RP- HPLC Method for Simultaneous Estimation of Paracetamol and Etoricoxib from Bulk and Tablets

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Abstract

A simple, selective, rapid, precise and economical reverse phase high-pressure liquid chromatographic method has been developed for the simultaneous estimation of Paracetamol and Etoricoxib from pharmaceutical formulation. The method was carried out on an inertsil ODS, 5µ, C8-3 column, with a mobile phase consisting of methanol: acetonitrile: phosphate buffer pH 3.5 (40:20:40 v/v) at a flow rate of 1.0 ml/min. Detection was carried out at 242. The retention time of Paracetamol and Etoricoxib were 3.27, 6.12 min. respectively. The developed method was validated in terms of accuracy, precision, linearity, Limit of detection, Limit of quantitation. The proposed method can be used for estimation of these drugs in combined dosage form for routine analysis.

Key Words: Paracetamol, Etoricoxib, RP-HPLC.

Introduction

Paracetamol is chemically 4-hydroxyacetanilide, is a centrally and peripherally acting non-opioid analgesic and antipyretic. Paracetamol is official in IP, BP and USP[1-3]. Etoricoxib is 5-chloro-2-(6-methylpyridin-3-yl)-3-(4-methylsulfonylphenyl) pyridine belongs to the group of non-steroidal anti-inflammatory drugs (NSAIDs) known as selective Cox-2 inhibitor. This drug is used for treatment in rheumatoid arthritis, osteoarthritis and pain[4-7]. A tablet formulation containing 500 mg of Paracetamol and 60 mg of Etoricoxib has been introduced in to clinical practice. A survey of literature revealed that few HPLC and spectrophotometric methods are reported for determination of Etoricoxib and Paracetamol individually[8-11]. However there is no HPLC method reported for simultaneous determination of Paracetamol and Etoricoxib from combine dosage form. The present work describes the simple, precise and accurate RP-HPLC method for simultaneous estimation of Paracetamol and Etoricoxib in tablets.

It is validated by ICH guidelines [12].

Experimental Section

Reagents and Chemicals:
Acetonitrile (HPLC grade) and Methanol (HPLC grade) was purchased from Merck specialties pvt. Ltd. (Worli, Mumbai, India) and Water (HPLC grade) was purchased from Loba Chemie (Mumbai, India). Potassium dihydrogen phosphate AR and phosphoric acid AR was purchased from Merck and S. D. Fine Chemicals, Mumbai respectively. All other reagents used were of HPLC grade. Working standard of Paracetamol and Etoricoxib were provided by Sanofi-aventis and Glenmark Generics Ltd.

*Pharmaceutical formulation***:**
Commercial tablets, each containing Paracetamol (500mg) and Etoricoxib (60 mg); were procured from the local market.

Method Development
Different mobile phases containing methanol, water, Acetonitrile, and different buffers in different proportion were tried and finally of methanol: acetonitrile: phosphate buffer pH 3.5 (40:20:40 v/v) was selected as moile phase which gave good resolution and acceptable peak parameters for both Paracetamol and Etoricoxib.

System Suitability Studies:
The resolution, number of theoretical plates and peak asymmetry were calculated for the standard solutions and is as shown in Table 1.

Table 1: System suitability studies

Sr. No.	Parameters	Paracetamol	Etoricoxib
1.	Theoratical plate/ meter	6475	8174
2.	Resolution factor	2.81	5.33
3.	Asymmetry	1.28	1.11
4.	LOD (µg/ml)	0.03	0.02
5.	LOQ (µg/ml)	0.1	0.08

The values obtained demonstrated the suitability of the system for the analysis of these drugs in combinations. The typical chromatogram of standard solution is as shown in Fig.1.

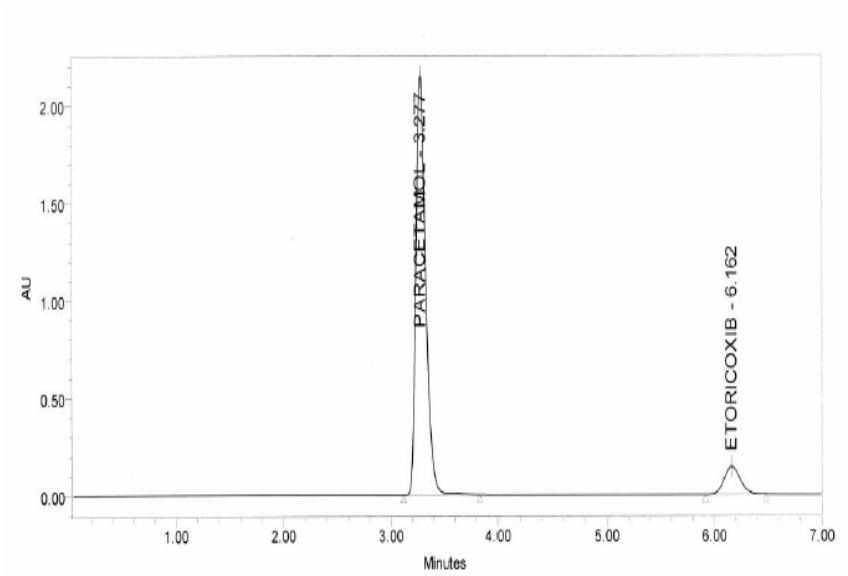

Figure 1: Chromatogram of Paracetamol (3.274min), Etoricoxib (6.149 min), respectively

Apparatus and chromatographic Conditions:
Chromatographic separation was performed on a Waters Alliance HPLC system 2695 Seperation module. The software used was Empower 2. ODS, C8-3 column (250 mm x 4.6 mm i.d, partical size 5 µ) was used for the separation; mobile phase of a mixture of methanol: acetonitrile: phosphate buffer pH 3.5 (40:20:40 v/v) was delivered at a flow rate of 1.0 ml/min with detection at 242nm. The mobile phase was filtered through a 0.2µ membrane filter and degassed. The injection volume was 20 µl; analysis was performed at ambient temperature.

Preparation of Standard Solutions:
Standard stock solution of Paracetamol and Etoricoxib was prepared in methanol. From the standard stock solutions, mixed standard solution was prepared containing 100 µg/ml of Paracetamol and 12 µg/ml of Etoricoxib.

Calibration Curve:
Linearity of the system was investigated by serially diluting the stock solutions to give concentrations in the range of 50 to 150 µg/ml for Paracetamol and 6-18µg/ml Etoricoxib. An aliquot (20µl) was injected using mixture methanol: acetonitrile: phosphate buffer pH 3.5 (40:20:40) v/v as mobile phase. Calibration curves were obtained by plotting the Peak area vs. concentration. The calibration curves are as shown in Fig.2 and Fig.3 for Paracetamol and Etoricoxib respectively. The equations of the regression lines are For Paracetamol y = 93740X-180754 (R^2= 0994)
For Etoricoxib y = 82936X-24335 (R^2= 0.995)

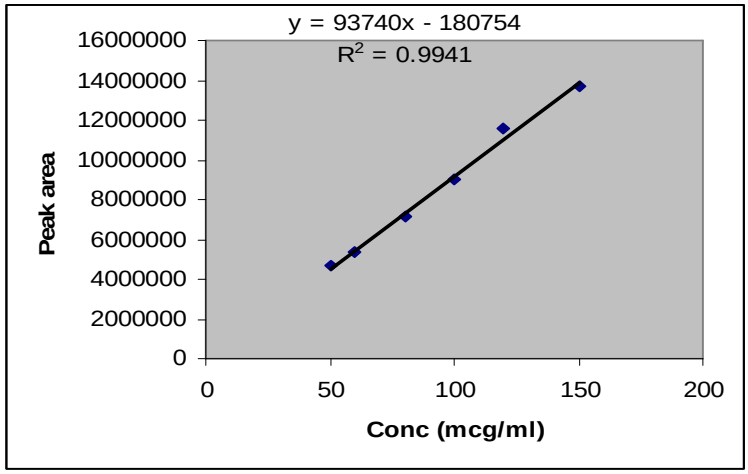

Figure2: Calibration curve for Paracetamol

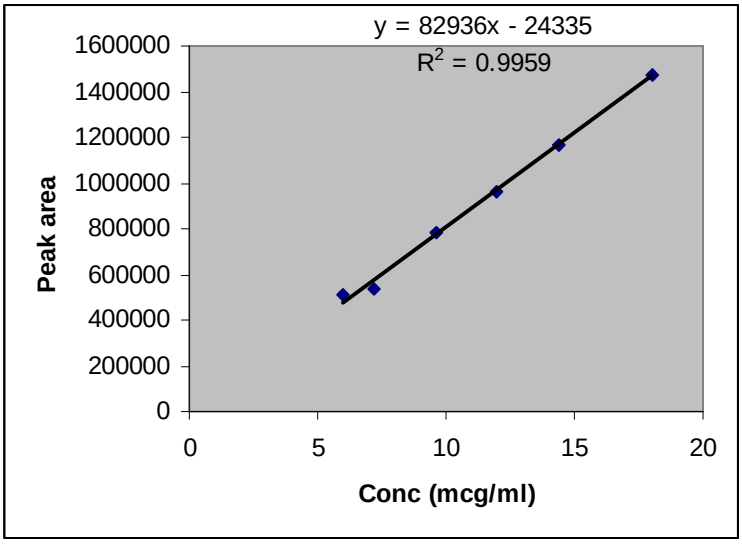

Figure 3: Calibration curve for Etoricoxib

Assay:

Preparation of Sample Solutions:

Twenty tablets, each containing 500 mg of Paracetamol and 60 mg of etoricoxib were weighed and finely powdered. A quantity of powder equivalent to 500 mg of Paracetamol and 60 mg of Etoricoxib was weighed and transferred to 200 ml volumetric flask containing 5 ml methanol and 140 ml of mobile phase. The mixture was sonicated for 20 min. The volume was made up to 200 ml with mobile phase. Further dilutions were made to get a concentration of 100 µg/ml of Paracetamol and 12 µg/ml of etoricoxib. The contents were filtered through 0.22 µ membrane filter. Twenty micro liters of the test and standard solutions were injected separately and chromatograms were recorded upto 8 min. The proposed method was found to be specific and no interference from common tablet excipents like lactose, starch etc was observed. The assay was

calculated from the equation of regression line for each drug. The percentage of individual drugs found in formulations was calculated and presented in table 2.
The results of analysis shows that the amounts of drugs were in good agreement with the label claim of the formulations.

Method Validation:
As per the ICH guidelines, the method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantitation and robustness.

Linearity and Range:
The linearity of the method was determined for the formulation at five concentration levels ranging from 50 to 150 µg/ml for paracetamol and 6-18µg/ml Etoricoxib. The equation for regression line was y = 93740X-180754 (R^2= 0994) for Paracetamol and
y = 82936X-24335 (R^2= 0.995) for Etoricoxib. The results show that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above.

Accuracy and Precision:
The accuracy of the method was determined by recovery experiments. The recovery studies were carried out at three levels of 80, 100 and 120% and the percentage recovery was calculated and presented in Table 2.

Table 2: Recovery studies of Paracetamol (PARA) and Etoricoxib (ETO)

Level of % Recovery	% Mean Recovery		% RSD	
	PARA	ETO	PARA	ETO
80	100.37	100.33	0.06993	0.33
100	99.87	99.41	0.045	0.4
120	99.82	98.23	0.076	0.28

Table 3: Inerday and Intraday precision studies of Paracetamol and Etoricoxib
(System Precision)

Conc. (µg/ml)	Paracetamol		Conc.	Etoricoxib	
	% RSD			% RSD	
	Intra- Day	Inter-Day		Intra- Day	Inter-Day
50.0	0.16	0.22	6.0	0.53	0.62
60.0	0.29	0.30	7.2	0.69	0.76
80.0	0.2	0.27	9.6	0.31	0.36
100.0	0.31	0.25	12.0	0.92	1.5
120.0	0.32	0.37	14.4	0.13	1.2
150.0	0.46	0.56	18.0	1.0	1.12

Recovery was within the range of 100 ± 2% which indicates accuracy of the method. The precision of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, 3 repeated injections of standard and sample solutions were made in a day and the response factor of drug peaks and percentage RSD were calculated and presented in Table 3. In the inter day variation studies, 3 repeated injections of standard and sample solutions were made on 3 consecutive days and response factor of drugs peaks and percentage RSD were calculated and presented in Table 3 and 4 for both system and method precision.

Table 4: Interday and Intraday precision studies of Paracetamol and Etoricoxib (Method Precision)

Conc. (µg/ml)	Paracetamol		Conc.	Etoricoxib	
	% RSD			% RSD	
	Intra- Day	Inter-Day		Intra- Day	Inter-Day
50.0	0.36	0.52	6.0	0.45	0.63
60.0	0.42	0.48	7.2	0.61	0.76
80.0	0.56	0.43	9.6	0.35	0.39
100.0	0.36	0.21	12.0	1.2	1.33
120.0	0.21	0.34	14.4	0.9	0.37
150.0	0.9	0.53	18.0	0.65	0.41

The data obtained, %RSD not more than 1.5%, indicates that the developed RP-HPLC method is precise.

Limit of Detection and Limit of Quantification:

The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response. LOD was calculated using the following formula

LOD = (3.3 x standard deviation)/ Slope of calibration curve

The LOD for Paracetamol and Etoricoxib were found to be 0.03µg/ml and 0.02 µg/ml, respectively.

The Limit of Quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. LOQ was calculated using the following formula

LOQ = (10 x standard deviation) / Slope of calibration curve.

The LOQ was 0.1µg/ml and 0.08µg/ml for Paracetamol and Etoricoxib respectively.

Robustness:

Robustness is checked by making slight deliberate change in the experimental procedures. In the present method a deliberate change of room temperature and pH was made and the effects were noted. The method was found to be robust with respect to change in room temperatures.

Results and Discussion

The proposed method was found to be simple and linear in the concentration range of 50-150 µg/ml for Paracetamol and 6-18 µg/ml Etoricoxib respectively. The method was found to be

accurate and precise as indicated by recovery studies and % RSD not more than 1.5. Moreover LOD and LOQ for Paracetamol were found to be 0.03µg/ml and 0.1µg/ml, respectively and for Etoricoxib were 0.02 and 0.08µg/ml, respectively. Thus the method is specific and sensitive.

Conclusion

The proposed RP-HPLC method for the simultaneous estimation of Paracetamol and Etoricoxib in combined dosage forms was found to be sensitive, accurate, precise, simple and rapid. Hence the present RP –HPLC method may be used for routine analysis of the raw materials and formulations.

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