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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC FOR SIMULTANEOUS ESTIMATION OF ETODOLAC & THIOCOLCHICOSIDE

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ABSTRACT

This paper elucidates development and validation of a high-performance liquid chromatographic analytical procedure for simultaneously Estimation of Etodolac and Thiocolchicoside in a tablet formulation. Chromatographic determination was performed on a C18 column (4.5mm x 250 mm, 5µm) using Methanol: Acetonitrile: Water 20:60:20 (v/v/v) as mobile phase by (85:15) v/v at a flow rate of 1.0ml/min with UV detection at 274 nm. The retention times of Etodolac and Thiocolchicoside were found to be at 7.86 and 5.49 minutes respectively. The method was validated for analytical parameters Specificity, linearity, precision, accuracy, LOD and LOQ.

KEYWORDS: Etodolac and Thiocolchicoside, High performance liquid chromatography, Method development, Validation.

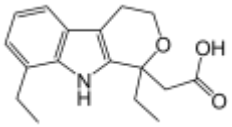
INTRODUCTION

Etodolac is a non-steroidal anti-Inflammatory drug (NSAID) with antipyretic and analgesic properties and mainly it is used to get relief from the signs and symptoms of Osteoarthritis and rheumatoid arthritis by inhibiting prostaglandin synthesis.

Thiocolchicoside it is a semi synthetic derivative of naturally occurring colchicoside from the seeds of various species of *Colchicum autumnale* (autumn crocus, meadow saffron, *Gloriosasuperba*) and it binds to GABA-A and Strychnine sensitive glycine receptors and acting as a GABA-A receptor antagonist. Primarily it acts as a Muscle relaxant with anti-

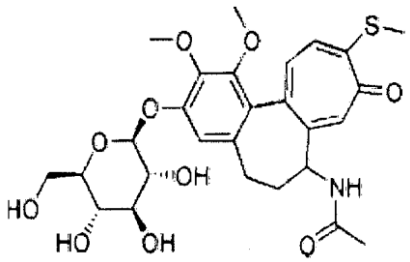
Inflammatory and analgesic properties in conditions like like Neuralgia, Parkinsonism, Backache, Pain, Sciatic pain etc.

Chemically



Etodolac: 2-{1,8-diethyl-1H,3H,4H,9Hpyrano
1H,3H,4H,9Hpyrano [3,4-b]indol-1-yl}acetic acid.

(C17H21NO3)



Thiocolchicoside

N-[(7S)-3-(beta-D-glucopyranosyloxy)-1,2dimethoxy
10(methylsulfanyl)9oxo5,6,7,9tetrahydrobenzo[a] heptalen-7-yl] acetamide

(C27H33NO10S)

The aim of the present study was to develop and validate a HPLC method for the simultaneous estimation of Etodolac and Thiocolchicoside in tablets. Till now, there is no simple RP-HPLC method has been reported using simple solvents. The method described complied with validation requirements of ICH and could be used for routine quality control of pharmaceutical formulations in Ordinary laboratories.

MATERIALS AND METHODS

Reagent and Chemicals

Etodolac and Thiocolchicoside, were obtained as gift sample from IPCA Laboratories. All solvent and reagent used were of HPLC and spectroscopic grade. Acetonitrile and Methanol HPLC grade obtained from Merck chemicals, Millipore water obtained from (Milli-Q) was used in all experiments. Etodolac and Thiocolchicoside are used as reference standards.

Instrument and Chromatographic Condition

The method development was performed with a High pressure liquid chromatographic system Consisting of an Agilent model PEAK LC-700 solvent delivery system. The system was controlled and data analysis were performed with the PEAK software. The detector was set at 274nm and injection volume 20µL at flow rate 1.0mL/minute and peak areas were integrated automatically by using software. Separation was carried out at ambient temperature using ZODIAC C18 column (4.5mm x 250 mm, 5µm).

Mobile phase Preparation

The Mobile phase contains Methanol: Acetonitrile: Water 20:60:20 (v/v/v) was prepared.

By mixing 200ml of Methanol, 600ml of Acetonitrile and 200ml of water. The above solution

Was filtered through 0.22µm nylon membrane filter and degassed by sonication.

Diluent Preparation: Mobile phase.

Standard stock Preparation

Weighed and transferred 50mg of Etodolac and 50mg of Thiocolchicoside into 50mL volumetric flask, added 35mL of Mobile phase and dissolved with sonication then diluted to volume with Mobile phase to get the concentration of 1000µg/mL.

The above stock used for Linearity Levels, LOQ and LOD.

Sample preparation: Crushed 20 Tablets with Motor and pestle then weighed 12 mg of drug into 200mL Volumetric flask, which is equivalent to 60µg/mL and added 150mL diluent and sonicated then cooled to room temperature then diluted to volume with diluent and mixed well.

Validation

1. System suitability
2. Linearity and Range
3. LOD and LOQ
4. Precision

Inter day and intraday precision.

5. Accuracy

6. Robustness

1. System Suitability

Injected Standard 50µg/mL Concentration and Calculated System suitability as fallows.

Table: 1.

System analysis parameter	Etodolac	Thiocolchicoside	Acceptance Criteria
Tailing Factor for (T)	1.6	1.6	NMT 2.0
Column efficiency-plate counts (N)	31867	11830	NLT 2000
Resolution (R)	5.8	-	NLT 2.0

2. Linearity and Range

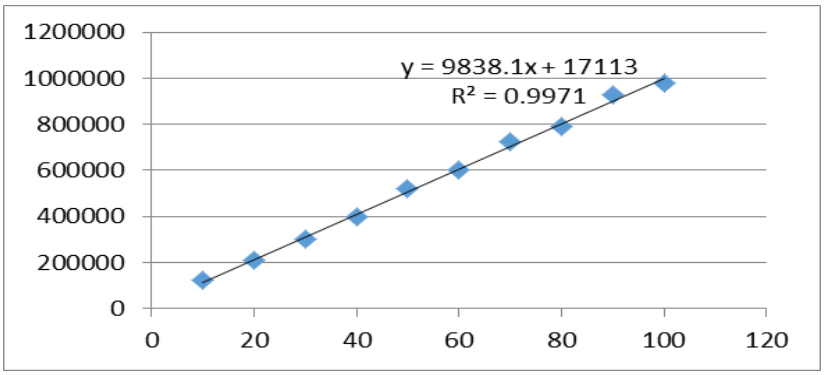
Linearity Stock Preparation: Prepared a solution contained 1000µg/mL of both Etodolac and Thiocolchicoside.

A series of solutions containing Etodolac and Thiocolchicoside at concentrations listed below were prepared. A single injection of each solution was performed. The peak areas of each level were plotted against the respective concentrations slope, y-intercept and coefficient of determination (r^2) were calculated from the regression analysis. The obtained results are presented in below table.

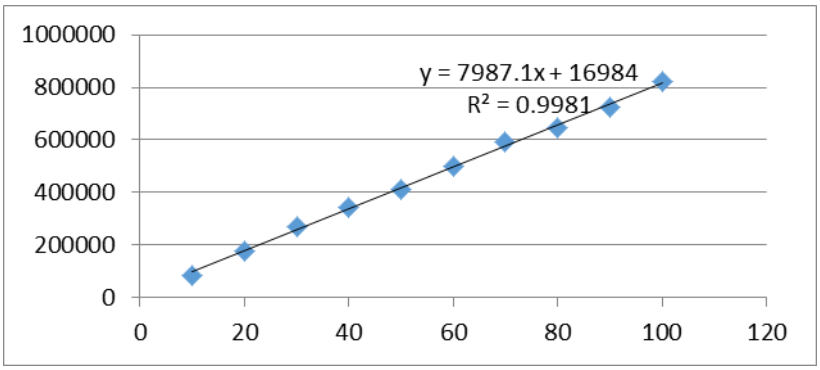
Table: 2.

% Level	Concentration (µg/mL)	Area Response	
		Etodolac	Thiocolchicoside
20%	10	125078	82448
40%	20	210827	176868
60%	30	299523	269243
80%	40	399246	342921
100%	50	519502	411646
120%	60	602788	500203
140%	70	726602	590757
160%	80	790556	644834
180%	90	928848	722754
200%	100	979092	821051
	Slope	**9838.1**	**7987.1**
	Y-Intercept	**17113.13**	**16984.2**
	r^2	**0.997**	**0.999**

Etodolac



Thiocolchicoside



3. Accuracy

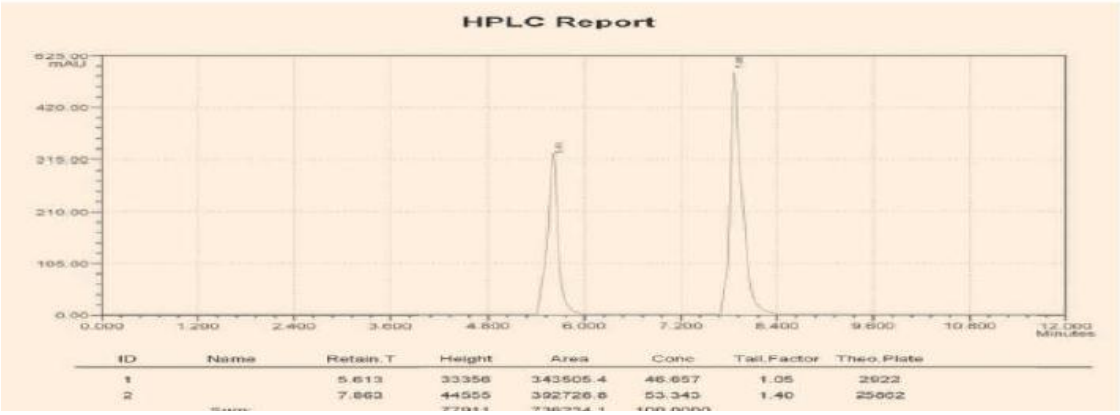
Recovery Studies of ETIODOLAC and THIOCOLCHISIDE

Recovery studies were carried out by applying the standard addition method.It was carried out by spiking the already analysied sample of the tablets with standard of ETD and THIO which contain of 50%, 100% and 150% of already analysed sample.

Table: 3.

% of Recovery	Target Con (µg/mL)	Spiked conc(µg/mL)	Final Conc (µg/mL)	Conc Obtanined in Etodolac	% of Assay Etodolac	Conc Obtained in Thio	% of Assay in Thio
50%	20	10	30	30.16	100.56	29.76	99.23
	20	10	30	30.11	100.39	29.63	98.77
	20	10	30	29.75	99.17	30.06	100.21
100%	20	20	40	39.34	98.36	40.05	100.17
	20	20	40	39.47	98.67	40.06	100.22
	20	20	40	40.33	100.83	39.75	99.17
150%	20	30	50	49.69	99.38	49.07	98.15
	20	30	50	49.02	98.04	49.74	99.48
	20	30	50	49.11	98.22	50.31	100.63

RECOVERY 100% CHROMATOGRAM



4. PRECISION

Precision is also called repeatability studies

1) Intraday Precision

2) Inter day Precision

Intraday Precision: Intraday precision was determined by analyzing same Concentration (60µg/ml) of Etodolac & Thiocolchicoside for six times in the same day.

Inter day Precision: Inter day precision was determined by analyzing the same Concentration (60µg/ml) of Etodolac & Thiocolchicoside on different days.

Table: 4.

S.NO	Concentration (µg/mL)	Peak Area(Intraday)		Peak Area(Inter day)	
		Etodolac	Thiocolchicoside	Etodolac	Thiocolchicoside
1	60	638844	499912	613297	484439
2	60	635493	491844	608745	484225
3	60	647323	496581	608590	480946
4	60	622522	497893	612504	481051
5	60	623603	493451	611335	481064
6	60	642767	491499	610547	499854
	Mean	**635092**	**495197**	**610836**	**485263**
	SD	**10126.631**	**3446.199**	**1928.111**	**7330.249**
	%RSD	**1.6**	**0.7**	**0.3**	**1.5**

5. Limit of Detection

The limit of detection of an individual analytical procedure is the lowest amount analyte in a sample which can be detected not necessarily quantitated.

Calculated as

LOD=3.3 x (SD/Slope)

Etodolac LOD=3.3 x 10216/9838.1

Thiocolchicoside LOD: 3.3*3446/7987.1

Table: 5.

S.No	Drugs	LOD
1	Etodolac	0.33µg/mL
2	Thioclchicoside	0.28µg/mL

6. Limit of Quantitation

The Limit of quantification is an analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy.

Quantitation limit (LOQ) may be expressed as

LOQ = 10 x (SD/Slope)

Etodolac LOD=10x 10216/9838.1

Thiocolchicoside LOD=10*3446/7987.1

Table: 6.

S.No	Drugs	LOQ
1	Etodolac	1.024µg/mL
2	Thioclchicoside	0.84µg/mL

7. Robustness

The robustness of an analytical procedure was tested by measuring its capacity of remain Unaffected by small, but deliberate variations in the method parameters and provides an indication of its reliability during the normal use.

Parameters of Robustness

Table: 7.

S.No	Condition	Mean Area of Etodolac	% Difference	Mean Area of Thiocolchicoside	% Difference
1	**Standard**	602788	0.0	500203	0.0
2	MP-Change-1	604925	0.35	500388	0.03
3	MP-Change-2	605752	0.49	507464	1.45
4	WL-Change-1	610994	1.3	507624	1.48
5	WL-Change-2	605932	0.52	503566	0.67
6	Flow changes-1	611448	1.4	504619	0.88
7	Flow changes-2	609013	1.03	508146	1.58

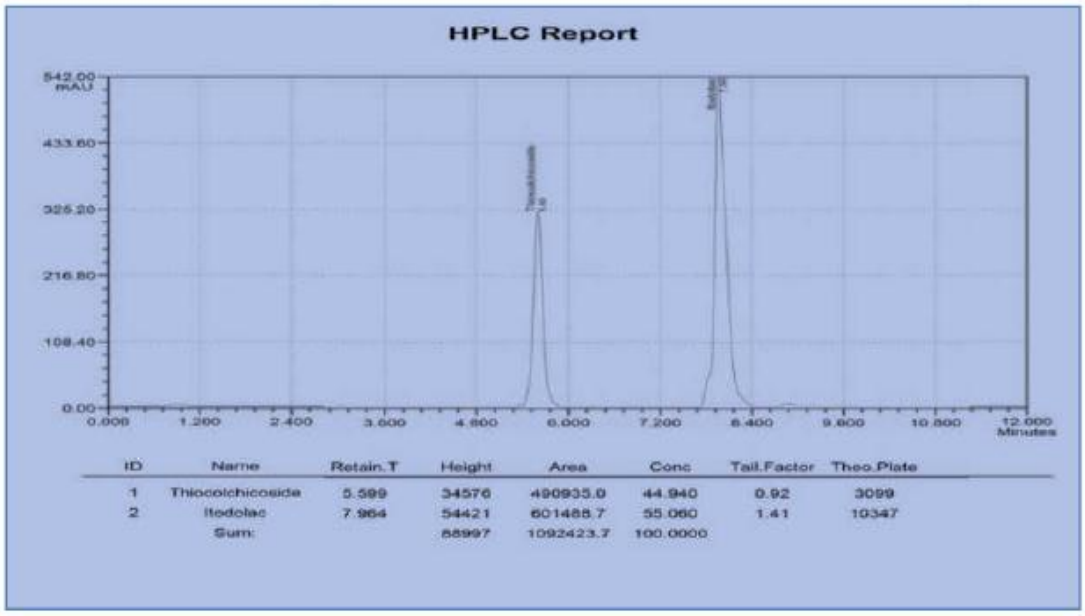
Robustness of the method was studied by making deliberate changes in the Chromatographic conditions and the effect of results were examined the mean difference is less than 2 indicating robustness of the method.

Assay Result of Marketed Formultion

Table: 8.

S.No	Drugs	Brand Name	Lable Claim	Concentration in (µg/mL)	Amount found	% of Assay	% RSD
1	Etodolac	Etova-MR	400mg	60µg/mL	99.85	99.70	1.00
2	Thiocolchicoside		4mg	60µg/mL	99.00	98.01	1.01

MARKETEDFORMULATION CHROMATOGRAM



Six replicates of the samples solutions were injected for quantitative analysis. The Amounts of ETO and THIO estimated were found to 99.7% and 98.01% respectively. A good Separation and resolution of both drugs indicate that there was no interference from Excipients commonly present in pharmaceutical formulations. Dosage form was accurate within This showed that the estimation of dosage form was accurate within the given acceptable level of 95% to 105%.

RESULTS AND DISCUSSION

To optimize the mobile phase, various proportions of Solvents were tested. Mobile phase composition was changed and the method development was started By ZODIAC C-18 Column (4.6×250mm, 5µm) column and with a flow rate of 1.0 mL/min, and detection

wavelength of 274 nm. Column temperature was maintained at 30°C. Injection volume is 20 µL, and run time is for 12 min. The mobile phase consists of Methanol: Acetonitrile: Water 20:60:20 (v/v/v) finally an Isocratic method was developed. The retention times of Etodolac and Thiocolchicoside Peaks are about 7.86 and 5.49 minutes respectively. Quantitative linearity was observed over the concentration range of 10.000 to 100.000 µg/mL for Etodolac and Thiocolchicoside. The regression equations of concentration of Etodolac and Thiocolchicoside are found to be Y=8229x+50020 and Y=10083x+602788 respectively, where y is the peak area and x is the concentrations of drugs (µg/mL). The numbers of theoretical plates obtained were 31867 and 11830 for Etodolac and Thiocolchicoside respectively, which indicates the efficiency of the column. The limit of detection and limit of quantitation were found to be 1.024µg/mL and 0.84µg/mL for Etodolac and Thiocolchicoside respectively, which indicates the sensitivity of the method. The high percentage recovery indicates that the proposed method is highly accurate.

CONCLUSION

To summarize, the validated reverse phase liquid chromatographic method was evaluated in a mass of facets, such as linear relation include correlation coefficient and proved to be accurate, simple, precise and sensitive and suitable for the Identification and Quantitative estimation of Etodolac and Thiocolchicoside for Routine analysis of Individual and combination of drugs. This Method was developed and validated as per the ICH Q2 (R1) as It can be used by analytical department.

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