K31 Method Validation for Simultaneous Identification and Quantification of Postmortem Volatiles in Whole Blood (WB), Vitreous Humor (VH), and Cerebrospinal Fluid (CSF) Using Gas Chromatography/Headspace/ Flame Ionization Detector (GC/HS/FID)

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Learning Overview: After attending this presentation, attendees will gain appreciation for the application of a validated GC/HS/FID methodology for simultaneous detection and quantification of seven volatiles as detected in stored whole blood, vitreous humor, and cerebrospinal fluid.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by helping toxicologists understand the intricacies and significance of analyzing many volatiles in single run using acetonitrile as an internal standard. This will also help attendees better understand the correlation of ethanol concentration between each body fluid.

Determination of blood alcohol content is a frequently requested assay in forensic toxicology laboratory. Although determination of alcohols in blood is a routinely used procedure, the results and methodologies are constantly improving. A highly sensitive, reproducible, and rapid GC method was validated for the analysis of seven volatiles frequently encountered in stored samples and samples collected within hours of autopsy. The extensive review of literature in this field yielded methods with extensive instrumentation and method preparation. Although the use of GC for the determination of blood alcohol concentration is a routinely used procedure, its validation for the use for stored and postmortem samples is an emerging research field. This presentation covers a method, using GC/HS/FID, capillary column (DB-624) and carrier gas-Nitrogen, validated to analyze ethanol, methanol, acetone, acetaldehyde, N-propanol, I-propanol, and N-butanol in different postmortem matrices such as femoral blood; vitreous humor and cerebrospinal fluid; with acetonitrile as an internal standard. Addition of an internal standard (acetonitrile) was ensured to authenticate results and compensate for any matrix changes. A good peak resolution between volatiles and internal standard was achieved. Linear correlation was achieved for peak area and concentration across the range of 3.95mg to 316mg/100ml with a correlation coefficient within the range of 0.988–0.999 for all matrixes. Limit of Quantitation (LOQ) and Limit of Detection (LOD) were within the range of 0.1mg%–1mg% for all matrixes. Reproducibility of samples and standards, inter- and intra-day, resulted in precision and accuracy within the range of acceptance as prescribed by validation guidelines. The sample volume required for validation was 1ml. With the developed method, no sample preparation or pre-treatment was required for volatile estimation. The total run time of the GC and HS cycle was 24.68min after sample sealing. The advantage of simultaneous screening of volatiles is important in forensic toxicology because of the possibility of identification of volatiles in single run, less sample utilized, and easy results for interpretation.

In conclusion, the proposed methodology serves to analyze the compounds, quantitatively and qualitatively utilizing minimum sample volume, and analysis of different biological fluids with the same method resulted in a methodology competent enough to be used in research and forensic samples.

Postmortem Alcohols, Gas Chromatography, Validation

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