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Reverse Phase High Performance Liquid Chromatography for Simultaneous Determination of Aceclofenac and Thiocolchicoside in Bulk and Pharmaceutical Dosage form

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Abstract

A simple, rapid and accurate high performance liquid chromatography method is described for simultaneous determination of aceclofenac and thiocolchicoside from combined dosage form i.e. tablets. The separation of drug was achieved on Polaris C18 (150 x 4.6 mm i.d.) with 5 µ particle size, column showed most favorable chromatographic pattern over the other columns. The mobile phase consisted of a mixture of buffer and acetonitrile (55:45 % (v/v)). The buffer was 0.01 M ammonium acetate solution adjusted the pH 3.4 with ortho-phosphoric acid. The detection was carried out at wavelength 270 nm. The mixture of buffer of pH 3.4 and acetonitrile (55:45% v/v) was used as a diluent. The method was validated for system suitability, linearity, accuracy, precision, robustness, stability of sample solution. The method has been successfully used to analyze aceclofenac and thiocolchicoside from combined dosage form i.e. tablets.

Keywords: Aceclofenac, Thiocolchicoside Acetonitrile, Ammonium acetate, Ortho phosphoric acid.

Introduction

Aceclofenac, chemically {[[2-[(2,6-Dichlorophenyl) amino] phenyl] acetyl]oxy}Acetic acid. It is the non steroidal anti inflammatory, analgesic and anti-inflammatory drug. It is used in treatment of relief in variety of painful condition.

Thiocolchicoside, a semi synthetic derivative of naturally occurring compound of colchicoside from the seeds of various species of colchicum antumnale (autumn crocus, meadow saffron, Gloriosa upuba), chemically, *N*-[(7*S*)-3-(β-D-Glucopyranosyloxy)-1,2-dimethoxy-10-(methylsulfanyl)-9-oxo-5,6,7,9-tetrahydrobenzo[*a*]heptalen-7-yl]acetamide It is centrally acting muscles relaxant and it also show analgesic activity. It is used in treatment of muscular pain and gout.

Literature survey reveals, UPLC[1], HPTLC[2], HPLC[3] and UV spectrophotometric methods[3,4] for simultaneous determination of thiocolchicoside and aceclofenac in combined dosage form. This proposed work presents simple, accurate and reproducible reverse phase high performance liquid chromatographic method for simultaneous determination of aceclofenac and thiocolchicoside in tablet dosage form.

Materials and Methods

Chemical and reagents: Reference standard of aceclofenac and thiocolchicoside were obtained from reputed firm with certificate of analysis. Ammonium acetate, acetonitrile and ortho phosphoric acid were used of analytical grade and the HPLC grade water was used from Millipore. Standard and sample solutions were prepared in diluent [mixture of buffer of pH 3.4 and acetonitrile (55:35 % (v/v)].

Instrumentation: The HPLC system used was MERCK Hitachi HPLC system equipped with auto sampler (D 7200 separation module) and UV detector (D- 7400). The chromatogram was recorded and peaks quantified by means of PC based EZ Chrom Elite software. A Shimadzu analytical balance (0.01 mg) was used.

Standard solution: A 100 mg of standard aceclofenac and 4 mg of thiocolchicoside and were weighted accurately and transferred in 100 ml volumetric flask. About 50 ml of diluent [mixture of buffer of pH 3.4 and acetonitrile [55:45 % (v/v)] was added and sonicated for 10 minutes. The volume was adjusted up to the mark with diluent to give concentration as 1000 µg /ml of aceclofenac and 4 µg /ml of thiocolchicoside respectively.

Sample preparation: Twenty tablets were weighed accurately and average weight of each tablet was determined. A powder equivalent to 100 mg of standard aceclofenac and 4 mg of thiocolchicoside were weighted accurately and transferred in 100 ml volumetric flask to give concentration as 1000 µg /ml of aceclofenac and 4 µg /ml. of respectively.

Chromatographic condition: Chromatographic separation was performed on a reverse phase Polaris C18 (150 x 4.6 mm i.d.) with 5 µ particle size column. The mobile phase was a mixture of buffer of pH 3.4 and acetonitrile [55:45 % (v/v)]. The buffer

was 0.01M ammonium acetate adjusted the pH 3.4 with ortho-phosphoric acid. The flow rate of the mobile phase was adjusted to 1 ml /min. The detection was carried out at wavelength 270 nm. (Figure-1). The injection volume of the standard and sample solution was set at 1.0 µl.

Method validation: System suitability: System performances of developed HPLC method were determined by injecting standard solutions. Parameter such as theoretical plates (N), asymmetry, resolution and area were determined. The results are shown in Table-1 which indicates good performance of the system.

Specificity: Specificity is the ability of the method to resolve the active ingredients. Hence blank, standard ofloxacin was injected to prove specificity. The typical chromatogram of the standard and sample assayed are given in Figure-2 and 3 respectively.

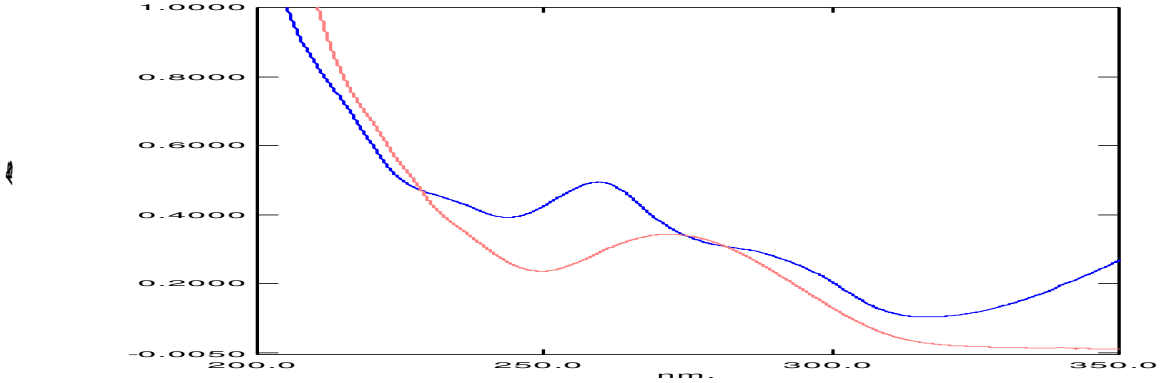

Figure-1
Overlay UV spectra of aceclofenac and thiocolchicoside

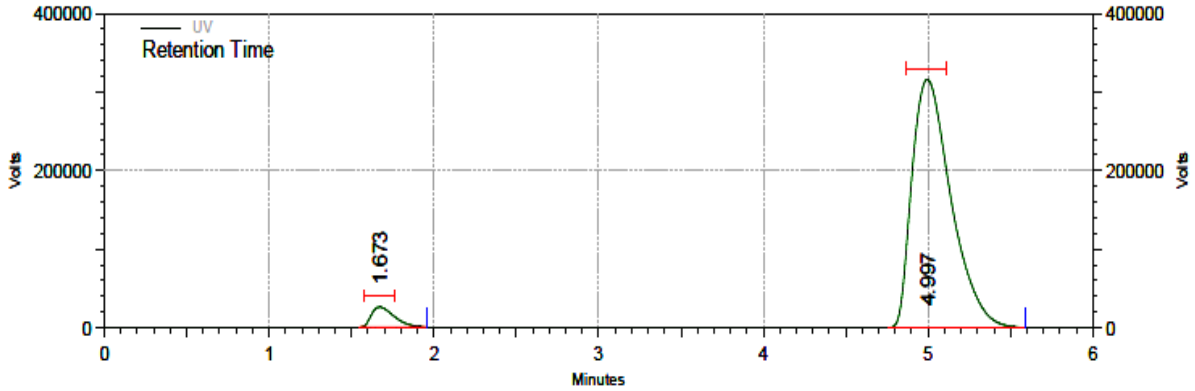

Figure-2
Typical chromatogram of Aceclofenac and thiocolchicoside (standard)

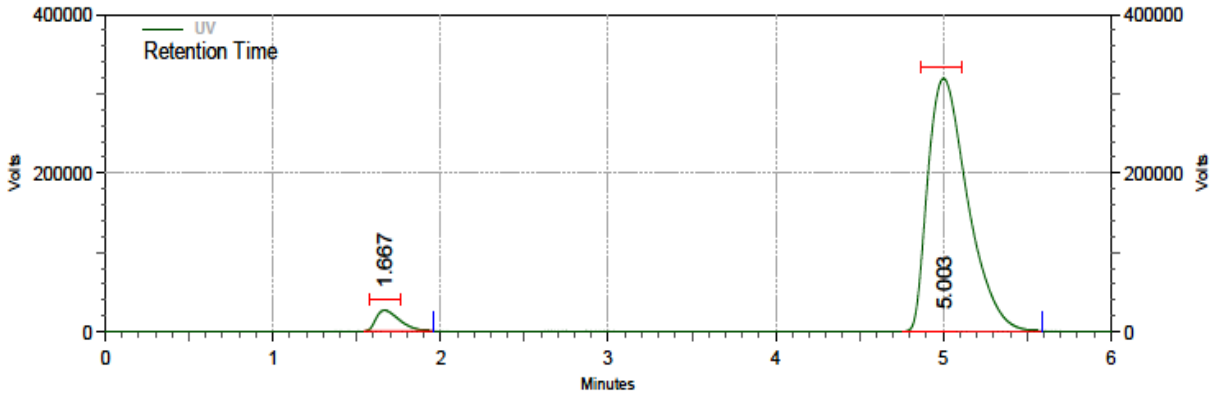

Figure-3
Typical chromatogram of Aceclofenac and thiocolchicoside (sample)

Linearity: Under the experimental conditions described above, linear calibration curve were obtained throughout the concentration range studied. Regression analysis was done on the peak area (y) v/s concentration (x). The regression analysis data obtained is tabulated in Table-2.

Accuracy: The accuracy method was determined by applying proposed method to synthetic mixture containing known amount of drug corresponding to 80 %, 100 % and 120 %. The accuracy was then calculated as the percentage of analyte recovered by the assay. The results of the recovery analysis are enclosed under Table-3, 4.

Precision: The method precision was established by carrying out the analysis of aceclofenac and thiocolchicoside. The assay was carried out of the drug using analytical method in five replicates. The value of relative standard deviation lies well with the limits. The results of the same are tabulated in the Table-5,6.

Robustness: The robustness of the method was determined to check the reliability of an analysis with respect to deliberate variations in method parameters.

The typical variations are given below:
Variation in the flow rate by $\pm$ 0.2 ml /min
Variation in mobile phase composition by $\pm$ 2 %
Variation in wavelength ± 5 nm

The results of the analysis of the samples under the conditions of the above variation indicated the nature of robustness of the method.

Method application: Twenty tablets were weighed accurately and average weight of each tablet was determined. A powder equivalent to 100 mg of standard aceclofenac and 4 mg of thiocolchicoside were weighted accurately and transferred in 100 ml volumetric flask to give concentration as 1000 µg /ml of aceclofenac and 4 µg /ml. of thiocolchicoside respectively. From this solution 1.0 µl was injected specific conditions. The analyte peak was identified by comparison with that of respective standard. The (%) assay results were expressed in Table-4, 5. It indicates the amount of aceclofenac and thiocolchicoside in the product meets the requirement.

Table-1
System suitability parameters evaluated on standard solution of Aceclofenac and thiocolchicoside

Name	Retention Time	Area	Asymmetry	Resolution
Thiocolchicoside	1.673	251164	1.63	-
Aceclofenac	4.997	5203102	1.55	9.595

Table-2
Statistical evaluation of the data subjected to regression analysis

Parameters	Aceclofenac	Thiocolchicoside
Correlation Coefficient (r)	0.9987	0.9961
% Intercept (y)	90305	3704.5
Slope (m)	51453	5819

Table-3
Statistical evaluation of the data subjected to accuracy of aceclofenac

Level	Test	weight in mg	Area	Quantity added in µg /ml	Quantity recovered in µg /ml	% recovery	Mean recovery
80%	1	10.09	4255410	80.96	82.05	101.35	**101.16**
	2	10.14	4221397	80.96	81.40	100.54	
	3	10.11	4265873	80.96	82.25	101.60	
100%	1	10.08	5287863	101.2	101.96	100.75	**100.52**
	2	10.11	5276604	101.2	101.74	100.54	
	3	10.12	5263162	101.2	101.48	100.28	
120%	1	10.10	6360489	121.44	122.64	100.99	**100.90**
	2	10.12	6320862	121.44	121.88	100.36	
	3	10.11	6383009	121.44	123.08	101.35	
					Mean recovery of all level		**100.86**

Table-4
Statistical evaluation of the data subjected to accuracy of thiocolchicoside

For thiocolchicoside							
Level	**Test**	**Weight in mg**	**Area**	**Quantity added in µg/ ml**	**Quantity recovered in µg/ ml**	**% recovery**	**Mean recovery**
80%	1	4.31	204703	34.4	34.54	100.41	**100.12**
	2	4.29	201436	34.4	33.99	98.80	
	3	4.28	206216	34.4	34.79	101.15	
100%	1	4.27	252820	43	42.66	99.21	**99.54**
	2	4.32	253322	43	42.74	99.40	
	3	4.26	254867	43	43.00	100.01	
120%	1	4.27	305317	51.6	51.52	99.84	**99.44**
	2	4.31	304822	51.6	51.43	99.68	
	3	4.32	302130	51.6	50.98	98.80	
					Mean recovery of all level		**99.70**

Table-5
Statistical evaluation of the data subjected to method precision of aceclofenac

Test	**Weight of test sample in mg**	**Area**	**% assay**
Test-1	10.11	5203102	99.23
Test-1	10.12	5204312	99.16
Test-13	10.09	5206628	99.50
Test-4	10.08	5237014	100.18
Test-5	10.09	5234712	100.04
Test-6	10.07	5233307	100.21
	Mean Assay		**99.72**
	SD		**0.479**
	RSD		**0.480**

Table-6
Statistical evaluation of the data subjected to method precision of thiocolchicoside

For thiocolchicoside			
Test	**Weight of test sample in mg**	**Area**	**% assay**
Test-1	4.28	251164	99.02
Test-1	4.29	252944	99.49
Test-13	4.31	253732	99.33
Test-4	4.30	256184	100.53
Test-5	4.27	255463	100.95
Test-6	4.32	253665	99.08
	Mean Assay		**99.73**
	SD		**0.809**
	RSD		**0.811**

Result and Discussion

The reproducibility, repeatability and accuracy of the proposed method were found to be satisfactory which is evidenced by low values of standard deviation and percent relative standard deviation. (Table-3,4) The accuracy and reproducibility of the proposed method was confirmed by recovery experiments, performed by adding known amount of the drug to the pre-analyzed active pharmaceutical ingredient and reanalyzing the mixture by proposed method.

Conclusion

Thus the proposed RP-HPLC method is used for estimation of aceclofenac and thiocolchicoside from active pharmaceutical ingredient and pharmaceutical combined dosage form. It is more economical, precise, accurate, linear, robust, simple and rapid method. Hence the proposed RP-HPLC method is strongly recommended for the quality control of the raw material, active pharmaceutical ingredient and pharmaceutical formulation.

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