Stability of propofol in sampled ante-mortem blood

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ABSTRACT The stability of propofol in whole blood preserved with fluoride oxalate and fluoride citrate mixtures was investigated at different storage temperatures. Neither additives, particularly the fluoride citrate additive, stabilised propofol in blood stored at -20°C. Propofol stability was higher at 5°C and -80°C. The stability at -20°C was improved by adding antioxidants to the preserved blood.

INTRODUCTION Propofol (2,6-diisopropyl-phenol) is an intravenous anaesthetic agent that is used for the induction and maintenance of anaesthesia. Propofol is also used recreationally due to its sedative and relaxing properties and has been involved in fatalities. Thus, for several reasons accurate determination of propofol concentrations in clinical and forensic samples is needed. Due to the time lag between sampling and analysis, which may be as long as several days, it is essential to be aware of the stability properties of propofol.

METHODS Propofol stability was investigated using both spiked donor blood and clinical samples with authentic contents of propofol and propofol glucuronide (propofol G). All samples were stored in two different blood collection tubes: Venosafe VF-054SFX tubes (Terumo Europe) containing 9 mg of sodium fluoride (NaF) and 9 mg of potassium oxalate (FO mixture) for a 4-mL draw volume of blood and Venosafe VF-053SFC32 tubes containing 6.8 mg of NaF and 15.7 mg of citrate-EDTA buffer ingredients (FC mixture) for a 3-mL draw volume of blood.

The spiked samples were prepared at propofol concentrations of 2-3 mg/L and propofol glucuronide concentrations of 4-6 mg/L. The solvents of the spiked solutions were evaporated before being mixed with the blood samples. The spiked samples were stored at ambient temperature, 5, -20 and -80°C for up to 4 months. The authentic samples were stored at -20°C for up to 1 year. Analyses were performed periodically during storage using a sensitive LC-MS/MS method that simultaneously determines the two analytes [1].

1

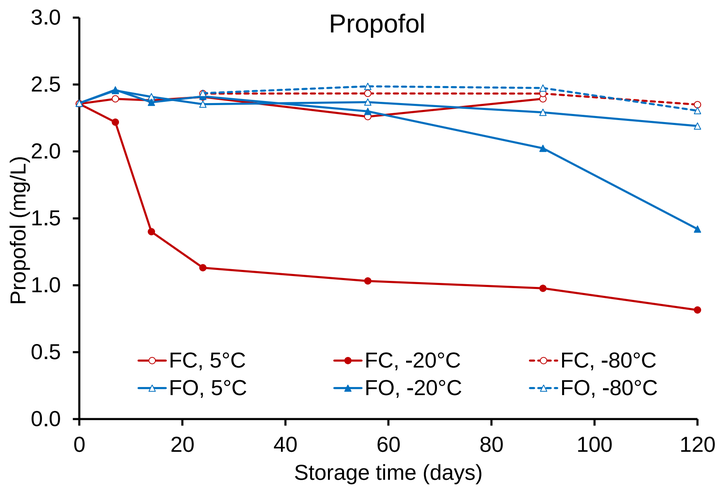

Figure 1 Stability of propofol in spiked donor blood. The blood was preserved with FC and FO mixtures and stored at 5, -20 and -80°C for 4 months. The samples were analysed periodically during the storage period. The sample pH values were 7.4 (FO preserved blood) and 6.0 (FC preserved blood)

Propofol was stable at 5 and -80°C but was unstable at -20°C.

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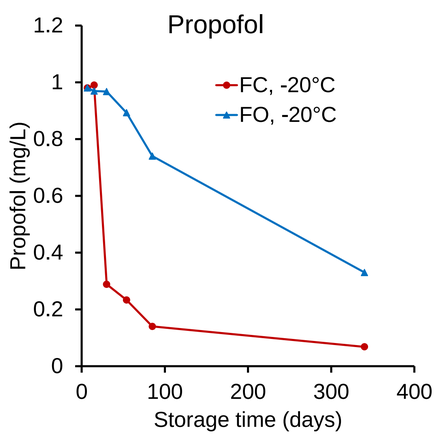

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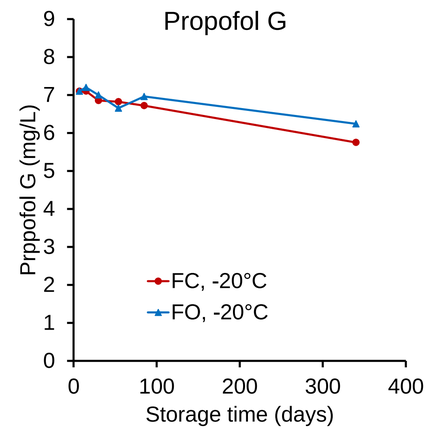

Figure 2 & 3 Stability of propofol and propofol G in an authentic sample. The blood from a person administrated propofol was collected in tubes containing FC and FO mixtures and stored at -20°C for 1 year. The first analyses were performed approximately 1 week after collection.

Propofol was unstable at -20°C, whereas the propofol G concentration only slightly decreased.

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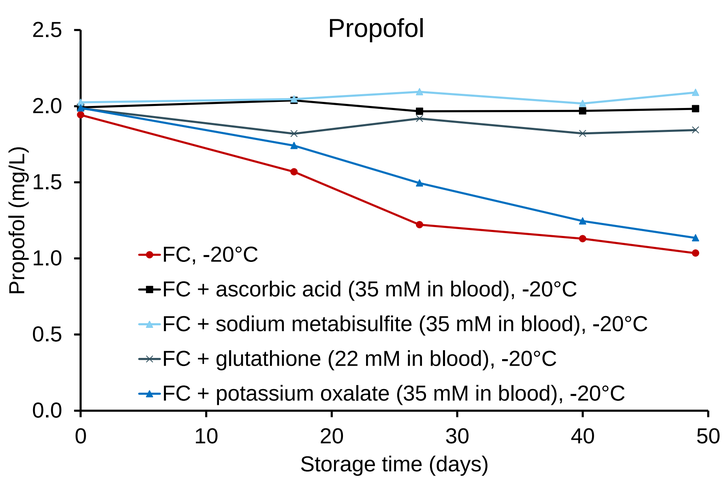

Figure 4 The effect of antioxidants on the stability of propofol. Donor blood was preserved with the FC mixture and FC mixtures combined with either ascorbic acid, sodium metabisulfite, glutathione or potassium oxalate. The samples were stored at -20°C. The pH values of the preserved blood were 6.0 without antioxidants and 5.5, 5.9, 5.7 and 6.1 for blood added ascorbic acid, sodium metabisulfite, glutathinone and potassium oxalate respectively.

Addition of the antioxidants ascorbic acid, metabisulfite and glutatione improved the stability of propofol significantly during the 50-days storage period.

RESULTS & DISCUSSION Samples spiked with propofol to concentrations of 2-3 mg/L showed a 20-40% reduction in concentration over a 2 week period when stored at -20°C in FC tubes (Fig. 1). The concentration of propofol also declined in the FO tubes but at a notably lower rate. Significant instability was not observed at 5°C and -80°C throughout 4 months of storage. At ambient temperature (20-22°C), propofol was stable for at least 2 weeks (data not shown). No pronounced instability of propofol G was observed during the 4 month storage period, irrespective of the storage conditions (data not shown).

The observed instability of propofol at -20°C was verified using several clinical samples with authentic contents of propofol. The samples were collected in both FC and FO tubes and stored at -20°C. Representative stability patterns are shown in Fig. 2 and 3.

Propofol acts as an antioxidant due to its phenolic moiety, and a hypothesis is that the atypical stability pattern of propofol is related to oxidative changes to the molecule. At -20°C, oxidative changes caused by autoxidation, for example, can still occur while the efficiency of the biochemical reduction system is diminished, especially at low pH. By adding antioxidants, such as ascorbic acid, sodium metabisulfite and glutathione to the FC-treated blood, the propofol content was in fact stabilised (Fig. 4).

CONCLUSION Propofol is unstable in whole blood preserved with FC and FO additives when stored at -20°C. In FC-preserved blood, the propofol concentration significantly declined after 2 weeks of storage. The stability of propofol was considerably improved by storing at -80 and 5°C . The stability was also improved by adding antioxidants to preserved samples stored at -20°C.

REFERENCES

1 L.K. Sørensen, J.B. Hasselstrøm. Simultaneous determination of propofol and its glucuronide in whole blood by liquid chromatography-electrospray tandem mass spectrometry and the influence of sample storage conditions on the reliability of the test results. J. Pharm. Biomed. Anal. 109 (2015) 158-163.