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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF DIACEREIN AND ACECLOFENAC IN DOSAGE FORM

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ABSTRACT: A simple Reverse phase liquid chromatographic method has been developed and subsequently validated for simultaneous determination of Diacerein and Aceclofenac in combination. The separation was carried out using a mobile phase consisting of dipotassium hydrogen phosphate buffer of pH 4.5, Acetonitrile and methanol in the ratio of 40: 40: 20. The column used was Phenomenex Luna C18, 5µ, 250 mm × 4.6 mm id with flow rate of 1.0 ml / min using UV detection at 265 nm. The described method was linear over a concentration range of 80-120 µg/ml and 160-240 µg/ml for the assay of Diacerein and Aceclofenac respectively. The retention times of Diacerein and Aceclofenac were found to be 1.780, and 3.035 min respectively. Results of analysis were validated statistically. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Diacerein and Aceclofenac bulk drug and in its pharmaceutical dosage form.
Keywords: Diacerein and Aceclofenac.

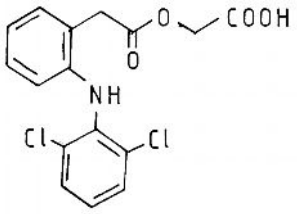
INTRODUCTION

Osteoarthritis (OA) is one of the most prevalent musculoskeletal conditions, which affect the joints. The degeneration of the cartilage that protects the ends of bones causes pain and inflammation [1]. Diacerein, (diacetylrhein; [4,5-bis (acetyloxy)-9,10-dihydro-9,10-dioxo-anthracene-2-carboxylic acid]; CAS no 13739-02-1) has been found to be effective in the treatment of OA as a synthetic chemical and also in native form from many plants. It is the pro-drug, which converts entirely to its main active metabolite, rhein (4, 5-dihydroxy-9, 10-dihydro-9,10-dioxo-anthracene-2-carboxylic acidbefore systemic absorption [2,3]. Rhein also occurs naturally in some plants. It belongs to the anthraquinone class of molecules. It is about 99% bound to plasma proteins. The primary route of elimination is through urine, where, 20% is removed in the native form, 60% as a glucoronide conjugate and 20% as sulfate.

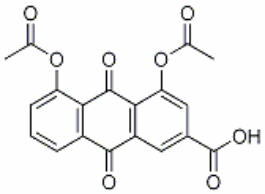
Aceclofenac, ([2-(2-6-dichlorophenyl) amino] phenyl acetoxy acetic acid; belongs to the class of non-steroidal anti inflammatory drugs (NSAIDs). It has pronounced anti-inflammatory, antipyretic, antirheumatoid and analgesic effect and an improved gastro-intestinal tolerance. It is well absorbed after oral administration and circulates mainly as unchanged drug. 70% of the administered dose is excreted in urine as gluconoride of aceclofenac and diclofenac [4].

This drug is also more than 99% bound to plasma proteins. Central Drug Standard Control Organization of India has approved a fixed dose combination formulation containing 50mg diacerein and 100mg aceclofenac for the treatment of OA. The

literature survey revealed that few methods have been reported for the estimation of Diacerein and Aceclofenac. So far, no method has been reported [5-10]. For estimation of Diacerein and Aceclofenac in combined dosage forms, hence we attempted to develop a simple, accurate, and economical analytical method. This paper describes validated RP-HPLC for simultaneous estimation of Diacerein and Aceclofenac in combination using phosphate buffer of pH 4.5 acetonitrile and methanol in the ratio of 40: 40: 20. The column used was Phenomenex Luna C-18 with flow rate of 1.0 ml /min using UV detection at 265 nm.



Diacerein



Aceclofenac

MATERIALS AND METHODS

Standard bulk drug sample Diacerein and Aceclofenac were provided by Micro Laboratories Ltd., Bangalore. Tablets of combined dosage form were procured from the local market. All other reagents used were of HPLC grade. HPLC (Shimadzu LC-20AT) method was developed using Phenomenex Luna C18, 5µ, 250 mm × 4.6 mm id. Mobile phase selected for this method contained 40 parts of phosphate buffer, 40 parts of acetonitrile and 20 parts of methanol adjusted to pH 4.5 with 0.1% orthophosphoric acid that was filtered through 0.45-micron membrane filter. Flow rate employed was 1.2 ml/min. Detection of eluent was carried out at 265 nm using UV detector. Method was developed. Standard stock solutions of pure drugs were made separately in mobile phase containing 80-120 µg /ml of Diacerein, and 160-240 µg /ml of Aceclofenac and filtered through a 0.45µ membrane filter. Each solution was injected and a chromatogram was recorded. Mean retention times Diacerein and Aceclofenac were found to be 1.780 and 3.035 min respectively.

ANALYSIS OF FORMULATION:

Twenty tablets of the formulation were weighed and the average weight per tablet was calculated. Twenty tablets were crushed and ground to a fine powder. Powder equivalent to 100 mg of Diacerein was weighed and transferred to a 100 ml volumetric flask. The tablet powder was dissolved in the mobile phase and filtered through a membrane filter (0.45µ). The sample solution was suitably diluted and used for the analysis. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solution was loaded in the 20 µl fixed - sample loop of the injection port. The solution was injected and a chromatogram was recorded. The injections were repeated six times and the peak areas were recorded. A representative chromatogram has been given in Figure-1. The peak area ratios of each of the drugs were calculated and the amount of each drug present per tablet was estimated from the respective calibration curves. The result of analysis reported in [Table – 1].

RESULTS AND DISCUSSION

The developed RP-HPLC method for simultaneous estimation of Diacerein and Aceclofenac from combined dosage form utilizing C18 column and 0.5 % phosphate buffer acetonitrile and methanol in the ratio of 40:40:20 as mobile phase. Detection of eluent was carried out using UV detector at 265 nm. The method was developed. The run time per sample is just 6 min. The excipients in the formulation did not interfere in the accurate estimation of Diacerein and Aceclofenac. The method was validated as per ICH guidelines in terms of linearity, accuracy, specificity, intraday and interday precision, repeatability of measurement of peak area as well as repeatability of sample application and the results are shown in [Table –2]. Since none of the methods is reported for simultaneous estimation of Diacerein and Aceclofenac from combined dosage form, this developed method can be used for routine analysis of two components in formulation.

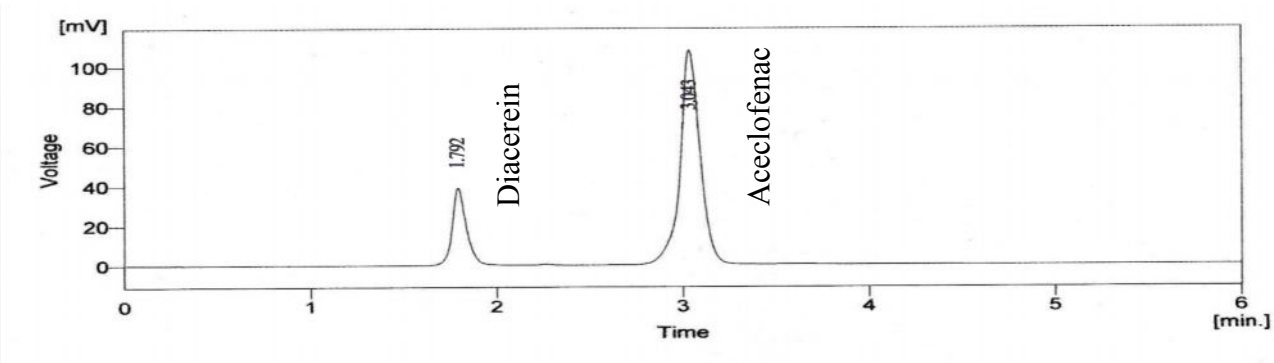
Table: 1 Analysis of formulation: DYACERIN -A
Label Claim: Diacerein 50 mg + Aceclofenac 100 mg

Drug	Std. wt (mg)	Sample wt (mg)	Avg. wt (mg)	LC (mg)	Std. Area	Sample Area	Amount Present (mg)	% Assay
Diacerein	100.4	473.6	239.5	50	207658	205680	50.22	100.44 %
Aceclofenac	201.0			100	823504	807892	99.47	99.47 %

Table: 2 Validation Parameters

	VALIDATION PARAMETER	DIACEREIN	ACECLOFENAC
S. Suitability	Theoretical Plates	3498	6460
	Tailing Factor	1.164	1
	Resolution	7.981	
Accuracy	% Recovery	98.74	99.28
Linearity	Co-eff. of variation	0.9992	0.9991
Precision	Intra day: % RSD	0.16	0.34
		0.27	0.22
Robustness	M.P.Ratio: % RSD	1.29	0.77
	F. RATE: % RSD	1.23	0.85

Fig.1: Chromatogram for Formulation



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