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SPECTROPHOTOMETRIC QUANTITATIVE DETERMINATION OF AMBROXOL HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS USING PDAB REAGENT

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Keywords:

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ABSTRACT: A simple, sensitive, cost-effective spectrophotometric method has been developed and validated for the determination of Ambroxol hydrochloride (AMB), in bulk drug and its pharmaceutical formulations. The method is based on the formation of a Schiff base with PDAB; the reaction of drug with reagent gives a bright yellow color. The so formed colored species absorbance was measured at its absorption maximum (λ_{max}) 423 nm. Beer's law has been obeyed in the concentration range 5-30 µg/ml. The optical parameters were calculated as 2.0702×10^4 (L/mol/cm), 0.0183 (µg/cm^2), molar absorptivity and Sandell sensitivity respectively. The LOD and LOQ of the proposed method were calculated 0.0844 (µg/ml), 0.2812 (µg/ml), respectively. All the variables were studied to optimize the reaction conditions. No interference was observed in the presence of common pharmaceutical excipients. The validity of the method was tested by analyzing AMB in its pharmaceutical formulations and critically tested for its accuracy by statistical tests. Good recoveries were obtained by the developed method; the obtained results were critically analyzed and successfully employed for the determination of AMB in its pharmaceutical dosage forms.

INTRODUCTION: Chemically Ambroxol hydrochloride (AMB) is [trans-(2-amino-3, 5-dibromobenzylamino) cyclohexanol hydrochloride] **Fig. 1**, a metabolite of bromhexine, as hydrochloride, is used as a bronchosecretolytic and expectorant drug [1]. It has also been reported to have a cough suppressing effect and anti-inflammatory action.

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Recently, the inhibition of nitric oxide-dependent activation of soluble guanylate cyclase was suggested as one of the molecular mechanism of the therapeutic action AMB, also used in pulmonary alveolar proteinosis in pulmonary distress and infant respiratory distress syndrome [2, 3]. It can be administered as a hydrochloric salt in daily doses of 30–120 mg using mostly oral formulations like tablets and syrups [4].

The extensive literature survey reveals that there are several spectrophotometric methods have been reported for the qualitative and quantitative determination of AMB in pharmaceutical formulations [5-9]. Various HPLC [10-14], GLC [15, 16], LC-MS/MS [17, 18] and electroanalytical [19] methods

are also reported for its determination in biological fluids.

In the present research work, an effort has been made to develop and validate a simple spectrophotometric determination of AMB in bulk and pharmaceutical formulations. Although, there are several highly sophisticated instrumental methods were reported but are suffered by the time of analysis, cost per analysis, sophistication, and most importantly, the skilled analyst to handle the instruments. The present method offers a simple, sensitive, cost-effective method for the determination of AMB in any common QC laboratory.

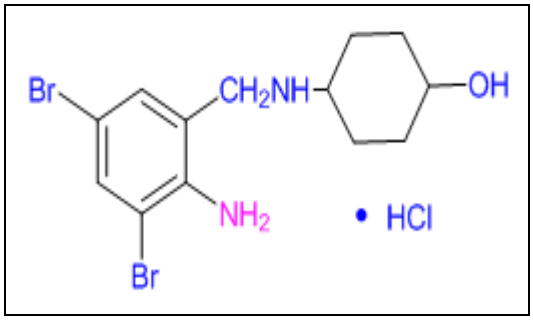


FIG. 1: CHEMICAL STRUCTURE OF AMBROXOL HYDROCHLORIDE

EXPERIMENTAL:

Apparatus: Spectral and absorbance measurements were carried out by using double beam UV-Spectrophotometer ELICO-SL-244.

Materials and Reagents: All the chemicals used were of analytical grade. All the solutions were prepared freshly, and deionized water is used throughout the experiment. Ambroxol hydrochloride bulk drug obtained from Sun Pharmaceutical (Mumbai, India) certified to contain 99.7% of the active ingredient, which has been used as a reference substance, as received without further purifications.

p-dimethylaminobenzaldehyde (PDAB) was procured from SD-Fine chemicals 99.5% purity, and HCl procured from SD-Fine chemicals 35% purity. Methanol AR grade procured from SD-Fine chemicals. Ambroxol hydrochloride tablets were purchased from the pharmaceutical store, different makes such as Ambcet® (30 mg AMB) and Mucolite® (60 mg AMB) from Fourrts (India) laboratory Ltd. and Dr. Reddy's laboratory Ltd respectively.

Preparation of 5% (w/v) PDAB Solution: Accurately weighed 5 g of PDAB in a 100 ml volumetric flask, added 20 ml of methanol swirled to mix then the solution made up to the mark with methanol.

Preparation of Standard Stock Solution: Weighed accurately near (0.001g) 10 mg of the reference standard in a 10 ml volumetric flask; added 5 ml deionized water swirled to mix and brought to the mark with deionized water. The apparent concentration has been reached to 1000 µg/ml. Further stepwise dilutions were made to obtain the working standard stock solution 100 µg/ml.

Preparation of Sample Solution:

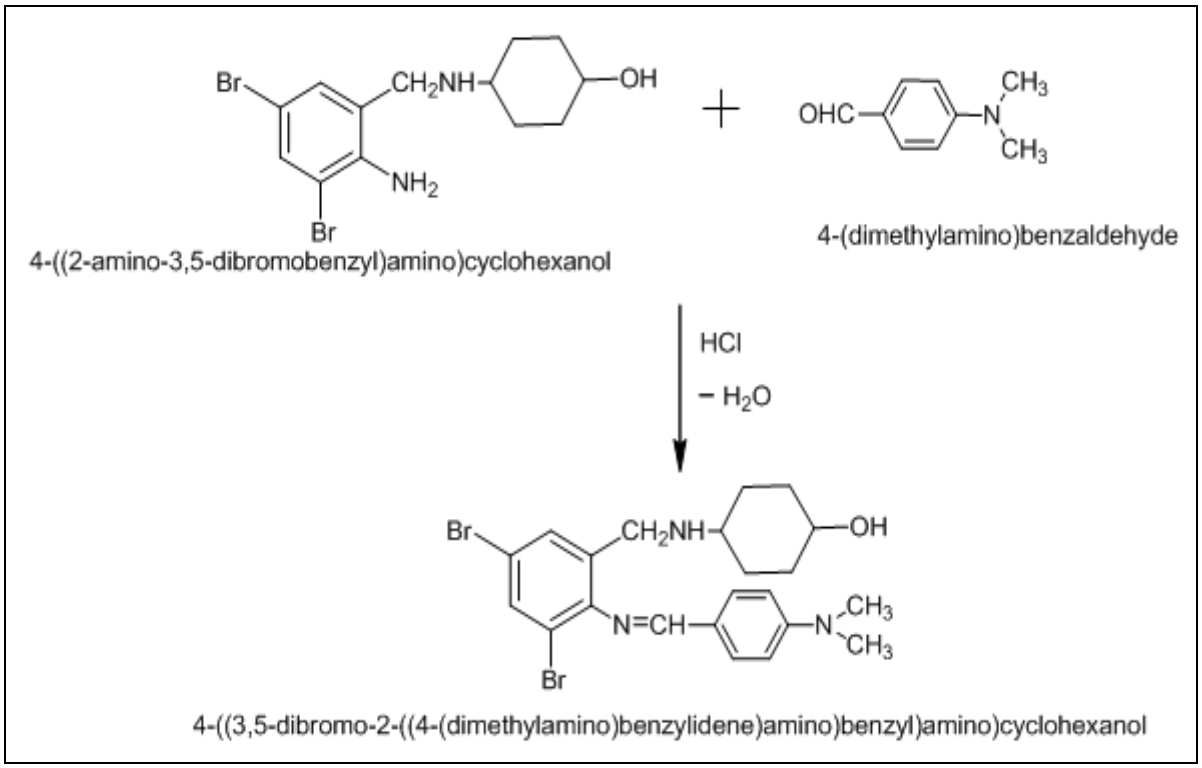
1. Mucolite® tablets (10 tablets) labeled claim to contain 30 mg of AMB, the average weight of each tablet was 216 mg were triturated and made a fine powder, mixed it well for homogeneity. A portion (720 mg) of the fine powder was transferred to contain 100 mg of the AMB into a beaker and dissolved with 20 ml of deionized water and mixed well. This solution was filtered through a Whatmann filter paper No.41, into a 100 ml volumetric flask. The filtrate was made up to the mark with deionized water.

2. Ambcet® tablets (10 tablets) labeled claim to contain 60 mg of AMB, the average weight of each tablet was 117 mg were triturated and made a fine powder, mixed it well for homogeneity. A portion (195 mg) of the fine powder was transferred to contain 100 mg of the AMB into a beaker and dissolved with 20 ml of deionized water and mixed well. This solution was filtered through a Whatmann filter paper No.41, into a 100 ml volumetric flask. The filtrate was made up to the mark with deionized water.

General Procedure for the Determination of Ambroxol Hydrochloride: Variable aliquots of working standard solution containing 5-30 µg/ml of AMB were transferred into series 10 ml volumetric flasks. To each flask 2 ml of concentrated HCl was added, mixed the solution mechanically and then

added 2 ml of 5% PDAB solution then the flasks are heated on a water bath for 10 min. There is a formation of yellow colored Schiff base, then the flasks are allowed to cool to room temperature, and the solutions made up to the mark with water. The colored species absorbance was measured at 423 nm using reagent as a blank. The formation of the Schiff base is shown in **Scheme 1**. The calibration graph was prepared by plotting absorbance versus concentration of drug, and the concentration of unknown was read from the calibration graph or computed from the regression equation derived from Beer's law data.

The same procedure was followed for the determination of AMB in the tablet formulations, and the content of the tablets was calculated by using the regression equation.



SCHEME 1

RESULT AND DISCUSSION:

Determination of Absorption Maxima (λ_{max}): To determine the λ_{max} of the colored species, 1 ml of 100 µg/ml of the AMB was added to a 10 ml volumetric flask and 2 ml of concentrated HCl, mixed the contents mechanically then added 2 ml of 5% PDAB solution then the flasks are heated on a water bath for 10 min. There is a formation of yellow colored Schiff base, then the flasks are allowed to cool to room temperature, and the solutions made up to the mark with water.

The colored species was measured against reagent blank in the range of 400 nm to 800 nm. The λ_{max} of the complex was found to be 423 nm. The absorption spectrum of the proposed method was shown in **Fig. 2**. Under the experimental conditions, each reagent blank showed a negligible absorbance at the corresponding λ_{max}.

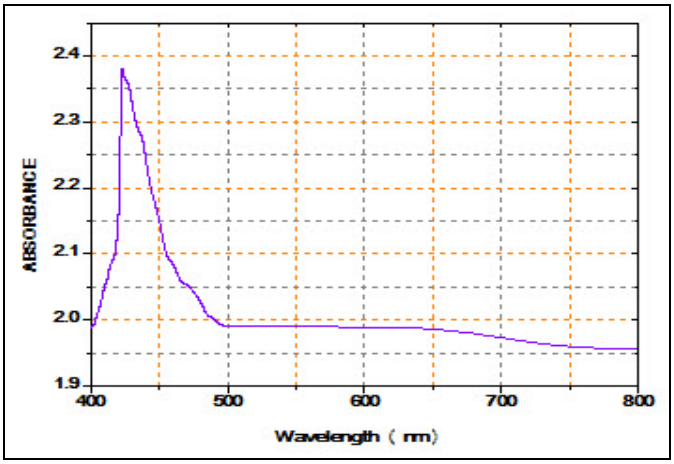


FIG. 2: ABSORPTION SPECTRUM OF THE COLOURED SPECIES

Investigation of Assay Parameters: Optimum reagent concentrations required for the formation of sensitive and quantitative colored products were

determined by varying one reagent concentration and fixing the concentrations of other reagents, and its effect on absorbance was measured at 423 nm.

Effect of Heating Time: To study the effect of heating time for the development of maximum color, the contents of the mixture were heated for up to 30 min. at 100 ± 1°C. The intensity of the color developed was measured at room temperature after the dilution to 10 ml with deionized water. It is apparent from the investigation that the maximum intensity of color was obtained after 10 min of heating and remained constant. Therefore the optimum heating time was fixed to 10 min.

Effect of Reagent Concentration: The effect of concentration of PDAB solution and HCl were studied on the related absorbance values. Different concentrations of PDAB solutions from 0.5% to 5% were studied. Volumes of 0.5–3.0 ml of PDAB (5%) and 0.5–3.0 ml of concentrated HCl were examined. The investigations showed that 2.0 ml of PDAB and 2.0 ml of concentrated HCl gave maximum absorbance. There is no change in intensity of the color any further with the increasing amounts of PDAB and concentrated HCl. So the 2.0 ml of PDAB and 2.0 ml of concentrated HCl were chosen throughout the experiment.

Interference Studies: To study the potential interference from the commonly used excipients and other additives such as glucose, lactose, starch, sodium starch glycolate, cellulose, magnesium stearate, and ascorbic acid recovery studies were carried out. Under the experimental conditions employed, to a known amount of drug. The recovery studies suggest that there was no significant interference from the excipients on the assay of the drug.

Validation of the Method:

Detection and Quantification Limits: According to the Analytical Methods Committee the detection limit (LOD) is the concentration of drug corresponding to a signal equal to the blank mean (Y_B) plus three times the standard deviation of the blank (S_B). Quantification limit (LOQ) is the concentration of drug corresponds to the blank mean plus ten times the standard deviation of the blank. The LOD and LOQ values for CP were found to be 0.0844 µg/ml and 0.2812 µg/ml, respectively.

Quantification: The optical characteristics such as Beer's law limits, Sandell sensitivity, and molar absorptivity were calculated for the proposed method, and the results are summarized in **Table 1**. Regression analysis of the Beer's law plot at their λ_{max} revealed a good correlation, as shown in **Fig. 3**. For the regression analysis, we have selected different concentration sets, but the best fit curve was obtained in the concentration range 5 to 30 µg/ml of standard AMB. For the verification of Beer's law, we have taken a series of 10 ml volumetric flasks and added the standard working solution (100 µg/ml) serially from 0.5 ml to 3.0 ml followed by added all the reagents as mentioned in the assay procedure. Graph of absorbance versus concentration plotted and are described by the regression equation Y= bx+a (where 'Y' is the absorbance, 'b' is the slope, 'x' is the concentration of the drug in µg/ml and 'a' is the intercept) obtained by least squares method. The results were summarized in **Table 1**.

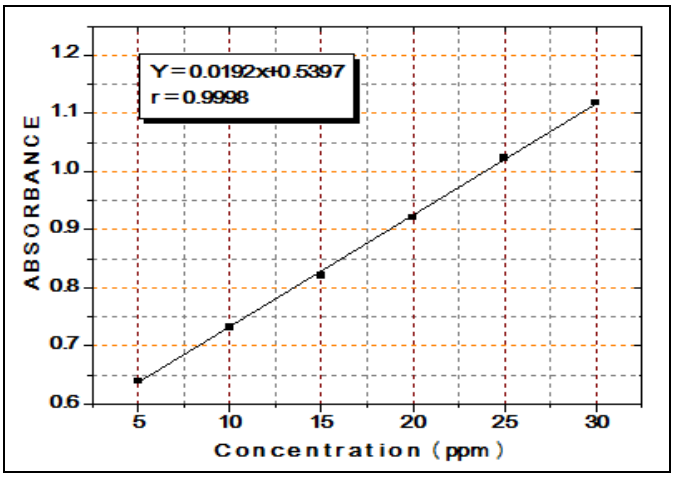


FIG. 3: BEER'S LAW CALIBRATION CURVE

Accuracy precision and recovery studies: The accuracy and precision of the proposed method were evaluated by performing five replicate determination of AMB in pure form at three different concentrations (10, 20 and 30 µg/ml) by short term (intra-day) precisions as shown in **Table 2**. The standard analytical errors, relative standard deviations (% RSD), and recoveries obtained in the intra-day analysis for the proposed method were found to be acceptable. Thus the proposed method is effective for the determination of AMB.

TABLE 1: OPTICAL AND REGRESSION CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHOD

S. no.	Parameters	Value
1	λ_{max} (nm)	423
2	Beer's law limit (µg/ml)	5-30
3	Sandell sensitivity (µg/cm^2/0.001 abs. unit)	0.0183
4	Molar absorptivity (L mole^{-1}.cm^{-1})	2.0702x10^4
5	Stability of Color (hours)	2
6	Regression equation	y = 0.0192x + 0.5397
7	Correlation coefficient	0.9995
8	% Relative standard deviation	0.06574
9	% Range of errors	0.8213±0.67x10^{-3}
	0.05 %, 0.01%	0.8213±0.90x10^{-3}
10	Limit of detection (µg/ml)	0.0844
11	Limit of quantification (µg/ml)	0.2812

TABLE 2: EVALUATION OF THE ACCURACY AND PRECISION OF THE PROPOSED METHOD BY INTRA-DAY ASSAY

Concentration of AMB (µg/ml)	Observed concentration of AMB (µg/ml)			
	Intra-day			
	Mean*	Error (%)	RSD (%)	Recovery (%)
10	10.042	0.008	0.026	100.4
20	19.886	0.007	0.017	99.43
30	30.130	0.013	0.026	100.4

*For five determinations

The accuracy of the proposed method was further checked by performing recovery experiments through the standard addition technique. For this purpose, a known amount of pure AMB was added to pre-analyzed dosage forms and then determined by the recommended procedure. The results are as shown in **Table 3**. The values of mean recovery and relative standard deviation (% RSD) were in the range of 99.99-100.0% and 0.032-0.033% respectively. This indicates the reproducibility of the method. No interference was observed from the common excipients of the tablet.

TABLE 3: DETERMINATION OF AMB IN PHARMACEUTICAL FORMULATION BY STANDARD ADDITION TECHNIQUE

Amount of drug before addition (µg)	Amount of drug added (µg)	Theoretical amount (µg)	Mean amount recovered (µg) (n=5)	Mean % of recovery (n=5)	RSD%
5	10	15	14.99	99.99	0.032
5	20	25	25.00	100.0	0.033

Applicability of the Method: The proposed method applied to the analysis of AMB in pharmaceutical dosage forms, and the results were statistically compared with the reference method by calculating the student's t-values.

The evaluated t-values were less than the tabulated values at the 95% confidence level for five degrees of freedom, as revealed by the results compiled in **Table 4**. This suggests that the proposed method is accurate and precise as the reference method.

TABLE 4: RESULTS OF ANALYSIS OF TABLET FORMULATION CONTAINING AMB

Formulation	Labelled amount (mg)	% Found ± SD			
		Reference method	Proposed method*	% Recovery of proposed method	t-test**
Mucolite®	30	29.96[a]	29.98 ±0.00036	99.92	1.558
Ambcet®	60	60.31[b]	59.98 ±0.00036	99.96	1.869

***** Recovery amount was the average of five determinations. **The t-value from the table is 2.776 at 95% level[21]. 'a' and 'b' are the contents of AMB in reference methods [20, 8].

CONCLUSION: The proposed method was quite simple and did not require any pre-treatment of the drug and tedious extraction procedure. The method has a wider range with good accuracy and precision. Hence, the data presented in the manuscript demonstrate that the proposed method was accurate, precise, linear, selective and offers advantages of reagent availability and stability, less time consumption, and highly sensitive. Thus, it can be extended for routine analysis of AMB in pharmaceutical industries and hospitals and research laboratories. Unlike the LC/MS procedure and HPLC procedures, the UV-Visible Spectrophotometer instrument is simple and not of highly expensive on the other hand in simplicity, and user-friendly the method could be considered superior in comparison with the previously reported methods. Moreover, the method is free from interferences by common additives and excipients.

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CONFLICT OF INTEREST: Nil

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