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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF DIACEREIN CAPSULES BY USING RP-HPLC

M.Suresh babu*, K.Ayyappa Dhana Raju, U.Ram Satish, K.Ramesh, A.Malleswara Rao, P.G.S.Prakash

Department of Pharmaceutical Analysis, JITS College of Pharmacy, Kalagampudi, West Godavari, Andhra Pradesh, India

ABSTRACT

Aim is to develop a new RP-HPLC method for the estimation of Diacerein in pharmaceutical dosage form. Linearity was observed in the range 20-120 µg /ml for Diacerein (r^2 =0.996) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The above results reveal that method is linear. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation.

Key Words: Diacerein, pharmaceutical dosage form, RP-HPLC method

Author for correspondence
M.Suresh babu,
Department of Pharmaceutical Analysis,
JITS College of Pharmacy, Kalagampudi,
West Godavari, Andhra Pradesh, India
Email: sureshbabu3377@gmail.com,
Ph no- 7013737123

INTRODUCTION

High-performance liquid chromatography (HPLC) is the fastest growing analytical technique for analysis of drugs. Its simplicity, high specificity and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids. According to IUPAC, chromatography is a physical method of separation in which components will be separated or distributed between stationary and mobile phases. High Performance Liquid Chromatography (HPLC) is the term used to describe liquid chromatography in which the liquid mobile phase is forced through the column at high speed as a result, the analysis time is reduced by 1-2 orders of the Magnitude relative to classical column chromatography and the use of much smaller particles of the adsorbent or support becomes possible increasing the column efficiency substantially. The importance of chromatography is increasing rapidly in pharmaceutical analysis for the exact differentiation, selective identification and quantitative determination of structurally closely related compounds. Another important field of application of chromatographic methods is the purity testing of final products and the intermediates. Adsorption chromatography employs high-surface area particles that absorb the solute molecules. Usually a polar solid such as a silica gel, alumina or porous glass beads and a non-polar mobile phase such as heptane, octane or chloroform are used in adsorption chromatography. In adsorption chromatography, adsorption process is described by competition model and solvent interaction model. Competition model assumes that entire surface of the stationary phase is covered by mobile phase molecules as result of competition for absorption site. In solvent interaction model the retention results from the interaction of solute molecule with the second

layer of adsorbed mobile phase molecules. The differences in affinity of solutes for the surface of the stationary phase account for the separation achieved. In partition chromatography, the solid support is coated with a liquid stationary phase. The relative distribution of solutes between the two liquid phases determines the separation. The stationary phase can either be polar or non polar. If the stationary phase is polar and the mobile phase is non polar, it is called normal phase partition chromatography. If the opposite case holds, it is called reversed-phase partition chromatography. In normal phase mode, the polar molecule partition preferentially in to the stationary phase and are retained longer than non-polar compounds. In reverse phase partition chromatography, the opposite behavior is observed. In 1960s, chromatographers started modifying the polar nature of the silanol group by chemically reacting silicon with organic silanes. A large number of chemically bonded silica based stationary phases are available commercially. Silica based stationary phases are still more popular in reversed phase chromatography; however other adsorbents based on polymer (styrene divinyl benzene copolymer) are slowly gaining ground. The less water-soluble compounds are better retained by the reversed phase surface. The retention time decreases in the following order: Aliphatic > induced dipoles (E.g. CCl_4) > permanent dipoles (E.g. $CHCl_3$) > weak Lewis bases (Ethers, aldehydes, ketones) > strong Lewis bases (amines) > weak Lewis acids (alcohols, phenols) > strong Lewis acids (carboxylic acids). Also the retention increases as the number of carbon atoms increases. As a general rule the retention increases with an increase in the contact area between sample molecule and stationary phase i.e., with an increase in the number of water molecules, which are released during the adsorption of a compound. Branched chain compounds are eluted more rapidly than their corresponding normal isomers. In reversed phase system the strong attractive forces between water molecules arising from the 3-dimensional intermolecular hydrogen bonded network present in the structure of water must be distorted or disrupted when a solute is dissolved. Only higher polar or ionic solutes can interact with the water structure. Now polar solutes are squeezed out of the mobile phase and are relatively insoluble in it but with the hydrogen carbon moieties of the stationary phase. Chemically bonded octadecyl silane (ODS) and alkane with 18 carbon atoms is the most popular stationary phase used in pharmaceutical industry. Since most pharmaceutical compounds are polar and water soluble, the majority of HPLC methods used for quality assurance, decomposition studies, quantitative analysis of both bulk drugs and their formulations use ODS (1-3).

Diacerein is anti-inflammatory, analgesic and antipyretic drug. Antiosteoarthritic and cartilage-stimulating properties have been demonstrated *in-vitro* and in animal models. Diacerein and rhein have been shown to inhibit the production of interleukin-1 beta by human monocytes and the effects of the cytokine on chondrocytes *in vivo*. They exert chondroprotective effects in cultured articular cartilage and reduce severity of cartilage, bone, and synovial membrane damage in osteoarthritis. There appear to be some inhibitory effects on leucocyte migration and activation, contributing to the weak anti-inflammatory activity of the drug. Diacerein stimulate prostaglandin synthesis, especially PGF-2 alpha, a prostaglandin with cytoprotective effect on the gastric mucosa. Diacerein in therapeutic doses inhibits the stimulation of interleukin-1 beta production and production of nitrous oxide. It also significantly reduces severity of pathological changes of osteoarthritis compared to placebo and increases the expression of transforming growth factor (TGF)-beta1 and TGF-beta 2, with potential cartilage repairing properties. Oral bioavailability of Diacerein is 35% to 56%. Concurrent intake of food delays the time to peak concentration from 2.4 hours to 5.2 hours (p less than 0.05), but is associated with a 25% increase in absorption. Therefore, diacerein is best given with food. Total protein binding of rhein is about 99% to plasma albumin and in a lesser Percentage to lipoproteins and gamma-immunoglobulins. It achieves synovial fluid concentration of 0.3 to 3.0 milligrams/liter.

Only very few HPLC methods have been reported in the literature for the estimation of Diacerein present in pharmaceutical dosage forms. There are few reported methods for the determination of Diacerein by HPLC in pharmaceutical dosage forms. Hence the author has

made an attempt to develop a HPLC method as per ICH guidelines for the determination of Diacerein in pharmaceutical formulations.

MATERIALS AND METHODS

Preparation of standard solution (4-7)

Weighed 50.01 mg of diacerein standard in to a 100ml volumetric flask, dissolve in 50mi of diluent ml mix well trough sonication and make up to the volume with the diluent. Transfer 10ml volumetric flask and diluted to volume with diluent.

Preparation of test solution

Weigh about 261.5mg of diacerein test sample in to a 100ml volumetric flask, dissolve in 50mi of diluent ml mix well trough sonication and make up to the volume with the diluent. Transfer 10ml volumetric flask and diluted to volume with diluent. Ensure that the system meets required system suitability by injecting the standard solution three times. Perform the analysis by injecting sample solution twice at different intervals of initial day and another day.

Method Precision

Weighed 50.01 mg of diacerein standard in to a 100ml volumetric flask, dissolve in 50mi of diluent ml mix well trough sonication and make up to the volume with the diluent. Transfer 10ml into 100ml volumetric flask and diluted to volume with diluent. Separately inject diluent as blank once and suitability solution five times, calculate % RSD of diacerein peak.

RESULTS AND DISCUSSION

The objective of this experiment was to optimize the assay method for estimation of diacerein based on the literature survey made and the methods given in official pharmacopoeias. So here the trials mentioned describes how the optimization was done. The retention time and shape was good, hence this method was finalized for the estimation of diacerein (Fig-1).

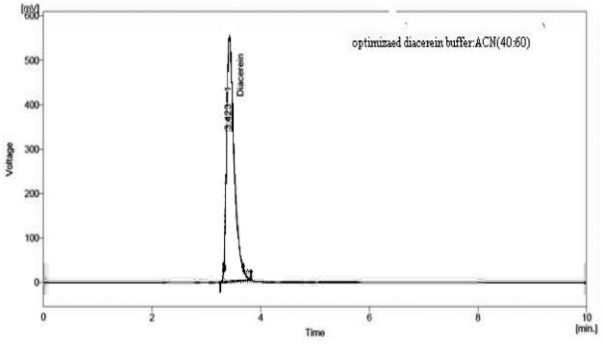

Fig-1 Optimized chromatography of Diacerein

Weighed 50.01 mg of diacerein standard in to a 100ml volumetric flask, dissolve in 50mi of diluent ml mix well trough sonication and make up to the volume with the diluent. The coefficient of linear regression should not be less than 0.999. The above results reveal that method is linear. As the results are within the acceptance limits 0f 98-102%. The % RSD for five replicate injections should not b more than 2.0 (Table-1).

Table-1 Assay of diacerein

S.No	Standard Rt	Standard area	Sample Rt	Sample area
1	3.427	5238.891	3.423	5355.753
2	3.423	5397.588	3.420	5418.734
MEAN	3.425	5316.74	3.4215	5387.2435
SD	0.002828	80.8485	0.002121	31.4905
%RSD	0.008258	1.520641	0.062	0.584538

The % RSD for five replicate injections should not b more than 2.0. The system meets the required system precision (Table-2).

Table-2 System Precision of Diacerein

Injection		RT	Area
Standard-1		3.437	5414.601
Standard-2		3.437	5429.090
Standard-3		3.437	5433.803
Standard-4		3.433	5436.684
Standard-5		3.430	5449.751
	Average	3.4348	5432786
	SD	0.003194	12.7381
	%RSD	0.092982	0.234351

The LOD for diacerein was found to be 2.0080. The LOD for diacerein was found to be 6.0851. The results are within the acceptance limit, the proposed method is found to be rugged (table-3).

Table-3 Robustness of Diacerein

Proposed variations		Retention time	Area
Variation in Flow rate	0.9 ml/min	3.697	4481.919
	1.5ml/min	3.137	3828.803
Viriation in wavelenth	250nm	3.393	36940.604
	258nm	3.390	3840.848

CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of Diacerein. The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, the method can be easily and conveniently adopted for routine analysis of Diacerein.

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