ISSN : 0974-7419 Volume 15 Issue 2

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 15(2) 2015 [049-053]

A new RP-HPLC-UV method for the simultaneous quantification of aceclofenac in bulk & its tablets

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ABSTRACT

A simple, selective, linear, precise, and accurate RP-HPLC method was developed and validated for the estimation of Aceclofenac from bulk drug. Chromatographic separation was achieved isocratically on a Phenomenex, C8 column (250×4.6 mm, 3 µ particle size) using a mobile phase, (0.01M ammonium acetate buffer with 2 ml triethylamine, (v/v)-acetonitrile (68:32 v/v) pH was adjusted to 6.5 with glacial Acetic acid. The flow rate was 1.2 ml/min and effluent was detected at 270 nm and 20µl of sample was injected. The retention time of Aceclofenac was 6.4 min. Linearity was observed in the concentration range of 8-16 µg/ml. Percent recoveries obtained for Aceclofenac was 99.65-99.93%. The percentage RSD for precision and accuracy of the method was found to be less than 1%. The method was validated according to the ICH guidelines with respect to specificity, linearity, accuracy, precision, LOD and LOQ. The method developed was successfully applied for the analysis & estimation of Aceclofenac in bulk drug.

KEYWORDS

Aceclofenac;
Tablet;
Bulk drug;
Acetate buffer.

INTRODUCTION

Aceclofenac, 2-[2-[2-[(2,6-dichlorophenyl) amino]-phenyl]acetyl] oxyacetic acid. The molecular formula of aceclofenac is $C_{16}H_{13}Cl_2NO_4$ and its molecular weight is 354.19 g/mole[1]. It is a white or almost white powder. It is practically insoluble in water, freely soluble in acetone and soluble in alcohol. The structural formula is as follows:

That shows analgesic properties and good tolerability profile in a variety of painful conditions[2,3]. It is used in the treatment of rheumatic disorders and soft tissue injuries. Aceclofenac inhibits the cyclooxygenase enzyme and thus exerts its anti-inflammatory activity by inhibition of prostaglandin synthesis. This effect seems to be correlated to the appearance of acute protocol it is associated with non-steroidal anti-inflammatory drug therapy[4-6].

The mode of action of aceclofenac is largely based on the inhibition of prostaglandin synthesis. Aceclofenac is potent inhibiter of the enzyme cyclooxygenase, which is involved in the production of prostaglandins. Aceclofenac has been shown to exert effects on a variety of mediators of inflammation. The drug inhibits synthesis of the inflammatory cytokines interleukin (IL)-1â and tumor necrosis factor and inhibits prostaglandin E2

(PGE2) production. Effects on cell adhesion molecules from neutrophils have also been noted. In vitro data indicate inhibition of cyclooxygenase (COX)-1 and 2 by aceclofenac in whole blood assays, with selectivity of COX-2 being evident.

In contrast to some other NSAIDs, aceclofenac has shown stimulatory effects on cartilage matrix synthesis, which may be linked to the ability of the drug to inhibit IL-1â activity. In vitro data indicates stimulation by the drug of synthesis of glycosaminoglycan in osteoarthritic cartilage. There is also evidence that aceclofenac stimulates the synthesis of IL-1 receptor antagonist in human articular chondrocytes subjected to inflammatory stimuli and that 4'-hydroxyaceclofenac has chondro protective properties attributable to suppression of IL-1â mediated pro matrix metallo proteinase production and proteoglycan release.

In patients with osteoarthritis of the knee, aceclofenac decreases pain, reduces disease severity and improves the functional capacity of the knee. It reduce joint reduces inflammation, pain intensity and the duration of morning stiffness in patients with rheumatoid arthritis. The duration of morning stiffness and pain intensity are reduced and spinal mobility improved, by aceclofenac in patients with ankylosingspondylitis.

Only few analytical methods for the quantitative determination of aceclofenac in pharmaceutical formulation drug analysis are described in the literature like. Simple HPLC method for quantification of aceclofenac in rat plasma (HPLC)[7], zone capillary electrophoresis method for the simultaneous determination of aceclofenac and diclofenac in human plasma[8], reversed phase HPLC method for the determination of aceclofenac in human plasma[9], HPLC assay method for the determination of aceclofenac in plasma and its pharmacokinetics in dogs[10].

No HPLC-UV method has been reported in the literature for the determination of aceclofenac in its pharmaceutical bulk drug and tablet form simultaneously. It would therefore be beneficial to provide accurate, precise, and reliable methods for the determination of aceclofenac in tablet formulation and bulk drug form. The present work describes an analytical procedure for the quantitation of aceclofenac using reversed phase HPLC.

MATERIALS AND METHODS

Chemicals

Aceclofenac API was obtained as a gift sample from Mepro Pharmaceuticals Pvt.Limited (Surendranagar, India) and its percentage purity was in between 99.78% and 100.00%. A commercial tablet formulation Acenext-P from Cadila Pharmaceuticals Ltd, (India) containing 100 mg of Aceclofenac was purchased from local market and used within its self-life period. HPLC grade acetonitrile was obtained from Merck Limited. Ammonium acetate was obtained from SD Fine (Mumbai, India). HPLC grade water was obtained by Rankem Limited. All other chemicals used were of pharmaceutical or analytical grade.

Chromatographic conditions

The HPLC system (LC 20AD, Shimadzu, Japan) consisted of binary gradient system, in-line degasser, UV detector (Shimadzu, FPD-20A model) and rheodyne injector (Shimadzu, 7725i). Data was processed using LC Solution Ver. 1.2 software (Shimadzu, Japan). Isocratic elution of the mobile phase Acetonitrile: buffer (0.01M ammonium acetate buffer with 2 ml triethylamine), (32:68 v/v) pH was adjusted to 6.5 with glacial Acetic acid with the flow rate of 1.2 ml/min. Separation was performed on a Phenomenex C8 (250 x 4.6 mm i.d, 3 µ particle size) analytical column and a pre-column to protect the analytical column from strongly bonded material. Integration of the detector output was performed using the Shimadzu LC Solution software to determine the peak area. The contents of the mobile phase were filtered through a 0.45 µ m membrane filter

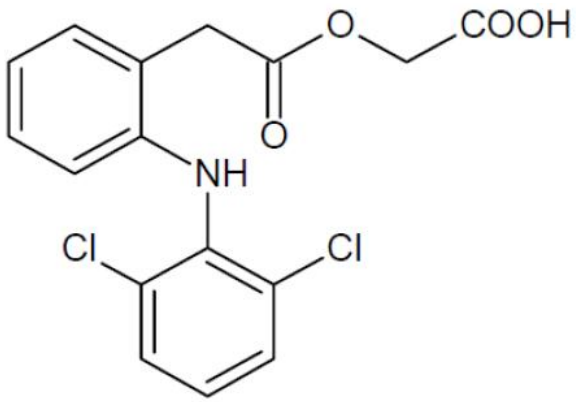

Figure 1 : Chemical structure of Aceclofenac

and degassed by sonication before use. Mobile phase was used as diluents. The flow rate of the mobile phase was optimized to 1.2 ml/min which yields a column back pressure of 122 bar. The run time was set at 14 min and a column temperature was maintained at 30°C. The volume of injection was 20 µl, prior to injection of the analyte, the column was equilibrated for 30–40 min with the mobile phase. The eluent was detected at 270 nm. The developed method was validated in terms of specificity, linearity, accuracy, limit of detection (LOD), limit of quantification(LOQ), intra-day and inter-day precision and robustness for the assay of Aceclofenac as per ICH guidelines[11].

Preparation of standard stock solutions of aceclofenac

Standard solution containing tramadol hydrochloride (0.0375 mg/ml) and aceclofenac (0.100 mg/ml) were prepared by dissolving 18.75 mg tramadol hydrochloride and 50 mg aceclofenac in 50 ml volumetric flask by diluent (stock standard solution). Pipette out 5 ml stock solution into 50 ml volumetric flask and dilute up to mark with diluent (standard solution).

Preparation of test Solution (Analysis of aceclofenac in tablet formulation)

Twenty tablets were weighed and the average tablet weight was determined. Tablets were crushed by mortar and pastel. Tablet powder was weighed equivalent to five times of average weight and transfer in to 500 ml volumetric flask. About50 ml methanol and 300 ml mobile phase was added and sonicated for of 20 min. time interval with intermittent shaking. Content was brought back to room temperature and dilute to volume with diluent (stock test solution). The stock solution was filtered through0.45 µm nylon syringe filter. Pipette out 5ml filtered stock solution in to 50 ml volumetric flask and dilute with diluent (test solution). The concentration obtain was 0.0375 mg/ml of tramadol hydrochloride and 0.100 mg/ml of aceclofenac.

RESULTS AND DISCUSSION

The present research work was designed at developing a rapid, sensitive, precise and accurate HPLC method for the estimation of aceclofenac in pharmaceutical tablet dosage forms. In order to affect analysis of the component peaks under isocratic conditions, mixtures of buffer and acetonitrile in different combinations with different pH were tested as mobile phase on a Phenomenex C8 stationary phase. A binary mixture of Acetonitrile: buffer pH adjusted to 6.5 with glacial acetic acid in the ratio of 32:68 v/v was proved to be the most suitable of all the combinations since the chromatographic peaks obtained were better defined and resolved and free from tailing. A flow rate of 1.2 ml/min of the mobile phase was found to be suitable.

Method development

After various trials, the following chromatographic conditions were finally optimized for the estimation of aceclofenac in a tablet drug form. Mobile phase constitutes of Acetonitrile: buffer pH adjusted to 6.5 with glacial acetic acid in the ratio of 32:68 v/v. Detection wave length 270 nm flow rate1.2 ml/min, after a steady baseline the standard solution were injected and chromatograms were recorded until the reproducibility of the peak areas were found and finally 100 µg/ml of the standard solution of the individual sample of aceclofenac and standard solution was injected and the chromatograms were recorded. The separation of aceclofenac with retention times of 6.4 min. The typical chromatogram of the standard solutions was recorded for the repeatability and the respective chromatogram was given in Figure 2.

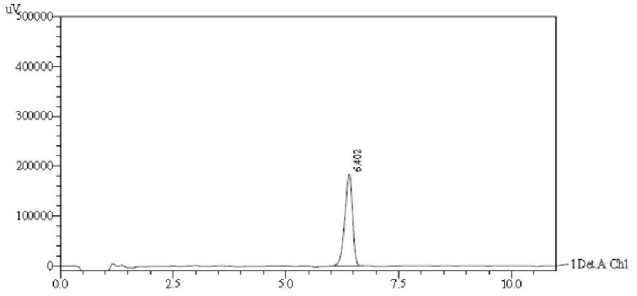

Figure 2 : Typical chromatogram of aceclofenac with detection at 270 nm.

Method validation

After development of method, validation of the method for the estimation of aceclofenac was performed in accordance with ICH guidelines (International Conference on Harmonization (ICH) 2000) which include System suitability, Linearity, Accuracy, Precision, LOD

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and LOQ, Specificity and Robustness[11].

Linearity

Calibration graph was constructed by plotting peak area vs. concentration of aceclofenac and the regression equation was calculated. The calibration graph was plotted over 5 different linear concentrations in the range of 0.030-0.120 mg/ml for aspirin and 0.015-0.060 mg/ml for clopidogrel. Aliquots (20 µl) of each solution were injected under the operating chromatographic condition described above [Number of replicates (n = 6)]. The method was found linear over the concentration range of 8-16 µg/ml. Linearity curve of aceclofenac was shown in figure 3.

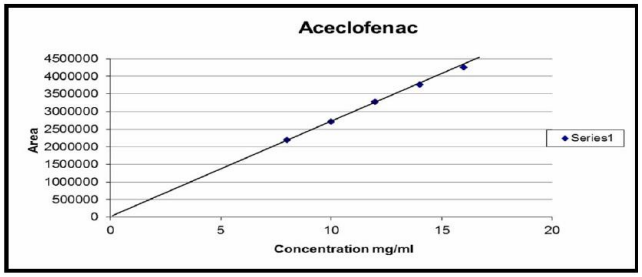

Figure 3 : Linearity of Aceclofenac

Accuracy

The accuracy of the method was established by recovery studies i.e., external standard addition method. The known amount of standard was added at three different levels to pre analyzed sample. Each determination was performed in triplicate. The mean recovery obtained was 99.65% and 99.93% for aceclofenac. The result of accuracy is tabulated in TABLE 1.

Precision

The intraday and interday precision of the proposed method was determined by analyzing mixed standard solution of aceclofenac at concentration 100 µ g/ml 3 times on the same day and on 3 different days. The results are reported in terms of relative standard deviation. The % RSD value for aceclofenac was found to be 0.45%.

TABLE 1 : Summary of validation parameters

S. No	Validation Parameters	Results
		Aceclofenac
1	Accuracy (% Recovery)	99-100
2	Precession (% RSD)	0.45
3	Interday precession (%RSD)	0.47
4	LOD	0.9 ng ml^{-1}
5	LOQ	0.25 ng ml^{-1}

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

The limit of detection (LOD) and limit of quantitation (LOQ) of aceclofenac was determined by calculating the signal-to noise (S/N) ratio of 3:1 and 10:1, respectively according to International Conference on Harmonization guidelines. LOD value for aceclofenac was found to be 0.9 ng ml^{-1}. LOQ value for aceclofenac was found to be 0.25 ng ml^{-1}.

Robustness

The robustness of the method was evaluated by assaying the test solutions after slight but deliberate changes in the analytical conditions like flow rate (0.1 ml min-1), and pH of the mobile phase (± 0.2). Stability of standard and test solution (prepared from the dosage form) was established by storage at 25 °C and 15 °C for 48 h. During the storage period, the test solutions were reanalyzed at intervals of 6, 12, 24, 36 and 48 h and assay was determined against appropriate fresh standard preparations.

Assay of the tablet dosage form

The proposed validated method was successfully applied to determine Aceclofenac in tablet dosage form. The result obtained was comparable with corresponding labeled amounts (TABLE 2).

The accuracy of the proposed method was assessed by recovery studies. All solutions were prepared and

TABLE 2 : Assay of the tablet dosage form

Sample No.	Aceclofenac		
	Labeled Amount (mg/tablet)	Amount Found (mg/tablet)	% Assay
1	100	101.04	101.04
2		100.70	100.70
3		99.60	99.60
4		101.05	101.05
5		100.99	100.99
6		100.72	100.72
	Average Assay		101
	STD		0.55
	% RSD		0.55

analysed in triplicate. The above procedure is adopted for both the drugs and a high recovery values obtained (TABLE-1) indicate that the proposed method is highly accurate. The method specificity was assessed by studying the chromatogram (Figure 2) obtained for a mixture of the drug and the common excipients. As none of the excipients interfered with the analytes of interest, the method was found to be suitable for analyzing the commercial formulation of these drugs.

CONCLUSION

The present results provide clear evidence that the proposed method can be successfully used for the quantification of aceclofenac content in tablet drug.

ACKNOWLEDGEMENT

Author PMC is grateful to UGC (F.No.47-2052/11 WRO) for providing financial support. Authors are also thankful to Mr. Avnish Mandli, Mepro Pharmaceuticals Pvt. Ltd, Surendranagar, for providing APIs and Pramukh Swami Science & H.D. Patel Arts College, Kadi for providing all the necessary facilities to carry out this research work.

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