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Reverse phase high performance liquid chromatography for simultaneous determination of diclofenac and thiocolchicoside in bulk drug 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 diclofenac potassium and thiocolchicoside from bulk drugs and their combined pharmaceutical dosage form i.e. tablets. The separation of drug was achieved on Intersil ODS 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 (66:34 % (v/v)). The buffer was 0.01 M sodium dihydrogen phosphate solution and tiethylamine. 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 diclofenac potassium and thiocolchicoside from combined dosage form i.e. tablets.

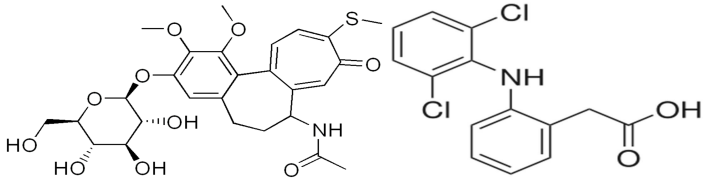
Keywords: Diclofenac potassium, thiocolchicoside acetonitrile, sodium dihydrogen phosphate, triethylamine.

INTRODUCTION

Diclofenac potassium, chemically potassium salt of 2-(2,6-dichloranilino) phenylacetic 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, UV spectrophotometric [1-7], HPLC [8, 9] and HPTLC [10, 11] methods for simultaneous determination of thiocolchicoside and diclofenac potassium in combined dosage form. This proposed work presents simple, accurate and reproducible reverse phase high performance liquid chromatographic method for simultaneous determination of diclofenac potassium and thiocolchicoside in tablet dosage form.



Structure of diclofenac **structure of thiocolchicoside**

MATERIALS AND METHODS

Chemical and reagents

Reference standard of diclofenac potassium and thiocolchicoside were obtained from reputed firm with certificate of analysis. Sodium dihydrogen phosphate and triethylamine were used of analytical grade 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 4 mg of thiocolchicoside and were weighted accurately and transferred in 10 ml volumetric flask. About 5 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 400 µg /ml of thiocolchicoside respectively.

A 5 mg of standard diclofenac potassium was weighed accurately and transferred in 10 ml volumetric flask. About 5 ml of diluent [mixture of buffer and acetonitrile [66:34 % (v/v)] was added, into this solution 1 ml of 400 µg /ml of thiocolchicoside was added. This solution was sonicated for 10 minutes. The volume was adjusted up to the mark with diluent to give concentration as 500 µg /ml of diclofenac potassium and 40 µg /ml of thiocolchicoside respectively. A 1ml of this solution was diluted to 10 ml with diluant. It gave 50 µg /ml of diclofenac potassium and 4 µg /ml. of respectively. Such solution was used for analysis.

Sample preparation

Twenty tablets were weighed accurately and average weight of each tablet was determined. A powder equivalent to 50 mg of standard diclofenac potassium and 4 mg of thiocolchicoside were weighted accurately and transferred in 100 ml volumetric flask to give concentration as 500 µg /ml of diclofenac potassium and 40 µg /ml. of respectively. A 1 ml of this solution was diluted to 10 ml with diluents. It gave 50 µg /ml of diclofenac potassium and 4 µg /ml. of respectively. This solution was used for analysis.

Chromatographic condition

Chromatographic separation was performed on a reverse phase Intersil ODS C18 (150 x 4.6 mm i.d.) with 5 µ particle size column. The mobile phase was a mixture of buffer and acetonitrile [66:34 % (v/v)]. The buffer was 0.01M sodium dihydrogen phosphate and 1 ml triethylamine. 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 10 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.

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

Name	Retention Time	Area	Asymmetry	Resolution (USP)
Thiocolchicoside	1.927	313359	1.63	-
Diclofenac potassium	5.160	1973870	1.55	9.79515

Specificity

Specificity is the ability of the method to resolve the active ingredients. Hence blank, standard were injected to prove specificity. The typical chromatogram of the standard and sample assayed are given in figure 1 and 2 respectively.

Figure 1: Typical chromatogram of Diclofenac potassium and thiocolchicoside (standard)

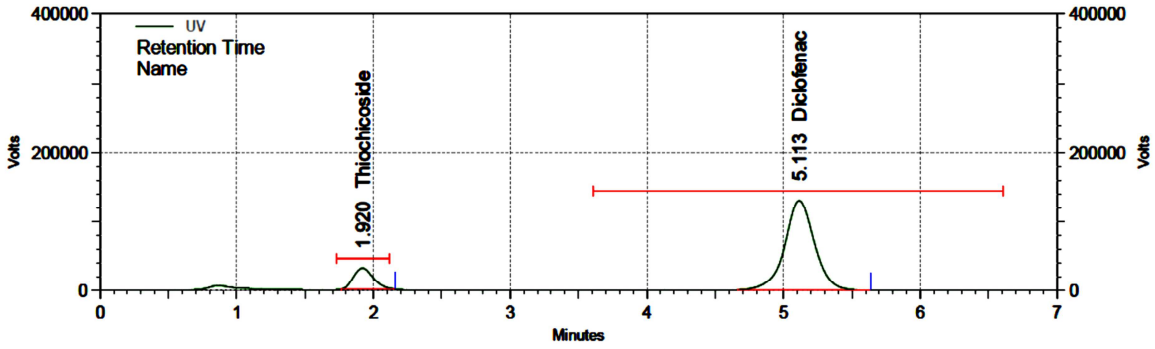
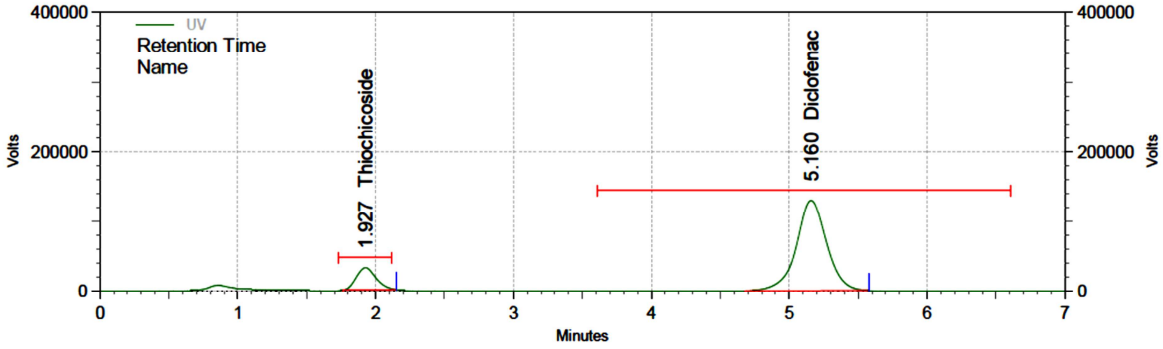


Figure 2: Typical chromatogram of Diclofenac potassium and thiocolchicoside (sample)



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	Diclofenac potassium	Thiocolchicoside
Correlation Coefficient (r)	0.9999	0.9998
% Intercept (y)	637.63	539.49
Slope (m)	38503	7401.1

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 diclofenac potassium

level	test	wt in mg	area	quantity added in µg /ml	quantity recovered in µg /ml	% recovery	mean recovery
80%	1	5.09	1572224	40.96	40.72	99.41	**99.57**
	2	5.14	1575025	40.96	40.79	99.58	
	3	5.11	1577200	40.96	40.85	99.72	
100%	1	5.08	1974353	51.2	51.13	99.86	**99.94**
	2	5.11	1976539	51.2	51.19	99.98	
	3	5.12	1976517	51.2	51.19	99.97	
120%	1	5.10	2366866	61.44	61.30	99.77	**99.75**
	2	5.12	2367828	61.44	61.32	99.81	
	3	5.11	2364497	61.44	61.23	99.67	
					Mean recovery of all level		**99.75**

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

level	test	wt in mg	area	quantity added in µg /ml	quantity recovered in µg /ml	% recovery	mean recovery
80%	1	4.11	245006	33.28	32.64	98.08	**97.82**
	2	4.19	244158	33.28	32.53	97.74	
	3	4.18	243911	33.28	32.50	97.64	
100%	1	4.17	311950	41.6	41.56	99.91	**99.85**
	2	4.12	312550	41.6	41.64	100.10	
	3	4.16	310826	41.6	41.41	99.55	
120%	1	4.17	367566	49.92	48.97	98.10	**98.09**
	2	4.11	367449	49.92	48.95	98.07	
	3	4.12	367566	49.92	48.97	98.10	
					Mean recovery of all level		**98.59**

Precision

The method precision was established by carrying out the analysis of diclofenac potassium 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 diclofenac potassium

Test	weight of test	Area	% assay
Test-1	5.11	1976252	100.16
Test-1	5.12	1976632	99.98
Test-13	5.09	1976966	100.59
Test-4	5.08	1977152	100.79
Test-5	5.09	1976564	100.57
Test-6	5.07	1974402	100.85
	Mean Assay		**100.49**
	SD		**0.349**
	RSD		**0.348**

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

Test	Weight of test	Area	% assay
Test-1	4.18	311804	99.38
Test-1	4.19	313586	99.71
Test-13	4.11	309832	100.43
Test-4	4.10	311273	101.15
Test-5	4.17	313380	100.12
Test-6	4.12	311273	100.66
	Mean Assay		**100.24**
	SD		**0.643**
	RSD		**0.641**

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 50 mg of standard diclofenac potassium and 4 mg of thiocolchicoside were weighted accurately and transferred in 100 ml volumetric flask to give concentration as 500 µg /ml of diclofenac potassium and 40 µg /ml. of respectively. A 1 ml of this solution was diluted to 10 ml with diluents. It gives 50 µg /ml of diclofenac potassium and 4 µg /ml. of respectively. This solution was used for analysis. 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 diclofenac potassium 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 diclofenac potassium 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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