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## Note

# Determination of ethoheptazine in human post mortem material

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Ethoheptazine, or 1-methyl-4-carboxy-4-phenylhexamethylene imine, is a synthetic analgesic which has been used clinically for over a decade in the treatment of mild to moderate pain of varied etiology. Reports [1—5] indicate that ethoheptazine is an effective analgesic which is well tolerated and non-addictive. The few clinical studies suggest that ethoheptazine provides effective analgesia following administration of doses ranging from 50 to 150 mg three of four times a day. After oral therapy the incidence of side effects is relatively low. The metabolic fate of ethoheptazine in man is unknown.

Evidence for metabolic pathways is mainly obtained from animal studies. Ethoheptazine is metabolized by at least three routes, including hydrolysis to the corresponding acid, oxidation to the hydroxy derivative which may further undergo hydrolysis, and a possible N-desmethylation to the corresponding nor-derivative which subsequently may be hydrolysed. Biotransformation was established by identifying respective metabolites by either tracer techniques, paper chromatography, infrared spectroscopy or electrophoresis.

In this paper a fatal case of drug overdose is described. An unknown amount of ethoheptazine was involved. No data have been reported on the concentrations of ethoheptazine in human biological material (plasma, urine, tissue) after therapeutic or toxic dosage. A gas chromatographic method was used to determine ethoheptazine in post mortem blood, liver, kidney, spleen and brain.

## MATERIALS AND METHODS

*Apparatus*

An Intersmat gas chromatograph Model 120 F was used, equipped with an alkali flame detector. The column was a 2 m × 3.2 mm I.D. glass column with 3% OV-17 on Chromosorb W HP. Chromatograms were recorded on a Varian instrument Model A 25.

*Conditions*

Column temperature 225°C; injector temperature 240°C; detector temperature 290°C; gas-flow 30 ml $N_2$/min.

## ANALYTICAL PROCEDURE

1. Prepare the following solutions:
   (A) Dissolve 230 mg of pethidine HCl as internal standard in 100 ml of distilled water. Dissolve 453.5 mg of ethoheptazine citrate in 100 ml of distilled water. Dilute 1 ml of this solution with 100 ml of distilled water.
   (B) Extraction solvent. Mix diethyl ether—*n*-hexane—isopropanol (4:1:0.1).
   (C) Ammonium hydroxide. Dilute 25 ml of 25% (w/v) $NH_4OH$ to 100 ml with distilled water.
2. Homogenize about 2 g of minced tissue with 5 ml of the internal standard at 0°C with a Potter Elvejehm homogeniser. Measure the volume of the homogenate (I).
3. Centrifuge at 3000 *g* for 10 min. Transfer to a clean test tube the clear supernatant and measure its volume (II).
4. Add 0.25 ml of the diluted ammonia solution for each milliliter of the supernatant (II), and 6.0 ml of the extraction solvent mixture.
5. Vortex for 2 min. Centrifuge at 3000 *g* for 10 min.
6. Take an aliquot of the organic layer and evaporate to dryness at room temperature. Dissolve the residue in 200 μl of ethanol and inject 1 μl into the gas chromatograph.

*Calibration curve*

Control specimens (2 g or 2 ml) of liver, kidney, spleen, brain and plasma containing 2—20 μg of ethoheptazine were prepared and analyzed according to the described method. Peak height ratios of ethoheptazine to the internal standard were calculated and plotted against the concentration of ethoheptazine. The equation of the calibration curve was $Y = 0.102X - 0.057$ with a correlation of 0.993.

## RESULTS AND DISCUSSION

Using the described procedure the peaks of ethoheptazine and internal standard were completely separated from those of the control plasma and solvent. Fig. 1 shows a typical chromatogram of ethoheptazine (a) and

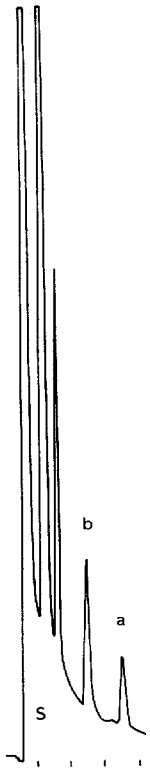


Fig. 1. Chromatogram of extract from plasma. S = solvent, b = pethidine, a = ethoheptazine.

TABLE I

CONCENTRATIONS OF ETHOHEPTAZINE FOUND IN VARIOUS POST MORTEM SPECIMENS

| Tissue | Ethoheptazine ($\mu$g/g) |
|---|---|
| Liver | 10.0 |
| Brain | 4.5 |
| Spleen | 3.1 |
| Kidney | 2.4 |
| Blood | 15.0 |

pethidine (b) in plasma. Peaks not assigned are from biological matrix or solvent.

The minimum detectable concentration of ethoheptazine in plasma was 1.0 $\mu$g/ml. The coefficients of variation over the concentration range 1.0—10 $\mu$g/ml plasma were measured to be 8.2—3.5%.

Recoveries of ethoheptazine from spiked plasma samples over the calibration range were 85 ± 7%. Also recovery studies were performed in the following tissues: liver, spleen, kidney and brain. After homogenizing and centrifuging the respective tissues a solid and a liquid layer are obtained. After extraction of

both the aqueous phase and the solid layer for each tissue, total recovery of ethoheptazine was 80 ± 18%.

The described method proved to be adequate to study the post mortem distribution of ethoheptazine in biological material. In our case, the respective concentrations of the drug in the different post mortem specimens are shown in Table I. Neither chromatographic nor mass spectrometric research revealed any possible metabolites or degradation products of ethoheptazine.

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