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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF PARACETAMOL AND ETORICOXIB IN PHARMACEUTICAL DOSAGE FORMS BY RP-HPLC

G. Lakshmi Priyanka*, P.V. Madhavi Latha

Department of Pharmacy, Viswanadha Institute of Pharmaceutical Sciences, JNTUK, Visakhapatnam - 530009, A.P, INDIA

*Corresponding author E-mail: priyankavarma9697@gmail.com

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ABSTRACT

A simple, Accurate, precise technique was developed for the simultaneous estimation of the Paracetamol and Etoricoxib in Tablet dosage form. Chromatogram was run through Inertsil-ODS C_{18} (250 x 4.6mm, 5µ) column. Mobile phase containing Methanol: Acetonitrile: Phosphate Buffer taken in the proportions 40:25:35v/v was pumped through column at flow rate of 1.0ml/min. Temperature was kept ambient. Optimised wavelength selected was 241nm. Retention time of Paracetamol and Etoricoxib were observed to be 2.5min and 4.3min. %RSD of the Paracetamol and Etoricoxib were and observed to be 0.362 and 0.129 respectively. %Recovery was obtained as 100.12% for Paracetamol and 99.73% for Etoricoxib respectively. LOD, LOQ values obtained from regression equations of Paracetamol and Etoricoxib were 0.33, 1.02 and 1.44, 3.27 respectively. Regression equation of Paracetamol is y = 51886x + 1315, and y = 55508x + 940.6 of Etoricoxib . Retention times were decreased and that run time was decreased, so the technique developed was simple and conservative that can be embraced in regular quality control test in industries.

INTRODUCTION

Etoricoxib is a non-steroidal anti-inflammatory drug (NSAID) used to treat Rheumatoid Arthritis , Gout and Osteoarthritis and Paracetamol is also NSAID used to relieve mild to moderate aches and pains associated with headache, migraine, cold n flu as well as anti-pyretic drug (fever reducer). The combination of Etoricoxib and Paracetamol work by blocking the release of certain chemical messengers in the brain that cause pain and fever and also used in the treatment of headaches , arthritis, backache and the symptoms of cold. [1-2]

MATERIALS and METHODS

Preparation of buffer: reparation of phosphate (KH_2PO_4 0.1M) buffer: Approximately weighed 3.8954g of di-sodium hydrogen phosphate and 3.4023g of potassium dihydrogen phosphate in to a beaker containing 1000 mL of distilled water and dissolved completely. Then pH was adjusted to 3.5 using orthophosphoric acid and then filtered through 0.45µm membrane filter.

Preparation of diluents: Depending on the nature of solubility of the selected drugs, Methanol, Acetonitrile and phosphate buffer in the ratio of 40:25:35 V/V was prepared after degassing and filtering the solution using 0.45 µm membrane filter. [3]

Stock solution:

Preparation of standard stock solution: The solution was prepared by dissolving 50mg of accurately weighed Paracetamol and 10mg Etoricoxib in Mobile phase, in two 100.0mL

volumetric flasks separately and sonicate for 20min.

Preparation of sample stock solution: 20 tablets were weighed and the average weight of each tablet was calculated, and a quantity of tablet powder equivalent to 50 mg Paracetamol and 10 mg Etoricoxib were weighed and dissolved in the 70 mL mobile phase with the aid of ultrasonication for 20 min. The mixture was diluted to 100 mL with mobile phase to furnish a sample stock solution.

Working solution:

Preparation of working standard solution: From the above stock solutions 10 mL from each stock solution was taken into a 50 mL volumetric then madeup with mobile phase and sonicated for 10min and filtered through 0.45µ membrane.

Preparation of sample working solution: The sample stock solution was filtered through a 0.45 µm Nylon syringe filter and 10 mL of the filtrate was diluted into a 50 mL volumetric flask to give a sample solution containing 100 µg/mL Paracetamol and 20 µg/mL Etoricoxib.

Procedure: Sample solutions (20µL) in duplicates were injected and the peak responses were measured. % assay were calculated for Paracetamol and Etoricoxib.

RESULTS AND DISCUSSION:

Method validation: Specificity, linearity, range, Accuracy, precision, Repeatability, Intermediate precision, limit of detection, limit of Quantification, Robustness.

Method development: Method development was performed by changing various chromatographic conditions like mobile phase ratios, buffers, flow rates .

SPECIFICITY: The system suitability for specificity was carried out to determine whether there is an interference of any impurities in retention time of analytical peak. the specificity study was performed by injecting blank. It was found that there was no interference of impurities in retention time of analytical peak.

LINEARITY: To establish the linearity of the method, serial dilutions were prepared to obtain the mixture of Paracetamol and Etoricoxib ranging from 25ppm to 150ppm and 5ppm to 30ppm level all the solutions were filtered through a 0.45µm Millipore filters. The final solution was injected in duplicate manner keeping the injection volume 20µl. Calibration curve was plotted between mean peak area and concentration. The correlation coefficient and slope were determined from the calibration curve. The linearity chats of Paracetamol and Etoricoxib was shown in figure no.5 and 6 . The correlation coefficient was found to be 0.999 for both drugs and hence the method was set to be linear. They were tabulated in table 1. [(4)]

ACCURACY: Accuracy was evaluated by standard addition method of three known concentration of the drug and the spiked solution were analysed. The recovery of the added drug was determined by calculating the pre-analysed drug concentration with concentration of spiked drug. The % recovery was calculated and the result was reported in table no. 2 & 3. [(5)]

PRECISION: The precision of the analytical method was studied by injecting six replicates of standard and sample concentration on the same day and another day. The concentration of Paracetamol and Etoricoxib were injected at two levels intra and method precision. The %RSD was calculated and results were reported and table no. 4 & 5. [(6)]

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ): The limit of detection (LOD) and limit of quantification (LOQ) were determined by injecting six replicates of mobile phase followed by three concentration of the drug. The LOD was defined as the concentration which yields a signal-to-noise ratio 3:1 while the LOQ was calculated to be the lowest concentration that could be measured with signal-to-noise ratio 10:1. The LOD & LOQ were calculated by measuring the standard deviation of the response and slope. The result of LOD &LOQ was tabulated in table no. 6 . [(7)]

ROBUSTNESS: The small deliberate changes in method like flow rate was made but there were no recognized change in the result and are within the range as per ICH guide lines. Robustness condition like flow minus (0.8ml/min), flow plus (1.2ml/min), temperature ambient was maintained and samples were injected in duplicate manner.

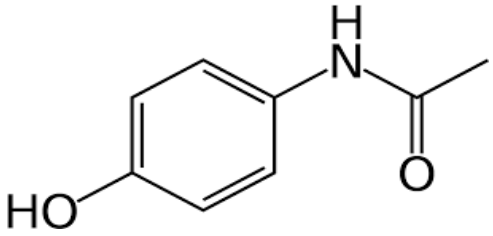

Fig-1: structure of Paracetamol

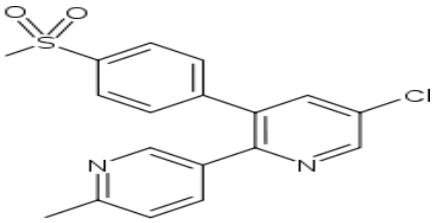

Fig-2: structure of Etoricoxib

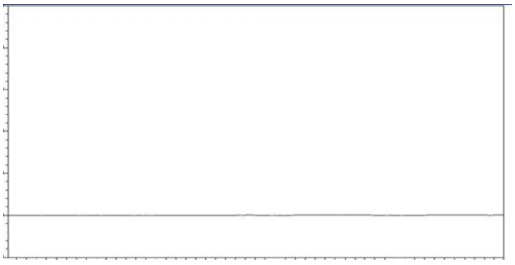

Fig-3: Chromatogram showing blank

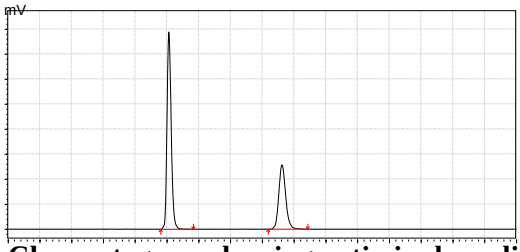

Fig-4: Chromatogram showing optimised condition

Table 1: Linearity results for Paracetamol and Etoricoxib

S.No	Concentration of Paracetamol (µg/mL)	Peak area	Concentration of Etoricoxib (µg/mL)	Peak area
1	25	1329217	5	268657
2	50	2611653	10	564910
3	75	3836211	15	843844
4	100	5160592	20	1102478
5	125	6505013	25	1394280
6	150	7806466	30	1660779

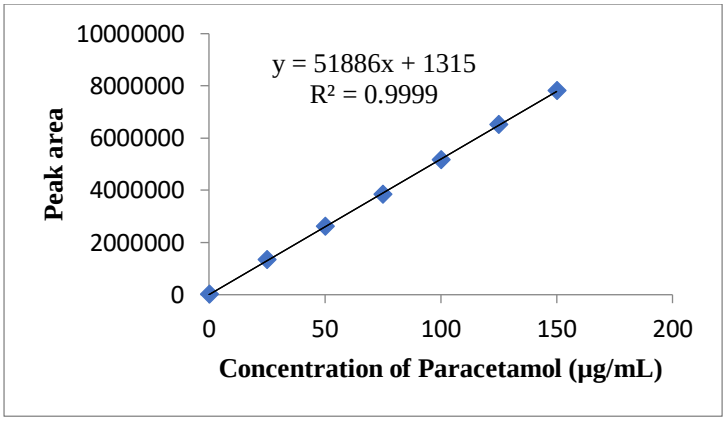

Fig-5: showing calibration curve of Paracetamol

Table 2 : Accuracy data for Paracetamol

% level	Amount Spiked(µg/mL)	Amount recovered(µg/mL)	% Recovery	Mean %Recovery	%RSD
50%	50	50.246	101.37	100.18%	0.16%
	50	50.189	101.05		
	50	50.203	101.13		
100%	100	99.921	99.78	99.97%	0.17%
	100	100.027	100.08		
	100	100.020	100.06		
150%	150	149.991	99.98	100.23%	0.24%
	150	150.142	100.26		
	150	150.247	100.46		

Table 3 : Accuracy data for Etoricoxib

% Level	Amount Spiked (µg/mL)	Amount recovered(µg/mL)	% Recovery	Mean %Recovery	%RSD
50%	10	9.935	99.79	100.43%	0.55%
	10	10.581	100.73		
	10	10.611	100.76		
100%	20	20.219	100.14	99.59%	0.48%
	20	19.763	99.23		
	20	20.055	99.41		
150%	30	30.312	98.88	99.18%	0.26%
	30	30.339	99.31		
	30	30.441	99.35		

Table-4: System precision data for Paracetamol and Etoricoxib

S.No	Peak Area	
	Paracetamol	Etoricoxib
1	5126679	1119139
2	5199898	1113185
3	5121125	1145538
4	5176603	1154077
5	5188957	1140696
6	5128855	1138440
Mean	5157019	1135179
Std dev	35339.78	15789.44
%RSD	0.68	1.39

Table-5: Method precision results for Paracetamol and Etoricoxib

S.No	Peak Area	
	Paracetamol	Etoricoxib
1	5165118	1132577
2	5131380	1136680
3	5160426	1132876
4	5174749	1134503
5	5165391	1134502
6	5160241	1132675
Mean	5159413	1134228
Std dev	16510	1636.819
RSD	0.3	0.14

Table-6: LOD and LOQ data for Paracetamol and Etoricoxib

Drug name	LOD	LOQ
Paracetamol	0.33	1.02
Etoricoxib	1.44	3.27

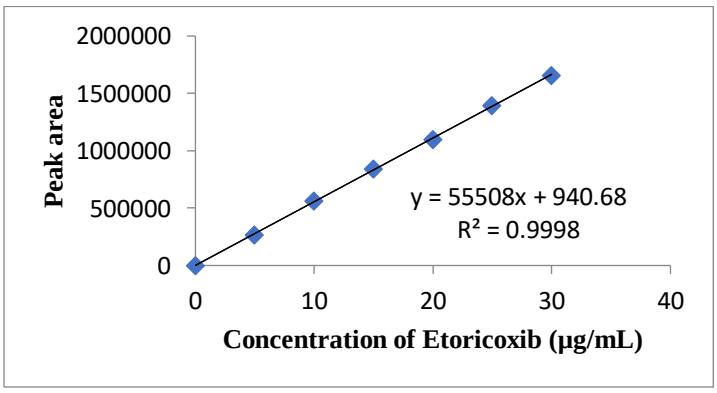

Fig-6: showing calibration curve of Etoricoxib

Table-7: Robustness data for Paracetamol and Etoricoxib

Paracetamol					
Flow rate (mL/min)	**Retention time**	**Peak area**	**Tailing factor**	**USP plate count**	**%RSD**
0.8	3.299	5838521	0.85	6443	0.3
1.2	2.115	4712855	0.88	6194	0.5
Etoricoxib					
0.8	5.273	1274497	0.85	6443	0.1
1.2	3.597	1023556	1.32	7327	0.6

System suitability parameters were not much affected and all the parameters were passed %RSD was found to be within the limits and results were tabulated in table no.7. **(8)**

CONCLUSION:

The developed method was validated as per ICH guidelines. All the system suitability parameters were within the range and satisfactory as per ICH guidelines. Interfering peaks in this method were not observed in blank, standard and sample chromatograms. So this method was said to be specific. Hence, a simple, precise, accurate, specific, sensitive and robust method was developed and validated which can be used in routine control analysis.

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