

CHROM. 17,299

Note

Simple precolumn sample enrichment in high-performance liquid chromatography for determination of phenoxy acid herbicides in water samples from exposure studies

MALIN ÅKERBLOM

National Laboratory for Agricultural Chemistry, S-750 07 Uppsala (Sweden)

(First received April 24th, 1984; revised manuscript received October 13th, 1984)

Trace enrichment of water pollutants is often a necessary step in the analytical procedure owing to their low concentrations. The use of a precolumn as an integrated part of a high-performance liquid chromatographic (HPLC) system has been described by several workers. Werkhoven-Goewie *et al.*, for instance, could detect less than 0.1 $\mu\text{g/l}$ of pentachlorophenol in water by pumping some 100 ml of water through special precolumns with a high capacity for hydrophobic substances¹.

In a broad study of exposure to herbicides, 33 spraymen were followed by air and urine sampling^{2,3}. The herbicides studied were MCPA, dichlorprop, mecoprop and 2,4-D (Fig. 1). MCPA and dichlorprop are the most used herbicides in Swedish agriculture. Airborne herbicides were sampled in glass scrubber flasks containing distilled water. The water was analysed by HPLC. The herbicides were concentrated on a small precolumn, which replaced the injection loop. The rest of the HPLC system was as originally used for the analysis of formulations of herbicides⁴. With this set-up the determination was completed in 10 min, and the detection limit was 5–10 $\mu\text{g/l}$ for a 2-ml sample. The method is also used in this laboratory for screening of polluted water for a number of herbicides.

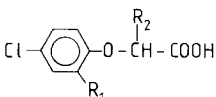
		R ₁	R ₂	k'
	2,4-D	Cl	H	18
	MCPA	CH ₃	H	24
	dichlorprop	Cl	CH ₃	34
	mecoprop	CH ₃	CH ₃	43

Fig. 1. Structure of herbicides studied and their capacity factors (k') in the chromatographic system.

EXPERIMENTAL

Apparatus

A Spectra-Physics Model 3500B liquid chromatograph (Spectra-Physics, Santa Clara, CA, U.S.A.) was used. It was equipped with a Valco six-way injector and a UV detector (Spectra-Physics SP 8200), operated at 254 or 280 nm, with a cell volume of 20 μl . The analytical column was of stainless steel, 25 cm \times 3.2 mm I.D., packed with Spherisorb ODS (5 μm) held at ambient temperature. A guard column, 2.2 cm

× 3.6 mm I.D. (Waters Assoc., Milford, MA, U.S.A.), which was dry-packed with 0.4 g of 37–50 μm pellicular C_{18} silica gel, replaced the injector loop. The guard column was installed so that the sample was eluted in the same direction as it had been applied. A disposable plastic syringe (2 ml) with a Luer fitting was used for the application of samples.

Chemicals

The eluent was 0.1 *M* acetic acid–methanol A.R. (50:50) at a flow-rate of 1.0–1.2 ml/min; 10 *M* acetic acid was used to acidify the samples before application. Reference substances of the four herbicides as acids were 98–100% pure and were kindly provided by Bayer, Leverkusen, F.R.G. Stock solutions of 1000 $\mu\text{g/ml}$ in methanol were prepared. Working solutions were made by diluting stock solutions to ca. 0.1 $\mu\text{g/ml}$ with 0.1 *M* acetic acid.

Sample application

The precolumn was conditioned by flushing with acetic acid (4 ml, 0.1 *M*). The water sample (5 ml) was acidified with acetic acid (50 μl , 10 *M*), and then 2 ml were withdrawn with the syringe and injected manually onto the precolumn with the injector in the load position. The herbicides were then trapped in the precolumn, and the water passed on to the waste. The injector valve was turned, and the substances started migrating with the eluent.

In some experiments the samples were made 1 *M* in sodium chloride as well, by adding solid sodium chloride. In these cases the precolumn was washed with acetic acid (0.5 ml, 0.1 *M*) after the application of sample and before turning the valve. Reference solutions were injected at intervals in the same way as the samples. Peak heights were used for calculation.

Trapping of airborne herbicides in scrubber flasks

In the exposure studies, a scrubber flask (20 ml) was placed in the sprayman's breathing zone³. The flask, which contained distilled water (15 ml) as the trapping liquid, was connected to a belt pump, giving an air flow-rate of 1 l/min. The flasks were changed once an hour.

It was determined in the following way whether the pH of the water would affect the trapping efficiency. The inlet tubes of two scrubber flasks were put in holes in the bottom of a big cardboard box (0.2 m³). The flasks were filled with phosphoric acid (0.05 *M*, pH 1.9) and phosphate buffer (0.005 *M*, pH 8), respectively. They were then connected to an air pump each via a second scrubber flask, containing, in both cases, phosphate buffer (pH 8). The pumps gave an air flow-rate of 1 l/min.

A formulation containing MCPA and dichlorprop was diluted with water to a concentration of 0.2 g/l, and 2 ml of this solution were sprayed every 12 min for 1 h with a reagent-spraying device through a hole in the box wall to create a spray mist. The liquid in the four scrubber flasks was analysed. The experiment was repeated once.

RESULTS AND DISCUSSION

Retention behaviour

The four herbicides are used as sodium, potassium or dimethylamine salts in

Swedish agriculture. Their pK_a values are *ca.* 3. In order to be trapped in the pre-column they had to be hydrophobic, *e.g.* in their acid form. This was achieved by acidifying the sample and by conditioning the precolumn with acid. They were also chromatographed to a great extent in their un-ionized form, since the pH of the eluent was low.

The capacity factors in the chromatographic system used are listed in Fig. 1. They show the effect of substituting a chlorine by a methyl group on the ring as well as of increasing hydrophobicity in the side-chain.

The same pattern of retention was reflected in the trace enrichment procedure. Table I shows that if the amount of sample applied was increased there was a significant loss of 2,4-D, owing to migration through the precolumn already with pure diluted acid. Mecoprop, the most hydrophobic of the herbicides, was well retained even when 16 ml were applied. The retention on the precolumn was increased when the ionic strength of the sample was increased by adding sodium chloride (Table I). This "salting out" effect was most pronounced for the most hydrophilic compound. The salting out can be used when a greater volume of sample has to be applied to enable determination of lower concentrations, *e.g.* in polluted water.

Repeatability and detection limit

On repeated injections of standard the peak heights deviated from the mean by 0–1.5%. Over a five-day period with 21–28 injections per day, 4–6 of which were standard solutions, the relative standard deviation for the standards was 3.3%. The performance of the trace enrichment with 2-ml injections as described above was tested from 0.01 to 0.8 $\mu\text{g/ml}$, and was found to be linear. When actual samples were fortified with the four herbicides the recoveries, relative to injections of standards, were *ca.* 100%. The detection limits for the 2-ml sample injections were 0.005–0.01 $\mu\text{g/ml}$ of water. This corresponds to a detection limit of *ca.* 0.001 mg/m^3 of air in the occupational health studies.

Position of the enrichment column

The guard column was chosen as an inexpensive alternative to other trace enrichment systems, which depend on extra pumps and valves. The low back-pressure of the stationary phase makes finger-tip application easy.

The guard column can be positioned in two ways. It can be placed either so

TABLE I

AMOUNT OF HERBICIDE RETAINED ON PRECOLUMN, AS A PERCENTAGE OF THE AMOUNT APPLIED

Herbicide	Applied volume of sample saturated with sodium chloride, ml				Applied volume of sample without sodium chloride, ml			
	2	4	8	16	2	4	8	16
2,4-D	100	92	85	57	74	74	59	33
MCPA	100	93	85	72	75	75	70	55
Dichlorprop	100	94	89	76	84	81	77	66
Mecoprop	100	98	90	82	85	83	81	75

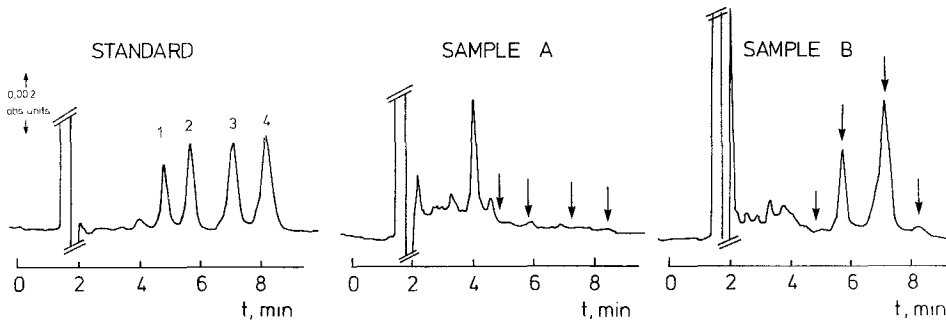


Fig. 2. Chromatograms of samples from air sampling of phenoxy acid herbicides. Peaks in standard: 1 = 2,4-D, 0.10 $\mu\text{g}/\text{ml}$; 2 = MCPA, 0.10 $\mu\text{g}/\text{ml}$; 3 = dichlorprop, 0.10 $\mu\text{g}/\text{ml}$; 4 = mecoprop, 0.13 $\mu\text{g}/\text{ml}$. Sample A: no herbicides detected. Sample B: MCPA, 0.09 $\mu\text{g}/\text{ml}$; dichlorprop, 0.14 $\mu\text{g}/\text{ml}$; mecoprop, 0.009 $\mu\text{g}/\text{ml}$.

that it acts as a guard column as well as an enrichment column, *e.g.* the eluent passes in the same direction as the water sample at application, or so that it acts as a true enrichment column that is eluted by a reversal of the flow. In the former case, peak broadening of 2–10% was observed when compared with direct injections of 10 μl of a 200-fold more concentrated solution of the herbicides. When the guard column was placed so that the sample was injected by back-flush, less band broadening was observed. The simultaneous action as guard column was, however, preferred when handling the “crude” samples in question.

Trapping of airborne herbicides in scrubber flasks

It was assumed that the herbicides might be lost as vapour from the flask containing phosphoric acid (pH 1.9), then being in their un-ionized form. There was, however, no significant difference in the concentration of herbicides in the flasks with low and high pH. Furthermore, no herbicides were detected in the secondary flasks, indicating that the capacity and efficiency of both absorbents were sufficient. Since the pH did not influence the trapping yield as investigated here, distilled water was chosen in practice for convenience.

Exposure studies

In all, more than 200 samples have been analysed in the studies performed^{2,3}. Fig. 2 shows some examples of the routine analysis of samples. The amounts of herbicides found in the water samples were low, generally from non-detectable to 0.2 $\mu\text{g}/\text{ml}$. Occasionally values of up to 3 $\mu\text{g}/\text{ml}$ were found. This corresponds to air concentrations from less than 0.001 up to 0.1 mg/m^3 in most cases^{2,3}. In the U.S.A. the threshold limit value (TLV) for 2,4-D is 10 mg/m^3 . (In Sweden no TLVs for phenoxy acid herbicides have been established.)

The scrubber flasks are carried during work and must therefore be light. Thus the water sample available for analysis is small. With the present precolumn concentration technique a detection limit sufficient for exposure studies is obtained.

REFERENCES

- 1 C. E. Werkhoven-Goewie, U. A. Th. Brinkman and R. W. Frei, *Anal. Chem.*, 53 (1981) 2072.
- 2 B. Kolmodin-Hedman, K. Erne and M. Åkerblom, *Studies Environ. Sci.*, 7 (1980) 73.
- 3 B. Kolmodin-Hedman, S. Höglund and M. Åkerblom, *Arch. Toxicol.*, 54 (1983) 257.
- 4 M. Åkerblom and B. Lindgren, *J. Chromatogr.*, 258 (1983) 302.