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Evaluation of sequential injection chromatography for reversed phase separation of triazine herbicides exploiting monolithic and core–shell columns

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ABSTRACT

This paper describes the development of reversed phase sequential injection chromatography (SIC) methods for separation of simazine (SIM) and atrazine (AT), as well as their metabolites deethylatrazine (DEA), deisopropylatrazine (DIA) and hydroxyatrazine (HAT) exploiting silica based monolithic (50 × 4.6 mm) and core–shell (30 × 4.6 mm, 2.7 μm particles) columns. The separation was made by stepwise elution with two mobile phases: MP_1 composed of 15:85 (v/v) acetonitrile: 2.5 mmol L^{-1} acetic acid/ammonium acetate buffer (pH 4.2), and MP_2, composed of 35:65 (v/v) acetonitrile: 2.5 mmol L^{-1} acetic acid/ammonium acetate buffer (pH 4.2).The less hydrophobic compounds (DIA, HAT and DEA) eluted with MP_1, whereas SIM and AT eluted with MP_2. The method using core–shell column exhibited better chromatographic efficiency compared with monolithic column for separation of SIM and AT, but failed to provide base line separation of DIA and HAT. The proposed composition of mobile phases enabled the monolithic column to separate all the studied compounds with resolution > 2.3 at flow rate of 35 μL s^{-1} and sampling throughput of 8 analyses per hour, whereas in the core–shell the maximum flow rate allowed in the SIC system was 8 μL s^{-1} (sampling throughput of 3 analyses per hour). The limits of detection were between 24 μg L^{-1} (AT) and 40 μg L^{-1} (DEA) using the monolithic column, and between 20 μg L^{-1} (SIM) and 38 μg L^{-1} (DEA) with the core–shell. Ultrasound-assisted extraction (80:20 v/v acetonitrile:water) of a soil sample enriched with the five triazines (250, 500 and 1000 μg kg^{-1}) resulted recoveries between 51% and 121% of the spiked concentrations.

1. Introduction

Sequential injection chromatography (SIC) [1] evolved from sequential injection analysis (SIA) [2], conferring to this versatile technique the capability to perform high performance separations. The sequential injection systems used in the early SIC applications were prone to leaking of mobile phase, but mechanical reconfigurations with high- and medium-pressure components of high performance liquid chromatographs enabled the construction of equipments suitable to handle pressures up to 1000 psi [3,4]. This medium-pressure configuration enhanced the separation capabilities of SIC, enabling the use of modern short columns packed with core–shell particles, as well as narrow-bore monolithic columns [5]. For instance, Chocholous et al. [5] described the separation of four estrogens and the internal standard using core–shell packed particle (30 × 4.6 mm, 2.7 μm particle diameters) and narrow bore (100 × 3 mm) RP-18 monolithic columns. The improved robustness of medium-pressure SIC systems enabled exploitation of diverse separation mechanisms provided by commercially available core–shell packed columns. The separation of seven phenolic compounds in core–shell particle columns with different surface chemistries (C_{18}, amide and phenyl-hexyl) was demonstrated by Chocholous et al. [6].

The morphology of monolithic and core–shell columns enhances the mass transfer (lower C term of the Van Deemter equation) in comparison with columns packed with totally porous particles. Whereas in monolithic column the mass transport is governed by convective rather than slow diffusive processes [7], in the core–shell particles the mass transfer is enhanced because the particle is constituted by a fused impenetrable silica nucleus (typically from 1.3 to 5.0 μm) recovered by a 0.23–0.5 μm thick layer of porous silica gel [8], that is, the diffusive paths are much shorter than those of totally porous particles. Both monolithic (especially the narrow bore ones) and core–shell columns provide faster separations and reduced consumption of mobile phase in comparison with columns packed with totally porous particles, attending the demand for cleaner analytical methods [9].

Sequential injection chromatography has been mostly applied to pharmaceutical and biological samples [10,11], although some few

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works have demonstrated its applicability to environmental analysis [12–15]. Atrazine (AT) and simazine (SIM) are herbicides widely used to stop pre- and post-emergence of broadleaf and grassy weeds in major crops such as sugar cane, maize, soybean and citrus fruits [16]. Atrazine and SIM were banned from European Community since 2004, but they are still used in large areas of Brazil and United States [17,18]. Microbial mediated oxidation of one or both of the side chains of triazines produces deethylatrazine (DEA) or deisopropylatrazine (DIA), compounds that are less hydrophobic than the parent herbicide and which toxicities are not so well known [19]. Deethylatrazine is the more frequently detected metabolite because the ethyl group of AT or SIM is more easily metabolized than the isopropyl group (present only in AT) by soil microorganisms [20,21]. Structures and physicochemical properties of the studied triazines is given in Table 1S [22,23].

The present paper describes the development of SIC methods for separation and quantification of AT and SIM, as well as the metabolites DEA, DIA and hydroxyatrazine (HAT) using a 50 × 4.6 mm monolithic column and a 30 × 4.6 mm core–shell (2.7 μm particle diameter, 0.5 μm thick shell) by stepwise elution [24] with acetonitrile: acetic acid/ammonium acetate mobile phases.

2. Experimental

2.1. Apparatus and reagents

A SIChrom™ – accelerated liquid chromatograph was provided by FIAlab® Instruments (Bellevue, WA, USA). The FIAlab 5.1 software synchronized the movements of the syringe pump and the selection valve (Fig. 1), as well as data acquisition from the UV detector. Details on the instrumental configuration and mode of operation were described elsewhere [4]. Spectrophotometric measurements were carried out with a 4-cm optical path SMA-Z-40 μvol PEEK flow-through cell (10 μL of internal volume) from FIAlab Instruments. Detection was made at 223 and 238 nm using a 200–850 nm USB 4000 spectrometer (Ocean Optics, Dunedin, FL, USA) with a grating of 600 lines mm^{-1}, resolution of 1.5 nm and slit width of 25 μm (height of 1000 μm). An integration time of 50 ms was used. A DH 2000 Deuterium Tungsten Halogen lamp (Mikropack GmbH, Germany) was used as light source. Two 600 μm diameter UV/vis optical fibers (20 in. long) were used to transmit radiation from the source to the flow cell and to the spectrometer.

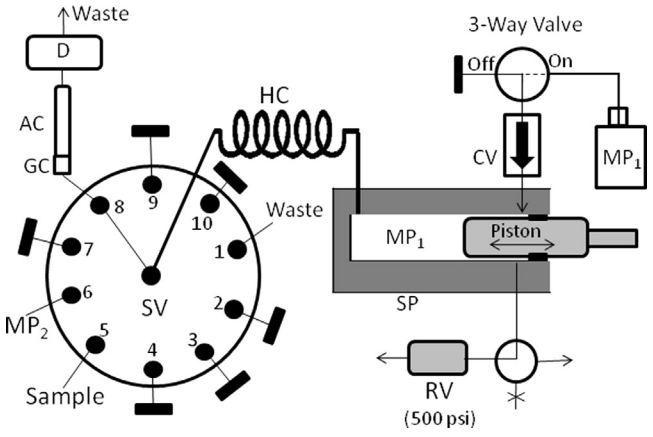

Fig. 1. Sequential injection chromatograph used for separation of triazines and metabolites. SP=Syringe pump; RV=Relief valve; CV=Check valve; HC=Holding coil (4 m × 0.8 mm i.d.); SV=selection valve; GC=guard column; AC=Analytical Column (50 × 4.6 mm C18 Phenomenex monolithic column or 30 × 4.6 mm Ascentis Express 2.7 μm C18 core–shell column); *D*=UV detector (223, 238 and 300 nm) with a 4-cm light pathlength 10-μL flow cell; MP_1=mobile phase 1: 13% or 15% (v/v) ACN: 2.5 mmol L^{-1} HAc/NH_4Ac (pH 4.2); MP_2=mobile phase 2: 35% (v/v) ACN: 2.5 mmol L^{-1} HAc/NH_4Ac (pH 4.2).

Separations were made in a 50 × 4.6 mm i.d. Onyx™ C_{18} monolithic column coupled to 5 × 4.6 mm C_{18} monolithic guard column from Phenomenex® (Torrance, CA, USA). Separations were also made in a 30 × 4.6 mm i.d. Ascentis Express C18 column packed with 2.7 μm core–shell particles, coupled to a 5 × 4.6 mm i.d. guard column packed with the same material, both acquired from Supelco Analytical (Bellfonte, PA, USA).

The analytical standards (Pestanal® grade) of DEA (6-chloro-*N*-(propan-2-yl)-1,3,5-triazine-2,4-diamine), DIA (6-chloro-*N*-ethyl-1,3,5-triazine-2,4-diamine), HAT (4-(ethylamino)-6-(isopropylamino)-1,3,5-triazin-2-ol), SIM (6-chloro-*N*,*N*′-diethyl-1,3,5-triazine-2,4-diamine) and AT (6-chloro-*N*-ethyl-*N*′-(propan-2-yl)-1,3,5-triazine-2,4-diamine) were purchased from Sigma-Aldrich (Sigma-Aldrich Brazil, São Paulo, SP). Stock solutions of these compounds were prepared at concentration of 500.0 mg L^{-1} in methanol. These standards, solids or solutions, were stored in freezer at −18 °C. Methanol (MeOH) and acetonitrile (ACN) of HPLC grade was supplied by J.T. Baker (Phillipsburg, NJ, USA). Ammonium acetate (NH_4Ac) and acetic acid (HAc) were purchased from Merck (Rio de Janeiro, RJ, Brazil). Water used in all experiments was distilled in an all-glass equipment and deionized using the Simplicity 185 system from Millipore (Billerica, MA, USA) coupled to an UV lamp. All other reagents used in this work were of analytical grade from Merck, Sigma or Aldrich.

Chromatographic separations on monolithic column employed two mobile phases (MP): MP_1=(15:85, v/v) ACN: 2.5 mM HAc/NH_4Ac buffer at pH 4.2, and MP_2=(35:65, v/v) ACN:HAc/NH_4Ac buffer (2.5 mM, pH 4.2). In most of experiments with the core–shell column the composition of MP_1 was (13:87, v/v) ACN: 2.5 mM HAc/NH_4Ac buffer at pH 4.2. All mobile phases were filtered through 0.45 μm regenerated cellulose acetate membranes, sonicated for 30 min and purged with high purity He for 20 min prior to use.

2.2. SIC procedure

The SIChrom™ system was modified to use a stainless steel high pressure selection valve (5000 psi) which configuration and mode of operation was described elsewhere [4]. The procedure is described in Tables 1 and 2 for the monolithic and core–shell column, respectively, based on the instrumental scheme shown in Fig. 1. In steps 1–3 (Tables 1 and 2) the system fills the syringe pump with the mobile phase and the sampling line with the sample solution to be analyzed. In the step 4 the sample solution is aspirated inside the holding coil (200 μL for the monolithic column and 80 μL for the core–shell column). The flow is reversed toward the detector flow cell passing through the separation column (step 5) while the spectrophotometer register the peaks corresponding to DIA, HAT and DEA. After that the system is programmed to fill the holding coil and part of the syringe with 4000 μL of MP_2 (step 6; 2500 μL in the case of core–shell column). The flow is reversed toward the column at 35 μL s^{-1} for the separation of SIM and AT (step 7). Finally the syringe pump is refilled with MP_1 (step 8) and the flow is reversed toward the column and flow cell, conditioning the system for the next analysis (step 9). To assure reproducible elution from sample-to-sample these reconditioning steps were repeated in steps 10 and 11. The major steps are similar for both procedures used for separation in the monolithic and in the core–shell columns, with differences in the sample volumes, mobile phase volumes and flow rates (Tables 1 and 2). Retention times, peak areas and peak heights were computed using the signal processing feature of the Origin 8.5.1 software.

2.3. Soil sample and soil extraction

The soil sample was collected at the experimental farm of the Escola Superior de Agricultura Luiz de Queiroz da Universidade de São