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Simultaneous Estimation of Diacerein and Aceclofenac in Bulk and Pharmaceutical Dosage form by UV Spectroscopy Method

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Abstract: An accurate and precise UV spectrophotometric method was developed for the simultaneous determination of Diacerein (DIA) and Aceclofenac (ACF) from combined solid dosage form. The absorbance maxima at 257nm and 274 nm were selected for the determination of Diacerein and Aceclofenac respectively. UV-Spectroscopy was carried out with a solvent system composition of Methanol: Phosphate buffer pH-6.8 in the ratio of (25:75). The method was linear over the concentration range of 1-10mcg/ml and 2-18mcg/ml for both drugs of Diacerein and Aceclofenac with a correlation coefficient of 0.999. The percentage label claim was found to be 99.03±0.87% and 101.05±0.85% for Diacerein and Aceclofenac respectively. The method was showed good reproducibility, recovery studies and successfully applied for the determination of different brands of pharmaceutical formulations.
Keywords: Diacerein, aceclofenac, simultaneous equation methods, Pharmaceutical dosage form.

INTRODUCTION:

Chemically Diacerein[1] (DIA) is known as 4, 5-Bis (acetyloxy)-9, 10-dioxo-2-anthracenecarboxylic acid **(Fig.1)**. Diacerein is a novel anti-inflammatory drug and used for the treatment of not only Osteoarthritis but also used for rheumatoid arthritis when used in combination[2, 3]. Diacerein is a di-acetylated derivative of rhein, a molecule with an anthraquinone ring which is actually the active metabolite of Diacerein[4]. DIA is a selective inhibitor of interleukin-1 having protective effect on granuloma-induced cartilage breakdown by a reduction in the concentration range of proinflammatory cytokines[5, 6]. However DIA lacks cyclooxygenase inhibitory activity and hence shows no effect on prostaglandin synthesis. Therefore it has been considered as a slow-acting anti-arthritic drug.

Chemically Aceclofenac (ACF) is [o-(2, 6-dichloroanilino) phenyl] acetate glycolic acid ester (Fig.2) with anti-inflammatory and analgesic property [7]. It is used in various pain conditions like Rheumatoid arthritis, Osteoarthritis

Literature survey revealed that, two stability indicating HPLC methods have been reported for the quantitative estimation of DIA in bulk drugs [8] and in capsule dosage forms [9]. Two Impurities from DIA bulk drug have been isolated and structurally elucidated by HPLC and LC-MS methods respectively [10]. DIA has been also found to be estimated by chemiluminescence Technique in pharmaceutical dosage forms [11].

Figure 1: Chemical Structure of Diacerein (DIA).

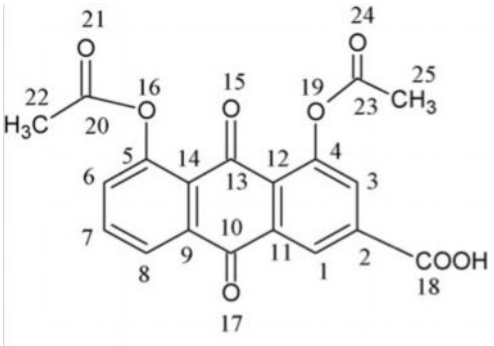
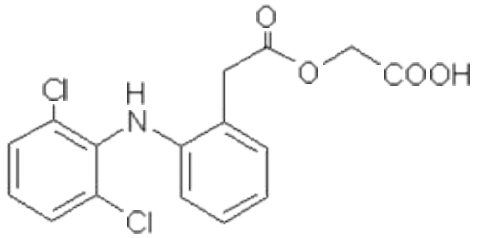


Figure 2: Chemical Structure of Aceclofenac (ACF)



Literature survey also revealed that Recently RP-HPLC method has been developed for the simultaneous estimation of rhein (the immediate metabolite of diacerein) and ACF in human plasma [12]. Fixed dose combination of DIA and ACF are available in market as tablets. After thorough literature review, it was found that, no simultaneous UV spectrophotometric method is available for the quantitative determination of DIA and ACF in pharmaceutical dosage form. A successful attempt has been made to estimate two drugs simultaneously by spectrophotometric analysis. The objective of the present work was to develop simple, rapid, accurate and specific UV spectrophotometric method for simultaneous determination of DIA and ACF in pharmaceutical dosage forms. The method had sufficiently good accuracy, precision and allowed a simple and cost effective assay for these compounds in mixtures.

MATERIALS AND METHODS:

Instrument:

A TECHOMP UV-visible spectrophotometer model 2310 was employed with matched quartz cell corresponding to 1cm path length and spectral band width of 1.5cm was used for the estimation. Digital balance (Sartorius BT 224S), Millipore direct Q3 System, digital pH meter MK-VI were employed.

Chemicals and Reagents:

DIA & ACF were kindly supplied as a gift samples by SAIN Pharma Pvt Ltd, Hyderabad and SHASHAN Pharmaceuticals Pondicherry respectively. DYCERIN-A tablets containing DIA (50mg) and ACF (100mg) were purchased from local pharmacy. All reagents were of Analytical grade.

Solvent composition used Methanol: Phosphate buffer P^H 6.8 (25:75)

Preparation of Standard Stock Solution:

DIA (25mg) and ACF (25mg) were accurately weighed and transferred to two separate 25ml volumetric flask, dissolved in the solvent composition of Methanol: Phosphate buffer p^H 6.8 (25:75) to obtain stock solution (1mg/ml). From the stock solutions, further dilutions were made to get concentration from 1-100 mcg/ml of DIA and ACF respectively. The two solutions were sonicated for 10 minutes and scanned in entire UV range to determine the λ max. DIA has λ max of 257nm and while ACF has the λ max at 274nm respectively. Both of these drugs show isobestic point at 284nm. The overlain spectrum of DIA and ACF was shown on **fig: 3**,from the stock solution, the Standard solutions were prepared having concentration range of 1-10mcg/ml and 2-18mcg/ml for DIA and ACF respectively.

The absorbance of these standard solutions was measured at 257nm and 274nm and calibration curves were plotted at these wavelengths. Optical characteristics and calibration curves of both these drugs were given in **Table: 1, 2. and fig: 4, 5**.

Table 1: Optical characteristics of Diacerein and Aceclofenac

PARAMETERS	DIA	ACF
Absorption maxima (λ max)	257	274
Beer's lamberts limit (mcg/ml)	1-10mcg/ml	2-18mcg/ml
Molar absorptivity	2.8387×10^5	1.0292×10^5
Co-efficient of correlation	0.999	0.999
Regression equation	Y=0.0034+0.08004x	Y=0.0269+0.0285x
Intercept (A)	0.0034	0.0269
Slope (B)	0.08004	0.0285
Sandell's sensitivity (µg/cm/0.001AU)	0.01297	0.033783
Stranded error	0.1000	0.0637

Table 2: Linearity range value of Diacerein and Aceclofenac

Linearity range of Diacerein			Linearity range of Aceclofenac	
S.No	**Concentration in (mcg/ml)**	**Absorbance at 257nm**	**Concentration in (mcg/ml)**	**Absorbance at 274nm**
1	1	0.0788	2	0.0694
2	2	0.1690	4	0.1224
3	3	0.2439	6	0.1912
4	4	0.3283	8	0.2558
5	5	0.3964	10	0.3116
6	6	0.4839	12	0.369
7	7	0.5647	14	0.4253
8	8	0.6434	16	0.4804
9	9	0.7258	18	0.547
10	10	0.8028	-	-

Figure 3: Overlain spectrum of Diacerein and Aceclofenac

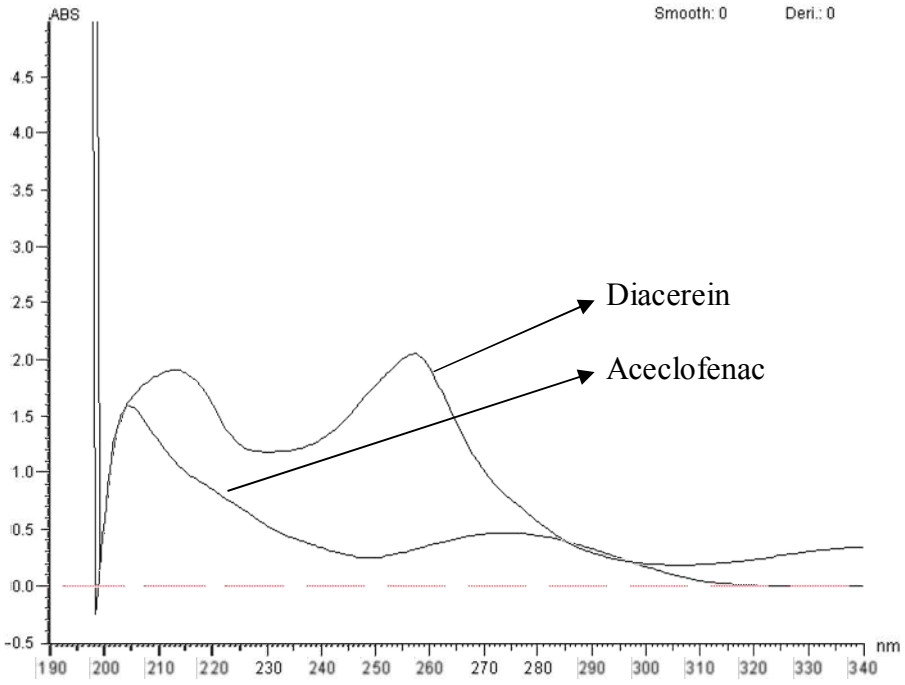
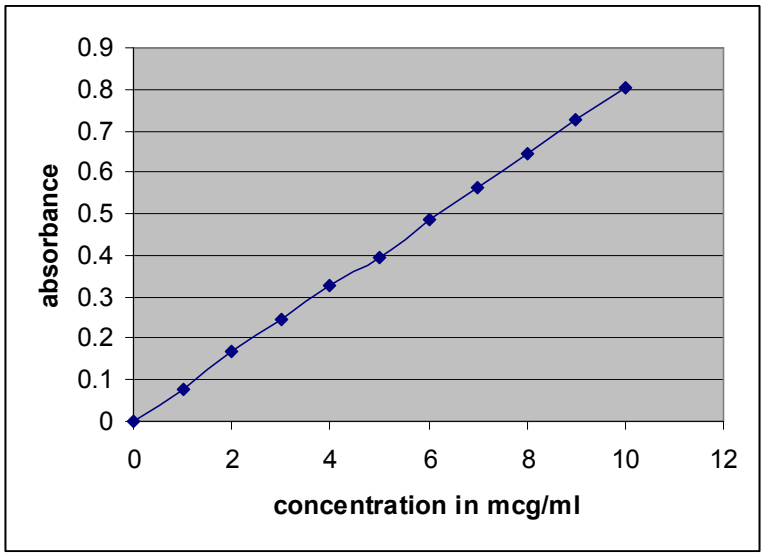


Figure 4: Calibration Curve of Diacerein



Optimization of concentration of the standards:
Concentration of 10mcg/ml of DIA and 10mcg/ml of ACF were used as standard 1 and standard 2. The simultaneous equations (in two variables C_x and C_y) were formed using these absorptivity coefficient values

$A_1 = (770.8)\ C_x + (335.9)\ C_y$ …….. 01
$A_2 = (204.6)\ C_x + (290.6)\ C_y$ …….. 02

Where C_x and C_y are the concentrations of DIA and ACF measured in g/100ml in the sample solutions. A_1 and A_2 are the absorbance mixture at selected wavelengths 257nm and 274nm respectively.

By applying the Cramens rule to equation 1& 2 the concentration CDIA and CACF can be obtained as follows.

$$C\ DIA = \frac{A2\ (204.6) - A1\ (290.6)}{335.9 \times 204.6 - 770.8 \times 290.6} \quad \ldots\ldots\ldots 03$$

$$C\ ACF = \frac{A1\ (335.9) - A2\ (770.8)}{335.9 \times 204.6 - 770.8 \times 290.6} \quad \ldots\ldots\ldots 04$$

Preparation and Analysis of tablet sample solution:
Twenty tablets each containing 50mg of Diacerein and 100mg of Aceclofenac were weighed and crushed to fine powder. An accurately weighed powder sample equivalent to 50mgof DIA &100mg of ACF was transferred to 50ml volumetric flask and the content was dissolved to 30ml with the composition of Methanol: Phosphate buffer p^H 6.8 (25:75). Shake well and the content was kept in ultrasonicator for 20 minutes finally the volume was made up to the mark with same solvent. The solution was filtered with membrane filter 0.45µ. The final concentration was made to 5mcg/ml Diacerein and 10mcg/ml Aceclofenac and was subjected to above method and the amount of DIA and ACF were determined from equation 3&4. Percentage label claim and standard deviation (S.D) was calculated and reported in **Table No. 3.**

Recovery studies:
In order to ensure the suitability and reliability of the proposed method recovery studies were carried out. Recovery studies were carried out by applying the method to pre-analyzed drug sample in tablet dosage form to which known amount of DIA and ACF corresponding to 80%, 100% and 120% of label claim was added (standard addition method).The mixed sample solutions were analyzed as per tablet formulation. At each level three determinations were performed. Results of recovery studies and statistical evaluation are shown in **Table No.4.**

Ruggedness:
The Ruggedness of an analytical method was determined by analysis of aliquots from homogenous lots by different analyst using operational and environmental conditions that may differ but were still within the specified parameters of the assay. The degree of reproducibility of the test results was then determined as a function of the assay variables. This reproducibility was assayed under normal conditions

to obtain a measure of the ruggedness of the analytical method.

The assay of Diacerein and Aceclofenac were performed in different conditions like different analyst on different days. The results of the ruggedness study were given in **Table No: 5**.

Table 3: Result of Marketed formulation Analysis

Product	Drug	Label Claim	% Label claim	S.D	R.S.D
DYCEREIN-A	DIA	50mg	99.03±0.37	0.3613	0.3662
	ACF	100mg	101.05±0.85	0.1911	0.1861

Mean of six Estimations: DIA= Diacerein, ACF = Aceclofenac.

Table 4: Result of recovery studies

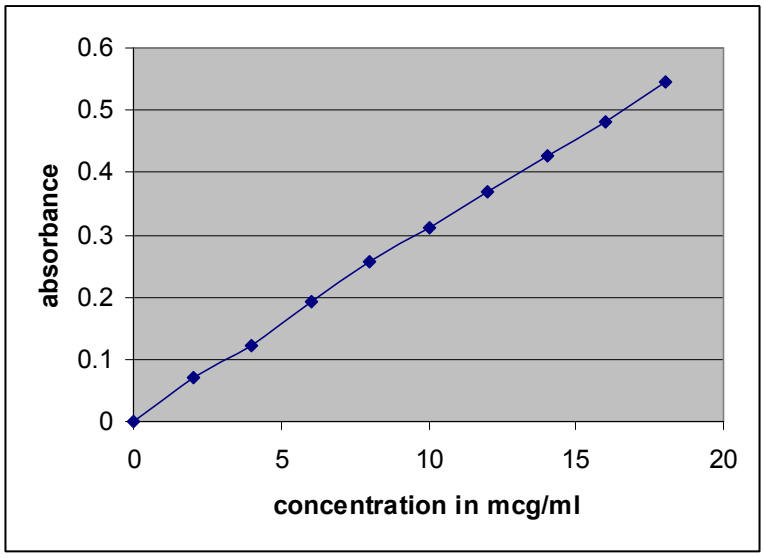
Level of Recovery	Amount of sample drug added (mcg/ml)	Amount of standard drug added (mcg/ml)	Drug	% Recovery	S.D±
80%	5	8	DIA	99.26	0.2531
	10	8	ACF	99.38	0.1268
100%	5	10	DIA	100.12	0.2472
	10	10	ACF	100.07	0.0223
120%	5	12	DIA	100.05	0.0316
	10	12	ACF	100.47	0.0264

Mean of 3 Estimations: DIA= Diacerein, ACF= Aceclofenac.

Table 5: Results of Ruggedness study

Day	% Label claim		S.D		R.S.D	
	DIA	ACF	DIA	ACF	DIA	ACF
Day-1	99.12±0.74	101.91±0.86	0.2913	0.3717	0.2960	0.3628
Day-2	99.50±0.56	100.98±0.91	0.0793	0.0806	0.0801	0.0786

Figure 5: Calibration Curve of Aceclofenac



RESULT AND DISCUSSION

The absorption maxima (λ_{max}) in UV spectra were observed at 257 nm and 274 nm for Diacerein and Aceclofenac. Diacerein and aceclofenac were showed linearity in the concentration range of 1-10µg/ml and 2-18 µg/ml. Absorptivity values for Diacerein and Aceclofenac were calculated. The quantitative estimation was carried out in tablet formulation by taking a concentration of 5µg/ml of Diacerein and 10 µg/ml of aceclofenac. The tablet formulation shows the percentage purity values 99.03±0.37% w/w for Diacerein 101.05±0.85% w/w for aceclofenac. The percentage deviation values were found to lie between ± 0.5 for Diacerein and +0.05 to 0.94 for Aceclofenac. The validation of the proposed simultaneous equation method was further confirmed by recovery studies. The percentage recovery values vary from 99.26 to 100.12% w/w for Diacerein and 99.38 to 100.11% w/w for Aceclofenac. This serves as a good index of accuracy and reproducing of the study. The quantitative results obtained were subjected to statistical analysis to find out standard deviation, relative standard deviation and standard error values. The relative standard deviation values are below 2% indicating the precision of the methodology and low standard error values show the accuracy of the method.

CONCLUSION

This method was found to be simple, specific and easy to perform and require very short time to analyze the pharmaceutical dosage forms. The solvent composition is simple to prepare and very economical when compare to other method of this drug analysis. The sample recoveries were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. The values of RSD are less than 2% indicating the accuracy and precision of the method. Linearity evaluated by the analyst, absorbance showed a good linear response over a wide range of concentration. The linearity, precision, accuracy of the method proves that the method is specific, accurate, easily reproducible and can be used for simultaneous estimation of diacerein and aceclofenac in pharmaceutical dosage form.

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REFERENCES:

[1] Tamura, T., Shirai, T., Kosaka, N., Ohmori, K., Takafumi, N., *Eur. J. Pharmacol.* 2002,*448*, 81-87.
[2] Toegel, S., Huang, W., Piana, C., Unger, F.M., Wirth, M., Gold ring, M.B. et al., *BMCMolecular Biology*. 2007**,** *8*, 13. DOI: 10.1186/1471-2199-8-13.
[3] Nicolas, P., Tod, M., Padoin, C., Petitjean,O., *Clin. Pharmacokinetic*. 1998, *35*, 347-359.
[4] Tamura, T., Ohmori, K., *Jpn. J. Pharmacol*. 2001**,** *85*, 101-104.
[5] Pelletier, J.P., Mineau, F., Fernandes, J.C., Duval, N., Martel-Pelletier, J., *J. Rheumatol.*1998, *25*, 2417-2424.
[6] Zawilla, N.H., Mohammad, M., Abdul, A., El Kousy, N.M., Ali, S.M., EI Moghazy*., J.Pharm. Biomed. Anal*. 2002, 243-251.
[7] La Villa G., Marra F., Laffi G., Belli B., Meacci E., Fascetti P. et al., *Eur J ClinPharmacol* .1989, *37, 1*-5.
[8] Reynolds, J.E., Prasad, B.A., *Martindale- The Extra Pharmacopoeia,* 30th ed.,Pharmaceutical Press: London, 1993, pp. 2.
[9] Brogden, R.N., Wiseman, L.R. Drugs, 1996**,** *52,* 113-124.
[10] Giannellini, V., Salvatore, F., Bartolucci, G., Coran, S.A., Bambagiotti-Alberti, M., *J Pharm Biomed Anal.* 2005**,** 776-780.
[11] Rao, J., Chauhan, K., Mahadik, K.R. and Kadam, S.S., *Indian J Pharm Sci.* 2009, 24-29.
[12] Chaudhari, A., Maikap, G., Deo, A., Vivek, K., Agrawal, H., Peshawe, U. et al, *J Pharm Biomed Anal.* 2009, 525-528.

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