B201 Investigating the Robustness of a Statistical Method to Compare Mass Spectra of Fentanyl Analogs

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Learning Overview: After attending this presentation, attendees will be familiar with a method for the statistical comparison of mass spectra that can be used as a tool to increase confidence in identifications. Positional isomers of Fluorobutyryl Fentanyl (FBF) and Fluoroisobutyryl Fentanyl (FIBF) will be used to demonstrate application of the method, taking into account the effect of concentration and mass spectral tune conditions on the association and discrimination of the isomers.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by describing a method by which two mass spectra can be compared statistically to increase confidence in controlled substance identification, which is especially useful for positional isomers and other structurally similar compounds.

The typical method for the identification of controlled substances is to analyze samples using Gas Chromatography/Mass Spectrometry (GC/MS) and to perform a visual comparison of the resulting mass spectrum to a suitable reference spectrum. However, for spectra of structurally similar compounds, visual comparison of spectra for identification can be challenging. Previous work in the Michigan State University laboratory focused on the development of a statistical method to compare the mass spectrum of an unknown sample to a suitable reference spectrum using an unequal variance *t*-test. In this approach, *t*-tests are used to statistically compare the mean abundances at every corresponding *m/z* value in the scan range for the two spectra at a user-defined confidence level. The null hypothesis (H_0) being tested is that the difference between the mean abundances of a given *m/z* value is equal to zero. The alternative hypothesis (H_a) states that the difference between the mean abundances of a given *m/z* value is not equal to zero. If H_0 is accepted at each *m/z* value, the two spectra are statistically indistinguishable and a random-match probability is calculated to estimate the probability that the fragmentation pattern occurred by random chance alone. However, if H_a is accepted at any *m/z* value in the scan range, the two spectra are statistically distinguishable, and the ions responsible for discrimination are identified.

The statistical comparison method has been applied to differentiate amphetamine-type stimulants as well as positional isomers of ethylmethcathinone and fluoromethamphetamine. While the method has shown potential for differentiation of isomers, in the current work, the robustness of the method is tested by investigating the effect of analyte concentration and the effect of mass spectral tuning on the ability to associate and discriminate isomers.

In this work, GC/MS was used to analyze two sets of fentanyl isomers, which included the *ortho-*, *meta-*, and *para-* forms of FBF and the *ortho-*, *meta-*, and *para-* forms of FIBF. Compounds within each set were initially analyzed under equivalent conditions over several days, and the resulting spectra were statistically compared to assess association of corresponding isomers with discrimination from the others.

Initial results indicate the potential of the method for differentiation of these isomers. For example, spectra of corresponding isomers of FBF were statistically associated at the 99.9% confidence level with discrimination from the other FBF isomers. More specifically, *ortho*-FBF was discriminated from *para*-FBF and *meta*-FBF at the 99% confidence level with nine ions responsible for discrimination in each case, including common ions of *m/z* 44, 71, 102, 118, 144, 164, and 171. Similarly, *para*-FBF was discriminated from *meta*-FBF at the 99% confidence level, with three ions responsible for discrimination, *m/z* 44, 176, and 234. A long-term study is currently being conducted to investigate the effect of concentration and the effect of mass spectral tune conditions on the association and discrimination of these isomers.

In this presentation, the statistical method and ions responsible for discrimination will be described in more detail. Further, the results of the long-term concentration and tune studies will be presented to demonstrate the robustness of the method.

Mass Spectral Comparison, Fentanyl Analogs, GC/MS

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