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Original Paper

Optimization of separation and online sample concentration of *N,N*-dimethyltryptamine and related compounds using MEKC

The optimal separation conditions and online sample concentration for *N*,*N*-dimethyltryptamine (DMT) and related compounds, including α-methyltryptamine (AMT), 5-methoxy-AMT (5-MeO-AMT), *N*,*N*-diethyltryptamine (DET), *N*,*N*-dipropyltryptamine (DPT), *N*,*N*-dibutyltryptamine (DBT), *N*,*N*-diisopropyltryptamine (DiPT), 5-methoxy-DMT (5-MeO-DMT), and 5-methoxy-*N*,*N*-DiPT (5-MeO-DiPT), using micellar EKC (MEKC) with UV-absorbance detection are described. The LODs (S/N = 3) for MEKC ranged from 1.0 ~ 1.8 μg/mL. Use of online sample concentration methods, including sweeping-MEKC and cation-selective exhaustive injection-sweep-MEKC (CSEI-sweep-MEKC) improved the LODs to 2.2 ~ 8.0 ng/mL and 1.3 ~ 2.7 ng/mL, respectively. In addition, the order of migration of the nine tryptamines was investigated. A urine sample, obtained by spiking urine collected from a human volunteer with DMT, was also successfully examined.

Keywords: MEKC / Sweeping-MEKC / Tryptamine

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1 Introduction

N,*N*-dimethyltryptamine (DMT legal status: class A, UK; schedule I, US), a powerful psychoactive substance, is both a naturally occurring tryptamine and a potent psychedelic drug. Structurally, it is analogous to the neurotransmitter serotonin and other psychedelic tryptamines, such as α-methyltryptamine (AMT), 5-methoxy-AMT (5-MeO-AMT), and 5-methoxy-*N*,*N*-diisopropyltryptamine (5-MeO-DiPT). These tryptamine derivatives are illegal drugs that are listed on Schedule I of the Controlled Substances Act (US). It should be noted that substitutions to the tryptamine molecule give rise to a group of related compounds; the degree of alkylation of the side-chain nitrogen of tryptamine has a significant effect on its psychoactivity. However, a number of tryptamine derivatives, the so-called designer drugs, such as *N*,*N*-diethyltryptamine (DET), *N*,*N*-dipropyltryptamine (DPT), *N*,*N*-dibutyltryptamine (DBT), DiPT, and 5-methoxy-*N*,*N*-DMT (5-MeO-DMT) remain unscheduled. Hence, detection of these tryptamine-like drugs, which may be produced in underground laboratories and sold on the streets, is an analytical challenge, not only because their chemical structures are similar, but also because their levels in biological fluids are very low. From the point of view of screening for and confirmation of illicit tryptamine derivatives, more detailed information on separation and detection of these compounds is highly desirable. Currently, GC-EI/MS [1 – 7], LC-ESI/MS [8 – 11], and CE [12 – 13] are the most popular and well-developed methods for identification of tryptamine derivatives. Each of these methods has unique advantages and disadvantages with respect to sensitivity, precision, and simplicity of use. In our previous research, we have ever compared the analytical sensitivity, selectivity, time, cost, and the order of migration based on different separation techniques (GC, HPLC, and CE, respectively) [14]. In this study, mixtures of DMT, and related compounds, including AMT, 5-MeO-AMT (primary amines), DET, DPT, DiPT, DBT (various degree of alkylation of the side-chain nitrogen in tryptamine), 5-MeO-AMT, 5-MeO-DMT, and 5-MeO-DiPT (5-methoxy-tryptamines) were used in the CE separation experiments. Using MEKC, a set of operational conditions was selected for investigation of migration order. Furthermore, in attempts to improve the sensitivity of

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Abbreviations: AMT, α-methyltryptamine; **BGS,** background solution; **CSEI-sweep-MEKC,** cation-selective exhaustive injection-sweep-micellar EKC; **DBT,** *N*,*N*-dibutyltryptamine; **DET,** *N*,*N*-diethyltryptamine; **DiPT,** *N*,*N*-diisopropyltryptamine; **DMT,** *N*,*N*-dimethyltryptamine; **DPT,** *N*,*N*-dipropyltryptamine; **5-MeO-AMT,** 5-methoxy-AMT; **5-MeO-DMT,** 5-methoxy-DMT

detection, online sample concentration techniques, including sweeping-MEKC and cation-selective exhaustive injection-sweep-micellar EKC (CSEI-sweep-MEKC) [15 – 24], were applied. A urine sample was obtained by spiking urine collected from a human volunteer with DMT. After liquid – liquid extraction, the sample was examined using the sweeping-MEKC mode under optimized conditions. The extraction procedures used for the urine sample and the CE conditions were also optimized and are reported here.

2 Experimental

2.1 Reagents

DMT, AMT, 5-MeO-AMT, and 5-MeO-DiPT were generously donated by the Military Police Command, Forensic Science Center, Taiwan. DET, DPT, DBT, DiPT, and 5-MeO-DMT were synthesized in this study. The procedures for their synthesis have been described previously by Shulgin and Shulgin, TIHKAL (Tryptamines I Have Known and Loved) [25]. Following synthesis, the final products were identified using NMR and IR, and were verified using GC/MS. SDS was purchased from Acros (Belgium). All other chemicals were of analytical grade and were obtained from commercial sources.

2.2 Apparatus

The CE setup was identical to that used in our previous study [26]. Briefly, a high-voltage power supply (Model RR30-2R, Gamma, FL, USA) was used to drive electrophoresis, and a 50 μm id fused-silica capillary column (J&W Scientific, CA, USA) was used for separation (total/effective length of capillary: 80/67 cm). The sample solution was hydrodynamically injected by raising the sample reservoir to provide the height difference for the injection length. A UV-detector (CE-971 UV, Jasco, Japan) was used for analyte determination at a detection wavelength of 280 nm.

2.3 Urine extraction procedure

In a glass tube, a 1 mL aliquot of a clean urine sample obtained from a human volunteer, was spiked with 25 ng DMT. The spiked urine sample was made alkaline by the addition of 2 mg of potassium carbonate and then was shaken for 1 min. 4 mL of hexane/dichloromethane (v/v 3:1) were added, and gently mixed for 5 min, followed by centrifugation. The upper layer was collected (3 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 extraction efficiency of this procedure for DMT was 56.8 ± 6.2% (n = 5).

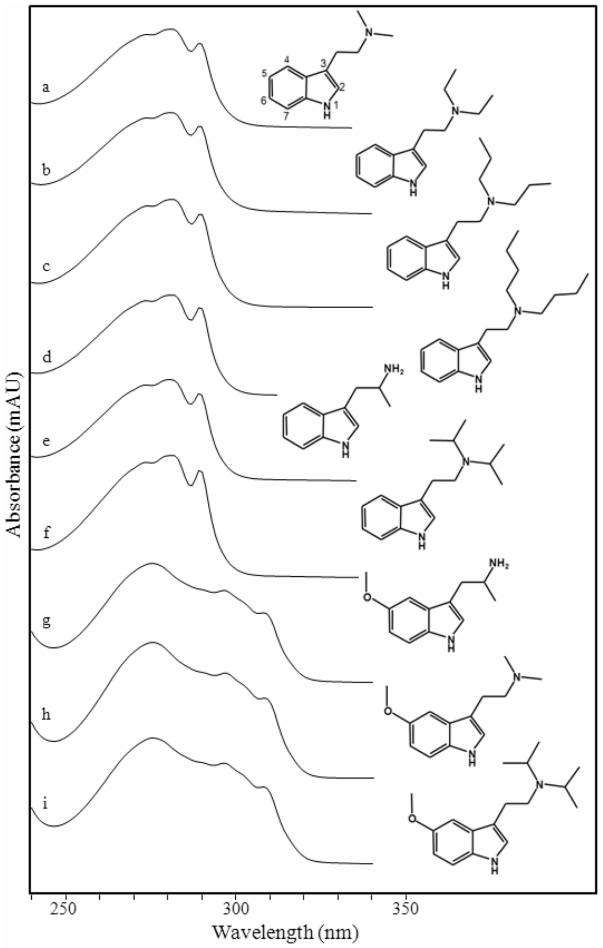

Figure 1. UV absorption spectra and molecular structures of the nine tryptamines, and the abbreviations used in this paper.

3 Results and discussion

3.1 MEKC

Figure 1 shows the UV absorption spectra and molecular structures of the nine tryptamines used in this study; spectra a – i correspond to DMT, DET, DPT, DBT, AMT, DiPT, 5-MeO-AMT, 5-MeO-DMT, and 5-MeO-DiPT, respectively. Maximum absorption was observed at ~280 nm. The 5-MeO-AMT, 5-MeO-DMT, and 5-MeO-DiPT produced spectral patterns that differed from those of the other compounds. These spectra are useful for online identification if a CCD detector is available. Figure 2 shows typical MEKC/UV-absorption electropherograms for the nine tryptamines, each measured at a concentration of 10 μg/mL, using SDS as the surfactant. The CE buffer consisted of a mixture of 75 mM SDS and 50 mM NaH_2PO_4. The volumetric ratio of water, methanol, and ACN (*i.e.*, water/methanol/ACN by volume) for electropherograms a – d, were as follows: a, 80:20:0; b, 70:30:0; c, 70:0:30; d,

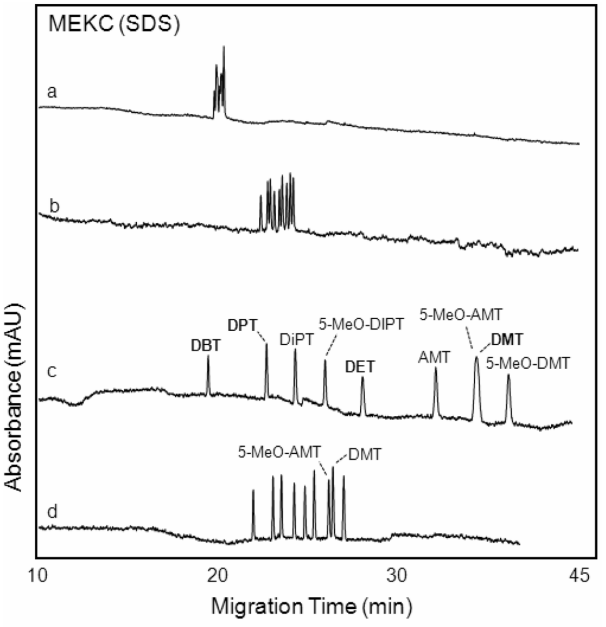

Figure 2. Typical MEKC/UV-absorption electropherogram for the nine tryptamines using SDS as surfactant; sample concentration, 10 μg/1 mL of methanol. The CE buffer consisted of 75 mM SDS and 50 mM NaH_2PO_4 in a mixture of water/methanol/ACN (electropherograms a – d, by volume a, 80:20:0; b, 70:30:0; c, 70:0:30; d, 65:30:5, respectively); the pH of which was adjusted to ~2.2* using H_3PO_4. The applied voltage was –15 kV.

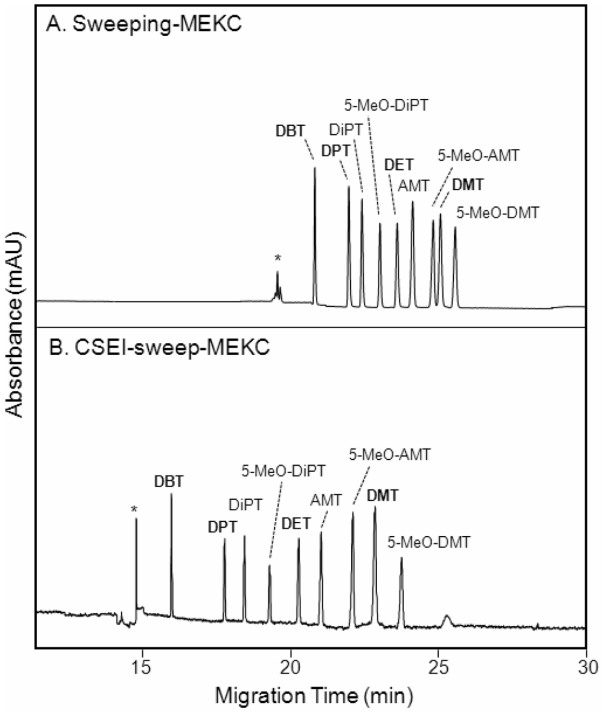

Figure 3. (A) Sweeping-MEKC/UV-absorption electropherogram for the nine tryptamines. Sample concentration, 0.5 μg in BGS (without SDS). Conditions: Phosphate buffer (50 mM, pH 2.2) in an ACN/methanol/water (5:30:65 by volume) solution containing 75 mM SDS. The applied voltage was –15 kV. The samples were prepared in matrices (electropherograms, 50 mM NaH_2PO_4 aqueous solution). Sample injection length, 45 cm; total/effective length of the capillary, 80/67 cm. (B) CSEI-sweep-MEKC electropherogram for the nine tryptamines. Sample concentration, 50 ng in 1 mL BGS (without SDS). Conditions: The capillary was first filled with background buffer, followed by injection of a high conductivity buffer (approximately a 10 cm length of capillary with 200 mM NaH_2PO_4 solution. System peak indicated by "*".

65:30:5. The pH of the water/organic solvent mixture was adjusted to ~2.2*, using H_3PO_4. As seen in electropherograms a and b, use of methanol alone does not result in complete separation. When ACN was used instead of methanol, separation efficiency improved, although the separation time increased. Optimal separation was observed when a mixture of water/methanol/ACN (65:30:5 by volume) was used, as shown in electropherogram d. The use of various concentrations of SDS (50, 100, and 150 mM) was investigated, but separation efficiency cannot be further improved. Using MEKC, migration order is determined by effective charge, degree of protonation, effective electrophoretic mobility, and electro-osmotic mobility [27]. When the buffer is acidic and a negative potential is applied, EOF is suppressed, and the major force driving separation is derived from the SDS-micelles (negative charge), which carry the tryptamines toward the outlet. For this reason, both large cations, such as tertiary tryptamine (DBT > DPT = DiPT > DET > DMT; Fig. 2c) or molecules that interact strongly with SDS-micelles move toward the outlet quickly, leading to shorter migration times. After DMT use (Fig. 2c), the observed migration order was as predicted: DBT, DPT (DiPT), DET, and DMT. The rate of DiPT migration was slower than that of DPT due to stereo-obstruction, which leads to weaker interactions with the SDS-micelles. The interactions between AMTs and SDS-micelles are stronger than that of cationic tryptamines. As a result, AMT and 5-MeO-AMT move faster than DMT and 5-MeO-DMT, respectively. On the other hand, the interaction between 5-MeO-tryptamine and the SDS-micelles is weak because of stereo-obstruction. This is the reason why 5-MeO-DiPT, 5-MeO-AMT, and 5-MeO-DMT migrate at slower rates than DiPT, AMT, and DMT, respectively. It should be noted that adjustment of the pH of the solution to 7.0 and application of a positive voltage (+15 kV) completely reversed the order of migration (data not shown). This information is useful, especially for analysis of home-made tablets that may contain complicated components, including tryptamines, phenethylamines, and related designer drugs. The LODs (S/N = 3) obtained using MEKC ranged from 1.0~1.8 μg/mL. To further enhance the LOD for tryptamines, sweeping-MEKC was applied, as described below.

Table 1. LOD values (at S/N = 3), plate numbers, and RSD ($n = 3$) for the nine tryptamine standards obtained using MEKC, sweeping-MEKC, and CSEI-sweep-MEKC

	DMT	DET	DPT	DBT	DiPT	AMT	5-MeO-DMT	5-MeO-DiPT	5-MeO-AMT
MEKC									
LOD (μg/mL)	1.5	1.8	1.2	1.3	1.3	1.2	1.5	1.0	1.5
Plate number ($\times 10^5$)	4.6	5.4	5.8	5.8	5.1	5.0	4.2	5.8	4.9
Sweeping-MEKC									
LOD (ng/mL)	4.8	4.8	2.7	8.0	2.4	2.4	5.0	2.2	4.6
Plate number ($\times 10^5$)	5.0	5.5	7.7	13.4	8.0	5.3	4.9	6.8	5.1
RSD (%)									
Migration time									
Intraday	0.68	0.71	0.72	0.72	0.72	0.70	0.71	0.71	0.69
Interday	6.61	6.77	7.92	6.88	6.28	6.71	6.55	6.84	6.59
Peak area									
Intraday	5.46	5.11	2.04	3.72	5.53	3.05	1.36	4.83	3.67
Interday	6.49	9.59	3.66	5.17	8.74	3.74	7.02	6.91	6.80
CSEI-sweep-MEKC									
LOD (ng/mL)	1.3	2.0	2.4	1.6	1.9	1.5	2.2	2.7	1.4
Plate number ($\times 10^5$)	4.1	8.9	9.8	15.0	9.9	7.1	5.0	11.0	4.4

3.2 Sweeping-MEKC

Figure 3A shows a typical sweeping-MEKC electropherogram for the nine tryptamines. The background solution (BGS) consisted of 75 mM SDS and 50 mM NaH_2PO_4 in a mixed ACN/methanol/water solution (5:30:65 by volume), as described above. The nine tryptamines (each 500 ng/mL) were first dissolved in a phosphate buffer (50 mM NaH_2PO_4) resulting in a nonmicellar buffer. Hydrodynamic injection was achieved by raising the reservoir for injection. The capillary dimensions were as follows: id, 50 μm; total/effective length, 67/80 cm; and, injection length, 45 cm. After the injection was complete, −15 kV was applied to drive the CE separation. The effects of injection length on the corresponding signal intensity when the sweeping-MEKC technique was used under the same experimental conditions were investigated. Various column lengths of the sample solution (30, 40, 45, and 50 cm) were injected into the capillary and detection performance was evaluated. Use of the 45 cm column length provided both superior detection sensitivity and separation efficiency. The order of migration observed for sweeping-MEKC was the same as that observed using MEKC. The LOD values (at S/N = 3), plate numbers, and RSD (%) for the nine tryptamine standards, as measured under optimal conditions, are summarized in Table 1. This method is simple and efficient, so we selected sweeping-MEKC for the urinalysis experiment, as described below.

3.3 CSEI-sweep-MEKC

Figure 3B shows typical CSEI-sweep-MEKC electropherogram for the nine tryptamines. Initially, the capillary was filled with background buffer, followed by injection of a high conductivity buffer (approximately a 10 cm length of capillary with 200 mM NaH_2PO_4 solution). Using electrokinetic injection at +10 kV, cationic analytes were injected for a period of 7 min. Meanwhile, the cationic analytes were concentrated at the interface between the water and buffer zones. Finally, the cationic analytes focus or stack at the interface between the water zone and the high conductivity buffer. The injection was then stopped and micellar BGSs were placed at both ends of the capillary. Next, the voltage was quickly shifted to −15 kV. The high negative voltage permitted entry of micelles from the inlet vial into the capillary to sweep the stacked analytes and to introduce the analytes into the narrow brands. The LOD values (at S/N = 3) and plate numbers are summarized in the Table 1. Electrokinetic injection efficiency for the nine tryptamines was low. As a result, LOD could not be further improved. It should be noted that the plate number obtained using CSEI-sweep-MEKC was greater than that measured for sweeping-MEKC. This result suggests that the electrokinetic injection method provides a narrower sample zone, which facilitates high-efficiency separation.

3.4 Application of the sweeping-MEKC technique to real sample

Figure 4 shows typical sweeping-MEKC electropherograms of human urine extracted using a liquid – liquid extraction method, as described above. Electropherogram a in Fig. 4 shows the results for a mock urine sample that was obtained by spiking a clean urine sample collected from a human volunteer with the DMT standard

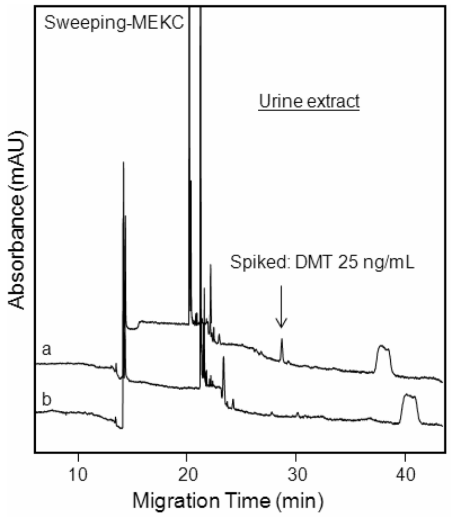

Figure 4. Electropherogram obtained from extracted urine collected from a human volunteer after spiking the sample with 25 ng DMT/mL urine. Electropherograms a and b show the results obtained from the spiked and clean urine extracts, respectively.

(spiked concentration, 25 ng/1 mL urine); electropherogram b shows the results for the clean urine sample. Thus, this approach can be applied to detection of tryptamine and related drugs in urine obtained from suspects.

4 Conclusions

The CE method, despite its popularity, is a simple, fast, and relatively inexpensive method that also is sensitive to tryptamines. Using online sample concentration techniques, a 1000-fold improvement was achieved. The methods discussed herein may also be applied to forensic and clinical analysis of various illegal drugs, including both natural and synthetic tryptamines, phenethylamines, and related compounds.

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The authors declared no conflict of interest.

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