K55 Sample Preparation of Cannabiniods in Urine Using Dispersive Solid Phase Extraction and Clean Up

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After attending this presentation, attendees will understand how to increase sample throughput using a simplified sample clean up method for analyzing cannabinoids in urine as well as understand how to derivatize and analyze cannabinoids by GC-MS.

The sample preparation and analysis methodology discussed will impact the forensic community by providing an alternate means of processing and analyzing cannabinoids compared to current sample preparation and analysis methodologies.

The main psychoactive component in marijuana, Δ^9– tetrahydrocannabinol (Δ^9–THC), is quickly absorbed and metabolized to 11-hydroxy-Δ^9-tetrahydrocannabinol (hydroxy-THC), an active metabolite. The hydroxy-THC is further metabolized (rapidly) to 11- nor-9-carboxy-Δ^9-tetrahydrocannabinol (carboxy-THC), an inactive metabolite commonly found in urine, blood, hair, and other tissues. GC- MS (Gas Chromatography-Mass Spectrometry) often is used for confirming and quantifying Δ^9–THC and carboxy-THC. However, GC-MS methods require time-consuming steps like sample clean up to obtain acceptable chromatography. Using a dispersive solid phase extraction and clean up technique saves time without sacrificing reproducibility and sensitivity.

This study included developing a sample clean up method for analyzing cannabinoids in urine using a dispersive solid phase extraction and clean up method (dSPE). The dSPE method employs a quick extraction followed by a cleanup of the sample. Small polypropylene centrifuge tubes are prefilled with precise weights of $MgSO_4$ and SPE (solid phase extraction) adsorbents to remove excess water and unwanted contaminants from the samples. After agitation and centrifugation, the cleaned extracts are ready for further processing. Samples may be derivatized, pH adjusted to protect sensitive compounds and/or solvent-exchanged to improve analysis by GC-MS. Internal standards can also be added. The samples are then ready for analysis by GC-MS. Also, dSPE and sample clean up process can be used for HPLC-MS (high performance liquid chromatography-mass spectrometry) applications.

A reproducible, quantitative GC-MS method for analyzing cleaned- up, derivatized cannabinoids in urine was developed. Two goals were the focus in this study: (1) to reduce sample clean up time for cannabinoids in urine, and (2) to provide a reliable and reproducible sample preparation method for quantification data in the low ng range (< 10ng). Compounds analyzed for were Δ^9–THC and carboxy-THC. The internal standard used was deuterated THC. Derivatizing reagents experimented with included a silylation reagent and an acylation reagent. The instrument used was a Shimadzu GC-MS.

Results showed that the proper dispersive solid phase extraction and sample clean up method coupled with the proper derivatization reagent produced reproducible data with linearity across a broad range of concentrations. The limit of detection (LOD) reached was as low as 5ng on-column, and sample preparation time was reduced. The use of GC- MS allowed for identification of derivatized cannabinoids, particularly Δ^9–THC and carboxy-THC, relative to their unique mass spectra. Analysis time was kept under 10 minutes since run conditions were optimized.

In conclusion, the methods developed in this study can benefit analysts by providing a simple and short extraction and clean-up procedure, by providing a reproducible derivatization procedure and by providing reduced analysis times using GC-MS for cannabinoids in urine.

Sample Preparation, GC-MS, THC

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