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Original scientific paper

Determination of dimethoxyphenethylamine derivatives in urine by deuterium labeled internal standards

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Abstract: The use of gas chromatography–mass spectrometry (GC–MS) in forensic analysis is increasing. To exploit fully the capabilities of MS, labeled standards, that can be used to improve the performance of the quantitative analysis, and to increase accuracy and precision, are required. A series of deuterated internal standards, corresponding to the 2C-series of phenethylamine derivatives, including 4-bromo-2,5-dimethoxyphenethylamine-d_6 (2C-B), 4-chloro-2,5-dimethoxyphenethylamine-d_6 (2C-C), 4-iodo-2,5-dimethoxy-phenethylamine-d_6 (2C-I), 4-ethylthio-2,5-dimethoxy-phenethylamine-d_6 (2C-T-2) and 2,5-dimethoxy-4-*n*-propylthiophenethylamine-d_6 (2C-T-7), were synthesized. These deuterated compounds were used to analyze for the corresponding unlabeled compounds in urine. The analysis was performed using GC–MS, with the selected ion monitoring (SIM) technique, whereby good results were achieved.

Keywords: phenethylamine; designer drugs; 2C-C; 2C-B; 2C-I; 2C-T-2; 2C-T-7.

INTRODUCTION

The increased availability of the 2C-series of phenethylamine derivatives on the illicit market has become a serious social problem.[1] Shulgin *et al.,* in their publication, *Phenethylamines I have Known and Loved* (PiHKAL), documented 179 phenethylamine derivatives, including 3,4-methylenedioxymethamphetamine (MDMA), mescaline, 2C-B, 2C-C, 2C-I, 2C-T-2 and 2C-T-7. They also described relevant synthetic procedures.[2] Phenethylamine derivatives are increasingly abused psychoactive drugs, the abuse of which is well documented.[3–8] The series of homologous designer drugs continues to be explored and their widespread consumption has led to increasing number of reports of abuse and intoxication. The abuse of psychoactive drugs from the phenylethylamine and phenylisopropylamine groups has become a very serious social problem in Taiwan over the last decade.[9–22]

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Unknown drugs are typically detected and identified by gas chromatography–mass spectrometry (GC–MS) because this approach is highly sensitive and can separate organic compounds in complex mixtures.[23–26] The compounds are often derivatized on the amine to yield more specific fragmentation information. This process seriously influences the ability to detect novel amphetamine controlled substance analogs.[27,28] Many studies have addressed the preparation of deuterium-labeled control drugs as internal standards for GC–MS analysis.[29–39] The synthetic routes to 2C-C-d_6, 2C-B-d_6, 2C-I-d_6, 2C-T-2-d_6 and 2C-T-7-d_6 have been described elsewhere.[31] The present investigation explores the applications of 2C-C-d_6, 2C-B-d_6, 2C-I-d_6, 2C-T-2-d_6 and 2C-T-7-d_6 as internal standards.

EXPERIMENTAL

Reagents

Methanol and ethyl acetate (EA) were purchased from Mallinckrodt (Paris, KY, USA). Trifluoroacetic anhydride was purchased from Fluka (Buchs, Switzerland). Stock solutions of the analytes (100 μg mL^{-1}) were prepared in methanol. Subsequent working solutions of calibration samples were prepared by diluting the stock solutions with blank urine. An internal standard (IS) solution of 2C-C-d_6, 2C-B-d_6, 2C-I-d_6, 2C-T-2-d_6 and 2C-T-7-d_6, each at 20 μg mL^{-1}, was prepared in methanol. The preparation of 2C-B-d_6, 2C-C-d_6, 2C-I-d_6, 2C-T-2-d_6 and 2C--T-7-d_6 has been described elsewhere.[31] The structures of 2C-B-d_6, 2C-C-d_6, 2C-I-d_6, 2C-T--2-d_6 and 2C-T-7-d_6.are presented in Fig. 1.

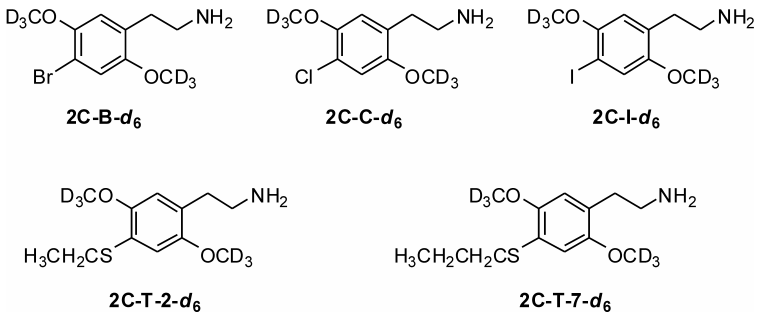


Fig. 1. The structures of 2C-B-d_6, 2C-C-d_6, 2C-I-d_6, 2C-T-2-d_6 and 2C-T-7-d_6.

Procedure

Blank urine samples, which had been collected from volunteer laboratory personnel, were used for the development of the method. Blank urine samples were spiked with appropriate amounts of analytes at concentrations of 0, 50, 100, 500, 1000 and 2000 ng mL^{-1} to prepare calibration curves. Samples were maintained in the refrigerator at 4 °C until analysis.

Instrumentation

A Hewlett Packard 6890 gas chromatograph was coupled to a Hewlett Packard 5973 quadrupole mass spectrometer under EI conditions. Injection was performed in the splitless mode. The flow rate of the carrier gas (He) was 0.60 mL min^{-1}. An HP-5MS column (12.5 m×0.20 mm ID, 0.33 μm film thickness; Agilent Technologies, Palo Alto, CA, USA) was used. The injection port temperature was maintained at 250 °C. The GC oven temperature program started at 70 °C, which was maintained for 0.5 min, and then increased at 30 °C min^{-1} to 255 °C,

which was maintained for 0.5 min. One μL was injected for GC–MS analysis in the full scan monitoring mode. The total analysis time was 12 min per sample with a solvent delay of 3.0 min. The transfer line temperature and MS source temperature were 280 and 230 °C, respectively. The electron energy of the MS was set to 70 eV. Full scan mass spectra of analytes and their deuterium analogs were collected in the *m*/*z* range 50–450 at a scan rate of 3.62 scan/s. The data were collected using Hewlett-Packard ChemStation software.

Sample preparation

To a clean 12–mL screw-cap glass tube was added 2.0 mL of urine sample and 50 μL of IS solution. The mixture was alkalinized with 2.0 mL of 1.0 M NaOH, and extracted with 3.0 mL of EA after vortexing and subsequent centrifugation at 3000 rpm for 5 min. The organic layer was carefully transferred to a clean tube. The mixture was evaporated to dryness under a stream of nitrogen gas at 50–60 °C. The dried extract was dissolved in 50 μL of EA and derivatized with 50 μL of trifluoroacetic anhydride for 30 min at 60 °C. The samples were then cooled to room temperature, evaporated to dryness, and reconstituted with 50 μL of EA. One μL was injected for GC–MS analysis.

RESULTS AND DISCUSSION

The total ion current chromatogram (monitored in the full scan mode) of five phenethylamine designer drugs and four structurally related sympathomimetic amines [amphetamine (A, AMP), methamphetamine (MA), 3,4-methylenedioxyamphetamine (MDA), and MDMA] are presented in Fig. 2. All compounds were chromatographically well separated. The retention times of the drugs and their deuterated analogues are given in Table I. Although the retention times according to the GCs of five sets of labeled and unlabeled compounds vary very little (0.01 min), the selected ion monitoring (SIM) technique discriminates the labeled and unlabeled compounds. Therefore, these deuterium-labeled compounds have the potential to be used as internal standards in GC–MS analysis. The $[M]^+$ of the labeled and unlabeled compounds did not overlap each other and no interference from the urine samples was observed. Accordingly, the 14 $[M]^+$ were monitored using GC–MS with a SIM. The SIM chromatogram was obtained from 2.0 mL of urine sample with 1.0 μg of A, MA, MDA, MDMA and IS. Although A, MA, MDA and MDMA were not the standard samples in this study, generally these compounds were analogous to the IS samples, and could be distinguished in the GC–MS chromatogram.

The electron impact mass spectra of 2C-B-TFA, 2C-B-d_6-TFA, 2C-C-TFA, 2C-C-d_6-TFA, 2C-I-TFA, 2C-I-d_6-TFA, 2C-T-2-TFA, 2C-T-2-d_6-TFA, 2C-T-7--TFA and 2C-T-7-d_6 are presented in Figs. 3–7. Very high $[M]^+$ peaks were observed for 2C-B-TFA, 2C-C-TFA, 2C-I-TFA, 2C-T-2-TFA and 2C-T-7-TFA at *m*/*z* 355, 311, 403, 337 and 351, respectively. For 2C-B-d_6-TFA, 2C-C-d_6-TFA, 2C-I-d_6-TFA, 2C-T-2-d_6-TFA and 2C-T-7-d_6-TFA, very strong $[M]^+$ peaks appeared at *m*/*z* 361, 317, 409, 343 and 357, respectively.

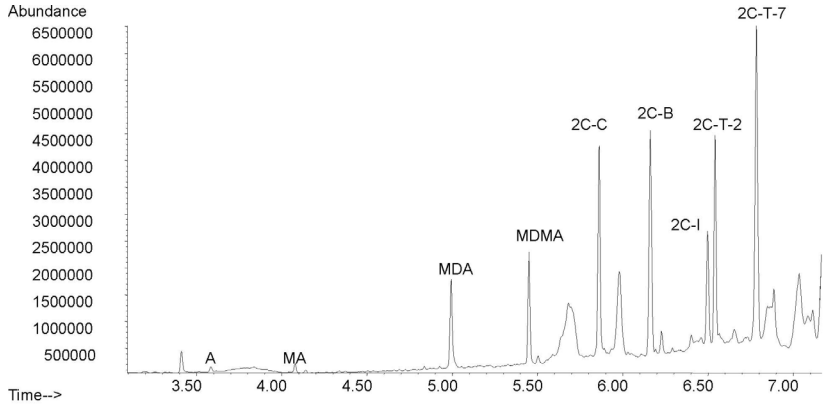


Fig. 2. Total ion chromatography (time in min). The concentrations of the analytes were 0.50 μg mL^{-1}.

TABLE I. Retention times and ions monitored for GC/MS analysis

Compound	Retention time, min	Ions monitored[a] (relative intensity, %)
2C-C-d_6	5.98	204, 191 (69.4), 317 (67.8)
2C-C	5.99	198, 185 (78.7), 311 (74.2)
2C-B-d_6	6.26	248, 361 (76.3), 235 (61.2)
2C-B	6.27	242, 355 (75.6), 229 (77.7)
2C-I-d_6	6.61	409, 296 (80.9), 283 (50.8)
2C-I	6.62	403, 290 (85.6), 277 (53.1)
2C-T-2-d_6	6.66	217, 343 (82.6), 230 (26.5)
2C-T-2	6.67	211, 337 (74.1), 224 (23.8)
2C-T-7-d_6	6.93	231, 357 (78.1), 244 (23.5)
2C-T-7	6.94	225, 351 (89.6), 238 (23.9)
AMP	3.64	140, 118 (91.9), 91 (40.6)
MA	4.14	154, 118 (29.9), 110 (22.7)
MDA	5.05	162, 275 (49.0), 135 (234.4)
MDMA	5.51	154, 162 (78.4), 135 (56.1)

[a]Quantification ions underlined

The EI mass spectrum of 2C-B-d_6-TFA (Fig. 3) has a base peak ion at *m*/*z* 248. This odd electron ion was formed by a McLafferty rearrangement to eliminate trifluoroacetamide (Scheme 1). The ion *m*/*z* 235 was also generated by eliminating an *N*-ethylidene-2,2,2-trifluoroacetamide from the molecular ion *m*/*z* 361. The ion *m*/*z* 203 was formed from the ion *m*/*z* 235 by the well-known specific six-center H-rearrangement of a *γ*-D-atom of the methoxy-d_3 (OCD_3) side chain to the benzylic part, eliminating neutral formaldehyde-d_2 (CD_2O). This rearrangement was proven by comparing the mass spectrum of 2C-B-TFA and 2C-B-d_6--TFA (Fig. 3), *i.e.*, the ion *m*/*z* 199 corresponds to ion *m*/*z* 203 in 2C-B-d_6-TFA and the ion *m*/*z* 203 has four deuterium atoms. 2C-B-d_6-TFA contains a bromine and thus the ions *m*/*z* 361, 248, 235 and 203 always have corresponding isotopic ions *m*/*z* 363, 251, 237 and 205. The ion *m*/*z* 151 was generated by eliminating a

bromomethane ($BrCD_3$) from the molecular ion *m*/*z* 248. Compounds 2C-B, 2C-C, 2C-I, 2C-C-d_6 and 2C-I-d_6 have the same fragmentation pathway, and their corresponding mass spectra are shown in Fig. 3–5.

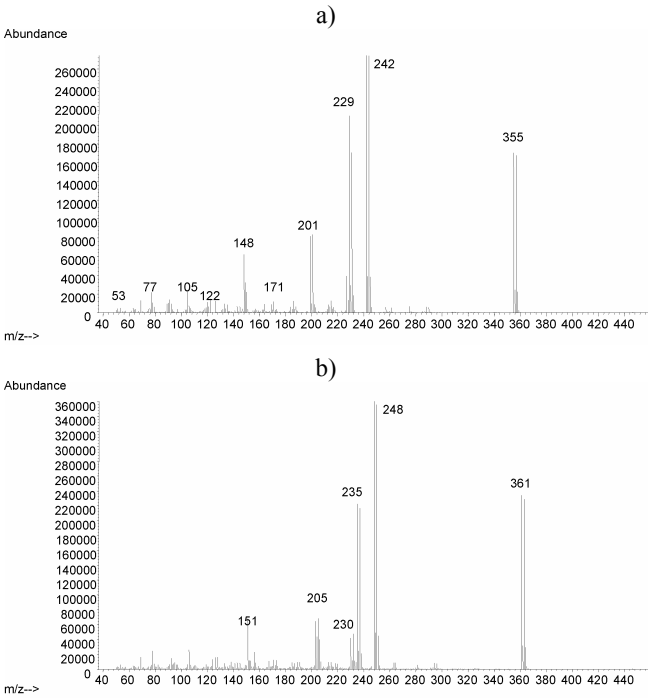
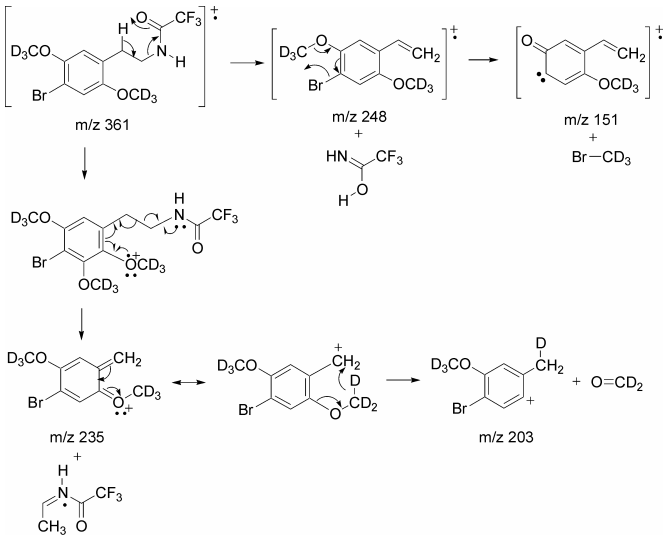


Fig. 3. The mass spectra of TFA derivatives of 2C-B (a) and 2C-B-d_6 (b).



Scheme 1. Spectral interpretation of *N*-[2-(4-bromo-2,5-dimethoxyphenyl)ethyl]--2,2,2-trifluoroacetamide-d_6 (2C-B-d_6-TFA).

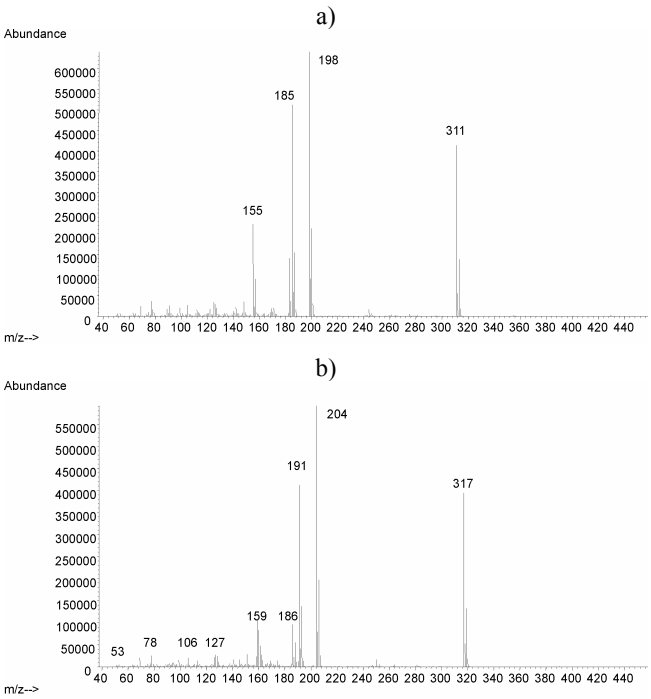


Fig. 4. The mass spectra of TFA derivatives of 2C-C (a) and 2C-C-d_6 (b).

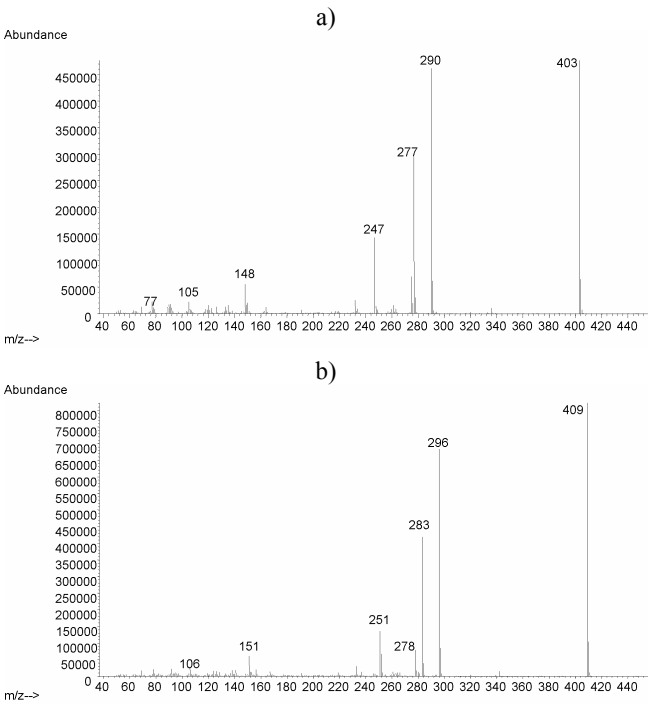


Fig. 5. The mass spectra of TFA derivatives of 2C-I (a) and 2C-I-d_6 (b).

The EI mass spectrum of 2C-T-2-d_6-TFA (Fig. 6) has a base peak ion at *m*/*z* 217. The ion *m*/*z* 217 was generated by eliminating *N*-ethylidene-2,2,2-trifluoroacetamide from the molecular ion *m*/*z* 343. This odd electron ion *m*/*z* 230 was formed by a McLafferty rearrangement to eliminate a trifluoroacetamide (Scheme 2). The ion *m*/*z* 185 was formed from the ion *m*/*z* 217 by the well-known specific six-center H-rearrangement of a γ-D-atom of the methoxy-d_3 (OCD_3) side chain to the benzylic part, eliminating neutral formaldehyde-d_2 (CD_2O). This rearrangement was proven by comparing the mass spectrum of 2C-T-2-TFA and 2C-T--2-d_6-TFA (Fig. 6), *i.e.*, the ion *m*/*z* 181 corresponds to ion *m*/*z* 185 in 2C-T-2-d_6--TFA, and ion *m*/*z* 185 has four deuterium atoms. The ion *m*/*z* 157 was generated by eliminating an ethylene (CH_2=CH_2) from the molecular ion *m*/*z* 185. Compounds 2C-T-2, 2C-T-7 and 2C-T-7-d_6 have the same fragmentation pathway and their corresponding mass spectra are shown in Figs. 6 and 7.

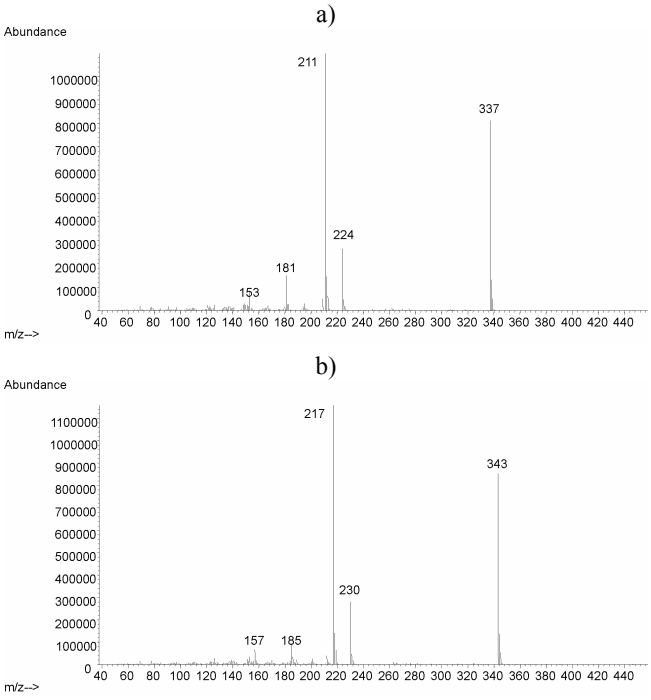
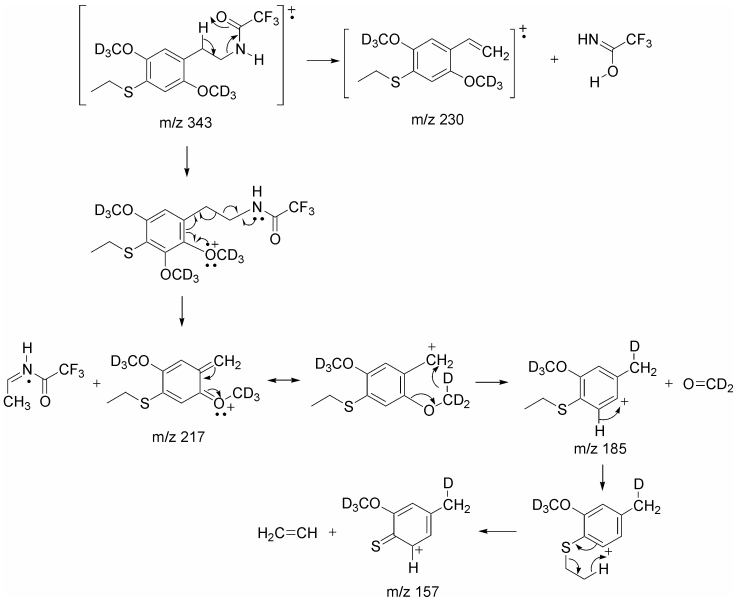


Fig. 6. The mass spectra of TFA derivatives of 2C-T-2 (a) and 2C-T-2-d_6 (b).

The extraction and derivatization were performed using ethyl acetate as solvent. Due to the need to heat for derivatization, a solvent with boiling point above 60 °C was selected. The boiling point of ethyl acetate is 77 °C. The derivatization time (30 min) and temperature (60 °C) were selected, because these derivatization conditions are well established.[16]

The linearity of the quantification method was determined using calibration standards at 0, 50, 100, 500, 1000, 2000 ng of analyte. The selected ion monitoring (SIM) mode was employed throughout the study. Linear regression of the



Scheme 2. Spectral interprettation of *N*-[2-(4-ethylsulfanyl-2,5-dimethoxyphenyl) ethyl]-2,2,2-trifluoroacetamide-d_6 (2C-2-T-*d*6-TFA).

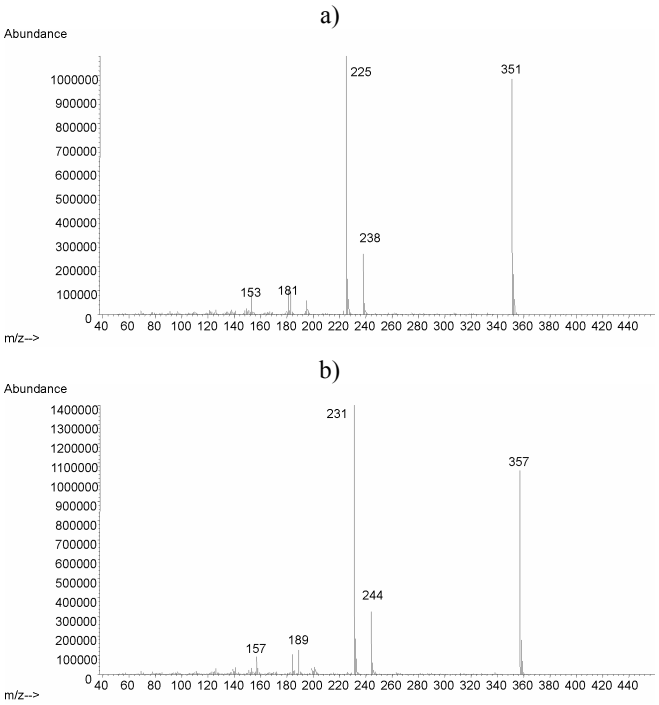


Fig. 7. The mass spectra of TFA derivatives of 2C-T-7 (a) and 2C-T-7-d_6 (b).

calibration curves gave r^2 values between 0.9946 and 0.9999, with most values > 0.9990. The limit of detection (*LOD*) was defined as the signal noise ratio, equal to 3. The *LOD* values of the target compounds are given in Table II, from which it can be seen that the calibration curves for the studied compounds gave excellent straight lines over the range of 0–2000 ng mL^{-1}. Thus, excellent accuracy and precision could be obtained by this method.

Table II. Linear regression of the calibration curves

Compound	Coefficient of correlation (r^2)	Regression line	*LOD* (*S*/*N* = 3) ng mL^{-1}
2C-C	0.9998	$y = 0.0026x - 0.0084$	5.1
2C-B	0.9946	$y = 0.0023x + 0.0259$	8.0
2C-I	0.9999	$y = 0.0015x - 0.0061$	6.7
2C-T-2	0.9991	$y = 0.0018x - 0.0128$	1.6
2C-T-7	0.9999	$y = 0.0015x - 0.0061$	2.9

CONCLUSIONS

This study demonstrates the applications of 2C-B-d_6, 2C-C-d_6, 2C-I-d_6, 2C-T-2-d_6 and 2C-T-7-d_6 as internal standards in GC–MS. Although the GC retention times of the five sets of labeled and unlabeled compounds vary very little (0.01 min), quantification by MS with the selected ion monitoring (SIM) technique enhances the performance of the quantitative analysis. Therefore, five deuterium-labeled compounds possess the potential to be used as the internal standard for GC–MS analysis.

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ИЗВОД

ОДРЕЂИВАЊЕ ДЕРИВАТА ДИМЕТОКСИФЕНЕТИЛАМИНА У УРИНУ ПРИМЕНОМ ДЕУТЕРИСАНИХ ИНТЕРНИХ СТАНДАРДА

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У форензичкој анализи примена гасне хроматографије са масеном спектрометријом (GC–MS) је све већа. Употреба обележених стандарда може побољшати перформансе квантитативне хемијске анализе, повећањем тачности и прецизности одређивања методом GC–MS. Синтетисана је серија деутерисаних интерних стандарда, која одговара 2C серији деривата фенетиламина, укључујући 4-бром-2,5-диметоксифенетиламин-d_6 (2C-B), 4-хлор-2,5-диметоксифенетиламин-d_6 (2C-C), 4-јод-2,5-диметокси-фенетиламин-d_6 (2C-I), 4-етилтио-2,5-диметокси-фенетиламин-d_6 (2C-T-2) и 2,5-диметокси-4-*n*-пропилтиофенетиламин-d_6 (2C-T-7). Деутерисана једињења су коришћена у анализи одговарајућих необележених једињења у узор-

цима урина. Анализа је изведена применом GC–MS у SIM (техника мониторинга одабраних јона) моду, при чему су добијани аналитички поуздани резултати.

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