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Colorimetric method for the determination of hydralazine hydrochloride in pharmaceutical formulations using vanillin as chromogen

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Abstract

A colorimetric method was developed for the quantitative determination of hydralazine hydrochloride in dosage forms. The method was based on the formation of a hydrazone following a coupling reaction between hydralazine and vanillin in acidic condition and the spectrophotometric determination at the absorption maximum. The hydrazone has an absorption maximum (λ_{max}) at 390 nm with a molar absorptivity of 1.429×10^4 L mol $^{-1}$cm^{-1}. Beer's law was obeyed in the concentration range of 4-20 µg/mL at the wavelength of maximum absorption. The reaction product was stable for at least 24 hours. Overall relative standard deviation and recovery were 1.26% and 100.11% respectively. Excipients used in the pharmaceutical formulation did not interfere with the analysis. The technique offers the advantages of high sensitivity, rapidity and simplicity without the need for extraction, heating or cooling to low temperatures.

Keywords: Hydralazine hydrochloride; Vanillin; Hydrazone; Colorimetric determination.

Introduction

Hydralazine (1-hydrazinophthalazine) is a potent vasodilator that has been used for many years, chiefly in the treatment of ambulatory patients with primary hypertension of moderate severity (Westfall, 1982). In therapeutic doses, hydralazine produces little effect on nonvascular smooth muscle or on the heart. Its pharmacological actions are largely confined to vascular smooth muscle and occur predominantly on the arterial side of circulation.

The official methods (WHO, 1988; British Pharmacopoeia, 1993) for the assay of hydralazine hydrochloride utilize redox titration with potentiometric end point detection. In spite of the official protocols, the drug has been assayed by a variety of methods, such as UV-visible spectrophotometry (Dutt *et al.*, 1983; Stewart and Parks, 1983; Ibrahim *et al.*, 1986; Bedair *et al.*, 1986; Marr *et al.*, 1991); spectrofluorometry (Naik *et al.*, 1976); oxidimetry (Gaidukevich *et al.*, 1988); polarography (Fijalek and Szysko 1983) and high performance liquid chromatography (Di-Pietra *et al.*, 1993). Some of these techniques are not sufficiently sensitive or they are laborious and require highly sophisticated instrumentation not available in most quality-control laboratories. Zak *et al.* (1974) have reported a method for the determination of

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hydralazine in biological fluids using a technique that is based on the reaction with p-methoxybenzaldehyde. This technique was quite sensitive and specific for unchanged hydralazine, but not suitable for routine laboratory screening because the derivatization reaction requires elevated temperatures for completion. A condensation reaction involving hydralazine and 2-hydroxy-1-naphthaldehyde and subsequent spectrophotometric measurement of the coloured product has been reported (Marr *et al.*, 1991).

Aldehydes and ketones react with compounds containing hydrazino moiety to form hydrazones. These derivatives are usually well- defined solids and are useful for the detection of carbonyl compounds (Finar, 1994). The conversion of an aldehyde or ketone to the corresponding hydrazone is generally quantitative and the product is usually intensely coloured. The procedure has been exploited as a colorimetric method for estimating pharmaceuticals or other organic molecules containing the hydrazino function. For example, isoniazid (isonicotinic acid hydrazide, INH) has been determined colorimetrically by its derivatization to hydrazones with piperonal (Eidus and Little, 1962) and transcinnamaldehyde (Eidus and Harnanasingh, 1971).

Vanillin (*p*-hydroxy-*m*-methoxy benzaldehyde) has been used frequently as a chromogen in the field of pharmaceutical analysis (Plakogiannis and Saad, 1978; Shingbal and Khandeparkar, 1987). Recently, we reported the use of vanillin for the determination of chloramphenicol in pharmaceutical formulations (Ahmed and Onah, 2003). The inherently laborious and time-consuming official method (B.P., 1993) and the limitations that are associated with the other existing methods prompted this work. This paper for the first time reports the use of vanillin for the quantitative determination of hydralazine hydrochloride in pharmaceutical formulations.

Experimental

Apparatus. All spectral measurements were carried out with a spectrophotometer (Eagle Scientific Ltd, England).

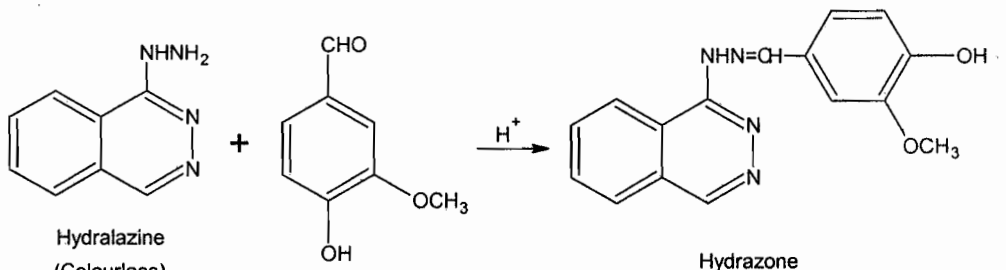
Materials and reagents. The following reagents used were of analytical grade and were used without further purification: Hydrochloric acid (May & Baker); Ethanol (AnalaR, BDH Chemicals, England); vanillin powder (AnalaR, BDH, England); Hydralazine hydrochloride pure powder (CIBA Laboratories, Harsham, West Sussex, England); commercial pharmaceutical formulations of hydralazine hydrochloride were purchased from drug stores in Jos, Nigeria.

Hydralazine hydrochloride stock solution was prepared by dissolving 100 mg of the pure sample in water and then diluting to volume in a 100 mL volumetric flask. The solution was protected from light before use. Working solution of hydralazine hydrochloride containing 100 μg/mL was prepared by further dilution of the stock solution with 0.5M ethanolic HCl solution and then standardized according to the B.P (1993) procedure. The solution was prepared as and when required.

Determination of absorption maximum. A 5 mL aliquot of hydralazine hydrochloride stock solution was transferred into a 25 mL volumetric flask and 4 mL of 3% vanillin solution was added. The solution was diluted to volume with 0.5M ethanolic HCl and mixed thoroughly. After allowing the solution to stand for 5 minutes for the colour to fully develop, the absorption maximum was determined against a reagent blank. The equation of reaction is illustrated below.

Optimization of analytical technique. The analytical technique was first optimized by determining the concentration of vanillin required. The limits of detection and

quantitation and selectivity were similarly investigated. The effect of temperature on the stability of the colour was also studied in the range of 20 – 40°C. The product was found to be stable for more than 12 hours at 40°C, and the results were reproducible so a temperature range of 25 – 30°C was employed for the present study.



Analytical procedure. Aliquot of hydralazine hydrochloride stock solution was transferred into 25 mL volumetric flask, and 4 mL of 3% vanillin solution added. The solution was diluted to mark with 0.5M ethanolic HCl solution and mixed thoroughly. After allowing it to stand for 5 minutes, the absorbance of the solution was determined at 390 nm Vs a reagent blank prepared simultaneously.

Preparation and validation of calibration curve. In order to construct a calibration curve, serial volumes of hydralazine hydrochloride solution containing equivalents of 0.1 to 0.5 g were transferred into series of 25 mL volumetric flask and the analytical procedure followed. Replicate preparations for each concentration was made and repeated on different days. The mean absorbances were plotted against drug concentrations. The calibration curve was then regressed by the method of least squares (Bauer, 1971). Identical analytical procedure was used to validate the calibration curve except that the serial concentrations of the solution of the drug used were changed. The means of absorbance readings obtained during the validation process were converted directly to their corresponding concentrations using the calibration curve. The relative recoveries and their errors with respect to the calibration curve were determined by comparing the concentrations obtained from the calibration curve with the actual concentrations of the solutions of the drug prepared.

Hydralazine hydrochloride assay in the pharmaceutical formulations. Twenty (20) tablets were randomly selected, weighed and finely powdered. An amount of power equivalent to 100 mg of hydralazine hydrochloride was weighed and dissolved (by gentle warming) in 20 mL of water and the content filtered directly into 100 mL volumetric flask. The residue was washed with water until the entire drug was extracted. The combined filtrate and washings was diluted to 100 mL with water. 10 mL of the resulting solution was further diluted to 100 mL with 0.5M ethanolic HCl and appropriate portions (between 0.1 to 0.5 g) treated as described in the analytical procedure.

For injection formulation, 10 ampoules were randomly selected and the weight of the contents determined. Powder for reconstitution equivalent to 100 mg hydralazine hydrochloride was then weighed, dissolved, filtered and the filtrate diluted as for the tablets. Aliquots equivalent to 0.1 to 0.5 g of the solution was treated as described in analytical procedure.

The content of the tablets and ampoules were determined directly from the calibration curve. The Official British Pharmacopoeia (1993) method was also used to determine the drug concentrations. Five (5) formulations of hydralazine hydrochloride were used in the assays.

The assay results obtained using the two methods (the proposed method and the official method) were compared using the student's t-test. A two-tailed probability value less than 95% confidence interval was considered to be significant.

Results and discussion

Hydralazine was found to react with vanillin at room temperature in acidic condition to produce a yellow compound that was stable for at least 24 hours. The absorption profile of the product is shown in Figure 1. Coupling of hydralazine with vanillin therefore produced bathochromic shift in the absorption spectrum relative to hydralazine (λ_{max} =260 nm). This is indicative of the formation of a product containing highly conjugated chromophores. The peak at 390nm was selected as the wavelength for all determinations because at this wavelength, optimum sensitivity of the method was achieved. Optimum colour intensity was obtained with 3 – 5 mL of 3% vanillin made up to 25 mL. An excess of vanillin over hydralazine HCl had no effect on colour intensity. The colour was stable at 40°C for 12 h. The calibration curve obtained from this method was linear and passed through the origin and so shows that Beer's law was obeyed. The molar absorptivity was calculated to be 1.429×10^4 l $Mol^{-1}cm^{-1}$. Limits of detection (LOD) and quantitation (LOQ) were 0.495 µg/mL and 1.650 µg/mL respectively. Linear regression analysis of the calibration data gave the following equation.

$$A_{390} = 0.0124 + 0.0603X$$

where A_{390} is absorbance at 390nm and X is the concentration of the analyte in the final assay solution in µg/mL. Correlation coefficient (r) value was 0.9989, suggesting a near perfect linearity between the absorbance and concentration of the drug. Regression analysis indicated that the value of the slope of the curve suggest high analytical sensitivity (Olaniyi and Fasanmade, 2000).

The accuracy of the method was evaluated by calculating the percentage recovery and error of each of the four (4) concentration levels of the drug. The overall percent recovery and percent error were 100.11 and 0.92% respectively. The precision was determined by calculating the relative standard deviation (RSD) for all replicate determinations. Table 1 shows the results obtained from the evaluation of the accuracy and precision of the proposed method. The RSD, expressed in percentage for each concentration was between 1.02 and 1.63%, while the average RSD for all the 4 determinations was 1.26%. The low value of the overall RSD is an indication of the high precision (reproducibility) of the proposed method.

After the method was validated, it was applied to the quantitative analysis of hydralazine hydrochloride in five (5) of its commercial product formulations. The results obtained by the proposed method were statistically compared with those obtained by applying the official British Pharmacopoeia (1993) method (Table 2). The student t-test was used to assess the performance of the proposed method at the 95% confidence level. The calculated t-value for each of the formulations (1.3459 - 2.5934) did not exceed the theoretical value (2.776), indicating that there is no significant difference between the proposed method and the official (BP.1993) method. The observation that the presence of common excipients did not interfere with the absorbance of the drug confirms the selectivity of the proposed method.

Table 1: Evaluation of accuracy and precision of the proposed method

Conc. prepared (μg/ml)	Conc. recovered* (μg/ml)	% RSD (n = 8)	% Recovery	% Error
6	6.07 ± 0.09	1.63	101.17	1.17
10	10.09 ± 0.13	1.31	100.90	0.90
14	13.89 ± 0,14	1.02	99.21	0.79
18	17.85 ± 0.19	1.06	99.17	0.83

*mean value of eight (8) determinations at each concentration ± standard deviation.

Table 2: Analysis of hydralazine hydrochloride in pharmaceutical formulations by the proposed method and the B.P. (1993) method.

Preparation	Sample No.	Label claim	Percent of labeled content found*	
			B.P. Method	Proposed method
Apresoline Injection	1	20mg	101.25 ± 0.58	100.98 ± 0.46
Apresoline 25 tablet	2	25mg	100.27 ± 0.62	101.03 ± 0.49
Apresoline 50 tablet	3	50mg	99.17 ± 0.68	98.83 ± 0.75
Cesoline tablet	4	25mg	98.67 ± 0.48	99.33 ± 0.55
Generic tablet	5	25mg	99.52 ± 0.60	99.35 ± 0.71

* Average of five (5) determinations ± standard deviation

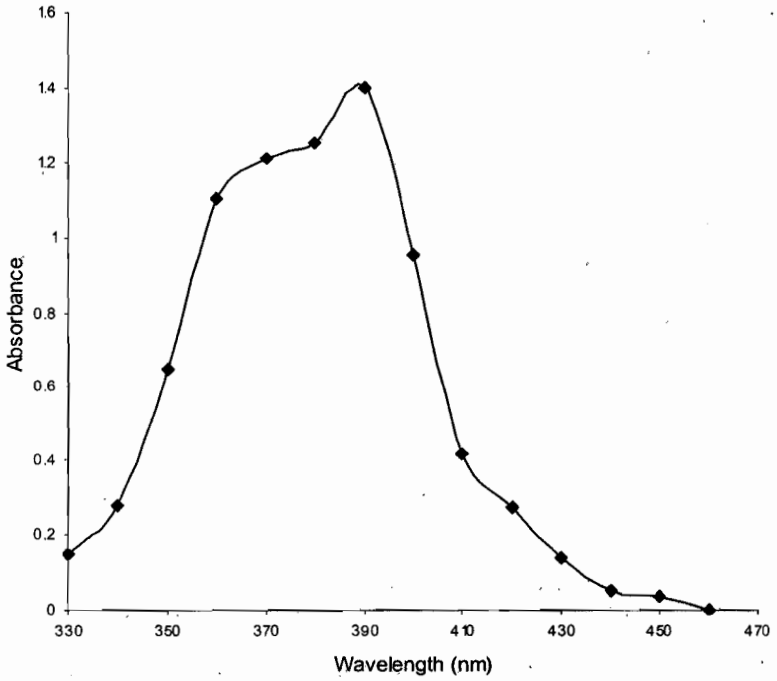


Fig.1: Absorption spectrum of the hydrazone of hydralazine with vanillin.

An attractive feature of the method is its relative freedom from interference by the usual tablet excipients in amounts far in excess of their normal occurrence in formulated products. However, the major limitation is that contaminants containing aromatic hydrazino group or aromatic primary amino group can interfere with the analytical method. Pharmaceutical grade hydralazine hydrochloride would not be expected to contain these impurities.

Conclusion.

The proposed method allows for the determination of hydralazine hydrochloride in pure form and in pharmaceutical formulations with an accuracy and precision comparable to the Official methods. This proposed method has an advantage of speed, sensitivity and specificity that is comparable to the Official technique. Unlike other techniques the instrumentation here is simple, inexpensive and economical and hence could be used for routine quality control of hydralazine hydrochloride in less endowed laboratories.

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