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Validated RP-HPLC method for estimation of diacerein and aceclofenac in tablet dosage form

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ABSTRACT

In the present study, a reverse phase high performance liquid chromatographic method was developed and validated for the determination of Diacerein (DIA) and Aceclofenac (ACE) in tablet dosage form with the use of Paracetamol (PCM) as an internal standard (IS). Chromatographic separation was carried out on a RP-18 column using a mobile phase consisting of acetonitrile : water (60:40, v/v) 0f pH 3.0,adjusted with o-phosphoric acid (1%). The flow rate was maintained at 1.0ml/min and UV detection was carried at 268nm. The calibration curve was found linear over the range 10-150µg/ml for DIA and 20-300µg/ml. Relative Standard deviation (RSD) for precision was found to be less than 3%. The results of accuracy study were observed in the range of 99.50% to100.18% with RSD less than 1%. Limit of detection (LOD) was found to be 0.9839µg/ml for DIA and 1.9479µg/ml for ACE. Limit of quantification (LOQ) was found to be 2.9816µg/ml for DIA and 5.9030µg/ml for ACE. The developed analytical method was found to be simple, rapid, and easy to apply, making it suitable for routine analysis of ACE and DIA in tablet dosage form.

KEYWORDS

Diacerein;
Aceclofenac;
Paracetamol;
RP-HPLC;
Validation.

INTRODUCTION

Diacerein (DIA) is 9,10-dihydro-4,5-dihydroxy-9,10-dioxo-2-anthranoic acid diacetate and has anti-rheumatic activity(Figure 1)[1]. It works by inhibiting interleukin-1. This drug is used for the relief of pain and inflammation in rheumatoid arthritis, osteoarthritis[2].

Aceclofenac (ACE) is [(2,6-dichlorophenyl) amino] phenylacetoxyacetic acid has analgesic and anti-inflammatory activity(Figure 1)[1]. It is a cytokine inhibitor and works by blocking the action of cyclo-oxygenase that is involved in the production of prostaglandins which causes pain, swelling and inflammation[3].

DIA and ACE both are official in Indian Pharmacopoiea 2010. The combination of DIA and ACE are available in tablet dosage form in the ratio of 1:2.

Simultaneous HPLC determination of Rhein and Aceclofenac in human plasma[4], Aceclofenac and its three metabolites in plasma(LCMS)[5], Aceclofenac, Paracetamol and Chlorzoxazone in tablet dosage form[6], Paracetamol and Aceclofenac in tablets[7, 8] and stability-indicating spectrophotometric and densitometric method for determination of Aceclofenac[9] have been

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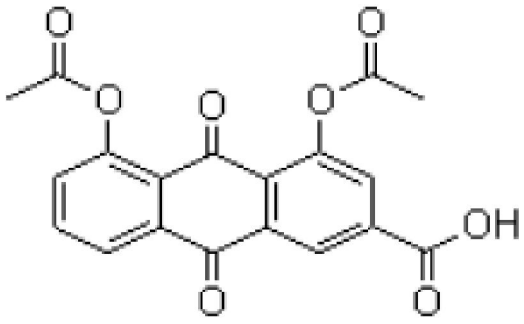
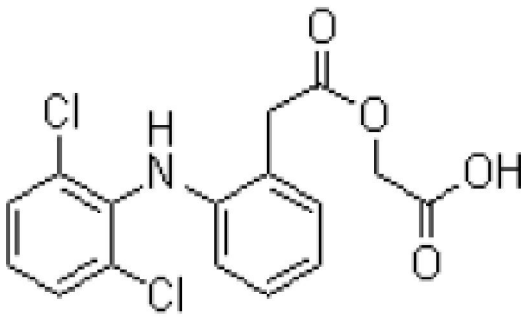


DIA



ACE

Figure 1 : Chemical Structure of Diacerein (DIA) and Aceclofenac (ACE)

reported. Many spectrophotmetric methods like absorption ratio, absorption correction and derivative spectroscopy methods are available for simultaneous estimation of DIA and ACE[10]. Literature review revealed that none of the RP-HPLC method is reported for the determination of DIA and ACE using an internal standard in tablet dosage form. Hence in present work attempt has been made for the development and validation of simple, rapid, sensitive, and precise HPLC method, using an internal standard.

EXPERIMENTAL

Reagents and chemicals

DIA and ACE were kindly provided by Micro Labs Ltd., Bangalore (India). PCM (used as an IS) was provided by Juggat Pharma (India). Acetonitrile, o-phosphoric acid and Water used were of HPLC grade. Tablets containing DIA and ACE (Dycerin A, Glenmark Pharmaceutical Ltd.) were purchased from local pharmacy.

Instrumentation

The HPLC system used was Shimadzu LC-20AT pump, Rheodyne injector (20µl), SPD-20A UV detection and the system was controlled through Spinchrome software. Analytical column used for this method was Gracesmart RP18 (250 X 4.6mm, 5µm). Sartorius digital Balance, Digisun 7007 pH meter, RC Systems sonicator and vacuum pump were also used in the experiment.

Chromatographic conditions

The composition of the mobile phase used was acetonitrile : water (60:40, v/v) (adjusted to pH 3.0 with o-phosphoric acid). The mobile phase was vacuum-filtered through 0.45µm nylon Millipore membranes (Millipore, USA), and degassed by ultrasonication for 10min before use. The mobile phase flow rate was set at 1.0ml/min. After equilibration with the solvent to obtain a stable baseline, aliquots of samples (20µl) were injected through Rheodyne injector in the column. The total run time was kept about 12min. The absorbance of the eluent was monitored at 268nm with a detection sensitivity of 0.100aufs. Paracetamol (10µg/ml) was used as an internal standard.

Preparation of standards and sample solutions

Mixed standard stock solutions of DIA (500µg/ml) and ACE (1000µg/ml) and standard stock solutions PCM (IS) (100µg/ml) were prepared in HPLC grade methanol. These solutions were kept and stored under refrigeration (4.0±0.5ºC). Working standard solutions were freshly prepared daily by appropriate dilution of the stock solutions with mobile phase. Sample solutions were prepared using 30 tablets of Dycerin A. The tabltes were accurately weighed and finely powdered. The powder equivalent to 5mg of DIA was taken in a 50ml volumetric flask, about 40ml of HPLC grade methanol was added and kept in ultrasonic bath for 10min then made up to volume 50ml. The resulting solution was filtered through whatman paper (No. 41). Exact 1ml of this filtrate was transferred into 10ml volumetric flask along with 1ml of PCM solution (100µg/ml) and made up to the volume with mobile phase (50µg/ml DIA, 100µg/ml ACE and 10µg/ml PCM). The standard and sample solutions were filtered through

0.45μm nylon millipore membranes (Millipore, USA) before injecting into HPLC system. The chromatogram of standard solution is as shown in Figure 2 while assay results of the marketed product are as shown in TABLE 1.

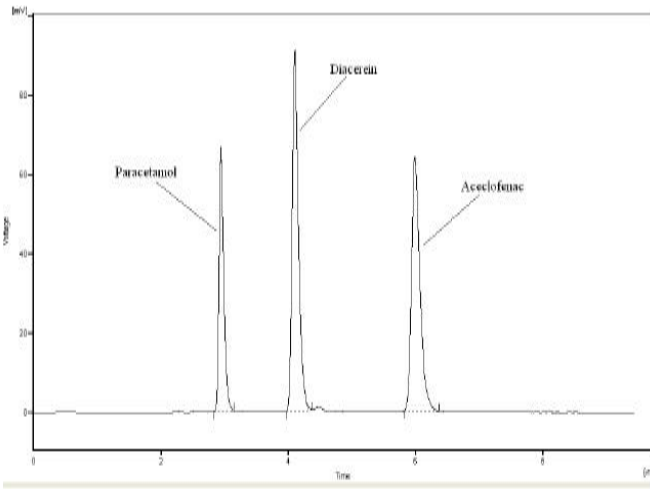


Figure 2 : Chromatogram of DIA (tR 4.08 min), ACE (tR 5.95 min) with PCM (tR 2.98 min)

TABLE 1 : Assay of marketed formulation

Tablet content	Label claim (mg/tablet)	% Amount Found (n=6)	±SD	% RSD
DIA	50	99.66	0.21	0.42
ACE	100	99.95	0.27	0.27

Method validation

Method validation was carried out following the guidelines of International Conference on Harmonization (ICH)[11, 12]. The developed method was validated with respect to linearity, precision, accuracy, sensitivity and robustness.

Linearity

Calibration curves were obtained from injecting the six sets of eight serial dilutions of mixed standard solution (10, 15, 20, 40, 50, 100 and 150μg/ml of DIA; 20, 30, 40, 50, 100, 200, 300μg/ml of ACE; 10μg/ml of PCM). The curves of DIA were generated by plotting the peak area ratios between DIA and PCM against DIA concentration. The curves of ACE were generated by plotting the peak area ratios between ACE and PCM against ACE concentration Linearity was evaluated by linear regression using ANOVA.

Precision

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day) and was expressed as RSD. Repeatability was determined by performing nine determinations from triplicate injections of three different mixture of concentrations of DIA (10, 20 and 100μg/ml) and ACE (20, 40 and 200μg/ml) on the same day at different time intervals and on three different days for inter-day precision.

Accuracy/Recovery

In this study, accuracy was determined based on the recovery (percentage) of known amounts of standard DIA and ACE added in the assay samples. This was performed by analyzing DIA and ACE at three different concentration levels 80%, 100% and 120% to the assay sample, with a constant concentration of 10μg/ml of IS. Samples were prepared in triplicate. The accuracy of the assay was determined by comparing the found concentration with the added concentration.

Sensitivity

Sensitivity of the method was determined by means of the LOD and LOQ. The LOD and LOQ were measured based on the method described by the International Conference on Harmonization. Calculations for LOD and LOQ were based on the standard deviation of the intercepts (σ) and the slope of curve (S), using the equation LOD= 3.3×σ/S and the equation LOQ= 10×σ/S.

Robustness

Robustness of the method was evaluated by the analyzing DIA and ACE solution under different experimental conditions such as variation in pH of the mobile phase and flow rate. The flow rate and pH were varied by 3% and their effects on the retention time (tR), tailing factor (T), resolution of the peaks (R) and repeatability were studied.

RESULTS AND DISCUSSION

Optimization of the chromatographic method

The chromatographic conditions were adjusted to provide the best performance of the assay. For system

optimization the important parameters such as type and concentration of organic solvents, pH and mobile phase flow rate were investigated.

Effect of pH

Different pH values of the mobile phase were checked to establish the optimum separation and highest analytical sensitivity for DIA, ACE and PCM. The pH values tested were 4.5 and 3.0. Finally, the best results were obtained at pH 3.0±0.2 by using 1% o-phosphoric acid. The choice of this pH for the mobile phase is justified by the excellent symmetry of the peaks and the adequate retention times of DIA, ACE and PCM.

Effect of mobile phase composition

Different mobile phase composition like methanol : water and acetonitrile : water were tried first on RPTLC plates for separation of DIA, ACE and PCM. Methanol : water and acetonitrile : water were further tried on HPLC system. It was observed that the acetonitrile : water system gave a better resolution and peak symmetry than the methanol : water system.

As the percentage of acetonitrile and water changed (40:60, 50:50, 60:40v/v), retention time of DIA, ACE and PCM was varied in the range of 2min to 35min. The standard solutions of DIA, ACE and PCM showed symmetric and well-defined peaks, with an average retention time of 4.083min for DIA, 5.957min for ACE and 2.933min for PCM in acetonitrile : water (60:40 v/v) (adjusted to pH 3.0±0.2with1% o-phosphoric acid) was chosen as a mobile phase. Resolution between peaks of PCM, DIA and ACE were found 7.019 and 8.842, respectively while tailing factor was found about 1.583 for DIA, 1.545 for ACE and 1.667 for PCM. Hence the mobile phase composition used for present study was acetonitrile : water (60:40 v/v) (adjusted to pH 3.0±0.2with1% o-phosphoric acid).

Effect of flow rate

Different mobile phase flow rates (0.5, 1.0 and 1.2ml/min) were investigated. The optimum flow rate for which the column plate number (N) was maximum, with the best resolution between all components and with a short run time (<10min) was found to be 1.0ml/min.

Internal standard

Different compounds were tested as an IS for the chromatographic procedure. Among them, PCM eluted before 5 min of the analysis and has a better symmetry and resolution with respect to DIA and ACE. Therefore, PCM has been chosen as an IS.

Method validation

Precision

The %R.S.D. of repeatability (intra-day) was found between 0.10 to 0.60 for DIA and 0.06 to 0.86 for ACE. The %R.S.D. of intermediate precision (inter-day) was found between 0.16 to 1.44 for DIA and 0.14 to 1.86 for ACE. These values show a low variability between the values obtained for each concentration. The results of precision are as shown in TABLE 2.

TABLE 2 : Summary of precision determined during method validation

Drug	Concentration (µg/ml)	Intra-day (n=3) % RSD	Inter-day (n=3) % RSD
DIA	10	0.60	1.44
	20	0.30	0.78
	100	0.10	0.16
ACE	20	0.86	1.86
	40	0.52	1.11
	200	0.06	0.14

Accuracy

As shown in TABLE 3, the results of accuracy study was observed in the range of 99.50% to100.18% with RSD less than 1% for DIA and 99.83% to 100.12% with RSD less than 1% for ACE.

TABLE 3 : Accuracy of the method determined according to ICH Q2 guidelines.

Level of Recovery	Drug	% Recovery	±SD
80%	DIA	100.18	0.55
	ACE	100.09	0.49
100%	DIA	99.88	0.38
	ACE	100.12	0.62
120%	DIA	99.50	0.47
	ACE	99.83	0.25

Linearity

The standard calibration curve was found linear over the concentration range 10-150µg/ml for DIA (Figure

TABLE 4 : Statistical analysis of linearity.

Multiple regression analysis							
Parameter	**Drug**	**Coefficient**	**Standard Error**	**t-statistic**	**p-value**	**Lower 95%**	**Upper 95%**
Intercept	DIA	-0.04193	0.04004	-1.04743	0.33524	-0.13991	0.05603
	ACE	-0.03789	0.03376	-1.12246	0.30456	-0.12050	0.04471
Slope	DIA	0.15540	0.00059	261.622	210E-13	0.15395	0.15685
	ACE	0.082813	0.00025	330.690	5.16E-14	0.08220	0.08342

ANOVA					
Parameter	**Drug**	**Sum of square**	**Degree of freedom**	**Mean of square**	**F**
Regression	DIA	0.009	5	0.0018	0.000026
	ACE	0.112	5	0.0225	0.000294
Residual	DIA	2438	36	67.720	
	ACE	2759	36	76.650	
Total	DIA	2438	41		
	ACE	2759	41		
Multiple R	DIA			0.99995	
	ACE			0.99997	
R square	DIA			0.99991	
	ACE			0.99994	
Adjusted R square	DIA			0.99989	
	ACE			0.99993	
Standard Error	DIA			0.08238	
	ACE			0.06946	

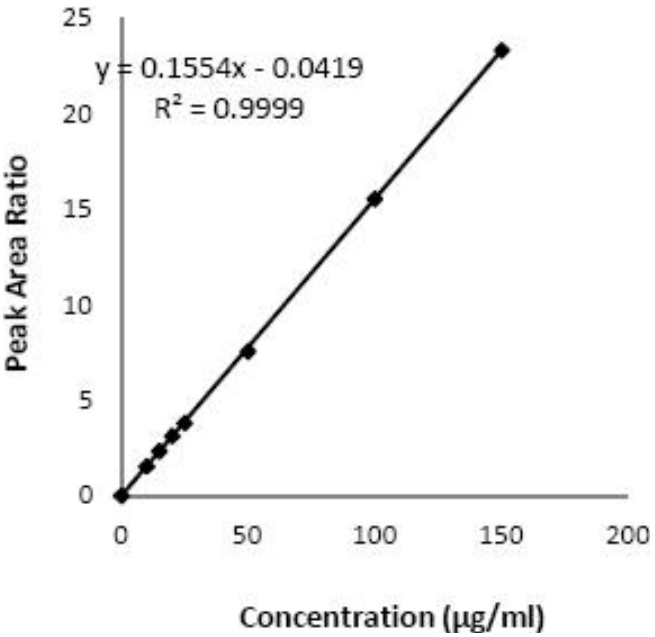


Figure 3 : Calibration Curve of DIA

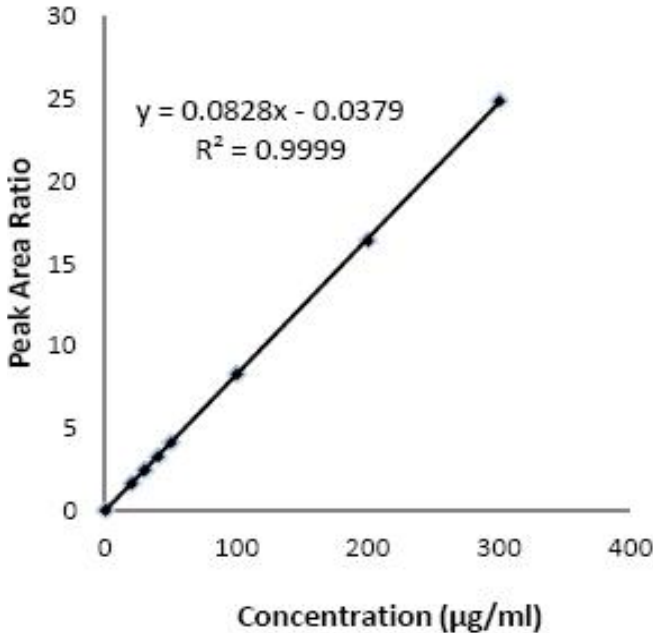


Figure 4 : Calibration Curve of ACE

3) and 20-300µg/ml for ACE (Figure 4). The correlation coefficient obtained after linear regression analysis was 0.9999 for DIA and ACE. The linearity of developed method was also confirmed by multiple regression analysis and ANOVA (TABLE 4).

Sensitivity

LOD was found to be 0.9839µg/ml for DIA and 1.9479µg/ml for ACE. LOQ was found to be 2.9816µg/

ml for DIA and 5.9030µg/ml for ACE. These values are adequate for the detection and quantification of DIA and ACE.

Robustness

During the robustness study, it was observed that peak symmetry (T) and the retention times (tR) were not significantly changed as shown in TABLE 5. These facts suggest that the method did not change with time and experimental conditions. However, it could be noted that organic composition of the mobile phase can influence the method performance.

TABLE 5 : Robustness of the method.

Parameter	Drug	Value	T[a]	tR[b]	
				min	%RSD[c]
pH		3.09	1.747	4.070	
	DIA	3.0	1.652	4.087	0.23
		2.91	1.708	4.085	
		3.09	1.797	5.897	
	ACE	3.0	1.645	5.953	0.71
		2.91	1.541	5.981	
Flow rate (ml/min)		1.03	1.485	3.976	
	DIA	1.0	1.652	4.087	2.90
		0.97	1.424	4.214	
		1.03	1.709	5.797	
	ACE	1.0	1.645	5.953	2.87
		0.97	1.589	6.136	

a : tailing factor, b: retention time, c: relative standard deviation.

System suitability

System suitability was performed to confirm that the equipment was adequate for the analysis to be performed. The test was carried out by making replicate injections of a standard solution containing 50µg/ml of DIA, 100µg/ml of ACE and 10µg/ml of PCM (IS), and analyzing each solute for their peak area, theoretical plates (N), resolution (R) and tailing factor (T). The results of system suitability in comparison with the required limits are shown in TABLE 6. The proposed method fulfils these requirements; the results of system suitability are within the accepted limits.

TABLE 6 : System suitability results of the proposed method.

Analyte	R[a]	N[b]	T[c]
PCM	--	3441	0.600
DIA	5.709	8137	1.652
ACE	9.052	10503	1.645
Required limits	>2	> 2000	< 2

a: Resolution, b: No. of theoretical plate, c: Tailing factor.

CONCLUSION

In the present research work to achieve highest precision in quantitative estimation of DIA and ACE in pharmaceutical dosage form, a reverse phase liquid chromatography method for DIA and ACE using IS was developed and validated. The method was validated in terms of linearity, precision, accuracy, detection limit, quantification limit and robustness. It involves a simple procedure for the preparation of the samples, shorter run times for analytical procedure (less than 12min) and a low percent of organic solvent (acetonitrile 60% and water 40%) was used in the composition of the mobile phase. Hence the present RPHPLC method can be considered as simple, rapid, suitable and easy to apply for routine analysis of DIA and ACE in combined pharmaceutical dosage form.

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