Research Article

Simultaneous Estimation and Quantification of three main ingredients, Aceclofenac, Thiocolchicoside & Paracetamol in Finished Dosage Form by using Reversed Phase High Performance Liquid Chromatography

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ABSTRACT

A simple, accurate, and reproducible high-pressure liquid chromatographic method has been developed for the simultaneous estimation of Paracetamol, Aceclofenac and Thiocolchicoside from pharmaceutical formulation. The method was carried out on a Thermo Hypersil CPS column, 25 cm × 4.6 mm, 5 µm column, with a mobile phase consisting of buffer : methanol (80: 20, v/v) at flow rate of 0.5 ml/min. Detection was carried out at 220 nm. There was no interference observed from blank and placebo at the retention time of active ingredients in method. The method was linear over wide concentration range of 1.32ppm-6.72ppm for Thiocolchicoside 165-840ppm for Paracetamol, 3.3- 16.8ppm for Aceclofenac. The method was validated for precision, robustness and recovery. Statistical analysis showed that the method is repeatable and selective for the estimation of Paracetamol, Aceclofenac and Thiocolchicoside and can be can be employed for assessing the quantitative determination of these drugs as a bulk and also for its dosage form.

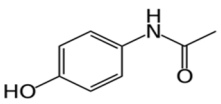
Keywords: Paracetamol, Aceclofenac, Thiocolchicoside, RP-HPLC, ICH, Validation.

INTRODUCTION

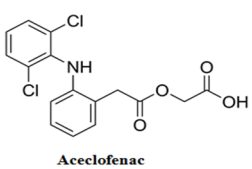
Paracetamol or Acetaminophen is a widely used as an analgesic (pain reliever) and antipyretic (fever reducer).[1,2] It is chemically known as *N*-(4-hydroxyphenyl) acetamide.

Thiocolchicoside is a muscle relaxant with anti-inflammatory and analgesic effects.[3-6] It acts as a competitive $GABA_A$ receptor antagonist and also glycine receptor antagonist with similar potency and nicotinic acetylcholine receptors to a much lesser extent.[7,8] It is chemically known as *N*-[(7*S*)-3-(β-D-Glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide.

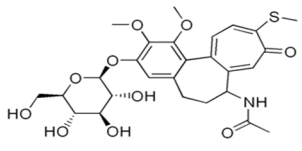
Aceclofenac is a non-steroidal anti-inflammatory drug (NSAID) analog of Diclofenac. It is used for the relief of pain and inflammation in rheumatoid arthritis, osteoarthritis and ankylosing spondylitis.[7] It is chemically known as 2-[2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetyl]oxyacetic acid.



Paracetamol



Aceclofenac



Thiocolchicoside

Literature survey revealed that, no method is available in the official compendia using HPLC for analyzing Paracetamol, Aceclofenac and Thiocolchicoside in tablets. The present proposed method was compared with the reported method in the literature and comparison is shown in Table-1.

MATERIALS AND METHODS

Chemicals and Reagents

Standards for Paracetamol, aceclofenac, thiocolchicoside and excipients were provided by Medley Pharma, Mumbai, India.

HPLC grade, Acetonitrile, potassium dihydrogen phsophate and triethylamie were obtained from Merck chemicals. Distilled water was prepared using a Milli-Q system (Millipore). Nylon syringe filters (0.45 µm) were from Millipore.

Equipment's Used

Chromatographic separation was achieved using HPLC System (Waters Alliance 2695 Separation Module) containing binary solvent manager, a sample manager and UV detector. The output signal was monitored and processed using Empower Software. The analytical balance used was from Sartorius, Model – CPA225D. UV spectrophotometer used was from Shimadzu, UV-1800.

Selection of UV wavelength

For paracetamol, aceclofenac and thiocolchicoside, a solution of 10ppm each was prepared separately in methanol. UV scan of the above solutions were carried out over a wavelength range of 200–400 nm by using the Shimadzu UV spectrophotometer, Model- UV-1800. The

detection wavelength was set at 220 nm to obtain good UV responses of all analytes.

HPLC instruments and analytical conditions

A Thermo Hypersil CPS column (250 mm X 4.6 mm id and 5 µm particle size) was used as the stationary phase. Mobile phase containing, Phosphate Buffer pH 5.5 and methanol in ratio 80:20 v/v with isocratic elution was used. The mobile phase was delivered at a flow rate of 0.5 mL/min. The column temperature was kept at 25°C. The detector was set at the wavelength of 220 nm. Injection volume kept was 20 µL. Sample and standard preparation was done in a mobile phase.

Solutions and sample preparation

Preparation of reference standard solution

Reference standard solutions of different concentrations containing paracetamol (0.50 mg/mL), aceclofenac (0.010 mg/mL), and thiocolchicoside (0.004 mg/mL) were prepared from working standards and diluted with the mobile phase. 20µL of this reference standard solution was injected in HPLC System.

Preparation of Sample solutions

Synthetic mixture containing all these actives at the concentration level available in its available marketed formulation was prepared (1 tablet equivalent to Paracetamol 500 mg, Aceclofenac 100 mg and Thiocolchicoside 4 mg). To these analytes, the basic excipients were added.[11]

Synthetic mixture equivalent to 1 tablet was weighed and transferred to 100 mL volumetric flask. Added 50 mL of diluent to this mixture and sonicated the solution for approximately 10 minutes. Cooled to room temperature and diluted to the mark with diluent. 5 mL aliquot of this sample stock solution was transferred to 50 mL volumetric flask and diluted to the mark with diluent to obtain a test solution of paracetamol (0.50 mg/mL), aceclofenac (0.010 mg/mL), and thiocolchicoside (0.004 mg/mL). The solution was filtered through Nylon 0.45 µm membrane filter. 20 µL of this synthetic mixture solution was injected in HPLC System.

Preparation of Placebo solution

Placebo was prepared with excipients containing 54.0 mg of MCC, 20 mg of HPC, 10 mg of cross Carcarmellose sodium 2.0 mg of Mg stearate 4 mg of colloidal silicon dioxide and 0.4 mg of iron oxide 10.4 mg of instacoat & purified water.

Placebo equivalent to 5 tablets was weighed and transferred to 500 mL volumetric flask. Added 150 mL of diluent to this mixture and sonicated the solution for approximately 10 minutes. Cooled to room temperature and diluted to the mark with diluent. 5 mL aliquot of this sample stock solution was transferred to 50 mL volumetric flask and diluted to the mark with diluent to obtain placebo solution. The solution was filtered through Nylon 0.45 µm membrane filter. 20µL of this placebo solution was injected in HPLC System.

Calculation

All active ingredients were quantified with the following calculation:

$$\%\ Assay = \frac{Sample\ Area \times Standard\ dilution\ factor \times 100}{Standard\ area \times Sample\ dilution\ factor}$$

Method Validation

The developed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guideline, VALIDATION OF ANALYTICAL PROCEDURES: Q2(R1), for the parameters like system suitability, linearity and range, precision (repeatability), intermediate precision (ruggedness), specificity, accuracy and robustness.[12,13]

System suitability

The system suitability test performed according to USP36.[14]

The standard solution was injected six times and results were recorded to find the adequate peak separation (resolution), percentage relative standard deviation for area, and retention time. The results obtained were compiled in Table-1.

Table 1: Comparison of the performance characteristics of the present method with the published methods

S. No.	Method/Column	Mobile phase	Detection Wavelength/flow rate	Column	Reference
1	HPLC	Acetonitrile, Water (30:70)v/v with Isocratic elution	263 nm/1.0 mL/min	HiQ Sil C18 (250 mm × 4.6 mm, 5.0 µ)	[9]
2	HPLC	15mM phosphate buffer pH 3.25 and acetonitrile with gradient elution	230 nm /1.1 mL/min	Kromasil C18 (250 × 4.6 mm, 5 μm)	[10]
3	HPLC	Phosphate Buffer pH 5.5 and methanol in ratio 80:20 v/v	220 nm/0.5 mL/min	Thermo Hypersil CPS (250 mm × 4.6 mm, 5 µm)	Present work

Table 1: System suitability – Percentage relative standard deviation for area and Retention time

	Thiocolchicoside	Paracetamol	Aceclofenac
Reference solution Peak Area for n=6			
%RSD	0.23	0.26	0.21
Acceptance Criteria	Not more than 2.0%		
Reference solution Peak retention time (min), for n=6			
%RSD	0.072	0.052	0.025
Acceptance Criteria	Not more than 1.0%		

Table 2: Precision and Intermediated precision results

	Thiocolchicoside	Paracetamol	Aceclofenac
Intra Day Precision –Assay %			
Average	99.9	99.9	99.6
%RSD	0.24	0.31	0.38
Inter Day Precision – Assay %			
Average	99.7	99.8	99.9
%RSD	0.26	0.28	0.11
Mean differences between day-1 and day-2	0.2	0.1	0.3

Table 3: Summary of Linearity and range results in assay method for simultaneous determination of Paracetamol

Active Ingredient	Concentration Range (mg/mL)	correlation coefficient
Thiocolchicoside	1.32 to 6.72	0.99993
Paracetamol	165 to 840	0.99992
Aceclofenac	3.3 to 16.8	1.00000

Aceclofenac and thiocolchicoid

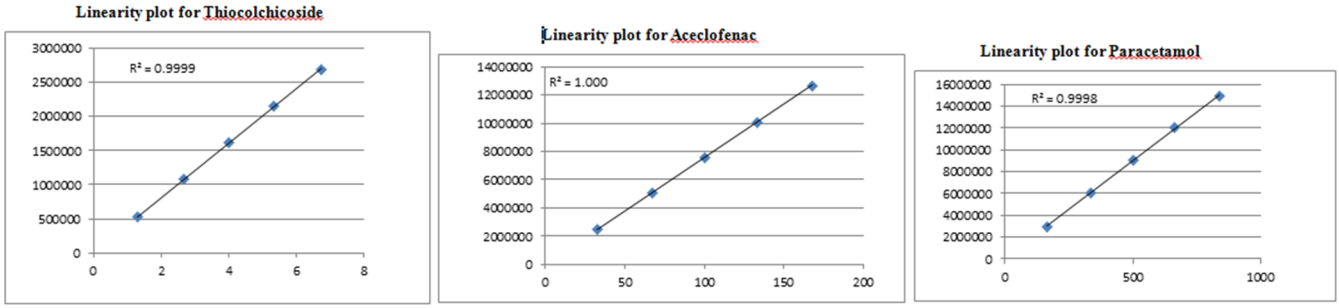


Figure 3: Calibration curves of Paracetamol, Aceclofenac and thiocolchicoid showing linearity

Table 4: Accuracy (Recovery)

Active Ingredient Name	Concentration (%)	Amount Added (mg/mL)	Amount found (mg/mL)*	Mean Recovery (%)**	Average Recovery (%)
Thiocolchicoside	65	2.631	2.696	102.5	101.8
	100	4.050	4.043	99.8	
	130	5.239	5.407	103.2	
Paracetamol	65	330.72	333.50	100.9	100.7
	100	501.20	497.72	99.3	
	130	651.56	663.95	101.9	
Aceclofenac	65	6.504	6.672	102.6	101.4
	100	10.001	9.961	99.6	
	130	13.008	13.259	101.9	
Acceptance criteria	**The mean and individual recoveries should be within 95.0 – 105.0%**				

* mean of 3 readings for individual level; ** Average recovery for all levels

Table 5: Robustness results (Resolution, symmetry factor and Theoretical plates)

Summary of system suitability Parameters												
Sr. No.	Change in Temp. (°C)	Change in Flow rate mL/min	Resolution			Symmetry Factor			Theoretical plates			
			Thiocolchicoside	Paracetamol	Aceclofenac	Thiocolchicoside	Paracetamol	Aceclofenac	Thiocolchicoside	Paracetamol	Aceclofenac	
Normal	25°C	0.5	-	2.58	12.72	1.09	1.10	1.02	2745	3702	4432	
A1	20°C	-	-	2.70	12.88	1.04	1.09	1.03	2933	3770	4529	
A2	30°C	-	-	2.36	11.57	1.07	1.12	1.01	2847	3808	4204	
B1	-	0.6	-	2.70	12.88	1.12	1.03	1.06	3006	4038	4379	
B2	-	0.4	-	2.47	12.93	1.01	1.12	1.01	2524	3509	4614	
Acceptance Criteria			**Not less than 2.0**			**Not less than 2.0**			**Not less than 2000**			

Table 6: Solution Stability results

Time (Hrs.)	Area of Thiochlolchicoside	% Assay	% Change w.r.t. Initial	Area of Paracetamol	% Assay	% Change w.r.t. Initial	Area of Aceclofenac	% Assay	% Change w.r.t. Initial
Initial	1614173	99.6	0.00	9022400	99.5	0.00	7556112	99.5	0.00
12hrs	1623164	100.2	0.20	9062600	99.9	0.2	7521116	99.1	0.20
24hrs	1620180	100.0	0.40	9060397	99.9	0.1	7575020	99.8	0.10
Acceptance Criteria :			% Change of the active ingredient ≤ 1.0% w.r.t. initial						

Specificity

Specificity was performed to detect the presence of interfering peak (blank and placebo peaks) at the retention time of the analyte peak. The specificity of the method was checked by comparison of chromatograms obtained from synthetic mixture and the corresponding placebo. The interference of excipients was detected by preparing placebo solution equivalent to about the weight in proportion of synthetic mixture preparation as per the test method and was injected into the HPLC system. The interference of blank was detected by injecting diluent as per the test method. The representative chromatogram obtained for standard solution is shown in Figure 1.

Precision and Ruggedness (Intermediate precision)

Method precision was evaluated by injecting six different sample preparation of synthetic mixture. Different analyst from the same laboratory evaluated the intermediate precision of the method. The assay of these samples was determined. Precision and intermediate precision of the method was evaluated by calculating the %RSD. The values were given in Table-2.

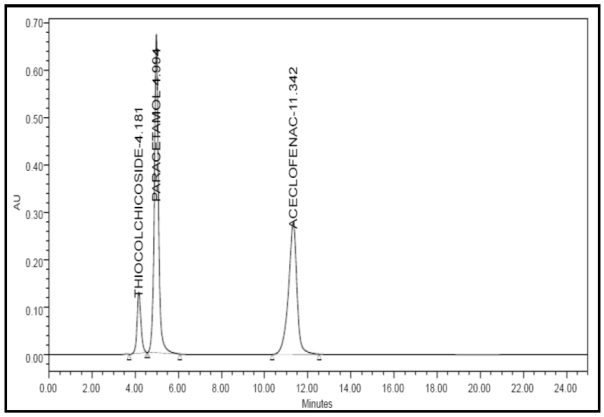


Figure 1: Typical Chromatograms of Combined Standard Solution containing Paracetamol, Aceclofenac and thiocolchicoid

Linearity and Range

The linearity of detector response was determined by preparing a series of solution of the working standards (mixture of all active ingredients) over the range of 65% to 130% of targeted concentration. These solutions were

injected into the chromatographic system and response area was recorded.

Calibration curve was constructed by plotting area against concentration and regression equation was computed. The values were given in Table-3.

Accuracy (Recovery)

To study the accuracy of the method recovery experiments were carried out. The accuracy of the test method was determined by preparing recovery samples (spiking method) at the level of 65%, 100% and 130% of targeted concentration. The recovery samples were prepared in triplicate at each level. The contents were determined from the respective chromatograms. The samples at different levels were chromatographed and the percentage recovery for the amount added was calculated. The values were given in Table-4.

Robustness-Effect of variation in Temperature and variation in flow rate

Small, deliberate changes were made to the chromatographic condition. A study was performed to determine the effect of variation in the temperature and flow rate. Standard solution prepared as per the test method and was injected into the HPLC system at 20°C and 30°C temperature. Flow rate change was done by varying flow rate at from 0.5 mL/min to 0.6 mL/min and 0.4 mL/min. The system suitability parameters were evaluated. The values were given in Table-5.

Solution Stability

To study solution stability, reference standard was stored at ambient condition for 25 °C for 24 hours, and injected in HPLC system at predetermined time interval. The percentage change with respect to initial for test and reference solutions were evaluated. The values were given in Table-6.

RESULTS AND DISCUSION

The RP-HPLC method was developed for the simultaneous estimation of Thiocolchicoside Paracetamol & Aceclofenac in bulk drug and synthetic mixture prepared(as per tablet formulation) and validated as per ICH guidelines for the following parameters: system suitability, linearity and range, precision (repeatability), intermediate precision (ruggedness), specificity, accuracy and robustness. The observations and results obtained for each of the parameters lies well within the acceptance criteria. So the developed method is simple, specific, linear, precise, accurate, robust and rugged and could be extensively used for the simultaneous estimation of Thiocolchicoside Paracetamol &Aceclofenac in bulk drug and solid dosage form.

System suitability parameters proved that the proposed method suits for the simultaneous estimation of Thiocolchicoside Paracetamol & Aceclofenac Chromatogram for Thiocolchicoside Paracetamol &Aceclofenac was found satisfactory on Thermo Hypersil CPS column, 25 cm × 4.6 mm, 5 µ. Drug peak was found to be symmetrical as observed from asymmetry factor. Resolution of the proposed method was found to be satisfactory. Sensitivity of the method was good and also linearity was observed over a wide concentration range of 1.32ppm-6.72ppm for Thiocolchicoside 165-840ppm for Paracetamol, 3.3- 16.8ppm for Aceclofenac, Accuracy of the method was determined by recovery with spiked concentration of pure drug at three levels for Thiocolchicoside Paracetamol & Aceclofenac. Recovery of drug was well within the acceptance limits of 95.0-105.0%. Method was robust as observed from insignificant variation in the results of analysis on changes in flow rate, temperature and analysis being performed by different analysts, in different days using different columns respectively.

The solution was found to be stable up to 24hours, at 25°C (laboratory temperature).

CONCLUSION

The RP-HPLC method developed for quantitative determination of Paracetamol, Aceclofenac and Thiocolchicoside is novel, rapid, precise, accurate and selective and is suitable for its intended purpose. The method was validated as per ICH guidelines, showing satisfactory data for all the method validation parameters tested.

The developed method was found “specific” to the drug and for the dosage form, as the peaks of the excipient did not interfere with the drug peak. Hence, the proposed method can be employed for assessing the quantitative determination of Paracetamol, Aceclofenac and Thiocolchicoside in as a bulk drug and also for its dosage form.

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