

## QUANTITATIVE DETERMINATION AND VALIDATION OF ETORICOXIB AND PARACETAMOL COMBINED TABLET DOSAGE FORM BY REVERSE PHASE-HPLC

Nandini Goudar<sup>1</sup>, Bharathi Tejas<sup>2</sup>, Muddukrishna Badamane Sathyanarayana<sup>3</sup>, Aravind Pai<sup>4</sup> and Vasudev Pai<sup>5</sup>, ✉

<sup>1</sup>Department of Pharmaceutical Chemistry, Vivekananda College of Pharmacy, Bengaluru-560055, Karnataka, India.

<sup>2</sup>Department of Quality Assurance, Analytica Chemie Inc. Peenya, Bengaluru-560058, Karnataka, India

<sup>3</sup>Department of Pharmaceutical Quality Assurance Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal-576104, Karnataka, India

<sup>4</sup>Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal-576104, Karnataka, India

<sup>5</sup>Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal-576104, Karnataka, India

✉Corresponding Author: [pai.vasudev@manipal.edu](mailto:pai.vasudev@manipal.edu)

### ABSTRACT

An RP-HPLC technique was used to produce a cost-effective simultaneous quantification of Etoricoxib and Paracetamol in combined pharmaceutical tablet dosage forms. The approach was validated for Etoricoxib and Paracetamol using ICH recommendations over a range of 20-120ppm and 20-200ppm, respectively. At a temperature of 25°C±0.5°C, an analytical column PURITAS™ EXIMIUS C18, 250mmx4.6mm, 5 microns was utilized. At a flow rate of 1.0 ml/min, the mobile phase was acetonitrile and 0.1 percent acetic acid in water in a 70:30V/V ratio. The elution was examined using a PDA detector with a detection wavelength of 235nm. The retention times for Etoricoxib and paracetamol are 4.2 and 2.1 minutes, respectively. The percentage recoveries for Etoricoxib and paracetamol were 98.28% and 102.1%, respectively. The RSD values are not greater than 2%.

**Keywords:** Quantification, Etoricoxib, Paracetamol, Reverse Phase-HPLC, Tablet Dosage Form.

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### INTRODUCTION

Etoricoxib is a COX-2 selective inhibitor comprised of 5-chloro-2-(6-methyl pyridin-3-yl)-3-[4-(trideuteriomethylsulfonyl) Phenyl] pyridine that has been approved in several countries throughout the world (e.g. some Latin American countries and the UK).<sup>1-5</sup> Rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, chronic low back pain, acute pain, and gout are all treated with it.<sup>6,7</sup> Etoricoxib, like any other COX-2 selective inhibitor, inhibits isoform 2 of the cyclooxygenase enzyme, which decreases the generation of prostaglandins (PGs) from arachidonic acid (COX-2).<sup>8-10</sup> Pain, edema, and inflammation are all caused by prostaglandins. This limits the effect of analgesics and anti-inflammatory drugs in the peripheral nervous system. Rheumatoid arthritis, osteoarthritis, gout, postoperative tooth pain, acute and chronic lower back pain (including chronic musculoskeletal pain), and primary dysmenorrhoea are among the indications.<sup>11,12</sup> In well-designed studies, Etoricoxib is effective in the treatment of various arthritis like osteoarthritis, rheumatoid arthritis, lower back pain, acute gout, postoperative tooth pain, primary dysmenorrhoea, and haemophilic arthropathy. Etoricoxib has well tolerated and has a greater GI profile than the non-COX selective non-steroidal anti-inflammatory drugs (NSAIDs).<sup>13</sup> Paracetamol is a non-opioid analgesic comprised of N-(4-hydroxyphenyl) acetamide that is used to treat moderate to mild pain and pyrexia as first-line therapy.<sup>14,15</sup> It is widely recommended for low back pain, orthopedic, traumatic, and rheumatologic illnesses since it is less sedative than other centrally acting muscle relaxants Fig.-1. Chemical Structures of Etoricoxib and Paracetamol.

Despite its modest effectiveness, paracetamol is frequently used for analgesia in osteoarthritis, owing to a lack of effective or well-tolerated alternatives and its relative safety.<sup>16</sup> Paracetamol is an anti-inflammatory and analgesic muscle relaxant.<sup>17</sup> As a result, a unique combination of both Etoricoxib and paracetamol works as a powerful anti-inflammatory and analgesic medicine for the management of the pain.<sup>18</sup> The identification of these drugs in pharmaceutical dose forms, either alone or in combination with other pharmaceuticals, has been documented using spectrophotometric methods, HPLC methods stability-indicating methods, and plasma extraction methods, according to a literature review with the combination of the above-mentioned medicines, a few UV, HPLC methods.<sup>19-22</sup> There are options with smaller linearity ranges and/or longer retention times. The author attempted to design and validate a low-cost and precise RP-HPLC estimation method for Etoricoxib and Paracetamol in formulated dosage forms. The new technique has been verified according to ICH and all applicable requirements for a wider linearity range than any other method now available, as well as improved retention periods and shorter run times, and compatibility with LCMS.<sup>23</sup>

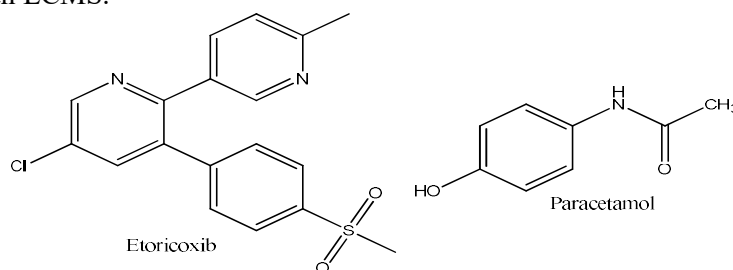


Fig.-1: Chemical Structures of Etoricoxib and Paracetamol

## EXPERIMENTAL

Reference standard of Etoricoxib and paracetamol gift samples provided from Analytica Chemi private limited Bangalore. Tablets containing 60mg of Etoricoxib and 325mg of Paracetamol were purchased from the pharmacy. S.D fine chemical, Bangalore, provided HPLC quality acetonitrile, water, and acetic acid. Water series No. 2996 Photodiode array detector connected to water HPLC 2695 series with Hamilton syringe and autosampler for chromatography. The system additionally includes a degasser for removing dissolved air and a column oven for maintaining the required temperature. The chromatographic conditions were a mobile phase of acetonitrile: 0.1 % acetic acid in water 70:30 v/v with 1.0ml flow rate as well as a stationary phase of Puritas Eximius C18, 250X4.6mm, 5 microns with an injection volume of 5 microliters. The wavelength of the detectors was set at 235nm. Throughout the separation process, the column temperature was kept at room temperature. The freshly prepared mobile phase was filtered via a 0.45 $\mu$  nylon filter.

### Preparation of the Buffer Solution

The buffer solution was prepared by dissolving 1 mL of acetic acid in 1 liter of distilled water and filtering the solution through a 0.45  $\mu$  nylon filter.

### Preparation Standard Solution

10mg of Etoricoxib and Paracetamol reference standards were weighed using a sensitive weighing balance and transferred to volumetric flasks with a capacity of 25mL, where it was dissolved in diluent (mobile Phase). To facilitate dissolution, the solutions were sonicated for 5 minutes before being built up to volume with the same diluent. The dilutions and concentrations of the stock solution are shown in Table-1.

### Preparation of Sample

Weighed and pulverized into fine powder ten tablets containing 60mg Etoricoxib and 325mg Paracetamol. A powder containing 60mg Etoricoxib and 325mg Paracetamol was weighed using a sensitive weighing balance and transferred to a 50 mL volumetric flask and a few ml of diluent was also added. The above solution is sonicated for 30 minutes to solubilize completely and 5ml of the solution was transferred to a 100ml volumetric flask, also diluent was added to make up the volume. The final solution was filtered using a 0.45 $\mu$  membrane syringe filter.

Table-1: Standards Calibration Curve Data Used

Etoricoxib stock solution conc.(PPM)	Volume taken (ml)	Final Volume	Conc. of Etoricoxib ( $\mu\text{g/ml}$ )	Paracetamol stock solution Conc.(PPM)	Volume taken (ml)	Final Volume	Conc. of Paracetamol ( $\mu\text{g/ml}$ )
400	0.5	10	20	400	0.5	10	20
	1.0		40		1		40
	1.5		60		2		80
	2		80		3		120
	2.5		100		4		160
	3		120		5		200

## RESULTS AND DISCUSSION

Method development begins with the selection of a prescription combination, dissolving both drugs in a suitable HPLC grade diluent to give a clean solution. As per the literature of Dr. Chaple *et al.* who reported one UV technique for Simultaneous quantification of Etoricoxib and Paracetamol in combination tablet dosage form. Furthermore, three HPLC techniques for the simultaneous measurement of Etoricoxib and Paracetamol have been published. In the first paper, S. R. Pattan and S. G. Jamdar presented a 7-minute run approach with a phosphate-added buffer as the mobile phase. Maitreyi zaveri and Amit khandhar reported a 20-minute run with the gradient method in the second paper. Third, Krishna R. Gupta and Amruta Likhar of a 10-minute run of the isocratic method. As a result, the current research was designed to optimize the chromatographic conditions for the suggested investigation. During the experimental experiments, the mobile phase composition and pH conditions were varied. After experimenting with various combinations, we eventually settled on a mobile phase composition. Acetonitrile: 0.1% Acetic acid (70:30) as a result of good peak form and improved resolution. Furthermore, throughout the study, it was discovered that the acetic acid reduced the tailing effect in the chromatogram. This combination of mobile phases was selected for the development of the chromatogram. The approach was validated by ICH Q2 (R1) guideline and the following parameters were considered for the study appropriateness of the system precision, accuracy, specificity, robustness, linearity, LOD, and LOQ and all critical factors considered. Six replicate injections of a 100% target solution of Etoricoxib and Paracetamol were used to assess the appropriateness of the system. The number of theoretical plates, area, and peak tailing were all calculated, and all parameters were found to be within the limits. (Fig.-2, Table-2) shows the HPLC Chromatogram and displays the results.

Table 2 shows the System Appropriateness Parameter

Parameters	Etoricoxib	Paracetamol
USP Tailing	1.34	1.1
Theoretical Plates	4485	10237

### Specificity

Specificity refers to the capacity to verify the analyte in the presence of components that may be expected to be present. Impurities, deterioration, and matrix are examples of these. There was no interaction with the drug peak from excipients or other components. As a result, the method followed has been found to be very specific.

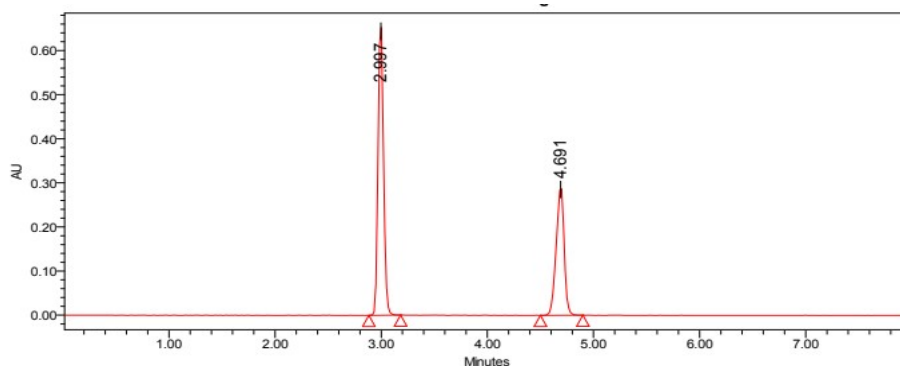


Fig.-2. HPLC Chromatogram

**Linearity**

The method's linearity was tested by producing concentration ranges of 20-120ppm and 20-200ppm. Under the chromatographic conditions mentioned above, 9.95-29.84 Etoricoxib and paracetamol stock solutions were prepared and injected. The drug concentration was plotted against the individual 235nm peak regions to create calibration curves. Within the concentration range, the results demonstrated a substantial link between detector response and drug concentration level. Both drugs exhibited a linear response with the equation  $Y=mx+C$ . (Fig.-3, Fig.-4) show the calibration curve of Etoricoxib and Paracetamol.

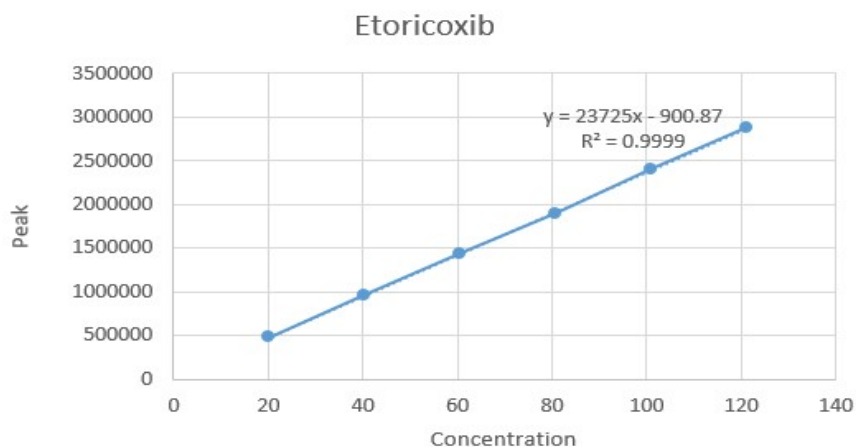


Fig.-3: Etoricoxib Calibration Curve

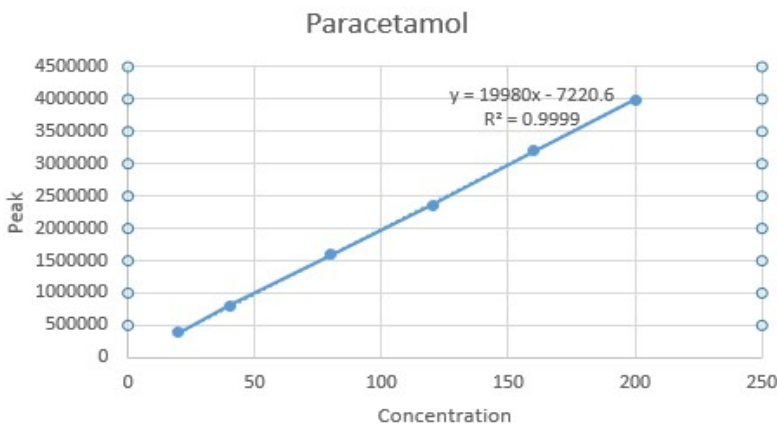


Fig.-4: Paracetamol calibration curve

**Accuracy**

The accuracy was determined by analyzing the tablet and standard at three concentration levels: low, medium, and high. The accuracy was evaluated from three replicate injections and calculated to be 50%, 100%, and 150% of the label claim once Etoricoxib and Paracetamol tablet solution were added. (Table-3) summarises the outcomes of the approach, which was proven to be accurate.

Table 3: Paracetamol Etoricoxib Accuracy Data

Level	Area		%Recovery	
	Etoricoxib	Paracetamol	Etoricoxib	Paracetamol
50%	367224	19587	98.45	98.70
	368828	19753	98.88	98.80
	368001	19460	98.66	99.36
100%	740951	36928	98.72	98.45
	741950	36653	98.85	98.80
	745782	36559	99.36	98.60

150%	1124447	54580	99.88	99.88
	1133086	55240	100.64	98.86
	1130273	54879	100.39	99.30
Average			99.32	98.97
STD Deviation			0.803987	0.452554
RSD			0.809519	0.457252

### Repeatability

The precision of the development approach was assessed for intraday (precision) and interday (precision) (by varying the analyst and HPLC column called intermediate precision). The % RSD for Etoricoxib and Paracetamol was determined to be within acceptable ranges. (RSD<2), as shown in (Table-4).

Table-4: Precision statistics for Etoricoxib and Paracetamol

Validation Parameter	Intra-Day		Inter-Day	
	Etoricoxib	Paracetamol	Etoricoxib	Paracetamol
% Mean	100.37	99.62	98.32	98.99
STD Deviation	0.88	0.03	0.47	0.87
%RSD	0.88	0.03	0.48	0.88

### Robustness

By altering the experimental and the conditions of the chromatogram the robustness of a procedure was evaluated. Changing the flow rate ( $1 \pm 0.2$  ml/min), the column temperature ( $25 \pm 5$  °C), the composition of the mobile phase ( $\pm 5$ ), and the allowed limitations from the real chromatographic situation. There was no discernible change in mean Retention time or Relative standard deviation, and all values were within the limit of  $\leq 2$ . Results are given in (Table-5).

Table-5: Etoricoxib and Paracetamol Robustness Data

Validation Parameter	Changed value	Retention time		%Assay	
		Etoricoxib	Paracetamol	Etoricoxib	Paracetamol
Column Temperature	20°C	4.01	2.14	98.9	98.0
	25°C	4.06	2.15	98.4	99.6
	30°C	4.13	2.20	98.9	100.4
Flow rate	0.8ml/min	5.34	2.83	98.7	98.8
	1.0ml/min	4.45	2.36	101.2	100.5
	1.2ml/min	3.95	2.09	100.2	100.8
Mobile Phase Acetonitrile:0.1%Acetic acid	75:25	4.91	2.79	98.3	99.2
	70:30	5.23	2.78	99.6	99.8
	65:35	5.49	2.70	99.8	98.8
Average				99.32	98.97
STDEV				0.803987	0.452554
% RSD				0.809519	0.457252

### LOD and LOQ

The LOD and LOQ were estimated as per ICH guidelines using the equations  $LOD = 3\sigma/S$  and  $LOQ = 10\sigma/S$ , where  $\sigma$  is the response's standard deviation, and "s" is the calibration curve's slope. The least quantity of analyte necessary to induce a substantive response is termed the LOD and the smallest number of analysts that could be quantified reproducibly was defined as the LOQ. Table-6 displays the results depending on the response's standard deviation and slope. LOD and LOQ data results are given in (Table-6).

Table-6: Sample Solution LOD and LOQ Data

Parameter	Etoricoxib	Paracetamol
LOD	0.33 $\mu$ g/ml	0.54 $\mu$ g/ml
LOQ	1.00 $\mu$ g/ml	1.65 $\mu$ g/ml

### Sample Solution Stability Data

The stability investigations were after 24 hours at room temperature, and the chromatographic conditions specified above were conducted in the diluent. These investigations demonstrated that Etoricoxib and

Paracetamol were stable in the diluent for at least 24 hours, supporting the analytical reliability of the suggested technique. Etoricoxib and Paracetamol Stability Data are given in (Table-7).

Table-7: Etoricoxib and Paracetamol Stability Data

Drug	% of Assay	
	% Assay at 0 hour	% Assay at 24-hour
Etoricoxib	98.28	99.58
Paracetamol	98.19	98.38

## CONCLUSION

A new Reverse Phase-HPLC method for simultaneous quantification of Etoricoxib and Paracetamol combined tablet dosage form was developed and also validated. The calibration curve was found to be linear in the concentration ranges of 20-200 $\mu$ g/ml for Etoricoxib and 20-200 $\mu$ g/ml for Paracetamol, respectively. A linear equation was created to offer the best fit for the concentration versus detector response. During the validation, the Correlation coefficient is equal to 0.99. The acquired percent RSD value of <2 demonstrates that the proposed approach is highly exact. Furthermore, the separation of the analyses took only 8 minutes, making the suggested RP-HPLC method suitable for regular quality control analysis of combination formulations including these medications.

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