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Research

Rp-Hplc Method Development And Validation For Estimation Of Thiocolchicoside And Aceclofenac In Pure And Pharmaceutical Dosage Form

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Abstract

A simple, reproducible and efficient reverse phase high performance liquid chromatographic method was developed for simultaneous determination of Aceclofenac and Thiocolchicoside in pure form and marketed combined pharmaceutical dosage forms. A column having Symmetry (C18) (150mm x 4.6mm, 5µm) in isocratic mode with mobile phase containing Methanol: Phosphate Buffer (pH-3.8) (28:72% v/v) was used. The flow rate was 1.0 ml/min and effluent was monitored at 252 nm. The retention time (min) and linearity range (ppm) for Aceclofenac and Thiocolchicoside were (1.794, 3.440min) and (10-30, 10-50), respectively. The method has been validated for linearity, accuracy and precision, robustness and limit of detection and limit of quantitation. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.86µg/ml and 2.58µg/ml for Aceclofenac and 1.28µg/ml 3.84µg/ml for Thiocolchicoside respectively. The developed method was found to be accurate, precise and selective for simultaneous determination of Aceclofenac and Thiocolchicoside in tablets.

Keywords: Aceclofenac and Thiocolchicoside, RP-HPLC, Validation, Accuracy, ICH Guidelines.

INTRODUCTION

Analytical chemistry[1] is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative identification or detection of compounds and the quantitative measurement of the substances present in bulk and pharmaceutical preparation.

Measurements of physical properties of analytes such as conductivity, electrode potential, light absorption or emission, mass to charge ratio, and fluorescence, began to be used for quantitative analysis of variety of inorganic and biochemical analytes. Highly efficient chromatographic and electrophoretic techniques began to replace distillation, extraction and precipitation for the separation of components of complex mixtures prior to their qualitative or quantitative determination. These newer methods for separating and determining chemical species are known collectively as instrumental methods of analysis. Most of the instrumental methods fit into one of the three following categories viz spectroscopy, electrochemistry and chromatography.

Introduction to HPLC

HPLC[3] is a type of liquid chromatography that employs a liquid mobile phase and a very finely divided stationary phase. In order to obtain satisfactory flow rate liquid must be pressurized to a few thousands of pounds per square inch.

The rate of distribution of drugs between Stationary and mobile phase is controlled by diffusion process. If diffusion is minimized faster and effective separation can be achieved .The techniques of high performance liquid chromatography are so called because of its improved performance when compared to classical column chromatography advances in column chromatography into high speed, efficient ,accurate and highly resolved method of separation.

For the recent study Clonazepam and Propranolol was selected for estimation of amount of analyte present in formulation and bulk drug. The HPLC method is selected in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages

- Speed many analysis can be accomplished in 20min (or) less.
- Greater sensitivity (various detectors can be employed).
- Improved resolution (wide variety of stationary phases).
- Re usable columns (expensive columns but can be used for many analysis).
- Ideal for the substances of low viscosity.
- Easy sample recovery, handling and maintenance.
- Instrumentation leads itself to automation and quantification (less time and less labour).
- Precise and reproducible.
- Integrator itself does calculations.
- Suitable for preparative liquid chromatography on a much larger scale.

HPLC components

The essential components[4] of a complete HPLC system are solvent delivery system (Pump), detector, fixed volume injector loop or auto sampler, solvent reservoirs, packed column, data system and recorder. A schematic of a simplified HPLC system is shown in Figure 1.

Column

The column is probably the heart of HPLC system. The development of this column technology leads to the evolution of the HPLC instrumentation systems used today. The conventionally used HPLC columns are particle packed columns. The key of column selection when previous separation is not available resides in knowing the chemistry of the sample. Columns should never be dry. A dry column will eventually have voids because the packing will shrink away from the wall, which would result in band broadening. Before running a sample in HPLC the column should be equilibrated. Usually column equilibrium is achieved after passage of 10 – 20 column volumes of the new mobile phase through the column. Insufficient column equilibrium usually leads to retention difference.

Pump

The solvent delivery system or as it is commonly called the pump includes two major types, constant volume or flow and constant pressure. Constant volume pumps are mechanically driven systems, most commonly using screw

driven syringes or reciprocating pistons. On the other hand, constant pressure pumps are driven or controlled by gas pressure.

Injector or Auto sampler

Samples are usually introduced by syringe injection via a manual injector into the mobile phase stream or by the use of an auto sampler. The important aspects in sample introduction are precise and reproducible injections. This is especially important with quantitative analysis where the reproducibility of the peak response is dependent on the precision of the sample introduction. Direct syringe injection through a manual injector was the first popular method of sample introduction. As HPLC instrumentation evolved, many auto sampler techniques were applied so that sample introduction has become more precise and rapid.

Detector

HPLC detectors include ultraviolet-visible, fluorescence, electrochemical, refractometer, mass spectrometer and others. The UV visible absorption detector is the most widely used detector in liquid chromatography, since most organic compounds show some useful absorption in the UV region. This detector is fairly universal in application, although sensitivity depends on how strongly the sample absorbs light at a particular wavelength.

Solvent reservoir

Different containers are used as a solvent delivery system reservoir. The best material from which the containers are made is glass. Plastic containers are not recommended as it leads to plasticizer leaching. The container should be covered to prevent solvent evaporation. The tubing from the reservoir can be made of stainless steel or Teflon, and both are satisfactory.

Data handling and analysis

Data handling in HPLC is as important to the success of any experiment or analysis as any other components in the system. It is part of good HPLC techniques to properly label and document the analytical results. The advanced computer softwares used now in data handling and analysis allow easy recording and storage of all chromatographic data.

Normal phase chromatography

In normal phase mode the stationary base (eg; silica gel) is polar in nature and the mobile phase is non polar. In this technique, non polar compound travel faster and are eluted first. This is because less affinity between solute and stationary phase and take more time to elute.

Reverse phase chromatography

The popularity of reversed phase liquid chromatography is easily explained by its unmatched simplicity, versatility and scope. Neutral and ionic analytes can be separated simultaneously. Retention in RPLC is believed to occur through nonspecific hydrophobic interaction of the solute with the stationary phase. The near universal application of RPLC stems from the fact that almost all organic compounds have hydrophobic regions in their structure and are capable of interacting with the stationary phase.

A decrease in the polarity of the mobile phase leads to a decrease in retention. It is also generally observed in RPLC that branched chain compounds are retained to a lesser extent than their straight chain analogues and that unsaturated compounds are eluted before their fully saturated analogs. A wide variety of RP-HPLC columns are available. Most columns are silica based. Silica offers good mechanical stability. A typical stationary phase is formed by chemically bonding a long-chain hydrocarbon group to porous silica. Typical ligands are n-octadecyl (C18), n-octayl (C8), n-butyl (C4), diphenyl (C2), and cyano propyl.

Parameters affecting separation[6]:

Separation in reversed phase chromatography is affected by stationary phase type and column length. It is also affected by organic solvent type and percentage in the mobile phase and by mobile phase pH. Flow rate could also affect separation in reversed phase chromatography; however it is usually limited by the developed backpressure. Moreover temperature of the column also has an effect on separation.

MATERIALS AND METHODS

Aceclofenac-Sura labs, Thiocolchicoside-Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC-Merck.

HPLC method development
Trails
Preparation of standard solution: Accurately weigh and transfer 10 mg of Aceclofenac and Thiocolchicoside working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.
Further pipette 0.2ml of the above Aceclofenac and 0.3ml of the Thiocolchicoside stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization: Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: Phosphate Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Bufferin proportion 28:72 (pH-3.8) v/v respectively.

Optimization of Column: The method was performed with various columns like C18 column, Symmetry and Zodiac column. Symmetry (C18) (150mm x 4.6mm, 5µm) Column 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 PDA Detector 996 model.
Temperature : Ambient
Column : Symmetry (C18) (150mm x 4.6mm, 5µm) Column
Buffer : Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.8 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.
pH : 3.8
Mobile phase : Methanol: Phosphate Buffer (28:72% v/v)
Flow rate : 1ml/min
Wavelength : 252 nm
Injection volume : 20 µl
Run time : 8 min

Method validation
Preparation of buffer and mobile phase
Preparation of Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-3.8)
Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.8 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.
Preparation of mobile phase: Accurately measured 280 ml (28%) of Methanol, 720 ml of Phosphate buffer (72%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.
Diluent Preparation: The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)
Mobile phase ratio : Methanol: Phosphate Buffer (pH-3.8) (28:72% v/v)
Column : Symmetry (C18) (150mm x 4.6mm, 5µm) Column
Columntemperature : Ambient
Wavelength : 252nm

Flowrate : 1.0ml/min
Injection volume : 20µl
Run time : 8minutes

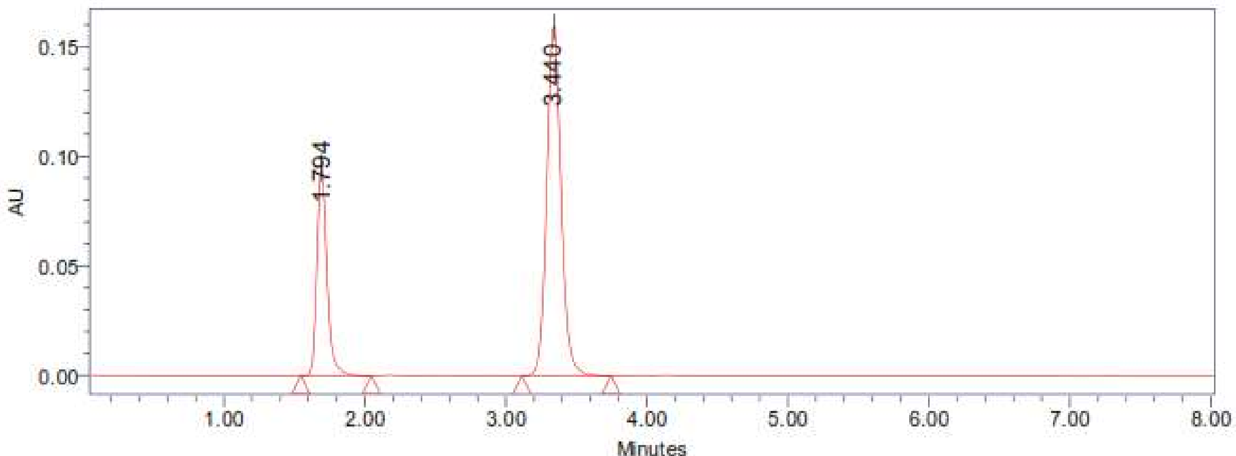

Fig 1: Optimized Chromatogram (Standard)

Table 1: Optimized Chromatogram (Standard)

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Aceclofenac	1.794	545265	7462	1.09	7564
2	Thiocolchicoside	3.440	7768545	43652	1.12	8695

Optimized Chromatogram (Sample)

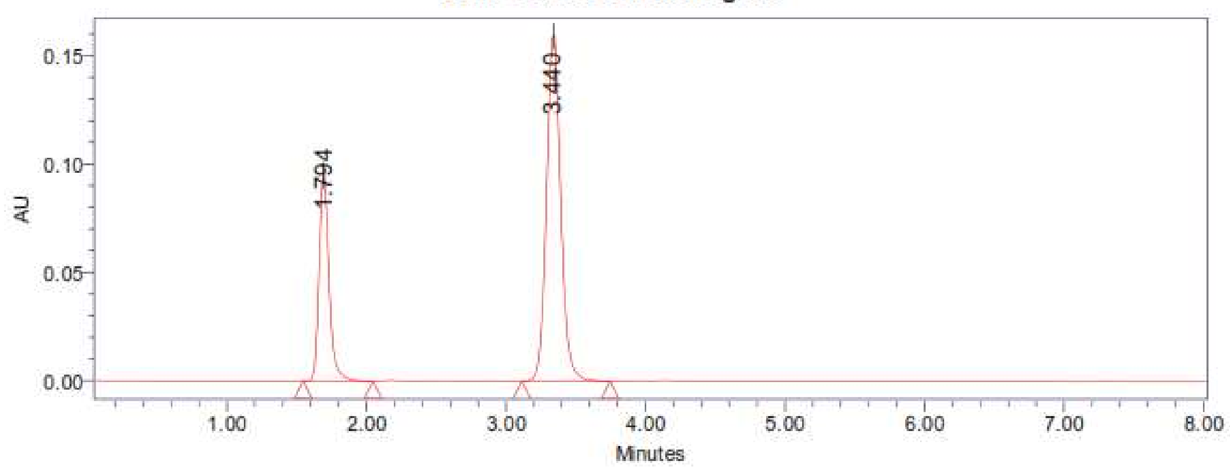

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.No	Name	RT	Area	Height	USPTailing	USPPlateCount
1	Aceclofenac	1.794	558659	7584	1.10	7659
2	Thiocolchicoside	3.440	7856985	44658	1.13	8743

Theoretical plates must be not less than 2000, Tailing factor must be not less than 2.It was found from above data that all the system suitability parameters for developed method were within the limit.

Assay (Standard)

Table 3: Peak results for assay standard of Aceclofenac

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Aceclofenac	1.788	545698	7458	7595	1.09
2	Aceclofenac	1.792	548765	7469	7548	1.10
3	Aceclofenac	1.793	548965	7428	7563	1.09
4	Aceclofenac	1.788	548783	7495	7592	1.10
5	Aceclofenac	1.787	548752	7461	7543	1.09
Mean			548192.6			
Std.Dev.			1397.209			
%RSD			0.254876			

%RSD of five different sample solutions should not more than 2.
The %RSD obtained is within the limit, hence the method is suitable.

Table 4: Peak results for assay standard of Thiocolchicoside

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Coun	USP Tailing
1	Thiocolchicoside	3.438	7785698	43652	8652	1.12
2	Thiocolchicoside	3.446	7786354	43698	8674	1.13
3	Thiocolchicoside	3.444	7786942	43587	8692	1.13
4	Thiocolchicoside	3.465	7785464	43698	8649	1.12
5	Thiocolchicoside	3.465	7785986	43568	8625	1.12
Mean			7786089			
Std.Dev.			581.3667			
%RSD			0.007467			

%RSD of five different sample solutions should not more than 2.
The %RSD obtained is within the limit, hence the method is suitable.

Table 5: Peak results for Assay sample of Aceclofenac

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Aceclofenac	1.794	556985	75895	1.10	7698	1
2	Aceclofenac	1.791	558742	75468	1.10	7682	2
3	Aceclofenac	1.791	559683	75426	1.11	7649	3

Table 6: Peak results for Assay sample of Thiocolchicoside

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Thiocolchicoside	3.440	7856859	44586	1.14	8759
2	Thiocolchicoside	3.442	7826594	44658	1.15	8726
3	Thiocolchicoside	3.434	7854879	44859	1.14	8794

$$\%ASSAY = \frac{\text{Samplearea}}{\text{Standardarea}} \times \frac{\text{Weight ofstandard}}{\text{Dilution ofstandard}} \times \frac{\text{Dilution ofsample}}{\text{Weight ofsample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight oftablet}}{\text{Labelclaim}} \times 100$$

The % purity of Aceclofenac and Thiocolchicosidein pharmaceutical dosage form was found to be 100.154%

Linearity

Chromatographic data for linearity study for aceclofenac

Concentration µg/ml	Average PeakArea
10	292985
15	430752
20	565265
25	693487
30	821584

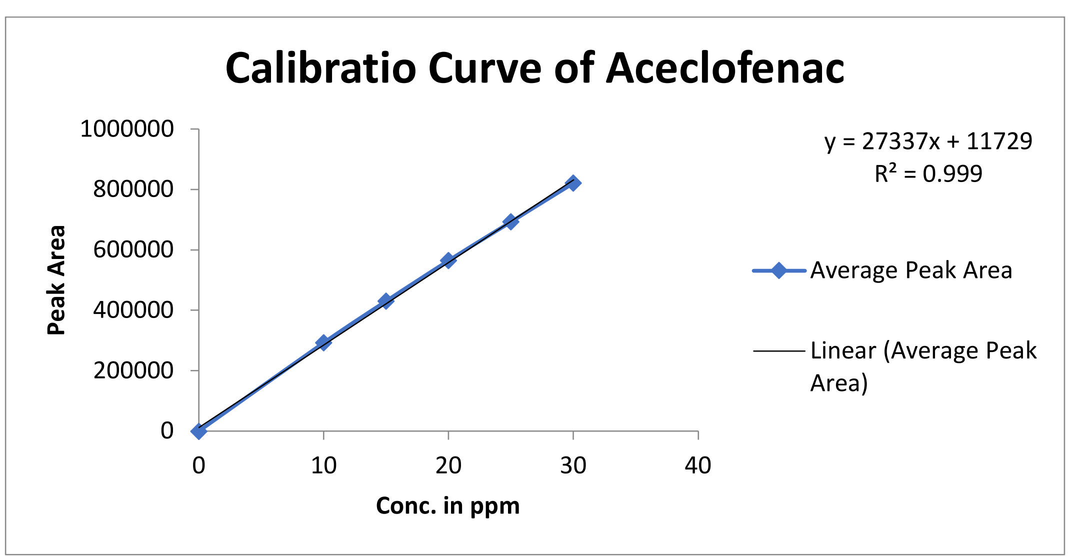

Fig 3: Chromatogram showing linearity level

Chromatographic data for linearity study for thiocolchicoside

Concentration µg/ml	Average PeakArea
10	2828756
20	5485784
30	7999859
40	10656542
50	13085985

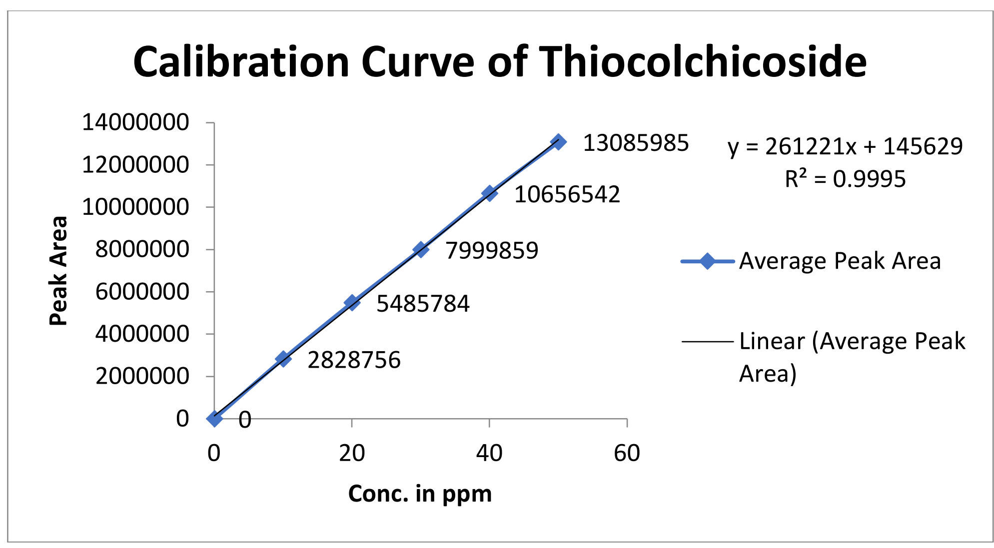

Fig 4: Chromatogram showing linearity level

Repeatability

Table 7: Results of Repeatability for Aceclofenac:

S. No.	Peak Name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Aceclofenac	1.792	548698	7458	7569	1.10
2	Aceclofenac	1.791	548955	7485	7546	1.10
3	Aceclofenac	1.790	548745	7469	7592	1.09
4	Aceclofenac	1.790	549856	7463	7519	1.10
5	Aceclofenac	1.789	546587	7495	7535	1.09
Mean			548568.2			
Std.dev			1202.217			
%RSD			0.2191554			

%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 Repeatability for Thiocolchicoside

S. No.	Peak Name	Retention time	Area(µV*sec)	Height (µV)	USP PlateCount	USP Tailing
1	Thiocolchicoside	3.435	7768958	43659	8659	1.12
2	Thiocolchicoside	3.428	7765984	43856	8647	1.13
3	Thiocolchicoside	3.419	7785469	43658	8675	1.12
4	Thiocolchicoside	3.414	7785498	43549	8652	1.12
5	Thiocolchicoside	3.408	7769852	44526	8692	1.13
Mean			7775152			
Std.dev			9539.236			
%RSD			0.122689			

%RSD forsampleshould be NMT 2.
The %RSD for the standardsolution is below 1, which is within the limits hence method is precise.

Intermediate precision

Table 9: Results of Intermediate precision day1forAceclofenac

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
	Aceclofenac	1.787	556985	75986	7695	1.11
2	Aceclofenac	1.789	558649	75986	7642	1.12
3	Aceclofenac	1.789	557847	75689	7683	1.12
Mean			557827			
Std.Dev.			832.1803			
%RSD			0.149183			

%RSD ofthree different sample solutions should not more than 2.

Table 10: Results of Intermediate precision day 1 forThiocolchicoside

S. No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Thiocolchicoside	3.482	7856982	44586	8758	1.13
2	Thiocolchicoside	3.477	7845285	44758	8769	1.14
3	Thiocolchicoside	3.477	7854633	44986	8728	1.13
Mean			7852300			
Std.Dev.			6187.659			
%RSD			0.078801			

%RSD ofthree different samplesolutionsshouldnotmorethan2.

Day 2

Table 11: Results of Intermediate precision Day 2 forAceclofenac

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Platecount	USP Tailing
1	Aceclofenac	1.790	536598	7365	7459	1.08
2	Aceclofenac	1.789	534875	7358	7436	1.07
3	Aceclofenac	1.793	534698	7349	7482	1.08
Mean			535390.3			
Std.Dev.			1049.608			
%RSD			0.196045			

%RSD ofthree different sample solutions should not more than 2.

Table 12: Results of Intermediate precision Day 2 forThiocolchicoside

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Platecount	USP Tailing
1	Thiocolchicoside	3.474	7698521	42568	8582	1.11
2	Thiocolchicoside	3.473	7685985	42698	8546	1.10
3	Thiocolchicoside	3.478	7645897	42365	8574	1.10
Mean			7676801			
Std.Dev.			27487.83			
%RSD			0.358064			

%RSD ofthree different samplesolutionsshouldnotmorethan 2.

Accuracy

Table 13: The accuracy results for Aceclofenac

%Concentration (at Specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	286080.7	10.035	10	100.350%	100.291%
100%	561215	20.100	20	100.500%	
150%	833959.7	30.077	30	100.023%	

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 14: The accuracy results forThiocolchicoside

%Concentration (at Specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	408328	15	15.074	100.493%	100.163%
100%	798306.3	30	30.003	100.010%	
150%	1189915	45	44.994	99.986%	

The percentage recovery was foundto be within the limit (98-102%).
The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table 15: Results for Robustness -Aceclofenac

Parameter used for sample analysis	PeakArea	Retention Time	Theoretical plates	Tailing factor
Actual Flowrate 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 More Flow rate of 0.9mL/min	526548	1.744	7425	1.12
Less organic phase (about 5 % decrease in organic phase)	536548	1.831	7265	1.06
More organic phase (about 5 % Increase in organicphase)	514875	1.874	7169	1.08

Table 16: Results for Robustness-Thiocolchicoside

Parameter used for sample analysis	PeakArea	Retention Time	Theoretical plates	Tailing factor
ActualFlowrateof 1.0mL/min	7768545	3.440	8695	1.12
Less Flowrateof 0.9mL/min	7985695	3.721	8948	1.13
MoreFlowrateof 1.1mL/min	7458642	3.097	8452	1.12
Less organicphase (about 5 % decrease in organicphase)	7685421	6.242	8365	1.10
Moreorganicphase (about 5 % Increase in organicphase)	7569864	2.402	8254	1.09

The Tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Thiocolchicoside And Aceclofenac in bulk drug and pharmaceutical dosage forms. Aceclofenac was found to be soluble in the organic solvents ethanol, DMSO, and dimethyl formamide (DMF) and slightly soluble in water and Acetonitrile. Thiocolchicoside was found to be soluble in water, methanol, 0.1N HCl, 0.1N NaOH. Methanol: Phosphate Buffer (pH-3.8) (28:72% v/v) was chosen as the mobile phase. The solvent system

used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tablesfor RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Aceclofenac and Thiocolchicosidein bulk drug and in Pharmaceutical dosage forms.

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