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Simultaneous Determination of Ceitrizine Hydrochloride and Ambroxol Hydrochloride in Combined 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 Cetirizine hydrochloride and Ambroxol hydrochloride 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 methanol, acetonitrile and water in the ratio of (30:30:40v/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 Cetirizine hydrochloride and Ambroxol hydrochloride was found to be around 2.27 ± 0.12 min and 4.70 ± 0.14 min respectively. Excellent linearity range was found between 1-10 µg/ml for cetirizine hydrochloride and 10-100 µg/ml for Ambroxol hydrochloride. The method was validated with respect to linearity, robustness, precision and accuracy and was successfully applied for the simultaneous determination of Cetirizine hydrochloride and Ambroxol hydrochloride from the combined dosage formulation.

Key words: Cetirizine hydrochloride; Ambroxol hydrochloride; RP-HPLC method.

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INTRODUCTION

Ambroxol hydrochloride is semisynthetic derivative of vasicine obtained from Indian shrub *Adhatoda vasica* . It is the metabolic product of Bromhexine. It is official in Martin Dale-The Extra Pharmacopoeia. Chemically it is Trans-4-(2-amino-3, 5-dibromobenzylamino) cyclohexanol hydrochloride. It acts as a bronchosecretolytic and expectorant drug. It stimulates the transportation of the viscous secretions in the respiratory organs and reduces the accumulation of the secretions. Several spectrophotometric methods have been used for the qualitative and quantitative determination of ambroxol hydrochloride in pharmaceutical formulations. Different high-performance liquid chromatographic (HPLC) methods have been reported for determination of Ambroxol hydrochloride in pharmaceutical formulations and biological fluids. Cetirizine is the carboxylated metabolite of Hydroxyzine, and it has high specific affinity for histamine H_1 receptor. Cetirizine is chemically known as 2-[4-(4-chlorobenzhydryl) piperazine-1-yl] ethoxy acetic acid. Several spectrophotometric methods have been reported for determination of Cetirizine in pharmaceutical formulations and in human plasma. Different HPLC methods (Walily *et al.* , 1998) have been reported for determination of Cetirizine in pharmaceutical formulations and biological fluids. Literature survey reveals that high-performance thin-layer chromatography has been reported for the simultaneous determination of Ambroxol hydrochloride and Cetirizine hydrochloride in pharmaceutical formulations.

MATERIALS AND METHODS

Equipment and chromatographic conditions

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).

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 mixture is methanol, acetonitrile and water in the ratio of (30:30:40v/v) and was delivered at a flow rate of 1 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. Optimized chromatographic conditions.

Materials and reagents

Cetirizine hydrochloride and Ambroxol hydrochloride pure powder were gift samples supplied from Aurabindo Pharma Ltd, India. Acetonitrile (HPLC grade) was purchased from Merck Ltd, India...Methanol (HPLC grade) was purchased from Merck Ltd, India. Water for HPLC was prepared by triple glass distillation and filtered through a 0.45 µ membrane filter (Gelman Laboratory, India).

Preparation of standard solutions

Cetirizine hydrochloride and Ambroxol hydrochloride 10 mg were dissolved in 100 ml of mobile phase to obtain a standard stock solution of (100 µg/ml) of each drug. Further working standard solutions of Cetirizine hydrochloride and Ambroxol hydrochloride, 1-10 µg/ml and 10-100 µg/ml respectively, were prepared by suitable dilution of the stock solution with mobile phase.

Preparation of sample solutions

For analysis of commercial formulations, 20 tablets were weighed, powdered and weight equivalent to 5 mg and 60 mg of Cetirizine hydrochloride and Ambroxol hydrochloride 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.

RESULT AND DISCUSSION

Optimization of 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 mixture is methanol, acetonitrile and water in the ratio of (30:30:40v/v) and was delivered at a flow rate of 1 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. Optimized chromatographic conditions are listed in table -1.

Method Validation

Linearity

The linearity measurement was evaluated by analysing different concentrations of the standard Solutions of Cetirizine hydrochloride and Ambroxol hydrochloride. The Beer lamberts concentration was found to be between 1-10 µg/ml for cetirizine hydrochloride and 10-100 µg/ml for Ambroxol hydrochloride. Calibration curve was constructed by plotting peak area against concentration and regression equation was computed. The results are presented in table 1.

Table 1: Summary for RP-HPLC Method

S.no	Parameter	Acceptance criteria	Results obtained
1	System suitability	Theoretical Plates-NLT2000	CET-2370 DUTA- 3625
2	System Precision	%RSD of CET NMT2% %RSD of AMB NMT2%	CET -1.026 AMB- 0.28
3	Method Precision	%RSD of CET NMT2% %RSD of AMB NMT2%	CET -1.07 AMB- 0.116
4	ID System Precision	%RSD of CET NMT2% %RSD of AMB NMT2%	CET -0.56 AMB- 0.85
5	ID method Precision	%RSD of CET NMT2% %RSD of AMB NMT2%	CET -0.62 AMB- 0.132
6	Linearity	Correlation coefficient NLT 0.996	CET -0.999 AMB- 0.998
7	Accuracy	Percentage Recovery 98-102%	CET -99.6 AMB- 99.8

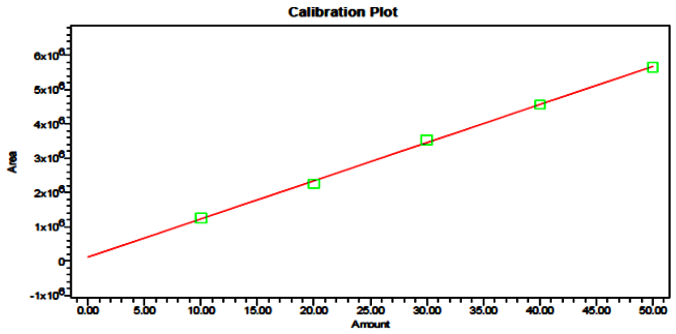

Figure 1: Linearity for Cetirizine HCl:

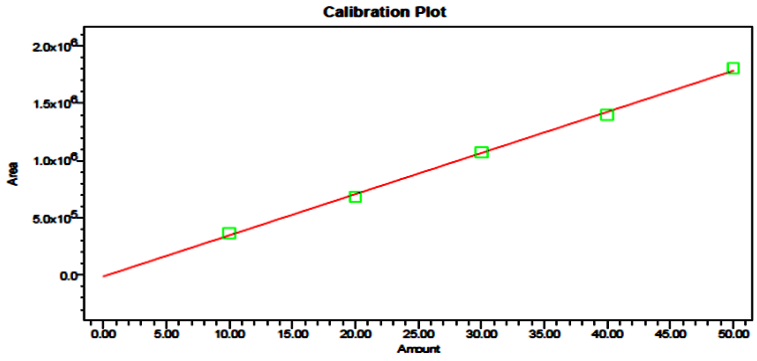

Figure 2: Linearity for Ambroxol HCl:

Specificity (Selectivity)

The method specificity was assessed by comparing the chromatograms obtained from the drug and the most commonly used excipients mixture with those obtained from blank (excipients solution in water without drug). The method was specific as none of the excipients interfered with the analytes of interest.

Accuracy and 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 1

Limits of Detection and Quantitation

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. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3).The LOD values were found to be 0.1 µg/ml and 1 µg/ml for Cetirizine hydrochloride and Ambroxol hydrochloride respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ values were found to be 1 µg/ml and 10 µg/ml for Cetirizine hydrochloride and Ambroxol hydrochloride respectively. The results are presented in table 1.

Analysis of Pharmaceutical Dosage Form (Tablets)

AMBCET tablet was taken and analysied the tablet. The values of analysis of capsules obtained by the proposed method were between 99.86% and 99.78(table 2), which showed that the estimation of dosage forms were accurate within the acceptance level of 95% to 105%(refer table 2).

Table 2: Results of analysis of formulation and recovery studies

Drug	Quantity Claimed	Quantity Found	%Recovery
Cetirizine HCl	5	5.01	100.2
ambroxol HCl	60	60.14	100.23

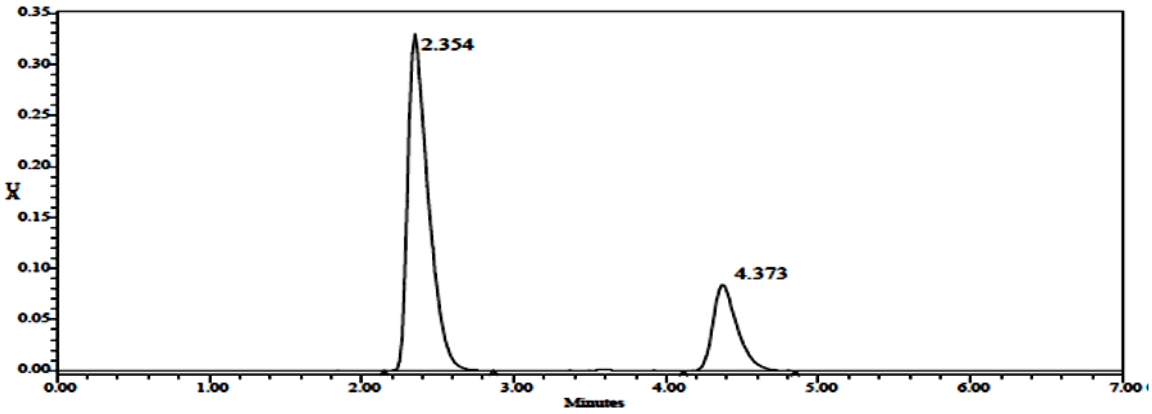

Figure 3: Retention time for both the drug

Table 3:

S.No	Name	Retention time(min)	Area	Height	Plate count	Tailing
1	Cetirizine HCl	2.354	3061716	329220	2370.1	1.6
2	Ambroxol HCl	4.37	925851	84168	3625.0	1.4

CONCLUSION

A convenient and rapid RP- HPLC method has been developed for estimation of Cetirizine hydrochloride and Ambroxol hydrochloride in combined dosage form. The assay provides a linear response across a wide range of concentrations. Low intra-day and inter-day % RSD coupled with excellent recoveries. Hence, this method can be conveniently adopted for routine analysis of Cetirizine hydrochloride and Ambroxol hydrochloride in pure form and its dosage forms and can also be used for dissolution or other similar studies.

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