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 ETORICOXIB AND SERRATIOPEPTIDASE BY RP-HPLC METHOD IN BULK AND PHARMACEUTICAL FORMULATIONS

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KEYWORDS:

Etoricoxib, Serratiopeptidase, RP-HPLC.

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ABSTRACT

A simple, rapid, specific, precise and accurate RP-HPLC method have been developed for simultaneous estimation of Etoricoxib and Serratiopeptidase in bulk drug and Pharmaceutical formulation. The separation was achieved by RP-C18 column with Phosphate buffer: Acetonitrile (pH 4.4) in the ratio of 70:30 was used as mobile phase. Flow rate was maintained at 1ml/min and UV detection was carried out at 278nm. Retention time for Etoricoxib and Serratiopeptidase was found to be 7.45min and 2.5min respectively. The method has been validated for linearity, accuracy and precision. Linearity for Etoricoxib and Serratiopeptidase were found in the range of 18-90µglml and 3-15µglml respectively. Recovery and assay studies of Etoricoxib and Serratiopeptidase were within 99% to 102% indicating that the proposed method is suitable for routine analysis of tablet formulation.

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INTRODUCTION:

Etoricoxib (Fig:1) is a 5-chloro-2-(6-methylpyridin-3-yl)-3-(methylsulfonylphenyl) pyridine.[1] It is COX-2 selective inhibitor. It is used for treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, chronic low back pain, acute pain and gout. It selectively inhibits isoform 2 of the enzyme cyclooxygenase (COX-2). It has approximately 106-fold selectively for COX-2 inhibition over COX-1. This reduces the generation of prostaglandins (PGs) from arachidonic acid. [2-4]

Serratiopeptidase (Fig:2) is a proteolytic enzyme produced by enterobacterium serratia.[5] This anti-inflammatory proteolytic enzyme binds to the alpha-2-macroglobuline in the blood, which helps to mask its antigenicity. Then it is slowly transferred to the site of inflammation. Serratiopeptidase hydrolyse bradykinin, histamine, serotoxin responsible for oedema. It reduces swelling improves microcirculation & expectoration of sputum. Due to this Serratiopeptidase has anti-inflammatory, antioedemic & fibrinolytic activity & act rapidly on localized inflammation.[6] It is official in Indian Pharmacopoeia.[7] The combination of Etoricoxib & Serratiopeptidase is used for joint swelling, bones & joints pain, muscles pain, joint inflammation, muscles aching, migraine & other conditions.

On literature survey, Etoricoxib alone has been estimated & simultaneous estimation in combination with other drugs has been reported.[8-10] Serratiopeptidase alone has been estimated & simultaneous estimation in combination with other drugs has been reported.[11-13] It was found that no method has been reported for the simultaneous estimation of Etoricoxib & Serratiopeptidase in combined dosage form & no method is available in pharmacopoeia. In the view of the need for a suitable method for routine analysis in combined formulations, attempts are being made to develop simple, precise & accurate analytical methods for simultaneous estimation of titled drugs & extend it for their determination in formulations.

MATERIALS AND METHODS:

Instrument used:

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 and all other chemical reagents were of analytical grade.

Drug sample:

Standard Etoricoxib was procured from Yarrow chem products, Mumbai. Standard gift sample of Serratiopeptidase was obtained from IPCA laboratories limited, Mumbai.

Preparation of mobile phase:

The mobile phase was prepared by mixing of buffer solution and acetonitrile in the ratio of 70:30v/v and pH was adjusted to 4.4 with triethylamine. The mobile phase was filtered through a 0.45µm filter paper and then it was sonicated for 10min.

Preparation of Buffer solution:

2.72g of Potassium dihydrogen phosphate was dissolved in 1000ml of HPLC grade distilled water (0.02M).

Preparation of Standard stock solution:

50 mg each of ETOR and SERR were weighed separately and transferred in two different 50 mL volumetric flasks. Both the drugs were dissolved in 25 mL of mobile phase by sonication and then volume was made upto 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 10 ml of aliquot was pipetted out into a 100 ml volumetric flask and the volume was made up to the mark with mobile phase to obtain a concentration of 100µg/ml of each component (stock B and B' solution). From the stock solution B and B’ further dilution were made to get the concentration of 18-90µg/ml for Etoricoxib and 3-15 µg/ml for Serratiopeptidase respectively.

Sample Solution:

Twenty tablet which contain both Etoricoxib and Serratiopeptidase were taken and crushed into fine powder. An accurately weighed amount of powder equivalent to 100mg of Etoricoxib was taken and dissolved in a mobile phase and made up to the mark of a 100ml volumetric flask. The solution was filtered through 0.4 µm membrane filter paper. The contents were sonicated for 20minutes and the final volume was made upto the mark with mobile phase. From this, required dilutions are made to get final concentration within in their range.

RESULTS AND DISCUSION

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

Specificity and selectivity

The specificity of the HPLC method was determined by comparing the chromatograms of the standard and sample solutions. The parameters like retention time, resolution and tailing factor were calculated. Good correlation was found between the results of standard and sample solution. The method was selective, showed no interference peaks around the retention time, base line showed no significant noise.

Linearity

Linearity was demonstrated for Etoricoxib in the range of 18-90µg/ml and for Serratiopeptidase 3-15µg/ml, the Correlation coefficient (r^2) values were >0.999. Typically, the regression equations for the calibration curve was found to be y=32170x-86.095 and y = 35161x-1984.2 for Etoricoxib and Serratiopeptidase respectively. Summary of validation parameters by developed methods are shown in Table 1.

Accuracy

Recovery studies were carried out by adding 80%, 100% and 120% of the standard drug solution of ETOR and SERR to the known amount of sample solution by standard addition method. The sample response was obtained and drug concentrations of ETOR and SERR were calculated by using statistical data. The mean % recoveries were within 99-102% and are shown in Table 2.

Precision

The intra-day and inter-day precision of ETOR and SERR was determined by three times on the same day and on three different days respectively. The precision of the method was expressed in terms of % relative standard deviation. The summary of Intra-day and Inter-day precision methods are shown in Table 3 and Table 4.

LOD and LOQ

The limit of detection (LOD) is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value. The limit of quantification (LOQ) is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

Based on the standard deviation of the response and the slope the LOD and LOQ are expressed as:

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.

LOD and LOQ was found to be 0.234µg/ml and 0.708µg/ml for Etoricoxib and 0.391µg/ml and 1.185µg/ml for Serratiopeptidase respectively.

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 36µg/ml of Etoricoxib 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 36µg/ml of Etoricoxib and 6µg/ml of Serratiopeptidase was injected into sample injector of HPLC two times by different analysts. The results are tabulated in Table 7.

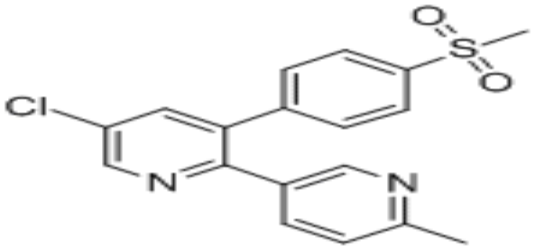

Fig 1: Structure of Etoricoxib

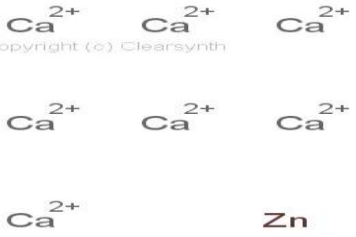

Fig 2: Structure of Serratiopeptidase

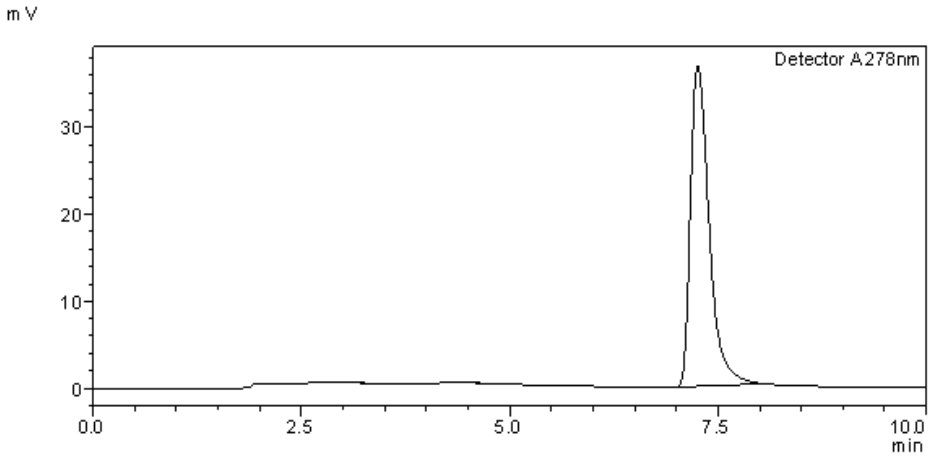

Fig. 3: Chromatogram of Etoricoxib

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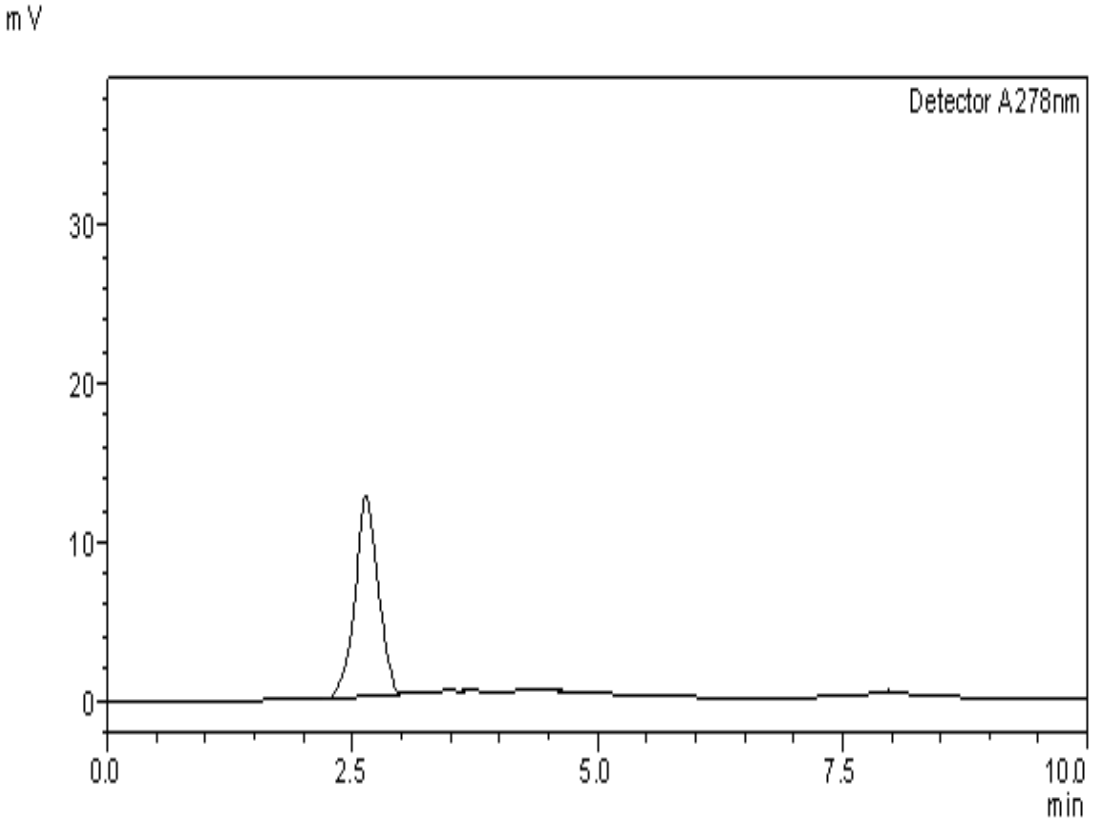

Fig. 4: Chromatogram of Serratiopeptidase

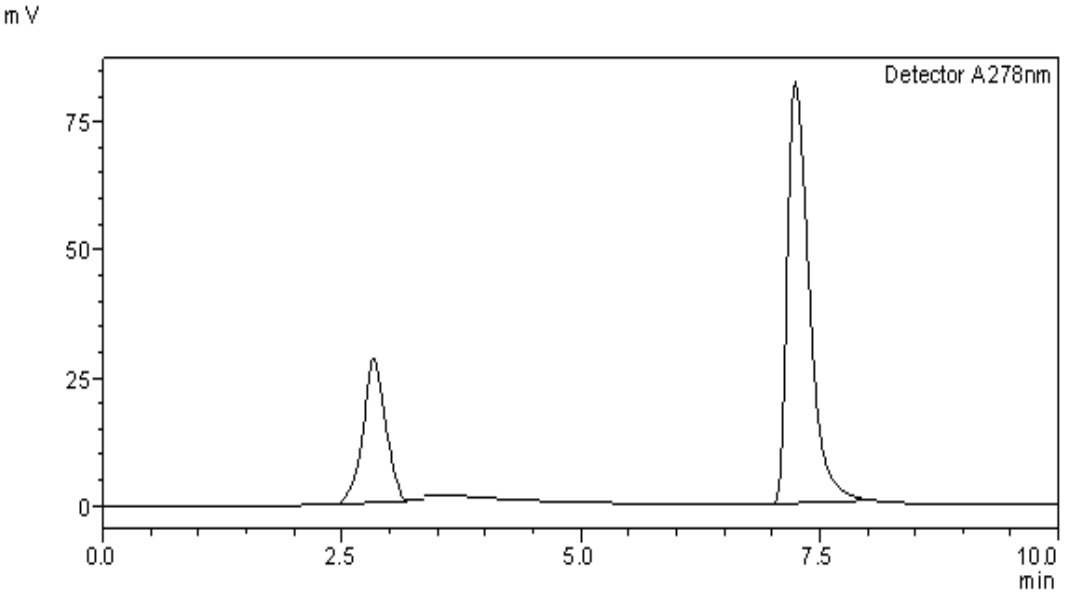

Fig. 5: Chromatogram of Etoricoxib and Serratiopeptidase

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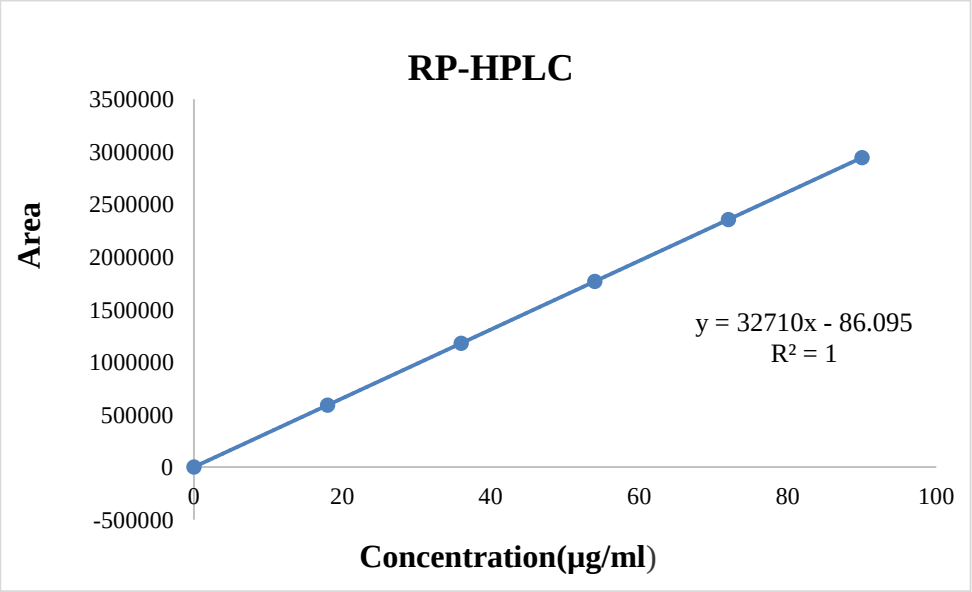

Fig 6: Calibration curve of ETOR at 278nm by RP-HPLC method

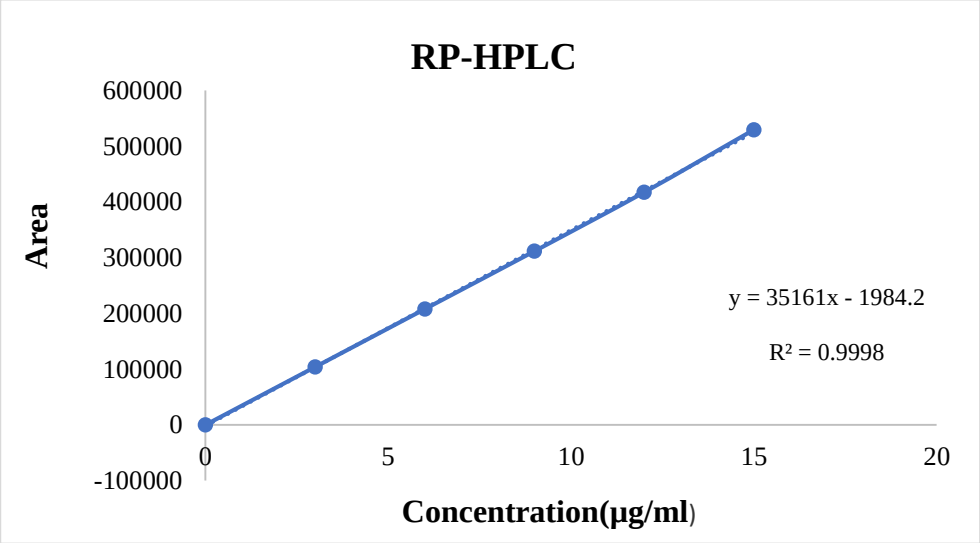

Fig 7: Calibration curve of SERR at 278nm by RP-HPLC method

Table 1: Summary of validation and system suitability parameters of ETOR and SERR

Parameters	ETOR	SERR
Linear range(µg/ml)	18-90 µg/ml	3-15 µg/ml
Slope	32710	35161
Intercept	86.095	1984.2
Regression coefficient (r2)	1	0.9998
Limit of Detection (µg/ml)	0.234	0.391
Limit of Quantification (µg/ml)	0.708	1.185
Retention time (min)	7.454	2.581
Tailing factor	1.430	1.063
Resolution factor	12.987	
Theoretical plate	3452	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%	ETOR	36	28.8	43.18	99.97	0.0388
	SERR	6	4.8	7.20	99.93	0.07652
100%	ETOR	36	36	47.91	99.96	0.0765
	SERR	6	6	7.9	99.5	0.3838
120%	ETOR	36	43.2	52.75	99.97	0.0811
	SERR	6	7.2	8.76	99.82	0.2665

Table 3: Statistical validation data for Intra-day precision.

Components	Method A	
	ETOR	SERR
Mean	99.86	99.33
Standard Deviation	0.1916	0.2981
Relative Standard Deviation	0.1919	0.3001
Standard Error	0.0785	0.1221

n* = 6

Table 4: Statistical validation data for Inter-day precision.

Components	Method A	
	ETOR	SERR
Mean	99.98	99.62
Standard Deviation	0.15508	0.5527
Relative Standard Deviation	0.1553	0.5547
Standard Error	0.3191	0.0895

n* = 3

Table 5: Robustness results for variations in flow rate(ml/min)

Method parameter	Level	Retention Time		Tailing factor	
Flow rate (ml/min)		ETOR	SERR	ETOR	SERR
0.9	-1	7.520	2.657	1.467	1.085
1.0	0	7.454	2.581	1.439	1.063
1.1	+1	7.382	2.559	1.418	1.075

Table 6: Robustness results for variations in wavelength (nm)

Method parameter	Level	Retention Time		Tailing factor	
Wavelength (nm)		ETOR	SERR	ETOR	SERR
280	+2	7.621	2.498	1.489	1.079
278	0	7.454	2.581	1.439	1.063
276	-2	7.407	2.601	1.398	1.104

Table 7: Ruggedness results for variations in Analysts

Parameter	Retention time		Tailing factor	
Analysts	ETOR	SERR	ETOR	SERR
Analysts 1	7.454	2.581	1.439	1.063
Analysts 2	7.459	2.584	1.440	1.067

CONCLUSION:

An accurate, sensitive and precise HPLC method with UV detection for the simultaneous estimation of Etoricoxib and Serratiopeptidase was developed and validated for quality control analysis in combined tablets. The proposed method is rapid, where the total analytical run time for both drugs are less than 10min and shows high degree of accuracy and precision. It is convenient for laboratory quality control of tablet dosage forms containing both substances.

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.

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