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Journal of Chromatography A, 1101 (2006) 319–323
JOURNAL OF CHROMATOGRAPHY A
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Short communication

Optimization of the separation and on-line sample concentration of phenethylamine designer drugs with capillary electrophoresis–fluorescence detection

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Received 17 August 2005; received in revised form 9 November 2005; accepted 11 November 2005

Abstract

Five 2C-series of phenethylamine designer drugs, including 2,5-dimethoxy-4-ethylthio-phenethylamine (2C-T-2), 2,5-dimethoxy-4-(*n*)-propylthiophenethylamine (2C-T-7), 4-chloro-2,5-dimethoxyphenethylamine (2C-C), 4-bromo-2,5-dimethoxy-phenethylamine (2C-B), 2,5-dimethoxy-4-iodo-phenethylamine (2C-I), were synthesized and standard GC/MS and fluorescence spectra are reported for them. A mixture of the five drugs was separated and detected by means of capillary electrophoresis (CE) with native fluorescence and light emitting diode (LED)-induced fluorescence (LIF) detection, respectively, for comparison. In the former case, exciting at a wavelength of 300 nm from a Xe lamp was used. The detection limits were found to be only in the range of $\sim 10^{-4}$ M by the micellar electrokinetic chromatography (MEKC) mode but were improved to $\sim 10^{-7}$ M when the sweeping-MEKC mode was used. For a highly sensitive analysis, LED-induced fluorescence detection was examined by derivatizing the compounds with a fluorescent dye, fluorescein isothiocyanate isomer I (FITC). A blue-LED ($\sim$2 mW) was used as the fluorescence excitation source. The detection limits were improved to $\sim 10^{-7}$ and $\sim 10^{-8}$ M, respectively, when the MEKC and stacking-MEKC modes were applied. A mimic urine sample was obtained by spiking urine from a volunteer with the five standards, and after liquid–liquid extraction, the sample was examined by means of the MEKC–LIF mode. The extraction procedures used for the urine sample and the CE conditions for the separation were optimized.

Keywords: Phenethylamine; Designer drugs; 2C-T-2; 2C-T-7; 2C-C; 2C-B; 2C-I; Urine; LED; FITC; LED-induced fluorescence (LIF); Native fluorescence

1. Introduction

The increased availability of 2C-series of phenethylamine derivatives on the illicit market has become a serious social problem [1]. However, little information is available concerning their bioactivities, detection and metabolic fate [2–4]. Shulgin et al, in their publication Phenethylamines I Have Known And Loved (PiHKAL), documented over 250 phenethylamine derivatives, including MDMA (3,4-methylenedioxymethamphetamine), mescaline, 2,5-dimethoxy-4-ethylthio-phenethylamine (2C-T-2), 2,5-dimethoxy-4-(*n*)-propylthiophenethylamine (2C-T-7), 4-chloro-2,5-dimethoxyphenethylamine (2C-C), 4-bromo-2,5-dimethoxy-phenethylamine (2C-B), 2,5-dimethoxy-4-iodo-phenethylamine (2C-I) and many others in 1991; synthetic procedures were also reported in their monograph [5]. However, information with reference to their standard spectra, GC/MS and fluorescence spectra, CE-separation/detection method is still limited. 2C-series phenethylamine designer drugs were introduced as drugs-of-abuse in the Taiwan illicit markets in 2004 (2C-B, 2004; 2C-C and 2C-I, 2005 found by the Forensic Science Center, Military Police Command, Ministry of National Defense). Thus far, 2C-B was permanently placed in Schedule III in 2005 in Taiwan. No evidence for the domestic abuse of the other 2C-series designer drugs has surfaced as of this writing. Thus, synthesizing standards of these 2C-series designer drugs, establishing a reliable analytical procedure, and evaluating their bioactivities would be highly desirable. This is not only with respect to providing valuable information for use

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doi:10.1016/j.chroma.2005.11.029

in forensic analysis but also would be useful in clinical studies. In a previous research project, four metabolites of 2C-T-2 were detected and their metabolism in the rat was outlined, based on a gas-chromatography/mass spectrometry (GC/MS) selected ion monitoring method for the analysis of in vivo urine samples from rats [6]. In this study, we report on the separation and detection of 2C-series phenethylamine designer drugs by means of CE separation with native fluorescence and light emitting diode (LED)-induced fluorescence (LIF) detection, respectively. A method for separating the five designer drugs in a human urine sample by means of MEKC–LIF was optimized. The detection limits and precision of these methods are discussed and complete data on them are reported herein.

2. Materials and methods

2.1. Reagents

All chemicals were of analytical grade and were obtained form commercial sources. Ethyl acetate, methanol, ethanol (EtOH), diethylether, 1,4-dimethoxybenzene, chlorosulfonic acid, zinc, sulfuric acid, ethyl bromide, potassium hydroxide, *N*-methylformanilide, nitromethane, ammonium acetate anhydrous, fluorescein isothiocyanate isomer I (FITC), acetonitrile, sodium dodecyl sulfate (SDS), sodium tetraborate, phosphoric acid, were all purchased from Acros (NJ, USA). Hydrochloric acid was acquired from Tedia company Inc. (Ohio, USA). Anhydrous sodium sulfate, potassium carbonate and sodium hydroxide were purchased from Yakuri (Osaka, Japan) and J. T. Baker (NJ, USA), respectively. The chemical structures of 2C-T-2, 2C-T-7, 2C-C, 2C-B and 2C-I are shown in the inset in Fig. 1A. These compounds are not commercially available, and synthetic procedures for preparing them have been described previously in the literature [5]. Following the synthesis, the final products were identified by NMR, IR and verified by GC/MS.

2.2. Apparatus

2.2.1. Native fluorescence detection system

The CE set-up used is similar to that described previously [7]. The excitation source was selected by a monochromator (Acton Research Corporation; Model SP-150) connected to a Xe lamp (Muller Elektronik Optik, SVX/LAX 1450). Fluorescence data were collected at a right angle to the light source and dispersed by a second monochromator, followed by detection by means of a photomultiplier tube. The fluorescence excitation and fluorescence emission wavelengths were 300 and 340 nm, respectively.

2.2.2. LED-induced fluorescence detection system

The blue-LED light source (∼2 mW), CE set-up and data acquisition system used was the same to that described previously [8] and are abbreviated herein. A high-voltage power supply was used to drive the electrophoresis and a 50-μm i.d. fused silica capillary column was used for the separation

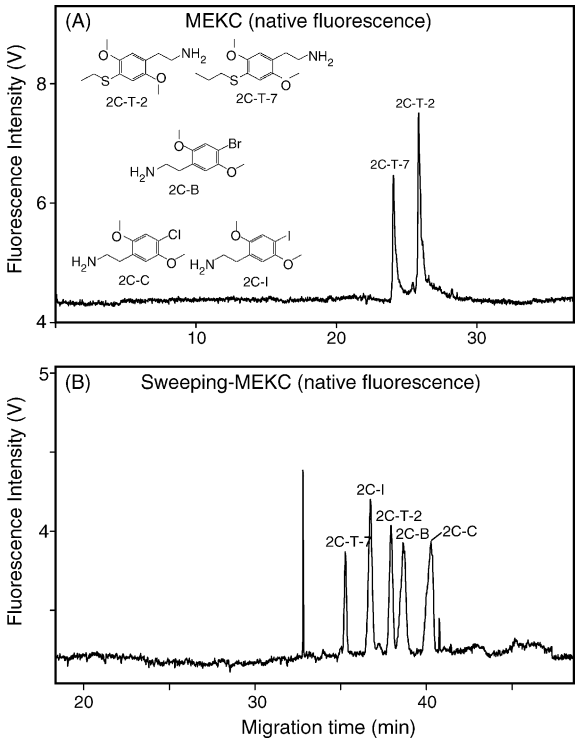

Fig. 1. Native fluorescence detection. Electropherograms of a mixture of the five analytes by various separation modes (frame A, MEKC; frame B, sweeping-MEKC modes, respectively). CE conditions: (A) a solution of acetonitrile/methanol/water (15/20/65, v/v), containing 30 mM H_3PO_4 and 120 mM of SDS. The voltage used was −20 kV (current, −40 to −50 μA); (B) a background electrolyte consisted of acetonitrile/methanol/water (15/10/75, v/v), containing 30 mM H_3PO_4, 100 mM of SDS, 100 mM β-CD and 2.3 M urea. Sample matrix, 30 mM H_3PO_4; sample injected length, 32 cm. The voltage used was −25 kV (current, ∼−40 μA). Sample concentration: frame A, 10^{-3} M for 2C-T-2 and 2C-T-7; frame B, 1.0×10^{-6} M, 1.0×10^{-6} M, 2.3×10^{-5} M, 9.6×10^{-6} M and 8.1×10^{-6} M for 2C-T-2, 2C-T-7, 2C-C, 2C-B and 2C-I, respectively.

(total/effective length: 96/90 cm in the case of sweeping-MEKC and 100/94 cm in the case of stacking, respectively). The sample was hydrodynamically injected by raising the reservoir 48 cm relative to the exit reservoir (at this height, the flow rate for the sample injection was 2.3 cm/min) to provide the injection length (depending on the specific situations).

2.2.3. GC/MS

A gas chromatograph (GC 6890 Hewlett-Packard, Avondale, PA, USA) equipped with a mass spectrometer (Hewlett-Packard 5973 mass selective detector) and was used for the detection. A capillary column (30 m × 0.25 μm i.d.) with an HP-5MS (cross-linked 5% PH ME siloxane) bonded stationary phase film, 0.25 μm in thickness (Agilent Technologies, USA) was used. The inlet temperature was maintained at 250 °C. The column oven was held at 70 °C for 1 min, then programmed from 70 to 200 °C at 15 °C/min, held for 2 min, and then programmed from 200 to 260 °C at 20 °C/min, finally, held for 8.84 min (carrier gas: helium, flow-rate 1 mL/min). The mass spectrometry conditions were as follows: ionization energy, 70 eV; ion source temperature, 230 °C. Data were collected using the Hewlett-Packard Chem-Station software.

2.3. Urine extraction procedure

A 1-mL aliquot of urine in a glass tube was made alkaline by the addition of 2 mg of potassium carbonate and briefly shaken for 2 min. Three milliliters of ethyl acetate were added, and the sample and solvent gently mixed for 10 min, after which, the tube was centrifuged. The upper layer was collected (1.5 mL) and the organic phase was evaporated to dryness. The residue was dissolved in 0.05 mL of ethanol for the subsequent CE separation. The concentration of the sample used for recovery testing was 4.0×10^{-6} M. In a comparison of the five standards, the liquid-liquid extraction efficiency for 2C-T-2, 2C-T-7, 2C-C, 2C-B and 2C-I were determined to be $67.0 \pm 2.3\%$, $68.8 \pm 3.2\%$, $78.5 \pm 6.1\%$, $78.3 \pm 2.0\%$ and $73.2 \pm 3.8\%$, respectively.

2.4. Fluorescence derivatization

FITC is a common fluorescence dye and widely used for in the LIF method [9–12,17]. Herein, to 250 μL solution containing 100 μL of 20 mM sodium tetraborate and 20 mM boric acid solution, 50 μL the 2C-series drugs was added (each 2×10^{-5} M in EtOH). The reaction was initiated by the addition of 100 μL FITC (5×10^{-5} M in an acetone solution) to give concentrations of [2C-series of designer drugs] = 4×10^{-6} M and [FITC] = 2×10^{-4} M. The concentration ratio of FITC/total-analytes was 10/1. The reaction solution was allowed to stand at room temperature in the dark for 10 h. At this moment, the reaction could be completed finished. The resulting derivative was directly used for the subsequent CE separation.

3. Results and discussion

3.1. Native fluorescence detection

The observed fluorescence excitation maxima were at 312.2, 311.8, 299.1, 297.6, and 297.9 nm for 2C-T-2, 2C-T-7, 2C-C, 2C-B and 2C-I; the observed fluorescence emission maxima are 339.8, 338.5, 324.0, 322.8 and 323.5 nm, respectively. The information is useful for fluorescence detection and identification using either CE or HPLC separation. The ratios of fluorescence intensity for 2C-T-2, 2C-T-7, 2C-C, 2C-B and 2C-I are 0.91: 1: 0.03: 0.12: 0.23, respectively. This is because that the heavy-atom effect (Cl, Br, and I atoms on 2C-C, 2C-B and 2C-I, respectively) could cause a weaker fluorescence intensities. For such a weaker fluorescent, an on-line sample concentration technique or a fluorescence derivatization is recommended. Fig. 1 shows typical electropherograms of these analytes by various separation modes (frame A, MEKC; frame B, sweeping-MEKC [13,14] modes, respectively). In frame A, the concentrations of 2C-T-2 and 2C-T-7 are 10^{-3} M, respectively. Since these analytes are not strongly fluorescent, the detection limits are poor; 2C-C, 2C-B and 2C-I are detected with difficulty with this method. However, this can be improved when the sweeping-MEKC mode is used. In such a case, the optimized background electrolyte (BGE) consisted of 100 mM SDS, 100 mM β-CD, urea 2.3 M and 30 mM H_3PO_4 in a mixed acetonitrile–methanol–water solution (15/10/75, v/v). The samples were dissolved in a 30 mM H_3PO_4 solution (a non-micelle matrix). The results obtained by the sweeping-MEKC mode under optimized conditions are summarized in Table 1. In order to investigate the effects of organic solvents in BGE, various ratios of acetonitrile (0, 10, 15 and 20% in water, v/v) and methanol (0, 5, 10 and 15% in water, v/v) were examined. However, the findings show that a mixed acetonitrile–methanol–water solution provided better separation efficiency. Furthermore, since the chemical structures of the five analytes are similar, the addition of 100 mM β-CD to the BGS is necessary. In order to investigate the effects of sample injection length and the corresponding signal intensity when the sweeping-MEKC mode was applied, several different sample injection lengths (15, 24, 32, 40 and 48 cm in capillary length) were examined under exactly the same experimental conditions.

Table 1
Limit of detection (LOD) values and plate number for the 2C-series of phenethylamine designer drugs based on CE/native fluorescence detection method

Compounds	2C-T-2	2C-T-7	2C-C	2C-B	2C-I
MEKC					
LOD	9.6×10^{-5} M	9.0×10^{-5} M	–	–	–
Sweeping-MEKC					
LOD (S/N = 3)	1.1×10^{-7} M	2.2×10^{-7} M	4.7×10^{-7} M	1.1×10^{-6} M	3.3×10^{-7} M
Plate number	4.3×10^{5}	17.8×10^{5}	3.7×10^{5}	2.4×10^{5}	5.1×10^{5}

Table 2
Limit of detection (LOD) values and plate number for the 2C-series of phenethylamine designer drugs based on CE/fluorescence derivatization detection method

Compounds	2C-T-2	2C-T-7	2C-C	2C-B	2C-I
MEKC					
LOD (S/N = 3)	1.2×10^{-7} M	2.0×10^{-7} M	1.1×10^{-7} M	1.4×10^{-7} M	2.7×10^{-7} M
Plate number	2.3×10^{5}	1.5×10^{5}	2.1×10^{5}	1.9×10^{5}	1.7×10^{5}
Stacking-MEKC					
LOD (S/N = 3)	4.0×10^{-8} M	4.2×10^{-8} M	1.8×10^{-8} M	1.9×10^{-8} M	2.2×10^{-8} M
Plate number	9.1×10^{4}	6.8×10^{4}	9.8×10^{4}	9.6×10^{4}	7.1×10^{4}

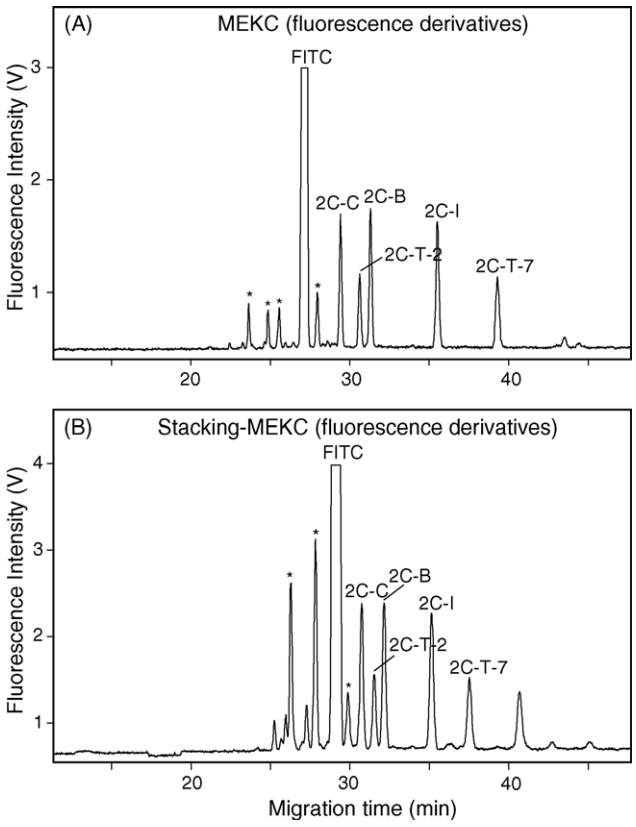

Fig. 2. Fluorescence derivatization detection. Electropherograms of a mixture of the five analytes by various separation modes (frame A, MEKC; frame B, stacking-MEKC modes, respectively). CE conditions: (A) a solution of acetonitrile/water (6/94, v/v), containing 30 mM sodium tetraborate and 60 mM of SDS. The voltage used was +20 kV (current, ~+42 μA); (B) a background electrolyte consisted of acetonitrile/water (8/92, v/v), containing 30 mM sodium tetraborate, 60 mM of SDS; sample injected length, 32 cm. The voltage used was +21 kV (current, ~+26 μA). Sample concentration: frame A, 4.0×10^{-6} M for each; frame B, 4.0×10^{-7} M for each.

The optimal length for sample injection was 32 cm (~1/3 to capillary total length); the longer injection length did not provide a high sensitivity.

3.2. Fluorescence derivatization detection

The derivatization procedures used were as described above. After labeling with FITC, the derivatives are well suited either for an Argon-laser (488 nm) or a blue LED (emitting range, 476 ± 10 nm) for fluorescence exciting. Fig. 2 shows typical electropherograms of the five analytes by various separation modes (frame A, MEKC; frame B, stacking-MEKC [15,16] modes, respectively). In the case of the MEKC mode, the separation buffer consisted of 60 mM SDS and 30 mM sodium tetraborate in a mixed acetonitrile–water solution (6/94, v/v). Compared to the native fluorescence method, the detection limits were improved to $\sim 10^{-7}$ M (details are summarized in Table 2). The peaks indicated as "*" correspond to degradation products of FITC, which are typically seen when FITC is used [12]. These derivatives are stable when stored in an alkaline solution. This is recommended when the stacking-MEKC mode is used for on-line sample concentration rather than the sweeping-MEKC mode. Herein, the optimized BGS consisted of 60 mM SDS and 30 mM sodium tetraborate in a mixed acetonitrile–water solution (8/92, v/v), the conductivity of which was 5.4 mS/cm. The samples were dissolved in a low conductivity solution (0.03 mM aqueous sodium tetraborate solution; conductivity, 2.2 μS/cm). In order to determine the adequate length for sample stacking and the corresponding signal intensity, several different sample injection lengths (4–7 cm in capillary length) were examined. As a result, when the stacking mode was applied, the detection limits were improved to $\sim 10^{-8}$ M. The results obtained for the stacking-MEKC mode under the optimized conditions are also summarized in Table 2. It should be noted that the LODs obtained by the conventional GC/MS method were 2.1×10^{-5}, 2.0×10^{-5}, 2.3×10^{-5}, 1.9×10^{-5} and 1.6×10^{-5} M for 2C-T-2, 2C-T-7, 2C-C, 2C-B and 2C-I without any derivative steps. The CE/fluorescence detection methods can provide better results if the on-line sample concentration techniques were used.

3.3. Analysis of 2C-series drugs in a urine sample

For convenience, the MEKC mode, providing a detection limit of $\sim 10^{-7}$ M, was selected for the analysis of a urine sample. At the time of this study, an actual urine sample from a suspect could not be obtained. As a result, a mimic urine sample was

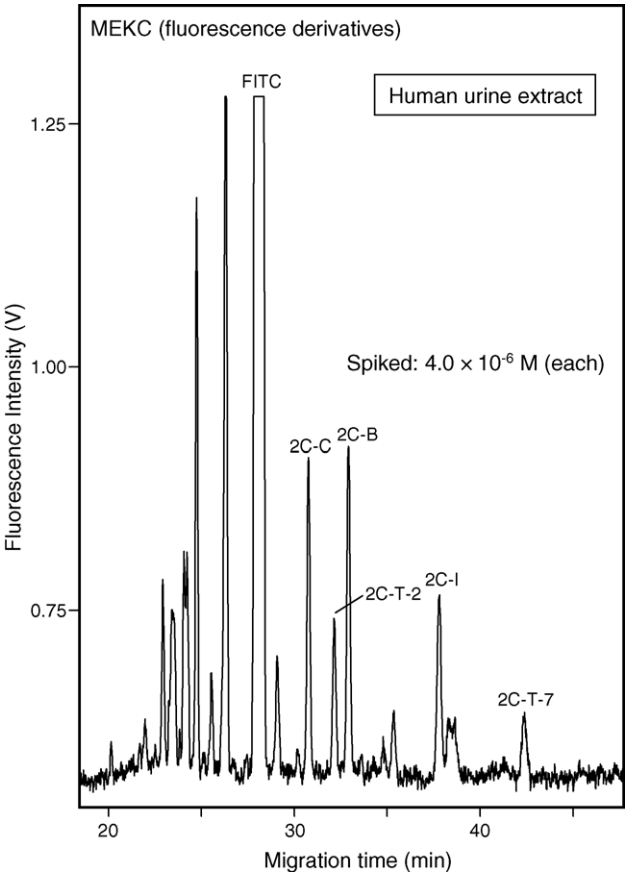

Fig. 3. Electropherogram obtained from a urine extract from a human volunteer after spiking with 4.0×10^{-6} M of 2C-C, 2C-B, 2C-I, 2C-T-2 and 2C-T-7 standards by the MEKC–LIF method.

obtained by spiking a normal sample from a volunteer with the five 2C-series of phenethylamine designer drugs (concentration of each, 4.0×10^{-6} M). Fig. 3 shows typical electropherogram of a human urine extract obtained by liquid–liquid extraction (extraction procedures as described above) using of the MEKC method. The CE conditions were the same as those described above. For a comparison of the migration times and a standard spiking method, the five analytes can be identified and are indicated in the figure. The urine sample extract was examined by means of GC/MS (data not shown) and the findings were in agreement with the results obtained by the CE method. Thus, by applying this approach to humans, if the urine sample of a suspect can be obtained, it could be used in determining the quantity of 2C-series drugs taken.

4. Conclusions

We demonstrate here that CE/fluorescence detection methods can be successfully used for the separation and on-line concentration of five similar analytes of the 2C-series of phenethylamine designer drugs. The methods used were based on native fluorescence and LED induced fluorescence detection, respectively. The former method is simple but results in a poor LOD; whereas the latter provides a much better LOD. When the MEKC mode in conjunction with fluorescence derivatization was applied to a urine extract containing the five 2C-series drugs, the can be determined successfully. This method provides a sensitive, accurate, simple, and economic complementary method to GC/MS for use in forensic and clinical analysis, as well as in related work.

Acknowledgment

This work was supported by a grant from the National Science Council of Taiwan under Contract No. NSC-94-2113-M-003-017.

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