G78 Estimation of Postmortem Interval: Determination of Hypoxanthine in Vitreous Humor by Mass Spectrometry

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After attending this presentation, attendees will understand the potential contribution of a new method for confirmation and quantification of hypoxanthine in vitreous humor for determining the postmortem interval.

This presentation will impact the forensic science community by showing this a method and how it can increase the accuracy to calculate the postmortem interval, creating new possibilities, especially if a combined study is done taking into account the weight and the temperatures of the body and the environment.

Currently there are a number of procedures for determining the postmortem interval (PMI). Numerous methods have been used, some pseudoscientific, others merely descriptive, many without practical value, and most of them without any corroborating mathematical support. Others suffer from poor reproducibility and consequent uncertainty of their results exacerbated by failure to carry out field tests. Those using a methodology based on body cooling rate, although accompanied by statistical support, include certain clearly subjective factors.

The best results derive from the biochemistry of the vitreous humor (VH) and are based on the simultaneous determination of potassium (K) and hypoxanthine (Hx), correlating the value of both substances with postmortem interval. New statistical approaches have led to more precise and robust methodological processes which have helped clarify the interpretation of results. However, the analytical methodology for the determination of hypoxanthine has remained unchanged and therefore subject to certain limitations of validity and quantification in particular.

Objective: The goal of this presentation was to develop and fully validate a new method for confirmation and quantification of hypoxanthine in vitreous humor, applying liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)

Methods: Vitreous humor samples, take in fresh bodies, were subjected to solid-phase extraction. Chromatographic separation was performed using an (2.1x100mm, 3µM) analytical column, working in gradient mode, with acetonitrile and ammonium acetate 10mM (pH=4.5) as mobile phase. A tandem mass spectrometer was employed for the detection of hypoxanthine. Mass spectrometer worked in electrospray positive mode (ESI +) and MRM mode, monitoring two precursor-product transitions: 137> 110, and 137> 119 for identification and quantification of hypoxanthine. For method validation, linearity, precision, accuracy, limit of detection and quantification, matrix effect, extraction efficiency, and process efficiency were studied. All the studied validated parameters were within the accepted criteria, and LOQ of hypoxanthine was 10µM.

Discussion: It is a well known fact that hypoxanthine alone cannot provide an estimate of the time of death with any more accuracy than the potassium. However, it is necessary to use different variables to lend precision to the estimates. Hypoxanthine is one of the variables validated as an estimator for postmortem interval. The improvement in the statistical and analytical methods must be used in estimation methodology, reducing the confidence interval and increasing the accuracy and thereby provide the forensic pathologist with an objective validated method for postmortem interval estimation. With this method new studies could be made, for example taking into account the weight and the temperatures of the body and the environment.

PMI, Hypoxanthine, LC-MS/MS

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