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(RESEARCH ARTICLE)



## Concurrent analysis of ambroxol HCl and salbutamol sulphate from tablet formulation by RP-HPLC

Wrushali A. Panchale<sup>1</sup>, Neha A. Badukle<sup>2</sup>, Amar F. Sabhadinde<sup>1</sup>, Ravindra L. Bakal<sup>1</sup> and Jagdish V. Manwar<sup>2,\*</sup><sup>1</sup> IBSS's Dr. Rajendra Gode Institute of Pharmacy, Mardi Road, Amravati-444 602, MS, India.<sup>2</sup> IBSS's Dr. Rajendra Gode College of Pharmacy, Mardi Road, Amravati-444 602, MS, India.

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### Abstract

RP-HPLC method was developed for concurrent analysis of ambroxol HCl and salbutamol sulphate from tablet formulation. Analytes were separated with mobile phase consisting of mixture of methanol and water (0.1% triethylamine) in the ratio 50: 50 at a flow rate of 0.7 ml/min with Nucleosil (4.6 mm I.D x 250 mm) C18 column. The retention time of ambroxol HCl and salbutamol sulphate was found to be 3.61 and 6.20 min, respectively. The detection was carried out at 224 nm. The dynamic range for ambroxol HCl and salbutamol sulphate observed was 15-75 µg/ml and 1-5 µg/ml, respectively. The percent recovery obtained for ambroxol HCl and salbutamol sulphate were close to 100%. Obtained statistical data of results was found to satisfactorily.

**Keywords:** RP-HPLC; Ambroxol HCl; Salbutamol sulphate; Validation

### 1. Introduction

Ambroxol HCl (trans-4-[(2-amino-3,5-dibromobenzyl)amino]cyclohexanol hydrochloride), an mucolytic agent official in Indian Pharmacopoeia [1-2]. Salbutamol sulphate ((RS-1-(4-hydroxy-3-hydroxymethylphenyl)-2-(tert-butylamino)ethanol sulphate) is beta adrenoceptor agonist, official in British Pharmacopoeia [3-4]. It used in management of asthma [5]. There are number of analytical methods for the analysis of pharmaceutical drugs from different formulations [6-19]. Literature survey revealed various analytical methods have been reported for estimation of Ambroxol HCl alone and in combination with other drugs [20-25]. Likewise, in literature there are two of UV-spectroscopic methods and one HPLC available for simultaneous analysis of ambroxol HCl and salbutamol sulphate in combined dosage form [26-28]. However nobody has enclosed the complete validation as per ICH guidelines. Therefore, attempts were made to develop new RP-HPLC method for the concurrent analysis of ambroxol HCl (AMB) and salbutamol sulphate (SLS) from tablet the formulation.

### 2. Material and methods

#### 2.1. Instrumentation and chemicals

Chromatography was performed with Youngline ACME 9000 (Autochro-3000 software) system coupled with Nucleosil (4.6 mm I.D x 250 mm) C18 column and UV 730 detector. A Rheodyne injector (manual loading) with a 20 µL external loop was used. All chemicals and reagents used in method were of HPLC grade. Standard drugs were obtained as gift samples from Grandix Pharma Ltd., Mumbai and tablet formulations (Sal Mucolite™, contents- AMB- 30 mg &SLS - 2 mg) were purchased from local medical shop.

\*Corresponding author Jagdish V Manwar  
IBSS's Dr. Rajendra Gode College of Pharmacy, Mardi Road, Amravati-444 602, MS, India.

## 2.2. Selection of wavelength and chromatographic conditions

Wavelength for analysis of both the drugs was selected by scanning the individual drug's standard solutions in methanol (i.e. AMB 300 µg/ml, SLS 20 µg/ml). From overlain spectra (Fig. 1), wavelength 224 nm was selected for further experimental work. Mobile phase for separation of drugs from mixed standard solution (containing AMB 30 µg/ml & SLS- 2 µg/ml) was consists of mixture of methanol and water (0.1% triethylamine) (50:50 v/v) in isocratic mode with flow rate 0.7 ml/min using 20 µl injection volume.

## 2.3. Evaluation of system suitability parameters

The system suitability test was performed by collecting data from five replicate injections (20 µl) of mixed standard solution (containing AMB 30 µg/ml & SLS- 2 µg/ml in methanol) at selected chromatographic conditions. The studied parameters includes retention time, resolution, area under curve (AUC), height equivalent to theoretical plates (HETP) and tailing factor.

## 2.4. Tablet formulation assay

Sample solution containing AMB (30µg/ml) and SLS (2µg/ml) was prepared in methanol as per the procedure mentioned in literature [28]. About 20µl sample solution was injected into the system and concentration of each drug was calculation from respective regression equation prepared for individual drug using AUC.

## 2.5. Validation of method [29]

Studied validation parameters includes accuracy and precision, linearity & range, LOD (limit of detection) & LOQ (limit of quantitation) and robustness.

### 2.5.1. Accuracy & precision

To study the accuracy and precision, recovery study was carried out by addition of standard drugs solutions to preanalysed sample. Recovery study was undertaken at three levels i.e. 80%, 100% and 120%.

### 2.5.2. Linearity & range

Linearity was studied by injecting a series of dilutions of mixed standard stock solution in the concentration range 15-75 µg/ml (AMB) and 1-5 µg/ml (SLS) into the HPLC system using 20µl volume. Calibration graph was plotted as concentration versus AUC.

### 2.5.3. LOD & LOQ

The LOD & LOQ were confirmed by diluting known concentrations of drug until the average AUC were approximately 3 or 10 times the standard deviation of AUC of the blank for five replicate determinations. The signal/noise ratios 3:1 and 10:1 were taken as the LOD and LOQ, respectively [30].

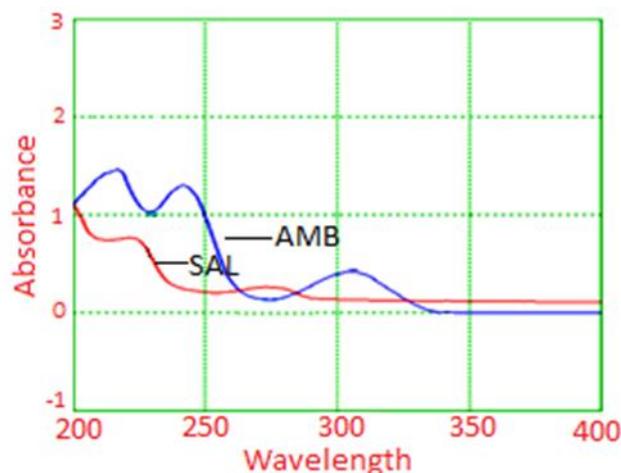
### 2.5.4. Robustness

Robustness was studied by making changes in the chromatographic conditions, such as slight change in mobile phase composition ( $\pm 1\%$ ), change in mobile phase flow rate ( $\pm 0.1$  ml/min), and change in wavelength ( $\pm 1$  nm). Percent contents of drugs were measured in preanalysed tablet formulation.

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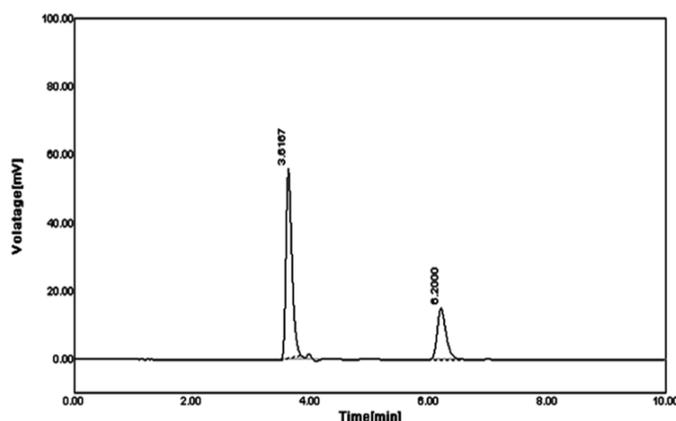
## 3. Results and discussion

Based on the literature survey, combination of AMB and SLS were selected for RP-HPLC method development. Solvent methanol was used to prepare standard and sample solutions as it dissolved both the drugs at selected concentration. Wavelength for detection selected was 224 nm because at this wavelength both the drug showed better sensitivity (see Fig. 1).



**Figure 1** Overlain UV-vis spectra of AMB and SLS

Concentration selected were 30  $\mu\text{g/ml}$  for AMB and 2  $\mu\text{g/ml}$  for SLS. At selected chromatographic conditions i.e. mobile phase consisting of mixture of methanol and water (0.1% triethylamine) in the ratio 50: 50 at a flow rate of 0.7 ml/min with Nucleosil (4.6 mm I.D x 250 mm) C18 column at ambient temperature, retention time obtained for AMB and SLS was 3.61 and 6.20 min, respectively. 0.1% triethylamine was used to adjust the pH so as to get sharp peak with minimum tailing and fronting. Herein, AMB elutes first as it is more polar followed by less polar SLS [31]. Chromatogram of mixed standard solution is shown in Fig. 2



**Figure 2** Chromatogram of AMB (first peak) and SLS (second peak)

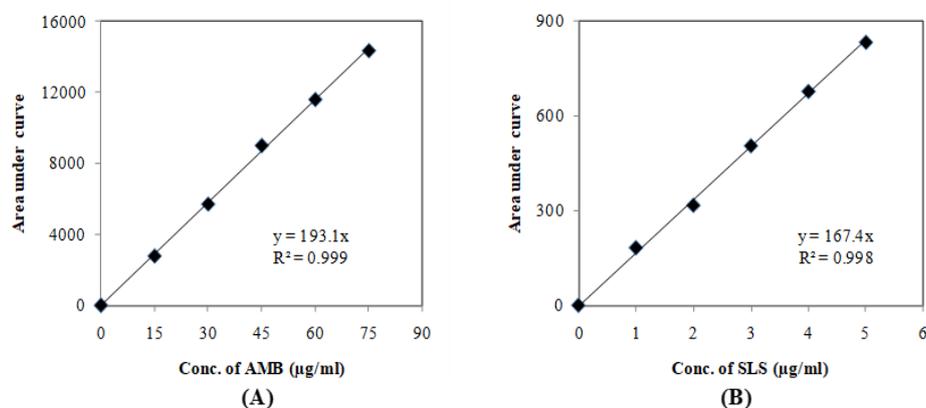
The validation was performed as per ICH guidelines. Linearity and range was studied by using the series of dilution of each drug solution. From this, concentration for AMB and SLS were selected. The LOD & LOQ were checked by diluting known concentration of standard drug until the mean responses were approximately 3 or 10 times the standard deviation of the responses of the blank for five replicate measurements. The signal/noise ratios 3:1 and 10:1 were considered as the LOD and LOQ, respectively. LOD and LOQ values obtained are given in Table 1 and linearity graphs are shown in Fig. 3.

Precision of the method was checked by measuring system suitability parameter by replicate injection of mixed standard solution. The results are expressed % RSD. Accuracy of the method was performed by recovery study by standard addition method at three levels i.e. 80, 100 and 120 %. The percentage recovery for both the drug was closed to 100% w/w for both drugs. Precision was determined by studying system suitability parameters by injecting standard solution (Table 1)

**Table 1** Results of the method

Sr. No.	Study	Parameters	Result	
			AMB	SLS
1	Label claim*		30 mg	2 mg
2	% Recovery*	80% level	98.12	97.822
		100% level	101.37	99.552
		120% level	101.62	99.41
3	Linearity and range	Range **	15-75	1-5
		% RSD**	1.85	1.64
		Slope	193.1	167.4
		Corr. Coeff. (R <sup>2</sup> )	0.999	0.998
		LOD	0.191	0.152
		LOQ	0.367	0.002
4	System suitability parameters	Ret. Time	3.61	6.20
		Resolution	-	9.45
		AUC	5825	316
		HETP	106453	2905
		Tailing Factor	1.37	1.20
5	Robustness#			
	(i) Mobile phase (a: b)##	51:49	99.18	100.65
		49:51	100.54	99.09
	(ii) Flow rate	0.6 ml/min	101.62	101.54
		0.8 ml/min	98.97	99.11
	(iii) Intra- & Inter-day variation	Intra-day	100.52	100.70
		Inter-day	99.39	99.78

\* Amount in mg/tablet; \*\*Mean of three results; # % contents of drugs were measured in preanalysed tablet formulation; ## a- Methanol, b-water (0.1% TEA); ♣ Results are expressed in percentage w/w; ♣♣ Concentration in µg/ml

**Figure 3** Calibration curves for ambroxol HCl (A) and salbutamol sulphate (B)

The capacity of developed method was checked by performing robustness study. The conditions were changed deliberately in mobile phase composition ( $\pm 1$ ), flow rate ( $\pm 0.1$ ), and wavelength ( $\pm 1$ ), Intraday and inter-day variation and percent contents in formulation were estimated. The result showed develop method remain unaffected. The percent contents of drugs were measured in preanalysed tablet formulation (Table 1)

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#### 4. Conclusion

Developed new RP-HPLC method for concurrent analysis of ambroxol HCL and salbutamol sulphate from tablet formulation was found to be simple, sensitive and accurate and does not get affected upon smaller difference in experimental condition. Thus, the method could be used for routine quality control analysis of bulk drugs and tablet formulation.

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#### Compliance with ethical standards

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##### *Disclosure of conflict of interest*

The authors declare no conflicts of interest.

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