Asian Journal of Chemistry; Vol. 23, No. 12 (2011), 5531-5534

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Method Development and Validation of Stability Indicating Method for Assay of Diacerein and Aceclofenac by High Performance Liquid Chromatography

MADHUKAR A. BADGUJAR* and KIRAN V. MANGAONKAR

Analytical Chemistry Research Laboratory, Mithibai College, Vile Parle (W), Mumbai-400 056, India

*Corresponding author: E-mail: bmbadgujar@yahoo.com

(*Received*: 14 April 2011; *Accepted*: 26 August 2011) AJC-10322

A simple, fast, precise, specific, accurate and stability-indicating reversed phase high performance liquid chromatographic (HPLC) method was developed and validated for the simultaneous determination of diacerein and aceclofenac in tablets. The column used was Inertsil ODS 3V C_{18}, 250 × 4.6 mm, *i.d.* 5 µm and a mobile phase composed of methanol:acetonitrile:buffer (0.02 M KH_2PO_4) (50:20:30), pH 5.9 adjusted with 0.1 % triethylamine. The retention times of diacerein and aceclofenac were found to be 3.2 and 6.2 min, respectively. Linearity was established for diacerein and aceclofenac in the range of 20-100 µg/mL and 40-200 µg/mL, respectively. The percentage recoveries of diacerein and aceclofenac were found to be in the range of 99.99 to 101.34 % and 97.90 to 100.35 % respectively. Both these drugs were subjected to acid, alkali, oxidation and thermal degradation. The degradation study shows 22.58 and 13.49 % degradation for diacerein and aceclofenac respectively, under alkali degradation condition. The degradation products of diacerein and aceclofenac were well resolved from the pure drug with significant differences in their retention time values. This method can be successfully employed for simultaneous quantitative analysis of diacerein and aceclofenac in bulk drugs and formulations.

Key Words: Diacerein, Aceclofenac, RP-HPLC, Validation.

INTRODUCTION

Osteoarthritis also known as degenerative arthritis or degenerative joint disease, is a group of mechanical abnormalities involving degradation of joints including articular cartilage and subchondral bone. Symptoms may include joint pain, tenderness, stiffness, locking and sometimes an effusion. A variety of causes-hereditary, developmental, metabolic and mechanical-may initiate processes leading to loss of cartilage. When bone surfaces become less well protected by cartilage, bone may be exposed and damaged[1]. As a result of decreased movement secondary to pain, regional muscles may atrophy and ligaments may become more lax[2]. diacerein, (diacetylrhein; [4,5-*bis* (acetyloxy)-9,10-dihydro-9,10-dioxo-anthracene-2-carboxylic acid]; CAS no 13739-02-1) [Fig. 1A] has been found to be effective in the treatment of osteoarthritis as a synthetic chemical and also in native form from many plants. It belongs to anthraquinone class of molecules.

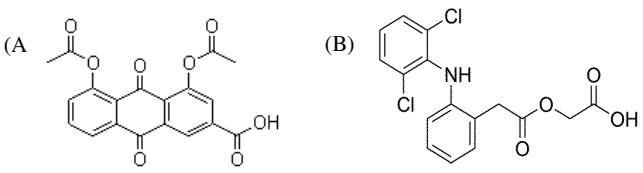


Fig. 1. Chemical structures of (A) Diacerein and (B) Aceclofenac

Aceclofenac, ([2-(2'6'-dichlorophenyl)amino]phenyl acetoxy acetic acid; CAS no. 89796-99-6) [Fig. 1B] belongs to the class of non-steroidal anti inflammatory drugs (NSAIDs). It has pronounced antiinflammatory, antipyretic, antirheumatoid and analgesic effect and an improved gastro-intestinal tolerance. It is applicable in various pain conditions like rheumatoid arthritis, osteoarthritis and ankylosing spondylatis. It is well absorbed after oral administration and circulates mainly as unchanged drug. 70 % of the administered dose is excreted in urine as gluconoride of aceclofenac and diclofenac[3].

Literature survey reveals that various analytical techniques *viz*, UV spectrophotometry[4,5], high performance thin layer chromatography (HPTLC)[6] and high performance liquid chromatography (HPLC) were reported for the analysis of diacerein and aceclofenac in pharmaceuticals. Few high performance liquid chromatography[7-12] methods have been reported for the simultaneous determination of diacerein and aceclofenac. But there was no any stability indicating HPLC method reported for the analysis of these drugs. Hence our aim is to develop stability indicating method for the estimation of diacerein and aceclofenac in combined dosage form. Present paper describes validated stability indicating reverse phase HPLC method for simultaneous determination of diacerein and aceclofenac.

EXPERIMENTAL

Diacerein and aceclofenac working standard were obtained from Glenmark (Mumbai, India), tablets of Integrace® containing diacerein (50 mg) and aceclofenac (100 mg) were obtained from Glenmark (Mumbai, India), high performance liquid chromatography grade methanol and acetonitrile were purchased from Baker (Mumbai, India), AR grade potassium dihydrogen phosphate, triethylamine, sodium hydroxide (NaOH), hydrochloric acid (HCL), hydrogen peroxide (H_2O_2) were purchased from Merck (Mumbai, India).

Standard stock preparation: Each of diacerein E (50 mg) and aceclofenac (100 mg) working standards were weighed separately and transferred in to the 100 mL volumetric flask. Dissolved the content with 10 mL of dimethyl sulfoxide and finally made the volume upto the mark with diluent. Further 5 mL of the above stock solution was diluted to 50 mL with diluent to give the concentration of 50 ppm for diacerein and 100 ppm for aceclofenac.

Preparation of sample solution: Twenty tablets were weighed and average weight was calculated. These tablets were crushed and weight equivalent to 1 tablet was taken in a 100 mL volumetric flask. To this 10 mL of dimethyl sulfoxide was added and sonicated for 20 min and shaken by mechanical means for 20 min at 250 rpm. Further the solution was diluted with diluent upto the mark. The solution was mixed and allowed settling for 5 min. Then the solution was filtered through 0.45 µ syringe filter. 5 mL of the filtrate was diluted to 50 mL with diluent and mixed. The concentrations obtained were 50 µg/mL of diacerein and 100 µg/mL of aceclofenac. 20 µL of standard and sample solutions were injected in triplicate under the optimized chromatographic conditions.

Chromatographic conditions: The chromatographic system consist of a waters HPLC system having waters 501 isocratic pump equipped with waters™ 717plus autosampler and a waters 486 tunable absorbance UV-detector. The data was recorded using Millenium chromatographic software. Separation was performed on a 250 mm × 4.6 mm *i.d.*, 5 µ particle size Inertsil C_{18} column. Mobile phase consisted of a mixture of methanol : acetonitrile : buffer (50:20:30), pH 5.9 adjusted with 0.1 % triethylamine. Flow rate was kept at 1.0 mL/min. Wavelength was set at 265 nm.

Method validation: The method was validated as per ICH guidelines[13] for specificity, linearity, quantification limit, precision, accuracy, recovery and stability. Specificity was investigated by analyzing the blank diluents and samples of 100 % level for any interference of the exciepients at the retention times of diacerein and aceclofenac. It was also investigated by performing the force degradation studies. The accuracy of the method was determined by recovery experiments. The precision of the method was demonstrated by interday and intraday variation studies, six repeated injections of standard and sample were made and percentage RSD was calculated. In the intraday variation studies six repeated injections of standard and sample solution was carried out by injecting on the same day at different intervals and percentage RSD was calculated. In the interday variation studies six repeated injections of standard and sample solution were made for three consecutive days and percentage RSD was calculated. For stability indicating method the standard drugs were subjected for force degradation studies. The linearity of the method was demonstrated at seven concentration levels of the mixed standards of diacerein and aceclofenac.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions: In order to develop an isocratic reverse phase stability indicating HPLC method for the simultaneous determination of diacerein and aceclofenac in combined dosage form, the chromatographic conditions were optimized. For better separation and resolution the different buffers were tried. It has been found that potassium dihydrogen phosphate buffer, pH 5.9 adjusted with 0.1 % triethylamine gave better peak shape than other buffers. The different compositions of mobile phase were changed for getting better separation of these analytes. Thus the mobile phase composed of the mixture of methanol, acetonitrile and buffer (0.02 M potassium dihydrogen phosphate, pH 5.9 adjusted with 0.1 % triethylamine) in the ratio of (50:20:30 v/v) was finalized. The better separation, peak symmetry and reproducibility were obtained with Inertsil C_{18}, 250 mm × 4.6 mm, 5 µm column compared to Thermo BDS Hypersil C_8, 150 mm × 4.6 mm, 5 µm column. Both these analytes gave better response at 265 nm wavelength using UV detector. The flow rate kept was 1.0 mL/min. There was no peak tailing observed under these optimized chromatographic conditions. The retention times of diacerein and aceclofenac were found to be 3.2 and 6.2 mins respectively. These analytes were subjected for forced degradation under different conditions. It has been found that there was no any interference of the exciepients and degraded products of the analytes at the retention times of the analytes. Thus the developed method was stability indicating method.

Validation: The proposed stability indicating method was shows short elution time and good separation between diacerein and aceclofenac. The system suitability test was performed as per the international conference of harmonization (ICH) [13] guidelines to confirm the suitability and the reproducibility of the method. Six consecutive injections of the standard solution were performed and evaluated for repeatability, tailing factor, theoretical plates and resolution. % RSD values were found to be 0.30 and 0.28 for diacerein and aceclofenac, respectively. The tailing factor and theoretical plates were found to be within the limits.

The method was linear over the range 20-100 and 40-200 µg/mL for diacerein and aceclofenac respectively. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was $Y = 4.47e + 004X + 3.23e + 004$ ($r_2 = 0.9992$) for diacerein and $Y = 2.60e + 004X - 5.06e + 003$ ($r_2 = 0.9994$) for aceclofenac. The results show that an excellent correlation between response factor and concentration of drugs.

The limit of quantification (LOQ) and limit of detection (LOD) were established at a signal-to-noise ratio. The limit of quantification and limit of detection of diacerein and aceclofenac were experimentally determined. The limit of detection of diacerein and aceclofenac was found to be 0.0025 and 0.020 µg/mL respectively. The limit of quantification of

diacerein and aceclofenac was found to be 0.005 and 0.045 µg/mL respectively.

The developed method was validated for system precision (repeatability) and method precision. Six injections of mixed standards of 50 µg/mL of diacerein and 100 µg/mL of aceclofenac were injected and % RSD calculated for injection repeatability. Six samples were prepared at 100 % levels and assayed according to the procedure. The average assay of three replicate analysis was found to be 102.12 % for diacerein and 99.91 % for aceclofenac with a relative standard deviation of 0.02 and 0.00 % respectively.

The accuracy of the method was determined by the standard addition method at three different levels. The sample solution of 100 % level was considered as a zero level and 10, 20 and 30 % of the standard drug of analytes were added respectively. Each determination was performed in triplicates. The accuracy was then calculated as the percentage of the standard drug recovered by the recovery study. Mean recoveries for diacerein and aceclofenac from the combination formulation are shown in Table-1. The results are well within the acceptance limit and hence the method is accurate.

TABLE-1
ACCURACY STUDY (ANALYTE RECOVERY)

Analyte	Theoretical recovery level[a] (%)	Amount added (ppm)	Amount recovered (ppm)	Observed recovered (%)	RSD (%)
DIA	10	55	55.73	101.33	0.010
	20	60	60.05	100.08	0.081
	30	65	65.55	100.85	0.095
ACF	10	110	108.44	98.58	0.483
	20	120	117.70	98.08	0.179
	30	130	129.25	99.42	1.032

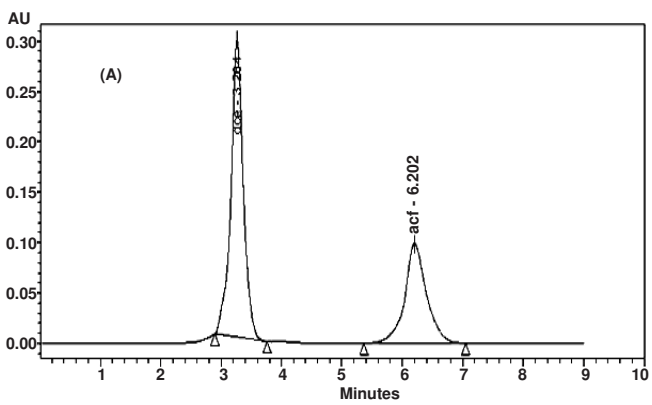
n = 3 determination; a. Recovery procedure: The active pharmaceutical ingredient(API) was spiked with 100 % level sample solution at 10, 20 and 30 % level of target analyte concentration and analyzed as per the proposed method.

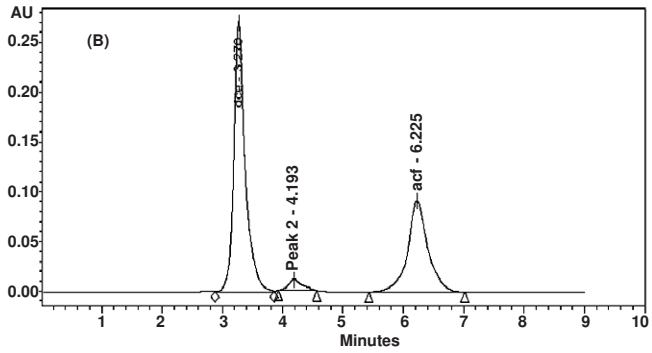
The stability of both the standard and the samples was determined by monitoring the peak area responses of the standard solution and the sample solution of diacerein and aceclofenac at 6, 12 and 24 h at room temperature. The results showed that there were no significant difference.

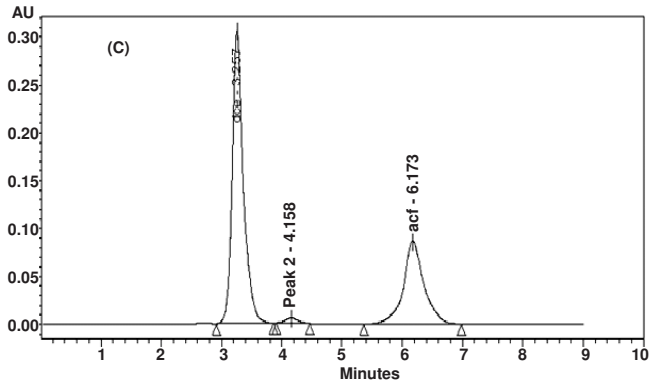
The specificity of the method was determined by exposing 100 % sample solution of diacerein and aceclofenac to stress conditions *i.e*. 0.1 M sodium hydroxide, 0.1 M hydrochloric acid, 10 % hydrogen peroxide and thermal degradation. Typical chromatograms obtained from the assay of pure sample and stressed samples are shown in the Fig. 2. The degradation products were separated from their parent compounds. The results of the forced degradation studies indicated a high degree of selectivity and specificity of this method for diacerein and aceclofenac. Diacerein and aceclofenac were found to be stable under dry heat conditions, but in acid, alkali and 10 % hydrogen peroxide the drugs undergoes degradation. Under acid and alkali degradation the degradation product of diacerein shows the peak at 4.15 min while in oxidative degradation, the peak appears at 2.60 min. The details are given in Table-2.

TABLE-2
FORCED DEGRADATION STUDY OF DIACEREIN AND ACECLOFENAC

Conditions	Temp. (ºC)	Time	% Degradation	
			Diacerein (%)	Aceclofenac (%)
0.1 N NaOH	70	15 min	22.58	13.49
0.1 N HCL	70	15 min	15.34	16.91
10 % H_2O_2	70	15 min	10.47	12.15
Thermal	105	24 h	No degradation	No degradation







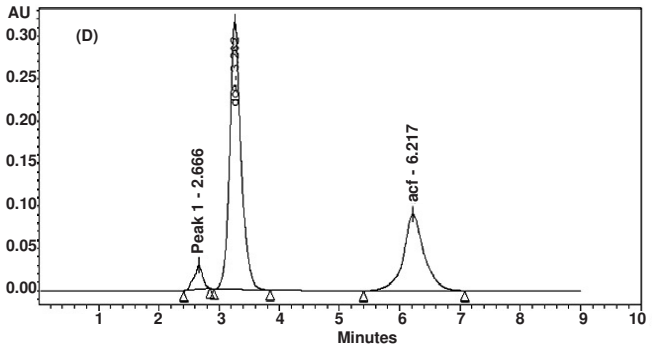


TABLE-3
ANALYSIS OF FORMULATION: DYACERIN-A (LABEL CLAIM: DIACEREIN 50 mg + ACECLOFENAC 100 mg)

Drug	Std wt (mg)	Avg. wt mg	Label claim (mg)	Mean std area	Mean sample area	Amount present	% Assay
Diacerein	50 mg	232.5	50 mg	4508395	4604313	51.06 mg	102.12 %
Aceclofenac	100 mg		100 mg	2577797	2575580	99.91 mg	99.91 %

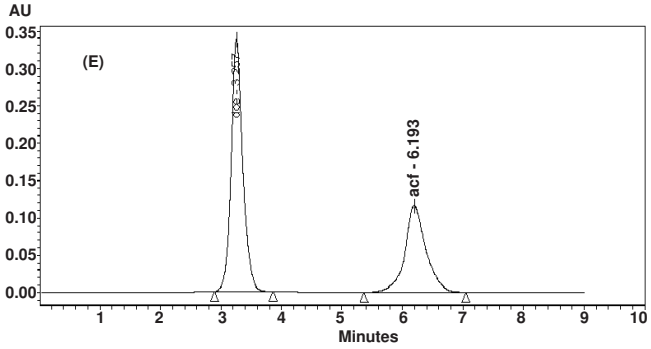


Fig. 2. HPLC Chromatograms of (A) 100 % sample solution, (B) alkali degradation (0.1N sodium hydroxide), (C) acid degradation (0.1 N Hydrochloric acid), (D) Oxidative degradation(10 % Hydrogen peroxide) and (E) Thermal degradation (at 105 ºC)

Applications: The validated stability indicating HPLC method was applied to the simultaneous determination of diacerein and aceclofenac in tablet dosage form. The samples were analyzed and the assay results are as per the label claim shown in Table-3.

Conclusion

The isocratic RP- HPLC method has proved to be simple, specific, precise and accurate and is suitable for simultaneous quantification of diacerein and aceclofenac. The proposed method gives a good resolution among these analytes. High percentage of recovery shows that the method is accurate. The forced degradation study shows that there is no any interference of the excipients and the degraded products at the retention times of diacerein and aceclofenac.

ACKNOWLEDGEMENTS

The authors are thankful to Department of Chemistry, Mithibai College, Mumbai for their support.

REFERENCES

1. P. Nicolas, M. Tod, C. Padoin and O. Petitjean, *Clin. Pharmecokinet.*, **35**, 347 (1998).
2. T.S.A. Fidelix, B.G.D.O. Soares and V.F.M. Trevisani, *Cochrane Database Syst. Rev.,* **1**, CD005117 (2006).
3. A.C. Moffat, M.D. Osselton, E.G.C. Clarke, B. Widdop and L.Y. Galichet, Clarke's Analysis of Drugs and Poisons, Pharmaceutical Press, edn. 3, Vol. 2, p. 570 (2004).
4. S. Narade, S. Patil, S. Surve, D. Shete and Y. Pore, *J. Pharm. Sci. Res.*, **2**, 137 (2010).
5. K.S. Topagi, P.K. Sinha, R.M. Jeswani, M.C. Damlela, *Int. J. Chem. Tech. Res.*, **1**, 991 (2009).
6. S. Bhalerao, S. Tambe, V. Pareek, R. Shinde and L. Gupta, *Asian J. Pharm. Clin. Res.*, **3**, 25 (2010).
7. J.R. Bhinge, R.V. Kumar and V.R. Sinha, *J. Chromatogr. Sci.*, **46**, 440 (2008).
8. R. Siva, N. Srisutherson, W.D. Sam Solomon, N.P. Kumar and R. Venkatnarayanan, *Int. J. Pharm. Tech. Res.*, **2**, 940 (2010).
9. V. Giannellini, F. Salvatore, G. Bartolucci, S.A. Coran and M. Bambagiotti-Alberti, *J. Pharm. Biomed. Anal.*, **39**, 776 (2005).
10. A. Goyal and S. Jain, *Acta Pharm. Sci.*, **49**, 147 (2007).
11. A. Ojha, R. Rathod and H. Padh, *J. Chromatogr. B; Anal. Technol. Biomed. Life Sci.*, **877**, 1145 (2009).
12. V. Sekar, S. Jayaseelan, E.U. Kumar, N. Subash, M. Prakash and P. Perumal, *Int. J. Chem. Tech. Res.*, **2**, 168 (2010).
13. ICH Q2A, Text on Validation of Analytical Procedures, International Conference on Harmonization Tripartite Guidelines, Adapted 27 October (1994).