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Validated Spectrophotometric Method for the Assay of Diacerein in Bulk and Pharmaceutical Formulations

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Abstract:

The objective of this research was to develop a spectrophotometric method for determination of diacerein in pure form and pharmaceutical formulations. The method was based on the reaction of carboxylic acid group of the drug with a mixture of potassium iodate (KIO_3) and potassium iodide (KI) in aqueous medium at room temperature. The reaction followed yellow colored chromogen spectrophotometrically measured absorbance at 352 nm. Beer's law was obeyed in the concentration range 2-12 µg/ml with molar absorptivity and Sandell's sensitivity of 2.8339 x 10^4 L mol/cm and 0.001360 µg/cm^2 per 0.001 absorbance units, respectively.

Key Words: *spectrophotometry, diacerein, potassium iodate (KIO_3), potassium iodide (KI), dimethyl acetamamide, validation.*

Introduction:

Diacerein is an anthraquinone derivative drug, it is chemically, 4, 5-bis(acetyloxy)-9, 10-dihydro-9, 10-dioxo-2-anthracenecarboxylic acid (1-3) (Fig. 1).

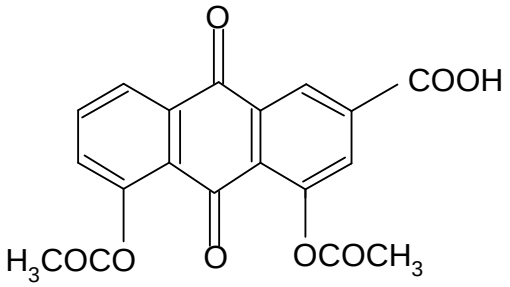


Fig 1: Structure of Diacerein

The diacerein is an osteoarthritis drug used as an anti-inflammatory activity by inhibiting the interleukin-1 (IL-1) without inhibiting prostaglandin synthesis. It has been proposed that diacerein is a slow acting, symptom modifying and perhaps disease structure modifying drug for osteoarthritis. It has a gold level of evidence that, it has small consistent benefit in improvement in pain (4). Diacerein and its active metabolite affect the catabolic and anabolic process in the osteoarthritic cartilages, principally by inhibiting the production and activity of interleukin-1 (5). The inhibitory effect on interleukin-1 has an immense effect on the pathophysiological mechanism of osteoarthritis (6-7). It is effective in the treatment osteoarthritis- a degenerative process of the joints which is characterized by progressive destruction and erosion of the cartilage. It has also used to treat and prevent vascular diseases (8). It is not official in any pharmacopoeia. In literature, only one method were reported (HPLC) for stability- indicating methods for diacerein in bulk drug substance (9).

This paper describes simple and sensitive validated spectrophotometric methods have been developed for quantitative estimation of diacerein in pure and dosage form (capsule) is based on formation of yellow colored, chromogen, which absorbs maximally at 352 nm. This method has been successfully extended to the pharmaceutical dosage forms. The proposed methods are optimized and validated as per the international conference on Harmonization (USA) guidelines (10).

Materials and methods:

Apparatus

A Shimadzu UV- Visible double beam spectrophotometer (model 1700, Japan) with 1cm matched quartz cell were used for spectral measurements.

Reagents and Standards

All chemicals and reagents used were of analytical grade or pharmaceutical grade. All solutions were prepared in doubly distilled water. A 0.15 M potassium iodide (S.D. Fine Chem., Mumbai, India) solution was prepared in distilled water. The solution was standardized by the recommended procedure (11). A 0.1 M potassium iodate (S.D. Fine Chem.,

Mumbai, India) was also prepared in distilled water.

Working Standard of drug and sample solutions

About 100 mg of diacerein (pure and formulation) was accurately weighed and dissolved in a 50 ml of dimethyl acetamide to ensure complete solubility of drug. The mixture was sonicated and then makes up to 100 ml with methanol (1mg/ml) the final concentration of diacerein was brought to 100 µg/ml with methanol. In case of formulation, different brand (Dycerin, Icerin-50) of commercially available capsules were analyzed by the proposed methods. Ten capsules of diacerein each containing 50 mg were accurately weighed drug powder equivalent to 100 mg of diacerein taken.

Recommended Procedure

Aliquots of diacerein ranging from 0.2 – 1.2 ml (1 ml = 100 µg ml^{-1}) were transferred into a series of volumetric flasks. To each volumetric flask, 2.2 ml 0.1 M potassium iodate (KIO_3) followed by 3.3 ml of 0.15 M potassium iodide (KI) were added. After 45 min, the volume was made up to the mark with doubly distilled water. The content of each flask were mixed well and the absorbance of the yellow colored chromogen was measured at 352 nm (Fig. 2) against reagent blank prepared similarly. The amount of diacerein present in the sample computed from calibration curve or regression equation.

Analysis of Pharmaceutical Formulation

The sample solution containing diacerein at a concentration of 100 µg/ml was prepared by the using ten capsules of diacerein 50 mg strength were obtained by gentle tapping and the hard gelatin shells were discarded. Diacerein capsules powder was extracted by dissolved in water, and then all water soluble additives were removed by filtration using Whattman No.42 filter paper. Then drug can be extracted with dimethyl acetamide and transferred to a 100 ml standard volumetric flask, diluted with methanol. It was further diluted according to the need and subjected to the recommended procedure for the determination of diacerein.

Results:

Spectral studies

The spectrum of reference pure drug of diacerein in aqueous solution shows absorption bands at 343 nm (Fig. 2).

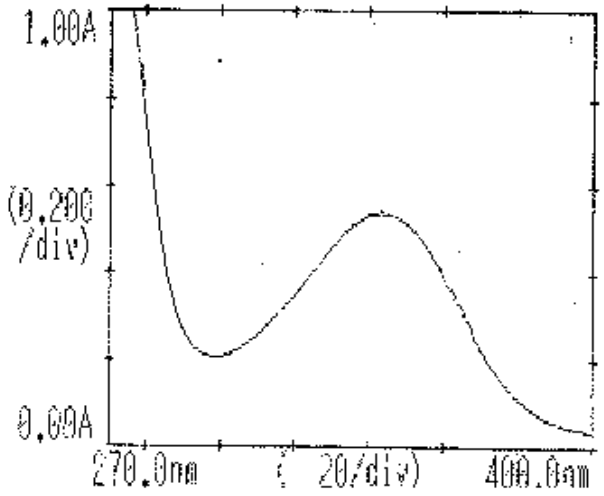


Fig 2: Absorption spectrum Diacerein in aqueous solution

The addition of aqueous solutions of KI and KIO_3 to the drug solution causes change in the absorption spectrum with new characteristic bands peaking at 285 and 352 nm (Fig. 3).

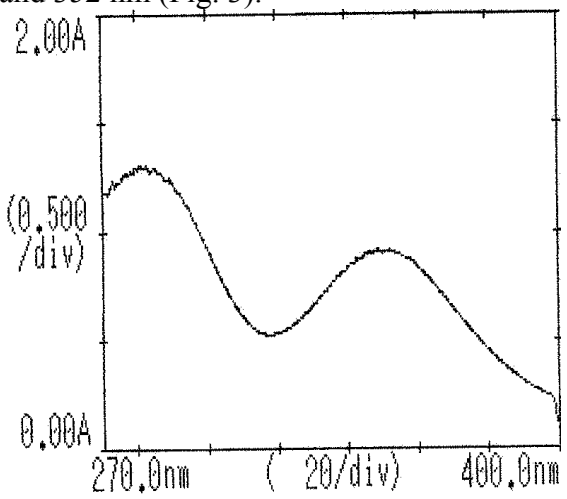
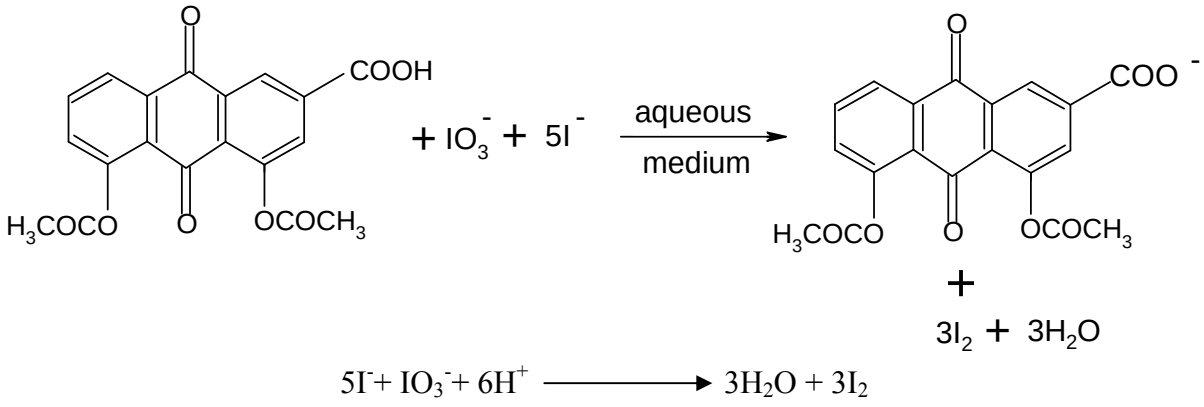


Fig 3: Absorption spectrum Diacerein in potassium iodate (KIO_3) Potassium iodide (KI) in aqueous solution

The reagent blank solution containing KI and KIO_3 shows one peak at 285 nm and a negligible absorbance at 352 nm when measured against distilled water as a



$$5I^- + IO_3^- + 6H^+ \longrightarrow 3H_2O + 3I_2$$

Scheme 1

Table 1: Analytical parameters of spectrophotometric methods

Parameter	Method
$\lambda_{max,}$ nm	352
Beer' s law limits, µg/ml	2– 12
Molar absorptivity, L/mol/cm	2.8339 x 10^4
Sandell sensitivity, µg/cm^2	0.001360
Regression equation (Y*)	
Slope (b)	0.077
Intercept(a)	0.0041
Correlation coefficient (r)	0.9998
% RSD	0.241
Range of errors**	
Confidence limits with 0.05 level	0.00154
Confidence limits with 0.01 level	0.002289

*Y=a + bX where Y is the absorbance at the respective λ_{max}, a intercept, b slope and X Concentration in µg/ml, ** for five measurements.

Table 2: Evaluation of Diacerein in Pharmaceutical Dosage Forms.

Sample (capsules)	Labelled Amount (mg)	Amount obtained (mg)* Proposed methods	% Recovery** Proposed methods
Dycerin	50	49.94±0.03	99.88±0.05
Icerin-50	50	49.89±0.05	99.78±0.06

*Mean and standard deviation of five determinations; **Mean and standard; Deviation of five determinations; (50 mg of diacerein was added and recovered)

reference. The absorbance obtained at 352 nm is higher than the absorbance at 285 nm, thus showing higher sensitivity at 352 nm. Therefore, the absorbance measurements for the determination of diacerein were made at 352 nm. The equilibrium is attained at about 45 min. Therefore, a spectrophotometric method was developed for the quantitative determination of diacerein measuring the in absorbance at 352 nm after 45 minutes.

Mechanism of the color reaction

It has been suggested that water-soluble acidic compounds liberate iodine from a solution containing both KIO_3 and KI according to the reaction (12). The change of solution color to yellow reveals the occurrence of the reaction. The color of the solution is due to the formation of I_2, which immediately converted into triiodide ions in the presence of iodide ions ($I_2 + I^- \longrightarrow I_3^-$) exhibiting absorption maxima at 290 nm and 360 nm (13). Diacerein, possesses -COOH group in its moiety and undergoes a similar reaction with iodide-iodate mixture resulting in the evolution of iodine. The liberated iodine immediately reacts with potassium iodide to give triiodide ions showing absorption maxima at 285 nm and 352 nm. The reaction mechanism was proposed and given in Scheme 1.

The confirmatory test for the presence of iodine in the final solution of the drug is established by the blue color, which appears on addition of starch solution (14).

Solution Stability

The solution stability of diacerein in bulk and capsule solutions was monitored by keeping the solutions at room temperature (25 ± 1^0 C) under darkness for several days and then recording the absorption spectra of the solutions, and also by performing TLC analysis. The standard drug and quality control sample solutions showed no change in the absorption spectra for at least five days.

Study of Interferences

A study of interferences of some common excipients has been made during determination of 20 µg/ml and 5.0 µg/ml diacerein. However, the drug content from the powder of capsules diacerein was extracted by dissolve in water all water soluble additives can remove by filtration using Whattman No. 42 filter paper. Then the diacerein was extracted with dimethyl acetamide, which was completely eliminates any interference by the common excipients found in drug formulations. Thus, the proposed method is stability indicating assay for the analysis of diacerein in drug formulations in the presence of various excipients found in formulations.

Optimization of variables

The optimum reaction conditions for the quantitative estimation diacerein of were established via a number of preliminary experiments. The optimum conditions for the assay procedures method have been established by studying the reactions as a function of reaction time, concentration of reagents, solution stability and the stability of the colored species.

Accuracy and Precision

The accuracy and precision of the methods were evaluated by performing seven replicate analyses on pure drug solutions at three different amount/concentration levels (within the working ranges). The relative error (%), an indicator of accuracy was within 2.5 % and intra day precision which is also called the repeatability expressed in relative standard deviation (RSD) (%) was also less than 2.5 %, indicating the high accuracy and precision of the methods. The results of this study are compiled in Table 1. The reproducibility of the methods, also called the day-to-day precision, was assessed by performing replicate analyses on pure drug solutions at three levels over a period of five days preparing all solutions afresh each day. The day-to-day RSD values were less than 4 % reflecting the usefulness of the methods in routine use.

Ruggedness

The method ruggedness was evaluated by a second analyst using a different instrument (A Shimadzu 1601 UV-visible spectrophotometer, Japan) and freshly prepared standard and sample solutions. The analysis of the diacerein capsules were carried out five times at one concentration level by the ruggedness chemist and developing chemist following the recommended procedures. The results agreed well within the acceptable limits and no degradation was found to interfere with the determination process. These

results demonstrated acceptable method ruggedness for routine quality control analysis of active drug in pharmaceutical formulations.

The operational *Robustness*

Parameters of method for the assay of diacerein capsules were found to be very robust. The operational parameters investigated were:

- 2.2 ml (± 0.1 ml) of 0.1 M potassium iodate (KIO_3) in aqueous solution.
- 3.3 ml (±0.1ml) of 0.15 M potassium iodide (KI) in aqueous solution.
- Solution reacting time was 45 min (± 5 min).

The robustness of the proposed methods relative to each operational parameter was examined by analyzing the diacerein capsules under variable experimental conditions. For this, a sample solution of different drug formulations containing 50 µg/ml of active drug was assayed five times (Table 2). The results showed good mean recovery with low relative standard deviation. Thus the proposed methods were found to be very robust for routine quality control analysis of drug in pharmaceutical formulations.

Discussion:

Analytical Data

Under the optimized experimental conditions, calibration graphs were constructed by plotting the absorbance against the concentration of diacerein. Beer's law was obeyed in the concentration range 2.0–12.0 µg/ml with molar absorption coefficients and Sandell's sensitivity of 2.8339 x 10^4 L mol/cm and 0.001360 µg/cm^2 per 0.001 absorbance units respectively. Table 1 summarizes the optical characteristics and the results of statistical analysis of the experimental data such as linear regression equations for method along with correlation coefficient, standard deviation of slope (S_b) and intercept (S_a), confidence interval of slope (tS_b) and intercept (tS_a), detection limit and quantitation limit. The limit of detection (LOD) and quantitation (LOQ) were calculated using the following relation (Ermer, 2001)

$$LOD = 3.3 \times S_0/b, \text{ and } LOQ = 10 \times S_0/b$$

Where S_0 is the standard deviation of the calibration curve and b is the slope. The small value of variance suggested negligible scatter of experimental data points around the line of regression.

The accuracy and precision of the propose method was evaluated by performing five replicate determinations of diacerein in pure forms at three different concentrations by short term (intra day) and daily (inter day) precisions (Table 2). The standard analytical errors, relative standard deviations and recoveries obtained in the intra day and inter day analyses for method found to be acceptable. Thus were found to be acceptable. Thus the proposed method is effective for the determination of diacerein. The accuracy of the proposed methods was also checked by performing recovery experiments through standard addition technique. For this purpose, a known amount of pure diacerein was added to pre-analyzed dosage forms and then determined by the recommended procedures. The results (Table 2) showed that the mean recovery and relative standard deviation were in the range of 99.78–99.88 and 0.241 % for respectively. No interference from the common excipients was observed. It is clear from the table that the proposed methods are sensitive with acceptable values of relative standard deviations. Thus the proposed methods can use full for determination of the drug at lower concentrations.

Conclusions:

These proposed method found to be more sensitive and it does not require any pretreatment of the drug and tedious extraction procedure prior to its determination. The true bias of all samples is less than ± 2 %, confirming acceptable recovery and precision. The method was successfully applied to enable estimation of drug in pharmaceutical dosage forms at a lower concentration level (each capsule

claiming 50 mg), and can be used for routine quality control analysis of diacerein in pharmaceutical formulations.

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References:

[1] Martindale, The Extra Pharmacopoeia (2002) 33rd ed, pp. 28, Royal Pharmaceutical Society, London.
[2] J.O. Maryadele, The Merck Index (2001) 13th ed, pp. 521, Merck & Co, Inc, NJ.
[3] A. Tschirch, K. Heuberger, Arch Pharm, 240 (1902) 596.
[4] T.S Fedilix, B.G.Soares, V.F.Trevisani Diacerhein for the osteoarthritis. Cochrane database Syst Rev, 1 (2006) CD005117.
[5] M. Solignac, Press Med**,** 33(2004) 10-12.
[6] M. Yaron, I.Shirazi, I. Yaron, Osteoarthritis Cartilage, 7 (1999) 272-280.
[7] N. Felisaz, K. Boumebine, C. Ghayor, Osteoarthritis Cartilage, 7 (1999), 255-264.
[8] T. Cruz and A. Pastrak, PCT Int. Appl**.** (2003), 26.
[9] G. Valerio, S. Francesco, B. Gianluca, A.C. Silvia, B.A., J Pharm Biomed Analysis, 39 (2005) 776.
[10] International Conference on Hormonisation, ICH Harmonised Tripartite Guideline-Text on Validation of Analytical Procedures, Fed Regist, 60 (1995),11260.
[11] F.Sierra-Jemmenez, C.Sanchez-Pedreno, Anales real Soc Espan Fis y quim, 54b (1958), 541-552.
[12] F. Feigl Spot test in organic analysis chapter 3: Preliminary (Exploratory) tests, 6th ed, Amsterdam, Elsevier Publiting company, (1960), 117 - 118.
[13] A.I. Popov, W.A. Deskin Study of chemisrtry halogens and of polyhalides, XV. Iodine Halide complex with acetonitril, J. Am. Chem. Soc, 80 (1958) 2976 - 2979.
[14] J. Zhang, D. Thickett, L. Green, JAIC, 33 (1994), 47 - 53.