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RESEARCH ARTICLE

Estimation of Diacerein using hydrotropic agent and dye drug reaction

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ABSTRACT

This study was designed to develop and validate hydrotropic agent and dye drug reaction methods for estimation of diacerein in capsule formulation. Hydrotropic solubilization is a technique used to increase the aqueous solubility of poorly water-soluble drugs and the present study was aimed at developing a hydrotropic technique to increase the solubility of diacerein. The analysis of tablets indicated good correlation between the amounts estimated and label claim. The study results indicate good sensitivity of the proposed method. The results of analysis were validated statistically and by recovery studies. The developed methods were validated according to ICH guidelines and values of accuracy, precision and other statistical analysis were found to be in good accordance with the prescribed values. Thus the proposed method was found to be simple, environmentally friendly, accurate and cost effective and can be successfully employed in routine analysis of diacerein in capsule.

Keywords: Hydrotropic Solubilizing Agents, Diacerein, Dye Drug Reaction, ICH, validation

Introduction

Various techniques have been employed to enhance the aqueous solubility of poorly water-soluble drugs. Hydrotropic solubilization is one such method. Hydrotropes are a class of chemical compounds which affect an increased aqueous solubility by several folds to certain solutes which are sparingly soluble in water under normal conditions. Increasing the aqueous solubility of insoluble and slightly soluble drugs is of major importance and hydrotropy can be considered as a potential and industrially attractive technique because of easy recovery of dissolved solute and possible reuse of hydrotrope solutions [1-3].

Diacerein also known as diacetylrhein, is a drug used in the treatment of steoarthritis [4]. Diacerein is the drug to be proved as disease modifying agent. Diacerein [4,5- bis[acetyloxy]-9,10-dioxo-2–anthracene carboxylic acid] is an anthracine derivative. It is converted to active metabolite "Rhein" which has anti inflammatory effects through inhibition of interleukin-1B. It reduces the fibrinolytic synovial fibroblasts. It also dose-dependently inhibits chemotaxis and super oxide anion production. It consequently reduces collagenase production in the intraarticular cartilage which spontaneously occurs in the body during destructive inflammation [5].

The spectrophotometric methods available for diacerein in literature reveal the use of dimethyl acetamide (DMA) [6] and dimethyl sulfoxide (DMSO) [7] to solubilize diacerein. Drawbacks of organic solvents include their higher cost, toxicity, and pollution. In addition, diacerein is poorly soluble in water. Therefore, hydrotropic solution may be a suitable alternative to exclude the use of organic solvents. Special systems are required to solubilize poorly water-soluble drugs.

Therefore, the objective of the present investigation was to develop simple, precise, and accurate methods for determination of diacerein in the capsule dosage form using hydrotropic solubilizing agents and dye drug reaction.

Materials and Methods

Reagents and standards

Reference standard of *diacerein* was a generous gift from scan research laboratories, Bhopal (M.P.), Sodium acetate obtained from Merck Chemical Division, Mumbai. Commercial formulation of *diacerein* was procured from the local drug market. Label claim of *diacerein* in capsule is 50 mg. Reverse osmosis water was used throughout the study. All chemicals used in the study were of analytical grade.

Instrument

The proposed work was carried out on a Labindia UV-Visible Spectrophotometer (Model: 3000+), which possesses a double beam double detector configuration with matched 1 cm quartz cells. Electronic balance (Wencer) was used for weighing purpose in the experimental. Sonicator was used for sonication of drug.

Solubility

Solubility of diacerein was determined at 25±1°C. Accurately weighed 10 mg NAP diacerein was added in different 10 ml volumetric flask containing different solvent and placed at mechanical shaker for 8 hrs. After 8 hrs filter both solution were filtered through whatman filter paper No. 41. The filtrates were diluted suitably and analyzed spectrophotometrically against water.

The developed methods were validated as per the ICH guidelines [8-10].

Method I

Selection of solvent system

Diacerein was scanned in various hydrotropic agents in the spectrum mode over the UV range (200-400) and 2M sodium acetate was found to be most appropriate because drug was soluble in it (50%), drug was stable in it, both drugs exhibit good spectral characteristics in it and sodium acetate solution has no interference with the λ_{max} of drugs.

Establishment of stability profile

Stability of drug was observed by dissolving diacerein in sodium acetate (2M) solution used as solvent. Solution of daicerine was prepared in the conc. of 5 μg/ml and scanned under time scan for 30 min. Spectra of drug under time scan shows that the drug is stable in hydrotropic solution.

Preparation of standard stock solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 80`mL mixed hydrotropic solution containing 2M sodium acetate and the flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark with mixed hydrotropic agent to get a concentration of 1000 μg/ml (Stock-A) for drug.

Preparation of sub stock solution (Stock-B)

Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution A of diacerein and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with RO Water that gave concentration of 100 μg/ml (Stock-B).

Preparation of working standard solution

Aliquots of 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml and 0.5 ml withdrawn with help of pipette from standard stock solution (Stock-B) separately in 10 ml volumetric flask and volume was made up to 10 ml with RO water. This gave the solutions of 1 μg/ml, 2 μg/ml, 3 μg/ml, 4 μg/ml and 5 μg/ml respectively for diacerein.

Selection of wavelength for linearity

Solution of 50 μg/ml of diacerein was prepared and the solution was scanned in the spectrum mode from 200 nm to 400 nm. The maximum absorbance of diacerin was observed at 228.0 nm. Diacerein showed linearity in the concentration range of 1-5 μg/ml at their respective maxim. Calibration curve was plotted, absorbance versus concentration (Figure 1, 2).

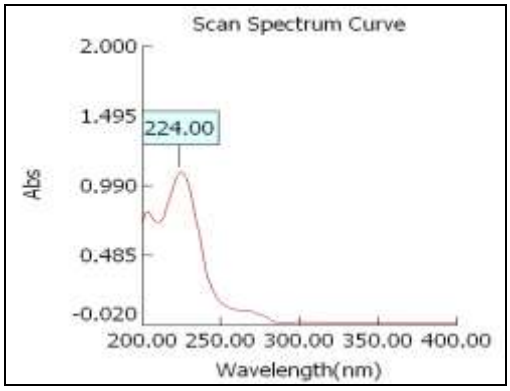

Fig 1. Determination of λ_{max} of diacerin

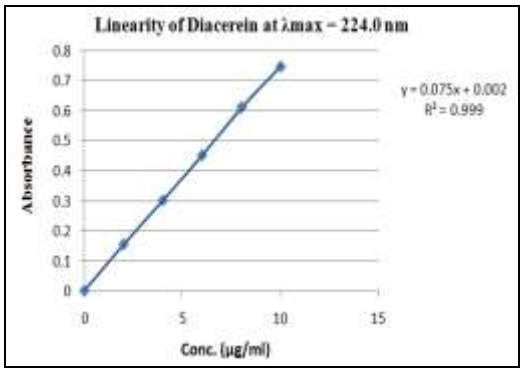

Fig 2. Calibration curve of diacerin

Validation of method

Linearity

Linearity of drug was established by response ratios of drugs. Response-ratio of diacerin was calculated by dividing the absorbance with respective concentration. Then a graph was plotted between concentration and response ratio.

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100%, 120%. The recovery studies were carried out by adding known amount of standard solution of diacerein to preanalysed capsule solutions. The resulting solutions were then re-analysed by proposed methods. Whole analysis procedure was repeated to find out the recovery of the added drug sample. This recovery analysis was repeated at 3 replicate of 5 concentrations levels.

Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times. Day to day was performed by analyzing 5 different concentration of the drug for three days in a week. The results are shown in tables.

Robustness

Robustness of the analytical method was performed by changing the concentration and ratio of hydrotropic solution for check the analytical methods capacity to remain unchanged. For the robustness of the analytical method we changed the ratio of hydrotropic solution. Instead the 2M ratios of sodium acetate, 1.8M sodium acetate used as solvent.

Analysis of capsule sample

Twenty marketed capsules of DIA was weighed and ground to a fine powder; amount equal to 50 mg of DIA was taken in 10 ml volumetric flask. The DIA present in this amount of capsule powder was 50 mg. Then 4 ml of sodium acetate solution was added and the flask was sonicated for about 10 min to solubilize the drug present in capsule powder and the volume was made up to

the mark with hydrotropic solution. After sonication filtration was done through Whatman filter paper No. 41. Filtrate was collected and further diluted with RO water to get the final concentrations of both drugs in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from simultaneous equation method. The procedure was repeated for five times.

Method II

Preparation of standard stock solution (Stock-A)

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in 60 mL of 7.2 pH phosphate buffer solution and the flask was sonicated for about 10 min to solubilize the drug and the volume was made up to the mark with mixed buffer to get a concentration of 1000 μg/ml (Stock-A) for drug.

Preparation of sub stock solution (Stock-B)

Aliquots of 2.5 ml withdrawn with help of pipette from standard stock solution A of diacerein and transferred into 25 ml volumetric flask separately and diluted up to 25 ml with buffer that gave concentration of 100 μg/ml (Stock-B).

Preparation of working standard solution

Aliquots of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml and 2.5 ml withdrawn with help of pipette from standard stock solution (Stock-B) separately in 10 ml volumetric flask and volume was made up to 10 ml with RO water. This gave the solutions of 5 μg/ml, 10 μg/ml, 15 μg/ml, 20 μg/ml and 25 μg/ml respectively for diacerein.

Dye drug reaction using 2% bromo thymol blue

From the aliquots of 5 μg/ml, 10 μg/ml, 15 μg/ml, 20 μg/ml and 25 μg/ml, take 2 ml of drug standard solution from each aliquot and add 2 ml of 2% Bromo thymol Blue dye solution and 3 ml of Chloroform, In same manner prepare blank and scanned between 200-400nm.

Selection of wavelength for linearity

Solutions of 50 μg/ml of diacerein were prepared and the solutions were scanned in the spectrum mode from 400 nm to 800 nm. The maximum absorbance of diacerin was observed at 228.0 nm. Diacerein showed linearity in the concentration range of 5-25 μg/ml at their respective maxim. Calibration curve was plotted, absorbance versus concentration (Figure 3, 4).

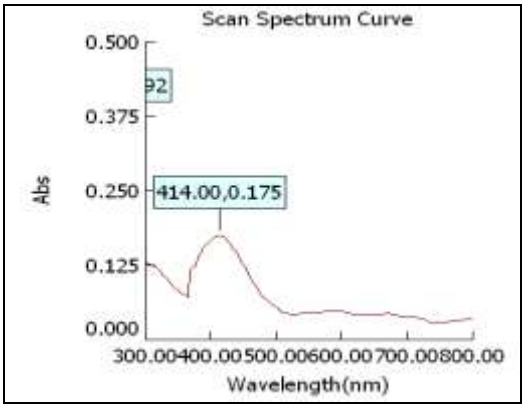

Fig 3. Determination of λ_{max} of diacerin

Linearity

An initial spectrum of the stock solutions were scanned in order to ascertain the wavelength for

detection of the drug complex and 414.0 nm was selected as the wavelength of detection.

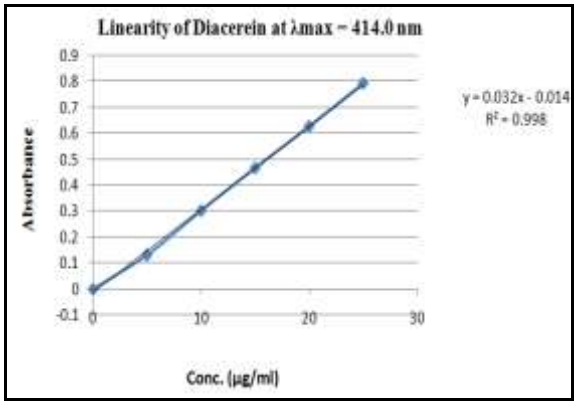

Fig 4. Calibration curve of diacerin

Accuracy

The accuracy of the proposed method was assessed by recovery studies at three different levels i.e. 80%, 100% and 120%. Three replicate studies to determine the concentration of added analyte was performed at five concentration levels.

Precision

Precision of the methods was studied at three level as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility. Repeatability was performed by analyzing same concentration of drugs for five times. Day to day was performed by analyzing 5 different concentration of the drug for three days in a week.

Robustness

Robustness of the analytical method was performed by changing the concentration and ratio of hydrotropic solution for check the analytical methods capacity to remain unchanged. For the robustness of the analytical method we changed the ratio of hydrotropic solution. Instead the 2% dye, 2.1% dye is used.

Analysis of capsule sample

Twenty marketed Capsules of DIA was weighed and ground to a fine powder; amount equal to 50 mg of DIA was taken in 10 ml volumetric flask. The DIA present in this amount of Capsule powder was 50 mg. Then 2 ml of dye solution was added and the extracted with chloroform. The absorbance of final dilutions was observed at selected wavelengths and the concentrations were obtained from simultaneous equation method. The procedure was repeated for five times.

Results and discussion

The study revealed that estimations of diacerein can be done within 24 hours without any detrimental effect on drug stability as no precipitation was observed.

Enhancement of solubility was more than 50 for Diacerein respectively in mixed hydrotropic solution. The enhancement of solubility of diacerein was due to the hydrotropic solubilization phenomenon. Results of solubility in different solvent for both the drug were shown in Table 1.

Based on the solubility, stability and spectral characteristics of the drugs, 2M Sodium acetate was selected as hydrotropic agent. Presence of hydrotropic agent do not shows any significant interference in the spectrophotometric assay thus further confirming the applicability and reproducibility of the developed method.

The developed methods were found to be linear. The values of mean percent recoveries were found to shown in and results of validation are shown in table 2.

Table 1: Solubility of drug in different solvents

S. No.	Solvents	Solubility Diacerein
1	Water	-
2	Hot water	-
3	Cold water	-
4	2M Sodium acetate	-
5	8M Urea	-
6	2M Sodium citrate	-
7	2M Sodium benzoate	-
8	2M Sodium acetate	+

The mean percent label claims of tablets by the proposed methods were close to 100, indicating the accuracy of the proposed method and low values of standard deviation, percent coefficient of variation and standard error further validated the proposed method as shown in table 3.

Table 2: Results of linearity of diacerein

	Method-I	Method-II
PARAMETER	**DIA**	**DIA**
Working λ	224 nm	414 nm
Beer's law limit (µg/ml)	1-5	5-25
Correlation Coefficient (r2)*	0.998	0.998
Slope (m)*	0.075	0.032
Intercept (c)*	0.002	0.014

*Average of five determination

Method I: Calibration curve Method, Method II: Using dye drug reaction

Table 3: Results of recovery studies on marketed formulations

Recovery level %	% Recovery (Mean±SD)*	
	Method I	Method II
	DIA	DIA
80	99.04±0.258	98.87±0.323
100	99.05±0.400	98.62±0.789
120	99.82±0.105	99.32±0.434

Method I: Calibration curve Method, Method II: Using dye drug reaction

Table 4: Results of validation (%R.S.D.)

Parameter		**Method - I**	**Method - II**
		DIA	**DIA**
Precision (%R.S.D.)*	Repeatability	0.571	0.570
	Day to Day	1.071	0.321
	Analyst to Analyst	0.656	0.121
	Reproducibility	0.984	0.516
Robustness*		0.510	0.490

*Average of five determination

Method I: Simultaneous Equation Method, Method II: Using dye drug reaction

Main criteria for the selection of hydrotropic agents in spectrophotometric methods include sufficient concentration and volume of hydrotropic agents, which completely solubilize content of drug' and these hydrotropic agents should not interfere in analyzes. Hydrotropic solutions selected for this work in spectrophotometric methods have not shown any interference. These values are very close to 100, indicating the accuracy of the proposed method. The amount of the drug estimated by using these

two methods was found to be in good agreement with the label claim, which indicates that there was no interference from the excipients commonly present in the capsule formulation. These methods were validated for accuracy, precision and robustness. Both these methods are simple, economical, and rapid and can suitably be used for determination of diacerein in bulk and in capsule formulation.

Conclusion

Developed spectrophotometric diacerein by using different hydrotropic agents was found to be the best alternative for estimations of poorly water-soluble drugs and to minimize the use of organic solvents. Thus a new method has been developed that is precise, simple, cost effective, accurate, and safe which has been validated. This proposed method which was developed on the principle of hydrotropic solubilization concept can be well employed in routine analysis of diacerein in capsule formulation.

References

1. Shete AS, Yadav AV, Dabke AP, Sakhare SS. Formulation and evaluation of hydrotropic solubilization based suspensions of griseofulvin. International Journal of Pharma Sciences and Research 2010; 1(1): 51–57.
2. Vinnakota SNA, Deveswaran R, Bharath S, Basavaraj BV, Madhavan V. Application of mixed hydrotropic solubilization in spectrophotometric estimation of aceclofenac in tablets. Journal of Current Pharmaceutical Research 2011; 1(3): 223–226.
3. Gurumurthy V, Deveswaran R, Bharath S, Basavaraj BV, Madhavan V. Application of hydrotropic solubilisation in simultaneous estimation of atenolol and amlodipine besylate. Asian Journal of Research Chemistry 2012; 5 (1): 57–60.
4. Renapurkar DK, Mathur S, Rao KJ. Evaluation of efficacy and safety of diacerein in osteoarthritis of knee joint, International Journal of Pharma and Bio Sciences 2010; 1(3):1-12.
5. Yao HC, Yang XF. Sensitive determination of nanogram levels of diacerein in a pharmaceutical formulation by flow injection chemiluminescence analysis. Journal of the Chinese Chemical Society, 2007, 54, 949-956.
6. Gupta KR, Samrit VE, Thakur V, Hemke AT. UV-spectrophotometric estimation of diacerein in pharmaceutical formulation. J Chem Pharm Res. 2010;2:467–72.
7. Sivakumar R, Nallasivan PK, Saranya KC, Sam Solomon WD, Akelesh T, Venkatnarayanan R. Visible spectrophotometric estimation of diacerein in bulk and pharmaceutical dosage forms. Pharm Anal. 2010;2:414-416.

8. ICH, Q2A: Text on Validation of Analytical Procedures, International Conference on Harmonization. 1994.
9. ICH, Q3B: Validation of Analytical Procedures: Methodology, International Conference on Harmonization. 1996.
10. ICH Harmonized Tripartite Guideline, Q2 (R1): Validation of Analytical Procedures: Text and Methodology. 2005.