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Final Summary Overview

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Statistical and Mass Spectral Tools for the Identification and Characterization of Synthetic Phenethylamines

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Ruth Smith 07/12/18

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Purpose of the Project

Synthetic designer drugs appeared on the market in 2008 and have continued to pose problems in forensic laboratories since then, primarily in terms of identification and characterization. These compounds are manufactured to maintain the psychoactive effects of a currently controlled substance but with a slight structural change that circumvents current legislation. This results in a variety of compounds that contain the same core structure but which differ in the position or the identity of a single substituent. Current methods of identification rely on gas chromatography-mass spectrometry (GC-MS), typically using electron ionization (EI) and a single quadrupole mass analyzer. While EI affords sufficient fragmentation for structural elucidation, the single quadrupole generates low-resolution, nominal mass data. Thus, definitive identification of structurally similar compounds based on a visual comparison of low-resolution mass spectral data is difficult. Further, with the rapid emergence of new analogs, oftentimes no reference material is immediately available for comparison to aid in the identification.

In this work, methods were developed to aid in both the identification and characterization of synthetic designer drugs, initially focusing on three structural subclasses of the synthetic phenethylamines. For identification, a statistical comparison method was refined and applied to demonstrate comparison of two mass spectra with a statistical assessment of the veracity of the identification (Goal 1). For characterization, mass spectral features of the three synthetic phenethylamines subclasses were probed to identify those features that were sufficiently characteristic of the subclass. These features formed the basis of a flow-chart style characterization scheme with the intention that such a scheme could be used to characterize a new analog according to structural subclass (Goal 2). Further, the utility of high-resolution mass spectrometry for designer drug characterization was demonstrated. With the accurate mass data

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afforded, the mass defect of an ion can be determined. These mass defects were exploited to increase specificity in the characterization (Goal 3). Finally, project findings will be disseminated as a webinar through the Forensic Technology Center of Excellence, most likely during summer 2018 (Goal 4).

Project Design and Methods

Synthetic phenethylamine reference standards were purchased from Cayman Chemical (Ann Arbor, MI) throughout this project. These standards included representative compounds from three structural subclasses: the 2,5-dimethoxy (2C-), the aminopropylbenzofuran (APB-), and the 2,5-dimethoxy-N-(2-methoxybenzyl) (NBOMe-) phenethylamines. Particularly for the 2C- and NBOMe-phenethylamines, care was taken to ensure that a range of possible substituents (*e.g.*, halogens, sulfur, and nitro groups) was included. These compounds were primarily used to develop and initially test the developed methods. Additional phenethylamines from other subclasses, as well as cathinones and tryptamines, were also purchased to further test the robustness of the methods developed. All compounds included in this study are shown in Appendix 1.

Each standard was analyzed by gas chromatography-mass spectrometry, with both a single quadrupole mass analyzer (GC-QMS) to generate low-resolution data and with a time-of-flight mass analyzer (GC-TOFMS) to generate high-resolution data. Instrument and method parameters are given in Appendix 2. The low-resolution data were used to refine and test the statistical comparison method and to develop the first characterization scheme (Goals 1 and 2), while the high-resolution data were used to define mass defect filters and hence, increase specificity of the characterization scheme (Goal 3). The data processing methods are included in Appendix 3.

Project Findings

Goal 1. Further develop and validate a method for the statistical comparison of mass spectra

A statistical approach for the comparison of two mass spectra was previously developed in our laboratory [1]. The method is automated in a Microsoft Excel worksheet (Microsoft Corp., Redmond, WA) and uses the unequal variance *t*-test to compare the abundance of corresponding ions in the two spectra across the mass scan range. In doing so, the null hypothesis (H_0) tested is that the abundances are statistically similar whereas, the alternative hypothesis (H_1) is that the abundances are statistically distinct. If H_0 is accepted at all *m/z* values in the mass scan range, then the two spectra are statistically indistinguishable and a random-match probability (RMP) is calculated. The RMP provides the probability that the mass spectral fragmentation pattern in question occurred by random chance alone. If H_1 is accepted at any *m/z* value in the mass scan range, then the two spectra are statistically distinguishable and in these cases, the number and identity of the ions responsible for discrimination are determined.

In this project, the original Excel worksheet was modified to minimize inadvertent errors. Logical tests were incorporated to highlight ions that round to the same integer value and residuals were then calculated to determine if these ions were, in fact, the same ion [2]. The RMP calculation was also modified to include a maximum probability by assuming that the occurrence of every ion is a dependent event. With this modification, a range of RMP values is now reported to provide a more realistic estimate of the RMP.

Spectra of compounds within each phenethylamine subclass and within the tryptamine class were compared using the modified statistical comparison template. For each comparison, one spectrum of the sample was compared to triplicate spectra of the reference standard. For the four APB-phenethylamine isomers (Appendix 4, Table A4.1), 6-APB was distinguished from the other three isomers at the 99.9% confidence level, with one ion (*m/z* 132) responsible for

discrimination. Casale and Hays previously reported that 6-APB could be distinguished from 4-, 5-, and 7-APB based on the ratio of *m/z* 131:132 – in 6-APB, the ratio of these two ions is approximately 1.2:1 whereas, in the other three isomers, this ratio is approximately 2.6:1 [3]. Thus, the statistical comparison method is sufficiently sensitive to distinguish 6-APB from the other three isomers based on the difference in abundance of this one ion.

For the 2C-phenethylamines (Appendix 4, Table A4.3), each sample was statistically indistinguishable from the corresponding reference spectra at the 99.9% confidence level, with zero discriminating ions. Discrimination of each 2C-phenethylamine from all other reference spectra was observed, with the number of discriminating ions ranging from 2 (for comparison of 2C-C to 2C-I) to 20 (for comparison of 2C-N to 2C-C). For the NBOMe-phenethylamines (Appendix 4, Table A4.5), there was unexpected association of 25B-NBOMe, 25C-NBOMe, and 25N-NBOMe at the 99.9% confidence level. For these comparisons, the calculated *t*-values for certain ions were close to the critical value (t_{crit} = 31.6 at 99.9% confidence level, 2 degrees of freedom) with the result that discrimination was not achieved (*i.e.*, H_0 accepted). However, at the 99% confidence level, these three compounds were discriminated due to the higher t_{crit} value (9.9 at 99% confidence level, 2 degrees of freedom).

For the tryptamines, expected association and discrimination was observed at the 99.9% confidence level (Appendix 4, Table A4.7), with two exceptions: comparison of 4-methyl-α-ethyl-tryptamine to itself and comparison of 5-methoxy-dimethyltryptamine to itself. For 4-methyl-α-ethyl-tryptamine, the two discriminating ions were *m/z* 86 and *m/z* 87; however, this is a rounding error in the statistical comparison template. In the sample, the ion was measured as *m/z* 86.4, which was rounded down to *m/z* 86 whereas, in the standards, the ion was measured as *m/z* 86.5, which was rounded up to *m/z* 87. Although these are likely the same ion, the rounding

error means that the comparison method reads these as two different ions, leading to discrimination. For 5-methoxy-dimethyltryptamine, the discriminating ion was the molecular ion at *m/z* 218, with the difference in abundance between the sample and the two standards leading to discrimination.

In the statistical comparison method, Pearson product-moment correlation (PPMC) coefficients are used as a measure of the degree of similarity (or otherwise) of the two spectra being compared. PPMC coefficients are calculated based on the relative intensity spectra as well as the binary spectra that are generated for subsequent calculation of the random-match probability. To generate the binary spectrum, a zero is returned for a given *m/z* value if the ion is not present or the *m/z* value corresponds to a known impurity ion (*e.g.*, *m/z* 207 or 281, which are common column bleed ions).

PPMC coefficients based on binary spectra are shown in Appendix 4 for comparison of the APB-, NBOMe-, and 2C-phenethylamines, as well as the tryptamines. For the three NBOMe-phenethylamines (25B-, 25C-, and 25N-NBOMe) previously associated at the 99.9% confidence level, the PPMC coefficients calculated based on the binary spectra offered distinction (Appendix 4, Table A4.6), with higher PPMC coefficients observed in cases of true association. Further, for the tryptamines (Appendix 4, Table A4.8), PPMC coefficients indicated strong correlation between spectra of 4-methyl-α-ethyl-tryptamine and between spectra of 5-methoxy-dimethyltryptamine, both of which were previously distinguished based on one discriminating ion. Thus, the PPMC coefficient based on binary spectra can be used as an additional tool to increase confidence in the association and discrimination observed.

Currently, the statistical comparison method is being applied to compare mass spectra of positional isomers and once completed, error rates associated with the method will be evaluated.

Goal 2. Develop a spectral interpretation scheme for structural class characterization of synthetic phenethylamines based on gas chromatography-mass spectrometry

Spectra obtained for each compound by GC-QMS and GC-TOFMS were initially probed to identify characteristic mass spectral features. Using the GC-TOFMS data, elemental formulae for characteristic ions were assigned based on the accurate mass and ion structures corresponding to these formulae were proposed. Characteristic spectral features included isotope ratios to indicate the presence of halogen substituents, even-mass molecular ion indicating the presence of a nitro-group substitution, and neutral losses common to the 2C- and NBOMe-phenethylamines. The spectra obtained by GC-QMS were then interrogated to confirm the previously identified characteristic spectral features and neutral losses.

These features were then incorporated into a flow-chart style characterization scheme. The scheme is designed based on the low-resolution data obtained by GC-QMS as these data are more conventionally generated in forensic laboratories. However, to highlight the utility of high-resolution methods, the scheme also incorporates the additional information, in the form of elemental formulae and mass defect filters (see Goal 3) obtained from accurate mass data. The mass spectra of several phenethylamine and non-phenethylamine compounds were then characterized using the scheme in both low-resolution and high-resolution mode. The full scheme is shown as Appendix 5 and characterization examples based on the low-resolution version of the scheme are given in Appendix 6.

The low-resolution spectra were also used to develop multivariate statistical classification models that, in the future, may also be incorporated into the characterization scheme. Principal components analysis (PCA) was first used as a variable selection method to identify those variables (*i.e.*, *m/z* values) that were discriminating among the phenethylamine subclasses. It should be noted that the tryptamines were also included in the data set to demonstrate the ability

to distinguish phenethylamines from structurally similar classes. A total of 9 variables was selected and used to develop linear discriminant analysis (LDA) models for classification. Following internal validation of the model, a test set of compounds was classified, resulting in a successful classification rate of 87%. A second LDA model was developed, this time using variables selected *via* an informed chemical approach, rather than using the unsupervised PCA method. In this approach, ions known to be characteristic of each compound class were selected, resulting in a total of 13 variables, and the subsequent LDA model generated a successful classification rate of 93%. The variables selected using each method and the LDA classification plots are shown in Appendix 7.

Goal 3. Develop mass defect filters as a tool for rapid classification of synthetic phenethylamines

The accurate mass data obtained by GC-TOFMS were also used to develop mass defect filters characteristic of each phenethylamine subclass. The absolute mass defect of an ion is calculated as the difference between the nominal and the accurate mass of the ion. Three filters were initially developed, one for each phenethylamine subclass. For each filter, representative compounds from that subclass formed the training set and the absolute mass defect of each molecular ion in the training set compounds was calculated. The mean mass defect of the training set compounds was determined and an associated tolerance was calculated as a confidence interval, which was calculated at the confidence level encompassing approximately two standard deviations around the mean mass defect. The mass defect filters were then tested to assess the efficacy of the filters in correctly characterizing compounds according to structural subclass.

Compounds with a halogen, sulfur, or nitro substituent initially fell outside the filters due to the large negative mass defect associated with these substituents. As the nature of the

substituent is often determinable from the mass spectrum (*e.g.*, doublet of peaks, in an approximate 3:1 ratio, spaced 2 Da apart indicates presence of Cl substituent), the mass of the molecular ion can be adjusted to account for the substituent and the mass defect can be re-calculated based on this adjusted mass. With this adjustment, the mass defects of compounds with non-alkyl substitutions were within the defined filters.

However, initial filters based on absolute mass defect failed to distinguish compounds within the three phenethylamine subclasses and perhaps more importantly, failed to distinguish phenethylamines from cathinones. Hence, additional mass defect filters based on Kendrick mass defect were investigated. The Kendrick mass of an ion is calculated according to Eq. 1 and the Kendrick mass defect (KMD) is further calculated according to Eq. 2 [4, 5].

$$Kendrick\ Mass = IUPAC\ mass\ \times \left(\frac{14.00000}{14.01565}\right) \qquad \text{Eq. 1}$$

$$Kendrick\ Mass\ Defect = Nominal\ KM - Exact\ KM \qquad \text{Eq. 2}$$

The Kendrick mass scale normalizes the mass of a methylene group to exactly 14.0000 Da, with the result that members of a homologous series that differ only in the number of methylene groups have the same theoretical KMD. Thus, KMD filters based on the molecular ions of compounds in the training sets were defined and tested. To increase specificity, additional KMD filters were developed based on fragment ions remaining after common neutral losses in the 2C- and NBOMe-phenethylamines.

The KMD filter based on the molecular ion of the APB-phenethylamines was defined as 96.5 ± 0.5 mDa. The APB-phenethylamine in the test set was correctly characterized as an APB-phenethylamine using this filter. Further, correct distinction of 2C-phenethylamines, NBOMe-phenethylamines, structurally similar phenethylamines, and cathinones was demonstrated.

Similarly, the KMD filter for NBOMe-phenethylamines based on the molecular ion was defined as 171.5 ± 3.2 mDa and again, correct characterization was achieved with this filter.

For the 2C-phenethylamines, the KMD filter based on the molecular ion was defined as 92.1 ± 2.2 mDa. Other 2C-phenethylamines in the test set were correctly characterized, with distinction from APB-phenethylamines, NBOMe-phenethylamines, structurally similar phenethylamines, and cathinones. However, there were two exceptions: the KMDs of the molecular ions of two of the structurally similar phenethylamines, 2,5-dimethoxy-4-methylamphetamine and 4-bromo-2,5-dimethoxyamphetamine, were within the range defined by the filter (Figure 1). Although neither of these compounds is defined as a 2C-phenethylamine, both compounds are in fact in a homologous series with the 2C-phenethylamines, with additional methyl substitutions on the α-carbon (structures shown in Appendix 1).

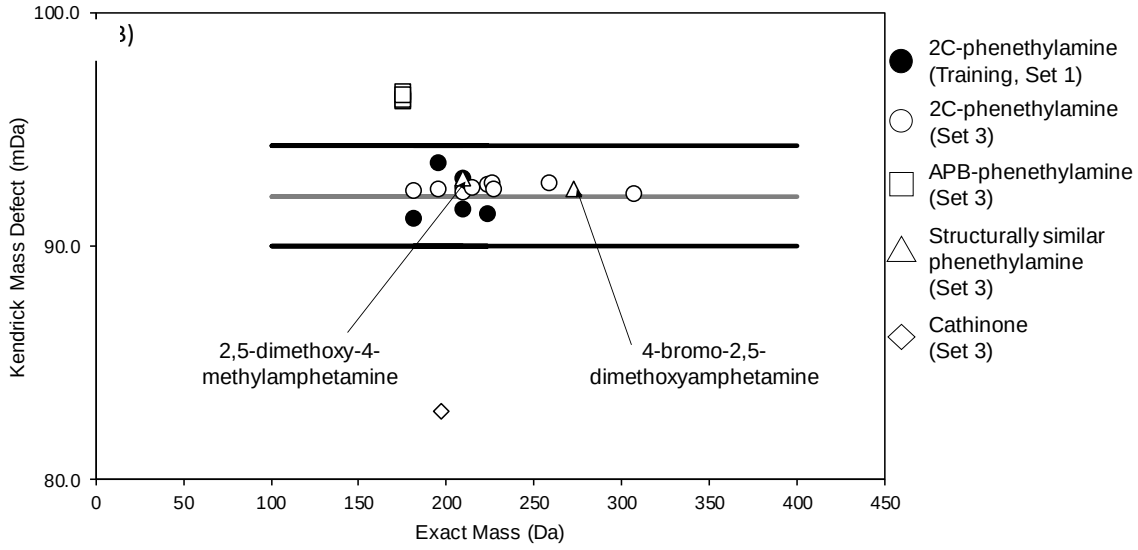

Figure 1. Kendrick mass defect filter defined at 92.1 ± 2.2 mDa based on the molecular ion of 2C-phenethylamines in the training set showing test set compounds in the third test set.

To increase specificity, additional KMD filters were defined based on the fragment ions remaining after the two common neutral losses observed in the 2C-phenethylamines: $[M\text{-}CH_3N]^+$ and $[M\text{-}C_2H_6NO]^+$. For $[M\text{-}CH_3N]^+$, the filter was defined as 85.4 ± 2.4 mDa at the 99.9%

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confidence level and for $[M-C_2H_6NO]^+$, the filter was defined as 69.5 ± 4.0 mDa at the 99% confidence level. These two losses were not observed in 2,5-dimethoxy-4-methylamphetamine and 4-bromo-2,5-dimethoxyamphetamine and thus, incorporating filters based on ions remaining after common neutral losses increased the specificity and enabled distinction of the structurally similar phenethylamines from the 2C-phenethylamines. Thus, correct characterization of compounds to the corresponding subclass with distinction from other subclasses was demonstrated using the KMD filters based on the molecular ion as well as fragment ions remaining after the common neutral losses. Examples of characterization based on the high-resolution version of the scheme are given in Appendix 8.

Implications for Criminal Justice Policy and Practice in the US

In this research, tools to enhance the identification and characterization of synthetic designer drugs have been developed and their successful application demonstrated. A mass spectral comparison method provides a statistical assessment of the veracity of identifications being made based on comparison of two mass spectra. A characterization scheme has been developed that uses mass spectral features to assign compounds to one of the three synthetic phenethylamine subclasses. Further, the characterization scheme includes a high-resolution mode, incorporating mass defect filters to enhance specificity in characterization and to demonstrate the utility of high-resolution mass spectrometry for characterization of designer drugs. The tools developed in this work can be used to increase confidence in the identification and characterization of synthetic designer drugs, including newly emerging analogs for which no reference material exists.

References

[1] M.A. Bodnar-Willard, R. Waddell Smith, V.L. McGuffin, Statistical approach to establish equivalence of unabbreviated mass spectra, *Rapid Communications in Mass Spectrometry* 28 (2014) 83-95.

[2] M.A. Bodnar-Willard, V.L. McGuffin, R. Waddell Smith, Statistical comparison of mass spectra for identification of amphetamine-type stimulants, *Forensic Science International* 270 (2017) 111-120.

[3] J.F. Casale, P.A. Hayes, The characterization of 6-(2-aminopropyl)benzofuran and differentiation from its 4-, 5-, and 7-positional analogues *Microgram Journal* 9 (2012) 61-74.

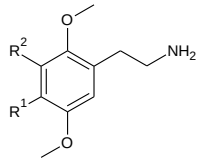
[4] C.A. Hughey, C.L. Hendrickson, R.P. Rodgers, A.G. Marshall, Kendrick mass defect spectrum: A compact visual analysis for ultrahigh-resolution broadband mass spectra, *Analytical Chemistry* 73 (2001) 4676-4681.

[5] L. Sleno, The use of mass defect in modern mass spectrometry, *Journal of Mass Spectrometry* 47 (2012) 226-236.

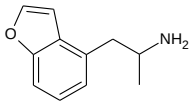
Appendices

Appendix 1. Compounds included in this study

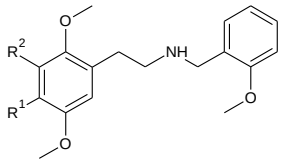
Appendix 1A. Phenethylamines



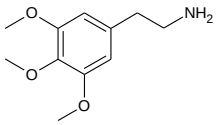
2C-phenethylamine
(see Table 1 for substitutions)



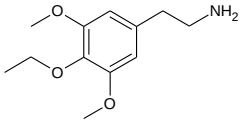
4-APB



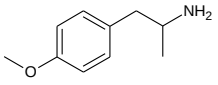
NBOMe-phenethylamine
(see Table 2 for substitutions)



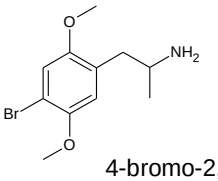
Mescaline



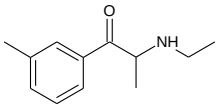
Escaline



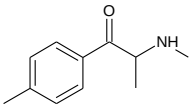
4-methoxyamphetamine



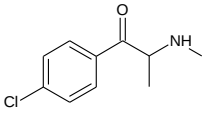
4-bromo-2,5-
dimethoxyamphetamine



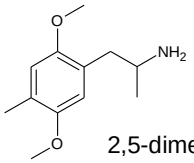
3-methyl-
ethcathinone



4-methyl-
methcathinone



4-chloro-
methcathinone



2,5-dimethoxy-4-
methylamphetamine

Table 1. 2C-phenethylamines included in this work

Compound	R^1	R^2	Compound	R^1	R^2
2C-B	Br	H	2C-H	H	H
2C-C	Cl	H	2C-I	I	H
2C-D	CH_3	H	2C-N	NO_2	H
2C-E	C_2H_5	H	2C-P	C_3H_7	H
2C-G	CH_3	CH_3	2C-T	SCH_3	H

Table 2. NBOMe-phenethylamines included in this work

Compound	R^1	R^2	Compound	R^1	R^2
25C-NBOMe	Cl	H	25H-NBOMe	H	H
25D-NBOMe	CH_3	H	25N-NBOMe	NO_2	H
25E-NBOMe	C_2H_5	H	25T-7-NBOMe	SC_3H_7	H
25G-NBOMe	CH_3	CH_3			

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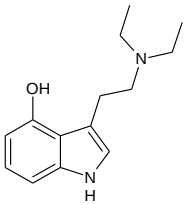
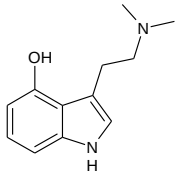
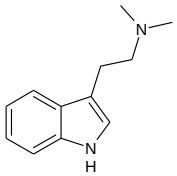
Appendix 1B. Tryptamines



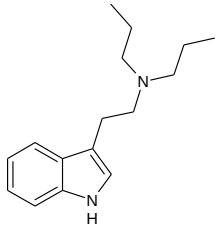
4-hydroxy-diethyltryptamine
(4-hydroxy-DET)



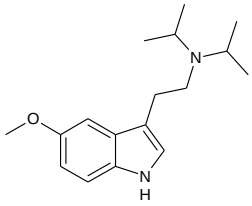
4-hydroxy-dimethyltryptamine
(4-hydroxy-DMT)



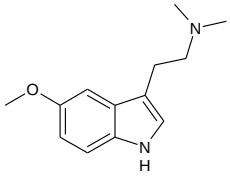
N,N-dimethyltryptamine
(N,N-DMT)



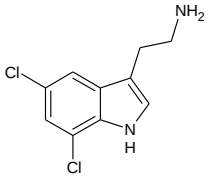
N,N-dipropyltryptamine
(DPT)



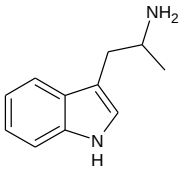
5-methoxy-N,N-diisopropyltryptamine
(5-MeO-DiPT)



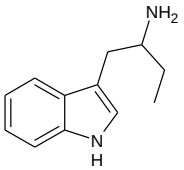
5-methoxy-N,N-dimethyltryptamine
(5-MeO-DMT)



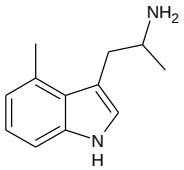
5,7-dichlorotryptamine
(5,7-DCT)



α-methyl tryptamine
(α-MT)



α-ethyl tryptamine
(α-ET)



4-methyl-α-ethyl tryptamine
(4-methyl-α-ET)

Appendix 2: GC-MS instrument and method parameters

All standards were analyzed by both low-resolution and high-resolution GC-MS and were prepared in methanol (ACS grade, Sigma-Aldrich, St. Louis, MO) at a concentration of 1 mg/mL prior to analysis.

For low-resolution GC-MS, an Agilent 6890N gas chromatograph coupled to an Agilent 5975C mass spectrometer with an Agilent 7683B injector (Agilent Technologies, Santa Clara, CA) was used. The GC contained a capillary column with a 5% diphenyl-95% dimethylpolysiloxane stationary phase (RTX-5, 30 m x 0.25 mm x 0.25 μm, Restek, Bellefonte, PA). The injection port temperature was 250 °C and 1 μL of each standard was injected in splitless mode. The carrier gas was ultra-high purity helium at a flow rate of 1 mL/min. The oven temperature program was as follows: 40 °C for 1 min, 20 °C/min to 280 °C with a final hold of 7 min. The transfer line temperature was 280 °C. Electron ionization at 70 eV was used, the ion source temperature was 230 °C and the mass analyzer temperature was 150 °C. The mass scan range was 35 – 550 u, with a scan rate of 2.83 scans/s.

For analysis by high-resolution GC-MS, the standards were divided into three sets. Set 1 contained 2C-phenethylamines (2C-B, 2C-D, 2C-E, 2C-G, 2C-H, 2C-P, and 2C-T), 3C-phenethylamines (mescaline and escaline), two cathinones (3-methylethcathinone and 4-methylmethcathinone). Standards in this set were used to develop and initially test the mass defect filters. These standards were analyzed on an Agilent 6890N gas chromatograph equipped with an Agilent 7683B autosampler (Agilent Technologies, Santa Clara, CA) and coupled to a Waters GCT Premier TOFMS (Waters Corporation, Milford, MA). The GC contained a capillary column with a 5% diphenyl-95% dimethylpolysiloxane stationary phase (RTX-5, 30 m x 0.25 mm x 0.25 μm, Restek, Bellefonte, PA). The injection port temperature was held at 210 °C and 1

μL of each standard was injected. Appropriate split ratios (splitless, 50:1, or 100:1) were used to ensure acceptable chromatographic peak shape for each standard. Ultra-high purity helium was used as the carrier gas at a constant flow rate of 1.3 mL/min. The GC oven temperature program was as follows: 50 °C for 1 min, then 15 °C/min to 280 °C, with a final hold of 2 min. The transfer line temperature was 280 °C and the mass spectrometer was operated in electron ionization mode (70 eV), with the ion source temperature set to 180 °C. Mass spectra were collected across the range *m/z* 35 – 300, with a scan rate of 5.00 scans/s, and a mass resolution (M/ΔM) at full width half-maximum of approximately 6,000 for the m/z 219.9856 fragment ion from the mass calibrant perfluorotributylamine (PFTBA), which was constantly infused throughout the analysis as a lock mass to ensure good mass accuracy.

The second set of standards consisted of NBOMe-phenethylamines that were all analyzed on an Agilent 7890N gas chromatograph (Agilent Technologies) equipped with a Gerstel MPS2 autosampler (GERSTEL, Inc., Linthicum Heights, MD) and coupled to a LECO Pegasus HRT mass spectrometer (LECO Corp., St. Joseph, MI). The GC contained a capillary column with a 1,4-bis(dimethylsiloxy)phenylene dimethyl-polysiloxane stationary phase (Rxi-5sil ms, 20 m x 0.18 mm x 0.18 μm, Restek, Bellefonte, PA). The injection port temperature was held at 250 °C and 1 μL of each standard was injected with a 100:1 split ratio. Ultra-high purity helium was used as the carrier gas at a constant flow rate of 0.85 mL/min. The GC oven was temperature programmed as follows: 60 °C for 0.5 min, then 36 °C/min to 340 °C with a final hold of 4 min. The transfer line temperature was 295 °C and the mass spectrometer was operated in electron ionization mode (70 eV), with the ion source temperature set to 250 °C. Mass spectra were collected across the range *m/z* 35 – 510, with a scan rate of 10 scans/s, and a mass resolution

(M/ΔM) at full width half-maximum of up to 50,000. A constant infusion of PFTBA was used during each analysis to ensure good mass accuracy via lock mass correction.

The third set of standards consisted of APB-phenethylamines (4-, 5-, 6-, and 7-APB), 2C-phenethylamines (2C-B, 2C-C, 2C-D, 2C-E, 2C-G, 2C-H, 2C-I, 2C-N, 2C-P, 2C-T), structurally similar phenethylamines (2,5-dimethoxy-4-methylamphetamine, 4-bromo-2,5-dimethoxyamphetamine, 4-methoxyamphetamine), and one cathinone (4-chloro-methcathinone).

Standards in Set 3 were also analyzed on the LECO GC-HRT instrument, although under different conditions than for the second set of standards. The GC contained a capillary column with a 5% diphenyl-95% dimethylpolysiloxane stationary phase (Rxi-5ms, 28.5 m x 0.25 mm x 0.25 μm, Restek, Bellefonte, PA). The injection port temperature was held at 250 °C and 1 μL of each standard was injected with a 150:1 split ratio. Ultra-high purity helium was used as the carrier gas at a constant flow rate of 1 mL/min. The GC oven was temperature programmed as follows: 40 °C for 1 min, then 20 °C/min to 280 °C with a final hold of 7 min. The transfer line temperature was 275 °C and the mass spectrometer was operated in electron ionization mode (70 eV), with the ion source temperature set to 250 °C. Mass spectra were collected across the range *m/z* 35 – 510, with a scan rate of 4 scans/s, and a mass resolution (M/ΔM) at full width half-maximum of up to 50,000. As before, a constant infusion of PFTBA during each analysis was used to ensure good mass accuracy via lock mass correction.

Appendix 3: Mass spectral data processing methods

Spectra collected on the Waters GCT Premier were processed using Waters MassLynx software (version 4.1, Waters Corporation) while those spectra collected on the LECO Pegasus HRT instruments were processed using LECO ChromaTOF software (version 4.2.3.1, LECO Corporation). In all cases, spectra were obtained by scanning across the chromatographic peak and were subsequently processed to subtract background and calibrant ions. The elemental composition tools in each software program were used to assign elemental formulae to all ions, within a tolerance of 50 ppm for spectra collected using the Waters GCT Premier and within 10 mDa for spectra collected using the LECO Pegasus HRT (note that 10 mDa is equivalent to 50 ppm for *m/z* 200). Spectral lists were then exported to Microsoft Excel (version 12.0, Microsoft Corporation, Redmond, WA) and each spectrum was normalized to the abundance of the corresponding base peak.

Appendix 4 Statistical comparison of APB-phenethylamines, 2C-phenethylamines, NBOMe-phenethylamines, and tryptamines

Table A4.1. Number of discriminating ions for comparison of APB-phenethylamines at the 99.9% confidence level

Sample	Reference Standard			
	4-APB	**5-APB**	**6-APB**	**7-APB**
4-APB	**0**	0	0	0
5-APB	0	**0**	1	0
6-APB	1	1	**0**	1
7-APB	0	0	1	**0**

Bold entries indicate comparison of corresponding spectra. As the APB-phenethylamines are positional isomers, there is association between non-corresponding spectra.

Table A4.2. Pearson product-moment correlation coefficients for comparison of APB-phenethylamines based on binary mass spectra

Sample	Reference Standard			
	4-APB	**5-APB**	**6-APB**	**7-APB**
4-APB	**0.9298**	0.9298	0.9490	0.9298
5-APB	0.9313	**0.9657**	0.9832	0.9313
6-APB	0.9313	0.9313	**0.9494**	0.8970
7-APB	0.9672	0.9672	0.9508	**0.9338**

Bold entries indicate comparison of corresponding spectra. As the APB-phenethylamines are positional isomers, high coefficients are observed for all comparisons, ranging from 0.8970 – 0.9832

Table A4.3. Number of discriminating ions for comparison of 2C-phenethylamines at the 99.9% confidence level

Sample	Reference Standard									
	2C-B	**2C-C**	**2C-D**	**2C-E**	**2C-G**	**2C-H**	**2C-I**	**2C-N**	**2C-P**	**2C-T**
2C-B	**0**	3	4	4	6	5	3	10	6	8
2C-C	3	**0**	3	4	5	3	2	12	6	7
2C-D	4	3	**0**	4	5	4	3	11	7	8
2C-E	4	4	4	**0**	3	5	4	12	4	7
2C-G	6	5	5	2	**0**	5	5	7	6	8
2C-H	5	3	4	4	6	**0**	3	9	6	6
2C-I	3	2	3	4	5	3	**0**	11	5	6
2C-N	11	20	13	10	8	9	13	**0**	16	11
2C-P	6	8	7	4	7	6	5	15	**0**	10
2C-T	8	7	8	8	9	6	6	10	9	**0**

Bold entries indicate comparison of corresponding spectra.

Table A4.4. Pearson product-moment correlation coefficients for comparison of 2C-phenethylamines based on binary mass spectra

Sample	Reference Standard									
	2C-B	**2C-C**	**2C-D**	**2C-E**	**2C-G**	**2C-H**	**2C-I**	**2C-N**	**2C-P**	**2C-T**
2C-B	**0.9526**	0.5730	0.5279	0.4393	0.4254	0.4721	0.4926	0.5166	0.3949	0.3973
2C-C	0.5794	**0.9545**	0.3987	0.3118	0.2864	0.3977	0.4418	0.3847	0.2912	0.3253
2C-D	0.4762	0.4009	**0.9624**	0.6998	0.7061	0.6370	0.5333	0.7061	0.6907	0.5406
2C-E	0.4463	0.3132	0.6989	**0.9885**	0.8467	0.6289	0.4544	0.5802	0.7664	0.4676
2C-G	0.4133	0.2872	0.6812	0.8457	**0.9682**	0.5439	0.4248	0.5436	0.7003	0.3951
2C-H	0.5163	0.4009	0.6860	0.6273	0.5443	**0.9484**	0.5852	0.7061	0.4809	0.5624
2C-I	0.5045	0.4729	0.5177	0.4654	0.4357	0.5673	**0.9607**	0.5525	0.4177	0.3293
2C-N	0.5348	0.3800	0.7193	0.5945	0.5579	0.7028	0.5375	**1.000**	0.5224	0.5676
2C-P	0.3948	0.2822	0.7293	0.7609	0.7176	0.5155	0.3931	0.5432	**0.9456**	0.3926
2C-T	0.4377	0.3663	0.5639	0.4914	0.4581	0.5685	0.3713	0.5753	0.3857	**0.9818**

Bold entries indicate comparison of corresponding spectra. Comparisons between non-corresponding spectra result in coefficients of 0.8467 or less, whereas, comparisons between corresponding spectra result in coefficients of greater than 0.9456.

Table A4.5. Number of discriminating ions for comparison of NBOMe-phenethylamines at the 99.9% confidence level

Sample	Reference Standard								
	25B-NBOMe	**25C-NBOMe**	**25D-NBOMe**	**25E-NBOMe**	**25G-NBOMe**	**25H-NBOMe**	**25N-NBOMe**	**25P-NBOMe**	**25T-NBOMe**
25B-NBOMe	0	0 (46)	5	9	6	5	0 (27)	7	2
25C-NBOMe	0 (48)	0	6	9	4	4	0 (36)	6	2
25D-NBOMe	3	3	0	8	5	5	2	7	3
25E-NBOMe	9	8	8	0	5	10	5	10	8
25G-NBOMe	7	7	7	5	0	9	6	9	8
25H-NBOMe	5	4	7	11	10	0	4	10	3
25N-NBOMe	0 (27)	0 (36)	4	6	6	4	0	6	1
25P-NBOMe	7	5	9	10	8	10	5	0	7
25T-NBOMe	2	3	5	9	7	5	1	9	0

Bold entries indicate comparison of corresponding spectra. Numbers in parentheses indicate number of discriminating ions at the 99% confidence level.

Table A4.6. Pearson product-moment correlation coefficients for comparison of NBOMe-phenethylamines based on binary mass spectra

Sample	Reference Standard								
	25B-NBOMe	**25C-NBOMe**	**25D-NBOMe**	**25E-NBOMe**	**25G-NBOMe**	**25H-NBOMe**	**25N-NBOMe**	**25P-NBOMe**	**25T-NBOMe**
25B-NBOMe	**0.9803**	0.5264	0.4917	0.4635	0.4992	0.4851	0.6358	0.4805	0.4972
25C-NBOMe	0.5800	**0.9563**	0.4851	0.4232	0.4561	0.5191	0.5929	0.4389	0.4872
25D-NBOMe	0.5137	0.4958	**0.9803**	0.6528	0.6619	0.5800	0.6906	0.6385	0.5511
25E-NBOMe	0.4464	0.4309	0.6498	**0.9522**	0.8827	0.5062	0.5679	0.6222	0.4422
25G-NBOMe	0.4915	0.4389	0.6972	0.8709	**0.9662**	0.5153	0.6154	0.6333	0.4504
25H-NBOMe	0.4958	0.4754	0.5678	0.4975	0.5348	**1.0000**	0.5929	0.5153	0.6656
25N-NBOMe	0.6493	0.5500	0.7169	0.5583	0.6380	0.5929	**1.0000**	0.6154	0.5634
25P-NBOMe	0.4826	0.4309	0.6142	0.6966	0.6457	0.5062	0.6048	**0.9837**	0.4422
25T-NBOMe	0.5255	0.4642	0.5547	0.4855	0.4836	0.6357	0.5794	0.4656	**0.9572**

Bold entries indicate comparison of corresponding spectra. Comparisons between non-corresponding spectra result in coefficients of 0.8827 or less, whereas, comparisons between corresponding spectra result in coefficients of greater than 0.9522. Entries in red indicate previous comparisons that were associated at the 99.9% confidence level.

Table A4.7. Number of discriminating ions for comparison of tryptamines at the 99.9% confidence level

Sample	Reference Standard									
	4-hydroxy-DET	**4-methyl-α-ET**	**5,7-DCT**	**α-ET**	**α-MT**	**4-hydroxy-DMT**	**5-MeO-DiPT**	**5-MeO-DMT**	**DPT**	***N*,*N*-DMT**
4-hydroxy-DET	**0**	24	30	4	7	10	12	16	13	18
4-methyl-α-ET	24	**2**	44	3	9	32	58	40	53	40
5,7-DCT	28	44	**0**	18	20	34	43	46	39	39
α-ET	3	3	18	**0**	1	2	4	2	3	1
α-MT	4	9	20	1	**0**	6	6	5	5	5
4-hydroxy-DMT	11	31	33	3	6	**0**	21	13	23	19
5-MeO-DiPT	12	55	41	5	7	20	**0**	29	23	46
5-MeO-DMT	16	45	45	3	6	13	30	**1**	35	34
DPT	15	41	41	3	5	21	21	34	**0**	39
***N*,*N*-DMT**	19	40	40	1	5	18	47	35	41	**0**

Bold entries indicate comparison of corresponding spectra. Entries bolded in red indicate unexpected discrimination. Abbreviations are as follows: 4-hydroxy-DET = 4-hydroxy-diethyltryptamine; 4-methyl-α-ET = 4-methyl-α-ethyl-tryptamine; 5,7-DCT = 5,7-dichlorotryptamine; α-ET = α-ethyltryptamine; α-MT = α-methyltryptamine; 4-hydroxy-DMT = 4-hydroxy-dimethyltryptamine; 5-MeO-DiPT = 5-methoxy-diisopropyltryptamine; 5-methoxy-DMT = 5-MeO-dimethyltryptamine; DPT = *N*,*N*-Dipropyltryptamine; *N*,*N*-DMT = *N*,*N*-dimethyltryptamine.

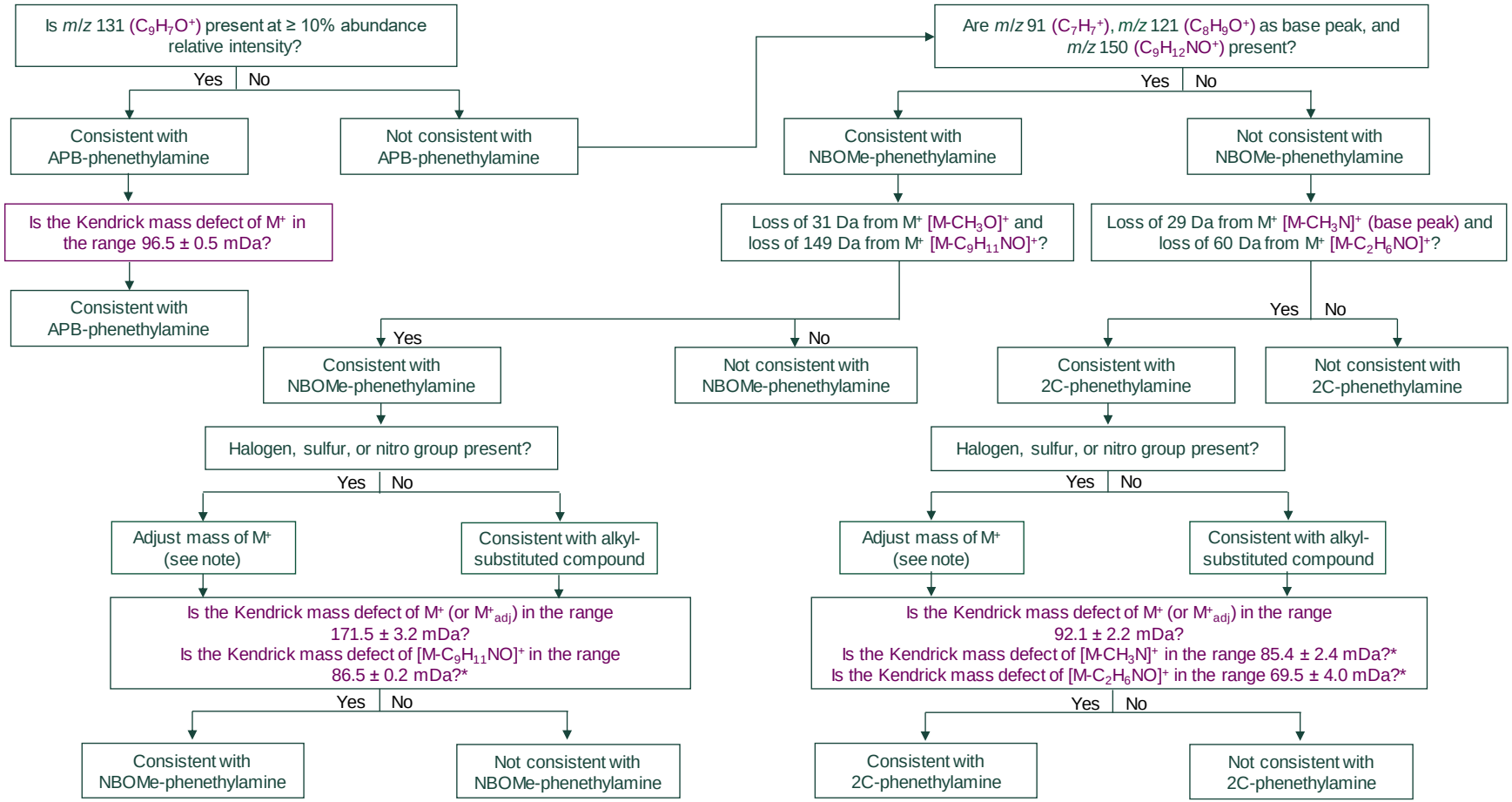
Table A4.8. Pearson product-moment correlation coefficients for comparison of tryptamines based on binary mass spectra

Sample	Reference Standard									
	4-hydroxy-DET	**4-methyl-α-ET**	**5,7-DCT**	**α-ET**	**α-MT**	**4-hydroxy-DMT**	**5-MeO-DiPT**	**5-MeO-DMT**	**DPT**	***N*,*N*-DMT**
4-hydroxy-DET	**0.8670**	0.4096	0.0379	0.3348	0.2813	0.5883	0.4014	0.3918	0.2719	0.2879
4-methyl-α-ET	0.3909	**0.9646**	0.1272	0.4942	0.4039	0.4784	0.3250	0.5736	0.4789	0.6284
5,7-DCT	0.0462	0.1250	**0.9473**	0.0516	0.1774	0.0551	-0.0074	0.0119	0.0769	0.1114
α-ET	0.2519	0.4643	0.0587	**0.8486**	0.4973	0.3790	0.2090	0.3464	0.3673	0.4861
α-MT	0.1571	0.3375	0.1988	0.5142	**0.8601**	0.3930	0.2173	0.3594	0.3804	0.5571
4-hydroxy-DMT	0.4981	0.4886	0.0611	0.4204	0.4671	**0.9629**	0.3389	0.6735	0.3617	0.5699
5-MeO-DiPT	0.4113	0.3135	-0.0103	0.2514	0.2514	0.3661	**0.9708**	0.5243	0.4674	0.2657
5-MeO-DMT	0.4159	0.5736	0.0134	0.3819	0.3819	0.6604	0.4927	**0.9603**	0.4622	0.6238
DPT	0.2803	0.4647	0.0727	0.4541	0.4541	0.3867	0.4690	0.4485	**0.9737**	0.5403
***N*,*N*-DMT**	0.2986	0.6135	0.1351	0.5095	0.6099	0.5452	0.2683	0.6091	0.5430	**0.9376**

Bold entries indicate comparison of corresponding spectra. Comparisons between non-corresponding spectra result in coefficients of 0.6735 or less, whereas, comparisons between corresponding spectra result in coefficients of greater than 0.8486.

Abbreviations are as follows: 4-hydroxy-DET = 4-hydroxy-diethyltryptamine; 4-methyl-α-ET = 4-methyl-α-ethyl-tryptamine; 5,7-DCT = 5,7-dichlorotryptamine; α-ET = α-ethyltryptamine; α-MT = α-methyltryptamine; 4-hydroxy-DMT = 4-hydroxy-dimethyltryptamine; 5-MeO-DiPT = 5-methoxy-diisopropyltryptamine; 5-methoxy-DMT = 5-MeO-dimethyltryptamine; DPT = *N*,*N*-Dipropyltryptamine; *N*,*N*-DMT = *N*,*N*-dimethyltryptamine.

Appendix 5: Characterization scheme to assign compounds to APB-, 2C-, or NBOMe-phenethylamine subclasses



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Notes for adjusting mass of $[M]^+$

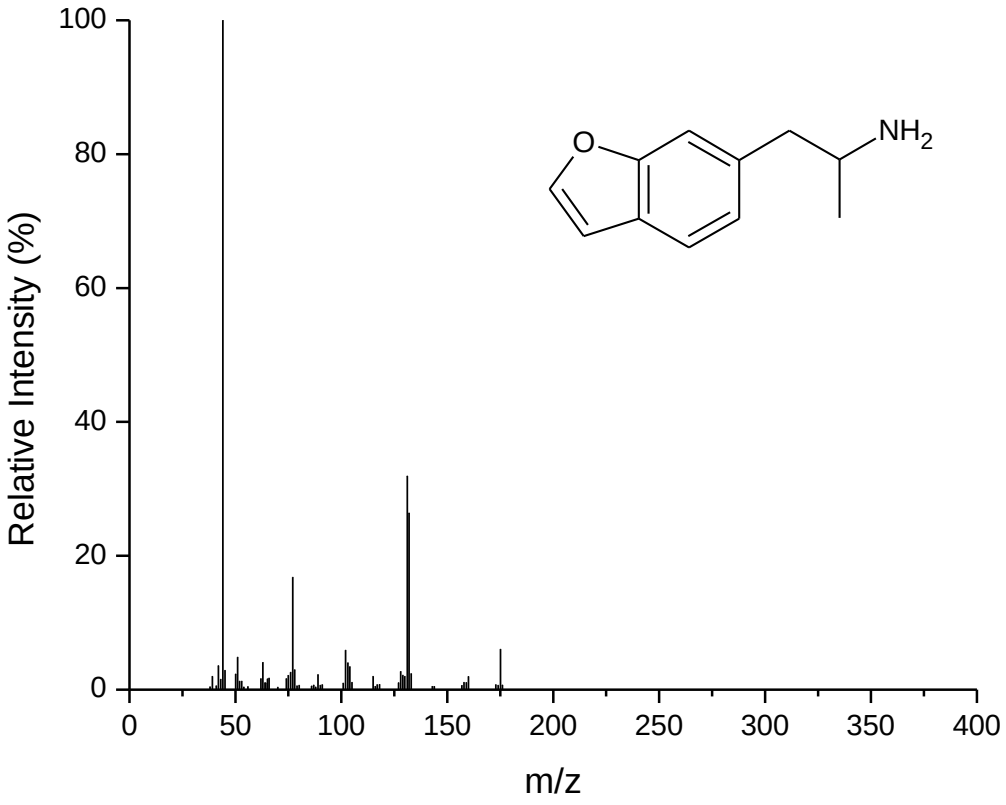
If Br, Cl, or I are present, subtract the mass of the halogen (78.9183, 34.9689, 126.9045 Da, respectively) from the mass of the molecular ion and add the mass of hydrogen (1.0078 Da).

If the compound has an even-mass M^+, subtract the mass of NO_2 (45.9929 Da) and add the mass of hydrogen (1.0078 Da).

If S is present, subtract the mass of sulfur (31.9721 Da) and add the mass of CH_2 (14.0157 Da).

Appendix 6: Characterization examples based on low-resolution mass spectral data

6-APB

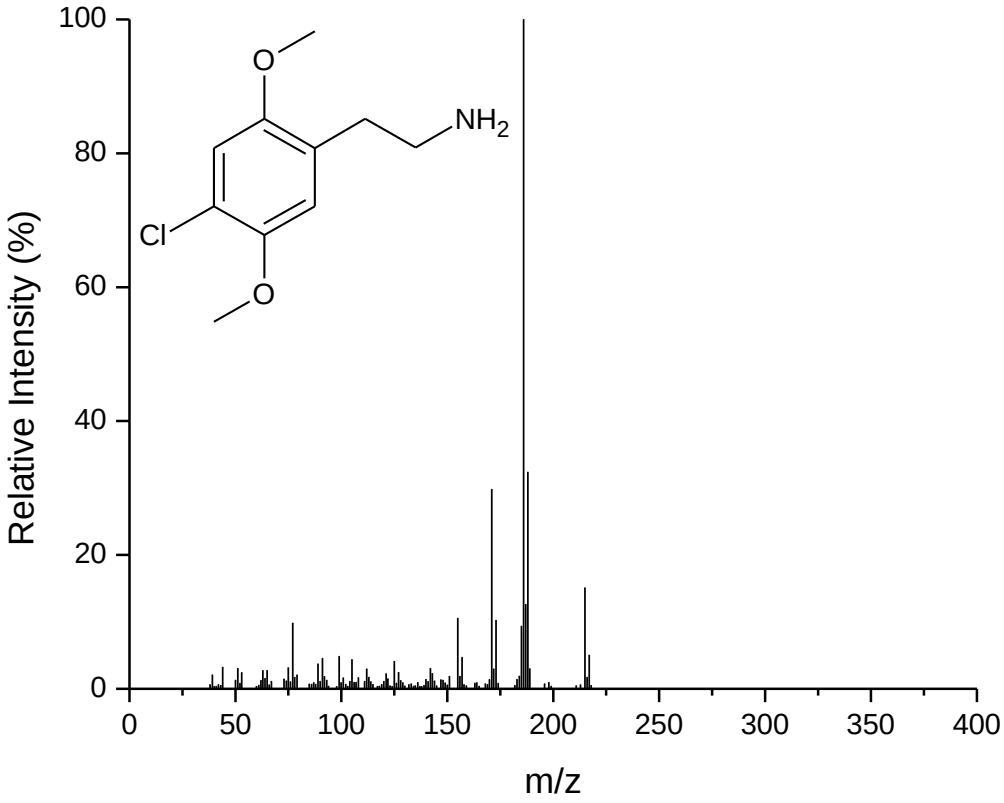


m/z 131 present ≥ 10% relative abundance? Yes

Consistent with APB-phenethylamine

Conclusion: Consistent with APB-phenethylamine

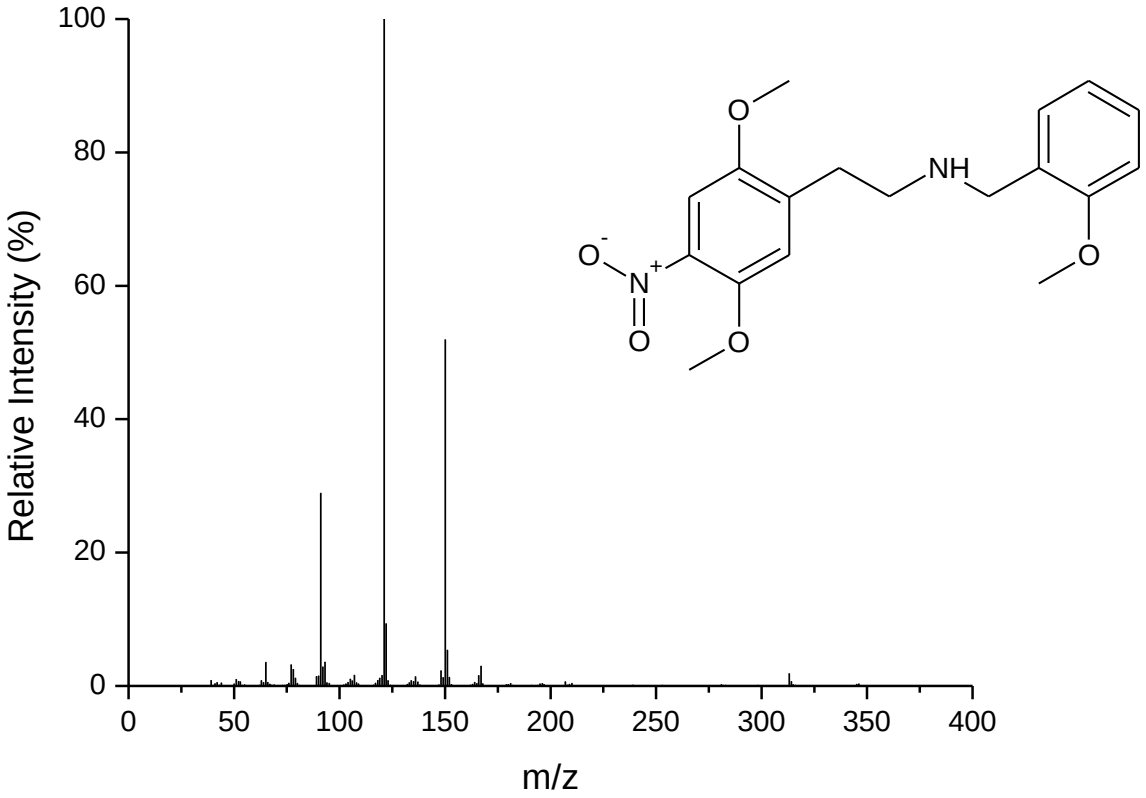
2C-C



m/z 131 present ≥ 10% relative abundance?	No
	Not consistent with APB-phenethylamine
m/z 91, 121 (base peak), and 150 present?	No
	Not consistent with NBOMe-phenethylamine
Loss of 29 Da from M^+ (base peak) and loss of 60 Da from M^+?	Yes **Consistent with 2C-phenethylamine**
Halogen, sulfur, or nitro group present?	Yes, 3:1 ratio indicates Cl
	Consistent with 2C-phenethylamine with Cl substitution

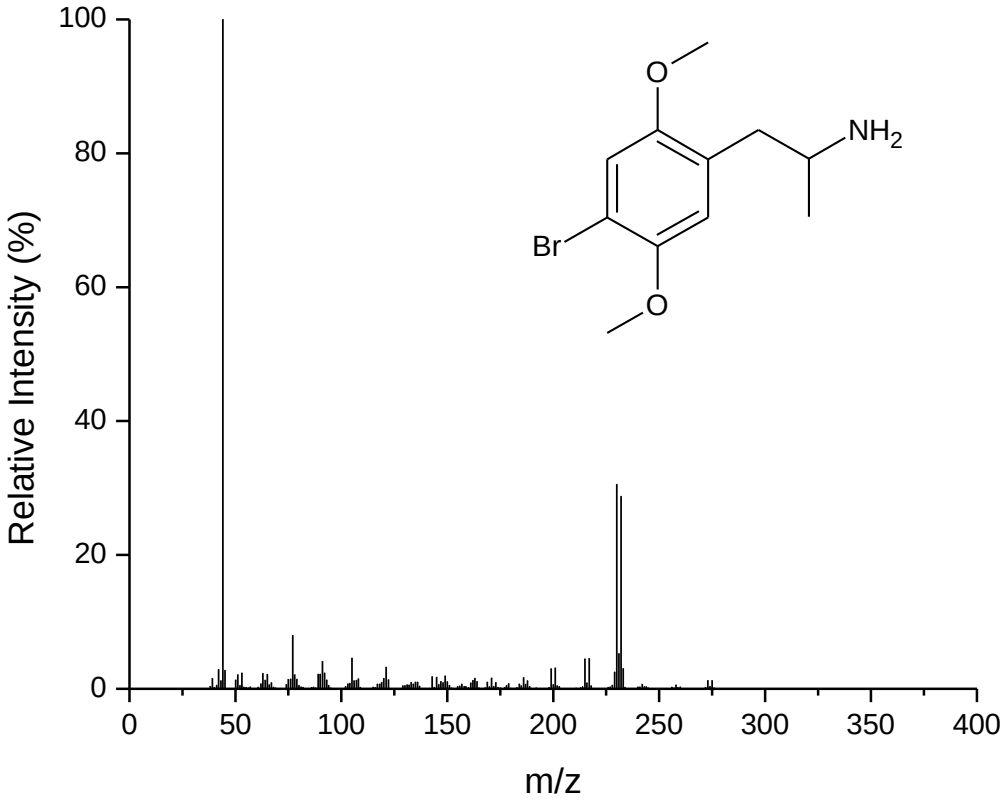
Conclusion: Consistent with 2C-phenethylamine with chlorine substitution

25N-NBOMe



m/z 131 present ≥ 10% relative abundance?	No
	Not consistent with APB-phenethylamine
m/z 91, 121 (base peak), and 150 present?	Yes
	Consistent with NBOMe-phenethylamine
Loss of 31 Da from M^+ and loss of 149 Da from M^+?	Yes **Consistent with NBOMe-phenethylamine**
Halogen, sulfur, or nitro group present?	Yes, even mass $[M]^+$ indicates NO_2 group
	Consistent with NBOMe-phenethylamine with NO_2 substitution

4-Br-2,5-dimethoxyamphetamine



m/z 131 present ≥ 10% relative abundance?	No
	Not consistent with APB-phenethylamine
m/z 91, 121 (base peak), and 150 present?	No
	Not consistent with NBOMe-phenethylamine
Loss of 29 Da from M^+ (base peak) and loss of 60 Da from M^+?	No **Not consistent with 2C-phenethylamine**

Conclusion: Not consistent with APB, NBOMe, or 2C-phenethylamine

Appendix 7: Classification of phenethylamines and tryptamines using principal components analysis and linear discriminant analysis

Appendix 7.1 Feature selection using PCA

The first three principal components (PCs) accounted for 44.65% of the total variance in the dataset. From the scores plot of PC1 vs PC2 (Fig. A7.1), the NBOMe-phenethylamines are separated from the other three classes on PC1 while the APB-phenethylamines are mostly separated from the 2C-phenethylamines and tryptamines on PC2. The 2C-phenethylamines and tryptamines overlap on PC2 but gain some separation on PC1 and PC3. The loadings plots for the first 3 PCs were used to identify the *m/z* values contributing to the variance in the data set. As an example, for PC1 (Fig. A7.2), *m/z* 91, 121, and 150 dominate, with additional contributions from *m/z* 44, 58, 130 and 131. Three sets of variables were identified based on the loadings for the first 3 PCs: those with relative loadings greater than 30%, 20%, and 15% (Table A6.1) across all three PCs.

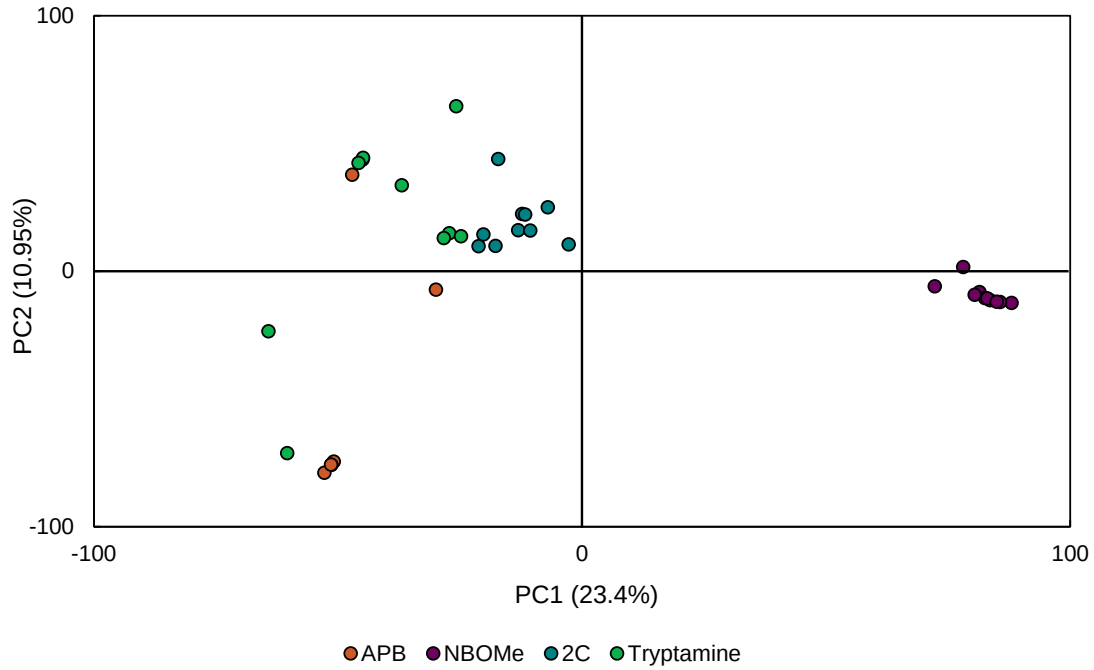


Figure A7.1 PCA scores plot for the first two principal components showing association and discrimination of the APB-phenethylamines, the 2C-phenethylamines, the NBOMe-phenethylamines, and the tryptamines.

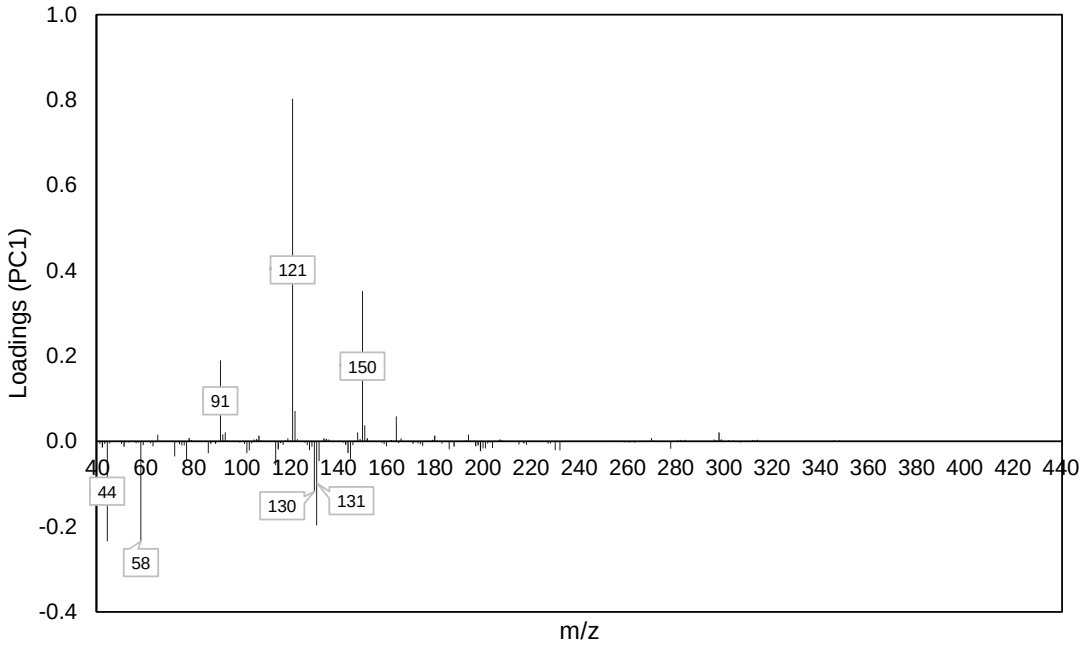


Figure A7.2 PCA loadings plot for the first principal component showing the *m/z* ratios contributing most to the variance described.

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Table A7.1 *m/z* values selected based on PCA

m/z values present at relative loadings greater than		
30%	**20%**	**15%**
44	44	44
58	58	58
	91	91
121	121	121
		130
131	131	131
		132
150	150	150
		165
		180
		197
198	198	198
199	199	199
	200	200
		201

Appendix 7.2 Feature selection using an informed chemical approach

Mass spectra of compounds from each class were probed to identify characteristic spectral features. In Figs. A7.3-7.6, peaks are labeled for *m/z* values considered characteristic of that class. It should be noted that not all identified characteristic ions for each class (listed in tables to the right of each spectrum) are present in every compound.

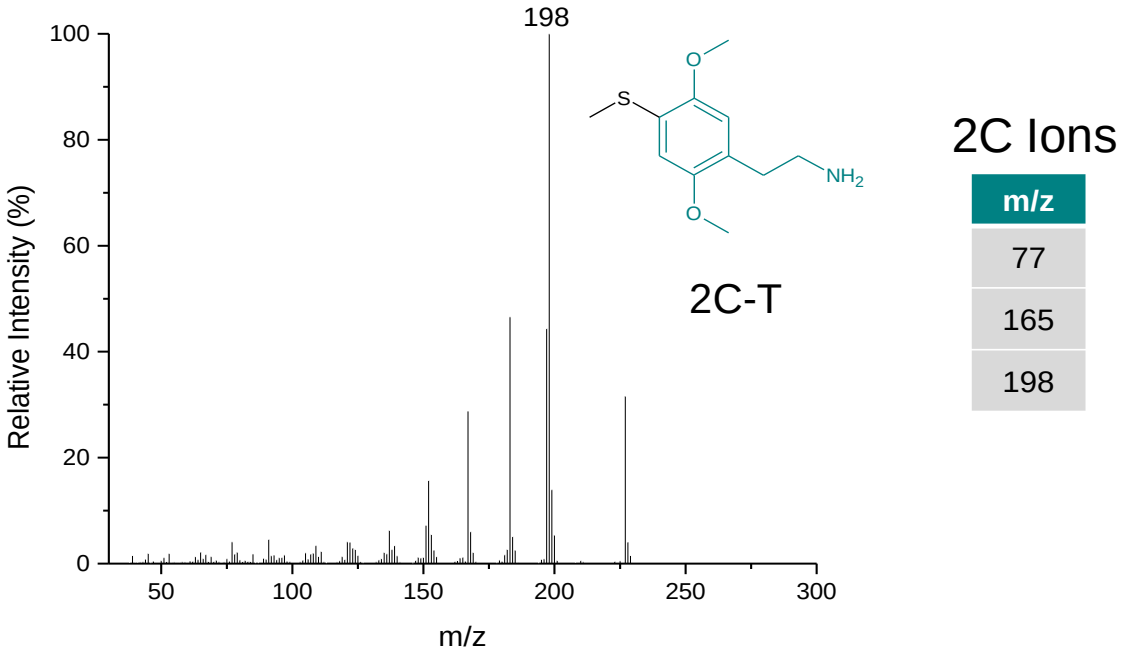
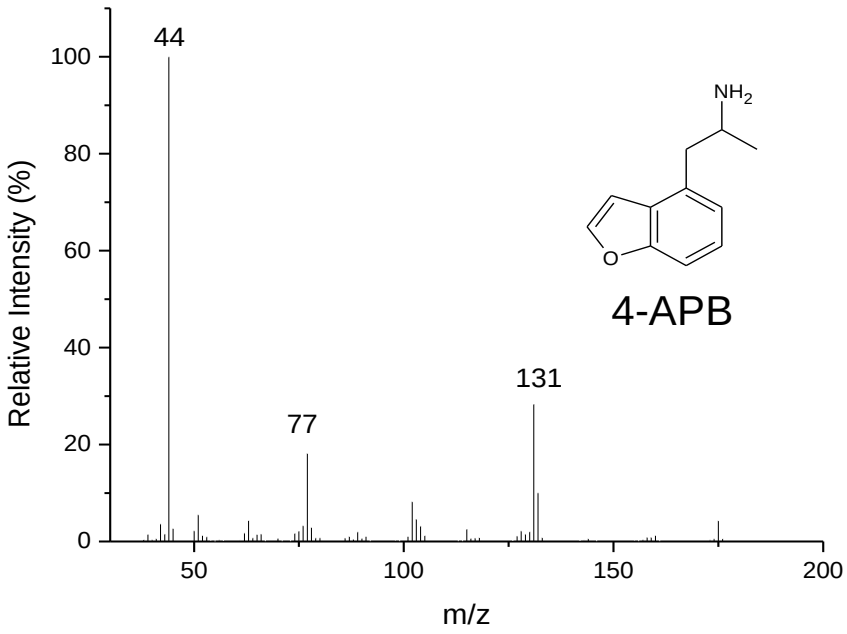


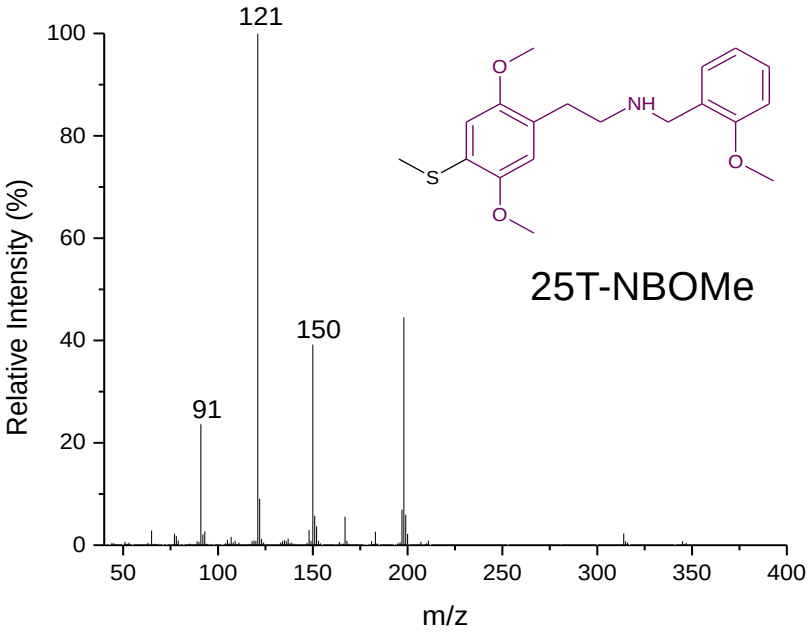
Figure A7.3 Representative spectrum of 2C-T, with ions considered characteristic of the 2C-phenethylamine subclass shown in the table to the right of the spectrum.



APB Ions

m/z
44
58
77
131

Figure A7.4 Representative spectrum of 4-APB, with ions considered characteristic of the APB-phenethylamine subclass shown in the table to the right of the spectrum.



NBOMe Ions

m/z
91
121
150

Figure A7.5 Representative spectrum of 25T-NBOMe, with ions considered characteristic of the NBOMe-phenethylamine subclass shown in the table to the right of the spectrum.

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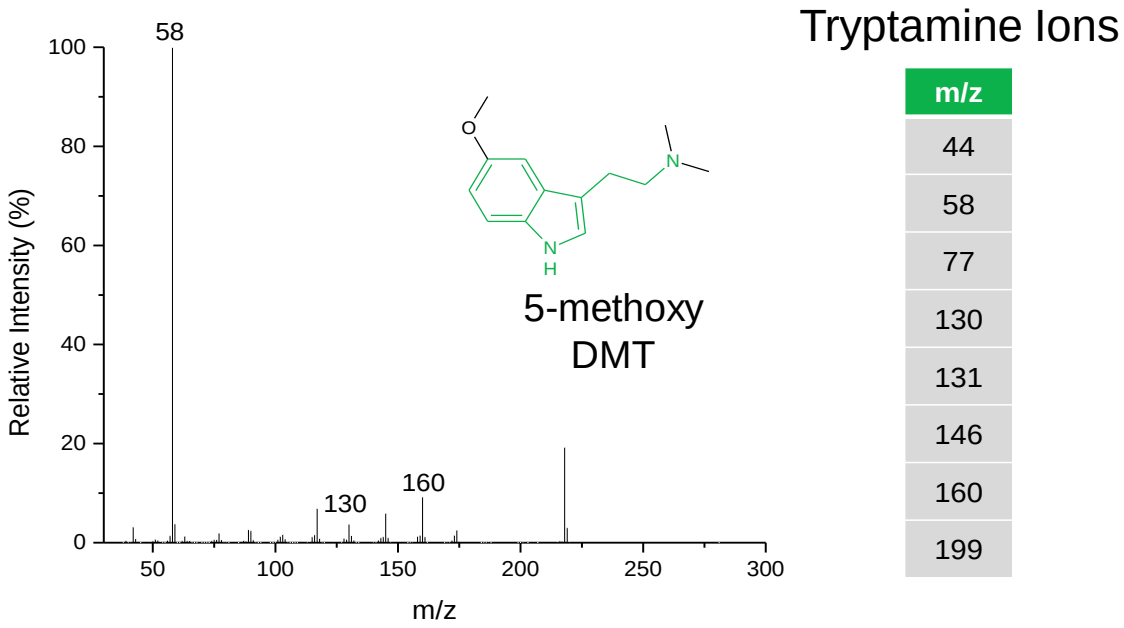


Figure A7.6 Representative spectrum of 5-methoxy-dimethyltryptamine, with ions considered characteristic of the tryptamine class shown in the table to the right of the spectrum.

Appendix 7.3 Classification models based on linear discriminant analysis

Linear discriminant analysis (LDA) models were developed and cross validated for each of the variable sets selected using PCA (Table A7.1). The 9-variable dataset, containing ions with >20% relative loadings, performed best (86% successful classification using cross validation with the training set) and was used in further comparisons with the informed chemical approach.

LDA scores plots with variables chosen by the informed chemical approach show four distinct groups with NBOMe-phenethylamines well distinguished from the others (Fig. A7.7). Tryptamines and APB-phenethylamines score similarly on LD1 while APB- and 2C-phenethylamines score similarly on LD2. However, separation between APB-phenethylamines and tryptamines is achieved on LD3 while APB- and 2C-phenethylamines are separated on LD2.

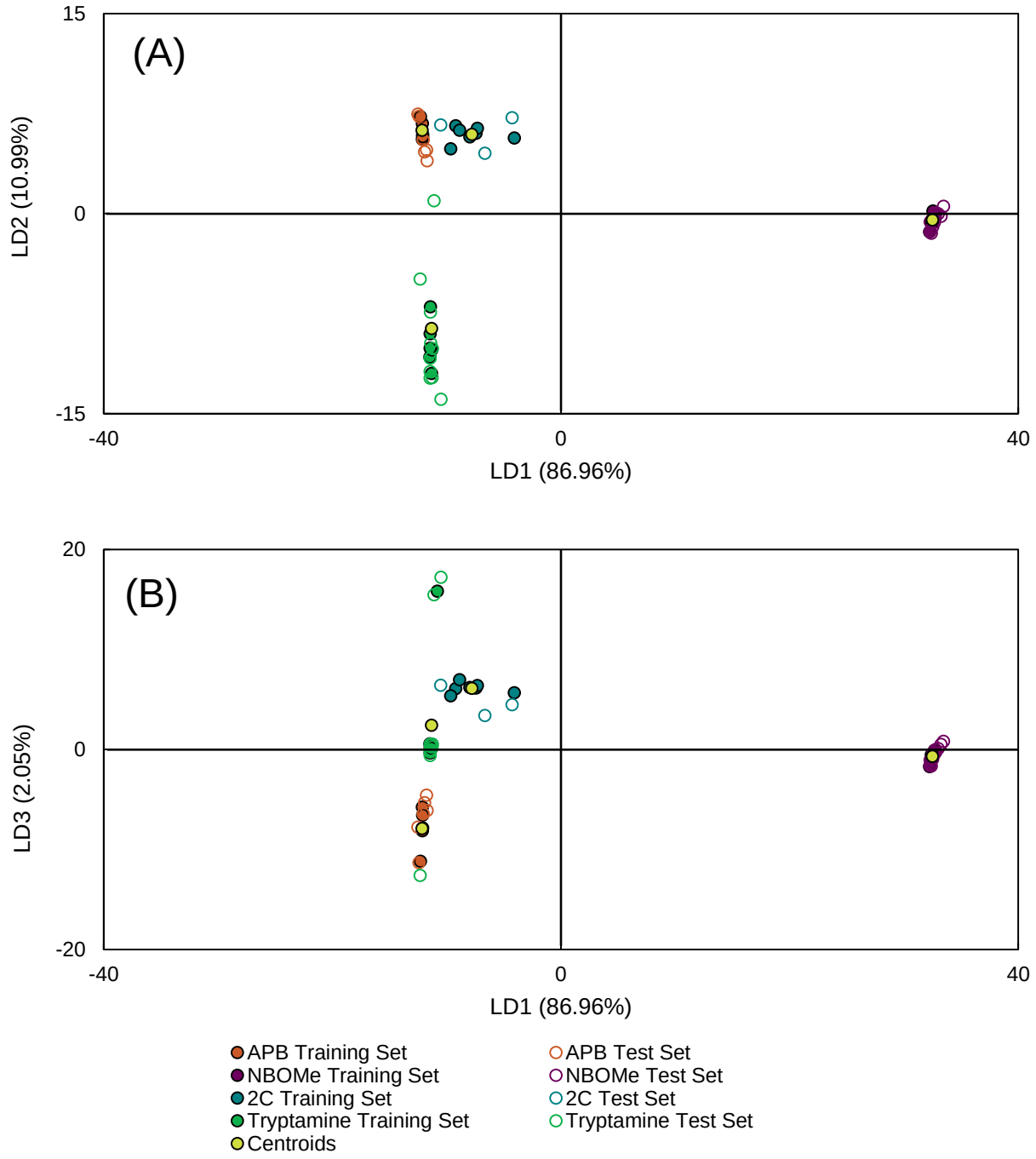


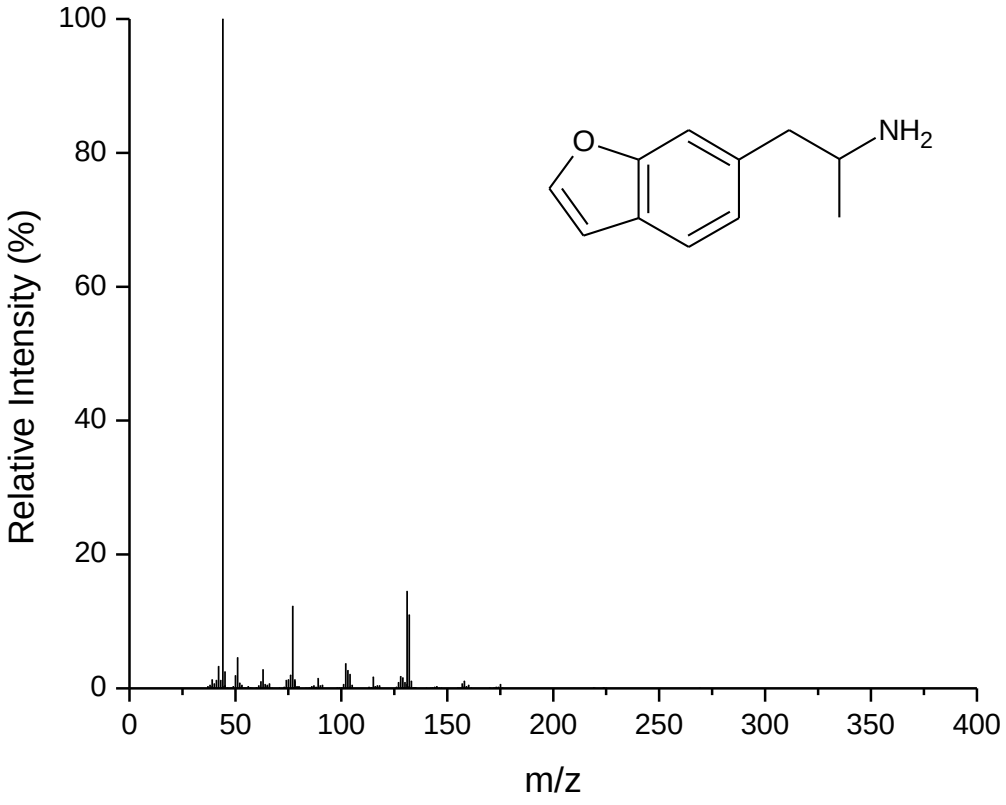
Figure A7.7 Linear discriminant analysis scores plot for (A) LD1 vs LD2 and (B) LD1 vs LD3.

With the PCA feature selection method and using the 9-variable data set, four compounds in the test set were misclassified, yielding an 87% classification success rate. With the informed chemical approach, two compounds from the test set were misclassified, yielding a 93%

classification success rate. The two compounds misclassified were α-ethyl tryptamine and N,N-dimethyltryptamine. The former was misclassified as an APB-phenethylamine due to the high abundance of *m/z* 44 and 131, that are also dominant ions in the APB-phenethylamines. N,N-dimethyltryptamine was misclassified as a 2C-phenethylamine due to a lack of *m/z* 146 and 160 which are common ions for methoxy- and hydroxy- substituted tryptamines. Nonetheless, successful classification was achieved with each feature selection method, with classification rates of 87% and 93%.

Appendix 8: Characterization examples based on high-resolution mass spectral data

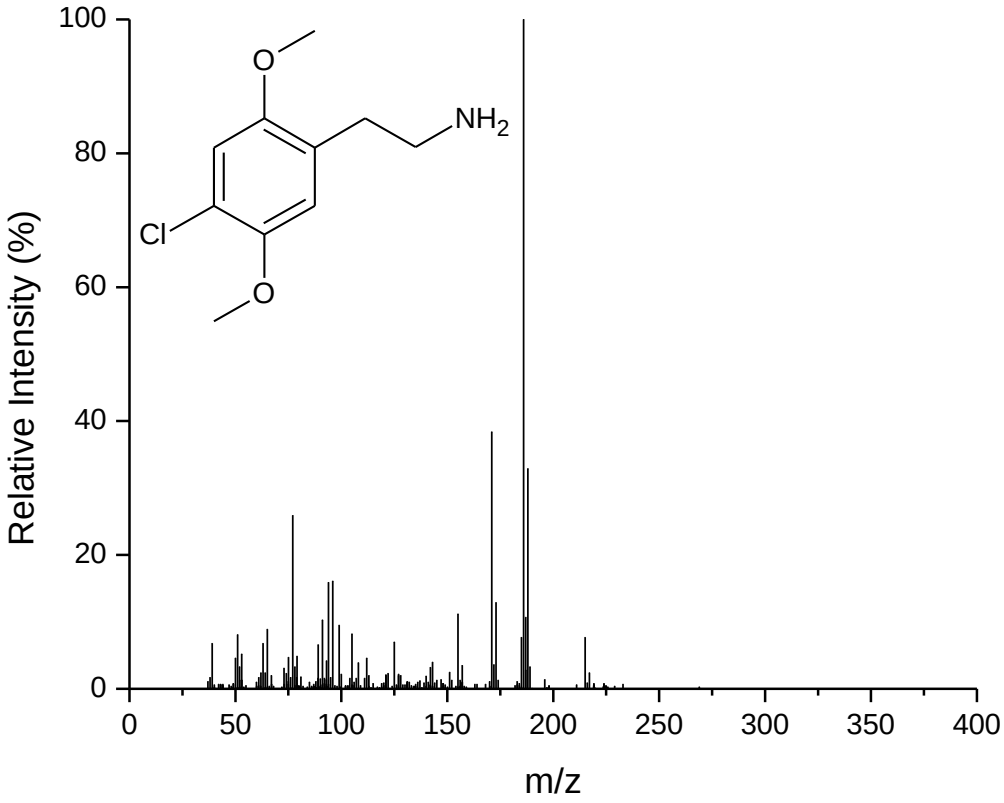
6-APB



m/z 131 ($C_9H_7O^+$) present ≥ 10% relative abundance?	Yes **Consistent with APB-phenethylamine**
Kendrick mass defect (KMD) $[M]^+$ 96.5 ± 0.5 mDa?	Yes, KMD $[M]^+$ = 96.3 mDa **Consistent with APB-phenethylamine**

Conclusion: Consistent with APB-phenethylamine

2C-C

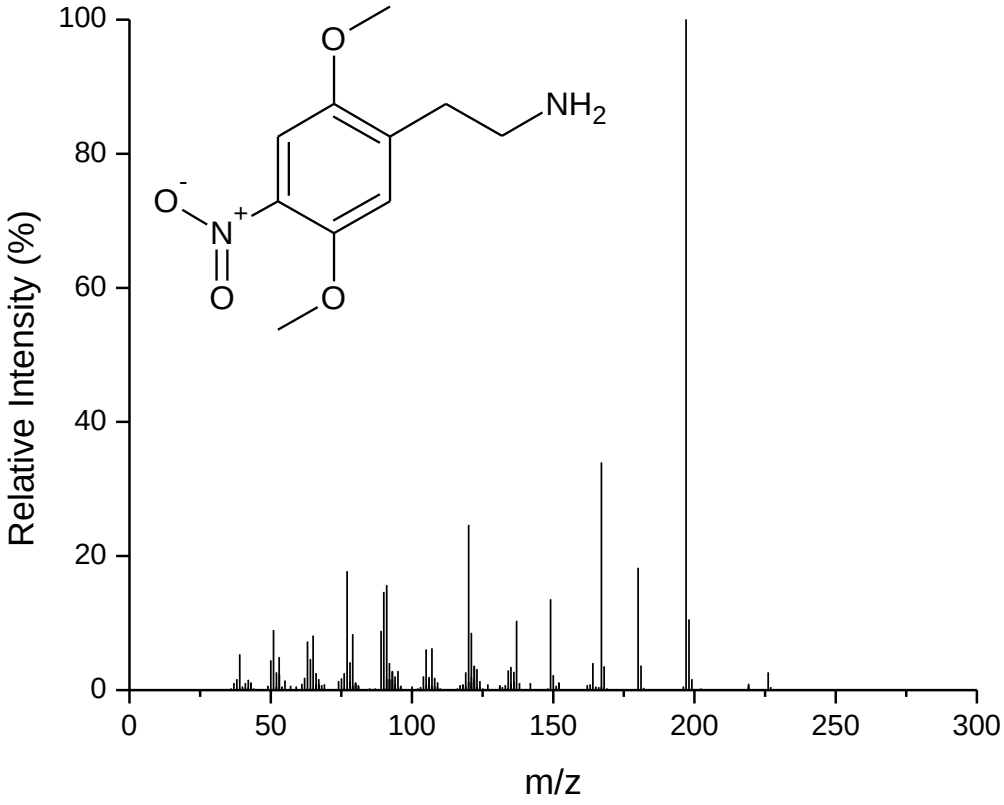


m/z 131 ($C_9H_7O^+$) present ≥ 10% relative abundance?	No **Not consistent with APB-phenethylamine**
m/z 91 ($C_7H_7^+$), 121 (base peak, $C_8H_9O^+$), and 150 ($C_9H_{12}NO^+$) present?	No **Not consistent with NBOMe-phenethylamine**
Loss of 29 Da from M^+ [M-CH_3N]$^+$ (base peak) and loss of 60 Da from M^+ [M-C_2H_6NO]$^+$?	Yes **Consistent with 2C-phenethylamine**
Halogen, sulfur, or nitro group present?	Yes, 3:1 ratio indicates Cl $[M]^+_{adj}$ = 180.9074 Da
KMD $[M]^+_{adj}$ 92.1 ± 2.2 mDa?	Yes, KMD $[M]^+_{adj}$ = 92.6 mDa

KMD $[M\text{-}CH_3N]^+$ 85.4 ± 2.4 mDa?	Yes, KMD $[M\text{-}CH_3N]^+$ = 86.4 mDa
KMD $[M\text{-}C_2H_6N]^+$ 69.5 ± 4.0 mDa?	Yes, KMD $[M\text{-}C_2H_6N]^+$ = 70.2 mDa

Conclusion: Consistent with 2C-phenethylamine with chlorine substitution

25N-NBOMe

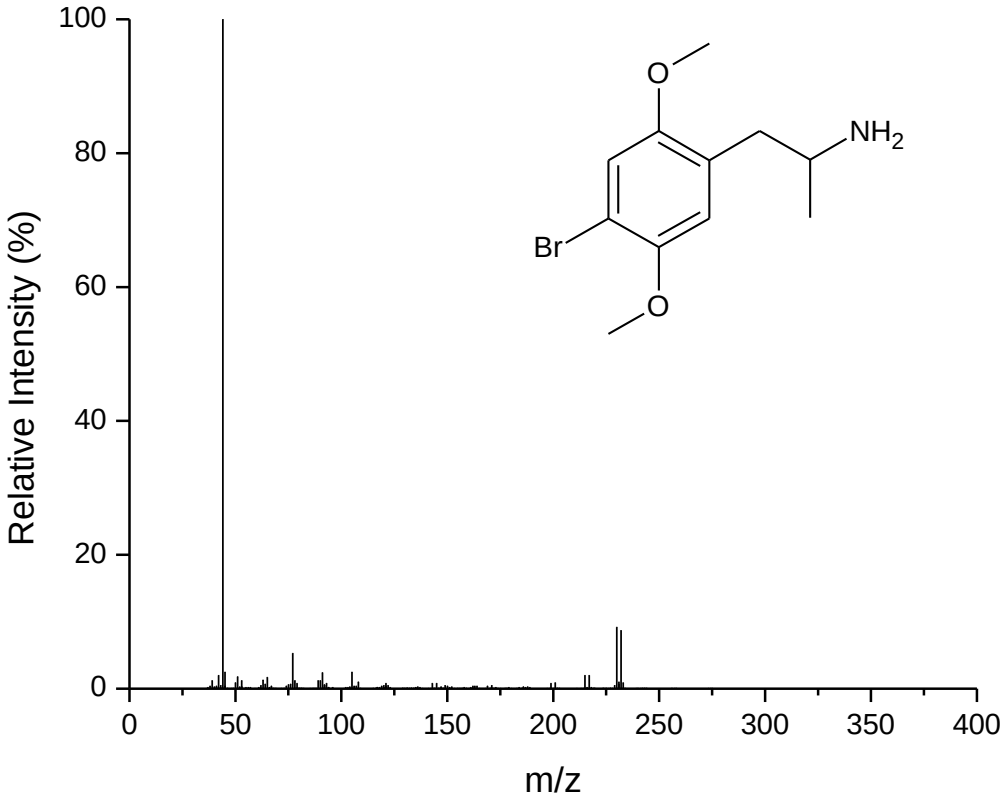


m/z 131 ($C_9H_7O^+$) present ≥ 10% relative abundance?	No **Not consistent with APB-phenethylamine**
m/z 91 ($C_7H_7^+$), 121 (base peak, $C_8H_9O^+$), and 150 ($C_9H_{12}NO^+$) present?	Yes **Consistent with NBOMe-phenethylamine**
Loss of 31 Da from M^+ $[M\text{-}CH_3O]^+$ and loss of 149 Da from M^+ $[M\text{-}C_9H_{11}NO]^+$?	Yes **Consistent with NBOMe-phenethylamine**

Halogen, sulfur, or nitro group present?	Yes, even mass $[M]^+$ indicates NO_2 group $[M]^+_{adj}$ = 300.8279 Da
KMD $[M]^+_{adj}$ 171.5 ± 3.2 mDa?	Yes, KMD $[M]^+_{adj}$ = 172.1 mDa
KMD $[M\text{-}C_9H_{11}NO]^+$ 86.5 ± 0.2 mDa?	Yes, KMD $[M\text{-}C_2H_6N]^+$ = 86.3 mDa

Conclusion: Consistent with NBOMe-phenethylamine with NO_2 substitution

4-Br-2,5-dimethoxyamphetamine



m/z 131 ($C_9H_7O^+$) present ≥ 10% relative abundance?	No **Not consistent with APB-phenethylamine**

m/z 91 ($C_7H_7^+$), 121 ($C_8H_9O^+$), and 150 ($C_9H_{12}NO^+$) present?	No **Not consistent with NBOMe-phenethylamine**
Loss of 29 Da from M^+ $[M\text{-}CH_3N]^+$ (base peak) and loss of 60 Da from M^+ $[M\text{-}C_2H_6NO]^+$?	No **Not consistent with 2C-phenethylamine**

Conclusion: Not consistent with APB, NBOMe, or 2C-phenethylamine

Appendix 9: Scholarly products

A8.1 Publications

Bodnar-Willard MA, McGuffin VL, Waddell Smith R. Statistical Comparison of Mass Spectra for Identification of Amphetamine-Type Stimulants. *Forensic Science International* **2017**, 270, 111-120.

Anstett AL, Chu F, Alonso DE, **Waddell Smith R**. Characterization of 2C-Phenethylamines using High-Resolution Mass Spectrometry and Kendrick Mass Defect Filters. *Forensic Chemistry* **2018**, *7*, 47-55.

A8.2 Presentations

Alexandria Anstett, Fanny Chu, and Ruth Waddell Smith. Characterization of Synthetic Phenethylamines using High-Resolution Mass Spectrometry. Oral presentation at the American Academy of Forensic Sciences Annual Meeting, Las Vegas, NV. February 2016.

Alexandria Anstett, Fanny Chu, David E. Alonso and Ruth Waddell Smith. Characterization of Synthetic Phenethylamines using High-Resolution GC-TOFMS and Mass Defect Filters. Oral presentation at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Atlanta, GA. March 2016.

Fanny Chu, A. Daniel Jones, and Ruth Waddell Smith. Strategies for Classification and Annotation of Novel Synthetic Designer Drugs. Poster presentation at the American Society for Mass Spectrometry Annual Meeting, San Antonio, TX. June 2016.

Melissa A. Bodnar-Willard, Ruth Waddell Smith, and Victoria L. McGuffin. Frequentist Approach for Statistical Comparison of Mass Spectra. Oral presentation at the 45th Annual Meeting of the Midwestern Association of Forensic Scientists, Branson, MO. October 2016.

Alexandria L. Anstett, David E. Alonso, A. Daniel Jones, and Ruth Waddell Smith. Development of a Characterization Scheme for Emerging Synthetic Phenethylamines. Poster presentation at the 69th American Academy of Forensic Sciences Annual Meeting, New Orleans, LA. February 2017.

Alexandria L. Anstett, Fanny Chu, David E. Alonso, A. Daniel Jones, and Ruth Waddell Smith. Mass Spectral Tools for Characterization of Synthetic Phenethylamines. Oral presentation at the 68th Annual Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (Pittcon), Chicago, IL. March 2017.

Alexandria Anstett, Fanny Chu, David E. Alonso and Ruth Waddell Smith. Mass Spectral Tools for Characterization of Synthetic Phenethylamines. Oral presentation at the 46th Annual Meeting of the Midwestern Association of Forensic Scientists, Cincinnati, OH. September 2017.

Amanda L. Setser and Ruth Waddell Smith. Classification of Synthetic Phenethylamines According to Structural Subclass using Multivariate Statistical Procedures. Poster presentation at the 70th American Academy of Forensic Sciences Annual Meeting, Seattle, WA. February 2018.

Alexandria L. Anstett, Fanny Chu, David E. Alonso, Victoria L. McGuffin, and Ruth Waddell Smith. Statistical and Mass Spectral Tools for the Identification and Characterization of Synthetic Phenethylamines. Oral presentation at the 69th Annual Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (Pittcon), Orlando, FL. March 2018.

A8.3 Planned future products

Publications

Setser AL and Waddell Smith R. Comparison of Variable Selection Methods Prior to Statistical Classification of Synthetic Phenethylamines and Tryptamines. Manuscript submission to *Forensic Science International, Journal of Forensic Sciences,* or *Forensic Chemistry.* Anticipated submission: July 2018.

Stuhmer E, McGuffin VL, Waddell Smith, R. Statistical Comparison of Mass Spectra to Identify Designer Drug Positional Isomers. Manuscript submission to *Forensic Science International, Journal of Forensic Sciences,* or *Forensic Chemistry.* Anticipated submission: September 2018.

Presentations

Emma L. Stuhmer, Victoria L. McGuffin, and Ruth Waddell Smith. Statistical Comparison of Designer Drug Positional Isomers. Anticipated oral presentation at the Midwestern Association of Forensic Scientists Fall Meeting in Indianapolis, IN. September 2018.

Amanda L. Setser and Ruth Waddell Smith. Development and Application of Multivariate Statistical Models for Designer Drug Classification. Invited oral presentation at SCIX in Atlanta, GA. October 2018.

Emma L. Stuhmer, Victoria L. McGuffin, and Ruth Waddell Smith. Further Validation of a Statistical Method to Compare Mass Spectra with Applications for the Identification of Synthetic Phenethylamines. Anticipated oral presentation at the American Academy of Forensic Sciences Annual Meeting in Baltimore, MD. February 2019.