



B193 Using Silica-Hydride-Based Stationary Phases for Dual-Mode Ultra High Performance Liquid Chromatography (UHPLC) Separation of Synthetic Cathinone Positional Isomers

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Learning Overview: After attending this presentation, attendees will understand the great utility of silica-hydride-based LC columns for the separation of synthetic cathinone positional isomers.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing methodology that will enhance the separation power of traditional LC technology employed for the separation of positional isomers of emerging drugs by involving the use of silica-hydride-based stationary phases, allowing for more accurate identification of these compounds.

Synthetic cathinones are a class of “emerging drug” commonly referred to and marketed as “bath salts.” Synthetic cathinones provide users similar psychostimulant and hallucinogenic effects as amphetamine, cocaine, and MDMA, but at a much cheaper price and with possibly lower legal liability. Like many other classes of emerging drugs (fentanyl, synthetic cannabinoids, and phenethylamines), many positional isomeric forms of synthetic cathinones exist, making identification of these drugs difficult for forensic laboratories.

Tandem techniques, such as Gas Chromatography/Mass Spectrometry (GC/MS) and LC/MS are employed for the analysis of emerging drugs. The identification of positional isomers using MS detection can be difficult, especially when substitution occurs on the benzene ring. Photo Diode Array Ultraviolet (PDA UV), vacuum-UV, and Fourier Transform Infrared (FTIR) detection for GC and LC have proved to be capable of distinguishing between some or all isomeric forms of these compounds. Due to the high degree of structural similarity between positional isomers, separation is often difficult. GC is commonly employed by laboratories to analyze synthetic cathinones, but derivatization and/or liquid-liquid extraction is normally required. LC (either characterized as High-Performance Liquid Chromatography [HPLC] or UHPLC) uses Reversed Phase Chromatography (RPC) or Hydrophilic Interaction Chromatography (HILIC) that allows for the direct analysis of these compounds. Due to the presence of a water layer formed during the chromatographic process, equilibration and repeatability in HILIC is challenging.

For Silica-Hydride (SiH) -based stationary phases, under Aqueous Normal Phase (ANP) conditions, a negligible water layer exists surrounding the stationary phase. ANP chromatography, similar to HILIC, employs an aqueous mobile phase with high organic content. Unlike HILIC, equilibration times are shorter and run times exhibit good repeatability. Another favorable aspect of SiH columns is that they can be operated under both RP and ANP modes. This dual-mode capability is highly advantageous in that a single sample can be run under both chromatographic modes using the same solvent reservoir solutions without the need to change columns. This methodology yields orthogonal retention times and dual UV wavelength detection, thereby significantly increasing the reliability of compound identification.

A mixture of eight synthetic cathinone positional isomers was analyzed by UHPLC-PDA/MS. Several SiH columns (C18, Phenyl Hydride, UDC Cholesterol, Diamond Hydride, and Silica-C) were run under RP and ANP conditions. Results were compared to the use of classical RPC and HILIC columns: C18, Silica, and Pentafluorophenyl (PFP). The PFP column also exhibits dual mode capability. “Optimum” separation conditions were determined for each column, and column selectivity, reversibility, and repeatability were explored.

Significant selectivity differences were observed using bi-modal columns and between different stationary phases. The SiH Silica-C column, which contains no derivatized ligands attached to the silica-hydride backbone, not only gave the most orthogonal separations of the bi-modal columns, but provided a unique separation of all 8 positional isomers (resolution>1) using ANP. Although the combination of RPC and HILIC columns gave the most orthogonal separations (with slightly greater selectivity than Silica-C stationary phase), allowing for a separation of all the positional isomers, poor repeatability was obtained using HILIC. In contrast, the Silica-C column, under ANP conditions, produced great separation and repeatability. The ability to use a single column to perform two types of orthogonal chromatographic separations with good repeatability would be beneficial for the separation of positional isomers of emerging drugs.

Silica-Hydride Stationary Phases, Emerging Drugs, Positional Isomers