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# Development and Validation of analytical methods for Simultaneous Estimation of Diacerein and Aceclofenac in Bulk and Tablets using UV-visible spectroscopy

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**Abstract:** Three simple spectrophotometric methods have been developed for simultaneous estimation of Diacerein and Aceclofenac from tablet dosage form. 0.1M acidic methanol was used as solvent. First method, Simultaneous equation method, involves the measurement of absorbances at two wavelengths 256.0 nm (λmax of Diacerein) and 276.0 nm (λmax of Aceclofenac), Second method is First order derivative spectroscopy, wavelength selected for quantitation were 250.0 nm for Diacerein (zero cross for Aceclofenac) and 256.8 nm for Aceclofenac (zero cross for Diacerein) and third method is Area under curve method, area under curve in the range of 251.0-261.0 nm (for Diacerein) and 271.0-281.0 nm (for Aceclofenac) were selected for the analysis. The linearity lies between 2-30 µg/mL for Diacerein and Aceclofenac for the simultaneous equation and area under the curve method. For the first derivative method the linearity range is 5-50 µg/mL for both the drugs. The accuracy and precision of the methods were determined and validated statically. All the methods showed good reproducibility and recovery with % RSD less than 1. The proposed methods were found to be rapid, specific, precise, accurate and can be successfully applied for the routine analysis of Diacerein and Aceclofenac in bulk and combined dosage form.

**Key Words:** Diacerein, Aceclofenac, Simultaneous equation method, First order derivative spectroscopy, Area under curve method.

## Introduction:

Diacerein (DIA) 4,5-diacetoxy-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid ($C_{19}H_{12}O_8$) is a symptomatic slow acting drug for osteoarthritis. It shows efficacy on functional manifestations of osteoarthritis and on structural component[1,2]. In vitro experimentation revealed that rhein (4,5-dihydroxy-9,10-dihydro-9,10-dioxo-anthracene-2-carboxylic acid)- the active metabolite of Diacerein exerts its pharmacologic action by inhibiting IL-1 synthesis, release and down modulating its induced activities which in turn plays a fundamental role in osteoarthritis pathophysiology and cartilage destruction[3]. Methods reported in the literature to determine Diacerein in pharmaceutical formulations include spectrophotometric[4], RP-HPLC with UV detection[5,6] and flow Injection Chemiluminescence[7].

Aceclofenac (ACE) 2-[2-[2-[(2,6 dichloro phenyl) amino]phenyl]-acetyl]oxyacetic acid ($C_{16}H_{13}Cl_2NO_4$) is used as an effective non-steroidal anti-inflammatory drug (NSAID) derived from the phenylacetic acid with pronounced antirheumatic, anti-inflammatory, analgesic and antipyretic properties. Methods for analysis of Aceclofenac in tablet formulation include UV-visible spectrophotometric[8-11], spectro-fluorometric[12], adsorptive stripping voltammetric

techniques on conventional and surfactant chemically modified carbon paste electrodes[13]. Several spectrophotometric[14,15] methods have been developed for the determination of Aceclofenac in the presence of its degradation product, Diclofenac. HPLC[16-20] methods for analysis of Aceclofenac or in combination with other drugs in dosage forms have been described. UV detector has been used with most of the HPLC methods described.

A combination of these drugs, DIA (50 mg), and ACE (100 mg) is available as tablets for clinical practice. This combination is used for the treatment of osteoarthritis.

## Materials and Methods:

A double-beam Shimadzu 1700 UV-Visible spectrophotometer, 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 the resulting solution. Pure drug samples of Diacerein and Aceclofenac were obtained as gift sample from Lupin Pharmaceuticals Ltd. and Emcure Pharmaceuticals Ltd. Pune respectively. Combined dose Diacerein and Aceclofenac tablets (DYCERIN-A, 50 mg Diacerein and 100 mg Aceclofenac; manufactured by Glenmark Pharmaceuticals Ltd.), were purchased from local market. 0.1M acidic methanol was used as solvent.

**Preparation of stock solution:** Standard stock solution of Diacerein and Aceclofenac were prepared by dissolving 10 mg of each drug in 50 mL of 0.1M acidic methanol, sonicated then volume made upto100 mL with the same solvent to get a concentration of 100 µg/mL solution.

**Determination of Absorption Maxima:** By appropriate dilution of two standard drug solutions with 0.1M acidic methanol, solutions containing 10 µg/mL of Diacerein and 20 µg/mL of Aceclofenac were scanned separately in the range of 200-400 nm to determine the wavelength of maximum absorption for both the drugs. Diacerein showed absorbance maxima at 256 nm and Aceclofenac at 276 nm (Fig. 1).

**Simultaneous equation method (Method I):** From the stock solution (100 µg/mL), working standard solutions of drugs were prepared by appropriate dilution and were scanned in entire UV range to determine the λmax. Standard solutions were prepared having concentration 2-20 µg/mL for Diacerein and Aceclofenac. The absorbance of these standard solutions were measured at 256.0 nm and 276.0 nm and calibration curves were plotted. Two simultaneous equations (in two variables $C_x$ and $C_y$) were formed using these absorptivity coefficient values.

$A1 = 0.1120\ C_x + 0.0183\ C_y$ (1)

$A2 = 0.0262\ C_x + 0.0338\ C_y$ (2)

Where,

$C_x$ and $C_y$ are the concentration of DIA and ACE measured in µg/mL, in sample solutions. A1 and A2 are the absorbance of mixture at 256.0 nm and 276.0 nm wavelength respectively. By applying the Cramer's rule to equation 1 and 2, the concentration $C_{DIA}$ and $C_{ACE}$, can be obtained as follows,

$$C_{DIA} = \frac{A2\ (0.0183) - A1\ (0.0338)}{0.00330614}$$

$$C_{ACE} = \frac{A1\ (0.0262) - A2\ (0.1120)}{0.00330614}$$

**First order derivative spectroscopy (Method II):** In this method solutions of DIA (10 µg/mL) and ACE (20 µg/mL), were prepared separately by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. The absorption spectra thus obtained were derivatized from first to fourth order. First order derivative spectra were selected for analysis of both drugs. From the overlain spectra of both drugs (Fig. 2), wavelength selected for quantitation were 250.0 nm for DIA (zero cross for ACE) and 255.8 nm for ACE (zero cross for DIA). The calibration curves for DIA and ACE were plotted in the concentration range of 5-50 µg/ml at wavelength 250.0 nm and 255.8 nm, respectively. The concentration of the individual drug present in the mixture was determined against the calibration curve in quantitation mode.

**Area under curve method (Method III):** From the overlain spectra of both drugs (Fig. 3), area under the curve in the range of 251.0-261.0 nm (for Diacerein) and 271.0-281.0 nm (for Aceclofenac) were selected for the analysis. The calibration curves for Diacerein and Aceclofenac were prepared in the concentration range of 2- 20 µg/ml at their respective AUC range. The 'X' values of the drugs were determined for both the drugs at the selected AUC range. The 'X' is the ratio of area under the curve at selected wavelength ranges with the concentration of component in gm/lit. These 'X' values were the mean of six independent determinations. A set of two simultaneous equations obtained by using mean 'X' values are given below.

$A1 = 1092.6626\ C_{DIA} + 220.7456\ C_{ACE}$ -(at $\lambda_{251.0\text{-}261.0}$ nm) -- (3)

$A2 = 310.0899\ C_{DIA} + 388.8992\ C_{ACE}$ -(at $\lambda_{271.0\text{-}281.0}$ nm) -- (4)

Where A1 and A2 were area under curve of sample at the wavelength range 251.0-261.0 nm and 271.0-281.0 nm, respectively, 1092.6626 and 310.0899 were 'X' values of DIA at wavelength range 251.0-261.0 nm and 271.0-281.0 nm, respectively. Similarly 220.7456 and 388.8992 were 'X' values of ACE at the wavelength range 251.0-261.0 nm and 271.0-281.0 nm, respectively. $C_{DIA}$ and $C_{ACE}$ were concentration of Diacerein and Aceclofenac, respectively. The concentration of Diacerein and Aceclofenac in sample was determined by using the equation (3) and (4).

**Application of the proposed method for the determination of DIA and ACE in tablets**

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 10 mg DIA was transferred to 100.0 ml volumetric flask and volume made up to the mark with 0.1M acidic methanol and ultrasonicated for 10 minutes. The solution was then filtered through a Whatmann filter paper (No. 41). From the filtrate 1.0 ml was transferred to a 10.0 ml volumetric flask and diluted to the mark with the same solvent to obtain 10 µg/ml DIA and 20 µg/ml of ACE. Absorbance of sample solutions were recorded at 256.0 nm and 276.0 nm and the concentration of two drugs in the sample were determined by using eqns. 1 and 2 (Method-I).

The concentration of both DIA and ACE were determined by measuring the absorbance of the sample at 250.0 nm and 255.8 nm in first order spectrum mode. The results of the tablet analysis were calculated against the calibration curve in quantitation mode (Method II).

For Method-III, the concentration of both DIA and ACE were determined by measuring area under curve in the range of 251.0-261.0 nm (for DIA) and 271.0-281.0 nm (for ACE) and values were substituted in the respective formula to obtain concentrations. The analysis procedure was repeated for 6 times with tablet formulations. The results are reported in Table- 1.

**Validation**

The methods were validated with respect to linearity, accuracy, precision and selectivity.

**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% (table 2). The mean percent recovery for DIA and ACE by all the three methods was found in the range of 99.86 % to 100.36 %.

**Linearity** The linearity of measurement was evaluated by analyzing different concentration of the standard solution of DIA and ACE. For simultaneous equation and area under the curve method, the Beer-Lambert's concentration range was found to be 2-30 µg/ml and for derivative spectrophotomety 5-50 µg/mL for both DIA and ACE.

**Precision** The reproducibility of the proposed method was determined by performing tablet assay at different time intervals (morning, afternoon and evening) on same day (Intraday assay precision) and on three different days (Interday precision). Result of intraday and interday precision is expressed in % RSD (table 3). Percent RSD for Intraday assay precision was found to be 0.1753 (for DIA) and 0.0539 (for ACE) in simultaneous equation method; 0.1392 (for DIA) and 0.1174 (for ACE) in first derivative spectrophotometric method and 0.1825 (for DIA) and 0.0794 (for ACE) in area under the curve method. Interday assay precision was found to be 0.1649 (for DIA) and 0.0683 (for ACE) in simultaneous equation method; 0.1196 (for DIA) and 0.0983 (for ACE) in first derivative spectrophotometric method and 0.1470 (for DIA) and 0.0816 (for ACE) in area under the curve method.

**Table No. 1: Results of Analysis of Tablet Formulation**

| Method | Tablet content | Label claim (mg/tab) | Amount Found* | | ±SD* | RSD (%)* |
|---|---|---|---|---|---|---|
| | | | (in mg) | (in %) | | |
| I | DIA | 50.0 | 49.9748 | 99.9496 | 0.0912 | 0.2282 |
| | ACE | 100.0 | 100.0537 | 100.0537 | 0.0230 | 0.0117 |
| II | DIA | 50.0 | 49.9945 | 99.9890 | 0.0793 | 0.1625 |
| | ACE | 100.0 | 100.0161 | 100.0161 | 0.1169 | 0.1703 |
| III | DIA | 50.0 | 50.1662 | 100.3324 | 0.1155 | 0.2317 |
| | ACE | 100.0 | 99.9167 | 99.9167 | 0.0913 | 0.0958 |

*denotes n = 6, average of six determinations; DIA = Diacerein; ACE = Aceclofenac

**Table No. 2: Results of recovery studies**

| Level of recovery | Amount of drug added µg/mL | Drug | Method I | | Method II | | Method III | |
|---|---|---|---|---|---|---|---|---|
| | | | Recovery (%)* | ±SD* | Recovery (%)* | ±SD* | Recovery (%)* | ±SD* |
| 80% | 8.0 | DIA | 99.94 | 0.0927 | 100.36 | 0.0438 | 100.02 | 0.0483 |
| | 16.0 | ACE | 99.96 | 0.2281 | 100.25 | 0.0604 | 100.10 | 0.0729 |
| 100% | 10.0 | DIA | 100.05 | 0.1013 | 99.98 | 0.1307 | 99.93 | 0.0914 |
| | 20.0 | ACE | 99.89 | 0.1261 | 100.01 | 0.0735 | 100.31 | 0.0362 |
| 120% | 12.0 | DIA | 100.21 | 0.0859 | 100.02 | 0.0819 | 99.86 | 0.0973 |
| | 24.0 | ACE | 100.08 | 0.1463 | 99.98 | 0.1003 | 99.90 | 0.0711 |

*Mean of six estimations; DIA = Diacerein; ACE = Aceclofenac

**Table No. 3: Results of intermediate precisions**

| Day | Method I | | Method II | | Method III | |
|---|---|---|---|---|---|---|
| | % Label claim estimated* (Mean ± % R.S.D.) | | % Label claim estimated* (Mean ± % R.S.D.) | | % Label claim estimated* (Mean ± % R.S.D.) | |
| | DIA | ACE | DIA | ACE | DIA | ACE |
| Intraday | 49.9608 ± 0.1753 | 100.0917 ± 0.0539 | 49.9735 ± 0.1392 | 100.0416 ± 0.1174 | 50.0479 ± 0.1825 | 99.9418 ± 0.0794 |
| Interday | 49.9327 ± 0.1649 | 100.0630 ± 0.0683 | 50.0491 ± 0.1196 | 100.0657 ± 0.0983 | 50.0619 ± 0.1470 | 99.9619 ± 0.0816 |

**Table No. 4: Linear regression analysis of calibration curves with their respective absorptivity values.**

| Parameters | Method I | | Method II | | Method III | |
|---|---|---|---|---|---|---|
| | DIA | ACE | DIA | ACE | DIA | ACE |
| Beer's law limit (µg/mL) | 2-30 | 2-30 | 5-50 | 5-50 | 2-30 | 2-30 |
| Correlation coefficient (r) | 0.9989 | 0.9995 | 0.9991 | 0.9996 | 0.9984 | 0.9987 |
| Molar absorptivity (L/mole/cm) | 41234 | 11957 | 1118 | 22954 | 17391 | 21036 |
| Sandell's sensitivity (mcg/Sq.cm/0.001) | 0.0089 | 0.0296 | 0.3295 | 0.0201 | 0.0232 | 0.0218 |
| Slope | 0.1114 | 0.0334 | 0.0030 | 0.001 | 1.0881 | 0.3863 |
| Intercept | 0.0044 | 0.0004 | 0.0007 | 0.0003 | 0.0258 | 0.0277 |
| Limit of Detection (µg/mL) | 0.019 | 0.023 | 0.049 | 0.057 | 0.020 | 0.019 |
| Limit of Quantitation (µg/mL) | 0.060 | 0.072 | 0.151 | 0.176 | 0.061 | 0.060 |

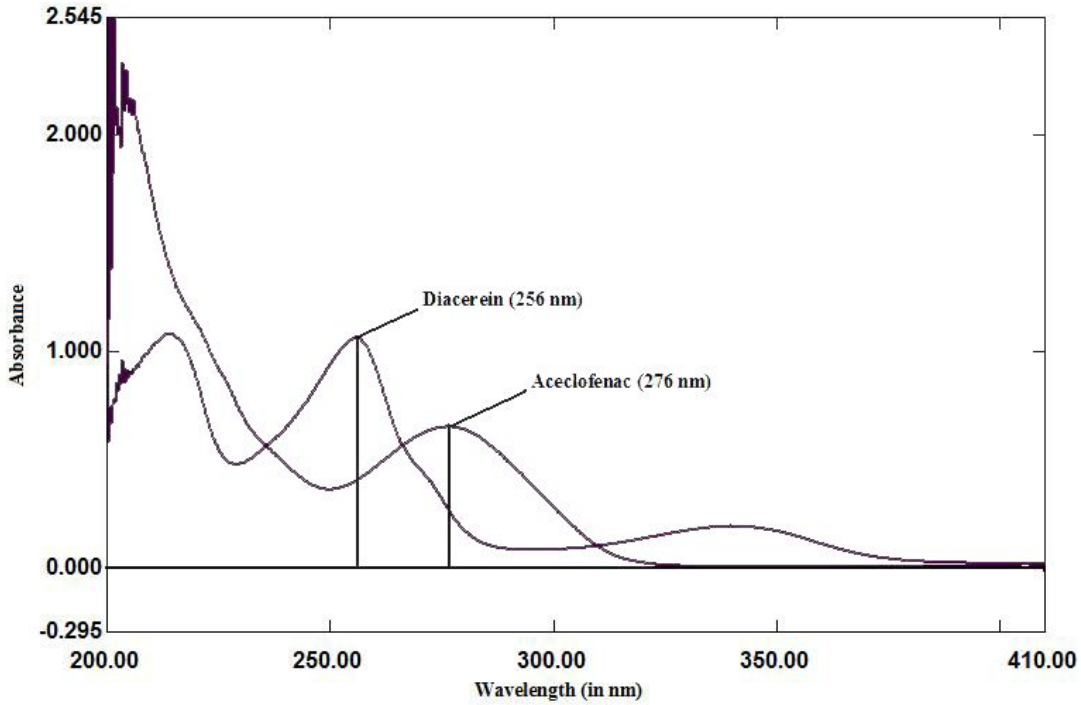


**Fig. 1: Overlain spectra of Diacerein (DIA) and Aceclofenac (ACE).**

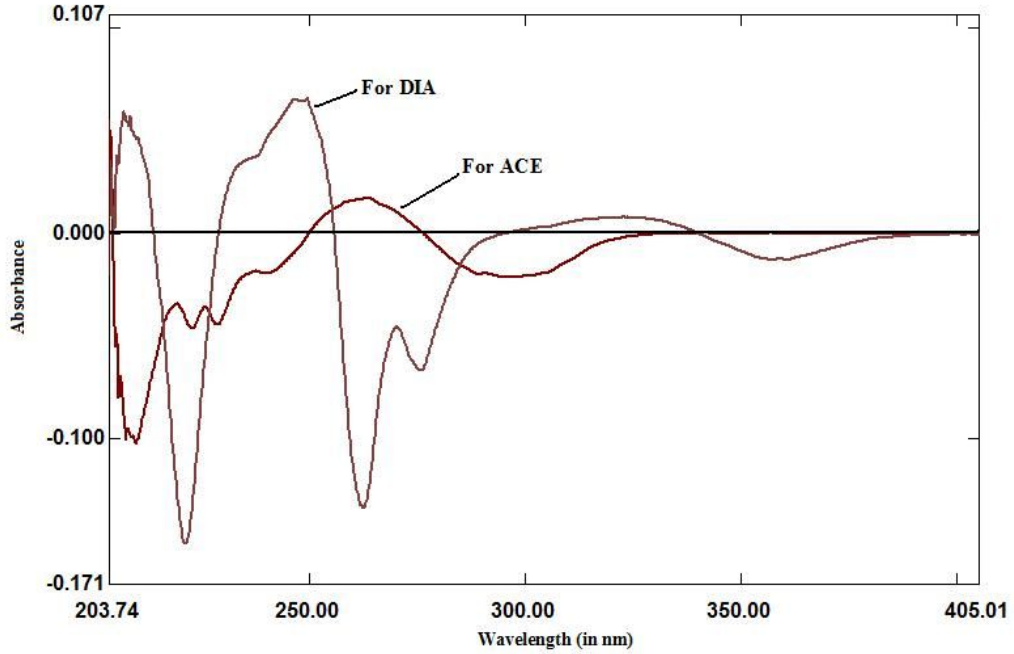


**Fig. 2: Overlain First order derivative Spectra of Diacerein and Aceclofenac.**

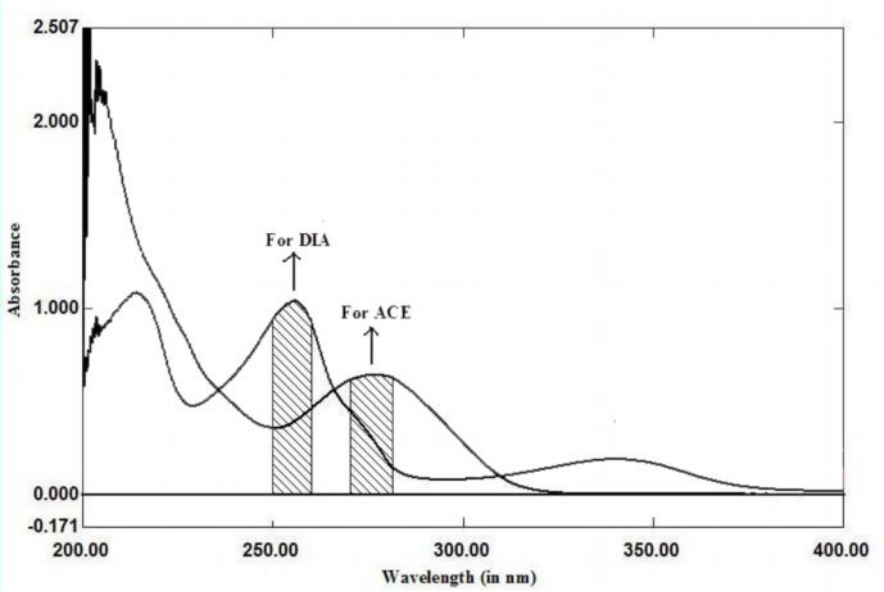


**Fig. 3: Overlain spectra of Diacerein (DIA) and Aceclofenac (ACE) in Method III.**

## Results and Discussion

The methods discussed in the present work provide a convenient and accurate way for simultaneous analysis of DIA and ACE. In simultaneous equation method, wavelength selected for quantitation were 256.0 nm (λmax of DIA) and 276.0 nm (λmax of ACE). In first order derivative spectroscopy, wavelengths selected for quantitation were 250.0 nm for DIA (zero cross for ACE) and 255.8 nm for ACE (zero cross for DIA). In area under curve method, the area under curve in the range of 251.0-261.0 nm (for DIA) and 271.0-281.0 nm (for ACE) were selected for the analysis. The optical characteristics such as Beer's law limits, molar absorptivities and Sandell's sensitivities are presented in [Table 4]. Percent label claim for DIA and ACE in tablet analysis, by all the three methods, was found in the range of 99.9167 % to 100.3324 %. Standard deviation and coefficient of variance for six determinations of tablet sample, by all the methods, was found to be less than ± 2.0 indicating the precision of both the methods. Accuracy of proposed methods was ascertained by recovery studies and the results are expressed as % recovery. Percent recovery for DIA and ACE, by all the methods, was found in the range of 99.86 % - 100.36 %, values of standard deviation and coefficient of variation was satisfactorily low indicating the accuracy of both the methods. Based on the results obtained, it is found that the proposed methods are accurate, precise, reproducible & economical and can be employed for routine quality control of Diacerein and Aceclofenac in combined dose tablet formulation.

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