K19 Quantitative Analysis of Methylphenidate (Ritalin®) and Ritalinic Acid in Oral Fluid by Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS)

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The goal of this presentation is to introduce attendees to the use of human oral fluid for the quantitation of Methylphenidate (MPH) and its major metabolite, Ritalinic Acid (RA), through chromatographic and mass spectral analysis. Attendees will be familiarized with the advantages of a non-conventional biological matrix when applied to the forensic and clinical monitoring of these drugs.

This presentation will impact the forensic science community by informing attendees about an efficient, simple, and fast analytical procedure utilizing a non-invasive technique for monitoring MPH and RA in human oral fluid via the introduction of a dilute-and-shoot LC/QqQ/MS method of analysis.

MPH is an amphetamine derivative commonly used in the treatment of Attention-Deficit Hyperactivity Disorder (ADHD) in children, adolescents, and adults, a condition affecting 3%-5 % of the United States population. The 2011 National Survey of Children's Health reported that 11% of children aged 4-17 years were diagnosed with ADHD by a health care provider in the United States, with 6.1% of children in that age group having been prescribed medication for treatment of ADHD. In recent years, methylphenidate HCl has become one of the most frequently abused prescription drugs due to its psychostimulant effects. In addition, the drug has become popular with college students as a "study drug." According to the Drug Abuse Warning Network (DAWN) Report, the number of emergency department visits involving the non-medical use of ADHD-stimulant medications increased from 5,212 to 15,585 between 2005 and 2010. When administered orally, MPH is rapidly absorbed and hydrolyzed to its inactive metabolite RA. Current analytical methods for analysis of MPH and RA involve the analysis of blood and urine matrices using liquid-liquid extraction, solid-phase extraction, and Gas Chromatography/Mass Spectrometry (GC/MS). These methods are time consuming and sample collection is invasive and tedious. Due to the rising abuse of MPH, development of quick and non-invasive monitoring methods is important.

This presentation will demonstrate human oral fluid as a suitable matrix for analysis of MPH and RA using Electrospray Ionization (ESI) Liquid Chromatography/Mass Spectrometry (LC/MS) as a sensitive and specific method for screening and quantitation. Oral fluid samples were collected using an oral fluid collection device containing a cotton pad. They were vortexed, centrifuged for 30min at 3,200rpm and filtered into polypropylene serum tubes. The extracts were stored at -20°C until use. An Agilent 6460 triple quadrupole MS-equipped with Jet Stream ESI technology was used in the triggered Multiple Reaction Monitoring (MRM) mode for the characterization of the analytes. The ESI source was operated at 325°C in positive mode, with an optimized fragmentor voltage of 75V. The tMRM transitions were 234→84 and 234→56 for MPH and 220→84 and 220→56 for RA, with a cycle time of 500ms. The advantage of using multiple transitions is suggested as a fingerprint mass spectral tool for unambiguous identification of the target compounds. Gradient elution was performed on a Poroshell 120 EC-C18 column (2.1 x 100mm x 2.7µm) using MeOH+0.1% formic acid as organic phase and 5mM ammonium formate + 0.1% formic acid as phase modifier, with a total run time of 5min. Six-point linear calibrations with a weight of 1/x based on the internal standards (+)-methylphenidate-d9 HCl and (+)-threo-Ritalinic acid-d10 HCl, ($r^2$3$0.99) were obtained for concentrations between 0.50ng/mL and 100.00ng/mL for the drugs of interest, with quality controls showing an RSD<13%. The limits of detection for MPH and RA were 0.20ng/mL and 0.30ng/mL, respectively, indicating the validity of the method for identification and confirmation at low concentrations.

Methylphenidate, LC/QqQ/MS, Oral Fluid

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