

SIMULTANEOUS ESTIMATION OF AMBROXOL HYDROCHLORIDE AND CETIRIZINE HYDROCHLORIDE IN TABLET DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous determination of ambroxol hydrochloride and cetirizine hydrochloride in tablet dosage forms. A Princeton C-8 (4.6 × 250 mm, 5 μm) column with mobile phase containing methanol-potassium dihydrogen phosphate buffer 80:20 (v/v) (10 mM, pH 3.5 ± 0.02, adjusted with orthophosphoric acid) was used. The flow rate was 1.0 ml/min and effluents were monitored at 276 nm. The retention times of ambroxol hydrochloride and cetirizine hydrochloride were 2.7 min and 4.2 min, respectively. The linearity for both the drugs was in the range of 2-12 μg/ml. The % recoveries of ambroxol hydrochloride and cetirizine hydrochloride were found to be in the range of 100.34±0.67 and 101.75±0.41, respectively. The proposed method was validated and successfully applied to the estimation of ambroxol hydrochloride and cetirizine hydrochloride in combined tablet dosage forms.

Keywords: Ambroxol hydrochloride, cetirizine hydrochloride, simultaneous estimation, RP-HPLC

INTRODUCTION

Ambroxol hydrochloride (AB) chemically, 1-((2-bromo-3,5-dibromophenyl)-methyl)amino)cyclohexanol monohydrochloride is a semi synthetic derivative of vasicine obtained from the Indian shrub "Adhatoda vasica". It is a mucolytic agent. Cetirizine hydrochloride (CT) or 2-[2-[4-[(4-chlorophenyl)phenylmethyl]-piperazin-1-yl]ethoxy]acetic acid dihydrochloride, is used for symptomatic relief of hypersensitivity reactions including rhinitis and chronic urticaria (1-3). The structures of ambroxol hydrochloride and cetirizine hydrochloride are shown in Figure 1. Numerous UV, HPLC and HPTLC methods have been reported for estimation of these drugs alone as well as in combination with other drugs in pharmaceutical dosage forms and/or in biological fluids (4-18). The previously published methods also make use of complicated mobile phase systems and need more investigation for method development and validation. Therefore, the main aim of present work was to develop and validate RP HPLC method for simultaneous estimation of AB and CT in pharmaceutical dosage forms.

MATERIALS AND METHODS

Chemicals and Reagents

Reference standards of AB and CT were obtained from Sun Pharma, Mumbai, India. HPLC grade acetonitrile, water and triethylamine were obtained from Rankem, Ranbaxy Fine Chemical Limited, New Delhi, India. Potassium dihydrogen orthophosphate AR and ortho-

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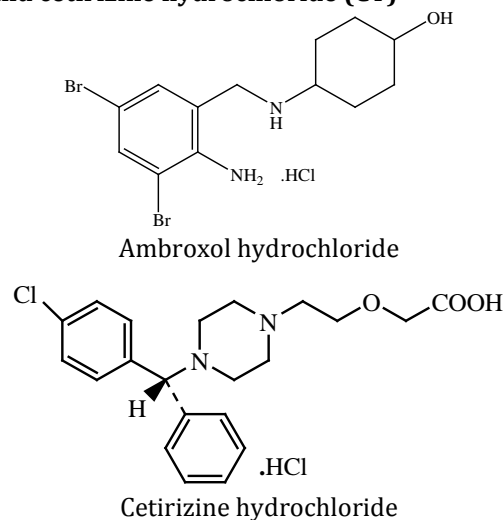
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Figure 1. The structures of ambroxol hydrochloride (AB) and cetirizine hydrochloride (CT)



phosphoric acid AR grade were procured from local sources unless specified.

Instrumentation

The HPLC (PerkinElmer series 200) instrument was equipped with a model series 200 pump, vacuum degasser, rheodyne injector with a 20 μl loop, UV-Visible detector and C-8 column.

Chromatographic Conditions

The isocratic mobile phase consisting of methanol-potassium dihydrogen phosphate buffer 80:20 (v/v) (10 mM, pH 3.5 ± 0.02, adjusted with orthophosphoric acid) was used at a flow rate of 1.0 ml/min. The variable wavelength UV-visible detector was set at 276 nm. All analyses were performed at ambient temperature.

Preparation of Mobile Phase

Mobile phase was prepared by mixing 800 ml of methanol with 200 ml of potassium dihydrogen phosphate buffer. The pH was adjusted to 3.5 ± 0.02 with ortho phosphoric acid. The mobile phase was sonicated for 15 min and filtered through a 0.45μ membrane filter paper.

Preparation of Standard Stock Solution

10 mg AB and 10 mg CT were accurately weighed and transferred to 100 ml volumetric flasks separately and dissolved in the mobile phase. The volume was adjusted with mobile phase to give stock solutions of $100 \mu\text{g/ml}$ each of AB and CT.

Preparation of Sample Solution

Twenty tablets (TRILERT-AX, Sun Pharma) were weighed and finely powdered. Tablet powder equivalent to 25 mg of AB and 5 mg of CT was transferred to a 100 ml volumetric flask and dissolved in 50 ml of mobile phase. The solution was ultrasonicated for 15 min and filtered through $0.45 \mu\text{m}$ membrane filter. The solutions were further diluted with mobile phase to obtain concentration of $25 \mu\text{g/ml}$ of AB and $2.5 \mu\text{g/ml}$ of CT and were subjected to HPLC analysis as described earlier. From the peak area of AB and CT, the amount of drugs in samples was computed (19).

Method Validation

The method was validated in terms of linearity, range, specificity, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ) (20).

Linearity and Range

Six different concentrations (2, 4, 6, 8, 10 and $12 \mu\text{g/ml}$) of mixture of two drugs were prepared for linearity studies. The responses were measured as peak area. The calibration curves obtained by plotting peak area against concentration showed linearity in the concentration range of 2 to $12 \mu\text{g/ml}$ for both the drugs. The linear regression equations for AB and CT were found to be $y = 14.233x + 32.164$, and $y = 15.602x + 35.822$, respectively. The regression coefficient values (r^2) were found to be 0.9998 and 0.9999 respectively indicating a high degree of linearity.

Specificity

The specificity studies proved the absence of interference, since none of the peaks of AB and CT appeared at the same retention time. The interaction study in standard solution was also carried out by comparing peak of each drug individually and in drug mixture.

Precision

From the standard stock solutions, mixed standards containing AB and CT were prepared. Standard solutions ($n=6$) were injected using a universal rheodyne injector with injection volume of $20 \mu\text{l}$. The intraday and interday precisions were determined.

Accuracy

Recovery studies were carried out by applying the standard addition method. A known amount of standard AB and CT corresponding to 80%, 100%, and 120% of the label claim was added to preanalysed sample of tablet dosage form separately. The recovery studies were carried out six times, at each level of recovery.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

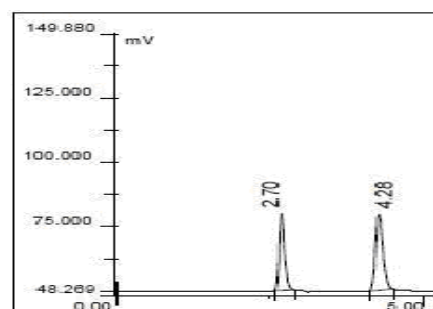
The LOD and LOQ were separately determined on the

basis of standard calibration curve. The residual standard deviation of the regression line or the standard deviation of y-intercepts of regression lines was used to calculate LOD and LOQ. Following formulae were used; $\text{LOD} = 3.3 \times D/S$ and $\text{LOQ} = 10 \times D/S$, where, D is the standard deviation of the y-intercepts of regression line and S is the slope of the calibration curve.

RESULTS AND DISCUSSION

Several mobile phase compositions were tried to resolve the peaks of AB and CT. The optimum mobile phase containing methanol-potassium dihydrogen phosphate buffer 80:20 (v/v) was selected because it could resolve the peaks of AB ($\text{RT} = 2.70 \pm 0.02$) and CT ($\text{RT} = 4.28 \pm 0.03$) with a resolution factor of 9.0. The pH was adjusted to 3.5 ± 0.02 with orthophosphoric acid. Quantification was achieved with UV detection at 276 nm on the basis of peak area. A typical HPLC chromatogram obtained during simultaneous determination of AB and CT is given in Figure 2.

Figure 2. HPLC chromatogram obtained during simultaneous determination of AB and CT



Linear regression data showed a good relationship over a concentration range of 2-12 $\mu\text{g/ml}$ for AB and CT. The correlation coefficients (r^2) were found to be 0.9998 and 0.9999 for AB and CT respectively. The limit of detection and limit of quantification were found to be 0.60 and $1.5 \mu\text{g/ml}$ for AB and 0.20 and $0.90 \mu\text{g/ml}$ for CT. The values indicate that the method is sensitive. The intra-day and inter-day precisions were assessed by analyzing standard solutions. The % RSD was found to be 0.51 and 0.62 for AB and CT respectively. The lower values of % RSD indicate that the method is precise.

Analysis of marketed tablets (TRILERT-AX) was carried out using optimized mobile phase. The % drug content of tablets obtained by the proposed method was found to be between 99.35% and 101.62%, which showed that the estimation of dosage forms were accurate within the acceptance level of 95% to 105%. The results are given in the Table 1.

Table 1. Analysis of marketed tablets

Drug	Label claimed (mg/tablet)	Quantity found (mg/tablet) (n = 3)	RSD (%)	% Assay
Ambroxol hydrochloride	60	60.35	0.94	100.58
Cetirizine hydrochloride	5	4.99	0.56	99.64

To study accuracy of the developed method, recovery study was carried out using standard addition method at three different levels. The average % recoveries for AB and CT in marketed formulation were found to be between 99.91 ± 0.67 and 100.29 ± 0.41 . The results revealed that there was no interference of excipients. The results of accuracy are shown in Table 2.

Table 2. Percent recovery data

Drug	% simulated dosage nominal	% Mean (n=6)	±SD	RSD (%)
AB	80	99.12	0.46	0.41
CT	80	100.50	0.78	0.63
AB	100	99.95	0.97	0.91
CT	100	99.38	0.67	0.55
AB	120	100.67	0.87	0.70
CT	120	100.98	0.52	0.45

System Suitability Parameters

For system suitability parameters, six replicate injections of mixed standard solution were injected and parameters such as the resolution, capacity factor, tailing factor,

CONCLUSION

Proposed study describes an RP-HPLC method for the estimation of AB and CT combination. The method has been found to be better than previously reported method, because of use of an economical and readily available mobile phase and UV detection. The method gives good resolution for both the drugs with a short analysis time (<10 min). The method was validated and found to be simple, sensitive, accurate and precise. Percentage recovery shows that the method is free from interference

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theoretical plate, retention volume and asymmetry factor of the peaks were calculated. The results are shown in Table 3.

Table 3. System suitability data

Parameters	Ambroxol Hydrochloride	Cetirizine Hydrochloride
Resolution	-	7.98
Capacity factor	0.15	0.69
Tailing factor	1.0	1.05
Theoretical plates	11020	15678
Asymmetry factor	1.0	1.02

of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of AB and CT in combined dosage form.

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