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METHOD DEVELOPMENT AND VALIDATION OF OFLOXACIN AND ORNIDAZOLE IN BULK AND IN ITS PHARMACEUTICAL DOSAGE FORMS USING RP HPLC

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ABSTRACT

Aim of the Study is to develop simple, rapid, specific and sensitive RP-HPLC method for the determination of Ornidazole and Ofloxacin. The Specificity of Ofloxacin and Ornidazole was found there was no interference in the method and good separation between all peaks; it means no impurity was interfered and also reveals that commonly used excipients and additives present in the pharmaceutical formulation were not interfering in the proposed methods. The precision was found to be within the limits. The limit were not more than RSD <2%.Precision RSD was found to be 0.9937for Ofloxacin and 0.8048 for Ornidazole.The low %RSD value for intraday and inter day precision reveled that the proposed method is robust and rugged. From the linearity table it was found that , the drug obeys beer's law and from the linearity range 22 to 66ug/ml for ofloxacin and 55 to 165ug/ml for ornidazole. In Robustness parameter in both conditions the RSD was less than 2%. Hence the method was better for pharmaceutical formulation analysis.

Key words: Ornidazole and Ofloxacin, sensitive RP-HPLC method.

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INTRODUCTION

Analytical chemistry may be defined as the science and art of determining the omposition of material in terms of elements or compounds contained in it. Analytical chemistry is divided into two branches quantitative and qualitative. A qualitative method is the information about the identity of atomic or molecular species or functional groups in sample. A quantitative method provides numerical information as to the relative amount of one or more of these components. Drug analysis reveals identification characterization & determination of the drugs in mixtures like dosage forms & biological fluids. The number of drugs introduced in to the market has been increasing at very fast rate. These drugs may be either new entities in the market or partial structural modification of the existing drugs. Newer analytical methods are developed for these drugs or drug combination of the below reasons. The development of a method of analysis of an any compound is usually based on existing literature, using same or quite similar instrumentation. But now days HPLC based method is not similar as compare to existing literature based approaches. The development of new or any improved method should be beneficial in any way than the existing method. Method development usually requires selecting the method requirement and deciding the instrumentation to utilize for what purpose. Analytical Method validation according to

USP is performed to ensure that an analytical methodology is accurate, specific, reproducible, precise and rugged over the specified range that an analyte will be analysed. Validation of method is the process by which a method is tested by a developer or user for reliability, accuracy, and preciseness of it intended purpose. The international conference on Harmonization (ICH) of technical requirement for the registration of Pharmaceutical for human use has developed suitable text on the validation of analytical procedure. The document includes definitions for eight validation parameter. The United States Food and Drug Administration (USFDA) have proposed guidelines on submitting samples and analytical data for method validation. The United States Pharmacopoeia (USP) has published specific guidelines for method validation for compound evaluation (1-4).

Aim of the Study is to develop simple, rapid, specific and sensitive RP-HPLC method for the determination of Ornidazole and Ofloxacin in Tablet formulation**.** According to the literature survey it was found that simultaneous estimation of Ornidazole and Ofloxacin by RP-HPLC was not developed.

MATERIALS AND METHODS (5-8)

Preparation of Standard solution

Ofloxacin- Weigh 22.38gm of Ofloxacin standard and dissolve in 75ml of 100% methanol and sonicate for 10 min.Make up the volume to 100ml with 100% methanol, 5ml of above taken & make up the volume to 25ml with 100% methanol.

Ornidazole- Weigh 55.89gm of Ornidazole standard and dissolve in 75ml 100% methanol and sonicate for 10 min.Make up the volume to 100ml with 100% methanol, 5ml of above taken & make up the volume to 25ml with 100% methanol.

Accuracy

Twenty tablets were weighed and finely powdered. The average weight of tablets is determined with help of 20 tablets weight. A portion of powder equivalent to weight of 50mg , 100mg and 150mg was taken into three 100ml volumetric flask and add 70ml of mobile phase into each flask and sonicate for 10 min's to effect complete dissolution of the drugs, the solution was made upto the volume 100ml with mobile phase . The solution was filtered through 0.45um Durapore PVDC hdrophillic membrane filter. 5ml of above taken and the volume made upto 25 ml of mobile phase, The final concentration for 50% is Ofloxacin 22ug/ml and Ornidazole 55ug/ml, for 100% is Ofloxacin 44ug/ml and Ornidazole 110ug/ml, for 150% is Ofloxacin 66ug/ml and Ornidazole 165ug/ml.

Linearity

Stock solution of (770ug/ml) of the standard drug was prepaed by mixing 22mg ofloxacin and 55mg of ornidazole in 100ml volumetric flask and make up the volume to 100ml with 100% methanol. The stock solution was suitabily diluted to 22 to 66ug/ml for ofloxacin and 55 to 165ug/ml for ornidazole respectively. 10ml of resulting solution was injected to HPLC system.

RESULTS AND DISCUSSION

Chromatographic conditions for the optimized method

The Chromatographic conditions for the optimized method are given in table-1 and fig-1.

Table-1 Chromatographic conditions for the optimized method

Parameters	Description
Column	L1 Column (C-18)
Mobile Phase	Acetonitrile : Buffer(18:82) pH adjusted to 3.0 with dil.orthophosphoric acid
Injection volume	10 µL
Flow rate	1.0ml/min
Detector Wavelength	254nm
Temperature	25 °C
Run Time	20 min

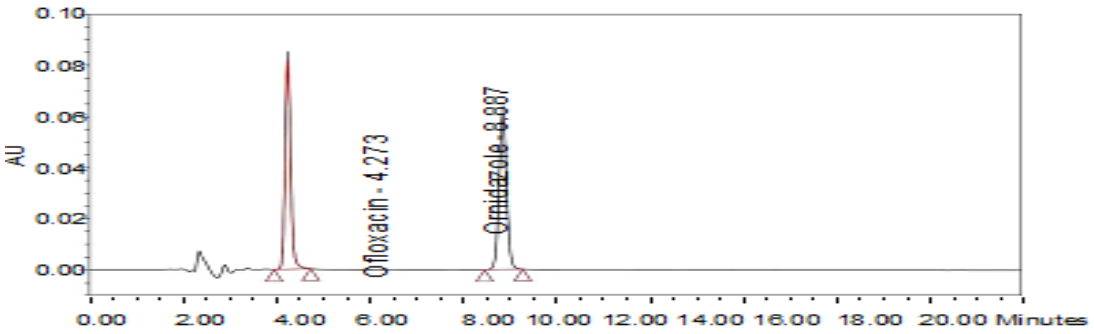

Fig-1 Chromatogram for optimized method

Validation Parameters

Specificity- Prepare standard as per test procedure and make Five injections. Evaluate System suiability parameters as per the test procedure and tabulate the results in the table-2.

Table-2 Parameters for Specificity

System suitability	Obseved value	Acceptance criteria
Tailing factor for		
1.Ofloxacin	1.51	NMT 2.0
2.Ornidazole	1.16	NMT 2.0
Relative standard deviation for		
1.Ofloxacin	0.99	NMT 2.0%
2.Ornidazole	0.80	NMT 2.0%
From five injections of standard		

Robustness- The robustness of an analytical procedure is tested by measuring its capacity to remain unaffaected by small , but deliberate variations in the method parameters and provides an indication of its reliability during normal use (Table-3).

Table-3 Parameters for Robustness

S.No	Parameters	Below normal range	Above normal range
1	Flow rate	0.8ml/min	1.2 ml/min
2	Mobile phase pH	2.8	3.1

Linearity

The data of Linearity is given in table-4 and fig-2 and 3.

Table-4 Data for Linearity

STD's	Ofloxacin	Ornidazole	CONC	Ofloxacin	Ornidazole
STD-1	670569	705123	50	353317	363126
STD-2	648682	670728	60	413862	429382
STD-3	661988	694169	75	517798	530019

STD-4	663038	695147	100	668252	686130
STD-5	673496	693814	125	856835	875377
AVG	**663554.**	**691796.2**	**150**	**984968**	**1003659**
STDEV	**9642.98**	**12672.8273**	**CORR**	**0.999**	**0.99997**
%RSD	1.453	1.8319	B_STD	659396	682256

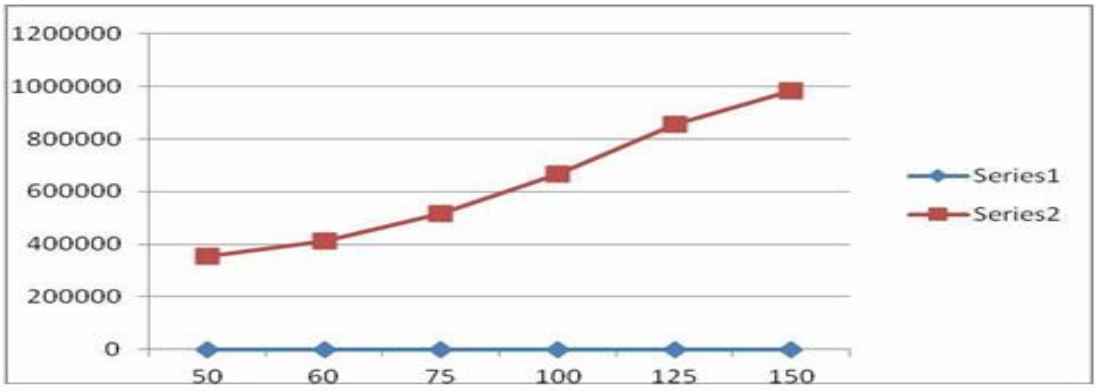

Fig-2 Linearity curve for Ofloxacin

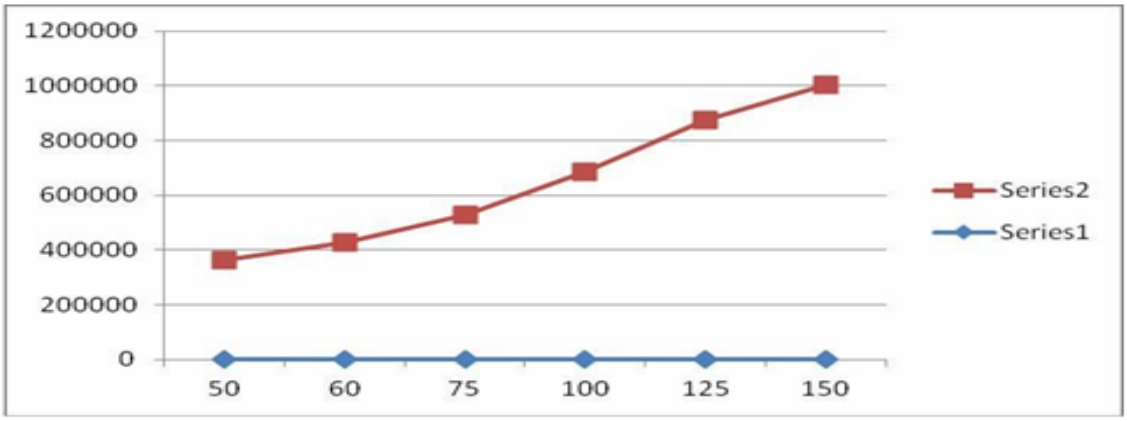

Fig-3 Linearity curve for Ornidazole

CONCLUSION

On the basis of the experiments, we can conclude that the RP-HPLC method developed for the Simultaneous Determination of Ofloxacin and Ornidazole can be used for routine analysis. The system suitability was found to be within the limits. The limit were not more than RSD <2%. This indicates that the method is suitable. The Specificity of Ofloxacin and Ornidazole was shown in fig and found there was no interference in the method and good separation between all peaks; it means no impurity was interfered and also reveals that commonly used excipients and additives present in the pharmaceutical formulation were not interfering in the proposed methods. The precision was found to be within the limits. The limit were not more than RSD <2%.Precision RSD was found to be 0.9937 for Ofloxacin and 0.8048 for Ornidazole.The low %RSD value for intraday and inter day precision reveled that the proposed method is robust and rugged. From the linearity table it was found that , the drug obeys beer's law and from the linearity range 22 to 66ug/ml for ofloxacin and 55 to 165ug/ml for ornidazole From the results shown in the accuracy table ,it was found that the recovery value of pure drug from the solution

were between 98.0% to 103.0% which indicate that the method is accurate. In Robustness parameter in both conditions the RSD was less than 2%. Hence the method was better for pharmaceutical formulation analysis.The developed RP-HPLC method for simultaneous assay of Ofloxacin and Ornidazole in combined tablets dosage forms is simple,precise,specific,and highly accurate and less time consumption for analysis could be recorded.So it can be employed for the routine analysis for simultaneous estimation .Hence RP-HPLC method is suitable for quality control of raw materials and formulations.

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