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

Research Article

DEVELOPMENT AND VALIDATION FOR UV SPECTROMETRIC ESTIMATION OF AMBROXOL HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM USING AREA UNDER CURVE METHOD

Ranjale Amol Rangnath[1], Jain Hemant Kumar[1]*, Gujar Kishore Namdeorao[2]
[1]Department of Quality Assurance Techniques, Sinhgad College of Pharmacy, Vadgaon (Bk.), Pune, Maharashtra, India
[2]Principal, Sinhgad College of Pharmacy, Vadgaon (Bk.), Pune, Maharashtra, India
*Corresponding Author Email: hemantkjain2001@yahoo.co.in

Article Received on: 24/05/14 Revised on: 17/06/14 Approved for publication: 07/07/14

DOI: 10.7897/2230-8407.0507118

ABSTRACT
This paper describes development and validation of UV spectrophotometric method for the estimation of Ambroxol hydrochloride in bulk and pharmaceutical formulation using area under curve method. The standard and sample solutions of Ambroxol hydrochloride were prepared in methanol. The area under curve in between 238 to 258 nm was measured. The method followed linearity in the range of 2-12 µg/ml with correlation coefficient value of 0.999. This method was validated for various parameters according to ICH guideline Q2 (R1). Satisfactory values of Percent relative standard deviation for the intra-day and inter-day precision indicated that method was precise. The mean percentage recovery studies were found to be 100.69 %. Limit of Detection and Limit of Quantitation were calculated as 0.0841 µg/ml and 0.2551 µg/ml, respectively. Validation results suggest that the further developed method can be used for routine quality control analysis studies for Ambroxol hydrochloride in bulk and tablet dosage form.
Keywords: Estimation, UV spectrophotometry, Area under curve, Validation, Ambroxol hydrochloride.

INTRODUCTION

Ambroxol hydrochloride[trans-4-(2-Amino-3,5-dibrombenzylamino)cyclohexanolhydrochloride] is a mucolytic agent used in the treatment of respiratory disorders associated with viscid or excessive mucus, it also decreases bronchial hyper-reactivity, increases cellular surfactant production, stimulates the amount of antibiotic penetration and thus decreases daily dose of them and exhibits anti-inflammatory properties as well[1]. This drug is official in IP 2010, BP 2009[2,3]. Few methods such as UV Spectroscopy[4], liquid chromatography[5], HPLC potentiometric estimation[6], RP-UPLC[7] are reported for the estimation of Ambroxol hydrochloride. Only few papers are available in literature which describes spectrophotometric estimation of Ambroxol hydrochloride as single drug and in combined dosage forms. In this context, we wish to further explore UV spectrophotometric technique using area under curve method for estimation of Ambroxol hydrochloride in bulk and tablet dosage form.

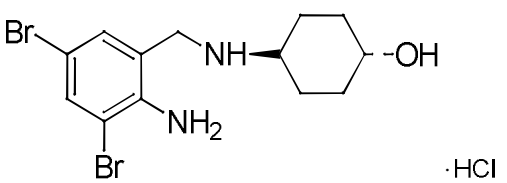

Figure 1: Chemical Structure of Ambroxol hydrochloride

MATERIALS AND METHODS

Instrumentation and Apparatus

Double beam spectrophotometer (UV 1800, Shimadzu, Japan) using two identical 1 cm quartz cells, connected to computer loaded with UV Prob Software, was employed in this work. Single pan electronic balance (AX 200, Shimadzu, Japan) was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonic Cleaning Bath (UCB 40, Spectra lab, India). All the glass wares (borosil®) were calibrated before use.

Chemical and Reagents

Reference standard of Ambroxol hydrochloride was obtained as gift sample by Dr. Reddy lab Ltd, Mumbai, India. Commercially available tablets (Mucolite® Label claim – 30 mg, Dr. Reddy, Batch No – 20013011) were obtained from local pharmacy. Methanol was obtained from Merck, Mumbai, India.

Preparation of standard solution

10 mg of Ambroxol hydrochloride was accurately weighed and transferred to a 100 ml volumetric flask containing 30 ml of methanol and sonicate for 15 minutes. This was further diluted up to the mark with methanol to obtain drug concentration of 100 µg /ml. From this solution, 1 ml was further diluted using same solvent to obtain drug concentration solution of 10 µg/ml as a working standard solution.

Selection of wavelength range

For the selection of analytical wavelength range for area under curve method, 10 µg/ml solution of Ambroxol hydrochloride was scanned in the spectrum mode from 400 nm to 200 nm by against methanol as blank. Wavelength range was selected around wavelength maxima (248 nm). Different working standards were prepared between 2-12 µg/ml. Various wavelength range were tried and final range between 228-258 nm was selected on the basis of linear relationship between area and corresponding concentration (Figure 2).

Area under curve (Area calculation)

Area under curve method involves the calculation of integrated value of absorbance with respect to the wavelength

between two selected wavelengths such as λ1 and λ2 representing start and end point of curve region. The area under curve between λ1 and λ2 was calculated using UV probe software. In this study area was integrated between wavelength ranges from 238 to 258 nm.

Preparation of calibration curve

Adequate dilutions were made from standard stock solution to obtain concentration of 2, 4, 6, 8, 10, 12 µg/ml respectively. These solutions were scanned from 400 to 200 nm and area under curve (AUC) values was integrated[8] in the range of 238-258 nm. The calibration curve was plotted between areas under curve values against concentration (Figure 3).

Assay of tablet formulation

Twenty tablets each containing 30 mg of Ambroxol hydrochloride were weighed, powdered and average weight was calculated. Powder equivalent to 10 mg of Ambroxol hydrochloride was transferred in 100 ml of volumetric flask containing 30 ml methanol and solution was sonicated for 15 minutes. This was further diluted up to the mark with methanol. The solution was filtered with whatmann filter paper No. 41. The first 5 ml of filtrate was discarded and suitable aliquot was diluted to obtain solution of 10 µg/ml concentration. This procedure was repeated in triplicate (Table 1).

Method validation

The objective of validation of an analytical procedure is to demonstrate whether the procedure is suitable for its intended purpose. The proposed method was validated for various parameters such as Linearity, Accuracy, Precision, Limit of detection (LOD) and Limit of Quantitation (LOQ) according to ICH Q2 (R1) guideline[9].

Linearity and Range

From standard stock solution adequate dilutions were prepared to obtain solution containing 2, 4, 6, 8, 10, 12 µg/ml of Ambroxol hydrochloride respectively. Solutions were scanned between 400 nm to 200 nm in spectrum mode. The area under curve was determined and calibration curve was plotted (Figure 3). Regression equation and correlation coefficient were determined.

Precision

Intermediate Precision (Reproducibility)

The intra-day and inter-day precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days for 10 µg/ml standard solution of Ambroxol hydrochloride, respectively. This experiment was performed in triplicate. The percentage relative standard deviation (% RSD) values were calculated (Table 2).

Accuracy

The accuracy of the method was determined by recovery study carried out using standard addition method at three different levels. The resulting spiked sample solutions were assayed in triplicate. The accuracy was determined at 80 %, 100 % and 120 % levels of 10 µg/ml standard solution. Area under curve was measured in the range of 238 - 258 nm. The percentage recovery was calculated for each level. The results are tabulated in (Table 3).

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

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations-

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

Where σ is the standard deviation of the signal to noise ratio of the sample and S is the slope of the related calibrations graphs

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability. Six sets of each known concentrations (2-12 µg/ml) were prepared and scanned. By using these spectra's, regression equation was obtained. By taking average of slopes and standard deviation of y – intercept LOD and LOQ were calculated. The values of LOD and LOQ are given in Table 4.

RESULTS AND DISCUSSION

An attempt was made to develop a simple AUC spectrophotometric method for the determination of Ambroxol hydrochloride in tablet dosage form. The generated regression equation was

$$\int_{238}^{258} A d\lambda = 0.098x + 0.009\ (R^2 = 0.999).$$

Where, $\int_{238}^{258} A d\lambda$ is area under curve between 238 to 258 nm, C is concentration and R^2 is correlation coefficient.

The R^2 value as 0.999 and drug concentrations was found to be linear in the range of 2-12 µg/ml. For intra-day and inter-day precision results in terms of percent relative standard deviation values were found to be 0.73951 and 0.73969, respectively. The results were satisfactory and method was precise. The mean percentage recovery was found to be 100.69 % which indicated good recovery of the drug. The limit of Detection and limit of Quantitation values were found to be 0.08418 µg/ml and 0.2551 µg/ml respectively. The result of the analysis of pharmaceutical formulation by the developed method was consistent with the label claim, which indicates method is highly reproducible and reliable. The validation parameters are summarized in Table 4.

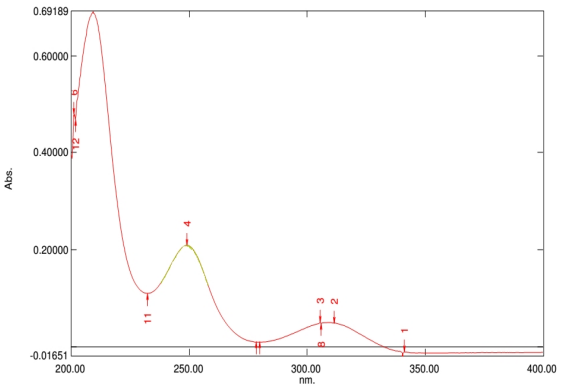

Figure 2: Area under curve for Ambroxol hydrochloride

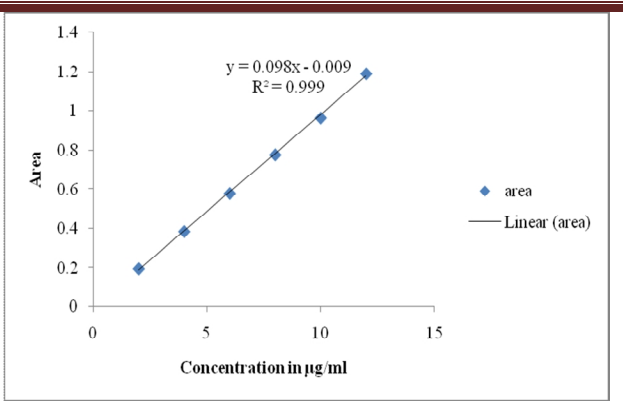

Figure 3: Calibration curve of Ambroxol hydrochloride

Table 1: Assay of Tablet Dosage Form (mucolite®)

S. No.	Sample concentration (µg/ml)	Amount Found (%)	Mean ± SD (%)*	% RSD*
1	10	100.77	101.29 ± 0.4578	0.4520
2	10	101.62		
3	10	101.49		

*n = 3, % RSD = % Relative Standard

Table 2: Precision data for Ambroxol hydrochloride

Drug	Concentration of drug (µg/ml)	Absorbance (Mean ± SD)*	% RSD*
Intraday (n = 3)	10	1.3363 ± 0.0071	0.53443
Inter day (n = 3)	10	1.3242 ± 0.0040	0.30801

*n = 3

Table 3: Accuracy Results for Ambroxol hydrochloride

Accuracy Level	Sample conc. (µg/ml)	Standard spiked (µg/ml)	Total added Amount (µg/ml)	% Recovery ± SD*	Mean Recovery (%)*	% RSD*
I (80 %)	10	8	18	100.48 ± 0.12343	100.45	0.140579
II (100 %)	10	10	20	100.70 ± 0.05131		
III (120 %)	10	12	22	100.19 ± 0.24846		

*n = 3

Table 4: Summary of Validation Parameters

Parameter	Results
λ max	248
Linearity range (µg/ml)	2-12
Regression Equation (y = mx + c)	y = 0.098x + 0.009
Correlation Coefficient (R^2)	0.999
Precision (% R.S.D)*	
Intraday (*n=3)	0.73951
Inter day (*n=3)	0.73969
Accuracy (Mean % Recovery)	100.45
Limit of Detection (LOD) µg/ml	0.08418
Limit of Quantitation (LOQ) µg/ml	0.2551

CONCLUSION

It can be concluded from the results that the proposed UV spectrophotometric method was accurate, precise and consistent for the determination of Ambroxol hydrochloride in tablet dosage form. This method was validated as per ICH guidelines. Results suggest that this method can be used for routine estimation of Ambroxol hydrochloride in bulk and pharmaceutical dosage forms.

ACKNOWLEDGEMENT

The authors would like to convey regards to Dr. Reddy Lab Ltd, Mumbai, India for providing the gift sample of the pure drug and Dr. K. N. Gujar, Principal, Sinhgad college of Pharmacy, Vadgaon (Bk.), Pune, India for providing the necessary facilities for carrying out the research work

REFERENCES

1. Shaikha KA, Patil SD, Devkhile AB. Development and validation of a reversed-phase HPLC method for simultaneous estimation of ambroxol hydrochloride and azithromycin in tablet dosage form, Journal of Pharmaceutical and Biomedical Analysis 2008; 48: 1481–1484. http://dx.doi.org/10.1016/j.jpba.2008.09.031
2. Indian Pharmacopoeia, Government of India, Ghaziabad, The Indian Pharmacopoeia Commission, Vol. II; p. 91.
3. British Pharmacopeia, Her Majesty's Stationary Office, London, Vol I; 2009. p. 265.
4. Lakshmana Prabu S, Thiagarajan S, Srinivasan M, Queeni Marina. Simultaneous Estimation of Gatifloxacin and Ambroxol hydrochloride by UV-Spectrometery, International Journal of Pharmaceutical Sciences Review and Research 2010; 3(suppl. 2): 123-126.
5. Meiling Qi, Peng Wang, Ruihua Congb, Jianjun Yang. Simultaneous determination of roxithromycin and ambroxol hydrochloride in a new tablet formulation by liquid chromatography, Journal of Pharmaceutical

and Biomedical Analysis 2004; 35: 1287–1291. http://dx.doi.org/10.1016/j.jpba.2004.04.001
6. Grzegorz Bazylak, Nagels Luc J. Simultaneous high-throughput determination of clenbuterol, ambroxol and bromhexine in pharmaceutical formulations by HPLC with potentiometric detection, Journal of Pharmaceutical and Biomedical Analysis 2003; 32: 887-903. http://dx.doi.org/10.1016/S0731-7085(03)00191-2
7. Rakshit Kanubhai Trivedi, Patel Mukesh C, Jadhav Sushant B. A Rapid, Stability Indicating RP-UPLC Method for Simultaneous Determination of Ambroxol Hydrochloride, Cetirizine Hydrochloride and Antimicrobial Preservatives in Liquid Pharmaceutical Formulation, Scientia pharmaceutiaca 2011; 79: 525–543.
8. Dahivadkar Manish Sudhakar, Jain Hemant Kumar, Gujar Kishore Namdeorao. Development and Validation of UV Spectrophotometric Estimation of Ebastine, in bulk and tablet dosage form using Area Under Curve Method, Int. Res. J. Pharm 2013; 4(6): 201-204. http://dx.doi.org/10.7897/2230-8407.04645
9. Bhangale Pallavi Raghunath, Jain Hemant Kumar, Spectrophotometric method for Simultaneous Estimation of Formoterol Fumarate and Mometasone Furoate in Respicaps, Int. Res. J. Pharm 2013; 4(6): 220-223. http://dx.doi.org/10.7897/2230-8407.04650
10. Shimadzu Corporation Kyoto Japan, Analytical and Measuring Instruments Division, Instruction Manual Operation Guide-UV 1800 2008; 13: 21-13.25.
11. ICH Harmonized Tripartite Guidelines, Validation of Analytical Procedure: Text and Methodology Q2 (R1); 2005.

Cite this article as:
Ranjale Amol Rangnath, Jain Hemant Kumar, Gujar Kishore Namdeorao. Development and validation for UV spectrometric 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

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