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INNOVATIVE TRIAD: RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF LEVOSALBUTAMOL SULPHATE, AMBROXOL HYDROCHLORIDE AND GUAIPHENESIN IN ORAL LIQUID DOSAGE FORM

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ABSTRACT

Levosalbutamol Sulphate, Ambroxol Hydrochloride, and Guaiphenesin together provide a comprehensive approach to the treatment of different respiratory disorders, symptom relief, and improved respiratory health. RP-HPLC has become a highly effective method with great resolution, sensitivity, and repeatability for analyzing complex mixtures. This paper describes the development and estimation of the RP-HPLC approach for the concurrent quantification of guaiphenesin, ambroxol hydrochloride, and levosalbutamol sulphate in bulk and medicinal dose form. The chromatographic separation was performed with HPLC agilent 1100 series with DAD detector, EZ Chromelite software was used for the chromatogram identification. The stationary phase used was C-18 inertsil column of dimension 250 × 4.6 mm with particle sizes of 5µ. The mobile phase used was buffer, acetonitrile and methanol in the ratio of (65:10:25). The developed method produced chromatogram with perfect sharp peaks, and with good resolution and minimal tailing. The retention time of Levosalbutamol sulphate, guaiphenesin and ambroxol hydrochloride was found to be 2.853, 8.273 and 12.273 respectively. The method was linear with the correlation coefficient value of 0.9999. The system suitability parameters were found to be within the acceptable criteria. In summary, we have developed a robust analytical approach that can reliably and accurately quantify these active components through rigorous method development and validation procedures. The findings of the technique validation, carried out in compliance with ICH principles, have shown that the approach is suitable for routine quality control analysis and that the results fall within acceptable bounds.

Keywords: RP-HPLC, Levosalbutamol Sulphate, Ambroxol Hydrochloride, Guaiphenesin, Methanol, Acetonitrile, ICH Guidelines

1. INTRODUCTION

Levosalbutamol Sulphate is an agonist of the β2-adrenergic receptor that has bronchodilatory properties that help with bronchoconstriction and breathing in diseases including asthma and chronic bronchitis (Prabha Thangavelu *et al.,* 2019). The mucolytic drug ambroxol hydrochloride helps to remove respiratory secretions, which lessens the symptoms of productive cough. As an expectorant, guaiphenesin facilitates mucus discharge from the respiratory tract, relieves symptoms associated with respiratory conditions. A powerful therapeutic alternative for the treatment of a number of respiratory disorders, such as cough, bronchitis, and asthma, is the pharmacological combination of levosalbutamol sulphate, ambroxol hydrochloride, and guaiphenesin (Arkoti Chaitanya *et al.,* 2019).

Levalbuterol, also known as levosalbutamol, is a β2 adrenergic receptor agonist which is used in the treatment of asthma and other respiratory conditions (Nirav Patel C *et al.,* 2013). Its chemical formula is $C_{13}H_{21}NO_3$, and its weight is 239.311 g/mol. Its IUPAC name is 4-[(1R)-2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl) phenol; sulfuric acid. Levosalbutamol belongs to the category of bronchodilators. Levosalbutamol has drug interactions with other short-acting sympathomimetic bronchodilators or epinephrine (Ahmad Kantar *et al.,* 2020).

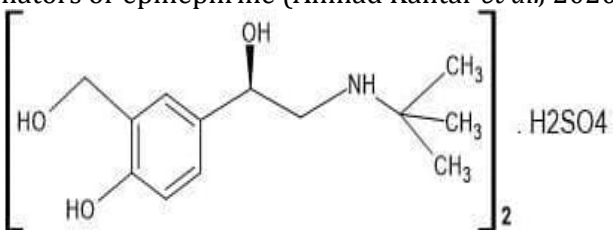

Fig. 1: Chemical structure of Levosalbutamol sulphate

Guaiphenesin is used to treat respiratory conditions, coughing and congestion caused on by regular colds, and bronchitis (Gagandeep *et al.,* 2012). The Chemical formula and weight of Guaiphenesin were $C_{10}H_{14}O_4$ and 198.2 g/mol respectively. Its IUPAC name is 3-(2-methoxyphenoxy) propane-1,2-diol. Guaiphenesin belongs to the category of Expectorants. While administering Guaiphenesin the rate of absorption of paracetamol may increase which causes drug interactions (Helmut Albrecht H *et al.,* 2017).

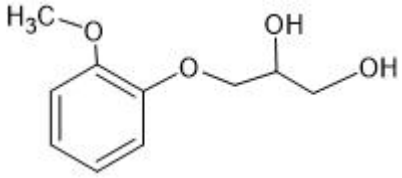

Fig. 2: Chemical structure of Guaiphenesin

Ambroxol hydrochloride allows patients to breathe deeply by assisting with mucus expulsion, facilitating expectoration, and reducing active coughing **(**M. Sumithra *et al.,* 2016). Its IUPAC name is 4- [(2-amino-3,5- dibromobenzyl) amino] cyclohexanol Hydrochloride. Its molecular formula is $C_{13}H_{19}Br_2ClN_2O$ and its molecular weight is 414.566 g/mol. Ambroxol hydrochloride belongs to the category of mucolytic agent. The possible drug interactions after taking ambroxol includes, an increase in the amounts of antibiotics such as erythromycin, cefuroxime, and amoxycillin in sputum and bronchial secretions **(**Rakesh Kumar *et al.,* 2020**).**

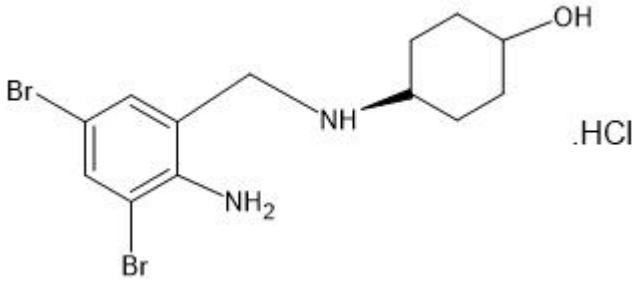

Fig. 3: Chemical structure of Ambroxol Hydrochloride

In pharmaceutical analysis, evaluating the potency and quality of combination therapies requires the simultaneous assessment of numerous medicines in a single analytical approach. Because it can separate compounds according to how hydrophobic they are, HPLC in reverse phase is a flexible and commonly used technique for such multi-drug analyses. This makes it especially useful for the simultaneous estimation of drugs with a variety of physicochemical properties (Teja Kumar Reddy Konatham *et al.,* 2019).

The simultaneous determination of guaiphenesin, ambroxol hydrochloride, and levosalbutamol sulphate presents analytical difficulties because of variations in their spectrum interferences and physicochemical characteristics (Padmavathi Prabhu P *et al.,* 2018). Consequently, there's a rise in demand for analytical techniques that is able to quantify those chemicals in pharmaceutical formulations simultaneously. With its excellent resolution, sensitivity, and repeatability, RP-HPLC has become a potent method for the examination of complicated mixtures. To provide precise quantification, a strong analytical technique for the simultaneous determination of these active components must be developed and validated (Itagimatha N and Manjunatha DH, 2019).

However, there is only one method available for the simultaneous quantification of levosalbutamol sulphate, ambroxol hydrochloride, and guaiphenesin in combination, efforts are made to develop a method with cost efficient and simple for routine quality control analysis.

2. MATERIAL AND METHODS:

MATERIALS AND TECHNIQUES:

The separation achieved by chromatography was performed with HPLC agilent 1100 series with DAD detector. The stationary phase used was C-18 inertsil column of dimension 250 × 4.6 mm with particle size of 5µ. The mobile phase used was buffer, acetonitrile and methanol in the ratio of (65:10:25). A Syrup containing 50 mg of Guaiphenesin, 30 mg of ambroxol hydrochloride and 1 mg of levosalbutamol sulphate in each 5ml of syrup was procured from local market which is used as formulation.

CHEMICALS AND REAGENTS:

Sodium dihydrogen orthophosphate, Acetonitrile, methanol, triethylamine, orthophosphoric acid, and Distilled Water.

PREPARATION OF BUFFER SOLUTION:

1.56 grams of sodium dihydrogen orthophosphate was taken in a 1000ml beaker and dissolved into 1000ml pf distilled water. To this add 3ml of triethylamine and in order to lower the pH to approximately 3.0, orthophosphoric acid was used.

PREPARATION OF MOBILE PHASE:

The mobile phase was prepared by mixing the phosphate buffer, acetonitrile and methanol in the ratio of 65:10:25 and the mixture was degassed in ultrasonicator for 10 min. The mixture was filtered through 0.45µ nylon filter under vacuum filtration.

CHROMATOGRAPHIC CONDITIONS:

Table. 1: Optimized chromatographic conditions

Mobile phase	Phosphate buffer:Acetonitrile:Methanol (65:10:25)
Column	C-18
Injection volume	10µL
Flow rate	1.0 ml/min
Ph	3.0 adjusted with orthophosphoric acid
Run time	20 min
Detection wavelength	276 nm

PREPARATION OF STANDARD SOLUTION:

Weigh 50 mg of Levosalbutamol sulphate and transfer into 100 ml volumetric flask and disperse with mobile phase of about 25 ml and the volume was made up along with mixture of mobile phase. Take 2ml from this solution and mix it with accurately weighed 50 mg of Guaiphenesin, 30 mg Ambroxol hydrochloride and transfer it into 100 ml volumetric flask, dissolve it and makeup with mobile phase. Filter, it using 0.45µ filter and 10 µL of the resultant solution was injected for HPLC analysis.

PREPARATION OF SAMPLE SOLUTION:

Accurately weigh, 5.8015g amount equivalence of syrup formulation which contains 50 mg of Guaiphenesin, 30 mg of Ambroxol hydrochloride and 1 mg of Levosalbutamol was transferred it into a 100 ml volumetric flask and dissolve it with 25 ml of mobile phase. The solution was kept under sonicator for complete dissolving of drugs for 15 min. then the solution was filled upto the mark with mixture of mobile phase. Filter the solution using 0.45µ filter and 10 µL of the resultant solution was injected for HPLC analysis.

3. RESULTS AND DISCUSSION

METHOD VALIDATION:

According to ICH criteria, the technique developed has been validated for the following parameters such as., System suitability, specificity, accuracy, precision, linearity, limit of detection and quantification, robustness.

SYSTEM SUITABILITY:

A standard chromatogram was used to assess the system appropriateness parameters, such as resolution, retention duration, plate number (N), and peak asymmetry factor (Tailing), and the results indicated.

Table.2: System suitability results

Parameters	Levosalbutamol	Guaiphenesin	Ambroxol hydrochloride	Acceptance criteria
Retention time	2.853 min	8.273 min	12.273 min	NA
Resolution	NA	26.57	9.86	NLT 2
No. of. Theoretical plates	9696	12851	8960	NLT 2000
Tailing factor	1.1	0.9	1.1	0.8-1.8

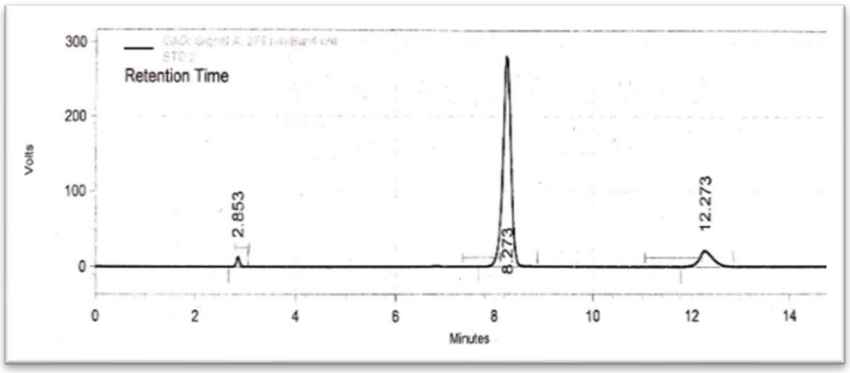

Fig. 4: Chromatogram for Levosalbutamol sulphate, Guaiphenesin, Ambroxol hydrochloride

SPECIFICITY

The specificity was assessed by comparing the retention times in standard solution of levosalbutamol, ambroxol, and guaiphenesin with the sample solution, blank and placebo.

Levosalbutamol, ambroxol, and guaiphenesin retention time have not shown any interference in the mobile phase, blank or placebo chromatograms. This ensures accurate identification and quantification of each compound. The results showed that the developed method is specific.

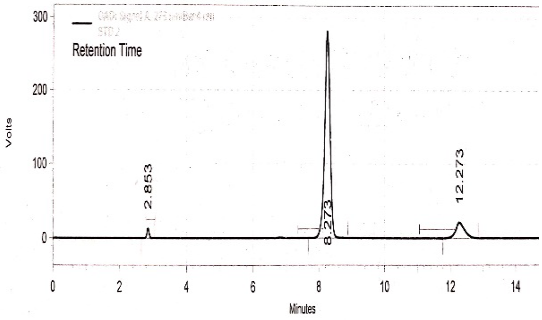

Fig.5: Standard chromatogram

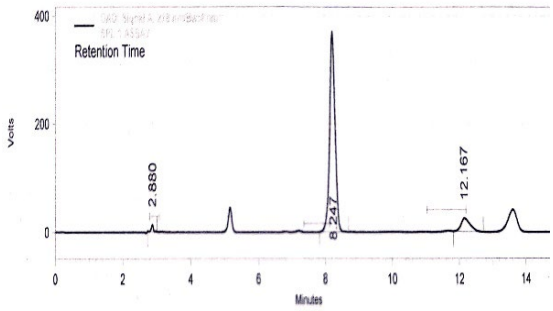

Fig.6: Sample chromatogram

LINEARITY

Levosalbutamol sulphate, Ambroxol hydrochloride and Guaiphenesin showed the linearity in the range of 5-15, 150-450, 250-750 (µg/mL) respectively. The calibration graphs were plotted by taking X-axis as concentration of standard solution and the Y-axis as peak area.

Table 3: Linearity of Levosalbutamol sulphate, Ambroxol hydrochloride and Guaiphenesin

Levosalbutamol sulphate		Guaiphenesin		Ambroxol hydrochloride	
Conc of std. soln (µg/ml)	Peak area	Conc of std. soln (µg/ml)	Peak area	Conc of std. soln (µg/ml)	Peak area
5	6880	250	326235	150	41150
7.5	9380	375	486235	225	62127
10	11880	500	646235	300	82627
12.5	14380	625	806235	375	103127
15	17000	750	960235	450	123627

Coefficient of correlation (R^2)	0.999955	Coefficient of correlation (R^2)	0.999971	Coefficient of correlation (R^2)	0.999989

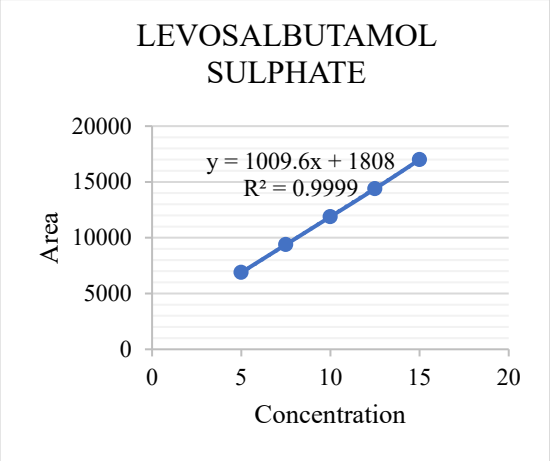

Fig.7: Calibration graph of Levosalbutamol sulphate

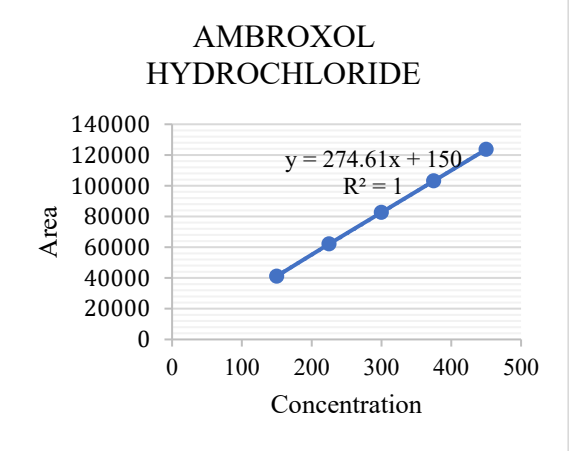

Fig.8: Calibration graph of ambroxol hydrochloride

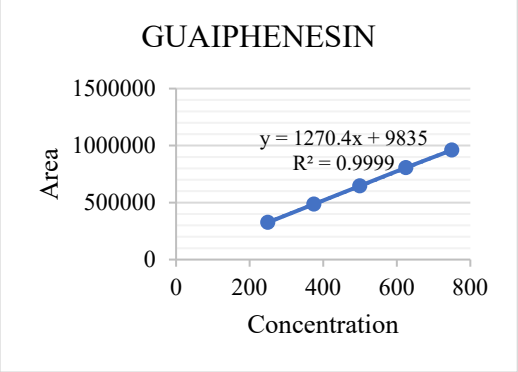

Fig.9: Calibration graph of Guaiphenesin

ACCURACY

The recovery research was conducted and the contents were assessed, using the appropriate chromatogram. The 50%, 100% and 150% concentration levels were used in the recovery tests to assess accuracy.

Table 4: Accuracy data

Accuracy	Levo Sal Added	Levo Sal Found	% Recovery	Mean recovery	SD	% RSD	Guai Added	Guai found	% Recover	Mean recovery	SD	% RSD	Amb. Hyd Added	Amb. Hyd Found	% recovery	Mean Recovery	SD	% RSD

50%	5.03	4.99	100.8	100.9	0.6	0.6	249.96	249.63	100.13	100.01	0.12	0.12	149.98	149.47	100.34	100.35	0.21	0.2
		4.95	101.6					250.23	99.89					149.11	100.57			
		5.01	100.3					249.81	100.06					149.77	100.14			
100%	9.97	9.93	100.4	100.4	0.2	0.19	500.09	499.13	100.19	100.06	0.11	0.1	300.13	299.77	100.12	100.06	0.08	0.08
		9.91	100.6					500.13	99.99					299.78	100.11			
		9.95	100.2					500.03	100.01					300.25	99.96			
150%	15.13	15.03	100.6	100.1	0.3	0.3	749.99	749.42	100.07	100.05	0.11	0.1	449.93	449.13	100.17	100.1	0.06	0.06
		15.11	100.1					750.43	99.94					449.73	100.04			
		15.15	99.86					748.73	100.16					449.52	100.09			

4. PRECISION

SYSTEM PRECISION

The system precision is achieved, when injecting solution of standard preparation into the analytical column six times, measuring the peak area, and then calculating the area's percentage relative standard deviation.

REPEATABILITY

The method precision or the repeatability was achieved by doing an experiment on six replicates to determine sample preparation in the test concentration level, and the % RSD was estimated.

Ruggedness:

The "intermediate precision" or the "Ruggedness" has been done with different analyst in a same day by using different HPLC system, with same column type and dimensions.

Table 5: Results for precision data

S.NO	SYSTEM PRECISION			METHOD PRECISION			INTERMEDIATE PRECISION		
	LEVOSAL (Area)	GUAI (Area)	AMB. HYD (Area)	LEVOSAL % assay	GUAI % assay	AMB. HYD % assay	LEVOSAL % assay	GUAI % assay	AMB. HYD % assay
1	11850	646235	82473	100.95	100.08	100.01	100.94	100.01	100.1533
2	11770	646553	82553	100.77	100.08	100.12	100.31	100.03	100.1989
3	11880	646353	82627	100.86	100.02	100.19	100.32	100.01	100.0632
4	11893	646666	82373	100.94	100.11	100.17	101.14	100.07	100.1165
5	11823	646290	82773	100.39	100.01	100.19	100.92	100.03	100.1245
6	11803	646170	82235	100.68	100.03	100.12	100.33	100.11	100.1103
Mean	11836.5	646377.8	82505.67	100.76	100.05	100.16	101.83	100.04	100.1278
SD	46.8903	192.6919	189.9249	0.208747	0.04135	0.036515	0.4965	0.03823	0.045436
%RSD	0.39	0.02	0.23	0.20	0.04	0.03	0.49	0.03	0.04

ROBUSTNESS

A method's robustness is assessed by adjusting its parameters, such as flow rate, wavelength, etc., and seeing any impact on the method's output.

Table 6: Results for robustness for the change of wavelength

NAME	WAVE LENGTH	Ret. Time (min)	Tailing	Plate count	Resolution	FLOW RATE	Ret. Time (min)	Tailing	Plate count	Resolution
LEVOSAL	274 nm	2.880	1.0	12365	NA	0.8 ml	3.580	1.0	12715	NA
	276 nm	2.859	1.1	9696	NA	1.0 ml	2.859	1.1	9696	NA
	278 nm	2.873	1.0	10686	NA	1.2 ml	2.407	1.2	8905	NA
GUAI	274 nm	7.987	0.8	13172	26.20	0.8 ml	10.200	0.9	14867	28.68

	276 nm	8.273	0.9	12851	26.57	1.0 ml	8.273	0.9	12851	26.57
	278 nm	7.947	0.9	13159	26.13	1.2 ml	6.693	0.9	11154	24.11
AMB. HYD	274 nm	11.773	1.4	11290	10.49	0.8 ml	14.987	1.6	9679	10.14
	276 nm	12.273	1.1	8960	9.86	1.0 ml	12.273	1.1	8960	9.86
	278 nm	11.707	1.2	9856	10.04	1.2 ml	9.953	1.2	8080	9.36

5. CONCLUSION

In conclusion, developing and determining an analytical technique to simultaneously measure the concentrations of levosalbutamol sulphate, ambroxol hydrochloride, and guaiphenesin is an essential step in ensuring the safety, efficacy, and quality of drug formulations used to treat respiratory conditions. The developed method produced chromatogram with perfect sharp peaks, and with good resolution and minimal tailing. The retention time of Levosalbutamol sulphate, guaiphenesin and ambroxol hydrochloride was found to be 2.853, 8.273 and 12.273 respectively. The method was linear with the correlation coefficient value of R^2=0.9999 for each drugs respectively. The system suitability parameters were found to be within the acceptable criteria.

We have effectively developed a robust analytical tool that can accurately and reliably quantify these active components. The created technique's specificity, linearity, accuracy, precision and robustness have all been confirmed to meet the standards by carrying out the method validation studies, which were carried out in compliance with ICH recommendations. This confirms the method's suitability for regular quality control analysis.

CONFLICT OF INTEREST

The authors declare no conflicts of interest relevant to this article.

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