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Research Article

SIMULTANEOUS ESTIMATION OF SALBUTAMOL, AMBROXOL AND GUAIFENESIN IN TABLET DOSAGE FORMS BY USING RP-HPLC

Srinivas Sumanth Kamatham*[1], Srinivas Kolli[1], Devi Deepika Joga[1], Kumari Ramya Vasa[1], Bharathi Devi Yanamadala[1].

[1]Sri vasavi institute of pharmaceutical sciences, Tadepalligudem, West Godvari (Dist), Andhra Pradesh-534101

***Corresponding author E-mail:** sumanth.kamatham222@gmail.com

ABSTRACT

A simple and reproducible method for simultaneous estimation of salbutamol (SAL), guaifenisin (GUA) and ambroxol (AMB) by high performance liquid chromatography (HPLC) in formulation was developed. The analysis was performed with a mobile phase containing acetonitrile (ACN) and potassium di-hydrogen phosphate (PDHP) adjusted to a pH- 4 in the ratio of 70 : 30 (% v/v) at a flow rate of 1.0 ml/min. A SHISEIDO C_{18} column (250 x 4.6mm i.d; 5µm) was used. A UV spectrum of salbutamol, guaifenisin and ambroxol was recorded by scanning between 200-400 nm, from the overlapping spectra a wavelength of 215 nm is selected and simultaneous estimation is carried out. The analysis was performed in the linearity range of 2-16 µg/ml for Salbutamol, 0.5-4 µg/ml for Ambroxol and 5-40 µg/ml for Guaifenisin respectively. The correlation coefficient was 0.996 (SAL), 0.994 (AMB), 0.998 (GUA). Thus the developed and validated chromatographic method for Salbutamol, Ambroxol and Guaifenisin is said to be rapid, precise (RSD<=2%), simple, accurate (% recovery=90-101%).

KEY WORDS

Salbutamol, Ambroxol, Guaifenesin, Acetonitrile and potassium di-hydrogen phosphate buffer(pH-4).

INTRODUCTION

Ambroxol hydrochloride (AMB) [trans-4-(2-amino-3,5dibromobenzylamino)Cyclohexanol Hydrochloride] [1] is semi-synthetic derivative of vasicine obtained from Indian shrub *Adhatoda vasica.* It is a metabolic product of bromhexine. It is used as broncho secretolytic and expectorant drug [2]. It stimulates the transportation of the viscous secretions in the respiratory organs and reduces the stand stillness of the secretions. Several spectrophotometric methods have been reported for the qualitative and quantitative determination of AMB from pharmaceutical formulations [3-6]. Various HPLC [7-10], GLC [11-12], LC-MS [13] and Capillary electrophoretic methods [14] are also reported for its determination from biological fluids.

Salbutamol is chemically 2-(hydroxymethyl)-4-[1-hydroxy- 2-(tert-butylamino) ethyl] phenol. It is a short-acting β2-adrenergic receptor agonist used for the relief of bronchospasm in conditions such as asthma and COPD. It is usually given by the inhaled route for direct effect on bronchial smooth muscle. Salbutamol Sulphate in pharmaceuticals has been assayed using visible spectrophotometric methods based on reactions such as redox, reducing and then chelating, oxidative coupling, diazotization

and coupling, nitrosation, nitration, nitration followed by Meisenheiner complex formation and charge-transfer complex formation. A number of analytical methods exist for the determination of Salbutamol in biological fluids, including reversed phase high-performance liquid chromatography [15-18] equipped with ultraviolet [19,20], fluorescent detection [21], electrophoresis [22-24], amperometric [25,26], thin layer chromatography [27], cation exchange [28], direct conductivity [29], gamma radiation [30] and liquid chromatography mass spectrometric detection [31].

Guaifenesin (glyceryl guaiacolate) has the chemical name 3-(2-methoxyphenoxy)-1,2-propanediol. Its molecular formula is $C_{10}H_{14}O_4$ with a molecular weight of 198.21. It is a white or slightly gray crystalline substance with a slightly bitter aromatic taste. One gram dissolves in 20 mL water at 25°C; it is freely soluble in ethanol. Guaifenesin is readily absorbed from the GI tract and is rapidly metabolized and excreted in the urine. Guaifenesin has a plasma half-life of one hour. The major urinary metabolite is β-(2-methoxyphenoxy) lactic acid [32-34]. Different methods have been reported for the analysis of Guaifenesin including HPLC [35-41], GC [42-43], capillary electrophoresis mass spectrometry [44], X-ray diffraction[45], voltammetry[46]. Guaifenesin is an expectorant available for oral administration.

The present aim of the study is to develop a method for simultaneous estimation of Salbutamol, Ambroxol and Guaifenesin.

2. MATERIALS AND METHODS

2.1 Materials used:

2.1.1. Chemicals and Reagents:

Acetonitrile of HPLC grade was supplied by Merck Limited, Mumbai. Water HPLC grade was supplied by Merck Limited, Mumbai. Potassium di-hydrogen phosphate was supplied by Fisher scientific (Qualigens). Working Standards of Salbutamol, Guaifenesin, Ambroxol were obtained from Yarrow Chem. Products, Mumbai.

2.1.2. Instruments used:

Cyberlab HPLC system having the configurations, LC-20AD solvent delivery system, Rheodyne 7725*i* injector with 20 µl loop, SPD 20A dual wavelength detector, LC Solutions data station, a SHISEIDO C_{18} column (250 x 4.6mm i.d; 5µm).

2.2. Optimization of chromatographic conditions:

Proper selection of the chromatographic method depends upon the nature of the sample (ionic or neutral molecule), its molecular weight and solubility. The drugs selected for the present study is polar in nature and hence either reverse phase or ion pair or ion exchange chromatography can be used. For the present study reverse phase HPLC method is considered. Wavelength for Salbutamol, Guaifenesin and Ambroxol was selected by scanning between 200 - 400 nm and the UV spectrum was recorded. From the spectra, detection wavelength 215 nm was selected for Salbutamol, Guaifenesin and Ambroxol.

2.2.1. Optimized chromatographic conditions:

Stationary phase : SHISEIDO C18 (250 x 4.6 mm i.d., 5µ)
Mobile Phase : Acetonitrile : 20 mM potassium di-hydrogen Orthophosphate
pH : 4
Mobile phase ratio : 30:70
Flow rate : 1.0 ml/min
Sample volume : 20 µl using Rheodyne P/N 7725i injector
Detection : 215nm using SPD- 20A wavelength detector

Data station : LC Solution data station

The retention times of Salbutamol, Ambroxol and Guaifenesin were 2.57, 7.1 and 5.85 min respectively. The chromatograms were shown in figures 1 and 2.

2.3. Preparation of standard and sample solutions:

a. Standard stock solution of Salbutamol, Guaifenesin, Ambroxol:

10 mg of Salbutamol, Guaifenesin and Ambroxol working standard were accurately weighed and transferred into a 10 ml volumetric flask individually and dissolved in Acetonitrile and Phosphate buffer (pH-4) (30 : 70) made up to the volume with the same solvent to produce a 1mg/ml of Salbutamol, Guaifenesin and Ambroxol. The stock solution was diluted to suitable concentrations to obtain calibration curve (CC) standards and quality control (QC) samples.

b. Calibration curve standards and quality control samples:

Working solutions for calibration and controls were prepared from the stock solution by an adequate dilution using Acetonitrile and Potassium di-hydrogen ortho phosphate buffer (pH-4). Calibration standards for Salbutamol were prepared by the stock solution to obtain the concentration levels of 2, 4, 6, 8, 10, 12, 14 and 16 µg/ml. Quality control samples were prepared as bulk, at a concentration of 2 µg/ml (LQC), 8 µg/ml (MQC) and 16 µg/ml (HQC).

Calibration standards for control for Ambroxol were prepared by the stock solution to obtain the concentration levels 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 µg/ml. Quality control samples were prepared as bulk, at concentration of 0.5 µg/ml (LQC), 2 µg/ml (MQC) and 4 µg/ml (HQC).

Calibration standards for Guaifenesin were prepared by the stock solution to obtain the concentration levels 5, 10, 15, 20, 25, 30, 35 and 40 µg/ml. Quality control samples were prepared as bulk, at concentration of 5 µg/ml(LQC), 20 µg/ml (MQC) and 40 µg/ml (HQC).

2.4. Validation

2.4.1. Selectivity/ Specificity

A method is said to be specific when it produces a response only for a single analyte. Method selectivity is the ability of the method to produce a response for the analyte in the Presence of other interferences. In order to prove that the method chosen was specific and selective the following two sets of samples were processed and injected into the HPLC using the extraction procedure.

2.4.2. Sensitivity

It is expressed as limit of detection and limit of quantification. It is the lowest amount of analyte in a sample matrix that can be detected and that can be quantifiable.

2.4.3. Linearity

Linearity and range of the methods were analyzed by preparing calibration curves using different concentrations of the standard solution containing the internal standard. The calibration curve was plotted using peak area and concentration of the standard solutions. Linearity was established over the range of 2 to 16µg/ml for Salbutamol, 5 to 40µg/ml for Guaifenesin and 0.5 to 4µg/ml for Ambroxol using the weighted least square regression analysis.

2.4.4. Precision

Precision is expressed as the percentage coefficient of variation (%CV) which is calculated as:

%CV= (Standard Deviation/Mean) x 100.

Both Intra-day precision, Inter-day precision and Inter-week precision were performed.

2.4.5. Accuracy

Accuracy is reported as % nominal or % Recovery of the analyzed concentration which is calculated as:

% Nominal = (Measured Concentration /Actual Concentration) x 100.

It is performed by measuring the response obtained from a certain amount of analyte added to and extracted from the matrix, expressed as a percentage of the response obtained for the true concentration of the pure authentic standard which has not been subjected to the extraction procedure. To determine recovery of this method, six replicates of aqueous quality control samples (unextracted) with concentrations close to spiked Quality Control sample at Low concentration (LQC), Quality Control sample at Middle concentration (MQC) and Quality Control sample at High concentration (HQC), concentration (extracted) were prepared and injected.

2.5. Stock solution stability

2.5.1. Short term stock dilution stability

The stability of stock dilutions of analyte was evaluated at room temperature. Aqueous stock dilutions of the analyte were prepared. One portion of the stock dilution was placed in the refrigerator between 2-8°C, while the other portion was placed at room temperature for 24 hours. Stock dilution stored at room temperature (stability samples) was compared with refrigerated stock dilutions considered as 'comparison samples'. Six replicate injections of the above solutions were made.

2.5.2 Long term stock solution stability

The stability of the stock solution when stored for a given period of time was determined. Stock solutions of the analyte were prepared and stored in the refrigerator between 2 - 8°C for 7 days (stability stock). The stock solution stabilities of the analyte were determined with a comparison stock solution, which was prepared freshly. Five replicate injections of the above solutions were made. The response of comparison samples were corrected by multiplying with correction factor to nullify the difference between the measured weights or the dilutions made.

2.5.3. Long-term (LT) stability

To assess the stability of the analyte in the sample matrix under the same conditions of storage as that of the study samples for the time period between the date of first sample collection and the date of last sample analysis, the following test was performed. Six samples of each quality control samples at low and high concentrations were stored and the stability of the analyte was evaluated by comparing each of the back calculated concentrations of stability Quality Control sample (QCs) with the mean concentrations of the respective QCs analysed in the first accepted precision and accuracy batch.

2.5.4. Ruggedness

Ruggedness of the method was studied by changing the experimental conditions such as operators, instruments, source of reagents, solvents and column of similar type.

2.5.5. Robustness

Robustness of the method was studied by injecting the standard solutions with slight variations in the optimized conditions namely, ± 1% in the ratio of Acetonitrile in the mobile phase, varying pH range ±1 and ± 0.1 ml of the flow rate.

3. RESULTS AND DISCUSSION

3.1. SYSTEM SUITABILITY

System was evaluated for reproducibility by finding the concentration of six replicates of Salbutamol, Ambroxol and Guaifenesin (10 mg/ml) dilution. The coefficient of variation

was obtained. The results obtained are given in **Table: 1**.

3.2. METHOD VALIDATION (ICH GUIDELINES):

3.2.1. SENSITIVITY:

The Limit of Detection for Salbutamol was 0.5 µg/ml, for Ambroxol was 0.1 µg/ml and for Guaifenesin was 1 µg/ml.The Limit of Quantification for Salbutamol was 2 µg/ml, for Ambroxol was 0.5 µg/ml, and for Guaifenesin was 5 µg/ml.

3.2.2.. LINEARITY:

The linearity and range was performed over a range of 2 to 16 µg/ml for Salbutamol, 5 to 40 µg/ml for Guaifenesin and 0.5 to 4 µg/ml for Ambroxol. The Correlation coefficient [R^2] was obtained as 0.996 for Salbutamol, 0.994 for Ambroxol and 0.998 for Guaifenesin. The graphs were shown in **Fig: 3, 4 and 5**. The peak area values for linearity are mentioned in the **Table: 2**.

3.2.3. PRECISION:

By the precision studies the relative standard deviation values were obtained as less than 2 %. The results were shown in **Table No: 3**. The Inter-day precision studies were carried out and the values of relative standard deviation were shown in **Table No: 4**.

3.2.4. ACCURACY:

The mean absolute recovery of Salbutamol, Ambroxol and Guaifenesin in this method was 90-101%. The results were shown in **Table: 5, 6 And 7** respectively.

3.2.5. stability:

The stock solution stability studies were carried out and the relative standard deviation values of the drugs are shown in **Table No: 8**. The long term stability studies were carried out and the values of relative standard deviation were shown in **Table No: 9**.

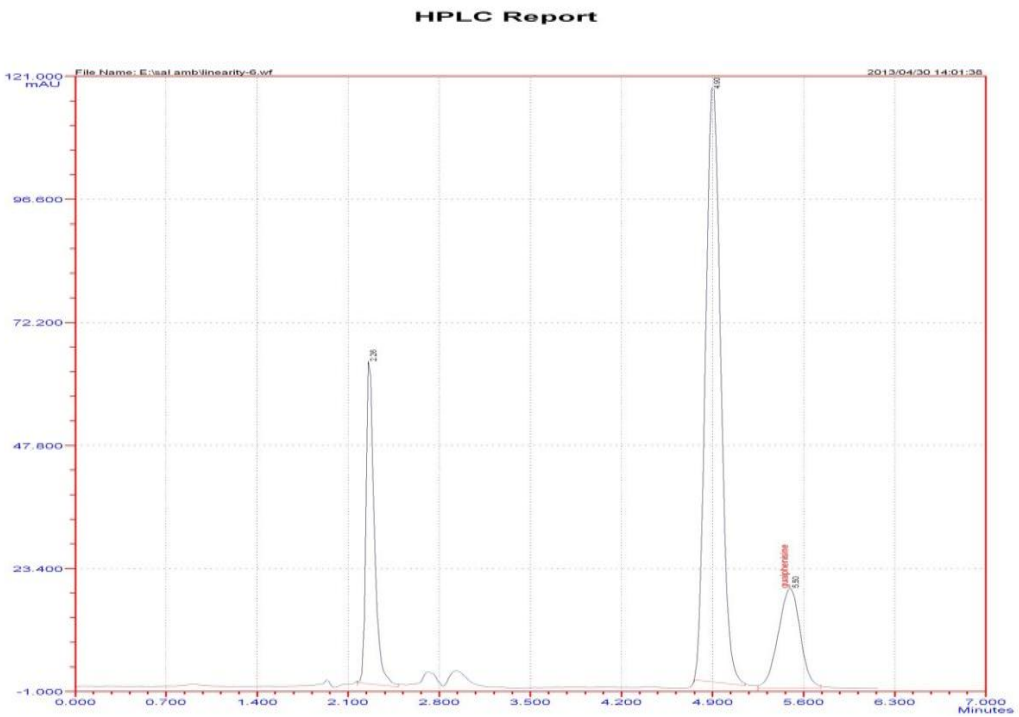

Figure 1: Representative chromatogram of standard

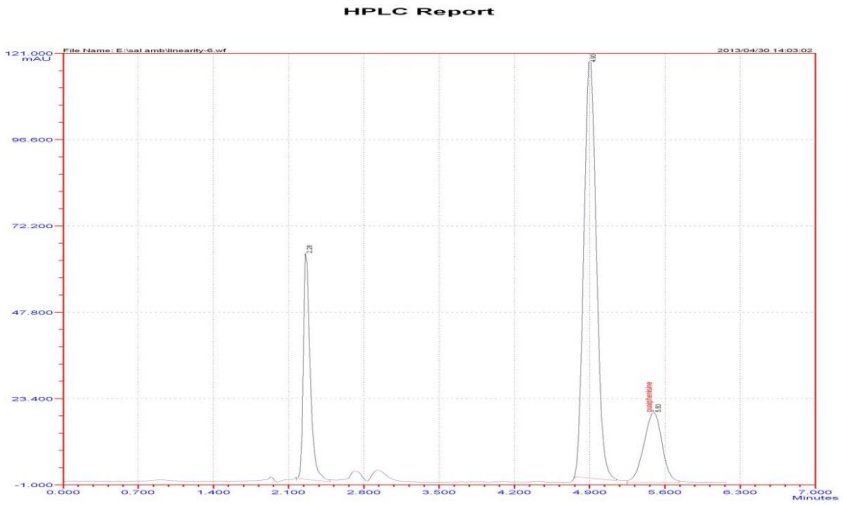

Figure 2: Representative chromatogram of sample

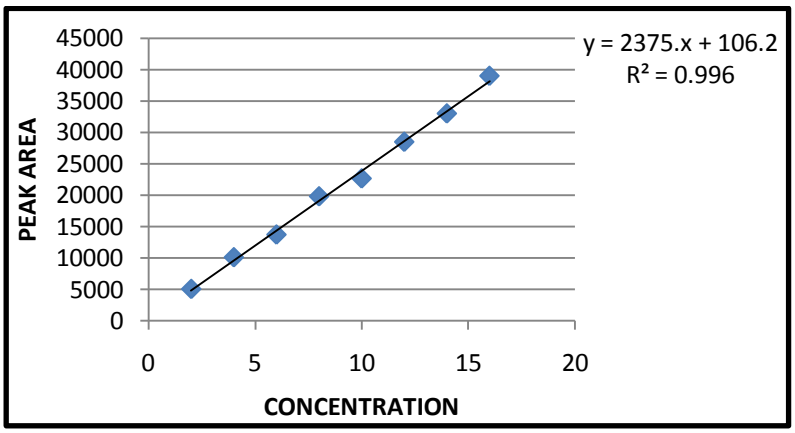

Figure 3: Linearity of Salbutamol

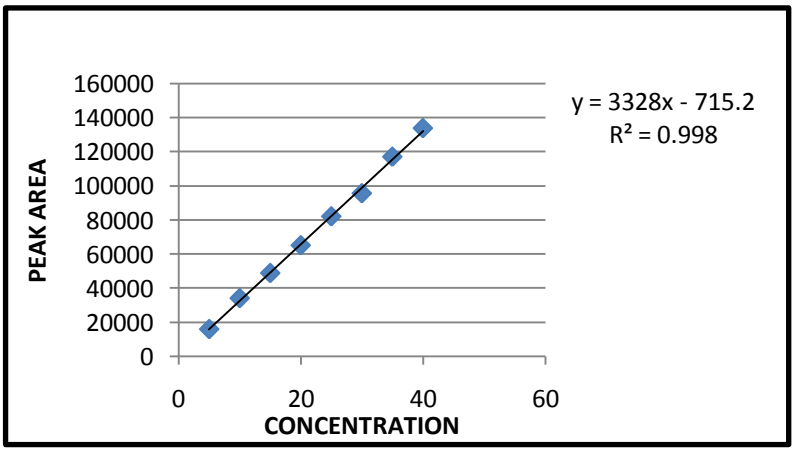

Figure 4: Linearity of Ambroxol

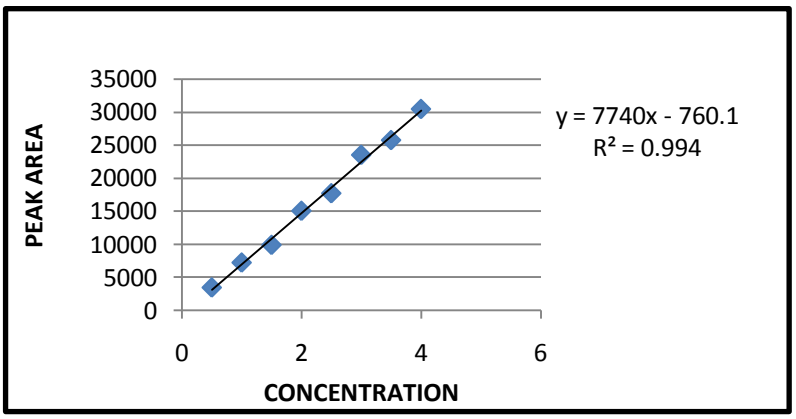

Figure 5: Linearity of Guaiphenesin

Table 1: System suitability of Salbutamol, Ambroxol and Guaifenesin

Salbutamol		Ambroxol		Guaifenesin	
Injection no.	Peak area	Injection no.	Peak area	Injection no.	Peak area
1	23465.10	1	81284.60	1	17872.90
2	24375.30	2	83721.82	2	18772.98
3	21985.22	3	82827.32	3	18982.67
4	23487.41	4	83678.92	4	17927.38
5	24763.21	5	82789.37	5	17247.67
6	22867.89	6	84121.67	6	18489.69
AVG	23490.68	AVG	83070.62	AVG	18215.55
STD. DEV	1005.66	STD. DEV	1022.44	STD. DEV	649.86
%RSD	4.28	%RSD	1.23	%RSD	3.56

Table: 2 Linearity table of Salbutamol, Ambroxol and Guaifenesin

SALBUTAMOL		AMBROXOL		GUAIFENESIN	
Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area	Concentration (µg/ml)	Peak area
2	5071.9	5	16024.1	0.5	3479.0
4	10118.5	10	34160.7	1.0	7249.8
6	13728.0	15	48916.9	1.5	9921.0
8	19818.4	20	65216.3	2.0	15074.8
10	22665.0	25	82184.6	2.5	17732.9
12	28493.0	30	95717.6	3.0	23533.7
14	33014.4	35	117163.8	3.5	25772.2
16	39004.2	40	133935.0	4.0	30474.9
R^2	0.996	R^2	0.994	R^2	0.998
Slope	2375	Slope	7740	Slope	3328
Intercept	106.2	Intercept	760.1	Intercept	715.2

Table: 3 Intra-day precision for Salbutamol, Ambroxol and Guaifenesin

Inj No.	Salbutamol			Ambroxol			Guaifenesin		
	LQC	MQC	HQC	LQC	MQC	HQC	LQC	MQC	HQC
1st	3214.25	17346.21	36897.21	13654.25	69209.70	131881.80	3892.01	14883.30	28875.50
2nd	3312.56	17343.90	35896.45	13698.96	71128.00	136260.30	3872.56	14775.00	29598.50
3rd	3254.89	16968.70	35467.29	13547.89	71395.20	134102.10	3782.12	15060.70	29263.00
AVG	3260.56	17219.60	36086.98	13633.70	70577.63	134081.40	3848.89	14906.33	29245.67
SD	49.40	217.29	733.75	77.60	1192.17	2189.32	58.64	144.23	361.81
%RSD	1.51	1.26	2.03	0.56	1.68	1.63	1.52	0.96	1.23

Table: 4 Inter-day precision for Salbutamol, Ambroxol and Guaifenesin.

DAY	Salbutamol			Ambroxol			Guaifenesin		
	LQC	MQC	HQC	LQC	MQC	HQC	LQC	MQC	HQC
1	4192.10	16886.40	37254.70	19291.40	70577.60	134081.0	3692.86	14906.30	38045.70
2	4193.98	16822.80	37372.70	19918.9	71500.37	130998.0	3618.23	14832.90	37886.90
3	4287.70	16960.20	38501.50	19701.10	72686.50	132361.0	3738.31	14702.3	37441.10
AVG	4224.59	16889.81	37709.64	19637.12	71588.14	132480.4	3683.13	14813.84	37791.22
SD	54.66	68.76	688.30	318.61	1057.17	1544.94	60.62	103.34	313.43
%RSD	1.29	0.40	1.82	1.62	1.47	1.16	1.64	0.69	0.82

Table: 5 Accuracy estimation of Salbutamol

	Amount of drug taken(µg/ml)	**Amount of drug added(µg/ml)**	**Amount of drug recovered**	**% Recovery**
LQC	10	10	18	90.00
MQC	30	10	39	97.50
HQC	50	10	57	95.00

Table: 6 Accuracy estimation of Ambroxol

	Amount of drug taken(µg/ml)	**Amount of drug added(µg/ml)**	**Amount of drug recovered**	**% Recovery**
LQC	20	10	27	90.00
MQC	40	10	48	96.00
HQC	60	10	67	95.70

Table: 7 Accuracy estimation of Guaifenesin

	Amount of drug taken (µg/ml)	**Amount of drug added(µg/ml)**	**Amount of drug recovered**	**% Recovery**
LQC	5	10	14	93.33
MQC	15	10	23	92.00
HQC	25	10	34	97.10

Table: 8 Stock solution stability studies of Salbutamol, Ambroxol and Guaifenesin

DAYS	Salbutamol			Ambroxol			Guaifenesin		
	LQC	MQC	HQC	LQC	MQC	HQC	LQC	MQC	HQC
1	5192.10	16886.43	37354.73	18291.37	70577.63	134081.40	3692.86	14906.33	39245.67
2	4593.00	16822.80	33572.70	19918.90	68500.30	130998.40	3518.00	14332.90	27886.90
3	4187.70	15160.20	31801.50	16701.10	63686.50	122361.30	3138.00	12102.30	24441.10
AVG	4657.60	16289.81	34242.98	18303.79	67588.14	129147.00	3449.62	13780.51	27191.22
SD	505.30	978.78	2836.64	1608.93	3534.96	6075.43	283.67	1481.38	2476.68
%RSD	10.84	6.01	8.28	8.79	5.23	4.70	8.22	10.74	9.10

Table: 9 Long -term stability studies of Ambroxol, Salbutamol and Guaifenesin

week	Salbutamol			Ambroxol			Guaifenesin		
	LQC	MQC	HQC	LQC	MQC	HQC	LQC	MQC	HQC
1	4657.60	16289.81	34242.98	18303.79	67588.14	129147.00	3449.62	13780.51	27191.22
2	4892.20	16897.23	33897.32	17895.23	66987.21	128965.50	3789.21	14772.30	28714.59
3	4789.35	17895.23	34285.89	19864.71	67856.13	131325.30	3678.89	13897.54	29487.36
AVG	4779.71	17027.42	34142.06	18687.91	67477.16	129812.60	3639.24	14150.12	28464.39
SD	117.59	810.59	213.04	1039.41	444.96	1313.13	173.23	541.99	1168.33
%RSD	2.46	4.76	0.62	5.56	0.659429	1.01	4.76	3.83	4.10

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