International Standard Serial Number (ISSN): 2319-8141
International Journal of Universal Pharmacy and Bio Sciences 8(2): March-April 2019
INTERNATIONAL JOURNAL OF UNIVERSAL PHARMACY AND BIO SCIENCES
*IMPACT FACTOR 4.018****
*ICV 6.16****
Pharmaceutical Sciences **RESEARCH ARTICLE……!!!**

SIMULTANEOUS DETERMINATION AND VALIDATION OF OFLOXACIN AND SERRATIOPEPTIDASE BY RP-HPLC IN BULK AND PHARMACEUTICAL FORMULATIONS

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KEYWORDS:

Ofloxacin, Serratiopeptidase, RP-HPLC, Validation.

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ABSTRACT

An accurate, simple, reproducible and sensitive method for the determination of Ofloxacin and Serratiopeptidase was developed and validated as per ICH Guidelines. Ofloxacin and Serratiopeptidase were separated by HPLC using a Shimadzu RP-18 column (5µm,250mm × 4.6mm i.d) and isocratic elution with a flow rate of 1 ml/min. Mixture of Phosphate buffer and Acetonitrile (pH=4.4) (70:30) was used as mobile phase. The detection was at 278 nm wavelength. The retention time of OFLO and SERR was found to be 6.523 and 2.581 respectively. The linearity of developed method was achieved in the range of 20-100 µg/mL (r^2 = 0.9994) and 2-10 µg/mL (r^2 = 0.9998) for Ofloxacin and Serratiopeptidase respectively. LOD of both the drugs were 0.7549 µg/mL and 0.2883 µg/mL and LOQ was found to be 2.2878 µg/mL and 0.8738 µg/mL for Ofloxacin and Serratiopeptidase respectively. The developed method is suitable for routine quality control analysis of titled drugs in combination of tablet formulations.

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INTRODUCTION:

Ofloxacin[1-5] is a quinolone antimicrobial agent. Chemically Ofloxacin is (*RS*)-9-fluoro-3-methyl 10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3,-*de*]-1,4-benzoazeine-6 carboxylic acid. It acts by inhibiting the bacterial topoisomerase IV and DNA gyrase (both of which are type II topoisomerases), enzymes required for DNA replication, transcription, repair and recombination. By inhibiting their function the drug there by block the normal cell function.

Serratiopeptidase[6-10] is an enzyme derived from the bacteria belonging to genus Serratia. The mechanism of action of Serratiopeptidase appears to be hydrolysis of histamine, bradykinin and serotonin. Serratiopeptidase also has a proteolytic and fibrinolytic effect. This is achieved by dissolving the complement (specific proteins responsible for inflammation) and increasing the plasmin activity by inhibiting the plasmin inactivators.

The combination of Ofloxacin and Serratiopeptidase is prescribed for certain types of bacterial infection such as chronic bronchitis, pneumonia, skin and skin structure infections.

On literature survey, Ofloxacin alone has been estimated individually and simultaneous estimation in combination with other drugs has been reported [11-16]. Serratiopeptidase alone has been estimated and simultaneous estimation in combination with other drugs has been reported [17-19]. It was found that no method has been reported for the simultaneous estimation of Ofloxacin and Serratiopeptidase in combined dosage forms. In the view of the need for a suitable method for routine analysis in combined formulations, attempts are being made to develop simple, precise and accurate analytical methods for simultaneous estimation of titled drugs and extend it for their determination in formulations.

MATERIALS AND METHODS:

Instrument

A high performance liquid chromatographic system (SHIMADZU Corporation, LC-20 AD), a Shimadzu SPD-20A UV/VIS detector was used for analysis. The data was recorded using Lab Solutions Software.

Chemicals and reagents:

Acetonitrile (HPLC grade) was procured from Merck Ltd, double distilled water (HPLC grade), Potassium dihydrogen O-Phosphate (AR grade). All other chemical reagents were of analytical grade.

Drug Sample:

Standard Ofloxacin was obtained as gift sample from Micro labs, Bangalore. Standard Serratiopeptidase was procured from IPCA Laboratories, Mumbai.

Preparation of mobile phase:

The mobile phase (1000 ml) was prepared by mixing phosphate buffer and Acetonitrile solution in the ratio of 70:30, pH was adjusted to 4.4 with triethylene amine. The mobile phase was filtered through a 0.45 µm filter paper and then sonicated for 10mins.

Preparation of buffer solution:

2.72g of Potassium dihydrogen O-Phosphate was dissolved into 1000 ml of HPLC water (0.02M).

Preparation of standard stock solution:

100 mg each of OFLO and SERR were weighed separately and transferred into two different 100 mL volumetric flasks. Both the drugs were dissolved in 50 mL of mobile phase by sonication and then volume was made up to the mark with mobile phase to get a concentration of 1000 µg/mL of each component (stock A and A' solution).

From the above stock A and A' solution 10mL of aliquot was pipetted out into a 100mL volumetric flask and the volume was made upto the mark with mobile phase to obtain a concentration of 100 µg/mL of each component (stock B and B' solution).

From the above stock B and B' solution further dilutions were made to get concentration from 20-100µg/mL for Ofloxacin and 2-10 µg/mL for Serratiopeptidase.

Preparation of sample solution

Twenty tablets of OFLO and SERR in combination were weighed and their average weight was determined. The tablets were crushed to fine powder and a tablet powder equivalent to 100mg of OFLO was weighed and transferred to 100 mL volumetric flask, dissolved in sufficient quantity of mobile phase. The solution was filtered through 0.4 µm membrane filter paper. The contents were sonicated for 20 minutes and the final volume was made up to the mark with mobile phase to get the concentration of 1000 µg/mL of OFLO and this solution was used as stock “A” solution of the sample.

From the above solution, further dilutions were made to bring the concentration of the drugs within the range.

A 20 µL volume of each sample mixture was injected in to the sample injector of HPLC system and their chromatograms were recorded under the same chromatographic conditions as described earlier. The area of each peak was determined at 278nm and the amount of drug present in the sample mixture was determined.

RESULTS AND DISCUSSIONS

The developed method for determination of Ofloxacin and Serratiopeptidase was further validated by using following parameters:

Linearity:

Linearity was established by least square regression analysis of the calibration curve. The constructed calibration curves were linear over the concentration range of 20-100 µg/mL for OFLO and 2-10 µg/mL for SERR respectively. Peak areas of OFLO and SERR were plotted with their respective concentrations and linear regression analysis was performed on the resultant curves. (fig 6 &7) The regression equation was found to be y = 24314x + 14095(r^2 = 0.9994) for OFLO and y = 51347x + 1619(r^2 = 0.9998) for SERR.

LOD and LOQ

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision. In this study, LOD and LOQ were determined based on the standard deviation of the response and the slope of the corresponding curve using the following equations.

LOD = 3.3 SD/Slope and LOQ = 10 SD/Slope.

Where, SD is the standard deviation of the absorbance of the sample and the slope of the related calibrations curve.

The LOD and LOQ of OFLO and SERR were found to be 0.7579 µg/mL and 2.2878 µg/mL, 0.2883 µg/mL and 0.8738 µg/mL respectively.

Accuracy

Accuracy studies were done as percent recovery, it was performed by adding constant amount of the standard drug to the sample taken from formulations at levels of 80%, 100% and 120% of the test concentration. The results are tabulated in (table 2).

Precision

The Intraday and Interday precisions of the proposed method were determined by estimating the corresponding responses three times on the same day and on 3 different days over a period of one week for 3 different concentration and 3 replicates of OFLO and SERR and reported in terms of relative standard deviation (RSD). Statistical validation of data for Intraday and Inter day precision methods as shown in (table 3) and (table 4).

Robustness:

The evaluation of robustness should be considered during the development phase and depends upon the type of procedure under study. It should show the reliability of analysis with respect to deliberate variations in method parameters. The solution containing 60µg/ml of Ofloxacin and 6µg/ml of

Serratiopeptidase was injected into sample injector of HPLC three times under different parameters like deliberate variations in flow rate (table 5) and wavelengths (table 6).

Ruggedness:

The evaluation of robustness should be considered during the development phase and depends upon the type of procedure under study. It should show the reliability of analysis with respect to deliberate variations in analyst or instrument. The solution containing 60µg/ml of Ofloxacin and 6µg/ml of Serratiopeptidase was injected into sample injector of HPLC two times by different analysts.(table 7).

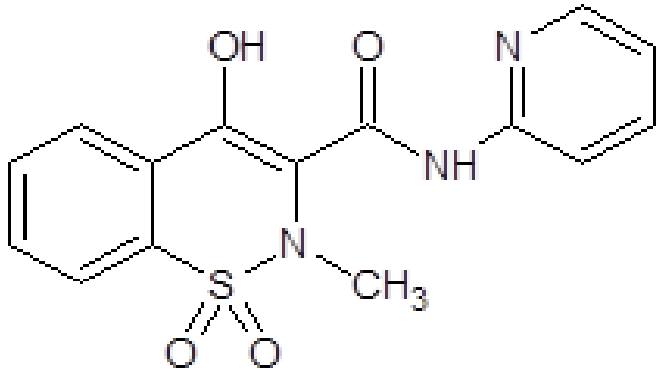

Fig 1: Chemical structure of Ofloxacin.

Fig 2: Chemical structure of Serratiopeptidase

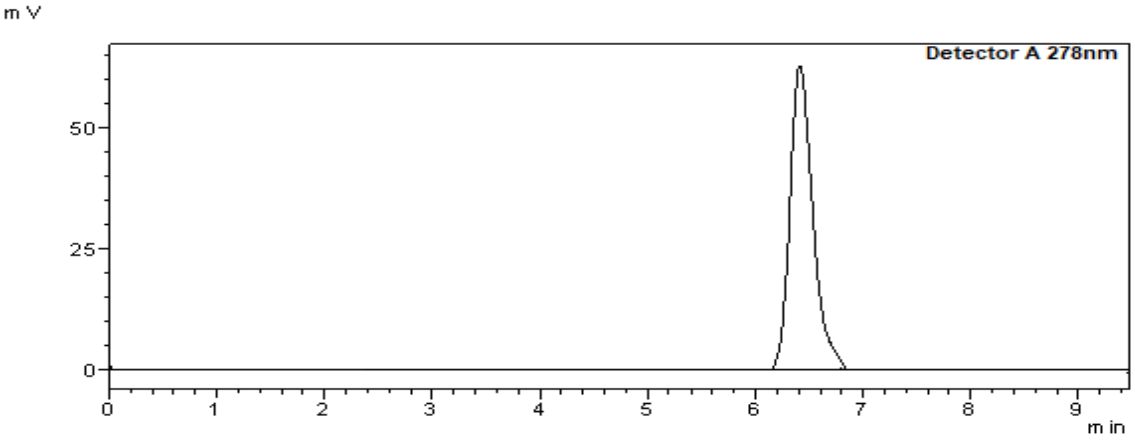

Fig 3: Chromatogram of Ofloxacin

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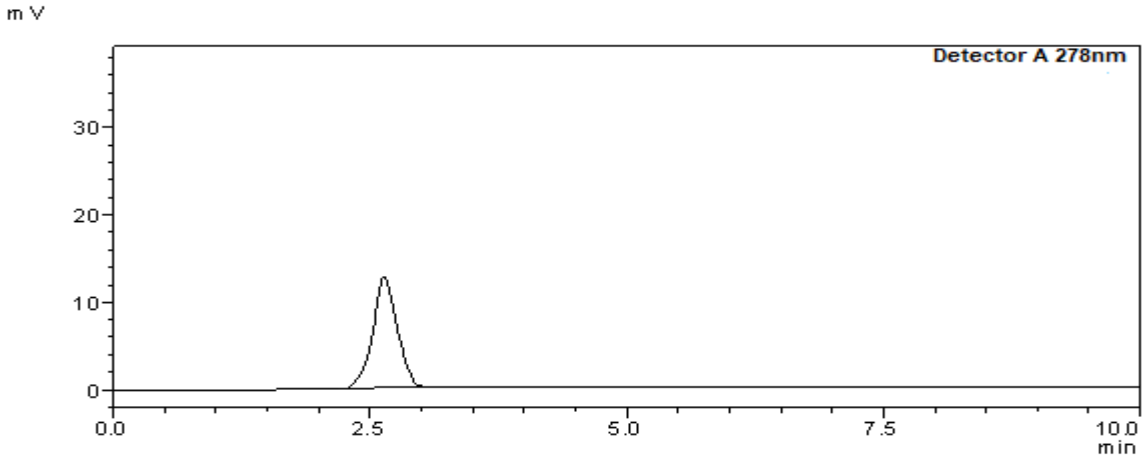

Fig 4: Chromatogram of Serratiopeptidase

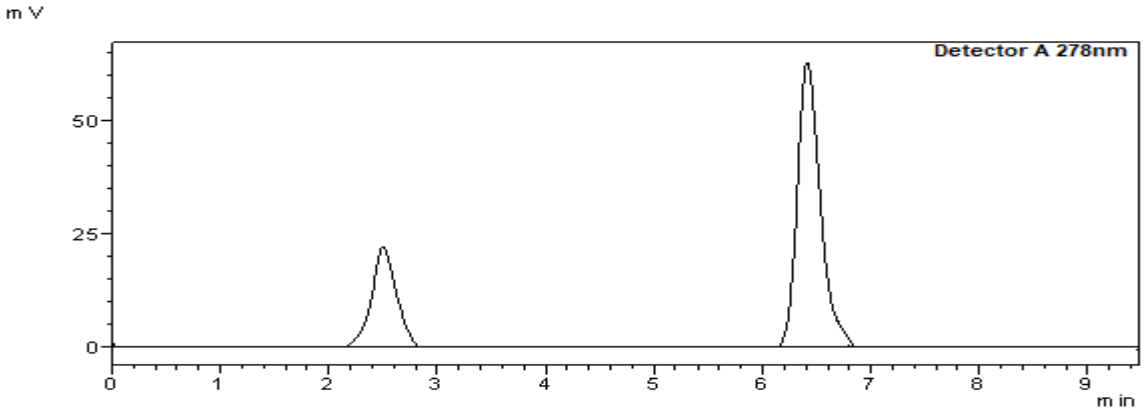

Fig 5: Chromatogram of mixture of Ofloxacin and Serratiopeptidase

Fig 6: Calibration Curve of Ofloxacin at 278nm by HPLC method

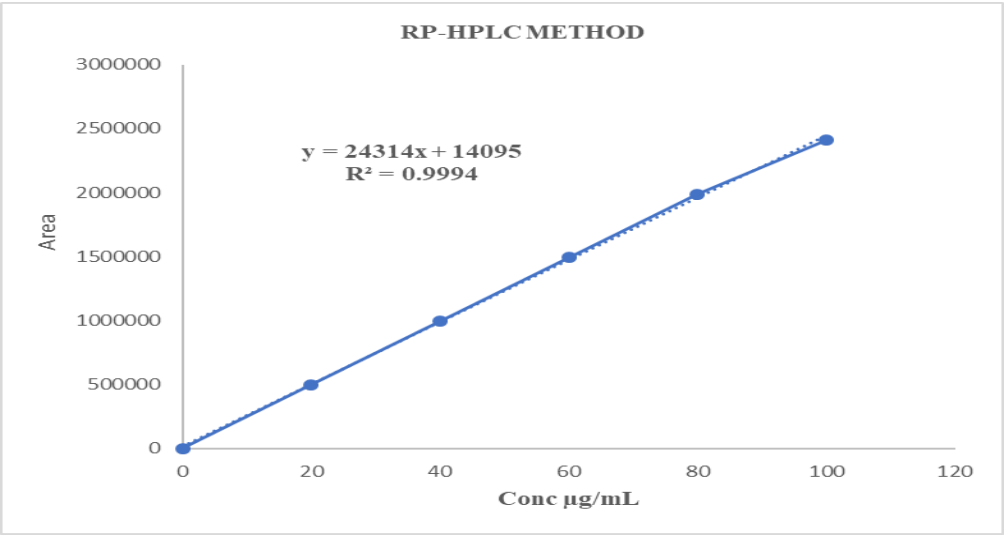

Fig 7: Calibration Curve of Serratiopeptidase at 278nm by HPLC method

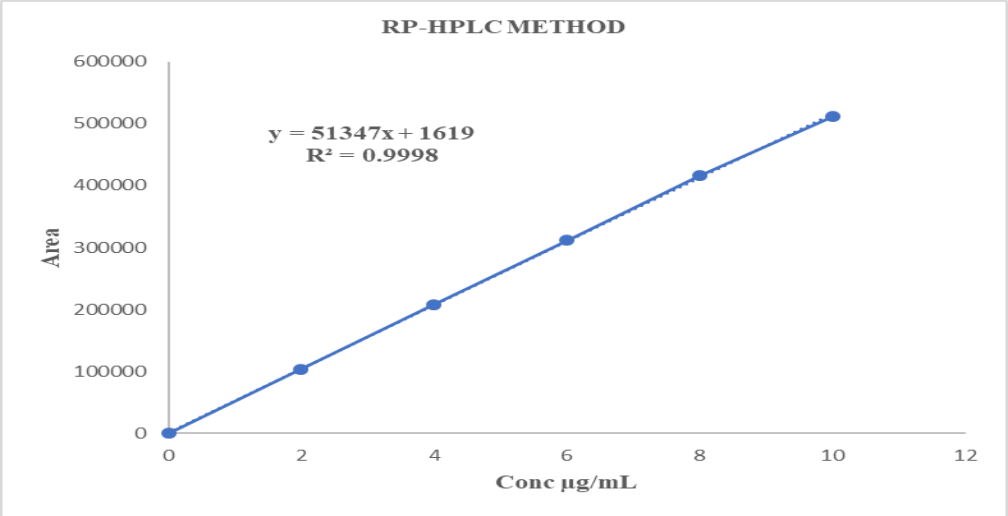

Table 1: Summary of Validation Parameters by Developed Methods

Parameters	OFLO	SERR
Linearity Range (µg/mL)	20-100	2-10
Slope	24314	51347
Intercept	14095	1619
Regression Coefficient (r^2)	0.9994	0.9998
Limit of Detection (µg/mL)	0.7549	0.2883
Limit of Quantification(µg/mL)	2.2878	0.8738
Retention time (min)	6.523	2.581
Tailing factor	1.439	1.063
Resolution factor	11.236	
Theoretical plate	6810	2750

Table 2: Statistical Validation Data for Accuracy Determination

Level of % Recovery	Components	Amount present (µg/ml)	Amount of Standard drug added (µg)	Total amount recovered (µg)	% Recovery	RSD
80%	OFLO	40	32	71.95	99.93	0.1259
	SERR	4	3.2	7.18	99.75	0.7133
100%	OFLO	40	40	80.05	100.0	0.1110
	SERR	4	4	7.95	99.37	0.9665
120%	OFLO	40	48	87.96	99.95	0.1316
	SERR	4	4.8	8.75	99.43	0.5855

Table 3: Statistical Validation Data for Intra-day Precision.

Components	Mean	Std.deviation	Co-efficient of variation	Standard error
OFLO	99.98	0.1244	0.1244	0.0508
SERR	99.75	0.7051	0.7069	0.2879

$n^* = 6$

Table 4: Statistical Validation Data for Inter-day Precision.

Components	Mean	Std.deviation	Co-efficient of variation	Standard error
OFLO	99.99	0.1290	0.1290	0.0745
SERR	99.84	0.7574	0.7584	0.4373

$n^* = 3$

Table 5: Robustness result for variations in Flow Rate (mL/min).

Method Parameter	Level	Retention Time		Tailing factor	
Flow rate (mL/min)		**OFLO**	**SERR**	**OFLO**	**SERR**
0.9	-1	6.608	2.605	1.456	1.071
1.0	0	6.523	2.581	1.439	1.063
1.1	+1	6.492	2.562	1.428	1.058

Table 6: Robustness result for variations in Wavelength (nm).

Method Parameter	Level	Retention Time		Tailing factor	
Wavelength(nm)		**OFLO**	**SERR**	**OFLO**	**SERR**
276	-2	6.492	2.563	1.424	1.058
278	0	6.523	2.581	1.439	1.063
280	+2	6.592	2.602	1.456	1.072

Table 7: Ruggedness result for variations in Analyst.

Method Parameter	Retention Time		Tailing Factor	
Analysts	**OFLO**	**SERR**	**OFLO**	**SERR**
Analyst 01	6.523	2.581	1.439	1.063
Analyst 02	6.482	2.558	1.437	1.061

CONCLUSION

An simple, accurate, sensitive and precise HPLC method with UV detection for the simultaneous estimation of Ofloxacin and Serratiopeptidase was developed and can be used for routine analysis. Above method was validated as per ICH guidelines.

ACKNOWLEDGEMENT:

Authors express sincere thanks to the Principal and staff Department of Pharmaceutical Analysis, National College of Pharmacy, Shimoga, for guidance, encouragement and providing laboratory facilities.

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