

Simple RP-HPLC method for Aceclofenac quantitative analysis in pharmaceutical tablets

Suriya Sharmin¹, Md. Hossain Sohrab¹, Fatema Moni¹, Farhana Afroz¹, Satyajit Roy Rony¹, Shammi Akhter¹

¹ Pharmaceutical Sciences Research Division, BCSIR Laboratories Dhaka, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dr. Quadrat-I-Khuda Road, Dhanmondi, Dhaka-1205, Bangladesh

Corresponding author: Md. Hossain Sohrab (mhsohrab@bcsir.gov.bd)

Received 25 August 2020 ♦ Accepted 1 September 2020 ♦ Published 27 November 2020

Citation: Sharmin S, Sohrab MH, Moni F, Afroz F, Rony SR, Akhter S (2020) Simple RP-HPLC method for Aceclofenac quantitative analysis in pharmaceutical tablets. *Pharmacia* 67(4): 383–391. <https://doi.org/10.3897/pharmacia.67.e57981>

Abstract

A reverse phase liquid chromatographic method for estimation of Aceclofenac in bulk drug and tablet dosage form was developed and validated. The chromatographic conditions to achieve the highest performance parameters using octylsilyl column with guard filter were optimized. The separation was carried out using a mobile phase containing 10 mM Phosphate Buffer, pH 2.1 and methanol (30:70% v/v) pumped at a flow rate of 1.0 mL/min with detection at 272 nm. The method was shown to be linear in 19.8–148.5 µg/mL concentration range (regression coefficient of 0.999). The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.0692 µg/mL and 0.2076 µg/mL, respectively. The accuracy of the method was assessed by adding fixed amount of pre-analyzed sample to different standard solutions (80%, 100%, and 120% of the tested concentration) in triplicate. The percentage mean recoveries were 97.91% to 100.39% with %RSD values of 0.64–0.79. The method was found to be precise with %RSD value of 1.13 and 1.60 for intraday and interday precision study, respectively. The method specificity and robustness were also established. New and sensitive HPLC method for estimation of Aceclofenac has been developed, in respect to the reviewed analytical methods.

Keywords

Aceclofenac, Octylsilyl column, 10 mM Phosphate Buffer, Method validation, Robustness

Introduction

Aceclofenac (2-[(2,6-dichlorophenyl)amino]phenylacetoxyacetic acid) is a nonsteroidal anti-inflammatory drug (NSAID) of the phenylacetic acid group. Aceclofenac (ACF) [Fig. 1] selectively inhibit Cyclooxygenase (COX)-2 enzyme to inhibit generation of inflammatory mediators and in turn suppress pro-inflammatory prostaglandins and cytokines production resulting in analgesic, antipyretic activity by both central and peripheral actions (Arslan and Tirnaksiz 2010).

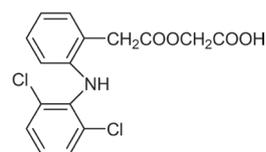


Figure 1. Aceclofenac.

Several methods utilized techniques like HPLC, UV-VIS Spectroscopy, GC, TLC etc. for quantification of ACF have been published as shown in Table 1.

Table 1. Analytical methods of Aceclofenac.

Sl No	Analytical Method	Method Condition/Mobile Phase/ Stationary Phase/Retention Time (Approximately)	Wavelength/ Detector	Linearity Range	Reference
1	HPLC	Methanol and 0.02% of orthophosphoric acid in the ratio of 70:30 (% v/v); C18; 10 min	275 nm	1–100 µg/mL	Bhingre et al. 2008
2	UV spectroscopy	Phosphate buffer saline of pH 7.4 as diluent	273 nm	0–20 µg/mL	Shah et al. 2008
3	HPLC	Acetonitrile, methanol and water in the ratio of 60:28:12 (% v/v) and pH of 7.0 adjusted with either glacial acetic acid and sodium hydroxide; C18; 6 min	274 nm	0.0138–0.370 µg/mL	Sherikar et al. 2011
4	HPLC	Acetonitrile, methanol and phosphate buffer of pH 7.0 in the ratio 30:17:53 (% v/v); C18; 13.8 min	280 nm	2–10 µg/mL	Kumar et al. 2008
5	GC	Injector temperature: 260 °C; Detector temperature: 300 °C; N ₂ flow rate: 5.0 mL/min; Make up flow: 30 mL/min; Split ratio: 10:1. Caffeine was used as internal standard (IS)	FID Detector (30 m X 0.53 mm; 1.5 µm)	10–110 µg/mL	Zeng-ri and Fu-jun 2010
6	Spectrophotometric method	Aceclofenac was reacted with 0.25% w/v solution of <i>p</i> -dimethyl-aminocinnamaldehyde (PDAC) in 1% v/v perchloric acid solution at 75 °C for 20 min and then diluted with methanol.	665.5 nm	20–100 µg/mL	Zawilla et al. 2002
	HPLC	Methanol, acetonitrile and acetic acid (2% solution in deionized water) in the ratio of 100:150:250 (% v/v/v) containing 0.3 ml triethylamine; C18; 8.8 min	275 nm	20–70 µg/mL	
	Densitometric method	Chloroform, ethyl acetate and acetic acid in the ratio of 75:25:5, (% v/v/v). Calibration curve is obtained from area under the peak against the concentrations.	254 nm and 275 nm	1–10 µg/spot	
7	Spectrophotometric method	Aceclofenac was reacted with <i>p</i> -dimethylamino-cinnamaldehyde and 1% perchloric acid at 90 °C for 10 min and then diluted with methanol.	658 nm	1–200 µg/mL	Bose et al. 2010
		Aceclofenac was reacted with 3-Methyl-2-benzothiazolinone hydrazine hydrochloride and 0.1% ferric chloride for 20 min and then diluted with water.	592 nm	1–100 µg/mL	
8	HPLC	Sodium phosphate buffer pH 5.0 and Acetonitrile in the ratio of 60:40 (% v/v); C18; IS: Etoricoxib (ETC); 8 min	275 nm.	25–125 µg/mL	Paul et al. 2011
9	HPLC	Mixed phosphate buffer pH 6.8 and acetonitrile in the ratio of 50:50 (% v/v); C18; 8.5 min	278 nm	2–10 µg/mL	Ravisankar et al. 2013
10	UV spectrophotometry	Diluent: Methanol	276 nm	0–120 µg/mL	Valambhia 2013
11	Third-derivative spectrophotometry (D3)	Calibration curve was constructed from peak amplitude (height) against corresponding concentration for the linearity range of Aceclofenac solution in methanol.	283 nm	4–24 µg/mL	El-Saharty et al. 2002
	Ratio-spectra first-derivative (RSD1) spectrophotometry	The absorption spectra of Aceclofenac in the linearity range were divided by that of diclofenac sodium (25 mg/mL), and the ratio spectra were differentiated with respect to wavelength. Calibration curve was obtained by plotting the first-derivative values at 252 nm against the corresponding concentration.	252 nm	4–32 µg/mL	
	Spectrodensitometric method of Thin-layer chromatogram	Chloroform, methanol and ammonia in the ratio of 48:11.5:0.5 (% v/v/v) were used for TLC development. Calibration curve was constructed by plotting the area under the peak against the corresponding concentrations to develop the regression equation.	274 nm	2–10 µg/spot	
	Third derivative spectrophotometry	UV-spectrum of Aceclofenac solution was measured against absolute ethanol as a blank. The peak height at 242 nm was measured. The calibration curve was constructed with the measured peak height against the corresponding concentration.	242 nm	5–40 µg/mL	
12	Ratio-spectra first-derivative (RSD1) spectrophotometry	Absorption spectra of Aceclofenac solutions were divided by the absorption spectrum of 5 mg/ml of the degradate. The ratio spectra thus obtained were smoothed and differentiated to determine first derivatives of the ratio spectra. The calibration curve was constructed between the measured first derivative values at 245 nm against the corresponding concentration.	245 nm	10–40 µg/mL	Hasan et al. 2003
	pH-induced difference (ΔA) spectrophotometry	The difference in absorbance was observed between 0.1 N sodium hydroxide and 0.1 N hydrochloric acid solution of Aceclofenac. Calibration curve was constructed by plotting the difference in absorbance against respective concentration.	273 nm	15–50 µg/mL	
	Quantitative densitometric evaluation of thin layer chromatogram	Tetrahydrofuran and methanol (90:10, % v/v) was used for TLC development. Calibration curve was constructed by plotting the area under the peak against the corresponding concentrations.	275 nm	50–200 µg/mL	
	HPLC	Methanol and water in the ratio of 60:40 (% v/v); C18; 8 min	230 nm	1–50 µg/mL	
13	HPLC	0.01 M ammonium acetate buffer with 2 ml (% v/v) triethylamine and acetonitrile in the ratio of 68:32 (% v/v) and pH was adjusted to 6.5 with glacial acetic acid; C8; 6.5 min	270 nm	8–16 µg/mL	Chatrabhuji et al. 2015
14	HPLC	0.07% of orthophosphoric acid and acetonitrile in the ratio of 68:32 (% v/v) at pH 7.0 ± 0.05; C18; 6 min	275 nm	160–240 µg/mL	Hossain et al. 2013
15	Microwave assisted spectrophotometry	Aceclofenac was reacted with ammonium molybdate in presence of sulfuric acid under microwave irradiation for 5 min.	740 nm	50–250 µg/mL	Mumtaz et al. 2013

Moreover, several HPLC methods for simultaneous estimation of ACF in present of other active constituents have been established as summarized in Suppl. material 1: Table S1. In respect to these findings a new specific HPLC method for rapid, accurate and precise estimation of ACF in bulk drug and in pharmaceutical dosage form has been developed.

Experimental

Instruments

All weighing were done on Electronic balance (A & D Company Ltd, Japan). Digital pH meter (SENSION+, Spain), bath sonicator (Wisd Laboratory Instrument,

Table 2. Placebo Constituents.

Lactose	:	480 mg
Microcrystalline cellulose	:	500 mg
Sodium Starch Glycolate	:	200 mg
Povidone K-30	:	125 mg
Magnesium Stearate	:	100 mg
Talc	:	100 mg

Germany) were also used in this study. UV-Vis spectra were recorded on a Specord 250 plus PC double beam spectrophotometer using 1.0 cm quartz cells. High purity deionized water was obtained from Millipore, Milli-Q (Merck KGaA, Darmstadt, Germany) water purification system. Assay test was performed with a HPLC (Hitachi High – Tech Science Corporation, Tokyo, Japan) machine with pump (Hitachi chromaster 5110), autosampler (Hitachi chromaster 5210) and PDA Detector (Hitachi chromaster 5430). LC separations were performed on a C8 column (250 × 4.6 mm i.d., 5 μm particle size), LaChrom, Hitachi, Japan with C8 guard column (23 mm X 4 mm; 3 μm), LaChrom, Hitachi, Japan. Data was integrated using Agilent open lab control panel CDS software. The mobile phase consisted of 10 mM Phosphate Buffer, pH 2.1 and methanol in 40:60%, v/v. The flow rate was set to 1.0 mL/min and UV detection was carried out at 272 nm at 25 °C.

Reagents

The 0.25-μm PTFE filters were obtained from Chromafil Xtra (Macherey Nagel GmbH & Co. AG., Germany). Working standards of pharmaceutical grade ACF (batch no. 28296) was supplied as a gift sample by Beximco Pharmaceutical Ltd (Dhaka, Bangladesh). Marketed ACF 100 mg tablets (Square Pharmaceuticals Ltd.) were purchased from local drug store. All chemicals and reagents of analytical grade were purchased from Active Fine Chemicals, Dhaka, Bangladesh.

Buffer Preparation

10 mM phosphate buffer was prepared by dissolving 0.64 gm potassium dihydrogen phosphate and 0.4 mL phosphoric acid to 900 mL deionized water. pH of the

solution was adjusted to 2.1 with dilute phosphoric acid solution if necessary. The final volume was then adjusted to 1000 mL with deionized water.

Placebo preparation

Commonly used excipients were mixed at appropriate amount to obtain the placebo mixture as Table 2. Placebo stock solution was prepared by mixing average tablet placebo content with mobile phase to obtain concentration of approximately 1.5 mg/mL.

Method Optimization

Stock solution of ACF was prepared in methanol (~50 μg/mL) and the UV spectrum was taken in the range of 200–400 nm to obtain the wavelength for maximum absorbance (λ_{max}) of Aceclofenac. As Aceclofenac (pKa≈3.4) is a strongly acidic drug, pH 2.1 was chosen as the buffer pH for mobile phase preparation. Mobile phase was taken as 10 mM phosphate buffer of pH 2.1 and methanol in the ratio of 30:70 (% v/v). Solvent stability was checked at approximately 100 μg/mL concentration for consecutive five days in the mobile phase by determining the relative standard deviation (%RSD) of response and peak purity of the drug. The described method has been validated for response function, accuracy, repeatability and intermediate precision.

Standard and Sample preparation

Standard stock solution at 1 mg/mL concentration was prepared in mobile phase by dissolving it first on not more than 5% of methanol. Sample stock solution was prepared by crushing randomly selected 10 tablets. Average weight equivalent sample was taken in volumetric flask to obtain concentration of approximately 1 mg/mL. Sample was also first dissolved in not more than 5% of methanol. The final volume was adjusted with mobile phase. The concentration for assay preparation was approximately 100 μg/mL. Drug content was determined using Equation 1.

$$\text{Drug content (mg/tablet)} = \frac{\text{sample peak area}}{\text{standard peak area}} \times \frac{\text{standard weight (mg)}}{\text{sample weight (mg)}} \times \text{average weight (mg)} \times \frac{\text{Potency of standard(\%)}}{100} \quad (\text{Eq. 1})$$

Linearity

The linearity of the method was established by determining linear regression equation from the calibration curve of ACF. The calibration curve was prepared using six different concentration levels in triplicate ranging from 20% to 150% of the assay preparation of analyte. For this, a stock solution (1 mg/mL) of the drug was prepared in mobile phase using not more than 5% of methanol initially. A range of concentrations (19.8–148.5 μg/mL) were then prepared after suitable dilution of the prepared stock with mobile phase.

LOD and LOQ

LOD and LOQ were separately determined based on the signal to noise ratio as per ICH guidelines (ICH Q2 R1, 2005).

Accuracy

Accuracy of the method was determined by performing the recovery experiment of standard addition method at three concentration levels in triplicate (Chakraborty et al. 2018). Different amount of standard stock solution was taken at 80% to 120% of assay concentration and mixed with

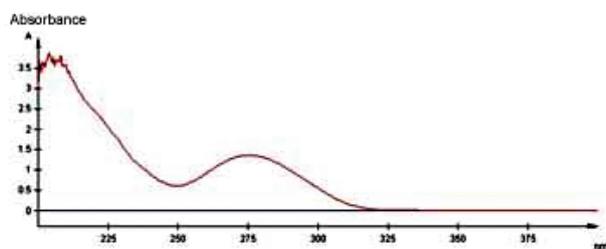


Figure 2. Absorbance maxima of Aceclofenac.

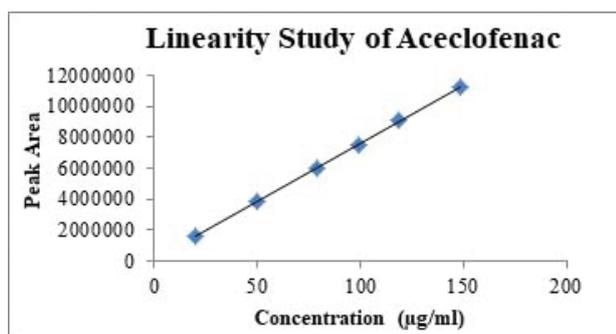


Figure 3. Calibration curve of linearity study.

fixed amount of previously analyzed sample stock solution of 1 mg/mL concentration to obtain final concentration of approximately 101–140 µg/mL.

Precision

Repeatability

The precision of the instrument (RSD) was checked by repeated scanning of samples ($n = 6$) for ACF standard without changing the parameter of the proposed method.

Intermediate precision

To determine the intra-day and inter-day precision of the method, the drug solution at assay concentration (100 µg/mL) was prepared ($n = 6$) in one laboratory on the same day (1st, 3rd, and 6th hour) and also on five different days from the same standard stock solution. The concentration was calculated from the areas obtained and the results were expressed as relative standard deviation (%RSD).

Specificity

Specificity was determined by checking the chromatograms of blank, placebo, standard and sample solution for interference with analyte peak, as well as through determination of peak purity for the drug in the presence of degradation products.

At first different placebo concentration were spiked with nominal concentration of drug substance and then different concentration level of drug were spiked with fixed placebo concentration to determine the peak res-

Table 3. Evaluation of linearity, Limit of Detection (LOD) and Limit of Quantitation (LOQ).

λ_{max} (nm)	Regression equation ($y = mx + c$)	Linearity range (µg/mL)	Residual sum of squares	Correlation coefficient	Baseline Noise in rms	S/N for LOD	S/N for LOQ
275	$74894x + 94097$	19.8 to 148.5	2.213	0.999	218.7	3.005	9.36

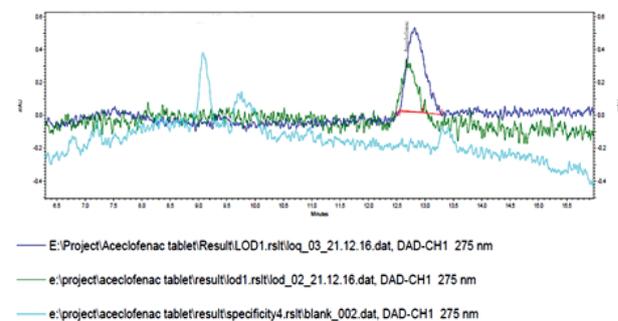


Figure 4. Sensitivity study.

ponse (Chakraborty et al. 2018). The responses of the standard ACF, marketed product and excipient of stressed condition (kept at 80 °C for 48 hrs) were also compared with the response of the same samples of unstressed condition at the assay concentrations to establish the stability indicating nature of the developed method as part of the forced degradation studies.

Robustness

Robustness was determined by changing the different method parameters like mobile phase composition, pH of buffer, column temperature, mobile phase flow rate and detector wavelength.

System Suitability

Six replicate injection of ACF standard solution at assay concentration was checked for tailing factor, theoretical plate, retention time, capacity factor and relative standard deviation of response to establish suitability of the method in the instrument.

Results and discussion

The wavelength for maximum absorbance of ACF was found to be at approximately 275 nm (Fig. 2).

Marketed tablets were analyzed through the developed method which showed 101.78% of ACF with 0.186% %RSD. The proposed method was found to be linear with a correlation coefficient of 0.999 (Table 3 and Fig. 3).

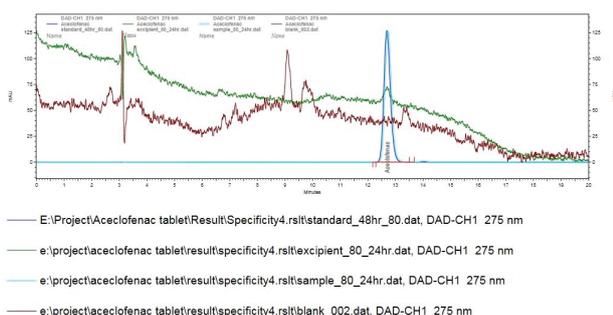
The baseline noise value was obtained from the software (Table 3). The minimum concentration levels at which ACF can be reliably detected (LOD) and quantified (LOQ) were found to be 0.0692 µg/mL and 0.2076 µg/mL respectively (Fig. 4 and Table 3) which was more sensitive

Table 4. Evaluation data of accuracy study.

Level	Theoretical Conc. (µg/mL)	Peak area	Actual Conc. (µg/mL)	% Recovery	% RSD
80%	101.16	7553140	100.64	99.48	0.64
	101.16	7618998	101.52	100.36	
	101.16	7525643	100.28	99.13	
100%	121.36	8984771	119.72	98.65	0.66
	121.36	8917608	118.83	97.91	
	121.36	9034943	120.39	99.2	
120%	141.55	10664687	142.11	100.39	0.79
	141.55	10737296	143.07	98.94	
	141.55	10644064	141.83	100.2	

Table 5. Evaluation data of precision study.

Sample No.	Repeatability	Intermediate Precision			
		Intra - day		Inter - day	
1	102.83	1 st hour	102.57	1 st day	103.70
2	99.86	3 rd hour	101.40	2 nd day	101.22
3	101.73	8 th hour	102.68	3 rd day	102.53
4	102.48	–	–	4 th day	99.86
5	101.73	–	–	5 th day	103.59
6	101.69	–	–	–	–
Mean	101.721		102.216		102.181
SD	1.025		0.579		1.464
%RSD	1.007		0.567		1.432

**Figure 5.** Specificity study-response of the standard, sample, excipient and mobile phase.

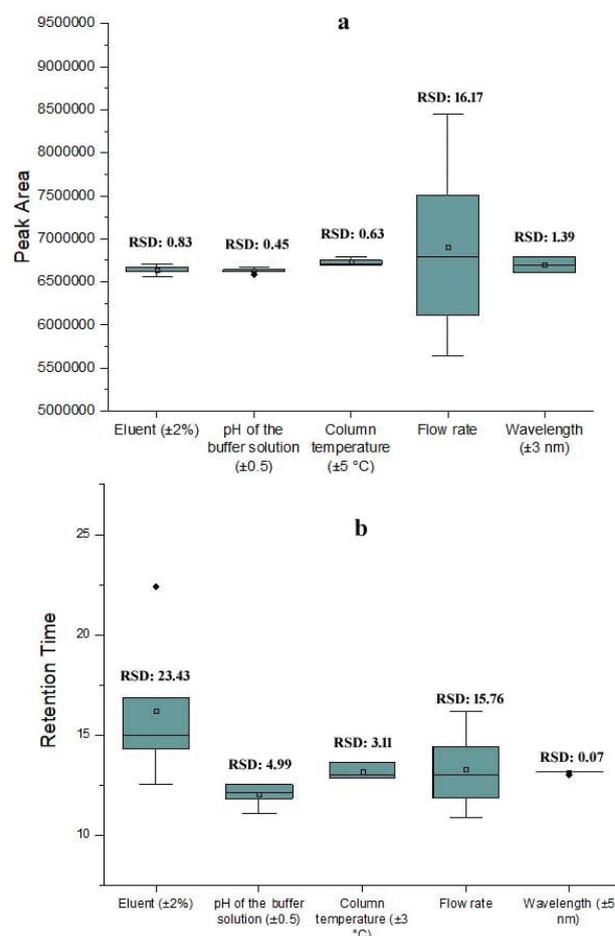
than some of the other published methods (Ravisankar et al. 2013, Balan and Kannappan 2014). Signal to noise (S/N) ratio for LOD and LOQ was shown in Table 3 which was also within acceptable limits.

The mean recoveries were 97.91% to 100.39% substantiated the method as accurate (Table 4). The method was also found to be precise with <2% of RSD value (Table 5) for both repeatability and intermediate precision study.

The developed analytical method should be specific for ACF assay in presence of all the potential matrix components which was checked by evaluating the peak responses and peak purity of the standard, sample, excipient and mobile phase (blank) solutions in assay concentration (Fig. 5). Specificity of the proposed method is also evaluated by the stress study of the samples. The % assays of ACF standard and marketed product were unaffected by the stressed condition as compared to the initial result at different observation days (Table 6). So

Table 6. Assay at Accelerated State of 80 °C.

	Concentration (%)			
	Initial	8 hour (80 °C)	24 hour (80 °C)	48 hour (80 °C)
Standard :	100.16	100.72	103.28	103.13
Sample :	96.18	100.49	97.81	97.48

**Figure 6.** Robustness Study (a) Variance of peak area for change in different method parameters with %RSD; (b) Variance of Retention Time for change in different method parameters with %RSD. *Method was robust for change in pH of mobile phase (±0.5), wavelength (±3 nm) and column temperature (±3 °C).

the placebo effect was checked in peak response as described in the method. The response obtained with the mixture showed no interference with the standard response (Table 7).

The change in organic solvent of ±2% [Buffer/68–72(% v/v) Methanol] significantly changed the peak retention time from 22.40–12.41 min, although the %RSD of the peak response was found to be 0.83. The method was found to be robust for pH variation of the buffer solution from 1.7 to 2.5 (%RSD of 0.45 for the peak response), although the retention time (R_t) changed from 11.09 to 12.52 min. For the change in column temperature from 20–25 °C the retention time changed from 14.23–12.27 min with acceptable %RSD (0.63) of the peak response.

Table 7. Specificity study of Aceclofenac-standard spiked with excipients.

Fixed drug substance spiked with different concentration of excipient			Fixed excipient spiked with different concentration of drug substance		
Nominal drug substance concentration ($\mu\text{g/mL}$)	Excipient concentration ($\mu\text{g/mL}$)	Absorbance	Nominal excipient concentration ($\mu\text{g/mL}$)	Drug substance concentration ($\mu\text{g/mL}$)	Absorbance
87.693	128.72	6705872	160.9	70.154	5428265
	144.81	6664433		78.923	5898186
	160.9	6707371		87.693	6873075
	176.99	6784787		96.462	7506926
	193.08	6805847		105.231	8074567
RSD = 0.881%			Regression equation, $y = 78699x - 145122$ $R^2 = 0.9892$		

Flow rate of mobile phase was changed from 0.8 to 1.2 mL/min. The R_f and peak response of ACF both changed significantly for change of flow rate from 0.9 to 1.1 mL/min. Robustness of the method was checked for change in wavelength from 270–280 nm. The method was found to be robust for 272–278 nm with %RSD of 1.39 for peak response. The method conditions at which response were most stable was shown in Fig. 6.

Conclusion

In this study a simple RP-HPLC-DAD method for use in routine estimation of ACF in bulk drug and tablet dosage form has been developed and validated. The accuracy, precision and specificity of the method have been established, with determination of the method parameters up to which the method was found to be stable.

Reference

- Ardha VS (2016) RP-HPLC Method development and validation for the simultaneous estimation of aceclofenac and rabeprazole sodium in the bulk and marketed formulation. *Indian Journal of Pharmacy and Pharmacology* 3(3): 146–151. <https://doi.org/10.5958/2393-9087.2016.00031.5>
- Arslan SA, Tirnaksiz FA (2010) A nonsteroidal antiinflammatory drug: aceclofenac. *FABAD Journal of Pharmaceutical Sciences* 35(2): 105–118. <http://dergi.fabad.org.tr/tr/2010-volume-35-issue-2/>
- Ashrafur Islam SM, Abuzar SM, Paul PK (2011) Validation of UV-Spectrophotometric and RP-HPLC methods for the simultaneous analysis of paracetamol and aceclofenac in marketed tablets. *International Journal of Pharmacy & Life Sciences* 2(12): 1267–1275. <https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.1074.4833&rep=rep1&type=pdf>
- Azhilwar S, Ravi TK (2011) Stability indicating HPLC method for simultaneous determination of drotaverine and aceclofenac. *International Journal of Pharmacy and Pharmaceutical Sciences* 3(1): 245–250. <https://innovareacademics.in/journal/ijpps/Vol3Issue1/1056.pdf>
- Badgujar MA, Mangaonkar KV (2011) Method development and validation of stability indicating method for assay of diacerein and aceclofenac by HPLC. *Asian Journal of Chemistry* 23(12): 5531–5534. http://www.asianjournalofchemistry.co.in/user/journal/viewarticle.aspx?ArticleID=23_12_73
- Balan P, Kannappan N (2014) Development and validation of stability-indicating RP-UPLC method for simultaneous estimation of thiocolchicoside and aceclofenac in combined dosage form. *International Current Pharmaceutical Journal* 3(7): 296–300. <https://doi.org/10.3329/icpj.v3i7.19078>
- Bawazeer S, Badr-Eldin KM, Abdel-Megied AM (2018) Development and validation of a versatile UPLC-PDA method for simultaneous determination of paracetamol, tizanidine, aceclofenac, and nimesulide in their new combinations. *Journal of Analytical Methods in Chemistry* 2018: e7463914. <https://doi.org/10.1155/2018/7463914>
- Bhinghe JR, Kumar RV, Sinha VR (2008) A simple and sensitive stability-indicating RP-HPLC assay method for the determination of aceclofenac. *Journal of Chromatographic Science* 46(5): 440–444. <https://doi.org/10.1093/chromsci/46.5.440>
- Bordoloi R, Baishya H, Gogoi B, Gogoi P (2018) A validated reversed phase UHPLC method for simultaneous estimation of aceclofenac and paracetamol in bulk and pharmaceutical dosage form. *Asian Journal of Pharmaceutical Research and Development* 6(2): 1–5. <https://doi.org/10.22270/ajprd.v6i2.365>
- Bose A, Dash PP, Sahoo MK (2010) Simple spectrophotometric methods for estimation of aceclofenac from bulk and formulations. *Pharmaceutical Methods* 1(1): 57–60. <https://doi.org/10.4103/2229-4708.72233>
- Chakraborty S, Sharmin S, Rony SR, Ahmad SA, Sohrab MH (2018) Stability-indicating UV/Vis Spectrophotometric Method for Diazepam Development and Validation. *Indian Journal of Pharmaceutical Sciences* 80(2): 366–373. <https://doi.org/10.4172/pharmaceutical-sciences.1000366>
- Chandra P, Rathore AS, Lohidasan S, Mahadik KR (2012) Application of HPLC for the simultaneous determination of aceclofenac, paracetamol and tramadol hydrochloride in pharmaceutical dosage form. *Scientia Pharmaceutica* 80(2): 337–351. <https://doi.org/10.3797/sci-pharm.1108-04>
- Chatrabhuji PM, Pandya CV, Patel MC (2015) A new RP-HPLC-UV method for the simultaneous quantification of aceclofenac in bulk & its tablets. *Analytical Chemistry: An Indian Journal* 15(2): 49–53. <https://www.tsijournals.com/journals/archive/tsac-volume-15-issue-2-year-2015.html>
- Chitlange SS, Shinde PS, Pawbake GR, Wankhede SB (2010) Simultaneous estimation of thiocolchicoside and aceclofenac in pharmaceutical dosage form by spectrophotometric and LC method. *Der Pharmacia Lettre* 2(2): 86–93. <https://www.scholarsresearchlibrary.com/abstract/simultaneous-estimation-of-thiocolchicoside-and-aceclofenac-in-pharmaceutical-dosage-form-by-spectrophotometric-and-lc-m-9492.html>
- Choudhari V, Ingale K, Sahoo M, Syal P, Ingale S, Shinde S, Sutar A, Kuchekar B (2010) Development and validation of a RP-HPLC-PDA method for simultaneous estimation of drotaverine and aceclofenac in a combined dosage form. *International Journal of Research in Pharmaceutical Sciences* 1: 253–258. <https://www.pharmascope.org/~pharmascope/index.php/ijrps/article/view/790>

- Choudhari VP, Raut RP, Sahoo M, Syal PK, Shinde SN, Hable AA, Kuchekar BS (2011) Development and validation of stability-indicating RP-HPLC-PDA method for simultaneous analysis of thiocolchicoside and aceclofenac in pharmaceutical dosage form. *Journal of Pharmacy Research* 4(6): 1820–1823. <https://www.semanticscholar.org/paper/Development-and-Validation-of-Stability-Indicating-Choudhari-Raut/4435029d70b557a78c18ae1da090a2f3cc7a4761>
- El-Saharty YS, Refaat M, El-Khateeb SZ (2002) Stability-indicating spectrophotometric and densitometric methods for determination of aceclofenac. *Drug Development and Industrial Pharmacy* 28(5): 571–582. <https://doi.org/10.1081/DDC-120003453>
- Gandhi S, Deshpande P, Rajmane V, Dodal T, Parab J (2010) Method development and validation for simultaneous estimation of drotaverine hydrochloride and aceclofenac in tablet dosage form by RP-HPLC. *International Journal of Pharmaceutical Sciences Review and Research* 4(3): 49–52. <http://globalresearchonline.net/journalcontents/volume4issue3/Article%20009.pdf>
- Gandhi SP, Dewani MG, Borole TC, Damle MC (2011) Development and validation of stability indicating HPLC method for determination of diacerein and aceclofenac as bulk drug and in tablet dosage form. *International Journal of Research in Pharmacy and Chemistry* 1(4): 799–806. <https://www.ijrpc.com/files/00007.pdf>
- Gandla K, Lalitha T, Harika R (2015) Development and validation of RP-HPLC method for simultaneous estimation of aceclofenac and tramadol in tablet dosage form. *Asian Journal of Research in Pharmaceutical Sciences* 5(3): 135–138. <https://doi.org/10.5958/2231-5659.2015.00021.1>
- Gelani HD, Chauhan PP, Shah SK (2014) Practical implication of chromatographic method for estimation of Aceclofenac and pregabalin in bulk and pharmaceutical dosage forms. *Chromatography Research International* 2014: e2014. <https://doi.org/10.1155/2014/643027>
- Gharge D, Dhabale P (2010) Simultaneous estimation of aceclofenac and paracetamol in solid dosage form by RP-HPLC Method. *International Journal of ChemTech Research* 2: 942–946. http://sphinxσαι.com/s_v2_n2/CT_V.2No.2/chemTech_vol2_no.2_index4.htm
- Godse VP, Deodhar MN, Bhosale AV, Sonawane RA, Sakpal PS, Borkar DD, Bafana YS (2009) Reverse phase HPLC method for determination of aceclofenac and paracetamol in tablet dosage form. *Asian Journal of Research in Chemistry* 2(1): 37–40. <https://ajrconline.org/AbstractView.aspx?PID=2009-2-1-8>
- Gousuddin M, Sengupta P, Chatterjee B, Das SK (2017) Stability-indicating RP-HPLC method for simultaneous quantitation of tramadol and aceclofenac in presence of their major degradation products: Method development and validation. *Journal of Liquid Chromatography & Related Technologies* 40(17): 887–893. <https://doi.org/10.1080/10826076.2017.1381849>
- Hasan NY, Abdel-Elkawy M, Elzeany BE, Wagieh NE (2003) Stability indicating methods for the determination of aceclofenac. *Il Farmaco* 58(2): 91–99. [https://doi.org/10.1016/S0014-827X\(02\)01271-5](https://doi.org/10.1016/S0014-827X(02)01271-5)
- Hossain MF, Bhadra S, Kumar U, Rouf AS (2013) The ICH guidance in practice: Stress degradation studies on aceclofenac and development of a validated stability-indicating reversed-phase HPLC assay in tablet dosage form. *Der Pharma Chemica* 5(4): 131–146. <https://www.derpharmachemica.com/pharma-chemica/the-ich-guidance-in-practice-stress-degradation-studies-on-aceclofenac-and-development-of-a-validated-stabilityindicatin.pdf>
- Jadhav S, Gosar A (2016) Development and validation of new analytical method for the simultaneous determination of five different drug substance in drug product using sub two microncolumn. *World Journal of Pharmaceutical Research* 5(12): 1029–1035. https://wjpr.net/dashboard/abstract_id/6234
- Jamil S, Talegaonkar S, Khar RK, Kohli K (2008) Development and validation of a stability-indicating LC method for simultaneous analysis of aceclofenac and paracetamol in conventional tablets and in microsphere formulations. *Chromatographia* 68(7–8): 557–565. <https://doi.org/10.1365/s10337-008-0797-x>
- Jinde RN, Chipade VD, Shinde VA, Kshirsagar MD, Chandewar AV (2013) Development and validation of RP-HPLC-DAD method for estimation of aceclofenac and rabeprazole sodium in bulk and combined dosage form. *Research Journal of Pharmacy and Technology* 6(2): 162–165. <https://rjptonline.org/AbstractView.aspx?PID=2013-6-2-17>
- Joshi R, Sharma R (2008) Development and validation of RP-HPLC method for simultaneous estimation of three-component tablet formulation containing acetaminophen, chlorzoxazone, and aceclofenac. *Analytical Letters* 41(18): 3297–3308. <https://doi.org/10.1080/00032710802515086>
- Kachhadia PK, Doshi AS, Ram VR, Joshi HS (2008) Validated LC method for simultaneous analysis of tramadol hydrochloride and aceclofenac in a commercial tablet. *Chromatographia* 68(11–12): e997. <https://doi.org/10.1365/s10337-008-0829-6>
- Karbhari PA, Joshi SJ, Bhoir SI (2014) RP-LC gradient elution method for simultaneous determination of thiocolchicoside, aceclofenac and related impurities in tablet formulation. *Journal of Pharmacy & Bio-Allied Sciences* 6: 246–252.
- Khanage SG, Kale DS, Mohite PB, Deshmukh VK (2014) Reversed phase high performance liquid chromatographic method for simultaneous estimation of pregabalin and aceclofenac in tablet formulation (Acenac-N). *Journal of Reports in Pharmaceutical Sciences* 3(2): 184–192. <http://www.jrpsjournal.com/article.asp?issn=2322-1232;year=2014;volume=3;issue=2;page=184;epage=192;aulast=Khanage;type=0>
- Kumar AC, Deveswaran R, Madhavan V (2008) Reversed-phase HPLC determination of aceclofenac in bulk powder and its pharmaceutical tablets. *Asian Journal of Chemistry* 20(1): 811–813. http://www.asianjournalofchemistry.co.in/User/ViewFreeArticle.aspx?ArticleID=20_1_122&fileType=HTML
- Kumar RS, Nathan PS, Nallasivan PK, Solomon WS, Venkatnarayanan R (2010) Validated RP-HPLC method for the simultaneous estimation of aceclofenac and diacerein in bulk and formulation. *International Journal of PharmTech Research* 2(1): 940–944. [http://sphinxσαι.com/sphinxσαιVol_2No.1/PharmTech_Vol_2No.1/PharmTech_Vol_2No.1PDF/PT=141%20\(940-944\).pdf](http://sphinxσαι.com/sphinxσαιVol_2No.1/PharmTech_Vol_2No.1/PharmTech_Vol_2No.1PDF/PT=141%20(940-944).pdf)
- Kumar RS, Senthil Kumaran P, Kumar Nallasivan P, Sam Solomon WD, Venkatnarayanan R (2010) A validated reversed phase HPLC-method for the determination of aceclofenac and tizanidine in tablets. *Asian Journal of Pharmaceutical Research and Health Care* 2(1): 84–94. <http://www.informaticsjournals.com/index.php/ajprhc/article/view/603>
- Momin MY, Yeole PG, Puranik MP, Wadher SJ (2006) Reverse phase HPLC method for determination of aceclofenac and paracetamol in tablet dosage form. *Indian Journal of Pharmaceutical Sciences* 68(3): 387–389. <https://doi.org/10.4103/0250-474X.26672>
- Mumtaz A, Sattar AA, Nazir R, Naseem A (2013) Development and implication of methodology for aceclofenac in pure and pharmaceutical formulations by microwave assisted spectrophotometry. *Pakistan Journal of Pharmaceutical Sciences* 26(1): 169–173. <https://pubmed.ncbi.nlm.nih.gov/23261744/>
- Muralidharan S (2014) Simple and sensitive method for the simultaneous estimation of drotaverine HCL and aceclofenac using RP-HPLC. *Journal of Pharmacy and Pharmaceutics* 1(1): 1–3. <https://doi.org/10.15436/2377-1313.14.001>

- Navneet Kumar U, Afroze A, Pradeepti C, Shailendra K, Naik KK (2017) Development and validation of a new chromatographic method for the simultaneous estimation of serratiopeptidase, aceclofenac and paracetamol by RP-HPLC. *Pharmaceutical Analytical Chemistry* 3(2): e122. <https://doi.org/10.4172/2471-2698.1000122>
- Nikalje APG, Gadikar R (2018) A simple liquid chromatographic method for simultaneous determination of aceclofenac, methyl salicylate, and benzyl alcohol in pharmaceuticals. *Journal of Pharmacy Research* 12(3): 283–287. http://jprsolution.info/article_detail.php?article_id=2010
- Nyola NK, Jeyabalan G, Kalra N, Parveen G, Choudhary S (2012) Development and validation of a RP-HPLC method for simultaneous estimation of diacerein and aceclofenac in pharmaceutical dosage form. *Research and Reviews: Journal of Pharmaceutical Analysis* 1(1): 1–8. <http://www.rroj.com/open-access/development-and-validation-of-a-rphplc-method-for-simultaneous-estimation-of-diacerein-and-aceclofenac-in-pharmaceutical-dosage-form-.php?aid=34634>
- Padma A, Girija D, Reddy AT, Reddy BV (2019) Validated stability indicating RP-HPLC method for the simultaneous estimation of drotaverin HCl and aceclofenac in bulk and formulation. *PharmaTutor* 7(2): 55–60. <https://doi.org/10.29161/PT.v7.i2.2019.55>
- Patel PN, Samanthula G, Shrigod V, Modh SC, Chaudhari JR (2013) RP-HPLC Method for determination of several NSAIDs and their combination drugs. *Chromatography Research International* 2013. <https://doi.org/10.1155/2013/242868>
- Paul K, Nagarajan JS, Chandan RS (2011) Standardization an a RP-HPLC method for the estimation of Aceclofenac in dosage form. *International Journal of Research in Pharmacy and Chemistry* 1(4): 853–859. <http://www.ijrpc.com/files/000015.pdf>
- Pawar UD, Naik AV, Sulebhavikar AV, Datar TA, Mangaonkar K (2009) Simultaneous determination of aceclofenac, paracetamol and chlorzoxazone by HPLC in tablet dose form. *Journal of Chemistry* 6(1): 289–294. <https://doi.org/10.1155/2009/139484>
- Pinnamaneni P, ElphinePrabahar A, Suresh PV, Nagamalleswari G, Nandendra RR (2015) Method development and validation for simultaneous estimation of drotaverine hydrochloride and aceclofenac in bulk and formulation by RP-HPLC. *World Journal of Pharmacy and Pharmaceutical Sciences* 4: 1351–1358. https://www.wjpps.com/Wjpps_controller/abstract_id/4332
- Podili B, Seelam M, Kammela PR (2017) Analytical method development and validation of simultaneous estimation of paracetamol, aceclofenac and serratiopeptidase by RP-HPLC. *International Journal of Ophthalmology & Visual Science* 2(3): 69–74. <http://www.sciencepublishinggroup.com/journal/paperinfo?journalid=230&doi=10.11648/j.ijovs.20170203.12>
- Pravallika KE, Naidu JB, Parimi R (2016) Development and validation of a novel stability indicating RP-HPLC method for simultaneous determination of Aceclofenac and Misoprostol in bulk and from their combined dosage form. *Der Pharm Lettre* 8(19): 92–99. <https://www.scholarsresearchlibrary.com/articles/development-and-validation-of-a-novel-stability-indicating-rphplc-method-for-simultaneous-determination-of-aceclofenac-a.pdf>
- Rajamahanti SG, Santosh T, Durga B, Raziya SK (2017) Evaluation of a new stability indicating method for the determination of aceclofenac and thiocolchicoside in pharmaceutical dosage form by RP-HPLC. *Oriental Journal of Chemistry* 33(3): 1337–1346. <http://dx.doi.org/10.13005/ojc/330334>
- Ravisankar P, Devadasu C, Rao GD, Rao MN (2013) Development and validation of RP-HPLC method for Simultaneous determination of paracetamol, aceclofenac sodium and chlorzoxazone in combined dosage form. *World Journal of Pharmacy and Pharmaceutical Sciences* 3(1): 667–681. https://www.wjpps.com/Wjpps_controller/abstract_id/743
- Ravisankar P, Devala Rao G (2013) RP-HPLC method for the separation of paracetamol, tizanidine, aceclofenac, chlorzoxazone: application to tizanidine determination in tablet dosage form. *International Research Journal of Pharmacy* 4(6): 156–163. <https://doi.org/10.7897/2230-8407.04635>
- Ravisankar P, Rao GD, Saibabu S, Devadasu C, Babu PS, Sricharitha P, Kumar KV (2013) Development and validation of a reverse phase HPLC method for the determination of Aceclofenac in bulk and pharmaceutical dosage forms. *International Journal of Research IN Pharmacy and Chemistry* 3: 483–494. <https://www.ijrpc.com/files/43-3128.pdf>
- Rele RV, Mali RN (2014) Advance simultaneous determination of paracetamol, thiocolchicoside and aceclofenac in tablets by reverse phase high performance liquid chromatography. *Der Pharmacia Sinica* 5(1): 34–39. <https://www.imedpub.com/abstract/advance-simultaneous-determination-of-paracetamol-thiocolchicoside-and-aceclofenac-in-tablets-by-reverse-phase-high-performance-liquid-chromatography-11618.html>
- Samanthula G, Shrigod VV, Patel PN (2014) Validated stability-indicating assay method for simultaneous determination of aceclofenac and thiocolchicoside using RP-HPLC. *Drug Research* 64(8): 429–435. <https://doi.org/10.1055/s-0033-1361128>
- Sekar V, Jayaseelan S, Udhaya Kumar E, Subash N, Prakash M, Perumal P (2010) Development and validation of RP-HPLC method for the simultaneous estimation of diacerein and aceclofenac in dosage form. *International Journal of ChemTech Research* 2(1): 168–171. [http://sphinxnsai.com/sphinxnsaiVol_2No.1/ChemTech_Vol_2No.1/ChemTech_Vol_2No.1PDF/CT=28%20\(168-171\).pdf](http://sphinxnsai.com/sphinxnsaiVol_2No.1/ChemTech_Vol_2No.1/ChemTech_Vol_2No.1PDF/CT=28%20(168-171).pdf)
- Selvan PS, Gopinath R, Saravanan VS, Gopal N, Kumar AS, Periyasamy K (2007) Simultaneous estimation of paracetamol and aceclofenac in combined dosage forms by RP-HPLC method. *Asian Journal of Chemistry* 19(2): 1004–1010. http://www.asianjournalofchemistry.co.in/user/journal/viewarticle.aspx?ArticleID=19_2_25
- Shah R, Magdum C, Patil SK, Chougule DK, Naikwade N (2008) Validated spectroscopic method for estimation of aceclofenac from tablet formulation. *Research Journal of Pharmacy and Technology* 1(4): 430–432. <https://rjptonline.org/AbstractView.aspx?PID=2008-1-4-84>
- Shaikh KA, Devkhile AB (2008) Simultaneous determination of aceclofenac, paracetamol, and chlorzoxazone by RP-HPLC in pharmaceutical dosage form. *Journal of Chromatographic Science* 46(7): 649–652. <https://doi.org/10.1093/chromsci/46.7.649>
- Shaikh KA, Patil AT, Ingole AB (2012) Sensitive LC method for the simultaneous determination of diacerein and aceclofenac in tablet dosage form. *International Journal of Industrial Chemistry* 3(1): e3. <https://doi.org/10.1186/2228-5547-3-3>
- Sharma G, Bansal N, Jain DK, Verma S, Jha AK (2012) Reversed-phase high-performance liquid chromatographic and mass spectrophotometric methods for simultaneous determination of paracetamol, aceclofenac and tramadol in combined tablet dosage form in presence of its degradation products. *Asian Journal of Research in Chemistry* 5(7): 854–858.
- Sherikar OD, Puranik MP, Yeole PG (2011) A validated reversed phase high performance liquid chromatographic (RP-HPLC) method for simultaneous estimation of aceclofenac drug substance and its related traces impurities in the solid dosage form. *International Journal of ChemTech Research* 3(2): 547–554.

- Somasekhar V, Gowrisankar D (2011) HPLC Method for the simultaneous determination of paracetamol, aceclofenac and tizanidine hydrochloride. *Asian Journal of Research in Chemistry* 23(4): 1651–1654.
- Suganthi A, Lakshmi CHS, Vinod S, Ravi TK (2014) Development of validated RP-HPLC method for bosentan in formulation and its application to in vitro interaction study with aceclofenac. *World Journal of Pharmaceutical Research* 3: 2897–2909. https://wjpr.net/dash-board/abstract_id/707
- Suganthi A, Ravi TK (2010) Stability indicating HPLC method for simultaneous determination of diacerein and aceclofenac. *Research Journal of Pharmacy and Technology* 3(2): 600–603. <https://rjptonline.org/AbstractView.aspx?PID=2010-3-2-57>
- Vaidya VV, Singh GR, Choukekar MP, Kekare MB (2010) Simultaneous RP HPLC determination of aceclofenac, paracetamol and tizanidine in pharmaceutical preparations. *Journal of Chemistry* 7(1): 260–264. <https://doi.org/10.1155/2010/323410>
- Valambhia KR (2013) Validation of UV spectroscopic method of analysis for assay and dissolution of aceclofenac tablet. *World Journal of Pharmacy and Pharmaceutical Sciences* 2(5): 3977–3983. https://www.wjpps.com/Wjpps_controller/abstract_id/457
- Validation of analytical procedures: text and methodology Q2 (R1) (2005) International conference on harmonization, Geneva, Switzerland. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdf
- Ventrapragada B, Kumar SA, Debnath M, Krishna NS, Saravanan J, Greeshma V (2014) Method development and validation for the simultaneous estimation of aceclofenac and paracetamol in solid dosage form by using RP–HPLC. *International Journal of Biopharm* 5(2): 109–118. <https://pdfs.semanticscholar.org/d6ba/2a2f3dbf732fcd97b44641e26c269c002a77.pdf>
- Zawilla NH, Mohammad MAA, El Kousy NM, El-Moghazy Aly SM (2002) Determination of aceclofenac in bulk and pharmaceutical formulations. *Journal of Pharmaceutical and Biomedical Analysis* 27(1–2): 243–251. [https://doi.org/10.1016/S0731-7085\(01\)00518-0](https://doi.org/10.1016/S0731-7085(01)00518-0)
- Zeng-ri WA, Fu-jun PE (2010) Determination of aceclofenac by GC. *Qilu Pharmaceutical Affairs* 8: 468–470. https://en.cnki.com.cn/Article_en/CJFDTOTAL-SDYG201008013.htm

Supplementary material 1

Table S1. HPLC Analytical methods for simultaneous estimation of Aceclofenac with other constituents

Authors: Suriya Sharmin, Md. Hossain Sohrab, Fatema Moni, Farhana Afroz, Satyajit Roy Rony, Shammi Akhter

Data type: HPLC Analytical methods

Explanation note: Comparison of published simultaneous HPLC analytical method of Aceclofenac with other drugs.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/PlantSociology.67.57981.suppl1>