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Note

Gas chromatographic analysis of pentazocine

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Pentazocine is an analgesic drug with low abuse potential. Brotell *et al.*[1] have developed an electron-capture gas chromatographic assay for pentazocine which is both sensitive and reproducible. This paper describes several modifications of that assay which make it substantially faster and more convenient, while retaining the sensitivity and reproducibility of the original procedure. The method is applicable to whole blood, plasma or urine.

EXPERIMENTAL

Fig. 1 illustrates the steps in the assay procedure. Levallorphan, a close structural homolog of pentazocine with similar solvent partitioning characteristics, was used as an internal standard. An internal standard solution of concentration 50 ng per 100 μl was prepared by diluting a 1 mg/ml pharmaceutical preparation of levallorphan tartrate (Lorfan) in 0.1 *M* sulfuric acid. Each sample was assayed as follows: 0.2–1.0 ml of whole blood, plasma or urine and 50 ng of internal standard were added to a 100 × 13 mm culture tube with PTFE-lined screw-cap, which contained 150 μl of carbonate buffer (pH 10.2, 1.0 *M*). A 3-ml volume of toluene was then added. Each tube was mixed by tilting for 5 min on a Labquake Shaker and centrifuged at 1000 *g* for 5 min. The organic and aqueous phases were separated by freezing each tube in a dry-ice–acetone bath, and pouring the unfrozen organic phase into a second tube containing 200 μl of 0.1 *M* sulfuric acid. This tube was mixed on the labquake shaker for 5 min, centrifuged for 5 min, frozen in dry-ice–acetone and the toluene discarded. To the remaining aqueous phase in each tube were added 100 μl of 12 *M* sodium hydroxide solution, 500 μl of 0.1 *M* tetrabutylammonium hydrogen phosphate (TBA phosphate) and 200 μl of a 0.4% solution of pentafluorobenzyl bromide (PFB bromide) in dichloroethane. This mixture was shaken and derivatized at room temperature for 20 min. Then 2 ml of *n*-hexane were added to the mixture and the tube was vortexed for 10 sec, centrifuged for 5 min and frozen in dry-ice–acetone. The

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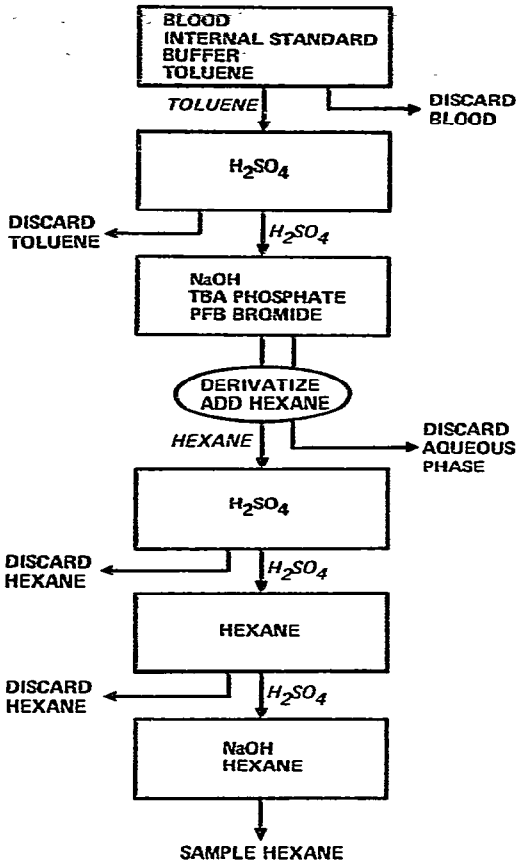

Fig. 1. Flow diagram of sample preparation procedure.

organic phase was poured into a third 100 × 13 mm culture tube containing 0.5 ml of 0.5 *M* sulfuric acid. This mixture was vortexed for 1 min, centrifuged for 1 min and the organic phase discarded. This procedure was repeated with an additional 2 ml of *n*-hexane. Finally, 100 μl of 6 *M* sodium hydroxide solution and 50 μl of *n*-hexane were added to the tube, mixed for 5 min and centrifuged for 5 min. Approximately 5 μl of *n*-hexane was sampled from the tube with a 10-μl syringe. Desmethylimipramine (DMI) was injected with the sample according to the technique described by Brotell *et al.*[1].

A 1.8 m × 3.2 mm glass column, packed with 3% Dexsil 300 on Gas-Chrom Q, 100–120 mesh, was used in a Hewlett-Packard Model 5710A gas chromatograph fitted with a ^{63}Ni electron-capture detector. The column was conditioned at 300° for 48 h before use. The oven temperature was 265°, the injector port 250° and the detector 350°. The carrier gas (5% methane in argon) flow-rate was 50 ml/min.

The assay was calibrated by analysing samples containing 5, 10, 20, 30, 40 and 50 ng of pentazocine together with 50 ng of the internal standard. For each sample, the ratio of the peak height of pentazocine to that of the internal standard was determined. Each ratio was divided by the amount of pentazocine in each sample

to give a normalized peak-height ratio. The normalized peak-height ratios were averaged, and the mean was used to determine the amounts of pentazocine in unknown samples. Variation in the assay was estimated by calculating the coefficient of variation of the five normalized peak-height ratios.

The reproducibility of the assay was measured by determining the coefficient of variation for five samples, each containing the same amount of pentazocine. The reproducibility was determined in this way for 5-, 10- and 30-ng amounts of pentazocine.

The effect of varying the blood volume of the samples was determined. Four samples, containing 100, 200, 500 and 1000 μl of plasma with 30 ng of pentazocine in each, were analysed. The peak-height ratios were measured, and the coefficient of variation between all of the samples was calculated.

RESULTS AND DISCUSSION

Retention times under the conditions described were 4.8 min for pentazocine and 6.2 min for the internal standard. Fig. 2 shows a chromatogram of a 0.5-ml control blood sample (A), and a blood sample containing 21.8 ng of pentazocine and 50 ng of the internal standard (B). The two compounds are well separated, and there were no significant peaks that interfered with the peaks of interest in samples of whole blood, plasma or urine from twelve individuals.

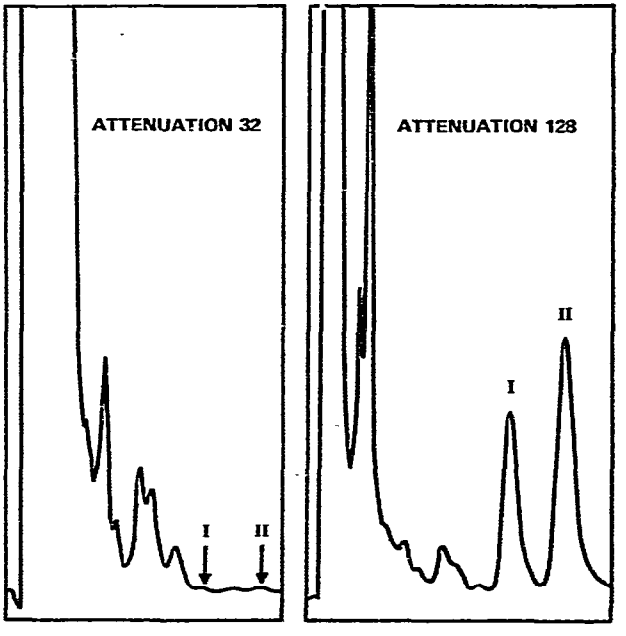

Fig. 2. A, Chromatogram of a control blood sample. B, Chromatogram from a sample containing 21.8 ng of pentazocine (I) and 50 ng of internal standard (II). I and II in A indicate the locations of the pentazocine and internal standard peaks in the control samples. Note that the sensitivity for the control chromatogram is four times as great as that for the chromatogram shown in B.

Fig. 3 shows a typical calibration graph for pentazocine from whole blood. The line through the five standard points was rectilinear and passed through the origin. The coefficient of variation in the range 5–50 ng was 6.0%. Table I summarizes data from 17 calibration graphs for blood, plasma and urine.

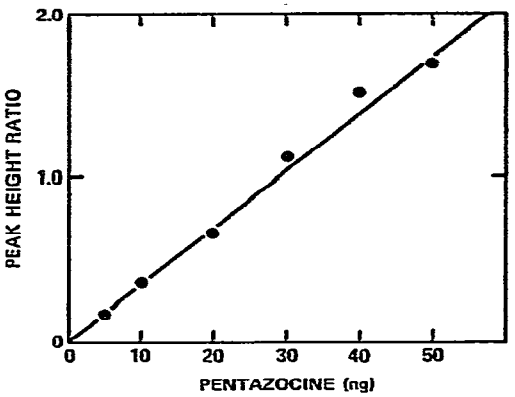

Fig. 3. Calibration graph for pentazocine assay. $R^2 = 0.99$.

TABLE I

CALIBRATION GRAPH DATA

Parameter	*Biological fluid*		
	Blood	*Plasma*	*Urine*
Number of studies	12	4	1
Mean normalized peak-height ratio	0.0402	0.0350	0.0323
Mean coefficient of variation (%)	6.7	7.0	7.5

The reproducibility of the assay at a given concentration was comparable to the reproducibility of the original method. The coefficients of variation of the peak-height ratios were 1.9% at 30 ng/ml, 2.3% at 10 ng/ml and 5.7% at 5 ng/ml; Brotell *et al.*[1] obtained 7.0% at 25 ng/ml and 5.5% at 5 ng/ml.

Differences in sample volume had no detectable effect on the peak-height ratio. The coefficient of variation between the four tubes with different volumes was 4.3%, and there was no consistent trend in the data.

The sample preparation outlined in Fig. 1 has several advantages over the original assay. The internal standard is added to the sample prior to the initial extraction, thereby eliminating the need for quantitative transfers of solvents, and making the assay more convenient. This method is considerably more rapid than the original one following the modifications in the extraction times and techniques. We found that only one third of the extraction times suggested in the earlier method are necessary, and the time-consuming evaporation step has been replaced with a faster and more selective aqueous solvent partitioning. Because of this cleaner solvent partitioning, it is necessary to wash the derivatization mixture only twice instead of four times with *n*-hexane. Consequently, the total preparation time is only about

90 min compared with an estimated 150 min for the sample preparation of Brotell *et al.*[1]. In the interest of laboratory personnel safety, it is of note that toluene is less toxic than the benzene employed in the original assay. Toluene (m.p. −95°) is also more suitable than benzene (m.p. 5.5°) for separations from aqueous systems by freezing. The clearance of pentazocine is high in relation to hepatic blood flow, and it may therefore be desirable to measure total blood concentrations in pharmacokinetic studies. Our method measures plasma, whole blood and urine, whereas the original method reported data from plasma only. This assay has been applied to a study of pentazocine kinetics in twelve subjects, and was found to be sufficiently sensitive and reproducible.

ACKNOWLEDGEMENTS

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