K45 The Methylecgonine to Cocaine Ratio in Blood Samples and the Effectiveness of Preservation With Sodium Fluoride

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After attending this presentation, attendees will understand the use of the methylecgonine to cocaine concentration ratio in blood samples as an indicator of enzymatic cocaine hydrolysis and effectiveness of preservation with sodium fluoride.

This presentation will impact the forensic science community by providing data on the methylecgonine to cocaine concentration ratio in blood samples as an indicator of enzymatic hydrolysis and effectiveness of preservation with sodium fluoride.

The limited stability of cocaine in forensic samples has been a problem for several decades. In unpreserved whole blood, cocaine (COC) will hydrolyze to methylecgonine (ME) by the action of cholinesterase and to benzoylecgonine (BE) by pH-dependent chemical hydrolysis. ME and BE are further converted into ecgonine (ECG). Fluoride inhibits plasma cholinesterase and as a result the conversions of COC to ME and of BE to ECG are reduced. The conversions of COC to BE and ME to ECG can be inhibited by acidification. Moreover, cooling slows all conversions. Addition of fluoride is generally recommended to prevent cocaine hydrolysis. In postmortem blood, acidification may occur, which makes the conversions of COC to BE and ME to ECG less important. However, enzymatic conversions continue after death. As a result, a large part of ME measured in postmortem blood may originate from postmortem hydrolysis of cocaine.

In the Netherlands, whole blood samples in cases of driving under the influence (DUI) of alcohol and/or drugs are collected in glass tubes containing sodium heparin and sodium fluoride (NaF). The samples are then sent to the Netherlands Forensic Institute (NFI) by regular mail, which generally takes one to two days, without cooling. After receipt at the NFI, blood samples are kept at 4ºC for a maximum of two weeks and at -18ºC thereafter. In autopsy cases, the interval between the finding of the body and the autopsy is generally one to two days. Preservation of (femoral) blood samples takes place after the autopsy, by using the same tubes as in DUI cases and by freezing at -18ºC. Until the end of 2005, blood tubes contained 0.8% NaF and 700 IU/mL sodium heparin. From 2006 until mid-2007, these tubes were gradually replaced by tubes containing 0.4% NaF and 143 IU/mL sodium heparin, for commercial reasons.

In this paper, the ME/COC and BE/COC concentration ratios in whole blood samples of DUI cases with 0.8% NaF, DUI cases with 0.4% NaF, and autopsy cases were compared. The goal of this study was to obtain more insight in the role of NaF as preservative and to investigate if the ME/COC or the BE/COC concentration ratio is indicative for hydrolysis of cocaine.

Electronic data files of the NFI were searched for concentrations of COC, BE and ME in blood samples from 1999 through 2010. The ME/COC ratios and BE/COC were calculated and statistically evaluated. Cases of DUI as well as autopsy cases were investigated. COC, BE, and ME were analyzed by using GC-MS, after solid phase extraction and derivatization, or by LC/MS/MS, after protein precipitation.

The results show that the median ME/COC concentration ratio increased over the years after 2006 in cases of DUI. This increase coincided with a gradual change from blood tubes containing 0.8% NaF to tubes containing 0.4% NaF from 2006 until mid-2007. A trend was not observed in the BE/COC concentration ratios or in autopsy cases over the years. The median ME/COC concentration ratios (and 95% range) were respectively 0.8 (0-2.1) for cases of DUI (0.8% NaF), 1.5 (0-4.6) for cases of DUI (0.4% NaF), and 1.9 (0-5.5) for autopsy cases. The observed increase in ME/COC concentration ratio is in line with the order of decreasing preservation in DUI cases. In autopsy cases, the median BE/COC concentration ratio was significantly lower than in DUI cases.

In conclusion, the results show that the ME/COC concentration ratio is probably a useful indicator of enzymatic cocaine hydrolysis in cases of DUI as well as in autopsy cases. A ME/COC ratio greater than 2.1 is indicative of (enzymatic) cocaine hydrolysis. This information will improve the interpretation of forensic results. Furthermore, the data show that a concentration of 0.4% NaF was insufficient to prevent decomposition of cocaine in blood during more than one or two days under the practical circumstances in DUI cases in the Netherlands. The relatively low BE/COC ratio in autopsy cases points to the protective role of postmortem acidification in the chemical hydrolysis of cocaine.

Cocaine, Hydrolysis, Sodium Fluoride

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