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Research Article

Validated Simultaneous Derivative Spectrophotometric Estimation of Azithromycin, Fluconazole and Secnidazole in Bulk and Pharmaceutical Formulation

M.A. Raskar*[1], P.A. Kate[1], S.S. Mungse[1], G. R. Godge[2]

[1] *Department of Pharmaceutical Chemistry, Dr. Vithalrao Vikhe Patil Foundation's College of Pharmacy, Vilad Ghat, Ahmednagar*

[2] *Department of Pharmaceutics, Dr. Vithalrao Vikhe Patil Foundation's College of Pharmacy, Vilad Ghat, Ahmednagar*

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*Address for Correspondence:

Prof. M.A. Raskar, Department of Pharmaceutical Chemistry, Dr. Vithalrao Vikhe Patil Foundation's College of Pharmacy, Vilad Ghat, Ahmednagar, Maharashtra-414111.

Abstract

Two simple, sensitive, accurate and precise spectrophotometric methods were developed and validated for the quantitative determination of Azithromycin (AZI), Fluconazole (FLU) and Secnidazole (SEC) in bulk and tablet dosage form. Method A is based on the first-order derivative (D1) and Method B is based on the second-order derivative (D2) spectrophotometric method. In Method A, absorbance was measured at 215nm, 275nm and 333nm being the zero crossing points for AZI, FLU and SEC respectively. In Method B, absorbance was measured at 220nm, 225nm and 211nm being the zero crossing points for AZI, FLU and SEC respectively. For Method A, all three drugs obey Beer's law in the concentration range 5-30µg/ml for AZI, 5-60 µg/ml for FLU and 5-40 µg/ml for SEC with correlation coefficients 0.995, 0.998 and 0.998 respectively. For Method B, all three drugs obey Beer's law in the concentration range 5-35 µg/ml for AZI, 5-40 µg/ml for FLU and 5-40 µg/ml for SEC with correlation coefficients 0.995, 0.999 and 0.998 respectively. Both methods can be used for routine analysis of these three drugs in their pharmaceutical dosage form. Results for analysis of both methods were tested and validated for various parameters according to ICH guidelines.

Keywords: Derivative, Azithromycin, Fluconazole, Secnidazole

INTRODUCTION

Azithromycin (AZI) chemically is (2R,3S,4R,5R,8R,10R,11R, 12S,13R,14R)-13-[2,6-dideoxy-3-C-methyl-3-O-methyl-α-L-ribohexopyranosyl)oxy]-2-ethyl-3,4,10-trihydroxy-3,5,6,8,10, 12,14-heptamethyl-11-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylohexopyranosyl]oxy]-1-oxa-6-azacyclopentadecan-15-one monohydrate or dehydrate. It is a semisynthetic macrolide antibiotic used to treat certain bacterial infections, such as bronchitis and pneumonia, and infections of the ears, lungs, skin, and throat. It is official in Indian Pharmacopoeia[1]. The most commonly used techniques for determining AZI in pharmaceutical dosage forms are UV-Visible spectrophotometric[2-5], RP-HPLC[6-8], HPTLC[9-10], LC-MS[11-13], microbiological[14], differential pulse voltametric[15-17], amperometric[18], IR spectroscopy [19], diffuse reflectance near-infrared spectroscopy[20] and UPLC[21].

Fluconazole (FLU) chemically is 2-(2,4-Diflurophenyl)-1,3-bis (1H-1,2,4-triazo-1-yl)propan-2-ol. It belongs to the antifungal triazole class. It is widely used to treat fungal infections caused by cryptococci, candida and coccidia, and it is official in Indian Pharmacopoeia[1]. The most commonly used techniques for determining FLU in pharmaceutical dosage forms and biological fluid are the UV-Spectrophotometric[22-23], HPLC[24-27], UPLC[28] and LC-MS[29-30].

Secnidazole (SEC) chemically is (RS) - 1-(2-methyl-5-nitroimidazole-1-yl) propan-2-ol. It is used to treat protozoal infections and anaerobic bacterial infections. It is official in Indian Pharmacopoeia[1]. The most commonly used techniques for determining SEC in pharmaceutical dosage forms are UV spectrophotometric[31-32], HPLC[33-35], supercritical fluid chromatography[36] and electrochemical method[37]. Other method has been performed to determine SEC in biological fluids, such as polarography[38]

As per our knowledge, no derivative UV spectrophotometric method has been reported for simultaneous estimation of Azithromycin, Fluconazole and Secnidazole in tablet formulation. Hence we have developed two derivative spectrophotometric methods for simultaneous estimation of these three drugs from bulk and pharmaceutical formulation.

MATERIALS AND METHODS

Chemicals and Reagents

AZI, FLU and SEC were purchased from Balaji Drugs, Surat(Gujarat). The tablet dosage form of FLU, AZI and SEC

Combikit, Hetero Healthcare Limited, Assam, India (Label Claim: 150mg FLU, 1gm AZI and 1gm SEC) was procured from the local market. AR grade methanol was used throughout the analysis.

Instrument

A double-beam UV-Visible Spectrophotometer (Jasco, Model V-630) was employed with a pair of 1cm quartz cells for all analytical work.

Selection of Common Solvent

For all three drugs, methanol was used as a common solvent for developing spectral characteristics by assessing the solubility in various solvents.

Preparation of Standard Stock Solution

The standard stock solutions of AZI, FLU and SEC were prepared separately by dissolving 10mg of each drug in 40ml of methanol. The final volume was adjusted with methanol to get a solution containing 100 µg/ml of each drug. For the selection of analytical wavelength, a standard solution of 20 µg/ml of each AZI, FLU and SEC was prepared separately by appropriate dilution of standard stock solution with methanol and scanned in the entire UV range of 200-400nm. The spectral data were processed to obtain each drug's first-order derivative spectrum, and the above process was repeated for the second-order derivative method.[39-41]

Derivative Spectrophotometric Method

Method 1: First-Order Derivative Method

Each pure drug's first-order derivative (D1) overlain spectra showed zero crossing points (ZCP). They assisted in their simultaneous estimation, as shown in **Fig.1**. The first-order derivative wavelength considered for AZI was 215nm, at which FLU and SEC show zero absorbance. Similarly, the estimation of FLU and SEC was carried out at 275 and 333nm, at which the other two drugs show zero absorbance. Calibration Curves were plotted between absorbance observed at D1 for three drugs at selected wavelengths against the concentration in the ranges of 5-30, 5-60 and 5-40 µg/ml for AZI, FLU and SEC respectively.[42-45]

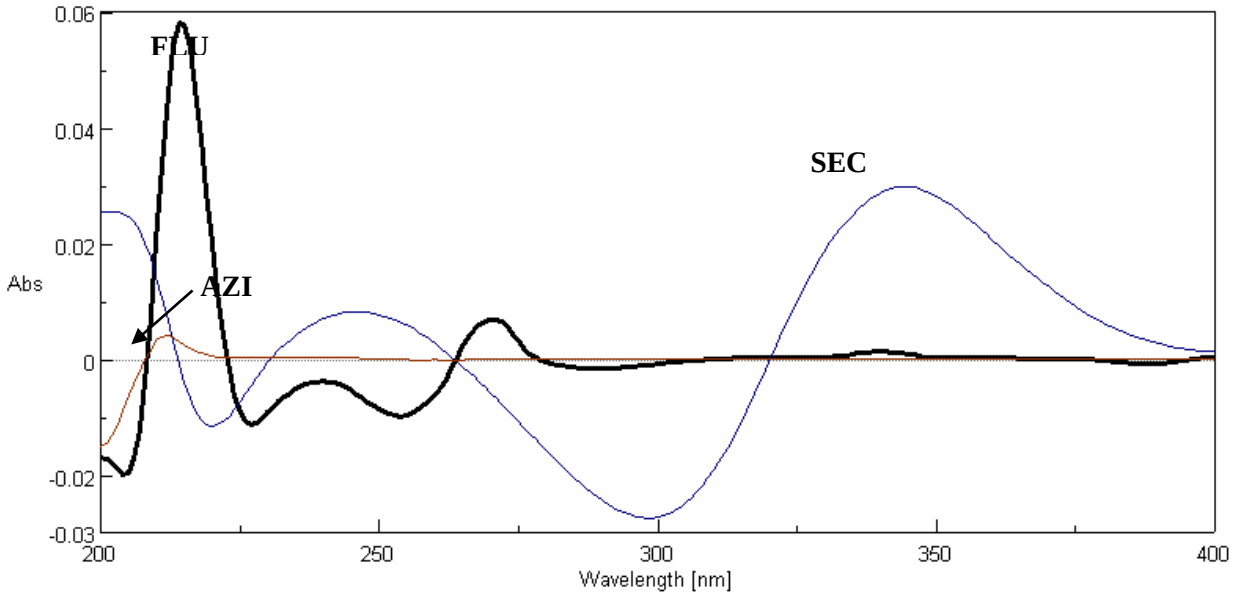

Figure 1: First-order derivative overlain spectra of AZI, FLU and SEC

Method 2 – Second Order Derivative Method

The second order derivative (D2) overlain spectra of each pure drug was found to show Zero Crossing Point (ZCP) and assisted in their simultaneous estimation, as shown in **Fig.2**. The second derivative wavelength considered for AZI was 220nm at which FLU and SEC show zero absorbance. Similarly, the estimation of FLU and SEC was carried out at 225 and 211nm, at which the other two drugs show zero absorbance. Calibration Curves were plotted between absorbance observed at D2 for three drugs at selected wavelengths against the concentration in the ranges of 5-35, 5-40 and 5-40 µg/ml for AZI, FLU and SEC respectively.[46]

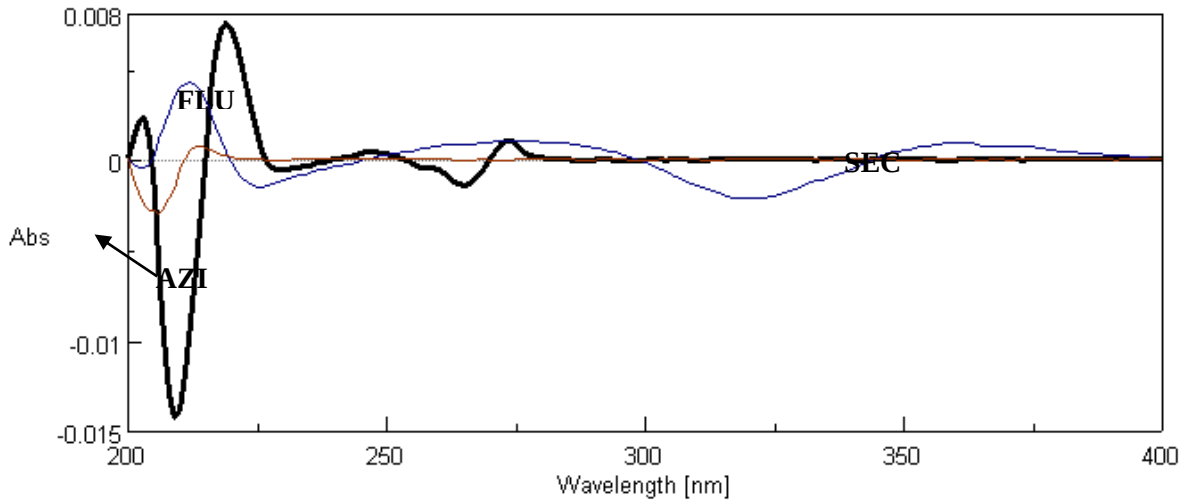

Figure 2: Second-order derivative overlain spectra of AZI, FLU and SEC

Analysis of Tablet Formulation

Twenty tablets of each AZI, FLU and SEC (FAS-Kit) were weighed, and the average weight of each tablet was determined individually. The tablets of each drug were crushed into a fine powder, accurately weighed tablet powder equivalent to 1000mg (14.4 mg) of AZI, 150 mg (24.2 mg) of FLU and 1000 mg (14 mg) of SEC respectively and dissolved in methanol, sonicated for 10 min and diluted to 100ml with methanol and transferred into three individual 100ml volumetric flasks. The tablet solution of each drug was filtered through Whatman filter paper (no.41). After appropriate dilution, the absorbance of sample solutions was recorded at corresponding wavelengths and the results were recorded as shown in **Table no.1**.[47, 48]

Table 1: Result of Tablet Analysis

Parameters	Method 1			Method 2		
	AZI	FLU	SEC	AZI	FLU	SEC
%Drug Content	99.23	97.65	98.15	99.95	98.52	97.4
SD*	0.0072	0.0023	0.0025	0.0036	0.0012	0.00113
%RSD	0.099	0.12	1.11	0.04	0.26	0.19

*Mean of three determinations

Validation

The methods were validated according to International Conference on Harmonization (ICH) Q2B guidelines for validation of analytical procedures to determine linearity, precision and accuracy of each analyte. Both precision and accuracy were determined with standard samples prepared in triplicates at different concentration levels covering the entire linearity range[39].

RESULTS AND DISCUSSION[39-47]

Linearity

The linearity for the first-order derivative method was determined from 5-30, 5-60 and 5-40 µg/ml for AZI, FLU and SEC respectively. For the second-order derivative method, linearity ranges from 5-35, 5-40 and 5-40 µg/ml for AZI, FLU and SEC respectively.

Precision

Precision was determined by studying repeatability and intermediate precision. The experiment was repeated three times a day for intra-day and on three different days for inter-day precision. The results of the precision study are presented in **Table No. 2**. In both methods, SD in the intra- and inter-day precision study was not more than 2.0%, indicating excellent repeatability and intermediate precision.

Table 2: Optical Characteristics and Validation Parameters

Parameters	AZI		FLU		SEC	
	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2
Working wavelength(nm)	215 nm	220 nm	275 nm	225 nm	333 nm	211 nm
Beer-Lambert's Law range(µg/ml)	5-30	5-35	5-60	5-40	5-40	5-40
Precision* Interday precision (SD)	0.22	0.29	0.47	0.24	0.05	0.36
Intraday precision (SD)	0.03	0.25	0.20	0.4	0.03	0.32
LOD(µg/ml) *	0.16	0.21	0.39	0.16	0.03	0.25
LOQ(µg/ml) *	0.49	0.65	1.2	0.50	0.11	0.76
Regression Values						
Slope*	0.031	0.023	0.005	0.005	0.0448	0.031
Intercept*	0.035	0.037	0.009	0.002	0.0091	0.028
Regression Coefficient(R^2)	0.995	0.995	0.998	0.999	0.998	0.998

*Mean of three determinations

Accuracy

Recovery studies by standard addition method assessed the validity and reliability of the proposed methods. The results are shown in **Table No.3**. The SD for mean of recovery (%) values was found to be < 2.0 for both methods.

Table 3: Results of Recovery Studies

Drug	Recovery Level	% Recovery ± SD*	
		Method 1	Method 2
AZI	50%	99.8± 0.10	97.2 ± 0.15
FLU		99.2 ± 0.18	98 ± 0.67
SEC		99 ± 0.23	96.13± 0.91
AZI	100%	99± 0.099	99.95± 0.04
FLU		97.65± 0.12	98± 0.26
SEC		98.15±1.11	97.4± 0.19
AZI	150%	98.32±0.074	99.68 ± 0.07
FLU		100± 0.06	98.4±0.46
SEC		98.96 ± 0.27	97.32± 0.15

*Mean of three determinations

CONCLUSION

The proposed UV spectrophotometric derivative methods for estimation of AZI, FLU and SEC were found to be simple, accurate and precise. The results obtained were found to be within the acceptable limit. The developed methods are applicable for estimating AZI, FLU and SEC in pure and tablet dosage forms. The good validation criteria of the proposed methods allow their use in quality control laboratories.

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CONFLICT OF INTEREST

There is no conflict of interest involved by the authors.

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