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Simultaneous determination of psychotropic phenylalkylamine derivatives in human hair by gas chromatography/mass spectrometry

Jin Young Kim*, Kyu Sung Jung, Min Kyoung Kim, Jae Il Lee and Moon Kyo In
Drug Analysis Laboratory, Forensic Science Division, Supreme Prosecutors' Office, Seoul, Korea

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A gas chromatography/mass spectrometric (GC/MS) method was developed and validated for the determination of thirteen psychotropic phenylalkylamine derivatives (amphetamine; AP, phentermine; PT, methamphamine; MA, cathinone; Khat, methcathinone; MCAT, fenfluramine; FFA, desmethylselegiline; DSEL, 3,4-methylenedioxyamphetamine; MDA, 3,4-methylenedioxymethamphetamine; MDMA, 3,4-methylenedioxyethylamphetamine; MDEA, norketamine; NKT, mescaline; MES, 4-bromo-2,5-dimethoxyphenethylamine; 2CB) in human hair. Hair samples (20 mg) were washed with distilled water and acetone, cut into small fragments (<1 mm), and incubated in 0.25 M methanolic HCl under ultrasonication at 50°C for 1 h. The resulting solutions were evaporated to dryness, derivatized using trifluoroacetic anhydride (TFAA) at 70°C for 30 min, and analyzed by GC/MS. The linear ranges were 0.02–25.0 ng/mg for AP, PT, Khat, FFA, DSEL, MDMA, and 2CB; 0.05–25.0 ng/mg for MA, MCAT, and MES; 0.05–12.5 ng/mg for MDA; and 0.1–25.0 ng/mg for MDEA and NKT, with good correlation coefficients ($r^2 > 0.9985$). The intra-day, inter-day, and inter-person precisions were within 12.7%, 14.8%, and 16.8%, respectively. The intra-day, inter-day, and inter-person accuracies were between −10.7 and 13.4%, −12.7 and 11.6%, and −15.3 and 11.9%, respectively. The limits of quantifications (LOQs) for each compound were lower than 0.08 ng/mg. The recoveries were in the range of 76.7–95.6%. The method proved to be suitable for the simultaneous qualification and quantification of phenylalkylamine derivatives in hair specimens.

Phenylalkylamine derivatives such as amphetamine (AP), phentermine (PT), methamphamine (MA), cathinone (Khat), fenfluramine (FFA), methcathinone (MCAT), selegiline, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine (MDEA), ketamine, mescaline (MES), and 4-bromo-2,5-dimethoxyphenethylamine (2CB) are abused drugs possessing stimulant or hallucinogenic properties.[1–3] All these drugs except for selegiline are regulated under the Controlled Substances Act in Korea. MA, a powerful stimulant of the central nervous system,[4] is currently the most used illegal drug of abuse in Korea. The abuse of MA and its methylenedioxy derivatives increased dramatically in the late 1990s and has become a serious social problem. Recently, there has been a growing tendency for the illegal circulation and abuse of ketamine, 2CB, FFA and PT. Ketamine is used as a veterinary and medical anesthetic and its major metabolite is norketamine (NKT). Although ketamine is not as common as other recreational drugs such as Ecstasy (MDMA), there has been a recent tendency for KET abuse in East Asia as well as in Korea.[5–7] 2CB is a new psychoactive phenylethylamine drug that has recently been discovered in seizures of illicit drugs sold on the Korean black market.[8] 2CB has a similar chemical structure to the hallucinogenic drug MES. FFA and PT are also stimulants used primarily for weight loss that have been associated with serious health problems. They were frequently found to be adulterants in imported diet products from China, causing valvular heart disease and primary pulmonary hypertension.[9,10] Therefore, FFA has been banned in five Asian countries including Korea.[11] Selegiline, an irreversible and selective inhibitor of monoamine oxidase type B, is metabolized into desmethylselegiline (DSEL), levomethamphetamine, and levoamphetamine. Levomethamphetamine is not considered psychoactive and has little abuse potential.[12,13] Because of its metabolites, selegiline can result in positives for AP/MA on drug tests. The increasing abuse and harmfulness of these psychotropic phenylalkylamines

**Correspondence to*: J. Y. Kim, Drug Analysis Laboratory, Forensic Science Division, Supreme Prosecutors' Office, 706, Banporo, Seocho-gu, Seoul 137-730, Korea.
E-mail: paxus@spo.go.kr
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require rapid and simultaneous analytical methods for their detection in biological samples and this has become a focus of attention in forensic and clinical toxicology.

The matrices commonly investigated for phenylalkylamine derivatives are urine, blood, and hair in forensic toxicology. Analysis of hair as an alternative or complementary matrix has several advantages over urine testing. Conventional urine or blood analysis informs on recent or current exposure to drug use, while hair analysis provides a relatively long-term window depending on the length of hair shaft and allows distinction between repeated use and not.[14–17] To date, the determination of psychotropic phenylalkylamine derivatives has been based on immunoassay,[18,19] gas chromatography (GC),[20] GC/mass spectrometry (MS),[21–24] and liquid chromatography (LC)/MS.[25–27] Because of its specificity and sensitivity, GC/MS with selected ion monitoring (SIM) has routinely been employed for the determination of psychotropic phenylalkylamines of abuse.

However, all these methods covered only single substances or mixtures of a few phenylalkylamine derivatives. The objective of the study was to develop a method to simultaneously detect and quantify phenylalkylamine derivatives in monitoring for the abuse of single or multiple drugs. The simultaneous determination of several kinds of abused drugs was advantageous because of the limitations in the size of the sample and the consumption of polydrugs. A new digestion and extraction solution of methanolic HCl was applied to achieve effective extraction of the analytes from the hair matrix.

In this study, a GC/MS method was described for the simultaneous determination of thirteen psychotropic phenylalkylamines in human hair, as substrate for the detection of residues of illicitly administered substances. The method was validated and its applicability to hair checked with samples obtained from suspected abusers.

EXPERIMENTAL

Reagents and materials

The reference compounds Khat, FFA, MCAT, DSEL, NKT, MES and 2CB were obtained from Cerilliant (Austin, TX, USA) at a concentration of 1000 μg/mL in methanol. A mixed methanolic solution of AP, PT, MA, MDA, MDMA, and MDEA in a vial at a concentration of 250 μg/mL and methanolic solutions of the deuterated internal standards AP-d_8, MA-d_{11}, FFA-d_{10}, MDA-d_5, MDMA-d_5, MDEA-d_6, NKT-d_4 and MES-d_9 in each vial at a concentration of 100 μg/mL were also obtained from Cerilliant. HPLC-grade ethyl acetate, methanol, and acetone were supplied by J.T. Baker (Phillipsburg, NJ, USA). Trifluoroacetic anhydride (TFAA) was obtained from Alltech (Deerfield, IL, USA). Acetyl chloride was supplied by Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade. Extract-Clean™ empty reservoirs and polyethylene frits for a 4.0-mL reservoir were obtained from Alltech. The water was purified with a Maxima water purification system (ELGA, High Wycombe, UK).

Preparation of solutions

Working standard solutions (0.1, 1.0, and 10.0 μg/mL) of each compound were prepared by appropriate dilution with methanol. Internal standards were prepared in methanol to give a working standard solution of 1.0 μg/mL. All these solutions were stored at −20°C in the absence of light until use.

Methanolic HCl solution (0.25 M) was prepared by the dropwise addition (on ice) of 8.9 mL of acetyl chloride to approximately 150 mL methanol with magnetic stirring in a 250-mL Quick-fit Büchner filter flask and cooled in an ice-water bath. The methanolic HCl was then added to a 500-mL volumetric flask and diluted to volume with methanol. This solution was used within 2 months of the date of preparation.

Hair specimens

Drug-free hair to be used as a matrix for control and calibration samples was obtained from a 39-year-old male volunteer. Head hair samples were received from the Narcotics Department at the Seoul District Prosecutors' Office between October and November 2006. A total of 141 samples were collected from possible drug abusers including samples that had tested positive for its use during a screening test of urine samples by GC/MS. These hair samples were generally cut as close as possible to the skin from the posterior vertex. The total length of each hair was measured and special treatments such as coloring and bleaching were noted.

Comparison of the efficiencies of digestion and extraction procedures

Five different digestion and extraction procedures were tested with the pool of hair samples that had tested positive for MA. Three digestions were performed in acidic conditions as follows: 20 mg hair samples were placed in test tubes with 1 mL of 1 M HCl, 2mL of 0.25 M methanolic HCl or 2 mL of 5 M HCl/methanol (1:20, v/v).[28,29] After digestion with 1 mL of 1 M HCl overnight at 50°C, the samples were adjusted to pH 12 with 1 M NaOH. The samples were then subjected to liquid-liquid extraction with 5 mL of ethyl acetate. After centrifugation, the organic phase was transferred to a test tube. In the case of extraction of hair samples with 2 mL of 0.25 M methanolic HCl or 5 M HCl / methanol (1:20, v/v), hair samples were incubated at 50°C for 1 h under ultrasonication. After the hair had been filtered with a fritted reservoir (Extract-Clean™ empty reservoir with a bottom polyethylene frit installed), the filtrate was collected in a new test tube. The forth extraction procedure was as follows: after digestion with 1 mL of NaOH at 95°C for 10 min, 5 mL of ethyl acetate was added to the incubation mixture. The samples were extracted by mechanical shaking for 20 min at approximately 60 cycles/min. After centrifugation, the organic phase was transferred to a test tube.[30,31] The fifth extraction procedure was performed by a direct extraction of analytes using methanol. The hair sample was sonicated for 1 h (50°C) with 2 mL of methanol in a test tube and then allowed to stand overnight. After the hair had been filtered with a fritted reservoir, the filtrate was collected in a new test tube.[32]

The solutions obtained after five different extraction procedures were each evaporated to dryness under a nitrogen stream at 45°C and 30 kPa. Trifluoroacetyl (TFA)

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derivatives were formed by reaction with 50 μL of ethyl acetate and 50 μL of TFAA as a derivatization agent in a dry heating block at 70°C for 30 min. The residue was reconstituted with 40 μL of ethyl acetate. An aliquot (1 μL) of sample solution was injected into the GC/MS system.

Sample preparation

Hair samples (20 mg) were washed with water (10 mL) and acetone (10 mL) twice, air-dried and cut with scissors into small fragments (<1 mm) before transfer to a test tube (12 × 100 mm) containing 75 μL of the combined internal standard solution (1.0 ng/mL). The hair samples were extracted with 2 mL of 0.25 M methanolic HCl at 50°C for 1 h under ultrasonication. The hair was then filtered with a fritted reservoir and the filtrate was dried under a nitrogen stream at 45°C and 30 kPa. TFA derivatives of each compound were formed by reaction of the sample with 50 μL of TFAA and 50 μL of ethyl acetate in a dry heating block at 70°C for 30 min, followed by drying under a nitrogen stream. The residue was reconstituted with 40 μL of ethyl acetate. An aliquot (1 μL) of the sample solution was injected into the GC/MS system.

GC/MS analysis

GC/MS analyses were performed with an 5975i mass spectrometer (Agilent Technologies, Foster City, CA, USA) equipped with a 6890N gas chromatograph and 7683B autosampler. Data acquisition and analysis were performed using standard software supplied by the manufacturer (Agilent Techologies, MSD Chemstation D.02.00). Separation was achieved on a capillary column (DB-5MS, 30 m × 0.25 mm i.d., 0.25 μm; J&W Scientific, Folsom, CA, USA) with helium as the carrier gas at a flow rate of 1.1 mL/min. The GC temperature program was as follows: initial temperature was 90°C for 3 min, increased to 170°C at a rate of 15°C/min, held for 3.0 min, increased to 210°C at a rate of 25°C/min, held for 1.5 min, then increased to 230°C at a rate of 20°C/min, held for 0.5 min, finally increased to 300°C at a rate of 35°C/min, and held for 0.5 min. The splitless injection mode was used with a purge-on time of 0.1 min. The injector and the GC interface temperatures were 260 and 280°C, respectively. The mass spectrometer was operated at 70 eV in the electron ionization (EI) mode with selected ion monitoring (SIM) for quantification. The quantifier and qualifier ions were monitored in respective groups for each compound that changed with elution time; the ions are listed with the elution order in Table 1.

Table 1. Retention times (RT), molecular weights (MW), and ions monitored for GC/MS analysis of TFA derivatives

			Ions monitored (*m*/*z*)		
Compound	RT (min)	MW	Quantifier ions	Qualifier ions	
AP-d_8-TFA	7.68	231	143	—	—
AP-TFA	7.71	239	118	140	91
PT-TFA	7.90	245	154	91	132
MA-d_{11}-TFA	8.80	256	160	—	—
MA-TFA	8.86	245	154	118	110
Khat-TFA	8.96	245	105	77	—
FFA-d_{10}-TFA	9.01	337	177	—	—
FFA-TFA	9.07	327	168	140	159
MCAT-TFA	9.36	259	105	154	77
DSEL-TFA	10.04	269	178	118	91
MDA-d_5-TFA	11.50	280	136	—	—
MDA-TFA	11.53	275	162	135	77
MDMA-d_5-TFA	12.61	294	158	—	—
MDMA-TFA	12.64	289	154	162	135
MDEA-d_6-TFA	13.01	309	174	—	—
MDEA-TFA	13.05	303	162	168	135
NKT-d_4-TFA	13.23	323	288	—	—
NKT-TFA	13.25	319	284	239	256
MES-d_9-TFA	13.44	316	190	—	—
MES-TFA	13.50	307	181	307	179
2CB-TFA	14.56	355	244	231	357

Validation of analytical method

The method was validated and tested according to the accepted protocol before the application to real samples.[33,34] Selectivity, matrix effect, linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy, and recovery were assayed as reported for the analysis of psychotropic phenylalkylamines of abuse in human hair.[22–24,26]

To evaluate selectivity, a drug-free hair sample was extracted and analyzed to evaluate potential interferences released from the hair matrix. The apparent response at the retention times of the analytes under investigation was compared with the response of analytes at the LOQ. Potential interferences from five phenylalkylamine derivatives (norephedrine, ephedrine, methylephedrine, methoxyphenamine, phendimetrazine, and dextromethorphan) were evaluated by spiking 20 mg of drug-free hair with 100 ng of the aforementioned substances and carrying them through the entire procedure.

The potential for carryover was evaluated by injecting the highest point of the calibration curve, followed by solvent blank, and measuring the area of the peaks present at the retention times of analytes under investigation. For the routine analysis of hair samples, ethyl acetate blanks were run between each pair of samples.

Calibration curves were constructed over the LOQ for all the analytes. Linear regression analysis was performed on the peak area ratios of analyte to internal standard versus analyte concentrations. The limits of detection (LODs) and quantification (LOQs) for each compound were calculated based on the concentration corresponding to a signal plus 3 and 10 standard deviations from the mean of six replicates of drug-free hair, respectively.

Six replicates at the three different quality control (QC) sample concentrations (0.3, 6.0, and 20.0 ng/mg) added to drug-free hair samples and extracted as described above were analyzed for the determination of intra-day precision and accuracy. The inter-day precision and accuracy were determined for three independent experimental days of the aforementioned replicates, and the inter-person precision and accuracy were determined for three different operators of the aforementioned replicates. To determine the precision, the coefficients of variations (% CV) were calculated for the replicate measurements. Accuracy (% bias) was expressed as the relative error of the calculated concentrations. It was

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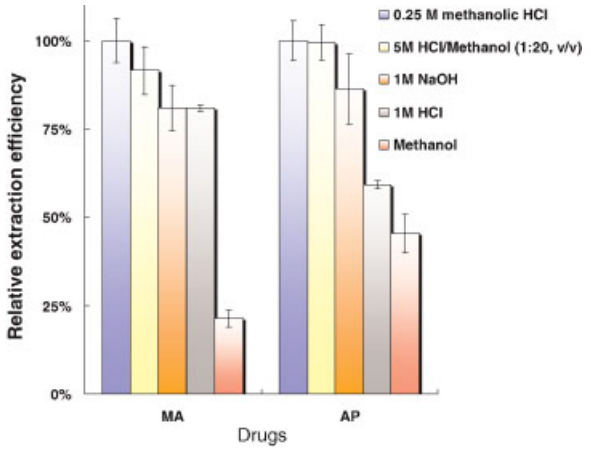

Figure 1. Comparison of the mean efficiencies of five digestion and extraction procedures.

calculated by the degree of agreement between the measured and nominal concentrations of the fortified samples.

Analytical recoveries were calculated by comparing the peak areas obtained when calibration samples were analyzed by adding the reference substances and the internal standards in the extract from drug-free hair sample prior to and after the extraction procedure. The recoveries were assessed by QC samples using five replicates for each QC sample concentration.

RESULTS AND DISCUSSION

Effect of five procedures on the extraction efficiency

Figure 1 demonstrates the relative extraction efficiency of various digestion and extraction procedures. It was determined by assaying hair known to be positive for MA in the presence of deuterated internal standards with three replicates at each procedure. These hair samples were processed by each of the digestion and extraction procedures as described above. The relative extraction efficiency was calculated from the integrated peak areas using the quantification ions of AP and MA.

The results exhibited significant differences among the five procedures. AP and MA were extracted with the highest extraction efficiency in 0.25 M methanolic HCl solution, while methanol had very poor extraction efficiency. In 5 M HCl/methanol (1:20, v/v) mixture, 1 M NaOH, and 1 M HCl, the relative extraction efficiencies of AP and MA varied widely. These data demonstrate that the effect of the digestion conditions should be considered when interpreting the results of the analysis in hair samples of phenylalkylamine derivatives, which are structurally similar to AP and MA. The 0.25 M methanolic HCl solution was selected as the best digestion and extraction solution.

Mass spectral characteristics of derivatives

The chemical structures, molecular weights and EI full-scan mass spectra, recorded with the quadrupole mass spectrometer, of the derivatized analytes and corresponding internal standards, are shown in Fig. 2.

The main feature of derivatization is the significant increase in the molecular weight, leading to new compounds with altered polarity and volatility properties; this allows improved overall chromatographic selectivity and non-tailing peak shapes. The relative abundance of the molecular ions in the EI mass spectra of the derivatives depend on their chemical structures. Under the conditions used for analysis (nominal electron energy 70 eV), molecular ions of most analytes were not observed except for MDA, MDMA, MDEA, MES, and 2CB in which the molecular ion was of low relative abundance. The base peak of the EI mass spectrum and some of key fragment ions were used to indicate the presence of each analyte and internal standard. The characteristic fragment ions indicative of each analyte were used as quantifier and qualifier ions (Table 1).

Decontamination

Passive drug absorption by hair must be considered a possible source of false-positive results. Thus, before proceeding with analysis, it was necessary that a step to remove external contaminants was included. To confirm that drugs adsorbed onto hair surface would be eluted with washing, positive samples were washed using the described washing procedure and then washed again with 10 mL acetone. The efficiency of the acetone wash after the normal wash of hair samples was evaluated. Of the analytes only AP, PT, MA, MDA, MDMA, MDEA, and NKT were examined; these substances were not detected in an acetone wash after the normal washing procedure. As positive hair samples for Khat, FFA, MCAT, DSEL, MES, and 2CB have not yet been obtained, washing efficiencies for these compounds could not be measured.

The efficiency of the wash procedure for these compounds will be explained hereafter.

Evaluation of validation data

Representative chromatograms obtained following the extraction of drug-free hair, drug-fortified hair, and drug-user hair are shown in Fig. 3. All the analytes are well separated chromatographically after derivatization. Comparison of the SIM GC/MS chromatograms (the sums of the signal intensities of the monitored ions in SIM mode) for the blank hair sample and for a hair sample spiked with analytes and internal standard showed that no chemical interferences were observed from the hair matrix. No additional peaks due to phenylalkylamine derivatives, which might have interfered with the detection of the analytes were observed.

The linearity of the method was checked in the concentration range of 0.02–25.0 ng/mg for AP, PT, Khat, FFA, DSEL, MDMA and 2CB; 0.05–25.0 ng/mg for MA, MCAT and MES; 0.05–12.5 ng/mg for MDA; and 0.1–25.0 ng/mg for MDEA and NKT. Using 20-mg samples of drug-free hair spiked with methanolic solutions of each compound, six-point for MDA, MDEA and NKT; seven-point for MA, MCAT and MES; and eight-point for AP, PT, Khat, FFA. DSEL, MDMA and 2CB, calibration curves were established with three replicates at each concentration. Mean calibration curves ($n = 3$) presented the parameters of slope, intercept, and coefficient of correlation. Table 2 gives the results

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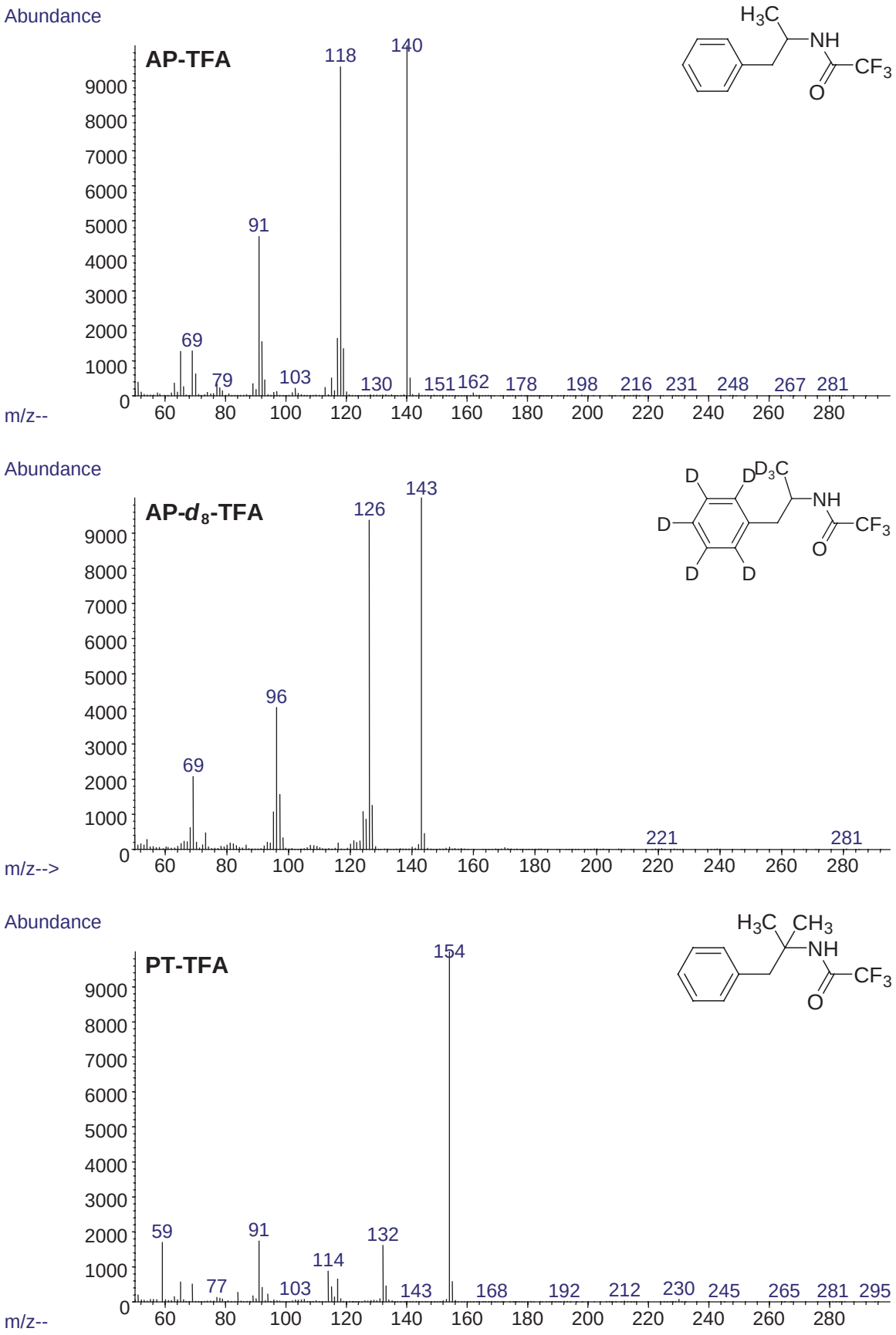

Figure 2. EI mass spectra of trifluoroacetylated (TFA) analytes and internal standards.

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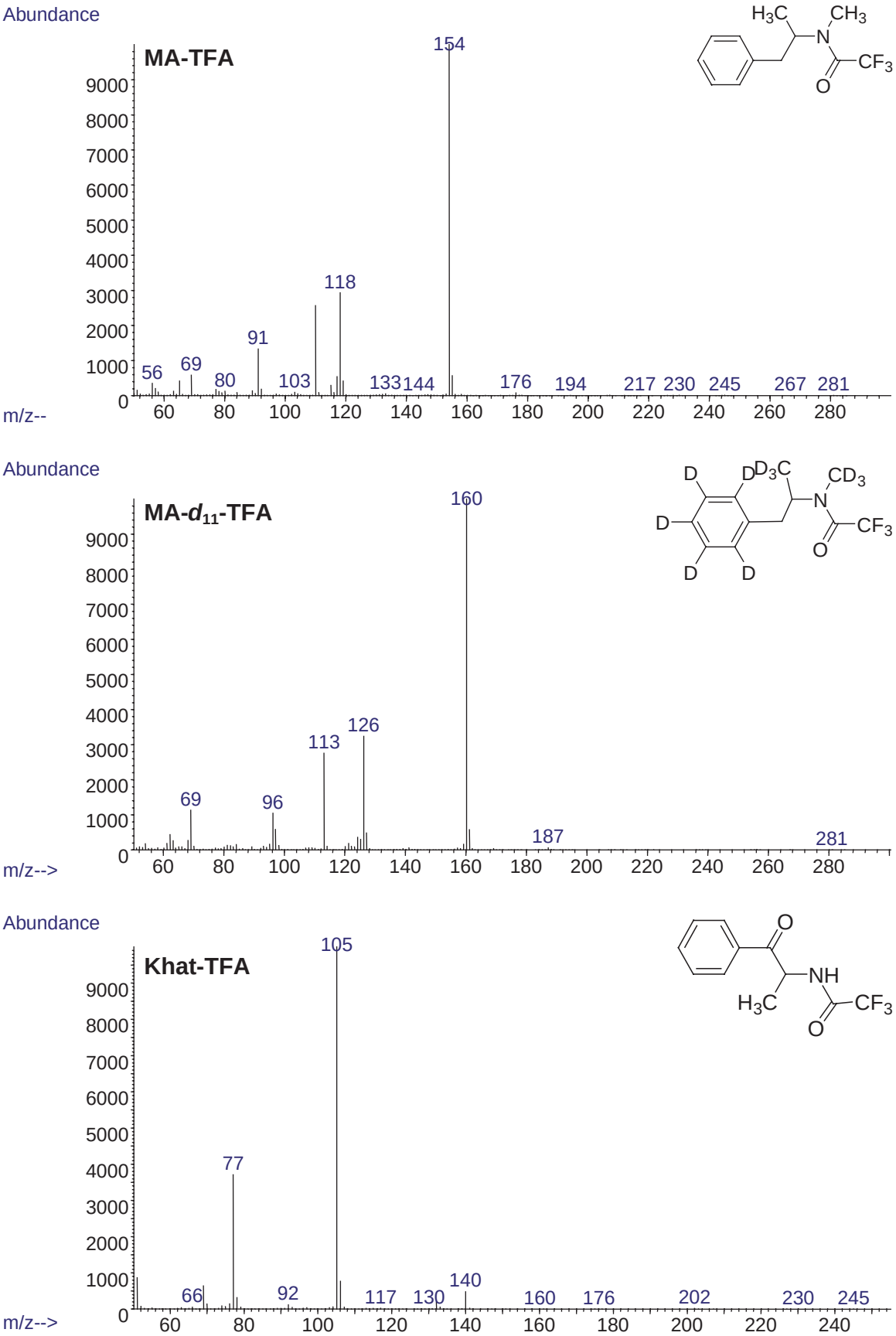

Figure 2. (Continued).

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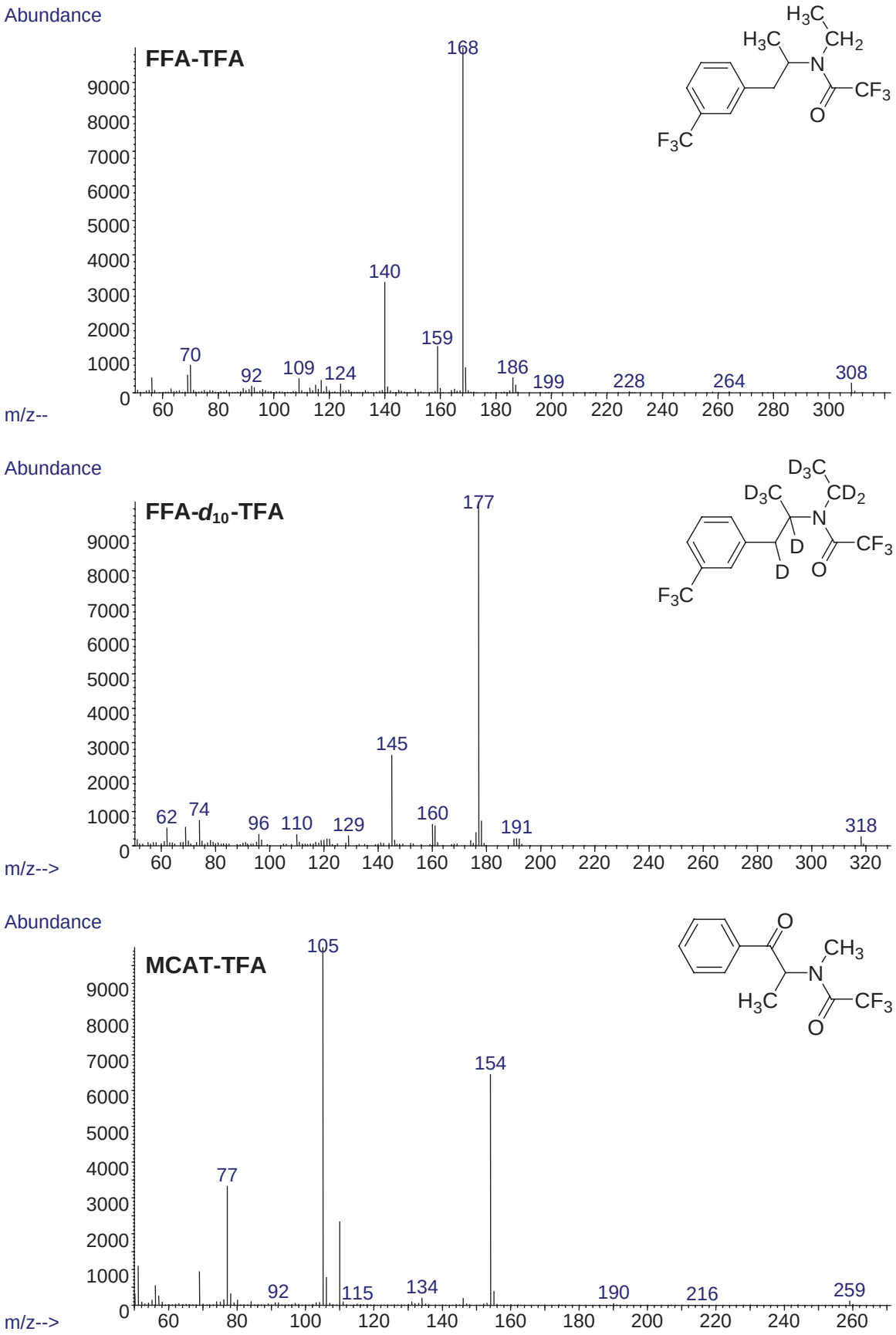

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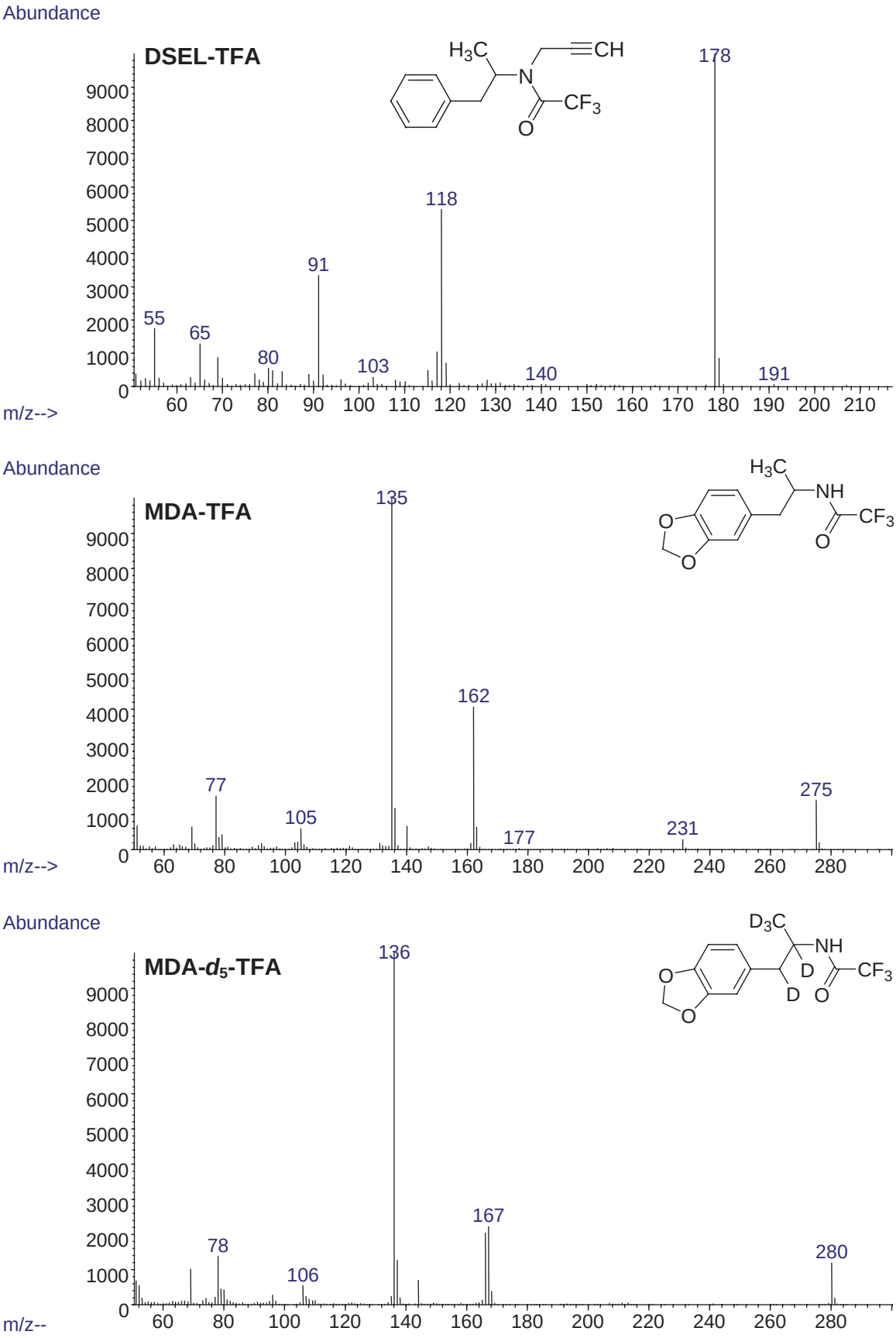

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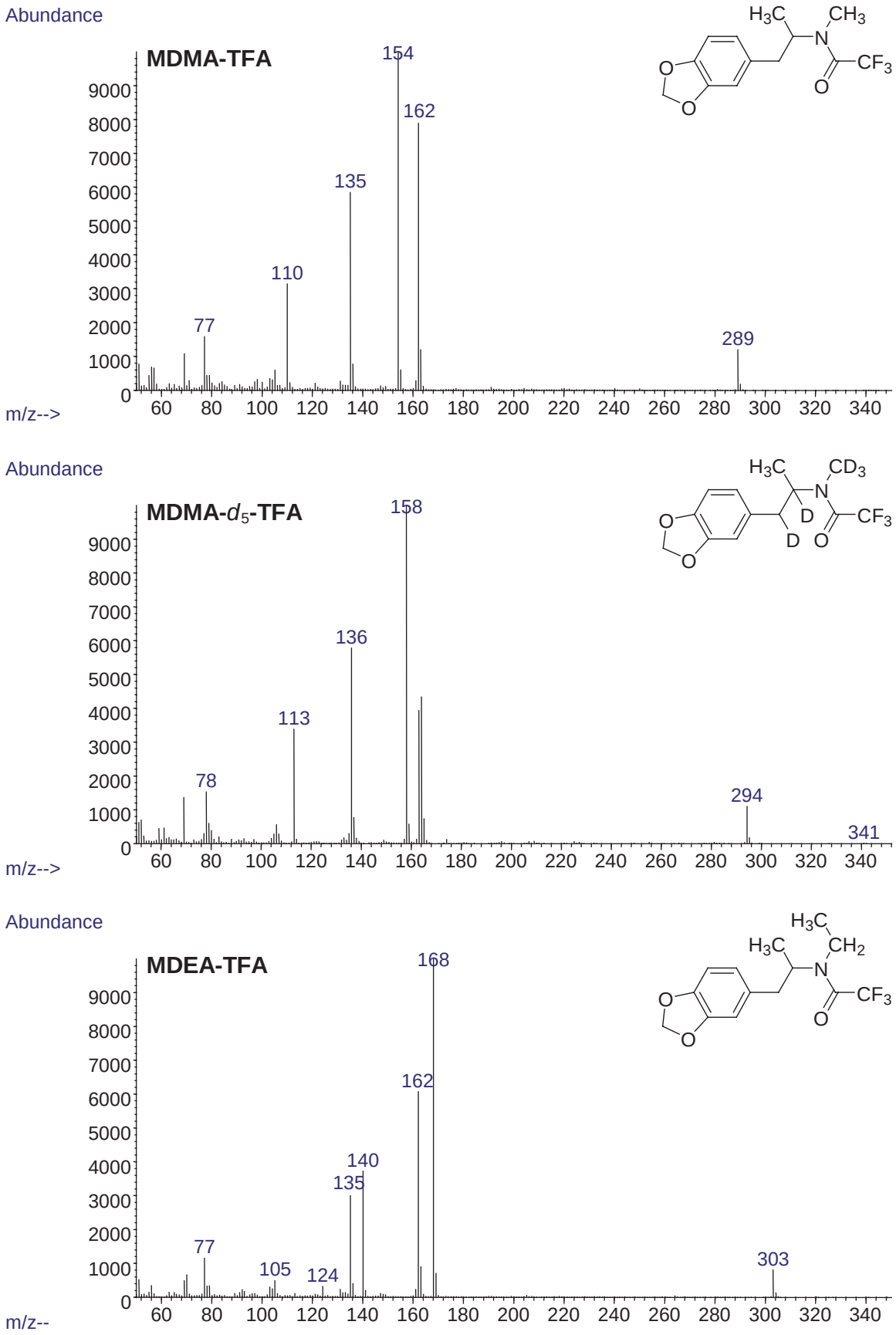

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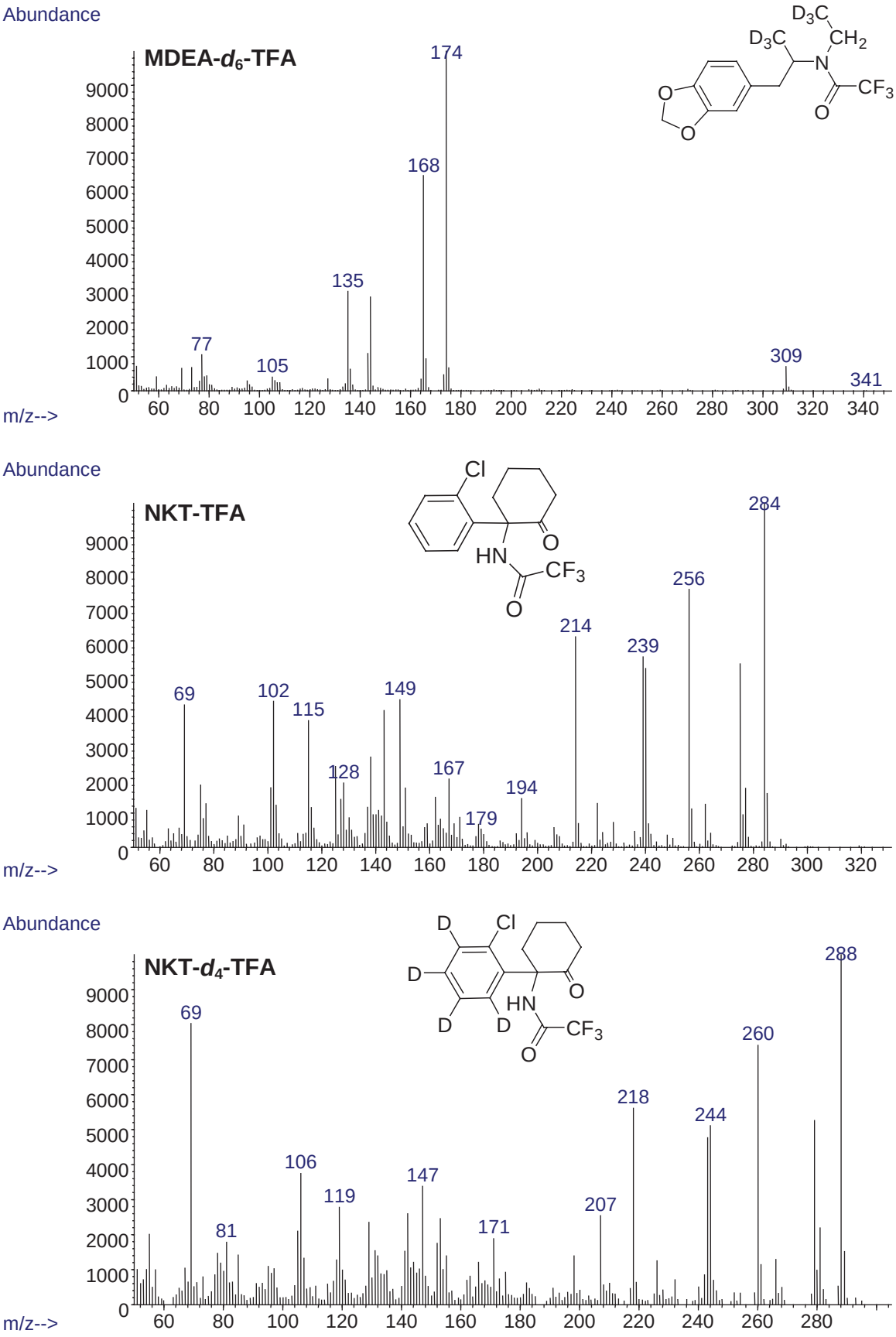

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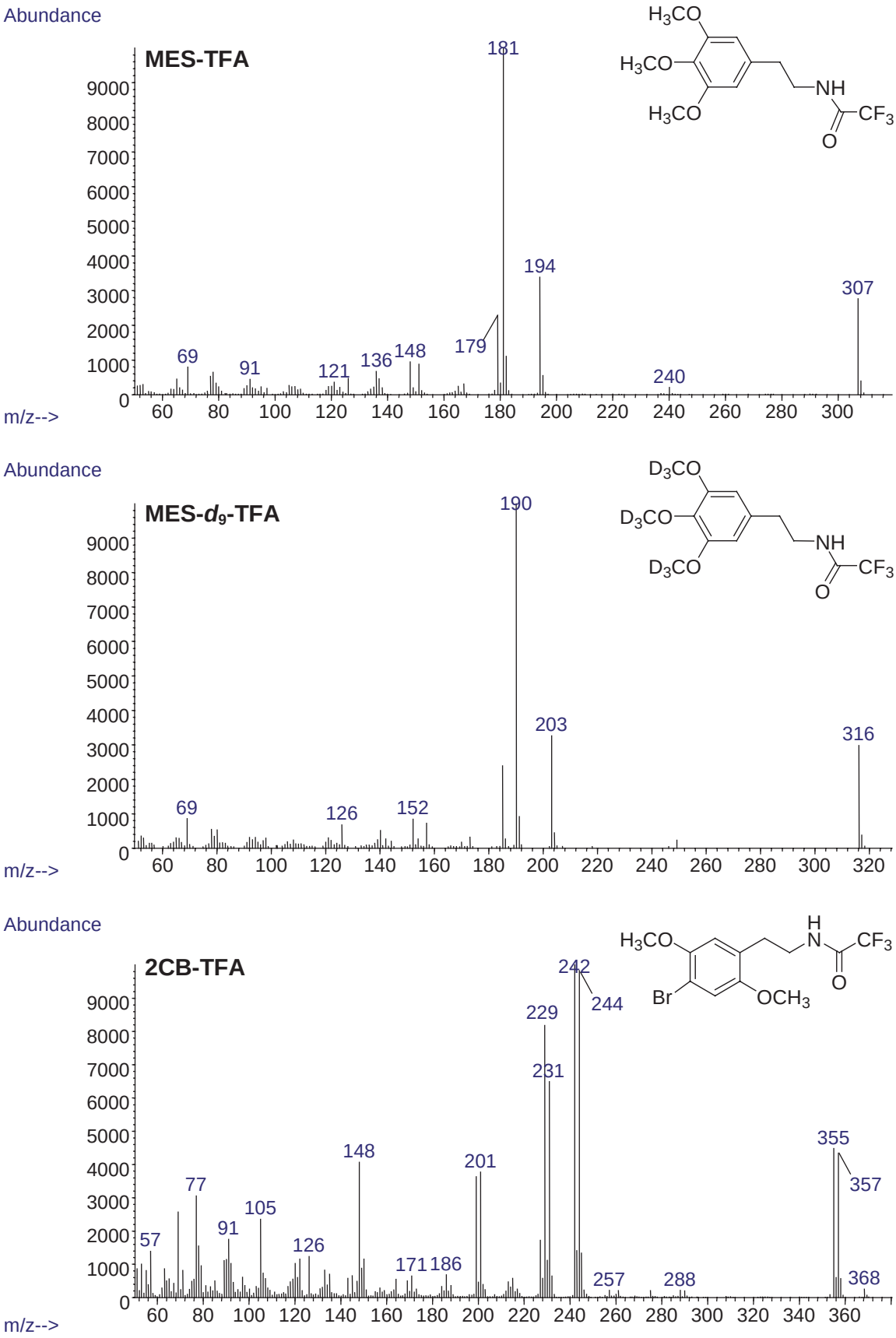

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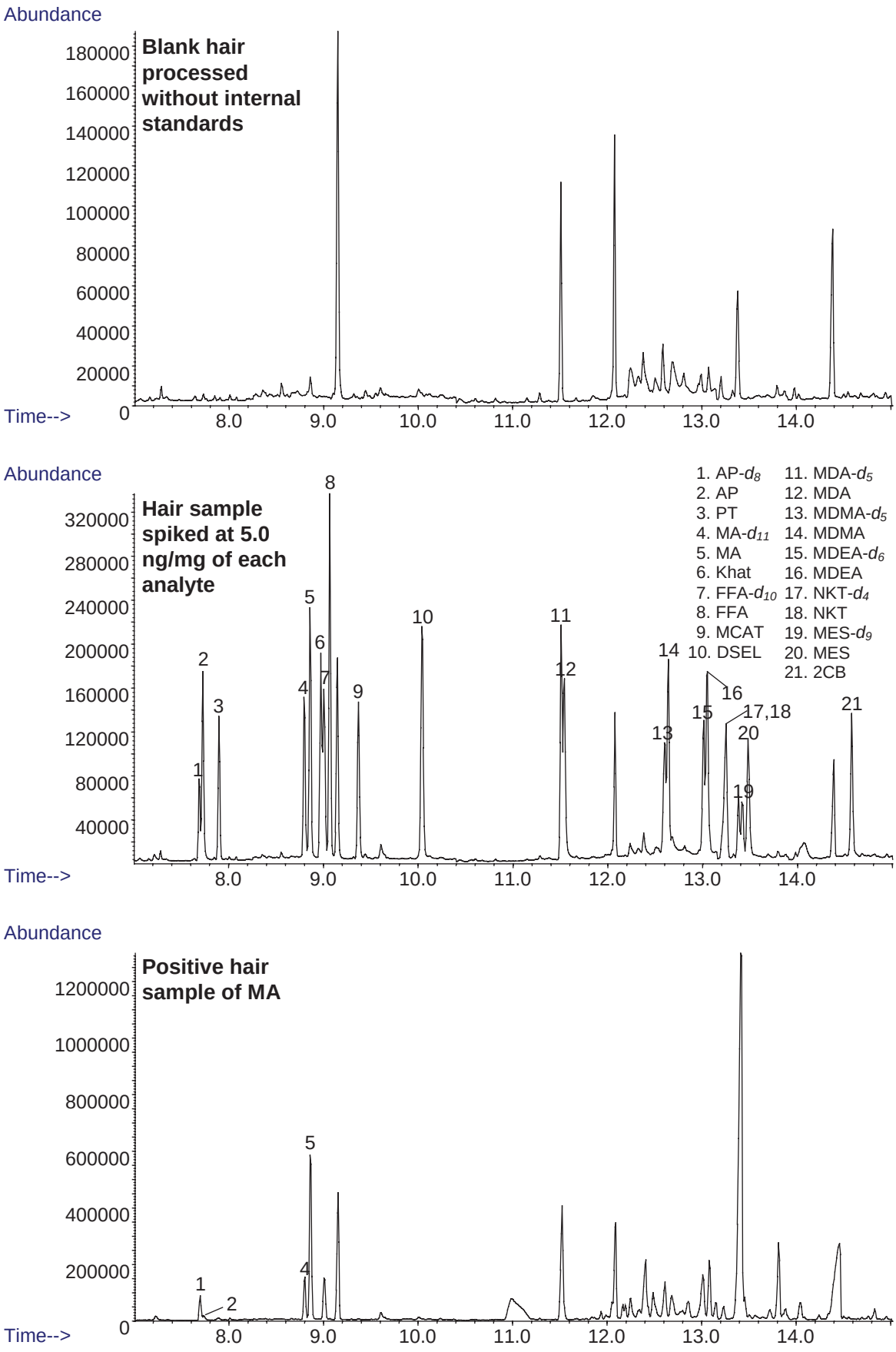

Figure 3. GC/MS chromatograms for phenylalkylamine TFA derivatives including drug-free hair, drug-fortified hair at 5.0 ng/mg of each analyte, and drug-user hair samples.

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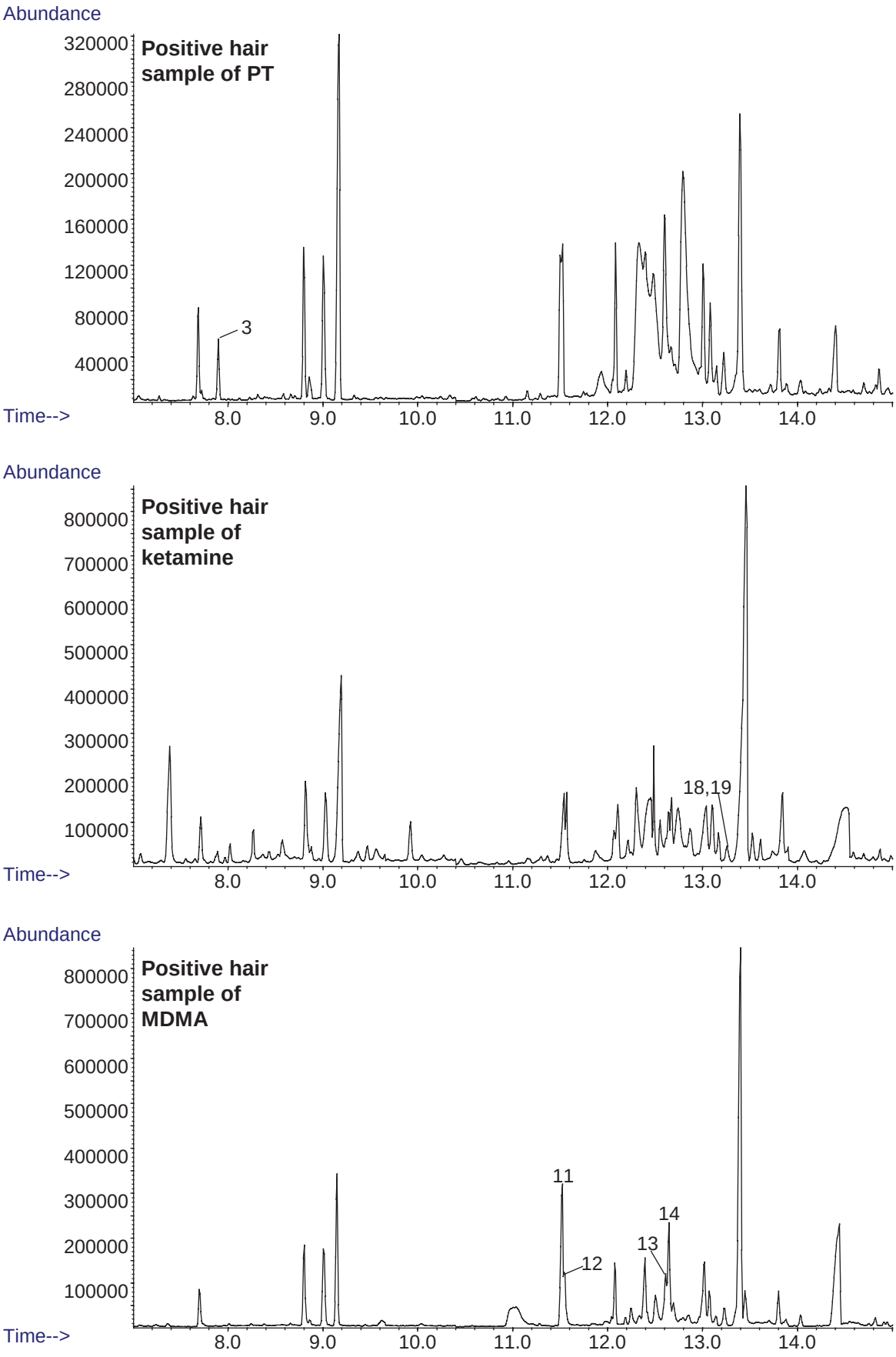

Figure 3. (Continued).

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Table 2. Method calibration

Analyte	Internal standard	Concentration range (ng/mg)	Slope	y-intercept	Linearity[a] (r^2)	LOD[b] (ng/mg)	LOQ[c] (ng/mg)
AP	AP-d_8	0.02–25.0	0.2473	0.0026	0.9991	0.002	0.01
PT	AP-d_8	0.02–25.0	0.1692	0.0021	0.9998	0.002	0.01
MA	MA-d_{11}	0.05–25.0	0.2206	0.0117	0.9998	0.007	0.03
Khat	MA-d_{11}	0.02–25.0	0.2653	0.0023	0.9998	0.003	0.02
FFA	FFA-d_{10}	0.02–25.0	0.2807	0.0043	0.9999	0.005	0.02
MCAT	FFA-d_{10}	0.05–25.0	0.1592	0.0044	0.9997	0.011	0.04
DSEL	FFA-d_{10}	0.02–25.0	0.1973	−0.0052	0.9998	0.006	0.02
MDA	MDA-d_5	0.05–12.5	0.0546	0.0140	0.9985	0.007	0.03
MDMA	MDMA-d_5	0.02–25.0	0.1750	−0.0102	0.9996	0.006	0.02
MDEA	MDEA-d_6	0.10–25.0	0.1163	0.0008	0.9997	0.024	0.08
NKT	NKT-d_4	0.10–25.0	0.2580	−0.0025	0.9994	0.021	0.08
MES	MES-d_9	0.05–25.0	0.2823	0.0005	0.9996	0.009	0.04
2CB	MES-d_9	0.02–25.0	0.0840	−0.0014	0.9993	0.004	0.02

[a] Linearity is described by the correlation coefficient for the calibration curve.
[b] Limit of detection (LOD).
[c] Limit of quantification (LOQ) were based on the concentration corresponding to a signal plus 3 and 10 standard deviations from the mean of six replicates of drug-free hair, respectively.

obtained in the calibration curves. Correlation coefficients were >0.9985 for all thirteen analytes, indicating good linear regression.

The sensitivity of the method was evaluated by determining the LOD and the LOQ for each analyte. The LOD and LOQ of the analytical method – defined as the concentration, respectively, giving a signal plus 3 and 10 standard deviations from the mean of six replicates of drug-free hair – were, respectively, determined to be in the range of 0.002–0.024 and 0.01–0.08 ng/mg for each compound.

The extraction recoveries were determined at three concentration levels in replicates of five. The peak-area ratios for each analyte were compared between those for the samples that had been spiked with analytes prior to extraction and those for the samples to which the same levels of analytes were added after extraction. The recoveries of the analytes were 76.7–95.6% (Table 3).

To evaluate the potential carryover, the highest extracted calibrator was injected into the GC/MS instrument, followed by an ethyl acetate blank to see if the results of solvent blank injections were affected; no carryover effect was detected. Subsequently, ethyl acetate blanks were used throughout the sample sequence to verify that no sample-to-sample contamination occurred.

Table 4 presents the summarized quantitative validation parameters. Intra-day, inter-day, and inter-person precision and accuracy were obtained by analyzing six replicates of three different spiked hair samples (0.3, 6.0, and 20.0 ng/mg) according to the procedure described. The intra-day, inter-day, and inter-person precisions were within 12.7%, 14.8%, and 16.8%, while the intra-day, inter-day, and inter-person accuracies were between −10.7 and 13.4%, −12.7 and 11.6%, and −15.3 and 11.9%, respectively. Considering the criteria for validation and acceptance, we regard these results as satisfactory.[33,34]

Application to hair of suspected drug abusers

In order to demonstrate the applicability on real samples, the method has been used to analyze hair samples obtained from suspected illicit drug abusers. Figure 3 shows the representative chromatograms of drug-free hair, drug-fortified hair, and drug-user hair samples of PT, MA, MDMA and ketamine. A total of 141 samples obtained during the 2 months were analyzed and quantified. The results are

Table 3. Analytical recoveries (% mean ± SD[a], $n = 5$)

	Concentrations (ng/mg)		
Analyte	0.3	6.0	20.0
AP	90.3 ± 3.1	88.1 ± 2.7	87.4 ± 5.6
PT	92.7 ± 3.3	92.6 ± 1.4	88.0 ± 6.9
MA	90.9 ± 3.5	87.0 ± 3.5	86.4 ± 4.5
Khat	85.4 ± 2.4	76.7 ± 2.6	85.8 ± 6.2
MCAT	89.4 ± 1.6	78.3 ± 3.0	86.0 ± 5.8
FFA	88.6 ± 1.1	87.3 ± 2.1	85.8 ± 5.4
DSEL	88.6 ± 1.3	82.6 ± 2.0	83.6 ± 6.8
MDA	88.5 ± 0.2	86.0 ± 3.1	—
MDMA	87.7 ± 1.0	86.1 ± 2.5	82.1 ± 7.7
MDEA	95.6 ± 2.2	91.5 ± 2.2	83.1 ± 8.6
NKT	87.7 ± 2.9	85.4 ± 1.9	82.3 ± 8.2
MES	90.8 ± 2.9	87.2 ± 3.4	81.1 ± 8.4
2CB	86.9 ± 3.1	86.2 ± 2.7	80.6 ± 8.4

[a] Standard deviation.

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Table 4. Intra-day, inter-day, inter-person precision and accuracy

Analyte	Nominal concentration (ng/mg)	Intra-day ($n=3$)		Inter-day ($n=3$)		Inter-person ($n=3$)	
		Precision[a] (% CV)	Accuracy[b] (% bias)	Precision (% CV)	Accuracy (% bias)	Precision (% CV)	Accuracy (% bias)
AP	0.3	2.6	6.3	0.7	4.1	2.2	7.0
	6.0	1.6	−2.8	2.9	0.4	2.4	−2.8
	20.0	2.7	0.0	2.3	1.3	2.9	-0.5
PT	0.3	5.3	12.3	3.9	7.8	5.9	10.8
	6.0	5.7	2.8	3.6	6.1	5.5	5.1
	20.0	6.7	−8.8	4.2	0.1	4.2	2.0
MA	0.3	0.7	1.1	3.0	1.3	1.0	3.2
	6.0	3.4	1.6	3.2	1.8	2.8	0.9
	20.0	0.8	1.1	4.1	−0.9	3.7	2.1
Khat	0.3	9.2	1.4	4.5	3.7	7.1	8.6
	6.0	12.7	1.0	8.5	4.2	3.5	−3.4
	20.0	5.7	−1.2	2.2	2.0	4.1	−1.0
FFA	0.3	6.0	6.2	8.1	4.4	1.3	4.1
	6.0	2.1	−1.1	2.5	1.3	0.6	0.5
	20.0	1.4	0.6	2.8	−0.1	4.6	2.3
MCAT	0.3	1.1	13.4	6.3	6.3	3.8	6.7
	6.0	10.1	7.4	11.6	5.7	2.7	−2.1
	20.0	1.9	3.2	2.4	0.8	1.8	−0.3
DSEL	0.3	10.3	4.6	4.5	1.1	3.0	1.8
	6.0	9.4	6.1	1.3	−0.8	2.4	−2.7
	20.0	2.5	3.4	5.4	−0.6	3.8	−2.6
MDA	0.3	7.1	−10.7	9.3	−12.7	2.2	−15.3
	6.0	2.3	5.9	2.2	9.1	5.0	6.5
MDMA	0.3	2.9	13.4	14.8	7.3	13.9	−7.1
	6.0	4.3	−5.1	5.8	−1.4	5.5	−4.6
	20.0	3.0	0.9	0.3	3.6	5.4	−2.6
MDEA	0.3	4.0	1.6	5.9	1.0	4.5	−0.7
	6.0	2.1	−4.1	4.7	−1.3	2.0	−2.3
	20.0	1.5	−0.7	1.5	−0.1	2.1	−0.2
NKT	0.3	2.1	11.2	3.0	5.4	7.0	11.1
	6.0	5.7	−4.1	4.6	−4.9	0.9	−1.7
	20.0	0.8	1.9	8.7	−0.1	3.1	3.6
MES	0.3	5.2	10.7	5.6	11.6	6.6	11.9
	6.0	4.7	0.4	2.8	−4.0	3.7	−3.4
	20.0	1.8	2.3	2.3	0.3	3.5	−0.2
2CB	0.3	9.1	8.0	9.4	6.4	16.8	3.2
	6.0	7.1	6.8	4.1	0.1	4.1	3.8
	20.0	4.2	−2.8	1.8	−1.6	7.2	3.9

[a] Expressed as the coefficient of variance of the peak area ratios of analyte/internal standard.
[b] Calculated as [(mean calculated concentration–nominal concentration)/nominal concentration] × 100.

reported as the number of analyzed samples that tested positive for MA, AP, PT, MDMA, MDA and NKT (Table 5). MA was the most frequently detected compound both alone and in association with its major metabolite AP. Other drugs including PT, MDMA, MDA and NKT were also detected. PT or MDMA use could be characterized by a relatively high frequency of polyconsumption.

The described methodology allows the detection and quantification of the mentioned phenylalkylamine derivatives simultaneously in hair samples. The simultaneous detection and quantification of several kinds of abused drugs can be a considerable advantage as toxicologists are frequently confronted with limitations in the size of the sample, and also because of multiple drug use.

Table 5. Concentration of analytes in positive hair samples

Analyte	Analyzed samples	Positive samples	Range (ng/mg)	Mean (ng/mg)
MA	141	79	0.05–24.02	6.12
AP	141	67	0.04–6.89	0.87
PT	141	2 (2)[a]	0.13–1.92	1.03
MDMA	141	2 (1)[b]	0.05–1.38	0.36
MDA	141	1 (1)[b]	0.09	—
NKT	141	1	0.34	—

Polyconsumption cases of [a](MA + AP + PT) and [b](MA + AP + MDMA + MDA) are indicated in parentheses.

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CONCLUSIONS

This study describes a sensitive and reliable GC/MS method for the simultaneous determination of thirteen psychotropic phenylalkylamine derivatives in human hair. The direct extraction method using 0.25 M methanolic HCl solution was coupled with TFA derivatization to qualify and quantify thirteen phenylalkylamine derivatives in human hair without significant interferences from matrix components. Improved extraction efficiency was obtained using 0.25 M methanolic HCl as a digestion and extraction solution compared with previous extraction methods. The proposed method has been validated and applied to the effective analysis of hair samples collected from drug abusers.

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