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Research article

DETERMINATION AND ESTIMATION OF AMBROXOL HCL, GUAIPHENESIN BY USING UV- VISIBLE SPECTROPHOTOMETERIC METHOD

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ABSTRACT

In this project we are going to analyse the four simple, rapid, accurate, precise, reliable and economical spectrophotometric methods have been proposed for simultaneous determination of Ambroxol, guaiphenesin in pure and pharmaceutical formulations without any prior separation or purification step. The methods are first derivative zero crossing spectrophotometry, simultaneous equation, derivative ratio spectra zero crossing and double divisor ratio spectra derivative method. Developed methods show best results in terms of linearity, adsorption for standard laboratory mixtures of pure drugs and marketed formulations. Common excipients and additives did not interfere in determinations of these APIs. The results obtained by the proposed methods have been statistically compared by means of student t-test.

Keywords: Simultaneous equation method; Ambroxol HCL; Guaiphenesin.

INTRODUCTION

Ambroxol HCl (AB) chemically known as trans-4-(2-Amino-3,5- dibrombenzylamino)-cyclohexanol hydrochloride, is a secretolytic agent used in the treatment of respiratory diseases associated with viscid or excessive mucus. Guaifenesin, also known as Glyceryl Guaiacolate, is an expectorant medication that aids in the elimination of the sputum from the respiratory track. Chemically it is an either of guaiacol and glycerine.it is often used in combinations with other medication. It is taken by mouth. Guaifenesin has been used medically since at least 1933. It is available as a generic medication and over the counter. Guaifenesin (GF) chemically known as (RS)-3-(2-methoxyphenoxy) propane-1,2-diol, an expectorant that also has some muscle relaxing action. AB [1] and GF [2] [3] are official in IP and BP. The official methods involve determination of AB [4] and GF [5] using potentiometer for pure drug but method has not been described for formulation. Some procedures have been described for the assay of AMB or GUF in single dosage forms [4].

AB, GF mixture is not yet official in any pharmacopoeia but few formulations are available in generic market. As per literature, no analytical methods could be traced for the analysis of AB, GF combination in pharmaceutical dosage forms. Therefore simple, rapid, and reliable methods for simultaneous estimation of these drugs in mixture seemed to be necessary. The aim of this work is to investigate the utility of derivative spectrophotometry and to develop reliable spectrophotometric procedures for the simultaneous determination of AB, GF either in laboratory samples, or in commercial dosage forms without any prior separation of individual drugs. AB, GF have closely overlapped spectra, which prevents the use of zero-order UV-VIS spectrophotometry for their determination. Derivative spectrophotometry is a very useful tool for overcoming this problem. This technique has been successfully

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applied in pharmaceutical and environmental analysis for determination of drugs in multicomponent systems [5]. In this work, various orders of derivative and different kinds of measurements were assayed, i.e., zero-crossing first derivative [6], simultaneous equation method [7], ratio-spectra first derivative zero crossing and double divisor ratio spectra derivative method [8]. Four methods have been successfully developed for mentioned combination and satisfactory results were obtained.

MATERIALS AND METHODS

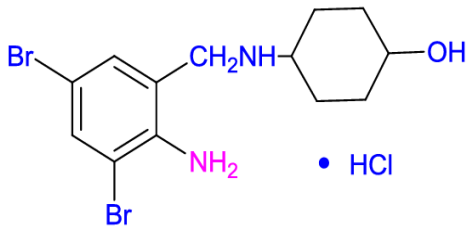
An UV-Visible single beam spectrophotometer with 1 cm matched quartz cells was used for spectrophotometric measurements. All weighing were done on electronic balance (Model ShimadzuAUW-220D). Methanol used was of analytical grade purchased from Loba Chemie Pvt. Ltd. Ambroxol hydrochloride and Guaiphenesin were obtained as gift samples from Synthokem Labs Private Limited- Hyderabad.

Preparation of Stock Solutions and Sample Solution:

Accurately weighed quantities of AMH and GUF were dissolved separately in 20 mL of methanol and volumes were made up to 100 mL with methanol (100 mcg / mL). These solutions of AMH and GUF were used as working standards. Aliquot portions of stock solutions of AMH and GUF were diluted appropriately with methanol to obtain concentration of 20 mcg / mL of AMH and GUF. The working wavelengths selected for each formulation for the use of simultaneous equation were 242 and 272 nm for AMH and GUF respectively as shown in Figure 1. For calibration, series of solutions were prepared containing AMH 10, 15, 20, 25, 30 mcg/mL: GUF 10, 20, 30, 40, 50 mcg/mL by diluting the stock standard solution with methanol in standard volumetric flasks (10mL). Similarly, mixed standard solutions were used for UV- spectrophotometric analysis by simultaneous equation method.

DRUG PROFILE:

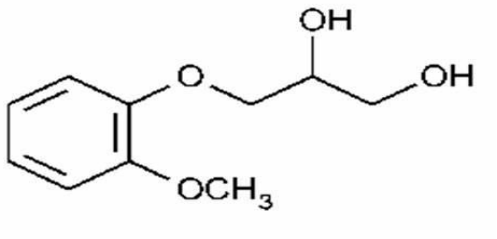
AMBROXOL HCL:



IUPAC Name: 4-[(2-amino-3,5-dibromophenyl) methylamino]cyclohexan-1-ol; hydrochloride
Molecular Weight: 414.56
Molecular Formula: $C_{13}H_{18}Br_2N_2O$
Mechanism of Action:

The substance acts on mucus membranes, restoring the physiological clearance mechanisms of the respiratory tract (which play an important role in the body's natural defence mechanisms) through several mechanisms, including breaking up phlegm, stimulating mucus production, and stimulating synthesis and release of surfactant by type II pneumocytes.[7][8] Surfactant 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.

GUINAPHENESIN:



IUPAC Name: 3-(2-methoxyphenoxy) propane-1,2-diol
Molecular Weight: 198.22
Molecular Formula: $C_{10}H_{14}O_4$

Mechanism of Action:

Guaifenesin is thought to act as an expectorant by increasing the volume and reducing the viscosity of secretions in the trachea and bronchi. It may aid in the flow of respiratory tract secretions, allowing ciliary movement to carry the loosened secretions upward toward the pharynx. Thus, it may increase the efficiency of the cough reflex and facilitate removal of the secretions.

All reagents were tested for stability in solution and during the actual analysis. The behaviour of the analytes remained unchanged up to about 24 h from their preparation at the room temperature. All the three drugs were found to be stable during each kind of experimental measurements. Each measurement was done at room temperature.

Third Derivative Zero Crossing Spectrophotometry (Method 1):

Absorption spectra of the samples were recorded between 220- 400 nm against a reagent blank (same samples without compounds to be determined) using a 1.0 cm quartz cell. Zero order spectra of pure drugs were stored individually within above concentration ranges and were derivatized in third order using delta lambda 4 and scaling factor 10 for all three drugs. The third derivative amplitudes were recorded at 252.2 nm, 282.6 nm, and 285 nm for determination of AB and GF respectively. Standard laboratory mixtures of AB, and GF in 15: 50: 0.5 ratios were prepared, and absorbance was measured at 252.2 nm, 282.6nm and 285nm for AB, GF respectively.

Simultaneous Equation Method (Method 2):

This method is based on third derivative and wavelengths selected for estimation of AB, GF and 282.6 nm and 285 nm respectively. However, in contrast to first method, this method utilized simultaneous equations (Verdot's method) on derivative spectra to overcome spectral interference at selected wavelength. The first derivative absorptivity coefficients were determined at the selected wavelengths. A set of three equations framed using these coefficient values were listed below: CAB = DAAB 1980.009 (1) CGF = DAGF 395.8294 (2) CLS = DALS 1902.973 (3) J. Chill. Chem. Soc., 57, Nº 4 (2012) 1437 Where, CAB, CGF and CLS are the concentration of AB, GF respectively; These equations were directly utilized for simultaneous estimation of AB and GF standard laboratory mixture as well as the marketed formulations.

Derivative Ratio Spectra Zero Crossing Spectrophotometry (Method 3):

Absorption spectra of pure drugs and their ternary mixtures were recorded between 210 – 320 nm. Absorption spectra of pure AB and their ternary mixture were divided by a standard spectrum of 20 µg mL-1 of LS, absorption spectra of pure GF and their ternary mixture were divided by a standard spectrum of 20 µg mL-1 of LS and absorption spectra of pure LS and their ternary mixture were divided by a standard spectrum of 20 µg mL-1 of GF and first derivative of the ratio spectra were plotted using delta lambda 8 nm and scaling factor 10. In the ternary mixture, concentration of AB and GF were proportional to the first derivative ratio signals at 297 nm (zero crossing point for GF where 20 µg mL-1 of LS was used as divisor), 275.6 nm (zero crossing point for AB where 20 µg mL-1 of LS was used as divisor) and 227.5 nm (zero crossing point for AB where 20 µg mL-1 of GF was used as divisor) respectively. Calibration graphs were obtained by measuring the derivative ratio amplitudes against increasing concentration of pure AB, pure GF and pure using respective divisors. Contents of AB and GF in standard laboratory mixture and commercial formulation were determined by use of above-mentioned procedure.

Double Divisor Ratio Spectra Derivative Method (Method 4):

Absorption spectra of the pure drugs and their ternary mixtures were recorded between 210 – 320 nm. The absorption spectra of AB and their ternary mixture were divided by a standard spectrum obtained by the addition of stored spectrum of 20 µg mL-1 of GF, and first derivative of the ratio spectra was plotted using delta lambda 4 nm and scaling factor 1. In the ternary mixture, concentration of AB was proportional to first derivative ratio signals at 240.4 nm. Calibration graph was obtained by measuring derivative ratio amplitudes against increasing concentration of pure AB by using same divisor described above. Content of AB was determined by use of above-mentioned calibration graph. Similarly, for determination of GF, a standard spectrum obtained by the addition of stored spectrum of 20 µg mL-1 of AB was used as determination, a standard spectrum obtained by the addition of stored spectrum of 20 µg mL-1 of GF and 20 µg mL-1 of AB used as divisor. First derivative of the ratio spectra were plotted using delta lambda were 4 nm and scaling factor 1. In the ternary mixture, the concentration of GF and LS were proportional to the first derivative ratio signals at 262.4 nm and 289.1 nm respectively.

Recovery Study:

To check the accuracy of the developed method and to study the interference of formulation, additives, analytical recovery experiments were carried out by standard addition method. The precision of an analytical method is expressed as SD or RSD of a series of measurements. It was ascertained by replicate estimation of drug by the proposed method. Test for ruggedness was carried out by repeating the procedure under different days, at different time and by different analyst. Linearity and range study was done by preparing concentration in the range of 80-120% of the test concentration and absorbance values were recorded at 242 and 272 nm. The plot of linearity of AMH and GUF was shown in Figure 2 and 3 respectively.

RESULTS AND DISCUSSION

Table 1. Optical Characteristics Data For Ambroxol And Guiaphenesin

Paramerers	AMH	GUF
Absorption maxima (nm)	242	272
Beer's law limit(mcg/ml)	5-50	10-80
Absorptivity A (1%,1cm)	235.3	107.6
Molar absorptivity (It mole -1 cm-1)	9742.36	2132.87
Regression equation	Y=0.00228 X -0.0063	Y=0.01 X + 0.0063
Correlation coefficient	0.9984	0.9993
Intercept	0.0063	0.0063
Slope	0.0228	0.01

Table 2. Result of analysis of powder formulation and statistical data

Drug	Labelled chain (mg)	Amount found (mg)*	%Labelled	SD	RSD	SE
AMB	30	29.97	99.96	0.177	0.0017	0.0794
GUF	100	99.77	99.77	0.513	0.0051	0.2294

Table 3. Results of Ruggedness Study

Condition	Amount found (mg)*		% Labelled claim		SD		RSD		SE	
	AMH	GUF	AMH	GUF	AMH	GUF	AMH	GUF	AMH	GUF
Intra day	29.90	100.01	99.68	100.01	0.2902	0.1856	0.0029	0.001	0.118	0.075
Inter day	30.04	99.98	100.15	99.68	0.1375	0.2284	0.0013	0.0024	0.0561	0.093
Different analysis	29.94	99.94	99.84	99.94	0.1596	0.2048	0.0015	0.002	0.0651	0.083

Table 4. : Absorbance of Ambroxol Drug

S.No	RANGE (nm)	Concentrations	Absorbance
1	242.0	2.5ml	0.5496
2	242.0	5ml	0.3836
3	242.0	7.5ml	-0.2329
4	242.0	10ml	0.0598
5	242.0	12.5ml	0.2575

Table 5. Absorbance of Guaiphenesin Drug

S.No	RANGE (nm)	Concentrations	Absorbance
1	272.0	2.5ml	0.0933
2	272.0	5ml	-0.3010
3	272.0	7.5ml	-0.3010
4	272.0	10ml	-0.3010
5	272.0	12.5ml	-0.3010

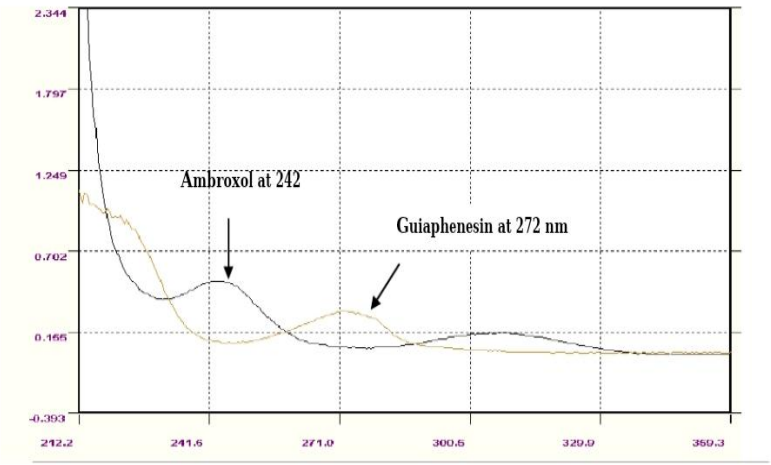


Figure 1: Overlain spectra of Ambroxol hydrochloride and Guiaphensin

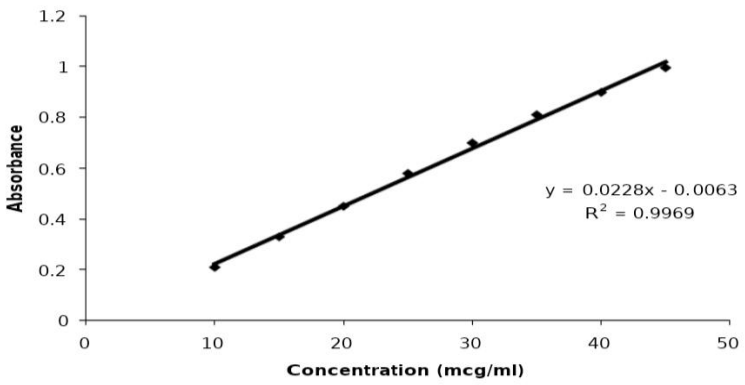


Figure 2: Linearity of Ambroxol hydrochloride at 242 nm

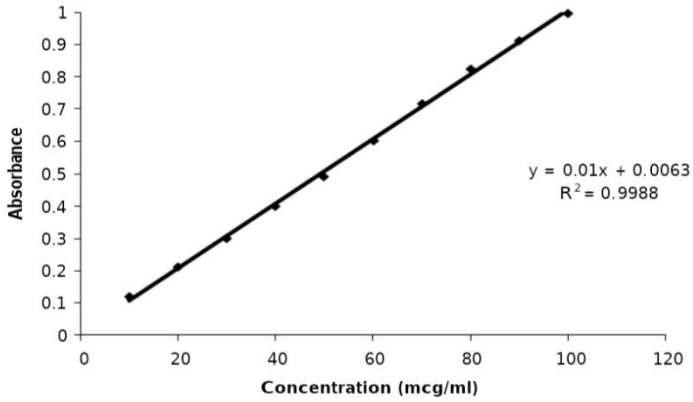


Figure 3: Linearity of Guiaphensin at 272 nm

An attempt has been made to develop a fast, sensitive, precise, reproducible and economical analytical method for simultaneous estimation of AMH and GUF in their combined dosage form. UV absorption spectrum exhibited maximum absorbance for AMH and GUF at 242 and 272 nm respectively.

In this method, AMH and GUF obey Beer's law in the concentration range of 5-50 mcg/mL and 10-80 mcg/mL respectively. Standard 1% solutions were prepared and measured absorbance at both the wavelengths of each respective content of formulations.

For AMH, molar absorptivity was found to be 9742.34±0.894 and 1015.68±0.707 at 242 and 272 nm respectively, for GUF 741.39±0.769 and 2132.87±0.852 at 242 and 272 nm respectively. Recovery studies carried out for the method by spiking standard drug as per the ICH guidelines.

The results of the recovery study were found to be with in the limit of 98.5-102%, providing the accuracy and showing that the method is free from interference from excipients. For ruggedness, proposed method was repeated under different conditions like at different time, on different days and by different analysts.

From the study of validation parameters namely accuracy, precision, ruggedness (inter day, intraday and different analyst), specificity, linearity and range, it was observed that the method can employed for routine quantitative analysis of tablet dosage form containing AMH and GUF..

CONCLUSION

The UV absorption of Ambroxol HCl and Guaifenesin maximum absorption was found to be at 242 nm and 272 nm. The results obtained by the proposed

methods have been statistically compared by statistical analysis. The developed methods showed best results in terms of linearity, adsorption for standard laboratory mixtures of pure drugs and marketed formulations. The proposed method was statistically validated in accordance with ICH guidelines and results were found to be satisfactory for selected parameters. The various analytical techniques available for the quantification of single and multicomponent dosage form. The UV spectroscopy and other analytical techniques like chromatography, electrophoresis and hyphenated technique are available for the are widely used are most extensively used for analysis of Ambroxol HCL(AMB) and Guaifenesin (GF). The study suggests that UV spectroscopy analysis could be employed for routine quantitative analysis of various dosage forms containing ambroxol HCl and Guaifenesin. Hence, it can be applied for the routine estimation of ambroxol hydrochloride and Guaifenesin in bulk and pharmaceutical dosage form even at very low concentration in formulation of various dosage forms.

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