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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ETORICOXIB AND THIOCOLCHICOSIDE IN PHARMACEUTICAL DOSAGE FORMS

Suresh Kumar S*[1], Natraj D[2], Asadulla Khan[3], Kalyan Kumar B[4] and venkateswara Rao J[5]

[1]Sri Vekateshwara College of Pharmacy, Madhapur, Hyderabad, Andhra Pradesh, India.
[2]Sree Vishnu College of Pharmacy, Bhimavaram, Andhra Pradesh, India.
[3]PRRM College of Pharmacy, Shabad, (R.R.Dist), Andhra Pradesh, India.
[4]Vikas College of B.Pharmacy, Suryapet, Nalgonda, Andhra Pradesh, India.
[5]Director. Sultan ul-uloom College of Pharmacy, Hyderabad, Andhra Pradesh, India.

*Corresponding Author: sudamsuresh@gmail.com

ABSTRACT

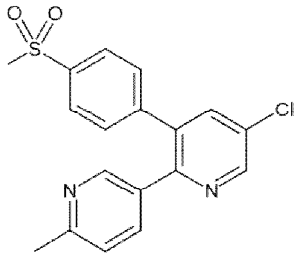
Simple, precise and economical RP-HPLC method was developed for the simultaneous estimation of Etoricoxib (ETR) and Thiocolchicoside (THC) in bulk and combined tablet dosage form. Chromatography was performed on C18 stainless steel column (InertSil ODS-3, 250 mm x 4.6 mm ID, particle size 5µm), the mobile phase used was a mixture of phosphate buffer (P^H6, adjusted with orthophosphoric acid) and methanol (30:70 v/v). The wavelength used for detection of Etoricoxib and Thiocolchicoside was 255 nm and flow rate of 1.2 ml/min. The retention times were 2.506 min. and 4.600 min. for Etoricoxib and Thiocolchicoside, respectively. Linearity was determined for Etoricoxib in the range of 40-80 µg/ml and for Thiocolchicoside 2-6 µg/ml. The correlation coefficient ('r') values were found to be >0.999. The method was validated with respect to accuracy, precision, linearity and robustness as per the ICH Guidelines. The proposed method can be successfully used to determine the drug content of marketed formulation.

Keywords: Etoricoxib, Thiocolchicoside, RP-HPLC, Simultaneous Estimation.

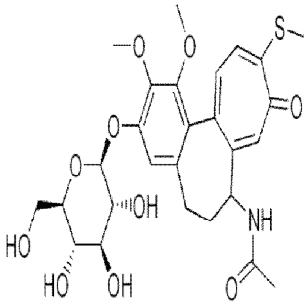
INTRODUCTION

Etoricoxib is chemically, 5-chloro-3-(4-methanesulfonylphenyl)-2-(6-methylpyridin-3-yl) pyridine. It is a new COX-2 selective inhibitor and used in the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, chronic low back pain, acute pain and gout. Thiocolchicoside is chemically, *N*-[(7*S*)-3-(beta-D-glucopyranosyloxy)-1, 2-dimethoxy-10-(methylsulfanyl)-9-oxo-5, 6, 7, 9-tetrahydrobenzo[a]heptalen-7-yl] acetamide and it is used as a muscle relaxant with anti-inflammatory and analgesic effects. It acts as competitive $GABA_A$ receptor antagonist and also inhibits glycine receptors with similar potency and nicotinic acetylcholine receptors to a much lesser extent. Literature survey reveals that there are lots of HPLC and U.V Spectroscopy methods reported for Determination of Etoricoxib and Thiocolchicoside in individual dosage forms. To the best of our knowledge there is no HPLC method reported that can

simultaneously determine both drugs in combined dosage form. Hence there was the need to develop a new RP-HPLC (Willard JH *et al*., 1986. p. 170) method for the analysis of ETR & THC in combined dosage form. Therefore the aim of the present study was to develop a sensitive, precise, accurate and specific HPLC method for the determination of Etoricoxib and Thiocolchicoside simultaneously in Pharmaceutical dosage forms.



Structure of Etoricoxib



Structure of Thiocolchicoside

MATERIALS AND METHODS

Reagents & chemicals

Etoricoxib and Thiocolchicoside were gift samples obtained from Sun Pharma, Mumbai. Potassium dihydrogen orthophosphate and Potassium Hydroxide were of Analytical grade (Merck) and water of HPLC grade (Merck). Commercially available Tablets claimed to contain 60 mg of Etoricoxib and 4 mg of Thiocolchicoside (ETOSHINE MR) were procured from local market. Quantitative HPLC (Ahuja S et al., 2001.) was performed on a high-pressure liquid chromatography (Younglin YL9100) with YL9110 pump, UV detector (YL9120), and C_{18} stainless steel column (InertSil ODS-3, 250 mm x 4.6 mm ID, particle size 5µm). HPLC (Skoog DA *et al*., 5th ed.) system was equipped with data acquisition and processing software "Autochro-3000" electronic balance (Shimadzu) was used for weighing the sample.

Preparation of Mobile Phase

Mobile phase comprised of Potassium dihydrogen orthophosphate (Adjusted to PH 6±0.05 with Ortho phosphoric acid) and methanol (30:70 v/v), diluents used was Phosphate buffer and methanol in ratio of 30:70 v/v. Mobile phase was filtered through a 0.45µm membrane filter, degassed with a Helium spurge for 20 min and pumped from the respective solvent reservoir to the column (flow rate, 1.2 ml/min), which yields a column back pressure of 538-543 psi. Run time was set as 8 min; column was equilibrated for 60 min with mobile phase flowing through the system. Eluents were monitored at 255 nm and data were acquired, stored and analyzed with the software"Autochro-3000" (Young Lin).

Preparation of standard stock solution

Accurately about 10 mg of Etoricoxib and 10 mg of Thiocolchicoside were weighed and transfer into separate 100 ml volumetric flasks. To them about 70 ml of mobile phase (Phosphate buffer and methanol in ratio of 30:70 v/v) was added and sonicated for 20min to dissolve it completely. Then the volume was made up to the mark with the mobile phase. Further working standard solution of mixer of Etoricoxib and Thiocolchicoside (60µg/ml and 4µg/ml) was prepared with mobile phase.

Preparation of sample solution

Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 60 mg of Etoricoxib and 4mg of Thiocolchicoside was weighed and dissolved in 80 ml of methanol. Then it is sonicated for 30 min and solution was filtered through 0.45 µ membrane filter into a 100 ml volumetric flask. Filter paper was washed with the solvent, adding washings to the volumetric flask and volume was made up to mark. Further working sample solutions of 60µg/ml and 4µg/ml of Etoricoxib and Thiocolchicoside was prepared with mobile phase respectively.

Assay procedure

20µl of the standard stock solutions were injected and the retention time and peak area were determined. The sample solutions were also analyzed by injecting 20µl of the solution and the peak area were determined. The amount of Etoricoxib and Thiocolchicoside present in commercial tablets was calculated by comparing the peak area of standard and sample (Fig 2).

Evaluation of Analytical methods

Linearity

Linearity (Snyder LR *et al*., 1997) was determined for Etoricoxib (ETR) in the range of 40-80 µg/ml and for Thiocolchicoside (THC) 2-6 µg/ml, The correlation coefficient ('r') values were >0.999. Typically, the regression equations for the calibration curve was found to be y = 286640x + 545838 for ETR and y = 249635x + 11579 for THC.

Precision

The intraday and inter-day variations of the method were determined using five replicate injections of same concentrations and analyzed on the same day and three different days. The result revealed the precision with %RSD (0.41% and 0.3%for ETR) and (0.26% and 0.25% for THC), respectively for intraday and inter day.

Accuracy

To check the accuracy of the method, recovery studies were carried out at three different levels 50%, 100% and 150% solutions made from standard solution. In this method involves spiking the standard stock solution in placebo at different levels. The sample responses were obtained and drug concentrations of ETR and THC were calculated by using statistical data. The mean % recoveries were in between 99.3-99.7 and were given in (Table 2).

Detection limit and quantitation limit

A calibration curve was prepared by using concentrations in the range of 2-6 µg/ml for ETR and 40-60 µg/ml for THC. The standard deviation of y-intercepts of regression lines were determined and kept in following equation for the determination of detection limit and quantitation limit. Detection limit= $3.3\sigma / s$; quantitation limit=$10\ \sigma/s$; where σ is the standard deviation of y-intercepts of regression lines and s is the slope of the calibration curve. LOD and LOQ values for ETR was found to be 0.0115, 0.0348 µg/ml and for THC was found to be 0.0132, 0.04 µg/ml respectively.

Specificity and selectivity

The specificity (ICH QIA (R2), 2001) of the HPLC method was determined by comparing the chromatograms of the standard and sample solutions. The parameters like retention time, resolution and tailing factor were calculated. Good correlation was found between the results of standard and sample solution. The method was selective, showed no interference peaks around the retention time, base line showed no significant noise.

Robustness

The robustness of a method is its ability to remain unaffected by small deliberate changes in parameters. Robustness (ICH, 1996, p.1-8) of the method was determined by carrying out the analysis under conditions during which mobile phase ratio, flow rate and temperature were altered. There is no significant impact on retention time and peak area was found.

RESULTS AND DISCUSSION

The present study was carried out to develop a sensitive, precise and accurate RP-HPLC method for the analysis of Etoricoxib and Thiocolchicoside in pharmaceutical dosage forms. The retention times for Thiocolchicoside and Etoricoxib was found to be 2.50 and 4.60 respectively. Each sample was injected five times and the similar retention times were observed in all cases. The peak area for drug solution was reproducible as indicated by low coefficient of variation. A good linear relationship (r = 0.999) was observed between the concentrations and the respective peak areas. In intra and inter day variation studies, Inter day and intraday precision was determined by analyzing the drug sample at different concentration levels. The results are presented in the form of %RSD which is below 1.00 and shows that the proposed HPLC method was highly precise. The amount of drug recovered was shown in Table (1).The method was robust as observed from insignificant variation in the results of analysis by changes in flow rate, Mobile phase composition and temperature. The drug

content in the Tablet was quantified using the proposed analytical method. The absence of additional peaks indicates no interference of the excipients used in the capsules. The proposed reversed phase HPLC method was found to be simple, precise, highly accurate, specific and less time consuming.

CONCLUSION

The developed method was found to be simple, sensitive, accurate, precise and reproducible. It can be used for dissolution studies as well as routine quality control analysis of Etoricoxib and Thiocolchicoside in tablet formulation.

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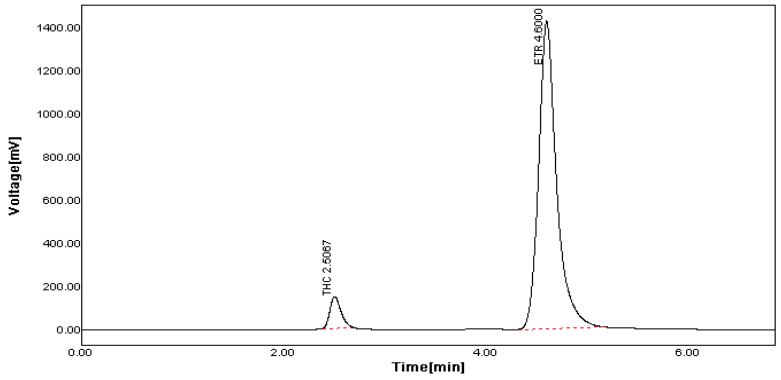

Fig. 2: Typical Chromatogram of THC and ETR obtained from Sample

Table 1: Optical Characteristics of Etoricoxib and Thiocolchicoside by HPLC method

Parameters	Thiocolchicoside	Etoricoxib
Correlation coefficient (r)	0.9991	0.9997
Retention time	2.50	4.60
Resolution	0	8.3
No. of Theoretical plates	2488.3	3759.4
Tailing factor	1.26	1.33
LOD	0.0132	0.0115
LOQ	0.04	0.0348

Table 2: Assay and Recovery studies for THC and ETR

Drug	conc.	Label claim (mg)	Amt. Added (mg)	Amt. Found (mg)	% Recovery	Mean % Recovery
THC	50	4	2.1	6	98.36	99.32
	100	4	4	8.1	101.2	
	150	4	5.8	9.65	98.4	
ETR	50	60	29.7	88.77	98.8	99.70
	100	60	60	121.7	101.4	
	150	60	90.5	148.9	98.9	

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