K21 The Analysis of Benzodiazepines in Dried Blood Spots (DBS) Using Liquid Chromatographic/Tandem Mass Spectrometry (LC/MS/MS)

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After attending this presentation, attendees will better understand the use of DBS as a substrate for collection of blood for the purpose of detecting specific benzodiazepines.

This presentation will impact the forensic science community by providing a method that determines the sensitivity and stability of common benzodiazepines in blood samples using FTA®DMPK-C (untreated) cards by GE Healthcare as the substrate.

Benzodiazepines are central nervous system depressants and are commonly prescribed medications in the United States today. They are classified as Schedule IV in the Controlled Substances Act and have a high potential for abuse due to sedative properties, especially when mixed with other depressants. This is important because the detection of benzodiazepines is pertinent to Driving Under the Influence (DUI) toxicology. Drug-impaired drivers harm or kill thousands of people each year in the United States and there is a growing body of scientific evidence confirming that driving under the influence of prescribed medications has become a significant problem worldwide. Therefore, a selective and sensitive analytical method for the detection and stability of benzodiazepines in biological samples would be exceedingly beneficial to the field of forensic toxicology. Among the benzodiazepines available, analyses of alprazolam, clonazepam, 7-aminoclonazepam, diazepam, lorazepam, nordiazepam, nitrazepam, and flunitrazepam in blood samples in addition to the deuterated internal standards alprazolam-d5, clonazepam-d4, 7-aminoclonazepam-d4, and lorazepam-d4 have been used in this research utilizing DBS.

The use of DBS on cards as a substrate for collection of blood for the purpose of detecting drugs in DUI cases has many advantages. The technique uses less blood, typically 10µL-50µL of capillary blood, which can be obtained through minimally invasive procedures. The cards are easy to handle, easy to transport, and can be stored at ambient temperature in the laboratory with minimal analyte loss. This makes sampling simpler, faster, and less invasive.

A selective LC/MS/MS method was developed for the analysis of eight benzodiazepines. The method showed good linearity for each benzodiazepine with R^2 values of >0.99 and was able to detect down to 50ng/mL of each analyte in 10µL of blood from a DBS with a simple methanol extraction. The analysis was performed using an ABI® SCIEX™ 3200 Qtrap® triple quadrupole mass spectrometer interfaced with a Shimadzu® LC system consisting of two Shimadzu® LC-20AD Prominence liquid chromatography binary pumps, two Shimadzu® DGO-20A_3 Prominence degassers, and a Shimadzu® SIL-20AC Prominence auto sampler. Chromatographic separation was achieved using an Ultra® Biphenyl LC Column (5.0cm x 3.0mm i.d., 2.7µm particle size). The High-Performance Liquid Chromatography (HPLC) method was isocratic with 30:70 0.1% (v/v) formic acid in methanol and the total run time was 9.50 minutes. A retention time versus temperature optimization study provided the most favorable separation conditions at 20°C.

Benzodiazepines, Dried Blood Spots, LC/MS/MS

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