

## Research Article

# Development of New Analytical Method and Its Validation for the Determination of Guaiphenesin in Bulk and Marketed Formulations

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## ABSTRACT

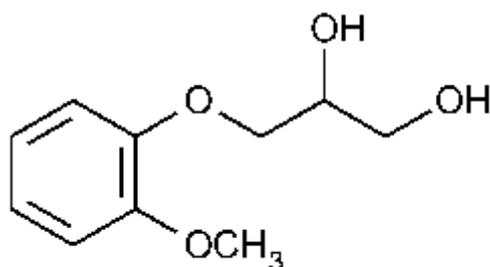
A simple and accurate colorimetric method has been developed for the estimation of Guaiphenesin in bulk and pharmaceutical dosage forms. Colorimetric method was based on oxidation-reduction reaction. In this method ceric ammonium sulphate was made to react with Guaiphenesin in excess amount, in presence of sulphuric acid. Unreacted amount of oxidizing agent oxidizes standard amount of crystal violet which produces violet colour. Absorbance maxima were found to be 579 nm. Linearity was found in the range of 5-30 µg/ml of drug concentration. The method has been validated according to ICH Guidelines.

**Keywords:** Guaiphenesin, Ceric ammonium sulphate, Sulphuric acid, Crystal violet.

## INTRODUCTION

A study of the interaction of light (or other electromagnetic radiation) with matter is an important and versatile tool for the chemist. Indeed, much of our knowledge for chemical substances comes from their specific absorption or emission of light. In this experiment, we are interested in analytical procedures based on the amount of light absorbed (or transmitted) as it passes through a sample.<sup>1</sup>

Guaiphenesin is thought to act as an expectorant by increasing the volume and reducing the viscosity of secretions in the trachea and bronchi.



Its IUPAC name is (RS)-3-(2-methoxyphenoxy) propane-1, 2-diol and formula is C<sub>10</sub>H<sub>14</sub>O<sub>4</sub>. It is freely soluble in alcohol. Up to now there are HPLC and

spectrophotometric methods developed on Guaiphenesin<sup>4-12</sup>. The USP has published specific guidelines for method validation for compound evaluation. USP defines eight steps for validation: Accuracy, Precision, Specificity, Limit of detection, and Limit of quantitation, Linearity and range, Ruggedness, Robustness<sup>13-14</sup>.

## MATERIALS AND METHODS

### INSTRUMENTS

- (1) The instrument used for the present study was PC based Jasco V-630 UV-Visible double beam Spectrophotometer with 1 cm matched pair quartz cell and spectral bandwidth of 1.5 nm.
- (2) SHIMADZU UV-Visible 1700spectrophotometer.

### MATERIALS

Pure drug of Guaiphenesin was obtained from Yarrow chem Ltd. and commercial formulations were procured from local market. All the chemicals used were of analytical grade.

### REAGENTS

- Ceric ammonium sulphate (0.5%w/v)
- Crystal Violet (0.01%w/v)
- Distilled water

- Sulphuric acid (4M)
- Distilled ethanol

## EXPERIMENTAL

Guaiphenesin was determined spectrophotometrically in bulk and marketed formulation by using crystal violet dye and ceric ammonium sulphate (CAS) as a strong oxidizing agent in presence of H<sub>2</sub>SO<sub>4</sub>.

### Preparation of stock solution

Preparation of standard stock solution of Guaiphenesin: Standard stock solution was prepared by accurately weighing 100 mg of Guaiphenesin in 100 ml calibrated volumetric flask and made up the volume with distilled alcohol up to 100 ml.

Preparation of working standard solution of Guaiphenesin: Working standard was prepared by transferring 10 ml standard stock solution into 100 ml calibrated volumetric flask and made up the volume with distilled alcohol to get concentration of 100µg/ml.

## METHOD

A series of concentration of 5-30µg/ml of drug solution were prepared from Stock Solution (B) in 10ml volumetric flasks. To each appropriately labelled flask, 0.5ml of 0.5%ceric ammonium sulphate solution and 1ml of 4MH<sub>2</sub>SO<sub>4</sub> was added. The flask was kept aside for 20 minutes and 1.5ml of 0.01% Crystal violet solution was added and the absorbance was taken at 579 nm. The graph and the absorbance for the above are given in **Table no: 1 and Graph no: 1**

### Method validation

Calibration curve was plotted over a concentration range of 5-30 µg/ml for Guaiphenesin. Accurately measured standard working solutions of Guaiphenesin (0.5, 1, 1.5, 2, 2.5 and 3ml) were transferred to one set of a series of 10 ml volumetric flasks. Solutions were made up to volume with distilled ethanol. A spectrum was recorded by placing drug solutions and diluent in sample and reference cells respectively. The absorbance was measured at 579nm (Peak maxima) and was plotted vs. concentration to give calibration curve, and regression equation and correlation coefficient was calculated and presented in **Table no: 1 and Fig no: 1**. The calibration curve of amplitude of absorbance against concentration of the drug showed linearity.

### (a) Accuracy

Recovery studies by the standard addition method were performed to study the accuracy of the proposed method. Preanalysed samples

of Guaiphenesin (10µg/ml) were spiked with 80, 100 and 120 % extra Guaiphenesin standard and the mixture were analyzed with the proposed method. Accuracy was assessed as the % Recovery at each concentration level. Data obtained from accuracy study were given in **Table no: 3**

### (b)Sensitivity

The sensitivity of the proposed method for measurement of Guaiphenesin was estimated in terms of limit of detection [LOD] and limit of quantification [LOQ]. The LOD and LOQ were calculated by using the slope and SD of response (intercept). The mean slope value and SD of response were obtained after plotting six calibration curves. The LOD and LOQ obtained are reported in **Table no: 3**

### (c) Precision

The precision of the method was established by system precision and method precision. System Precision was subjected to intraday and inter-day variation studies.

### i) System Precision

Intraday precision was determined by using three different levels of drug concentrations (5, 10, 15 µg/ml) prepared from stock solution-II and each level was analyzed three times in a day. Same procedure was followed for three different days to study the Inter-day precision. Data obtained are given in the **Table no: 3**

### ii) Method Precision

Method precision was determined by using sample solution of drug concentrations (5, 10, 15, 20, 25, 30µg/ml) and it was analyzed six times in a day by the same analyst. Data obtained are given in the **Table no: 3**

### (d) Ruggedness

To establish ruggedness of the proposed method, assays for two different concentrations of Guaiphenesin were performed by two different analysts. The results of assays are represented as % Recovery with SD and % RSD showing the ruggedness of the proposed method were illustrated in **Table no: 3**

### (e) Specificity:

Refers to the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other components of similar behaviour. The data obtained were given in the **Table no: 3**.

### (f) Reproducibility

The absorbance readings of 10µg/ ml were measured at different laboratory using different

spectrophotometer by another analyst and the %RSD values obtained to verify their reproducibility. Data obtained were given in **Table no: 3**

#### Determination of Guaiphenesin from Dosage form:

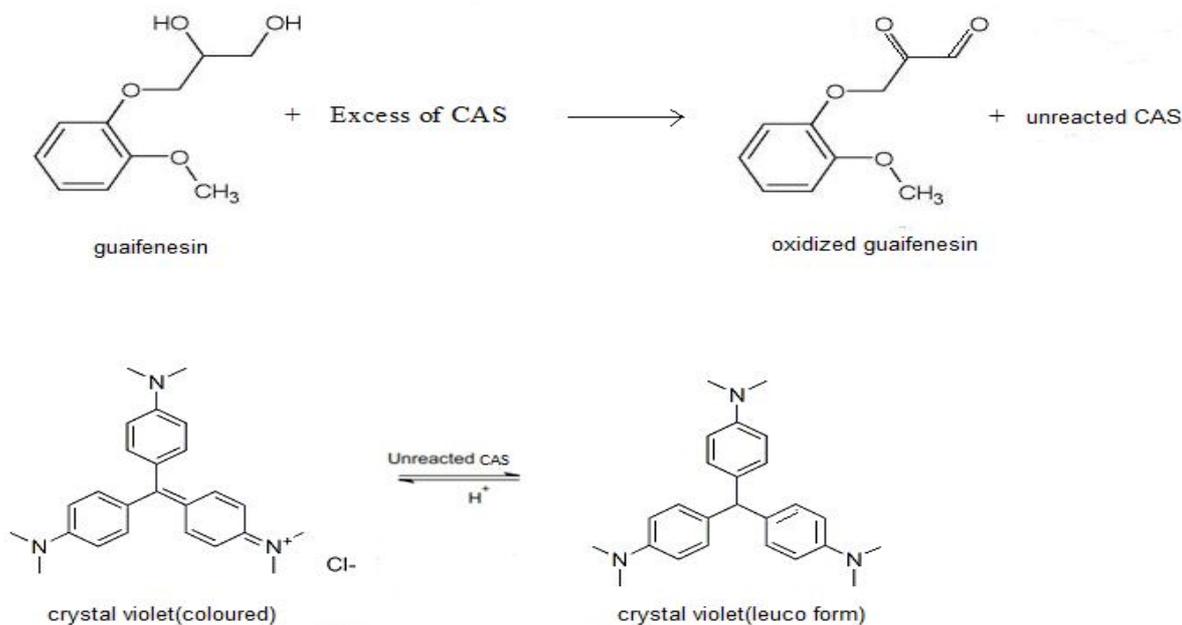
20 tablets of Barfeit (marketed product) containing 200mg Guaiphenesin was obtained for all analytical study. Powder equivalent to 100mg of Guaiphenesin was weighed accurately and transferred into 100 ml volumetric flask. The volume was made up to 100ml using distilled ethanol. The flask was shaken and volume was made up to the mark with distilled ethanol to give a solution of 1000 µg/ml (Stock Solution A).

From the above Stock solution A 1ml was pipetted out and added to a 100 ml volumetric flask. (Stock solution B) and the volume was made up to 10ml with distilled ethanol.

From the stock solution B, 2ml was pipetted into 10ml volumetric flask. To the flask 0.5ml of 0.5% ceric ammonium sulphate solution and 1ml of 4M $H_2SO_4$  were added. The flask was kept aside for 20 minutes and 1.5ml of 0.01% Crystal violet solution was added and made up to the mark with distilled ethanol. The absorbance reading was taken at 579 nm against reagent blank. The absorbance for the above is as given in **Table no: 2**

#### RESULT AND DISCUSSION

Ceric ammonium sulphate is a strong oxidizing agent. It reacts with Guaiphenesin in presence of acidic medium. When CAS was added in excessive amount it oxidises Guaiphenesin and remaining CAS reacts with crystal violet & crystal violet was oxidised by CAS. Crystal violet which was left after oxidization produces different colours accordingly which indirectly indicates the amount of drug present.



**Table 1: Absorbance of different concentration of Guaiphenesin**

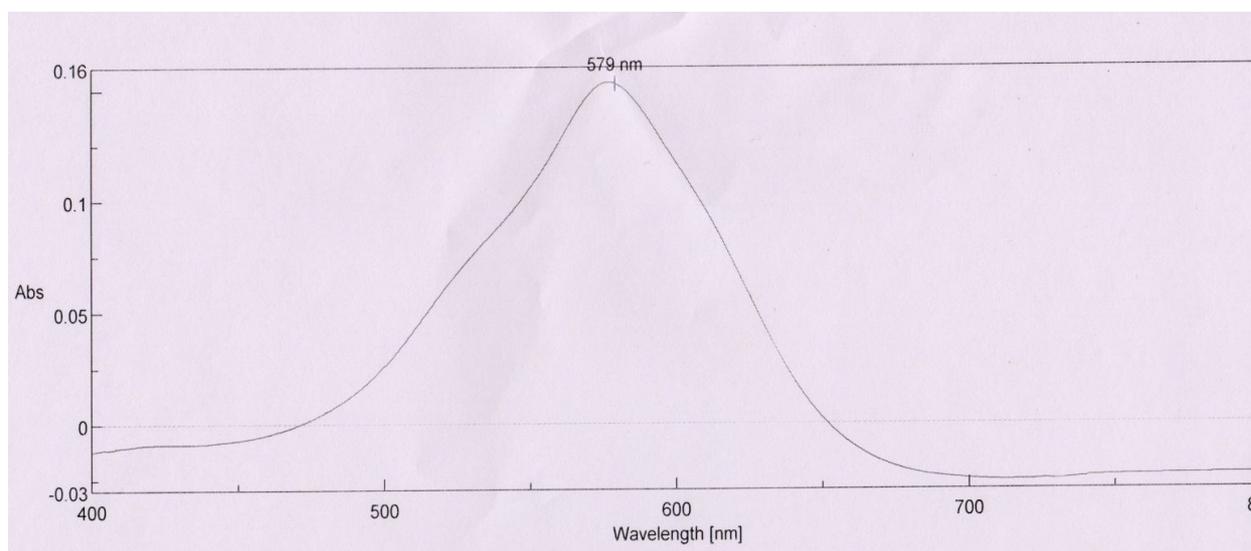
SL No.	Volume of drug taken (100 µg/ml)	Concentration in µg/ml	Absorbance At 579 nm
1.	0.5	5	0.142
2.	1.0	10	0.282
3.	1.5	15	0.423
4.	2.0	20	0.569
5.	2.5	25	0.707
6.	3.0	30	0.84

**Table 2: Assay Results of Marketed Formulation**

Formulation	Actual concentration of Guaiphenesin ( $\mu\text{g/ml}$ )	Amount obtained of Guaiphenesin ( $\mu\text{g/ml}$ )	% Guaiphenesin
Tablet	20	19.91	99.55

**Table 3: Summary of parameters of spectrophotometry**

Parameter	Result
$\lambda_{\text{max}}(\text{nm})$	579 nm
Beer's law limits ( $\mu\text{g/ml}$ )	5-30 $\mu\text{g/ml}$
Molar absorptivity ( $1/\text{mol.cm}$ )	$3.09 \times 10^{-2}$
Sandell's sensitivity ( $\mu\text{g.cm}^2/0.001 \text{ Au}$ )	0.0725
Regression equation ( $y=a+bc$ )	
Slope (b)	$b=0.0281$
Intercept (a)	$a=0.0015$
Correlation coefficient ( $r^2$ )	0.9999
% recovery	1) At Level-1 (50%)=99.94 2) At Level-2 (100%)=99.88 3) At Level-3 (150%)=100.05
Repeatability (%RSD)	0.13 to 0.52
Limit of Detection ( $\mu\text{g/ml}$ )	0.0160
Limit of Quantitation ( $\mu\text{g/ml}$ )	0.0485
Specificity	Specific
Selectivity	Selective
Reproducibility (n=6)	
Instrument 1 (%RSD)	0.16
Instrument 2 (%RSD)	0.13
Precision (n=3)	
Intraday precision (%RSD)	0.07-0.17
Inter day precision (%RSD)	0.04-0.24



**Guaiphenesin with Crystal violet and CAS**  
 $\lambda_{\text{max}}$  of colored complex

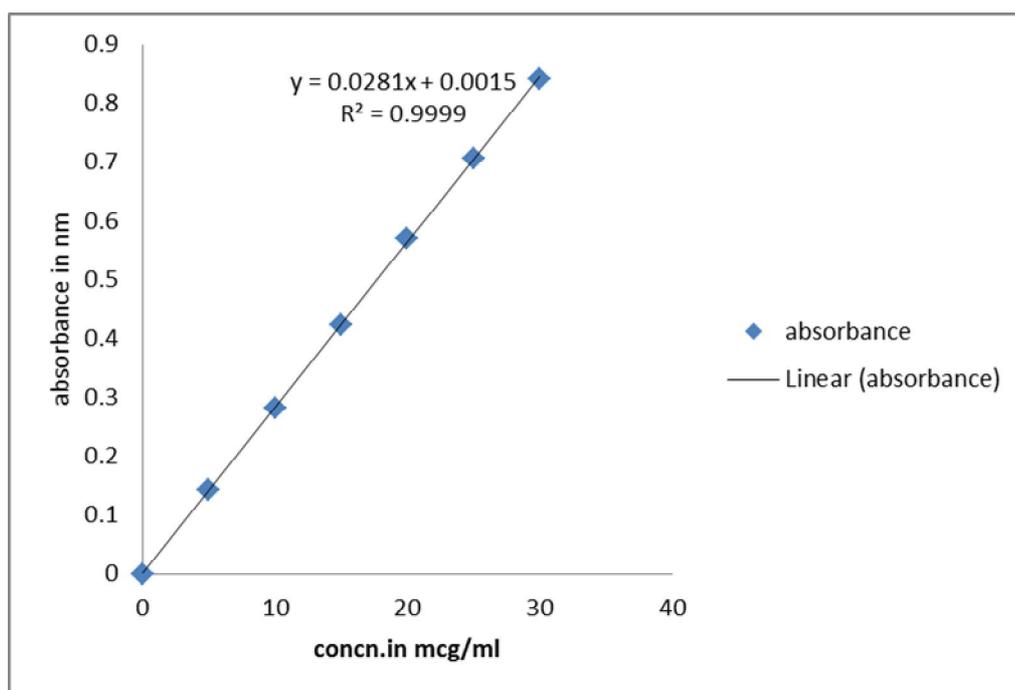


Fig. 1: Standard curve of Guaiphenesin

## CONCLUSION

For routine analytical purpose, it is always necessary to establish methods capable of analysing huge number of samples in a short time period with due accuracy and precision. A very few analytical method appeared in the literature for the determination of Guaiphenesin. In view of the above fact, some simple analytical method was planned to develop with sensitivity, accuracy, precision and economical. In the present investigation, colorimetric method for the quantitative estimation of Guaiphenesin in bulk drug and pharmaceutical formulations has been developed.

## ACKNOWLEDGEMENT

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