Research Article

Simultaneous spectrophotometric determination of azithromycin and levofloxacin from solid dosage form

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ABSTRACT

Aim: Simple, precise, and economical spectrophotometric methods have been developed for the simultaneous estimation of azithromycin (AZI) and levofloxacin (LFI) in tablet formulations. **Methods:** The first method is based on the use of multicomponent mode analysis, the second method is based on the absorbance ratio of the components, that is, absorbance ratio method, and the third is partial least squares calibration method. **Results:** The proposed methods do not require any prior chemical separation for their estimation from the dosage form. The Beer's law range was varying from 20–100 µg/ml in Methods I and II and 2–10 µg/ml for method III for both the drugs AZI and LFI. **Conclusion:** All three proposed methods were successfully applied to the determination of AZI and LFI in several synthetic and real sample matrices.

KEY WORDS: Absorbance ratio method, Azithromycin, Levofloxacin, Multicomponent analysis, Partial least squares regression

INTRODUCTION

Azithromycin (AZI) is chemically (2R, 3S, 4R, 5R, 8R, 10R, 11R, 12R, 13R, and 14R)13-[(2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl) oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10,12,14-hepta methyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one.[1] AZI prevents bacterial growth by interfering with their ability to make proteins. Due to differences in the way, proteins are made in bacteria and humans the macrolide antibiotics do not interfere with the production of proteins in humans. It is an unusual antibiotic that it stays in the body for a quiet while (has a long half-life), allowing for once a day dosing and for shorter treatment courses for most infections[Figure 1]. Levofloxacin (LFI) is chemically(-)-(S)-9-fluoro2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid hemihydrate[Figure 2].[2] LFI is an antibiotic used for treating bacterial infections. It stops multiplication of bacteria by preventing the reproduction and repair of their genetic material, DNA.

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Only few methods appear in the literature for determination of AZI based on spectrophotometric,[3,4] high-performance liquid chromatography,[5-9] and high-performance thin-layer chromatography methods[10] individually and in combination with other drugs.[11-13] There have been several reports on the determination of LFI individually and in combinations using spectrophotometry and chromatography techniques. Spectrophotometry being a relatively easy method for the simultaneous determination of two drugs, authors report three simple and sensitive methods for the estimation of the mentioned analyses of interest.

EXPERIMENTAL

Instrument

A Shimadzu 1601 UV-VIS Spectrophotometer with bandwidth of 2nm, wavelength accuracy of ±0.5nm and matched quartz cells were used for all measurements

Reagents and Chemicals

Standard gift samples of AZI and LFI were obtained from Indian Drugs, Kukatpally, Hyderabad. Tablets containing AZI and LFI were procured from local pharmacies.

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Standard and Sample Solutions

Standard solutions

The stock solutions (1000 µg/ml) of AZI and LFI were prepared separately by dissolving accurately about 100 mg of drug in 20 ml 0.1 N HCl, and the volume was made up to the mark with distilled water.

Sample preparation

For the preparation of sample solution, 20 tablets were weighed and powdered in a mortar. An amount equivalent to one tablet containing AZI and LFI was placed in a 100 ml volumetric flask along with 20 ml of 0.1 N HCl. The content of the flask was mechanically shaken for 30 mins and filtered through a 0.45 µm membrane filter. The obtained solution was diluted to the respective working concentration range. Appropriate volumes of standard stock solutions at three different concentration levels were added to the analyzed sample solution for recovery studies, and the procedure was repeated three times at each concentration levels.

Individual Calibrations

To verify Beer's law calibration graphs were prepared for the determination of AZI and LFI [Table 1]. The correlation coefficients were obtained at the selected wavelengths by overcoming the mutual interference using the proposed Method I.

Method I: Multiwavelength spectroscopy

Using the overlain spectra of AZI and LFI in water, the wavelength maxima of both drugs, that is, 245.0 nm and 290.0 nm, were selected as two sampling wavelengths for this method. Six mixed standards of two drugs in methanol were prepared to contain 20–100 µg/ml of AZI and LFI. All mixed standard solutions were scanned over the range of 350 nm to 200 nm in the multicomponent mode of spectrophotometer using 245.0 nm and 290.0 nm as two sampling wavelengths. The spectral data from these scans were used to determine the concentration of two drugs in the sample solution.

Method II: Absorbance ratio/Q-analysis method

From the overlain spectrum of AZI and LFI, two wavelengths were selected, one at 245.0 nm, the λ_{max} of AZI and other at 209.60 nm, the iso-absorptive point for both the drugs. The absorbance values of the prepared solutions were measured at the selected wavelengths.

Table 1: Optical characteristics of AZI and LFI at the selected wavelengths (Method I)

Parameters	AZI	LFI
Wavelength maximum (nm)	245.0	290.0
Beer's law limit (µg/ml)	20-100	20-100
Regression coefficient (r)	0.9971	0.9975
Slope	0.0018	0.0605
Intercept	0.0024	0.0112

AZI: Azithromycin, LFI: Levofloxacin

The concentration of each component was calculated by mathematical treatment of the following equation

Concentration of AZI= $Q_0 - Q_A/Q_C - Q_A \times A/\varepsilon_1$

Concentration of LFI= $Q_0 - Q_C/Q_A - Q_C \times A/\varepsilon_2$

Where, A = Absorbance of sample solution at isosbestic wavelength

ε_1 and ε_2 = absorptivities of AZI and LFI at isosbestic wavelength

Q_A = Absorbance of AZI at 245nm/Absorbance of AZI at 209.60 nm

Q_C = Absorbance of LFI at 245nm/Absorbance of LFI at 209.60 nm

Q_0 = Absorbance of sample solution at 245nm/ Absorbance of sample solution at 209.60 nm.

Method III: Partial least squares (PLS) regression

Calibration and prediction sets were designed with 25 and 10 binary mixtures of the cited drugs, respectively. Concentrations of AZI and LFI in calibration and prediction solutions were in the range of 2–10 µg/ml. Each calibration and prediction mixture was prepared by diluting the appropriate aliquot of stock solution with distilled water. Each solution was recorded against the blank in the range of 200–350 nm with the wavelength interval of 1 nm as shown in Figure 3.

RESULTS AND DISCUSSION

The proposed methods were found to be simple, accurate, economical, and rapid for routine simultaneous estimation of two drugs. Recovery studies were found close to 100 % that indicates accuracy and precision of the proposed methods.

The first method employing multicomponent mode is very simple and can be easily applied for routine analysis. Once the mixed standards are scanned, very little time is required for the analysis as it would only require determination of absorbances of the sample solution at selected wavelengths, and concentration is given by the microprocessor of the instrument without employing any manual calculations.

In the second method, the calculations have been minimized by taking one of the measurements at isosbestic wavelength, that is, at 209.60 nm, as ratio is fixed for a specific mixture, the degree of dilution of two substances by solution do not alter the value of "Q_o" within the limit of accurate absorptiometric measurements. The value of the standard deviation was satisfactorily low indicating the reproducibility and accuracy of the proposed methods.

PLS Method

The PLS calibration method is performed by composing both concentration and absorbance matrices into latent variables, $A = TP^T + E$ and $C = UQ^T + F$. Vector b is given as $b = W (P^TW)^{-1}Q$, where W represents a weight

matrix. The next step is to use the linear regression C = a + bA, where constant "a" has the form $a = C_{mean} - A^{T}_{mean} \times b$. The ability of the PLS calibration of resolving overlapped spectra was examined by selecting calibration and prediction sets. Sixteen binary mixtures were selected as the calibration set for model construction. For evaluation of the constructed model, a prediction set with eight samples was selected randomly. Composition of calibration and prediction standards is summarized in Table 2. A total of 16 data points were recorded between 205 and 220 nm. The number of latent variables (factors) was determined by the cross-validation method. The prediction error was calculated in the prediction set. This error was expressed as the prediction residual error sum of squares (PRESS). PRESS was calculated for the first latent variable, which was built by the PLS modeling in the calibration set. Then, another factor was added and PRESS was calculated again. Calculations were repeated and the corresponding PRESS values were estimated. The optimum number of latent variables was nine since it gave the minimum PRESS value for both drugs.

Validation of the Proposed PLS Method

In case of chemometric calibrations, the ability of the calibration model is defined in various ways. The most general expression is the standard error of prediction (SEP) and standard error of calibration (SEC) given as:

$$\text{SEC or SEP} = \sqrt{\frac{\sum_{i=0}^{n}\left(\hat{C}|Ci^{2}\right)}{n}}$$ [

Where $\hat{C}$ denotes the added drug concentration, C_i is the predicted drug concentration and *n* represents the total number of synthetic mixtures. SEC and SEP values were calculated and are represented in Table 3.

Table 2: Different concentrations in calibration and prediction sets (Method 3)

Calibration set (µg/ml)		Prediction set (µg/ml)	
AZI (µg/ml)	**LFI (µg/ml)**	**AZI (µg/ml)**	**LFI (µg/ml)**
2	6	2	2
2	6	4	2
2	8	6	4
2	10	8	2
4	4	8	4
4	6	8	8
4	8	10	6
4	10	10	8
6	2		
6	4		
6	8		
6	10		
8	6		
8	10		
10	2		
10	4		

AZI: Azithromycin, LFI: Levofloxacin

Table 3: Statistical parameters of the PLS method

Step	Parameter	AZI	LFI
Calibration	SEC	0.4061	0.5547
	Slope	1.003	0.999
	Intercept	0.024	0.049
	R	0.999	1.003
Prediction	SEP	1.4806	0.2094
	Slope	0.990	1.003
	Intercept	0.108	0.009
	R	0.997	0.996

R: Correlation coefficient, SEP: Standard error of prediction, SEC: Standard error of calibration, AZI: Azithromycin, LFI: Levofloxacin

Recovery and Precision Studies

Accuracy and precision for the analysis of AZI and LFI in the proposed synthetic mixtures at three different concentrations were tested in intraday and interday experiments. Good accuracy and precision were obtained in all the proposed methods [Table 4]. The standard addition method was used to observe the selectivity of the proposed methods. Appropriate volumes of standard stock solutions of AZI and LFI at three different concentrations were added to the analyzed tablet solutions and reanalyzed by the proposed methods. The procedure was repeated six times for each concentration level. The recovery results at three concentration levels were calculated and are given in Table 5.

Analysis of Commercial Tablets

Results obtained by the application of the proposed methods to the analysis of AZI and LFI in the formulation are summarized in Table 4 and Table 6.

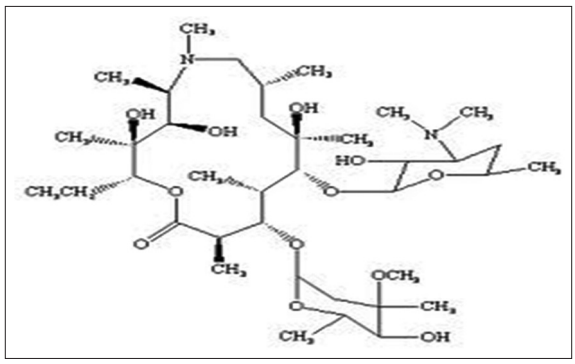

Figure 1: Structure of Azithromycin

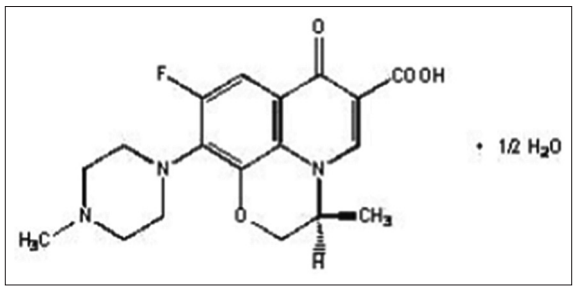

Figure 2: Structure of Levofloxacin

Table 4: Results of evaluation of precision

Drug	Amount present in µg/ml	Method 1		Method 2		Amount present in µg/ml	Method 3	
		A	B	A	B		A	B
AZI	40	1.21	1.32	1.11	1.12	6	1.02	0.97
		0.98	1.01	0.96	0.95		0.95	0.96
		0.97	1.12	1.04	1.02		0.86	0.88
LFI	40	1.01	1.11	0.93	0.94	6	1.21	0.93
		0.92	0.93	0.92	0.04		1.22	0.94
		0.97	0.94	1.03	1.04		1.23	1.02

A: Intraday precision, (CV %, *n*=3), B: Interday precision, (CV %, *n*=3), AZI: Azithromycin, LFI: Levofloxacin

Table 5: Results of recovery studies

Brand name	Drug	Recovery level (%)	Initial Amount (µg/ml)	Amount added (µg/ml)	Recovery (%)	CV (%)
X	AZI	80	32	0	99.23	1.02
		100	40	20	98.45	0.95
		120	48	40	101.33	0.96
	LFI	80	32	0	98.75	1.06
		100	40	20	98.55	0.93
		120	48	40	100.87	0.87
Y	AZI	80	32	0	97.86	0.93
		100	40	20	98.56	0.95
		120	48	40	100.67	0.96
	LFI	80	32	0	100.54	0.94
		100	40	20	99.74	1.03
		120	48	40	99.65	1.02

X: Label claim – 250 mg each, Y: Label claim – 500 mg each, AZI: Azithromycin, LFI: Levofloxacin

Table 6: Results from assay of AZI and LFI in tablets by proposed methods

Method	Label claim (mg)		Amount found (mg/tab±SD, *n*=3)		Percentage of label claim (±SD)	
	AZI	LFI	AZI	LFI	AZI	LFI
I	250	250	249.6±0.55	247.5±0.76	99.84±0.77	99.01±0.67
II			248.2±0.71	251.2±0.81	99.28±1.03	100.48±0.97
III			249.42±0.66	250.42±0.72	99.76±0.65	100.16±0.73
Method	**Label claim (mg)**		**Amount found (mg/tab±SD, *n*=3)**		**Percentage of label claim (±SD)**	
	AZI	**LFI**	**AZI**	**LFI**	**AZI**	**LFI**
I	500	500	499.1±0.52	498.3±0.72	99.82±0.56	99.66±0.43
II			500.8±0.82	501.3±1.02	100.16±0.76	100.26±0.94
III			493.8±0.72	501.8±1.07	98.76±0.66	100.36±1.07

AZI: Azithromycin, LFI: Levofloxacin, SD: Standard deviation

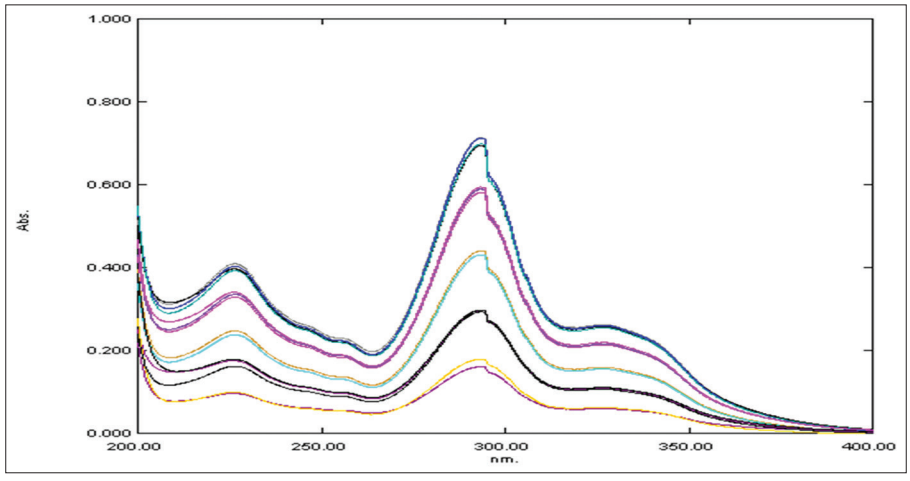

Figure 3: UV spectra of AZI and LEVO at different concentrations

CONCLUSION

Three simple, rapid, accurate, and precise methods were developed for simultaneous estimation of AZI and LFI. The methods were validated and demonstrated a wide range of linearity with good accuracy, precision, and specificity. The proposed methods have simple and rapid procedure and hence can be used for routine quality control analysis.

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