Urinary Excretion Profiles of 5-Methoxy-*N,N*-diisopropyltryptamine and Its Relevant Metabolites in Humans

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5-Methoxy-*N,N*-diisopropyltryptamine (5-MeO-DIPT), a psychotomimetic tryptamine derivative, and its relevant metabolites have been determined in eleven urine specimens from six 5-MeO-DIPT users, and their excretion profiles have been investigated by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS). Three metabolites, 5-hydroxy-*N,N*-diisopropyltryptamine (5-OH-DIPT), 6-hydroxy-5-methoxy-*N,N*-diisopropyltryptamine (6-OH-5-MeO-DIPT), and 5-methoxy-*N*-isopropyltryptamine (5-MeO-NIPT) were determined in the urine specimens. Urinary conjugated metabolites, both sulfates and glucuronides of 5-OH-DIPT and 6-OH-5-MeO-DIPT, were hydrolyzed completely by the use of *Helix pomatia* sulfatase/*β*-glucuronidase. Degradation of 6-OH-5-MeO-DIPT during incubation for hydrolysis was successfully prevented by the addition of ascorbic acid. The hydrolysis treatment increased the detection amounts of 5-OH-DIPT and 6-OH-5-MeO-DIPT in most of the specimens, and the increase in 6-OH-5-MeO-DIPT was more drastic than that in 5-OH-DIPT. The concentrations of 5-MeO-DIPT (< 1.7 μg/ml) and 5-MeO-NIPT (< 3.5 μg/ml) were lower than those of 5-OH-DIPT (0.01–47 μg/ml) and 6-OH-5-MeO-DIPT (< 69 μg/ml) detected after hydrolysis (the totals of their free and conjugated forms). These metabolites were detectable over longer periods post intake than the parent drug; 35 hr for 5-MeO-DIPT, 80 hr for 5-OH-DIPT, and 60 hr for 6-OH-5-MeO-DIPT and 5-MeO-NIPT.

Key words —— 5-methoxy-*N,N*-diisopropyltryptamine, excretion profile, enzymatic hydrolysis, urine analysis, forensic science

INTRODUCTION

5-Methoxy-*N,N*-diisopropyltryptamine (5-MeO-DIPT) (Fig. 1) is a psychotomimetic tryptamine derivative first synthesized and reported by Shulgin and Carter in 1980.[1] It causes mydriasis, and high dosage produces nausea, jaw clenching, muscle tension, and overt hallucinations with both auditory and visual distortions.[2] 5-MeO-DIPT has been abused since the 1990s in many countries under the name "Foxy" or "Foxy Methoxy." In 2005, this drug was banned in Japan under the Narcotics and Psychotropics Control Law to prevent its rapid spread as a "recreational drug."

For the control of drugs of abuse, careful investigation on the metabolism and excretion of the drug and relevant metabolites in humans, as well as the establishment of the analytical procedure is indispensable to prove the intake of the drug by urine analysis. However, only a few reports have been published about the analysis of 5-MeO-DIPT and its metabolites in biological samples,[3,4] and very little information was available about the excretion of this drug and its metabolites.

In our previous study paper, we determined 5-MeO-DIPT and its three relevant metabolites (reflecting the structure of the parent drug/substance) in the users' urine, and reported that 5-MeO-DIPT is readily metabolized in the human body.[5] The metabolites identified are: 5-hydroxy-*N,N*-diisopropyltryptamine (5-OH-DIPT), demethylated metabolite; 6-hydroxy-5-methoxy-*N,N*-diisopropyltryptamine (6-OH-5-MeO-DIPT), aromatic ring hydroxylated metabolite; and 5-methoxy-*N*-isopropyltryptamine (5-MeO-NIPT), deisopropylated metabolite. Figure 1 shows their chemical structures. Of these metabolites, 5-OH-DIPT and 6-OH-5-MeO-DIPT, both of which have the hydroxyl group, were found to be excreted into urine partially as their sulfates and glucuronides. Thus, the complete hydrolysis of these conjugates before extraction was considered to be indispensable to quantitate the total amounts of these analytes in their free and conjugated forms.

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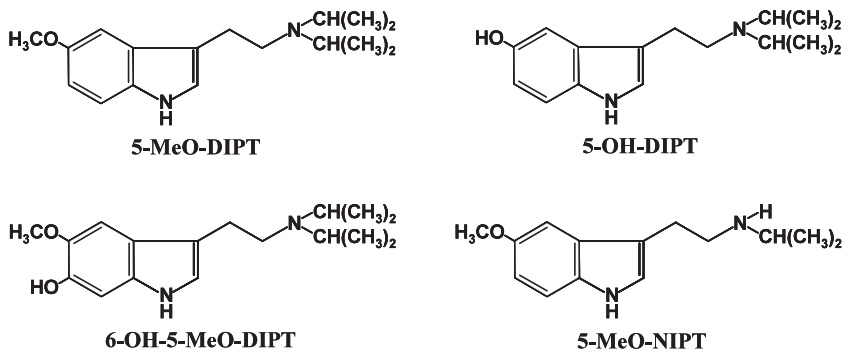

Fig. 1. Chemical Structures of 5-MeO-DIPT and Its Metabolites

The present paper reports a quantitative investigation of 5-MeO-DIPT and its metabolites in eleven urine specimens from six 5-MeO-DIPT users. The conditions for the enzymatic hydrolysis of the conjugated metabolites were carefully investigated, and the above-mentioned four substances were determined before and after the hydrolysis treatment. The excretion profiles of 5-MeO-DIPT and its metabolites into urine were investigated in humans, and the detectable periods of these analytes in urine were examined.

MATERIALS AND METHODS

Materials —— 5-MeO-DIPT hydrochloride, 5-OH-DIPT hydrochloride, 6-OH-5-MeO-DIPT, and 5-MeO-NIPT hydrochloride were synthesized in our laboratory according to previously published methods.[5] The internal standard (I.S.) 5-methyltryptamine (5-MT) hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). *N*-Methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) used for trimethylsilyl (TMS) derivatization was obtained from Wako Pure Chemicals (Osaka, Japan). Sulfatase Type H-1 from *Helix pomatia* (*H. pomatia*; 15.1 units/mg with 437-unit β-glucuronidase activity/mg) was purchased from Sigma-Aldrich. Methanol was of HPLC-grade, and other chemicals used were of analytical grade. The acetate buffer (0.5 M) was prepared by adjusting a 0.5 M sodium acetate aqueous solution to pH 5 with 3% (v/v) acetic acid. An I.S. solution (200 ng/ml) was prepared in 25% methanol-10 mM ammonium formate (adjusted to pH 3.5 with formic acid in advance).

Instrumentation —— Gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS) were performed on a GCMS-QP2010 (Shimadzu, Kyoto, Japan) and a Micromass ZMD system equipped with an Alliance 2690 pump (Waters, Milford, MA, U.S.A.), respectively, according to our previous paper.[5]

TMS Derivatization for GC/MS —— Methanolic solutions of each synthesized standards and the extracts of urine specimens were transferred into screw-capped glass vials. These were evaporated to dryness under a gentle stream of nitrogen at 40°C. After the addition of MSTFA, the vials were capped and heated at 60°C for 30 min.

Urine Specimens from 5-MeO-DIPT Users —— Eleven urine specimens were voluntarily provided from six 5-MeO-DIPT users (Users A–F). Users A and B are identical with Users A and B in our previous paper,[5] respectively. Additional specimens from these users were analyzed in the present study. Users C–F confessed that they orally consumed 5-MeO-DIPT but their exact doses could not be specified.

Extraction of 5-MeO-DIPT and Its Metabolites —— Liquid-liquid extraction: Liquid-liquid extraction was carried out with a chloroform-isopropyl alcohol mixture (3 : 1, v/v) as previously described.[5] The residue obtained was dissolved in methanol for GC/MS or the initial mobile phase for LC/MS, and was subjected to the respective systems.

Solid-phase extraction: One milliliter of urine was applied to a polymeric strata^{TM}X cartridge (polymer-based reversed-phase sorbent, 60 mg/3 ml, Phenomenex, Torrance, CA, U.S.A.) preconditioned by sequential addition of 1 ml methanol and 1 ml water. The cartridge was then

washed with 2 ml of 5% methanol, dried under full vacuum for 30 sec, and the retained analytes were eluted with 2 ml of methanol. The eluent was evaporated to dryness under a gentle stream of nitrogen. The obtained residue was analyzed in the same manner as described above.

Enzymatic Hydrolysis of Conjugated Metabolites —— For hydrolysis treatment, 200 μl of distilled water and 10 μl of 0.3 M ascorbic acid were added to a 100 μl urine specimen, and the mixture was adjusted to pH 5 with 2.8% ammonia solution. After adding 60 μl of 0.5 M acetate buffer (pH 5.0) and 300 units of sulfatase (with 8680-unit β-glucuronidase activity), the mixture was incubated at 45°C for 3 hr.

Quantitative Analysis —— A 100 μl of urine specimen was used for each assay. 5-MeO-DIPT and its metabolites were extracted by liquid-liquid extraction described in the experimental section with/without the hydrolysis treatment. The residue obtained by evaporation was dissolved in 100 μl of the I.S. solution and analyzed by LC/MS as previously described.[5)]

RESULTS AND DISCUSSION

GC/MS and LC/MS of 5-MeO-DIPT and Its Metabolites

The GC/MS spectra with electron ionization of 5-MeO-DIPT and its metabolites obtained are shown in Fig. 2. The analytes were well separated with their retention indices of 2382 for 5-MeO-DIPT, 2480 for 5-OH-DIPT, 2576 for 6-OH-5-MeO-DIPT, and 2179 for 5-MeO-NIPT. However, tailing was observed in each peak on the chromatogram. Therefore, the TMS derivatization with MSTFA was attempted to improve their peak shapes and the intensities of their molecular ions in their mass spectra. TMS derivatization provided sharp peaks and good peak separations. Their retention indices were 2411 for 5-MeO-DIPT-TMS, 2451 for 5-OH-DIPT-*di*-TMS, 2585 for 6-OH-5-MeO-DIPT-*di*-TMS, and 2207 for 5-MeO-NIPT-TMS. The EI mass spectra of their TMS derivatives are shown in Fig. 3. Contrary to GC/MS spectra, their electrospray ionization mass spectra by LC/MS were characterized by the predominant protonated molecules. Thus, it was concluded that GC/MS would be preferable for their qualitative analysis because plural ions reflecting their chemical structures were found in their EI mass spectra, though a combination of GC/MS and LC/MS provides their indisputable duplicate identification.

Extraction of Urinary 5-MeO-DIPT and Its Metabolites

Liquid-liquid extraction (with chloroform-isopropyl alcohol) and solid-phase extraction using a strata^{TM}X cartridge (polymer-based reversed-phase sorbent) were evaluated for the extraction of 5-MeO-DIPT and its metabolites from urine spiked with these analytes at 1 μg/ml each (n = 5). The extracts obtained by the methods described in the experimental section were analyzed and evaluated by both GC/MS and LC/MS. Recoveries with the strata^{TM}X cartridge were 100% for 5-MeO-DIPT, 99.3% for 5-OH-DIPT, 92.4% for 6-OH-5-MeO-

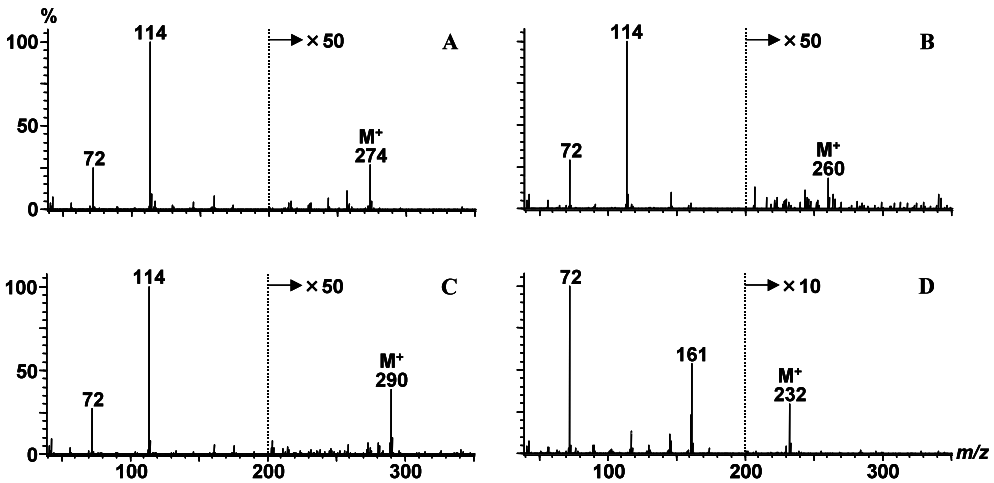

Fig. 2. GC/EI MS Spectra of 5-MeO-DIPT (A), 5-OH-DIPT (B), 6-OH-5-MeO-DIPT (C), and 5-MeO-NIPT (D)

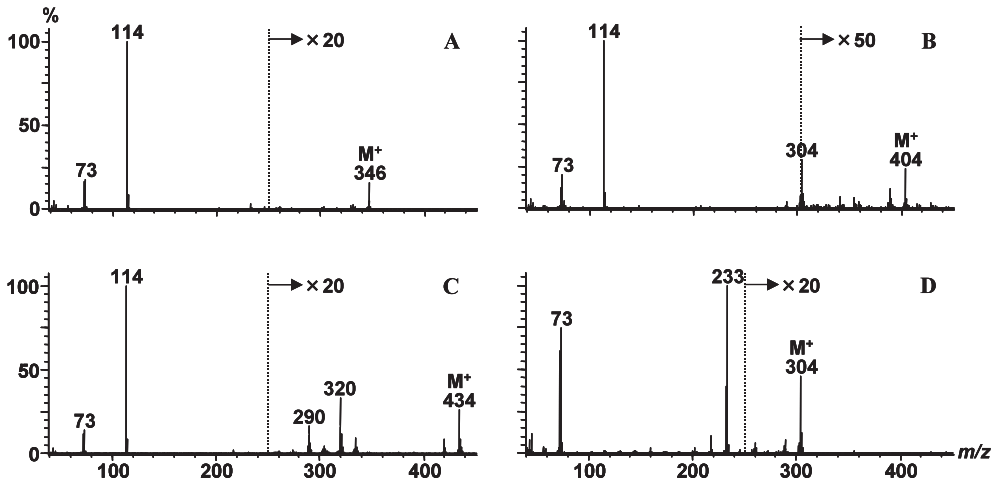

Fig. 3. GC/EI MS Spectra of TMS Derivatives of 5-MeO-DIPT (A), 5-OH-DIPT (B), 6-OH-5-MeO-DIPT (C), and 5-MeO-NIPT (D)

DIPT, and 98.1% for 5-MeO-NIPT, and they were approximately equivalent to the results with liquid-liquid extraction previously reported (92.3–105%).[5] Both methods provided sufficient recoveries for all of the analytes, but liquid-liquid extraction led to somewhat more accurate results than solid-phase extraction. Contrarily, solid-phase extraction provided cleaner chromatograms in GC/MS. Thus, liquid-liquid extraction would be preferable for the quantitative analysis, and solid-phase extraction would be recommended for the qualitative analysis of 5-MeO-DIPT and its metabolites in urine, especially for the identification of trace-levels of these analytes in urine.

Enzymatic Hydrolysis of Conjugated Metabolites

In order to determine drug metabolites in urine, hydrolysis treatment is one of the most essential processes, when the metabolites tend to be excreted as their conjugates. Particularly for metabolites having a hydroxyl group, the effective hydrolysis treatment of such conjugates is indispensable for quantitating the total amounts of their free and conjugated forms. In the present study, enzymatic hydrolysis was employed and optimized. This was because 6-OH-5-MeO-DIPT was unstable even in its neutral aqueous solution at room temperature, and thus the acid or alkali hydrolysis was expected to accelerate its degradation. Our previous study[5] showed that *H. pomatia* sulfatase/β-glucuronidase was effective for the hydrolysis of conjugated metabolites of 5-MeO-DIPT. Also, 6-OH-5-MeO-DIPT sulfate was more difficult to hydrolyze than the others. Therefore, the amount of the *H. pomatia* enzyme and incubation conditions were first optimized focusing on 6-OH-5-MeO-DIPT sulfate, using the urine specimen from User A sampled at 11 hr post intake (hereafter, A-11 hr). Varied amounts of the enzyme were added to the urine samples that had been adjusted to pH 5, and the mixtures were incubated at 37°C or 45°C for 1–5 hr. To simultaneously monitor both liberated free-form metabolites and the remaining conjugates, LC/MS was performed after extraction with methanol as described in our previous paper[5] because such conjugates were difficult to extract and determine by GC/MS after liquid-liquid extraction due to their high polarity. The complete hydrolysis of 6-OH-5-MeO-DIPT sulfate was achieved within 3 hr by incubation at 45°C, using 3000 units of the sulfatase (with 86800 units of β-glucuronidase activity)/ml urine, which was preceded by dilution of urine to dissolve large amount of the enzyme. The other three conjugates were also completely hydrolyzed under the same conditions. In addition, no remaining conjugates were detected after 3 hr incubation under the same conditions in any of the specimens analyzed here. Under the above-mentioned conditions, the amount of ascorbic acid in the reaction mixture was investigated to improve the recovery of unstable 6-OH-5-MeO-DIPT. The hydrolysis treatments with varied amounts of ascorbic acid (10–100 μmol/ml urine) were performed. As a result, the addition of 30 μmol/ml urine of ascorbic acid was found to be sufficient to completely prevent its degradation during the hydrolysis treatment.

The hydrolysis conditions optimized for the uri-

nary metabolites of 5-MeO-DIPT are very different from those for urinary psilocin (4-hydroxy-*N,N*-dimethyltryptamine) glucuronide, the metabolite of psilocybin, established in our previous study,[6] although both drugs are psychotomimetic tryptamine derivatives. β-Glucuronidase was used for conjugated psilocin because only glucuronide was detected as its conjugate. On the contrary, a sulfatase was employed in this study because a substantial amount of sulfate was detected for the metabolites of 5-MeO-DIPT, in addition to their glucuronides. No additional β-glucuronidase was required because the sulfatase (Sigma Type H-1) chosen in this study also has an activity as β-glucuronidase at 86800 units/ml urine. The major metabolisms of both psilocybin and 5-MeO-DIPT convert them into aromatic-hydroxydated *N,N*-dialkyltryptamines, but significant differences were revealed between their phase II metabolisms. In addition, considerably effective hydrolysis conditions (*i.e.*, longer incubation at a higher temperature, with a larger amount of stronger enzyme) should be applied for the conjugated metabolites of 5-MeO-DIPT because its abundant sulfate metabolites were much more difficult to hydrolyze. Thus, even if hydrolysis conditions could be available for similar compounds, even among the same drug family, careful investigation for the optimization of the hydrolysis conditions should be carried out for each drug and metabolite in order to accurately quantitate the total amount of free-form and conjugated metabolites.

Urinary Excretion of 5-MeO-DIPT and Its Metabolites

The established analytical procedures for the determination of 5-MeO-DIPT and its metabolites in urine were applied to the eleven specimens before and after the hydrolysis treatment. For A-11 hr, A-35 hr, and B-18 hr described in our previous paper,[5] 5-OH-DIPT and 6-OH-5-MeO-DIPT were requantitated under the hydrolysis conditions in the present study. The concentration levels of the four analytes after the hydrolysis treatment and those of 5-OH-DIPT and 6-OH-5-MeO-DIPT before the treatment are summarized in Table 1. The hydrolysis treatment of urine increased the detected concentrations of the metabolites having the hydroxyl group, 5-OH-DIPT and 6-OH-5-MeO-DIPT, in eight specimens (A-11 hr, A-35 hr; B-18 hr; C-12 hr, C-60 hr; D-12 hr; E-10 hr; and F-12 hr). The 6-OH-5-MeO-DIPT concentrations detected in A-11 hr and B-18 hr after the treatment were higher than those in our previous paper.[5] These results were considered to be led mainly by hydrolysis of 6-OH-5-MeO-DIPT sulfate remained under the conditions in the paper.[5] Additionally, increased ascorbic acid in the reaction mixture possibly contributed to the results because 10 μmol/ml urine was not sufficient to prevent the degradation of 6-OH-5-MeO-DIPT. The increase in 6-OH-5-MeO-DIPT was more drastic than that in 5-OH-DIPT. Especially in the specimens B-45 hr, C-60 hr, and F-12 hr, the hydrolysis treatment led to successful detection of 6-OH-5-MeO-DIPT. The conjugation rates for 6-OH-5-

Table 1. Urinary Concentration Levels of 5-MeO-DIPT and Its Metabolites in Specimens from Six 5-MeO-DIPT Users

	Time post intake	Concentrations (μg/ml)			
	(hr)	5-MeO-DIPT	5-OH-DIPT	6-OH-5-MeO-DIPT	5-MeO-NIPT
User A	11	1.7	47 (32)	69 (2.6)	1.7
	35	trace*	0.73 (0.71)	0.52 (trace)	0.03
	80	n.d.	0.01* (0.01)	n.d. (n.d.)	n.d.
User B	18	0.03	2.7 (2.4)	3.9 (n.d.)	0.45
	45	n.d.	0.04 (0.04)	n.d. (n.d.)	trace
User C	12	0.54	28 (20)	11 (0.52)	1.3
	60	n.d.	0.05 (0.05)	trace* (n.d.)	trace*
User D	12	1.2	38 (20)	3.8 (0.26)	0.31
	60	n.d.	0.03 (0.03)	n.d. (n.d.)	trace*
User E	10	0.85	34 (22)	5.9 (0.30)	3.5
User F	12	trace	5.5 (4.2)	0.16 (n.d.)	0.04

*: The latest detection of each analyte. trace: < 0.01 for 5-MeO-DIPT and 5-MeO-NIPT; < 0.1 for 6-OH-5-MeO-DIPT. n.d.: not detected. The parenthesized values for 5-OH-DIPT and 6-OH-5-MeO-DIPT represent the concentrations before hydrolysis.

MeO-DIPT were higher than 90% in every specimen in which 6-OH-5-MeO-DIPT was detected before the hydrolysis treatment (A-11 hr, C-12 hr, D-12 hr, and E-10 hr).

In the comparison of the levels of the four analytes in each specimen, the levels of 5-MeO-DIPT (< 1.7 μg/ml) were lower than those of 5-OH-DIPT (0.01–47 μg/ml), 6-OH-5-MeO-DIPT (< 69 μg/ml), and 5-MeO-NIPT (< 3.5 μg/ml). Also, these metabolites were detectable in longer periods than the parent compound. The longest detectable periods were 35 hr for 5-MeO-DIPT, 80 hr for 5-OH-DIPT, and 60 hr for 6-OH-5-MeO-DIPT and 5-MeO-NIPT. These results indicate that 5-OH-DIPT, 6-OH-5-MeO-DIPT, and 5-MeO-NIPT are target analytes, in addition to the parent drug, in the urine analysis for proving 5-MeO-DIPT use.

Of these three metabolites, however, only 6-OH-5-MeO-DIPT retains both the methoxy and the diisopropylamino groups which are structural characteristics of the parent drug 5-MeO-DIPT. This suggests that 6-OH-5-MeO-DIPT would be the most specific metabolite and thus the most useful indicator of 5-MeO-DIPT intake, which can discriminate 5-MeO-DIPT intake from those of other tryptamines, such as 5-methoxy-*N,N*-dipropyltryptamine (5-MeO-DPT) and 5-methoxy-*N*-methyl-*N*-isopropyltryptamine (5-MeO-MIPT). On the contrary, 5-OH-DIPT is somewhat inferior to 6-OH-5-MeO-DIPT in specificity for the parent drug. However, 5-OH-DIPT is also detectable at high levels, either almost equal to or more than those of 6-OH-5-MeO-DIPT, and it provides the longest detectable period. The wide variety of 5-OH-DIPT/6-OH-5-MeO-DIPT ratio among the specimens analyzed in this study might indicate large individual differences on 5-MeO-DIPT metabolism. These show that both 6-OH-5-MeO-DIPT and 5-OH-DIPT would be the useful target analytes in determining the use of 5-MeO-DIPT. However, it should be noted that an appropriate hydrolysis treatment should be performed prior to the extraction because these metabolites are excreted partially as conjugates.

In conclusion, this study provides a reliable method for the determination of 5-MeO-DIPT and its metabolites in urine, which is indispensable for proving its use. To our knowledge, the time-courses of urinary levels of 5-MeO-DIPT and its metabolites have not been fully investigated. This study would also provide information about the metabolism of 5-MeO-DIPT and the excretion profiles of this drug and its metabolites. After 5-MeO-DIPT was banned in 2005, several of its analogs such as 5-MeO-MIPT have increasingly been abused[7] as its substitute in Japan and many other countries. This study can also provide useful information for establishing analytical procedures for determining the use of such drugs, as well as for considering their metabolisms.

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