Research Article

Estimation of Ambroxol Hydrochloride in Bulk and Pharmaceutical Formulations by Simple Visible Spectrophotometry

Prof.T. Sreenivasulu Reddy*, K. Nagabhushana Reddy, A.Giri
Department of Chemistry, S.K.University, Anantapur, Andhra Pradesh, India.
***Corresponding author's E-mail:** tsrreddychem@gmail.com

ABSTRACT

The present paper reports a simple, accurate, precise, highly sensitive and selective visible, Spectrophotometric method for the estimation of Ambroxol hydrochloride in bulk and pharmaceutical formulations. The method is based on the colour reaction between Ambroxol and Palladium (II) in the pH range 3.0 – 9.0. They form a yellow colored complex solution. Colored species has maximum intensity at 410 nm. Studies were carried at pH 6.0 where the absorbance is maximum. A 0.1% sodiumdodesylsulphate (SDS) keeps the complex in solution. The colour intensity attains maximum value after 30 minutes of mixing the various components. Under the optimum conditions Beer's law is obeyed in the range 4.0-65.0 μg/ml. The straight line plot obeyed the equation A = 0.0146 C - 0.0008. The molar absorptivity and sandell's sensitivity are 5.500×10^3 lmol^{-1} cm^{-1} and 0.0687 μg cm^{-2} respectively. The standard deviation of the method for ten determinations of 30 μg/ml Ambroxol is 0.0033. The correlation coefficient (γ) of the experimental data of the calibration plot is 0.9996. The effect of various excipients was studied. The composition of the complex is established as 2:1[(Pd(II) : Ambroxol]. The stability constant of the complex is 2.090×10^{11}. The developed method was validated according to ICH guidelines and was found to be accurate and precise. The validation parameters are linearity, accuracy, precision, LOD, LOQ and ruggedness. Hence the proposed method is successfully applied for the determination of Ambroxol hydrochloride in pharmaceutical formulation.

Keywords: Ambroxol, Method validation, Pd(II), Visible spectrophotometry.

INTRODUCTION

Ambroxol is 4-[[2-amino-3,5-dibromophenyl) – methyl]amino] cyclohexanol (or) N – (trans –P – hydroxy cyclohexyl) – (2 – amino -3,5 – dibromobenzyl) amine. It is a white crystalline powder freely soluble in water and its molecular formula is $C_{13}H_{18-}Br_2N_2O$ (M. W. = 378.11). Its molecular structure is;
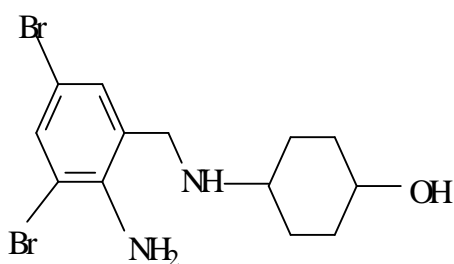
Ambroxol hydrochloride is an active ingredient in a number of pharmaceutical preparations. Ambroxol is a highly substituted aniline derivate metabolite of bromohexine. Ambroxol is one of the most popular medicines used to relieve the symptoms of cough asthma and colds. It is widely used in the treatment of chronic diseases of the respiratory tract as a broncho secretolyticum. This is a mucolytic agent that increases respiratory tract secretions, enhances pulmonary surfactant productions and stimulates ciliary activity, which results in an improved mucus flow and transport. The enhancement of fluid secretions and mucociliary clearance facilities expectoration and there by causes coughing. Ambroxol stimulates the transportation of viscous secretions in the respiratory organs and reduces secretion stagnation. It is administered as the hydrochloride in daily doses of 30 –120 mg and is available commercially as syrups, tablets and granules similarly doses have been given by inhalation, injection.

Joao L.M. Santos et al determined spectrophotometrically Ambroxol in an automated multi – pumping pulsed flow system.[1] Nondestructive determination of Ambroxol content in tablets by Raman Spectroscopy[2] is reported. Derivative UV Spectrophotometric and HPLC methods are reported for quantitative determination of Ambroxol in tablets by Zafer et al.[3] A sensitive and selective liquid chromatographic method coupled with tandem mass spectrometry (LC – MS / MS) is developed for the quantitative determination of Ambroxol in human plasma.[4] Quantitative determination of Ambroxol in commercial tablets using Partial Least Squares (PLS) treatment of FT – Raman spectroscopic data is carried out by Roman Szostak and Sylwester Mazurek.[5] Simultaneous determination of Roxithromycin and Ambroxol hydrochloride in a new tablet formulation by liquid chromatography is reported.[6] A new sensitive HPLC – UV method is developed and validated for the determination of Ambroxol in dog plasma.[7] Spectrophotometric determination of trace amounts of Ambroxol is carried out by liquid – liquid extraction using bromothymol blue with a flow – injection system.[8] Derivative UV Spectrophotometric method for the simultaneous determination of Ambroxol and preservatives in syrups is developed by Hasan Basan et al.[9] Kothekar et al reported a quantitative determination of levofloxacin and Ambroxol in pharmaceutical dosage forms by RP-HPLC method.[10] Prabhu et al reported Simultaneous UV-

Spectrophotometric method for the estimation of Ambroxol and leavocitrizine.[11] Makarand et al reported a simultaneous UV-Spectrophotometric method for the determination of levofloxacin and Ambroxol in tablets.[12] A simultaneous UV-Spectrophotometric estimation of Ambroxol hydrochloride and guiaphensin in tablet dosage forms using simultaneous equations was reported.[13] Prabu et al reported a simultaneous estimation of Gatiflaxicin and Ambroxol hydrochloride by UV-spectrophotometric.[14] A simultaneous estimation of Ambroxol and Cetrizine hydrochloride in tablet dosage form was reported by RP-HPLC method was reported.[15] A rapid stability indicating RP-UPLC method for simultaneous determination of Ambroxol hydrochloride, Cetrizine hydrochloride antimicrobial preservatives in liquid pharmaceutical formulation was reported.[16] Asha et al reported a simultaneous UV-Spectrophotometric estimation of Ambroxol and loratadine tablet dosage forms.[17] Simultaneous UV-Spectrophotometric analysis of Ambroxol hydrochloride, guaifenesin and terbutaline sulphate in liquid dosage forms was reported.[18] Patel et al reported a simultaneous UV-Spectrophotometric estimation of Ambroxol and salbutamol in fixed dosage combination.[19]

The above survey of literature shows no report of a direct visible Spectrophotometric method for the determination of Ambroxol. This paper reports a simple, sensitive and precise visible Spectrophotometric procedure for the determination of Ambroxol hydrochloride in bulk and pharmaceutical formulation.

MATERIALS AND METHODS

All chemicals and solvents used were of analytical reagent grade.

Solutions

Palladium (II) solution

1 g of palladium chloride (Loba Chime Ltd.) is dissolved in distilled water in a 100ml standard flask and standardized.[20] working solution is prepared by suitably diluting the stock solution.

Ambroxol solution

100 mg of Ambroxol was weighed accurately and transferred into a 100 ml standard flask, dissolved and made up to the mark in double distilled water. This solution was diluted as required.

Buffer solutions were prepared by adopting the standard procedures reported in the literature.[21] The solutions employed for the preparation are given below.

pH	Constituents
0.5 – 3.0	1 M Sodium acetate + 1 M Hydrochloric acid
3.0 – 6.0	0.2 M Sodium acetate + 0.2 M Acetic acid
7.0	1.0 M Sodium acetate + 0.2 M Acetic acid
8.0 – 12.0	2.0 M Ammonia + 2.0 M ammonium chloride

Instruments

A Shimadzo UV-Visible recording spectrophotometer (UV-160A) measuring wavelength at 200-1100 nm, and wave length accuracy $\pm$ 0.5nm with automatic wavelength correction.

An ELICO digital pH meter was used for measuring the pH of buffer solutions. The reproducibility of measurements is within $\pm$ 0.01 pH.

Procedures

Preparation of pharmaceutical sample (Tablets)

A known number of tablets are weighed and ground to a fine powder. A portion of the powder containing 100 mg of the active component is accurately weighed into a 100 ml calibrated flask, 60 ml of distilled water are added and shaken thoroughly for about 20 minutes to extract the drug. The contents are diluted to the mark, mixed well and filtered using quantitative filter paper to remove the insoluble residue. The filtrate is diluted to get required concentration of drug

Absorbance spectrum

5ml of buffer solution of pH 6.0, 1ml of Pd(II) [5×10^{-3}M] solution and 1 ml of Ambroxol [2×10^{-4}M] were taken in a 10 ml volumetric flask and made up to the mark with distilled water. The absorbance of the solution was measured in the wavelength region 300-600nm against a blank consisting of 5 ml of buffer solution made up to the mark with distilled water in a 10 ml volumetric flask.

Determination of Ambroxol

5ml of buffer solution of pH 6.0, 1ml of Pd(II) [1×10^{-2}M] solution and varying volumes of Ambroxol [2×10^{-3}M] solution were taken in a series of 10 ml volumetric flasks and the contents of each flask were made up to the mark with distilled water. The absorbance of the solution was measured at 410 nm using buffer blank.

Determination of Ambroxol in tablets

5ml of buffer solution of pH 6.0, 1ml of Pd(II) [5×10^{-3}M] solution and known aliquot of the tablet solution were taken in a series of 10 ml volumetric flasks and the contents were made up to the mark with distilled water. The absorbance of the solutions was measured at 410 nm using buffer blank.

Effect of SDS Concentrations

In order to improve the sensitivity of the method and to avoid precipitation of the complex species, the effect of various surfactants on the Ambroxol–Pd(II) complex solution was studied .Of all the surfactants studied sodiumdodesylsulphate (SDS) is found to enhance the absorbance. The effect of varying concentration of SDS on the absorbance studies are reported in Table 1. The study reveals that 0.1% of SDS gives maximum absorbance for the system.

Interference studies

In order to assess the applicability of the method, the effect of presence of various excipients that are generally associated with Ambroxol in its pharmaceutical compounds are added to a fixed amount of Ambroxol (20 µg/ml) solution and the absorbance measurements are carried out under optimal conditions. The concentration (µg/ml) at which various excipients do not cause an error of more than ± 4% in absorbance is taken as the tolerance limit of excipients and the results are given in Table 2.

RESULTS AND DISCUSSION

Ambroxol reacts with Pd(II) in the pH range 3.0-8.0 forming an yellow colored complex solution. The absorption spectrum of the yellow colored Pd(II) – Ambroxol complex shows(Figure 1) absorption maximum at 410 nm. At this wave length either Pd(II) or Ambroxol has no significant absorbance. The colour intensity of the complex is maximum in pH range 5.5-6.5. Hence studies were carried at pH 6.0. The colour formation attains maximum intensity after 30 minutes of mixing the various components. There after the colour of the complex remains stable for more than 24 hours. A fivefold molar excess of Pd(II) is sufficient to produce maximum absorbance. The absorbance varied linearly with the concentration of Ambroxol. Beer's law is obeyed in the range 4.0-5.0 µg/ml of Ambroxol. The straight line plot obeyed the equation $A = 0.0146\ C - 0.0008$. The molar absorptivity and Sandell's sensitivity are 5.500×10^3 lmol^{-1} cm^{-1} and 0.0687 µg cm^{-2} respectively. The standard deviation of the method for ten determinations of 30µg/ml Ambroxol is 0.0033. The correlation coefficient (γ) of the experimental data of the calibration plot is 0.9996. The effective range of concentration for accurate determination of Ambroxol as ascertained from Ringbom's plot is 5.0 - 62.0 µg/ml.
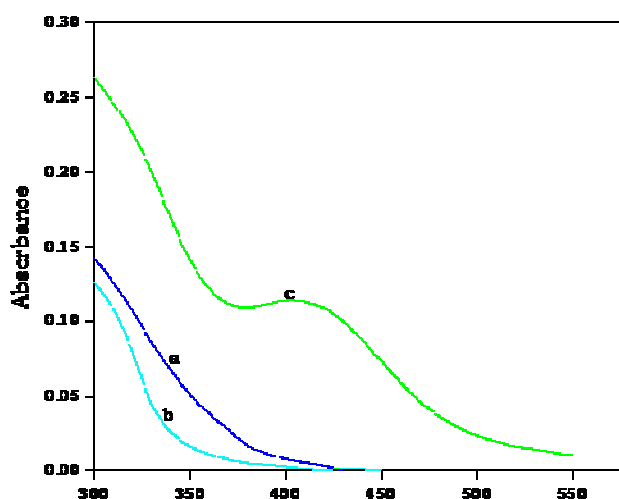
Figure 1: Absorption spectra of (a) Pd (II) vs. buffer blank; (b) ABX vs. buffer blank; (c) ABX – Pd(II) vs. buffer blank [Pd(II)] = 5.0 x 10^{-4}M ; [ABX]= 2.0 X 10^{-5}M

Table 1: Effect of SDS Concentration on the absorbance

[ABX] = 6 x 10^{-5} M; λ = 410 nm; [Pd(II)] = 1.25 x 10^{-4} M pH = 6.0.

Volume of 1% SDS solution	Absorbance
0.20	0.316
0.50	0.438
1.00	0.450
1.50	0.451
2.0	0.449

Table 2: Tolerance limits of excipients

Amount of ABX = 20 µg/ml; pH = 6.0

Excipients	Tolerance limit (µg/ml)
Fructose	1534
Glucose	1105
Sucrose	1672
Lactose	2079
Gelatin	2211
Starch	1738
Sodium Alginate	1617
Boric Acid	2310
Magnesium stearate	1925

Table 3: Optical and regression characteristics, precession and accuracy of the Proposed method for Ambroxol

Parameter	Ambroxol
Analytical Wavelength (nm)	410
Beer's law limits (µg/ml)	4 – 65
Limits of detection (µg/ml)	0.7458
Limits of quantization (µg/ml)	2.2376
Molar absorptivity (l.mo1^{-1}cm^{-1})	5500
Sandell's Sensitivity (µg/cm^2)	0.0687
Regression equation (y = a + b x)	
Slope (b)	0.0146
Intercept (a)	-0.0007
Correlation coefficient (γ)	0.9995
Standard deviation (SD)	0.0033

Table 4: Assay of Ambroxol in pharmaceutical formulation

Sample (Manufacturer – Formulation)	Label Claim (mg)	Amount found *(mg)	Error (%)
BRAND I: (Ambrodil-Aristo Pharma Pvt.Ltd.,– Tablet)	30.00	30.20	+0.67
BRAND II: (ACOCONTIN-Modi Mundi Pharma Ltd., – Tablet)	75.00	74.89	-0.14

Table 5: Intra and Inter day precision studies of Ambroxol (n=3, p=0.05)

Concentration (µg/ml)	Mean absorbance $\pm$ SD		% RSD		Calculated value of t
	Day-1	Day-2	Day-1	Day-2	
20	0.300$\pm$0.001	0.301$\pm$0.001	0.51	0.50	0.092
30	0.460$\pm$0.002	0.462$\pm$0.001	0.33	0.22	0.137
40	0.612$\pm$0.001	0.614$\pm$0.002	0.25	0.32	0.070

Table 6: Recovery studies for Ambroxol in tablets

Tablet	Amount of sample (µg/ml)	Amount of drug added (µg/ml) Amount	Amount Recovered (µg/ml)	% Recovery $\pm$ SD
BRAND-I (Ambrodil-Aristo Pharma Pvt.Ltd.– Tablet)	30	20	45.75	100.40$\pm$0.003
	30	30	60.19	99.7$\pm$0.002
	30	40	75.12	100.1$\pm$0.003
BRAND-II (ACOCONTIN-Modi Mundi Pharma Ltd. – Tablet)	20	20	34.96	100.5$\pm$0.001
	20	30	51.14	99.6$\pm$0.002
	20	40	65.20	100.30$\pm$0.002

Table 7: Ruggedness result for the Ambroxol in tablets

Tablet	Analyst -I			Analyst- II		
	Label Claim (mg)	Amount found *(mg)	(%) Recovery $\pm$SD	Amount found *(mg)	(%) Recovery $\pm$ SD
BRAND-I	30.00	29.18	97.30$\pm$0.001	30.14	100.47$\pm$0.004
BRAND- II	75.00	75.10	100.13$\pm$0.002	74.90	99.9$\pm$0.001

The composition of the complex was studied by Job's method and molar ratio method. Both the methods confirm the ratio of [Pd(II) : Ambroxol] as 2:1. The stability constant of the complex as evaluated from the Jobs method is 2.090×10^{11}. Linearity, sensitivity, LOD, LOQ and regression equation are summarized in Table-3. The method was applied successfully for the assay of Ambroxol in pharmaceutical formulation. The data are presented in Table 4.

Method Validation and Statistical Analysis

The developed method was validated as per official specifications of ICH guidelines. The validation parameters were found to be accurate and precise. Statistical results are expressed interms of mean $\pm$ SD, % RSD and student t-test and are calculated with aid of Excel 2007. Differences were considered significant at the 95% confidence limit. Repeatability of the method was verified by intraday and interday precision studies (Table 5). Accuracy of the method was studied by recovery studies and the results are summarized in Table 6. Ruggedness studies were carried out by changing the analyst and the results are shown in Table 7.

CONCLUSION

The present method for the determination of Ambroxol is a simple Visible Spectrophotometric procedure which is not only fairly rapid, precise and sensitive but also is within the reach of an ordinary clinical laboratory. The linearity parameter and the corresponding regression data indicated excellent linear relationship (γ =0.9996).

Survey of literature shows no report of a simple, sensitive Visible Spectrophotometric procedure for the estimation of Ambroxol. Methods reported for its determination either use costly instrumentation or suffer from interference of excipients.

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