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SIMULTANEOUS ESTIMATION OF OFLOXACIN AND TINIDAZOLE IN SOLID DOSAGE FORM BY U V SPECTROPHOTOMETRY USING MIXED SOLVENCY CONCEPT

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ABSTRACT

In present research work a new simple, specific, precise, accurate, robust and economical UV-Spectrophotometric method for simultaneous estimation of ofloxacin and tinidazole in tablet dosage form using mixed solvency concept was developed and validated. In the present work 10% phenol and 20% sodium benzoate blend was used as a hydrotropic solvent to increase the solubility of poorly water soluble ofloxacin and tinidazole. The analytical wavelength for ofloxacin and tinidazole are 330nm and 318 nm respectively. The developed method was validated as per ICH guidelines in terms of linearity and range, specificity, accuracy, precision and sensitivity. The percent drug estimated in tablet formulation was 95.60±0.338 for tinidazole and 97.10±0.499 for ofloxacin by method B. and similarly it was 95.26±0.504 for tinidazole and 95.33±0.594 for ofloxacin by method A respectively. The range of percent recoveries varied from 95.63±0.349 to 97.60±0.457 for tinidazole and 96.00±0.999 to 97.55±0.133 for ofloxacin. Based on the results obtained the proposed method can be regarded a simple, precise, accurate, reliable, cost effective and eco friendly for simultaneous estimation of ofloxacin and tinidazole.

KEYWORDS: Ofloxacin, Tinidazole, UV-Spectrophotometry, solid dosage formulation, mixed solvency concept.

INTRODUCTION

Increasing the aqueous solubility of Insoluble and slightly soluble drugs has been done by various methods to avoid the usage of organic solvents. Because of toxicity, volatility, and also high cost of organic solvents, an alternative method has been developed. Mixed solvency concept is one of the methods to enhance the aqueous solubility of less water soluble drugs. Mixed solvency concept may be a proper choice to preclude the use of organic solvents. So there is a broad scope for mixed solvency concept in quantitative estimation of other less water soluble drugs. By application of this concept, innumerable solvent system can be developed. Maheshwari[1-6] is one of the opinions that each substance possesses solubilizing power. He has given several ecofriendly methods in the area of drug estimations and formulations precluding the use of toxic organic solvents. The solubility of large number of poorly soluble drugs has been enhanced by mixed solvency concept.[1-23]

The present research work also provides an ecofriendly method for simultaneous estimation of ofloxacin and tinidazole in solid dosage form by UV Spectrophotometry by using mixed solvency concept. Ofloxacin is (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1, 2, 3-de]-1,4-benzoxazine-6-carboxylic acid. Tinidazole is 1-[2- (Ethyl sulfonyl) ethyl]-2-methyl-5-nitro-1H-imidazole. Ofloxacin is Antibacterial, ocular anti-infective. Tinidazole is Antiprotozoal, antibacterial agent.

EXPERIMENTAL

Chemicals and Reagents

Pharmaceutical grade Ofloxacin and Tinidazole was a gift from Modern Laboratories Pvt. Ltd. Indore and its dosage formulation OfloxTZ was purchased from local market. All other chemicals were of analytical grade.

Instrumentation

UV Visible spectrophotometer (Model 1800, Shimadzu, Japan) with 10 –mm path length connected to a computer was used for spectrophotometric analysis.

Selection of solvent: 10% phenol and 20% sodium benzoate blend was selected as the solvent after considering the solubility and stability factor of both the

drugs as well as the interference due to excipients present in the formulation.

Preparation of stock solution: The 50 mg each ofloxacin and tinidazole were accurately weighed and transferred in 100 ml volumetric flasks separately, dissolved in 20 ml of 10% phenol and 20% sodium benzoate blend and volume was adjusted to 100 ml with distilled water to obtain solution (500µg/ml) of each drug.

Selection of appropriate wavelength:
Standard solution of appropriate concentration of ofloxacin and tinidazole were prepared separately and scanned in the range of 400 to 200 nm at a slow scan speed. The absorption maximas of ofloxacin was found at 330 nm while for tinidazole at 318 nm. The overlain spectra of ofloxacin and tinidazole were recorded as shown in fig.1.

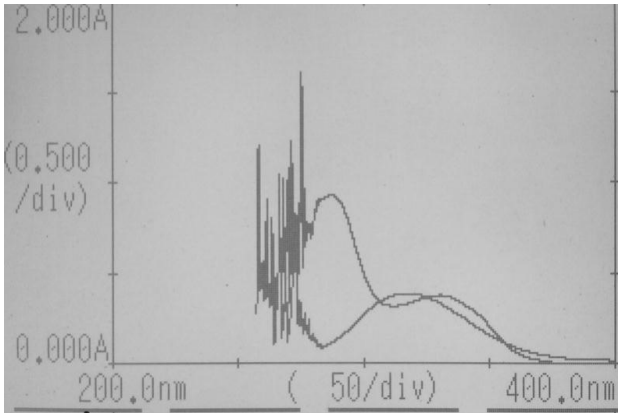

Fig. 1: Overlain spectra of ofloxacin and tinidazole with 10% phenol and 20% sodium benzoate blend.

Calibration curve
Appropriate volumes of stock solution were further diluted with distilled water to obtain final concentrations in the range of 10-50 µg/ml for ofloxacin and tinidazole separately. The absorptions of these standard solutions were noted at 318 nm, 330 nm, and 325 nm (Isobestic point) against respective reagent blanks. A calibration curve of absorbance against concentration was plotted and the regression coefficient (R^2) was determined for both the drugs separately as shown in Fig.2. The aborptivity of for both the drugs is presented in Table-I.

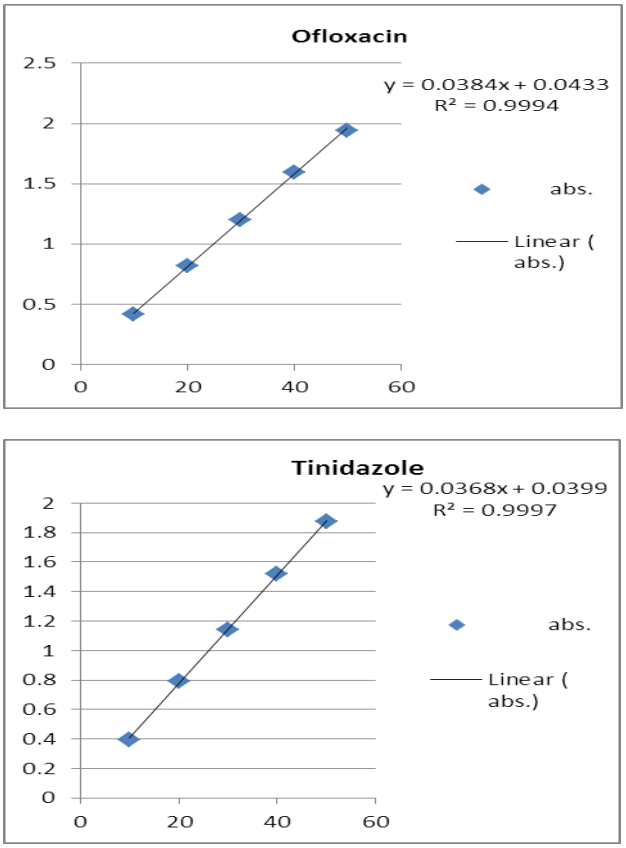

Fig. 2: calibration curves of absorbance against concentration.

Table. I0: Absorbtivity* values for ofloxacin and tinidazole.

Concentration (µg/ml)		Absorptivity at 325 nm		Absorptivity at 318 nm		Absorptivity at 330 nm	
ofloxacin	**Tinidazole**	**ofloxacin**	**Tinidazole**	**ofloxacin**	**Tinidazole**	**ofloxacin**	**Tinidazole**
10	10	365	365	374	397	415	348
20	20	388	354	374	396	408	345
30	30	355	372	367	381	401	333
40	40	372	373	367	380	400	331
50	50	388	381	356	375	389	327
mean	mean	380	369	368	386	403	330

Absorbtivity* =Absorbance/Con. In gm per 100ml.

The assay of above multicomponent system is done by A- Simultaneous equation Method: This spectrophotometric technique is employed when the two absorbing drugs in the sample X and Y absorbs at the λ max of each other provided that the following criteria are fulfilled;

The criteria are that the ratios between

$$\frac{A_2}{A_1} / \frac{ax_2}{ax_1} \text{ And } \frac{ay_2}{ay_1} / \frac{A_2}{A_1}$$

Should lie outside the range 0.1-0.2 for precise determination of Y and X respectively.

Where ax_1 and ax_2 are the absorptivities of drug X at λ_1 and λ_2, ay_1 and ay_2 are the absorptivities of drug Y at λ_1 and λ_2. A_1 and A_2 are the absorbances of the diluted sample at λ_1 andλ_2.

Based on the fact that the absorbance of the mixture is the sum of the individual absorbance's of X and Y, two simultaneous equation can be built for the system as;-

$$A_1=a_{x1}.b.Cx+a_{y1}.b.Cy \quad \text{..... 6.}$$

$$A_2=a_{x2}.b.Cx+a_{y2}.b.Cy \quad \text{..... 7.}$$

When the path length is 1cm, the equation 2 can be rearranged in terms of

$$C_y = \frac{A_2 - ax_2 C_X}{ay_2}$$

Substituting the value of Cy in equation (6), we get

$$C_X = \frac{A_2 ay_1 - A_1 ay_2}{ax_2 ay_1 - ax_1 ay_2} \quad \text{..... 8.}$$

Similar,

$$C_Y = \frac{A_1 ax_2 - A_2 ax_1}{ax_2 ay_1 - ax_1 ay_2} \quad \text{..... 9.}$$

Procedure for analysis of Tablet formulation

Twenty tablets weighed accurately. The average weight was determined and then ground to a fine powder. A quantity equivalent to 50 mg of tinidazole and 16.66mg of ofloxacin were transferred to a 100ml volumetric flask. The contents were ultra sonicated for 10 min with blend made to volume and filtered through Whatmann filter paper no.41. Six ml of the above solution diluted to 100 ml with distilled water to give concentration of 10 µg/ml of ofloxacin and 30µg/ml of tinidazole (expected) respectively. Absorbances of these solutions were measured at 318 nm (tinidazole) and 330 nm (ofloxacin) as A_1 and A_2 respectively and concentration of these two drugs in the sample were calculated using equation 6 and 7.Results of the analysis of tablet formulations are reported intable2.

Table. 2: Determination of ofloxacin and tinidazole in combined tablet dosage form.

Component	Methods	Labelled Drug(mg/tablet)	Amount obtained(mg)	%Amount Found	S.D.*	%R.S.D.*
OF	A	200	190.66	95.33	0.594	0.622
	B	200	194.20	97.10	0.499	0.514
TZ	A	600	571.56	95.26	0.504	0.529
	B	600	573.60	95.60	0.338	0.353

S.D.*=Standard deviation, n=3, R.S.D. *=Relative standard deviation

Tablet Formulation OfloxTZ, manufactured by-CIPLA LTD.

Recovery studies

To perform the recovery studies standard ofloxacin and tinidazole drugs were mixed to from a uniform mixture of both the drugs in the ratio of 1:3 respectively and added in the quantity of 53.28mg, 66.66mg and 79.92mg separately to the pre-analyzed tablet powder equivalent to 50 mg of tinidazole and 16.66mg of ofloxacin and the drug contents were determined by the proposed method. Results of analysis were reported in Table 3.

Table. 3: Statistical validation of Recovery studies.

Level of % recovery	Methods	% Recovery*		% R.S.D.*	
		OF	TZ	OF	TZ
80	A	96.00	95.81	1.041	0.148
	B	96.92	96.22	0.480	0.259
100	A	97.00	95.63	0.515	0.365
	B	97.25	96.16	0.927	0.345
120	A	96.57	96.66	0.311	1.17
	B	97.55	97.60	0.137	0.469

***Denotes average of three estimations at each level of recovery.**

METHOD VALIDATION

The developed UV-spectrophotometric method was validated as per ICH guidelines in terms of linearity, and range, specificity, precision, sensitivity and accuracy.

In order to determine linearity range of developed method a series of solutions were prepared using ofloxacin and tinidazole stock solution at concentration range of 10-50µg/ml.The absorbances of the resultant solutions were measured at 318 and 330 nm against reagent blank. The calibration curves were constructed by plotting concentration on X axis and absorbance on Y axis. R^2 value not less than0.999 was regarded as acceptance criteria (Figure 1).

Table. 4: Developed UV method specification.

Instrument and specification	UV-Spectrophotometer Shimadzu 1800
Scanning Range	200 nm to 400 nm
Solvent Used	Hydrotropic Solvent
Strength of Solvent	10% phenol and 20% sodium benzoate
Composition of Solvent	10% phenol and 20% sodium benzoate
Wavelength Maxima of ofloxacin and tinidazole	318 nm and330 nm

Specificity was performed to exclude the possibilities of interference of solvent in the region of maximum absorbance peaks of ofloxacin and tinidazole. The specificity of the method was tested under the normal conditions and results of the tests proved that the components other than ofloxacin and tinidazole did not produce the deductable peaks at the maximum absorbance peaks of the drug.

Accuracy of the developed method was determined by recovery studies at three different levels. The pre analyzed samples were spiked with 80, 100 and 120% of mixed standard solution. The mixtures were analyzed and the recoveries were determined. The recovery study was carried out in triplicate. The mean % recovery of the ofloxacin and tinidazole at each level should not be less than 98% and not more than102% was considered as the acceptance criteria.

Precision was studied to find out intra- day and inter-day variations in the test method of ofloxacin and tinidazole, Intra- day assay precision was found by analysis of standard drug thrice on the same day in different intervals of time. Inter-day assay precision was carried out on three different days and percentage relative standard deviation (%RSD) was calculated. The %RSD should not be more than 2.0%.

Sensitivity of proposed method was estimated in terms of limit of Detection (LOD)and Limit of quantification(LOQ). The LOD and LOQ of ofloxacin and tinidazole by proposed methods were determined using calibration standards. LOD and LOQ were calculated as 3.3s/S and 10s/S respectively .where S is the slope of calibration curve and s is standard deviation of response.

Table. 5: Optical character and Validation Data of Ofloxacin and Tinidazole.

Parameters	Ofloxacin		Tinidazole	
	Method A	**Method B**	**Method A**	**Method B**
Maximum Absorbance	330nm	325nm	318nm	325nm
Linearity	10-50µg/ml	10-50µg/ml	10-50µg/ml	10-50µg/ml
Correlation coefficient	0.999	--------	0.999	--------
Precision	0.622	0.514	0.529	0.353
% Recovery	96.52	97.24	96.03	96.66
LOD	1.215	------	0.953	------
LOQ	3.647	--------	2.859	-------
Tablet Assay	95.33	97.10	95.26	95.60

B-Absorbance ratio or Q analysis method-

From the overlain spectrum of ofloxacin and tinidazole, two wavelengths were selected, one at 325 nm Isoabsorptive point for both the drugs and the other at 318 nm max of tinidazole. The absorbances of the standard and the sample solutions were measured in same manner as in previous method. The aborptivity values for both the drugs at the selected wavelengths are presented in table. The methods employ Q values; the concentrations of drugs in sample solution were determined by using the following formula.

For ofloxacin

$C_x = Q_m - Q_y / Q_x - Q_y * A_1 / ax_1$

For Tinidazole

$C_y = Q_m - Q_x / Q_y - Q_x * A_1 / ay_1$

Q_m= Absorbance of sample at318 nm/ Absorbance of sample at305 nm

Q_x= Absorptivity of ofloxacin at318 nm/ Absorptivity of ofloxacin at 305 nm

Q_y= Absorptivity of tinidazole at318 nm/ Absorptivity of tinidazole at 305 nm

A_1=Absorbance of sample at Isoabsorptive point

ax_1 =Absorptivity of ofloxacin at Isoabsorptive point

ay_1 = Absorptivity of tinidazole at Isoabsorptive point

RESULTS AND DISCUSSION

The solubility of ofloxacin and tinidazole in distilled water was found to be 0.02 % at room temperature. Approximate solubility of ofloxacin and tinidazole in aqueous solution of 10% phenol and 20% sodium benzoate was 1.0%w/v. It is evident from table-2 that the percent drug estimated in tablet formulation was 95.60±0.338 for tinidazole and 97.10±0.499 for ofloxacin by method B. and similarly it was 95.26±0.504 for tinidazole and 95.33±0.594 for ofloxacin by method A respectively. These values are very close to100, indicating the precision of the proposed analytical method. Further table-3 shows that the range of percent recoveries varied from 95.63±0.349 to 97.60±0.457 for

tinidazole and 96.00±0.999 to 97.55±0.133 for ofloxacin which are again very close to 100, indicating the accuracy of the proposed method. Proposed analytical method is further supported significantly by small values of statistical parameters viz. standard deviation, percent coefficient of variation and standard error (table3). The limit of detection and the limit of quantification were found to be 1.215µg/ml and 3.647µg/ml for ofloxacin and 0.953 and 2.859 for tinidazole.

CONCLUSION

A rapid, simple, and non toxic UV spectrophotometric method has been developed for simultaneous estimation of ofloxacin and tinidazole in solid dosage form by UV Spectrophotometry.

The present method also validated as per ICH guidelines for linearity, precision, accuracy. The results of all these parameter shows that the present UV spectrophotometric methods found to be precise, linear, rapid, and accurate and can be used for routine quality control analysis of ofloxacin and tinidazole in tablet dosage formulation in any laboratory. Phenol does not interfere above 300nm.A further research can be done to improve the percent of drug estimated and percent recoveries.

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