INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

DEVELOPMENT AND VALIDATION OF ABSORBANCE CORRECTION METHOD FOR SIMULTANEOUS DETERMINATION OF LEVOCETIRIZINE AND AMBROXOL IN COMBINED TABLET DOSAGE FORM

Nancy J. Thakkar*, Satish A. Patel
Department of Pharmaceutical Quality Assurance, Shree S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat Vidyanagar, Mehsana, Gujarat, India
*Corresponding Author Email: nancy.thakkar92@gmail.com

Article Received on: 21/01/15 Revised on: 20/02/15 Approved for publication: 17/03/15

DOI: 10.7897/2230-8407.06349

ABSTRACT

A new, simple, accurate and sensitive UV-Spectrophotometric absorbance correction method has been developed and validated for simultaneous estimation of Levocetirizine (LEV) and Ambroxol (AMB) in a combined tablet dosage form. Distill water was used as solvent. In this method, two wave lengths on Ambroxol curve were selected where it showed same absorbance, which were 261 nm and 303 nm. At 303 nm, Levocetirizine showed zero absorbance while at 261 nm it gave considerable absorbance. At 261 nm, both the drugs showed some absorbance. The absorbance of Levocetirizine was corrected at 261 nm. The method was found to be linear between the range of 4-28 µg/ml for Levocetirizine and Ambroxol. The mean percentage recovery was found in the range of 99.4 ± 1.11 % and 100.2 ± 1 % for Levocetirizine and Ambroxol respectively at three different levels of standard additions. The precision (intra-day, inter-day) of method were found within limits (RSD < 2 %). The method was validated according to ICH guidelines. Thus the proposed method was simple, precise, economic, rapid and accurate and can be successfully applied for simultaneous determination of Levocetirizine and Ambroxol in combined tablet dosage form.

Keywords: Levocetirizine, Ambroxol, Absorbance correction method, UV spectrophotometric, Validation.

INTRODUCTION

Levocetirizine (Figure 1) is the active R enantiomer of cetirizine. It is third generation antihistamine and chemically is, (2-{4-[(R)-(4-Chlorophenyl) (phenyl) methyl]-1-piperazinyl}ethoxy)acetic acid, which is a selective H_1 -receptor antagonist. This drug is official in Indian Pharmacopoeia. IP describe LC and potentiometry methods for its estimation[1]. Literature survey reveals that LC[2], spectrophotometry[3,4] and charge transfer complexation[5,6] methods have been reported for its determination in combination and single dosage form. Ambroxol (Figure 2) is metabolite of bromhexine, a derivative of the alkaloid vasicine obtained from *Adhatoda vasica* (Vasaka). It is potent mucolytic and mucokinetic (expectorant) and chemically is trans-4-(2-Amino-3,5-dibrombenzylamino)-cyclohexanol. This drug is official in British Pharmacopoeia, Indian Pharmacopoeia and European Pharmacopoeia. The above three pharmacopoeias describe potentiometry method for its estimation[7-9]. Literature survey reveals that diffuse reflectance spectroscopy[10], spectrophotometry[11,12], RP-HPLC[13], stability indicating HPTLC[14] and acid dye methods[15] methods have been reported for the estimation of Ambroxol from pharmaceutical formulations and in biological fluids.

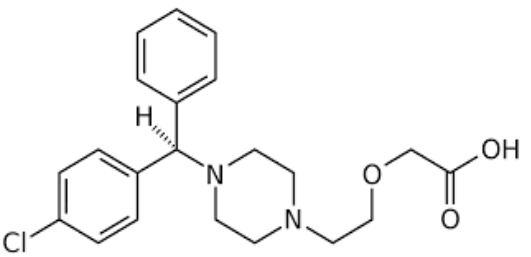

Figure 1: Chemical Structure of Levocetirizine

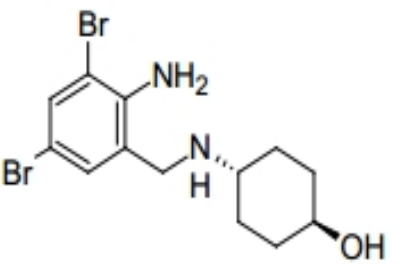

Figure 2: Chemical Structure of Ambroxol

The combination of Levocetirizine and Ambroxol is not official in any pharmacopoeia; hence no official method is available for estimation of these two drugs in combined dosage form. Literature survey reveals that several RP-HPLC[16-18] and spectrophotometric[19] methods have been reported for estimation of Levocetirizine and Ambroxol in combined dosage form. So far no UV-visible spectroscopic method was reported by absorbance correction method of quantitative estimation of Levocetirizine and Ambroxol in combined dosage form. So, it was thought of interest to develop a new, simple, precise, accurate and cost effective method of analysis for the simultaneous estimation of Levocetirizine and Ambroxol in combined tablet dosage form by absorbance correction method.

MATERIALS AND METHODS

Levocetirizine and Ambroxol pure powder was obtained as a gift sample from Camper Pharmaceutical Limited (Kherva, Gujarat, India). All the reagents used were of AR grade and procured from S. D. Fine chemicals Ltd., Mumbai, India. A double beam UV-visible

spectrophotometer (UV–1800, Shimadzu, Japan) attached to computer software UV Probe 2.0 with a spectral width of 2 nm, wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells. A Sartorius (CP224S) analytical balance and ultrasonic cleaner (Frontline FS-4) sonicator were used during the study. Tablet of Levocetirizine and Ambroxol were purchased from local pharmacy.

Preparation of Standard Solutions

A standard stock solution of Levocetirizine and Ambroxol (100 µg/ml) was prepared by dissolving 10 mg of pure drug powder to 100 ml volumetric flask separately in distill water. Aliquots of standard stock solution of Levocetirizine and Ambroxol were suitably diluted with distill water to obtain the final concentration in the range of 4-28 µg/ml. The solution was scanned in the range of 200 nm to 400 nm against distill water as a blank, to obtain the absorbance. The absorbance measured at 261 nm for Levocetirizine and at 303 nm for Ambroxol. The calibration curve was prepared by plotting concentration of Levocetirizine or Ambroxol vs. Absorbance of solution. Absorptivity of Ambroxol was calculated at 303 nm (a_1) and 261 nm (a_2) and absorptivity of Levocetirizine was calculated at 261 nm (a_3).

Preparation of Sample Solution

For analysis of Levocetirizine and Ambroxol in tablet dosage form, twenty tablets were accurately weighed and powdered. A quantity of the accurately weighed tablet powder equivalent to 1 mg of Levocetirizine and 12 mg of Ambroxol was transferred to a 100 ml volumetric flask. To this powder mixture 10 mg of Levocetirizine pure drug was added to get the considerable result. From the result obtained, the content of pure Levocetirizine drug was subtracted to get the content of sample. The content was mixed with distil water (30 ml) and sonicated for 20 min. to dissolve the drug as completely as possible. Finally volume was made up to the mark with distill water and further shaken for 15 min for complete extraction of from its matrix. Above solution filtered through wattman filter paper No.42 and diluted up to mark with distill water. Aliquot (1 ml) of above prepared sample solution was suitably diluted with methanol to obtain solution of Levocetirizine (12 µg/ml) and Ambroxol (10 µg/ml) and analyzed by absorbance correction method calculated as following,

$$A = a * b * c$$
$$C_X = A_1 / a * b$$
$$C_X = A_1 / a_1 * b \quad \text{..... 1}$$
$$A_1 = a_2 * b * C_X$$
$$A\,(Mix) = A_1\,(AMB) + A_2\,(LEV)$$
$$A_2 = A - A_1$$
$$C_Y = A_2 / a_3 * b \quad \text{..... 2}$$

Where, A_1 = Absorbance of sample solution at 303 nm, A_2 = Absorbance of sample solution at 261 nm, a_1 = Absorptivity of Ambroxol at 303 nm, a_2 = Absorptivity of Ambroxol at 261 nm, a_3 = Absorptivity of Levocetirizine at 261 nm

RESULTS AND DISCUSSION

Under the experimental conditions, linearity, precision, accuracy and assay, LOD and LOQ were estimated. Linearity was obtained over the concentration range of 4-28 µg/ml for Levocetirizine and Ambroxol. Correlation coefficient was found to be > 0.995. The values of S.D. and R.S.D. were found to be less than 2.0, which indicate precision of the method. The results are presented in Table 1. The % assay was found to be 99.63 % for Levocetirizine and 100.1 % for Ambroxol. No interference was observed from the pharmaceutical adjuvants /excipients. The proposed method was accurate, precise, sensitive and reproducible for simultaneous determination of Levocetirizine and Ambroxol in bulk and combined dosage form. The method utilizes easily available and cheap solvent for analysis; hence the method was also economic for estimation.

Method Validation

The developed method was validated as per ICH guideline[20] and validation parameters are summarized in Table 1.

Linearity

The drugs obey beer's law in the concentration range of 4-28 µg/ml for Levocetirizine and Ambroxol respectively. The high value of correlation coefficient suggested that the proposed method is linear in the stated range.

Precision

The % RSD value for repeatability study of Levocetirizine and Ambroxol were found to be 1.47 and 0.58. The low values (< 2 %) of RSD indicate that the proposed method is repeatable. The low values of % RSD for intraday (0.23 – 1.31 and 0.11 – 1.87) and interday (0.76 – 1.85 and 0.26 – 1.69) precision respectively for Levocetirizine and Ambroxol indicate that the proposed method is precise and reproducible.

LOD and LOQ

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations as per International Conference on Harmonization (ICH) guidelines[19].

$$LOD = 3.3 \times \sigma/S$$
$$LOQ = 10 \times \sigma/S$$

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

LOD for Levocetirizine and Ambroxol were found to be 1.05 µg/ml and 0.24 µg/ml, respectively. LOQ for Levocetirizine and Ambroxol were found to be 3.18 µg/ml and 0.74 µg/ml, respectively (Table 1). Low values of LOD and LOQ describe the method is sensitive.

Accuracy

Recovery studies were done by standard addition method by adding known quantity of standard solution (50 %, 100 % and 150 % levels) to preanalyzed sample solution and the mixtures were reanalyzed by proposed method. The mean recovery obtained was 99.4 ± 0.15 % and 100.2 ± 0.18 % for Levocetirizine and Ambroxol, respectively (Table 1). The high values indicate that the method is accurate.

Assay

The assay results obtained was 99.63 ± 0.74 for Levocetirizine and 100.1 ± 0.96 for Ambroxol (Table 2); indicate that the excipients do not interfere during the analysis normally present in the tablet.

Table 1: Regression analysis Data and Summary of Validation Parameters by Proposed Absorption Correction Method

Parameters	Absorbance correction method	
	LEV	AMB
Wavelength	261 nm	303 nm
Beer's Law Limit	4-28 µg/ml	4-28 µg/ml
Regression equation (y = mx + c)	y = 0.0011x + 0.0024	y = 0.0067x - 0.0017
Slope	0.0011	0.0067
Intercept	0.0024	0.0017
Correlation coefficient (R^2)	0.9992	0.9984
LOD[a] (µg/ml)	1.05	0.24
LOQ[b] (µg/ml)	3.18	0.74
Accuracy (% recovery, n = 6)	99.4 ± 0.15	100.2 ± 0.18
Repeatability (% RSD[c], n = 6)	1.47	0.58
Precision (% RSD, n = 3)		
Intraday	0.23-1.31	0.11-1.87
Interday	0.76-1.85	0.26-1.69

[a]Limit of detection, [b]Limit of quantification, [c]Standard deviation, [d]Relative standard deviation

Table 2: Assay Results for the Tablet Dosage Form

Brand	Label Claim (mg)		Amount found (mg)		% Label Claim ± S.D. (n = 6)	
	LEV	AMB	LEV	AMB	LEV	AMB
Brand I	5	60	4.98	60.05	99.63	100.1

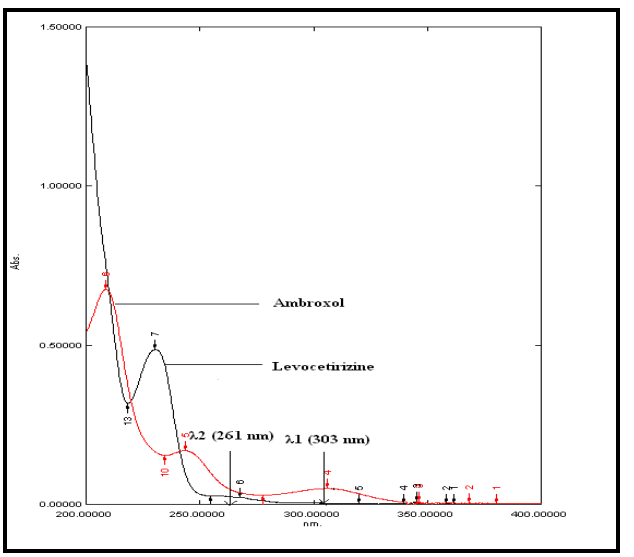

Figure 3: Overlain UV Absorption Spectra of Levocetirizine (20 µg/ml) and Ambroxol (20 µg/ml) in Distill Water

CONCLUSION

The proposed absorbance correction method was found to be linear between the range of 4-28 µg/ml for Levocetirizine and Ambroxol. The mean percentage recovery was found 99.4 % and 100.2 % for Levocetirizine and Ambroxol, respectively at three different levels of standard additions. The precision (repeatability, intra-day and inter-day) of methods were found within limits (RSD < 2 %). It could be concluded from the results obtained in the present investigation that the proposed method for the simultaneous estimation of Levocetirizine and Ambroxol in tablet dosage form is simple, rapid, accurate, precise and economical and can be used, successfully in the quality control of pharmaceutical formulations and other routine laboratory analysis.

ACKNOWLEDGEMENT

The authors are thankful to Camper Pharmaceutical Limited (Kherva, Gujarat, India) for providing gift sample of Levocetirizine and Ambroxol for research. The authors are thankful to Shree S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat Vidyanagar, Mehsana, India for providing all the facilities to carry out this work.

REFERENCES

1. Indian Pharmacopoeia. Government of India, Ministry of Health and Family Welfare. Published by the Controller of Publication, New Delhi 2010; 2: 1573-1575.
2. Chaitanya Prasad MK, Vidyasagar G, Madhusudhanareddy I, Sambasiva Rao KRS, Ramanjeneyulu S. Development of Validated Liquid Chromatographic Method for Estimation of Levocetirizine from Pharmaceutical Dosage Forms. Journal of Applied Pharmaceutical Science 2011; 1(10): 95-97.
3. Prashant S, Virag S, Dhananjay G, Rohit S, Shital P, Dhanya C. Validation of UV Spectrophotometric Method for Estimation of Levocetirizine Dihydrochloride in Bulk and Pharmaceutical Formulation. Journal of Pharmacy Research 2010; 3(10): 2386 -2387.

4. Vishakha VD, Dipmala DW, Swetal PV, Kishore NG. Development of First Order Derivative Ultraviolet Spectrophotometric Method for Simultaneous Estimation of Levocetirizine Hydrochloride and Phenylephrine Hydrochloride in Bulk and Combined Dosage Form. Int. Res. J. Pharm 2013; 4(5): 115-119. http://dx.doi.org/10.7897/2230-8407.04523
5. Basavaiah K, Raghu MS, Vinay KB. Simple and Rapid Spectrophotometric Assay of Levocetirizine in Pharmaceuticals Through Charge-transfer Complexation Using Chloranilic Acid and 2,3-dichloro-5,6-dicyanoquinone as π- Acceptors. Bull Chem Soc Ethiop 2012; 26(3): 319-328. http://dx.doi.org/10.4314/bcse.v26i3.1
6. Raghu MS, Basavaiah K. Optimized and Validated Spectrophotometric Methods for the Determination of Levocetirizine in Pharmaceuticals Based on Charge Transfer Reaction. Journal of the Association of Arab Universities for Basic and Applied Sciences 2012; 12: 33-41. http://dx.doi.org/10.1016/j.jaubas.2012.02.002
7. Indian Pharmacopoeia. Government of India, Ministry of Health and Family Welfare. Published by the Controller of Publication, New Delhi 2010; 2: 792-793.
8. British Pharmacopoeia. The Department of Health, Social Services and Public Safety 2010; 2: 118-119.
9. European Pharmacopoeia. 6th Edition. Starboury, Council of Europe 2008; 2: 1156-1157.
10. Jose LR, Joel MS, Mayra AR, Helena RP, Leonardo P. Determination of Ambroxol in Syrups Using Diffuse Reflectance Spectroscopy. Quim Nova 2009; 32(6): 1513-1516. http://dx.doi.org/10.1590/S0100-40422009000600029
11. Amol RR, Hemant J, Kishore NG. Development and Validation for UV Spectrophotometric Estimation of Ambroxol Hydrochloride in Bulk and Tablet Dosage Form Using Area Under Curve Method. Int. Res. J. Pharm 2014; 5(7): 580-583. http://dx.doi.org/10.7897/2230-8407.0507118
12. Rajan RV, Pankaj GJ. Simple Spectrophotometric Methods for Determination of Ambroxol Hydrochloride from Pharmaceutical Formulation. International Journal of PharmTech Research 2012; 4(3): 994-998.
13. Pasha K, Patil CS, Vijaykumar K, Sadath A, Chimkod VB. RP-HPLC Method for the Determination of Ambroxol in Pharmaceutical Dosage Forms. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2010; 1(3): 250-254.
14. Jain PS. Stability-Indicating HPTLC Determination of Ambroxol Hydrochloride in Bulk Drug and Pharmaceutical Dosage Form. Journal of Chromatographic Science 2010; 48(3): 45-50. http://dx.doi.org/10.1093/chromsci/48.1.45
15. Vidyasagar G, Sastry BS. Acidic dyes as Ion-Pairing Reagents for the Determination of Ambroxol. International Journal of Chemical Science 2003; 1(1): 49-52.
16. Venkateswari P, Kumar GVS, Puranik SB, Srinivas S, Ramprasad R, Ramya G *et al*. Development of Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Ambroxol Hydrochloride and Levocetirizine Dihydrochloride. International Journal of Advances in Pharmaceutical Analysis 2012; 2(2): 34-40.
17. Kamarapu SK, Vijayanthi, Bahlul ZEA, Venisetty RK. Development of RP-HPLC Method for the Analysis of Levocetirizine Dihydrochloride and Ambroxol Hydrochloride in Combination and its Application. International Journal of Pharmaceutical Sciences and Nanotechnology 2010; 3(1): 893-896.
18. Farida MSE, Nahla SI, Rasha EG, Omnia IA, Anwar W. Development and Validation of RP-HPLC Method for the Simultaneous Determination of Levocetirizine Dihydrochloride and Ambroxol Hydrochloride in Bulk Drug and Pharmaceutical Dosage Form. American Journal of Pharmacy and Health Research 2013; 1(8): 85-97.
19. Jyostna SP, Chandan RR, Radhika DI. Simultaneous Spectrophotometric Methods for Estimation of Levocetirizine and Ambroxol in Pharmaceutical Dosage Form. Journal of Chemical, Biological and Physical Sciences 2013; 3(2): 1115-1122.
20. The International Conference on Harmonization. Q2 (R1). Validation of Analytical Procedure Text and Methodology; 2005.

Cite this article as:
Satish A. Patel, Nancy J. Thakkar. Development and validation of absorbance correction method for simultaneous determination of levocetirizine and ambroxol in combined tablet dosage form. Int. Res. J. Pharm. 2015; 6(3):223-226 http://dx.doi.org/10.7897/2230-8407.06349

Source of support: Nil, Conflict of interest: None Declared