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DETERMINATION OF PHENOXYACID HERBICIDES IN WATER

POLYMERIC PRE-COLUMN PRECONCENTRATION AND TETRABUTYLAMMONIUM ION-PAIR SEPARATION ON A PRP-1 COLUMN

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SUMMARY

Optimum conditions for preconcentration of phenoxyacid herbicides from water on polymeric material and separation on an analytical column (PLRP-S and PRP-1 respectively) have been achieved. The method consists of (a) an on-line preconcentration at pH 3, (b) clean-up with four (precolumn) bed volumes of acetonitrile–water (30:70) at pH 3 and (c) isocratic analytical separation at pH 11 with 0.01 mol/l tetrabutylammonium as the ion-pair reagent and acetonitrile–water (30:70) as the eluent. Data on the repeatability of the method, sample flow dependence and sorption capacity are reported. For reliable integration of the chromatogram a clean-up step was introduced. This washing procedure however is more effective for tap-water than for surface water samples. The results of the method are promising and indicate that 10–50 ml of surface water can be applied to the precolumn without breakthrough. Detection limits in surface water samples are 0.1–0.5 μg/l whereas those in tap-water are 10–50 ng/l. The applicability of the method was tested. The results of this method were in good agreement with gas chromatographic–mass spectrometric results, and only *ca.* 0.1 μg/l lower over the entire range, whereas the analysis time was much shorter. The potential of this technique for automation was demonstrated using a microprocessor-controlled column-switching unit, resulting in a reduction of the total analysis time to *ca.* 20 min.

INTRODUCTION

Phenoxyacid herbicides are widely used to control the growth of broad-leaf weeds. Although they are not extremely poisonous and/or environmentally persistent, their widescale production necessitates an analysis method capable of low detection limits of these herbicides.

In our institute the chlorophenoxy carboxylic acid herbicides 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) and (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T) are determined in

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industrial waste-water by gas chromatography (GC) after conversion into the corresponding methyl esters using methanol–BF_3. This method is very cumbersome, poorly repeatable and time consuming because of the many steps such as liquid–liquid extraction, concentration and derivatization with or without additional clean-up. Solid-phase extraction is an attractive alternative for enrichment of environmental samples prior to quantitation. Effective preconcentration is demonstrated for many components or groups[1–4] and detection limits for real (water) samples are often in the low μg/l range.

Åkerblom[5] reported upon a simple precolumn sample enrichment system for acid herbicides in which the sample loop was replaced by a precolumn. Samples were introduced by a syringe into the precolumn, which was packed with *ca.* 40 μm C_{18} silica gel. Smith and Pietrzyk[6] used an auxiliary pump to deliver the sample to the precolumn which was packed with 10- or 20-μm PRP-1. Separations were performed on a PRP-1 analytical column which provided good efficiencies for separation of complex mixtures like chlorophenols and phenoxyacetic acid herbicides.

In a previous paper[7] the simultaneous preconcentration on various materials of fourteen polar (chloro- and methyl-substituted) anilines from surface water was reported. In this paper, a procedure is described for the preconcentration and analysis of some polar phenoxyacid herbicides from water. Enrichment conditions have been studied on a macroporous polystyrene–divinylbenzene copolymer (PLRP-S). This material was chosen because of its good adsorption properties. Desorption and analysis were performed at high pH using tetrabutylammonium (TBA) ion-pair separation on a PRP-1 analytical column. The automated method developed utilizes a microprocessor-controlled valve-switching unit by which the total analysis time is reduced to *ca.* 20 min. To overcome an high background in the chromatogram after the desorption step, a "washing" step was introduced prior to desorption of the herbicides.

EXPERIMENTAL

Reagents

HPLC-grade acetonitrile and water were obtained from Mallinckrodt (St. Louis, MO, U.S.A.) and HPLC-grade methanol, sodium hydroxide and perchloric acid from Baker (Deventer, The Netherlands). All chlorophenoxy carboxylic acids were obtained from Riedel-de Haën (Hannover, F.R.G.) and tetrabutylammonium hydrogensulphate from (Fluka, Buchs, Switzerland).

Apparatus

The HPLC equipment to deliver the wetting solvent, the aqueous sample and the precolumn washing solvent included two LKB (Bromma, Sweden) Model 2150 pumps; an LKB system controller Model 2152, LKB low- and high-pressure mixing valves, Models 2040-203 and 2154-400. A Pye-Unicam (Philips, Eindhoven, The Netherlands) LC-XPC pump was used to deliver the mobile phase. The variable-wavelength UV absorbance detector was a Pye-Unicam Model 4110, set at 230 or 280 nm. The Model SPH 99 column thermostat was from Spark (Emmen, The Netherlands).

The microprocessor-controlled autoinjector (Spark, Model Promis) with two additional valves was used to inject standard samples and to control the flow scheme during analysis. Chromatograms were recorded and integrated by a data station (Millipore, Milford, MA, U.S.A.) with Baseline 810 software.

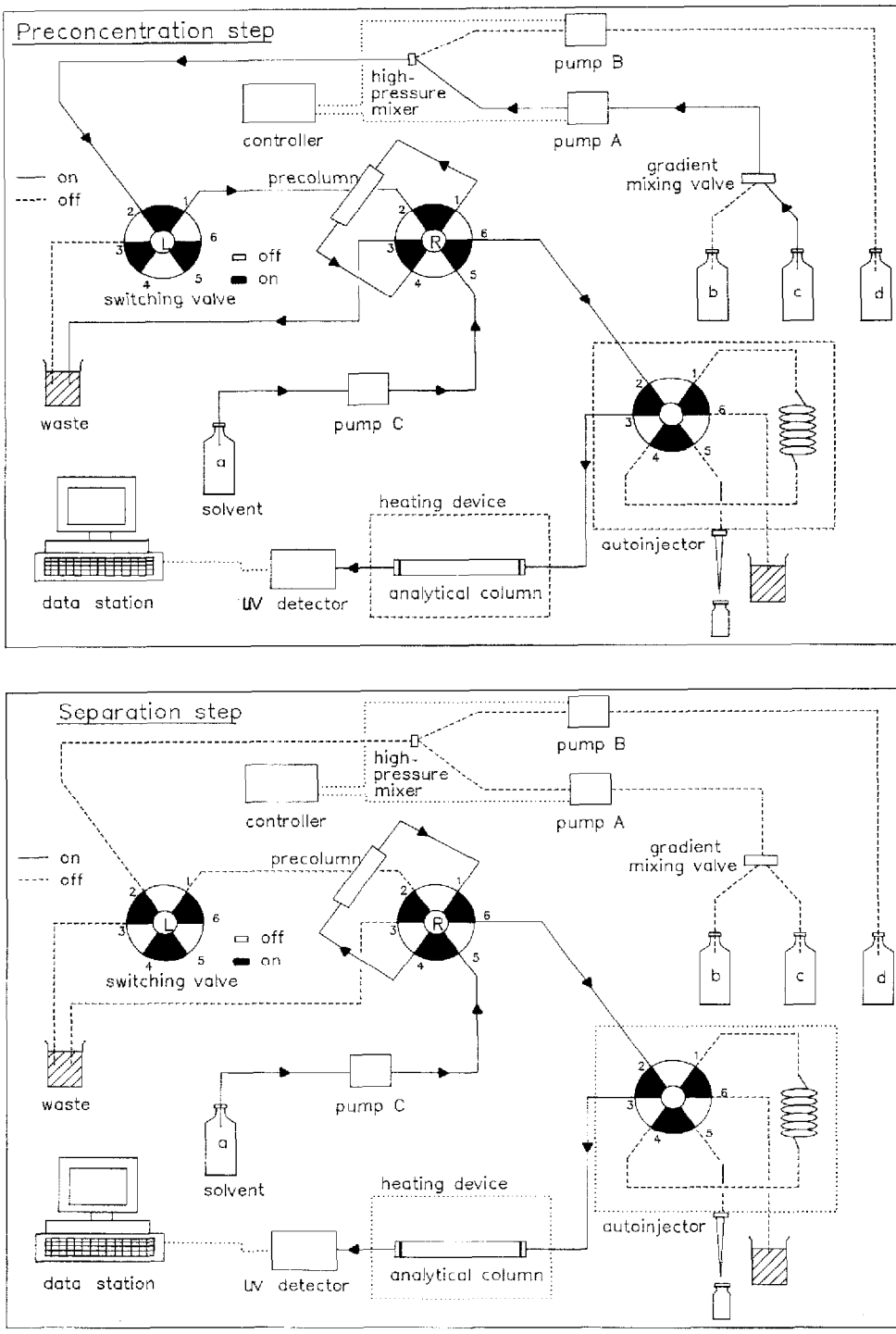

Fig. 1. Experimental set-ups for the on-line enrichment and separation of water samples: a = mobile phase, acetonitrile–water (30:70), 0.010 mol/l at pH 11; b = wetting solvent, 0.02 mol/l $HClO_4$; c = water sample; d = washing solvent, acetonitrile–water (30:70) at pH 3. Analytical column: 150 mm × 4.1 mm I.D. PRP-1; precolumn 10 mm × 2 mm I.D. PLRP-S. The autoinjector is used to inject a standard (loop) sample.

The experimental set-up for the on-line enrichment and separation of water samples is shown in Fig. 1.

Procedures

Stock solutions of the chlorophenoxy herbicides were prepared by weighing *ca.* 5 mg of each component followed by dissolution in 50 ml of methanol. These solutions were diluted in tap-water to obtain standard solutions as well as mixed standard solutions.

Sample solutions were preparad by diluting the stock solutions in tap-water or surface water and acidified to pH 3 with 0.1 mol/l $HClO_4$. Precolumns (10 mm × 2 mm) were manually packed using a microspatula with 15–25 μm PLRP-S (Polymer Laboratories, Shropshire, U.K.) and activated with 30 ml (2 ml/min) of 0.02 mol/l $HClO_4$ and 30 ml (2 ml/min) of 0.001 mol/l $HClO_4$ prior to preconcentration of the samples.

Separations were carried out at 50°C on a 150 mm × 4.1 mm I.D. column, prepacked with 10-μm PRP-1 (Hamilton, Reno, NV, U.S.A.) using acetonitrile–water (30:70), 0.010 mol/l of tetrabutylammonium (TBA), pH 11 (adjusted with 5 mol/l NaOH) at a flow-rate of 1.0 ml/min as the mobile phase, which also served as the desorption phase of the precolumn. Conditions during sample concentration are described below, and illustrated in Fig. 1. Time programming of the automated autoinjector and system controller is described in ref. 8.

RESULTS AND DISCUSSION

Chromatography

Solid phase extraction of polar compounds, such as acid herbicides with polymeric materials, has been shown to be successful from acidified samples[5,6]. Earlier experiments[8,9] showed that desorption of the acid herbicides from PLRP-S is favoured by (a) a high eluent pH and/or (b) a high eluent content of organic solvent. Separation, however, on analytical columns with a related type of material (PRP-1) was hardly achieved up to now because of the general lack of efficiency of those columns in general.

Optimization of the separation conditions, for the acetic acid derivatives MCPA and 2,4-D and the propionic acid derivatives MCPP and 2,4-DP, was adequately effected only using TBA as an ion-pair reagent. Other ion-pair reagents such as ammonium, caesium, tetradecyltrimethylammonium (cetrimide) and tetramethylammonium were not suitable in this respect. High molarity (0.05 mol/l) TBA increased the capacity factor, k', but did not improve the separation, while low molarity (0.010 mol/l) TBA gave higher selectivity, especially for the aforementioned pairs. Raising the temperature to 50°C further improved the separation. Separations therefore were carried out under the optimum isocratic conditions described in Experimental.

Sample flow dependence

In order to determine the dependence of the adsorption of acid herbicides from tap-water on the sample flow-rate, the flow-rate was varied between 1 and 20 ml/min. Recovery percentages of the acid herbicides were determined by comparing the peak areas of a loop injecton with those after enrichment and desorption from the

TABLE I

DEPENDENCE OF THE RECOVERY FROM TAP-WATER ON THE SAMPLE FLOW-RATE AND THE CONTACT TIME

Conditions: flow-rate = 1, 2 or 5 ml/min, sample = 5 ml, 50 μg of component/l; flow-rate = 10, 15 or 20 ml/min, sample = 10 ml, 25 μg of component/l.

Flow-rate (ml/min)	*Contact time[a] (s)*	*Recovery (%)*				
		MCPA	*2,4-D*	*MCPP*	*2,4-DP*	*2,4,5-T*
1	1.20	103	107	89	105	95
2	0.60	103	106	84	108	96
5	0.24	112	122	90	109	99
10	0.12	86	86	85	85	100
15	0.08	107	108	87	89	99
20	0.06	113	101	84	100	100

[a] Contact time $= \frac{\text{precolumn void volume}}{\text{flow-rate}} = 0.64\pi r^2 L/F$

where 0.64 = column porosity factor, ε_u[10], r = inner radius (dm), L = column length (dm) and F = flow-rate (l/s).

precolumn. From Table I it is seen that, even with a very short contact time of 0.06 s, in the case of a flow-rate of 20 ml/min, the recoveries of all five acid herbicides investigated are over 84%. Moreover no breakthrough occurred in the case of a 10-ml sample. The precolumn was the same during all these experiments. During all other experiments, however, the sample flow-rate was kept constant at 5 ml/min, and new precolumns were used.

Sample pH dependence

The dependence of the retention on sample pH was studied in the range pH 3–7 in order to determine the affinity of the acid herbicides for the hydrophobic adsorbent surface. It might be expected that the acid herbicides (pK_a 2.6–3.0) are more efficiently trapped in their acidic forms by PLRP-S and related materials than are their conjugated bases. Surprisingly, from Table II it is seen that the recoveries from

TABLE II

DEPENDENCE OF RECOVERY ON SAMPLE pH

Conditions: sample = 10 ml of tap-water, 25 μg of component/l; flow-rate = ml/min.

pH	*Recovery (%)*				
	MCPA	*2,4-D*	*MCPP*	*2,4-DP*	*2,4,5-T*
3	97	104	70	89	100
4	96	102	68	88	102
5	100	106	68	94	99
6	101	107	73	82	96
7	94	109	72	95	99

tap-water for all five herbicides investigated, over the entire pH range, are good. Recoveries from surface water (not shown in the table) with a sample pH 7 however are poor (11–41%), whereas at pH 3 the recoveries from surface water for all five herbicides are good ($\geq$70%). This means that PLRP-S is able to adsorb charged and uncharged organics, but that the adsorption of the neutral solute is stronger. Breakthrough at pH 7 with surface water might also be aided by competitive sorption by humic substances[11]. Recoveries at pH 3 might further be improved by adding sodium chloride (salting out effect) to the sample[5,12].

Repeatability and washing procedure

The repeatability was tested by applying five spiked tap-water samples on a precolumn. From Table III it is seen that the repeatability is satisfactory, *e.g.*, standard deviation (S.D.) $\approx$12%. The mean recovery of MCPP however is 70%. This can be attributed to peak integration errors of the chromatogram, obtained after desorption of the acid herbicides from the precolumn: a chromatogram of a blank shows, especially in the region of MCPP, a sharp irregular dip resulting in a less reliable integration in this region.

In the case of sample loop injections, baselines are straight and chromtogram integration is easily achieved.

During the enrichment step, not only the (spiked) acid herbicides and other unknown compounds are adsorbed, but water is also trapped by the macroporous resin. Desorption of the components and, more slowly, the release of water from the precolumn causes a large unretained peak, which only slowly and irregularly returns to the baseline. Therefore a washing step has been introduced to clean-up the precolumn and to remove interfering compounds and to "recalibrate" the polymer with the organic phase. This is done by delivering acetonitrile–water (30:70) at pH 3 at a constant, but low flow-rate by time programming the LC pump during the preconcentration step (see also Fig. 1A).

From Table IV it is seen that with washing volumes of 70 and 140 μl the recoveries are good and, moreover, the recovery of MCPP is in the same order, due to reliable integration.

TABLE III

REPEATABILITY OF PRECONCENTRATION FROM TAP-WATER

Conditions: as in Table II.

n	*Recovery (%)*				
	MCPA	*2,4-D*	*MCPP*	*2,4-DP*	*2,4,5-T*
1	82	81	79	91	119
2	104	102	77	97	109
3	102	105	72	95	136
4	106	110	52	94	103
5	108	108	70	98	104
Mean	100	101	70	95	114
S.D.	10	12	11	3	14

TABLE IV

DEPENDENCE OF RECOVERY ON WASHING VOLUME

Washing solvent: acetonitrile–water (30:70), pH 3, flow-rate = 0.1 ml/min. Other conditions as in Table II.

No. of bed volumes	*Volume (µl)*	*Recovery (%)*				
		MCPA	*2,4-D*	*MCPP*	*2,4-DP*	*2,4,5-T*
2	70	81	81	79	80	79
4	140	81	81	93	92	87
6	210	48	33	79	77	70

At this point it is also noticeable that manually packing of the precolumns contributes to some extent to the recovery percentages. Commercial, disposable cartridges showed better reproducibility[13] and will be used in our future work.

The low flow-rate of the washing solvent (0.1 ml/min) not only ensures reproducibility of the total volume applied to the precolumn, but also displacement of the water from the inner polymer structure. With 210 µl of washing solvent (six bed volumes), breakthrough of MCPA and 2,4-D is apparent. Fig. 2 demonstrates the effect of the washing step on the chromatogram baseline.

Sorption capacity and detection limits from tap-water and surface water

The sorption capacity of the PLRP-S polymer was measured by applying equal volumes of sample to the precolumn with varying herbicide concentrations (25–5000

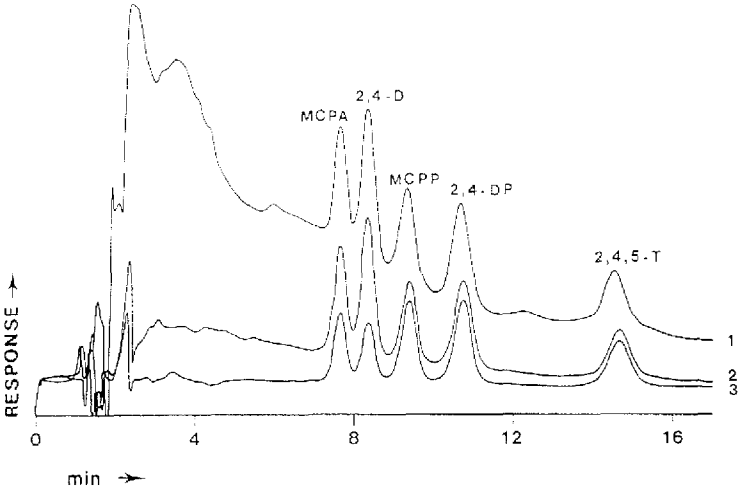

Fig. 2. HPLC chromatograms of acid herbicides after preconcentration: (1) without clean-up step; (2) after clean-up with four precolumn bed volumes and (3) after clean-up with six precolumn bed volumes. Preconcentration conditions: 10 ml of tap-water, 25 µg of component/l; sample flow-rate 5 ml/min; washing solvent, acetonitrile–water (30:70) pH 3, flow-rate 0.1 ml/min. System: 10 mm × 2 mm I.D. precolumn packed with 20 µm PLRP-S, 150 mm × 4.1 mm I.D. analytical column packed with 10 µm PRP-1; eluent, acetonitrile–water (30:70), 0.010 mol/l TBA, pH 11; flow-rate = 1.0 ml/min; UV detection at 280 nm.

TABLE V

RECOVERIES FROM TAP-WATER

Conditions: sample = 10 ml; flow-rate = 5 ml/min.

Concn. (mg of component/l)	*Absolute amount (μg of component)*	*Recovery (%)*				
		MCPA	*2,4-D*	*MCPP*	*2,4-DP*	*2,4,5-T*
0.025	0.25	104	102	79	89	92
0.05	0.50	91	87	75	75	89
0.10	1.00	95	94	88	88	99
0.20	2.00	93	91	84	82	101
0.40	4.00	101	100	91	85	98
1.00	10.00	98	96	90[a]	90[a]	96
2.00	20.00	89	93	89[a]	89[a]	102
5.00	50.00	88[a]	88[a]	88[a]	88[a]	95

[a] Recovery estimated by dividing the peak area of two coinciding components by two.

μg/l). From Table V it is seen that the capacity of the precolumn, determined by the affinity of the herbicides to PLRP-S, is good and not a limiting factor in enrichment of the herbicides from tap-water.

Comparing Tables V and VI, it is seen that breakthrough of MCPA from surface water occurs at concentrations 50 times lower than for tap-water. For surface water, also the other herbicides, except 2,4-D and 2,4,5-T, tend to breakthrough at higher concentrations.

In environmental surface waters, however, the concentrations are commonly below 1 μg/l and so the concentration of 0.5 μg of component applied to the precolumn, at which concentration, *i.e.*, 50 μg/l recoveries are still good, is far above the analyte concentration in practical environmental analysis. This means that the capacity of the PLRP-S material for surface water is sufficient.

With real surface water samples however the clean-up procedure as outlined above is not sufficient to eliminate the "injection peak" as in the case of tap-water samples (see also Fig. 2). This means that the first peaks eluted are more difficult to integrate, due to this injection peak. Moreover, if the sample volume is raised from 10 to 50–100 ml, the injection peak will also increase too.

TABLE VI

RECOVERIES FROM SURFACE WATER

Conditions as in Table V.

Concn. (mg of component/l)	*Absolute amount (μg of component)*	*Recovery (%)*				
		MCPA	*2,4-D*	*MCPP*	*2,4-DP*	*2,4,5-T*
0.025	0.25	95	101	86	85	95
0.05	0.50	94	93	95	98	99
0.10	1.00	51	90	91	97	103
0.20	2.00	40	75	75	77	101
0.40	4.00	32	95	63	64	99

TABLE VII

DEPENDENCE OF RECOVERY ON SAMPLE VOLUME (TAP-WATER)

Flow-rate = 1 ml/min.

Concn. (μg of component/l)	*Preconcn. volume (ml)*	*Recovery (%)*				
		MCPA	*2,4-D*	*MCPP*	*2,4-DP*	*2,4,5-T*
5	10	91	87	75	75	89
0.5	100	71	77	67	81	83
0.12	400	67	46	53	67	56

From Table VII it is seen that the recovery of the phenoxyacid herbicides decreases only slowly with increasing sample volume. With an 100-ml sample and 0.5 μg component/l, the recoveries are acceptable and detection limits of 10–50 ng/l (signal-to-noise ratio 3) were calculated. This, of course, is true only for tap-water samples. Surface water samples were, up to now, limited to 50 ml with detection limits of 0.1–0.5 μg/l (signal-to-noise ratio 3). However, without (off-line) clean-up procedures like gel permeation chromatography (GPC), ultrafiltration or dialysis before concentration of the analytes, lower detection limits for surface water are not expected.

Currently, the behaviour of humic substances (1–5 mg/l surface water) is studied with on-line GPC and off-line SPE techniques in order to improve the sample to be concentrated and to improve the efficiency of the clean-up procedure. The results will be presented in a future paper.

Application to surface water samples

On September 9th, 1988 an accidental spill of 2,4-DP (dichlorprop) (0.5–1 ton) in the river Rhine near Ludwigshafen (F.R.G.) was reported to the authorities. About half of the Dutch drinking water is prepared from surface water, so a monitoring

TABLE VIII

CONCENTRATION AND CALCULATED AMOUNT OF 2,4-DP IN RHINE SAMPLES AFTER ACCIDENTAL SPILL

Sample[a]	*Date*	*Time (h)*	*Concn. (μg/l)*	*Amount (ton)*
1	12-09-88	20.00–24.00	0.5	0.01
5	13-09-88	08.00–12.00	1.5	0.04
7	13-09-88	12.00–16.00	2.2	0.05
9	13-09-88	16.00–20.00	3.0	0.07
11	13-09-88	20.00–24.00	2.7	0.06
13	14-09-88	00.00–04.00	3.2	0.07
15	14-09-88	04.00–08.00	3.1	0.06
16	14-09-88	08.00	2.5	——
			Total =	0.36

[a] Composite or grab samples.

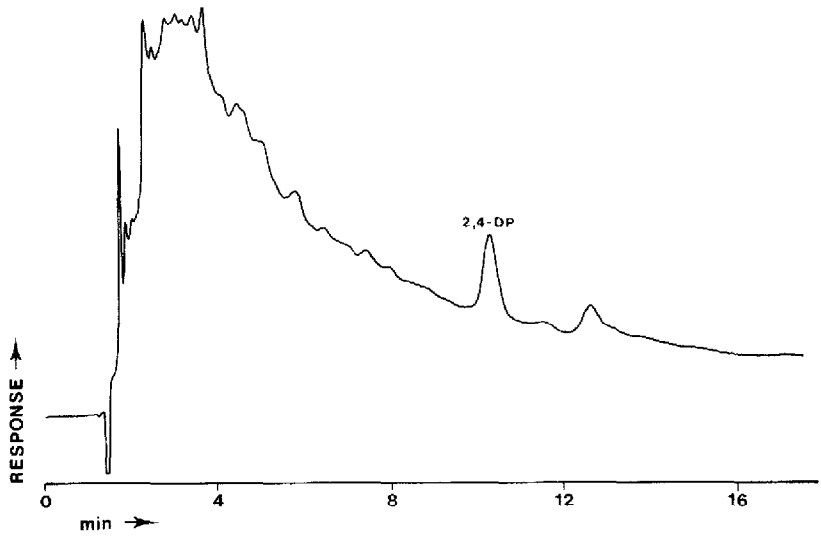

Fig. 3. HPLC chromatogram of a river Rhine sample after an accidental spill. Conditions: see text and Fig. 2; UV detection at 230 nm.

programme, with the aforementioned method, was started. At Lobith, the entrance of the Rhine in The Netherlands, samples were collected and analyzed in our laboratory. From the results the total amount of 2,4-DP can be determined.

From Table VIII it is seen that the maximum concentration was *ca.* 3.2 μg/l 2,4-DP and that the measured amount of 2,4-DP, during the measuring time, was *ca.* 0.4 ton; this fits well with the statement from the factory.

The results were in good agreement with the results of the Water Transport Company[14] (WRK, Nieuwegein) and only slightly lower (*ca.* 0.1 μg/l over the entire range) than their gas chromatographic–mass spectrometric (GC–MS) method, which essentially consists of (a) preconcentration of 0.5 l surface water on a C_{18} cartridge, (b) drying of the cartridge with nitrogen, (c) desorption with 2 ml methanol, (d) methylation with diazomethane, (e) evaporation with nitrogen to 50 μl and (f) injection of 2 μl for GC–MS.

In Fig. 3 a chromatogram is shown of the river Rhine sample using the aforementioned method, except for the washing step, which was omitted.

CONCLUSIONS

Separation of the acid herbicides MCPA, 2,4-D, MCPP, 2,4-DP and 2,4,5-T on a styrene–divinylbenzene copolymer (PRP-1) analytical column has been achieved with the ion-pair reagent tetrabutylammonium under isocratic conditions.

Solid phase extraction was studied on the polymeric material PLRP-S. The herbicides were applied to the precolumn in tap-water or surface water matrices. The results for the tap-water samples indicate that the sorption capacity of the precolumn (with a volume of *ca.* 35 μl) is excellent and that recoveries at pH 3 with various flow-rates (1–20 ml/min) are over 80%. The repeatability of the method is acceptable (S.D. $\approx$12%; 25 μg of component/l).

To overcome the interference of a large unretained peak, which results in a partly less reliable integration of the chromatogram, a clean-up step was introduced which essentially consists of a slow delivery of four bed volumes of acetonitrile–water (30:70) at pH 3. This procedure however, is not sufficient to eliminate the injection peak in the case of surface water samples. Due to a high humic content, an additional clean-up step before preconcentration is considered necessary.

Also the results for the surface water samples indicate that the competitive sorption capacity of the sample matrix is a limiting factor for the sorption capacity of the precolumn. Breakthrough of the first herbicides eluted occurs for surface water 50 times more rapidly than for tap-water samples. Still at least 10–50 ml of surface water can be applied to the precolumn without breakthrough. Detection limits from tap-water are calculated to be 10–50 ng/l (signal-to-noise ratio 3) and those from surface water are 0.1–0.5 μg/l (signal-to-noise ratio 3).

The easy handling of aqueous samples, the low detection limits obtainable and the potential of this technique for automation are very promising.

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