K33 The Analysis of N,N-Dimethyltryptamine (DMT) in Plasma by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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After attending this presentation, attendees will better understand, through actual demonstration, an LC/MS/MS method to analyze DMT in plasma.

Hallucinogenic drugs such as DMT are popular drugs of abuse. It is important that there are effective analytical methods to test for these drugs in biological samples such as plasma. This presentation will impact the forensic science community by illustrating how to analyze for DMT in plasma by LC/MS/MS.

DMT is an indole compound that produces hallucinogenic effects. It is a **Drug Enforcement Administration** (DEA) Schedule I controlled substance. DMT is found in South American hallucinogenic plants such as *Piptadenia peregrina*. DMT is structurally similar to the neurotransmitter serotonin and may exert it effects on neuro-mechanisms that involve serotonin. Small amounts of endogenous DMT (generally less than 1ng/mL) have been detected in human plasma, but typical DMT use results in plasma concentrations in the 10ng/mL to 100ng/mL range. A method utilizing LC/MS/MS was developed to determine DMT in rat plasma samples. An effort to achieve a 1.0ng/mL DMT Limit Of Quantitation (LOQ) for the analysis seemed reasonable.

For the analysis, a 0.1mL volume of calibrators (range 1ng/mL to 500ng/mL) and controls (3ng/mL, 25ng/mL, and 400ng/mL) were prepared in separate, clean 16mm x 100mm culture tubes. Each tube was fortified with 30ng/mL of 5-methyl-DMT (30µL of 0.1ng/µL) as the internal standard. Liquid-liquid extraction was used to prepare the samples for analysis: 0.5mL water, 0.1ml ammonium hydroxide, and 3mL of 1-chlorobutane:acetonitrile (4:1) were added to each tube. The tubes were mixed for 20 minutes on a reciprocating shaker and centrifuged. The organic layer from each tube was collected into separate, clean 13mm x 100mm glass culture tubes. A 0.1mL volume of 0.1% hydrochloric acid in methanol was added to each extract tube. The extracts were evaporated to dryness using a Turbovap® evaporator, reconstituted with 0.2mL of 10mM ammonium acetate, pH 5:methanol (85:15), and transferred to separate conical polypropylene autosampler vials. The LC/MS-MS analysis used an Agilent® 1100 LC interfaced with an Access TSQ Quantum® MS and was operated by Xcalibur™ version 2.0 SR2 software. The LC conditions involved the use of a Selectra® DA 100 x 2.1mm, 3µm column. The isocratic mobile phase was 10mM ammonium acetate, pH 5.0:methanol (55:45) at a 0.2mL/minute flow rate. The MS/MS analysis employed positive ion electrospray ionization and Selected Reaction Monitoring (SRM). For each compound, the precursor ion was the protonated molecular ion. The SRM transitions that were monitored were: DMT: 189®144 (quant.), 189®58; 5-methyl-DMT: 203®158. The DMT and 5-methyl-DMT were chromatographically separated with good peak shape. Ion suppression evaluation showed that blank plasma extracts did not suppress the MS/MS signal for DMT and 5-methyl-DMT. For six different blank plasma sources, blank matrix selectivity studies showed that peak area ratios from endogenous compounds in blank plasma were well below 20% of the 1ng/mL DMT (LOQ) peak area ratio. Precision and accuracy were evaluated by analyzing the control samples. The intra-run accuracy ranged from 90.3% to 106.9% of target and the intra-run precision ranged from 1.7% to 3.7%. The inter-run accuracy ranged from 101.8% to 113.0% of target and precision ranged from 4.6% to 10.4%. The extracts were stable for at least six days at room temperature and 19 days at 4°C. In conclusion, this study developed an LC/MS/MS method that was capable of analyzing for DMT in rat plasma. Cross-validation to human plasma is planned.

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N,N-Dimethyltryptamine, LC/MS/MS, Analysis