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Research Article

DETERMINATION OF SOME ANTIBLOCKERS THROUGH SPECTROPHOTOMETRIC, HPLC AND TLC-DENSITOMETRIC METHODS

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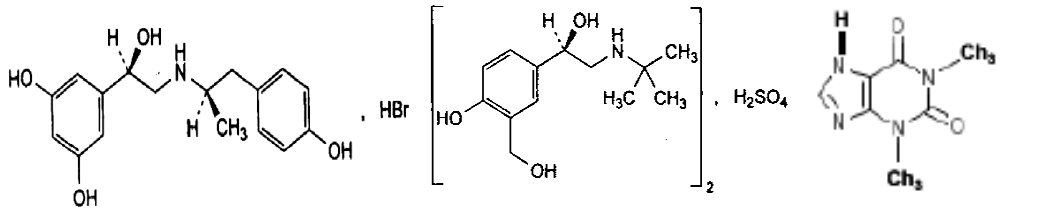
ABSTRACT

Three simple, accurate and precise methods were developed for determination of fenoterol HBr I, salbutamol sulphate II and theophylline III in pure forms and in pharmaceutical preparations. The first method based on ion-pair formation between I, II and hematoxylin in alkaline medium pH 9.5 to give a stable colored product that showed maximum absorption at 559 nm. The linearity range was found to be 20-100 and 15-100 µg/ml for I and II respectively with recovery found to be 99.1 and 99.1for I and II respectively. Sandle sensitivity found to be and 0.44 µg/cm^2 for I and II respectively. The second one was TLC scanning method for I and II using silica gel plates in Ethyl acetate: Methanol and Ammonia (26:4:1) and the spots was measured by densitometer at 280 nm for both drugs. The limits of Beer's law was 5-40 and 5-50 µg/spot in case of I and II respectively. Recovery found to be 101.12±1.17 and 100.20 ± 1.10 for I and II respectively. The third method was separated by HPLC for II and III using Lidocaine as internal standard and Luna 5-CN 150 X 4.6 mm column, the mobile phase was (0.1% Heptane sulphonic acid soudium salt + 0.1ml triethylamine + 1 ml glacial acetic acid): Methanol (35: 65 v/v) and detected by UV/Visible spectrometric at 244 nm. The liner relationship between area under beaks and concentration was given over a range from 20-400 and10-100 µg/ml for drugs II and III respectively. The three methods were successfully applied on the raw materials and in pharmaceutical preparations.

Keywords: Fenoterol HBr, HPLC, Spectrophotometric, TLC-Densitometer.

INTRODUCTION



(I) FenoterolHBr[2] **(II) Salbutamol[3]** **(III) Theophyillin[3]**

Fig. 1: Chemical structure of Fenoterol, Sulbutamolsulphate and theophylline

The drugs I, II and III are a group of bronchodilator which a direct-acting sympathomimetic agent with a relatively selective action on beta-adrenoceptors. It's main clinical uses were in the management of reversible airways obstruction [1] .

The official methods for the determination of drugs I,II and III have been described in British pharmacopeia[2,3]. Several methods have been reported for the determination each drug.

For drug I: Spectrophotometric methods [4,5] ,chromatographic methods including HPLC[6-10] ,GC [11,12] , spectrofluorimetry [13] , voltammetry [14] and MS[11,12].
For drug II: Spectrophotometric methods [15-17], chromatographic methods including HPLC [7,18,19] and GC[11].
For dug III: Spectrophotometric methods [20-22], chromatographic methods including HPLC[23-25] and TLC[26,27].

MATERIALS AND METHODS

Instrumentation

The present work was carried out on each of the following instruments:

i. Shimadzu model 160A UV/Vis. double beem spectrophotometer with 1.0 cm quartz cells.
ii. Shimadzu-Dual wavelength lamp flying CS 9301 densitometer.
iii. Ultraviolet short wavelength lamp 254 nm
iv. HPLC system, Helwett Packard 1100 Equipped with quaternary pump, diode array detector and manual injector 20 µl loop.

Reagents and Solutions

Fenoterol HBr was supplied from Cid-Bohringer, Egypt, Salbutamol sulphate from Galaxo-Welcome, Egypt and theophylline from Medical Union pharmaceuticals, Egypt. Hematoxylline from Merck, Egypt. All solvents: acetonitrile, methanol, ethanol and 1, 4 dioxane from B. D. H. Salbovent tab. 2 mg from Alexandria for pharmaceuticals, Alexadria, Egypt, Perotic tab. 2.5 mg from Cid-Bohringer, Egypt, Methanol (HPLC grade) obtained from Merck, glacial acetic acid, ethyl acetate, Dihydrogen phosphate and ammonia (33%) from B. D. H.
Drugs I, II (1 mg/ml) in methanol, hematoxylline (2 mg/ml) in water, Universal Buffer pH 2-12. For TLC method drugs I and II (4 µg/ml) and for HPLC method drugs II and III (0.25 mg/ml).
Solutions of drugs in pharmaceutical preparations: twenty tablets were accurately weighed and determine the average weight of the tablet, crush the tablets well to a fine powder. A powder equivalent to 100 mg of the active material was dissolved well in 100 ml methanol and filtered off.

General procedure for ion-pair complex

(0.1- 1) ml of drug solutions I, II was pipetted into 10.0 ml volumetric flask, then 2.0 ml of hematoxyllin solution and 2 ml of buffer solution pH 9.5 and the solutions mixed well. After 10 minutes complete to the volume by water and measure the color formed at 559 nm for each drugs against blank solution.

Procedure for TLC-densitometer

Aliquots of 20 µl of each solution were applied to a separate precoated thin layer chromatographic plate (20x20 cm) using 50 µl micropipette. The chromatographic chamber was equilibrated with 5-40µg/spot and from 5-50µg/spot (26: 4: 1v/v) for 1.0 h. The chromatograms were developed at room temperature by ascending migration of the mobile phase over distance 16 cm. The plate was removed, dried and the spots were visualized under UV lamp at 254 nm. The chromatograms were scanned with the spectrodensitometer at maximum 280 nm. The calibration curves were plated representing the relation between the recorded area under the beak and the corresponding concentration.

Assay of pharmaceutical preparations:

Performed as procedure mentioned before and calculate the concentration of each drug from the corresponding rgression equation.

Procedure for HPLC

Different aliquots of the standard solutions equivalent to (20-400 µg/ml**)** of II or (10-100 µg/ml) for III were transformed to 10 ml volumetric flask, 1 ml of lidocaine standard equivalent to (40 µg) was added as internal standard and the volume was completed with the mobile phase, the solution were filtered using 0.45 µm membrane filter. Triplicates of 20 µl injections were injected to each concentration. The mobile was (0.1 Heptansulphonic acid): methanol (35: 65 v/v) using Luna 5 CN 150X4.6 m column and detected by UV/visible spectrometric at 244 nm. The flow rate was 1.5 ml/minute.
For tablets: The solutions of tablets were prepared at different concentrations and the experiments were completed as standard solution.

VALIDATION

Validation of the developed method was done according to ICH guidelines.

Linearity

The linearity of the method is its ability to elicit test results that are directly proportional to the concentration of analyte in samples. The calibration curves were taken in the ranges of 20-100 and 15-100µg/ml for drugs I and II at λ_{max} 559 nm using spectrophotmetric method ,5-40, 5-50 and 10-50 µg/spot for drugs I,II and III respectively; using TLC method at λ_{max} 280 nm. For HPLCmethod the linearity ranges were 20-400 and 10-100 µg/ml for drugs II and III respectively and at λ_{max} 244nm.

Precision and accuracy

The precision of analytical methods is determined by assaying a sufficient number of aliquots of a homogeneous sample to be able to calculate statistically valid estimate of % Relative Standard Deviation (%RSD). Intermediate precision was done to express within laboratory variation, on different days. (% RSD) was found less than 2% .

Specificity

Results of tablets solutionsshowed that there is no interference of the excipients when compare with the working standard solutions. Thus the methods were said to be specific.

RESULTS AND DISCUSSIONS

Spectrophotometric method

This work is undertaken in the view that an ion-pair are formed between drugs I, II with hematoxyllin to form ion-pair complex at alkaline medium pH 9.5 which had a characteristic color having maximum absorption at 559 nm for the two drugs, Fig.(2).

The absorption increases till concentration limit and the spectra are unaffected by the increase of the reagent concentration. The best formation of the ion-pair complex are after 10 minutes and the color formed is stable at more than 24 hours. The stoichiometry of the ratio between drugs and reagent were studied by continuous variation and molar ratio between drug: reagent was 1:1 for the two drugs with the reagent.

Abeyance to Beer's law: the drugs I, II complexes with hematoxyllin at pH 9.5 were obeyed to Beer's law at limits 15-100 and 20-100 µg/ml for drugs I, II respectively. The molar absorptivities and Sandell's sensitivities were calculated, Table (1).

Accuracy and Precision: There replicates of five different concentrations of each drug under the experimental conditions, Table (2). These values indicate the high accuracy and precision of the proposed method. The performance of the proposed methods was assessed by comparison the British Pharmacopoeia showed equivalency of the method.

Application of the proposed methods on the determination of the drugs on the pharmaceutical preparations:

Three proposed concentration are applied for the specrophotometric determination of drugs I, II in dosage forms, Table (3). The official method (non-aqueous titration) requires a high concentration of the drugs while the proposed method worked well in micro concentration ranges.

TLC- Densitometeric method

In the TLC method, the separation of the cited drugs was obtained using ethyl acetate: methanol: ammonia (33%) in the ratio 26:4:1 as developing system. The R_f values were 0.49 and 0.67 for II and I respectively. The spot were scanned by densitometer at 280 nm.

A linear correlation between the concentration and the corresponding peak area was obtained within the range 5-50 µg/spot and 4-40 µg/spot for II and I respectively. The linear regression equation calculated and results are shown in Table (4). The method was successfully applied for the determination of each of II and I in pure form and pharmaceutical preparation and results are shown in tables (5, 6). To assess the efficacy of the proposed method and pharmaceutical preparation of both II and I in laboratory prepared mixture with mean percentage recoveries 101.12±1.17 and 100.2±1.10 for the proposed method and 100.40±1.10 and 100.3±1.04 for the pharmaceutical preparation respectively. The validity of the method was assessed by applying the standard addition technique are no interference from additives and excipients which are normally present in the used tables.

HPLC method

HPLC assay parameters were tested such as

Mobile phase: the mobile phase composition was studied for good separation and determination and it was taken as (0.1% Heptane sulphonic acid): methanol (35: 65 v/v) after several trials to choose the best one.

Type of column: Different types of columns were tested. The best one was Luna 5CN 150X4.6 m column gave a good separation.
Wavelength: To maximum detector sensitivity, the response at various wavelengths at 285, 250, 244 and 230 nm were tested; good quantification was achieved using UV-detector at 244 nm for II and III.
Calibration curves were constructed representing the relationship between the ratio of area under beak and the corresponding concentration in the range 20-400 µg/ml for I and 10-100 µg/ml for III respectively. The characteristic of the parameter was illustrated in table (7).
For accuracy and permission of the proposed procedure different concentrations were tested and gave good results for the standard samples and the pharmaceutical preparations table (8). The validity, recovery was carried out applying the standard addition technique. Recovery of laboratory prepared mixture for II and III are shown in table (9).

CONCLUSION

The three proposed methods provide simple, accurate, sensitive, selective and reproducible quantitative analysis in bulk powder and in pharmaceutical dosage forms. The proposed methods gave a high sensitivity that needs a very small amount of the drugs than that the official one which needs a high concentration to be determined. The proposed methods were successfully applied on the raw material of drugs and in their pharmaceutical dosage form in quality control laboratories.

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Table 1: Regression Data of Fentrol HBr-hematoxyline complex and Salbutamol sulphat-hematoxyline complex

Parameter	Fenoterol	Salbutamol
Wavelength nm	559	559
Beer's law µg/ml	20-100	15-100
Molar absorbation L/Mol/cm	3.021	4.09
Sandle sensitivity µg/cm²	0.4333	0.443
Slope	0.36	0.43
Intercept	0.01	0.0
r	0.99	0.99

Table 2: Evaluation of accuracy and precision of the proposed method in the determination of Salbtamol sulfate and FenoterolHBr by using hematoxyline

Salbutamol					Fenoterol				
Taken µg/ml	Found µg/ml	SD	RSD%	% Recovery	Taken µg/ml	Found µg/ml	SD	RSD%	% Recovery
30	29.6	0.10	0.34	98	30	29.5	0.20	0.60	98.3
50	49.5	0.10	0.30	99	50	49.8	0.20	0.20	99.4
70	70.0	0.12	0.21	100	70	69.6	0.10	0.20	99.4
90	89.6	0.17	0.20	99.5	90	89.7	0.10	0.10	99.6
Mean±SD				99.1±1	Mean±SD				99.1±1

M: Mean of % recovery SD: Standard deviation of % recovery

Table 3: Evaluation of accuracy and precision of the proposed method in the determination of Salbovent and Berotic by using hematoxyline

Salbovent					Berotic				
Taken µg/ml	Found µg/ml	SD	RSD%	% Recovery	Taken µg/ml	Found µg/ml	SD	RSD%	% Recovery
30	29.5	0.20	0.60	98.3	30	29.8	0.10	0.30	99.3
50	49.5	0.20	0.40	99.0	50	49.6	0.30	0.60	99.2
70	69.7	0.30	0.40	99.6	70	69.6	0.20	0.30	99.4
90	89.7	0.30	0.30	99.6	90	89.0	0.20	0.20	98.9
Mean±SD				99.1±1	Mean±SD				99.2±1

Table 4: Regression data of FenoterolHBr and Theophylline and Salbutamol sulphate by TLC and HPLC methods

Parameter	TLC		HPLC	
	Fenoterol HBr	Salbutamol	Theophylline	Salbutamol
Linearity range	5-40µg/spot	5-50µg/ml	10-100µg/spot	20-400µg/ml
Regression equation	y=-367.16x+31.913	y=307x-60.986	y=4.11x+4.07	y=30.67x+6.50
Regression coefficient	R^2= 0.9999	R^2= 0.9928	R^2= 0.9994	R^2= 0.9998
intercept				
LOD	0.75 µg /spot	0.5 0µg /spot	2.00 µg /ml	1.50 µg /ml
LOQ	2.50 µg /spot	2.00µg /spot	6.50 µg /ml	5.00 µg /ml

LOD: limit of detection LOQ: Limit of quantization

Table 5: Determination of pharmaceutical preparations of fenoterol and salbutamol using the TLC densitometric method

FenotrolBeroteic				Salbutamol Salbovant			
Amount taken (µg)	AUPx^{-1}	Amount found	% Accuracy	Amount taken (µg)	AUPx^{-1}	Amount found	% Accuracy
10	3900	10.08	100.8	6	3900	5.9	98.3
20	7513	20.02	100.1	8	5300	8.1	101
30	10921	30.3	101	10	6000	10.08	100.8
40	13872	40.07	100.1	20	12700	20.4	102
50	19002	49.8	99.6	30	20800	30.02	100.06
Mean			100.30±1.04	Mean			100.40±1.1

M: Mean of % recovery SD: Standard deviation of % recovery

Table 6: Determination of pure samples of fenoterol and salbutamol using the TLC densitometric method

Fenotrol				Salbutamol			
Amount taken µg/ml	AUPx^{-1}	Amount found	% Accuracy	Amount taken µg/ml	AUPx^{-1}	Amount found	% Accuracy
5	1838	5.01	100.2	5	1597	5,07	101.4
10	3702	10.09	100.9	10	3394	9.9	99
20	7352	20.5	102.5	15	4805	15.05	100.3
30	11128	30.6	102	25	8109	25.03	100.1
40	14518	39,8	99.5	35	10179	35.5	101.4
50	18458	50.8	101.6	50	15698	49.7	99.4
Mean±SD			101.12±1.172				100.2±1.191

M: Mean of % recovery SD: Standard deviation of % recovery

Table 7: Determination of pure samples of Theophylline and salbutamol sulphate using the HPLC method

Theophylline			Salbutamol sulphate		
Taken µg/ml	Found µg/ml	% Recovery	Taken µg/ml	Found µg/ml	% Recovery
10	9.98	99.80	20	20.20	101.00
20	20.25	101.25	40	39.70	99.25
30	30.45	101.50	80	80.80	101.00
40	39.83	99.56	120	118.90	99.08
60	60.60	101.00	160	161.30	100.81
80	79.57	99.46	200	199.20	99.60
100	100.47	100.47	280	281.50	100.54
			360	360.80	100.22
		100.43±0.840			100.188±0.782

M: Mean of % recovery SD: Standard deviation of % recovery

Table 8: Application of standard addition technique for the determination of Theophylline tablet and Salbutamol sulphate tablet using HPLC method

Theophylline				Salbutamol sulphate			
Taken µg/ml	Added µg/ml	Found of added µg/ml	% Recovery of added	Taken µg/ml	Added µg/ml	Found of added µg/ml	%Recoveryof added
20	-				-	-	-
	10	9.96	99.60.		20	19.90	99.00
	20	20.10	100.50		40	39.52	98.80
	30	30.35	101.18	50	80	80.80	101.00
	50	48.41	98.40		160	160.80	100.50
	60	59.88	99.80		240	244.32	101.80
M ±SD			99.90 ±0.817				100.22±1.327

M: Mean of % recovery SD: Standard deviation of % recovery

Table 9: Determination of laboratory prepared mixture of Theophylline and Salbutamol Salphate by HPLC methods

Theophylline			Salbutamol		
Taken µg/ml	Found µg/ml	% Recovery	Taken µg/ml	Found µg/ml	% Recovery
10	10.125	101.25	20	20.23	101.16
20	19.83	99.17	40	40.83	102.08
40	39.95	99.88	80	79.00	98.75
60	60.75	101.30	120	120.76	100.63
80	79.20	99.00	240	243.43	101.43
100	101.34	101.43	320	319.49	99.84
			400	401.00	100.25
Mean ±SD		100.34±1.124			100.59±0.820

M: Mean of % recovery SD: Standard deviation of % recovery

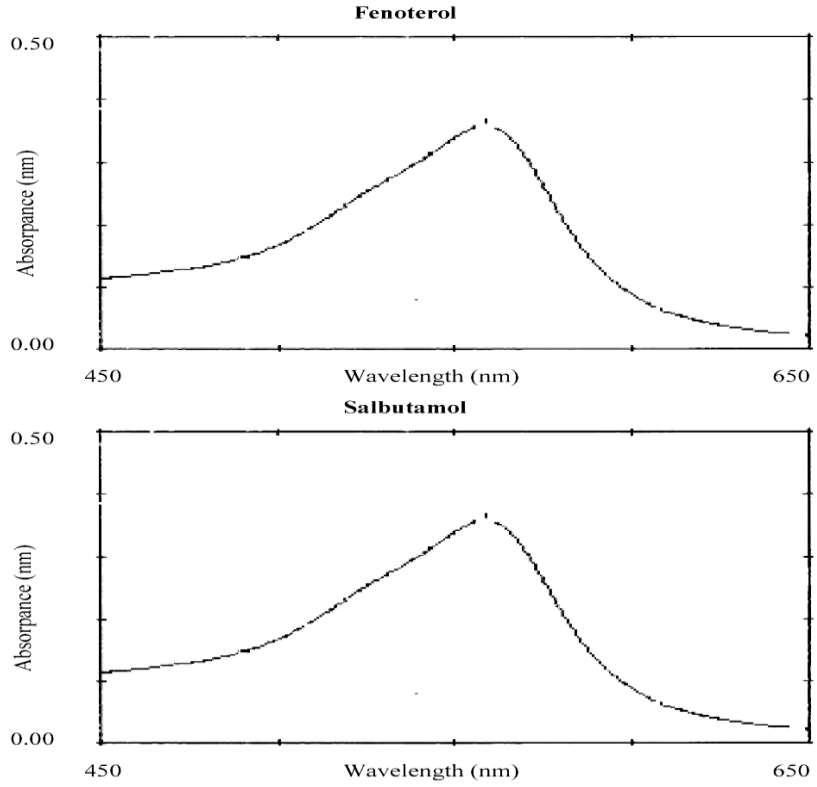

Fig. 2: Absorption spectra of fenoterol-hematoxylin complex (100µg/ml) and salbutamol complex (100 µg/ml)

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