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Simultaneous Determination of aceclofenac and thiocolchicoside by reverse phase high performance liquid chromatography form bulk drug and pharmaceutical dosage form

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ABSTRACT

A simple, economical 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 Intersil ODS 3V C18 (150 x 4.6 mm i.d.) with 5 µ particle size, column. It showed most favorable chromatographic pattern over the other columns. The mobile phase consisted of a mixture of buffer and acetonitrile (66:34 %(v/v)). The buffer was 0.01 M sodium dihydrogen phosphate solution and 1 ml triethyl amine. The detection was carried out at wavelength 263 nm. The mixture of buffer and acetonitrile (66:34% 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, sodium dihydrogen phosphate, triethyl amine.

INTRODUCTION

Aceclofenac, chemically {[[2-[(2,6-Dichlorophenyl)amino]phenyl]acetyl]oxy}Acetic acid. It is the non steroidal anti inflammatory and analgesic 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.

Chemical and reagents

Reference standard of aceclofenac and thiocolchicoside were obtained from reputed firm with certificate of analysis. Sodium dihydrogen phosphate and triethylamine were used of analytical grade and acetonitrile was used of HPLC

grade. The HPLC grade water was used from Millipore. Standard and sample solutions were prepared in diluent [mixture of buffer and acetonitrile (66:34 % (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.

Preparation of Standard preparation

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 and acetonitrile [66:34 % (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 40 µg /ml of thiocolchicoside respectively.

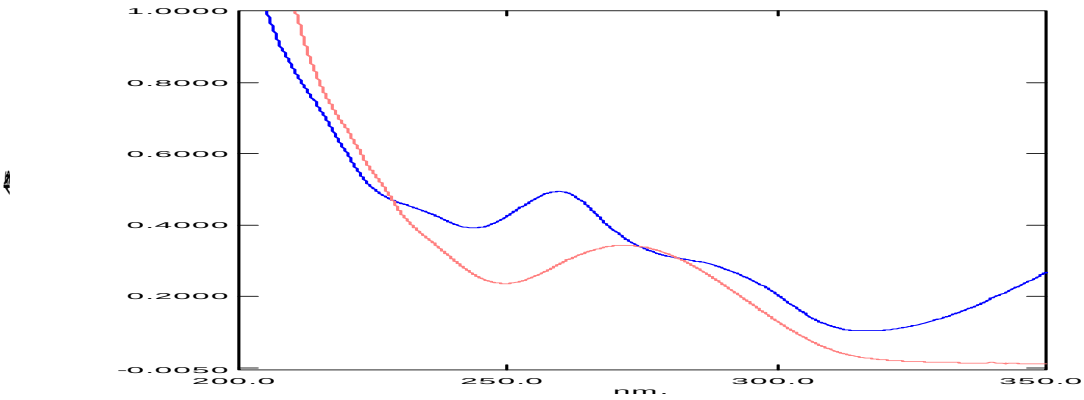
Sample preparation

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 40 µg /ml. of respectively. A 1.0 ml of this solution was diluted to 10 ml with diluent to give 100 µg /ml of aceclofenac and 4 µg /ml of thiocolchicoside respectively.

Chromatographic condition

Chromatographic separation was performed on a reverse phase Intersil ODS 3V C18 (150 x 4.6 mm i.d.) with 5 µ particle size column. The mobile phase was a mixture of buffer and acetonitrile [65:34 % (v/v)]. The buffer was 0.01M sodium dihydrogen phosphate with 1 ml of triethyl amine. The flow rate of the mobile phase was adjusted to 1 ml /min. The detection was carried out at wavelength 263 nm. (Fig.1) The injection volume of the standard and sample solution was set at 1.0 µl.

Figure 1: Overlay UV spectra of aceclofenac and thiocolchicoside



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.

Table 1: System suitability parameters evaluated on standard solution of aceclofenac and thiocolchicoside

Name	Retention Time	Area
Thiocolchicoside	1.920	310972
Aceclofenac	7.827	383814

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 2: Typical chromatogram of Aceclofenac and thiocolchicoside (standard)

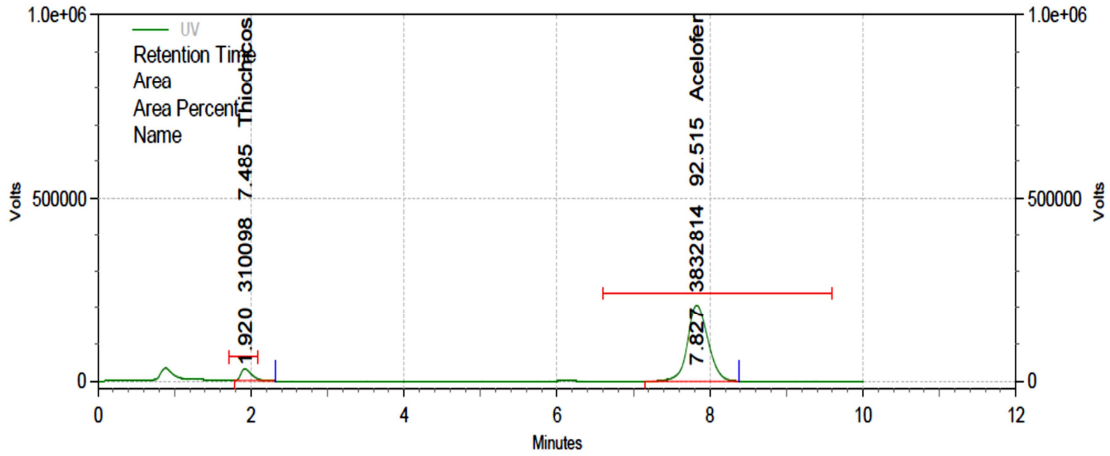
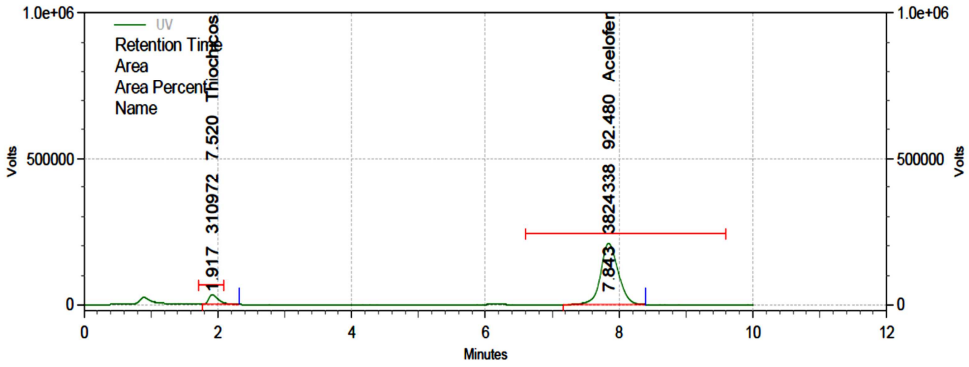


Figure 3: Typical chromatogram of Aceclofenac and thiocolchicoside (sample)



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 no. 2

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

Parameters	Aceclofenac	Thiocolchicoside
Correlation Coefficient (r)	0.9981	0.9994
% Intercept (y)	22227	11409
Slope (m)	37089	7578.3

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 no.3, 4.

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	10.19	3051917	80.96	80.85	99.87	**99.79**
	2	10.24	3028085	80.96	80.22	99.09	
	3	10.21	3068280	80.96	81.29	100.40	
100%	1	10.25	3840110	101.2	101.73	100.53	**100.51**
	2	10.01	3837772	101.2	101.67	100.47	
	3	10.22	3840518	101.2	101.74	100.54	
120%	1	10.07	4664887	121.44	123.58	101.76	**101.58**
	2	10.35	4653030	121.44	123.27	101.51	
	3	10.32	4651460	121.44	123.23	101.47	
					Mean recovery of all level		**100.63**

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

level	test	weight in mg	area	quantity added in µg/ ml	quantity recovered in µg/ ml	% recovery	mean recovery
80%	1	4.2	253272	34.4	34.87	101.38	**100.90**
	2	4.19	248875	34.4	34.27	99.62	
	3	4.25	254071	34.4	34.98	101.70	
100%	1	4.22	310656	43	42.77	99.48	**99.65**
	2	4.35	311965	43	42.95	99.89	
	3	4.28	310972	43	42.82	99.58	
120%	1	4.37	379919	51.6	52.31	101.38	**100.57**
	2	4.15	372996	51.6	51.36	99.53	
	3	4.12	377708	51.6	52.01	100.79	
					Mean recovery of all level		**100.37**

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 no.5, 6.

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

Test	weight of test	Area	% assay
Test-1	10.05	3735152	99.43
Test-1	10.02	3773035	100.74
Test-3	10.09	3817288	101.22
Test-4	10.30	3821172	99.25
Test-5	10.15	3820163	100.69
Test-6	10.07	3794756	100.82
	Mean Assay		**100.36**
	SD		**0.811**
	RSD		**0.808**

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

Test	weight of test	Area	% assay
Test-1	4.25	307851	100.90
Test-1	4.32	311913	100.57
Test-3	4.3	310541	100.59
Test-4	4.32	311904	100.57
Test-5	4.38	310972	98.89
Test-6	4.36	315690	100.86
	Mean Assay		**100.40**
	SD		**0.751**
	RSD		**0.748**

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 ± 0.2 ml /min
Variation in mobile phase composition by ± 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 40 µg /ml. of thiocolchicoside respectively. A 1.0 ml of this solution was diluted to 10 ml with diluent. 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 no. 4, 5. It indicates the amount of aceclofenac and thiocolchicoside in the product meets the requirement.

RESULTS AND CONCLUSION

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. 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. Thus the proposed RP-HPLC method is used for estimation of aceclofenac and thiocolchicoside from active pharmaceutical ingredient. It is more 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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