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On-line hyphenation of solid-phase extraction to chromatographic separation of sulfonamides with fused-core columns in sequential injection chromatography

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ABSTRACT

On-line sample pretreatment (clean-up and analyte preconcentration) is for the first time coupled to sequential injection chromatography. The approach combines anion-exchange solid-phase extraction and the highly effective pentafluorophenylpropyl (F5) fused-core particle column for separation of eight sulfonamide antibiotics with similar structures (sulfathiazole, sulfanilamide, sulfacetamide, sulfadiazine, sulfamerazine, sulfadimidine, sulfamethoxazole and sulfadimethoxine). The stationary phase was selected after a critical comparison of the performance achieved by three fused-core reversed phase columns (Ascentis® Express RP-Amide, Phenyl-Hexyl, and F5) and two monolithic columns (Chromolith® High Resolution RP-18 and CN). Acetonitrile and acetate buffer pH 5.0 at 0.60 mL min^{-1} were used as mobile phase to perform the separations before spectrophotometric detection. The first mobile phase was successfully used as eluent from SPE column ensuring transfer of a narrow zone to the chromatographic column. Enrichment factors up to 39.2 were achieved with a 500 μL sample volume. The developed procedure showed analysis time < 10.5 min, resolutions > 1.83 with peak symmetry ≤ 1.52, LODs between 4.9 and 27 μg L^{-1}, linear response ranges from 30.0 to 1000.0 μg L^{-1} ($r^2 > 0.996$) and RSDs of peak heights $< 2.9\%$ ($n=6$) at a 100 μg L^{-1} level and enabled the screening control of freshwater samples contaminated at the 100 μg L^{-1} level. The proposed approach expanded the analytical potentiality of SIC and avoided the time-consuming batch sample pretreatment step, thus minimizing risks of sample contamination and analyte losses.

1. Introduction

Aiming multidetermination, sequential injection analysis (SIA) was coupled with chromatographic monolithic columns, introducing the sequential injection chromatography (SIC). This approach combines the versatility of SIA for solutions handling with the potential of chromatography for highly efficient separations [1]. Monolithic columns, which operate at pressures within 300–750 psi, were until recently the only option for SIC separations. The major hindrance was the lack of different stationary phases, being the RP-C18, RP-C8 and silica phases, the only commercially available options.

The introduction of chromatographic columns with fused-core particle technology increased the applicability of SIC [2]. These columns are filled by 2.7-μm diameter solid fused-silica core particles which 1.7-μm core is impermeable to the mobile phase (as well as to the analytes) and a 0.5-μm thick layer shell of porous silica gel that acts as stationary phase. Thus, the mobile phase has shorter diffusion path in the particle, which reduces axial dispersion of the analytes and minimizes peak broadening. Short fused-core particle columns with lower dead volumes then provides better separation performance than longer monolithic columns [2] and it is possible to exploit different commercially available stationary phases to improve selectivity [3].

The F5 stationary phase is composed by pentafluorophenylpropyl groups that provide a stable, reversed-phase packing with electron-deficient phenyl rings due to the presence of electronegative fluorines, which can retain compounds by forming p–p and polar interactions [4]. This phase exhibits higher ion-exchange character compared to its alkyl counterparts (*i.e.* C18 and C8) and thus it provides excellent chromatographic separations of analytes with different ionization grades. Then, F5 columns can show a dual-mode retention (reversed-phase and hydrophilic interaction).

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As sulfonamides are widely used for treatment of bacterial infections in human and animals, there is a growing concern about their effect in the environment [5–7] and food [8,9]. Because the organism poorly absorbs antibiotics, most of them are excreted in the unchanged form through urine and feces to water bodies. The US Geological survey has found that up to 20% of the surface water is contaminated by sulfonamides [10] and even low levels of these substances can favor the proliferation of resistant bacteria. High performance liquid chromatography (HPLC) with detection by spectrophotometry or mass spectrometry are the main techniques used for the analysis of sulfonamides in environmental matrices [7,11–17]. These procedures are often time-consuming and require highly sophisticated and expensive equipment. For screening of environmental contamination, simpler, portable and less expensive approaches are needed.

Because sulfonamides are found in environmental samples at low concentrations, analyte preconcentration is often required before analysis. This is a critical step, which is susceptible to losses of analyte and external contaminations. The procedures usually require high volumes of samples and organic solvents, often including solvent evaporation and sample reconstitution in the mobile phase. When solid-phase extraction (SPE) is used, the cartridges are usually discarded after a single use, which increases waste generation and analysis cost.

The column-switching approach allows the selective on-line transfer of analytes from the first column (sample pretreatment) to the second one (chromatographic column). It automates the analytical process, with improvements in precision and sample throughput. For sample pretreatment, typical SPE sorbents enable loading of large sample volumes (some milliliters) with high retention of the analytes but low retention of the matrix. A suitable eluent solution assures the transference of a narrow sample zone to the chromatographic column. This concept was introduced in HPLC almost three decades ago [18,19] and some applications were recently presented [20,21]. In spite of its inherent characteristics for solutions handling, this approach has not been exploited in SIC.

The aim of this work was to develop a two-step SPE–SIC method for on-line sample pretreatment before chromatographic separation of sulfonamides. To this aim, the performance of different

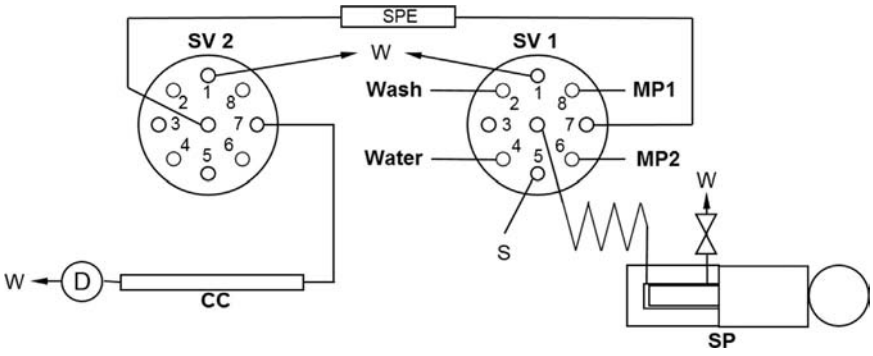


Fig. 1. Scheme of SIC setup with on-line SPE for determination of sulfonamides. SV1 and SV2: selection valves; SP: syringe pump; CC: chromatography column; SPE: extraction column; S: sample, Wash: 0.1 mol L^{-1} $NaHCO_3$; Water: water; MP1: first mobile phase 1; MP2: second mobile phase 2; W: waste; D: spectrophotometric detector.

Table 1
Steps of the SPE-SIC control program for on-line extraction, preconcentration and separation of sulfonamides.

Action	Unit	Parameter
Aspiration of wash solution	Selection valve 1	Valve port 2
	Pump	Volume: 500 μL/Flow rate: 50 (μL s^{-1})
Dispense of wash solution to SPE column	Selection valve 1	Valve port 7
	Pump	Volume: 500 μL/Flow rate: 10 (μL s^{-1})
Aspiration of water	Selection valve 1	Valve port 4
	Pump	Volume: 500 μL/Flow rate: 50 (μL s^{-1})
Aspiration of sample	Selection valve 1	Valve port 5
	Pump	Volume: 500 μL/Flow rate: 50 (μL s^{-1})
Dispense of sample and water to SPE column	Selection valve 1	Valve port 7
	Pump	Volume: 700 μL/Flow rate: 10 (μL s^{-1})
Dispense to waste	Selection valve 1	Valve port 1
	Pump	Volume: 300 μL/Flow rate: 50 (μL s^{-1})
Aspiration of MP1	Selection valve 1	Valve port 8
	Pump	Volume: 3800 μL/Flow rate: 70 (μL s^{-1})
	Selection valve 1	Valve port 7
Dispense of MP1 to SPE and CC	Selection valve 2	Valve port 7
	Pump	Volume: 3800 μL/Flow rate: 10 (μL s^{-1})
Aspiration of MP2	Selection valve 1	Valve port 6
	Pump	Volume: 2500 μL/Flow rate: 70 (μL s^{-1})
Dispense of MP2 to SPE and CC	Selection valve 1	Valve port 7
	Pump	Volume: 2500 μL/Flow rate: 10 (μL s^{-1})
Aspiration of MP1	Selection valve 1	Valve port 8
	Pump	Volume: 2500 μL/Flow rate: 70 (μL s^{-1})
Dispense of MP to SPE and CC	Selection valve 1	Valve port 7
	Pump	Volume: 2500 μL/Flow rate: 10 (μL s^{-1})

monolithic and fused-core particle columns was critically evaluated. An on-line SPE procedure was for the first time implemented in SIC for sample clean-up and analyte preconcentration.

2. Experimental

2.1. Apparatus

A SIChrom™ instrument (FIAlab® Instruments, Bellevue, WA, USA) equipped with an S17 PDP syringe pump with a 4.0 mL reservoir (Sapphire™ Engineering, MA, USA) and two 8-port high-pressure stainless-steel selection C5H valves (Valco Instrument Co., Houston, TX, USA) were used in the presented work. The flow lines were made of 0.25 mm and 0.50 mm i.d. PEEK tubing. The manifold was coupled to an USB4000 fiber-optic CCD UV–vis detector (Ocean Optics, Dunedin, FL, USA), with a DH-2000 deuterium UV light source (Ocean Optics, Dunedin, FL, USA) and SMA connector ended optical fibers with a core diameter of 600 μm (CeramOptec®, East Longmeadow, MA, USA). Measurements were carried out in an Ultem® micro-volume 9-μL Z-flow cell with a 20-mm optical path (FIAlab®, Bellevue, WA, USA). In one of the pump outlets, an Alltech AP19258 1/16″ (Czech Republic) manometer with 0–3000 psi gauge and a system pressure safety 750-psi relief valve were mounted, which enabled real-time monitoring of the system pressure and to set the pressure limit of the system. The whole SIC system was controlled by a PC equipped with FIAlab® 5.9 software (FIAlab® Instruments, Bellevue, WA, USA).

Chromatographic separations were performed on three fused-core particle reversed phase columns with different stationary phases: RP-Amide, Phenyl-Hexyl, and F5 (Ascentis® Express, 30 mm × 4.6 mm, core-shell particle size 2.7 μm, Supelco, USA) and two monolithic columns with High Resolution RP-18 and CN (Chromolith® 50 mm x 4.6 mm, Merck, Germany) stationary phases. Three different SPE anion-exchange resins were evaluated: 2-diethylamino-ethyl (Iontosorb DEAE, 80–100 μm particle, Czech Republic), aminopropyl (Applied Separations, Spe-ed cartridge, USA) and 3-trimethylamino-2-hydroxypropyl (Iontosorb TMAHP, 80–100 μm particle, Czech Republic) packed into a Cheminert® column with 20 mm length and 1.6 mm i.d. (VICI Valco Instruments, TX, USA). All measurements were performed at ambient temperature (25 °C).

2.2. Reagents and solutions

Analytical grade chemicals (from Sigma-Aldrich®) and ultra-pure water (Millipore™, Czech Republic) were used throughout the experiments. Reference solutions were prepared from sulfanilamide (SAD), sulfacetamide (SCT), sulfadiazine (SDZ), sulfathiazole (STZ), sulfamerazine (SMR), sulfadimidine (SDM), sulfamethoxazole (SMX) and sulfadimethoxine (SDT) with purity ≥ 98%. Stock 1.0 g L^{-1} solutions were prepared in methanol and stored at 5 °C. Working standard solutions with eight sulfonamides were daily prepared in the first mobile phase. A 10.0 mg L^{-1} solution of each sulfonamide was used for optimization of the chromatographic separation and evaluation of the different columns. Optimization of the SPE step was performed with an 1.0 mg L^{-1} solution of each analyte. A 0.1 mol L^{-1} potassium hydrogen carbonate solution was used for washing and conditioning the SPE column.

Table 2
Characterization of the SIC process performed on monolithic and fused core particle columns.

	Stationary phase	SAD	SCT	SDZ	STZ	SMR	SDM	SMZ	SDT
Retention time (min)	RP-18	1.87	2.78	–	3.87	–	–	–	–
	CN	1.62	2.07	–	2.95	–	–	–	–
	RP-Amide	0.93	1.93	–	3.88	–	–	–	–
	Phenyl-Hexyl	0.98	1.90	2.53	3.27	3.68	5.22	7.36	8.16
	F5	1.08	2.23	2.65	3.23	3.73	4.82	7.79	8.44
Peak symmetry	RP-18	1.78	1.55	–	1.44	–	–	–	–
	CN	1.36	1.67	–	1.46	–	–	–	–
	RP-Amide	1.43	1.09	–	1.10	–	–	–	–
	Phenyl-Hexyl	1.55	1.28	1.22	1.20	1.10	1.07	1.30	1.23
	F5	1.52	1.31	1.29	1.20	1.10	1.13	1.46	1.26
Peak resolution	RP-18	2.91[a]	3.20[b]	–	–	–	–	–	–
	CN	1.83[a]	2.97[b]	–	–	–	–	–	–
	RP-Amide	4.51[a]	5.34[b]	–	–	–	–	–	–
	Phenyl-Hexyl	4.31[a]	2.39[b]	2.26[c]	1.13[d]	3.30[e]	6.23	3.52[g]	
	F5	5.09[a]	1.64[b]	2.07 [c]	1.54 [d]	2.48 [e]	8.53 [f]	3.51 [g]	–
Number of theoretical plates	RP-18	1944	3027	–	6153	–	–	–	–
	CN	2260	2862	–	3624	–	–	–	–
	RP-Amide	1202	2527	–	3144	–	–	–	–
	Phenyl-Hexyl	1310	3427	3055	4268	3987	4388	N/A	N/A
	F5	1380	3547	4994	4977	5575	3741	N/A	N/A
Height equivalent to a theoretical plate (μm)	RP-18	25.72	16.51	–	8.13	–	–	–	–
	CN	22.11	17.46	–	13.79	–	–	–	–
	RP-Amide	22.32	12.68	–	10.15	–	–	–	–
	Phenyl-Hexyl	22.90	8.76	9.82	7.03	7.52	6.84	N/A	N/A
	F5	21.75	8.46	6.01	6.03	5.38	8.02	N/A	N/A

[a] SAD/SCT.
[b] SCT/SDZ.
[c] SDZ/STZ.
[d] STZ/SMR.
[e] SMR/SDM.
[f] SDM/SMZ.
[g] SMZ/SDT.

The chromatographic separation was initially evaluated under isocratic conditions. The mobile phases were composed by acetonitrile and diluted acetic acid (pH 3.0), whose exact ratio depended on the column used: acetic acid/acetonitrile (90/10, v/v) for the Phenyl-Hexyl, F5 and RP-18 columns; 95/5 (v/v) for the RP-Amide and 98/2 (v/v) for the CN column.

The separation of the eight sulfonamides by F5 fused-core particle column was performed under gradient elution: 0.1 mol L^{-1} acetate buffer pH 5.0/acetonitrile (92/8) for 6.3 min (volume 3.8 mL) and then 0.1 mol L^{-1} acetate buffer pH 5.0/acetonitrile (75/25) until 10.5 min (volume 1.7 mL). The mobile phases were degassed before use by sonication for 5 min.

2.3. Procedure

The SIC system (Fig. 1) was operated according to the sequence described in Table 1. Initially the SPE column was conditioned with water and $NaHCO_3$ solution and then loaded with 500 μL of sample. The elution of the analytes from the SPE column was performed by the first mobile phase (acetate buffer pH 5.0/acetonitrile, 92/8) which was also used for separation of six sulfonamides (SAD, SCT, SDZ, STZ, SMR, and SDM). Then the second mobile phase (acetate buffer pH 5.0 /acetonitrile, 75/25) was aspirated by the syringe pump and used for the chromatographic separation of the two sulfonamides that remained in the column after the elution with the first mobile phase (SMX and SDT). Detection was simultaneously carried out at 285 nm (absorption maximum for STZ) and 263 nm (absorption maximum for the other evaluated sulfonamides). Measurements were based on peak heights as in previous works with SIC [2,3]. The chromatographic parameters (retention time, peak symmetry, peak resolution, number of theoretical plates and height equivalent to a theoretical plate) were calculated from experimental data as recommended by FDA [22].

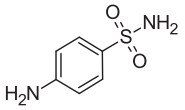
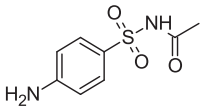
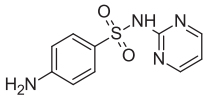
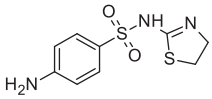
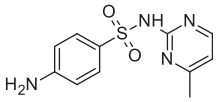
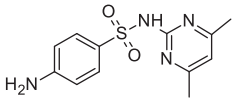
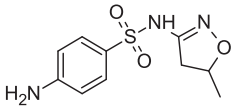
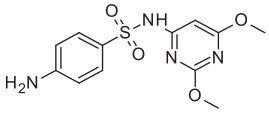
3. Results and discussions

3.1. Chromatographic characteristics

The chromatographic performance of five different columns was evaluated for the separation of eight sulfonamides. SAD, STZ and SCT were selected for the initial experiments to evaluate the chromatographic behavior of different stationary phases, by using the manifold presented in Fig. 1 with direct injection of the sample on the chromatographic column (*i.e.* without the SPE step). The chromatographic parameters for the different columns are shown in Table 2.

Columns with F5 and Phenyl-Hexyl stationary phases allowed the complete separation of the eight sulfonamides, whose structures and the respective pK_a values are shown in Table 3 [23–25]. The F5 phase presented the best chromatographic characteristics among the tested columns (Resolution >1.54, peak symmetry <1.52 and HETP from 5.38 to 21.75 μm for all species.) and it was used in the SPE-SIC procedure. The successful separation of the sulfonamides is not attributed only to the best physical properties of the columns (*i.e.* shorter diffusion path and partial porosity) but to the different chemistry of stationary phases that enabled differential interactions with the analytes. Some of the sulfonamides evaluated in this study present similar substituents (*e.g.* SDZ and SMR, Table 3), which makes the chromatographic separation difficult and the selection of a proper stationary phase is then extremely important.

Table 3
Chemical structures and dissociation constants of analyzed sulfonamides.

Analyte	Molecular structure	pK_a
Sulfanilamide		1.78; 11.19
Sulfacetamide		1.76; 5.22
Sulfadiazine		2.10; 6.28
Sulfathiazole		2.08; 7.07
Sulfamerazine		2.17; 6.77
Sulfadimidine		2.28; 7.42
Sulfamethoxazole		1.83; 5.57
Sulfadimethoxine		1.87; 5.86

3.2. On-line solid-phase extraction

The SPE–SIC manifold presented in Fig. 1 enabled on-line transfer of a pre-treated sample to the chromatographic column, according to the steps showed in Table 1. Sulfonamides are amphoteric species with weakly basic and acid properties, which make feasible the use of anion-exchange for extraction and preconcentration. Three resins were evaluated to this aim, and their performance at different pH of sample and mobile phase is presented in Fig. 2.

The strongly basic anion-exchange resin with 3-trimethylamino-2-hydroxypropyl groups presented the best performance. The active site of this sorbent is a tertiary amine substituted by three methyl groups, which provide a positive charge to the nitrogen atom, increasing the affinity to the anionic sulfonamides. The weakly basic anion-exchange resin with 2-diethylamino-ethyl functional group did not yield good extraction efficiency due to the longer amine substituents that hinder the interaction with the anionic sulfonamides. The anion-exchange resin with aminopropyl group presented an intermediary performance because of its shorter substituents.

The sample pH critically affects the interaction of analytes with the active surface of SPE sorbent as observed in Fig. 2. Samples were maintained at pH 11.0 to keep the species at anionic form and increase the adsorption efficiency (see the pK_a values in Table 3). The first mobile phase was used for elution of the analytes and its pH controlled release of the analytes from the anion-exchange resin, forming a narrow zone for injection

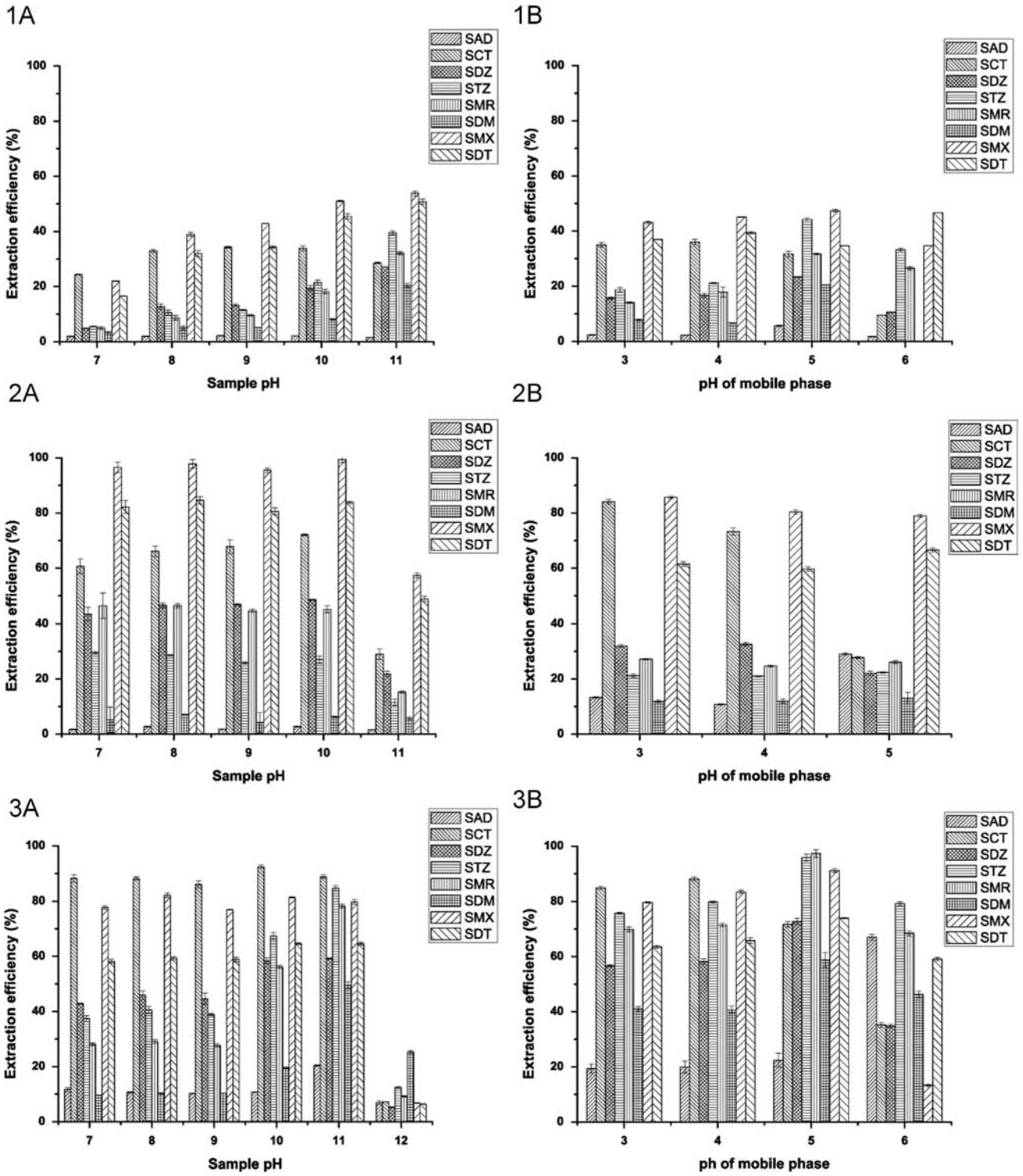


Fig. 2. Comparison of SPE efficiency with different anion exchange resins at different pH of mobile phase and sample (A: pH 7–11; B: pH 3–5). 1: 2-diethylamino-ethyl resin; 2: aminopropyl resin; 3: 3-trimethylamino-2-hydroxypropyl resin.

on the chromatographic column, *i.e.* avoiding peak broadening. The best elution was observed at pH 5.0, in which all sulfonamides were predominantly at their non-charged form (see Fig. 2). The efficiency of the column switching can be observed in the chromatogram presented in Fig. 3, which shows that the SPE step did not hinder the chromatographic separation.

3.3. Analytical features and application

Under the optimized conditions, the analytical characteristics of the proposed system were evaluated for eight sulfonamides (Table 4). Enrichment factors up to 39.2 were achieved even with only 500 μL of the sample. Sulfanilamide presented a poor

enrichment factor because it is not predominantly dissociated in pH 11 ($pK_a = 11.19$). Wide linear working ranges were observed for all analytes. The developed procedure consumed just 900 μL of organic solvent (acetonitrile) and 500 μL of sample per analysis and avoid using organic solvent in the SPE step, thus following the trend to the development of more environmentally friendly procedures [26]. The complete analysis (including extraction, separation and column re-equilibration) of all analytes was achieved in 10.5 min. Characterization of separation process of sulfonamides spiked to freshwater samples (500 μg L^{-1} each) is showed in Table 5.

The analytical features were better in comparison to the achieved in previously described procedures (Table 6). A chromatographic procedure with batch SPE, for example, consumed 1000 mL of sample and 33.0 mL of methanol for the separation of six sulfonamides using a 150 mm column [17]. Lower sample volume is needed for the single-drop liquid-liquid microextraction procedure, however, low reproducibility was reported due the mechanical instability of the drop [11]. Most of the reported works performed batch extraction procedures that have some drawbacks, as susceptibility to both analyte losses and contamination. In addition, this step is often ignored when the analysis time is estimated. The on-line SPE procedure is performed in a closed system that avoids these problems. The high enrichment factors achieved with a low sample volume and without hinder the sample throughput demonstrate the efficiency of the SPE step. It was achieved the highest concentration efficiency value (an enrichment factor of 11.2 can be achieved in one minute), demonstrating the improvement of sample throughput by on-line SPE. The low consumptive index in comparison to procedures listed in Table 6 demonstrates the feasibility of exploiting higher sample volumes to achieve higher enrichment factors.

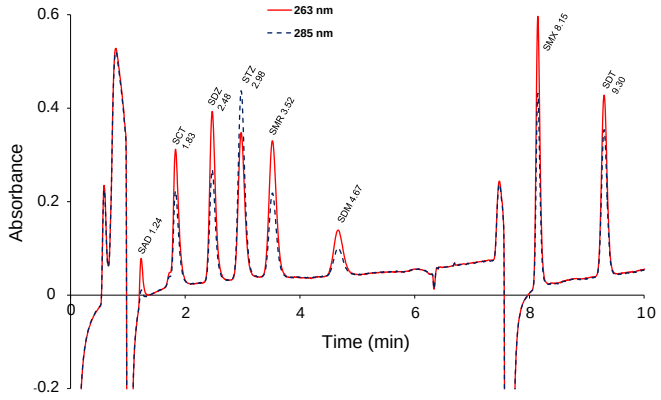


Fig. 3. Chromatogram obtained from the on-line SPE of a solution of sulfonamides (500 μg L^{-1} each). Absorbance values measured at 263 nm (continuous line) and 285 nm (dashed lines).

Four spiked river water samples were analyzed to demonstrate the applicability of the on-line SPE–SIC procedure. Samples were spiked with the target compounds at 100 μg L^{-1} which correspond to the high contamination of waters. The chromatograms obtained from the samples did not show any unknown peak, indicating that the SPE step was efficient for removing matrix components. Fig. 4 shows that SAD and SDM were quantitatively recovered, while SCT, STZ and SMR showed recoveries better than 80%. Low recoveries were observed for SDZ, SMX and SDT, which indicate that they can interact with matrix components (*e.g.* organic matter), interfering in the adsorption of the analytes on the anion-exchange resin. Low recoveries values for this species from freshwaters were previously observed on SPE procedures, even employing molecularly imprinted polymer as sorbent (recoveries from 37.6% to 61.0%) [27].

Table 4
Analytical features of the on-line SPE and chromatographic separation by SIC with F5 fused core particle column.

	Calibration equation[a]	R^2	Linear range (μg L^{-1})	LOD (μg L^{-1})	RSD (%), $n=6$	Enrichment factor
SAD	$y=(1.850\pm0.027)\times10^{-4}C-(1.361\pm0.102)\times10^{-2}$	0.999	100–1000	27	2.5	2.90
SCT	$y=(5.847\pm0.089)\times10^{-4}C-(0.741\pm0.200)\times10^{-2}$	0.999	30–1000	9	2.8	34.2
SDZ	$y=(7.245\pm0.125)\times10^{-4}C-(0.365\pm0.230)\times10^{-2}$	0.999	30–1000	7.7	2.2	39.2
STZ	$y=(8.028\pm0.085)\times10^{-4}C-(0.260\pm0.070)\times10^{-2}$	0.999	30–1000	6.8	2.9	28.8
SMR	$y=(5.319\pm0.640)\times10^{-4}C-(0.341\pm0.141)\times10^{-2}$	0.996	30–1000	6.8	2.8	27.6
SDM	$y=(1.913\pm0.083)\times10^{-4}C-(0.331\pm0.052)\times10^{-2}$	0.999	100–1000	27	2.7	21.0
SMX	$y=(1.160\pm0.009)\times10^{-3}C+(0.633\pm0.322)\times10^{-2}$	0.998	30–1000	4.9	2.8	28.7
SDT	$y=(7.808\pm0.026)\times10^{-4}C-(0.310\pm0.089)\times10^{-2}$	0.998	30–1000	6.7	2.2	34.7

[a] y=peak height and C=sulfonamide concentration (μg L^{-1}).

Table 5
Characterization of separation process of river water spiked with eight sulfonamide antibiotics performed on SPE-SIC.

	SAD	SCT	SDZ	STZ	SMR	SDM	SMX	SDT
Retention time (min)	1.24	1.83	2.48	2.98	3.52	4.67	8.15	9.30
Peak symmetry	1.37	1.18	1.45	1.17	1.40	1.03	1.14	1.09
Resolution	6.25[a]	5.04[b]	3.07[c]	3.13[d]	7.14[e]	25.7[f]	9.04[g]	–
Retention factor	1.12	2.17	3.24	4.13	5.05	7.03	13.05	15.03

[a] SAD/SCT.
[b] SCT/SDZ.
[c] SDZ/STZ.
[d] STZ/SMR.
[e] SMR/SDM.
[f] SDM/SMZ.
[g] SMZ/SDT.

Table 6
Analytical features of some procedures for sulfonamides determination in water samples.

Procedure	Chromatographic column	Analytes	Sample treatment	Analysis time (min)	Organic solvent consume (mL)	Sample consume (mL)	Consumptive index (mL)[a]	Concentration efficiency (min^{-1})[a]	RSD (%)	Reference
HPLC/UV	TC-C18 (4.6 mm × 150 mm, 5 μm)	6 sulfonamides	SD-LPME	10.0	3.4	15.0	0.25	3.0	< 8.2 (n=5)	[11]
HPLC/UV	Eclipse XDBC18 (4.6 mm × 250 mm, 5 μm)	3 sulfonamides	Ionic liquid-salt aqueous two-phase extraction	15.0	3.2	9.0	–	–	< 3.2 (n=6)	[12]
UHPLC/DAD	Hypersil Gold C18 (2.1 mm × 100 mm, 1.9 μm)	11 sulfonamides; 14 quinolines	DLLME	20.0	2.0	5.0	0.15	2.2	< 1.7 (n=5)	[13]
HPLC/UV	XTerra RP18 (4.6 mm × 250 mm, 5 μm)	8 sulfonamides	LLLME	35.0	5.2	12	0.040	9.9	< 5.3 (n=7)	[14]
HPLC/DAD/FLU	Eclipse XDB-C18 (3.0 mm × 150 mm, 3.5 μm)	4 sulfonamides; 5 metabolites	HF-LPME	32.0	3.1	50.0	0.05	2.66	< 1.8 (n=4)	[16]
LC/MS	Luna C18 (4.6 mm × 150 mm, 5 μm)	6 sulfonamides; trimethoprim	SPE	18.0	32.0	1000	1.0	3.0	< 4.9 (n=10)	[17]
SIC/UV	Ascentis Express F5 (4.6 mm × 30 mm, 2.7 μm)	8 sulfonamides	On-line SPE	10.5[b]	0.9	0.5	0.013	11.2	< 2.9 (n=6)	This work

DLME—Dispersive liquid-liquid microextraction; HF-LPME—Hollow fiber based-liquid phase microextraction; LLLME—Liquid–liquid–liquid microextraction; SDME—Single drop–liquid phase microextraction.

[a] Values estimated for the sulfonamide with best analytical performance.
[b] Time elapsed for SPE plus SIC separation.

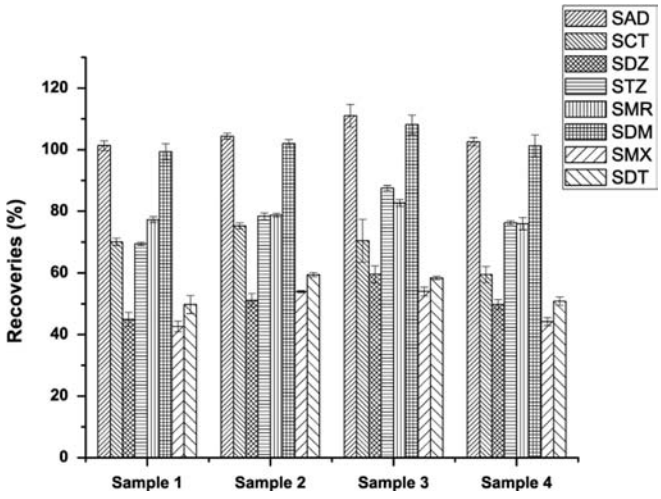


Fig. 4. Recoveries of sulfonamides from different river water samples after on-line SPE.

4. Conclusions

A hyphenated two-step method using on-line SPE coupled to sequential injection chromatography was developed. The anion-exchange resin with a tertiary amine group showed the best results for on-line SPE, by exploiting the acid–base properties of the analytes for sample loading and elution, and compatibility with chromatographic step. Fused-core and monolithic columns were compared in terms of separation efficiency in SIC. A F5 fused-core column achieved the complete separation of a mixture of eight sulfonamides with similar structure. This is the first report of the application of this sorbent on SIC and it demonstrates the need for proper selection of the stationary phase in chromatographic separation of closely related analytes. The procedure provided fast, fully automated SPE/chromatographic separation, with low consumption of organic solvent. The main advantages of the on-line SPE were reuse of the resin, increase of sample throughput as well as low risks of contaminations and analyte losses in comparison to the manual off-line methods of sample pretreatment. The developed procedure yielded suitable sample clean-up and recoveries of most of the sulfonamides spiked to freshwater samples. However, the detection limits achieved enabled only the screening of highly contaminated waters. Aiming the monitoring of sulfonamides at the concentrations typically found in freshwaters, further development will be focused on injection of higher sample volumes (*i.e.* some milliliters) to increase the enrichment factors as well as exploitation of more sensitive detectors, including fluorescence and mass spectrometry.

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