Back to *Microgram Journal* Index | Next

Analytical Profiles for 3,4,5-, 2,4,5-, and 2,4,6-Trimethoxyamphetamine

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ABSTRACT: Analytical profiles (Marquis color testing, infrared spectroscopy, nuclear magnetic resonance, thin layer chromatography, high-performance liquid chromatography, and gas chromatography/mass spectrometry) are presented for 3,4,5-trimethoxyamphetamine, 2,4,5-trimethoxyamphetamine, and 2,4,6-trimethoxyamphetamine. The data allows identification and differentiation of these positional isomers.

KEYWORDS: 3,4,5-Trimethoxyamphetamine, 2,4,5-Trimethoxyamphetamine, 2,4,6-Trimethoxyamphetamine, TMA, Positional Isomers, Marquis, IR, NMR, TLC, HPLC, GC/MS, Forensic Chemistry

Introduction

Most of the trimethoxyamphetamines (TMAs) are hallucinogens (1). There are six different positional isomers, that differ only in the respective positions of the three methoxy groups on the benzene ring (see Figure 1, next page). Of the six isomers, 3,4,5-trimethoxyamphetamine (TMA-1), 2,4,5-trimethoxyamphetamine (TMA-2), and 2,4,6-trimethoxyamphetamine (TMA-6) are more important than other three isomers, both from the perspective of their legal status and their circulation in Japanese drug markets. Unlike in the United States, positional isomers of hallucinogenic phenethylamines are not automatically controlled under Japanese statutes. Thus, TMA-1 is controlled by the Narcotics and Psychotropics Control Law in Japan, while TMA-2 is currently uncontrolled (but is anticipated to be scheduled in the near future), and TMA-6 is currently uncontrolled. In Japan, TMA-1 is usually sold as a solid, while TMA-2 and TMA-6 are more commonly sold in liquid forms, usually mixed with pigments, flavors, and sometimes other psychoactive compounds. Currently, abuse of 2,3,4-trimethoxyamphetamine (TMA-3), 2,3,5-trimethoxyamphetamine (TMA-4), and 2,3,6-trimethoxyamphetamine (TMA-5) have not been reported in Japan.

Because the legal status of the TMAs vary by structure in Japan, it is important to be able to identify and differentiate between (at least) TMA-1, TMA-2, and TMA-6. To our knowledge, no methods have been reported for such differentiation. Herein, we present analytical data (color testing, infrared spectroscopy (IR), nuclear magnetic resonance (NMR), thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas chromatography/mass spectrometry (GC/MS)) for TMA-1, TMA-2, and TMA-6.

Experimental

Syntheses: Authentic standards of hydrochloride salts of TMA-1, TMA-2, and TMA-6 were synthesized in our laboratory using previously reported procedures (1). All other chemicals used were of analytical grade.

Color Testing: Marquis reagent was prepared by adding one drop of formaldehyde to 1 mL of concentrated sulfuric acid (2). The sample was placed in a depression of spot plates, and 3 drops of the reagent were added. [The TLC spray reagents are reported below.]

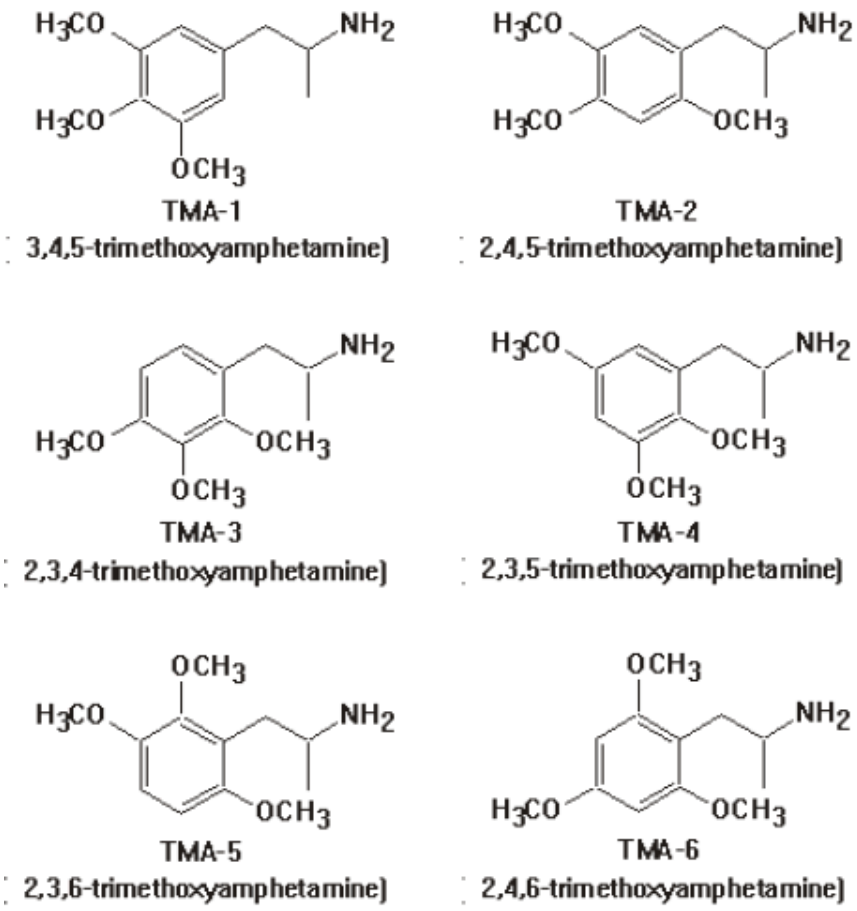

Figure 1. The TMA Positional Isomers.

FTIR: A Shimadzu FTIR-8900 Fourier Transform Infrared Spectrophotometer was used. The substrates were analyzed using the standard potassium bromide method. Thirty-two scans were collected between 4000 and 450 cm^{-1}, with a resolution of 4.0 cm^{-1}.

NMR: Proton NMR analyses were performed on a JEOL JNM-ECP600 NMR spectrometer. The samples were prepared at approximately 10 mg/mL in methanol-d_4 (CD_3OD), using added tetramethylsilane (TMS) as the 0.0 ppm reference.

TLC: TLC analyses were performed using the method of Takahashi *et al.* (3), with a minor modification. The analyses were carried out on silica gel plates (10 x 10 cm) containing a fluorescent indicator (254 nm) on glass support (Merck, Darmstadt, Germany). The respective hydrochlorides of each TMA were dissolved in methanol at concentrations of 10, 1, and 0.1 mg/mL. These were applied manually on the plates with a microsyringe. A solvent mixture of chloroform/methanol/25 % aqueous ammonia (75:25:3 v/v/v) was used as the mobile phase. After development and evaporation of the mobile phase, the compounds were detected by UV (254 nm) and by spraying with Dragendorff or fluorescamine reagents (prepared as follows):

> Dragendorff reagent: Bismuth hydroxide (0.9 g) was dissolved in concentrated hydrochloric acid (2 mL), and potassium iodine (3 g) dissolved in water (3 mL) and 70 % aqueous acetic acid (45 mL) were then added (4).
>
> Fluorescamine reagent: Fluorescamine (0.5 mg) was dissolved in acetone (1 mL) (2). The spots were observed under UV (365 nm).

HPLC: HPLC analyses were performed using the method of Kikura-Hanajiri *et al*. (5), with a minor modification. A Shimadzu LC-10ADvp series equipped with an SPD-M10Avp diode array detector set at 230 nm was used. The column was a Symmetry C18 column (Waters, 150 mm x 2.1 mm i.d., 3.5 µm) protected by an OptiGuard C18 guard column (Optimize technology), and was operated at 40 °C. The mobile phase, delivered at a flow rate of 0.2 mL/min, was a gradient of a mixture of acetonitrile-methanol (7:3 v/v) (B) in 10 mM ammonium formate (pH 3.5) (A): 0-1 min, 10 % B; 1-24 min, from 10 % to 33 % linear gradient of B in A. Sample Prep: A volume of 20 µL containing 10 µg/mL of each

trimethoxyamphetamine hydrochloride dissolved in distilled water was injected.

GC/MS: GC/MS analyses were performed using a GCMS-QP5050A (Shimadzu) equipped with a DB-5MS capillary column (Agilent technologies, 30 m x 0.25 mm i.d., 0.25 µm film thickness). The temperature of the injector and the interface was set at 250 °C. The oven temperature was held at 50 °C for 1 min, then raised to 300 °C at 15 °C/min, then held for 3 min. Helium was used as the carrier gas (head pressure 52.8 kPa, column flow 1.0 mL/min at 50 °C, constant pressure). The mass spectrometer was operated under electron ionization (EI) mode. One microliter samples were injected in the splitless mode. Sample Prep: For the free bases, the respective hydrochloride salts (200 µg) were dissolved in 1 mL of distilled water, basified to pH 12 with 1 M sodium hydroxide, and extracted with 1 mL of ethyl acetate. The extract was transferred to a GC vial. For the trifluoroacetylated derivatives, 100 µL of trifluoroacetic anhydride and 100 µL of ethyl acetate was added to 50 µg of the respective hydrochloride salt, and the mixture heated at 55 °C for 20 min. After evaporation of excess reagents, the residue was redissolved in 1 mL of ethyl acetate, and transferred to a GC vial.

Results and Discussion

Color Testing: The Marquis reagent reacted the three TMAs to give the following colors: TMA-1: Red; TMA-2: Pale yellow; and TMA-6: Orange. Different ring substitution patterns are known to give different colors with the Marquis reagent (6); however, the color differences between TMA-1, TMA-2, and TMA-6 were distinct and (somewhat) unexpected.

IR: The IR spectra of the three TMA hydrochloride salts are shown in Figure 2. The spectral patterns in the fingerprint region (< 1500 cm^{-1}) were completely different, and could therefore be used to unambiguously identify and differentiate the compounds.

NMR: The Proton NMR spectra are shown in Figure 3. The splitting patterns in the aromatic region were different for TMA-2 (two singlet peaks) versus TMA-1 and TMA-6 (one singlet peak). TMA-2 has two chemically nonequivalent protons, while TMA-1 and TMA-6 have two chemically equivalent protons. TMA-1 and TMA-6 could be distinguished by chemical shifts of their aromatic protons. The respective values for TMA-1 and TMA-6 were 6.56 ppm and 6.25 ppm. These values did not agree with those predicted from the empirical rule (7) (6.13 ppm for TMA-1 and 6.00 ppm for TMA-6), but the relative difference was consistent.

TLC: The R_f values of TMA-1, TMA-2, and TMA-6 using the described system were 0.69, 0.65, and 0.59, respectively. Although the spots were very close, they could be differentiated from one another. Table 1 (next page) shows the detection limits by the UV (254 nm) and various detection reagents. The sensitivities of the reagents were in decreasing order: Fluorescamine reagent, Marquis reagent, and Dragendorff reagent. However, the fluorescamine and Dragendorff reagents gave minimal color differences between the three isomers (green fluorescence under UV (365 nm) for the fluorescamine reagent, and orange for the Dragendorff reagent). On the other hand, spraying with Marquis reagent gave different colors, as follows: TMA-1: Orange but immediately fading; TMA-2: yellow; and TMA-6: Orange then changing to purple-red.

Table 1. Detection Limits (milligrams) of the TMAs.

Compound	Detection			
	UV (254 nm)	Dragendorff	Fluorescamine	Marquis
TMA-1	2	10	0.05	5
TMA-2	1	5	0.2	0.05
TMA-6	2	10	0.2	0.5

HPLC: Figure 4 shows the HPLC chromatogram of a mixture of the three TMAs.

GC/MS: Figure 5 shows the total ion chromatograms (TICs) of the nonderivatized and trifluoroacetylated (TFA-derivatized) TMAs. The nonderivatized TMAs all displayed tailing, and TMA-1 and TMA-2 were not baseline resolved. However, the TFA-derivatives displayed improved peak shapes and enhanced separation between TMA-1 and TMA-2.

Figure 6 shows the EI mass spectra. The spectra of nonderivatized TMAs were similar, and it was especially difficult to discriminate between TMA-1 and TMA-2. However, the TFA-derivatives (though also similar) were sufficiently different for differentiation.

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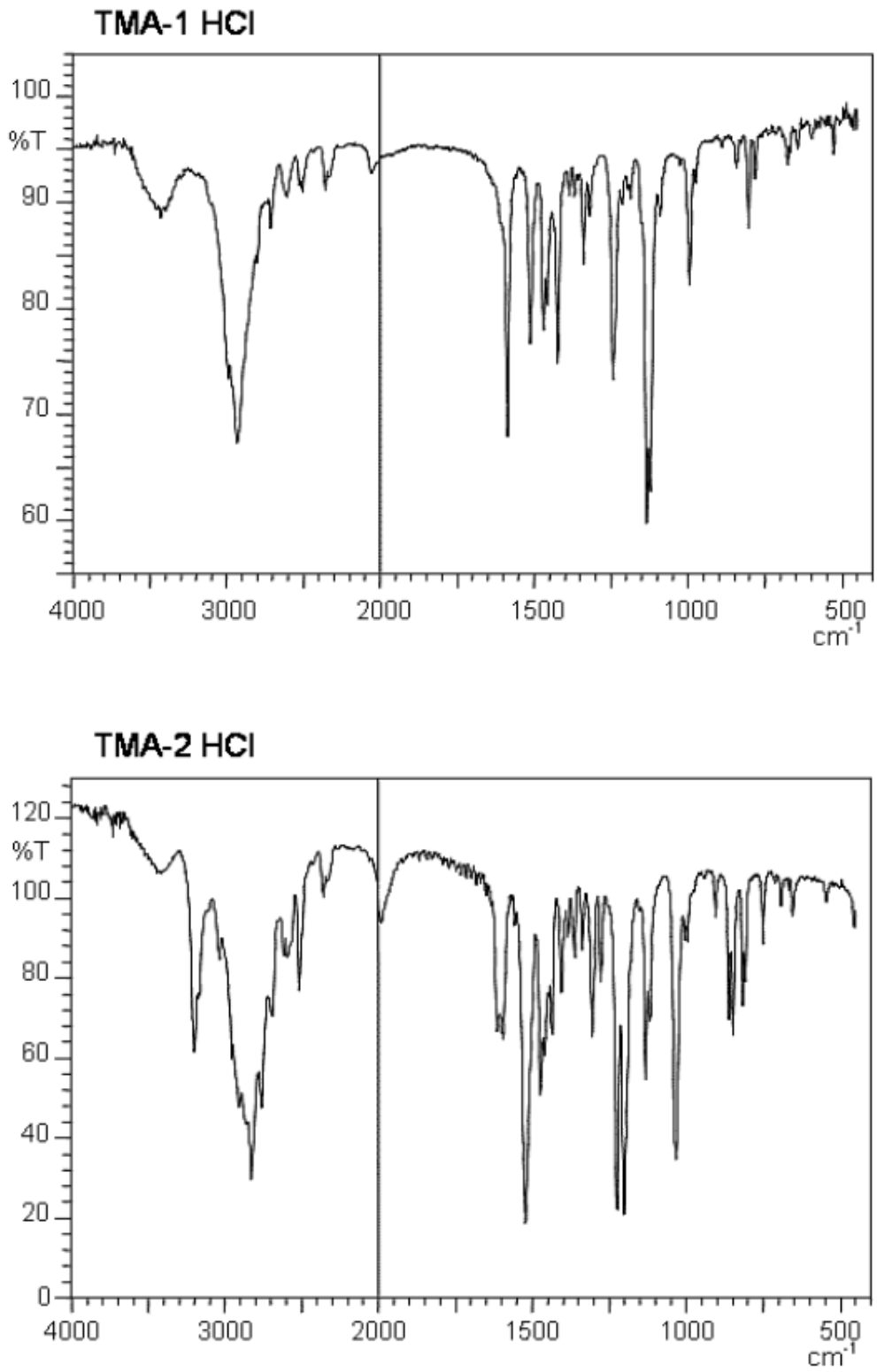
[Figures 2 - 6 Follow (Note: Figure 4 is Between Figures 2c and 3a in Order to Improve Layout).]



Figures 2a-b. IR Spectra of the Hydrochloride Salts of TMA-1 and TMA-2.

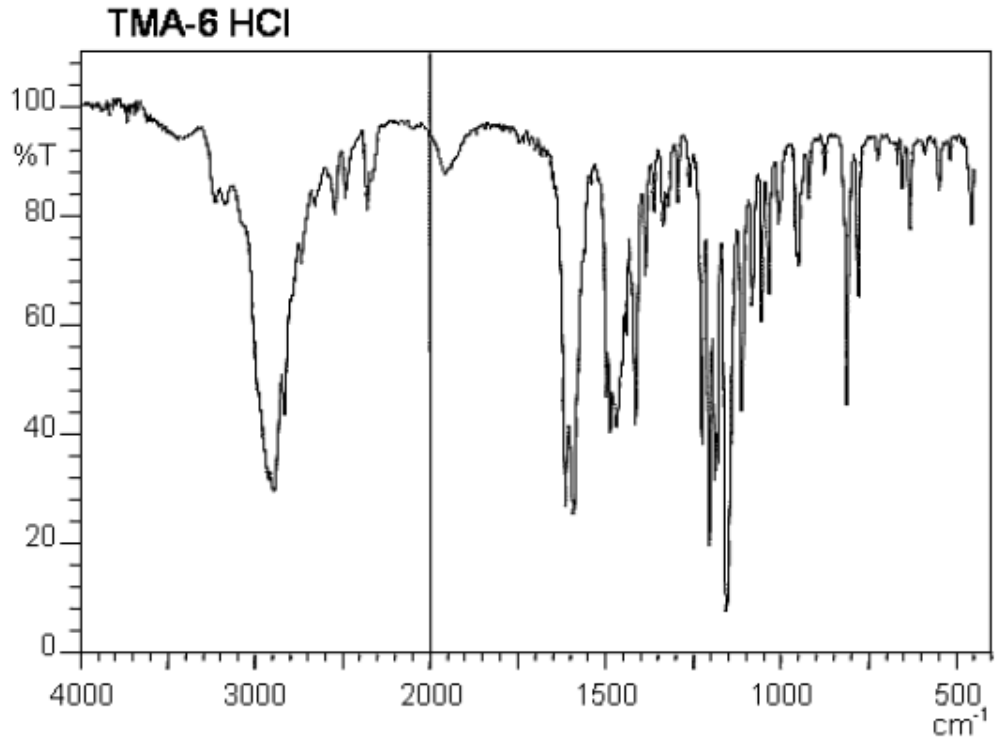


Figure 2c. IR Spectra of the Hydrochloride Salt of TMA-6.

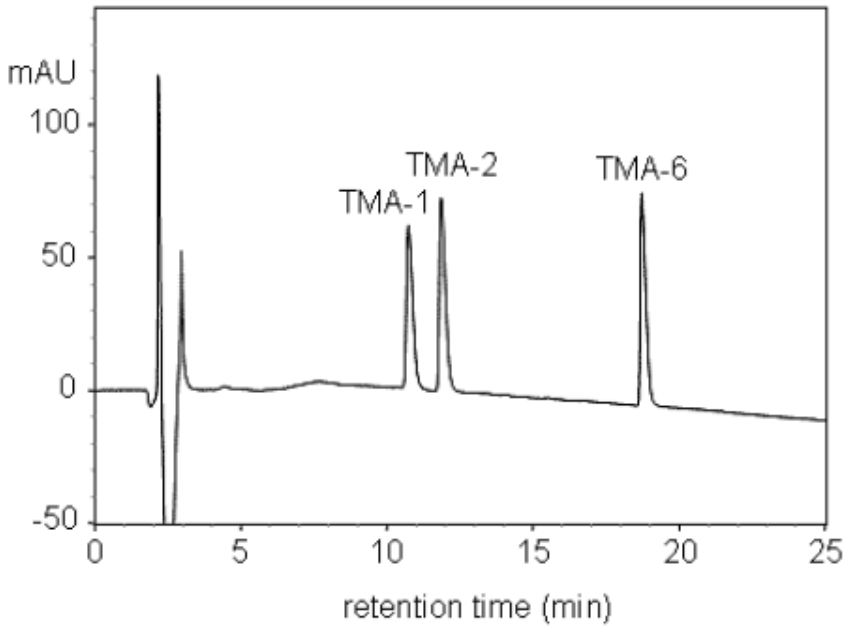


Figure 4. HPLC Chromatogram of a Mixture of the Three TMAs (Detection: UV 230 nm). Retention times (Minutes): TMA-1 - 10.7, TMA-2 - 11.9, and TMA-6 - 18.7.

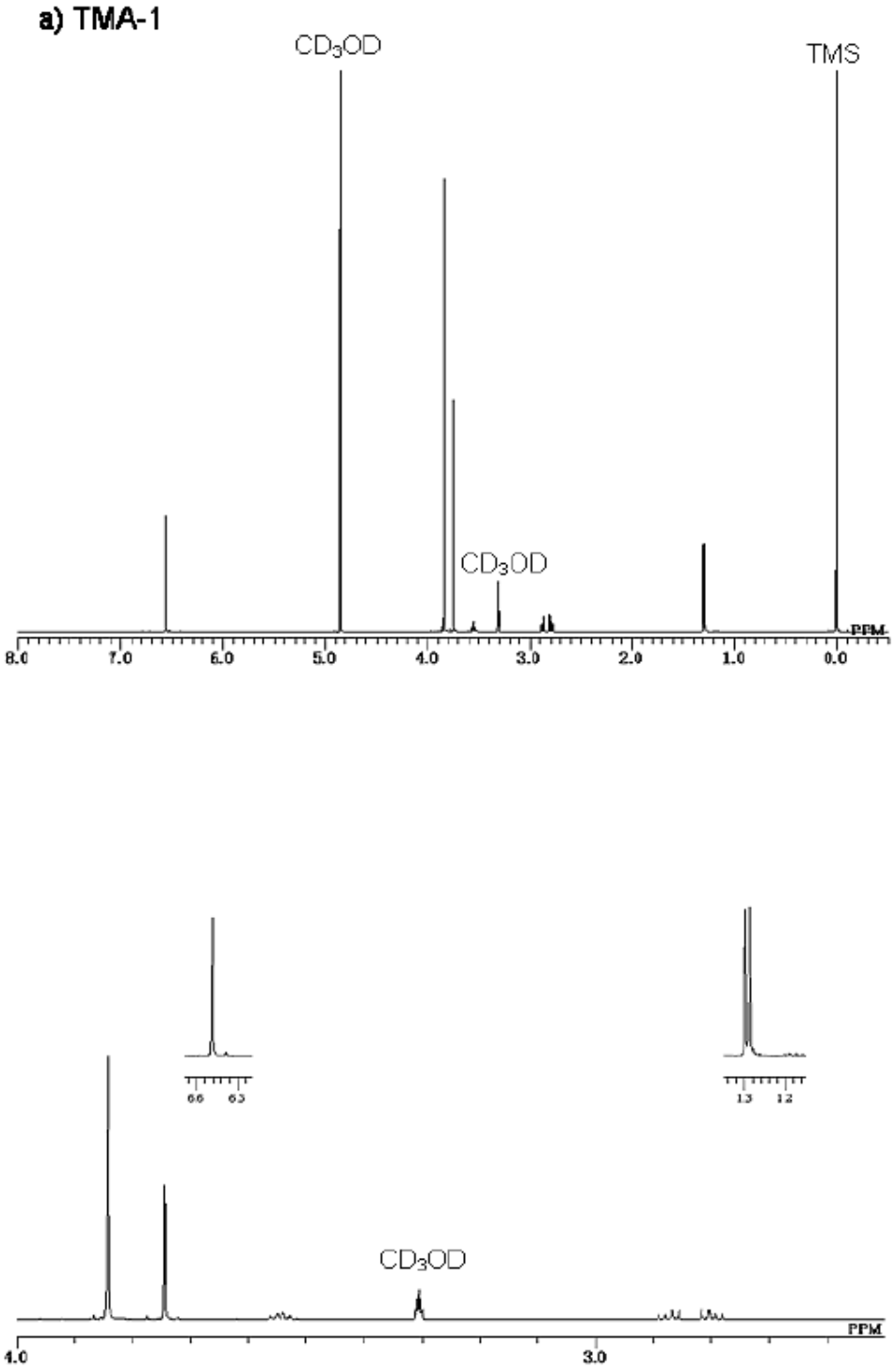


Figure 3a. Proton NMR (600 MHz) of TMA-1: ^{1}H-NMR (CD_3OD) δ: 6.56 (2H, s), 3.84 (6H, s), 3.74 (3H, s), 3.58-3.51 (1H, m), 2.87 (1H, dd, *J* = 13.7, 7.1 Hz), 2.80 (1H, dd, *J* = 13.5, 7.4 Hz), 1.29 (3H, d, *J* = 6.6 Hz).

b) TMA-2

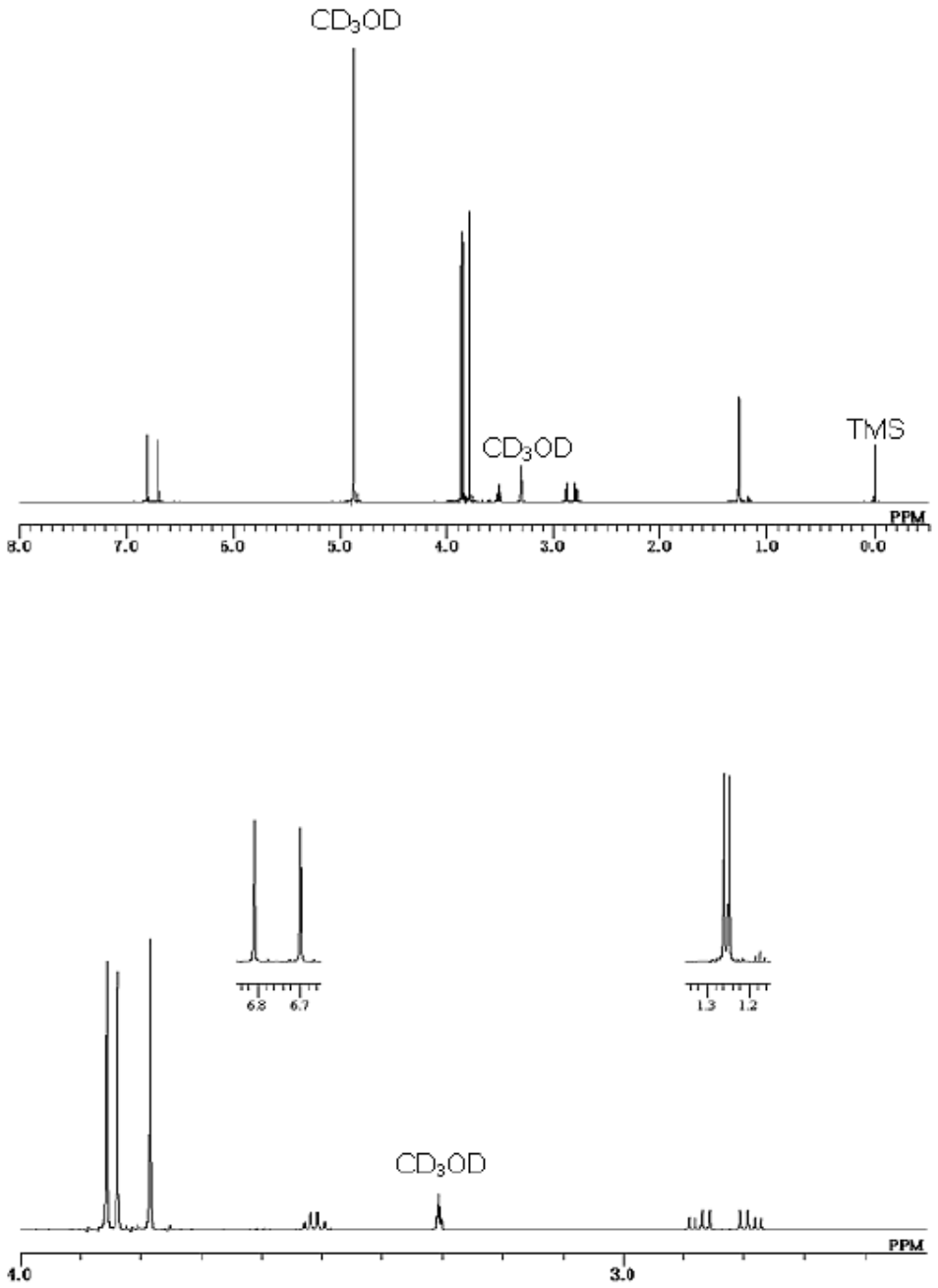


Figure 3b. Proton NMR (600 MHz) of TMA-2: ^{1}H-NMR (CD_3OD) δ: 6.81 (1H, s), 6.70 (1H, s), 3.86 (3H, s), 3.84 (3H, s), 3.78 (3H, s), 3.54-3.48 (1H, m), 2.87 (1H, dd, *J* = 13.6, 6.9 Hz), 2.79 (1H, dd, *J* = 13.7, 6.9 Hz), 1.25 (3H, d, *J* = 6.7 Hz).

c) TMA-6

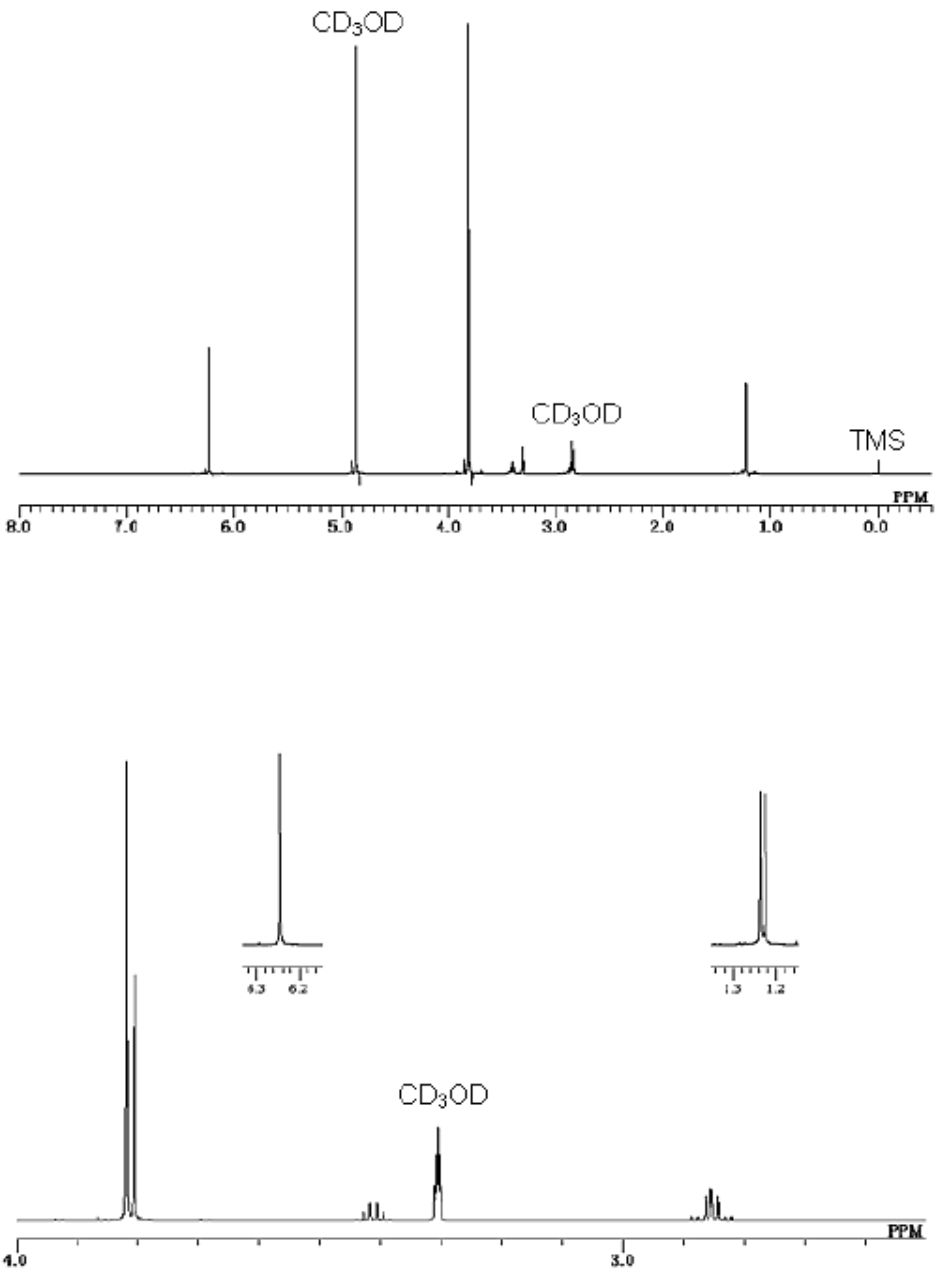
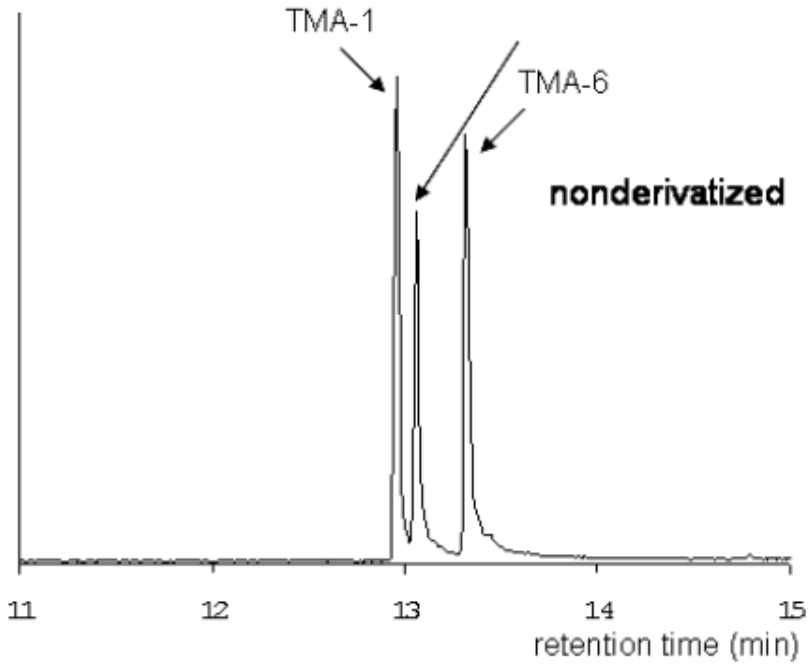


Figure 3c. Proton NMR (600 MHz) of TMA-6: ^{1}H-NMR (CD_3OD) δ: 6.25 (2H, s), 3.82 (6H, s), 3.81 (3H, s), 3.44-3.38 (1H, m), 2.87 (1H, dd, *J* = 13.0, 6.0 Hz), 2.84 (1H, dd, *J* = 13.3, 6.8 Hz), 1.23 (3H, d, *J* = 6.7 Hz).



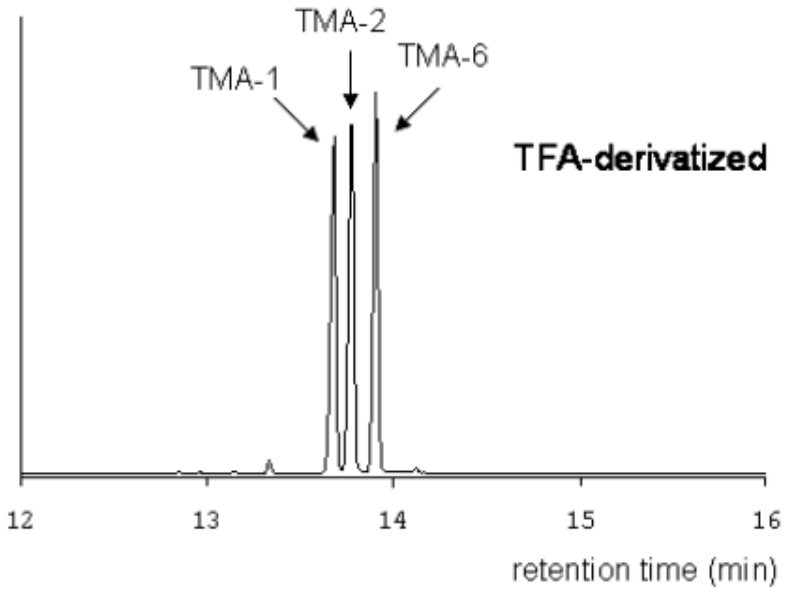


Figure 5. Total Ion Chromatograms of the Three Non-Derivatized and TFA-Derivatized TMAs.

[Retention Indices: Non-Derivatized: TMA-1 - 1724; TMA-2 - 1739; TMA-6 - 1771;
TFA-Derivatized: TMA-1 - 1814; TMA-2 - 1830; TMA-6 - 1849.]

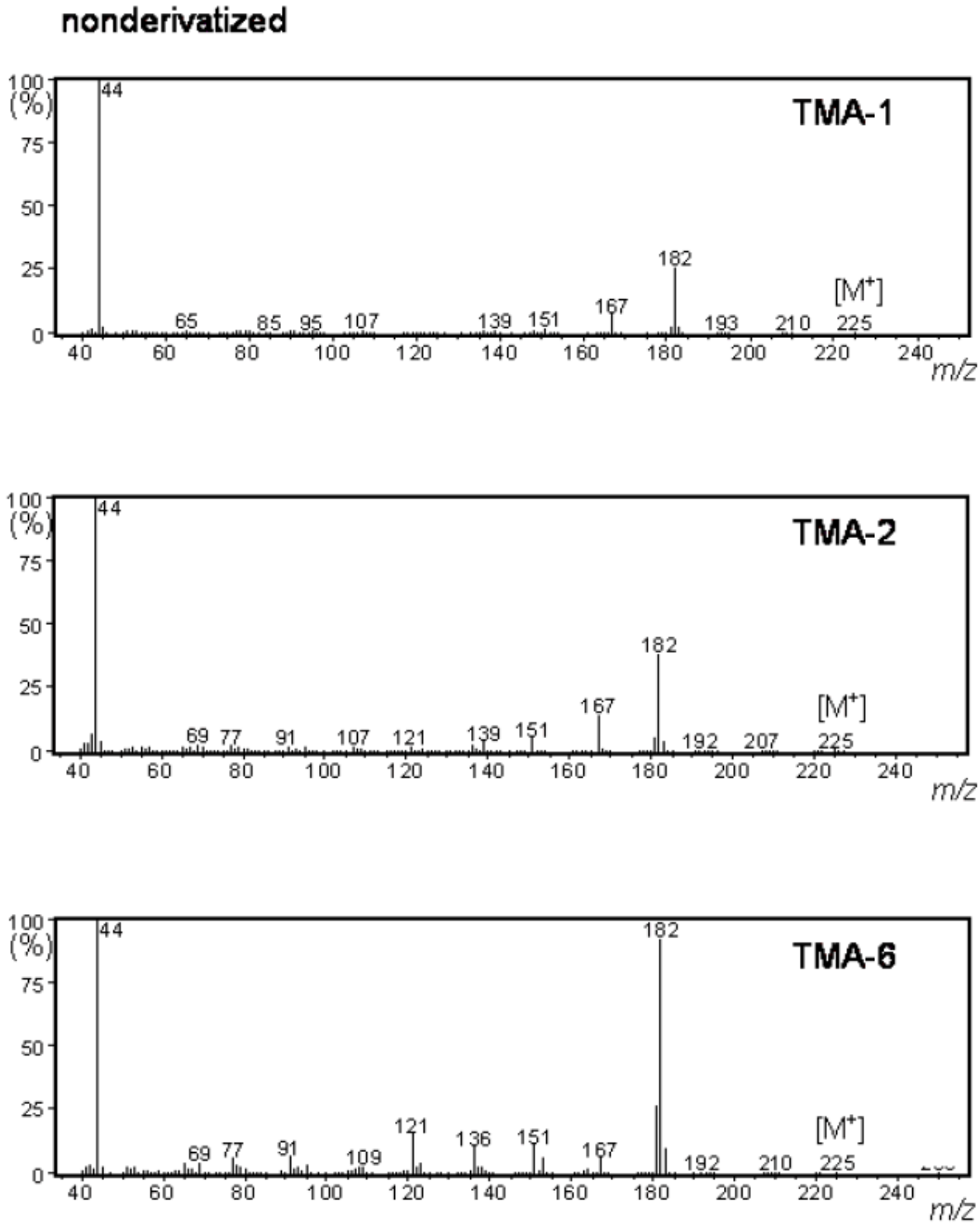


Figure 6a. EI Mass Spectra of the Three Non-Derivatized TMAs.

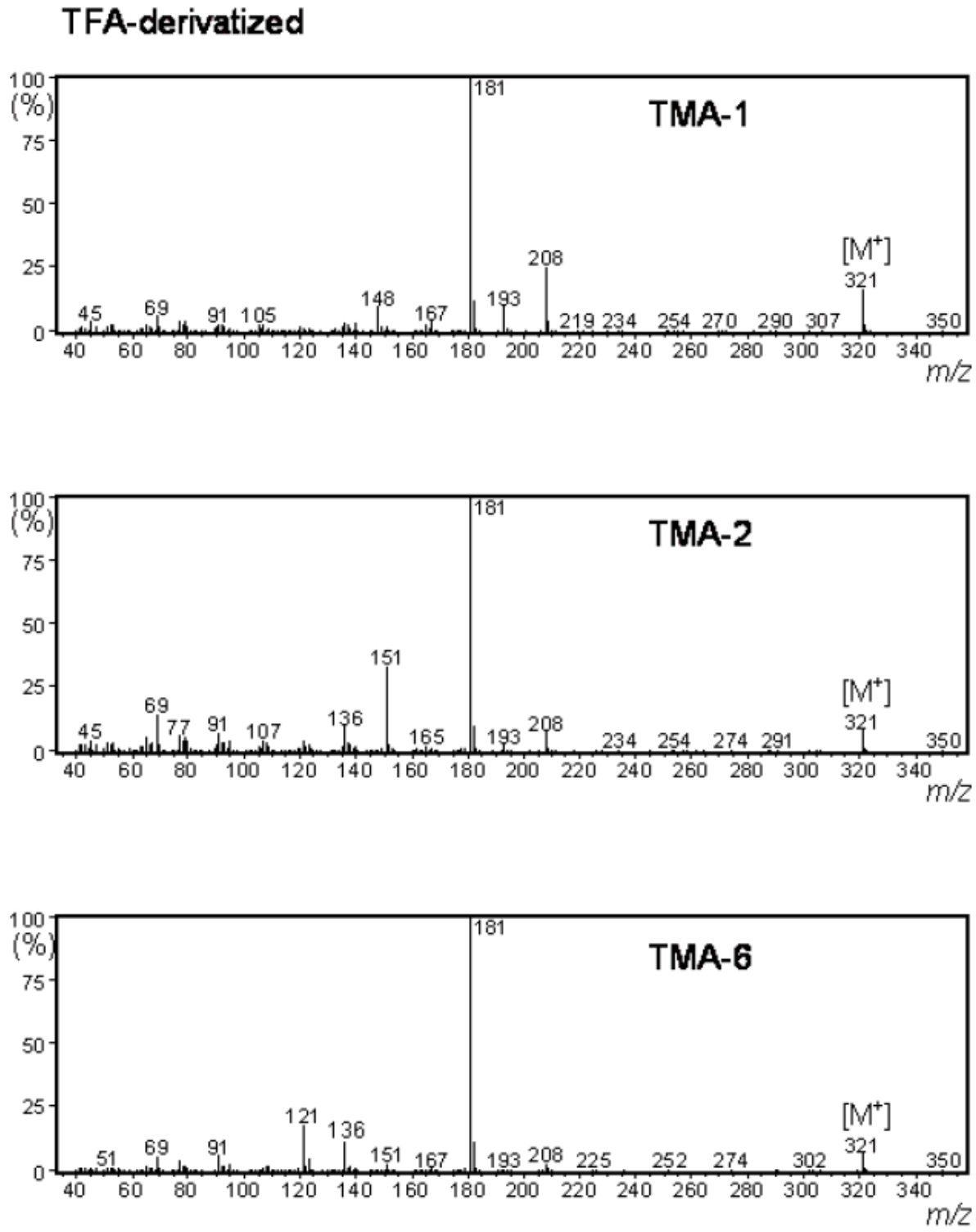


Figure 6b. EI Mass Spectra of the Three TFA-Derivatized TMAs.

Back to *Microgram Journal* Index | Next