B180 The Application of Ultra High-Performance Liquid Chromatography-Time-of-Flight/Mass Spectrometry (UHPLC-TOF/MS) to the Analysis of Phenethylamine Derivatives

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Learning Overview: After attending this presentation, attendees will understand the methods of analysis for selected substituted phenethylamines (the 2C-x series and 25x-NBOMe series) based on ultra-high-performance liquid chromatography-time of flight-mass spectrometry (UHPLC-TOF/MS).

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating UHPLC-TOF/MS as a valuable analysis method for substituted phenethylamine compounds. Separation and identification of phenethylamine positional isomers using optimized conditions could significantly increase sample throughput in a forensic drug testing laboratory.

An ever-growing concern to the public health and safety worldwide is the threat posed by an increase in the popularity of emerging drugs. To evade drug laws, synthetic analogues or "designer drugs" are being created by altering the chemical structure of known illicit compounds to mimic the desired effect. Subsequently, the plethora of new drugs appearing on the drug market are being created at a rate that challenges law enforcements efforts to curb their consumption. Phenethylamines are one of the most broad and diverse classes of drugs. Compounds within the class are composed of a core phenethylamine structure consisting of a phenyl ring joined to an amino group via a two-carbon sidechain. The opportunities for derivatization at the aromatic ring, side chain, or amino group are almost endless. For example, the 2C-x series refers to a group of ring-substituted phenethylamines with a methoxy group at the 2 and 5 positions, and generally a lipophilic substituent at the 4 position. In contrast, the 25x-NBOMe series are N-benzyl derivatives of the corresponding 2C-x series, with the substitution of 2-methoxybenzyl on the amine resulting in an increase in the potency.

Due to the high degree of structural similarities, substituted phenethylamines present a challenge when analyzed using traditional techniques such as gas chromatography-mass spectrometry (GC/MS). While GC/MS is the main technique utilized for general forensic drug screening, the analysis of positional isomers poses an important challenge. The lack of discrimination in the fragmentation pathways, in addition to the MS detection yielding little or no molecular ion, results in heavy reliance being placed on retention time for identification. To increase the confidence in the analysis, UHPLC-TOF-MS may be used as an orthogonal identification technique as it provides pseudo molecular ion information and complementary retention time data.

Derivatives of phenethylamines contain substituents which offer varying degrees of size, polarity, and lipophilicity. However, positional isomers differ only in the location of the substituent on the molecule. Hence, the main analysis problem encountered is the discrimination and subsequent identification between the different positional isomers.

In this study, a total of twenty-five substituted phenethylamines were subjected to analysis via UHPLC-TOF/MS. Four different superficially porous (SPP) 150mm x 2.1mm x 2.7µm columns were studied either in reversed phase chromatographic (RPC) mode, hydrophilic interaction liquid chromatographic (HILIC) mode or both. Three columns (C18, Phenyl-Hexyl, and Dimethylpentafluorophenylpropyl (PFP)) were utilized in RPC mode and two columns (HILIC and PFP) were utilized in HILIC mode. For each column, the mobile phase was optimized by either isocratic or gradient conditions to yield optimal separation. Effectiveness of separation was judged based on the ability to resolve the most compounds with special emphasis on the separation of positional isomers. Of the four columns employed, it was determined that the most efficient column was the C18 in RPC mode, which separated sixteen out of the twenty-five compounds. Furthermore, all positional isomers were resolved at a resolution greater than 1.

All positional isomers were separated, and nineteen out of the twenty-five phenethylamine derivatives were resolved by GC/MS. GC/MS provides a higher resolving power but relies heavily on retention time due to lack of discrimination between mass spectra of isomers. UHPLC-TOF/MS uses electrospray ionization (ESI) which yields a pseudo molecular ion and provides accurate mass information. This is an orthogonal technique which provides complementary data to that produced via GC/MS and increases the confidence in the identification of substituted phenethylamines.

Phenethylamines, UHPLC-TOF/MS, Positional Isomers