K27 A Rapid Analysis of Pharmaceuticals in Human Tissues by 2D-Liquid Chromatography/ Tandem Mass Spectrometry (2D-LC/MS/MS) Technology

Malorie Mella, BA, Waters Corporation, 34 Maple Street, Milford, MA ; Claude Mallet, PhD, Waters Corporation, 34 Maple Street, Milford, MA ; Sabra R. Botch-Jones, MS, MA, Boston University School of Medicine, Biomedical Forensic Sciences, 72 E Concord Street, Boston, MA 02118; Brendan Nolan Schweitzer, BS, Boston University, 20782 Medley Lane, Topanga, CA 90290; Dennis V. Canfield, PhD, FAA, Civil Aeromedical Inst, Bioaeronautical Sciences, AAM-610, PO Box 25082, Oklahoma City, OK 73125-5066; Philip M. Kemp, PhD, Bioaeronautical Research Lab, 6500 S MacArthur Boulevard, FAA, CAMI Bldg, Oklahoma City, OK 73169-6901; and Kacey Cliburn, MS, FAA, AAM-610, PO Box 25082, Oklahoma City, OK*

After attending this presentation, attendees will better understand a rapid sample preparation method for use when extracting drugs from human tissues prior to analysis via LC/MS/MS.

This presentation will impact the forensic science community by showcasing a quick sample prep method of a complex matrix without any evaporation-to-dryness step as well as the advantages of using multidimensional chromatography.

Introduction: In postmortem forensic toxicology, tissues such as heart, lung, liver, spleen, kidney, brain, and stomach muscle are often utilized for testing due to the state of human remains or lack of available blood or urine. However, detection and quantitation of drugs in complex matrices, such as these tissues, is challenging due to time-consuming extraction processes and at times the inability to detect an analyte at trace concentrations. Additionally, an analytical method capable of screening a large number of compounds is time-consuming to develop and difficult to optimize for every compound.

Objective: A robust extraction and clean-up methodology, in which a homogenization step precedes extraction, is required to efficiently extract drugs from complex matrices, to reach a target Limit Of Detection (LOD), and to maintain instrumental performance. Traditional solid phase extraction techniques require a lengthy evaporation step, which can take hours. The objective of this study was to develop a micro extraction protocol combined with multi-dimension chromatography to decrease sample preparation time without sacrificing the quality seen with current single dimension chromatography techniques.

Method: For this study, in collaboration with the Federal Aviation Administration, de-identified human tissue samples consisting of brain, heart, lung, kidney, liver, spleen, and muscle were analyzed. The method described includes 21 compounds and metabolites including: zolpidem, citalopram, norbuprenorphine, oxycodone, normeperidine, dextrorphan, dextromethorphan, diazepam, diltiazem, quetiapine, diphenhydramine, buprenorphine, promethazine, dihydrocodeine, doxylamine, flecainide, hydromorphone, nordiazepam, temazepam, n-desmethylcitalopram, and oxazepam. Samples were homogenized using a Precellys Evolution tissue homogenizer (Bertin Technologies, Montingny-le-Bretonneux, France) incorporating a mixed mode reversed-phase/ion exchange sorbent. The use of a 2D-LC/MS/MS technology eliminated the need for a lengthy evaporation step in the extraction method as eluents were transferred directly to the LC vials for analysis. The chosen 2D-LC/MS/MS (Acquity 2D UPLC with Xevo TQD, Waters, Milford, MA, USA) used in this application was selected using a 6x6 automated method development protocol.

Results: The manual extraction of tissue samples was completed in less than 30 minutes. The analysis was performed using 100uL of the final organic solvent (methanol or acetonitrile) extracts. The Limit Of Quantitation (LOQ) for all drugs was measured at 100ng/g from a 1g sample mass. The limit of detection LOD for most drugs was determined to be 10ng/g (10ppt) with some as low as 1ng/g (1ppt).

Conclusion: The micro extraction protocol combined with multi-dimension chromatography used in this study decreased sample preparation time significantly without sacrificing the quality seen with current single dimension chromatography techniques. The procedure developed in this study can be utilized on various human tissues and completed in less than 30 minutes before injection of 100uL final extract into the 2D-LC/MS/MS system.

LC/MS/MS, Multidimensional Chromatograph, Pharmaceuticals