

# 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

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**Abstract:** A reversed-phase high performance liquid chromatographic method is developed and validated for the simultaneous evaluation of aceclofenac and its related impurities in tablet dosage form. The chromatographic separation achieved on a C<sub>18</sub> Inertsil reversed-phase column using an isocratic elution, being mobile phase acetonitrile: methanol: water (60:28:12) and pH of final mobile phase was adjusted to 7.0 with glacial acetic acid and sodium hydroxide. The limit of quantitation ( $S/N = 10:1$ ) is in the range of 0.0138-0.370  $\mu\text{g/ml}$  for each impurity. The coefficient of variations is less than 3% for intraday and variation in analysts. The recovery of aceclofenac spiked samples ranged from 99-102 % and the mean recovery for each level ranges from 99-101 % for the detected impurities. The related impurities (methyl and ethyl esters) are synthesized by refluxing acids in appropriate alcohol as described in the literature and used as a reference in analysis. Their structures are confirmed by spectral (IR, NMR, MASS) and elemental analysis while their purity confirmed by HPLC analysis. The proposed validated method is suitable for the analysis of different brands of aceclofenac tablets available in market, hence can be employed in the routine quality control analysis of aceclofenac and its solid dosage form.

**Keywords:** RP-HPLC, Aceclofenac, Impurities, Tablet dosage form.

## Introduction:

The presence of minute quantities of unwanted chemicals in pharmaceutical substances which may influence the drug safety and efficacy and is a major concern in the overall quality of pharmaceuticals. The challenges to analytical chemist are identification and quantification of impurities.

2-[(2,6-Dichlorophenyl) amino] benzene acetic acid carboxymethyl ester (Aceclofenac; CAS 89796-99-6), non-steroidal anti-inflammatory drug (NSAID) indicated in the symptomatic treatment of pain and inflammatory or degenerative arthropathies such as osteoarthritis, rheumatoid arthritis, ankylosing

spondylitis, abarticular inflammations, post trauma or postoperative inflammations.<sup>1</sup> Aceclofenac finds place in Martindale-Extra Pharmacopoeia<sup>2</sup>

Merck Index<sup>3</sup> and reported to be analytically estimated by various methods, such as, spectrophotometric estimation of aceclofenac by forming a coloured complex with

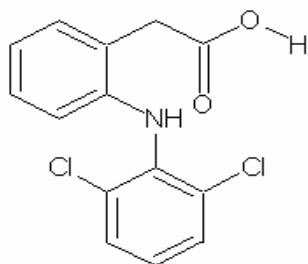
*p*-dimethylaminocinnamaldehyde (PDAC) and measuring absorption characteristics at the 665.5 nm<sup>4</sup>; Reverse – phase HPLC determination of aceclofenac by using C<sub>18</sub> column and methanol and water in the ratio of 55:45 as a mobile phase in isocratic mode. The detection wavelength was 274 nm with flow rate of 0.8

ml min<sup>-1</sup> 5; stability indicating assay with densitometric determination of aceclofenac<sup>6</sup>; UV – spectrophotometric determination of aceclofenac in alcohol at absorption maxima of 276.5 nm.<sup>7</sup>

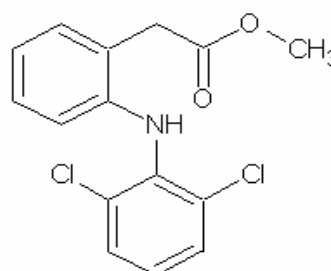
The various process related organic impurities that may be present in aceclofenac drug substance are 2-{2-[(2, 6-dichlorophenyl) amino] phenyl} acetic acid (impurity1), ethyl 2-[(2-[(2, 6 dichlorophenyl)

amino] phenyl} acetyl oxy] acetate (impurity2),methyl 2- {2-[(2, 6- dichlorophenyl) amino] phenyl} acetate (impurity3), methyl 2-[(2-[(2-[(2, 6 dichlorophenyl) amino] phenyl} acetyl) oxy] acetate (impurity 4), ethyl 2- {2-[(2, 6-dichlorophenyl) amino] phenyl} acetate (impurity 5)

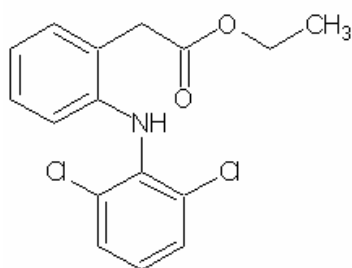
,Figure 1.



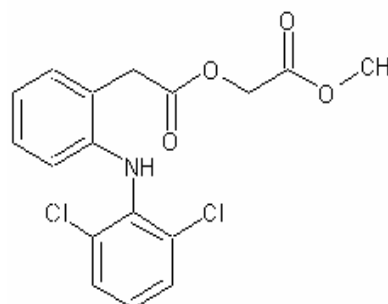
**Diclofenac (Imp.1)**



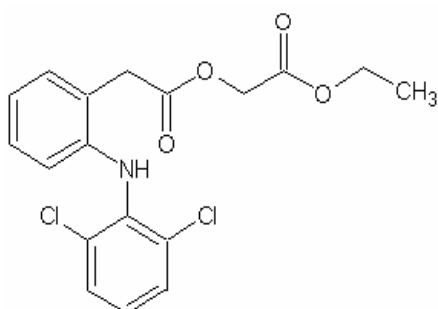
**Methyl ester of Diclofenac (Imp.3)**



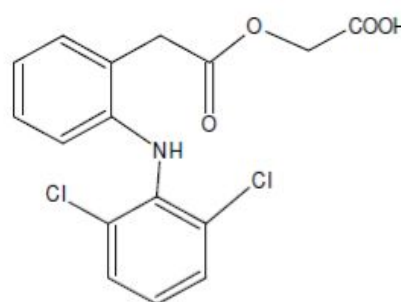
**Ethyl ester of Diclofenac (Imp.5)**



**Methyl ester of Aceclofenac (Imp.4)**



**Ethyl ester of Aceclofenac (Imp.2)**



**Aceclofenac**

**Figure 1: Structure of aceclofenac and its related impurities.**

Their profiles are influenced by the choice of synthetic route, the quality of starting materials, reagents and solvents, the reaction conditions, the work-up and final purification and the design of process equipment. Since impurities can have safety and efficacy implications, they are the subject of considerable attention by both the manufacturer and regulatory agencies. Thus, an analytical method to go hand-in-hand with process design is needed to detect and identify these impurities.

The present compendial monographs like European Pharmacopeia<sup>8</sup>, British Pharmacopeia<sup>9</sup> include HPLC methods for the potential impurities most likely to arise during the syntheses and which can contribute to the impurity profile of the drug substance. The official method for Aceclofenac along with its related impurities has following chromatographic specifications. C<sub>18</sub> column with *l*=0.25m and i.d of 4.6 mm with column temperature 40<sup>0</sup> c. Elution mode is gradient with two mobile phases A and B having composition 1.12gm/lit solution of phosphoric acid adjusted to p<sup>H</sup> 7.0 using 42gm/lit solution of sodium hydroxide and water : acetonitrile (1:9 v/v) respectively. Detection with spectrophotometer at 275nm using flow rate of 1ml min<sup>-1</sup>.

The aim of the present work was to develop and validate sensitive, specific and reproducible method for determination of related impurities of aceclofenac in tablet dosage form in a single chromatographic run. This could potentially improve the efficiency of the analysis and reduce laboratory supply costs associated with revalidating and testing of methods for individual impurities. To the best of our knowledge, for the first time in the present paper we report RP-HPLC method for the simultaneous determination of aceclofenac drug substance and its related impurities in the solid dosage form. The proposed validated method was found to be suitable and sensitive and useful in the quality control analysis of aceclofenac and its related impurities in solid dosage form.

#### **Experimental: Instruments:**

- (1) Infra red (IR) spectra were recorded using KBr disk on a Shimadzu (8400 S) spectrophotometer (Japan); <sup>1</sup>H-NMR spectra were recorded at 400 MHz on a Bruker AM spectrometer (USA) and their chemical shifts are reported in δ ppm units with respect to TMS as internal standard. The MASS spectrum was recorded on Autospec Mass spectrometer and UV spectra were recorded on UV-Visible Spectrophotometer (Shimadzu UV 2401 PC, Japan).
- (2) Chromatography was performed with a Jasco PU-1580 HPLC system (Japan) provided with

precision loop injector Rheodyne (20 μl) and variable wavelength UV-visible detector (Jasco UV-VIS 1575). Data were collected and analyzed using Borwin software. The separation of analytes was accomplished using C<sub>18</sub> Inertsil reversed-phase column (250 mm x 4.6 mm id) maintained at room temperature. Final chromatographic conditions involved an isocratic elution, using mobile phase acetonitrile: methanol: water (60:28:12) and pH of final mobile phase was adjusted to 7.0 with glacial acetic acid and sodium hydroxide. The pump flow rate was 0.5 ml/min.

#### **Chemicals:**

All the chemicals were of either analytical or HPLC grade purchased from Loba Chemicals, Mumbai, India. Reference standard for aceclofenac EP was obtained as a gift sample from Amoli Organics Ltd., Vapi, India and Aarti Drugs Ltd., Mumbai, India. Reference standard Diclofenac EP was obtained as a gift sample from Neon Labs Ltd., Palghar, India. The different tablet dosage forms were purchased from the local market.

#### **General procedure for the synthesis of related impurities of aceclofenac<sup>10</sup>:**

Mixture of acid (i.e. diclofenac or aceclofenac) 0.01 mole and 35 ml of methanol or ethanol was placed in 250 ml of dry round bottom flask and contents were mixed thoroughly. Then hydrogen chloride was passed through it till the increased in the weight of contents which was indicated by cloudiness in the reaction mixture. The contents were refluxed for 1 hour in water bath, cooled to room temperature; the crystals were collected after filtration and purified by recrystallisation from methanol or ethanol to obtain pure methyl or ethyl ester of acid. Physicochemical and spectral data for the synthesized related impurities are as follows:

#### **1)Ethyl 2-[(2-{2-[(2, 6-dichlorophenyl) amino] phenyl} acetyl) oxy] acetate (Imp.2):**

It was synthesized using aceclofenac and ethanol as white crystals. Yield: 82 %; m.p. 122-126 °C ; λ max: 274.50 nm

**IR (KBr, cm<sup>-1</sup>):** 3352, 2990, 1731, 1612, 1456, 1434, 1280, 748

**<sup>1</sup>H-NMR:** δ 7.65-7.62 (d, 2H, Ar), 7.55-7.52 (d, 1H, Ar), 7.42-7.38 (d, 1H, Ar), 7.24-7.19 (t, 1H, Ar), 7.11-7.08 (m, 2H, Ar), 6.39-6.37 (d, 1H, Ar), 3.81 (s, 2H, -CH<sub>2</sub>-), 3.73 (s, 1H, -OCH<sub>2</sub>-), 3.31 (s, 1H, NH)

**MASS m/z:** 351, 277, 260, 244, 242, 216, 214, 178, 152, 150;

**C, H, N analysis:** C=56.09 % (calculated 56.51 %), H=4.29 % (calculated 4.44 %), N=3.40%

(calculated 3.66 %)

**Purity by HPLC:** 99.99 %, solvent System : acetonitrile : methanol : water (60:30:10) and pH of final mobile phase was adjusted to 7.0 with glacial acetic acid and sodium hydroxide.

### **2) Methyl 2- {2-[(2, 6-dichlorophenyl) aminol phenyl] acetate (Imp. 3):**

It was synthesized using diclofenac and methanol as white crystals. Yield: 75 %; m.p.120-122 °C (literature : m.p. 122– 124 °C)<sup>11</sup> ;  $\lambda$  max: 275.80 nm

**IR (KBr, cm<sup>-1</sup>):** 3350, 2950, 1737, 1600, 1587, 1448, 1149, 750, 713

**<sup>1</sup>H-NMR:**  $\delta$  7.42-7.39 (d, 2H, Ar), 7.22-7.19 (d, 1H, Ar), 7.10-7.04 (m, 2H, Ar), 6.93-6.88 (t, 1H, Ar), 6.42-6.39 (d, 1H, Ar), 3.80 (s, 2H, -CH<sub>2</sub>-), 3.73 (s, 3H, OCH<sub>3</sub>), 3.31 (s, 1H, NH)

**MASS m/z:** 309, 242, 214, 179, 178, 151

**C, H, N analysis:** C=57.82 % (calculated 58.25 %), H=4.20 % (calculated 4.24 %), N= 4.0 % (calculated 4.53 %)

**Purity by HPLC:** 99.99 % , solvent system : acetonitrile : methanol : water (60:30:10) and pH of final mobile phase was adjusted to 7.0 with glacial acetic acid and sodium hydroxide.

### **3) Methyl 2-[(2-{2-[(2, 6-dichlorophenyl) aminol phenyl] acetyl oxy] acetate (Imp.4)**

It was synthesized using aceclofenac and methanol as white crystals. Yield: 80 %; m.p. 100-102 °C ;  $\lambda$  max : 275.80 nm

**IR (KBr, cm<sup>-1</sup>):** 3352, 2959, 1737, 1506, 1450, 1431, 1278

**<sup>1</sup>H-NMR:**  $\delta$  7.65-7.62 (d, 2H, Ar), 7.55-7.52 (d, 1H, Ar), 7.40-7.38 (d, 1H, Ar), 7.24-7.19 (t, 1H, Ar), 7.11-7.08 (m, 2H, Ar), 6.39-6.37 (d, 1H, Ar), 3.81 (s, 2H, -CH<sub>2</sub>-), 3.31 (s, 1H, NH); **MASS m/z:** 355, 341, 309, 277, 242, 216, 214, 179, 178, 151

**C, H, N analysis:** C=55.31 % (calculated 55.40 %), H=4.07 % (calculated 4.29 %), N= 3.42 % (calculated 3.80 %)

**Purity by HPLC:** 99.99 % , solvent system : acetonitrile : methanol : water (60:30:10) and pH of final mobile phase was adjusted to 7.0 with glacial acetic acid and sodium hydroxide.

### **4) Ethyl 2- {2-[(2, 6-dichlorophenyl) aminol phenyl] acetate (Imp. 5)**

It was synthesized using diclofenac and ethanol as white crystals. Yield: 70 %; m.p.: 124- 126 °C ;  $\lambda$  max: 275.60 nm

**IR (KBr, cm<sup>-1</sup>):** 3294, 3074, 2988, 1710, 1600, 1577, 1056, 1454, 1286, 745

**<sup>1</sup>H-NMR:**  $\delta$  7.42-7.39 (d, 2H, Ar), 7.22-7.19 (d, 1H, Ar), 7.10-7.04 (m, 2H, Ar), 6.93-6.88 (t, 1H, Ar), 6.43-6.40 (d, 1H, Ar), 4.22- 4.15 (m, 2H, OCH<sub>3</sub>), 3.79 (s, 2H, -CH<sub>2</sub>-), 3.312 (s, 1H, NH), 1.29-1.24 (t, 3H, CH<sub>3</sub>)

**MASS m/z:** 308, 279, 277, 242, 214, 179, 151

**C, H, N analysis:** C=59.14 % (calculated 59.44 %), H=4.84 % (calculated 4.64 %), N = 4.05 % (calculated 4.33 %)

**Purity by HPLC:** 99.99 % , solvent system : acetonitrile : methanol : water (60:30:10) and pH of final mobile phase was adjusted to 7.0 with glacial acetic acid and sodium hydroxide.

### **Analysis of aceclofenac and its related impurities:**

#### **Standard solutions and sample preparation:**

A stock solution of aceclofenac and its five impurities was prepared separately by dissolving 50 mg of each with mobile phase. The mixture was sonicated for 10 min and adjusted to the 50 ml mark with mobile phase in order to obtain the solution of 1 mg/ml. The working standards (1-100  $\mu$ g/ml) for each impurity were prepared from the stock solution by serial dilution with mobile phase. 0.2 ml of individual stock solution was taken in same 10 ml volumetric flask and volume was made up to mark with mobile phase to get the concentration of 20  $\mu$ g/ml.

For preparation of aceclofenac samples, twenty tablets were weighed separately of each brand and average weight was determined, it was then finely powdered. Accurately weighed tablet powder equivalent to 50 mg of aceclofenac was transferred into five different 50 ml volumetric flasks and then about 20 ml of mobile phase was added. It was sonicated for 10 min, volume was adjusted to mark with mobile phase and finally filtered through Whatman paper No. 41 and diluted with mobile phase to get concentration of 20  $\mu$ g/ml of aceclofenac.

**Table 1: Impurities detected in aceclofenac tablet dosage form**

Brand <sup>a</sup>	Average weight (mg)	Concentration of impurities (mg/100 mg)					Total amount of impurities (% w/w)
		Imp. 1	Imp. 3	Imp. 5	Imp. 4	Imp. 2	
B-1	191.9	2.0	b	0.45	b	b	2.45
B-2	213.1	b	b	b	b	1.025	1.025
B-3	307.9	0.331	b	b	b	b	0.331

<sup>a</sup>Label claim 100 mg; <sup>b</sup>Not detected

B-1, B -2 and B-3 are the different brands procured from local market.

### Results and Discussion:

The related impurities of aceclofenac (methyl/ethyl esters of aceclofenac and diclofenac) were synthesized in good yield and purity by refluxing diclofenac or aceclofenac in methanol or ethanol to obtain methyl or ethyl ester of acids. Their structures were confirmed by spectral (IR, NMR, and MASS), elemental (CHN) analysis and the purity was confirmed by HPLC analysis. The synthesized impurities Imp. 2, Imp. 3, Imp. 4 and Imp.5 were used as a reference in analyses.

The RP-HPLC method was developed for the aceclofenac and related impurities. The separation of analytes was carried out using C<sub>18</sub> Inertsil reversed-phase column maintained at room temperature using acetonitrile: methanol: water (60:28:12) as isocratic mobile phase at pH 7.0. Representative chromatogram obtained by the described method for the standard aceclofenac and all five impurities is shown in Figure 2. The retention time for aceclofenac, Imp. 1, Imp. 2, Imp. 3, Imp. 4 and Imp. 5 were 6.38, 7.31, 9.0, 12.40, 13.05 and 15.45 min respectively. Chromatograms for each brand of aceclofenac are shown in Figures 3 (A-C).

Validation of the method was conducted in two phases: the analytical development phase in which the method conditions were defined and application to the actual analyses of tablet samples of various marketed brand of aceclofenac was carried out. The specificity was evaluated by individual injection of five

impurities, the blank mobile phase, the working standard solution mixture and unspiked and spiked aceclofenac tablet samples. In regard to specificity, there was evidence that the substances being quantitated were the intended analytes. No interference was observed at the same or at  $\pm 5\%$  of retention time of each known impurity when the analytes were individually analyzed and all of the impurities were well resolved from the drug. The relationship between response and concentration was demonstrated to be linear and reproducible. The correlation coefficients between peak area and concentration for each impurity were  $>0.999$ . A calibration curve was generated for each analytical run and was used to calculate the concentrations of each impurity in tablet samples of aceclofenac.

System suitability was evaluated by injecting the middle level of standard solutions ( $n = 5$ ). The system was deemed to be suitable if the number of theoretical plates for each peak was not less than 14,500. The tailing (peak asymmetry) factor was less than 1.3 and the resolution between all adjacent peaks more than 2. The limits of detection based on the signal to noise ratio 3:1 were 0.115  $\mu\text{g/ml}$  for Imp.1, Imp. 2 and Imp.5, 0.46 $\mu\text{g/ml}$  for Imp.3, 0.122 $\mu\text{g/ml}$  for Imp.4. The limits of quantitation as determined by precision and accuracy and were 0.35  $\mu\text{g/ml}$  for Imp.1, Imp. 2 and Imp 5, 0.138 $\mu\text{g/ml}$  for Imp. 3 and 0.370  $\mu\text{g/ml}$  for Imp. 4.

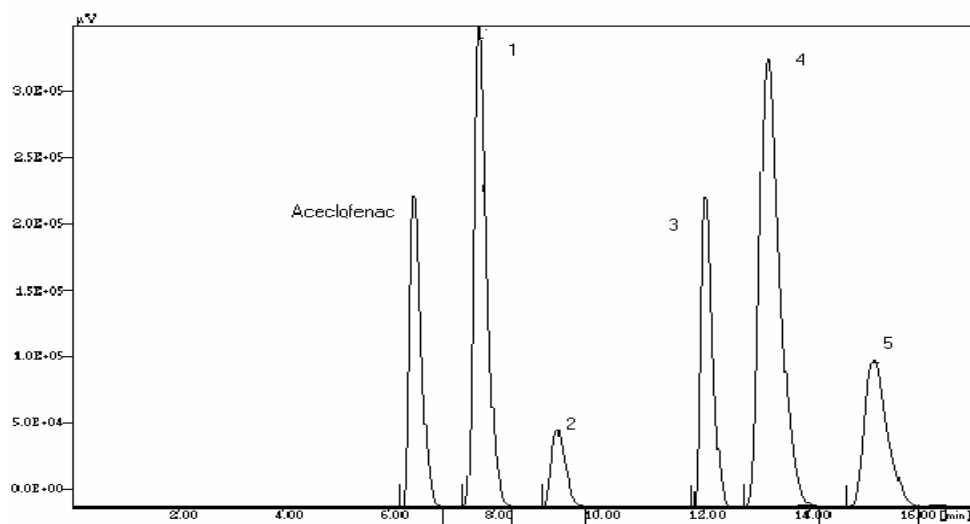


Figure 2: Chromatogram for the standard aceclofenac and all five impurities (<sup>1</sup>Imp. 1, <sup>2</sup>Imp. 2, <sup>3</sup>Imp. 3, <sup>4</sup>Imp. 4, <sup>5</sup>Imp. 5)

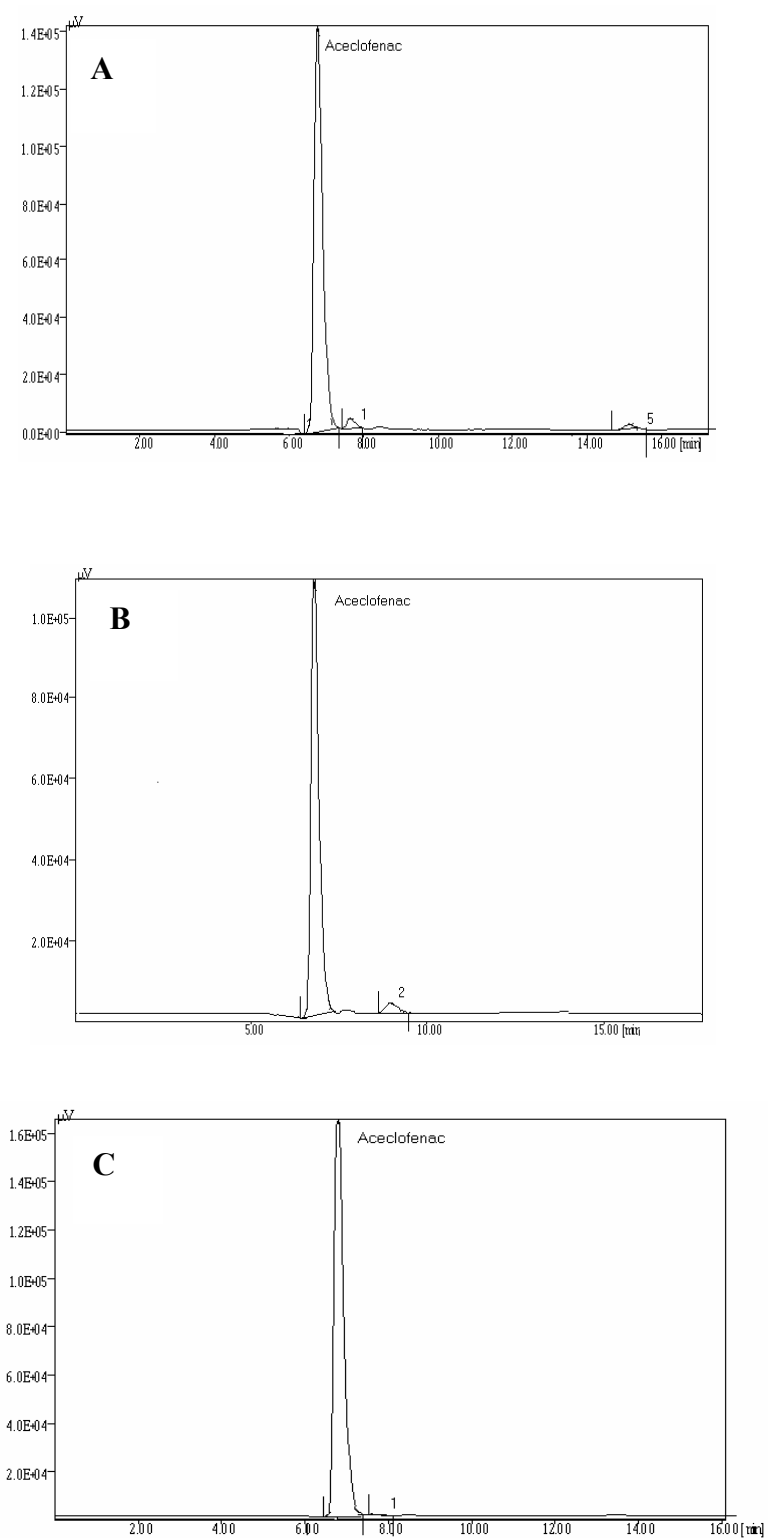


Figure 3: Chromatograms for commercial brands of aceclofenac (<sup>A</sup>Brand B-1, <sup>B</sup>Brand B-2, <sup>C</sup>Brand B-3).

**Table 2: Accuracy studies for the detected impurities in aceclofenac tablets.**

Brand and Impurity Detected	Added Concentration ( $\mu\text{g/ml}$ )	Mean* Recovery (%)	% RSD
B 1: Imp. 1	5	100.4	0.36
	10	100.0	0.28
	15	100.1	0.25
B 1: Imp. 5	5	100.5	0.39
	10	100.6	0.47
	15	99.3	0.23
B 2: Imp. 2	5	99.7	0.45
	10	100.1	0.65
	15	99.9	0.44
B 3 : Imp.1	5	100.7	0.47
	10	101.3	0.23
	15	99.9	0.56

\*Mean value represents three spiked samples for each concentration.  
(n = 3)

Table 1 describes the impurities detected in each marketed aceclofenac tablet. The accuracy and precision with which known concentrations of each impurity in tablet dosage form of aceclofenac of each brand can be determined were evaluated as depicted in Table 2.

The recovery assessment was performed by analyses of aceclofenac samples spiked with known amounts of impurity which was found in it during its analysis at three concentration levels.

Mean average recoveries in percent and the percent relative standard deviations for the detected impurities are shown in Table 2. The robustness of the method was evaluated by deliberate variation in the method parameters, such as pH ( $\pm 0.2$  units), and flow rate ( $\pm 10\%$ ). The changes in the chromatographic results of the system suitability were monitored by varying these parameters, and it was found that there were no changes in the resolution, selectivity, peak width, or symmetry.

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### Conclusion:

The RP-HPLC method described in the present paper was successfully employed in the analysis of commercially available tablet dosage form of aceclofenac without any interference. The developed RP-HPLC was found to be linear, reproducible, sensitive, selective and robust; therefore it can be used to analyze simultaneously five known impurities in aceclofenac drug substance and its tablet dosage form. The proposed validated method useful in the quality control analysis of aceclofenac bulk drug and its solid dosage form manufacturing in order to build the overall quality of pharmaceutical dosage form.

### Acknowledgement:

The authors are very much grateful to Amoli Organics Ltd., Vapi, India, Aarti Drugs Ltd., Mumbai, India and Neon Labs Ltd., Palghar, India for generously providing gift sample of Aceclofenac and Diclofenac pure drug for present study.

Authors are also thankful to SAIF Lucknow, for providing necessary facility for spectroscopic characterization of impurities.

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