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Sensitive determination of alpha-methyltryptamine (AMT) and 5-methoxy-*N*,*N*-diisopropyltryptamine (5MeO-DIPT) in whole blood and urine using gas chromatography–mass spectrometry

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Abstract

We devised a sensitive and simple method to determine alpha-methyltryptamine (AMT) and 5-methoxy-*N*,*N*-diisopropyltryptamine (5MeO-DIPT) in whole blood and urine, using gas chromatography–mass spectrometry (GC–MS). AMT and 5MeO-DIPT were extracted using an Extrelut® column with an internal standard, bupivacaine, followed by derivatization with acetic anhydride. The derivatized extract was used for GC–MS analysis of EI-SIM mode. The calibration curves of AMT and 5MeO-DIPT were linear in the concentration range from 10 to 750 ng/ml in both blood and urine samples. The method detection limit (MDL) of AMT and 5MeO-DIPT were 1 ng/ml each in whole blood and 5 ng/ml each in urine. This method should be most useful to accurately determine the presence of these drugs in blood and urine in clinical and forensic cases.

Keywords: Alpha-methyltryptamine; 5-Methoxy-*N*,*N*-diisopropyltryptamine

1. Introduction

Many analogues of a class of tryptamines have hallucinogenic properties [1–14]. The following five drugs are controlled in Japan: *N*,*N*-dimethyltryptamine (DMT), *N*,*N*-diethyltryptamine (DET), alpha-ethyltryptamine (AET), psilocin and psilocybin. On the other hand, non-controlled tryptamines known as "designer drugs" became popular [3], because they can be easily obtained through internet and portable phones [14]. Among them, alpha-methyltryptamine (AMT) and 5-methoxy-*N*,*N*-diisopropyltryptamine (5MeO-DIPT) are the most popular among young people; AMT is called "Day Tripper" or "IT-290", and 5MeO-DIPT is called "FOXY" or "FOXY Methoxy" or "5MEO" in the streets and are sold for reasonable prices at web sites and on the streets [14]. Thus, abuse of these drugs is increasing significantly.

AMT contains a methyl group at alpha-position of nitrogen and 5MeO-DIPT have a diisopropyl group on the nitrogen in tryptamine structure (Fig. 1). The steric hindrance by these alkyl groups slow the metabolism of AMT and 5MeO-DIPT, and they became orally active [11,12]. AMT was initially studied as a monoamine oxidase inhibitor and was used as an antidepressant. There is a documentation of the usefulness of AMT as a psychotropic drug [7]. In animal studies, AMT has 3,4-methylenedioxymethamphetamine (MDMA)-like properties [1]. 5MeO-DIPT is known to have hallucinogenic effects, but there seems to be only one preliminary psychopharmacology study [12].

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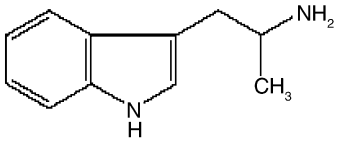
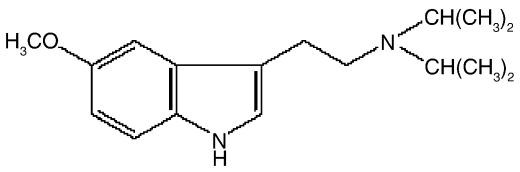
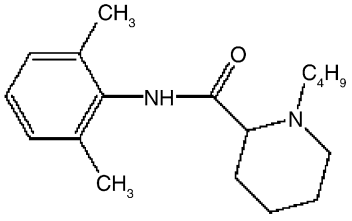


alpha-methyltryptamine (AMT)



5-methoxy-*N,N*-diisopropyltryptamine (5MeO-DIPT)



bupivacaine (IS)

Fig. 1. Chemical structures of AMT, 5MeO-DIPT and bupivacaine (IS).

In addition to the hallucinogenic effects, users of AMT or 5MeO-DIPT experienced euphoria, dilated pupils, empathy, visual and auditory distortions, "feeling of love" and emotional distress. Some users even experience nausea, vomiting and diarrhea (http://www.dea.gov).

Although these designer drugs are widely abused, only two case reports, in a man who ingested white powder of AMT [6] and in a man who ingested "FOXY" tablet [11], are available and no validated analytical procedures with evaluated data of method detection limit (MDL), recovery and reproducibility are reported concerning AMT and 5MeO-DIPT.

We developed a sensitive and selective gas chromatography–mass spectrometry (GC–MS) method for the simultaneous determination of AMT and 5MeO-DIPT in human blood and urine.

2. Experimental

2.1. Reagents

AMT was purchased from Aldrich Chemical Company (Milwaukee, WI, USA). 5MeO-DIPT was synthesized by Chemical Soft R&D Inc. (Kyoto, Japan). Bupivacaine hydrochloride was provided by Astra-Japan Ltd. (Osaka, Japan). Urease, from Jack Bean, ethyl acetate and hydrochloric acid were purchased from Wako Pure Chemical Industries (Osaka, Japan). Urease (200 mg) was dissolved in 10 ml of distilled water. Pyridine (silylation grade) was purchased from Pierce (Milwaukee, WI, USA), acetic anhydride was from Sigma–Aldrich Co. (St. Louis, MO, USA) and Extrelut® NT Refill Pack was from Merck (Darmstadt, Germany). The powder (2.5 g each) was packed in a 1.5 cm diameter glass column, and each sample was directly applied to the column without any steps of conditioning. Ethyl acetate was distilled prior to use and other chemicals were of analytical reagent grade.

2.2. Biological samples

Whole blood and urine samples obtained from healthy Japanese volunteers were kept at −20 °C until analysis.

2.3. Standard solutions

AMT (5.0 mg) and 5MeO-DIPT hydrochloride (5.7 mg) were dissolved in methanol and the volume was adjusted to 5 ml, to obtain a concentration of 1000 ng/μl as a free base. This solution was further diluted in methanol to 100, 10 and 1 ng/μl. Bupivacaine hydrochloride (5.6 mg) was dissolved in methanol, in the same manner.

2.4. Extraction and derivatization procedure

One milliliter sample of whole blood was mixed with 1 ml of 0.01 M hydrochloric acid and 3 μl IS solution (300 ng bupivacaine) in a centrifuge tube (10 ml). The mixture was vortex-mixed for 10 s and centrifuged at 850 × *g* for 15 min. After transferring the supernatant to another centrifuge tube (10 ml), the pH was adjusted to 9.5 by adding 1 ml of 1 M sodium carbonate–sodium hydrogencarbonate buffer (pH 9.5). The mixture was vortex-mixed for 10 s, then applied to an Extrelut® NT column. After standing for 20 min, AMT, 5MeO-DIPT and IS were eluted with 12 ml of ethyl acetate. Then the elute was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 50 μl of pyridine, and 50 μl of acetic anhydride was added to the solution for acetylation. The mixture was kept at 60 °C for 30 min, then the solvent was evaporated to dryness. The residue was dissolved in 60 μl of ethyl acetate, and a 2-μl aliquot of the solution was injected onto a GC–MS apparatus.

One milliliter of urine sample was mixed with 3 μl IS solution (300 ng bupivacaine) in a centrifuge tube (10 ml), then was digested with 200 units of urease at 37 °C for 20 min. To the mixture was added 1 ml of 0.5 M hydrochloric acid, and the preparation was vortex-mixed and centrifuged at 850 × *g* for 15 min. After transferring supernatant to another centrifuge tube (10 ml), the pH was adjusted to 9.5 by adding 1 ml of 1 M sodium carbonate–sodium hydrogen carbonate buffer (pH 9.5). The mixture was then applied to an Extrelut® NT column, derivatized and injected onto a

GC–MS apparatus under the same process used for blood samples.

2.5. GC–MS conditions

The apparatus used was a Hewlett–Packard 5972A GC–MS system. An HP-1ms fused-silica capillary column (30 m × 0.25 mm i.d., 0.25-μm film thickness) coated with 100% dimethylpolysiloxane stationary phase was used. Splitless injection mode was selected with a valve off time of 2 min. The GC–MS conditions were as follows: the initial temperature 60 °C was held for 2 min, the temperature was programmed to 300 °C at a rate of 20 °C/min; this temperature being maintained for 5 min. Injection port and transfer line temperatures were 250 and 280 °C, respectively. Helium with a flow rate of 1 ml/min was used as the carrier gas.

The selected ion-monitoring (SIM) mode was used. The following ions were used as quantifier and qualifier ions: *m*/*z* 130 and 157 for AMT and *m*/*z* 114 and 160 for 5MeO-DIPT for whole blood samples, *m*/*z* 157 and 130 for AMT and *m*/*z* 114 and 160 for 5MeO-DIPT for urine samples, respectively. The ion of *m*/*z* 140 was used for IS.

2.6. Preparation of calibration curves

Whole blood and urine samples were prepared to contain AMT and 5MeO-DIPT at concentrations of 10, 50, 100, 250 and 750 ng/ml, each containing 300 ng IS. These samples were extracted as described above. The calibration curve was obtained by plotting the peak-area ratio of AMT derivative (or 5MeO-DIPT) to IS versus the amount of AMT (or 5MeO-DIPT).

3. Results and discussion

3.1. Extraction procedure

Many abused drugs, such as amphetamine, methamphetamine, MDMA and opiates have been analyzed using solid-phase extraction (SPE) procedures and whole blood and urine samples [15–19]. We therefore first extracted AMT and 5MeO-DIPT using several SPE columns with a silica or polymer base. However, the procedures required many steps in order to obtain colorless extracts especially for urine samples and recoveries were low. Thus, we used an Extrelut® NT column that yields colorless extracts in various matrices [20–23]. Several eluting solvents, ethyl acetate, dichloromethane and mixtures of dichloromethane–ethyl acetate at different ratios were examined, and ethyl acetate led to the best recovery of AMT and 5MeO-DIPT. In urine samples, a large amount of urea interfered with analysis of these drugs. Shinka et al. successfully removed urea by urease for the analysis of 4-hydroxybutyric acid (GHB) [24], and when we applied their method we obtained sharp and symmetrical peaks of AMT-acetate and 5MeO-DIPT.

3.2. Derivatization procedure

Trimethylsilylation (TMS) and acetylation were examined for the derivatization of AMT and 5MeO-DIPT. As TMS-derivatized extracts gave interfering peaks on the chromatogram, probably due to biological matricies, and as only one large peak was obtained on the mass spectra of AMT and 5MeO-DIPT, acetylation was selected. Although, 5MeO-DIPT and bupivacaine were not derivatized, sharp peaks were obtained for these compounds without derivatization.

3.3. Selection of internal standard

It is general to use IS having a similar structure to target drugs. Lindenblatt et al. [25] determined psilocin, a

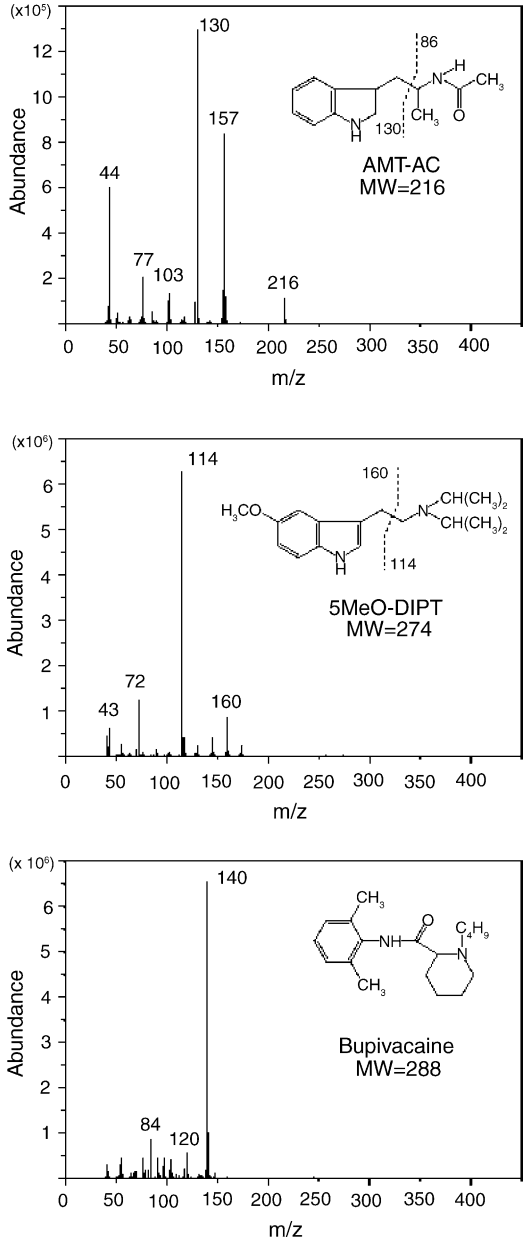


Fig. 2. EI mass spectra of AMT-AC, 5MeO-DIPT and IS.

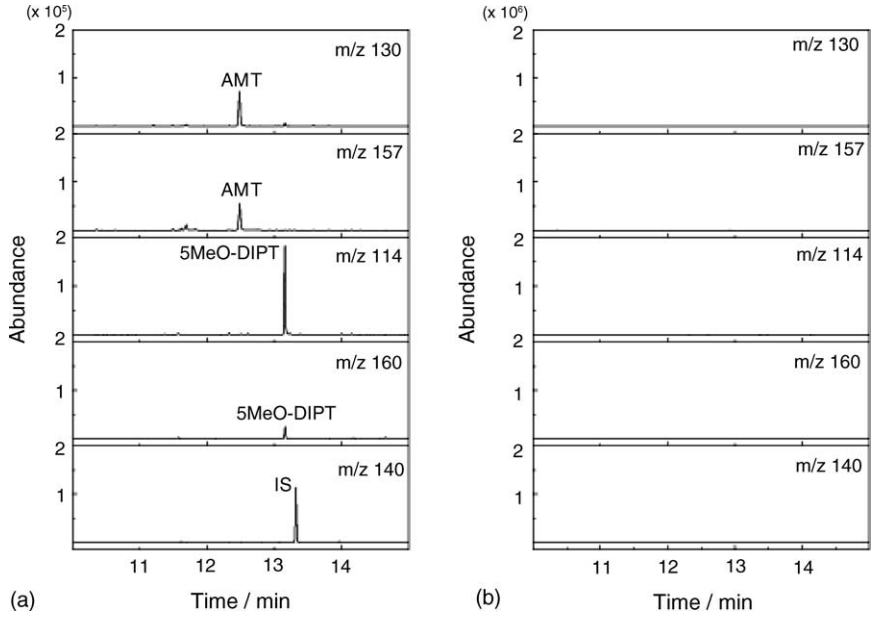


Fig. 3. SIM chromatograms of derivatized extracts from whole blood spiked with 750 ng/ml each of AMT, 5MeO-DIPT and 300 ng/ml of IS (a), and from control whole blood (b).

component of magic mushroom, in human plasma using 5-hydroxy-indole as IS. We examined several indole compounds such as 5-hydroxy-indole, 5-methoxy-indole and indole as IS, but they did not yield sharp peaks. Morano et al. [2] analyzed ethyltryptamine by GC using mepivacaine as IS. As mepivacaine was partially derivated in our condition, it was not proper as IS. When we used bupivacaine, a local anesthetic with a structure similar to mepivacaine, we obtained a satisfactory reproducibility for determination of AMT and 5MeO-DIPT. Therefore, this IS was considered to be suitable for the determination of AMT and 5MeO-DIPT using GC–MS.

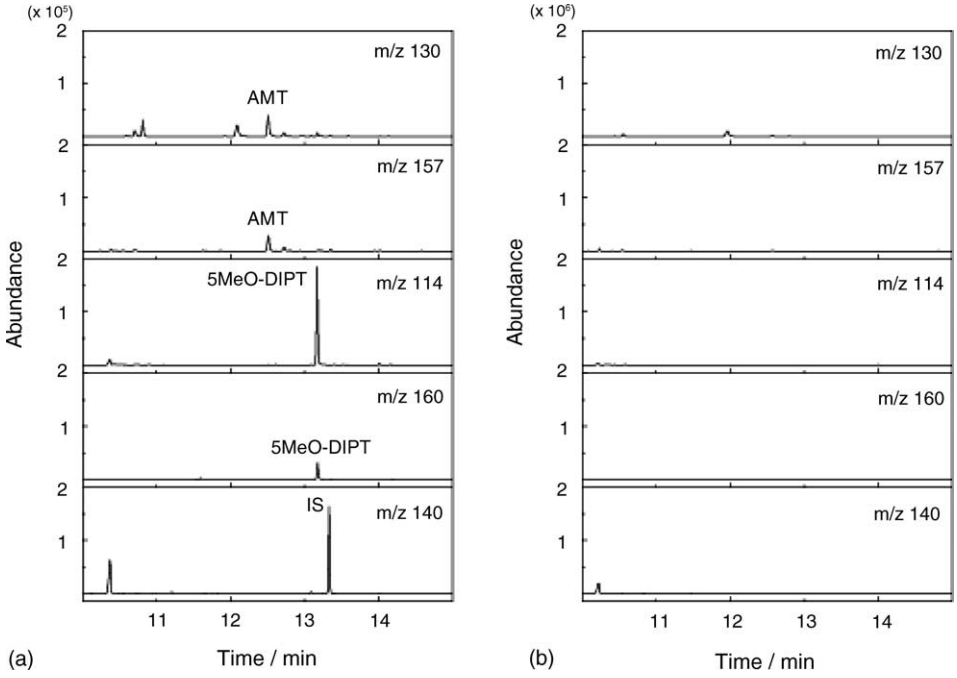


Fig. 4. SIM chromatograms of derivatized extracts from urine spiked with 750 ng/ml each of AMT, 5MeO-DIPT and 300 ng/ml of IS (a), and from control urine (b).

3.4. GC–MS determination of AMT and 5MeO-DIPT in whole blood and urine

Election impact (EI) mass spectra of AMT-AC, 5MeO-DIPT and IS are shown in Fig. 2. Major fragment ions were observed at *m*/*z* 216, 130, 157 for AMT-AC, *m*/*z* 114, 160, 72 for 5MeO-DIPT and *m*/*z* 140 for IS. Each ion was examined and ions of *m*/*z* 130 for AMT-AC and *m*/*z* 114 for 5MeO-DIPT were selected as quantifiers in whole blood sample and ions of *m*/*z* 157 for AMT-AC and *m*/*z* 114 for 5MeO-DIPT were selected as quantifier ions in urine samples. Fig. 3 shows SIM chromatograms of derivatized extracts from whole blood spiked with 750 ng/ml each of AMT, 5MeO-DIPT and 300 ng of IS and from blank whole blood. Each peak was clearly separated on the chromatograms with retention times of 12.50, 13.17 and 13.33 min, respectively. There were no interfering peaks on the chromatograms of blank samples. In urine samples, the similar chromatograms were obtained, as shown in Fig. 4. The calibration curves were linear in the concentration range from 10 to 750 ng/ml in whole blood and urine samples. Linear regression analyses gave the equations, $y = 0.0015x + 0.0051$ (blood) and $y = 0.0006x - 0.003$ (urine) for AMT, and $y = 0.0024x - 0.0368$ (blood) and $y = 0.0017x - 0.0238$ (urine) for 5MeO-DIPT with correlation coefficients exceeded 0.99 (x = the analyte concentration (ng/ml), y = peak area ratio). The method detection limit for AMT and 5MeO-DIPT, at a signal-to-noise ratio of 3, were 1 ng/ml each in whole blood and were 5 ng/ml each in urine. These values are sufficiently sensitive and useful because quantitative analysis results in a urinary concentration of 1.7 μg/ml in a man who ingested "FOXY" tablet [11]. The absolute recoveries of AMT and 5MeO-DIPT in whole blood and urine at two different concentrations, 10 and 100 ng/ml, were determined by comparing the peak areas of derivatives of AMT acetate or 5MeO-DIPT in samples with those in standard solutions. The calculated recoveries for AMT and 5MeO-DIPT in whole blood samples were 29.3 and 79.2% at 10 ng/ml and 78.7 and 73.0% at 100 ng/ml and those in urine samples were 29.9 and 49.6% at 10 ng/ml and 102.6 and 108.4% at 100 ng/ml, respectively. The recovery of AMT and 5MeO-DIPT at 10 ng/ml was low probably due to adsorption of drugs onto the column. However, each peak was clearly detected without any interfering peaks. The recovery of AMT and 5MeO-DIPT in a urine sample at 100 ng/ml exceeded 100% and was not due to overlapping impurity peaks. This phenomenon can be explained by the possibility that certain biological components contained in the urine extract stabilize the drugs or at this concentration prevent them from adsorption onto the column. Within-day precision of this method in whole blood and urine at concentrations of 10 and 100 ng/ml is summarized in Table 1. The relative standard deviations ($n = 5$) were 5.5–7.5% for AMT and 4.3–7.1% for 5MeO-DIPT.

Thus, this method should be useful for confirmation of even minor intake of AMT and 5MeO-DIPT in emergency medicine and forensic toxicology.

Table 1
Precision and accuracy of the method

Samples	AMT		5MeO-DIPT	
	10[a]	100[a]	10[a]	100[a]
Whole blood	6.9	6.3	5.7	7.1
Urine	7.5	5.5	4.3	4.4

Within-day variations for AMT and 5MeO-DIPT in human samples (R.S.D. (%), $n = 5$).
[a] Concentration (ng/ml).

4. Conclusions

We developed a highly sensitive and selective GC/MS method for the simultaneous determination of AMT and 5MeO-DIPT in whole blood and urine. This is the first validated procedure, which can be used in clinical and forensic toxicological cases.

Acknowledgments

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