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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF SALBUTAMOL SULPHATE, GUAIFENESIN, AND AMBROXOL HYDROCHLORIDE BY RP-HPLC METHOD IN COMMERCIAL ORAL LIQUID DOSAGE FORM

Prabha Thangavelu^{*}, Arul Caroline Grace and Jagadeeswaran Murugesan

Department of Pharmaceutical Analysis, Nandha College of Pharmacy, Koorapalayam Pirivu, Pitchandam Palayam Post, Erode - 638052, Tamil Nadu, India.

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Correspondence to Author:

Dr. T. Prabha

Professor,
Department of Pharmaceutical
Analysis, Nandha College of
Pharmacy, Koorapalayam Pirivu,
Pitchandam Palayam Post, Erode -
638052, Tamil Nadu, India.

E-mail: drtpappa@yahoo.com

ABSTRACT: Salbutamol is a bronchodilator, Guaifenesin is an expectorant and Ambroxol is a mucolytic. Combination of these drugs is used in the formulation of cough syrups. In the literature, there is no method reported for the simultaneous estimation of the drugs in oral liquid dosage form. Hence, the present work is aimed to develop reverse phase HPLC method for the simultaneous determination of Salbutamol sulfate (SAL), Guaifenesin (GUA) and Ambroxol hydrochloride (AMB) in oral liquid dosage form and validation of the developed method. The chromatographic separation of the drugs was achieved with the mobile phase system sodium dihydrogen phosphate buffer pH 3.0: acetonitrile: methanol in the ratio of 65:10:25 with the flow rate of 1 mL/min and injection volume 10 μ L. An Inertsil C8-3 (250 \times 4.6 mm, 5 μ m) column was used, and the detection wavelength was 276 nm. This system produced sharp peaks with good resolution, minimum tailing and satisfactory retention times of SAL, GUA and AMB were found to be 3.157, 9.949 and 11.883 min respectively indicating the suitability of the system. The developed method was validated for various parameters accuracy, precision, linearity, robustness, and specificity as per ICH guidelines.

INTRODUCTION: Salbutamol sulfate (SAL) is a selective beta₂-adrenergic receptor agonist. It works by binding of Salbutamol to beta (2) receptors in the lungs results in relaxation of bronchial smooth muscles¹. Salbutamol is chemically designated as (RS)- 4-[2-(tert Butyl amino)-1-hydroxyethyl]-2-(hydroxymethyl) phenol **Fig. 1** and its molecular formula is (C₁₃H₂₁NO₃)₂.

Guaifenesin (GUA) chemically designated as 3-(2-methoxy phenoxy) propane-1, 2-diol **Fig. 1** and its molecular formula are C₁₀H₁₄O₄, mainly used as an expectorant which will reduce the viscosity of sputum. It may act as an irritant to the gastric vagal receptor, and recruit efferent parasympathetic reflexes that cause glandular exocytosis of a less viscous mucus mixture².

Ambroxol hydrochloride (AMB) is a mucolytic agent chemically designated as Trans-4-[(2-amino-3, 5-dibromo benzyl) amino] cyclohexyl Hydrochloride **Fig. 1** and its molecular formula is C₁₃H₁₉Br₂ClN₂O. Ambroxol produces their action by inhibiting the NO-dependent activation of soluble guanylate cyclase can suppress the

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excessive mucus secretion. Therefore it lowers the phlegm viscosity and improves the mucociliary transport of bronchial secretions. It promotes the

removal of tenacious secretions in the respiratory tract and reduces mucus stasis³.

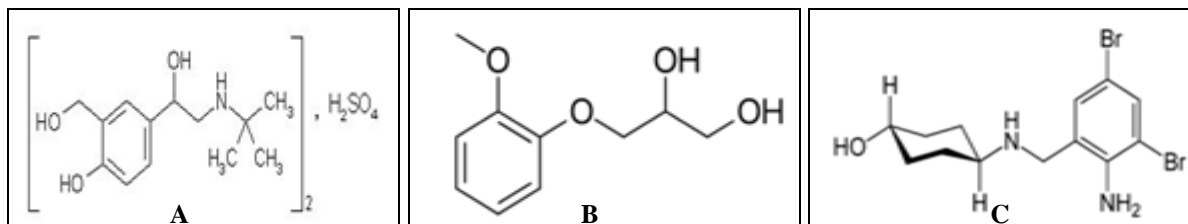


FIG. 1: (A) SALBUTAMOL (B) GUAIFENESIN (C) AMBROXOL

There are few methods have been reported for simultaneous estimation of SAL, AMB, and GUA and in combination with some other drugs^{4, 5}. Among those, the only one method is available which is a simple and precise Liquid Chromatography method for the simultaneous estimation of SAL, GUA, and AMB in the combined tablet dosage form and was developed, validated by Mukesh *et al.*, Several methods such as HPLC⁶⁻²², Spectrophotometric^{23, 24}, Gas Chromatography^{25, 26, 27} and stability indicating LC Method²⁸ have been reported for the analysis of SAL, GUA and AMB as an individual drug or in combination, either in pure or in pharmaceutical forms as well as in biological fluids and tissues.

However, there is no method reported for the simultaneous estimation of SAL, GUA and AMB in the oral liquid dosage form. So it was felt that there is a need to develop the RP-HPLC method for the determination of SAL, GUA and AMB simultaneously in a single step process. Hence the present work is aimed to develop and validate a simple RP-HPLC method for the simultaneous estimation of SAL, GUA and AMB in the liquid oral dosage form.

MATERIALS AND METHODS: The chromatographic technique performed on HPLC Agilent separation module 1200 with UV detector with reversed phase inertsil C₈ column (250 × 4.6 mm, 5 μ) as stationary phase, Jasco double beam UV-VIS spectrophotometer was used as a detector for this study. CRMS Syrup containing 500 mg of Guaifenesin, 150 mg of Ambroxol and 25 mg of Salbutamol was procured from local market. All other AR and LR grade chemicals such as sodium dihydrogen phosphate, acetonitrile, methanol and, triethylamine were procured from Rankem Limited, New Delhi.

Chromatographic Conditions: The sample separation was achieved on an Inertsil C₈ column (250 mm × 4.6 mm, 5 μ) with ambient temperature, the injection volume was set as 10 μL, UV detection at 276 nm, with the flow rate of 1.0 mL/min, the mobile phase aided by mobile phase mixture of Buffer: acetonitrile: methanol (65:10: 25% v/v).

Preparation of Phosphate Buffer: An amount of 1.56 gm of NaH₂PO₄ taken into a 1000 mL flask, dissolved and made up to 1000 mL with water and added 3 mL of triethylamine and adjusted the pH to 3.0 with ortho-phosphoric acid.

Preparation of Mobile Phase: Mixed the mixture of above buffer 650 mL (65%), 100 mL of Acetonitrile (10%) and 250 mL of Methanol (25%) and degassed in ultrasonicator for 5 min.

Finally, filtered through 0.45 μ nylon membrane filter under vacuum filtration.

Standard Solution (Preparation of SAL, GUA and AMB Stock Solution): Accurately weighed quantity of 500 mg of Guaifenesin, 150 mg Ambroxol and 25 mg of Salbutamol was transferred to three different 100 mL volumetric flask, dissolved in 25 mL of mobile phase, and sonicated for 5 min and the volume was made up with mobile phase.

Preparation of Working Standard: Working standard for Guaifenesin, Ambroxol and Salbutamol were prepared by pipetting out 10 mL, 10 mL, and 4 mL respectively from each of the above stock solutions into a 100 mL volumetric flasks and the volume was made up with the mobile phase.

Sample Preparation: An amount equivalent to 500 mg of Guaifenesin, 150 mg of Ambroxol and 25 mg of Salbutamol from liquid formulation was accurately weighed and taken in three different 100 mL volumetric flask, and 25 mL of mobile phase was added. The mixture was subjected to sonication for 15 min with frequent shaking for the complete dissolving of drugs.

Cooled to room temperature and the solution was made up with mobile phase and filtered through a 0.45 μ membrane filter. From the above of each SAL, GUA and AMB solution, 4 ml, 10 ml, and 10 ml respectively were pipetted out into 100 ml volumetric flask, and the volume was made with mobile phase. Then 10 μ l of this solution was injected for HPLC analysis.

Determination of Working Wavelength (λ_{max}):

The stock solution of SAL, GUA, and AMB, each 10 μ g/mL in methanol, were scanned over the wavelength range of 200 - 400 nm against blank. After a thorough examination of the spectra, the wavelength of 276 nm was chosen for further analysis.

RESULT AND DISCUSSION:

Method Validation (System Suitability): The system suitability parameters like resolution,

retention time, plate number (N), peak asymmetry factor (Tailing) were evaluated with the help of standard chromatogram and showed in **Table 1**.

TABLE 1: RESULTS FOR SYSTEM SUITABILITY PARAMETERS

Parameters	SAL	GUA	AMB	Acceptance criteria
Resolution	NA	29.90	4.69	NLT 2
Tailing factor	1.1	1.1	1.3	NLT 2
No. of theoretical plate	14133	13119	9978	NLT 2000
Retention time	3.157	9.949	11.883	NA

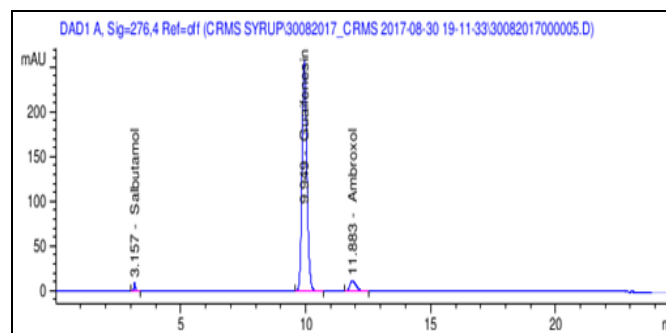


FIG. 2: CHROMATOGRAM FOR SAL, GUA AND AMB

Accuracy: The recovery study was carried out, and the contents were determined from the respective chromatogram. Recovery studies did the accuracy at 50%, 100%, and 150% concentration level. The recoveries at three different concentrations were found to be within the range of 98.4 to 101.6%, and the results were shown in **Table 2**.

TABLE 2: RESULTS FOR RECOVERY STUDY FOR SAL, GUA AND AMB

Accuracy	SAL Added (μ g)	SAL Found (μ g)	% Recovery	% Mean Recovery	SD	% RSD	GUA Added (mg)	GUA Found (mg)	% Recovery	% Mean Recovery	SD	% RSD	AMB Added (μ g)	AMB Found (μ g)	% Recovery	% Mean Recovery	SD	% RSD
50%	4.99	4.91	101.6	101.5	0.23	0.23	4.98	4.83	98.6	98.6	0.06	0.06	74.85	74.15	100.6	100.5	0.25	0.25
		4.93	101.2					4.84	98.7					73.89	100.9			
		4.91	101.6					4.84	98.6					74.25	100.4			
100%	9.98	9.87	101.1	101.3	0.15	0.15	9.94	9.79	98.6	98.8	0.06	0.06	149.70	148.42	100.8	100.6	0.26	0.26
		9.85	101.3					9.77	98.8					148.62	100.7			
		9.84	101.4					9.76	98.8					149.12	100.3			
150%	14.97	14.78	101.3	101.2	0.15	0.15	14.96	14.86	98.6	98.6	0.06	0.06	224.55	222.58	100.9	100.7	0.15	0.15
		14.82	101.0					14.88	98.8					222.96	100.7			
		14.79	101.2					14.84	98.4					223.16	100.6			

Precision: System Precision, Method Precision, and Intermediate Precision / Ruggedness: The system precision was performed by injecting standard solution for six times to the analytical column and the peak area was measured then the % RSD for the area was calculated.

The method precision was done by performing an assay on six replicate determination of sample preparation at test concentration level (as per the method of analysis), and the relative standard deviation of % assay of results was calculated. To evaluate the intermediate precision of the method,

the precision was performed on a different day by using different column make of same dimensions. The below **Table 3** shows the overall data precision.

Linearity and Range: SAL showed linearity in the range of 5-15 (μ g/mL), GUA showed linearity in the range of 250-750 (μ g/mL) and AMB showed linearity in the range of 75-225 (μ g/mL). The calibration graphs were plotted with peak area in the Y-axis and concentration of the standard solution in the X-axis **Fig. 3**. The degree of linearity was estimated by calculating the

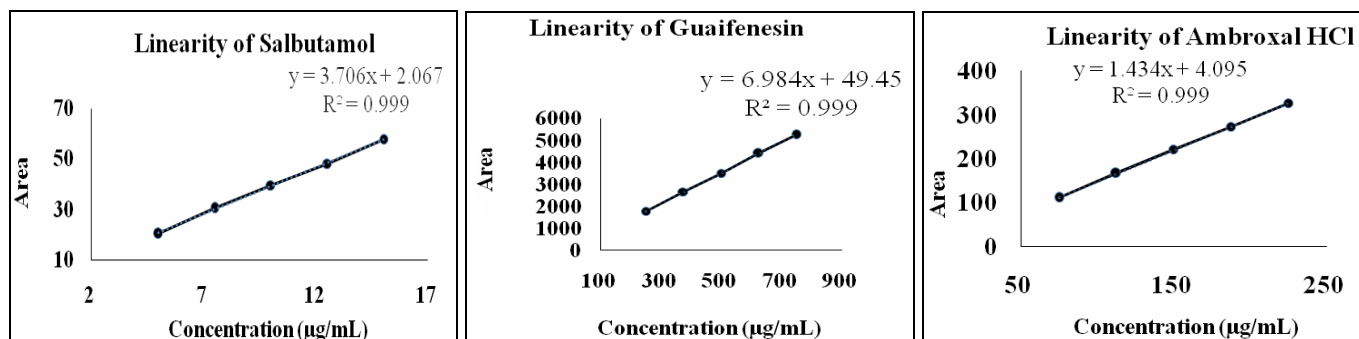
correlation coefficient. The correlation coefficient values for SAL, GUA and AMB, were found to be 0.9994, 0.9999 and, 0.9998 respectively and shown in **Table 4**.

TABLE 3: DATA FOR PRECISION OF SAL, GUA AND AMB

S. no.	System precision			Method precision			Intermediate precision		
	Area of SAL	Area of GUA	Area of AMB	% Assay SAL	% Assay GUA	% Assay AMB	% Assay SAL	% Assay GUA	% Assay AMB
1	38.112	3488.011	214.053	101.86	98.43	100.28	101.47	98.29	100.44
2	38.998	3498.539	216.653	101.56	98.54	100.34	101.33	98.23	100.35
3	38.620	3507.698	214.790	101.31	98.49	100.28	101.23	98.09	100.39
4	38.148	3506.391	215.290	101.20	98.39	100.27	101.06	98.34	100.28
5	38.613	3510.320	216.647	101.08	98.46	100.21	100.97	98.29	100.27
6	38.618	3507.696	214.791	100.93	98.39	100.18	100.95	98.24	100.29
Mean	38.498	3502.012	215.491	101.32	98.45	100.26	101.17	98.25	100.34
S.D	0.37	9.06	1.15	0.34	0.06	0.06	0.21	0.09	0.07
% RSD	1.0	0.3	0.5	0.33	0.06	0.06	0.31	0.09	0.07

TABLE 4: RESULTS FOR LINEARITY DATA

Concentration of SAL ($\mu\text{g/mL}$)	Peak area	Concentration of GUA ($\mu\text{g/mL}$)	Peak area	Concentration of AMB ($\mu\text{g/mL}$)	Peak area
05	20.298	250	1784.443	75	110.443
7.5	30.306	375	2676.681	112.5	166.149
10	39.608	500	3520.368	150	220.646
12.5	48.196	625	4422.826	187.5	272.420
15	57.828	750	5267.516	225	326.137
Correlation coefficient - 0.9994		Correlation coefficient - 0.9999		Correlation coefficient - 0.9998	

**FIG. 3: LINEARITY PLOT FOR SAL, GUA AND, AMB**

Robustness: The robustness of a method is evaluated by varying method parameters such as flow rate, wavelength, etc., and determining the effect (if any) on the results of the method. The overall percentage relative standard deviation in the various parameters was found to be less than the acceptance limit of % RSD which is within 2%.

The result indicated that the method was robust and is represented in **Table 5** and **6**.

Solution Stability: Solution stability study was performed at different days against the freshly prepared standard, and sample solution and the results were represented in **Table 7** and **8**.

TABLE 5: RESULTS FOR EFFECT OF FLOW RATE AND WAVELENGTH

Name	Flow rate mL/min	RT	Plate count	Tailing	Resolution	Wavelength (nm)	RT	Plate count	Tailing	Resolution
SAL	0.9	3.49	11482	0.8	NA	274	3.56	11882	0.8	NA
	1.0	3.15	14133	1.1	NA	276	3.15	14133	1.1	NA
	1.1	2.80	10403	0.8	NA	278	2.86	11403	0.8	NA
GUA	0.9	10.88	13848	1.1	29.52	274	10.98	12848	1.1	29.22
	1.0	9.94	13119	1.1	29.90	276	9.94	13119	1.1	29.90
	1.1	9.01	12690	1.1	28.52	278	9.31	11690	1.1	28.98
AMB	0.9	12.86	10421	1.3	4.52	274	12.46	10521	1.3	4.82
	1.0	11.88	9978	1.3	4.69	276	11.88	9978	1.3	4.69
	1.1	10.93	9477	1.3	4.48	278	11.13	9677	1.3	4.28

TABLE 6: RESULTS FOR CHANGE IN FLOW RATE AND WAVELENGTH

Flow rate (mL/min)	Area of SAL	Area of GUA	Area of AMB	Wavelength (nm)	Area of SAL	Area of GUA	Area of AMB
0.9	38.011	3488.011	214.053	274	39.021	3504.258	216.687
	38.108	3498.539	216.653		39.254	3508.654	216.458
Mean	38.056	3493.275	215.353	Mean	39.138	3506.456	216.573
S.D	0.07	7.44	1.84	S.D	0.16	3.11	0.16
% RSD	0.18	0.21	0.85	% RSD	0.42	0.09	0.07
1.0	38.684	3506.425	215.243	276	38.698	3498.254	215.124
	38.298	3510.122	215.987		38.386	3501.568	215.354
Mean	38.491	3508.274	215.615	Mean	38.542	3499.911	215.239
S.D	0.27	2.61	0.54	S.D	0.22	2.34	0.16
% RSD	0.71	0.07	0.24	% RSD	0.57	0.07	0.08
1.1	37.854	3486.218	213.458	278	38.054	3488.478	214.689
	38.024	3488.452	213.853		38.114	3492.854	214.287
Mean	37.939	3487.335	213.655	Mean	38.084	3490.666	214.488
S.D	0.12	1.58	0.28	S.D	0.04	3.09	0.28
% RSD	0.32	0.05	0.13	% RSD	0.11	0.09	0.13

TABLE 7: RESULTS FOR STANDARD SOLUTION STABILITY

Interval	Room Temperature (23 °C - 27 °C)					
	SAL area	GUA area	AMB area	Similarity factor		
				SAL	GUA	AMB
Initial	38.620	3507.698	214.790	1.01	1.00	1.00
24 h	37.557	3497.494	213.358	1.00	1.01	1.01
36 h	38.998	3526.216	215.165	0.99	1.01	1.01
48 h	40.356	3598.583	216.639	1.01	1.00	1.00

TABLE 8: RESULTS FOR SAMPLE SOLUTION STABILITY

Interval	Room temperature (23 °C - 27 °C)								
	SAB area	GUA area	AMB area	% Assay			Absolute % difference		
				SAB	GUA	AMB	SAB	GUA	AMB
Initial	38.256	3557.256	215.011	101.58	99.56	100.88	NA	NA	NA
24 h	38.557	3602.494	214.058	101.15	99.15	100.56	0.4	0.4	0.3
36 h	39.987	3687.216	214.665	100.96	98.56	100.05	0.2	0.6	0.5
48 h	40.998	3714.583	216.139	100.85	98.21	99.87	0.1	0.3	0.2

CONCLUSION: The developed method for simultaneous estimation of Salbutamol sulfate, Guaifenesin and Ambroxol hydrochloride said to be rapid, simple, accurate, precise, sensitive, robust and specific that can be successfully applied for the routine analysis of Salbutamol sulfate, Guaifenesin and Ambroxol hydrochloride in their marketed oral liquid dosage form.

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