Fluorescence of Hydralazine in Concentrated Sulfuric Acid

DATTA V. NAIK, BRIEN R. DAVIS, KATHLEEN M. MINNET, and STEPHEN G. SCHULMAN ˣ

Abstract □ Hydralazine shows intense fluorescence at 353 nm in concentrated sulfuric acid when excited at 320 nm. This fluorescence can be utilized for the quantitative analysis of the drug in dosage forms.

Keyphrases □ Hydralazine—fluorometric analysis, dosage forms □ Fluorometry—analysis, hydralazine in dosage forms

Hydralazine (1-hydrazinophthalazine) has been used in the treatment of hypertension for many years. It apparently lowers blood pressure by inhibiting the enzymes responsible for the decarboxylation of amino acids to pressor amines and exerts a peripheral vasodilating effect through a direct relaxation of vascular smooth muscle.

Most methods for hydralazine analysis are spectrophotometric and are based on (*a*) its UV absorption in the 260–270-nm region (1), (*b*) condensation of carbonyl compounds with the reactive hydrazino group to yield hydrazone derivatives (2–8), or (*c*) the complexing and reducing property of hydralazine (9–11). The spectrophotometric method of analysis based on the reaction of hydralazine with ninhydrin (5) gave incomplete recovery and variable results (6), so an alternative method was developed based on the condensation of hydralazine with *p*-hydroxybenzaldehyde. This procedure was modified later to obtain reproducible results (7).

Recently, Zak *et al.* (8) found that neither the original (6) nor the modified (7) method afforded satisfactory reproducible results. They developed a new spectrophotometric method based on the reaction of hydralazine with *p*-methoxybenzaldehyde. In this procedure, the absorbance values were proportional to the concentration of hydralazine in the 0.1–5.0-μg/ml range, but the derivatization reaction required 30 min at elevated temperature for completion.

To date, no fluorometric method for hydralazine analysis has been reported. Because of the usual sensitivity and simplicity of fluorescence methods, the present study was undertaken to develop such a method for hydralazine.

EXPERIMENTAL

Reagents and Equipment—The pH measurements were made on a pH meter[1] with a combination silver–silver chloride glass electrode. Electronic absorption spectra were taken in 1-cm silica cells on a grating-type spectrophotometer[2]. Fluorescence spectra and intensity measurements were taken on a fluorescence spectrophotometer[3] whose monochromators were calibrated against the xenon line emission spectrum and whose output was corrected for instrumental response by means of a rhodamine-B quantum counter.

Hydralazine hydrochloride[4] was purified by recrystallization from 95% ethanol. Sulfuric acid (18 *M*) and sodium hydroxide were of analytical grade[5], and anhydrous methanol[5] was of spectroscopic quality. Formaldehyde solution[6], acetaldehyde[7], and benzaldehyde[6] were used as supplied. Sulfuric acid solutions were prepared by dilution with distilled deionized water, and the corrected Hammett acidity scale of Jorgenson and Hartter (12) was employed to calibrate these solutions. Solutions in the 0–3 pH region were prepared by appropriate dilution of 1 *N* sulfuric acid. Trace amounts of the acid or sodium hydroxide were used to adjust the pH of solutions in the pH 4–10 region. Solutions with higher pH were obtained by appropriate dilution of 1 *N* NaOH.

Standard Curves—A 1.0×10^{-2} *M* stock solution of pure hydralazine hydrochloride was prepared in 50% methanol. Appropriate dilutions of the stock solution were made with 50% methanol to give a range of concentrations. For the fluorescence standard curve, 0.1 ml of hydralazine hydrochloride solution of the appropriate concentration (concentration = 100 times the final desired concentration) was delivered to a 10-ml glass-stoppered volumetric flask, which was then filled to the mark with concentrated sulfuric acid.

The acid solution was shaken and transferred to a stoppered 1-cm² silica cell, and its fluorescence intensity was measured at 353 nm with the excitation monochromator set at 320 nm. The background intensity due to concentrated sulfuric acid alone was also measured and subtracted from the intensity obtained for the drug solution. The corrected intensities for different concentrations were plotted against the respective concentrations.

For the absorption (UV) standard curve, 0.1 ml of hydralazine hydrochloride solution of the appropriate concentration (concentration = 100 times the final desired concentration) was delivered to a 10-ml glass-stoppered volumetric flask. The flask was then filled to the mark with 50% methanol. The absorption of this solution at 270 nm was measured with 50% methanol as a reference. The absorption of several standard solutions were measured and plotted against their respective concentrations.

Tablet Extraction and Analysis—For individual tablet analysis, a tablet of hydralazine[8] was crushed in a glassine paper and the powder was quantitatively transferred to a 250-ml erlenmeyer flask. Exactly 100 ml of 50% methanol then was added, and the flask was shaken in an ultrasonic bath for 20 min to effect the dissolution of hydralazine hydrochloride. The solution was filtered through a sintered-glass funnel, and the first 5 ml of the filtrate was discarded. The filtrate thus obtained for a tablet whose labeled content is 50 mg of the drug would be 0.5 mg/ml.

For fluorometric analysis, 0.1 ml of the tablet extract was transferred to a 10-ml glass-stoppered volumetric flask, which was then filled to volume with concentrated sulfuric acid. The fluorescence intensity of the acid solution was measured in the same way as for the standard solutions. For absorptiometric analysis, 0.5 ml of the tablet extract was transferred to a 25-ml glass-stoppered volumetric flask, which was then filled to volume with 50% methanol. The absorption of this solution at 270 nm was measured with 50% methanol as a reference.

Analysis of Hydralazine Hydrochloride Injection—Exactly 0.5 ml of hydrazine hydrochloride injection[8] with a labeled content of 20 mg was transferred to a 25-ml volumetric flask and diluted to

[1] Research model 12-B, Corning Scientific Instruments, Corning, N.Y.
[2] Model DB-GT, Beckman Instruments, Fullerton, Calif.
[3] Model MPF-2A, Perkin-Elmer Corp., Norwalk, Conn.
[4] Pfaltz & Bauer, Flushing, N.Y.
[5] Mallinckrodt Chemical Works, St. Louis, Mo.
[6] J. T. Baker Chemical Co., Phillipsburg, N.J.
[7] Matheson, Coleman & Bell, East Rutherford, N.J.
[8] Apresoline, 50 mg, Ciba-Geigy Corp.

volume with 50% methanol. The expected concentration of this solution would be 0.4 mg/ml. For fluorometric analysis, 0.1 ml of the solution was added to a 10-ml glass-stoppered volumetric flask, which was then filled to the mark with concentrated sulfuric acid. The intensity measurements on the acid solution were carried out in the same way as for the acid solution of the tablet extract. For absorptiometric analysis, 0.5 ml of the solution of the injection was transferred to a 25-ml glass-stoppered volumetric flask, which was then filled to volume with 50% methanol. The absorption of this solution was measured at 270 nm with 50% methanol as a reference.

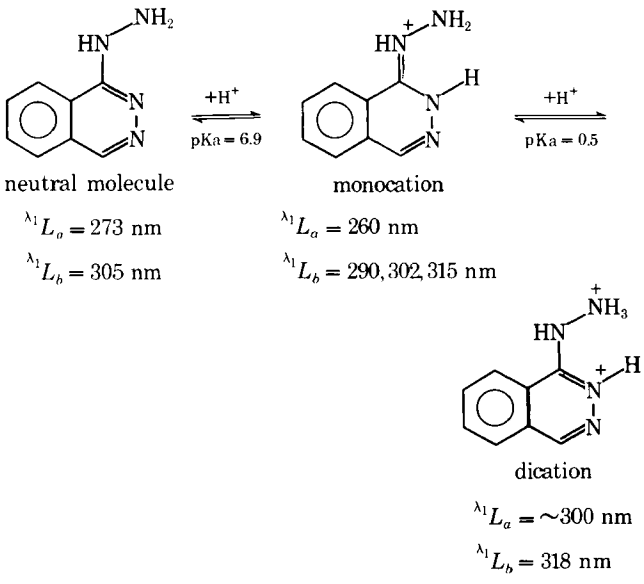
The background fluorescence intensity near 350 nm largely depends on the concentration of sulfuric acid because of a fluorescing impurity in the latter. This impurity is present in the analytical reagent grade sulfuric acid from essentially all commercial suppliers. Moreover, the sulfuric acid concentration affects the amount of fluorophore produced (Fig. 2); therefore, to avoid absorption of atmospheric moisture by the acid, it should be kept tightly stoppered in a dry place. When a fresh bottle of sulfuric acid is used or if the concentration of sulfuric acid is suspected to have changed, a new standard curve should be prepared. It is desirable to carry out the intensity measurement on at least one standard solution (preferably having a final concentration close to that of the unknown solution) of the drug at the same time that the unknowns are measured, so as to check any variation in the concentration of sulfuric acid.

RESULTS AND DISCUSSION

Since hydralazine does not fluoresce in aqueous solution in the pH 0–14 range, an attempt was made to make a fluorescent derivative of hydralazine. First, the benzaldehyde hydrazone of hydralazine was prepared by condensation of hydralazine with benzaldehyde in water. The product obtained had fluorescence at 358 nm when excited at 296 nm. However, the reaction was very slow, was dependent on the initial concentration of hydralazine in the reaction mixture, and required the use of heat for completion in a short time. Therefore, it was unsatisfactory for analytical use.

Next, the acetaldehyde hydrazone was prepared by condensation of hydralazine with acetaldehyde using a 1% aqueous solution of the latter. The product obtained *in situ* had fluorescence at 410 nm when excited at 320 nm. The reaction could be taken to completion with concentrations of hydralazine as low as 2 μg/ml by heating at 70° for 20 min. The fluorophore obtained was stable for 24 hr. Fluorescence intensity varied linearly with the concentration of hydralazine over the 2–10-μg/ml range. However, the main disadvantage of this procedure was the instability of the aqueous acetaldehyde solution, whose concentration was found to affect the sensitivity of the analysis.

An attempt was made to prepare the formaldehyde hydrazone of hydralazine. When hydralazine was directly reacted with a 10% aqueous solution of formaldehyde at room temperature, no fluorescence resulted. On heating the reaction mixture, the hydrazone was generated and showed a broad fluorescence band at 345 nm when excited at 275 nm. However, the reaction did not go to completion even after heating at 80° for 40 min. The fluorescence intensity kept increasing with the amount of heat, which made it unsuitable for analytical use.



Scheme I

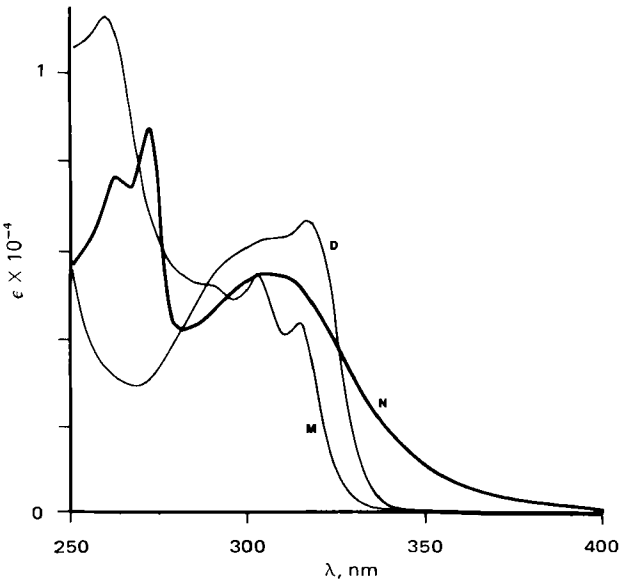


Figure 1—*Absorption spectra of hydralazine (1 × 10⁻⁴ M). Key: N, neutral; M, monocation; and D, dication.*

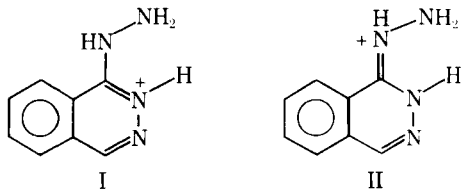
Hydralazine has four basic nitrogen atoms. However, only two of them show protolytic equilibria, involving three distinct species, in the acidity range of −10–10. These species, along with their ^1L_a and ^1L_b absorption bands, and the pKa values corresponding to their interconversion are presented in Scheme I. The absorption spectra of the neutral molecule, monocation, and dication are shown in Fig. 1.

The neutral molecule and doubly protonated cation yield broad long wavelength absorption bands with blurred vibrational structure. The spectral features of these species, given in the scheme, correspond to the band maxima. The monocation, however, exhibits some vibrational structure in the absorption band, but the most distinct and intense vibrational features of the absorption bands are given. The ground-state pKa's were determined absorptiometrically.

The ^1L_b band in hydralazine is broad and occurs in the same spectral region as in phthalazine (13). However, the ^1L_a band moves from 260 nm in the latter to 273 nm in the former. This difference indicates that the substitution of the hydrazino group in the 1-position does not affect the ^1L_b band of phthalazine but, as expected, shifts the ^1L_a band to a longer wavelength due to the charge transfer from the hydrazino group to the ring.

In the first protonation, the proton undoubtedly goes on the more basic ring nitrogen. In the case of 1-hydroxy- and 1-mercaptophthalazine, Albert and Barlin (14) showed that the first proton goes on the ring nitrogen in the 2-position of phthalazine. In hydralazine, however, the positive charge can be located at the protonated ring nitrogen (I) or at the imino nitrogen of the hydrazino group (II).

If the positive charge was on the ring nitrogen, a red shift in both the ^1L_b and ^1L_a bands would be expected. Alternatively, if the positive charge resided on the amino nitrogen of the hydrazino



I II

Table I—Results of Analysis of Commercial Hydralazine Hydrochloride Preparations

	Labeled Contents, mg	Fluorometric Method		UV Absorption Method (NF XIII)	
		Average Found[a], mg	%	Average Found[a], mg	%
Hydralazine hydrochloride[b] tablet	50	49.2 ± 2	98.2 ± 4	48.8 ± 2	97.6 ± 4
Hydralazine hydrochloride[c] injection (0.5 ml analyzed)	20	20.0 ± 0.5	100 ± 2.5	20.2 ± 0.5	101 ± 2.5

[a] Based on five samples from a 10-tablet or a 10-injection batch. [b] Apresoline, Lot 10064. [c] Apresoline, Lot 37022-402.

group, the ^1L_a band should show a blue shift because the conjugation of the hydrazino group with the ring is less extensive in the excited state than in the ground state of the monocation. The observed blue shift of 13 nm in the ^1L_a on the first protonation of hydralazine indicates that II is the more plausible representation of the monocation. A similar amidine-type structure was also suggested for 2-aminoquinoline (15).

In the second protonation, which would be the protonation of the terminal nitrogen of the hydrazino group, there is no ambiguity about the location of the two positive charges, and the dication can be represented as shown in Scheme I. Just as in the case of the protonation of quinoline, the second protonation of hydralazine causes the ^1L_a band to red shift and overlap the ^1L_b band (Fig. 1). The red shift, which is anomalous for the protonation of the hydrazino group (16), is caused by the restoration of the aromaticity of the heterocyclic ring by partial withdrawal of the lone pair of the hydrazino group due to attraction by the positive charge on the terminal nitrogen of the same group.

No fluorescence could be detected from the neutral molecule or the singly protonated species. However, a slight fluorescence was detected in the hydralazine solution of Hammett acidity of −5. Therefore, a fluorometric titration in the Hammett region was carried out, and the titration curve is shown in Fig. 2. Since the absorption spectra showed no change in very concentrated sulfuric acid, the fluorescence must occur as a result of an excited-state protolytic process. The fluorescence band at 353 nm is thus due to either the doubly protonated hydralazine, which is more acidic in the excited state, or a triply protonated hydralazine, which is more basic in the excited state. Which one of the two species is responsible for fluorescence at 353 nm cannot be ascertained at this time.

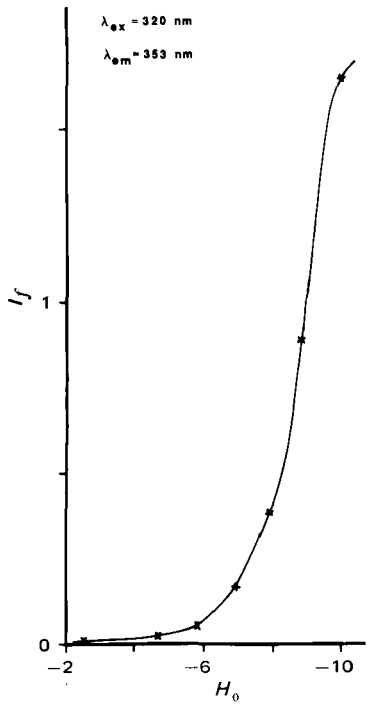


Figure 2—*Relative fluorescence intensity,* I_f, versus *the Hammett acidity of the hydralazine solutions* (1×10^{-4} M).

The fluorophore can be generated instantly in concentrated sulfuric acid; the amount of the fluorophore produced and, hence, the fluorescence intensity at 353 nm directly depend on the concentration of sulfuric acid (Fig. 2). The fluorescence is quite strong at Hammett acidity of −10, and the intensity remains unchanged at least for 24 hr. Heating the acid solution of hydralazine followed by cooling at room temperature did not alter the fluorescence intensity. Strict temperature control of the sample compartment was not necessary because of the insensitivity of the fluorescence intensity to small changes in the temperature of the solution.

The relative fluorescence intensity of the acid solutions varied linearly with the concentration of hydralazine hydrochloride in the 2–8-μg/ml range. The limit of detection, based on the amount of hydralazine hydrochloride required to yield a fluorescent intensity greater than twice that of the background, was 0.15 μg/ml.

Table I shows the results of analysis of hydralazine hydrochloride tablets and injections. The method is very reproducible, and the agreement between the analyses of several tablets and injections is excellent. To confirm the results, the same tablets or injections were analyzed by the UV absorption method similar to that recommended in NF XIII (1) (Table I). The agreement between the fluorometric method and the UV method is excellent.

This method of analysis is very simple and is the first reported method using a fluorescence technique. It is relatively fast and involves only one easily available reagent. This fluorometric method should be useful for doing fast assays on small quantities of hydralazine.

REFERENCES

(1) "The National Formulary," 13th ed., Mack Publishing Co., Easton, Pa., 1970, p. 349.

(2) R. Ruggieri, *Farmaco, Ed. Prat.*, **11**, 571(1956).

(3) B. Wesley-Hadzija and F. Abaffy, *Croat. Chem. Acta*, **30**, 15(1958).

(4) G. I. Luk'yanchikova, *Byul. Izobret., No. 14*, **1962**, 44.

(5) H. M. Perry, H. A. Schroeder, and J. D. Morrow, *Amer. J. Med. Sci.*, **228**, 405(1954).

(6) A. R. Schulert, *Arch. Int. Pharmacodyn. Ther.*, **13**, 420(1972).

(7) R. Zacest and J. Koch-Weser, *Clin. Pharmacol. Ther.*, **13**, 420(1972).

(8) S. B. Zak, M. F. Bartlett, W. E. Wagner, T. G. Gilleran, and G. Lukas, *J. Pharm. Sci.*, **63**, 225(1974).

(9) S. Fallab and H. Erlenmeyer, *Helv. Chim. Acta*, **45**, 1957(1962).

(10) P. Cooper, *Pharm. J.*, **177**, 495(1956).

(11) T. Urbanyi and A. O'Connell, *Anal. Chem.*, **44**, 565(1972).

(12) M. J. Jorgenson and D. R. Hartter, *J. Amer. Chem. Soc.*, **85**, 878(1963).

(13) S. F. Mason, *J. Chem. Soc.*, **1962**, 493.

(14) A. Albert and G. B. Barlin, *ibid.*, **1962**, 3129.

(15) P. J. Kovi. A. C. Capomacchia, and S. G. Schulman, *Anal. Chem.*, **44**, 1611(1972).

(16) A. Albert and G. Catterall, *J. Chem. Soc., C*, **1967**, 1533.

ACKNOWLEDGMENTS AND ADDRESSES

Received September 30, 1974, from the *College of Pharmacy, University of Florida, Gainesville, FL 32610*

Accepted for publication May 28, 1975.

[x] To whom inquiries should be directed.