K17 The Separation of Chemically Similar and Isobaric Novel Psychoactive Substances (NPS) Using 2D-Liquid Chromatography (2D-LC)

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After attending this presentation, attendees will understand the principles of 2D-LC and its application for the separation of isobaric NPS that would otherwise co-elute in conventional 1D-Liquid Chromatography (1D-LC) separations.

This presentation will impact the forensic science community by demonstrating how 2D-LC can be a useful technique for the separation of NPS, including those from the cannabinoid class of drugs, which represents a large area of interest for detection and identification in forensic toxicology.

The constant emergence of NPS provides a significant challenge to detecting and identifying compounds of interest. New compounds are often isomers or analogs of existing NPS, and since such compounds are often structurally related, there is a risk of co-elution during 1D chromatographic analyses. If the coeluting compounds are unknown or previously unreported, a problem could arise in identification if the compounds are indistinguishable using mass spectral data alone (e.g., isobaric derivatives). To solve this problem, a better separation approach must be used to ensure that all compounds are resolved prior to identification.

Two-dimensional liquid chromatography (2D-LC) has been proposed as a method to improve separation and resolution of co-eluting and isobaric compounds prior to further mass spectral analysis. 2D-LC uses two orthogonal separation systems, or dimensions, to improve the resolving power of the overall separation by combining the power of each dimension.

This research focused on development of a comprehensive 2D-LC method in which two reverse-phase columns were used, one in each dimension, with the entire eluent from the first dimension transferred to the second dimension. An Agilent Poroshell 120 Bonus-RP column (2.1mm x 150mm, 2.7µm) was used in the first dimension, and a Supelco Ascentis Express Biphenyl column (2.1mm x 100mm, 2.7µm) was used in the second. An Agilent Infinity 1290 Diode Array Detector was used between the first and second dimensions, and an Agilent 6530 Quadrupole Time-of-Flight (qTOF) mass spectrometer was used after the second dimension. Two simple NPS mixes were created in methanol for use in development of the 2D-LC method. These mixes, called co-elution (CE) mixes, contained five co-eluting compounds each—CE Mix 1 contained isobaric compounds from the JWH-019 family of cannabinoids and CE Mix 2 contained non-isobaric compounds from other cannabinoid families that co-eluted in 1D-LC separations.

It was hypothesized that a 2D-LC system would enable separation of all five components in each mix in a single, continuous analytical run. Method development involved the optimization of each of the two dimensions, first as traditional 1D systems, then together as a continuous 2D system. Once optimized, each of the complete mixes and individual mix components were analyzed using the 2D methods. The first dimension was operated with a very narrow gradient and slow flow rate over 25 minutes. The second dimension was operated under a much faster flow rate using a shifted gradient to promote separation of co-eluting compounds after the first dimension analysis.

Initial 1D analysis using the bonus-RP column demonstrated that only three components of the five-component CE Mixes could be separated, with minimal resolution. In contrast, using the 2D-LC method, full separation of all five compounds in both CE Mixes 1 and 2 was achieved with good resolution. The separated and resolved peaks could then be analyzed by mass spectrometry. These studies confirmed that the use of 2D-LC can be a powerful technique when attempting to separate co-eluting and isobaric NPS.

Work is continuing to increase the number of compounds contained in each mix that can be successfully separated via 2D-LC. The method will also be further optimized so that separation of co-eluting and isobaric compounds from additional classes of NPS can be achieved.

2D-LC, Cannabinoids, NPS