International Journal of Research in Pharmacy and Pharmaceutical Sciences
www.pharmacyjournal.in
ISSN: 2455-698X
Received: 15-08-2023, Accepted: 30-08-2023, Published: 16-09-2023
Volume 8, Issue 3, 2023, Page No. 44-47

Simultaneous UV-analytical method development and validation of ambroxol & cetrizine in combined dosage form

Ranjeet Kokate[1*], Tanaji Surushe[2], Mahesh Chitmalwar[3], Suryakant Bokhare[4], Chincholkar Nandini[5]
[1] Associate Professor, Department of Pharmaceutical Chemistry, Saraswati Institute of Pharmacy, Kurtadi, Hingoli, Maharashtra, India
[2] Associate Professor, Head of Department, Department of Pharmaceutical Chemistry, Saraswati Institute of Pharmacy, Kurtadi, Maharashtra, India
[3] Associate Professor, Department of Pharmaceutics, Saraswati Institute of Pharmacy, Kurtadi, Maharashtra, India
[4] Assistant Professor, Department of Pharmaceutical Chemistry, Saraswati Institute of Pharmacy, Kurtadi, Maharashtra, India
[5] Saraswati Institute of Pharmacy, Kurtadi, Maharashtra, India

Abstract
The aim of present work is to develop and validate simple, sensitive, economical and accurate Spectrophotometric method has been developed for simultaneous estimation of Ambroxol and Cetrizine. Ambroxol and Cetrizine in Methanol show maximum absorbance at 244 nm & 230 nm. The drug obeyed Beer's law in the concentration range of 0.1 -0.6µg/ml in methanol. The proposed methods were successfully applied for the determination of drug in commercial tablet preparations. The results of the analysis have been validated statistically and by recovery studies.

Keywords: Ambroxol, cetrizine, method validation, ultraviolet spectroscopy

Introduction

Development of simple and reproducible analytical methods for estimation of multicomponent drugs is very important part of quality control and assurance .Multicomponent formulations in market are increasing therefore, it is very essential that two or more number of drugs should be estimated simultaneously. Chemically Ambroxol HCl is trans-4-(2-Amino-3,5-dibrombenzylamino)-cyclohexanol, sodium salt. Ambroxol is a mucolyte, used in the treatment of respiratory disease. Ambroxol is a mucolytic .It breaks down the acid mucopolysaccharides fibres which makes the sputum thinner and less viscous and therefore promotes the mucous clearance. its also facilities expectoration and eases productive cough. Ambroxil also has mild anesthetic effect and therefore used to relieve the pain in sore throat /pharyngitis. The structure is shown in figure1

Chemically Cetrrizine HCl (±)-[2-[4-[(4-Chlorophenyl) phenylmethyl]-1-piperazinyl]ethoxy] acetic acid. CTZ is a Histamine Antagonists and it competes with free histamine for binding at H1-receptors in the GI tract, uterus, large blood vessels, and bronchial smooth muscle. These blocks the reduce action of endogenous histamine, which subsequently leads to temporary relief of the negative symptoms (e.g.nasal congestion, watery eyes) brought on by histamine. The structure is shown in figure 2.

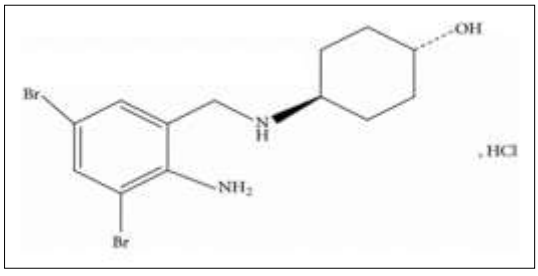

Fig 1: Structure of Ambroxol HCL

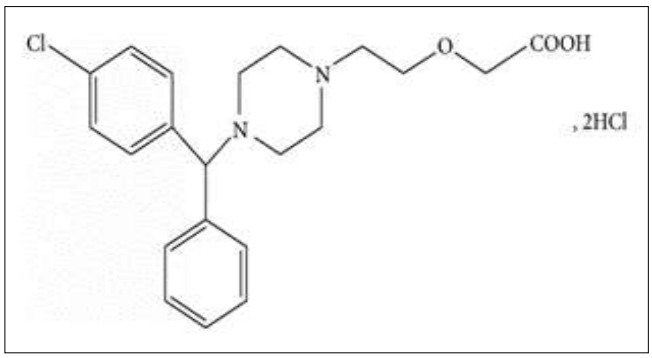

Fig 2: Structure of Cetrizine HCl

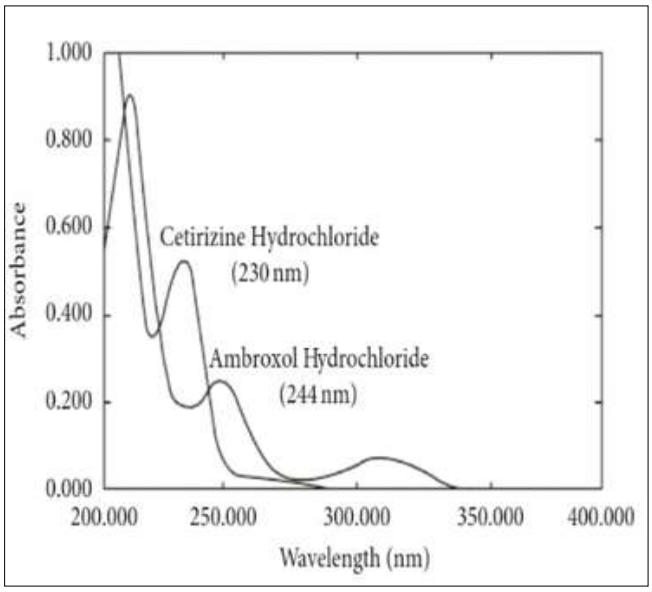

Fig 3: Overlay Spectrum of mixture CTZ and AMB

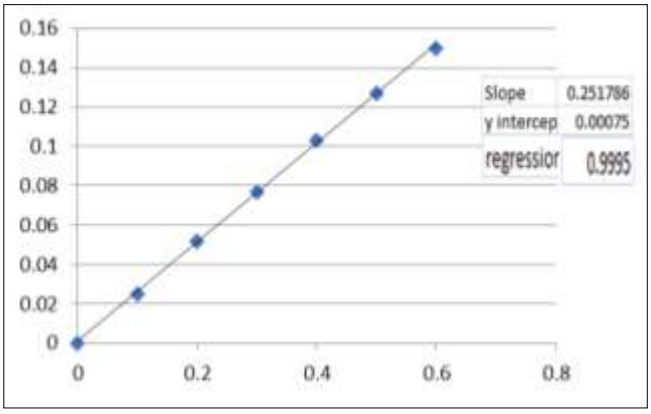

Fig 4: Calibration curve for CTZ

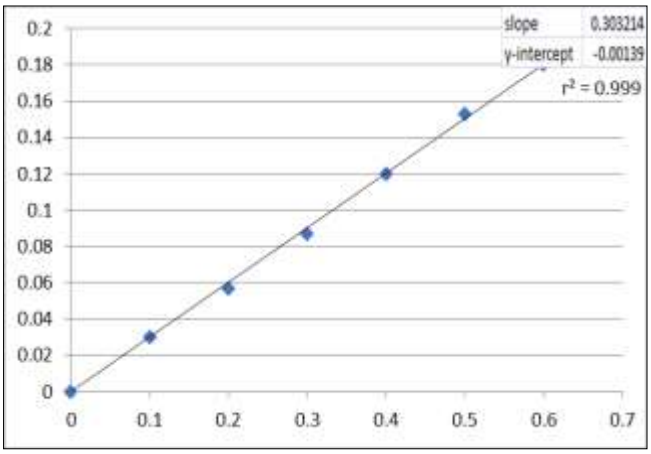

Fig 5: Calibration curve for AMB

Materials

Pharmaceutical grade Ambroxol HCl and Cetrizine HCl were pursued as a gift sample from Bidwai Chemicals Private Limited, Nanded (MH) India. All chemicals and solvents of AR grade and were purchased from Qualigens fine Chemicals, Mumbai, India. UV-spectrophotometer UV-1800 (Shimadzu, Japan) with spectral bandwidth of 2 nm and 10 mm matched quartz cells were used for development analytical method over the range of 200-400 nm. Marketed formulation Relent M tablet containing AMB 50 mg and CTZ 5 mg was used as sample; purchased from local pharmacy. Calibrated glassware was used throughout the work.

Procedure

Method used for simultaneous estimation of CTZ and AMB

CTZ (5 mg) and AMB (10 mg) were separately dissolved in methanol to get 50 µg/ml and 100 µg/ml solution respectively. This solution further diluted with methanol and scanned for maximum absorbance (λmax) in a UV-spectrophotometer between a U.V ranges from 200 to 400 nm against methanol as blank. For the estimation of both drugs, wavelength maxima of CTZ and AMB were determined and found to be 230 nm (λ1) and 244nm (λ2) respectively where there was no interference among the drugs. Calibration curve was plotted between absorbance and its nominal concentration in the range of 0.1- 0.6 µg/ml for CTZ and 0.1-0.6 µg/ml for AMB at their respective maxima.

Calibration curve of CTZ and AMB

Stock solutions of CTZ and AMB were prepared by dissolving 10 mg of CTZ and 10 mg AMB separately dissolved in methanol and then the volume was adjusted to 100 ml with methanol separately. Stock solutions of CTZ and AMB were subsequently diluted with methanol to get 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 µg/ml and 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 µg/mL respectively. Then the absorbance of these diluted solutions were measured at 230 nm (λ1) for CTZ and 244nm (λ2) for AMB by using double beam U.V. spectrophotometer against a blank of methanol. Average of six replicates readings was taken and tabulated. Regression equation was derived from the slope of the curve Y= 0.205X-0.001; r 2 = 0.998 for CTZ and For AMB regression equation is Y=0.106X-0.001; r2 = 0.998.

Preparation of sample solution

Twenty tablets were accurately weighed and crushed to get the fine powder. Powder equivalent to 5 mg of CTZ and 50 mg of AMB was weighed and dissolved in methanol, sonicated for 20 min and filtered. Then different concentrations of tablet sample were prepared by serial dilution technique and used for analysis.

Method Validation

The method validation parameters linearity, precision, accuracy, repeatability, limit of detection and limit of quantization were checked as per ICH guidelines.

Linearity and Range

The linearity for CTZ and AMB were determined at six concentration levels, ranging both from 0.1-0.6 µg/ml respectively using working standards.

Precision

Precision of the method was evaluated by interday and intraday variation studies. In intraday studies, working solutions of standard and sample were analysed thrice in a day and percentage relative standard deviation (% RSD) was calculated. In the interday variation studies, working solution of standard and sample were analysed on two consecutive days and percentage relative standard deviation (% RSD) was calculated. The data is reported in Table 4.

Limit of Detection and Limit of Quantitation

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula and shown in Table 4.

$$LOD = 3.3 (\sigma / S)$$

Where, S = slope of calibration curve, σ = standard deviation of the response.

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives a response that can be accurately quantified. LOQ was calculated using the following formula and shown in Table 4.

$$LOQ = 10 (\sigma / S)$$

Where, S = Slope of calibration curve, σ = standard deviation of the response.

Table 1: Analysis of tablet formulation

Sr.no.	Lable Claim (mg/tab)		Amount found (mg/tab)		% of Label Claim	
	CTZ	AMB	CTZ	AMB	CTZ	AMB
1	5	60	5.09	60.16	101.78	100.26
2	5	60	4.98	60.20	99.63	100.33
3	5	60	4.98	60.20	99.63	100.33
4	5	60	4.98	60.20	99.63	100.33
5	5	60	5.00	60.02	99.60	100.03
6	5	60	5.02	59.84	100.40	99.73
				Mean*	100.11	100.16
				SD*	0.8745	0.2441
				RSD*	0.8738	0.2437

*Denotes average of 6 determinations

Table 2: Linearity study of CTZ

Sr. No.	Concentration (µg/mL)	Absorbance(nm)	Regression Data		
1.	0.1	0.025	m=0.251	C=0.00075	r^2=0.999
2.	0.2	0.052			
3.	0.3	0.077			
4.	0.4	0.103			
5.	0.5	0.127			
6.	0.6	0.150			

Table 3: Linearity study AMB

Sr. No.	Concentration (µg/ml)	Absorbance (nm)	Regression Data		
1	0.1	0.030	m=0303	C=-0.00139	r^2=0.999
2	0.2	0.057			
3	0.3	0.087			
4	0.4	0.120			
5	0.5	0.153			
6	0.6	0.180			

Table 4: Intraday precision data

Component	% Mean.	S.D.	% R.S.D.
CTZ	100.15	0.8410	0.8397
AMB	101.02	1.4795	1.4647

Table 5: Intraday precision data

Component	% Mean.	S.D.	% R.S.D.
CTZ	100.15	0.8410	0.8397
AMB	101.02	1.4795	1.4647

Table 6: Repeatability data

Component	% Mean*	Standard Deviation*	Relative Standard Deviation*
CTZ	99.75	0.7385	0.7403
AMB	99.63	0.5991	0.6013

Table 7: Summary of validation parameters for dual wavelength method

Parameters		CTZ	AMB
Linearity range (µg/ml)		0.1-0.6	0.1-0.6
Correlation coefficient (r^2)		0.999	0.999
Precision (RSD)	Intraday*	0.8397	1.4647
	Interday*	0.8397	1.4647
Accuracy (%)	80%@	101.04	100.16
	100%@	100.46	100.20
	120%@	99.36	100.36
Repeatability (RSD)*		0.7403	0.6013
LOD (µg/ml)		0.0355	0.015
LOQ(µg/ml)		0.1071	0.043

Results and Discussion

In the present work, new estimation method was developed for the simultaneous spectroscopic estimation of CTZ and AMB in commercially available tablet dosage form. The concentrations in the range of 0.1-0.6 µg/ml of CTZ and 0.1-0.6 µg/ml of AMB mixed working standard and two set of wavelengths gave optimum accuracy, precision, economy, and sensitivity for this method. The proposed procedure was successfully applied to the determination of CTZ and AMB in the commercially available tablets dosage form, and the results are reported in Table No. 1. The recovery studies were carried out at different concentrations by spiking a known concentration of standard drug to the pre-analyzed sample and contents were pre analyzed by proposed method. The method was validated statistically for range, linearity, precision, accuracy, repeatability, LOD, and LOQ (Table No. 7). Accuracy was ascertained on the basis of recovery studies. Precision was calculated as Interday and Intraday variation for both drugs. The content estimated using the proposed method was found in agreement with labelled amount. The relative standard deviations were found to be within the limit, indicating good accuracy, precision, and repeatability of the proposed method.

Conclusion

The simultaneous estimation method permits simple, rapid and direct determination of CTZ and AMB in commercially available tablet dosage form without previous separation. The results of analysis of two drugs from tablet formulation using method was found close to 100%, standard deviation was satisfactorily low indicating accuracy and reproducibility of the method. Recovery studies were satisfactory which showed that there is no interference of excipients. The most striking feature of this method is its simplicity and rapidity, non- requiring consuming sample preparations such as extraction of solvents, heating, degassing which are generally needed for HPLC analysis. It is a new and novel method and can be employed for routine quality control analysis. The described method gives accurate and precise results for determination of CTZ and AMB in Tablets.

Acknowledgement

Authors are thankful to Bidwai Chemicals Pvt. Ltd Nanded, Maharshtra for providing us the drug sample. Also thankful to Director RUSA Centre Swami Raman and Teerth Marathwada University, Nanded for providing necessary Instruments facilities, also Management and Principal of Saraswati Institute of Pharmacy, Kurtadi Hingoli for providing research platform and support.

References

1. Chatwal Gurudeep R, Anand Sham. Instrumental Method of Chemical Analysis, Himalaya Publishing House pp. 2.149-2.184.
2. Indian Pharmacopoeia, Volume-II, Govt. of India, Ministry of Health and Family Welfare. The controller of publication, Ghaziabad, 2014, Cetrizine Hydrochloride pp. 1339-1340 And Ambroxol Hydrochloride, 2014, 1025-1026.
3. ICH guideline, Q2 (R1) Step 4, Validation of Analytical Procedures: Text and Methodology, 2005.
4. Chakraborty D Chakraborty R, Kundu S, Rakesh Kumar R. Development and validation of a new HPLC-UV Method for simultaneous estimation of Cetrizine and Phenylpherine Hydrochloride in tablet formulation, International Journal of Pharmaceutical Science and Research.2018:10(7):3349-3353.
5. Sharma Deepak, Singh Mankkaran, Kumar Dinesh, Singh Gurmeet. Spectroscopic method development and validation for simultaneous estimation of Salbutamol sulphate & Ambroxol HCl in combined dosage form, International Journal of Drug Development & Research.2013:5(4):124-132.
6. Wankhede S.B, Lad K.A, Chitlange S.S. Development and validation of UV Spectrophotometric method for simultaneous estimation of Cetrizine hydrochloride and Phenylpherine hydrochloride in tablet. International Journal of Pharmaceutical Science & Drug Research,2012:4(3):222-226.
7. ICH, Q2B Validation of Analytical Procedures: Methodology, Consensus, Consensus Guidelines.