Short Communication

Colorimetric determination of prenalterol hydrochloride in dosage forms

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Introduction

Prenalterol hydrochloride is a sympathomimetic agent with stimulant effects on 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 or open heart surgery or shock [1]. In spite of the clinical importance of prenalterol hydrochloride, few methods have been described for its determination.

Prenalterol hydrochloride has been determined as the raw material and in certain dosage forms by colorimetric, spectrophotometric and fluorimetric methods [2]. In biological fluids it was determined by gas–liquid chromatography [3] and high-performance liquid chromatography (HPLC) [4–6].

The present paper describes the use of 2,6-dichloroquinone chlorimide (DCQ) and 4-amino-antipyrine (AAP) for the sensitive and rapid colorimetric determination of prenalterol hydrochloride, either in pure form or in certain dosage forms.

Experimental

Apparatus

A Perkin–Elmer 550 S spectrophotometer with matched 10-mm quartz cells was used.

Materials

A pure drug sample was kindly supplied by Boehringer Ing. (Germany). Dosage forms containing prenalterol hydrochloride were purchased from commercial sources.

Reagents

All the reagents used were of analytical reagent grade. 2-6-Dichloroquinone chlorimide solution (DCQ): 0.1% (w/v) in isopropanol. Sodium acetate solution: 20% aqueous solution. Ammonia buffer: 40 g of ammonium chloride and 40 ml of concentrated ammonium hydroxide were dissolved in water to give 170 ml of solution. 4-Aminoantipyrine solution (AAP): 0.2% methanolic solution. Sodium carbonate solution: 0.1% aqueous solution. Potassium hexacyanoferrate (III) solution: 0.4% aqueous solution.

Sample preparation

A stock solution containing 1.0 mg ml^{-1} of prenalterol hydrochloride in water was prepared and diluted further to contain 200 µg ml^{-1}.

Construction of the calibration curves.

DCQ method. An aliquot containing the drug in the working concentration range (0.8–11 µg ml^{-1}) was transferred into a set of 25-ml standard flasks and 1 ml of DCQ solution; 1 ml of sodium acetate solution and 1 ml of ammonia buffer were added. The solution was diluted to volume with water and the absorbance measured at 595 nm against a reagent blank. The absorbance was plotted against the final concentration in order to obtain a calibration graph.

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AAP method. An aliquot containing the drug in the working concentration range (2–22 μg ml) was transferred into a set of 25-ml standard flasks and 1.5 ml of sodium carbonate solution; 0.6 ml of AAP solution and 1 ml of potassium hexacyanoferrate (III) solution were added. The solution was diluted to volume with water and the absorbance was measured at 500 nm against a reagent blank. The absorbance was plotted against the final concentration to obtain a calibration graph.

Analysis of tablets

Twenty tablets were weighed and powdered. An accurately weighed amount of the powder equivalent to 20 mg of prenalterol hydrochloride was transferred into a small conical flask and extracted with 3 × 30 ml portions of water. The extract was transferred into a 100-ml standard flask and diluted to the mark with water. The analysis was continued as described above using the DCQ and AAP methods. The nominal content was calculated from the corresponding calibration graph or regression equation.

Analysis of ampoules

The contents of 20 ampoules were mixed. An accurately measured volume equivalent to 20 mg of prenalterol hydrochloride was transferred into a 100-ml standard flask and diluted to the mark with water. The analysis was continued as described for construction of the calibration curves using the DCQ and AAP methods. The nominal content was calculated from the corresponding calibration graph or regression equation.

Results and Discussion

Prenalterol hydrochloride is a phenolic compound and can therefore react with DCQ in the presence of sodium acetate and ammonia buffer to give a coloured reaction product with a λ_{max} at 595 nm.

The different experimental parameters affecting the colour development were extensively studied to determine the optimal conditions for the assay procedure. The reaction was studied as a function of the volume of reagent, reaction time, stability and alkalinizing agents. Maximum absorbance was attained using 1 ml of 0.1% DCQ solution and a mixture of 1 ml of 20% sodium acetate solution and 1 ml of ammonia buffer as alkalinizing agent. The colour developed immediately and remained stable for more than 2 h.
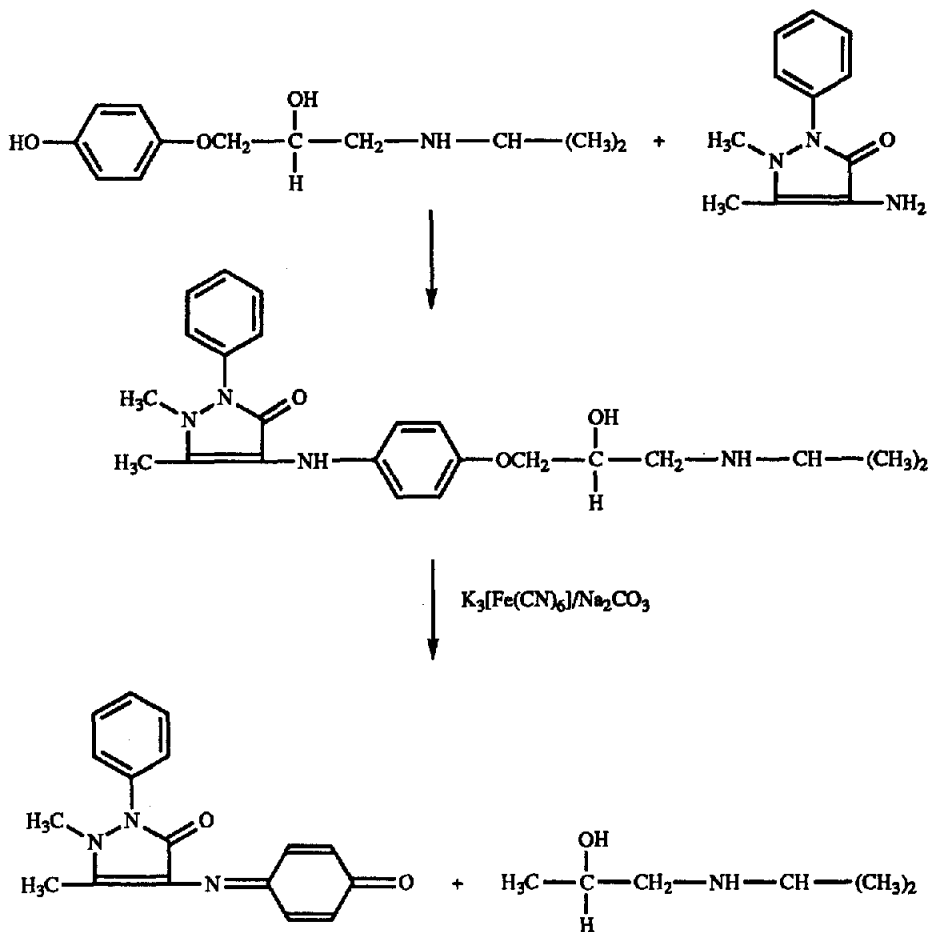
Sweeney and Hall [7] reported that the use of a mixture of 1 ml of 20% sodium acetate solution and 1 ml of ammonia buffer as an alkalinizing agent resulted in a 50% increase in colour intensity and an increase in stability of the colour.

Under the described experimental con-
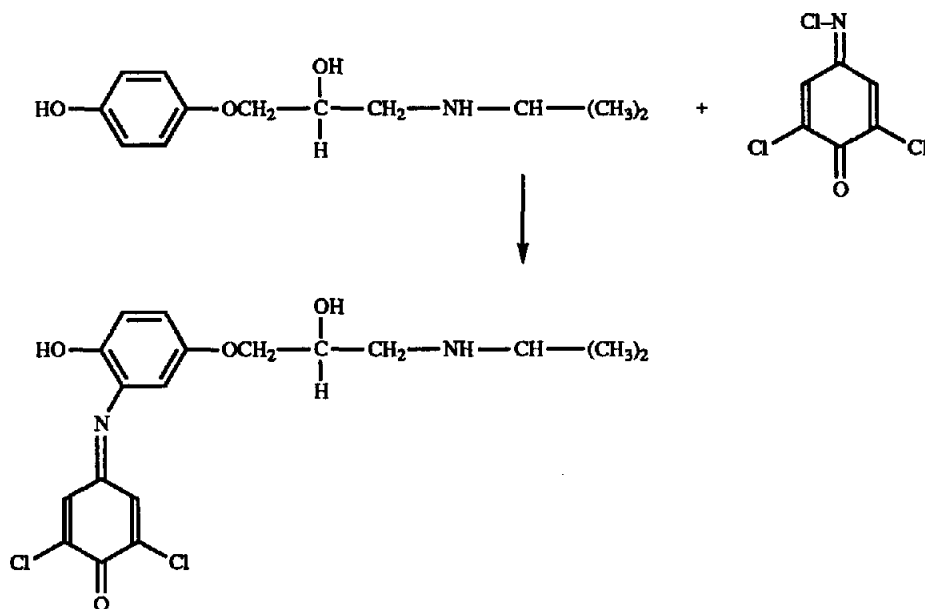
Scheme 1
Proposed reaction pathway between DCQ and prenalterol hydrochloride.

Scheme 2
Proposed reaction pathway between AAP and prenalterol hydrochloride.

ditions, a standard calibration curve was constructed by plotting absorbance versus concentration. Conformity with Beer's law was evident in the concentration range of the final dilution of $0.8-11$ μg ml^{-1}. The linearity between absorbance, A, at 595 nm and concentration, c, in μg ml^{-1} was found to be expressed by the following equation:

$$A = 0.0864c + 0.0078 \ (r = 0.9999).$$

To study the reaction further the molar ratio of DCQ to prenalterol hydrochloride in the reaction mixture was determined by the molar ratio method [8]. The molar ratio was found to be 1:1. According to Scudi [9] DCQ reacts via the chlorine atom of the chlorimide group. Accordingly, the reaction proceeds as proposed in Scheme 1, whereby indophenols are formed [10].

Prenalterol hydrochloride was also found to react with AAP in the presence of an alkaline

oxidizing agent to form a red colour with λ_{max} at 500 nm. The colour developed immediately and remained stable for more than 3 h. Beer's law was obeyed over the concentration range of $2-22$ μg ml^{-1}. The linearity between absorbance, A, at 500 nm and concentration, c, in μg ml^{-1} was found to be expressed by the following equation:

$$A = 0.0407c - 0.0005 \ (r = 0.9999).$$

It is reported that AAP reacts with phenolic compounds giving a quinonoid structure [11] of the following type:
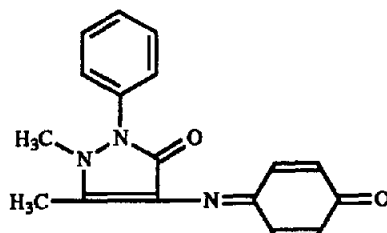
Table 1
Determination of prenalterol hydrochloride and its dosage forms by the DCQ and AAP methods and by a difference spectrophotometric method

	% Found ± SD†		
	DCQ method	AAP method	Difference spectrophotometric method [2]
Pure samples	100.3 ± 1.10	100.1 ± 0.92	100.2 ± 1.09
Prenalterol hydrochloride tablets (prepared tablets containing 10 mg per tablet)	100.4 ± 1.11	100.1 ± 0.77	100.8 ± 0.50
Varbian ampoules* (1 mg prenalterol hydrochloride per ml)	100.7 ± 0.78	100.2 ± 0.39	100.9 ± 0.64

* Product of Ciba (UK).
† $n = 5$ for pure samples and $n = 4$ for dosage forms.

This necessitates that the position para to the phenolic group be either free or substituted with a group that can be expelled during the reaction. In the case of prenalterol hydrochloride, it is postulated that an alkoxy group in the para position is expelled under the reaction conditions described.

To study the reaction further, the molar ratio of AAP to prenalterol hydrochloride in the reaction mixture was studied by the molar ratio method [8] and found to be 1:1. Accordingly, the reaction was postulated to proceed as proposed in Scheme 2, whereby an antipyrine dye is formed [11].

The appreciable values of molar absorptivities (2.3×10^4 and 1.1×10^4 l mol^{-1} cm^{-1} for the DCQ and AAP methods, respectively) and the stability of the products permit the successful application of the proposed methods to the determination of prenalterol hydrochloride, either in pure form or in its dosage forms (Table 1). These results were compared with those obtained by the difference spectrophotometric method [2]. The proposed methods were shown to be precise and reproducible.

Statistical analysis [12] of the results by DCQ, AAP and difference spectrophotometric methods [2] using Student's t-test and the variance ratio F-test showed no significant difference between the performance of the three methods in respect of accuracy and precision.

Conclusions

DCQ and AAP are sensitive chromogenic reagents for the determination of prenalterol hydrochloride in pure form or in its dosage forms. The suggested methods are simple, rapid, sensitive and suitable for routine analysis in control laboratories. In addition, a colorimeter is the only instrumentation required, and this is an advantage of the method in developing and underdeveloped countries.

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