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SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF DIACERHEIN AND ACECLOFENAC

Kirti S. Topagi[1], Purushotam K. Sinha[1], Rajesh M. Jeswani[1], Mrinalini C. Damle*
[1]Department of Pharmaceutical Chemistry ,AISSMS College of Pharmacy,Kennedy road, Near RTO,Pune – 411001, Maharashtra, India

Corres.author: mcdamle@rediffmail.com
Phone. No. – 9860230912
Fax No. – +91-020-26058208

ABSTRACT: Two simple spectrophotometric methods have been developed for simultaneous estimation of Diacerhein and Aceclofenac from tablet dosage form. Method I involves Absorbance correction method in which absorbance is measured at two wavelengths 340nm at which Aceclofenac has no absorbance and 275nm at which both the drugs have considerable absorbance. Method II Dual wavelength method which involves two wavelengths at which the drug has same absorbance are selected. The method was found linear between the range of 10µg/ml - 50µg/ml for both Diacerhein and Aceclofenac for both the methods. The accuracy and precision were determined and validated statistically. Both the methods showed good reproducibility and recovery with % RSD less than 1. The methods were found to be rapid, specific, precise and accurate and can be successfully applied for the routine analysis of Diacerhein and Aceclofenac in bulk and combined dosage form.
KEY WORDS: Diacerhein, Aceclofenac, Absorbance Correction method, Dual Wavelength method.

INTRODUCTION AND EXPERIMENTAL

Diacerhein is chemically known as 4,5-Bis(acetyloxy)-9,10-dioxo-2-anthracenecarboxylic acid[1]. Aceclofenac is chemically known as 2-[2-[2-(2,6-Dichlorophenyl)aminophenyl]cetyl] oxyacetic acid[2]. Diacerhein is used treatment of osteoarthritis. 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 Diacerhein 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 Diacerhein 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 Diacerhein and Aceclofenac in combination therefore, in the present work, a successful attempt has been made to estimate both these drugs simultaneously by two simple UV spectrophotometric methods (absorbance correction method, and Dual wavelength method). Instrument used is an UV-Visible double beam spectrophotometer Make Jasco (model V-550) with 1cm matched quartz cells. All weighing was done on Shimadzu balance (Model AY-120). Working standards of Diacerhein was obtained as gift sample from Lupin Research Park, Pune and Aceclofenac was obtained from NuLife Pharmaceuticals, Pune. Combined tablet formulation (DycerinA) was procured from local market. Methanol AR was used as solvent.

PREPARATION OF STOCK SOLUTION:

Accurately weighed quantity of Diacerhein (10 mg) and Aceclofenac (10 mg) was transferred to two separate 10 mL volumetric flasks, dissolved in 1mL

Diamethylacetamide because Diacerhein is only soluble in Diamethylacetamide and Dimethylsulfoxide. Later it was diluted to the mark with the methanol (stock solution:1000µg/mL).

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. From the stock solutions, working standard solutions of Diacerhein (20µg/ml) and Aceclofenac (20µg/ml) were prepared by appropriate dilution and were scanned in the entire UV range to determine the wavelengths. Diacerhein (20µg/ml) and Aceclofenac have λmax at 340nm and at 275nm, respectively. Both the drugs were found to have considerable absorbance at 275nm. The wavelengths selected for analysis were 275nm and 340nm respectively for Aceclofenac and Diacerhein. A series of standard solutions ranging from 10-50µg/mL for Diacerhein and Aceclofenac both were prepared and the absorbance of solutions was recorded at 275nm and 340nm to plot a calibration curve of absorbance versus concentration. The calibration
curves were found to be linear in the concentration range under study and are presented in Table-1.

The concentration of two drugs in mixture was calculated by using following equations:

$$C_{dia} = \frac{A_1}{a_{x1}} \qquad (1)$$

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

$$C_{Aceclo} = \frac{A_2 - a_{X2}C_x}{a_{Y2}} \qquad (2)$$

Where A1 and A2 are the absorbances of mixture at 340nm and 275nm and ax1, ax2 and, ay2 are absorptivities of two drugs at 340 nm and 275 nm.

DUAL WAVELENGTH METHOD (METHOD II)

From the stock solution of 1000µg/mL, working standard solutions of drugs were prepared by appropriate dilution and were scanned in entire UV range to determine the λmax. In this method, Aceclofenac was determined by plotting the difference in absorbance at 250.2 and 259.4 nm (difference is zero for Diacerhein) against the concentration of Aceclofenac. Similarly for the determination of Diacerhein, the difference in absorbance at 271.6nm and 279.8 nm (difference is zero for Aceclofenac) was plotted against the concentration of Diacerhein. Standard solutions were prepared having concentration 10-50 µg/mL for both drugs. The difference in absorbances at 250.2 and 259.4 nm were plotted against the concentration of Aceclofenac and that at 271.6nm and 279.8 nm were plotted against the concentration of Diacerhein to construct two separate calibration curves for Diacerhein and Aceclofenac. The method shows good linearity in concentration range of 10-50 µg/mL for both Diacerhein and Aceclofenac. The concentration of two drugs in mixture was calculated by using the calibration curve equation given in table 2.

ASSAY OF TABLET FORMULATION BY METHOD I & II:

20 tablets were weighed and crushed to obtain fine powder. An accurately weighed tablet powder equivalent to about 10mg of Diacerhein and 20mg of Aceclofenac was transferred to 10mL volumetric flask, dissolved in 1 mL Diamethylacetamide and sonicated for 15 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 filterate then filtered through membrane filter paper. Filtrate was appropriately diluted to get concentration of 10µg/mL of Diacerhein and 20µg/mL of Aceclofenac. Absorbances of sample solutions were recorded at 275nm and 340 nm and the concentration of two drugs in the sample were determined by using eqns. 1 and 2 (Method-I).

The tablet sample solution was also subjected to analysis by Dual Wavelength Method. Absorbances of sample solutions were recorded at selected wavelengths, differences were calculated and concentration of two drugs in the sample were determined by using calibration curve equation.

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.

RECOVERY STUDIES:

To study the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three 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 presented in Table-3.

RESULTS AND DISCUSSION

For both the two methods linearity was observed in the concentration range of 10-50 µg/ml for both Diacerhein and Aceclofenac. Marketed brand of tablet was analyzed and amount of drug determined by proposed methods ranges from 98 to 102% as shown in Table 4. 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 99.42 to 101.82 for both the two methods. Precision was calculated as interday and intraday variations (%RSD is

less than 1.5) for both drugs. The proposed methods were found to be simple, accurate and rapid for the routine determination of Diacerhein and Aceclofenac in tablet formulation. The two methods can be successfully used for simultaneous estimation of Diacerhein and Aceclofenac in combined dosage form.

Table 1: Validation Parameters for Absorbance Correction Method

PARAMETERS	Diacerhein	Aceclofenac
Beer's law range	10-50µg/mL	10-50µg/mL
Wavelength (nm)	340 nm	275 nm
Correlation Coefficient	0.9997	0.9963
Linearity equation = y = mx+c		
Slope	0.0141	0.0304
Intercept	0.0051	0.0383
LOD[1] (µg/mL)	0.391	0.308
LOQ[2] (µg/mL)	1.183	0.935
% RSD Intraday precision Interday precision	 0.8-1.17 1.08-1.44	 0.62-1.19 1.2-1.45

Table 2: Validation Parameters for Dual Wavelength Method

PARAMETERS	DIACERHEIN	ACECLOFENAC
Beer's law range	10-50µg/mL	10-50µg/mL
Wavelength (nm)	271.6nm and 279.8 nm	250.2 and 259.4 nm
Correlation Coefficient	0.9988	0.9958
Linearity equation = y = mx+c		
Slope	0.0206	0.0067
Intercept	0.0863	0.0036
LOD[1] (µg/mL)	0.88	0.55
LOQ[2] (µg/mL)	2.68	1.67
% RSD Intraday precision Interday precision	0.39 1.13	 0.31 1.33

Table 3: Recovery Studies

Level of Recovery	Amt. of Drug added µg/ml	Drug	Method I		Method II	
			Recovery (%)*	± SD*	Recovery (%)*	± SD*
80%	16	Dia	101.82	0.94	99.79	1.34
	16	Aceclo	100.13	0.52	99.42	1.45
100%	20	Dia	101.09	0.92	99.59	1.28
	20	Aceclo	99.94	0.57	99.52	0.87
120%	24	Dia	100.72	0.71	100.43	1.38
	24	Aceclo	100.68	0.04	99.95	0.70

*Mean of six estimations

Table 4: Results of simultaneous estimation of marketed formulation for Method I & II

Method	Label claim mg/tablet		%of label claim estimated ± S.D.	
	DIA	ACECLO	DIA	ACECLO
Method-I	50	100	98.82±0.11	100.12±0.68
Method-II	50	100	99.66±1.74	100.92±0.95

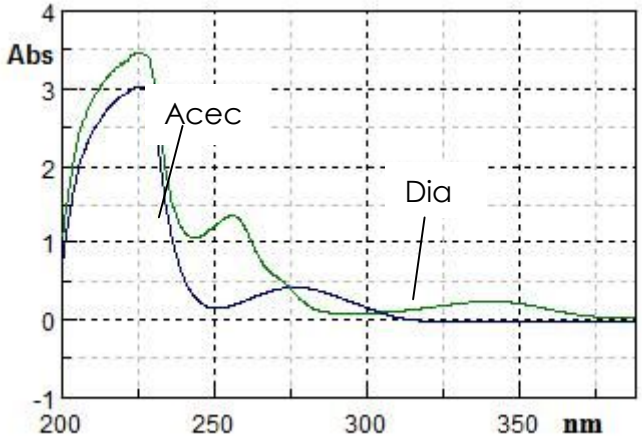

Figure 1:- Overlain spectra of Diacerhein and Aceclofenac

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