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UV-Spectrophotometric Methods for Determination of Aceclofenac and Diacerein in Pharmaceutical Formulation

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Abstract:

Three simple, specific, reproducible methods have been developed and validated for the simultaneous estimation of Aceclofenac and Diacerein in pharmaceutical formulation by UV-Spectrophotometric methods viz; Method I, Absorbance Correction method, Method II, Simultaneous Equation method and Method III, Absorbance Ratio method. For development of Method I, wavelengths selected were 277.0 nm λ_{max} for ACE and 341.5 nm λ_{max} for DIA. For method II, wavelengths selected were 256.5 nm and 277.0 nm for estimation of DIA and ACE respectively, while for Method III, 277.0 nm λ_{max} for ACE and 267.5 nm an isoabsorptive point of ACE and DIA. The two drugs follow Beer-Lambert's law over the concentration range of 10-50 µg/mL for ACE and 5-25 µg/mL for DIA for all three methods. The percent recovery of the drugs was found to be nearly 100 % representing the accuracy of the all three methods. Validation of the proposed methods was carried out for its accuracy, precision, specificity and ruggedness according to ICH guidelines. The proposed methods can be successfully applied in routine work for the determination of ACE and DIA in combined dosage form.

Keywords: *Aceclofenac, Diacerein, Absorbance Ratio method, Absorbance Correction method, Simultaneous Equation method.*

Introduction:

Diacerein is chemically known as 4, 5-Bis (acetyloxy) - 9, 10-dioxo-2-anthracene carboxylic acid [1]. Aceclofenac is chemically known as 2-[2-[2-(2, 6-Dichlorophenyl) amino phenyl] cetyl] oxyacetic acid [2]. Diacerein is used treatment of osteoarthritis while Aceclofenac is used as anti-inflammatory drug. Literature survey reveals that assay of Aceclofenac in bulk and dosage form is official in British Pharmacopoeia 2007[3] and Indian Pharmacopoeia 2007[4]. Several analytical methods that have been reported for estimation of Aceclofenac are Spectrophotometry [5-6], HPLC [7-10], Thin layer chromatography [11-12], LC-MS [13] and Fluorimetry [14]. Analytical methods reported for the estimation of Diacerein are Spectrophotometry [15], HPLC [16] and flow injection chemiluminescence [17]. To the best of our knowledge, there is no published spectrophotometric method for this combination. The present paper describes a simple, accurate and precise method for simultaneous estimation of Diacerein and Aceclofenac in combined tablet dosage form. The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [18]. Since no Spectrophotometric method is reported for simultaneous estimation of Diacerein and Aceclofenac in combination therefore, in the present work, a successful attempt has been made to estimate both these drugs simultaneously by three simple UV Spectrophotometric methods (absorbance correction method, Simultaneous equation method and Absorbance ratio method).

Materials and Methods:

Instrument:

SHIMADZU double beam UV-visible spectrophotometer (model 1700) with 1 cm matched quartz cuvettes were used for all absorbance measurements. Shimadzu AUX220 balance was used for weighing the samples**.** All the chemicals used were of AR grade. Double distilled water and Whatmann filter paper (no.41) were used throughout the experimental work.

Materials:

Multicomponent tablet Diacerein A (DIA 25.0mg and ACE 50.0mg) manufactured by Glenmark, Goa. All chemicals and reagents used were of AR grade.

Preparation of standard stock solution

An accurately weighed quantity of DIA and ACE was transferred to two different 50.0

mL volumetric flask, dissolved in 5.0 mL Dimethylacetamide, volume was made up to the mark with methanol to get standard stock solution of concentration 250µg/mL and 500µg/mL respectively by ultrasonicating for 20 min. Aliquots of the above solution was appropriately diluted in a 50.0 mL volumetric flask with methanol to get final concentration 5µg/mL and 10µg/mL for DIA and ACE. The standard solutions of both DIA (5µg/mL) and ACE (10µg/mL) were scanned in the range of 400-200nm in 1.0 cm cell against solvent methanol and spectra was recorded as shown in Fig. 1.

Absorbance correction method (Method I)

Absorbance correction method uses the absorbances at two selected wavelengths, one at λmax of one drug where other drug also shows considerable absorbance and other being the wavelength at which the first drug has practically nil absorbance. The study of spectra showed that Diacerein and Aceclofenac have λmax at 341.5 nm and at 277.0 nm, respectively. Both the drugs were found to have considerable absorbance at 277.0 nm. The wavelengths selected for analysis were 277.0 nm and 341.5 nm for Aceclofenac and Diacerein respectively. A series of standard solutions ranging from 10-50µg/mL for Aceclofenac and 5-25µg/mL for Diacerein both were prepared by diluting the aliquots of stock solutions and the absorbance of solutions was recorded at 277.0 nm and 341.5 nm. Calibration curve of absorbance versus concentration was plotted. The Calibration curves were found to be linear in the concentration range under study. The concentration of two drugs in mixture was calculated by using following equations:

$$Cdia = \frac{A_1}{ax_1} \qquad (1)$$

The concentration of the other drug is calculated by using the formula obtained by rearranging the equation 2 i.e.

$$Caceclo = \frac{A_2 - ax_2Cx}{ay_2} \qquad (2)$$

Where A_1 and A_2 are the absorbances of mixture at 341.5nm and 277.0nm and ax_1, ax_2 and, ay_2 are absorptivities of two drugs at 341.5 nm and 277.0 nm

Simultaneous equation method (Method II)

For the simultaneous equation method wavelengths selected were λmax of both the drugs, at the λmax of the Aceclofenac, Diacerein shows the considerable absorbance and at the λmax of Diacerein, Aceclofenac shows considerable absorbance. The study of spectra also reveals that Diacerein and Aceclofenac have λmax at 256.5 nm and at 277.0 nm respectively. Both the drugs were found to have considerable absorbance at λmax of each other. The wavelengths selected for analysis were 256.5 nm and 277.0 nm respectively for Diacerein and Aceclofenac. A series of standard solutions ranging from 5-25µg/mL for Diacerein and from 10-50µg/mL for Aceclofenac both were prepared and the absorbance of solutions was recorded at selected wavelengths. Calibration curve of absorbance versus concentration was plotted. The Calibration curves were found to be linear in the concentration range under study. The concentration of two drugs in mixture was calculated by using following equations:

$$Caceclo = \frac{A_2ay_1 - A_1ay_2}{ax_2ay_1 - ax_1ay_2} \qquad (3)$$

$$Cdia = \frac{A_1ax_2 - A_2ax_1}{ax_2ay_1 - ax_1ay_2} \qquad (4)$$

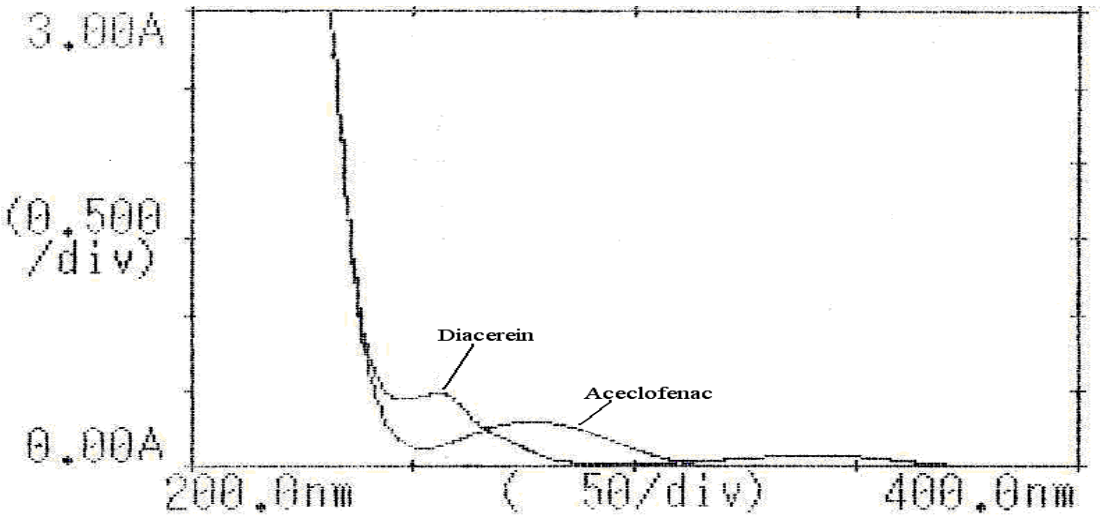

Fig. 1: Overlain spectra of Diacerein and Aceclofenac

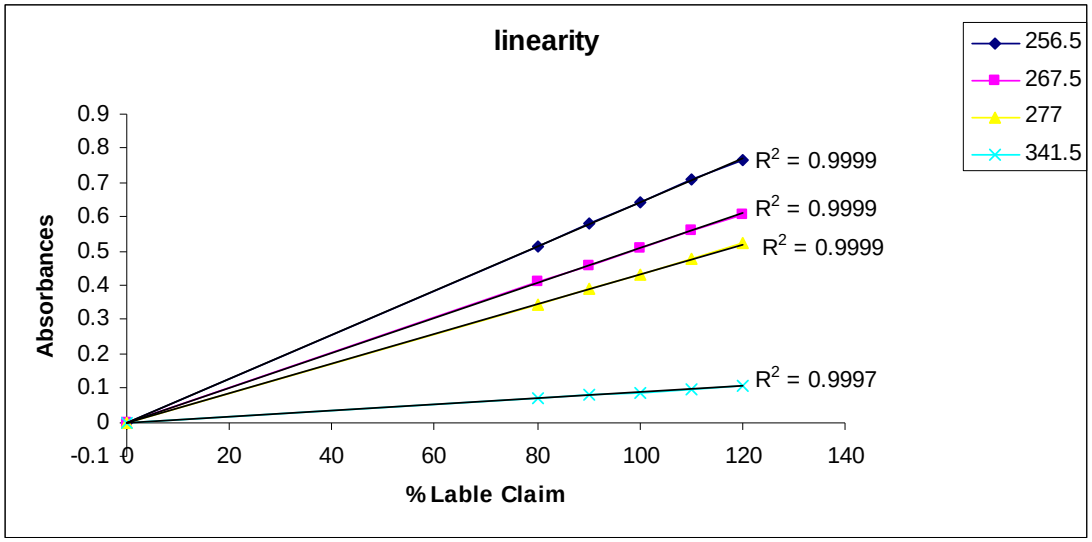

Fig. 2: Plot of Linearity and Range

Where A_1 and A_2 are the absorbances of mixture at 277.0nm and 256.5nm and ax_1, ay_1, ax_2 and ay_2 were absorptivity of Aceclofenac and Diacerein at 277.0nm and 256.5nm respectively.

Absorbance ratio method (Method III)

For the Absorbance ratio method two wavelengths selected were one λmax of Aceclofenac and another wavelengths at isobestic point were both the drugs shows same absorbance. The wavelength selected were an isobestic point at 267.5 nm and λmax at 277.0 nm of ACE. Both the drugs were found the same absorbance at 267.5 nm. The wavelengths selected for analysis were 267.5 nm and 277.0 nm for estimation of Diacerein and Aceclofenac.

Table 1: Optical properties and validation parameters

Beer's law range	Method I	Method II	Method III
Diacerein	5-25 µg/mL	5-25 µg/mL	5-25 µg/mL
Aceclofenac	10-50µg/mL	10-50µg/mL	10-50µg/mL
Wavelength (nm)	341.5 nm, 277 nm	256.5nm, 277nm	267.5nm, 277nm
Correlation Coeffi.	0.9999, 0.9999	0.9995, 0.9999	1, 0.9999
Linearity equation = y = mx+c			
Slope	0.0183, 0.0324	0.105, 0.0324	0.0359, 0.0324
%RSD			
Intraday precision	0.0, 0.18	0.007, 0.364	0.002, 0.208
Interday precision	1.95, 7.35	6.13, 10.01	5.03, 9.9

Method I; Absorbance correction method, Method II: Simultaneous equation method and Method III: Absorbance ratio method
*in case of Correlation Coeffi., slope & %RSD value Diacerein, Aceclofenac is mentioned

Table 2: Results of estimation of marketed formulation for Method I, II & III

Sr. No.	Wt. taken (In mg)	% Label Claim					
		Method (I)		Method (II)		Method (III)	
		DIA	ACE	DIA	ACE	DIA	ACE
1	59.5	99.55	98.47	100.58	98.09	98.45	98.88
2	59.49	101.11	97.95	97.92	99.13	98.23	98.99
3	59.08	100.67	100.52	99.28	100.03	100.69	100.5
4	63.59	100.72	99.11	99.63	99.51	100.50	99.19
5	59.8	100.63	100.4	101.37	100.44	100.13	100.88
Avg.		100.54	99.29	99.75	99.64	99.61	99.69
±SD		0.58	1.14	1.31	1.14	1.15	0.835
CV		0.59	1.13	1.31	1.14	1.15	0.83

Method I; Absorbance correction method, Method II: Simultaneous equation method and Method III: Absorbance ratio method

Table 3: Results of Recovery studies

Sr. No.	Amount of pure Drug added		% Recovery					
			Method (I)		Method (II)		Method (III)	
	DIA	ACE	DIA	ACE	DIA	ACE	DIA	ACE
1	2.8	5.3	100.39	99.81	98.18	98.81	100.19	98.48
2	5.23	10.2	99.39	100.31	100.28	100.86	98.91	100.34
3	7.8	15.3	98.78	99.48	97.98	98.78	99.57	99.29
4	10.1	20.6	99.79	99.87	98.79	99.15	100.48	99.6
Avg.			99.51	98.56	99.04	98.88	98.5	99.03
±SD			0.699	0.78	0.414	0.658	0.787	0.606
CV			0.696	0.769	0.409	0.651	0.776	0.599

Method I; Absorbance correction method, Method II: Simultaneous equation method and Method III: Absorbance ratio method

A series of standard solutions ranging from 10-50µg/mL for Aceclofenac and from 5-25µg/mL for Diacerein both were prepared and the absorbances of solution were recorded at selected wavelengths. Calibration curve of absorbance versus concentration was plotted. The Calibration curves were found to be linear in the concentration range under study and correlation coefficient values for method I, II and III are presented in Table 1. The concentration of two drugs in mixture was calculated by using following equations:

$$\text{Caceclo} = \frac{Qm - Qy}{Qx - Qy} \times \frac{A}{a_x} \quad (5)$$

$$\text{Cdia} = \frac{Qm - Qx}{Qy - Qx} \times \frac{A}{a_y} \quad (6)$$

Where, Qm was ratio of absorbance of laboratory mixture at λmax & isobestic point and Qx, Qy were ratio of absorptivity at λmax & isobestic point of Aceclofenac and Diacerein respectively and ax, ay absorptivity of Aceclofenac and Diacerein at isobestic point and 'A' was absorbance of mixture at isobestic point.

Assay of tablet formulation by method I, II & III

Twenty tablets were weighed and crushed to obtain fine powder. An accurately weighed tablet powder equivalent to about 12.5mg of Diacerein was transferred to 50.0 mL volumetric flask, dissolved in 5mL Diamethylacetamide and sonicated for 20 min. The volume was then made up to the mark using methanol as solvent. The resulting solution was filtered first through Whatmann filter paper and if particles found in filtrate then filtered through membrane filter paper. Filtrate was appropriately diluted to get concentration of 5µg/mL of Diacerein and 10µg/mL of Aceclofenac. Absorbances of sample solutions were recorded at 256.5 nm, 267.5 nm, 277.0 nm and 341.5 nm and the concentration of two drugs in the sample were determined by using eqns. 1 and 2 for Method-I, using eqns. 3 and 4 for Method-II and by using eqns. 5 and 6 for Method-III. Results of estimations are shown in Table 2.

Recovery studies

To study the accuracy of the proposed methods, recovery studies were carried out by standard addition method at four different levels. A known amount of drug was added to preanalyzed tablet powder and percentage recoveries were calculated. The results of recovery studies were satisfactory and are recorded in Table 3.

Precision

Precision was checked out by performing interday variation and intraday variation studies. In interday variation the absorbance for standard solution was measured on three consecutive days. In intraday variation the absorbances were measured three times in a day. The results of precision studies were satisfactory and are recorded in Table 1.

Linearity and Range

Accurately weighed quantities of tablet powder equivalent to 80, 90, 100, 110, and 120 % of label claim of DIA/ACE were taken and dilutions were made as described under marketed formulation. The absorbances of the resulting solutions were measured at 256.5 nm, 267.5 nm, 277.0 nm and 341.5 nm against blank. The graphs of concentration vs. absorbance were plotted and were found to be linear as shown in Fig 2.

Results and Discussion:

For all three methods linearity was observed in the concentration range of 5-25µg/mL for Diacerein and 10-50 µg/mL for Aceclofenac. Marketed brand of tablet was analyzed and amount of drug determined by

proposed methods ranges from 98 to 102% as shown in Table 2. The proposed methods were validated as per ICH guideline. The accuracy of method was determined at 80, 100 and 120 % level. The % recovery ranges from 98.50 to 99.56 for all the three methods. Precision was calculated as interday and intraday variations (% RSD found to be 9.9) for both drugs. From the interday and intraday studies it is supposed that the drug in solution state is stable for a period of 4h. The changes in percent label claims of Diacerein and Aceclofenac indicating some sort of degradation might have taken place. No attempt has been made to identify the degradation. The proposed methods were found to be simple, accurate and rapid for the routine determination of Diacerein and Aceclofenac in tablet formulation. All three methods can be successfully used for simultaneous estimation of Diacerein and Aceclofenac in combined dosage

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