Stability of Cathinones in Whole Blood Samples

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Introduction

The stimulating alkaloid cathinone is found in the leaves of the shrub khat (Catha edulis). Chewing of khat has spread from Africa and the Arabian Peninsula and gained an increasing global prominence. In addition to the native cathinone that is available from the khat plant, a broad range of cathinone derivatives have been synthesized. Therefore, testing for cathinones in the blood of vehicle drivers is pertinent and requires understanding of the stability of these drugs.

Experimental

Sample materials

Ante-mortem whole blood samples were preserved with sodium fluoride/potassium oxalate (FO) and sodium fluoride/citrate-EDTA (FC) buffer in Venosafe tubes (Terumo Europe, Leuven, Belgium) and fortified with cathinones (100 µg/L) and ephedrines (100 µg/L) (Fig. 1). Each sample were divided into subsamples that were stored at 20 ± 2°C and 5 ± 2°C until analysis.

Analytical method

A 300-µL volume of whole blood was mixed with 100 µL of an internal standard solution and 600 µL of methanol. The mixture was centrifuged at 10,000×g for 5 min. A 300-µL volume of clear supernatant was acidified with 10 µL of formic acid and then ultra-filtrated through a 30,000-kDa regenerated cellulose membrane. The filtrate was diluted with water (1:1) and then analysed using LC-ESI-MS/MS. A detailed description is provided in ref. 1.

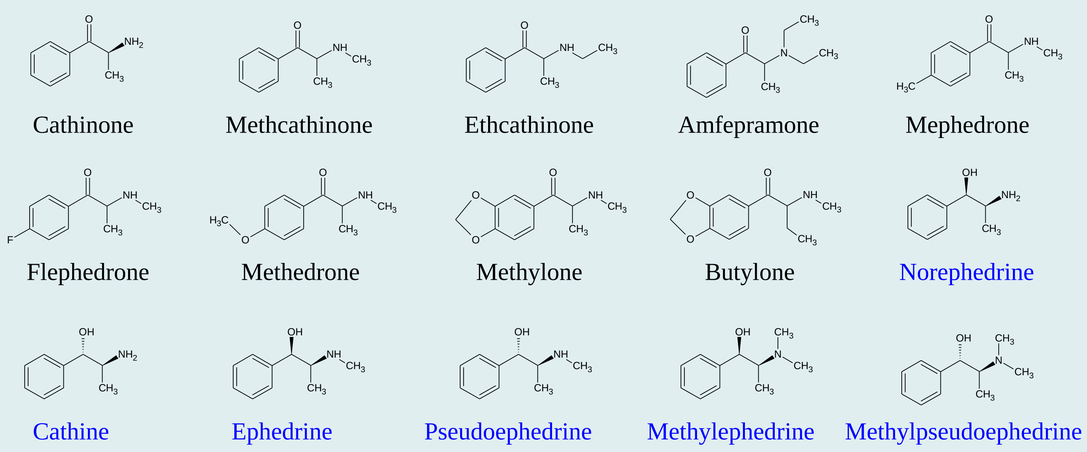

Fig. 1
Cathinones and ephedrines included in the current study

Results

In blood samples that were preserved with the FO additive, the measured concentrations of cathinone, methcathinone, ethcathinone, mephedrone and flephedrone declined by approximately 30% after two days of storage at 20°C (Fig. 2). When the blood samples were preserved with the FC additive, the loss was reduced to approximately 10% (Fig. 3). The concentrations of amfepramone, methedrone, methylone and butylone also decreased but with slower kinetics. The sample pH was approximately 7.4 in the tubes containing the FO additive and 5.9 in the tubes containing the FC additive. At a storage temperature of 5°C, the decomposition proceeded at a markedly lower rate. However a trend was still observed after three to six days of storage, especially for samples that were preserved with the FO additive (Fig. 4). The related ephedrines, which contain a hydroxyl group instead of the ketone group at the β position, were stable over the same storage periods regardless of pH and temperature.

The stability of cathinones in the final sample extracts, which were obtained following protein precipitation was pH-dependent (Fig. 5). In the pH range of 2.5-3.5, no significant degradation was observed after a week of storage at 20 ± 2°C. A pH in that range was obtained by adding 10 µL of formic acid to a 300-µL extract.

Conclusion

This study revealed that sample pH and temperature during shipment and storage of whole blood samples and extracts are critical variables to be considered for the determination of cathinones. If precautions are not taken, the accuracy and applicability of analytical results may be affected.

[1] L.K. Sørensen. Determination of cathinones and related ephedrines in forensic whole-blood samples by liquid-chromatography-electrospray tandem mass spectrometry. J. Chrom. B, 879, 727-736 (2011)

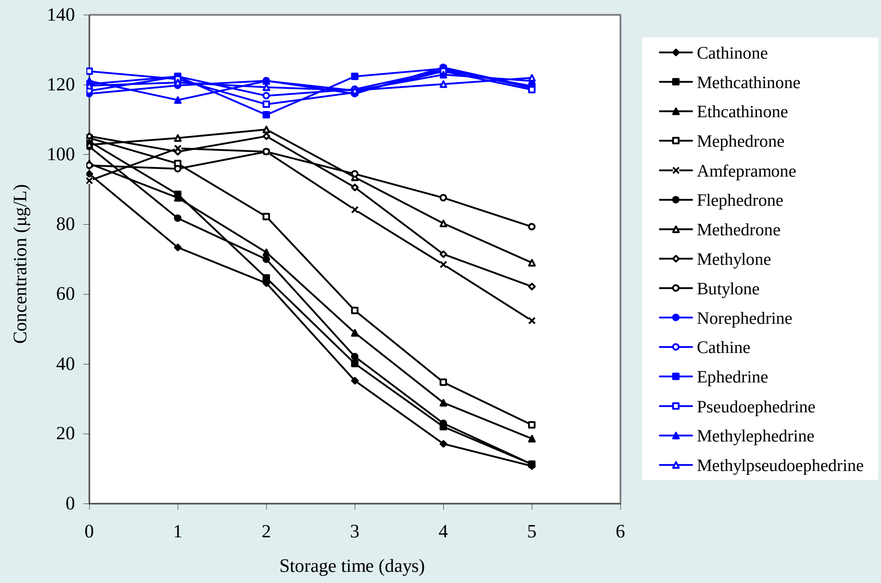

Fig. 2
The stability of cathinones and ephedrines in whole blood samples at a pH of approximately 7.4 when stored at an ambient temperature (20 ± 2°C) in tubes containing the FO additive. The concentration curves of the ephedrines were biased +20 µg/L in the plot.

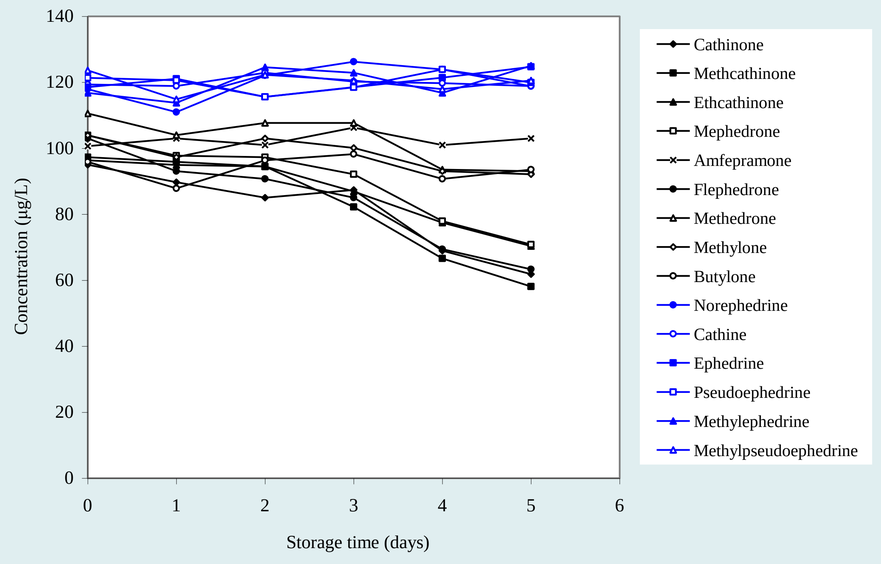

Fig. 3
The stability of cathinones and ephedrines in whole blood samples at a pH of approximately 5.9 when stored at an ambient temperature (20 ± 2°C) in tubes containing the FC additive. The concentration curves of the ephedrines were biased +20 µg/L in the plot.

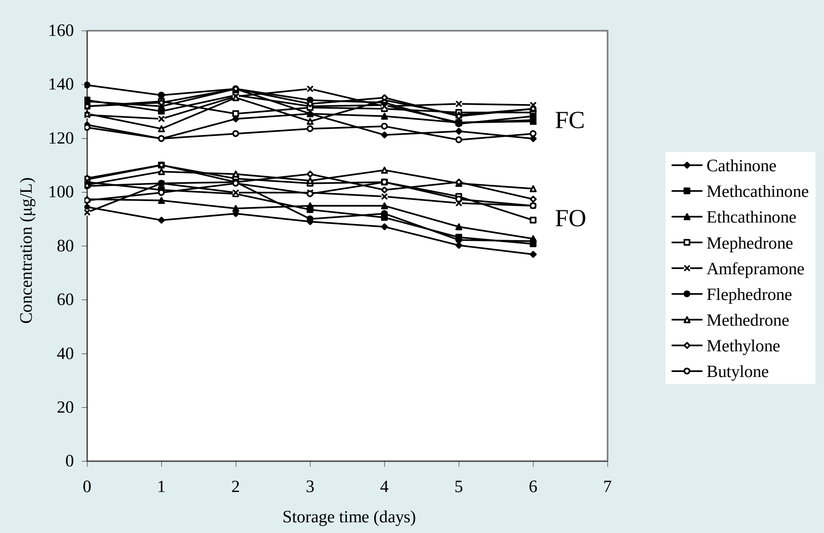

Fig. 4
The stability of cathinones in whole blood samples when stored at 5 ± 2°C in tubes containing FO or FC additives. The concentration curves of the samples that were preserved with the FC additive were biased +30 µg/L in the plot.

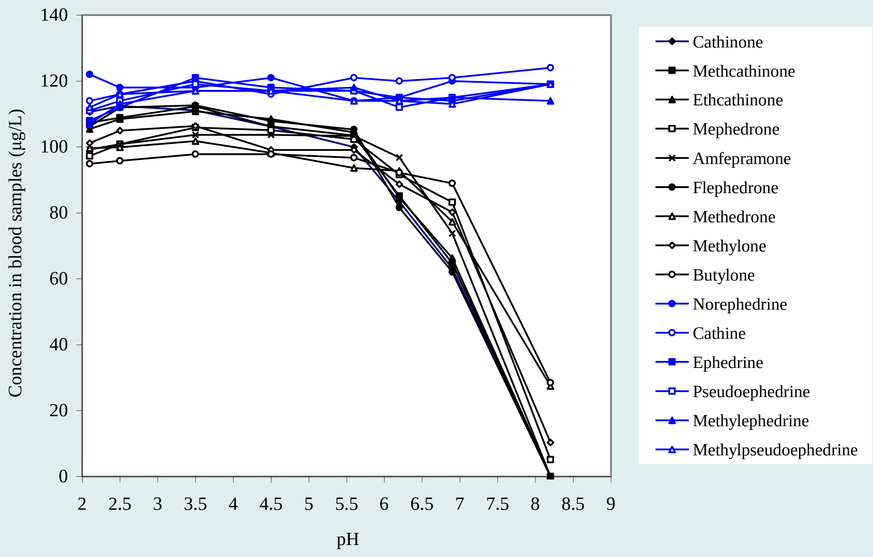

Fig. 5
The stability of cathinones and ephedrines in the final extracts of whole blood samples that were fortified with 100 µg/L of each substance and then stored at 20 ± 2°C for seven days as a function of the pH of the final extract. The extracts were acidified using formic acid. The concentration curves of the ephedrines were biased +20 µg/L in the plot.