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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

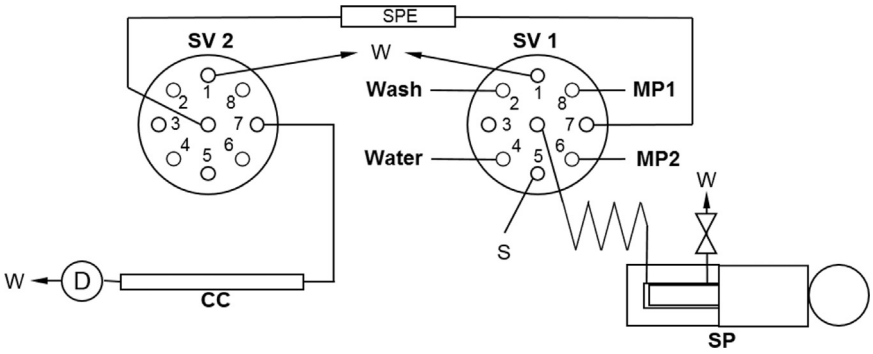

Fig. 1. Scheme of SIC setup with on-line SPE for determination of sulfonamides. SV1 and SV2: selection valves; SP: syringe pump; CC: chromatography column; SPE: extraction column; S: sample, Wash: 0.1 mol L^{-1} $NaHCO_3$; Water: water; MP1: first mobile phase 1; MP2: second mobile phase 2; W: waste;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