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Simultaneous separation and on-line concentration of amitrole and benzimidazole pesticides by capillary electrophoresis with a volatile migration buffer applicable to mass spectrometric detection

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Abstract

This study used capillary electrophoresis (CE) to investigate the simultaneous separation and on-line concentration of five pesticides: amitrole (AMT), carbendazim (MBC), 2-aminobenzimidazole (ABI), thiabendazole (TBZ) and 1,2-diaminobenzene (DAB). A volatile migration buffer was used for the investigation because of the applicability to mass spectrometric (MS) detection. They were separated completely at pH 4.0 as a result of changing pH using formic acid–ammonium formate buffer. Values of the dissociation constant for MBC and DAB estimated from the changes in the mobility with pH showed good agreement with values in the literature. Dissociation constants for AMT and TBZ were estimated. Limits of detection (LODs) for the analytes were on the ppm level with UV detection under the optimized separation condition. On-line concentration by simple stacking mode was not effective except to 2-aminobenzimidazole because of the peak tailing. The addition of formic acid to sample matrix improved the peak shapes. That improvement may be attributed to transient isotachophoretic effect. The concentration factors obtained from the comparison of the LODs were in the range of 7.6–27-fold. This concentration method was applied preliminarily to CE with MS detection.

Keywords: Buffer composition; Amitrole; Benzimidazole; Pesticides; Endocrine disruptors

1. Introduction

Pesticides with different properties are widely used. Gas chromatography–mass spectrometry (GC–MS) is used generally for analysis of volatile pesticides because of its high resolution and identification efficiency. Nevertheless, some pesticides are ionic, polar, or thermally degradable compounds. They cannot be analyzed directly by GC–MS. The "Strategic Programs on Environmental Endocrine Disruptors'98" (SPEED'98) arranged by the Ministry of the Environment, Japan, lists 44 suspected pesticides [1]. For three of them, amitrole (AMT), benomyl, and methomyl, liquid chromatography (LC), or GC–MS after derivatization are regulated as their respective analytical methods [2]. Capillary electrophoresis (CE) can be applied easily for analysis of non-volatile or thermally degradable chemicals. There are some reviews for the analysis of pesticides by CE [3–6]. AMT and benomyl are considered to be more suitable for CE analysis than that of LC because of their high basicity. In water, benomyl promptly decomposes to carbendazim (methyl-2-benzimidazole carbamate, MBC), another benzimidazole pesticide [7]. For that reason, MBC was determined instead of benomyl in environmental waters. Although determination of AMT [8,9] or MBC [10–13] by CE with UV detection has been reported, their simultaneous determination has not been investigated, as we know. Moreover, two degradation products of MBC [7], 2-aminobenzimidazole (ABI) and 1,2-diaminobenzene (DAB) are also considered to be suitable for CE analysis,

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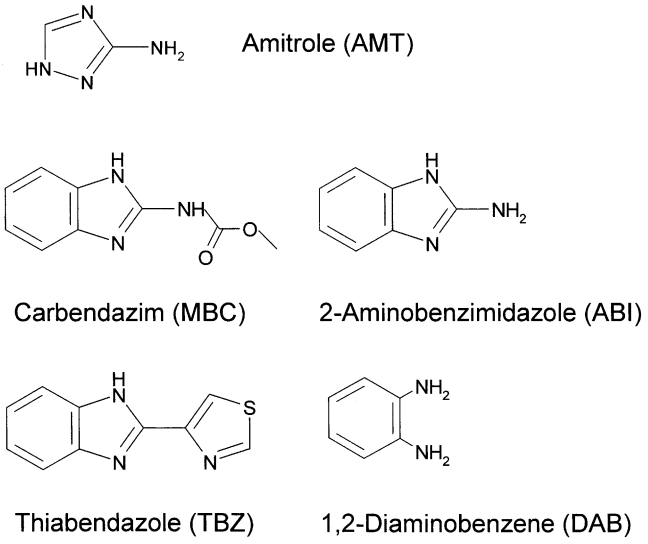

Fig. 1. Structures of target pesticides.

but their CE analysis has not been reported. The simultaneous determination of MBC and degradation products considered being important for the prediction of MBC degradation behavior in environmental waters. Another benzimidazole pesticide, thiabendazole (TBZ) were analyzed by CE–MS [14] or CE with diode-array detection [15]. The simultaneous separation of MBC and TBZ has been investigated by LC–MS [16], on the other hand, not yet by CE. It seems to be useful that these substances can be analyzed simultaneously by one measurement. Although the off-line concentration method has been sometimes used for their CE analysis because of low concentration sensitivity, it is complex and time-consuming. Therefore, we investigated simultaneous separation and on-line concentration of those five pesticides by CE. MS detection is desirable for CE as well as for GC and LC because of their identification in environmental samples. We used volatile migration buffer for their analysis because of its applicability to MS detection.

2. Experimental

2.1. Chemicals

AMT, MBC, ABI and TBZ were obtained from Wako (Osaka, Japan). DAB was obtained from Nacalai (Kyoto, Japan). Structures of these compounds were shown in Fig. 1. Ammonium formate was obtained from Wako (Osaka, Japan). Formic acid was obtained from Yoneyama (Osaka, Japan). Methanol was obtained from Kanto (Tokyo, Japan). All reagents and solvents were of analytical grade and used without further purification. Distilled water for buffer preparation was prepared by Milli-Q Jr. (Millipore, CA, USA).

2.2. Equipment

A CAPI-3300 CE system (Otsuka Electronics, Osaka, Japan) was used for the CE separation and UV detection. For the MS detection, a M-8000 3DQMS system (Hitachi, Tokyo, Japan) was used. Their combination was performed by a laboratory-made electrospary ionization (ESI) interface whose details were described former publication [17]. A Model 11 syringe pump (Harvard Appratus, MA, USA) was used when direct mode MS detection or for the delivery of sheath liquid. A high-voltage power supply for the ESI was a HCZE-30PN0.25 power supply (Matsusada, Shiga, Japan).

A 50 μm i.d. × 375 μm o.d. fused silica capillary (Polymicro Technologies, AZ, USA) of 62 cm total length was used for UV detection. The effective length was 50 cm to the detector. A 50 μm i.d. × 192 μm o.d. fused silica capillary (Polymicro Technologies, AZ, USA) of 110 cm total length was used for direct injection of samples to MS, or CE–MS.

2.3. Procedure

CE running solutions were prepared by mixing 50 mM formic acid and 50 mM ammonium formate adjusted to appropriate pH. These solutions were filtered through a 0.45 μm pore size membrane filter prior to use. Stock solutions of pesticides were prepared in methanol.

In CE separation with UV detection, a 50 mM formate buffer was used for the inlet buffer, and a mixture of 50 mM formate buffer–methanol (1:1) was used for the outlet buffer. When the running solution was changed, the capillary was rinsed with 0.1 M ammonia solution for 10 min, followed by subsequent rinses of distilled water for 3 min and running buffer for 3 min. After each run, the capillary was rinsed with running buffer for 3 min. The concentration of sample solution was 50 mg/l for the investigation of separation, or 5 mg/l for the investigation of on-line concentration. Sample injections were made by pressure (5 kPa). The set-up voltage and temperature were 20 kV and 25 °C, respectively. Migrated samples were detected by on-column measurement at 210 nm.

Each sample solution (150 mg/l) was directly introduced into the ESI interface at 2 μl/min with a syringe pump to investigate sheath liquid composition for MS detection. In the case of CE–MS, the flow rate of sheath liquid was 2 μl/min. The flow late of nitrogen gas was set to 1.4 l/min. ESI applied voltage, MS focus voltage and MS drift voltage were set to 4.5 kV, 30, and 20 V, respectively. Other conditions were as the same as those for UV detection.

3. Results and discussion

3.1. Simultaneous separation and pK_a estimation of pesticides

The effect of pH was investigated for the simultaneous separation of five pesticides. The respective dependencies of

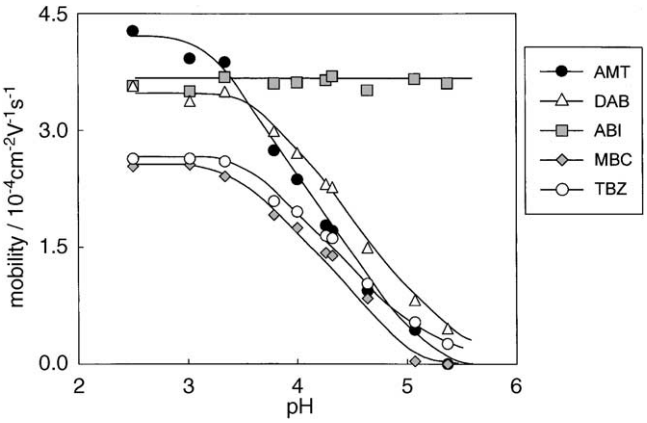

Fig. 2. Dependence of the mobilities of pesticides on pH. Conditions: capillary, 50 μm i.d. × 62 cm (50 cm effective length); running solution, 50 mM formate (inlet) and 50 mM formate–methanol (1:1) (outlet); sample concentration, 50 mg/l; injection, pressure (5 kPa) × 2 s (ca. 3.1 nl); separation voltage, 20 kV; temperature 25 °C; detection wavelength, 210 nm.

their mobilities on pH are shown in Fig. 2. Those mobilities, except for ABI, varied considerably in this pH range. Complete separation was achieved at pH 4.0. Mobility variations in CE can be related to their dissociation constants (pK_a) [18–21]. The relation are estimated roughly as follows:

$$\mu \approx \frac{[H^+]}{[H^+] + K_a}\mu_{BH^+}$$

where μ is the observed mobility and μ_{BH^+} the mobility of completely conjugated acid form. This equation is not considered the contribution of activity coefficient. Table 1 shows the estimated values of pK_a from the least-square fitting of the experimental data along with values from the literature [22]. Values for MBC and DAB almost agree. Therefore, values for AMT and TBZ estimated from the experimental data seem to be valid. Estimation of pK_a values is important for assessment of their environmental fate using quantitative structure-activity relationship [23].

Under optimized separation conditions, the limits of detection (LODs) of five pesticides calculated from the calibration data were in the range of 0.25–1.0 mg/l. A combination with some concentration method is necessary for their detection at lower concentration.

Table 1
pK_a values for the pesticides estimated from experimental data and the literature

	pK_a (estimated value)	pK_a (literature value)
AMT	4.07	No data
DAB	4.49	4.57[a]
MBC	4.46	4.48
TBZ	4.59	No data

[a] This value is the pK_{a2} value ($pK_{a1} = 0.80$).

3.2. On-line concentration: simple stacking

Various on-line concentration methods were investigated in CE [24]. Very high concentration efficiency was obtained by some methods, such as sweeping in micellar electrokinetic chromatography [25]. However, methods using non-volatile buffer or surfactant are not suitable for the combination of MS.

For that reason, we first investigated simple stacking using distilled water as the sample matrix. When migration buffer was used as the sample matrix, the shapes of all peaks were distorted along with the increase of injection time. When distilled water was used, the peak of ABI was relatively sharp (N is ca. 10^5), but other peaks were remarkably tailed. Those peak tailings may result from slower mobilities of samples, except ABI, than that of the background co-ion (ammonium ion) [26,27].

3.3. On-line concentration: addition of formic acid to sample matrix

Next, we investigated the addition of formic acid to the sample matrix. The increased sample mobilities in the sample matrix that resulted from the decreased pH were expected to improve the peak shapes. Therefore, we investigated three different concentration solutions of formic acid (50, 200 mM and 1 M). The theoretical plate numbers of all peaks as injection time were not so decreased in contrast to the case in which distilled water was used as the sample matrix. Peak sharpness and separation were acceptable even when injection time was increased to 30 s. Therefore, we set the optimized injection time to 30 s.

Pherograms at large volume injection (30 s, injection volume was ca. 46 nl) are shown in Fig. 3. The peak sharpness, except that for ABI, improved as the formic acid concentration increased. This effect appeared strongly for pesticides with relatively low mobilities. This phenomenon was identical to the case of on-line concentration using transient isotachophoresis (ITP) for nitrite and nitrate ion analysis in seawater [28]. For these pesticides, as well as the analysis of rare earth ions [29], ammonium ion in migration buffer was considered to possibly correspond to leading ion; also, hydrogen ion in the sample matrix may correspond to terminating ion in transient ITP. The peak resolution using 1 M formic acid was lower than that using 50 or 200 mM formic acid. Furthermore, unknown small peak appeared in front of ABI when formic acid concentration was 200 mM or 1 M. For that reason, we used 50 mM formic acid as the optimized sample matrix.

Calibration data of pesticides were calculated using standard samples (0.2–5 mg/l) with optimized sample matrix. Although calibration curves using peak heights were not linear, calibration curves using peak area had good linearity. Calibration data with peak area, relative standard deviations (R.S.D.s) and LODs, are shown in Table 2. The LODs at normal injection (2 s) are also shown there. Concentration

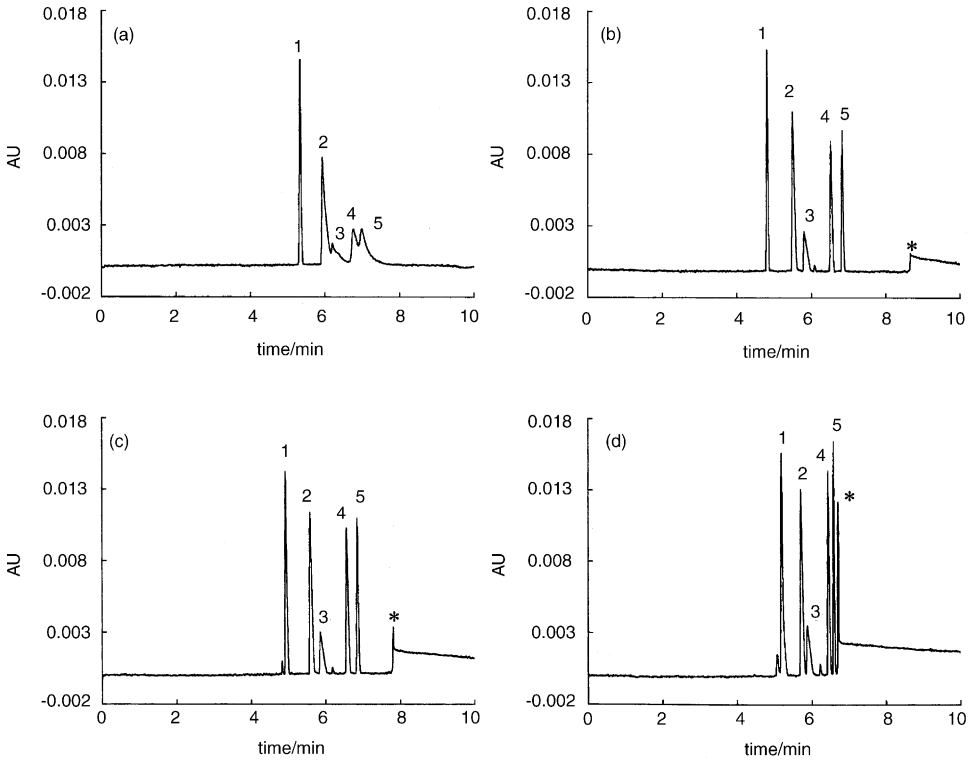

Fig. 3. Pherograms changing formic acid concentration in the sample matrix with UV detection in large volume injection. Sample matrix: (a) distilled water; (b) 50 mM formic acid; (c) 200 mM formic acid; (d) 1 M formic acid. Conditions: sample concentration, 5 mg/l; injection, pressure (5 kPa), 30 s (ca. 46 nl). Other conditions as in Fig. 2. Peak identification: 1 = ABI; 2 = DAB; 3 = AMT; 4 = TBZ; 5 = MBC; (*) formic acid.

factors obtained from comparison of the LODs were in the range of 7.6–27-fold.

3.4. Preliminary application to CE–MS

Initially, the sheath liquid composition was investigated by direct injection. Three sample matrices were used in 1:1 mixtures of methanol with 50 mM formic acid, 50 mM formate buffer (pH 4.0), or 50 mM ammonium formate. Molecular ion peaks ($[M + H]^+$) were observed for all pesticides (*m*/*z* 85 for AMT, *m*/*z* 109 for DAB, *m*/*z* 134 for ABI, *m*/*z* 192 for MBC and *m*/*z* 202 for TBZ). The highest signals were obtained using 50 mM formic acid–methanol (1:1). Therefore, we used this solution as the sheath liquid for CE–MS.

Fig. 4 shows results obtained for normal (5 s, a) and large volume injection (60 s, b and c) of samples. Two sample matrices, distilled water (b) and 50 mM formic acid (c), were used in the case of large volume injection. Sample concentrations were 50 mg/l (a) or 5 mg/l (b and c), respectively. Similar peak heights were obtained in the case of large volume injection even though the concentrations were one-tenth that of the case of normal injection. Furthermore, separation efficiency was maintained using 50 mM formic acid as the sample matrix.

Regarding MS detection with our CE–MS system, sensitivity was about one-tenth that with UV detection and R.S.D.s of peak area exceeding 10% because of the high noise level originated from the instability of the electrospray at the laboratory-made interface. Although the obtained re-

Table 2
Calibration data, limits of detection (LODs) and relative standard deviations (R.S.D.s) of five pesticides in large volume injection (30 s, ca. 46 nl), LODs in normal injection (2.0 s, ca. 3.1 nl), and concentration factors, with UV detection (210 nm)

	ABI	DAB	AMT	MBC	TBZ
Calibration line	$y = 7.3x + 0.22$	$y = 6.9x - 0.011$	$y = 2.8x + 0.83$	$y = 5.9x + 0.24$	$y = 3.6x + 0.12$
Correlation coefficient	1.000	1.000	0.999	1.000	1.000
LOD (S/N = 3) (mg/l) [*a*]	0.014	0.016	0.13	0.047	0.033
R.S.D. (1 mg/l, $n = 3$)					
Migration time (%)	0.1	0.3	0.7	0.2	0.3
Peak area (%)	2.9	4.9	4.6	4.3	4.1
LOD (S/N = 3) in normal injection (mg/l) [*b*]	0.35	0.25	1.0	0.73	0.89
Factor (=[*a*]/[*b*])	24	15	7.6	16	27

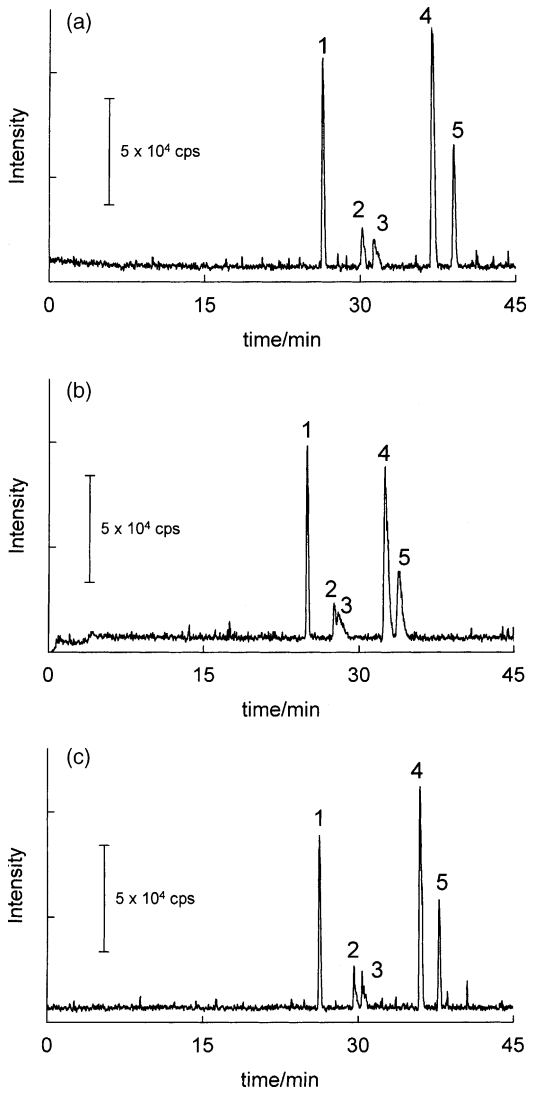

Fig. 4. Ion pherograms in normal and large volume injection with MS detection. Sample matrix: (a) running buffer; (b) distilled water; (c) 50 mM formic acid. Conditions: capillary, 50 μm i.d. × 110 cm; sample concentration, (a) 50 mg/l; (b and c) 5 mg/l; injection, (a) pressure (5 kPa), 5 s (ca. 4.3 nl); (b and c) pressure (5 kPa), 60 s (ca. 52 nl); sheath liquid flow-rate, 2 μl/min; nitrogen gas flow-rate, 1.4 l/min; CE separation voltage, 15.5 kV; ESI voltage, 4.5 kV. Other conditions as in Fig. 2. Included ion ranges in pherograms; *m/z* 84–86, 108–110, 133–135, 191–193 and 201–203.

sults do not allow strictly quantitative comparison, it was clear that on-line concentration by addition of formic acid was as successful as in the case of UV detection.

4. Conclusion

Simultaneous separation and on-line concentration of five basic pesticides by CE were achieved with volatile migration buffer. The procedure for on-line concentration was simple: formic acid was only added to the sample matrix. This developed technique may be useful for measurement of real samples by a more reliable CE–MS system.

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