Research Article

SIMULTANEOUS ESTIMATION OF LEVOCETIZINE DIHYDROCHLORIDE AND AMBROXOL HYDROCHLORIDE IN SYRUP DOSAGE FORM BY USING UV SPECTROPHOTOMETRIC METHODS. S. Pekamwar^{1*}, T. M. Kalyankar¹, Madhav Shelke²¹Pharmaceutical Chemistry Department, School of Pharmacy, S. R. T. M. University, Nanded, Maharashtra, India²Quality Assurance Department, School of Pharmacy, S. R. T. M. University, Nanded, Maharashtra, India

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DOI: 10.7897/2230-8407.068111**ABSTRACT**

A simple, rapid, accurate, precise and economical UV Spectrophotometric method developed for simultaneous estimation of Levocetizine dihydrochloride (LCTZ) and Ambroxol hydrochloride (AMB) in bulk and Syrup formulation and validate as per ICH guidelines. Determination of Levocetizine dihydrochloride and Ambroxol hydrochloride in bulk and syrup was conducted by using simultaneous equation method based on measurement of absorbance at two wavelengths. The solvent used for proposed method was methanol: water (1:1). The λ_{max} of Levocetizine dihydrochloride and Ambroxol hydrochloride were found to be 231 nm and 246 nm, respectively. The linearity for LCTZ and AMB was in the range of 5-35 $\mu\text{g/ml}$ and 5-50 $\mu\text{g/ml}$, respectively. The % recovery was found to be in the range of 99.52- 99.82% and 100.37- 100.72% for LCTZ and AMB, respectively. Percentage RSD for precision and accuracy of the method was found to be less than 2%. LOD values for LCTZ and AMB were found to be 3.0891 $\mu\text{g/ml}$ and 3.1645 $\mu\text{g/ml}$, respectively. LOQ values for LCTZ and AMB were found to be 10.224 $\mu\text{g/ml}$ and 10.474 $\mu\text{g/ml}$, respectively. Proposed method can be successfully employed for routine analysis of Levocetizine dihydrochloride and Ambroxol hydrochloride in bulk and Syrup formulation.

Keywords: Levocetizine dihydrochloride, Ambroxol hydrochloride, Simultaneous Equation Method, UV Spectrophotometry, Syrup, Validation.**INTRODUCTION**

Levocetirizine is a third generation non-sedative antihistamine. Chemically, levocetirizine is the active enantiomer of cetirizine. It is L-enantiomer of the cetirizine racemate. Levocetirizine works by blocking histamine receptors. Levocetirizine is chemically (R)-2-[2-[4-[(4-chlorophenyl)phenylmethyl] piperazin-1-yl] ethoxy] acetic acid dihydrochloride.

Ambroxol is an active N-desmethyl metabolite of mucolytic bromhexine. Ambroxol is used as a bronchosecretolytic and expectorant drug. Ambroxol has been shown to increase the quantity and decrease the viscosity of respiratory tract secretions. It enhances pulmonary surfactant production (surfactant activator) and stimulates ciliary motility. These actions result in improved mucus flow and transport (mucociliary clearance). Enhancement of fluid secretion and mucociliary clearance facilitates expectoration and eases cough. Ambroxol is chemically trans-4-[(2-Amino-3,5-dibromobenzyl)amino] cyclohexanol hydrochloride.¹⁻⁴

Literature survey revealed that various analytical methods involving UV Spectrophotometry, HPLC and HPTLC have been reported for LCTZ and AMB alone and in combination with other drugs. However, there is no method developed for simultaneous estimation of Levocetizine dihydrochloride and Ambroxol hydrochloride in liquid dosage form (Syrup). The objective of present work was to develop simple, rapid, economic, accurate and precise analytical method for simultaneous estimation of Levocetizine dihydrochloride and Ambroxol hydrochloride in bulk and in liquid dosage form (Syrup). The developed method was validated in accordance with ICH guidelines and successfully employed for the assay of LCTZ and AMB in combined liquid dosage form (Syrup).

MATERIALS AND METHODS

Chemicals and Reagents: Bidwai Chemicals Pvt. Ltd. Nanded and Glenmark Pharmaceuticals, Mumbai, Maharashtra provided analytically pure LCTZ and AMB as gift sample, respectively. Syrup of LCTZ and AMB, Mzic D[®] manufactured by Monad Pharmaceuticals, was purchased from local pharmacy. Methanol AR grade (Molychem) and Distilled water was used throughout the experimental work.

Instruments: A UV-Visible Spectrophotometer: Shimadzu, Model: UV-1800 with 1 cm matched quartz cell and UV Probe 2.33 software was used. Calibrated analytical balance Anamed was used for weighing purpose. Digital Ultrasonic Cleaner (Sonicator): HMG India and Vacuum Pump Assembly: Millipore was used for the experimental work.

Method Development**Ambroxol hydrochloride standard stock solution**

Accurately weighed reference standard of AMB (10 mg) was transferred to 100 ml volumetric flask and dissolved in 50 ml solvent (Water, methanol 1:1) and sonicated for 5 min. then volume was made up to the mark with same solvent, to obtain standard stock solution (100 $\mu\text{g/ml}$) of drug. For the preparation of working standards, suitable aliquots of stock solution were pipette out and volumes were made up to the mark with solvent (Water, methanol 1:1), to get required concentrations.

Levocetizine dihydrochloride standard stock solution

Accurately weighed reference standard of LCTZ (10 mg) was transferred to 100 ml volumetric flask and dissolved in 50 ml solvent (Water, methanol 1:1) and sonicated for 5 min. then volumes was

made up to the mark with same solvent to obtain standard stock solution (100µg/ml) of drug. For the preparation of working standards, suitable aliquots of stock solution were pipette out and volumes were made up to the mark with solvent (Water, methanol 1:1), to get required concentrations.

Selection of analytical wavelength

Sample solutions were scanned over the wavelength range of 200 nm to 400 nm. λ_{max} for AMB and LCTZ were found at 246 nm and 231 nm. Representative absorption spectra of AMB and LCTZ are shown in figure 3 & 4.

Preparation of Calibration curve of AMB and LCTZ

By appropriate dilution of standard stock solution, different dilutions were prepared ranging from 5µg/ml to 35µg/ml for LCTZ and 5 µg/ml to 50 µg/ml for AMB. Absorbances of all the dilutions were plotted against the respective concentrations to obtain the calibration curve. The absorption-overlain spectrum of AMB and LCTZ is shown in figure 5.

Analysis of marketed syrup formulation

As per label claim syrup contain LCTZ 5 mg/ 5 ml and AMB 30 mg/ 5 ml, so 5 ml syrup was pipette out in 100 ml volumetric flask and dissolved in water: methanol (1:1) solution, sonicated for 10 min and filtered. Then different concentrations of syrup sample were prepared

by serial dilution method and used for analysis. Prepared syrup sample was analysed on UV spectrophotometer using Simultaneous Equation Method.

Method Validation

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

Precision: Precision of the method was verified by using syrup stock solution, the repeatability indicates the performance of the UV instrument. Inter-day and intra-day precision was determined by repeating the assay three times in same day for intraday precision and on different day for inter-day precision studies.

Accuracy (Recovery Study): To check the accuracy of proposed method, recovery studies were carried out at 80%, 100% and 120% of the test concentrations as per ICH guidelines.

To perform recovery study at 100%, 5 ml syrup pipette out in volumetric flask and to this standard 30 mg AMB and 5 mg LCTZ were added. The volume was made up to the mark with water: methanol (1:1) solution and further dilution with same solvent to obtain sample solution Similarly, for 80% recovery study, 26 mg AMB and 4 mg LCTZ were added, and for 120% recovery study, 36 mg AMB and 6 mg LCTZ were added respectively.

Table 1: Analysis of Syrup Formulation

Sr. No.	Label claim (mg/5ml)		Amount found (mg/5ml)		% of Label claim	
	AMB	LCTZ	AMB	LCTZ	AMB	LCTZ
1	30	5	30.21	4.97	100.70	99.57
2	30	5	30.10	4.96	100.34	99.35
3	30	5	29.95	4.96	99.84	99.28
4	30	5	30.20	4.97	100.68	99.54
5	30	5	29.93	4.96	99.78	99.25
6	30	5	30.14	4.97	100.47	99.42

Table 2: Statistical validation analysis of syrup formulation

Name of the drug	Mean*	SD	%RSD
AMB	100.30	0.4040	0.4027
LCTZ	99.40	0.1328	0.1336

* Indicates average of six determinations

Table 3: Intra-day Precision data of marketed formulation

Sr. No.	Interval of Time	Concentration (µg/ml)		% Recovery	
		AMB	LCTZ	AMB	LCTZ
I	Intra-day	30.10	4.96	100.34	99.35
II		29.95	4.95	99.84	99.28
III		30.20	4.97	100.68	99.54

Table 4: Statistical validation of intra-day precision data

Name of the drug	Mean*	SD	%RSD
AMB	100.28	0.4225	0.4213
LCTZ	99.40	0.1345	0.1353

* Indicates average of three determinations

Table 5: Inter-day Precision Data

Sr. No.	Interval of Time	Concentration (µg/ml)		% Recovery	
		AMB	LCTZ	AMB	LCTZ
I	Inter-day	30.14	4.98	100.45	99.79
II		30.09	4.98	100.32	99.72
III		29.96	4.98	99.87	99.63

Table 6: Statistical validation of inter-day precision data

Name of the drug	Mean*	SD	%RSD
AMB	100.21	0.3043	0.3036
LCTZ	99.71	0.0802	0.0804

* Indicates average of three determinations

Table 7: Recovery study data

Level of Recovery	Amount present (mg/5ml)		Added concentration (mg)		Amount recovered (mg)		% Recovery	
	AMB	LCTZ	AMB	LCTZ	AMB	LCTZ	AMB	LCTZ
80%	30	5	24	4	54.31	8.97	100.58	99.74
	30	5	24	4	54.20	8.95	100.38	99.52
	30	5	24	4	54.38	8.98	100.72	99.82
100%	30	5	30	5	60.28	9.96	100.47	99.68
	30	5	30	5	60.32	9.97	100.54	99.70
	30	5	30	5	60.22	9.95	100.38	99.54
120%	30	5	36	6	66.31	10.96	100.48	99.69
	30	5	36	6	66.36	10.97	100.56	99.72
	30	5	36	6	66.24	10.95	100.37	99.58

Table 8: Statistical validation of recovery study data

Level of recovery	% Mean recovery		S.D.		% R.S.D.	
	AMB	LCTZ	AMB	LCTZ	AMB	LCTZ
80%	100.56	99.70	0.1708	0.1553	0.1698	0.1557
100%	100.46	99.64	0.0802	0.0871	0.0798	0.0874
120%	100.47	99.67	0.0953	0.0737	0.0948	0.0951

Table 9: Summary of validation parameters of proposed UV method

Parameters		AMB	LCTZ
Detection wavelength (nm)		246	231
Linearity range ($\mu\text{g/ml}$)		5 – 50	5 – 35
Correlation coefficient (r^2)		0.9997	0.9998
Slope (m)		0.029	0.036
Intercept (c)		0.029	0.025
Precision (%RSD)	Intra-day	100.28	99.40
	Inter-day	100.21	99.71
Accuracy (%)	80% $\pm$ %RSD	100.56 $\pm$ 0.1698	99.70 $\pm$ 0.1557
	100% $\pm$ %RSD	100.46 $\pm$ 0.0798	99.64 $\pm$ 0.0874
	120% $\pm$ %RSD	100.47 $\pm$ 0.0948	99.67 $\pm$ 0.0951
Repeatability(%RSD)		1.0111 $\pm$ 0.2571	0.8843 $\pm$ 0.3392
LOD ($\mu\text{g/ml}$)		3.1645	3.0891
LOQ ($\mu\text{g/ml}$)		10.474	10.224

Figure 1: Chemical Structure of Levocetirizine dihydrochloride

Figure 2: Chemical Structure of Ambroxol hydrochloride

Figure 3: Absorption Spectra of LCTZ

Figure 4: Absorption spectra of AMB

Figure 5: Overlay Spectra of LCTZ & AMB

Figure 6: Calibration Curve of LCTZ

Figure 7: Calibration Curve of AMB

RESULTS AND DISCUSSION

Linearity

From the calibration plot of LCTZ and AMB at their respective absorption maximas, the linearity was observed in the concentration range of 5-35 µg/ml for LCTZ and 5-55 µg/ml for AMB. Coefficient of correlation (R) was found to be 0.9998 for LCTZ and 0.9997 for AMB. The high value of Coefficient of correlation (R) also indicates good linearity of calibration curve for the drugs as shown in fig. 6 and 7.

Precision

Precision studies were carried out to study the intra-day and inter-day variations of the response. Intraday precision (%RSD) was assessed by analyzing standard drug solutions within the calibration range, three times on same day. Inter-day precision (%RSD) was assessed by analyzing drug solutions within the calibration range on three different days. The intra-day and inter-day precisions were determined, results of which are given in table 3-6.

Accuracy (Recovery study)

In order to check the accuracy, reproducibility and precision of the proposed method, recovery study was carried out by taking standard mixture solution of LCTZ and AMB at 231 nm and 246 nm, respectively. Known amount of LCTZ (4, 5, 6 mg) and AMB (24, 30, 36 mg) were added to a pre-quantified sample solution and the amount of LCTZ and AMB were estimated by proposed method.

LOD and LOQ

LOD and LOQ of the drug were calculated as per ICH guidelines, using following formulas.

$$\text{LOD} = 3.3 \times \sigma / S \text{ and } \text{LOQ} = 10 \times \sigma / S$$

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

LOD values for LCTZ and AMB were found to be 3.0891 µg/ml and 3.1645 µg/ml, respectively. LOQ values for LCTZ and AMB were found to be 10.224 µg/ml and 10.474 µg/ml, respectively.

DISCUSSION

The proposed UV spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of LCTZ and AMB in syrup dosage form. The method utilizes easily available and cheap solvent for analysis of LCTZ and AMB in syrup dosage form. The common excipients and other additives are usually present in syrup dosage form do not interfere in the analysis of LCTZ and AMB in method, hence it can be conveniently adopted for routine quality control analysis of the drugs in combined pharmaceutical formulation.

CONCLUSION

The method developed is simple, rapid and does not involve the use of complex instrument. Low value of standard deviation showed that the method is precise and high percentage of recovery (as shown in table) showed that the method is accurate. Thus from the results it is concluded that above developed UV method is suitable for estimation of AMB and LCTZ in syrup formulation. The method is sensitive, precise, accurate, specific and economic. Hence, the method can be used successfully for quality control and routine analysis of finished pharmaceutical dosage form.

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ABBREVIATIONS

LCTZ: Levocetirizine Dihydrochloride, AMB: Ambroxol Hydrochloride, RSD: Relative Standard Deviation, SD: Standard Deviation, LOD: Limit of Detection, LOQ: Limit of Quantitation

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