Separation of Sympathomimetic Amines of Abuse and Related Compounds by Micellar Electrokinetic Chromatography

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Separation of twelve sympathomimetic amines and related compounds by micellar electrokinetic chromatography (MEKC) with UV absorbance detection is described. These amines were well separated within 25 min using 50 mM sodium tetraborate solution containing 15 mM sodium dodecylsulfate (SDS) of pH 9.3 as a running solution and detected at 210 nm. MEKC was performed with an applied voltage of 13 kV at 25 °C using a fused-silica capillary (50 cm×75 mm i.d.) with effective length of 37.5 cm. The detection limits of these compounds were in the range from 4 to 97 fmol/injection at a signal-to-noise ratio (S/N) of 3. The reproducibility of the method expressed as relative standard deviation (RSD) for within-day ($n=6$) and between-day ($n=5$) assays was less than 4.8 and 8.8%, respectively. The proposed method could be applied to the determination of an anorectic drug, phentermine, in Chinese tea with a detection limit of 99 μg/g (105 fmol/injection, S/N=3).

Key words micellar electrokinetic chromatography; sympathomimetic amine; phentermine; Chinese tea

Methamphetamine (MP) and amphetamine (AP) are well-known drugs of abuse and the illicit use of MP is spreading and causing serious social problems in Japan. The use of these stimulants is controlled by the Stimulants Control Law for drug offenses. 4-Bromo-2,5-dimethoxyphenylethylamine (2C-B) which has an intensely hallucinogenic effect was also designated as a "narcotic" and has been controlled by the Narcotics and Psychotropic Control Law since 1998. In addition to these drugs, anorectic drugs such as phentermine (PT) and fenfluramine (FF) were illegally used as adulterants in commercial Chinese tea a few years ago in Japan.[1] Due to the increasing diversity of drugs of abuse, a rapid and simple analysis which can be applied to a wide range of analytes for qualitative and quantitative purposes is required in forensic and toxicological studies.

For the analysis of some of these drugs, gas chromatography (GC),[2—4] GC–mass spectrometry (GC–MS)[5—9] and high-performance liquid chromatography (HPLC)[10—14] are exclusively employed. GC–MS methods are generally highly sensitive but require a derivatization procedure prior to the analysis.[5—9] HPLC methods with fluorescence (FL),[11,14] chemiluminescence (CL)[12] and UV–Vis[10,13] detections also require derivatization to increase the sensitivity and a rather long time to separate analytes. As an analytical technique to meet the requirements for the applicability to diverse analytes, capillary electrophoresis (CE) has been developed.[15] We previously described the usefulness of CE with UV absorbance detection for simultaneous determination of six kinds of stimulants and related compounds in urine samples.[16] In this study, we have expanded the analytes to include other amines [*i.e.*, *l*-deprenyl (DPN, a prescription drug for Parkinsonism that is metabolized to *R*(−)-AP and *R*(−)-MP), methylephedrine (ME, a starting material of stimulants), 2C-B (a narcotic) and PT, FF and Mazindol (MZ) (anorexics)], and investigated their separation and detection using micellar electrokinetic chromatography (MEKC). Since the present method is expected to be effective as a rapid and simple qualitative and quantitative analysis for seized drugs and their adulterated foods, its applicability was investigated by applying it to the determination of PT spiked into Chinese tea leaves.

Experimental

Chemicals and Materials 1-Phenylethylamine (1-PA), 2-phenylethylamine (2-PA), PT and FF were purchased from Sigma-Aldrich Japan (Tokyo). MP hydrochloride was obtained from Dainippon Pharmacy (Osaka, Japan). AP sulfate, 4-hydroxyamphetamine (4-HAP), and 4-hydroxymethamphetamine (4-HMP) were synthesized in our laboratory and 2C-B was synthesized by one of the authors. ME and MZ were the gifts of Dainippon Pharmacy and Sandoz Pharmaceutical, Ltd. (Tokyo, Japan), respectively. DPN was supplied by Fujimoto Pharmaceutical Co. (Osaka, Japan). These compounds were dissolved in water to give 0.01 M solutions and then diluted with water to appropriate concentrations prior to use. Sodium tetraborate and sodium dodecylsulfate (SDS) from Wako Pure Chemical Industries (Osaka, Japan) were used as received. Water was deionized and passed through a Pure line WL21 water purification system (Yamato Kagaku, Tokyo, Japan). All other reagents and solvents were of analytical reagent grade.

Apparatus and Conditions The CE analysis was performed on a CAPI-3100A CE system equipped with a photodiode array detector (Otsuka Electronics, Osaka, Japan). A fused-silica capillary (Otsuka Electronics, 50 cm×75 μm i.d.) with an effective length of 37.5 cm was used for the separation. The analytes were injected by siphoning (20 s with 20 mm height differential which corresponded to 8-nl injection) and detected at 210 nm. The separation was carried out at 25 °C with an applied voltage of 13 kV using 50 mM sodium tetraborate solution (pH 9.3) containing 15 mM SDS as a running solution. The capillary was rinsed with the running solution for 5 min before each run.

Sample Preparation for the Determination of PT in Chinese Tea An accurately weighed 10-mg portion of Chinese tea (Genpi-cha) leaves was dried in a desiccator for 12 h at ambient temperature. To this was added 5 ml of methanol (MeOH) and the tea leaves were extracted for 40 min in an ultrasonic bath. After centrifugation (1000 ***g***, 5 min), a 4-ml portion of MeOH was evaporated and 400 μl of water was added to the residue. The resultant solution which passed through a 0.45 μm filter was applied to the CE system. For the preparation of a calibration curve, an appropriate volume of the standard PT solution was spiked into an accurately weighed sample of Chinese tea to give the desired concentration and then the mixture was subjected to the procedure for sample preparation described above.

Results and Discussion

Analytical Conditions In a previous study,[16] MEKC with SDS was better able to separate six kinds of stimulant

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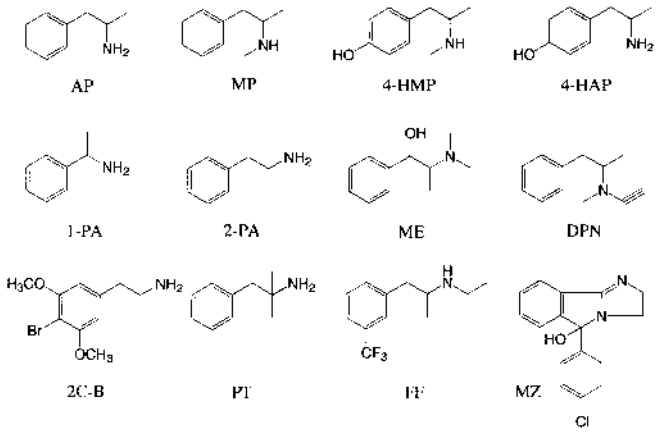

Fig. 1. Structures of Sympathomimetic Amines and Related Compounds Examined

related compounds than was capillary zone electrophoresis (CZE). Separation conditions for twelve sympathomimetic amines (Fig. 1) were thus examined based on the previous MEKC. The effect of pH on the separation was investigated with the following solutions containing 15 mM SDS: 50 mM sodium tetraborate–sodium dihydrogen phosphate (pH 8.8—9.0), sodium tetraborate (pH 9.3) and sodium tetraborate–sodium hydroxide (pH 9.5). The best separation was obtained at pH 9.3 and this pH was selected in this study.

The migration time for FF, 2C-B, MZ, PT, MP and DPN increased with an increase in the concentration of sodium tetraborate in the solution from 25 to 65 mM. In the other amines, the migration times slightly decreased in the presence of sodium tetraborate at more than 50 mM, and the peaks for ME and 2-PA and those for DPN and AP could not be well separated from each other at 65 mM. Therefore, 50 mM of sodium tetraborate was selected for further investigation.

The migration time for each peak increased with an increase in SDS concentration from 10 to 20 mM and 15 mM was employed for further experiments by considering rapid separation.

The applied voltage for the separation also affected the separation of amines. The migration time for all peaks decreased with an increase in the voltage from 12 to 15 kV; 13 kV was recommended for the separation.

Figure 2 shows a typical chromatogram. The twelve sympathomimetic amines could be completely separated from each other within 25 min.

Calibration Curve, Detection Limit and Reproducibility Calibration curves were prepared using known concentrations of standard amines. Table 1 summarizes the calibration curve parameters and the detection limits. A peak-area method providing slightly better precision than a peak-height method was employed to prepare the calibration curves. The relationship between peak-area and concentration was linear from 0.04 to 0.40 pmol/injection for MZ, from 0.08 to 0.80 pmol/injection for 2C-B, and from 0.16 to 1.60 pmol/injection for the other amines. The detection limits calculated were in a range from 4 to 97 fmol/injection at a signal-to-noise ratio (S/N) of 3. This sensitivity is almost comparable to that for an HPLC-UV method employing sodium naphthoquinone-4-sulphonate as a derivatizing reagent[13] and a GC method with a nitrogen-phosphorus detector,[3] but lower than that of GC–MS[7,8] and HPLC–FL methods.[11,14]

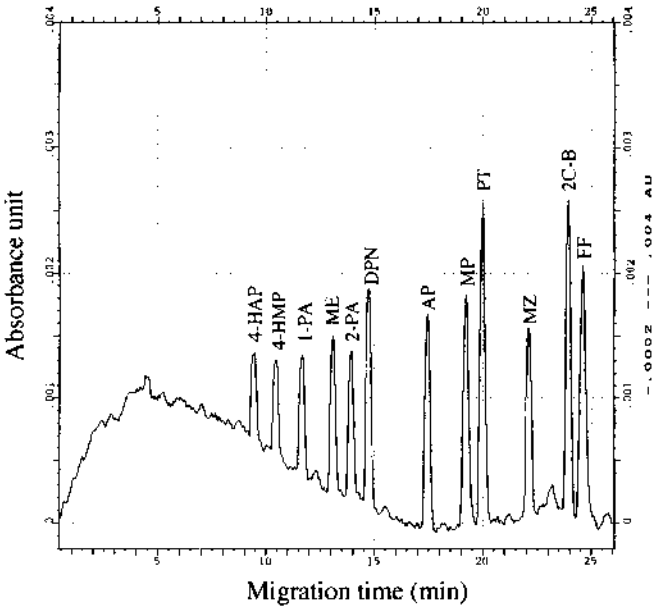

Fig. 2. Chromatogram of Sympathomimetic Amines and Related Compounds

Sample concentrations (pmol/injection): 2C-B (0.40), MZ (0.20) and the other compounds (0.80); other CE conditions are as in Experimental.

Table 1. Calibration Curves and Detection Limits for Sympathomimetic Amines and Related Compounds

Compound	Range (pmol/injection)	Equation[a]	r	Detection limit (S/N=3) (fmol/injection)
4-HAP	0.16−1.60	$y=1.78x+1.29$	1.000	57
4-HMP	0.16−1.60	$y=2.04x-2.85$	0.998	60
1-PA	0.16−1.60	$y=2.36x+0.38$	1.000	97
ME	0.16−1.60	$y=3.12x-0.49$	1.000	62
2-PA	0.16−1.60	$y=2.88x-2.59$	0.999	55
DPN	0.16−1.60	$y=3.92x-0.22$	1.000	42
AP	0.16−1.60	$y=4.32x-9.04$	1.000	29
MP	0.16−1.60	$y=4.78x-11.49$	0.999	23
PT	0.16−1.60	$y=6.62x-13.83$	1.000	15
MZ	0.04−0.40	$y=16.64x-9.26$	0.999	4
2C-B	0.08−0.80	$y=13.03x-15.05$	0.999	5
FF	0.16−1.60	$y=6.39x-15.21$	1.000	50

a) y=peak-area (10^8 AU·min); x=concentration (pmol/injection).

The reproducibility of the proposed method was examined at the 0.20, 0.40 and 0.80 pmol/injection levels for MZ, 2C-B and the other amines, respectively. The reproducibility expressed as relative standard deviation (RSD) obtained with the peak-area method was from 1.0 to 4.8% (within-day, $n=6$) and from 1.4 to 8.8% (between-day, $n=5$), and that with the peak-height method was from 1.2 to 5.8% (within-day) and from 3.4 to 7.7% (between-day). Good precision for migration time was also obtained in within-day assays (RSD: 1.1—1.7%) and between-day assays (RSD: 2.4—3.6%).

Application to the Determination of PT in Chinese Tea The present method is expected to be effective as a rapid and simple qualitative and quantitative analysis for seized drugs and their adulterated foods. However, because of the diffi-

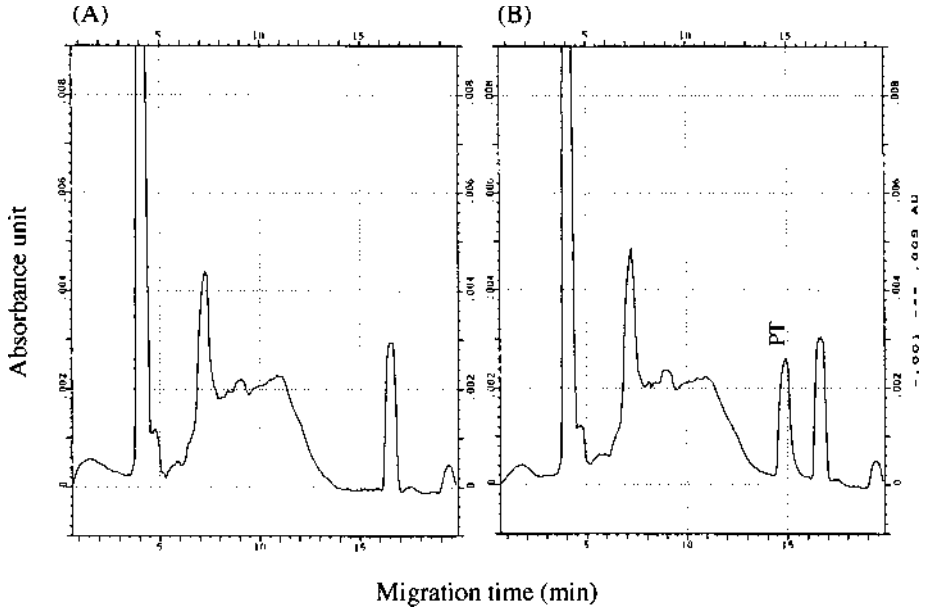

Fig. 3. Typical Chromatograms of Chinese Tea (A) and PT-Spiked Chinese Tea (B)
Chinese tea and that spiked with PT (3.0 mg/g) were treated as in Experimental.

culty in obtaining seized illicit drugs and adulterated samples, Chinese tea spiked with PT and FF were selected as analytes in this study. Both these anorexics, which are not permitted to be used in Japan, were reportedly found as adulterants in Chinese tea[1] and traditional Chinese medicines.[17] To evaluate the applicability of the proposed method, an attempt was made to identify PT and FF spiked into Chinese tea. In a preliminary study, PT and FF extracted from Chinese tea with hot water and MeOH were subjected to the CE analysis. Extraction with hot water did not allow identification of either substance because of interference by a large unknown peak derived from the tea sample. The peak for FF obtained from the sample with MeOH extraction also overlapped with an unknown peak. Therefore, an investigation on the determination of PT in Chinese tea was carried out using the extraction with MeOH. Since no information on adulterated levels of PT in Chinese tea was available, 3 mg/g tea leaves of PT were spiked into Chinese tea as done with FF (4.64—7.59 mg/g tea leaves)[1] to optimize the extraction conditions.

Five milliliters of MeOH was used to extract PT from Chinese tea and the extractability was examined over different periods (10—60 min) using an ultrasonic bath. The recovery of PT reached the maximum (*ca.* 60%) and remained constant at an extraction time of 40 min or more. The number of repeated extractions was further investigated using 40 min for each extraction period. Additional recoveries obtained with 2nd and 3rd extractions were 12% and 5% of the 1st extraction, respectively. Extraction was thus decided to be once for 40 min to simplify the assay procedure.

Typical chromatograms obtained with Chinese tea and tea spiked with PT are demonstrated in Fig. 3. When compared to the separation of standard PT (migration time: 19.7 min), a relatively short migration time of PT (14.8 min) was observed in the sample from Chinese tea. It is known that migration times of analytes are influenced by several factors related to sample matrix such as a surfactant.[18] Coexisting compounds like saponins in Chinese tea might cause the decrease in migration time of PT.

A calibration curve was prepared using spiked Chinese tea with known concentrations of PT. A linear relationship was obtained in the concentration range of 0.3—9.0 mg/g; the regression equation and correlation coefficient were $y=6.56x-39.04$ [where y is peak-area (10^8 AU·min and x is the concentration in μg/g] and $r=0.999$, respectively. The detection limit at S/N=3 was 99 μg/g (105 fmol/injection). The reproducibility of the method was investigated using Chinese tea spiked with PT at the concentration of 1.5 mg/g; RSDs for within-day ($n=5$) and between-day assays ($n=4$) were 4.8% and 5.3%, respectively.

The proposed method was applied to the determination of PT in five kinds of commercially available Chinese teas. Of these samples, components were specified in two samples [black tea (Pou-earl), Coicis Semen, Cassiae Semen, Sweetbrier, Eucommiaceae, Jasmine, Lonicerae Flos, Plantaginis Semen and Sennae Folium; black tea (Pou-earl), Coicis Semen, Cassiae Semen, Eucommiaceae, Hordei Semen, Garcinia, Genmai, Gymnema Sylvestre and Aloe], while the other samples did not state the components. Results of the assay showed none of them to contain PT.

In conclusion, twelve sympathomimetic amines and related compounds could be separated within 25 min using MEKC. The sensitivity as a detection limit for these amines was in the range from 4 to 97 fmol/injection. Although the proposed method is not as highly sensitive as the GC–MS and HPLC–FL methods, its simplicity, rapidity and reliability are indispensable advantages. The method thus might be useful for the rapid identification and determination of a wide range of sympathomimetic amines in seized drugs. As to the applicability of the method, we have shown that it is profitable for the determination of PT in Chinese tea using a simple extraction procedure. However, detailed investigations on sample clean-up might be required when the method is applied to the determination of FF in Chinese tea or other samples that have complicated matrices such as body fluids,

because the CE separation tends to suffer unfavorable effects due to electrolytes and coexisting substances in samples.

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