A novel method to distinguish β-methylphenylethylamines from isomeric α-methylphenylethylamines by liquid chromatography coupled to electrospray ionization mass spectrometry

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Abstract

Purpose Various phenylethylamines have been detected lately in dietary or sports supplements. N-Methyl-2-phenylpropan-1-amine (phenpromethamine) and 2-phenylpropan-1-amine (β-methylphenylethylamine, BMPEA) are known to produce mass spectra almost identical to those produced by methamphetamine (MA) and amphetamine (AP), respectively, when analyzed by liquid chromatography/mass spectrometry (LC/MS). They may interfere with the analysis of MA and AP. The aims of the present study were to determine whether some substances other than phenpromethamine and BMPEA give mass spectra similar to those given by MA or AP and to develop an analytical method of distinguishing phenpromethamine from MA and BMPEA from AP by derivatization.

Methods Twenty isomers of MA or AP were selected to be analyzed using LC/MS. Six reagents were examined for derivatization of MA, AP, phenpromethamine, and BMPEA. Three mass spectrometers from two manufacturers were evaluated for their ability to reproduce the data.

Results All isomers except phenpromethamine and BMPEA were shown to be distinguishable from MA and AP by their mass spectra. For the discrimination of isomeric pairs, derivatization using N-succinimidyl-4-nitrophenylacetate was found to be the best for tandem mass spectrometry and that using 4-nitrobenzoyl chloride was the best for in-source collision-induced dissociation. One or more ions from each pair of isomers gave adequate difference in their relative intensities according to the World Anti-Doping Agency criteria.

Conclusions The newly developed method was proved to be usable for discriminating among those phenylethylamines.

Keywords Liquid chromatography/mass spectrometry · β-Methylphenylethylamine · Phenpromethamine · Methamphetamine · Amphetamine

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Introduction

In recent years, many phenylethylamines have been detected in dietary or sports supplements [1–4]. Some of them are isomers of methamphetamine (MA, 16 in Fig. 1) or amphetamine (AP, 15). MA is a substance of significant abuse around the world and the most prevalent illicit drug in Japan. AP is not as prevalent as MA, but is frequently detected in human urine, because it is one of the major metabolites of MA. In Japan, both are regulated by the Stimulants Control Act. As the act regulates the intake of MA and AP for recreational purposes, their detection in seized urine samples is of great importance for law enforcement. Phenylethylamines in supplements raise concern because they may interfere with MA and AP detection efforts.
other countries [1, 2]. The database of the Early Warning detected in supplements sold in the United States [3] and the Netherlands [14], and Spain [11]. BMPEA has been AP, gives a mass spectrum almost identical to that given (β-methylphenylethylamine, BMPEA, 13), an isomer of recently in supplements sold in Australia [13], 1940s as an active ingredient of Vonedrine. It has been 2-phenylpropan-1-amine (phenpromethamine, 14), and 18 other amines (16), β-methylphenylethylamine (BMPEA, 13), phenpromethamine (14), and 18 other amines.

High-resolution mass spectrometry with a time-of-flight mass spectrometry (TOF–MS) instrument has a strong ability to discriminate compounds [5], but it is not effective for discriminating isomers. Collision-induced dissociation (CID) by tandem mass spectrometry (MS/MS) provides useful information for distinguishing isomers. In-source CID with a single-quadrupole mass spectrometer is also used [6–9]. However, certain kinds of isomers are not distinguishable even with MS/MS or in-source CID. N-Methyl-2-phenylpropan-1-amine (phenpromethamine, 14) is an isomer of MA and gives a mass spectrum almost identical to that given by MA, when analyzed by liquid chromatography/mass spectrometry (LC/MS) with electrospray ionization [10, 11]. Similarly, 2-phenylpropan-1-amine (β-methylphenylethylamine, BMPEA, 13), an isomer of AP, gives a mass spectrum almost identical to that given by AP [10, 12]. Phenpromethamine was prevalent in the 1940s as an active ingredient of Vonedrine. It has been detected recently in supplements sold in Australia [13], the Netherlands [14], and Spain [11]. BMPEA has been detected in supplements sold in the United States [3] and other countries [1, 2]. The database of the Early Warning Advisory on New Psychoactive Substances administered by the United Nations Office on Drugs and Crime (UNODC) [15] also includes numerous cases of these two substances from Europe and Canada. Both substances are prohibited by the World Anti-Doping Agency (WADA) [16]. As phenpromethamine and BMPEA are not regulated in Japan and are commercially available via the internet, they may be contained in seized urine samples. MA and AP are α-methylphenylethylamines, whereas phenpromethamine and BMPEA are β-methylphenylethylamines.

These β-methylphenylethylamines are clearly distinguishable from α-methylphenylethylamines by gas chromatography/mass spectrometry (GC/MS) [10]. However, if LC/MS was the only technique adopted, misidentification may occur. Brown et al. [10] reported that these four amines gave ions of different intensities in multiple reaction monitoring (MRM) after benzoylation with pentafluorobenzyl chloride (PFB-Cl). The differences in the ratios were around 30–40%. If some other methods were developed to enhance the differences, those methods would be helpful for the analysis of MA and AP in various matrices.

The first aim of our study is to determine whether some substances other than phenpromethamine and BMPEA give mass spectra similar to those given by MA or AP. We selected 18 additional amines (Fig. 1) to be analyzed in tandem mode (LC/MS/MS) or single mode (LC/MS). The second aim is to develop an analytical method that can distinguish amines better than Brown’s method. Three mass spectrometers from two manufacturers were used to evaluate the reproducibility of the data.

Materials and methods

Amines
d-Methamphetamine (HCl salt) and d-amphetamine (H2SO4 salt) were the products of Sumitomo Dainippon Pharma (Osaka, Japan). Phentermine (21, HCl salt) was a reference material used in the laboratory of the Kinki Narcotics Control Department. The other amines were purchased from vendors as follows. N-Benzyl-N-propylamine (2), 1-phenylpropan-1-amine (7), (S)-1-phenylbutylamine (9), N-methyl-2-phenylethan-1-amine (11), N,N-dimethyl-2-phenylethan-1-amine (12), N-methyl-2-phenylpropan-1-amine (phenpromethamine), 4-phenylbutan-2-amine (19), and 4-phenyl-1-butynamine (20) were from Sigma-Aldrich (St. Louis, MO, USA); 1-phenylbutan-2-amine (17, HCl salt) and 4-methylamphetamine (22, HCl salt) were from Cayman Chemical (Ann Arbor, MI, USA); N-benzylpropan-2-amine (3), N-methyl-1-phenylethan-1-amine (5), N-ethyl-1-phenylethanamine (6), N-methyl-1-phenylpropan-1-amine (8),
2-methyl-1-phenylpropylamine (10), 3-phenylpropan-1-amine (18) and N-benzylethanamine (1) were from Fujifilm Wako Pure Chemical (Osaka, Japan); N-benzyl-N-methyleneaniline (4) was from Tokyo Chemical Industry (Tokyo, Japan); and 2-phenylpropan-1-amine (BMPEA) was from Ark Pharm (Arlington Heights, IL, USA).

Each amine was dissolved in methanol or distilled water. The units of concentration were µg/mL (w/v) for solids (salts) and nL/mL (v/v) for liquids (free amines).

**Derivatizing reagents and other chemicals**

Propionyl chloride, 4-methylvaleraloyl chloride, 4-nitrobenzoyle chloride (PNBC), N-succinimidyld-4-nitrophenylacetate (SNPA), 3,5-dinitrobenzoyl chloride (DNBC), 4-(4,5-diphenyl-1H-imidazol-2-yl) benzoyl chloride hydrochloride (DIB-Cl) were purchased from Tokyo Chemical Industry. DIB-Cl was dissolved in acetonitrile. The other derivatizing reagents were dissolved in tetrahydrofuran (THF). The units of concentration were µg/mL (w/v) for PNBC, SNPA, and DNBC, and nL/mL (v/v) for propionyl chloride and 4-methylvaleraloyl chloride. THF was organic synthesis grade, whereas NaHCO₃ and 25% NH₃ were special grade. Methanol and distilled water were HPLC grade.

**Preparation of test solutions**

*Free amines:* a methanol solution of amines (10 µg/mL or 10 nL/mL) was used for the measurements.

*Derivatized amines other than DIB derivatives:* the preparation procedure was based on the literature [17]. Fifty µL of amine in methanol (1000 µg/mL or 1000 nL/mL) was taken into a vial and dried by a gentle N₂ stream. Then 0.2 mL THF and about 2 equivalent mass of derivatizing reagent was added. The vial was kept at 60 °C for 1 h and the mixture was dried by a N₂ stream. The residue was dissolved in 1 mL acetonitrile and the solution was filtered by a membrane filter (0.20 µm). For SNPA derivatization, AP and MA salts were dissolved in water followed by the addition of 25% NH₃ and extraction into n-hexane. The n-hexane layers were dried and used for the reaction.

*DIB-derivatized amines:* the preparation procedure was based on the literature [18]. After 20 µL of amine in methanol (100 µg/mL or 100 nL/mL) was taken into a vial and dried by a gentle N₂ stream, 20 µL of NaHCO₃ buffer (10 mmol/L, pH 9.0) and 170 µL of DIB-Cl solution (0.1 mmol/L in acetonitrile) were added. The vial was left at room temperature for 10 min, after which 10 µL of 25% NH₃ was added.

**Liquid chromatography/mass spectrometry**

Three mass spectrometers were used. Two of them were quadrupole time-of-flight mass spectrometry (Q-TOF–MS) instruments and the third was a triple-quadrupole mass spectrometry (QqQ-MS) instrument. Each of them was connected to a liquid chromatograph. All mass spectrometers were operated in electrospray ionization (ESI)-positive mode. A Xevo G2-S Q-TOF (Waters, Milford, MA, USA) was used to obtain detailed data of 22 amines in their free form. A 6530 Accurate-Mass Q-TOF (Agilent Technologies, Santa Clara, CA, USA) was used to investigate the mass spectra of derivatized amines. A Xevo TQD (Waters) was also used. All mass spectrometers were used to confirm the reproducibility of the data for free and derivatized amines. Scan mode was used for all data acquisition. MRM and SIM (selected ion monitoring) were not used. Precursor ions for MS/MS were protonated molecules ([M + H]+) for all compounds. The operating conditions of the instruments were as follows.

**Xevo G2-S Q-TOF**: the LC instrument was an ACQUITY UPLC H class (Waters) equipped with an ACQUITY UPLC HSS T3 column (2.1 mm i.d. × 100 mm, 1.8 µm, Waters). The parameters for the mass spectrometer were as follows: desolvation gas: N₂ (1000 L/h, 500 °C); capillary voltage: 0.8 kV; scan range: m/z 50–600; corn voltage (CV) for LC/MS: 10, 20, 30, 40 V; CV for LC/MS/MS: 10 V; collision energy (CE) for LC/MS/MS: 5, 10, 15, 20 eV. Ion peaks of 2% or more abundance compared to the base peak in each mass spectrum were considered for the identification of compounds.

**6530 Accurate-Mass Q-TOF**: the LC instrument was a 1290 Infinity (Agilent Technologies) equipped with a ZORBAX Eclipse Plus C18 Rapid Resolution HD column (2.1 mm i.d. × 100 mm, 1.8 µm, Agilent Technologies). The parameters for the mass spectrometer were as follows: desolvation gas: N₂ (600 L/h, 300 °C); capillary voltage: 3.5 kV; scan range: m/z 50–1000; fragmentor voltage for LC/MS: 100, 130, 160, 190 V; fragmentor voltage for LC/MS/MS: 100 V; CE for LC/MS/MS: 5, 10, 15, 20 eV. Ion peaks of 1% or more abundance compared to the base peak in each mass spectrum were considered for the identification of compounds.

**Xevo TQD**: the LC instrument was an ACQUITY UPLC I class (Waters) equipped with an ACQUITY UPLC HSS T3 column (2.1 mm i.d. × 100 mm, 1.8 µm, Waters). The parameters for the mass spectrometer were: capillary voltage: 2.0 kV; scan range: m/z 50–500; CV for LC/MS: 20, 30, 40, 50 V; CV for LC/MS/MS: 30 V; CE for LC/MS/MS: 5, 10, 15, 25 eV.

The LC instrument was operated in the following conditions for all the instruments.
For free amines: mobile phase A was ammonium formate in water (5 mmol/L, pH 3) and mobile phase B was 0.1% formic acid in acetonitrile. A: B was 90:10. The flow rate was 0.4 mL/min and the column oven temperature was 40 °C. The injection volume was 1 µL.

For derivatized amines: mobile phase A was 0.1% formic acid and 10 mmol/L ammonium formate in water. Mobile phase B was acetonitrile. A: B was 40:60 for DIB derivatives and 55:45 for the other derivatives. The flow rate was 0.2 mL/min. The other conditions were the same as those for free amines.

Data processing for LC/MS(/MS)

Comparison among derivatizing reagents: each derivative was analyzed once and the relative intensities of ions were calculated based on the intensity of the largest ion in each mass spectrum. Diagnostic ions for discrimination were chosen considering the extent of difference between the relative intensities of ions from pairs of isomers.

Evaluation of discrimination ability for isomer pairs: each derivative was analyzed five times. The peak area of diagnostic ions for individual CE and m/z in the extracted ion chromatogram were used for calculation.

Diagnostic ions for discrimination of AP and BMPEA using Xevo TQD: relative intensities of m/z 299→181 to 299→299 at CE 10 eV and m/z 299→136 to 299→91 at CE 15 eV were used for NPA derivatives with LC/MS/MS. Relative intensities of m/z 150–91 and m/z 167–91 were used for PNB derivatives with LC/MS.

Diagnostic ions for discrimination of MA and phenpromethamine using Xevo TQD: relative intensities of m/z 313→150 to 313→313 at CE 10 eV and m/z 313→150 to 313→91 at CE 15 eV were used for NPA derivatives with LC/MS/MS. Relative intensities of m/z 150–299 and 181–299 were used for PNB derivatives with LC/MS.

Table 1 Retention times (tR) and observed ions for amphetamine, methamphetamine and their isomers by LC/MS/MS using Xevo G2-S Q-TOF

| No | Compound                                  | tR (min) | Ions (m/z)  |
|----|------------------------------------------|---------|-------------|
| [Amphetamine and its isomers]  |                                           |         |             |
| 1  | N-Benzylethanamine                       | 2.02    | 91, 136     |
| 5  | N-Methyl-1-phenylethan-1-amine           | 2.78    | 105, 136    |
| 11 | N-Methyl-2-Phenylethan-1-amine           | 2.82    | 105, 136    |
| 15 | Amphetamine (AP)                         | 3.95    | 91, 119, 136|
| 13 | BMPEA                                    | 4.20    | 91, 119, 136|
| 7  | 1-Phenylpropan-1-amine                   | 4.23    | 91, 119, 136|
| 18 | 3-Phenylpropan-1-amine                   | 5.13    | 91, 119, 136|
| [Methamphetamine and its isomers] |                                           |         |             |
| 4  | N-Benzyl-N-methylethanamine              | 2.37    | 91, 150     |
| 3  | N-Benzylpropan-2-amine                   | 2.86    | 91, 150     |
| 12 | N,N-Dimethyl-2-phenylethan-1-amine       | 3.31    | 105, 150    |
| 2  | N-Benzyl-N-propylamine                   | 3.69    | 91, 150     |
| 6  | N-Ethyl-1-phenylethanamine               | 3.79    | 105, 150    |
| 14 | Phenpromethamine                         | 4.75    | 91, 119, 150|
| 16 | Methamphetamine (MA)                    | 4.92    | 91, 119, 150|
| 8  | N-Methyl-1-phenylpropan-1-amine          | 6.18    | 91, 119, 150|
| 21 | Phentermine                              | 6.50    | 91, 133, 150|
| 17 | 1-Phenylbutan-2-amine                    | 7.54    | 91, 133, 150|
| 10 | 2-Methyl-1-phenyl-propylamine            | 8.94    | 91, 150     |
| 19 | 4-Phenylbutan-2-amine                    | 9.33    | 91, 133, 150|
| 22 | 4-Methylamphetamine                      | 10.92   | 105, 133, 150|
| 9  | 1-Phenylbutylamine                       | 11.73   | 91, 133, 150|
| 20 | 4-Phenyl-1-butanamine                    | 11.79   | 91, 133, 150|

Numbers correspond to those in Fig. 1. Major ions observed at any collision energy of 5, 10, 15, and 20 eV are listed.

Fig. 2 Breakdown curves of amphetamine (AP), methamphetamine (MA) and their isomers by Xevo G2-S Q-TOF. The numbers 7, 8, and 18 correspond to those in Fig. 1.
Comparison among mass spectrometers: diagnostic ions were basically the same as those for Xevo TQD with exceptions as follows: relative intensities of $m/z$ 150–285 and $m/z$ 167–285 were used for PNB derivatives of AP and BMPEA instead of $m/z$ 150–91 and $m/z$ 167–91. Relative intensities of $m/z$ 150–299 were not used for PNB derivatives of MA and phenpromethamine.

Calculation of identification windows referring the WADA criteria: the average relative intensity of each ion in the extracted chromatogram was used. With an ion of 50–100% relative intensity, the window was average ± 10% (absolute).
In the same manner, with an ion of 25–50% or 1–25% relative abundance, the window was ±20% (relative) or ±5% (absolute), respectively.

Results

LC/MS/MS and LC/MS of free amines

Retention times ($t_R$) and mass spectra of free amines were thoroughly examined using Xevo G2-S Q-TOF and the results were confirmed by the other two instruments. Table 1 shows $t_R$ and observed ions for AP, MA and their isomers by LC/MS/MS with Xevo G2-S Q-TOF. BMPEA (13), 1-phenylpropan-1-amine (7), and 3-phenylpropan-1-amine (18) gave the same set of product ions ($m/z$ 91, 119, 136) as that given by AP (15). Phenpromethamine (14) and N-methyl-1-phenylpropan-1-amine (8) gave the same set of product ions ($m/z$ 91, 119, 150) that MA gave. All other isomers gave different sets of ions. The other two mass spectrometers produced essentially the same sets of ions for each amine that Xevo G2-S Q-TOF did (data not shown).

Figure 2 shows the breakdown curves of AP, MA and their isomers measured by Xevo G2-S Q-TOF. Compounds 7 and 18 were distinguishable from AP with their characteristic patterns of breakdown curves. Compound 8 was distinguishable from MA in a manner similar to those of 7 and 18. Only two compounds, BMPEA and phenpromethamine, gave very similar breakdown curves as those given by AP and MA, respectively. The chromatograms and mass spectra of the four amines were shown in Fig. S1.

Mass spectrometry of each compound by in-source CID was also investigated. Essentially the same pattern was shown as MS/MS using 6530Q-TOF and Xevo TQD (data not shown). In-source CID with Xevo G2-S was tried at cone voltages of 10, 20, 30, and 40 V, showing no fragmentation.

Derivatization of amines

With very similar breakdown curves for AP and BMPEA, as well as for MA and phenpromethamine, we tried to analyze them after derivatization. Six reagents were used for the investigation. PNBC, SNPA, and DNBC were chosen because they are the common derivatizing reagents in LC [17]. DIB-Cl [18] is one of the frequently used reagents for detection of MA and AP from human urine in our laboratories. Propionyl chloride and 4-methylvaleroyl chloride were also chosen as they have smaller molecular weight and were expected to give different results from those given by the other reagents. As SNPA derivatization of AP and MA salts gave poor yield, these two salts were dissolved in water and extracted into n-hexane. The n-hexane layers were dried and used for the reaction.

Retention times and mass spectra of derivatives were thoroughly examined using 6530 Accurate-Mass Q-TOF. Table 2 shows the results. Both the isocratic and gradient conditions were examined; the isocratic condition gave a greater signal-to-noise ratio. As derivatives of DIB had significantly longer $t_R$, their mobile phases differed from those
of the other derivatives. Each pair of derivatives of isomeric amines was distinguishable by \( t_R \), although \( t_R \) of AP and BMPEA derivatives were close to each other.

**LC/MS/MS of derivatives**

The product ion scan spectra for all derivatives were acquired. The precursor to collision was \([M + H]^+\) of each derivative. Table 2 shows observed ions for each derivative at CE 15 eV. All derivatives except for those of DIB gave 3–7 product ions of 1% or higher relative intensity. DIB derivatives gave only 1 or 2 product ions even at the highest collision energy of 20 eV. No pair of derivatives gave a characteristic set of ions to be used for the discrimination of \( \beta \)-methylphenylethylamines and \( \alpha \)-methylphenylethylamines. However, some of the intensity ratios of each pair of ions differed greatly. SNPA gave the best results and seemed to be a suitable derivatizing reagent for MS/MS. The transitions 299 → 181 at CE 10 eV and 299 → 136 at CE 15 eV were selected for AP-NPA and BMPEA-NPA. The transitions 313 → 150 at CE 10 and 15 eV were selected for MA-NPA and phenpromethamine-NPA. Some of the reagents other than SNPA were suitable for only one of the two pairs. Both AP and MA should be targeted, because they are detected in urine simultaneously. Some of derivatives produced by the other reagents gave ions of insufficient intensity.

The reproducibility of selected ion sets for NPA derivatives was examined using three mass spectrometers. Each measurement was repeated five times and the peak area of extracted ion chromatogram was recorded. Table 3 shows the results. The intensity ratio of each pair was similar between the instruments. For example, relative intensities of \( m/z \) 299 → 136 ions of BMPEA-NPA were about three times greater than those of AP-NPA in all cases. Xevo TQD gave fragment ions of greater intensity than those of the other two instruments. Repeatability was also sufficient for discrimination. Relative standard deviations (RSD) of intensities were below 20% for all ions except \( m/z \) 150 ion of MA-NPA acquired by Xevo G2-S Q-TOF at CE 10 eV. The intensity of that ion was very small (data not shown). Figure 3 shows the chromatograms and mass spectra of the NPA derivatives obtained by Xevo TQD.

Brown et al.\(^{10}\) reported that pentafluorobenzoyl derivatives of these four amines gave ions of different intensities in MRM. Differences were reported to be 30–40%. Our new method gave a greater difference and hence better discrimination ability.

**LC/MS of derivatives**

In-source CID of derivatives was also investigated using 6530 Accurate-Mass Q-TOF without collision at the quadrupole. Fragmentor voltage was set at four steps. At 100, 130, and 160 V, almost none of the derivatives gave a fragment ion of relative intensity higher than 1%. At 190 V, several fragment ions were observed. Ion sets similar to those of MS/MS (Table 2) were observed (data not shown). DIB derivatives gave no fragment ion even at 190 V. As all pairs of derivatives gave same sets of ions, the intensity ratios of all the ions were compared. PNBC gave the best results and was chosen as the derivatizing reagent for in-source CID.

Fig. 4 Extracted ion chromatograms (\([M + H]^+\)) and mass spectra (CV 50 V) for in-source CID of 4-nitrobenzoyl (PNB) derivatives of the four compounds measured by Xevo TQD. Each test solution of a derivative was measured separately and the chromatograms were merged. Ions for the discrimination of isomers are indicated by asterisks.

![Fig. 4](image-url)
The intensities of m/z 150 and 167 ions were used for the discrimination of AP-PNB and BMPEA-PNB. The intensity of m/z 181 was used for the discrimination of MA-PNB and phenpromethamine-PNB. The m/z 150 ions of MA-PNB and phenpromethamine-PNB were so small that this ion was not considered a diagnostic ion. SNPA also showed good results, but the intensities of m/z 167 for MA and phenpromethamine were smaller than those of PNB derivatives.

The reproducibility of the intensity of selected ions was examined using two mass spectrometers. Xevo G2-S Q-TOF was not used because it did not adequately fragment analytes. Each measurement was repeated five times and the peak area of the extracted ion chromatogram was recorded. Table 3 shows the results. The differences were at least three times greater for 6530 Accurate-Mass Q-TOF and at least 2.5 times greater for Xevo TQD. Relative standard deviations of intensity were below 20% for all ions acquired by both spectrometers (data not shown). Figure 4 shows the chromatograms and mass spectra of the four PNB derivatives.

### Discussion

#### Criteria for discrimination

Criteria for discrimination were investigated using the dataset obtained by XEVO-TQD, as it gave fragment ions of greatest intensity among the three instruments. We evaluated the dataset referring to the criteria by WADA [19], which gives the maximum tolerance windows for relative abundances to ensure appropriate confidence in identification. It says that maximum tolerance windows are ±10% (absolute), ±20% (relative), and ±5% (absolute) with peaks of 50–100%, 25–50%, and 1–25% relative abundance, respectively. Table 4 shows the variation range of relative abundance of diagnostic ions obtained by XEVO-TQD with the maximum tolerance windows based on the criteria by WADA. Relative abundance was calculated by dividing the area of the ion trace of each diagnostic ion by the area obtained from the ion trace of the base peak. The range of variation is expressed as average ± 3 × SD.

Some of the diagnostic ions, e.g., m/z 299 → 136 in MS/MS or m/z 150 in MS gave completely separated windows for isomeric pairs of α-methylphenylethylamine and β-methylphenylethylamine. They are indicated by bold characters in Table 4. These ions are considered to be suitable for identification. Both of the isomeric pairs have at least one diagnostic ion suitable for identification in both modes, MS/MS and MS. On the other hand, m/z 299 → 181 and 313 → 150 in MS/MS at CE 10 eV gave overlapping windows, although their ranges of variation did not overlap. Some other criteria can be defined for these diagnostic ions based on additional experiments.
The mechanism for different intensities of diagnostic ions has been speculated. Figure 5 shows proposed fragmentation pathways for NPA derivatives of the four amines in MS/MS. Brown et al. [10] suggested a common fragmentation pathway involving the formation of a phenonium ion of \(m/z\) 119 in MS/MS of underivatized and PFB-derivatized amines. The same ion was observed with NPA derivatives in our research. The ion was further fragmented to produce the \(m/z\) 91 ion. They were the two dominant ions in all the spectra of four derivatives (see Fig. 3). The other ions were observed at \(m/z\) 136, 181, 150, and 195. The relative intensities of \(m/z\) 181 ion for AP-NPA and those of \(m/z\) 195 for MA-NPA were greater than those for BMPEA-NPA and phenpromethamine-NPA, respectively. It indicates that the pathway b is more preferable for the \(\alpha\)-methylphenylethylamines-NPA than that for the \(\beta\)-methylphenylethylamines-NPA. The pathway b probably consists of McLafferty rearrangement and loss of neutral phenylpropene, which requires at least one \(\gamma\)-hydrogen. \(\alpha\)-Methylphenylethylamines-NPA have twice as many \(\gamma\)-hydrogens than \(\beta\)-methylphenylethylamines-NPA. (The \(\gamma\)-position in McLafferty rearrangement means a relative location to the carbonyl group, not to the nitrogen.)

The relative intensities of \(m/z\) 136 ion for BMPEA-NPA and those of \(m/z\) 150 ion for phenpromethamine-NPA were greater than those for AP-NPA and MA-NPA, respectively (see Fig. 3). This phenomenon indicates the different stability of each set of ions or different preferences of pathway a between the two groups of amines. Position of methyl group might influence the stability of \(m/z\) 136 and 150 ions.

The fragmentation pathways for PNB derivatives of four amines in in-source CID was considered to be similar to those of the NPA derivatives with an exception of \(m/z\) 150 ions in all spectra (Fig. S2). The formula of the ion was identified as \([C_7H_4NO_3]^+\) by 6530 Q-TOF. This ion was considered as \([O_2N(C_6H_4)CO]^+\) produced by cleavage of the 4-nitrobenzoyl group.

**Conclusions**

Twenty amines including BMPEA and phenpromethamine were analyzed using LC/MS/MS and LC/MS. All amines except BMPEA and phenpromethamine were distinguishable from AP and MA by mass spectra. Derivatization using six reagents was investigated and SNPA for MS/MS and PNBC for MS were the best solutions for the discrimination of these compounds. Three mass spectrometers from two manufacturers were used to evaluate the reproducibility of the data. They gave similar intensity ratio for each pair of \(\alpha\)-methylphenylethylamine and \(\beta\)-methylphenylethylamine derivatives. With the novel method, \(\beta\)-methylphenylethylamines were distinguishable from isomeric \(\alpha\)-methylphenylethylamines by their mass spectra with adequate differences in relative abundance of diagnostic ions according to the WADA criteria.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest other than the grant mentioned above.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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