International Journal of Enhanced Research in Science, Technology & Engineering
ISSN: 2319-7463, Vol. 12 Issue 6, June-2023, Impact Factor: 7.957

Development and Validation of UV Spectrophotometric Method for the Simultaneous Estimation of Itraconazole and Amoxicillin in Combined Dosage form using Simultaneous Equation Method in Bulk and Ophthalmic Dosage form

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ABSTRACT

Two validated UV-Vis Spectrophotometric method for the simultaneous estimation of Itraconazole and Amoxicillin in pure powder and in two component dosage forms have been developed, utilizing Simultaneous equation method. The method employs formation and solving of simultaneous equation using 239 nm and 262 nm as two analytical wavelengths (λ_{max} of the drugs) of detection. Both the drugs obeyed Beer-Lambert's law over the concentration range 2-12 µg/mL for Itraconazole and 3-18 µg/mL for Amoxicillin, respectively. The developed method was validated for Accuracy, Precision, Limit of Detection and Limit of Quantification as per ICH guidelines and results of analysis were validated statistically in bulk drug and its formulation.The developed methods were economical in terms of time taken and amount of solvent consumed for analysis.

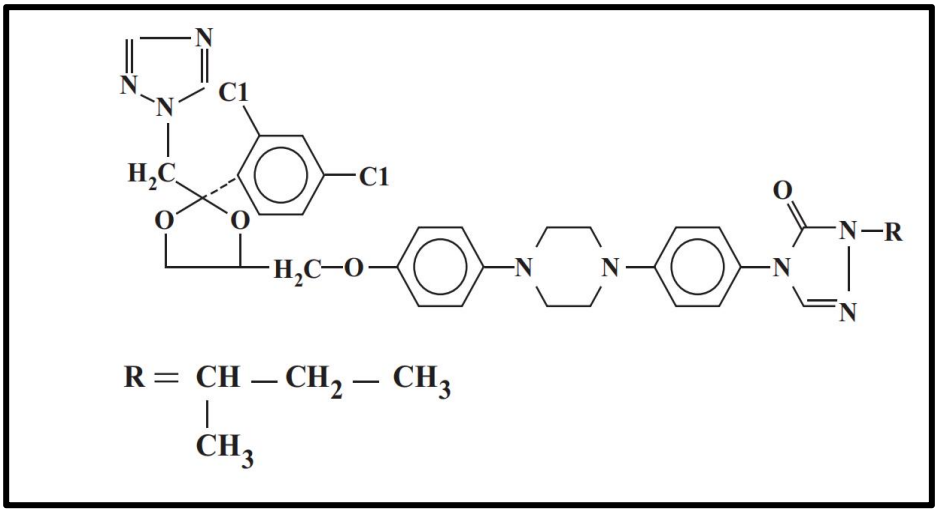
Key Words: UV-Vis Spectrophotometric; Itraconazole; Amoxicillin; Simultaneous Equation Method, Validation.

INTRODUCTION

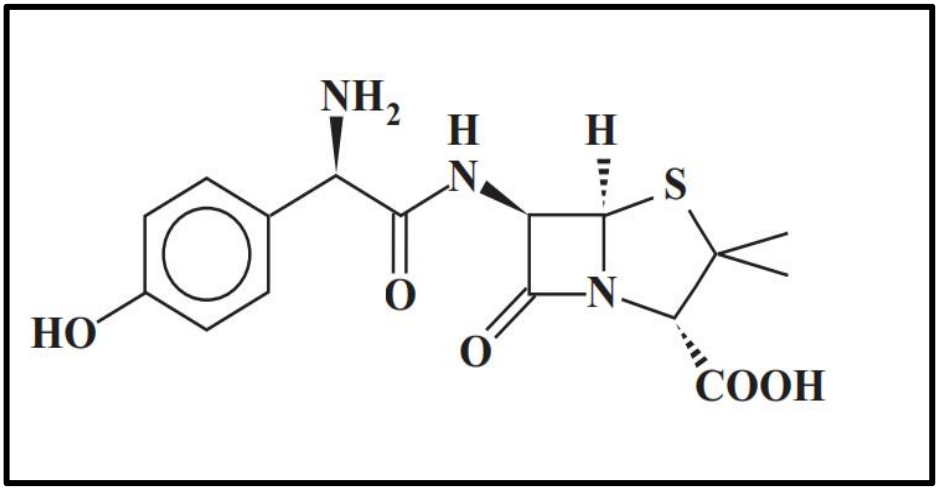
Itraconazole (Figure. 1-A), chemically is(cis-4-[4-[4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-5-methyl-2-(3-methylbutyl)-3H1,2,4-triazol-3-one). It is official in Indian Pharmacopoeia.[1]It has a molecular formula $C_{35}H_{38}Cl_2N_8O_4$ and a molecular weight is 705.64.[2-4]

Amoxicillin (Figure. 1-B), chemically is. It is also official in Indian Pharmacopoeia.[2]It has molecular formula (2S,5R,6R)-6-[(R)-2-amino-2-(4-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0.] heptane-2-carboxylic acid trihydrate.It is also official in Indian Pharmacopoeia.[1]It has a molecular formula $C_{16}H_{25}N_3O_8S$ and a molecular weight is 419.5.

Various spectrophotometric, HPLC, HPTLC and stability indicating HPLC methods are reported in the literature for the estimation of Itraconazole[5-8] and Amoxicillin trihydrate[9-15]individually and in combination with other drugs. According to literature survey no stability indicating UV spectrophotometric method has yet been reported for simultaneous estimation of Itraconazole and Amoxicillin in combination by using Dimethyl sulfoxide (DMSO) and water as solvent.



(A)



(B)

Figure 1: Chemical structure of (A) Itraconazole (B)Amoxicillin

MATERIAL & METHODS

Reagents & Instruments

A UV-VIS spectrophotometer UV-1800 Shimadzu(Shimadzu, Japan),over the range of 200-400 nm with a spectral bandwidth of 2 nm and 10 mm matched quartz cells was utilized to create an analytical procedure.Itraconazole (ITZ) and Amoxicillin (AMX) standard materials were obtained as gift sample from Glenmark Pharmaceuticals Ltd. and Aurobindo,Telangana(India) respectively. All chemicals were of analytical reagent grade and solutions were prepared with water AR grade.

Preparation of Standard stock solution of Itraconazole and Amoxicillin

Itraconazole(ITZ) and Amoxicillin(AMX) 50 mg each were accurately weighed and dissolved separately in DMSO. Shake and sonicate it for 20 minutes. Adjust the final volume to 50 ml with DMSO to get a concentration of 1000 µg/ml. 10 ml of each from the above prepared solutions were further separately diluted to 100 ml to get a concentration of 100 µg/ml of each ITZ and AMX. These were used as stock solutions.

Preparation of mixed standard solution

Accurately 100 mg of ITZ and 50 mg of AMX were weighed into 100 mL volumetric flask and DMSO was added. The solution was further sonicated for 20 minutes and then the volume was made up to 100 mL with DMSO to get a concentration of 100 µg/ml of ITZ and 50 µg/ml of AMX simultaneously.

Determination of λ_{max} of Individual Components

By appropriate dilution of standard solutions of ITZ and AMX with water, solutions containing 10 µg/mL of both drugs were scanned separately in the range of 200-400 nm against water as blank. ITZ shows λ_{max} at 262 nm and AMX at 239 nm.

2.5 Study of Beer-Lamberts Law

An aliquot portion of stock solutions of ITZ and AMX were diluted appropriately with distilled water to get a series of concentration between 2-12 µg mL^{-1} for ITZ and 3-18 µg mL^{-1} for AMX, respectively. Similarly, aliquot portions of stock solutions were mixed (Std. laboratory mixture) and diluted with distilled water to get series of concentration between 2-12 µg mL^{-1}ITZ and 3-8 µg mL^{-1}AMX. The absorbance of each solution was measured at 262 nm and 239 nm in 1 cm cell against solvent blank.The graphs were plotted as concentration vs. absorbance at selected wavelengths.

Determination of absorptivity values of drugs at selected wavelengths

Aliquot portions of ITZ and AMX stock solutions were diluted with distilled water to obtain different concentrations of each drug.The absorbance of each solution was measured at 262.00 nm and 239.00 nm. A (1%, 1cm) values were calculated using the following formula;

$$\textbf{Absorptivity} = \frac{\textbf{Absorbance}}{\textbf{Concentration (g/ lit)}}$$

METHODOLOGY

Analysis of laboratory mixture by the proposed method:

In order to see the feasibility of the proposed method for simultaneous estimation of ITZ and AMX in pharmaceutical formulation, the aliquot portions of stock solutions were mixed (Std. laboratory mixture) and diluted with distilled water to get a fixed concentration in the ratio 2:1. The absorbance of each solution was measured at 262.00 nm and 239.00 nm in 1cm cell against blank. The amount of each drug was determined using a simultaneous equation as follows;[16]

$$Cx = \frac{A_2ay_1 - A_1ay_2}{ax_2ay_1 - ax_1ay_2} \qquad Cy = \frac{A_1ax_2 - A_2ax_1}{ax_2ay_1 - ax_1ay_2}$$

Where,
Cx = Concentration of Itraconazole in g/100 ml.
Cy = Concentration of Amoxicillin in g/100 ml.
A1 = Abs. of the mixture at the wavelength of 1st drug.
A2 = Abs. of the mixture at the wavelength of 2nd drug.
ay1 = Absorptivity value of 2nd drug on wavelength of 1st drug.
ay2 = Absorptivity value of 2nd drug on its own wavelength.
ax1 = Absorptivity value of 1st drug on its own wavelength.
ax2 = Absorptivity value of 1st drug on wavelength of 2nd drug.

$$\textbf{\% Estimation} = \frac{C \times d}{W} \times 100$$

Where,
C = Cx or Cy = Conc. of ITZ or AMX in g/100mL.
d = Dilution factor.
W = Weight of drug either ITZ or AMX in laboratory mixture.

Analysis of ophthalmic formulation by the proposed method:

Accurately weighed quantities of ophthalmic formulation equivalent to 100 mg of ITZ and 50 mg of AMX was transferred to a 100 ml volumetric flask and dissolved in sufficient quantity of DMSO. It was sonicated for 30mins and solution was filtered through the Whatman filter. The aliquot portion of the filtrate was further diluted with water to get a final concentration of about 10µg/ml ITZ and 5 µg/ml of AMX. The absorbance of sample solution was measured at 262.00 nm and 239.00 nm in 1cm cell against blank. The content of ITZ and AMX in ophthalmic formulation was calculated using the following formula;[16]

$$\% \text{ Label claim} = \frac{\text{Cx or Cy x d x W}}{\text{Wm x L}} \times 100$$

Where,
Cx or Cy = Conc. of ITZ or AMX in g/100ml.
W = Total volume of formulation (g).
Wm = Actual weight of taken formulation (g).
L = Labeled claim of the sample taken.
d = Dilution factor.

Recovery study
Recovery study was carried out by standard addition method.

Preparation of standard solution:
An accurately weighed 50 mg of pure ITZ and AMX were dissolved in 50 ml DMSO separately and volume was made up to the mark to obtain concentration of 1 mg/ml. The stock solution was serially diluted by withdrawing 10 ml from the above solution to give concentrations of 100 µg/mL.

Preparation of sample solution:
An accurately weighed quantity of pre-analysed eye drop equivalent to 100 mg of ITZ was taken in a 100 ml volumetric flask; to it standard solutions of ITZ and AMX were added in different proportions. Then volume was adjusted up to the mark with distilled water. Solution was filtered through Whatman filter paper. The aliquot portions of the filtrate were further diluted to get final concentration. The absorbances of sample solutions were measured at 262.00 nm and 239.00 nm in 1cm cell against blank. The content of the drug was calculated using same formula as in proposed formulation. The % recovery was then calculated by using formula;

$$\% \text{ Recovery} = \left[\frac{C_{Spiked} - C_{Non\text{-}spiked}}{C_{Added}}\right] 100$$

Where,
C_{Spiked} = Concentration assessed for the spiked sample.
$C_{Non\text{-}spiked}$ = Concentration assessed for the unspiked sample.
C_{Added}= Amount of pure drug added.

VALIDATION

The described methods have been validated for the assay of both the major components of bulk drug using following ICH parameters.[17]

Linearity
Linearity was studied by preparing standard solutions at different concentration levels. Calibration curves were prepared using the standard solutions of 2-12 µg/ml for ITZ and 3-18 µg/ml for AMX and mixed solution for concentration 2-12 µg mL^{-1} and 3-8µg mL^{-1}, of ITZ and AMX respectively at selected wavelengths.Calibration curves were constructed by plotting absorbance versus concentrations and
regression equations were calculated for both the drugs.

Precision
Repeatability
The precision of the instrument was checked by repeated scanning and measurement of absorbance of mixed solutions (n = 6) of ITZ and AMX without changing the parameter of the proposed spectrophotometry method. The absorbance were measured at 262 nm and 239 nm. The standard deviation and %RSD was calculated.

Intermediate Precision
The intra-day and inter-day precision of the proposed method was determined by analyzing the corresponding responses three times on the same day and on three different days three different concentrations of standard solutions of ITZ&AMX.

Limit of Detection (LOD) and Limit of Quantification (LOQ)
The LOD and LOQ of the developed method was assessed by analyzing ten replicates of standard solutions containing concentrations 10 µg/ml for ITZ and 5 µg/ml for AMX.

The LOD may be calculated as

LOD = 3.3 × SD/ Slope

The LOQ may be calculated as

LOQ = 10 × SD/ Slope

Where, SD = Three replicates of absorbance

Slope = the mean slope of the 3 calibration curves

Robustness It is the capacity of a method to remain unaffected by small, deliberate but slight variations in method parameters. These factors includes analyst to analyst variation and instrument to instrument variation (±2).

RESULT AND DISCUSSION

Study of Beer-Lamberts Law

The absorbances were recorded skillfully and graphs were plotted between concentration vs absorbance for drugs ITZ and AMX as well as laboratory mixture for concentration 2-12 µg mL^{-1} and 3-8 µg mL^{-1}, respectively at selected wavelengths **(Table 1)**. In laboratory mixture, at 262 nm, the regression equation was found to be 0.0768x + 0.0265, and at 239 nm, equation was 0.01443 x - 0.2916. In both the cases, r^2 values are 0.999, which indicated desired linearity of the proposed method.

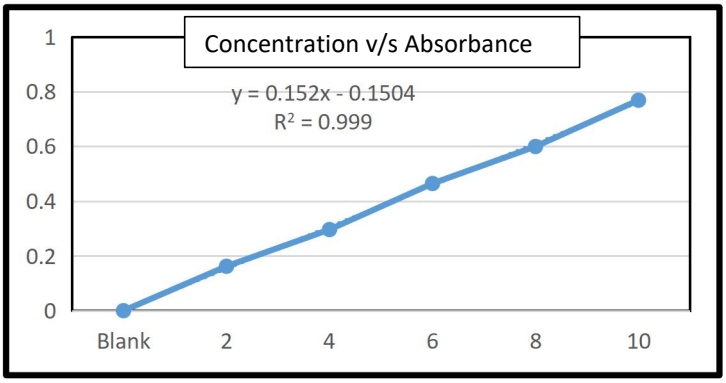


Figure 2: Calibration Curve Of ITZ

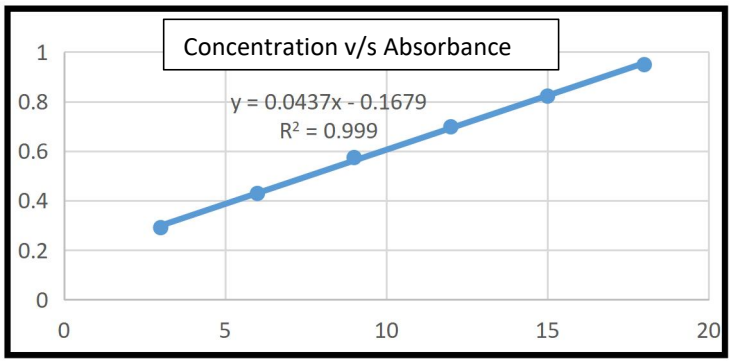


Figure 3: Calibration curve of AMX

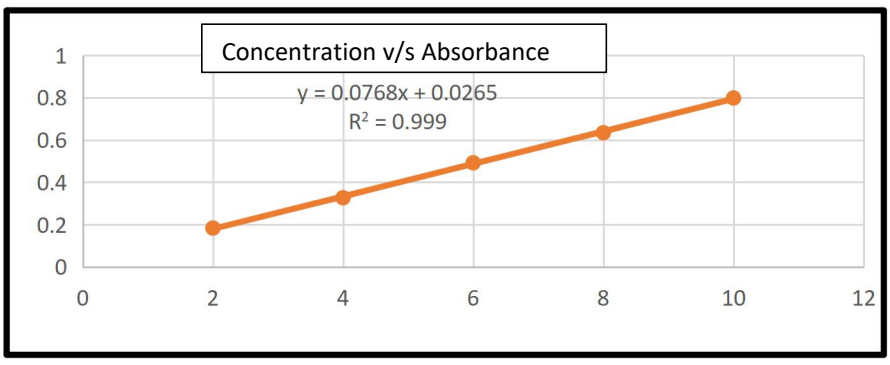


Figure 4: Plot of Beer-Lambert study for laboratory mixture of ITZ at 262 nm

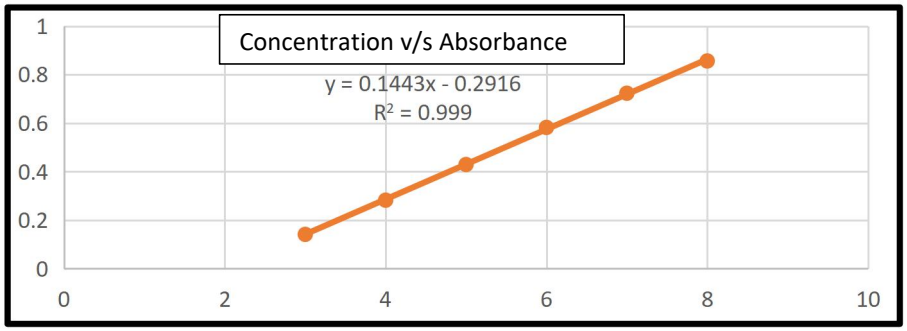


Figure 5: Plot of Beer-Lambert study for laboratory mixture of AMX at 239 nm

Table 1: Absorbance and Absorptivity value of ITZ and AMX

Concentration (µg mL^{-1})		Absorbance(nm)				Absorptivity(nm)			
		ITZ		AMX		ITZ		AMX	
		262	239	262	239	262	239	262	239
2	3	0.162	0.011	0.033	0.291	0.081	0.006	0.011	0.097
4	6	0.296	0.019	0.056	0.429	0.074	0.005	0.009	0.0715
6	9	0.464	0.028	0.069	0.574	0.077	0.005	0.005	0.0638
8	12	0.599	0.100	0.173	0.698	0.075	0.013	0.013	0.0581
10	15	0.768	0.102	0.181	0.822	0.077	0.010	0.010	0.0548
12	18	0.897	0.107	0.264	0.949	0.075	0.009	0.009	0.0527

Analysis of bulk drug by proposed method

The proposed method was found to be very sensitive for simultaneous determination of ITZ and AMX. The method successfully detected both the drugs in microgram concentrations. The drug estimation for ITZ and AMX was found to be in range of 98.50 to 99.08% and 98.30 to 98.54%, respectively. These results represented that the method is precise enough to determine both the components in bulk. The results are demonstrated in **Table 2**.

Table 2. Simultaneous estimation of ITZ and AMX in bulk

Sr. No	Amount of pure drug taken (g)		Absorbance (nm)		% Drug estimation	
	ITZ	AMX	262nm	239nm	262nm	239nm
1	0.12	0.050	0.809	0.404	98.81	98.43
2	0.11	0.051	0.810	0.405	99.08	98.54
3	0.12	0.051	0.809	0.403	98.50	98.46
4	0.13	0.052	0.809	0.401	97.89	98.50
5	0.12	0.051	0.808	0.404	98.84	98.30
			Mean		98.62	98.45
			S.D.		0.4609	0.0925
			R.S.D		0.0046	0.0009
			%R.S.D		0.4673	0.0940

***n = 5**

S.D. standard deviation; R.S.D. relative standard deviation; %R.S.D. % relative standard deviation

Analysis of ophthalmic formulation by proposed method

The proposed method was simple, rapid and precise and do not suffer from any interference due to excipients of the formulation. Various optical characteristics are shown in the **Table 3**. The % RSD was found to be 0.0725 in case of ITZ and 0.0885 for AMX, which demonstrated that the method is acceptable (limit; <2%).

Table 3. Simultaneous estimation of ITZ and AMX in ophthalmic formulation
Total volume = 10 ml, Weight taken= 4.84 g

Sr. No	Wt. Of eye drop (g)	Absorbance (nm)		% Label claim*	
		262nm	239nm	ITZ	AMX
1	0.4841	0.998	0.707	98.36	98.36
2	0.4841	0.999	0.708	98.46	98.46
3	0.4840	0.997	0.707	98.25	98.25
4	0.4840	0.998	0.708	98.35	98.35
5	0.4840	0.998	0.707	98.36	98.36
		Mean		98.36	98.40
		S.D.		0.0713	0.0871
		R.S.D		0.000725	0.00088
		%R.S.D		0.0725	0.0885

***n = 5**

S.D. standard deviation; R.S.D. relative standard deviation; %R.S.D. % relative standard deviation

Recovery Studies

Accuracy

The % recovery for this analytic method for all the three concentration levels ranged 98.05 % to 98.13 % with standard deviation of 0.01 for ITZ and 0.04 for AMX showing that any small change in the drug concentration can be accurately determined with high accuracy. Thus, values of recovery greater than 98.0% indicated that proposed method was accurate for the analysis of the drug. The recovery data for accuracy studies are given in **Table 4**.

Table 4. Results of recovery studies of ITZ and AMX

Sr. No	Level of % Recovery	Test Concentration(ppm)		Amount spiked		% Recovery*	
		ITZ	AMX	ITZ	AMX	ITZ	AMX
1	80%	6	3	4.8	2.4	98.06	98.15
2	100%	6	3	6.0	3.0	98.05	98.08
3	120%	6	3	7.2	3.6	98.04	98.16
					Mean	98.05	98.13
					S.D.	0.01	0.04
					R.S.D	0.00010	0.00044
					%R.S.D	0.0101	0.0444

***n = 6**

S.D. standard deviation; R.S.D. relative standard deviation; %R.S.D. % relative standard deviation

Table 5. Method Validation Parameters

Parameters	**Method Simultaneous equation method**	
	ITZ	AMX

Linearity	2-12 µg/ml	3-18µg/ml
Precision (%Drug estimated)		
Repeatability	98.75	98.94
Intraday	98.75	98.93
Interday	98.56	98.72
Different analyst	98.64	98.32
Correlation Coefficient(r^2)	0.999	0.999
Intercept	0.1504	0.1679
Slope	0.152	0.0437
LOD	0.48975	1.48410
LOQ	0.11271	0.34155

Robustness

The result of robustness study of the developed assay method was established. The result shows that during all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. It showed the reliability of an analysis with respect to deliberate variations in method parameters. System suitability parameters were also found satisfactory; hence the analytical method would be concluded as robust.

CONCLUSION

Proposed study describes method for the estimation of ITZ and AMX combination in mixture. The method was validated and found to be simple, sensitive, accurate and precise as per ICH guidelines .The method was successfully used for determination of drugs in ophthalmic formulation.

ACKNOWLEDGMENTS

The authors are thankful to Glenmark Pharmaceuticals Ltd. and Aurobindo, Telangana(India) for providing gift sample of Itraconazole and Amoxicillin. The authors are very thankful to Principal and Management of H.K College of Pharmacy for providing necessary facilities to carry out research work.

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