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Development and Validation of Stability Indicating UV-Visible Spectrophotometric Method for Simultaneous Determination of Salbutamol Sulphate and Ambroxol Hydrochloridein Liquid Dosage Form

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Abstract*---Purpose:*** A new, simple, precise and validated UV spectrophotometric method has been developed for simultaneous estimation of salbutamol sulphate and ambroxol hydrochloride in liquid dosage form. *Method:* The method employed was simultaneous equation method which involves solving simultaneous equations based on measurement of absorbance at two wavelengths 224 nm and 244 nm, the λmax of salbutamol sulphate and ambroxol

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hydrochloride, respectively. Specificity of the method was determined by subjecting the drugs to various stress conditions like acid, alkali, thermal and photolytic degradation. *Results:* The linearity was obtained in the concentration range of 0.1-0.6 µg/mL for salbutamol sulphateand 1.5-09 µg/mL for ambroxol hydrochloride. The proposed method was effectively applied to liquid dosage form for estimation of both drugs. The accuracy and reproducibility results are close to 100% with ≤2% RSD. The results of proposed method have been validated as per ICH guidelines. *Conclusion:* A novel, precise and accurate stability indicating spectrophotometric method has been developed for the estimation of salbutamol sulphate and ambroxol hydrochloride in pharmaceutical syrup formulation.

***Keywords*---**Salbutamol sulphate, Ambroxol hydrochloride, Simultenous equation, Stability indicating, Validation, Spectrophotometric method.

Introduction

Salbutamol sulphateis a chemically bis [(1RS)-2-[(1, 1-dimethylethyl) amino]-1-[4-hydroxy-3 (hydroxymethyl) phenyl] ethanol] sulphate (Fig. 1), it is beta-adrenoceptor agonist used for the relief of broncho-spasm in conditions such as asthma and COPD (chronic obstructive pulmonary disease)[1-4]. It stimulates beta (2)-adrenergic receptors located in the lungs, which ultimately results in relaxation of bronchial smooth muscles[5]. Literature survey reveals that salbutamol sulphate in combination with other drugs has been estimated by UV spectrophotometric methods [6-10], HPTLC [11], RP-HPLC [12], TLC method[13].Ambroxol hydrochloride is a chemically trans-4-((2-amino-3,5-dibromobenzyl) amino) cyclohexanol hydrochloride (Fig. 2).Ambroxol hydrochloridereduces bronchial hyper-reactivity and acts as a mucolytic and cough suppressant. Both salbutamol sulphateand ambroxol hydrochloride are official in Indian pharmacopoeia. Combination of salbutamol sulphateand ambroxol hydrochloride is used for the treatment of asthma and bronchitis[14-16]. Literature survey also reveals UV spectrophotometric[17-20], RP-HPLC [21-25], HPTLC [26] and LC-MS/MS [27]methods are reported for estimation of ambroxol hydrochloride with other drugs in combination.Only UV Spectrophotometric[28], RP-HPLC[29] and HPTLC[30]methods have been found to be reported for the simultaneous estimation of salbutamol sulphate and ambroxol hydrochloride in combination for solid dosage form and no any stability indicating UV spectrophotometric method reported yet for liquid dosage form of same drug combination. Therefore, the objective of the present work is to develop new stability indicating UV spectrophotometric method for estimation of salbutamol sulphate and ambroxol hydrochloride in liquid dosage form with good accuracy, simplicity, precision and reproducibility over other spectrophotometric methods and which can be used for routine analysis methods for simultaneous determination of salbutamol sulphateand ambroxol hydrochloride. The proposed methods were optimized and validated as per ICH guidelines[31].

Fig. 1: Structure of Salbutamol sulphate

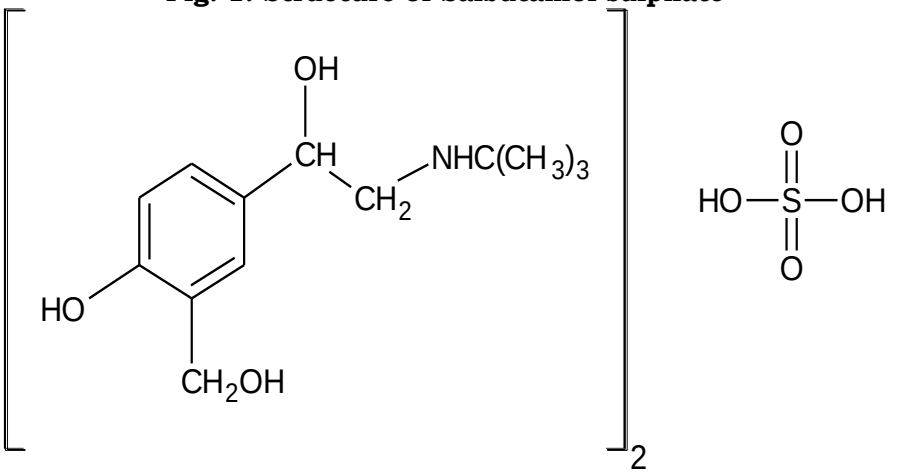
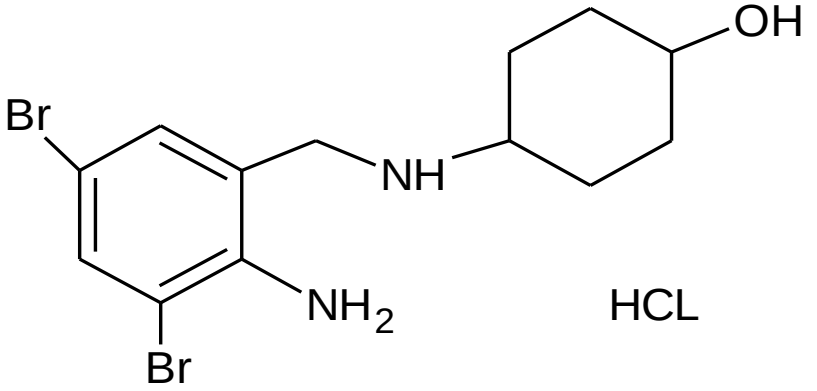


Fig. 2: Structure of Ambroxol hydrochloride



Materials and Methods

The instrument used in the present study was UV- spectrophotometer UV-1800 (Shimadzu, Japan) with spectral bandwidth of 2 nm and 10 mm matched quartz cells over the range of 200-400 nm. All weighing was done on digital weighing balance (Anamed, AA-2200).

Reagents and Chemicals

Sample of salbutamol sulphateand ambroxol hydrochloride were obtained as a gift sample from Orex Pharma Pvt. Ltd., Thane, India and Erica Pharma Pvt. Ltd., Mumbai, India respectively. Marketed formulation Ambrodil-S syrup containing salbutamol sulphate 01 mg and Ambroxol hydrochloride 15mg for each 5 mL of syrup was purchased from local market. All solvents used were of AR grade.

Preparation of Stock Standard Solutions

Standard stock solutions of both salbutamol sulphate and ambroxol hydrochloride were prepared by dissolving 10 mg of salbutamol sulphate and 10 mg of ambroxol hydrochloride separately in 10 mL of 0.1N HCL in 100 mL volumetric flasks. Final volume was made up to 100 mL with 0.1N HCL to get working standard solution of each containing100µg/mL of both salbutamol sulphate and ambroxol hydrochloride.

Determination of Absorption Maxima

By appropriate dilution of standard stock solutions of salbutamol sulphate and ambroxol hydrochloride with 0.1N HCL, solutions containing 10 µg/mL of salbutamol sulphate and 10 µg/mL of ambroxol hydrochloride were scanned separately in the range of 200-400 nm. Wavelength of absorption maxima's was determined for both drugs. Salbutamol sulphate showed maximum absorbance at 224 nm and ambroxol hydrochloride at 244 nm.

Development of Simultaneous Equation

From the stock solution, working standard solution of drugs was prepared by appropriate dilution and was scanned from 400nm to 200nm. Two wavelengths were selected for this method i.e. 224 nm and 244 nm that are absorption maximas of salbutamol sulphate and ambroxol hydrochloride respectively in 0.1N HCL. Series of dilution were prepared from standard solutions of salbutamol sulphate and ambroxol hydrochloride. The linearity was observed in the concentration range of 0.1-0.6 µg/mL for salbutamol sulphate and1.5- 09 µg/mL for AMB. The absorbance values were measured at the selected wavelengths and absorptivities for both the drugs at both wavelengths were determined. The calibration curves for salbutamol sulphateand ambroxol hydrochloride were plotted in the concentration range of 0.1-0.6 µg/mL and 1.5-09 µg/mL. The concentrations of drugs in sample solution were determined by using the formula 3 and 4,

$A_1 = ax_1Cx + ay_1Cy$ .....(1)
$A_2 = ay_1Cx + ay_2Cy$.....(2)
For the measurements in 1 cm cells, b =1
Rearranging above equations,

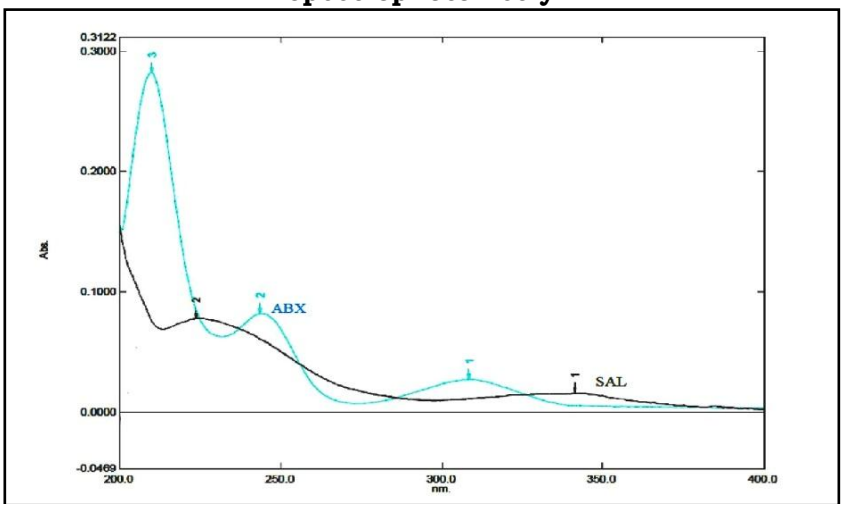
$$c_x = \frac{A_2 ay_1 - A_1 ay_2}{ax_2 ay_1 - ax_1 ay_2} \text{.....(3)}$$

$$c_y = \frac{A_1 ax_2 - A_2 ax_1}{ax_2 ay_1 - ax_1 ay_2} \text{.....(4)}$$

Where, A_1 and A_2 = Absorbance of sample at λ_1 and λ_2
Cx and Cy = Concentrations of salbutamol and ambroxol in sample matrix.
ax_1 and ax_2 = Absorptivities of salbutamol sulphateat λ_1 and λ_2
$ay_1$and ay_2= Absorptivities of ambroxol hydrochloride at λ_1 and λ_2

By solving the two simultaneous equations, the concentrations of salbutamol sulphate and ambroxol hydrochloride in sample solutions were obtained. Overlain spectrum of salbutamol and ambroxol by UV Spectrophotometry is shown in (Fig.3)

Fig. 3: Overlain spectrum of salbutamol and ambroxol by UV Spectrophotometry



Analysis of marketed formulation

Accurately 5 mL of ambrodil-S syrup (Label claim: Each 5mL of syrup contains salbutamol sulphate 01 mg and ambroxol hydrochloride 15 mg) was measured and taken into a 100mL volumetric flask. It is then dissolved in 0.1N HCL and is made up to the mark. The solution is then filtered through Whatman filter paper no. 41. The concentrations of the two drugs in the sample solutions were determined by simultaneous equation (Table 1).

Table 1: Analysis of marketed syrup formulation

* Indicates average of six determinations

Sr. no.	Label claim (mg/5 ml)		Amount of drug found (mg/5 ml)		% Label Claim	
	SAL	**ABX**	**SAL**	**ABX**	**SAL**	**ABX**
1	01	15	1.01	15.04	101.34	100.32
2	01	15	1.00	15.15	100.22	101.02
3	01	15	0.99	14.99	99.82	99.98
4	01	15	0.99	14.98	99.97	99.91
5	01	15	0.98	14.79	98.93	98.65
6	01	15	1.00	15.24	100.25	101.65
				Mean*	100.08	100.25
				SD	0.779	1.030
				% RSD	0.778	1.027

Method Validation

Validation of proposed method was done as per ICH guidelines[31]by studying the following method validation parameters.

Linearity:

As per ICH guidelines the linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample[31]. The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of salbutamol sulphate and ambroxol hydrochloride. For linearity study, stock solutions were subsequently diluted with 0.1 N HCL to get concentrations from 0.1-0.6µg/ml and 1.5-09µg/ml of salbutamol sulphate and ambroxol hydrochloride respectively. Then the absorbance of these diluted solutions were measured at 224 nm (λ_1) for salbutamol sulphate and 244 nm (λ_2) for ambroxol hydrochloride by double beam UV spectrophotometer against a blank of 0.1 N HCL. Average of six replicates readings was taken and tabulated (Table 2-4). For both the methods, the Beer law was obeyed in the concentration range 0.1-0.6 µg/mL and 1.5-09 µg/mL for salbutamol sulphate andambroxol hydrochloride respectively. Calibration curve of salbutamol sulphate and ambroxol hydrochloride is shown in (Fig. 4) and (Fig. 5) respectively.

Fig. 4: Calibration curve of salbutamol sulphate by UV Spectrophotometry

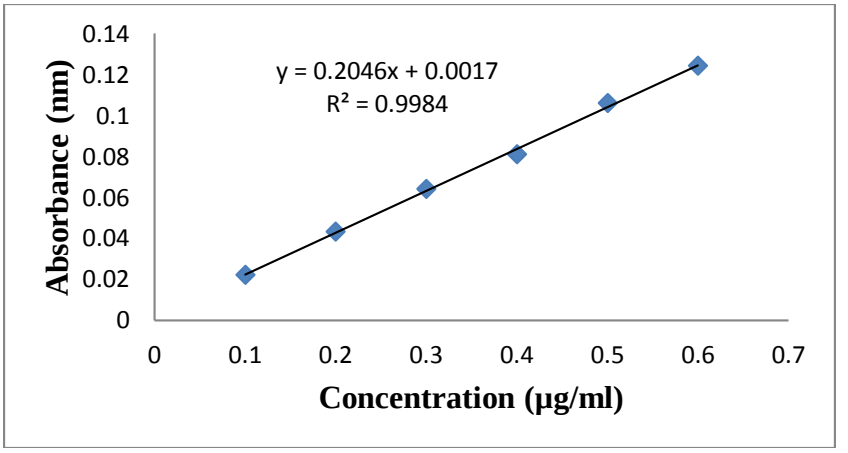


Fig. 5: Calibration curve of ambroxol hydrochloride by UV Spectrophotometry

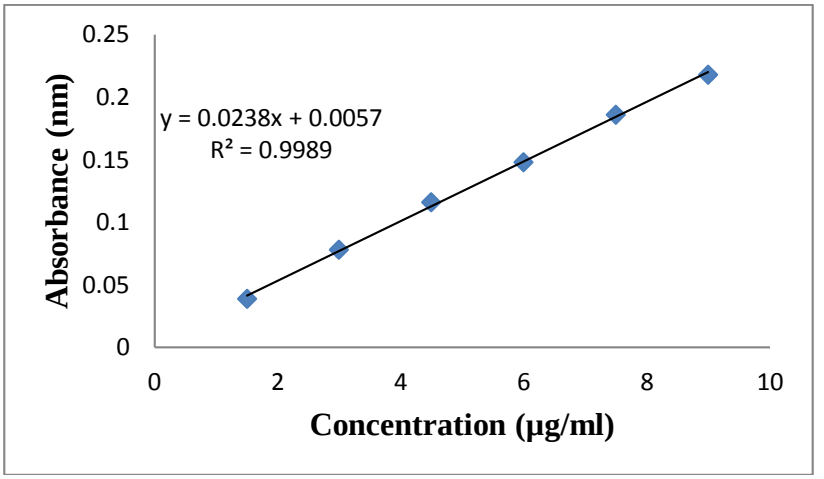


Table 2: Linearity study data of salbutamol sulphate

Sr. No.	Conc. (µg/ml)	Absorbance at 224 nm
1	0.1	0.022
2	0.2	0.043
3	0.3	0.064
4	0.4	0.081
5	0.5	0.106
6	0.6	0.124

Table 3: Linearity study data of ambroxol hydrochloride

Sr. No.	Conc. (µg/ml)	Absorbance at 244 nm
1	1.5	0.039
2	3.0	0.078
3	4.5	0.116

4	6.0	0.148
5	7.5	0.186
6	9.0	0.218

Table 4: Optical characteristics and other parameters

Parameters	SAL	ABX
Absorption maxima (nm)	224	244
Linearity range (µg/ml)	0.1-0.6	1.5-9.0
Limit of detection (µg/ml)	0.015	0.135
Limit of quantitation (µg/ml)	0.048	0.411
Slope (m)	0.204	0.023
Intercept (c)	0.001	0.005
Regression coefficient (R^2)	0.998	0.998

Repeatability (Intra-assay precision):

To check the degree of repeatability of the methods, suitable statistical evaluation was carried out. Six samples of the syrup formulation were analyzed for the repeatability study. The standard deviation and coefficient of variance was calculated (Table 5).

Table 5: Repeatability Data

No. of Sample	% Recovery	
	SAL	ABX
1	99.24	99.15
2	99.24	99.15
3	101.7	101.6
4	99.24	99.15
5	99.24	101.6
6	99.24	99.15

Mean*	100	100
SD *	1.312	1.841
%RSD*	1.312	0.841

***** Indicates average of six determination

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions[31]. The reproducibility of the proposed methods was determined by performing tablet assay at different time intervals on same day (Intraday precision) and on three different days (Inter day precision) (Table 6).

Table 6: Precision data of marketed formulation

Samples	**Intraday**		**Interday**	
	SAL	**ABX**	**SAL**	**ABX**
1	98.80	98.31	98.41	99.15
2	101.19	100.00	98.80	100.84
3	98.80	101.68	99.60	99.71
Mean*	99.59	99.99	99.60	99.90
SD*	1.379	1.685	0.606	0.860
%RSD*	1.385	1.685	0.613	0.861

***** Indicates average of three determinations

Accuracy (Recovery studies):

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found[31]. To ascertain the accuracy of proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). Percent recovery for salbutamol sulphate andambroxol hydrochloride, by both the methods, was calculated (Table 7-8).

Table 7: Recovery study data for method validation of Salbutamol and Ambroxol

Level of Recovery	Amount present (mg)		Added concentration (mg)		Amount recovered (mg)		% Recovery	
	SAL	**ABX**	**SAL**	**ABX**	**SAL**	**ABX**	**SAL**	**ABX**
	01	15	0.8	12	1.772	26.88	98.47	99.59
80%	01	15	0.8	12	1.793	26.88	99.66	99.59
	01	15	0.8	12	1.764	26.66	98.04	98.75
	01	15	01	15	1.997	29.58	99.85	98.63
100%	01	15	01	15	1.968	29.79	98.42	99.31
	01	15	01	15	1.996	29.79	99.81	99.31
	01	15	1.2	17	2.212	32.53	100.57	101.68
120%	01	15	1.2	17	2.230	32.43	101.38	101.36
	01	15	1.2	17	2.212	31.98	100.57	99.94

Table 8: Statistical validation of recovery study data

Level of Recovery	% Mean recovery*		SD		% RSD	
	SAL	**ABX**	**SAL**	**ABX**	**SAL**	**ABX**
80%	98.72	99.31	0.839	0.484	0.850	0.488
100%	99.36	99.08	0.814	0.392	0.819	0.396
120%	100.84	100.99	0.467	0.926	0.463	0.917

***** Indicates average of three determinations

LOD and LOQ:

According to ICH guidelines the detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Limit of detection can be calculated using following equation.

$LOD = 3.3 \times N/S$

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Limit of quantification can be calculated using following equation.

$LOQ = 10 \times N/S$

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

Forced degradation study

Specificity of the method was determined by calculating percent amount of possible degradation products produced during the forced degradation study. The forced degradation was carried out with 0.1 NHCL,0.1 NNaOH, thermal (80 °C) and UV photolysis (365 nm) as per ICH guidelines[32-34].

Results

The absorbance of the salbutamol sulphate andambroxol hydrochloride was measured at 224 nm and 244 nm respectively and calibration curves were plotted as concentrations versus absorbance. The Relative Standard Deviation (RSD) for intra-day analysis of salbutamol sulphate and ambroxol hydrochloride was found to be 1.385 and 1.685 respectively. RSD for Inter-day analysis of salbutamol sulphate and ambroxol hydrochloride was found to be 0.613 and 0.861 respectively. The accuracy and reproducibility is evident from the data as results are close to 100 % and the value of standard deviation and % R.S.D. were found to be < 2 %; shows the high precision of the method. The summary of validated parameters is shown in (Table 09). The proposed method is simple, economical, rapid, precise and accurate. Hence it can be used for routine analysis of salbutamol sulphate and ambroxol hydrochloride in pharmaceutical formulation. The proposed method was found to be specific as there is no interference from other excipients. The LOD for salbutamol sulphate and ambroxol hydrochloride was found to be 0.015 µg/mL and 0.135µg/mL respectively. The LOQ for salbutamol sulphate and ambroxol hydrochloride was found to be 0.048µg/mL and 0.411µg/mL respectively. Marketed brand of syrup (Ambrodil-S) was analyzed, the amounts of salbutamol sulphate and ambroxol hydrochloride determined by proposed method were found to be 100.08 % and 100.25 % respectively. The stress degradation studies showed that salbutamol sulphateundergoes degradation in acidic, alkaline, photolytic and thermal condition and ambroxol hydrochloride undergoes degradation in acidic, alkaline, photolytic and thermal condition respectively. The results for forced degradation are shown in (Table 10).

Table 9: Summary of validation parameters.

Parameters	Salbutamol sulphate	Ambroxol hydrochloride
Linearity Range (µg/mL)	0.1-0.6	1.5-9.0
Correlation coefficient (r^2)	0. 998	0. 998
Precision (%RSD)		
Intraday	1.385	1.685
Interday	0.613	0.861
Accuracy (%)		
80% ± RSD	98.72 ± 0.850	99.31 ± 0.488
100% ± RSD	99.36 ± 0.819	99.08 ± 0.396

120% ± RSD	100.84 ± 0.463	100.99 ± 0.917
Repeatability (%RSD)	1.312	1.841
LOD (µg/mL)	0.015	0.135
LOQ (µg/mL)	0.048	0.411
Solution stability	Stable for 24 hrs.	Stable for 24 hrs.

Table 10: Summary of results of stress degradation studies

Sr. no.	Condition	% Degradation		% Assay	
		SAL	ABX	SAL	ABX
1	Acid hydrolysis (0.1 N HCL, 80°c, 6 hrs.)	38.09	47.61	61.91	52.95
2	Base hydrolysis (0.1 N NaOH, 80°c, 2 hrs.)	20.23	19.32	79.77	80.68
3	Photolytic degradation (UV Radiation, 3 hrs.)	38.09	45.65	61.91	54.35
4	Thermal degradation (80°c, 5 hrs)	17.85	16.52	82.15	83.48

Discussion

In simultaneous equation method, the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all the wavelength, which was fulfilled in case of both these drugs. The wavelengths used for the analysis of the drugs were 224 nm (λ-max of salbutamol sulphate) and 244 nm (λ-max of ambroxol hydrochloride) at which the calibration curves were prepared for both the drugs. In 0.1 N HCL, salbutamol sulphate and ambroxol hydrochloride obeyed linearity in the concentration range of 0.1-0.6 µg/mL and 1.5-09µg/mL respectively at their respective λmax with correlation coefficient (r^2> 0.99) in both the case. In proposed method precision was studied as repeatability (%RSD<2) and inter and intra-day variations (%RSD<2) for both drugs; shows the high precision of the method. The accuracy of method was determined by calculating mean percentage recovery. It was determined at 80, 100 and 120 % level and data tabulated. The developed method for estimation of salbutamol sulphate and ambroxol hydrochloride in liquid dosage form was found to be simple, accurate, reproducible, sensitive and economic. For projected method we used easily available and cheap solvent like 0.1 NHCL (AR grade). The proposed method of simultaneous estimation not required any expensive and satisfactory apparatus in contrast to reported chromatographic and hyphenated techniques. So it shows projected method is simple, economic and rapid for estimation of salbutamol sulphate and ambroxol hydrochloride in combined liquid

dosage forms. Hence the developed method for estimation of salbutamol sulphate and ambroxol hydrochloride can be constructive in the routine analysis.

Conclusion

The developed and validated stability indicating UV spectrophotometric method reported here is rapid, simple, accurate, sensitive and specific in accordance to the ICH guidelines showing linearity, accuracy, precision, selectivity, stability and system suitability for the routine determination of salbutamol sulphate and ambroxol hydrochloride. The developed method was successfully used for quantitative estimation and analysis of salbutamol sulphate and ambroxol hydrochloride in combined liquid dosage form. Stress degradation results of show that the method is selective and stability indicating. Thus the reported method is of substantial importance and has great industrial applicability for quality control and analysis of salbutamol sulphate and ambroxol hydrochloride in liquid dosage forms. By observing validation parameter and statistical data, the proposed method was found to be satisfactory over other reported spectrophotometric and chromatographic methods.

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