Stability of drugs in whole blood - from sampling to testing

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Introduction

When a blood sample has been collected from a person, e.g., in cases of suspected driving under the influence of drugs, several days may elapse before the sample is received at the forensic laboratory. The blood is collected in vessels containing an anticoagulation mixture, which usually also includes a preservative agent. Although the blood sample is stabilised by these additives, enzymatic and chemical reactions may not be prevented in all cases. To evaluate this sampling problem, approximately 50 commonly detected drugs and metabolites were tested for their short-term stability at different temperatures in blood that had been stabilised with different additives..

Experimental

Materials

FO tubes: Venosafe evacuated blood collection tubes (VF-109SFX07) containing 100 mg of sodium fluoride (NaF) and 22.5 mg of potassium oxalate ($K_2C_2O_4$) for a 9 mL draw volume of blood (Terumo Europe, Leuven, Belgium). **FC tubes**: Venosafe evacuated tubes (VF-053SFC32) containing 6.8 mg NaF and 15.7 mg citrate buffer/Na_2EDTA mixture for a 3 mL draw volume. **EDTA tubes**: Venosafe evacuated tubes (VF-052SDK) containing 3.9 mg K_2EDTA for a 2 mL draw volume.

Preparation of blood samples

An aliquot of a mixed standard solution in methanol was evaporated to bare dryness at 30°C under a stream of nitrogen. The residue was re-dissolved in whole blood from the local blood bank. The obtained concentrations were approximately 0.008 mg/L for buprenorphine, fentanyl, 6-monoacetylmorphine (6-MAM), norbuprenorphine and noscapine, and in the range 0.1-0.4 mg/L for the other substances. The blood was transferred to FO, FC and EDTA tubes in aliquots corresponding to their specified draw volumes. The sample tubes were stored at 20°C, 5°C and -20°C for 2 weeks. To prevent the repeated thawing and freezing, sub-samples of preserved blood were also transferred to polypropylene vials for uninterrupted storage at -20°C.

Chemical analysis

The samples were analysed in duplicate shortly after preparation and again after 1, 2, 3, 5, 7 and 14 days of storage using an ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method validated on ante-mortem and post-mortem blood [1]. Quantitative analysis was obtained by a combined use of matrix-matched calibrants and the isotope dilution of each substance.

Statistical analysis

The results were obtained in two independent replicate experiments using different batches of blood. The mean results were evaluated from plots (measured concentration *versus* storage time), and a linear regression analysis was performed on the data from 0-3, 0-7 and 0-14 days of storage when the analyte concentration change was less than 50%. Instability was considered significant when the p-value of the first order term was less than 0.05. In most cases a concentration loss of 5-15% could be measured with statistical significance.

Results and discussion

For many of the investigated drugs, no significant changes in the concentrations were observed after 3-7 days of storage at ambient temperature, irrespectively of the composition of the additive mixture (Table 1). However, olanzapine, methylphenidate, zopiclone, cocaine and the metabolites 6-MAM and benzoylecgonine were clearly unstable at ambient temperatures in one or more of the blood collection tubes (Fig. 1). In FO tubes, a significant amount of benzoylecgonine was produced from cocaine at ambient temperature (Fig. 2), which resulted in atypical plots when the blood was fortified with both substances (Fig. 3).

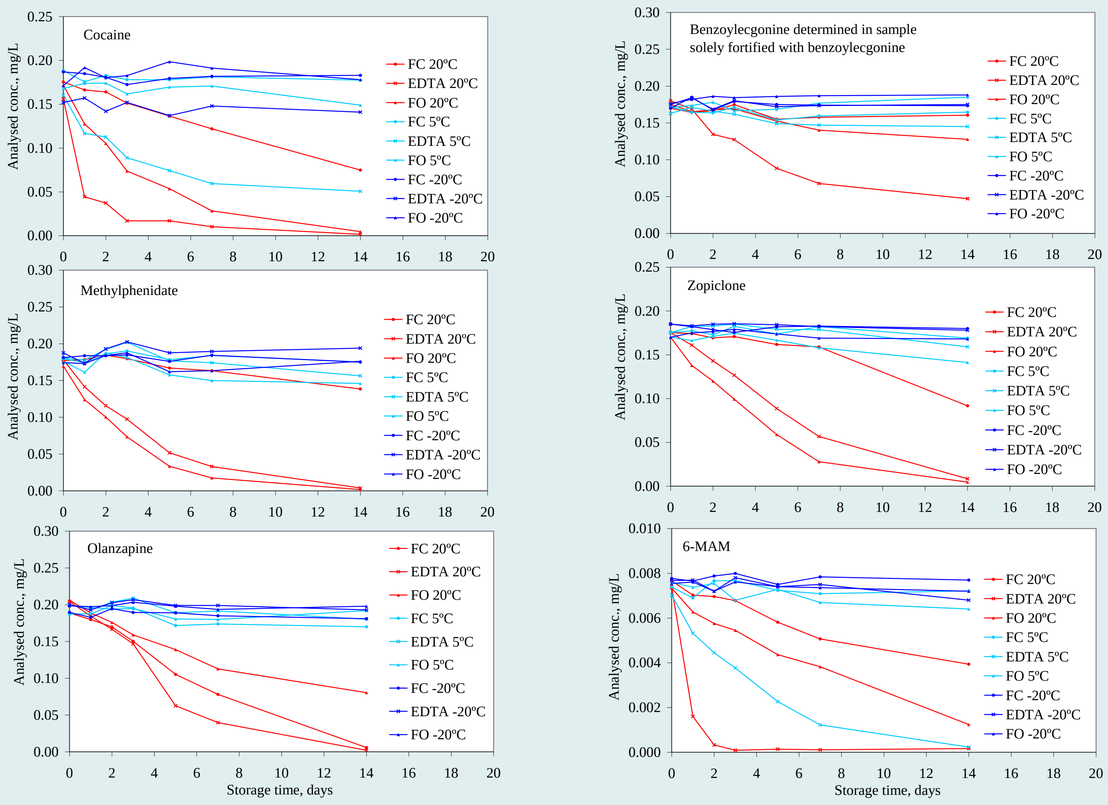

Fig. 1
Stability plots of cocaine, benzoylecgonine, methylphenidate, zopiclone, olanzapine and 6-MAM. The -20°C results were obtained from uninterrupted storage at that temperature. The benzoylecgonine results were obtained from blood solely fortified with this substance.

The overall order of stabilisation power was FC > FO > EDTA. In contrast to the EDTA tubes, the FC and the FO tubes contained NaF. The FC mixture was characterised by reducing the pH of blood to approximately 5.9, whereas the other additives maintained a more neutral pH. The lower pH is less favourable for many enzymatic reactions. In general, the stability of the labile drugs was improved considerably when the samples were kept at 5°C or lower. However, even at 5°C, cocaine and 6-MAM showed distinct instability in the EDTA tubes. At -20°C, no significant changes in concentrations were observed during uninterrupted storage for 2 weeks. When samples were thawed by natural air convection at ambient temperature and frozen again repeatedly (6 times) over the two weeks period, a significant reduction in concentrations was observed for olanzapine, cocaine and 6-MAM in EDTA tubes (20-50%) and olanzapine in FC tubes (approximately 20%).

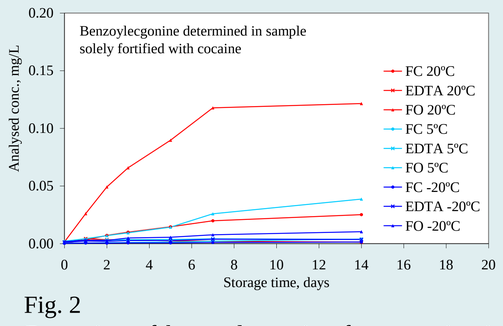

Fig. 2
Formation of benzoylecgonine from cocaine in samples fortified with 0.2 mg/L cocaine.

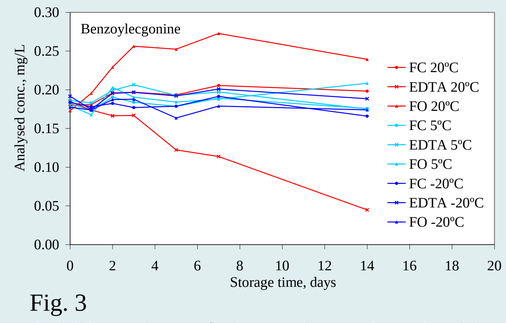

Fig. 3
Stability plot of benzoylecgonine in blood fortified with both benzoylecgonine and cocaine (0.2 mg/L of each).

Table 1
Drug stability in blood stored at 20, 5 and -20°C for 3-14 days. Drugs that appeared stable in FO, FC and EDTA tubes are marked with a "√". If an additive could not stabilise the drug, the additive is specified and the observed mean loss (in percent) is indicated in the bracket. The -20°C results were obtained from uninterrupted storage.

Substance	20ºC, 3 days	20ºC, 7 days	5ºC, 7 days	5ºC, 14 days	-20ºC, 14 days
Alprazolam	√	√	√	√	√
Amphetamine	√	√	√	√	√
Benzoylecgonine	EDTA(29)	EDTA(57),FO(17)	EDTA(9)	EDTA(11)	√
Bromazepam	√	√	√	√	√
Buprenorphine	√	√	√	√	√
Chlordiazepoxide	√	√	√	√	√
Chlorprothixene	√	√	√	√	√
Citalopram	√	√	√	√	√
Clonazepam	√	√	√	√	√
Clonazepam, 7-amino	√	√	√	√	√
Cocaine	EDTA(89),FO(57),FC(14)	EDTA(93),FO(79),FC(28)	EDTA(63)	EDTA(68), FO(13)	√
Codeine	√	√	√	√	√
Demoxepam	√	√	√	√	√
Diazepam	√	√	√	√	√
Fentanyl	√	√	√	√	√
Flunitrazepam	√	√	√	√	√
Flunitrazepam, 7-amino	√	√	√	√	√
Hydromorphone	√	√	√	√	√
Ketamine	√	√	√	√	√
Lidocaine	√	√	√	√	√
Lorazepam	√	EDTA(13)	√	√	√
MDA	√	√	√	√	√
MDEA	√	√	√	√	√
MDMA	√	√	√	√	√
Methadone	√	√	√	√	√
Methamphetamine	√	√	√	√	√
Methylphenidate	FO(57),EDTA(45)	FO(90),EDTA(81),FC(9)	√	√	√
Mirtazapine	√	√	√	√	√
Mirtazapine, desmethyl	√	√	√	√	√
6-MAM	EDTA(99),FO(26)	EDTA(99),FO(48),FC(34)	EDTA(83)	EDTA(97),FO(16)	√
Morphine	√	√	√	√	√
Nitrazepam	√	√	√	√	√
Norbuprenorphine	√	√	√	√	√
Nordazepam	√	√	√	√	√
Noscapine	√	√	√	√	√
Olanzapine	EDTA(28),FO(23),FC(20)	EDTA(79),FC(57),FO(41)	√	√	√
Oxazepam	√	√	√	√	√
Oxycodone	√	√	√	√	√
Papaverine	√	√	√	√	√
Quetiapine	√	√	√	√	√
Sertraline	√	√	√	√	√
Tramadol	√	√	√	√	√
Tramadol, o-desmethyl	√	√	√	√	√
Venlafaxine	√	√	√	√	√
Venlafaxin, o-desmethyl	√	√	√	√	√
Zolpidem	√	√	√	√	√
Zopiclone	FO(42),EDTA(28)	FO(80),EDTA(64),FC(8)	√	FO(16),EDTA(12)	√
Zuclopenthixol	√	√	√	√	√

Conclusion

Short-term stability over a 14-day period is not an issue when blood samples are kept uninterrupted at -20°C in FC, FO or EDTA tubes. However, repeated thawing and freezing should be avoided, especially for samples collected in EDTA tubes. At 5°C, fair drug stability was obtained in FC and FO tubes over a 7-day period. At ambient temperature, the FC additive appears to be the most suitable as an all-round preservation mixture, most likely because of the lower pH induced by the mixture. However, the FC additive is not ideal for all substances.

References

[1] L.K. Sørensen, J.B. Hasselstrøm. A high-throughput multi-class liquid chromatography tandem mass spectrometry method for quantitative determination of licit and illicit drugs in whole blood. Analytical methods 5 (2013) 3185-3193.