The bioactivity of 2,5-dimethoxy-4-ethylthiophenethylamine (2C-T-2) and its detection in rat urine by capillary electrophoresis combined with an on-line sample concentration technique

Yu-Chih Chiu[a], Shiu-Huey Chou[b], Ju-Tsung Liu[c], Cheng-Huang Lin[a],*

[a] *Department of Chemistry, National Taiwan Normal University, 88 Sec. 4, Tingchow Road, Taipei, Taiwan*
[b] *Department of Life Science, Fu-Jen University, 510 Chung Cheng Road, Hsinchuang, Taipei, Hsien 24205, Taiwan*
[c] *Forensic Science Center, Command of the Army Force of Military Police, Department of Defense, Taipei, Taiwan*

Abstract

The bioactivity of 2,5-dimethoxy-4-ethylthiophenethylamine (2C-T-2) on nitric oxide (NO) production and the proliferation of spleen and thymus lymphocytes to mitogen stimulation in mice are reported for the first time. NO production by T and B lymphocytes in spleen and T cells in the thymus of mice decreased after the oral administration of 2C-T-2. This indicates that 2C-T-2 intake may perturb both neural and immune activity since a decrease in NO production is indicative of a weakened defense function. 2C-T-2 (the parent drug) in rat urine samples was detected by means of capillary electrophoresis/UV absorbance combined with an on-line sample concentration technique. When the CZE and MEKC modes were employed, the detection limit was found to be 4.5 and 5.0 µg/mL (at a 92.1% confidence level); whereas when on-line sample concentration methods, including stacking and sweeping-micellar electrokinetic chromatography were used, the detection limits were improved to 19.2 and 9.1 ng/mL, respectively. In an analysis of some actual samples from animal experiments, three male rats were administered 20 µg/g of body weight of 2C-T-2 by intra-peritoneal injection. The first- and second-day urine fractions were collected after the administration, for use in the analysis. As a result, 2.9 µg/mL and 0.25 µg/mL of 2C-T-2, respectively, were detected after ingestion of the doses.
© 2004 Elsevier B.V. All rights reserved.

Keywords: 2,5-Dimethoxy-4-ethylthiophenethylamine (2C-T-2); Nitric oxide (NO); Lymphocytes; Capillary electrophoresis; Urine

1. Introduction

β-Phenethylamine is a natural biochemical found in both plants and animals. The increased availability of β-phenethylamine derivatives in the illicit market represents a serious social problem. 2,5-Dimethoxy-4-ethylthiophenethylamine (2C-T-2), a ring-substituted phenethylamine, was first synthesized by Shulgin et al.; the synthetic procedures were also reported in their monograph [1]. Although the dosages (12–25 mg) of 2C-T-2 were generally mentioned in this publication, little information is available concerning its

bioactivity, detection and metabolic fate. Nitric oxide (NO) has been identified as an important molecule that is involved in the regulation of biological activities in the vascular, neural, and immune system [2]. It has been reported that psychostimulants have immunomodulatory effects [3,4]. In this work, the bioactivity of 2C-T-2 was investigated by NO production and the proliferation of spleen and thymus lymphocytes in response to mitogen stimulation in mice. In order to understand the effects of 2C-T-2 bioactivity in animals, a detailed knowledge of the metabolic pathways associated with 2C-T-2 in the animals is required. GC/MS remains the official method of analysis and is useful for the examining the metabolic fate of a compound such as 2C-T-2. However, it is necessary to derivatize phenethylamines prior

to their injection into a GC system and these processes are time consuming. Hence, a rapid and complementary method would be highly desirable. Capillary electrophoresis (CE) represents a rapidly growing separation technique that might well meet this need, since it is now well established and is a widely used analytical method in many fields, such as bioscience, pharmaceutical, environmental studies, food science and forensic research. A series of reports on on-line sample concentration techniques recently appeared, concerning the so called "stacking" and "sweeping" technique [5–16]. A dramatic increase in sensitivity can be obtained using these techniques. In our previous studies, four metabolites of 2C-T-2 were detected and the metabolic pathway in the rat was outlined, based on a gas chromatography–selected ion monitoring–mass spectrometry (GC–SIM–MS) method for the analysis of in vivo urine samples from rats [17]. In this study, we report on a simple and specific method for the detection of 2C-T-2 in urine samples using UV absorption combined with on-line sample concentration techniques. The extraction procedures used for the urine samples and several electrophoretic parameters, including buffer conditions, SDS concentration, and the injection length required for the separation were optimized. These data are reported herein.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals used were of analytical grade. Ethyl acetate, methanol, diethylether, 1,4-dimethoxybenzene, chlorosulfonic acid, zinc, sulfuric acid, ethyl bromide, potassium hydroxide, N-methylformamide, nitromethane, ammonium acetate anhydrous, sodium dodecyl sulfate (SDS), sodium tetraborate, methanol and phosphoric acid were all purchased from Acros (NJ, USA). Anhydrous sodium sulfate and sodium hydroxide were purchased from Yakuri Pure Chemicals Co. Ltd. (Osaka, Japan) and J.T. Baker (NJ, USA), respectively. The complete culture media used for all assays consisted of RPMI-1640 or Dulbecco's modified eagle medium (DMEM) supplemented with L-glutamine (2 mM), penicillin (100 U/mL), streptomycin (100 µg/ml), Hepes (10 mM) (Gibco, Life Technologies, USA) and 10% fetal bovine serum (HyClone, USA). The mitogens Concanavalin A (Con A), lipopolysaccharide (LPS) from *Escherichia coli*, heparin (100 U/mL) and MTT (3-[4,5-dimethylthiazolzyl]-2,5-diphenyltetrazolium bromide) were purchased from Sigma (St. Louis, MO, USA). Thymocytes and splenocytes were collected using Dulbecco's medium without calcium and magnesium (cmf-DPBS) supplemented with 10 mM Hepes, 2 mM EDTA, 100 U/mL penicillin, 100 mg/mL streptomycin, and 2% heat inactivated foetal bovine serum (HIFBS). Red blood cells were disrupted using an osmotic lysing buffer: 150 mM ammonium chloride, 10 mM sodium bicarbonate, and 1 mM EDTA (Sigma, St. Louis, MO, USA).

Nitric oxide (NO) assays were prepared in flat-bottomed tissue culture plates (Nalge Nunc, USA).

2.2. Animals

A BALB/c strain of inbred male mice (10–12 weeks) or Wistar strain rats (220–250 g) were purchased from Laboratory animal center in National Taiwan University College of Medicine (Taipei, Taiwan). Upon arrival, the mice and rats were caged in a separate colony room where a 12-h light–dark cycle was maintained through artificial illumination. The mice received free access to both food and water throughout the experiment, except as noted, and a 2-week acclimation period prior to experimental manipulation. The animals were also handled on a bidaily basis to prevent the hyperactivity that occurs when they are left untouched for a prolonged period.

2.3. Drug administration and urine sampling

For urine collection, Wistar rats were housed in a metabolic study cage one week before the 2C-T-2 administration. Blank urine samples were collected 24 h before drug administration. An intra-peritoneal injection (10 mg/kg) was then performed. All urine samples were collected over a 24 h period and stored at −20 °C until used. For nitric oxide and the proliferation analysis of immune tissues, mice were randomly assigned to a control or an experimental group (*n* = 3–4 mice/group). 2C-T-2 (10 mg/kg) was fed to the test animals by gavage. Control mice received a sterile phosphate buffer saline solution (PBS). The experimental groups of animals were fed a solution of 2C-T-2. The 2C-T-2 fed group of animals were sacrificed two hours after gavage. Each subject was anesthesized with carbon oxide gas. One ml of blood was harvested by a heart puncture. After the blood was collected, the mice were rapidly sacrificed by cervical dislocation and immune tissues were then harvested.

2.4. Spleen and thymus sample preparation

Tissue and blood were collected as described previously [18]. After sacrifice, the animal was placed on its back. Whole blood (1 mL) was collected from the heart of each animal by using a 22-gauge needle into a heparinized syringe. Serum was separated and transferred 1.5 mL microcentrifuged tubes and stored at −20 °C until used. Each spleen and thymus was dissociated into a single-cell suspension by grinding the spleen between the frosted ends of two glass slides. A single-cell suspension was collected after removal of cell debris and clumps. Splenocytes and thymocytes were enumerated and then diluted to 5×10^6/mL with complete culture medium.

2.5. Mitogen proliferation assay

A mitogen proliferation assay was performed with thymocytes and splenocytes to assess lymphocyte proliferation as

described by Chou et al. [19] with minor modifications. Con A was used as a T cell mitogen. The proliferation response was evaluated by the reduction in the ratio of MTT by mitochondrial dehydrogenase of viable cells to blue formzan products which can be measured spectrophotometrically. In a typical experiment, 5×10^6/mL splenocytes or thymocytes were incubated with an optimal concentration of Con A (0.625, 1.25, 2.5 µg/mL) for 48 h, after which 5 mg/mL of MTT solution was added to the solution, followed by incubation for 30–60 min. The formzan was dissolved with acid-isopropanol. Optical densities were measured in a microplate spectrophotometer (Spectramx 340PC384, Molecular Device, USA) at 570 nm.

2.6. Nitric oxide assay

Nitric oxide concentrations were determined as nitrite by a microplate assay [19,20]. One hundred microliters of 5×10^6 splenocytes or thymocytes from control or experimental animals were incubated with Con A (0.625, 1.25 and 2.5 µg/mL) or LPS (0 and 2.5 µg/mL) for 48 h. At the end of the incubation, 100 µL of culture supernatant was harvested from each culture of mitogen-stimulated lymphocytes. The 100 µL sample aliquots, were mixed with 100 µL of Griess reagent (0.5% sulfanilamide/0.05% naphthylethylene diamine dihydrochloride/2.5% H_3PO_4) and incubated at room temperature for 10 min. The optical density was measured in an ELISA microtiter plate reader at 550 nm against air as a blank. A sodium nitrite ($NaNO_2$) standard curve (0–100 µmol/mL) was generated in parallel. The results are expressed as the mean concentration of nitrate in the supernatants from triplicate wells and analyzed as repeated-measures ANOVA using the no-mitogen control and all concentrations of a given mitogen.

2.7. Urine extraction

One milliliter of urine sample was made alkaline by the addition of excess K_2CO_3. The free bases were then extracted into 2 mL of a hexane/CH_2Cl_2 (3:1, v/v) solution by stirring the suspension for 1 min. After centrifugation, the upper layer (1.5 mL) was collected and evaporated to dryness. The residue was dissolved in 50 µL of matrix (water/acetonitrile, 85/15) for the subsequent sweeping-MEKC separation.

2.8. Apparatus and instrumental techniques

The apparatus used is identical to that described previously [17]. Briefly, a nuclear magnetic resonance spectrometer (NMR; Bruker, Avance 500 MHz), an infrared (IR; Perkin-Elmer, FT-IR Paragon 500) spectrometer and a gas chromatograph (GC 6890 Hewlett-Packard, Avondale, PA, USA) equipped with a mass spectrometer (Hewlett-Packard 5973 mass selective detector) were used for the identification of the products produced during the different synthesis processes and for the final product. The CE set-up was fabricated in-house [21] where a high-voltage power supply (model RR30-2R, 0–30 kV, 0–2 mA, reversible, Gamma, FL, USA) was used to drive the electrophoresis and 50 or 75 µm i.d. fused silica capillary columns (J&W Scientific, CA, USA) were used for the separation. A UV detector (CE-971 UV, Jasco, Japan) was used for the determination of analytes and the wavelength used for the detection was 290 nm. A laboratory-built operational amplifier was used for signal amplification, which contained a single integrated LM1458 circuit chip. The analog signal was converted to a digital signal by an ac/dc converter (ADAM-4012 module, Advantech Co. Ltd., Taiwan). Electropherograms were collected with a data acquisition system connected to a personal computer.

2.9. Statistical analysis

A Student's t-test was used to assess the effect of 2C-T-2 on NO production or T cell proliferation in activated lymphocytes. In the experiment to characterize the effects of 2C-T-2, an ANOVA comparison was used to assess significance of the differences among the experimental and control groups. The level of significance for the t-test was set at a value of P less than or equal to 0.05.

3. Results and discussion

3.1. Synthesis

The synthetic procedures for 2C-T-2 have been previously described in the literature [1]. Following these processes, the final product was identified by NMR, IR and verified by GC–MS. As a result, specific fragments derived from the product (m/z of 153, 183, 197, 212 and 241) permitted its characterization, and the fragments were subsequently analyzed by GC/MS in the electron impact (EI) mode. The chemical structure of 2C-T-2 is shown in the inset in Fig. 3 (frame A).

3.2. Bioactivity

In the proliferation assays, nitric oxide after mitogen stimulation was measured as an indicator of functional potential. NO production was measured by converting NO to the more easily detectable form, nitrite. The results of the nitrite assays of supernatants from spleen mononuclear cells stimulated with mitogens, Con A or LPS, and thymocytes stimulated with Con A are shown in Fig. 1. 2C-T-2 markly inhibited NO production in both Con A and LPS stimulated splenocytes ($p < 0.05$). Exposure of 2C-T-2 also inhibited NO release in Con A stimulated thymocytes ($p < 0.05$). However, there is no significant difference in T cell proliferation to mitogen Con A in the spleen and thymus between controls and 2C-T-2 treated mice (Fig. 2, $p > 0.1$). Psychostimulants have been reported to modulate several physiological and behavioral activities. Some studies reported that psychostimulants, such as amphetamine, posses immunomodulatory effects [3,4]. In this
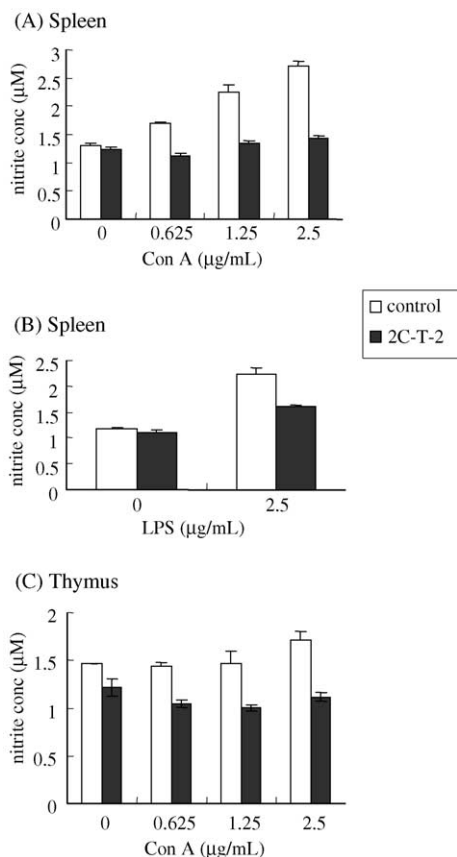
Fig. 1. The effect of 2C-T on nitric oxide production by splenocytes and thymocytes incubated with the optimal concentration of mitogens Con A and LPS as indicated in (A)–(C). Nitrite was measured as an indicator of nitric oxide production in the supernatant of mitogen-induced blastogenesis cultures. Mice were orally administered either (0.9%) saline or 2C-T (10 mg/kg). The results are expressed as the mean (±S.E.) μM; n = 3/group.
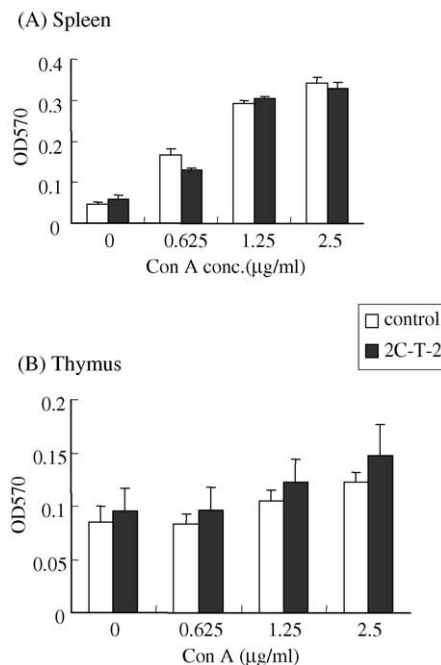

Fig. 2. Proliferation of splenocytes and thymocytes to optimal concentrations of the mitogen Con A as noted in (A) and (B). Mice were orally administered either (0.9%) saline or 2C-T (10 mg/kg). The spleen and thymus were collected after 2 h. The proliferative response was measured by an MTT assay. The results are expressed as the mean (±S.E.) MTT optical absorbency at 570 nm; n = 3/group.

study, we evaluated the proliferation of splenic or thymic T lymphocytes to mitogen stimulation on mice receiving orally administered 2C-T-2. The oral intake of 2C-T-2 had no effect on the proliferation of lymphocytes to mitogen stimulation in either the thymus or spleen but significantly inhibited NO production in Con A or LPS stimulated lymphocytes. NO, a versatile molecule, plays an important role in vascular tone and neurotransmission [22]. Importantly, NO produced by activated macrophages and lymphocytes has been shown to mediate host defense functions, including antimicrobial and antitumor activities. Accumulated data indicate that the long-term intake of psychostimulants and its analogs can cause an increase in NO production which then induces a decline in neural chemical events and behaviorial changes [23,24]. In contrast to the increase in NO production in nerve tissues, 2C-T-2 inhibited NO production in T and B lymphocytes in spleen and T cells in the thymus. The decline in NO production is indicative of weakened defense functions. The difference in the effect of 2C-T-2 on neural and immune cells may be due to the functional roles or regulatory mechanism of both tissues, however, to verify this, further study will be required. These results suggest that 2C-T-2 intake may exhibit differ-

ential effects on both neural and immune activity. The major difference between 2C-T-2 and amphetamine is the presence of a C_2H_5S-group on the former which may be responsible for the change in NO production. Additional bioactivity experiments concerning 2C-T-2 related compounds, including 2C-T-7 (4-propylthio-2,5-dimethoxy-phenethylamine), 2C-B (4-bromo-2,5-dimethoxy-phenethylamine), 2C–Cl (4-chloro-2,5-dimethoxy-phenethylamine) and 2C-I (4-iodine-2,5-dimethoxy-phenethylamine) are currently in progress.

3.3. Detection

The metabolites of 2C-T-2 in rat urine samples were detected by GC/MS previously [17]. Herein, the CE/UV absorbance combined with an on-line sample concentration technique (a complementary method to GC/MS) was investigated. Fig. 3 shows typical electropherograms of a 2C-T-2 standard by various separation modes (electropherograms a–d; CZE, MEKC, stacking and sweeping-MEKC modes, respectively). The concentrations of 2C-T-2 used in electropherograms a–d were 100, 100, 0.5 and 0.5 μg/mL, respectively. Details of the CE conditions are shown in the caption of Fig. 3. In the case of the CZE and MEKC modes, the detected signal intensities were 0.48 and 0.54 V. However, after applying stacking (sample injection length: 16.4 cm) and sweeping-MEKC (sample injection length: 30.3 cm) methods, the detection limits were dramatically improved (0.12 and 1.68 V, respectively). Compared to the
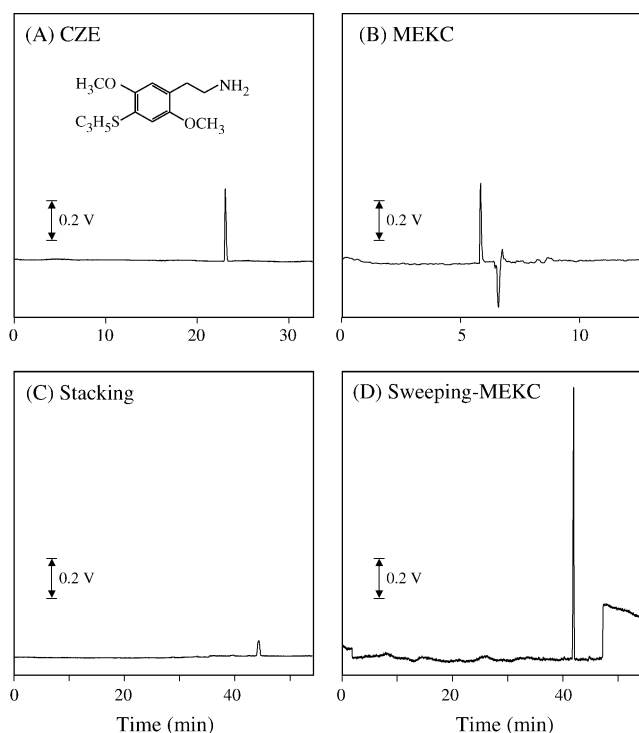
Fig. 3. CE electropherograms (frames, A–D) obtained using different CE separation modes (CZE, MEKC, stacking and sweeping-MEKC, respectively). CE conditions: (A) buffer consisting of 75 mM H_3PO_4, 12.6 mM Na_2HPO_4 and 13.25 mM NaH_2PO_4 in a mixed water–acetonitrile solution (95:5, v/v); applied voltage 15 kV; currents, 40–50 μA; (B) buffer consisting of 25 mM SDS, 3 mM HBO_3 and 25 mM $NaHPO_4·7H_2O$ in a mixed water–acetonitrile solution (70:30, v/v); applied voltage 15 kV; current, 70–80 μA; (C) buffer consisting of 75 mM H_3PO_4, 12.6 mM Na_2HPO_4 and 13.25 mM NaH_2PO_4 in a mixed water–acetonitrile solution (95:5, v/v); matrix, 0.75 mM H_3PO_4, 0.126 mM Na_2HPO_4 and 0.1325 mM NaH_2PO_4 in a mixed water–acetonitrile solution (95:5, v/v); applied voltage 15 kV; currents, 10–50 μA; (D) buffer consisting of 120 mM SDS and 30 mM H_3PO_4 in a mixed water–acetonitrile–methanol solution (65:15:20, v/v/v); matrix, 30 mM H_3PO_4 in a mixed water–acetonitrile solution (85:15, v/v); applied voltage −20 kV; currents, −20 to −14 μA. UV absorption, 290 nm.

CZE and MEKC modes (normal injection methods), a ∼50- and ∼600-fold improvement was realized when the on-line sample concentration techniques (stacking, sweeping-MEKC, respectively) were applied. Table 1 summarizes these results as well as the equation used in constructing for the calibration curves, the coefficient of variation values,
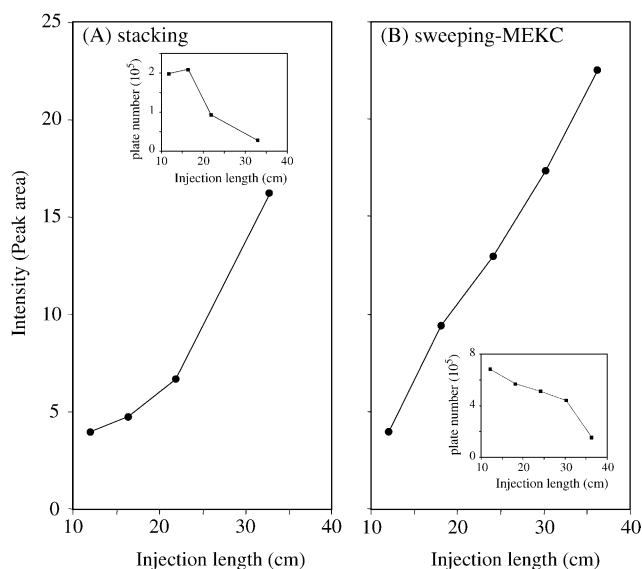

Fig. 4. Relationship between the length (cm) of the sample injection and the corresponding peak area for the stacking (A) and sweeping-MEKC (B) modes. The insets show the relationship between the length of the sample injection and the number of theoretical plates, respectively. Capillaries used: (A) i.d. 75 μm; (B) i.d. 50 μm.

the limit of detection and the plate numbers for 2C-T-2 for the CZE, MEKC, stacking and sweeping-MEKC modes, respectively, for the above experiment. When CZE and MEKC were used, the detection limits were 4.5 and 5.0 μg/mL (at the 92.1% confidence level); whereas when on-line sample concentration techniques were used, including stacking and sweeping-MEKC, the detection limits were improved to 19.2 and 9.1 ng/mL, respectively. To investigate the effects of injection length and the corresponding signal intensity when the stacking and sweeping-MEKC techniques were used under exactly the same experimental conditions, various column lengths (stacking: 12, 16, 22, 33 cm; sweeping-MEKC: 12, 18, 24, 30 and 36 cm) for the sample solution were injected into the capillary and the results were plotted, as shown in Fig. 4 (frames, A and B). Basically the signal intensity (peak area) increased with increasing injection length. The insets in frames A and B show the relationship between the length of the sample injection and plate number. Although reasonable plate numbers (∼10^5) can be obtained

Table 1

Limit of detection (LOD) values, linearity of peak areas, coefficients of variation and plate numbers for 2C-T-2 for the CZE, MEKC, stacking and sweeping-MEKC modes[a]

| Method	CZE	MEKC	Stacking	Sweeping-MEKC
Capillary i.d.	75 μm i.d.	75 μm i.d.	75 μm i.d.	50 μm i.d.
Effective/total length (cm)	72/85	47/60	72/85	105/118
Injected length (cm)	0.3	0.3	16.4	30.3
Concentration ranges	4.1×10^{-4} to 4.1×10^{-6} M	4.1×10^{-4} to 2.1×10^{-5} M	4.1×10^{-6} to 1.0×10^{-7} M	2.1×10^{-6} to 6.5×10^{-8} M
Equation of line	$y = 0.049x + 0.014$	$y = 0.020x + 0.056$	$y = 0.006x - 0.135$	$y = 0.038x + 0.138$
Coefficient of variation	$r^2 = 0.9942$	$r^2 = 0.993$	$r^2 = 0.999$	$r^2 = 0.999$
LOD (at the 92.1% CL)	2.0×10^{-5} M (4.5 μg/mL)	2.1×10^{-5} M (5.0 μg/mL)	8.0×10^{-8} M (19.2 ng/mL)	3.8×10^{-8} M (9.1 ng/mL)
Plate number (N)	1.5×10^5 to 1.2×10^5	7.2×10^4 to 5.3×10^4	1.2×10^5 to 6.6×10^4	3.3×10^5 to 1.1×10^6

[a] UV absorption at 290 nm.
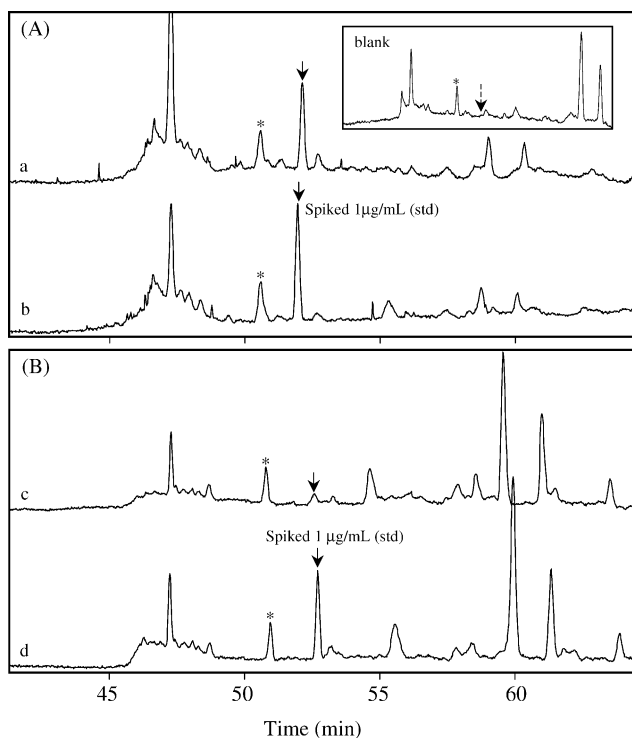
Fig. 5. (A) electropherogram a, urine extract (after ingestion of 2C-T-2 for 24 h) from the 2C-T-2 dosed mouse; electropherogram a, spiked with 1.0 μg/mL of 2C-T-2 before extraction as a single-point standard addition. The inset shows the CE electropherogram of a control mouse (blank urine sample extract). (B) Electropherograms for the second day of urine collection (electropherograms a and b, before and after 1.0 μg/mL of 2C-T-2 standard addition).

in all of these conditions, the sweeping-MEKC mode provides better detection sensitivity and better separation efficiency. For this reason, the sweeping-MEKC mode was used in subsequent experiments.

Fig. 5 shows typical CE electropherograms of urine sample extracts from the 2C-T-2 test mouse (frame A, the first day of urine collection after the ingestion of the 2C-T-2; frame B, the second day of urine collection) by applying the sweeping-MEKC technique. Herein, the background solution used in the sweeping-MEKC mode consisted of 120 mM SDS and 30 mM H_3PO_4 in a mixed water–acetonitrile–methanol solution (65:15:20, v/v/v). The sample extracts were dissolved in the matrix solution which consisted of 30 mM H_3PO_4 in a mixed water–acetonitrile solution (85:15, v/v) resulting a non-micelle buffer. Hydrodynamic injection was achieved by raising the reservoir 45 cm relative to the exit reservoir for 1200 s. Using this procedure, 24 cm column lengths of sample solution were injected into the capillary. Electropherograms a and b (in frame A) show the separation results obtained before and after a single-point standard addition (1 μg/mL of 2C-T-2) for the first day urine sample extracts, respectively. For convenience, the indicated peak (marked as (*) in frames A–D) was selected for comparison. As shown in electropherogram a, the 2C-T-2 parent drug (arrow mark) appears to the right of the indicated peak. In order to determine if this

peak (arrow mark) is 2C-T-2 or not, we spiked 1 μg/mL of a 2C-T-2 standard (before extraction) and the peak indeed increased, as shown in the electropherogram b. We assigned this peak as the 2C-T-2 parent drug and its concentration was determined to be 2.9 μg/mL. In order to confirm that this peak appeared only for the 2C-T-2 test mouse, a urine extract from a control mouse (without 2C-T-2 dosage) was examined. No peak was detected having this migration time, as shown in the inset in frame A (broken arrow). Using the same procedure, frame B shows CE electropherograms (c, before spiking; d, 1 μg/mL of 2C-T-2 spiking) for the second day urine sample extracts from the same 2C-T-2 test mouse. A minor peak is visible in the electropherogram c (arrow peak). We assigned this peak as the 2C-T-2 parent drug and its concentration was determined to be 0.25 μg/mL. Thus, we conclude that the 2C-T-2 parent drug can be detected in urine samples of this test mouse at levels of 2.9 and 0.25 μg/mL, respectively, from the first day and the second day urine collections. Using the same experimental procedures, including
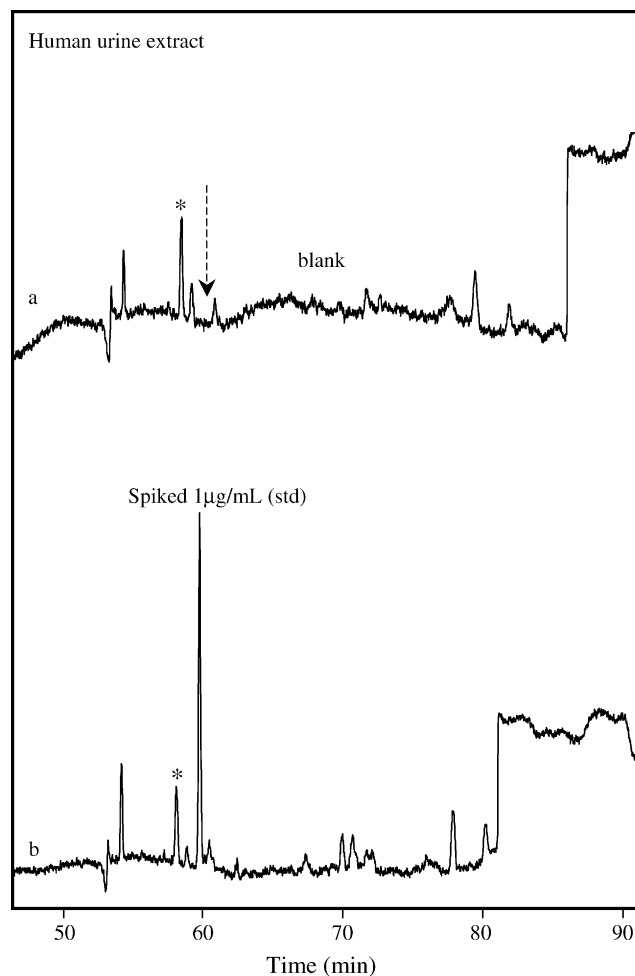

Fig. 6. CE electropherograms obtained from urine extracts from a human male volunteer (electropherogram a, blank urine extract; electropherogram b, after spiking with 1 μg/mL 2C-T-2 std.) by means of sweeping-MEKC method. The CE conditions and method used were the same as those described above.

the liquid-liquid extraction procedures, with the CE running buffer and the sweeping-MEKC mode, a spiked urine sample from a human volunteer was also tested. The spiked 2C-T-2 (1 μg/mL) peak was clearly and easily detected (as shown in the inset). Fig. 6 show the results obtained from sample extracts from the male volunteer (electropherogram a, blank urine extract; electropherogram b, after spiking with 1 μg/mL 2C-T-2 std.) by means of the sweeping-MEKC method. The CE conditions were the same as those described above. For a comparison of the separation efficiency, an indicated peak (marked as (*) in electropherograms a and b) was selected for evaluation. In electropherogram a, the arrow marked indicated the expected migration time for 2C-T-2, but it did not show up in the blank urine sample extract. This indicates that no peaks exist in the extract that could overlap with the 2C-T-2 analyte. Using a standard addition method, the results were compared and the findings show that a peak (marked spiking peak in electropherogram b) clearly appeared. Thus, by applying this approach, to humans, if the relationship between 2C-T-2 concentration in the urine and various dosing times can be determined, it would be of use in determining the quantity of 2C-T-2 ingested, after a period of days, using the method developed here. Furthermore, a blood sample extract from the 2C-T-2 test mouse (6 h after ingestion) also was examined. However, the 2C-T-2 parent drug was not detectable, either by applying the sweeping-MEKC technique or by GC/MS.

4. Conclusions

The bioactivity of 2C-T-2 by an assays for NO production and the proliferation of spleen and thymus lymphocytes to mitogen stimulation was investigated. The decline in NO production is indicative of a weakened defense function and this may be the reason for why 2C-T-2 is classified as a drug of abuse. The detection of 2C-T-2 in urine was successful using capillary electrophoresis/UV absorbance combined with an on-line sample concentration technique. This method provides a sensitive, accurate, rapid, simple, and economic complementary method to GC/MS for use in forensic and clinical analysis, as well as in related work.

Acknowledgment

References

[1] A. Shulgin, A. Shulgin, A. PiHKAL, A Chemical Love Story, fifth printing, Transform Press, Berkeley, CA 94712, 2000, p. 577.
[2] S. Moncada, R. Palmer, E. Higgs, Pharmacol. Rev. 43 (1992) 109.
[3] R. House, P. Thomas, H. Bhargava, Immunopharm. Immunot. 16 (1994) 1.
[4] M. Bubera, M. Filip, A. Basta-Kaim, E. Nowak, B. Budziszewska, M. Tetich, V. Holan, B. Korzeniak, E. Przegalinski, J. Physiol. Pharmacol. 53 (2002) 232.
[5] D.S. Burgi, R.L. Chien, Anal. Biochem. 202 (1992) 306.
[6] R.L. Chien, D.S. Burgi, J. Chromatogr. 559 (1991) 141.
[7] C.X. Zhang, W. Thormann, Anal. Chem. 68 (1996) 2523.
[8] J.P. Quirino, S. Terabe, Anal. Chem. 70 (1998) 149.
[9] Z.K. Shihabi, J. Capillary Electrophor. 6 (1995) 267.
[10] Y. Xiong, S. Park, S. Swerdlow, Anal. Chem. 70 (1998) 3605.
[11] J.L. Beckers, J. Chromatogr. 641 (1993) 363.
[12] L. Krivankova, A. Vrana, P. Gebauer, P. Bocek, J. Chromatogr. A 772 (1997) 283.
[13] P. Gebauer, W. Thormann, P. Bocek, J. Chromatogr. 608 (1992) 47.
[14] J.P. Quirino, S. Terabe, Science 282 (1998) 465.
[15] J. Palmer, N.J. Munro, J.P. Landers, Anal. Chem. 71 (1999) 1679.
[16] P. Britz-McKibbin, D.D.Y. Chen, Anal. Chem. 72 (2000) 1242.
[17] L.-C. Lin, J.-T. Liu, C.-H. Lin, J. Chromatogr. B 798 (2003) 241.
[18] S.-H. Chou, L.D. Kojic, J.E. Cunnick, Brain Behav. and Immun. 11 (1997) 79.
[19] S.H. Chou, L.D. Kojic, K. Nordyke-Messingham, J.E. Cunnick, Brain Behav. Immun. 10 (1996) 399.
[20] D. Stuehr, C. Nathan, J. Exp. Med. 169 (1989) 1543.
[21] M.-G. Chen, S.-H. Chou, C.-H. Lin, J. Chromatogr. B 801 (2004) 347.
[22] C. Nathan, FASEB J. 6 (1992) 3051.
[23] J. Gibb, M. Johnson, G. Hanson, NeuroToxicology 11 (1990) 317.
[24] T. Taraska, K. Finnegan, J. Pharmacol. Exp. Ther. 280 (1997) 941.