OPT SPE cartridges.

Table 3
Characteristic parameters of the calibration curve equations of the analytes studied.

Analyte	R^2	Slope	Intercept
Sulfanilamide	0.9961	2.7	2.2
Theophylline	0.9973	2.7	4.1
Acetaminophen	0.9989	10.9	5.7
Sulfacetamide	0.9990	3.4	6.9
Caffeine	0.9989	1.7	6.4
Sulfadiazine	0.9992	3.6	4.4
Sulfathiazole	0.9989	8.5	5.8
Sulfapyridine	0.9950	5.6	3.0
Sulfamerazine	0.9998	3.2	4.2
Sulfamethazine	0.9986	1.0	4.7
Sulfamethoxypyridazine	0.9993	3.0	2.4
Sulfamonomethoxine	0.9974	2.8	1.3
Sulfamethoxazole	0.9979	2.5	1.5
Methylparaben	0.9980	3.4	4.7
Sulfadimethoxine	0.9967	2.0	4.8
Sulfaphenazole	0.9974	2.7	4.8
Ethylparaben	0.9979	1.4	4.8
Propylparaben	0.9990	1.1	1.3
Ketoprofen	0.9989	3.1	6.0
17 α-ethinyl estradiol	0.9956	1.1	6.5
Estrone	0.9956	0.9	7.5
Fenoprofen	0.9992	1.4	4.6
Flurbiprofen	0.9998	1.3	4.6
Diclofenac	0.9990	1.8	5.1

(ICH) recommendations [43], the approach based on signal to noise ratio. LODs and LOQs were experimentally estimated from the injections of standard solutions serially diluted until the signal-to-noise ratio for any single analyte reached a value of ten for LOQ and three for LOD. The detection and quantitation limits determined in the study are given in Table 5. The detection limits of the method ranged from 1.5 to 15 $\mu g\,L^{-1}$, while limits of quantification were in the range from 5 to 50 $\mu g\,L^{-1}$ with UV detection.

The low limits of detection confirmed the applicability of the proposed method for the analysis of real samples. The detection range of $\mu g\,L^{-1}$ has often been reported in studies based on using UV detection for the determination of pharmaceuticals in environmental samples (e.g. [44–48]). Detection limits could be decreased even further by using mass spectrometry detection; however, the matrix effect might be a serious issue, as matrix components such as humic and fulvic acids in the aqueous matrix may reduce or enhance the analyte signals and might affect the reproducibility and accuracy of the assay [49]. These effects are particularly pronounced when very complex samples such as wastewater are analyzed. In addition, MS detection systems are not available in many laboratories due to high cost.

3.3.4. Accuracy

The recovery of the studied analytes was evaluated by calculating the ratio of the peak area obtained from the extraction of spiked deionized water sample to the peak area of the corresponding standard solution at two concentration levels. The recoveries were within the range of 67.5% to 97.0% with standard deviations not higher than 5.7%, except for SND, APH and ASA, for which low recovery was obtained. The recoveries of the analytes using Oasis HLB cartridges are listed in Table 5.

The recoveries of SND and APH were low due to the fact that the pKa values for these compounds are 9.5 and 10.4 respectively, which means that the extraction should be performed under alkaline conditions. However, should this condition be met, the recoveries of the remaining analytes would be reduced. The low recoveries found for these compounds were also reported in other studies carried out with Oasis HLB cartridges for APH [36,50,51] and for SND [17,34]. On the other hand, ASA has a pKa value of 2.97, which requires strongly acidic conditions. However, adjusting the pH to a low value could again result in reduced recoveries of the remaining analytes. Carrying out the extraction without adjusting the sample pH was a compromise solution to accommodate as many analytes as possible. The low recovery of ASA ($<15\%$) introduced high uncertainty in its quantitation at very low concentrations, but detection was still possible. The low recovery of ASA was reported in the literature under similar extraction conditions [35].

3.3.5. Selectivity

Unlike LC–MS, HPLC with UV detection lacks the ability to provide compound confirmation. Therefore selectivity of the method was evaluated by comparison of the analytes' retention times obtained from the chromatogram of spiked water samples to the chromatogram of unspiked samples (blank samples). The results indicated that there was no overlap between the analyte peaks and the peaks of interfering components; and the signal measured was not influenced by other substances.

Also, the specificity and the selectivity of the method were evaluated by studying the peak purity index values including purity factor (similarity factor), threshold limit and purity ratio (similarity ratio) for each analyte (Table 6). Agilent Chemstation software was used for calculating the purity factor and the threshold limit. A similarity factor of 0 indicates no match between spectra and 1000 indicates identical spectra. Generally,

Table 4
Intra- and inter-day precision of the analysis.

Analyte	Intra-day precision (RSD)		Inter-day precision (RSD)	
	100 μg/L	1000 μg/L	100 μg/L	1000 μg/L
Sulfanilamide	3.1	3.0	4.5	3.9
Theophylline	2.7	1.6	2.8	2.7
Acetaminophen	2.2	3.4	5.1	4.2
Sulfacetamide	2.9	2.5	2.6	2.2
Caffeine	5.4	3.5	3.5	5.2
Sulfadiazine	2.7	2.1	5.2	2.8
Sulfathiazole	2.2	2.1	5.2	1.4
Sulfapyridine	3.8	1.1	5.4	2.1
Sulfamerazine	5.0	1.9	4.3	2.8
Sulfamethazine	5.0	1.8	5.4	3.5
Sulfamethoxypyridazine	3.4	3.5	4.9	1.9
Sulfamonomethoxine	4.6	3.0	3.6	4.2
Acetyl salicylic acid	8.7	7.6	11.3	10.2
Sulfamethoxazole	2.8	2.4	5.2	2.1
Methylparaben	3.4	3.4	4.8	4.1
Sulfadimethoxine	4.5	3.1	4.0	4.3
Sulfaphenazole	3.3	4.0	5.2	3.0
Ethylparaben	2.9	3.2	4.3	4.0
Propylparaben	3.4	4.4	3.3	3.2
Ketoprofen	3.4	4.7	3.0	2.8
17 α-ethinyl estradiol	4.8	1.6	4.8	4.2
Estrone	4.4	1.9	2.5	4.0
Fenoprofen	5.4	2.1	4.5	4.2
Flurbiprofen	3.3	2.3	2.7	3.7
Diclofenac	5.2	2.3	4.5	5.2

RSD: relative standard deviation (%).

Table 5
Recovery on Oasis HLB cartridges, LOD and LOQ for the analytes studied.

Analyte	Recovery (% ± SD)		LOD (μg/L)[a]	LOQ (μg/L)[b]
	100 μg/L	1000 μg/L		
Sulfanilamide	32.2 ± 4.8	33.9 ± 2.6	10	33
Theophylline	74.8 ± 2.5	76.9 ± 1.3	12	40
Acetaminophen	31.6 ± 2.4	32.2 ± 2.9	9	30
Sulfacetamide	71.5 ± 2.6	73.7 ± 2.3	6	20
Caffeine	75.6 ± 1.7	75.8 ± 2.1	12	40
Sulfadiazine	81.3 ± 2.1	81.5 ± 2.5	7.5	25
Sulfathiazole	71.7 ± 2.7	72.2 ± 2.0	9	30
Sulfapyridine	90.6 ± 1.7	90.1 ± 1.6	3	10
Sulfamerazine	84.6 ± 2.5	85.8 ± 1.9	3	10
Sulfamethazine	95.1 ± 4.8	95.3 ± 3.5	1.5	5
Sulfamethoxypyridazine	91.6 ± 2.1	90.6 ± 2.7	7	23
Sulfamonomethoxine	89.9 ± 2.2	91.2 ± 5.7	7	23
Acetyl salicylic acid	12.7 ± 3.6	14.9 ± 2.6	15	50
Sulfamethoxazole	71.5 ± 2.8	70.5 ± 2.6	7.5	25
Methylparaben	81.4 ± 2.3	80.8 ± 2.5	7.5	25
Sulfadimethoxine	92.8 ± 3.9	95.2 ± 1.9	6	20
Sulfaphenazole	70.5 ± 5.3	71.4 ± 4.1	12	40
Ethylparaben	69.4 ± 4.1	72.8 ± 1.9	7.5	25
Propylparaben	77.8 ± 2.5	79.2 ± 2.2	7.5	25
Ketoprofen	82.9 ± 3.8	84.0 ± 1.9	8	26
17 α-ethinyl estradiol	67.5 ± 3.1	68.5 ± 2.3	15	50
Estrone	95.6 ± 3.0	97.0 ± 2.3	15	50
Fenoprofen	85.4 ± 4.6	86.5 ± 4.2	7	24
Flurbiprofen	76.3 ± 3.5	76.9 ± 4.2	3	10
Diclofenac	77.4 ± 3.8	78.4 ± 4.0	12	40

SD: standard deviation.

[a] $S/N \geq 3$.

[b] $S/N \geq 10$.

values very close to the ideal similarity factor (greater than 995) indicate that the spectra are very similar; values lower than 990 but higher than 900 indicate some similarity.

Table 6
Peak purity index values for the analytes studied in deionized water and wastewater.

Analyte	Purity factor (similarity factor)	Threshold	Purity ratio (similarity ratio)
Sulfanilamide	998.147	958.741	0.044911
Theophylline	999.958	957.508	0.000988
Acetaminophen	999.978	996.293	0.005935
Sulfacetamide	999.983	941.612	0.000291
Caffeine	999.985	976.352	0.000634
Sulfadiazine	999.954	969.769	0.001522
Sulfathiazole	999.97	940.295	0.000502
Sulfapyridine	999.965	938.686	0.000571
Sulfamerazine	999.981	918.085	0.000232
Sulfamethazine	999.877	896.727	0.001191
Sulfamethoxypyridazine	999.913	907.17	0.000937
Sulfamonomethoxine	999.992	958.035	0.000191
Acetyl salicylic acid	999.989	994.056	0.001851
Sulfamethoxazole	995.283	883.456	0.040474
Methylparaben	999.917	978.354	0.003834
Sulfadimethoxine	999.96	960.926	0.001024
Sulfaphenazole	999.998	963.899	5.54E-05
Ethylparaben	999.993	969.648	0.000231
Propylparaben	999.986	969.094	0.000453
Ketoprofen	999.98	988.742	0.001777
17 α-ethinyl estradiol	999.999	997.038	0.000338
Estrone	999.996	995.73	0.000937
Fenoprofen	999.999	998.963	0.000964
Flurbiprofen	999.982	994.108	0.003055
Diclofenac	999.984	977.663	0.000716
Wastewater sample			
Acetaminophen	999.363	745.163	0.0025
Sulfaphenazole	999.554	387.234	0.000728

Similarity ratio or purity ratio was calculated from the following equation:

ratio = (1000 − similarity)/(1000–threshold)

For a spectrally pure peak the ratio values are below unity, and for spectrally impure peaks the values are above unity. For all the studied analytes, similarity ratios were below unity, so no significant differences between spectra could be detected. As a result, there was no evidence of coelutions and the peaks were considered pure. The peak purity index values listed in Table 6 indicate that the chromatographic peaks of the studied analytes were pure and were not attributable to more than one analyte.

3.4. Analysis of real water samples

The method developed was successfully applied to the analysis of different pharmaceuticals and preservatives in real water samples. None of the target analytes were found in the analyzed river and lake water samples; however, in raw wastewater samples two of the studied analytes, sulfapyridine and sulfaphenazole, could be detected at their maximum absorption wavelength of 272 nm. Other analytes were below their LODs. Fig. 4 shows the chromatogram of the analyzed wastewater sample. The identities of these pharmaceuticals were confirmed based on the retention times, UV spectra and the increase in the response after spiking the samples with the authentic standards of these analytes.

4. Conclusions

A fast HPLC method with UV diode-array detection for the analysis of 25 emerging contaminants in different types of water samples was developed using a C18 reversed-phase column packed with fused-core particles. Separation of all compounds was achieved in 10 min with acceptable reproducibility, resolution and selectivity. Columns packed with fused-core particles are

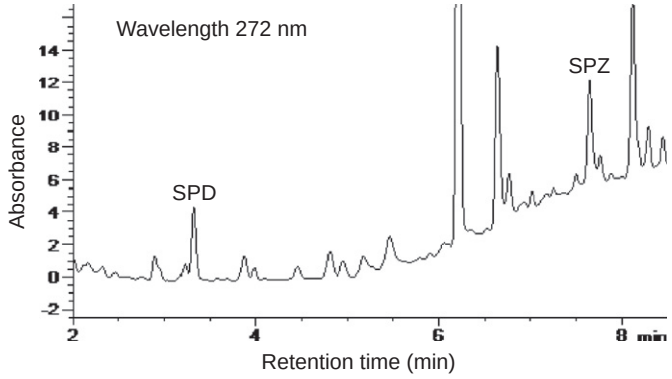


Fig. 4. HPLC–UV chromatogram of wastewater sample extract separated on the column packed with fused-core particles using Oasis HLB cartridges showing sulfapyridine and sulfaphenazole at a detection wavelength of 272 nm.

capable of fast and efficient separations at conventional pressure limits. These particles produce low back pressure, which allows much higher flow rates to be used. Fused-core particles can be used as an alternative to sub-2 μm particles for fast separations in environmental analysis. The method developed using the column packed with fused-core particles was used for the determination of selected analytes in river, lake and wastewater samples. Two of the studied analytes were detected in the analyzed wastewater samples.

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