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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF AMBROXOL HYDROCHLORIDE AND LEVOCETIRIZINE IN TABLET DOSAGE FORM

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ABSTRACT

RP-HPLC method was developed and validated as per ICH guidelines for the estimation of Ambroxol HCl and Levocetirizine. Simultaneous Estimation of Ambroxol HCl and Levocetirizine.2HCl were carried out by RP- HPLC using sodium phosphate buffer (P^H 3.0): Methanol (30:70) and column Phenomenex Luna C-18 (250x4.6 mm, 5um) as a stationary phase and peak was observed at 230 nm which was selected as a wavelength for quantitative estimation. After the development of the method, it was validated for specificity, linearity, precision, accuracy, robustness and ruggedness studies. The system suitability parameter also reveals that the values within the specified limit for the proposed method. Theoretical plate for Ambroxol HCl was found to be 5232, for Levocetirizine it was found to be 6590. The precision of the System and Method were checked and found to be within limits. This indicates that the method is precise. From the linearity studies, the specified range for Ambroxol HCl and Levocetirizine.2HCl was found to be 20% to 120%. It was evaluated by the visual inspection of the plot of Peak area vs. Concentration. Validation revealed the method is specific, rapid, precise, reliable, and reproducible.

Keywords: Method Development, Simultaneous Estimation, Validation, Ambroxol, Levocetirizine, Solid Dosage Form.

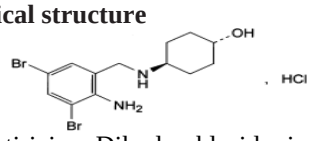
INTRODUCTION

Ambroxol HCl is mucolytic agent and expectorant. Sparingly soluble in water, soluble in methanol. Ambroxol is an active N-desmethyl metabolite of the mucolytic, bromhexine. Ambroxol is shown to exert several activities, as follows: Its mucolytic activity by which it facilitates breakdown of acid mucopolysaccharide fibres in the mucous making it thinner and less viscous and, therefore, easy for expectoration; it stimulates the ciliary activity thereby improving mucokinesis (transport of mucous); it stimulates production of pulmonary surfactant, a substance found to play a major role in the lung host defence mechanism, thereby further protecting against lung inflammation and infection; also exhibits anti-inflammatory and antioxidant activity. When administered orally, onset of action occurs after about 30 minutes. Ambroxol is rapidly absorbed (70-80%) after oral administration. The time to reach peak plasma concentration is approximately 2 hours. The distribution half-life of Ambroxol is around 1.3 hours [1].

Chemical name

trans-4-[(2-Amino-3,5-dibromobenzyl) amino]cyclohexanol hydrochloride.

Chemical structure



Levocetirizine Dihydrochloride is H_1-histamine receptor antagonist. Soluble in water and methanol. Levocetirizine, the (R) enantiomer of cetirizine, is a potent and selective antagonist of peripheral H_1-receptors.

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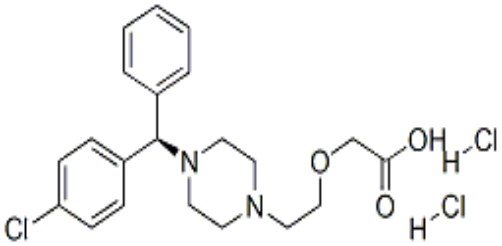
Binding studies revealed that levocetirizine has high affinity for human H_1 -receptors. Levocetirizine is rapidly and extensively absorbed following oral administration. Peak plasma concentrations are achieved 0.9 hours after dosing. Steady state is +achieved after 2 days. Peak concentrations are, typically, 270ng/ml and 308ng/ml following a single and a repeated 5 mg O.D. dose, respectively. The extent of absorption is dose independent and is not altered by food, but the peak concentration is reduced and delayed. No tissue distribution data are available in humans. Levocetirizine is 90% bound to plasma proteins. The distribution of levocetirizine is restrictive, as the volume of distribution is 0.4 l/kg [2].

Chemical name

(2-(4-[(R)-(4-Chlorophenyl)(phenyl)methyl] piperazin-1-yl)ethoxy)aceticacid dihydrochloride

Chemical structure



MATERIALS AND METHODS [3-5]

Reference Standards

1. Ambroxol.HCl % purity – 99.53%

2. Levocetirizine.2HCl % purity –99.41%

Preparation of solvents

Preparation of sodium phosphate buffer

15.61 gm of Sodium Dihydrogen phosphate is dissolved in 1000 ml of water and final concentration made up to 0.1 M. then it was adjusted to pH 3.0 with Ortho phosphoric acid .

Tablet Brand Used: BOROX-L

Label claim:

Ambroxol.HCl: 50mg

Levocetirizine.2HCl: 5mg

Optimized Chromatographic Conditions

Stationary phase: Phenomenex Luna C_{18} (250 × 4.6 mm, 5µm)

Mobile phase: Methanol : 0.1M Sodium Phosphate buffer (70: 30 % v/v)

P^H: 3

Flow rate: 1 ml/min

Column Temperature: Room temperature

Volume of Injection loop: 20µl

Detection of Wavelength: 230 nm

Run time: 8 min

Mode of Operation: Isocratic

Preparation of Solutions

Preparation of 0.1 M Sodium hydrogen phosphate buffer solution

15.61 gm of Sodium Dihydrogen phosphate is dissolved in 1000 ml of water and final concentration made up to 0.1 M. then it was adjusted to pH 3.0 with Ortho phosphoric acid

Preparation of Standard Stock Solution

Accurately weighed quantity of 50mg Ambroxol.HCl and 5mg Levocetirizine.2HCl were transferred in to 50 ml volumetric flask, dissolved in 20ml mobile phase and sonicated for 15 min and volume was made up with mobile phase.

Diluted Standard Solution

From the standard stock preparation 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1.0ml and 1.2ml were drawn by pipette and made up to 10 ml with mobile phase and this solution is used for further purpose.

Preparation of Sample Solution

For estimating the tablet dosage form, 20 tablets from each batch were randomly selected and powdered, crush the tablets. From the powdered tablets, weigh accurately 203.4mg of powdered tablets (equivalent to 50mg Ambroxol.HCl and 5mg of Levocetirizine.2HCl) transfer it in 50 ml of volumetric flask 15ml of mobile phase was added and make up to 50ml with mobile phase. The mixture was subjected to sonication for 10 min with intermediate shaking for complete extraction of drugs. Cool to room temperature and shake well and filter the solution. Take 1 ml solution and dilute it to 10 ml with mobile phase. The sample was centrifuged in tight enclosure for 10 min at 3000 RPM.

METHOD VALIDATION [6-11]

After the method development, the method was validated in terms of parameters like accuracy, precision, linearity and range, robustness, stability etc.

System suitability studies

Ambroxol HCl and Levocetirizine standard solution as per test method were prepared make five replicate injections were given Then evaluated the system suitability parameters like plate number (N),Peak asymmetry factor (As),were studied with the help of standard chromatogram.

Linearity and Range

Ambroxol.HCl showed linearity in the range of 20-120µg/ml and Levocetirizine.2HCl showed linearity in the range of 2-12µg/ml. The calibration graph was plotted with peak area in the Y axis and concentration of standard solution in the X axis. The degree of linearity was estimated by calculating the correlation coefficient. Y-

Intercept, slope of the regression line. The slope, intercept and correlation coefficient values for Ambroxol.HCl were found to be 17.13, 24.35 and 0.998 respectively. The slope, intercept and correlation coefficient values for Levocetrizine.2HCl were found to be 20.05, 0.347 and 0.999 respectively.

Accuracy:

Accuracy expresses the closeness of agreement between the value, which is accepted either as conventional true value or and accepted reference value (International Standard e.g. pharmacopoeial standard) and the value found (mean value) obtained by applying the test procedure a number of times. To study reliability, suitability and accuracy of the method, recovery studies were carried out, by adding a known quantity of the standard to the pre analyzed sample and recovery study was done. The recovery was carried out at 80%, 100% and 120% level and the contents were determined from the respective chromatogram. From the results obtained we can conclude that the method was accurate.

Precision

Repeatability of Injection

The precision of test method was done by performing assay on six replicate determination of sample preparation at test concentrations level (as per method of analysis) and calculated relative standard deviation of assay results. Six 20ml injection from a standard solution were injected on to the analytical column and the peak area data obtained and %RSD was calculated. And System Precision and Method Precision were calculated.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, etc. To perform the specificity parameter, stressed samples (sample heated to 60^0 C for 2 h, sample treated with 1N HCl for 2h, and sample treated with 1N NaOH for 2 h) and working standard were injected separately. The analyte did not have any interference with the degraded components and was well resolved from them. The retention time of the degraded products peak was different from that of the analyte peak.

Robustness

For demonstrating the robustness of the developed method experimental conditions were purposely altered and evaluated. The method must be robust enough to withstand such slight changes and allow routine analysis of the sample.

Effect of flow rate

Robustness of assay method was carried out with variation of flow rate ($\pm$0.2ml/minute of set value .i.e. 0.9ml and 1.1 ml/minute).Standard preparation was prepared and performed analysis as per test method and evaluated the system suitability parameters.

Effect of Wave Length

Robustness of assay method was checked with the system suitability parameters by injecting standard preparation with different wave length i.e. one is nm -225 and other one nm is - 235).

Ruggedness

Defined by the USP as the degree of reproducibility of results obtained under a variety of conditions, such as different laboratories, analysts, instruments, environmental conditions, operators and materials. Ruggedness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst.

Limit of Detection (LOD) [12]

Limit of detection is the lowest concentration of the analyte that can be detected by injecting decreasing amount, not necessarily quantity by the method, under the stated experimental conditions.

The minimum concentration at which the analyte can be detected is determined from the linearity curve by applying the formula.

$$\text{Limit of detection} = \frac{\sigma}{S} \times 3.3$$

The lowest concentration of Ambroxol.HCl that can be detected, was determined from standard curve was 0.204176µg/ml. The lowest concentration of Levocetrizine.2HCl that can be detected was determined from standard curve was 0.101878µg/ml.

Limit of Quantitation (LOQ)

Limit of quantitation is the lowest concentration of the analyte in a sample that can be estimated quantitatively. By injecting decreasing amount of drug, with acceptable precision and accuracy under the stated experimental conditions of the method. Limit of quantitation can be obtained from linearity curve by applying the following formula.

$$\text{Limit of quantitation} = \frac{\sigma}{S} \times 10$$

The lowest concentration at which peak can be quantified is called LOQ, was found to be 0.618714µg/ml for Ambroxol.HCl. And for Levocetrizine.2HCl was found to be 0.308722µg/ml.

Stability studies

Stability of the sample and standard used in HPLC method is required for a reasonable time to generate reproducible and reliable results. The stability of the

sample spiked with drug was subjected to short term stability at room temperature for 0-hour and 8-hour.

Assay Procedure for the Proposed Method

Preparation of Sample Solution

For estimating the tablet dosage form, 20 tablets from each batch were randomly selected and powdered, crush the tablets. From the powdered tablets, weigh accurately 203.4mg of powdered tablets (equivalent to 50mg Ambroxol.HCl and 5mg of Levocetirizine.2HCl) transfer it in 50 ml of volumetric flask 15ml of mobile phase was added and make up to 50ml with mobile phase. The mixture was subjected to sonication for 10 min with intermediate shaking for complete extraction of drugs. Cool to room temperature and shake well and filter the solution. Take 1 ml solution and dilute it to 10 ml with mobile phase. The sample was centrifuged in tight enclosure for 10 min at 3000 RPM.

Separately Blank, Standard and test preparation were injected into liquid chromatographic system and the areas for major peaks were recorded. The amount of Ambroxol.HCl and Levocetrizine.2HCl present in each tablet were calculated by comparing the peak area of the standard.

Assay

$$\frac{\text{Sample Area X Standard weight X Purity of Working Standard X Average weight}}{\text{Standard Area X Sample weight X Label claim}}$$

Standard area of Ambroxol.HCl is found to be 1737.167 and the standared area of levocetirizine.2HCl was found to be 204.406. The mean recovery for assay result was found to be 98%-101.09% for Ambroxol.HCl and Levocetirizine.2HCl.

Table 1. System Suitability Parameters

System suitability parameters	Ambroxol.HCl	Levocetirizine.2HCl
Resolution	5.623	
Tailing factor	1.45	1.17
Number of theoretical plate	5232	6590
Retention time	4.200	5.633
Repeatability % RSD	0.479	1.210

Acceptance Criteria: % RSD not more than 2%

Table 2. Linearity Data for Ambroxol.HCl

S. No	Concentration of Ambroxol.HCl (µg/ml)	Area of Ambroxol.HCl (mV)
1	20	385.439
2	40	709.868
3	60	1063.083
4	80	1434.557
5	100	1680.373
6	120	2093.511

Table 3. Linearity Data for Levocetrizine.2HCl

S. No	Concentration of Levocetrizine.2HCl (µg/ml)	Area of Levocetrizine.2HCl (mV)
1	2	42.495
2	4	80.281
3	6	117.699
4	8	161.443
5	10	201.526
6	12	241.273

Table 4. Analytical Performance Parameters for Ambroxol.HCl and Levocetrizine.2HCl

Parameters	Ambroxol.HCl	Levocetrizine.2HCl
Linearity Dynamic Range	20-120µg/ml	2-12µg/ml
Correlation Coefficient	0.998	0.999
Slope (m)	17.13	20.05
Intercept	24.35	0.347

Table 5. Recovery Studies for Ambroxol.HCl and Levocetrizine.2HCl

S. No	Inj. Sample	Spike level	Amount Present	Amount Recovered	% Recovered
1	**Ambroxol.HCl**	80 %	80mcg	79.9961mcg	99.99%
2		100 %	100mcg	99.757mcg	99.75%
3		120 %	120mcg	119.752mcg	99.79%
4	**Levocetrizine.2HCl**	80 %	8mcg	7.980mcg	99.76%
5		100 %	10mcg	9.996mcg	99.96%
6		120 %	12mcg	11.963mcg	99.69%

Table 6. Mean Average Recovery of Ambroxol.HCl and Levocetrizine.2HCl for Accuracy

Accuracy level	Mean recovery of Ambroxol.HCl	Mean recovery of Levocetrizine.2HCl
Accuracy 80%	99.99%	99.76%
Accuracy 100%	99.75%	99.96%
Accuracy 120%	99.79%	99.69%

Table 7. System Precision for Ambroxol.HCl and Levocetrizine.2HCl

S. No	Area of Ambroxol.HCl (mV)	Area of Levocetrizine.2HCl (mV)
1	1673.669	192.662
2	1683.577	198.716
3	1677.01	195.866
4	1688.32	193.454
5	1693.429	195.383
MEAN	1683.201	195.2162
S.D	8.064506	2.362623
% R.S.D	0.479117	1.21026

Acceptance Criteria: % RSD not more than 2 %

Table 8. Method Precision of Ambroxol.HCl and Levocetrizine. 2HCl

Sample No	Area of Ambroxol.HCl (mV)	Area of Levocetrizine.2HCl (mV)
1	1624.913	192.16
2	1624.756	185.563
3	1610.697	186.624
4	1651.328	183.828
5	1648.038	185.494
MEAN	1631.946	186.7338
SD	15.4097	3.194028
% RSD	0.944253	1.710471

Acceptance Criteria: % RSD not more than 2%

Table 9. Data For Specificity for Ambroxol.HCl and Levocetirizine.2HCl

Sample	RT of analyte (min) of Ambroxol.HCl	RT of analyte (min) of Levocetirizine.2HCl
Acid treated	4.250	5.683
Alkali treated	4.260	5.697
Heat treated	4.276	5.707

Table 10. Effect of Flow Rate

Flow rate	Retention time of Ambroxol.HCl	Retention time of Levocetrizine.2HCl
0.9ml/min	4.70 min	6.28 min
1.1ml/min	3.81 min	5.10 min

Table 11. Effect of Wavelength

Wave Length	Area of Ambroxol.HCl (mV)	Area of Levocetrizine.2HCl (mV)
225	2318.883	173.814
235	1600.984	177.294

Table 12. Ruggedness

Analysts	Area of Ambroxol.HCl (mV)	Area of Levocetrizine.2HCl (mV)
Analysts I	1492.24	169.808
Analysts II	1503.153	175.187

Table 13. LOD and LOQ

Sample	LOD	LOQ
Ambroxol.HCl	0.204176µg	0.618714µg
Levocetrizine.2HCl	0.101878µg	0.308722µg

Table 14. Stability Studies

Stability	Area of Ambroxol.HCl	Area of Levocetrizine.2HCl
At o hour	2093.511	248.273
At 8 hour	2091.283	246.175

Table 15. Assay Result for Replicate Injections of Sample

S. No	Area of Ambroxol.HCl (mv)	Area of Levocetirizine.2HCl (mv)	% of Ambroxol.HCl	% of Levocetirizine.2HCl
1	1728.341	206.051	99.32461	100.0651
2	1732.501	205.276	99.56367	99.68869
3	1732.769	203.075	99.57907	98.61981
Mean	1731.204	204.8007	99.48912	99.45787
SD	2.482761	1.543891	0.142675	0.749784
%RSD	0.143412	0.753851	0.143408	0.753871

The mean recovery for assay result was found to be 98%-101.09% for Ambroxol.HCl and Levocetirizine.2HCl

Fig. 1. Linearity Plot For Ambroxol.HCl

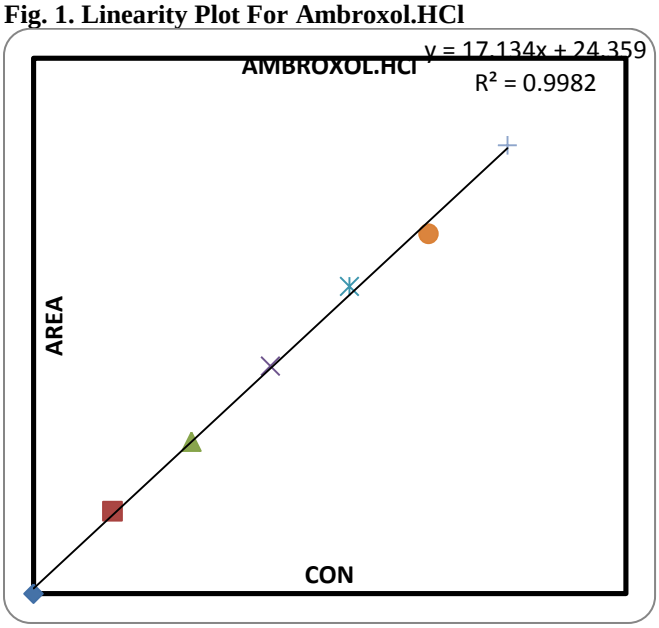
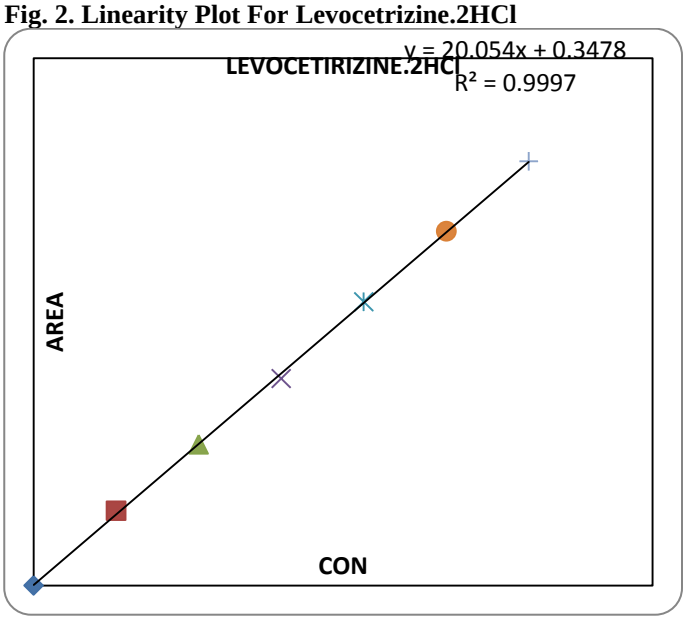
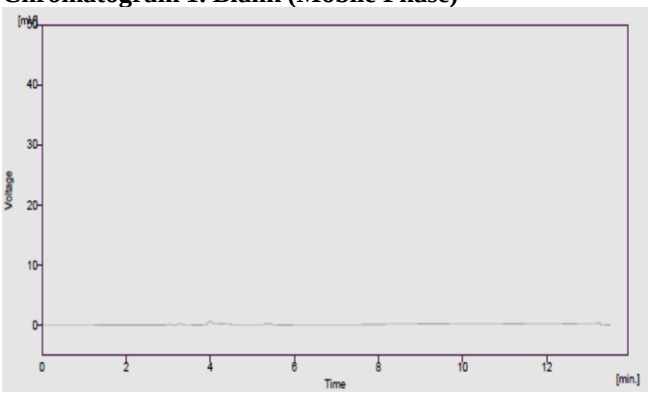


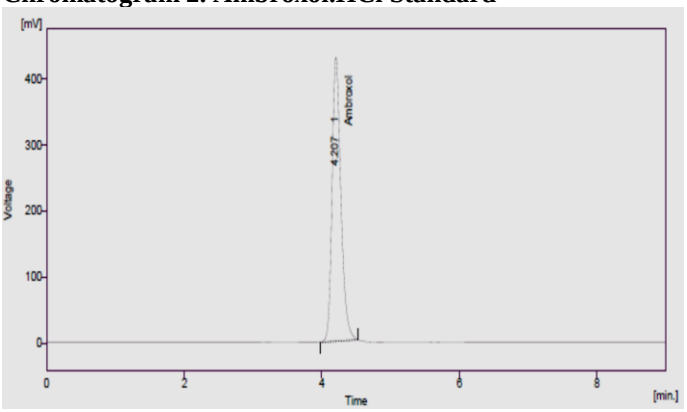
Fig. 2. Linearity Plot For Levocetrizine.2HCl



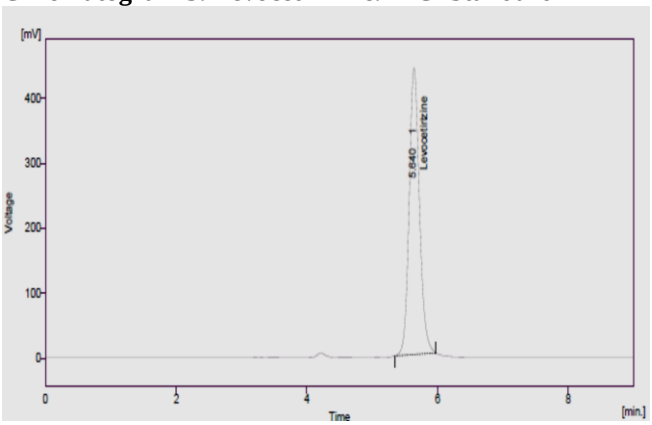
Chromatogram 1. Blank (Mobile Phase)



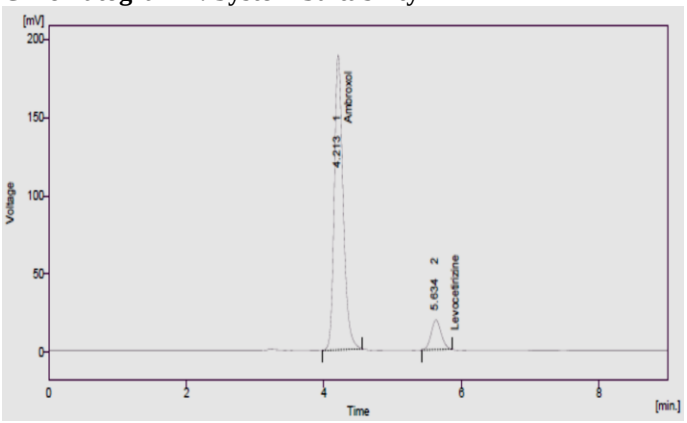
Chromatogram 2. Ambroxol.HCl Standard



Chromatogram 3. Levocetirizine.2HCl Standard



Chromatogram 4. System Suitability-1



RESULTS AND DISCUSSION

In the present study, a simple, precise, accurate and rapid high performance liquid chromatographic method has been developed and validated for the determination of Ambroxol.HCl and Levocetirizine.2HCl in tablet dosage formulation. The developed method was validated in terms of specificity, linearity, precision, accuracy, robustness, system suitability, etc.

In this method the estimation of the Ambroxol.HCl and Levocetirizine.2HCl in tablet dosage form is done by using the mobile phase containing the methanol and 0.1M sodium dihydrogen phosphate buffer in the ratio of 70:30v/v with pH 3. In this method Phenomenex Luna C_{18} (250x4.6) column is used and the flow rate is 1ml/min.

The linearity data for both the drugs were given in the table 2 and 3 and corresponding calibration graphs were shown in figure 1 and 2.The linearity of the method was determined at the concentration levels ranging from 20-120 µg/ml for Ambroxol.HCl and 2 - 12µg/ml for Levocetirizine.2HCl. The correlation coefficient of Ambroxol.HCl and Levocetirizine.2HCl was found to be 0.997 and 0.999 these are within limit.

System suitability parameters such as resolution, tailing factor and number of theoretical plates are presented in Table.1.

The percentage of drug recovered is estimated by accuracy. The recover studies were calculated and the data is available in table 5. From the obtained data method was found to be accurate.

To precise the method system precession and method precession were carried out. It was demonstrated by repeatability of injections. All the solutions were injected in to the chromatogram system the peak area and percentage relative standard deviation were calculated and present in the tables 7 and 8.

To specify the method the sample was treated with acid, alkali and heated. The specificity values were reported in the table 9.

The robustness of the method was performed by flow rate changes and wavelength change and the data given in the table 10 and 11.

The ruggedness of the method checked by the analysing the prepared solution with different analyst and the data were reported in table 12.

The LOD and LOQ were calculated and presented in table 13. Stability studies were carried out and the test results were presented in table 14.

Table 16. Results of the method

S. No	Parameters	Ambroxol.HCl	Levocetirizine.2HCl
1	Accuracy	% Recovery=99.88	% Recovery=99.87
2	System Precession	%RSD=0.479117	%RSD=1.21026
3	Method Precession	%RSD=0.944253	%RSD=1.710471
4	Linearity	R_2=0.997	R_2=0.999
5	Range	20-120 µg/ml	2-12 µg/ml
6	LOD	0.204176µg	0. 101878µg
7	LOQ	0.618714µg	0. 308722µg

Estimation of Ambroxol.HCl and Levocetirizine.2HCl in tablet dosage forms by the developed RP-HPLC method was carried out. The standard and sample solutions were prepared and the chromatograms were recorded.

The assay was performed and the percentage was calculated. The assay percentage of individual drugs and amount present per tablet were calculated and presented in Table 15

As per the validation parameters, the developed HPLC method for the simultaneous estimation of Ambroxol.HCl and Levocetirizine.2HCl in tablet dosage forms was accurate, precise, linear, robust, simple and rapid.

SUMMARY AND CONCLUSION

RP-HPLC method was developed and validated as per ICH guidelines for the estimation of Ambroxol.HCl and Levocetirizine.2HCl.

Simultaneous Estimation of Ambroxol.HCl and Levocetirizine.2HCl were carried out by RP- HPLC using sodium phosphate buffer (P^H 3.0): Methanol (30:70) and column Phenomenex Luna C-18 (250x4.6 mm, 5um) as a stationary phase and peak was observed at 230 nm which was selected as a wavelength for quantitative estimation. After the development of the method, it was validated for specificity, linearity, precision, accuracy, robustness and ruggedness studies.

The system suitability parameter also reveals that the values within the specified limit for the proposed method. Theoretical plate for Ambroxol.HCl was found to be 5232, for Levocetirizine.2HCl it was found to be 6590.

The precision of the System and Method were checked and found to be within limits. This indicates that the method is precise.

From the linearity studies, the specified range for Ambroxol.HCl and Levocetirizine.2HCl was found to be 20% to 120%. It was evaluated by the visual inspection of the plot of Peak area vs. Concentration.

From the results shown in the accuracy table, it was found that recovery value of pure drug were between 98.0 % to 102%. Which indicates that the method is accurate and also reveals that commonly used excipients and additives present in the pharmaceutical formulations were not interfering in the proposed methods.

The ruggedness of the method was checked by different analysts and found that the results were nearly same which indicates that the method is rugged.

The robustness of the method was checked by varying flow rate, wavelength and found that the system suitability parameters were within limit at all variable conditions, hence the method is robust.

Based on the results observed, it was concluded that proposed method can be used for routine analysis of Ambroxol.HCl and Levocetirizine.2HCl.

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