International Journal of
Medicinal Chemistry & Analysis
www.ijmca.com
e ISSN 2249 - 7587
Print ISSN 2249 - 7595

SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF GEMIFLOXACIN AND AMBROXOL FROM TABLET FORMULATION

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ABSTRACT

Two simple, accurate and precise UV methods were developed for the estimation of Gemifloxacin (GEM) and Ambroxol hydrochloride (AMB) from its tablet dosage form. Both the drugs are used in treatment of chronic bronchitis and mild to moderate pneumonia. Method I is Simultaneous equation method, wavelengths selected for Quantitation are 271.0 nm and 245.5 nm for c respectively which are the λ_{max} of both the drugs. Method II is Q –Analysis method, wavelengths selected were 271nm (λmax of GEM) and 244.0 nm (Isobestic point) for the analysis. In both the methods linearity for detector response was observed in the concentration range of 10-60 µg/ml for GEM and2-12 µg/ml for AMB respectively. The results of tablet analysis for method I is found to be 99.97% ± 0.041 S.D for GEM and 99.93% ± 0.21 S.D for AMB and results obtained for Method II is 99.94% ± 0.080 S.D for GEM and 99.90% ± 0.15 S.D for AMB. The proposed methods were successfully applied for the simultaneous determination of both the drugs in commercial tablet preparation. The results of the analysis have been validated statistically.

Keywords: Gemifloxacin mesylate, Ambroxol *HCl*, UV-Spectrophotometry, Simultaneous equation method, Q-Analysis method.

INTRODUCTION

Gemifloxacin (GEM) chemically R,S-7-(3 amino methyl 4- syn methoxyimino-1pyrrolidinyl)-1cyclopropy l-6-flouro 1, 4, dihydro 4- oxo-1, 8 napthyridine-3-carboxylic acid methane- sulphonate [1-3] is a new flouroquinolone antibacterial compound with enhanced affinity for bacterial topoisomerase-IV and is being used for the treatment of respiratory and urinary tract infections, [4-5]light brown powder, freely soluble in water and slightly soluble in Methanol. Ambroxol hydrochloride (AMB) (Fig. 1B) Chemically, 4-[(2-amino-3,5-dibromophenyl)-methyl]-amino] cyclohexanol hydrochloride is a mucolytic expectorant and used to reduce the viscosity of mucous [6].

Literature survey revealed that few analytical method have been reported for the estimation of Gemifloxacin, rapid and sensitive LC method for analysis of Gemifloxacin in human plasma [7] , spectrophotometric determination of Gemifloxacin mesylate in pharmaceutical formulation trough ion-pair complexation [8] and validated stability indicating assay of Gemifloxacin and lomefloxacin in tablet formulation by capillary electrophoresis [9]. No analytical method is available for analysis of ambroxol and Gemifloxacin in combination.

Fig 1A. Stature of Gemifloxacin

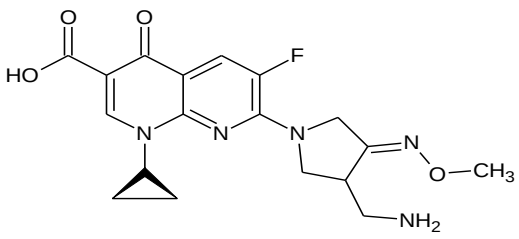

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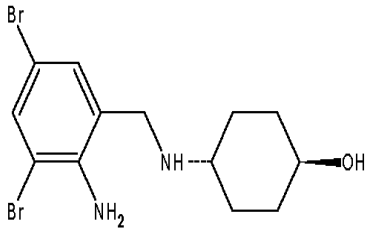
Fig 1B. Stature of Ambroxol



MATERIAL AND METHODS

Instruments

UV-Visible Spectrophotometer (Double Beam)
Make: Jasco
Model: UV V-630 Spectrophotometer
Spectral Bandwidth: 2nm

Materials

Standard gift sample of Gemifloxacin and Ambroxol were provided by Hetero Drugs Ltd., H.P.
Solvent used: Doubled distilled water used as solvent.

Stock solution

Stock solution of both the drugs100mcg/ml is prepared by dissolving 10mg each drug in100ml volumetric flask and the volume is make up by distilled water [10].

Procedure

Method I - Simultaneous equation method

In this method, the stock solution of both the drugs100mcg/ml is prepared by dissolving 10mg each drug in100ml volumetric flask and the volume is makeup by distilled water. By appropriate dilution of standard stock solutions of both the drugs to 20 µg /ml dilution respectively is scanned in the spectrum mode from 400nm to 200 nm. The absorption spectra thus obtained is selected for analysis, from the overlain spectra of both the drugs (fig.1), wavelength selected for Quantitation are271 nm and 244.50 nm for Gemifloxacin and Ambroxol and which are the λ_{max} of both the drugs. The calibration curves for Gemifloxacin and Ambroxol concentration range of 10-60 µg /ml for GEM and 2-12 µg /ml for Ambroxol exhibiting the Beer's and Lamberts range. The concentration of individual drug present in the mixture was determined by using the simultaneous equation calculations [11].

Method II- Q Analysis method

For the selection of Analytical wavelength, solution of GEM and AMB (10 mcg/ml, each) were prepared separately by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. From overlain spectra of both the drugs (fig.1), wavelengths selected were 271 nm and 244.0 nm (Isobastic point) for the analysis. The Q values of both the drugs were determined at the selected wavelength. The Q value is the ratio of Absorbance of std.1 at 271.0 nm to the Absorbance of std.2 at 244.50 nm. Molar Absorptivities for both the drugs were calculated by Absorbance of mix at 244.0 nm with the concentration in gm/lit. A set of two simultaneous equations obtained by using 'Q' values are given below.

$C_{GEM} = Q0 - Q_{AMB}/ Q_{GEM} - Q_{AMB} x\ A / a_{GEM}$ ---------- (1)

$C_{AMB} = Q0 - Q_{GEM}/ Q_{AMB} - Q_{GEM} x\ A / a_{AMB}$---------- (2)

C_{AMB} and C_{GEM} were concentration of AMB and GEM, respectively. The concentration of AMB and GEM in sample was determined by using the equation (1) and (2).

VALIDATION

The methods were validated with respect to linearity, accuracy, precision and selectivity.

Accuracy

To ascertain the accuracy of the proposed, methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% and120%). Percent recovery for GEM and AMB, by methods, was found in the range of 99.46% to 100.01%.

Linearity

The linearity of measurement was evaluated by analyzing different concentration of standard solution of GEM and AMB For both the methods, the Beer-Lamberts concentration range was found to be 2-12 µg /ml for AMB, and10-60 µg /ml for GEM.

Precision

The reproducibility of proposed method was determined by performing tablet assay at different time intervals (morning, afternoon, and evening) on same day (Intra-day precision) and on three different days (Inter-day precision) Results of precision is expressed in %RSD. Result for precision was found to be 0.0416 (for GEM) and 0.2148(for AMB) in simultaneous equation method; 0.0810 (for GEM) and 0.157 (for AMB) in Q-Analysis method [12, 13].

RESULTS AND DISCUSSION

The methods discussed in the present work provide a convenient and accurate way for simultaneous analysis of GEM and AMB. In simultaneous equation method wavelength selected for Quantitation were 271.0 nm for GEM and 244.50 nm for AMB. In Q-Analysis method the wavelength selected were 271.0 nm and 245 nm (Isobastic point). In both the methods linearity for detector response was observed in the concentration range of 2-12mcg/ml for GEM and AMB both. In method I, concentration of individual drug present in the mixture was determined against calibration curve in Quantitation mode .In method II; Q values were calculated for both the

drugs at selected wavelengths and substituted in equations for determining the concentration of GEM and AMB in Bulk drug sample solution. Percent label claim for GEM and AMB in tablet analysis by both the methods was found in the range of 99.79% to 100.02%.

Figure 2. Overlain spectra of GEM and AMB Showing Isobestic Point

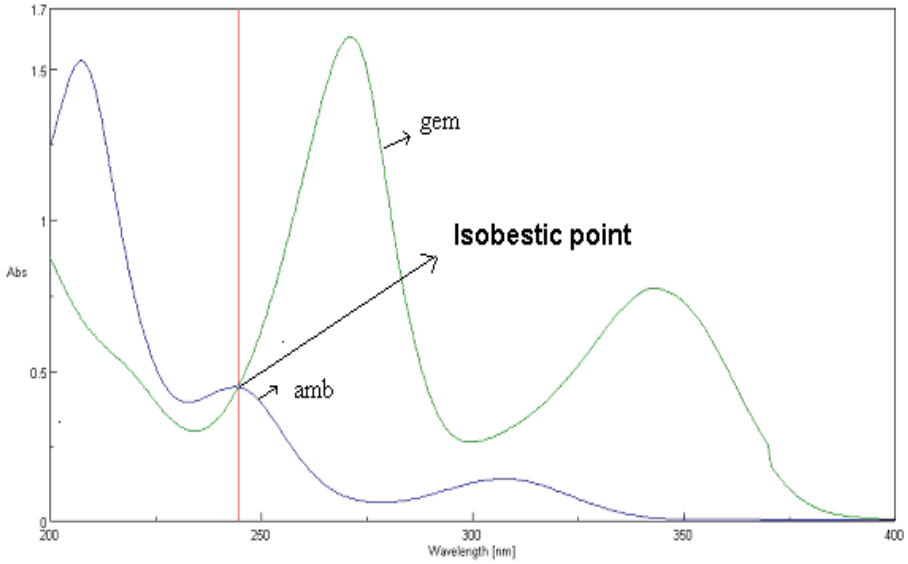


Table1. Spectral Characteristics and Linearity Data

Parameters	Method I		Method II	
	GEM	AMB	GEM	AMB
λ_{max}	271	244.5	271	244
Linearity (µg/ml)	10-60	2-12	10-60	2-12
Regression Equation(y=a+bx)	0.054x+0.007	0.10x+0.008	0.059x+0.003	0.255x+0.016
Slope(b)	0.054	0.10x	0.059	0.255
Intercept(a)	0.007	0.008	0.003	0.016
Correlation coefficient	0.998	0.999	0.999	0.995
LOD	0.42	0.26	0.16	0.48
LOQ	1.2	0.8	0.50	0.62

Table 2. Analysis of Commercial Formulations (precision)

Method		Method I		Method II	
S.No		% Label Claim		% Label Claim	
		GEM	AMB	GEM	AMB
1		99.94	100.08	99.96	99.68
2		99.97	99.69	99.85	99.85
3		99.93	99.98	99.89	99.93
4		100.03	99.81	100.06	99.98
5		100	100.12	99.97	100.1
	Mean	99.97	99.93	99.94	99.90
	S.D	0.0415	0.2147	0.080	0.156
	%RSD	0.0416	0.2148	0.081	0.157
	± S.E	0.0186	0.0960	0.036	0.069

*n=5 S.D.=standard deviation, %R.S.D.=percentage standard deviation, S.E. = standard error

Table 3. Recovery Study

Method I								
Level of % Recovery	**Amount present (mg/tab)**		**Amount of standard added (mg)**		**Total amount recovered (mg)**		**%Recovery**	
	GEM	**AMB**	**GEM**	**AMB**	**GEM**	**AMB**	**GEM**	**AMB**
80	320	75	256	64	576.65	134.46	99.94	99.60
100	320	75	320	75	639.80	149.73	99.97	99.82
120	320	75	384	90	703.57	164.57	99.94	99.74
		GEM				**AMB**		
Mean		99.95				99.72		
SD		0.0173				0.111		
%RSD		0.0174				0.112		
Method II								
Level of % Recovery	**Amount present (mg/tab) Amount of standard added (mg)**				**Total amount recovered (mg)**		**%Recovery**	
	GEM	**AMB**	**GEM**	**AMB**	**GEM**	**AMB**	**GEM**	**AMB**
80	320	75	256	64	575.88	135.14	99.98	100.11
100	320	75	320	75	640.38	149.83	100.06	99.89
120	320	75	384	90	704.07	164.90	100.01	99.94
		GEM				**AMB**		
Mean		100.01				99.98		
SD		0.040				0.115		
%RSD		0.041				0.116		

*n=3

CONCLUSION

Standard deviation and coefficient of variance for six determination of tablet sample, by both the methods was found to be less than ±2.0 indicating precision of both the methods. The result of analysis shows that the developed methods are accurate, precise, reproducible and economical and can be employed for routine quality control analysis off Gemifloxacin and Ambroxol Hydrochloride in combined dose formulation

ACKNOWLEDGEMENTS

The authors are very much thankful to the Chairman, Mrs.Fatma Rafiq Zakaria, Maulana Azad Educational Trust, for providing necessary facilities for the project work. The authors are also thankful to Hetero Drugs Ltd. H.P for providing gift samples of Gemifloxacin and Ambroxol Hydrochloride.

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