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Note

Quantitation of hydralazine hydrochloride in pharmaceutical dosage forms by high-performance liquid chromatography

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Hydralazine hydrochloride is a widely prescribed anti-hypertensive[1]. Several high-performance liquid chromatographic (HPLC) methods have been developed, which utilize the derivatization product of hydralazine with *p*-hydroxybenzaldehyde[2] or *p*-anisaldehyde[3–5]. Derivatization methods are generally time consuming and less precise. A method which could chromatograph hydralazine hydrochloride underivatized would be preferable. Only a few HPLC methods have been found in the literature, which chromatograph hydralazine hydrochloride in the underivatized form[6–8]. Two of these methods[6,7] were not validated as stability indicating. For our purposes, stability-indicating methods are defined as those which are capable of monitoring peak decomposition. The other method[8] was an ion-exchange HPLC procedure, which is less commonly used than reversed-phase HPLC[9].

This note describes a reversed-phase HPLC method for the quantitative analysis of hydralazine in pharmaceutical dosage forms. The method is precise, accurate, and stability indicating.

EXPERIMENTAL

Materials

Hydralazine hydrochloride reference standard was obtained from USP (Rockville, MD, U.S.A.) and phthalazine from Aldrich (Milwaukee, WI, U.S.A.). The methanol, acetic acid, and water were HPLC grade.

Equipment

Two separate liquid chromatographs were used in the analyses. Both systems contained a WISP Model 710B (Waters, Milford, MA, U.S.A.) and a Column Compartment Oven Model 860 (Dupont, Wilmington, DE, U.S.A.). The system used for most of the experimentation utilized a reciprocating pump Model 590 (Waters) and a Spectroflow UV–VIS absorbance detector Model 757 (Kratos, Ramsey, NJ, U.S.A.). The second system was used for the interlaboratory evaluation of the method. This system utilized a reciprocating pump Model 510 and a UV–VIS absorbance detector Model 440 (both from Waters). The hydralazine capsule powder was stressed in a Sunlighter 150 (Engler, Jersey City, NJ, U.S.A.) to generate deg-

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radation products for a proof of method specificity. All data were collected and processed by a Model 3357 laboratory data system (Hewlett-Packard, Sunnyvale, CA, U.S.A.). Spectral scans of chromatographic peaks were done "on the fly" with a 1040A photo diode array detector (Hewlett-Packard).

HPLC method

The chromatographic parameters were as follows; analytical column: μBondapak Phenyl, 10 μm (Waters) 30 cm × 3.9 mm I.D., mobile phase: methanol–2% acetic acid solution (60:40, v/v); column temperature: 35°C; flow-rate: 1.0 ml/min; wavelength: 295 nm; injection concentration: 20 μg/ml; injection volume: 50 μl.

Standard preparation

Approximately 20 mg of hydralazine hydrochloride reference standard was accurately weighed into a 10-ml volumetric flask. Methanol was used to dissolve and dilute the sample to volume. A 1.0-ml aliquot was transferred to a 100-ml volumetric flask and diluted to volume with water. This solution was used for injection.

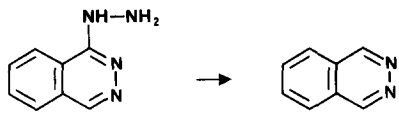
Sample preparation

A number of capsule contents were quantitatively transferred to an appropriate volumetric flask, such that the theoretical concentration of hydralazine hydrochloride in the solution was about 1 mg/ml. About 2/3 volume of methanol was added. The sample was sonicated 5 min. This was followed by 10 min of mechanical shaking. The sample was then diluted to volume with methanol and centrifuged. A 1.0-ml aliquot was transferred to a 50-ml volumetric flask and diluted to volume with water. This solution was used for injection. (This sample preparation was used for hydralazine hydrochloride capsules. Other dosage forms such as tablets or solutions could be used with the appropriate modifications.)

RESULTS

Specificity

Hydralazine hydrochloride was shown to separate from its primary breakdown product (phthalazine):



hydralazine phthalazine

A chromatogram of this separation is shown in Fig. 1. Hydralazine capsule powder was extracted into methanol and exposed to intense light for one week. Several degradation products formed and they were all separated from the hydralazine peak (Fig. 2). As illustrated, the retention time of the hydralazine peak in Fig. 2 (7.4 min) was greater than that in Fig. 1 (5.5 min). Presumably, this difference in retention time was due to column ageing, as all operating conditions for both Figs. 1 and 2

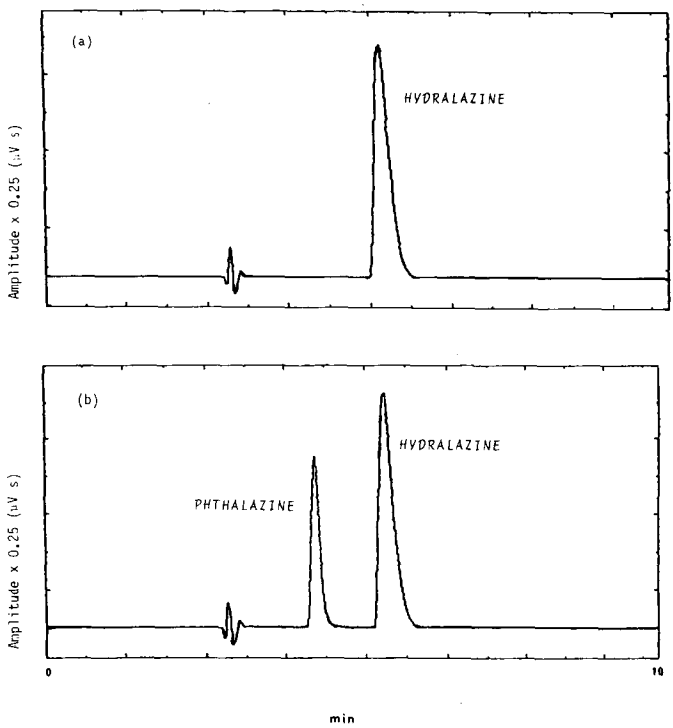


Fig. 1. (a) Chromatogram of hydralazine hydrochloride in water. (b) Chromatogram of hydralazine hydrochloride spiked with phthalazine (in water).

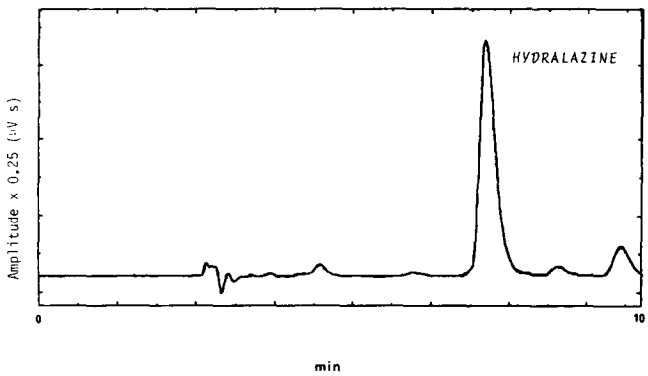


Fig. 2. Chromatogram of hydralazine capsule powder extracted into methanol and exposed to intense light one week.

were identical. The identity and purity of the hydralazine peak were ascertained by spectral overlay using a photo diode array detector (Fig. 3).

Linearity

The linearity of the calibration curve was investigated over the hydralazine

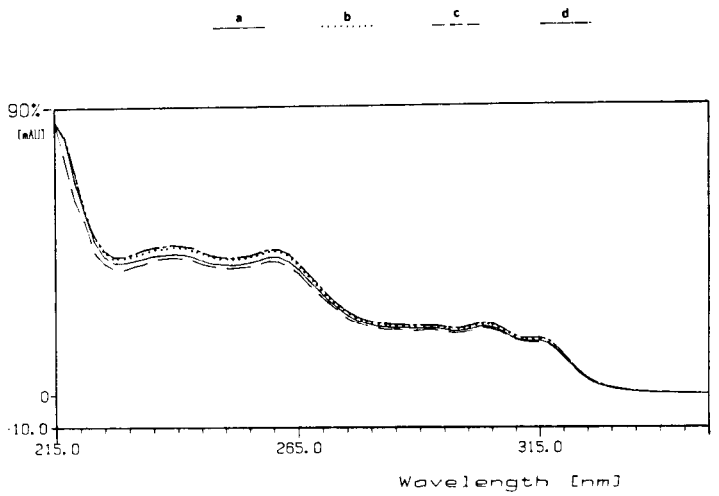


Fig. 3. UV spectra of hydralazine hydrochloride reference standard (d), and of upslope (a), apex (b), and downslope (c) of hydralazine peak shown in Figure 2.

hydrochloride concentration range of 9.7 to 29.1 μg/ml. The following results were obtained; correlation coefficient = 0.999, y-intercept = −1740, slope = 2686.

Precision

The precision of the method was demonstrated by replicate assays performed by two individual laboratories. Both laboratories reported a relative standard deviation (R.S.D.) of 0.8% on five replicates each.

Accuracy

Standard addition was used as a means of accuracy. The powder from the dosage form was spiked with accurately weighed amounts of hydralazine hydrochloride standard material (10%, 20%, 30% over claim). A recovery of 100.9% with an R.S.D. of 0.8% was achieved (Table I).

TABLE I

RESULTS OF THE STANDARD ADDITION EXPERIMENTS

Amount of hydralazine hydrochloride per capsule (mg)		*Recovery (%)*
Theoretical (including spike)	*Actual*	
11.49	11.54	100.4
11.20	11.32	101.1
12.19	12.20	100.1
12.28	12.29	100.1
13.20	13.38	101.4
13.14	13.42	102.1
Mean		100.9
R.S.D. (%)		0.8

DISCUSSION

From the data shown, the method described above for the quantitation of hydralazine hydrochloride is specific, accurate, precise, and easy to use. Derivatives of hydralazine used in other methods mentioned in this paper are perhaps more stable than hydralazine itself. However, we found that if hydralazine was extracted from the dosage form in methanol and diluted with water within 2 h, no significant degradation occurred. Solutions of hydralazine hydrochloride in water are stable for several days[10]. In clinical and stability studies where multiple sample assays are needed, this method is adequate and more convenient than derivatization procedures.

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