SPECTROPHOTOMETRIC DETERMINATION OF FENOTEROL IN PURE FORM AND PHARMACEUTICAL FORMULATION.

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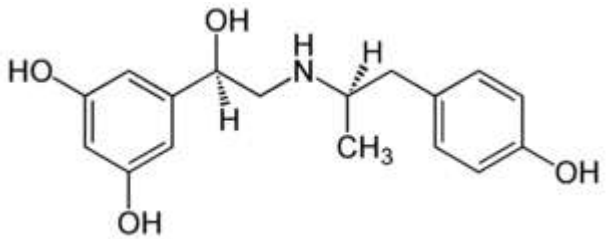
ABSTRACT

Four simple and selective spectrophotometric methods were developed for quantitative determination of Fenoterol in pure forms as well as in its pharmaceutical formulation. **Method [A]** is based on the nitration and subsequent complexation with a nucleophilic reagent forming a yellow colour with λ_{max} at 411 nm, Beer's law was obeyed in the concentration range 1-6 μg ml^{-1}. LOD and LOQ were found to be 0.024 and 0.08 μg ml^{-1} respectively. **Method [B]** is based on the coupling of the drug as a phenolic compound with the diazonium salt of o-nitroaniline forming red azodye with λ_{max} at 505 nm. Good linearity obtained in the range of 3-21 μg ml^{-1}. LOD & LOQ were found to be 0.12 and 0.41, respectively. **Method [C]** is based on coupling with diazo reagent (method B) and subsequent chelation with copper sulphate and extraction of the resulting chelate into chloroform and measuring the chloroformic layer at 412 nm. Beer's low was obeyed in the concentration range 3-21 μg ml^{-1}. LOQ & LOQ were found to be 0.056 and 0.188 μg ml^{-1}. respectively. **Method [D]** involves the reduction of follin ciocalteu's phenol reagent (FCP) by the drug to be a blue colored product which exhibited an absorption maximum at λ_{max} 655 nm. Regression analysis of Beer's plot showed good correlation in the concentration range of 2-14 μg ml^{-1}. LOD & LOQ were found to be 0.011 and 0.038 μg ml^{-1}. respectively. The optimization of the reaction conditions was investigated, the methods were successfully applied to the analysis of Fenoterol in its pharmaceutical formulation with good recovery.

INTRODUCTION

Fenoterol (Fig. 1) is a direct acting sympathomimetic agent with predominantly beta-adrenergic activity and a selective action on β_2 receptors. It is used as bronchodilator, with its bronchodilating action being relatively more prominent than its effect on the heart. It is used in the treatment of bronchial asthma, prevention of exercise-induced bronchspasm and in the management of premature labour (**Kathleen, 1999)** .



1-(3, 5-dihydroxyphenyl)-2-(4-hydroxy-methylphenethylamino) ethanol hydrobromide

Fig.1

Different methods have been reported for the determination of Fenoterol such as spectrophotometric **(El-Shabrawy *et al.*, 2003; Negussie *et al.*, 2004; Zamuner *et al.*, 2008)**, spectrofluorimetric **(Manal, 2007; El-Tarras *et al.*, 2005)**, HPLC **(Danuta *et al.*, 2008; Ingolfe *et al.*, 2002)**, LC-MS (**Hee Seung, *et al.*, 2008)**, Capillary zone electrophoresis **(Somsak and Proespichaya, 2008)**, and electrochemical methods (**Belal *et al.*, 2000)**. The aim of the present study is to develop new, simple, and accurate quantitative methods for determination of fenoterol in both pure and pharmaceutical forms.

Experimental

Pure sample

Fenoterol hydrobromide (99.67%) was kindly supplied by Chemical Industries Development Company, (CID), Giza, Egypt.

Market sample

Berotec® Tablets: Product of Chemical Industries Development Company (CID) Cairo, Egypt. Batch No. (503104), labeled to contain 0.5 mg Fenoterol hydrobromide per tablet and purchased from local pharmacies.

Chemical and reagent

All reagents used were of analytical grade, solvents were of spectroscopic grade, and water was freshly double distilled.

- Acetone, potassium hydroxide, copper sulphate, sodium hydroxide, chloroform, hydrochloric acid, (ADWIC, Egypt).
- o- nitroaniline (Aldrich U.S.A) 0.1% in 1 N HCl
- Nitric acid (Riedel- Germany)
- Sulphuric acid (Merck – Germany)
- Sodium nitrite (Winlab, UK) 1% aqueous solution
- Follin ciocaletu's phenol (FCR) 2N solution (Sigma chemical Co. USA) used directly for investigation.
- 0.125 M Copper sulphate aqueous solution
- 10% aqueous potassium hydroxide solution
- 1N aqueous sodium hydroxide solution
- 1N aqueous sulphuric acid.

4. Apparatus:

Shimaolzu, UV-Vis 1650PC spectrophotometer, equipped with 10 mm matched quartz cells.

5. Standard Solutions:

For all methods, A, B, C and D, stock solutions of Fenoterol (1 mg ml^{-1}) were prepared by dissolving 100 mg powder of Fenoterol in 100 ml water. Solutions with different concentrations were prepared from the stock solution by suitable dilutions.

6. Procedures:

Method A (Nitration and subsequent complexation)

Aliquots of standard solution of the drug (1 mg ml^{-1}) were evaporated to dryness in small beaker at about $60^{o}C$. The residues were treated with 2ml of nitric acid and 2 ml of sulphuric acid, left to stand for10 min., cooled transferred quantitatively into 100 ml calibrated flasks, and diluted to volume with water, then 1 ml volume was transferred into a 10 ml calibrated flask and treated with 3 ml acetone followed by 3 ml of potassium

hydroxide (10%) aqueous solution and diluted to volume with H_2O. The absorbance of the resulting yellow colour was measured against a reagent blank at 411 nm.

Method B: (Diazo-coupling with o-nitro aniline)

A volume of 1.5ml of o-nitroaniline (0.1% in 1 N HCl) was mixed with 2.5 ml of $NaNO_2$ solution (1%) in a 10 ml calibrated flask and the mixture was left to stand for 10 min. an aliquot of the standard solution of Fenoterol (equivalent to 150 μg) was added to the diazo reagent followed by 3 ml of 1N NaOH solution and the mixture was left to stand for 5 min. the solution was then diluted to volume with water and the absorbance of the red coloured formed was measured at 505 nm against the reagent blank.

Method C: (Diazo-coupling and subsequent chelation)

A volume of 1.5 ml of o-nitroaniline (0.1% in 1 N HCl) solution and 2.5 ml of $NaNO_2$ solution (1%) were mixed and left to stand for 10 min. accurately measured aliquots of standard Fenoterol (0.25 mg ml^{-1}) was added followed by 3 ml of 1N NaOH solution. The mixture was allowed to stand for 5 min. and then treated with 5 ml of $CuSO_4$ solution (0.125 M), 6 ml of 1N H_2SO_4 and extracted three times with a total volume of 25 ml chloroform. The extracts were collected in a 25 ml calibrated flasks, the absorbance of the chloroform extract was measure at 412 nm. against the reagent blank.

Method D: (FCP method)

Aliquots of the drug solution (0.1 mg ml^{-1}) were transferred to a series of 10 ml volumetric flasks followed by 0.5 ml 2 N FCP reagent and 1 ml of 1N NaOH solution. The contents were mixed and after 25 min. the volumes were completed to the mark with water and the absorbencies were measured at 655 nm against the reagent blank.

7. Analysis of tablets:

For all methods, the contents of 10 tablets of Fenoterol were weighed and powdered. A quantity of the powder equivalent to 100 mg was transferred into 100 ml volumetric flask and shaked with 20 ml water for about 10 min. the volumes were adjusted with water and then filtered. Analysed aliquots of the clear filtrate, labeled to contain (1 mg ml^{-1}), suitably diluted, and subjected to procedures A, B, C, and D.

RESULTS AND DISCUSSION

Method A (Nitration and subsequent complexation)

Nitration of phenolic drug (Fenoterol) results in the formation of the dinitro derivatives in the two ortho positions to the two phenolic groups. The formed complex between the electron-deficient polynitro derivative and the created acetone carbanion will produce intensely coloured complex (**Hesham 2004)**. This complex is of Meisenheimer type (**Strauss 1970)** and differs from those obtained by adding only an alkali to nitroso-derivative. The absorption spectrum of the complex is shown in fig 2.

Different conditions affecting the reaction were studied. Different reagents for nitration were tried such as $NaNO_2$ in presence of H_2SO_4 or mixture of concentrated HNO_3 and H_2SO_4. The best result were obtained on using 2 ml mixture of concentrated HNO_3 and H_2SO_4 of 1 : 1 mixture.

Maximum absorbance was obtained when using 3 ml of acetone Fig. 3 and 3 ml of potassium hydroxide Fig. 4. The absorbance was not much affected outside the specified

ranges. The colour formed was stable for at least one hour. The suggested pathway of Fenoterol with nitroso derivative is shown in Scheme.

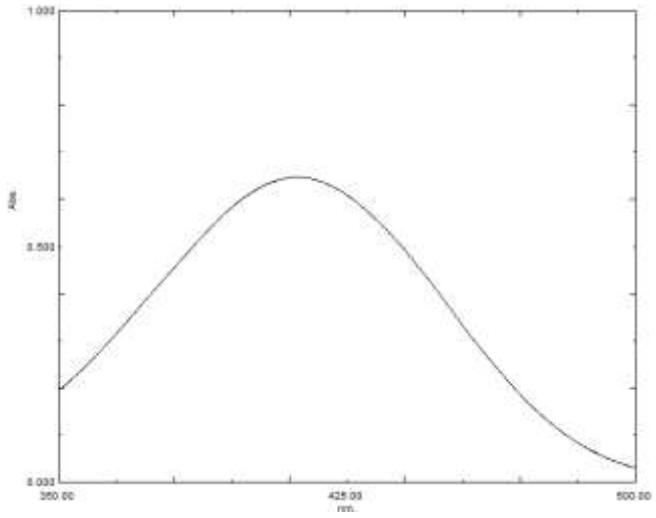


Fig. 2. Absorption spectra of Fenoterol (4 μg ml^{-1}) nitro derivative.

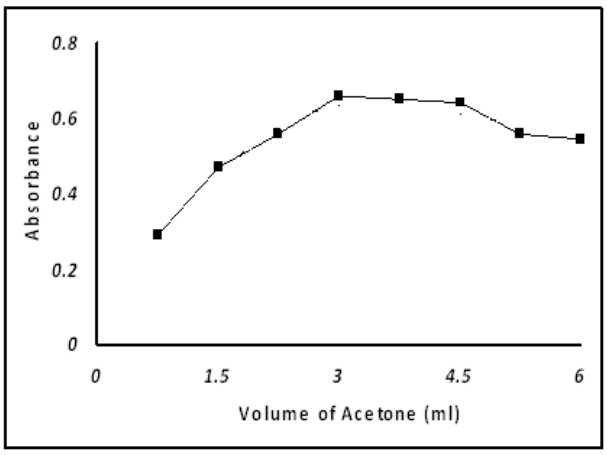


Fig. 3. Effect of acetone volume on the absorbance of the reaction product with Fenoterol.

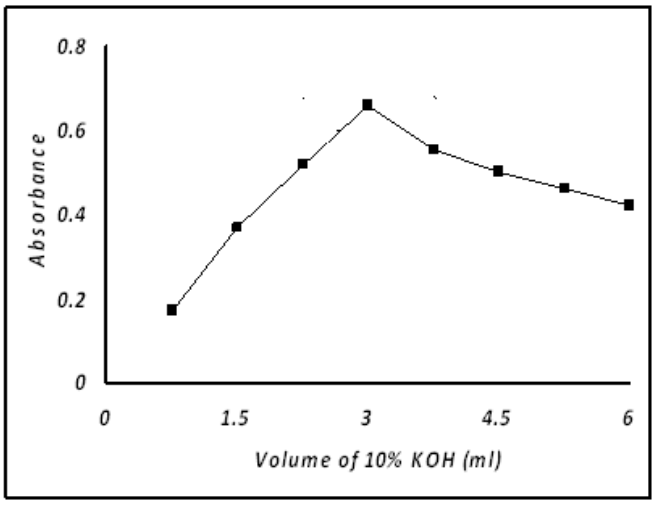
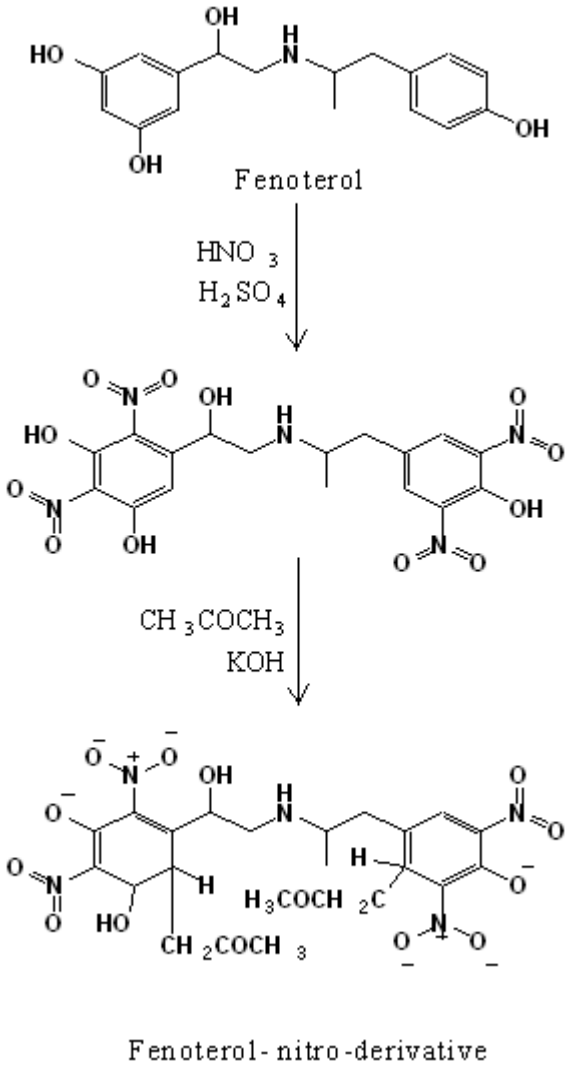


Fig. 4. Effect of KOH volume on the absorbance of the reaction product with Fenoterol

Reaction mechanism was suggested to be as follow (Strauss 1970)



Scheme 1. The suggested reaction pathway of Fenoterol by nitration in presence of alkali.

Method B (Diazo coupling with o-nitro aniline)

The utility of diazotized o-nitroaniline as a chromogenic reagent for the determination of the phenolic drug was investigated in the present study. The stability of the complex maintained by the use of 1.5 ml of 0.1% of o-nitroaniline solution (Fig. 5), 2.5 ml of 1% $NaNO_2$ solution (Fig. 6) making the medium alkaline with 3 ml 1N NaOH solution (Fig. 7). The reaction mixture was allowed to stand for 5 min. before adjusting volumes with distilled water and measuring the absorbance at its λ_{max}, which was 505 nm (Fig. 8). The colour intensity was found to be stable for more than one hour. The reaction pathway is shown in scheme 2.

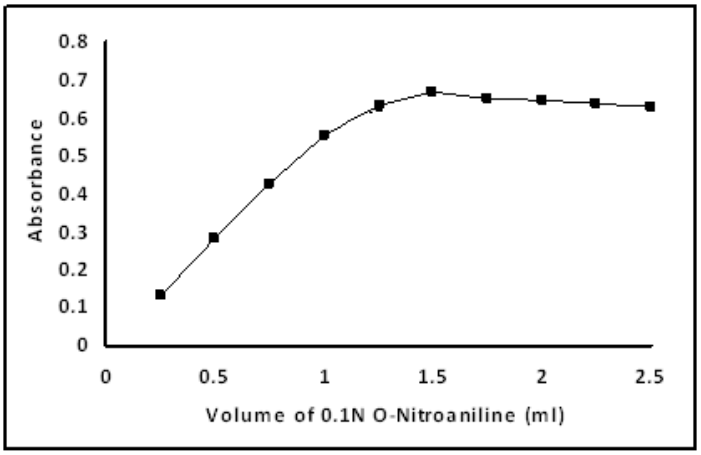


Fig.5. Effect of volume of O-nitroaniline on the absorbance of the reaction product with Fenoterol.

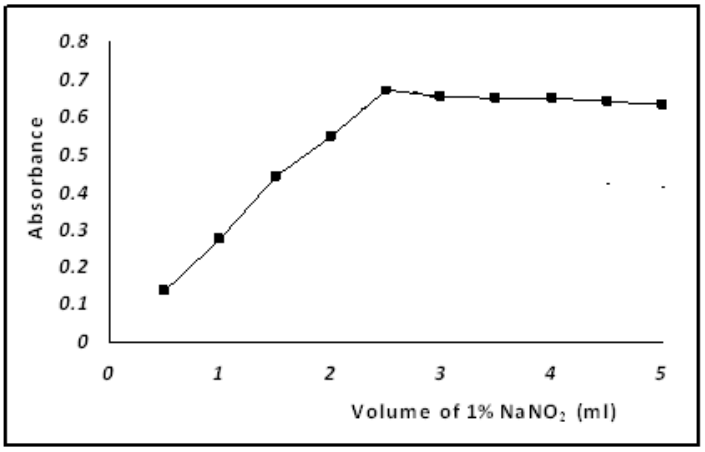


Fig. 6. Effect of volume of nitrite on the absorbance of the reaction product with Fenoterol.

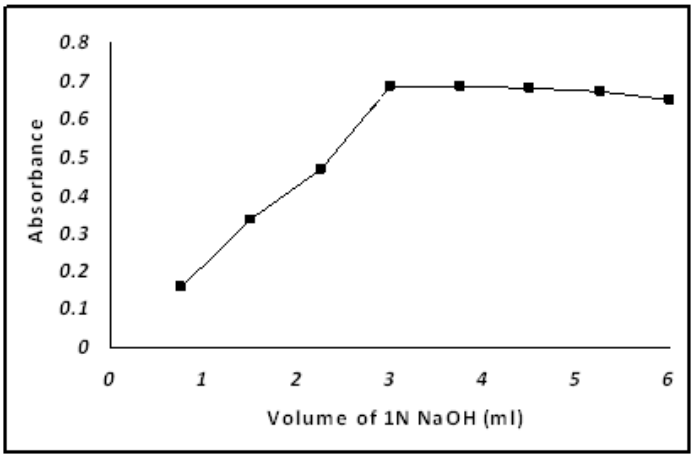


Fig.7. Effect of volume of NaOH on the absorbance of the reaction product with Fenoterol.

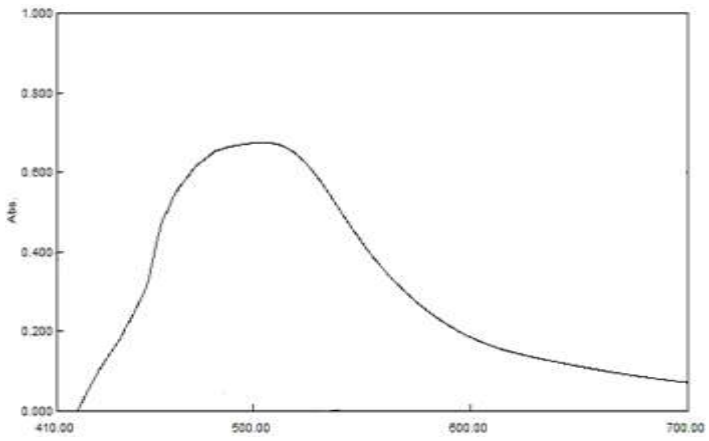
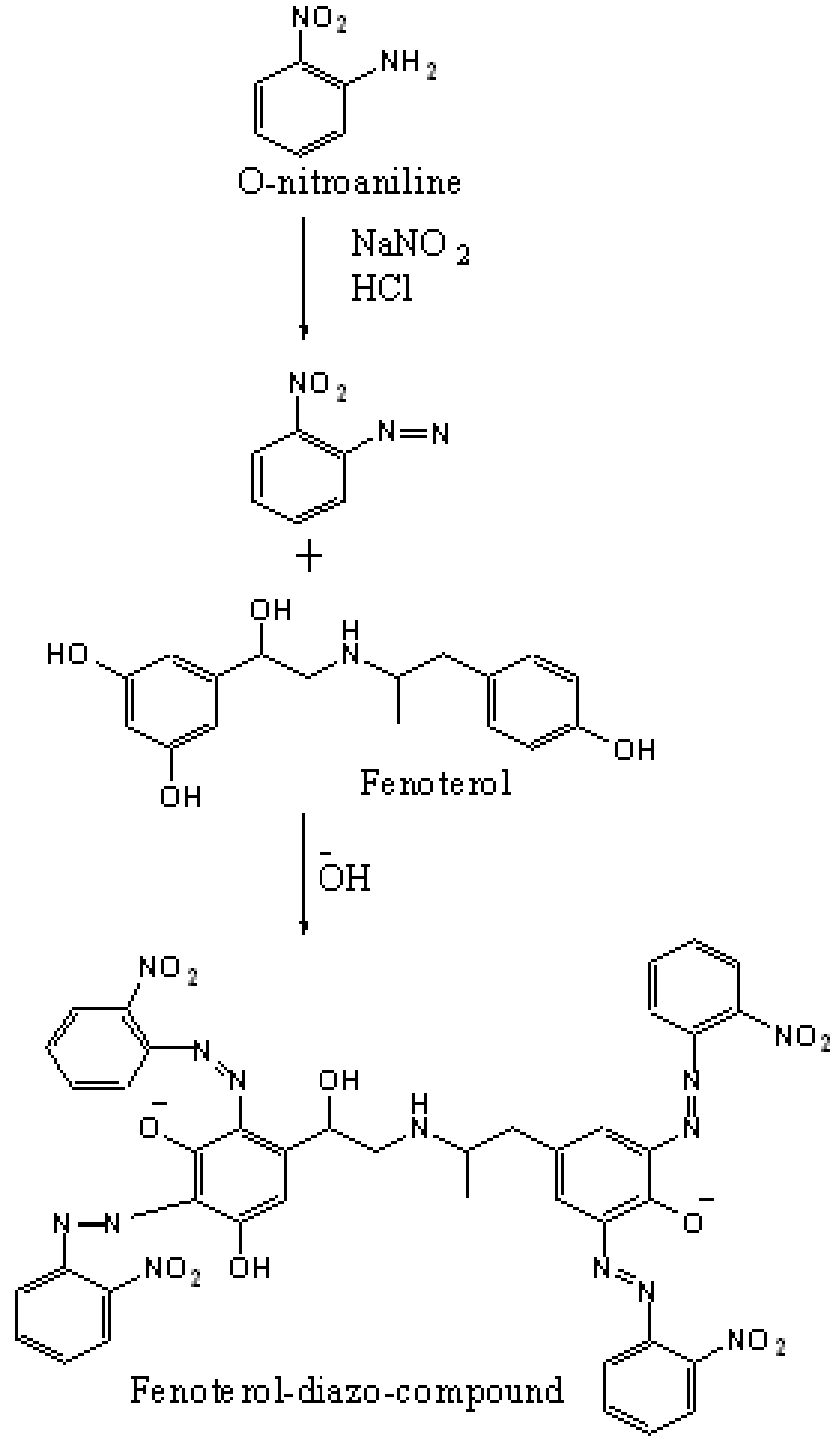


Fig. 8. Absorption spectra of Fenoterol (15 µg ml^{-1}) with Diazotized o-nitroaniline.

Reaction mechanism was suggested to be as follow (Strauss 1970)



Scheme 2. The suggested reaction pathway of Fenoterol with diazotized 0.1% o-nitroaniline.

Method C (diazo-coupling and subsequent chelation)

This method was extended to utilize the coupled compound as a chelating agent for copper (II) as the former contains a phenolic hydroxy group with an easily replaceable

proton and a diazo group (–N = N–) that offers a lone pair of electrons, and the two groups are so arranged as to include copper (II) ion in a six membered ring in the chelate **(Strauss 1970)**.

The copper (II) chelate was hardly soluble in water but was easily extractable into chloroform to give a yellow solution (λ_{max} at 412 nm) (Fig. 9). The optimum conditions involve using 5 mL of 0.125 M copper sulphate which give highest absorbance as shown in Fig. 10

The chelate was stable for at least 4 h after extraction. A slightly acidic medium favoured quantitative copper (II) chelate formation and quantitative extraction. It has been found that the use of 6 ml of 6 N H_2SO_4 after the addition of the copper (II) sulphate solution gave maximum absorbance, indicating quantitative chelation as shown in Fig.11.

Moreover, the order of reagent addition, copper (II) sulphate followed by 1N sulphuric acid was essential as addition of 1N sulphuric acid before copper (II) sulphate solution destroyed the diazo- compound. The reaction pathway for the proposed technique is illustrated in scheme 3.

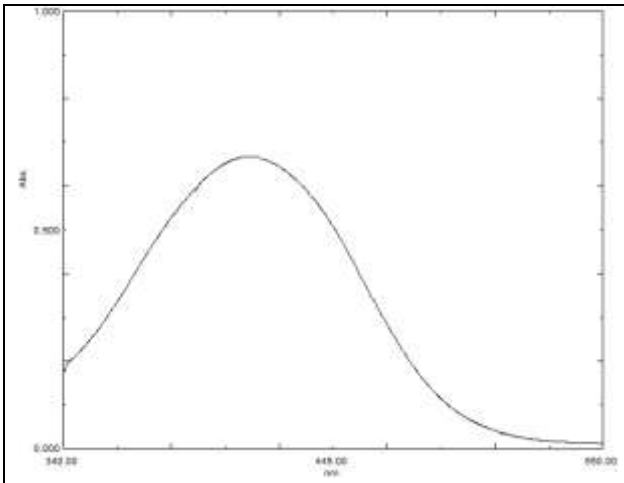


Fig. 9. Absorption spectra of chelation product of Fenoterol (15 µg ml-1) with $CuSO_4$

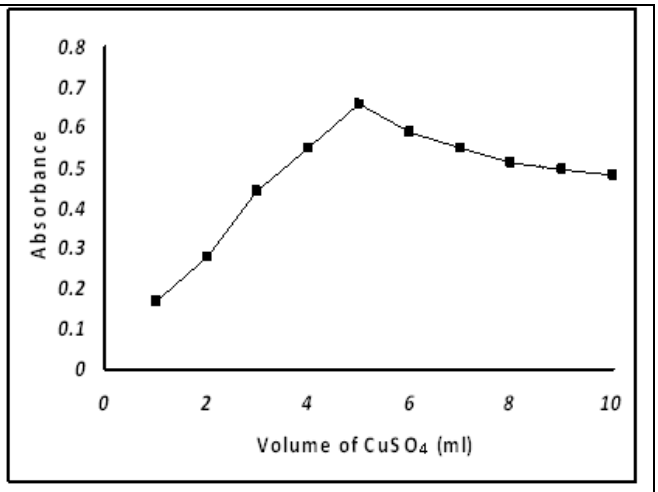


Fig. 10. Effect of volume of $CuSO_4$ on the absorbance of the reaction product with Fenoterol.

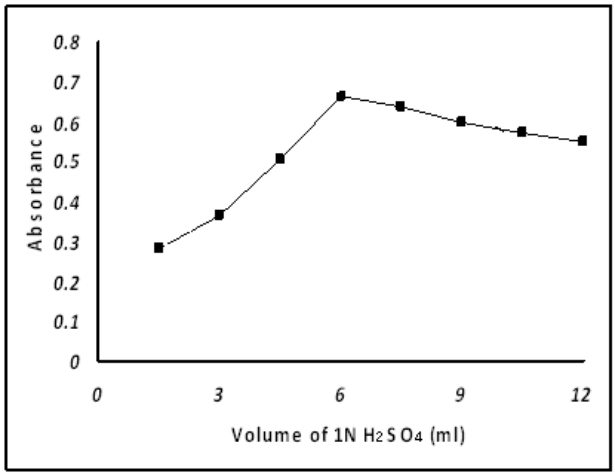
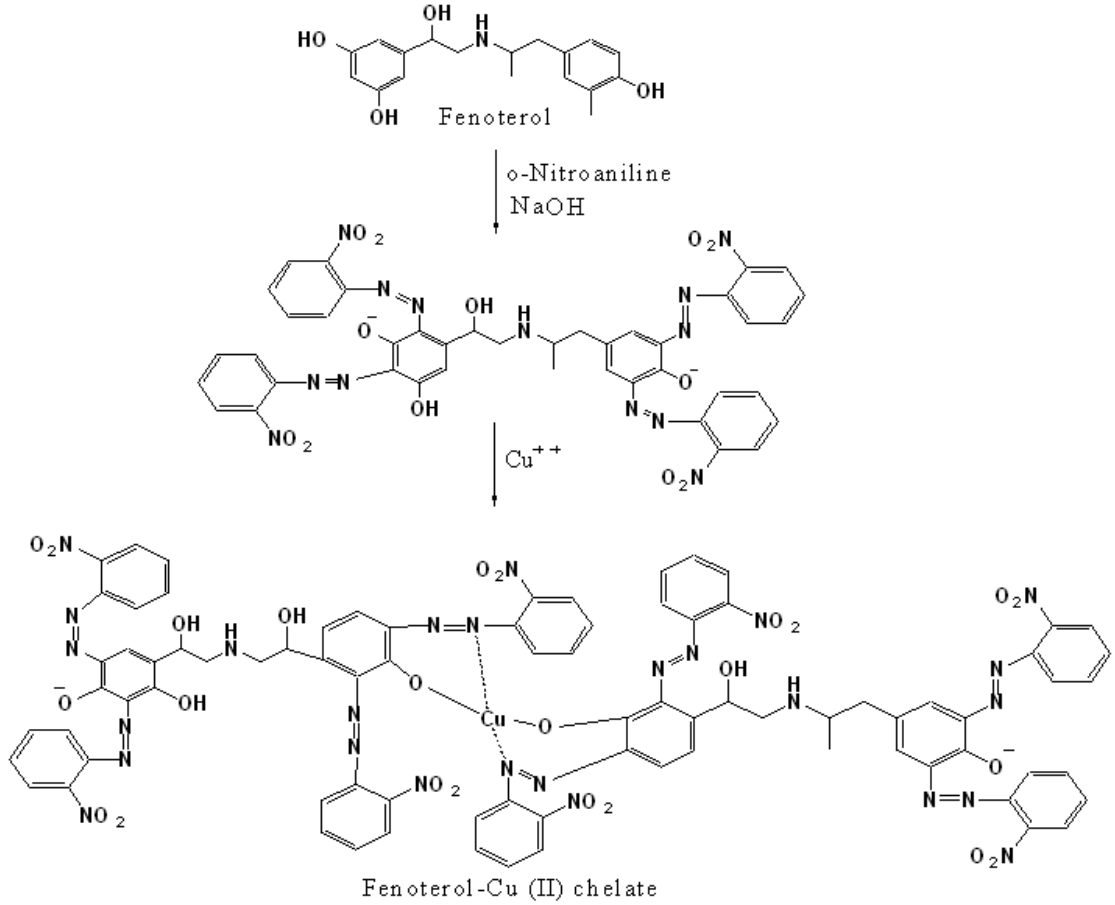


Fig. 11. Effect of volume of H_2SO_4 on the absorbance of the reaction product with Fenoterol.

Reaction mechanism was suggested to be as follow



Scheme 3. The suggested reaction pathway of fenoterol with 0.1 % o-nitroanailine and copper sulphate.

Method D (FCP method)

The absorption spectrum of the coloured species results from the reaction of fenoterol with FCP reagent showed a maximum absorbance at 655 nm (Fig. 12). The experimental conditions were established by varying each parameter individually and observing the effect on absorbance of the colour species. A volume of 1 ml of FCP reagent (Fig. 13), 5 ml of 1N NaOH solution (Fig. 14) and standing for 20 min (Fig. 15) were found to be the optimum conditions. The colour formed was stable for at least 1 hour.

The color formation by FCP reagent with Fenoterol may be explained in the following manner, the mixed acids in the FCR involve the following chemical species.

$3H_2O.P_2O_5\ 13Wo_3.5Mo\ O_3.10H_2O$

$3H_2O.P_2O_5\ 14Wo_3.4Mo\ O_3.10H_2O$

The drug affects reduction of 1,2, or 3 oxygen atoms from tungestate and/or molybdate in FCP reagent producing one or more possible reduced species which have a characteristic intense blue colour (**Sastry *et al.*, 1996)**.

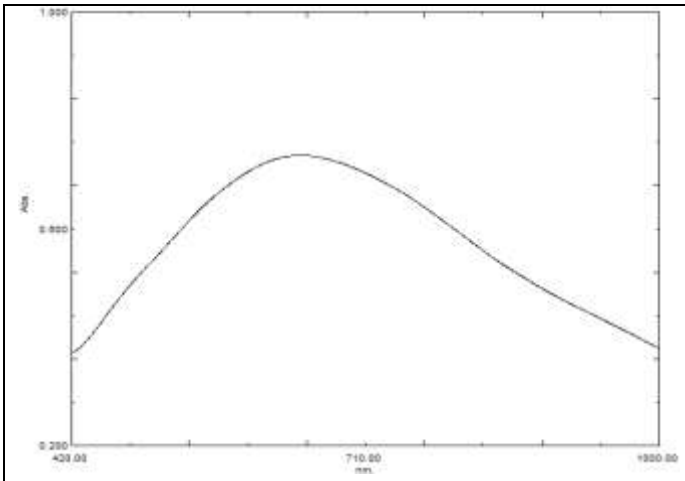


Fig. 12. Absorption spectrum of reduction product of Fenoterol (10 μgml^{-1}) with Follin ciocalteu's phenol reagent.

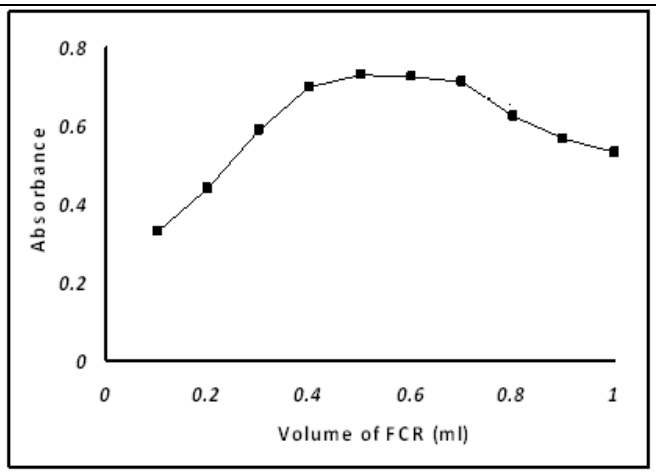


Fig. 13. Effect of 2 N FCR on the absorbance of the reaction product with Fenoterol.

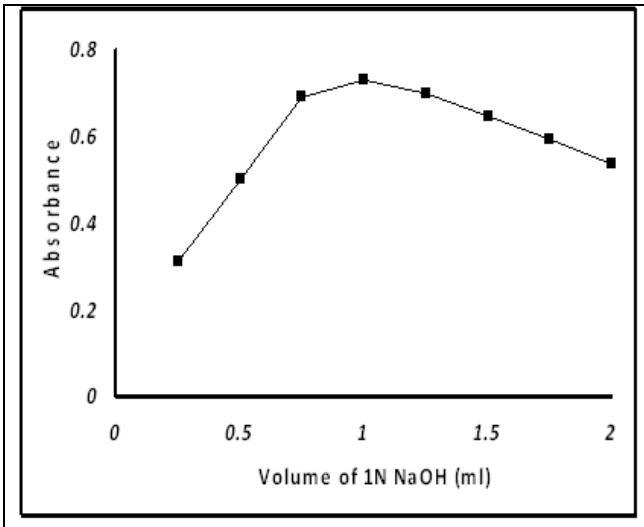


Fig. 14. Effect of volume of NaOH on the absorbance of the reaction product with Fenoterol.

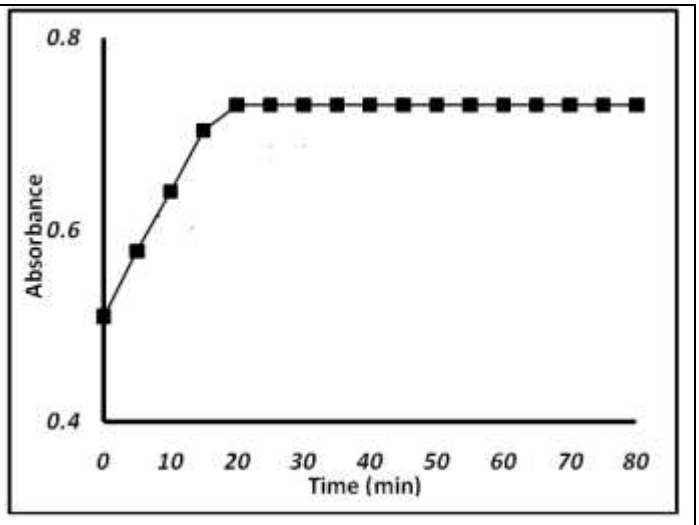


Fig. 15. Effect of time on the absorbance of the reaction product with Fenoterol.

Validation of the procedures :

Linearity :

The linearity range of the drug was validated, where good correlation between the absorbencies and the corresponding drug concentrations for method A, B, C, and D in the range of 1 – 6, 3 – 21, 3 – 21, 2 – 14 μg ml^{-1}, respectively.

LOD and LOQ

The experimental LOD and LOQ for the described procedures were determined according to the USP **(United state pharmacopeia, 2000)**, (Table 1).

TABLE 1. Selected spectral data for the determination of Fenoterol by the proposed procedures.

Method	*A*	*B*	*C*	*D*
λ_{max} nm	411	505	412	655
Linearity range (µg ml^{-1})	1 – 6	3 – 21	3 – 21	2 – 14
LOD (µg ml^{-1})	0.024	0.123	0.056	0.0114
LOQ (µg ml^{-1})	0.081	0.410	0.188	0.0381
Working range (µg ml^{-1})	1-6	3 - 21	3 - 21	2- 14
Response factor ± SD	0.161 ±0.001	0.045 ±0.0004	0.44 ±0.008	0.069 ±0.0009
A (1%, 1 cm)	1610.18	454.38	441.94	738.5
Regression Parameters- Slope	0.158	0.04467	0.04415	0.0711
- Intersept	0.0058	0.00509	0.00084	0.0136
Correlation Coefficient (r^2)	0.9996	0.9998	0.9998	0.9997

Accuracy and precision

Intraday and interday accuracy and precision of the proposed procedures were calculated. Table 2 revealed the accuracy and precision of the developed methods.

TABLE 2. Intraday* and interday* accuracy and precision for the determination of Fenoterol by the proposed procedures:

Method	*Conc. µgml⁻¹*	*Intraday*			*Interday*		
		Found Conc. +SD	*Accuracy (R%)*	*Precision (RSD%)*	*Found Conc. ±SD*	*Accuracy (R%)*	*Precision (RSD%)*
A	3	3±0.006	100	0.762	3.05±0.002	100.66	0.551
	4	3.89±0.05	99.5	1.3	3.99±0.02	99.75	0.5
	5	5±0.02	100	0.4	5±0.002	100	0.401
B	9	9.01±0.004	100.11	0.591	9±0.001	100	0.916
	12	12.11±0.004	100.91	0.717	12.12±0.005	101	0.86
	15	15.13±0.003	100.88	0.55	14.89±0.004	99.24	0.74
C	12	12.05±0.002	100.41	0.762	12±0.01	100	0.654
	15	15±0.003	100	0.432	15.3±0.06	102	0.623
	18	18.23±0.005	101.27	0.341	18.3±0.003	101.66	0.72
D	8	7.95±0.002	99.37	0.651	7.98±0.002	99.75	0.65
	10	10±0.02	100	0.321	10.10±0.003	101	0.346
	12	11.85±0.031	98.75	0.72	12±0.006	100	0.567

* n = 5

Specificity

Owing to the phenolic character of the drug investigated, the reaction was found to be specific for Fenoterol in presence of its excipients and diluents.

Stability of standard solutions

The stability of Fenoterol solution was evaluated and was found to be stable for 7 days at room temperature and 3 weeks in refrigerator.

Analysis of pharmaceutical preparations

The proposed procedures were also adopted for the determination of Fenoterol in Berotc® tablets. It should be pointed out that no interference by excipients and additives in Berotc® tablets.

The recovery of the proposed methods was assured by applying the standard addition technique (Table 3).

The results obtained by the proposed procedures were statistically compared with those obtained by the reported spectrophotometric methods[3] after reaction with 4-aminoantipyrine. The data in Table 4 shows that the calculated "t" and "F" values are less than the tabulated ones, indicating no significant difference between the proposed methods and reported one, confirming accuracy and precision at 95% confidence limits.

TABLE 4. Statistical analysis of results obtained by the proposed and reported method for the determination of Fenoterol in its pharmaceutical preparation

Methods	**A**	**B**	**C**	**D**	**Reported*** method**
*** N**	**6**	**6**	**7**	**7**	**5**
Mean	99.76	100.63	99.7	99.27	100.60
SD	0.55	0.85	0.83	0.31	0.77
RSD%	0.55	0.85	0.83	0.31	0.77
t**	1.43 (2.16)	0.06 (1.83)	0.356 (2.26)	1.34 (2.16)	
F**	3.05 (3.73)	1.23 (6.26)	1.39 (6.26)	3.04 (4.21)	

* Number of experimental.

** The values in parenthesis are tabulated values for "t" and "F" at $P < 0.05$.

*** The reported method involves spectrophotometric method[3]

CONCLUSION :

The proposed methods are simpler, faster and more sensitive than the reported method.

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تعيين فينوتيرول فى صورته النقية والمستحضر الصيدلى بأستخدام طرق طيف ضوئية

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فى هذا البحث تم استنباط اربع طرق طيف ضوئية لتقيم عقار فينوتيرول هيدروكلوريد سواء فى الصورة النقية أو المستحضر الصيدلى

الطريقة الأولى: تم استنباط طريقة لتعيين عقار فينوتيرول بطريقة النيترة بإستخدام حمض الكبرتيك المركز وحمض النيتريك المركز ثم إضافة الاسيتون فى وجود هيدروكسيد البوتاسيوم مكونا لونا أصفر يمكن قياسة عند طول موجى ٤١١ ن.م وبتركيز يتراوح مابين ١ ـ ٦ ميكروجرام /مل .

الطريقة الثانية: تم استباط طريقة لتعيين عقار فينوتيرول وذلك بترابطه كمادة فينولية مع ملح الديازونيوم المكون من مادة الارثوفينالين مكونا لونا أحمريمكن قياسه عند طول موجى ٥٠٥ ن.م وبتركيز يتراوح مابين ٣ ـ ٢١ ميكروجرام/مل.

الطريقة الثالثة: تعتمد على تعيين عقار فينوتيرول وذلك باستخدام الطريقة الثانية ثم اضافة كبريتات النحاس واستخلاص اللون الأصفر بإستخدام مادة الكلوروفورم ثم قياس هذا اللون عند طول موجى ٤١٢ ن.م وبتركيز يتراوح مابين ٣ ـ ٢١ ميكروجرام/مل

الطريقة الرابعة : تعتمد على إختزال كاشف الفولين سيوكالتس فينول بواسطة عقار فينوتيرول فى وجود محلول هيدروكسيد الصوديوم وقياس اللون الأزرق الناتج عند طول موجى ٦٥٥ ن.م وقد وجد أن هناك علاقة طردية بين الامتصاص والتركيز فى مدى ٢ـ١٤ ميكروجرام/مل

هذا وقد تم تطبيق هذه الطرق بنجاح لتقييم عقار فينوتيرول كمادة خام وفى المستحضر الصيدلى كما تم مقارنة النتائج التى تم الحصول عليها بهذه الطرق بنتائج الطريقة المنشورة ووجد انه لاتوجد فروق تذكر بين نتائج تلك الطرق