K5 Analysis of a Group of Volatile Compounds With Forensic Interest: Validation of an Analytical Method by HS-GC/FID

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After attending this presentation, attendees will understand the potential contribution of a new method for detection and quantification of volatile substances in different biological matrices with interest in forensic contexts.

This presentation will impact the forensic science community by allowing toxicology experts to understand the specificities and difficulties of validating an analytical method developed for the analysis of volatile compounds with different solubilities like toluene or acetone.

Although pharmaceutical products, drugs of abuse and ethanol (alcohol) are the most common poisons encountered in clinical and forensic toxicology, the possibility of poisoning with a wide range of other compounds has to be taken into account. These include pesticides, volatile substances, metals and anions, and natural toxins.

The purpose of this work was the optimization and validation of a sensitive and rapid analytical procedure to the detection and quantification of some volatile organic compounds (acetaldehyde, ethyl acetate, acetone, acetonitrile, 1-butanol, diethyl ether, methanol, 2-propanol, chloroform, toluene and xylene) in different matrices (blood, urine and vitreous humor) using a gas chromatograph, equipped with a flame ionization detector coupled to a headspace injector of fixed volume (1mL loop) Headspace/Gas Chromatograph/Flame Ionization Detector (HS/GC/FID).

The substances under study were divided and grouped according to their solubility and working range. For substances with high water solubility, a mixture was created (acetaldehyde, ethyl acetate, acetone, acetonitrile, 1-butanol, diethyl ether, methanol, 2-propanol). The other substances, whose solubility in water was practically non-existent but had a good solubility in methanol, were divided according to the working range.

Prior to gas chromatographic analysis, all specimens, including the calibrators, were diluted 1:10. By volume, i.e., 100µL of urine, vitreous humor, or blood were diluted with 1mL aqueous solution of n-propanol (100mg/L), used as internal standard.

The chromatographic separation was performed using two capillary columns with different polarities, in order to ensure fulfillment of the identification criteria recommended for this type of analysis (Flanagan *et al*., 1997; Kugelberg *et al*., 2007).[1,2] Chromatographic analysis conditions were as follows: an initial oven temperature of 40°C, held for 5 min, followed by a rise to 130°C with a gradient of 10°C/min. At the end of each analytical cycle, the initial conditions were resumed and maintained for 3 min. The injector temperature was maintained at 150°C, with a split ratio of 4:1, with detectors set at 250°C. The carrier gas was helium at a constant flow rate of 2.7mL/min.

All compounds studied, including n-propanol (internal standard), eluted in a time interval of 15 min and were all well resolved with no interference of metabolites, degradation products, or other substances, such as t-butanol, formaldehyde, ethylene glycol, methyl and ethyl formate, etc. In the concentration ranges analyzed, and for all compounds, the analytical response proved to be linear with a correlation coefficient greater than 0.9962. The limits of detection varied between 1mg/L (1-butanol, toluene, and xylene) and 10mg/L (chloroform) and the limits of quantification between 2mg/L (xylene) and 31mg/L (chloroform). The coefficients of variation obtained for intermediate precision varied from 0.8% (acetonitrile) to 7.0% (xylene). The accuracy of the method varied between 87.8% (acetaldehyde) to 106.3% (xylene).

The study focused on all parameters included in the validation procedure for quantitative methods, in place at the forensic toxicology laboratory of Centre Branch—Portuguese National Institute of Legal Medicine. These included the study of selectivity, linearity, limits of detection and quantification, precision, accuracy, robustness and carryover, having the method shown to be suitable for the intended purpose.

References:

1. Flanagan, R.J.; Streete, P.J.; Ramsey, J.D. (1997), Volatile substance abuse; practical guidelines for analytical investigation of suspected cases and interpretation of results, UNDCP *Technical Series* No 5, United Nations Drug Control Programme, Vienna.
2. Kugelberg, F.C.; Jones, A.W. (2007), Interpreting results of ethanol analysis in postmortem specimens: a review of the literature, Forensic Science International 165, 10-29.

HS-GC/FID, Volatile, Validation

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