Sarika Narade et al /J. Pharm. Sci. & Res. Vol.2(2), 2010, 137-142.

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Simultaneous UV spectrophotometric method for the determination of diacerein and aceclofenac in tablets

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Abstract:
A simple, rapid, accurate and precise UV spectrophotometric method was developed for the simultaneous determination of diacerein (DCN) and aceclofenac (ACF) in tablet dosage forms. The method involved solving simultaneous equations based on measurement of absorbance at two wavelengths, 258 nm and 274 nm, λmax of diacerein and aceclofenac respectively. The linearity for both diacerein and aceclofenac was in the range of 1-10 µg/ml and 5-40 µg/ml respectively. The % recovery was found to be 99-101 % and 99-100 % for diacerein and aceclofenac respectively indicating proposed method is accurate and precise for simultaneous estimation of DCN and ACF in tablets.

Keywords: Aceclofenac, diacerein, dosage form, simultaneous equation, UV spectrophotometry

Introduction:

Diacerein (DCN), chemically, 4,5-diacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid (Fig. 1A) is a chondroprotective agent used for the treatment of osteoarthritis [1, 2]. DCN is the di-acetylated derivative of rhein, a molecule with an anthraquinone ring which is actually the active metabolite of diacerein [3]. DCN is a selective inhibitor of interleukin-1 having protective effect on granuloma-induced cartilage breakdown by a reduction in the concentrations of proinflammatory cytokines [4, 5]. However, DCN lacks cyclooxygenase inhibitory activity and hence shows no effect on prostaglandin synthesis [6, 7]. Therefore, it has been considered as a slow-acting anti-arthritic drug not belonging to the NSAIDs that may interfere with the pathological course of osteoarthritis [3].

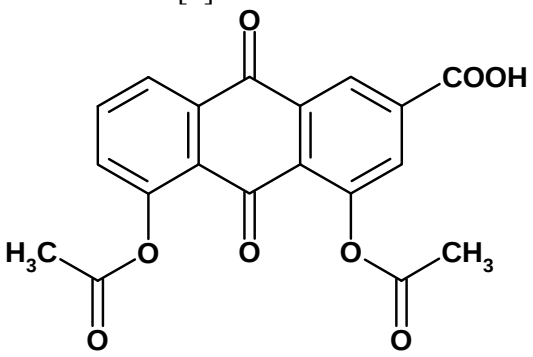

Fig. 1A: Chemical structure of diacerein (DCN).

Aceclofenac (ACF) is [o-(2,6-dichloroanilino)phenyl] acetate glycolic acid ester (Fig. 1B) with anti-inflammatory and analgesic properties [8]. It is used in various pain conditions like rheumatoid arthritis, osteoarthritis and ankylosing spondylatis [9].

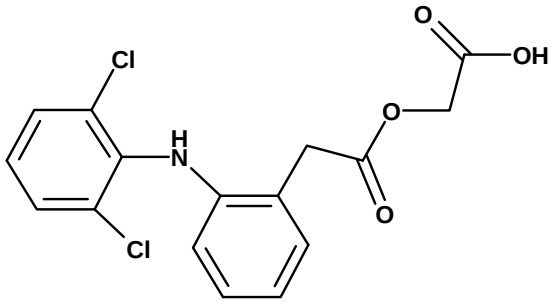

Fig. 1B: Chemical structure of aceclofenac (ACF).

Literature survey revealed that, two stability indicating HPLC methods have been reported for the quantitative estimation of DCN in bulk drugs [10] and in capsule dosage forms [11]. Two impurities from DCN bulk drug have been isolated and structurally elucidated by HPLC and LC-MS methods respectively [12]. DCN has been also found to be estimated by chemiluminescence technique in pharmaceutical dosage forms [13].

Literature survey also revealed that several analytical methods like colourimetric [14] spetrophotometric [15-17], spectroflurimetric [18] densitometric [19], HPLC [20-25] and stripping voltametric [26] have been reported for the estimation of ACF alone or in combination with certain drugs. Recently RP-HPLC method

has been developed for the simultaneous estimation of rhein (the immediate metabolite of diacerein) and ACF in human plasma [27]. Fixed dose combination of DCN 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 DCN 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 DCN 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:

Instrumentation

A Shimadzu 1700 UV (Shimadzu, Japan) spectrophotometer with 1 cm matched quartz cells was used for the estimation.

Chemicals and reagents

DCN and ACF were kindly supplied by Glenmark Pharmaceuticals Ltd., and Ajanta Pharmaceuticals Ltd., Mumbai, India, respectively as gift samples. Tablets containing DCN and ACF were procured from local pharmacy. All the reagents were of analytical grade. Glass double distilled water was used throughout the experiment.

Standard Preparation

Accurately weighed quantities (5 mg each) of DCN and ACF were dissolved separately in 10 ml of dimethylformamide (DMF) in a 50 ml volumetric flask. The solutions were shaken vigorously and the volume was adjusted up to the mark with distilled water to obtain a stock solution of 100 µg/ml; each of DCN and ACF. The solution was then filtered through Whatman filter paper No. 41. For the selection of analytical wavelength, the stock solutions of DCN and ACF were separately diluted in distilled water, to get concentrations of 20 µg/ml each, and scanned in the wavelength range of 200-400 nm. From the individual spectra of both drugs, wavelengths 258 nm (λmax of DCN) and 274 nm (λmax of ACF) were selected for the formation of simultaneous equation. For calibration curves, stock solutions of DCN and ACF were appropriately diluted to obtain concentration range of 1-10 µg/ml and 5-40 µg/ml respectively. The absorbance of both the drugs was measured at these two wavelengths and calibration curves were plotted. The absorptivity values (A1%, 1 cm) of each drug at both the wavelengths were calculated.

Sample preparation

For the estimation of drugs from the commercial formulations, twenty tablets of two brands Dycerin (Glenmark Ltd., Mumbai, India) and Arthocerin (Pulse Ltd., Secunderabad , India) containing 50 mg of DCN and 100 mg of ACF were weighed, and finely powdered. Quantity of powder equivalent to 5 mg of DCN and 10 mg of ACF was transferred to 50 ml volumetric flask, dissolved in 10 ml of DMF. The solution was shaken vigorously and the volume was adjusted up to the mark with distilled water to obtain a stock solution of 100 µg/ml of DCN and 200 µg/ml of ACF. The solution was then filtered through Whatman filter paper No. 41 and the filtrate was appropriately diluted to obtain final concentrations 5, 7.5 and 10 µg/ml of DCN and 10, 15, and 20 µg/ml of ACF. Absorbance of this solution was measured at appropriate wavelengths, and values were substituted in the respective formulae to obtain concentrations.

Results and Discussion:

A solvent system, dimethyl formamide (DMF): distilled water (1:4) was selected for the estimation of DCN and ACF, as both drugs are soluble in this composition of solvents. The UV spectra of standard solutions of DCN and ACF (20 µg/mL

each) were determined separately in DMF: distilled water (1:4) (Fig 2A and 2B). The λ_{max} of DCN and ACF were found to be 258 nm (λ_1) and 274 nm (λ_2) respectively. Both the solutions were found to be sufficiently stable throughout the experiment.

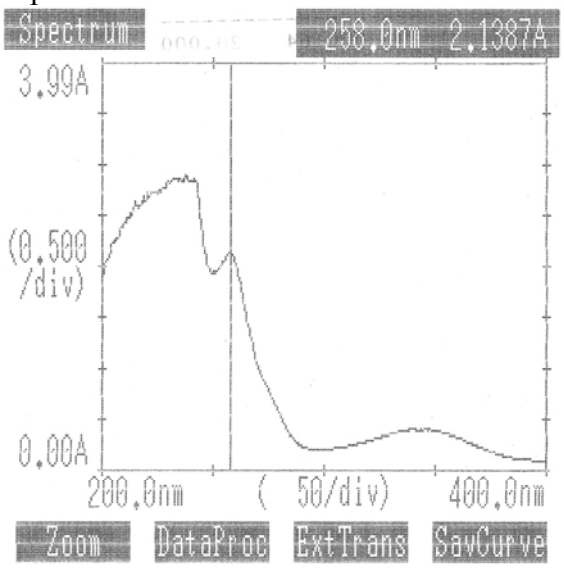

Fig. 2A: λ_{max} of diacerein (DCN) in DMF: distilled water.

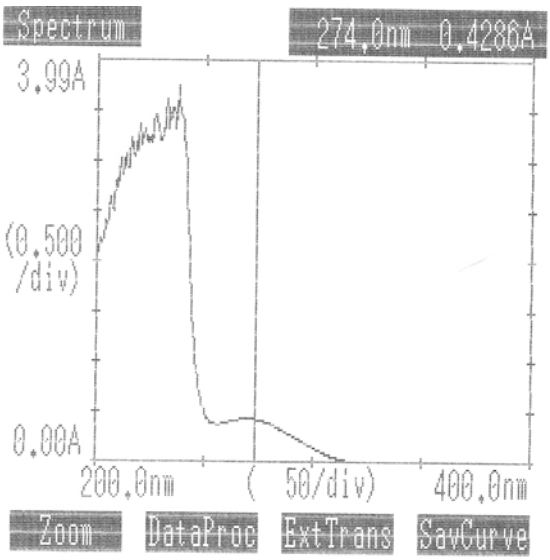

Fig. 2B: λ_{max} of aceclofenac (ACF) in DMF: distilled water.

Both the drugs DCN and ACF interfere with the UV absorption of each other. However Fig 2A and 2B show different absorption maxima. Therefore, modified Beer's law [28] (law of additivity) was used for their estimation in combined dosage form. The developed method was further validated according to ICH guidelines [29].

The method involved solving of simultaneous equations using Cramer's rule and matrices. The absorptivity values of both the drugs at λ_1 and λ_2 were determined by measuring the absorbance of different concentrations of drugs at these wavelengths. The absorptivity "*a*" was calculated from the equation

$$a = A/C \quad \ldots (1)$$

where, *a* is the extinction coefficient (absorptivity), *A* is the absorbance and *C* is the concentration (g/100ml). Two simultaneous equations were constructed from equation 1 as follows:

$$A_1 = a_{x1} C_X + a_{y1} C_Y \quad \ldots (2)$$

$$A_2 = a_{x2} C_X + a_{y2} C_Y \quad \ldots (3)$$

where, C_X and C_Y are the concentrations of DCN and ACF, respectively, A_1 is the absorbance of sample (mixture) at λ_1 i.e. 258 nm (λ_{max} of DCN), A_2 is the absorbance of sample (mixture) at λ_2 i.e. 274 nm (λ_{max} of ACF), a_{x1} is the absorptivity of DCN at 258 nm, a_{x2} is the absorptivity of DCN at 274 nm, a_{y1} is the absorptivity of ACF at 258 nm and a_{y2} is the absorptivity of ACF at 274 nm. Substituting the mean absorptivity values (Table 1) in equations 2 and 3, two simultaneous equations were framed for DCN and ACF at λ_1 and λ_2.

$$A_1 = 99.33\ C_X + 20.06\ C_Y \quad \ldots (4)$$

$$A_2 = 35.19\ C_X + 25.88\ C_Y \quad \ldots (5)$$

Solving the equations 4 and 5, the concentration of individual component was determined.

$$C_X = a_{y2}\ A_1 - a_{y1}\ A_2 / a_{x1}\ a_{y2} - a_{y1}\ a_{x2} \quad (6)$$

$$C_Y = a_{x2}\ A_1 - a_{x1}\ A_2 / a_{y1}\ a_{x2} - a_{x1}\ a_{y2} \quad (7)$$

The calibration curves were constructed for both DCN and ACF at both the selected wavelengths using appropriate dilutions of stock solutions. The precision and reproducibility of the method was checked by analyzing six replicate samples of the same concentration (n=6) of DCN

Table 1: Absorptivity values at 258 nm (λmax of DCN) and 274 nm (λmax of ACF).

Absorptivity at 258 nm (Mean ± S.D.) *		Absorptivity at 274 nm (Mean ± S.D.) *	
DCN	ACF	DCN	ACF
a_{x1}	a_{y1}	a_{x2}	a_{y2}
99.33 ± 0.85	20.06 ± 1.08	35.19 ± 1.11	25.88 ± 0.94

DCN: Diacrein; ACF: Aceclofenac; * Indicates mean of six experiments (n=6); S.D.: Standard deviation.

Table 2: Validation parameters for standard DCN and ACF.

Parameter	**DCN (258 nm)**	**ACF (274 nm)**
Molar absortptivity (l/mol/cm)	3.6555×10^4	9.161×10^3
Sandell's sensitivity ($\mu g/cm^2/0.001AU$)	0.010067	0.038642
Linearity range (µg/ml)	1-10	5-40
Correlation coefficient (r^2)	0.9993	0.9996
Intercept	0.005867	– 0.00139
Slope	0.09933	0.02588
Regression equation	$y = 0.09933x + 0.005867$	$y = 0.02588x - 0.00139$
LOD (µg/ml)	0.035	0.06
LOQ (µg/ml)	0.10	0.19
Precision (% RSD)*	0.75	1.01

DCN: Diacerein; ACF: Aceclofenac; * Indicates mean of six determinations (n=6).

Table 3: Analysis of dosage forms and recovery studies.

Product	Drug	Label claim	% Estimated *	% RSD	% Recovery *	% RSD
Dycerin A	DCN	50 mg	99.79	0.97	100.53	1.36
	ACF	100 mg	100.46	0.68	98.48	0.38
Arthocerin A	DCN	50 mg	98.63	0.89	99.33	1.52
	ACF	100 mg	98.51	1.15	99.53	0.74

DCN: Diacerein; ACF: Aceclofenac; * Indicates mean of three determinations (n=3).

and ACF. The low % RSD values obtained for DCN (0.75) and ACF (1.01) indicated that the method had high precision and reproducibility. The molar absortivity, Sandell's sensitivity, regression equation, slope, intercept, correlation coefficient, precision and linearity range are given in Table 2.

The sensitivity of the method was checked by determination of limit of detection (LOD) and limit of quantification (LOQ). LOD is the lowest detectable concentration of the analyte by the method while LOQ is the minimum quantifiable concentration. LOD and LOQ of DCN and ACF were calculated according to the equations 8 and 9 respectively at their respective λ_{max}

where, δ is the standard deviation of blank and s is slope of calibration [30].

$$\text{LOD} = \frac{3.3\sigma}{S} \qquad (8)$$

$$\text{LOQ} = \frac{10\sigma}{S} \qquad (9)$$

LOD and LOQ for DCN were 0.035 and 0.1 µg/ml respectively while LOD and LOQ for ACF were 0.06 and 0.19 µg/ml. (Table 2). These values indicate high sensitivity of the UV method.

For the determination of DCN and ACF from pharmaceutical tablet formulations by simultaneous equation method, the absorbance of sample solutions (mixture) were measured at λ_1 and λ_2 and values were substituted in the equations (equations 6 and 7) to obtain the concentrations of two components. The results obtained from analysis of dosage forms are presented in Table 3. It was observed that the developed method has estimated 98.63-99.79 % of DCN and 98.51-100.46 % of ACF from the marketed tablets giving satisfactory results for the estimation of individual components.

The accuracy and specificity of the proposed method was confirmed by recovery experiments. Recovery studies were carried out at 50, 100 and 150 % level by adding a known quantity of pure drug to the preanalyzed formulation and the proposed method was followed. From the amount of drug found, percentage mean recovery was calculated (Table 3). The % recovery for DCN and ACF were found to be in the range of 99.33-100.53 % (% RSD 1.52-1.36) and 98.48-99.53 % (% RSD 0.38-0.74) respectively for both the formulations analyzed. The high recovery rate with low % RSD values suggested that the method had a good accuracy and specificity, as there was no interference from the excipients present in formulations.

Intra-day precision and accuracy were determined by analyzing three samples of two different concentrations, prepared on same day. Inter-day variability was assessed by analyzing two concentrations on three different days. No significant difference was found in these experiments, indicating accuracy and reproducibility of the method. The % RSD values reported in Table 4 indicates that proposed method provides acceptable intra-day and inter-day variation of DCN and ACF.

Table 4: % RSD values for repeatability, intra- day, inter-day variation and ruggedness (n=3).

Parameter	DCN	ACF
Repeatability	1.35	0.36
Precision		
Intra-day	1.05	0.27
Inter-day	0.46	0.52
Ruggedness		
Analyst 1	0.71	0.58
Analyst 2	0.42	0.65

DCN: Diacerein; ACF: Aceclofenac.

Ruggedness of the proposed method was tested by analyzing DCN and ACF by different analysts, using similar operational and environmental conditions; the % RSD values are reported in Table 4 and found to be less than 2 %.

Robustness of the proposed method was determined by minor changes on the selected wavelength. Since the absorbance was not significantly affected, the proposed method could be considered as robust.

Conclusions:

The proposed method was successfully applied to the simultaneous determination of DCN and ACF from pharmaceutical tablet formulation. The presented method was found to be simple, accurate, precise, rugged and robust. It can be directly and easily applied to the analysis of the combined pharmaceutical tablet formulation of DCN and ACF. The present method is rapid and cost-effective. Therefore, it can be concluded that the proposed method provides an alternative procedure for the quality control of DCN and ACF in pharmaceutical formulations.

Acknowledgements:

We are grateful to Glenmark Pharmaceuticals Ltd., and Ajanta

Pharmaceuticals Ltd., Mumbai, India, for providing gift samples of drug for research work. We are thankful to Principal, Govt. College of Pharmacy, Karad for providing laboratory facilities and constant encouragement.

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