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HPLC based method using sample precolumn cleanup for the determination of triazines and thiolcarbamates in hemodialysis saline solutions

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Abstract

Solid-phase extraction (SPE) procedures for cleanup and preconcentration followed by HPLC-UV method were investigated for the simultaneous determination of seven low-dosed pesticides in saline concentrates for hemodialysis. The target compounds were ametryn, desmetryn, prometryn, terbutryn, molinate, triallate and butylate. Polyethylene (three different types), teflon, polyurethane and polystyrene, in powder form, were investigated as adsorbents for solid-phase extraction of the analytes from the saline samples. Quantification was performed at 222 nm and the analytes were separated on a LiChrosorb RP-18 (5 μm, 125 mm × 4 mm i.d.) column using gradient elution with water/acetonitrile as mobile phase. The duration each chromatographic run was 18 min including column reconditioning. The efficiency of the different SPE substrates for retaining the analytes from the highly concentrated saline (HCS) samples was discussed. The best performance was achieved with polystyrene as SPE material considering preconcentration factor, precolumn clogging, reusing capability and similarity between the mobile phases for SPE and HPLC procedures. Analyte concentrations as low as 1 $\mu g\,L^{-1}$ could be determined in spiked HCS samples after preconcentration on polystyrene SPE precolumns. Recoveries between 98.7 and 102.2% were obtained from commercial spiked samples. Detection limits ranging from 4.8 (for prometryn) to 46 $\mu g\,L^{-1}$ (for butylate) were calculated (without preconcentration). The within-day relative standard deviations ($n = 9$) ranged from 2.3 to 4.8%.

Keywords: HPLC; SPE; Saline concentrates; Triazines; Thiolcarbamates

1. Introduction

The negative environmental impact from the widespread use of pesticides has led to a number of examinations for the development of different analytical methods. Among them, chromatographic measurements are world wide used employing different detectors as fluorimetric [1–7], photometric [8–10] and electrochemical [11–13]. Chromatographic methods, despite high selectivity and sensitivity, show frequently drawbacks owing to matrix interference. Investigations of substrates for solid-phase extraction (SPE) aiming sample cleanup and analyte preconcentration are important to improve chromatographic separations, mainly considering the cost, efficiency relative to the target matrices and reuse possibility. Chromatographic determination of pesticides in soils, plants and natural waters, including samples moderately saline like seawater (total salt concentration about of 30 $g\,L^{-1}$), is well documented. However, there is a lack of studies to assay these analytes in highly saline media as solutions used for the preparation of hemodialysis fluids (total salt concentration about of 400 $g\,L^{-1}$). In these samples, the saline media interfere drastically in the chromatographic separation by decreasing the column performance. Moreover, it leads to an excessive increase of the retention times of non polar analytes due to a significant salting out effect in reversed-phase separations.

Renal patients on hemodialysis treatment are subject to some disturbances due to the transference of contaminants

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from the dialysis fluid directly into the circulatory system [14–17]. Different kinds of contaminants have been detected in dialysis fluids, not only chemicals as metallic [18–20] and non metallic [21] species, but also microorganisms [22–25].

The dialysis fluid or dialysate is prepared by mixing purified water with a highly concentrated saline solution containing acetic acid, sodium bicarbonate, glucose and chlorides of sodium, calcium and magnesium. The saline concentrate is mixed with water during the hemodialysis session in such a proportion that until the end of the session 120 L of pure water is turned into a solution containing these electrolytes in the same concentration as in blood. Since these saline concentrates are commercialized in 3.4 or 4.0 L bottles, the total saline concentration reaches ca. 400 g L^{-1}.

Nowadays, machines that automatically mix saline concentrate with the water do not allow dialysate manipulation, therefore, the quality control of these concentrates, besides the regular water control, should be done before they are connected to the machine.

Analytical methods to access directly the quality of the highly concentrated saline (HCS) solutions are relatively rare. In part because the water is considered the main source of contamination for dialysates and in part due to analytical drawbacks associated to the analysis of HCS matrices. To overcome difficulties of analyzing such matrices, large samples dilution are often the only choice, despite prejudice on sensitivity. The loss in sensitivity is a problem for pesticides determination because their limit in drink water, established by EPA [26], ranged from 3 to 10 μg L^{-1} (herbicides) for oral intake. Considering that through dialysate pesticides would be delivered directly into the circulatory system, the allowed level should be even lower.

This paper describes the simultaneous determination of ametryn, desmetryn, prometryn, terbutryn, molinate, triallate and butylate by HPLC with spectrophotometric detection in HCS solutions. To overcome interferences of the HCS matrix and also to improve the sensitivity, SPE columns filled out with different substrates were investigated for analyte separation.

2. Experimental

2.1. Reagents and solutions

The water used in this work was distilled, de-ionized and further purified using a Milli-Q high-purity water device (Millipore, Bedford, USA). Aqueous stock solutions of ametryn (50 mg L^{-1}), desmetryn (80 mg L^{-1}), prometryn (22 mg L^{-1}), terbutryn (20 mg L^{-1}), molinate (20 mg L^{-1}), triallate (1 mg L^{-1}) and butylate (15 mg L^{-1}) were prepared by dissolving the triazines and thiolcarbamates (Riedel-de Haën, Hannover, Germany) in water with 10 min manually vigorous agitation followed by 10 min sonication with a commercial ultra-sound batch (Sonorex, RK 510 H, Berlin). The obtained solutions were stable for long periods (>60 days) without phase separation. Work solutions were prepared by adequate dilution of the stock solutions with water.

Artificial HCS solution containing K^+, Ca^{2+}, Mg^{2+}, Na^+ ions in a total concentration about of 400.0 g L^{-1} were prepared from their chloride salts (all Merck). Glucose and $NaHCO_3$ or $KHCO_3$ were also added to compose some HCS solutions. The commercial HCS solutions had the following composition: concentrate I: Solurin (JP Indústrias Farmacêuticas, São Paulo, Brazil) with 684.82 g NaCl; 598.94 g CH_3COONa; 18.06 g $MgCl_2$, 22.20 g $CaCl_2$ and 300.20 g of glucose in 3.6 L; concentrate II: HB (Salbego, São Paulo, Brazil) with 864.40 g NaCl; 13.41 g KCl; 6.27 g $MgCl_2$, 23.10 g $CaCl_2$ and 108.00 g of glucose in 3.4 L; concentrate III: HB Bic (Salbego, São Paulo, Brazil) with 947.6 g NaCl; 18.0 g KCl; 12.4 g $MgCl_2$, 46.8 g $CaCl_2$ and 352.8 g of $NaHCO_3$ in 4.0 L; concentrate IV: HD 3.5 (B. Braun, São Paulo, Brazil) with 864.40 g NaCl; 13.41 g KCl; 6.27 g $MgCl_2$, 23.10 g $CaCl_2$, 352.8 g $NaHCO_3$ and 108.00 g of glucose in 3.4 L.

2.2. Chromatographic separation

The chromatographic equipment consisted of a DX-300 gradient chromatography system (Dionex, Sunnyvale, United States) with an UV–Vis detector SPD-10AV (Shimadzu, Kyoto, Japan) set at 222 nm. The data were processed with a C-R6A data processor (Shimadzu). Chromatographic separations were performed on an octadecylsilane modified silica column (250 mm × 4 mm i.d., LiChrospher, Merck). The column was kept at 25 ± 2 °C and the injection volume was 100 μL.A linear gradient profile with an ACN–water eluent was established by using the following steps. 0–6 min: isocratic 60:40 (v/v) ACN–water. 6–6.5 min: gradient from 60:40 to 90:10 (v/v) ACN–water. 6.5–15 min: isocratic 90:10 (v/v) ACN–water. 15–18 min: gradient from 90:10 to 60:40 (v/v) ACN–water.

2.3. Solid-phase extraction

Low density polyethylene (LDPE) and high density polyethylene (HDPE) were obtained as powder by PPH (Pólo Petroquímico de Triunfo, Brazil). Polystyrene (PS) (725 m^2 g^{-1} surface area, 20–60 mesh), ultrahigh molecular mass polyethylene (UHPE) and polytetrafluorethylene (PTFE) (1 μm particle size) were obtained from Sigma–Aldrich (USA). Polyurethane (PUF) was a commercial, open-cell, polyether-type polyurethane foam (Vulcan of Brazil-VCON 202, 42% resilience and 10–12 cells/linear cm) and was treated as described previously [27]. The SPE substrates were sieved before using and the 60 mesh fraction was used throughout the work. An octadecylsilane modified silica (C-18) was also assayed as SPE material.

The columns were prepared by filling glass tubes (10.0 cm length × 2 mm i.d.) individually with the polymers. The packing material was fixed in the columns with small pieces

of glass wool. The amount of each polymer necessary to fill the columns was 0.8 g for PTFE and 1.2 for the others.

A Minipuls peristaltic pump (Gilson, Villiers-le-Bel, France) was used to transport the samples through the SPE columns before the chromatographic analysis. The individual components were connected with (4.0 mm i.d.) Tygon tubing.

The conditioning of the SPE columns was carried out as described elsewhere by Dupas et al. [28] for C-18 and by Lepane et al. [29] for the PS material. The other substrates were conditioned with 10 mL water, 5 mL ethanol and 10 mL water again. All at a flow rate of 1.0 mL min^{-1}.

For the preconcentration studies, solutions containing from 1 to 100 $\mu g\,L^{-1}$ of each analyte were pumped through the SPE columns at a flow of 3.5 mL min^{-1} to separate the analytes from the matrix. The effluent was discharged to waste. Subsequently, 1 mL of a 60:40 (v/v) ACN–water solution was used to elute the analytes at 0.5 mL min^{-1} flow rate for the following HPLC analysis. Between each separation, the SPE column was washed with 10 mL water at a flow rate of 5.0 mL min^{-1}.

2.4. Analysis of commercial saline concentrates

Seven different commercial concentrates for hemodialysis were analyzed following the procedure above described. 0.1 L sample was pumped through the PS column at a flow rate of 3.5 mL min^{-1}. The column was washed with 1 mL water and the elution carried out with 1 mL ACN/water (60:40) at a flow rate of 0.5 mL min^{-1} followed by HPLC analysis. These samples were also analyzed after spiking with 1.0 $\mu g\,L^{-1}$ of each analyte.

3. Results and discussion

3.1. Determination of triazines and thiolcarbamates by HPLC-UV detection

In a previous work [30] we studied the solubility of triazines in organic solvents and mixtures aiming their chromatographic separation. In spite of pure ACN has been the best option, its mixture with up to 40% water was necessary for chromatographic purposes.

In the present work, to choose the best eluent for the SPE and mobile phase for HPLC analysis we extended the solubility study to the target thiolcarbamates. It was observed a similar behavior between triazines and thiolcarbamates relative to their solubility in organic solvents, probably because their polarities do not differ considerably. Therefore the use of ACN–water mixtures assured as much the best HPLC performance considering peak reproducibility, baseline noise, peak shape and retention time as the elution of the pesticides from the SPE columns.

For the simultaneous chromatographic separation of triazines and thiolcarbamates neither ACN nor water were good eluents. Mobile phase containing only ACN produced poor peak resolution. Therefore, the elution started with a mobile phase 60:40 ACN–water but had to be increased up to 90:10 ACN–water to elute triallate, which is practically insoluble in water. With this two-steps gradient the analytes were separated in 15 min, however, the entire chromatographic run lasted 18 min in order to recondition the column for the next injection. With this approach, relative standard deviations <5% ($n = 9$) were obtained for all analytes. A typical chromatogram obtained from an aqueous solution spiked with the analytes is shown in Fig. 1.

The calibration graphs ($y = a + bx$) were obtained by linear regression for two sets of concentration because the analytes presented different molar absorptivities (ϵ) at 222 nm. Whereas ametryn, desmetryn and prometryn presented ϵ values higher than 25,000 $mol^{-1}\,L\,cm^{-1}$, for butylate, molinate, terbutryn and triallate ϵ values lower than 10,000 $mol^{-1}\,L\,cm^{-1}$ were observed. For the first group the calibration graph started at 20 $\mu g\,L^{-1}$ and for the second at 100 $\mu g\,L^{-1}$. For both groups concentrations up to 700 $\mu g\,L^{-1}$ were in the linear range. Table 1 shows the analytical characteristics of the chromatographic method including detection (LOD) and quantification (LOQ) limits defined as the concentration of the analytes that produces a peak with a signal-to-noise ratio of 3 and 10, respectively.

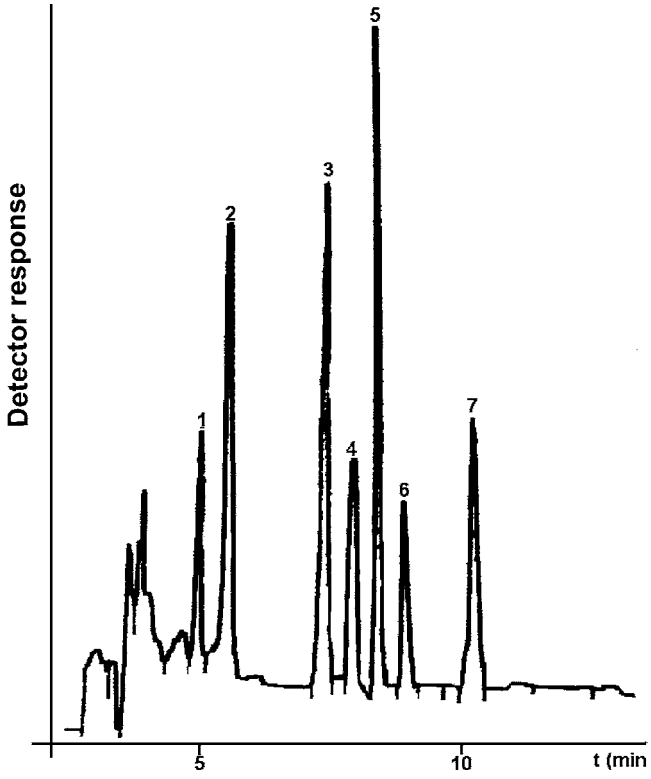

Fig. 1. Chromatogram of terbutryn (1), desmetryn (2), ametryn (3), butylate (4), prometryn (5), molinate (6) and triallate (7) obtained from a spiked (250 $\mu g\,L^{-1}$) aqueous sample. For gradient and chromatographic conditions see text.

Table 1
Analytical characteristics of the chromatographic method

Analyte	*$y = bx + a$		r	LOD ($\mu g\,L^{-1}$)	LOQ ($\mu g\,L^{-1}$)
	b	a			
Ametryn	0.39	+0.06	0.9996	5.5	18.2
Desmetryn	0.23	+0.04	0.9990	8.0	26.4
Prometryn	0.59	−0.04	0.9996	4.8	15.8
Terbutryn	0.22	−0.06	0.9980	13.0	42.9
Butylate	0.06	+0.02	0.9992	46.0	151.8
Molinate	0.13	−0.09	0.9994	21.0	69.3
Triallate	0.16	+0.06	0.9981	20.0	66.0

* x: Concentration [$\mu g\,L^{-1}$]; y: UV-detector response at 222 nm [a.u].

3.2. Sample clean up and preconcentration

The analytes could not be directly determined by HPLC in the HCS samples mainly due to the baseline increase observed after successive injections, clogging of the chromatographic system and changes in the retention times of the analytes. Additionally, the more hydrophobic compounds were hardly eluted probably due to the salting-out effect originated by the high salt content of the samples.

The use of SPE in this work not only avoided the introduction of sample matrix into the analytical column but also permitted to concentrate efficiently traces of the analytes. However, this procedure always requires optimization of a series of variables involved in both adsorption and desorption steps [31–38]. Since the method must be used to assay trace levels of low polarity analytes in samples containing high concentrations of ionic species, a range of non polar substrates for the SPE procedures was investigated. C-18, LDPE, HDPE, UHDP PTFE, PUF and PS were tested as adsorbents. They were evaluated considering the efficiency to retain only the analytes (and not the matrix), preconcentration factors, reuse capability and similarity between eluent for SPE desorption and HPLC mobile phase.

To access the adsorption yields for the pesticides by the SPE procedures, molecular absorption spectra of spiked samples obtained before and after the SPE runs were compared. 0.1 L solutions containing 100 or 1000 $\mu g\,L^{-1}$ each analyte individually in water and also in artificial HCS samples were assayed. The flow rate was 3.5 mL min^{-1}. As during these tests some SPE columns became partially blocked owing to the saline matrix, the polymers were also investigated for the maximum sample volume allowable without changes in the SPE column back pressure. Artificial HCS solutions were pumped through the columns at a flow rate of 3.5 mL min^{-1} until they get blocked (even partially) or until a maximum volume of 1000 mL passed through the SPE column. The results showed that all analytes were totally retained from aqueous samples (100 to 1000 mL) by the polymers HDPE, LDPE, UHPE and PS. By using the saline samples instead of the aqueous ones, the adsorption yields increased as PUF and PTFE also retained all the analytes present in the 100 $\mu g\,L^{-1}$ samples (but not in the 1000 $\mu g\,L^{-1}$). The increasing in the adsorption yields can be attributed to the ionic strength of the HCS samples. The salting out effect enhanced the interaction analyte/substrate [39]. The C-18 material used as SPE substrate showed only a moderate adsorption capability as the analyte signals were observed in all effluents.

For the desorption of the analytes from the SPE columns the HPLC mobile phase was tested. With exception of the C-18 SPE material, fast and complete desorption with 1 mL 60:40 (v/v) ACN–water mixture was obtained. However, PTFE, HDPE, LDPE and PUF showed an important disadvantage. Beside the analytes, some not identified compounds were co-eluted from the SPE columns, and caused interference in the following HPLC analysis. Many attempts were done to cleanup the SPE material prior the column preparation, however, the interference was not avoided. With the PS material this problem was not observed. The good performance of the PS material to preconcentrate triazines from polar samples was also pointed out elsewhere [40,41], mainly in comparison with the C-18 cartridges that are more widely used.

Since the PS used as SPE material showed, besides an adequate analyte adsorption/desorption process, no high column back pressure and no co-elution of interferents, it was selected to clean up the samples and also to preconcentrate the analytes before the HPLC determinations. Preconcentration factors and the best (highest possible) flow rate for analyte retention were investigated by testing sample volumes ranging from 20 to 500 mL solutions containing 1 μg each analyte. The flow rate of 3.5 mL min^{-1} was the highest possible for total retention of the analytes from 500 mL HCS samples. Complete elution from the SPE columns was achieved with 1 mL of 60:40 (v/v) ACN–water solution, so that with an elution flow rate of 0.5 mL min^{-1} a preconcentration factor of 500 was obtained before the HPLC analysis.

3.3. Analysis of commercial samples

Table 2 shows the results of the analysis of different concentrates for hemodialysis. None of them presented pre-

Table 2
Recovery by the HPLC SPE-PS method of triazines and thiolcarbamates in 100 mL spiked commercial saline concentrates for hemodialysis

HCS sample[a]	Analyte spiked 1.0 $\mu g\,L^{-1}$ each	Recovery[b] (%)
1	Am + De + Te + Pr	98; 96; 102; 95
2	Mo + Tr + Bu	93; 98; 95
3	Am + De + Tr + Bu	97; 96; 102; 93
4	Mo + Te + Tr + Pr	95; 99; 101; 95
5	Bu + De + Te + Pr	97; 92; 96; 90
6	Am + Mo + Te + Tr	92; 97; 104; 90
7	Am + De + Tr + Pr	94; 90; 97; 95;
	+ Mo + Tr + Bu	97; 92; 93

s_r ranging from 2.2 to 4.8% ($n = 9$); Am: ametryn; De: desmetryn; Pr: prometryn; Te: terbutryn; Mo: molinate; Tr: triallate; Bu: butylate.

[a] HCS solutions: JP Indústrias Farmacêuticas, Salbego, B. Braun.

[b] Values calculated from the calibration function (see text).

Table 3
Regression characteristics obtained by the correlation between proposed and reference methods [30] applied to dialysis fluid samples spiked individually with triazines and thiolcarbamates

Analyte	Spiked range ($\mu g\,L^{-1}$)	$y = bx + a$		r
		b	a	
Ametryn	20.0–100.0	1.06	0.35	0.998
Desmetryn	20.0–100.0	0.99	0.32	0.982
Prometryn	20.0–100.0	0.96	0.24	0.995
Terbutryn	50.0–250.0	0.91	0.53	0.998
Butylate	200.0–600.0	0.98	1.12	0.974
Molinate	50.0–250.0	1.00	0.66	0.995
Triallate	50.0–250.0	0.97	0.42	0.984

viously these pesticides as contaminant. The analysis of 100 mL spiked samples revealed that after sample cleanup and preconcentration on SPE-PS columns, concentrations as low as $1\,\mu g\,L^{-1}$ could be determined in commercial HCS samples with recoveries ranging from 90 to 104%.

3.4. Reference method

For the determination of triazines or thiolcarbamates in saline concentrates for hemodialysis, reference standard methods are not available. Therefore, spiked HCS samples were analyzed by the proposed method and by a voltammetric one (reference method) described in a previous paper [30]. The voltammetric method was developed to assay the total content of triazines (without speciation) in HCS solutions after extraction of the analytes in ACN. The triazines were detected at pH 3.0 in the potential range of −800 to −1200 mV (versus Ag/AgCl, KCl 3.0 $mol\,L^{-1}$) at the hanging mercury drop electrode after a preconcentration time of 30 s at −800 mV. To determine the thiolcarbamates the method was adapted by changing the potential window (−600 to 0 mV), the preconcentration potential (−600 mV) and the electrolyte (pH = 6.5). Since the speciation among the analytes was not possible by the voltammetric method, the proposed and reference methods were compared by using artificial HCS solutions spiked only with one analyte at a time. The regression characteristics (Table 3) indicate that similar results were obtained by both methods in the investigated concentration ranges as the slope was near 1 for all curves, the intercepts were not significantly different from 0 (considering the concentration values) and linear correlation coefficients were around 1 in all cases.

4. Conclusions

The association of the HPLC method with a clean up/preconcentration step in precolumns filled with polystyrene powder as SPE material enabled the determination of triazines and thiolcarbamates in HCS solutions used in hemodialysis. Among the tested substrates as SPE material PS showed the best performance to extract the analytes from the HCS samples because neither clogging effects nor co-elution of unknown compounds were observed. Additionally, very high preconcentration factors (500 times) could be reached with SPE-PS column. The analytes were promptly adsorbed from the saline samples and easily eluted with 1 mL of the mobile phase used in the following HPLC separation. Moreover, PS column showed the highest reusing capability. The same column was used throughout the work.

The SPE-HPLC method for the determination of pesticides in HCS media was developed for hemodialysis fluids but can be also adapted for other saline matrices as seawater.

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