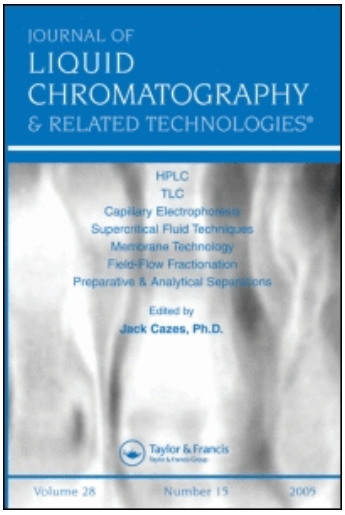

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY OF PHENYLBUTAZONE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, rapid and reliable high performance liquid chromatographic procedure for the quantitation of phenylbutazone in pharmaceutical dosage forms was developed, and compared with the U.S.P. XXI method and a spectrophotometric assay developed in this laboratory. A comparison of the three methods indicated that the HPLC method is the most rapid, simple and reproducible. The recoveries based on six placebo samples were 100.2, 99.2, and 99.4% by HPLC, UV and the U.S.P. method, respectively, and their

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respective CVs were 0.39, 0.73, and 1.5%. Replicate regression analyses of three standard plots in the concentration range of 0.02-0.12 mg/ml obtained using the HPLC assay on three different days yielded a correlation coefficient >0.999 and the coefficient of variation for the three slopes was 1.05%. It is suggested that the proposed HPLC method should be used for routine quality control and dosage form assay of phenylbutazone.

INTRODUCTION

Phenylbutazone, a synthetic pyrazolone derivative chemically related to aminopyrine, has antiinflammatory, antipyretic, analgesic, and mild uricosuric properties (1). Several methods for quantitation of phenylbutazone in pharmaceutical dosage forms have been reported. A GC method using flame ionization detection (2), quantitative IR spectroscopic method (3), differential spectrophotometry relying on difference in absorption between acidic and basic species of phenylbutazone (4) are available. Other assay techniques have included separation on an ion exchange column followed by titration with NaOH in the presence of phenolphthalein (5), or acid hydrolysis followed by oxidation and spectrophotometric assay (6). The U.S.P. XXI for the assay of phenylbutazone in tablets entails an ether-based extraction, followed by a UV spectrophotometric determination. All of these approaches require either lengthy sample preparation steps and/or non-specific quantitation.

The purpose of the present work was to develop an HPLC assay for the measurement of phenylbutazone in pharmaceutical formulations. A direct spectrophotometric assay without separating the drug by a HPLC column was also developed for the quantitation of phenylbutazone in dosage forms. The HPLC, spectrophotometric and the U.S.P. XXI procedures were compared with respect to their accuracy, simplicity and reproducibility.

EXPERIMENTAL

Chemicals and Reagents: Phenylbutazone (7) and hexylparaben (8) were used without further purification. Acetonitrile (9) was HPLC grade. Water was distilled and deionized. All other chemicals and reagents were USP or ACS quality and were used as received.

Chromatography: Analyses were performed on a liquid chromatograph equipped with a variable wavelength UV detector (10) set at 254 nm and operated at ambient temperature. A stainless steel reversed-phase C_{18} column (15 cm x 3.9 mm) containing 5 u spherical silica (11) was used with a guard column. Samples were introduced into the column through a fixed volume injector (12) with 20 ul sample loop. Chromatograms were traced on a strip-chart recorder (13) at a speed of 0.5 cm/min.

Mobile Phase: The eluting medium consisting of 0.05 M acetate buffer (pH 5.2) and acetonitrile (40:60% V/V) was

prepared and degassed by bubbling helium gas for 5 min prior to use. Column equilibration with the eluting solvent was established by pumping (14) the mobile phase at a rate of 0.2 ml/min for overnight. The flow rate was set at 1.0 ml/min during analysis.

Calibration Marker: A stock solution of hexylparaben 0.2 mg/ml acetonitrile was prepared weekly and stored at 4°C.

Preparation of Standard Solutions of Phenylbutazone: A stock solution was prepared by dissolving 100 mg of phenylbutazone in 100 ml acetonitrile. Seven aliquots equivalent to 0.2, 0.3, 0.4, 0.6, 0.8, 1.0 and 1.2 mg of phenylbutazone were added to 10 ml volumetric flask. After an aliquot of the calibration marker equivalent to 200 ug was added to each 10 ml flask, the flasks were brought to volume by acetonitrile and mixed thoroughly. Three 20 ul injections of each standard solution of phenylbutazone containing the calibration marker were made to prepare standard plots. The peak height ratios of phenylbutazone/hexylparaben were plotted against phenylbutazone concentrations. Least squares linear regression analysis was used to determine the slope, y-intercept, and the correlation coefficients of the standard plots.

Sample Preparation: Tablets - Individual phenylbutazone tablets (100 mg labelled) were pulverized using a mortar and pestle, and completely transferred to a 50 ml centrifuge tube containing 25.0 ml of acetonitrile. After vigorously shaking for

5 min, the undissolved contents were allowed to settle down. Five ml of the supernatant was pipetted into a 15 ml screw capped tube and centrifuged at 2500 r.p.m. for 5 min. An aliquot was taken for both the HPLC and spectrophotometric assay.

Capsules - The contents of each capsule (100 mg labelled) were placed in a 50 ml centrifuge tube containing 25.0 ml of acetonitrile followed by the subjection to the sample preparation procedure described for tablets.

Quantitation of Phenylbutazone: HPLC Method - One hundred ul of the sample solution prepared for tablets or capsules was transferred to a 10 ml volumetric flask containing 1.0 ml of the stock solution of calibration marker (0.2 mg/ml) and diluted to volume with acetonitrile. Twenty ul was injected onto the column for quantitation. Ten replicate tablets or capsules were simultaneously analyzed for statistical evaluation.

Spectrophotometric Method - One hundred ul of the solution prepared for tablets or capsules was diluted with acetonitrile (1:400) and assayed spectrophotometrically at 240 nm using acetonitrile as reference.

USP Method - The USP XXI procedure was carried out for ten replicate samples of tablets or capsules. The method involves pulverization of the dosage forms followed by shaking vigorously for 45 minutes with 150 ml sodium hydroxide solution (0.4%) and completing to 250 ml with 0.4% sodium hydroxide solution. An aliquot of the sample was filtered rejecting the first 20 ml of

the filterate. A five ml portion of the filterate was rendered acidic with hydrochloric acid, and extracted with ether for three times. The combined etherial layer was reextracted with 0.4% sodium hydroxide solution. The solution was aerated with nitrogen to remove residual ether, then determined spectrophotometrically at 264 nm.

Calculation: The amount of phenylbutazone in the sample was determined from the following equation:

$$Q = \left(\frac{R}{a} + b\right) \times \text{dilution factor}$$

Where Q is the amount of phenylbutazone in dosage form, R is the peak height ratio (drug/calibration marker), a is the slope of the calibration curve and b is the y-intercept.

Recovery of phenylbutazone from placebo samples: Placebo samples containing 100 mg of phenylbutazone and 100 mg each of starch and lactose were similarly subjected to the proposed HPLC, UV and USP method to compare the accuracy and precision of the procedures.

RESULTS AND DISCUSSION

Figure 1 shows a typical chromatogram of the sample containing phenylbutazone and the calibration marker. Using the chromatographic conditions described, phenylbutazone and hexylparaben were well separated and their retention times were

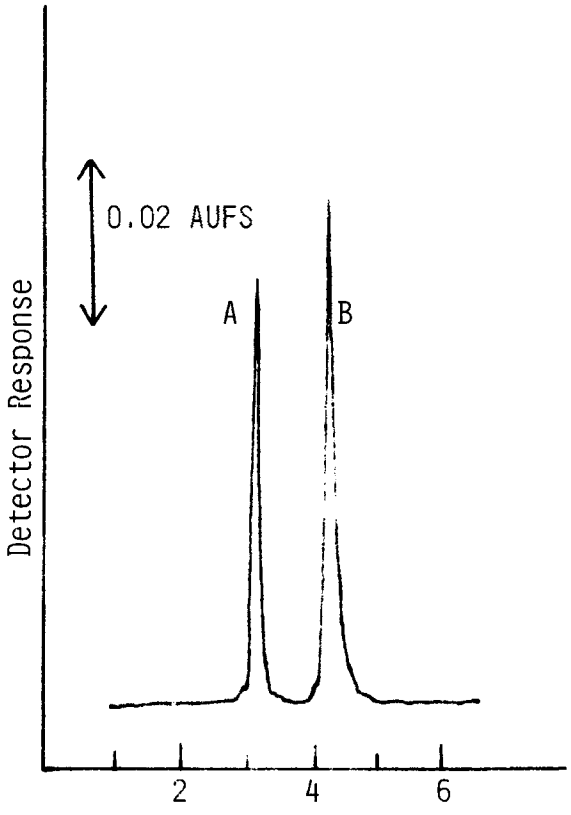

Figure 1. Chromatogram of phenylbutazone tablet.
Key: A - Phenylbutazone, B - Hexylparaben

3.2 and 4.4 min, respectively. The peaks are sharp and symmetrical with good baseline resolution, thus facilitating the accurate measurement of the peak height ratios. No interfering peaks were found due to tablet excipients, although no sample clean-up steps were employed. Figure 2 shows a calibration plot for the peak height ratios of varying amounts of phenylbutazone

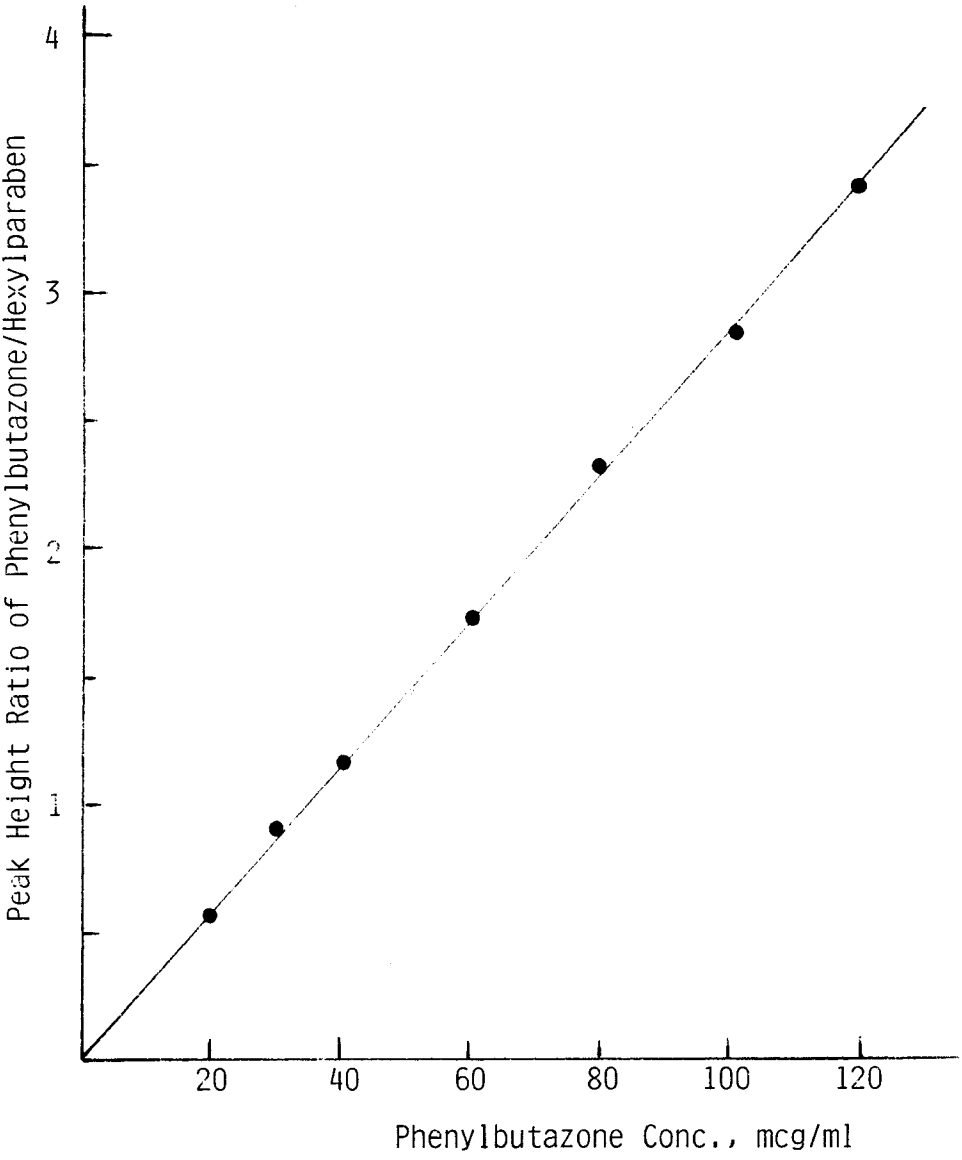

Figure 2. Standard Calibration Plot of Phenylbutazone

(0.02-0.12 mg/ml) to a constant amount of hexylparaben (20 ug/ml).

The plot was highly linear (r = 0.9999) and the regression analysis of the data yielded the slope and intercept as:

$$y = 28.38x + 0.026$$

where y and x are the peak height ratio and phenylbutazone concentration (mg/ml), respectively. Three replicate analyses of phenylbutazone at concentrations of 0.02-0.12 mg/ml were assayed at three different days over one week period. The results of this evaluation are summarized in Table I. The average correlation coefficient was 0.9997 and the coefficient of variation of the slopes of the three lines was 1.05%. Analysis of variance of the data showed no detectable difference in the slopes of the three standard plots ($F = 3.4$, $P > 0.01$). The similarities in the slopes and the high correlation coefficients indicate that the assay possesses excellent reproducibility and linearity. Thus, the method should be accurate and precise within the assay day as well as between assay days.

Recovery from Placebo Samples: Table II compares the average recoveries of phenylbutazone from placebo samples containing 100 mg phenylbutazone and 100 mg each of lactose and starch, using the HPLC, UV and the USP method. The average recoveries were 100.2, 99.2 and 99.4% for the HPLC, UV and the USP method, respectively and their respective relative standard deviations were 0.39%, 0.73% and 1.5%. The values obtained by

TABLE I

Regression Analyses of Three Standard Plots of Phenylbutazone

Standard[a] Plot	Slope[b]	Intercept[b]	Correlation Coefficient
1	28.529	0.0017	0.99965
2	28.380	0.0260	0.99990
3	28.960	0.0150	0.99970

a - obtained in three different days
b - the mean of 3 determinations at each drug concentration

TABLE II

Recovery of Phenylbutazone from Spiked Placebo Samples with the HPLC, UV and USP

Method	n	Amount Added mg	Amount Recovered mg	CV%
USP	6	100	99.4	1.5
HPLC	6	100	100.2	0.39
UV	6	100	99.2	0.73

n = number of replicates

the HPLC method compared favorably with those obtained by the U.S.P. and spectrophotometric procedures. In most cases, however, the HPLC assay gave slightly higher values than the USP method. The difference may have been caused by a loss of sample during several extraction steps in the USP method. The U.S.P. XXI assay for phenylbutazone is much time consuming as it requires shaking with 0.4% sodium hydroxide solution for 45 min, then extraction with ether for three times. The etherial layer was then reextracted with the sodium hydroxide solution for three

TABLE III

Comparison of the HPLC, UV and USP Methods for Determination of Phenylbutazone in Pharmaceutical Dosage Forms

Sample	Content[a] (CV) mg (%)		
	USP	HPLC	UV
Capsule A	97.2 (2.3)	100.2 (1.1)	99.9 (1.7)
Capsule B	92.3 (3.0)	98.5 (1.3)	97.4 (1.5)
Tablet A	96.0 (1.2)	95.9 (0.4)	95.1 (1.1)
Tablet B	97.4 (2.1)	103.5 (1.4)	101.4 (1.8)
Tablet C	94.7 (1.3)	99.7 (0.9)	100.5 (1.2)
Tablet D	92.9 (3.4)	95.7 (1.8)	95.4 (2.4)
Mean	95.1 (2.2)	98.9 (1.2)	98.3 (1.6)

[a] Mean of 10 replicate samples

times, followed by spectrophotometric determination at 264 nm. As such the method requires many hours of analytical time (i.e., around 6 hours for the analysis of six placebo tablets compared to 1 hour by the proposed HPLC or UV method).

Analysis of Phenylbutazone in Dosage Forms: Table III presents the results comparing recoveries of phenylbutazone from tablets or capsules by the HPLC, UV and the U.S.P. XXI assay procedure. The average recovery and coefficients of variation from different dosage forms were 95.1, 98.9, 98.3 mg and 2.2, 1.2, 1.6% for the USP, HPLC and UV procedure, respectively. The requirements for content uniformity of phenylbutazone tablets or capsules in the U.S.P. specify that the potency must fall within 93-107% and 90-110%, respectively, of the label claim. Thus, the tablets or capsules selected randomly in this determination met the U.S.P. requirements for the content uniformity.

Conclusion: The HPLC method developed in this study has the advantage of simplicity, precision and reliability. It allows for the direct determination of phenylbutazone bypassing several tedious steps involved in the USP method. It should be useful for routine analytical and quality control assay of phenylbutazone in dosage forms.

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