B131 Differentiation of Cathinone Isomers Using High Resolution Collision-Induced Dissociation Mass Spectrometry (CID/MS)

Cynthia Kaeser, MS, 162 Martin Avenue, Livermore, CA 94551; A. Daniel Jones, PhD, 219 Biochemistry, East Lansing, MI 48824; and Ruth Waddell Smith, PhD, Michigan State University, School of Criminal Justice, 560 Baker Hall, East Lansing, MI 48824*

After attending this presentation, attendees will understand how CID/MS fragmentation of cathinones can be used to differentiate isomers and how the underlying fragmentation mechanisms lead to the observed differences.

This presentation will impact the forensic science community by demonstrating the differentiating power of CID/MS for isomer identification. This presentation will provide better understanding of fragmentation mechanisms that can also be applied to analogs when no standard is available for comparison.

With the increased availability and subsequent regulation of synthetic designer drugs such as cathinones, clandestine chemists continue to produce new unregulated analogs of these compounds. These analogs keep many of the same structural features that yield the desired biological response but contain slight structural differences to avoid regulation. Isomers are especially difficult to distinguish because these analogs have the same chemical formula but with different structural arrangements. Typical analysis of a suspected cathinone by a forensic laboratory uses Gas Chromatography/Mass Spectrometry (GC/MS) with the Electron Ionization (EI) MS spectra compared to reference standards.

However, positive identification of new analogs by GC/MS is complicated by the lack of molecular ion and available reference standards. In these cases, several analytical techniques are necessary for identification, a difficult requirement to meet given the high caseloads and lack of advanced instrumentation available in many laboratories. As an alternative to using multiple instruments, CID/MS can be performed with high-mass resolution while using multiple collision energies for fragmentation. The main advantages of this technique over EI/MS are twofold. First, varying the collision energy used produces both the intact ion and a series of increasingly smaller fragments as the energy is increased. This reveals more details about the structural arrangement of an isomer than using a single collision energy. Second, high-mass resolution improves confidence in molecular formula assignment for positive identification.

While part of a larger study of cathinone fragmentation, three cathinone isomers were selected for this presentation: butylone, ethylone, and the 2,3-ethylone isomer. Butylone and ethylone are in Schedule I of the Controlled Substances Act while the 2,3-ethylone isomer is currently unregulated. Butylone and ethylone differ in the length of hydrocarbon chains at the alpha position and on the amine while the 3,4-methylenedioxy substitution on the aromatic ring is the same for both. The 2,3-ethylone isomer has the same hydrocarbon arrangement as ethylone, but the methylenedioxy group is at the 2,3-position.

Each standard was prepared at a concentration of 5ug/mL in methanol and directly injected into a quadrupole time-of-flight mass analyzer with Electrospray Ionization (ESI) and a leucine-enkephalin reference compound to improve mass accuracy. Fragmentation of each isomer was observed using collision energies of 10eV, 20eV, and 40eV. Resulting mass spectra were interrogated and the molecular formulas of major fragments were determined using their exact masses (four decimal places, <20ppm mass error). The molecular formulas and the voltage of appearance for each isomer's fragments were used to generate flowcharts for identification of common fragmentation pathways. These pathways provide insight into the positions of functional groups on the molecule allowing isomers to be distinguished.

While some differences in fragmentation were observed at the lowest collision energy, more discriminating features were observed at the higher energies. For example, at 10eV the loss of the amine group from the intact molecule distinguished butylone from the ethylone isomers. The fragment masses alone could not distinguish between the two ethylone isomers, although some differences in the relative abundances of each fragment were present. At higher voltages, additional fragments were identified by their exact masses to include losses of both odd and even electron species. While radical (odd electron) losses are less common for ESI than for EI, such species are resonantly stabilized by the aromatic ring. Radical fragments, including losses of radical methyl and ethyl groups, were the most differentiating between the two ethylone isomers.

This presentation will demonstrate the use of CID/MS as a single technique for successful isomer differentiation of cathinones. While this presentation focuses on a limited subset of cathinones, the process for using the observed fragmentation mechanisms for the identification of new cathinone analogs will also be discussed.

Cathinones, Mass Spectrometry, Designer Drugs

*Presenting Author