Journal of Pharmaceutical & Biomedical Analysis
Vol. 11, No. 8, pp. 647–650, 1993
Printed in Great Britain

0731–7085/93 $6.00 + 0.00

Spectrophotometric determination of dihydralazine in pharmaceuticals after derivatization with 2-hydroxy-1-naphthaldehyde

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Abstract: A sensitive and selective colorimetric assay has been developed for the determination of dihydralazine. The method is based on the interaction of dihydralazine with an ethanolic solution of 2-hydroxy-1-naphthaldehyde to yield a water-insoluble yellow product, 1,4-bis[(2-hydroxy-1-naphthyl)methylene hydrazine]phthazine. This colour can be quantified spectrophotometrically at 420 nm. The calibration curve was linear between 0.4 and 8 μg ml^{-1} of dihydralazine. The molar absorptivity at 420 nm is 24000 l mol^{-1} cm^{-1}. The method was successfully applied to the determination of dihydralazine in mixtures containing other drugs (reserpine, hydrochlorothiazide, oxprenolol, xanthinol, rutoside, chlorthalidone and bietaserpine).

Keywords: *Dihydralazine determination; 2-hydroxyl-1-naphthaldehyde; spectrophotometry; pharmaceuticals.*

Introduction

Dihydralazine (1,4-phthalazinedione-2,3-dihydro-dihydrazone) is a phthalazine derivative used in the treatment of severe or moderate hypertension [1] because of its direct relaxing effect on vascular smooth muscle.

Several methods have been described for the determination of dihydralazine in pharmaceuticals and biological fluids, including titrimetry [2–9], polarography [10, 11], spectrophotometry [3, 4, 12–18], gas chromatography [19, 20] and high-performance liquid chromatography (HPLC) [21–23]. Several colorimetric methods are based on the reaction of dihydralazine with aromatic aldehydes such as *p*-dimethylaminobenzaldehyde [14], vanillin [15], 3-methyl-3-nitro-pyridine-6-carboxaldehyde [16] and sodium 1,2-naphthoquinone-4-sulphonate [17].

The synergistic effects produced by the concomitant administration of dihydralazine with diuretics (hydrochlorothiazide, chlorthalidone) and β-adrenergic blocking agents (oxprenolol) are useful since the dose of dihydralazine can be reduced and adverse side-effects induced by prolonged administration of the drug can be minimized. Therefore, the complex pharmaceutical formulations of dihydralazine can only be analysed by more selective methods based on derivatization and extraction procedures.

In the present communication, a novel spectrophotometric method for the determination of dihydralazine is described. The method is based on coupling of the antihypertensive drug with an aromatic aldehyde, 2-hydroxy-1-naphthaldehyde, to form a yellow dihydrazone, 1,4-bis[(2-hydroxy-1-naphthyl)-methylene hydrazine]phthazine. This yellow reaction product can be extracted with dichloromethane and its maximum absorption at 420 nm used for determination of dihydralazine.

2-Hydroxy-1-naphthaldehyde has been proposed previously as a derivatization reagent for hydrazine and isoniazid determination in aqueous solution [24, 25] and isoniazid and hydralazine in plasma [26, 27].

Experimental

Apparatus

A Shimadzu UV-240 spectrophotometer, with 1 cm quartz cells; a Perkin–Elmer 843 infrared-spectrometer, a Perkin–Elmer LC 55 chromatograph equipped with a UV–vis spectrophotometer detector, a Crison 2002 pH

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meter; a Heidolph Reax 2000 shaker and a S.B.S. water bath were used.

Reagents and solutions

All chemicals used were of analytical-reagent grade. Dihydralazine stock solution 100 μg ml^{-1} was prepared from dihydralazine sulphate (99.5%) kindly provided by Ciba–Geigy Co. (Barcelona, Spain). This solution is stable for at least 6 weeks at 4°C. Dihydralazine standard solution (10 μg ml^{-1}) was prepared by appropriate dilution of the stock solution immediately before use. 2-Hydroxy-1-naphthaldehyde (Ega-Chemie) 0.01 M in ethanol, hydrochloric acid (0.1 and 0.01 M) and dichloromethane were used.

Procedure

A portion of the sample solution containing up to 40 μg of dihydralazine was transferred by pipette into a borosilicate glass-stoppered tube and diluted with distilled water to 5 ml. A 1 ml volume of 0.1 M hydrochloric acid and 1 ml of reagent solution were added. The reactants were heated in a 25°C water bath for 60 min. The reaction product was extracted by mechanical shaking for 1 min with 5 ml of dichoromethane; the mixture was transferred into a borosilicate separation funnel and, immediately after phase separation the absorbance of the organic layer was measured at 420 nm against a reagent blank.

Determination of dihydralazine in formulations

Twenty units of the solid dosage form (tablets, capsules or pellets) were weighed and powdered. A weighed portion of powder equivalent to about 10 mg of dihydralazine was transferred to a beaker and extracted with 25 ml of 0.01 M HCl for 15 min; the mixture was filtered and diluted to 250 ml with distilled water; 10 ml of the latter solution was diluted to 100 ml in a calibrated flask. The sample was then ready for treatment as described under the procedure section.

Results and Discussion

Characterization and properties of the reaction product

The reaction product was obtained by reaction of one part of dihydralazine with two parts of 2-hydroxy-1-naphthaldehyde in aqueous alcoholic solution for 12 h at 25°C, followed by filtration of the suspension to yield a residue yellow.

HPLC with a column (250 × 4.6 mm i.d.) packed with 5 μm Spherosorb ODS-2 and a mobile phase of acetonitrile–water–phosphoric acid (80:20:0.1, v/v/v), (pH 3.0) at 0.8 ml min^{-1} showed the presence of a single peak with a retention time of 14 min. Provided this product is protected from sunlight, it is stable up to 50°C.

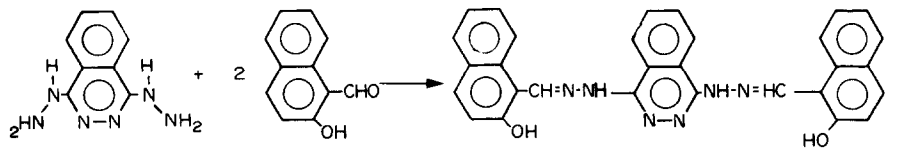
In the IR spectrum of the product the most outstanding feature was the absence of the bands characteristic of the aldehyde group, namely combination vibrations at 2890 cm^{-1} and stretching vibrations C=O at 1639 cm^{-1} and the appearance of a new band at 1608 cm^{-1} associated with the C=N stretching vibration.

The UV–vis spectrum of the derivative in dichloromethane showed an absorption maximum at 420 nm (ϵ = 24000 l mol^{-1} cm^{-1}). The reagent blank did not absorb at this wavelength. Similar results were obtained employing as extractive solvents diethyl ether, isobutanol and chloroform.

The reaction between dihydralazine and 2-hydroxy-1-naphthaldehyde is shown in Scheme 1.

Effect of 2-hydroxy-1-naphthaldehyde concentration

Increasing the reagent concentration with a constant dihydralazine concentration of 10 μg ml^{-1}, resulted in an increase of absorbance up to a reagent concentration of 0.005 mM (Fig. 1). Above this reagent concentration the blank absorbance increased but this did not affect the sample absorbance.



Scheme 1

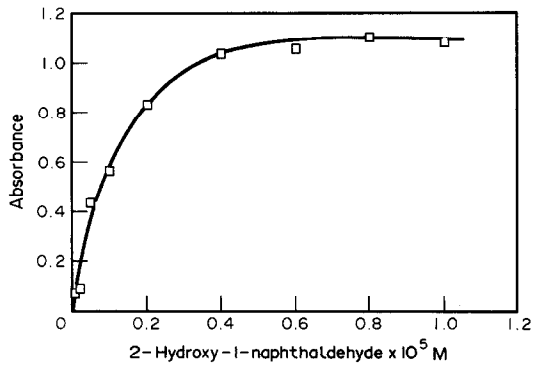


Figure 1
Effect of reagent concentration on the derivative absorbance at 420 nm.

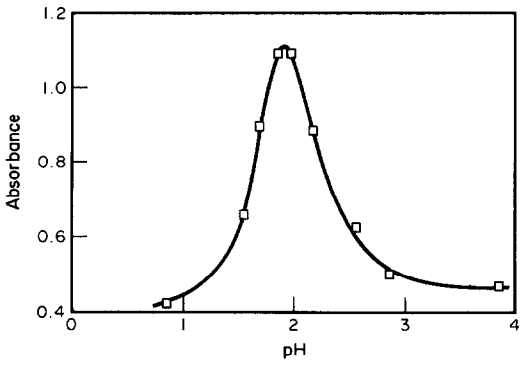


Figure 2
Variation of absorbance with the pH of the reaction mixture.

Effect of pH and ionic strength

The effect of pH on the reaction was investigated over the range 0.7–4. As shown in Fig. 2 the optimum pH value is 1.8. No significant effect of ionic strength was observed.

Colour development

The interaction between 2-hydroxy-1-naphthaldehyde and dihydralazine depends on temperature and time of heating. The process is endothermic but the reaction product is not stable at 50°C. The most precise analytical results (RSD = 2.12%) were obtained for heating at 25°C for 1 h.

Effect of shaking and standing time

The extraction time was varied from 10 s to 5 min, extraction being complete after 30 s.

The colour of the reaction product in dichloromethane was stable for more than 24 h at 4°C when stored in the dark or in diffuse light.

Analytical parameters

Absorbances at 420 nm were linearly related to the concentration of dihydralazine ranging from 0.4 to 8 μg ml^{-1} using 5 ml of the solution. The correlation coefficient was 0.998 and the equation of the calibration graph was: $y = -0.0121(\pm 0.0120) + 0.0821(\pm 0.0013)\ x$, where y is the measured absorbance at 420 nm and x the concentration of dihydralazine solution in μg ml^{-1}.

The detection limit, defined as the sample concentration giving an absorbance that is three times the standard deviation of the absorbance of the blank solution, is 0.08 μg ml^{-1}.

Under the optimized conditions, reproducibility of the method was checked by performing 15 replicate determinations of 4 μg ml^{-1} of dihydralazine over a period of 1 week. The SD, the RSD, the confidence limits and the relative error ($P = 0.05$) were: 0.13 μg ml^{-1}, 2.02%, ±0.07 μg ml^{-1} and 1.18%, respectively.

Table 1
Determination of dihydralazine sulphate in pharmaceuticals by proposed method and a colorimetric method using *p*-dimethyl aminobenzaldehyde (*p*-DAB)

Sample	Other active substances	Dihydralazine declared (mg)	Dihydralazine found (mg ± SD)*		
			Proposed method	*p*-DAB method	*t*†
Tablets	Oxprenolol	25	24.875 ± 0.017	24.569 ± 0.007	1.17
Tablets	Reserpine	10	10.619 ± 0.009	10.915 ± 0.010	1.55
Tablets	Reserpine Hydrochlorothiazide	10	10.701 ± 0.010	11.057 ± 0.017	1.89
Pellets	Rutoside Xanthinol Chlorthalidone	10	10.377 ± 0.021	11.152 ± 0.022	2.92
Capsules	Hydrochlorothiazide Bietaserpine	10	10.077 ± 0.009	9.701 ± 0.008	0.77

*Mean of 10 determinations ± standard deviation.
†Student *t*-value ($P = 0.05$) = 2.101.

Application of the method

To test the applicability of the proposed method, the dihydralazine content of some pharmaceuticals was determined. No interference is observed from additives and excipients commonly used in formulations.

The results are summarized in Table 1 and are compared with those obtained by *p*-dimethylaminobenzaldehyde (*p*-DAB) method [27]. Statistical analysis of the results using the Student's *t*-test for paired data revealed no significant differences between the two methods at the 95% confidence level for tablets and capsules. In the case of pellets the differences are most commonly due to the presence of dyes used in this dosage form, which interfere with the *p*-dimethylaminobenzaldehyde method whereas such interferences are eliminated in the proposed method by the extraction process. Thus, the proposed method can be used for the routine determination of dihydralazine in simple and complex dosage forms.

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[Received for review 15 May 1992; revised manuscript received 30 July 1992]