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DETERMINATION OF HYDRALAZINE HYDROCHLORIDE AND HYDROCHLOROTHIAZIDE IN DOSAGE FORMS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A high performance liquid chromatographic method is presented for the simultaneous determination of hydralazine hydrochloride and hydrochlorothiazide in combination dosage forms. The compounds are chromatographed on a radialpak cyanopropylsilane cartridge with a mixture of methanol, water and dibutylamine phosphate as mobile phase and UV detection at 254 nm. The hydralazine hydrochloride and hydrochlorothiazide showed linear detector responses over a range of 50-150% of label claim with correlation coefficients of 0.999. Assay recoveries (n=5) were found to contain an average of 98.9% $\pm$ 1.3 of hydralazine hydrochloride and 98.8% $\pm$ 1.1 of hydrochlorothiazide. The proposed method showed excellent resolution and reproducibility. It will be helpful in routine quality control analysis of such combination dosage forms.

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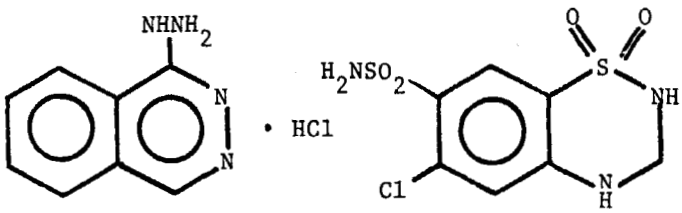
INTRODUCTION

Hydralazine hydrochloride, (1-hydrazinophthalazine, monohydrochloride) I, and hydrochlorothiazide (6-chloro-3,4-dihydro-7-sulfamyl-2H-1,2,4-benzothiadiazine-1,1-dioxide) II, have been widely used as diuretic and antihypertensive agents. These agents have been formulated as single and combination dosage forms.



I II

The quantitation of hydralazine hydrochloride and hydrochlorothiazide has been reported using a number of high performance liquid chromatography procedures (1-5). Most of these procedures describe the determination of hydralazine hydrochloride and/or hydrochlorothiazide in pharmaceutical dosage forms. One procedure (4) was tried for the simultaneous determination of hydralazine hydrochloride and hydrochlorothiazide in a combination dosage form. This procedure required a diphenyldichlorosilane (phenyl) column, and acetonitrile-0.1% ammonium acetate mixture (20:80), pH 7.35 as mobile phase and UV detection at 254 nm.

Although the two components were separated by this procedure (4), hydralazine hydrochloride peak showed significant tailing and upon reanalysis of the same sample after few hours, a significant splitting of this peak was also noted. The USP XXI (6) has

separate monographs on hydralazine hydrochloride and hydrochlorothiazide dosage forms, however, it has a monograph on a combination of reserpine, hydralazine hydrochloride and hydrochlorothiazide dosage forms. The USP XXI procedures make use of high performance liquid chromatography for the determination of hydralazine hydrochloride and hydrochlorothiazide as single drug products. The combination products (reserpine, hydralazine hydrochloride and hydrochlorothiazide) are assayed using three different procedures.

This report describes a method that can be used for the determination of hydralazine hydrochloride and hydrochlorothiazide in combination as well as single drug products.

MATERIALS

Apparatus

A. Liquid Chromatograph - Waters system equipped with 710B autosampler, model 510B pump, Lambda 480 variable wavelength UV detector and Z-Module (all from Waters, Milford, Mass.)

B. Integrator and Recorder - HP3390A integrator (Hewlett-Packard, Palo Alto, CA) and Fisher Recordall®, series 5000 chart recorder (Fisher Scientific Co., Fair Lawn, NJ)

C. Columns - Radialpak cyanopropylsilane cartridge, 10 micron, 10 cm x 8 mm i.d. (Waters, Milford, Mass.)

Chemicals and Reagents

Hydralazine hydrochloride and hydrochlorothiazide were USP reference standards. Methanol (HPLC grade) and dibutylamine phosphate (purchased as D_4 from Waters, Milford, Mass.) were used as received.

METHODS

Mobile Phase

A solution of methanol and water (65:35) was prepared. Ten mL

of 1M dibutylamine phosphate per liter of mobile phase was added as modifier. The mobile phase was vacuum filtered and degassed before use.

Standard Solution

Standard solution containing 0.25 mg/mL of hydralazine hydrochloride and 0.25 mg/mL of hydrochlorothiazide were prepared in methanol for composite assay and content uniformity test. This standard solution can also be used for the assay of dissolution samples by making one to ten dilution with the dissolution medium.

Calibration Standard Solutions (Linearity)

Five solutions containing 50, 75, 100, 125 and 150% of label claim of hydralazine hydrochloride and hydrochlorothiazide standards were prepared in methanol. Duplicate 5-uL aliquots of these solutions were chromatographed to determine the linearity of detector response to concentration.

Analysis of Dosage Forms

Composite Assay - A composite sample of 20 units (tablets or capsules) was finely powdered. A quantity of powdered material equivalent to one dose unit was transferred to a 100 mL volumetric flask. The sample was dissolved in methanol and ultrasonicated for 10 minutes. A portion was filtered through a 0.45 micron porosity membrane filter for chromatographic determination.

Content Uniformity

A single dosage unit (tablet or capsule) was finely powdered, transferred to a 100-mL volumetric flask and dissolved in methanol by ultrasonication for 10 minutes. Samples of 10 dosage units were prepared in this manner. A portion of each sample was filtered through a 0.45 micron membrane filter for chromatographic determination.

Dissolution Test

The dissolution test was performed on 12 units by the procedure described in USP XXI using Method I (baskets) at 100 rpm (7). Each dissolution vessel contained 900 mL, 0.1N HCl kept at 37 ± 0.5°C as dissolution medium. The samples were withdrawn from each vessel after 60 minutes, filtered and analyzed.

Chromatography

System Suitability

With all system components in place and UV-detector set at 254 nm, the degassed mobile phase was passed through the column at a flow rate of 2.0 mL per minute until a stable baseline was obtained. Five replicate injections of 5 uL portions of standard solution were made. The peaks were recorded by an integrator or a chart recorder. The major peaks were completely resolved and symmetrical. The relative standard deviation (RSD) calculated from peak area response of five replicate injections was less than 2.0% for each component.

Procedure

Equal volumes (about 5 uL) of the filtered portions of standard solution and sample solution were chromatographed for composite assay and content uniformity test.

For dissolution test, 50 uL portions of standard and sample solutions were chromatographed.

Calculations

The percent label claim of hydralazine hydrochloride or hydrochlorothiazide was calculated from:

$$\% \text{ Label Claim} = \frac{Au}{As} \times \frac{W(std)}{W(label)} \times 100$$

The percent of each drug dissolved in dissolution test, was determined by the following equation:

$$\% \text{ Dissolved} = \frac{Au}{As} \times \frac{W(std)}{W(label)} \times \text{Volume of dissolution medium} \times 100$$

Where:

Au	=	Peak area response from sample solution
As	=	Peak area response from standard solution
W(std)	=	Weight in milligrams of respective standard
W(label)	=	Label claim in milligrams of respective component

RESULTS AND DISCUSSION

The separation of hydralazine hydrochloride and hydrochlorothiazide is shown in Figure 1. Hydrochlorothiazide elutes at about 1.95 minutes and hydralazine hydrochloride elutes at about 2.70 minutes. The reproducibility of the method is established by the relative standard deviation of less than 2.0% for five replicate injections of standard solution. A typical chromatographic run is complete within 5 minutes. However, by decreasing the amount of methanol in the mobile phase, the chromatographic run time can be increased.

The specificity of the procedure is tested by chromatographing samples of excipient blend and a common impurity in hydrochlorothiazide, 1-amino-3-chloro-4,6- benzenedisulfonamide. Neither the excipient blend nor 1-amino-3-chloro-4,6-benzenedisulfonamide showed any interference.

The standard curves are linear from 0.125 mg/mL to 0.375 mg/mL range with correlation coefficients of 0.999 for each component.

The time required for constant extraction of hydralazine hydrochloride and hydrochlorothiazide from the dosage mass is determined by ultrasonicating a finely powdered representative sample of the formula in methanol for 5, 10 and 15 minutes. In each case, the sample is filtered after the appropriate time and a

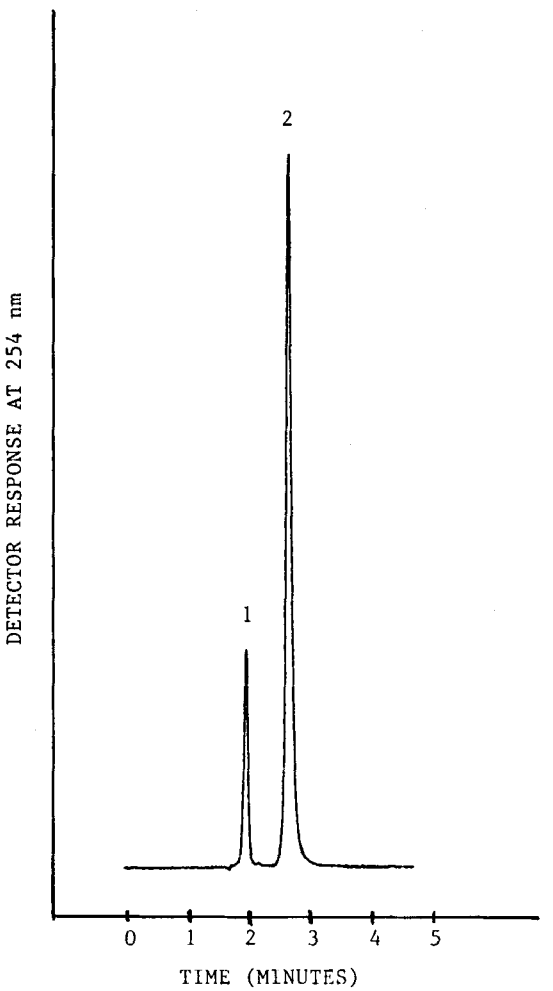


Figure 1. A typical chromatogram from standard solution. Peaks 1 and 2 are from hydrochlorothiazide and hydralazine hydrochloride, respectively.

TABLE 1. Assay Results by Proposed HPLC Method and USP XXI Procedures

	Proposed HPLC Method (n=5)		USP XXI Procedures (n=5)	
	Label, mg	Found % of Label	Label, mg	Found % of Label
Hydralazine Hydrochloride	25	98.9% ± 1.3	25	96.6 ± 2.1
Hydrochlorothiazide	25	98.8% ± 1.1	25	98.5 ± 1.6

TABLE 2. Content Uniformity Results

	Hydralazine Hydrochloride % of Label	Hydrochlorothiazide % of Label
1	102.9	100.9
2	101.6	100.1
3	100.6	102.5
4	99.7	102.1
5	100.8	104.1
6	102.2	102.1
7	101.5	104.6
8	100.2	104.1
9	105.9	106.2
10	103.0	105.0
Mean	101.8	103.2
±SD	1.8	1.9
RSD	1.8	1.8

5 uL portion chromatographed. The peak area comparisons of these samples are identical in all three cases, indicating constant extraction of both compounds within 5 minutes. However, extraction time of 10 minutes is most desirable.

To ascertain the recovery, a commercial batch of hydralazine hydrochloride and hydrochlorothiazide capsules, labeled to contain 25 mg of each drug, is analyzed (n=5) by proposed HPLC method and

TABLE 3. Dissolution Results

	Hydralazine Hydrochloride % of Label Dissolved in 60 Minutes	Hydrochlorothiazide % of Label Dissolved in 60 Minutes
1	93.6	88.1
2	97.5	91.3
3	95.0	89.4
4	93.2	89.2
5	96.9	88.3
6	96.4	89.3
7	93.0	91.6
8	95.5	93.1
9	93.2	90.0
10	97.3	91.8
11	96.9	91.9
12	102.9	97.1
Mean	96.0	90.9
±SD	2.8	2.5
Range	93.0 - 102.9	88.1 - 97.1

by USP XXI method. The results are comparable and are presented in Table 1. The mean recoveries by proposed method, expressed as a percent of the label claim, are 98.9 ± 1.3 and 98.8 ± 1.1 for hydralazine hydrochloride and hydrochlorothiazide, respectively.

The method is used to determine the content uniformity of capsules. The content uniformity results of one commercial batch are summarized in Table 2.

The dissolution results are presented in Table 3. It is important to point out that hydralazine hydrochloride peak invariably showed band broadening and splitting when dissolution samples were pulled using syringes with steel cannulas. It is speculated that in presence of acidic dissolution medium, hydralazine hydrochloride probably forms a metal complex while passing through steel cannulas into the syringe. This problem is

not investigated fully; instead, the glass pipets are used for withdrawing the dissolution samples with success.

The proposed method is applicable to the determination of hydralazine hydrochloride and hydrochlorothiazide in combination as well as single component dosage forms. This method can also be successfully used in the analysis of hydralazine hydrochloride and hydrochlorothiazide portions of reserpine, hydralazine hydrochloride and hydrochlorothiazide combination dosage forms, since reserpine did not show any interference. The proposed method may be used for stability assays since hydrochlorothiazide impurity, 1-amino-3-chloro-4,6-benzenedisulfonamide and formulation excipients did not interfere with the peaks of interest. However, the method is most suitable for quality control analysis of such combination products because of its fast, accurate and reproducible features and also its compatibility of results with USP XXI procedures.

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