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Short Communication

Determination of phenoxyacid herbicides in water

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ABSTRACT

Novel clean-up techniques for a polymeric precolumn (PLRP-S) for the subsequent determination of bentazone and eight phenoxy acid herbicides in surface water samples are described. After preconcentration of the components at pH 3 on a 10 × 2 mm I.D. precolumn, the technique consists of a clean-up with 1000 μl of 0.1 mol/l sodium hydroxide solution (pH 12.5) and of a heartcut consisting of four precolumn bed volumes of eluent directed to waste followed by ten precolumn bed volumes of eluent directed to the analytical column. Analytical separation is performed with acetonitrile–water (30:70) containing 0.005 mol/l of tetrabutylammonium hydrogensulphate (pH 8.3) (which is also the desorption eluent during heartcutting) on a polymeric analytical column (PLRP-S). With 25 ml of surface water, spiked at 0.25 and 1 μg/l, applied to the precolumn, recoveries for all components were over 85% with a relative standard deviation (n = 5) of *ca.* 9% at 0.25 μg/l and *ca.* 2% at 1 μg/l. Detection limits in surface water samples are 0.05–0.1 μg/l. Owing to automation, the total analysis time is *ca.* 30 min.

INTRODUCTION

Trace enrichment of (medium) polar pesticides on porous polymeric sorbents [1–4] such as PRP-1 and PLRP-S, in order to isolate and concentrate them prior to their separation and detection, has become a widely used approach. These hydrophobic porous polymers, compared with other hydrophobic sorbents such as C_{18} types, show better retention of the analytes and also a greater sorption capacity. Their poor selectivity, however, remains a major disadvantage, *i.e.*, part of the sample matrix may also be adsorbed. In natural waters this matrix consists of DOC (dissolved organic carbon), 30–50% of which is generally composed of humic acids, fulvic acids and hydrophobic low-molecular-weight acids [5], and the concentration of which is often in the mg/l range. Breakthrough volumes of surface water samples therefore depend on the sample matrix constituents rather than on the concentration of the analytes.

In a previous paper [1] the preconcentration and separation of five phenoxy acids by a fully automated method with a total analysis time of *ca.* 20 min were

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reported. Detection limits in surface water were 0.1–0.5 μg/l and those in tap water were 0.01–0.05 μg/l. The clean-up procedure in this method, consisting of delivering to waste four precolumn bed volumes of acetonitrile–water (30:70) (pH 3), was not sufficient to eliminate interfering chromatographic peaks with surface water samples.

In this study alternative procedures for scavenging the precolumn and to eliminate the interference of the large matrix peak were investigated. Washing the precolumn with 0.1 mol/l sodium hydroxide solution and heartcutting (transferring to the analytical column the fraction that contains the solutes of interest) turned out to be reliable and effective in removing the humic matrix constituents. A fully automated method applicable to bentazone, 4-chloro-2-methylphenoxyacetic acid (MCPA), (2,4-dichlorophenoxy)acetic acid (2,4-D), 2-(4-chloro-2-methylphenoxy)propionic acid (MCPP), 2-(2,4-dichlorophenoxy)propionic acid (2,4-DP), (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T), 4-(dichlorophenoxy)butyric acid (2,4-DB), 4-(4-chloro-2-methylphenoxy)butyric acid (MCPB) and (2,4,5-trichlorophenoxy)propionic acid (2,4,5-TP), including data on repeatability, is described here and the scope of the method is discussed.

EXPERIMENTAL

Reagents

High-performance liquid chromatographic (HPLC)-grade acetonitrile and water were obtained from Mallinckrodt (St. Louis, MO, USA), HPLC-grade methanol and dichloromethane, sodium hydroxide, ethyl acetate and perchloric acid from Baker (Deventer, Netherlands), buffer solutions of pH of 7.0 and 10.0 from Merck (Darmstadt, Germany) and low-UV PIC A reagent (a buffered solution of tetrabutylammonium hydrogensulphate) from Millipore (Bedford, MA, USA). Chlorophenoxy carboxylic acids were obtained from Riedel-de Haën (Hannover, Germany) and Promochem (Wesel, Germany) and bentazone from Promochem.

Apparatus

The HPLC apparatus consisted of a Waters Assoc. (Millipore, Bedford, MA, USA) Model 600E pump to deliver the wetting, conditioning and washing solvents, two LKB (Bromma, Sweden) Model 2150 pumps and an LKB Model 2152 system controller to deliver the mobile phase and the aqueous samples. A Pye Unicam Model 4110 variable-wavelength UV absorbance detector obtained from Philips (Eindhoven, Netherlands) was set at 230 nm. Model SPH 99 a column thermostat was obtained from Spark (Emmen, Netherlands). PROSPEKT fully automated cartridge exchange system (Spark) with two additional valves was used to control the flow scheme during analysis. Chromatograms were recorded and integrated by a data station (Millipore) with Baseline 810 software.

Procedures

Stock solutions of the chlorophenoxy acids and bentazone were prepared by weighing *ca.* 5 mg of each component followed by dissolution in 50 ml of methanol. These solutions were diluted with tap water to obtain standard solutions and mixed standard solutions (tap water is relatively pure except for the presence of some natural humic substances, in this respect the matrix is already very much like surface water, which is an advantage for these experiments).

Sample solutions were prepared by diluting the stock solutions with tap water or surface water and acidified to pH 3 with 0.1 mol/l perchloric acid.

Precolumns (10 × 2 mm I.D.) were prepacked with 15–25-μm PLRP-S (Polymer Labs., Shropshire, UK), wetted with 2 ml of methanol (2 ml/min) and activated with 2 ml of 0.001 mol/l perchloric acid (1 ml/min) prior to preconcentration of the samples.

Separations were carried out at 50°C on a 250 × 4.6 mm I.D. column, prepacked with 5-μm, 100 Å PLRP-S, using acetonitrile–water (30:70) containing 0.005 mol/l of tetrabutylammonium hydrogensulphate (TBA) (pH 8.3) [in practice one bottle of low-UV PIC A was added to 1 l of acetonitrile–water (30:70)] at a flow-rate of 1.0 ml/min as the mobile phase, which also served as the desorption eluent for the precolumn. Conditions during sample concentration, heartcutting and precolumn washing are described below.

RESULTS AND DISCUSSION

Heartcut technique

Heartcutting in liquid chromatography is not a common technique, probably because additional microprocessor-controlled valves are required. Several years ago [6,7], the so-called "venting-technique" was introduced; 10 μl of untreated blood plasma were injected into a precolumn, and by carefully regulating the precolumn eluent with the aid of a three-port valve and a back-pressure regulator, interfering matrix components could be vented to waste. Goewie and Hogendoorn [8] transferred the analytes of interest in a small volume of mobile phase to the analytical column after a washing procedure with a solvent of low eluotropic strength [9]. Both techniques are known as heartcutting.

In our laboratory, we investigated the possibility of taking a heartcut from the precolumn eluent that exclusively and instantaneously is desorbed by the mobile phase. To do so we used the set-up in Fig. 1, which is the basis of the PROSPEKT apparatus. With switch moments of valve 2 at 10, 20 or 40 s with mobile phase flow-rates of 1, 0.5 and 0.25 ml/min, respectively, the recoveries are still very good ($\geqslant$85%) for all components. These results indicate that the first five bed volumes (*ca.* 170 μl) can be directed to waste without significant loss. The end-point (switching valve 1 again) is between 20 and 30 s. This means that the analytes of interest are desorbed from the precolumn in a narrow band of *ca.* 300 μl (*ca.* 10 bed volumes).

Desorption and heartcutting of the precolumn, to which a surface water sample had been applied, resulted in a decrease of the interfering matrix peak, now becoming relatively small and sharp. A humic peak, however, remained in the middle of the chromatogram coming from humic substances, which was confirmed by LC with diode-array detection. Therefore, although the large interfering matrix peak can be removed or decreased, other clean-up techniques are still required to remove all interfering humic substances.

Clean-up procedure

As outlined above and previously [1], it was not possible to remove the surface water matrix constituents from the precolumn with the "normal" washing procedures, *i.e.*, even 30% acetonitrile at pH 3 did not eliminate the interfering matrix

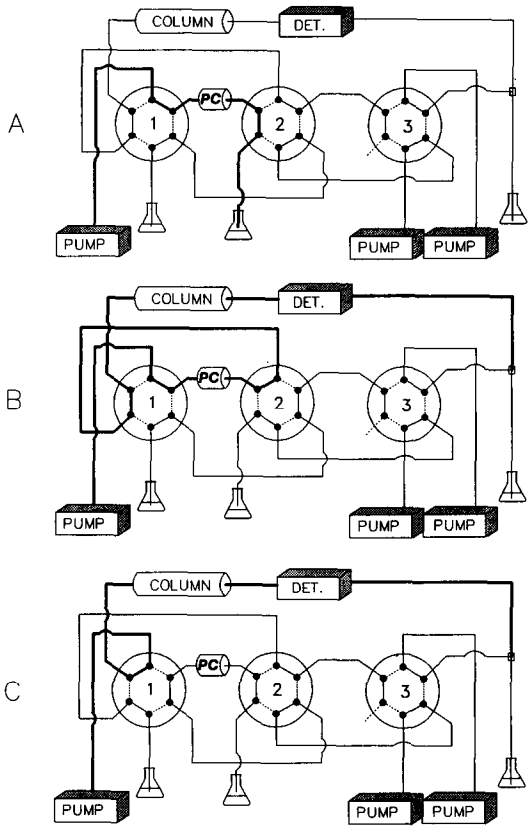

Fig. 1. Flow diagram of the heartcutting set-up. (A) Desorption from precolumn to waste; (B) first point of heartcut is reached; desorption to analytical column; (C) second point of heartcut is reached; end desorption, start of separation.

peak. During desorption at higher pH, however, the matrix components causing this interfering peak mostly elute prior to the analytes of interest. This suggested the development of an extraneous washing procedure with solvents of high pH.

Initial experiments indicated that washing with HPLC-grade water of pH 9.7 and 11.3 gave better results with increasing pH. Clean-up with 7 ml of 0.1 mol/l sodium hydroxide solution (pH 12.5), however, showed no breakthrough of the phenoxy acids. Also, interfering matrix peak decreased considerably and became sharp, and moreover the humic peak in the middle of the chromatogram disappeared completely. Increasing the volume of washing solvent, however, did not result in a continuous decrease in the remaining interfering matrix peak, *i.e.*, after 1 ml of washing solvent there was no further improvement in the chromatogram. Bentazone, however, will elute (breakthrough) with *ca.* 2 ml of washing solvent. At pH 12.5 bentazone is a neutral component ($pK_B \approx 10$). The difference in behaviour of the phenoxy acids and the apparently "similar" humic substances might be explained in the following way.

At very high pH values the humic substances, which can be imagined as "large

complex spheres", substituted with acidic (CO_2H) and phenolic (OH) groups, are negatively charged not only on the outside but also on the inside. They are wholly polar phases now and, consequently, will elute easily from the precolumn without dragging along the analytes of interest. The phenoxy acids, being carboxylic acids, will also be negatively charged at this pH. The major part of their molecules, however, still remains apolar and will be attracted by the polymer. At this pH bentazone is a neutral component, being attracted by the polymer, yet still fairly polar.

Apparently a high pH value is necessary to charge the whole humic "phase" negatively, *i.e.*, to make it wholly polar. At lower pH there is apparently still available an apolar humic "phase", being contained in the mobile phase, which is why the analytes of interest show an earlier breakthrough.

Optimization and repeatability

Using surface water spiked at 1 μg/l with all components of interest, we now combined both techniques and determined the optimum conditions for automated measurements. These optimum conditions turned out to be a washing volume of 1000 μl of 0.1 mol/l sodium hydroxide solution and a switching moment for valve 2 of 8 s. Chromatograms obtained from spiked surface water, tap water and HPLC-grade water are shown in Fig. 2.

Under these optimum conditions, the repeatability of the method was tested using spiked surface water samples at 0.25 μg/l and 1 μg/l for all components. The repeatability at both levels is excellent, *i.e.*, the relative standard deviation (R.S.D.) at the 1 μg/l level was *ca.* 2% (2,4,5-TP 8%) and at the 0.25 μg/l level *ca.* 9% (2,4,5-TP 16%) (n = 5). Detection limits for surface water samples were calculated to be 0.05–0.1 μg/l (signal-to-noise ratio = 3).

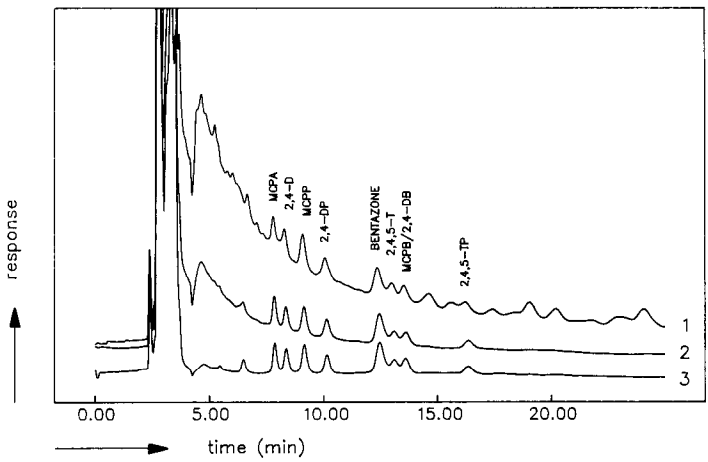

Fig. 2. Chromatograms for 25 ml of spiked water samples (1 μg/l) under optimum conditions. (1) Surface water; (2) tap water; (3) HPLC-grade water. Mobile phase, acetonitrile–water (30:70) containing 0.005 mol/l TBA (pH 8.3); flow-rate, 1 ml/min; analytical columns, 250 × 4.6 mm I.D. PLRP-S (50°C); precolumn, 10 × 2 mm I.D. PLRP-S; 0.1 mol/l NaOH (pH 12.5) wash volume, 1000 μl; heartcut first 130 μl to waste, second 300 μl to analytical column; UV detection at 230 nm, 0.1 a.u.f.s.

Scope

As outlined above humic acid constituents elute easily from the precolumn at very high pH without dragging along the analytes of interest. The phenoxy carboxylic acids were retained even after applying 10 ml of 0.1 mol/l sodium hydroxide washing solvent. The basic component bentazone also elutes slowly. From these results we concluded that, in principal, all basic, neutral and acidic components having an apolar part in their molecule can be applied with this method.

We are currently investigating another approach, namely preconcentration of a broad range of components (acidic, basic and neutral pesticides) from alkaline surface water samples.

CONCLUSION

Clean-up procedures using a polymeric precolumn (PLRP-S) in the determination of bentazone and phenoxy acid herbicides in surface water samples have been studied. Combining both clean-up techniques, *i.e.*, washing of the precolumn with 0.1 mol/l sodium hydroxide solution at pH 12.5 followed by heartcutting, resulted in a considerable decrease in the interfering matrix peak and consequently reliable integration of the chromatogram.

Surface water samples spiked at 0.25 and 1 μg/l showed excellent recoveries (>85%) with R.S.D.s at 1 μg/l of *ca.* 2% and 0.25 μg/l of *ca.* 9% ($n = 5$). Detection limits for surface water samples were calculated to be 0.05–0.1 μg/l. Owing to the automation, the total analysis time was only 30 min, indicating promising potential for this technique.

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