Open Access Contents
Int. J. Drug Dev. & Res.
| October - December 2013 | Vol. 5 | Issue 4 | ISSN 0975-9344 |
www.ijddr.in

Full Length Original Research Paper

Spectrophotometric method development and validation for simultaneous estimation of salbutamol sulphate and Ambroxol Hydrochloride in combined dosage Forms

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Abstract:
Salbutamol Sulphate and Ambroxol Hydrochloride is used for the treatment of bronchitis, cough and asthma. A simple, economical, accurate and precise method for simultaneous estimation of Salbutamol Sulphate and Ambroxol Hydrochloride in combined dosage form has been developed. Simultaneous equation method based on measurement of absorbance at two wavelengths i.e. 244 nm and 276 nm, λ_{max} of Ambroxol Hydrochloride and Salbutamol Sulphate in 6.8 pH phosphate buffer. Both these drugs obeyed Beer Lambert's law in the concentration range of 2-18 µg/ml for Ambroxol Hydrochloride and 10-100 µg/ml for Salbutamol Sulphate. The high values of correlation coefficient (R^2) indicated good linearity of calibration curve for both the drugs. The accuracy and precision of method was determined and the method validated statistically. Result of percentage recovery study confirms the accuracy of proposed method. As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, precision and assay of drug formulation. Based on the results obtained, it can be concluded that the proposed UV-Spectrophotometric methods for simultaneous determination of Salbutamol Sulphate and Ambroxol Hydrochloride is rapid, economical, accurate, precise and reproducible. Hence, the proposed method can be employed for quantitative determination of Salbutamol Sulphate and Ambroxol Hydrochloride in combined dose tablet formulation. Simultaneous equation method can be used to carry out dissolution study in combination tablet formulation of these drugs.

Keywords: Salbutamol Sulphate, Ambroxol Hydrochloride, UV Spectrophotometric method, Simultaneous equation, λ_{max}.

Introduction

Salbutamol Sulphate (SAL), (Fig. 1) chemically known as (RS)-1-(4-hydroxy-3-hydroxy-methylphenyl)-2-(tert-butylamino) ethanol sulphate. It is a white or almost white, crystalline powder. It is freely soluble in water, slightly soluble in ethanol (95 %) and in ether; very slightly soluble in dichloromethane. The drug is official in Indian Pharmacopoeia and British Pharmacopoeia [1, 2]. Salbutamol sulphate is a short-acting ß2-adrenergic receptor agonist used for the relief of bronchospasm in conditions such as asthma and COPD (Chronic obstructive pulmonary disease)[3].

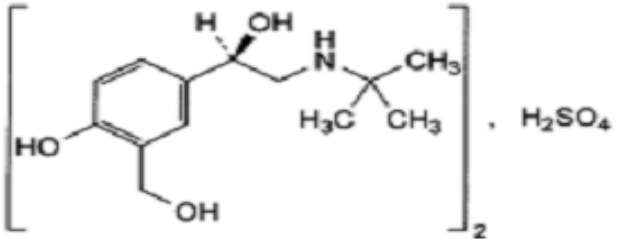

Fig. 1: Chemical Structure of Salbutamol Sulphate

Ambroxol Hydrochloride [AMB HCl] (Fig. 2) official in Indian Pharmacopoeia and British Pharmacopoeia, is chemically Trans-4-[(2-amino-3, 5-dibromobenzyl) amino]-cyclohexanol hydrochloride. It is a white or yellowish crystalline

powder. It is sparingly soluble in water; soluble in methanol; practically insoluble in methylene chloride [4, 5]. Ambroxol hydrochloride is a potent mucolytic & mucokinetic, capable of inducing bronchial secretion. It depolymerises mucopolysaccharides directly as well as by liberating lysosomal enzymes network of fibres in tenacious sputum is broken. It is particularly useful in if mucus plugs are present. Ambroxol hydrochloride (AMB) is semi-synthetic derivative of vasicine obtained from Indian shrub Adhatoda vasica. It is a metabolic product of bromhexine. Used in a variety of respiratory disorders including chronic bronchitis, also used in the treatment of cough [6].

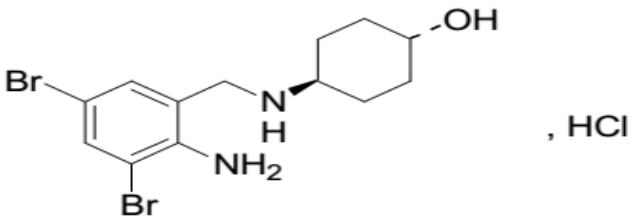

Fig. 2: Chemical Structure of Ambroxol Hydrochloride

The combination of these two drugs is not official in any pharmacopoeia; hence, no official method is available for the simultaneous estimation of Salbutamol Sulphate and Ambroxol Hydrochloride in their combined dosage forms. Literature survey does not reveal any simple spectrophotometric method for simultaneous estimation of Salbutamol Sulphate and Ambroxol Hydrochloride in combined dosage form. The present research paper describes simple, sensitive, rapid, accurate, precise and economical spectrophotometric method based on simultaneous equation method for estimation of both drugs in the combined dosage forms is developed and validated.

Ultraviolet and Visible Spectrophotometric is one of the most frequently employed analytical tools in the pharmaceutical industry. Spectrophotometry is mainly concerned with the following regions of spectrum: ultraviolet, visible and infrared [7]. Ultraviolet and Visible absorption spectrophotometry involves the measurement of the absorption of monochromatic radiation by solutions of chemical substances, in the range of 185 nm to 380 nm, and 380 nm to 780 nm of the spectrum, respectively [8].

Simultaneous Equation Method

If a sample containing two absorbing drugs (X and Y) each of which absorbs at the λ_{max} different from the other, it may be determine both the drugs by the technique of simultaneous equations. The absorptivities of drug X at λ_1 and λ_2 are ax_1 and ax_2 respectively, absorptivities of drug Y at λ_1 and λ_2 are ay_1 and ay_2 respectively and the absorbances of diluted sample at λ_1 and λ_2 are A_1 and A_2 respectively. Let C_x and C_y be the concentrations of X and Y respectively in the diluted sample. L is the path length.

Absorbance of pure compound X at λ_1 and λ_2

At λ_1 $A_1 = ax_1C_xL$

At λ_2 $A_2 = ax_2C_xL$

Absorbance of pure compound Y at λ_1 and λ_2

At λ_1 $A_1 = ay_1C_yL$

At λ_2 $A_2 = ay_2C_yL$

Absorbance of mixture of compound X and Y at λ_1 and λ_2

At λ_1 $A_1 = ax_1C_xL + ay_1C_yL$ ..... (1)

At λ_2 $A_2 = ax_2C_xL + ay_2C_yL$..... (2)

Criteria for obtaining maximum precision, based on absorbance ratios, have been suggested that place limits on the relative concentrations of components of the mixture. The

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criteria are that the ratios should lie outside the range 0.1 – 2.0 for the precise determination of Y and X respectively.

$\frac{A_2/A_1}{ax_2/ax_1}$ and $\frac{ay_2/ay_1}{A_2/A_1}$

These criteria are satisfied only when the λ_{max} of tw o component are reasonably dissimilar. An additio nal criterion is that the two components don't interact chemically, thereby negating the initial assumption that the total absorbance is the sum of the individual absorptions. The additivity of absorbances should always be confirmed in the development of a new application of this technique [9].

Materials and Methods

Apparatus

A double UV Visible Spectrophotometer (UV- 1800 Shimadzu, Japan) was used. Absorption and overlain spectra of both test and standard solutions were recorded over the wavelength range of 200-400 nm using 1cm quartz cell at fast scanned speed and fixed slit width of 1.0 nm. All weighing of ingredients were done on digital weighing balance (DV 215 CD Ohaus, USA) and bath sonicator (PCI Analytical Pvt. Ltd) was also used in study. Glassware used in each procedure were soaked overnight in a mixture of chromic acid and sulphuric acid rinsed thoroughly with double distilled water and dried in hot air oven.

Reagents and Materials

Salbutamol Sulphate and Ambroxol Hydrochloride were supplied as gift sample by Trojan Pharma Baddi, India. All other chemicals and reagents used were of analytical grade.

Selection of Common Solvent

Phosphate buffer of pH 6.8 was selected as common solvent for developing spectral characteristics of drug. The selection was made after assessing the solubility of both the drugs in different solvents.

Preparation of Standard Stock Solution

Standard stock solution of both Salbutamol Sulphate and Ambroxol Hydrochloride were prepared by dissolving 10 mg of SAL and 10 mg of AMB separately in 10 ml of 6.8 pH Phosphate buffer solution and sonicated for 15 minutes in bath sonicator and filtered through whatman filter paper in order to get dilution of 1 mg/1 ml i.e. 1000 µg/ml.

Determination of Absorption Maximas

By appropriate dilution of standard stock solutions of Salbutamol Sulphate and Ambroxol Hydrochloride with 6.8 pH phosphate buffer, solution containing 10 µg/ml of Salbutamol Sulphate and 10 µg/ml of Ambroxol Hydrochloride was scanned separately in the range of 200-400 nm. Wavelength of absorption maximas was determined for both drugs. Salbutamol Sulphate showed absorption maximas one at 224 nm and other at 276 nm respectively (Fig. 3). 276 nm was selected as λ_{max} of Salbutamol Sulphate. Ambroxol Hydrochloride showed maximum absorbance at 244 nm and other at 308 nm respectively (Fig. 4). 244 nm was selected as λ_{max} of Ambroxol Hydrochloride.

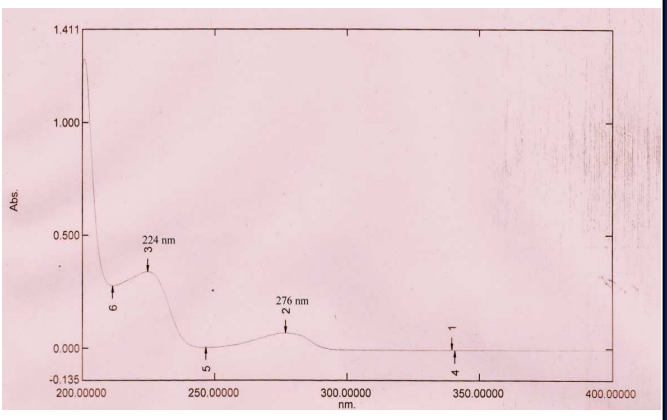

Fig. 3: UV Scan of Salbutamol Sulphate

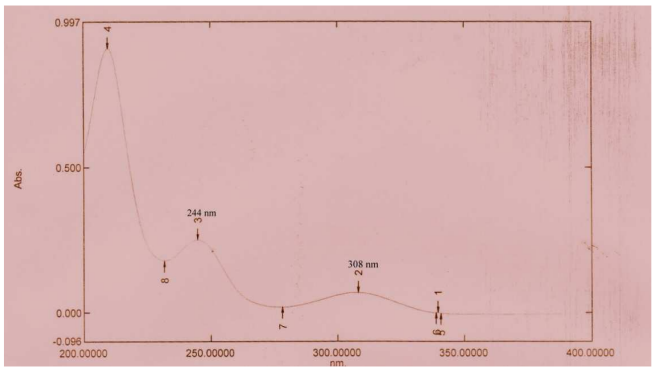

Fig. 4: UV Scan of Ambroxol Hydrochloride

Development of Simultaneous Equation

From the overlain spectra of Salbutamol Sulphate and Ambroxol Hydrochloride (Fig. 5), two wavelengths namely 276 nm and 244 nm, λ_{max} of Salbutamol Sulphate and Ambroxol Hydrochloride were selected.

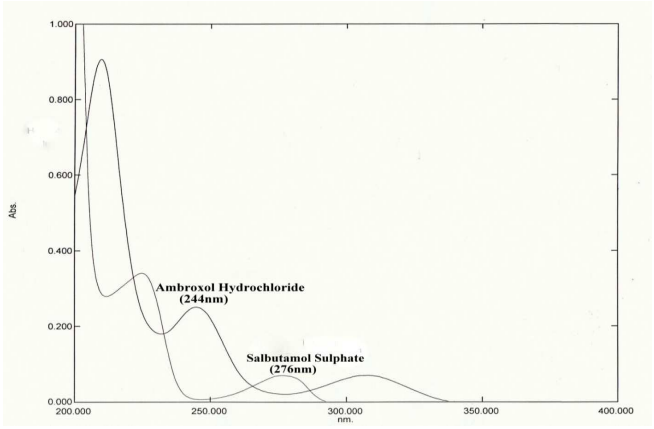

Figure 5: Overlay Spectra of Salbutamol Sulphate and Ambroxol Hydrochloride for Simultaneous Equation Method

The calibration curves were constructed in concentration range of 10-100 µg/ml for Salbutamol Sulphate (Fig. 6, Fig. 7) and 2-18 µg/ml for Ambroxol Hydrochloride at each wavelength i.e. 276 nm and 244 nm **(**Fig. 8, Fig. 9). The linearity was observed in the concentration range of 10-100 µg/ml for Salbutamol Sulphate and 2-18 µg/ml for Ambroxol Hydrochloride. The absorbances were measured at the selected wavelengths and absorptivities for both drugs were determined at both wavelengths. The concentrations of drugs in sample solution were determined by using following formula

At 244 nm $A_1 = ax_1 C_a + ay_1 C_s$ 1

At 276 nm $A_2 = ax_2 C_a + ay_2 C_s$ 2

Where C_a and C_s are the concentration of Ambroxol Hydrochloride and Salbutamol Sulphate respectively, A_1 and A_2 are absorbance at 244 nm and 276 nm respectively, ax_1 and ax_2 are absorptivities of Ambroxol Hydrochloride at 244 nm and 276 nm respectively; ay_1 and ay_2 are absorptivities of Salbutamol Sulphate at 244 nm and 276 nm respectively.

By solving the two simultaneous equations, the co ncentrations of Ambroxol Hydrochloride and Salbutamol Sulphate in sample solutions were obtained.

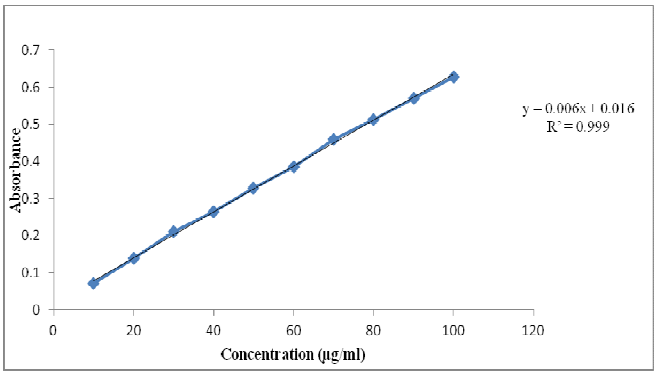

Fig. 6: Standard calibration plot of Salbutamol Sulphate at 276 nm in 6.8 pH Phosphate buffer solution

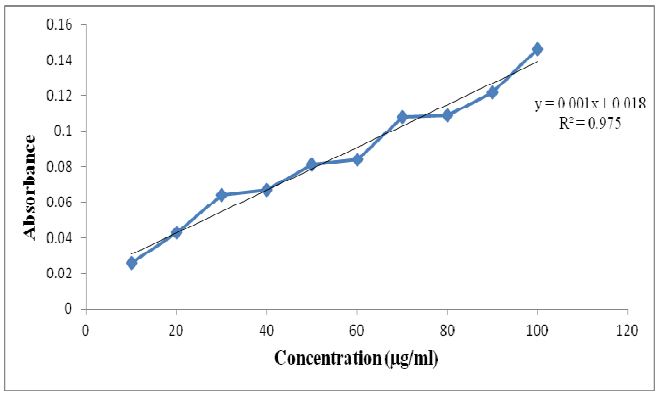

Fig. 7: Standard calibration plot of Salbutamol Sulphate at 244 nm in 6.8 pH Phosphate buffer solution

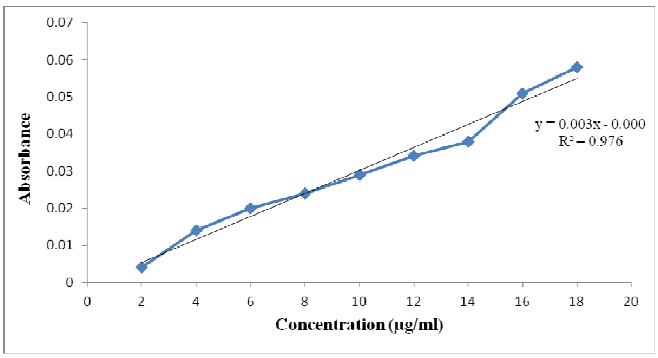

Fig. 8: Standard calibration plot of Ambroxol Hydrochloride at 276 nm in 6.8 pH Phosphate buffer solution

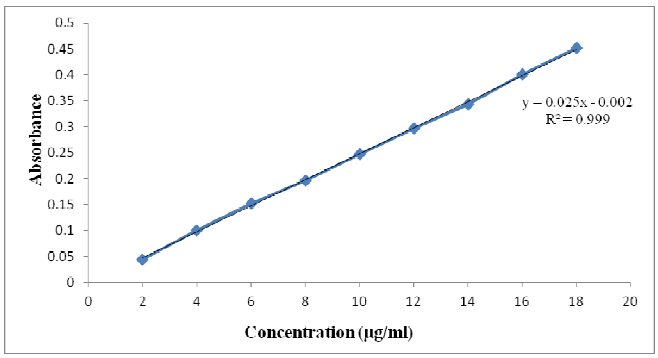

Fig. 9: Standard calibration plot of Ambroxol Hydrochloride at 244 nm in 6.8 pH Phosphate buffer solution

The absorbances were measured at the selected wavelengths and absorptivities for both drugs (Table 1, Table 2) were determined at both wavelengths. The concentrations of drugs in sample solution were determined by using following formula.

Table 1: Absorbance and Absorptivity of Ambroxol Hydrochloride at λ_{max} 244 nm and 276 nm respectively

Sr. No.	Concentration (µg/ml)	*Absorbance at 244 nm± S.D	*Absorbance at 276 nm ± S.D	Absorptivity At 244nm	Absorptivity at 276 nm
1	2	0.044 ± 0.001	0.004 ± 0.002	0.022	0.0020
2	4	0.101 ± 0.002	0.014 ± 0.001	0.025	0.0035
3	6	0.152 ± 0.003	0.020 ± 0.004	0.025	0.0033
4	8	0.197 ± 0.001	0.024 ± 0.003	0.025	0.0030
5	10	0.248 ± 0.002	0.029 ± 0.002	0.025	0.0029
6	12	0.297 ± 0.004	0.034 ± 0.001	0.025	0.0028
7	14	0.343 ± 0.002	0.038 ± 0.002	0.025	0.0027
8	16	0.401 ± 0.002	0.051 ± 0.001	0.025	0.0032
9	18	0.452 ± 0.003	0.058 ± 0.003	0.025	0.0032
			Average	ax_1 = 0.025	ax_2 = 0.0030

*Each value is the average of three determinations

Table 2: Absorbance and Absorptivity of Salbutamol Sulphate at λ_{max} 244 nm and 276 nm respectively

Sr. No.	Concentration (µg/ml)	*Absorbance at 244 nm± S.D	*Absorbance at 276 nm ± S.D	Absorptivity At 244nm	Absorptivity at 276 nm
1	10	0.026 ± 0.003	0.071 ± 0.002	0.0026	0.0071
2	20	0.043 ± 0.004	0.138 ± 0.003	0.0022	0.0069
3	30	0.064 ± 0.002	0.211 ± 0.002	0.0021	0.0070
4	40	0.067 ± 0.001	0.264 ± 0.001	0.0017	0.0066
5	50	0.081 ± 0.002	0.327 ± 0.002	0.0016	0.0065
6	60	0.084 ± 0.003	0.385 ± 0.003	0.0014	0.0064
7	70	0.108 ± 0.002	0.459 ± 0.001	0.0015	0.0066
8	80	0.109 ± 0.002	0.512 ± 0.001	0.0014	0.0064
9	90	0.122 ± 0.003	0.571 ± 0.001	0.0014	0.0063
10	100	0.146 ± 0.002	0.628 ± 0.002	0.0015	0.0063
			Average	ay_1 = 0.0017	ay_2 = 0.0066

*Each value is the average of three determinations

Table 3: Mean Absorptivity of Salbutamol Sulphate and Ambroxol Hydrochloride at two λ_{max}

Drug	λ_{max} (nm)	Absorptivity (Mean)
Ambroxol Hydrochloride	244	0.025
Ambroxol Hydrochloride	276	0.0030
Salbutamol Sulphate	244	0.0017
Salbutamol Sulphate	276	0.0066

Substituting the values of ax_1, ax_2, ay_1 and ay_2, the equation could be rearranged as:

At 244 nm $A_1 = 0.025\ C_a + 0.0017\ C_s$ 1

At 276 nm $A_2 = 0.0030\ C_a + 0.0066\ C_s$ 2

Where C_a and C_s are the concentration of Ambroxol Hydrochloride and Salbutamol Sulphate in µg/ml

By putting the values of A_1 and A_2 at their respective wavelengths, the concentration of Ambroxol Hydrochloride and Salbutamol Sulphate can be obtained in dosage form.

Validation of Proposed Method: The method was validated according to ICH guidelines to study linearity, accuracy, precision and drug assay.

Linearity

The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of Salbutamol Sulphate and Ambroxol Hydrochloride. For simultaneous equation method, the Beer Lambert's law was obeyed in the concentration range 10-100 µg/ml and 2-18 µg/ml for Salbutamol Sulphate and Ambroxol Hydrochloride respectively. The correlation coefficient (R^2) was found to be 0.999 for both Salbutamol Sulphate and Ambroxol Hydrochloride at their λ_{max} respectively as shown in Fig. 6 and Fig. 9.

Precision (Repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 3) for Ambroxol Hydrochloride and Salbutamol Sulphate (10 µg/ml for both drugs) without changing the parameter of the proposed spectrophotometry method.

Table 4: Optical Characteristics of Ambroxol Hydrochloride and Salbutamol Sulphate

Optical Characteristics	Ambroxol Hydrochloride	Salbutamol Sulphate
Wavelength (nm)	244	276
Beer Lambert's law limit (µg/ml)	2-18	10-100
Regression equation (y = mx + c)	y = 0.025x - 0.002	y = 0.006x + 0.016
Slope (m)	0.025	0.006
Intercept (c)	0.002	0.016
Correlation coefficient (R^2)	0.999	0.999
Precision (n = 3)	0.677	0.018

Recovery (Accuracy) Studies

In order to check the accuracy, reproducibility and precision of the proposed method, recovery study was carried out by taking standard mixture solution of both Salbutamol Sulphate and Ambroxol Hydrochloride and absorbances was determined at 276 nm and 244 nm respectively .Mean percentage recovery was determined. Recovery values were calculated and shown in Table 5.

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Table 5: Recovery studies for Ambroxol Hydrochloride and Salbutamol Sulphate

Ambroxol Hydrochloride (µg/ml)	Salbutamol Sulphate (µg/ml)	Ambroxol Hydrochloride (Mean percentage recovery)	Salbutamol Sulphate (Mean percentage recovery)
8	20	92.85 ± 2.53	98.63 ±1.76
12	40	97.35 ± 3.14	95.93 ± 2.65
16	60	99.30 ± 1.15	103.5 ± 3.27

Results are shown in ±S.D (n=3)

Drug content Uniformity (Assay)

Ten tablets (200 mg) were powdered in mortar pestle and the blend equivalent to 2 mg of Salbutamol Sulphate and 7.5 mg of Ambroxol Hydrochloride was weighed and dissolved in 100 ml of 6.8 pH phosphate buffer solutions. The solution was sonicated, filtered through whatman filter paper, suitably diluted with 6.8 pH phosphate buffer and the drug content was analyzed form simultaneous equation method by using Double beam UV spectrophotometer at 244 nm and 276 nm respectively. Each sample was analyzed in triplicate.

At 244 nm $A_1 = 0.025\ C_a + 0.0017\ C_s$ 1

At 276 nm $A_2 = 0.0030\ C_a + 0.0066\ C_s$ 2

Where C_a and C_s are the concentration of Ambroxol Hydrochloride and Salbutamol Sulphate in µg/ml

Table 6: Drug Content Uniformity of Salbutamol Sulphate, Ambroxol Hydrochloride

Sr. No.	Absorbance at 244 nm ± S.D (A_1)	Absorbance at 276 nm ± S.D (A_2)	C_a (µg/ml)	C_s (µg/ml)	Drug Content of Ambroxol Hydrochloride (%age) ± S.D	Drug Content of Salbutamol Sulphate (%age) ± S.D
1	0.203	0.036	7.99	1.82	106.5	90.5
2	0.201	0.037	7.90	2.01	105.3	100.5
3	0.205	0.036	8.08	1.78	107.7	89
Mean (n=3)	0.203 ± 0.002	0.036 ± 0.001			106.5 ± 1.53	93.33 ± 6.25

Results and Discussion

The method discussed in the present work provides a convenient and accurate way for simultaneous analysis of Salbutamol Sulphate and Ambroxol Hydrochloride. In simultaneous equation method, wavelengths selected for analysis were 244 nm for Ambroxol Hydrochloride and 276 nm for Salbutamol Sulphate. Ambroxol Hydrochloride and Salbutamol Sulphate showed linearity with absorbance in the range of 2-18 µg/ml and 10-100 µg/ml at their respective absorption maxima, which were validated by least square method. Coefficient of correlation was found to be 0.999 for both Ambroxol Hydrochloride and Salbutamol Sulphate. The observations are presented in Fig. 9, Fig. 6 and in Table 1, Table 2. The Absorptivity were found approximately same for all the concentrations hence both drugs obeyed Beer Lambert's law in indicated concentration range. The high value of correlation coefficient (R^2) also indicates good linearity of calibration curve for both the drugs. The validation parameters were studied at all the wavelengths for the proposed method. Accuracy was determined by calculating the recovery and the mean was determined in Table 5. The result of validation parameters indicates the accuracy of proposed methods for estimation of Ambroxol Hydrochloride and Salbutamol Sulphate. Simultaneous equation method can be employed for routine analysis of Ambroxol

Hydrochloride and Salbutamol Sulphate in combined dosage form. A critical evaluation of proposed method was performed by statistical analysis of data where slope, intercept, correlation coefficient are shown in Table 4. As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, precision and assay of drug formulation in Table 6. Simultaneous equation method was applied for dissolution study and percentage release during dissolution study was always greater than 85% within 30 minutes for both drugs in the tablet formulation.

Based on the results obtained, it can be concluded that the proposed UV-Spectrophotometric methods for simultaneous determination of Ambroxol Hydrochloride and Salbutamol Sulphate is rapid, economical, accurate, precise and reproducible. The utility of the developed methods have been demonstrated by analysis of combined dose tablet formulation. Hence, the proposed method can be employed for quantitative determination of Ambroxol Hydrochloride and Salbutamol Sulphate in combined dose tablet formulation. Simultaneous equation method can be used to carry out dissolution study in combination tablet formulation of these drugs.

Conclusions

The developed UV Spectrophotometric method i.e. simultaneous equation method is found to be simple, sensitive, accurate and precise and can be used for routine analysis of Ambroxol Hydrochloride and Salbutamol Sulphate. The developed method was validated as per ICH guidelines. The results demonstrated that simultaneous equation method by UV/Visible spectrophotometer could be useful technique for determination of Ambroxol Hydrochloride and Salbutamol Sulphate when they both drugs are given in same dosage form.

Conflict of Interests

The authors declare that they do not have any financial and personal relationships with other people or any other organizations that could inappropriately influence this research work.

Acknowledgement

The authors thankful to Director, Dr. A.K. Sharma and Head of department, Dr. Mahendra Singh Rathore, CT Institute of Pharmaceutical Sciences, Jalandhar for encouragement and stimulating discussions and also thankful to the Library Department of Pharmaceutical Sciences of Institute, providing all necessary facilities for preparation of this Research Paper.

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Article History:----------
Date of Submission: 21-06-2013
Date of Acceptance: 21-10-2013
Conflict of Interest: NIL
Source of Support: NONE