



(RESEARCH ARTICLE)



First-order derivative spectrophotometric estimation of gemifloxacin mesylate and ambroxol HCl in tablet dosage form

Wrushali A. Panchale * and Ravindra L. Bakal

IBSS's Dr. Rajendra Gode Institute of Pharmacy, Amravati-444 602, MS, India.

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Abstract

Aim of present work was to develop and validate a simple, precise and accurate uv-vis spectrophotometric method for the simultaneous estimation of gemifloxacin mesylate (GEMI) and ambroxol HCl (AMB) in their combined tablet dosage form. The method is based on first-order derivative spectroscopy. For determination of sampling wavelengths, each of GEMI and AMB were scanned in the wavelength range of 200–400 nm in the spectrum mode and sampling wavelengths were selected at 360 nm (zero crossing of GEMI) where AMB showed considerable absorbance and at 221.6 nm (zero crossing of AMB) where GEMI showed considerable absorbance. The linearity was obtained in the concentration range of 32-192 µg/ml for GEMI and 7.5-45 µg/ml for AMB. The correlation coefficients were found to be 0.9987 and 0.9992 for GEMI and AMB, respectively. The method was validated as per ICH guidelines. Mean recoveries were found satisfactory. All the data of validation study was found to be satisfactorily. The proposed method can be applied for simultaneous estimation of both the drugs

Keywords: Gemifloxacin mesylate; Ambroxol HCl; First order derivative

1. Introduction

Gemifloxacin mesylate (GEMI), chemicaly is (R, S)-7-[(4Z)-3-(aminomethyl)-4-(methoxyimino)-1-pyrrolidinyl]-1-cyclopropyl-6-fluoro-1, 4-dihydro-4-oxo-1, 8-naphthyridine-3-carboxylic acid (Fig. 1). Gemifloxacin acts by inhibiting bacterial DNA synthesis by binding to bacterial topoisomerases II (DNA gyrase) and IV, resulting in bacterial death. It has potent activity against most Gram-positive bacteria, particularly *Streptococcus pneumoniae* [1, 2]. Ambroxol HCl (AMB) is (Fig. 2) is mucolytic agent act by breaking the acid mucopolysaccharide fibres which makes the sputum thinner and less viscous. It is used as mucolytic agent [3, 4]. Individually both drugs are used in respiratory disorders, but the combination of these two drugs is approved for the treatment of lower respiratory tract infection mainly pneumonia and bronchitis. The combination provides better therapeutic management in suffering individual. This combination is banned in India in 2017, but being in used in other countries like Bangladesh, Nepal and in some European countries [5]. Both the drugs are official in Indian Pharmacopoeia [6].

The derivative spectrophotometric method is one of the modern spectrophotometric method that offer a useful way for extracting both qualitative and quantitative information from the spectra composed of overlapped bands. It is based on using the first- or higher-order derivatives of absorbance with respect to wavelength from parent zero-order ones. Derivatization can lead to the separation of unresolved signals and reduction of spectral background interferences in the presence of others without initial separation or purification. The application of derivative spectrophotometry in pharmaceutical analysis has been critically reviewed [7, 8].

* Corresponding author: Ms. Wrushali Arjun Panchale
IBSS's Dr. Rajendra Gode Institute of Pharmacy, Amravati-444 602, MS, India.

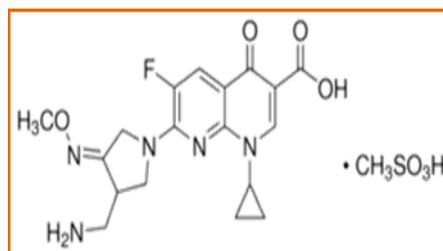


Figure 1 Chemical structure of gemifloxacin mesylate

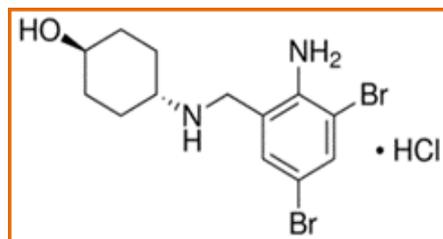


Figure 2 Chemical structure of ambroxol HCl

Analytical methods are used for qualitative and quantitative analysis of drugs alone or in combination with other drugs from various pharmaceutical formulations and even for analysis of non-pharmaceutical molecules or impurities in ayurvedic or pharmaceutical products [9-28]. Literature survey reveals that GEMI alone and in combination with AMB can be estimated by some methods [29-39]. However, there is no report of First-order derivative Spectrophotometric method for simultaneous estimation of GEMI and AMB in their combined dosage form. Therefore, it was thought of interest to develop and validate first-order derivative spectrophotometric method for the simultaneous estimation of both drugs in their combined tablet dosage form.

2. Material and methods

2.1. Instrumentation and materials

Double beam UV-visible spectrophotometer (Simadzu model- UV 2401 PC, Shimadzu Corp., Kyoto, Japan) with spectral width of 2nm, quartz cell (1.0 cm path) was employed to measure absorbance of solutions. Pure gift sample of GEMI and AMB were obtained from Glenmark pharmaceuticals Ltd., Hosur and Dr. Reddy's laboratories, Hyderabad, respectively. Tablet G-CIN A (GEMI 320 mg, AMB 75mg) was used in experimental work. All chemicals were of analytical reagent grade and solutions were prepared with water AR grade.

2.2. Methodology

Standard stock solution of GEMI and AMB were prepared individual containing 640 $\mu\text{g/ml}$ of GEMI and 150 $\mu\text{g/ml}$ of AMB in methanol. UV-vis spectrums were made and overlapped to decided wavelengths (Fig. 2-A). These absorption spectra were converted to first-order derivative spectra by using the instrument mode. After observing the overlain first-order derivative spectra with scaling factor = 4 and $\Delta\lambda = 4$ for GEMI and AMB (Fig. 2-B), zero crossing points of drugs were selected for the analysis of other drugs. The first wavelength selected was 360 nm (zero crossing of GEMI), where AMB showed considerable absorbance.

The second wavelength selected was 221.6 nm (zero crossing of AMB), where GEMI showed considerable absorbance. The spectrums obtained was derivatised to obtain first derivative spectrum. These two wavelengths can be employed for the estimation of GEMI and AMB without any interference from the other drugs in their combined formulation. Calibration curves were prepared separately by using series of individual solutions 32-192 $\mu\text{g/ml}$ (i.e. 32, 64, 96, 128, 160, 192 $\mu\text{g/ml}$) for GEMI and 7.5-45 $\mu\text{g/ml}$ (i.e. 7.5, 15, 22.5, 30, 37.5, 45 $\mu\text{g/ml}$) for AMB. Calibration curves were plotted by taking $dA/d\lambda$ on Y-axis and concentrations on X-axis. The overlapping spectras of calibration study are shown in Fig. 2-C.

2.3. Sample solution

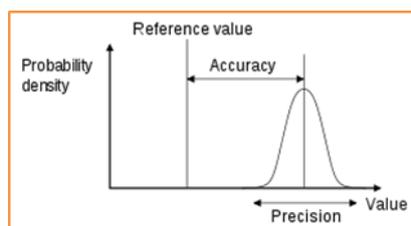
Sample solution in methanol was prepared using marketed tablet formulation contained 64 µg/ml of GEMI and 15 µg/ml of AMB. Spectrum of sample also derivatised and absorbance of both drug were measured at selected wavelengths and concentration of both drug calculated from calibration curve. The procedure was repeated six times for sample analysis.

2.4. Validation of method

In measurement of a set, accuracy is closeness of the measurements to a specific value, while precision is the closeness of the measurements to each other. Validation was performed as per ICH guidelines.[40]

2.5. Accuracy (Recovery study)

Accuracy is the proximity of measurement results to the true value; precision is the degree to which repeated (or reproducible) measurements under unchanged conditions show the same results.



A recovery study was carried out by the addition of known amount of the standard drug in the preanalysed tablet formulation in 80, 100, and 120% of the label claim. At each level of amount, three determinations were performed.

2.6. LOD and LOQ

The determination of LOD (the limit of detection) of instrumental procedures is carried out by determining the signal-to-noise ratio by comparing test results from the samples with known concentration of analyte with those of blank samples and establishing the minimum level at which the analyte can be reliably detected. A signal-to-noise ratio of 2:1 or 3:1 is generally accepted. The signal-to-noise ratio is determined by dividing the base peak by the standard deviation of all data points below a set threshold. Limit of detection is calculated by taking the concentration of the peak of interest divided by three times the signal-to-noise ratio. Other approaches depend on the determination of the slope of the calibration curve and the standard deviation of responses as shown below.

$$LOD = 3.3 \times \frac{s}{S}$$

Where, s = standard deviation of the response,

S = slope

The LOQ (limit of quantification) is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy. It is usually expressed as the concentration of analyte in the sample. It can be determined by comparing measured signals of samples with known low concentrations of analyte with those of blank samples. The minimum concentration at which the analyte can reliably be quantified is established. A typical acceptable signal-to-noise ratio is 10:1. Other approaches depend on the determination of the slope of the calibration curve and the standard deviation of responses.

$$LOQ = 10 \times \frac{s}{S}$$

Where, s = standard deviation of the response

S = slope

2.7. Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. It is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements. Precision may be a measure of either the degree of reproducibility or of repeatability of the analytical method. Reproducibility refers to the use of the analytical procedure in different laboratories, or on different days or with different analysts. Repeatability refers to the use of the analytical procedure within the same laboratory over a short period of time using the same analyst with the same equipment.

Precision was determined by assaying a sufficient number of samples using standard deviation or coefficient of variation for set of n value. The RSD values are important for showing degree of variation expected when the analytical procedure is repeated several time in a standard situation. (RSD below 1% for built drugs, RSD below 2% for assays in finished product). The ICH documents recommend that repeatability should be assessed using a minimum of 9 determinations covering the specified range for the procedure (i.e. 3 concentrations and 3 replicates of each concentration).

3. Results and discussion

First-order derivative spectrophotometric method for simultaneous estimation of GEMI and AMB in tablet dosage form. GEMI and AMB both drugs were soluble in methanol; hence methanol was selected as solvent. zero-order overlain spectra of GEMI and AMB at 64 $\mu\text{g/ml}$ and and the spectra showed λ_{max} of 360 nm and 221.6 nm for GEM and AMB, respectively. Also, both absorbs at the λ_{max} of the each other hence, simultaneous equation method was used to estimate GEM and AMB in presence of each other. For first order derivative method measurement zero crossing were used. Both the methods were validated statistically as per ICH guideline for parameters like linearity, precision and accuracy. Various overlain spectras of GEMI and AMB are shown in Fig. 3-A - 2-C.

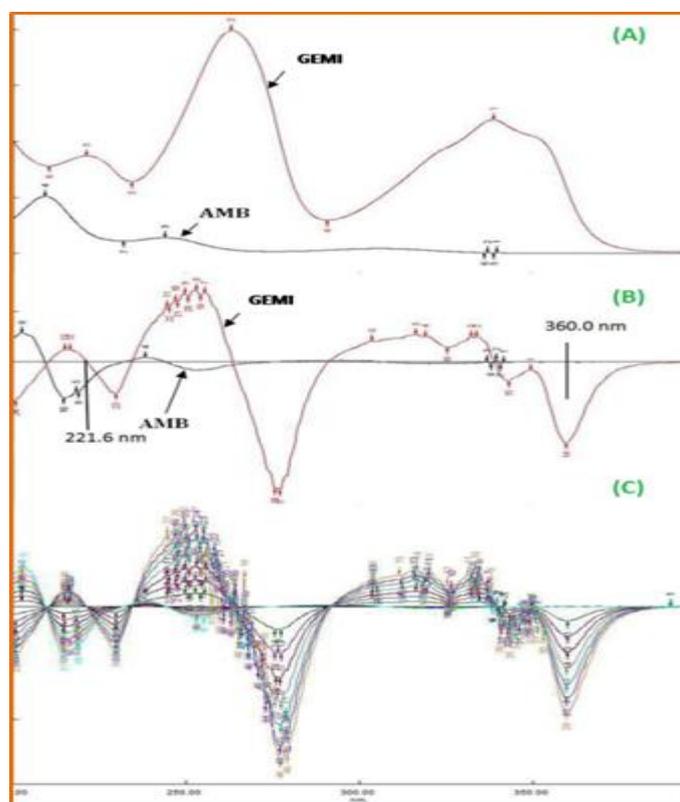


Figure 3 Spectras of GEMI and AMB i.e. (A) overlain, (b) first order derivative, (c) overlapping calibration

For each drug, linearity was observed by diluting appropriate aliquots of the working standard stock solution to get a final concentration range of 32-192 $\mu\text{g/ml}$ and 7.5-45 $\mu\text{g/ml}$ for both GEMI and AMB, respectively. From the calibration curves, concentration selected for further experimental work were 64 $\mu\text{g/ml}$ for GEMI and 15 $\mu\text{g/ml}$ for AMB.

In sample solution analysis, the samples were scanned in the wavelength range 200-400 nm, and the first-order derivative of the spectrum was taken. The $dA/d\lambda$ of each of these solutions was measured at the selected wavelength and plotted against concentration to obtain the calibration graph. The statistical parameters of the calibration curve, such as correlation coefficient, regression equation, LOD, and LOQ, for GEMI and AMB are given in Table 1.

Validation was performed in terms of determination of accuracy by recovery study. Accuracy was performed by recovery study at three level 80%, 100%, and 120% of the test concentration. The RSD values for intraday and interday precision are less than 2%. The recovery was between 99.65-102.08 %. So the developed method is accurate and precise. All data are as shown in Table 1. The amounts in terms of % label claim obtained by proposed methods are presented in Table 1.

Table 1 Characteristics of the proposed method (first-order derivative method)

Parameter	GEMI	AMB
λ (WL)	360 nm	221.6 nm
Linearity	32-192	7.5-45
Slope	0.00355	0.0036
Intercept	- 0.001	0.0004
R ²	0.9987	0.9992
LOD	3.7 $\mu\text{g/mL}$	6.1 $\mu\text{g/mL}$
LOQ	9.91 $\mu\text{g/mL}$	18.75 $\mu\text{g/mL}$
Assay of tablet		
Label claim	320 mg/tablet	75 mg/tablet
Amt. estimated	100.17 %w/w	99.88 %w/w
Recovery at		
80%	99.65	100.13
100%	101.02	102.08
120%	101.64	101.31
Precision (%RSD)		
Intra-day	99.33	100.85
Inter-day	100.49	101.21

*Average of three determination

4. Conclusion

The proposed UV-vis spectrophotometric method was found to be simple, accurate, precise and linear. Hence, it can be directly used for the analysis of GEMI and AMB from combined dosage form

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflicts of interest.

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