The stability of diazepam in plasma samples when stored under varying conditions

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Diazepam a popular tranquillizer, is frequently involved as a cause of deliberate poisoning. Little is known of its stability when stored under varying conditions. Gamble, Assaf & others (1975a) recommended that plasma samples be stored at −20°, although this does not appear to be essential for the first week. In view of the observations of Wilkinson & Way (1969) that morphine is adsorbed on to the glass of storage containers it seemed useful to investigate the stability of diazepam samples under varying conditions.

Blood samples were taken from patients 60 min after a single clinical dose of diazepam (5–20 mg). These were part of a study on the relation of clinical effect to plasma concentrations (Gamble, Dundee & Assaf, 1975b). After centrifugation the plasma was divided into two aliquots, the first being analysed on the day of sampling and the other stored in clear polystyrene containers under one of the following conditions: five batches of 20 samples were stored for 1 year at −20° and then re-analysed. Ten samples were stored for 8 weeks in a domestic refrigerator (4°) and re-analysed at weekly intervals. Ten samples were stored on the bench at room temperature (15–22°) for 20 days and re-analysed on each of the first six days and on days 8, 10, 18 and 20.

All analyses were by a single stage benzene extraction and gas-liquid chromatography as described by Gamble & others (1975a). Plasma (200 μl) is extracted with benzene (4 ml Ultrar grade) and 2 ml of the benzene extract evaporated to dryness, reconstituted with 50 μl of benzene and 5 μl injected on to the column of a Perkin-Elmer Fll g.l.c. fitted with a ^{63}Ni electron capture detector; column: borosilicate glass length 2 m, i.d. 4 mm; packing 3% OV17 on 100/200 mesh Gas Q; carrier gas: oxygen free nitrogen at 207 kNm^{2} (30 lbs inch^{-2}); temperatures: oven 240°, injection port 270°, detector 260°; internal standard: 5-(2-flurophenyl 1)-1,3 dihydro-7-nitro-1-methyl 1-2H-1,4 benzodiazepine-2-one (flunitrazepam).

Table 1. *Average concentrations of plasma diazepam before and after storage (ng mg^{-1}).*

Batch	Control concentration	After storage	t	P
1	80·2 ± 11·04	88·9 ± 10·58	0·569	<0·60
2	124·7 ± 17·79	130·4 ± 17·15	0·233	<0·90
3	182·4 ± 19·44	183·5 ± 17·93	0·040	<0·95
4	204·5 ± 19·69	210·0 ± 18·56	0·203	<0·80
5	202·3 ± 20·31	215·4 ± 19·67	0·463	<0·60

Statistical analysis was carried out on all groups, comparing results before and after storage.

Table 1 gives the average readings for each batch before and after storage at −20° for 1 year. Diazepam concentrations in the stored samples were not significantly different from the control values.

A similar result was obtained with the samples stored on the bench. Over the 20 days there was no significant variation in the readings (16·2 ± 4·4, s.e.m. n = 10).

The refrigerated samples did not show any significant variation from the control concentrations (4·6 ± 3·6, s.e.m. n = 10). However samples stored on the bench showed evidence of decomposition of the plasma and had an offensive smell but despite this the chromatograms were free from contaminant peaks.

The present findings may be of significance in legal cases where it may be argued that the concentration of drug in the sample tested could be high or low because of the period of time between collection and analysis.

With overdosage involving several drugs, a sample containing diazepam would remain virtually unchanged if stored over a period of time whereas other drugs may show significant loss (Wilkinson & Way, 1969).

It was thought that with the decomposition of the plasma by fermentation and bacterial invasion some metabolism of the diazepam may have occurred when they were stored on the bench and at 4°. There was no evidence of this.

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