K24 A Rapid Quantitative Analysis of Stimulants by Ultra High-Pressure Liquid Chromatography-Mass Spectrometry (UHPLC-MS)

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After attending this presentation, attendees will have a deeper understanding of a UHPLC-Tandem Mass Spectrometric (MS/MS) method for analyzing blood specimens for common stimulants, including prevalent drugs of abuse, such as amphetamine, methamphetamine, 3,4 Methylenedioxymethamphetamine (MDMA), Phencyclidine (PCP), and cocaine.

This presentation will impact the forensic science community by providing a quick, comprehensive method for stimulant confirmation performed on UHPLC-MS/MS. This method is capable of analyzing compounds often analyzed in separate methods and creates a green waste stream.

The presented assay is used for the quantitative analysis of amphetamine, methamphetamine, MDMA, MDA, MDEA, phentermine, phenylpropanolamine, pseudoephedrine, ephedrine, cocaine, benzoylecgonine, and PCP. These drugs all exhibit stimulating effects on the central nervous system, such as euphoria, excitation, feelings of strength, hallucinations, and feelings of well-being or calmness.[1-3] All of these drugs are classified as stimulants with the exception of PCP, which is a hallucinogen (all drugs of interest will be referred to as "stimulants" for convenience throughout this presentation).

Stimulants have been used to treat a variety of medical ailments over time, including asthma, obesity, narcolepsy, ADHD, and as an anesthetic. As stimulant use grew, the abuse of these drugs grew as well and their medical use decreased to only a few specific cases.[4] Stimulants are seen in forensic toxicology as one of the most prevalent impairing compound groups, with an estimated 1.7 million users of one or more of the drugs of interest in 2012.[5,6] The Substance Abuse and Mental Health Services Administration estimates approximately 3.6 million users of nonmedical stimulants, methamphetamine, MDMA, cocaine, and PCP, with trends of steady or slowly declining usage over the last 10-15 years. The 2015 Drug Enforcement Agency National Drug Threat Assessment shows methamphetamine and cocaine as readily available in most areas.[7,8] The need for accurate and efficient analysis of these drugs as well as other stimulants will persist for the foreseeable future.

Blood specimens analyzed using this method are prepared using solid phase extraction on SPEware Trace-B columns. The columns are conditioned prior to sample addition, and washed with water, dilute acetic acid, methanol and ethyl acetate. Ethyl acetate, ammonium hydroxide, and isopropanol are used for the elution. This method produces a green waste stream as halogenated solvents are not used and only 9mL total are necessary to condition and wash the columns.

This assay utilizes an Agilent 1290 UHPLC equipped with an Agilent phenyl hexyl 2.5µm X 100mm analytical column and phenyl hexyl guard column interfaced with an Agilent 6410 triple quadrupole mass spectrometer, equipped with a electrospray ionization source operated in the positive mode. The method achieves chromatographic separation of all 12 analytes over a 10 minute analytical run time. Two MS^2 fragmentation transitions are monitored

for mass spectral identification of each analyte. This method proposes linear quantification ranges of 5ng/mL-500ng/mL for all analytes except cocaine and benzoylecgonine, which will be 10ng/mL-1000ng/mL.

A method has been presented that provides quantitative analysis of 12 stimulant analytes via UPLC/MS^2. This method provides clear chromatographic separation of all compounds, including isomers pseudoephedrine and ephedrine, and large linear dynamic ranges. This assay combines drugs that are normally analyzed separately, which saves time and materials allowing for rapid analysis of samples. By utilizing UPLC/MS^2 this method removes the need for derivatization, which again minimizes prep time and also minimizes loss of these volatile compounds through the heating process. Validation of this method will follow SWGTOX guidelines to ensure the quality of data obtained.

Reference(s):

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