JOURNAL OF FORENSIC SCIENCES

*J Forensic Sci,* July 2011, Vol. 56, No. 4
doi: 10.1111/j.1556-4029.2011.01753.x
Available online at: onlinelibrary.wiley.com

**TECHNICAL NOTE**

**TOXICOLOGY**

*Ming J. Jin,*[1] *Ph.D.; Changbae Jin,*[1] *Ph.D.; Jin Y. Kim,*[2] *Ph.D.; Moon K. In,*[2] *Ph.D.; Oh S. Kwon,*[1] *Ph.D.; and Hye H. Yoo,*[3] *Ph.D.*

# A Quantitative Method for Simultaneous Determination of 5-Methoxy-*N,N*-Diisopropyltryptamine and its Metabolites in Urine Using Liquid Chromatography–Electrospray Ionization–Tandem Mass Spectrometry

**ABSTRACT:** 5-Methoxy-*N,N*-diisopropyltryptamine (5-MeO-DIPT) is a designer hallucinogen derived from tryptamine and is reportedly abused and involved in criminal activities. For the detection of 5-MeO-DIPT use, a liquid chromatography–tandem mass spectrometric method for 5-MeO-DIPT and its metabolites, 5-hydroxy-*N,N*-diisopropyltryptamine (5-OH-DIPT) and 5-methoxy-*N,N*-isopropyltryptamine (5-MeO-IPT) was developed and validated in rat urine. The urine samples were pretreated by protein precipitation with acetonitrile and introduced into a BDS HYPERSIL $C_{18}$ column (50 × 2.0 mm, 5 μm) for chromatographic separation. Mobile phases consisted of methanol, water, and 1% formic acid, and gradient elution was used at a flow rate of 0.2 mL/min. For the MS detection, multiple-reaction monitoring analysis was adopted. The linear range was 0.01–10 μg/mL, and the lower limit of quantification was 10 ng/mL for all analytes. The intra- and interday accuracies and precisions met the criteria (<15%). The developed method was successfully applied to the drug-treated rat urine.

**KEYWORDS:** forensic science, 5-MeO-DIPT, 5-OH-DIPT, 5-MeO-IPT, urine, LC/MS/MS

5-Methoxy-*N,N*-diisopropyltryptamine (5-MeO-DIPT) is a designer hallucinogen derived from tryptamine (1). 5-MeO-DIPT is also called "FOXY" or "FOXY methoxy" and is used recreationally as a psychedelic drug. 5-MeO-DIPT use is known to cause hallucinations, mydriasis, hypertension, tachycardia, confusion, tremors, and seizures (2). Because of its abuse, many countries, including several European countries, Japan, and the United States, have banned the use of this drug since 1999 (2–4). The Korean government also registered 5-MeO-DIPT on the official list of controlled substances in 2006. Therefore, convenient and reliable analytical methods for 5-MeO-DIPT and its metabolites in biologic samples have been demanded for the purposes of forensic toxicological study.

There have been many investigations of the metabolism of 5-MeO-DIPT. 5-MeO-DIPT is mainly metabolized to 5-hydroxy-*N,N*-diisopropyltryptamine (5-OH-DIPT) and 5-methoxy-*N*-diisopropyltryptamine (5-MeO-IPT) in human and rat (4–7) (Fig. 1). The chemical structure of the metabolites were detected and characterized in urine samples of human via gas chromatography–mass spectrometry (GC-MS) (8,9) and liquid chromatography–mass spectrometry (LC-MS) (4,5,10). However, to our knowledge, there has been no report regarding a method for simultaneous quantification of 5-MeO-DIPT and its metabolites using liquid chromatography–tandem mass spectrometry (LC-MS/MS) for routine application in the forensic field. Therefore, we developed a quantitative method for simultaneous determination of 5-MeO-DIPT and its major metabolites, 5-OH-DIPT and 5-MeO-IPT, in urine by LC–electrospray ionization (ESI)–MS/MS.

## Methods

### *Chemicals and Reagents*

5-MeO-DIPT, 5-OH-DIPT, 5-MeO-IPT, 5-MeO-DIPT-$d_4$, 5-OH-DIPT-$d_4$, and 5-MeO-IPT-$d_4$ were provided by the Pesticide Chemistry & Toxicology Laboratory (Seoul National University, Seoul, Korea). The chemical purities of all six compounds were more than 95% (HPLC). Acetic acid and corn oil were purchased from Sigma Chemical (St. Louis, MO). HPLC-grade acetonitrile was purchased from J.T. Baker (Phillipsburg, NJ). HPLC-grade water was prepared using a Milli-Q purification system (Millipore, Bedford, MA). High-purity nitrogen (99.999%) was obtained from Shin Yang Gas (Seoul, Korea). All other chemicals were of analytical grade.

[1]Doping Control Center, Korea Institute of Science and Technology, Seoul 130-650, South Korea.
[2]Drug Analysis Laboratory, Supreme Prosecutors' Office, Seoul 137-730, South Korea.
[3]College of Pharmacy, Hanyang University, Ansan-si, Gyeonggi-do 426-791, South Korea.

Received 25 Mar. 2010; and in revised form 28 May 2010; accepted 13 June 2010.

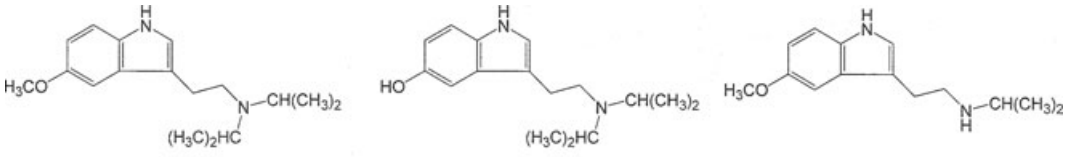


FIG. 1—*Chemical structures of 5-MeO-DIPT, 5-OH-DIPT, and 5-MeO-IPT.*

*Preparation of Standard Solutions*

Standard stock solutions of 5-MeO-DIPT, 5-OH-DIPT, and 5-MeO-IPT were prepared at a concentration of 1 mg/mL in methanol. The standard solutions were serially diluted with methanol to obtain working standard solutions at several concentrations. The internal standard (IS) was prepared to contain 30 μg/mL of 5-MeO-DIPT-$d_4$, 5-OH-DIPT-$d_4$, and 5-MeO-IPT-$d_4$ mixture. The standard solutions were stored at −20°C.

*Preparation of Calibration Standards and Quality Control (QC) Samples*

The standard mixture solution (30 μL) of 5-MeO-DIPT, 5-OH-DIPT, and 5-MeO-IPT was added to 270 μL of blank rat urine to prepare calibration standards and QC samples at final concentrations of 0.01–10 μg/mL. After the addition of 10 μL IS solution, the urine sample was deproteinized with acetonitrile (600 μL). The sample was vigorously vortex-mixed, and the resulting mixture was then centrifuged at 10,000 × *g* for 10 min. The supernatant was subsequently evaporated to dryness under a gentle stream of nitrogen. The residue was reconstituted in 100 μL of mobile phase, and a 5-μL aliquot of the reconstituted solution was injected into the LC-MS/MS system for analysis.

*Sample Preparation*

The urine sample (300 μL) was taken and added with 10 μL of IS solution. The sample was then prepared as described earlier.

*Method Validation*

The calibration curves of 5-MeO-DIPT, 5-OH-DIPT, and 5-MeO-IPT in rat urine were generated by plotting the peak area ratio for the analyte to each corresponding IS vs. the analyte concentration in the calibration standard by least square linear regression. QC samples at four different concentrations (0.02, 0.1, 1, and 10 μg/mL) in rat urine were assayed by replicated analysis ($n = 5$) to determine intraday accuracy and precision. The same method was used over 5 days for interday validation. The precision was expressed as the relative standard deviation (%RSD), and the accuracy was represented as the ratio of the measured concentration to the nominal concentration multiplied by 100. The lower limit of quantification (LLOQ) was determined as the lowest concentration with precision within 20% and accuracy between 80% and 120% on the standard calibration curve.

*Recovery*

The recovery was evaluated by comparing the MS response for the QC samples at three concentrations (at 0.2, 1, and 5 μg/mL) to the standard mixture solutions without matrix. For each run, the samples were analyzed in triplicate.

*Stability*

The stability of 5-MeO-DITP, 5-OH-DIPT, and 5-MeO-IPT was evaluated by analyzing QC samples at a concentration of 0.2 μg/mL in three sets of replicates. For short-term stability, the QC sample was stored at room temperature for 6 h and then analyzed. For long-term stability, the QC sample was stored at −70°C for 14 days, thawed, and then analyzed. For freeze and thaw stability test, the QC sample was stored at −70°C for 24 h and thawed at room temperature. When completely thawed, the sample was refrozen for 24 h at −70°C. The freeze/thaw cycle was repeated two more times, and the sample was analyzed after the third cycle.

*Animal Treatment*

Rats were housed in a temperature- (23 ± 2°C) and moisture (55 ± 10%)-controlled room, exposed to a controlled 12-hr cycle of light and darkness, and allowed free access to food and water. Rats were fasted overnight before administration of the drug. 5-MeO-DIPT dissolved in corn oil was orally administered to rats by gastric intubation. Urine samples were collected 4 h after administration of 5-MeO-DIPT (20 mg/kg) and stored at −70°C until analysis.

*LC-MS/MS Instrumentation and Conditions*

The HPLC system consisted of LC-10ADvp binary pump, SIL-10ADvp autosampler and CTO-10ASvp oven (Shimadzu, Kyoto, Japan). Chromatographic separation was achieved on a BDS HYPERSIL $C_{18}$ column (50 × 2.1 mm, 5 μm; Thermo Fisher Scientific Inc., Worcester, MA). The HPLC mobile phases consisted of 0.1% formic acid (A) and methanol (B). The flow rate was 0.2 mL/min. The gradient program began with 10% B, which was increased to 90% in 1 min, was maintained to 3.5 min, and followed by 2.5 min re-equilibration. Total run time was 6 min. The HPLC system was coupled to an SCIEX API2000 triple-quadrupole mass spectrometer (Applied Biosystems, Concord, Ontario, Canada) equipped with a turbo ion spray source. ESI was performed in positive mode with an ion spray voltage at 5500 V. Nitrogen gas was used as the nebulizing, turbo spray, and curtain gases with the optimum values set at 40, 75, and 40, respectively (arbitrary units). Nebulizer temperature was set at 400°C. Multiple-reaction monitoring (MRM) detection was employed using nitrogen as the collision

TABLE 1—*Retention time ($t_R$), precursor–product ion pairs, and collision energies for multiple-reaction monitoring detection.*

| Compound | $t_R$ (min) | Precursor Ion | Product Ion | CE (V) |
|---|---|---|---|---|
| 5-MeO-DIPT | 2.40 | 275 | 174 | 29 |
| 5-MeO-DIPT-$d_4$ | 2.40 | 279 | 178 | 31 |
| 5-OH-DIPT | 2.06 | 261 | 160 | 29 |
| 5-OH-DIPT-$d_4$ | 2.06 | 265 | 164 | 33 |
| 5-MeO-IPT | 2.28 | 233 | 174 | 25 |
| 5-MeO-IPT-$d_4$ | 2.28 | 237 | 178 | 23 |

gas (4 arbitrary units) with a dwell time of 150 ms for each transition. The precursor–product ion pairs and collision energy values used in the MRM mode were tabulated in Table 1. Data acquisition and analysis were controlled using the analytical software provided by the manufacturer (Version 1.3).

## Results and Discussion

### *Chromatography and Spectrometry*

The ESI conditions were optimized by direct infusion of the reference standard solutions into a mass analyzer. All analytes, which

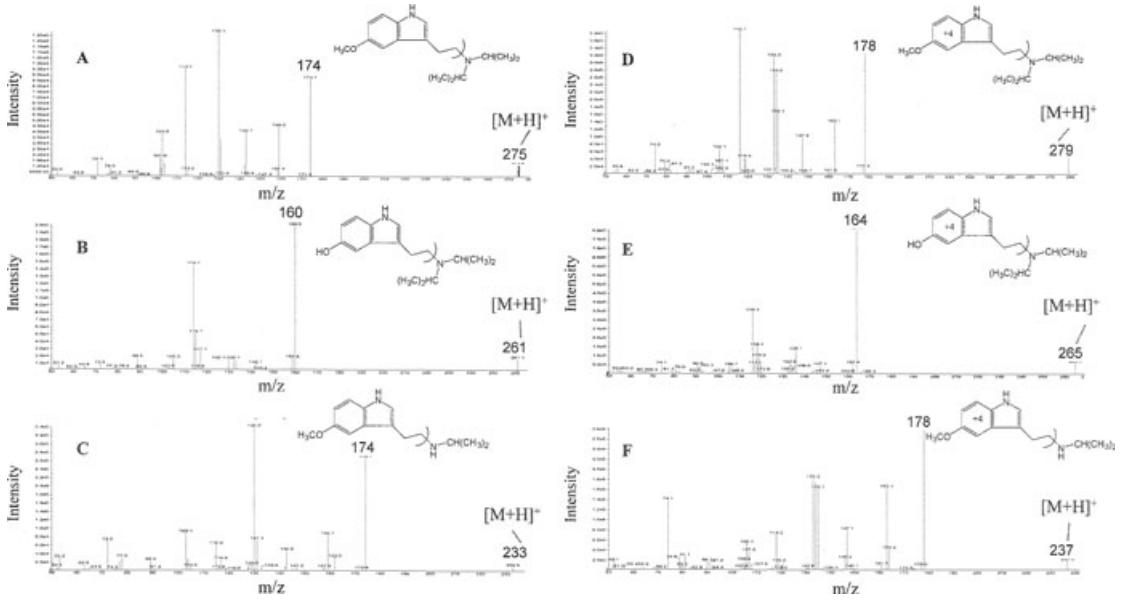


FIG. 2—*MS/MS spectra of (A) 5-MeO-DIPT, (B) 5-OH-DIPT, (C) 5-MeO-IPT, (D) 5-MeO-DIPT-$d_4$, (E) 5-OH-DIPT-$d_4$, and (F) 5-MeO-IPT-$d_4$.*

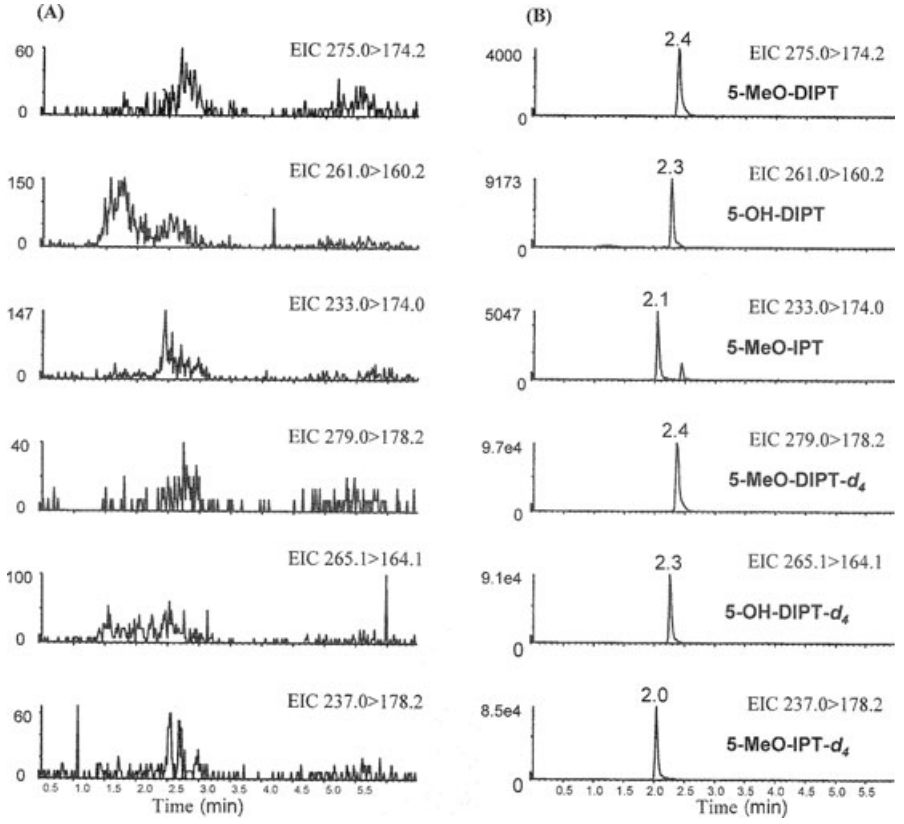


FIG. 3—*Typical MRM chromatograms of (A) blank rat urine and (B) urine spiked with 5-MeO-DIPT, 5-OH-DIPT, and 5-MeO-IPT standards (0.1 μg/mL) and their corresponding deuterated internal standards.*

were dissolved in methanol, produced stable protonated ions in positive ion mode when introduced into the mass spectrometer. The product ion mass spectra for the protonated molecular ions and the postulated fragmentation patterns of 5-MeO-DIPT, 5-OH-DIPT, 5-MeO-IPT, and IS compounds are shown in Fig. 2. All analytes showed a common MS/MS fragmentation pattern such that the predominant product ion was produced by loss of an alkyl amine moiety. Based on these MS/MS fragmentations, the MRM conditions were optimized (Table 1). The chromatographic separation was conducted on a BDS $C_{18}$ column using gradient elution. Mobile phases consisted of methanol and water with 0.1% formic acid. All analytes showed good resolution and peak shape and were eluted within 3 min. The retention times of 5-MeO-DITP, 5-OH-DIPT, 5-MeO-IPT, 5-MeO-DIPT-$d_4$, 5-OH-DIPT-$d_4$, and 5-MeO-IPT-$d_4$ were 2.4, 2.1, 2.3, 2.4, 2.1, and 2.4 min, respectively (Fig. 3).

*Specificity*

Representative MRM chromatograms of blank and standard-spiked urine samples are shown in Fig. 3*A*,*B*. No interference from the matrix was observed at the retention time of any analyte. Therefore, this method showed good specificity for 5-MeO-DITP, 5-OH-DIPT, and 5-MeO-IPT in urine samples.

*Linearity and Lower Limit of Quantification (LLOQ)*

The calibration curves for 5-MeO-DITP, 5-OH-DIPT, and 5-MeO-IPT were obtained using 10-point calibration standards at concentrations between 0.01 and 10 μg/mL ($n = 3$). The correlation coefficients ($r^2$) for all calibration curves were >0.998, and the intercepts were near zero (Table 2). Thus, the calibration curves showed good linearity. The LLOQ was determined as the lowest concentration analyzed with accuracy between 80% and 120% and precision (%RSD) <20%. According to this criterion, the LLOQ of 5-MeO-DITP, 5-OH-DIPT, and 5-MeO-IPT was 0.01 μg/mL.

*Precision and Accuracy*

The precision and accuracy of the method were determined by replicate analyses ($n = 5$) of QC samples prepared at four concentration levels (0.02, 0.1, 1, and 10 μg/mL) spanning the calibration range. The intraday precision was lower than 5.8% and the accuracy remained between 98.9% and 106.8% for all compounds. The interday precision was lower than 7.4% and accuracy remained between 96.6% and 107.4%. The results are tabulated in Table 3. These results proved that the developed method was reproducible and precise enough for the quantification of 5-MeO-DIPT, 5-MeO-IPT, and 5-OH-DIPT in urine.

*Recovery*

The recovery of 5-MeO-DIPT, 5-MeO-IPT, and 5-OH-DIPT from rat urine was found to be 99.3–110.5% at three QC levels (0.2, 1, and 5 μg/mL) (Table 4). Such recovery values around 100% reflect that there was not any significant ion suppression or enhancement caused by matrix ions.

*Stability*

The stability of 5-MeO-DITP, 5-OH-DIPT, and 5-MeO-IPT in rat urine was investigated under a variety of storage and process conditions. All analytes, 5-MeO-DITP, 5-OH-DIPT, and 5-MeO-IPT, were stable in rat urine with acceptable accuracies (92.0–112.6%) and precisions (<10.6%) after 6 h at room temperature, 14 days at −70°C, and three freeze/thaw cycles. Therefore, the present method was considered applicable for routine analysis.

TABLE 2—*Calibration data for 5-MeO-DIPT, 5-OH-DIPT, and 5-MeO-IPT in rat urine.*

| Compound | Concentration Range (μg/mL) | Slope, A* | Intercept, B* | Correlation Coefficient, $r^2$ |
|---|---|---|---|---|
| 5-MeO-DIPT | 0.1–10 | 0.4940 | $-1.510 \times 10^{-4}$ | 0.9989 |
| 5-OH-DIPT | 0.1–10 | 0.5160 | $2.460 \times 10^{-3}$ | 0.9987 |
| 5-MeO-IPT | 0.1–10 | 1.010 | $1.210 \times 10^{-3}$ | 0.9992 |

*Values are mean of three calibration curves: slope and intercept refer to the regression equation, $y = Ax + B$.

TABLE 4—*Recoveries of 5-MeO-DIPT, 5-MeO-IPT, and 5-OH-DIPT in rat urine (*n *= 3).*

| | Recovery (%) | | |
|---|---|---|---|
| Nominal Conc. (μg/mL) | 5-MeO-DIPT | 5-MeO-IPT | 5-OH-DIPT |
| 0.2 | 104.6 ± 2.8 | 98.9 ± 0.6 | 110.9 ± 5.4 |
| 1 | 109.5 ± 1.1 | 99.5 ± 1.8 | 107.2 ± 0.6 |
| 5 | 106.4 ± 1.2 | 99.5 ± 2.6 | 105.2 ± 1.7 |

TABLE 3—*Intra- and interday precision and accuracy for the determination of 5-MeO-DIPT, 5-OH-DIPT, and 5-MeO-IPT in rat urine.*

| | | Intraday ($n = 5$) | | | Interday ($n = 5$) | | |
|---|---|---|---|---|---|---|---|
| Analytes | Nominal Concentration (μg/mL) | Concentration Found | Precision (%) | Accuracy (%) | Concentration Found | Precision (%) | Accuracy (%) |
| 5-MeO-DIPT | 0.02 | 0.02 ± 0.00 | 4.64 | 106.80 | 0.02 ± 0.00 | 3.89 | 107.40 |
| | 0.1 | 0.10 ± 0.01 | 5.75 | 102.82 | 0.10 ± 0.01 | 3.20 | 103.88 |
| | 1 | 1.00 ± 0.04 | 4.09 | 100.36 | 1.06 ± 0.05 | 4.67 | 105.60 |
| | 10 | 10.38 ± 0.22 | 2.09 | 103.80 | 10.46 ± 0.25 | 2.40 | 104.60 |
| 5-OH-DIPT | 0.02 | 0.02 ± 0.00 | 5.61 | 99.84 | 0.02 ± 0.00 | 6.96 | 98.70 |
| | 0.1 | 0.10 ± 0.01 | 5.43 | 100.54 | 0.10 ± 0.00 | 4.66 | 95.56 |
| | 1 | 0.99 ± 0.05 | 4.83 | 98.80 | 1.03 ± 0.07 | 7.10 | 102.50 |
| | 10 | 10.21 ± 0.54 | 5.28 | 102.14 | 10.64 ± 0.23 | 2.16 | 106.40 |
| 5-MeO-NIPT | 0.02 | 0.02 ± 0.00 | 4.55 | 104.24 | 0.02 ± 0.00 | 4.00 | 101.86 |
| | 0.1 | 0.10 ± 0.01 | 2.00 | 101.74 | 0.10 ± 0.01 | 7.11 | 101.92 |
| | 1 | 1.03 ± 0.05 | 4.50 | 102.84 | 1.02 ± 0.05 | 4.44 | 102.64 |
| | 10 | 10.23 ± 0.43 | 4.16 | 102.34 | 10.09 ± 0.75 | 7.44 | 100.84 |

Data were represented as the mean ± SD ($n = 5$)

*Application to Urine Samples from 5-MeO-DIPT Dosed Rats*

The developed method was applied to analysis of urine collected 4 h after oral administration of 5-MeO-DIPT to rats. The peaks of 5-MeO-DIPT and the metabolites were all obviously detected and sufficient to determine the drug use. The concentrations of 5-MeO-DIPT, 5-OH-DIPT, and 5-MeO-IPT were 6.3, 7.1, and 18.7 μg/mL, respectively.

## Conclusion

In conclusion, a sensitive, simple, and rapid LC-MS/MS method for the simultaneous detection of 5-MeO-DIPT, 5-OH-DIPT, and 5-MeO-IPT in rat urine was developed and validated. Although the developed method was not applied to human urine samples because of unavailability of urine samples from 5-MeO-DIPT users, this method could be useful for detection of 5-MeO-DIPT users using urine samples.

*Acknowledgments*

This work was supported by a grant from National R&D Program of Ministry of Education, Science and Technology (MEST) and Korea Science and Engineering Foundation (KOSEF).

## References

1. Shulgin AT, Cater MF. N,N-Diisopopyltryptamine (DIPT) and 5-methoxy-N,N-diisopropyltriptamine (5-MeO-DIPT), two orally active tryptamine analogs with CNS activity. Commun Psychopharmacol 1980;4(5): 363–9.
2. Smolinske SC, Rastogi R, Schenkel S. Foxy methoxy: a new drug of abuse. J Med Toxicol 2005;1(1):22–5.
3. Drug Enforcement Administration, U.S. Department of Justice. Alpha-methyltryptamine (AMT) and 5-methoxy-N,N-diisopropyltryptamine (5-MeO-DIPT, ''FOXY'') are emergency scheduled. Microgram Bull 2003;36(5):96–7.
4. Kamata T, Katagi M, Kamata HT, Miki A, Shima N, Zaitsu K, et al. Metabolism of the psychotomimetic tryptamine derivative 5-methoxy-N,N-diisopropyltriptamine in humans: identification and quantification of its urinary metabolites. Drug Metab Dispos 2006;34(2):281–7.
5. Narimatsu S, Yonemoto R, Saito K, Takaya K, Kumamoto T, Ishikawa T, et al. Oxidative metabolism of 5-methoxy-N,N-diisopropyltriptamine (FOXY) by human liver microsomes and recombinant cytochrome P450 enzymes. Biochem Pharmacol 2006;71(9):1377–85.
6. Kanamori T, Kuwayama K, Tsujikawa K, Miyaguchi H, Iwata Y, Inoue H, et al. In vivo metabolism of 5-methoxy-N,N-diisopropyltryptamine in rat. J Health Sci 2006;52(4):425–30.
7. Narimatsu S, Yonemoto R, Masuda K, Katsu T, Asanuma M, Kamata T, et al. Oxidative of 5-methoxy-N,N-diisopropyltriptamine in rat liver microsomes and recombinant cytochrome P450 enzymes. Biochem Pharmacol 2008;75(3):752–60.
8. Ishida T, Kudo K, Kiyoshima A, Inoue H, Tsuji A, Ikeda N. Sensitive determination of alpha-methyltryptamine (AMT) and 5-methoxy-N, N-diisopropyltryptamine (5MeO-DIPT) in whole blood and urine using gas chromatography-mass spectrometry. J Chromatogr B 2005;823(1): 47–52.
9. Wilson JM, McGeorge F, Smolinske S, Meatherall R. A foxy intoxication. Forensic Sci Int 2005;148(1):31–6.
10. Tanaka E, Kamata T, Katagi M, Tsuchihashi H, Honda K. A fatal poisoning with 5-methoxy-N,N-diisopropyltryptamine. Forensic Sci Int 2006;2:152–4.

Additional information and reprint requests:
Hye H. Yoo, Ph.D.
College of Pharmacy
Hanyang University
1271, Sa 3-dong, Sangnok-gu, Ansan-si
Gyeonggi-do 426-791, Korea
E-mail: yoohh@hanyang.ac.kr