## B185 Multivariate Statistics for the Masses: A Novel, User-Friendly Method for Positional Isomer Differentiation Using Mass Spectral Data

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**Learning Overview:** After attending this presentation, attendees will be aware of a novel method of positional isomer differentiation using only mass spectral data that is commonly generated during the analytical scheme of controlled substances. Additionally, attendees will be introduced to an easy-to-use user interface that has been developed with the typical forensic scientist in mind.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by showing an alternative method of positional isomer differentiation using multivariate statistical analysis of mass spectral data rather than other, more time-consuming methods, such as additional chemical or instrumental analysis.

Mass spectral analysis is the confirmatory method of choice for seized drug analysis in forensic laboratories. However, spectra of positional isomers are often too visually similar to allow for differentiation, which leads to identification challenges for forensic laboratories that are inundated with novel psychoactive substances. Scientists now find themselves in a position to make a choice between reporting an ambiguous conclusion that does not identify the specific isomer or resorting to additional chemical or instrumental analysis.

This presentation builds on previously published work that used multivariate statistical analysis of Fluoromethcathinone (FMC) and fluorofentanyl to offer a feasible alternative means of differentiating between electron ionization mass spectra of positional isomers, saving laboratory resources while still resulting in specific compound identification.[1]

Mass spectral data was collected from the four state regional laboratories across the Commonwealth of Virginia. Data from the past two to three years for any primary standards of commonly encountered positional isomers was requested. Isomer groups included pseudoephedrine/ephedrine, novel cathinone isomers, and several fentalogs. All data was collected on instruments that were tuned daily using either an autotune or etune procedure. A starting library of more than 950 spectra was collected from more than ten different Gas Chromatography/Mass Spectrometry (GC/MS) instruments.

The isomer groups were analyzed using the proposed method of spectral comparison which utilizes Principal Component Analysis (PCA) for dimension reduction, followed by Linear Discriminant Analysis (LDA) for isomer classification. For PCA, the original variables were the individual *m/z* ratios, and the data for each sample was its normalized abundance at each particular *m/z* ratio. The variables that were used to derive the LDA discriminant functions are the scores that resulted from projecting the original *m/z* variables onto the first several principal component axes. A user interface was developed that allows the user to dictate the number of principal components to utilize for LDA. Once selected, a Leave-One-Sample-Out Cross Validation (LOOCV) error rate can be calculated that shows how accurately the method can classify an unknown sample based on the available library.

Once the library is shown to be robust by resulting in a LOOCV error rate below a chosen threshold, the user interface allows a chemist to upload an unknown spectrum as a text file for comparison to the chosen group of isomers, and the program will use the above method to classify the compound based on the posterior probability. A blind study was conducted to show the accuracy of this classification method.

The results indicate multivariate statistical analysis, especially when combined with an easy-to-use user interface, is a promising addition to the analytical scheme of the identification of positional isomers. This would allow for higher confidence in the final identification of a compound without the need for additional chemical or instrumental analysis, saving laboratories both time and money.

**Reference(s):**

1. Bonetti J. Mass spectral differentiation of positional isomers using multivariate statistics. *Forensic Chemistry* 2018:9;50-61.

**Designer Drugs, Mass Spectrometry, Multivariate Statistics**



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