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Herbicide analysis by micellar electrokinetic capillary chromatography

Kumar V. Penmetsa, Ross B. Leidy, Damian Shea*

Department of Toxicology, 3709 Hillsborough Street, North Carolina State University, Raleigh, NC 27607, USA

Abstract

Capillary electrophoresis with ultraviolet detection (CE–UV) and laser-induced fluorescence detection (CE–LIF) was used for analysis of a group of herbicides that have widespread use in the USA. CE–UV was employed for simultaneous determination of atrazine, simazine, alachlor and metolachlor in water. In addition, CE–UV was also suitable for analysis of dicamba, 2,4-D and chlorimuron ethyl. Dicamba, 2,4-D and chlorimuron ethyl were also analyzed using CE–LIF following derivatization with fluorescent reagents. Dicamba and 2,4-D were derivatized with 4-bromomethyl-7-methoxycoumarin and chlorimuron ethyl was derivatized with dansyl chloride following hydrolysis. The detection limit with CE–UV for atrazine, simazine and metolachlor was 0.1 $\mu\text{g/l}$ and for alachlor was 1.0 $\mu\text{g/l}$. The estimated detection limit with CE–LIF for dicamba, 2,4-D and chlorimuron ethyl was 10 ng/l. Our results demonstrate that CE provides a powerful new analytical tool for herbicide analysis.

Keywords: Environmental analysis; Water analysis; Laser-induced fluorescence detection; Pesticides

1. Introduction

Herbicides are widely used for controlling weeds in a number of agricultural crops in the USA. Residues of these herbicides are frequently found in surface and ground water because of their persistence and water solubility [1–3]. A variety of analytical methods have been used for analysis of these herbicides in water including GC–MS [4], GC–nitrogen–phosphorus detection (GC–NPD), GC–electron-capture detection (GC–ECD) [5–7] and HPLC [8–10]. GC–MS and GC with NPD and ECD have proven to be very reliable for the analysis of herbicide mixtures in water, but often these methods are time consuming and expensive because of the sample preparation and sophisticated equipment that is required. Thus, there is a need for rapid, simple

and reliable analytical methods for measuring herbicides in natural waters.

A relatively new analytical technique that is complementary (and orthogonal) to GC and HPLC and offers many advantages over these conventional techniques is capillary electrophoresis (CE) [11,12]. CE is well suited for analysis of complex mixtures, due to high separation efficiency and a separation mechanism that often precludes the use of extensive sample preparation. Different modes of CE, such as capillary zone electrophoresis (CZE), micellar electrokinetic capillary chromatography (MEKC) and capillary gel electrophoresis (CGE) are used for separation of a wide array of molecules.

At present, CE is undergoing a period of rapid expansion and increasingly is finding applications in many fields of biochemical, pharmaceutical and environmental analysis [13,14]. A few reports have been published describing herbicide analysis by CZE

*Corresponding author.

[15–17] and MEKC [18–20] with ultraviolet detection. However, detection sensitivity is a limitation with CE–UV. The sensitivity of CE can be improved by using sample concentration techniques or by using more sensitive modes of detection, such as laser-induced fluorescence (LIF) detection. The latter approach was used in our laboratory to analyze benzo[*a*]pyrene–DNA adducts at the attomole (10^{-18} mol) level [21]. To date, only one report has been published that describes the application of CE–LIF for analysis of herbicides (phenoxy acids) following derivatization with a fluorescent agent [14].

This paper describes CE–UV methods for analysis of selected herbicides (atrazine, simazine, alachlor, metolachlor, dicamba, 2,4-D and chlorimuron ethyl). In addition, CE–LIF methods are described for analysis of dicamba, 2,4-D and chlorimuron ethyl. To our knowledge, this is the first report describing the application of CE–LIF for analysis of benzoic acid herbicides (dicamba) and sulfonylurea herbicides (chlorimuron ethyl).

2. Experimental

2.1. Materials

Atrazine, simazine and alachlor (each 99%) were obtained from Supelco (Bellefonte, PA, USA). Metolachlor (99%) was obtained from Ciba-Geigy (Greensboro, NC, USA). Dicamba and 2,4-D (each 99%) were obtained from Chem Service (West Chester, PA, USA). Chlorimuron ethyl (99%) was obtained from DuPont (Wilmington, DE, USA). Monobenzene, dansyl chloride and 4-bromomethyl-7-methoxycoumarin (BMC) were obtained from Aldrich (Milwaukee, WI, USA). All buffers and Sudan III were obtained from Sigma (St. Louis, MO, USA). All solvents (HPLC grade) and Empore C_{18} extraction disks were obtained from J.T. Baker (Phillipsburg, NJ, USA). The fused-silica capillary used for CE was obtained from Polymicro Technologies (Phoenix, AZ, USA).

2.2. Apparatus

A Beckman P/ACE 2200 unit equipped with an ultraviolet and a laser-induced fluorescence detector

was employed for CE analysis. An Omnichrome model 3056-8M He/Cd laser was used for LIF excitation at 325 nm. The capillary used for separation was 50 cm \times 50–75 μ m I.D., housed in a cartridge configured for either UV or LIF detection. Standard/sample injections were made using pressure injection (3.45 kPa) for 1 or 2 s. The separation voltage ranged from 20–25 kV. All operations of the P/ACE unit were controlled by an IBM personal computer with Beckman Gold Software. At the beginning of each day, the capillary was rinsed for 10 min with 0.1 M NaOH, followed by 5 min with deionized water and 15 min with run buffer. Before each sample injection, the capillary was rinsed for 2 min with 0.1 M NaOH followed by 2 min with run buffer. At the end of each day, the capillary was rinsed for 10 min with 0.1 M NaOH, followed by a 5-min rinse with deionized water. When the instrument was not in use, the electrodes were left immersed in deionized water.

2.3. Sample fortification and extraction

Deionized and pond-water samples (1 l) were fortified by adding a mixture of herbicides dissolved in methanol (atrazine, simazine, alachlor and metolachlor) to obtain a concentration of 2 μ g/l of each compound. Unfortified deionized and pond-water samples served as the blank controls. The fortified deionized water samples and the control sample were extracted using Empore C_{18} extraction disks, according to US Environmental Protection Agency (EPA) method 525.1, revision 2.2. Water was extracted by vacuum filtration through the disk and the disk was then eluted with 5 ml of ethyl acetate, 5 ml of ethyl acetate–methylene chloride (1:1, v/v) and 5 ml of methylene chloride. The combined eluent was dried using anhydrous sodium sulfate and evaporated to dryness using a gentle stream of nitrogen at room temperature. Prior to CE–UV analysis, the dried samples were resuspended using 20 μ l of methanol containing 80 ng/ μ l of the internal standard (I.S.), monobenzene.

The fortified pond water samples and the control sample were extracted using liquid–liquid extraction. Briefly, 1 l of fortified water was extracted three times with 60 ml of methylene chloride. The methylene chloride extracts were combined, dried using anhydrous sodium sulfate and evaporated to dryness

using a rotary evaporator at 40°C. The dried samples were redissolved in 4 ml of methanol and the methanol was evaporated to dryness under a gentle stream of nitrogen at room temperature. Prior to CE–UV analysis, the dried samples were resuspended using 20 μl of methanol containing 80 ng/ μl of monobenzene.

2.4. Derivatization

The derivatization of dicamba and 2,4-D was carried out according to an earlier reported procedure, with minor modifications [22]. A 1-ml solution of dicamba and 2,4-D (100 $\mu\text{g}/\text{ml}$ in acetone) was transferred to a 15-ml PTFE-capped vial. To this solution, 10 mg of cesium carbonate was added followed by the addition of 1 ml of BMC (0.5 mg/ml in acetone) and the solution was incubated at 35°C for 1 h, with occasional shaking in the dark. After derivatization, the reaction was stopped by adding 100 μl of glacial acetic acid after which the volume was made up to 5 ml with deionized water. The mixture was then extracted twice with 5 ml of light petroleum and the extract was filtered through a 0.45- μm filter. The filtered extract was then evaporated to dryness using nitrogen at room temperature. The dried extract was redissolved in 1 ml of methanol and the derivatized sample was analyzed by CE–LIF. A sample without dicamba and 2,4-D was derivatized using the procedure described above and served as the control.

The derivatization of chlorimuron ethyl was carried out according to an earlier reported procedure for sulfonylurea drugs, with minor modifications [23]. A 100- μl volume of chlorimuron ethyl (1 mg/ml in methanol) was transferred to a 15-ml PTFE-capped vial and the methanol was evaporated using nitrogen at room temperature. To this vial, 1 ml of 5 M NaOH was added and the vial was heated for 2 h at 100°C in a Lab-line block heater. The

solution was allowed to cool and was neutralized with HCl (approximately 850 μl). The neutralized solution was then mixed with 2 ml of 1 M NaHCO_3 – Na_2CO_3 buffer (pH 9.5), followed by the addition of 2 ml of dansyl chloride solution (2 mg/ml). The solution was then incubated at 45°C for 30 min in the dark, after which time the acetone was evaporated with nitrogen at 45°C. The aqueous residue was then extracted twice with 2 ml of hexane and the extract was filtered through a 0.45- μm filter. The filtered extract was then evaporated to dryness using nitrogen at room temperature and the dried extract was redissolved in 1 ml of methanol and analyzed by CE–LIF. A sample without chlorimuron ethyl was derivatized using the procedure described above and served as the control.

3. Results and discussion

Several buffers were tested during method development to identify the best buffers for optimum separation of the herbicides. Table 1 lists the optimum buffers used for analysis of the selected herbicides. A method was developed to simultaneously analyze the herbicides atrazine, simazine, alachlor and metolachlor using a buffer consisting of 10 mM Na_2HPO_4 and 30 mM sodium dodecyl sulfate (SDS) with 8% methanol, pH 9.0 (Fig. 1). The peak shape for alachlor shown in Fig. 1 is poor. The reason for the poor peak shape is not known, but it could be due to instability of alachlor at high pH [24] or band broadening due to diffusion as a result of an increase in capillary temperature or interaction with the capillary wall [25].

To validate this method with actual samples, deionized and pond-water samples fortified with a known concentration of these herbicides were extracted and analyzed by CE–UV using the above buffer solution. Fig. 2 shows a typical electropherog-

Table 1
Optimum buffers used in herbicide analysis

Buffer Composition	Analyte(s)
(1) 10 mM Na_2HPO_4 +30 mM SDS+8% MeOH, pH 9.0	Atrazine, simazine, alachlor and metolachlor
(2) 10 mM $\text{Na}_2\text{B}_4\text{O}_7$ +30 mM SDS+5 mM Brij 35, pH 9.2	Dicamba and 2,4-D
(3) 10 mM Na_2HPO_4 +30 mM SDS, pH 9.0	Chlorimuron ethyl
(4) 10 mM $\text{Na}_2\text{B}_4\text{O}_7$ +100 mM NaCh, pH 9.3	Dicamba and 2,4-D
(5) 10 mM $\text{Na}_2\text{B}_4\text{O}_7$ +30 mM SDS, pH 9.2	Chlorimuron ethyl

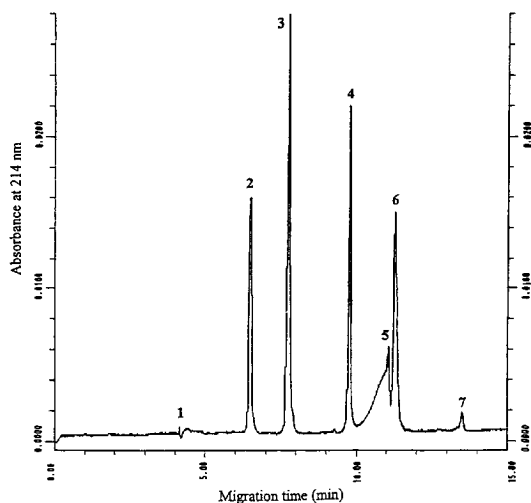


Fig. 1. Electropherogram of standard herbicide mixture: (1) methanol (t_0), (2) simazine, (3) atrazine, (4) monobenzene (I.S.), (5) alachlor, (6) metolachlor, (7) sudan III (t_{mc} marker). Analysis conditions: 50 cm \times 75 μ m I.D. capillary column; pressure injection (1 s = 6 nl); 10 mM Na_2HPO_4 plus 30 mM SDS buffer with 8% MeOH, pH 9.0; 20 kV (45 μ A); 214 nm UV absorbance.

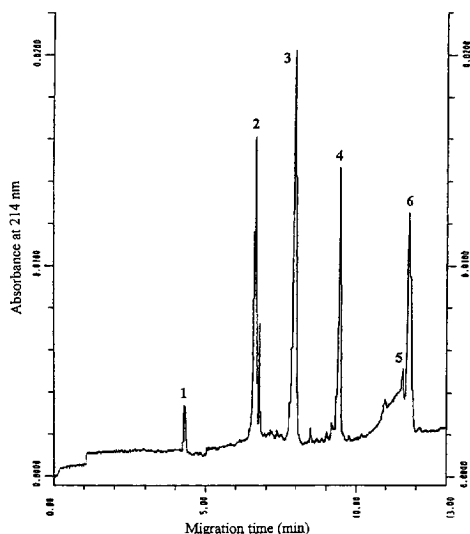


Fig. 2. Electropherogram of a deionized water sample fortified with 2 μ g/l of atrazine, simazine, alachlor and metolachlor, followed by extraction with an Empore C_{18} extraction disk: (1) methanol, (2) simazine, (3) atrazine, (4) monobenzene (I.S.), (5) alachlor, (6) metolachlor. Analysis conditions: same as in Fig. 1.

ram of a deionized water sample fortified with 2.0 μ g/l of atrazine, simazine, alachlor and metolachlor, followed by extraction with Empore C_{18} extraction disks and analysis by CE–UV. The Empore C_{18} extraction disks were not suitable for extraction of pond water samples, due to clogging of the disks with suspended particles. The use of Empore Filter Aid 400 (glass beads; average diameter 40 μ m) did not show any improvement in flow-rates. Therefore, liquid–liquid extraction (separatory funnel technique) was used for extraction of fortified pond water samples. Fig. 3 shows a typical electropherogram of a pond water sample fortified with 2.0 μ g/l of atrazine, simazine, alachlor and metolachlor, followed by liquid–liquid extraction and analysis by CE–UV. Based on the results obtained from CE–UV analysis of fortified deionized and pond-water samples, the detection limits for atrazine, simazine and metolachlor were estimated to be 0.1 μ g/l and that for alachlor was estimated to be 1.0 μ g/l ($S/N=3$).

Dicamba and 2,4-D are widely used in the lawn care industry in the USA and chlorimuron ethyl is used for weed control in soybean crops. Dicamba and 2,4-D were analyzed using CE–UV. Fig. 4 shows a typical electropherogram for the separation of these two herbicides using a buffer consisting of

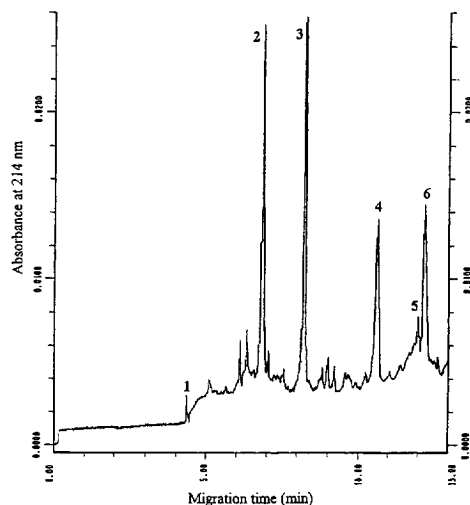


Fig. 3. Electropherogram of a pond-water sample fortified with 2 μ g/l of atrazine, simazine, alachlor and metolachlor, followed by liquid–liquid extraction: (1) methanol, (2) simazine, (3) atrazine, (4) monobenzene (I.S.), (5) alachlor, (6) metolachlor. Analysis conditions: same as in Fig. 1.

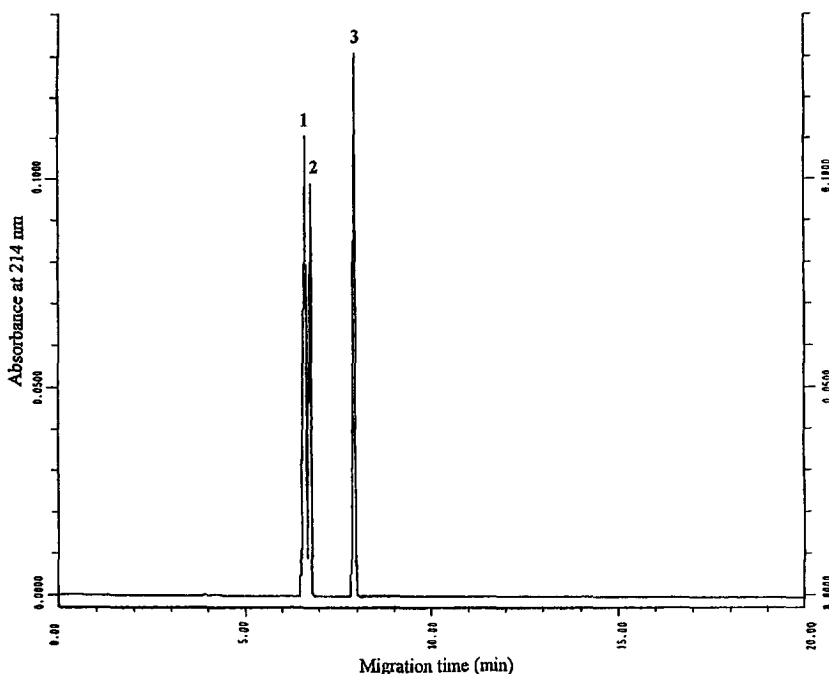


Fig. 4. Electropherogram of a standard herbicide mixture: (1) dicamba, (2) 2,4-D, (3) benzoic acid (I.S.). Analysis conditions: 50 cm \times 75 μ m I.D. capillary column; pressure injection (1 s=6 nl); 10 mM Na₂B₄O₇-30 mM SDS-5 mM Brij 35 buffer, pH 9.2; 20 kV (51 μ A); 214 nm UV absorbance.

10 mM Na₂B₄O₇-30 mM SDS-5 mM Brij 35, pH 9.2. Chlorimuron ethyl was analyzed using CE-UV with a buffer solution consisting of 10 mM Na₂HPO₄ with 30 mM SDS, pH 9.0 (Fig. 5).

The electropherograms shown in Figs. 1–5 demonstrate the potential of CE to separate commonly used herbicides. However, the relatively high detection limits achieved with UV detection (0.1 to 1.0 μ g/l) limits the application of CE-UV for trace analysis of pollutants. Detection in CE is mass sensitive because it measures the absolute on-column amount of analyte, but the concentration limits of detection are high because very small injection volumes (normally 1 to 10 nl) are used for analysis. A few approaches can be taken to improve detection sensitivity in CE. Because CE typically requires very small samples for analysis, the sample can be preconcentrated (for example, we extracted 1 l of water and concentrated the extract to 20 μ l) prior to CE-UV analysis. In addition, more sensitive detection schemes can be used. Our laboratory is interested in developing sensitive LIF detection for

use in environmental and toxicology studies. Three herbicides (dicamba, 2,4-D and chlorimuron ethyl) were selected for CE-LIF method development. Because these three herbicides do not possess native fluorescence, they required derivatization with a fluorescent reagent prior to CE-LIF analysis. Dicamba and 2,4-D can be labelled with BMC [22]. We modified this procedure slightly to derivatize dicamba and 2,4-D and measured the UV absorbance spectrum of the methylmethoxycoumarin esters (MMCE) of dicamba and 2,4-D. The maximum absorbance wavelength for MMCE of dicamba and 2,4-D was determined to be between 325 and 340 nm, near the output wavelength of a 325 nm He/Cd laser. Fig. 6 shows the analysis of the MMCE of dicamba and 2,4-D using the He/Cd laser CE-LIF method. Optimum separation was obtained with a buffer consisting of 10 mM sodium borate with 100 mM sodium cholate (NaCh), pH 9.3. The two peaks of interest (1 and 2 with migration times of 11.7 and 12.2 min, respectively) are well resolved from the excess BMC (migration time, 8.9 min) and other

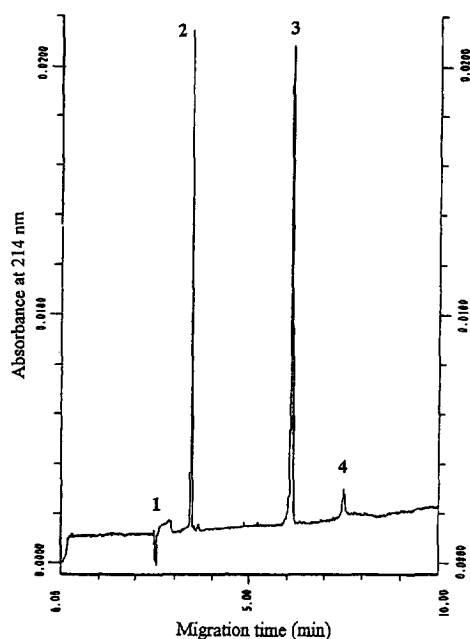


Fig. 5. Electropherogram of chlorimuron ethyl: (1) methanol, (2) chlorimuron ethyl, (3) monobenzene (I.S.), (4) sudan III. Analysis conditions: 50 cm \times 75 μ m I.D. capillary column; pressure injection (1 s=6 nl); 10 mM Na₂HPO₄ plus 30 mM SDS buffer, pH 9.0; 25 kV (75 μ A); 214 nm UV absorbance.

side-products that elute prior to the compounds of interest. A separate HPLC method was developed to separate the MMCE of dicamba and 2,4-D to determine the derivatization efficiency. Fig. 7 shows an HPLC chromatogram of MMCE of dicamba and 2,4-D. The elution pattern observed in HPLC is similar to that of CE and the peaks of interest are well resolved from the excess BMC and other side-products. The derivatization efficiency was estimated to be 90%, which is almost identical to the value obtained by previous investigators [22]. The detection limit in water samples is estimated to be 10 ng/l for dicamba and 2,4-D using the CE-LIF method.

Chlorimuron ethyl belongs to the sulfonylurea group of herbicides and can undergo acid and base hydrolysis giving rise to a sulfonamide, a heterocyclic amine and carbon dioxide [26]. The resulting amine can be derivatized with reagents such as dansyl chloride. This principle was used for derivatizing a sulfonylurea drug with dansyl chloride

following hydrolysis [23]. We modified this procedure slightly to derivatize chlorimuron ethyl with dansyl chloride following hydrolysis and measured the UV absorbance spectrum of the dansyl derivative (data not shown). The maximum absorbance for the dansyl derivative of chlorimuron ethyl was determined to be around 340 nm. The 325 nm He/Cd laser was found to be suitable for excitation in CE-LIF experiments. A similar wavelength laser was used for detecting dansylated nucleotides that have an excitation maximum of 340 nm [27]. Fig. 8 shows a typical electropherogram of the dansyl derivative of chlorimuron ethyl using the CE-LIF method. A buffer consisting of 10 mM sodium borate with 30 mM SDS, pH 9.2, was found to be suitable for analysis. The detection limit in water samples is estimated to be 10 ng/l for chlorimuron ethyl using the CE-LIF method.

4. Conclusions

The results reported herein demonstrate that atrazine, simazine, alachlor and metolachlor can be determined simultaneously using CE with ultraviolet detection. In addition, CE-UV was found to be suitable for analyzing dicamba, 2,4-D and chlorimuron ethyl. The detection sensitivity in CE can be improved by employing sample concentration techniques and/or by using sensitive means of detection, such as LIF. The latter approach was tested for three herbicides (dicamba, 2,4-D and chlorimuron ethyl). The results show that dicamba, 2,4-D and chlorimuron ethyl can be derivatized with fluorescent tags and analyzed using CE-LIF with a He/Cd laser. The derivatization schemes described in this report can be used for other phenoxy/benzoic acid herbicides and sulfonylurea herbicides, to permit analysis by CE-LIF. The reported CE methods are simple, rapid and efficient; separation of compounds can be carried out in a relatively short amount of time. Our results show that CE provides a powerful new analytical tool for herbicide residue analysis in surface and ground water samples. These methods are being used for analysis of these selected herbicides by CE-UV and CE-LIF in water, soil and crop samples obtained from different locations in North Carolina, USA.

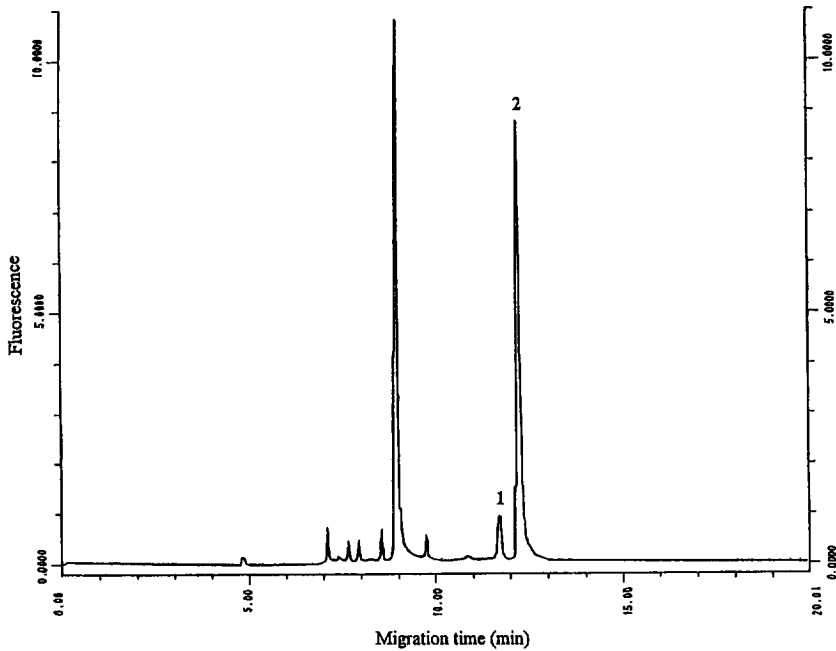


Fig. 6. Electropherogram of methylmethoxycoumarin esters (MMCE) of dicamba and 2,4-D: (1) MMCE of 2,4-D, (2) MMCE of dicamba. Analysis conditions: 50 cm \times 50 μ m I.D. capillary column; pressure injection (2 s=2.4 nl); 10 mM Na₂B₄O₇ plus 100 mM NaCh buffer, pH 9.3; 20 kV (47 μ A); He/Cd laser fluorescence, λ_{ex} 325 nm, λ_{em} 400 nm.

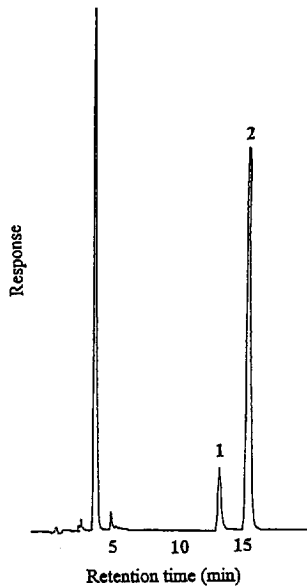


Fig. 7. HPLC chromatogram of methylmethoxycoumarin esters (MMCE) of dicamba and 2,4-D: (1) MMCE of 2,4-D, (2) MMCE of dicamba. HPLC conditions: HP ODS Hypersil column (5 μ m, 200 \times 4.6 mm); mobile phase, acetonitrile–water (55:45, v/v) plus 0.125% acetic acid (isocratic); 5 μ l injection volume; 340 nm UV absorbance.

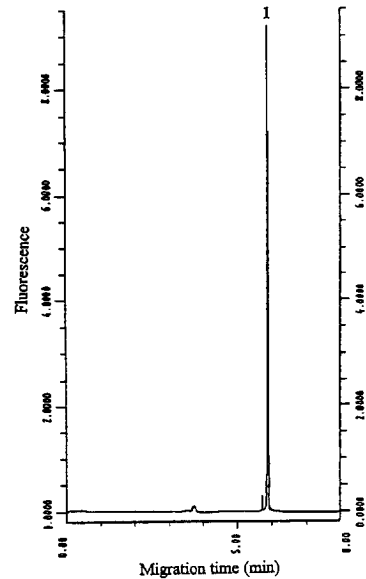


Fig. 8. Electropherogram of the dansyl derivative of chlorimuron ethyl. (1) Dansylated chlorimuron ethyl. Analysis conditions: 50 cm \times 75 μ m I.D. capillary column; pressure injection (1 s=6 nl); 10 mM Na₂B₄O₇ plus 30 mM SDS buffer, pH 9.2; 20 kV (50 μ A); He/Cd laser fluorescence, λ_{ex} 325 nm, λ_{em} 520 nm.

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