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METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF AMBROXOL HCl IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT: Rapid, sensitive, specific and validated colorimetric methods have been developed for the quantitative estimation of ambroxol HCl in bulk and dosage form. The current method was developed based on oxidation of ambroxol with Gibb's reagent (method I) and p-diethyl amino benzaldehyde in toluene (method II). The formed intense colour complex was measured at 537.2 nm and 438.4 nm respectively. Under optimized experimental conditions, Beer's law is obeyed in concentration range 5 - 30 µg/ml for both the methods with regression co-efficient 0.9994 and 0.9992. Recovery studies were conducted by standard addition method to confirm the accuracy of the method. The LOD and LOQ for the estimation of ambroxol were found as 0.0773, 0.2343 for method I and 0.0667, 0.2021 for method II respectively. The proposed method was validated as per ICH guidelines.

INTRODUCTION: Spectrometric methods are a large group of analytical methods that are based on atomic and molecular spectroscopy [1]. Colorimetric assays generally consists of adding a reagent to the assay preparation or to the substance being tested, to produce a colour that is compared with that of a standard preparation that has been prepared simultaneously and contains approximate quantity of the reference standard [2]. In general, in analysis the first step is to determine the nature of the sample that is complete qualitative information and then further proceed for quantitative information by accuracy, LOD, LOQ *etc.* [3] Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use [4].

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The parameters for method validation have been defined in different working groups of national and international committees and are described in the literature [5]. Ambroxol is a clinically proven systematically active mucolytic agent. It is freely soluble in water, practically insoluble or very slightly soluble in ethanol (96 per cent) and in methylene chloride [6, 7, 8]. The literature survey reveals that few methods are reported for the determination of Ambroxol Hydrochloride such as thin layer chromatography, RP isocratic HPLC, UHPLC *etc*. [10].

Validation characteristics evaluated are Specificity, Accuracy, Precision, Limit of detection, Limit of quantitation, Linearity, Range, Ruggedness and Robustness. The method is based on formation of a purple coloured chromogen by the interaction of Ambroxol Hydrochloride with Gibb's reagent (Method IA) and light greenish yellow colour chromogen with p-dimethylamino benzaldehyde reagent and toluene (Method IB) under acidic condition.

MATERIALS AND METHODS: Shimadzu UV spectrophotometer 1700 and Jasco V-630 spectrophotometer with 1 cm matched quartz cells was used for all spectral and absorbance measurements. Pure drug was procured from local pharmaceutical industry. Basic apparatus like calibrated volumetric flasks, pipette, beakers and graduated pipettes were used.

Experimental:

Preparation of Stock Solutions: Accurately weighed 100 mg of Ambroxol HCl was dissolved in 100 ml ethanol to give a concentration of 1000 µg/ml. The final concentration was brought to 100 µg/ml for Methods A and B.

Method IA:

- 0.1% Gibb's reagent
- 0.2% Borax

Method IB:

- 1% Paradimethyl amino benzaldehyde
- Toluene

Assay Procedure for the Determination of Ambroxol HCl:

Method IA: Seven 10 ml volumetric flasks were taken. 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6 ml of working standard of Ambroxol HCl was added in each volumetric flask. Then 1.0 ml 0.1% of Gibbs reagent solution and 1 ml of 0.2% Borax were added and left for 10 min. Volume was made up to mark with Ethanol. Absorbance was taken at 537.2 nm.

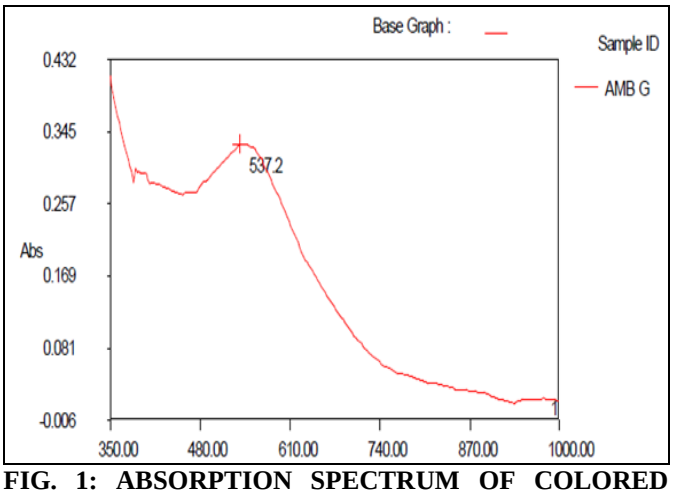

FIG. 1: ABSORPTION SPECTRUM OF COLORED CHROMOGEN IN METHOD IA

Method IB: Seven 10 ml volumetric flasks were taken. 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6 ml of working standard (10 µg/ml) of Ambroxol HCl was added in each volumetric flask. To flask, 2 ml of 1% PDAB, 0.3 ml of toluene and 0.02 ml of H_2SO_4 were added. The volume in flask was made up to the mark with ethanol. The absorbance was measured against the reagent blank at 438.4 nm.

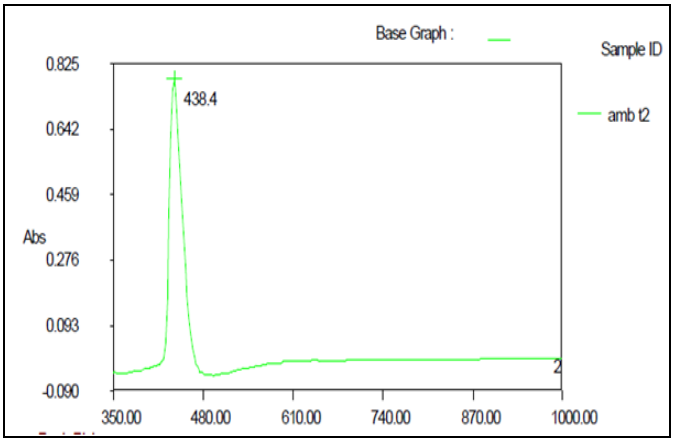

FIG: 2 ABSORPTION SPECTRUM OF COLORED CHROMOGEN IN METHOD IB

Assay of Pharmaceutical Formulations: Weighed accurately tablet powder equivalent to 100 mg and transferred into 100 ml volumetric flask and Ambroxol HCl was extracted in 10 ml of ethanol. The solution was then filtered and the filtrate was then made up to 100 ml with ethanol to get 1000 µg/ml concentration. This solution was further diluted to get concentration of 10 µg/ml.

Appropriate aliquots of drug solution were taken. The individual assay procedure was carried out for the estimation of drug contents in tablets. The concentration of the drug in the tablets was calculated using calibration curve. The recovery experiment was carried out by standard addition method. The values of optical and regression terms of analysis are given in **Table 1**.

RESULTS AND DISCUSSION: Method IA, Ambroxol HCl gives purple coloured chromogen with Gibb's reagent, which showed λ_{max} at 537.2 nm.

In Method IB, the drug was reacted with Paradimethyl amino benzaldehyde which produce greenish yellow colour chromogen which showed λ_{max} at 438.4 nm.

The optical characteristics such as absorption maxima and Beer's law limits for these methods are presented in **Table 1**. The regression analysis using the method of least squares was made for the slope (a) and intercept (b) obtained from different concentrations are summarized in **Table 1**. The precision and accuracy were found by analyzing six replicate samples containing known amounts of the drug and the results are summarized in **Table 1**.

TABLE 1: ABSORPTION MAXIMA AND BEER's LAW LIMITS

Parameter	Result of Method IA	Result of Method IB
λ_{max} (nm)	537.2	438.45 - 30
Beer's law limit	5-30	
Regression Equation* (y)	y = bx + a : y = 0.0324x - 0.0014	y = bx + a : y = 0.0207x - 0.0005
Slope (b)	0.0324	0.0207
Intercept (a)	0.0014	0.0005
Correlation coefficient (R^2)	0.9994	0.9992
Limit of Detection (µg/ml)	0.0101	0.0318
Limit of quantitation (µg/ml)	0.0308	0.0966
Accuracy (% Recovery ± SD)	98.88 ± 0.015	96.83 ± 0.0152
Precision (Reproducibility)		
Intraday (% Recovery ± SD)	0.330267 ± 0.000208	0.19933 ± 0.000251
Interday (% Recovery ± SD)	0.3303 ± 000321	0.19927 ± 000351

y = bx + a, where y is the absorbance and x is the concentration of N-acetylcysteine in µg/ ml.

Recovery Studies: Weighed accurately tablet powder equivalent to 100 mg and transferred into 100ml volumetric flask and Ambroxol HCl was extracted in 10 ml of ethanol. The solution was then filtered and the filtrate was then made up to 100 ml with ethanol to get get 1000 µg/ml concentration. This solution was further diluted to get concentration of 10 µg/ml. To keep an additional check on accuracy of developed assay method, analytical recovery experiments were performed. The different solutions of different concentrations like 2, 4 and 6 µg/ml were prepared in case of both pure drug solution and the formulation extract solution and these solutions were subjected to analysis by above developed method. The six such samples were prepared and average of that readings taken for calculation of % recovery. This is reported in following **Table 2**.

TABLE 2: RECOVERY STUDIES

Method	Sample	Labelled amount (mg)	Amount found (mg)	% Recovery
IA	Ambroxol HCl	30	28.85	96.16
IB	Ambroxol HCl	30	29.15	97.16

CONCLUSION For routine analytical purpose, it is always necessary to establish methods capable of analyzing huge number of samples in a short time period with due accuracy and precision. Few analytical methods appeared in the literature for the determination of Salbutamol Sulphate. Proposed method makes use of simple reagent, which an ordinary analytical laboratory can afford. The method was found to be simple, precise, economic and less time consuming. In the present investigation, colorimetric method for the quantitative estimation of Salbutamol Sulphate in bulk drug and pharmaceutical formulations has been developed.

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CONFLICT OF INTEREST: Nil

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