ELSEVIER

Journal of Chromatography A, 731 (1996) 115–122

JOURNAL OF CHROMATOGRAPHY A

On-line preconcentration, cleanup and high-performance liquid chromatographic determination of chlorophenoxy acid herbicides in water

Luz E. Vera-Avila*, Patricia C. Padilla, Maira G. Hernandez, Jose Luis L. Meraz

Departamento de Química Analítica, Facultad de Química, Universidad Nacional Autónoma de México, 04510 México D.F., Mexico

Received 11 January 1995; revised 25 September 1995; accepted 28 September 1995

Abstract

A rapid and simple technique for the determination of six chlorophenoxy acid herbicides in water is described. After extraction and preconcentration of the analytes at pH≈1 in a small 20×2 mm I.D. polymeric reversed-phase precolumn, an on-line cleanup procedure is performed by a selective transfer of the ionized compounds to a second precolumn packed with an anion exchanger. An acetonitrile–sodium hydroxide (pH 10; 10:90, v/v) solution is used for this step. The anion-exchange precolumn is further on-line analyzed by reversed-phase chromatography with gradient elution and UV detection at 230 nm. The recovery of the examined herbicides is nearly 100% with a R.S.D.<10%. The method is linear in the range 1–80 μg/l. Application of this methodology to the analysis of tap water and river water shows the effectiveness of the cleanup procedure.

Keywords: Water analysis; Environmental analysis; Sample preparation; Pesticides; Chlorophenoxy acids

1. Introduction

Chlorophenoxy acids, formulated as salts or alkyl esters, have been extensively used for the control of weeds in dams and cereal fields. Their persistency in soil is about 1 to 5 months but higher persistence is to be expected in water with a lower biological activity [1]. The high solubility of phenoxy acids in neutral water promotes their entry into surface or ground waters by natural drainage or infiltration [2]. This fact and the evidence on their toxicity demand the development of selective and sensitive methods for their trace determination in natural and potable waters.

Standard methods for the determination of these pollutants in water matrixes involve liquid–liquid extraction of the sample, concentration and derivatization, with or without additional cleanup, prior to GC analysis [3]. Recently, these cumbersome and time-consuming methods have been successfully replaced by solid-phase extraction (SPE), in off-line [2,4] or on-line [5–7] modes, followed by HPLC analysis with UV detection.

Due to their high sorption capacity, polystyrene-divinylbenzene copolymers have been the adsorbents of choice for the extraction and trace enrichment of moderately polar analytes from water [8]. In the case of solutes with acid-base properties, this material offers the advantage of greater stability over the entire pH range than other reversed-phase adsorbents

*Corresponding author.

0021-9673/96/$15.00
SSDI 0021-9673(95)01115-3

as alkyl bonded silicas. Thus, phenoxy acids may be extracted in their molecular form from acidified water samples of very low pH and be desorbed as ionized species by means of an alkaline hydro-organic solution. However, the poor selectivity of reversed phases remains a major disadvantage. Additional cleanup procedures must be set up to eliminate interfering substances in the analysis of natural waters. Geerdink et al. [7] proposed a heartcutting cleanup procedure for the on-line trace enrichment and determination of bentazone and eight phenoxy acid herbicides in surface water. After the preconcentration step, the precolumn was washed with a sodium hydroxide solution and only the fraction containing the solutes of interest was transferred to the analytical column.

In this study, an alternative procedure, using a two-precolumn setup, is proposed for the on-line preconcentration, cleanup and determination of chlorophenoxy acid herbicides in water. The compounds of interest are trapped on a polymeric reversed-phase adsorbent in the first precolumn, while inorganic interferences are eliminated. Further sample cleanup is performed by a selective transfer of the ionized analytes to the second precolumn packed with an anion exchanger. The latter is finally on-line analyzed by reversed-phase chromatography with gradient elution and UV detection.

2. Experimental

2.1. Reagents

HPLC-grade acetonitrile was from Prolabo (Paris, France). Reagent water was obtained from a Nanopure (Barnstead Thermolyne, Dubuque, IA, USA) deionizer. All other chemicals were analytical-grade reagents obtained from various furnishers: methanol from Prolabo, perchloric acid from Química Dinámica (Monterrey, Mexico), formic acid from Merck (Darmstadt, Germany) and sodium hydroxide from Baker (Phillipsburgh, NJ, USA). All chlorophenoxy carboxylic acids were purchased from Chem Service (West Chester, PA, USA) with certified degree of purity between 97 and 99%.

Stock solutions of the herbicides (1000 mg/l) were prepared by weighting and dissolving each solute in methanol. These solutions were used to prepare mixed standards of different concentration which were also dissolved in methanol.

2.2. Apparatus

A Beckman (Berkeley, CA, USA) 110 B isocratic pump (SPE pump) was used to deliver the aqueous sample, the solvents to equilibrate and flush the precolumns and the transfer solvent. The HPLC system consisted of two Gilson (Villiers-le-Bel, France) Model 305 and 306 pumps (gradient LC pumps), a Gilson 805 manometric module, a Gilson 811 B dynamic mixer and a Gilson 115 variable-wavelength detector set at 230 nm. Chromatograms were recorded and integrated by a Hewlett-Packard (Avondale, PA, USA) 3396 Series II integrator. Quantitation was based on peak-area measurements. A 7125 Rheodyne (Berkeley, CA, USA) valve with a 20-μl calibrated loop was used for the injection of the herbicide standards. Loop calibration was performed in situ as described in Ref. [9]. Two 7000 Rheodyne valves, with the reversed-phase and the anion-exchange precolumns respectively placed in the position corresponding to the loop, were inserted between the injector and the HPLC column for the column switching operations. The diagram in Fig. 1 shows the experimental setup.

2.3. Stationary phases and columns

Stainless steel precolumns, 20×2 mm I.D., from Upchurch Scientific (Oak Harbor, WA, USA) were home-packed with a thin slurry of the respective adsorbent using a Haskel (Burbank, CA, USA) Model 29426 column packing system. The reversed-phase precolumn (RP precolumn in Fig. 1) was packed with an ethanolic slurry of the styrene–divinylbenzene copolymer CHP-3C, 10 μm, from Mitsubishi (Tokyo, Japan) at a constant pressure of 207 bar. The anion-exchange precolumn (AX precolumn, Fig. 1) was packed with a methanol–NaOH (pH 13; 60:40, v/v) slurry of the polymeric anion exchanger PRP-X100, 10 μm, from Hamilton (Reno, NV, USA) at a constant pressure of 117 bar.

Separations were carried out at ambient temperature on a 150×4.6 mm I.D. stainless steel cartridge (RP-HPLC column in Fig. 1), prepacked with 5 μm

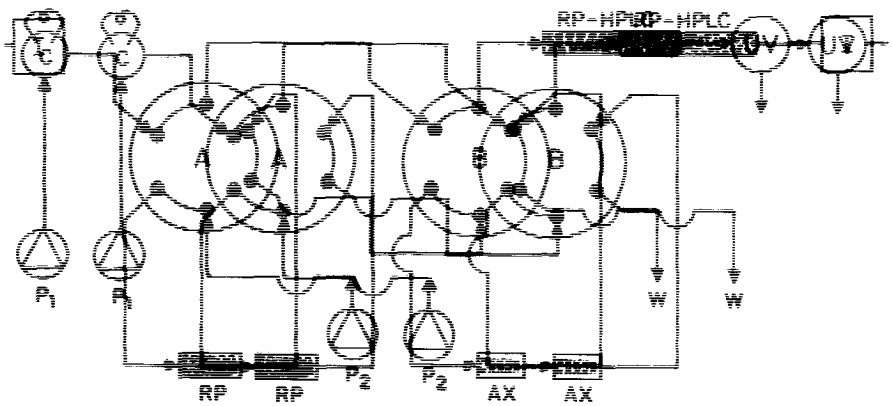

Fig. 1. Diagram of the experimental setup for the on-line preconcentration, sample cleanup and HPLC analysis of chlorophenoxy acid herbicides in water. P_1=gradient LC pump; P_2=isocratic SPE pump; A,B=high-pressure switching valves; C=injector valve; UV=detector; I=integrator; W=waste. Columns: RP, 20×2 mm I.D. reversed-phase precolumn packed with 10 μm CHP-3C; AX, 20×2 mm I.D. anion-exchange precolumn packed with 10 μm PRP-X100; RP-HPLC, 150×4.6 mm I.D. analytical column prepacked with Spherisorb ODS-2, 5 μm.

Spherisorb ODS-2 (Phase Separations, Deeside, UK), using a mobile phase gradient at a flow-rate of 1 ml/min. Eluents A and B contained 20% (v/v) and 80% (v/v) of acetonitrile, respectively. The aqueous part in both eluents consisted of a 0.01 *M* $HClO_4$ and 0.01 *M* formic acid solution with the pH adjusted to 3.8 with NaOH. The gradient system was: eluent B, 0 min=0%, 15 min=7%, 30 min=20%, 55 min=70%.

2.4. Procedures

2.4.1. Sample preparation

For the application of this method, aqueous samples must be collected at the sampling site in 250-ml amber glass bottles with Teflon-lined caps (sampling bottle).

The sampling bottle was filled with 250 ml of reagent water, tap water or river water spiked with a known volume of an herbicide standard mixture. A Millipore (Bedford, MA, USA) glass filter holder with a nylon 66 membrane (0.4 μm pore size) was used for sample filtration. Before use, the membrane was soaked for 1 or 2 h in methanol, then it was placed on the filter holder and rinsed with 10 ml of fresh methanol and 20 ml of reagent water. After filtering the sample, the sampling bottle was sequentially rinsed with 12.5 ml of methanol and 5 ml of reagent water. The rinsing solvents were passed through the membrane used to filter the sample and were collected in the same flask. The mixture was acidified by addition of 4 ml of diluted perchloric acid (1:1, v/v) directly in the flask. This recipient with the prepared sample was used as reservoir of the isocratic SPE pump.

2.4.2. Preconcentration and analysis

Steps 1–5 described in the following general procedure were carried out with the SPE pump (pump P_2, Fig. 1) while holding the gradient LC pumps switched off. Before each step, the pump lines and tubing were carefully rinsed and filled up with the corresponding solvent, keeping the switching valves A and B in the 'inject' position. Steps 6–8 were performed with the gradient LC pumps (pump P_1, Fig. 1). The positions of switching valves A and B and of the injector C in each step are reported in Table 1.

General procedure:

1. Condition RP-precolumn with 25 ml of a mixture methanol–$HClO_4$ (pH≈1) 5:95 (v/v).
2. Load RP-precolumn with 75 ml of the prepared sample and flush it with 0.2 ml of reagent water.
3. Condition AX-precolumn with 25 ml of a mixture acetonitrile–NaOH (pH≈10) 10:90 (v/v).
4. Transfer the preconcentrated sample from the RP-

Table 1
Position of switching valves during the trace enrichment and determination of chlorophenoxy acids

Step	Operation	Valve A	Valve B	Injector C
1	RP conditioning	L	I	L/I
2	RP loading and flushing	L	I	L/I
3	AX conditioning	I	L	L/I
4	RP to AX transfer	L	L	L/I
5	AX flushing	I	L	L/I
6	RP-HPLC equilibrium	L	L	L/I
7	Sample analysis	L	I	L/I
8	Standard injection	L	I	I

RP=reversed-phase precolumn; AX=anion-exchange precolumn; RP-HPLC=reversed-phase analytical column; L=load; I=inject.

precolumn to the AX-precolumn with 5 ml of the previous mixture.

5. Flush AX-precolumn with 0.2 ml of reagent water.
6. Equilibrate the analytical column with 10 ml of mobile phase A.
7. Analyze the sample by coupling AX-precolumn to RP-HPLC column.
8. Inject a standard for quantitation.

After this procedure, the RP-precolumn is regenerated with 10 ml of reagent water and 10 ml of methanol. This regeneration as well as the conditioning of the RP-precolumn (step 1) can be performed while steps 7 and 8 are carried out. The AX-precolumn needs no extra regeneration (it is regenerated by the gradient elution), but it is recommended to activate it after a 10-sample cycle with an aqueous NaOH solution of pH 12 followed by an abundant rinsing with water.

For the development of the method and to optimize all the experimental conditions, in this work the switching valves and pumps were manually controlled. However, all the on-line operations can efficiently be automated and made suitable for routine screening of series of samples. For this purpose, an automatic selector valve should be adapted at the inlet of the SPE pump and a third switching valve, coupling the LC-pumps to the HPLC column and the precolumn circuit, would also be convenient. With the extra valve it could be possible to equilibrate the analytical column (step 6) at the same time that the sample is being pretreated in the precolumn circuit (steps 1–5).

3. Results and discussion

3.1. Sample preparation

Chlorophenoxy acids in their molecular form are highly hydrophobic and can easily be adsorbed on the walls of vessels or the surface of filters and other materials which may be in contact with their aqueous solutions. Therefore, it is necessary to take some precautions during sample preparation to avoid losses of analytes. First, the sample must be filtered before pH adjustment in order to keep all solutes of interest in their ionized form. Then, the sampling bottle and the membrane filter have to be rinsed with an organic solvent, like methanol, to recover adsorbed solutes. However, we found that the membrane filter releases organic substances, which may interfere with the analysis of target compounds, when pure methanol is passed through it; previously soaking the filter in methanol and rinsing it with reagent water eliminates this contamination problem. Finally, the sample is acidified in the same flask where it was collected after filtering and this flask is used as the reservoir of the pump for the preconcentration step. In this way, the sample is in contact with the minimum number of recipients and the risk of losses by solute adsorption decreases.

The sample prepared as indicated in the experimental section contains 4.6% (v/v) of methanol in a total volume of 271.5 ml. The pH of the solution is about 1.1.

3.2. Chromatography

Optimization of the separation conditions for the six phenoxy acids was carried out with the analytical column coupled to the anion-exchange precolumn. This was necessary because solute retention and peak shape were completely different with the single HPLC column and with the set of two columns. This means that the anion-exchange precolumn participates in solute retention by a mechanism different from the retention mechanism in C_{18} phases. Probably, at the pH of the mobile phase (3.8), a mixed mechanism with electrical and hydrophobic interactions is responsible for solute retention in this adsorbent. In fact, we found that the addition of perchloric acid to the eluent, followed by readjust-

ment of pH to 3.8, significantly improved the peak shapes and decreased the retention times of chlorophenoxy acids. This can be explained by the strong affinity of perchlorate ions for anion exchangers.

Complete separation of the solutes was achieved with the acetonitrile gradient described in section 2. Formic acid was used to fix the pH at 3.8, which was found to be an optimal value for the separation of Mecoprop and 2.4.5-T. The gradient was designed for solute elution to begin at ~10 min because, in the analysis of natural waters, there is always a large peak at the beginning of the chromatogram which only slowly returns to the baseline.

On the other hand, the performance of our detector at 230 nm with the acetonitrile gradient was rather poor. The limits of detection for the herbicides in injected samples were ~50 ng except for 2.4-DB that elutes at the end of the gradient where the baseline perturbations render more difficult its detection at low concentrations. For the latter the limit of detection was 130 ng. However, it has been reported [4] that sub-nanogram amounts of the chlorophenoxy acids can be detected in injected samples using an acetonitrile–phosphate buffer mobile phase of constant composition. Therefore, in cases where it is not necessary to analyze the six compounds, it should still be possible to substantially lower the detection limits using isocratic conditions and a better detection device.

3.3. Preconcentration and analysis

Preliminary experiments with solutions of the less hydrophobic solute, 2.4-D, in reagent water acidified to pH 1.5 showed that it was possible to percolate more than 200 ml of sample through the reversed-phase precolumn without breakthrough of the analyte. However, when the sample contained about 5% (v/v) of methanol and in the presence of other organic compounds, the breakthrough volume of 2.4-D considerably decreased. In this case, it was necessary to reduce the volume of sample loaded on the precolumn to 75 ml for a complete recovery of this solute.

Considering the amount of each herbicide that can be detected in our experimental conditions, the dilution of the sample during its preparation and the volume of sample that can be concentrated in the RP-precolumn, the attainable limits of detection of the method are between 0.7 and 2 μg/l.

On-line solid-phase extraction on polymeric reversed-phase adsorbents is actually a widely used technique for the analysis of pollutants at trace concentration levels in water. However, the lack of selectivity of this adsorbent is a severe drawback, specially when it is combined with a low selectivity detection mode as UV absorbance at low wavelengths. It is therefore necessary to effect an additional cleanup of the preconcentrated sample. For compounds with acid-base properties (phenols, anilines etc.), a selective transfer of the ionized solutes from the reversed-phase precolumn to a second precolumn packed with an ion exchanger has been proposed [10,11]. In this way, neutral compounds of low and medium polarity remain in the first precolumn and only the solutes capable of being ionized and desorbed from the RP-adsorbent by the transfer solvent will reach the second precolumn. This interesting approach was used in this work because chlorophenoxy acids are easily ionizable (pK_a 2–4).

The desorption of the herbicides from the RP-adsorbent was assayed with NaOH solutions of pH 10–11, but this solvent was not capable of completely desorbing the most retained solutes. Addition of a small volume of acetonitrile to the basic solution gave better results. With only 5 ml of a mixture acetonitrile–NaOH (pH 10; 10:90, v/v), it was possible to completely transfer all the analytes from the first to the second precolumn. Besides, no breakthrough of the herbicides occurred from the anion-exchange precolumn with this small transfer volume.

In the general procedure, the RP and AX precolumns are flushed with 200 μl of reagent water after loading the acidified sample or transferring the herbicides, respectively (steps 2 and 5). A very small flushing volume, equivalent to about two or three precolumn void volumes, was used in both cases to avoid breakthrough of the analytes due to the pH modifications induced by water. In the RP-precolumn, the flushing step was necessary to sweep out the remaining acidic solution before the passage of the alkaline transfer solvent; neutralization of strong acids by strong bases may provoke undesirable effects like heating. In the case of the AX-pre-

Table 2
Accuracy and precision of the method

Compound	Recovery (%)	R.S.D. (%)
2.4-D	100	4.3
MCPA	101	5.0
Mecoprop	118	8.1
2.4.5-T	102	5.3
Silvex	102	2.5
2.4-DB	101	5.8

Conditions: reagent water samples spiked at 5 μg/l of each herbicide were filtered and acidified to pH 1.1; 75 ml of the prepared sample were analyzed according to the 8-step general procedure described in section 2. Results are the averages from nine independent samples. Data are based on peak-area measurements.

column, it was necessary to eliminate the NaOH solution remaining after the transfer of the herbicides because in the next step the precolumn is coupled to the analytical C_{18} column which cannot stand an alkaline pH. The 200-μl flush and the use of a buffered mobile phase for the on-line elution of the precolumn are sufficient to protect the HPLC column; this has been demonstrated by the use of the same column for eight months without appreciable loss of efficiency.

Table 2 shows the accuracy and precision obtained when nine identical samples were analyzed using the general procedure described in section 2 and the setup of Fig. 1. Samples were prepared from reagent water spiked with a mixed standard to give concentrations of ca. 5 μg/l for each phenoxy acid. Recoveries were calculated by comparison with a direct loop injection of the standard. These results show that all the herbicides are completely recovered using the proposed methodology. The precision of recovery is fairly good for this concentration level. Only Mecoprop, which coelutes with a small interfering peak, gives a less satisfactory result.

The linear concentration range and the detection limits of the method were determined from the analysis of reagent water samples fortified with the six chlorophenoxy acids at various concentrations. Results from these experiments show that the method is linear from concentrations of 1 or 2 μg/l until ~80 μg/l, for all the compounds examined. Higher concentrations deviate from linearity probably because of incomplete solubility of the herbicides in the acidified water sample. The limits of detection, reported in Table 3, correspond to the compound concentration in reagent water that produces a signal of three times the baseline noise when the sample is analyzed by this method. These limits are practically the same as the 'attainable detection limits' mentioned before, which is explained by the fact that all six herbicides are completely recovered during the different steps of the method.

Recovery calculations for the samples containing different concentrations of the herbicides are also presented in Table 3. These are reported as relations of recovered amount vs added amount. It can be seen that intercepts are near zero and slopes, which represent the fraction of analyte recovered, are around 1. This confirms the good accuracy of the method in the range of concentrations from 1 to 80 μg/l.

Chromatograms obtained from the analysis of reagent water, tap water and river water spiked at 10 μg/l of each herbicide are shown in Fig. 2. The

Table 3
Relations of recovered amount vs added amount

Compound	Intercept (μg)	Slope	r	Range (μg)	MDL[a] (μg/l)
2.4-D	−0.029	0.968	0.999	0.14–5.35	1
MCPA	−0.033	0.957	0.999	0.07–5.35	1
Mecoprop	−0.037	0.982	0.997	0.07–5.81	1
2.4.5-T	0.007	0.968	0.999	0.07–5.61	1
Silvex	−0.015	0.953	0.999	0.14–5.59	1
2.4-DB	0.000	0.937	0.998	0.14–5.49	2

Detection limits of the method. Conditions: reagent water samples fortified with the herbicides at different concentrations (n=8) were filtered and acidified to pH 1.1; 75 ml of the prepared sample were analyzed according to the 8-step general procedure described in section 2. Data are based on peak-area measurements.

[a] MDL=method detection limit, defined for S/N=3.

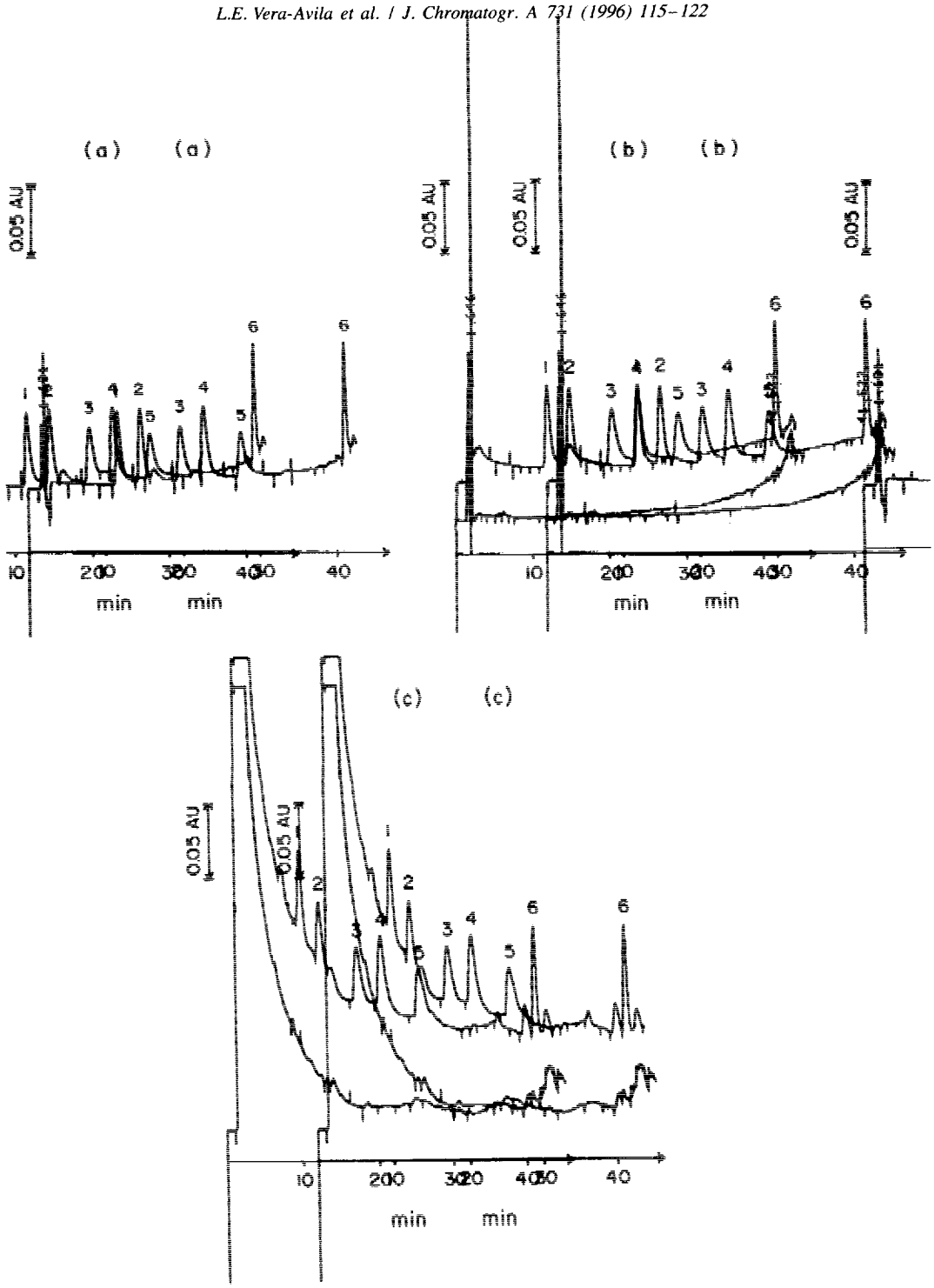

Fig. 2. Chromatograms corresponding to the analysis of different water samples: (a) spiked reagent water, (b) unspiked and spiked tap water and (c) unspiked and spiked river water. Spikes at 10 μg/l of each herbicide. Solutes: (1) 2.4-D, (2) MCPA, (3) Mecoprop, (4) 2.4.5-T, (5) Silvex, and (6) 2.4-DB. Preconcentration of 75 ml of the prepared sample on the reversed-phase precolumn. Cleanup by transfer of solutes to the anion-exchange precolumn with 5 ml of acetonitrile–NaOH (pH 10; 10:90, v/v). Chromatographic conditions described in section 2.

chromatograms corresponding to the analysis of the blank real samples are also shown in Fig. 2, demonstrating the high efficiency of the cleanup procedure.

The chromatograms from the spiked tap water and reagent water are very similar and the recoveries of the six compounds in both samples are identical. On the other hand, the big matrix peak at the beginning of the chromatogram from river water screens part of the peak area of the first eluting solutes, 2.4-D and MCPA. Therefore, their recoveries in this sample decrease to 78% and 70%, respectively, relative to the reagent water sample. The other four compounds, however, are completely recovered and the chromatogram in general is rather clean.

Experiments with several spiked surface water samples have shown that the recoveries of 2.4-D and MCPA at the 10 μg/l level may vary from 70% for some river waters to 100% for source waters. Nevertheless, the precision of recovery in repeated analysis of the same sample remains at ~5%. The limits of detection for the two analytes also are highly dependent on the sample and especially on the size of the matrix peak. Thus, limits of detection varying from 1 to 3 μg/l have been observed with different samples.

The reliability and robustness of the proposed system have been proved for almost one year through the analysis of a great variety of water samples. In this period the frits of the RP-precolumn have been changed several times but the precolumns themselves have been re-packed only once. The same analytical column has been used throughout.

4. Conclusions

This work demonstrates the high potential of on-line precolumn technologies for the determination of trace amounts of pesticides in water matrices.

The combination of a polymeric reversed-phase precolumn with a more selective ion-exchange precolumn allows an efficient preconcentration and cleanup of the aqueous sample. The optimization of all the experimental conditions, from sample filtration to chromatographic separation has resulted in the proposition of a rapid, simple and accurate method for the determination of chlorophenoxy acids at low μg/l concentrations in water.

Acknowledgments

Financial support for this work was provided by grants from 'Dirección General de Asuntos del Personal Académico, Universidad Nacional Autónoma de México' and 'Consejo Nacional de Ciencia y Tecnología de México'.

References

[1] R. Hamann and A. Kettrup, Chemosphere, 16 (1987) 527
[2] R. Schuster and A. Gratzfeld-Hüsgen, Analusis, 19 (1991) i45.
[3] A.E. Greenberg, J.J. Connors and D. Jenkins (Editors), Standard Methods for the Examination of Water and Waste, 15th edn., American Public Health Association, Washington, DC, 1981.
[4] A. Betti, G. Lodi and S. Coppi, J. Chromatogr., 513 (1990) 219.
[5] R.L. Smith and D.J. Pietrzyk, J. Chromatogr. Sci., 21 (1983) 282.
[6] R.B. Geerdink, C.A.A. Van Balkom and H.-J. Brouwer, J. Chromatogr., 481 (1989) 275.
[7] R.B. Geerdink, A.M.B.C. Graumans and J. Viveen, J. Chromatogr., 547 (1991) 478.
[8] M.W.F. Nielen, U.A.Th. Brinkman and R.W. Frei, Anal. Chem., 57 (1985) 806.
[9] L.E. Vera-Avila and R. Covarrubias, Int. J. Environ. Anal. Chem., 56 (1994) 33.
[10] M.W.F. Nielen, J. de Jong, R.W. Frei and U.A.Th. Brinkman, Int. J. Environ. Anal. Chem., 25 (1986) 37.
[11] M.-C. Hennion, P. Subra, V. Coquart and R. Rosset, Fresenius J. Anal. Chem., 339 (1991) 488.