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[Research article]

Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Aceclofenac and Thiocolchicoside in Tablet Dosage Form

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ABSTRACT

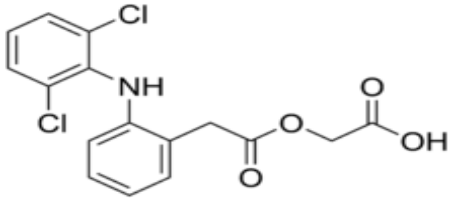
A simple reversed-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for simultaneous determination of Aceclofenac and Thiocolchicoside in tablet dosage form. Chromatographic analysis was performed on a Symmetry Nucleosil C8 (150X4.6 mm,5µm) column ambient temperature with a mixture of mixed phosphate buffer and Acetonitrile in the ratio 40:60(buffer preparation: Prepare 0.01M Disodium hydrogen orthophospate as mobile phase, at a flow rate of 0.80 mL min^{-1}. UV detection was performed at 261 nm. The method was validated for accuracy, precision, specificity, linearity and sensitivity. The retention times of Aceclofenac and Thiocolchicoside were 2.167 and 4.866 min, respectively. The Limit of detection was 0.33 and 3.9 µg mL^{-1} and the quantification limit was 1.002 µg mL^{-1} and 11.9 µg mL^{-1} for Aceclofenac and Thiocolchicoside respectively. The accuracy of the proposed method was determined by recovery studies and found to be 99.94% to 99.81%.

Keywords: Aceclofenac, Thiocolchcicoside, RP-HPLC, Validation.

INTRODUCTION

Aceclofenac is a non-steroidal anti-inflammatory drug (NSAID).Aceclofenac has higher anti-inflamatory action than conventional NSAID. It works by blocking the action of a substance in the body called cyclo-oxygenase. Cyclo-oxygenase is involved in the production of prostaglandins, which cause pain, swelling and inflamation.

ACECLOFENAC



Aceclofenac

Aceclofenac is an NSAID known to exhibit multifactor mechanism of action. Aceclofenac was developed in order to provide a highly effective pain relieving therapy with a reduced side effect profile.

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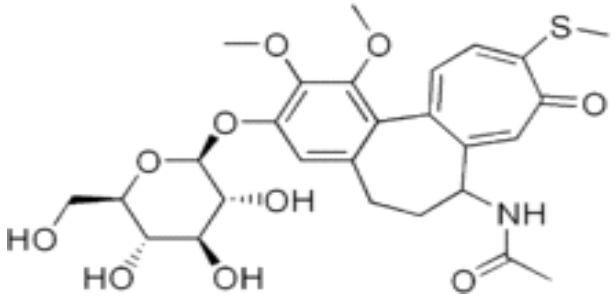
Aceclofenac directly blocks PGE 2 secretion at the site of inflammation by inhibiting IL-Beta & TNF in the inflammatory cells (Intracellular Action). Aceclofenac has been demonstrated to inhibit cyclooxygenase (COX) activity and to suppress the PGE 2 production by inflammatory cells, which are likely to be a primary source of PGE 2. Inflammatory cells release IL-1 and TNF, which produce PGE 2 by induction of COX-2. Aceclofenac and 4`-hydroxyaceclofenac penetrate the inflammatory cells like polymorphonuclears, monocytes and rheumatoid synovial cells and get hydrolyzed to the active metabolites diclofenac and 4`-hydroxydiclofenac which inhibit IL-1 and TNF released by the inflammatory cells and therefore suppress production of PGE 2 at the site of inflammation.

2. Aceclofenac stimulates the synthesis of the extracellular matrix of the Human Articular Cartilages. Aceclofenac blocks degeneration and stimulates synthesis of extracellular matrix of cartilages by inhibiting the action of different cytokines. Aceclofenac and the metabolites inhibit IL-6 production by human chondrocytes. This leads to inhibition of increase of inflammatory cells in synovial tissue, inhibition of IL-1 amplification, inhibition of increased MMP synthesis and thus ensuring proteoglycan production. Aceclofenac also inhibits IL-1 and TNF production by human chondrocytes, inflammatory cells and synovial cells and therefore blocks suppression of GAG and collagen synthesis and stimulates growth factor mediated synthesis of GAG and collagen. 4`-hydroxyaceclofenac, a metabolite of aceclofenac inhibits pro MMP1 and pro MMP3 produced by synovial cells (Rheumatoid Synovial Cells) in serum and in synovial fluid and thus inhibits progressive joint destruction by MM

THIOCOLCHICOSIDE[1,6]:

Thiocolchicoside is a muscle relavant and analgesic effects. It acts as a competitive GABA receptor antagonist and also glycine antagonist with similar potency and nicotinic acetylcholine receptors to a much lesser extent. It has powerful convulsion activity and should not be used in seizure-prone individuals.

THIOCOLCHICOSIDE[5]



$C_{27}H_{33}NO_{10}S$.

Mechanism of action[1,6]

Thiocolchicoside is a muscle relaxant which has been claimed to possess GABA mimetic and glycernic action

MATERIALS AND METHODS

Chemicals

Aceclofenac and Thiocolchicoside obtained from Bio Leo.lab.Pvt.Ltd, Hyderabad, as a gift samples. Disodium hydrogen orthophosphate (AR Grade), ortho-phosphoric acid (AR Grade), Acetonitrile (HPLC Grade), were purchased from Merck (India) Ltd., Worli, Mumbai, India. Tablet formulation (Equiflex) was purchased from local market, containing Aceclofenac (100mg), Thiocolchicoside (8mg). Double distilled water was used throughout the experiment.

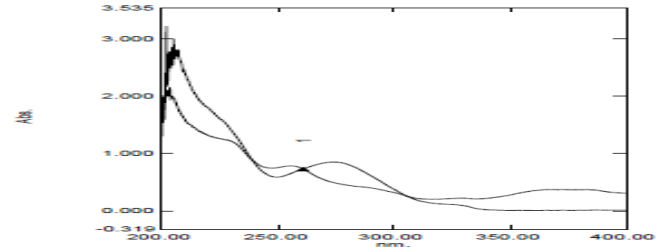
Instruments

Waters HPLC 2 2695 series consisting 4 pump. Auto sampler with 5 racks, each rack has 24 vials holding capacity with temperature control. Auto injector has capacity to inject 5µL to 500µL. UV-Vis Detector with PDA. Thermostat column compartment connected it has a capacity to maintain 5°C to 60°C column temperature. Waters (alliance) HPLC System is equipped with Empower-2 software.

ANALYTICAL METHOD DEVELOPMENT

Optimization of UV conditions

Figure-3 Isobestic point of Aceclofenac and Thiocolchoside

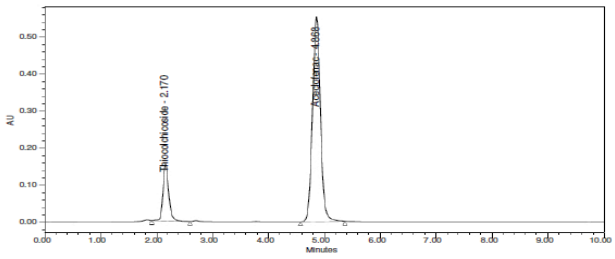


No.	Wavelength	Absorbance	Description
1	261.40	0.726	Aceclo&Thio
2			

Chromatographic Conditions

A waters symmetry C-18 column (150 mm x 4.6 mm i.d., 5-µm) was used for chromatographic separation. The mobile phase composed of Acetonitrile and mixed phosphate buffer (60:40 *v/v*);at a flow rate of 0.8 mL min-1 with run time of 8min. Mobile phase and sample solutions were filtered through a 0.45 µm membrane filter and degassed. The detection of both drugs was carried out at 261nm.

Figure-4 Optimized Chromatogram



	Peak Name	RT	Area	Height	% Area	USP Plate Count	USP Resolution	USP Tailing
1	Thiocolchicoside	2.170	989642	153221	16.54	3520		1.12
2	Aceclofenac	4.868	5462465	552361	83.46	5812	12.19	1.05

METHODOLOGY

Mobile phase preparation

Buffer preparation: Prepare 0.01 M Disodium hydrogen orthophosphate.

Mix buffer and Acetonitrile at 40:60 ratios sonicate the resulting solution and degas it using vacuum filtration through 0.4 micron membrane filter.

Standard stock solution preparation

Weigh and transfer 8 mg of Thiocolchicoside working standard and 100 mg of Aceclofenac working standard into 100 mL volumetric flask, add 50 mL of diluent and sonicate to dissolve and dilute to volume with diluent.

Standard preparation

Transfer 10 mL of standard stock solution into 100 mL volumetric flask and dilute to volume with diluent.

Sample Preparation

Finely grind pre weighed 20 tablets. Transfer grinded sample quantitatively equivalent to 8 mg of Thiocolchicoside and 100 mg of Aceclofenac in to 100 mL volumetric flask add 50 mL of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filter the solution through filter paper. Dilute 10 ml of filtrate to 100 ml with mobile phase.

Procedure

Inject 20 µL of blank solution, placebo solution, Standard solution, Disregard peaks due to blank and placebo if any.

VALIDATION OF METHOD

The HPLC method was validated in accordance with ICH guidelines.

Precision

The system precision of the method was verified by six replicate injections of standard solution containing Aceclofenac and Thiocolchicoside. The method precision ws carried out the analyte six times using the proposed method. Repeatability was measured by multiple injections of a

homogenous sample of Aceclofenac and Thiocolchicosie.

Accuracy

Accuracy was carried out by % recovery studies at three different concentration levels. To the pre-analyzed sample solution of Aceclofenac and Thiocolchicoside; a known amount of standard drug powder of Aceclofenac and Thiocolchicoside were added at 80, 100 and 120 % level.

Specificity and Selectivity

Specificity of the method was determined through study of resolution factor of drug peak from the nearest resolving peak. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix.

Limit of detection and Limit of quantitation

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD = 3.3 x ASD/S and LOQ = 10 x ASD/S, Where, 'ASD' is the average standard deviation and 'S' is the slope of the line.

Robustness

Robustness was evaluated by making deliberate variations in few method parameters such as variation of wave length; flow rate and change in mobile phase composition. The robustness of the method was studied for Aceclofenac and Thiocolchicoside

RESULTS AND DISCUSSION

LINEARITY DATA

The Linear detector response for Aceclofenac and Thiocolchicoside is demonstrated by concentration versus Area. Over the range of 25 to 150% with respect to the target concentration (Dosage).

Table-1 For Peak Area of Thiocolchicoside

	Thiocolchicoside	
%	**Conc(mcg)**	**Area**
25	2.000	229393
50	4.000	469953
75	6.000	733223
100	8.000	995643
125	10.000	1265073
150	12.000	1545641

Figure- 5 Calibration curve for Thiocolchicoside

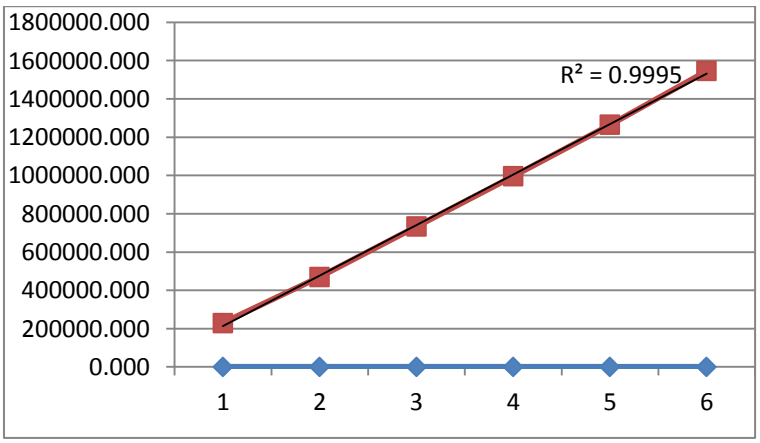


Table-2 For Peak Area of Aceclofenac

%	Conc(mcg)	Area
25	25	1359281
50	50	2700678
75	75	4039435
100	100	5371488
125	125	6700613
150	150	8049989

Figure-6 CalibrationcurveforAceclofenac

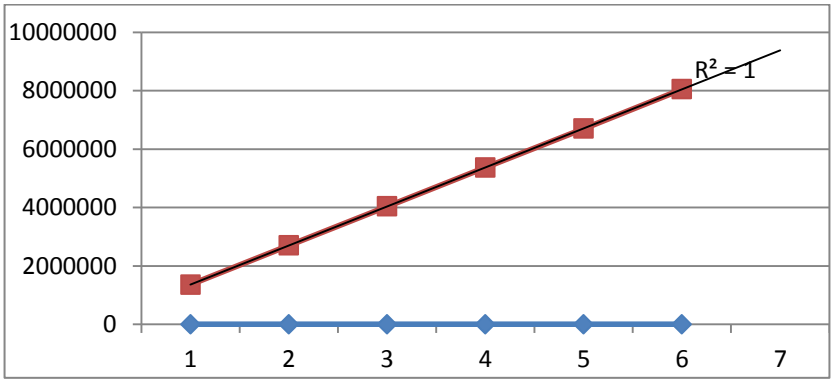


Table-3 PRECISION

	Thio & Para System Precision				
S No	**Name**	**Thio**		**Aceclo**	
		RT	Area	RT	Area
1	S-Precision-1	2.170	1070911	4.868	5387326
2	S-Precision-2	2.171	1074413	4.870	5422851
3	S-Precision-3	2.170	1079399	4.868	5434761
4	S-Precision-4	2.169	1087466	4.868	5450533
5	S-Precision-5	2.170	1075912	4.868	5429502
6	S-Precision-6	2.172	1075825	4.865	5422462
	Average	2.170	1077321	4.868	5424573
	Standard Deviation	0.0010	5674.63	0.002	20960.8
	RSD	0.048	0.527	0.033	0.386

Table-4 Method Precision

Method Precision					
S No	**Name**	**Thio**		**Aceclo**	
		RT	**Area**	**RT**	**Area**
1	M-Precision-1	2.169	1080521	4.870	5385345
2	M-Precision-2	2.170	1081413	4.868	5442833
3	M-Precision-3	2.171	1080399	4.869	5435462
4	M-Precision-4	2.170	1078545	4.870	5452425
5	M-Precision-5	2.172	1085844	4.871	5434531
6	M-Precision-6	2.168	1069845	4.868	5442356
	Average	2.170	1079428	4.869	5432159
	Standard Deviation	0.0014	5288.8	0.001	23818.94
	RSD	0.0652	0.490	0.025	0.438

Table-4 System Precision & Method Precision

System precision & Method Precision					
S No	**Name**	**Thio**		**Aceclo**	
		RT	**Area**	**RT**	**Area**
1	S-Precision-1	2.170	1070911	4.868	5387326
2	S-Precision-2	2.171	1074413	4.870	5422851
3	S-Precision-3	2.170	1079399	4.868	5434761
4	S-Precision-4	2.169	1087466	4.868	5450533
5	S-Precision-5	2.170	1080911	4.868	5429502
6	S-Precision-6	2.172	1075825	4.865	5422462
7	M-Precision-1	2.169	1080521	4.870	5385345
8	M-Precision-2	2.170	1081413	4.868	5442833
9	M-Precision-3	2.171	1080399	4.869	5435462
10	M-Precision-4	2.170	1078545	4.870	5452425
11	M-Precision-5	2.172	1085844	4.871	5434531
12	M-Precision-6	2.168	1069845	4.868	5442356
	Average	2.170	1078791	4.869	5428366
	Standard Deviation	0.001	5329.743	0.002	21755.110
	% RSD	0.055	0.494	0.032	0.401

RESULT

System and Method precision**:**

THIOCOLCHICOSIDE

% of RSD for RT = 0.055%

Area = 0.494%

ACECLOFENAC

% of RSD for RT = 0.032%

Area = 0.401%

Acceptance criteria

The % of RSD for Area and RT from Repeated injections should not be more than 2.0**%.**

ACCURACY

The accuracy of the test method is demonstrated by % of recovery. The sample preparations are spiked with known amount of standard at three concentration levels and injected three times (Like 80% 100% and 120%).

Accuracy data.

Table-5 Standard area

Standard Area		
S No	**Thio**	**Aceclo**
	Area	Area
1	994825	5365422
2	993982	5385251
Avg	994404	5375337

Table-6 Placebo

Placebo		
S No	**Thio Area**	**Aceclo Area**
1	0	0
2	0	0
Avg	0	0

Table -7 Accuracy for Paracetamol and Tramadol hydrochloride

	Accuracy-- 80%		**Accuracy-- 100%**		**Accuracy-- 120%**	
S No	**Thio Area**	**Aceclo Area**	**Thio Area**	**Aceclo Area**	**Thio Area**	**Aceclo Area**
Injection-1	787245	4239345	989642	5362465	1201152	6421625
Injection-2	788832	4336125	991625	5381152	1155053	6390515
Injection-3	789545	4237245	992525	5372214	1212142	6428642
Avg	**788541**	**4270905**	**991264**	**5371944**	**1189449**	**6413594**
Amt added(mg)	**6.40**	**80.00**	**8.00**	**100.00**	**9.60**	**120.00**
Amt Recoverd(mg)	**6.34**	**79.45**	**7.98**	**99.94**	**9.57**	**119.32**
%Recovery	**99.12**	**99.32**	**99.81**	**99.94**	**99.68**	**99.43**

Results : (% Of Recovery)

THIOCOLCHICOSIDE

At 80% = 99.12%
At 100% = 99.81 %
At 120% = 99.68 %

ACECLOFENAC :

At 80% = 99.32 %
At 100% = 99.94%
At 120% = 99.43 %

Acceptance criteria

The % of recovery should be between 98 to 102%.

LIMIT OF DETECTION (LOD)

Table-8 Limit Of detection results.

S.NO	Name	LOD Value (µg/ml)
1.	Aceclofenac	0.33
2.	Thiocolchicoside	3.9

Table-9 Limit of Quantitation (LOQ) results.

S.NO	Name	LOQ Value(µg/ml)
1.	Aceclofenac	1.002
2.	Thiocolchicoside	11.9

RUGUDNESS

Ruggedness Day-1

S No	Name	Thio		Aceclo	
		RT	Area	RT	Area
1	Ruggedness-(Day-1)-1	2.170	1070911	4.868	5387326
2	Ruggedness-(Day-1)-2	2.171	1074413	4.870	5422851
3	Ruggedness-(Day-1)-3	2.170	1079399	4.868	5434761
4	Ruggedness-(Day-1)-4	2.169	1087466	4.868	5450533
5	Ruggedness-(Day-1)-5	2.170	1075912	4.868	5429502
6	Ruggedness-(Day-1)-6	2.172	1075825	4.865	5422462
	Average	2.170	1077321	4.868	5424573
	Standard Deviation	0.0010	5674.6	0.002	20960.79
	RSD	0.0476	0.527	0.03	0.39

Ruggedness Day-2

S No	Name	Thio		Aceclo	
		RT	Area	RT	Area
1	Ruggedness-(Day-2)-1	2.171	1080521	4.870	5485315
2	Ruggedness-(Day-2)-2	2.172	1081121	4.872	5402925
3	Ruggedness-(Day-2)-3	2.172	1082021	4.869	5415662
4	Ruggedness-(Day-2)-4	2.170	1081141	4.870	5421545
5	Ruggedness-(Day-2)-5	2.171	1082832	4.865	5428512
6	Ruggedness-(Day-2)-6	2.170	1084105	4.867	5425435
	Average	2.171	1081957	4.869	5429899
	Standard Deviation	0.0009	1327.4	0.002	28610.71
	RSD	0.0412	0.123	0.05	0.53

Ruggedness Day-1 & Day-2

S No	Name	Thio		Aceclo	
		RT	Area	RT	Area
1	Ruggedness-(Day-1)-1	2.170	1070911	4.868	5387326
2	Ruggedness-(Day-1)-2	2.171	1074413	4.870	5422851
3	Ruggedness-(Day-1)-3	2.170	1079399	4.868	5434761
4	Ruggedness-(Day-1)-4	2.169	1087466	4.868	5450533
5	Ruggedness-(Day-1)-5	2.170	1075912	4.868	5429502
6	Ruggedness-(Day-1)-6	2.172	1075825	4.865	5422462
7	Ruggedness-(Day-2)-1	2.171	1080521	4.870	5485315
8	Ruggedness-(Day-2)-2	2.172	1081121	4.872	5402925
9	Ruggedness-(Day-2)-3	2.172	1082021	4.869	5415662
10	Ruggedness-(Day-2)-4	2.170	1081141	4.870	5421545
11	Ruggedness-(Day-2)-5	2.171	1082832	4.865	5428512
12	Ruggedness-(Day-2)-6	2.170	1084105	4.867	5425435
	Average	2.171	1079639	4.868	5427236
	Standard Deviation	0.0010	4615.10	0.0021	24073.28
	% RSD	0.045	0.427	0.042	0.444

RUGGEDNESS

The ruggedness of test method is demonstrated by carrying out precision studies with different analysts and on different days.

Results

Thiocolchicoside

% of RSD on Day-1& Day-2

RT= 0.045%

Area= 0.427%

Aceclofenac

% of RSD on Day-1& Day-2

RT= 0.042%

Area= 0.444%

Acceptance criteria

The % of RSD of areas from six injections should not be more than 2.0**%**

ASSAY

Assay for Aceclofenac and Thiocolchicoside

Standard preparation

Transfer 10 ml of standard stock solution in to 100 mL volumetric flask and make up to volume with diluent.

Sample Preparation

Transfer sample quantitatively equivalent to 100 mg of Aceclofenac and 8 mg of Thiocolchicoside in to 200 mL volumetric flask add 50 mL of diluent, sonicate to dissolve for 10 minutes and dilute to volume with diluent. Further filter the solution through filter paper. Dilute 10 ml of filtrate to 50 ml with mobile phase.

Procedure

Inject 20 µL of blank solution, standard solution, and sample solution record the chromatogram. And calculate percentage of assay.

Table-11

Thio	8-mg
Aceclofenac	100-mg
Avg wt	154-mg

Table-12

S No	Name	Thio RT	Thio Area	Aceclo RT	Aceclo Area
1	Standard-1	2.171	1084412	4.871	5421852
2	Standard-2	2.172	1080399	4.868	5434765
Avg		2.172	1082406	4.870	5428309
3	Sample-1	2.170	1079825	4.870	5430812
4	Sample-2	2.171	1083465	4.871	5436825
Avg		2.171	1081645	4.871	5433819

Table-13 Results for Aceclofenac

Aceclofenac								
5433819	100	10	100	100	99.91	154	**mg/tab**	**% Assay**
5428309	100	100	154	10	100		**100.10**	**100.10**

Table- 14 Results for Thiocolchicoside

Thiocolchicoside								
1081645	8	10	100	100	99.82	154	**mg/Tab**	**%Assay**
1082406	100	100	154	10	100		**7.99**	**99.93**

Assays result

Aceclofenac = 100.00 %

Thiocolchicoside = 99.93 %

SYSTEM SUITABILITY PARAMETERS

Table-15 System suitability parameters results for Aceclofenac and Thiocolchicoside

Parameters	Results	
	Aceclofenac	**Thiocolchicoside**
Tailing factor	1.05	1.12
Theoretical plates per column	5812	3520
Resolution	12.19	

CONCLUSION

The developed RP-HPLC method is simple, precise, accurate, selective and reproducible. The method has been found to be adequately rugged and robust and can be used for simultaneous determination of Aceclofenac and Thiocolchicoside in tablet formulation. The method was validated as per ICH guidelines.

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