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Colorimetric determination of hydralazine hydrochloride with 9-methoxyacridine

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Summary

A colorimetric assay for hydralazine hydrochloride based on the interaction of the drug with 9-methoxyacridine is described. The highly colored substituted 9-aminoacridine product shows an absorption maximum at 455 nm. Color development is affected by time of heating or standing at ambient temperature and by the quantity of acridine reagent used. The method will determine hydralazine in the 0.1–12 μg/ml range with good accuracy and precision. It can be used to analyze for hydralazine hydrochloride concentration in pharmaceutical dosage forms.

Introduction

The interaction of hydralazine hydrochloride, a widely prescribed antihypertensive agent, with 9-methoxyacridine to give a highly colored substituted 9-aminoacridine has been observed in this laboratory. This led to the development of a colorimetric method which can be utilized to determine hydralazine hydrochloride in pharmaceutical dosage forms.

Existing analytical methods for hydralazine include titrimetry (United States Pharmacopeia XX, 1980; Goryacheva and Prikhodkina, 1968, Perelman and Evstratova, 1963), polarography (Modras, 1973), and spectrophotometry (Solomonova et al., 1973; Schulert, 1961; Zak et al., 1974), fluorometry (Naik et al., 1976), and gas and high-performance liquid chromatography (Jack et al., 1975; Smith et al., 1977; Proveaux et al., 1979; Ludden et al., 1979). Many of the spectrophotometric methods used reagents such as tetrazolium chloride, ninhydrin or aldehydes to produce derivatives that absorbed in the visible spectrum. These

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procedures were subject to such problems as inadequate sensitivity and precision, long derivatization reaction times, multiple extraction and solvent evaporation steps, and multiple pH adjustments. The fluorometric assay was based on generation of the hydralazine fluorophore in concentrated sulfuric acid. It did not, however, provide a significant increase in sensitivity compared to the spectrophotometric methods. The gas and high-performance liquid chromatographic methods offer ng/ml sensitivity and the advantage of separation of hydralazine and its metabolites especially in biological samples.

This laboratory (Stewart and Chang, 1979) has reported on a colorimetric assay for hydralazine hydrochloride that was based on derivative formation with 9-chloroacridine. The method was not only comparable in sensitivity to the better spectrophotometric procedures, but it was more accurate and precise and involved fewer manipulative steps. A few problems were encountered with the assay that needed improvement. The acridine stock solution was only stable for approximately 12 h and it was desirable to decrease the 1 h heating time needed for maximum color development. An investigation into these problems led us to examine the usefulness of 9-methoxyacridine as a suitable alternative reagent.

This paper describes an improved colorimetric method for determining microgram quantities of hydralazine hydrochloride with 9-methoxyacridine. A stock solution of the methoxyacridine is stable for at least a week without evidence of decomposition and maximum color development requires only 5 min at 50°C (or 15 min at ambient temperature). The procedure has been successfully applied to the analysis of hydralazine hydrochloride in pharmaceutical dosage forms.

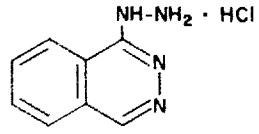
Materials and Methods

Apparatus

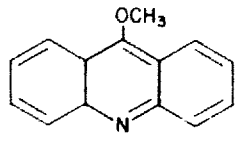
Spectra and absorbance measurements were made in a Bausch and Lomb Model 2000 spectrophotometer using matched cells with a 1-cm optical path. An American Optical shaking water bath (Model 02156) was also used in the analytical procedure.

Reagents and chemicals

9-Methoxyacridine was synthesized and recrystallized (mp 103°C) (Barber et al.,



HYDRALAZINE HYDROCHLORIDE



9-METHOXYACRIDINE

1947). Hydralazine hydrochloride, hydrochlorothiazide and reserpine powders were obtained from Ciba-Geigy, Summit, NJ, U.S.A. Propranolol hydrochloride powder was supplied by Ayerst Laboratories, New York, NY, USA.

Preparation of stock solutions

Stock solutions were prepared by dissolving weighed quantities of each drug powder and 9-methoxyacridine in absolute methanol with appropriate dilutions where necessary. The resulting concentrations were: hydralazine hydrochloride (0.2 mg/ml), hydrochlorothiazide (0.3 mg/ml), reserpine (0.06 mg/ml), propranolol hydrochloride (0.26 mg/ml) and 9-methoxyacridine (10 mg/ml).

Assay procedure

A quantity (5–600 μl) of the methanolic hydralazine hydrochloride solution (0.2 mg/ml) is placed in a 10-ml volumetric flask. 9-Methoxyacridine stock solution (200 μl) is added to the flask and the mixture heated in a shaking water bath at 50 ± 1°C for 5 min (or shaken at ambient temperature for 15 min). Upon cooling, absolute methanol is added to volume, the solution poured into a cuvette, and the absorbance measured at 455 nm. A blank determination is run concurrently.

Analysis of solid dosage form

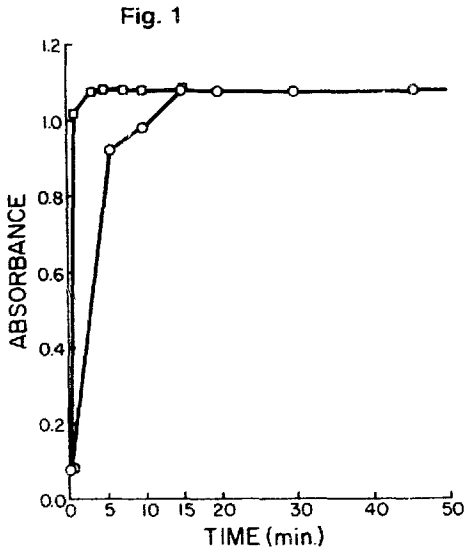
Tablets were dissolved with the aid of sonication in absolute methanol. In the case of capsules, the powdered contents were dissolved in absolute methanol with sonication, where necessary. The resulting solutions were filtered and diluted with methanol to give 0.2 mg/ml solutions of hydralazine hydrochloride. Microliter aliquots of the methanolic extract were then assayed according to the Assay Procedure above.

Results and Discussion

9-Methoxyacridine interacts with hydralazine hydrochloride in the analytical procedure to yield a highly colored yellow solution. The absorption curve in the visible spectrum for an analytical solution of the drug shows an absorption maximum at 455 nm.

The interaction between the acridine and drug is affected by time of heating or standing at ambient temperature and the quantity of acridine reagent utilized. When the analytical solutions are heated at 50 ± 1°C for a minimum of 5 min or allowed to stand at ambient temperature for at least 15 min, maximum absorbance readings are obtained (Fig. 1).

The intensity of the color produced is also directly affected by the concentration of methoxyacridine employed. Higher absorbance values were obtained up to a 16–20-fold molar excess of the reagent. Quantities of the acridine beyond this excess did not effectively change the absorbance readings for the drug as shown in Fig. 2. Under the conditions stated in the procedure, the color is stable for at least 1 day after development. In addition, the reagent blank is colorless.



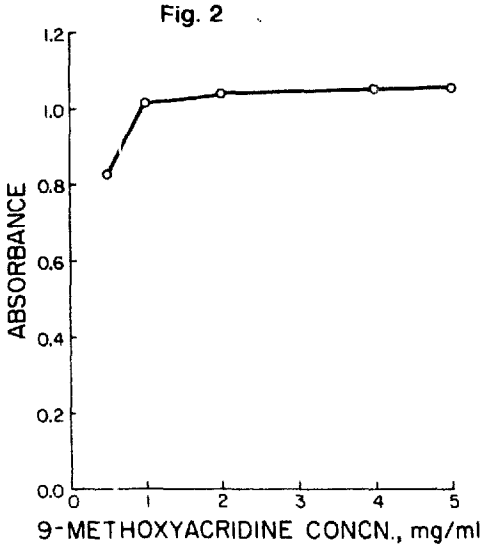


Fig. 1. Effect of time of heating or time at ambient temperature on color development. Key: □, shaking water bath at 50 ± 1°C; ○, ambient temperature. The final hydralazine and 9-methoxyacridine concentrations were 10 and 200 μg/ml, respectively.

Fig. 2. Effect of 9-methoxyacridine concentration on color development. The final hydralazine concentration was 10 μg/ml. Each analytical solution was heated in a shaking water bath at 50 ± 1°C for 5 min.

A typical standard curve for hydralazine hydrochloride was prepared in the 0.1–12 μg/ml range at 455 nm. Linear regression analysis of absorbance versus drug concentration gave a typical slope 0.096, intercept 0.005, and correlation coefficient (r) 0.9993 (n = 22). Table 1 shows recovery and precision data for various hydralazine concentrations run as "unknowns".

Data in Table 2 from several drug mixtures containing hydralazine and other drugs which are either present in its commercial dosage forms or used in concurrent therapy reveal that the procedure shows good specificity for hydralazine. Other data generated in this laboratory revealed that reserpine will interfere with the assay if it is present in a molar concentration greater than one-tenth that of the hydralazine concentration.

TABLE 1

ANALYSIS OF SPIKED HYDRALAZINE HYDROCHLORIDE SAMPLES BY THE 9-METHOXYACRIDINE METHOD

Final conc. (μg/ml)	Conc. found (μg/ml)	Recovery (%)	RSD (%)
0.16	0.158 ± 0.005 [a]	98.8	3.29
0.50	0.501 ± 0.007	100.2	1.41
1.00	0.999 ± 0.007	99.9	0.69
2.00	2.023 ± 0.007	101.1	0.35
4.00	3.99 ± 0.007	99.8	0.17
6.00	6.014 ± 0.009	100.2	0.15

[a] Mean ± standard deviation based on triplicate determinations of each concentration.

TABLE 2

ANALYSIS OF HYDRALAZINE HYDROCHLORIDE MIXTURES FOR HYDRALAZINE HYDROCHLORIDE

Mixture	Components [a]	Hydralazine hydrochloride	
		Recovery (%)	RSD (%)
1	Hydralazine hydrochloride + reserpine [c]	99.00 ± 0.70 [b]	0.70
2	Hydralazine hydrochloride + hydrochlorothiazide [d]	99.38 ± 0.48	0.48
3	Hydralazine hydrochloride + propranolol hydrochloride [d]	99.18 ± 0.79	0.80
4	Hydralazine hydrochloride + reserpine [c] + hydrochlorothiazide [d]	99.87 ± 0.40	0.40

[a] The final concentration of hydralazine hydrochloride in each mixture was 10 μg/ml.
[b] Mean ± standard deviation based on quadruplicate determination of each mixture.
[c] The reserpine concentration used was 1/10 molar with respect to hydralazine hydrochloride.
[d] The concentration used was equimolar with respect to hydralazine hydrochloride.

Results obtained by applying the assay procedure to commercial dosage forms containing hydralazine hydrochloride are shown in Table 3. The data indicate that the method can be successfully used to analyze for hydralazine concentration in these kinds of samples with good recovery and precision.

The color produced from the interaction of hydralazine with 9-methoxyacridine suggests that a primary amino function in a drug molecule is necessary for the reaction. This finding is in agreement with Barber et al. (1947) who showed that 9-alkoxyacridines react with primary amine salts under relatively mild conditions to product substituted 9-aminoacridines in good yield. It has also been suggested that the reaction proceeds more rapidly with an amine salt than its free base. It should be possible, therefore, to utilize this analysis procedure for any primary amine-containing compound preferably in the salt form.

TABLE 3

ANALYSIS OF HYDRALAZINE HYDROCHLORIDE IN COMMERCIAL DOSAGE FORMS BY THE 9-METHOXYACRIDINE METHOD

Dosage form	Components [a]	Hydralazine found (mean % of labeled amt. [b])	RSD (%)
Tablet	Hydralazine hydrochloride	99.87 ± 1.65	1.66
Capsule	Hydralazine hydrochloride + hydrochlorothiazide [c]	100.25 ± 0.30	0.30
Tablet	Hydralazine hydrochloride + reserpine [d]	100.67 ± 0.84	0.83
Tablet	Hydralazine hydrochloride + hydrochlorothiazide [c] + reserpine [d]	103.27 ± 0.23	0.22

[a] The labeled amount of hydralazine hydrochloride in each dosage form was 25 mg.
[b] Mean ± standard deviation based on 2–4 determinations of each dosage form.
[c] Labeled amount was 25 mg.
[d] Labeled amount was 0.1 mg.

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