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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF IN AMBROXOL AND LEVOCETIRIZINE BULK AND PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC METHOD

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ABSTRACT

A simple, accurate, economical and reproducible reverse phase high performance liquid chromatographic (RP-HPLC) method was developed and validated for the determination of Ambroxol and Levocetirizine in bulk and pharmaceutical formulations. The separation was achieved on a phenomenex C18 column (150 × 4.6 mm i.d, particle size of 5µ) using a mixture of 0.01M Potassium dihydrogen orthophosphate (pH 5.0 ± 0.05) & Acetonitrile (60:40 v/v) as mobile phase in an isocratic elution mode, at a flow rate of 1 ml/min. The detection was monitored at 230 nm. The retention time of was found to be around 3.60min (Levocetirizine) 4.68min (Ambroxol) respectively. Excellent linearity range was found between12-120 µg/ml for Ambroxol and 1-10µg/ml for Levocetirizine, n. The method was validated with respect to linearity, robustness, precision and accuracy and was successfully applied for the simultaneous determination of Ambroxol and Levocetirizine from the combined dosage formulation.

1. INTRODUCTION

Amroxol (AMB) is chemically Trans-4-(2-Amino-3,5- dibromobenzylamino)- cyclo hexan-1-ol AMB is Mucolytic ,respiratory agent and used in the treatment of the upper respiratory tract diseases. With its mucolytic activity, AMB facilitates the breakdown of acid muco polysaccharide fibres in the mucous thus making it thinner and less viscous for expectoration. As well it stimulates the production of pulmonary surfactant, a substance fiund to play a major role in the lung defense mechanism and thereby further protect it against inflammation and infection. Levocetirizine(LCTZ) is chemically (2-(4-[(R)-(4-chlorophenyl) (phenyl)methyl]- piperazin-1-yl) ethoxy)acetic acid.Levocetirizine is a third generation non-sedative antihistamine developed from the second generation antihistamine cetirizine. It is the R-enantiomer of the cetirizine which functions to block histamine receptors. More specifically, LCTZ does not prevent the actual release of histamine from mast cells but prevents its binding to its receptors. This in turn prevents the release of other allergy chemicals and increases the blood supply to the area providing relief from the symptoms of hay fever. Literature survey revealed that AMB and LCTZ has been estimated individually or in combination using UV, HPLC and HPTLC. The present work describes the development of a simple, precise, accurate and reproducible spectrophotometric method for the

simultaneous estimation of AMB and LCTZ in Pharmaceutical dosage form[1- 14].

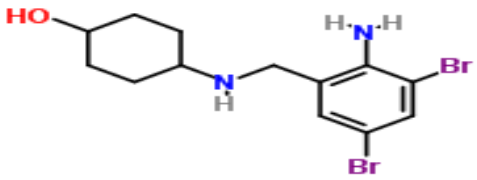

Fig. 1: Structure of Ambroxol (AMB)

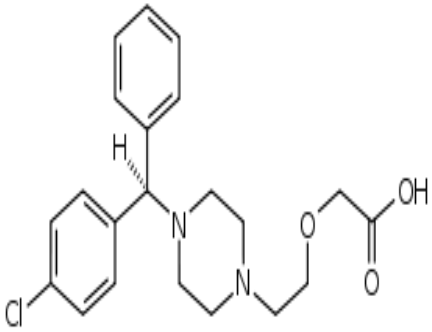

Fig. 2: Structure of Levocetirizine(LCTZ)

2. Experimental

2.1. Apparatus

A SHIMADZU (Japan) HPLC instrument (LC-20AD) equipped with a UV-Visible detector, rheodyne injector with 20 µL loop, phenomenex C18 column (150 mm x 4.6 mm i.d, 5µ particle size) and LC-Solution software were used. Other instruments included are SHIMADZU electronic balance, BL-220H (SHIMADZU corp., Japan), fast clean ultrasonic cleaner and value 1 stage vaccum pump (model: VE115).

2.2 CHEMICALS AND REAGENTS

Methanol obtained from local market, Pure ambroxol and levocetirizine were obtained as gift sample from Suraksha pharmaceutical Pvt.Ltd.,Roorki.

2.3 Pharmaceutical formulation

The tablet dosage form COLDTHROW-AM(claim: 60mg ambroxol and 5mg levocetirizine) was procured from local market.

2.4 Chromatographic conditions

Chromatographic separation was performed on shimadzu HPLC with phenomenox C18 column (150 x 4.6 mm i.d, particle size of 5 µ) and constant flow pump. Rheodyne injector with 20 µl loop. The composition of the mobile phase was in the ratio of 0.05% TEA (pH adjusted to 6.0 with 0.1% ortho phosphoric acid) and and acetonitrile (90:10 % (v/v) and was delivered at a flow rate of 0.8 ml /min. The mobile phase was filtered through a 0.45 µ membrane filter and sonicated for 5 min. Analysis was performed at ambient temperature. Chromatogram of standard drugs was shown in fig 3. Optimized chromatographic conditions are listed in table - 1.

2.5. Preparation of mobile phase

The selected mobile was prepared by dissolving 0.136 g of potassium di-hydrogen orthophosphate in 100 ml of tripled distilled water, mixed thoroughly and pH of the solution was at 5.0. The buffer and acetonitrile were mixed in the ratio of 60:40 v/v.

2.6 Preparation of standard AMB and LCTZ solutions

Stock solutions were prepared by dissolving 1 mg of Ambroxol, 1mg of Levocetirizine in 10 ml of mobile phase separately. Aliquots of the standard stock solutions of Ambroxol And Levocetirizine were transferred into 10 ml volumetric flasks and solution was made up to the volume to yield required concentrations of both drugs within the linearity range., were prepared by suitable dilution of the stock solution with mobile phase.

2.7. Preparation of sample solution

For analysis of commercial formulations (Coldthrow –AM) , 20 tablets were weighed, powdered and weight equivalent to 60 mg and 5 mg of Ambroxol and Levocetirizine respectively was taken and transferred into 100 ml volumetric flask and dissolved in 100 ml mobile phase, filtered through a whatmann filter paper and the solution was further diluted stepwise with mobile phase to get the concentration within the linearity range.

2.8 Analysis of formulation

The amount of drug present in the pharmaceutical formulation was calculated through peak area by making use of the standard calibration curve (Concentration in µg/ml on x-axis and peak area on Y-axis) the results were shown in table-2.

3. RESULTS AND DISCUSSIONS

Once the HPLC method development was over, the method was validated in terms of parameters like linearity, precision, LOD, LOQ, recovery studies etc,. The proposed HPLC method was validated as per ICH guidelines

3.1. Linearity and Range

Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curves were linear over the concentration range of 12-120 µg/ml for Ambroxol and 1-10µg/ml for Levocetirizine, Fig3-4. Peak areas were plotted versus respective concentrations and linear regression analysis was performed on the resultant curves. The slope, intercept and correlation coefficient were found to be 26266, 7608 and 0.9991 respectively for Ambroxol and 51822, 5191.9 and 0.999 respectively for Levocetirizine.

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3.2. Precision

The precision of the method was ascertained separately from the peak areas obtained by actual determination of three replicates of a fixed amount of drug. The intra and inter-day variation in the peak areas of the drug solution was calculated in terms of percent RSD and the results are presented in table 3-4 .

3.3. Recovery Studies

Recovery studies of the drug were carried out for determining accuracy parameter. It was done by mixing known quantity of standard drugs with the analyzed sample formulation and the contents were reanalyzed by the proposed method. This was carried out at 50 and 100% levels. Results of recovery are shown in Table 5

3.4. Limit of Detection and Limit of Quantification

The limit of Detection (LOD) and limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. LOD and LOQ were calculated mathematically. The LOD of Ambroxol and Levocetirizine were found to be 4.309µg/ml and 0.357µg/ml respectively. The LOQ of Ambroxol and Levocetirizine were found to be 13.05µg/ml and 1.822µg/ml respectively.

3.5 System suitability studies

System suitability parameters like Retention time, number of theoretical plates (N), Tailing factor, resolution (Rs) etc., were studied, and results are given in Table 6

3.6. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was investigated under a variety of conditions including changes of composition of buffer in the mobile phase and flow rate. % RSD of assay was calculated for each condition. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust shown in Fig no 7

3.7. Ruggedness

The ruggedness of the method was assessed by comparison of the intra-day and inter-day assay results for Ambroxol and Levocetirizine that has been performed by two analysts. The % RSD values for assays performed in the same laboratory by two analysts did not exceed 2, indicating the ruggedness of the method.

4. CONCLUSION

The direct Reverse Phase-HPLC method development for the simultaneous analysis of Ambroxol and Levocetirizine can be applied for the routine analysis of formulation. The developed Reverse Phase-HPLC method offers simplicity, selectivity, precision and accuracy. In this proposed method symmetrical peaks with good resolution were obtained. These methods can be used for the simultaneous analysis of Ambroxol and Levocetirizinein combined dosage form.

Table 1: Optimized Chromatographic Conditions

Parameters	Method
Stationary phase (Column)	Phenomenex C18 (150 x4.6 mm i.d, 5 µ size)
Mobile phase	0.01M Potassium dihydrogen orthophosphate (pH 5.0 ± 0.05) & Acetonitrile (60:40 v/v)
Flow rate (ml/min)	1
Pressure (kgf)	194
Run time (min)	10
Column temperature(^{0}c)	Ambient
Detection wavelength (nm)	230
Drugs Retention time (min)	3.60min(Levocetirizin) 4.68min(Ambroxol)

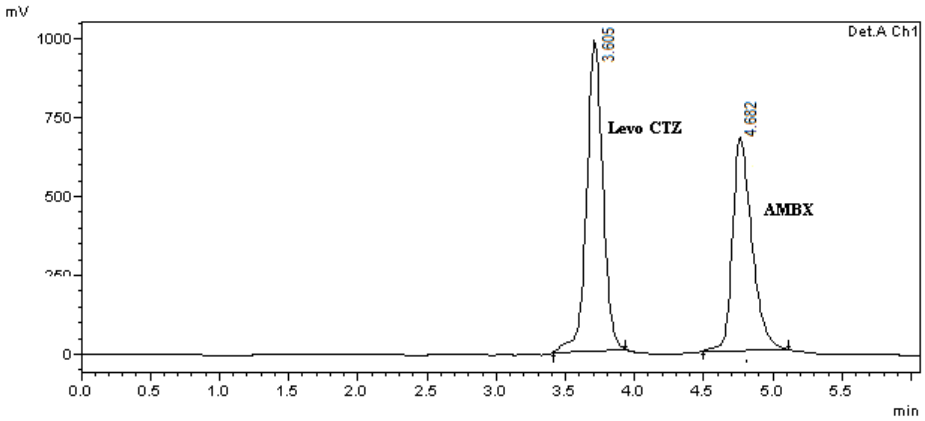

Fig. 3: Optimized chromatogram

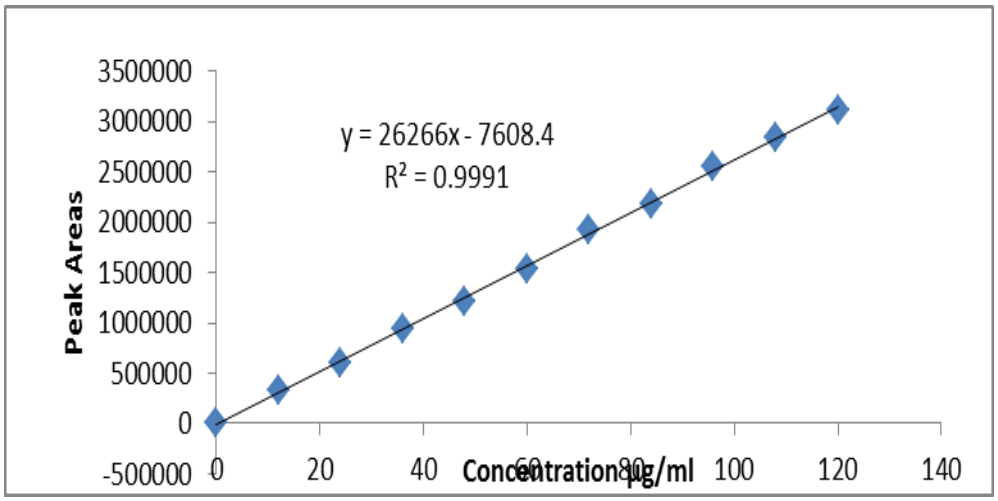

Fig. 4: Calibration Graph of Ambroxol(12µg/ml-120µg/ml)

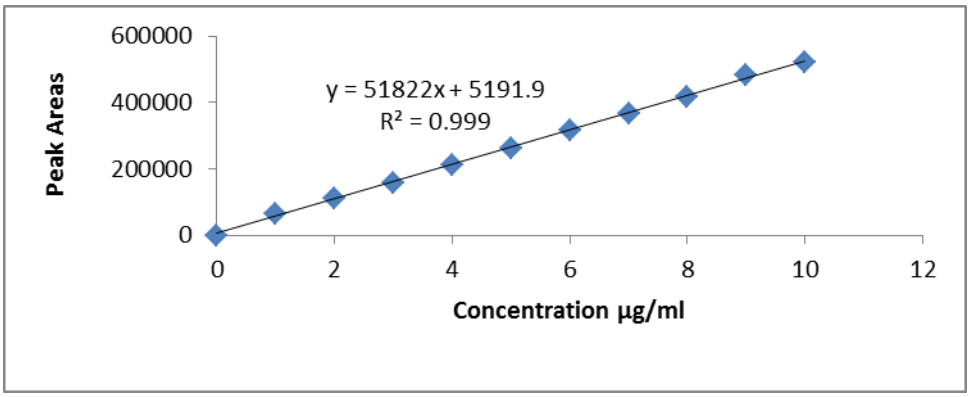

Fig. 5: Calibration Graph of Levocetirizine (1µg/ml-10µg/ml)

Table 2: Analysis of marketed formulation

Drugs	Labeled amount(mg)	Amount found(mg)	% Label claim	*%RSD
AMB	60	60.12	100.2	0.652
LCTZ	5	4.91	98.2	0.715

Table 3: Intraday precision

Drug	Concentration (µg/ml)	Peak Area (Avg*)	% RSD
Amroxol	60	1539361	0.991
	72	340432	0.945
Levocetirizine	5	2027943	0.961
	6	326824	0.938

* mean of six observations

Table no 4: Inter day precision

Drug	Concentration (µg/ml)	Peak Area (Avg*)	%RSD
Ambroxol	60	1539361	1.122
	72	340432	0.923
Levocetirizine	5	2027943	0.942
	6	326824	0.825

* mean of six observations

Table 5: Accuracy studies

Drug	Amount added (µg/ml) (%)	Amount recovered (µg/ml)	% Recovery	*% RSD
AMB	30	29.4	98%	0.657
	60	59.7	99.5%	0.555
LCTZ	2.5	2.6	104%	0.293
	5	5.1	102%	0.278

Table 6: System suitability studies

Drug	Theoretical plates (N)	Retention time(R_t)	Tailing factor
AMB	4265	4.68	1.5
LCTZ	3157	3.60	0.9

Table 7: Robustness

Parameters	Retention time(min)	
Mobile phase composition	**AMB**	**LCTZ**
88:12%v/v	4.92	3.33
90:10%v/v	4.68	3.60
92:08%v/v	4.99	4.18
pH of buffer		
pH 5.8	4.52	3.22
pH 6.0	4.68	3.60
pH 6.2	5.10	4.34

*mean of three observations

4. REFERENCES

1. International Conference on Harmonization, Validation of analytical procedures: methodology. Federal Register, 1996.
2. ICH harmonized tripartite guideline, Text on validation of analytical procedures, Recommended for adoption at step 4 of the ICH process by the ICH steering committee.
3. http://www.drugbank.ca/drugs/DB00967.
4. http://www.drugbank.ca/drugs/DB00852.
5. Venkateshwari P and Suresh kumar GV. Development of stability indicating RP-HPLC method for the simultaneous estimation of Ambroxol hydrochloride and Levocetirizinedihydrochloride. Int J Pharm Anal. 2012;2(2):34-40.
6. Sekar V* , Bajivali SK, Vanitha C and Permaul. RP-HPLC Method Development and Validation for the Simultaneous Estimation of Ambroxol and Levocetirizine in Solid Dosage Forms. Asian J Res che. 2011;4.
7. Lakshamana Prabhu S, Shirwaikar AA and Annie Shirwaikar. Simultaneous UV Spectrophotometric method for the estimation of Ambroxol hydrochloride and Levocetirizinedihydrochloride. Indian J Pharm. Sci. 2008;70(2).
8. Jyostna S Patil, Chandan R Rao and Radhika D Ingala. Simultaneous Spectrophotometric method for the estimation of Ambroxol hydrochloride and Levocetirizinedihydrochloride in pharmaceutical dosage forms. J Chem Bio Sci Sec C. 2013;3(2).
9. Kamarapu SK and Vaijayanthi. Development of RP-HPLC method for the estimation of Ambroxol hydrochloride and Levocetirizinedihydrochloride in combined dosage form and its applications. Int J Pharm Sci Nano. 3(1).