K34 Chiral Separation of Methylphenidate, Ethylphenidate, and Ritalinic Acid in Blood

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Learning Overview: After attending this presentation, attendees will understand chiral separation of cognitive stimulants and how to effectively isolate and extract them from biological samples.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing the attendees with a unique approach to extraction of cognitive stimulants from blood. With this, a novel approach to chiral separation will be presented to the forensic community.

There has been a recent trend of abusing cognitive stimulants. Drugs such as Methylphenidate (MPH) are commonly prescribed for Attention-Deficit/Hyperactivity Disorder. MPH exists as two enantiomers, though the Dextro (D) configuration is more potent. Analytically, separation of the isomers is essential. However, chiral analysis poses challenges to researchers. Currently, there are no methods available to detect multiple chiral stimulants, and their metabolites in a single analysis. While Ritalinic Acid (RA) is the primary metabolite, Ethylphenidate (EPH) is also produced when alcohol is co-administered. Due to limited assays, this study sought to develop a method that separates the entantiomers of MPH and its metabolites following isolation and extraction from blood samples. Methods such as this are critical to understanding the pharmacokinetics of such cognitive stimulants.

Blood (0.25mL) was fortified with deuterated internal standards (0.025mL). The samples were diluted with phosphate buffer (pH 6) and allowed to stand for five minutes before centrifugation. Following conditioning with methanol and phosphate buffer, samples were loaded onto DAU clean screen extract Solid-Phase Extraction (SPE) cartridges. Cartridges were washed with 0.1M acetic acid and methanol. Analytes were eluted with 2% ammonium hydroxide in methanol, dried, and reconstituted in mobile phase. An Agilent® Technologies 1290 Infinity Liquid Chromatograph coupled to an Agilent® 6470 Triple Quadrupole spectrometer was used for detection of the analytes. Chiral separation was achieved using an Agilent® Poroshell Chiral-V column (2.1 x 100mm, 2.7u) with a flow rate of 0.6mL/min. Mobile phase consisted of deionized water (A) and trifluoroacetic acid (0.0125%, v/v) and ammonium acetate (0.025%, w/v) in methanol (B) at a ratio of 2:98 (A:B) with an isocratic elution. The run time was four minutes.

When developing this method, various parameters were assessed in order to optimize chiral separation. Different gradient elution systems with different buffers and mobile phase composition were investigated in hopes of achieving full baseline separation of the enantiomers. With the final mobile phase and column selections (discussed above), full baseline resolution between the D- and L-MPH enantiomers and the D- and L-EPH enantiomers was achieved. In the same injection, this study was also able to detect and separate RA. Various sample preparation techniques such as protein precipitation, liquid-liquid, supported liquid, and other SPE chemistries were investigated. Optimal isolation and extraction of the analytes in blood was achieved in the extraction discussed above. Recoveries were >90% and matrix effects were <±22% with the exception of RA (80%). All matrix effects and extraction recoveries were compensated by matched deuterated internal standards.

This is the first known method that chromatographically separates the enantiomers of MPH and EPH, in addition to RA. This method also utilized simple SPE to isolate the drug and metabolites from a single sample of blood (0.25mL).

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