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A New RP-HPLC Method Development and Validation of Simultaneous Estimation of Levosalbutamol, Ambroxol and Guaiphenesin by Syrup Formulation

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Abstract

A procedure using RP-HPLC is developed, validated and employed for concurrent estimation of ambroxol, guaiphenesin and levosalbutamolin syrup formulation. The procedure involves separation and analysis of ambroxol, guaiphenesin and levosalbutamolin Waters C18 column (stationary phase) and 60% 0.1M sodium dihydrogen phosphate (pH 5.0) and 40% acetonitrile mixture (mobile phase). The levosalbutamol, guaiphenesin and ambroxol were eluted at 4.767 min, 6.409 min and 9.850 min, respectively. Linear ranges are 0.5-1.5 µg/ml, 50-150 µg/ml and 15-45 µg/ml with regression coefficient values of 0.9999, 0.9994 and 0.9991 for levosalbutamol, guaiphenesin and ambroxol, respectively. The determined LOD values are: levosalbutamol – 0.022 µg/ml, guaiphenesin – 0.1115 µg/ml and ambroxol – 0.072 µg/ml, and determined LOQ values are: levosalbutamol – 0.072 µg/ml, guaiphenesin – 0.3716 µg/ml and ambroxol – 0.239 µg/ml. Validation parameters tested following guidelines of ICH are good enough for the intended assay. The method demonstrated as appropriate procedure for assay of ambroxol, guaiphenesin and levosalbutamolin syrup formulation with excellent assay percentage values.

Keywords
Ambroxol, Guaiphenesin, Levosalbutamol, RP-HPLC Method.

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INTRODUCTION

Levosalbutamol

Chemically it is 4-[(1R)-2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl)phenol.

Levosalbutamol's bronchodilator properties give it indications in treatment of COPD (chronic obstructive pulmonary disease also known as chronic obstructive lung disease) and asthma. Activation of β2 adrenergic receptors on airway smooth muscle leads to the activation of adenylate cyclase and to an

increase in the intracellular concentration of 3', 5'-cyclic adenosine monophosphate (cyclic AMP). The increase in cyclic AMP is associated with the activation of protein kinase A, which in turn, inhibits the phosphorylation of myosin and lowers intracellular ionic calcium concentrations, resulting in muscle relaxation. Levosalbutamol relaxes the smooth muscles of all airways, from the trachea to the terminal bronchioles. Increased cyclic AMP concentrations are also associated with the inhibition of the release of mediators from mast cells in the airways. Levosalbutamol acts as a functional agonist that relaxes the airway irrespective of the spasmogen involved, thereby protecting against all Broncho constrictor challenges. It gets eliminated in urine completely and has half-life of 3-4 hr.

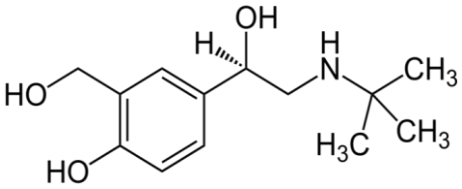


Figure 1: Levosalbutamol structure

Ambroxol

Chemically it is 4-[(2-amino-3,5-dibromophenyl) methyl amino] cyclohexan-1-ol. Ambroxol is a drug that breaks up phlegm, used in the treatment of respiratory diseases associated with viscid or excessive mucus. Ambroxol is indicated as "secretolytic therapy in bronchopulmonary diseases associated with abnormal mucus secretion and impaired mucus transport. It promotes mucus clearance, facilitates expectoration and eases productive cough, allowing patients to breathe freely and deeply. The substance acts on mucus membranes, restoring the physiological clearance mechanisms of the respiratory tract (which play an important role in the body's natural defence mechanisms) through several mechanisms, including breaking up phlegm, stimulating mucus production, and stimulating synthesis and release of surfactant by type II pneumocytes. Surfactant acts as an anti-glue factor by reducing the adhesion of mucus to the bronchial wall, in improving its transport and in providing protection against infection and irritating agents. It has 70-80% of bioavailability and half-life of 7-12 hr.

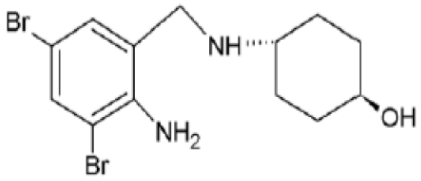


Figure 2: Ambroxol structure

Guaiphenesin:

Chemically it is 3-(2-methoxyphenoxy) propane-1,2-diol. Guaiphenesin is thought to act as an expectorant by increasing the volume and reducing the viscosity of secretions in the trachea and bronchi. It has been said to aid in the flow of respiratory tract secretions, allowing ciliary movement to carry the loosened secretions upward toward the pharynx. [13] Thus, it may increase the efficiency of the cough reflex and facilitate removal of the secretions. Guaifenesin has muscle relaxant and anticonvulsant properties and may act as an NMDA receptor antagonist. [14] It has bioavailability of approximately 70%. It is eliminated in urine and have half-life of 1-5 hr.A

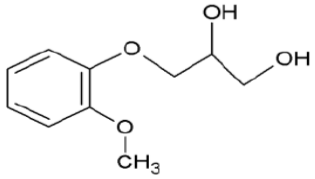


Figure 3: Guaiphenesin structure

Ambroxol, Levosalbutamol and Guaiphenesin syrup:

The above drug combination is specified to control, treat, improve and prevent the symptoms and conditions of below stated diseases:

Bronchitis, Cough, Common cold, Asthma, Breathing illnesses, Respiratory tract diseases, Pain in arthritis, Pain in muscle strains or sprains, Lung Disorder, Congestion, Thickened mucus cough, Pain in tendons.

MATERIALS AND METHODS

Materials:

- Levosalbutamol, guaiphenesin and ambroxol hydrochloride was obtained from Rainbow pharma training labs, Telangana, India.
- Levosalbutamol Sulphate Ambroxol Hcl, GuaiphenesinSyp (Bionext Pharma Private Limited, Mumbai, India): Label strength**:** Each 5 ml contains: 0.5 mg levosalbutamol, 15 mg ambroxol hydrochloride and 50 mg guaiphenesin.
- Acetronitrile - HPLC grade, Merck specialties Ltd, India
- Sodium dihydrogen phosphate - Analytical grade, Sd Fine-Chem Limited, India.

Instrumentation

- HPLC system – Waters Alliance 2695 model
- Column – Waters C18 column (250 × 4.6) mm, particle size 5 µm
- Detector – photodiode array detector

Preparation of Mobile phase

- 60% 0.1M sodium dihydrogen phosphate and 40% acetonitrile. pH is 5.0.

Assaying chromatographic conditions:

Column oven temperature - 25 ^{o}C Volume of injection - 10 µl. Flow rate -1.0 ml\min. Wavelength for detection - 237 nm.

Preparation of levosalbutamol, ambroxol and guaiphenesin standard solutions:

0.5 mg of levosalbutamol, 15 mg of ambroxol hydrochloride and 50 mg of guaiphenesin were measured and transferred to volumetric flask (100 ml). Thirty ml of mobile phase have been added and for 20 min have been sonicated. Mobile phase was further added to make 100 ml volume (Concentration - 5 µg/ml levosalbutamol, 150 µg/ml ambroxol hydrochloride and 500 µg/ml guaiphenesin). This solution is levosalbutamol, ambroxol hydrochloride and guaiphenesin stock solution.

Levosalbutamol, ambroxol hydrochloride and guaiphenesin solutions for linearity curve are made by stock solution dilution properly to ten ml by mobile phase to have following concentrations:

- 0.5 µg/ml, 0.75 µg/ml, 1.0µg/ml, 1.25 µg/ml and 1.5 µg/ml – Levosalbutamol
- 50 µg/ml, 75 µg/ml, 100 µg/ml, 125 µg/ml and 150 µg/ml – guaiphenesin
- 15.0 µg/ml, 22.50 µg/ml, 30.0 µg/ml, 37.5 µg/ml and 45.0 µg/ml – ambroxol hydrochloride

To check validation contents, levosalbutamol, ambroxol hydrochloride and guaiphenesin stock solution was properly diluted to ten ml by mobile phase (Concentration - 1 µg/ml levosalbutamol, 30 µg/ml ambroxol hydrochloride and 100 µg/ml guaiphenesin).

Preparation of levosalbutamol, ambroxol and guaiphenesin syrup solutions:

Five ml of syrup equal to 0.5 mg of levosalbutamol, 15 mg of ambroxol hydrochloride and 50 mg of guaiphenesin were measured and transferred to volumetric flask (100 ml). Mobile phase of 30 ml was added and 20 min sonicated. Mobile phase was additional added to make total volume to 100 ml (Concentration - 5 µg/ml levosalbutamol, 150 µg/ml ambroxol hydrochloride and 500 µg/ml guaiphenesin). This is levosalbutamol, ambroxol hydrochloride and guaiphenesin syrup stock solution. For analysis, 2 ml of levosalbutamol, ambroxol hydrochloride and guaiphenesin syrup stock solution is diluting to 10 ml using mobile phase (Concentration - 1 µg/ml levosalbutamol, 30 µg/ml ambroxol hydrochloride and 100 µg/ml guaiphenesin).

RESULT AND DISCUSSION

Development of method:

Table 1: Method development

Composition and ratio in mobile phase	:	**0.1 M Na_2HPO_4 : Acetonitrile (60:40, *vol/vol*)**
Column tested	:	Waters, C18, 250 mm × 4.6 mm, particle size 5 µm
Flow rate within column	:	1.0 ml/min
Column's temperature	:	25˚C
Sample quantity injected	:	10 µl
Time of run	:	10 min
Detection at	:	237 nm
pH	:	5.0

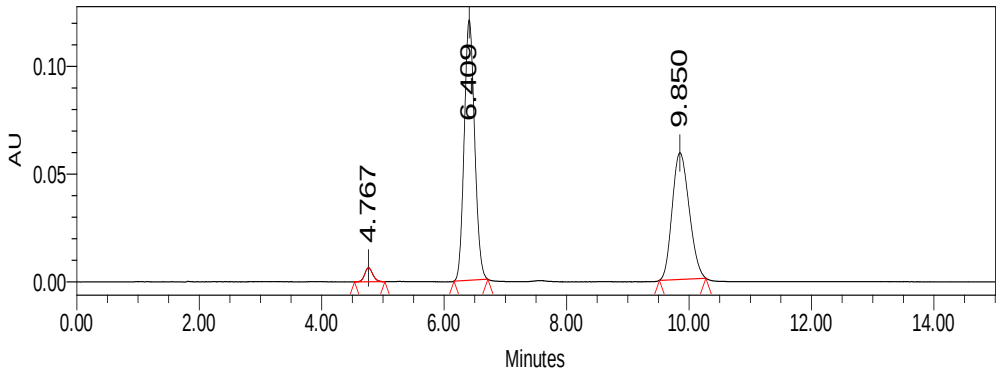


Figure 4: Chromatogram of levosalbutamol, guaiphenesin and ambroxol hydrochloride

Method validation:

The method developed for the determination of levosalbutamol, ambroxol hydrochloride and guaiphenesin in syrup was validated for linearity, system suitability, accuracy, LOD and LOQ, robustness and precision.

System suitability:

In order to ascertain that the entire HPLC system performs well, system suitability variables were calculated. Testing variables (% RSD peak area- acceptable value ≤2.0%; tailing factor- acceptable value ≤2.0; resolution factor - acceptable value ≥2.0, plate count - acceptable value 2000) for system suitability for the method established were computed and acceptable results were gained. This test was done through introducing standard solution (1 µg/ml levosalbutamol, 30 µg/ml ambroxol hydrochloride and 100 µg/ml guaiphenesin) into system five times.

Table 2: System suitability results for levosalbutamol, guaiphenesin and ambroxol

S No	Levosalbutamol Sulphate			Guaiphenesin			AmbroxolHCl		
	RT(min)	PC	Tailing	RT(min)	PC	Tailing	RT(min)	PC	**Tailing**
1	4.755	6629	1.07	6.392	6861	1.11	9.950	5229	8.20
2	4.754	6653	1.05	6.389	6861	1.11	9.982	5210	8.22
3	4.785	6611	1.05	6.421	6788	1.10	10.176	5188	8.44
4	4.823	6625	1.06	6.464	6763	1.10	10.315	5339	8.64
5	4.834	6611	1.04	6.469	6779	1.10	10.301	5296	8.58

Selectivity:

Through comparison of retention time values of levosalbutamol, ambroxol and guaiphenesin in standard with those of placebo, syrup sample and mobile phase blank, the selectivity was tested. Interference at retention times of levosalbutamol, ambroxol and guaiphenesin was not observed in mobile phase and placebo chromatograms. The results specified method's selectivity.

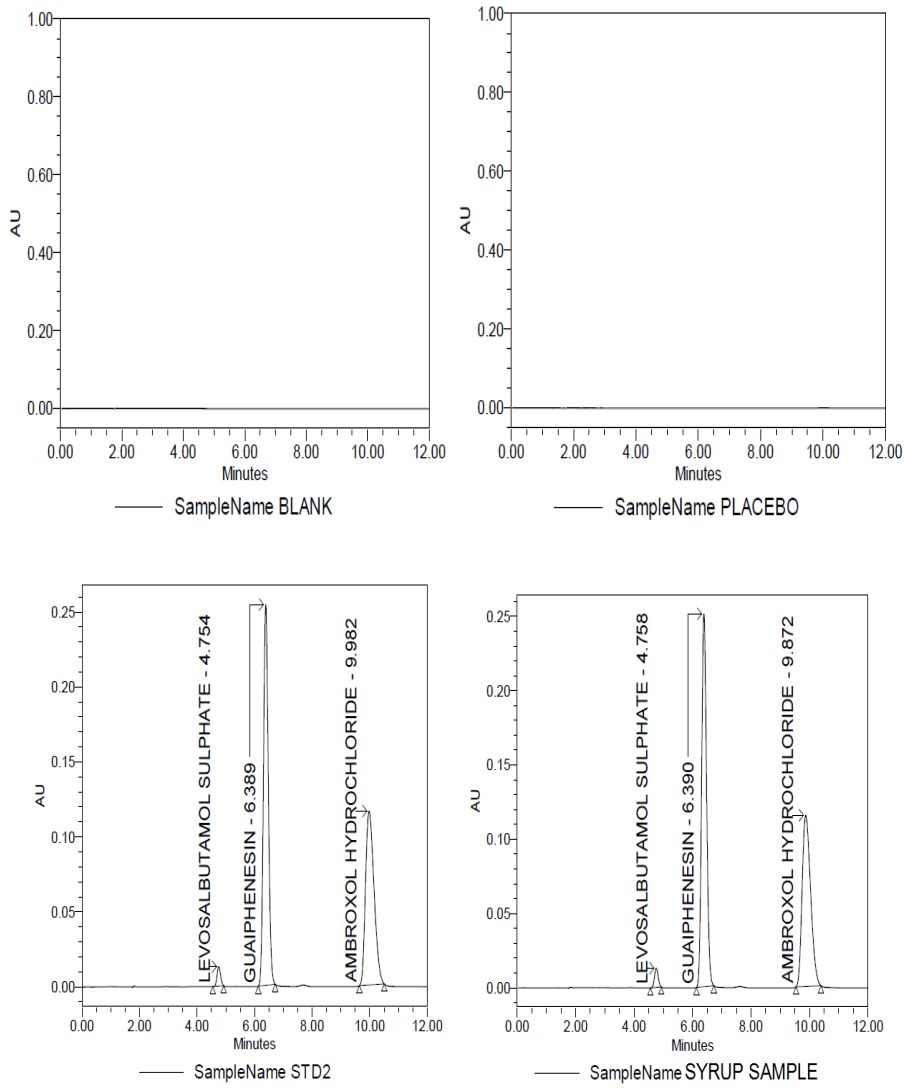


Figure 5: Chromatograms of mobile phase, placebo, standard and syrup sample

Linearity:

Linearity of levosalbutamol, ambroxol and guaiphenesin were checked by analyzing five concentrations by the proposed method. Linearity of the calibration curves plotted was validated by the high value of the regression coefficients and good values of slope and intercept. The linearity was in the range of 0.5-1.5 µg/ml, 50-150 µg/ml and 15-45 µg/ml for levosalbutamol, guaiphenesin and ambroxol, respectively.

Table 3: Linearity data of levosalbutamol, ambroxol and guaiphenesin

Lev osalbutamol Concentration (µg/ml)	Levosalbutamol peak area	Ambroxol Concentration (µg/ml)	Ambroxol peak area	Guaiphenesin Concentration (µg/ml)	Guaiphenesin peak area
0.5	55979	15	1189181	50	1464166
0.75	83990	22.5	1777164	75	2208833
1.0	111988	30	2363344	100	2933956
1.25	139511	37.5	2961412	125	3674788
1.5	167785	45	3557384	150	4406521

Table 4: Regression equation of levosalbutamol, ambroxol and guaiphenesin

Drug name	Y = mx + C	Regression coefficient
Levosalbutamol	y = 11176x + 70.5	0.9999
Ambroxol	y = 78969x + 512.6	0.9991
Guaiphenesin	y = 29388x - 933.2	0.9994

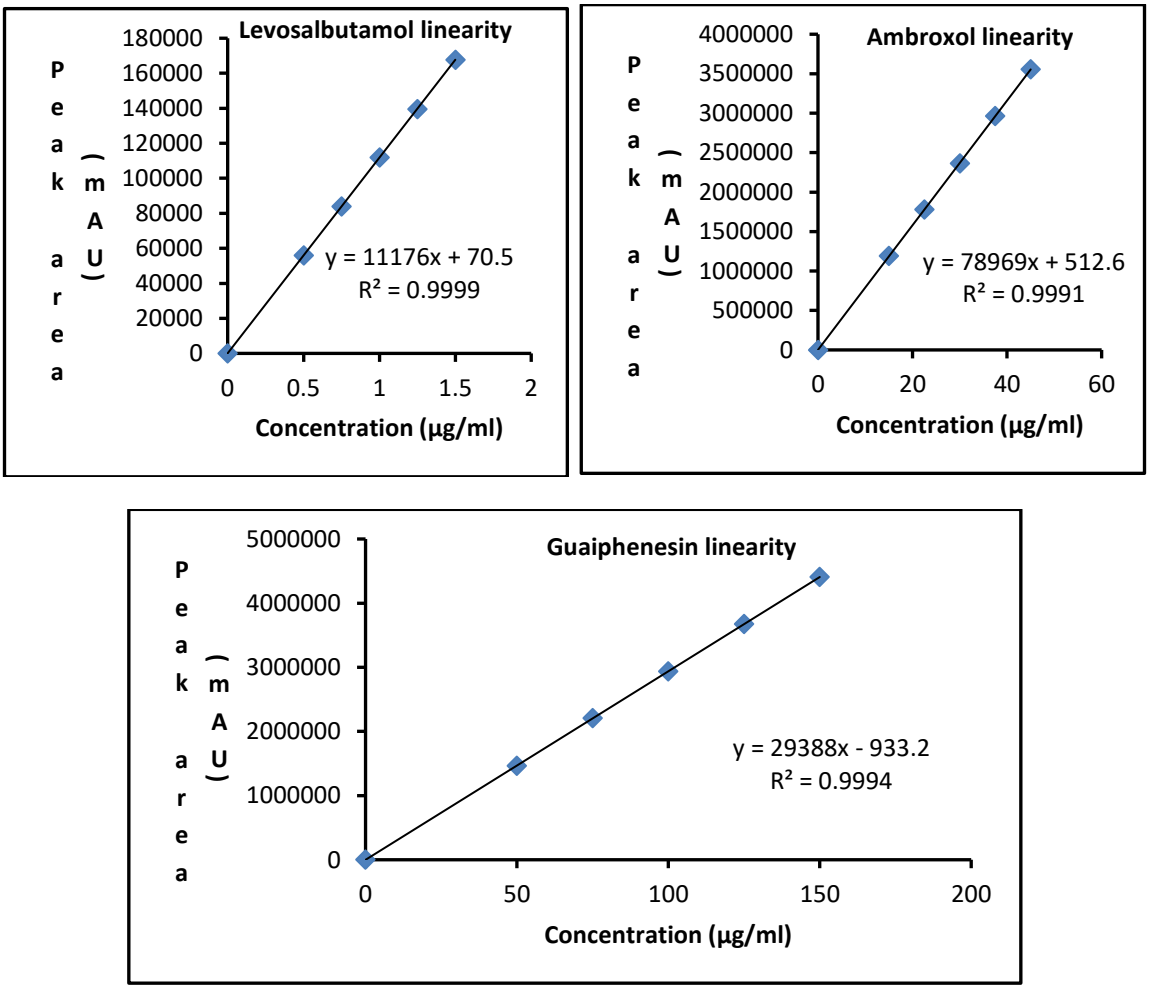
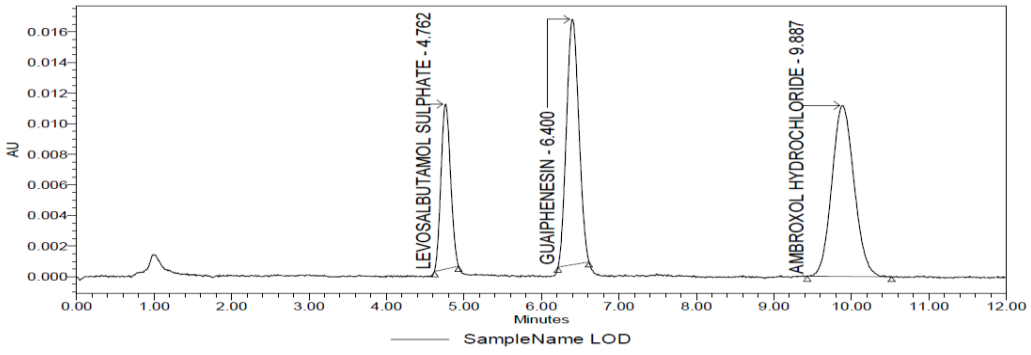


Figure 6: Linearity curves of levosalbutamol, ambroxolhydrochloride and guaiphenesin

Limit of detection and limit of quantification:
Signal-to-noise level of 3 was determined for LOD (levosalbutamol – 0.022 µg/ml, guaiphenesin – 0.1115 µg/ml and ambroxol – 0.072 µg/ml) and Signal-to-noise level of 10 was determined for LOQ (levosalbutamol – 0.072 µg/ml, guaiphenesin – 0.3716 µg/ml and ambroxol – 0.239 µg/ml).



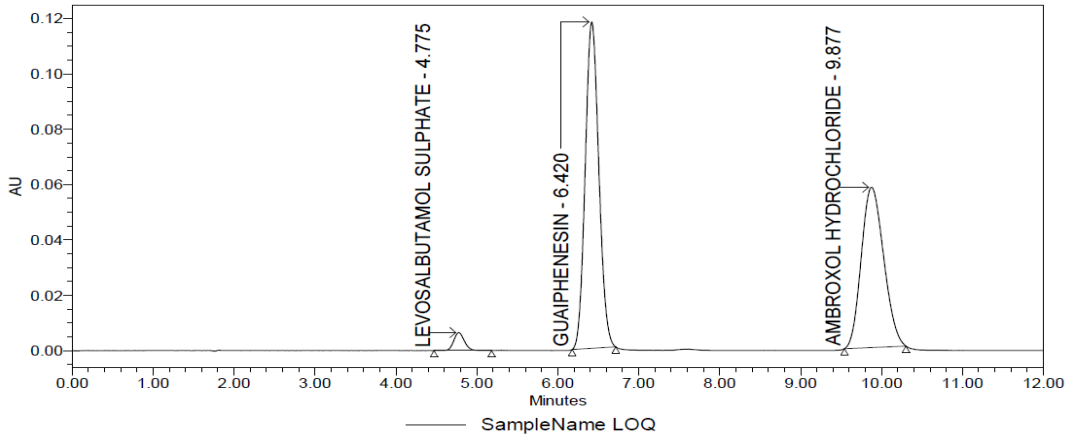


Figure 7: Chromatograms of levosalbutamol, guaiphenesin andambroxol hydrochloride at LOD and LOQ concentration

Precision:

It was monitored by repeated analysis of levosalbutamol (1 µg/ml), ambroxol (30 µg/ml) and guaiphenesin (100 µg/ml) standard 6 times and then results were disclosed as %RSD of peak areas. The test results were proved by relatively low RSD values calculated from peak areas.

Table 5: Precision test results

Levosalbutamol peak area	Sta tistical study	Ambroxol peak area	Statistical study	Guaiphenesin peak area	Statistical study
111888	Mean:	2936365	Mean:	2367713	Mean:
111110	111509	2938136	2936548	2361782	2364322
111040	SD:	2932788	SD:	2365863	SD:
111929	4.2.648	2934916	2409.285	2365961	2600.31
111310	RSD:	2939468	RSD:	2363461	RSD:
111777	0.361	2937616	0.082	2361151	0.110

SD – standard deviation; RSD - relative standard deviation

Accuracy:

The method's accuracy was used to be examined by way of determination of levosalbutamol, ambroxol and guaiphenesin recovery at three levels of quantity. Preanalyzed syrup samples have been spiked with extra levosalbutamol, ambroxol and guaiphenesin (50, 100, and 150%). The mixtures had been reanalysed. The accuracy test results were proved by good recovery of levosalbutamol, ambroxol and guaiphenesin at studied levels.

Table 6: Recovery results of levosalbutamol

Level (%)	Amount of levosalbutamol spiked (µg/ml)	Amount of levosalbutamol recovered (µg/ml)	Recovered percent levosalbutamol (%)	Mean recovered levosalbutamol (%)
50	0.495	0.498	100.64	
50	0.495	0.498	100.59	100.62
50	0.495	0.498	100.63	
100	0.990	0.991	100.11	
100	0.990	0.997	100.67	100.30
100	0.990	0.991	100.13	
150	1.485	1.487	100.15	
150	1.485	1.495	100.69	100.34
150	1.485	1.488	100.17	

Table 7: Recovery results of ambroxol

Level (%)	Amount of ambroxol spiked (µg/ml)	Amount of ambroxol recovered (µg/ml)	Recovered percent ambroxol (%)	Mean recovered ambroxol (%)
50	50	49.74	99.49	
50	50	49.83	99.66	99.49
50	50	49.66	99.32	
100	100	99.57	99.57	
100	100	99.77	99.77	99.64
100	100	99.58	99.58	
150	150	149.45	99.63	
150	150	149.52	99.68	99.64
150	150	149.40	99.60	

Table 8: Recovery results of guaiphenesin

Level (%)	Amount of guaiphenesin spiked (µg/ml)	Amount of guaiphenesin recovered (µg/ml)	Recovered percent guaiphenesin (%)	Mean recovered guaiphenesin (%)
50	15	15.00	100.00	
50	15	15.02	100.17	100.17
50	15	15.05	100.34	
100	30	29.91	99.70	
100	30	29.88	99.59	99.73
100	30	29.97	99.90	
150	45	44.96	99.91	
150	45	45.00	100.00	99.97
150	45	45.00	100.01	

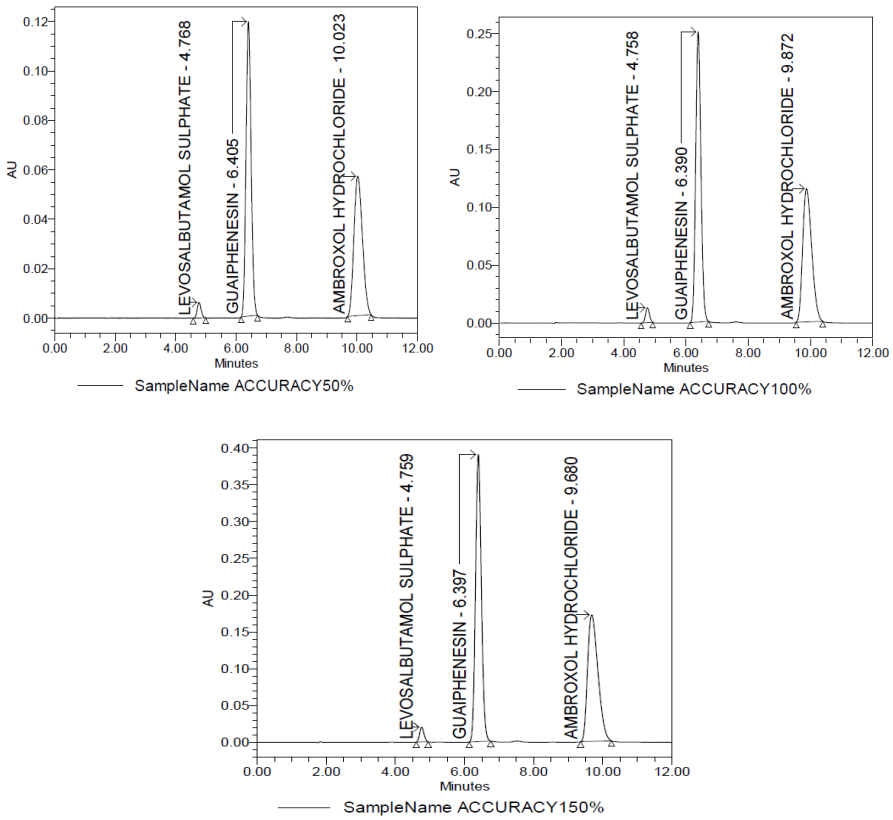


Figure 8: Chromatograms of levosalbutamol, guaiphenesin and ambroxol hydrochloride in accuracy test

Robustness:
The method's robustness was analyzed with standard levosalbutamol (1 µg/ml), ambroxol (30 µg/ml) and guaiphenesin (100 µg/ml) by introducing relatively minor deliberate adjustments in the ratio of mobile phase composition, flow rate, temperature and pH. The robustness test results were proved by insignificant changes on tailing, peak area, resolution and plate count of levosalbutamol, ambroxol and guaiphenesin peaks at studied changes.

Table 9: Robustness of levosalbutamol, guaiphenesin, ambroxol

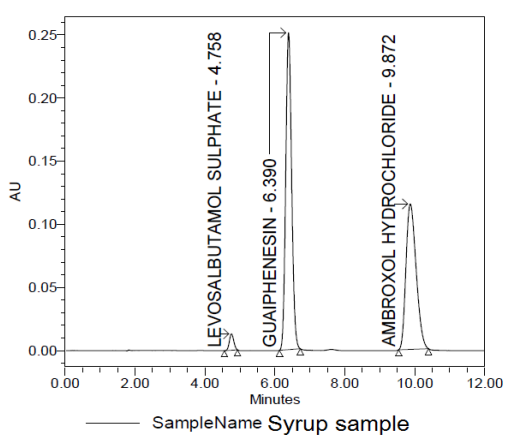
Parameter	Tested values for levosalbutamol, guaiphenesin, ambroxol
Flow rate(ml/min)	0.9
	1.1
Temperature in column(°C)	23
	27
pH in mobile phase	4.8
	5.2
Acetonitrile ratio(%)	35
	45

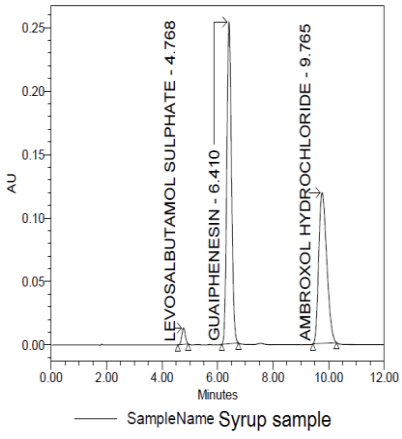
Assay in syrup formulation:
The method's validity was tested by assaying levosalbutamol, ambroxol and guaiphenesin simultaneously in syrup formulation. The outcomes proved the accuracy, precision and non-interference from excipients.

Table 10: Levosalbutamol, ambroxol and guaiphenesin assay in syrup

Drug claimed (mg/5ml) Levosalbutamol	Determined content (mg)	Statistical study
0.5	0.503	Mean: 0.502 mg
0.5	0.502	SD: 0.000872
0.5	0.502	RSD: 0.174
Guaiphenesin		
50	49.745	Mean: 49.80 mg
50	49.820	SD: 0.048218
50	49.835	RSD: 0.097
Ambroxol		
15	15.026	Mean: 14.994 mg
15	14.960	SD: 0.033045
15	14.996	RSD: 0.220

SD – standard deviation; RSD – relative standard deviation





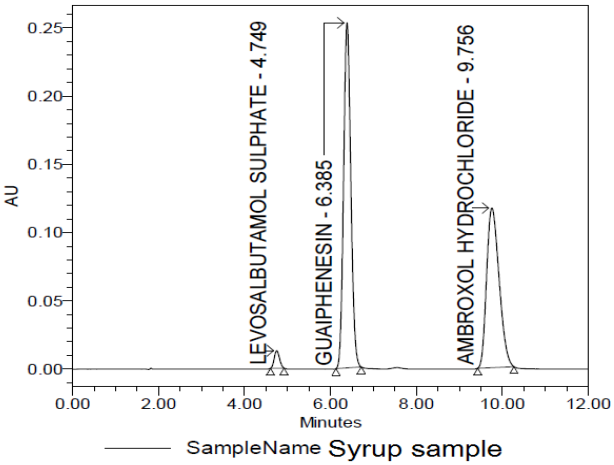


Figure 9: Chromatograms of levosalbutamol, guaiphenesin and ambroxolin syrup assay test

CONCLUSION

A simple, rapid, selective, precise, robust and accurate RP-HPLC technique was developed, which allowed quantitative evaluation of levo salbutamol, ambroxol and guaiphenesin in combination syrup formulation. Mobile phase developed for this method effectively resolved levo salbutamol, ambroxol and guaiphenesin. The validity data will be helpful in the correct quantification of levosalbutamol, ambroxol and guaiphenesin combination. This method is useful for routine analysis of levo salbutamol, ambroxol and guaiphenesin combination and quality control of levo salbutamol, ambroxol and guaiphenesin combination syrup formulation along with its excipients.

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