K14 An Analysis of Stimulants and Metabolites From Dried Blood Spots (DBS) Using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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After attending this presentation, attendees will gain knowledge and insight into the advantages of the usage of DBS as a sample collection technique for Driving Under the Influence of Drugs (DUID) cases as well as an appropriate procedure that allows for the extraction and analysis of stimulant-type drugs from DBS.

This presentation will impact the forensic science community by providing new information and methods pertaining to the field of drug testing and forensic toxicology. This proposed method reveals multiple advantages for the use of DBS compared to typical DUID sample collection procedures and could ultimately be adapted as a means of roadside sampling during these investigations. These DBS samples allow for a less invasive sample collection, lower amounts of blood needed, and an easier means of storage and transportation.[1]

The majority of DUID investigations rely on blood testing to determine the types and quantities of drugs present in a sample due to the fact that blood allows for the analysis of parent compounds as well as their metabolites.[2] During a typical DUID process, blood samples are collected from the individual once the stop is completed and the person is transported to the location of sample collection. Often times there is a long time in between the time of the stop and the time the sample is collected, which typically involves large volumes of blood being collected. This large time span can cause decreased levels of the drugs present in the sample, and it is possible for these samples to fall below screening cut-off values.[3]

In this report, a selective liquid chromatography tandem mass spectrometry (LC/MS/MS) method was developed in order to analyze stimulant type drugs that have been extracted from DBS. FTA DMPK-C blood cards were used as the medium to collect and store the blood samples. The extracts from 30µL of blood deposited on blood cards were analyzed using a Shimadzu LC system connected to an ABI Sciex 3200 QTRAP triple quadrupole mass spectrometer operating in positive-ion mode. The liquid chromatographic separation of the compounds was completed and optimized using a Restek Ultra® C_{18} column (5.0cm x 2.1mm, 3.0µm). The HPLC method used a binary mobile phase system consisting of a 0.1% formic acid weak phase and a 0.1% formic acid in acetonitrile strong phase and the total run time was 6.5 minutes. A retention time versus temperature optimization study provided the most favorable separation conditions at 25°C. Optimum MS conditions (Q1 and Q3 ions, collision energy, declustering potential) were determined for each of the compounds as well as their internal standards. Amphetamine, benzoylecgonine, cocaethylene, cocaine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), and methamphetamine were the seven stimulants chosen as the focus for this study. The extraction procedure for the DBS was optimized through testing multiple extraction solvents, mechanical mixing techniques, blood spot sizes, and drying down techniques. The optimized method was used to analyze authentic blood samples from toxicology laboratories to test its fit for purpose.

In conclusion, this developed method has potential to be used in forensic toxicology testing during DUID investigation. This method could allow for roadside sampling onto the DBS cards which will decrease the time between the DUID stop and sample collection, allowing for more accurate levels of the drug present in these samples to be determined during the forensic testing.

Reference(s):

1. Li W, Tse F. Dried blood spot sampling in combination with LC-MS/MS for quantitative analysis of small molecules. Biomed Chromatogr 2010;24:49-65.
2. Logan BK, et al. Recommendations for toxicological investigation of drug-impaired driving and motor vehicle fatalities. J Anal Tox 2013:1-7.
3. Mercolini L, et al. Dried blood spots: liquid chromatography-mass spectrometry analysis of Δ^9-tetrahydrocannabinol and its main metabolites. J Chromatogr A 2013;1272:33-40.

Stimulants, Dried Blood Spots, LC/MS/MS