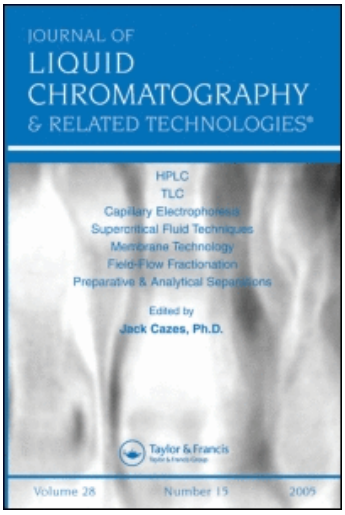

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Determination of Phenoxy Acid Herbicides in Drinking Waters by HPLC and Solid Phase Extraction

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DETERMINATION OF PHENOXY ACID HERBICIDES IN DRINKING WATERS BY HPLC AND SOLID PHASE EXTRACTION

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ABSTRACT

An HPLC procedure for determining phenoxy acid herbicides in waters is described. A LichroSpher RP select B octadecyl-silane analytical column and spectrophotometric detection at 230 nm were used. Adequate retention was achieved with a mobile phase containing MeOH/phosphate buffer 10^{-2} M pH 2.5/PnOH (50/42/8, v/v). The herbicides were isolated from water samples by using a single solid phase extraction procedure with C_{18} solid-phase columns. An enrichment factor of 500 is achieved. The coefficients of variation of the method were generally lower than 2.7% at 0.4 μg L^{-1} herbicide concentration levels. Recoveries ranged between 93 and 118%. The results obtained indicate that the proposed method is well suitable for monitoring phenoxy acid herbicides in compliance with the European Community standard for drinking water.

INTRODUCTION

Chlorinated phenoxy acid herbicides are widely used to control the growth of broad-leafs weeds in crops because of their reactive cheapness and efectiveness. This class of herbicides is not extremely poisonous, or environmentally persistent; they are considered to be moderately toxic to man and aquatic organisms, but their increasing agricultural consumption has led to greater possibilities of contamination of soil, water, and food by their residues and their phenolic metabolites. For this reason, there is a need for the development of sensitive methods for the analysis of water samples for the presence of these herbicides.

Phenoxy acid herbicides in environmental water samples have been determined by gas chromatography (GC)[1-4] and high performance liquid chromatography (HPLC)[5-15] with UV at 230 or 280 nm, or particle beam mass spectrometry detection. GC analysis generally requires esterification[1-2] or silylation[3-4] of phenoxy acids because of their high polarity and low volatility. HPLC procedures usually require the use of gradient concentrations of the organic solvents in order to achieve enough resolution and adequate time of analysis.

Different analytical procedures for isolating phenoxy acid herbicides from water samples as liquid-liquid extraction[16-19] or solid-phase extraction (SPE) using C_{18},[10,14] anion strong exchanger,[6] or polymeric materials[9] cartridges have been proposed.

This paper describes a new, rapid, simple, and sensitive chromatographic procedure for determining residues of bentazone, dicamba, and six phenoxy acid herbicides in drinking waters after preconcentration of the samples on solid-phase columns. The addition of pentanol in the hydro-organic mobile phase eliminates the use of complicated elution gradients and reduces the time of analysis.

MATERIALS AND METHODS

Apparatus

A Hewlett-Packard HP 1100 series high performance liquid chromatograph with an isocratic pump and UV-visible detector was used (Palo Alto, CA, USA). Data acquisition and processing were performed on a computer HP Vectra XM (Amsterdam, The Netherlands) provided with HP

Chemstation software (A0402, 1996). The solutions were injected into the chromatograph through a Rheodyne valve (Cotati, CA, USA) with a 20 µL loop. A LichroSpher RP select B octadecyl-silane column (5 µm, 250 x 4 mm) and a guard column of similar characteristics (30 x 4 mm) (Scharlau, Barcelona, Spain) were used. The mobile phase flow rate was 1 mL min^{-1}. The detection was performed in UV at 230 nm. All the assays were carried out at room temperature.

A solid phase extraction vacuum station Vac Elut 20 (Varian Sample Preparation products, Harbor City, CA, USA) was used.

Reagents and Standards

Mobile phases were prepared by mixing 0.01 M phosphate buffer solution (pH 2.5), methanol, MeOH, and pentanol, PnOH, (analytical grade, Scharlau, Barcelona, Spain) to obtain the working concentration. A mobile phase containing MeOH/phosphate buffer/PnOH, (50/42/8, v/v) was recommended. The phosphate buffer was prepared with disodium hydrogen phosphate and phosphoric acid (analytical grade, Panreac, Barcelona, Spain).

Stock standard solutions of the herbicides 2-methoxy-3,6-dichlorobenzoic acid (dicamba, DC), bentazone (BZ), 2,4-dichlorophenoxyacetic acid (2,4-D), 4-chloro-2-methylphenoxyacetic acid (MCPA), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2-(2,4-dichlorophenoxy)propionic acid (dichlorprop, DP), 2-(2-methyl-4-chlorophenoxy)propionic acid (mecoprop, MP), and 4-chloro-2-methylphenoxybutiric acid (MCPB), (>99.3 %, Dr Ehrenstorfer GmbH, Augsburg, Germany) were prepared in methanol (10 mg L^{-1}) and stored at -18°C in the dark.

Working solutions were prepared by dilution of the stock standard solutions in methanol or in mobile phase. Figure 1 shows the structure of the phenoxy acid herbicides studied.

Bond Elut C_{18} 3CC/500MG solid phase extraction columns (Varian Sample Preparation products, Harbor City, CA, USA) were used.

Barnstead E-pure, deionized water (Sybron, Boston, MA, USA) was used throughout. The mobile phase and the solutions injected into the chromatograph were vacuum-filtered through 0.45 µm and 0.22 µm Nylon membranes, respectively (Micron Separations, Westboro, MA, USA).

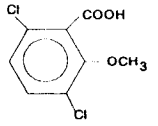
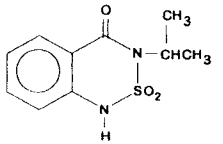
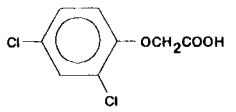
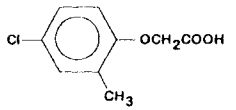
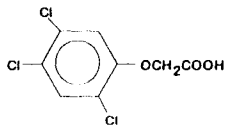
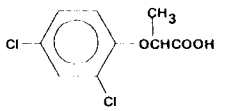
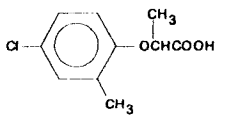
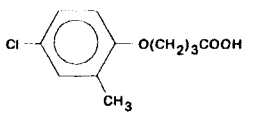
Herbicide	Structure
DC	
BZ	
2,4-D	
MCPA	
2,4,5-T	
DP	
MP	
MCPB	

Figure 1. Structure of phenoxy acids studied.

Sample Preparation

SPE columns were conditioned by washing with 4 mL of MeOH/phosphate buffer, 10^{-2} M, pH = 2.5/PnOH (50/42/8, v/v) mixture and 6 mL of deionized water acidified at pH 2.5 with phosphoric acid. 500 mL of water sample were forced through the C_{18} SPE column using vacuum at a flow rate of 9 mL min^{-1}. The compounds were eluted using 1 mL of MeOH/phosphate buffer, 10^{-2} M, pH = 6.9/PnOH (50/42/8, v/v) mixture.

RESULTS

Chromatographic Conditions

A study to select the adequate composition of the mobile phase (pH and acetonitrile or methanol concentration) was performed. Different mobile phases containing ACN or MeOH as modifiers and phosphate buffer 10^{-2} M at pH 3.0 or 2.5 were tested. In all cases the retention factors of compounds were obtained as mean of triplicate injections.

When the ACN content in the mobile phase was lower than 35% at pH 3.0, the retention factors obtained for the highly hydrophobic compounds were too high, (i.e. for MCPB k = 27.6 and 15.6, for 30/70 and 35/65, ACN/phosphate buffer pH 3.0, v/v, respectively). In contrast, the retention factor obtained for dicamba, the least hydrophobic herbicide, was too low and it overlapped with the refractometric perturbation at the begining of the chromatogram. On the other hand, when a mobile phase at pH 2.5 containing ACN/phosphate buffer 10^{-2}M, (48/52 v/v) was used the retention factor of dicamba increased and it did not overlap with the refractometric perturbation. This behaviour may be due to the diferences between the protonation constants of compounds. The use of this mobile phase produced a decrease on the retention factors of the rest of the phenoxy acids decreased, but the chromatografic peak of 2,4-D overlapped with MCPA and the corresponding peaks to DP and 2,4,5 T were also overlapped.

When MeOH was used as modifier the retention factors were too high. For example the retention factor of MCPB was 22.9 when MeOH/phosphate buffer pH 2.5 (50/50, v/v) was used as mobile phase. The increase of methanol concentration (up to 60%) produced a large decrease in the retention factors (k = 7.6 for MCPB), but important peak overlapping appeared.

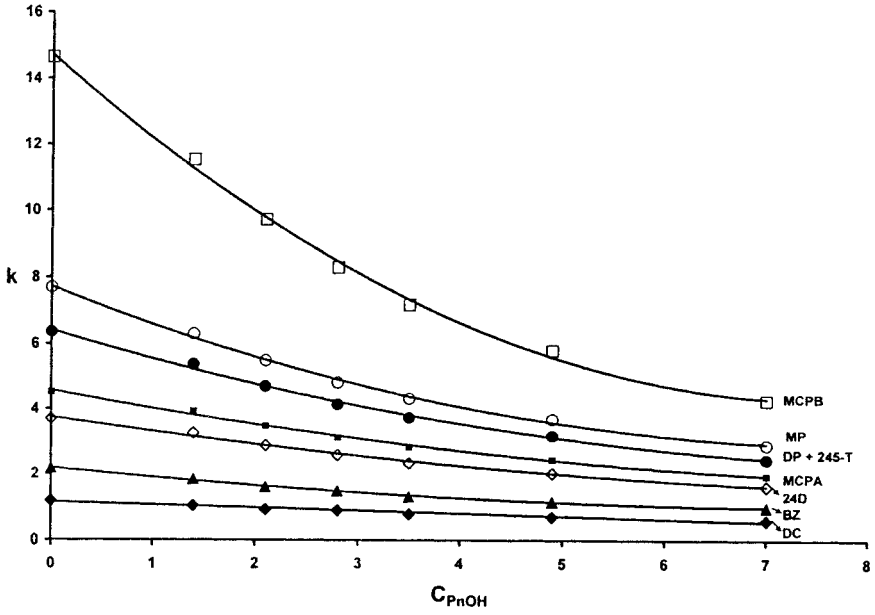


Figure 2. Effect of the pentanol concentration in the mobile phase on the retention of the phenoxy acid herbicides. Mobile phase composition: MeOH/phosphate buffer, pH 2.5 /PnOH (50/50-x/x , v/v).

In order to achieve adequate resolution and retention factors pentanol was added to the mobile phase. The concentration of pentanol added to the mobile phase (x) containing MeOH/phosphate buffer 10^{-2} M pH 2.5/PnOH (50/50-x/x, v/v) ranged between 1.4 and 10%. The addition of 10% of pentanol produced overlapping of the dicamba peak and the refractometric perturbation at the begining of the chromatogram. Figure 2 shows the effect of the pentanol concentration in the mobile phase on the retention of compounds. As can be observed, the increase of the concentration of the pentanol in the mobile phase caused important decreasing in retention factors, mainly for highly hydrophobic herbicides (Figure 3). Except for DP and 2,4,5 T, the chromatogram showed an adequate resolution even for the highest concentrations of PnOH. Pentanol is an alcohol with high eluent strength that produces a decrease on the hydrophobicity of the stationary phase and the polarity of the mobile phase and, in consequence, it produces a diminution on the retention factors of the compounds. The decrease on the retention factors is larger as the hydrophobicity of compounds increase.

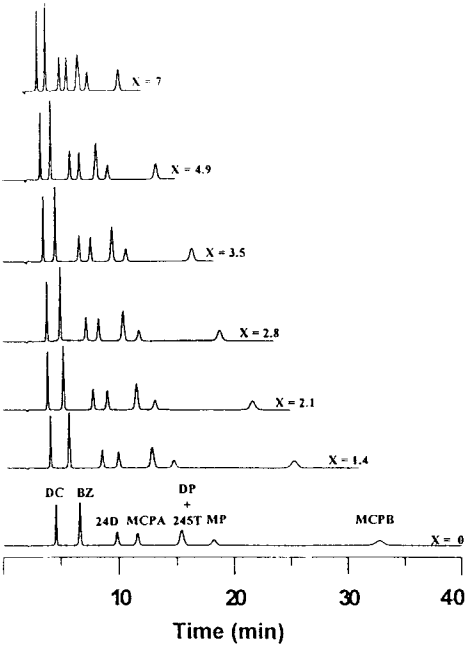


Figure 3. Chromatograms corresponding to different concentrations of pentanol. Mobile phase composition: MeOH/phosphate buffer pH 2.5/PnOH (50/50-x/x, v/v). Flow rate 1 mL min^{-1}. Wavelength 230 nm.

A mobile phase composition of MeOH/phosphate buffer 10^{-2} M pH 2.5 / PnOH (50/42/8, v/v) was selected. Using this mobile phase, the retention times of the more hydrophobic herbicides were low enough (i.e. the retention time for MCPB was 9 min) and adequate resolution was achieved.

Analytical Data

The calibration curves of compounds were obtained by triplicate injections of standard solutions containing different concentrations of the analytes in the 0.1-0.5 mg L^{-1} range. The peak area was used as dependent variable. Linear relationships were obtained in the working interval. In all cases, the calibration curves showed adequate regression coefficients ($r > 0.99$) and significance levels.

Table 1

Repeatability and Limits of Detection of the Phenoxy Acids

Compound	C.V. (%), n= 5	LOD ($\mu g\ L^{-1}$)
DC	3.0	17
BZ	3.4	12
2,4-D	2.7	25
MCPA	3.9	38
2,4,5-T	0.5	9.4
DP	5.2	107
MP	0.8	4.9
MCPB	6.2	67

Table 2

Recovery of Phenoxy Acids from C_{18} Columns and Reproducibility of the Proposed Method

Compound	Recovery (%)	C.V. (%), n= 5
DC	99.9	9.5
BZ	118	1.0
2,4-D	94.5	1.5
MCPA	97.5	2.4
2,4,5-T	97.7	1.2
DP	93.5	2.7
MP	107	1.2
MCPB	100	1.6

The repeatability was evaluated from five injections of standard solutions of phenoxy acid herbicides in concentration 0.1 mg L^{-1}. Table 1 shows the coefficient of variation values (CV) found for each compound. As can be observed the CV values were ranged between 0.5 and 6.2%.

Limits of detection (LODs) were calculated from the standard deviation corresponding to five-fold injections of 0.1 mg L^{-1} solutions of each herbicide (3σ criteria). The LODs values for each herbicide are shown in Table 1 and they were ranged between 4.9 and 107 $\mu g\ L^{-1}$. These values were too high for determining this kind of herbicides in drinking water samples. Consequently a

preliminary preconcentration step is necessary in order to achieve sufficiently high enrichment factors, which enable phenoxy acid herbicides to be monitored in drinking waters samples at 0.1 μg L^{-1} level.

Sample Preconcentration

Owing the detection limits values obtained, the analytes should be extracted from a relatively large volume of water sample (500 mL was assayed) and eluted with a small volume of eluent (1 mL was assayed). In these conditions, an enrichment factor of 500 would be achieved, that assures the procedure is suitable for determining bentazone, dicamba, and phenoxy acid herbicides in drinking water at lower concentration levels than the admissible concentrations by the European Union.

The ability of the Bond Elut C_{18} 3CC/500MG cartridges to retain quantitatively the compounds and the composition of the eluent was studied. To carry out these studies, spiked E-pure water samples containing a concentration of 0.4 μg L^{-1} of each herbicide were prepared. Phenoxy acid herbicides were extracted from 500 mL of the spiked water sample according to the proposed procedure (see experimental section).

Acetonitrile and methanol was assayed as eluents. The use of these eluents produced a large noise in the initial part of the chromatogram, which made the determination of less hydrophobic compounds impossible. Bentazone, dicamba, and phenoxy acid herbicides were adequately eluted using 1 mL of MeOH/phosphate buffer 10^{-2} M pH = 6.9/PnOH (50/42/8, v/v) mixture. Except for the pH value, the eluent composition is like the mobile phase. When the mobile phase was used to elute herbicides from the EPS columns, the elution was not quantitative.

The recovery achieved for each herbicide in the preconcentration step was determined. The recovery values were obtained by comparing the peak areas corresponding to the extracts with those obtained by direct injection of standard solutions containing 0.2 mg L^{-1} of each herbicide (0.2 mg L^{-1} is the concentration of the herbicides in the eluates assuming a recovery equal to 100 %). Figure 4 shows the chromatograms corresponding to a sample of herbicides after the preconcentration step together with the corresponding of the standard solutions containing 0.2 mg L^{-1} of each herbicide. Table 2 shows the recovery values and the reproducibility (CV) obtained for each phenoxy acid herbicide from five independent analysis. The recovery values ranged between 93.5 and 118.0%.

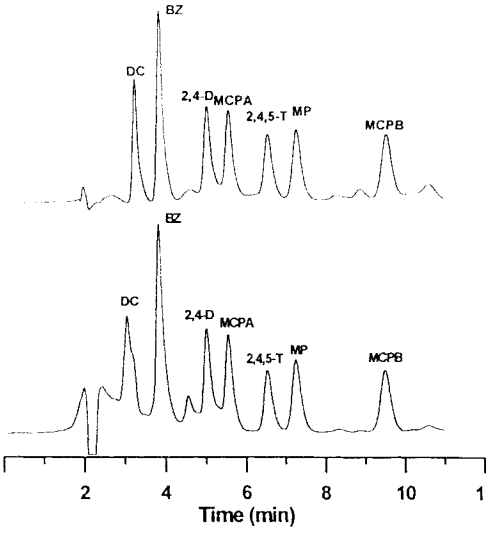


Figure 4. Chromatograms corresponding to a mixture of bentazone, dicamba and phenoxy acid herbicides containing 0.2 mg L^{-1} of each compound (upper part) and a eluate obtained by EPS of solutions containig 0.4 μg L^{-1} of each compound (lower part).

The uncertainty of the method, including preconcentration and chromatographic analysis steps was evaluated. The method showed adequate reproducibility; the values of the coefficients of variation obtained ranged between 1.0 to 9.5% (Table 2). By comparing the variance values corresponding to the chromatographic analysis step (ranged between $8x10^{-5}$ and $2x10^{-3}$; average $6x10^{-4}$) with the corresponding global procedure (ranged between $6x10^{-5}$ and $6x10^{-3}$, average = $1x10^{-3}$) it can be concluded that the uncertainty associated to the preconcentration step was, in general, slightly higher than that associated to the chromatographic step.

The results showed above indicate that the proposed method is adequate for monitoring bentazone, dicamba, and phenoxy acid herbicides in drinking waters in compliance with the European Union standard for drinking water. The addition of pentanol to the mobile phase is not usual in liquid chromatography.

The result shown in this paper indicate that the addition of pentanol to the hydro-organic mobile phase could be an alternative to the use of complicate gradient chromatographic steps.

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