http://dx.doi.org/10.5935/0103-5053.20150227

J. Braz. Chem. Soc., Vol. 26, No. 11, 2331-2338, 2015.
Printed in Brazil - ©2015 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Evaluation of Monolithic and Core-Shell Columns for Separation of Triazine Herbicides by Reversed Phase High Performance Liquid Chromatography

Ricardo P. Urio and Jorge C. Masini*

Instituto de Química, Universidade de São Paulo, CP 26077, 05513-970 São Paulo-SP, Brazil

Monolithic and core-shell columns were comparatively evaluated for separation of deisopropylatrazine (DIA), deethylatrazine (DEA), hydroxyatrazine (HAT), simazine (SIM) and atrazine (ATR) under similar conditions of elution, flow rate and sample volume. Although both columns provided separation of the analytes with resolution > 1.5, the core-shell column exhibited better efficiency, leading to narrower peaks. It has height equivalent to the theoretical plates (HETP) for DEA, DIA and HAT, eluted under isocratic elution, about 2.5 times smaller than those of the monolithic column. A soil sample was studied by ultrasound assisted extraction with 80:20 (v/v) acetonitrile:water, revealing the presence of HAT and ATR. This sample was enriched with 0.25, 0.50 and 1.0 mg kg^{-1} of the five triazine compounds and analyzed, leading to recovery percentages between 45 and 86%.

Keywords: atrazine, simazine, ultrasound extraction, monolithic column, core-shell column

Introduction

Herbicides have been used in agriculture since 1940, promoting an increase in agricultural productivity. On the other hand, they caused contamination of soil, water and food, leading to problems of toxicity for living beings gradually exposed to these compounds.[1]

Atrazine (ATR) and simazine (SIM) are triazine herbicides which have been banned from European Community since 2004. However, they are widely used in large areas of Brazil and United States in cultures of soybean, corn, sugar cane, etc. Soil microorganisms breakdown ATR and SIM producing metabolites such as 2-hydroxyatrazine (HAT), deethylatrazine (DEA) and deisopropylatrazine (DIA).[2-4] These compounds are less hydrophobic than the parent herbicides and their toxicities are not so well known.[5]

Management of environmental quality and safety requires frequent monitoring of herbicide residues and herbicide metabolites in waters, soils and food, so that efficient analytical methods for extraction, separation, identification and quantification of these compounds have been continuously developed.[1,2] Analyses of soils require an extraction step aiming the enrichment of analytes and the removal, as much as possible, of interfering species,[6] followed by the separation, identification and quantification step. Most of the analytical methods for the analysis of residues of pesticides and their metabolites in soil extracts are based on gas chromatography and/or reversed phase liquid chromatography coupled to mass spectrometry. In recent years the application of tandem mass spectrometry in LC-MS/MS-based methods has increased thanks to the enhanced selectivity and sensitivity of these techniques, minimizing or even removing interferences in selected reaction monitoring (SRM) mode.[1,2]

Methods based on ultrahigh-pressure liquid chromatography (UHPLC) coupled to MS/MS have been described for ultra-fast separations and sensitive determination of triazines.[6-8] However, they are of high cost, being prohibitive for many laboratories. Additionally to the totally porous silica particles (1.8 μm diameter) modified with C_{18} used in reversed phase UHPLC, recent advances in the technology of chromatographic columns produced monolithic and core-shell stationary phases which enable the use of conventional HPLC pressures.[9,10]

The morphology of monolithic and core-shell columns enhances the mass transfer in comparison with columns packed with totally porous particles. In monolithic column the mass transport is governed by convective rather than slow diffusive processes.[11] 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

*e-mail: jcmasini@iq.usp.br

5.0 μm) recovered by a 0.23 to 0.5 μm thick layer of porous silica gel,[12] that is, the diffusive paths are much shorter than those of totally porous particles. Both monolithic and core-shell columns provide faster separations and reduced consumption of mobile phase in comparison with columns packed with totally porous particles.[9]

The present paper describes the development of HPLC methods for separation and quantification of ATR and SIM, as well as the metabolites DEA, DIA and HAT using 50 × 4.6 mm monolithic and 30 × 4.6 mm core-shell (2.7 μm particle diameter, 0.5 μm thick shell) columns in a matrix of ultrasound soil extract.

Experimental

Apparatus and reagents

An LC 9A Shimadzu (Tokyo, Japan) high performance liquid chromatograph coupled to a Shimadzu SPD 6 AV UV detector (223 nm) was used. Sample injection was made with a rotary Rheodyne (Rohnert Park, CA, USA) valve using a 20 μL sample loop. Data acquisition and data processing was made by the LC Workstation Class-LC 10 software from Shimadzu (Tokyo, Japan). An ultrasonic bath Retsch GmbH & Co. (Haan, Dusseldorf, Germany), with frequency of 35 KHz was used for soil extraction.

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 ATR (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, Brazil). Stock solutions of these compounds were prepared at concentration of 500 mg L^{-1} in methanol. These standards, solids or solutions, were stored in a freezer at –18 °C. Methanol (MeOH) and acetonitrile (ACN) of HPLC grade were supplied by J.T. Baker (Phillipsburg, NJ, USA). Ammonium acetate (NH_4Ac) and acetic acid were purchased from Merck (Rio de Janeiro, RJ, Brazil). Deionized water (resistivity > 18.2 MΩ cm) was obtained from a Simplicity 185 system from Millipore (Billerica, MA, USA) coupled to an UV lamp.

The following chromatographic columns were used: (*i*) 50 × 4.6 mm internal diameter (i.d.) Onyx™ C_{18} monolithic column coupled to 5 × 4.6 mm C_{18} monolithic guard column from Phenomenex® (Torrance, CA, USA); (*ii*) 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).

Soil sample

The soil sample was classified as an oxisol, and was kindly provided by Prof Gilberto Abate from the Departamento de Química of the Universidade Federal do Paraná, Brazil. The soil was collected in an agricultural area of Pato Branco City located in the southwest of the Paraná State, Brazil, 430 km distant from the capital, Curitiba.[13] Maize is the main crop cultivated in the area. The samples were collected in October 4th, 2013, two days after application of the atrazine formulation named Primoleo® (Syngenta Brasil, São Paulo, SP, Brazil). Sampling was made at depth of 0-10 cm from different points, and mixed to compose a single sample. The sample was dried in vacuum oven at 50 °C until constant weight, gently ground to pass through a 250 μm sieve, and stored in a desiccator.

Soil extraction

Soil samples (2 g) were weighted (precision of ± 1 mg) inside test tubes and ultrasonically extracted with 3.0 mL of 80:20 (v/v) ACN:water for 30 min at 35 kHz. The resulting suspension was centrifuged at 5000 rpm for 10 min and the supernatant was transferred to a 10.0 mL volumetric flask. This procedure was repeated two more times and the extracts were combined and then evaporated inside a desiccator under vacuum. The residues were dissolved in water, filtered through 0.45 μm syringe filters and analyzed by HPLC.

The accuracy of the method was evaluated by spike and recovery studies. Aliquots of the soil sample were spiked with 0.25, 0.50 and 1.0 mg kg^{-1} of DEA, DIA, HAT, SIM and ATR. After 24 h, the spiked samples were extracted as described in the previous paragraph.

Chromatographic analyzes

Reversed phase separation of the five triazines was made using two mobile phases: mobile phase A was pure 2.5 mmol L^{-1} NH_4Ac/HAc buffer (pH 4.2) and mobile phase B was pure ACN. The flow rate used in both monolithic and core-shell columns was 1.5 mL min^{-1}. In the core-shell column the elution program was as follows: 0 to 5 min, isocratic at 10% B, 5 to 7 min, linear gradient from 10 to 35% B, 7 to 9.5 min: isocratic at 35% B, 9.5 to 12 min, gradient from 35 to 10% B, and finally, 12 to 15 min, isocratic at 10% B. For the monolithic column the time program was similar and the only difference was that elution started and ended with 13% B. The values refer to volumetric fractions.

The presence of HAT and ATR was confirmed by HPLC-MS/MS using a Shimadzu HPLC system consisted

of two LC-20AD pumps, a SIL 20AC automatic injector, a CBM-20A controller, a CTO-20A column oven and an UV SPD-20 detector coupled to an ion trap Amazon Speed ETD-Bruker Daltonics mass spectrometer operating with electron spray ionization (ESI) in the positive mode. Other experimental conditions were: capillary voltage = 4.5 kV, nebulizer pressure = 40 psi, dry gas flow rate = 9 L min^{-1}, dry temperature = 300 °C. The gradient composition and conditions were the same as those described for HPLC-UV for both monolithic and core-shell columns. For detection the ion trap was scanned at *m/z* 75-600 in full scan mode.

Results and Discussion

Method development

Different compositions of mobile phase and program gradient were studied for separation of the five compounds (Figures 1 and 2). In the core-shell column the separation of DIA and HAT was only possible when the initial concentration of ACN in the mobile phase was < 13% (v/v) (Figures 1a-c). Base line separation of the three metabolites DIA, HAT and DEA was achieved by isocratic elution using a mobile phase composed of 10% (v/v) ACN: 2.5 mmol L^{-1} NH_4Ac/HAc (Figure 1d). As SIM and ATR are much less polar than the metabolites, the gradient of ACN from 10 to 35% was necessary (Figure 1).

In the monolithic column the separation was studied starting the elution with compositions of 10, 13 and 15% (v/v) ACN (in 2.5 mmol L^{-1} NH_4Ac/HAc buffer, pH 4.2). Separation of the five compounds was achieved in all studied conditions (Figure 2). Efficient separation of DIA, DEA and HAT was achieved starting the elution with 15% ACN, but the peak of SIM was significantly widened (Figure 2a). Starting the elution with 10% ACN caused strong retention and peak widening for DEA and HAT (Figure 2c), so that a composition of 13% ACN was chosen as a compromise (Figure 2b).

Despite the fact that the monolithic and core-shell columns operated under the reversed phase mode and used silica based materials modified with C_{18}, the elution order of DEA and HAT was different in the two columns. Hydroxyatrazine (HAT) was more strongly retained than DEA in the monolithic column and the contrary was verified in the core-shell (Figures 1 and 2). The explanation for this finding is not clear but may be related to the presence of residual silanol groups and some degree of ion exchange retention of HAT in the monolithic column.[14] The pKa of HAT is 5.4, so that at pH 4.2 the molecule is mostly protonated and positively charged, behaving as stronger

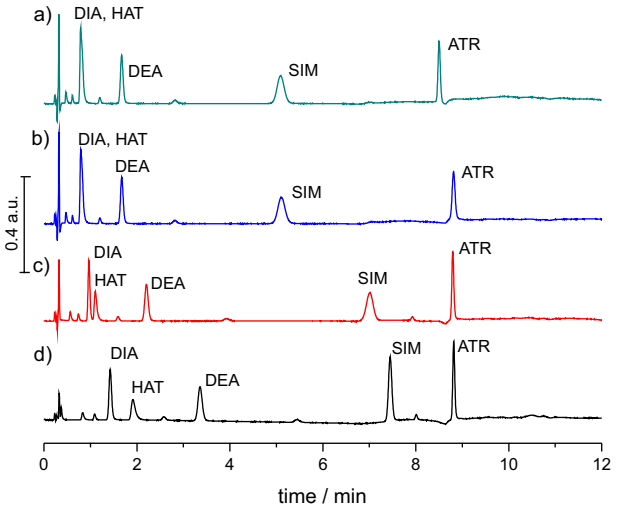

Figure 1. Chromatograms showing the influence of composition of mobile phase on the separation of the five triazines in the core-shell column. Chromatographic conditions: (a) 0 to 5 min, isocratic at 15% ACN, 5 to 7 min, linear gradient of 15 to 35% ACN, 7 to 9.5 min: isocratic at 35% ACN, 9.5 to 12 min, gradient of 35 to 10% ACN, and finally, 12 to 15 min, isocratic at 15% ACN; (b) 0 to 5 min, isocratic at 15% ACN, 5 to 7 min, linear gradient of 15 to 30% ACN, 7 to 9.5 min: isocratic at 30% ACN, 9.5 to 12 min, gradient from 30 to 15% ACN, and finally, 12 to 15 min, isocratic at 15% ACN; (c) similar as (a) but starting and finishing the elution with 13% ACN; (d) similar as (c) but starting and finishing the elution with 10% ACN. Flow rate = 1.5 mL min^{-1}, sample volume = 20 µL, detection at 223 nm.

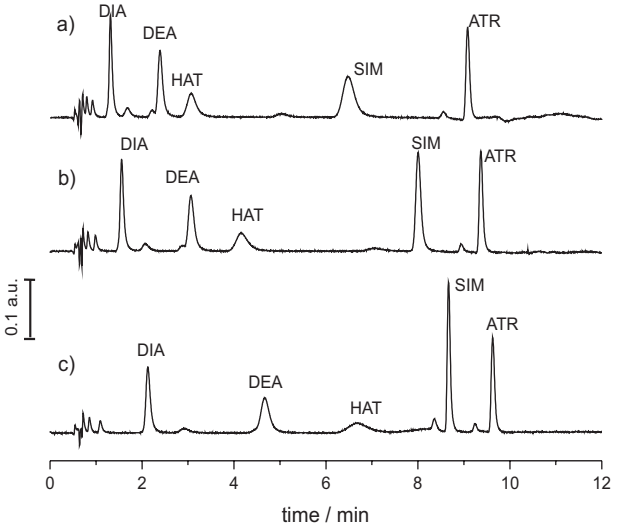

Figure 2. Chromatograms showing the influence of composition of mobile phase on the separation of the five triazine compounds in the monolithic column. Chromatographic conditions: (a) 0 to 5 min, isocratic at 15% ACN, 5 to 7 min, linear gradient of 15 to 35% ACN, 7 to 9.5 min: isocratic at 35% ACN, 9.5 to 12 min, gradient of 35 to 15% ACN, and finally, 12 to 15 min, isocratic at 15% ACN; (b) similar as (a) but starting and finishing the elution with 13% ACN; (c) similar as (a) but starting and finishing the elution with 10% ACN. Flow rate = 1.5 mL min^{-1}, sample volume = 20 µL, detection at 223 nm.

base than the other triazine compounds which have pKa < 2.[15] On the other hand, residual silanol groups have not been verified in Chromolith monolithic columns, so

that the behavior of HAT could also be attributed to the different phase structure of the monoliths rather than silica activity under the studied conditions.[16]

Chromatographic parameters and column efficiency

Retention time (t_R), resolution (Rs), retention factor (k), peak asymmetry (As), plates *per* meter and height equivalent to the theoretical plate (HETP) for both monolithic and core-shell columns are shown in Table 1. Comparison of the chromatographic parameters was made under similar elution conditions, described in Figures 1c and 2c, that is, starting the elution with the 13% (v/v) ACN in 2.5 mmol L^{-1} NH_4Ac/HAc buffer (pH 4.2) mobile phase.

Excepting HAT, it is clear that the core-shell column provided higher retention factors than the monolithic column, even with shorter retention times for all compounds. In reversed phase liquid chromatography, a retention factor > 2 imply that the interference of highly polar compounds is not likely to occur. DIA was the triazine with lowest retention factor (Table 1), so that this is the only compound which determination may suffer interference of humic substances and organic acids[17,18] that may be co-extracted from soils. Interference of polar compounds in the determination of DIA is more likely to occur in the monolithic column because of the lower k value obtained for this compound in comparison with that obtained in the core-shell column. Values of k > 5 suggest that the separation method can be developed further in both columns,[19] using, for instance, a steeper gradient of ACN to reduce the retention time of SIM and ATR, thus shortening the total time of analysis, especially in the case of the core-shell column which had k values ≥ 20 for these compounds. Additionally to the slope of the gradient, the total time of analysis could be significantly reduced by increasing the flow rate of the mobile phase, especially in the monolithic column, exploiting further the high permeability of this column. For instance, at the flow rate of 1.5 mL min^{-1} used in the present comparison, the back pressures were 28 and 130 bar in the monolithic and core-shell columns, respectively.

Resolution > 1.5 was obtained in both columns if elution started with ACN concentration ≤ 13% (v/v). The asymmetry factors were between 1.1 and 2.1, indicating that no significant peak tailing occurred in both columns. Column efficiency was estimated from HETP for DIA, HAT and DEA, which were eluted under isocratic conditions in the first 5 min of the chromatographic run. The HETP values obtained in the core-shell column were significantly lower than those obtained in the monolithic column. The better efficiency of the core-shell column can be also qualitatively deduced from the narrower peaks observed in the chromatograms (Figures 1 and 2). Besides to better efficiency, narrow peaks imply in better selectivity and faster analyses, features which favors the core-shell column in comparison with the monolithic.

The bimodal structure of macro and mesopores of silica based monolithic columns favors fast mass transport between the stationary and mobile phase by rapid convective processes instead of slow diffusion.[20]

Table 1. Chromatographic parameters related to separation of triazines in both core-shell and monolithic columns

Triazine	t_R[a] / min	R_S[b]	K[c]	A_S[d]	Plates[e] / m^{-1}	HETP[f] / μm
			Core-shell			
DIA[g]	0.97 ± 0.01		1.8	1.3	78312	12.8
HAT[h]	1.1 ± 0.03	1.6	2.3	2.1	70884	14.1
DEA[i]	2.2 ± 0.02	9.2	5.9	1.2	122168	8.19
SIM[j]	7.0 ± 0.01	21	20	1.1	–	–
ATR[k]	8.8 ± 0.01	9.1	26	1.2	–	–
			Monolithic			
DIA[g]	1.5 ± 0.01		1.2	1.5	35388	28.2
DEA[i]	3.1 ± 0.01	8.2	3.3	1.4	50090	19.9
HAT[h]	4.2 ± 0.02	3.1	4.8	1.2	25390	39.4
SIM[j]	8.0 ± 0.01	11.3	10.3	1.6	–	–
ATR[k]	9.4 ± 0.01	7.1	12.2	1.5	–	–

[a]Retention time (t_R); [b]resolution was computed as $2(t_2 - t_1) / 1.7(w_{1/2,1} + w_{1/2,2})$; [c]computed as $K = (t_R - t_M) / t_M$ where t_M is the time of the unretained substance (0.1% (v/v) acetone); [d]peak asymmetry (As); [e]number of plates *per* meter was computed as $N = 5.55 t_R^2 / w_{1/2}^2$ considering column lengths of 50 mm (monolithic) and 30 mm (core-shell); [f]height equivalent to the theoretical plate (HETP); [g]deisopropylatrazine (DIA); [h]hydroxyatrazine (HAT); [i]deethylatrazine (DEA); [j]simazine (SIM); [k]atrazine (ATR).

Although this conformation provides the high permeability for the monolithic column, it limits the chromatographic efficiency of these columns, as a consequence of large size distribution, random size distribution and variable geometry of the interskeleton, additionally to the radial heterogeneity of the monolith.[21,22] The better efficiency of the core-shell column may be attributed to two factors: (*i*) the short diffusion path provided by the superficially porous layer allowing fast mass transfer of solutes between the stationary and mobile phase, minimizing the axial dispersion and the peak broadening; and (*ii*) dense and uniform packing of 2.7 μm particles with exceptionally narrow size distribution, reducing the eddy diffusion.[10,23]

Analytical features

The linearity of the peak areas as a function of triazine concentrations were studied in the concentration range between 100 and 1000 μg L^{-1} (Table 2). The limits of detection (LOD) and quantification (LOQ) were computed as LOD = $3S_d/m$ and LOQ = $10S_d/m$ where m is the slope of the calibration curves (peak heights) and S_d is the standard deviation of the noise in the base line, measured around the retention time (t_R). The LOD values varied between 1.1 μg L^{-1} for DIA (monolithic column) and 16 μg L^{-1} for HAT (core-shell column). These values are mostly higher than the maximum concentration levels allowed in drinking waters defined by the United States Environmental Protection Agency (US-EPA) of 3 μg L^{-1}, or by the Brazilian Environment National Council resolution (CONAMA 354/2005) of 2 μg L^{-1}, so that a pre-concentration step would be necessary for monitoring these concentration levels. On the other hand, the LOD and LOQ values obtained for both monolithic and core-shell columns enable the determination of the studied triazines in soil extracts, as well as the monitoring of triazine concentrations in adsorption and degradation studies.[13,24,25]

The linearity, sensitivity and detectability of both monolithic and core-shell columns were of similar magnitude, as well as the intraday and interday relative standard deviations of retention times and slope of the calibration curves. Sensitivity and detectability of HAT could be significantly improved by monitoring the absorbance at 238 nm, but this aspect was not investigated further because the goal of the work was the evaluation of the chromatographic columns and not to investigate the maximum sensitivity and the lowest LOD and LOQ.

Application to a soil sample

The chromatograms of the soil extract superposed to the chromatograms of the soil spiked with 1 mg kg^{-1} of a mix of the five triazines suggest that the sample had detectable amounts of both HAT and ATR (Figures 3 and 4). Several other peaks were observed corresponding to co-extractives. The large peak of unretained solutes can be assigned to polar humic substances or low molar mass carboxylic acids, which are of high abundance in soils.[26] The retention factor of DIA was high enough to provide efficient separation from the pool of weakly retained polar substances. The high efficiency of the columns, especially, that of the core-shell column, enabled the characterization of well-defined peaks in a crude soil extract which was not subjected to any cleanup procedure. Triplicate of extractions resulted concentrations of ATR of 273 ± 12 and 331 ± 16 μg kg^{-1}, obtained in the core-shell and monolithic columns, respectively. The higher concentration of ATR provided by the monolithic column may be attributed to some co-extractive affecting the peak integration. As the efficiency of the core-shell column is better, it can improve the accuracy of peak integration, leading to better column selectivity. However, a larger number of extractions and quantification experiments should be made to prove this hypothesis.

Table 2. Calibration curve parameters and limits of detection and quantification obtained for monolithic and core-shell columns

Triazine	Column									
	Core-shell					Monolithic				
	Slope[a,b] / (10^{-6} L μg^{-1})	Intercept / (10^{-5})	R^2	LOD[c] / (μg L^{-1})	LOQ[d] / (μg L^{-1})	Slope / (10^{-6} L μg^{-1})	Intercept / (10^{-5})	R^2	LOD[c] / (μg L^{-1})	LOQ[d] / (μg L^{-1})
DIA[e]	1.99 ± 0.01	-0.0 ± 0.4	0.9995	2.2	7.5	1.92 ± 0.02	−1.3 ± 0.2	0.9997	1.1	3.7
HAT[f]	0.86 ± 0.01	−2.4 ± 0.9	0.9993	16	53	0.72 ± 0.03	−1.7 ± 0.5	0.994	5.3	17
DEA[g]	1.88 ± 0.01	−0.4 ± 0.2	0.9998	3.3	11	1.96 ± 0.04	−0.7 ± 0.2	0.9999	2.3	8.0
SIM[h]	2.73 ± 0.01	0.8 ± 0.4	0.9998	5.1	15	2.75 ± 0.02	0.9 ± 1.0	0.9999	4.4	15
ATR[i]	2.71 ± 0.01	−4 ± 5	0.9998	7.1	24	2.22 ± 0.02	−0.3 ± 0.7	0.9998	12	41

[a]Mean and standard deviation of triplicates; [b]for these cases the slope was determined for concentrations between 100 and 1000 μg L^{-1}; [c]limit of detection (LOD); [d]limit of quantification (LOQ); [e]deisopropylatrazine (DIA); [f]hydroxyatrazine (HAT); [g]deethylatrazine (DEA); [h]simazine (SIM); [i]atrazine (ATR).

For HAT, the concentrations found were 437 ± 12 μg kg^{-1} (core-shell) and 428 ± 18 μg kg^{-1} (monolithic), without any evidence of statistically significant differences between the results at the 95% confidence level.

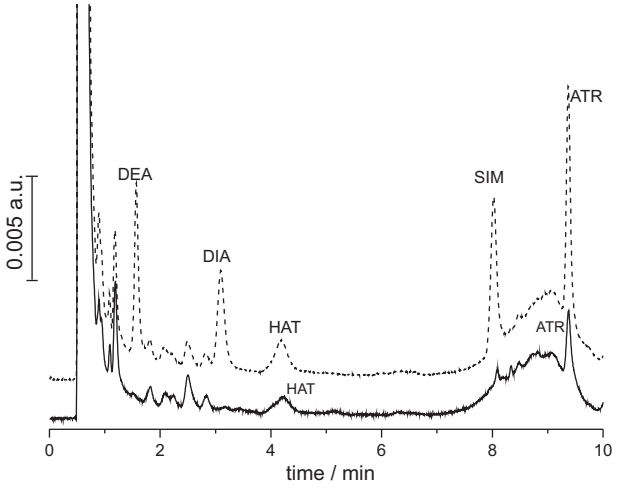

Figure 3. Chromatograms of a soil extract (continuous line) superposed to the chromatogram of an extract obtained from the 1 mg kg^{-1} spiked soil (dashed line) obtained in the monolithic column. Chromatographic conditions were described in caption of Figure 2b.

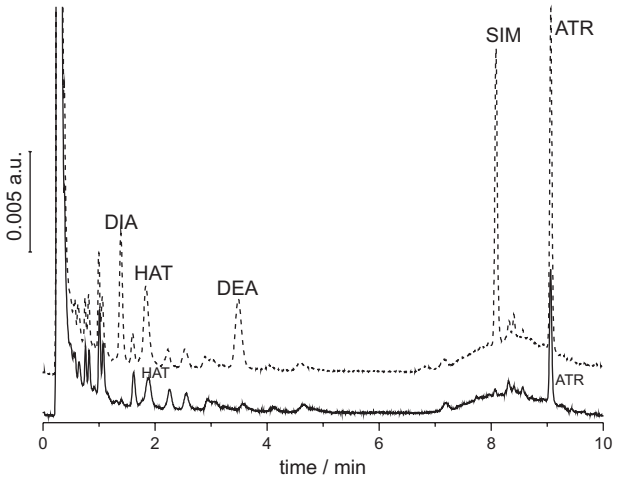

Figure 4. Chromatograms of a soil extract (continuous line) superposed to the chromatogram of an extract obtained from the 1 mg kg^{-1} spiked soil (dashed line) obtained in the core-shell column. Chromatographic conditions: were described in caption of Figure 1d.

The presence of ATR and HAT was confirmed by HPLC-MS/MS using full scan at *m/z* = 75-600. Atrazine was confirmed by the precursor ion $[M + H]^+$ with *m/z* = 216 and the fragment ion $[M + H\text{-}C_3H_6]^+$ with *m/z* = 198, derived from the loss of isopropyl moiety. Hydroxyatrazine was confirmed by the peaks of the precursor ion $[M + H]^+$ at *m/z* = 198 and the fragment ion $[M + H\text{-}C_3H_6]^+$ at *m/z* = 156 (Figures S1 and S2).[27] Both monolithic (Figure S1) and core-shell (Figure S2) columns enabled efficient separation of the analytes avoiding interferences of soil co-extractives on the ionization of the studied triazines.

The accuracy of the studied methodology was evaluated by spike and recovery studies showed in Figures 5a and 5b. Recoveries from the 0.25 mg kg^{-1} spiked soil were consistently higher than those obtained from soils spiked with 0.50 and 1.0 mg kg^{-1} of the five triazines, independent of the chromatographic column. Recoveries obtained with the core-shell column were systematically higher than those obtained with the monolithic, probably as a consequence of the better efficiency of the former, leading to narrow peaks which facilitate the peak integration, minimizing interference of co-extractives. Recoveries from 45 to 86% (Figures 5a and 5b) were consistent with those obtained by Amadori *et al.*[13] studying soil samples from the same region as the one studied in the present work, using a similar extraction method. Those authors spiked the soil with 2.5 mg kg^{-1} of ATR, DIA and DEA, obtaining recoveries of 40-65% for ATR, 45-70% for DIA and 60-80% for DEA using an ACE C_{18} 100 × 4.6 mm i.d., 5 μm particle column.

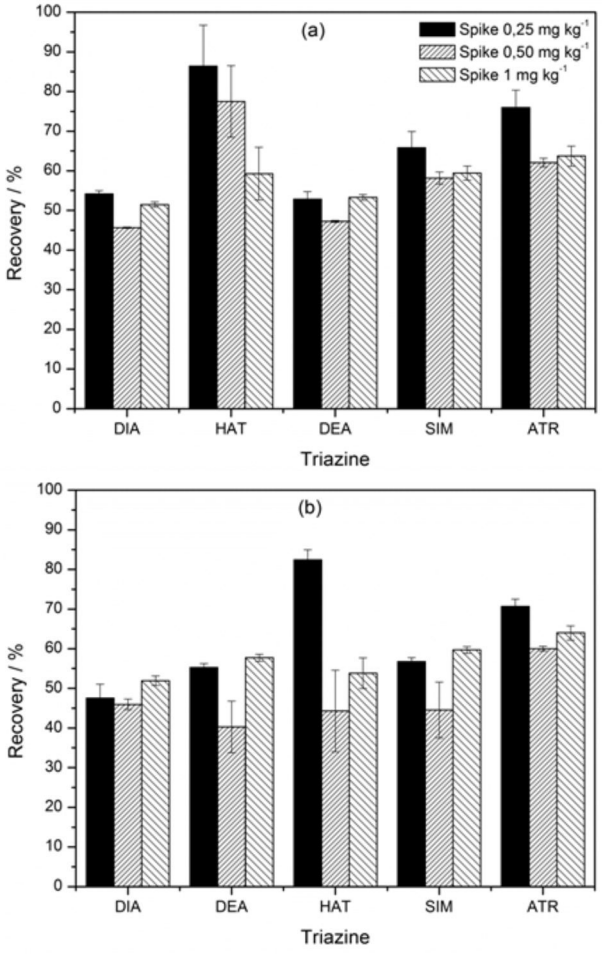

Figure 5. Recoveries found in extracts obtained in the (a) core-shell; (b) monolithic columns after triplicates of extraction of soil samples spiked with 0.25, 0.50 and 1.0 mg kg^{-1} of the five triazines.

Stipicevic *et al*.,[28] employing ultrasound extraction of soils enriched with 14 to 600 ng g^{-1} obtained recoveries between 40-56% for DIA, 80-94% for DEA, 82-98% for SIM and 58-88% for ATR using a 250 × 4 mm i.d., 5 μm particle, Hypersil ODS column. Lesuer *et al*.[29] compared several extraction methods, finding that ultrasound extraction from soils enriched with 500 ng g^{-1} led to recoveries of 15-30% for DEA, 30-50% for ATR and 45-50% for SIM employing a Zorbax SB-C18 150 × 2.1 mm i.d., 3.5 μm particle size, and MS/MS detection. Thus the recoveries between 50 and 86% found in the present work are consistent with several other recovery rates described in the literature for complex soil samples.

Conclusions

The monolithic and core-shell columns provided efficient separation of the studied triazines, but the core-shell column performed better, providing narrower peaks, higher resolution and smaller peak asymmetry. As a consequence, in the chromatographic analyses of the soil extracts, the core-shell column gave higher recovery percentages than those obtained with the monolithic column. The features of these modern columns can be exploited further to improve the sampling throughput. For instance, faster analysis can be achieved exploiting the high permeability of the monolithic column using flow rates much higher than those reported here, without serious backpressure problems, but at the cost of high consumption of mobile phase. Regarding the core-shell column, steeper gradient of ACN could be exploited to reduce the analysis time without significant loss in efficiency. Both columns were easily adapted for use in HPLC-MS/MS system.

Supplementary Information

Supplementary information on the mass spectra obtained in the HPLC-MS/MS experiments is available free of charge at http://jbcs.sbq.org.br as a PDF file.

Acknowledgments

This work was funded by grants 306075/2013-0 from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and grant 2013/18507-4 from the São Paulo Research Foundation (FAPESP). A fellowship from FAPESP to Ricardo P. Urio (contract 2011/08309-5) is acknowledged. Authors acknowledge PhD Vânia A. B. Bueno Silva for her helpful comments on the LC-MS/MS experiments.

References

1. Rissato, S. R.; Libanio, M.; Giafferis, G. P.; Gerenutti, M.; *Quim. Nova* **2004**, *27*, 739.
2. Benvenuto, F.; Marin, J. M.; Sancho, J. V.; Canobbio, S.; Mezzanotte, V.; Hernandez, F.; *Anal. Bioanal. Chem.* **2010**, *397*, 2791.
3. Sass, J. B.; Colangelo, A.; *Int. J. Occup. Environ. Health* **2006**, *12*, 260.
4. Nogueira, E. N.; Dores, E. F. G. C.; Pinto, A. A.; Amorim, R. S. S.; Ribeiro, M. L.; Lourencetti, C.; *J. Braz. Chem. Soc.* **2012**, *23*, 1476.
5. Ralston-Hooper, K.; Hardy, J.; Hahn, L.; Ochoa-Acuna, H.; Lee, L. S.; Mollenhauer, R.; Sepulveda, M. S.; *Ecotoxicology* **2009**, *18*, 899.
6. Hernandez, F.; Marin, J. M.; Pozo, O. J.; Sancho, J. V.; Lopez, F. J.; Morell, I.; *Int. J. Environ. Anal. Chem.* **2008**, *88*, 409.
7. Ibanez, M.; Sancho, J. V.; McMillan, D.; Rao, R.; Hernandez, F.; *TrAC, Trends Anal. Chem.* **2008**, *27*, 481.
8. Marin, J. M.; Gracia-Lor, E.; Sancho, J. V.; Lopez, F. J.; Hernandez, F.; *J. Chromatogr. A* **2009**, *1216*, 1410.
9. Chester, T. L.; *Anal. Chem.* **2013**, *85*, 579.
10. Borges, E. M.; Rostagno, M. A.; Meireles, M. A. A.; *RSC Adv.* **2014**, *4*, 22875.
11. Svec, F.; Huber, C. G.; *Anal. Chem.* **2006**, *78*, 2100.
12. Kirkland, J. J.; Langlois, T. J.; DeStefano, J. J.; *Am. Lab.* **2007**, *39*, 18.
13. Amadori, M. F.; Cordeiro, G. A.; Reboucas, C. C.; Peralta-Zamora, P. G.; Grassi, M. T.; Abate, G.; *J. Braz. Chem. Soc.* **2013**, *24*, 483.
14. McCalley, D. V.; *J. Chromatogr. A* **2002**, *965*, 51.
15. Vermeulen, N. M. J.; Apostolides, Z.; Potgieter, D. J. J.; Nel, P. C.; Smit, N. S. H.; *J. Chromatogr.* **1982**, *240*, 247.
16. Herrero-Martinez, J. M.; Mendez, A.; Bosch, E.; Roses, M.; *J. Chromatogr. A* **2004**, *1060*, 135.
17. Infante, C. M. C.; Urio, R. D.; Masini, J. C.; *Anal. Lett.* **2011**, *44*, 503.
18. dos Santos, L. B. O.; Infante, C. M. C.; Masini, J. C.; *J. Sep. Sci.* **2009**, *32*, 494.
19. Christian, G. D.; *Analytical Chemistry*, 6th ed.; Wiley: Hoboken, NJ, 2004.
20. Miyazaki, S.; Takahashi, M.; Ohira, M.; Terashima, H.; Morisato, K.; Nakanishi, K.; Ikegami, T.; Miyabe, K.; Tanaka, N.; *J. Chromatogr. A* **2011**, *1218*, 1988.
21. Ghanem, A.; Ikegami, T.; *J. Sep. Sci.* **2011**, *34*, 1945.
22. Guiochon, G.; *J. Chromatogr. A* **2007**, *1168*, 101.
23. Fekete, S.; Fekete, J.; Ganzler, K.; *J. Pharm. Biomed. Anal.* **2009**, *49*, 64.
24. Urio, R. D.; Infante, C. M. C.; Masini, J. C.; *J. Agric. Food Chem.* **2013**, *61*, 7909.

25. Konda, L. N.; Pasztor, Z.; *J. Agric. Food Chem.* **2001**, *49*, 3859.
26. Baziramakenga, R.; Simard, R. R.; Leroux, G. D.; *Soil Biol. Biochem.* **1995**, *27*, 349.
27. Hu, E.; Cheng, H.; *Microchim. Acta* **2013**, *180*, 703.
28. Stipicevic, S.; Fingler, S.; Zupancic-Kralj, L.; Drevenkar, V.; *J. Sep. Sci.* **2003**, *26*, 1237.
29. Lesueur, C.; Gartner, M.; Mentler, A.; Fuerhacker, M.; *Talanta* **2008**, *75*, 284.

Submitted: July 4, 2015
Published online: September 4, 2015

FAPESP has sponsored the publication of this article.