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Original Article

DEVELOPMENT AND VALIDATION OF SPECTROSCOPIC METHOD FOR SIMULTANEOUS ESTIMATION OF SALBUTAMOL SULPHATE, AMBROXOL HYDROCHLORIDE AND CETIRIZINE HYDROCHLORIDE IN COMBINED PHARMACEUTICAL TABLET FORMULATION: A NOVEL TECHNIQUE FOR IN-VITRO DISSOLUTION STUDIES

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ABSTRACT

Objective: To develop a simple, rapid, accurate, precise and economical spectroscopic method for the simultaneous determination of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride in combined pharmaceutical tablet formulation and validate as per ICH guidelines.

Methods: In this method, the 6.8 pH phosphate buffer was selected for the developing spectral characteristics of the three drugs. The overlay spectra of Salbutamol Sulphate, Ambroxol hydrochloride and Cetirizine hydrochloride were resolved by making the use of simultaneous equation method based on measurement of absorbance at three wavelengths.

Results: The λ_{max} of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine hydrochloride were found to be 276 nm, 244 nm and 230 nm. The method obeyed Beer Lambert's law in the concentration range of 10-100 μg/ml for SAL, 2- 18 μg/ml for AMB HCl and 2-20 μg/ml for CET HCl. The high values of correlation coefficient (R) indicated good linearity of calibration plot for the drugs. Result of percentage recovery study confirms the accuracy of proposed method. Percentage RSD for precision and accuracy of the method was found to be less than 2%. LOD values for SAL, AMB, and CET were found to be 0.523 μg/mL, 0.450 μg/mL and 0.457 μg/mL, respectively. LOQ values for SAL, AMB, and CET were found to be 1.372 μg/mL, 1.424 μg/mL and 1.386 μg/mL, respectively.

Conclusion: A rapid, economical, accurate, precise and reproducible simultaneous equation spectroscopic method was developed and validated. The proposed method can be employed for routine analysis of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride in combined pharmaceutical tablet formulation.

Keywords: Salbutamol Sulphate, Ambroxol Hydrochloride, Cetirizine Hydrochloride, Simultaneous equation, Absorbtivity, Absorbance, Absorption Maxima., ICH guidelines, Validation.

INTRODUCTION

Salbutamol Sulphate (SAL), (fig. 1) official in Indian Pharmacopoeia and British Pharmacopoeia, is 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 ß$_2$-adrenergic receptor agonist used for the relief of bronchospasm in conditions such as asthma and COPD (Chronic obstructive pulmonary disease) [3].

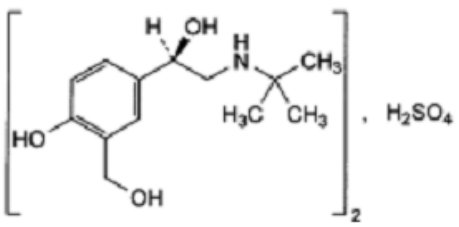

Fig. 1: Chemical Structure of Salbutamol Sulphate

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].

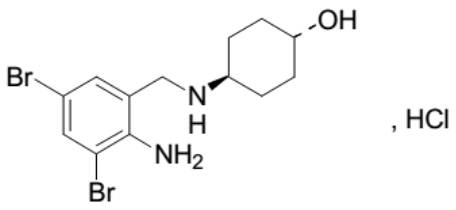

Fig. 2: Chemical Structure of Ambroxol Hydrochloride

Cetirizine hydrochloride [CET HCl] (fig. 3) official in Indian Pharmacopoeia and British Pharmacopoeia, is chemically [2-[4-[(4-chlorophenyl) phenylmethyl]-1-piperazinyl]ethoxy]acetic acid and the active metabolite of the piperazine H_1- receptor antagonist hydroxyzine. It is white or almost white powder, freely soluble in water, practically insoluble in acetone and in methylene chloride [7, 8]. It is a non-sedative second generation anti-histamine drug used in the treatment of seasonal allergic rhinitis, perennial allergic rhinitis, chronic urticaria also used as adjuvant in seasonal asthma. Cetirizine inhibits the release of histamine and of cytotoxic mediators from platelets, as well as eosinophil chemotaxis during the secondary phase of allergic response [9].

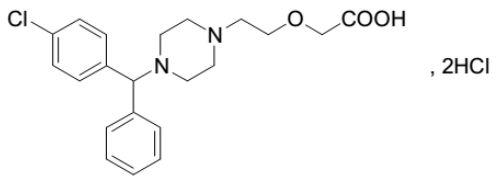

Fig. 3: Chemical Structure of Cetirizine Hydrochloride

The combination of these three drugs is not official in any pharmacopoeia; hence, no official method is available for the simultaneous estimation of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride in combined tablet dosage form. Literature survey revealed that there are several methods that have been reported for the estimation of these drugs individually or in combination with other drugs by using UV spectrophotometry, [10-21], chromatographic methods such as RP-HPLC [22–33], RP-UPLC [34], HPTLC [35-37] and LC-MS [38]. As per literature, no analytical method could be traced for the analysis of SAL, AMB, and CET in combined tablet dosage form. Therefore, simple, rapid, and reliable method for simultaneous estimation of these drugs in combination seemed to be necessary. Spectrophotometric methods of analysis are more economic and simpler, compared to methods such as chromatography and electrophoresis. Hence an attempt has been made to develop new simultaneous equation spectrophotometric method which is simple, rapid, reproducible, and economical method for simultaneous estimation of SAL, AMB, and CET in combined tablet dosage form. The presently developed method was validated as per International Conference on Harmonization guidelines (ICH) [39-40].

MATERIALS AND METHODS

Apparatus and Instrument

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 1 cm 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. Glasswares 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

Pure drug samples of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine hydrochloride were supplied as gift sample by Trojan Pharma Baddi, Himachal Pradesh, 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 the drugs in different solvents.

Preparation of standard stock solution

Standard stock solution of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride were prepared by dissolving 10 mg of Salbutamol Sulphate, 10 mg of Ambroxol Hydrochloride and 10 mg of Cetirizine Hydrochloride separately in 10 ml of pH 6.8 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 (λ) maximas

By appropriate dilution of standard stock solutions of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride with pH 6.8 phosphate buffer, solution containing 10 μg/ml of Salbutamol Sulphate, 10 μg/ml of Ambroxol Hydrochloride and 10 μg/ml of Cetirizine Hydrochloride was scanned separately and then in mixture form in the range of 200-400 nm.

Calibration plots for salbutamol sulphate, ambroxol hydrochloride and cetirizine hydrochloride

The calibration plots were constructed for Salbutamol Sulphate, Ambroxol Hydrochloride and for Cetirizine hydrochloride using pH 6.8 phosphate buffer solutions at their respective absorption maximas in order to find the linearity range of drugs at their respective absorption maximas.

Development of simultaneous equation

The absorbances were measured at all the selected wavelengths and absorptivities for three drugs were determined at all three wavelengths. The concentrations of drugs in sample solution were determined by using following formula:

At 276 nm $A_1 = ax_1C_S + ay_1C_A + az_1C_C$ ..... (1)

At 244 nm $A_2 = ax_2C_S + ay_2C_A + az_2C_C$ ..... (2)

At 230 nm $A_3 = ax_3C_S + ay_3C_A + az_3C_C$ .....(3)

Where, C_S, C_A and C_C are the concentration of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride, A_1, A_2 and A_3 are absorbance at 276 nm, 244 nm and 230 nm respectively, ax_1, ax_2 and ax_3 are absorptivities of Salbutamol Sulphate at 276 nm, 244 nm and 230 nm respectively; ay_1, ay_2 and ay_3 are absorptivities of Ambroxol Hydrochloride at 276 nm, 244 nm and 230 nm respectively; az_1, az_2 and az_3 are absorptivities of Cetirizine Hydrochloride at 276 nm, 244 nm and 230 nm respectively.

Validation of proposed method

The optimized UV spectrophotometric method was completely validated according to the procedure described in ICH guidelines. The performance parameters evaluated for the method were linearity, precision, accuracy, limits of detection and quantitation, and assay of drug.

Linearity

The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride.

Precision

Repeatability

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 6) for Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride (10 µg/ml for each drug) without changing the parameter of the proposed spectrophotometry method.

Intermediate precision

Precision of method was determined in terms of intraday and interday variations (%RSD). Intraday precision (%RSD) was assessed by analyzing standard drug solutions within the calibration range, three times on the same day. Interday precision (%RSD) was assessed by analyzing drug solutions within the calibration range on three different days.

Limit of detection and Limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (4) and (5), respectively.

$$\frac{LOD}{S} = 3.3\,\delta \quad \text{..... (4)}$$

$$\frac{LOQ}{S} = 10\,\delta \quad \text{..... (5)}$$

Where, δ: standard deviation of y-intercept and S: slope of calibration curve.

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 SAL, AMB and CET and absorbances were determined at 276 nm, 244 nm and 230 nm respectively.

Assay (Analysis) of Drug

Ten tablets (200 mg) were powdered in a mortar pestle and the blend equivalent to 2 mg of Salbutamol Sulphate, 7.5 mg of Ambroxol Hydrochloride and 5 mg of Cetirizine Hydrochloride were weighed and dissolved in 100 ml of pH 6.8 phosphate buffer solutions.

The solution was sonicated for 15 minutes, filtered through whatman filter paper, suitably diluted with pH 6.8 phosphate buffer and the drug content was analyzed form simultaneous equation method by using double beam UV spectrophotometer at 276 nm, 244 nm and 230 nm respectively. Each sample was analyzed in triplicate, the result of which was given in table 8

RESULTS AND DISCUSSION

Selection of Absorption maximas

Wavelengths of absorption maximas were determined for three drugs. SAL showed absorption maxima at 276 nm, AMB HCl showed absorption maximas at 244 nm, CET HCl showed maximum absorbance at 230 nm with reference to British Pharmacopoeia and Indian Pharmacopoeia.

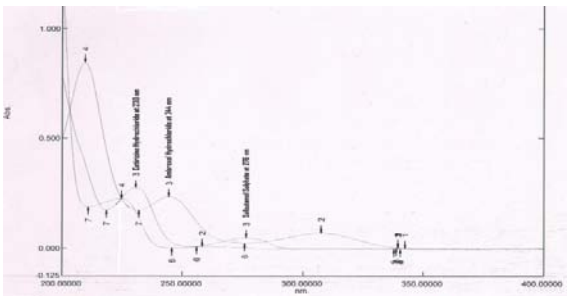

Fig. 4: Overlain UV Spectra of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride Solution (10μg/mL concentration of each drug solution)

From the overlain spectra of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride **(**fig. 4); three wavelengths 276 nm, 244 nm and 230 nm, λ_{max} of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine hydrochloride were selected for further spectroscopic studies. Therefore, for simultaneous equation method, wavelengths selected for analysis were 276 nm for SAL, 244 nm for AMB HCl and 230 nm for CET HCl.

Linearity of Calibration curves

From the calibration plot of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride at their respective absorption maximas, the linearity was observed in the concentration range of 10–100 μg/mL for Salbutamol Sulphate, 2-18 µg/ml for Ambroxol Hydrochloride and 2-20 µg/ml for Cetirizine Hydrochloride at all three wavelengths, which were validated by least square method. Coefficient of correlation (R) was found to be 0.999 for Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride as given in table 4. The high value of correlation coefficient (R) also indicates good linearity of calibration curve for the drugs as shown in fig. 5, fig. 6 and fig. 7.

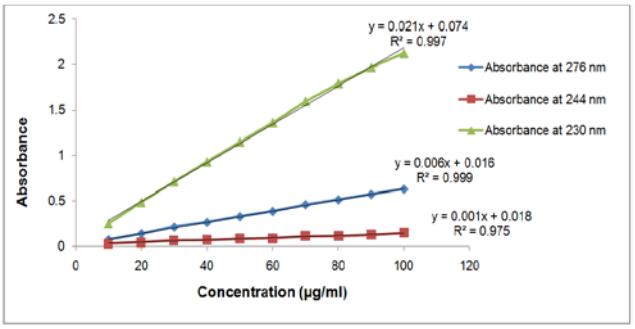

Fig. 5: Standard Calibration Plot of Salbutamol Sulphate at 276 nm, 244 nm and 230 nm in pH 6.8 Phosphate Buffer

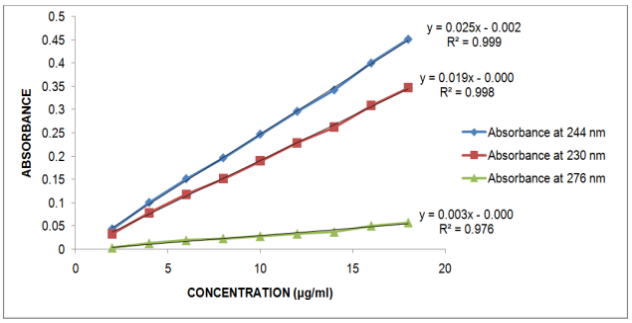

Fig. 6: Standard Calibration Plot of Ambroxol Hydrochloride at 244 nm, 230 nm and 276 nm in pH 6.8 Phosphate Buffer

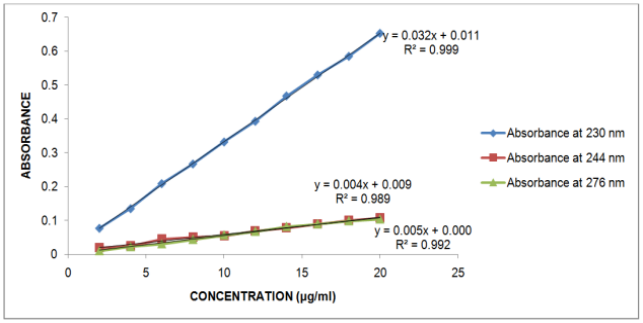

Fig. 7: Standard Calibration Plot of Cetirizine Hydrochloride at 230 nm, 244 nm and 276 nm in pH 6.8 Phosphate Buffer

Table 1: Absorbance and Absorptivity of Salbutamol Sulphate at 276 nm, 244 nm and 230 nm respectively

Concentration (μg/ml)	*Absorbance at 276 nm± S. D	*Absorbance at 244 nm± S. D	*Absorbance at 230 nm ± S. D	Absorptivity at 276 nm	Absorptivity at 244 nm	Absorptivity at 230 nm
10	0.071 ± 0.002	0.026 ± 0.003	0.250 ± 0.003	0.0071	0.0026	0.025
20	0.138 ± 0.003	0.043 ± 0.004	0.485 ± 0.002	0.0069	0.0022	0.024
30	0.211 ± 0.002	0.064 ± 0.002	0.713 ± 0.001	0.0070	0.0021	0.024
40	0.264 ± 0.001	0.067 ± 0.001	0.930 ± 0.002	0.0066	0.0017	0.023
50	0.327 ± 0.002	0.081 ± 0.002	1.151 ± 0.003	0.0065	0.0016	0.023
60	0.385 ± 0.003	0.084 ± 0.003	1.362 ± 0.002	0.0064	0.0014	0.023
70	0.459 ± 0.001	0.108 ± 0.002	1.599 ± 0.002	0.0066	0.0015	0.023
80	0.512 ± 0.001	0.109 ± 0.002	1.794 ± 0.004	0.0064	0.0014	0.022
90	0.571 ± 0.001	0.122 ± 0.003	1.969 ± 0.002	0.0063	0.0014	0.022
100	0.628 ± 0.002	0.146 ± 0.002	2.125 ± 0.003	0.0063	0.0015	0.021
			Mean	ax_1 = 0.0066	ax_2 = 0.0017	ax_3 = 0.023

*Each value is the average of three determinations

Table 2: Absorbance and Absorptivity of Ambroxol Hydrochloride at 276 nm, 244 nm and 230 nm respectively

Concentration (μg/ml)	*Absorbance at 276 nm ± S. D	*Absorbance at 244 nm± S. D	*Absorbance at 230 nm ± S. D	Absorptivity at 276 nm	Absorptivity at 244 nm	Absorptivity at 230 nm
2	0.004 ± 0.002	0.044 ± 0.001	0.034 ± 0.002	0.0020	0.022	0.017
4	0.014 ± 0.001	0.101 ± 0.002	0.079 ± 0.004	0.0035	0.025	0.020
6	0.020 ± 0.004	0.152 ± 0.003	0.119 ± 0.002	0.0033	0.025	0.020
8	0.024 ± 0.003	0.197 ± 0.001	0.152 ± 0.003	0.0030	0.025	0.019
10	0.029 ± 0.002	0.248 ± 0.002	0.190 ± 0.002	0.0029	0.025	0.019
12	0.034 ± 0.001	0.297 ± 0.004	0.229 ± 0.001	0.0028	0.025	0.019
14	0.038 ± 0.002	0.343 ± 0.002	0.262 ± 0.001	0.0027	0.025	0.019
16	0.051 ± 0.001	0.401 ± 0.002	0.309 ± 0.003	0.0032	0.025	0.019
18	0.058 ± 0.003	0.452 ± 0.003	0.347 ± 0.002	0.0032	0.025	0.019
			Mean	ay_1 = 0.0030	ay_2 = 0.025	ay_3 = 0.019

*Each value is the average of three determinations

Table 3: Absorbance and Absorptivity of Cetirizine Hydrochloride at 276 nm, 244 nm and 230 nm respectively

Concentration (μg/ml)	*Absorbance at 276 nm ± S. D	*Absorbance at 244 nm± S. D	*Absorbance at 230 nm ± S. D	Absorptivity at 276 nm	Absorptivity at 244 nm	Absorptivity at 230 nm
2	0.010 ± 0.004	0.019 ± 0.004	0.077 ± 0.002	0.0050	0.0095	0.0385
4	0.022 ± 0.003	0.025 ± 0.003	0.135 ± 0.003	0.0055	0.0063	0.0375
6	0.031 ± 0.002	0.045 ± 0.002	0.209 ± 0.003	0.0051	0.0075	0.0348
8	0.044 ± 0.005	0.051 ± 0.005	0.267 ± 0.001	0.0055	0.0064	0.0334
10	0.057 ± 0.003	0.054 ± 0.003	0.332 ± 0.004	0.0057	0.0054	0.0332
12	0.068 ± 0.002	0.069 ± 0.002	0.393 ± 0.002	0.0056	0.0058	0.0328
14	0.082 ± 0.001	0.078 ± 0.001	0.468 ± 0.002	0.0058	0.0056	0.0334
16	0.090 ± 0.002	0.089 ± 0.002	0.530 ± 0.003	0.0061	0.0056	0.0331
18	0.099 ± 0.001	0.100 ± 0.003	0.585 ± 0.001	0.0057	0.0056	0.0325
20	0.104 ± 0.003	0.107 ± 0.001	0.653 ± 0.002	0.0054	0.0054	0.0327
			Mean	az_1 = 0.0050	az_2 = 0.0063	az_3 = 0.0338

Development of simultaneous equation

The absorptivity values were found approximately same for all the concentrations hence all three drugs obeyed Beer Lambert's law in an indicated concentration range as given in table 1, table 2 and table 3.

Substituting the values of ax_1, ax_2, ax_3, ay_1, ay_2, ay_3, az_1, az_2, az_3 from table 1, table 2 and table 3 in eqn. 1, 2 and 3, the following equation was designed for the simultaneous estimation of Ambroxol Hydrochloride and Cetirizine Hydrochloride in tablet dosage form:

At 276 nm $A_1 = 0.0066\ C_S + 0.0030\ C_A + 0.0050\ C_C$ …….. (6)

At 244 nm $A_2 = 0.0017\ C_S + 0.025\ C_A + 0.0063\ C_C$ …….. …. (7)

At 230 nm $A_3 = 0.023\ C_S + 0.019\ C_A + 0.0338\ C_C$ …….. …. (8)

Where C_S, C_A and C_C are the concentration of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride respectively, A_1, A_2 and A_2 are absorbance values at 276 nm, 244 nm and 230 nm respectively, 0.0066, 0.0017 and 0.023 are absorptivities of Salbutamol Sulphate at 276 nm, 244 nm and 230 nm respectively, 0.0030, 0.025 and 0.019 are absorptivities of Ambroxol Hydrochloride at 276 nm, 244 nm and 230 nm respectively; 0.0050, 0.0063 and 0.0338 are absorptivities of Cetirizine Hydrochloride at 276 nm, 244 nm and 230 nm respectively. By solving equation (6), (7) and (8) simultaneously, the concentration of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride in combined tablet dosage form can be calculated.

Validation of Proposed Method

The validation parameters were studied at all the wavelengths for the proposed method. The optical characteristics such as λ_{max}, Beer's Lambert's law limit, Regression equation, Slope, Intercept, correlation coefficient, were calculated and are summarized in table 4.

Linearity

The linearity was observed in the concentration range of 10–100 μg/mL for Salbutamol Sulphate, 2-18 μg/ml for Ambroxol Hydrochloride and 2-20 μg/ml for Cetirizine Hydrochloride at all three wavelengths, which were validated by least square method. Coefficient of correlation (R) was found to be 0.999 for Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride. Regression parameters are mentioned in table 4.

LOD and LOQ

LOD and LOQ of the drug were calculated as per ICH guideline. LOD values for SAL, AMB, and CET were found to be 0.523 μg/mL, 0.450 μg/mL, and 0.457 μg/mL, respectively. LOQ values for SAL, AMB, and CET were found to be 1.372 μg/mL, 1.424 μg/mL, and 1.386 μg/mL, respectively (Table 4). These data show that the proposed method is precise and sensitive for the determination of SAL, AMB, and CET.

Repeatability

Instrumental precision study was carried out by repeatability study. The % RSD values for SAL, AMB, and CET were found to be 1.6609, 1.9596, and 1.8547 respectively given in table 5. Low relative standard deviation (<2) indicates that the proposed method is repeatable.

Intermediate precision (Reproducibility)

Precision studies were carried out to study the intra-day and inter-day variations of the responses. The intraday and interday precisions were determined, results of which are given in table 6

Recovery (Accuracy) studies

Known amounts of Salbutamol Sulphate (20, 40, and 60 μg/ml), Ambroxol Hydrochloride (4, 8, and 12 μg/ml) and Cetirizine Hydrochloride (6, 10 and 14 μg/ml) were added to a pre-quantified sample solution, and the amount of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride were estimated by proposed method.

Assay of Drugs

Based on the above results obtained, it can be concluded that the proposed spectroscopic method for simultaneous estimation of

Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride is rapid, economical, accurate, precise and reproducible. The utility of the developed method has been demonstrated by analysis of combined dose tablet formulation.

Hence, the proposed method can be employed for quantitative estimation of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride in combined tablet dosage form. Simultaneous equation method can be used to carry out *in-vitro* dissolution study in combined tablet formulation of these drugs.

Table 4: Regression analysis data and summary of validation parameters for the proposed method

Optical Characteristics	Salbutamol Sulphate	Ambroxol Hydrochloride	Cetirizine Hydrochloride
$\lambda_{max.}$ (nm)	276	244	230
Linearity range (µg/ml)	10-100	2-18	2-20
Regression equation (y = mx + c)	y= 0.006x+ 0.016	y = 0.025x - 0.002	y = 0.032x + 0.011
Slope (m)	0.006	0.025	0.032
Intercept (c)	0.016	0.002	0.011
Correlation coefficient (R)	0.999	0.999	0.999
LOD (μg/ml)	0.523	0.450	0.457
LOQ (μg/ml)	1.372	1.424	1.386
Precision (% RSD)			
Repeatability (n=6)	1.6609	1.9596	1.8547
Intra-day (n=3)	1.2877	1.6166	1.7930
Inter-day (n=3)	1.4785	1.6238	1.4048

As per the ICH guidelines, the method validation parameters checked were linearity, LOD and LOQ, repeatability, intermediate precision, accuracy (recovery) studies and assay of drug.

Table 5: Repeatability Data for Proposed Method

Serial No.	Concentration (µg/ml)	Absorbance of SAL at 276 nm	Absorbance of AMB at 244 nm	Absorbance of CET at 230 nm
1	10 ppm	0.069	0.043	0.076
2	10 ppm	0.070	0.041	0.074
3	10 ppm	0.072	0.041	0.073
4	10 ppm	0.070	0.042	0.072
5	10 ppm	0.071	0.041	0.074
6	10 ppm	0.069	0.042	0.073
	Mean	0.070	0.042	0.074
	SD	0.001169	0.00081	0.00137
	% RSD	1.6609	1.9596	1.8547

SD: Standard deviation, RSD: Relative Standard deviation

Table 6: Intra-day and inter-day precision data of SAL, AMB, and CET

Drug	Amount Labeled [200 mg Tablet]	Percentage obtained (n=3)		SD		% RSD	
		Intraday	Interday	Intraday	Interday	Intraday	Interday
	2 mg	98.24	99.43				
SAL	2 mg	99.57	96.56	1.2656	1.4472	1.2877	1.4785
	2 mg	97.04	97.67				
	Mean	98.28	97.89				
	7.5 mg	99.46	98.29				
AMB	7.5 mg	96.36	98.89	1.5800	1.6158	1.6166	1.6238
	7.5 mg	97.38	101.34				
	Mean	97.73	99.51				
	5 mg	97.33	99.45				
CET	5 mg	99.67	96.78	1.7526	1.3757	1.7930	1.4048
	5 mg	96.24	97.54				
	Mean	97.75	97.92				

SD: Standard deviation, RSD: Relative Standard deviation

Table 7: Recovery studies

Salbutamol Sulphate (μg/ml)	Ambroxol Hydrochloride (μg/ml)	Cetirizine Hydrochloride (μg/ml)	Salbutamol Sulphate % recovery ±S. D (n=3)	Ambroxol Hydrochloride % recovery ±S. D (n=3)	Cetirizine Hydrochloride % recovery ±S. D (n=3)
20	4	6	97.44 ± 2.01	96.67 ± 1.53	98.63 ± 0.76
40	8	10	100.33 ± 1.12	95.43 ± 0.41	99.79 ± 0.25
60	12	14	96.80 ± 1.45	98.80 ± 1.15	101.5 ± 1.29

SD: Standard deviation

Table 8: Assay of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride

Parameters	Salbutamol Sulphate	Ambroxol Hydrochloride	Cetirizine Hydrochloride
Amount Present (mg) (200 mg Tablet)	2	7.5	5
% Drug Content ± SD	97.37 ± 1.885	95.41 ± 0.828	99.75 ± 1.324
% RSD	1.9364	0.8679	1.3278

SD: Standard deviation, RSD: Relative Standard deviation

CONCLUSION

The developed spectroscopic method i. e. simultaneous equation method is found to be simple, sensitive, accurate and precise and can be used for routine analysis of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride in combined table dosage form. The developed method was validated as per ICH guidelines. The results demonstrated that simultaneous equation method by spectrophotometer could be useful technique for estimation of Salbutamol Sulphate, Ambroxol Hydrochloride and Cetirizine Hydrochloride in combined tablet dosage form and cost of analysis is less as compared to RP-HPLC, HPTLC, RP-UPLC and LC-MS method. Hence simultaneous equation method can be conveniently used for routine quality control analysis of SAL, AMB HCl and CET HCl in its combined pharmaceutical tablet formulations.

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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.

ABBREVIATIONS

SAL: Salbutamol Sulphate

CET HCl: Cetirizine Hydrochloride

AMB HCl: Ambroxol Hydrochloride

RSD: Relative Standard Deviation

SD: Standard Deviation

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