Simultaneous Estimation of Aceclofenac and Paracetamol in Solid Dosage Form by RP-HPLC Method

Deepali Gharge*, Prof. Pandurang Dhabale

Govt. College of Pharmacy, Karad, Dist-Satara. Pin - 415124. M.S. India.

Corres.author : deepali_gharge@rediffmail.com

ABSTRACT: A simple, specific, accurate and precise reverse phase high pressure liquid chromatographic method has been developed for the simultaneous determination of aceclofenac and paracetamol from tablets by reverse phase C18 column (HiQsil C18, 250 mm x 4.6 mm). The sample was analyzed using Methanol: Ammonium acetate buffer in the ratio of 80:20, (pH adjusted to 3.4 with triethylamine) as a mobile phase at a flow rate of 1.0 ml/min and detection at 267.0 nm. The retention time for paractetamol and aceclofenac was found to be 2.978 and 4.647 min respectively, and recoveries from tablet were between 98 and 102 %. The method can be used for estimation of combination of these drugs in tablets.

Key words: Aceclofenac, Paracetamol, RP-HPLC.

INTRODUCTION

Aceclofenac and Paracetamol are available in tablet dosage form in the ratio of 1:5. Aceclofenac is a non steroidal anti-inflammatory drug with good analgesic and anti-rheumatic properties1. 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.[1,2,3,4] It is official in British Pharmacopoeia.[4] Paracetamol is N-(4 – hydroxyphenyl) acetamide has analgesic and antipyretic activity. Paracetamol is official in Indian Pharmacopoeia and British Pharmacopoeia. [4] B.P. suggests a potentiometric assay method for aceclofenac in bulk drugs. The I.P. & B.P. both suggest titrimetric and UV spectrophotometric assay method for paracetamol in bulk and tablet formulations.

Several analytical techniques like titrimetric[4,5], colourimetric[7], spectroflurimetric[7], densitometric[8,9], HPLC[9-11], RP–HPLC[12,13], spectrophotometric[14,15,16,17] and stripping voltametric[18] have been reported for assay of aceclofenac and paracetamol. This paper describes two simple, rapid, accurate, reproducible and economical methods for the simultaneous estimation of aceclofenac and paracetamol in tablet formulations using simultaneous equation and absorbance ratio methods.

EXPERIMENTAL

Chemicals and Reagents

Aceclofenac and paracetamol were obtained from, Aristo Pharma. Ltd. Mumbai. Water (HPLC grade), methanol (HPLC grade), triethylamine and ortho-phosphoric acid were of reagent grade. The pharmaceutical preparations of combination of aceclofenac and paracetamol that is Aceclo-P tablet (Aristo Pharma. Ltd. Mumbai).

Instrumentation

A Gradient HPLC PU 2080 Plus (JASCO) with UV-2075 Plus detector and RP-C18 column was used. A Rheodyne injector with a 20 μl loop was used for the injection of sample. The HPLC system was equipped with Borwin software for data processing.

Chromatographic Condition

The mobile phase containing methanol: ammonium acetate buffer (80:20), pH adjusted to 3.4 with triethylamine was found to resolve aceclofenac and paracetamol. Triethylamine was used for pH adjustment of buffer. The mobile phase was filtered on a 0.45 micron membrane filter and then ultrasonicated for 15 min. The flow rate was set to 1.0 ml/min. The 267.0 nm wavelength was selected for analysis. All

determinations were performed at constant column temperature (25 ± 2^0C).

Preparation of Ammonium acetate buffer:
3.85gm of ammonium acetate dissolved in 50ml water then volume made upto 1000ml with water. From this solution 50ml diluted to 1000ml with 0.05M acetic acid and pH adjusted to 3.4 with triethylamine.

Preparation of Stock Solutions
Standard stock solutions containing aceclofenac and paracetamol was prepared by dissolving 10 mg of aceclofenac and 10 mg of paracetamol in 80ml of methanol in two separate 100ml volumetric flasks. It was then sonicated for 15 minutes and the final volume of the solution made up to 100 ml with ammonium acetate buffer to get stock solutions containing 100 µg/mL of aceclofenac and paracetamol each.

Calibration curve
Calibration curves were prepared by taking appropriate aliquots of standard aceclofenac and paracetamol stock solutions in different 10 ml volumetric flask and diluted up to the mark with mobile phase to obtain final concentrations of 2-10 µg/ml of aceclofenac and 2-10 µg/ml paracetamol. Standard solutions were injected through 20 µl loop system and chromatograms were obtained using 1.0 ml/min. flow rate. The effluent was monitored at 267.0nm. Calibration curve was constructed by plotting average peak area against concentration and regression equation was computed.

Validation of the method
The developed method was validated in terms of linearity, accuracy, specificity, limit of detection, limit of quantification, intra-day and inter-day precision and repeatability of measurement.

Analysis of marketed tablet formulation:
A total of 20 tablets were accurately weighted and triturated with glass mortar and pestle. An amount equivalent to one tablet (containing 100 mg of aceclofenac and 500mg of paracetamol) was transferred to a 100ml volumetric flask; 400mg of pure aceclofenac added to it for making 1:1 ratio aceclofenac and paracetamol, 50 ml of mobile phase was added and the flask was kept in an ultrasonic bath for 15 min. The volume was made up to mark with mobile phase and the solution was filtered through 0.45 micron membrane filter. The final volume of the solution was made up to 100 ml with mobile phase to get stock solutions containing 5000 µg/mL aceclofenac and 5000 µg/mL paracetamol. The diluted solution (20 µg/mL) was analyzed under optimized chromatographic conditions and chromatogram is depicted in Fig. No.1.

RESULT AND DISCUSSION
To develop a precise, accurate and suitable RP- HPLC method for the estimation of aceclofenac and paracetamol, different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination. System suitability tests were carried out as per USP XXIV and parameters are summarized in Table.1. The results obtained by the analysis of marketed formulation are summarized in Table.2.

Method Validation [12]
The proposed HPLC method was validated as per ICH guidelines.

Specificity
The peak purity of aceclofenac and paracetamol were assessed by comparing the retention time (Rt) of standard aceclofenac and paracetamol. Good correlation was obtained between the retention time of standard and sample of aceclofenac and paracetamol.

Linearity
Linearity was studied by preparing standard solutions at different concentration levels. The linearity range for aceclofenac was found to be 2-10 µg/ml and for paracetamol was found to be 2-10 µg/ml. The regression equation for aceclofenac was found to be y = 36295x + 2485.134 and for paracetamol was found to be y = 53635x − 18443.842 with coefficient of correlation 0.9994 and 0.9997 respectively.

Precision
Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise.

Accuracy (Recovery studies)
To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120%. Known amounts of standard mixture of aceclofenac and paracetamol was added to pre-analyzed samples and was subjected to the proposed HPLC method. Results of recovery studies are shown in Table 3.

Robustness of method
To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate, pH and mobile phase ratio on the retention time and tailing factor were studied. The method was found to be unaffected by small changes like ± 0.1 change in pH, ± 0.1 change in flow rate and ± 1 change in mobile phase.

CONCLUSION
The most striking feature of this method is its simplicity and rapidity, non- requiring- consuming sample preparations such as extraction of solvents, heating, degassing which are needed for HPLC procedure. These methods can be employed for routine quality control analysis. The described methods give accurate and precise results for determination of aceclofenac and paracetamol mixture in marketed formulation.

Table 1: System suitability parameters

Parameter	Paracetamol	Aceclofenac
Linearity range (µg/ml)	2-10	2-10
Correlation coefficient	0.9997	0.9994
Slope	53635	36295
Retention time (min.)	2.978	4.647
Resolution factor	-	3.155
Tailing factor	0.95	1.15

Table 2: Results of analysis of marketed tablet formulation.

Tablet	Aceclo-P	
Analyte	Paracetamol	Aceclofenac
% Estimated	96.51	99.93
S.D.	0.01277	0.02909
%R.S.D.	0.4287	0.6261
Amount found in mg	482.55	99.93
%LOD	0.00011	0.00026
%LOQ	0.00023	0.00080

S.D.= Standard Deviation, R.S.D.= Relative Standard Deviation

Table 3: Recovery study data

% Standard Addition	% Estimated		S. D.		% R.S.D.	
	P	A	P	A	P	A
80	99.79	100.22	0.3338	1.4089	0.3345	1.4058
100	99.14	100.82	1.8186	1.7811	1.8344	1.7666
120	99.45	98.78	1.0231	1.1600	1.0288	1.1743

S.D.= Standard Deviation, R.S.D.= Relative Standard Deviation

Fig.1. Typical chromatogram of Paracetamol (P) and Aceclofenac (A)
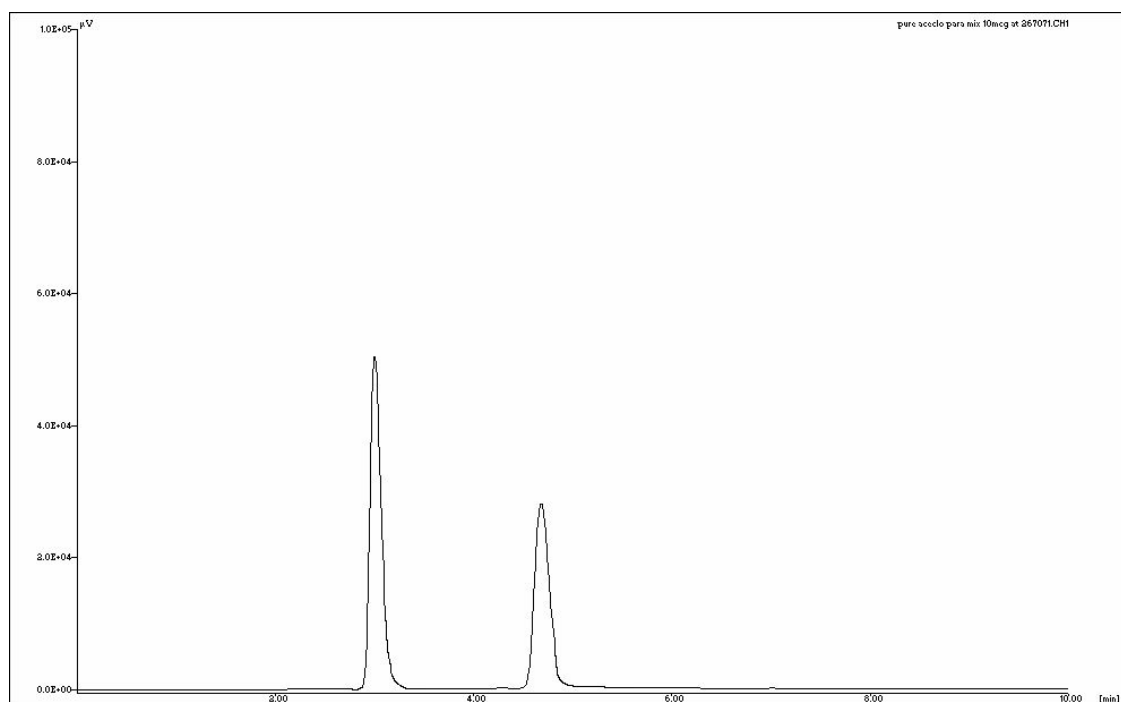
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