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Simultaneous Separation and Detection of 18 Phenethylamine/ Tryptamine Derivatives by Liquid Chromatography–UV Absorption and –Electrospray Ionization Mass Spectrometry

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The optimal conditions for the separation and detection of a mixture of 18 phenethylamine/tryptamine derivatives were determined, using liquid chromatography/UV-absorption (LC/UV) and liquid chromatography/electrospray ionization mass spectrometry (LC/ESI MS) methods, respectively. Complete separation could be achieved within ~25 min using gradient elution (A, 0.1% formic acid aqueous solution/pH 2.5; B, acetonitrile). The limit of detection (LOD at $S/N = 3$) obtained by LC/UV-absorption (absorption wavelength, 280 nm) was in the range from 0.3 to 3 μg/mL. In contrast, when the LC/ESI MS method was used, the LODs for primary, secondary and tertiary amines were in the ranges 0.1 – 3.0, 0.1 – 0.2, and 0.05 – 1.8 μg/mL, respectively. The lower LOD obtained for a tertiary amine can be attributed to the fact that its ionization efficiency (during the ESI process) is better than the others. In order to improve the LOD of a primary/secondary amine, a derivatization procedure was used in which the chemical structure was altered to a secondary/tertiary amine, *via* a reaction with acetic anhydride. As a result, the LODs for primary/secondary amines could be significantly improved. The characteristic mass fragmentations of the 18 phenethylamine/tryptamine derivatives, as well as the products of the reaction with acetic anhydride, were investigated, and the data were reported. A urine sample was obtained by spiking urine from a volunteer with the 18 derivatives, and after liquid–liquid extraction the sample was examined by LC/UV and LC/ESI MS, respectively. The extraction procedures used for the urine sample and the experimental conditions for the separation and detection were optimized.

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Introduction

Thus far, methods for the synthesis and dosage conditions for 179 phenethylamine and 55 tryptamine derivatives have been qualitatively described by Ann and Alexander Shulgin in their books entitled PiHKAL (Phenethylamines i Have Known And Loved) and TiHKAL (Tryptamines i Have Known and Loved), respectively.[1,2] Unfortunately, these compounds have been abused since the mid-1980s, and their increased availability in the illicit market has become a serious social problem. It should be noted that most clandestine tablets, which are produced in underground labs and sold on the street, contain multiple components. Thus, a method that can be used for the simultaneous determination of these would be highly desirable. A number of analytical methods have been commercially developed for their identification, including a fluorescence polarization immunoassay,[3] an immunochromatographic assay[4] and thin-layer chromatographic analysis.[5,6] Simpler methods, including the use of drug/narcotic detection kits, aerosol sprays/cans or collection paper dispensers, are also commercially available. However, these tests provide only a quick cursory examination and are not legally acceptable as scientific proof. For analyses of these compounds, the method used should have a high degree of accuracy and high sensitivity, not only because the chemical structures of phenethylamine/ tryptamine derivatives are similar, but also because their levels in biological fluids are usually very low. GC/MS (gas chromatography/mass spectrometry) analysis meets this need, since it constitutes to be the most popular and powerful technique for the analysis of illicit drugs, and is also the officially prescribed method.[7-17] However, the major ionization source used in GC/MS is electron impact (EI) and, in many cases, acquiring parent ions of the analytes is a difficult task. Hence, a rapid and soft-ionization method, such as liquid chromatography/electrospray ionization mass spectrometry (LC/ESI MS) methods,[18-21] which is also reliable and complementary to GC/MS for use in forensic analysis, would be highly desirable. In this paper, we report on a simple and specific method for the separation and detection for phenethylamine/tryptamine derivatives, using liquid chromatography/UV-absorption (LC/UV) and LC/ESI MS methods, respectively. Eighteen phenethylamine/tryptamine derivatives were selected as model compounds. The optimal conditions for their separation and detection were determined, and the results are reported herein. Furthermore, a urine sample obtained by spiking urine collected from a human volunteer with the 18 phenethylamine/tryptamine derivatives was also examined. After liquid–liquid extraction, the urine extract was examined using LC/ESI MS under optimized conditions. The extraction procedures used for urine samples and the MS conditions were also optimized, and are reported here.

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Table 1 Abbreviations used for the phenethylamine and tryptamine standards (tabulated by structure) in this study and drug schedules[a] (legal status, US)

Phenethylamine									
Abbreviation	R	R_2	R_3	R[b]	R_4	R_5	R_6	$R_{N1,N2}$	Schedule[a]
DMMDA	H	H		$-OCH_2O-$		H	H	CH_3,CH_3	—
3,4-BDB	CH_2CH_3	H		$-OCH_2O-$		H	H	H,H	—
3,4-MDMA	CH_3	H		$-OCH_2O-$		H	H	H,CH_3	I
3,4-MBDB	CH_2CH_3	H		$-OCH_2O-$		H	H	H,CH_3	—
2C-T-7	H	H	OCH_3		$SCH_2CH_2CH_3$	H	OCH_3	H,H	I
2C-T-2	H	H	OCH_3		SCH_2CH_3	H	OCH_3	H,H	—
2C-D	H	H	OCH_3		CH_3	H	OCH_3	H,H	—
2C-B	H	H	OCH_3		Br	H	OCH_3	H,H	I
2C-E	H	H	OCH_3		CH_2CH_3	H	OCH_3	H,H	—
Tryptamine									
Abbreviation		R_5		R	R_{N1}		R_{N2}		Schedule
AMT		H		CH_3	H		H		I
DMT		H		H	CH_3		CH_3		I
5-MeO-AMT		OCH_3		CH_3	H		H		I
DET		H		H	CH_2CH_3		CH_2CH_3		I
DPT		H		H	$CH_2CH_2CH_3$		$CH_2CH_2CH_3$		—
DBT		H		H	$CH_2CH_2CH_2CH_3$		$CH_2CH_2CH_2CH_3$		—
5-MeO-DMT		OCH_3		H	CH_3		CH_3		—
DiPT		H		H	$CH(CH_3)_2$		$CH(CH_3)_2$		—
5-MeO-DiPT		OCH_3		H	$CH(CH_3)_2$		$CH(CH_3)_2$		I

See text for addreviations. a. Drug schedules: I, a law made for the comprehensive drug abuse prevention and control act; —, none scheduled by legal status. b. A ring formed with both R_3 and R_4.

Experimental

Apparatus

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 280 nm, and the mobile phase was pumped at a rate of 1.0 mL/min. A mass spectrometer (Finnigan LCQ Classic LC/MS/MS) was hyphenated and an electrospray ionization (ESI) probe was operated in the positive ion mode. The mass signal was recorded under the full-scans mode (*m*/*z*, 50 - 2000), in which the data recording speed was ~0.63 dot/s. An Xcalibur data system was used for data collecting, which was converted to an ASCII text file. The scan mode used was SIM; the capillary temperature and spray voltage were set to 300°C and 4.5 kV, respectively. The tube lens offset and the capillary voltage were set at –10 and 6 V, respectively; the sheath gas and auxiliary gas flow rates were 70 and 20 (arb), respectively.

Reagents

DMMDA (3,4-methylenedioxy-*N*,*N*-dimethylphenethylamine), 3,4-BDB (3,4-methylenedioxy-α-ethylphenethylamine), 3,4-MDMA (3,4-methylenedioxymethamphetamine), 3,4-MBDB (*N*-methyl-1-(1,3-benzodioxol-5-yl)-2-butylamine), 2C-T-7 (4-propylthio-2,5-dimethoxy-β-phenethylamine), 2C-T-2 (4-ethylthio-2,5-dimethoxy-β-phenethylamine), 2C-D (4-methyl-2,5-dimethoxy-β-phenethylamine), 2C-B (4-bromo-2,5-dimethoxy-β-phenethylamine), 2C-E (4-ethyl-2,5-dimethoxy-β-phenethylamine), AMT (α-methyltryptamine), DMT (*N*,*N*-dimethyltryptamine), 5-MeO-AMT (5-methoxy-α-methyltryptamine), DET (*N*,*N*-diethyltryptamine), DPT (*N*,*N*-dipropyltryptamine), DBT (*N*,*N*-dibutyltryptamine), 5-MeO-DMT (5-methoxy-*N*,*N*-dimethyltryptamine), DiPT (*N*,*N*-diisopropyltryptamine) and 5-MeO-DiPT (5-methoxy-*N*,*N*-diisopropyltryptamine) were generously donated by the Military Police Command, Forensic Science Center, Taiwan. The procedures for their synthesis were described previously by Ann and Alexander Shulgin in their momagraphs.[1,2] Following the synthesis, the final products were identified by NMR, IR and GC/MS. All other chemicals were of analytical grade, and were obtained from commercial sources.

Urine derivatization and extraction procedure

A 1-mL aliquot of a urine sample obtained from a human volunteer was placed in a glass tube and then spiked with 18 phenethylamine/tryptamine derivatives (5 μg each in 1.0 mL urine); research on humans was conducted with observing the code of ethics of the World Medical Association. A spiked urine sample was made alkaline by the addition of 1 g of potassium carbonate, and was then shaken for 1 min. Four milliliters of a hexane–dichloromethane mixture (v/v: 3/1) was added, followed by gently mixing for 5 min. The upper layer was collected (3 mL), and the derivatization procedure was then performed *via* a reaction with acetic anhydride (100 μL). The mixture was shaken for 5 min, followed by centrifugation. The upper layer was collected (3 mL) and evaporated to dryness. The residue was dissolved in 100 μL of methanol, and filtered through a 0.45-μm PVDF (polyvinylidene fluoride) filter for subsequent LC/MS experiments. The extraction efficiency of this procedure was 56.8 ± 6.2% (n = 5).

Results and Discussion

LC/UV-absorption

Table 1 gives abbreviations of the 9 phenethylamine and 9 tryptamine derivatives (tabulated by structure) used in this study (part A, phenethylamines; part B, tryptamines) and the drug schedules (legal status, US); the chemical structures of some are very similar. Figure 1 shows a typical HPLC/UV-absorption chromatogram for the 18 analytes (concentration of each sample, 50 μg in 1 mL methanol; sample injection volume, 10 μL). A gradient system was used with mobile phase A (H_2O; pH 2.5 with

Table 2 Limit of detection ($S/N = 3$; μg/mL) of 18 phenethylamine/tryptamine derivatives before and after a reaction with acetic anhydride based on UV-absorption (absorption wavelength, 280 nm) and ESI/MS methods, respectively

Abbreviation		Analtye		After reaction with acetic anhydride	
		UV-absorption	ESI/MS	UV-absorption	ESI/MS
1°	2C-T-7	0.8 ± 0.2	0.25 ± 0.20	1.0 ± 0.2	0.03 ± 0.02
1°	2C-T-2	3.0 ± 0.8	0.10 ± 0.06	0.7 ± 0.5	0.02 ± 0.01
1°	2C-D	1.5 ± 0.8	0.20 ± 0.10	0.5 ± 0.4	0.03 ± 0.01
1°	2C-B	2.0 ± 0.4	3.00 ± 1.16	1.2 ± 0.5	0.13 ± 0.04
1°	2C-E	1.3 ± 0.4	0.10 ± 0.07	0.6 ± 0.1	0.06 ± 0.02
1°	AMT	0.5 ± 0.1	3.00 ± 0.86	0.2 ± 0.1	0.08 ± 0.01
1°	5-MeO-AMT	0.5 ± 0.1	0.80 ± 0.46	0.2 ± 0.0	0.06 ± 0.04
1°	3,4-BDB	1.2 ± 0.1	0.20 ± 0.08	0.6 ± 0.1	0.03 ± 0.02
2°	3,4-MDMA	0.9 ± 0.1	0.10 ± 0.09	0.7 ± 0.0	0.08 ± 0.02
2°	3,4-MBDB	1.5 ± 0.4	0.20 ± 0.09	0.7 ± 0.1	0.33 ± 0.02
3°	DMMDA	1.2 ± 0.4	0.20 ± 0.06	1.2 ± 0.3	0.18 ± 0.01
3°	DMT	0.6 ± 0.0	1.50 ± 0.06	0.8 ± 0.3	2.21 ± 0.08
3°	DET	0.8 ± 0.1	1.80 ± 0.72	1.0 ± 0.2	2.70 ± 0.06
3°	DPT	0.5 ± 0.1	0.25 ± 0.05	0.7 ± 0.2	0.38 ± 0.02
3°	DBT	0.3 ± 0.1	0.05 ± 0.04	0.4 ± 0.2	0.06 ± 0.03
3°	5-MeO-DMT	0.5 ± 0.1	0.90 ± 0.10	0.7 ± 0.1	1.15 ± 0.13
3°	DiPT	0.8 ± 0.1	0.30 ± 0.16	0.9 ± 0.4	0.46 ± 0.06
3°	5-MeO-DiPT	0.8 ± 0.5	0.70 ± 0.12	1.0 ± 0.2	1.06 ± 0.06

0.1% formic acid) and mobile phase B (acetonitrile) delivered at 1.0 mL/min; A:B, 90:10 (0 min) – 80:20 (15 min) – 60:40 (20 min) – 55:45 (22 min) – 55:45 (35 min). As can be seen, the 18 analytes could be completely separated under these conditions. Herein, a reversed-phase C18 column was used in which the separation efficiency was mainly dependent on the ratios of the solvents used and the pH of the solvents. We found that the use of acetonitrile was superior to methanol, due to a shorter retention time and a sharper peak width. Thus, water and acetonitrile were selected as solvents A and B, respectively. We also found that these analytes would elute faster when the pH values were lower. This is because, when an acidic aqueous solution was used, the analytes tended to form cations that had a strong affinity to water, leading to their moving through the column rapidly. The retention times for 3,4-MDMA (M_w, 193) and its isomers (DMMDA and 3,4-BDB) were in the following order: DMMDA (3°; 6.67 min), 3,4-MDMA (2°; 7.46 min) and 3,4-BDB (1°; 11.90 min), respectively. Various pH values were investigated, and the findings showed that a solution with pH 2.5 (adjusted with 0.1% formic acid) was optimal. The LODs of the 18 analytes are summarized in Table 2 (column 3), based on the LC/UV-absorption method. It can be seen that the LODs are in the range from 0.3 – 3 μg/mL; approaching the limitation of the LC/UV-absorption method.

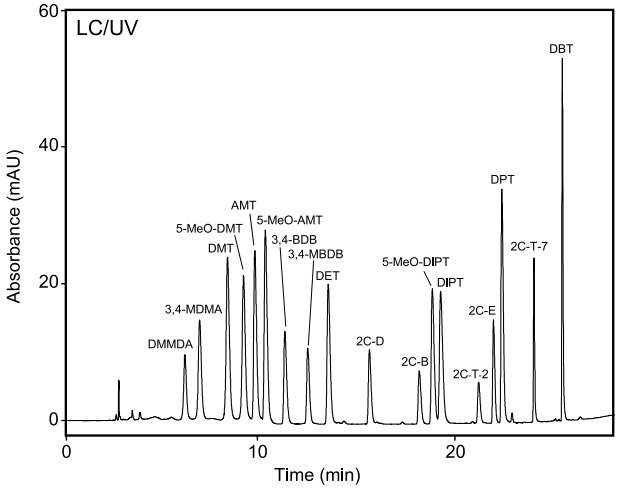

Fig. 1 Typical HPLC/UV-absorption (λ_{abs}, 280 nm) chromatogram of a standard solution containing 9 phenethylamine and 9 tryptamine derivatives (concentration of each sample, 50 μg/mL; injection volume, 10 μL). Separation gradient system: mobile phase A (H_2O; pH 2.5 with 0.1% formic acid)/mobile phase B (acetonitrile) delivered at 1 mL/min; A:B, 90:10 (0 min) – 80:20 (15 min) – 60:40 (20 min) – 55:45 (22 min) – 55:45 (35 min).

LC/ESI MS method

Figure 2 shows a typical LC/ESI-total ion current (TIC) mass chromatogram (upper) for the 18 analytes in the full-scan mode (concentration of each sample, 50 μg in 1 mL methanol; sample injection volume, 10 μL), using the same LC separation conditions as described in Fig. 1. As can be seen, the analytes were not only completely separated, but their major mass fragments could also be observed. The chromatograms obtained in the SIM (selected-ion monitoring at m/z = 50 – 400 amu) mode are shown below the TIC chromatogram ($[M+H]^+$, m/z: DMMDA, 194; 3,4-MDMA, 194; DMT, 189; 5-MeO-DMT, 219; AMT, 175; 5-MeO-AMT, 205; 3,4-BDB, 194; 3,4-MBDB, 208; DET, 217; 2C-D, 196; 2C-B, 261; 5-MeO-DiPT, 275; DiPT, 245; 2C-T-2, 242; 2C-E, 210; DPT, 245; 2C-T-7, 256; DBT, 273). It is clear that each individual peak could be effectively detected in the SIM mode. Furthermore, if the MS/MS mode was applied, the characteristic mass fragmentation of these 18 derivatives could also be established (data not shown). Table 3 (in columns 3 – 5) summarizes the ESI/MS patterns for the 18 analytes, including fragments produced by β-cleavage, α-cleavage, and the stable protonated parent ion ($[M+H]^+$), respectively. This information can serve as fingerprints for the 18 analytes and can be very useful for sample identification. As can be seen in Table 2 (column 4), the LODs for primary, secondary and tertiary amines are in the range from 0.1 – 3, 0.1 – 0.2, 0.05 – 1.8 μg/mL, respectively. Within-day and between-day RSD values of the 18 analytes ranged over 0.2 – 0.8% (retention time)/2.0 – 4.9% (peak area) and 0.5 – 1.3% (retention time)/ 3.3 – 5.7% (peak area), respectively. In order to simplify the experimental procedure in this study, despite the presence of primary, secondary or tertiary amines, a derivatization procedure was performed *via* a reaction with acetic anhydride,[22,23] although tertiary amines are unreactive. After completing of the reaction,

Table 3 Mass fragmentations (*m/z* values) of 18 phenethylamine/tryptamine derivatives before and after a reaction with acetic anhydride based on ESI/MS

Abbreviaton		Analtye			After reaction with acetic anhydride		
		β-Cleavage	α-Cleavage	$[M+H]^+$	β-Cleavage	α-Cleavage	$[M+H]^+$
1°	2C-T-7	—	239	256	72	239	298
1°	2C-T-2	—	225	242	72	225	284
1°	2C-D	—	179	196	72	179	238
1°	2C-B	—	243	260	72	243	302
1°	2C-E	—	193	210	72	193	252
1°	AMT	—	158	175	114	158	217
1°	5-MeO-AMT	—	188	205	114	188	247
1°	3,4-BDB	58	177	194	100	177	236
2°	3,4-MDMA	58	163	194	100	163	236
2°	3,4-MBDB	72	177	208	114	177	250
3°	DMMDA	58	149	194	—	—	—
3°	DMT	58	144	189	—	—	—
3°	DET	86	144	217	—	—	—
3°	DPT	114	144	245	—	—	—
3°	DBT	142	144	273	—	—	—
3°	5-MeO-DMT	58	174	219	—	—	—
3°	DiPT	114	144	245	—	—	—
3°	5-MeO-DiPT	114	174	275	—	—	—

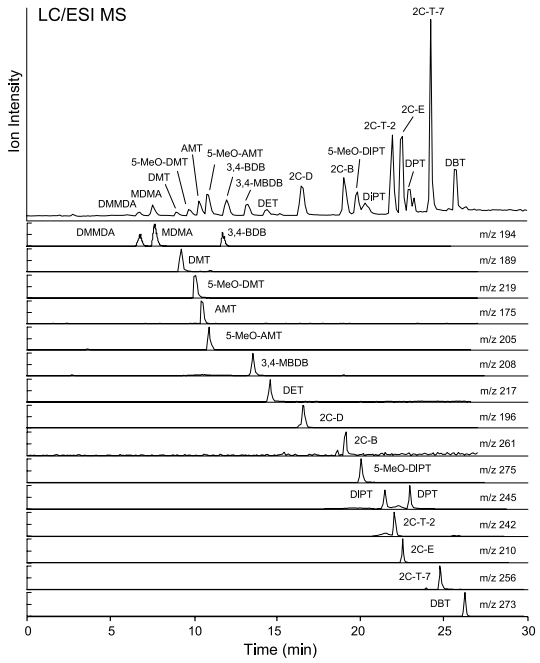

Fig. 2 Typical LC/ESI-total ion current (TIC) chromatogram of a standard solution containing 9 phenethylamine and 9 tryptamine derivatives (concentration of each sample, 50 μg in 1 mL methanol; sample injection volume, 10 μL).

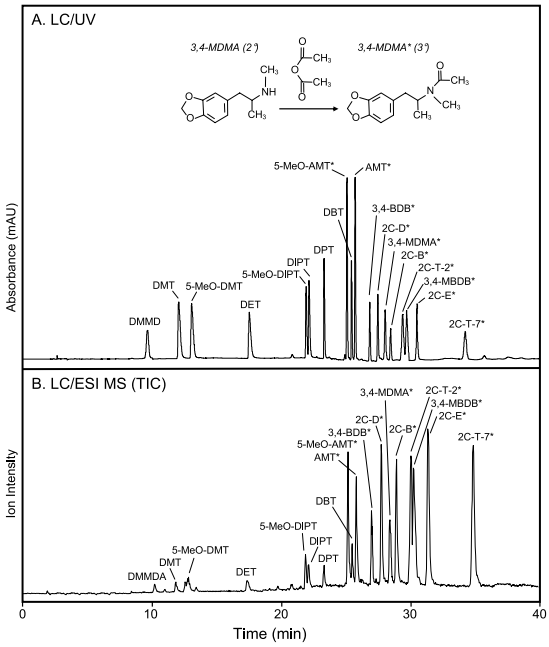

Fig. 3 Typical LC/UV-absorption chromatogram (frame A) and LC/ESI MS chromatogram (frame B) of a standard solution containing 9 phenethylamine and 9 tryptamine derivatives (concentration of each sample, 50 μg in 1 mL methanol; sample injection volume, 10 μL). Separation gradient system: mobile phase A (H_2O; pH 2.5 with 0.1% formic acid)/mobile phase B (acetonitrile) delivered at 1 mL/min; A:B, 89:11 (0 min) – 87:13 (5 min) – 87:13 (7 min) – 60:40 (15 min) – 55:45 (18 min) – 50:50 (40 min).

the reactant was first examined by LC/UV-absorption, and then by the LC/ESI MS method; the results are shown in Figs. 3A and 3B, respectively; the analytes after the reaction are indicated by "*" symbols. The inset in Fig. 3A shows one example of a reaction formula before and after derivatization, using 3,4-MDMA as a model compound. In this case, the gradient system was used with mobile phase A (H_2O; pH 2.5 with 0.1% formic acid) and mobile phase B (acetonitrile) delivered at 1.0 mL/min; A:B, 89:11 (0 min) – 87:13 (5 min) – 87:13 (7 min) – 60:40 (15 min) – 55:45 (18 min) – 50:50 (40 min). It can be seen that the separation was complete, although the orders of retention were altered. Herein, a full-scan mode was also used *at m/z* = 50 – 400 amu ($[M+H]^+$, *m/z*: 3,4-MDMA*, 236; AMT*, 217; 5-MeO-AMT*, 247; 3,4-BDB*, 236; 3,4-MBDB*, 250; 2C-D*, 238; 2C-B*, 302; 2C-T-2*, 284; 2C-E*, 252; 2C-T-7*, 298). Table 2 (in columns 5 and 6) gives the LOD values obtained for the LC/UV-absorption and LC/ESI MS methods, respectively. It can be seen that the LODs were dramatically improved for the primary amines, which were in the range from 0.02 – 0.13 μg/mL; an improvement for secondary amine is not clear. Various LODs were found for tertiary amines (in columns 4 and 6). This is because during the

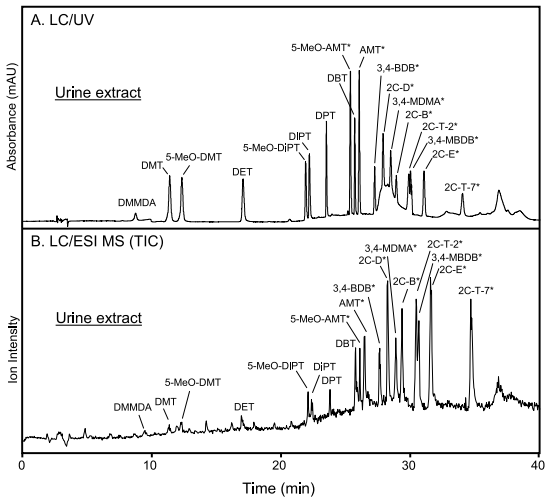

Fig. 4 Typical LC/UV-absorption chromatogram (frame A) and LC/ESI MS chromatogram (frame B), respectively, for a urine extract, under the same separation conditions, as described in Fig. 3.

derivatization procedure tertiary amines could not be completely collected. Table 3 (in columns 6 – 8) summarizes the ESI/MS patterns for the 18 analytes. Thus, we conclude that, based on the LC/ESI MS method, a derivatization procedure, as applied in this study, is a very useful, simple and sensitive method for the simultaneous determination of phenethylamine/tryptamine derivatives.

Application to a urine sample

A urine sample was obtained from a human volunteer. After spiking the sample with 18 analytes (spiked concentration, 5.0 μg each in 1 mL urine), a simple derivatization procedure was performed *via* a reaction with acetic anhydride, and the liquid–liquid extraction method was then applied, as described above. Figure 4 shows a typical LC/UV-absorption chromatogram (frame A) and an LC/ESI MS chromatogram (frame B), respectively, for the urine extract. As can be seen, broad background signals can be seen in both chromatograms due to unknown matrix effects. However, all of the 18 phenethylamine/tryptamine derivatives could be found and identified. Thus, this approach can be applied to the detection and identification of phenethylamine/tryptamine derivatives and related drugs in urine obtained from suspects.

Conclusions

We have demonstrated that the use of LC/ESI MS is a reliable and complementary method for GC/MS, especially for phenethylamine/tryptamines, which have a difficulty to acquire parent ions. The method discussed herein can be applied to forensic and clinical analyses of various illegal drugs, including both natural and synthetic tryptamines, phenethylamines, and related compounds. Furthermore, the method is also sufficiently reliable to serve as a complementary alternative to the officially prescribed method, GC/MS, for use in this field.

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