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JMS Letter

Dear Sir,

A technique combining trifluoroacetyl derivatization and gas chromatography–mass spectrometry to distinguish methamphetamine and its 4-substituted analogs

The abuse of drugs has become a serious problem throughout the world. One synthetic psychotropic drug that has been extensively abused is methamphetamine (MA). Although MA is controlled around the world, many newly emerging designer drugs are modified from MA. For example, the 4-substituted psychoactive analogs of MA, 4-methoxymethamphetamine (4-MMA) and 4-fluoromethamphetamine (4-FMA), have recently emerged on the illicit drug market.[1–3] In addition to 4-FMA, other 4-halogenated analogs include 4-chloromethamphetamine (4-CMA), 4-bromomethamphetamine (4-BMA) and 4-iodomethamphetamine (4-IMA).

So far, there have been no reports of the abuse of 4-CMA, 4-BMA, 4-IMA or 4-nitromethamphetamine (4-NMA). However, their abuse is expected since Ledgard[4] has reported the psychotomimetic properties of 4-CMA, 4-BMA and 4-NMA along with their synthetic processes. Furthermore, like 4-iodo-2,5-dimethoxy-phenethylamine (2C-I) and 4-iodo-2,5-dimethoxy-amphetamine (DOI), which have been widely abused as designer drugs,[5–7] 4-IMA also has a 4-iodinated aromatic part. Because of their psychotomimetic effects, it is expected that novel 4-substituted analogs of MA will be encountered in the future.

In order to control the abuse of such MA drugs, new methods of identifying and discriminating them must continually be developed. In Japan, 4-MMA and 4-FMA have been controlled as designated substances by the Pharmaceutical Affairs Law since April 2007 and January 2009, respectively. However, the other 4-substituted analogs of MA are not currently classified as illegal drugs. Therefore, there is a pressing need for methods to clearly distinguish each drug.

This paper reports a successful method that combines trifluoroacetyl (TFA) derivatization and gas chromatography–mass spectrometry (GC–MS). Based on previous studies in which TFA derivatization improved the differentiation of phenethylamine-type drugs,[1,2,5,8–10] we examined the effects of TFA derivatization of MA and its 4-substituted analogs on the peak shapes of their GC chromatograms and the fragmentation patterns of their mass spectra. TFA derivatization resulted in a significant improvement in the peak shapes of the chromatograms and the properties of the mass spectra. This in turn enabled the clear differentiation of MA and six of its 4-substituted analogs.

MA was purchased from Dainippon Pharmaceutical Co., Ltd (Osaka, Japan). 4-MMA was obtained from Sigma–Aldrich Corp. (St Louis, MO, USA). 4-Chlorophenylacetone, 4-bromophenylacetone and sodium cyanoborohydride were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). 4-Fluorophenylacetone, 4-nitrophenylacetone, 4-iodophenylacetic acid, 40% methylamine methanol solution and acetic anhydride were purchased from Wako Pure Chemicals Industries, Ltd (Osaka, Japan). Trifluoroacetic anhydride was obtained from Nacalai Tesque, Inc. (Kyoto, Japan). Other common chemicals were purchased from commercial sources and used without further purification.

Five 4-substituted analogs of MA (Fig. 1) were synthesized. They were obtained by the reductive amination of 4-substituted phenylacetones in methanol with sodium cyanoborohydride.[11] The synthesized compounds were ascertained by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) and nuclear magnetic resonance spectroscopy (NMR).

4-FMA: A mixture of 4-fluorophenylacetone (152 mg, 1 mmol), 40% methylamine methanol solution (120 µl, 1.5 mmol), acetic acid (120 µl, 2 mmol) and sodium cyanoborohydride (95 mg, 1.5 mmol) was stirred overnight at room temperature in methanol (3 ml). After the reaction, methanol was evaporated *in vacuo*, and 2 M hydrochloric acid solution (3 ml) was added to the residue. The aqueous solution was stirred for 0.5 h at room temperature, after which 1 M aqueous sodium hydroxide (6 ml) was added to make the solution alkaline. The aqueous layer was extracted with dichloromethane (5 ml × 2), and the combined organic layer was washed with brine (5 ml), dried over anhydrous sodium sulfate and concentrated. Ethereal hydrochloric acid (1 ml) was added to the residue. The solution was allowed to stand at 5 °C until precipitation occurred. The precipitate was then filtered, and the residue was washed with pre-chilled diethyl ether (5 ml) and air dried to afford 4-FMA hydrochloride (129 mg, 63%).

4-CMA: The same procedure as that used to obtain 4-FMA hydrochloride was carried out on 4-chlorophenylacetone (168 mg, 1 mmol), which was used as the starting material to obtain 4-CMA hydrochloride (86 mg, 39%).

4-BMA: The same procedure as that used to obtain 4-FMA hydrochloride was carried out on 4-bromophenylacetone (213 mg, 1 mmol), which was used as the starting material to obtain 4-BMA hydrochloride (96 mg, 36%).

4-IMA: 4-Iodophenylacetone was prepared by refluxing 4-iodophenylacetic acid (524 mg, 2 mmol) and acetic anhydride (1 ml) with pyridine (1 ml).[12] The same procedure as that used to obtain 4-FMA hydrochloride was carried out on 4-iodophenylacetone, which was used as the starting material to obtain 4-IMA hydrochloride (106 mg, 17%).

4-NMA: The same procedure as that used to obtain 4-FMA hydrochloride was carried out on 4-nitrophenylacetone (179 mg, 1 mmol), which was used as the starting material to obtain 4-NMA hydrochloride (107 mg, 47%).

The synthesized compounds (4-FMA, 4-CMA, 4-BMA, 4-IMA and 4-NMA) were identified by MALDI-TOF/MS and NMR. The spectral data are listed in Table S1 (Supporting Information). The MALDI-TOF/MS data were obtained using an Applied Biosystems Voyager RP mass spectrometer in the reflector mode with 2,5-dihydroxybenzoic acid (DHB) as the matrix. The ^{1}H and ^{13}C-NMR spectra were obtained using a Varian Unity-300 spectrometer.

The GC–MS analysis of MA and six 4-substituted MAs was performed as follows. The instrument, a Shimadzu 17-A gas chromatograph equipped with a QP-5050A mass spectrometer (Kyoto, Japan), was operated under the following conditions: ionization mode set to electron ionization (EI); an ionization energy of 70 eV; helium as the carrier gas; a flow rate of 1.9 ml/min; a DB5-MS capillary column (Agilent, Santa Clara, CA, USA, 30 m × 0.25 mm i.d., 0.25 µm film thickness); an injector temperature of 200 °C; the injection mode set to the splitless mode; an oven temperature of 60 °C (2-min hold) followed by an increase to 250 °C (3-min hold) at a rate of 20 °C/min; and a scan range of *m/z* 35–375.

The TFA derivatives of the analytes were prepared by adding 100 µl of trifluoroacetic anhydride to 100 µl of standard solutions of MA and six 4-substituted MAs prepared in ethyl acetate at five different concentrations, after which the mixture was reacted at 65 °C for 10 min. The reaction mixture was carefully evaporated and dried under a gentle nitrogen stream and then reconstituted in 100 µl of ethyl acetate. Subsequently, 1 µl of the aliquots were automatically injected into the GC–MS system. A quantitative analysis was performed with *N*-butylbenzylamine (15 µg/ml) as the internal standard. Linearity was examined at 1, 3, 10, 30 and 100 µg/ml for each compound using calibration curves based on the peak-area ratios in the total ion chromatograms.

To examine the impact of the urine matrix, blank urine samples spiked with MA and six 4-substituted MAs were extracted as follows. Fifty microliters of *N*-butylbenzylamine (150 µg/ml), the internal standard, was added to 0.5 ml of urine. The mixture was alkalinized with 5% sodium carbonate and extracted three times with 0.5 ml of isopropanol/chloroform (1 : 3). The organic layer obtained was evaporated to dryness under a stream of nitrogen, and the residue was reconstituted in 100 µl of ethyl acetate. Then, the same procedure as that used to obtain the TFA derivatives of standard solutions was carried out. Subsequently, 1 µl of the aliquots was

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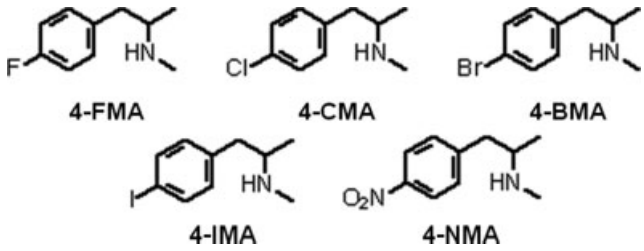

Figure 1. Structures of five 4-substituted analogs of MA synthesized in this study: 4-fluoromethamphetamine (4-FMA), 4-chloromethamphetamine (4-CMA), 4-bromomethamphetamine (4-BMA), 4-iodomethamphetamine (4-IMA) and 4-nitromethamphetamine (4-NMA).

Table 1. Retention indices of MA and six 4-substituted MA analogs with or without TFA derivatization

Compound	Free base	TFA derivative
MA	1188	1411
4-MMA	1445	1652
4-FMA	1199	1419
4-CMA	1391	1607
4-BMA	1487	1703
4-IMA	1605	1821
4-NMA	1669	1882

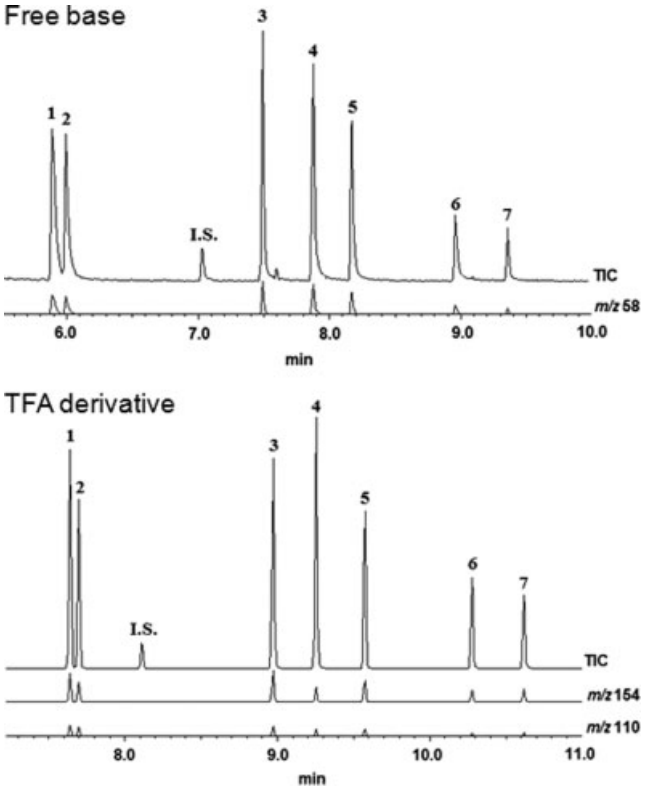

Figure 2. Total ion and extracted mass chromatograms of mixed solution of MA and six 4-substituted analogs of MA with or without TFA derivatization. Peak identification: 1, MA; 2, 4-FMA; 3, 4-CMA; 4, 4-MMA; 5, 4-BMA; 6, 4-IMA and 7, 4-NMA.

automatically injected into the GC–MS system. Seven calibration curves were established in the range 1–100 μg/ml by mass chromatography; the peak-area ratios of the base peak ions, *m/z* 154 or 121 to 91, were used for quantitation.

MA and six 4-substituted MAs were analyzed by GC–MS with and without TFA derivatization. The retention indices of each compound under the abovementioned conditions are listed in Table 1. Without TFA derivatization, all seven compounds could be separated from each other within 10 min in the following order: MA, 4-FMA, 4-CMA, 4-MMA, 4-BMA, 4-IMA and 4-NMA (Fig. 2). However, all were eluted with somewhat tailing peaks.

To improve the shape of the peaks, we subsequently performed the TFA derivatization of the compounds. As shown in Fig. 2, after TFA derivatization, all compounds could be separated within 11 min. Most importantly, the tailing peaks of the analytes observed for the free bases were overcome.

Figure 3 shows the fragmentation patterns of MA and the six 4-substituted MAs. Each EI mass spectrum of the seven nonderivatized compounds was characterized by the predominant ions at *m/z* 58, produced by an α-cleavage reaction.[13] With the exception of 4-NMA, fragment ions corresponding to the aromatic part of the molecule were exhibited by MA at *m/z* 91; 4-FMA at *m/z* 109; 4-CMA at *m/z* 125 and 127; 4-MMA at *m/z* 121; 4-BMA at *m/z* 90 and 4-IMA at *m/z* 90. In the mass spectra of 4-BMA and 4-IMA, the fragment ions at *m/z* 90 may be generated by an α-cleavage benzyl bond and subsequent halogen elimination. The mass spectra of the seven nonderivatized compounds were almost indistinguishable because the fragment ions derived from the aromatic part exhibited an abundance of ~10% or less. Therefore, the mass spectral information was insufficient to unambiguously identify the seven nonderivatized compounds.

In previous studies, TFA derivatization has been successfully applied to the discrimination of the phenethylamine-type drugs. Therefore, we

Table 2. Validation data based on total ion chromatograms for quantitative analysis of MA and six 4-substituted MA analogs by GC–MS after TFA derivatization

Compound	Correlation coefficient[a]	Intra-day CV (%)[b]			Inter-day CV (%)[b]		
		1 μg/ml	10 μg/ml	100 μg/ml	1 μg/ml	10 μg/ml	100 μg/ml
MA	0.9993	6.2	3.1	0.7	4.3	7.0	5.8
4-MMA	0.9984	6.7	12.5	11.8	17.1	16.3	9.7
4-FMA	0.9984	1.2	5.1	3.0	3.0	10.8	6.2
4-CMA	0.9988	3.4	10.3	8.7	9.5	11.4	5.1
4-BMA	0.9987	21.1	13.5	11.9	20.7	15.5	10.6
4-IMA	0.9988	13.8	16.7	14.6	26.9	21.4	15.2
4-NMA	0.9898	20.4	20.7	17.5	7.6	27.7	13.2

CV, coefficient of variation.
[a] Linearity range: 1–100 μg/ml.
[b] Each value was obtained from three determinations.

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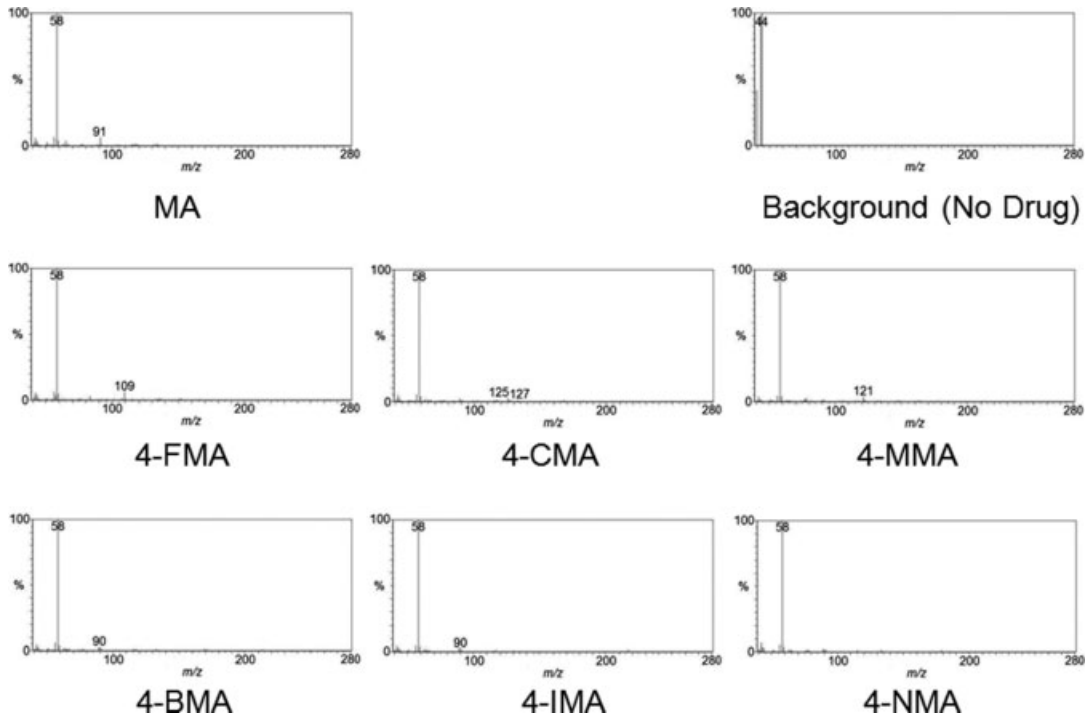

Figure 3. EI mass spectra of nonderivatized MA and six 4-substituted analogs of MA and background at 6.5 min.

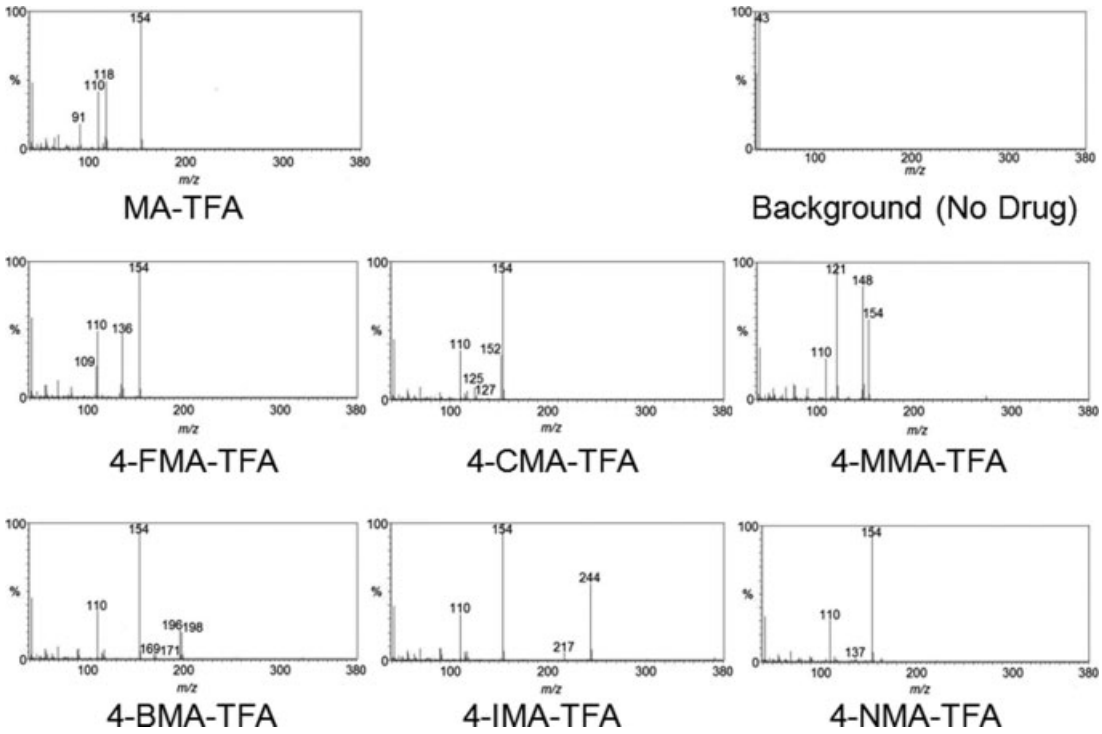

Figure 4. EI mass spectra of TFA derivatized MA and six 4-substituted analogs of MA and background at 8.0 min.

explored the TFA derivatization of MA and its analog compounds. As shown in Fig. 4, the ions at *m/z* 110 and 154 were common in the mass spectra of the seven trifluoroacetylated compounds. The diagnostic ions derived from the substituted aromatic part, exhibited by MA-TFA at *m/z* 91 and 118; 4-FMA-TFA at *m/z* 109 and 136; 4-CMA-TFA at *m/z* 125, 127 and 152; 4-MMA-TFA at *m/z* 121 and 148; 4-BMA-TFA at *m/z* 169, 171, 196 and 198; 4-IMA-TFA at *m/z* 217 and 244 and 4-NMA-TFA at *m/z* 137, made it easy to distinguish the seven trifluoroacetylated compounds. These results show that TFA derivatization is highly effective for differentiating the mass spectra of MA and the six 4-substituted MAs.

All quantitative analyses were performed after TFA derivatization. The validation data for the analysis of the seven compounds after derivatization are listed in Table 2. Good linearity of the response was confirmed between 1 and 100 μg/ml for all compounds. As shown in the table, the precision of the quantitative analysis by GC–MS after TFA derivatization was satisfactory. Further, Table 3 listing the analysis results

Table 3. Validation data based on mass chromatograms for quantitative analysis of blank urine samples spiked with MA and six 4-substituted MA analogs by GC-MS after TFA derivatization

Compound	Correlation coefficient[a]	Intra-day CV (%)[b]			Inter-day CV (%)[b]		
		1 μg/ml	10 μg/ml	100 μg/ml	1 μg/ml	10 μg/ml	100 μg/ml
MA[c]	0.9994	4.4	5.7	1.5	28.6	6.9	4.7
4-MMA[d]	0.9958	13.4	4.7	9.2	14.2	17.2	13.5
4-FMA[c]	0.9976	8.3	3.9	5.9	11.3	7.7	10.1
4-CMA[c]	0.9952	13.8	7.8	8.8	13.5	15.0	13.3
4-BMA[c]	0.9945	12.6	7.3	9.9	11.4	15.8	12.6
4-IMA[c]	0.9962	19.5	5.4	10.2	16.4	21.4	13.4
4-NMA[c]	0.9977	16.8	13.7	11.9	11.7	25.2	15.8

CV, coefficient of variation.
[a] Linearity range: 1–100 μg/ml.
[b] Each value was obtained from three determinations.
[c] The peak area of the base peak ion at *m/z* 154 was used.
[d] The peak area of the base peak ion at *m/z* 121 was used.

of blank urine samples spiked with the seven compounds proves that there is no interference from the urine matrix.

We synthesized five 4-substituted MAs and developed a reliable GC–MS method for the discrimination of MA and six 4-substituted MAs. Additionally, quantitative reliability was tested for the TFA-derivatized forms of these compounds. TFA derivatization improved the peak shape of the chromatograms and the fragmentation patterns in the mass spectra of all the compounds, allowing both chromatographic as well as mass spectrometric discrimination of MA and its six 4-substituted analogs. The proposed GC–MS technique should be especially helpful in forensic toxicological investigations.

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Supporting information

Supporting information may be found in the online version of this article.

Yours,

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