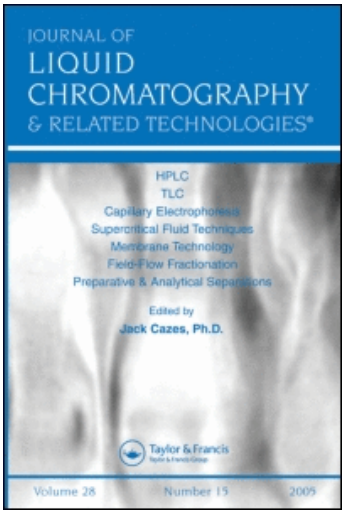

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A HPLC Method for the Determination of Butaperazine in Solutions, Tablets, Plasma and Bile

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A HPLC METHOD FOR THE DETERMINATION OF BUTAPERAZINE IN SOLUTIONS, TABLETS, PLASMA AND BILE

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ABSTRACT

A specific HPLC method for the determination of the antipsychotic drug butaperazine (B) in solutions, tablets, plasma and bile has been developed. The instrument used was a Waters HPLC equipped with a Model 440 Spectrometer and a μ-Bondapak-NH_2 column. The mobile phase, chloroform-methanol (100:3.5) was pumped at a rate of 1.5 ml per minute. Ultraviolet absorbance at a wave length of 280 nm was used for detection. The procedure involved the extraction of the drug from the dosage forms with chloroform and from plasma and bile with hexane-isopropanol (9:1). Hydrocortisone acetate was used as the internal standard. Retention times of 2.4 and 3.9 minutes were obtained for the internal standard and B respectively. Analytical calibration yielded a linear relationship from 0.1-25 μg per ml, with r^2 value of 0.99. The percentage recovery of B averaged 99% from the dosage forms and 94% from the biological fluids. An improvement in the method for determining B in bile and plasma was later developed. It involved the use of a different mobile phase and detecting the drug fluorometrically.

INTRODUCTION

Butaperazine is 2-butyryl-10-[3-(4-methylpiperazin-1-yl)-propyl] phenothiazine. It is a commonly used antipsychotic medication for the treatment of patients with emotional or mental disorders (1). Several methods for butaperazine determination were reported. Included among the methods are Colorimetric, Titrimetric, UV absorption, Spectrophotometric and Chromatographic procedures (2,3). The thin layer chromatographic (TLC) method (2) is not sensitive enough to measure butaperazine

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in the therapeutic range. Although the gas chromatographic (GC) method (3) was found sensitive enough to determine B in biological fluids yet, some investigators (2) observed that because of its high boiling point and low thermal stability, butaperazine was too low in volatility to be eluted quantitatively without extensive thermal decomposition. High reproducibility and sensitivity were reported for the fluorometric methods (4-6) used to determine butaperazine. In this study, a specific, simple and sensitive HPLC method for the determination of butaperazine in solutions, tablets, plasma and bile is reported.

MATERIALS

Instrumentation

The instrument used was a Waters Associates (Milford, MA) liquid chromatograph, equipped with a 6000A pump, a 440 spectrometer, a 420C fluorometer and a data module with dual pen recorder. A micro BONDAPAK-NH_2 column (3.9mm X 30cm, Waters Associates) was used for all separation.

Chemicals

The solvents used for drug extraction and in the mobile phases were chloroform, B.D.H. Chemicals Ltd. (Poole, England), methanol and hexane, Merk (Darmstadt, Germany), and isopropanol and methylene chloride, Reidel-De Haen AG (Seelze-Hannover, Germany). The mobile phases were prepared by mixing the corresponding solvents and filtering the mixture through a 0.5 µm pore size membrane filter obtained from Millipore Corporation (Bedford, Massachusetts) before degassing. Butaperazine tablets and solutions were obtained from the local market. A reference sample was also obtained from A.H. Robins Company (Richmond, Virginia, USA) for standardization. All other chemicals were of analytical grade.

METHODS

Development of HPLC Conditions

Based upon the semipolar nature of butaperazine, the μ-Bondapak NH_2 column was chosen for this study. Preliminary trials with other columns of different polarities were unsatisfactory. Mobile phase selection was based upon achieving good resolution for the drug within a reasonable retention time. Upon using chloroform-methanol mixture (100:3.5) retention time of 3.9 minutes was obtained for butaperazine. By examining the integrated areas under the peaks obtained for two different drug concentrations, proper drug resolution was confirmed. Hydrocortisone acetate obtained from Sigma Chemical Company (St. Louis, Missouri, USA) was used as the internal standard. It was found to have a well defined peak separated from that of the drug with a retention time of 2.4 minutes. Ultraviolet absorption at a wave length of 280 nm was used for detection. Solutions of different concentrations of butaperazine in the mobile phase were prepared and used to calibrate this method of analysis.

Determination of Butaperazine in Tablets and Solutions

A number of B tablets obtained from the local market was crushed. A known weight of the powder equivalent to 10 mg B was treated with 20 ml 5N solution of sodium hydroxide and mixed thoroughly in a separatory funnel. Two portions of chloroform, each 50 ml, were used to extract B from the mixture. Ten milliliters of the chloroform extract were evaporated to dryness and the residue was dissolved in 50 ml mobile phase containing 200 μg/ml of the internal standard. Exactly, 20 μl of the product were injected in the HPLC for B determination. A second injection of 30 μl was carried for confirmation. Essentially the same procedure was followed for B determination in solutions beginning with

the addition of 20 ml 5N sodium hydroxide solution to a volume containing 10 mg of B.

Determination of Butaperazine in Presence of Bile and Plasma

A stock solution of known concentration of B was prepared by dissolving 10 mg pure butaperazine maleate in 20 ml of water acidulated with dilute hydrochloride acid. To a 0.5 ml of freshly obtained bile or plasma, 0.2 ml of the stock solution of B was added. The mixture was shaken with 2 ml of 5N sodium hydroxide solution, then extracted with 10 ml of hexane-isopropanol (9:1). Two milliliters of the extract were evaporated to dryness and the residue was dissolved in 2 ml mobile phase containing the internal standard before injection into the HPLC.

Determination of Butaperazine in Bile and Blood of Rats Administering the Drug

Rats (350-400 gm) anaesthesized with urethane were injected intravenously with 2.5 ml of B solution in normal saline (100 µg/ml). Bile was collected through a cannula for about 4 hours. Before the animals were sacrificed, a blood sample was obtained from the heart from which the plasma was separated. Both plasma and bile were extracted in the same way as before except that the residue after evaporating the organic solvent was dissolved in 1 ml of a mobile phase consisting of hexane-chloroform-methylene chloride (1:3:6). This new mobile phase when used at a flow rate of 1.2 ml/min., was found to better separate B from other extracted constituents including B metabolites. The drug was detected both by the UV detector (280 nm) and fluorescence detector (280 nm activation and 530 nm fluorescence).

RESULTS AND DISCUSSION

Figure 1 shows typical chromatograms for drug formulations and a placebo mixture in which the internal standard and the butaperazine peaks appear at 2.4 and 3.9 minutes respectively. The chromatograms show no interference from the excipients in either of the solutions or tablets.

A plot of the ratio of butaperazine peak area to the internal standard peak area versus the butaperazine concentration in the solution was linear up to at least 25 µg/ml and essentially passed through the origin ($r^2 = 0.99$). Butaperazine recovery from both solutions and tablets ranged from 97.1 to 101%. This result was obtained through the addition of known amounts of B to either placebo formulations or previously assayed dosages containing the active ingredient before analysis. Replicate analysis of five individual sample preparations of different concentrations showed high precision. The relative standard deviation was found to be in the range of ± 0.4 to ± 1.5%.

Similar results were obtained when B was added to bile and plasma then extracted. Recovery of B from both bile and plasma ranged from 91-96.5%. Concentrations down to 0.5 µg/ml were detected. This procedure was found, however, to be unsatisfactory to determine B in bile and blood collected from animals administering the drug.

Two major and three minor B metabolites were previously (7) separated from biological fluids obtained from rats and dogs. It is beleived that under the conditions used in this procedure B was not properly separated from its metabolites. Through the modification in the mobile phase and the flow rate complete separation of B was achieved. The new retention time of B was 5.15 minutes. Also, by using the fluorescence detector, the sensitivity was improved by a factor of 6-7. Concentrations of B in bile and blood down to 0.1 µg/ml were determined.

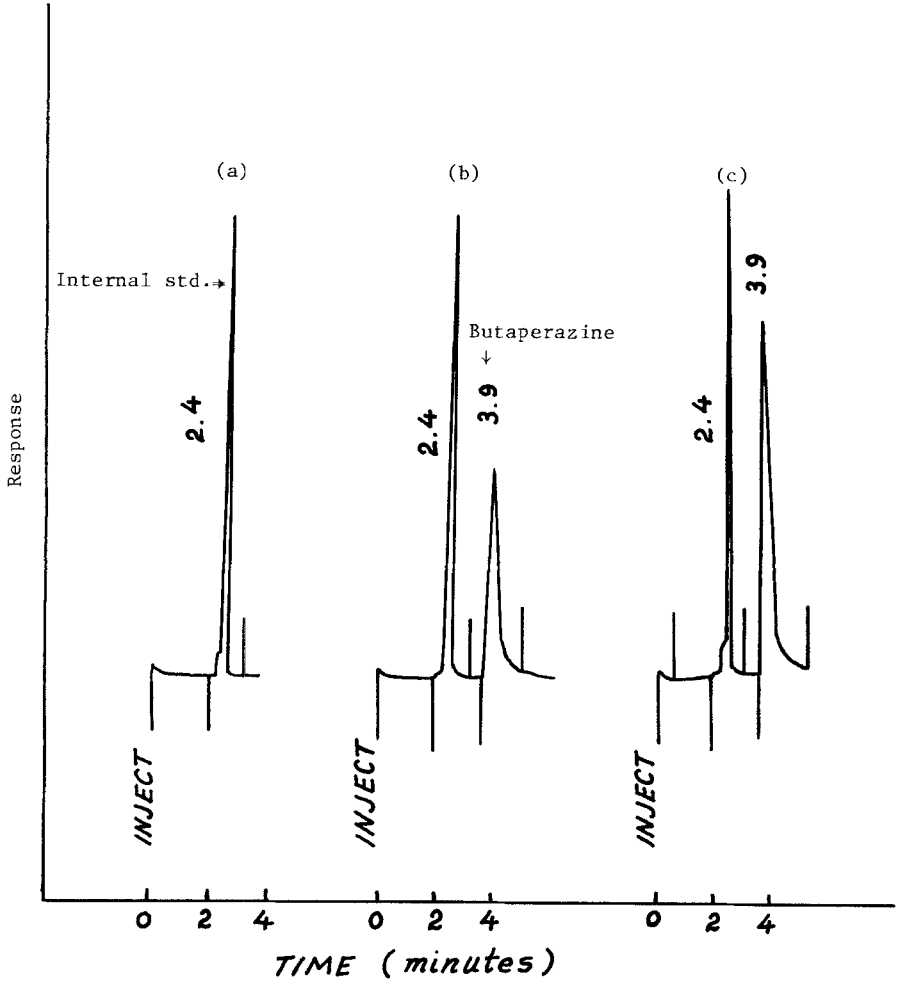

Figure 1: Typical butaperazine chromatogram with retention times, a, Internal standard; b, Butaperazine extracted from solutions; c, Butaperazine extracted from tablets.

This method of determining B has proved to be simple and accurate. A high performance liquid chromatograph equipped with a fluorescence detector would be more useful for B determination in biological fluids. The separation of B from its metabolites in biological fluids should enable the study of the drug pharmacokinetics under different physiological and nonphysiological conditions.

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