Determination of Nanogram Quantities of Diphenhydramine and Orphenadrine in Human Plasma Using Gas-Liquid Chromatography

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Received: May 14, 1973, accepted: August 8, 1973

Summary. A method is described for the assay of nanogram quantities of diphenhydramine and orphenadrine in human plasma. The procedure employs gas-liquid chromatography and a high sensitivity nitrogen detector. It has been used to assay diphenhydramine in plasma after oral administration of therapeutic doses.

Key words: Diphenhydramine, orphenadrine, gas-liquid chromatography, N-selective detector, pharmacokinetics.

The only current method [1, 2] for determination of the antihistamine diphenhydramine is based on formation of a fluorescent complex with Tinopal GS (Ciba-Geigy) after extraction from plasma. The sensitivity of this method, which is reported to be 0.2 μg, is insufficient for measuring therapeutic blood levels. The need for a large volume of plasma is an additional disadvantage.

These problems have now been overcome by development of a specific GLC method of assay.

In preliminary trials with a standard FID-detector it was found impossible to determine quantitatively nanogram/ml amounts of diphenhydramine in plasma. With a nitrogen selective detector, however, concentrations less than 1 ng/ml plasma could be detected. Orphenadrine was chosen as an internal standard for the assay because its chemical structure resembles that of diphenhydramine and it behaves similarly in extraction procedures and on gas chromatography.

Method

1. Extraction

Extraction from plasma was performed as described by Dill and Glazko [2] with minor modifications.

Standard solutions of 10 μg free base/ml were prepared by dissolving 11.43 mg diphenhydramine-hydrochloride (Scherer, Eberbach), or 11.35 mg orphenadrine-hydrochloride (Boehringer, Mannheim), in 1000 ml deionized water. n-Heptane (Merck, Darmstadt) and methanol (Merck, Darmstadt) were of analytical reagent quality and chloroform (Merck, Darmstadt) was of spectral quality.

Experiments were carried out in 25 ml stoppered glass tubes with conical bottoms and 300 μl reactivials (Pierce Chemical Company, Rockford, Ill.) with screw caps containing a teflon-faced rubber disc (Machery-Nagel, Düren).

Into a 25 ml glass tube were pipetted successively 2 ml plasma from heparinized blood, 15 μl of the orphenadrine-hydrochloride standard solution and 2 ml 0.1 N NaOH. 12 ml n-heptane were layered carefully over the mixture. After shaking vigorously for 30 min, the phases were separated by centrifuging the tubes for 10 min. 8 ml of the organic phase was transferred to a 25 ml tube and evaporated under nitrogen at 50°C. The residue was dissolved in 500 μl chloroform and transferred to a reacti-vial where the solution was evaporated at 40°C and the vials closed by screw caps containing a teflon-faced rubber disc.

A blank sample was extracted in the same way. Three standards were prepared by adding 100, 200 or 300 ng diphenhydramine from the standard solution to the plasma blank.

2. Gas Chromatography

A gas-liquid chromatograph (model 7620 A, Research Chromatograph, Hewlett-Packard) equipped with a high sensitivity nitrogen detector (model 15161 B, Hewlett-Packard) was used. The helium carrier gas had a flow rate of 60 ml/min. Hydrogen, at a flow rate of 32 ml/min, and synthetic air at a flow rate of 180 ml/min, were purified by filtration through moisture traps filled with a molecular sieve 5A. The glass column (6ft, 2 mm I.D.) was cleaned with chromic acid, siliconized with 5% dimethylchlorosilane in toluene, rinsed with methanol and dried, before being packed with 3% OV-17 on Chromosorb W AW DMCS High Performance. The working temperature of the injection port was 260°C and of the detector 380°C; the column was held at a constant temperature of 220°C. Other working details were: range 10²; attenuation 16; the recorder chart was
operated at 0.25 inch/min; the residue of each reactive was dissolved in 10 μl methanol and 4 μl were injected for each determination.

Results

1. Retention Times

Under these operating conditions diphenhydramine (Fig. 1b, D) and orphenadrine (Fig. 1b, O) had retention times of 1.6 and 2 min, respectively. Fig. 1a shows a gas-chromatogram of a plasma sample taken from an untreated control subject; it lacks any detectable peaks with the retention times of diphenhydramine and orphenadrine. It should be compared with the chromatogram of a plasma sample from the same person after a dose of diphenhydramine hydrochloride (Fig. 1b).

![Chromatogram of blank sample of human plasma.](image)

Fig. 1. a) Chromatogram of blank sample of human plasma. b) Chromatogram of plasma sample containing 56 ng diphenhydramine (peak D) and orphenadrine as internal standard (peak O).

2. Detector Response Curve

The sharpness of the peaks corresponding to diphenhydramine and orphenadrine permitted measurement of the peak heights and the linearity of the detector response is apparent in Fig. 2. The peak height ratio of diphenhydramine to orphenadrine was proportional to the weight ratio ($r = 0.999$).

The response of the detector was linear over a concentration range of 10 to 100 ng (Fig. 2), but the slope of the calibration curve changed from day to day depending on various parameters, such as operating temperature and gas flow.

![Detector response curve from 10 to 100 ng diphenhydramine.](image)

Fig. 2. Detector response curve from 10 to 100 ng diphenhydramine. The mean values ($n=5$) and standard deviations are shown.

3. Reproducibility and Accuracy of the Method

Two principal sources of error were adsorption of the amines onto glass surface that made it very difficult to transfer the total residue from the tubes to the vials and the impossibility in the last step of