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**Pharmaceutical Sciences** **RESEARCH ARTICLE……!!!**

# SIMULTANEOUS DETERMINATION AND VALIDATION OF ETORICOXIB AND SERRATIOPEPTIDASE BY ZERO ORDER AND SECOND ORDER DERIVATIVE METHODS IN BULK AND PHARMACEUTICAL FORMULATIONS

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**KEYWORDS:**

Etoricoxib, Serratiopeptidase, Zero order method, Second order derivative, Validation.

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**ABSTRACT**

Two simple, accurate and rapid UV Spectrophotometric methods (Zero order method and Second order derivative method) have been developed for the quantitative estimation of Etoricoxib and Serratiopeptidase in combined pharmaceutical dosage forms by using water as a solvent. The two wavelengths 285nm and 223nm were selected for Etoricoxib and Serratiopeptidase in Zero order method. The Beer's law obeyed in the concentration range of 12-60µg/ml and 2-10µg/ml with correlation coefficient $r^2$ = 0.9996 and $r^2$ = 0.9997 for Etoricoxib and Serratiopeptidase respectively. LOD and LOQ was found to be 0.3963 µg/ml and 1.2009µg/ml for Etoricoxib and 0.0679µg/ml and 0.2060µg/ml for Serratiopeptidase respectively. Second order derivative method involves measurements of absorbance at the wavelength of 289nm and 228nm for Etoricoxib and Serratiopeptidase respectively. Linearity range was found to be 12-60 µg/ml and 2-10 µg/ml with correlation coefficient $r^2$ = 0.9999 and $r^2$ =0.9998 for Etoricoxib and Serratiopeptidase respectively. LOD and LOQ was found to be 0.5497µg/ml and 1.6658µg/ml for Etoricoxib and 0.0577µg/ml and 0.17506µg/ml for Serratiopeptidase respectively. In both the methods the % RSD for intra-day and inter-day precision were found within 2%.

## 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.[8-9] Serratiopeptidase alone has been estimated & simultaneous estimation in combination with other drugs has been reported.[10-13] 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

A double-beam Shimadzu UV/Vis spectrophotometer, 1800 with spectral bandwidth of 1± 0. 2 nm, wavelength accuracy of ±0.3 nm and a pair of 1-cm matched quartz cells was used to measure absorbance of the resulting solution.

### Chemicals

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

## Methods

### Preparation of Standard solution

100mg each of Etoricoxib (ETOR) and Serratiopeptidase (SERR) were weighed separately and transferred into two different 100ml Volumetric flasks. ETOR was dissolved in minimum quantity of methanol and SERR in 10ml distilled Water for dissolution, and then final volume of both the solution was made upto 100ml with distilled water to obtain final concentration of 1000µg/ml of each component (Stock A solution)

From the above Stock A solution, 10ml was pipetted out into two different 100ml volumetric flask and the final volume was made upto the mark with distilled water to obtain the concentration of 100µg/ml (Stock B solution). From the stock B solution further dilution were made to get concentration from 12-60µg/ml for Etoricoxib and 2-10µg/ml for Serratiopeptidase.

### Preparation of 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 distilled water and made up to the mark of a 100ml volumetric flask. The solution was filtered through Whatmann filter paper no. 41. From this, required dilutions are made to get final concentration within in their range.

### Method of estimation

### Method A (Zero order method)

For the estimation of ETOR and SERR by Zero order method, both the solutions were prepared separately and were scanned in the spectrum mode from 400 to 200nm. The analysis of both the drugs was done at wavelengths 285nm and 223nm for ETOR and SERR respectively.

### Method B (Second order derivative)

The most characteristic feature of a second order derivative is a negative band with minimum at the same wavelength as the maximum on the Zero order band. From the Second order derivative spectra of standard ETOR and SERR wavelength selected for their estimation was 289nm and 228nm respectively.

## VALIDATION PARAMETER

### Linearity

In Method A (Fig.3) overlay spectra of the mixture and Fig.4 and Fig.5 were shown the linearity of both the drugs in their respective wavelengths. The responses for both drugs show linear concentration range of 12-60µg/ml and 2-10µg/ml for ETOR and SERR respectively.

The regression equation calculated by least square method was y =0.0086x + 0.0053 and y = 0.1018x + 0.0029 with correlation coefficient of both drugs was $r^2$ = 0.9996 and $r^2$ = 0.9997.

In Method B (Fig: 6) overlay spectra of the mixture and Fig.7 and Fig 8 were shown the linearity of both the drugs in their respective wavelengths. The responses of second derivatives for both drugs show linear concentration range of 12-60µg/ml and 2-10µg/ml for ETOR and SERR respectively. The regression equation calculated by least square method was y = -0.0031x – 0.0003 and y = -0.043x + 0.0025 with correlation coefficient of both drugs was $r^2$= 0.9999 and $r^2$ = 0.9998. Summary of validation parameters by developed methods as shown in Table no 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 results are tabulated in Table no 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 as shown in Table no 3 and Table no 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.

**RESULTS AND DISCUSSION**

The methods discussed in the present work provide a convenient, precise and accurate way for simultaneous estimation of Etoricoxib and Serratiopeptidase in its bulk and pharmaceutical dosage form. The methods were validated for all validation parameters as per ICH guidelines. The linearity was obtained in concentration range of 12-60µg/ml and 2-10 µg/ml for Etoricoxib and Serratiopeptidase respectively. The % RSD for repeatability (n=6), intra-day and inter-day (n=3) precision was found to be less than 2% indicating the precision of method. Accuracy of proposed methods was ascertained by

recovery studies and the results are expressed as % recovery. % recovery for ETOR and SERR was found within the range of 98 % and 102%. The assay results are found to be within the limits.

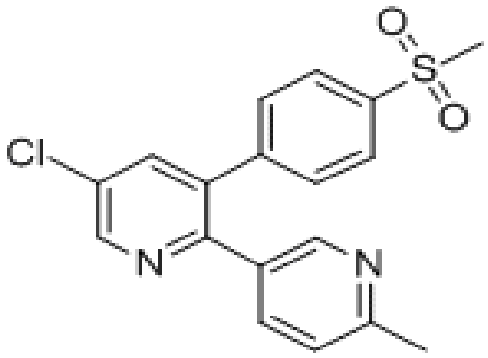


**Fig 1: Structure of Etoricoxib**

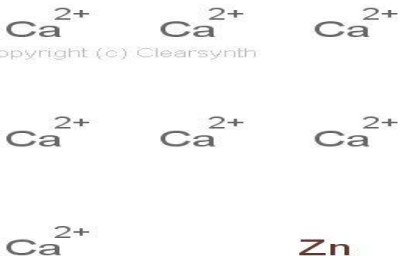


**Fig 2: Structure of Serratiopeptidase**

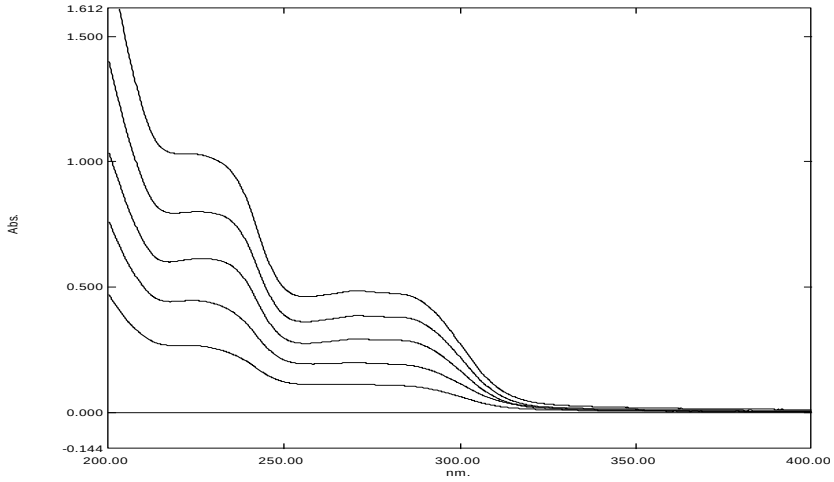


**Fig 3: overlay Spectra of ETOR and SERR by Zero order method.**

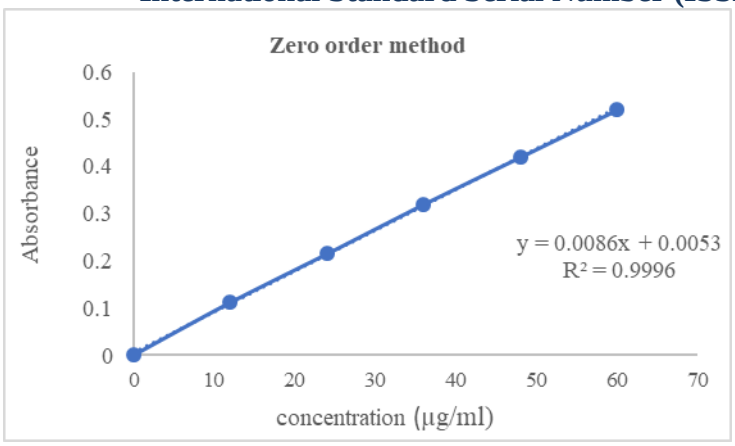


**Fig 4: Calibration curve of ETOR at 285nm**

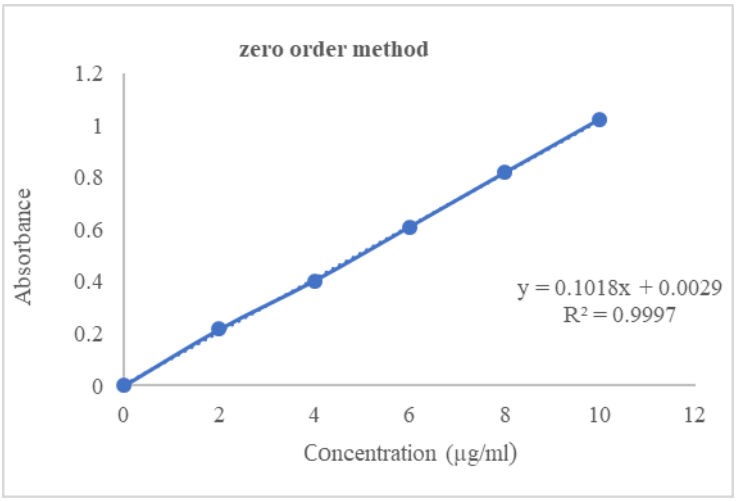


**Fig 5: Calibration curve of SERR at 223nm**

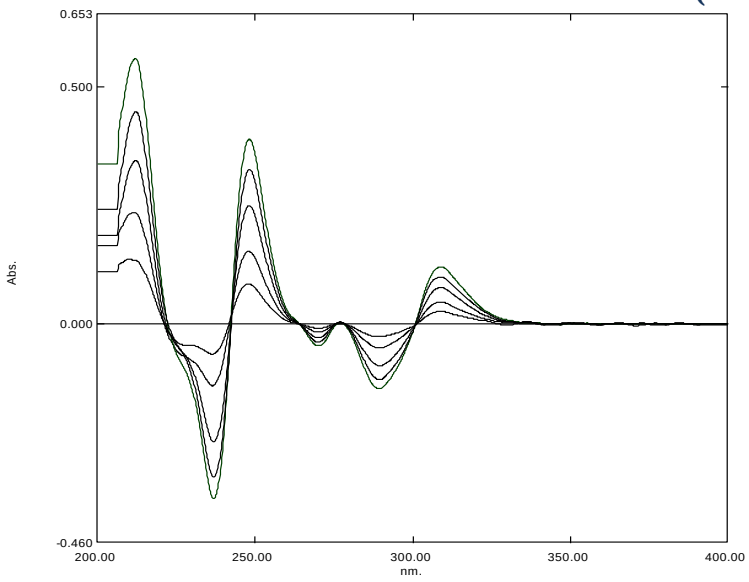


**Fig 6: overlay Spectra of ETOR and SERR by Second order method.**

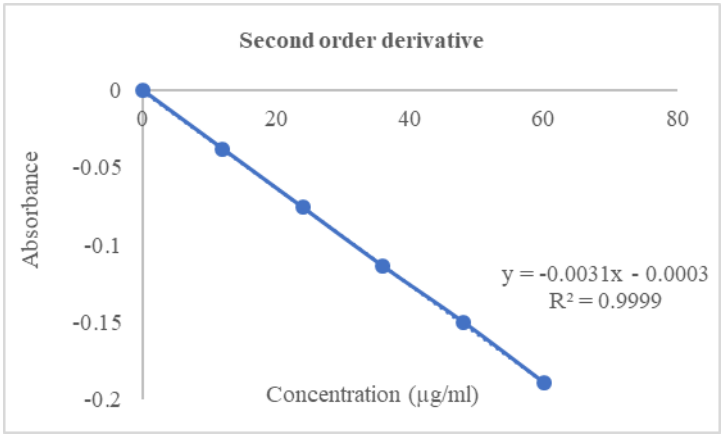


**Fig 7: Calibration curve of ETOR at 289nm**

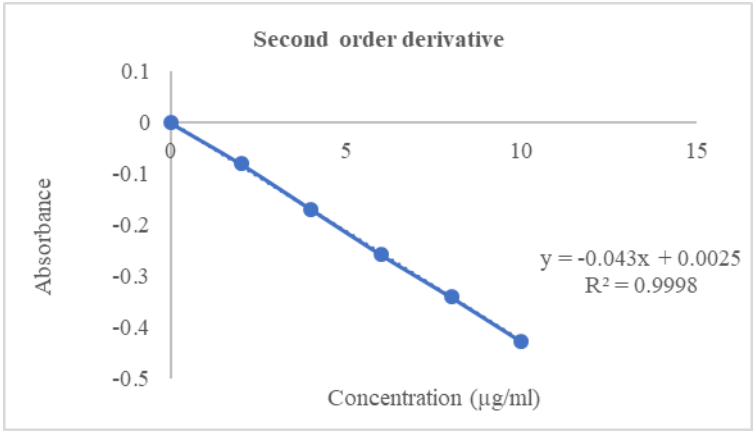


**Fig 8: Calibration curve of SERR at 228nm**

**Table 1: Summary of Validation Parameters by Developed Methods.**

| Parameter | Method A | | Method B | |
|---|---|---|---|---|
| | ETOR | SERR | ETOR | SERR |
| Wavelength (nm) | 285 | 223 | 289 | 228 |
| Linearity Range (µg/ml) | 12-60 | 2-10 | 12-60 | 2-10 |
| Regression equation (y = a + bc) | y = 0.0086x + 0.0053 | y = 0.1018x + 0.0029 | y = -0.0331x - 0.0003 | y = -0.043x + 0.0025 |
| Slope (b) | 0.0086x | 0.1018x | -0.0331x | 0.043x |
| Intercept (a) | 0.0053 | 0.0029 | -0.0003 | 0.0025 |
| Correlation Coefficient ($r^2$) | 0.9996 | 0.9997 | 0.9999 | 0.9998 |
| LOD (µg/ml) | 0.3963 | 0.0679 | 0.5497 | 0.0577 |
| LOQ (µg/ml) | 1.2009 | 0.2060 | 1.6658 | 0.1750 |

**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% | ETOR | 24 | 19.2 | 43.18 | 99.86 | 0.2141 | 43.16 | 99.81 | 0.2598 |
| | SERR | 4 | 3.2 | 7.20 | 100 | 0.8126 | 7.19 | 99.7 | 0.8126 |
| 100% | ETOR | 24 | 24 | 47.91 | 99.81 | 0.3022 | 47.89 | 99.77 | 0.3014 |
| | SERR | 4 | 4 | 7.9 | 99.24 | 0.7516 | 7.94 | 99.25 | 0.7023 |
| 120% | ETOR | 24 | 28.8 | 52.75 | 99.79 | 0.1028 | 52.65 | 99.71 | 0.1158 |
| | SERR | 4 | 4.8 | 8.76 | 99.46 | 0.2883 | 8.72 | 99.09 | 0.2985 |

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

| Components | Method A | | Method B | |
|---|---|---|---|---|
| | ETOR | SERR | ETOR | SERR |
| Mean | 99.82 | 99.91 | 99.75 | 99.93 |
| Standard Deviation | 0.2390 | 0.2581 | 0.2487 | 0.2754 |
| Relative Standard Deviation | 0.2394 | 0.2597 | 0.2499 | 0.2763 |
| Standard Error | 0.0979 | 0.1058 | 0.1023 | 0.1345 |

**n*=6**

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

| Components | Method A | | Method B | |
|---|---|---|---|---|
| | ETOR | SERR | ETOR | SERR |
| Mean | 99.71 | 99.47 | 99.89 | 99.52 |
| Standard Deviation | 0.2768 | 1.0311 | 0.2832 | 1.151 |
| Relative Standard Deviation | 0.2776 | 1.0365 | 0.2658 | 1.255 |
| Standard Error | 0.1598 | 0.5953 | 0.1756 | 0.6553 |

**n*=3**

## CONCULSION:

The developed Zero order and Second order derivative methods were found to be simple, precise, specific, and accurate and can be used for routine analysis of Etoricoxib and Serratiopeptidase. Both methods were validated as per ICH guidelines.

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