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(RESEARCH ARTICLE)

Simultaneous estimation of salbutamol sulphate and ambroxol HCl from their combined dosage form by UV-VIS spectroscopy using simultaneous equation method

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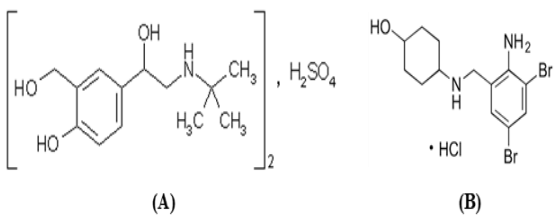
Abstract

A simple UV-Vis Spectrophometric method was developed for the simultaneous determination of salbutamol sulphate and ambroxol HCl (AMB) from their combined dosage form. The method employs formation and solving of simultaneous equation using 242 nm and 272 nm as two analytical wavelengths (λ_{Max} of the drugs) of detection. Both the drugs obeyed Beer-Lambert's law over the concentration range 1-50 µg/mL for salbutamol sulphate and 10-50 µg/mL for ambroxol HCl, respectively. The developed method was validated for Accuracy, Precision, Limit of Detection and Limit of Quantification as per ICH guidelines and results of analysis were validated statistically.

Keywords: UV-Vis Spectroscopy; Simultaneous Equation Method; Salbutamol sulphate; Ambroxol HCl

1. Introduction

Salbutamol Sulphate (Fig. 1), chemically is [(1RS)-2-[(1,1-dimethylethyl)amino]-1-[4-hydrxy-3-(hydroxymethyl) phenyl] ethanol] sulphate [1]. It is official in Indian Pharmacopoeia [2]. It is beta-2 adrenergic agonist and used in management of asthma [3-5]. Ambroxol HCl (Fig. 1), chemically is 4-((2-amino-3,5-dibromobenzyl)amino)cyclohexan-1-ol hydrochloride [6]. It is also official in Indian Pharmacopoeia [2]. It is mucolytic agent act by breaking the acid muco polysaccharide fibres which makes the sputum thinner and less viscous. It is used as mucolytic agent [7].



(A) (B)

Figure 1 Chemical structure of (A) Salbutamol Sulphate and (B) Ambroxol HCl

There are number of analytical methods for the determination of various drugs from bulk and various formulations like tablets, capsules, injections, etc. These methods include Uv-spectrophotometry, HPLC, UPLC, Gas chromatography, etc [8-23]. Literature survey revealed various analytical methods have been reported for estimation of salbutamol sulphate alone and in combination with other drugs [24-29]. Similarly, there are two of analytical methods reported for Ambroxol

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HCl alone and in combination with other drugs [30-31]. However nobody has covered the complete validation as per ICH guidelines [32].

2. Material and methods

2.1. Chemicals and reagents

Salbutamol Sulphate (SAL) and Ambroxol HCl (AMB) standard materials were obtained as gift sample from Grandix Pharma Ltd., Mumbai (India). Tablets (Sal Mucolite™) make Dr Reddy's Laboratories Ltd containing Salbutamol (2mg) and Ambroxol (30mg) were purchased from the local market. All chemicals were of analytical reagent grade and solutions were prepared with water AR grade.

2.2. Instrumentation

A double beam UV-visible spectrophotometer (Simadzu model UV 2401 PC, Shimadzu Corporation, Kyoto, Japan) with spectral width of 2nm, quartz cell (1.0 cm path) was employed to measure absorbance of solutions. On the basis of solubility study water was selected as the solvent for dissolving SAL and AMB.

2.3. Standard Stock Solutions of SAL and AMB

Standard stock solution of drugs were prepared individually containing 1000 µg/mL of each drug. The solutions were filtered through 0.45 µm Whatman filter paper.

2.4. Determination of λmax of Individual Components

By appropriate dilution of standard solutions of SAL and AMB with water, solutions containing 10µg/mL of both drugs were scanned separately in the range of 200-400nm against water as blank. SAL shows λmax at 242m and AMB at 272 nm (see Fig. 2).

2.5. Overlay Spectra of SAL and AMB

The overlain spectra of SAL and AMB was recorded (Fig. 2) and two wavelengths 242nm (λmax of SAL) and 272nm (λmax of AMB) were selected for subsequent study.

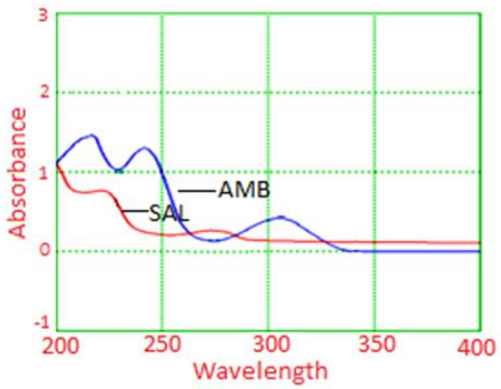

Figure 2 Overlain spectra of SAL and AMB

2.6. Methods: Simultaneous Equation Method

Standard Stock solutions of SAL and AMB in the concentration range 1-10 µg/mL and 15-75µg/ml were made in the water and absorbance of these solutions was measured at 242nm and 272nm. Calibration curves were plotted to confirm the Beer's law and the absorptivity values calculated at the respective wavelengths for both the drugs. Two simultaneous equations as below were formed using these absorptivity values A (1%, 1 cm).

$$\text{At } \lambda 1 \; A1 = ax1bCx + ay1bCy \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$\text{At } \lambda 2 \; A2 = ax2bCx + ay2\,bCy \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

For measurements in 1 cm cells b=1

Rearrange eq. (2)

Cy= A2- ax2Cx/ ay2

Substituting for Cy in eq (1) and rearranging

Cx = A2ay1-A1 ay2/ax2 ay1-ax1 ay2…….. (3)

Cy = A1ax2-A2 ax1/ax2 ay1-ax1 ay2……..(4)

Where Cx and Cyare the concentration of SAL and AMB, respectively, A1 and A2 are absorbance at 242 nm and 272 nm respectively, ax1 and ax2 are absorptivities of SAL at 242 nm and 272 nm respectively; ay1 and ay2 are absorptivities of AMB at 242 nm and 272 nm respectively. By solving the two simultaneous equations, the concentrations of SAL and AMB in sample solutions were obtained.

2.7. Analysis of tablet formulation

Average weight of 20 tablets was determined and were then crushed to fine powder. Average power equivalent to 30 mg of SAL (also contain 2 mg of AMB) was weighed accurately and was transferred to 100 ml volumetric flask. To this 20 ml of water was added and shaken for 30 min and sonicated for 10 min. Final volume was added up to 100 ml with same solvent. The solution was filtered the whatman filter paper.10 ml of above solution was diluted to 100 ml with methanol. The contained 30µg/ml of SAL and 2µg/ml of AMB. The absorbance of the solution was measured at 242 nm and 272 nm. The absorbances were measured at the selected wavelengths and absorptivities for both drugs (Table 1) were determined at both wavelengths.

2.8. Validation of proposed method

The method was validated according to ICH guidelines for validation of analytical procedures in order to determine linearity, sensitivity, accuracy and precision for each analyte [32].

2.9. Linearity

Appropriate dilutions of working standard solutions for SAL and AMB were prepared in the concentration range of 10-50µg/mL and 2.5-12.5µg/ml, respectively and analyzed as per the developed method. Calibration curves were prepared and the linearity was measured by the least square regression method.

2.10. Precision

Precision was checked as intra-day and inter-day variations. Intra-day precision was determined by analysingSAL (1, 2, 3 µg/mL) and AMB (15, 30, 45 µg/mL) for three times on the same day. Inter day precision was determined by analysing same concentration of solutions for three different days.

2.11. Accuracy (Recovery studies)

To study the accuracy, recovery study was carried out byaddition of standard drug solutions at three concentration levels (80 %, 100 %, and 120%) to preanalysed sample.

2.12. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of the developed method was assessed by analyzing ten replicates of standard solutions containing concentrations 2 µg/ml for SAL and 30 µg/ml for AMB.

The LOD may be calculated as

LOD = 3.3 × SD/ Slope

The LOQ may be calculated as

LOQ = 10 × SD/ Slope

Where, SD = Three replicates of absorbance
Slope = the mean slope of the 3 calibration curves

2.13. Robustness

It is the capacity of a method to remain unaffected by small, deliberate but slight variations in method parameters. These factors includes analyst to analyst variation and instrument to instrument variation (±2)

3. Results and discussion

In the present study, we have to develop UV-vis spectrophotometric method for the simultaneous estimation of SAL and AMB from combined dosage form. The developed method was validated as per the ICH guidelines. Linear relationship was found in the concentration range of 1-5 µg/mL for SAL (Fig. 3, Fig. 4) and 15-75 µg/mL for AMB at each wavelength i.e. 242nm and 272 nm (Fig. 5, Fig. 6). The linearity was observed in the concentration range of 1-5 µg/mL for SAL and 15-75 µg/mL for AMB. 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 (R2) also indicates good linearity for both the drugs. The absorbances were measured at the selected wavelengths and absorptivities for both drugs were determined at both wavelengths (Table 1).

Table 1 Absorbance and Absorptivity of SAL and AMB at two wavelengths

Conc. of sol. (µg/mL)		Absorbance				Absorptivity			
		SAL		AMB		SAL		AMB	
SAL	AMB	242nm	272nm	242nm	272nm	242nm	272 nm	242 nm	272 nm
1	15	0.01	0.05	3.33	1.10	0.0100	0.0450	0.2220	0.0733
2	30	0.05	0.09	3.49	1.82	0.0250	0.0450	0.1163	0.0607
3	45	0.10	0.13	3.62	2.55	0.0333	0.0433	0.0804	0.0567
4	60	0.15	0.17	3.79	3.20	0.0375	0.0425	0.0632	0.0533
5	75	0.20	0.22	3.92	3.80	0.0400	0.0440	0.0523	0.0507
					Avg.	ax1= 0.0292	ax2= 0.0440	ay1= 0.1068	ay2= 0.0589

*Each value is mean of three observations

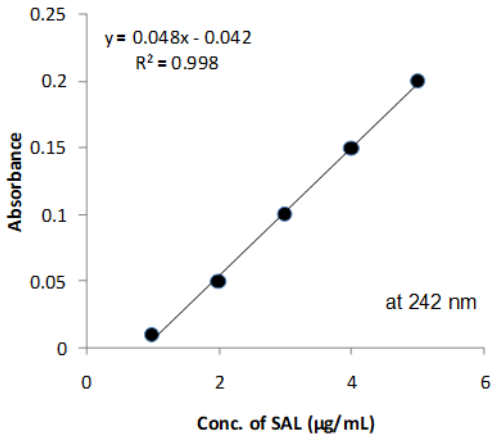

Figure 3 Calibration curves of SAL at 242nm

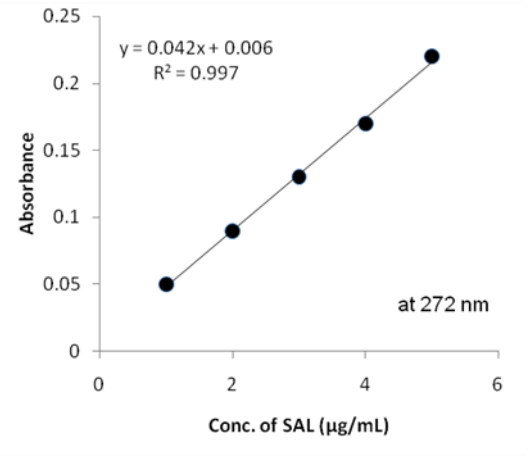

Figure 4 Calibration curves of SAL at 272 nm

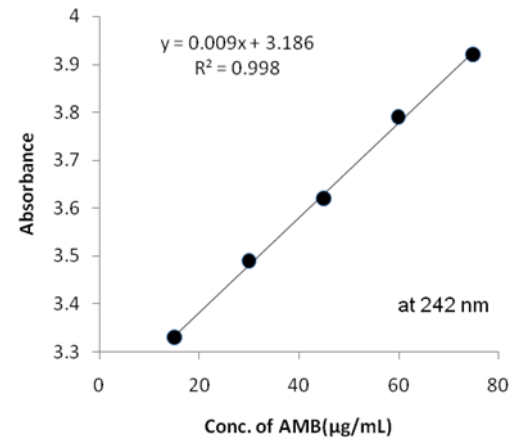

Figure 5 Calibration curves of SAL at 242 nm

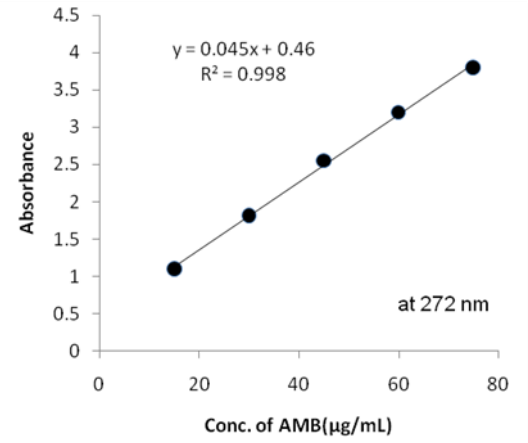

Figure 6 Calibration curves of AMB at 272 nm

The concentrations of drugs in sample solution were determined by using following formula.

Substituting the values of ax1, ax2, ay1 and ay2, the equation could be rearranged as:

At 242 nm, A1= 0.0292Cx + 0.0440 Cy …….1

At 272 nm, A2= 0.1068Cx + 0.0589 Cy …….2

Where Cx and Cy are the concentration of SAL and AMB in µg/mL

The method was validated according to ICH guidelines to study linearity, accuracy, and precision. Results of recovery studies are shown in Table 3. Percentage recovery for SAL and AMB by this method was found in the range of 98.83% to 99.83% and 99.16% to 99.85%, respectively. The value of % Recovery within the limit indicated that the method is accurate and percentage recovery shows that there is no interference from the excipients (Table 2).

Table 2 Recovery study of SAL and AMB

Level	Conc.	SAL		Conc.	AMB	
		% Recovery	%RSD		% Recovery	%RSD
80%	1.6	98.83	1.32	24	99.85	1.83
100%	2.0	99.17	0.93	30	99.44	0.99
120%	2.4	99.83	1.25	36	99.16	1.58
Mean		99.66	1.16	Mean	99.48	1.46

*Concentration in µg/mL

The LOD and LOQ of the developed method was assessed by analyzing five replicates of standard solutions containing concentrations 2 µg/ml for SAL and 30 µg/ml for AMB. The values for LOD and LOQ are given in (Table 3).

Table 3 LOD and LOQ

Drugs	Wavelength	LOD (µg/ml)	LOQ (µg/ml)
SAL	242nm	0.95	0.18375
AMB	272 nm	0.95	0.18375

Robustness of method was studied by analysing the tablet formulation at various conditions likes such as analyst to analyst variation and instrument to instrument variation (Table4).

Table 4 Results of robustness study

			SAL		AMB	
No	Factor	Term	% Drug estimated	% RSD	% Drug estimated	% RSD
1.	Analyst to analyst variation	Anal.1	99.32	1.62	99.63	1.11
		Anal.2	98.97	1.95	100.65	1.37
2	Instrument to instrument variation	Instr.1	99.15	1.06	98.44	1.65
		Instr.2	99.54	1.49	99.77	1.82

* Concentration of SAL (2 µg/ml) and AMB (30 µg/mL) (n=3)

4. Conclusion

The proposed UV-vis spectrophotometric method was found to be simple, accurate, precise and linear. Hence, it can be directly used for the analysis of SAL and AMB from combined dosage form.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Authors have declared no conflict of interest exist.

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