Forensic Toxicol (2008) 26:6–12

ORIGINAL ARTICLE

Simultaneous analysis of six phenethylamine-type designer drugs by TLC, LC-MS, and GC-MS

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Received: 4 December 2007 / Accepted: 31 January 2008 / Published online: 13 March 2008

Abstract Extensive data are presented for simultaneous analysis of six phenethylamine-type designer drugs. The drugs are 2,5-dimethoxyphenethylamine (2C-H), 2,5-dimethoxyamphetamine (2,5-DMA), 4-bromo-2,5-dimethoxyphenethylamine (2C-B), 4-bromo-2,5-dimethoxyamphetamine (DOB), 4-iodo-2,5-dimethoxyphenethylamine (2C-I), and 4-iodo-2,5-dimethoxyamphetamine (DOI). The data include their proton nuclear magnetic resonance (NMR) spectra, infrared spectra, retention times detected by liquid chromatography (LC) and gas chromatography (GC), thin-layer chromatography (TLC) data using seven solvent mixture systems, electrospray ionization (ESI) mass spectra without derivatization, and electron ionization (EI) mass spectra with trifluoroacetyl (TFA) derivatization. Quantitative reliability was also tested for these compounds by LC-mass spectrometry (MS) and GC-MS in high concentration ranges. The data concerned is intended to serve for screening, identification, and quantitation of the drugs contained in seized tablets, crystals, and powder; the data are very useful for such purposes in actual forensic toxicological practice.

Keywords 2,5-Dimethoxyphenethylamine analogues · Designer drugs · 2C-B · DOB · 2C-I · DOI

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Introduction

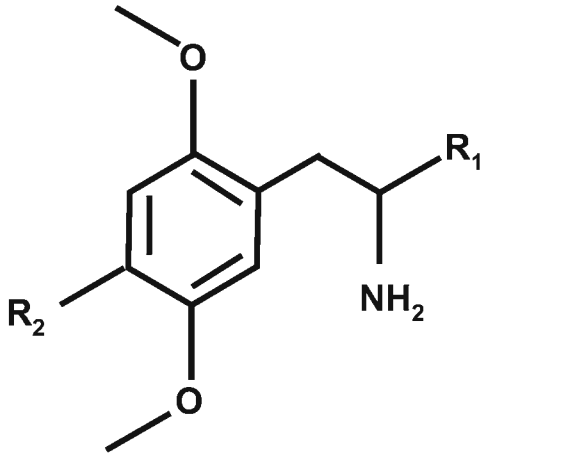
Recently, the abuse of designer drugs has become a serious social problem worldwide. 2,5-Dimethoxyphenethylamine (2C-H) and its α-methyl analogue 2,5-dimethoxyamphetamine (2,5-DMA) are known as psychoactive drugs [1]. Their 4-halogenated analogues are also psychoactive and widely abused in some countries. Among these types of analogues, 2,5-DMA and 4-bromo-2,5-dimethoxyamphetamine (DOB) [1–3] were listed in Schedule 1 of the Controlled Substances Act in 1971. 4-Bromo-2,5-dimethoxyphenethylamine (2C-B) [1], which was first synthesized by Alexander Shulgin [4] and appeared on the drug market in the 1980s [5–7], is also listed in Schedule 1. In Japan, 2,5-DMA, DOB, and 2C-B are controlled by the Narcotics and Psychotropic Control Law, and 4-iodo-2,5-dimethoxyphenethylamine (2C-I) [8] is controlled only by the Pharmaceutical Affairs Law. However, 2C-H and 4-iodo-2,5-dimethoxyamphetamine (DOI) [1] are not yet controlled by any law in Japan. Thus, each drug should be clearly distinguished according to Japanese laws. For such purpose, analytical data must be obtained to identify each drug. In this study, we synthesized 2C-H, 2,5-DMA, and their 4-halogenated analogues, and measured their proton nuclear magnetic resonance (^{1}H NMR) spectra, infrared (IR) spectra, and mass spectra. In addition, we attempted simultaneous analyses of these hallucinogens by

thin-layer chromatography (TLC), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS). To our knowledge, such a comprehensive study has not been reported, although some reports dealing with a single or a few drugs are available [2,3,5–8].

Materials and methods

Chemical synthesis and their identification

The 2,5-dimethoxyphenethylamine analogues (Fig. 1) were synthesized in our laboratory according to the method of Shulgin and Shulgin [9] with minor modifications. All compounds were obtained as HCl salts and identified by ^{1}H NMR (400 MHz) spectroscopy using a JEOL ECP-400 spectrometer (Tokyo, Japan). IR spectra were measured on a Shimadzu FT-IR8600PC (Kyoto, Japan) with the NaCl-tablet method. Acquired IR spectra of these compounds are shown in Fig. 2. Melting points (m.p.) were determined with a micro-melting point apparatus MP-500D (Yanaco, Kyoto, Japan).



R_1	R_2	
H	H	2C-H
CH_3	H	2,5-DMA
H	Br	2C-B
CH_3	Br	DOB
H	I	2C-I
CH_3	I	DOI

Fig. 1 Structures of 2,5-dimethoxyphenetylamine analogues synthesized in this study. *2C-H*, 2,5-Dimethoxyphenethylamine; *2,5-DMA*, 2,5-dimethoxyamphetamine; *2C-B*, 4-bromo-2,5-dimethoxyphenethylamine; *DOB*, 4-bromo-2,5-dimethoxyamphetamine; *2C-I*, 4-iodo-2,5-dimethoxyphenethylamine; *DOI*, 4-iodo-2,5-dimethoxyamphetamine

2C-H. m.p. 130°–135°C; ^{1}H NMR (D_2O) δ: 7.12 (1H, d, J = 9.0 Hz, H-C_3), 7.04 (1H, dd, J = 9.0, 2.1 Hz, H-C_4), 6.99 (1H, br.s, H-C_6), 3.91 (3H, s, CH_3O), 3.88 (3H, s, CH_3O), 3.32 (2H, t, J = 6.9 Hz, CH_2N), 3.05 (2H, t, J = 6.9 Hz, $ArCH_2$); MS (EI) *m*/*z*: 30 (base peak, BP), 32, 137, 152, 181 (M^+).

2,5-DMA. m.p. 115°–117°C; ^{1}H NMR (D_2O) δ: 7.13 (1H, d, J = 9.0 Hz, H-C_3), 7.05 (1H, dd, J = 9.0, 3.1 Hz, H-C_4), 6.97 (1H, d, J = 3.1 Hz, H-C_6), 3.90 (3H, s, CH_3O), 3.89 (3H, s, CH_3O), 3.74 (1H, q, J = 6.8 Hz, CH-N), 3.01 and 2.99 (each 1H, d, J = 2.0 Hz, $ArCH_2$), 1.37 (3H, d, J = 6.7 Hz, CH_3); MS (EI) *m*/*z*: 44(BP), 152.

2C-B. m.p. 176°–180°C; ^{1}H NMR (D_2O) δ: 7.41 (1H, s, H-C_3), 7.11 (1H, s, H-C_6), 3.95 (3H, s, CH_3O), 3.91 (3H, s, CH_3O), 3.31 (2H, t, J = 6.9 Hz, $ArCH_2$), 3.05 (2H, t, J = 6.9 Hz, CH_2N); MS (EI) *m*/*z*: 30 (BP), 230, 232.

DOB. m.p. 182°–187°C; ^{1}H NMR (D_2O) δ: 7.41 (1H, s, H-C_3), 7.09 (1H, s, H-C_6), 3.95 (3H, s, CH_3O), 3.90 (3H, s, CH_3O), 3.74 (1H, q, J = 6.6 Hz, CH-N), 3.01 and 2.99 (each 1H, s, $ArCH_2$), 1.37 (3H, d, J = 6.6 Hz, CH_3); MS (EI) *m*/*z*: 44 (BP), 230, 232.

2C-I. m.p. 212°–215°C; ^{1}H NMR (D_2O) δ: 7.58 (1H, s, H-C_3), 7.04 (1H, s, H-C_6), 3.93 (3H, s, CH_3O), 3.90 (3H, s, CH_3O), 3.31 (2H, t, J = 7.0 Hz, $ArCH_2$), 3.05 (2H, t, J = 7.0 Hz, CH_2-N); MS (EI) *m*/*z*: 30 (BP), 278.

DOI. m.p. 182°–189°C; ^{1}H NMR (D_2O) δ: 7.59 (1H, s, H-C_3), 7.01 (1H, s, H-C_6), 3.93 (3H, s, CH_3O), 3.89 (3H, s, CH_3O), 3.74 (1H, q, J = 6.5 Hz, CH-N), 3.01 and 2.99 (each 1H, s, $ArCH_2$), 1.37 (3H, d, J = 6.5 Hz, CH_3); MS (EI) *m*/*z*: 44 (BP), 278.

Other chemicals

Methamphetamine-HCl was purchased from Dainippon Pharmaceuticals (Osaka, Japan). Amphetamine sulfate and methylenedioxymethamphetamine (MDMA)-HCl were obtained from the Ministry of Health, Labor, and Welfare as standard compounds for criminal identification. Other common chemicals used were of the highest purity commercially available.

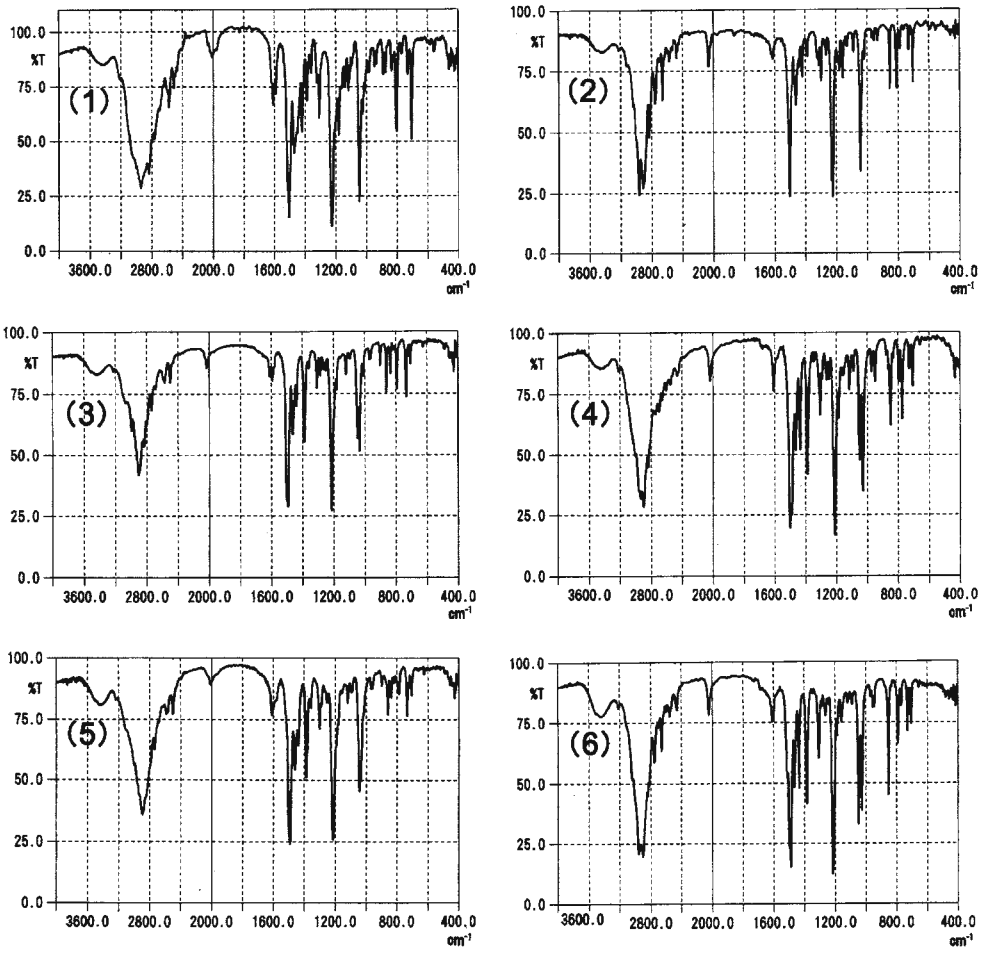


Fig. 2 Infrared (IR) spectra of 2,5-dimethoxyphenethylamine analogues. *1*, 2,5-DMA HCl salt; *2*, 2C-H HCl salt; *3*, DOB HCl salt; *4*, 2C-B HCl salt; *5*, DOI HCl salt; *6*, 2C-I HCl salt

TLC conditions

The sample solution for TLC (1 µl) was applied to Silica Gel 60 F254 plates (Merck, Darmstadt, Germany) and developed with each of seven solvent mixtures as shown in Table 1. Spots were detected under ultraviolet (UV) radiation at 254 nm. In addition, fluorescamine reagent was sprayed evenly on the plates and fluorescence spots were visualized under UV radiation at 365 nm [10]. Methamphetamine, a secondary amine, was detected with Simon's reagent [10].

LC-MS conditions

LC-MS analysis of the 2,5-dimethoxyphenethylamine analogues was performed as follows. The instrument, a Waters Acquity Ultra Performance LC system connected with a Micromass Quattro Premier XE mass instrument (Waters, Milford, MA, USA); column, Waters Acquity UPLC BEH-C18, 50 × 2.1 mm i.d., 1.7 µm (Waters); column temperature, 40°C; mobile phase, acetonitrile and 10 mM ammonium acetate with a linear gradient of acetonitrile from 10% to 25% in 13 min; total flow rate, 0.2 ml/min; injection volume, 5 µl; ionization mode, electrospray ionization (ESI) in the positive mode; desolvation gas, nitrogen at a flow rate of 800 l/h at 350°C; capillary voltage, 4.5 kV; cone voltage, 25 V; cone gas flow, 50 l/h. MS data were recorded in the full scan mode (*m/z* 100–400). A quantitative analysis was carried out by mass chromatography with each protonated molecular ion ($[M+H]^+$) in the positive ion mode. The monitoring ions were as follows: 2C-H (*m/z* 182), 2,5-DMA (*m/z* 196), 2C-B (*m/z* 262), DOB (*m/z* 276), 2C-I (*m/z* 308), and DOI (*m/z* 322). Chromatographic peaks were detected and integrated by a Waters Mass Lynx system (Waters). Linearity was examined at 0.5, 1, 2, 5, 10, 20, 50, and 100 µg/ml of each compound, and the calibration curves were based on the peak areas of mass chromatograms.

GC-MS conditions

GC-MS analysis of the 2,5-dimethoxyphenethylamine analogues was performed as follows. The instrument, a

Shimadzu 17-A gas chromatograph with a QP-5050A mass spectrometer (Kyoto, Japan); ionization, electron ionization (EI) mode; ionization energy, 70 eV; carrier gas, helium; flow rate, 0.9 ml/min; column, DB5-MS capillary (Agilent, Santa Clara, CA, USA, 30 m × 0.25 mm i.d., 0.25 μm film thickness); injector temperature, 250°C; injection mode, split injection (split ratio 20:1); oven temperature, 120°C (3-min hold), followed by 10°C/min raise to 250°C (3-min hold); scan range, *m*/*z* 30–500.

Trifluoroacetyl (TFA)-derivatization of compounds was performed as follows. Fifty microliters of trifluoroacetic anhydride was added to 50 μl of sample solution diluted with ethyl acetate and incubated at 55°C for 15 min. After careful evaporation, the residue was dissolved in 50 μl ethyl acetate, and an aliquot (1 μl) of the derivatized sample was injected into the GC-MS. Quantitative analysis was performed with *N*-butylbenzylamine as internal standard. Linearity was examined at 2, 3, 5, 10, 30, 50, and 100 μg/ml of each compound, and the calibration curves were based on the peak-area ratios of total ion chromatograms.

Results and discussion

Separation of 2,5-dimethoxyphenethylamine analogues by TLC

The R_f values of eight compounds are shown in Table 1. The developing solvents (2) and (6) caused spot tailing for all drugs. Other solvents gave improved spot shapes, but good separation of 2,5-dimethoxyphenethylamine analogues could not be achieved for all solvent systems. Moreover, there was no identifiable solvent system capable of separating the 2,5-dimethoxyphenethylamine analogues from both methamphetamine and amphetamine.

Analysis of 2,5-dimethoxyphenethylamine analogues by LC-MS

The 2,5-dimethoxyphenethylamine analogues were analyzed by LC-MS. The retention times of the compounds were 2.9, 4.0, 7.0, 8.5, 9.0, and 10.5 min for 2C-H, 2,5-DMA, 2C-B, DOB, 2C-I, and DOI, respectively. Good separation of these compounds was achieved under the present LC conditions except for DOB and 2C-I (Fig. 3). In their mass spectra, the $[M+H]^+$ ions appeared as base peaks for all compounds (Fig. 4).

Quantitative analysis was made using mass chromatograms with each $[M+H]^+$ ion. Validation data for quantitation are shown in Table 2 and are generally acceptable for all compounds in high concentration ranges. The linearity of the response was confirmed in the range of 1 to 100 μg/ml for 2C-H, 2,5-DMA, 2C-I, and DOI, and in the range of 2 to 100 μg/ml for 2C-B and DOB. The detection limits by mass chromatography were 2.5 ng per injection for 2C-H, 2,5-DMA, 2C-I, and DOI, and 5 ng per injection for 2C-B and DOB. However, the detection limit calculated from the total ion chromatograms was about 25 ng per injection for all compounds. The precision data of the quantitative analysis by LC-MS are also indicated in Table 2.

Table 1 R_f values of 2,5-dimethoxyphenethylamine analogues using thin-layer chromatography (TLC)

Compound	Developing solvent systems[a]						
	(1)	(2)	(3)	(4)	(5)	(6)	(7)
2C-H	0.60	0.46[b]	0.08	0.64	0.39	0.27[b]	0.44
2,5-DMA	0.59	0.53[b]	0.07	0.70	0.49	0.21[b]	0.51
2C-B	0.60	0.39[b]	0.07	0.64	0.37	0.24[b]	0.41
DOB	0.59	0.53[b]	0.08	0.71	0.48	0.23[b]	0.51
2C-I	0.60	0.39[b]	0.07	0.64	0.37	0.24[b]	0.42
DOI	0.59	0.53[b]	0.10	0.71	0.49	0.23[b]	0.52
Methamphetamine[c]	0.27	0.46[b]	0.06	0.66	0.51	0.11[b]	0.52
Amphetamine	0.60	0.59[b]	0.08	0.69	0.54	0.23[b]	0.58

2C-H, 2,5-Dimethoxyphenethylamine; 2,5-DMA, 2,5-dimethoxyamphetamine; 2C-B, 4-bromo-2,5-dimethoxyphenethylamine; DOB, 4-bromo-2,5-dimethoxyamphetamine; 2C-I, 4-iodo-2,5-dimethoxyphenethylamine; DOI, 4-iodo-2,5-dimethoxyamphetamine

[a] (1) Acetone/toluene/28% ammonia solution (20:10:1, v/v/v); (2) 2-propanol/28% ammonia solution (95:5, v/v); (3) methyl ethyl ketone/dimethylformamide/28% ammonia solution (13:1.9:0.1, v/v/v); (4) chloroform/methanol (95:5, v/v)/28% ammonia solution (lower layer); (5) chloroform/dioxane/ethyl acetate/28% ammonia solution (25:60:10:5, v/v/v/v); (6) acetone/chloroform/methanol (3:3:1, v/v/v); (7) methanol/28% ammonia solution (100:1.5, v/v)

[b] Tailing

[c] Detected by Simon's reagent

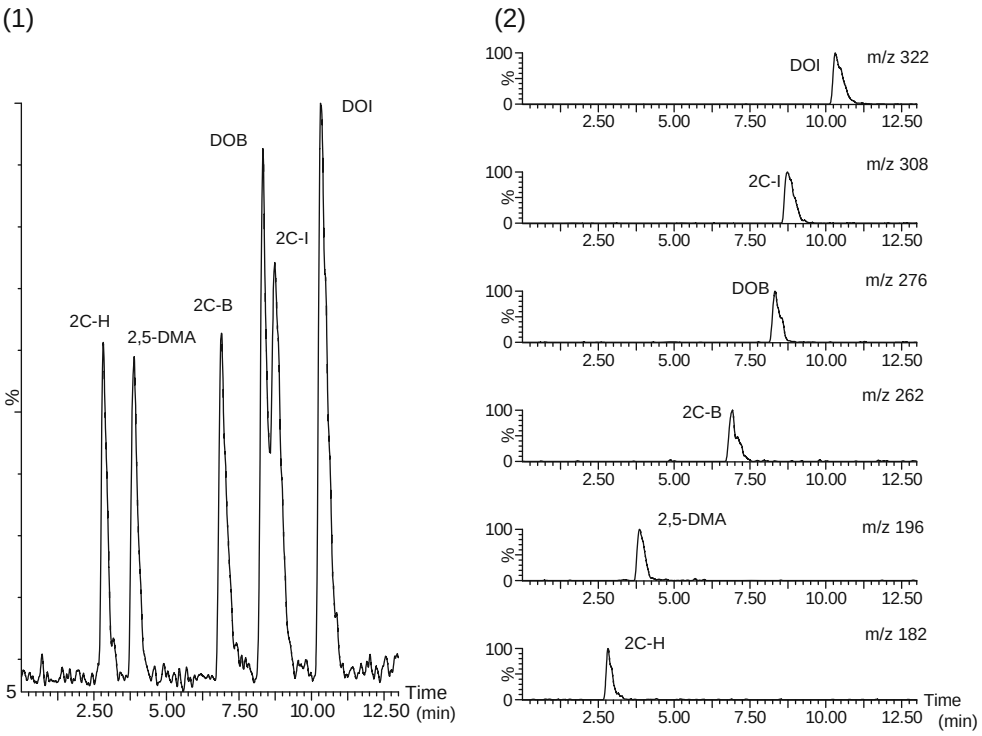


Fig. 3 Liquid chromatography-mass spectrometry (LC-MS) analysis of a mixed standard solution of 2,5-dimethoxyphenethylamine analogues. Total ion chromatogram (*1*) and mass chromatograms (*2*) of a standard solution of the six drugs (100 μg/ml each). $[M+H]^+$ ions were used as monitoring ions for mass chromatography

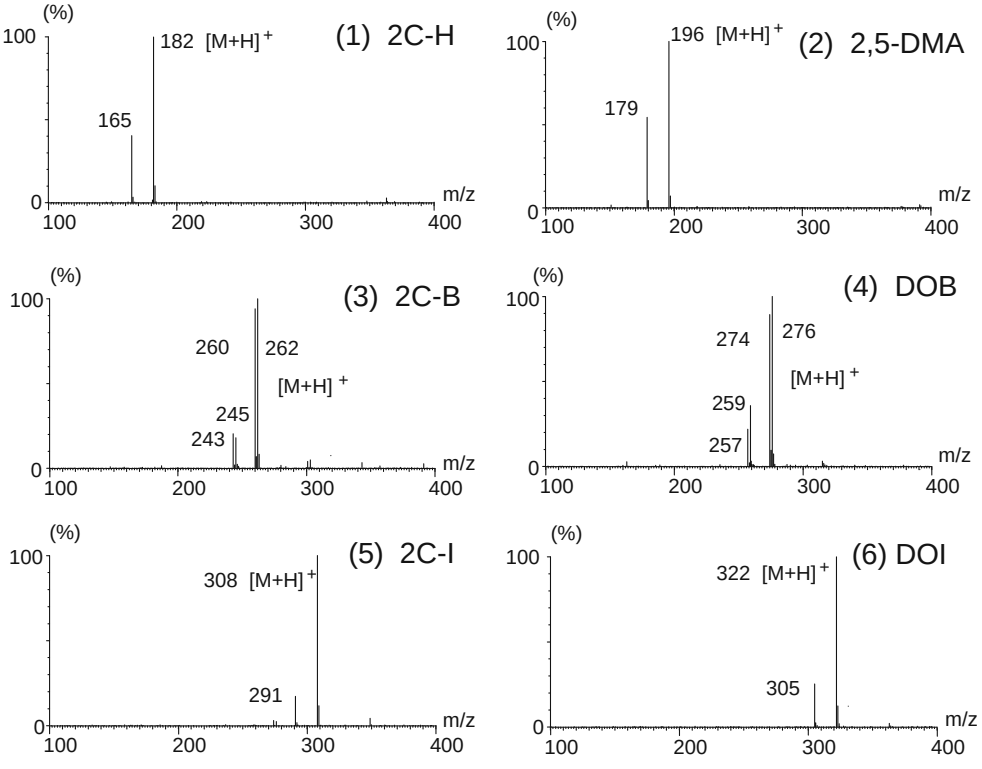


Fig. 4 Mass spectra of 2,5-dimethoxyphenethylamine analogues by LC-MS

Table 2 Validation data for quantitative analysis of 2,5-dimethoxyphenethylamine analogues in aqueous solution by liquid chromatography-mass spectrometry (LC-MS)

Compound	Correlation coefficient[a]	Intraday CV(%)[c]			Interday CV(%)[c]		
		2 μg/ml	10 μg/ml	50 μg/ml	2 μg/ml	10 μg/ml	50 μg/ml
2C-H	0.9823	19.8	10.3	1.7	17.0	8.8	9.4
2,5-DMA	0.9927	11.1	10.6	1.4	8.5	7.8	7.8
2C-B	0.9905	11.6	4.7	1.0	15.7	6.7	5.2
DOB	0.9904	10.2	4.6	5.5	15.6	9.1	6.0
2C-I	0.9902	22.4	5.1	6.3	21.8	8.9	4.7
DOI	0.9894	11.9	7.5	2.7	16.0	6.1	4.6

Determinations performed by mass chromatography
CV, Coefficient of variation
[a] Linearity range: 1–100 μg/ml for 2,5-DMA, 2C-H, DOI, 2C-I; 2–100 μg/ml for DOB and 2C-B
[c] Each value obtained from three determinations

Table 3 Retention times of 2,5-dimethoxyphenethylamine analogues by gas chromatography-mass spectrometry (GC-MS)

Compound	Retention time (min)	
	Free base	TFA derivative
2C-H	9.69	11.4
2,5-DMA	9.83	11.3
2C-B	13.1	14.4
DOB	13.2	14.1
2C-I	14.3	15.5
DOI	14.3	15.2
Amphetamine	3.85	5.98
Methamphetamine	4.51	7.62
MDMA	9.54	12.2

GC conditions described in the Materials and methods section
TFA, Trifluoroacetyl; MDMA, methylenedioxymethamphetamine

Analysis of 2,5-dimethoxyphenethylamine analogues by GC-MS

2,5-Dimethoxyphenethylamine analogues were analyzed by GC-MS with and without TFA derivatization. The retention times of each compound under our conditions are indicated in Table 3. After TFA derivatization, all drugs were completely separated (Fig. 5). However, without derivatization 2C-B and DOB were not well separable; this was also the case for 2C-I and DOI (Table 3). Moreover, methylene artifacts [11] were observed when very highly concentrated solution of the 2,5-dimethoxyphenethylamine analogues in methanol was injected into the injection port at over 200°C; much more of the unidentified artifacts was generated accord-

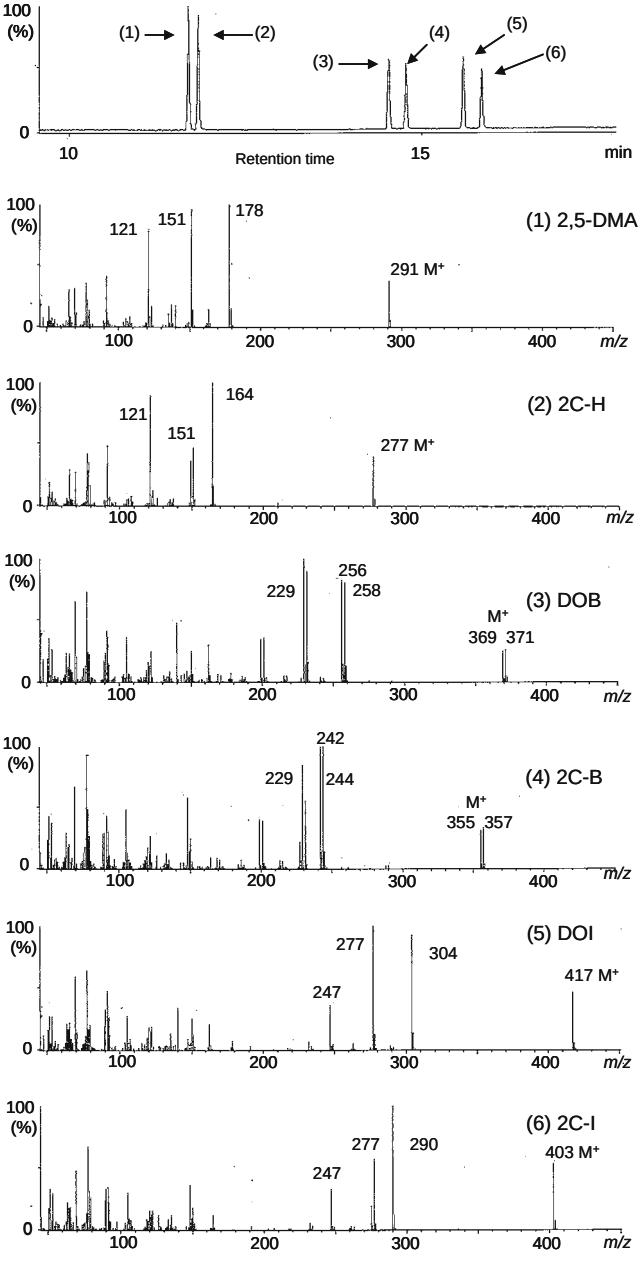


Fig. 5 Total ion chromatograms of a mixed solution of 2,5-dimethoxyphenethylamine analogues after trifluoroacetyl derivatization (50 μg/ml each) and mass spectra of each compound by gas chromatography-mass spectrometry

Table 4 Validation data for quantitative analysis of 2,5-dimethoxyphenethylamine analogues by GC-MS after TFA derivatization

Compound	Correlation coefficient[a]	Intraday CV(%)[b]			Interday CV(%)[b]		
		3 μg/ml	30 μg/ml	100 μg/ml	3 μg/ml	30 μg/ml	100 μg/ml
2C-H	0.9994	12.1	2.0	3.1	30.3	6.1	8.1
2,5-DMA	0.9995	11.5	0.92	0.29	29.6	6.6	5.5
2C-B	0.9994	24.2	5.2	0.66	31.8	9.8	3.4
DOB	0.9995	11.4	2.0	0.59	19.9	13.9	1.5
2C-I	0.9988	16.9	2.2	1.3	24.8	12.1	1.6
DOI	0.9983	16.8	2.7	0.58	24.3	9.2	3.0

Determinations based on total ion chromatograms
[a] Linearity range: 3–100 μg/ml
[b] Each value obtained from three determinations

ing to the higher temperatures of the injection port. From the above results, we concluded that the GC-MS analysis of underivatized free bases is not suitable for the simultaneous analysis of 2,5-dimethoxyphenetylamine analogues.

All of the quantitative analysis was thus made after TFA derivatization. The validation data for the analysis of the six drugs after derivatization are shown in Table 4. Good linearity of the response was confirmed between 3 and 100 μg/ml for all compounds. The detection limit by this method was 2 ng per injection using total ion chromatograms. The precision data of the quantitative analysis by GC-MS after TFA derivatization was satisfactory, as shown in Table 4.

Conclusions

We synthesized six 2,5-dimethoxyphenethylamine analogue designer drugs, and their NMR spectra, IR spectra, TLC data, ESI mass spectra without derivatization, and GC-MS mass spectra with TFA derivatization are presented. Quantitative reliability was also tested for these compounds by LC-MS (underivatized forms) and GC-MS (TFA-derivatived forms). These extensive data aimed to serve for screening, identification, and quantitation of the drugs in seized tablets, crystals, and powder. Our data seem very useful for such purposes. Therefore, calibration ranges were set at high levels, and highly sensitive detection techniques such as selected-ion monitoring were not employed. However, the present study will give useful basic information for creating new methods for highly sensitive analysis of 2,5-dimethoxyphenethylamine designer drugs in human body fluids and tissues.

Acknowledgments We are grateful to Dr. Kenji Tsujikawa (National Research Institute of Police Science) for technical suggestions on the LC-MS analysis.

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