Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol. 4, Suppl 1, 2011 ISSN - 0974-2441

Research Article

STABILITY AND REVERSE PHASE HPLC ASSAY METHOD FOR DETERMINATION OF DIACEREIN AND ACECLOFENAC IN TABLET DOSAGE FORM - APPLICATION TO DISSOLUTION STUDIES

M. C.SHARMA [A], SMITA SHARMA *

a School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore (M.P) 452001, India, * Department of Chemistry Chodhary Dilip Singh Kanya Mahavidyalya, Bhind (M.P) 477001, India. Email: drsmita.sharma@rediffmail.com

ABSTRACT

The proposed method is precise, accurate, selective and rapid for the simultaneous determination of Diacerein and Aceclofenac. A simple, rapid and selective HPLC method has been developed for quantitation of Diacerein and Aceclofenac from bulk drug and pharmaceutical formulations using a mobile phase consisting mixture of methanol and water (80:20 v/v) at the flow rate of 1mL/min. An ODS C18 RP-Column (Intersile 4.6 mm x 25 cm, 10 µm) column was used as stationary phase. The retention time of Diacerein and Aceclofenac were 4.6 min. and 7.3 min. respectively. Linearity was obtained in the concentration range of 5-35µg/ml for Diacerein, 5-35 µg/ml for Aceclofenac. Gatifloxacin was used as an internal standard. The method was statistically validated and RSD was found to be less than 2% indicating high degree of accuracy and precision of the proposed HPLC method. Due to its simplicity, rapidness, high precision and accuracy, the proposed HPLC method may be used for determining Diacerein and Aceclofenac in bulk drug samples or in pharmaceutical dosage form.

Keywords: Drug interactions, Antidepressants, Drug utilization, Severity, Prescriptions.

INTRODUCTION

Diacerein chemically is 4,5-diacetyloxy- 9,10- dioxo- anthracene – 2-carboxylic acid. Diacerein is anti-arthrtic activity without inhibiting prostaglandin synthesis. It is novel antinflammatory drug with pharmacological properties different from those of chemical non-steroidal antiflammatory drug clinical studies have suggested that diacerein exerts a beneficial effect on the symptomatic treatment of osteoarthrisis[1,2] Aceclofenac {[2-(2', 6'-dichlorophenyl) amino] phenyl acetoxyacetic acid} is a new phenyl acetic acid derivative with potent analgesic and anti-inflammatory properties and improved gastric tolerance[3,4].

Aceclofenac is an orally administered phenyl acetic acid derivative with effect on a variety of inflammatory mediators. It is the non steroidal antinflammatory drug. Aceclofenc provides symptomatic relief in a variety of painful condition. A reduction in the stimulated generation of reactive oxygen species which may play a role in a joint damage, aceclofenac is inhibition of prostaglandin synthesis, aceclofenac is potent inhibitor of the enzyme cycloxygenase. The drug inhibits synthesis of the inflammatory cytokines interleukin (IL) -1 and tumour necrosis factor and prostaglandin E2 (PGE2) production, Invitro drug indicate inhibition of cyclooxygenase (Cox)-1 and 2 by aceclofenac in whole blood assays with selectively for Cox -2 being evident.

Literature survey reveals that few UV, HPLC, HPTLC and colorimetric methods have been reported for the estimation of Diacerein and Aceclofenac as single component formulation and combination with other drugs in bulk samples, formulations and biological fluids[5-13]. Author of the article and his research team has developed a HPLC method development in different pharmaceutical dosage form [14-18].

The method was validated according to procedures and acceptances criteria based on FDA guidelines and recommendations of ICH. However, no method has been developed for estimation of these drugs in combined dosage form. This paper presents simple, rapid, reproducible and economical method for RP-HPLC simultaneous estimation of Diacerein and Aceclofenac in tablet dosage form.

MATERIAL AND METHOD

Chromatographic conditions

All reagents were of HPLC grade unless otherwise specified. from E.Merck (Mumbai, India), Potassium Dihydrogen Phosphate and o-phosphoric acid were purchased from SD fine chemical Ltd (Ahmadabad, India) and were of analytical grade Water of HPLC grade was used. Potassium Dihydrogen Phosphate and o-phosphoric acid were purchased from SD fine chemical Ltd (Ahmadabad, India) and were of analytical grade Water of HPLC grade was used. Gatifloxacin was used as an internal standard. The LicATVphere C_{18} column was used 25°C temperature. Rheodyne injection syringe with 20 µl loop volume and windows based chrompass software. An ODS C18 RP-Column (Intersile 4.6 mm x 25 cm, 10 µm) was used for separation. The flow rate of the mobile phase was maintained at 1.2 ml/min and the column temperature 45°C. Detection was carried out at 210 nm and the injection volume was 50 µm. Run time was 9 min.The elution was carried out isocratically at flow rate of 1.2 mL/min using methanol: water (80:20 v/v) mobile phase. The mobile phase was passed through nylon 0.45 µm membrane filters and degassed before use.

Preparation of Standard Stock Solution

The standard stock solution 0.5 mg/ml of Diacerein and Aceclofenac were prepared separately by dissolving 50 mg of each drug in 50 ml mixture of methanol and water (80:20 v/v). From the standard stock solution, mixed standard solution was prepared to contain 50 µg/mL of Diacerein and 100 µg/mL of Aceclofenac. Gatifloxacin (100µg/ml) was used as an internal standard.

Preparation of Sample Solution

Twenty tablets each containing 50 mg of Diacerein and 100 mg of Aceclofenac were weighed and powder equivalent to 50 mg of Diacerein and 100 mg of aceclofenac was weighed accurately and transferred to a conical flask and extracted thrice with 20 ml mixture of methanol and water (80:20). The combined extracts were transferred to a volumetric flask and the volume adjusted to 100 ml with mobile phase. From this solution, 10 ml was pipette and transferred to 100 ml volumetric flask and made volume up to the mark with mobile phase to get the concentration50 µg/ml of Diacerein and 100 µg/ml of aceclofenac. Further dilutions were made using mobile phase to get the final concentration of 5 µg/ml of Diacerein and 10 µg/ml aceclofenac.

Dissolution study

For the dissolution study[19-20] of Diacerein and aceclofenac analysis was done by using above chromatographic conditions. For this study standard solution of Diacerein and aceclofenac was prepared in dissolution media. For sample preparation an intact tablet was dissolved in 0.1 N HCL media (RPM 70). Sample was collected in

dissolution vials after 2 hrs and then decanted the 0.1 N HCL media and the 6.8 pH phosphate buffer media was loaded and set RPM 70. Samples were collected in dissolution vials after different time intervals and filtered through 0.45 µm filter. Equal volumes (50 µL) of these solutions were injected into the chromatograph by auto sampler and peak areas were measured.

Effect of pH

The effect of changing the pH of the mobile phase on the selectivity and retention times of the test solutes was investigated using mobile phases of pH ranging from 4.5-6.1. Shows that a pH of 4.7 was the most appropriate one giving well-resolved peaks and highest no. of theoretical plates. There were always asymmetric and broad peaks of Diacerein and aceclofenac at pH values > 6.0.

Effect of Flow rate

The effect of flow rate on the formation and separation of peaks was studied by varying the flow rate from 1 - 1.5; a flow rate of 1.5 mL min^{-1} was optional for good separation and resolution of peaks in a reasonable time.

Effect of Temperature

The effect of Temperature on the formation, separation and resolution was studied by varying the Temperature from 16 – 24 ^{0}C; we found that at lower Temperatures the peaks are not well resolved.

RESULTS AND DISCUSSION

Method Validation

Linearity

Calibration graphs were constructed by plotting peak area Vs concentration of Diacerein and Aceclofenac.The calibration graphs were plotted over six different concentrations in the range of 5-35µg/ml for both drugs. Accurately measured mixed standard solution aliquots of Diacerein and Aceclofenac (1, 2.0, 3.0, 4.0, 5.0 ml) were transferred to series of 10 ml volumetric flasks and diluted to mark with methanol. Aliquots (10µl) of each solution were injected under the operating chromatographic condition described above [Number of replicates (n=6)].

Accuracy

The accuracy of the method was established using recovery technique i.e. external standard addition method. The known amount of standard was added at three different levels to preanalysed sample. Each determination was performed in triplicate. The result of recovery study is presented in (table 2).

Method precision (repeatability)

The precision of the instrument was checked by repeatedly injecting (n = 6) mixed standard solution of Diacerein and Aceclofenac.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing mixed standard solution of Diacerein and Aceclofenac at concentration 5µg/ml and 25µg/ml three times on the same day and on three different days. The results are reported in terms of relative standard deviation.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD with signal to noise (S/N) ratio of 6:2 and LOQ with (S/N) ratio of 2:1 were calculated for both drugs using the following equations according to International Conference on Harmonization guidelines.

$LOD = 8 \times \sigma/S$, $LOQ = 4.5 \times \sigma/S$

Where σ = the standard deviation (SD) of the response and S = the SD of the y-intercept of the regression line.To ensure the reliability and accuracy of the method, recovery studies were carried out in triplicate at three concentration levels (50%, 100% and 150%) of test concentration. Several aliquots of standard solutions of Diacerein and Aceclofenac were taken in different 10ml volumetric flasks and diluted up to the mark with mobile phase such that the final concentration of Diacerein and Aceclofenac is 5-35 µg/mL and5-45 µg/mL respectively. Evaluation of two drugs were performed with PDA detector at 266 nm, peak area recorded for all the peaks and are given in the Table I.

The slope and intercept value for calibration curve was y =7654.80 x + 0.543 (r^2 = 0.9993) for Diacerein and y =3276.65x + 26.858 (r^2 = 0.9988) for aceclofenac. The results show that an excellent correlation exists between peak area and concentration of drugs within the concentration range indicated above. The limit of Detection (LOD) and limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 8). The LOD Diacerein and Aceclofenac were found to be 23 ng/ml and 15 ng/ml, respectively.

The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed, are rugged and robust. The mean (n = 6) percentage dissolution of Diacerein and Aceclofenac in 6.8 pH phosphate buffer in 2 hrs from tablet dosage form was found within the limit.

Analytical RP-HPLC method was developed and validated for the determination of Diacerein and Aceclofenac in bulk and dosage form. The advantages of the method are short run time, simplicity of sample preparation, no need of derivative formation, which require longer time for analysis. The other advantage of the method is the common chromatographic conditions adopted for both the assay and dissolution studies. As a result, the proposed method reduces the time required for switch over of chromatographic conditions, equilibration of column and post column flushing; that are typically associated when different chromatographic conditions are used.

CONCLUSION

To study the accuracy and reproducibility of the proposed method recovery experiments were carried out. A fixed amount of pre-analyzed sample was taken and standard drug was added at 50% and 100% levels. Each level was repeated three times. The lower values of RSD of assay indicate the method is accurate. The mean recoveries of Diacerein and Aceclofenac were in range of 100.77% and 100.66% that shows there is no interference from excipients.

ACKNOWLEDGEMENT

The authors are thanking the referees for their valuable suggestions.

Table 1: System suitability test parameter for Diacerein and Aceclofenac

Property (n*=6)	DIA	ACE
Retention time(min)	4.6	7.3
Tailing factor ATV	1.16	1.26
Capacity factor ATV	2.05	4.11
Theoretical plates number	7693	9836
Resolution	1.94	2.29

* n = Number of determination, DIA-Diacerein, ACE-Aceclofenac

Table 2: Recovery Study Diacerein and Aceclofenac

Diacerein				Aceclofenac			
Label claimed	**%Amount added**	**Found in(µg/ml)**	**%recovery**	**Label claimed**	**%Amount added**	**Found in(µg/ml)**	**%recovery**
	80	50.05	100.07		80	100.22	100.01
50	100	51.13	101.03	100	100	100.09	100.19
	120	50.31	100.26		120	101.13	101.03

DIA-Diacerein ,ACE-Aceclofenac

Table 3: Regression Analysis of Calibration Graph for Diacerein and Aceclofenac

Parameter	Diacerein	Aceclofenac
Concentration range	5-35 µg/ml	5-45 µg/ml
Slope	43276	176544
SD[$] of the slope	1.076	3.965
Intercept	36532	776432
SD[a] of the intercept	2.765	8.6276
Correlation coefficient	0.9993	0.9988

[$] SD = Standard Deviation

Table 4: Summary of validation parameter Diacerein and Aceclofenac

Parameter	Diacerein	Aceclofenac
LOD[a]	33.2ng/ml	25.01ng/ml
LOQ[b]	10.02ng/ml	10.07µg/ml
Accuracy, %	99.97± 0.21	101± 0.17%
Repeatability(RSD[c] %, n =6)	0.327	0.517
Precision (RSD, %)		
Intraday (n =3)	0.28	0.36
Interday (n = 3)	0.15	0.22

ATV- Atorvastatin Calcium, PIO-Pioglitazone

Table 5: Dissolution parameters and HPLC Condition Diacerein and Aceclofenac

Dissolution parameters	
Medium	Phosphate buffer pH 6.8, 0.1 N HCL buffer pH-1.2 and Water.
Volume	900 mL
Apparatus	Paddle
RPM	70
Temperature	37 ± 0.5^{0}C
Time	120 minutes

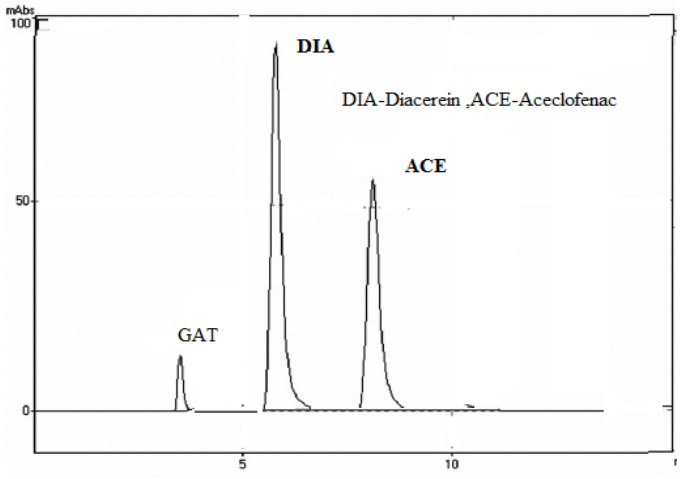

Fig. 1: Chromatogram of Diacerein and Aceclofenac

REFERENCES

1. Beckett A H ,Stenlake JB, Practical Pharmaceutical Chemistry, 4th ed., Part II, CBS Publisher and Distributor, New Delhi, (1997) 1.
2. Martindale, the Extra pharmacopoeia, 31stedition, edited by James E. F. Reynolds. p. 35.
3. European Pharmacopeia 6.0. 2, 1093-1095.
4. Borgmann SH, Parcianello LM, Arend M.Z, Cardoso S G.Direct Spectrophotometric determination of Diacerein in capsules Bioinfo bank library, Pharmazie. 2007; 62:483-5.
5. Giannellini V, Salvatore F, Bartolucci G, Coran SA, Bambagiotti-Alberti M. A validated HPLC stability-indicating method for the

determination of Diacerein in in bulk drug substance.Bioinfo bank library, J .Pharm. Biomed. Anal. 2005 ; 15:39 (3-4).

6. Musmade P, Subramanian G, Srinivasan KK.High-performance liquid Chromatography and pharmacokinetics of aceclofenac in rats, Analytica Chimica Acta. 2007;585(1): 103-109.
7. Gopinath R, Rajan S, MeyyanathanSN, Krishnaveni N, Suresh B.A RP-HPLC method for simultaneous estimation of Paracetamol and Aceclofenac in tablet, Indian. J. Pharm sci.2007; 69(1):137-140.
8. N. H. Zawilla NH, Abdul Azim MM, El Kousy NM, El-Moghazy Aly SM.Determination of aceclofenac in bulk and pharmaceutical formulations" J. Pharma and BiomedAnal.2002;27(1-2): 243-251.
9. El Kousy N M.Spectrophotometric and spectrofluorimetric determination of etodolac and aceclofenac, J . Pharma and Biomed Anal.1999; 20(1-2): 185-194.
10. Kousey E, Zawilla NH , Abdul AM. Determination of aceclofenac in bulk and pharmaceutical formulation by HPLC, J. Pharm Biomed. Anal. 2002; 9(4):243-251.
11. Shanmugam S, Cendil KA, Vetrichelvan J, Manavalan R, Venkappayya D and Pandey V. Spectrophotometric method for estimation of Aceclofenac in tablets, Indian Drugs.2005;42:106-107.
12. Sane RT, Menon SN, Mote M and Inamdar S. HPTLC determination of aceclofenac in bulk drug and in pharmaceutical preparation. J. Planer Chromatogr.2004; 17(3):241-245.
13. Sharma M.C, Sharma S.Development and Validation of An HPTLC Method for Determination of Oseltamivir phosphate in pharmaceutical dosage form,Indian Drugs.2010; 47(11)68-72.
14. Sharma S, Sharma MC, Kohli DV, Chaturvedi SC. Isocratic Reverse Phase HPLC estimation method of Torsemide and Spironolactone in pharmaceutical combined dosage form, Optoelectronics and Advanced Materials - Rapid Communications .2010;4,ISS.2, ,234-237.
15. Sharma S, Sharma MC, Kohli DV, Chaturvedi SC. Study of stressed degradation behaviour of Pioglitazone hydrochloride in bulk and pharmaceutical formulation by HPLC assay method, J. Optoel. and Biomed. Mate.2010; 1(1):17 – 24.
16. Sharma, S., Sharma, M.C., Chaturvedi, S.C.Development and validation of isocratic reversed-phase HPLC method for simultaneous estimation of torsemide and spironolactone in bulk and pharmaceutical combined tablet dosage form"Optoelectronics and Advanced Materials-Rapid Communications .2010; 4 ISS. 3,427- 430.
17. Sharma, S., Sharma, M.C., Chaturvedi, S.C.Simultaneous estimation of Rosuvastatin calcium and Ezetimibe in tablet dosage form by reverse phase high performance liquid chromatography,Optoelectronics and Advanced Materials-Rapid Communications. 2010; 4 ISS.2,238 – 241.
18. Morre JW, Flanner HH. Mathematical comparison of dissolution profiles, Pharm Techno.1996; 6: 64.
19. Shah VP, Tsong Y, Sathe P, Williams RL. Dissolution profile comparison using similarity factor, f_2, Dissolution Techno.1999; 6: 15.