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VALIDATED ANALYTICAL METHOD DEVELOPMENT FOR DIACEREIN AND ACECLOFENAC IN COMBINED TABLET DOSAGE FORM BY RP-HPLC

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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, Acetonitrile and methanol in the ratio of 60: 40 %v/v. The column used was Phenomenex Luna C18 Column (150mm× 4.6 mm id 5μ) with flow rate of 1 ml / min using UV detection at 267 nm. The described method was linear over a concentration range 2-10 µg/ml and 20 μg/ml of 2for the assay of Diacerein and Aceclofenac respectively. The retention times of Diacerein and Aceclofenac were found to be 0.9927 and 0.88322 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 by RP-HPLC Method.

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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 acid before 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, antrheumatoid 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, Acetonitrile and methanol in the ratio of 60: 10: 30. The column used was Phenomenex Luna C-18 with flow rate of 1 ml /min using UV detection at 267 nm.

MATERIALS AND METHODS

Diacerein and Aceclofenac were obtained as a souvenir samples from Glen mark Pharma Ltd, Hyderabad. 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 AUX -220 Digital balance method was developed using Phenomenex Luna C18 Column x4.6 mm id 5µ 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 with 0.1% orthophosphoric acid that was filtered through 0.45-micron membrane filter. Flow rate employed was 1 ml/min. Detection of eluent was carried out at 267nm using UV detector. Method was developed. Standard stock solutions of pure drugs were made separately in mobile phase containing 1 to 10 µg/ml and 20µg/ 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 0.9927 and 0.8832 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 50 mg of Diacerein was weighed and transferred to a 50 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 No.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.

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 60:10:30 as mobile phase. Detection of eluent was carried out using UV detector at 267 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 Since none of the methods is reported for Validated Analytical method development for Diacerein and Aceclofenac combined tablet dosage form- by RP-HPLC, Method this developed method can be used for routine analysis of two components in formulation.

Table No.1: Analysis of Formulation (Dycerin-A)-RP-HPLC

Lable Claim: Diacerein 50mg+Aceclofenac 100mg

S.No	Drug	Sample No.	Labeled amount (mg/tab)	Amount found (mg)	Percentage obtained	Average (%) ± S.D	% R.S.D.
1	DIA	1	50	49.58	99.16	99.95±1.023	1.0235
		2	50	50.23	100.1		
		3	50	49.20	98.4		
		4	50	5.00	100.00		
		5	50	50.64	101.28		
		6	50	50.22	100.44		
2	ACE	1	100	299.62	99.58	98.72 ±0.538	0.5458
		2	100	298.81	98.91		
		3	100	297.07	98.14		
		4	100	297.46	98.14		
		5	100	301.13	98.74		
		6	100	295.17	98.78		

Table No.2: Recovery Analysis of Formulation (Dycerin-A) By RP - HPLC

S.No	Drug	Sample No.	Amount present (µg/ml)	Amount added (µg/ml)	Amount estimated* (µg/ml)	Amount recovered (µg/ml)	% Recovery	± S.D	% R.S.D	S.E.
1	DIA	1	2.05	2	4.078	2.028	101.4	±1.0050	0.9927	0.5802
		2	2.05	4	6.136	4.086	102.15			
		3	2.05	6	8.06	6.01	100.16			
2	ACE	1	4.01	2	6.033	2.02	101.00	±0.9023	0.8832	0.5209
		2	4.01	4	8.08	4.19	102.75			
		3	4.01	6	10.17	6.16	102.67			

Table No.3: System Suitability Parameters for the Optimized Chromatogram by RP-HPLC

S.No	Parameters	Diacerein	Aceclofenac
1	Tailing factor	1.29	1.25
2	Asymmetrical factor	1.48	1.41
3	Theoretical plates	5885	8192
4	Theoretical plate per unit Length	5885.43	8192.29
5	Resolution	Between DIA and ACE 10.88	

Table No.4: Optical Characteristics of Diacerein and Aceclofenac by RP - HPLC

S.No	Parameters	Diacerein	Aceclofenac
1	λ_{max} (nm)	267	267
2	Beers law limit (µg/ml)	2 -10	2 - 10
3	Correlation coefficient (r)	0.9997	0.9992
4	Regression équation (y=mx+c)	y= 766360.2905x + 68230.8254	y=507342.3334 x + 52266.7142
5	Slope (m)	766360.2905	325022.6429
6	Intercept (c)	68230.8254	37298.1746
7	LOD (µg/ml)	0.16068	0.02535
8	LOQ (µg/ml)	0.486923	0.07683
9	Standard Error	7590.6563	4872.3455

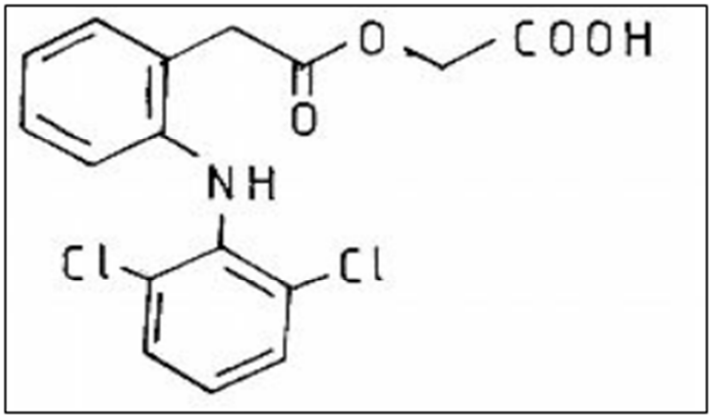


Figure No.1: Chemical Structure of Diacerin

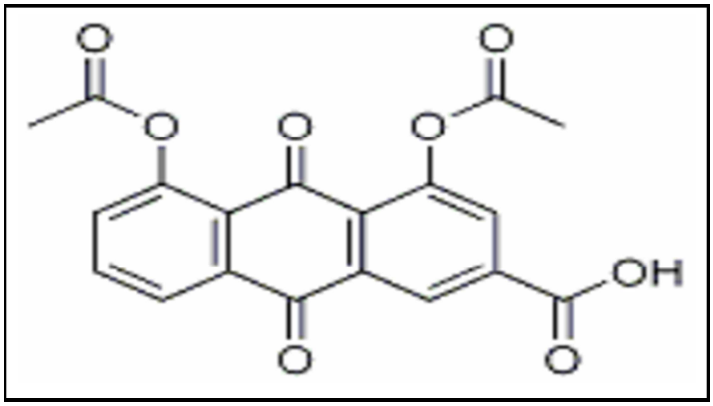
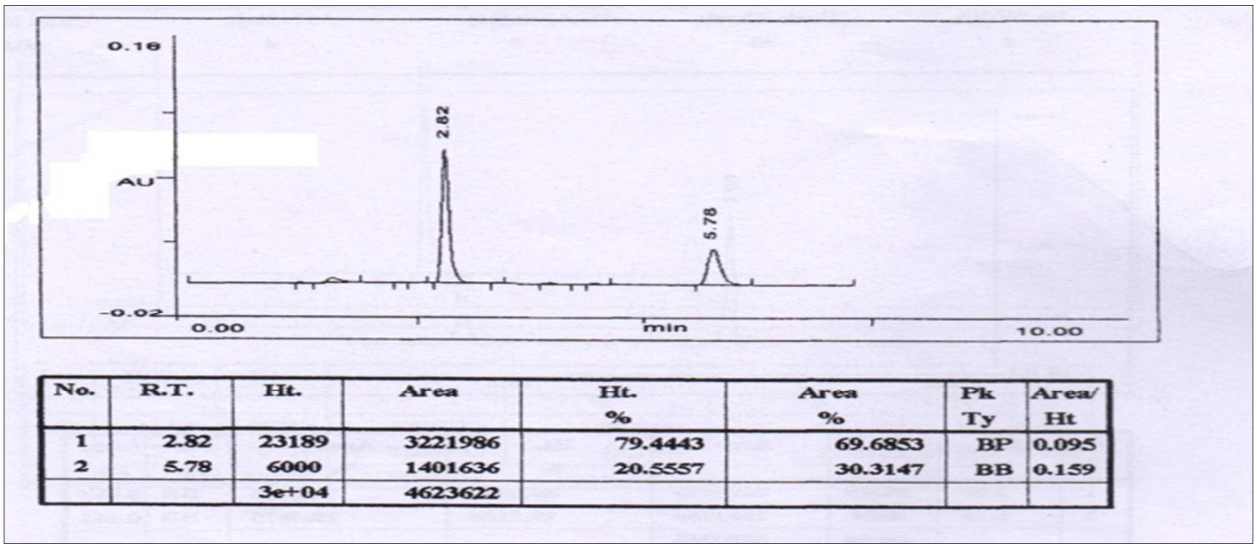
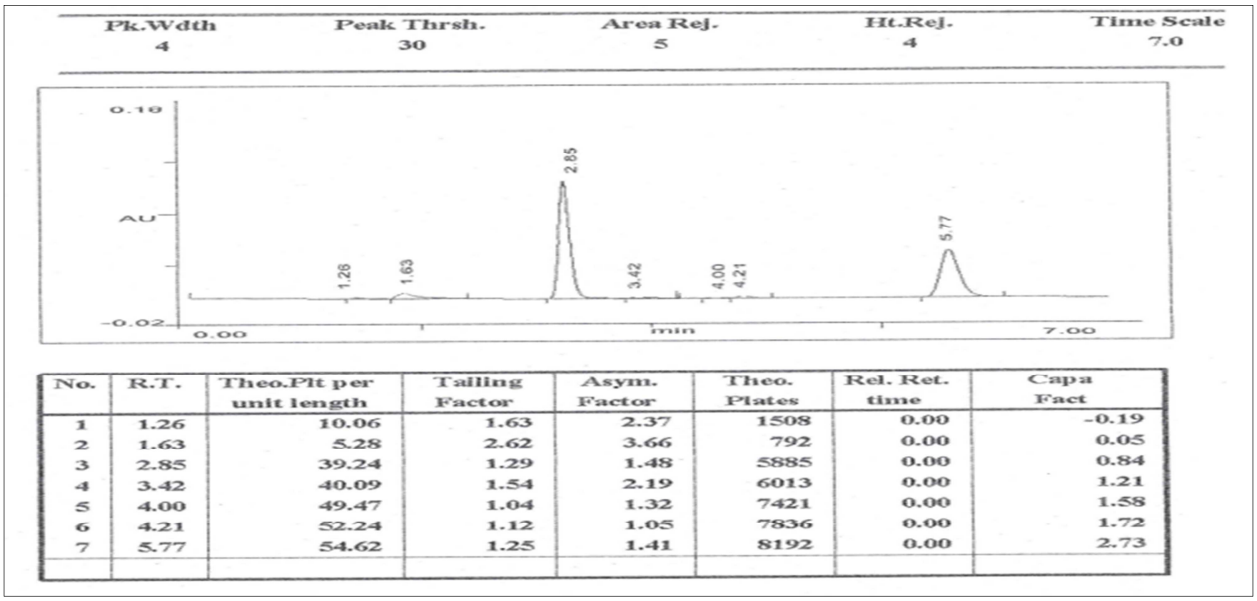


Figure No.2: Chemical Structure of Aceclofenac



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.82	23189	3221986	79.4443	69.6853	BP	0.095
2	5.78	6000	1401636	20.5557	30.3147	BB	0.159
		3e+04	4623622				

Figure No.3: Chromatogram for analysis of formulation - Dycerin-a



No.	R.T.	Theo.Plt per unit length	Tailing Factor	Asym. Factor	Theo. Plates	Rel. Ret. time	Capa Fact
1	1.26	10.06	1.63	2.37	1508	0.00	-0.19
2	1.63	5.28	2.62	3.66	792	0.00	0.05
3	2.85	39.24	1.29	1.48	5885	0.00	0.84
4	3.42	40.09	1.54	2.19	6013	0.00	1.21
5	4.00	49.47	1.04	1.32	7421	0.00	1.58
6	4.21	52.24	1.12	1.05	7836	0.00	1.72
7	5.77	54.62	1.25	1.41	8192	0.00	2.73

Figure No.4: Optimized Chromatogram for Diacerein and Aceclofenac Report

CONCLUSION

An exercise has been made for a simple, rapid, accurate and precise method for the estimation of Diacerein and Aceclofenac in pure form and in formulation by an isocratic RP – HPLC method. The optimization was done by changing the ratio of mobile phase. The ratio of mobile phase tried was Acetonitrile: 20mM potassium dihydrogen phosphate buffer pH 3 adj with H_3po_4 (50:50 %v/v), Acetonitrile: Methonal: 10mM potassium dihydrogen phosphate buffer pH 5 adj with H_3po_4 (60: 5: 35 %v/v), Acetonitrile: Methanol: Water (60: 10: 30, 50: 10: 40 % v/v), Acetonitrile: Water (60:40 pH 3 and pH 5 %v/v) and Acetonitrile: Water pH 3 (80: 20 % v/v) From this Acetonitrile: Water pH 3 (60: 40 % v/v)ratio was selected for further analysis. The retention time of Diacerein and Aceclofenac were found to be 2.82 and 5.79, respectively. The retention time of both drugs indicate that the drugs were separated with better resolution of 10.88.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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