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Stability indicating validated HPLC method for determination of RP-HPLC method development for Etofylline, Bromhexine hydrochloride and salbutamol sulphate in bulk and pharmaceutical dosage form

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ABSTRACT

A simple, rapid, precise and accurate stability indicating Reverse phase high performance liquid chromatography method was developed and validated for the quantitative determination of ET, BH and SS in bulk drug and Pharmaceutical dosage form. Separation was achieved on a ZORBAX CN 250X 4.6MM, 0.5µm, particle size column at a detection wavelength of 225 nm for all compounds, using a mobile phase consists of 0.1%OPA: METHANOL 800:500 in a Isocratic elution mode at a flow rate of 1.0ml per min. The ET peak was observed at 3.9min with peak area 93286, tailing factor 1.482 and resolution 3.647. BH peak was observed at 8.023min, with peak area 1453626, tailing factor 2.189 and resolution 5.943. SS peak was observed at 2.129 min with peak area 1875999, tailing factor 2.436. Because of the satisfactory results, less retention time, this trial was optimized. An attempt has been made to develop a new stability indicating validated RP-HPLC method for the simultaneous estimation of ET, BH and SS & UV-methods for estimation of ET, BH and SS in bulk and in dosage form. The proposed RP-HPLC, UV-Spectrophotometric methods were suitable methods for the determination of Etofylline, Bromhexine hydrochloride, and salbutamol sulphate in combination dosage forms. All the parameters of developed methods met the criteria of ICH guidelines for method validation.

Keywords: Etofylline, Bromhexine Hydrochloride, Salbutamol Sulphate, Method validation, ICH Guidelines

INTRODUCTION

Etofylline (ET), chemically known as 7-(2-hydroxyethyl)-1, 3-dimethyl-3, 7-dihydro-1, 4-purine-2, 6-dione, is a xanthine bronchodilator used for the treatment of respiratory diseases and asthma in combination with SS [1].

Bromhexine hydrochloride (BH), chemically known as N-(2-amino-3, 5-dibromobenzyl)-N methyl cychlohexanamine hydrochloride, is an expectorant use in the treatment of various respiratory disorders.

Salbutamol sulphate (SS), chemically known as bis [(1RS)-2-[(1, 1-dimethylethyl) amino]-1-[4-hydroxy3-(hydroxymethyl) phenyl] ethanol] sulphate, is beta adenocepter agonist. It is used for

the relief of bronchospasm in condition such as asthma and chronic obstructive pulmonary disease. The smooth muscles are relaxed by the increase in the intracellular cyclic adenosine monophosphate.

SS, BH and ET are official in BP. Official methods involve determination of SS, BH and ET using Potentiometry. SS, BH and ET as component of a multi ingredient formulation and has been reported to be estimated by some spectroscopic methods either single or in combination simultaneously. One spectroscopic method has been reported for the determination of SS along with BH and ET in combined dosage forms. The tertiary combination SS, BH and ET, is not yet official in any pharmacopoeia. Therefore simple, rapid, economical and reliable UV spectroscopic method for estimation of these drugs in mixture seemed to be necessary. All the analytical and validation procedures followed in the present study were as per ICH guidelines.

Literature survey reveals information that some of the methods like UV-visible spectrophotometry [1-3], HPLC [4-14], Methods for all the individual drugs. But yet not a single method has been reported for all the drugs in a single combination. This study was designed to develop a simple and reliable method to quantitative ET, BH and SS in a relatively short time with high sensitivity. Therefore, this study focused on the development of simple and rapid RP-HPLC method which can be employed for the routine analysis of ET, BH and SS in bulk drug and formulation and the method was validated as per ICH guidelines.

MATERIALS AND METHODS

Instrumentation

Reverse phase high performance liquid chromatography equipped with Auto Sampler and UV detector. RP-HPLC experiments were carried out on Waters 717plus- Empower software, with waters UV detector using Auto sampler. Data collections and processing was done using EMPOWER software. The analytical column used for the separation was ZORBAX CN 250X 4.6MM, 0.5µM particle size column, Analytical balance (Denver), pH meter (Eutech), Sonicator (Unichrome) [9-11].

Chemicals and reagents

SS, BH and ET were supplied as gift sample from **PVS Laboratories Limited** and **Koch organics in vijayawada** and marketed formulation of Eto-salbetol-10 (Batch Number–BTK0017, Kare Labs Pvt. Ltd., Goa, India) was procured from the local drug store, Moga, Punjab. Acetonitrile (AR Grade; MERCK), orthophospharic acid (HPLC grade, MERCK), methanol and HPLC grade water were used for the entire study [2-5].

Chromatographic condition

Use suitable High Performance Liquid Chromatograph equipped with UV-visible detector. The chromatographic column used was ZORBAX CN 250X 4.6MM, 0.5µM particle size. The mobile phase consists of 0.1%OPA: METHANOL 800:500 at a flow rate of 1.0mL/min at an ambient temperature and the chromatograms were monitored at a detector wavelength of 225 nm using UV-Detector. The injection volume was 20µL Retention time of Salbutamol is about 2 to 3.5 min. Retention time of Etofylline is about 3.7 to 4 min. Retention time of Bromhexine is about 8 to 9 min [6-8].

PREPARATION OF STANDARD & SAMPLE SOLUTIONS

Preparation of standard solution

Weigh accurately about 20 mg of salbutamol, 50mg of ethofylline and 80mg of Bromhexine working standards into a 50 mL volumetric flask. Add 30 mL of diluent, sonicate to dissolve and dilute to volume with diluent. Further dilute 5mL of Solution to 50 mL with the diluent.

Preparation of Sample Solution

Weigh 1 tablet and taken into a 50 mL volumetric flask. Add 30 mL of diluent, sonicate to dissolve and dilute to volume diluent.

Preparation-1

Filter through 0.45µ Nylon syringe filter and used for quantification of Salbutamol & Bromhexine.

Preparation-2

Further dilute 2.5 mL to 100 mL with the diluent for Etofylline. Filter through 0.45µ Nylon syringe filter.

PROCEDURE

Inject 20µL of Standard preparation five times and Sample preparations-1& 2 in the Chromatograph. Record the chromatograms and measure the peak responses for Salbutamol & Bromhexine from Preparation-1 & etofylline from Preparation-2. The System suitability parameters should be met. From the peak responses, calculate the content of Salbutamol, Bromhexine & Etofylline in the sample.

Method development

To saturate the column, the mobile phase was pumped for about 30 minutes thereby to get the base line corrected. The standard calibration line was constructed for drug. A series of aliquots were prepared from the above stock solution using diluent to get the concentration 5-30 µg/mL for ET, 10-40µg/mL for BH and 10-120 µg/mL for SS. Each concentration 6 replicates were injected in to chromatographic system. Each time peak area and retention time were recorded separately for the drugs. Calibration curves were constructed by taking average peak area on Y-axis and concentration on X-axis. From the calibration curve regression equation were calculated, this regression equation were used to calculate drug content in formulation as shown in the **(figure 9-13).**

Assay of Sample Solution

Crush 20 tablets into a fine powder. Transfer a powder equivalent to 100mg of ETO- Salbetol into 200ml volumetric flask, add 150 mL of diluent & sonicate for 15 min with continuous vigorous shaking at a temp NMT 20°C. Dissolve and dilute to volume diluent. Filter through 0.45µ Nylon syringe filter. This solution was estimated by above developed method. The assay procedure was repeated 6 times (n=6) the drug content was estimated using above calculated regression equation; the results of tablet dosage form are shown in the **(Table-3).**

%Assay of Formulation

Assay calculations

For Salbutamol,

$$= \frac{AT}{AS} \times \frac{\text{Std wt (mg)}}{50ml} \times \frac{5ml}{50ml} \times \frac{(P)\ \%\ \text{Potency of Std}}{\text{wt taken}} \times \frac{}{100} \times 100$$

For Bromhexine,

$$= \frac{AT}{AS} \times \frac{\text{Std wt (mg)}}{50ml} \times \frac{5ml}{50ml} \times \frac{(P)\ \%\ \text{Potency of Std}}{\text{wt taken}} \times \frac{}{100} \times 100$$

For Etofylline,

$$= \frac{AT}{AS} \times \frac{\text{Std wt (mg)}}{50ml} \times \frac{5ml}{50ml} \times \frac{(P)\ \%\ \text{Potency of Std}}{\text{wt taken}} \times \frac{100}{2.5} \times \frac{}{100} \times 100$$

Assay (%): Assay (mg/tab) x 100/LC

Where,

AT= Average area count of SS, ET & BH peaks in the chromatogram of sample solution.

AS= Average area count of SS, ET & BH peaks in the chromatogram of standard solution.

P=Percent potency of SS, ET & BH working standard on as is basis.

LC= Label claim of ET, BH and SS in mg.

Method validation

The analytical method was validated for various parameters as per ICH guidelines.

System suitability parameters

For assessing system suitability, six replicates of working standard samples of the ET, BH and SS peaks Were injected and studied the parameters like

plate number(N), tailing factor(K), resolution, relative retention time and peak asymmetry of samples**. (Table-2)**

Specificity and selectivity

Specificity is the degree to which the procedure applies to a single analyte and is checked in each analysis by examining blank matrix samples for any interfering peaks. The HPLC chromatograms recorded for the drug matrix showed almost no interfering peaks with in retention time ranges. Fig.4, 5 & 6 show the chromatogram for ET, BH and SS. The figures shows that the selected drugs were cleanly separated. Thus, the RP-HPLC method proposed in this study was selective.[12-14].

Linearity

Linearity of an analytical method is its ability to elicit the test results that are directly, or by well-defined mathematical transformation, proportional to the concentration of analyte in sample within a given range. Linear correlation was obtained between peak area Vs concentration of ET, BH and SS were in the range of 20 – 150µg/mL, 32 – 240and 8 – 58µg/mL. The linearity of the calibration curve was validated by the high value of correlation co-efficient of regression equation. The solution was injected in six replicates. The average peak area versus concentration data of drug was treated by least squares linear regression analysis and the results obtained as shown in **Table-4**

Accuracy

Accuracy is expressed as the closeness of the results from standard samples to that of the actual known amounts. Accuracy was evaluated in three replicates, at three different concentration levels equivalent to 50%, 100%, and 150% of the target concentration of active ingredient, by adding a known amount of each of the Standard to a pre-analysed concentration of drugs (ET, BH and SS) and calculating the % of recovery, and the results obtained were shown in **Table-5(5a-5c).**

Precision

Precision is the degree of repeatability of an analytical method under normal operation conditions.

Method precision was achieved by repeating the same procedure of preparation solution six times and injected. The % RSD was calculated.

System precision is checked by using standard substance to ensure that the analytical system is working properly. In this peak area and % of drug of six determinations is measured and % RSD was calculated. The results are shown in the **Table-6(6a, 6b).**

Ruggedness and robustness

Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective peak areas were noted. The result was indicated by % RSD **(Table 7)**.

Robustness of the method was determined by carrying out the analysis at two different P^H of mobile phase (i.e. 7.0±0.5) and three different flow rates (i.e. 1±0.2 mL/min)

The high % RSD values of robustness and for ET, BH and SS with change in flow rate indicates that the method is not robust for change in flow rate.

The low % RSD values of robustness and for ET, BH and SS with change in P^H reveal that the proposed method is robust **(Table 8a-8c)**.

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) limit of quantification (LOQ) of the drug carry was calculated using the following equation as per international conference harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma / S$

LOD for ET was found to be 0.015µg/mL and LOQ for ET was found to be 0.0495µg/mL, LOD for BH was found to be 0.2000µg/mL and LOQ for BH was found to be 0.660µg/mL and LOD & LOQ for SS was found to be 0.1500µg/mL and0.4950 µg/mL (**Table 9)**.

Stability study

40µg/mL of ET, 64µg/mL of BH and 15µg/mL of SS were prepared and stability study was carried out at 0hrs and 24hrs and the results were recorded. The results reveal that the sample solutions are stable and accurate without interference **(Table 10)**.

Degradation study

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the ET, BH and SS using the proposed method **(Table 11)**.

RESULTS AND DISCUSSION

The conditions tested for method development indicates that all the system suitability parameters according to ICH guidelines were achieved by using ZORBAX CN 250X 4.6MM, 0.5µm, particle size column using mobile phase 0.1%OPA: METHANOL 800:500 in a Isocratic elution mode with a flow rate of 1 mL per min throughout the Isocratic program with a detection wavelength of 225 nm for all the compounds with an injection volume of 20 µL.

To validate the RP-HPLC method, a series of tests were made using the most promising conditions. A calibration curve was made and concentration examined within the detection range of 30 µg/mL for Etofylline, 10-40 µg/mL for Bromhexine Hydrochloride, 10-120 µg/mL for Salbutamol Sulphate and correlation coefficient was found to be 0.999 for all the compounds respectively. The precision (expressed as the relative standard deviation RSD) was determined for ET, BH and SS for repeated analysis and the values are presented in **Table**

The assay values obtained by proposed method and recovery experiment values obtained were performed by adding different amounts placebo to preanalysed concentration summarized.

The stability of sample was checked by forced degradation in different conditions and % of degradation was calculated. The peak purity of the analyte was passed in all conditions (purity angle should be less than the threshold value). The results as shown in Table-9 indicate that any other impurity is not merging with the main peak. The analyte sample solution was stable up to 24hrs at refrigerated conditions. A method was developed for the determination of ET, BH and SS in Tablets which is rapid, stable & specific. The results indicate that the described method can be used for quantitative analysis of the compounds.

Table 1: Optimized chromatographic conditions

PARAMETERS	OBSERVATION
Elution	Isocratic
Temperature	Ambient
Mobile Phase	0.1%OPA: METHANOL 800:500
P^H	7.0(with OPA)
Column	ZORBAX CN 250X 4.6MM, 0.5µM.
Detection Wave Length	225 nm
Flow Rate	1 mL/min
Runtime	12 min

Table 2: Results of system suitability parameters for ET, BH and SS

S.NO	Name	Retention time	Peak area	Tailing factor	Resolution
1	ET	3.909	93286	1.482	3.647
2	BH	8.091	1453626	2.189	5.943
3	SS	2.150	1875999	2.436	-

Table 3: Results of tablet dosage form

Drug	Avg std area(n=6)	Avg sample area(n=6)	Avg wt of tab. (mg)	Std wt (mg)	Sample wt(mg)	Lable amount(mg)	Std purity	Amount found (mg)	% assay
ET	3579795	3543324	298.4	50	298.5	200	100	197.8	98.9
BH	5098850	5130207	298.4	80.3	298.5	8	100	8.08	101

SS	1524714	1422330	298.4	20.3	298.5	2		100	1.894	94.7

Table 4: Linearity results of ET, BH and SS

S.NO	Etofylline		Bromhexine		Salbutamol	
	Conc.(µg/mL)	Peak area	Conc.(µg/mL)	Peak area	Conc.(µg/mL)	Peak area
1	0.00	0	0.00	0	0.00	0
2	20.08	707589	7.76	647256	32.08	840177
3	40.16	1453626	15.52	932826	64.16	1875999
4	60.24	2169899	23.28	1207243	96.24	3039940
5	80.32	2894003	31.04	1503585	128.32	3946200
6	100.40	3585006	38.80	1747360	160.40	5087562
7	120.48	4377428	46.56	2036610	192.48	657599
8	150.6	5373100	58.20	2447016	240.60	7681175
Regression equation	y = 1263645+34x-39606		y = 1263645+34x-39606		y = 1263645+34x-39606	
Slope	35882.26		33081.54		35582.56	
Intercept	3244.06		149842.30		378922.48	
R^2	0.99990		0.9991		0.99987	

Table 5: Accuracy results for ET, BH and SS

Table 5 a: accuracy results of ET by RP-HPLC method

%Accuracy Level	Amount of API Added (mg)	Actual API Added (mg)	Area Counts	Amount Recovered(mg)	% Recovery	Mean Recovery	% RSD
50 %	7.60	7.60	1799146	7.52	98.9	99.6	0.6
	7.60	7.60	1821030	7.61	100.1		
	7.60	7.60	1811770	7.57	99.6		
100 %	15.20	15.20	3653208	15.27	100.5	100.6	0.230
	15.20	15.20	3653538	15.27	100.5		
	15.20	15.20	3668498	15.33	100.9		
150%	22.80	22.80	5441262	22.74	99.7	100.0	0.340
	22.80	22.80	5449976	22.78	99.9		
	22.80	22.80	5477481	22.89	100.4		

Table 5 B: Accuracy Results Of BH by RP-HPLC Method

%Accuracy Level	Amount of API Added (mg)	Actual API Added (mg)	Area Counts	Amount Recovered (mg)	% Recovery	Mean Recovery	% RSD
50 %	4.18	4.18	2466833	4.13	98.9	98.8	0.740
	4.18	4.18	2446893	4.09	98.0		
	4.18	4.18	2479235	4.15	99.4		
100 %	8.35	8.35	5126629	8.57	102.6	102.7	0.080
	8.35	8.35	5127581	8.58	102.8		
	-	-	-	-	-		
150%	12.53	12.53	7736619	12.94	103.3	103.7	0.290
	12.53	12.53	7771142	13	103.8		
	12.53	12.53	7780965	13.01	103.9		

Table 5c: Accuracy Results of SS By RP-HPLC Method

%Accuracy Level	Amount of API Added	Actual API Added (mg)	Area Counts	Amount Recovered	% Recovery	Mean Recovery	% RSD

	(mg)			(mg)			
50 %	1.12	1.12	1039848	1.12	100.0	100.0	0.890
	1.12	1.12	1021932	1.11	99.1		
	1.12	1.12	1042929	1.13	100.9		
100 %	1.96	1.96	1772664	1.92	98.0	97.1	1.090
	1.96	1.96	1736091	1.88	95.9		
	1.96	1.96	1767358	1.91	97.4		
150%	2.58	2.58	2423995	2.62	101.7	102.7	0.950
	2.58	2.58	2446217	2.65	102.9		
	2.58	2.58	2467889	2.67	103.6		

Table 6: Precision results for ET, BH and SS

Table 6A: method precession for ET, BH and SS

S.NO	Method Precision	Etofylline	Bromhexine	Salbutamol
1	MP 1	98.9	101	96.8
2	MP 2	99.6	102.4	96.9
3	MP 3	99.4	101.3	96.9
4	MP 4	99.7	100.9	97.0
5	MP 5	99.6	101.9	96.5
6	MP 6	101.7	100.1	96.5
Mean		99.8	101.3	96.8
SD		0.966	0.807	0.216
% RSD		0.97	0.8	0.22

Table 6 B: System Precession for ET, BH and SS

S.NO	System Precision	Etofylline	Bromhexine	Salbutamol
1	Injection- 1	3682459	5152104	1776803
2	Injection- 2	3672481	5191451	1766961
3	Injection- 3	3640104	5074564	1778513
4	Injection- 4	3728661	5298419	1796981
5	Injection- 5	3759092	5260884	1815952
6	Injection- 6	3642534	5070548	1798291
Mean		3687555	5174662	1788917
% RSD		1.291	1.82	1.006

Table 7: Ruggedness studies for ET, BH AND SS

S.NO	Parameter	ET	BH	SS	Limit
1	Mean	3717583	5602640	1539425	NMT 2.0%
2	%RSD	1.919	2.706	1.416	

Table 8: Robustness studies for ET, BH AND SS

(a) Robustness results of etofylline BY RP-HPLC

Parameter	ET					
	Retention time	Peak area	Resolution	Tailing	Plate count	% RSD
Method Precision - Control	4.014	3588749	5.914	1.482	1117	0.397
Wave Length Plus	4.014	3043313	6.048	1.481	1117	0.433
Wave Length Minus	4.014	5301188	5.852	1.480	1112	0.450
Organic Plus	4.293	3728567	6.250	1.235	1936	0.169
Organic Minus	4.538	3901894	7.248	1.238	1898	1.480
Flow Plus	3.728	3277805	5.672	1.868	1188	0.911

Flow Minus	5.019	4235088	7.044	1.233	1859	0.325

(b) Robustness results of bromhexine hydrochloride by RP-HPLC

Parameter	**BH**					
	Retention time	**Peak area**	**Resolution**	**Tailing**	**Plate count**	**% RSD**
Method Precision - Control	8.432	5107093	3.647	2.147	1213	0.284
Wave Length Plus	8.432	3313041	3.632	2.021	1273	1.047
Wave Length Minus	8.432	9543571	3.609	2.185	1191	0.360
Organic Plus	8.389	5284248	4.470	1.993	1373	0.710
Organic Minus	10.124	5448261	5.292	2.024	1333	0.211
Flow Plus	3.728	4709398	3.226	2.220	1087	1.049
Flow Minus	10.970	6017536	5.293	1.852	1319	1.258

(b) Robustness results of salbutamol sulphate by RP-HPLC

(c)

Parameter	**SS**					
	Retention time	**Peak area**	**Resolution**	**Tailing**	**Plate count**	**% RSD**
Method Precision - Control	2.741	1522614	-	2.436	3903	1.359
Wave Length Plus	2.742	1286940	-	2.436	3750	1.323
Wave Length Minus	2.742	1437613	-	2.449	3654	1.329
Organic Plus	2.976	1597196	-	1.950	4605	0.085
Organic Minus	3.025	1561146	-	2.241	9743	0.192
Flow Plus	2.608	1380261	-	2.769	2097	0.859
Flow Minus	3.351	1740142	-	2.021	9909	0.534

Table 9: Sensitivity parameters (LOD & LOQ) by RP-HPLC

parameter	**ET**		**BH**		**SS**	
	µg/mL	**Area**	**µg/mL**	**Area**	**µg/mL**	**Area**
LOD	0.0150	10398	0.2000	242399	0.1500	103989
LOQ	0.0495	31195	0.6600	727198	0.4950	311967

Table 10: Results of stability study

(a) Stability study results of etofylline

Time period (hours)	**Etofylline**				
	Retention Time	**Peak Area**	**Tailing Factor**	**Plate Count**	**Resolution**
6	4.340	3888396	1.221	1686	4.338
12	4.350	3804225	1.264	1804	4.449
18	4.362	3811135	1.253	1785	4.570
24	4.366	3801338	1.217	1708	4.341

(b) Stability study results of bromhexine hydrochloride

Time period (hours)	**Bromhexine Hydrochloride**				
	Retention Time	**Peak Area**	**Tailing Factor**	**Plate Count**	**Resolution**
6	8.673	5501665	1.925	1248	6.171
12	8.719	5490052	1.763	1301	6.208
18	8.784	5479477	1.711	1246	6.207
24	8.821	5442238	1.654	1282	6.337

(c) Stability study results of salbutamol sulphate

Time period (hours)	**Salbutamol Sulphate**				
	Retention Time	**Peak Area**	**Tailing Factor**	**Plate Count**	**Resolution**

6	3.007	1570954	1.887	4868	5.067
12	3.020	1544009	1.921	5706	5.089
18	3.027	1558372	1.894	5481	5.272
24	3.029	1579345	1.974	4784	5.345

Table 11: degradation study results of ET, BH AND SS

Degradation	**sample**	**Etofylline**		
		Mean area (n=6)	**% label claim**	**% degradation**
ACID	375.5	4389776	97.4	2.4
ALKALI	330.4	3866004	97.5	2.3
PEROXIDE	330.7	3903178	98.4	1.4
REDUCTION	330.9	3907967	98.4	1.4
sHEAT	300.5	3560670	98.8	1
HYDROLYSIS	300.1	3585850	99.6	0.2
PHOTO	307.9	3661702	99.1	0.7
THERMAL	300.4	3536997	98.1	1.7

Degradation	**sample**	**Bromhexine Hydrochloride**		
		Mean area (n=6)	**% label claim**	**% degradation**
ACID	375.5	119010	74.5	26.8
ALKALI	330.4	87164	62	39.3
PEROXIDE	330.7	90354	64.2	37.1
REDUCTION	330.9	93458	66.4	34.9
HEAT	300.5	74762	58.5	42.8
HYDROLYSIS	300.1	100459	78.7	22.6
PHOTO	307.9	118892	90.7	10.6
THERMAL	300.4	65937	51.6	49.7

Degradation	**sample**	**Salbutamol Sulphate**		
		Mean area (n=6)	**% label claim**	**% degradation**
ACID	375.5	176358	9.3	86.4
ALKALI	330.4	161992	9.7	86
PEROXIDE	330.7	210071	12.6	83.1
REDUCTION	330.9	171739	10.3	85.4
HEAT	300.5	170993	0	95.7
HYDROLYSIS	300.1	167771	11.1	84.6
PHOTO	307.9	171335	11.1	84.6
THERMAL	300.4	169180	11.2	84.5

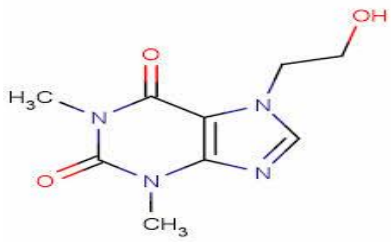

Figure 1: Molecular structure of Etofylline

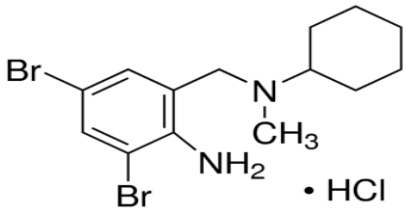

Figure 2: Molecular structure of Bromhexine Hydrochloride

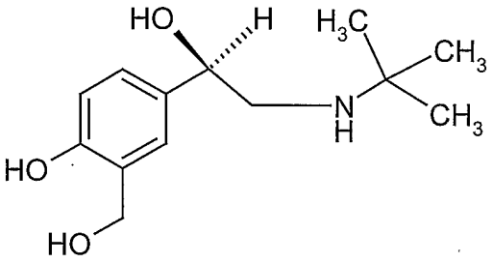

Figure 3: Molecular structure of Salbutamol Sulphate

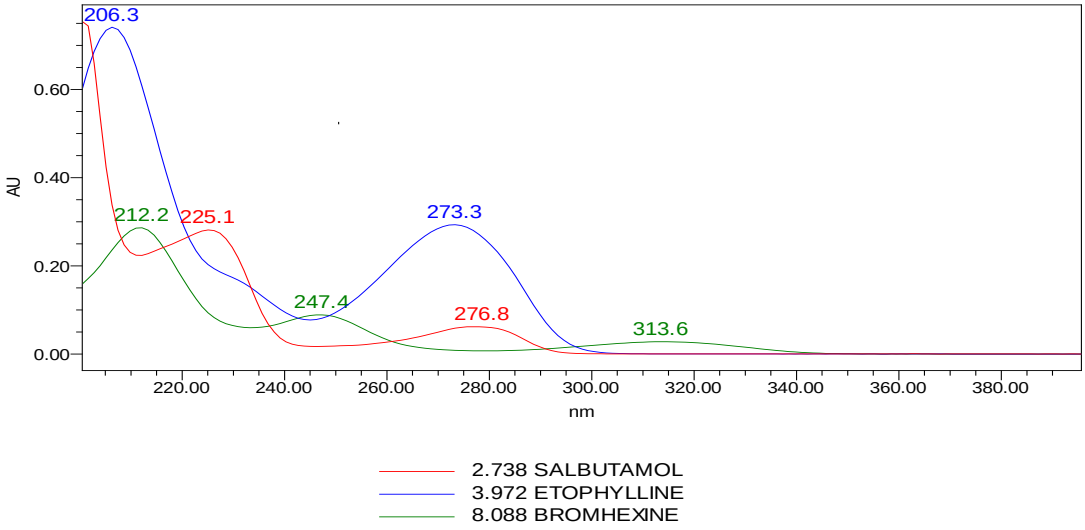

Fig.4 Chromatogram of uv- spectum

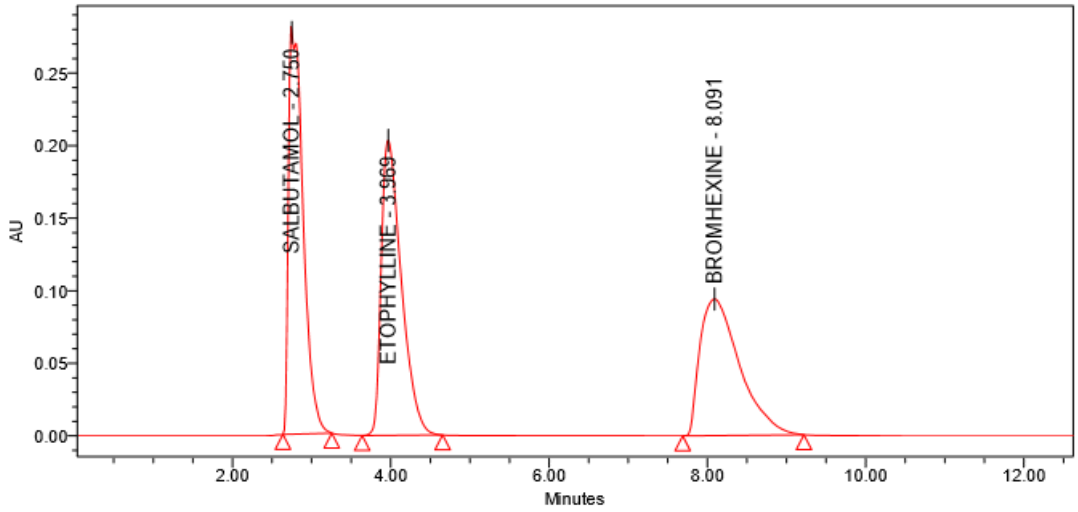

Fig.5 Chromatogram of standard

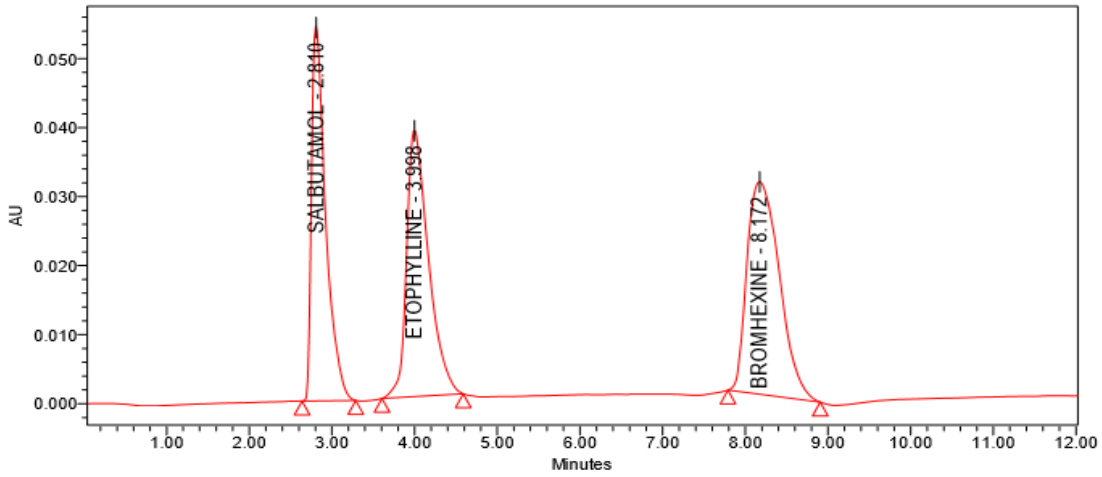

Fig.6 Chromatogram of sample

Calibration Curve for ET, BH and SS

Calibration Curve of Etofylline

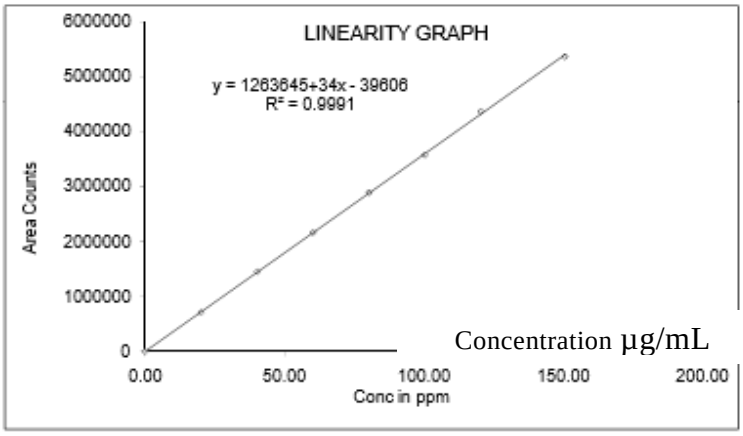

Figure 7: Calibration Curve of Etofylline at 225 nm.

Calibration Curve of Bromhexine Hydrochloride

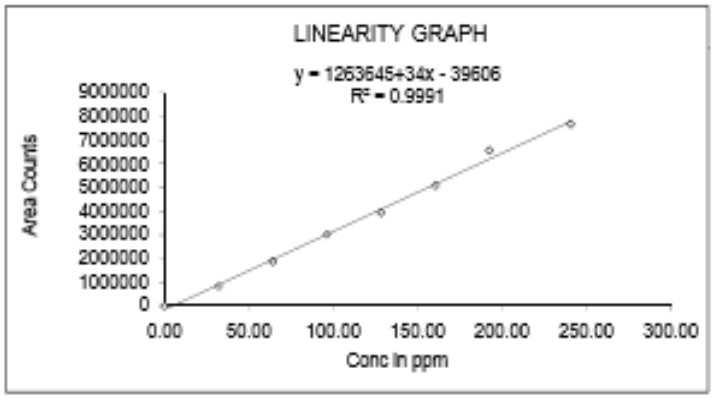

Figure 8: Calibration Curve of Bromhexine Hydrochloride at 225 nm.

Calibration Curve of Salbutamol sulphate

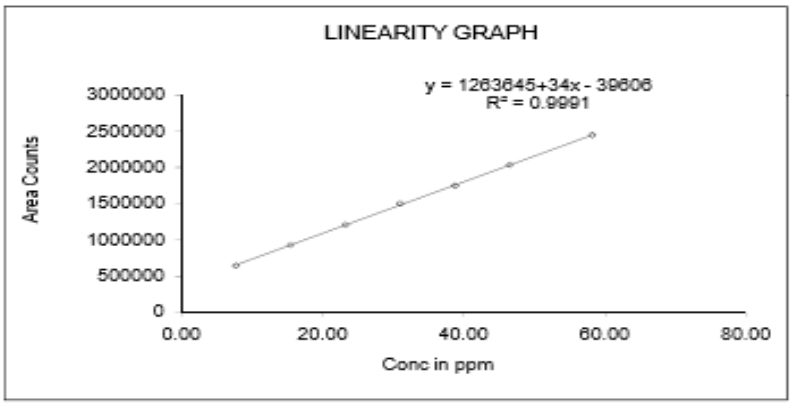

Figure 9: Linearity of Salbutamol sulphate at 225 nm.

CONCLUSION

The proposed RP-HPLC, UV-Spectrophotometric methods were suitable methods for the determination of etofylline, Bromhexine hydrochloride, and salbutamol sulfate in combination dosage forms. All the parameters of developed methods met the criteria of ICH guidelines for method validation.

The developed HPLC method has the following advantages

- No tedious extraction procedures were involved.
- These methods are also having an advantage than reported method of good resolution and with retention time.
- The developed method has good recovery and sensitivity.
- The run time required for recording chromatogram was below 8.0 mins.
- Suitable for the analysis of raw materials and formulations.
- Hence, the developed chromatographic method for etophylline, Bromhexine hydrochloride, and salbutamol sulfate are said to be rapid, simple, precise, accurate, specific and cost effective that can be effectively applied for the routine analysis.

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