

Research Article



SPECTROPHOTOMETRIC SIMULTANEOUS ANALYSIS OF AMBROXOL HYDROCHLORIDE, GUAIFENESIN AND TERBUTALINE SULPHATE IN LIQUID DOSAGE FORM (SYRUP)

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ABSTRACT

This study proposes a method for simultaneous estimation of Ambroxol HCl, Guaifenesin and Terbutaline sulphate in syrup form. The study was done by combining three spectrophotometric methods viz use of specific absorbance [A 1%, 1cm], second order derivative and colorimetry. Absorption of Guaifenesin and Terbutaline sulphate were found to be zero at 307.5nm, thus enabling the measurement of Ambroxol HCl, using specific absorbance in zero order spectrum. Applying the second order derivative, the amplitude of Guaifenesin was measured at 279.4nm, while Ambroxol HCl and Terbutaline sulphate were at zero cross point. For colorimetric measurement of Terbutaline Sulphate, a colored substance was obtained by coupling the oxidized product of Terbutaline sulphate with 4-aminoantipyrine and its absorption was measured at 550nm. The proposed method was statistically validated in accordance with ICH guidelines and results were found to be satisfactory for accuracy, precision and specificity.

Keywords: Ambroxol HCl, Guaifenesin and Terbutaline sulphate, second order derivative, colorimetric method.

INTRODUCTION

Ambroxol hydrochloride (AB) [*trans*-4-[(2-amino-3,5-dibromobenzyl)amino]cyclohexanol hydrochloride] is a semi-synthetic derivative of vasicine obtained from Indian shrub *Adhatodavastica*. 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)¹. Guaifenesin (GF), (*RS*)-3-(2-methoxyphenoxy) propane-1, 2-diol reportedly increases the volume and reduces the viscosity of tenacious sputum². Terbutaline sulfate (TBS) [(*tert*-butylamino) methyl]-3, 5-dihydroxybenzyl alcohol sulfate is a synthetic 2-adrenoceptor which predominantly stimulates β 2-receptors and is used as a bronchodilator in the treatment of bronchial asthma³.

The above combination is indicated for clinical relief of cough associated with bronchitis, bronchial asthma, emphysema and other bronchopulmonary disorders where bronchospasm, mucous plugging and problems of expectoration co-exist.

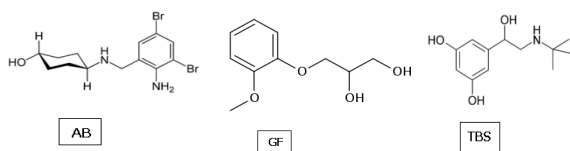


Figure 1: Chemical structures of AB, GF and TBS.

AB, GF and TBS are the subjects of monographs in Indian Pharmacopoeia (IP)⁴. Several methods such as spectrophotometric⁵⁻⁸, HPLC⁹⁻¹⁷, GC^{18,19}, fluorimetric²⁰, capillary electrophoresis^{21,22}, ELISA²³ and colorimetric method²⁴ have been reported for the analysis of AB, GF,

TBS as an individual drug or in combination, either in pure or in pharmaceutical forms as well as in biological fluids and tissues. Literature survey reveals that there is no single spectrophotometric analysis reported for the determination of AB, GF and TBS simultaneously in liquid formulation like syrup.

Hence the objective of the present study was to develop spectrophotometric analysis for simultaneous estimation of AB, GF and TBS in syrup.

MATERIALS AND METHODS

Instrumentation

A Shimadzu UV-Visible spectrophotometer model 1700 (Japan) with 1 cm matched quartz cells connected to a PC computer running UV- Probe 2.32 Software was used for all the absorbance measurements and treatment of data. Digisun electronics digital pH meter 7007 was used along with Sartorius digital balance.

Chemicals and reagents

AB, GF and TBS were kindly supplied by Juggat Pharma, Bangalore, India. Axalin syrup, Cipla (AB 15mg, GF 50mg, TBS 1.25mg per 5 ml) was used as marketed formulation. Freshly prepared 3.63% Tris (hydroxymethyl) aminomethane buffer solution (adjusted pH between 9.4 and 9.6), 8% w/v of potassium ferricyanide solution, 2% w/v of 4-aminoantipyrine solution and distilled water were used throughout the experiment. All reagents were used of an analytical grade.

Preparation of standard stock solution

Standard stock solution of AB, GF and TBS were prepared separately by dissolving 100 mg each drug in distilled water, made up the volume up to 100ml.



Determination of wavelengths of absorbance

The stock solutions of AB, GF and TBS were further diluted with distilled water to get concentration of 30 μ g/ml. These solutions were scanned in the range 200-400nm.

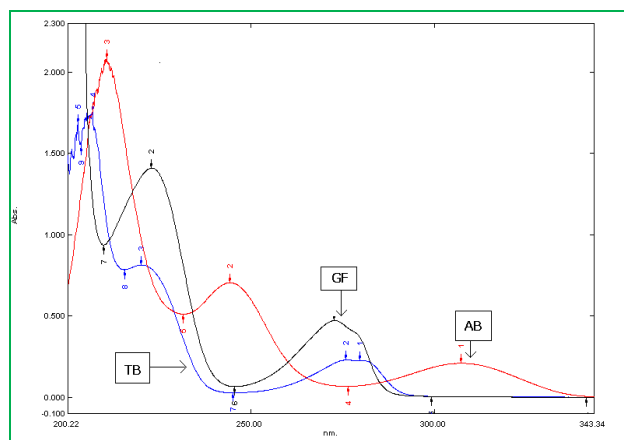


Figure 2: Overlay of AB, GF, TBS

Preparation of calibration curve

The stock solution of AB was appropriately diluted with distilled water to obtain concentration range of 20-120 μ g/ml and the absorbance were measured at 307.5nm.

The solutions of GF were prepared in the range of 10-80 μ g/ml and scanned in range 200 - 350nm and all the spectra were converted to second order derivative spectra ($\Delta\lambda=1$, scaling factor=10), and absorbance were measured at 279.4nm.

Assay of marketed formulation

25ml of marketed syrup (Axalin, Cipla) was measured and transferred in 50ml volumetric flask and made up to the mark with distilled water. This solution was filtered through whatman filter paper. This stock solution further diluted to get final concentration 15 μ g/ml of AB and 50 μ g/ml of GF. This solution was scanned in UV-Visible region of 200-350nm for estimation of AB and GF.

For determination of TBS concentration in syrup 4ml of stock solution was transferred in 50ml volumetric flask, 35 ml of a buffer solution and 1.0 ml of 4-aminoantipyrine solution were added and mixed. Further 1.0 ml of potassium ferricyanide solution was transferred with vigorous swirling and then sufficient buffer solution was added to produce 50.0 ml. TBS standard solution (0.0125%) was prepared, diluted simultaneously in similar manner. Exactly after 75 seconds of the addition of potassium ferricyanide solution, the absorbance of the resulting solution was measured at the maximum of 550 nm using blank, prepared in the same manner omitting TBS. (Table 1)

Method Validation

Recovery studies: To study the accuracy of the proposed method, recovery studies were carried out

by standard addition method of drug at three different levels (50%, 100% and 150%). A known amount of AB, GF and TBS were added to pre-analysed syrup separately and % recoveries were calculated. (Table 2)

Precision: The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day) and expressed as relative standard deviation (RSD). Intra-day precision was evaluated by analyzing concentration of AB (15 μ g/ml), GF (50 μ g/ml) and TBS (10 μ g/ml) at three different time intervals under the same experimental conditions on the same day. Intermediate precision (inter-day precision) was determined by analyzing above mentioned concentrations of each drug on three consecutive days. (Table 3)

Specificity of colorimetric method: This was carried out by analyzing the standard TBS solutions after spiking with appropriate amount of AB and GF. The concentration of TBS solution was determined by performing the assay as per the procedure mentioned in assay of marketed formulation (Table 4).

RESULTS AND DISCUSSION

First and foremost, the selection of solvent for the analysis was carried out by preparing the standard solution of AB, GF, and TBS in traditional solvents like 0.1N sodium hydroxide, 0.1N hydrochloric acid, and distilled water. Since the overlain spectra in all solvents was found to be almost identical, double distilled water chosen as it offered better solubility and stability.

As shown in figure 2, the wavelengths of absorption for AB were found to be at 244.5nm and 307.5nm. Since the absorbance of GF and TBS were found to be almost zero in their wide range of concentration at 307.5nm, the content of AB was determined by taking 70 as the value of A (1%, 1cm) at about 307.5nm. The linearity of AB at 307.5nm was found to be in the range of 20-120 μ g/ml. The percentage assay (99.23%) and recovery (101.4%) of AB in syrup were found to be satisfactory as tabulated in Table 1 and 2.

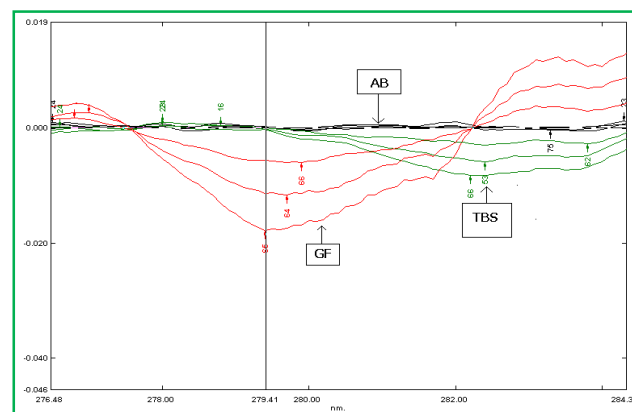


Figure 3: Second derivative spectrum showing common zero point of AB and TBS and GF absorbing prominently at 279.4nm.

Table 1: Assay of marketed formulation (Axalin syrup)

	Label claim (mg/5ml)	Mean Amount found (mg/5ml)	Mean Amount found (%)	R.S.D. (%) n=3
AB	15	14.88	99.23	0.052039
GF	50	45.22	90.45	1.265881
TBS	1.25	1.29	103.26	0.6651

Table 2: Recovery Studies

	Percent of amount of the drug found in preanalysed syrup solution(n=3)		
	50 % Add ⁿ ± RSD	100% Add ⁿ ± RSD	150 % Add ⁿ ±RSD
AB	102.76± 2.1547	101.16±1.7413	101.36±1.2413
GF	105.26±3.9504	106.80±3.5084	105.36±2.6169
TBS	96.86±2.8154	95.41±1.7621	95.41±1.3241

Table 3: Results of Precision Studies (Intra-day and Inter-day)

Component	Concentrations (µg/ml)	Intra-day precision		Inter-day Precision	
		Conc. Found µg/ml (n=3)	% RSD	Conc. Found µg/ml (n=3)	% RSD
TBS	10	10.2	1.879	8.92	5.86
AB	15	30.09	3.09	30.04	3.93
GF	50	49.27	1.26	48.98	1.15

Table 4: Results of Specificity

Sr. No	Con of AB (µg/ml)	Con of GF (µg/ml)	Con of TBS (µg/ml)	Absorbance at 550nm (n=3) ± RSD
1	0	0	10	0.478
2	0	50	0	0.00
3	15	0	0	0.005
4	15	50	10	0.474

Table 5: Analytical Validation Parameters

Parameters	AB	GF	TBS
Wavelengths (nm)	307.5	279.4*	550
Beer's law range(µg/ml)	20-120	10-80	5-25
Standard regression equations (n=9)	y= 0.007059x+ 0.002683	y = -0.004423x- 0.0008	y = 0.0452x + 0.0074
Standard absorbance value, A [1%, 1cm]	70.5	-	452.00
Correlation Coefficient	0.9998	0.9994	0.9993
LOD(µg/ml)	0.00035	0.000177	0.00002
LOQ(µg/ml)	0.00106	0.000536	0.000063
Sandel's sensitivity(µg/ml)	0.1418	0.22609	0.00185

*Results of second order derivative

While GF showed prominent absorption in the range of 210-293nm, AB and TBS were also found to be absorbing, making it difficult to determine the exact concentration of GF. Therefore, to measure GF, zero order spectrum of AB, GF and TBS were converted to its first, second and higher derivatives. Wavelength of 279.4nm of second order derivative was chosen for estimation of GF as TB and AB were showing zero cross point at this wavelength.

To optimize second order derivative method, different wavelength intervals ($\Delta\lambda=1, 2, 4, 8$) and scaling factors (1, 5, 10, 20) were studied. Quantitative estimation of GF in syrup was performed by second order derivative at about

279.4nm ($\Delta\lambda=1$, scaling factor=10). Linearity of GF was found to be in the concentration range of 10-80 µg/ml. While percentage recovery was found to be 105.8% (table 2), the assay of GF in syrup was found to be 90.45% (table 1).

It is a well known fact that liquid formulation like syrup contains many excipients viz colors, preservative, solubilizing agent. These excipients, mainly color may interfere in estimation of GF. There was no improvement in assay of GF despite adopting methods like derivative spectrophotometry and derivative ratio



spectrophotometry, where the blank was prepared assuming the excipients.

While the proposed study addresses the issue of estimation of GF in presence of AB and TBS in syrup, there is definite scope for further analysis and research to improve upon the proposed method.

Estimation of TBS was a challenging task as the concentration of TBS in the mixture was very low (1.25mg/5ml) when compared to GF (50mg/5ml) and AB (15mg/5ml). Due to low concentration of TBS, derivative spectroscopy could not be applied for the current composition. Increasing the concentration levels of TBS in sample solution resulted in proportional increase in the concentration of GF and AB, thus failing the Beer-Lambert's law.

Hence colorimetric method was adopted for estimation of TBS in presence of AB and GF. In this proposed method TBS was converted into colored complex by alkaline oxidation of phenol group by ferricyanide and then coupled with 4-aminoantipyrine. This reaction was carried out in tris buffer in the pH range of 9.4-9.6. The wavelength of absorption was found to be at about 550nm. The method was validated for specificity by adding calculated amount of AB and GF in the same ratio as in the original formulation. No interference of AB and GF were noted.

It was observed that TBS obeyed Beer-Lambert's law in the range of 5-25 µg/ml: %assay and recovery were found in the range 103.62% and 95.54% respectively (Table 1 and 2).

Precision was determined by inter- and intra-day studies of the solution of GF, TBS and AB. For inter- and intra-day studies of TBS, the stock solution was stored and converted into coloured product at the time of measurement. RSD of observations was found to be less than 3%.

CONCLUSION

To conclude, the proposed analytical method is simple, specific, accurate and precise for simultaneous analysis of AB, GF and TBS in syrup formulation.

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