

International Journal for Pharmaceutical Research Scholars (IJPRS)



ISSN No: 2277 - 7873

RESEARCH ARTICLE

Estimation of Ambroxol Hydrochloride and Gold (Iii) by Simple, Visible Spectrophotometry

Sreenivasulu Reddy Thummala^{*}, K. Nagabushan Reddy, A. Giri

Department of Chemistry, S. K. University, Anantapur, Andhra Pradesh, India. Manuscript No: IJPRS/V2/I4/00245, Received On: 21/12/2013, Accepted On: 28/12/2013

ABSTRACT

This paper describes a new, simple, accurate, and selective visible spectrophotometric method for the estimation of Ambroxol Hydrochloride in bulk drugs and pharmaceutical dosages. It is a specific method for the determination of Gold (III) in Gold alloys .Gold (III) reacts with Ambroxol in the pH range 1.0-5.0 forming a pink colored complex shows an absorption maximum at 520nm. A study of the colour reaction carried out at pH 2.5. Under the optimum conditions Beer's law is obeyed in the range 5.0-100.0 µg/ml. The straight line plot obeyed the equation A = 0.0122 C + 0.0019. The correlation coefficient (r) of the experimental data of the calibration plot is 0.9999. The method is applied successfully for the determination of Ambroxol in pharmaceutical formulation. Keeping Ambroxol in excess, Au (III) is determined. Beer's law is obeyed in the range 2.0-40.0 µg/ml of Au (III). The straight line plot obeyed the equation coefficient (r) of the experimental data of the calibration coefficient (r) of the experimental. Beer's law is obeyed in the range 2.0-40.0 µg/ml of Au (III). The straight line plot obeyed the equation of Ambroxol in generation coefficient (r) of the experimental data of the calibration coefficient (r) of the experimental data of the calibration plot is 0.9999. The effect of various foreign ions associated with Au (III) is studied. The method is applied for the determination of Au (III) in Egyptian gold alloy. 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 are studied. Thus the proposed method can be successfully applied for the estimation of Ambroxol and Gold (III).

KEYWORDS

Ambroxol, Au (III), Visible spectrophotometric determination, Method validation

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). 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.

*Address for Correspondence: Sreenivasulu Reddy Thummala Department of Chemistry, S. K. University, Anantapur, A.P, India. E-Mail Id: tsrreddychem@gmail.com



Ambroxol hydrochloride is an active ingredient in a number of pharmaceutical preparations. 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.

L. M. Joao Santos et al determined spectrophotometrically ambroxol in an automated multi – pumping pulsed flow system¹. Nondestructive determination of Ambroxol content in tablets by Raman Spetroscopy² is reported. Derivative U.Vspectrophotometric and HPLC methods are reported for quantitative determination of Ambroxol in tablets by Zafer et al³. 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 plasma⁴. 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 Mazurek⁵. Sylwester Simultaneous determination of roxithromycin and Ambroxol hydrochloride in a new tablet formulation by liquid chromatography is reported⁶. A new sensitive HPLC – UV method is developed and validated for the determination of Ambroxol in dog plasma⁷. Spectrophotometric determination of trace amounts of Ambroxol is carried out by liquid - liquid extraction using bromothymol blue with a flow – injection system⁸. Derivative U.V 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 levoflaxicin and Ambroxol in pharmaceutical dosage forms by RP-HPLC method¹⁰. A simultaneous UVspectrophotometric determination of Ambroxol hydrochloride and levocetirizine dihydrochloride was reported¹¹. Makarand et al reported a simultaneous UV-spectrophotometric method for the determination of levoflaxicin and Ambroxol in tablets¹². A simultaneous UV-

spectrophotometric estimation of Ambroxol hydrochloride and guiaphensin in tablet dosage forms using simultaneous equations was reported¹³. Prabu et al reported a simultaneous estimation of gatiflaxicin and Ambroxol hydrochloride by UV-spectrophotometric¹⁴. A simultaneous estimation of Ambroxol and cetrizine hydrochloride in tablet dosage form was reported by RP-HPLC method was reported¹⁵. A rapid stability indicating RP-UPLC method for simultaneous determination Ambroxol hydrochloride, cetirizine of hydrochloride antimicrobial preservatives in liquid pharmaceutical formulation was reported ¹⁶. Asha et al reported a simultaneous UVspectrophotometric estimation of Ambroxol and loratadine tablet dosage forms¹⁷. Simultaneous UV-spectrophotometric analysis of Ambroxol hydrochloride, guaifenesin and terbutaline sulphate in liquid dosage forms was reported¹⁸. Patel et al reported a simultaneous UVspectrophotometric estimation of Ambroxol and solbutamol in fixed dosage combination¹⁹.

The above survey of literature shows no report of a direct visible spectrophotometric method for the determination of Ambroxol. This paper reports a simple and sensitive visible spectrophotometric procedure for the determination of Ambroxol and a specific colorimetric procedure for the determination of Au (III).

MATERIALS AND METHOD

Experimental

All chemicals and solvents used were of analytical reagent grade.

Solutions

Gold (III) Solution

1g of chloroauric acid (Johnson Mathews, materials technology, U.K.) was dissolved in distilled water after adding few drops dilute HCl. The solution was made upto the mark in 100 ml volumetric flask. The gold content of the solution was determined by rhodamine B method²⁰. The working solutions were prepared by 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. The solutions employed for the preparation are given below.

pН	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 Shimadzu UV-Visible recording spectrophotometer (UV-160A) was used for absorbance measurements.

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 Alloy, Steel and Industrial Samples

A 0.1 - 0.5 g of the sample is dissolved in a mixture of 2 ml HCl and 10 ml HNO₃. The resulting solution is evaporated to a small volume. To this 5 ml of 1:1 [H₂O: H₂SO₄] mixture is added and evaporated to dryness. The residue is dissolved in 15 ml of distilled water and filtered through Whatman filter paper No. 41. The filtrate is collected in a 100 ml volumetric flask and made up to the mark with distilled water. The solution is further diluted as required.

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 drug²¹.

Absorbance Spectrum

5ml of buffer solution of pH 2.5, 1ml of Au (III) $[5 \times 10^{-3}M]$ solution and 1 ml of Ambroxol $[5 \times 10^{-4}M]$ 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 300-700 nm 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 2.5, 1ml of Au(III) $[5 \times 10^{-3}M]$ solution and varying volumes of Ambroxol $[5 \times 10^{-4}M]$ 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 520 nm using buffer blank.

Determination of Au (III)

5ml of buffer solution of pH 2.5, 1ml of Ambroxol $[5 \times 10^{-3}M]$ solution and varying volumes of Au(III) $[5 \times 10^{-4}M]$ 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 520 nm using buffer blank.

Determination of Ambroxol in Tablets

5ml of buffer solution of pH 2.5, 1ml of Au(III) $[5 \times 10^{-3}M]$ solution and known aliquot of the tablet solution were taken in a series of 10 ml

volumetric flasks and the contents was made up to the mark with distilled water. The absorbance of the solution was measured at 520 nm using buffer blank.

Determination of Au (III) in alloys

5ml of buffer solution of pH 2.5, 1ml of Ambroxol $[5 \times 10^{-3}M]$ solution and known aliquot of the solution of Egyptian gold alloy were taken in a series of 10 ml volumetric flasks and the contents of the flask were made up to the mark with distilled water. The absorbance of the solution was measured at 520 nm using buffer blank.

RESULTS AND DISCUSSION

Ambroxol reacts with Au(III) in the pH range 1-5 forming a pink colored complex solution. The absorption spectrum of the pink colored Au (III) – Ambroxol complex (Fig 1) shows an absorption maximum at 520 nm. The color intensity of the complex is maximum in pH range 2.0-3.0. Hence studies were made at pH 2.5.





 $[Au(III)] = 5.0 \times 10^{-4} M; [ABX] = 5.0 \times 10^{-5} M$

The colour formation attains maximum intensity after 30 minutes of mixing the various components. A fivefold molar excess of Au (III) is sufficient to produce maximum absorbance. The absorbance varied linearly with the concentration of Ambroxol. Beer's law is obeyed in the range 5.0-100.0 µg/ml of Ambroxol. The straight line plot obeyed the equation A = 0.0122 C + 0.0019. The molar Absorptivity and Sandell's sensitivity are $4.616 \times 10^3 \text{ Imol}^{-1} \text{ cm}^{-1}$ and 0.818 µg cm^{-2} respectively. The standard deviation of the method for ten determinations of 40 µg/ml Ambroxol is 0.0033. The correlation coefficient (r) of the experimental data of the calibration plot is 0.9999. The effective range of concentration for accurate determination of Ambroxol as ascertained from Ringbom's plot is 8.0 - 90.0 µg/ml.

The composition of the complex was studied by Job's method and molar ratio method. Both the methods confirm the ratio of Au (III): Ambroxol as 1:1. The stability constant of the complex as evaluated from the Jobs method is 6.63×10^3 .

The effect of various excipients was studied and presented in Table 1. The data shows that generally associated excipients in pharmaceutical formulations do not interfere in the determination of Ambroxol. The method was applied successfully for the Assay of Ambroxol in pharmaceutical formulation the data are presented in Table 2.

 Table 1: Tolerance limit of excipients

Amount of ABX = $40 \mu g/ml$

pH = 2.5

Excipients	Tolerance limit (µg/ml)
Fructose	1395
Glucose	1000
Sucrose	1520
Lactose	1890
Gelatin	2010
Starch	1580
Sodium Alginate	1470
Boric Acid	2100
Mg. stearate	1750

Sample (Manufacturer – Formulation)	Label Claim (mg)	Amount found * (mg)	Error (%)
BRAND-I (Ambrodil-Aristo Pharma Pvt. Ltd.,– Tablet)	30.00	30.22	0.72
BRAND- II (Acroex-Apex Pharma Pvt. Ltd- Tablet)	30.00	30.15	0.50

Table 2: Assay of Ambroxol in pharmaceutical formulation

.* Average of seven determinations

It was also observed that when Ambroxol concentration was kept in excess, the pink colored complex still shows maximum absorbance at 520 nm. The absorbance is also found to be maximum in the pH range 2.0-3.0. A fivefold molar excess of Ambroxol was sufficient to produce maximum absorbance at 520 nm. Under these conditions the absorbance of the complex varied linearly with the concentration of Au (III). Beer's law is obeyed in the range 2.0-40.0 µg/ml. The straight line plot obeyed the equation A = 0.0243 C +0.0007. The molar absorptivity and Sandell's sensitivity were 4.830×10^3 lmol⁻¹ cm⁻¹ and $0.408 \ \mu g \ cm^{-2}$ respectively. The standard deviation of the method for ten determinations of 20 µg/ml of Au (III) is 0.0024. The correlation coefficient (r) of the experimental data of the calibration plot is 0.9999. The effective range of concentration for accurate determination of Au (III) as ascertained from Ringbom's plot is 5.0 - 35.0 µg/ml.

The effect of various foreign ions associated with Au (III) was studied and presented in the Table 3. The data shows that none of the metal ions that are associated with Au (III) interfere even when present in large excess. Thus Ambroxol is a specific reagent for the colorimetric determination of Au (III). The method was applied for the determination of Au (III) in Egyptian gold alloy and the data is presented in Table 4.

Table 3: Tolerance limit of foreign ions

$$[Gold (III)] = 20 \ \mu g/ml \quad pH = 2.5$$

Ion	Tolerance Limit (µg/ml)	Ion	Tolerance Limit (µg/ml)
Iodide	25	Mo(VI)	144
Bromide	80	Ir(III)	288
Chloride	532	Co(II)	88
Fluoride	380	Ba(II)	205
Carbonate	600	Zn(II)	130
Sulphate	1443	Ce(IV)	210
Nitrate	1240	W(VI)	230
Phosphate	1424	Mn(II)	83
Oxalate	1054	V(V)	51
Thiocyan ate	290	Ag(I)	108
EDTA	2204	U(VI)	357
Tartrate	1363	Zr(VI)	137
Citrate	430	Se(IV)	158
Acetate	738	Th(IV)	348
Ascarbate	450	Te(IV)	127
Na(I)	283	Pb(II)	207
K(I)	780	Ga(III)	139
Mg(II)	480	In(III)	230
Ca(II)	800	Y(III)	267

Estimation of Ambroxol Hydrochloride and Gold (Iii) by Simple, Visible Spectrophotometry

Ru(III)	152	La(III)	555
Cr(VI)	78	Ti(IV)	192
Fe(III)	84	Ni(II)	235
Cu(II)	95	Al(III)	108
Cd(II)	169	NH ₄ (I)	360
Hg(II)	301	Pd(II)	Interfere

Method Validation

The results are subjected to statistical treatment and optical characteristics such as Beers law limit, molar absorptivity and other parameters are summarized in Table 5.

Table 4: Determination of Gold (III) in Egyptian Gold Alloy

Egyptian Gold Alloy	Amount of Gold (III) (%)		Error	S
(Sample Nos)	Certified	Found [#]	(%)	
1 *	87.66	87.93	0.30	
2 *	74.92	75.02	0.13	
3 *	55.15	55.09	-0.10	1

* Certified Composition (%)

- 1. Au (87.66); Cu (6.81); Ag (6.12),
- 2. Au (74.92); Cu (12.53); Ag (12.48),
- 3. Au (55.15); Cu (20.82); Ag (20.82).
- [#] Average of seven determinations
- Table 5: Optical characteristics and Statisticaldata of the Regression equation,

Parameter	Ambroxol	Gold(III)	
$\lambda_{max}(nm)$	520	520	
Beer's law limits (µg/ml)	5.0 - 100	2.0-40.0	

Limits of detection (µg/ml)	0.8926	0.3213
Limits of quantization (µg/ml)	2.6778	0.9640
Molar absorptivity (l.mo1 ⁻¹ cm ⁻¹)	4.616 x 10^3	4.830 x 10^3
Sandell's Sensitivity (µg/cm ²)	0.0818	0.0408
Regression equation	ion ($y = a$ -	+ b x)
Regression equation Slope (b)	tion ($y = a - 0.0122$	+ b x) 0.0243
Regression equation Slope (b)	ion ($y = a - 0.0122$ 0.0019	+ b x) 0.0243 0.0007
Regression equation Slope (b) Intercept (a) Correlation coefficient (γ)	ion ($y = a - 0.0122$ 0.0019 0.9999	+ b x) 0.0243 0.0007 0.9999

The intra and inter day precision of the proposed method confirm adequate sample stability and method reliability over a 24 h period. (Table- 6) which are reported. This is because for the three selected concentrations which are within the linearity range, the observed RSD's were all<5% and the calculated values of t were less than the tabulated values of t at 95% confidence limit. Accuracy was reported by the results of recovery studies. The recovery studies are carried out by adding the known amount of standard Ambroxol to the sample solution of the tablets results were shown in Table-7. Ruggedness studies were carried out by changing the analyst and results were shown in table Table-8.

CONCLUSION

The present method for the determination of Ambroxol is a simple Visible spectrophotometric procedure which is not only fairly rapid, précis and sensitive but also is within the reach of an ordinary clinical laboratory. The linearity parameter and the

Conc.	Mean absort	Dance <u>+</u> SD	D % RSD		Calculated
(µg/ml)	DAY-1	DAY-2	DAY-1	DAY-2	value of t
25	0.324 <u>+</u> 0.001	0.323 <u>+</u> 0.002	0.31	0.47	0.13
35	0.430 <u>+</u> 0.001	0.402 <u>+</u> 0.001	0.50	0.39	0.41
45	0584 <u>+</u> 0.001	0.583 <u>+</u> 0.001	0.35	0.45	0.52

Table 6: Intra- and Inter- day precision studies of Ambroxol (n=3,p=0.05)

 Table 7: Recovery studies for Ambroxol in tablets

Tablet	Amount of sample (µg/ml)	Amount of drug added (µg/ml)Amount	Amount Recovered (µg/ml)	%Recovery <u>+</u> SD
Brand-I	30	15	45.75	101.68 <u>+</u> 0.003
(Ambrodil- Avisto-pharma	30	30	60.19	100.31 <u>+</u> 0.003
Ltd)	30	45	75.12	100.27 <u>+</u> 0.002
Brand- II	20	15	34.96	99.98 <u>+</u> 0.003
(Acorex- Apexpharmaa	20	30	51.14	102.28 <u>+</u> 0.001
Ltd.)	20	45	65.20	100.30 <u>+</u> 0.004

Table-8: Ruggedness result for the Ambroxol in tablets

	Analyst -I			Analyst- II	
Tablet	Label Claim (mg)	Amount found* (mg)	(%) Recovery <u>+</u> SD	Amount found* (mg)	(%) Recovery <u>+</u> SD
BRAND-I	30.00	30.22	100.73 <u>+</u> 0.003	30.14	100.47 <u>+</u> 0.004
BRAND- II	30.00	30.15	100.50 <u>+</u> 0.002	30.20	100.65 <u>+</u> 0.003

regression corresponding data indicated excellent linear relationship ($r^2 = 0.999$). All the methods reported for its determination employ either expensive instrumentation or suffer from lack of sensitivity and interference from excipients. Further а simple Visible spectrophotometric method is proposed for the determination of Au (III). This is, though less sensitive is a specific method as no other metal ions associated with it react with Ambroxol.

ACKNOWLEDGEMENT

The authors thank the department of Chemistry of S. K. University Anantapur - 515003 for providing the necessary facilities.

REFERENCES

- Santos JLM, Clausse A, Lima JLFC, Saraiva MLMFS, Rangel AOS, Analytical Science, 2005, 21, 461.
- 2. Min-Sik Hwang, Cho S, Jung H, Woo Y, Journal of Pharmaceutical and Biomedical Analysis, 2005, 38, 210.
- 3. Dincer Z, Hasan B, Goger NB, J. Journal of Pharmaceutical and Biomedical Analysis, 2003, 31, 867.
- 4. Kim H, Yoo JY, Han SB, Lee HJ, Lee HR, Journal of Pharmaceutical and Biomedical Analysis, 2003, 32, 209.
- 5. Szostak R, Mazurek S, Journal of Molecular Structure, 2004, 704, 229.
- 6. Qi M, Wang P, Cong R, Yang J, J. Pharm. Biomed. Anal chim., 2004, 35, 1287.
- Kiss BD, Baloghnemes K, Urmos I, Szunyog J, Klebovich I, Chrom. Supl., 2000, 51, 217.
- Perez-Ruiz T, Martinez-Lozano C, Sanz A, M, Teresa San Miguel, Talanta, 1996, 43, 1029.
- 9. Basan H, Dincer Z, Goger NB, Chem. Anal., (Warsaw), 2005, 50, 465.

- 10. Kothekar KM, Jayakar B, Khandhar AP, Mishra RK, Eurasian Journal of Analytical Chemistry, 2007, 2(1), 21.
- 11. Prabu SL, Shirwaikar AA, Shirwaikar A, Kumar CD, Kumar GA, Indian Journal of Pharmaceutical Sciences, 2008, 70(2), 236.
- 12. Avhad M, Bonde CG, International Journal of ChemTech Research, 2009, 1(4), 873.
- 13. Prasanthi NL, Krishna M.Ch, Manikiran SS, Rama RN, International Journal of Research in Ayurveda & Pharmacy, 2010, 1(1), 140.
- 14. Prabu SL, Thiagarajan S, Srinivasan M, Marina Q, International Journal of Pharmaceutical Sciences Review and Research, 2010, 3(2), 123.
- 15. Maithani M, Raturi R, Gautam V, Kumar D, Gaurav A, Singh R, Pharmacie Globale, 2010, 1(2), 1.
- 16. Trivedi RK, Patel MC, Jadhav SB, Scientia Pharmaceutica, 2011, 79, 525.
- 17. Ponnilavarasan I, Kumar CSN, Asha P, International Journal of Pharm and Biosciences, 2011, 2, 338.
- 18. Kimbahune R, Sunil K, Kbra P, Delvadiya K, Surani S, International Journal of Pharmaceutical Sciences Review and Research, 2011, 8, 24.
- 19. Patel PA, Dole MN, Sawant SD, P.S. Shedpure PS, International Journal of Pharmaceutical Sciences and Research, 2011, 2, 1225.
- 20. Marczenko Z, Spectrophotometric determination of elements, 1st Edn, 1976, 282.
- 21. Basavaiah K, Manjunathaswamy J, Farmaco IL, 2001, 56, 579.