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# THE DETERMINATION OF ETHYL GLUCURONIDE AND ETHYL SULPHATE IN VITREOUS HUMOUR AS BIOMARKERS OF RECENT ALCOHOL USE IN POST-MORTEM FORENSIC CASES USING UPLC-MS/MS

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**ABSTRACT**

*Alcohol abuse is a major problem within societies all around the world. The use of alcohol contributed to 5.3% of deaths worldwide in 2016 while in South Africa, approximately 6.4% of all deaths are associated with alcohol consumption. In post-mortem investigations, the degree of alcohol intoxication is important to understand the circumstances of death. The gold standard method used to quantify alcohol intoxication analyses ethanol in blood. Despite this being a simple, accurate and specific analytical method, the interpretation and evaluation of the blood ethanol concentration (BEC) results is complicated by the behaviour of ethanol in a decedent prior to sampling. These complications are associated with the natural decomposition processes, circumstances of death, contamination and the short half-life of ethanol. The exploration into alternate biological specimens and alternate biomarkers has led to the scientific investigation of vitreous humour and analytes, such as ethyl glucuronide (EtG) and ethyl sulphate (EtS), as means to evaluate ethanol use and abuse. Vitreous humour is a simple biological matrix that is rarely contaminated by post-mortem processes. EtG and EtS are highly specific and sensitive ethanol biomarkers that are used to detect and quantify ethanol intoxication. The aim of this study was to develop and validate a method to simultaneously analyse EtG and EtS in vitreous humour using Ultra-Performance Liquid Chromatography couple with tandem Mass Spectrometry (UPLC-MS/MS) as well as to validate a method to analyse ethanol in vitreous humour using Headspace Gas Chromatography coupled with a Flame Ionization Detector (HSGC-FID). These methods were used to analyse 112 post-mortem vitreous humour samples obtained from the Johannesburg Forensic Pathology Services Medico-Legal Laboratory (JHB FPS MLL). EtG and EtS method development involved the optimization of sample preparation procedures, UPLC conditions and tandem MS conditions. The optimal sample preparation used protein precipitation with 0.1% formic acid in acetonitrile. EtG and EtS were simultaneously analysed using a UPLC-MS/MS system with a Phenyl-Hexyl column (150 mm length, 2.1 mm inner diameter and 1.7 µm particle size) and a gradient elution profile. Ethanol was analysed on a HSGC-FID system with a SUPELCOWAX® 10 GC column (30 m length, 0.25 mm internal diameter and 0.50 µm film thickness). According to European Medicines Agency (EMA) guidelines, both methods were deemed fit for purpose across the following linear ranges: ethanol (0.01-0.5 g/100mL), EtG (50–2500 ppb) and EtS (5-1000 ppb) and all analytical validation acceptance criteria were met. Of the 112 vitreous humour samples, 33 samples contained no ethanol, EtG or EtS. The remaining 79 samples had median ethanol, EtG and EtS concentration of 0.048 (0.000-0.175) g/100mL, 127.85 (54.26-932.16) ppb and 102.00 (8.44-705.25) ppb, respectively. Spearman's rank-order correlation test results suggested that, in vitreous humour, EtS is a more suitable analyte to predict ethanol consumption prior to death than EtG however, the*

*low correlation coefficients of 0.800 and 0.606 indicated that various other factors play a role in the relationship between ethanol and these alcohol biomarkers in vitreous humour. The pilot study results highlighted the various benefits, analysing EtG and EtS in post-mortem VH samples including to differentiate between ante mortem ethanol consumption and post-mortem formation and to clarify circumstances of death. The study also identified the short comings of solely using EtG and EtS results to determine ethanol intoxication for forensic purposes. In conclusion, the analysis of EtG and EtS in either post-mortem blood or vitreous humour samples should be considered as a routine test when pathologist request the analysis of BEC in order to differentiate between ante-mortem ethanol consumption and post-mortem formation as well as to providing additional information on the series of events prior to the death of a decedent.*