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SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF FENOTEROL HYDROBROMIDE IN PURE AND DOSAGE FORMS

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ABSTRACT

Two sensitive, simple and rapid spectrophotometric methods for determination of fenoterol either in pure or pharmaceutical preparation are described. The first proposed method (A) is based on coupling fenoterol with 3-Methylbenzothiozolin-2-one-hydrazone-hydrochloride (MBTH) in presence of ferric chloride hexahydrate in acid medium. The resulting stable coloured product showed an absorption maximum at 485 nm against reagent blank. The second proposed method (B) is based on the oxidative coupling of fenoterol with 4-Aminoantipyrine (4-AAP) in presence of potassium dihydrogenphosphate and potassium ferricyanide. The stable coloured product showed an absorption maximum at 528 nm. Appropriate conditions were established for both methods. Beer's law is obeyed in the concentration range 4-12 μgml^{-1} and 10-20 μgml^{-1} for method A & B respectively. Molar absorptivity and Sandell sensitivity for method A 8.05×10^{4} $l.mol^{-1}$ cm^{-1} and 4.8×10^{-3} μgcm^{-2}, while for method B is 3.194×10^{4} l $mol^{-1}cm^{-1}$ and 1.202×10^{-2} μgcm^{-2}. The detection and quantification limits are calculated. The developed methods are applied successfully for the determination of fenoterol in pure and in pharmaceutical formulation without interference from common excipients.

INTRODUCTION

Fenoterol, 1-(3,5-Dihydroxyphenyl)-2(4-hydroxy-α-methylphene-thylamino) ethanol, is a direct-acting sympathomimetic agent with predominantly β-adrenergic activity and a selective action on β_2-receptors. It is used as a bronchodilator, and its bronchodilating action is relatively more prominent than its effect on the heart. It is also used in the treatment of asthma, exercise-induced asthma and premature labor[1]. Several analytical methods are available in the literature for the determination of fenoterol in dosage from and biological fluids. These methods include spectrophotometery[2], GC/MS[3,4], fluoroimmunoassy[5,6], HPLC[7-9], Voltammetry[10], coulometry[11], colorimetric – flow injection[12], isotope – labeling[13], electrophoresis[14,15] and isotachophoresis[16].

All these methods are either insufficiently sensitive or tedious and expensive. Moreover, fenoterol weakly absorbs light in the UV region of the spectrum, the value of A 1% 1 cm 275 nm is 167[17] therefore, direct measurement of the absorbance is not reliable for its determination. It is obvious that there is still a need for fast simple and sensitive method, specially for routine analysis of pharmaceutical products containing fenoterol. This work describes two spectrophotometric methods for the determination of fenoterol hydrobromide, based on reaction with MBTH and 4-AAP. The methods were applied successfully to the determination of fenoterol in its dosage forms.

EXPERIMENTAL

1 Apparatus :

Shimadzu UV and visible recording spectrophotometer (UV-260) with 10 mm matched quartz cells was used for all spectral measurements.

2 Materials and Reagents :

All reagents were of analytical grade and all solvents were of spectroscopic grade. Authentic sample of fenoterol hydrobomide was kindly provided by Boehringer, Ingelheim, Germany :

1. MBTH solution, 0.4% w/v and 3×10^{-3} M (0.106%) solutions in distilled water were freshly prepared. Sigma (USA):
2. Ferric chloride hexahydrate solution 0.4% w/v in distilled water. (Merck Germany):
3. 4-Aminoantipyrine (4-AAP) solution, 0.1% w/v and 3×10^{-3} M (0.0203% w/v) in distilled water was freshly prepared obtained from Aldrich (USA).
4. Potassium dihydrogen phosphate, 0.05% aqueous solution. (Merck-Germany).
5. Potassium ferricyanide solution 0.1% w/v was freshly prepared in distilled water from Aldrich USA.

3 Standard Drug Solutions :

Solutions of 1 $mgml^{-1}$ were prepared by dissolving 100 mg of fenoterol hydrobromide in distilled water in a 100 ml volumetric flask and diluting to volume. The solutions were stable for at least 48 h in a cool (4°C) and dark place. Standard solution were prepared by diluting the appropriate volume of stock solutions with distilled water to give concentrations mentioned in table (1).

4 Pharmaceutical formulation :

1. Berotec® tablets from Boehringer Ingelheim company each tablet labeled to contain 2.5 mg fenoterol hydrobromide (Batch No 216117).
2. Berotec® metered aerosol from Boehringer Ingelheim company for oral inhalation each metered dose is labeled to contain 200 µg fenotenol hydrobromide (Batch No. 115801).

5. General Procedures

5.1 Procedure using MBTH :

Appropriate volumes of standard solution in concentration range 4-12 of fenoterol were placed in 10 ml volumetric flasks, with the appropriate volumes of the reagent 1ml 0.4% MBTH solution and 2ml 0.4% ferric chloride. mixed well, stand for 10 min at room temperature, diluted to 10 ml with distilled

water. The absorbance was measure at 485 nm against a reagent blank, prepared simultaneously omitting the drug (Table 1).

5.2 Procedure using 4-AAP:

Appropriate volumes of solutions prepared from the standard drug solution, in concentration range 10-20 of fenoterol were placed in 10 ml volumetric flasks followed by 2 ml 0.05% potassium dihydrogen phosphate, 1 ml 4-AAP solution, then 2 ml of potassium ferricyanide solution. Mixed well, stand for 10 min at room temperature and complete to 10 ml with distilled water. Measure the absorbance at 528 nm against a blank which was prepared simultaneously omitting the drug, Table (1).

5.3 Procedure for pharmaceutical preparations :

5.3.1 For tablets :

Twenty tablets were weighed, finely powdered and a portion of the powder equivalent to 25 mg of the drug were taken into a 25 ml volumetric flask. Make up to volume with water, shake for 15 minutes then filter. Analyze the solution as described above (Tables 4, 5)

5.3.2 For aerosols :

Shake the aerosol container then place its mouth-piece under 80 ml of water in a 100 ml beaker and spray 25 metered doses. After mixing, transfer to a 100 ml volumetric flask and make up to volume using water. Shake for about 15 minutes then filter. The methods were continued as mentioned above:

An additional check on the accuracy of the proposed method, recovery experiments were performed by adding known amounts of drugs to already analyzed pharmaceutical preparations (Tables 6, 7).

6. Mechanism of reactions

6.1 Reaction using MBTH and 4-APP :

The proposal methods depending upon the presence of phenolic groups in the tested drugs, so MBTH and 4-AAP in presence of ferric chloride and potassium ferricyanide respectively were loses two electrons and produce electrophillic intermediate which is the active coupling species [18-19]. It is suggested that the intermediate undergos electrophilic substitution with the drug. Molar ratio study showed that the drug interact with MBTH and 4-AAP in ratio 1:1 (Figs 5, 10).

The absorption spectra of the colored reaction product at the optimum conditions recorded in the general procedures showed a characteristics λ_{max} at 485 nm and λ_{max} 528 respectivly (Fig. 1)

7. Stiochiometry of the reaction :

Job's method of contineous variation was applied. A series of standard solutions of (fenoterol "3×10^{-3} M) was transferred into 10 ml measuring flasks,then MBTH solution (3×10^{-3} M for method (1) and 4-AAP solution (3×10^{-3} M) for method (2), solutions were added in complementary proportions of total volume of 2 ml (from 0+1 to 1+0 inclusive), each was treated with the reported volumes of other reagents. After complete reaction, the absorbance was measured at the appropriate λ_{max} against a blank prepared simultaneously omitting the drug.

RESULTS AND DISCUSSION

The spectra of the coloured reaction products of drug and the corresponding reagent in the two methods were shown in Fig. (1). The spectra of the reaction products show characteristic λ_{max} at 485 nm and 528 nm for methods (1) and (2) respectively. The experimental condition were established by varying the different parameters.

1 Method (A) using MBTH :

Various parameters affecting the reaction process to obtain the most intense and stable color were studied by varying one parameter while the others were kept constant concerning to the amount of MBTH.

1.1 Effect of MBTH concentration :

When different volumes of 0.4% MBTH solution were added to a fixed concentration of the drug, 1 ml was found to be sufficient for maximum color intensity. Increasing the reagent volume will decrease the colour intensity (Figure 2).

1.2 Effect of oxidizing agent :

Different oxidizing agents were studied such as ferric chloride, iron (III) ammonium sulphate, potassium per sulphate and hydrogen peroxide, ferric chloride was used as it gave the maximum development of the coloured products. Various concentration of ferric chloride were studied. It was found that 2 ml of 0.4% $FeCl_3$ gave maximum color intensity (Figure 3).

1.3 Effect of sequence of addition :

It was found that addition of drug solution followed by reagent solution then oxidant solution is the proper sequence for the determination fenoterol hydro-bromide.

It was found that standing for 10 min at room temperature were optimum for maximum absorption intensity of the colored product (Fig. 4).

The intensity will be slightly decreased with the time increase.

2 Method (B)Using 4-AAP:

The volumes of 4-AAP was studied by measuring the absorbancies at the specified wavelength for solutions containing a fixed concentration of the studied drug and varying the amount of 0.1% w/v 4-AAP solution, 1ml showed maxium colour intensity (Figure 6)

2.1 Effect of oxidizing agent :

Several oxidizing agents were investigated e.g. iron ammonium sulphate, hydrogen peroxide, potassium dichromate, potassium chromate, ferric ammonium sulphate, potassium periodate, potassium ferricyanide and ferric chloride. Potassium ferricyamide succeeded

to perform the oxidation process and stable colour. Increasing the volume of potassium ferricyanide was found to produce a proportional increase the absorbance till to 2 ml will give stable colour (Fig. 7). It is evident from (Fig. 8) that 1 ml of potassium dihydrogen phosphate is appropriate for the reaction larger volume would not increase the absorbance colour. The time required for complete reaction of fenoterol was found to be 10 min, longer times caused slightly decrease in absorbance (Fig. 9).

3. Stoichiometric relationship :

The investigation of Job's method (Fig. 5 & 10) showed that drug interact with MBTH and 4-AAP in the ratio 1:1.

Under the specified reaction condition, linear correlations were found between the abosrbance at 485 nm for method A using MBTH and absorbance at 528 nm for method B using 4-AAP. Beer's law limits, Sandel's sensitivity regression. The results were given in (Table 1).

4. Quantification, sensitivity, Accuracy and Precision :

A linear correlation was found between absorbance and concentration at specific λ_{max} for reagents in range in table (1). Intercepts, slops for the calibration data, molar absorptivities, coefficient of variation and Sandel's sensitivity for the cited drug were shown in table (1). The proposed methods were applied for the analysis of fenoterol hydrobromide in tablets and aerosal (Tables 4-7). Statistical analysis of the results obtained by the proposed methods and the official BP[20] reference method was done using student's (t-test) and the variance ratio (F-test). The calculated values didn't exceed the theoretical one, indicating no significant difference between the compared methods (Table 8-9). The proposed MBTH and 4-AAP methods are reproducible, accurate and precise need no special apparatus, determines low concentrations in comparison to the reference method. The recovery of the drugs was also tested by the standard addition method (Table 2,3) & pharmaceutical formulation tablets and aerosols (Tables 4-7) were found to be almost quantitative.

CONCLUSION

The proposed methods are rapid , simple and accurate. Thus it's recommended for direct determination of the cited drugs in pure and in pharmaceutical formulation without prior separation. Furthermore, the results obtained were encouraging to use the proposed methods for the determination of the cited drug in pharmaceutical formulations.

Tabel 1: Analytical parameters and Statistical data of regression equation for the determination of Fenoterol-hydrobromide using MBTH method (1) and 4-AAP method (2).

Analytical parameters and Statical data	Method (1) using MBTH	Method (2) using 4-AAP
Linear rang Beer' s Law ug ml^{-1}	4-12	10-20
λ_{max} nm	485	528
Reagent used	1 ml 0.4% MBTH	1 ml 0.1%4-AAP
Oxidising agent	2 ml 0.4% $FeCl_3$	2ml 0.1% $K_3Fe(CN)_6$
Other Reagent	--	2ml 0.05%KH_2PO_4
Temperature C°	Ambient 25 C°	Ambient 25 C°
Diluting solvent	Distilled Water	Distilled Water
Time For complete reaction (min)	10	10
Regession Eqation: a b r	 0.0006 0.02085 0.9996	 0.00199 0.00828 0.99920
Molar absorptivity L $mol^{-1}cm^{-1}$	8.05×10^4	3.194×10^4
Sandell' s Sensitivity ug cm^{-2}	4.8×10^{-3}	1.202×10^{-2}

Abs =b x C + a
Where C is the unknown concentration in ug ml^{-1}

Table 2: Determination of fenoterol using MBTH method (1).

Taken ug ml^{-1}	Found ug ml^{-1}	Recovery %
4.0	3.98	99.50
5.0	4.99	99.80
6.0	6.01	100.16
7.0	7.02	100.82
8.0	7.99	99.87
9.0	8.98	99.87
10.0	9.99	99.77
11.0	10.98	99.99
12.0	12.01	99.81
Mean	--	99.95±0.348
N	--	9
V	--	0.121
S.D	--	0.348
RSD	--	0.348
S.E	--	0.116

Table 3: Determination of fenoterol using 4-AAP method (2).

Taken µg ml^{-1}	Found µg ml^{-1}	Recovery %
10	10.1	101.00
11	10.98	99.80
12	12.05	100.40
13	13.07	100.50
14	13.99	99.92
16	16.04	100.25
17	16.95	99.70
18	18.05	100.28
19	19.07	100.36
20	19.99	99.95
Mean	--	100.216± 0.368
N	--	10
V	--	0.135
S.D	--	0.368
R.S.D	--	0.368
S.E	--	0.116

Table 4: Determination of Berotec® tablets using suggested MBTH method (1).

Taken µg ml^{-1}	Added µg ml	Found µg ml^{-1}	Recovery %
2	--	1.95	97.50
2	2	3.98	99.50
2	4	6.01	100.20
2	6	7.98	99.75
2	8	10.10	100.10
2	10	12.03	100.25
Mean	--	--	99.55± 0.954
N	--	--	6
V	--	--	0.910
S.D	--	--	0.954
R.S.D	--	--	0.958
S.E	--	--	0.389

Table 5: Determination of Berotec® tablets using suggested 4-AAP method (2).

Taken ug ml^{-1}	Added ug ml^{-1}	Found ug ml^{-1}	Recovery %
5	5	10.2	102.00
5	7	12.3	102.50
5	9	13.95	99.65
5	11	15.90	99.40
5	13	18.10	100.56
5	15	20.2	101.00
Mean	--	--	100.85± 1.132
N	--	--	6
V	--	--	1.282
S.D	--	--	1.132
R.S.D	--	--	1.122
S.E	--	--	0.462

Table 6: Determination of Berotec® aerosol using suggested MBTH method (1).

Taken µg ml^{-1}	Added µg ml^{-1}	Found µg ml^{-1}	Recovery %
1	2	2.95	98.33
1	4	5.1	102
1	6	7.2	102.8
1	8	9.1	101.1
1	10	10.99	99.90
1	11	12.1	100.83
Mean	--	--	100.83± 1.439
N	--	--	6
V	--	--	2.073
S.D	--	--	1.439
R.S.D	--	--	1.428
S.E	--	--	0.588

Table 7: Determination of Berotec® aerosol using suggested 4-AAP method (2).

Taken µg ml^{-1}	Added µg ml	Found µg ml^{-1}	Recovery %
4	6	10.1	101.0
4	8	11.95	99.6
4	10	13.90	99.3
4	12	16.2	101.3
4	14	18.3	101.7
4	16	20.2	101.0
Mean	--	--	100.65± 0.885
N	--		6
V	--	--	0.782
S.D	--	--	0.885
R.S.D	--	--	0.879
S.E	--	--	0.361

Table 8: Determination of fenoterol using suggested method (1) MBTH and method (2) Using 4-AAP compared with reference B.P method[20]

Item	Method 1 using MBTH	Method 2 using 4-AAP	Official B.P method
Mean	99.95	100-216	100.22
N	9	10	6
V	0.121	0.135	0.0915
S.D	0.348	0.368	0.302
R.S.D	0.348	0.368	0.123
S.E	0.116	0.166	0.301
t	2.418 (4.77)	0.125 (4.77)	--
F	1.322 (5.05)	1.475 (5.05)	--

Table 9: Determination of pharmaceutical formulation using suggested method (1) MBTH and method (2) 4-AAP compared with reference B.P method[20].

	Item	Proposed Method (1) MBTH	Proposed Method (2) 4AA-P	Official B.P Method [20]
Berotec® tablets	Mean % Recovery	99.55	100.85	100.33
	N	6	6.000	9
	Variance	0.91	1.282	0.570
	S.D	0.954	1.132	0.752
	S.E	0.389	0.462	0.252
	R.S.D	0.958	1.122	0.752
	t 0.5%	2.068 (3.37)	1.189 (3.37)	--
	F	1.596 (4.82)	2.249 (4.82)	--
	Item	Proposed Method (1) MBTH	Proposed Method (2) 4-AAP	Official B.P Method [20]
Berotec® aerosol	Mean % Recovery	100.83	100.65	100.28
	N	6	6	9
	Variance	2.073	0.782	1.846
	S.D	1.439	0.885	1.358
	S.E	0.588	0.361	0.452
	R.S.D	1.428	0.879	1.355
	t	1.139(3.37)	1.229 (3.37)	--
	F	1.123(4.82)	2.360 (4.82)	--

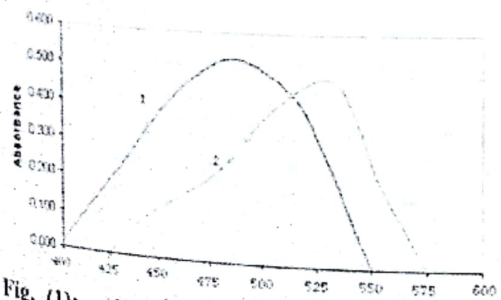

Fig. (1): (1) Absorption spectera of the reaction product of fenoterol using MBTH .(method 1) λmax 485 nm (2) Absorption spectera of the reaction product of fenoterol using 4-AAP (method 2) λmax 528 nm.

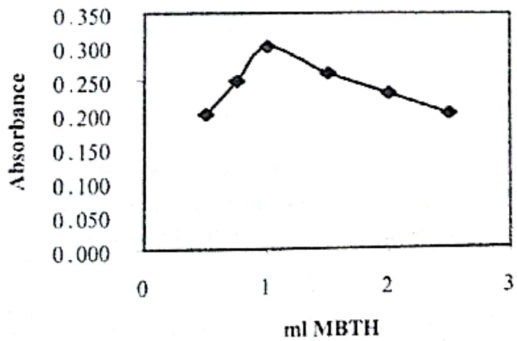

Fig. (2): Effect of volume of MBTH on the reaction of fenoterol

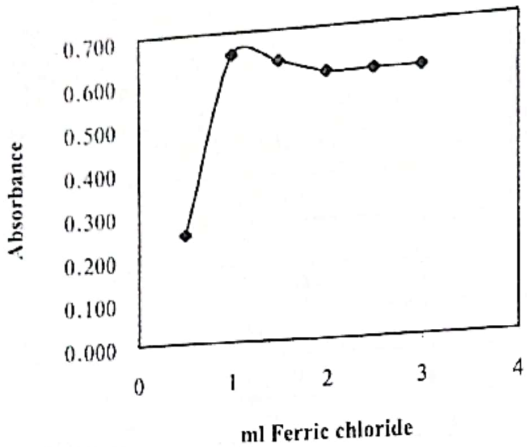

Fig. (3): Effect of volume of ferric chloride on the reaction of fenoterol using MBTH (method 1)

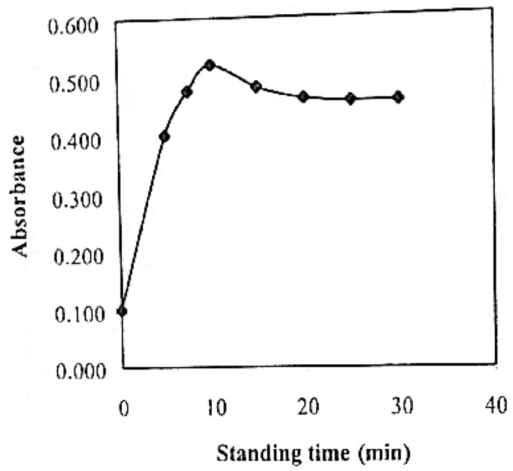

Fig. (4): Standing time (min) on the reaction of fenoterol using MBTH (method 1)

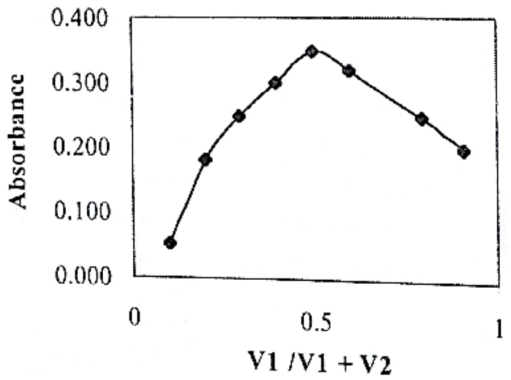

Fig. (5): Determination of the stoichiometry on the reaction between (3×10^{-3} M) fenoterol and (3×10^{-3} M) MBTH by continuous variation method.

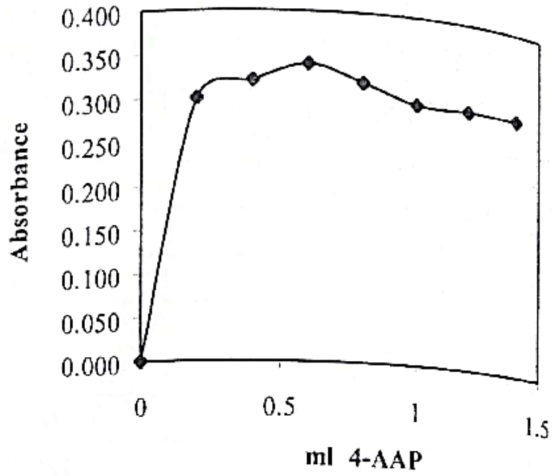

Fig. (6): Volume of 4-AAP on the reaction of fenoterol (method 2).

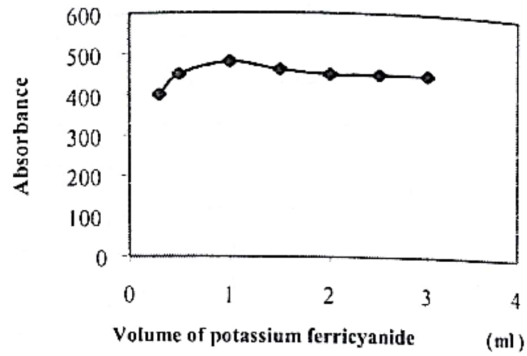

Fig. (7): Effect of volume potassium ferricyanide on the reaction between fenoterol using 4-AAP (method 2).

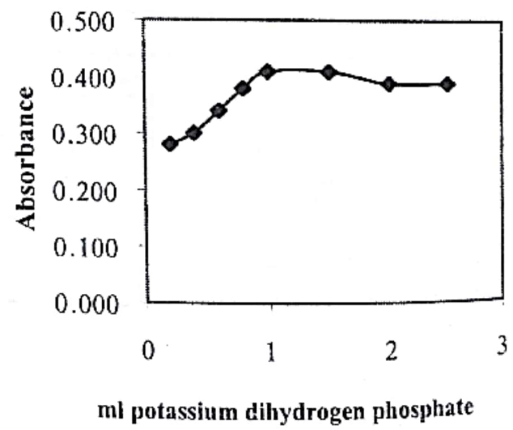

Fig. (8): Effect of volume of the potassium dihydrogen phosphate on the reaction between fenoterol using 4-AAP (method 2)

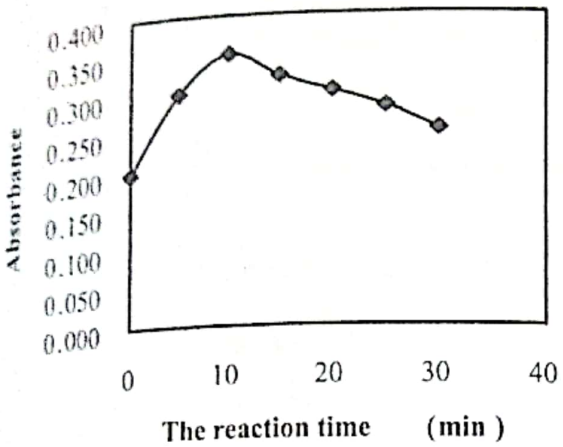

Fig. (9): Reaction time (min) between fenoterol using 4-AAP (method 2).

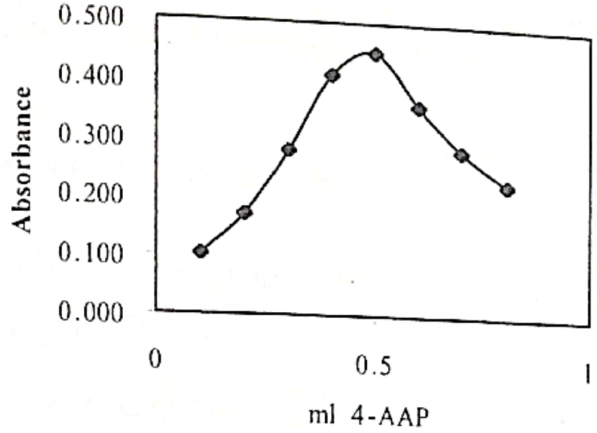

Fig. (10): Determination of the stoichiometry of the reaction between (3×10^{-3} M) fenoterol and (3×10^{-3} M) 4-AAP by continuous variation (method).

REFERENCES

1. J.E.F. Reynolds, editor, in "Martindale". The Extra Pharmacopoeia, 31st Edition. The Pharmaceutical Press, London, 1577 (1996).
2. M.A. Abounassif and E.A. Abdel-Moety, **Acta. Pharm Jugosl**, 39, 359 (1989).
3. F.J. Couper and O.H. Drummer, **J. Chromatogr**, B 685, 265 (1996).
4. J.G. Leferink, J. Dankers and R.A.A. Maes, **J. Chromatgor.** B, 229, 217 (1982).
5. C.T. Elliott, A. Baxter, W. Hasnoot, A. Lommen and W.J. McCaughey, **Food Agric. Immunol**, 8, 219 (1996).
6. W. Hassnoot, P. Stouten, A. Lommen, G. Gazemier, D. Hooijernik and R. Schilt, **Analysis**, 119, 2675 (1994).
7. A. Polettini, M. Montagna, E.A. Hogendoorn, E. Dijkman, P. Van-Zoonen and L.A. Van-Ginkel, **J. Chromatogr**, 695, 19 (1995).
8. G.A. Jacobson and G.M. Peterson, **J. Pharm. Biomed. Anal.**, 12, 825 (1994).
9. D.C. Jones, K. Dost, G. Davidson and M.W. George, **Analysis**, 124, 827 (1999).
10. D. Boyd, J.R.B. Rodriguez, A.J.M. Ordieres, P.T. blanco and M.R. Smyth, **Analysis**, 199, 1979 (1994).
11. K. Nikolic, L. Arsenijevec and M. Bogavac, **J. Pharm. Biomed. Anal.**, 11, 207 (1993).
12. S. Tanabe, T. Togawa and K. Kawanabe, **Anal. Sci.**, 5, 513 (1989).
13. D. Kohler, W. Fleischer and H. Matthys, **Respiration**, 53, 65 (1988).
14. M. Mazereeuw, A.J.P. Hofie, U.R. Tjaden and J. Van-Der-Greef, Rapid. **Commun. Mass. Spectrom**, 11, 981 (1997).
15. T. Wachs, R.L. Sheppard and J. Henion, **J. Chromatogr.** B, 685, 335 (1996).
16. M.H.Lamore, N.J. Reinhoud, U.R. Tjaden, W.M.A. Niessen and J. Van-Der-Greef, **Biol. Mass. Spectrom**, 23; 339 (1994).
17. A.C. Moffat, "Clarke's isolation and identification of drugs", 2nd ed. The Pharmaceutical Press, London, pp. 693 (1986).
18. Pospisilova, M., Polasek, M., and Suobodova, D. **Mikrochimica. Acta**, 129 (3-4), 201. (1998)
19. Revanasiddappa, H.D. and Manju, B.G., **J AOAC International**, 83(6), 1440. (2000)
20. British Pharmacopoeia. The Stationary Office, London, volume 1 (2001).

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طريقتان طيفيتان لتعيين مركب الفينوترول وذلك في صورته النقية او المستحضرات الصيدلية

محمد نجيب محمد البلقيني

قسم الكيمياء التحليلية - كلية الصيدلة – جامعة الزقازيق – الزقازيق - مصر

تـم اسـتحداث طريقتين طيفيتين سريعتين وبسيطتين وذلك لتعين مركب فينوترول الطريقة الأولى عن طريق دمج المستحضر الصيدلي في مادة ٣مثيل بنزوثيلزولين هيدرازون (MBTH)

وذلك في وجود مادة مؤكسدة هي كلوريد الحديديك في وسط حمضي وقياس اللون الناتج عند درجة امتصاص ٤٨٥ نـم . الطـريقة الثانـية هـي قـياس اللون الناتج عن دمج المركب الصيدلي مع مادة ٤-امينوأنتي بيرين (4-AAP) وذلـك في وجود بوتاسيوم فوسفات ثنائي الهيدروجين وكذلك بوتاسيوم فرايسينيد وقياس الطيف الناتج عـند درجة امتصاص ٥٢٨ نم وقد تم تجربة الطريقتين ومقارنتهما بالطرق الدستورية الأخرى (كدستور الأدوية الانجليزي) ووجدت النتائج الصادرة طيبة.