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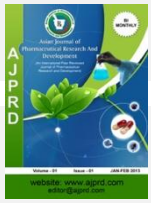

Open Access Research Article

A VALIDATED REVERSED PHASE UHPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ACECLOFENAC AND PARACETAMOL IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The purpose of this present work is to develop and validate a simple, linear, accurate and precise reversed phase U-High Performance Liquid Chromatography method for the simultaneous estimation of Paracetamol and Aceclofenac in tablet dosage form (fixed dose combination formulation). For this, the analysis was carried out by High Performance Liquid Chromatography using U-HPLC System (Thermo- Dionex) equipped with an UV detector and stainless steel column (25cm × 4.6mm × 5µm) packed with octadecylsilane chemically bonded to porous silica column. The mobile phase contain buffer , adjusted to pH 7.5 with 1(M) sodium hydroxide and Acetonitrile in the ratio of 60:40 v/v with a flow rate of 1.5 mL/min and detection wavelength 272 nm. The retention time of Paracetamol and Aceclofenac was found to be 1.698 and 2.548 respectively .The method was found to be linear in the concentration range of 10-100 µg/mL for Aceclofenac, 100-500 µg/mL for Paracetamol with correlation coefficient of 0.999 for both drugs respectively. The %RSD of 0.660 and 0.689 for intra-day and 0.897 and 0.856 for inter-day precision, respectively for of Paracetamol and Aceclofenac suggest the precision of the method as all these values are less than 2%. The method has shown good, consistent recoveries for Paracetamol (99.48%) and Aceclofenac (99.42%) which are close to 100%. Thus, the current study showed that the developed reverse-phase liquid chromatography method is sensitive and selective for the estimation of Paracetamol and Aceclofenac in combined dosage form.

Keywords: UHPLC; Validation, Paracetamol, Aceclofenac

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INTRODUCTION

Paracetamol and Aceclofenac are non-steroidal anti-inflammatory drugs (NSAID) available as over the counter drug commonly used as analgesic and antipyretic in the management of fever and relief from mild to moderate pain. Paracetamol (acetaminophen) has weak anti-inflammatory effects since it has poor ability to inhibit cyclooxygenase (COX) in the presence of high concentration of peroxides, as are found at sites of inflammation. Chemically Paracetamol is 4- hydroxy acetanilide. Aceclofenac [[2-(2', 6'-dichlorophenyl) amino] phenyl acetoxyacetic acid] is a phenyl acetic acid derivative belongs to the group of non-steroidal anti-inflammatory drug (NSAID) [1-3].

All conventional NSAIDs inhibit the conversion of arachidonic acid (AA) into prostaglandin H - PGH2. The stage is catalyzed by prostaglandin H synthase (PGHS), at present referred to as cyclooxygenase (COX) within which iso enzymes COX-1 (PGHS-1) and COX-2 (PGHS-2) occur.PGHS is a bifunctional enzyme and possesses two different enzymatic activities: cyclooxygenase and peroxidase (POX). The conversion of AA→PGH2

involves two reactions: cyclization of AA to unstable 15-hydroxyperoxide (PGG2) with the involvement of a cyclooxygenase component and double oxidation in position 9 and 11; whereas the reduction of PGG2 molecule to its 15- hydroxy analogue, unstable structure of PGH2, takes place due to peroxidase activity of PGHS (POX). Prostaglandin H2 (PGH2) is a substrate for specific synthases, tissue-dependent isomerases catalyzing its further conversions into different endogenous regulators, namely: prostaglandins of the D (PGD2), E (PGE2), F (PGF2) series and prostacyclin (PGI2; prostacyclin is not a prostaglandin and a commonly used abbreviation is historically conditioned) and thromboxanes (TXA2 and TXB2). They all are characterized by different biological activity and many of them have anti-inflammatory properties. Thus, the action of NSAIDs, which inhibits the stage of conversion AA→PGH2, and also the formation of the mentioned regulators, have some favorable (anti-inflammatory, analgesic and antipyretic) and side effects (associated with the inhibition of synthesis of particular regulators in different tissues). A precise mechanism of NSAID action together with therapeutic and side effects has been presented in the recently published large study by Nowak and Dzielska-Olczak and Nowak [4-5].

Materials and Methods

Paracetamol and Aceclofenac W/S were obtained as gift samples from Reddy's Laboratories, Hyderabad, India. Acetonitrile and Water (HPLC grade), sodium hydroxide and orthophosphoric acid were obtained from Merck Chemicals, Mumbai, India. Commercially available tablets (Acecloder TAB, Paracetamol 325 mg, Aceclofenac 100 mg) were procured from local retail pharmacy..

Chromatography instruments and conditions

The Separation was performed by High Performance Liquid Chromatography using U-HPLC System (Thermo-Dionex) equipped with an UV detector and stainless steel column (25cm × 4.6mm × 5µm) packed with octadecylsilane chemically bonded to porous silica column.The mobile phase contain buffer , adjusted to pH 7.5 with 1(M) sodium hydroxide and Acetonitrile in the ratio of 60:40 v/v with a flow rate of 1.5 mL/min and detection wavelength 272 nm. All the solutions were filtered through a 0.45 micron membrane filter paper and degassed before use.

Preparation of standard solution

Stock solutions were prepared by dissolving 10mg of Aceclofenac and Paracetamol in 10ml of the mobile phase to obtain a final concentration of 1000mcg.

Preparation of sample solution

Twenty commercially available tablet containing Aceclofenac and Paracetamol (100mg and 325mg respectively) were weighed. The average weight was calculated and powdered. A quantity equivalent to 10mg of Aceclofenac was accurately weighed and transferred to a 100ml of volumetric flask. The sample was sonicated with the mobile phase. Further dilutions were made to get a concentration equivalent to the linearity range.

Results and Discussion

Optimization of chromatographic conditions

To develop a method for the analysis of the drugs, preliminary tests were performed in order to select adequate and optimum conditions. Parameters such as detection wavelength, ideal mobile phase and its combination, optimum pH and concentration of the standard solutions were studied. Mobile phase consist of buffer and Acetonitrile (60:40 v/v). Flow rate was adjusted to 1.5 mL/min and UV detection at 272 nm was selected for analysis. Retention time of Paracetamol and Aceclofenac were found to be 3.84 and 1.92 min respectively.

Method validation

System suitability

Six replicate of sample containing Paracetamol and Aceclofenac were given to evaluate equipment, analytical operations and samples suitability. Parameters calculated for system suitability were %Relative Standard deviation (RSD), Retention time, Number of theoretical plates and Tailing factor.

Linearity:

Linearity for this analysis was established by least squares linear regression analysis. The calibration curves were found to be linear over the concentration range of 20-100 mg/ml for Aceclofenac and 100-500 mg/ml for paracetamol. Further the peaks were plotted versus their respective concentrations and linear regression analysis was performed. The correlation coefficients were found to be 0.999 and 0.999 for Aceclofenac and Paracetamol respectively. (Table 1).

Precision:

The precision of the method was determined by intra-day and inter-day precision studies at 100% test concentration by measuring the sample three times on the same day at intervals of 1 hour and on three different days for intra and inter day studies respectively. Further standard deviation and relative standard deviation were calculated (Table 2).

Accuracy

The accuracy of the method was proven by recovery test. Known amounts of Paracetamol and Aceclofenac standard (50, 100 and 150% level were added to the already analyzed sample solutions and the analysis was carried out. The method has shown good, consistent recoveries for Paracetamol and Aceclofenac (99.48% and 99.42%)which are close to 100% as shown (Table 3).

Robustness: The robustness of the method was checked by deliberately varying the mobile phase composition, wavelength and flow rate which shows that the small changes of the method parameters do not interfere the performance of the method. All the results obtained were in accordance with the results for original conditions. The %RSD value obtained for the assay in the changed condition was less than 2% which indicates the robustness of the proposed method. **Solution stability**:

The %RSD of the peak areas of the test samples were less than 1% for 24 hrs which indicates that the sample was stable under proposed mobile phase condition within this period only.

LOD and LOQ:

LOD for Paracetamol and Aceclofenac were found to be 0.08 µg/Ml and 0.02 µg/mL respectively. LOQ for Paracetamol and Aceclofenac were found to be 0.25 µg/mL and 0.06 µg/mL respectively as shown in Table 4.

Analysis of commercial formulations

Developed method was applied for the determination of Paracetamol and Aceclofenac in pharmaceutical dosage form and the results obtained were presented in Table 5. The assay value of 99.70%, 99.94% for Paracetamol and Aceclofenac indicates that the method is selective for the assay of Paracetamol and Aceclofenac without interference from any of the excipients of the tablet dosage form .

Table 1: Linearity and Correlation coefficient

Parameters	Paracetamol	Aceclofenac
Regression equation	**y = 0.252x - 0.034**	**y = 0.411x - 0.147**
Linearity (µg/ml)	**100-500**	**20-100**
Correlation coefficient	**0.999**	**0.999**

Table 2: Precision studies

Drug	Concentration µg/ml	Intraday Precision n=3 %RSD	Interday Precision n=3 %RSD
Paracetamol	100 µg/ml	0.660	0.589
Aceclofenac	100 µg/ml	0.897	0.856

Table 3: Accuracy

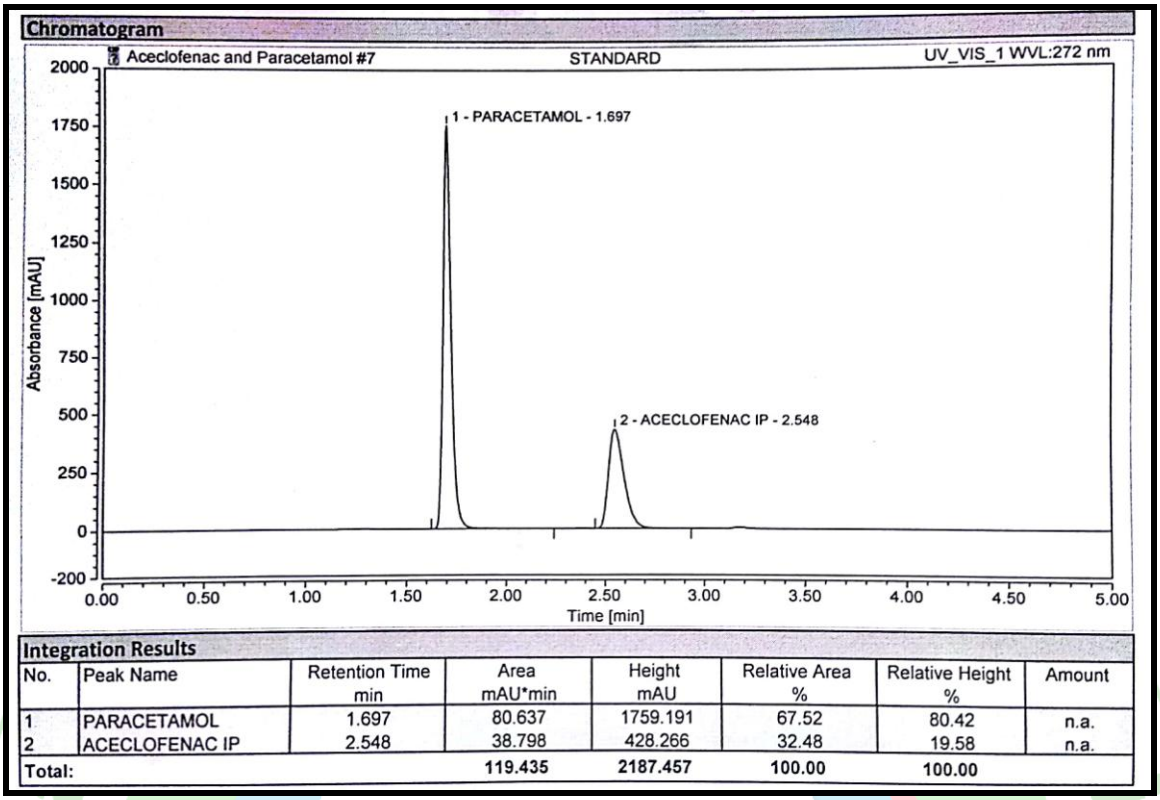
Drug	Amount taken	Amount added	Amount recovered	% Recovery % *RSD	% Recovery % *RSD
Paracetamol		162.5	161		
	325	325	323.2	99.48	0.653
		487.5	486		
Aceclofenac		50	48.6		
	100	100	98	99.42	0.543
		150	147.5		

Table 4: LOD and LOQ studies

Validation parameters	Paracetamol	Aceclofenac
LOD µg/ml	0.08	0.02
LOQ µg/ml	0.25	0.06

Table 5: Analysis of Formulation

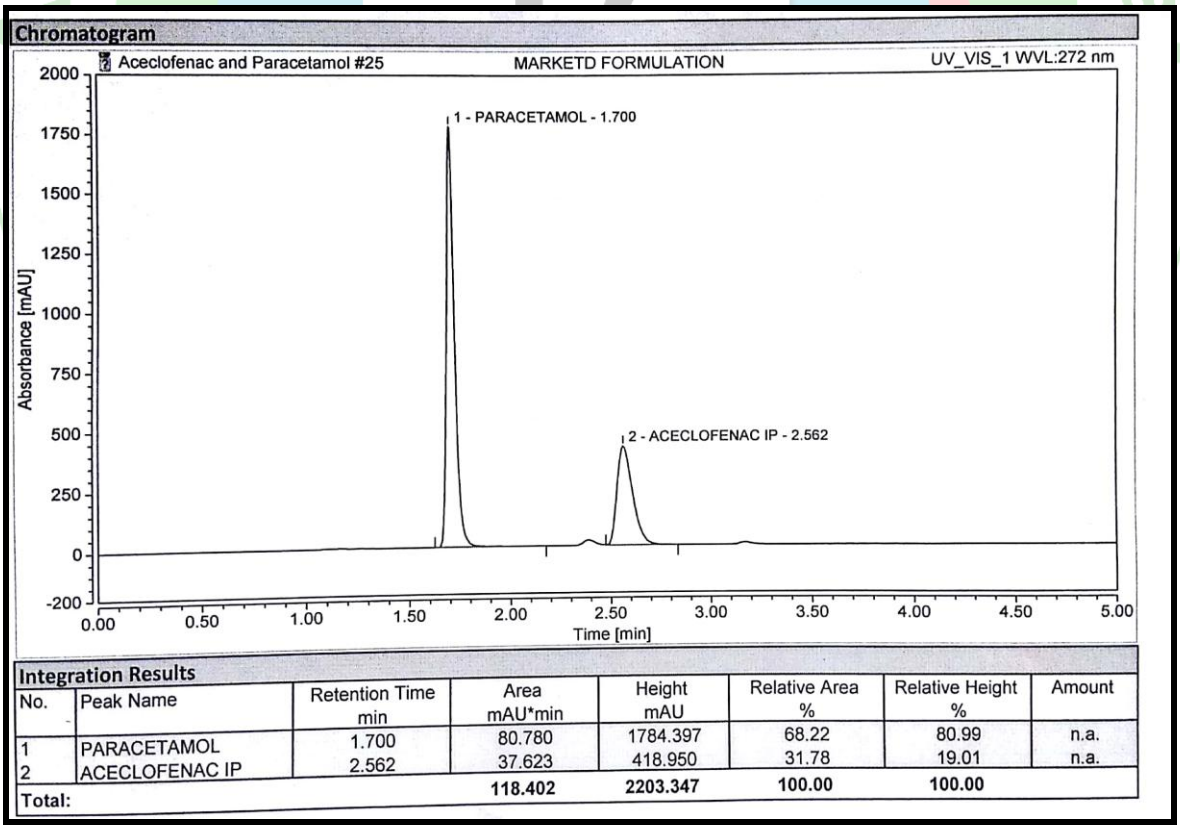
Drug	Labelled amount (mg/tablet)	Amount found (mg/tablet)	%Label claim	%*RSD
Paracetamol	325	311.6	95.8	0.30
Aceclofenac	100	96.5	96.5	0.28



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount
1	PARACETAMOL	1.697	80.637	1759.191	67.52	80.42	n.a.
2	ACECLOFENAC IP	2.548	38.798	428.266	32.48	19.58	n.a.
Total:			119.435	2187.457	100.00	100.00	

Fig 1: HPLC Chromatogram of Standard Paracetamol and Aceclofenac



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount
1	PARACETAMOL	1.700	80.780	1784.397	68.22	80.99	n.a.
2	ACECLOFENAC IP	2.562	37.623	418.950	31.78	19.01	n.a.
Total:			118.402	2203.347	100.00	100.00	

Fig 2: HPLC Chromatogram of Marketed Formulation

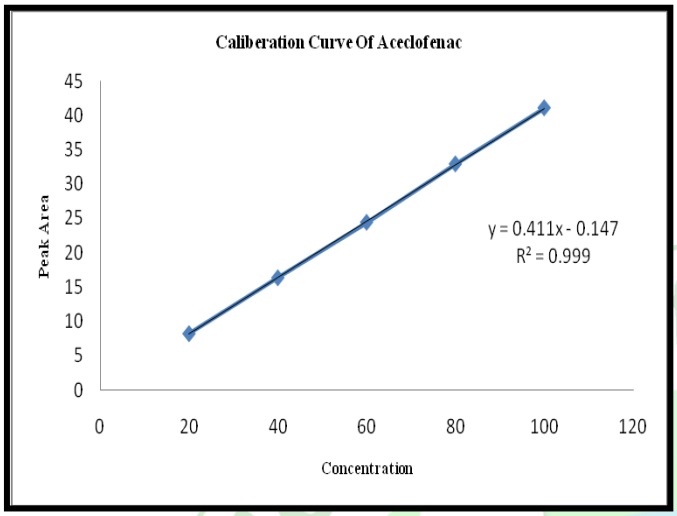


Fig 3: Caliberation Curve of Aceclofenac

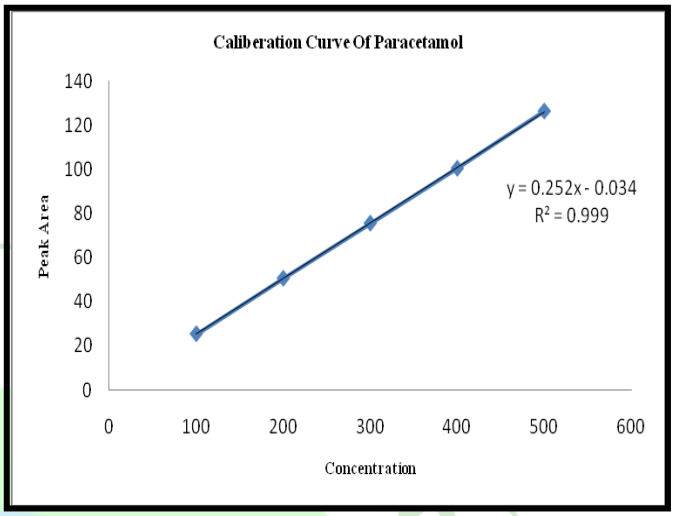


Fig 4: Caliberation Curve of Paracetamol

CONCLUSION

The proposed U-HPLC method was found to be simple, precise, accurate and specific for the simultaneous estimation of Paracetamol and Aceclofenac .The newly developed method is simple and cost effective as it uses simple mobile phase without ion-pairing reagent which was previously unreported .The separation was done in 5 minutes only. The method was validated as per ICH guidelines. All other parameters such as specificity, linearity, precision, accuracy, robustness passes the criteria set forth by ICH guidelines. It is seen that was no interference from any components of the formulation. Hence, this method can be easily used for routine quality control analysis of Paracetamol and Aceclofenac in pure and its combined dosage form.

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