

Short Communication

A Stability Study of Atropine in Post-Mortem Peripheral Blood and Liver at -20°C, +4°C, +10°C and +20°C Over 12 Months. Peripheral Blood and Liver Concentration Ratios

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Introduction

At the judicial inquiry of a criminal case involving atropine, we were required to carry out toxicological analyses on a body exhumed 31 months after death. It was obviously not possible to take samples of peripheral blood or any other fluid at the autopsy of the victim. However, we were able to analyse various organs, including the liver, so we considered it important to determine the stability of atropine in a post-mortem sample in order to better understand the concentration that could have been measured 31 months earlier. We therefore carried out such a study on liver and also on peripheral blood at different temperatures: -20°C, +4°C, +10°C and +20°C. As well as the standard laboratory storage temperatures (-20°C and +4°C) we added room temperature in temperate climates (+20°C) and the minimum soil temperature two meters underground, the depth at which a body is buried. In southern France this temperature is actually between +10°C and +18°C according to the season [1], with an average temperature between 14.1 and 14.6°C.

There is very little research on the stability of atropine in biological samples. A study on plasma was published by Kozeli et al. [2]. Twenty real cases were analysed immediately, then frozen for a year and reanalysed. Atropine showed good thermal stability (less than 10% degradation). Some authors of published assay methods have

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looked at short-term stability (24 hours in plasma at room temperature or 3 months at -20°C) [3], and 2 days at room temperature in urine and 9 months at -20°C [4], with results showing good stability, but there are no studies on real cases in putrefied matrices and at various temperatures.

The assays were carried out with liquid phase chromatography coupled with high-resolution mass spectrometry using techniques already published by our laboratory and fully validated [5,6], on a test sample of 1 mL of blood or 1 gram of liver. The linearity of the method was validated from 0.1 to 500 ng/mL for blood and from 0.5 to 500 ng/g for liver. The limits of quantification were 0.05 ng/mL for blood and 0.2 ng/g for liver, while the detection limits were 0.02 ng/mL for blood and 0.1 ng/g for liver.

We analysed samples from 32 deceased people whose peripheral blood and liver were available after their autopsies and where atropine had been measured at concentrations of over 5 ng/mL in blood and 10 ng/g in liver. These values were considered sufficiently high for us to still be reasonably able to detect atropine with good precision after several months of storage. The assays were carried out in duplicate: on day zero (D0), then after 1 month (M+1), 3 months (M+3), 6 months (M+6), 9 months (M+9) and 12 months (M+12).

Table 1 shows the average degradation values in blood and liver of the 32 samples after different storage times.

	Months of Storage	Percentage (%) of Degradation				
		M + 1	M + 3	M + 6	M + 9	M + 12
Peripheral Blood	-20°C	6	9	12	16	17
	+4°C	10	12	15	23	29
	+10°C	12	16	20	31	39
	+20°C	20	34	39	58	70
Liver	-20°C	7	10	13	18	20
	+4°C	10	14	16	20	27
	+10°C	14	16	22	30	39
	+20°C	22	33	42	60	74

Table 1: Average degradation values in blood and liver of the 32 samples after different storage times.

The various assays carried out in liver and peripheral blood enabled us to calculate a ratio between the two matrices. The average ratio was 1.6 between liver and peripheral blood, with extremes from 0.23 to 7.3.

Atropine therefore presents substantial instability which should be taken carefully into account in order to best interpret post-mortem concentrations measured in autopsy samples after long periods of interment.

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