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THE DETERMINATION OF BENZOMORPHAN DERIVATIVES IN PLASMA BY GAS CHROMATOGRAPHY

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Summary

A specific, sensitive and simple procedure for the extraction from plasma and determination by gas chromatography of benzomorphan derivatives is presented.

Benzomorphans (BM) derivatives are compounds with strong analgesic properties (1). In recent years pentazocine (1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(3-methyl-2-butenyl)-2, 6-methano-3-benzazocin-8-ol), a BM derivative is being increasingly used therapeutically. The estimation of this drug was achieved using spectrophotofluorometry (2) and gas chromatography (3). This communication describes an analytical procedure for the determination of several BM derivatives: pentazocine, cyclazocine (1,2,3,4,5,6-hexahydro-6, 11-dimethyl-3-cyclopropylmethyl-2, 6-methano-3-benzazocin-8-ol), phenazocine (1,2,3,4,5,6-hexahydro-6, 11-dimethyl-3-phenethyl-2, 6-methano-3-benzazocin-8-ol) and etazocine (1,2,3,4,5,6-hexahydro-6, 11-diethyl-3-methyl-2, 6-methano-3-benzazocin-8-ol) from human plasma.

Experimental Procedure

Pentazocine, cyclazocine, phenazocine and etazocine were obtained as bases. Stock solutions were prepared in 0.9% NaCl by adjusting the pH to 4 with HCl. Cyclazocine was used as the internal standard for pentazocine,

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phenazocine and etazocine; pentazocine was used as the internal standard for cyclazocine. All chemicals were of analytical reagent grade.

Blood was obtained from human volunteers, heparinized (2 μg/10 ml), centrifuged and the plasma separated. After addition of the drugs (pentazocine, cyclazocine, phenazocine and etazocine) to give a final concentration of 0.5, 1, 2, 3, 4 and 8 μg/ml, the plasma was kept at room temperature for 30 min.

Plasma (1 ml), distilled water (4 ml) and internal standard (0.2 ml of 10 μg/ml) were mixed and the pH adjusted to 8 with 0.15 ml of 0.05 N NaOH. The drugs were then extracted with benzene (8 ml) on a horizontal shaker for 7 min at medium speed.* The samples were centrifuged at 1900 x g for 20 min; 6 ml of the organic layer were transferred to conical tubes and evaporated to dryness on a water bath at 65°C under a stream of nitrogen. Just prior to analysis the residue was dissolved in 25 μl acetone and 1-2 μl injected into the gas chromatograph.

The amount of drug recovered from a sample was determined by measuring the peak height ratio of the drug to internal standard and relating this to previously constructed standard curves for each drug. These standard curves were linear over a wide range (20 to 300 ng).

A dual column gas chromatograph (Hewlett-Packard, Model 402) equipped with flame ionization detectors was used. Glass columns 6 feet long, 1/4 inch outside diameter, were packed with 3% OV-1 gas chrom Q (100/120 mesh). The columns were conditioned for 48 hours at 260°C. Operating conditions were: column temperature 210°C, injection port 250°C and flame detector 260°C. Nitrogen flow was 35 ml/min; hydrogen and air were adjusted to give optimum detector response.

Results

Fig. 1 shows the gas chromatographic separation of the four BM derivatives

*In case of low drug concentrations the volume of plasma can be doubled and the amount of distilled water decreased accordingly.

after extraction from plasma. The retention times for etazocine, cyclazocine, pentazocine and phenazocine were 3.8, 5.0, 5.7 and 19.0 min, respectively. The mean recoveries ± SD of pentazocine, phenazocine, cyclazocine and etazocine were 104 ± 8, 100 ± 2, 99 ± 7 and 97 ± 3%, respectively.

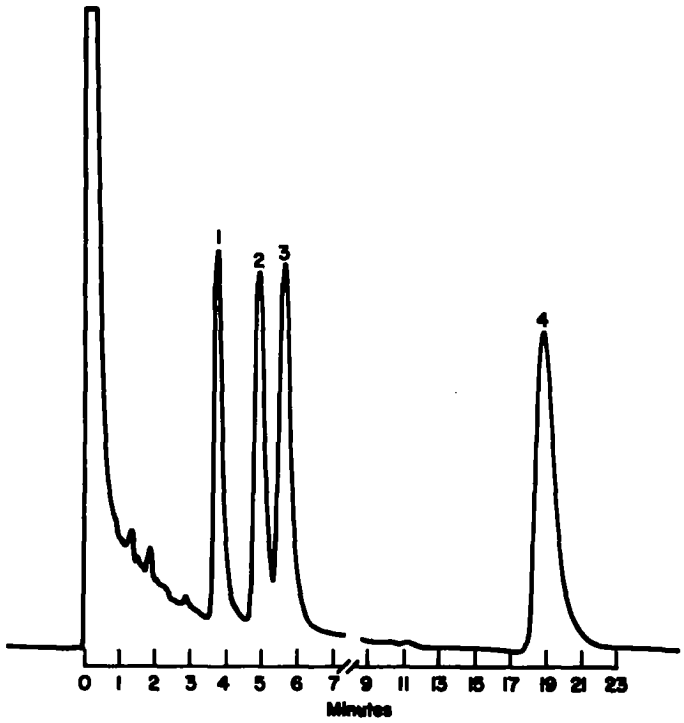

Fig. 1

Gas chromatographic separation of 150 ng each of etazocine (1), cyclazocine (2), pentazocine (3), and phenazocine (4) extracted from human plasma after the addition of the drugs.

Discussion

Pentazocine has been determined in biological materials by spectrophotofluorometry (2). In this method the specificity of the obtained fluorescence is of critical importance, since interference from the biological material is possible. In the reported gas chromatographic procedure for the estimation of pentazocine in biological samples (3), the authors presented only few analytical results: no figures on resolution, linearity and sensitivity were given.

The procedure involves multiple extractions with organic solvent, acid and alkali. With the method developed in the present study nearly quantitative recoveries were obtained for pentazocine, cyclazocine, phenazocine and etazocine after a single extraction from plasma. The smallest amount measured was 20 ng. The procedure allows the determination of one or more of the drugs from a single sample (Fig. 1).

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