β-Methylphenylethylamines: common fragmentation pathways with amphetamines in electrospray ionisation-collision induced dissociation

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Abstract

β-Methylphenylethylamines are positional isomers of amphetamines and have been discovered in sporting supplements. Although the fragmentation of the *β*-methylphenylethylamine and N-methyl-*β*-methylphenylethylamine in GC-EI-MS systems is significantly different to their amphetamine and methylamphetamine isomers, under electrospray ionisation commonly used in LC-MS systems, the fragmentation of each of the isomeric pairs is almost identical. The similarities in fragmentation make it possible for the misidentification of the *β*-methylphenylethylamines as the illicit amphetamines. It is proposed that the similarities are due to a fragmentation pathway involving a common phenonium ion intermediate. By careful control of fragmentation energies in LC-MS-MS systems and/or close examination of the relative abundances of product ions formed by collision induced dissociation (qualifier ratios) it is possible to distinguish the *β*-methylphenylethylamines from the amphetamines, even if significant retention time separation is not achieved. In LC-ESI-QTOF systems the mass spectra of the *β*-methylphenylethylamines are identical to their amphetamine isomers. In such systems retention time separation of the isomers is critical to avoid misidentification. During this study *β*-methylphenylethylamine and N-methyl-*β*-methylphenylethylamine have been identified in commercially available sporting supplements and oral fluid samples taken during the course of road-side drugs-in-drivers and workplace testing programs.

Keywords: Sporting supplements; beta-methylphenylethylamines; stimulants; amphetamines

Introduction

Dietary supplements, including weigh loss, "pre-workout" and "sporting" supplements have received significant attention in recent years due to the inclusion of compounds with potential stimulant properties in their formulations. In many cases the ingredients listed on these products do not specifically name the included stimulants. This case was no better highlighted than by Cohen *et al.* who identified the amphetamine derivative *N,α*-diethylphenylethylamine in the supplement Craze.[1-2] Numerous athletes have tested positive to this stimulant,[1, 3] which is banned by the World Anti-Doping Agency (WADA).[4]

Another such class of compounds that have been detected in sporting supplements are the *β*-methylphenylethylamines, specifically *β*-methylphenylethylamine (**1**),[5-8] *N*-methyl-*β*-methylphenylethylamine **(2)**[9-10] and *N,N*-dimethyl-*β*-methylphenylethylamine.[11] During the mid-20th century these compounds were explored for their medicinal vasopressor and sympathomimetic properties, particularly **2**.[12-14] Recent interest in the case of **2** has focused on its use as a performance-enhancing drug in sport (doping), and in the case of **1** its appearance in dietary supplements.[5-7, 15] The compounds **1** and **2** are positional isomers of amphetamine (**3**) and methylamphetamine (**4**), respectively, which are *α*-methylphenylethylamines. These compounds have potential stimulant properties and are banned by WADA (**2** specifically listed as phenpromethamine).[4] Two different issues are present with these compounds, firstly, they have been detected in supplements even though they do not appear on the ingredients list. For example, studies by Pawar *et al.,* and more recently Cohen *et al.,* have highlighted that fact that a number of supplements claiming to contain an "*Acacia rigidula* extract" contain *β*-methylphenylethylamine **(1)**.[5, 7] The second, and significant, issue relating to the *β*-methylphenylethylamines is that they appear to behave almost identically to their isomeric amphetamines under LC-MS (liquid chromatography-mass spectrometry) conditions. A review of recent articles focused on screening and confirmation methods for doping control that include **2** in their compound lists highlight the potential problems. In the most part these publications have used LC-MS based instrumentation with either HPLC (high performance liquid chromatography) or UHPLC (ultra high performance/pressure liquid chromatography) systems coupled with a variety of mass spectrometer (MS) detectors [e.g. quadrupole-time-of-flight (QTOF), time-of-flight (TOF), orbitrap, triple-quadrupole (QqQ)]. The data provided in these publications is varied: where parent or product ion data is presented for **2** and **4** it is identical (parent *m/z* 150, product ions *m/z* 119 and 91);[16] in a number of cases no retention time data for **2** and **4** is provided;[16] where retention time details are

provided for **2** and **4** in some cases it is very similar (ΔRT 0.01-0.04 min.),[17-20] or identical![21] Of these doping-based papers, only one provided discussion about the need to achieve retention time separation to distinguish **2** and **4**.[19] Interestingly, one paper reported the identification of **2** when testing a screening method with an automated search capacity using a certified reference material containing methylamphetamine (**4**).[22] When GC-MS (gas chromatography-MS) techniques are used for doping analysis then the fragmentation ions for **2** and **4** are different.[23] A number of recent publications have highlighted the similarity of the fragmentation of *β*-methylphenylethylamine (**1**) and amphetamine **(3**) by electrospray ionisation (ESI)-mass spectrometry.[5-6, 15] The parent ions for **1** and **3** under LC-ESI-MS conditions (ESI-Q-orbitrap, ESI-QTRAP or ESI-QTOF) have a *m/z* of 136 (M+1), and both produce product ions with *m/z* values of 119, 91 and 65 depending on applied collision energy,[5-6, 15] in almost identical intensities.[5-6] Pawar *et al.*[5] and Vaclavik *et al*.[15] reported retention time separations of **1** and **3**, however baseline-separation could not be achieved. A more recent UPLC method reported by Chołbiński *et al*. achieved *ca.* 0.5 min. separation of **1** and **3**.[6] None of above publications have provided an explanation to the mass spectral similarity for the *β*-methylphenylethylamines and their isomeric amphetamines. The similarities in mass spectra and possible similarities of retention times, highlighted above, leads to the possibility of miss identifying *β*-methylphenylethylamines as their isomeric amphetamines.

Within our laboratories we routinely quantify illicit amphetamines in urine, blood and oral fluid matrices, for the purposes of post mortem toxicology, drugs in driving offenses, and work-place testing. Our GC-MS, LC-ESI-QqQ and LC-ESI-QTOF analyses of these analytes follow on from liquid-liquid extraction, solid-phase extraction (SPE), and extractive-benzoylation methods. In this paper we explore the mass spectral and chromatographic properties of the *β*-methylphenylethylamines **1** and **2** both derivatised and underivatised and provide an explanation for the observed similarities in the fragmentation of these compounds with their isomeric amphetamines **3** and **4** under ESI-CID (electrospray ionisation - collision induced dissociation) conditions. We also present some methods for distinguishing these compounds using ESI-QqQ systems, crucially by the careful analysis of qualifier ratios and control of collision energies.

During the review of this manuscript the US Food and Drug Administration (FDA) released a statement on the presence of *β*-methylphenylethylamine (**1**) in dietary supplements, and highlighted action taken by the FDA towards a number of manufacturers of supplements containing *β*-methylphenylethylamine (**1**).[8] In Australia, dietary supplements including "sports-related food products" are regulated by Food Standards Australia New Zealand, through the Australia New

Zealand Food Standards Code. With regard individual compounds that may be found in supplements, the Therapeutic Good Administration (TGA) regulates medicines and poisons and maintains the *Standard for the Uniform Scheduling of Medicines and Poisons* (SUSMP). Though they do not regulate food products, individual compounds discovered in supplements may be banned by the TGA and added as a poison to the SUSMP.[24]

Methods

Standards of amphetamine and methylamphetamine (both D0 and D5) were purchased from Cerilliant, while samples of *β*-methylphenylethylamine (*β*-methylphenethylamine) and N-methyl-*β*-methylphenylethylamine [methyl-(2-phenyl-propyl)-amine] were purchased from Sigma-Aldrich. Sporting and dietary supplements BMP Labs Lipodrive tablets (received mid-2013), BMP Labs Lipodrive powder (received early-2014) and APS Mesomorph 2.0 (received early-2014), were all purchased locally. Table 1 lists the ingredients of these supplements, based on their labelling.

Extractive benzoylation was performed with pentafluorobenzoyl chloride (PFB-Cl) following standard procedures.[25] Both derivatised and underivatised samples were reconstituted in methanol or methanol:pH 9 buffer (70:30) prior to analysis on an Agilent 1200 Series HPLC coupled to an Agilent 6410 Triple Quadrupole Mass Spectrometer. QTOF analysis was performed on an Agilent 1260 Infinity HPLC coupled to an Agilent 6540 QTOF Mass Spectrometer. The following buffers were used for chromatographic analysis: 10 mM ammonium formate buffered to pH 9 with ammonia solution ("pH 9 buffer"); 10 mM ammonium formate buffered to pH 3 with formic acid ("pH 3 buffer"). Electron ionisation mass spectra were obtained on an Agilent 6890 GC-5975 MSD system with a *m/z* range of 40 - 400.

Preparation of supplement solutions for analysis: Supplement powders were dissolved in water based on the label instructions. The resulting solutions were then diluted 100 times with water. A Lipodrive tablet was dissolved in 500 mL water and the resulting solution was diluted 100 times with water. The diluted supplement solutions were extracted as per the extractive benzoylation (derivatised) and oral fluid extraction (underivatised) procedures below.

Extraction of oral fluid and supplement samples (underivatised): To 0.2 mL of oral fluid (either neat or with buffer/solvents from Dräger or Cozart/Alere oral fluid collection devices) was added 10 µL of a mixed internal standard solution containing *D*5-amphetamine and *D*5-methylamphetamine (1 µg/mL), 0.2 mL of concentrated ammonia solution (28%) and 5 mL cyclohexane. The mixture was vortexed for 5 mins, centrifuged and the organic phase separated. To the organic phase was added 1

drop of a methanolic HCl solution (5% 0.1 M HCl in methanol) and the mixture was evaporated to dryness without heat. The residue was reconstituted in 100 uL methanol. Samples were analysed on the Agilent 6410 Triple Quadrupole Mass Spectrometer in MRM (multiple reaction monitoring) mode, using a pH9 buffer/methanol elution solvent. Fragmentor voltage 110 V; MRM Transitions *m/z* 150 -> *m/z* 119 (CE 10 V), *m/z* 150 -> *m/z* 91 (CE 14 V); *m/z* 136 -> *m/z* 119 (CE 9 V); *m/z* 136 -> *m/z* 91 (CE 14 V).

Extractive benzoylation (derivatisation) of urine and supplement samples: To 0.2 mL of solution (urine or supplement solution) was added 0.2 mL of concentrated ammonia solution (28%), 1 mL water and 5 mL cyclohexane solution containing *D*5-amphetamine and *D*5-methylamphetamine (100 ng per 5 mL). While mixing, 75 µL of a pentafluorobenzoyl chloride solution (5% in butyl chloride) was added. After mixing for 5 minutes the organic phase was separated, and then evaporate to dryness without heat. The residue was reconstituted in 100 uL methanol:pH 9 buffer (70:30). Samples were analysed on the Agilent 6410 Triple Quadrupole Mass Spectrometer in MRM mode, using a pH9 buffer/methanol elution solvent. Fragmentor voltage 110 V; MRM Transitions *m/z* 344 -> *m/z* 119 (CE 5 V), *m/z* 344 -> *m/z* 91 (CE 5 V); *m/z* 330 -> *m/z* 119 (CE 5 V); *m/z* 330 -> *m/z* 91 (CE 5 V).

Chromatographic conditions.
Underivatised (pH 9*)*: pH 9 buffer : methanol; flow rate 0.5 mL/min; 50 °C pH 9 buffer 85% 0-1 min, 15% 3 min, 5% 4-7 min, 85% 7.1-12 min; using a Phenomenex Kinetex 2.6 µm, 100 x 3.0 mm C18 column.
Derivatised: pH 9 buffer : methanol; flow rate 0.5 mL/min; 50 °C ; pH 9 buffer 60% 0-1 min, 15% 2 min, 5% 3-5 min, 60% 5.1-10 min; using a Phenomenex Kinetex 2.6 µm, 100 x 3.0 mm C18 column.
Underivatised (pH 3): pH 3 buffer : acetonitrile; flow rate 0.6 mL/min; 30 °C ; pH 3 buffer 90% 0-2 min, 80% 4 min, 5% 6.7-10 min, 90% 10.1-13 min; using a ACE Excel 3 SuperC18 3 µm, 100 x 3.0 mm column.

Comparing qualifier ratio data obtained from LC-ESI-QqQ data: Since absolute qualifier ratios can vary with changes in the tuning of the QqQ mass spectrometer we determined percentage differences of qualifier ratios of the *β*-methylphenylethylamines (**1** or **2**) compared with the relevant amphetamine standards (**3** or **4**). For individual sets of data the qualifier ratios of each amphetamine standard was averaged, covering the full calibration range. This average value was used as the reference when determining the percentage difference for the qualifier ratios of the *β*-

methylphenylethylamines.

Results and Discussion

The structures of the *β*-methylphenylethylamines **1** and **2** and the amphetamines **3** and **4** are different enough to expect some significant fragmentation differences, based on conventional fragmentation pathways. When electron ionisation (EI) mass spectra, obtained from a GC-MS, were compared the differences were obvious (see Figure 1 as an example). The EI fragmentation of amphetamine (**3**) displayed the ions *m/z* 134, 120, 91, 64 and 44, whereas *β*-methylphenylethylamine (**1**) displayed numerous fragments, including a *m/z* 105 ion (and an intense *m/z* 30 ion if the mass spectrum is measured lower than *m/z* 40, see below). Conventional fragmentation pathways, with formation of a benzylic cation (or corresponding tropylium ion) readily explain the formation of the *m/z* 91 ion for **3** and the *m/z* 105 ion for **1**. The EI mass spectra for the N-methyl analogues **2** and **4** displayed analogous differences, including a *m/z* 105 ion for **2** (see Supporting Information). The EI mass spectra of the pentafluorobenzoyl (**PFB**) derivatised amines showed similar key differences with *m/z* 105 ions for the *β*-methylphenylethylamines (**1-PFB** and **2-PFB**) and *m/z* 91 ions for the amphetamines (**3-PFB** and **4-PFB**) (see Supporting Information, Figures S2 and S3), again consistent with conventional fragmentation pathways. Each of the amines, both derivatised and underivatised, displayed ions in the EI mass spectra that were consistent with the formation of iminium ions ($R_2C{=}NR_2^+$). In the case of the underivatised amines **1** - **4** the iminium ion is the most intense ion that forms following electron ionisation. For *β*-methylphenylethylamine (**1**) the iminium ion *m/z* 30 does not appear in Figure 1 as the instrument was set to measure *m/*z values greater than *m/z* 40, however, in the NIST mass spectral library the mass spectrum for **1** displays the *m/z* 30 ion as the base peak, with the next most intense ion being *m/z* 91 at <20% relative intensity.[26] Other than the absence of the ions less than *m/z* 40 the mass spectrum of **1** in Figure 1 is identical to that in the NIST mass spectral library.[26] The iminium ions for amphetamine **(3**) and methylamphetamine (**4**) are *m/z* 44 (Figure 1(b)) and *m/z* 58 (Figure S1(b)) respectively. These spectra are also identical to the spectra in the NIST mass spectral library.[26] In the case of *N*-methyl-*β*-methylphenylethylamine **(2**) the iminium ion is *m/z* 44 (Figure S1(a)). The ions that are consistent with the formation of iminium ions for the derivatised amines **1-PBF** (*m/z* 224), **2-PFB** (*m/z* 238), **3-PFB** (*m/z* 238) and **4-PFB** (*m/z* 252) were also present in their mass spectra (see Supporting Information). The EI-MS spectra for **3-PFB** and **4-PFB** presented in Figures S2 and S3 are consistent with published data.[27] As a consequence of the EI mass spectra it is easy to distinguish the *β*-methylphenylethylamines **1** and **2** from their isomeric amphetamine **3** and **4** by conventional GC-MS (derivatised or underivatised).

When the *β*-methylphenylethylamines **1** and **2** were fragmented under electrospray ionisation (ESI) conditions the ions that were formed were very different from those under EI conditions. Fragmentation of **1** or **2** using a ESI-QqQ with collision-induced dissociation (CID) of 5 V afforded product ions with only *m/z* values of 119 and 91 (see Figure 2(a)(i) and Figure S4(a)(i) in Supporting Information). These fragmentation ions were *identical* to those formed from the amphetamines **3** and **4** (see Figure 2(a)(ii) and Figure S4(a)(ii) in Supporting Information). The *m/z* 91 and 119 ions were also observed in the ESI-QqQ CID fragmentation of the pentafluorobenzoyl (**PFB**) derivatised amines **1-PFB** , **2-PFB**, **3-PFB** and **4-PFB** (see Figure 2(b) and Figure S4(b) in Supporting Information). In the case of the derivatised amines, the additional ions formed from fragmentation, *m/z* 212 and 226, involve the pentafluorobenzamide moiety. When the CID energy was increased then a product ion with an *m/z* of 65 formed in the CID of **1**, **2**, **3** and **4** (and their PFB derivatives). The observation that **1** and **3** formed the product ions *m/z* 119, 91 and 65 following CID is consistent with the MS/MS spectra reported by Pawar *et al.* using a LC-Qtrap system[5] and Chołbiński *et al*. using LC-QTOF and LC-tandem MS systems,[6] although the relative intensities of the ions differed due to different CID energies. Interestingly, no *m/z* 105 was observed in the ESI-CID of the *β*-methylphenylethylamines **1** and **2**, either underivatised or derivatised (i.e. **1-PFB** and **2-PFB**) (*cf.* EI-mass spectra in Figures 1, S1 and S2). Selective fragmentation by CID of the *m/z* 119 ion for each of the analytes examined afforded a 91 product ion. None of the ESI-CID spectra for the derivatised (**1-PFB** - **4-PBF**) or underivatised (**1** - **4**) amines displayed ions consistent with the formation of iminum ions.

The observed similarities in the ions that formed from the ESI-CID fragmentation of the *β*-methylphenylethylamines **1** and **2** and the amphetamines **3** and **4** (and their PFB derivatives) suggested a very different fragmentation process than those that occurred under EI conditions. We believe the similarities arise from a common fragmentation pathway involving the formation of a phenonium ion, in the first instance, following an intramolecular cyclisation fragmentation process (Scheme 1). Similar phenonium ions have been proposed in the fragmentation of phenylalanine derivatives[28] and gas-phase rearrangement mechanisms involving phenylpropanols.[29] Even though it was possible to measure the parent ion -> *m/z* 91 "transition", we believe that this fragmentation process goes *via* the phenonium ion (*m/z* 119) which further fragments to the *m/z* 91 ion.

Closer examination of the relative abundance (*i.e.* qualifier ratios: *m/z* M+1-> *m/z* 119 *vs m/z* M+1-> *m/z* 91) of the product ions that formed for each of the isomeric pairs of compounds: *β*-

methylphenylethylamine (**1**) and amphetamine (**3**); and N-methyl-*β*-methylphenylethylamine (**2**) and methylamphetamine (**4**), indicated a potential method for discrimination of these compounds. When the pentafluorobenzoyl derivatives **1-PFB**, **2-PFB**, **3-PFB** and **4-PFB** were fragmented with a fragmentor voltage of 100 V and a CID energy of 15 V the relative abundances of each of the major transitions *m/z* M+1 -> *m/z* 119 and *m/z* M+1-> *m/z* 91 were very similar (see Figure 3(a)). In the case of **1-PFB** the ratio of the *m/z* 330 -> *m/z* 91 and *m/z* 330 -> *m/z* 119 transitions were within 5-12% of the ratio for the equivalent transitions for the amphetamine isomer **3-PFB**, across a significant calibration range (10-1250 ng/mL). For **2-PFB** the ratio of the *m/z* 344 -> *m/z* 91 and *m/z* 344 -> *m/z* 119 transitions were not as close, but were still within 17-22% of the ratio for the equivalent transitions for the amphetamine isomer **4-PFB**. The similarities of the "qualifier ratios" is of significance since commonly accepted guidelines for the identification of compounds by mass spectrometry in a forensic context suggest one criteria for confirming the identification of compound is that the qualifier ratios are within 20% of that of a standard.[30-31] The information presented above clearly indicates the potential for the miss identification of *β*-methylphenylethylamines as their isomeric amphetamines (based on their mass spectra). When the same transitions were examined with a lower CID energy of 5 V the relative abundances of each of the major transitions became significantly different between the *β*-methylphenylethylamine and the amphetamines (Figure 3(b)). The ratios of the *m/z* 330 -> *m/z* 91 and *m/z* 330 -> *m/z* 119 transitions for **1-PFB** and the *m/z* 344 -> *m/z* 91 and *m/z* 344 -> *m/z* 119 transitions for **2-PFB** were greater than 30% (30-40%) different from the ratios for the equivalent transitions for the amphetamine isomers **3-PFB** and **4-PFB**. Using a lower CID energy allowed for these compounds to be distinguished based on the commonly accepted guidelines mentioned above. The use of a lower CID to differentiate the *β*-methylphenylethylamines from the amphetamines is also consistent with the proposed fragmentation pathways in Scheme 1. The amount of energy required to form the phenonium ion intermediate would be subtly different for the two different classes of compounds. With low CID energies not all of the parent ions would fragment to the *m/z* 119 ion, and for each of the different compounds this would vary. It is this variation that was critical for the differences in the qualifier ratios. For instance, when higher CID energies were used, it was possible to also examine the *m/z* M+1 -> *m/z* 65 transition for each of the four compounds. Through comparison of the *m/z* M+1 -> *m/z* 91 *vs* *m/z* M+1 -> *m/z* 65 transitions for each of the isomeric pairs of compounds, it was not possible to distinguish the *β*-methylphenylethylamines from the amphetamines, however, if the *m/z* M+1 -> *m/z* 119 transition (with low CID energy) was used versus either of the *m/z* M+1 -> *m/z* 91 or *m/z* M+1 -> *m/z* 65 transitions (with high CID energy) then it was possible to use qualifier ratios to distinguish the *β*-methylphenylethylamines from the amphetamines.

Unlike the derivatised compounds, decreasing the CID energies to 5 V for the analysis of the underivatised amines **1** - **4** did not produce a significant change in the relative abundances of the *m/z* M+1 -> *m/z* 119 and *m/z* M+1 -> *m/z* 91 transitions. For *β*-methylphenylethylamine (**1**), the ratio of the *m/z* M+1 -> *m/z* 119 and *m/z* M+1 -> *m/z* 91 transitions, determined with CID energies of 9 and 14 V respectively, was 17-23% different than the equivalent ratio for amphetamine (**3**), across a calibration range of 10-500 ng/mL (Figure 3(c)). This difference in ratio was significant enough to distinguish **1** and **3** relatively easily, provided the qualifier ratio ratios were examined closely. However, in the case of N-methyl-*β*-methylphenylethylamine (**2**) the equivalent ratio was typically only 9-15% different from that of methylamphetamine (**4**). In this case the possibility of the misidentification of N-methyl-*β*-methylphenylethylamine (**2**) as methylamphetamine (**4**), if retention time separation is not achieved, becomes significant.

Other than their mass spectral features, the other key aspect in the identification of compounds by LCMS is retention time. Due to the similarities in the structures of **1** and **2** with their amphetamine isomers **3** and **4** it is possible that their retention times could also be very similar. In our own studies, using pH 9 buffer-methanol based gradient elution programs the retention times of the *β*-methylphenylethylamines and the amphetamines were very similar, both derivatised and underivatised (Figure 3). Other researchers have also identified this problem, and suggested solutions with pH 3 solvent systems and particular columns.[5-6] We could obtain full baseline separation of the underivatised amines with a pH 3 buffer-acetonitrile gradient program (Figure 4). Analysis of the qualifier ratio data obtained from the pH 3 buffer-acetonitrile separation did not indicate a significant change in the relative qualifier ratios between the *β*-methylphenylethylamines and the amphetamines when compared to the data (above) obtained from the pH 9-methanol system. When investigating the derivatised compounds we could not adequately separate the isomeric pairs of pentafluorobenzoyl-derivatised amines using various chromatographic systems, including pH 3 buffer-acetonitrile, pH 3 buffer-methanol and pH 9 buffer-acetonitrile gradient programs, in addition to the pH 9 buffer-methanol gradient program detailed in the methods section.

There is significant interest in using LC-ESI-QTOF systems as a "one-stop" screening tool across many areas of analytical chemistry, e.g. post mortem toxicology, work place testing and doping control. Due to the interest in this technique we examined the capability of the Agilent QTOF system to distinguish between the isomeric *β*-methylphenylethylamines and amphetamines. In our preliminary efforts we found that the QTOF detector could not distinguish the isomeric pairs (**1** *vs* **3**, and **2** *vs* **4**). The MS/MS spectra obtained using the conventional 10, 20 and 40 V CID energies

were practically identical such that software analysis could not identify any significant differences (See Figures S5 and S6 in the Supporting Information). The MS/MS spectra reported here for *β*-methylphenylethylamines **(1)** and amphetamines **(3)** is similar to that obtained by Chołbiński *et al*., albeit using a different QTOF system.[6] We could only distinguish the isomers if retention time separation was first achieved with an appropriate solvent system and gradient program (e.g. Figure 4). This suggested that only with the inclusion of standards into an analysis batch, or good confidence on repeatability of retention times could an LC-ESI-QTOF system reliably distinguish between the *β*-methylphenylethylamines and amphetamines.

During casework within our Forensic Toxicology Section, including drugs in drivers analyses for the Western Australia Police and work place testing, we have identified both *β*-methylphenylethylamine **(1)** and N-methyl-*β*-methylphenylethylamine **(2)** in multiple oral fluid samples (covering an approximate range of <25 - 450 ng/mL) and **1** in a urine sample. It should be noted that our quantification of **1** and **2** in these samples was only approximate. These values were determined using a calibration based on amphetamine and methylamphetamine. The values have been included here since they are above the reporting cut-off of 25 ng/mL for amphetamines used by the Western Australian Police and the Australian work place testing standard "Drugs in Oral Fluid AS4760-2006". In all cases involving the detection of **1** and **2** in oral fluid or urine samples our analyses were perform on samples that had tested "positive" to amphetamines in a saliva or urine presumptive "on-site"/"on-roadside" testing device (such as Securetec DrugWipe®, Dräger DrugTest® 5000, and Cozart DDS/Alere DDS®2 oral fluid testing devices, or urine drug test cups containing test strips). During the course of both casework and this project we have also analysed a number of different "fat-burning" and "pre-workout" supplements (see Table 1). In one case, tablets of the BPM Labs Lipodrive tested positive to **1**, but not **2**, despite both compounds being listed in the supplement ingredients list, however, in a powder formulation of the same product, with the same ingredients list, both **1** and **2** were identified. In a different branded supplement (Mesomorph 2.0) both **1** and **2** were detected though neither was specified in the ingredient list, however, in this case the supplement claimed to contain an "*Acacia rigidula* extract". Supplements listing "*Acacia rigidula* extract" in the ingredients have been shown to contain **1**.[5, 7] Figures S7, S8 and S9 in the Supporting Information provide examples of the data obtained from analyses of the supplement materials, using LC-QqQ methods (derivatised and underivatised) as well as GCMS data of derivatised extracts. Figure S10 (Supporting Information) is an example of data obtained from an oral fluid sample analysed using an underivatised method on a pH 9-methanol gradient system, highlighting how similar the data for **1** and **2** may appear to **3** and **4**. In this example qualifier ratio indicated that the oral fluid did not contain the illicit amphetamines **3** and **4**. The analyses of the

supplements in this study focused on the detection of **1** or **2** in the materials, and not the specific quantification of each of the individual compounds in the supplements.

Conclusions

We have proposed a common fragmentation pathway for the *β*-methylphenylethylamines and amphetamines when fragmented in ESI-CID systems. We believe it is this pathway that makes isomeric pairs of these classes of compounds nearly indistinguishable by MS detectors commonly used in LCMS systems. As a consequence, without careful regard for retention times, and qualifier ratios (for MRM analyses) there is a possibility to miss identify *β*-methylphenylethylamines as amphetamines. For QTOF-based systems it is imperative to ensure retention time separation between the isomeric pairs *β*-methylphenylethylamine and amphetamine, and N-methyl-*β*-methylphenylethylamine and methylamphetamine as the QTOF analyser could not distinguish the isomers based on their mass spectral properties.

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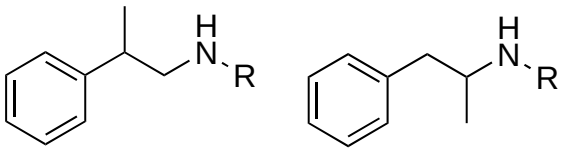
Tables and Figures

Table 1. The components listed on the labels of the supplement products analysed in this study

Product (Manufacturer)	Components*	Comments from this study
Lipodrive (BPM Labs, tablets)	Caffeine (27.5 g), R-beta-methylphenylethylamine.HCl (**1**.HCl) (3.4 g), green tea (Camellia sinensis) leaf extract (4.8 g), methylsynephrine (8.4 g), theobromine (13.5 g), N-methyl-B-methylphenylethylamine (**2**) (2.8 g), synephrine.HCl (8.3 g), Naringen (8.3 g), Citrus Aurantium (blood orange) extract (8.3 g), evodiamine (2.8 g), Yerba mate (5.5 g), Phenylethylamine.HCl (3.7 g), taurine (5.5 g)	**1** detected; **2** not detected
Lipodrive (BPM Labs, powder)	Proprietary blend containing: Taurine, caffeine, R-beta-methylphenylethylamine.HCl (**1**.HCl), green tea (Camellia sinensis) leaf extract, theobromine, N-methyl-B-methylphenylethylamine (**2**), Naringen, bitter orange, evodiamine, Yerba mate concentrate, Phenylethylamine.HCl	**1** and **2** detected
Mesomorph 2.0 (APS)	Beta alanine, L-citrulline, arginine alpha ketoglutarate, DL-creatine malate, L-taurine, creatine nitrate, ascorbic acid, creatinol-O-phosphate, agmatine sulfate, glucuronolactone, caffeine, theobromine, Naringen, Acacia Rigidula extract	**1** and **2** detected

* Nutritional information from label (Masses per 100 g where provided).
Note: The analyses conducted in this study only focused on compounds that may cause interferences in the analysis of methylamphetamine and amphetamine.



1 R = H
2 R = CH_3

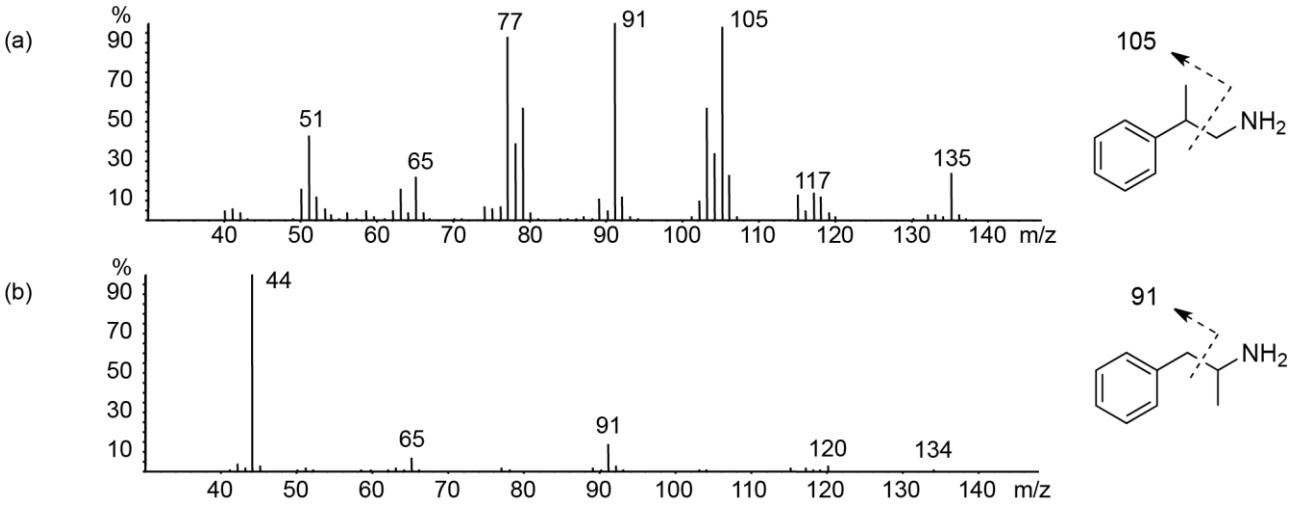


Figure 1. The electron ionisation mass spectra for (a) *β*-methylphenylethylamine **1** and (b) amphetamine **3**.

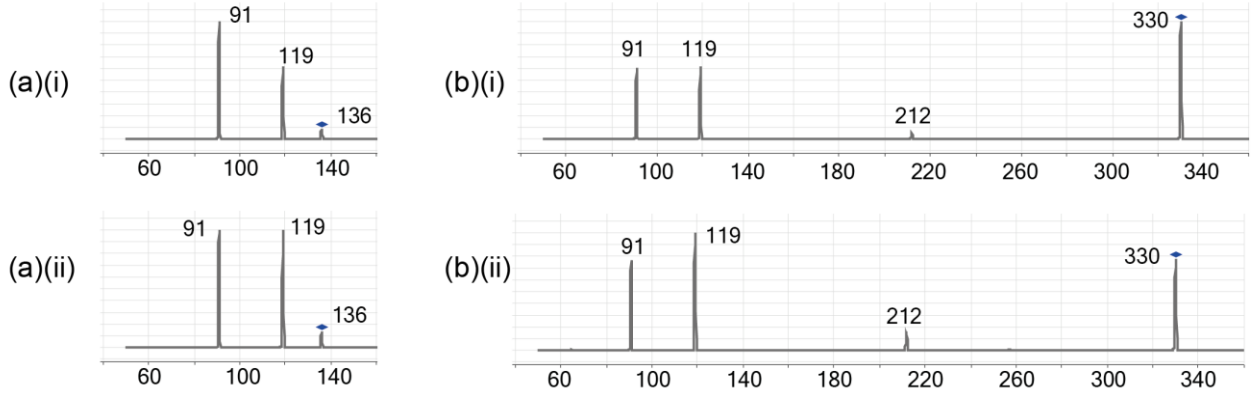
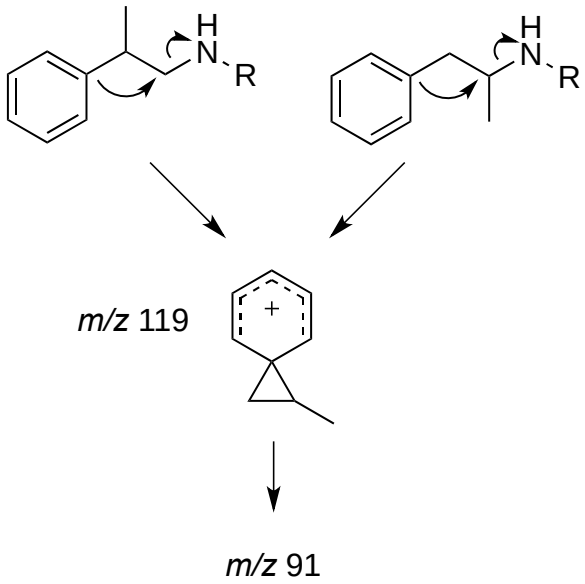


Figure 2. Product ion scans for: (a)(i) *β*-methylphenylethylamine **1** (M+1 = *m/z* 136); (a)(ii) amphetamine **3** (M+1 = *m/z* 136); and the pentafluorobenzoyl derivatives (M+1 = *m/z* 330) (b)(i) **1-PFB** and (b)(ii) **3-PFB** (Fragmentor 100 V, CID 5V).



Scheme 1. A proposed common ESI-CID fragmentation pathway for the *β*-methylphenylethylamines and amphetamines.

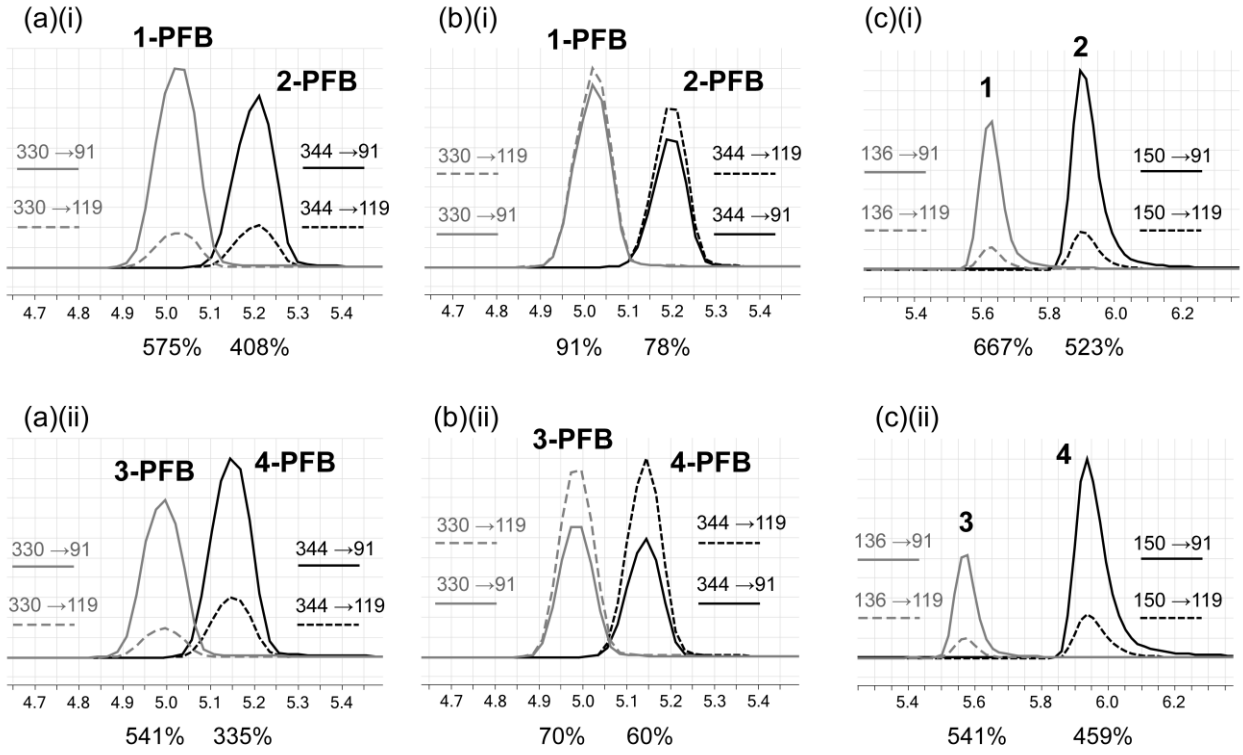


Figure 3. Chromatograms, with individual ion transitions, for: (a) (i) **1-PFB** and **2-PFB** , and (ii) **3-PFB** and **4-PFB** , with a 15 V CID energy; (b) (i) **1-PFB** and **2-PFB** , and (ii) **3-PFB** and **4-PFB** , with a 5 V CID energy; and (c) (i) **1** and **2**, and (ii) **3** and **4** (see Experimental section for chromatographic conditions). Each set of chromatograms (e.g. (i) and (ii)) are on the same retention time scale. Below each chromatogram are the ratios of the two transitions (*m/z* M+1 -> *m/z* 91 *vs.* *m/z* M+1 -> *m/z* 119; as a percentage) for each compound.

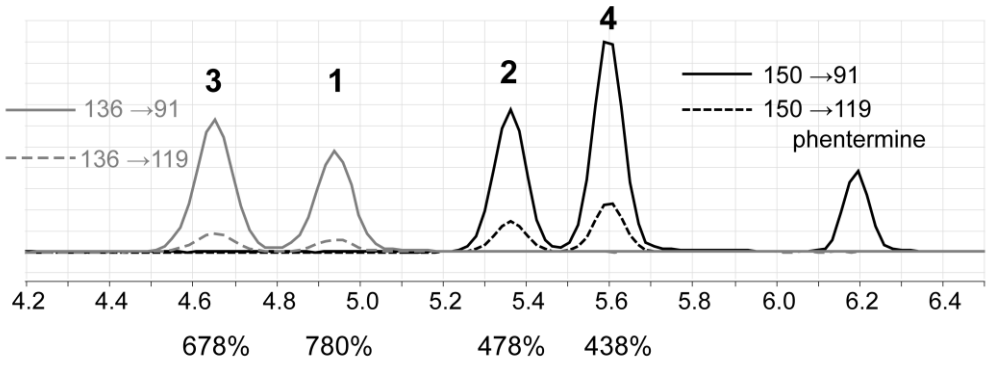


Figure 4. Chromatogram showing *m/z* M+1 -> *m/z* 119 and *m/z* M+1 -> *m/z* 91 transitions, separation of the underivatised amines **1-4**, and phentermine using a pH 3 buffer-acetonitrile gradient. Below the chromatogram are the ratios of the two transitions (*m/z* M+1 -> *m/z* 91 *vs. m/z* M+1 -> *m/z* 119; as a percentage) for each compound.