A201 Statistical Comparison of Mass Spectral Data in the Identification of Amphetamine- Type Stimulants

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After attending this presentation, attendees will be familiar with a statistical procedure that has been developed for the comparison of mass spectral data.

This presentation will impact the forensic science community by providing a method to assign statistical confidence to the comparison of mass spectra. Specifically, application of the statistical procedure in the comparison of mass spectral data generated for standards and case samples containing amphetamine-type stimulants will be presented.

Gas chromatography-mass spectrometry is commonly used for the analysis of controlled substance samples. While this technique provides both retention time and mass spectral data for the comparison of the submitted sample to a suitable reference standard, the identification of amphetamine-type stimulants can be challenging. Firstly, since many of these compounds have the same phenethylamine base structure, the resulting mass spectra are very similar, especially in the lower mass region. Additionally, these compounds readily fragment under typical electron ionization conditions and, oftentimes, the molecular ion is not present in the spectrum, making identification based on molecular mass difficult. These challenges can be overcome by derivatizing the sample or by using alternative ionization procedures; however, both of these options increase the time necessary for sample analysis, which is not desirable in a forensic laboratory. As a result, amphetamine-type stimulants are typically analyzed in the underivatized form and under electron ionization conditions.

The resulting mass spectrum of the controlled substance in the submitted sample is then compared visually to the spectrum of a known reference standard, either analyzed by the laboratory or obtained from a mass spectral database. However, since the publication of the National Academy of Sciences Report in 2009, there has been a trend toward a more statistical evaluation of forensic evidence, which is not commonplace in controlled substance analysis and identification. The aim of this research was to develop a suitable procedure that provides such a statistical basis for the comparison of mass spectral data, thereby addressing the current limitations.

The developed procedure uses Student's t-tests and classical probability theory to compare two mass spectral fragmentation patterns. Briefly, the Student's t-test is used to compare the two spectra at every mass-to-charge ratio (m/z) in the scan range. The calculated t-statistic (t_{calc}) is then compared to the critical t-value (t_{crit}) at the required confidence level. If, at any m/z in the range, t_{calc} is greater than t_{crit}, then the two spectra are considered to be statistically distinguishable. If t_{calc} is less than t_{crit} at all m/z, the two spectra are considered to be statistically indistinguishable. In these cases, classical probability theory is used to determine the random match probability, which is the probability that the spectral pattern occurs by chance.

In this presentation, the procedure is applied for the comparison of mass spectra of amphetamine-type stimulants. Spectra of case samples containing amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine, and phentermine were obtained from an accredited forensic laboratory and compared to spectra of appropriate reference standards. Each case sample spectrum was statistically associated to the corresponding reference standard at the 99.9% confidence level. Random match probabilities were also calculated and, in each case, the probability of the spectral pattern occurring by chance was infinitesimally small (e.g., 1.7×10^{-39} for the comparison of a case sample containing amphetamine to an amphetamine reference standard).

Mass spectra of the case samples were also compared to all other reference standards, using the same procedure. In general, statistical discrimination of the samples from unrelated standards was observed at the 99.9% confidence level, which is the most rigorous confidence level for discrimination. However, a case sample containing methamphetamine was discriminated from the phentermine reference standard at the slightly lower confidence level of 99.0%. These two substances have the same molecular mass and, since they are structural isomers, distinction of the two based on mass spectral data is challenging. Despite the similar fragmentation patterns, methamphetamine and phentermine were distinguished with high confidence.

While demonstrated for the comparison of controlled substances data, the statistical procedure developed can be readily applied to other types of forensic evidence in which the comparison of mass spectral data is necessary.

Mass Spectral Data, Statistics, Amphetamines

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