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Simultaneous Determination of Aceclofenac and Thiocolchicoside in Formulation by Reversed Phase High Performance Liquid Chromatography

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ABSTRACT

Rapid and accurate isocratic reverse phase high performance liquid chromatography method is described for simultaneous determination of aceclofenac and thiocolchicoside in the combination dosage form. The separation of two drugs was achieved on Thermo Hypersil BDS C18 (250 mm X 4.6 mm) column of 5 µm particle size. Mobile phase consisted of 42:58 of acetonitrile and buffer of pH 6 respectively. Detection was carried out at 261 nm. Thermo Hypersil BDS C18 column showed most favorable chromatographic parameters for analysis. The method was validated for system suitability, linearity, accuracy, precision, robustness and stability of sample solution. The linear range for aceclofenac and Thiocolchicoside was 25-125 µg/ml and 1-6 µg/ml respectively.

Keywords: Thiocolchicoside, Aceclofenac, Pharmaceutical dosage form, High pressure liquid chromatography.

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INTRODUCTION

In this communication the present work proposes a new RP-HPLC method for assay of two drugs i.e. aceclofenac and thiocolchicoside in combined dosage form. Aceclofenac is chemically described as [(2(2',6'-dichlorophenyl amino)phenyl] acetoxy acetic acid. It is non steroidal anti-inflammatory drug which also shows good effect of analgesic properties and good tolerability profile in variety of painful conditions. Thiocolchicoside is chemically described as N-[(7*S*)-3-(beta-D-glucopyranosyloxy)-1, 2-dimethoxy-10-(methylsulfanyl)-9-oxo-5, 6, 7, 9-tetrahydrobenzo[a]heptalen-7yl] acetamide which is used as a muscle relaxant. It acts as a competitive $GABA_A$ receptor antagonist and also inhibits glycine receptors with similar potency and nicotinic acetylcholine receptors to a much lesser extent. Aceclofenac is officially reported in BP[1]. Thiocolchicoside is not been yet reported in any of the pharmacopoeia. Literature survey reveals spectrophotometric[2], HPLC[3] and HPTLC[4] methods for assay of this combination of drugs. In this communication a simple, rapid and reliable RP-HPLC method is proposed for simultaneous determination of aceclofenac and thiocolchicoside in combination dosage. Newly developed method can be easily used for the routine analysis in quality control laboratories. In the proposed work development, optimization and validation of the method are presented.

MATERIALS AND METHODS

Materials

Reference standards of aceclofenac and thiocolchicoside were obtained from reputed firms with certificate of analysis. HPLC grade of acetonitrile from Merck was used for analysis. HPLC grade water was obtained using Millipore system. Standard sample solutions were prepared in diluent such as 50:50 with acetonitrile and water.

Instrumentation

The HPLC system, Merck La-chrome Elite HPLC system, equipped with auto sampler (L-2200), isocratic pump (L-2100) and diode array detector were used. The chromatogram was recorded and peaks were quantified by means of PC based Ezchrome Elite software.

Preparation of standard stock solutions

Standard stock solutions

The About 100 mg of thiocolchicoside was weighed accurately and transferred into 100 ml volumetric flask. About 60 ml of diluent (acetonitrile: water as 50:50 v/v) was added and sonicated for 5 min to dissolve thiocolchicoside. Volume was adjusted by diluent to give concentration as 1000 µg/ml of thiocolchicoside. Further 1ml of this solution was diluted to 100

ml with diluents to give 10µg/ml. The 4 ml of the resulting solution was then dissolved to 10 ml to get 4 µg/ml of thiocolchicoside solution.

About 100 mg of aceclofenac was weighed accurately and transferred into 100 ml volumetric flask. About 60 ml of diluent (acetonitrile: water as 50:50 v/v) was added and sonicated for 5 min to dissolve aceclofenac and volume was adjusted by diluents to give concentration as 1000 µg/ml solution. Further 10 ml of this solution was diluted with diluent to 100 ml to get concentration as 100 µg/ml of aceclofenac solution.

Sample Solution.

Twenty tablets of the dosage under study were accurately weighed and average weight of each tablet was determined. Tablets were crushed into fine powder form and from such powder portion equivalent to 100 mg of aceclofenac and 4 mg of thiocolchicoside was weighed accurately. It was transferred into 100 ml volumetric flask with 60 ml of diluent. Solution was sonicated at 60^{o}C for five minutes. Volume was adjusted to 100 ml with the diluent. Sample was then filtered through Whatman filter paper No.41. The 10 ml of this solution was then diluted to 100 ml with the diluent to obtain final concentration as 100 µg/ml of aceclofenac and 4.0 µg/ml of thiocolchicoside.

Chromatographic conditions:

Chromatographic separation was performed at room temperature on Thermo Hypersil BDS C18 (250 mm X 4.6 mm) column of 5 µm particle size. Mobile phase consisted acetonitrile and buffer of pH as 6 in the ratio of 58:42. Buffer solution was made up of 0.01 M potassium dihdyrogen phosphate. The pH of the solution was adjusted to 6.0 with orthophosphoric acid. The mobile phase was filtered and degassed. The flow rate of the mobile phase was adjusted to 0.6 ml/min. The detector wavelength was set at 261 nm. The injection volume of the standard and sample solution was 10 µl.

Method Development.

Different columns containing different packaging material and different dimensions were tried for effective separation and resolutions. It was found that Hypersil BDS C18 column offered more advantage over the other columns. Individual drug Solution was injected into column. Elution and resolution parameters of all the two drugs were recorded at the wavelength range of 200 to 400 nm and their response optimization was compared. From the overlain spectra of two drugs, a suitable wavelength as 261 nm selected for the detection of the two drugs with adequate sensitivity (**figure 1).** The pH study showed that pH 6.0 was most suitable pH for assay of these two drugs. It gave well shaped peaks for the assay of all the drugs. A typical chromatogram of

the drugs assayed is depicted in **figure. 2**. The good chromatographic separation indicated that these drugs could be used as internal standards for the assay of other drugs.

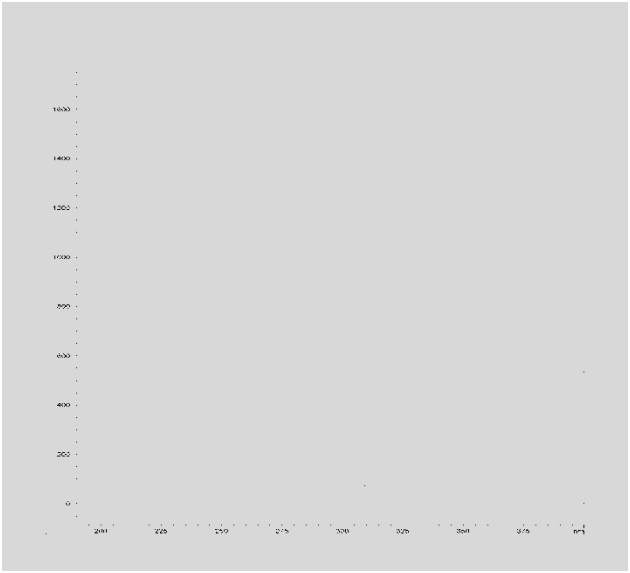
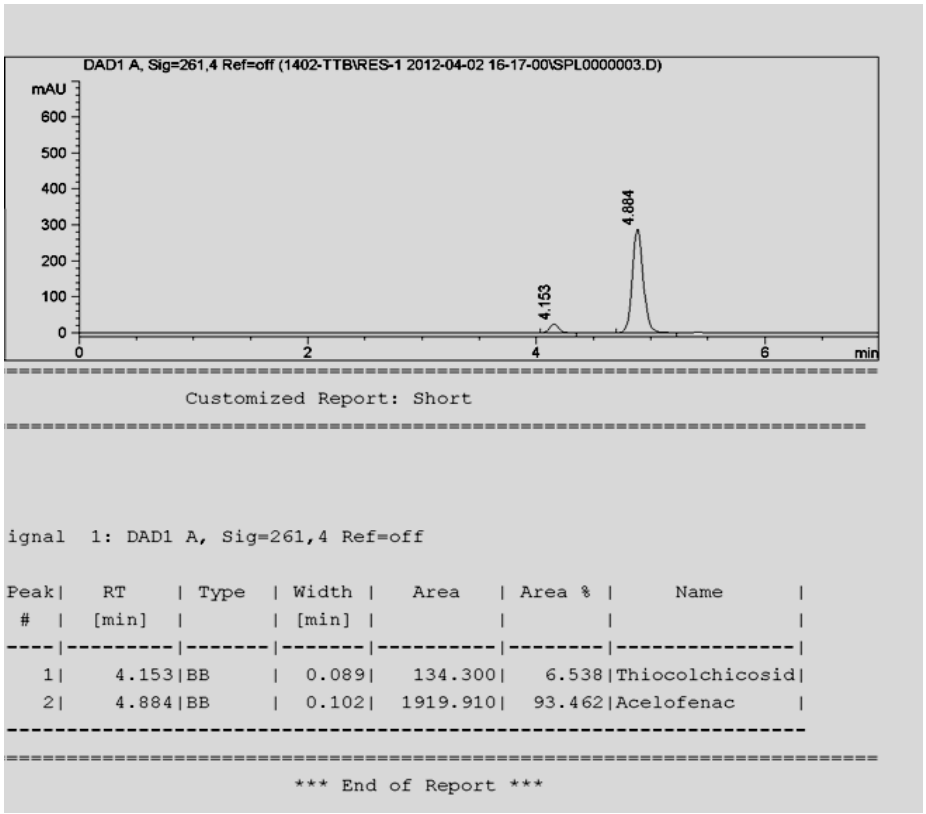


Figure 1: Overlain spectra of Aceclofenac and thiocolchicoside



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Peak #	RT [min]	Type	Width [min]	Area	Area %	Name
1	4.153	BB	0.089	134.300	6.538	Thiocolchicosid
2	4.884	BB	0.102	1919.910	93.462	Acelofenac

*** End of Report ***

Figure 2 : A Typical chromatogram of mixture of Aceclofenac and Thiocolchicoside.

RESULTS AND DISCUSSION

The results of analysis showed that the method was in precise and reproducible. Method validation parameters such as system suitability, precisions, accuracy, linearity, robustness,

solution Stability, etc. were ascertained.

METHOD VALIDATION

System suitability:

System performance parameters of developed HPLC method were determined by injecting standard solution in replicates. Parameters such as number of theoretical plates, tailing factor resolution and capacity factor were determined. The results are shown in **Table .1.** It indicated good performance of system.

Table 1: System performance parameters for Aceclofenac and Thiocolchicoside. (n = 6).

Drug Substances	Retention time	USP Tailing	No.of plates	Resolution factor
Aceclofenac	4.884	1.121	12369	-----
Thiocolchicoside	4.153	1.138	11874	3.94

Linearity

Under the experimental conditions described above, linear calibration curves for the two drugs were obtained throughout the concentration ranges studied. Regression analysis was done on the peak areas of the three drugs i.e.(Y) v/s concentration (X). The regression analysis data is represented in **Table 2**. The liner range of concentration was 25-125 µg/ml for aceclofenac and 1.0-6.0 µg/ml for thiocolchicoside.

Table 2: Linearity – regression analysis data

Parameters	Aceclofenac	Thiocolchicoside
Correlation Coefficient (r)	0.999	0.999
Intercept (y)	28.80	3.272
Slope (m)	18.88	33.53

Accuracy

Accuracy of the method was determined by applying the above method to synthetic mixtures. Synthetic mixtures contained 75%, 100%, and 125% of aceclofenac and thiocolchicoside of the label claim respectively. The accuracy was then calculated as the percentage of analyte recovered by the assay. The results of the analysis are given **in Table 3.**

Table 3: Accuracy - %Recovery of each analyte

Drug	level	Amount of drug added (mg)	Total amount of drug found (mg)	%Recovery
Acelofenac	75	75.09	77.11	102.78
	100	100.49	101.50	101.01
	125	125.34	127.47	101.58
	75	3.21	3.25	101.6
Thiocolchicoside	100	4.12	4.18	100.2
	125	4.84	4.89	101.32

** Average of triplicate analysis.*

Precision

The method precision was established by carrying out the analysis of tablets powder blend containing two drugs. The assay was carried out for the drugs using proposed analytical method in six replicates. The values of relative standard deviation were well within limits 0.28% and 4.18% for aceclofenac & thiocolchicoside respectively indicating the sample repeatability of the method. The results obtained are tabulated in **Table 4.**

Table 4: Precision – method precision.

Experiment No.	(%)Assay per Tablet	
	Aceclofenac	Thiocolchicoside
1	100.08	97.2
2	100.02	95.1
3	99.64	102.6
4	100.12	92.9
5	100.47	97.5
6	100.31	91.2
%RSD	0.28	4.18

Robustness

The robustness of the method is determined by small variation in method parameters.

The different variations are as given bellow:

Variation in flow rate by ± 0.2 ml/min.

Variation in pH by ± 0.2 units

Variation in wavelength ± 0.2 nm

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

Stability of solution

The stability of the solutions under study was established by keeping the solutions at room temperature for 24 hours. The results indicated no significant change in the assay results of the solutions. It confirmed the stability of the drug in the solvents used for the analysis.

Method application

The validated high performance liquid chromatographic method was applied to simultaneous determination of aceclofenac and thiocolchicoside. Twenty tablets were crushed into fine powder form and from such powder portion equivalent to 100.0 mg of aceclofenac and 4.0 mg of thiocolchicoside was weighed accurately. It was dissolved in 100 ml of diluent and further 10 ml of this solution was diluted in 100 ml of diluent to obtain 100 µg/ml of aceclofenac & 4.0 µg/ml of thiocolchicoside solution. From this solution 10 µl was injected into chromatograph under

specified conditions. The analyte peaks were identified by comparison with those of respective standards. The assay results expressed as mg/tablet are shown in Table 4. It indicates the amount of each drug in the product meets the requirement.

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 drugs to the pre-analyzed formulation and reanalyzing the mixture by proposed method. The percent recovery indicated non-interference from the excipients used in the formulations. Thus the proposed RP-HPLC method is novel method for the simultaneous estimation of aceclofenac and thiocolchicoside in combined dosage forms. It is precise, accurate, linear, robust, simple and rapid. Hence the proposed RP-HPLC method is strongly recommended for the quality control of formulations and dissolution studies.

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