CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.8205837

Available online at: http://www.iajps.com *Research Article*

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION THIOCOLCHICOSIDE AND ACECLOFENAC IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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Article Received: May 2023 **Accepted:** June 2023 **Published:** July 2023

Abstract:

The estimation of Thiocolchicoside and Aceclofenac was done by RP-HPLC. The mobile phase was optimized with consists of ACN : Phosphate buffer (pH-4.6) mixed in the ratio of 60:40 % v/ v. A Xterra column C18 (4.6 x 150mm, 5□m) or equivalent chemically bonded to porous silica particles was used as stationary phase. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. The linearity range of Thiocolchicoside and Aceclofenac were found to be from 1-5ppm, 25-125□g/ml respectively. Linear regression coefficient was not more than 0.999, 0.999.

Keywords: *Thiocolchicoside, Aceclofenac, RP HPLC*

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Please cite this article in press Killari Divya Rekha et al, ***Analytical Method Development And Validation For Simultaneous Estimation Thiocolchicoside And Aceclofenac In Pharmaceutical Dosage Form By RP-HPLC.,*** *Indo Am. J. P. Sci, 2023; 10 (07).*

INTRODUCTION:

Analytical chemistry [1]

Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behaviour of matter. The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts. Quality control in manufacturing industries, the monitoring of clinical and environmental samples, the assaying of geological specimens, and the support of fundamental and applied research are the principal applications. Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter.

Qualitative analysis is the identification of elements, species and/or compounds present in sample.

Quantitative analysis is the determination of the absolute or relative amounts of elements, species or compounds present in sample.

Structural analysis is the determination of the spatial arrangement of atoms in an element or molecule or the identification of characteristic groups of atoms (functional groups). An element, species or compound that is the subject of analysis is known as analyte. The remainder of the material or sample of which the analyte(s) form(s) a part is known as the matrix.

The gathering and interpretation of qualitative, quantitative and structural information is essential to many aspects of human endeavour, both terrestrial and extra-terrestrials. The maintenance of an improvement in the quality of life throughout the world and the management of resources heavily on the information provided by chemical analysis. Manufacturing industries use analytical data to monitor the quality of raw materials, intermediates and finished products. Progress and research in many areas is dependent on establishing the chemical composition of man-made or natural materials, and the monitoring of toxic substances in the environment is of ever increasing importance. Studies of biological and other complex systems are supported by the collection of large amounts of analytical data. Analytical data are required in a wide range of disciplines and situations that include not just chemistry and most other sciences, from biology to zoology, butte arts, such as painting and sculpture, and archaeology. Space exploration and clinical diagnosis are two quite desperate areas in which analytical data is vital. Important areas of application include the following.

Quality control (**QC**) in many manufacturing industries, the chemical composition of raw materials, intermediates and finished products needs to be monitored to ensure satisfactory quality and consistency. Virtually all consumer products from automobiles to clothing, pharmaceuticals and foodstuffs, electrical goods, sports equipment and horticultural products rely, in part, on chemical analysis. The food, pharmaceutical and water industries in particular have stringent requirements backed by legislation for major components and permitted levels of impurities or contaminants. The electronic industry needs analyses at ultra-trace levels (parts per billion) in relation to the manufacture of semi-conductor materials. Automated, computer-controlled procedures for process-stream analysis are employed in some industries.

Monitoring and control of pollutants:

The presence of toxic heavy metals (e.g., lead, cadmium and mercury), organic chemicals (e.g., polychlorinated biphenyls and detergents) and vehicle exhaust gases (oxides of carbon, nitrogen and sulphur, and hydrocarbons) in the environment are health hazards that need to be monitored by sensitive and accurate methods of analysis, and remedial action taken. Major sources of pollution are gaseous, solid and liquid wastes that are discharged or dumped from industrial sites, and vehicle exhaust gases.

Clinical and biological studies:

The levels of important nutrients, including trace metals (e.g., sodium, potassium, calcium and zinc), naturally produced chemicals, such as cholesterol, sugars and urea, and administered drugs in the body fluids of patients undergoing hospital treatment require monitoring. Speed of analysis is often a crucial factor and automated procedures have been designed for such analyses.

Geological assays:

The commercial value of ores and minerals are determined by the levels of particular metals, which must be accurately established. Highly accurate and reliable analytical procedures must be used for this purpose, and referee laboratories are sometimes employed where disputes arise.

Fundamental and applied research:

The chemical composition and structure of materials used in or developed during research programs in numerous disciplines can be of significance. Where new drugs or materials with potential commercial value are synthesized, a complete chemical

characterization maybe required involving considerable analytical work. Combinatorial chemistry is an approach used in pharmaceutical research that generates very large numbers of new compounds requiring confirmation of identity and structure.

Analytical techniques:

There are numerous chemical or physico-chemical processes that can be used to provide analytical information. The processes are related to a wide range of atomic and molecular properties and phenomena that enable elements and compounds to be detected and/or quantitatively measured under controlled conditions. The underlying processes define the various *analytical techniques*. The more important of these are listed in Table.No.1 together with their suitability for qualitative, quantitative or structural analysis and the levels of analyte(s) in a sample that can be measured. *Atomic, molecular spectrometry* and *chromatography*, which together comprise the largest and most widely used groups of techniques, can be further subdivided according to their physico-chemical basis. *Spectrometric techniques* may involve either the *emission or absorption* of *electromagnetic radiation* over a very wide range of energies, and can provide qualitative, quantitative and structural information for analytes from major components of a sample down to ultra-trace levels. The most important atomic and molecular spectrometric techniques and their principal applications are listed in Table.No.2.

Chromatographic techniques provide the means of separating the components of mixtures and simultaneous qualitative and quantitative analysis, as required. The linking of chromatographic and spectrometric techniques, called *hyphenation*, provides a powerful means of separating and identifying unknown compounds.

Electrophoresis's another separation technique with similarities to chromatography that is particularly useful for this parathion of charged species. The principal separation techniques and their applications are listed in Table.No.3.

Analytical methods:

An analytical method consists of a detailed, stepwise list of instructions to be followed in the qualitative, quantitative or structural analysis of a sample for one or more analytes and using a specified technique. It will include a summary and lists of chemicals and reagents to be used, laboratory apparatus and glassware, and appropriate instrumentation. The quality and sources of chemicals, including solvents, and the required performance characteristics of instruments will also be specified as will the procedure for obtaining a representative sample of the material to be analyzed. This is of crucial importance in obtaining meaningful results. The preparation or pre-treatment of the sample will be followed by any necessary standardization of reagents and/or calibration of instruments under specified conditions. Qualitative tests for the analyte(s) or quantitative measurements under the same conditions as those used for standards complete the practical part of the method. The remaining steps will be concerned with data processing, computational methods for quantitative analysis and the formatting of the analytical report. The statistical assessment of quantitative data is vital in establishing the reliability and value of the data, and the use of various statistical parameters and tests is widespread. Many *standard analytical methods* have been published as papers in analytical journals and other scientific literature, and in textbook form. Collections by trades associations representing, for example, the cosmetics, food, iron and steel, pharmaceutical, polymer plastics and paint, and water industries are available standards organizations and statutory authorities, instrument manufacturer's applications notes, the Royal Society of Chemistry and the US Environmental Protection Agency are also valuable sources of standard methods. Often, laboratories will develop their own *in-house methods* or adapt existing ones for specific purposes.

Method development [16] forms a significant part of the work of most analytical laboratories, and *method validation and* periodic revalidation is a necessity. Selection of the most appropriate analytical method should take into account the following factors:

- The purpose of the analysis, the required time scale and any cost constraints;
- The level of Analyte(s) expected and the detection limit required;
- The nature of the sample, the amount available and the necessary sample preparation procedure;
- The accuracy required for a quantitative analysis;
- The availability of reference materials, standards, chemicals and solvents, instrumentation and any special facilities;
- Possible interference with the detection or quantitative measurement of the analyte(s) and the possible need for sample clean-up to avoid matrix interference;
- The degree of selectivity available − methods may be selective for a small number of analytes or specific for only one.
- Quality control and safety factors.

Table 1: Analytical techniques and principal applications

Technique	Property measured	Principal areas of application
Gravimetry	Weight of pure analyte or compound of known as stoichiometry	Quantitative for major or minor components
Titrimetry	Volume of standard reagent solution reacting with the analyte	Quantitative for major or minor Component
Atomic molecular spectrometry	Wavelength and intensity of electromagnetic radiation emitted/ absorbed by the analyte	Qualitative, quantitative or structural or for major down to trace level components
Mass spectrometry	Mass of analyte or fragments of it	Qualitative or structural for major down to trace level components isotope ratios
Chromatography and electrophoresis	Various physicochemical properties of separated analytes	Qualitative and quantitative separations of mixtures at major to trace levels
Thermal analysis	Chemical/physical changes in the analyte when heated or cooled	Characterization of single or mixed major/minor compounds
Electrochemical analysis	Electrical properties of the analyte in solution	Qualitative and quantitative for major to trace level components
Radiochemical analysis	Characteristic ionizing nuclear radiation emitted by the analyte	Qualitative and quantitative at major to trace levels

Table 2: Spectrometric Techniques and Principal Applications

Technique	Basis	Principal applications
Plasma emission spectrometry	Atomic emission after excitation in high temperature gas plasma	Determination of metals and some non-metals mainly at trace levels
Flame emission spectrometry	Atomic emission after flame excitation	Determination of alkali and alkaline earth metals

MATERIALS AND METHODS:

Thiocolchicoside from Sura labs, Aceclofenac from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck, Phosphate buffer from Finar chemicals

Hplc method development:

Mobile Phase Optimization:

Initially the mobile phase tried was Water: Methanol and ACN: Methanol with varying proportions. Finally, the mobile phase was optimized to phosphate buffer (pH 4.6), Methanol in proportion 60:40 v/v respectively.

Optimization of Column:

The method was performed with various columns like Symmetry C18 column, Zodiac column. Xterra C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions:

Instrument used :Waters HPLC with auto sampler and PDAdetector 996 model.

Temperature : Ambient

Column : X bridge C18 (4.6×150mm) 5 µ

Buffer: Phosphate buffer (pH-4.6)-Dissolve 0.9g of anhydrous di hydrogen phosphate and 1.298 g of Citric acid mono hydrate in sufficient water to produce 1000ml. Adjust the pH4.6 by using ortho phosphoric acid.

pH : 4.6

Mobile phase : Methanol: Phosphate Buffer pH4.6 (60:40v/v)

Flow rate : 1.0 ml per min

Wavelength : 310 nm

Injection volume : 10 µl

Run time : 10 min.

Optimized chromatogram, blank, System suitability parameters are shown in the figure and the results are shown in Table.

Validation:

5.5.1: Preparation of Phosphate buffer (pH-4.6):

Dissolve 0.9g of anhydrous di hydrogen phosphate and 1.298 g of Citric acid mono hydrate in sufficient water to produce 1000mL .Adjust the p H 3 by using ortho phosphoric acid.

5.5.2 Preparation of mobile phase:

Accurately measured 600 ml (60%) of ACN and 400 ml of Phosphate buffer (40%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

5.5.3 Diluent Preparation:

The Mobile phase was used as the diluent

RESULTS AND DISCUSSION:

Optimized Chromatogram (Standard)

Mobile phase : Phosphate buffer (0.05M) pH 4.6: ACN (40:60%v/v)

Column : X Terra C18 (4.6×250mm, 5 µ)

Flow rate : 1 ml/min

Wavelength : 310 nm

Column temp : Ambient

Injection Volume : 10 µl

Run time : 10 min

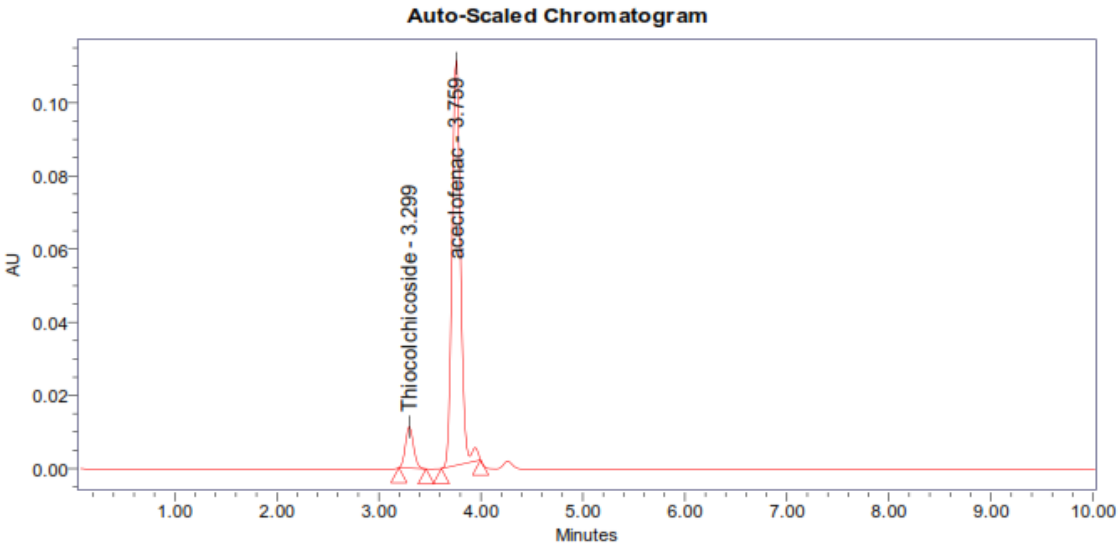

Figure 1: Optimized Chromatogram (Standard)

Table 3: - peak results for trail 5

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Thiocolchicoside	3.299	62468	11323		1.13	7889
2	Aceclofenac	3.759	637769	110584	3.07	1.06	10243

Observation:
From the above chromatogram it was observed that the Thiocolchicoside and Aceclofenac peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.
Retention time of Thiocolchicoside– 3.299min
Retention time of Aceclofenac –3.759min

Table 4: Optimized Chromatogram (Sample)

S.No	Name of compound	Label claim	Amount taken(from combination tablet)	%purity
1	Thiocolchicoside	4mg	3.98	99.5%
2	Aceclofenac	100mg	99.97	99.9%

The retention time of Aceclofenac and Thiocolchicoside was found to be 2.669min and 3.855mins respectively. The % purity of Thiocolchicoside and Aceclofenac in pharmaceutical dosage form was found to be 99.5% and 99.9% respectively

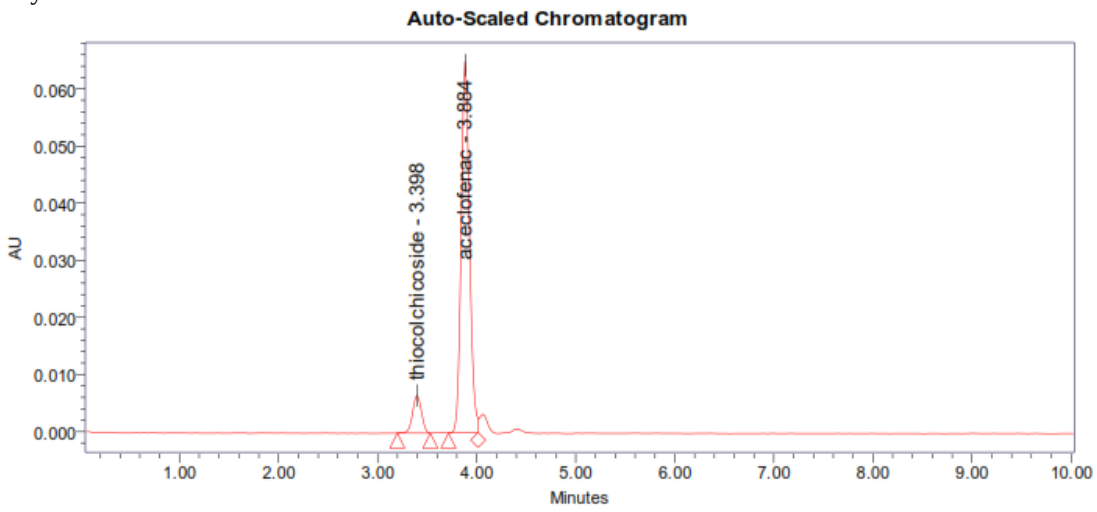

Fig 2: Chromatogram showing assay of sample

Assay (Standard):

Table 5: Results of system suitability parameters for Thiocolchicoside and Aceclofenac

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Thiocolchicoside	3.307	64818	11489		1.09	8134
2	Aceclofenac	3.769	633009	111818	3.04	1.06	10179

Acceptance criteria:

- Resolution between two drugs must be not less than 2
- Theoretical plates must be not less than 2000
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

Linearity:

Linearity Results: (for Thiocolchicoside)

S.No	Linearity Level	Concentration(ppm)	Area
1	I	1	12551
2	II	2	22343
3	III	3	33459
4	IV	4	43657
5	V	5	54491
Correlation Coefficient			0.999

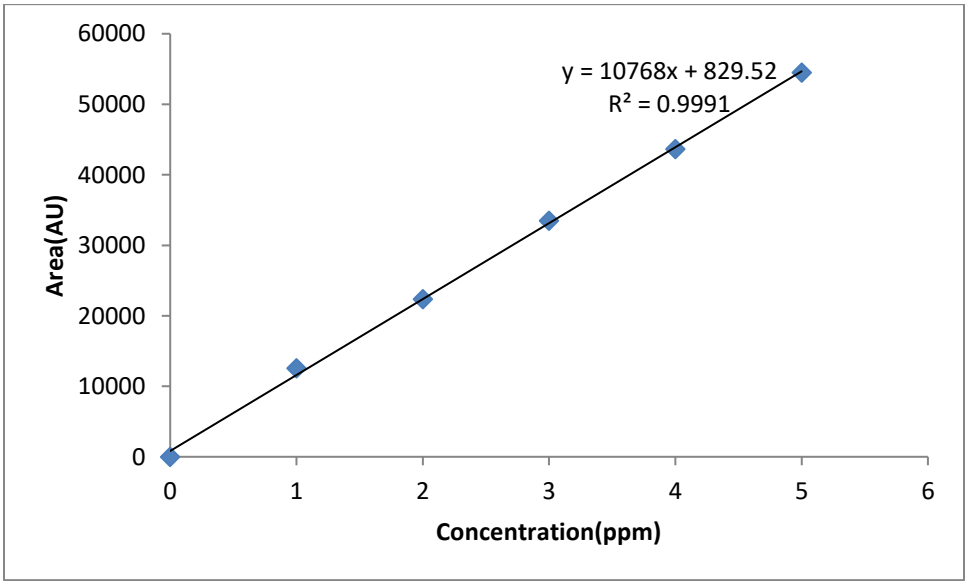

Figure 3: calibration graph for Thiocolchicoside

Acceptance Criteria: Correlation coefficient should be not less than 0.999

Linearity Results: (for Aceclofenac)

S.No	Linearity Level	Concentration(ppm)	Area
1	I	25	109709
2	II	50	206934
3	III	75	304864
4	IV	100	405694
5	V	125	502716
Correlation Coefficient			0.999

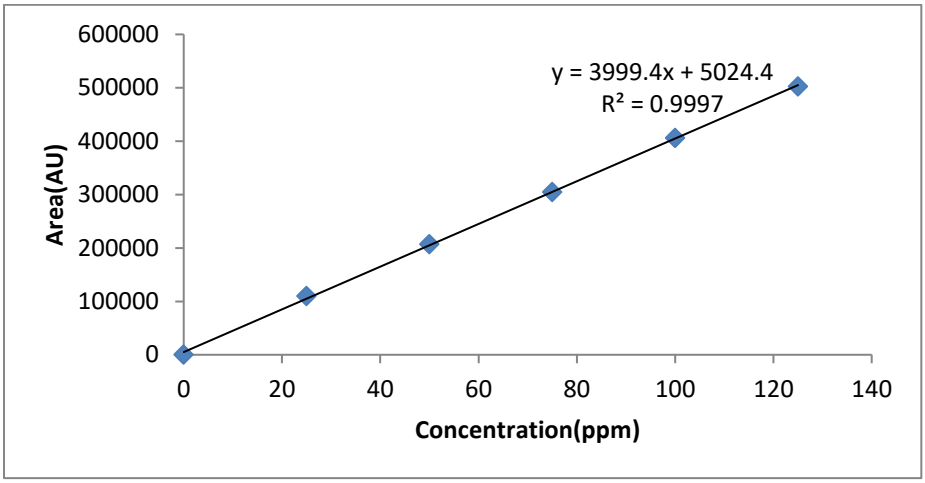

Figure 4: calibration graph for Aceclofenac

Acceptance Criteria:

- Correlation coefficient should be not less than 0.99.

Intermediate precision:

Table 6: Results of method precession Day 1 for Thiocolchicoside:

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Thiocolchicoside	3.378	39520	6588	6929.5	1.0
2	Thiocolchicoside	3.364	40090	6630	6886.4	1.0
3	Thiocolchicoside	3.397	40325	6580	6886.6	1.0
4	Thiocolchicoside	3.390	40429	6598	6776.1	1.0
5	Thiocolchicoside	3.384	40803	6667	6671.5	1.0
Mean			40233.5			
Std. Dev			474.3			
% RSD			1.2			

Table 7: Results of method precession Day 1 for Aceclofenac:

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Aceclofenac	3.838	378669	64578	10161.1	1.1	2.9
2	Aceclofenac	3.879	395452	64370	10006.6	1.1	3.0
3	Aceclofenac	3.872	396603	64721	9942.0	1.1	3.0
4	Aceclofenac	3.856	396896	64912	9954.6	1.1	3.0
5	Aceclofenac	3.864	397283	65123	10009.7	1.1	3.0
Mean			392980.7				
Std. Dev			8029.5				
% RSD			2.0				

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 8: Results of Intermediate precision Day 2 for Thiocolchicoside:

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Thiocolchicoside	3.375	40603	6630	6789.2	1.0
2	Thiocolchicoside	3.382	40938	6637	6714.4	1.0
3	Thiocolchicoside	3.371	41043	6689	6816.0	1.0
4	Thiocolchicoside	3.333	39951	6564	6718.8	1.0
5	Thiocolchicoside	3.341	40099	6594	6722.3	1.0
6	Thiocolchicoside	3.359	40315	6626	6850.4	1.0
Mean			40491.5			
Std. Dev			445.7119			
% RSD			1.1			

Table 9: Results of Intermediate precision Day 2 for Aceclofenac:

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Aceclofenac	3.855	354069	63308	10454.7	1.0	3.0
2	Aceclofenac	3.850	360532	64042	10263.0	1.1	3.0
3	Aceclofenac	3.846	361216	63997	10265.3	1.1	3.0
4	Aceclofenac	3.829	356725	63505	10179.4	1.0	2.9
5	Aceclofenac	3.798	363973	64094	9951.0	1.1	2.9
6	Aceclofenac	3.809	365768	64524	9809.6	1.0	2.9
Mean			360380.5				
Std. Dev			4378.354				
% RSD			1.2				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2

The %RSD obtained is within the limit, hence the method is rugged

Accuracy:

Table 10: accuracy (recovery) data for Thiocolchicoside

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	49720	5	4.97	99.4%	99.6%
100%	63697	10	9.99	99.9%	
150%	74472	15	14.96	99.7%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Table 11: accuracy (recovery) data for Aceclofenac

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	459246	5	4.98	99.6%	99.7%
100%	606482	10	9.99	99.9%	
150%	682043	15	14.97	99.8%	

Acceptance Criteria:

- The % Recovery for each level should be between 98.0 to 102.0%.

CONCLUSION:

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Thiocolchicoside and Aceclofenac was done by RP-HPLC. The mobile phase was optimized with consists of ACN : Phosphate buffer (pH-4.6) mixed in the ratio of 60:40 % v/ v. A Xterra column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. The linearity range of Thiocolchicoside and Aceclofenac were found to be from 1-5ppm, 25-125µg/ml respectively. Linear regression coefficient was not more than 0.999, 0.999.

The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 99.5%, 99.9% of Thiocolchicoside and Aceclofenac. LOD and LOQ were found to be within limit.

The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Acknowledgement:

The Authors are thankful to the Management and Principal, Department of Pharmacy Pydah College of Pharmacy , Kakinada, Andhra Pradesh , for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

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