Analysed conc., mg/L
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0 2 4 6 8 10 12 14 16 18 20
Storage time, days
-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-week period, a significant reduction in concentrations was observed for cocaine, benzoylecgonine and 6-MAM in EDTA tubes (20-50%) and clonazepam in FC tubes (approximately 20%).

![Graph](image)

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

### 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


**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-1099FX07) containing 100 mg of sodium fluoride (NaF) and 22.5 mg of potassium oxalate ($K_2(C_2)O_4$) for a 8 ml draw volume of blood. (Terumo Europe, Leuven, Belgium).

FC tubes: Venosafe evacuated tubes (VF-053F) containing 6.8 mg NaI and 15.7 mg citrate buffer/NaEDTA mixture for a 3 ml draw volume. EDTA tubes: Venosafe evacuated tubes (VF-053SDK) containing 3.9 mg K$_2$EDTA for a 2.5 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), nordopamine and norguanidine, 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 samples 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 anti-mortem and post-mortem blood [1]. Quantitative analysis was obtained by a combination of matrix-matched calibrants and the isotope dilution of each substance.

**Statistical analysis**

The results were analysed 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 in two from 0.3, 0.7 and 6-14 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, irrespective of the composition of the additive mixture (Table 1). However, clonazepam, 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).