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Comparison of the separation of nine tryptamine standards based on gas chromatography, high performance liquid chromatography and capillary electrophoresis methods

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Abstract

Nine tryptamines, including α-methyltryptamine (AMT), *N*,*N*-dimethyltryptamine (DMT), 5-methoxy-α-methyltryptamine (5-MeO-AMT), *N*,*N*-diethyltryptamine (DET), *N*,*N*-dipropyltryptamine (DPT), *N*,*N*-dibutyltryptamine (DBT), *N*,*N*-diisopropyltryptamine (DIPT), 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeO-DMT), and 5-methoxy-*N*,*N*-diisopropyltryptamine (5-MeO-DIPT) were selected as model compounds. Comparisons of their sensitivity, selectivity, time, cost and the order of migration are described based on different separation techniques (GC, HPLC and CE, respectively). As a result, the limit of detection (S/N = 3) obtained by GC/MS and LC/UV-absorption ranged from 0.5 to 15 μg/mL and 0.3 to 1.0 μg/mL, respectively. In contrast to this, based on the CZE/UV-absorption method, the limit of detection (S/N = 3) was determined to 0.5–1 μg/mL. However, when the sweeping-MEKC mode was applied, it dramatically improved to 2–10 ng/mL. In the case of GC, HPLC and CE, migration times of the nine standards ranged from 11 to 15 min and 8 to 23 min by GC and HPLC, respectively; ranged from 20 to 26 min by sweeping-MEKC. The order of migration of DMT, DET, DPT and DBT follows the molecular weight, whereas the order of migration of AMT and 5-MeO-AMT (primary amines), DIPT (an isomer of DPT) and 5-methoxy-tryptamines (5-MeO-AMT, 5-MeO-DMT and 5-MeO-DIPT) can be altered by changing the separation conditions.

Keywords: GC; HPLC; CE; Tryptamine

1. Introduction

Tryptamine alkaloids are normally used by humans for their psychotropic effects. The degree of alkylation of the side chain nitrogen in a tryptamine has a significant effect on its psychoactivity. Substitutions to the tryptamine molecule give rise to a group of compounds, and, as a result, a number of tryptamine-like illegal drugs are produced in underground labs and sold on the street. From the point of view of screening and confirmation of tryptamine derivatives on the illicit market, more detailed separation and detection information would be 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 their identification. Each of these methods has unique advantages and disadvantages with respect to sensitivity, precision, and simplicity of use. In this study, a mixture of nine tryptamines (Fig. 1), including α-methyltryptamine (AMT), *N*,*N*-dimethyltryptamine (DMT), 5-methoxy-α-methyltryptamine (5-MeO-AMT), *N*,*N*-diethyltryptamine (DET), *N*,*N*-dipropyltryptamine (DPT), *N*,*N*-dibutyltryptamine (DBT), *N*,*N*-diisopropyltryptamine (DIPT), 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeO-DMT), and 5-methoxy-*N*,*N*-diisopropyltryptamine (5-MeO-DIPT) were selected as model compound. Using GC/MS, HPLC/UV-absorption and CE/UV-absorption (including CZE, MEKC and sweeping-MEKC modes) methods, respectively, the separation conditions were optimized, and the results of comparisons of their sensitivity, selectivity, time, cost and the order of migration are reported herein.

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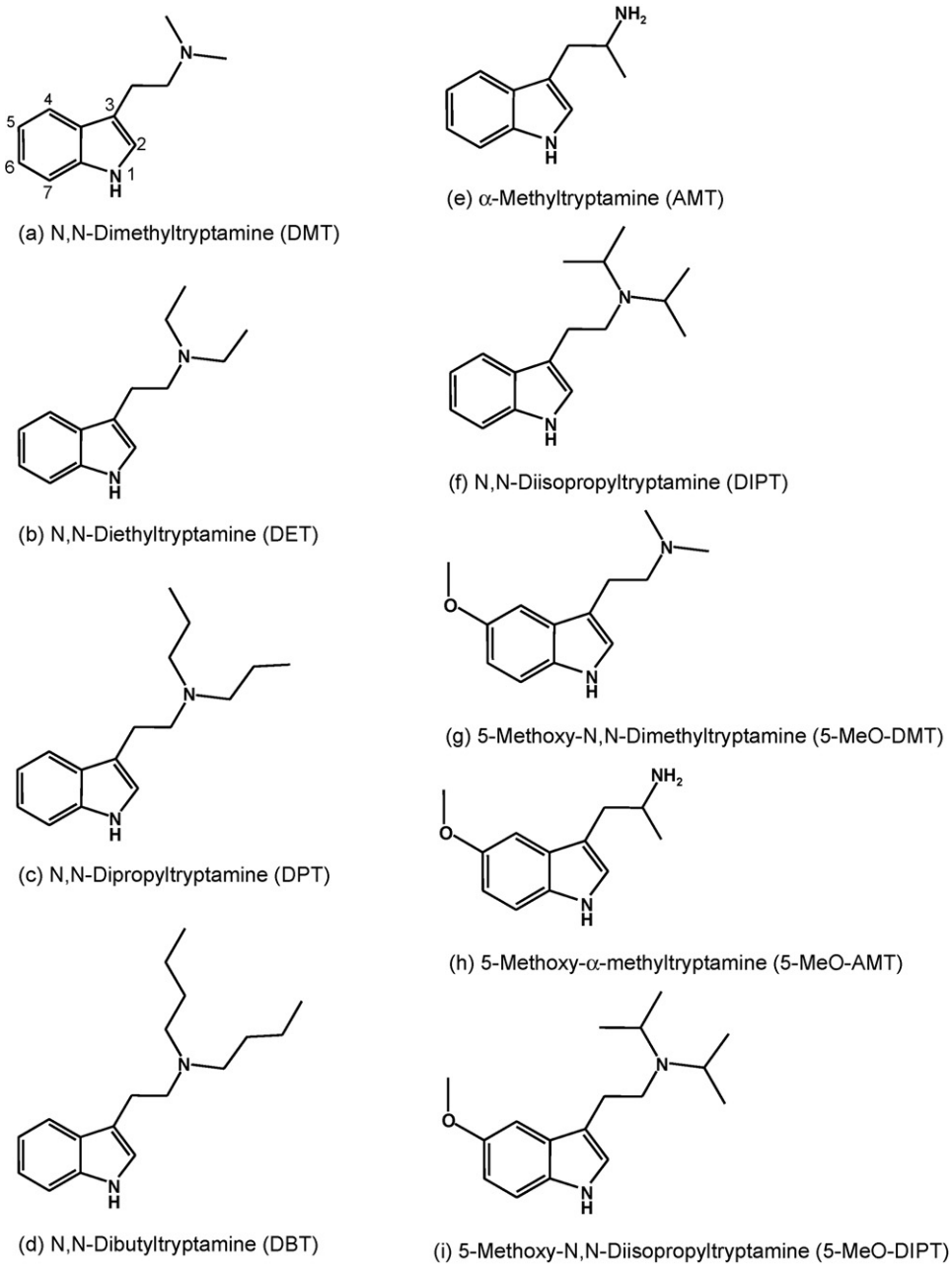

Fig. 1. Molecular structures of the 9 tryptamines and abbreviations used in this study.

2. Experimental

2.1. Reagents

α-Methyltryptamine (AMT), *N*,*N*-dimethyltryptamine (DMT),5-methoxy-α-methyltryptamine (5-MeO-AMT), *N*,*N*--diethyltryptamine (DET), *N*,*N*-dipropyltryptamine (DPT), *N*,*N*-dibutyltryptamine (DBT), *N*,*N*-diisopropyltryptamine (DIPT), 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeO-DMT), and 5-methoxy-*N*,*N*-diisopropyltryptamine (5-MeO-DIPT) were generously donated by the Military Police Command, Forensic Science Center, Taiwan. The procedures for their synthesis have been described previously by Ann and Alexander Shulgin in their book entitled TiHKAL [14]. Following the synthesis-steps, the final products were identified by NMR, IR and verified by GC/MS. Sodium dodecyl sulfate (SDS) was purchased from Acros (Belgium). All the other chemicals were of analytical grade and were obtained from commercial sources.

2.2. Apparatus

The CZE/UV-absorption (λ_{abs} = 280 nm) set-up was identical with that used in our previous studies [15] and is abbreviated herein. A gas chromatograph (GC 5890 Hewlett-Packard, Avondale, PA, USA) equipped with a mass spectrometer

(Hewlett-Packard 5972 mass selective detector) was used for the separation and detection; a capillary column (30 m × 0.25 μm I.D.) with an HP-5MS (cross-linked 5% phenyl and 95% dimethylpolysiloxane) bonded stationary phase film, 0.25 μm in thickness (Agilent Technologies, USA) was used. The LC/UV-absorption system consisted of a Constametric 4100 solvent delivery system (LDC Analytical, Gelnhausen, Germany), a manual injection valve from Shimadzu, a reversed phase column (Cosmosil 5C18-MS, 5 μm, 25 cm × 4.6 mm I.D; Nacalai Tesque, Kyoto, Japan) and a SpectraSystem SCM1000 ultraviolet detector. Ultraviolet detection was performed at 270 nm and mobile phase was pumped at a rate of 1.0 mL/min.

3. Results and discussion

3.1. Limit of detection (LOD)

Thus, far, GC/MS is the officially prescribed method and constitutes the most popular and powerful technique for the analysis of illicit drugs and analogs thereof [16–24]. Thus, optimization of the GC separation for tryptamines would be useful for investigators who are interested in identifying tryptamines in actual forensic cases. Fig. 2A shows a typical GC/total ion current chromatogram for the 9 tryptamines (concentration of each sample, 10 μg in 1 mL methanol; injection volume, 1 μL). The inlet temperature was maintained at 250 °C; the column oven was held at 100 °C for 3 min, then programmed from 100 to 250 °C at 15 °C/min, held for 5 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. As can be seen, the analytes are completely separated. The intensities of these tryptamines are different since their ionization efficiencies are different. Based on the electron impact (EI) mode, the ionization efficiency is poor for primary amines (AMT and 5-MeO-AMT) but better for tertiary amines (the others). The limit of detection (LOD) for primary amine was determined to ∼15 μg/mL; for tertiary amines, LOD was determined to ∼0.5 μg/mL. A 1–2 order of magnitude difference can be found for primary and tertiary amines. This would cause problems if a real sample were examined. Similar results were obtained from the LC/MS method, since the ionization efficiency for primary amines also is poor during the electrospray ionization (ESI) process. In contrast to this, UV absorption is the most popular detection scheme used in CE. Despite primary and tertiary amines, the sensitivities of the nine tryptamines are similar. Fig. 2B shows a typical HPLC/UV-absorption chromatogram for the nine tryptamines (concentration of each sample, 10 μg in 1 mL methanol; injection volume, 10 μL). The LOD was determined to 0.3–1 μg/mL. Fig. 3A shows a typical CZE/UV-absorption electropherogram for the nine tryptamines (pH, 2; applied voltage, +10 kV). In this case, the LOD was determined to 0.5–1 μg/mL. In order to further improve the limit of detection of tryptamines, a sweeping-MEKC mode was applied. Fig. 3B shows a typical sweeping-MEKC electropherogram for the nine tryptamines, using SDS as the surfactant. Herein, the background solution (BGS) consisted of 75 mM SDS and 50 mM NaH_2PO_4 in a mixed acetonitrile–methanol–water solution (5:30:65, v/v/v) the pH of which was 2.2*, adjusted by H_3PO_4. The nine tryptamines (each 500 ng/mL) were first dissolved in a phosphate buffer (50 mM NaH_2PO_4) resulting in a non-micelle buffer. Hydrodynamic injection was achieved by raising the reservoir for injection. The capillary I.D. was 50 μm; total/effective length was 67/80 cm; injection length was 45 cm. When the injection was completed, −15 kV was applied to power the CE separation. As a result, compared to the data obtained from the normal MEKC mode, a ∼300-fold improvement was achieved. In order to investigate the effects of injection length and the corresponding signal intensity when the sweeping-MEKC technique was used under the same experimental conditions, various (30, 40, 45 and 50 cm) column lengths of the sample solution were injected into the capillary, and these results show that the use of 45 cm in the column length provides better sensitivity in detection. Finally, the limit of detection (LOD) values (at S/N = 3) and plate numbers for the nine tryptamine standards obtained by the GC/MS, HPLC/UV-absorption and CE (MEKC and sweeping-MEKC), respectively, under optimized conditions are summarized in Table 1.

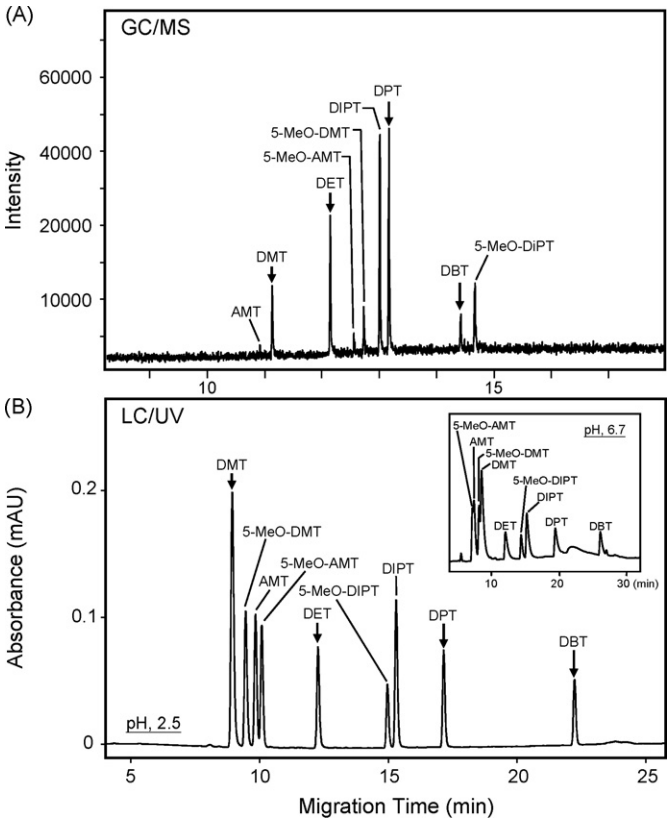

Fig. 2. (A) Typical GC/total ion current chromatogram for the 9 tryptamines (concentration of each sample, 10 μg/1 mL; injection volume, 1 μL). Inlet temperature, 250 °C; column oven, 100 °C for 3 min, then programmed from 100 to 250 °C at 15 °C/min, held for 5 min (carrier gas: helium, flow-rate 1 mL/min). Mass spectrometry conditions: ionization energy, 70 eV; ion source temperature, 230 °C. (B) typical HPLC/UV-absorption (λ_{abs}, 280 nm) chromatogram for the 9 tryptamines (concentration of each sample, 10 μg/1 mL; injection volume, 10 μL). Separation gradient system: mobile phase A (H_2O; pH 2.5 with 0.1% formic acid)/mobile phase B (acetonitrile) delivered at 1 mL/min; A:B, 90:10 (0 min)–62:38 (20 min)–55:45 (22 min)–40:60 (32 min); flow-rate, 1 mL/min. Inset, same conditions as described but pH was adjusted to 6.7.

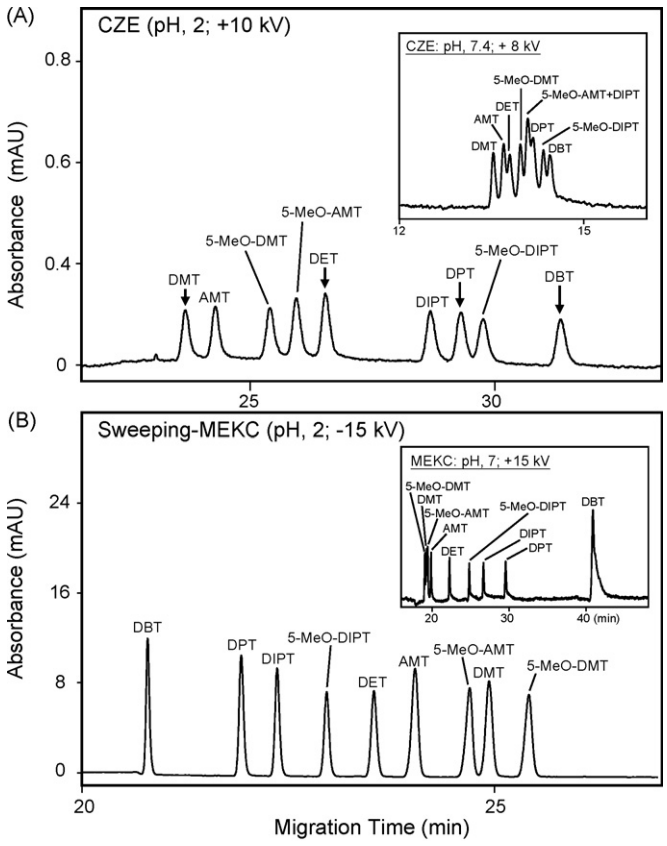

Fig. 3. (A) Typical CZE/UV-absorption electropherogram for the 9 tryptamines; pH, 2 by using 30 mM H_3PO_4; applied voltage, +10 kV. Capillary: I.D., 50 μm; total/effective length, 80/60 cm. Sample concentration, each 10 μg in 1 mL methanol. Inset, electropherogram: pH, 7.4 by using 15 mM NaH_2PO_4 and Na_2HPO_4; solution: water/acetonitrile: 65/35, v/v; applied voltage, +8 kV. (B) sweeping-MEKC/UV-absorption electropherogram for the 9 tryptamines. Sample concentration, each 0.5 μg/1 mL. Conditions: phosphate buffer (50 mM, pH, 2.2) in an acetonitrile–methanol–water (5/30/65, v/v) solution containing 75 mM SDS. The applied voltages used were −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. Inset, MEKC/UV-absorption electropherogram. Conditions: SDS, 70 mM; pH, 7; solution: water/acetonitrile: 70/30, v/v; applied voltage, +15 kV. Sample concentration: DBT, 50 μg/1 mL; the others, each 10 μg/1 mL.

3.2. Migration order

In the case of GC separation, according to the migration time of the analyte, in general, it depends on the temperature of the GC oven, the flow-rate of the carrier gas, and the interaction force between the analyte and the column-coating (stationary phase: polar or nonpolar). In order to simplify these experimental parameters, we selected DMT, DET, DPT and DBT (see Fig. 1, compounds a–d; Fig. 2A, marked as 4 solid arrows) as four reference points for discussion. As a result, we found that three basic requirements for the nine tryptamines based on the GC separation. First, the order of migration is basically proportional to the molecular weight, especially in the cases of DMT, DET, DPT and DBT, a linear relationship can be obtained. Elution of the *iso*-isomer in front of the n-isomer is a phenomenon generally observed in GC as the boiling point of the *iso*-isomer will be lower than that of the n-isomer. Thus, the *iso*-propyl-tryptamine moves faster than *n*-propyl-tryptamine. Finally, when the interaction force between the 5-MeO-tryptamine and column-coating is increased (or decreased), the migration time would be longer (or shorter). These requirements are simple and are useful for the discussion when HPLC and CE are applied, as described below.

Table 1
Limit of detection (LOD) values (at S/N = 3) and plate numbers for the nine tryptamine standards based on GC/MS, HPLC/UV-absorption and CE (MEKC and sweeping-MEKC) methods

	1° amines	3° amines
GC/MS		
LOD	12–15 μg/mL	0.5–6 μg/mL
Plate number	1.2×10^6 to 1.5×10^6	1.7×10^6 to 2.4×10^6
HPLC/UV-absorption		
LOD	0.4–0.5 μg/mL	0.3–1 μg/mL
Plate number	2.5×10^5 to 2.8×10^5	3.7×10^5 to 5.2×10^5
CE		
MEKC		
LOD	0.9–1.3 μg/mL	0.9–1.7 μg/mL
Plate number	4.2×10^5 to 4.4×10^5	5.1×10^5 to 5.8×10^5
Sweeping-MEKC		
LOD	5–8 ng/mL	2–10 ng/mL
Plate number	4.9×10^5 to 5.3×10^5	5.5×10^5 to 1.3×10^6

1° amines: α-methyltryptamine (AMT) and 5-methoxy-α-methyltryptamine (5-MeO-AMT). 3° amines: *N*,*N*-dimethyltryptamine (DMT), *N*,*N*-diethyltryptamine (DET), *N*,*N*-dipropyltryptamine (DPT), *N*,*N*-dibutyltryptamine (DBT), *N*,*N*-diisopropyltryptamine (DIPT), 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeO-DMT) and 5-methoxy-*N*,*N*-diisopropyltryptamine (5-MeO-DIPT).

The gradient system of HPLC separation (Fig. 2B) was used with mobile phase A (H_2O; pH 2.5 with 0.1% formic acid) and mobile phase B (acetonitrile) delivered at 1 mL/min; A:B, 90:10 (0 min)–62:38 (20 min)–55:45 (22 min)–40:60 (32 min); the flow-rate was maintained at 1 mL/min. As shown in Fig. 2B, the order of migration is basically proportional to the molecular weight, especially in the cases of DMT, DET, DPT and DBT; the order of AMT and 5-MeO-AMT, primary amines, are hard to be predicted. When the pH of the solution was adjusted to 6.7, the order of migration follows the molecular weight now.

In the case of CZE separation, the electrophoretic mobility (μ_e) is the ratio of electrical force to frictional force, i.e., $\mu_e = q/6\pi r\eta$ (q, ion charge; r, ion radius; η, solution viscosity). In this case, an acidic buffer was used (to suppress the EOF) and the separation was performed under the same electrical force. In the case of AMT and 5-MeO-AMT, they are almost neutral compounds in an acidic solution, and as a result, the charge effect is minor, leading to longer migration times. DIPT moves faster than DPT because its size is smaller. 5-MeO-DMT and 5-MeO-DIPT moves faster than the predicted times since their ionic volumes are smaller. When the solution pH was adjusted to 7.4, we found that the separation is incomplete, as shown in the inset (in Fig. 3A). This is because, in a weak alkaline solution, the nine tryptamines would all be nearly neutral compounds, and thus difficult to separate by the CZE mode.

In the MEKC mode, migration is due to electrophoresis and distribution between the aqueous phase and the micellar phase. This is determined by effective charge, degree of protonation,

effective electrophoretic mobility and electro-osmotic mobility, respectively [25]. When the buffer is acidic and a negative charge is applied, the EOF would be suppressed, and the major driving force for the separation derives from the SDS-micelles (negative charge), carrying the tryptamines, move toward the outlet. For this reason, a cation (such as tertiary tryptamine), the mass of which is larger or the interaction force with the SDS-micelles is stronger should move toward the outlet, leading to a shorter migration time. The observed orders of migration for the MEKC and the sweeping-MEKC would be the same under the same buffer conditions. We found that, in either the MEKC or sweeping-MEKC mode, the migration time is as follows: DBT < DPT < DET < DMT. AMT and 5-MeO-AMT are nearly neutral compounds, so that the interaction forces between them and the SDS-micelles are stronger than that of cationic tryptamines. As a result, they move faster than DMT and 5-MeO-DMT, respectively. When the solution pH was adjusted to 7.0 and a positive voltage (+15 kV) was applied, the order of migration is totally reversed, as shown in the inset in Fig. 3B. In this case, the EOF is the major driving force for the separation (toward the outlet), meanwhile SDS micelles, carrying the tryptamines, move slowly toward the inlet. Thus, analytes whose molecular weights are smaller or the interaction force with the SDS-micelles is weaker should move faster toward the outlet, leading to a shorter migration time. It should be noted that in a solution of pH 7, all of the tryptamines are nearly neutral compounds. The interaction forces between them and the SDS-micelles are based the degree of alkylation of the side chain nitrogen, i.e., DBT > > DPT > DET > DMT. This is very different from that when an acidic solution is used. In an acidic solution, the interaction forces between cationic tryptamines and the SDS-micelles are quite similar. The interaction forces between the SDS-micelles and the 5-MeO-tryptamines are weak, resulting in their low observed velocity (reversed elution mode). As a result, 5-MeO-DIPT, 5-MeO-AMT and 5-MeO-DMT move slower than DIPT, AMT and DMT, respectively. On the other hand, the elution orders in Fig. 2B inset and Fig. 3B inset are very similar, which would be expected from theory as for the neutral solutes solvophobic forces will dominate the retention process. However, with the (partially) charged solutes in MEKC also Coulomb interactions between the positively charged solutes and the negatively charged micelles will come into play. In that case also the selectivity which would be expected for an ion-exchange process has to be regarded beside the electrophoretic separation and solvophobic interaction between the neutral and/or the charged species and the micelles.

3.3. Time and cost issues

GC/MS analysis has dominated the field of "drug-screening" for many years, and, as a result, a huge database is available in most science libraries. However, the sample preparation for GC/MS analysis is still complicated and time-consuming. Typically, various samples (such as clandestine tablets, hair, urine, etc.) must be pretreated to extract the analytes by either liquid–liquid or solid-phase extraction methods. Each analyte has unique properties for extraction, and the conditions required for the subsequent individual derivatization, prior to their injection into the GC/MS system, are also different. Thus, thousands of samples, especially those containing multiple components, are frequently involved in routine testing. Due to the large number of seized samples, a rapid, reliable and complementary method to GC/MS for use in forensic analysis, would be highly desirable. A HPLC/UV absorption method could be another choice, although this requires a large volume of organic solvents to accomplish. The CE/UV absorption method, despite its popularity, is a simple, fast and economic methodology and also is sensitive to amines. Using an on-line sample concentration technique, such as the sweeping-MEKC method used in this study, dramatic increases in sensitivity can be obtained.

3.4. Possibilities to apply the sweeping-MEKC technique to real samples

The MEKC and sweeping-MEKC modes, providing a detection limit of 1–2 μg/mL and 2–10 ng/mL, respectively, could be used for the routine analysis of real samples. 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 obtained by spiking a blank urine sample from a volunteer with the DMT standard (spiked concentration level, 100 ng/1 mL urine), and the finding shows that the spiked DMT can be identified (data not shown). 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 tryptamine and related drugs taken.

4. Conclusions

We compared the sensitivity, selectivity, orders of migration, and separation time of nine tryptamine standards obtained by various separation methods, including GC, HPLC and CE in this study. The methods discussed herein can also be regarded as a helpful tool for use in forensic and clinical analysis dealing with different types of illegal drugs, not only tryptamines but also phenethylamines, and related compounds, whether they are derived from a natural source or by synthesis.

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