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Research Article

DEVELOPMENT AND VALIDATION OF RPHPLC METHOD FOR NSAIDs IN COMBINED PHARMACEUTICAL TABLET DOSAGE FORM

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ABSTRACT

The present research work includes the development and validation of High performance liquid chromatography method for simultaneous estimation of Etirocoxib (ETX)and Paracetamol (PCM) which are very much of useful in multiple therapies rather than the use of single drug formulation, because of multiple actions, fever side effect ,and quicker relief. We aimed to design a quick and simple method, suitable for the determination of identity, strength. The UV spectroscopic method were developed on standard ETX and PCM using methanol as standard solution and also, the standard stock solution of both ETX and PCM were diluted with 2%hydrochloric acid to obtain concentration 2.0µg/ml and 16.6µg/ml.The spectrum was recorded in the range of 400-200nm.The RP- HPLC method were developed on standard ETX and PCM which performed on C-18 column using mobile phase composed by acetonitrile :water 50:50(v/v) at a flow rate of 1 ml/min, with injection volume 20µl and Ph 6.8 can be adjusted using orthophosphoric acid.

Keyword: Etirocoxib, Paracetamol, RP-HPLC

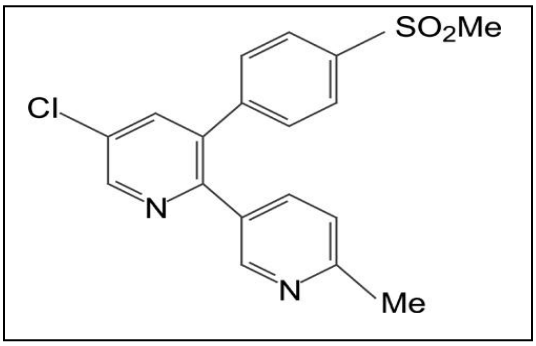
INTRODUCTION

Quality assurance and Quality control plays an important role in determining the safety and efficacy of medicines. Highly specific and sensitive analytical techniques hold the key to the design, development, standardization and quality control of medicinal products. Modern methods of analysis are extremely sensitive, providing precise and detailed information from small samples of material. The pharmaceutical dosage forms of combinational drugs are very much useful in multiple therapies rather than the use of single drug formulation because of multiple actions, fewer side effects, quicker relief etc. High Pressure Liquid Chromatography (HPLC), also known as high performance liquid chromatography, is a form of column chromatography in which the stationary phase consists of small particle (3-50µ) packing contain in column with a small bore (2-5 mm), one end of which is attached to a source of pressurized liquid eluant (mobile phase)[26]. HPLC is one of the most widely used analytical techniques today among different chromatographic procedures due to significant evolution in liquid chromatographic and unsurpassed reliability. Instrument provides superior qualitative and quantitative results, reproducibility, high detection and sensitivity[28] .HPLC is particularly well suited to compounds with reactive functional group such as hydro-peroxide group. Most importantly, HPLC can resolve a wide range of compound in a single analysis. For high specificity and sensitivity low range wavelength detector is used.

RP-HPLC is a very popular chromatographic technique. The term reversed phase implies that the stationary phase is a non-polar solid and the mobile phase is a more polar solvent. RP-HPLC column are efficient, stable and reproducible. In RP-HPLC, good resolution basis of complex and thermolabile compound can be obtained and it is simple to recover intact material for further analysis. Validation is documented evidence, which provide a high degree of assurance for specific method. It is done to ensure that an analytical method is accurate, reproducible and robust over the specific range, that an analyst is obliged to provide an assurance of reliability and for FDA compliance.

METHOD AND MATERIAL

1. Etoricoxib (ETX)

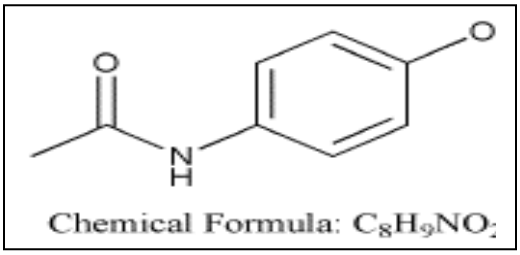


Molecular Formula	$C_{18}H_{15}ClN_2O_2S$
Molecular weight	358.84

Figure 1: IUPAC NAME:-5-chloro-6-methyl-3-[p-sulfonylphenyl]-2, 3-bipyridine

It is white crystalline powder. It is Soluble in propanol, methanol, acetonitrile phosphate dichloromethane and poorly soluble in water.

2. Paracetamol (PCM)



Molecular Formula	$C_8H_9NO_2$
Molecular weight	151.16

Figure 2: IUPAC NAME: - N-(4-Hydroxyphenyl) acetamide or 4-hydroxy acetanilide

It is white crystalline powder, soluble in methanol, ethanol, Ethylene dichloride, acetone, ethyl acetate, slightly soluble in ether.

Method Development:

Development of RP-HPLC method for simultaneous estimation of ETX and PCM in combined dosage form

A. Apparatus and Chromatographic conditions

HPLC model Chemito LC 6600 equipped with Knauer HPLC pump K-501 and Chemito UV- visible detector connected to Chemito Chrom C_{2000} data module is used for investigation and processing chromatogram. All chemicals used were of analytical grade and or HPLC grade. All HPLC solvents and solution were filtered through membrane filter (ultipore 'Nsi', Nylon 66, 0.45 um pore size) and degassed before use. The pure paracetamol (100.23 %) and Etoricoxib (99.83%) were donated by Zim Laboratories, Kalmeshwar`and Cadila Ltd, Raigad respectively and were used as reference standards.

The tablet preparations, Nucoxia-P were purchased from local market.

Trade name of tablet	label claim of tablet (mg)		Manufactured by
	ETX	PCM	
Nucoxia-P	60	500	Cadila Ltd.

B. Reagents and Solutions

Pure sample of ETX and PCM was kindly supplied by the Zim Laboratories, Kalmeshwar`and Cadila Ltd, Raigad. Methanol, acetonitrile and water used were of HPLC grade. All other reagents used in this study were of AR grade. Optimized chromatographic conditions are listed in Table 1.

Chromatograph	HPLC Chemito LC 6600
Colum	Eurosphere 100-5 µ C18 column (250 mm X 4.6mm)
Detection Wavelength	236 nm
Flow rate	1.0 mL/min
Temperature	Ambient (28-30^0 C)
Injection volume	20 µL
Mobile Phase	Acetonitrile: Water (50:50) TEA 0.2 mL pH 6.8 adjusted using orthophosphoric acid

Table 1: Chromatographic Conditions

C. Preparation of Standard stock solution

The both drugs (ETX and PCM) solution was prepared by dissolving 25 mg in acetonitrile upto volumetric flask (25 mL). An aliquot portion of stock solution was diluted with acetonitrile to get the final concentration.

D. Selection and preparation of mobile phase

The standard solution of ETX and PCM was run on HPLC C_{18} column using different mobile phases in order to get a good separation and stable peak. The mobile phase was filtered through (0.45 µm) membrane filter and vaccum degassed. The mobile phase containing acetonitrile and water wear mixed in the ratio (50:50) and 0.2 mL trimethylamine and pH adjusted up to 6.8 using of orthophosphoric acid.

E. Analysis of laboratory mixture by proposed mixture

The standard laboratory mixture were prepared by weighing accurately about 5 mg of ETX and 40 mg PCM and dissolving them in a diluent which is a mobile phase in volumetric flask (50 mL), make up volume up to the mark. The solution was filtered through 0.45-µ nylon filter. An aliquot portion of filtrate was further diluted to get the final concentration of 40 µg/mL of PCM hydrochloride and 5 µg/mL of ETX. The laboratory concentration of mixture was injected separately. The chromatograms wear recorded. The results of estimation of laboratory mixture are shown in Table 2.

Parameters	**ETX**	**PCM**
Linearity range	1-10 µg/Ml	8.2-80 µg/mL
Correlation coefficient	0.997	0.998
Slope	290.4	153.0
Y Intercept	163.2	324.4

Table 2.1: Validation Parameters

S. N.	Amount of drug taken for assay (µg/ml)		Amount of drug determined for assay (µg/ml)		% of drug estimated	
	ETX	PCM	ETX	PCM	ETX	PCM
1	5.0	40.0	5.01	40.0	100.2	100
2	5.0	40.0	0.496	39.96	99.28	99.9
3	5.0	40.0	0.498	39.98	99.60	99.95
4	5.0	40.0	5.01	39.96	100.2	99.9
Mean					99.93	100.0
±SD					±0.386	±0.141
%RSD					0.387	0.141

Table 2.2: Results of estimation ETX and PCM in laboratory mixture by HPLC method

F. Analysis of tablet by proposed method

Sample Preparation:

Twenty tablets were weighed and finely powered. The accurately weighed quantity of tablet powder equivalent to 50.0 mg of PCM into volumetric flask (25 mL) and diluent was added. The volumetric flasks were sonicated for 20 min to effect complete dissolution of the PCM and ETX. The solutions were then made up to volume with diluent. The solution was filtered through 0.45 µm nylon filter. The aliquot portion of the filtrate was further diluted to get final concentration of 40 µg/mL of PCM and 5 µg/mL of ETX. The amount of each component in tablet was calculated on the basis of concentration of sample obtained from chromatogram. The results are shown in Table 4.

G. Recovery Study

Accurately weighed quantities of preanalysed tablet powder equivalent to 50 mg of were taken in three 25.0 ml volumetric flasks. To these flasks add 40 µg/mL of PCM and 5 µg/mL of ETX standard solution were added and then sonicated with diluent for 15 min. The volume was made up to the mark with diluent and filtered through membrane filter paper. The aliquot portion of the filtrate was further diluted to get final concentration of 80 µg/mL of PCM hydrochloride and 10 µg/mL of ETX chromatograms were recorded. The concentration of each drug was estimated by comparing sample peak area with standard. The results of recovery study are shown in Table 3.

S.N.	Amount of drug added (µg\mL)		Amount of drug recovered (µg\mL)		% of drug recovery	
	ETX	PCM	ETX	PCM	ETX	PCM
1	5.0	40.0	4.9	40.2	98.0	100.5
2	5.0	40.0	5.1	40.5	102.0	101.25
3	5.0	40.0	5.0	39.7	100.0	99.25
				Mean	100.0	100.33
				± SD	2.00	1.010
				% RSD	2.00	1.006

Table 3: Data of Recovery Study

Nucoxia-P: Average weight of tablet = 0.6343 g (each tablet stated to contain 60 mg ETX and 500 mg PCM)

Method Validation:

Once the HPLC method development was over, the method was validated in terms of parameters like specificity, precision, accuracy, linearity and range, LOD, LOQ, ruggedness, robustness, stability etc. For all the parameters percentage relative standard deviation values were calculated. The proposed HPLC method was validated as per ICH guidelines.

Linearity and Range:

Aliquot portions of the mixed standard stock solution C were diluted to 25 mL with mobile phase to get conc. of 1µg/mL -10µg/mL ETX and 8.2µg/mL-80µg/mL for PCM respectively. The chromatographic conditions were set as per the optimized parameters and mobile phase was allowed to equilibrate with stationary phase as was indicated by the steady baseline. Standard solutions of different concentration were injected separately and the chromatograms were recorded. Peak areas were recorded for each injected concentration of drugs and the calibration curves, concentration verses peak area were constructed for both the drugs. The linearity of measurement was evaluated by analyzing different concentrations of the standard solutions of the ETX and PCM. Calibration curve was constructed by plotting average peak area against concentration and regression equation was computed..The slope, intercept and correlation coefficient values of ETX were found to be 290.4, 163.2 and 0.997 and PCM were found to be 153,324.4, 0.998. The results show that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated above regression graph was shown at Fig: 4a, 4b.

S. No	Amount of tablet powder taken for assay (g)	Wt. of tablet powder Determined for assay (g)		% Of drug found	
		ETX	PCM	ETX	PCM
1	0.05072	0.060	0.4995	100.0	99.9
2	0.05076	0.062	0.5002	103.3	100.04
3	0.05067	0.058	0.500	96.66	100.0
4	0.05068	0.059	0.499	98.33	99.80
			Mean	99.57	99.39
			±SD	±2.834	±0.107
			%RSD	2.846	0.107

Table 4.1: Results of analysis of marketed formulation (precision)

Nucoxia-P: Average weight of tablet = 0.6343 g (each tablet stated to contain 60 mg ETX and 500 mg PCM)

Conditions		% Labeled claim	
		ETX	PCM
Intraday	Time 1 hr	99.30	99.58
	Time 3 hr	98.98	98.71
	Mean	99.14	99.15
	±SD	±0.226	±0.615
	%RSD	0.227	0.620

Table 4.2: In Intraday

Accuracy:

Accuracy of proposed method was as certained on the basis of recovery studies performed by standard addition method. The recovery study method showed accuracy in Table 2.

Precision:

Precision of an analytical method is expressed as SD and % RSD of any measurements. Precision of estimation of ETX and PCM by proposed method was ascertained by replicate analysis of homogenous samples of tablet powder. The results are also shown in Table 3.

Limit of Detection and Limit of Quantification:

The limit of Detection (LOD) and limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal

to noise ratio of 3). The LOD for ETX were found to be 200 µg/mL and PCM were found to be 2 µg/mL respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ for ETX were found to be 800 µg/mL and PCM were found to be 4 µg/mL It was concluded that the developed method is sensitive.

Conditions		**% Labeled claim**	
		ETX	**PCM**
Interday	Day-1	100.25	100.58
	Day-2	99.36	98.12
	Mean	99.80	99.35
	±SD	±0.629	±1.739
	%RSD	0.630	1.750

Table 5: Different Days

Conditions		**% Labeled claim**	
		ETX	**PCM**
Different analyst	Analyst 1	101.75	99.00
	Analyst 2	101.51	98.22
	Mean	101.63	98.61
	±SD	±0.169	±0.551
	%RSD	0.166	0.558

Table 6: Different Analyst

Data of system suitability tests:

S.N.	A.U.C of Paracetamol	A.U.C of Etoricoxib
1	7286.24	1220.33
2	7284.23	1220.49
3	7285.13	1219.38
Mean	7284.25	1219.85
SD	1.490	1.225
Retention Time	2.180	7.820
Asymmetry	1.75	1.50
Theoretical plate/column	1830	6410

Ruggedness and Robustness:

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC and Agilent HPLC by different analyst using different columns of similar types. The %RSD values with two different instruments Shimadzu HPLC and Agilent HPLC, analysts and columns were 0.5- 0.5, 0.6- 0.5 and 0.4- 0.3% respectively. Robustness of the method was determined by making slight changes in the chromatographic conditions, such as change in mobile phase, flow rate and column temperature. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is rugged and robust. The robustness limit for mobile phase variation, flow rate variation, and temperature variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions and were within the acceptance criteria of not more than 2%. The results are also shown in Table 4, 5,6.

Specificity:

Condition of HPLC method like percentage of organic solvent in mobile phase, ionic strength, pH of buffer flow rate etc, was changed. In spite of above changes no additional peaks were found, although there were shift retention times or little changes in peak shapes.

Result and Discussion:

UV spectrum of PCM and ETX was recorded at 236 nm was selected as wavelength. Flow rate of 1ml/min was selected. The mobile phase containing acetonitrile and water wear mixed in the ratio (50:50)

and 0.2 mL trimethylamine and pH adjusted up to 6.8 using of orthophosphoric acid. The retention time of PCM and ETX was found to be 3.18 and 7.82 respectively. Hence the proposed method is simple, accurate, and rapid and can be employed for routine analysis. The low standard deviation and good percentage recovery indicates the reproducibility and accuracy of the method.

CONCLUSION

From the above studies it can be concluded that the tablet formulation containing both ETX and PCM can be successfully analyzed by UV spectrophotometer using multicomponent method and absorbance ratio method. The results obtained by RP-HPLC method for determination of ETX and PCM are reliable, accurate and precise. The method does not require prior separation of one drug from other. Hence, it can be employed for routine quality control analysis of ETX and PCM in combined tablet dosage form.

Hence at required column temperature and pH 6.8 of mobile phase, the sharp peaks were obtained. The flow rate was kept at 1.0 mL/min to achieve adequate retention time of PCM and ETX peaks. The linearity obtained for PCM at 1 -10µg/mL of ETX and 8.2-80 µg/mL. As per the validation studies accuracy was confirmed negative interference of excipients. The precision was confirmed by low values of ±S.D and % RSD less than 2% concluded that no changes in chromatographic parameters.

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