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A NEW RP HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF ACECLOFENAC AND THIOCOLCHICOSIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT
A simple and selective LC method is described for the determination of Aceclofenac and Thiocolchicoside in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 0.1M ammonium acetate:Methanol with detection of 248 nm. Linearity was observed for Aceclofenac r^2 =0.994) and for Thiocolcicoside(r^2 =0.995) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.
Key Words: Aceclofenac, Thiocolchicoside, tablet dosage forms

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INTRODUCTION
Pharmaceutical analysis simply means analysis of pharmaceuticals. Webster' dictionary defines a pharmaceutical is a medical drug. A more appropriate term for a pharmaceutical is active pharmaceutical ingredient (API) or active ingredient to distinguish it from a formulated product or drug product is prepared by formulating a drug substance with inert ingredient (excipient) to prepare a drug product that is suitable for administration to patients. Research and development (R&D) play a very comprehensive role in new drug development and follow up activities to ensure that a new drug product meets the established standards is stable and continue to approved by regulatory authorities,assuring that all batches of drug product are made to the specific standards utilization of approved ingredients and production method becomes the responsibility of pharmaceutical analysts in the quality control (QC) or quality assurance department. The methods are generally developed in an analytical R&D department and transferred to QC or other departments as needed. At times they are transferred to other divisions.Chromatography is a family of analytical chemistry techniques for the separation of mixtures. It involves passing the sample, a mixture that contains the analyte, in the "mobile phase", often in a stream of solvent, through the "stationary phase." The stationary phase retards the passage of the components of the sample. When

components pass through the system at different rates they become separated in time, like runners in a marathon. Ideally, each component has a characteristic time of passage through the system. This is called its "retention time." A physical separation method in which the components of a mixture are separated by differences in their distribution between two phases, one of which is stationary (stationary phase) while the other (mobile phase) moves through it in a definite direction. The substances must interact with the stationary phase to be retained and separated by it.A chromatograph takes a chemical mixture carried by liquid or gas and separates it into its component parts as a result of differential distributions of the solutes as they flow around or over a stationary liquid or solid phase. Various techniques for the separation of complex mixtures rely on the differential affinities of substances for a gas or liquid mobile medium and for a stationary adsorbing medium through which they pass; such as paper, gelatin, or magnesium silicate gel. Analytical chromatography is used to determine the identity and concentration of molecules in a mixture. Preparative chromatography is used to purify larger quantities of a molecular species (1-5). Aim is to develop new RP HPLC method for the simultaneous estimation of Aceclofenac and Thiocolchicoside pharmaceutical dosage form.

MATERIALS AND METHODS

Determination Of Working Wavelength (λmax)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution of Aceclofenac

10 mg of Aceclofenac was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of Thiocolchicoside

10mg of Thiocolchicoside was weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of samples for Assay

Preparation of mixed standard solution

weigh accurately 100 mg of aceclofenacand 4mg of thiocolchicosidein 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase.From above stock solution 100 µg/ml of aceclofenacand 4 µg/ml thiocolchicosideis prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10 tablets (each tablet contains100 mg of aceclofenacand 4mg of thiocolchicoside) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of thiocolchicosideand aceclofenac (µg/ml) were prepared by dissolving weight equivalent to 4 mg of Thiocolchicosideand100 mg of aceclofenac and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and diluted to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 4µg/ml of thiocolchicosideand 100µg/ml of aceclofenac was made by adding 1 ml of stock solution to 10 ml of mobile phase (6-8).

RESULTS AND DISCUSSION

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra are shown in the fig-1. The isobestic point was found to be 248nm for the combination.

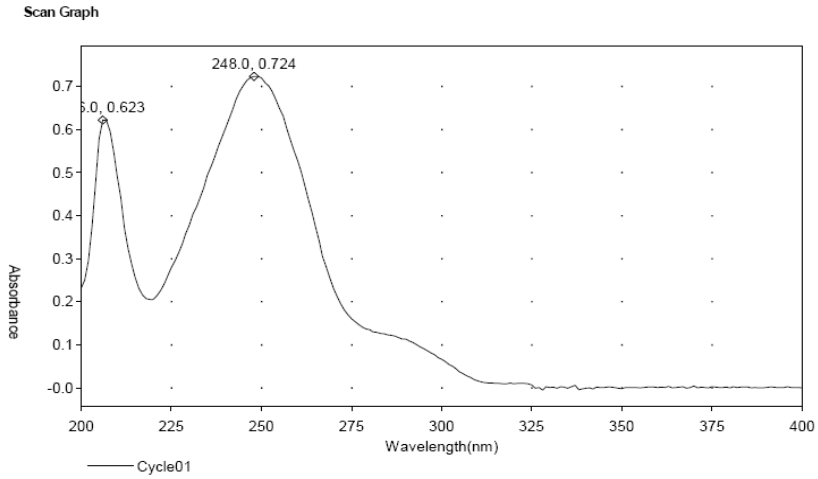

Fig-1 The Isobestic point was found to be 248nm for Aceclofenac and Thiocolchicosidein combination

The amount of aceclofenacand Thiocolchicosidepresent in the taken dosage form was found to be 100.912% and 100.842% respectively (Fig-2).

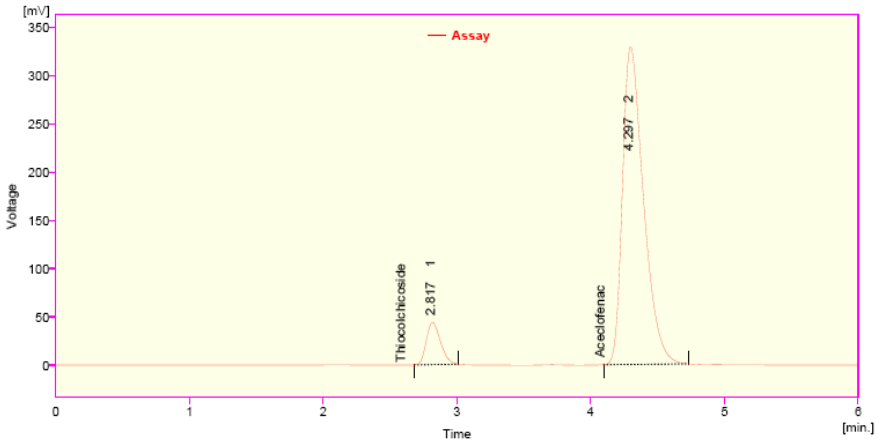

Fig-2 Chromatogram of Assay

The % RSD for the retention times and peak area of aceclofenacand thiocolchicosidewere found to be less than 2%. The plate count and tailing factor results were found to be satisfactory and are found to be within the limit.The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of aceclofenacand thiocolchicosideis 0.994 and 0.995. The relationship between the concentration of aceclofenacand thiocolchicosideand area of aceclofenacand thiocolchicosideis linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits (Table-1 and 2).

Table-1 Linearity of aceclofenac

S.No.	Conc.(µg/ml)	Area
1	60	2488.713
2	80	3252.121
3	100	3941.042
4	120	4690.891
5	140	5454.513

Table-2 Linearity of thiocolchicoside

S.No.	Conc.(µg/ml)	Area
1	3	231.895
2	4	312.713
3	5	385.139
4	6	446.353
5	7	540.788

The percentage mean recovery of aceclofenac and thiocolchicosideis 100.55% and 100.7% respectively.From the observation the between two analysts Assay values not greater than 2.0%, hence the method was rugged (Table-3).

Table-3 Results for Ruggedness

ACECLOFENAC	%Assay	THIOCOLCHICOSIDE	%Assay
Analyst 01	100.03	**Analyst 01**	109.98
Anaylst 02	97.76	**Anaylst 02**	99.96

CONCLUSION

The above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation Aceclofenac and Thiocolchicoside found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

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