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# Fluorimetric determination of prenalterol hydrochloride in pharmaceuticals and biological fluids based on its oxidation reaction by hexacyanoferrate(III)

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**Abstract**

A rapid and sensitive fluorimetric method for the determination of prenalterol hydrochloride is presented, based on the oxidation of the analyte with potassium hexacyanoferrate(III) in a slightly alkaline medium (pH 9.23). The different experimental parameters were carefully studied and incorporated into the procedure. The oxidation product exhibits a blue fluorescence with its emission maximum at 427 nm, and excitation maximum at 314 nm. Fluorescence intensity is a linear function of prenalterol hydrochloride concentration over the range of 0.2–3.6 μg $ml^{-1}$ in the solution finally measured. The method was successfully applied to the determination of prenalterol hydrochloride in pharmaceutical formulations and biological fluids. A proposal for the reaction pathway is suggested. 

*Keywords:* Prenalterol hydrochloride; Fluorimetry; Potassium hexacyanoferrate(III) oxidation; Pharmaceutical analysis; Biological samples

## 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 [2–6], spectrophotometric [2] and polarographic methods [3,7]. In biological fluids, it was determined by gas-liquid chromatography [8] and high-performance liquid chromatography [9–11]. Fluorimetrically, prenalterol hydrochloride in 0.1 N

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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]. Recently, a more sensitive spectrofluorimetric method was used for its determination in dosage forms and biological fluids over the concentration range 0.1–2.8 μg $ml^{-1}$. 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 and heating in a boiling water-bath for 1 h [12].

The present paper describes a simple, rapid and quite sensitive spectrofluorimetric method for the determination of prenalterol hydrochloride, whereby potassium hexacyanoferrate(III) is used as an oxidizing reagent yielding a fluorescent substance. The fluorescence is enhanced by the addition of dimethylformamide. The method has been satisfactorily applied to the determination of prenalterol hydrochloride in pharmaceutical preparations and biological fluids.

## 2. Experimental

### 2.1. Apparatus

An Aminco–Bowman Model J4-8960 spectrofluorimeter with the excitation and emission slit controls set at 5 mm and the intensity scale control set at 100 was used. The excitation and emission wavelengths used were 314 and 427 nm, respectively. Measurements were performed with a 1-cm silica cell.

### 2.2. Materials

Prenalterol hydrochloride of pharmaceutical grade (Boehringer, Germany) was used as the working standard. Dosage forms were obtained from commercial sources.

### 2.3. Reagents

All the reagents used were of analytical-reagent grade and the solutions were prepared with doubly distilled water:

1. An aqueous 0.25 g $l^{-1}$ hexacyanoferrate(III) solution was prepared from standard potassium hexacyanoferrate(III) (Riedel de Haen, Germany).
2. An aqueous 1.25 g $l^{-1}$ ascorbic acid was prepared from standard L-ascorbic acid (BDH, UK).
3. Dimethylformamide (Prolabo, France).
4. The 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 (Prolabo, France).
5. Michaelis borate buffers, pH 7.6–12.3 [13].

### 2.4. Sample preparation

A 1 mg $ml^{-1}$ stock standard solution of prenalterol hydrochloride in water was prepared. This solution was further diluted with water to give a 25 μg $ml^{-1}$ prenalterol hydrochloride working standard solution.

### 2.5. Recommended procedure for calibration

Known volumes (0.2–3.6 ml) of 25 μg $ml^{-1}$ prenalterol hydrochloride solution were transferred into separate 25-volumetric flasks. Then 5 ml of Michaelis borate buffer solution pH 9.23 and 1 ml of 0.25 g $l^{-1}$ hexacyanoferrate(III) solution were added to each of the volumetric flasks. After allowing the mixture to react at these oxidative conditions for 30 s, 1 ml of 1.25 g $l^{-1}$ ascorbic acid solution and 4 ml of dimethylformamide were added. The mixture was 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.

### 2.6. Procedure for dosage forms

#### 2.6.1. 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 $3 \times 30$ ml portions of water was performed. The contents were then transferred into a 100-ml

standard flask and diluted to the mark with water. One milliliter 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.

*2.6.2. 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.

*2.7. 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 solution and 10 ml diethyl ether was 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 3 ml of 0.1 M hydrochloric acid. The resulting solution was then transferred into a 10-ml standard flask and diluted to the mark with water. 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

*3.1. Spectral characteristics*

The oxidation of prenalterol hydrochloride by potassium hexacyanoferrate(III) in slightly alkaline medium was found to give a fluorescent product. This product shows native fluorescence with an excitation maximum at 314 nm and an emission maximum at 427 nm. Fig. 1 shows the excitation and emission spectra obtained.

*3.2. Effect of experimental variables*

The experimental conditions that were varied in order to optimise fluorescence included: pH, ascorbic acid concentration, hexacyanoferrate(III) concentration and time interval to react the oxidation reagent.

Michaelis borate buffer and ammonia-ammonium chloride buffer were tested. Michaelis borate buffer was found to be the best. Fig. 2 shows the effect of pH on the fluorescence intensity of oxidized prenalterol using Michaelis borate buffers. Maximum fluorescence intensity was obtained over the pH range 9.15–9.66.

The effect of hexacyanoferrate(III) concentration on the fluorescence intensity was studied by using several concentrations of this oxidizing solution ($2 \times 10^{-3}$–$6 \times 10^{-2}$ g $l^{-1}$) keeping the solution at pH 9.23. Fig. 3 shows that the highest fluorescence intensity was obtained over the concentration range 0.005–0.012 g $l^{-1}$. As a conse-

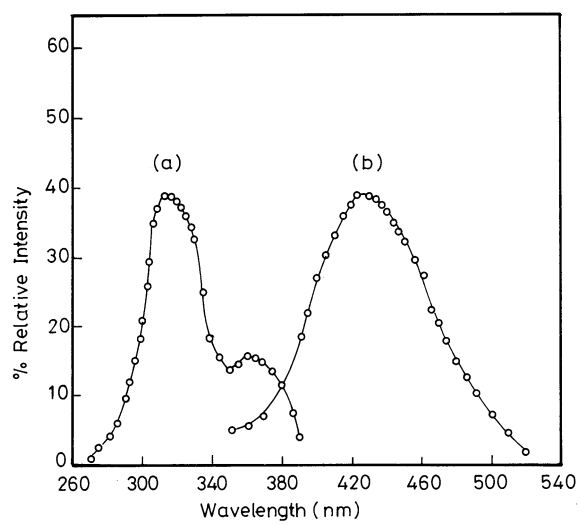



Fig. 1. Fluorescence spectra of the oxidation product of prenalterol hydrochloride (1.5 μg $ml^{-1}$). (a) Excitation spectrum; (b) emission spectrum.

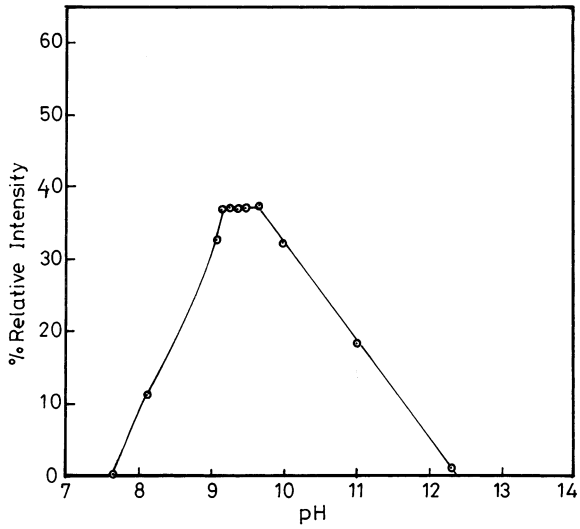



Fig. 2. Effect of pH on the fluorescence intensity of oxidised prenalterol hydrochloride (1.4 μg $ml^{-1}$) using Michaelis borate buffers.

quence, a 0.01 g $l^{-1}$ hexacyanoferrate(III) concentration was chosen for use throughout.

The stability of the solution containing the fluorescent product formed from prenalterol was studied. The results showed that the fluorescence

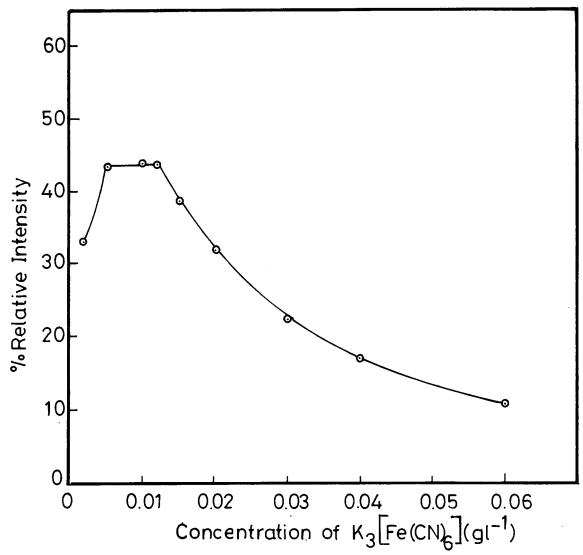



Fig. 3. Effect of potassium hexacyanoferrate(III) concentration on the fluorescence intensity of prenalterol hydrochloride (1.7 μg $ml^{-1}$) at pH 9.23.

intensity of the fluorophore rapidly deteriorates owing to the excess of hexacyanoferrate(III) present in the solution. This excess of reagent causes the oxidation process to continue, thus resulting in the disappearance of the fluorescence product obtained initially. For this reason, the use of ascorbic acid as a reducing agent is necessary to eliminate the excess of hexacyanoferrate(III). A concentration of 0.05 g $l^{-1}$ ascorbic acid was considered adequate for this purpose.

The time that prenalterol spend in contact with the oxidizing reagent, before the ascorbic acid was added, was studied and had a great effect on the height of the fluorescence intensity. This influence was tested with solutions containing several prenalterol concentrations and a time interval of 30 s was selected. At this conditions, the fluorescent product remained stable for more than 2 h.

The fluorescence of oxidized prenalterol was enhanced two times by addition of 4 ml of dimethylformamide.

### 3.3. Determination of prenalterol hydrochloride

Under the described experimental conditions, a standard calibration curve for prenalterol hydrochloride was constructed. The fluorescent intensity was linearly related to prenalterol hydrochloride concentration over the range 0.2–3.6 μg $ml^{-1}$. Linear regression analysis of the results gave the following equation:

$$C = 0.0351 + 0.0377\%\ \text{R.I.}\ (r = 0.9999,\ n = 7)$$

The standard deviation of slope and intercept was $2.8 \times 10^{-4}$ and $1.6 \times 10^{-2}$, respectively. The repeatability of the proposed method was checked with three series of 5 samples having a prenalterol hydrochloride concentration of 1, 2 and 3 μg $ml^{-1}$, respectively. The overall relative standard deviation (RSD) of fifteen determinations was 1.46%.

The precision of the method was evaluated by analyzing standard solutions of prenalterol hydrochloride. The results in Table 1 were in accord with those obtained by the different spectrophotometric method [2].

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 | |
| | 101.9 | |
| | 100.8 | |
| | 100.2 | |
| | 101.2 | |
| Mean ± S.D. | 100.7 ± 0.95 | 100.2 ± 1.09 |
| Prenalterol hydrochloride tablets[a](prepared tablets containing 10 mg per tablet) | 100.4 | |
| | 99.6 | |
| | 100.8 | |
| | 101.3 | |
| Mean ± S.D. | 100.5 ± 0.72 | 100.8 ± 0.50 |
| Varbian ampoules[b](1 mg prenalterol hydrochloride per ml) | 101.8 | |
| | 100.4 | |
| | 101.1 | |
| | 99.7 | |
| Mean ± S.D. | 100.8 ± 0.90 | 100.9 ± 0.64 |

[a] Prepared tablets containing the drug and the tablet excipients lactose (57 mg), starch (50 mg), magnesium stearate (0.9 mg) and talc (8.1 mg) per tablet.
[b] Product of Ciba (UK).

### 3.4. Investigation of the reaction product

The fluorophore produced by oxidation of prenalterol hydrochloride(I) is suggested to be 2,2′-dihydroxy-5,5′-di-3-isopropylaminopropane-2-ol biphenoxyl(II) (Scheme 1) by analogy with the oxidation of paracetamol with hexacyanoferrate(III) to yield the fluorophore 2,2′-dihydroxy-5,5′ diacetylaminobiphenyl [14,15].

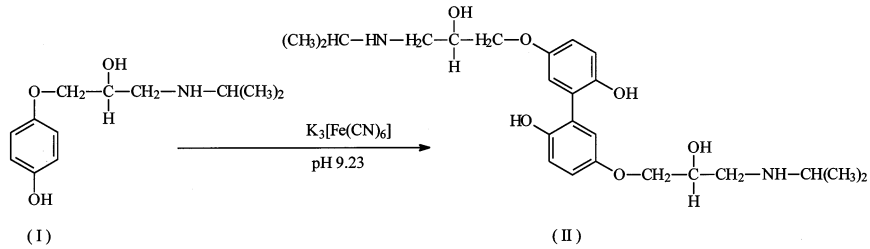
### 3.5. Analysis of pharmaceutical preparations

The proposed method was successfully applied to the analysis of some dosage forms containing prenalterol hydrochloride. The results in Table 1 agreed with those obtained by the different spectrophotometric method [2].

Statistical analysis [16] 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.

### 3.6. Analysis of spiked urine and plasma samples

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 approximately 20% is an unchanged drug [8]. 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 [8]. 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 by using diethyl ether at pH 10.8 [8]. Table 2 shows the results of the recovery studies of prenalterol hydrochloride from spiked urine and plasma. The between-day precision was also evaluated on several days up to three days. The percentage recoveries based on the average of five separate determinations were 98.4 ± 1.97 and 98.6 ± 1.82




Scheme 1. Proposed reaction pathway between prenalterol hydrochloride and potassium hexacyanoferrate(III).

for spiked urine and plasma samples, respectively. The corresponding RSD% was 2.00 and 1.85, respectively. These results indicate the good reproducibility and precision of the method.

### *3.7. Comparison of the proposed method with other reported methods*

Comparing the proposed method with other reported methods shows them all to be equally accurate and precise. The proposed method is more sensitive than most of the reported methods [2–7]. On the other hand the reported chromatographic methods [8–10] are more sensitive than the proposed method, but these methods require considerable skill to obtain reliable results.

The proposed method has some distinct advantages over other reported fluorimetric methods. It is more sensitive than the method reported by Wahbi et al (concentration range 2.5–12.5 μg $ml^{-1}$) [2] and it is more simple and rapid than the other method using 2-cyanoacetamide [12]. This method requires the preparation of nitroso-derivative of prenalterol to react with 2-cyanoacetamide in the presence of ammonia and heating in a boiling water bath for 1h.

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

| Concentration taken (μg $ml^{-1}$) | % Found | |
|---|---|---|
| | Urine | Plasma |
| 0.5 | 97.6 | 97.6 |
| 1.0 | 98.9 | 98.0 |
| 1.5 | 100.0 | 100.1 |
| 2.0 | 101.5 | 101.5 |
| 3.0 | 97.0 | 101.0 |
| Mean ± S.D. | 99.0 ± 1.82 | 99.7 ± 1.67 |

## 4. Conclusion

The proposed fluorimetric method is simple, rapid, accurate and sensitive. It can be readily adopted for use in the control laboratory. In addition, it can be considered as a stability indicating assay, since prenalterol, like other phenolic compounds, is prone to oxidation of the phenolic group to give the corresponding *p*-quinone. The method can be used for flow-injection spectrofluorimetric determination of prenalterol hydrochloride.

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