



Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF DICLOFENAC SODIUM AND THIOCOLCHICOSIDE IN TABLET DOSAGE FORM BY USING RP-HPLC

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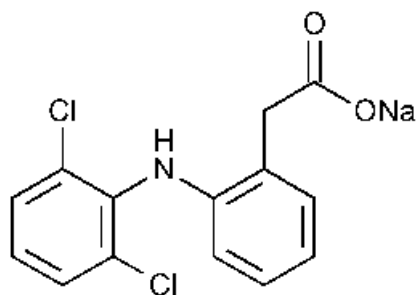
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Abstract: An isocratic, reversed phase-liquid-chromatographic method was developed for the quantitative determination of Diclofenac Sodium and Thiocolchicoside in combined-dosage form. A Waters Symmetry ShieldeRp18, (250*4.6*5 μ) column with mobile phase containing water pH 9.2 adjusted with di- Potassium hydrogen Phosphate: Methanol in the ratio of (60: 40, v/v) was used. The flow rate was 1.0 mL/min, column temperature was 30°C and effluents were monitored at 223 nm. The retention times of Diclofenac Sodium and Thiocolchicoside were 3.229 min and 4.999 min, respectively. The correlation co-efficient for Diclofenac Sodium and Thiocolchicoside was found to be 0.99 and 0.99, respectively. The proposed method was validated with respect to linearity, accuracy, precision, specificity, and robustness. Recovery of Diclofenac Sodium and Thiocolchicoside formulations was found to be in the range of 97-103% and 97-103% respectively confirms the non-interferences of the excipients in the formulation. Due to its simplicity, rapidness and high precision. The method was successfully applied to the estimation of Diclofenac Sodium and Thiocolchicoside in combined dosage form.

Key words: RP-HPLC, Diclofenac Sodium and Thiocolchicoside

INTRODUCTION

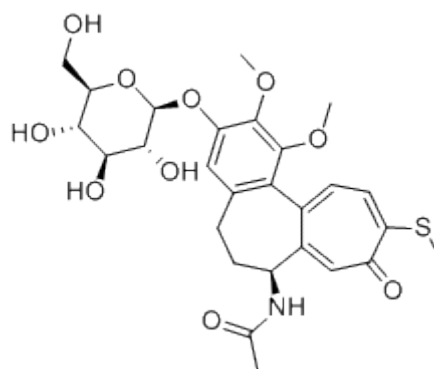
Diclofenac: Diclofenac is chemically named as 2-(2-(2,6-dichlorophenylamino) phenyl)acetic acid. Diclofenac belongs to a class of drugs called non-steroidal anti-inflammatory drugs (NSAIDs) that are used for the treatment of mild to moderate pain, fever, inflammation such as rheumatoid arthritis, gout, pyrophosphate arthropathy, osteoarthritis, and ankylosing spondylitis. Diclofenac sodium acts by potent cyclo-oxygenase inhibition, reduction of arachidonic acid release, and enhancement of arachidonic acid uptake. It thereby results in a dual inhibitory effect on both the cyclo-oxygenase and lipoxygenase pathways. The recommended dose for most conditions is 100-200 mg daily. Diclofenac (DIC) tablets can cause side effects like stomach pain, indigestion, heartburn and nausea.



Structure of Diclofenac Sodium

Thiocolchicoside: Thiocolchicoside is (THIO, (S)-N- [3-(B-D-glucopyranoxyloxy)-5, 6, 7, 9-tetrahydro-1, 2-dimethoxy-10-(methylthio) -9-oxobenzo [a] heptalen-7yl] acetamide

[Matrindale 3th, 2009; N.A. El-Ragehy et al., 2003]. Thiocolchicoside is a synthetic sulphur derivative of colchicoside. Thiocolchicoside has a selective affinity for γ -amino- butyric acid (GABA) receptors and acts on the muscular contracture by activating the GABA-nergic inhibitory pathways thereby acting as a potent muscle relaxant



Structure of Thiocolchicoside

Instrumentation: The separation was carried out on HPLC system with Waters 2695 alliance with binary HPLC pump, Waters 2998 PDA detector, Waters Empower2 software Waters Symmetry Shielde Rp18, (250*4.6*5 μ).

Chemicals and Reagents: Diclofenac Sodium and Thiocolchicoside was a gift sample by Dr. Reddy's Laboratories Ltd., Hyderabad. Methanol of HPLC grade was purchased from E. Merck (India) Ltd., Mumbai. di- Potassium hydrogen Phosphate of AR grade was obtained from S.D. Fine Chemicals Ltd., Mumbai and milli Q water.

HPLC Conditions: The mobile phase consisting of water (pH 9.2 adjusted with di- Potassium hydrogen Phosphate: Methanol (HPLC grade) were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 60:40v/v was pumped into the column at a flow rate of 1.0ml/min. The column temperature was 30°C. The detection was monitored at 285nm and the run time was 6min. The volume of injection loop was 10µl prior to injection of the drug solution the column was equilibrated for at least 15 min. with the mobile phase flowing through the system.

PREPARATIO OF STANDARD SOLUTION

DiclofenacSodium: Accurately weighed quantity, 50.0 mg of

Diclofenac Sodium was transferred into 100ml of volumetric flask and adds 30ml of mobile phase and sonicate for 15 min. make up the volume with mobile phase.

Thiocolchicoside: Accurately weighed quantity, 4mg of Thiocolchicoside was transferred into 100ml of volumetric flask and adds 30ml of mobile phase and sonicate for 15mins make up the volume with mobile phase

PREPARATION OF SAMPLE SOLUTION:

Accurately weighed 220 mg of sampel. Transfer the sampel into 100ml of volumetric flask added 25ml of mobile phase and sonicated for 30mins and make up the volume with mobile phase and filtered through the 0.45µm filter paper .

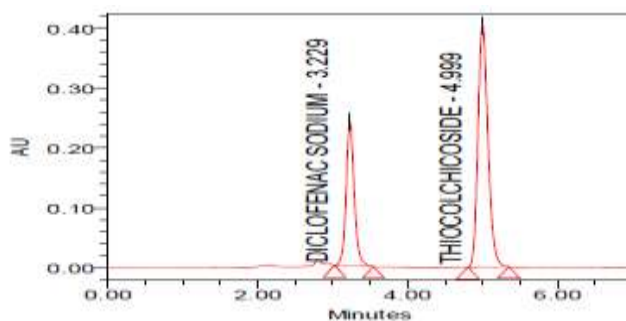


Fig. 1: Standard chromatogram for Diclofenac Sodium and Thiocolchicoside

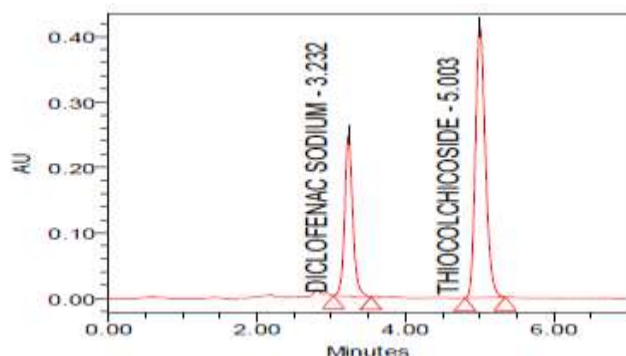


Fig. 2: Formulation chromatogram for Diclofenac Sodium and Thiocolchicoside

METHOD VALIDATION

System Suitability Studies: The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table 1). The values obtained demonstrated the suitability of

the system for the analysis of this drug combinations, system suitability parameters may fall within ± 3 % standard deviation range during routine performance of the method.

Table1: System Suitability Parameters

Parameters	Thiocolchicoside	Diclofenac Sodium
Correlation Coefficient	0.99	0.99
Regression Equation	y = 16616x	y = 19288x
LOD	3.260	0.0178
LOQ	9.867	0.0593
Theoretical plates	4601	6744
Tailing	1.157	1.212

Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc

ACCURACY AND PRECISION

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated. From the data obtained, added recoveries of standard drugs were found to be accurate

(Table-3&4). The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated. In the inter-day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drugs peaks and percentage RSD were calculated. The chromatograms of three different levels shown in Fig 3,4&5. From the data obtained, the developed RP-HPLC method was found to be precise (Table-2).

Table 2 : Precision Studies

SN O	Sample Wt(mg)	Area(dicl)	Area(Thio)	%Assya (dicl)	%Assya(Thio)
1	220.00	1831273	3795829	99	100
2	220.00	1833213	3797883	99	100
3	220.00	1830492	3790956	99	100
4	220.00	1831464	3797566	99	100
5	220.00	1831099	3796787	99	100
6	220.00	1833584	3792846	99	100

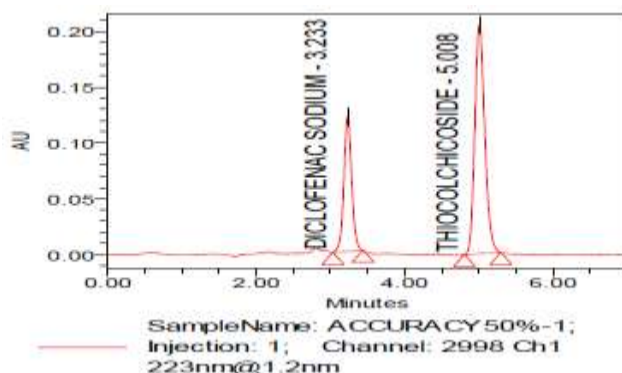


Fig. 3: Accuracy Chromatograms-50% of Diclofenac Sodium and Thiocolchicoside

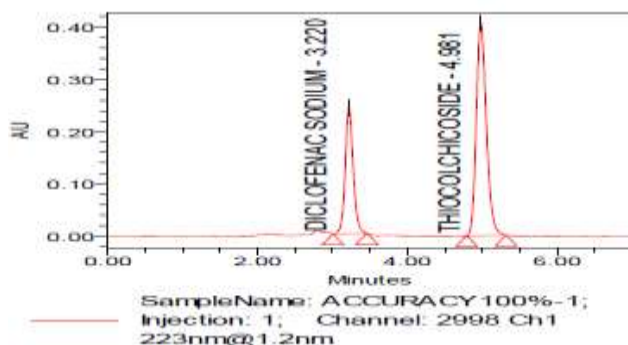


Fig. 4: Accuracy Chromatograms-100% of Diclofenac Sodium and Thiocolchicoside

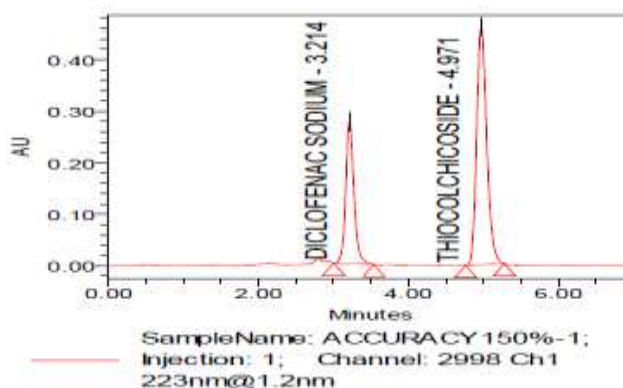


Fig. 5: Accuracy Chromatograms-150% of Diclofenac Sodium and Thiocolchicoside

Table 3: Accuracy for Diclofenac Sodium

Spiked Level	Sample Weight	Sample Area	µg/ml added	µg/ml found	% recovery	mean
50%	110.00	916760	2477.500	2477.08	100	100
50%	110.00	916193	2477.500	2475.55	100	
50%	110.00	916513	2477.500	2476.41	100	
50%	110.00	916261	2477.500	2475.73	100	
50%	110.00	916302	2477.500	2475.84	100	
50%	110.00	916417	2477.500	2476.15	100	
100%	220.00	1835474	4955.000	4959.44	100	100
100%	220.00	1837212	4955.000	4964.14	100	
100%	220.00	1831837	4955.000	4949.61	100	
150%	330.00	2753908	7432.500	7441.05	100	100
150%	330.00	2756576	7432.500	7448.26	100	
150%	330.00	2754974	7432.500	7443.93	100	
150%	330.00	2758254	7432.500	7452.79	100	
150%	330.00	2755427	7432.500	7445.15	100	
150%	330.00	2757259	7432.500	7450.10	100	

Table 4: Accuracy for Thiocolchicoside

Spiked level	Sample weight	Sample area	µg/ml added	µg/ml found	% recovery	mean
50%	110.00	1890923	19.940	19.88	100	100
50%	110.00	1891012	19.940	19.88	100	
50%	110.00	1890567	19.940	19.88	100	
50%	110.00	1895701	19.940	19.93	100	
50%	110.00	1893634	19.940	19.91	100	
50%	110.00	1892002	19.940	19.89	100	
100%	220.00	3794064.00	39.880	39.89	100	100
100%	220.00	3790494.00	39.880	39.86	100	
100%	220.00	3799041.00	39.880	39.95	100	
150%	330.00	5680034	59.820	59.72	100	100
150%	330.00	5687420	59.820	59.80	100	
150%	330.00	5688575	59.820	59.81	100	
150%	330.00	5687627	59.820	59.80	100	
150%	330.00	5684962	59.820	59.78	100	
150%	330.00	5681289	59.820	59.74	100	

LINEARITY AND RANGE

The linearity of the method was determined at five concentration levels. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was $y = 16616x$ ($R^2=0.99$) for Diclofenac Sodium and $y = 19288x$ ($R^2=0.99$) for Thiocolchicoside.

The results shows that an excellent correlation exists between areas and concentration of drugs within the concentration range indicated above. The overlay chromatograms of Linearity for Diclofenac Sodium and Thiocolchicoside shows in Fig 6 and the results for calibration curves are given in Fig 7&8.

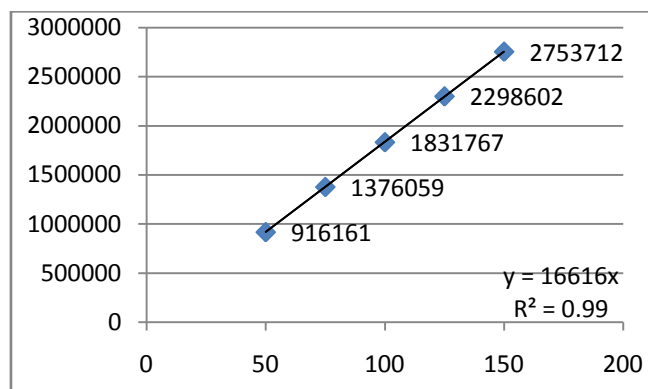


Fig. 7: Linearity Curve for Diclofenac Sodium

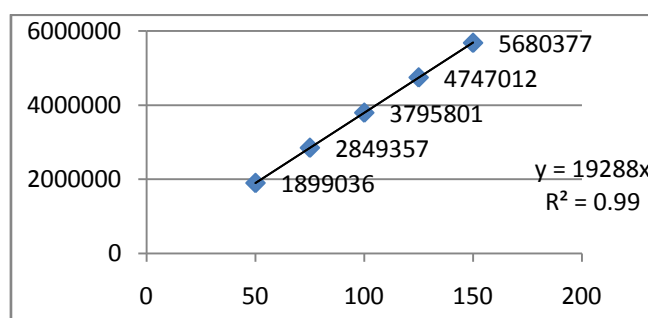


Fig. 8: Linearity Curve for Thiocolchicoside

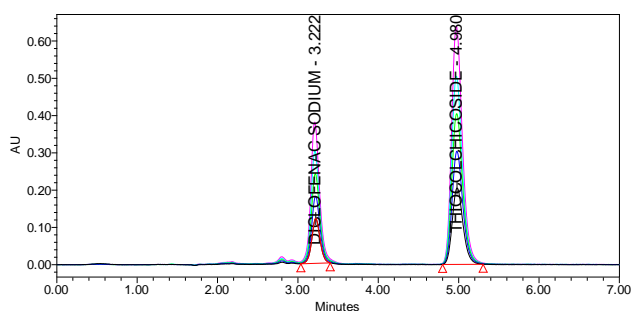
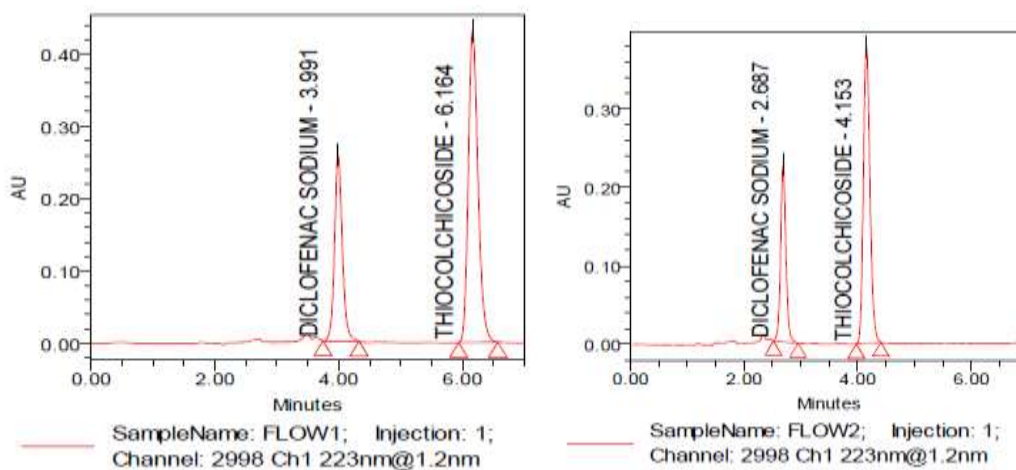


Fig. 9: Overlay chromatograms of Linearity For Diclofenac Sodium and Thiocolchicoside

ROBUSTNESS

Robustness of the method was determined by making slight changes in the chromatograph conditions. It was observed

that there were no marked changes in the chromatograms, which demonstrated that the RP HPLC method developed, is robust (Table-5&6).



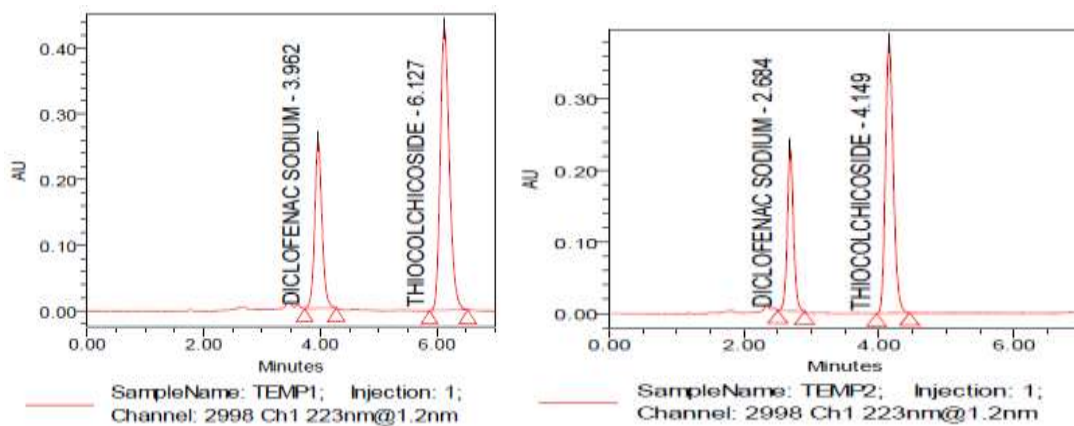


Table5:Robustness for Thiocolchicoside

	SAMPEL NAME	INJ	NAME	RT	AREA	USP TAILING	USP PLATECOUNT
1	TEMP-1	1	Thiocolchicoside	6.127	4688637	8.315	7371
2	TEMP-2	1	Thiocolchicoside	4.149	3078996	7.583	6027
3	FLOW-1	1	Thiocolchicoside	6.164	4687523	8.352	7546
4	FLOW-2	1	Thiocolchicoside	4.153	3058263	7.588	6086

Table6:Robustness for Diclfenac Sodium

	SAMPEL NAME	INJ	NAME	RT	AREA	USP TAILING	USP PLATECOUNT
1	TEMP-1	1	DiclfenacSodium	3.962	2217139	1.107	5127
2	TEMP-2	1	Diclfenac Sodium	2.684	1444374	1.095	4251
3	FLOW-1	1	Diclfenac Sodium	3.991	2257186	1.124	5090
4	FLOW-2	1	Diclfenac Sodium	2.687	1462684	1.120	4229

LOD &LOQ: Limit of quantification and detection were predicted by plotting linearity curve for different nominal concentrations of Diclofenac Sodium and Thiocolchicoside. Relative standard deviation (σ) method was applied, the LOQ and LOD values were predicted using following formulas (a) and (b). Precision was established at these predicted levels.

- (a) $LOQ = 10 \sigma / S$
- (b) $LOD = 3.3 \sigma / S$

Where σ = residual standard deviation of response
 S = slope of the calibration curve.

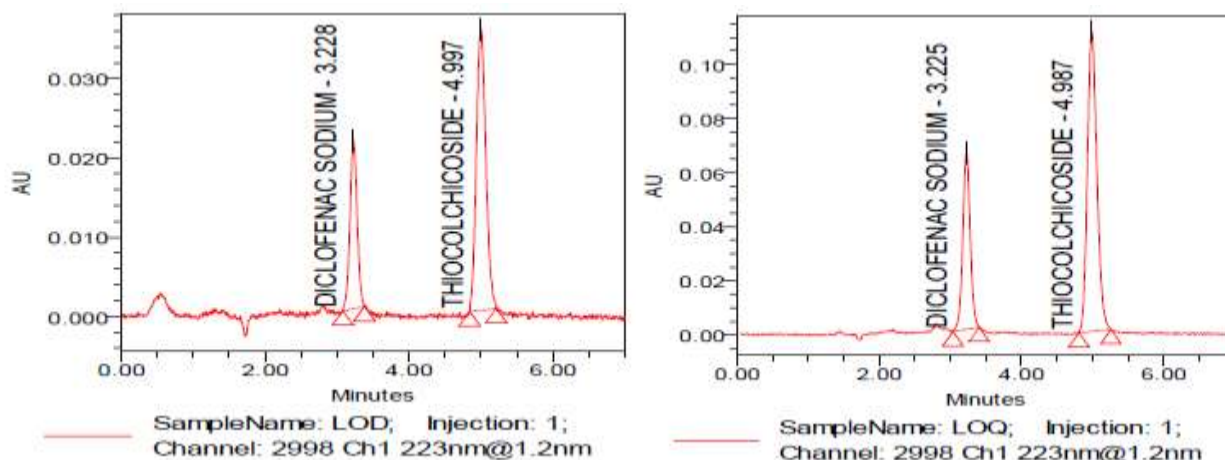


Table 7: LOD and LOQ For Diclofenac Sodium and Thiocolchicoside

	Sampel name	inj	Name	RT	Area
1	LOD	1	Diclo	3.228	147829
2	LOQ	1	Diclo	3.225	476743
1	LOD	1	Thio	4.997	326209
2	LOQ	1	Thio	4.987	1034334

RESULTS AND DISCUSSION

System suitability results were given by table 1 and system suitability parameters are retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 1 so we can say system is suitable for analysis method specificity was concluded by fig:1 and fig:2 those figures are Diclofenac Sodium and Thiocolchicoside standard chromatogram and other one is formulation they were not observed placebo and excipients peaks interference with standard and analytic peak so it proves method is selective. The result given in table 2 says that the method precision passed for both Diclofenac Sodium and Thiocolchicoside studies. The method accuracy was evaluated by recovery studies. Diclofenac Sodium and Thiocolchicoside recovery was founded 100% as per ICH 97%- 103% and also percentage RSD was very low so method is accurate shown in table 3&4. Linearity calibration curve was given below fig: 7&8 and plot the graph three different concentrations versus areas to construct the linear regression equation and to calculate the value of correlation co-efficient. Linear correlation was found to be $Y=16616$ for Diclofenac Sodium and $y = 19288$ for Thiocolchicoside. Method robustness results were given by table 5&6, LOQ and LOD Results were given by table 7.

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Diclofenac Sodium and Thiocolchicoside in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Diclofenac Sodium and Thiocolchicoside in pure and its pharmaceutical dosage forms.

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