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Research Article

SPECTROPHOTOMETRIC SIMULTANEOUS ESTIMATION OF SALBUTAMOL AND AMBROXOL IN BULK AND FORMULATION

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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 employed were simultaneous equation (I) and area under curve method (II). First method involves solving simultaneous equations based on measurement of absorbance at two wavelengths 223 nm and 244 nm, the λmax of salbutamol sulphate (SAL) and ambroxol hydrochloride (AMB), respectively. Second method is based on equation of area under curve (AUC) method. For the second method, the wavelength range 232-217nm was selected for SAL and 252-237nm for AMB. Both the methods showed linearity in the concentration range of 2-20µg/ml for salbutamol and 2-40 µg/ml for ambroxol. 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.

Key words: Salbutamol sulphate, Ambroxol hydrochloride, simultaneous equation, Area under curve.

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 simultaneous equation method and area under curve method. The proposed methods were optimized and validated as per ICH guidelines.

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).A pair of 1-cm matched quartz cells were used to measure absorbance of solution. The samples were weighed on electronic analytical balance (Contech Model CB-50)

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.

Determination of Absorption Maxima:

By appropriate dilution of standard stock solutions of SAL and AMB with 0.1N HCL, solutions containing 10 µg/ml of SAL and 10 µg/ml of AMB were scanned separately in the range of 200- 400 nm. Wavelength of maximum absorption was determined for both the drugs. SAL showed maximum absorbance at 223nm and AMB at 244nm.

Methods

Simultaneous Equation method (Method I)

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. 223 nm and 244 nm that are absorption maximas of SAL and AMB respectively in 0.1N HCL. Series of dilution were prepared from standard solutions of SAL and AMB. The linearity was observed in the concentration range of 2-20µg/ml for SAL and 2-40 µg/ml for AMB. The absorbances were measured at the selected wavelengths and absorptivities (A 1%, 1 cm) for both the drugs at both wavelengths were determined. The calibration curves for SAL and AMB were plotted in the concentration range of 2-20µg/ml and 2-40 µg/ml. The concentrations of drugs in sample solution were determined by using the following formula**,**

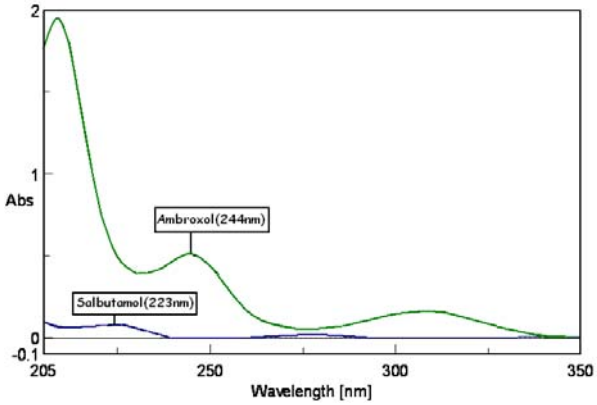

Fig. 1: Overlay Spectra of Sal and Amb for Simultaneous Equation Method

$$A_1 = ax_1Cx + ay_1Cy \quad \text{.....I}$$

$$A_2 = ay_1Cx + ay_2Cy \quad \text{.....II}$$

$$C_x = \frac{A_2 ay_1 + A_1 ay_2}{ax_2\, ay_1 - ax_1\, ay_2} \quad \text{.....III}$$

$$C_y = \frac{A_1 ax_2 + A_2 ax_1}{ax_2\, ay_1 - ax_1\, ay_2} \quad \text{.....IV}$$

A_1 and **A_2** = Absorbance of sample at λ_1 and λ_2
C_x and **C_y** = Concentrations of AMB and SAL in sample matrix.
ax_1 and **ax_2** = Absorptivities of AMB at λ_1 and λ_2
ay_1 and ay_2= Absorptivities of SAL at λ_1 and λ_2

By solving the two simultaneous equations, the concentrations of SAL and AMB in sample solutions were obtained.

Area under Curve (Method II)

For the selection of analytical wavelength standard solutions of SAL and AMB (10 µg/ml) were prepared separately by appropriate dilution of stock solution and scanned from 400 to 200 nm. From the overlay spectra of the two drugs (Fig. no.2), wavelength range of 232-217nm (for SAL) and 237-252nm (for AMB) were selected for the analysis. Series of dilutions of standard solutions of SAL and AMB were prepared. The linearity was observed in the concentration range of 2-20µg/ml for SAL and 2-40 µg/ml for AMB. The calibration curve for SAL and AMB was prepared in the concentration range of 2-20 µg/ml and 2-40µg/ml at their respective AUC range. The calibration curve was plotted with concentration v/s area.

$$\int_{217}^{232} Ad\lambda_1 = K_1C_1 + K_2C_2 \quad \text{.....V}$$

$$\int_{237}^{252} Ad\,\lambda_2 = K_3C_1 + K_4C_2 \quad \text{.....VI}$$

Where area of curve between 232-217nm is represented by $\int Ad\lambda_1$ and between 252-237nm by

$\int Ad\lambda_2$ respectively, C_1 and C_2 are concentrations (µg/ml) of AMB and SAL respectively k_1, k_2, k_3 and k_4 are constants. Final equations are summarized as follows:

$$\int_{217}^{232} Ad\lambda_1 = 0.5901C_1 + 0.4561C_2 \quad \text{.....VII}$$

$$\int_{237}^{252} Ad\,\lambda_2 = 0.521C_1 + 0.0151C_2 \quad \text{.....VIII}$$

The concentrations of both the components were calculated using above mentioned equations VII and VIII.

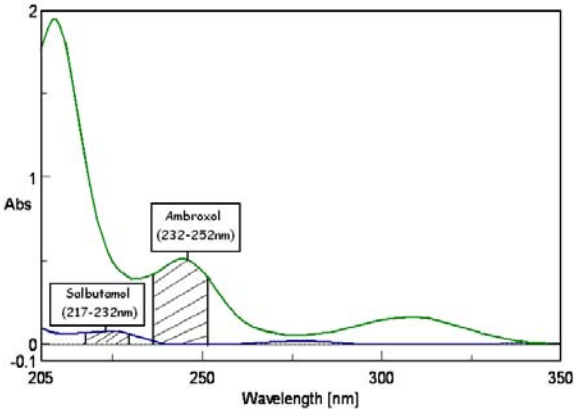

Figure 2: Overlain Spectra of Sal and Amb for Auc

Analysis of tablet formulation:

For the estimation of drugs in the commercial formulations, twenty 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 whatmann filter paper # 42. Further dilutions were made from this stock solution to get required concentration. In method I, the concentration of both SAL and AMB was determined by measuring absorbances of sample solutions at 223 nm (λ_{max} of SAL) and 244 nm (λ_{max} of AMB) using equations (III) and (IV). In method II, the concentration of both SAL and AMB was determined by measuring absorbances of sample solutions in wavelength range of 232nm-217nm (for SAL) and 252nm-237nm (for AMB) using equation VII and VIII Results of tablet analysis are shown in Table No. 1. The assay procedure was repeated six times (n=6).

Table 1: Result of marketed formulation analysis

Method	Label claim	% Label Claim* (Mean±SD)	%RSD
Simultaneous equation method	SAL 2mg	99.13±0.00485	0.5075
	AMB 30mg	98.16±0.00626	0.2912
Area Under Curve method	SAL 2mg	99.86±0.01328	0.1447
	AMB 30mg	98.86±0.00852	0.1258

* Mean of six estimations. SAL= Salbutamol, AMB=Ambroxol.

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 2-20 µg/ml and 2-40 µg/ml for SAL and AMB respectively. The correlation coefficient was found to be 0.9974 at 223nm for Salbutamol and 0.9972 at 244nm for Ambroxol.

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 No.2)

Table 2: Result 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	SAL	1	0.8	99.80	0.0039	0.3737	99.81	0.0682	0.5700
100		1	1	100.28	0.0779	0.6286	99.60	0.079	0.2562
120		1	1.2	99.52	0.0104	0.6900	99.48	0.0216	0.4234
80	AMB	15	12	99.25	0.0183	0.1728	99.86	0.0892	0.5544
100		15	15	100.25	0.0013	0.1390	99.55	0.0108	0.556
120		15	17	99.45	0.0018	0.1760	100.70	0.0224	0.4130

*Average of three determinations

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

The methods discussed in the present work provide a convenient and accurate way for simultaneous analysis of SAL and AMB. In simultaneous equation method, wavelengths selected for analysis were 223 nm for SAL and 244 nm for AMB. In area under curve method, the area under curve in the range of 232-217 nm (for SAL) and 252-237 nm (for AMB) were selected for the analysis. In both the methods linearity were observed in the concentration range of 2-20µg/ml and 2-40 µg/ml for SAL and AMB respectively. In method I, concentration of the individual drug present in the tablet matrix was determined by solving the simultaneous equation at 223 nm and 244 nm.

The absorptivities of the two drugs were used for the calculations. In method II, concentration of the individual drug present in the tablet matrix was determined by solving two equations at the range of 232nm-217nm and 252nm-237nm The absorptivities of the two drugs were used for the calculations. Assay values for SAL and AMB for tablet analysis, by both the methods, were found in the range of 98.10% to 100.43 %. 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. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as % recovery.

Percent recovery for SAL and AMB, by both the methods, was found in the range of 98.20% to 102 %. The results of validation parameters shown in table no.2 are satisfactory, indicates the accuracy of proposed methods for estimation of SAL and AMB. These methods can be employed for routine analysis of the two drugs in combined tablet dosage form.

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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