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Journal of Chromatography A, 719 (1996) 421–426

JOURNAL OF CHROMATOGRAPHY A

Sensitive and selective method for the determination of sodium monofluoroacetate by capillary zone electrophoresis

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First received 13 February 1995; revised manuscript received 13 June 1995; accepted 6 July 1995

Abstract

Sodium monofluoroacetate (SMFA) is a highly toxic substance and its determination is needed in several areas. Its chromatographic (GC or HPLC) determination is difficult because of its ionic and hydrophilic properties. A simple, sensitive and selective capillary zone electrophoretic method is described for the determination of SMFA. It is well separated from structurally related compounds (formate, acetate, chloroacetate, bromoacetate, etc.) in an acidic electrolyte buffer containing 5 mmol/l phthalate (pH 4.61) and 0.3 mmol/l cetyltrimethylammonium bromide (CTAB) or in a basic electrolyte solution containing 5 mmol/l 4-hydroxybenzoate, 0.3 mmol/l CTAB and 10 mmol/l ammonium (pH 9.49). Good resolution was achieved at relatively low electric field (200–250 kV/cm) and CTAB concentration (0.3 mmol/l). The separation selectivity was improved in acidic phthalate buffer. Using bromoacetate as internal standard, the calibration graph for SMFA was linear over the concentration range 1–10 μg/ml. The detection limit was 0.4 μg/ml. The method was applied to the determination of SMFA in rodenticide baits.

Keywords: Capillary electrophoresis; Optimization; Sodium monofluoroacetate; Pesticides

1. Introduction

High-performance capillary electrophoresis (HPCE) is a powerful separation technique developed in the last decade. More recently, capillary ion electrophoresis [1–5] has developed as a new branch in HPCE. A variety of analytes, including inorganic, organic anions and cations and ionic surfactants could be separated and determined by this technique. Capillary ion electrophoresis has several advantages over other ion analysis techniques: high separation efficiency, short run time, a wide range of analytes, very small sample take-up and direct sample injection with little pretreatment. These aspects make HPCE a unique technique for ion analysis, especially for the assay of anions [6,7].

Sodium monofluoroacetate (SMFA) is a highly toxic rodenticide, which is banned in urban and rural areas in many countries, including China, and its detection and determination are of importance for the enforcement of the regulations. GC [8–10], HPLC [11], ion chromatography [12] and NMR [13–15] have been reported for the determination of SMFA. However, the NMR method requires expensive instrumentation and the GC and HPLC methods are tedious because extraction or/and derivatization procedures are

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0021-9673/96/$15.00
SSDI 0021-9673(95)00752-0

required. Owing to the ionic and hydrophilic properties of SMFA, its chromatographic determination is difficult, but HPCE might be applicable. The determination of SMFA by capillary zone electrophoresis (CZE) was studied and the results are reported in this paper.

2. Experimental

2.1. Chemicals

All solutions were prepared from quartz-redistilled water. Stock solutions of 50 mmol/l potassium hydrogenphthalate (recrystallized), 4-hydroxybenzoic acid, Tris or ammonium buffer were prepared by dissolving the desired amount of these substances in water; dissolution of 4-hydroxybenzoic acid was accelerated by the addition of 1–2 ml of sodium hydroxide solution (1 mol/l). The pH was adjusted to the desired value with 1 mol/l NaOH or HCl. A surfactant stock solution of 5 mmol/l was prepared by dissolving 0.091 g of cetyltrimethylammonium bromide (CTAB) in water. Electrolytes containing indirect detection reagent, surfactant and buffer were obtained by diluting the stock solutions with water, and the pH was measured after dilution.

Pure SMFA, provided by Professor F.-G. Pan (Institute of Epidemiology and Microbiology), was used without further purification; other organic acids were of analytical-reagent or chemical grade. Stock solutions (1.00 mg/ml) were prepared by dissolving the required amount of these compounds in water. Sample solutions were prepared by dilution of the stock solutions with water to 500 μl.

2.2. Extraction of SMFA from rodenticide baits

Rodenticide bait, which were banned from sale and had been submitted for testing by an administration bureau, was weighed and 0.100 g was placed in a vortex tube. Water (4 ml) was added as an extraction solvent. The mixture was mechanically shaken for 2 min, extracted in an ultrasonic bath for 2 min and then filtered. Another 4 ml of water were added to the residues and the shaking, extraction and filtration process were repeated. The filtrate from the first extraction was diluted 100-fold. The dilute solution and the filtrate from the second extraction were subjected to CZE measurement.

2.3. Apparatus and electrophoresis

A BioFocus 3000 capillary electrophoresis system (Bio-Rad Labs., Hercules, CA, USA) equipped with a high-voltage power supply (30 kV) and control software was used for all separations; a domestic fused-capillary with polyimide-coated outer surface, 50 cm in total length (45.5 cm to detector) with 50 μm I.D. and 375 μm O.D., was used. A small section (ca. 0.5 cm) of the capillary coating, which was used as the window of the on-column UV detector cell, was burning off with a match. The unbounded capillary was washed with 0.1 mol/l NaOH for 1 h, rinsed with water, then equilibrated with the electrolyte for 10 min. The power polarity at the injection end of the capillary was set to negative by computer software and the other end near the detector was grounded. The electric field was 10 kV or 12.5 kV per 50 cm. Samples were injected electrophoretically or hydrodynamically by pressure difference between the capillary inlet and outlet. Injection conditions of 5 p.s.i. s related to a sample volume in the range 5–7 nl for a 50 cm $\times$ 50 μm capillary. Indirect detection was performed at 200 and 230 nm for phthalate background electrolyte or at 200 and 249 nm for 4-hydroxylbenzoate electrolyte, and peak integration was carried out with the data monitored at 230 or 249 nm. Electropherograms were recorded and evaluated on an IBM-compatible 486 computer with Bio-Rad Spectra and Integrator software.

3. Results and discussion

3.1. Selection of indirect detection reagents and electrolyte buffers

For the CZE separation of anions, two steps should be adopted to reduce run time. First, electroosmotic flow (EOF) is reversed by the

addition of a special substance to the electrolyte buffer. A tetraalkylammonium surfactant can react with the capillary inner wall and reverse the EOF. Second, the CE instrument is set in such a configuration that sample injection is at the negative terminal of the high-voltage power and the detector is near the positive terminal. The detection of non-absorptive anions is achieved by the indirect ultraviolet (UV) method. A UV-absorbing anion is added to the buffer to produce a high background absorbance. As each analyte of interest passes through the capillary detection window, a displacement of the background anion occurs to yield a negative peak, allowing the detection of the analyte. The UV-absorbing anion should have strong absorbance and have a similar electrophoretic mobility to that of analytes, so that maximum response and symmetrical peaks of analytes are produced.

Based on our preliminary experimental results, 5 mmol/l potassium hydrogenphthalate was chosen as both the buffer and background absorption electrolyte for the separation and detection of SMFA under acidic buffer conditions. Under basic buffer conditions, 5 mmol/l 4-hydroxybenzoate was selected as the indirect detection reagent and 10 mmol/l NH_4^+–NH_3 as the buffer, which was superior to Tris for the separation of SMFA and related compounds. CTAB was used as an EOF reverser.

3.2. Selection of detection wavelength

Three-dimensional graphs of absorbance, wavelength and time could be acquired and plotted with the BioFocus 3000 capillary electrophoresis system. From the three-dimensional graphs, UV absorption spectra of phthalate in acidic solution and 4-hydroxybenzoate in basic solution were extracted. As can be seen from the spectra, the maximum absorption wavelengths are 200 and 230 nm for phthalate and 200 and 249 nm for 4-hydroxybenzoate. These were chosen as the detection wavelengths for phthalate- and 4-hydroxybenzoate-containing electrolyte systems, respectively. The maximum absorption wavelengths of the background electrolytes were monitored in order to increase the responses of anions or decrease the detection limit.

3.3. Optimization of experimental conditions

The CZE determination of SMFA was performed under both acidic and basic buffer conditions, so that independent migration times were obtained and used for the determination of SMA in real samples with reduced chances of errors. In a basic electrolyte containing 5 mmol/l 4-hydroxybenzoate, 10 mmol/l ammonium and CTAB, the effects of CTAB concentration, buffer pH, run voltage and capillary temperature on the separation of SMFA and bromoacetate (internal standard) were examined by a trial and error method. The results suggested that the run voltage and CTAB concentration have a significant influence on the separation, whereas the capillary temperature has little effect. At lower run voltages and CTAB concentrations, better resolution could be achieved. Under the optimum experimental conditions (0.3 mmol/l CTAB, buffer pH 9.49, run voltage −12.5 kV and capillary temperature 25°C), SMFA was separated from structurally related monocarboxylates and dicarboxylates, such as formate, acetate, SMFA, chloroacetate and bromoacetate.

A statistic orthogonal design was employed to evaluate the effect of CTAB concentration, run voltage and capillary temperature on the separation of SMFA and bromoacetate under acidic buffer conditions. The experiments were arranged according to an $L_9(3^4)$ orthogonal design table, the resolution between SMFA and bromoacetate was taken as target function and the experimental results were calculated using Statistic Analysis System (SAS) software (Table 1). The statistical results indicated that the influence of these factors on the separation follow the order: run voltage > CTAB concentration > capillary temperature. Again we observed that lower run voltages and CTAB concentrations resulted in better resolution.

At pH 4.11 and the optimized CTAB concentration (0.3 mmol/l), run voltage (−10 kV) and capillary temperature (25°C), SMFA was well separated from bromoacetate and acetate, but overlapped with formate. To separate SMFA

Table 1
Optimization of experimental factors using orthogonal design

CTAB concentration (mmol/l)	Run voltage (kV)	Capillary temperature (°C)	Optimized factor level
A1 (0.1)	B1 (10)	C1 (15)	A2 (0.3 mmol/l)
A2 (0.3)	B2 (12.5)	C2 (20)	B1 (10 kV)
A3 (0.5)	B3 (15)	C3 (25)	C3 (25°C)

The experiments were conducted in phthalate buffer (pH 4.11) and electropherograms were monitored at 230 nm. The concentration of SMFA and bromoacetate was 10 μg/ml.

and formate, the buffer pH was changed because pH plays an important role in the CZE separation of organic acids. As the buffer pH changed from 4.11 to 4.61, the resolution between SMFA and formate increased from 0 to 2.22. At pH 4.61, SMFA was well separated from structurally related compounds, as shown in Fig. 1. The acetate peak is well behind the peaks of formate, SMFA, chloroacetate and bromoacetate because acetate migrates much more slowly at pH values smaller than its pK_a.

3.4. Effect of injection volume

Among the experimental parameters, injection volume has different effects on resolution and detection limit. The resolution and detection limit are high when a lower volume of sample is injected, and vice versa. A higher resolution and lower detection limit are always expected for an analytical method. Our strategy is to look for a reasonable injection volume at which the detection limit is low and the resolution is acceptable. In our experiments, the resolution between SMFA and bromoacetate decreased from 3.57 to 3.01 when the pressure injection volume increased from 5 to 15 p.s.i. s, whereas it dropped from 3.06 to 1.85 when the electrophoretic injection parameter varied from 5 kV·5 s to 10 kV·5 s. With an increase in injection volume, the theoretical plate number also dropped and the migration time decreased slightly. The latter phenomenon could be explained as described elsewhere [16]. In this investigation, an injection volume of 5–10 p.s.i. s or 7.5–10 kV·5 s was selected.

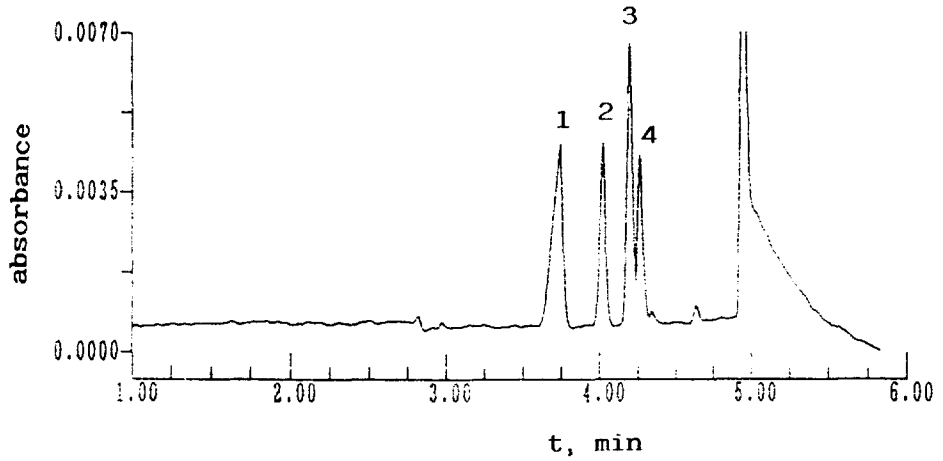

Fig. 1. Electropherogram of (1) formate, (2) SMFA, (3) chloroacetate and (4) bromoacetate in acidic buffer. The experiment was performed at 25°C in a buffer containing 5 mmol/l phthalate (pH 4.61) plus 0.3 mmol/l CTAB. The run voltage and detection wavelength were −10 kV and 230 nm, respectively, injection volume was 5 p.s.i. s and the concentration of the compounds was ca. 10 μg/ml.

3.5. Calibration graph

The internal standard calibration method was used for the quantification of SMFA, in order to minimize deviations. A good internal standard should be well separated from the test compounds, have the same or similar chemical or electrophoretic properties, be stable under the experimental conditions, not be present in the samples and be easily obtained. Bromoacetate meets these requirements fairly well. Using bromoacetate as an internal standard, the calibration graph for SMFA was plotted under acidic or basic buffer conditions. The ratio of migration time-calibrated peak area (A/t_m) of SMFA to that of bromoacetate showed a good linear relationship with the concentration ratio of SMFA to bromoacetate, the correlation coefficient (r) being greater than 0.98. The calibration range, regression equation and the detection limit (DL) are summarized in Table 2. With the hydrodynamic injection mode, the DL is lower in an acidic than a basic buffer.

3.6. Reproducibility

The within-run and between-run reproducibility of the method was examined. A sample containing 2 μg/ml of SMFA and 10 μg/ml of bromoacetate was successively measured five times in 500 μl of electrolyte containing 5 mmol/ml phthalate (pH 4.61) and 0.3 mmol/l CTAB. The R.S.D.s for absolute and relative migration times were small (0.42% and 0.12%, respectively), whereas that for the peak-area ratio was 7.4%. The latter relatively large value might result partly from the relatively low concentration of SMFA (2 μg/ml). The between-run reproducibility was examined by measuring six points of migration time or relative migration time on three days, two points each day. The R.S.D.s for migration time and relative migration time were 1.6% and 0.15%, respectively.

3.7. Determination of SMFA in rodenticide baits

The method was employed to determine SMFA in rodenticide baits. The bait was extracted, the supernatant of the extract was taken and diluted and the dilute solution was subjected to HPCE measurement after the addition of an internal standard and filtration through a 0.45-μm membrane. The electropherogram is shown in Fig. 2. As can be seen, there is no interference in the determination of SMFA. Two batches of baits were analysed and their contents of SMFA were determined to be 0.92% and 0.16%, respectively. Although the determined content of SMFA varied from one batch of baits to another, the calculated recovery was the same (90%). The recovery was calculated by extracting the baits separately twice, analysing the first and second extracts and calculating the recovery with the equation

$$\text{recovery} = [(A/t_m)_s/(A/t_m)_i]_{f.e.}/\{[(A/t_m)_s/(A/t_m)_i]_{f.e.} + [(A/t_m)_s/(A/t_m)_i]_{s.e.}\}$$

Table 2
Calibration range, regression equation, correlation coefficient and detection limit (DL)

Electrolyte[a]	Injection mode	Calibration range (μg/ml)	Regression equation[b]	r	DL (μg/ml)
Acidic	Hydrodynamic	1–10	$y = 1.10x + 0.080$	0.98	0.4
	Electrophoretic	0.2–1	$y = 1.10x + 0.080$	0.98	0.1
Basic	Hydrodynamic	2–10	$y = 1.72x + 0.086$	0.99	2
	Electrophoretic	0.1–1	$y = 6.15x + 1.21$	0.99	0.1

[a] Acidic electrolyte contained 5 mmol/l phthalate (pH 4.61) and 0.3 mmol/l CTAB and basic electrolyte contained 5 mmol/l 4-hydroxybenzoate plus 0.3 mmol/l CTAB and 10 mmol/l ammonium (pH 9.49).

[b] $y = [(A/t_m)_{SMFA}]/[(A/t_m)_{bromoacetate}]$; $x = C_{SMFA}/C_{bromoacetate}$; $n = 5$.

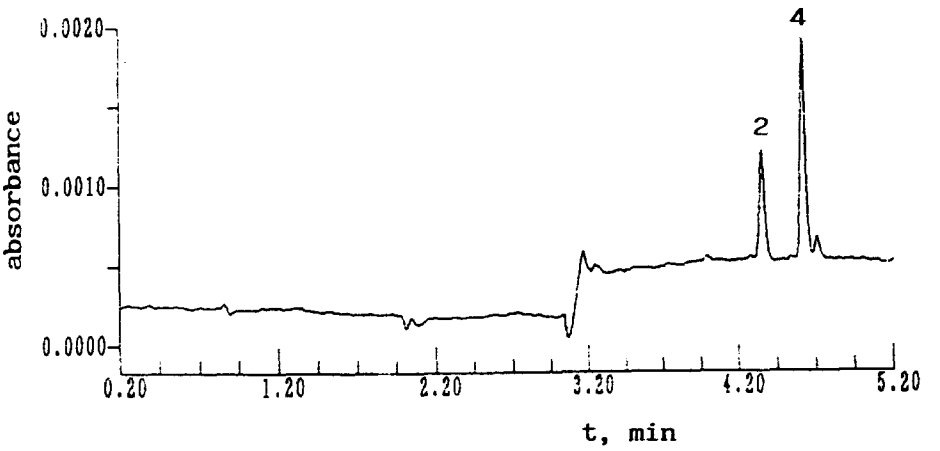

Fig. 2. Electropherogram of the extract of rodenticide bait. Corn bait (0.100 g) was extracted with 6 ml of redistilled water and the extract was diluted by 100-fold. The dilute solution was analyzed after 20 μg/ml of bromoacetate had been added as an internal standard. Other conditions and peak numbers as in Fig. 1.

where A is peak area, t is migration time (min) and the subscript s, i, f.e. and s.e. represent sample, internal standard and the first and second extraction, respectively.

In conclusion, this CZE method for the determination of SMFA is rapid and simple and requires no extraction and derivatization procedure, and hence has advantages over chromatographic methods for the determination of SMFA.

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