

## NOVEL AND VALIDATED SPECTROPHOTOMETRIC ESTIMATION OF DIACEREIN IN BULK AND CAPSULE FORMULATION USING MIXED HYDROTROPIC SOLUBILISATION APPROACH

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### ABSTRACT

In the present investigation, a poorly water-soluble drug, diacerein has been solubilized using hydrotropic agents containing sodium citrate (1, 3, 5 mM), sodium acetate (1, 3, 5 mM), urea (1, 3, 5 mM), sodium salicylate (1, 3, 5 mM) and sodium benzoate (1, 3, 5 mM) for the development of spectrophotometric analysis precluding the use of organic solvents. There was significant synergistic effect on enhancement in solubility of poorly water-soluble drug Diacerein by mixing two hydrotropic agents. Preliminary solubility studies revealed that 5mM sodium citrate solution and 3mM sodium acetate solution showed improvement in solubility over plain diacerein in water. 10 ml of 5mM sodium citrate solution and 10 ml of 3mM sodium acetate solution was mixed to observe the solubility of diacerein, it is carried out to study mixed hydrotropic solubilisation technique. The mixed hydrotropic solution was used to develop and validate UV visible spectroscopic method for bulk drug and marketed formulations. The method was validated statistically by low values of statistical parameters viz. standard deviation, percent coefficient of variation and standard error. The developed method is new, accurate, simple and economic.

**Keywords:** Mixed hydrotrophy, Diacerein, Solubility enhancement.

### INTRODUCTION

Therapeutic effectiveness of a drug depends upon the bioavailability and ultimately upon the solubility of drug molecules. Solubility is one of the important parameter to achieve desired concentration of drug in systemic circulation for pharmacological response to be shown. Currently only 8% of new drug candidates have both high solubility and permeability<sup>1-3</sup>. Hydrotrophy is a solubilisation process whereby addition of a large amount of second solute results in an increase in the aqueous solubility of another solute. The mechanism by which it improves solubility is more closely related to complexation involving a weak interaction between the hydrotropic agents like sodium benzoate, sodium acetate, sodium citrate, sodium salicylate, sodium ascorbate, sodium alginate, urea, niacinamide and the poorly soluble drug<sup>4-22</sup>.

Diacerein, chemically, 4, 5-diacetoxy-9, 10-dioxo-9, 10-dihydroanthracene-2-carboxylic acid is a chondroprotective agent used for the treatment of osteoarthritis. Literature survey revealed that several analytical methods like HPLC method, HPTLC method, LC-MS method and spectroscopic methods have been reported. In various spectroscopic determination which require use of costlier and toxic organic solvents like Dimethyl sulfoxide(DMSO), Dimethyl formamide, Acetonitrile, Methanol, Acetone etc<sup>23-29</sup>.

### MATERIALS AND METHODS

Diacerein was obtained as a gift sample from Glenmark pharmaceuticals, Mumbai. All other reagents used were of analytical grade. Commercial capsules of Diacerein (Orcerin, Macleods pharmaceuticals ltd) were procured from local market. A Shimadzu UV-spectrophotometer (UV-1800) with 1-cm match quartz cell was used for spectrophotometric analysis.

#### Preparation of calibration curve in water

Accurately weighed 10mg of Diacerein was added in 50 ml of volumetric flask, dissolved in organic solvent Dimethyl sulfoxide (DMSO) and volume was made with the DMSO. This stock solution was further diluted with water in concentration range of 2-14µg/ml. Absorbances were noted using UV-spectroscopy. The linear correlation was obtained between absorbance and concentration (Fig.1), which obeys Beer-Lambert's law.

#### Preliminary solubility study of drug

Determination of solubilities of the Diacerein in sodium citrate, sodium acetate, sodium citrate with sodium acetate in mMolar ratio

of 1, 3, 5 respectively and compared with distilled water. The study was carried out at room temperature. Excess amount of drug was added to screw capped 30 ml glass vials containing various concentration of hydrotropic solutions and distilled water. The vials were shaken mechanically for 12 hours at room temperature in rotary flask shaker. The solutions were allowed to equilibrate for 24 hours and centrifuged for 5 min at 2000 rpm. The supernatant of each vial were filtered separately through whatmann filter paper #41. Filtrates were diluted with distilled water and absorbance of each solutions were noted (Fig. 2).

#### Preparation of Calibration Curve

Accurately weighed 10 mg of Diacerein was added in 50 ml of volumetric flask, containing 10 ml of sodium citrate (5mM) and 10 ml of sodium acetate (3mM) solutions. The flask was then shaken for 10 minutes to solubilize the Diacerein and the volume was made up to 50ml with distilled water to get standard solution containing 200 µg/ml of Diacerein. This stock solution was divided into two parts A and B. Part A was kept at Room Temperature for 48 hrs to check its chemical stability and precipitation. Part B was further diluted with distilled water to get various dilutions containing 2, 4, 6, 8, 10 and 12 µg/ml of drug. Absorbances were recorded at 257.8 nm, against distilled water as blank (Fig. 3). The graph was constructed between absorbance and concentration. It obeys Beer Lambert's law within the concentration range of 2-12 µg/ml.

#### Analysis of Diacerein bulk drug by proposed method

Accurately weighed 10 mg of Diacerein was taken in a 50 ml of volumetric flask. 10 ml of each sodium citrate (5mM) and sodium acetate (3mM) solutions were added. The flask was shaken for 10 minutes to solubilize the drug and volume was made up to the mark with distilled water. It was filtered through whatmann filter paper #41. Filtered extract was appropriately diluted with distilled water and absorbance was noted at 257.8 nm and drug content was calculated.

#### Analysis of Diacerein in capsule by proposed method

Twenty capsules of Diacerein were emptied and weighed. Powder equivalent to 10 mg of Diacerein was taken in 50ml volumetric flask. 10ml of each sodium citrate (5 mM) and sodium acetate (3 mM) solutions were added. The flask was shaken properly for 10 minutes to solubilize the drug; the volume was made up to the mark with distilled water. It was filtered through whatmann filter paper #41. Filtered extract was appropriately diluted with distilled water and

absorbance was recorded at 257.8 nm and drug content was determined.

#### Recovery study

To evaluate the validity and reproducibility of the proposed method, recovery experiments were carried out. Recovery study was carried out as per ICH norms at three different concentration levels – 80%, 100% and 120% by replicate analysis. Here to a pre-analyzed capsule solution, standard drug solutions were added as a spiked concentration and drug contents were determined by proposed analytical method.

#### Validation of developed method

The method was validated statistically as per the ICH/USP guidelines for parameters like accuracy, precision, specificity, LOD, LOQ, ruggedness, linearity and range.

#### Accuracy

Accuracy of the developed method was confirmed by performing recovery study as per ICH norms at three different concentration levels- 80%, 100%, and 120% by replicate analysis (n=3). Here to a pre analysed capsule solution, standard drug solutions were added and the percentage of drug content was calculated.

#### Precision

Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicates the precision under the same operating conditions over a short interval of time and inter-assay precision. The standard deviation (SD), coefficient of variation was calculated. Repeatability was performed for six times with capsule formulation. The results of statistical evaluation are given in Table 2. Intermediate precision was studied as intra-day and inter-day variations. Intra-day precision was determined by analysing 6, 8 and 10 µg/ml of Diacerein for three times in the same day. Interday precision was determined by analysing the same concentration range of solutions daily for three days, results were recorded.

#### Specificity

The specificity of the method was checked for the interferences of impurities in the analysis of a drug solution.

#### Linearity and Range

Appropriate dilutions of standard stock solutions were analysed as per the developed methods. The Beer-Lambert's concentration range was found to be 2-12 µg/ml for Diacerein.

#### LOD and LOQ

As per ICH guidelines, limit of detection (LOD) and limit of quantitation (LOQ) were calculated as  $3.3\sigma/s$  and  $10\sigma/s$ , where  $\sigma$  is the SD of the response and  $s$  is the slope of the calibration curve. LOD is the smallest concentration of the analyte that gives a measurable response. The LOQ is the smallest concentration of the analyte which gives response that can be accurately quantified.

#### Ruggedness

The ruggedness of the method was determined by carrying out the experiments on different instruments and by different operators.

#### RESULTS AND DISCUSSION

Results of solubility studies indicate that, the enhancement in the solubility of Diacerein was increased in 5mM sodium citrate and 3mM sodium acetate solution as compared to its solubility in distilled water and other hydrotropic agents. Therefore, these two hydrotropic solutions were selected to see the synergistic effect on enhancement in solubility of Diacerein.

Part A solution of drug in hydrotropic solution was kept at room temperature for 48 hrs. There was no precipitation of drug in Part A solution within 48 hrs. In addition, drug contents of Part A solutions (after 48 hrs) were same as those of Part B solutions (fresh solutions). This study reveals that the estimations can be done within 48 hrs at least, without having any detrimental effect on drug stability.

As evident from Table 1, the mean percent Diacerein estimated in bulk drug sample by proposed method is 99.4067 %. The result of analysis by proposed method is very close to 100.0 %, indicating the accuracy of the proposed method. In the case of the proposed analytical method, the results are validated by low values of standard deviation (0.8286) and percent coefficient of variation (0.8336) (Table 1). It is evident from Table 2 that the value of mean percent drug estimated by proposed spectrophotometric method for capsule formulation is 99.1667 %. The amount of drug estimated, by the proposed method for capsule formulation is very close to 100.0, indicating the accuracy of the proposed method of analysis. Low values of standard deviation and percent coefficient of variation further validated the proposed method (Table 2). The percent recoveries estimated ranged from 98.42 % to 99.59 %. The values are close to 100 % indicating the accuracy of proposed method. The values of standard deviation and percent coefficient of variation are statistically low thus validating proposed method (Table 3).

**Table 1: Analysis data of Diacerein bulk drug sample with statistical evaluation**

S. No.	Amount of drug taken (mg)	% drug estimated* (mean ± SD)	% coefficient of Variation
1	10	99.4067 ± 0.8286	0.8336

\* Average of six determinations

**Table 2: Analysis data of commercial capsule of diacerein**

S. No.	Labelled claim (mg/ capsule)	% labelled claim estimated* (mean ± SD)	% coefficient of Variation
1	50	99.1667 ± 0.8210	0.8279

\* Average of six determinations

**Table 3: Recovery study for spiked concentration of diacerein added to preanalysed capsule formulation**

S. No.	Amount of drug in preanalyzed capsule powder (mg/ capsule)	Recovery level (added amount in %)	Percent recovery ± SD *	% coefficient of variation
1	50	80	99.4067 ± .7518	0.7563
2	50	100	99.5933 ± .8334	0.8369
3	50	120	98.42 ± .7136	0.7250

\* Average of six determinations

Table 4: Summary of optical characteristics and validation parameters

S. No.	Parameters	Acceptance criteria	Result
1	$\lambda_{max}$	-	257.8nm
2	Beer's law Range	-	2-12 $\mu\text{g/ml}$
3	Linearity	$r^2 = 0.995$ to $1.0$	0.9998
4	Specificity	No interference with placebo or impurity	Specific
5	Accuracy (Recovery study)	98.0-102.0 %	99.14 %
6	Precision	RSD not more than 2.0%	
	Intraday		0.27654 %
	Interday		0.2556 %
7	LOD	-	0.06226 $\mu\text{g/ml}$
8	LOQ	-	0.1887 $\mu\text{g/ml}$
9	Slope	-	0.106

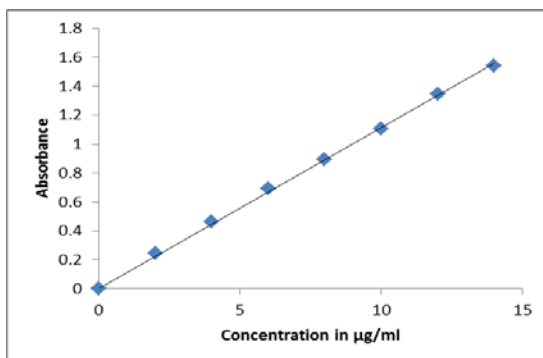


Fig. 1: Calibration curve of Diacerein in water

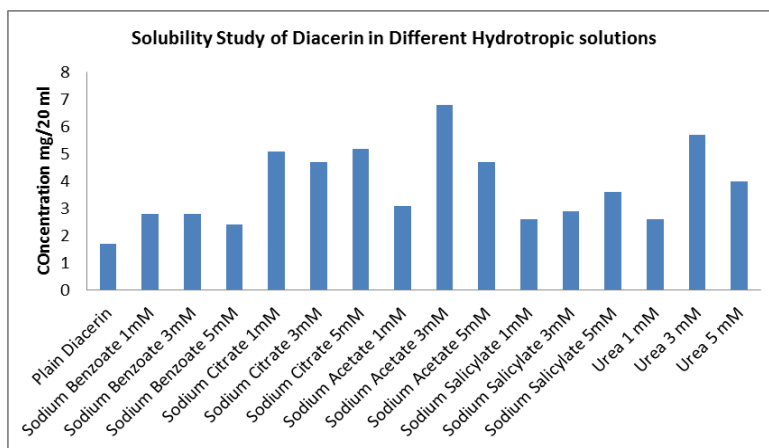


Fig. 2: Solubility study of Diacerein in different Hydrotrophic solution

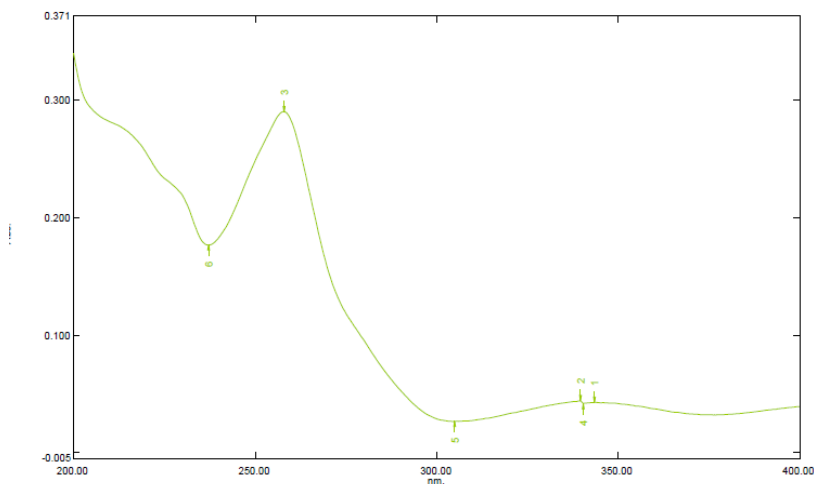


Fig. 3: UV-spectra of Diacerein in Sodium Citrate (5mM)

## CONCLUSION

It is concluded that the proposed method is new, simple, accurate, reproducible, eco- friendly and inexpensive. Advantage of this analytical method is that the organic solvents are precluded but not at the expense of accuracy. The proposed method can be successfully employed in the routine analysis of Diacerein in bulk drug sample as well as capsule dosage form. There is a good scope for other poorly soluble drugs which may be tried to get solubilized by suitable hydrotropic agents to carry out spectrophotometric analysis precluding use of costlier and unsafe organic-solvents. Mixed hydrotropy may find wide use in development of aqueous formulations of poorly water soluble drugs in future.

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