Research Article

Analytical method development and validation for the estimation of ambroxol HCL in its tablet dosage form by UV-spectrophotometry

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ABSTRACT

Objective: To develop and validate simple, accurate, rapid, precise, reproducible, and cost-effective spectrophotometric method for the quantitative estimation of ambroxol hydrochloride in its tablet dosage form. **Materials and Methods:** The developed ultraviolet spectrophotometric method for the quantitative estimation of ambroxol hydrochloride is based on measurement of absorption at maximum wavelength of 305 nm using methanolic water (1:9) as a solvent. The stock solution of ambroxol hydrochloride was prepared, and subsequent suitable dilution was made using distilled water to obtain calibration curve. The standard solution of ambroxol hydrochloride shows absorption maxima at 305 nm. **Results:** The drug obeyed beer lambert's law in the concentration range of 20-100 µg/ml with regression 0.999 at 305 nm. The overall % recovery was found to be present in between 100.17% and 100.38% which reflects that the method was free from the interference of the impurities and other excipients used in the formulation. The low value of % relative standard deviation (RSD) was indicative of accuracy and reproducibility of the method. The % RSD for interday and intraday precision was found to be 0.1179 and 0.1177, respectively, which is <2% hence proved that method is precise. **Conclusion:** The results of analysis have been validated as per International Conference on Harmonization guidelines. The developed method can be adopted in routine analysis of ambroxol hydrochloride in tablet dosage form as well bulk dosage form.

KEY WORDS: Ambroxol hydrochloride, International Conference on Harmonization Guidelines, Methanol, Method development, Ultraviolet spectrophotometry, Validation

INTRODUCTION

Ambroxol hydrochloride, 4-[(2-amino-3,5-dibromophenyl)methylamino]cyclohexan-1-ol hydrochloride (Figure 1). It is an antiasthmatic and mucolytic drug, and it is the drug of choice for treatment of bronchial asthma and cough. It is a metabolite of bromhexine that stimulates mucociliary action and clears the air passages in the respiratory tract.[1-4] It is a white crystalline powder, practically insoluble in ether, soluble in water, ethanol, methanol, and dimethyl sulfoxide.[5] As per investigation of literature, the ultraviolet (UV) spectrophotometric, high-performance liquid chromatography analytical method, and IR spectrometric methods were developed on different wavelength for analysis of ambroxol hydrochloride in pharmaceutical tablet dosage form or bulk drug samples.[6-17] The rational of this work is to develop a simple, accurate, rapid, precise, reproducible, and cost-effective spectrophotometric method for the direct quantitative determination of ambroxol hydrochloride. In this method, we developed a method for determination of ambroxol hydrochloride in bulk drug sample and tablet dosage form and validation as per International Conference on Harmonization (ICH) Guideline.[18,19]

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MATERIALS AND METHODS

Instruments

Electronic weighing balance - single pan balance, model axis liquid chromatography/gas chromatography. Sonicator - ultra sonicator - model-Bandelin Sonorex. Double Beam UV-visible spectrophotometer - model No-1800. A Schimadzu UV probe version 2.34 - double Beam UV-visible spectrophotometer. UV spectra of standard and sample solutions were recorded in 1 cm quartz cells at the wavelength ranges of 200-400 nm were used for this work.

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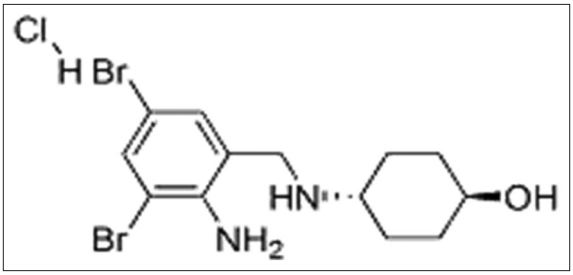

Figure 1: Chemical structure of ambroxol hydrochloride

Materials

Ambroxol hydrochloride was obtained as gift sample from Rachem Pharma Pvt. Ltd. Hyderabad, India. The commercially available tablets Mucolite 30 mg, Dr. Reddies Laboratories Ltd., India were obtained from the local market. Methanol (analytical grade) was used as a solvent was obtained from Fisher Scientific, India and Distilled water was used obtained from water purification unit.

Method Development

Preparation of standard solution

A standard stock solution was prepared by accurately weighed 100 mg of ambroxol hydrochloride in 100 ml of volumetric flask and dissolved in 10 ml methanol and make up to final volume with distilled water to obtain a concentration 1 mg/ml or 1000 µg/ml (standard Stock I). Further, dilution was made by pipetting 6 mL of stock solution to 100 ml with distilled water to get desired concentration of 60 µg/ml. This is the optimized concentration of standard drug.

Selection of Wavelength for Analysis of Ambroxol Hydrochloride

The solution of 60 µg/ml used for initial spectral scan in the UV range of 200-400 nm to detect maximum wavelength and was optimized at 305 nm. The further dilutions for linearity were prepared from the stock solution by allegation method.

Preparation of Serial Dilutions

The serial dilutions were prepared from the standard stock I solution to get a respective concentrations of 20, 30, 40, 50, 60, and up to 100 µg/ml.

Preparation of Sample Solution

20 tablets each containing 30 mg of ambroxol hydrochloride were weighed, powdered, and average weight was calculated. Tablet powder equivalent to 100 mg was transferred into 100 ml of volumetric flask, then 10 ml of methanol was added to dissolve the drug and make up to the final volume with distilled water. From the above, 6 ml was withdrawn and diluted up to 100 ml. The optimized concentration of ambroxol hydrochloride sample solution was 60 µg/ml. The solution was scanned in UV region in the wavelength range from 200 to 400 nm and λ_{max} was optimized at 305 nm.

Method Validation

The proposed method was validated for various parameters such as linearity and range, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), robustness, ruggedness, and sensitivity according to ICH Q2 (R1) guidelines.

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test result which is directly proportional to the concentration of an analyte in the sample. The range of an analytical procedure is the interval between the upper and lower concentration of an analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity. The linearity of the analytical method was demonstrated over the concentration range investigated by triplicate analysis (n = 3) at a concentration range of 20-100 µg/ml. The absorbance obtained at respective concentration was recorded, and the graph is plotted as concentration (µg/ml) versus absorbance. The linear regression equation and the coefficient correlation were obtained from the UV probe software.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. The accuracy is calculated by % recovery studies with known amount of sample solution. The determination of content of ambroxol HCL was performed at three levels by adding the calculated amount of ambroxol HCL. The sample was prepared in triplicate (9 determinations), i.e., 80%, 100%, and 120% of the working concentrations of the method. The percentage recovery was calculated in terms of percentage relative standard deviation (% RSD).

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the homogeneous sample under the prescribed conditions. The precision of the method was demonstrated by intraday and interday variation studies. In the intraday precision study, three different solutions of same concentration were prepared and analyzed in the same day (morning, noon, and evening), whereas in the interday precision study, the solutions of same concentration were prepared and analyzed, for two consecutive days, and the absorbance was

recorded. All study was performed in triplicates. The result was indicated by calculating RSD.

LOD

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected, but not necessarily quantitated as an exact value. The LOQ was calculated using the formula involving the standard deviation of response and the slope of the calibration curve.

$LOD = 3.3\ \sigma/S$

Where, σ = standard deviation, S = slope

LOQ

The detection limit is the lowest amount of analyte in a sample which can be detected but not quantitates. The LOQ was calculated using the formula involving the standard deviation of response and the slope of the calibration curve.

$LOQ = 10\ \sigma/S$

Where, σ = standard deviation, S = slope

Sensitivity

The sensitivity of the method was determined by calculating the different parameter such as molar absorptivity and Sandell's sensitivity.

Robustness

The robustness of an analytical procedure is a measure of its capacity remains unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The robustness of the proposed method involves the solutions of 60 µg/ml of sample ambroxol hydrochloride solution was prepared and analyzed by a change in wavelength. The wavelength was selected $\lambda_{max} \pm 1$, i.e., 304 and 306 nm, respectively, for sample ambroxol hydrochloride solution.

Ruggedness

The ruggedness is a degree of reproducibility of test result under verification of condition such as a different analyst, different instruments, and different days. To establish ruggedness of the proposed method, the solutions of 60 µg/ml of sample ambroxol hydrochloride solution was prepared and analyzed with the change in the different analyst.

RESULTS AND DISCUSSION

Selection of Wavelength

The UV spectrum of ambroxol hydrochloride in methanolic water (1:9) showed maximum absorption at 305 nm and was shown in Figure 2, which is complying with reported λ_{max}. Hence, it was selected as the λ_{max} of ambroxol hydrochloride in methanolic water for further use.

Linearity and Range

The linearity for the developed method was investigated by replicate analysis (n = 3) at 9 concentration levels (20-100 µg/ml) of reference standard ambroxol hydrochloride. The absorbance obtained at respective concentration was recorded, and graph was plotted shows good linear correlation coefficient from the UV probe software. The linearity data were shown in Table 1 and Figure 3.

Accuracy

The accuracy was determined in triplicate by analyzing % recovery of ambroxol hydrochloride

Table 1: Calibration curve data of ambroxol hydrochloride

Concentration (µg/ml)	Absorbance	
0	0.000	Y=0.007x+0.001
		R^2=0.999
20	0.151	
30	0.238	
40	0.323	
50	0.396	
60	0.487	
70	0.546	
80	0.618	
90	0.712	
100	0.795	

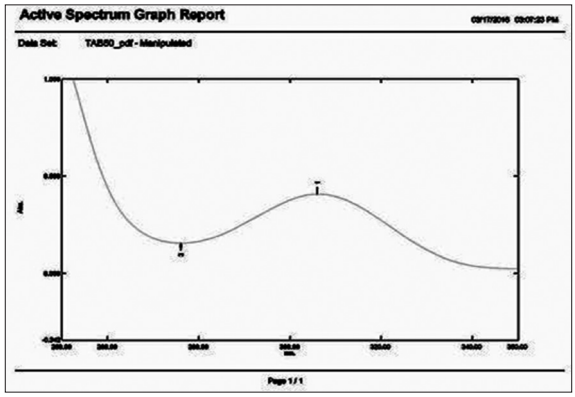

Figure 2: Ultraviolet spectrum of ambroxol hydrochloride

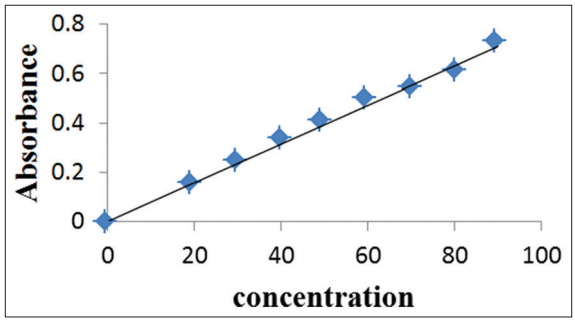

Figure 3: Calibration curve of ambroxol hydrochloride

Table 2: Accuracy study of ambroxol hydrochloride in its dosage form

% Recovery level	Absorbance	Mean % recovery	SD	% RSD
80	0.391 0.392 0.391	100.35	0.1443	0.14379
100	0.487 0.490 0.489	100.17	0.1855	0.18518
120	0.588 0.589 0.586	100.38	0.2596	0.25861

SD: Standard deviation, RSD: Relative standard deviation

Table 3: Method precision study of ambroxol hydrochloride

S. No	Sample absorbance values at 305 nm in distilled water	Percentage label claim (% w/w)
1	0.490	100.58
2	0.489	100.16
3	0.491	100.37
4	0.489	100.16
5	0.490	100.58
6	0.490	100.37
Mean±SD	0.489±0.00075	100.37±0.18783
% RSD	0.1539877	0.18713

SD: Standard deviation, RSD: Relative standard deviation

Table 4: Interday precision

Parameter	Ambroxol hydrochloride absorbance values at 305 nm			
	Day-1		Day-2	
	Standard absorbance	Sample absorbance	Standard absorbance	Sample absorbance
Day to day	0.487 0.488 0.487	0.490 0.489 0.490	0.488 0.487 0.488	0.490 0.489 0.490
Mean±SD	0.487±0.000577	0.489±0.000577	0.487±0.000577	0.489±0.000577
% RSD	0.11855	0.11799	0.118480	0.11799

SD: Standard deviation, RSD: Relative standard deviation

Table 5: Intraday precision

Parameter	Ambroxol hydrochloride absorbance values at 305nm	
	Standard	Sample
Absorbance at λ_{max}	0.487 0.487 0.488	0.490 0.491 0.490
Mean±SD	0.487±0.000577	0.490±0.000577
% RSD	0.118552	0.117755

SD: Standard deviation, RSD: Relative standard deviation

by standard addition method. The percent recovery obtained indicates non-interference from the excipients used in the formulation. The results were shown in Table 2.

The Acceptance Criteria for the percentage recovery according to ICH guidelines ranges from 98%-102%.

Method Precision

The precision of proposed method was determined by intraday and interday precision, and it was expressed in terms of % RSD. For interday and intraday %, RSD were found in the range of 0.15398 and 0.18713, respectively, as shown in Tables 3-5.

Ruggedness

In the ruggedness study, the influence of small, deliberate variations of the analytical parameters on the absorbance of the drug was examined. The factor selected was a change in the analyst. The result of ruggedness study indicates that the selected factor remained unaffected by small variations with % RSD of 0.11755-0.203665, which confirms the ruggedness of method. The results were summarized in Table 6.

Robustness

Robustness of this method was determined by analyzing the standard ambroxol hydrochloride solution of 60 µg/ml at a different wavelength (i.e., λ_{max} ± 1) and absorbance was measured. The standard deviation and % RSD were calculated. Results of robustness study indicate that the selected factor remained unaffected

Table 6: Ruggedness data for ambroxol hydrochloride

Parameter	Ambroxol hydrochloride standard			Ambroxol hydrochloride sample		
	Analyst 1	Analyst 2	Analyst 3	Analyst 1	Analyst 2	Analyst 3
Analyst to analyst	0.487	0.486	0.487	0.490	0.491	0.491
	0.488	0.487	0.489	0.491	0.490	0.492
	0.487	0.488	0.488	0.490	0.492	0.490
Mean±SD	0.487±0.000577	0.487±0.001	0.488±0.001	0.490±0.000577	0.491±0.001	0.491±0.001
% RSD	0.118480	0.205335	0.20491	0.11755	0.203665	0.203665

SD: Standard deviation, RSD: Relative standard deviation

Table 7: Robustness data for ambroxol hydrochloride

Wavelength (nm)	Ambroxol hydrochloride in methanolic water (1:9)	
	Standard	Sample
305	0.487	0.491
304	0.487	0.491
306	0.486	0.490
Mean±SD	0.486±0.000577	0.490±0.000577
% RSD	0.11872	0.117755

SD: Standard deviation, RSD: Relative standard deviation

Table 8: Optical characteristics of ambroxol hydrochloride

Parameters	Ambroxol hydrochloride in methanolic water (1:9)
Beers law limit (µg/ml)	20-100
Molar absorptivity (L mol^{-1} cm^{-1})	0.00811
	0.0152 µg/cm^2
Correlation coefficient (r^2)	0.999
Regression equation (y=mx+c)	y=0.007x+0.001
	R^2=0.999
Slope (m)	0.007
Intercept (c)	0.001
LOD (µg/ml)	0.3145
LOQ (µg/ml)	0.9532
Standard error	0.000307

by small variation with % RSD 0.11872-0.11775 confirms the robustness of the method. Results were shown in Tables 7 and 8.

CONCLUSION

A simple, sensitive, rapid, accurate, and precise spectrophotometric method was developed for the determination of ambroxol hydrochloride in its tablet dosage form using methanol water (1:9). The present analytical method was validated as per ICH Q2 (R1) guidelines, and it meets to the specific acceptance criteria. The statistical parameters and the recovery test data indicated the high reproducibility and accuracy of the proposed method. Analysis of authentic samples containing the studied drug showed no interference from common additives and auxiliary substances in general.

It is concluded that the developed analytical method was specific, precise, linear, accurate, robust, economic, and having stability indicating characteristics. The proposed method was successfully used for the routine quality control analysis of the ambroxol hydrochloride in marketed tablet dosage form.

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