ELSEVIER

Journal of Chromatography B, 717 (1998) 157–178

JOURNAL OF CHROMATOGRAPHY B

Review

Phenoxyacetic acids: separation and quantitative determination

Tibor Cserháti*, Esther Forgács

Central Research Institute for Chemistry, Hungarian Academy of Sciences, P.O. Box 17, H-1525 Budapest, Hungary

Abstract

The various chromatographic methods suitable for the separation and quantitative determination of phenoxyalkyl acid herbicides in environmental samples are reviewed, with special emphasis being placed on sample preparation methods such as liquid–liquid, solid-phase and supercritical fluid extractions. Techniques are classified (high-performance liquid chromatography, gas–liquid chromatography, capillary zone electrophoresis, micellar electrokinetic chromatography) and discussed separately. The advantages and disadvantages of the various preparation and separation methods and their combinations are evaluated.

Keywords: Reviews; Phenoxylacetic acid

Contents

1. Introduction

Herbicides of various chemical structure have been frequently used in up-to-date agrochemical practice to increase the yield of various crops [1]. However, the increasing use of herbicides has given rise to the problem of environmental pollution [2,3] and to undesirable side-effects in crops [4,5] and soil microflora [6,7].

Phenoxy acid herbicides showing auxin-like activity have been intensively used to control the

*Corresponding author.

0378-4347/98/$19.00
PII: S0378-4347(98)00028-0

growth of grass and broad-leaf weeds in many crops, such as rice [8], winter wheat [4,5], soybean [9], bermudagrass [10], etc. The molecular basis of the mode of action of phenoxyacetic acid herbicides is not entirely understood. It was assumed that they are uncouplers of the oxidative phosphorylation system and modify the structure of thylakoidal membranes [11]. Due to their solubility in water, they can move in agricultural ecosystems, causing the pollution of surface and ground waters [12]. The persistence of these compounds in the environment has been discussed vigorously. It was established that they are relatively less persistent in soil and water and can accumulate in river and lake sediments [13–15]. Phenoxy acid herbicides show moderate toxicity, however, some chlorinated metabolites can be toxic to human and aquatic organisms [16]. It was reported that they can cause soft tissue carcinoma in man [17,18] and show embryotoxicity in animals [19].

Due to their considerable practical importance, many efforts have been made to develop reliable and sensitive methods for analysing phenoxy acid herbicides in various matrices, such as formulations, soil surface and ground waters. Spectrophotometry and differential pulsed polarography have been used previously for the analysis of this class of herbicide, however, the separation power of these traditional methods is fairly low. The use of flow-injection analysis–thermospray tandem mass spectrometry for the fast screening of eight phenoxy acid herbicides has also been reported [20] and it was established that phenoxy acid herbicides can be determined at the low μg/l level with this fully automated procedure.

The objectives of this review are to enumerate and critically evaluate recent results obtained relating to the extraction, chromatographic separation and quantitative determination of phenoxy acid herbicides and to compare the efficacies of the various chromatographic techniques and procedures used, such as high-performance liquid chromatography (HPLC), gas–liquid chromatography, micellar electrokinetic capillary chromatography (MECC) and capillary zone electrophoresis (CZE). The newest achievements using hyphenated methods such as HPLC–mass spectrometry (HPLC–MS) and gas chromatography (GC)–MS are also discussed in detail.

2. Sample preparation

Chromatographic techniques have been developed for the separation and quantitative determination of organic and inorganic compounds that are present in low quantities in complicated matrices. The presence of a considerable quantity of accompanying mono-, oligo- and polymeric compounds can reduce the separation efficiency of any chromatographic method, and they can cause modified retention behavior and asymmetric peak shape. Two basically different methods were developed for the prepurification and preconcentration of solutes of interest: Liquid–liquid and liquid–solid (solid-phase, SPE) extractions. Both methods have advantages and disadvantages: Liquid–liquid extraction is easy to carry out, it does not need complicated instrumentation, however, the efficiency of this method is generally lower than that of SPE [21]. Liquid–liquid extraction gives rise to the production of a considerable amount of toxic waste and is also fairly time-consuming. SPE is a more rapid, more efficient and more solvent-sparing method, but special disposable cartridges are needed, which increases the cost of the analysis.

2.1. Liquid–liquid extraction for gas chromatographic analysis

As phenoxyalkyl acid herbicides are highly polar, they are soluble in water and in aqueous solutions and are less soluble (in their dissociated form) in water-immiscible apolar organic solvents. To overcome this difficulty, the aqueous phase has to be acidified before extraction, to suppress the dissociation of this class of herbicides and to facilitate the transfer of the undissociated molecular species to the organic phase. A wide variety of organic solvents, such as acetonitrile, acetone, diethyl ether and dichloromethane, have been used for the extraction of water-soluble acidic herbicides [22–26], however, the efficiencies of the various extracting solvents have never been compared. As the free phenoxyalkyl acids show very low volatility, their volatility has to be increased by derivatization before analysis by gas chromatography (GC). Derivatization is generally carried out as a separate step after preconcentration

and prepurification of the samples, but some elegant methods are suitable for the simultaneous extraction and derivatization of phenoxyalkyl acids.

Chlorophenoxyacetic acid herbicides [2,4-D (4-(2,4 dichlorophenoxy)butyric acid), 4-chloro-2-methylphenoxyacetic acid (MCPA) and 4-chloro-2-methylphenoxypropionic acid (MCPA or Mecoprop] have extracted from water and sediment using dichloromethane [27]. The pH of the samples was adjusted to pH 1 with 25% (v/v) sulfuric acid before extraction. The dichloromethane was evaporated, the residue was redissolved in 1 ml of acetone and derivatized with 2,3,4,5,6-pentafluorbenzylbromide. The reaction mixture was neutralized with aqueous K_2CO_3, the pentafluorobenzyl esters were extracted with petroleum ether, dried with sodium sulfate and further purified on a Florisil column. The concentrations of the herbicides were determined with GC electron capture detection (ECD). A similar method was employed for the extraction of the herbicides 2,4-D and MCPA from estuarine waters [28]. Water samples were firstly extracted with dichloromethane. This neutral fraction contained the neutral herbicides molinate, trifluralin, atrazine, simazine, alachlor, metolachlor, isoproturon, chlortoluron and linuron. After removing the neutral herbicides, the water sample was acidified with sulfuric acid and extracted again for bentazone, 2,4-D and MCPA. The extracts were analysed by GC with nitrogen–phosphorus detection (GC–NPD), GC–MS and HPLC diode array detection (HPLC–DAD).

Due to the importance of food quality control measurements, a multiresidue method was developed for the determination of chlorophenoxyalkyl acids and pentachlorophenol in fatty and nonfat foods [29]. Dairy products were treated by mixing 100 g of sample with 100 ml of methanol, 10 ml of 10% sulfuric acid, 2 g of sodium- or potassium oxalate and then were extracted with 50 ml of petroleum ether. Animal tissues (50 g) were blended with 50 ml of water, 100 ml of methanol, 10 ml of 10% sulfuric acid and 2 g of sodium- or potassium oxalate. After blending, the apparatus was rinsed with 50 ml of ethyl ether and the combined mixture was extracted with 50 ml of petroleum ether. Fats, shortening, vegetable oils, grains and cereal products, fruits, vegetables, beverages, sugars and high-sugar processed foods, water, legume vegetables and water were treated similarly, taking into consideration the composition of the sample. The organic phase of the extract was mixed with water, saturated NaCl solution and sulfuric acid and extracted twice with petroleum ether. The petroleum ether was evaporated and the residue was used for further GC analyses.

Liquid–liquid extraction has also been used to determine the concentration of phenoxy acid herbicides in soil and cereals [30]. Soil samples were mixed with acidified water and extracted with methylene chloride. The organic phase was extracted with alkaline water (0.05 *M* NaOH). The aqueous phase was acidified and extracted again with methylene chloride. The new extract was dried over anhydrous sodium sulphate and evaporated to dryness before derivatization. Plant samples were homogenized with 0.1 *M* NaOH [31], the extract was mixed with saturated sodium chloride, acidified with sulfuric acid and extracted with diethyl ether. The organic phase was extracted with a 0.5 *M* $NaHCO_3$ solution, the aqueous phase was acidified with sulfuric acid and reextracted with chloroform. The chloroform was dried with anhydrous sodium sulfate and evaporated to dryness. Derivatization was carried out using BF_3–methanol before GC analysis.

A simple method was developed for the simultaneous extraction from soil and methylation of chlorinated phenoxyacetic acids [2,3-D, 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T, MCPA)] before chromatographic analyses [32]. A 5-g amount of soil was mixed with 1.0–1.5 g of water and 0.5 g of 1% sulfuric acid and was extracted with 7 ml of 40% benzyltrimethylammonium derivatives in methanol. The sample was filtered and the liquid phase was concentrated to 1 ml at 35°C. The methyl esters were extracted with 25 ml of *n*-hexane, the hexane phase was concentrated again and purified on a Florisil column.

It has been proven on numerous occasions that liquid–liquid extraction methods are suitable for the extraction and partial purification of these types of herbicides from different matrices containing lipids, carbohydrates, proteins and inorganic constituents. The recoveries of these methods are equal to those of SPE methods, however, they are more time-consuming and are more complicated to perform.

2.2. Solid-phase extraction for GC analysis

Over the last few decades, SPE has found acceptance as a reliable and rapid extraction method and has been used to extract various pesticide residues from water [33,34]. Acidic pesticides can be extracted from water with a RP-18 cartridge [35]. A 1-g amount of support was conditioned with 5 ml of acetone, 5 ml of methanol, 10 ml of distilled water and 5 ml of distilled water that had been acidified to pH 1.5 with HCl and a 1-l water sample, acidified to pH 1.5 with HCl, was percolated at a flow-rate of 8 ml/min. After adsorption of the pesticides, the cartridge was dried in a stream of nitrogen, and the herbicides were eluted with 2 ml of methanol. Acidic esters were hydrolysed before derivatization with chloroformate. The dry extracts were dissolved in 100 μl of acetonitrile–ethanol–water–pyridine (5:2:2:1, v/v) and alkylated with 7 μl of methyl-, ethyl- or butyl chloroformates. The derivatives were dried in a flow of nitrogen and dissolved in 100 μl of toluene for GC separation. A similar extraction procedure was used to concentrate acid herbicides from surface waters [36]. RP-18 cartridges were conditioned with 10 ml of methanol and 10 ml of distilled water that had been acidified to pH 2.4–2.6 with HCl. Water samples (500 ml) were acidified to the same pH and percolated the cartridge at a flow-rate of 15 ml/min. After extraction, the cartridge was dried and then prewashed with 0.75 ml of methanol. Herbicides were eluted with 2×2 ml of methanol. Derivatization was carried out by drying the methanol extract, redissolving the residue in acetone, adding a 10% (v/v) solution of pentafluorbenzyl bromide and cesium carbonate. The mixture was refluxed at 90°C for 15 min and purified on a silica SEP-Pak cartridge. A highly similar extraction and derivatization procedure was employed for the determination of phenoxyalkanoic acids and other acidic herbicides at the low ppt level [37,38].

Although RP-18 supports have been used most frequently for the extraction of phenoxyalkyl acid herbicides, the use of other supports, such as PRP-1 [39] and Wofatit Y 77 [40] has also been reported. A quaternary ion exchanger (QAE Sephadex A-25) has also been used for the extraction of phenoxyacetic acids [41]. The ion exchanger was allowed to swell overnight in distilled water. The pH of the water samples was adjusted to pH>10 with 10 *M* NaOH, and 10 ml was shaken with 3 ml of ion exchanger for a minimum of 2 min. The slurry was centrifuged and the excess water was removed. The slurry was acidified (pH<2) with 9 *M* sulfuric acid and extracted with 3×2 ml of diethyl ether. The diethyl ether was evaporated, the residue was dissolved in 1 ml of 2,2,4-trimethylpentane, 0.5 ml of 2,2,2-trifluoroethanol and 25 μl of sulfuric acid. The mixture was stirred for 60 min at 70°C. After cooling the reaction mixture, 1 ml of 2,2,4-trimethylpentane containing the internal standard, aldrin, and 10 ml of 0.1 *M* phosphate buffer (pH 7) were added. After shaking, the sample was centrifuged and the organic phase was used for GC analysis. Filter disk extraction using RP-18 and polystyrene–divinyl benzene (PS–DVB) resin has also been employed for the extraction of acid herbicides from aqueous samples [42]. To increase the efficiency of extraction, 20% (w/w) anhydrous sodium sulfate was added to the samples and the pH was adjusted to 1.0±0.1 with sulfuric acid. Disks were conditioned with 20 ml of methanol–methyl *tert*.-butyl ether (MTBE) (10:90, v/v), 20 ml of methanol and 20 ml of water. Samples were passed through the disk and were eluted with methanol–MTBE. Derivatization was performed by purging the sample with diazomethane gas. The concentration of analyte was determined by GC. The recoveries and the relative standard deviation (R.S.D.) of the performance of the different disks and salting procedures are compiled in Table 1. The data clearly show that the addition of salt considerably enhances the recovery and decreases the differences between the extraction efficiency of C_{18} and resin disks.

The performances of traditional liquid–liquid extraction, SPE and liquid–solid disk extraction in the preconcentration of phenoxy acid herbicides from water have been compared [43]. Liquid–liquid extraction was carried out by shaking a 1-l water sample, acidified to pH 2, with 2×150 ml of dichloromethane. The volume of each organic phase was reduced to 10 ml under vacuum and the residue was dried over anhydrous sodium sulfate. The solution was further concentrated to 1 ml and 4 ml of a BF_3–methanol solution were added. After a reaction time of 12 h, the mixture was extracted with 2×10 ml of *n*-hexane, dried again with anhydrous sodium

Table 1
C_{18} and resin recoveries and effect of salting

Analyte	Recoveries±R.S.D. (%; n=3)			
	C_{18}[a]	Resin[a]	C_{18}[b]	Resin[b]
Acifluorfen	77±20	82±5	104±5	121±1
Bentazon	0	No data	90±13	71±5
Chloramben	8±11	3±15	72±14	77±7
2,4-D	86±12	83±6	81±8	94±15
Dalapon	0	42±25	12±75	31±30
2,4-DB	81±13	80±14	118±10	130±8
Dacthal	53±17	99±8	67±16	97±5
Dicamba	73±13	71±14	83±3	94±15
3,5-Dicholorobenzoic acid	70±17	76±2	86±25	107±20
Dichloprop	77±11	78±3	85±9	94±10
Dinoseb	72±16	75±5	92±26	85±6
Pentachlorophenol	69±14	70±2	65±15	73±8
Picloram	49±19	74±7	96±24	99±21
2,4,5-T	76±11	75±14	93±10	89±5
Silvex	73±14	74±14	82±9	80±5

[a]Fortified, unsalted reagent water. [b]Fortified reagent water with 20% (w/w) Na_2SO_4.
Reprinted with permission from Ref. [42].

sulfate and concentrated to 100 μl. RP-18 cartridges were conditioned with 10 ml of methanol and 10 ml of distilled water. Water samples (200 ml) were passed through the cartridge at a flow-rate of 4 ml/min. The cartridge was washed with 20 ml of water and dried under a stream of nitrogen. Herbicides were eluted with 20 ml of methanol, the methanol was evaporated and the herbicides were derivatized as described above. The recovery data for liquid–liquid extraction, for SPE and Empore disks are compiled in Table 2. It was concluded from the data that liquid–liquid extraction gave the highest recoveries. The lowest efficacy was with SPE methods and this was tentatively explained by the supposition that the affinity of herbicide to the support surface was not high enough or that they were not entirely eluted from the support using methanol.

Supercritical fluid extraction as an efficient has also found application as a rapid method for the enrichment of chlorophenoxy acid herbicides from both sediments [44] and soil [45]. As carbon dioxide is nontoxic, nonflammable, nonpolluting and relatively cheap, it represents an attractive alternative to other extraction procedures. A rapid method was developed for the simultaneous extraction and derivatization of chlorophenoxy acid herbicides from soil and the efficacy of the various derivatizing agents and experimental conditions were compared [46]. Spiked soil samples were mixed with the derivatizing agents in the extraction vessel. The conditions used for the extraction–derivatization procedure depended on the type of derivatizing agent. Soil samples containing trimethylphenylammonium hydroxide (TMPA), benzyltrimethylammonium chloride (BTMAC), benzyltriethylammonium chloride (BTEAC) and tetrabutylammonium hydroxide/methyliodide (TBA/MI) were extracted at 400 atm at varying extraction temperatures (80, 100°C). Extraction times were 15 min static followed by 15 min dynamic. Extracts were collected in 2 ml of methanol. Soils with added α-bromo-2,3,4,5,6-pentafluorotoluene (PFBBr) and triethylamine (TEA) were extracted at 5400 p.s.i. (at temperatures of 80 and 120°C, for 30 min static and 30 min dynamic). Analytes were collected in a trap packed with octadecylsilica and were removed with 1.5 ml of acetone. The esters were separated and quantitated by GC–ECD and GC–MS. The recoveries obtained using various types of derivatizing agent at different concentrations and temperatures are compiled in Tables 3 to 5. The use of BTMAC and BTEAC resulted in low recoveries and the simultaneous formation of benzyl-, methyl- and ethyl esters was observed, indicating that these derivatizing agents

Table 2
Recovery data (%)

Compound	Liquid–liquid extraction method					
	10 μg/l	5 μg/l	2.5 μg/l	0.5 μg/l	0.1 μg/l	0.05 μg/l
MCPA	98	97	80	78	79	77
2,4-D	95	94	78	72	75	65
Silvex	85	81	90	80	80	71
2,4,5-T	95	95	87	81	77	72
2,4-DB	83	75	70	76	71	70
	SPE method using cartridges					
Compound	10 μg/l	5 μg/l	2.5 μg/l	0.5 μg/l	0.1 μg/l	0.05 μg/l
MCPA	96	85	85	96	67	68
2,4-D	98	93	96	70	68	71
Silvex	86	83	65	71	75	66
2,4,5-T	96	94	85	77	64	63
2,4-DB	96	95	81	65	68	67
	SPE method using Empore disks					
Compound	10 μg/l	5 μg/l	2.5 μg/l	0.5 μg/l	0.1 μg/l	0.05 μg/l
MCPA	98	98	93	76	71	76
2,4-D	92	85	76	72	76	64
Silvex	86	84	82	70	68	66
2,4,5-T	94	93	82	88	70	69
2,4-DB	98	96	85	80	70	68

Reprinted with permission from Ref. [43].

are not suitable for the objectives of the study. The best recoveries were obtained using TMPA (Table 3). Additional experiments proved that the methyl group is derived from the TMPA and not from the alcoholic solvent (Table 4). It was further established that methylation takes place in the injector port, as indicated previously [47]. Recovery using the PFBBr–TEA derivatization process increased with increasing temperature of the reaction and with increasing concentrations of the derivatizing agents (Table 5). TBA–MI has also produced good recovery values, but these are slightly dependent on the type of soil used in the experiments.

The use of up-to-date extraction methods, such as SPE and supercritical fluid extraction, considerably increases the output of pesticide residue analyses. They simplify sample handling and markedly reduce solvent consumption; therefore, more frequent use of them can be expected in the near future.

2.3. Liquid–liquid extraction for HPLC analysis

The methods of sample preparation for HPLC are similar to those used for GC. However, the HPLC analysis of phenoxyalkyl acids does not require a derivatization step, which considerably simplifies the extraction of these herbicides from the accompanying matrices. However, derivatization of phenoxyalkyl acid herbicides for HPLC analysis has also been used to increase the selectivity and sensitivity of the separation process. As pesticide formulations contain active ingredients in considerable quantity, sample preparation is limited to the dissolving of active ingredients in an appropriate solvent or solvent mixture. Thus, formulations containing dicamba, 2,4-D and/or MCPP [2(4 chloro-2-methy(phenoxy)propionic acid] were dissolved in isopropanol–water prior to HPLC analysis [48].

Liquid–liquid extraction methods were employed

Table 3
Percent recoveries of the chlorophenoxy acid herbicides (as methyl esters) from spiked sand by SFE at 80 and 150°C with carbon dioxide using selected derivatizing agents[a]

Compound no.	Compound name	BTMAC[b]	BTEAC[c]	TMPA[d]
Temperature, 80°C				
1	Dicamba	1.8	ND[e]	89.1
2	MCPP	8.8	11.7	71.5
3	MCPA	14.8	ND	85.6
4	2,4-D	15.1	10.4	71.4
5	2,4,5-T	4.3	8.8	ND
6	MCPB	13.1	4.3	79.7
7	2,4-DB	11.0	7.8	68.9
Temperature, 150°C				
1	Dicamba	6.0	ND	73.5
2	MCPP	12.2	21.8	65.7
3	MCPA	27.3	47.9	73.5
4	2,4-D	29.1	39.4	62.9
5	2,4,5-T	27.8	27.2	13.4
6	MCPB[f]	11.5	9.1	95.7
7	2,4-DB	11.4	10.3	79.1

[a]The extraction was performed at 400 atm/80°C/15 min static, followed by 15 min dynamic, with the reagents added to the sample in the extraction vessel. Extraction vessel volume, 10 ml for BTMAC and BTEAC experiments and 2.5 ml for TMPA experiments. The sample size was 2 g and the spike level was 250 μg/g compound. The extracted material was collected in 2 ml of methanol and adjusted to 5 ml for GC–MS analysis. Single determinations.
[b]The volume of reagent was 3 ml of BTMAC solution (40% in methanol).
[c]The volume of reagent was 3 ml of BTEAC solution (40% in methanol).
[d]The volume of reagent was 1 ml of TMPA solution (10% in methanol).
[e]Not detected. Approximate limit was 10 μg/g.
[f]*MCPB*=4-(4-chloro-2-methylphenoxy)butyric acid.
Reprinted with permission from Ref. [46].

Table 4
Percentage recoveries of seven chlorophenoxy acid herbicides from spiked sand by SFE with carbon dioxide and 10% TMPA in methanol, ethanol or 2-propanol[a]

Compound no.	Compound name	TMPA in methanol	TMPA in ethanol	TMPA in 2-propanol
1	Dicamba	89.1	102	73.8
2	MCPP	71.5	52.9	61.0
3	MCPA	85.6	11.1	ND[b]
4	2,4-D	71.4	56.1	39.4
5	2,4,5-T	ND	ND	ND
6	MCPB	79.7	68.3	47.9
7	2,4-DB	68.9	38.4	14.5

[a]The values given are percent recoveries as methyl esters. The extraction was performed at 400 atm/80°C/15 min static, followed by 15 min dynamic, with 1 ml of TMPA solution added to the sample in the extraction vessel (10% methanol, ethanol or 2-propanol). Extraction vessel volume, 2.5 ml. The sample size was 2 g and the spike level was 250 μg/g compound. The extracted material was collected in 5 ml of solvent (methanol, ethanol or 2-propanol). Single determinations.
[b]Not detected. Approximate limit was 10 ng/μl.
Reprinted with permission from Ref. [46].

Table 5
Percentage recoveries of the chlorophenoxy acid herbicides from spiked topsoil by SFE with carbon dioxide and PFBBr–TEA[a]

Compound no.	Compound name	80°C, 250 μl of PFBBr and 50 μl of TEA	120°C, 250 μl of PFBBr and 50 μl of TEA	120°C, 1000 μl of PFBBr and 200 μl of TEA
1	Dicamba	12.2	78.2	82.9
2	MCPP	8.3	28.2	77.1
3	MCPA	3.7	4.3	36.7
4	2,4-D	3.6	1.9	23.7
5	2,4,5-T	6.5	2.8	33.7
6	MCPB	2.5	12.7	38.0
7	2,4-DB	2.9	8.7	32.5
8	Dichlorprop	7.8	22.5	73.7
9	2,4,5-TP	14.3	33.0	80.2

[a]The extractions were performed at 5400 p.s.i./30 min static, followed by 30 min dynamic, with a carbon dioxide flow-rate of 3 ml/min. Extraction vessel volume, 7 ml. The sample size was 5 g, and the spike levels were 1 μg/g for dicamba, MCPP, dichlorprop, and 2,4,5-TP; 2 μg/g for MCPA, 2,4,5-T, MCPB and 2,4-DP; and 3 μg/g for 2,4-D. The extracts were diluted tenfold prior to GC–ECD analysis, except for the experiment performed at 120°C with 1000 μl of PFBBr–50 μl of TEA, for which the dilution factor was 30. Single determinations. Reprinted with permission from Ref. [46].

for the extraction of phenoxy herbicides from water. Thus, a 20-ml water sample was acidified with 100 μl of 35% HCl, and extracted with three times with 2 ml of *n*-hexane ethyl acetate (20:80). The extracts were evaporated to ca. 0.5 ml. The residue was mixed with 10 ml of 0.25 mmol 9-anthryldiazomethane (ADAM) in acetone and allowed to react for 4 h at 40°C in the dark. After derivatization, the solvents were evaporated at 40°C and the fluorescent derivatives were purified on a silica column with *n*-hexane ethyl acetate (95:5, v/v) [49]. The efficiencies of liquid–liquid extraction and SPE for the extraction of chlorophenoxy acid herbicides from water were compared [50]. Water samples (20 ml each) were treated with Na_2SO_3, to remove free chlorine, and then acidified with HCl. Samples were extracted with 3 ml of benzene followed by 4 ml of ethyl acetate–*n*-hexane (8+2 ml). The organic phases were evaporated and derivatized with ADAM, as described above. RP-18 cartridges were conditioned with 3 ml of dichloromethane, methanol and 0.1 *M* HCl. Samples were passed through the cartridge at 4 ml/min and were removed with 0.5 ml of methanol and 3 ml of dichloromethane–methanol (8:2, v/v). The organic phase was evaporated and the residue was derivatized with ADAM. The recoveries achieved with liquid–liquid extraction and SPE, with and without preliminary dechlorination, are compiled in Table 6. It was concluded from the data that both liquid–liquid extraction and SPE can be successfully used for the extraction of phenoxy acid herbicides, with no significant differences being observed between the efficiencies of the extraction methods. It was further established that, in some instances, dechlorination considerably enhanced the efficacy of extraction, therefore, the dechlorination step is highly advocated.

The advantages and disadvantages of the use of liquid–liquid extraction for the HPLC analysis of phenoxyacetic acid derivatives are the same as for GC determination; they can be carried out with traditional laboratory equipment.

2.4. Solid-phase extraction for HPLC analysis

Similar SPE supports to those used for GC analysis were also employed for sample concentration in HPLC. Thus, the use of RP-18 [51], RP-8 [52], porous polymeric sorbent [53] and graphitized carbon black [54] has been reported. RP-18 support was used for the extraction of picloram and 2,4-D from water [55]. The support was conditioned with 5 ml of acetonitrile followed by 10 ml of 4% aqueous acetic acid. Samples were passed through the column at a flow-rate of 10–12 ml/min. Picloram was eluted with 9 ml of 25% acetic acid, and 2,4-D was removed from the support with 4.5 ml of methanol.

The recoveries of phenoxy acid herbicides from

Table 6
Recovery (%) of phenoxy acids and their ethyl esters from tap water processed with chlorine

Form	Phenoxy acid	Without dechlorination[a]		With dechlorination[b]	
		Liquid extraction	C_{18}-SPE	Liquid extraction	C_{18}-SPE
Acid	2,4-D	92–94	98–100	97±4	100±6
	MCPA	93–91	48–71	102±3	96±4
	2,4,5-T	93–98	96–100	98±6	98±6
	2,4-DP	95–98	92–95	99±4	101±4
	MCPP	70–83	29–56	95±4	98±4
	2,4-DB	94–97	92–94	98±4	96±3
	2,4,5-TP	95–98	98–100	93±3	93±3
	MCPB	<5	<5	97±4	95±4
	2,4,5-TB	94–96	94–96	92±4	97±5
Ester	2,4-D	92–96	93–95	99±4	98±3
	MCPA	82–91	64–91	95±4	97±3
	2,4,5-T	96–100	96–100	93±4	97±3
	2,4-DP	91–96	93–97	94±4	95±2
	MCPP	56–84	55–87	97±4	95±2
	2,4-DB	95–100	94–100	99±3	95±3
	2,4,5-TP	96–98	97–98	95±5	97±8
	MCPB	<5	<5	97±5	94±3
	2,4,5-TB	98–99	93–95	97±7	95±8

[a]Water samples contained 0.3–0.4 ppm free available chlorine. Concentrations of acids and esters were both 5 ppb. Each datum is the recovery range of three samples.
[b]Before addition of HCl, dechlorination was performed by the addition of 50 μl of 1% Na_2SO_3. Concentrations of acids and ethyl esters were 0.5 ppb each. Each value is the average of five samples±SD.
Reprinted with permission from Ref. [50].

water samples were compared by using two different RP-18 cartridges (Bakerbond SPE Octadecyl and Separcol SI C_{18}) [56]. Samples were acidified to pH 2.1 with 1 *M* HCl. Supports were conditioned with 2×2 ml of acetone, 2×2 ml of methanol and 2×2 ml of distilled water. Samples were passed through the supports at a flow-rate of 5 ml/min, then the column was washed with 2×2 ml of distilled water and dried. Solutes were eluted with 1 ml of methanol before HPLC analysis. The recoveries showed that both supports can be used for the extraction of phenoxy acid herbicides from water. Slightly different methods were used to condition RP-18 cartridges for the extraction of chlorophenoxy acids from water for HPLC particle beam mass spectrometric analysis [57]. The cartridge was treated with 5 ml of ethyl acetate, 5 ml of methanol, 20 ml of distilled water and 5 ml of water that had been acidified with HCl. After passing the sample through the cartridge, it was washed with 5 ml of acidified water. Analytes were eluted with 2 ml of methanol. The recovery values are compiled in Table 7. The recovery values were acceptable for distilled, deionized water, but they were high in each instance in tap water. This somewhat surprising result was not explained.

A RP-18 precolumn directly connected to the analytical column of a HPLC instrument has also been employed for the SPE extraction of phenoxy acid herbicides from water [58]. The precolumn was conditioned with 4 ml of 0.1 *M* acetic acid, then 2 ml of acidified water was injected into the precolumn, with the injector in the loading position. The water passed on to the waste, and analytes trapped on the precolumn were washed into the analytical column by the mobile phase used for the separation. The recovery values depended on the quantity of sample injected on to the precolumn and on the addition of sodium chloride, as demonstrated in Table 8. It was stated that sodium chloride enhanced recovery due to the 'salting out' effect.

On-line SPE using cartridges or membrane extraction disks offers special advantages over other

Table 7
SPE recovery for chlorophenoxy acids from water, as determined by HPLC–particle beam MS in the methane-enhanced ECNI[a] mode

Spiking level (μg/l)	Recovery (%)			Water
	2,4-D	2,4,5-T	Silvex	
20 (n=6)	89.3±10.5	109.1±15.4	99.5±8.7	DDI[b]
20 (n=6)	109.2±6.9	134.8±25.0	123.8±12.9	Tap
0 (n=3)	0	0	0	DDI[b]
0 (n=3)	0	0	0	Tap

[a]ECNI=Electron-Capture-Negative-Ionization.
[b]Distilled, deionized water.
Reprinted with permission from Ref. [57].

Table 8
Amount of herbicide retained on the 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

Reprinted with permission from Ref. [58].

extraction methods. It is more rapid, the validation parameters (recovery, day-to-day reproducibility, detection limit, etc.) are equal to, or higher than, similar parameters of the other extraction methods. It is highly probable that on-line extraction methods will find growing acceptance and application in the residue analysis of phenoxyacetic acid derivatives and related compounds.

3. Separation and quantitation

Due to their capacity to separate compounds with highly similar molecular structures and to the reliability of the quantitative evaluation, chromatographic methods play an outstanding role in the analysis of alkylphenoxy acid herbicides. Although these compounds can be successfully separated by paper chromatography [59] and thin-layer chromatography (TLC) [60], the low reproducibility attained with these methods has considerably limited their application. The present trends in the use of up-to-date chromatographic techniques in the analysis of herbicides have been discussed previously [61].

3.1. Gas chromatography

Due to its high separation efficiency, GC became the method of choice for the analysis of phenoxyalkyl acid herbicides. As the volatility of these types of compounds is fairly low, various derivatization processes were used to increase the volatility. Methyl esters, which exert the highest volatility, are used preferentially when a flame ionization detector is employed. As ECD is a sensitive method for the detection of halogenated analytes, derivatization for ECD is generally carried out with agents containing many halogens in the molecule. As derivatives with more halogens in the molecule are less volatile than methylated ones, considerably higher temperatures had to be used for the GC analysis of these derivatives.

A wide variety of columns, oven temperatures and coating agents have been used in the analysis of phenoxyalkyl acid herbicides. The use of a temperature gradient is dependent on the character and volatility of the compounds to be separated. Thus, isothermal separation of 2,4-D, mecoprop and MCPA as pentafluorobenzyl esters in water and sediments

was carried out on a 185 cm×2 mm I.D. glass column packed with 3% Silar 10 CP on Glas Chrom Q 100–120 mesh and on-column packed with 1.5% OV-17+1.95% QF-1 [27]. The use of packed columns instead of the more common capillary columns was motivated by the fact that packed columns need a less demanding clean-up procedure than the capillary columns. Nitrogen was used as the carrier gas (15 ml/min). The column, detector and injector temperatures were 200, 250 and 250°C, respectively. The detector was a ^{63}Ni ECD detector. The gas chromatogram of sediment analyzed on a 35 Silar 10 CP column is shown in Fig. 1. The chromatogram indicates that chlorophenoxyacetic acid herbicides can be separated under these conditions. It was established that ghost peaks interfering with the peaks of chlorophenoxy acids were observed on the column packed with 1.5% OV-17+1.95% QF-1, therefore, this column is not suitable for the analysis of these solutes. The chromatogram further indicates that many other compounds were coextracted during the liquid–liquid extraction, resulting in a drifting baseline. The quantitative results compiled in Table 9 support these qualitative conclusions. It was established that the detection limit was sufficiently low for this method to be used for monitoring the concentration of chlorophenoxyacetic acid herbicides in water and in sediments.

Various capillary columns have frequently been used for the separation of phenoxyalkyl acid herbicides. A nonpolar column (OV-101) and a ^{63}Ni ECD detector were employed for the analysis of methylated derivatives of chlorophenoxyalkyl acid herbicides [24]. This method allowed the determination of 2,4-D and 2,4,5-T in fatty and nonfat foods. Recoveries varied between 53 and 75% for 2,4-D at 200 ppb and between 61 and 93% for 2,4,5-T at 80 ppb. A fused-silica capillary column BP-1 (12 m× 0.22 mm I.D.; film thickness, 0.25 μm) was used for the determination of phenoxy acid herbicides as methyl esters in soil and cereals [30]. Helium was employed as the carrier gas. The temperature program was 85°C for 1 min, to 250°C at 25°C/min and held for 5 min. Selected ion monitoring (SIM) chromatograms of soil and plant extracts are shown in Fig. 2. Baseline separation of the chlorophenoxy acid herbicides was achieved in both soil and plant samples, the detection limits being 0.005 and 0.04 μg/g for soils and plants, respectively. The recoveries and R.S.D. values were between 80 and 106% and 1 and 12%, respectively. A different fused-silica capillary column (PTE-5; 30 m×0.25 mm I.D.; film thickness, 0.25 μm) was used for the analysis of methylated chlorophenoxy acid herbicides from soil by GC–MS [46]. Helium was used as the carrier gas. The injector temperature was 250°C, and the oven was programmed from 80 to 140°C at 20°C/min, then to 250°C (5 min hold) at 4°C. Acenaphtene-d_{10} and phenanthrene-d_{10} were used as internal standards.

Fig. 1. Gas chromatogram of the same sediment sample analyzed on 3% Silar 10 CP column (0, solvent; 1, Mecoprop; 2, MCPA traces; 3, 2.4-D traces). (Reprinted with permission from Ref. [27]).

Table 9
Comparison of the results obtained for chlorophenoxyacetic acid herbicides from the samples studied

		1.5% OV-17+1.95%QF-1			Silar 10 CP		
		Mecoprop	MCPA	2,4-D	Mecoprop	MCPA	2,4-D
Water	% of positive						
(26 samples)	samples	100	100	100	0	4	27
	Ranges (μg/l)	0.1–0.8	0.1–0.6	0.1–1.1	n.d.	0.1	0.1–1.0
	Averages (μg/l)	0.5	0.4	0.7	n.d.	0.1	0.5
	Detection limits (μg/l)	0.1	0.1	0.1	0.1	0.1	0.1
Sediments	% of positive						
(28 samples)	samples	89	89	50	0	0	11
	Ranges (ng/l)	n.d.–9.8	n.d.–14.5	n.d.–7.0	0	0	n.d.–3.8
	Averages (n/l)	3.0	4.2	3.7	0	0	2.6
	Detection limits (μg/l)	0.5	0.5	1.0	1.0	1.0	1.5

Reprinted with permission from Ref. [27].

As phenoxyalkyl herbicides are fairly soluble in water, there were many attempts to develop GC methods for their separation and quantitative determination in surface and ground waters. GC–MS and GC–ECD have been used for the detection of thirteen acidic herbicides as methyl-, ethyl- and butyl esters in water [35]. GC–ECD was carried out on a fused-silica capillary column (SE-54; 25 m×0.20 mm I.D.; 0.32 μm film thickness) using helium as the carrier gas. The injector and detector temperatures were 220 and 300°C, respectively. The oven temperature was 100°C for 1 min, increased to 150°C at 30°C/min, held for 2 min, increased to 205°C at 3°C, to 260°C at 10°C/min, then held for 25 min. Gas chromatograms of a ground water sample spiked with 100 ng/l of a mixture of thirteen acidic herbicides derivatized to their methyl, ethyl and butyl esters are shown in Fig. 3. The chromatorams clearly show that no interfering compounds were coextracted with the herbicides, making the method suitable for the analysis of these solutes in water at residue levels of 100 ng/l. GC–MS and GC–ECD were also used for the separation of the pentafluorobenzyl esters of acid herbicides extracted from water [36]. Dichlorobenzoic acid was used as the internal standard. The fused-silica capillary columns used were an Ultra-2 (cross-linked methyl siloxane; 25 m×0.32 mm I.D.; film thickness, 0.17 μm) and a DB-1701 (30 m×0.31 mm I.D.; film thickness, 0.25 μm). The injector and detector temperatures were 230 and 300°C, respectively. The initial column temperature was 60°C for 2 min, increased to 180°C at 25°C/min, held for 1 min, to 205°C at 2°C/min, held for 3 min, to 260°C at 10°C/min and, final, held for 12 min. ECD chromatograms of surface water, with and without spiking, are shown in Fig. 4. It was stated that this method is suitable for the quantitative determination of acid herbicides in surface water, the detection limit being 0.02–0.05 μg/l. A similar GC–MS method was employed for the determination of phenoxyalkanoic acids and other acidic herbicides in water [37]. Separations were carried out on a fused-silica HP-5 capillary column (25 m×0.2 mm I.D.;

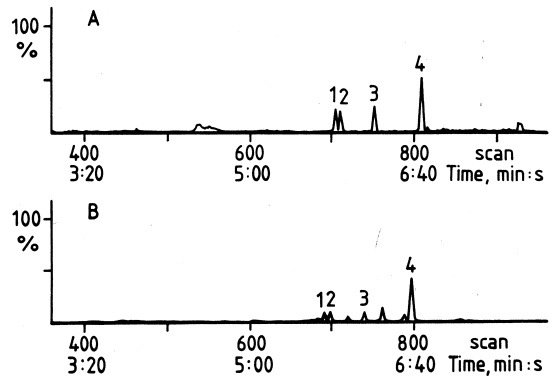

Fig. 2. SIM chromatograms of soil and plant extracts. (A) Soil sample (0.005 μg/g), (B) plant sample (0.04 μg/g). 1=MCPP, 2=MCPA, 3=2,4-D and 4=internal standard (MCPA propyl ester). (Reprinted with permission from Ref. [30]).

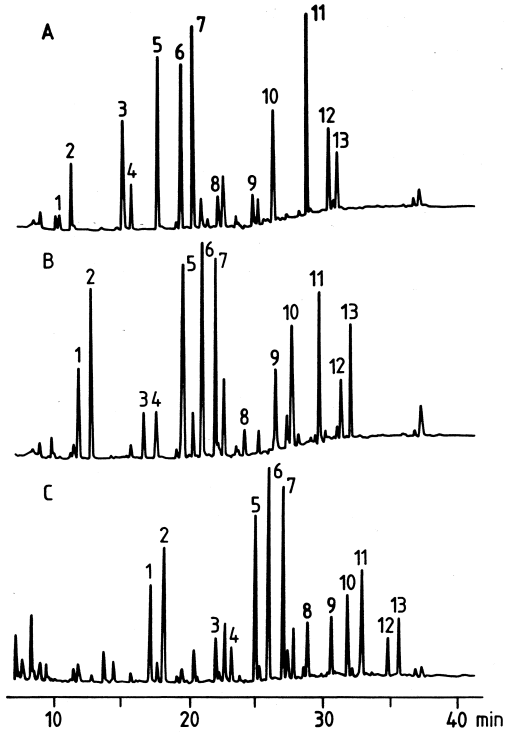

Fig. 3. Gas chromatogram of a ground water sample spiked with 100 ng/l of a mixture of thirteen acidic herbicides derivatized to their (A) methyl, (B) ethyl and (C) butyl esters. Detection was by ECD. Peaks: 1=dichlorobenzoic acid; 2=clopyralid, 3=dichlorprop; 4=2,4-D; 5=triclopyr; 6=fenoprop; 7=2,4,5-T; 8=2,4-DB; 9=picloram; 10=fluazifop; 11=haloxyfop; 12=flamprop and 13=acifluorfen. (Reprinted with permission from Ref. [35]).

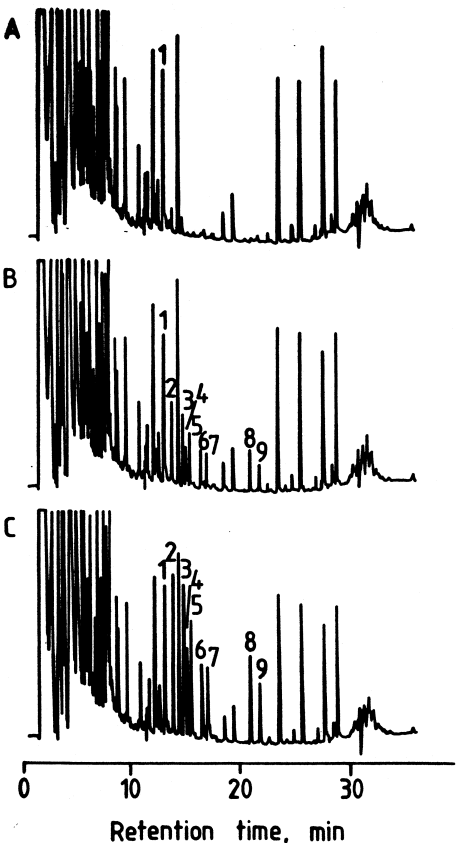

Fig. 4. ECD chromatograms of surface water obtained on an Ultra-2 fused-silica capillary column: (A) surface water; (B) fortified with 0.2 μg/l herbicides; (C) fortified with 0.5 μg/l herbicides. Pentafluorobenzyl (PFB) derivatives of (1) 2,4-dichlorobenzoic acid (internal standard); (2) mecoprop; (3) dicamba; (4) MCPA; (5) dichlorprop; (6) dikegulac; (7) 2,4-D; (8) bentazone and (9) MCPB. (Reprinted with permission from Ref. [36]).

film thickness, 0.33 μm) with a (1.5 m×0.32 mm I.D.; film thickness, 0.17 μm) HP-5 precolumn. Helium was the carrier gas. The initial column temperature was 100°C for 1 min, the temperature was then increased to 150°C at 30°C/min, held for 1 min, to 205°C at 3°C/min, to 260°C at 10°C and, then, held for 23 min. A multiple ion detection (MIP) chromatogram of spiked drinking water is shown in Fig. 5. The chromatogram proves that the herbicides are well separated under the chromatographic conditions employed, therefore, this method can be used for the analysis of drinking- and groundwater samples. The retention times and detection limits in a standard mixture and in spiked tap water samples are compiled in Table 10. The data indicate that the herbicides can be determined in water at detection limits of between 1 and 10 ng/l. The same method has been slightly modified and used for the determination of acid herbicides in water samples with a high matrix content [38].

The 2,2,2-trifluoroethyl esters of phenoxyacetic acid and phenoxypropionic acid herbicides extracted from water were separated and quantitated by GC–ECD (^{63}Ni detector) and GC–MS [41]. Separations were performed on a BP-5 (phenyl methyl silicon) fused-silica capillary column (25 m×0.33 mm I.D.; film thickness, 0.5 μm) using helium as the carrier gas. The injector and detector temperatures were 230 and 320°C, respectively. The initial column temperature was 80°C, this was increased rapidly to 130°C,

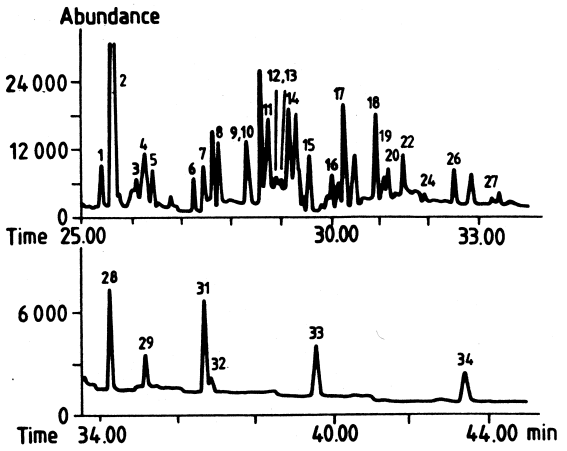

Fig. 5. Multiple ion detection (MID) chromatogram of an extract of a water sample spiked at 10 ng/l. Internal standard (20) added at a concentration of 200 ng/l. The asterisk indicates coeluting 3-indoleacetic acid pentafluorobenzyl ester. Numbers refer to the herbicides listed in Table 10. (Reprinted with permission from Ref. [37]).

then to 225°C at 5°C/min. Chromatograms of a standard mixture and a water sample from a polluted area are shown in Fig. 6. Phenoxyalkyl herbicides are well separated from the coextracted impurities and no interfering peak was observed on the chromatogram. The detection limits of the method were 0.4 and 4 nmol/l for 2,4,5-T and MCPA, respectively. This method was proposed for the analysis of the run-off waters from contaminated areas.

Water samples from the Ebro delta (Spain) were investigated using a different GC–MS method [43]. Methylated derivatives of phenoxy acid herbicides were separated on a DB-5 capillary column (25 m×0.25 mm I.D.; film thickness, 0.25 μm) with helium as the carrier gas. The initial oven temperature was 90°C (held for 0.7 min) and this was then increased to 285°C at 4°C/min. It was stated that this rapid and sensitive GC–MS method is suitable for the separation and detection of phenoxy acid herbicides in superficial water. A similar method was applied for the separation and determination of chlorinated acid herbicides in dechlorinated tap water, biologically active surface water and high humectant ground water [42].

GC–MS [61] and negative ionization GC–MS [62] have also been used for the analysis of chlorophenoxyacetic acids.

Due to its high separation capacity, capillary gas chromatography can be used successfully for the separation and quantitative determination of phenoxyacetic acids. However, it has to be borne in mind that the derivatization step required for the volatilization of this class of compounds necessarily increases the analysis time and introduces a new source of error.

Capillary GC combined with various mass spectrometric methods offers a unique possibility for the safe identification of various phenoxyacetic acid residues. These methods are reliable and may help, not only in the exact determination of pollution levels, but also in providing a better understanding of the fate of these pesticides under different environmental conditions.

3.2. High-performance liquid chromatography

HPLC has also been extensively used for the analysis of phenoxyalkyl acid herbicides and similar compounds. Due to the high degree of flexibility of reversed-phase HPLC (RP-HPLC) systems and to the lower toxicity of the mobile phase, RP-HPLC became a method of choice for the separation of this class of solutes. Many different detection modes have been used for phenoxyalkyl acids in HPLC. Thus, photoconductivity detection [63], simultaneous UV, fluorescence and electrochemical detection [64], and particle beam mass spectrometry [65] were successfully employed for monitoring the HPLC separation of chlorophenoxyacetic acids and other herbicides.

Dicamba, 2,4-D and MCPP were separated in pesticide formulations using a 150×4.6 mm I.D. C_{18} column (particle size, 5 μm). Acetonitrile (A) and 2% aqueous acetic acid (B) were the components of the mobile phase. Gradient elution was initiated with a 25% organic phase for 5 min; this was increased to 40% in 10 min, and held for 5 min. Peaks were detected at 280 nm. Chromatograms of typical formulations are shown in Fig. 7. It was established that possible impurities present in formulations did not interfere with the peaks of the herbicides of interest. The correlation coefficient of the relationship between detector response and the concentration of herbicide was, in each instance, over 0.9996, showing the good linearity of the correlation. The

Table 10
Detection limits (DL) of the target compounds as their pentafluorobenzyl derivatives in a standard mixture and in spiked tap water samples

No.	Compound (min)	t_R (pg)	DL (standard) (ng/l)[a]	DL (SPE)
1	Clofibric acid	25.42	2	<1
2	2,4-Dichlorbenzoic acid	25.65	4	<1
3	Clopyralid	26.18	20	<10
4	4-Chlorophenoxyacetic acid	26.28	10	<10
5	Mecoprop	26.43	10	<1
6	Dicamba	27.28	2	<1
7	MCPA	27.48	2	<1
8	Dichlorprop	27.79	1	<1
9	Chlorfurenol methyl[a]	28.31	10	<10
10	Flurenol butyl[b]	28.40	10	<10
11	2,4-D	28.72	20	<5
12	Chlorfenac	28.93	20	<10
13	Bromoxynil	29.07	20	<10
14	1-Naphthylacetic acid	29.17	2	<5
15	Triclopyr	29.56	2	<1
16	Fenoprop	30.03	2	<5
17	Fluazifop-*p*-butyl[b]	30.28	1	<1
18	Flamprop-isopropyl[b]	30.92	3	<1
19	2,4,5-T	31.12	20	<10
20	Bentazone	31.17	20	<10
21	3-Indolylacetic	acid	31.45	10
22	MCPB	31.54	20	<10
23	Chloramben	31.75	20	<25
24	Fluroxypyr	31.94	30	<10
25	Flurenol	32.15	30	<25
26	2,4-DB	32.87	30	<10
27	3-Indolylpropionic acid	33.28	10	–
28	Fluazifop	34.34	5	<1
29	Benazolin	35.22	10	<10
30	Picloram	35.48	30	–
31	Haloxyfop	36.85	10	<1
32	3-Indolybutyric	36.91	10	–
33	Flamprop	39.64	2	<1
34	Acifluorfen	42.96	100	<10

[a]Detection limits are not listed for compounds with unsatisfactory recoveries.
[b]Other esters.
Reprinted with permission from Ref. [37].

within-day and between-day precision of the method were 0.1–3.2% and 0.6–5.5%, respectively. The method was proposed for the analysis of pesticide formulations containing, simultaneously, dicamba, 2,4-D and MCPP. Nonderivatized picloram and 2,4-D were separated on a C_{18} column (150×4.6 mm I.D., 5 μm) using 4% aqueous acetic acid–acetonitrile (95:5, v/v and 60:40, v/v) mixtures for the elution of picloram and 2,4-D, respectively [55]. MCPA, MCPP, 2,4-D and 2,4,5-T were successfully separated on a C_{18} column using an isocratic eluent (methanol–0.01 *M* acetic acid, 61:39, v/v), as illustrated in Fig. 8. The flow-rate was 0.35 ml/min and the phenoxyalkyl acid herbicides were detected at 235 nm. No interference from the coextracted compounds was observed and the detection limit was 0.03 μg/l. The method was proposed for the analysis of drinking water. Another RP-HPLC method used a water–methanol (41:59, v/v) eluent containing 0.08% (v/v) trifluoroacetic acid (TFA) [34]. The use of TFA made the detection of herbicides at 230 nm possible, which considerably increased the sensitivity

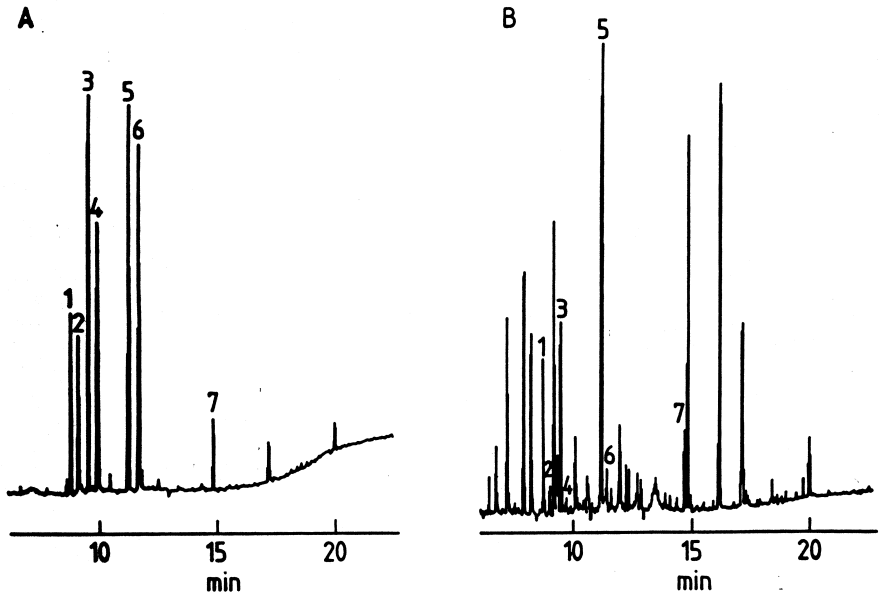

Fig. 6. Chromatogram from a standard mixture (A) and a water sample (B) analyzed as described. The sample is run-off from a polluted area that passed into a sewage plant. 1=MCPP; 2=MCPA; 3=2,4-D; 5=2,4,5-TP; 6=2,3,5-T and 7=aldrin. (Reprinted with permission from Ref. [41]).

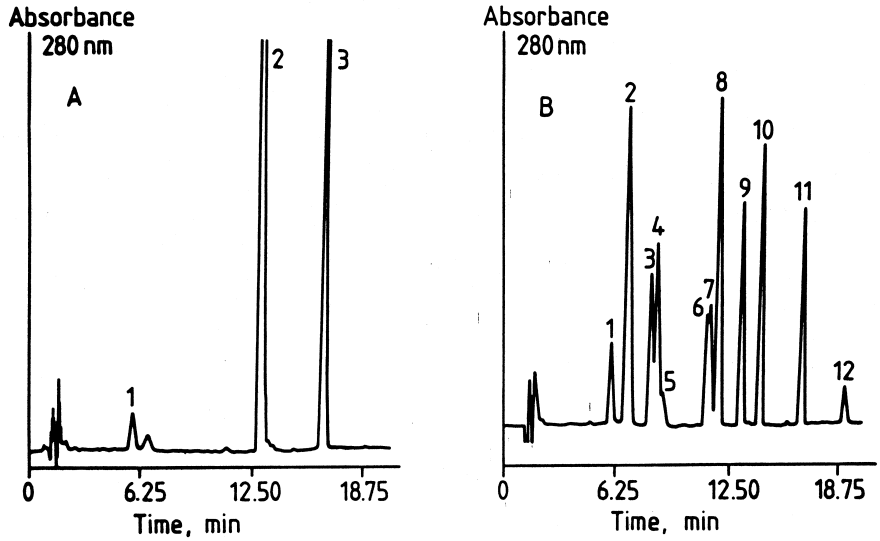

Fig. 7. (A) Chromatogram of a typical formulation containing dicamba at 2.5% (1), 2,4-D at 26.4% (2) and MCPP at 14.7% (3). (B) Separation of dicamba [10; 2,4-D (9) and MCPP (11)] from impurities that may exist in formulations containing these three herbicides. 2=*o*-chlorophenoxyacetic acid+*o*-chlorophenol; 3=*p*-chlorophenoxyacetic acid; 4=*p*-chlorophenol; 5=2,6-dichlorophenoxyacetic acid; 6=2-methylphenoxypropionic acid; 7=4-chlorophenoxypropionic acid; 8=2,6-dichlorophenol; 10=2,4-dichlorophenol; 12=2-methyl-4,6-dichlorophenoxypropionic acid. (Reprinted with permission from Ref. [48]).

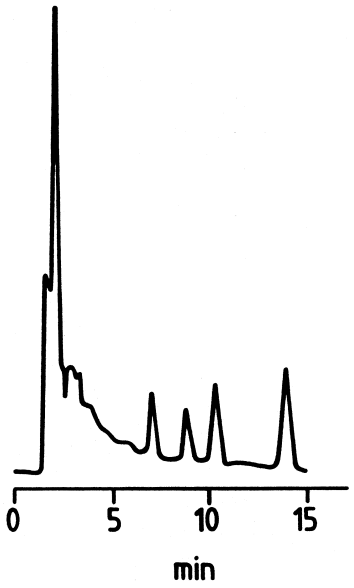

Fig. 8. HPLC of 2.5 μg of each phenoxyacid extracted from 300 ml of tap water using an optimized method on Separon SGX C_{18} with a mixture of methanol and 0.01 mol l^{-1} acetic acid (61:39, v/v) as the mobile phase. Phenoxy acids were eluted in the following order: (1) 2,4-D (7.10 min), (2) MCPA (8.80 min), (3) 2,4,5-T (10.23 min) and (4) MCPP (13.88 min). (Reprinted with permission from Ref. [56]).

of the method. The flow-rate was set to 1.5 ml/min. A typical chromatogram is shown in Fig. 9. The detection limit (signal-to-noise ratio=3) varied between 0.3 and 1.2 ng, indicating that the method can detect herbicides at levels below 0.1 μg/l. A microbore C_{18} column (300×2.1 mm I.D.) coupled with a particle beam mass spectrometer was also used for the separation of 2,4-D, 2,4,5-T and Silvex [57]. The mobile phase consisted of water–methanol (70:30, v/v) containing 1.7 ng/μl phenoxyacetic acid and 1% acetic acid. Phenoxyalkyl acid herbicides were separated in 10 min, as demonstrated in Fig. 10.

The detection limit of the method was in the low μg/l range for each herbicide. A C_{18} column (250× 3.2 mm I.D.; particle size, 5 μm) and 0.1 *M* acetic acid–methanol (50:50, v/v) were used for the separation of phenoxy acid herbicides (flow-rate, 1–1.2 ml/min; detection wavelength, 240 or 280 nm) [58]. This method was used successfully for the determination of 2,4-D (k'=1.8), MCPA (k'=2.4), dichlorprop (k'=3.4) and mecoprop (k'=4.3) in water and in airborne dust (Fig. 11). An interesting method

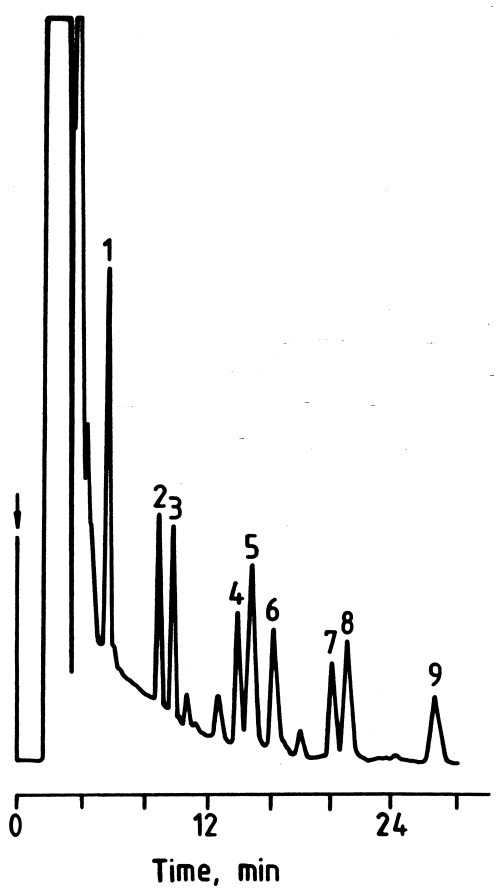

Fig. 9. Chromatogram obtained on sampling 100 ml of drinking water spiked with 0.3 μg/l concentrations of each herbicide. 1=Dicamba; 2=2,4-D; 3=MCPA; 4=2,4-DP; 5=MCPP; 6= 2,4,5-T; 7=2,4-DB; 8=MCPB and 9=2,4,5-TP. (Reprinted with permission from Ref. [34]).

was developed for the separation and indirect detection of 2,4-D, 2,4-DB and 2,4,5-T by adding iron(II)1,10-phenanthroline to the mobile phase [66]. It was assumed that the additive interacts with the chlorophenoxyalkyl acids by an ion-interaction mechanism. This interaction makes the detection of chlorophenoxyalkyl acids at 510 nm (absorption maximum of the additive) possible. Good separation of these solutes was achieved on a RP-2 column with methanol–water (40:60, v/v) as the eluent, containing 34 μM of additive.

Although the separation of phenoxyalkyl acids has considerable practical and theoretical importance, the number of papers dealing with the relationship

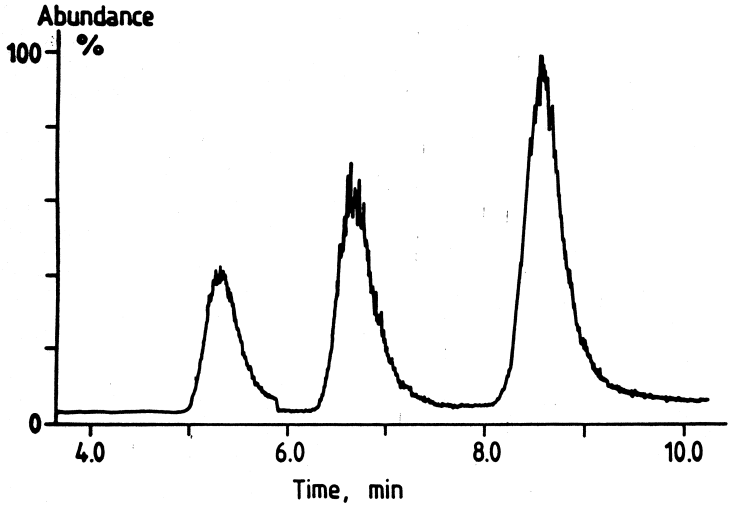

Fig. 10. Chromatogram obtained from HPLC–particle beam MS of 2,4-D, 2,4,5-T and Silvex in methane-enhanced electron-capture negative ionization mode under SIM conditions. (Reprinted with permission from Ref. [57]).

between retention characteristics and the molecular structure of solutes is surprisingly low. The retention of twelve phenoxyacetic acid derivatives was determined on a porous graphitized carbon column and the correlation between molecular structure and retention behaviour was elucidated [67]. It was established that the number of substituents on the benzene ring, their lipophilicity and the pH of the mobile phase exerted the highest impact on the retention.

Derivatization has also been used in the HPLC analysis of phenoxy acid herbicides. As the detection limit of solutes with a fluorescence chromophore in the molecule is significantly lower than that of the original compound, derivatization with a fluorescence agent considerably decreases the detection limit. Thus, ADAM was employed for the derivatization of 2,4-D, MCPA, MCPP and MCPB [49]. The fluorescence derivatives were separated on a TSK-gel ODS12T (250×4.6 mm I.D.) column using a mobile phase containing acetonitrile–water (75:25, v/v) with 3% tetrahydrofuran. The flow-rate was set to 1.0 ml/min. The excitation and emission wavelengths of the fluorescence detector were set to 365

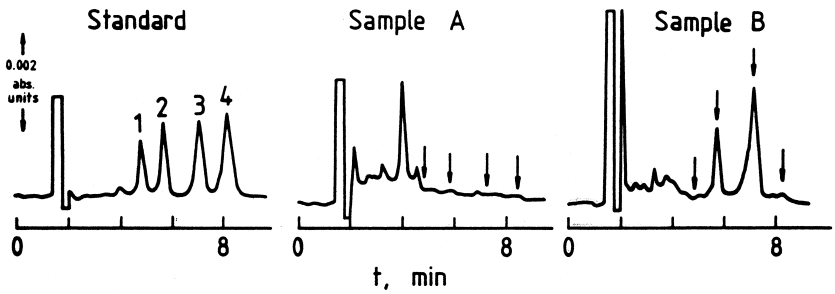

Fig. 11. Chromatograms of samples from air sampling of phenoxy acid herbicides. Peaks in the standard: 1=2,4-D, 0.10 μg/ml; 2=MCPA, 0.10 μg/ml; 3=dichlorprop, 0.10 μg/ml and 4=mecoprop, 0.13 μg/ml. No herbicides were detected in Sample A. Sample B: MCPA, 0.09 μg/ml; dichlorprop, 0.14 μ/ml and mecoprop, 0.009 μg/ml. (Reprinted with permission from Ref. [58]).

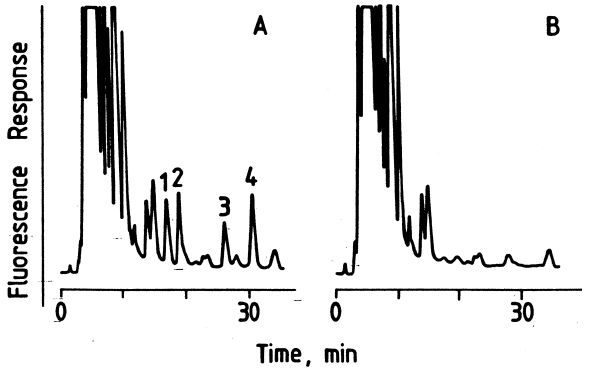

Fig. 12. Chromatograms of ground water extracts treated with 9-anthryldiazomethane. (A) Ground water sample spiked with 0.5 μg/l concentrations of phenoxy acid herbicides. Peaks: 1=2,4-D; 2=MCPA; 3=MCPP and 4=MCPB (Reprinted with permission from Ref. [49]).

and 412 nm, respectively. Typical chromatograms of spiked and unspiked water samples are shown in Fig. 12. The chromatograms clearly show that the peaks of coextracted impurities are well separated from those of the herbicides, indicating that the method can be safely used for monitoring water purity. The detection limit was 0.5 μg/l. The method was extended for the determination of other chlorophenoxy acid herbicides in water [50]. The same chromatographic system was used as in the previous study but the mobile phase was slightly modified (acetonitrile–water, 3:1, v/v). Some chromatograms, characterizing the separation capacity of the method, are shown in Fig. 13. Due to its very low detection limit, the method was proposed for the analysis of chlorophenoxy acid herbicides in drinking water.

As has been previously proved, both GC and HPLC methods have been successfully used for the separation and quantitative determination of phenoxyalkyl acid herbicides in different matrices. Surprisingly, the efficiencies of the methods have not been compared frequently. The separation characteristics of GC–MS and RP-HPLC for the analysis of chlorinated phenoxyacetic acids in soil were determined [32]. GC separation of the methyl esters of the herbicides was performed on a HP-5 cross-linked 5% phenylmethylsilicone capillary column (25 m×0.2 mm I.D.; film thickness, 0.33 μm) using a temperature program. RP-HPLC separation was carried out

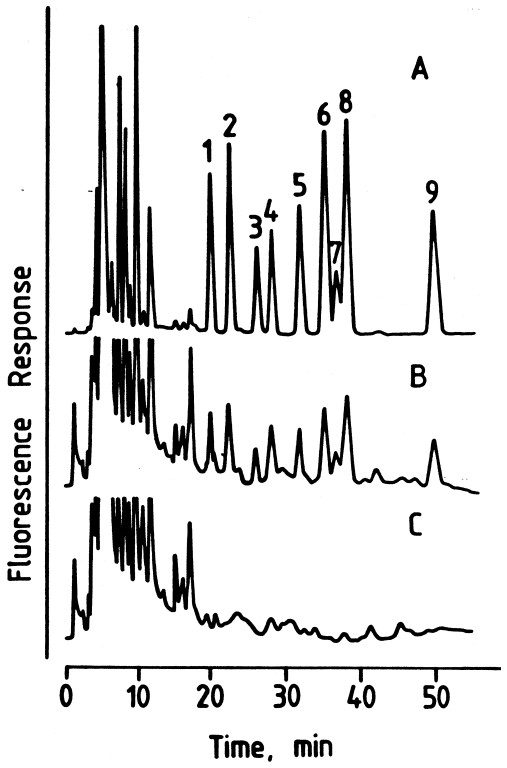

Fig. 13. Liquid chromatograms of ADAM-derivatized phenoxy acids. (A) Working standard solution: 1=2,4-D; 2=MCPA; 3=2,4,5-T; 4=2,4-DP; 5=MCPP; 6=2,4-DB; 7=2,4,5-TP; 8=MCPB and 9=2,4,5-TB (20 ng each per injection). (B) Groundwater sample spiked with 0.5 μg/l phenoxy acids. (C) Blank groundwater sample. (Reprinted with permission from Ref. [50]).

on a C_{18} column (150×4.6 mm I.D.) using methanol–water (70:30, v/v) as the mobile phase and UV detection at 220 nm. Both methods adequately separated the methyl esters of herbicides, as demonstrated in Fig. 14. The time of the separation was very similar both for GC and HPLC. It was concluded that the sample preparation is less tedious for HPLC, however, GC gave rise to better resolution and offered a unique possibility for the safe identification of the solutes.

Although various HPLC methods have also been used frequently for the separation and quantitative determination of phenoxyacetic acid herbicides, their separation capacity and the possibility of identification were not as high as for the capillary GC–MS methods.

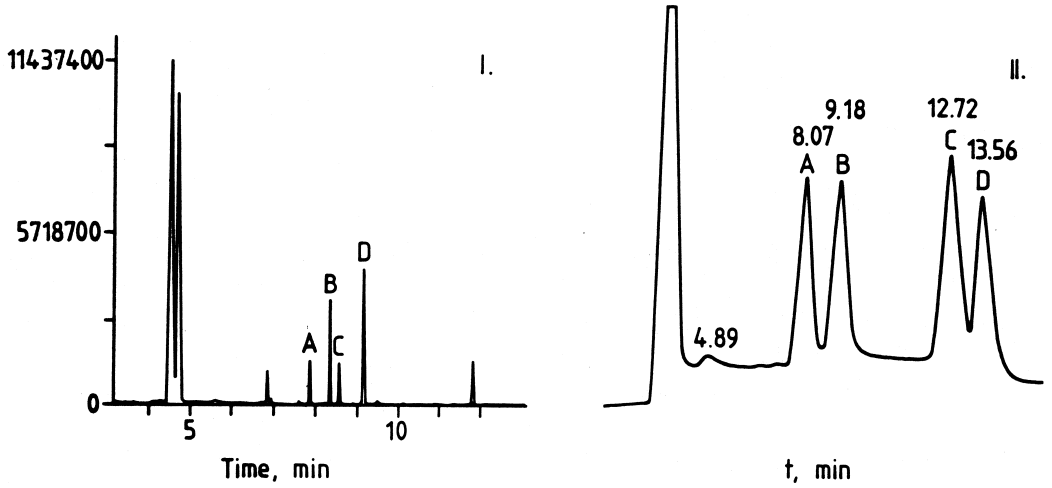

Fig. 14. (I) Gas chromatogram for a soil extract. A=MCPPME; B=2,4-DME; C=2,3-DME and D=2,4,5-TME. (II) LC of the four chlorophenoxyacetic acid methyl esters in soil. A=2,3-DME; B=2,4-DME; C=MCPPME and D=2,4,5-TME. (Reprinted with permission from Ref. [32]).

3.3. Capillary zone electrophoresis and micellar electrokinetic chromatography

CZE is a rapid and effective method for the separation of a wide variety of compounds. However, due to its lower sensitivity, CZE has not been used frequently in environmental analysis. To date, the separation of 2,4-D, 2,4,5-T and other herbicides was reported [68]. CZE has also been employed for the separation of phenoxy acid herbicides and their impurities as well for their chiral separation [69]. CZE was carried out on a fused-silica capillary (79.5 cm, 63.1 cm from the injector to the detector×50 μm I.D.) at +30 kV and 30°C. Solutes were detected at 200 nm. Buffers were aqueous solutions of lithium acetate (30 or 50 m*M*), with the pH adjusted to 4.80 with concentrated acetic acid. Cyclodextrins used for the chiral separations were dissolved in the buffer. Electropherograms of the separation of phenoxy acid herbicides and impurities are shown in Fig. 15. The relative standard deviation of the migration time and electrophoretic mobility were both less than 1%, indicating the good repeatability of the method. The detection limit was $1 \cdot 10^{-5}$ *M* at $S/N=2$. The addition of cyclodextrins markedly modified the retention of phenoxy acid herbicides and makes possible the chiral separation of the compounds, as demonstrated in Fig. 16. It was concluded from the

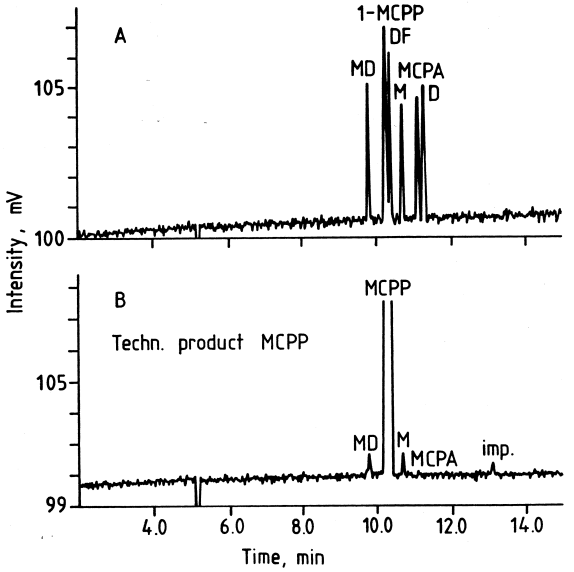

Fig. 15. Separation of (A) phenoxy acid herbicides and related impurities and (B) a real MCPP production sample by CZE using 50 m*M* lithium acetate buffer (pH 4.80) and +30 kV. Detection by UV absorbance at 200 mm (1 mAU/mV). Note that MCPP and i-MCPP co-migrate under these conditions. MD=2-(2-methyl-4,6-dichlorophenoxy)propionic acid; DP=2-(2,4-dichlorophenoxy)propionic acid; M=2-(2-methylphenoxy)propionic acid; D=2,4-dichlorophenoxyacetic acid. (Reprinted with permission from Ref. [69]).

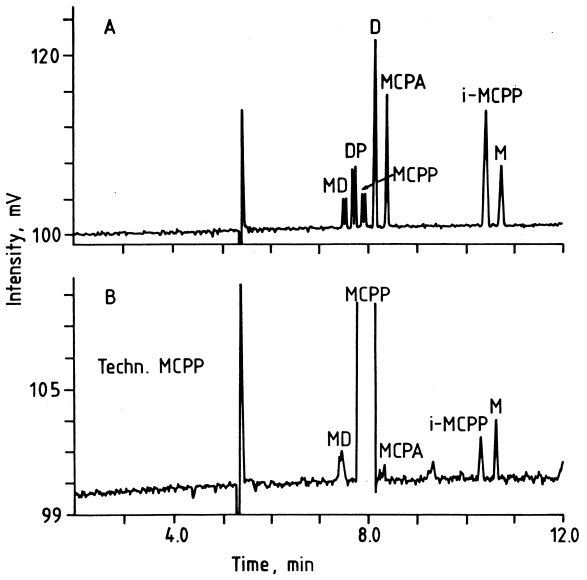

Fig. 16. Separation of (A) phenoxy acid herbicides and related impurities and (B) a real MCPP production sample by cyclodextrin (CD)-modified CZE using 50 m*M* lithium acetate buffer (pH 4.80) with the addition of 10 m*M* α-CD. MD=2-(2-methyl-4,6-dichlorophenoxy)propionic acid; DP=2-(2,4-dichlorophenoxy)propionic acid; M=2-(2-methylphenoxy)propionic acid; D=2,4-dichlorophenoxyacetic acid. (Reprinted with permission from Ref. [69]).

data that this CZE method leads to good separation of the phenoxy acid herbicides, it that it is stable, precise and shows good linearity.

The effect of nonionic (Brij 35), anionic [sodium dodecylsulfate (SDS)] and cationic [cetyltrimethylammonium bromide (CTAB)] surfactants on the micellar electrokinetic capillary chromatographic (MECC) separation of herbicides was studied in detail [70]. Separations were performed on fused-silica capillary columns (50 μm I.D.; total length 44 cm, effective length 37 cm) at 15 kV and a constant temperature of 25°C using multi-wavelength detection. It was established that the best separation can be achieved by the simultaneous addition of SDS, Brij 35 and methanol to the 0.02 *M* aqueous phosphate buffer. The R.S.D. values for migration time and peak area were about 1 and 2%, respectively.

The number of papers dealing with the CZE and MECC analysis of phenoxyacetic acid herbicides and related compounds is relatively low. Due to their high theoretical plate number and simplicity, both CZE and MECC can be successfully used in the future as alternatives to GC and HPLC.

4. Conclusions

The production and world-wide use of phenoxy acid herbicides is growing continually. As these herbicides in soil, and in ground and surface water can cause environmental pollution, accurate monitoring of them becomes more and more important. Various chromatographic methods, especially hyphenated ones such as GC–MS and HPLC–MS, offer a unique possibility for the separation and determination of these herbicides at very low concentrations. The high separation power of CZE and MECC has not been explored entirely in this interesting field of analysis, but use of them in the future is expected. The development of new, more selective supports for SPE, the use of microcolumn techniques and effective derivatization processes may further reduce the detection limit and will represent an exciting challenge for the chromatographers working in the area of herbicide residue analysis.

Acknowledgements

This work was supported by the grant OTKA T 023422.

References

[1] G.A. Best, A.D. Turhven (Editors), Pesticides — Developments, Impacts, and Control, Royal Society of Chemistry, Cambridge, 1995.
[2] J. Hajslová, L. Ryparová, I. Viden, J. Davidek, V. Kocourek, I. Zemanová, Int. J. Environ. Anal. Chem. 38 (1990) 105.
[3] C. Schlett, Z. Wasser-Abwasser-Forsch. 23 (1990) 32.
[4] A.G. Ogg Jr., F.L. Young, Weed Technol. 5 (1991) 291.
[5] D.C. Heering, T.F. Peeper, Weed Technol. 5 (1991) 317.
[6] G.W. Stratton, Rev. Environ. Toxicol. 3 (1987) 71.
[7] F. Leganés, E. Fernádez-Valiente, Arch. Environ. Contam. Toxicol. 22 (1992) 130.
[8] F. Leganés, E. Fernádez-Valiente, Arch. Environ. Contam. Toxicol. 22 (1992) 215.
[9] A.C. York, J.W. Wilcut, M.M. Keene, F.R. Walls Jr., Weed Technol. 5 (1991) 43.
[10] B.J. Johnson, T.R. Murphy, Weed Technol. 5 (1991) 607.

[11] M.H. Moreland, Ann. Rev. Plant Physiol. 31 (1980) 597.
[12] H.-B. Lee, T.E. Peart, J.M. Carron, H. Tse, J. Assoc. Off. Anal. Chem. 74 (1991) 835.
[13] R.D. DeLaune, L.M. Salinas, Bull. Environ. Contam. Toxicol. 35 (1985) 564.
[14] B.J. Finlayson, K.M. Verrue, Arch. Environ. Contam. Toxicol. 14 (1985) 153.
[15] J. Stenström, Toxicity Assessment 4 (1989) 405.
[16] R.W. Bovey, A. Youg, The Science of 2,4-D and Associated Phenoxy Herbicides, John Wiley, New York, 1980, p. 301.
[17] P. Vineis, B. Terracini, G. Ciccone, A. Cignetti, E. Colombo, A. Donna, L. Maffi, R. Pisa, P. Ricci, E. Zanini, P. Comba, Scand. J. Work Environ. Health 13 (1986) 9.
[18] E. Lynge, Br. J. Cancer 52 (1985) 259.
[19] R.D. Hood, B.L. Patterson, G.T. Thacker, G.L. Sloan, G.M. Szczech, J. Environ. Sci. Health C13 (1979) 189.
[20] R.B. Geerdink, P.G.M. Kienhuis, U.A.Th. Brinkman, J. Chromatogr. 647 (1993) 329.
[21] J. Rydberg, C. Musikas, G.R. Choppin (Editors), Principles and Practices of Solvent Extraction, Marcel Dekker, New York, 1992.
[22] R. Punkayastha, J. Agric. Food Chem. 22 (1974) 453.
[23] S.U. Khan, J. Assoc. Off. Anal. Chem. 58 (1975) 1027.
[24] D.W. Woodham, G. Mitchell, C.D. Loftis, C.W. Collier, J. Agric. Food. Chem. 19 (1971) 186.
[25] G.R. Leather, L.E. Forrence, J. Environ. Anal. 11 (1982) 345.
[26] A.E. Smith, J. Assoc. Off. Anal. Chem. 67 (1984) 794.
[27] S.M. Waliszewski, V.T. Pardo Sedas, Int. J. Environ. Anal. Chem. 49 (1992) 231.
[28] G. Durand, V. Bouvot, D. Barceló, J. Chromatogr. 607 (1992) 319.
[29] M.L. Hopper, B. McMahon, K.R. Griffitt, K. Cline, M.E. Fleming-Jones, D.C. Kendall, JAOAC Int. 75 (1992) 707.
[30] C. Sánchez-Brunete, A.I. Garcia-Valcárcel, J.L. Tadeo, J. Chromatogr. A 675 (1994) 213.
[31] A.J. Cessna, Pestic. Sci. 30 (1990) 141.
[32] L. Chiang, R.J. Magee, B.D. James, Anal. Chim. Acta 255 (1991) 187.
[33] S.H. Hoke, E.E. Bruggemann, L.J. Baxter, T. Trybus, J. Chromatogr. 357 (1986) 429.
[34] A. DiCorcia, M. Marchetti, R. Samperi, Anal. Chem. 61 (1989) 1363.
[35] S. Butz, H.-J. Stan, J. Chromatogr. 643 (1993) 227.
[36] M. Vink, J.M. van der Poll, J. Chromatogr. A 733 (1996) 361.
[37] S. Butz, Th. Heberer, H.-J. Stan, J. Chromatogr. A 677 (1994) 63.
[38] Th. Heberer, S. Butz, H.-J. Stan, Int. J. Environ. Anal. Chem. 58 (1995) 43.
[39] I. Liska, E.R. Brouwer, A.L. Ostheimer, H. Lingeman, U.A.Th. Brinkman, Int. J. Environ. Anal. Chem. 47 (1992) 267.
[40] W. Dedek, K.D. Wenzel, H. Oberländer, B. Mothes, J. Männig, Fresenius' Z. Anal. Chem. 339 (1991) 201.
[41] M. Adolfsson-Erici, L. Renberg, Chemosphere 23 (1991) 845.
[42] J. Hodgeson, J. Collins, W. Bashe, J. Chromatogr. A 659 (1994) 395.
[43] R. Alcaraz, J. Caixach, J. Garcia-Raurich, I. Espalader, J. Rivera, Quim. Anal. 13 (1994) 55.
[44] S.B. Hawthorne, D.J. Miller, D.E. Nivens, D.C. White, Anal. Chem. 64 (1992) 405.
[45] E.A. Rochette, J.B. Harsh, H.H. Hill Jr., Talanta 40 (1993) 147.
[46] V. Lopez-Avila, N.S. Dodhiwala, W.F. Beckert, J. Agric. Food Chem. 41 (1993) 2038.
[47] I. Brondz, I. Olsen, J. Chromatogr. 598 (1992) 309.
[48] R.D. Larson, J.E. Houglum, J. Assoc. Off. Anal. Chem. 74 (1991) 679.
[49] T. Suzuki, S. Watanabe, J. Chromatogr. 541 (1991) 359.
[50] T. Suzuki, S. Watanabe, J. Assoc. Off. Anal. Chem. 75 (1992) 720.
[51] P.R. Loconto, J. Liq. Chromatogr. 14 (1991) 1297.
[52] E.R. Bogus, T.L. Watschke, R.O. Mumma, J. Agric. Food Chem. 38 (1990) 142.
[53] R.B. Geerdink, A.M.B. Graumans, J. Viveen, J. Chromatogr. 547 (1991) 478.
[54] A. DiCorcia, M. Marchetti, Anal. Chem. 63 (1991) 580.
[55] M.J.M. Wells, J.R. Michael, Anal. Chem. 59 (1987) 1739.
[56] J. Frébertová, V. Tatarkovicová, Acta Univ. Palack. Olomuc. Fac. Rer. Nat. Chemica XXXII 112 (1993) 63.
[57] M.J.I. Mattina, J. Chromatogr. 542 (1991) 385.
[58] M. Akerblom, J. Chromatogr. 319 (1985) 427.
[59] H.S. Rathore, T. Begum, J. Planar Chromatogr. 4 (1991) 451.
[60] K. Erne, Acta Vet. Scand. 7 (1966) 77.
[61] H.-J. Stan, Th. Heberer, J. Chromatogr. A 653 (1993) 55.
[62] H.D. Meiring, G. den Engelsman, A.P.J.M. de Jong, J. Chromatogr. 644 (1993) 357.
[63] C.J. Miles, M.J. Zhou, J. Agric. Food Chem. 38 (1990) 986.
[64] W. Schüssler, Chromatographia 29 (1990) 24.
[65] I.S. Kim, F.I. Sasinos, R.D. Stephens, J. Wang, M.A. Brown, Anal. Chem. 63 (1991) 819.
[66] M. Fayyad, M. Alawi, T. El-Ahmad, J. Chromatogr. 481 (1989) 439.
[67] T. Cserháti, E. Forgács, J. Chromatogr. 643 (1993) 331.
[68] S.K. Yeo, H.K. Lee, S.F.Y. Li, J. Chromatogr. 594 (1992) 335.
[69] M.W.F. Nielen, J. Chromatogr. 637 (1993) 81.
[70] Q. Wu, H.A. Claessens, C.A. Cramers, Chromatographia 34 (1992) 25.