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DEVELOPMENT AND VALIDATION OF SIMULTANEOUS ESTIMATION OF DIACEREIN AND ACECLOFENAC IN BULK AND PHARMACEUTICAL TABLETS BY RP-HPLC

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ABSTRACT

The RP-HPLC method has been developed for the simultaneous estimation of Diacerein and Aceclofenac in pharmaceutical dosage form using C16 column (Phenomenex, 253 x 4.2mm, 5µm) in isocratic mode. The detection wavelength was carried out at 281nm. Aceclofenac 50-75µg/ml and for Diacerein 100-150µg/ml. The recoveries of Aceclofenac and Diacerein were found to be in the range of 99.23-100.39% and 99.45-100.56% respectively. The validation of method were carried out utilizing ICH-guidelines. The described RP-HPLC method were successfully employed for the analysis of pharmaceutical formulations containing combined dosage forms.

KEYWORDS

NSAID, Diacerein and Aceclofenac and Diacerein.

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INTRODUCTION

Diacerein is a selective inhibitor of interleukin-1 had protective effect on granuloma-induced cartilage breakdown by a reduction in the concentrations of pro-inflammatory cytokines[1]. Aceclofenac [(2, 6-dichlorophenyl) amino] phenylacetoxyacetic acid is used as an effective non-steroidal anti-inflammatory drug (NSAID) derived from the phenylacetic acid with pronounced anti-inflammatory, analgesic and antipyretic properties[2]. As per Literature survey reveals having some methods reported that diacerein viz. Spectrophotometric method[3], stability indicating High Performance Liquid Chromatographic (HPLC) methods[4,5] and stability indicating HPTLC method[6]. They Aceclofenac literature surveys reveals that many papers viz. Simple spectrometric methods[7], spectrofluorimetric method, stability indicating HPLC and HPTLC methods. Some research articles are also available for Diacerein and Aceclofenac combination as simultaneous UV spectrophotometric methods and HPTLC method.

MATERIAL AND METHODS

Reagents

All chemicals and reagents are used for HPLC grade. Authenticated of Diacerein and Aceclofenac was obtained as gift samples from Glenmerk Pharmaceutical Hyderabad. DYCERIN-A drug sample was employed in the study. The other chemicals and HPLC grade water from Ranbaxy chemicals, Mumbai, India.

Chromatographic Conditions

The mobile phase used as acetonitrile and water in the ratio of 55:45 (v/v) and filtered through 0.45µ membrane filter and degassed before use. The mobile phase was delivered isocratically at a flow rate of 1.5ml/min. Thermo Hypersil key stone C-18 ODS column 250x4.6mm i.d with 5µ particle size and the column was maintained at ambient temperature. The injection volume was 20µl and the total run time was 10 minutes. The detection was carried out at 275nm.

Preparation of Mobile Phase

The mobile phase was prepared by mixing solution acetonitrile and water in the ratio of 55:45 (v/v) and filter through 0.45µ membrane filter and sonicated for 30 min.

Preparation of Standard Stock Solution

Accurately weighed quantity of 60mg of Diacerein and 120mg of Aceclofenac is transferred in 100ml volumetric flask, dissolve with 20ml acetonitrile and make up with mobile phase having the concentration of 500µg/ml of Diacerein and 1000µg/ml of Aceclofenac.

Preparation of Standard Solution

From the above standard solution 5ml is pipette out into 50ml volumetric flask and make up to volume with mobile phase having the concentration of 50µg/ml of Diacerein and 100µg/ml of Aceclofenac.

Preparation of Sample Stock Solution

20 tablets were accurately weighed and powdered. An amount of powder equivalent to 50mg of Diacerein and 100mg of Aceclofenac were weighed and transferred in 100ml volumetric flask. Add about 20ml of acetonitrile and make up the volume with mobile phase up to 100ml. Then filtered through a 0.45µm membrane filter and sonicated for 30 min. make up to volume with mobile phase having the concentration of 50µg/ml of Diacerein and 100µg/ml of Aceclofenac.

Estimation Method

Aliquot of standard stock solution is diluted to the working concentration equivalent to that of sample. 20µl of standard and sample are injected separately and chromatograms are generated with peak area obtained for standard and sample the content of Diacerein and Aceclofenac in each tablet is calculated using the following formulae taken. The amount of drug present in tablet formulation was calculated by comparing the mean peak area from the standard and the assay values are given in Table No.2. The statistical validation was performed and the results were shown in Table No.3. A typical chromatogram of Diacerein was shown in Figure No.1 and also a typical chromatogram of Aceclofenac was shown in Figure No.2. The chromatogram of mixed standard shown in Figure No.3 and the assay chromatogram of sample formulations were shown in Figure No.4 and Figure No.5.

Linearity and Range

Linearity was assessed by performing single measurement at several analytes concentration varying quantities of stock standard solution diluted with the mobile phase to give a concentration of 50, 60, 62.5, 70 and 75µg/ml for Diacerein and 100, 120, 125, 140 and 150µg/ml for Aceclofenac. Linearity of Diacerein and Aceclofenac were found to exist between 50 to 75µg/ml and 100 to 150µg/ml respectively. The chromatograms were recorded and linearity graph was plotted by using peak area of individual drugs against respective concentrations to obtain the linearity range for individual drugs. The linearity data of Diacerein and Aceclofenac was shown in Table No.4 and Table No.5.

Accuracy

Accuracy of method was observed by recovery result from 3 placebos preparations accurately spiked with different concentration of the active ingredient.

Recovery assessment was obtained by using standard addition technique which was by adding known quantities of pure standards at three different levels in 100%, 110% and 120% to the pre analysed sample formulation.

Percentage recovery can be obtained by using following expression,

$$\text{Percentage recovery} = \frac{\text{Amount of drug found}}{\text{Amount of drug added}} \times 10$$

Recovery values were calculated and shown in Table No.6. The recovery chromatogram was shown in Figure No.4.

Precision

Reproducibility was performed by injecting five replicates concentrations of standard and sample solutions which were prepared and analyzed by same analyst on same day.

Ruggedness

The ruggedness were determined by using the data obtained by the analysis performed by different analyst. The result obtained is given in table.

Robustness

Robustness was carried out by varying two parameters from the optimized chromatographic condition which includes and values were shown in

Change in flow rate from 1.0ml/min to 1.5ml/min.

Change in detection wavelength from 260nm to 280nm.

RSD-Relative Standard deviation.

Specificity

The method was determined as specific by comparing test results obtained from analyses of sample solution containing placebo ingredients with that of test results those obtained from analyses of standard solution.

System Suitability Parameters

System suitability tests were carried out on freshly prepared standard stock solutions of Diacerein and Aceclofenac and it was calculated by determining the standard deviation of Diacerein and Aceclofenac standards by injecting a mixed standard containing all two standards in five replicates at 10 minutes interval and the values were recorded. The results of system suitability parameters were shown in Table No.10.

RESULTS AND DISCUSSION

HPLC Method

Method Development

The mobile phase was chosen after several trials with, acetonitrile and water in various proportions. The effects of flow rates in the range of 0.5 to 1.5ml/min were examined. The total time of analysis were less than 14 min. the solution containing Diacerein and Aceclofenac exhibited maximum absorption at 275nm and hence this wavelength was chosen for the analysis.

Linearity

The plot of peak area versus the respective concentration of Diacerein and Aceclofenac was found to be linear in the concentration range of 50-75µg/ml and 100-150µg/ml, respectively (Figure No.1 and Figure No.2). They were represented by the linear regression equation.

$Y_{\text{Diacerein}}$: 27935 x + 0.4225

$Y_{\text{Aceclofenac}}$: 19757 x + 7.8956.

Excellent linearity was obtained for both drugs between peak areas and concentrations with R^2=0.9999 and 0.9888 for Diacerein and Aceclofenac, respectively.

Accuracy

A standard working solution containing Diacerein and Aceclofenac, to give final concentrations respectively 50 and 100µg/ml was prepared. From the respective area counts, the concentrations of Diacerein and Aceclofenac were calculated using the detector responses. The accuracy defined in terms of percentage deviation of the calculated concentrations from the actual concentrations, the results are listed in Table No.6.

Specificity

The specificity of the HPLC method is illustrated in Figure No.3, where complete separation of Diacerein and Aceclofenac was noticed in presence of tablet excipients. The average retention time for Diacerein and Aceclofenac were found to be 5.8 and 13.4 min, respectively.

Precision

The precision of the method was demonstrated by repeatability studies. The precision of the proposed method was determined by assaying the standard solutions on the same day expressed as % RSD. Inter-day precision has depicted in Table No.7.

Analysis of Pharmaceutical formulation

The peaks at retention time 5.8 (for Diacerein) and 13.4 min (for Aceclofenac) were observed in the chromatogram of the samples extracted from tablets **(**Figure No.5). Experimental results of the amount of Diacerein and Aceclofenac in tablets, expressed as % of label claim were in good agreement with label claims, The drug content was found to be 50.554±0.1051 (% RSD =0.10501) and 100.606±0.5181 (% RSD =0.0.5181) for Diacerein and Aceclofenac, respectively. The low values of relative standard deviation indicated high precision of the method (Table No.2 and Figure No.3**)**. Recovery studies in this method were performed on the synthetic mixtures prepared by adding accurately weighed amounts of drugs (Table No.6). Mean recoveries and relative standard deviation were found to be 100.56 and 0.139 for Diacerein and 100.39 and 0.207 for Aceclofenac respectively.

Table No.1: Assay of Combined Tablet Dosage Form
Brand Used: Dycerin-A

S.No	Drug	Label Claim (mg/tablet)	Amount Estimated (mg/tablet)*	Percentage Label Claim (%)	% Deviation
1	Diacerein	50	50.554	101.108	+1.108
2	Aceclofenac	100	100.606	100.606	+0.606

*****Mean of five reading

Table No.2: Statistical Validation

S.No	Drug	Amount Estimated (mg/tablet)*	% Amount Estimated*	Standard Deviation	% RSD	Standard Error
1	Diacerein	50.554	101.108	0.1063	0.1051	0.047
2	Aceclofenac	100.606	100.606	0.5213	0.5181	0.233

*****Mean of five determinations, RSD - Relative Standard Deviation.

Table No.3: Linearity Studies of Diacerein and Aceclofenac

S.No	Diacerein (μg /ml)	Peak Area of Diacerein	Aceclofenac (μg /ml)	Peak Area of Aceclofenac
1	50	1396739	100	1975698
2	60	1676189	120	2370978
3	62.5	1745945	125	2469672
4	70	1955435	140	2765977
5	75	2095156	150	2963567

Table No.4: Analytical Performance Parameters of Diacerein

S.No	Parameters	Diacerein	Aceclofenac
1	Linear Dynamic Range	50-75μg/ml	100-150μg/ml
2	Correlation coefficient (r)	0.9999	0.9888
3	Slope (m)	27935	19757
4	Intercept (c)	0.4225	7.8956

Table No.5: Recovery Studies

S.No	Theoretical Amount	Obtained (μg) Diacerein	% Recovery	Obtained (μg) Aceclofenac	% Recovery
1	100	100.46	100.43	100.22	100.16
2	110	110.75	100.71	110.16	100.46
3	120	120.64	100.55	120.34	100.56
4	Mean % Recovery		100.564	Mean % Recovery	100.393
5	SD		0.1404	SD	0.2081
6	% RSD		0.1397%	% RSD	0.2073%

*Mean of three determinations in each level

Table No.6: Precision

S.No	Sample weight	Area for standard 50μg/ml Diacerein	Area Obtained Diacerein sample	Area for standard 100μg/ml Aceclofenac	Area Obtained Aceclofenac sample
1	235.74	1396744	1396739	1978572	1975698
2	236.23	1398749	1399746	1982998	1982247
3	234.99	1392754	1386744	1966053	1968029
4	235.99	1397763	1386738	1974784	1974777
5	235.64	1396733	1386732	1977714	1977718
6	Average		1391339	Average	1975693
7	STD DEV		6390.33	STD.DEV	5163.12
8	% RSD		0.4592%	% RSD	0.2613%

*Mean of five replicates of single concentration

Table No.7: Ruggedness for Diacerein

S.No	**Analyst-1**		**Analyst-2**	
1	1	50.51	1	50.549
2	2	50.56	2	50.526
3	3	50.51	3	50.509
4	4	50.64	4	50.487
5	5	50.55	5	50.612
6	Mean	50.554	Mean	50.536
7	STD DEV	0.05319	STD DEV	0.04788
8	% RSD	0.1051	% RSD	0.0947

*Mean of five replicates of single concentration RSD - Relative Standard Deviation

Table No.8: Ruggedness for Aceclofenac

S.No	**Analyst-1**		**Analyst-2**	
1	1	100.16mg	1	100.12
2	2	99.96	2	99.94
3	3	101.19	3	100.11
4	4	100.84	4	100.75
5	5	100.88	5	100.84
6	Mean	100.606	Mean	100.35
7	STD DEV	0.5213	STD DEV	0.4119
8	% RSD	0.5181	% RSD	0.4104

*Mean of five replicates of single concentration RSD - Relative Standard Deviation

Table No.9: Robustness

S.No	**Drug**	**Theoretical Concentration (mg/ml)**	**Measured Concentration***	
			Mean (mg/ml)	**% RSD**
1	Diacerein	50	50.5	0.1051
2	Aceclofenac	100	100.59	0.5179

* Mean of five replicates of single concentration

Table No.10: System Suitability Parameters

S.No	**Parameters**	**Diacerein**	**Aceclofenac**
1	Theoretical plate	3000	2500
2	Retention Time(min)	5.8	13.4
3	Resolution	3.48	
4	% RSD	0.1051	0.5181

Table No.11: Method Validation Parameters for HPLC

S.No	**Parameter**	**Results**	
		Diacerein	**Aceclofenac**
1	Linearity Range (µg/ml)	50-75	100-150
2	Correlation Coefficient	0.9999	0.9998
3	Accuracy	100.39	100.56
4	Precision (% RSD)	0.4592	0.2613
5	Specificity	Specific	Specific
6	LOD (µg)	0.004073	0.01342
7	LOQ (µg)	0.01235	0.02755

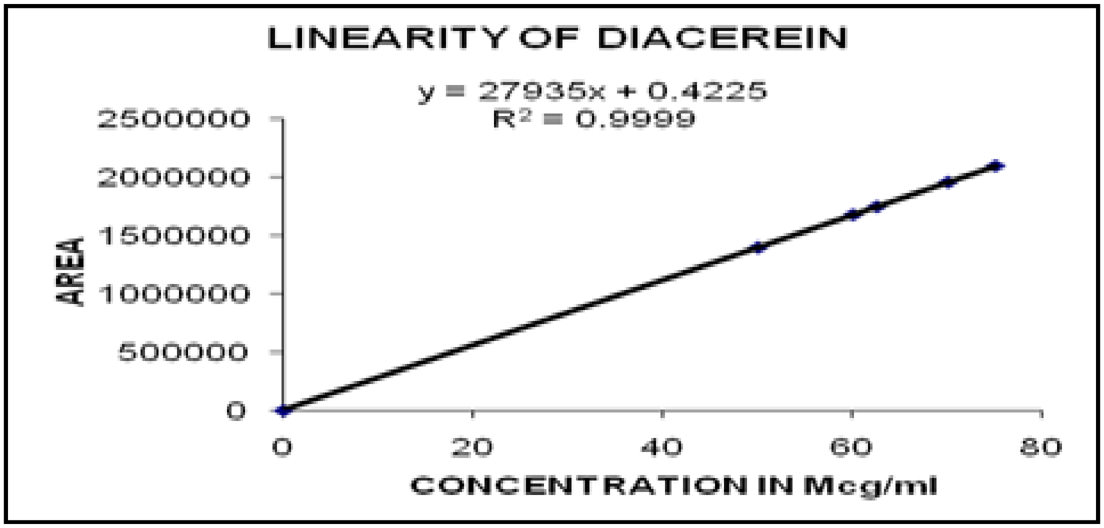

Figure No.1: Linearity of Diacerein by RP-HPLC Method

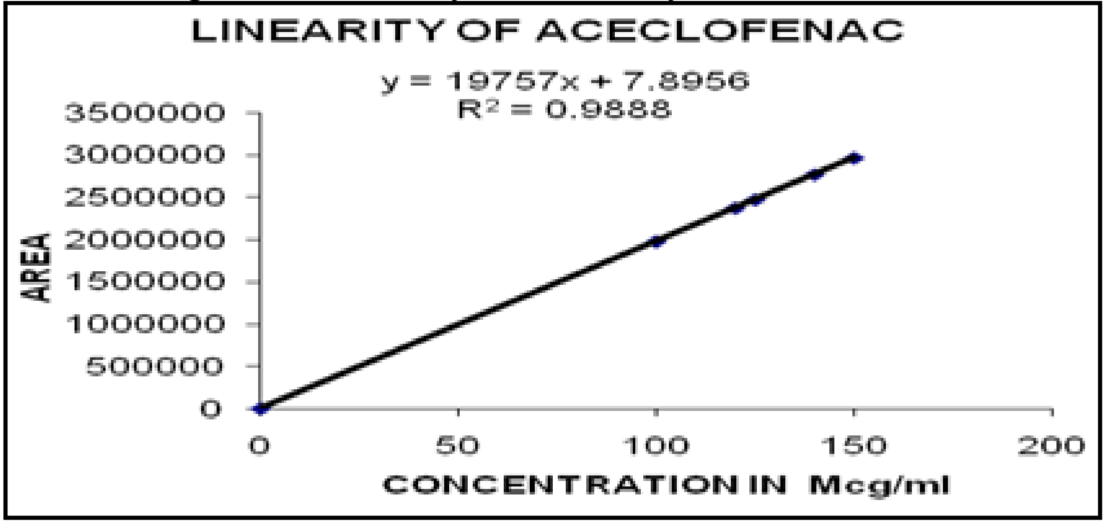

Figure No.2: Linearity of Aceclofenac by RP-HPLC Method

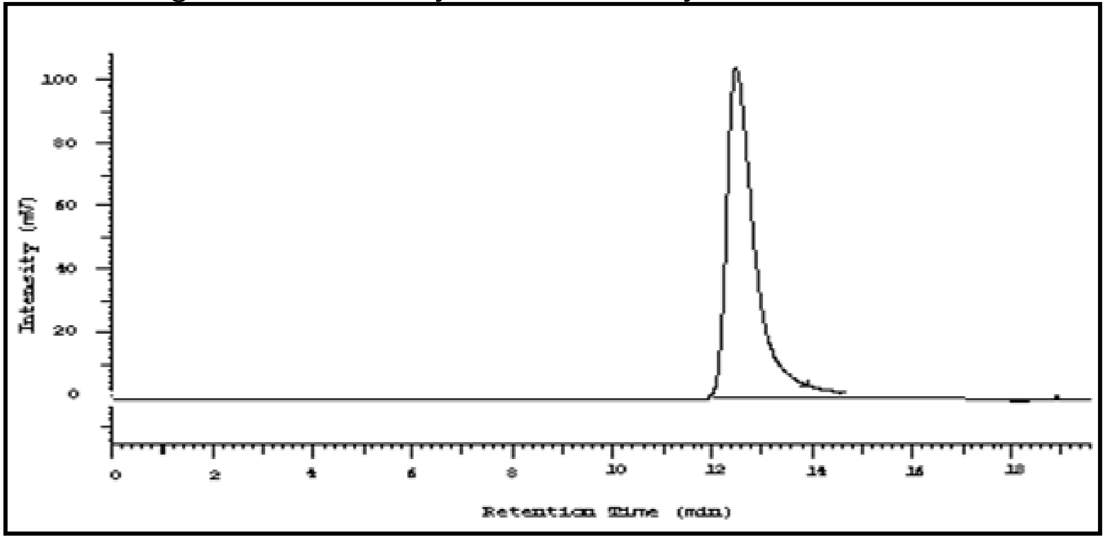

Figure No.3: Identification Chromatogram of Aceclofenac by HPLC Method

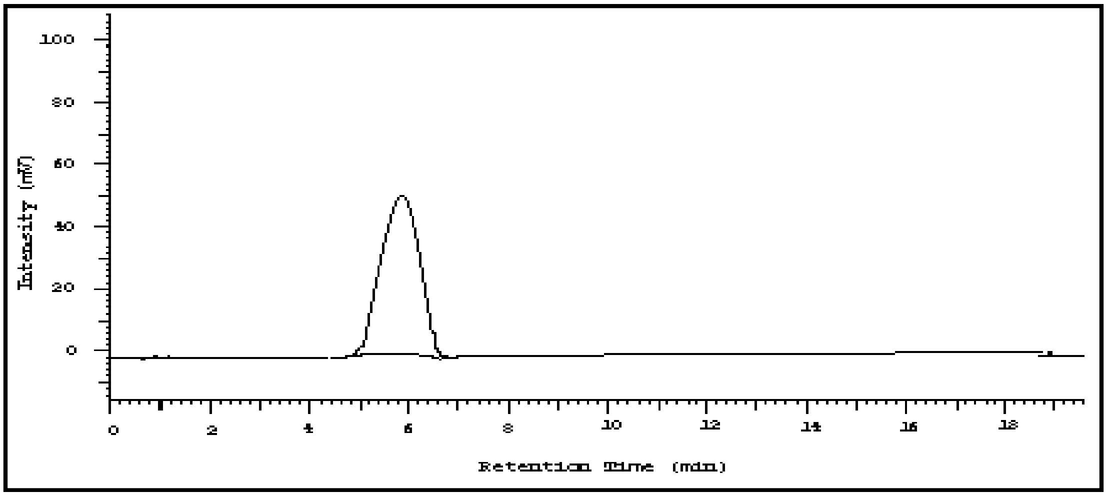

Figure No.4: Identification Chromatogram of Diacerein by HPLC Method

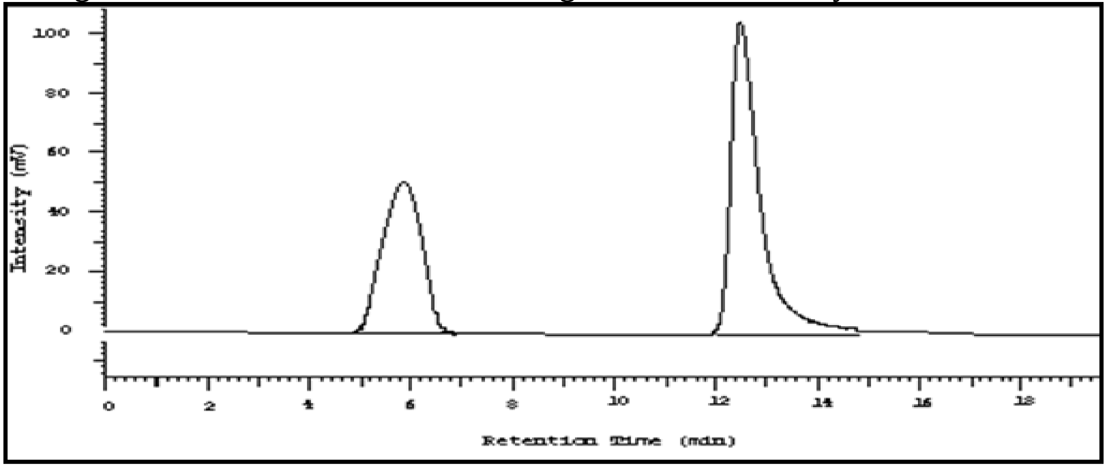

Figure No.5: Identification chromatogram of mixed standard by HPLC method

SUMMARY AND CONCLUSION

Although various methods have been developed for the estimation of Diacerein and Aceclofenac individually and in combination with other drugs, no method has been published till now with combination of these two drugs.

The present combination of Diacerein and Aceclofenac were marketed as one formulation.

Diacerein 50mg/tab
Aceclofenac 100mg/tab

HPLC INSTRUMENT DETAILS

Make : Shimadzu
Model : LC-20 ATVp
Detector : PDA detector
Column make : Thermo Hypersil keystone C-18 ODS column
Particle Size : 5μ
Column length : 240 x 4.3mm i.d
Injector Type : Rheodyne type injector.
Injection Volume : 20μl
Detection wavelength : 285nm
Mobile Phase Used : Acetonitrile and Water in the ratio of 55: 45 (v/v).

The concentrations of both like Diacerein and Aceclofenac were then determined by comparing the peak areas of sample with that of standard peak areas of Diacerein and Aceclofenac in mixture can be identified by their retention times being 5.8 minutes for Diacerein and 13.4 minutes for Aceclofenac. The results was obtained by HPLC method were reproducible and encouraging.

BIBLIOGRAPHY

1. British Pharmacopoeia, British pharmacopoeia commission, *Health Ministry, London,* 15th Edition, 2003, 38.
2. Satoskar R S. In; Pharmacology and pharmacotherapeutics, *Popular Prakashan, Mumbai,* 16th Edition, 1999, 427-563.
3. Text on validation of analytical procedures, *ICH harmonized tripartite guidelines, Adoption on 27 Oct*, 1994, 1-7.
4. Simone G C Ardoso and Silvia. Development and validation of a dissolution method with spectrophotometric analysis for Diacerein capsules, *Scien Pharm*, 76(3), 2008, 541-554.
5. Coran S A and Francesco Salvatore. A validated HPLC stability indicating method for the determination of diacerein in bulk drug substance, *Journal of Pharmaceutical and Biomedical Analysis*, 39(3-4), 2005, 776-780.
6. Yang X F. Sensitive determination of nanogram levels of diacerein in a pharmaceutical formulation by flow injection chemiluminescence analysis, *Journal of the Chinese Chemical Society*, 54(4), 2007, 949-956.
7. Vargas F. A fluorescence spectroscopic study, *Toxicology Mechanisms and Method,* 14(4), 2004, 227-231.
8. Lloyd R. Snyder, Joseph J. Kirkland, Joseph L. Glajch. In; Practical HPLC method development, *John Wiley and Sons, Inc, New York*, 2nd Edition, 1997, 800.
9. Beckett A H. In; Practical pharmaceutical chemistry, *CBS Publishers and Distributors, New Delhi,* 4th Edition, 2001, 157-167.
10. Sethi P D. In; HPLC quantitative analysis pharmaceutical formulation, *CBS Publisher and Distributors, New Delhi*, 1st Edition, 2001, 1-68.
11. Tripathi K D. In; Essentials of medical pharmacology, *Jaypee Brothers Medical Publishers (P) Ltd, New Delhi*, 6th Edition, 2008, 1-1020.
12. The Indian pharmacopoeia commission, *Indian Pharmacopoeia, Ghaziabad,* 5th Edition, 1, 2007, 681-683.

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