SPECTROPHOTOMETRIC DETERMINATION OF AMBROXOL HYDROCHLORIDE AND ITS FORMULATIONS BASED ON CHARGE TRANSFER COMPLEX REACTION

A. Narayana[1], C. Narasimha Rao[2], K. Sivakumar[1]*

[1]Dept. of Chemistry, S.V.Arts U.G. & P.G. College (TTD's), Tirupati, Andhra Pradesh, India, 517 502.
[2]Dept. of Chemistry, Sri Venkateswara University, Tirupati , Andhra Pradesh, India, 517 502.
E mail :sivakumark78@gmail.com
Ph.No.:09200808403

ABSTRACT:

A rapid, sensitive and accurate spectrophotometric method for the determination of ambroxol was described in this study. The current method was developed based on charge transfer complexation of ambroxolol (AMB) with the chloranilic acid (CAA). The formed intensed Colored complex was measured at 430 nm and used for determination of AMB and its formulations.Under optimized experimental conditions Beer's law is obeyed in the concentratin range of 5-40 µg/ml. Recovery studies were conducted by standard addition method to confirm the accuracy of the method. Molar absorptivity and Sandell's sensitivity were calculated and the developed method was validated as per ICH guidelines. The obtained results show that the present method can be successfully applied for the determination of AMB in pure and in pharmaceutical formulations.

Key words: Chloranilic acid, Ambroxol, Beer's law, ICH

INTRODUCTION:

Ambroxol hydrochloride (Figure 1) is a mucolytic agent, chemically known as trans-4-(2-amino-3,5dibromobenzylamino) cyclohexanol hydrochloride and it is used in the treatement of bronchial asthama and chronic bronchitis. It acts as an anti-glue factor by reducing the adhesion of mucus to the bronchial wall, in improving its transport and in providing protection against infection and irritating agents. Ambroxol is often administered as an active ingredient in cough syrup.

UV spectrophotometric [1-7], high performance liquid chromatographic [8-12], gas chromatographic [13-14] and liquid chromatography-mass spectrophotometry [15-18] methods were reported for quantitative determination of AMB in pure, in pharmaceutical formulations and in biological fluids. It is officially reported in British Pharmacopeia [19] and Indian pharmacopeia [20].

However no reliable methods were reported for determination of AMB based on charge transfer complex reaction. Hence the present work is aimed to develop a simple spectrophotometric method for the determination of AMB in pure and in pharmaceutical formulations. The current method is rapid, cost effective with good accuracy; hence this method can be utilized for regular quality control analysis.

MATERIALS AND METHODS:

Materials and reagents

Ambroxol hydrochloride was procured from M/s Malladi Drugs & Pharmaceuticals Limited, Inida as a gift sample. Mucolite, Ambrodil and Ambrolite were purchased in local market, Tiurpati. All the chemicals used were of analytical reagent grade.

Instrumentation

All measurements were carried out using a Shimadzu UV-Visible spectrophotometer (UV-160A) with a matched pair of 10 mm quartz cells. Mettler Toledo analytical balance (accuracy 0.1 mg) was used for weighing all the samples.

Preparation of standard solutions

A standard solution 1 mg/ml was prepared in 100 ml volumetric flask by dissolving 100 mg of AMB in methanol and solution was diluted to get appropriate concentration for the current investigation.

Preparation of reagents

1.0% (w/v) of CAA solution was prepared by dissolving 1g of compound in 100 ml of ethanol in standard volumetric flask, sonicated and used as such.

Analysis of tablets:

The pharmaceutical formulations containing AMB were purchased from local market and analysed by using developed methods. Ten tablets were grinded to make a fine powder; required quantity was transferred into the volumetric flask and dissolved in methanol with an aid of sonication. After soniaction, solution was filtered through 0.45µ paper and further diluted to get the required concentration. The above solution was analysed according to the procedure.

Method development

Different aliquots of standard drug solution were prepared in clean and dry volumetric flask in concentration range of 5-40 µg/ml.For these flasks, 5 ml of 1.0% CAA solution was added. The entire solution was mixed and kept aside for few minutes. The absorbance of pale yellow coloured complex was measured at 430 nm against the reagent blank.

RESULTS AND DISCUSSION :

Absorption spectrum

The ambroxol hydrochloride drug was allowed to react with the added 5.0 ml solution of 1% CAA solution in a series of volumetric flasks, pale yellow colour was developed which showed the maximum absorbance at 430 nm against balnk [Fig.2.]

Effect of reagent concentration

To study the optimum concentration of the reagent, various concentrations in the range of 1-5.4 ml were tested with standard drug solution and found that maximum absorbance was obtained with 5 ml of reagent. Hence, it was considered as the optimum concentration of the reagent for the further investigation.

Effect of concentration of drug

The effect of concentration of drug solution on the absorbance of coloured complex was studied by adding a standard volume of reagent to volumetric flask containing various alliquotes of drug in the range of 5-40 µg/ml of solution. Maximum absorbance was measured at 430 nm against reagent blank.

Analytical method validation

The developed method was validated according to the ICH guidelines [21-22] and the following characters of validation are addressed; Linearity, Accuracy, Precision, Specificity, LOD, LOQ and Robustness.

Standard calibration curve was constructed by plotting absorbance versus concentration [Fig.3.] and regression equation was derived from the calibration plot. The linearity of calibration graphs was proved by the high values of the correlation coefficient and the small values of the y – intercept of the regression equation. Recovery studies were conducted by using standard addition method and obtained results reported in Table 4 to proved accuracy of the method. To confirm the repetability, intra day and inter day analysis were performed and obtained results were reported in terms of % RSD in respective Table 2 and Table 3 and found no significanat variation. To assess the specificity and selectivity of developed method, the effect excipients like starch, lactose, glucose, sugar, talc etc. were studied. The results indicated in Table 5 that there was no effect of interference from the excipients on the developed methods.The Sandell's sensitivity, LOD, LOQ of the resulting colored complexes were also calculated and reported in respective Table 1.

CONCLUSION:

A simple, precise and accurate analytical method was developed for determination of AMB in bulk and in the form of pharmaceutical formulations. The linearity of the calibration standards of the drug by proposed method was good from the result of correlation coefficient. The overall recovery of the drug by the proposed method was ranged from 99.11 to 99.33 percent and found there is no interference due to the excipients. The developed method is simple, specific, accurate and precise than the existing methods for the estimation of AMB in pure and tablets. Hence this method can be utilized for routine analysis in laboratories.

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Table 1. Spectral characteristics of the drug with reagent

Parameter	CAA Method
λ_{max} (nm)	430
Beer's law limit (µg/ml)	5-40
Molar absorbance ($L.mol^{-1}$ cm^{-1})	5.4044
Sandell's sensitivity ($\mu g.cm^{-2}$/0.001 A.U)	0.0012
Correlation coefficient (r)	0.9980
Slope (m)	0.0119
Intercept (c)	0.0894
%RSD	0.5904
Colour	Pale yellow
LOD	0.8279
LOQ	2.5089

Table 2 Evaluation of accuracy and precision results of the proposed method

Method	Taken mg/ml	Intra day				Inter day			
		*Found	Recovery %	± SD	% RSD	*Found	Recovery%	± SD	% RSD
CAA	6	5.91	98.63	0.0435	0.7358	5.92	98.77	0.0225	0.3797
	8	7.92	98.98	0.0354	0.4476	7.93	99.08	0.0436	0.5508
	10	9.91	99.07	0.0592	0.5977	9.91	99.10	0.0252	0.2552

*****Average of six determinations

Table 3 Evaluation of accuracy and precision in pharmaceutical formulation

Method	Pharma-ceutical formu-lation	Taken mg/ml	Intra day				Inter day			
			*Found	Recovery %	± SD	% RSD	*Found	Recovery%	± SD	% RSD
CAA	Mucolite	4	3.93	98.45	0.0132	0.3374	3.92	98.16	0.0265	0.6769
	Ambrodil	6	5.89	98.19	0.0526	0.8943	5.92	98.77	0.0294	0.4967
	Ambrolite	8	7.88	98.56	0.0418	0.5305	7.85	98.12	0.0547	0.6977

*****Average of six determinations

Table 4 Recovery of ambroxol hydrochloride in pharmaceutical formulation

Name of the drug	Pharmaceutical formulation	Labeled amount (mg/ml)	*Found(mg/ml)	Recovery %	± SD	% RSD
Ambroxol hydrochlo-ride	Mucolite	30	29.75	99.11	0.1227	0.4392
	Ambrodil	30	29.76	99.33	0.1308	0.4427
	Ambrolite	30	29.77	99.22	0.1615	0.5583

*****Average of six determinations

Table 5 Determination of Ambroxol hydrochloride in presence of excipients

Excipients	Amount taken mg/ml	*Found mg/ml	Recovery %	±SD	RSD%
Glucose	2	1.96	98.41	0.0183	0.9321
Sucrose	3	2.95	98.5	0.0187	0.6331
Lactose	4	3.93	98.33	0.0121	0.3078
Dextrose	2	1.96	98.41	0.0098	0.4995
Talc	3	2.96	98.88	0.0136	0.4605
Starch	4	3.96	99.16	0.0186	0.4693

*Average of six determinations

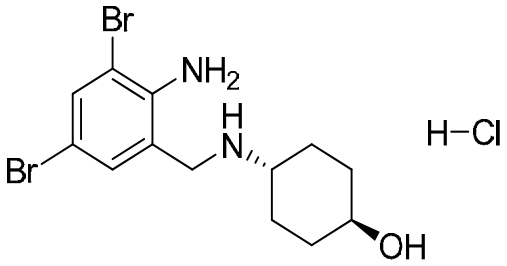

Fig. 1. Structure of ambroxol hydrochloride

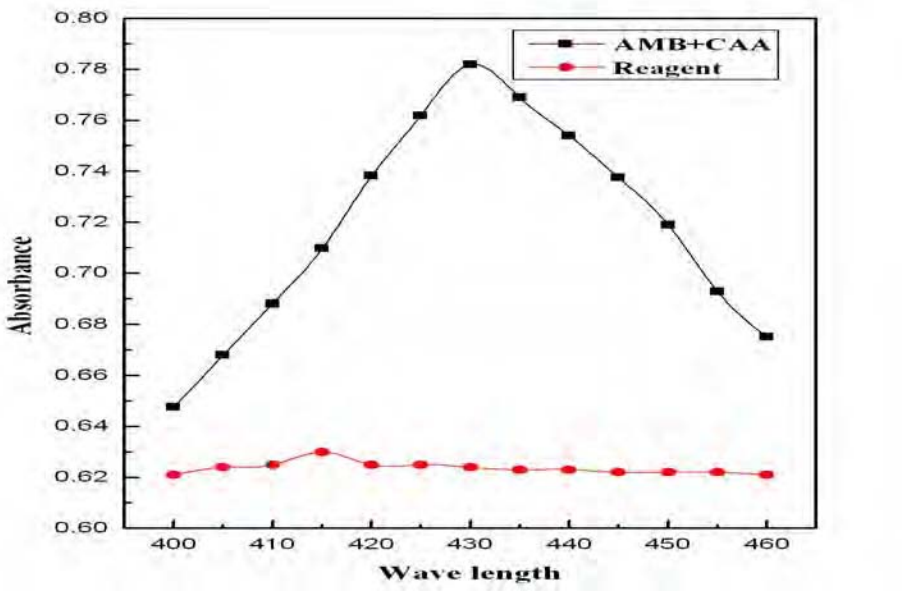

Fig.2. Absorption spectrum of ambroxol hydrochloride with CAA

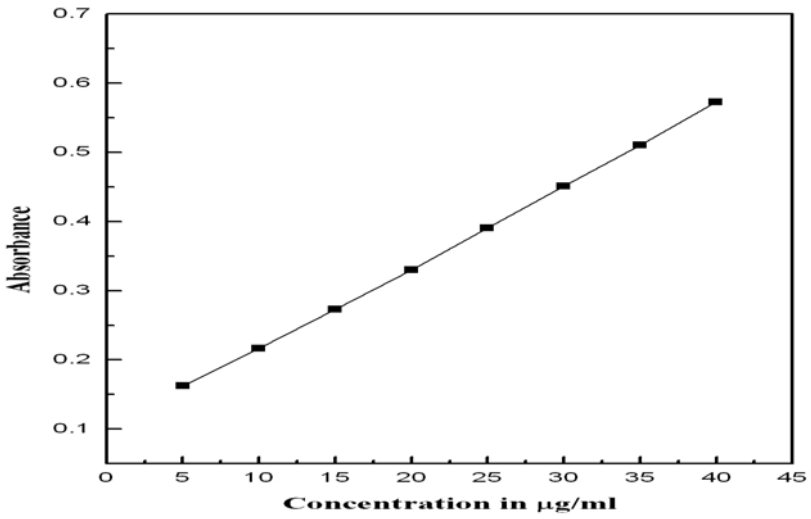

Fig.3. Calibration plot of ambroxol hydrochloride