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Absorption Ratio, Derivative Spectroscopy and RP-HPLC Methods for Estimation of Guaifenesin and Ambroxol hydrochloride in Tablet

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ABSTRACT

Present work describes a precise, accurate and reproducible absorption ratio, first order derivative spectroscopy and Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) methods for simultaneous estimation of Guaifenesin and Ambroxol hydrochloride. The absorption ratio (Q analysis) method was based on the measurement of absorbances at two wavelengths, one being the iso-absorptive point at 223nm (λ1) and other being λ max, 273nm (λ2) of one of the sample components. The second method was based on the use of first derivative spectroscopy, in which derivative amplitudes were measured at selected wavelengths (238 nm for guaifenesin and 255 nm for ambroxol hydrochloride), without mutual interference. In the RP-HPLC method, the drugs were resolved using a mobile phase of acetonitrile: 50mM potassium dihydrogen phosphate buffer pH adjusted to 3.2 using orthophosphoric acid (22:78 v/v) on HiQ Sil C18 (250 X 4.6 mm) 5µm column in isocratic mode. The retention times of guaifenesin and ambroxol hydrochloride were 7.5 and 9.5 min respectively. Recovery values with percentage relative standard deviation < 2 and correlation coefficient closed to 0.999 showed that the developed methods were accurate and precise. As per ICH guidelines the developed method were validated in terms of linearity, precision, accuracy, limit of detection and limit of quantification, and the results were found to be satisfactory.

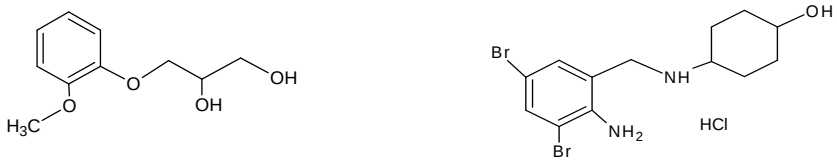
Key Words: Guaifenesin, Ambroxol hydrochloride, Absorption ratio, Derivative spectrophotometry, RP-HPLC.

INTRODUCTION

Guaifenesin (GF), (*RS*)-3-(2-methoxyphenoxy) propane-1, 2-diol reportedly increases the volume and reduces the viscosity of tenacious sputum and is used as an expectorant for productive cough. Ambroxol hydrochloride (AMB), [*trans*-4-[(2-amino-3, 5-dibromobenzyl) amino] cyclohexanol hydrochloride] is a semi-synthetic derivative of vasicine obtained from Indian shrub *Adhatoda vasica*. It is a metabolic product of bromhexine and possesses mucokinetic (improvement in mucus transport) and secretolytic (liquifies secretions) properties. It promotes the removal of tenacious secretions in the respiratory tract and reduces mucus stasis (arresting the secretion of mucus). Both GF and AMB are official in IP [1], BP [2] [Fig.1].

Literature survey reveals, spectrophotometry [3, 4], HPLC [5-9], colorimetric [10], GC [11], CE [12, 13], supercritical fluid chromatography [14], voltammetry [15] methods are reported for the estimation of GF alone and in combination with other anti-asthmatic agents. Methods such as HPLC [16-18], GLC [19], capillary electrophoretic [20], spectrophotometry [21, 22] are reported for estimation of AMB alone and in combination with other agents. Spectrophotometry [23, 24] and HPLC [24] methods have been reported for estimation of both the drugs in combined dosage form. The purpose of this research was to establish and validate, in accordance with International Conference on Harmonization (ICH) guidelines [25], a simple, accurate, precise and reproducible

absorption ratio, derivative spectroscopy and RP-HPLC method for quantitative analysis of GF and AMB in the bulk drug and pharmaceutical dosage forms.



Guaifenesin Ambroxol HCl

Fig. 1: Chemical structures of guaifenesin and ambroxol hydrochloride

MATERIALS AND METHODS

Authenticated standards of GF and AMB were kind gift samples from Elder Pharmaceuticals Ltd, Mumbai, India. Potassium dihydrogen phosphate, HPLC grade water and acetonitrile were procured from Merck Ltd, Mumbai, India. Orthophosphoric acid was purchased from Research lab, Mumbai. The commercial formulation of GF and AMB {Brutex tablet, GF 50 mg: AMB 15 mg} procured from local market.

Instrumentation:
Double beam UV/Vis spectrophotometer (JASCO V-530) with 1cm matched quartz cells was used to measure absorbance of resulting solution. The HPLC system, Jasco PU-2080 Plus, with manual Rheodyne injector facility operates at 20 µL loop capacity. The column used was HiQ Sil C18 (250 X 4.6 mm) 5µm and the detector, UV/VIS (Jasco UV 2075-Plus) operates at 262 nm. The data were acquired and processed using Borwin software version 1.5

Spectrophotometric methods:
Preparation of standard stock solution:
Weighed accurately 10 mg of GF and 10 mg of AMB, transferred to a 10 mL volumetric flask separately, add 5 mL of double distilled water to each flask and sonicate for 10 min. Finally the volume was made up to mark with the same solvent. From the resultant solutions 1 mL solution was pipetted out in 10 mL volumetric flask separately and volume was made up to the mark with double distilled water.

Development of the method
Q analysis method:
In Q analysis method the absorbances were measured at the isobestic point (223nm) and maximum absorption at wavelength (273nm) of GF.

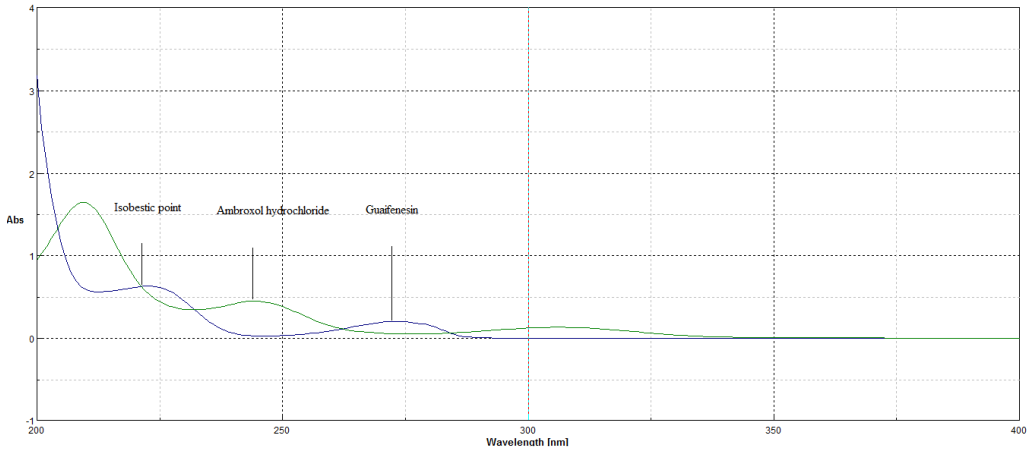


Fig. 2: Overlain zero order spectra of guaifenesin and ambroxol hydrochloride

The absorptivity coefficients of each drug at both wavelengths were determined. The concentration of each drug in laboratory mixture and tablet formulation was determined by substituting the absorbances and absorptivity coefficients in the following sets of equations.

$C_{GF} = (Qm-Q_{AMB}).\ A1 / (Q_{GF}-Q_{AMB}).\ a_{GF1}$
$C_{AMB} = (Qm-Q_{GF}).\ A1 / (Q_{AMB}-Q_{GF}).\ a_{AMB1}$

Where,
$Qm = A2 / A1$
$Q_{GF} = a_{GF2} / a_{GF1}$
$Q_{AMB} = a_{AMB2} / a_{AMB1}$
A2= Absorbance of Mixture at 273nm
A1= Absorbance of Mixture at 223 nm
a_{GF1}= absorptivity of GF at 223 nm
a_{AMB1}= absorptivity of AMB at 223 nm
a_{GF2}= absorptivity of GF at 273 nm
a_{AMB2}= absorptivity of AMB at 223 nm

First order derivative spectroscopy:
The absorbance of resulting solutions was measured at 273 and 245 nm for GF and AMB respectively, the calibration curves were plotted at these wavelengths. The overlain zero order spectra of GF and AMB (Fig.2) showed that the absorption maxima of GF and AMB lie in close proximity and at absorption maxima of one, another exhibits substantial absorbance. This clearly indicates the existence of spectral interference in estimation of GF and AMB. To overcome this, spectra of these two drugs were derivatised to first order between 200-400 nm. The overlain first derivative spectra of GF and AMB (Fig.3) reveal that ambroxol hydrochloride concentration can be estimated at 255 nm (zero-crossing point for guaifenesin) and guaifenesin can be estimated at 238 nm (zero-crossing point for ambroxol hydrochloride).

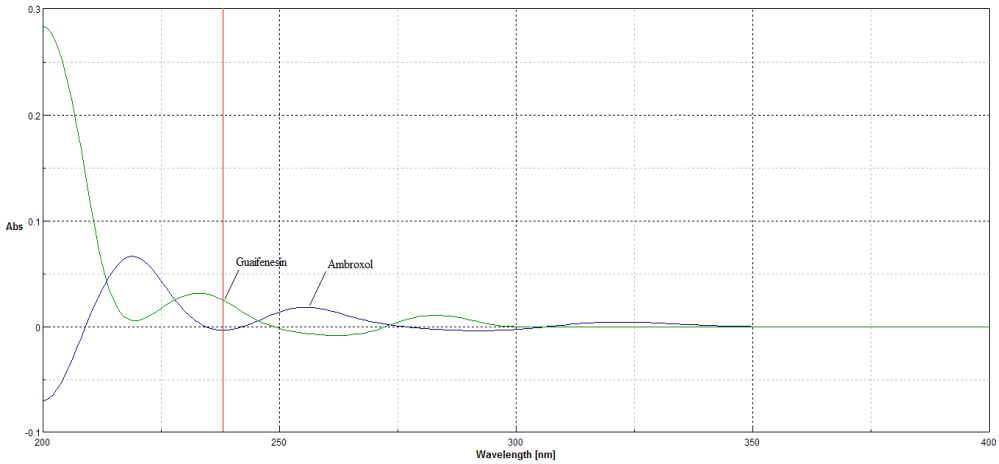


Fig.3 Overlain first order spectra of guaifenesin and ambroxol hydrochloride

Analysis of tablet formulation:
Twenty tablets were triturated and mixed thoroughly. Accurately weighed quantity of tablet powder equivalent to 50 mg of GF was transferred to 10 mL volumetric flask. Add 5 mL double distilled water and sonicate for 10 min. The resultant solution was filtered through 0.45µ membrane filter and finally diluted to volume with same solvent. From the resultant solution 0.03 mL was pipetted out and diluted upto 10 mL with distilled water. Absorbances were taken at 223 nm & 273 nm for Q-analysis and 238 nm & 255 nm for derivative spectroscopy.

RP-HPLC method:
Chromatographic Conditions:
Optimizations of chromatographic condition were carried out using acetonitrile: 50mM potassium dihydrogen phosphate buffer (pH 3.2 adjusted by using orthophosphoric acid) (22:78 v/v) as mobile phase. Prior to deliver into the system, the mobile phase was filtered through 0.45 µm filter and sonicate for 10 min. The samples were introduced by injector with a 20 µL sample loop. The analysis was carried out under isocratic conditions using flow rate 1.2 mL min^{-1} at 18^0C and chromatograms were recorded at 262 nm.

Preparation of standard stock solution:
Weighed accurately 50 mg of GF and 15 mg of AMB, transferred to a 10 mL volumetric flask, add 5 mL of mobile phase and sonicate for 10 min. Finally the volume was made up to mark with mobile phase.

Preparation of working standard solution:
From the resultant solution 0.1 mL solution was pipetted out in 10 mL volumetric flask and volume was made up to the mark with mobile phase.

Analysis of tablet formulation:
Accurately weighed quantity of tablet powder equivalent to 50 mg of GF was transferred to 10 mL volumetric flask, add 5 mL of mobile phase and sonicate for 10 min. The resultant solution was filtered through 0.45µ membrane filter, diluted to volume with mobile phase. 0.1 mL of resultant solution further diluted to 10 mL and injected to HPLC system.

System Suitability:
System suitability parameters were evaluated for retention times, asymmetry, capacity and theoretical plates of standard chromatograms (Table 1).

VALIDATION:
Limit of detection (LOD) and limit of quantification (LOQ):
Limit of detection and limit of quantification were calculated by the use of the equations LOD = 3.3 × *N*/*B* and LOQ = 10 × *N*/*B*, where *N* is the standard deviation of the peak area of the drug (n = 3), and taken as a measure of the noise, and *B* is the slope of the corresponding calibration plot.

Linearity:
To study the linearity of Q-analysis and derivative spectroscopic methods, series of diluted solutions were prepared by diluting standard stock solution with double distilled water so as to get final concentration in the range of 3, 6, 9, 12, 15, 18 µg $mL^{-1}$for both GF and AMB. Calibration graphs between absorbances against corresponding concentration were plotted at 223 nm & 273 nm for Q-analysis and 238 nm & 255 nm for derivative spectroscopy.
For HPLC method, diffrent standard solutions were prepared by diluting standard stock solution with mobile phase in concentration 5-180 µg $mL^{-1}$for GF and 1.5-54 µg $mL^{-1}$for AMB. The resultant solutions were injected and chromatograms were taken under standard chromatographic conditions.

Precision:
Variations of results within same day (intra-day) and between days (interday) were analyzed. Intra-day precision was determined by analyzing GF and AMB for three times in same day. Inter-day precision was determined by analyzing GF and AMB for three days.

Recovery:
To check the accuracy of the proposed method, recovery studies were carried out by applying standard addition method. A Known amount of standard GF and AMB, corresponding to 80, 100 and 120% of the label claim was added to preanalysed sample of tablet. The recovery studies were carried out in triplicate at each level.

RESULTS AND DISCUSSION

Wavelengths selected for Q analysis were, 223 nm (isobestic point) & 273 nm (λ max of GF) and for derivative spectroscopy 255 nm (zero-crossing point for guaifenesin) & 238 nm (zero-crossing point for ambroxol hydrochloride). Both spectrophotometric methods were found to be linear in the concentration range of 3-18 µg $mL^{-1}$for GF and AMB and correlation coefficient, 0.998 [Table 2 (Fig.4, 5)]. The results of commercial tablet formulation are presented in Table 3. Mean results of six determinations (Table 4) and percent recovery (Table 5) closed to 100% with relative standard deviation <2, concluded that the developed spectrophotometric methods are accurate, precise and can be employed successfully for routine estimation of GF and AMB in bulk and formulation.
In RP-HPLC method, the wavelength (262 nm) was selected for detection of better detector responses of drugs. Chromatographic conditions were optimized to obtain, an adequate separation of eluted compounds. Mobile phase and flow rate selection was based on system suitability parameters such as height, tailing, theoretical plates, capacity factor etc. The system with acetonitrile: 50 mM potassium dihydrogen phosphate buffer pH adjusted to 3.2 using orthophosphoric acid (22:78 v/v) as mobile phase with 1.2 µg mL^{-1} flow rate was found to be quite robust. A typical chromatogram for guaifenesin and ambroxol hydrochloride is shown in Fig 6. The average retention times for GF and AMB was found to be 7.5 and 9.5 min with linear range of 5-180 µg mL^{-1} (r^2 = 0.998) and 1.5-54 µg mL^{-1} (r^2 = 0.998) (Fig.7) respectively (Table 2). The method was found to be precise after quantification of six replicates of GF and AMB with RSD less than 2.0% (Table 4). The recovery values were found to be 99.05-101.61 % (Table 5).

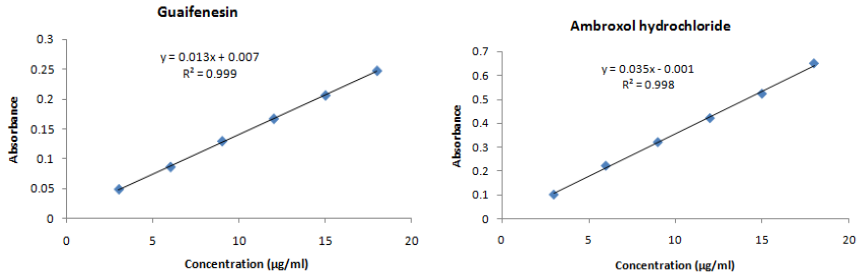


Fig.4 Linearity plots of guaifenesin and ambroxol hydrochloride (Q- Method)

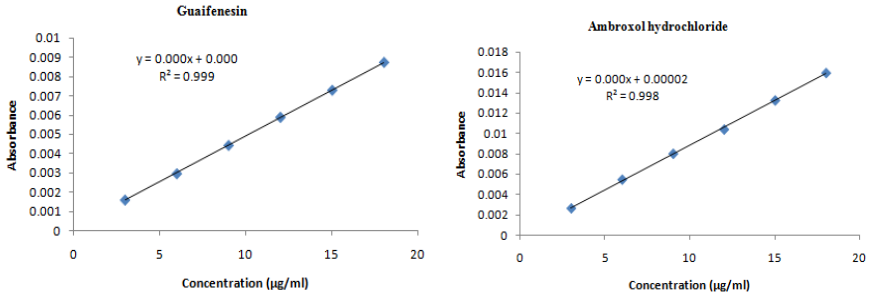


Fig.5 Linearity plots of guaifenesin and ambroxol hydrochloride (first order derivative)

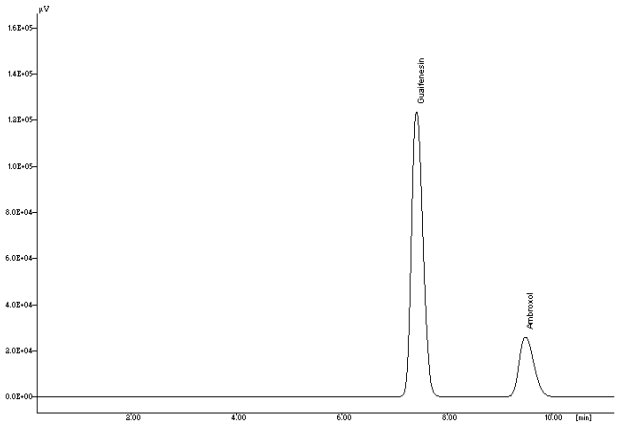


Fig.6: HPLC Chromatogram of guaifenesin and ambroxol hydrochloride

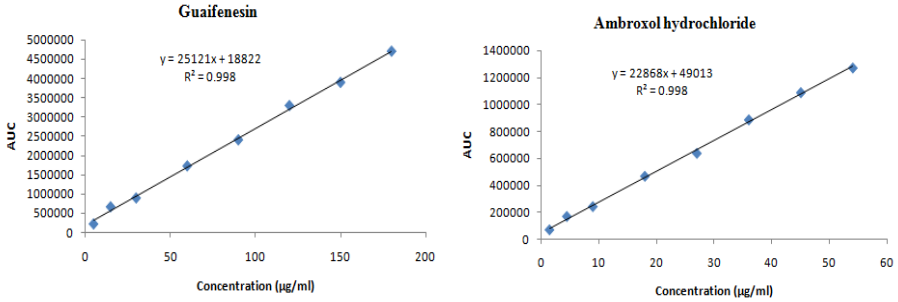


Fig.7: Linearity plots of guaifenesin and ambroxol hydrochloride (RP-HPLC)

Table 1: System suitability parameters for guaifenesin and ambroxol hydrochloride

Parameters	GF	AMB
Retention time	7.5	9.5
Asymmetry	1.12	1.18
Capacity	906	1161
Theoretical Plates	6872.53	6396.29

Table 2: Linear Regression data of guaifenesin and ambroxol hydrochloride

Parameters	Q-method		1st order derivative		RP-HPLC	
Calibration range (µg mL^{-1})	3-18	3-18	3-18	3-18	5-180	1.5- 54
Correlation coefficient (r^2)	0.999	0.998	0.999	0.998	0.998	0.998
Regression equation	0.013x +0.007	0.035x+ 0.001	0.00x+ 0.000	0.00x+ 0.00002	25121x +18822	22868x + 49013
Limit of detection (µg mL^{-1})	0.6	0.8	0.5	0.5	0.50	0.40
Limit of quantitation (µg mL^{-1})	2.0	2.5	1.5	1.5	1.60	1.25

Table 3: Analysis data of guaifenesin and ambroxol hydrochloride tablet

Sample	Labelled claim	Q-Method	1st order derivative	RP-HPLC
		% estimated ± S.D.	% estimated ± S.D.	% estimated ± S.D.
GF	50 mg	100.56 ± 0.8504	98.79 ± 0.9782	99.24 ± 0.5370
AMB	15 mg	99.92 ± 1.4035	99.74 ± 0.8704	98.76 ± 1.4919

S.D.- Standard deviation

Table 4: Precision data of guaifenesin and ambroxol hydrochloride

Parameter	Q-Method		1st order derivative		RP-HPLC	
	GF	AMB	GF	AMB	GF	AMB
Intra-day* (% estimated ± % RSD)	100.53± 1.78 101.50 ± 1.60 99.62 ± 1.58	98.74 ± 2.0 101.28 ± 0.26 101.20 ± 0.91	99.94± 0.85 100.54 ± 1.30 99.09 ± 0.92	99.91 ± 0.95 99.11 ± 0.81 101.05 ± 0.95	99.80 ± 1.32 100.70 ± 1.20 99.47 ± 1.91	100.21 ± 1.53 99.55 ± 0.95 100.87 ± 0.79
Inter-day* (% estimated ± % RSD)	100.02 ± 1.94 102.00 ± 0.61 99.41 ± 1.53	100.68 ± 1.27 98.02 ± 0.96 101.86 ± 0.37	101.48 ± 1.28 100.13 ± 1.49 101.76 ± 1.84	101.28 ± 0.36 100.27 ± 0.54 100.84 ± 1.94	101.36 ± 1.85 98.79 ± 1.65 99.43 ± 1.93	99.24 ± 1.58 100.40 ± 0.90 101.96 ± 1.30

**mean of three replicates, RSD-Relative Standard Deviation*

Table 5: Recovery study data of guaifenesin and ambroxol hydrochloride

Level of standard Addition (%)	Q-Method		1st order derivative		RP-HPLC	
GF	% Recovery*	S.D.	% Recovery*	S.D.	% Recovery*	S.D.
80	100.94	1.12	101.10	1.32	101.61	1.17
100	99.66	1.06	100.07	0.60	100.34	1.77
120	99.56	1.90	99.79	1.77	99.05	1.02
AMB						
80	101.45	0.51	99.80	0.71	99.23	0.49
100	99.92	0.55	99.69	1.18	100.18	0.99
120	99.27	0.48	100.16	1.37	101	0.83

**mean of three observations, S.D.-Standard Deviation*

CONCLUSION

The concept of this study was to develop accurate, precise and sensitive UV spectrophotometric and RP-HPLC methods for the determination of GF and AMB in commercial formulations without interference from tablet excipients. The advantages of the proposed methods were the ease of performance, reproducibility and devoid of complicated pre treatments before analysis. In addition, these methods have a potential for application in quality control laboratories.

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