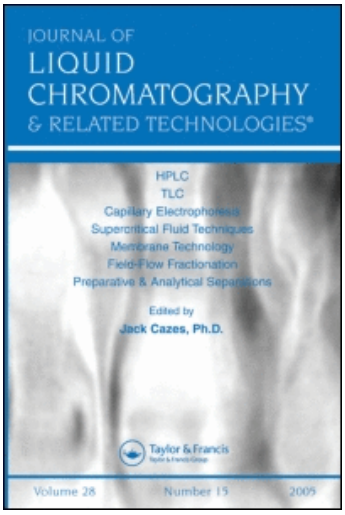

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QUANTITATION OF HYDRALAZINE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORMS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Stability-indicating assay methods for the quantitation of hydralazine hydrochloride based on high-performance liquid chromatography using two different columns have been developed. Phenylpropanolamine can be used as an internal standard with both columns (μC_{18} and μphenyl) while hydrochlorothiazide can be used only with μphenyl column. The method is accurate and precise with percent relative standard deviations based on 6 readings of less than 2. The excipients present in the dosage forms did not interfere with the assay method. In combination with hydrochlorothiazide, hydralazine can be quantified only with μphenyl column. Two oral liquid dosage forms prepared in a local hospital using commercially available strawberry syrup/simple syrup decomposed completely in one day.

INTRODUCTION

Hydralazine hydrochloride (Figure 1) is one of the most commonly used drugs against hypertension. The most common forms

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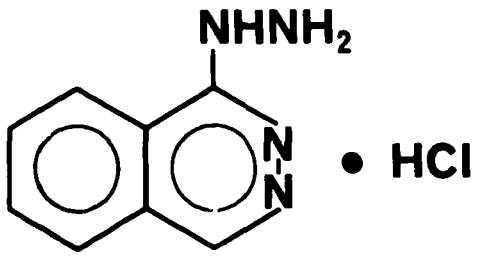

Figure 1 - Structure of hydralazine hydrochloride.

of commercial dosage forms available are tablets and injection. It is also available in combination with hydrochlorothiazide and reserpine.

The USP-NF method (1) for the analysis of hydralazine in tablets and injection is based on titration with potassium iodate. At the writing of this paper, the USP 21st ed.-N.F.-16th ed. has become available which includes a method based on high-performance liquid chromatography.

In the presence of reserpine and hydrochlorothiazide, the assay method (2) is a complicated colorimetric procedure. A different colorimetric method (3) based on reaction with 9-methoxyacridine has been reported. A GLC method (4) for the determination of hydrazine levels in the hydralazine dosage forms has been recommended. The purpose of these investigations was to develop a stability-indicating assay method based on high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Chemicals and Reagents - All chemicals and reagents were either USP-NF or ACS grade and used as received. Hydralazine hydrochlo-

ride (5) and hydrazine sulfate (5) powders were used without further purification.

Apparatus - A high-pressure liquid chromatograph (6) equipped with a multiple wavelength detector (7) and a recorder (8) was used. All pH values were determined using a pHmeter (9).

Columns - Two columns (10), a semipolar (μphenyl, 30 cm long x 3.9 mm i.d.) and a nonpolar (μC_{18}, 30 cm long x 3.9 mm i.d.) were used.

Chromatographic Conditions - A mobile phase containing 0.015M KH_2PO_4, 0.1% glacial acetic acid (V/V) and 0.5% V/V methanol (2% when column was nonpolar) in water was used. The flow rate was 3.0 ml/min. The detector was set at 256 nm, the sensitivity was 0.04 (AUFS), the temperature was ambient and the chart speed was 30.5 cm/hr.

Stock Solutions - A 2.0% aqueous solution of phenylpropanolamine hydrochloride (one of the internal standards) was prepared fresh every week. A 0.2% solution of hydrochlorothiazide (internal standard when assaying samples containing only hydralazine using μphenyl column) in methanol was prepared fresh every week. A 0.1% aqueous solution of hydralazine hydrochloride was prepared fresh daily.

Standard Soltuions - The standard solutions with or without the internal standard were prepared as needed by diluting the stock solution(s) with water.

Assay Solutions: - Injection - An appropriate quantity of the injection (usually 1.5 ml of 20.0 mg/ml injection) was diluted to

100 ml with water. A 10.0 ml quantity of this solution was mixed with either 8.0 ml quantity of stock solution of phenylpropanolamine hydrochloride or 3.0 ml quantity of the stock solution of hydrochlorothiazide and the mixture was diluted to 100 ml with water.

Tablets - Ten tablets (one for content uniformity) were/was ground to a fine powder. A quantity of the powder representing 10.0 mg of the powder was mixed thoroughly with 2 ml of ~0.5N HCl and the mixture was brought to volume (100 ml) with water. The mixture was shaken for about 2-3 minutes, filtered (11), first 15 ml of the filterate was rejected and then collected for further dilution. A 15.0 ml quantity of the filtrate was mixed with the stock solution of one of the internal standards (4.0 ml if phenylpropanolamine and 1.5 ml if hydrochlorothiazide) and the mixture brought to volume (50 ml) with water.

Assay Solution From Oral Liquid Dosage Forms Prepared in a Local Hospital - The dosage forms contained 1.0 mg/ml of hydralazine hydrochloride in either strawberry syrup or simple syrup for dispensing. For assay it was diluted with water to a concentration of 30.0 µg/ml of hydralazine hydrochloride based on the label claim. No internal standard was added to this solution.

Assay Procedure - A 20.0 µl aliquot of the assay solution was injected into the chromatograph using the described conditions. For comparison, an identical volume of the standard solution (containing same concentrations of hydralazine and the internal standard based on the label claim) was injected after the assay solution eluted.

Calculations - The result were calculated using:

$$\frac{Pha}{Phs} \times 100 = \text{Percent of the label claim}$$

where Pha is the ratio of peak heights of hydralazine and the internal standard of the assay solution and Phs that of the standard solution. Preliminary investigations indicated that concentrations versus ratio of the peak heights were linear between 15-45 µg/ml of hydralazine hydrochloride.

RESULTS AND DISCUSSION

The results (Table 1) indicate that hydralazine hydrochloride can be quantified in pharmaceutical dosage using the developed methods. The methods are precise and accurate with percent relative standard deviations based on 6 readings of 1.8 (with μC_{18} and phenylpropanolamine as the internal standard), 1.0 (with µphenyl and phenylpropanolamine as the internal standard), 1.1 (with µphenyl and hydrochlorothiazide as the internal standard and 1.9 (μC_{18}) without an internal standard. With μC_{18}, hydrochlorothiazide could not be used as an internal standard since its retention time was same as that of hydralazine (Figure 2). For the quantitation of hydralazine hydrochloride in the presence of hydrochlorothiazide only µphenyl column can be used (Figure 3). There was no interference from the excipients present in tablets and injection (Figures 2-4) using either one of the columns. In the presence of hydrochlorothiazide, the separation from hydralazine was complete using phenyl column (Figure 3). Using either column, the internal standard, phenylpropanolamine, eluted before

TABLE 1
Assay Results

Name of the Product or Synthetic Mixture	Claim (Hydralazine HCl)	Other ingredients if any	Percent of the Label Claim Found Using µC18 Column	Percent of the Label Claim Found Using µPhenyl Column
Tablets	50 mg	Excipients with light green color.	99.6	100.0
Tablets	25 mg	Excipients with dark blue color.	98.6	99.0
Tablets	10 mg	Excipients with yellow color.	99.2	99.2
Tablets	25 mg	Excipients with light orange color and 15 mg of hydrochlorothiazide and 0.1 mg of reserpine per tablet.	127.8[a]	99.5

Injection	20 mg/ml	Propylene glycol, 0.065% of methylparaben and 0.035% of propylparaben.	101.2	101.0
Oral dosage form in strawberry syrup (one day old)	1.0 mg/ml	Sodium benzoate 0.1% citric acid, artificial flavor and color.	2.6	2.9
Oral dosage form in simple syrup (one day old)	1.0 mg/ml	Sodium benzoate, 0.1% and citric acid.	2.1	2.0
Synthetic mixture #1	10 mg per 200 mg	Sorbitol	100.0	99.8
#2	25 mg per 200 mg	Mannitol	100.2	100.0
#3	25 mg per 200 mg	Hydrochlorothiazide 15 mg, reserpine 0.1 mg and mannitol.	101.0	100.8

[a]Hydrochlorothiazide interferes in the assay procedure. That is why results are higher.

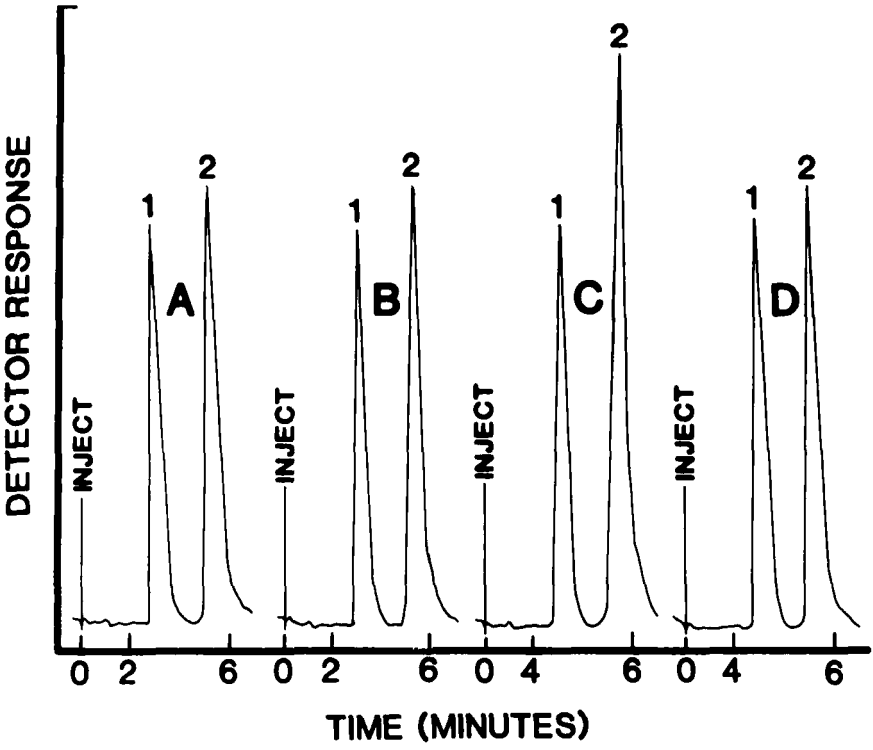

Figure 2 - Sample chromatograms using μC_{18} column. Peaks 1-2 are from phenylpropanolamine and hydralazine, respectively. Chromatogram A is from a standard solution; B from a 25 mg tablets; C from 25 mg tablets containing 15 mg per tablet of hydrochlorothiazide which eluted with hydralazine and 0.1 mg reserpine and D from an injection (20 mg/ml). For chromatographic conditions, see text.

hydralazine (peak 1 in Figures 2 and 3) and separated completely from hydralazine hydrochloride. With μphenyl column, hydrochlorothiazide eluted after hydralazine (peak 2 in Figure 3).

A wavelength of 256 nm was preferred since it is the wavelength of maximum absorption for phenylpropanolamine which is a very poor absorber of light. In spite of this, the concentration

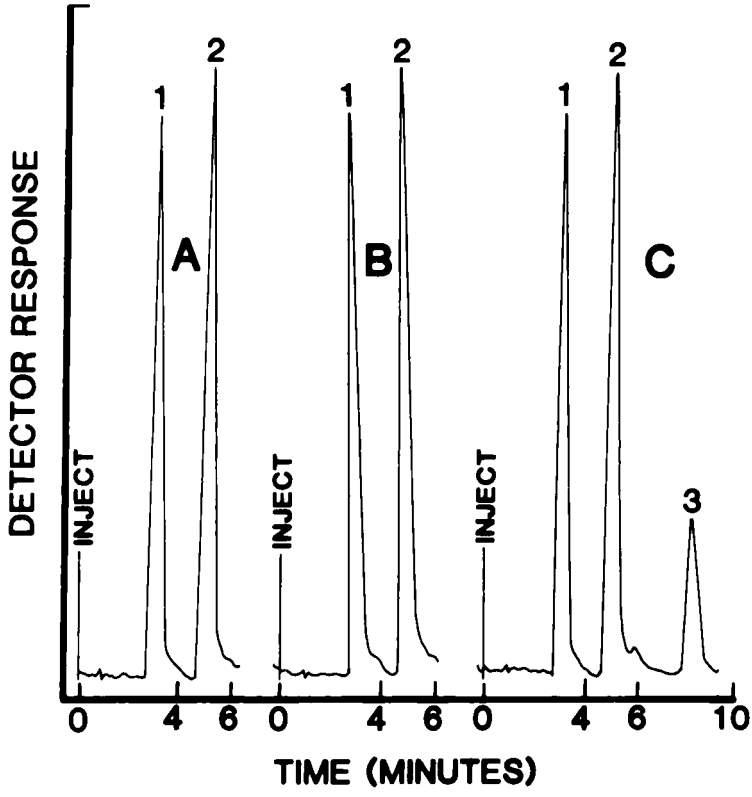

Figure 3 - Sample chromatograms using μphenyl column. Peaks 1-2 are from hydralazine and hydrochlorothiazide, respectively. Chromatogram A is from a standard solution and B from an injection (20 mg/ml). For chromatographic conditions, see text.

of phenylpropanolamine in standard solution was 1.6 mg/ml versus only 60 μg/ml for hydrochlorothiazide and 30 μg/ml for hydralazine.

The method can also be used to determine the content uniformity of tablets (Table 2). It is a stability-indicating method

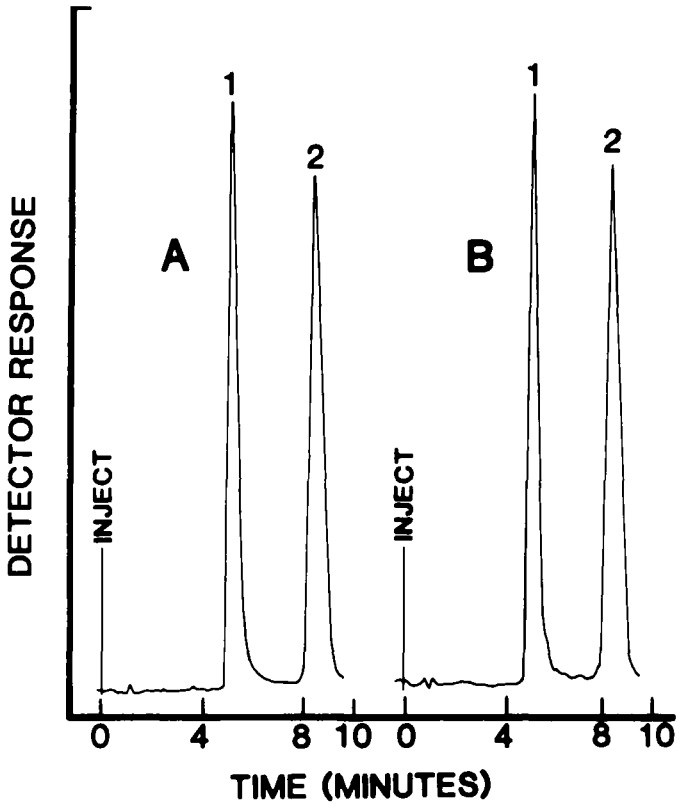

Figure 4 - Sample chromatograms using μphenyl column. Peaks 1-3 are from phenylpropanolamine, hydralazine and hydrochlorothiazide, respectively. Chromatogram A is from a standard solution; B from a 25 mg tablet and C from 25 mg tablet with hydrochlorothiazide and reserpine. For chromatographic conditions, see text.

since the product(s) of decomposition separated from the intact drug (Figure 5). The separation was better with μC_{18} column (Figure 5B) than with μphenyl column (Figure 5D). Therefore, for stability studies, μC_{18} column is recommended. When assaying oral liquid dosage forms, no internal standard was added in order to determine the presence of new peaks from the products of decompo-

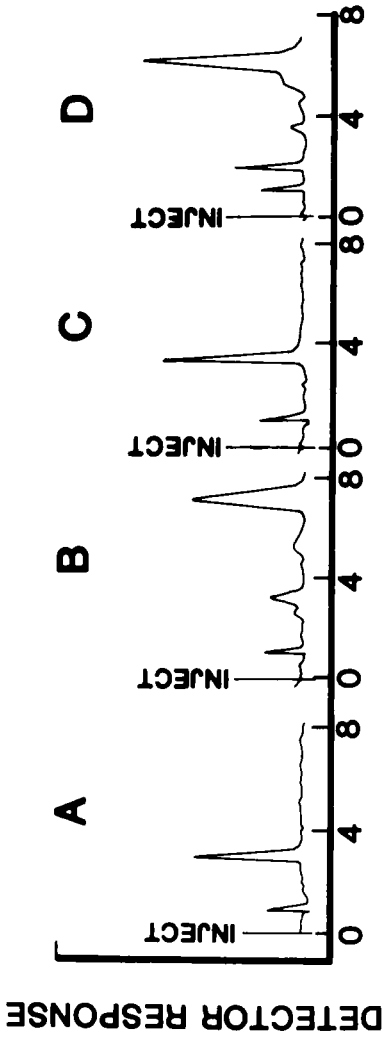

Figure 5 - Sample chromatograms using μC$_{18}$ (chromatograms A-B) and μphenyl (chromatograms C-D) columns. Peak 1 is from hydralazine. Chromatograms A and C are from a commercial strawberry syrup (diluted 1 in 25 with water) which was used to prepare oral dosage forms. Chromatograms B and D are from the oral dosage form (one day old) in strawberry syrup. For chromatographic conditions, see text.

TABLE 2
Contents Uniformity Results of 10 mg Tablets

Tablet No.	Percent of the Claim Found Using μC_{18} Column	μ Phenyl Column
1	92.8	92.1
2	99.2	99.0
3	104.4	103.7
4	96.2	95.7
5	107.4	107.6
6	97.0	97.0
7	93.8	94.5
8	96.7	96.3
9	94.8	94.1
10	105.2	105.8

sition. None of these peaks was from hydrazine as confirmed by injecting a solution (40 μg/ml) prepared from pure drug powder.

It is interesting to point out that the percent of intact hydralazine remaining after one day of storage at room temperature of samples prepared in simple syrup/strawberry syrup was almost nil (Figure 5 and Table 1). Thorough investigations of this problem are in progress in authors laboratory and will be published later on.

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(8) Omniscribe 1513-12, Houston Instruments, Austin, TX.

(9) Beckman Model 4500, Beckman Instruments, Fullerton, CA.

(10) μBondapak phenyl and μBondapak C_{18}, Waters Associates, Milford, MA.

(11) Fisher #9-801E, Fisher Scientific Co., Fairlawn, NJ.