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Validated RP-HPLC method for estimation of aceclofenac from bulk and microemulsion formulations

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ABSTRACT

Aceclofenac is a non steroidal anti-inflammatory drug with good analgesic and anti-rheumatic properties. Various methods for analysis of the same are available but are time consuming and expensive. Here we have developed a new, precise and simple RP-HPLC method for estimation of aceclofenac from bulk and microemulsion formulation using a UV detector. The selected mobile phase was composed of 50:50 v/v of acetonitrile and 25mM trishydroxylmethyl aminomethane in phosphate buffer pH 7.0. The wavelength selected was 276 nm. This method was validated according to ICH Guidelines. There was no significant difference in the intraday and interday analysis of aceclofenac determined for three different concentrations using this method. Linearity of the method was found to be 0 - 100µg/ml with regression coefficient of 0.9994. The detection limit and quantitation limit were 0.07µg/ml and 0.211µg/ml respectively. The method was found to be simple, accurate, precise, economical and robust.

KEYWORDS

Aceclofenac;
RP-HPLC;
Recovery;
Microemulsion.

INTRODUCTION

Aceclofenac is a non steroidal anti-inflammatory drug with good analgesic and anti-rheumatic properties[1]. Chemically it is [[[2-[(2, 6-Dichlorophenyl)-amino]-phenyl]-acetyl]-oxy]-acetic acid. It is used in various pain conditions like rheumatoid arthritis, osteoarthritis and ankylosing spondylatis[1-4]. It is official in British Pharmacopoeia[4]. Several analytical techniques like titrimetric[4,5], colourimetric[6], spectroflurimetric[7], densitometric[8,9], HPLC[9-11], RP–HPLC[12,13], spectro photometric[14,15]and stripping voltametric[16] have been reported for assay of Aceclofenac. However some of these methods are costlier and time consuming.

The main purpose of this investigation is to develop and validate reverse phase HPLC method which is simple, rapid, precise and sensitive RP-HPLC method for estimation of aceclofenac from microemulsion formulation. This method could also be easily used in routine analytical work and for dissolution and diffusion studies at very low concentrations of aceclofenac.

MATERIALS AND METHODS

Materials

Aceclofenac pure drug was obtained as a gift sample from Aarti Drugs Limited, Pune. Acetonitrile HPLC, Potassium dihydrogen orthophosphate AR, were procured from Merck Laboratories, Orthophosphoric acid AR, TRIS hydroxylmethyl aminomethane AR, were purchased from Research Laboratory, Mumbai. All the reagents used were of analytical grade and the reagent solutions were prepared using double distilled water.

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Instrument used

The HPLC system consisted of intelligent HPLC pump (model Jasco PU-2080 plus), intelligent UV-Vis detector model (Jasco UV-2075) and 20 µl sample loop injector (#7725i, Rheodyne, USA). The equipment was operated through software Borwin version 1.5, LC-Net II/ADC system.

Methods

Preparation of mobile phase

Phosphate buffer pH 7.0 was prepared in accordance with the guidelines of Indian Pharmacopoeia. 25mM of TRIS hydroxylmethyl amino methane was prepared by dissolving 1.5138 g in sufficient phosphate buffer to produce 500 ml. This was then mixed with 500 ml of Acetonitrile HPLC and adjusted to pH of binary mixture to 7.0. Sonicated the mixture for 15 min. and filtered the resultant mixture through 0.45µ nylon membrane filter (Whatman).

Determination of λmax

Weighed amount of Aceclofenac was dissolved in mobile phase to obtain a 100µg/ml solution. This solution was subjected to scanning between 200-400 nm and absorption maxima was determined.

Chromatographic condition

The separations were performed on Inertsil ODS, C_{18} column having dimensions 4.6mmϕ×250mm, 5µm particle size (GL. Sciences Inc., Japan). The mobile phase consisted of 50:50 v/v of acetonitrile and 25mM trishydroxymethyl aminomethane in phosphate buffer (pH 7.0). The pH of the binary solvent mixture was finally adjusted to 7.0 with o-phosphoric acid. The wavelength selected was 276 nm. The flow rate was 1.5 ml/min.

Linearity and calibration

Primary stock solution of aceclofenac was prepared by dissolving 50 mg of aceclofenac in mobile phase to produce 10 ml solution. Secondary stock solution of 500µg/ml was prepared by diluting the primary stock with mobile phase. The secondary stock solutions were diluted suitably to obtain calibration standards of 50, 100, 150, 200, 250, 500µg/ml. These solutions were injected into HPLC (20µl) in triplicate and peak areas were noted. Calibration curve for aceclofenac was then plotted between peak areas against concentration at 276 nm.

Method validation

The method was validated for accuracy and recovery, precision, detection limit, quantitation limit and robustness according to the ICH guidelines.

Accuracy

The accuracy of the method was determined by calculating the recovery of aceclofenac. The recovery studies were performed by standard addition method. Known amount of standard (100µg/ml) was taken in three 10 ml volumetric flask and to it added 50, 100, 150 µg/ml of working standard solution respectively and made the volume to mark. The amount of aceclofenac added was determined from the peak area ratios and fitting these values in calibration curve and percent recovery was determined.

Precision

The intra day and inter day precision study of aceclofenac was carried out by determining the corresponding responses. Different levels of drug concentration were prepared 3 times on the same day and on three different days and the responses were noted for evaluating the variability

Detection and quantitation limit

Based on the calibration curve plotted, the standard deviation of the y-intercepts of the regression line was determined and was placed in the following equation for determining detection limit and quantitation limit.

$$\text{Detection limit} = \frac{3.3\,\sigma}{s} \qquad \text{Quantitation limit} = \frac{10\,\sigma}{s}$$

Robustness

Robustness was evaluated for determining the system suitability to ensure the validity of analytical procedure. This was done by varying the composition of organic phase by ± 3% and pH by ± 0.2, of the mobile phase.

Analysis of microemulsion formulation

Accurately weighed 1.0g of aceclofenac micro emulsion was diluted to 10 ml in a calibrated volumetric flask with the mobile phase. The resulting mixture was filtered through 0.22 µ nylon membrane filter (Whatman). The filtered solution was injected into

HPLC and drug concentration was estimated from the calibration curve. The analysis was done in triplicate.

RESULTS AND DISCUSSION

For the optimisation of mobile phase different mobile phases comprising of phosphate buffers, acetate buffers, at different pH values and different combinations of organic phase (acetonitrile) were investigated. The mobile phase was optimised on the basis of asymmetry factor, peak area obtained, retention time and number of theoretical plates. Amongst the various compositions tried satisfactory separation, well resolved, short retention time, and good symmetrical peaks were obtained with the mobile phase comprising of 50:50 v/v of Acetonitrile and 25mM trishydroxymethyl aminomethane in phosphate buffer (pH=7). The pH of the binary mixture was adjusted to 7.0 with orthophosphoric acid. The UV-Vis scan of aceclofenac in mobile phase revealed absorption maximum at 276 nm. Hence this wavelength was selected for the further analysis. The scan is shown in figure 1.

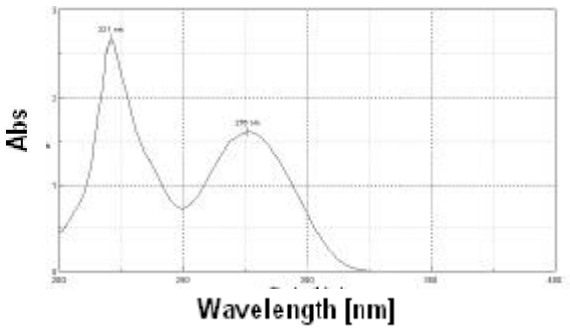

Figure 1: UV scan of aceclofenac in mobile phase

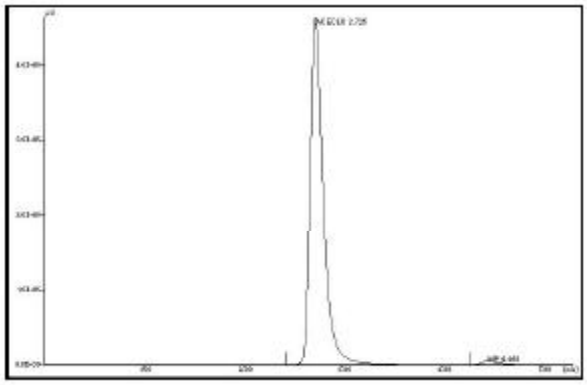

Figure 2: HPLC chromatogram of aceclofenac

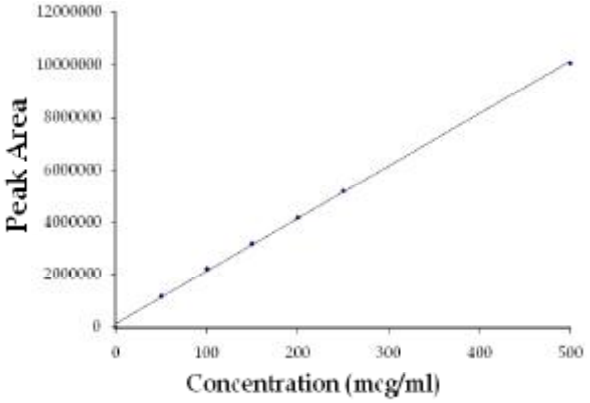

Figure 3: Calibration curve of aceclofenac

The peaks were highly resolved, the retention time was found to be 2.725 as depicted in figure 2. The asymmetry factor was 1.59 while number of theoretical plates was around 2425. All these factors revealed that the mobile phase was suitable for further analysis.

The calibration plot for aceclofenac was obtained by plotting the peak areas vs the concentration and was found to be linear. Regression analysis showed very good correlation. The calibration plot is shown in figure 3. The peak areas for the corresponding concentration of the calibration curve are depicted in TABLE 1. The standard deviation for all concentration levels were low and the % RSD also did not exceed 0.25 %.

TABLE 1: Aceclofenac calibration curve data

Concentration (µg/ml)	Peak area (mV-sec)*	% RSD
50	1201547.95 ± 3325.27	0.2768
100	2230726.963 ± 5545.427	0.2486
150	3185515.083 ± 7067.992	0.2219
200	4193335.15 ± 3279.587	0.0782
250	5209106.083 ± 6712.896	0.1289
500	10090602.17 ± 2199.939	0.0218

***Each value is average ± SD (n = 3)**

The statistical analysis of data obtained for the calibration curve of aceclofenac in pure solution indicated a high level of precision for the proposed method, as evidenced by low value of coefficient of variation. The coefficient of correlation was highly significant. The linearity range was observed between 0-100µg/ml. The plot clearly showed a straight line (Y= 19993X + 15993).

The accuracy of the method was judged by recovery studies. The recovery of aceclofenac was found to be 99.84-99.96. The results are showed in TABLE 2.

The precision of the method was evaluated by interday and intraday analysis which also showed good results with very low variations as revealed by very low %RSD values (< 0.25%). The results are shown in TABLES 3 and 4.

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TABLE 2: Recovery studies

Sr. no	Standard amount (µg/ml)	Amt of std. added (µg/ml)	Theoretical amount (µg/ml)	Amount recovered (µg/ml)	% Recovery
1.	100	50	150	149.94	99.96
2.	100	100	200	199.69	99.84
3.	100	150	250	249.72	99.89

TABLE 3: Intra-day variability of aceclofenac

Conc. (µg/ml)	Peak areas*	% RSD
100	2233995.75 ± 2067.764	0.0926
150	3184290.83 ± 4975.604	0.1563
200	4194216.21 ± 4069.697	0.0970

*Each value is average ± SD (n = 3)

TABLE 4: Inter-day variability

Conc. (µg/ml)	Peak areas*	% RSD
100	2234320.2 ± 5425.329	0.2428
150	3177339.5 ± 7925.395	0.2494
200	4195262.2 ± 7360.817	0.1755

*Each value is average ± SD (n = 3)

The detection limit for aceclofenac was 0.07µg/ml while quantitation limit was 0.211µg/ml, which suggests that a nanogram quantity of aceclofenac can be estimated accurately.

Analysis of formulation

The drug aceclofenac was estimated from microemulsion formulation using the method described. Different microemulsion formulations (containing 1% aceclofenac) prepared in the laboratory were tested for the drug content. Five different microemulsion formulations were used. The estimated content varied from 99.98-101.50 with % RSD < 0.8%. The assay values of the formulated formulations were very close to the label claim. This indicated that the excipients didn't interfere analysis method by proposed method. The HPLC chromatogram also reveals that the retention time is similar to that of the standard drug sample.

CONCLUSIONS

From the results and discussion it can be concluded that the method described for the estimation of aceclofenac from microemulsion formulation is simple, accurate, sensitive and reproducible. The proposed method requires very low time for separation and hence utilizes less solvent for separation. The proposed method could be employed even for routine analysis in quality control laboratories for different type of formulations.

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