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Application of Hydrotropic Solubilization Phenomenon for Estimating Diacerein in Capsule Dosage Form by Spectrophotometry Methods

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Abstract

To develop two safe, eco-friendly, sensitive and accurate UV-spectrophotometric methods by applying hydrotropic solubilization phenomenon for the estimation of diacerein in capsule dosage form. Preliminary solubility studies of drug, selection of hydrotrope, UV spectral studies, Optimization of hydrotrope, direct spectrophotometric and derivative method development, validation of proposed methods were performed as per ICH guidelines. Application of developed method on marketed formulation. The aqueous solubility of diacerein was increased by more than 270 folds by using 8M urea solution as hydrotropic agent in comparison to solubility in distilled water. The sample obeys the Beer's law in the concentration range of 1-15 µg/ml and 2-45 µg/ml with correlation coefficient of 0.9994 & 0.9997 for each method respectively. The accuracy was proved by recovery studies with mean recovery of 99.80 % and 99.08 % for each method respectively. Intermediate & repeatability precisions were performed on two consecutive days and analyst to analyst variation with %RSD obtained less than 2%. The LOD & LOQ results intricate the sensitivity of both the methods. It can be concluded that by applying the hydrotropic solubilization technique for estimating hydrophobic drugs provides a simple, sensitive, cheap & safe estimation. Moreover detrimental health effects & hazardous effects on our environment by using organic solvents can be overcome. Proposed method is less time consuming with two steps of analysis for estimating drug content in formulation.

Keywords: Spectrophotometry, Hydrotropic solubilization, Quantitative analysis, Diacerein
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1. Introduction

The number of potential drug candidates has been increasing by the advent of genomics, combinational chemistry and high throughput screening, forcing R & D organizations to accelerate attrition of compounds that do not have a high probability of successful development. Many of these new compounds are highly hydrophobic and poorly water soluble. Solubility is one of the most important physiochemical properties for the drug development since low solubility can hinder development of parenteral products and severely limit the bioavailability of orally administered dosage form. Independently of the intended route of administration of drug candidate, the requisite preclinical and toxicology studies make it necessary to prepare investigational formulations at relatively high concentrations. Such drug molecules are often obtained by using strong organic cosolvents like DMSO, which pose toxicological liabilities of their own and are not acceptable for use in clinical formulations [1]. There is a need for finding powerful solubilizing systems that are suitable for wide range of poorly soluble drugs. Among various techniques employed for enhancing solubility, hydrotropy is one of them. Hydrotropy is a molecular phenomenon whereby adding a second solute (the hydrotrope) results in an increase in aqueous solubility of poorly soluble solute [2, 3]. Typically, hydrotrope consist of a hydrophilic part and a hydrophobic part (like surfactants) but the hydrophobic

part is generally too small to cause spontaneous self-aggregation and do not have a critical concentration above which self aggregation 'suddenly' starts to occur [4]. Sodium salicylate, sodium benzoate, urea, nicotinamide, sodium citrate and sodium acetate are the most common examples of hydrotropic agents utilized to increase the water solubility of drug [5-17]. In this present investigation, we focus on the application of given hydrotropic system to work with diacerein molecules for more simplifying the quantification or estimation of drug content in formulation without the need of organic, toxic & costlier solvents.

Diacerein, chemically is 1,8-Diacetoxy-3-carboxyanthraquinone, widely used in the treatment of gout and is completely insoluble in water. It is also given in combination with glucosamine for severe gout conditions. Literature revealed number of assay method developments for diacerein in bulk and in formulations taking account of HPLC, UV spectrophotometry, Colorimetry, chemiluminescence [18-19]. A simple HPLC Method for quantitation of combined diacerein and glucosamine in tablet dosage form has also been reported [20]. Diacerein is known to have two impurities in bulk which can be isolated and elucidated [21]. However the reference method developments make the most of DMSO, acetonitrile, ethanol, methanol, dyes posing their own affects on the user. None of these methods are without their limitations so the need was felt to develop two new, simple, accurate, eco- friendly, cost effective, safe, sensitive spectrophotometric methods for estimation of diacerein in capsule dosage form by using aqueous solution of 8.0 M urea solution, as a hydrotropic agent. Thus the main aim of our present study is to explore the application of hydrotropy spectrophotometric analysis of hydrophobic drugs to make the analysis simpler.

2. Material and Methods

Reference diacerein was generous gift from Theon Pharmaceutical Pvt. Ltd., Nalagarh (India). Urea used in the study was of analytical grade. Commercial capsules of diacerein - Dycerin 50 (Glenmark) and Cartidin caps (Ranbaxy) were procured from local market. Shimadzu UV-visible spectrophotometer (model UV-1700 series), having double beam detector configuration with 1 cm matched quarts cells was used in the study.

Solubility of diacerein was determined at 28±2°C. An excess amount of drug was added to 25 ml volumetric flasks containing 15ml of different aqueous systems viz. distilled water, sodium benzoate (1, 2, 4, 6, 8M), Urea (1, 2, 4, 6, 8, 10M) and sodium acetate (1, 2, 4, 6, 8M) solution. Enhancement of solubility of drug was increased by 270 folds in 8M urea. This enhancement of solubility was due to the hydrotropic solubilization phenomenon. The enhancement ratio in solubility was determined by the following formula:

$$\eta_E = \frac{S_S}{S_W} \quad \text{(mg/ml)} \quad (1)$$

where S_S and S_W are the solubility of drug in hydrotropic solution and distilled water, respectively

Different available hydrotropic solubilizers including distilled water, sodium benzoate (1, 2, 4, 6, 8M), Urea (1, 2, 4, 6, 8, 10M) and sodium acetate (1, 2, 4, 6, 8M) solutions were used for optimization at room temperature.

In order to check any interaction between drug and the hydrotropic agent, UV spectral studies of diacerein were performed in different concentration of hydrotropic solutions. Possible spectroscopic changes in the structure of diacerein in the presence of hydrotropes were subsequently investigated.

Accurately weighed 50 mg of the diacerein drug sample was transferred into 50 ml volumetric flask containing 40 ml of 8M urea solution, shaken, sonicate for 7 min and diluted up to 50 ml with distilled water and filtered through Whatmann filter paper no.1. The 5 ml of filtered solution was further diluted to 50ml with distilled water to prepare stock solution (100µg/ml).

By using the proposed methods, the different optical characteristics of hydrotrope diacerein such as absorption maxima, Beer's law limit, molar absorptivity, sandle's sensitivity, Absorptivity ($A_{1\%, 1cm}$) were calculated. The regression analysis using the method of least squares was made for the slope (m), intercept (c) and correlation coefficient (r^2) obtained from different concentrations.

The fresh aliquot of 20µg/ml was prepared from stock solution and scanned in the spectrum mode from 200-400nm wavelength range on Shimadzu 1700 spectrophotometer. Fresh aliquots of standard stock solution (100µg/ml) were pipette out and suitably diluted with distilled water to get concentration of 40µg/ml for scanning of spectra. The scanned spectra were derivatized for 1st, 2nd, 3rd and 4th order of derivative.

Both the methods were validated in accordance of ICH (2005) and USP guidelines (2004) for validation of analytical procedures in order to substantiate linearity and range, precision, recovery, robustness, LOD and LOQ for each method [22,23].

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3. Results and Discussion

Diacerein, being insoluble in water, was selected for the application of hydrotropy phenomenon. The chemical structure of diacerein is shown in Fig. (1) revealing the hydrophobic property of drug with number of chromophores in it. After assessing their solubility pattern (Fig. 2), 8M urea was selected as working hydrotropic solubilizing agent for analysis. The pH of 8M urea was 8.56. The solubility enhancement of diacerein is not entirely due to pH effect, but is largely due to hydrotropy [24].

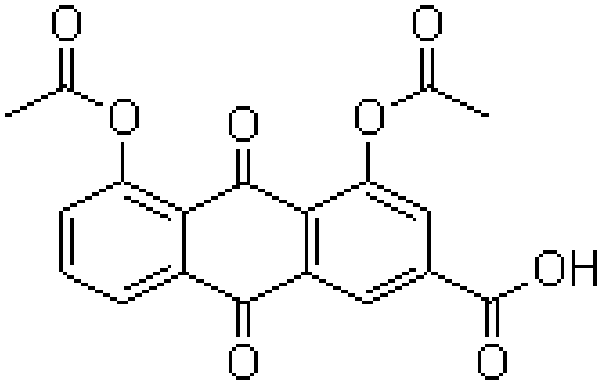

Fig. (1) Chemical structure of Diacerein

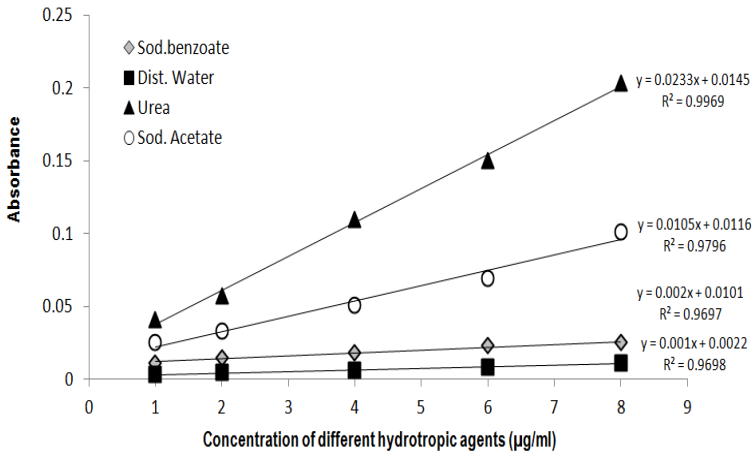

Fig. (2) Solubilities of hydrotropic solutions of 8M Sodium benzoate, 8M Sodium Acetate, 8M Urea and Distilled water

Urea does not show any absorbance above 240nm (Fig. 3). The other excipients (starch) in composition do not show any absorbance in analyzing range of diacerein (Fig. 4). Thus the hydrotropic agent as well as excipient did not interfere in the analysis of diacerein.

The different optical characteristics of hydrotrope diacerein were calculated for each proposed method and results are mentioned in Table (1).

On scanning, maximum absorbance was observed at 257.6nm and hence 257.6nm was selected as standard wavelength (Fig. 5). Calibration curve was plotted between concentration verses absorbance shows obeying the Beer-Lambert law in the range of 1-20µg/ml. Absorptivity of 776 was calculated from average of five concentrations against distilled water as blank. Drug content was calculated as per the following Beer-Lambert equation [25]:

$A = a\ b\ c$ (2)

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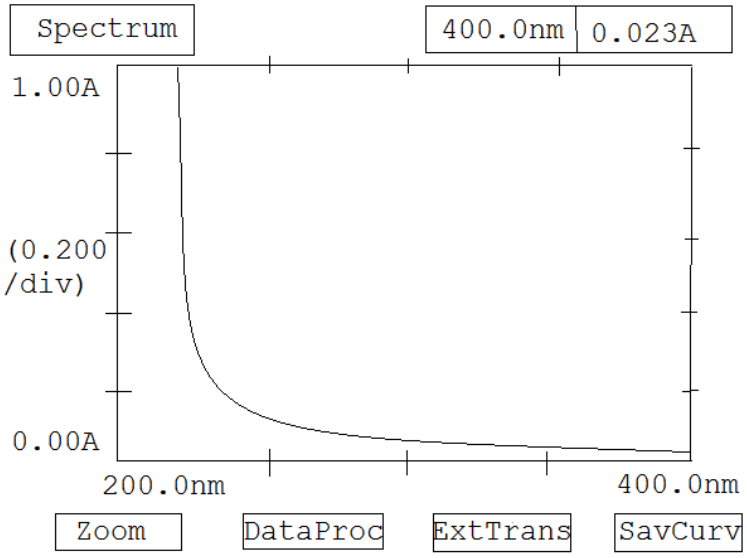

Fig. (3) Spectra of 8M Urea

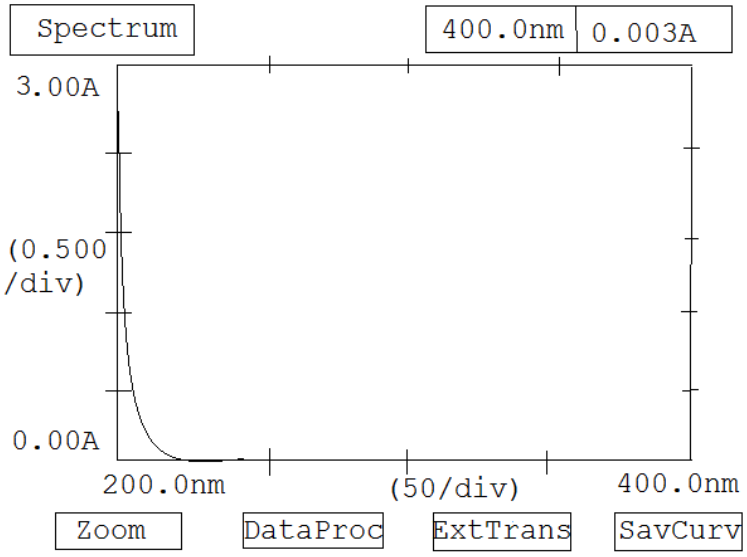

Fig. (4) Spectra of starch

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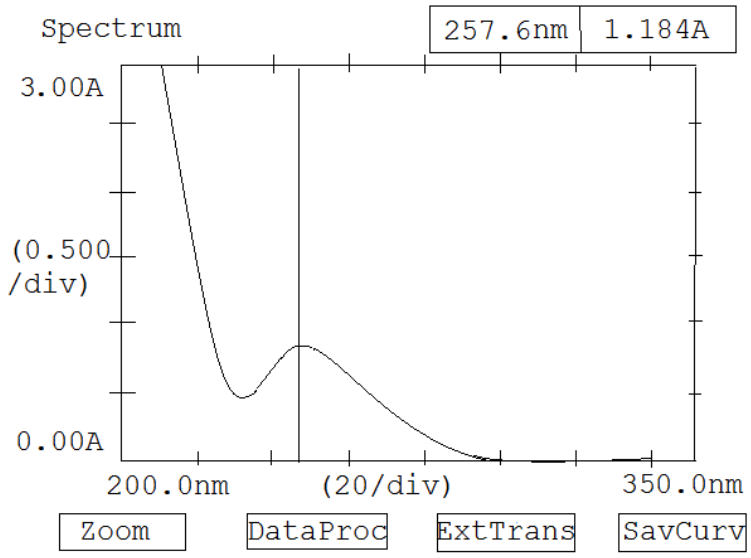

Fig. (5) Spectra of hydrotrope diacerein (15µg/ml)

On 1st order derivatization of spectra, a strong absorption minima was obtained at 280.5 nm (Fig. 6) while for 2nd, 3rd and 4th derivative no sharp peak was obtained (Fig. 7a, b, c). The calibration curve was plotted shows obeying the Beer-Lambert law in the concentration range of 2-45µg/ml with absorptivity of 251. Drug content was calculated as in Eq. (2).

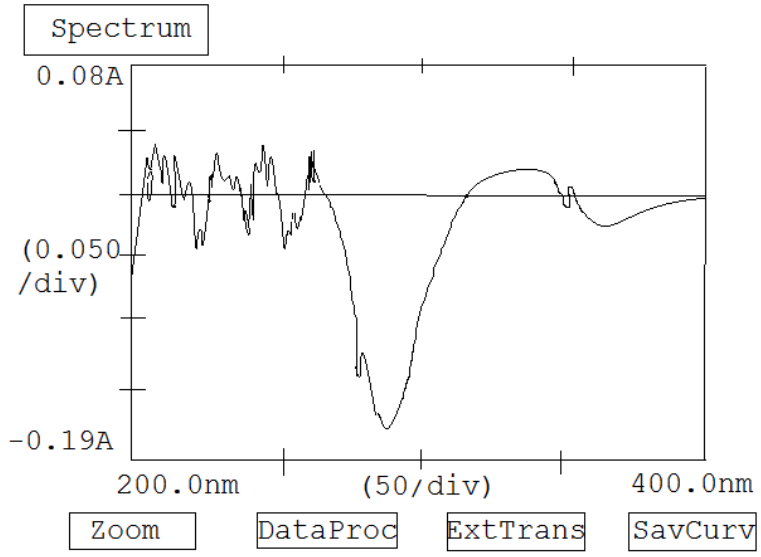

Fig. (6) First order derivative spectra of hydrotrope Diacerein (45µg/ml)

The validation of an analytical method confirms the characteristics of the method to satisfy the requirements of the application. Under the validation study the following parameters were studied and summarized results are shown in Table (2).

A linearity curve was plotted between concentration of hydrotrope diacerein and absorbance for each method. The absorbance were found to be linear over analytical range of 1-20µg/ml with regression coefficient value of 0.9994 at scanned wavelength of 257.6 nm for Method I (Fig. 8) and 2-45 µg/ml at 280.5 nm with regression coefficient value of 0.9997 for Method II (Fig. 9) against distilled water as the reagent blank.

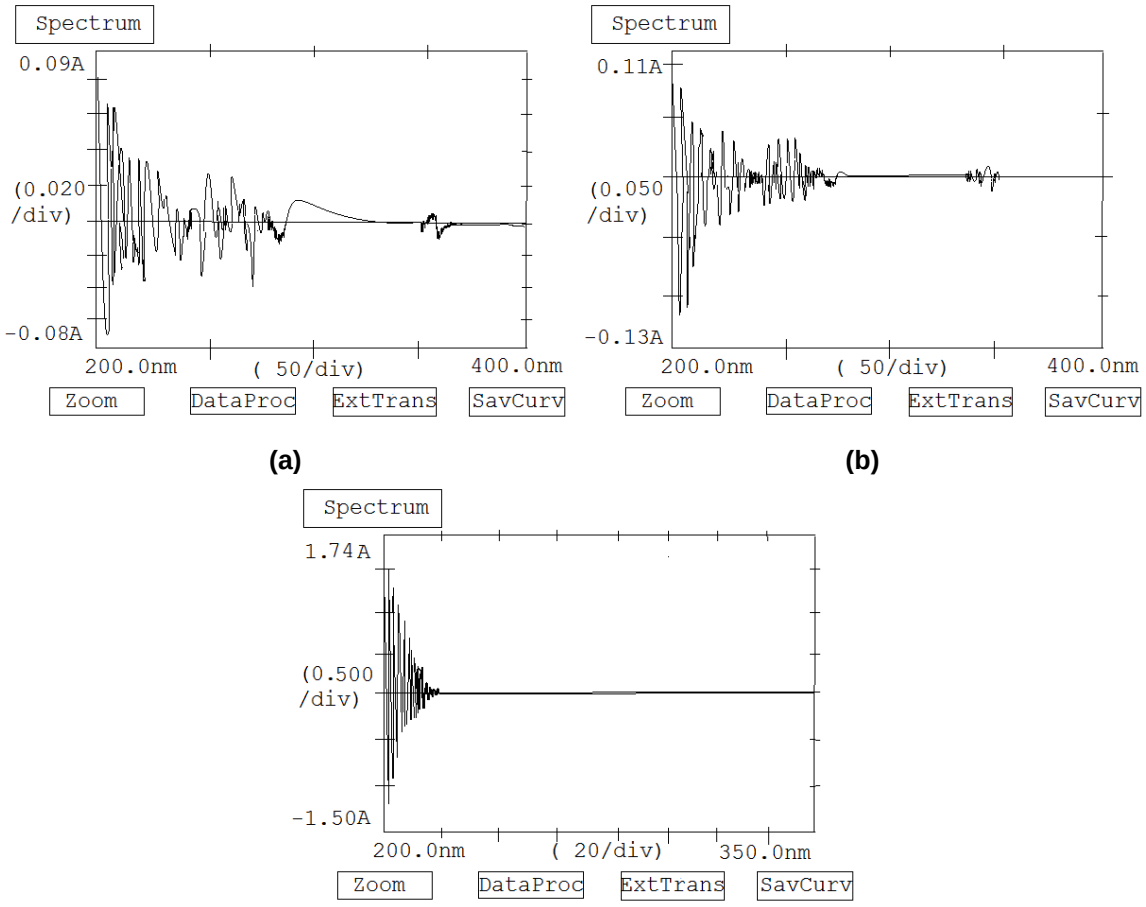

Fig. (7) Derivative spectra of hydrotrope diacerein (a) 2nd (b) 3rd and (c) 4th

Precision was evaluated as %RSD at two different parameters as repeatability and intermediate with three concentration and three replicates. The coefficient of variation and % mean ±Standard deviation of Intraday and interday precision, analyst to analyst precision for each method were calculated and found to be less than 2% respectively as shown in the Table (3).

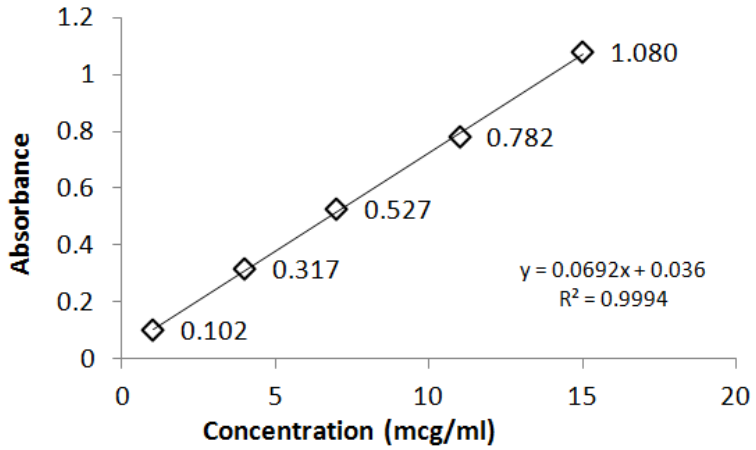

Fig. (8) Linearity curve of hydrotrope diacerein in direct spectrophotometry

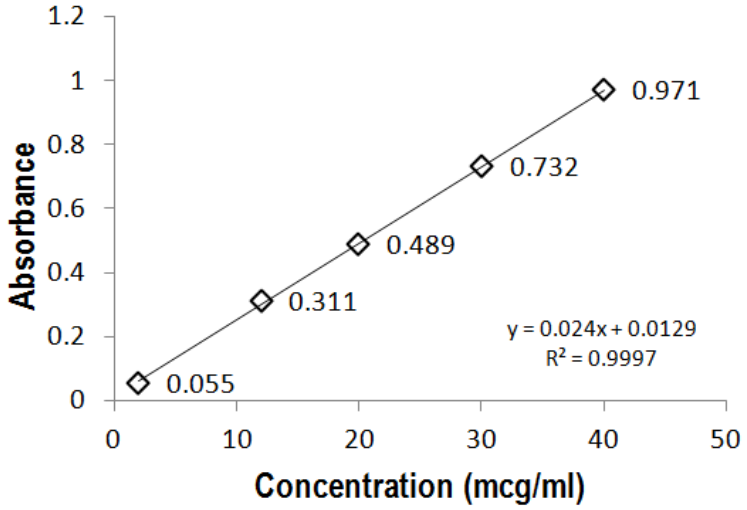

Fig. (9) Linearity curve of hydrotrope Diacerein in First derivative

Accuracy of each method was ascertained on the basis of recovery studies, were performed by the standard addition method at 5%, 10%, 15% level for method I and 40%, 80%, 120% level for method II, with minimum of nine determinations over three concentration levels within specified range. The percent recoveries, standard deviations, coefficient of variation were calculated for each method, respectively, as depicted in Table (4)**.** The mean recoveries for each method were found to 99.80% and 99.08%, respectively.

The LOD and LOQ for hydrotrope diacerein were calculated from the slope (m) of the calibration plots and the standard deviation (SD) of the blank using the following equations:

$$LOD = \frac{3.3\sigma}{S} \quad (3)$$

$$LOQ = \frac{10\sigma}{S} \quad (4)$$

where σ is the standard deviation and S is the slope

The LOD for hydrotrope diacerein for each method were 1.24µg/ml and 0.138µg/ml, while the values of LOQ were 3.75µg/ml and 0.417µg/ml, respectively.

The robustness was performed by making change of ±1nm in wavelength. The deliberate alteration of wavelength results in -0.25% and 0.52% deviation for method I and -0.80% and 0.80% deviation for II respectively. This demonstrates that the developed methods were robust and unaffected by minor changes. The results are given in Table (2).

The proposed method was applied to the determination of diacerein content in commercial pharmaceutical preparations (Capsules). Two marketed formulation Dycerin (Glenmark) and Cartidin cap (Ranbaxy) were selected for capsule analysis. Twenty capsules of each formulation were weighed and emptied for fine granules. An accurately weighed powder sample equivalent to 50mg of diacerein was transferred to a 50 ml of volumetric flask containing 40 ml of 8M Urea solution, shaken for about 7 min and volume was made up to the mark with distilled water. The solution was filtered through Whatmann filter paper. The filtrate was diluted appropriately with distilled water and analyzed on UV spectrophotometer at 257.6nm against distilled water as reagent blank. Drug content of capsule formulations were calculated for each formulation by proposed methods. The percent estimated by method I was found to be 100.84% (Dycerin 50) and 98.10% (Cartidin caps) while by method II it was found to be 101.48% (Dycerin 50) and 101.16% (Cartidin caps. The statistical evaluation of analytical data for each formulation was incorporated in Table (5).

Hydrotropy is one of the solubility enhancement techniques which enhance solubility to many folds with use of hydrotropes and do not require any chemical modification of hydrophobic drugs precluding the use of organic solvents like DMSO, DMF, methanol, ethanol, acetonitrile etc. As there is no hydrotropy work on diacerein was reported, UV Spectrophotometric determination of diacerein capsules using 8M Urea as hydrotropic solubilizing agent was developed. It is considered as simple, safe and cheapest method of estimation which can be useful in the routine analysis of hydrophobic drugs in formulation. To substantiate and authenticate the method results, validation of both the methods were performed as per ICH guideline. Quantitative analytical results are highly influenced by the quality of the calibration curve. Thus the linear regression analysis driven with acceptable

intercepts and correlation coefficients indicates a good correlation between concentration and absorbance within the concentration range tested for each method. The UV spectral studies were done divulging that there was not any interaction between drug and the hydrotropic agent. The results suggested a good precision of each method. The coefficient of variation at different levels for both the methods were found to be within acceptable limits (RSD<2%) suggesting methods are highly precise. The values of mean percent recoveries were also found to show variability in ranged from 98.80% to 98.08 % with %RSD values were found to be 0.636 and 0.463 for method I and II respectively. All these were very close to 100%. Low values of standard deviation, percent coefficient of variation and standard error further validated the proposed method statistically. The results of LOD and LOQ elaborate sufficient sensitivity of method. The robustness of the methods was shown no marked changes in the absorbance demonstrating that the UV spectrophotometric methods developed were robust. The developed methods were applied to the marketed formulation obtaining the results within validated range.

In conclusion, the present work was undertaken with a view to make the quantification of diacerein simpler, safe, eco-friendly, cost-effective, sensitive and accurate precluding the use of toxic, detrimental, costlier, organic solvents. Thus, exploring the application of hydrotropic solubilization phenomenon devoid of interaction between drug and hydrotropic agent.

Table (1) Optical parameters of hydrotrope diacerein for proposed methods

Parameters	***Spectrophotometry method***	***Spectrophotometry method***
	Direct	***Derivative***
Wavelength (ƛ)nm	257.6	280.5
Beer's law limit (µg/ml)	1 - 15 mcg/ml	1 - 45 mcg/ml
Molar absorptivity (L/mol.cm)	28580.08	9244.33
Sandel's sensitivity (µg/cm^2/0.001 absorbance unit)	0.012887	0.039841
Regression equation	y = 0.0693x + 0.0352	y = 0.024x + 0.0122
Slope (m)	0.0693	0.024
Intercept (C)	0.0352	0.0122
Correlation coefficient (r^2)	0.9994	0.9997
Absorptivity ($A_{1\%,1cm}$)	776	251

Table (2) Summary of method validation parameters for each method

No.	**Validation parameters**	**Limits**	**Results**	
			Direct Spectrophotometry Method	**Derivative Spectrophotometric Method**
1.	**Linearity (r^2)**	**0.9995 – 1.000**	0.9994	0.9995
2.	**Range**	**---------**	1 – 15 µg/ml	2 – 45 µg/ml
3.	**Precision**	**% RSD = NMT 2%**		
	(Day to Day)		0.78	0.69
	Intraday		0.14	1.20
	Interday			
	(Analyst to Analyst)		0.12	0.85
	Analyst I		0.45	0.28
	Analyst II			
4.	**Recovery studies** *(average mean recovery)*	**98 % - 102%**	99.80	99.08
5.	**LOD**	**-------**	1.24 µg/ml	0.138 µg/ml
	LOQ	**-------**	3.75 µg/ml	0.417 µg/ml
6.	**Robustness** *(Decreasing & Increasing)*	**%Deviation= NMT 1%**	-0.25% & 0.52%	-0.80% & 0.80%

Table (3) Results of Precision studies

Method	**Validation parameter**	**Percentage Mean ±S.D * (n = 9)**	**% RSD**
Direct Spectrophotometry	**Repeatability**	98.67 ± 1.02	1.03
	Intermediate Precision *(Day to day)*		
	Intra day	100.21 ± 0.78	0.78

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	Interday *(Analyst to analyst)* Analyst I Analyst II	99.85 ± 0.14 98.99 ± 0.12 99.87 ± 0.45	0.14 0.12 0.45
Derivative Spectrophotometry	**Repeatability** **Intermediate Precision** *(Day to day)* Intra day Interday *(Analyst to analyst)* Analyst I Analyst II	101.30 ± 0.26 100 ± 0.69 100.04 ±1.20 99.8 ±0.85 101.79 ± 0.28	0.30 0.69 1.20 0.85 0.28

**Mean of 9 determinations (3 replicates at 3 concentration level). SD = Standard deviation, RSD = Relative standard deviation*

Table (4) Result of recovery studies of capsule formulation with statistical evaluation

Method	Theoretical Concentration (µg/ml)	Amount Added (%)	Average Concentration Recovered (%)	Percentage recovery (mean ± SD) (n = 9)	Coefficient of Variation (%)	*Standard Error
Direct Spectrophotometry Method	100	----	99.91	99.91 ± 0.326	0.326	0.188
	100	5%	99.60	99.60 ± 0.694	0.697	0.401
	100	10%	99.82	99.82 ± 0.368	0.369	0.212
	100	15%	99.98	99.98 ± 0.842	0.842	0.486
Derivative Spectrophotometry Method	100	----	99.64	99.64 ± 0.467	0.469	0.270
	100	40%	99.06	99.06 ± 0.771	0.780	0.445
	100	80%	99.13	99.13 ± 0.406	0.410	0.234
	100	120%	99.04	99.04 ± 0.777	0.790	0.450

** n=9*

Table (5) Statistical evaluation of analysis of capsules

Parameter	***Direct spectrophotometry***		***Derivative spectrophotometry***	
	Dycerin 50	**Cartidin**	**Dycerin 50**	**Cartidin**
Mean % estimated	99.85	100.21	99.47	100.04
Standard Deviation	1.58	0.78	1.40	1.20
% Coefficient of variation	1.58	0.78	1.41	1.20
Standard error of mean	0.91	0.45	0.81	0.69

4. Conclusions

It can be concluded that by applying the hydrotropic solubilization technique for estimating hydrophobic drugs provides a simple, sensitive, cheap and safe estimation. Moreover detrimental health effects and hazardous effects on our environment by using organic solvents can be overcome. Proposed method is less time consuming with two steps of analysis for estimating drug content in formulation.

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