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Spectrophotometric determination of fenoterol hydrobromide in pure form and dosage forms

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Abstract

A sensitive and rapid spectrophotometric procedure has been investigated for the determination of fenoterol either per se or in pharmaceutical preparations. The proposed procedure is based on the reaction between the drug and 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) at pH 7.2, using borate buffer, to produce a yellow adduct. The latter has maximum absorbance at 400 nm and obeys Beer's law within the concentration range 5–30 μg/ml. Regression analysis of the calibration data showed a good correlation coefficient ($r = 0.9996$) with minimum detection limit of 0.24 μg/ml (6.2×10^{-8} M). The proposed procedure has been successfully applied to the determination of this drug in its tablets and in syrup, the mean percent recoveries were 97.45 ± 0.59 and 98.7 ± 0.64%, respectively. The results obtained are in good agreement with those given using a reference method. The pharmaceutical additives other than active ingredient did not interfere. A proposal of the reaction pathway has been postulated.

Keywords: Fenoterol; 4-Chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl); Spectrophotometry; Dosage forms

1. Introduction

Fenoterol is a sympathomimetic agent with predominantly β-adrenergic activity and a selective action on β_2-receptors. It is used as a bronchodilator. Its bronchodilating action being relatively more prominent than its effect on the heart [1]. Fenoterol is used in the treatment of bronchial asthma, prevention of exercise-induced bronchospasm and in the management of premature labour [1].

Several methods have been introduced for the determination of fenoterol in pharmaceutical preparations as well as biological fluids. These methods include titrimetry [2], spectrophotometry [3,4], flow-injection analysis [5], HPLC [6–11], GC [12,13], voltammetry [14–16], coulometry [17], electrophoresis [18,19] and immunoassay [20–22].

Most of the described methods are either insufficiently sensitive [2–4] or tedious and require highly dedicated instrumentation [6–22]. There is, therefore, a need for a reliable sensitive spectrophotometric method for its determination. The present work describes a simple and sensitive spectrophotometric method based on coupling between fenoterol and 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) to produce the corresponding NBD-fenoterol yellow adduct.

2. Experimental

2.1. Equipment

A Shimadzu (Model 1016 PC) UV–visible Spectrophotometer (Shimadzu, Kyoto, Japan) was used to measure the absorbance at 400 nm, using 1 cm quartz cells.

2.2. Materials and reagents

All the reagents used were of analytical reagent grade.

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Boehringer (Ingelheim, Germany) kindly supplied the authentic sample of Fenoterol hydrobromide. All pharmaceutical dosage forms (tablets and syrup) were obtained from commercial sources (Berotic® tablets and syrup, batch No. 1198105 and 701776, respectively). 4-Chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl) was purchased from Sigma (St. Louis, MO).

2.3. Standard solutions

A stock solution containing 0.2 mg/ml of fenoterol hydrobromide was prepared in distilled water and was further diluted as appropriate. This solution was stable for 1 week if kept in the refrigerator.

NBD-Cl solution 0.005% w/v was prepared in ethanol.

Borate buffer (0.1 M, pH 7.2) [2].

Concentrated hydrochloric acid (BDH, UK).

Ammonia solution 33% w/v (BDH, UK).

2.4. Procedures

2.4.1. Calibration curve

Transfer aliquot volumes of the stock solution containing 50–300 μg of fenoterol hydrobromide into a series of 10 ml volumetric flasks. Add 1 ml of NBD-Cl solution and 5 ml borate buffer. Mix well then heat using a thermostatically controlled water bath at 60 °C for 20 min then cool rapidly. Add 0.2 ml of hydrochloric acid, then complete to the mark with distilled water. Measure the absorbance at 400 nm against a reagent blank. Construct a calibration curve and derive the regression equation.

2.4.2. Determination of fenoterol hydrobromide in dosage forms

2.4.2.1. Procedure for tablets. Weigh and pulverize 10 tablets then transfer a quantity of the powder equivalent to 20 mg of fenoterol HBr into a small conical flask. Extract with 4 × 20 ml of distilled water, filter into a 100 ml volumetric flask and complete to the mark with distilled water. Transfer aliquot volumes into a 10 ml volumetric flask, then apply the procedure as described under calibration curve. Determine the nominal content of the tablet either from regression equation or using the calibration graph.

2.4.2.2. Procedure for syrup. Transfer an aliquot volume of syrup equivalent to 20 mg of fenoterol HBr into a separating funnel then add 5 ml of ammonia solution then mix well. Extract with 3 × 5 ml of chloroform, filter the chloroformic layer over anhydrous sodium sulfate and evaporate the chloroform to dryness. Dissolve the residue in 5 ml of ethanol. Transfer aliquot volumes into a 10 ml volumetric flask, then apply the procedure as described under Section 2.4.1. Determine the nominal content of the syrup either from the regression equation or using the calibration graph.

3. Results and discussion

NBD-Cl is a labeling reagent for primary and secondary amines. Several pharmaceutical compounds have been determined through this approach, such as, atenolol [23], vigabatrin and gabapentin [24].

Fenoterol hydrobromide is a secondary aliphatic amino derivative, that was found to react with NBD-Cl with the formation of the Meisenheimer complex resulting in yellow adduct [25]. Under the described experimental conditions, the yellow adduct has a characteristic absorption spectrum with maximum absorption at 400 nm as shown in Fig. 1.

The different experimental parameters affecting the produced color were extensively studied in order to determine the optimal conditions for the determination of the drug. First, the influence of pH on the absorption was studied; the maximum absorption occurs at ap-

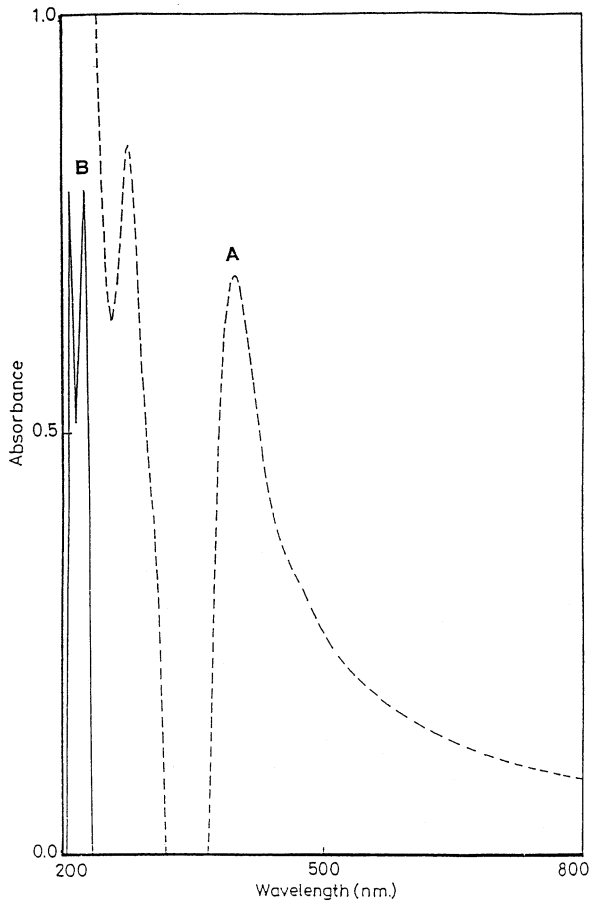

Fig. 1. Absorption spectra of: (A) The reaction product of fenoterol HBar (20 μg/ml) with NBD-Cl in borate buffer of pH 7.2. (B) Blank solution in the same medium.

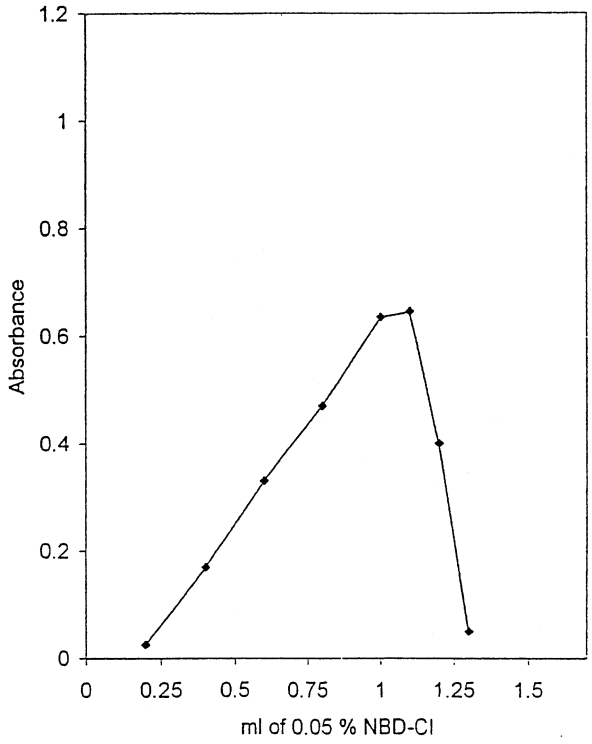

Fig. 2. Effect of pH on the development of the reaction product of fenoterol HBr (20 μg/ml) with NBD-Cl.

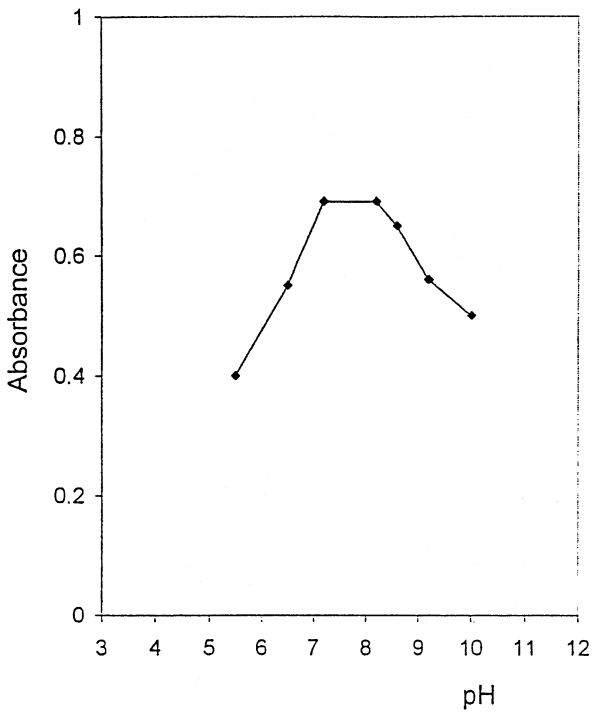

Fig. 3. Effect of volume of NBD-Cl (0.05%, w/v) on the development of the reaction product of fenoterol HBr (20 μg/ml) with NBD-Cl.

proximately pH 7.2 using borate buffer (Fig. 2). Other buffers having the same pH value such as carbonate or phosphate buffers were studied and compared with 0.1 M borate buffer which proved to be superior over carbonate and phosphate buffers because the absorbance readings were higher. This is due to the hydrolysis of NBD-Cl to 4-hydroxy-7-nitrobenzo-2-oxa-1,3-diazole (NBD-OH) using other buffers. These results are in agreement with that of Miyano et al. [26].

The effect of temperature on the produced adduct was studied, it was found that heating at 60 °C for 20 min was better than heating at a higher temperature for a shorter period. The adduct formed was found to be stable at room temperature for approximately one and half hour after which it faded slowly.

The most important factor affecting the formed yellow adduct was the volume of NBD-Cl, Fig. 3 shows that 1 ml of 5% w/v NBD-Cl solution gave maximum sensitivity. Increasing the volume of NBD-Cl leads to decrease in the absorbance, this may be due to the high background absorbance of the reagent. The absorption of the hydrolysis product of NBD-Cl, namely NBD-OH, completely disappeared at pH less than 1. [27] Therefore, acidification of the reaction solution prior to the measurement remarkably decreased the background absorbency without affecting the drug-reagent adduct, hence, the sensitivity of the procedure increased.

The formation constant of the reaction product K_f was calculated adopting the following formula: [28]

$$K_f = \frac{A/\mathrm{Am}}{(1 - A/\mathrm{Am})^{n+1} C^n n^n}$$

where A is maximum absorbance, Am is the Absorbance corresponding to intersection of the two tangents

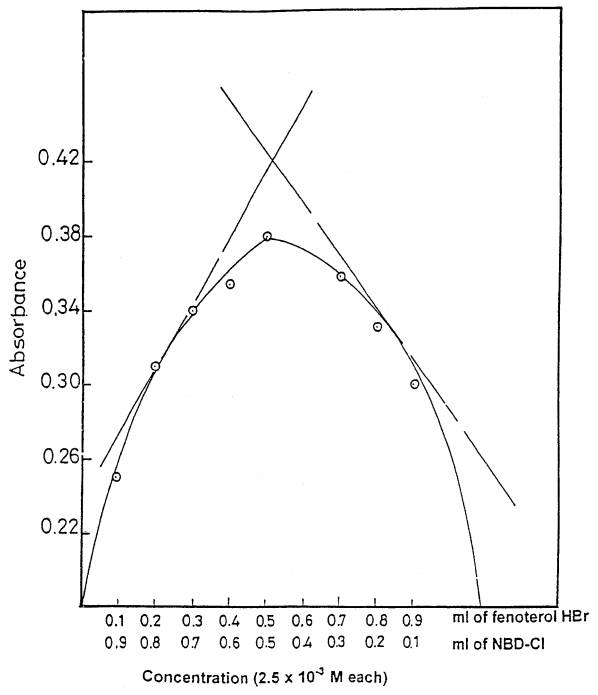

Fig. 4. The continuous variation plot for the stoichiometry of the reaction of fenoterol HBr and NBD-Cl.

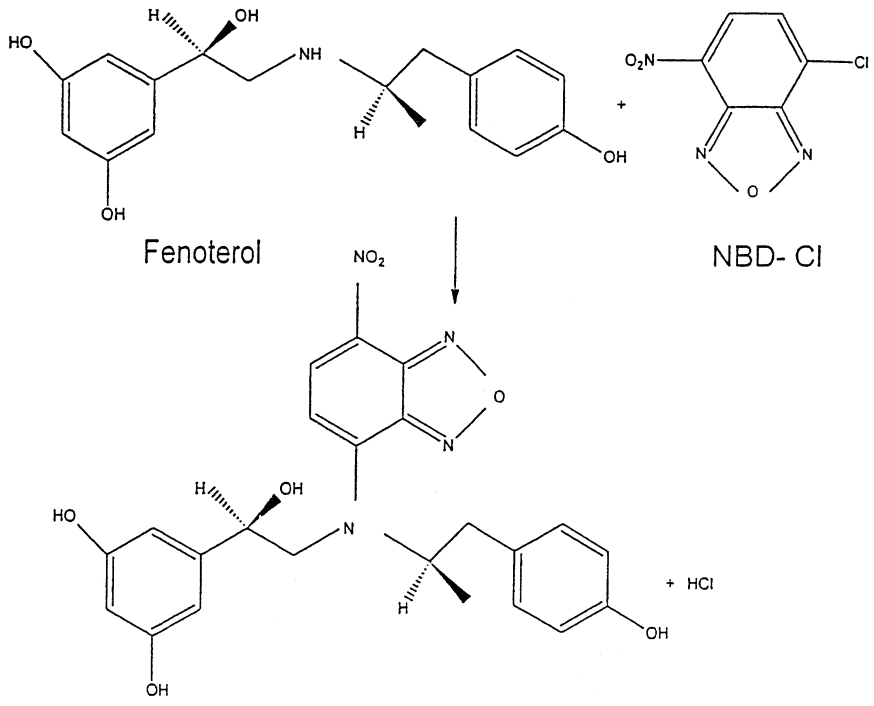

Fig. 5. Proposal of the reaction pathway between fenoterol and NBD-Cl.

of the curve in Fig. 4, C is the concentration corresponding to maximum absorbance, n is the amount of the drug in reaction product. Using this equation, K_f was found to be equal to 123.7.

The Gibbs free energy of the reaction was also calculated adopting the following equation [28]:

$$\Delta G = -2.303RT \log K_f$$

where ΔG is Gibbs free energy of the reaction, R is the universal gas constant, T is the absolute temperature and K_f is formation constant of the reaction.

The value of ΔG was found to be -13.33 Kcal/mol. The negative sign of ΔG points out to the spontaneous nature of the reaction.

The molar reactivity of NBD-Cl with fenoterol hydrobromide in the reaction product was determined by the molar ratio method [29], continuous variation method [29] and the limiting logarithmic method [30]. The molar ratio was found in all cases to be approximately 1:1, confirming that one molecule of fenoterol hyrobromide combines with one molecule of NBD-Cl. Based on the observed molar ratio, the reaction pathway shown in Fig. 5 is proposed.

Under the described experimental conditions, the relation between the absorbance at 400 nm with the concentration of fenoterol hydrobromide was linear over the range 5–30 μg/ml. Linear regression analysis of the concentration–absorption data gave the following equation:

$$A = 0.0068 + 0.028C, \qquad r = 0.9996$$

where C is the concentration in μg/ml, A is the absorbance and r is the correlation coefficient showing excellent linearity. The lower detection limit ($S/N = 2$) is 0.24 μg/ml (6.2×10^{-8} M).

Statistical analysis of the results obtained by the proposed and official methods revealed no significant difference between the performance of the two methods regarding the accuracy and precision [31] as shown in Table 1.

The proposed method was applied to the determination of fenoterol HBr in pharmaceutical preparations such as tables and syrup. Common tablet excipients

Table 1
Application of the proposed method and official method to the determination of fenoterol HBr in pure form

Taken (μg)	Proposed method		Official method [a]
	Found (μg)	Recovery (%)	Recovery (%)
5.0	4.97	99.40	100.1
10.0	10.10	101.00	98.5
15.0	14.90	99.33	99.2
20.0	19.80	99.10	
25.0	25.29	101.16	
30.0	30.11	100.37	
$\bar{X}$		100.06	99.27
±SD		±0.9	±0.655
t		1.240 (1.895) *	
F		1.88 (5.79) *	

[a] Each result is the average of three separate experiments.
* Tabulated values of t and F at $P = 0.05$.

Table 2
Application of the proposed method to the determination of fenoterol HBr in dosage forms

Preparation	Proposed method			Reference method [4]
	Taken (μg)	Found (μg)	Recovery (%)	Recovery (%)
Berotic tablets (2.5 mg fenoterol HBr per tablet) [a]	5.00	4.90	98.00	99.90
	10.00	9.83	98.30	99.80
	15.00	14.68	97.86	98.50
	20.00	19.47	97.30	
	25.00	24.58	98.32	
	30.00	29.76	99.19	
$\bar{X}$			98.45	99.40
±SD			0.662	0.654
t			1.346 (1.895)	
F			1.025 (5.79)	
Berotic syrup (2.5 mg fenoterol HBr per 5 ml) [b]	5.00	4.94	98.80	99.80
	10.0	9.76	97.60	98.50
	15.0	14.76	98.40	99.70
	20.0	19.9	99.50	
	25.0	24.65	98.60	
	30.0	29.83	99.40	99.33
$\bar{X}$			98.70	0.59
±SD			0.637	
t			0.967 (1.895)	
F			1.116 (5.79)	

[a] Product of Boehringer, Ingelheim, Germany, batch No. 1198105.
[b] Product of Boehringer, Ingelheim, Germany, batch No. 701776.

such as talc, maize starch, magnesium stearate, avisil, lactose and gelatin did not interfere with the assay. The results of analysis of tablets and syrup are shown in Table 2. Statistical analysis of the results obtained by both, the proposed method and a reference method [4] shows no significant difference between the two methods regarding accuracy (t-test) and precision (F-test).

4. Conclusion

A simple and reliable method has been proposed for the determination of fenoterol in dosage forms. The derivatization reaction resulted in an increase in the molar absorptivity from 4.1×10^3 to 1.1×10^4 l/(mol/cm). The lower detection limit (6.2×10^{-8} M) is comparable to those reported by chromatographic methods. The method can be readily adopted to routine quality control analysis.

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