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Simultaneous Spectrophotometric Estimation of Diacerein and Aceclofenac in tablet dosage form

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Abstract

Three UV spectrophotometric methods for the simultaneous determination of Diacerein (DIA), and aceclofenac (ACE) in tablets were developed in the present work. Method I is simultaneous equation method, wavelength selected are 258.5 nm (λ_{max} of Diacerein) and 274 nm (λ_{max} of aceclofenac). Method II involves multicomponent mode of analysis, wavelength selected are 258.5 nm (λ_{max} of Diacerein) and 274 nm (λmax of aceclofenac). Method III is area under curve method, wavelength range selected are 263.5-253.5 nm for Diacerein and 279-269 nm for aceclofenac respectively. All the methods were found linear between 2-14 µg/ml for Diacerein and 4-28 µg/ml for aceclofenac. The accuracy and precision of the methods were determined and validated stastically which showed no significant difference between the results obtained by the three methods. The proposed methods are simple, accurate and can be used for its intended purpose.

Key Words: Diacerein, aceclofenac, simultaneous equation method, multicomponent mode of analysis, area under curve method

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Introduction

Chemically, Diacerein (DIA) is known as 4,5-Bis(acetyloxy)-9,10-dioxo-2-anthracenecarboxylic acid [1]. Diacerein is novel anti-inflammatory drug and use for the treatment of not only osteoarthritis but also for rheumatoid arthritis when use in combination [2]. Diacerein alone or in combination with other drugs is reported to be estimated by spectrophotometry [3], HPLC [4], & flow injection chemiluminiscence [5]**.** Chemically, Aceclofenac (ACE) is 2-[2-[2-(2,6-Dichlorophenyl)aminophenylacetyl]oxyacetic acid. Aceclofenac is used as anti-inflammatory drug [6]. Aceclofenac alone or in combination with the

other drugs is reported to be estimated by TLC-densitometry, differential spectrophotometry [7-9], LC-MS [10], HPLC [11-13], and fluorimetry [14] .

Since only two spectrophotomtric methods (Absorbance correction method, Dual wavelength method) are reported for the simultaneous estimation of Diacerein & aceclofenac in combination [15]. In the present work, a successful attempt has been made to estimate both these drugs simultaneously by three simple UV-spectrophotometric methods (Simultaneous equation method, Multicomponent mode of analysis [16] Area under curve method [17]).The proposed methods were optimized & validated as per ICH guidelines [18].

Results and Discussion

For all the methods linearity was observed in the concentration range of 2-14 µg/ml and 4-28 µg/ml for Diacerein and aceclofenac, respectively. Commercial formulations containing Diacerein and aceclofenac were analyzed by the proposed methods. Six replicate analysis of formulation were carried out and the mean assay values were found in the range of 100.06 to 101.07 % .The proposed methods were validated as per the ICH guidelines. The accuracy of the proposed method was determined by recovery studies. Pure Diacerein and aceclofenac was added to the preanalyzed tablet powder at three spiking levels viz 80, 100, 120 %. Three replicate analyses were carried out at each level. The mean percent recovery was found in the range of 99.78 to 101.76 % for all the methods. Precision is calculated as interday and intraday variations for both the drugs. Percent relative standard deviations for estimation of Diacerein and aceclofenac under intraday and interday variations were found to be less than 1.

Materials and Methods

Instrument: A double-beam Shimadzu UV- Visible spectrophotometer, 1700 Pharmaspec, with spectral bandwidth of 2 nm, wavelength accuracy ± 0.5 nm and a pair of 1-cm matched quartz cells was used to measure absorbance of solution.

Material: Standard gift samples of Diacerein and aceclofenac were provided by Glenmark pharmaceuticals Ltd, Mumbai. Combined dose tablet formulation containing Diacerein and aceclofenac (DYCERIN –A, 50 mg of Diacerein and 100 mg of aceclofenac, Manufactured by Glenmark), were purchased from local market.

Solvent Used*:* Methanol- AR was used as solvent.

Preparation of stock solution*:* Accurately weighed quantity of Diacerein (5 mg) and aceclofenac (5 mg) was transferred to two separate 50.0 ml volumetric flask. Diacerein is dissolved in 5ml dimethyl sulfoxide and aceclofenac is dissolved in 5 ml methanol. Then both the drug solutions were diluted to the mark with the methanol (Stock solution 100 µg/ml).

Method I: Simultaneous Equation Method

For the selection of analytical wavelength, solutions of Diacerein (4 µg/ml) and aceclofenac (8 µg/ml) were prepared separately by appropriate dilution of standard stock solution (100 µg/ml) with methanol and scanned in the spectrum mode from 400 nm to 200 nm. Diacerein has λ_{max} of

258.5 nm and aceclofenac has λ_{max} of 274 nm. Standard solutions were prepared having concentrations 2-14 µg/ml for Diacerein and 4-28 µg/ml for aceclofenac. The absorbances of these standard solutions were measured at selected wavelength and calibration curves were plotted. Two simultaneous equations (in two variables C_1 and C_2) were formed using following absorptivity coefficient values.

$A_1 = (0.1210)\ C_1 + (0.0257)\ C_2$ (1)
$A_2 = (0.0427)\ C_1 + (0.0345)\ C_2$ (2)

Where C_1 and C_2 are the concentrations of Diacerein and aceclofenac measured in µg/ml, in sample solutions. A_1 and A_2 are the absorbances of mixture at selected wavelengths i.e., 258.5 nm and 274 nm.

By applying the Cramer's rule to equation 1 and 2, the concentration C_{DIA} and C_{ACE}, can be obtained as follows,

$$C_{DIA} = \frac{A_2\,(0.0257) - A_1\,(0.0345)}{-\ 0.003084} \quad (3)$$

$$C_{ACE} = \frac{A_1\,(0.0427) - A_2\,(0.1210)}{-0.003084} \quad (4)$$

Method II: Multicomponent Mode of Analysis

In this method, six mixed standard solutions with concentration of Diacerein and aceclofenac in the ratio of 4:8 µg/ml were prepared in methanol. All the standard solutions were scanned over the range of 400-200 nm, in the multicomponent mode, using two working wavelength 258.5 nm (λ_{max} of Diacerein) and 274 nm (λ_{max} of aceclofenac). The data from these scans was used to determine the concentrations of two drugs in tablet sample solutions.

Method III: Area under curve method

From the overlain spectra of both drugs, area under the curve in the range of 263.5-253.5 nm (for Diacerein) and 279-269 nm (for aceclofenac) were selected for the analysis (Fig.1). The calibration curves for Diacerein and aceclofenac were plotted in the concentration range of 2-14 µg/ml and 4-28 µg/ml, respectively. The 'X' values for both the drugs were determined at the selected AUC range. The 'X'value is the ratio of area under the curve at selected wavelength ranges with the concentration of component in g/lit. These 'X' values were the mean of six determinations. A set of two simultaneous equations obtained by using mean 'X' values are given below.

$A_1 = (1.1088)\ C_1 + (0.2973)\ C_2$ (at $\lambda_{263.5\text{-}253.5}$nm) (5)
$A_2 = (0.4830)\ C_1 + (0.3517)\ C_2$ (at $\lambda_{279\text{-}269}$ nm) (6)

Where A1 and A_2 were area under curve of sample at the wavelength range 263.5-253.5 nm and 279- 269 nm, respectively. 1.1088 and 0.4830 were 'X 'values of Diacerein at wavelength

range 263.5-253.5 nm and 279- 269 nm respectively. Similarly 0.2973 and 0.3517 were 'X 'values of aceclofenac at wavelength range 263.5-253.5 nm and 279- 269 nm, respectively. The concentration of Diacerein and aceclofenac in sample was determined by using the equations (5) and (6).

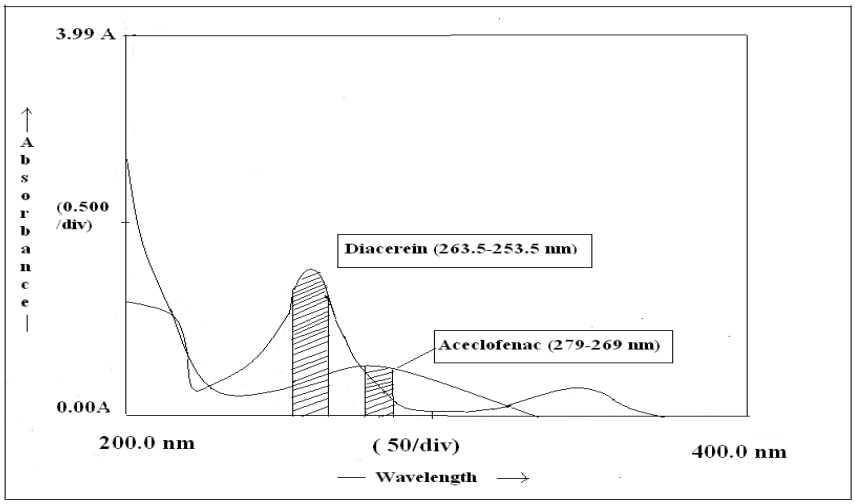

Fig.1: Overlain spectra of Diacerein and Aceclofenac.

Assay of tablet formulation by method I, II & III

For the estimation of drugs in the commercial formulations, twenty tablets were weighed and average weight was calculated. The tablets were crushed to obtain fine powder. Tablet powder equivalent to 4 mg Diacerein and 8 mg of aceclofenac was transferred to 50.0 ml volumetric flask; 5 ml dimethyl sulfoxide was added and sonicated for 20 min. The volume was then made up to the mark with methanol.

Table No – 1: Result of marketed formulation analysis

Method	Drug	Label Claim (mg/tablet)	Amount of drug estimated (mg/tablet)	% of label claim estimated ± S.D*
I	DIA	50	50.12	100.25 ± 0.89
	ACE	100	100.20	100.20 ± 0.65
II	DIA	50	50.25	100.50 ± 0.82
	ACE	100	100.06	100.06 ± 0.33
III	DIA	50	50.53	101.07 ± 0.49
	ACE	100	100.98	100.98 ± 0.89

* Mean of six estimation. DIA = Diacerein, ACE= Aceclofenac.

The resulting solution was filtered through Whatmann filter paper and filtrate was appropriately diluted to get approximate concentration of 4 µg/ml of Diacerein and 8 µg/ml of aceclofenac. In

method I, the concentration of both Diacerein and aceclofenac were determined by measuring absorbances of sample solutions at 258.5 nm (λ_{max} of Diacerein) and 274 nm (λ_{max} of aceclofenac) using equations (3) and (4). For method II, the same tablet sample solutions were subjected to analysis in the multicomponent mode of instrument, the concentration of both Diacerein and aceclofenac determined by analysis of spectral data of the sample solution with reference to the mixed standards at 258.5 nm (λ_{max} of Diacerein) and 274 nm (λ_{max} of aceclofenac). For method III, the concentration of both Diacerein and aceclofenac were determined by measuring area under curve in the range of 263.5-253.5 nm (for Diacerein) and 279-269 nm (for aceclofenac) and values were substituted in equations (5) and (6) to obtain concentration of both the drugs. Results of tablet analysis are shown in Table No. 1.

Validation

The proposed methods were validated as per ICH guidelines.

Accuracy

To ascertain the accuracy of the proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% &120%). The results of recovery studies were satisfactory and are presented in Table No.2.

Table No – 2: Result of recovery studies

Level of recovery	Amt. of Std. Drug added µg/ml	Drug	Method I		Method II		Method III	
			Recovery (%)*	± S.D*	Recovery (%)*	± S.D*	Recovery (%)*	± S.D*
80	3.2	DIA	100.41	0.14	100.84	0.30	100.50	0.48
	6.4	ACE	101.36	0.04	101.02	0.18	101.00	0.34
100	4.0	DIA	100.25	0.55	100.29	0.63	101.12	0.44
	8.0	ACE	101.21	0.41	100.27	0.15	100.09	0.68
120	4.8	DIA	100.94	0.17	100.03	0.23	99.78	0.01
	9.6	ACE	101.19	0.43	101.74	0.09	101.76	0.17

* Mean of three estimation. DIA = Diacerein, ACE= Aceclofenac.

Linearity

The linearity of measurement was evaluated by analyzing different concentration of the standard solution of Diacerein and aceclofenac. For all three methods, the Beer- Lambert's concentration range was found to be 2-14 µg/ml and 4-28µg/ml for Diacerein and aceclofenac respectively.

Precision

The reproducibility of the proposed methods were determined by performing tablet assay at different time intervals on same day (Intra-day assay precision) and on three different days (Inter-day assay precision). % RSD for intra-day assay precision was found to be 0.4085 (for Diacerein) & 0.8240 (for aceclofenac) in simultaneous equation method, 0.6496 (for Diacerein) & 0.4162 (for aceclofenac) in multicomponent mode method and 0.5352 (for Diacerein) & 0.5442 (for aceclofenac) in area under curve method. Inter-day assay precision % RSD was found to be 0.2939 (for Diacerein) & 0.8383 (for aceclofenac) in simultaneous equation method, 0.6037 (for Diacerein) & 0.3771 (for aceclofenac) in multicomponent mode method and 0.5291 (for Diacerein) & 0.4321 (for aceclofenac) in area under curve method.

Conclusion

The three proposed methods based on the spectrophotometry, were validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods are low, indicating high degree of precision of the methods. The results of the recovery studies performed show the high degree of accuracy of the proposed methods.
Hence, it can be concluded that the developed spectrophotometric methods are accurate, precise and selective and can be employed successfully for the estimation of Diacerein and aceclofenac in marketed formulation.

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