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 SIMULTANEOUS EQUATION AND FIRST ORDER DERIVATIVE METHODS IN BULK AND PHARMACEUTICAL FORMULATIONS

Rakshitha G R*, Vijaya krishna C Aradhya and A Satishkumar Shetty
Department of Pharmaceutical Analysis, National College of Pharmacy, Shimoga - 577201, Karnataka, India.

KEYWORDS:

Ofloxacin, Serratiopeptidase, Simultaneous equation method, First order derivative.

FOR CORRESPONDENCE:

Rakshitha G R*

ADDRESS:

Department of Pharmaceutical Analysis, National College of Pharmacy, Shimoga - 577201, Karnataka, India.

.

ABSTRACT

In the present work two simple and sensitive spectrophotometric methods were developed for the simultaneous estimation of Ofloxacin and Serratiopeptidase in bulk drugs and pharmaceutical dosage forms by using distilled water as a solvent. Method A: Simultaneous equation method is based on the measurement of absorbances at two selected wavelengths 287nm and 221nm for the estimation of Ofloxacin and Serratiopeptidase. Beer's law obeyed in the concentration range of 10-20µg/ml and 1-5µg/ml with (r^2 = 0.9995, %RSD = 0.4856-1.1075 and r^2 = 0.9999, %RSD = 0.4974-1.4709) for Ofloxacin and Serratiopeptidase respectively. LOD of both drugs were 0.2728µg/ml and 0.0480µg/ml and LOQ were found to be 0.8269µg/ml and 0.1455µg/ml for Ofloxacin and Serratiopeptidase respectively. Method B: First order derivative spectroscopic method is based on the measurement of absorbances at two selected wavelengths 277nm and 233nm for the estimation of Ofloxacin and Serratiopeptidase respectively. Linearity range was found 10-50µg/ml and 1-5µg/ml with (r^2 = 0.9996, %RSD = 0.4532-1.3240 and r^2 = 0.9998, %RSD = 0.6824-1.5977) for Ofloxacin and Serratiopeptidase respectively. LOD of both drugs were 0.4345µg/ml and 0.0511µg/ml and LOQ were found to be 1.3169µg/ml and 0.1548µg/ml for Ofloxacin and Serratiopeptidase respectively. In both the methods the % RSD for intra-day and inter-day precision was within 2%.

INTRODUCTION:

UV visible spectrophotometric method is very frequently employed in pharmaceutical analysis. It involves the measurement of the amount of ultraviolet (200 to 400nm) or visible (400 to 800nm) radiation absorbed by a substance in solution by an instrument which measures the ratio or a function of the ratio of the intensity of two beams of light in UV and Visible region. The basis of all spectrophotometric methods for multicomponent sample analysis is the property that the absorbance of a solution is the sum of absorbances of individual components or the measured absorbance is the difference between total absorbance of the solution in the sample cell and that of the solution in the reference (blank) cell. The various spectrophotometric methods which are used for estimation of drug in combined dosage forms include simultaneous equation method, absorbance ratio method, derivative spectrophotometry and dual wavelength method.

Ofloxacin[1-3] 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[2-5] 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[5-7].

On literature survey, Ofloxacin alone has been estimated individually and simultaneous estimation in combination with other drugs has been reported. Serratiopeptidase alone has been estimated and simultaneous estimation in combination with other drugs has been reported. It was found that no method has been reported for the simultaneous estimation of Ofloxacin and Serratiopeptidase in combined dosage forms and no method is available in the pharmacopoeias. 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[11-14].

MATERIALS AND METHODS:

Instrument:

For UV-Visible Spectroscopy methods, Shimadzu model 1800 double beam UV-Visible Spectrophotometer with spectral band width of 1 ± 0.2nm, wavelength accuracy of ± 0.3nm and a pair of quartz cuvettes having 1cm path length was used. Distilled water was used throughout the experimental work.

Chemicals

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

Methods[8-10]

Preparation of standard solutions

100mg of Ofloxacin(OFL) and Serratiopeptidase(SERR) was weighed and transferred to two different 100 ml volumetric flask. Drug was dissolved in 50 ml distilled water by ultra sonication and volume was made upto the mark with distilled water to obtained finally concentration of 1000µg/ml (stock A).

From the above stock A solution 10ml of aliquot was pipetted out into two different 100 ml volumetric flask and volume was made upto the mark with the distilled water to obtain a concentration of 100µg/ml (stock B). From the above stock B solution further dilutions were made to get concentration from 10-50µg/ml for Ofloxacin and 1-5 µg/ml for Serratiopeptidase.

Preparation of sample solution

20 tablets which contains both OFLO and SERR were weighed and powdered. The tablet powder equivalent to 100mg of Ofloxacin was weighed accurately and dissolves in 70 ml distilled water and sonicated for 15mins. The solution was filtered through Whatmann filter paper No. 41, finally the volume was made up to the mark with distilled water. Further dilutions were made to bring the concentration of the drugs within the range.

Method of estimation

Method A (Simultaneous equation method)

From the above standard solution both drugs were prepared and scanned in the wavelength range of 400-200nm using UV – Spectrophotometer. At 287nm OFLO showed maximum absorbance and at 221nm SERR shows maximum absorbance. Both drugs did not show any interference at either of the wavelength. Hence 287nm and 221nm for OFLO and SERR were selected as the working analytical wavelength.

$$C_x = \frac{A_1ay_2 - A_2ay_1}{ax_1ay_2 - ax_2ay_1}$$

$$C_y = \frac{A_2ax_1 - A_1ax_2}{ax_1ay_2 - ax_2ay_1}$$

Where,

C_x = absorbance of Sample at 287nm

C_y = absorbance of Sample at 221nm

ax_1 = absorptivity of Ofloxacin at 287nm

ax_2 = absorptivity of Ofloxacin at 221nm

ay_1 = absorptivity of Serratiopeptidase at 287nm

ay_2 = absorptivity of Serratiopeptidase at 221nm

Method B (First order derivative)

For the estimation of Ofloxacin and Serratiopeptidase by first order derivative spectroscopy, zero crossing point for both drugs were obtained and the wavelengths were selected in manner such that at the zero crossing of one drug, the other drug should show substantial absorbance. From the first order derivative spectra of standard Ofloxacin and Serratiopeptidase, zero crossing point of Ofloxacin was found at 287nm and zero crossing point of Serratiopeptidase was found at 221nm and wavelength selected for their estimation was 277nm for OFLO and 233nm for SERR.

VALIDATION PARAMETER:

Linearity:

In Method A (Fig. 3) overlay spectra of mixture were shown. Fig.4 and Fig.5 were shown linearity of both the drugs in their respective wavelengths. The responses of simulations equation for both drugs shows linear concentration range of 10-50µg/ml and 1-5µg/ml for OFLO and SERR respectively. The regression equation calculated by least square method was y = 0.0178x + 0.0011 and y = 0.2289x – 0.002 with correlation coefficient of both drugs was r^2 = 0.9995 and r^2 = 0.9999.

In Method B (Fig. 6) overlay spectra of both drugs and their mixtures were shown. Fig.7 and Fig 8 were shown linearity of both the drugs in their respective wavelengths. The responses of first derivatives both drug shows linear concentration range of 10-50µg/ml and 1-5µg/ml for OFLO and SERR respectively. The regression equation calculated by least square method was y = 0.0062x – 0.0011 and y = -0.0755x –

0.0001 with correlation coefficient of both drugs was r^2= 0.9996 and r^2 = 0.9998. Summary of validation parameters by developed methods as shown in Table no 1.

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 no 2.

Precision

The Intraday and Interday precisions of the proposed spectrophotometric methods 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 no 3 and Table no 4.

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.

RESULTS AND DISSCUSION:

The selected drugs Ofloxacin and Serratiopeptidase in Bulk and Formulation were estimated by using both simultaneous equation method and first order derivatives of UV spectrophotometric methods as per ICH guidelines. The methods were validated for all validation parameters as per ICH guidelines. The linearity range in both methods for OFLO tand SERR was 10-50µg/ml and 1-5µg/ml respectively. The % RSD for intraday and inter-day precision was found to be less than 2%. The methods have been validated in assay of active pharmaceutical ingredients. The accuracy of the methods were validated by recovery studies and was found to be significant and within specification limits, with % recovery 99-101%. The assay results were found to be within the acceptable limits.

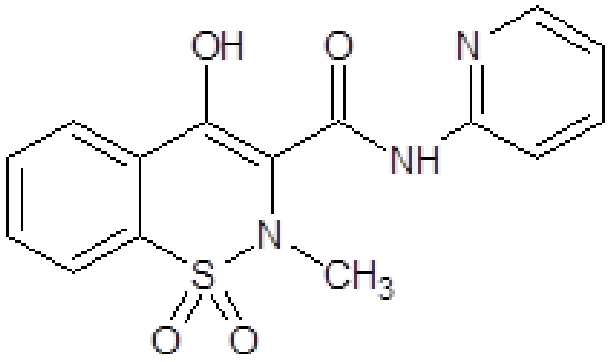

Fig 1: Chemical structure of Ofloxacin.

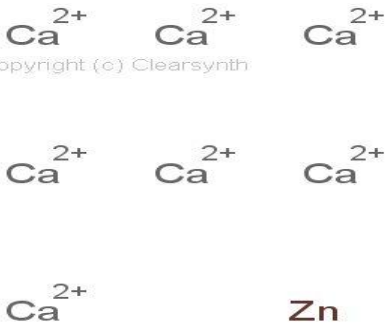

Fig 2: Chemical structure of Serratiopeptidase

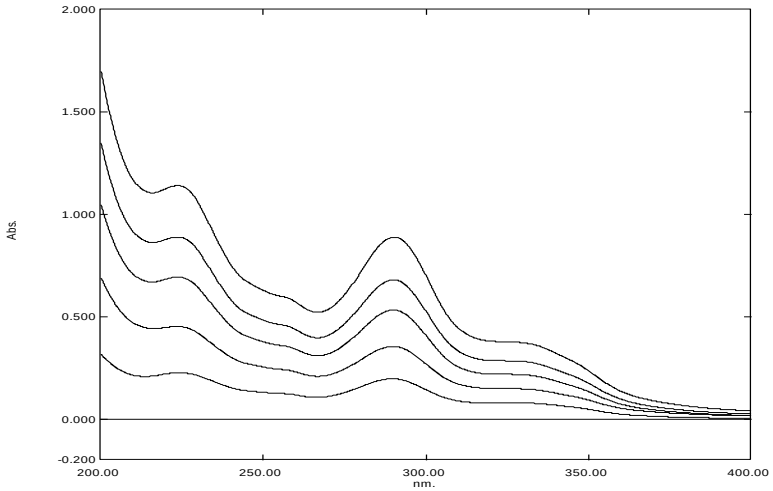

Fig 3: Overlay spectra of Standard Mixture

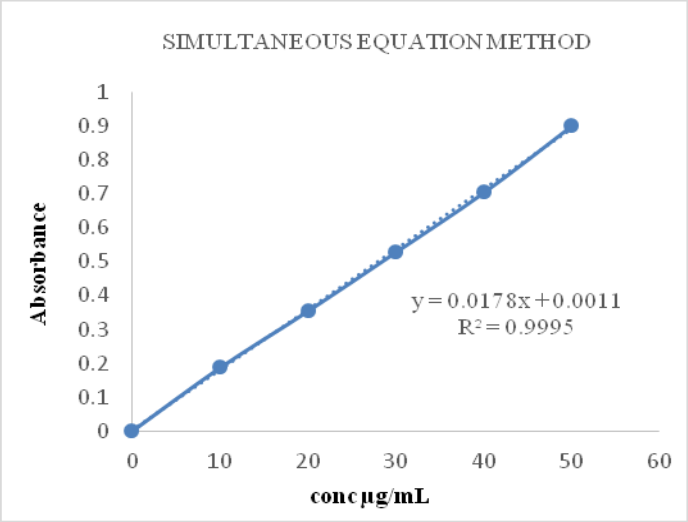

Fig 4: Calibration curve of Ofloxacin at 287nm

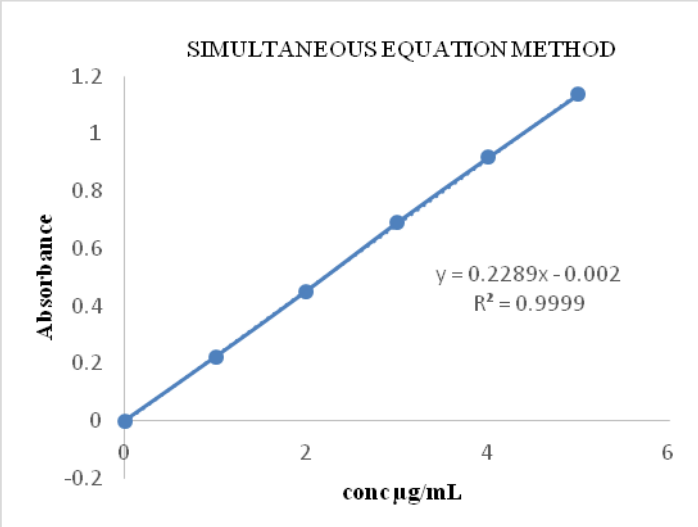

Fig 5: Calibration curve of Serratiopeptidase at 221nm

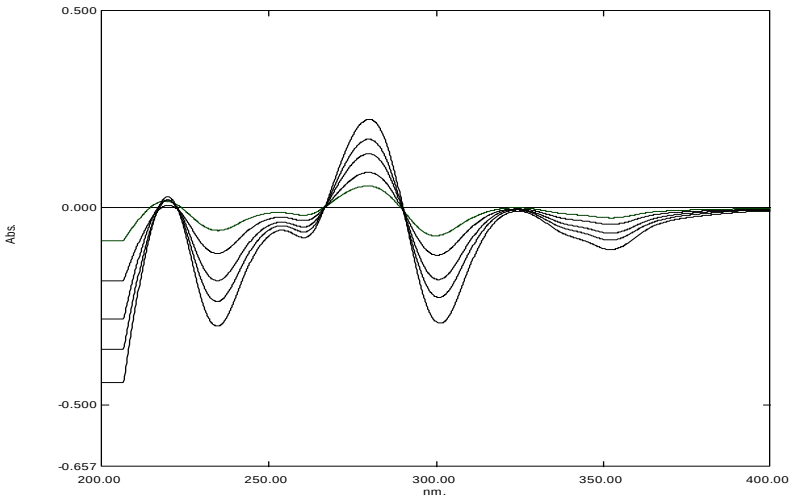

Fig 6: Overlay spectra of Standard Mixture

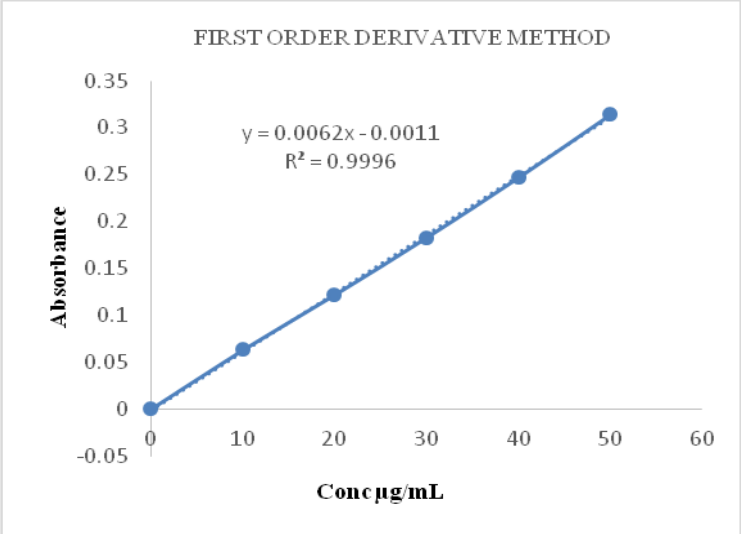

Fig 7: Calibration curve of Ofloxacin at 277nm

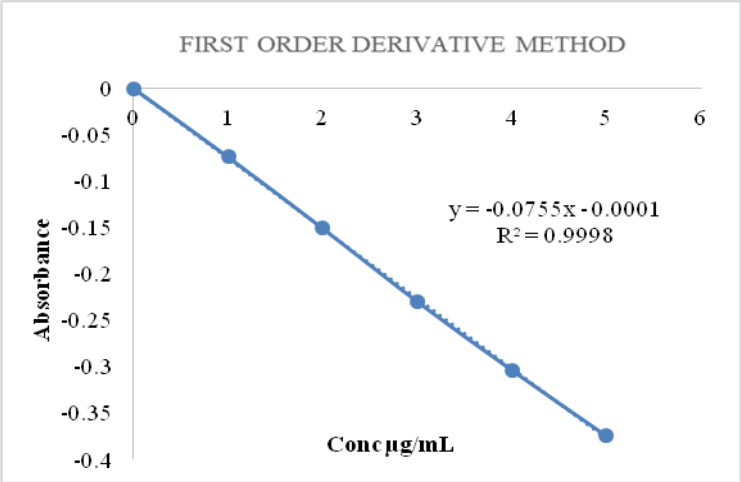

Fig 8: Calibration curve of Serratiopeptidase at 233nm

Table 1: Summary of Validation Parameters by Developed Methods.

Parameter	Method A		Method B	
	OFLO	SERR	OFLO	SERR
Wavelength (nm)	287	221	277	233
Linearity Range (µg/ml)	10-50	1-5	10-50	1-5
Regression equation (y = a + bc)	y = 0.0178x + 0.0011	y = 0.2289x – 0.002	y = 0.0062x- 0.0011	y = -0.0755x - 0.0001
Slope (b)	0.0178x	0.2289x	-0.0062x	0.0755x
Intercept (a)	0.0011	0.002	0.0011	0.0001
Correlation Coefficient (r^2)	0.9995	0.9999	0.9996	0.9998
LOD (µg/ml)	0.2728	0.0480	0.4345	0.0511
LOQ (µg/ml)	0.8269	0.1455	1.3169	0.1548

Table 2: Statistical Validation Data for Accuracy Determination.

Level of % Recovery	Components	Amount present (µg/ml)	Amount of Standard drug added (µg)	Method A			Method B		
				Total amount recovered (µg)	% Recovery	RSD	Total amount recovered (µg)	% Recovery	RSD
80%	OFL	20	16	35.99	99.97	0.1951	35.99	99.97	0.1951
	SERR	2	1.6	3.58	99.44	0.9710	3.58	99.44	0.9710
100%	OFL	20	20	39.98	99.95	0.1627	39.98	99.95	0.1627
	SERR	2	2	3.99	99.75	0.8809	3.99	99.75	0.8809
120%	OFL	20	24	43.95	99.88	0.3489	43.95	99.88	0.3489
	SERR	2	2.4	4.42	100.45	0.6787	4.42	100.45	0.6787

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

Components	Method A		Method B	
	OFLO	SERR	OFLO	SERR
Mean	100.16	100.00	100.16	100.00
Standard Deviation	0.4262	0.7745	0.6250	0.7745
Relative Standard Deviation	0.4255	0.7745	0.6239	0.7745
Standard Error	0.1740	0.3162	0.2552	0.3162

n*=6

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

Components	Method A		Method B	
	OFL	SERR	OFL	SERR
Mean	100.14	99.94	99.98	99.80
Standard Deviation	0.5698	0.8191	0.6643	0.7424
Relative Standard Deviation	0.5698	0.8195	0.6643	0.7430
Standard Error	0.2326	0.3344	0.2712	0.3031

n^*=3

CONCULSION:

The developed simultaneous equation method and first order derivative methods were found to be simple, precise, specific, and accurate and can be used for routine analysis of Ofloxacin and Serratiopeptidase. Both methods were validated as per ICH guidelines.

ACKNOWLEDGEMENT:

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

REFRENCES:

1. Indian Pharmacopoeia. India Pharmacopoeia commission, Ghaziabad, 2010; 3: 1808, 2010; 3:2097.
2. British Pharmacopoeia. 2003; 2: 1357.
3. United States Pharmacopoeia. 1995: 1213-14.
4. www.chemspider.com.
5. https://www.drugbank.ca/drugs/DB00397.
6. https://pubchem.ncbi.nlm.nih.gov/compounds.
7. https://en.wikipedia.org/wiki.
8. Laurenc LB, John SL, Keith LP. Goodman and Gilman's. The pharmacological basis of therapeutics; McGraw Hill, Newyork, U.S.A. 2005: 1119-21.
9. Satoskar RS, Nirmala NR, Bhandarkar SD. Pharmacology and Pharmacotherapeutics. 2013; 658, 1076.
10. Tripathi KD, Essential of Medical Pharmacology.2013: 134-37.
11. Sowmya C, Padmanabha Reddy Y, Ravindra Reddy J, Siva Maruti M, Roopesh GT, Santhosh Raja M. Simple UV- spectrophotometric methods for the estimation of Ofloxacin in pharmaceutical formulations. Int J Chem Sci, 2010; 8(2): 983 - 90.
12. Safila Naveed, Tanweer Alam, Asra Hameed, Neelam Sharif. UV- spectrophotometric method for estimation of Ofloxacin in tablet dosage form and comparative study of its two brands. J Bio equiv Availab, 2016; 8(3): 125-7.
13. Lobna A Hussein, Emad M Hussien, N Magdy, Heba S Mohamed. Simultaneous estimation of Ofloxacin and Cefixime in tablet form in presence of the inactive Ofloxacin USP Related compound A.Bulletin Faculty Pharm Cairo University, 2017; 55: 171–6.
14. Ekta Pandya J, Pankaj Kapupara, Ketan Shah V. Development and validation of simultaneous estimation of diclofenac potassium, paracetamol and serratiopeptidase by first order derivative UV- spectroscopy method in pharmaceutical formulation. J Chem and Ph Res, 2014; 6(5): 912 - 24.