The Simultaneous Estimation of Diacerein and Aceclofenac in Bulk Drugs and Pharmaceutical Dosage Forms Using a Novel Rp-Hplc Method

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***Abstract*—A reverse-phase high-pressure liquid chromatographic approach (RP-HPLC) was developed and validated to quantify Aceclofenac and Diacerein in pharmaceutical dosage forms and bulk pharmaceuticals. The chromatographic separation of the two drugs was investigated with a PHENOMENEX C18 (250 X 4.6mm I.D., 5µm) column. The mobile phase consists of 0.01M potassium dihydrogen orthophosphate and acetonitrile in a 75:25 ratio at a flow rate of 1.0 mlper minute. A dual wavelength UV detector operating at room temperature was used to measure absorbance at 241 nm. Diacerein and Aceclofenac had retention periods of 3.892 and 5.877 minutes, respectively. Aceclofenac and Diacerein had correlation values of 0.9973 and 0.9907, respectively. Linearity was seen in both drugs in the 5–50 µg/ml and 10-90 µg/ml ranges. Aceclofenac and Diacerein's respective percentage RSDs were as follows. Both within-day and between-day accuracy assessments revealed that the suggested approaches' relative standard deviation (%RSD) was less than the upper limit of 2.0. ICH principles were followed in the validation of the suggested approach. The quantitative analysis of aceclofenac and diacerein in pharmaceutical formulations and bulk pharmaceuticals was proven to be a straightforward, affordable, appropriate, precise, and dependable method.**

***Index Terms*—RP-HPLC, Diacerein, Aceclofenac, UV.**

I. INTRODUCTION

Diacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylic acid, or diacerein (DCN), is a chondroprotective drug used to treat osteoarthritis [1, 2]. As the active metabolite of diacerein, DCN is the di-acetylated derivative of rhein, a molecule having an anthraquinone ring [3]. Through a decrease in proinflammatory cytokine levels, DCN, a specific inhibitor of interleukin-1, protects against granuloma-induced cartilage degradation [4, 5]. However, DCN does not affect prostaglandin synthesis because it lacks cyclooxygenase inhibitory action [6, 7]. Aceclofenac, also known as [o (2,6- 2,6-dichloroaniline) phenyl] acetate glycolic acid ester, has analgesic and anti-inflammatory effects [8]. It is used for several pain diseases, including ankylosing spondylitis, osteoarthritis, and rheumatoid arthritis [9]. For the quantitative determination of DCN in bulk drugs [10] and in capsule dosage forms [11], two-stability indicating HPLC approaches have been reported.

The most used differentiation technique in HPLC today is reversed-phase chromatography because of its many uses. The reversed-phase approach is predicted to be used in more than 65 percent (and probably as much as 90 percent) of all HPLC separations [12]. The current study emphasizes the development of a straightforward, accurate, and dependable reversed-phase HPLC method for the simultaneous detection of diacerein and aceclofenac in pharmaceutical dosage forms and bulk drugs. Numerous analytical methods, such as high-performance liquid chromatography (HPLC), UV spectrophotometry, and high-performance thin-layer chromatography (HPTLC), have been described in the literature for the identification of aceclofenac and diacerein in pharmaceuticals [13].ACF and rhein, the immediate metabolite of diacerein, may now be measured simultaneously in human plasma using the RP-HPLC method [14].

II. MATERIALS AND METHODS

INSTRUMENT:

UV Win software and a UV-Visible Spectrophotometer with 1 cm matched quartz cells are features of the UV-3092 LABINDIA double beam.

UFLC Shimadzu The LC-20AD is a UV-visible dual absorbance detector that has a manual injector. Lab Solutions software was utilized to monitor and integrate the output signal. Separation is accomplished using a PHENOMENAX C18 column (250 x 4.6 mm I.D., 5µm).

Preparation of standard stock solution:

Weigh precisely and transfer 10 mg of Aceclofenac and 10 mg of Diacerein as a working standard into a 10 ml volumetric flask that has been cleaned and dried. To ensure thorough dissolution, add diluents and sonicate. Then, use the same solvent to adjust the volume. (Stock fix).

Additionally, pipette 1 ml of the stock solution mentioned above into a 10 ml volumetric flask, then dilute with the mixture of mobile phases. Diacerein (10 µg/ml) with Aceclofenac (10 µg/ml).

Preparation of sample solution:

A sample stock solution was made by crushing 10 randomly selected pills. An average weight equivalent sample was taken in a volumetric flask to reach a concentration of about 1 mg/ml.

10 tablets were precisely weighed and ground into powder. After being weighed, a quantity of powder equal to 50 mg of Diacerein and 100 mg of Aceclofenac was moved to a 10-milliliter volumetric flask. After dissolving it in a tiny quantity of diluent, make up the difference. After that, sonicate it for ten minutes after passing it through a 0.22 µ nylon filter. One milliliter of this solution is pipetted into a 10-milliliter volumetric flask, and the volume is filled with a mobile phase containing 25 µg/ml of Diacerein and 50 µg/ml of Aceclofenac.

Preparation of mobile phase:

Acetonitrile and 0.01M potassium dihydrogen orthophosphate are combined, filtered through a 0.45µ µm membrane filter to exclude any contaminants that might affect the final chromatogram, and then sonicated for five minutes.

Selection of Detection Wavelength:

The solution of each medication in acetonitrile was scanned in the 200–400 nm range. Both drugs were found to have considerable absorption at 241 nm. 241 nm was thus selected as the wavelength for detection.

III. RESULTS AND DISCUSSION

A. Method development and optimization:

Diacerein and aceclofenac's scanning absorption spectra were used to determine the detection wavelength. 10 ml of acetonitrile and 0.01M potassium dihydrogen orthophosphate were used to dissolve ten milligrams of the medicines, respectively. Aceclofenac and Diacerein's UV spectra were scanned independently in the 200–400 nm wavelength range. Following spectrum correlation, wavelengths of 241 nm and 277 nm were chosen for examination.

Several columns (S-Polar-18, Phenomenex, and Capcell PAK C18) were used for the trials. Acetonitrile and 0.01M potassium dihydrogen orthophosphate in a 75:25 ratio were used as the mobile phase to elute the medicines at a flow rate of 1.0 ml/min. Aceclofenac and Diacerein were shown to have retention periods of 5.8 and 3.9 minutes, respectively.

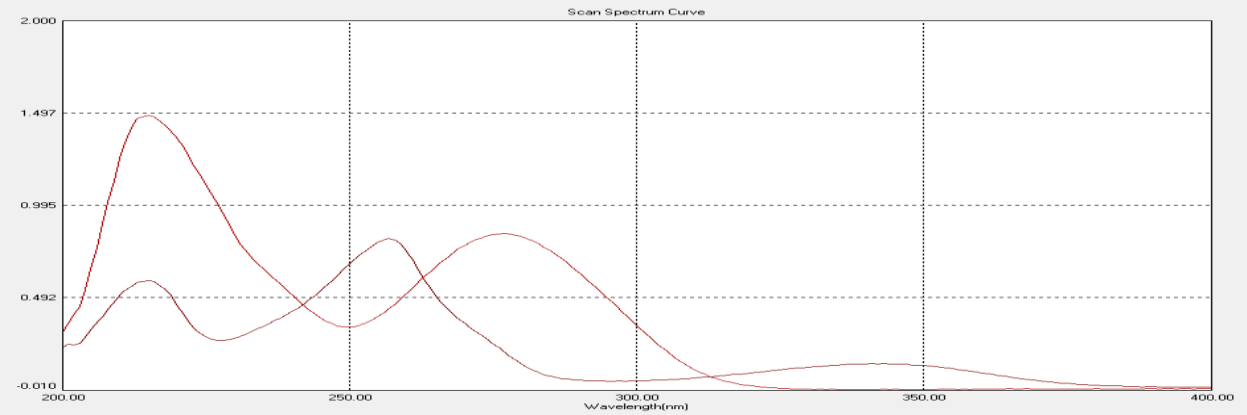

Fig 1: UV Overlay spectrum of Diacerein and Aceclofenac

B. VALIDATION

System suitability

In accordance with ICH rules, every system suitability parameter was within the approved range. After injecting 10 µl of solution into six replicates, the HPLC system was analyzed, with a focus on retention duration and standard deviations. The relative standard deviation was then calculated.

S.NO	Parameter	Diacerein	Aceclofenac
1.	Retention time	3.892	5.877
2.	Plate count	7400	11369
3.	Tailing factor	1.135	1.024
4.	Resolution	--	9.892
5.	%RSD	0.78	0.48

Acceptance Criteria: In accordance with ICH requirements, the resolution must be greater than 2, the tailing factor must be less than 2, and the plate count must be greater than 2000. Every appropriate system parameter was met and remained within the bounds.

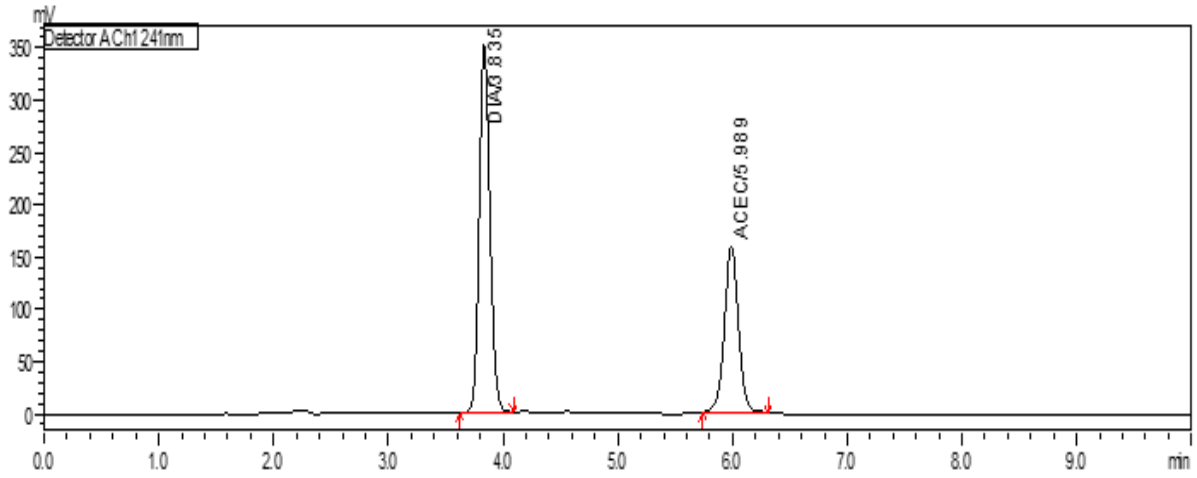

Figure 2: standard chromatogram

SENSITIVITY:

LOD and LOQ are used to express the method's sensitivity. Using the signal-to-noise ratio approach, it was computed. LOD is the concentration with a 3:1 S/N ratio, and LOQ is the concentration with a 10:1 S/N ratio.

DRUG	LOD	LOQ
Diacerein	0.5 µg/ml	0.1 µg/ml
Aceclofenac	0.1 µg/ml	1 µg/ml

LINEARITY:

Using the weighted least square regression analysis, linearity was established over the range of 5–50 µg/ml for Diacerein and 10–90 µg/ml for Aceclofenac. The findings are displayed in Table 2. The linearity graphs in Figures 3a and 3b show concentration on the y-axis and time on the x-axis.

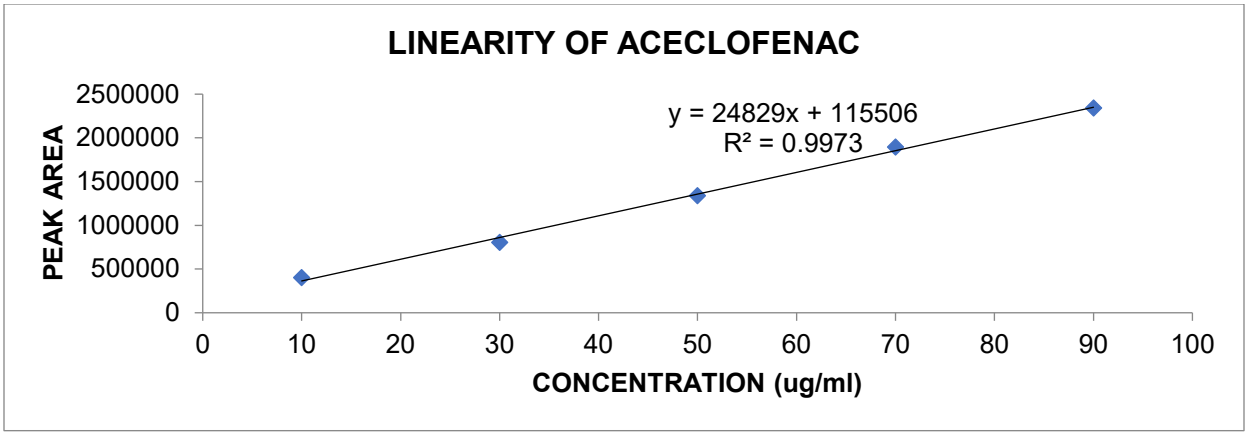

Fig 3a: Linearity plot of Aceclofenac

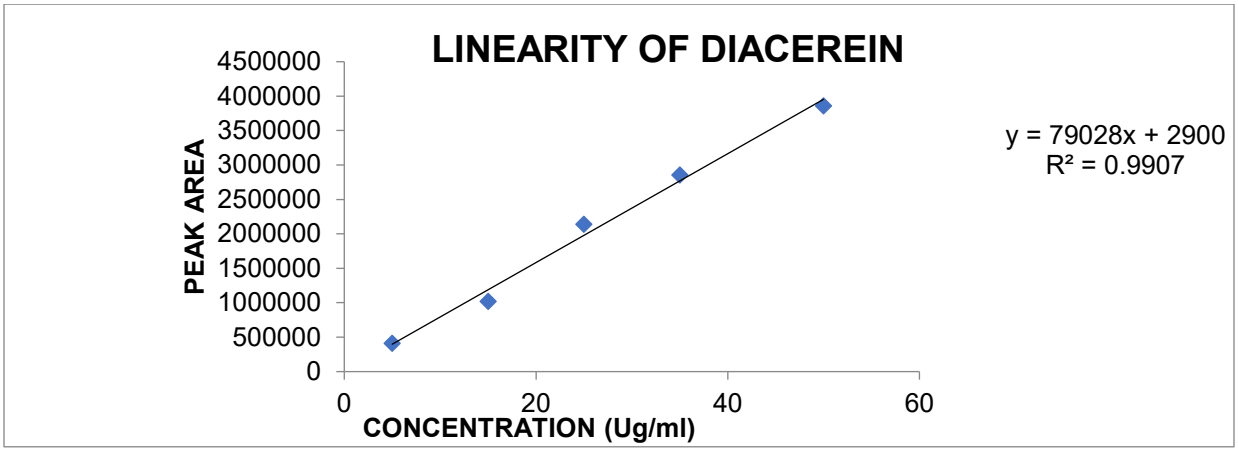

Fig 3b: Linearity plot of Diacerein

Table 2: Linearity of Diacerein and Aceclofenac

S.NO	Diacerein		Aceclofenac	
	Conc. (µg/ml)	Peak area	Conc. (µg/ml)	Peak area
1	5	411248	10	364372
2	15	1018648	30	649073
3	25	2139071	50	1364853
4	35	2857693	70	1914052
5	50	3861467	90	2353124

Table 2: graph properties

Regression equation	y = 79028x + 2900	y = 24829x + 115506
Slope	79028	24829
Intercept	2900	115506
R2	0.9907	0.9973

PRECISION:

A single volumetric flask of working standard solution was used to make three injections; the regions that resulted are mentioned above. The average area, standard deviation, and % RSD were calculated for two drugs. The percentage RSDs for Aceclofenac and Diacerein were 0.65% and 1.81%, respectively. This method was used to pass the system precision because the precision limit was less than "2." The system, technique, and intermediate precisions of aceclofenac and diacerein were found to be within acceptable limits. Tables 3a, 3b, 3c, 3d, 3e, and 3f present the results.

Table 3a: Intra-run precision of Diacerein

DIACEREIN			
SAMPLE NO.	LQC	MQC	HQC
1	442352	2098969	4015391
2	443676	2092354	4080478
3	445106	2030627	4074035
AVG	443711.3	2073983	4056635
SD	1377.34	37693.08	12334.16
RSD	0.310414	1.817424	0.304049

Table 3b: Intra-run precision of Aceclofenac

ACECLOFENAC			
SAMPLE NO.	LQC	MQC	HQC
1	434140	1301097	2444422
2	437692	1307202	2499199
3	431101	1290316	2492623
AVG	434311	1299538	2478748
SD	3298.826	8550.224	29908.47
RSD	0.759554	0.657943	1.206596

Table 3c: Intra-Day Precision of Diacerein

DIACEREIN			
PRECESION	LQC	MQC	HQC
1	424265	2013940	3321935
2	423338	2045441	3360228
3	425986	2018619	3358962
AVG	424529.7	2026000	3347042
SD	1097.121	13878.94	17760.62
RSD	0.258432	0.685042	0.530636

Table 3d: Intra-Day Precision of Aceclofenac

ACECLOFENAC			
SAMPLE NO	LQC	MQC	HQC
1	403437	1241060	1950183
2	405706	1271623	1919103
3	402369	1259658	1965874
AVG	403837.3	1257447	1945053

SD	1391.424	12574.86	19435.65
RSD	0.344551	1.000031	0.999235

Table 3e: Inter-Day Precision of Diacerein

ACECLOFENAC			
	LQC	MQC	HQC
DAY1	243840	1339144	2565165
DAY2	241562	1375166	2576880
DAY3	245869	1354698	2568946
AVG	243757	1356336	2570330
SD	1759.305	14751.46	4881.775
RSD	0.721745	1.087596	0.189928

Table 3f: Inter-Day Precision of Aceclofenac

DIACEREIN			
	LQC	MQC	HQC
DAY1	146264	2364235	4453114
DAY2	145264	2309001	4474016
DAY3	143695	2356984	4475896
AVG	145074.3	2343407	4467675
SD	1057.33	24507.91	10324.98
RSD	0.72882	1.045824	0.231104

ACCURACY

In the development of HPLC (High-Performance Liquid Chromatography) methods, accuracy is a crucial validation criterion that establishes how closely the measured results match the actual or recognized reference value. It guarantees that the technique can yield findings that accurately represent the analyte's true content in the sample. The degree to which the value discovered and the actual value or a standard (such as a reference material or known concentration) correspond is referred to as accuracy. To accomplish this, a known quantity of standard (spiked) analyte is added to the sample matrix. Use the following formula to get the % recovery after preparing the samples at the LQC, MQC, and HQC concentration levels.

Recovery (%) = (Added Concentration / Observed Concentration) ×100

Table 4a: Accuracy studies of Diacerin

Concentration level	Concentration of the sample	Amount of spiked standard	Amount recovered	% Recovery
LQC (µg/ml)	5	5	9.8±0.12	98.00±0.89
MQC (µg/ml)	25	5	29.2±0.54	97.33±0.78
HQC (µg/ml)	50	5	55.46±0.72	100.83±1.25

Table 4b: Accuracy studies of Aceclofenac

Concentration level	Concentration of the sample	Amount of spiked standard	Amount recovered	% Recovery
LQC (µg/ml)	10	10	19.85±0.52	99.25±0.49
MQC (µg/ml)	50	10	59.21±1.54	98.68±1.78
HQC (µg/ml)	90	10	101.46±1.72	101.46±1.45

ROBUSTNESS:

Optimal chromatographic conditions were purposefully changed to assess the method's robustness; the mobile phase B ratio (0.01M potassium dihydrogen orthophosphate) and flow rate (1 ml/min) were adjusted by ± 2%. Additionally, robustness was examined by varying the flow rate with the real ratio to 0.8 and 1.2 milliliters per minute, respectively. A robustness assessment was conducted twice, utilizing doses of 50 µg/ml of Aceclofenac and 25 µg/ml of Diacerein.

Table 5a: Robustness table of Diacerein

Parameter	Condition	Retention time(min)	Peak area	Tailing	Plate count
Flow rate Change (ml/min)	Less flow than actual (0.8ml)	4.814	3077308	1.142	8814
	More flow than actual (1.2ml)	3.233	1960499	1.140	6155
Organic Phase change	Less Org (32:68)	3.835	2281045	1.135	7103
	More Org (28:72)	3.842	2451806	1.153	7971

Table 5b: Robustness table of Aceclofenac

Parameter	Condition	Retention time(min)	Peak area	Tailing	Plate count
Flow rate Change (ml/min)	Less flow than actual (0.8ml)	7.300	1803633	0.998	13399
	More flow than actual (1.2ml)	4.897	1170485	1.030	9742
Organic Phase change	Less Org (32:68)	5.989	1360434	0.990	11005
	More Org (28:72)	5.550	1455608	1.016	12430

ASSAY:

Take five tablets of Aceclofenac and Diacerein, each having 50 mg of Diacerein and 100 mg of Aceclofenac, and place them in a 100 ml volumetric flask. 50 ml of mobile phase were taken in equal amounts, and they were sonicated for 15 minutes. Next, use the identical mobile phase to make up to 100ml. Put 1 ml of the stock solution mentioned above into a 10ml volumetric flask, add 100, and then do it again to get 10 µg/ml. Inject into the HPLC.

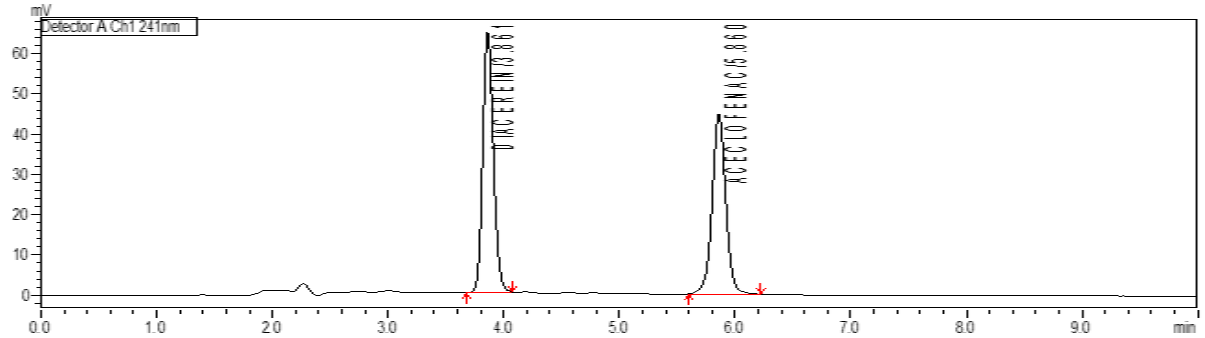

Fig 4: sample chromatogram

IV. CONCLUSION

The HPLC method is easy to use, quick, accurate, exact, dependable, and effective. It was created for the evaluation of different medications. Both the solvents and the mobile phase are sensitive, inexpensive, and easy to make. It is determined that the short and straightforward suggested methods are the most helpful for analysis purposes because the system validation parameters—linearity, precision, robustness, and assay of the HPLC method used for estimation of selected drugs in pure form—have also shown that they are adequate, precise, and,reproducible.

As a result, these can be used for regular analysis of aceclofenac and diacerein.

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