



B15 A Statistical Comparison of Mass Spectral Data for Positional Isomer Differentiation

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Learning Overview: After attending this presentation, attendees will know about a method for the statistical comparison of mass spectral data to increase confidence in controlled substance identification, and specifically the application of the method to compare mass spectra of positional isomers.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing a method to compare mass spectral data to identify and differentiate positional isomers with statistical confidence.

Suspected controlled substances are typically analyzed by gas chromatography-mass spectrometry (GC/MS) and the mass spectrum is compared to that of a reference material or library database for identification purposes. However, the identification is typically based on visual comparison of the spectra. With the increased prevalence of novel psychoactive substances, including positional isomers, identification in this manner is challenging due to high structural similarity among compounds.

Previous laboratory work developed a statistical comparison method to compare two mass spectra (e.g., spectrum of the submitted sample compared to spectrum of the reference material). The two spectra are compared using a Welch's *t*-test to assess the relative intensities at every mass-to-charge (m/z) value in the scan range. At each m/z value, the tested null hypothesis is that the relative intensities are not statistically different, whereas the alternative hypothesis is that the relative intensities are statistically different at the confidence level selected for the test. If the null hypothesis is accepted at every m/z value, the two spectra are statistically indistinguishable. In these instances, a random match probability (RMP) is calculated indicating the probability that the mass spectral fragmentation pattern occurred by random chance alone. In contrast, if the alternative hypothesis is accepted at any m/z value, the two spectra are statistically distinguishable and the ions responsible for discrimination are identified. Successful application of the method was previously demonstrated for the statistical association and discrimination of a set of structurally similar amphetamine-type stimulants.

The work presented demonstrates application of the statistical comparison method specifically for the differentiation of positional isomers. Two sets of isomers were considered: Set 1 contained 2-fluoromethamphetamine (2-FMA), 3-fluoromethamphetamine (3-FMA), and 4-fluoromethamphetamine (4-FMA); Set 2 contained 2-ethylmethcathinone (2-EMC), 3-ethylmethcathinone (3-EMC), and 4-ethylmethcathinone (4-EMC). Each set of isomers was analyzed by GC/MS using two different instruments and with different injection parameters (e.g., different split ratios). Isomers were also analyzed by GC-time of flight mass spectrometry to collect accurate mass data, which were used to determine elemental formulae of fragment ions.

Initial comparisons show promise in the ability of the method to statistically discriminate positional isomers within each set. For example, for the FMA isomers analyzed on the same instrument with a 50:1 split ratio, 2-FMA was discriminated from both 3-FMA and 4-FMA at the 99% confidence level with up to 10 ions responsible for discrimination. Ions responsible for discrimination included m/z 109 $[C_7H_6F]^+$ and m/z 117 $[C_8H_7N]^+$, with differentiation based on differences in relative intensities among the isomers. Discrimination of 3-FMA from 4-FMA was possible at the 99% confidence level, this time with 5 ions responsible for discrimination. At a 100:1 split ratio, discrimination was still observed among the isomers, albeit with fewer ions due to less representative spectra.

For the EMC isomers analyzed on the same instrument, differentiation was possible at the 99.9% confidence level. For example, with a 50:1 split ratio, 2-EMC was distinguished from 3-EMC with up to 12 discriminating ions and from 4-EMC with up to 16 discriminating ions. Discriminating ions included m/z 104 $[C_8H_8]^+$, m/z 131 $[C_9H_9N]^+$, and m/z 146 $[C_{10}H_{12}N]^+$, with differentiation based on differences in relative intensity among the three isomers. Differentiation between 3-EMC and 4-EMC was also observed at the 99.9% confidence level, albeit with only one ion (m/z 133, $[C_9H_9O]^+$) responsible for discrimination.

This presentation will discuss the application of the statistical comparison method to two sets of positional isomers and describe the chemical significance of the ions responsible for discrimination. While this research focuses on positional isomers, the statistical method can be applied to compare mass spectra of any controlled substance to increase confidence in identification.

Positional Isomers, GC/MS, Statistical Comparison