VALIDATED RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ACECLOFENAC AND DIACEREIN IN BULK AND FORMULATION

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ABSTRACT: A new simple, accurate, precise and reproducible RP-HPLC method has been developed for the simultaneous estimation of Aceclofenac and Diacerein in tablet dosage forms using C_{18} column (Phenomenex, 250 x 4.6 mm, 5 µm) in isocratic mode. The mobile phase consisted of 0.02 M phosphate buffer: acetonitrile with 5 ml of 0.4% triethylamine in ration of (35:65 v/v) and adjusted to pH 4. The detection wavelength was carried out at 254 nm. The method was linear over the concentration range for Aceclofenac 5-25 µg/ml and for Diacerein 2-10 µg/ml. The recoveries of aceclofenac and Diacerein were found to be in the range of 99.23-100.98% and 99.45-100.61% respectively. The validation of method was carried out using ICH-guidelines. The described HPLC method was successfully employed for the analysis of pharmaceutical formulations containing combined dosage form.
Key words: Simultaneous estimation, RP-HPLC, Aceclofenac, Diacerein, ICH guideline

INTRODUCTION

Aceclofenac, {2, (2,6dichlorophenyl) amino phenyl acetic acid.}, is a phenyl acetic acid derivative with the improved gastric tolerance and is used for relief pain and inflammation in rheumatoid arthritis[1]. Diacerein is chemically 4, 5-diacetyloxy-9, 10-dioxo-anthracene-2-carboxylic acid and is a disease modifying anti-rheumatoid drug used in the treatment of Osteoarthritis and chronic inflammatory arthritis[2]. Many methods have been described in the literature for the determination of aceclofenac with other drugs individually and in combination.[3-9] There are very few reports on analytical methods for estimation of diacerein[10-12] and so far one method has been reported for this combination in plasma[13]. The present work describes the development simple, precise and accurate reverse phase HPLC method for simultaneous estimation of Aceclofenac and Diacerein in bulk and pharmaceutical formulation. The method was validated as per ICH guidelines[14].

EXPERIMENTAL PROCEDURE
Drugs and chemicals:

The pharmaceutical grade pure sample of aceclofenac supplied by Healthcare Pharmaceuticals, Pondicherry, India and Diacerein was obtained from Micro labs, Hosur, India. Acetonitrile HPLC grade solvents; all analytical grade solvents obtained from E-Merck Ltd, Mumbai, India. Potassium dihydrogen ortho phosphate, triethylamine and ortho phosphoric acid AR grade were procured from Qualigens Fine Chemical, Mumbai, India. The HPLC grade water was obtained from a Milli-QRO water purification system.

HPLC apparatus and conditions:

The separation was performed by using Phenomenex C_{18} (250 × 4.6 mm, 5 µm) column on a Shimadzu liquid chromatographic system equipped with a Shimadzu LC 10 AT VP isocratic solvent delivery system, Shimadzu SPD 10A dual wavelength absorbance detector and Rheodyne injector with 20 µl loop volume. Mobile Phase consisted of 0.02 M phosphate buffer: acetonitrile with 5 ml of 0.4% triethylamine in ratio of (35:65 v/v) and its pH was adjusted to 4 with ortho phosphoric acid.

The mobile phase was prepared freshly, filtered, sonicated before use and delivered at a flow rate of 1.0 ml/min. and the detector wavelength was set at 254 nm. The injection volume was 20 µl (fixed loop).

Stock solutions and standards:
Standard stock solutions were prepared of 1000 µg/ml of Aceclofenac and Diacerein, separately using mobile phase. From the standard stock solution different concentrations of working standard solution were prepared ranging from 5-25 µg/ml for Aceclofenac and 2-10 µg/ml for Diacerein.

Calibration curve:
The calibration curves were constructed for the determination of the linearity and the curves were plotted with the concentration range verses area must obey Beer's law. The linearity was evaluated by analysis of the serially diluted sample in the range of 5-25 µg/ml for Aceclofenac and 2-10 µg/ml for Diacerein. An aliquot was injected using mixture of 0.02 M phosphate buffer: acetonitrile with 5 ml of 0.4% triethylamine in ratio of (35:65 v/v) adjusted to pH 4. The 20 µl mixture was injected for the estimation under the optimized chromatographic conditions. The typical chromatogram was recorded for standard as shown in Fig 1. The retention time of standard Aceclofenac and Diacerein were found to be 6.86 min. and 15.32 min respectively with a good resolution of 11.175 (Table 1).

Analysis of formulations:
Twenty tablets were weighed and finely powdered. A quantity equivalent to 50 mg of Diacerein and 100 mg of Aceclofenac were transferred to 100 ml volumetric flask and dissolved on about 50 ml of mobile phase. The solution was ultrasonicated for 10 min and filtered through Whatmann filter paper No.41 and the final filtration was done in 0.45 micron membrane and volume made up to mark with same solvent system. Above solution was taken to prepare a dilution of 10 µg/ml of Diacerein and 20 µg/ml of Aceclofenac. The amount of drug was determined and three replicate injections were done (Table 2).

The assay procedure was repeated for standard and sample six times and mean peak area ratio and concentration of drugs were calculated. The percentage of individual drugs found in formulation, mean, and % RSD in formulation were calculated and present in Table 2. Recovery study carried out for both the drugs was performed by spiking the known amount of pure drug in powdered formulations. It is usually done by adding 80 %, 100 % and 120 % of the pure drug with the formulation taken for analysis. The average % recovery for Aceclofenac and Diacerein was found to be 99.92 % to 100.20 % respectively. The results are tabulated below in Table 3.

RESULT AND DISCUSSION
Method development:
Several tests were performed in order to get satisfactory separation-resolution of Aceclofenac and Diacerein in different mobile phases with various ratios of organic phase and buffers by using C_{18} column. The ideal buffer was used 0.02 M phosphate buffer: acetonitrile (35:65 v/v) with 5 ml of triethylamine (0.4%) and adjusted to pH 4 by isocratic elution to obtain satisfactory and good resolution. Increasing or decreasing pH of mobile phase by ±0.2 dose not shows significant change in retention time of each analyte. The retention of Aceclofenac and Diacerein on analytical column was evaluated at a flow rate of 1.0 ml/min. and the injection volume was 20 µl. The retention time of standard and sample for Aceclofenac and Diacerein were satisfactory with good resolution.

Linearity:
The linearity for HPLC method was determined at six concentration levels. The linearity of Aceclofenac and Diacerein were determined by calibration curves and the linearity based on the area observed in the range of 2-10 µg/ml for Diacerein and 5-25 µg/ml for Aceclofenac. The % relative standard deviation of peak area and the retention time was within the limit of ± 2%. This indicates that the method was system suitable. The reports are tabulated below in Table 1. The regression co-efficient value (r^2) for Aceclofenac and Diacerein is 0.9995 and 0.9993 respectively.

Precision:
Precision was measured for both inter and intra-day, and checked with repeatability and the % RSD for the repeatability was found to be 0.521% to 0.361% and 0.433% to 0.576% respectively for Aceclofenac and Diacerein. The RSD was found to within the limit and tabulated in Table 1. The limit of quantification was determined by injecting minimum concentration of the drugs .The limit of quantification was found to be 2 µg /ml and 1 µg/ml for Aceclofenac and Diacerein.

Recovery studies:
Specificity and Selectivity:
Specificity was tested against standard compounds and against potential interferences. To determine specificity with respect to sample compounds the responses of standard and sample solution were compared. No interferences were detected at the retention times of either Aceclofenac or Diacerein in sample solution.

The limit of detection (LOD) was determined as lowest concentration giving response and limit of quantification was determined as the lowest concentration analyzed with accuracy method were determined by injecting progressively low

concentrations of the standard solutions using developed RP-HPLC method. The limit of detection (LOD) for Aceclofenac and Diacerein was found to be 1 µg /ml and 0.80 µg/ml respectively. The limit of quantification (LOQ) was 2 µg /ml and 1µg/ml for Aceclofenac and Diacerein respectively and reported in Table 1.

Stability:
In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 h at room temperature. The results show that for both solutions, the retention time and peak area of Aceclofenac and Diacerein remained almost similar (% RSD less than 2.0) and significant degradation within the indicated period, thus indicated that both solutions were stable for at least 24 h, which was sufficient to complete the whole analytical process.

Ruggedness and Robustness:
Ruggedness test was determined between two analysts, instruments and columns. Robustness of the method was determined by small deliberate changes in flow rate, mobile phase pH and mobile phase ratio. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was rugged and robust.

CONCLUSION
A selective, sensitive, precise and accurate method has been developed for the analysis of Aceclofenac and Diacerein in Tablet dosage form**.** Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.

Table 1: System Suitability Parameters

PARAMETERS	Aceclofenac	Diacerein
Calibration Range (µg /ml)	5-25	2-10
Correlation Coefficient(r2)	0.9995	0.9993
Retention time(Min)	6.86±0.2	15.32±0.2
Asymmetry	1.27	1.18
Theoretical Plates	4586	5347
Resolution factor	11.175	---
Tailing Factor	1.36	1.12
Repeatability %RSD (n=5)		
Intra day	0.433%	0.576%
Inter day	0.521%	0.361%
Limit of quantification (µg /ml)	2	1
Limit of detection (µg /ml)	1	0.8

Table 2: Analysis of Marketed Formulation

Formulation	Aceclofenac			Diacerein		
	Label claim mg/tab	Amount found* mg/tab ± RSD	% assay ± RSD	Label claim mg/tab	Amount found* mg/tab ± RSD	% assay ± RSD
DYCERIN A	100	99.48±0.423	99.48±0.852	50	50.82±0.891	101.64±0.728

Table 3: Recovery Studies of Aceclofenac and Diacerein in Combined Dosage Form

Formulation	Aceclofenac			Diacerein		
	% added	% recovered* ± RSD	% recovery ± RSD	% added	% recovered* ± RSD	% recovery ± RSD
DYCERIN A	80	79.38±0.421	99.23±0.018	80	80.44±0.591	100.55±0.382
	100	99.56±0.884	99.56±0.013	100	99.45±0.651	99.45±0.011
	120	121.18±1.124	100.98±0.068	120	120.73±0.632	100.61±0.223

*Mean of six estimations
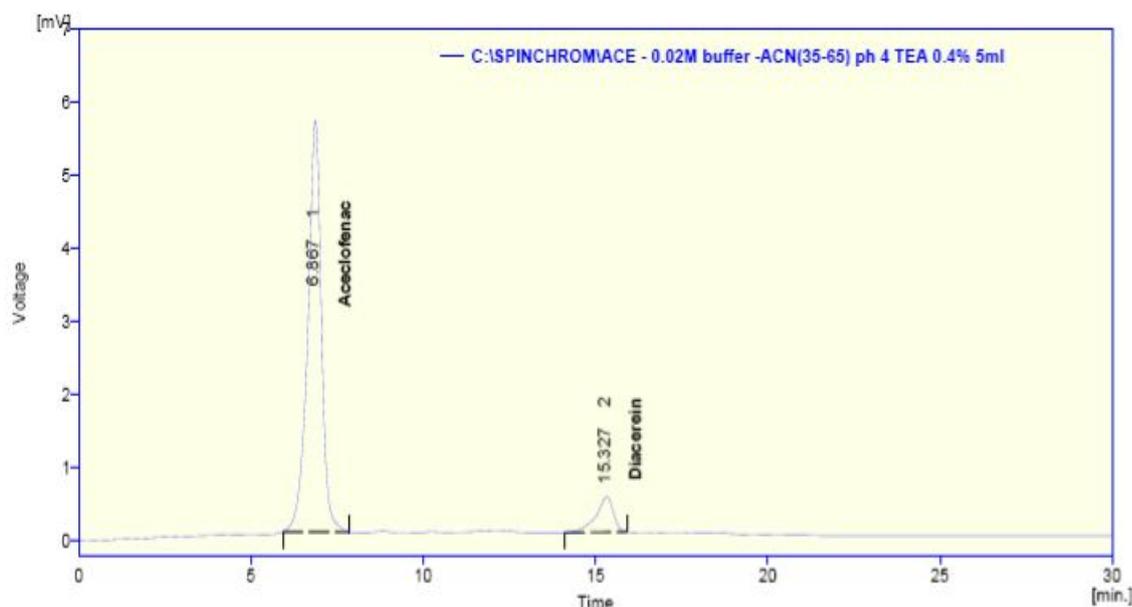
Figure 1:- A Typical Chromatogram for Aceclofenac and Diacerein

REFERENCES

1. Budavari S., *The Merck Index*, Merck & Co., INC., New Jersey, 2001, 22.
2. Oneil M.J., Heckelman P.E. and Koch C.B., In: The Merck Index. An Encyclopedia of Chemicals: Drugs and Biologicals. 14th ed., Whitehouse station, NJ: Merck and Co Inc.; 2006, 503.
3. Shanmugham S., Cendil Kumar A., Vetrichelvan T., Manavalan R., Venkappayya D. and Pandey V.P., Spectrophotometric method for the estimation of aceclofenac in tablets, Indian drugs, 2005, 42, 106- 108.
4. Srinivasan K.K., Alex J., Shirwaikar A.A., Jacob S., Sunil Kumar M.R. and Prabu S.L., Simultaneous derivative spectrophotometric estimation of aceclofenac and tramadol with paracetamol in combination solid dosage forms. Indian. J. Pharm.Sci., 2007, 69, 540-545.
5. El-Saharty Y.S., Refaat M. and El-Khateeb S.Z., Stability-Indicating Spectrophotometric and Densitometric Methods for Determination of Aceclofenac, Drug Development and Industrial Pharmacy, 2002, 28,571 – 582.
6. Gopinath R., Rajan S., Meyyanathan S.N., Krishnaveni N. and Suresh B. A., RP-HPLC Method for simultaneous Estimation of Paracetamol and Aceclofenac in tablets, Indian J Pharm Sci., 2007, 69, 137-140.
7. Shaikh K.A. and Devkhile A.B., Simultaneous Determination of Aceclofenac, Paracetamol, and Chlorzoxazone by RP-HPLC in

Pharmaceutical Dosage Form, J. of Chromatogr. Sci., 2008, 46, 649-652.

8. Garg G., Saraf S. and Saraf S., Simultaneous estimation of aceclofenac, paracetamol and chlorzoxazone in tablets, Indian. J. Pharm. Sci., 2007, 69, 692-694.

9. Bhinge J.R., Kumar R.V., and Sinha V.R., A Simple and Sensitive Stability-Indicating RP-HPLC Assay Method for the Determination of Aceclofenac, J. of Chromatogr. Sci., 2008, 46,440-444.

10. Borgmann S.H., Parcianello L.M., Arend M.Z. and Cardoso S.G., Direct spectrophotometric determination of diacerhein in capsules, Pharmazie, 2007, 62,483-485.

11. Layek B, Santosh Kumar T, Trivedi R, Mullangi R, Srinivas NR, Development and validation of a sensitive LC-MS/MS method with electrospray ionization for quantitation of rhein in human plasma: application to a pharmacokinetic study, Biomedical Chromatography, 2008, 22, 616 – 624.

12. Giannellini V., Salvatore F., Bartolucci G., Coran S.A., and Alberti M.B., A validated HPLC stability-indicating method for the determination of diacerhein in bulk drug substance, J. Pharm. Biomed. Anal., 2005, 39,776–780.

13. Ojha A., Rathod R. and Padh H., Simultaneous HPLC–UV determination of rhein and aceclofenac in human plasma, J. Chromatography B., 2009, 877, 1145-1148.

14. Siva kumar R., Kumar Nallasivan P., Saravanakumar S., Kandasamy C.S. and Venkatnarayanan R., Simultaneous RP-HPLC Estimation of Nitazoxanide and Ofloxacin in Tablet Dosage Forms, Asian J. Research. Chem., 2009, 2, 43-45.

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