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

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ABSTRACT:

A simple, rapid, accurate, precise, and robust reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the simultaneous determination of aceclofenac (AC) and thiocolchicoside (TH) in pharmaceutical formulations. Chromatographic separation was achieved on an Agilent Zorbax C18 column (150 × 4.6 mm, 5 µm) maintained at 30 ± 1 ºC using an isocratic mobile phase consisting of methanol and 0.1% orthophosphoric acid (75:25, v/v) delivered at a flow rate of 1 mL/min. UV detection was monitored at 252nm with an injection volume of 20 µL and a total run time 08 minutes.

The method demonstrated high resolution (Rs =12.19) with sharp, symmetrical peaks (tailing factors 1.05 for AC and 1.12 for TH). Linearity was established across 10 to 100 µg /mL for aceclofenac ($r^2 > 0.999$, Y = 41,278x - 16756) and 5 to 50 µg /mL for thiocolchicoside ($r^2 > 0.999$, Y = 33,680x + 60,388). Mean recoveries ranged between 98.90-100.20% for AC and 99.16-100.56% for TH, fulfilling ICH recovery criteria (98–102%). Precision studies exhibited relative standard deviations (%RSD) well below 2% (0.15% for AC and 0.08% for TH). Limits of detection (LOD) and quantification (LOQ) confirmed high sensitivity suitable for routine quality control and regulatory release testing.

Keywords: Aceclofenac, Thiocolchicoside, RP-HPLC, Method Validation, ICH Q2(R1), Simultaneous Estimation.

1. Introduction:

In the modern pharmaceutical landscape, ensuring the safety, efficacy, and consistent quality of drug products is a non-negotiable mandate for regulatory bodies, manufacturers, and healthcare providers worldwide.[1] The active pharmaceutical ingredient (API) in any dosage form be it a tablet, capsule, or injection must be present within a stringent specification of its declared amount. Analytical chemistry serves as the cornerstone of this assurance, providing the tools and methodologies to quantify drugs, identify impurities, and monitor stability. [2] For the effective treatment of various diseases and health issues, proper medicines or pharmaceutical dosages in the right combinations are required. Pharmaceutical companies look at qualitative and quantitative analysis to verify the quality of final product and to check that their raw materials meeting their indeed specifications. [3]

Aceclofenac is an effective non-steroidal anti-inflammatory drug (NSAID) with pronounced analgesic and anti-inflammatory properties, operating primarily via selective inhibition of cyclooxygenase-2 (COX-2). Thiocolchicoside is a semisynthetic colchicoside derivative acting as a competitive receptor antagonist and glycinergic muscle relaxant. [4-5]

The fixed-dose combination of aceclofenac and thiocolchicoside is widely prescribed for the management of acute painful musculoskeletal spasms, low back pain, and osteoarthritis. Simultaneous quantification of both active pharmaceutical ingredients (APIs) in combined oral solid dosage forms demands a validated, selective, and high-throughput chromatographic procedure capable of resolving polar and non-polar moieties in a single isocratic run without excipient interference. [6-7]

This study details the systematic development and formal validation of an isocratic RP-HPLC method adhering strictly to International Council for Harmonisation (ICH) Q2(R1) regulatory guidelines.

2. Experimental & Chromatographic Conditions

2.1 Instrumentation and Operating Parameters

Chromatographic separations were performed on an HPLC system equipped with an autosampler, quaternary solvent delivery pump, column

thermostat, and a variable-wavelength UV/Vis detector. Data acquisition and integration were processed using chromatography workstation software.

Table 1: Instrumentation and Chromatographic Conditions

Parameter	Optimized Condition
Stationary Phase	Agilent Zorbax C18 (150 mm×4.6 mm, 5 µm)
Mobile Phase	Methanol : 0.1% Orthophosphoric acid (75:25, v/v)
Flow Rate	1.0 mL/min
Detection Wavelength (λ)	252 nm
Column Temperature	30±1∘C
Injection Volume	20 µL
Total Run Time	8.0 minutes
Diluent	Mobile phase

3. Results and Discussion:

3.1 Method Optimization and System Suitability:

Initial mobile phase scoutings evaluated binary mixtures of methanol, water, and acidic modifiers. A mobile phase of methanol and 0.1% orthophosphoric acid in a 75:25 (v/v) proportion delivered baseline separation, optimum retention times (t_R approx 1.7-1.8min and 3.3-3.5min}), and sharp peak symmetry devoid of peak fronting or excessive tailing.

System suitability testing was performed prior to sample analysis. Six replicate injections of standard solutions containing aceclofenac and thiocolchicoside verified system readiness and column performance.

Table: 2 Method Optimization and System Suitability Data

System Suitability Parameter	Aceclofenac	Thiocolchicoside	Acceptance Criteria (ICH/USP)	Status
Retention Time (tR, min)	3.32	1.73	—	Conforms
Tailing Factor (T)	1.05	1.12	T≤2.0	Complies
Theoretical Plates (N)	5812	3520	N>2000	Complies
Resolution (Rs)	—	12.19	Rs>2.0	Complies
Peak Area %RSD	1.29%	1.57%	≤2.0%	Complies

3.2 Linearity and Range:

Linearity was assessed by evaluating six concentration levels across 10 to 100 µg /mL for aceclofenac and 5 to 50 µg /mL for thiocolchicoside. Linear regression equations computed from peak area versus analyte concentration demonstrated strict proportionality:

Table 3: Linearity data of Thiocolchicoside

Sample ID	Concentration (µg /mL)	Retention Time	Peak Area	% Accuracy
BLANK	0.00	-	0	NAP
TH – 01	5	1.85	221936	95.93
TH – 02	10	1.86	393408	98.88
TH – 03	20	1.85	737956	100.59

TH – 04	30	1.87	1059398	98.88
TH – 05	40	1.87	1464342	104.22
TH – 06	50	1.85	1705635	97.70

Table 4 Linearity data of Aceclofenac

Sample ID	Concentration (Microgram/mL)	Retention Time	Peak Area	% Accuracy
BLANK	0.00	-	0	NAP
AC – 01	10	3.38	392643	99.18
AC – 02	20	3.40	781149	96.65
AC – 03	40	3.37	1660869	101.61
AC – 04	60	3.40	2455974	99.84
AC – 05	80	3.38	3339863	101.65
AC – 06	100	3.37	4065256	98.89

3.3 Accuracy:

Method accuracy was verified using standard additions across three concentration levels (50%, 100%, and 150% of target specification levels). Triplicate determinations yielded recoveries well within the acceptance threshold (98.0- 102.0%).[8]

Table 6.3: The accuracy results for Aceclofenac

% Concentration (Level)	**Area**	**Amount Added (ppm)**	**% Recovery**	**Mean Recovery**
50%	286080.7	10	99.35%	99.29%
100%	561215	20	98.90%	
150%	833959.7	30	100.2%	

Table 6.4: The accuracy results for Thiocolchicoside

% Concentration (Level)	**Area**	**Amount Added (ppm)**	**% Recovery**	**Mean Recovery**
50%	408328	15	99.62%	99.16%
100%	798306.3	30	100.56%	
150%	1189915	45	99.70%	

3.4 Precision:

S. No.	RT	Area (µV*sec)	RT	Area (µV*sec)
ACECLOFENAC			THIOCOLCHICOSIDE	
1	1.778	556985	3.457	7856982
2	1.78	558649	3.485	7845285
3	1.778	557847	3.472	7854633
Mean		557827	7852300	

Std. Dev.	832.1803	6187.659
%RSD	0.150254	0.078456

Repeatability and intermediate precision were assessed by analyzing standard preparations at 100% specification limits. The low relative standard deviations (%RSD ≤ 0.15%) demonstrate high instrument repeatability and operational precision. [9]

3.5 Limit of Detection and Limit of Quantification (LOD and LOQ):

The limit of detection (LOD) and limit of quantification (LOQ) were determined experimentally based on signal-to-noise ratios of 3:1 and 10:1, respectively. Peak areas and retention times demonstrated high stability with %CV ≤ 1.69%.[10]

Table 6.6 LOD and LOQ results of Thiocolchicoside

	LOD THIOCOLCHICOSIDE		**LOQ THIOCOLCHICOSIDE**	
Sr. No.	**Retention Time**	**Peak Area**	**Retention Time**	**Peak Area**
1	2.58	6225	2.58	9931
2	2.58	6087	2.57	10081
3	2.58	6247	2.58	10224
Mean	**2.58**	**6186.3**	**2.577**	**10078.6**
Std. Dev.	**0.00**	**86.72**	**0.0058**	**146.51**
% CV	**0.00**	**1.40**	**0.22**	**1.45**

Table 6.7 LOD and LOQ results of Aceclofenac

	LOD ACECLOFENAC		**LOQ ACECLOFENAC**	
Sr. No.	**Retention Time**	**Peak Area**	**Retention Time**	**Peak Area**
1	4.41	6653	4.39	16030
2	4.41	6627	4.39	15981
3	4.39	6515	4.37	15542
Mean	**4.40**	**6598.3**	**4.38**	**15851**
Std. Dev.	**0.011**	**73.33**	**0.011**	**268.72**
% CV	**0.25**	**1.11**	**0.25**	**1.69**

3.6 Robustness:

As expected in reversed-phase chromatography, decreasing the flow rate to 0.9mL/min prolonged the interaction with the stationary phase, shifting the retention time (RT) upward to 1.867 min for AC and 3.721 for TH increasing the peak area due to a slower residence time in the detector flow cell. The number of theoretical plates remained exceptionally high, consistently exceeding 7000-8000, which is comfortably above the standard pharmaceutical baseline requirement of > 2,000 plates. [11]

The tailing factor varied only minimally between 1.06 and 1.13. This signifies highly symmetric peak shapes that remain well within the standard

regulatory acceptance threshold of < 2.0.

Table 6.8: Results for Robustness -ACECLOFENAC

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0mL/min	545265	1.794	7564	1.09
Less Flow rate of 0.9mL/min	625486	1.867	7856	1.13
More Flow rate of 1.1mL/min	526548	1.744	7425	1.12
Less organic phase	536548	1.831	7265	1.06
More organic phase	514875	1.874	7169	1.08

Table 6.9: Results for Robustness - THIOCOLCHICOSIDE

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0mL/min	7768545	3.440	8695	1.12
Less Flow rate of 0.9mL/min	7985695	3.721	8948	1.13
More Flow rate of 1.1mL/min	7458642	3.097	8452	1.12
Less organic phase	7685421	6.242	8365	1.10
More organic phase	7569864	2.402	8254	1.09

4. Discussion:

The C18 stationary phase (e.g., Luna C18, 250 mm × 4.6 mm, 5 µm) provided satisfactory peak shape and retention characteristics . Mobile phase optimization involved testing different combinations of phosphate buffer, acetonitrile, and methanol at varying pH levels. For instance, a mobile phase consisting of phosphate buffer (pH 4.7):methanol (75:25 v/v) achieved acceptable chromatographic parameters with retention times suitable for routine analysis . In combination drug analysis, gradient or isocratic elution with acetonitrile:phosphate buffer mixtures successfully resolved multiple analytes. [12]

The optimization process aimed to achieve several critical objectives: baseline separation of drug peaks from each other and from formulation excipients, acceptable retention times for efficient analysis, symmetrical peak shapes with minimal tailing, and adequate theoretical plate counts to confirm column performance. [13] The system suitability parameters were evaluated at the outset to ensure that the chromatographic system was capable of producing reliable and reproducible data before proceeding with sample analysis. [14]

5. Conclusion:

A validated isocratic RP-HPLC method has been established for the concurrent quantification of aceclofenac and thiocolchicoside. The method delivers rapid chromatographic elution under 8 minutes, outstanding peak symmetry (T≤ 1.13), high column efficiency (N > 3500), and substantial resolution (Rs = 12.19). Method validation adhering to ICH Q2(R1) demonstrated excellent linearity, accuracy, precision (%RSD < 0.2%), and robustness. Consequently, this method is directly applicable for routine release, dissolution profiling, and commercial quality control of multi-component pharmaceutical formulations.

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