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Research Article

QUANTITATIVE DETERMINATION OF ETORICOXIB AND PARACETAMOL IN PHARMACEUTICAL DOSAGE FORM AND IN-VITRO COMPARISON BY REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (RP-HPLC)

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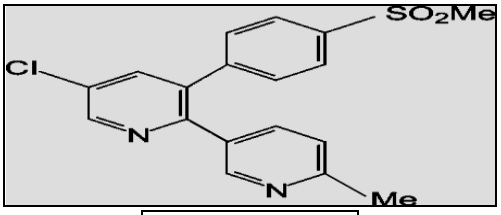
ABSTRACT: The objective of this present work was to develop and validate analytical method for quantitative determination of Paracetamol and Etoricoxib in a tablet formulation and also the comparison of invitro data with reference dosage form. Chromatographic separations of the two drugs were analyzed on a Kromasil C18 column (25cm X 4.6mm, 5µm). The mobile phase constituted of Buffer: Acetonitirile with gradient program was delivered at the flow rate 1.0 mL/min. Detection was performed at 220 nm. Separation was completed within 20 min. Calibration curves were linear with coefficient correlation between 0.99 to 1.0 over a concentration range of 48 to 146 µg/mL of Paracetamol and 6 to 19 µg/mL for Etoricoxib respectively. The relative standard deviation (R.S.D) was found to be less than 2.0%. Analysis for dissolution study was also performed by Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) method. Difference factor (f_1) were found to be 2.85 and 3.83 and similarity factor (f_2) were found to be 73.514 and 68.961 for Paracetamol and Etoricoxib respectively.

Key words: Paracetamol; Etoricoxib; RP-HPLC; In-vitro; Dissolution; HPLC; COX 2.

INTRODUCTION:

The combination tablet consists of Etoricoxib 60 mg and Parcetamol 500 mg. Non-Steroidal anti-inflammatory drugs are widely used for the treatment of pain, inflammation, and fever. Pharmacological action of drug of Etoricoxib is due to selective inhibition of cyclooxygenase-2 (COX-2), which block the prostaglandin synthesis[1-5]. Cyclooxygenase exist in two forms COX-1 and COX-2. COX –1 is having good action like protection of GI mucosa, maintenance of renal homeostasis, platelet aggregation. On the other hand COX-2 is responsible for prostaglandin synthesis, which leads to cause Pain, Inflammation, and Fever. Paracetamol (Acetaminophen) is Para-aminophenol derivative and is deethylated active metabolite of Phenactin. Paracetamol is commonly used for mild to moderate pain and having poor anti-inflammatory action. Paracetamol inhibit the cyclooxygenase not the periphery.



Structure-1 Etoricoxib

It is establish clinical practice to combine NSAIDS with Paracetamol for their synergistic analgesic activity. Since both the drugs have distinct mechanism of action. Further addition of Paracetamol provides additional antipyretic effect. Since fixed dose combinations of COX-2 inhibitors with Paracetamol are used for short –term treatment of acute pain only, their safety would not be matter of concern. Further US-FDA advisory committee has recommended the reintroduction of roficoxib in the US market[6]. The method for analyzing Etoricoxib in plasma[7, 8], impurity analysis and validation[9] by HPLC and UV[10] is already published in different journals while assay of paracetamol API and tablet is official in all Pharmacopoeia[11,12]. Simultaneous analysis of Etoricoxib with paracetamol is not published in any journal and its combination is yet to launch in the market. Dissolution test of conventional dosage forms provides useful recommendation for their evaluation. Different official apparatus are available. Assay method was validated as per the procedure and acceptance criteria based on FDA guideline[13] and recommendation of ICH [14]. The validation of dissolution test can be divided into two parts. The first regards equipment validation and second concerns method precision validation. There is no official method for

determination of dissolution rate of both these components in tablet dosage form. A review of the literature indicated that there was no reported dissolution method for this combination product.

MATERIAL AND METHODS:

Chemicals and Materials: Cadila Healthcare Limited, Patalganga supplied Etoricoxib and Granules India limited supplied Paracetamol. Acetonitrile and Trifluoroacetic acid were obtained from Spectrochem and E-Merck Limited respectively.

Instrumentation:

Shimadzu 2010C integrated high performance liquid chromatographic system was used for this experiment. Shimadzu 2010C system equipped with quaternary gradient pump, 2010C UV-VIS detector, 2010C Column Oven and 2010C programmable auto sampler controlled by CLASS-VP software. The Kromasil C18 (250X4.6 mm), 5µm was used as a stationary phase. Electrolab autosampler dissolution apparatus were used for comparative dissolution study.

HPLC Condition:

Column	Kromasil C18 (250X4.6 mm), 5µm
Detector	220 nm
Injection volume	20 µL (Assay) and 50 µL (Dissolution)
Flow rate	1.0 ml/min
Temperature	30˚ C
Run time	22 min
Diluent	Buffer: Acetonitrile (75: 25)
Mobile phase	Gradient programme

Time (minutes)	Buffer : Acetonitrile
0.01 ------> 9.0	90 : 10
9.01-------> 9.1	70 : 30
9.11-------> 15.0	70 : 30
15.01------> 15.1	90 : 10
15.10------> 22.0	90 : 10

Buffer preparation:

Take 1.0 ml of Trifluroacetic acid in to 2000 ml of Milli Q water and mix. Filter it through 0.45 um HVLP filter paper and degassed.

Media preparation:

Take 8.5 ml of concentrated hydrochloric acid in to 1000 ml of DM water and mix.

For Assay

Standard preparation:

Standard stock solutions were prepared in methanol and further for second dilution, dilute it with diluent to make final concentration Etoricoxib 12.5 µg and Paracetamol 100 µg respectively.

Sample preparation:

Weigh accurately tablets powdered equivalent to about 150 mg of Etoricoxib and 1225 mg of Paracetamol in to 250-ml volumetric flask. Add about 150-ml methanol and sonicate it for 45 minute to dissolve. Filtered it through 0.45 µ HVLP nylon filter and made further dilution 2.0 mL to 100.0 mL with diluent and mix.

For Dissolution

Standard preparation:

Standard stock solutions were prepared in methanol and further for second dilution, dilute it with media to make final concentration Etoricoxib 12.5 µg and Paracetamol 100 µg respectively.

Sample preparation:

Dissolution studies on the combination drug of Paracetamol and Etoricoxib were conducted using Apparatus II. The dissolution medium was 0.1 N Hcl at 37 ± 0.5 C and stirred at 100 rpm. In both the experiments 10-ml of sample were withdrawn at 30, 45, 60minutes and replacement with the equal volume of the fresh medium to maintain the constant volume total volume. Second dilutions were done by Transferring 2 ml the filtered test solution in 25 ml of volumetric flask and volume was make upto the mark with fresh media.

RESULTS:

The detection wavelength of 220 nm was chosen in order to achieve a good sensitivity for quantitative determination of Etoricoxib and Paracetamol in tablet dosage. The mobile phase consisting of Buffer: Acetonitirile offered a good separation at ambient temperature under these conditions using a flow rate of 1.0 mL/min and a runtime of 22 min. Paracetamol elutes at first and then Etoricoxib shown in the chromatogram, Fig. 1 and 2, which illustrate the separation of both active ingredients in this system. The model-independent method was applied to compare the dissolution data. By this method dissimilarity factor (f_1) were found to be 2.85 and 3.83 and similarity factor (f_2) were found to be 73.514 and 68.961 for Paracetamol and Etoricoxib respectively. The gradient program throughout HPLC method was adopted to analyze both components in a single run. The proposed method was simple and do not involve laborious time-consuming sample preparation.

System suitability and system precision:

System suitability and system precision was daily performed during entire validation of this method. The results of system suitability and system precision were presented in table 1.

Linearity and calibration curve:

The plot of peak area response against concentration was shown in Fig 3. The plot is linear over the concentration range of 48 to 146 µg/mL and 6 to 19 µg / mL for Paracetamol and Etoricoxib respectively. Linearity of the calibration curve was determined by weighed (1/c) least square regression analysis. The correlation coefficient was found to be 0.99 to 1.00. A linear relationship was found for all components. The results of linearity, limit of detection and limit of quantification were presented in table 2.

Specificity:

There was no interference from sample placebo and peak purity of Paracetamol and Etoricoxib were 1.000 and 1.000 respectively. It showed that developed analytical method was specific for the analysis of Paracetamol and Etoricoxib in tablet dosage form.

Standard and sample solution stability:

Standard and sample solution stability was evaluated at room temperature for 36 h. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution was stable up to 36 h at room temperature.

Method precision:

The precision of the method was established by carrying out the analysis of the analyte (n=6) using the proposed method. The low value of standard deviation showed that the method was precise. The results obtained were presented in table 3.

Method accuracy:

To ensure the reliability and accuracy of the method recovery studies were carried out at three different levels. The results of recovery studies were presented in table 4.

Method robustness:

Robustness of the method was determined by small deliberate changes in flow rate, mobile phase ratio and column oven temperature. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was robust. The results of robustness were presented in table 5.

Method Ruggedness:

Ruggedness test was determined between two different analysts, instruments and columns. The value of percentage RSD was below 2.0% showed ruggedness of developed analytical method. The results of ruggedness were presented in table 6.

Table 1.

SYSTEM SUITABILITY AND SYSTEM PRECISION

Compound	Retention Time (Mean ± SEM)	n	k'	R	T	α
Paracetamol	8.34 ± 0.0034	8428.63	1.76	-	1.08	-
Etoricoxib	18.45 ± 0.0036	97043.98	5.11	33.69	1.15	2.90

n= Theoretical plates
k'= Capacity Factor
R= Resolution
T= Asymmetry
α = Selectivity

Table 2.
CHARACTERISTICS OF THE ANALYTICAL METHOD DERIVED FROM THE STANDARD CALIBRATION CURVE

Compound	LOD µg/ml	LOQ µg/ml	Linearity range n=(5)	Correlation co-efficient µg/ml	Residual std. regression σ	Slope of regression S
Paracetamol	0.973	2.919	48-146	0.99970	5497.122	42796.091
Etoricoxib	0.250	0.750	6 -19	0.99979	11561.427	83977.007

LOD= Limit of detection
LOQ= Limit of quantification

Table 3.
METHOD PRECISION

Compound	Concentration µg/ml	Retention time Mean ± SEM (n=6)	% Assay Mean ± SEM (n=6)	% RSD of Assay (n=6)
Paracetamol	100	8.34 ± 0.1922	100.5 ± 0.1922	0.5
Etoricoxib	12.5	18.45 ± 0.0955	104.7 ± 0.09546	0.2

Table 4.
METHOD ACCURACY

Level	Drug Added (mg)	Drug recovered (mg)	% Assay (Mean ± SEM) (n=3)	% RSD of Assay (n=3)
For Paracetamol				
50%	613.6	625.37	101.8 ± 0.0577	0.1
100%	1224.2	1241.22	101.3 ± 0.1155	0.3
150%	1838.1	1849.27	100.4 ± 0.1202	0.2
For Etoricoxib				
50%	75.1	75.68	101.6 ± 0.3175	0.5
100%	150.3	151.5	101.4 ± 0.2332	0.4
150%	225.3	226.1	100. 5 ± 0.3175	0.3

Table 5.
METHOD ROBUSTNESS

Compound	% RSD in Normal and Changed condition (n=5)		
Temperature	**% RSD Normal**	**% RSD (-5°C)**	**% RSD (+5°C)**
Paracetamol	0.3	0.2	0.2
Etoricoxib	0.9	0.3	0.3
Flow Rate	**% RSD Normal**	**% RSD (-10%)**	**% RSD (+10%)**
Paracetamol	0.3	0.3	0.3
Etoricoxib	0.9	0.4	0.2

Table 6.
METHOD RUGGEDNESS

Compound	% Assay Mean ± SEM (n=6)	% RSD of Assay (n=6)
Day 1	**Analyst-1, Instrument-1 & Column-1**	
Paracetamol	100.5 ± 0.1922	0.5
Etoricoxib	104.7 ± 0.0946	0.2
Day 2	**Analyst-2, Instrument-2 & Column-2**	
Paracetamol	101.4 ± 0.0051	0.4
Etoricoxib	103.4 ± 0.0368	0.6

Table 7.

Comparative Dissolution Profile (Paracetamol)		
	Reference (Paracetamol Tablet 500 mg)	**Test (Etoricoxib+Paracetamol Tablet)**
Manufactured by	Zydus Cadila Limited	Zydus Cadila Limited
Batch No.	MD3290	PAET 500/F005
Apparatus	USP Type II, RPM- 50	
Method of Analysis	HPLC	
Dissolution medium (I)	900 ml, 0.1 N Hydrochloric acid	
% of Drug release for Paracetamol		
Time in minutes	**Reference**	**Test**
30	92.6	97.6
45	95.6	98.0
60	98.9	98.1
F_1 (Similarity factor)	2.856148	
F_2(Disimilarity factor)	73.51407	

Table 8.

Comparative Dissolution Profile (Etoricoxib)		
	Reference (Etoricoxib Tab 60 mg)	**Test (Etoricoxib+Paracetamol Tab)**
Manufactured by	Zydus Cadila	Zydus Cadila
Batch No.	MD-3124	PAET- 500/F005
Apparatus	USP Type II, RPM- 50	
Method of Analysis	HPLC	
Dissolution medium (I)	0.1 N Hydrochloric acid	
% of Drug release for Etoricoxib Tablet		
Time in min	**Reference**	**Test**
30	93.3	98.9
45	95.6	99.6
60	98.3	99.7
F_1 (Similarity factor)	3.830084	
F_2(Dissimilarity factor)	68.96134	

Fig.1: Chromatogram for Standard Solution for Assay

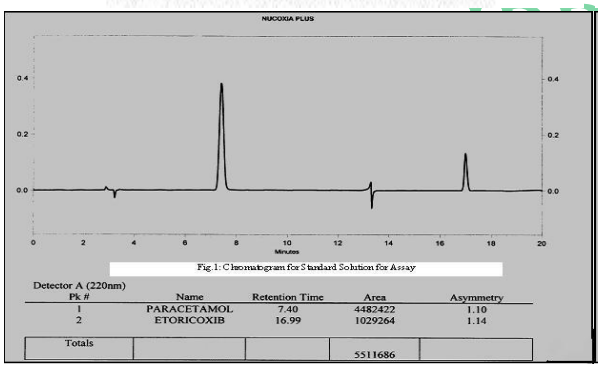


Fig 2: Chromatogram of test solution for Assay

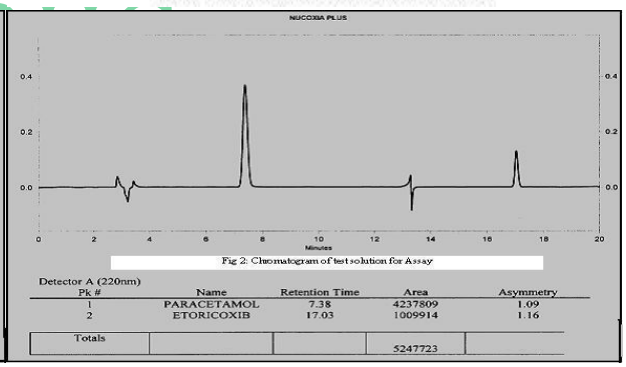
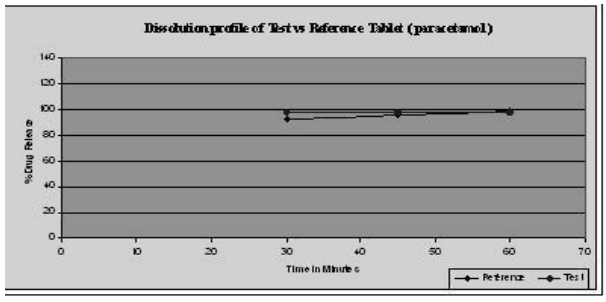
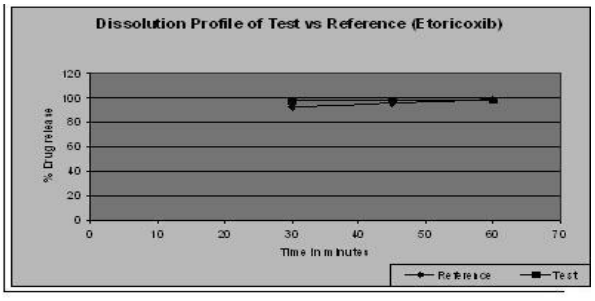


Fig 3: Dissolution profile





DISCUSSION:

The method described enables to the quantification of Paracetamol and Etoricoxib. The advantages lie in the simplicity of sample preparation and the low costs of reagents used. The proposed HPLC conditions ensure sufficient resolution and the precise quantification of the compounds. Results from statistical analysis of the experimental results were indicative of satisfactory precision and reproducibility. Hence, this HPLC method can be used for routine drug analysis.

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