B171 Decreasing the Uncertainty of Peak Assignments Using Multidimensional Ultra-High Performance Liquid Chromatography (UHPLC)

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After attending this presentation, attendees will understand the potential for multidimensional UHPLC to significantly reduce peak assignment uncertainty based on retention time.

This presentation will impact the forensic science community by demonstrating the utility of multidimensional UHPLC as a separation technique for drug analysis. Screening and identification of emerging drugs can be achieved with significantly decreased uncertainty using multiple dimensions compared to one-dimensional LC. Identification can be accomplished through retention times obtained in the first and second dimensions.

Chromatographic techniques, which are commonly employed in forensic analysis, utilize retention time as an identification parameter. Conventional single-dimension chromatographic techniques, such as Gas Chromatography (GC) and LC, inherently lack the separation power required to resolve the multitude of combinations possible when analyzing emerging drugs. The use of multidimensional chromatography, which significantly increases the resolving power, is a viable means to increase the utility of retention time measurements for compound identification.

One-dimensional UHPLC separations were conducted on mixtures of controlled emerging drugs and mixtures of positional isomers of certain of these solutes for either synthetic cannabinoids, synthetic cathinones, or phenethylamines in order to determine the most orthogonal combination for multidimensional chromatography. The separations utilized several stationary phases for both Reversed Phase Chromatographic (RPC) separations and Hydrophilic Interaction Liquid Chromatographic (HILIC) separations. All separations utilized 2.1mm x 100mm or 2.1mm x 50mm columns with 1.7µm or 1.8µm particle sizes, with either a 10min acetonitrile or methanol gradient (hold up to 5min) with a pH 2.3 formic acid or ammonium bicarbonate pH 11.6 additive, or identical isocratic mobile phases containing acetonitrile and water with an ammonium formate additive for up to 5min HILIC separations. HILIC and high pH separations were only applicable to the basic cathinone and phenethylamine solutes. Peaks were identified through their obtained Ultraviolet (UV) and Mass Spectrometry (MS) spectra. The retention times obtained for each separation were used to determine correlation coefficients (R^2) for two columns, which were then used to determine the Neue selectivity factor (S^2)[1], a measure of the orthogonality for multidimensional chromatography. The peak capacity (n_c) was determined for each separation and the theoretical peak capacity ${}^1n_c * {}^2n_c$ for a multidimensional separation that assumes full coverage of the possible separation space, which is difficult to obtain in practice. Since the actual separation space can be approximated by the S^2 value, the actual peak capacity can be estimated by the following equation: ${}^{2D}[n_c]_{actual}={}^1n_c[1+S^2({}^2n_c-1)]$ with $[1+S^2({}^2n_c-1)]$, which represents the gain factor in going from a one-dimensional separation to a multidimensional separation. In this work, the actual multidimensional peak capacity was also used to measure peak assignment uncertainty. Based on the one-dimensional separations performed, it was determined that a combination of a C8 and a PFP column produced the highest S^2 values, and were thus more orthogonal, for both the controlled synthetic cannabinoids and the JWH-018 positional isomers. For the above column combination, the peak capacity for the controlled synthetic cannabinoids mixture showed an increase from 69 for one-dimensional LC to 3352 for multidimensional chromatography. The gain factor was determined to be approximately 50; thus, the peak assignment uncertainty was decreased by 50x with the use of multidimensional chromatography. Likewise, the JWH-018 positional isomers, which elute in a narrower separation space, showed a peak capacity increase from 5 to 53 and a gain factor of 10. For synthetic cathinones and phenethylamines, it was determined that the best combination would be a C8 and PFP column operated in the RPC and HILIC modes, respectively. For these mixtures of controlled substances, similar peak capacities as synthetic cannabinoids were obtained in the first dimension, with gain factors of nine and five for the synthetic cathinones and phenethylamine, respectively, in going from one-dimensional to two-dimensional separations. Similar to the synthetic cannabinoids, smaller gain factors were obtained for positional isomers of synthetic cathinones and phenethylamines.

Examples of multidimensional separations will be presented. Through the use of multidimensional UHPLC, uncertainty in peak assignments would be significantly reduced, leading to increased accuracy in the identification of seized drugs. The number of chromatographic runs performed can also be reduced as only one chromatographic system would need to be employed to obtain orthogonal separations.

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Reference(s):

1. Neue U.D., O'Gara J.E., Mendex A. Selectivity in reversed-phase separations influence of the stationary phase. *Journal of Chromatography A.* 2006:1127:161-174.

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