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Spectrofluorimetric determination of prenalterol hydrochloride in pharmaceutical preparations and biological fluids

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Abstract

A simple and highly sensitive fluorimetric method was developed for the routine determination of prenalterol hydrochloride in bulk, in dosage forms and in biological fluids. The method is based on the fluorescence induced by reaction of the nitroso-derivative of prenalterol hydrochloride with 2-cyanoacetamide in the presence of ammonia. The different experimental parameters were carefully studied and incorporated into the procedure. The fluorescence is measured at 440 nm after excitation at 368 nm. Fluorescence intensity is a linear function of prenalterol hydrochloride concentration over the range of 0.1–2.8 μg ml^{-1} in the solution finally measured. A proposal for the reaction pathway was suggested. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Fluorimetry; Biological samples; Pharmaceuticals; 2-Cyanoacetamide; Prenalterol hydrochloride

1. Introduction

Prenalterol hydrochloride is a sympathomimetic agent with stimulant effects on the beta-adrenoceptors. It has an inotropic action on the heart with relatively little chronotropic effect. It is used in the treatment of heart failure associated with myocardial infarction, open heart surgery and shock [1].

In spite of the clinical importance of prenalterol hydrochloride, few methods have been described for its determination and no official methods for its assay have been reported. A review of the literature revealed that prenalterol hydrochloride content was determined in raw material and in certain dosage forms by colorimetric [2–5], spectrophotometric [2] and polarographic methods [3,6]. In biological fluids, it was determined by gas–liquid chromatography [7] and high-performance liquid chromatography [8–10]. Fluorimetrically, prenalterol hydrochloride in 0.1 N sulphuric acid was found to fluoresce at 325 nm with an excitation wavelength at 288 nm. This method was used over the concentration range 2.5–12.5 μg ml^{-1} for determination of prenalterol hydrochloride in its tablets [2].

2-Cyanoacetamide has been used for the fluorimetric determination of catecholamines [11], re-

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ducing carbohydrates [12,13] and reducing polysaccharides after enzymatic degradation [14].

The present paper describes a highly sensitive method for the determination of prenalterol hydrochloride that can be adopted for its determination in biological fluids. The method is based on the formation of blue fluorescence upon reaction of nitroso-derivative of prenalterol hydrochloride with 2-cyanoacetamide in the presence of ammonia.

2. Experimental

2.1. Apparatus

An Aminco-Bowman Model J4-8960 spectrofluorimeter was used with the excitation and emission slit controls set at 5 mm and the intensity scale control set at 30. The excitation and emission wavelengths used were 368 and 440 nm, respectively. Measurements were performed with a 1-cm silica cell.

2.2. Reagents and materials

The following reagents were used: hydrochloric acid (Prolabo, France), 0.1 M solution; sodium nitrite (Merck, Germany), 2% solution; ammonium sulphamate (Fluka, Switzerland), 5% solution; ammonia (Prolabo, France), 33% solution; 2-cyanoacetamide (Merck, Germany) 4% solution; buffer solution, pH 10.8 was made up from sodium carbonate (BDH, UK) 2 M solution and adjusted to pH 10.8 with 1 M hydrochloric acid; dosage forms containing prenalterol hydrochloride (Boehringer, Germany), were purchased from commercial sources.

2.3. General procedure

A 1.0 mg ml^{-1} stock standard solution was prepared of prenalterol hydrochloride in water. This solution was further diluted with water to give a 25.0 μg ml^{-1} prenalterol hydrochloride working standard solution. Portions (0.1–2.8 ml) of this solution, accurately measured, were transferred into separate 25-ml volumetric flasks; 1 ml of 0.1 M hydrochloric acid followed by 1 ml of 2% sodium nitrite solution were added, and the mixture was shaken well for 2 min; 2 ml of ammonium sulphamate solution were then added and the mixture was shaken well until no more nitrogen was evolved; 1 ml of 4% 2-cyanoacetamide and 3 ml of ammonia solution were added and the reaction mixture was heated in a boiling water bath for 1 h, then cooled and made up to volume with distilled water. The fluorescence was measured and the intensity was plotted against the concentration of prenalterol hydrochloride to obtain the standard calibration graph.

2.4. Procedure for tablets

Twenty tablets were weighed and powdered. An accurately weighed amount of the powder equivalent to 4.0 mg of prenalterol hydrochloride was transferred into a small conical flask. Extraction with 3 × 30 ml portions of water was performed. The contents were then transferred into a 100-ml standard flask and diluted to the mark with water;

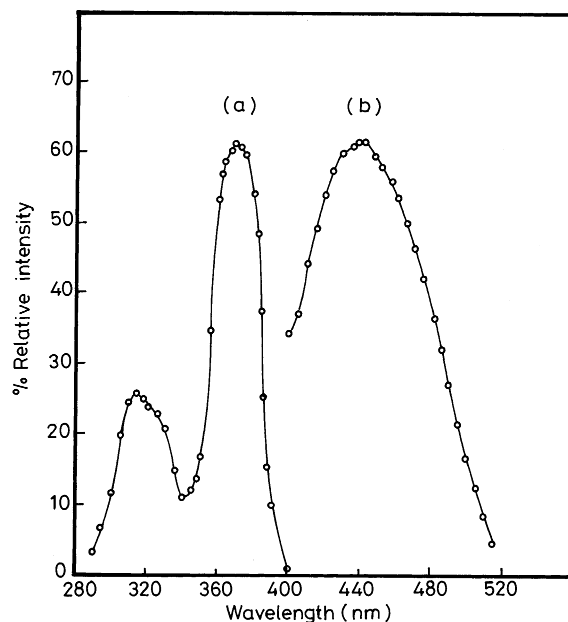


Fig. 1. Fluorescence spectra of the reaction product of prenalterol hydrochloride (1.7 μg ml^{-1}). (a) Excitation spectrum; (b) Emission spectrum.

Table 1
Fluorimetric determination of prenalterol hydrochloride and its dosage forms

Drug form	% Found	
	Proposed method	Spectrophotometric method [2]
Prenalterol hydrochloride (pure sample)	99.4 100.9 99.5 100.0 99.8	
Mean ± S.D.	99.9 ± 0.60	100.20 ± 1.09
Prenalterol hydrochloride tablets (prepared tablets containing 10 mg per tablet)	100.4 101.0 100.2 100.9	
Mean ± S.D.	100.6 ± 0.39	100.8 ± 0.50
Varbian ampoules[a] (1 mg prenalterol hydrochloride per ml)	101.3 100.5 99.5 100.2	
Mean ± S.D.	100.4 ± 0.75	100.9 ± 0.64

[a] Product of Ciba (UK).

1 ml of this solution was transferred into a 25-ml standard flask and the above procedure was then followed. The nominal content of the tablets was calculated either from a previously plotted calibration graph or using the regression equation.

Table 2
Fluorimetric determination of prenalterol hydrochloride in spiked urine and plasma

Concentration taken (μg ml^{-1})	% Found	
	Urine	Plasma
0.4	101.5	100.0
0.8	100.0	98.5
1.6	98.6	97.8
2.0	96.9	102.3
2.4	97.5	100.5
Mean ± S.D.	98.9 ± 1.87	99.8 ± 1.77

2.5. Procedure for ampoules

The contents of 20 ampoules were mixed; 4 ml of the mixed solution were transferred into a 100-ml calibrated flask and made up to the mark with water; 1 ml of this solution was then transferred into a 25-ml standard flask and the above-described procedure was followed. The nominal content of the ampoules was calculated either from a previously plotted calibration graph or using the regression equation.

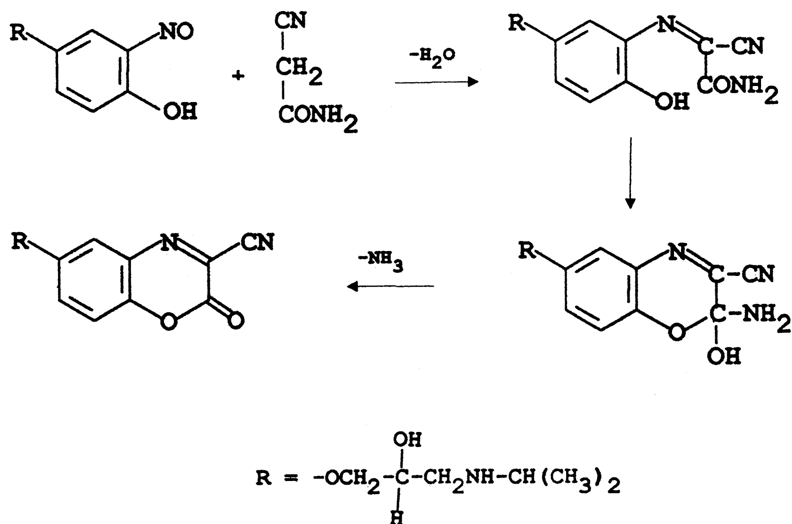
2.6. Procedure for spiked biological fluids

An aliquot of plasma or urine (1 ml) in a 15-ml centrifuge tube was spiked with an aliquot of aqueous solution of prenalterol hydrochloride containing 250 μg; 0.1 ml pH 10.8 buffer and 10 ml diethyl ether were added. The solution was vortexed at high speed for 2 min before being centrifuged at 2500 rpm for 5 min. The resulting supernatant was transferred into a small conical flask. The extraction was repeated with another 10 ml of diethyl ether. The combined extracts were evaporated to dryness under a stream of nitrogen at ambient temperature. The dry residue was dissolved in 10 ml of 0.1 M hydrochloric acid; 1 ml of this solution was transferred into a 25-ml standard flask and the above general procedure was then followed. A blank experiment was carried out adopting the above procedure.

3. Results and discussion

Treatment of the nitroso-derivative of prenalterol hydrochloride with 2-cyanoacetamide in the presence of ammonia was found to give a fluorescent product. Fig. 1 shows the excitation and emission spectra obtained. The nitroso-derivative of prenalterol hydrochloride was obtained rapidly at room temperature within 2 min using 1 ml of 0.1 M hydrochloric acid and 1 ml of 2% sodium nitrite.

The effect of experimental conditions on the fluorescence intensity was studied; 1.0 ml of 4%



Scheme 1.

2-cyanoacetamide was found to be sufficient to produce the maximum fluorescence intensity; a larger volume had no effect. Also 3.0 ml of 33% ammonia solution was found to be sufficient to produce the maximum fluorescence intensity; larger volumes of ammonia solution has no effect. The fluorophore is formed after heating in a boiling water-bath for 1 h and remained stable for more than 2 h.

The fluorescence intensity is linearly related to prenalterol hydrochloride concentration over the range 0.1–2.8 μg ml^{-1}. Linear regression analysis of the results gave the following equation:

$$C = -0.077 + 0.029\% \, R.I. \quad (r = 0.9998)$$

The precision of the method was evaluated by analysing standard solutions of prenalterol hydrochloride. The results in Table 1 were in accord with those obtained by the difference spectrophotometric method [2]. The method was also applied to some dosage forms containing prenalterol hydrochloride. The results in Table 1 agreed with those obtained by the difference spectrophotometric method [2]. Statistical analysis [15] of these results using Student's t-test and the variance-ratio F-test showed no significant difference between the performances of the two methods as regards accuracy and precision.

Tablet excipients such as talc, starch, gelatin, magnesium stearate and lactose did not interfere with the assay.

The high sensitivity attained by the proposed method allows the determination of prenalterol hydrochloride in biological fluids. About 50% of the prenalterol dose is excreted in urine as a sulphate conjugate and ca. 20% as unchanged drug [7]. Hydrolysis of the sulphate may be achieved either enzymatically or chemically by using 5 M hydrochloric acid and heating for 1 h at 60°C [7]. Thus the proposed method can be used for the determination of free prenalterol and total prenalterol (free and conjugated prenalterol) in human urine after hydrolysis in 5 M hydrochloric acid for 1 h at 60°C. For both plasma and urine, the extraction was made using diethyl ether at pH 10.8 [7]. Table 2 shows the results of the recovery studies of prenalterol hydrochloride from spiked plasma and urine.

As reported earlier [3,6], treatment of prenalterol hydrochloride with nitrous acid produces the corresponding nitroso-derivative. By analogy with the reaction of 2-cyanoacetamide with nitroso compounds [16], the reaction is proposed to proceed as shown in Scheme 1. Further cyclization involving the phenolic and the amide groups may occur producing the highly conjugated system.

The isolation and identification of the reaction product is the subject of a further communication.

Comparing the proposed method with the other reported fluorometric method [2], although the other method is simpler, the proposed method is more sensitive and has been applied successfully for the determination of prenalterol hydrochloride in biological fluids.

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