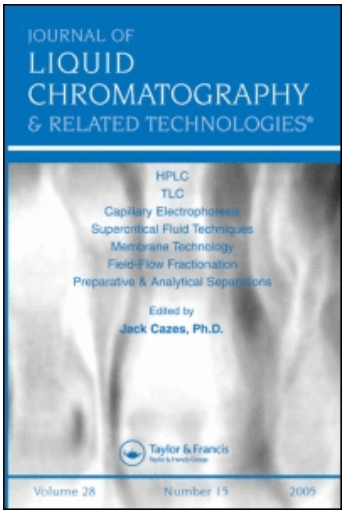

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ABSTRACT

A method for simultaneous determination of some herbicide residues in water has been developed. The method involves concentration with C18 solid phase extraction (SPE) cartridges followed by high performance liquid chromatographic (HPLC) analysis using a C18 column with diode array detection, a mobile phase of 50 : 50 (v/v) methanol : water at pH = 3.75 (phosphoric acid), and a flow rate of 0.8 mL/min. Method recovery was studied for four different levels of fortification: 0.2, 0.4, 2, and 4 μg/L. A plot of recovery mass x added mass verified the influence of fortification levels in recovery. The recoveries obtained were 58 to 118%, with RSD = 1–15% (3–7 replicates) for the herbicides bentazon, cyanazine, 2,4-D, atrazine, simazine, diuron, and linuron in water.

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INTRODUCTION

Environmental concerns have been growing in recent years, mainly in relation to contamination of water and soil. Among the pollutants, the most important and most studied are the organic pesticides, due to their apolar character, toxicity, great persistence, and accumulation in the environment.

The variety of compounds used as pesticides is very large, involving several different chemical classes, which turns the analysis of these compounds and their degradation products very complex.

The analytical techniques most used in the analysis of pesticides are liquid (LC) and gas (GC) chromatography (1–5). HPLC is favored over GC for acidic pesticides, with high polarities, low volatilities, and thermal instabilities, because GC can only be used following a prior derivatization step (4–11). In addition, since the majority of pesticides strongly absorb in the UV region, between 210–240 nm, they make excellent compounds for UV detection in liquid chromatography (4–14). Diode array detection (DAD) is a good option for detection of herbicides, because this also permits a confirmation of peak identity, utilizing the UV spectrum (6,15–18). Even so, most detectors used in LC still do not possess sufficient detectability to allow direct analysis of environmental samples, which possess very low concentration (trace and sub-trace) levels of the pollutants (19).

Solid phase extraction (SPE) is frequently used as a good alternative for pre-concentration and extraction of analytes from environmental samples, mainly water, and it has substituted traditional extraction methods, such as liquid-liquid extraction (LLE) (20–21). The main advantages of SPE over LLE are: reduces sample handling and labor, does not form emulsions, can be automated easily, and consumes a smaller volume of toxic organic solvents (2–4,19–25). However, SPE involves the investigation of many variables, which may affect the efficiency of the extraction (22,25–30). Of these, the most important are the adjusted ionic strength and the extraction volume.

Another important parameter, which must be determined in any method that involves extraction techniques, is recovery to evaluate the pollutant mass that might be present in a real sample in relation to a certain mass, which has been added to a fortified sample.

In the present work, recovery at four different levels of fortification, 0.2, 0.4, 2, and 4 μg/L, for the herbicides bentazon, cyanazine, simazine; 2,4-D, atrazine, diuron, and linuron, present in water together, was determined using HPLC (high performance liquid chromatography) and SPE. The influence of NaCl added for ionic strength adjustment, and the best volume for extraction utilizing 20, 60, 100, 200, 300, and 500 mL were also studied.

EXPERIMENTAL

Chemicals and Reagents

Standards were obtained from: Novartis (atrazine, 97.7%; simazine, 98.3%), Cyanamid (cyanazine, 98.0%), Basf (bentazon, 99.9%), Dow (2,4-D, 99%), Hoescht (linuron, 99.5%), and Du Pont (diuron, 99.27%). The methanol (Ominosolv, Merck) was chromatographic grade. Phosphoric acid (Synth) and sodium chloride (Nuclear) were analytical reagent grade. Water was purified with a Millipore Milli-Q Plus System.

The extraction cartridges were Envi C18, Supelclean (Supelco), packed with 500 mg silica-octadecyl C18.

Instrumentation

Chromatography was performed with a Waters HPLC system equipped with a Rheodyne 7725i injector with a 10 μL loop, a 515 pump, a PDA absorbance detector (Model 996), and Millennium system for data acquisition. The column (150 × 3.9 mm i.d.) and guard column (20 × 3.9 mm i.d.) were Waters Nova-Pak C-18, 4 μm.

The pH of the mobile phase was determined using a Digimed, model DM21, pHmeter, with glass and thermal compensation electrodes.

Procedure

The mobile phase was prepared volumetrically from individually measured aliquots of methanol and water. The pH was then adjusted to 3.75 with phosphoric acid. The mobile phase flow rate was 0.8 mL/min and detection was performed at Max Plot (maximum UV absorbance for individual compounds). All measurements were carried out at a temperature of 25°C.

Stock solutions for analytical curves were prepared in methanol at 0.1 g/L. The standard solutions were diluted in mobile phase at 50, 100, 250, 500, 1000, and 2000 μg/L and stored in the refrigerator (T = 4°C).

Milli-Q aqueous samples were fortified by addition of measured volumes of the stock solutions of the herbicides, resulting in four levels of fortification: 0.2, 0.4, 2, and 4 μg/L. After adjusting the pH to < 2, by addition of phosphoric acid, the samples were mixed well and percolated through the SPE column under vacuum at a rate of 3 mL/min. Before sample application, the SPE column was conditioned with 10 mL of methanol and equilibrated with 10 mL of Milli-Q water. After the sample had passed through the column, the column was washed

with 5 mL of Milli-Q water, the eluate discarded, and the sorbent bed dried under vacuum for 5 min. The analyte was then eluted with 1 mL of methanol. The solvent was evaporated to dryness under a stream of nitrogen and the residue was dissolved in 1 mL of mobile phase.

To verify the influence of added NaCl, the extractions were carried out both without and with 4 g of this compound, and recoveries were compared. To determinate the best volume of the sample, the extractions were made utilizing different volumes (20, 60, 100, 200, 300, and 500 mL) with a fixed herbicide mass ($\cong 0.2\,\mu g$ of each herbicide), and the recoveries were compared.

RESULTS AND DISCUSSION

Figure 1 shows a chromatogram and UV spectrum of the herbicides, obtained after SPE extraction and pre-concentration. The selected chromatographic conditions permitted the separation and identification of the herbicides peaks studied. The peak with retention time of 7.5 min was not identified.

The recovery tests were carried out with 3–7 replicates, permitting calculation of the relative estimated standard deviation (RSD). The recoveries were calculated using the equation: (31,32)

$$R = \frac{\text{Mass of analyte after extraction}}{\text{Mass of analyte added}} \times 100$$

Figure 2 presents the results of recoveries obtained with and without added NaCl. The figure shows that addition of NaCl does not enhance the recovery of any

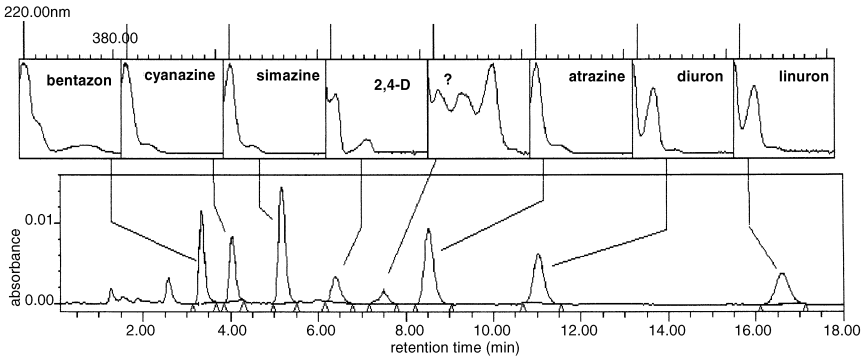

Figure 1. Chromatogram and UV absorption spectrum for herbicides in methanol : water (50 : 50, v/v, pH = 3.75, phosphoric acid), flow rate = 0.8 mL/min, taken with a DAD (max plot).

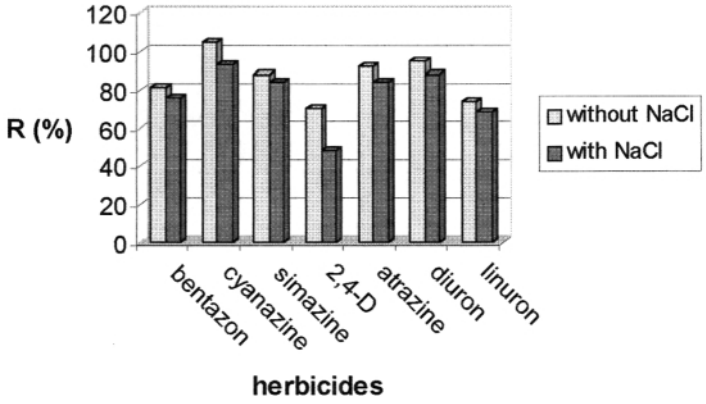

Figure 2. Influence of NaCl addition on herbicide recoveries.

herbicide; in fact the recoveries decreased after addition of NaCl. These results agree with results obtained by Ruiz et al. (33).

Figure 3 shows the results obtained utilizing different volumes for extraction of the herbicides. The results show, that the best volume for simultaneous herbicide extraction was 200 mL, because, with this volume the recovery results are greatest. Cyanazine seems to be an exception, because the best volume of extraction of this herbicide is 300 mL. Utilizing volumes higher than 200 mL, the overall recoveries decreased, because the volume approached the breakthrough volume.

Table 1 presents recoveries for herbicides at four different levels of fortification. The average results obtained for herbicide recoveries are very good,

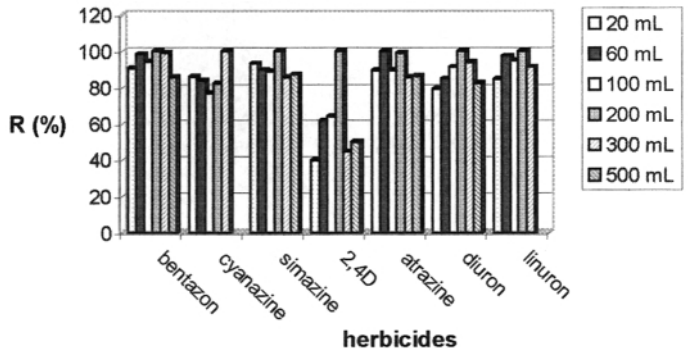

Figure 3. Plot showing the determination of the best volume for herbicide extraction.

Table 1. Recovery Results for the Studied Herbicides

	0.2 µg/L			0.4 µg/L			2 µg/L			4 µg/L		
Herbicides	R (%)	RSD (%)	N	R (%)	RSD (%)	N	R (%)	RSD (%)	N	R (%)	RSD (%)	N
Bentazon	111	7	3	103	5	6	77	9	6	77	3	3
Cyanazine	89	5	3	84	13	5	89	11	5	91	1	3
Simazine	80	6	3	91	8	6	83	15	6	70	4	3
2,4-D	93	7	3	90	11	7	61	15	5	59	3	3
Atrazine	98	10	3	87	8	6	80	15	5	65	8	3
Diuron	98	8	3	99	14	5	70	12	6	63	8	3
Linuron	118	8	3	104	14	7	77	14	5	58	10	3

N = number of replicates.

Table 2. Parameters of the Curves of Recovered Mass Versus Added Mass ($y = a + bx$)

Herbicides	a	b	r
Bentazon	0.02	0.75	0.9997
Cyanazine	−0.007	0.92	0.9999
Simazine	0.03	0.69	0.9971
2,4-D	0.04	0.57	0.9995
Atrazine	0.04	0.63	0.9963
Diuron	0.04	0.60	0.9997
Linuron	0.07	0.54	0.9937

a = linear coefficient.
b = angular coefficient.
r = correlation coefficient.

since a 50–120% recovery range is considered acceptable (34). The RSD values estimate the precision, and the results are very good, because acceptable precision is up to 15% (34).

The linear regression equation ($y = a + bx$) parameters for curves of recovered mass versus added mass are presented in Table 2. The results show that recoveries depend on the concentration levels and that 4 μg/L of fortification presents the worst results, perhaps due to saturation of the sorbent bed. The plot shows curves with good linearity and correlation coefficients ($r > 0.99$). These curves can be used to determine the expected recoveries, after a certain fortification (added mass).

CONCLUSION

The present work has shown that SPE can be utilized to concentrate the herbicides bentazon, cyanazine, simazine; 2,4-D; atrazine, diuron, and linuron present in water samples, prior to chromatographic analyses (HPLC). To obtain the best results for recovery, parameters such as sample volume and ionic adjustment were optimized.

The results obtained showed that the addition of NaCl, for adjustment of the ionic force, does not increase the recovery values of the herbicides studied.

The best extraction volume to obtain simultaneous SPE of the herbicides in this study was 200 mL, because with this volume, the best recovery values were obtained, except for cyanazine.

The recovery results show that there is a dependence of the recovery on the level of concentration of the samples of water submitted to SPE, and that the level

of fortification of 4 μg/L supplies the worst recovery results, perhaps due to a possible saturation of the active positions of the bed of the extraction sorbent.

The curves obtained for the mass recovered versus added mass present good linearity and correlation coefficients ($r > 0.99$), and can be used to determinate values of contamination of water samples submitted for SPE and HPLC.

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