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SIMULTANEOUS DETERMINATION OF SALBUTAMOL AND AMBROXOL IN FIXED DOSE COMBINATION BY SPECTROPHOTOMETRY

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ABSTRACT

Salbutamol (SAL) and Ambroxol (AMB) is used for the treatment of asthma and bronchitis. Two simple, economical, accurate, and precise methods for simultaneous estimation of Salbutamol Sulphate (SAL) and Ambroxol Hydrochloride (AMB) in tablet dosage form have been developed. The methods are absorbance correction method (I) and first order derivative method (II). In first method ambroxol hydrochloride (AMB) was determined directly from calibration plot by measuring absorbance at 300nm and salbutamol sulphate (SAL) was determined after correction for absorbance of ambroxol hydrochloride (AMB) at 223 nm. Second method is based on first order derivative spectroscopy to overcome the spectral interference from the other drug. Wavelength 252 nm was selected for SAL and 232 nm for AMB. Both the methods showed linearity in the concentration range of 2-10µg/ml for SAL and 2-20 µg/ml for AMB. The accuracy and precision of the methods were determined and the methods validated stastically. No significant difference was observed between the results obtained by the two methods.

INTRODUCTION: Salbutamol sulphate (SAL), chemically known as bis [(1RS)-2-[(1, 1-dimethylethyl) amino]-1-[4-hydroxy-3-(hydroxymethyl) phenyl] ethanol] sulphate, is beta-adrenoceptor agonist used for the relief of broncho-spasm in conditions such as asthma and chronic obstructive pulmonary disease. The drug is official in Indian pharmacopoeia [1-4]. Ambroxol hydrochloride (AMB) is chemically, trans-4-((2-amino-3, 5-dibromobenzyl) amino) cyclohexanol hydrochloride. Ambroxol reduces bronchial hyper-reactivity and acts as a mucolytic and cough suppressant. Combination of SAL and AMB is used for the treatment of asthma and bronchitis [5-7]

Literature survey reveals that salbutamol in combination with other drugs has been estimated by UV spectrophotometric methods [8-12], RP-HPLC methods [13-14], TLC method [15]. For simultaneous determination of Ambroxol in combination with other drugs, UV spectrophotometric methods [8, 16-20], RP-HPLC [20-24], HPTLC [25] and LC-MS/MS [26] are reported. Only one spectrophotometric method has been reported for the simultaneous estimation of salbutamol and ambroxol in combination [8]. Therefore, in the present work successful attempt has been made to estimate both the drugs simultaneously by two simple UV spectrophotometric methods i.e., absorbance correction method and first order derivative method. The proposed methods were optimized and validated as per ICH guidelines.
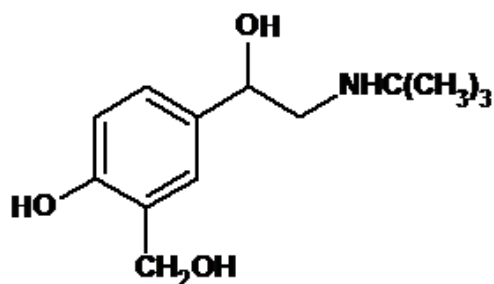

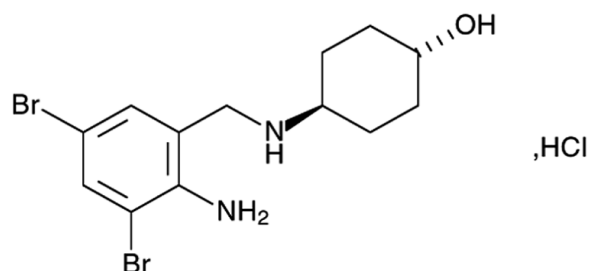**FIG. 1: SALBUTAMOL (4-[2-(TERT-BUTYLAMINO)-1-HYDROXYETHYL]-2-(HYDROXYMETHYL) PHENOL)**

FIG 2: AMBROXOL HYDROCHLORIDE (TRANS-4-((2-AMINO-3,5-DIBROMOBENZYL) AMINO) CYCLOHEXANOL)

MATERIAL AND METHODS:

Instrumentation: For the present study JASCO double beam UV/Visible spectrophotometer(Model V-630) was used with slit width fixed at 1.5nm ,equipped with spectra manager software (Version 1.5). Pair of 1-cm matched quartz cells were used to measure the absorbance of solution. The samples were weighed on electronic analytical balance (Contech Model CB-50) (**table 1**).

TABLE 1: INSTRUMENTS

Name	Model	Manufacture
UV/Visible spectrophotometer	V-630	Jasco
Electronic analytical balance	CB-50	Contech

Materials: Gift samples of Salbutamol sulphate and Ambroxol hydrochloride were provided by Glenmark Pharmaceuticals Limited, Nasik, India. The pharmaceutical dosage form used in this study was Sal Mucolite tablets (Cheminnova Remedies Pvt. Ltd). Each uncoated tablet contains 2mg SAL and 30mg AMB.

Solvent: Methanol Spectroscopic grade (Thomas Baker)

Preparation of stock solutions: Standard stock solutions of both Salbutamol sulphate and Ambroxol hydrochloride were prepared by dissolving 10 mg of SAL and 10mg of AMB separately in 20ml of 0.1N HCL in 100ml volumetric flasks. Final volume was made up to 100ml with 0.1N HCL to get working standard solution of each containing100µg/ml of both SAL and AMB.

Methods:

Absorbance correction method (Method 1): This method involves absorbance correction for SAL determination by subtracting absorbance of AMB from total absorbance of sample at 223nm (λ_{max} of SAL). AMB concentration was determined directly from calibration plot by measuring absorbance at 300nm where SAL shows zero absorbance. The equations obtained for the determination of concentration are,

$$C_{AMB} = A_{300nm}/ax_1 \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \text{[I]}$$

$$C_{SAL} = A_{223nm} - (ax_2 * C_{AMB})/ay_2 \dots\dots\dots\dots\dots\dots\dots\dots \text{[II]}$$

A_{300nm} and A_{223nm} = Absorbance of sample at 300nm and 223nm

C_{AMB} and C_{SAL} = Concentrations of AMB and SAL in sample matrix.

ax_1 and ax_2 = Absorptivities of AMB at 300 nm and 223 nm

ay_1 and ay_2 = Absorptivities of SAL at 300 nm and 223 nm

TABLE 2: ABSORPTIVITY VALUES OF SAL AND AMB

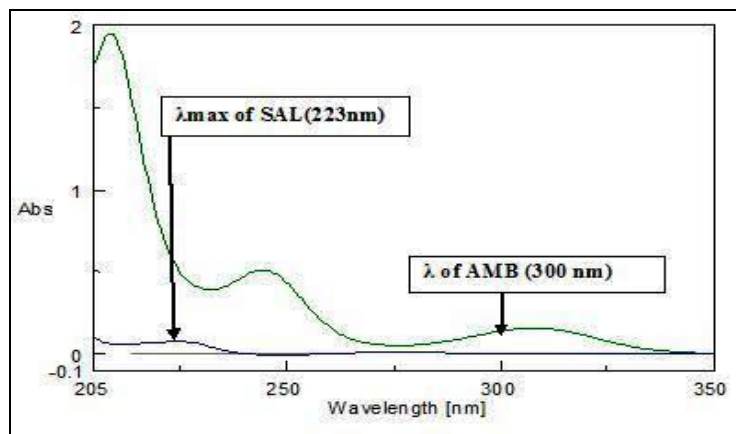| Drug	Absorptivity at 300nm	Absorptivity at 223nm
SAL	0.000 (ay_1)	0.0388 (ay_2)
AMB	0.008975 (ax_1)	0.03865 (ax_2)

FIG 3: OVERLAY SPECTRA OF SAL AND AMB FOR ABSORBANCE CORRECTION METHOD

First Order Derivative method (Method II): This method is based on first order derivative spectroscopy to overcome the spectral interference from other drug. Zero order spectrums of both the drugs are converted to first order derivative spectra with the help of spectra manager software of the instrument (**Figure 4**).

It was observed that SAL showed $dA/d\lambda$ zero at 252nm in contrast to AMB that has considerable $dA/d\lambda$ at this wavelength. Futher, AMB has $dA/d\lambda$ zero at 232nm while at this wavelength SAL has significant $dA/d\lambda$. Therefore, wavelengths 252nm and 232nm were employed for determination of AMB and SAL respectively without interference of other drug. The calibration curves were plotted at these wavelengths of concentrations against $dA/d\lambda$ separately. The equation of line obtained to determine concentrations of SAL and AMB is as follows;

$$C_{SAL} = dA/d\lambda_{252nm} - 0.000356/0.0035 \dots\dots\dots\dots\dots \text{[III]}$$

$$C_{AMB} = dA/d\lambda_{232nm} - (-0.00046)/0.00195 \dots\dots\dots\dots \text{[IV]}$$
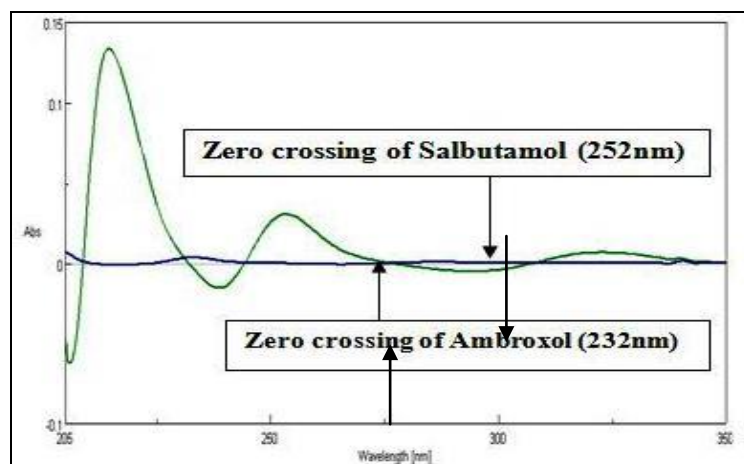

FIG 4: OVERLAY SPECTRA OF SAL AND AMB FOR FIRST OEDER DERIVATIVE METHOD

Analysis of tablet formulation: For the estimation of drugs in the commercial formulations, ten tablets containing 2 mg of SAL and 30 mg of AMB were weighed and average weight was calculated. The tablets were crushed and powdered in glass mortar. For the analysis of drugs, quantity of powder

equivalent to 1 mg of SAL and 15 mg of AMB was transferred to 100 ml volumetric flasks and dissolved in sufficient quantity of 0.1N HCL. It was sonicated for 30mins and volume was made up to obtain a stock solution of 10 µg/ml of SAL and 150 µg/ml of AMB. This solution was then filtered through whatman filter paper # 42. Further dilutions were made from this stock solution to get required concentration. In First method, AMB concentration was determined directly from calibration plot by measuring absorbance at 300nm where SAL shows zero absorbance and SAL was determined by subtracting absorbance of AMB from total aborbance of sample at 223nm (λ_{max} of SAL) by using equation I and II respectively. In second method, concentration of SAL and AMB was determined by measuring the dA/dλ at wavelength 252nm (zero crossing of AMB) and 232nm (zero crossing of SAL) using equation III and IV respectively. Results of tablet analysis are shown in **Table 3**. The assay procedure was repeated six times (n=6).

TABLE 3: RESULT OF MARKETED FORMULATION ANALYSIS

Method	Label claim	% Label Claim*(Mean±SD)	%RSD
Absorbance correction method	SAL 2mg	98.97±0.00172	0.2839
	AMB 30mg	98.41±0.000476	0.36033
First order Derivative method	SAL 2mg	100.99±0.1567	0.2328
	AMB 30mg	99.87±0.09987	0.1974

*Average of six determination

Validation: The method was validated according to ICH guidelines to study linearity, accuracy and precision [27].

Linearity: The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of SAL and AMB. For both the methods, the Beer law was obeyed in the concentration range 2-10 µg/ml and 2-20 µg/ml for SAL and AMB respectively.

For both the method, the correlation coefficient was found to be >0.998.

Accuracy (Recovery studies): 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 SAL and AMB, by both the methods, was found in the range of 98.20- 102% (**Table 4**).

TABLE 4: RESULTS OF RECOVERY STUDIES

Level Recovery	Drug	Conc. of Drug in µg/ml		Method I*			Method II*		
		Drug taken	Std drug added	%recovery	SD	%RSD	%recovery	SD	%RSD
80		1	0.8	99.44	0.0138	0.2516	99.88	0.0986	0.1476
100	SAL	1	1	98.68	0.0069	0.5742	101.5	0.2986	0.6563
120		1	1.2	99.25	0.0128	0.9447	101.6	0.1965	0.3232
80		15	12	99.57	0.0030	0.2709	99.79	0.2567	0.4123
100	AMB	15	15	98.57	0.0022	0.8510	99.80	0.1543	0.1986
120		15	18	99.86	0.0026	0.8825	100.33	0.1265	0.1756

Precision: The reproducibility of the proposed methods was determined by performing tablet assay at different time intervals on same day (Intra-day precision) and on three different days (Inter-day precision).

RESULTS AND DISCUSSION: Under experimental conditions described, linearity, assay of tablet, accuracy studies and precision were performed. In both the methods linearity was observed in the concentration range of 2-10µg/ml and 2-20 µg/ml for SAL and AMB respectively and correlation coefficient

was found to be > 0.998. The results of commercial tablet formulation are presented in Table 1. Results of accuracy studies are presented in Table 2. Percent recovery for SAL and AMB by both the methods was found in the range of 98.20% to 102 %. S.D. and R.S.D. for six determinations of tablet sample, by both the methods, was found to be less than 2.0 indicating the precision of both the methods. Hence, it can be concluded that the developed spectrophotometric methods are accurate, precise and can be employed successfully for the estimation of salbutamol and ambroxol in bulk and formulation.

CONCLUSION: The two spectrophotometric methods were developed and validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are within limits, indicating high degree of precision of the methods. The results of the recovery studies performed indicate the methods to be accurate. Hence, it can be concluded that the developed spectrophotometric methods are accurate, precise and can be employed successfully for the estimation of salbutamol and ambroxol in bulk and formulation.

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