Journal of Chromatography, 637 (1993) 81–90
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 899

(Enantio-)separation of phenoxy acid herbicides using capillary zone electrophoresis

M.W.F. Nielen

Akzo Research Laboratories Arnhem, Corporate Research, Analytical and Environmental Chemistry Department, P.O. Box 9300, 6800 SB Arnhem (Netherlands)

(First received November 24th, 1992; revised manuscript received January 7th, 1993)

ABSTRACT

Capillary zone electrophoretic (CZE) methods were developed for the separation of phenoxy acid herbicides and related impurities (including positional isomers) originating from production processes. In addition, chiral separations of phenoxypropionic acid herbicides were achieved by adding a suitable cyclodextrin-type chiral selector to the electrophoresis buffer. The selectivity of the separation can be controlled and fine-tuned by using different types of cyclodextrins. The presence of specific impurities could be confirmed after comparison of the electropherograms obtained with CZE systems having different selectivities. The CZE method shows good precision, linearity and long-term stability. The methods developed have been successfully applied to the analysis of real production samples and the determination of their enantiopurity. Both the identification and determination of the impurities compare with the results obtained with chromatographic methods. The CZE methods, however, are more flexible and simpler and will be more economical. The methods have potential for automated quality control assays.

INTRODUCTION

Phenoxy acids are widely used in agriculture as selective herbicides. Formulations of these herbicides can be analysed by capillary gas chromatography (GC) [1], but derivatization (esterification) of the carboxyl group will be required prior to the analysis. Liquid chromatography (LC) [1] can be applied directly but might not provide sufficient resolution for the determination of all related impurities. Trace analyses for these herbicides in environmental samples require preconcentration methods in order to meet the stringent requirements of the tolerance levels in, *e.g.*, drinking and ground water [2,3]. Phenoxypropionic acid herbicides are racemic mixtures and only the *d*-isomers are the active ingredient. Chiral separations of these herbicides are required in order to assess the enantiopurity of formulations and to optimize enantioselective production processes. LC using a chiral stationary phase can be applied for these purposes. The main disadvantages of LC approaches using chiral stationary phases are that the columns are relatively expensive, the performance is often much lower than that with regular LC columns and many different types of these LC columns are required in order to cover a relatively narrow range of racemic compounds.

Capillary zone electrophoresis (CZE) offers rapid and efficient separations of ionic and ionizable compounds [4,5]. Initially, CZE was applied mainly in biochemical analyses but in recent years its applicability has been demonstrated in all fields of chemical analysis including the determination of positional isomers in industrial products [6–9]. In addition, CZE has proved to be a very powerful and flexible technique for the separation of stereoisomers [10–15]. It should be noted, however, that CZE with UV absorbance detection lacks sufficient sensitivity for environmental trace analysis [16].

0021-9673/93/$06.00

Therefore, not surprisingly, there are only a few papers on the CZE of pesticides. Cai and El Rassi [17,18] used CZE for the separation of triazine herbicides and paraquat and diquat. Aguilar *et al.* [19] used CZE for the separation of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxypropionic acid (2,4,5-TP) and their corresponding phenols. Wu *et al.* [20] described the separation of different herbicides by micellar electrokinetic capillary chromatography (MECC). They found free solution electrophoresis (CZE) not to be selective enough for the separation of 2-(2,4-dichlorophenoxy)-propionic acid (2,4-DP) and 2-(2-methyl-4-chlorophenoxy)propionic acid (MCPP). Baseline resolution could only be realized after addition of SDS and Brij-35 to the electrophoresis buffer, thereby changing to a MECC separation mode. Yeo *et al.* [21] described the separation of several plant growth regulators by capillary electrophoresis, including 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Again, baseline resolution of these herbicides was only obtained with the help of additives in the electrophoresis buffer: a mixture of α-, β- and γ-cyclodextrins in the CZE buffer increased the selectivity of their system and provided good resolution for the compounds studied. The separations described so far relate to the analysis of aqueous standard solutions in the ppm range. Obviously, the electrophoretic separation of phenoxy acid herbicides without the use of additives, the analysis of real samples from production plants and, moreover, their chiral separation, are still a challenge in capillary electrophoresis.

In this study, we used CZE and cyclodextrin-modified CZE for the separation of structurally related phenoxy acid herbicides and the determination of trace impurities (including positional isomers) in real production samples. In addition, cyclodextrin-modified CZE was successfully applied to the determination of their enantiopurity.

EXPERIMENTAL

Apparatus

A Lauerlabs (Emmen, Netherlands) PRINCE capillary electrophoresis system was used, equipped with an F.u.G. (Rosenheim, Germany) HCN 35-35000 power supply and an Applied Biosystems (San Jose, CA, USA) Model 759 UV absorbance detector operated at 200 nm (1 mAU/mV) and a 0.5-s rise time. CZE was performed in a 79.5 cm (63.1 cm from injector to detector) $\times$ 50 μm I.D. fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA). The voltage was +30 kV (constant-voltage mode, ramp 6 kV/s), and the oven temperature 30°C. Samples were introduced using the controlled-pressure system (20 mbar for 0.1 min); the instrument injection performance was typically $\pm 0.5\%$ R.S.D. The coefficient of electroosmotic flow (μ_{eo}) was calculated using the migration time of the (negative) system peak. The electrophoretic mobilities (μ_{ep}) and the plate numbers were calculated using the equations in ref. 22. Data were recorded using a Fisons model VG-Multichrom integration system. Peaks were quantified by corrected peak areas (area divided by migration time). As a rule, our integration system did not provide acceptable baselines under the peaks, so the baselines had to be corrected interactively in the reprocess mode followed by recalculation of the peak areas.

Chemicals

Lithium acetate was obtained from Aldrich (Steinheim, Germany), α- and β-cyclodextrins from Fluka (Buchs, Switzerland) and heptakis(2,6-di-O-methyl)-β-cyclodextrin from Sigma (St. Louis, MO, U.S.A.). The following phenoxyacid herbicides and related impurities, kindly provided by Akzo Salt and Basic Chemicals (Hengelo, Netherlands), were investigated (Fig. 1): 2-(2-methyl-4-chlorophenoxy)propionic acid (MCPP), 2-(2-methyl-6-chlorophenoxy)propionic acid (i-MCPP), 2-(2-methyl-4,6-dichlorophenoxy)propionic acid (MD), 2-(2-methylphenoxy)propionic acid (M), 2-(2,4-dichlorophenoxy)propionic acid (DP), 2,4-dichlorophenoxyacetic acid (D) and 2-methyl-4-chlorophenoxyacetic acid (MCPA). All other chemicals were of analytical-grade from J.T. Baker (Deventer, Netherlands). Water was purified in an Alpha-Q apparatus (Millipore, Bedford, MA, USA).

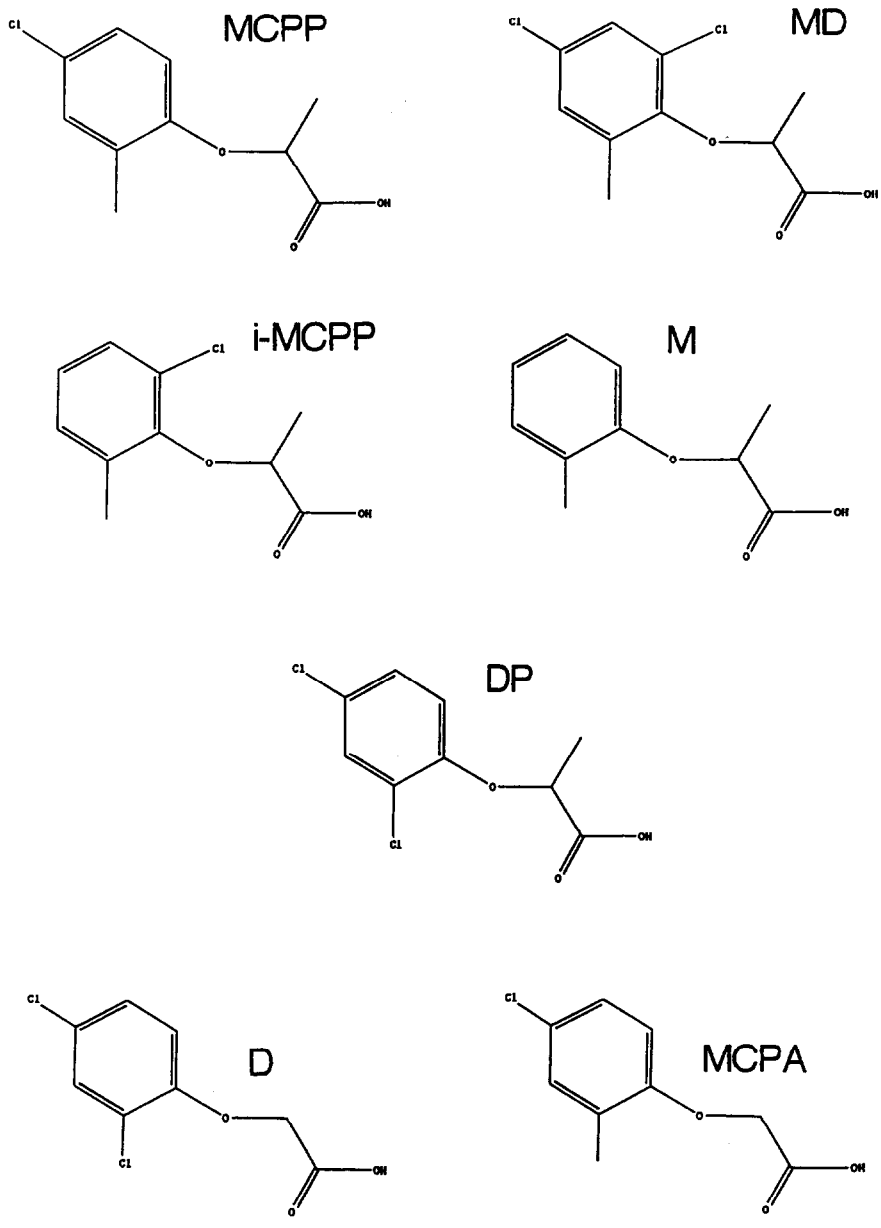

Fig. 1. Structures of the phenoxy acid herbicides and related impurities investigated.

Methods

The detection window in the CZE capillary was prepared by burning off a small part of the protective polyimide layer. Electrophoresis buffers were prepared by dissolving 30 or 50 m*M* lithium acetate in water and adjusting the pH to 4.80 with concentrated acetic acid. Cyclodextrins were dissolved in the lithium acetate buffer. Buffers were filtered through 0.45-μm Spartan 30/B membrane filters (Schleicher & Schüll, Dassel, Germany) prior to use. Stock solutions of the herbicides (0.01 *M*), the production samples (50 mg/ml) and the impurities (1 mg/ml) were prepared in methanol. Sample solutions were prepared by 50–200-fold dilution of the stock solutions with the electrophoresis buffer.

RESULTS AND DISCUSSION

Capillary zone electrophoresis

CZE without any additive (free solution zone electrophoresis) was evaluated for its separation power towards the structurally related herbicides MCPP, DP, MCPA and D. As can be seen in Fig. 1, these compounds differ by one substituent only, so it is not surprising that Wu *et al.* [20] found MCPP and DP difficult to separate. However, according to CZE theory [23], the best resolution would have been obtained at a pH value close to the pK_a values of the analytes, *i.e.*, at an acidic pH. Indeed the resolution increased from almost zero at pH 7.0 towards baseline separation at pH 4.80. Using 50 m*M* lithium acetate buffer (pH 4.80) and +30 kV, all the herbicides could be baseline resolved within 12 min, having plate numbers of 220 000, 240 000, 200 000 and 240 000 for MCPP, DP, MCPA and D, respectively.

The performance of this CZE system in terms of repeatability and linearity (range 0–250 × 10^{-5} *M*) was tested using a mixture of MD, DP, MCPA and D. The results are presented in Table I and it can be concluded that the migration times, the mobilities and the electroosmotic flow are very constant. This separation was found to be reproducible over several months. The relative standard deviations of the area/time data are fairly good and might be even better at higher concentrations, which will be less influenced by integration errors caused by the noisy baseline. Note that quantification was performed without any internal standard throughout this study. These repeatability and linearity data compare favourably with the data in refs. 19 and 20.

It can be seen that at 200 nm, the sensitivity (slope) for MCPA is about 20% less than that for the other compounds. This might be caused by a minor difference in the molar absorptivity at that wavelength. The detection limit of the phenoxy-acid herbicides was $1 \cdot 10^{-5}$ *M* (signal-to-noise ratio = 2). This value could be lowered tenfold while maintaining baseline resolution by using the sample stacking injection technique [24], yielding $1 \cdot 10^{-6}$ *M*. Obviously, this value is much too high for environmental trace analysis. It should be remembered, however, that the objective of this study was product analysis rather than environmental analysis.

Next, the applicability of this CZE system towards the separation and detection of related impurities, as known from chromatographic analyses, was studied. MCPP, DP, MCPA and D were mixed with MD, M and i-MCPP. The electropherogram obtained is shown in Fig. 2A. It can be seen that M and MD do not interfere with the separation. Only the positional isomer i-MCPP showed peak overlap with MCPP. The analysis of a real production sample is shown in Fig. 2B. In addition to the known impurities, an additional compound could be observed at 13

TABLE I

REPEATABILITY AND LINEARITY DATA

CZE system: 50 m*M* lithium acetate buffer (pH 4.80); voltage, +30 kV. The repeatabilities were determined at MD, DP, MCPA and D concentrations of $0.5 \cdot 10^{-4}$, $1.0 \cdot 10^{-4}$, $1.5 \cdot 10^{-4}$ and $2 \cdot 10^{-4}$ *M*, respectively, and are given as the relative standard deviations of the mean values of six replicates. The linearity data range from 0.0, $2.5 \cdot 10^{-5}$, $7.5 \cdot 10^{-5}$, $2.5 \cdot 10^{-4}$ and $7.5 \cdot 10^{-4}$ to $2.5 \cdot 10^{-3}$ *M*. Eof = system peak (electroosmotic flow marker); t_m = migration time; μ_{ep} = electrophoretic mobility. Other conditions as under Experimental.

Component	R.S.D. (%)			Regression (r^2)	Slope (×10^7)	Intercept
	t_m	μ_{ep}	Area/t_m			
MD	0.4	0.2	1.3	0.9998	1.17	195
DP	0.4	0.3	1.1	0.9999	1.25	169
MCPA	0.5	0.3	1.2	0.9998	0.96	177
D	0.5	0.3	1.4	0.9998	1.16	197
Eof	0.3	0.3				

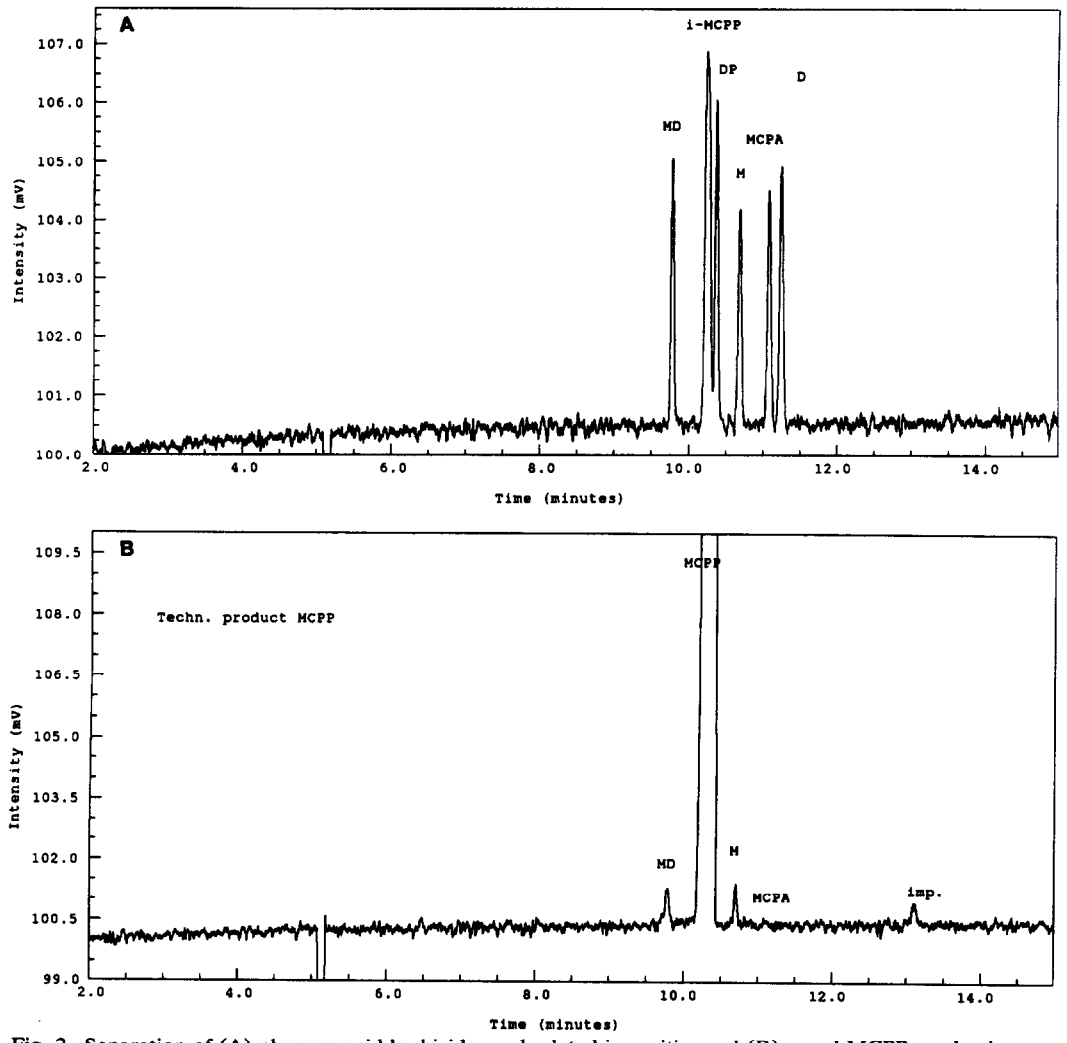

Fig. 2. Separation of (A) phenoxy acid herbicides and related impurities and (B) a real MCPP production sample by CZE using 50 m*M* lithium acetate buffer (pH 4.80) and +30 kV. Detection by UV absorbance at 200 nm (1 mAU/mV). Note that MCPP and i-MCPP co-migrate under these conditions. Other conditions as under Experimental.

min. The excess of MCPP co-migrated with i-MCPP (*cf.*, Fig. 2A) and partly overlapped with DP. The detection limit of DP relative to MCPP was determined experimentally and found to be 20 mg/g.

Cyclodextrin-modified capillary zone electrophoresis

Cyclodextrins (CDs) represent a range of cyclic glucopyranoses having a characteristic conical shape with a hydrophobic cavity and a polar exterior; α-, β- and γ-CDs have increasing diameters of the hydrophobic cavity. Alkylation of CDs induces significant changes in both the shape and the diameter. CDs and their alkylated derivatives are able to form inclusion complexes dynamically, with a variety of compounds. The optimum fit of the compounds studied (Fig. 1) will probably be a combination of inclusion of the aromatic ring and hydrogen bonding at the rim of the CD. Generally, the electrophoretic

mobility of the CD–analyte complex will be less negative than the mobility of the free (uncomplexed) compound. Consequently, the acidic herbicides will migrate closer to the system peak (electroosmotic flow marker), and the analysis time might be shortened considerably.

Initial experiments were carried out with β-CDs added to the electrophoresis buffer. The separation of a test mixture consisting of MD, DP, MCPA and D showed baseline resolution at 7 m*M* β-CD; in addition, partial chiral separation of the MD enantiomers could be obtained. In contrast to the chiral separation of basic compounds [15], the resolution of the enantiomers did not improve at lower field strengths. This was to be expected: from the resolution equation [4]

$$R_s = 0.177(\mu_{ep_1} - \mu_{ep_2})\left(\frac{VL_d}{D(\mu_{epm} + \mu_{osm})L_t}\right)^{1/2}$$

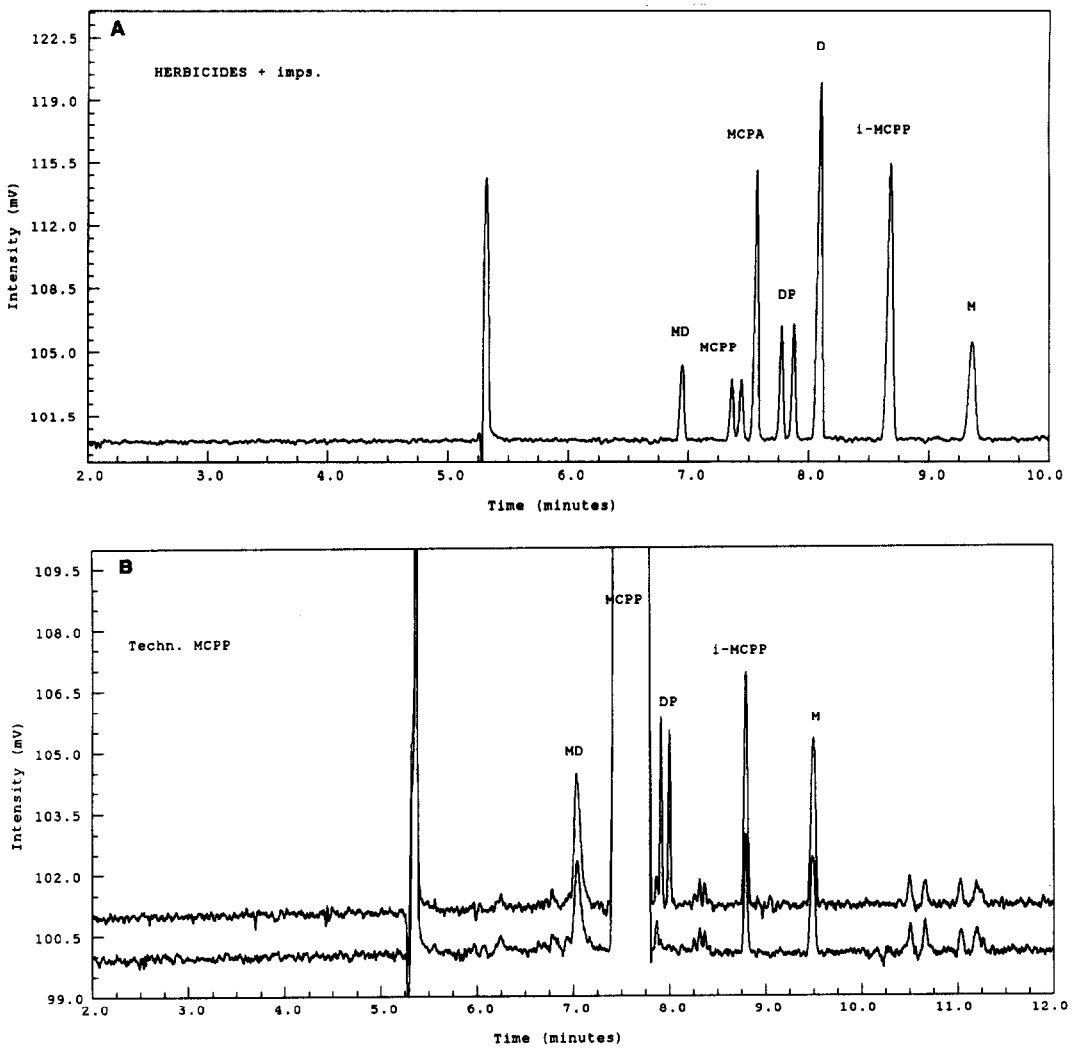

Fig. 3. Separation of (A) phenoxy acid herbicides and related impurities and (B) a real MCPP production sample by CD-modified CZE using 30 m*M* lithium acetate buffer (pH 4.80) with the addition of 20 g/l heptakis(2,6-di-O-methyl)-β-CD. For other conditions, see Fig. 2 and Experimental.

where μ_{ep} is the electrophoretic mobility of the analyte, μ_{epm} the mean of the two electrophoretic mobilities, V the applied voltage, D the diffusion coefficient, L_d and L_t the length of the capillary to the detection window and the total length, respectively, and μ_{osm} the coefficient of electroosmotic flow, it can be seen that small increases in electrophoretic and electroosmotic mobilities (*e.g.*, due to temperature rises at higher field strengths) counterbalance each other if they have opposite signs, as in the present study.

The addition of 20 g/l of heptakis(2,6-di-O-methyl)-β-CD to the electrophoresis buffer was more successful than the β-CD system; all herbicides were baseline resolved and chiral separations of both the MCPP and the DP enantiomers were obtained; in addition, none of the impurities were found to interfere with the separation (Fig. 3A). It should be noted that the selectivity changed dramatically on addition of the alkylated β-CD; compare, for example, Figs. 2A and 3A. The separation of the real production sample before and after addition of potential impurities is shown in Fig. 3B. In contrast to the unmodified CZE system, i-MCPP and DP could be separated from the excess of MCPP. Unidentified impurities can be observed between 10 and 12 min. Traces of MCPA, however, co-migrated with the huge MCPP peak.

The day-to-day reproducibility was studied by comparing duplicate analyses of the production sample on subsequent days. The results are presented in Table II. Except for the area/time reproducibility of MD, the day-to-day reproducibility was fairly good. It should be stressed that the separation of MCPP, i-MCPP, MD and M can also be peformed by LC, using a reversed-phase column and an acidified mobile phase [25]. However, the method requires 35 min and cannot be applied to the determination of the enantiopurity of MCPP and DP (see below).

An entirely different selectivity was obtained after changing to an α-CD-modified CZE system. Too high α-CD concentrations resulted in the co-migration of several peaks close to the system peak (electroosmotic flow marker). Addition of 10 mM α-CD was found to provide baseline resolution of the herbicides, including chiral separation of the DP and MCPP enantiomers. The potential impurities did not interfere with the separation and even the MD enantiomers could be baseline resolved (Fig. 4A). The separation of the production sample is shown in Fig. 4B. Herbicide D co-migrated with the excess of MCPP. Fortunately, the presence of D as an impurity in these MCPP samples is unlikely in practice.

TABLE II

DAY-TO-DAY REPRODUCIBILITY

Separation system and sample as in Fig. 3B. Reproducibilities are given as percentage deviations from the mean values ($n = 2$). For abbreviations, see Table I. Other conditions as under Experimental.

Component	Deviation from mean value (%)		
	t_m	μ_{ep}	Area/t_m
MD	0.8	0.4	6.5
MCPP	0.8	0.6	1.5
i-MCPP	1.0	0.2	2.2
M	1.1	0.2	1.1
Eof	0.7	0.7	

The main advantages of CD-modified CZE as compared with chromatographic approaches are its flexibility and its economy; one can easily and automatically switch to another CD–buffer system, having an entirely different selectivity. The changes in the peak order, the chiral selectivity and the analysis time are all reflected by the impact of the different CZE systems on the electrophoretic mobilities of the phenoxyacid herbicides, and are summarized in Fig. 5. The different selectivities can be utilized as confirmation criteria for impurities that are to be included in product specifications, thereby omitting complicated and expensive hyphenated identification techniques. Following this approach, the impurities in the MCPP production sample (Figs. 2B, 3B and 4B) were identified and determined after standard addition. The results are presented in Table III. The determination of the impurities are in acceptable agreement with each other, except for MD, which also showed the

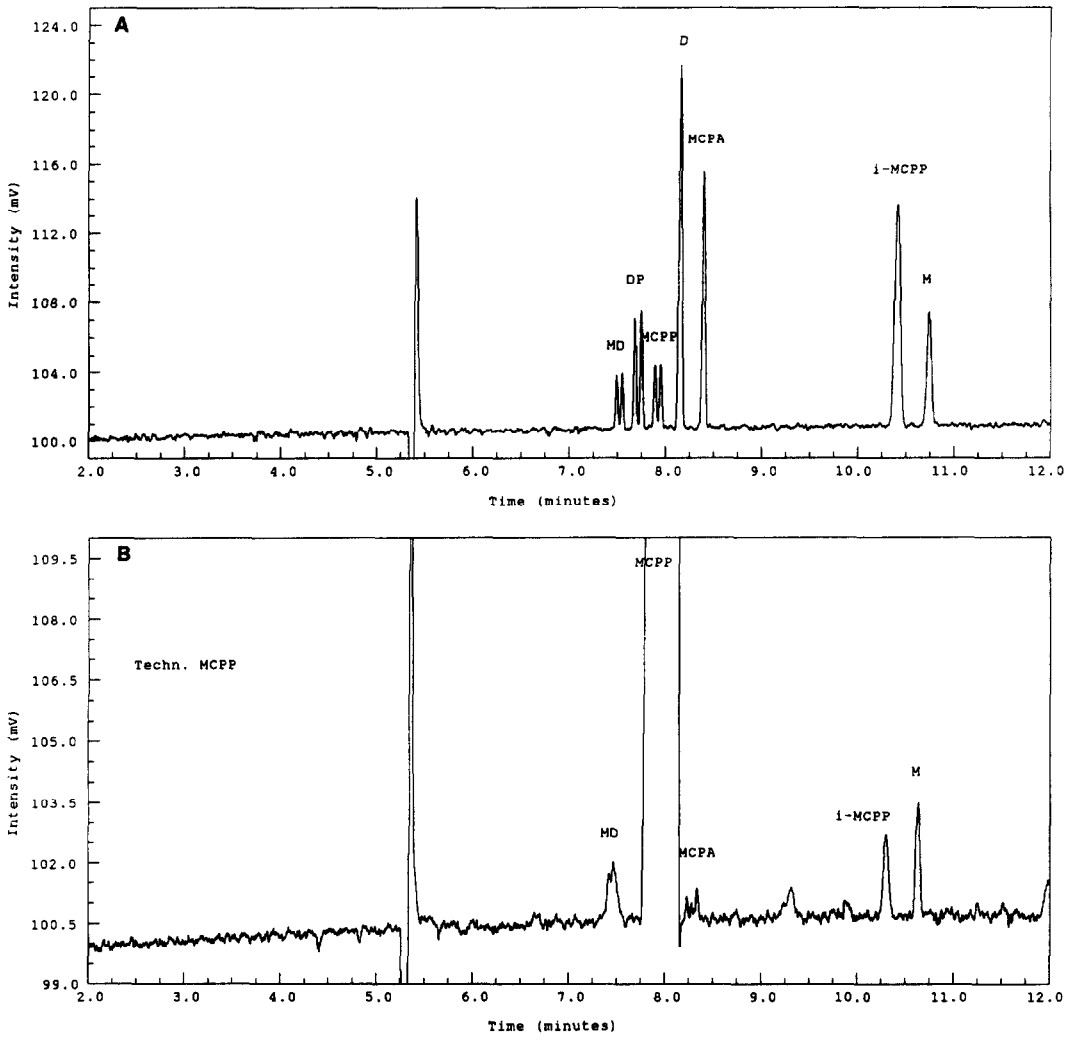

Fig. 4. Separation of (A) phenoxy acid herbicides and related impurities and (B) a real MCPP production sample by CD-modified CZE using 50 m*M* lithium acetate buffer (pH 4.80) with the addition of 10 m*M* α-CD. For other conditions, see Fig. 2 and Experimental.

worst day-to-day reproducibility and peak shapes throughout this study. The determination of the other impurities (*cf.*, Figs. 2B, 3B and 4B) might be carried out in a similar fashion provided that their identities have been assessed previously by, *e.g.*, CZE–MS.

Enantiopurity determination

The feasibility of the determination of the enantiopurity was demonstrated using the heptakis (2,6-di-O-methyl)β-CD-modified CZE system. A *d*-DP production sample was dissolved in methanol and diluted 100-fold with the CD-modified electrophoresis buffer. The electropherogram obtained is shown in Fig. 6A. It can be seen that the *d*-DP sample contained a relatively high amount of *l*-DP. In addition, an unidentified impurity can be observed at 9 min. The enantioimpurity, defined as $[l/(l+d)] \times 100\%$ was determined in duplicate and found to be $13.1 \pm 0.1\%$ ($n = 2$). This value is in good agreement with an LC determination using a CHIRAL-AGP column [25], which gave 12.7%. A *d*-MCPP production sample was analysed after

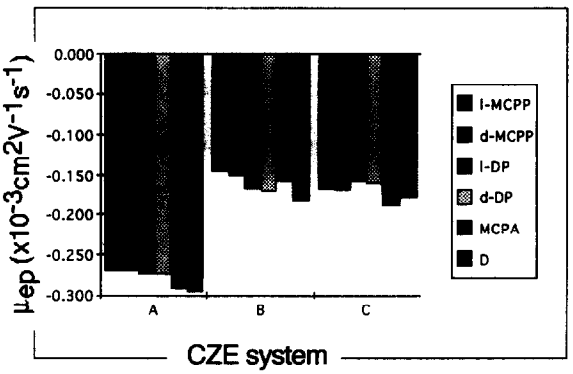

Fig. 5. Electrophoretic mobilities of phenoxy acid herbicides in the CZE systems of (A) Fig. 2, (B) Fig. 3 and (C) Fig. 4.

TABLE III

IMPURITIES IN THE MCPP PRODUCTION SAMPLE

Conditions: A, B and C correspond to Figs. 2B, 3B and 4B, respectively. For other conditions, see Experimental.

Component	Content relative to MCPP (mg/g)		
	A	B	C
MD	6	13	8
MCPA	1	n.d.[a]	1
i-MCPP	n.d.[a]	5	4
M	7	9	8
DP	<20	<1	<1
D	<1	<1	n.d.[a]

[a] Not determined because of peak overlap.

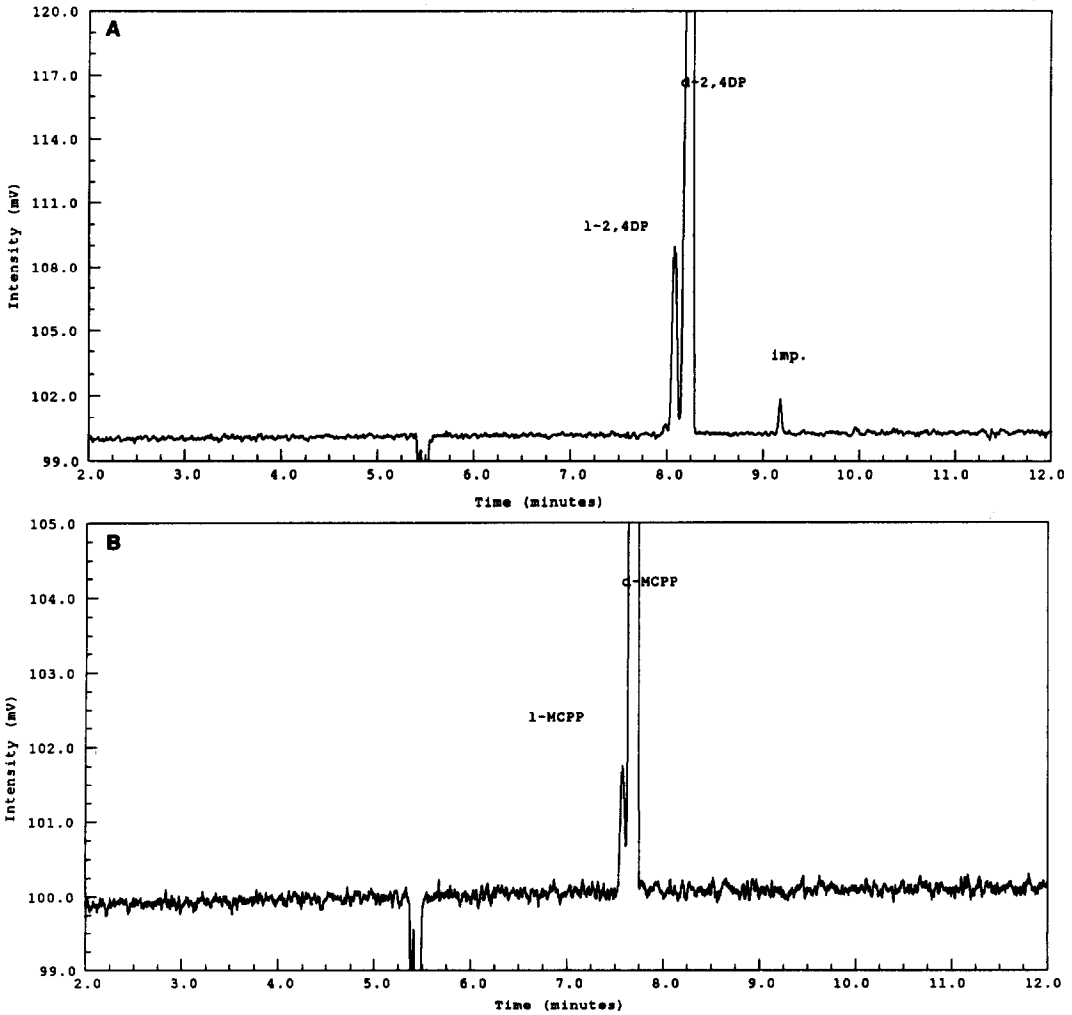

Fig. 6. Chiral separation of (A) *d*-DP and (B) *d*-MCPP samples originating from enantioselective production plants, using the CD-modified CZE system of Fig. 3. For other conditions, see Experimental.

200-fold dilution in a similar fashion. The electropherogram is shown in Fig. 6B. The enantioimpurity of this *d*-MCPP sample was found to be $3.2 \pm 0.1\%$ ($n = 2$), which again is in good agreement with the LC assay, which gave 3.5%. In contrast to the LC method, the enantiopurity of mixed *d*-MCPP–*d*-DP "cocktails" can be determined simultaneously, as the MCPP and DP peaks did not show any overlap. In addition, the same CD-modified CZE system can be used for the determination of related impurities in MCPP samples, as outlined above.

CONCLUSIONS

Phenoxy acid herbicides can be baseline separated by CZE provided that an appropriate buffer pH is selected. The CZE system thus obtained shows good stability, precision and linearity. Addition of CDs to this CZE system allows fine-tuning of the selectivity by using different types of CDs. One might easily and automatically switch to different CD-modified CZE buffers, and utilize the different selectivities to confirm the presence of specific impurities. The impurities could be determined in production samples at a level as low as 1 mg/g relative to the main component. Moreover, the dimethyl-β- and the α-CD systems can be used for chiral separations of phenoxypropionic acid herbicides and the determination of the enantiopurity of samples originating from enantioselective production plants.

REFERENCES

1 C. Sanchez-Brunete, S. Perez and J.L. Tadeo, *J. Chromatogr.*, 522 (1991) 235.
2 D. Barcelo, *Analyst*, 116 (1991) 681.
3 V. Coquart and M.-C. Hennion, *Sci. Total Environ.*, in press.
4 J. Jorgenson and K.D. Lukacs, *Anal. Chem.*, 53 (1981) 1298.
5 S.F.Y. Li, *Capillary Electrophoresis*, Elsevier, Amsterdam, 1992.
6 W.G. Kuhr and C.A. Monnig, *Anal. Chem.*, 64 (1992) 389R–407R.
7 W.G. Kuhr, *Anal. Chem.*, 62 (1990) 403R–414R.
8 M.W.F. Nielen, *J. Chromatogr.*, 625 (1992) 387.
9 M.J.A. Mensink and M.W.F. Nielen, *J. High Resolut. Chromatogr.*, 14 (1991) 417.
10 J. Snopek, I. Jelinek and E. Smolkova-Keulemansova, *J. Chromatogr.*, 452 (1988) 571.
11 J. Snopek, H. Soini, M. Novotny, E. Smolkova-Keulemansova and I. Jelinek, *J. Chromatogr.*, 559 (1991) 215.
12 S. Fanali, *J. Chromatogr.*, 474 (1989) 441.
13 M.J. Sepaniak, R.O. Cole and B.K. Clark, *J. Liq. Chromatogr.*, 15 (1992) 1023.
14 R. Kuhn, F. Stoeckun and F. Erni, *Chromatographia*, 33 (1992) 32.
15 M.W.F. Nielen, *Anal. Chem.*, 65 (1993) 7.
16 M.W.F. Nielen, presented at the *20th International Symposium on Environmental Analytical Chemistry, Strasbourg, 1990.*
17 J. Cai and Z. El Rassi, *J. Liq. Chromatogr.*, 15 (1992) 1179.
18 J. Cai and Z. El Rassi, *J. Liq. Chromatogr.*, 15 (1992) 1193.
19 M. Aguilar, A. Farran and V. Marti, *Sci. Total Environ.*, in press.
20 Q. Wu, H.A. Claessens and C.A. Cramers, *Chromatographia*, 34 (1992) 25.
21 S.K. Yeo, H.K. Lee and S.F.Y. Li, *J. Chromatogr.*, 594 (1992) 335.
22 M.W.F. Nielen, *J. Chromatogr.*, 542 (1991) 173.
23 S. Terabe, T. Yashima, N. Tanaka and M. Araki, *Anal. Chem.*, 60 (1988) 1673.
24 S.E. Moring, J.C. Colburn, P.D. Grossman and H.H. Lauer, *LC · GC Int.*, 3 (1990) 46.
25 B.H. Schel, Akzo Salt and Basic Chemicals, Hengelo, Netherlands, personal communication, 1992.