ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Development and validation of a RP-HPLC method for simultaneous determination of diacerein and aceclofenac in tablet dosage form

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ABSTRACT

A simple reverse phase high-performance liquid chromatographic method has been developed for the simultaneous determination of diacerein and aceclofenac in tablets. Chromatographic separations of the two drugs were analyzed on a Phenomenex C_{18} column (250 × 4.60 mm, 5 µ). The mobile phase constituted of 0.01 M potassium dihydrogen phosphate and acetonitrile 60:40 (*v*/*v*) and pH adjusted to 4.5 using glacial acetic acid was delivered at the flow rate 2.0 mL min^{-1}. Detection was performed at 280 nm. The retention time of diacerein and aceclofenac was 3.61 and 6.28 min, respectively. Calibration curves were linear with coefficient correlation between 0.99 to 1.0 over a concentration range of 80-120 µg mL^{-1} of diacerein and aceclofenac. The relative standard deviation (R.S.D) was found to be < 2.0 %.

Key words: RP-HPLC, Diacerein, Aceclofenac, Tablet

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ISSN: 0975-8585

INTRODUCTION

Diacerein, 4, 5-diacetoxy-9,10-dihydro-9,10-di oxo-anthracene-2-carboxylic acid (Fig.1), is inhibit the stimulation of interleukin-1 beta production and production of nitrous oxide [1]. It also significantly reduces severity of pathological changes of osteoarthritis compared to placebo and increases the expression of transforming growth factor (TGF) - beta 1and TGF-beta 2, with, potential cartilage repairing properties. Diacerein does not alter renal or platelet cyclo-oxygenase activity and may therefore be tolerated by patients with prostaglandin-dependent renal function. Diacerein is involved liquid chromatographic-tandem mass spectrometry (LC-MS/MS), Flow Injection Chemiluminescence method and spectrophotometric method [2-4].

Acelofenac, [(2-[(2,6-Dichlorophenyl)amino]phenyl]acetyl]oxy] acetic acid (Fig.1), is directly blocks PGE 2 secretion at the site of inflammation by inhibiting IL-Beta & TNF in the inflammatory cells (Intracellular Action) [5]. According to literature aceclofenac in UV Spectrophotometric method and RP-HPLC [6-10] method is available. Although various analytical methods have been developed for the determination of diacerein and aceclofenac individually in tablets. There has been no report in literature on the simultaneous determination of diacerein and aceclofenac in tablets. The present work describes the development of validated RP-HPLC method, which can quantify these components simultaneously from a combined dosage form. The proposed RP-HPLC method was validated in accordance with ICH guidelines [11,12], by assessing its selectivity, linearity, accuracy, precision, and limits of detection and quantification.

EXPERIMENTAL

Solvents and Reagents

Tetra hydro furan (THF), Glacial acetic acid (GAA) and acetonitrile (ACN) of HPLC grade were from Merck (Darmstadt, Germany) with highest purity (>99.95 %). Triethylamine (TEA) was obtained from S.D fine chemical ltd, Mumbai. HPLC grade water collected from Qualigens Pvt. Ltd., Mumbai. Working standard of diacerein and aceclofenac was obtained from MMC Health Care Pvt Ltd, Badhi (H.P) and Baafna Pharmaceuticals Pvt Ltd, Chennai.

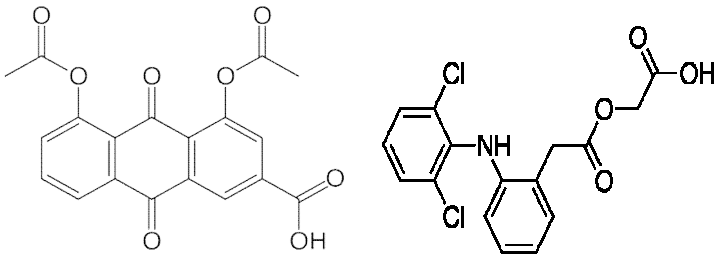
Chromatographic system and conditions

Chromatographic separation was performed with a Shimadzu (Japan) chromatograph equipped with an LC-10 AD *vp* solvent delivery module, an SPD 10A UV-VIS detector and a Rheodyne model 7125 injector valve with 20 µL sample loop. The column was used Phenomenex C_{18} (250 × 4.6 mm, 5 µ), the mobile phase consisting of a mixture of 0.01M Potassium dihydrogen phosphate: acetonitrile 60: 40 *v/v* adjusted the pH to 4.5 with glacial acetic acid, was delivered at a flow rate 2 ml min^{-1} with detection range performed at 280 nm. The mobile phase was filtered through a 0.45 µ membrane filter and sonicated. The injection volume was 20 µL analysis and was performed at 25ºC temperature. The equipment was controlled by PC workstation with shimadzu LC solution, Release 1.11SP1, chromatography software installed.

Sample Preparation

Stock solutions of diacerein and aceclofenac were prepared in HPLC grade tetra hydro furan (THF). The solutions were stored at 4º C until analysis. Series of standards of each of the substances were prepared by progressive dilution of stock solution. The solution was sonicated for 5 min, and diluted to 10 mL with THF; 20 µL was injected for chromatographic analysis.

ISSN: 0975-8585



A B

Fig.1: The chemical structures of diacerein (A), and aceclofenac (B)

RESULTS AND DISCUSSION

Method Development and optimization

Column chemistry, solvent selectivity, solvent strength, additive strength, detection wavelength, and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized so peak from the first-eluting compound did not interfere with those from the solvent, excipients, or plasma components. Other criteria, Viz. time required for analysis, appropriate κ range for eluted peaks, assay sensitivity, solvent noise, and use of the same solvent system for extraction of drug from formulation matrices during drug analysis, were also considered. After each change of mobile phase the column was re-equilibrated by passage of at least ten column volumes of the new mobile phase [13].

A set of columns of different length and particle size containing C_8, C_{18}, and RP-select B were tested. The final choice of the stationary phase giving satisfactory resolution and run time was the reversed-phase column Phenomenex C_{18} (250 × 4.6 mm 5µ). A series of different mobile phases containing methanol, water, buffer and actonitrile in different proportions were tried and finally 0.01 M Potassium dihydrogen phosphate: acetonitrile (60: 40 *v/v*) and adjusted the pH to 4.5 with glacial acetic acid was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for both diacerein and aceclofenac. All experiments were performed at 25º C. Elution was monitored over the whole UV range and 280 nm was selected for quantization because absorption by all the analytes was maximum at this wavelength (Fig.2-3).

Under the optimum chromatographic conditions, the retention times obtained for diacerein and aceclofenac were 3.613 and 6.280 min, respectively.

System Suitability Studies

The resolution, number of theoretical plates/meter and peak asymmetry were calculated for the standard solution. The resolution was more than 1.2, No. of theoretical plates were more than 5000/meter and peak asymmetry was less than 1.4 for both the drugs. The values were obtained and demonstrated the suitability of the system for the analysis of these drugs in combinations. A typical chromatogram of diacerein and aceclofenac is shown in Fig.4.

Method validation

The method was validated for selectivity, linearity, accuracy and precision as per the ICH guidelines.

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Linearity and Range

The result of the method was found to be linear in the range of 80 µg mL^{-1} to 120 µg mL^{-1} of diacerein and aceclofenac and peak areas recorded for all peaks and plotted as, peak *vs* concentration. Slope of diacerein and aceclofenac were 53.82 and 44.195, intercept of diacerein and aceclofenac was found to be 82.309 and 228.87, coefficient of correlation for diacerein was 0.9993 and aceclofenac was 0.9997.

Precision

The accuracy of the method was demonstrated by inter-day and intra-day variation studies. The intra-day studies performed at 5 repeated injections of standard and sample solutions were made in a day and the response of the factor of drug peaks and percentage RSD was calculated and found to < 0.213 % of diacerein and <0.266 % of aceclofenac. In the inter-day variation studies done at 5 repeated injections of standard and sample solutions were made on 3 consecutive days and the response of the factor of drug peaks and percentage RSD were calculated and found to < 0.213 % of diacerein and < 0.268 % of aceclofenac. The results obtained are listed in Table 1. The data obtained indicates that the developed RP-HPLC method is precise and reproducible, both during a single run and during different runs.

Accuracy

To ensure reliability and accuracy of the method recovery studies were carried out at three different levels. The results of recovery studies were presented in Table 2.

Solution stability

The standard solution prepared by using diacerein and aceclofenac pure as per the test method and injected initial and after 24 hours into the HPLC system. The solution stability parameters were evaluated and found to be within the limits. The deviations of the both initial and after 24 hrs 0.198 and 1.143 of diacerein and 0.048 and 3.099 of aceclofenac.

Robustness

Robustness of the method was determined by small deliberate changes in the experimental procedures. In the present method a deliberate change of flow rate and nanometer adjustment was made and the effects were noted. The method was found to be robust with respect to change in flow rate and nanometer.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) is the smallest concentration of the analyte that the measurable response. LOD was calculated using the following formula: LOD = 3.3σ / S. The LOD for diacerein and aceclofenac was found to be 11.40 µg mL^{-1} and 2.59 µg mL^{-1} respectively. The limit of Quantification (LOQ) is the smallest concentration of the analyte which gives response that can be accurately quantified. LOQ was calculated using the following formula: LOQ = 10σ / S. The LOQ for diacerein and aceclofenac was found to be 34.56 µg mL^{-1} and 7.87 µg mL^{-1} respectively.

ISSN: 0975-8585

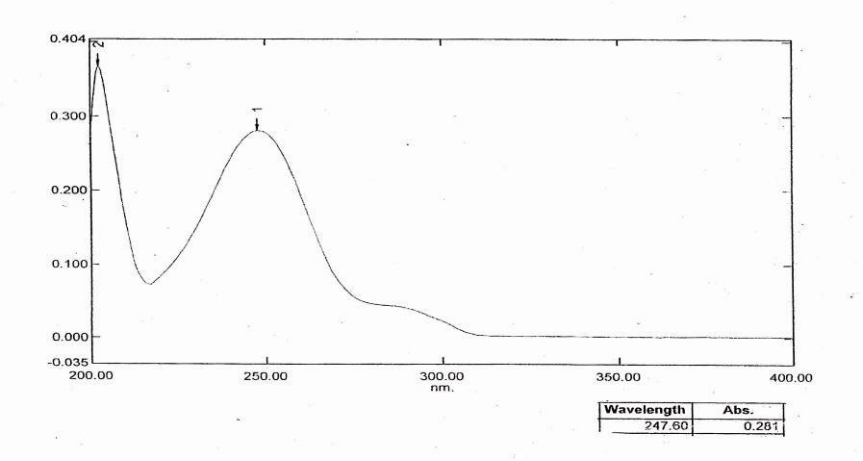


Fig.2. UV spectra of diacerein recorded under optimum HPLC condition

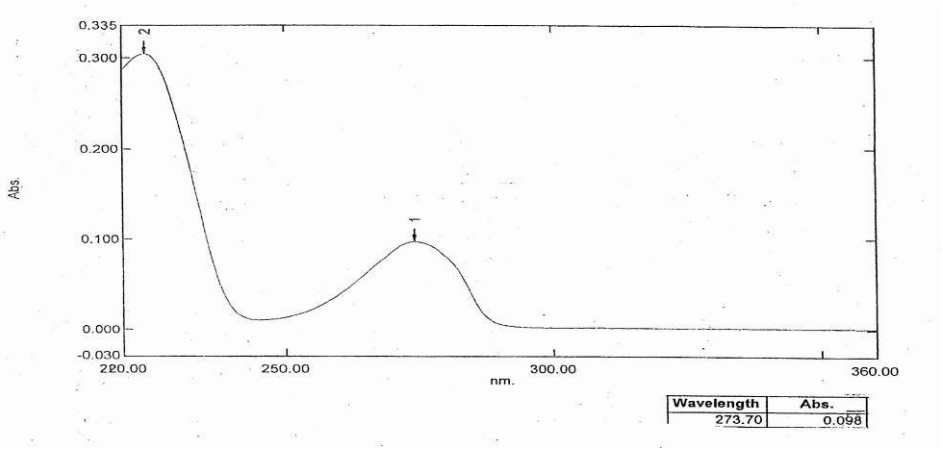
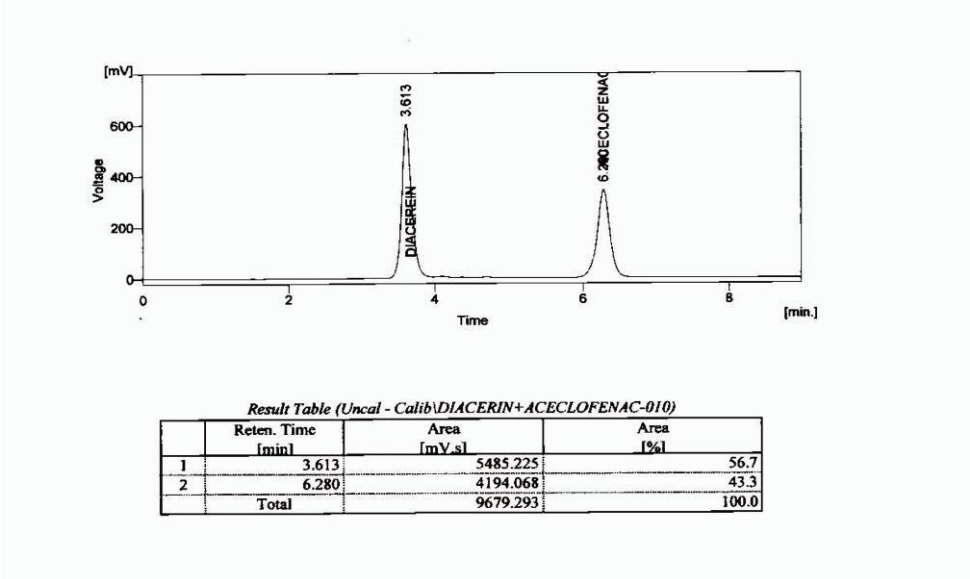


Fig.3. UV spectra of aceclofenac recorded under optimum HPLC condition



Result Table (Uncal - Calib\DIACERIN+ACECLOFENAC-010)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	3.613	5485.225	56.7
2	6.280	4194.068	43.3
	Total	9679.293	100.0

Fig.4. A typical chromatogram of diacerein (3.61 min) and aceclofenac (6.28 min)

ISSN: 0975-8585

Table 1. Result from method precision

Sample No	Area for Diacerein	Area for Aceclofenac	Test weight (mg)	Diacerein Assay		Aceclofenac Assay	
1	5488.389	4170.734	243.4	50.2	100.4	100.3	100.3
2	5473.364	4156.238	242.9	50.2	100.3	100.2	100.2
3	5488.493	4141.661	242.6	50.4	100.7	100.0	100.0
4	5481.961	4184.151	243.4	50.1	100.3	100.7	100.7
5	5465.866	4149.793	242.3	50.2	100.5	100.3	100.3
6	5482.407	4192.239	243.9	50.0	100.1	100.7	100.7
Average	5480.080	4165.803	-	50.2	100.4	100.4	100.4
RSD* (%)				0.213		0.268	

*Mean of six determinations

Table 2. Result from recovery studies

Sl. No	Spiking Level	Diacerin		Aceclofenac		Recovery (%)*	
		added (mg)	recovered (mg)	added (mg)	recovered (mg)	Diacerin	Aceclofenac
1	80%	40	40.4	80	79.6	101.1	99.5
2	100%	50	50.6	100	99.3	101.2	99.3
3	120%	60	59.8	120	121.7	99.6	101.4
% RSD*						0.0439	0.0502

*Mean of six determinations

CONCLUSION

A simple, reversed-phase HPLC method has been developed for the simultaneous estimation of diacerein and aceclofenac in tablets. The validation data indicative good precision, accuracy and reliability of the method. Hence the present RP-HPLC method used for routine analysis of the raw materials and formulations.

ACKNOWLEDGEMENT

The authors thank to the Management, Nandha College of Pharmacy, Erode, Tamil Nadu, for providing facilities for the research work. They also thank to MMC Health Care Pvt Ltd, Badhi (H.P) and Baafna Pharmaceuticals Pvt Ltd, Chennai, who kindly provided the gift sample of diacerein and aceclofenac.

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