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

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ABSTRACT

RP-HPLC method was developed and validated as per ICH guidelines for the estimation of Ambroxol.HCl and Levocetirizine dihydrochloride. Simultaneous estimation of Ambroxol.HCl and Levocetirizine dihydrochloride were carried out by RP- HPLC using sodium phosphate buffer (pH = 3.0): Methanol (30:70) and column Phenomenex Luna C_{18} (250 x 4.6 mm, 5µm) as a stationary phase and peak was observed at 230 nm which was selected as a wavelength for quantitative estimation. It was validated for specificity, linearity, precision, accuracy, robustness and ruggedness studies. Results reveals that recovery value of pure drug were between 98 % 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. Based on the results observed, it was concluded that proposed method can be used for routine analysis of Ambroxol HCl and Levocetirizine dihydrochloride.

Key words: Ambroxol HCl, Levocetirizine dihydrochloride, RP-HPLC method, ICH guidelines.

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INTRODUCTION

Pharmaceutical analysis may be defined as that branch of pharmaceutical chemistry which deals with the resolution, separation, identification, determination and purification of given sample of medicine or a pharmaceutical. The detection and estimation of impurities that may be present therein is also included. The quality of a pharmaceuticaldrug on being analysed should reflect the standards related to potency, safety and efficacy (1).

A drug may be defined as a substance meant for diagnosis, cure, mitigation, prevention, or treatment of diseases in human beings or animals or for alternating any structure or function of the body of human being or animals. Pharmaceutical chemistry is a science that makes use of general laws of chemistry to study drugs i.e., their preparation, chemical natures, composition, structure, influence on an organism and studies the physical and chemical properties of drugs, the methods of quality control and the conditions of their storage etc. It is necessary to find the content of each drug either in bulk or single or combined dosage forms for purity testing. It is also essential to know the concentration of the drug and it's metabolites in biological fluids after taking the dosage form for treatment.

The scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation.

A survey of literature reveals that good analytical methods are not available for the drugs like Ambroxol.HCl and Levocetirizine dihydrochloride. The existing phyisico-chemical methods are inadequate to meet the requirements; hence it is proposed to improve the existing methods and to develop new methods for estimation Ambroxol.HCl and Levocetirizine dihydrochloride in pharmaceutical dosage forms adopting different available analytical techniques (2-13). Hence, an attempt was made to develop a cheap, simple, rapid, accurate, precise and validated method for the estimation Ambroxol.HCl and Levocetirizine dihydrochloride in pharmaceutical dosage forms.

MATERIALS AND METHODS

Materials

Ambroxol HCl, Levocetirizine dihydrochloride (BOROX-L) were purchased from local market.

Methanols, Sodium Phosphate and Hydrochloric acid were purchased from Merck specialties, Pvt Ltd, Mumbai.

Optimized chromatographic conditions

Simultaneous estimation of Ambroxol.HCl and Levocetirizine dihydrochloride were carried out by RP- HPLC. Stationary phase-Phenomenex Luna C_{18} (250 × 4.6 mm, 5µm); Mobile phase- Methanol : 0.1M Sodium Phosphate buffer (70: 30 % v/v); pH-3; Flow rate-1 ml/min; Column Temperature- Room temperature; Volume of Injection loop-20 µl; Detection of Wavelength-230 nm; Run time- 8 min; Mode of Operation- Isocratic was used.

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 203.4 mg of powdered tablets (equivalent to 50 mg Ambroxol.HCl and 5 mg of Levocetirizine dihydrochloride) transfer it in 50 ml of volumetric flask 15 ml of mobile phase was added and make up to 50 ml 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.

Procedure

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

Assay

Sample Area X Standard weight X Purity of Working Standard X Average weight/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 was found to be 204.406.

Method validation

After the method development, the method was validated in terms of parameters like accuracy, precision, linearity and range, robustness, stability etc (14, 15).

Linearity and Range

Ambroxol HCl showed linearity in the range of 20-120µg/ml and Levocetirizine dihydrochloride showed linearity in the range of 2-12 µg/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.

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.

Six 20 ml injection from a standard solution were injected on to the analytical column and the peak area data obtained and % RSD was calculated.

Specificity is performed with the specificity parameter, stressed samples (sample heated to 60^0 C for 2 h, sample treated with 1N HCl for 2 h, and sample treated with 1N NaOH for 2 h) and working standard were injected separately.

RESULTS AND DISCUSSION

Good reproducibility was produced with Stationary phase- Phenomenex Luna C_{18} (250 × 4.6 mm, 5µm); Mobile phase- Methanol : 0.1M Sodium Phosphate buffer

(70: 30 % v/v); pH-3; Flow rate-1 ml/min; Detection of Wavelength-230 nm and Run time- 8 min (Fig-1).

Ambroxol HCl showed linearity in the range of 20-120µg/ml and Levocetirizine dihydrochloride showed linearity in the range of 2-12 µg/ml (Table-1). 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 Levocetirizine dihydrochloride were found to be 20.05, 0.347 and 0.999 respectively (Fig-2 and Fig-3).

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 (Table-2).

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. % RSD was found to be not more than 2 %.

The LOD of Ambroxol.HCl that can be detected, was determined from standard curve was 0.204176 µg/ml. The LOD of Levocetirizine dihydrochloride that can be detected was determined from standard curve was 0.101878 µg/ml. The lowest concentration at which peak can be quantified is called LOQ, was found to be 0.618714 µg/ml for Ambroxol HCl and for Levocetirizine dihydrochloride was found to be 0.308722 µg/ml.

Robustness of assay method was carried out with variation of flow rate ($\pm$0.2 ml/minute of set value i.e 0.9 ml/minute and 1.1 ml/minute). The results were found to be satisfactory.

Specificity was performed the specificity parameter, stressed samples. 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

The stability of the sample spiked with drug was subjected to short term stability at room temperature for 0-hour and 8-hour. The result was found to be satisfactory (Table-3).

Based on the results observed, it was concluded that proposed method can be used for routine analysis of Ambroxol.HCl and Levocetirizine dihydrochloride (Table-4).

Table- 1 Analytical performance parameters for Ambroxol Hcl and Levocetirizine dihydrochloride

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

Table-2 Recovery study results for Ambroxol HCl and levocetirizine dihydrochloride

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

Table-3 Results of stability studies

Stability	Area of Ambroxol.HCl	Area of Levocetirizine
At 0 hour	2093.511	248.273
At 8 hour	2091.283	246.175

Table-4 summary validation parameters for Ambroxol Hcl and levocetirizine dihydrochloride

S.No	PARAMETERS	Ambroxol.HCl	Levocetirizine
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.	Correlation coefficient	R^2=0.998	R^2=0.999
5.	Concentration Range	20-120 µg/ml	2-12 µg/ml
6.	Slope	17.13	20.25
7.	Intercept	24.35	0.347
8.	LOD	0.204176 µg	0. 101878 µg
9.	LOQ	0.618714 µg	0. 308722 µg
10.	Run time	8 min	8 min
11.	Retention time	4.200 min	5.633 min
12.	% RSD	0.479 %	1.210 %
13.	Tailing factor	1.45	1.17
14.	HETP	5232	6590

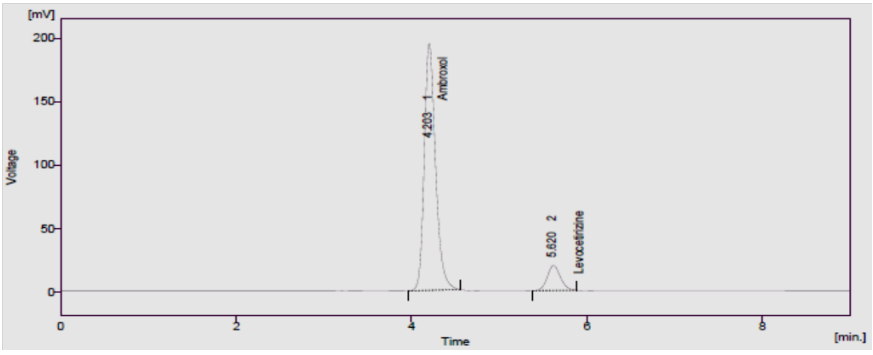

Fig-1 Chromatogram of Ambroxol HCl and Levocetirizine dihydrochloride

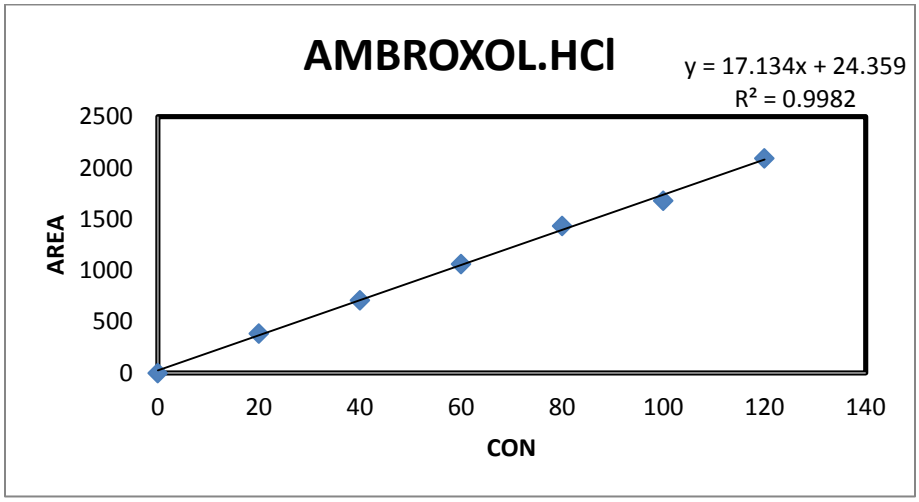

Fig-2 caliberation curve of Ambroxol Hcl

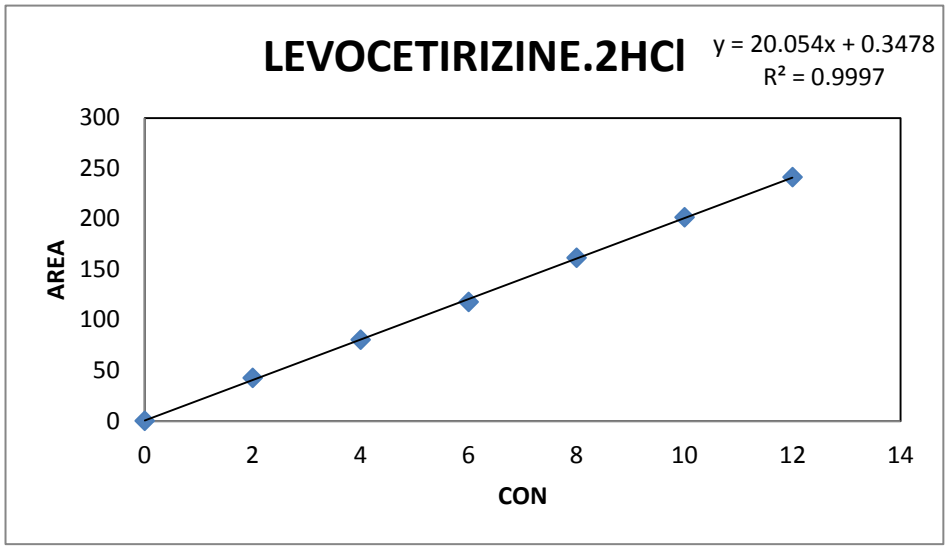

Fig-3 caliberation curve of Levocetirizine dihydrochloride

CONCLUSION

The proposed RP-HPLC is suitable for the estimation of Ambroxol HCL and Levocetirizine dihydrochloride in formulation. All the validation parameters for Ambroxol HCL and Levocetirizine dihydrochloride meet the criteria as per ICH guidelines. The analytical method was found to be simple, sensitive, accurate and precise. The developed method may be recommended for routine and quality control analysis of the investigated drug to provide reproducible quantitative analysis for the determination of Ambroxol HCL and Levocetirizine dihydrochloride in tablet formulation.

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