

# Discrimination and identification of the six aromatic positional isomers of trimethoxyamphetamine (TMA) by gas chromatography-mass spectrometry (GC-MS)

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A reliable and accurate GC-MS method was developed that allows both mass spectrometric and chromatographic discrimination of the six aromatic positional isomers of trimethoxyamphetamine (TMA). Regardless of the trifluoroacetyl (TFA) derivatization, chromatographic separation of all the investigated isomers was achieved by using DB-5ms capillary columns (30 m × 0.32 mm i.d.), with run times less than 15 min. However, the mass spectra of the nonderivatized TMAs, except 2,4,6-trimethoxyamphetamine (TMA-6), showed insufficient difference for unambiguous discrimination. On the other hand, the mass spectra of the TFA derivatives of the six isomers exhibited fragments with significant intensity differences, which allowed the unequivocal identification of all the aromatic positional isomers investigated in the present study. This GC-MS technique in combination with TFA derivatization, therefore, is a powerful method to discriminate these isomers, especially useful to distinguish the currently controlled 3,4,5-trimethoxyamphetamine (TMA-1) and 2,4,5-trimethoxyamphetamine (TMA-2) from other uncontrolled TMAs. Copyright © 2007 John Wiley & Sons, Ltd.

**KEYWORDS:** trimethoxyamphetamine; trifluoroacetyl derivatization; gas chromatography-mass spectrometry; designer drug; psychotomimetic property; forensic science

## INTRODUCTION

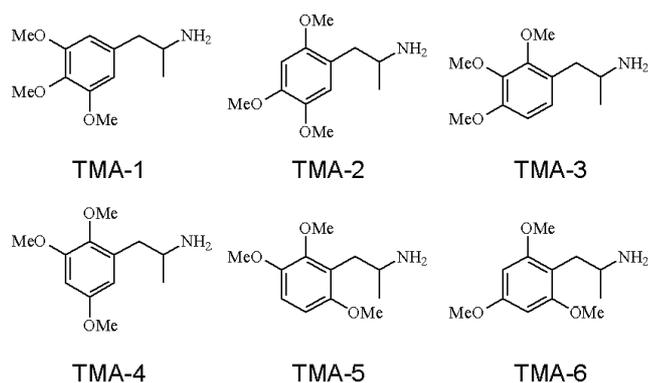
Drug abuse, which affects human behavior and causes numerous crimes, has become a serious problem throughout the world. Amphetamine and its analogs have been most extensively and increasingly abused as central nervous system stimulants, and are well documented in the literature.<sup>1–6</sup> There are hundreds of possible amphetamine analogs that modify the basic amphetamine structure and retain or vary the stimulant effects of the parent compound. The amphetamine analogs are known usually to be unexpectedly introduced onto the clandestine market as ‘designer drugs’, and their large number makes identification a difficult task in the forensic science field.<sup>7–9</sup>

More recently, trimethoxyamphetamines (TMAs) have emerged on the illicit drug markets in Europe, USA and even in Japan.<sup>10,11</sup> As shown in Fig. 1, there exist six possible aromatic positional isomers of TMA; i.e. 3,4,5-trimethoxyamphetamine (TMA-1), 2,4,5-trimethoxyamphetamine (TMA-2), 2,3,4-trimethoxyamphetamine (TMA-3), 2,3,5-trimethoxyamphetamine (TMA-4), 2,3,6-trimethoxyamphetamine (TMA-5) and 2,4,6-trimethoxyamphetamine (TMA-6).

TMA-1 was the first psychotomimetic drug synthesized in 1947 by modifying the structure of mescaline on the basis of the principles discovered in studying the relationships between chemical structures and biological activity, and has about twice the potency of mescaline.<sup>12</sup> TMA-2, the geometric isomer of TMA-1, was first synthesized in 1933, and its psychotomimetic properties were first reported by Shulgin in 1964. This was thus called TMA-2 as the second of the six possible positional isomers found to be psychotomimetic. TMA-2 was reported to possess some 20 times the potency of mescaline and is the most active of the six isomers of TMA.<sup>13</sup> The remaining isomers were numbered by Shulgin in the order of their progressive position of substitution, as abbreviated above. The values for relative human potencies of TMA-4, -5, and -6 compared to that of mescaline were evaluated to be 4, 10, and 10, respectively. TMA-3 was found to exhibit no psychotomimetic activity.<sup>14</sup> The isomers, except TMA-3, have both stimulant and psychedelic effects, and their qualitative effects on mood alteration and sensory enhancement are similar to those of mescaline. Although their physical health risks and toxic doses in humans have never been clarified, mental health risks are assumed to be similar to those of other hallucinogens.<sup>14</sup>

Recently, TMA-2 and TMA-6 have been encountered in the Japanese drug market as well as in Europe and USA and have been increasingly abused as new designer drugs. In

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**Figure 1.** Chemical structures of trimethoxyamphetamines. Abbreviations: TMA-1, 3,4,5-trimethoxyamphetamine; TMA-2, 2,4,5-trimethoxyamphetamine; TMA-3, 2,3,4-trimethoxyamphetamine; TMA-4, 2,3,5-trimethoxyamphetamine; TMA-5, 2,3,6-trimethoxyamphetamine; TMA-6, 2,4,6-trimethoxyamphetamine.

USA all the aromatic positional isomers of TMA have been controlled under U.S. Federal Law as Schedule I substances,<sup>15</sup> while TMA-1 has been controlled in Europe. More recently, TMA-2 has been controlled under most international laws in Europe,<sup>16</sup> and has also been placed in Schedule I by the U.N. Narcotics Commission.

On the other hand, in Japan, only TMA-1 had been controlled until Japanese authorities banned TMA-2 under the Narcotics and Psychotropics Control Law of 2006 because of a growing number of its encounter. The other aromatic positional isomers are not currently classified as illegal drugs, and nothing prevents their sale and abuse; thus there is much apprehension of its popularity in Japan.

As mentioned above, some positional isomers of TMA are currently not controlled in Japan and Europe. Also, a large variation in the psychoactive properties and toxicity of the isomers is expected. Discrimination of the six isomers is then indispensable for drug enforcement and forensic toxicology.

There are no full analytical data available on the aromatic positional isomers of TMA. Therefore, development of convenient and reliable analytical methods differentiating and identifying the isomers is strongly demanded, and has become an urgent subject in the forensic science field.

In the present study, we have synthesized authentic standards of the six aromatic positional isomers of TMA. Using these authentic standards, reliable and accurate methods for their unequivocal discrimination were developed by GC-MS.

## EXPERIMENTAL

### Materials

TMA-1, -2, -3, and -6 were synthesized according to the methods of Shulgin.<sup>1</sup> These were obtained by lithium aluminum hydride (Wako, Osaka) reduction of 2-nitro-1-(trimethoxyphenyl)propenes synthesized from the appropriate trimethoxybenzaldehydes (Aldrich, Milwaukee), and finally purified as their hydrochloride. TMA-4 and -5 were both synthesized as described in detail in the next section. The overall yields for TMA-1, -2, -3 and -6 were 14–59%, and those

for TMA-4 and -5 were 3 and 6%, respectively. Every synthesized compound was ensured to be of >98% purity, based on LC-MS by the flow-injection method. Stock standard solutions of these six compounds were prepared in methanol (1 mg/ml each), and these solutions were then diluted to the appropriate concentrations with distilled water, immediately prior to use.

The trifluoroacetyl (TFA) derivatives of the analytes were prepared by adding 200  $\mu$ l of trifluoroacetic anhydride (Wako, Osaka) and 200  $\mu$ l of ethyl acetate, followed by reacting at 60 °C for 20 min. The reaction mixture was carefully evaporated to dryness under a gentle nitrogen stream, and then reconstituted in 100  $\mu$ l of ethyl acetate. Then, 1  $\mu$ l aliquots were automatically injected into the GC-MS systems.

### Chemical synthesis

#### TMA-4

To concentrated nitric acid (80 ml) cooled in an ice bath, 2-hydroxy-5-methoxybenzaldehyde (7.60 g, 50 mmol) was added dropwise with stirring, and then the mixture was stirred for 6 h at 10–15 °C. The reaction mixture was poured into crushed ice and extracted with chloroform. The organic layer was successively washed with saturated sodium bicarbonate solution and brine. It was dried over sodium sulfate and evaporated to give a crude residue, which was chromatographed on silica gel (hexane–benzene–ethyl acetate, 5:1:0.1–5:1:0.5) to give the pure 2-hydroxy-5-methoxy-3-nitrobenzaldehyde (5.32 g, 54%).

To a mixture of 2-hydroxy-5-methoxy-3-nitrobenzaldehyde (9.85 g, 50 mmol) and potassium carbonate (6.90 g, 50 mmol) in dimethylformamide (100 ml), dimethyl sulfate (9.5 ml) was added dropwise. The mixture was stirred under an argon atmosphere at room temperature for 4 days. The reaction mixture was filtered, evaporated *in vacuo*, diluted with water and extracted with chloroform. The organic layer was washed with brine. Evaporation of the organic solvent gave a crude residue, which was chromatographed on silica gel (hexane–benzene–ethyl acetate, 5:1:0.1–5:1:0.5) to give the pure 2,5-dimethoxy-3-nitrobenzaldehyde (9.71 g, 92%).

A mixture of 2,5-dimethoxy-3-nitrobenzaldehyde (10.55 g, 50 mmol), powdered iron (2.80 g) and iron (II) sulfate heptahydrate (209 g) in an aqueous calcium carbonate (75 g) suspension (H<sub>2</sub>O, 1 l) was stirred for 6 h at 75 °C. The solid in the reaction mixture was removed by filtration and the filtrate was acidified to pH 1 with concentrated HCl in an ice bath.

To a solution of the crude 3-amino-2,5-dimethoxybenzaldehyde in aqueous hydrochloric acid, an aqueous sodium nitrite (3.45 g, 50 mmol) solution (10 ml) was added dropwise with stirring at 5 °C. After continuous stirring for 0.5 h, the aqueous mixture was increased to 80 °C and stirred for 1 h. The reaction mixture was filtered and then extracted with dichloromethane. The organic layer was successively washed with a saturated aqueous sodium bicarbonate solution and brine and dried over sodium sulfate. Evaporation of the organic solvent gave a crude residue, which was chromatographed on silica

gel (hexane–benzene–ethyl acetate, 5:1:0.1–5:1:0.5) to provide the pure 3-hydroxy-2,5-dimethoxybenzaldehyde (1.73 g, 19%).

Under an argon atmosphere, a mixture of 3-hydroxy-2,5-dimethoxybenzaldehyde (1.82 g, 10 mmol), methyl iodide (5.0 ml) and sodium carbonate (1.06 g, 10 mmol) in acetone (50 ml) was stirred under a gentle reflux for 6 h. The mixture was filtered and evaporated to give a crude residue, which was chromatographed on silica gel (hexane–benzene–ethyl acetate, 5:1:0.1–5:1:0.5) to give pure 2,3,5-trimethoxybenzaldehyde (1.61 g, 82%).

A solution of 2,3,5-trimethoxybenzaldehyde (1.96 g, 10 mmol) and anhydrous ammonium acetate (0.39 g, 5 mmol) in nitroethane (7.2 ml) was refluxed for 2 h under an argon atmosphere. The excess nitroethane was removed under vacuum, and the residue was dissolved in ethyl acetate. The organic layer was washed with brine and dried over sodium sulfate. Evaporation of the organic solvent gave a crude residue, which was chromatographed on silica gel (hexane–benzene–ethyl acetate, 5:1:0.1–5:1:0.5) to give pure 2-nitro-1-(2,3,5-trimethoxyphenyl)propene (1.77 g, 70%).

To a suspension of lithium aluminum hydride (1.52 g, 40 mmol) in dry tetrahydrofuran (30 ml) under an argon atmosphere, a solution of 2-nitro-1-(2,3,5-trimethoxyphenyl)propene (2.53 g, 10 mmol) in dry tetrahydrofuran (10 ml) was added dropwise at room temperature, and the mixture was kept at 40 °C with stirring for 12 h, and then the reaction mixture was cooled in an ice bath. The excess hydride was decomposed by adding water and a sodium hydroxide solution. The inorganic salts were removed by filtration, and the filter cake was washed with tetrahydrofuran. Evaporation of the combined filtrate and washings gave the crude base. This was dissolved in isopropyl alcohol, and then neutralized with methanolic hydrogen chloride. The solution was saturated with diethyl ether and allowed to stand for a few days to form a residue, which was recrystallized from isopropanol–ether to afford fine needles of TMA-4 hydrochloride (1.18 g, 54%).

#### TMA-5

To a stirred solution of 1,2,4-trimethoxybenzene (1.68 g, 10 mmol) in tetrahydrofuran (15 ml) was added dropwise a 1.0 M *n*-butyl lithium solution in tetrahydrofuran (11 ml) at –78 °C under an argon atmosphere. The reaction mixture was held at that temperature while stirring for 1 h, and then quenched by the addition of solid carbon dioxide. Aqueous ammonium chloride was added to the mixture, followed by extraction with ethyl acetate. The organic layer was washed with brine and dried over sodium sulfate. Evaporation of the organic solvent gave a crude residue, which was chromatographed on silica gel (hexane–ethyl acetate, 5:1) to give pure 2,3,6-trimethoxybenzoic acid (1.04 g, 49%).

To a suspension of lithium aluminum hydride (0.38 g, 10 mmol) in dry tetrahydrofuran (10 ml) under an argon atmosphere, a solution of 2,3,6-trimethoxybenzoic acid (2.12 g, 10 mmol) in dry tetrahydrofuran (30 ml) was added dropwise at room temperature. The mixture was kept at 40 °C with stirring for 12 h. Cooled in an ice bath, the excess hydride decomposed by the addition of water and a sodium

hydroxide solution. The inorganic material was removed by filtration and the filter cake was washed with tetrahydrofuran. Evaporation of the combined filtrate and washings gave the crude residue, which was chromatographed on silica gel (hexane–benzene–ethyl acetate, 5:1:0.1–5:1:0.5) to give the pure 2,3,6-trimethoxybenzyl alcohol (1.09 g, 55%).

The mixture of 2,3,6-trimethoxybenzyl alcohol (1.98 g, 10 mmol) and manganese dioxide (6.0 g) in benzene was refluxed for 8 h with vigorous stirring. The inorganic salts in the reaction mixture were removed by filtration, and the filter cake was washed with acetone. Evaporation of the combined filtrate and washings gave a crude residue, which was chromatographed on silica gel (hexane–benzene–ethyl acetate, 5:1:0.1–5:1:0.5) to provide pure 2,3,6-trimethoxybenzaldehyde (1.45 g, 74%).

According to the above-mentioned procedure for TMA-4, TMA-5 hydrochloride (0.70 g, 32%) was prepared, with a slight modification: instead of 2,3,5-trimethoxybenzaldehyde, 2,3,6-trimethoxybenzaldehyde was used.

#### NMR data for the synthesized compounds

The <sup>1</sup>H NMR spectra were obtained in CDCl<sub>3</sub> solutions using a Varian Gemini spectrometer operating at 300 MHz. The detailed spectral data are given below.

**TMA-1:** <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 6.41 (2H, s), 3.85 (6H, s), 3.83 (3H, s), 3.17 (1H, ddq, *J* = 4.8, 6.0, 8.3 Hz), 2.68 (1H, dd, *J* = 4.8, 13.4 Hz), 2.42 (1H, dd, *J* = 8.3, 13.4 Hz), 1.14 (3H, d, *J* = 6.0 Hz).

**TMA-2:** <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 6.69 (1H, s), 6.52 (1H, s), 3.88 (3H, s), 3.83 (3H, s), 3.79 (3H, s), 3.15 (1H, ddq, *J* = 5.4, 6.3, 8.0 Hz), 2.67 (1H, dd, *J* = 5.4, 13.1 Hz), 2.47 (1H, dd, *J* = 8.0, 13.1 Hz), 1.10 (3H, d, *J* = 6.3 Hz).

**TMA-3:** <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 6.82 (1H, d, *J* = 8.4 Hz), 6.61 (1H, d, *J* = 8.4 Hz), 3.874 (3H, s), 3.867 (3H, s), 3.84 (3H, s), 3.13 (1H, ddq, *J* = 5.4, 6.3, 7.8 Hz), 2.66 (1H, dd, *J* = 5.4, 13.2 Hz), 2.46 (1H, dd, *J* = 7.8, 13.2 Hz), 1.11 (3H, d, *J* = 6.3 Hz).

**TMA-4:** <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 6.39 (1H, d, *J* = 3.0 Hz), 6.28 (1H, d, *J* = 3.0 Hz), 3.83 (3H, s), 3.77 (3H, s), 3.75 (3H, s), 3.19 (1H, ddq, *J* = 5.7, 6.3, 8.1 Hz), 2.70 (1H, dd, *J* = 5.7, 12.9 Hz), 2.54 (1H, dd, *J* = 8.1, 12.9 Hz), 1.13 (3H, d, *J* = 6.3 Hz).

**TMA-5:** <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 6.73 (1H, d, *J* = 9.0), 6.55 (1H, d, *J* = 9.0), 3.82 (3H, s), 3.81 (3H, s), 3.76 (3H, s), 3.15 (1H, ddq, *J* = 5.9, 6.0, 7.8 Hz), 2.73 (1H, dd, *J* = 5.9, 12.5 Hz), 2.64 (1H, dd, *J* = 7.8, 12.5 Hz), 1.11 (3H, d, *J* = 6.0 Hz).

**TMA-6:** <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 6.13 (2H, s), 3.81 (3H, s), 3.78 (6H, s), 3.08 (1H, ddq, *J* = 5.6, 6.3, 7.8 Hz), 2.63 (1H, dd, *J* = 5.6, 12.9 Hz), 2.57 (1H, dd, *J* = 7.8, 12.9 Hz), 1.07 (3H, d, *J* = 6.3 Hz).

#### Instrumentation

GC-MS was carried out using a GCMS QP-2010 instrument (Shimadzu, Kyoto, Japan). Three different fused-silica capillary columns, DB-1MS, DB-5MS and DB-17MS (30 m × 0.32 mm i.d.; 0.25 – μm thickness; J&W Scientific, Rancho Cordova, CA, USA), were investigated for the chromatographic separation. Samples were automatically

injected in the splitless mode at 250 °C. The column oven temperature was maintained at 80 °C for 1 min, and then raised at 10 °C/min to 320 °C. The transfer line temperature was set at 250 °C. High-purity helium, at the flow rate of 3 ml/min, was used as the carrier gas. The electron ionization (EI) operating parameters were as follows: source temperature, 200 °C; electron energy, 70 eV; ion multiplier gain, 1.2kV. Mass spectra were collected from  $m/z$  40 to 500 at the scan rate of 0.5 s/scan.

**RESULTS AND DISCUSSION**

**Chromatographic separation**

In order to optimize the separation of the six aromatic positional isomers of TMA, three different column phases were explored, i.e. DB-1ms, DB-5ms and DB-17ms, under the same GC-MS operating conditions as described in the experimental section. As samples, artificial mixtures of the six TMAs as their free base in ethyl acetate (1 µg/ml) were first used.

With all columns, all six TMAs could be baseline-separated from each other within 15 min in the order TMA-3,

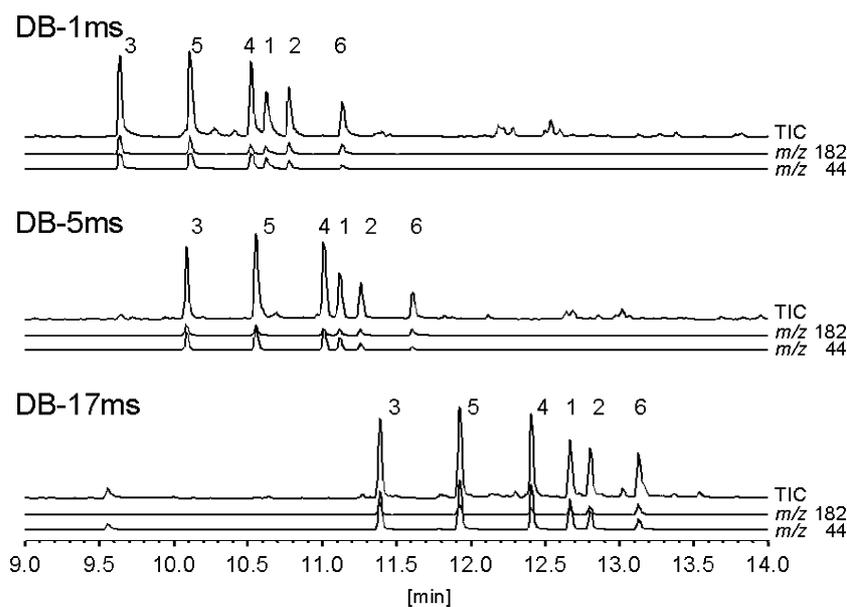
-5, -4, -1, -2 and -6. However, they were all eluted with somewhat tailing peaks (Fig. 2).

For improvement of the peak shapes, TFA derivatization of the TMAs was then examined. As depicted in Fig. 3, all TFA derivatives of the six TMAs eluted into the baseline-separated peaks in the same order as those of the nonderivatized TMAs with DB-1ms or DB-5ms within 15 min. Also, the tailing of the analytes observed for the free bases could be overcome. However, with DB-17ms, TMA-1, -2 and -6 were eluted with insufficient resolution. The calculated Kovats indices<sup>17</sup> are summarized in Table 1.

On the basis of the above comparison, we finally chose DB-5ms as the analytical column, which provided a better resolution among the free bases of TMA-4, -1 and -2 than from DB-1ms.

**Mass spectrometry**

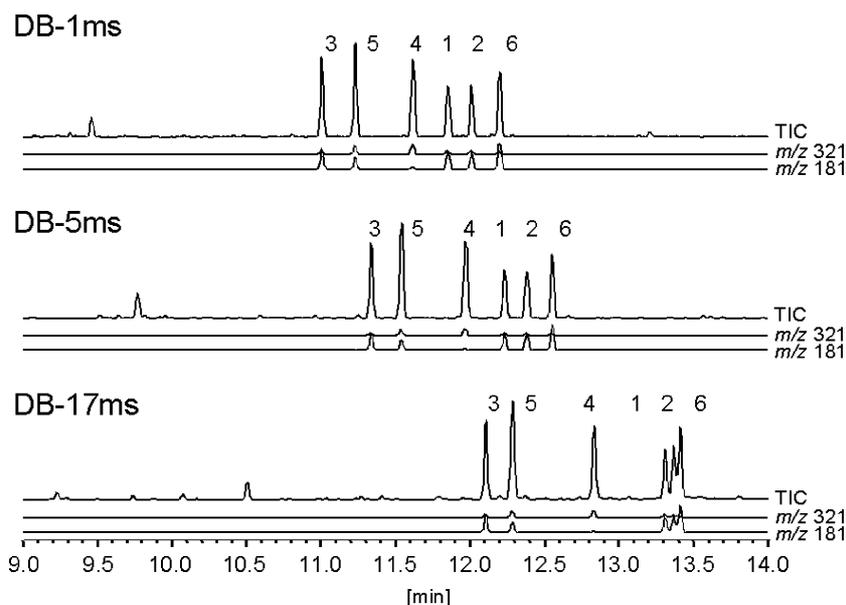
As shown in Fig. 4, each EI mass spectrum of the six nonderivatized TMAs is characterized by the predominant immonium ions at  $m/z$  44 produced by an  $\alpha$ -cleavage reaction, and the significant ions at  $m/z$  182 and 167. These fragment ions at  $m/z$  182 and 167 may be generated by



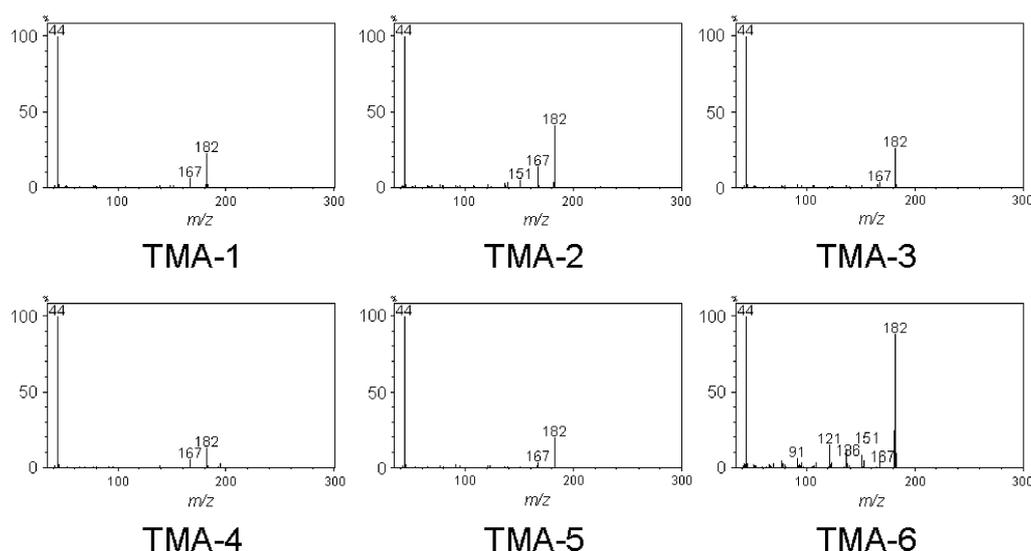
**Figure 2.** Total-ion and extracted-mass chromatograms obtained from a mixture of the nonderivatized TMAs using the different columns; top: DB-1ms, middle: DB-5ms, bottom: DB-17ms. Peak identification: 1, TMA-1; 2, TMA-2; 3, TMA-3; 4, TMA-4; 5, TMA-5; 6, TMA-6.

**Table 1.** Retention indices of the TMAs and the TFA-derivatized compounds

column type Compound form	DB-1ms		DB-5ms		DB-17ms	
	Free base	TFA derivative	Free base	TFA derivative	Free base	TFA derivative
TMA-1	1678	1788	1718	1817	1876	2118
TMA-2	1691	1802	1730	1830	1888	2124
TMA-3	1593	1712	1631	1737	1758	1996
TMA-4	1669	1767	1709	1793	1852	2069
TMA-5	1634	1732	1670	1755	1806	2014
TMA-6	1711	1820	1761	1846	1920	2128



**Figure 3.** Total-ion and extracted-mass chromatograms obtained from a mixture of the TFA-derivatized TMAs using the different columns; top: DB-1ms, middle: DB-5ms, bottom: DB-17ms. Peak identification: 1, TMA-1; 2, TMA-2; 3, TMA-3; 4, TMA-4; 5, TMA-5; 6, TMA-6.



**Figure 4.** EI mass spectra of the nonderivatized TMAs.

an H-rearrangement from the amino group to the aromatic part of the molecule with a subsequent  $\alpha$ -cleavage of the benzyl bond by the radical electron at the nitrogen, and the subsequent elimination of a methyl radical.<sup>18,19</sup> Especially, in the mass spectra of TMA-1, -2, -3, -4 and -5, the ions at  $m/z$  182 exhibited an abundance of about 15–45%, though TMA-6 showed the ion with an abundance of about 95%. It could be due to the stabilizing effect caused from the *ortho*-disubstitution of two methoxy groups on positive charge. At least, this higher relative abundance of the fragment ions at  $m/z$  182 seems to be a discriminating indicator for TMA-6.

However, there appears no significant fragment ion to allow the unequivocal discrimination between the five isomeric TMAs, and therefore the mass spectral information is not sufficient to unambiguously identify the five TMAs except TMA-6, without derivatization.

In our previous study, TFA derivatization was successfully applied to the discrimination of the *ortho*-, *meta*-, and *para*-methoxyamphetamines, which was able to be mass-spectral-differentiate with derivatization.<sup>4,5</sup> In the present study, TFA derivatization of the isomeric TMAs was explored.

As shown in Fig. 5, the mass spectra of the trifluoroacetylated TMA-1, -2, -3 and -6 were characterized by base peak ions at  $m/z$  181 owing to their trimethoxy-substituted benzyl or tropylium cations and their relatively intense molecular ions at  $m/z$  321 (5–15%).

Also, the spectra of the four isomeric TFA derivatives showed a significant intensity difference for the fragments at  $m/z$  208, 193, 166, 151, 136 and 121: TMA-1-TFA exhibited relatively intense ions at  $m/z$  208 (18%), and 193 (8%); TMA-2-TFA at  $m/z$  151 (26%), 136 (8%) and 208 (7%); TMA-3-TFA

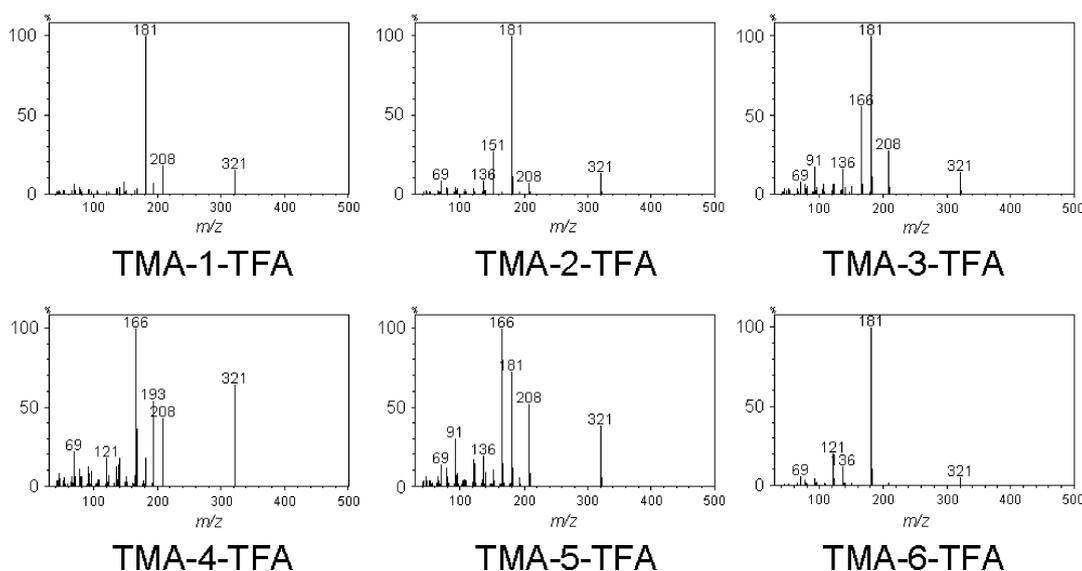


Figure 5. EI mass spectra of the TFA-derivatized TMAs.

Table 2. Relative intensities (%) of the ions in the EI mass spectra obtained from the TFA derivatives of the six TMA isomers

$m/z$	TMA-1-TFA	TMA-2-TFA	TMA-3-TFA	TMA-4-TFA	TMA-5-TFA	TMA-6-TFA
321	15	13	11	65	40	5
208	18	7	28	43	52	2
193	8	–	–	54	5	–
181	100	100	100	18	73	100
166	–	–	55	100	100	–
151	2	26	5	6	11	2
136	4	8	17	12	18	12
121	–	–	5	18	18	20

at  $m/z$  166 (55%), 208 (28%), 136 (17%) and 121 (5%); TMA-6-TFA at  $m/z$  121 (20%), 136 (12%) and 208 (2%). These mass spectrometric data for the TFA derivatives are summarized in Table 2.

The mass spectra of the trifluoroacetylated TMA-4 and -5, on the other hand, were characterized by base peak ions at  $m/z$  166 and intense molecular ions at  $m/z$  321 (40–65%). Also, the spectra of the two isomeric TFA derivatives showed a significant intensity difference for the fragments at  $m/z$  208, 193, 181, 151, 136 and 121: TMA-4-TFA exhibited relatively intense ions at  $m/z$  193 (54%), 208 (43%), 181 (18%), 121 (18%), 136 (12%) and 151 (6%), and TMA-5-TFA at  $m/z$  181 (73%), 208 (52%), 136 (18%), 121 (18%), 151 (11%) and 193 (5%).

The fragment ions at  $m/z$  208 would be generated by the inductive route of the McLafferty rearrangement, and the fragment ions at  $m/z$  151 and 121 would be produced from their trimethoxy-substituted benzyl cations at  $m/z$  181 by loss of a formaldehyde molecule and two formaldehyde molecules, respectively.<sup>18,19</sup>

These variations of the relative intensities between the significant fragment ions, as mentioned above, would allow discrimination of the six trifluoroacetylated TMAs, leading to the unequivocal identification of the positional isomeric TMAs. TFA derivatization, therefore, has been found to be very effective for the mass spectral differentiation of the positional isomers of TMA.

## CONCLUSIONS

A reliable and accurate GC-MS method was developed that allows both mass spectrometric and chromatographic discrimination of the six aromatic positional isomers of TMA. Regardless of the derivatization, chromatographic separation of all the investigated isomers was achieved by using the DB-5ms capillary column, with run times less than 15 min. The mass spectra of the nonderivatized TMAs, except TMA-6, showed insufficient difference for their unambiguous determination. The fragments of the mass spectra of their TFA derivatives, on the other hand, exhibited a significant intensity difference, which allowed the mass spectral differentiation of all the isomers investigated in the present study. GC-MS with TFA derivatization, therefore, is a powerful method for the discrimination of these isomers, especially useful to distinguish the currently controlled TMA-1 and TMA-2 from other uncontrolled TMAs.

## REFERENCES

- Shulgin AT. In *Pihkal, A Chemical Love Story*, Dan Joy (ed). Transform Press: Berkeley, 1991.
- Ellenhorn MJ (ed). *Amphetamines and designer drugs. Ellenhorn's Medical Toxicology*, 2nd ed. Williams & Wilkins: Baltimore, 1997; 340.
- Logan BK. Amphetamines: an update on forensic issues. *Journal of Analytical Toxicology* 2001; **25**: 400.

- Munehiro K, Hiroe T, Akihiro M, Kunio N, Hitoshi T. Analyses of clandestine tablets of amphetamines and their related designer drugs encountered in recent Japan. *Japanese Journal of Forensic Toxicology* 2002; **20**: 303.
- Munehiro K, Hitoshi T. Update on clandestine amphetamines and their analogues recently seen in Japan. *Journal of Health Science* 2002; **48**: 14.
- de Boer D, Bosman I. A new trend in drug-of-abuse; the 2C-series of phenethylamine designer drugs. *Pharmacy World and Science* 2004; **26**: 110.
- Marson C, Schneide S, Meys F, Wenning R. Structural elucidation of an uncommon phenethylamine analogue in urine responsible for discordant amphetamine immunoassay results. *Journal of Analytical Toxicology* 2000; **24**: 17.
- Koper C, Ali-Tolppa E, Bozenko JS, Dufey V, Puetz M, Weyermann C, Zrcek F. Identification of a new amphetamine type stimulant: 3,4-methylenedioxy-N-(2-hydroxyethyl)amphetamine (MDHOET). *Microgram Journal* 2005; **3**: 166.
- Rösner P, Quednow B, Girreser U, Junge T. Isomeric fluoro-methoxy-phenylalkylamines: a new series of controlled-substance analogues (designer drugs). *Forensic Science International* 2005; **148**: 143.
- Ewald AH, Fritschi G, Maurer HH. Designer drug 2, 4, 5-trimethoxyamphetamine (TMA-2): studies on its metabolism and toxicological detection in rat urine using gas chromatographic/mass spectrometric techniques. *Journal of Mass Spectrometry* 2006; **41**: 1140.
- Tsujikawa K, Kanamori T, Kuwayama K, Miyaguchi H, Iwata Y, Inoue H. Analytical profiles for 3, 4, 5-, 2, 4, 5-, and 2, 4, 6-trimethoxyamphetamine. *Microgram Journal* 2006; **4**: 12.
- Snyder SH, Richelson E. Psychedelic drugs: steric factors that predict psychotropic activity. *Biochemistry* 1968; **60**: 206.
- Shulgin AT. Profiles of psychedelic drugs: TMA-2. *Journal of Psychedelic Drugs* 1976; **8**: 169.
- Shulgin AT. Psychotomimetic drugs: structure-activity relationships. In *The Handbook of Psychopharmacology*, vol. 11, Stimulants, Iverson LL, Iverson SD, Snyder SH (eds). Plenum Publishing Company: New York, 1978; 243.
- Drug and Chemical Evaluation Section, Office of Diversion Control, Drug Enforcement Administration, U.S. Department of Justice. Scheduling Actions Controlled Substances Regulated Chemicals, April 2007.
- Council of the European Union, Council decision 2003/847/JHA of 27 November 2003 concerning control measures and criminal sanctions in respect of the new synthetic drugs 2C-1, 2C-T-2, 2C-T-7 and TMA-2, *official journal of the European union*, 2003; **6**: 64.
- Kovats ES. Gas chromatographic characterization of organic substances in the retention index system. *Advances in Chromatography* 1965; **1**: 229.
- McLafferty FW, Turecek F. *Interpretation of Mass Spectra*, 4th ed. University Science Books: Mill Valley, 1993.
- Smith RM, Busch KL. *Understanding Mass Spectra – A Basic Approach*. Wiley: New York, 1999.