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NOVEL METHOD FOR SIMULTANEOUS DETERMINATION OF TINIDAZOLE AND OFLOXACIN IN TABLET DOSAGE FORM BY RP- HPLC METHODS

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ABSTRACT

Validation is a process that comprises validating or showing that a method/system/analyst generates accurate and reproducible findings within a known and set range through laboratory testing. The purpose of this study is to create a method for analysing Tinidazole and Oflaxacin pills simultaneously utilising reverse phase high performance liquid chromatography. A simple, accurate, and exact approach for simultaneous measurement of Tinidazole and Ofloxacin in pharmaceutical dose form has been established. The devised approach was validated in accordance with ICH standards. Tinidazole and Ofloxacin had a percentage recovery of 100.51 and 100.73 percent, respectively, using RP-HPLC. The advantages stem from the ease with which samples are prepared and the cheap cost of the chemicals utilised. The HPLC settings provided here enable adequate resolution and exact quantification of the chemicals. The findings of statistical analysis of the experimental data indicated that they were of acceptable precision and repeatability. Thus, the suggested HPLC technique may be utilised to analyse Tinidazole and Ofloxacin in pharmaceutical dose form on a regular basis.

Keywords: RPHPLC, Validation, Method Development, Simultaneous Estimation.

INTRODUCTION

A pharmaceutical drug (medicine or medication and officially medicinal product) is any chemical substance formulated or compounded as single active ingredient or in combination of other pharmacologically active substance, it may be in a separate but packed in a single unit pack as combination product intended for internal, or external or for use in the medical diagnosis, cure, treatment, or prevention of disease. [1,2]

Instrumental methods of analysis use an apparatus to measure physical quantities of the analyte such as light absorption, fluorescence, or conductivity. The separation of materials is accomplished using chromatography, electrophoresis or field flow fractionation methods. Analytical method development and validation plays important role in the discovery, development and manufacture of pharmaceuticals. Analytical methods are required to characterized drug substances and drug products composition during all phases of pharmaceutical development. Development of analytical methods is to achieve the final goal of ensuring the quality of drug substances and drug products must be implemented in conjunction with an understanding of the chemical behavior and physicochemical properties of the drug substances. The aim of the present study is to develop method for the analysis of Simultaneous Estimation of Tinidazole and Oflaxacin tablets that can be performed by using Reverse Phase High Performance Liquid Chromatography (RP-HPLC).

MATERIALS AND METHODS

Choice of column

Phenomenex C_{18} (25 cm × 4.6 mm i.d., 5-µm particle size) was selected as the column owing to its robustness, reproducibility and reliability among diverse RP-HPLC columns. This column was found to be stable at the desired pH and temperature.

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It offers good peak symmetry. Columns with 5µm particle size give the best compromise of efficiency.

Choice of mobile phase

The preferred mobile phase binary mixture is acetonitrile: 0.1% Ortho Phosphoric acid (30:70) pKa of Tinidazole (4.7 ± 0.2), Oflaxacin (3.8 ±0.2) which ensures greater selectivity and interaction with the analyte. 0.1% w/v Ortho Phosphoric acid buffer separates the TINI and OFL in combined dosage form.

Choice of solvent

Owing to free solubility of the analyte in mobile phase it is used as solvent as it accomplishes enhanced miscibility with mobile phase.

Choice of wavelength for detection

Analysis of the analyte in solvent by UV Spectrophotometry revealed the isobestic point of the TINI and OFL were found to be 290 nm.

Preparation of mobile phase

% w/v Ortho Phosporic Acid Buffer Preparation:

Dissolve 1.0 gm of Ortophosporic acid in sufficient water to produce 1000 ml.

Mobile Phase Mixture:

Mix 30 volumes of Acetonitrile and 70 volumes of 0.1 % Orthophosporic acid and ultra sound for 15 minutes cool to room temperature and filter the mobile phase through 0.45 micron membrane filter.

Preparation of Tinidazole standard stock solution- I (1000 µg/ml)

50 mg of Tinidazole working standard was accurately weighed into 50 ml volumetric flask and dissolved in freshly prepared mobile phase and made up to the volume to get concentration of 1000 µg/ ml.

Preparation of Oflaxacin standard stock solution- I (1000 µg/ml)

50 mg of Oflaxacin working standard was accurately weighed into 50 ml volumetric flask and dissolved in freshly prepared mobile phase and made up to the volume to get concentration of 1000 µg/ ml.

Preparation of standard solution- II

Transfer 6.0 ml of Tinidazole and 2.0 ml of Oflaxacin from standard stock solution I to clean, dry 50 ml volumetric flask, dilute to 50 ml with the mobile phase to get the concentration range of 120 and 40 µg/ ml of Tinidazole and Oflaxacin. Filter through 0.45 micron membrane filter.

Preparation of sample solution

Take 20 tablets and find the average weight and crush the tablets into a fine powder Transfer half the tablet weight of powdered sample into a 100 ml clean, dry volumetric flask, add 50 ml mobile phase and ultra sound for 15 minutes to dissolve, make up to the volume with diluents. Dilute 2 ml of this solution to 50 ml with diluents. Filter through 0.45 micron membrane filter.

Analytical Method Validation [3]

System suitability parameters

System suitability parameters including USP Theoretical Plate Count, USP Tailing factor and Resolution, % RSD were assessed from 3 injections of Tinidazole and Oflaxacin standards (120 and 40 µg/ml).

Specificity

The interference of the blank with the chromatogram of Tinidazole and Oflaxacin was checked by recording and comparing the chromatograms of blank and that of Tinidazole and Oflaxacin.

Linearity and Range

Linearity for the concentration range 80%-120% was established by plotting concentrations on X- axis and corresponding peak area on Y- axis. Statistical parameters like correlation coefficient (R^2), line equation including slope (m), y- intercept (C) were determined. The specified range was derived from linearity studies by determining the difference between highest and lowest concentrations.

Precision

Intraday precision (Repeatability)

Repeatability of the developed method was assessed by 9 determinations covering 3 concentrations each of 3 replicates. % RSD was calculated for the results obtained.

Interday precision

Variation in the results for the developed method was assessed 3 different days (n=6). % RSD was calculated for the results obtained.

Robustness

Typical variations including change in flow rate (± 0.5 ml of optimized flow rate), change in the organic phase composition of mobile phase (± 10 ml) and change in wavelength (± 1 nm) were assessed.

Accuracy

Preparation of 50% solution:

Transfer 2 ml of sample (stock solution I) and each of 2.5 ml of Tinidazole and Oflaxacin working standard stock solution I into a 50 ml volumetric flask and diluted up to the mark with mobile phase.

Preparation of 100% solution:

Transfer 2 ml of sample (stock solution I)and each of 5ml of Tinidazole and Oflaxacin working standard (stock solution I) into a 50 ml volumetric flask and diluted up to the mark with mobile phase.

Preparation of 150% solution:

Transfer 2 ml of sample (stock solution I)and each of 7.5 ml of Tinidazole and Oflaxacin working standard (stock solution I) into a 50 ml volumetric flask and diluted up to the mark with mobile phase . Calculate the amount found and amount added for Tinidazole and Oflaxacin, also calculate the individual recovery and mean recovery values.

Applicability of Validated Method by RP- HPLC [4]

Assay of Formulation

Weigh 20 tablets and note the weight, divide it by 20 to find its average weight, and crush the tablets into a fine powder.

Sample preparation:

Shake a quantity of the powdered tablets containing half the weight of its average weight with 60 ml of mobile phase and ultra sound for 15 minutes, dilute to 100.0ml with mobile phase and allow settling for 10 minutes. The solution is cooled to room temperature and 2.0 ml of above solution is transferred into a 50 ml volumetric flask and diluted to volume with mobile phase and filtered through 0.45 micron membrane filter. This constitutes 120 µg/ ml of Tinidazole and 40 µg/ ml of Oflaxacin.

From the stock solution, aliquot corresponding to medium concentration of standard curve (TINI 120 µg/ ml and OFL 40 µg/ ml) was prepared and made up to the mark with the mobile phase.The chromatographic procedure is carried out using C18 ODS column (25 cm × 4.6 mm), 5µ and maintained at ambient temperature as the mobile phase with a flow rate of 1.0 ml per minute and a detection wavelength of 290 nm. Inject 20 µL of each solution separately. The peak area was noted and the corresponding concentration was then determined from the standard calibration curve [5].

Table 1: Chromatogram Optimized parameters

Name	Peak	Ret. Time	Area	Height
Oflaxacin	1	3.251	3795244	553269
Tinidazole	2	3.842	2703614	419986

System Suitability Parameters

Table 2: System Suitability Parameters for Tinidazole

Inj.No	RT	Area	Theoretical Plates	USP Tailing Factor
1	3.865	3087184	7655	1.433
2	3.880	3086122	7652	1.436
3	3.861	3087244	7665	1.433
4	3.867	3086953	7653	1.441
5	3.870	3089213	7657	1.416
Mean		3087343	7657	1.431
SD		1137.39	5.193	0.009
% RSD		0.0425	0.069	0.659

Table 3: System Suitability Parameters for Oflaxacin

Inj.No	RT	Area	Theoretical Plates	USP Tailing Factor
1	3.211	4412136	4902	1.357
2	3.214	4408331	4908	1.361
3	3.213	4409332	4904	1.349
4	3.217	4409332	4907	1.360
5	3.213	4408950	4898	1.346
Mean		4401696	4903	1.355
SD		1466.884	4.038	0.007
% RSD		0.0351	0.0854	0.5023

Table 4: Linearity Profile by RP- HPLC

Concentration	Tinidazole Peak Area	Oflaxacin Peak area
80	2489336	3572519
90	2768577	4029736
100	3085337	4466338

110	3386046	4872843
120	3644620	5359196

Table 5: Summary of Regression by RP- HPLC

Parameters	Tinidazole	Oflaxacin
Linear equation	Y=28985.03x+145268.315	Y=43525.628x+14625.38
Correlation coefficient (R^2)	0.9985	0.9968

Table 6: Intraday Precision by Tinidazole

Conc. (%)	Peak area			Average	SD	% RSD
	Day 1	Day 2	Day 3			
80	2489336	2493169	2486868	2489791	3175	0.0053
100	3084253	3082794	3078785	3081944	2832	0.0045
120	3644620	3635753	3639292	3639888	4463	0.0059

Table 7: Intraday Precision of Oflaxacin

Conc. (%)	Peak area			Average	SD	% RSD
	Day 1	Day 2	Day 3			
80	3492814	3493139	3492690	3492881	232	0.0057
100	4475366	4476284	4477483	4476378	1061	0.0169
120	5459925	5457395	5459517	5458946	1358	0.0145

Table 8: Robustness of the method for tinidazole

Parameter	Condition	System suitability parameters	
		Theoretical plates	USP Tailing factor
Change in flow rate (±0.2 ml/ min)	0.8 ml/ min	7475	1.40
	1.2 ml/ min	7656	1.47
Change in organic phase composition (±10 ml)	Methanol : Water (60:40)	7536	1.43
	Methanol : Water (80:20)	7656	1.45
Change in detector wavelength (± 2 nm)	292 nm	7583	1.45
	288 nm	7475	1.40

Table 9: Summary of Robustness for Ofloxacin

Parameter	Condition	System suitability parameters	
		Theoretical plates	USP Tailing factor
Change in flow rate (± 0.2 ml/ min)	0.8 ml/ min	4827	1.63
	1.2 ml/ min	4868	1.66
Change in organic phase composition (± 10 ml)	Methanol : Water (60:40)	4829	1.62
	Methanol : Water (80:20)	4826	1.65
Change in detector wavelength (± 2 nm)	292 nm	4837	1.64
	288 nm	4824	1.65

Table 10: Accuracy Data for tinidazole

Recovery levels	Accurate data for Tinidazole					
	Amount taken (µg/mL)	Amount added (µg/mL)	Area	Average area	Amount recovered (µg/mL)	% recovery
50%	100	50	4664469	4663588	150.92	100.61
	100	50	4662996			
	100	50	4663299			
100%	100	100	6160140	6158151	201.85	100.92
	100	100	6156172			
	100	100	6158142			
	100	150	7948540			

150%	100	150	7926569	7967092	249.21	103.27
	100	150	8026168			

Table 11: Accuracy Data for Ofloxacin

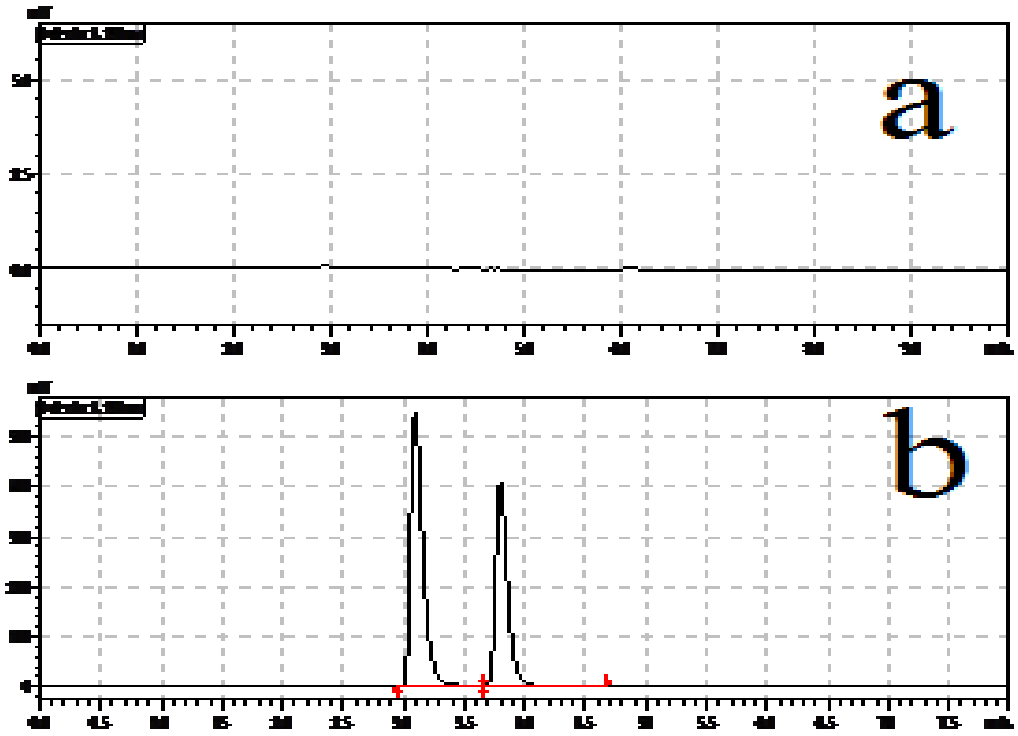
Recovery levels	Accurate data for Tinidazole					
	Amount taken (µg/mL)	Amount added (µg/mL)	Area	Average area	Amount recovered (µg/mL)	% recovery
50%	100	50	6371034	6469512	149.51	99.67
	100	50	6469442			
	100	50	6568060			
100%	100	100	8418078	8415670	201.58	100.79
	100	100	8411806			
	100	100	8417127			
150%	100	150	10977532	10964401	248.73	99.49
	100	150	10448820			
	100	150	11466852			

Table 12: Common test used to level of significance

Formulation	Peak area	Label claim	Amount found	% Assay ± SD*
Tinidazole	3087184	600mg	603.06	100.51
	3086122			
	3087244			
Ofloxacin	4412136	200mg	201.47	100.73
	4408331			
	4409332			

Figure 1: Chromatogram for Specificity

a. Blank; b. Formulation



RESULTS AND DISCUSSION

System Suitability Parameters

Acceptance criteria

Theoretical Plates- NLT 2000; USP Tailing factor- NMT 2.0; % RSD- NMT 2.0; Resolution-NLT 2.0. The system suitability parameters were within limits and hence the parameters for the optimized method could be applicable for the method to be validated.

Specificity

The method was found to be specific since the interference of blank with the chromatogram of Tinidazole and Oflaxacin was not observed.

Linearity and Range

The calibration set was linear with regression coefficient of 0.9985 for Tinidazole and 0.9968 for Oflaxacin.

Intraday Precision for Tinidazole

Intraday precision for Oflaxacin (Repeatability)

Robustness

Accuracy:

Acceptance Criteria:

The % Recovery for each level should be between 98.0 and 102.0% .The accuracy data was found to be within limits

ASSAY OF TABLETS BY RP- HPLC

Acceptance criteria: 95- 105%, Assay results were satisfactory and within limits

CONCLUSION

A simple, accurate, precise method was developed for the simultaneous estimation of the Tinidazole and Ofloxacin in pharmaceutical dosage form. The developed method was validated based on ICH guidelines. The percentage recovery was obtained as 100.51%and 100.73% for Tinidazole and Ofloxacin respectively by RP-HPLC. The advantages lie in the simplicity of sample preparation and the low costs of reagents used. The proposed HPLC conditions ensure sufficient resolution and the precise quantification of the compounds. Results from statistical analysis of the experimental results were indicative of satisfactory precision and reproducibility. Hence, the proposed HPLC method can be used for routine drug analysis of Tinidazole and Ofloxacin in pharmaceutical dosage form

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