K42 Chiral Separation and Analysis of Methylphenidate, Ethylphenidate, and Ritalinic Acid in Blood by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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Learning Overview: After attending this presentation, attendees will understand chiral separation and method validation of cognitive stimulants and how to effectively analyze them on an LC/MS/MS.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing attendees with a unique approach to the extraction of cognitive stimulants from blood. With this, a novel approach to chiral separation and analysis 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 isomers, threo and erythro, though the erythro isomer is the only one proven to give pharmacological effects. The erythro isomer is present as Dextro (D) or Levo (L) configuration, with the D configuration being more potent. However, many medications are sold as a racemic mixture. This makes analytic separation of the isomers essential. MPH metabolizes into Ritalinic Acid (RA) as well as Ethylphenidate (EPH) in the presence of ethanol. Chiral analysis poses challenges to researchers. Currently, there are no methods available to detect these chiral stimulants in a single analysis. Due to limited assays, this project aimed to develop a method that separates and quantifies the enantiomers of MPH and EPH as well as RA following isolation and extraction from blood samples. Methods such as this are critical to understanding the pharmacokinetics of such cognitive stimulants. The goal of this study was to fully develop and validate a method to separate the threo-enatiomers of d,l-MPH, d,l-EPH, and RA and quantify them in blood utilizing LC/MS/MS.

MPH, EPH, and RA were extracted from blood (250µL) using Solid Phase Extraction (SPE). Blood was fortified with calibrator or control solution (25µL) and internal standard (25uL), mixed with phosphate buffer (1mL, pH 6, 100mM), then centrifuged. The supernatant was loaded onto an UCT Clean Screen® DAU column (130mg/3mL) on an SPE cartridge previously conditioned with methanol and phosphate buffer. Following washes with 0.1M acetic acid and methanol, compounds were eluted with 2% ammonium hydroxide in methanol. Analysis was performed on an AGILENT® 1290 Infinity Liquid Chromatograph coupled to an AGILENT® 6470 Triple Quadrupole MS. Separation was achieved using an AGILENT® Chiral-V column (2.7um, 2.1x100mm) with an isocratic elution of 2:98 mobile phase A:B at 0.6mL/min. Mobile phase A was deionized water and mobile phase B was 0.025% ammonium acetate and 0.0125% trifluoroacetic acid in methanol. A Multiple Reaction Monitoring (MRM) method was used to detect the analytes with one transition for quantification and one for qualification. The method was validated according to Academy Standards Board (ASB) guidelines, including: precision and bias, linearity, carryover, interferences, matrix effects, Limit Of Detection (LOD), Limit Of Quantification (LOQ), dilution integrity, and stability.

The linear range for MPH and EPH was 0.5–200ng/mL and 0.5–500ng/mL for RA (R^2 >0.99 for five days). LOD was determined to be 0.25ng/mL for all analytes and LOQ was 0.5ng/mL. Extraction recovery was >79%. Matrix effects were determined via post-extraction addition and displayed enhancement for MPH and EPH (17%–58%) and suppression for RA (51%–58%). Bias ranged from -12.7% to -4.8% and maximum within run precision was 12.5% for all analytes. Stability was evaluated as a processed sample stability in the autosampler (48h, 4°C), refrigerated (48h, 4°C), and at room temperature (24h, 24°C), and all analytes were considered stable and quantified within 16%. No carryover was observed. Endogenous and exogenous interferences were evaluated and had no significant impact.

This method was developed and fully validated for the quantification of d,l-MPH, d,l-EPH, and RA in blood. This is the first method, per research, that successfully separates the enantiomers and quantifies all analytes within a single analysis without the use of chiral derivatization. Due to differing effects of these enantiomers as well as different rates of metabolism, it is essential to separate these compounds. This method can be utilized to help better understand the pharmacokinetics of these chiral stimulants.

Methylphenidate, Chiral Analysis, LC/MS/MS

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