JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ITRACONAZOLE AND SECNIDAZOLE IN BULK AND PHARMACEUTICAL FORMULATIONS

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Abstract: A simple, accurate and precise Ratio spectra derivative approach was devised and validated to simultaneously assess Itraconazole (ITZ) and Secnidazole (SEC). The ratio derivative spectroscopic approach that has been devised comprises measuring the amplitude of the ratio spectra at 262 nm for ITZ and 311 nm for SEC. The developed method used methanol as a solvent. The Beer's law complied in the concentration range of 3–15 µg/ml and 4–20 µg/ml for ITZ and SEC respectively. The proposed approach revealed that the percentage assay fell between 99.33 and 101.66 percent for ITZ and 99.5 and 101.25 percent for SEC. The results of analysis had been statistically evaluated in compliance with ICH recommendations. Recovery studies have verified the developed procedure's accuracy as well as reproducibility. ITZ and SEC were discovered to have LOD numbers of 0.01672 µg/ml and 0.01303 g/ml. ITZ and SEC were found to have LOQ numbers of 0.05067 µg/ml and 0.03949 µg/ml respectively.

Index Terms **- Itraconazole, Secnidazole, Ratio spectra derivative, Validation, UV spectrophotometry.**

I. INTRODUCTION

Itraconazole (Fig 1) is an antifungal drug, used to treat fungal infections of the toe and nails. Itraconazole oral solution is used to treat yeast infections of the mouth and throat or the oesophagus. Itraconazole is in a class of antifungals called triazoles. Chemically it is 2-butan-2-yl-4-[4-[4-[4-[[(2*R*,4*S*)-2-(2,4-dichlorophenyl) -2- (1,2,4-triazol-1-ylmethyl) - 1,3-dioxolan-4-yl] methoxy] phenyl]piperazin-1-yl]phenyl]-1,2,4-triazol-3-one. Itraconazole interacts with 14- α demethylase, a cytochrome p-450 enzyme necessary to convert lanosterol is an essential component of the fungal cell membrane, inhibition of its synthesis results in increased cellular permeability causing leakage of cellular contents[1].

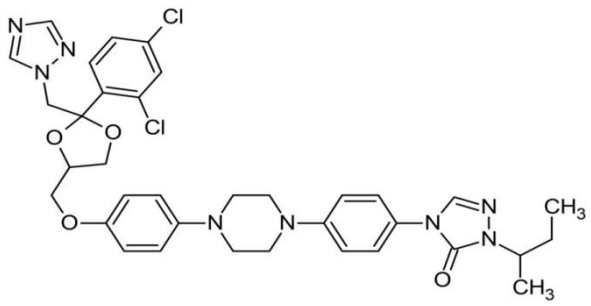

Fig. 1: Chemical structure of ITZ

Secnidazole (Fig 2) is a second-generation 5-nitroimidazole antimicrobial agent that is structurally related to other 5-nitroimidazoles. Chemically, Secnidazole is 1-(2-methyl-5-nitroimidazol-1-yl) propan-2-ol Secnidazole is used to treat intestinal

amoebiasis, fiardiasis, trichomoniasis and bacterial vaginosis. After entering into the microorganism by diffusion, its nitro group is reduced to intermediate compound which cause cytotoxicity, by damaging DNA[5].

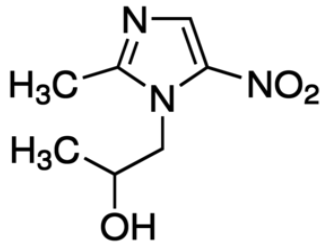

Fig. 2: Chemical structure of SEC

On literature survey, it has been discovered that ITZ and SEC have been assessed separately as well as simultaneously in combination with other drugs. It was found that a chemometric analysis has been employed for their simultaneous estimation in combined dosage form[6]. However, no other methods have been developed and neither any method is available in pharmacopoeia. In the view of the need for a suitable method for routine analysis in combined dosage form, attempts have been made to develop simple, precise and accurate analytical method for the simultaneous estimation of titled drugs.

II. EXPERIMENTAL

2.1 Reagents and Chemicals:

ITZ was procured as a gift sample from Anvik Biotech in Sonipat, Haryana, whereas SEC pure drug was obtained from Lincoln Pharmaceuticals in Gujarat, India. The combined formulation was purchased from a local market. All chemicals and reagents used were of analytical grade.

2.2 Instrumentation:

The developed method was performed on Shimadzu model 1800 double beam UV-Visible Spectrophotometer having a pair of quartz cuvettes with 1 cm path length.

III. MATERIALS AND METHODS

3.1 Preparation of standard stock solution ITZ as well as SEC:

100 mg of ITZ and 100 mg of SEC pure drugs were accurately weighed and transferred into separate 100 ml volumetric flasks and was dissolved and diluted up to the mark with methanol to get a concentration of 1000 µg/ml. From these stock solutions further dilutions were made to obtain 3, 6, 9, 12, 15 µg/ml and 4, 8, 12, 16, 20 µg/ml of ITZ and SEC respectively.

3.2 Preparation of sample stock solution:

20 tablets were taken and the average weight of one tablet was found out. All the tablets were finely powdered and the amount equivalent to 100 mg of ITZ was taken and transferred to 100 ml volumetric flask (which also contains SEC). Dissolved it in methanol and the volume was made up to 100 ml with methanol (1000 µg/ml). From this stock solution further dilutions were prepared to bring the drugs in their working concentration range.

IV. RATIO ORDER DEIVATIVE

This approach involves dividing the spectrum of mixture by the standardized spectra of each analyte and deriving the ratio of the obtained spectrum to the 1st order to obtain spectrum that is independent of the concentration of analyte used as a divisor[7].

Here, the absorption spectrum of ITZ (3-15 µg/ml) and SEC (4-20 µg/ml) were divided by SEC 8 µg/ml and ITZ 12 µg/ml as a devisor respectively. The ratio spectrum thus obtained were derivatized to first order derivative by taking delta lambda 5 and scaling factor 10 in order to remove interference of absorbing species. From the examination of ratio derivative spectrum of ITZ and SEC, 262 nm (λ_1) and 311 nm (λ_2) were selected as working wavelengths respectively. From the examination of ratio derivative spectrum of ITZ and SEC, 262 nm (λ_1) and 311 nm (λ_2) were selected as working wavelengths respectively.

V. VALIDATION PARAMETERS

The developed approach was validated for the following parameters in compliance with ICH recommendations[8].

5.1 Linearity:

The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample. Five different concentrations were used to evaluate the method's linearity; the ranges for ITZ and SEC were 3–15 µg/ml and 4–20 µg/ml respectively. The approach complies with Beer Lambert's law at a concentration range of 3–15 µg/ml and 4–20 µg/ml of ITZ and SEC respectively. The overlain spectrum and calibration curves for of ITZ and SEC along with the mixture are displayed in Fig. 3-8. The result of calibration curve for ITZ and SEC is furnished in Table No. 1.

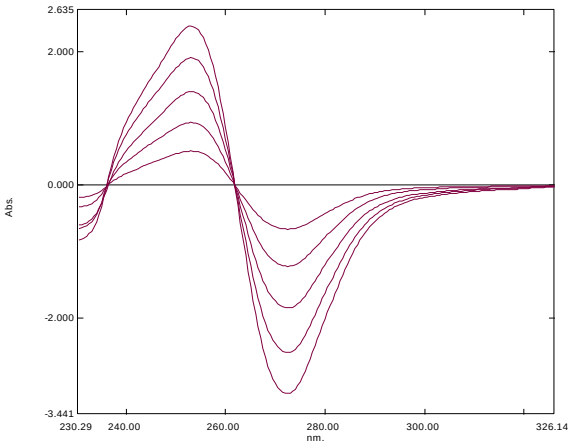

Fig. 3: Ratio derivative spectrum of ITZ at 262 nm using ITZ 12 µg/ml as a divisor

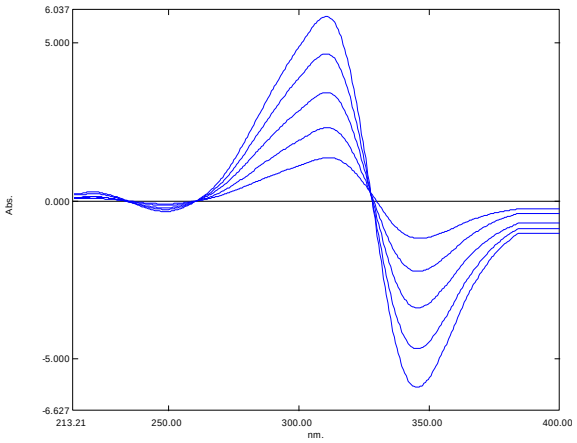

Fig. 4: Ratio derivative spectrum of SEC at 311 nm using SEC 8 µg/ml as a divisor

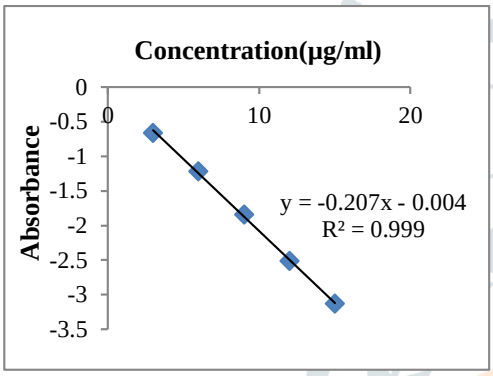

Fig. 5: Calibration curve of ITZ at 262 nm by Ratio spectra derivative method

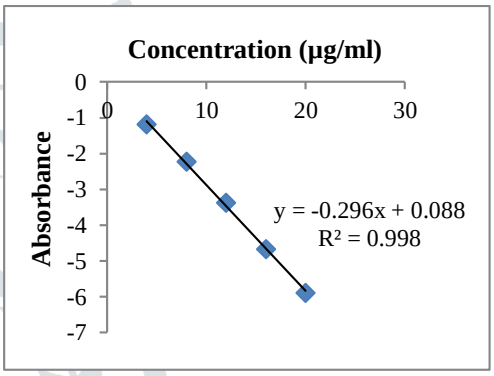

Fig. 6: Calibration curve of SEC at 311 nm by Ratio spectra derivative method

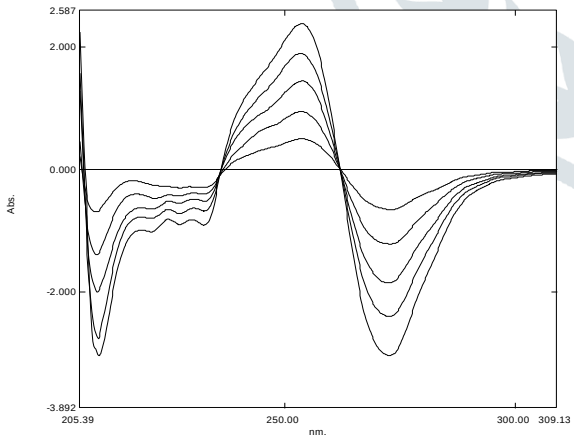

Fig. 7: Ratio derivative spectrum of mixture by using SEC 8µg/ml as a divisor

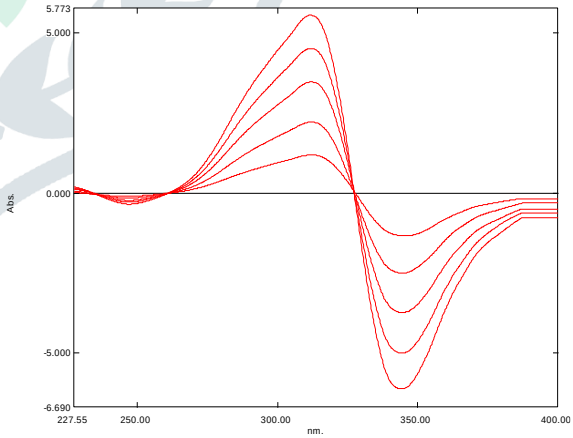

Fig. 8: Ratio derivative spectrum of mixture by using ITZ 12µg/ml as a divisor

Table No.1: Result of calibration curve for ITZ and SEC at 262 nm and 311 nm respectively

SI NO	Concentration (µg/ml)		Absorbance mean ± Standard deviation(n = 6)		%CV	
	ITZ	SEC	ITZ	SEC	ITZ	SEC
1	3	4	-0.660±0.00104	-1.180±0.00116	0.15879	0.09906
2	6	8	-1.218±0.00089	-2.228±0.00103	0.07343	0.04634
3	9	12	-1.845±0.00116	-3.371±0.00104	0.06336	0.03111
4	12	16	-2.508±0.00104	-4.671±0.00075	0.04181	0.01611
5	15	20	-3.131±0.00089	-5.88±0.00089	0.02857	0.0152

5.2 Accuracy:

The accuracy of an analytical procedure is the closeness of agreement between a conventional true value and the value found. Recovery studies were carried out by adding 80%, 100%, 120% of the standard drug solution of ITZ and SEC to the known amount of sample solution by standard addition method. The statistical validation data for accuracy determination and assay of formulation has been furnished in Table No. 2 and 3 respectively.

Table No.2: Statistical validation data for accuracy determination

Level of % Recovery	Mean*		Standard Deviation*		Co-efficient Variation*		Standard Error*	
	ITZ	SEC	ITZ	SEC	ITZ	SEC	ITZ	SEC
80%	99.72	99.90	0.30171	0.0450	0.00303	0.00045	0.1744	0.02607
100%	99.90	99.97	0.16802	0.0665	0.00168	0.00067	0.09713	0.03849
120%	99.81	99.97	0.26210	0.0556	0.00263	0.00056	0.15151	0.03218

*n=3

Table No.3: Statistical validation data for assay of formulation

Components	Mean*	Standard Deviation*	Co-efficient of Variation*	Standard Error*
ITZ	100.21	0.86006	0.00858	0.35105
SEC	99.98	0.14601	0.00146	0.05984

*n=6

5.3 Precision:

The precision of an analytical procedure is the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.
The intra-day precision was carried out by analyzing ITZ 15 µg/ml and SEC 20 µg/ml at 3 different time points on the same day. The inter-day precision was carried out by analyzing ITZ 15 µg/ml and SEC 20 µg/ml at 3 different time points on 3 different days. The statistical validation data for both intra-day and inter-day precision has been given in the Table No. 4.

Table No. 4: Statistical validation data for intra-day and inter-day precision

Components		Mean*	Standard Deviation*	Co-efficient of Variation*	Standard Error*
ITZ	Intra-day	99.89	0.37894	0.00379	0.15467
	Inter-day	99.73	0.2488	0.2494	0.1012
SEC	Intra-day	99.98	0.2273	0.00227	0.09278
	Inter-day	99.98	0.2336	0.00234	0.0550

*n = 6

5.4 Limit of Detection and Limit of Quantification:

LOD stands for the amount of the analyte that can be detected. LOQ is the least amount of the analyte that can be quantified. The drug's limit of detection and quantification was determined using the formula below;

LOD = 3.3* SD /Slope
LOQ = 10* SD /Slope

SD = standard deviation

VI. RESULTS AND DISCUSSION

It was discovered that the suggested procedure was simple, precise and accurate. The method was validated according to ICH guidelines. The linearity was discovered in the concentration between 3-5 µg/ml and 4-20 µg/ml for ITZ and SEC respectively. The regression equation of calibration curve for ITZ was y = -0.207x - 0.004 and that for SEC was y = -0.296x + 0.088. The regression coefficient values of calibration curve for ITZ and SEC were found to be 0.999 and 0.998 respectively which demonstrates the devised approach was linear. The standard deviation numbers were acceptable, and the recovery studies were close to 100%. The accuracy of the procedure was confirmed by the percentage recoveries for ITZ and SEC which were found to be in the range of 99.72 to 99.90% and 99.90 to 99.97 respectively. The repeatability and intermediate precision findings for ITZ and SEC fell within the permissible limit, in terms of % relative standard deviation which proves that the approach is precise. ITZ and SEC were discovered to have LOD numbers of 0.01672 µg/ml and 0.01303 µg/ml while LOQ numbers of 0.05067 µg/ml and 0.03949 µg/ml respectively (Table No. 5).

Table No. 5: Statistical data of ITZ and SEC at 262 nm and 311 nm respectively

Parameter	ITZ at 262 nm	SEC at 311nm
Linear range (µg/ml)	5-15	4-20
Slope	-0.207	-0.296
Intercept	-0.004	0.088
Limit of Detection (µg/ml)	0.01672	0.01303
Limit of Quantitation (µg/ml)	0.05067	0.03949

VII. CONCLUSION

The concomitant assessment of ITZ and SEC in bulk and pharmaceutical product has been executed using a straightforward, precise and accurate ratio order derivative method. The devised approach was validated in accordance with ICH guidelines and its findings were confirmed to be within the acceptable range. The approach demonstrated high recoveries and the %RSD values were discovered to be less than 2%, confirming its accuracy and precision. Therefore, this approach can be applied for the routine quality control analysis of the stated drugs.

VIII. ACKNOWLEDGEMENT

The authors are thankful to Anvik Biotech, Haryana and Lincoln Pharmaceuticals, Gujarat, India for providing the gift samples of Itraconazole and Secnidazole. The authors are also thankful to National College of Pharmacy, Shivamogga for providing necessary facilities to carry out research work.

IX. REFERENCE

[1] Ahmed M, Sanjana N R, Shetty S A. Development and validation of UV Spectrophotometric methods for the simultaneous estimation of Itraconazole and Terbinafine hydrochloride in bulk drug and pharmaceutical formulations. Int J Pharm Ph Res 2020; Vol. 18 (3): 486-495.

[2] Ajmera S R. Development and Validation of new RP-HPLC method for the estimation of Secnidazole in pure and pharmaceutical dosage forms. Asian J Res Chem. 2018; 11(1):134-138.

[3] Sharmin T, Akter M, Hossain M S. Analytical method development and validation of Secnidazole in the tablet dosage form by RP-HPLC method. Int Curr Pharm J, March 2016, 5(4): 41-44.

[4] Farooqui N A, Smith A A, Sharma H K, Manavalan R. Analytical Method Development and Validation of Secnidazole Tablets by RP-HPLC. J Pharm Sci Res. Vol.2 (7), 2010, 412-416.

[5] https://www.1doc3.com.

[6] Ibrahim AM. Nature inspired computation and ensemble neural network to build a robust model for spectral data. Spectrochim Acta A Mol Biomol Spectrosc. 2022 Feb 15; 267(Pt 1):120480.

[7] A R Rote, P D Bari. Ratio spectra derivative and zero-crossing difference spectrophotometric determination of olmesartan medoxomil and hydrochlorothiazide in combined pharmaceutical dosage form. AAPS Pharm Sci Tech. 2009; 10(4):1200-5.

[8] P P Prabhu, P Das et al., Spectrophotometric method for the development of Guaiphenesin in pharmaceutical dosage form. Int J Pharm Chem Bio Sci 2013, 3(3), 881-886.