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A New Simple Validated RP-HPLC-PDA Analytical Method for Estimation of Aceclofenec in Active Pharmaceutical Ingredients and Bulk Drug Form Used as Anti-Anti-Inflammatory Drug

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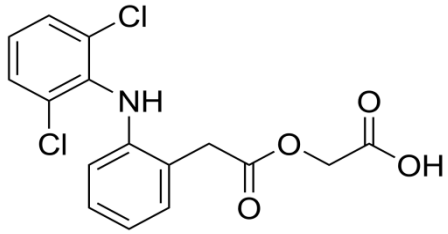
Abstract: *A simple, economical, user-friendly, precise, accurate, robust, rugged and linear analytical method developed and validated for estimation (Assay) of Aceclofenac in Active Pharmaceutical Ingredients or bulk drug form.Isocratic HPLC method developed, flow rate is 1.5 ml/min, and column used Hemochrom C18 Intsil (150mm X 4.6 mm X 5 µm), Column temperature used is 25ºC. Mobile phase used is made from 50 volume of buffer consist of 0.1 % orthophosphoric acid and 50 volumes of organic phase consist of 90% Acetonitrile. The UV detection wavelength is 275 nm. Linearity of the method was observed 50 µg/ml to 150 µg/ml. The retention time of Aceclofenac is ≈ 6.0 minute. This method was validated as per ICH guideline. (1) This method is very user-friendly and can be used for estimation of Aceclofenac in Active Pharmaceutical Ingredients and Bulk drug form because Analytical method validation of this method is successfully achieved in present study.*

Key Word*: Aceclofenac, Isocratic HPLC, Bulk drug, Pharmaceutical Ingredient*.

I. Introduction

Structure of Aceclofenac:(2)



IUPAC name of Aceclofenac is 2-[2-[2-[(2,6-dichlorophenyl) amino] phenyl] acetyl] oxyacetic acid. Its molecular weight 354.48 g mol-1. CAS no is 89796-99-6 chemical formula is C16H13Cl2NO4 Aceclofenac used as Anti-inflammatory, Analgesic drug in rheumatoid and osteoarthritis, bursitis, ankylosing spondylitis, toothache, dysmenorrhoea, post-traumatic and postoperative inflammatory conditions-affords quick relief of pain and wound edema.Also used as Antipyretic agent to treat fever.Aceclofenac also have some side effect like Nausea, headache, dizziness, rashes, gastric ulceration, epigasrtic pain. Aceclofenac is not given to breast feeding mothers, children's,

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pregnant women. Aceclofenac is sold under different trade name like Aceclo, Dolokind, Hifenac, Cincofen, Nacsiv, Acenacetc.

Different analytical technique used for estimation of Aceclofenac such as Spectrophotometric (3), Reverse phase-High Performance Liquid Chromatography(4-6), some of these methods are for estimation of Aceclofenac in tablet form and methods are complicated not much as user-friendly for Bulk drug estimation, so aim of present study to develop A simple, economical, user-friendly, precisious, accurate, robust, rugged and linear analytical method and validated as per ICH guideline for Aceclofenac Active Pharmaceutical ingredient.

II. Experimental Technique

Chemical and Reagents:

Sr. no.	Chemical Names	Grade	Make
1	Acetonitrile	HPLC grade	Merck
2	Orthophosphoric Acid	AR grade	Merck
3	Aceclofenac Working Standard	-	Shubham Biopharma
3	Aceclofenac Test Sample	-	Shubham Biopharama

Instrumentation and Analytical Conditions:

The analysis was carried out on Waters HPLC with photo diode array detector model-Aquity HPLC column used was Hemochrom C18 Intsil (150 mm X 4.6 mm X 5 µm). Mobile phase used as 50 volumes of buffer consist of 0.1 % orthophospharic acid and 50 volumes of organic phase consist of 90% Acetonitrile. Detection wavelength is 275 nm. Run time is 15 min. Buffer water and acetonitrile filter through 0.45 µm filter paper. Sonicate the Mobile phase for 15 minutes in sonicator. Equilibrate the column with mobile phase for 30 min.

Preparation of solution:

- **Diluent:** 50:50 (H_2O: Acetonitrile)
- **Standard Solution:** Weighed 10 mg Aceclofenac working standard and dissolve in diluent up to 100 ml in volumetric flask
- **Test Solution:** Weighed 10 mg Aceclofenac working standard and dissolve in diluent up to 100 ml in volumetric flask

Analytical Method Validation:

Analytical method validation is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications. “Methods validation is the process of demonstrating that analytical procedures are suitable for their intended use”.(7)

Typical Assay validation involves following steps:

1. Specificity
2. Precision
   - System Precision
   - Method Precision (Repeatability)
   - Intermediate precision
3. Linearity and Range
4. Accuracy
5. Robustness
6. Solution Stability

Specificity

Ability to assess unequivocally the analyte in the presence of components which may be expected to be present, such as impurities, degradation products, and matrix components. It is a measure of the degree of interference from such things as other active ingredients, excipients, impurities, and degradation products, ensuring that a peak response is due to an analyte only. No any elution observed at RT of principle peak in Blank (Shown in Figure 1) so specificity parameter passed.

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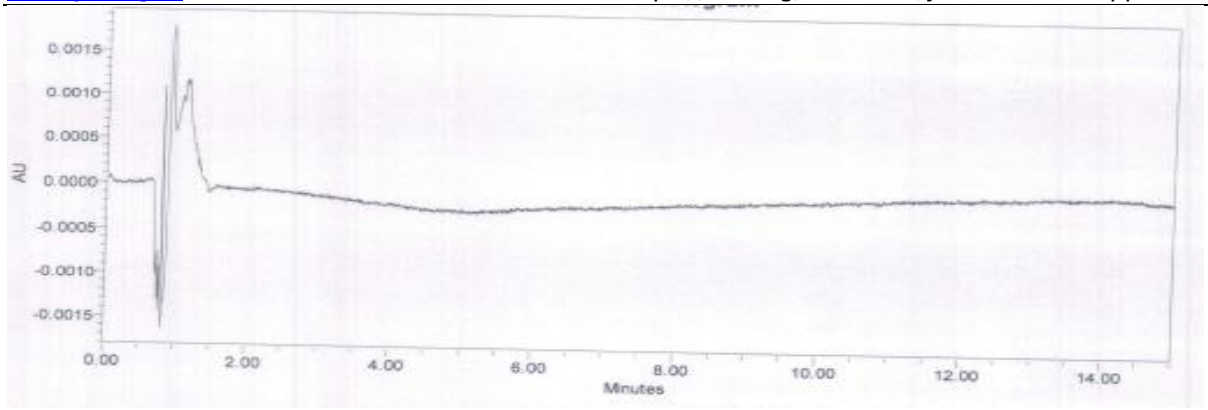


Figure 1: Typical chromatogram of Blank (Diluent)

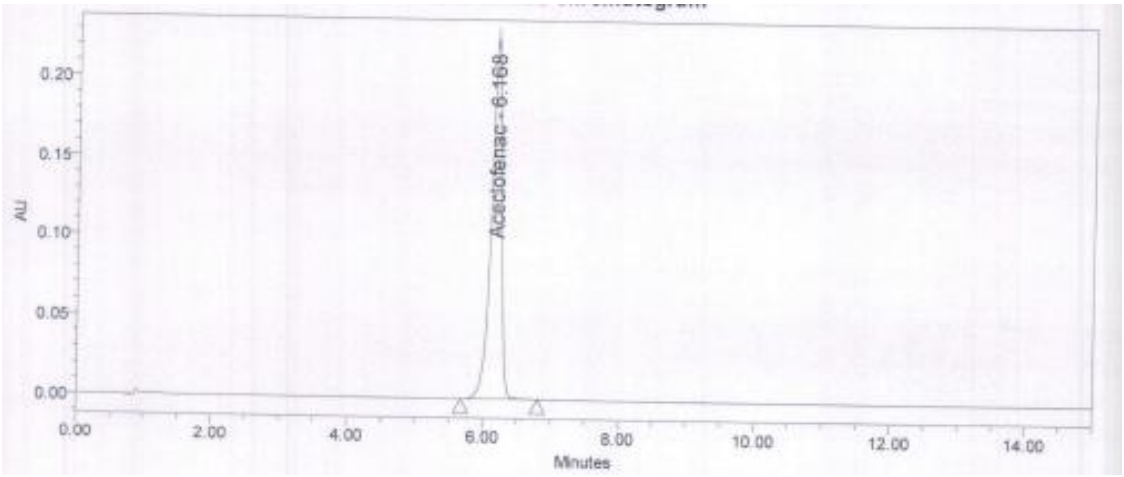


Figure 2: Typical chromatogram of Aceclofenac API.

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision has three types: repeatability, intermediate precision and reproducibility.

System suitability and System precision:

System suitability and System precision checked by injecting 0.1 mg/ml (100 ppm) sample solution in six replicates RSD found was 1.1 All injections having Theoretical plates is more 2000, All injections having Tailing factor less than 2 so method is system precious.

Table 1: System Suitability and System precision observations:

Standard Solution			
Injection	Theoretical plate	Tailing Factor	Area
1	8460	0.88	2377644
2	8486	0.88	2328863
3	8430	0.87	2381114
4	8395	0.87	2344043
5	8389	0.88	2394859
6	8440	0.87	2373903
Average			2366738

SD	24969
RSD	1.1
Wt of Std in mg	10.2
Purity in %	99.78

Method precision:
Method precision is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of same homogenous sample. Method precision checked by injecting 0.1 mg/ml (100 ppm) sample solution in six different preparations area found was 0.1 % and Assay RSD of six different preparation of same Batch sample is 0.0057 % so Method is Intermediate precious

Table 2: Method precision observations:

Standard Solution	
Injection	**Area**
1	2377644
2	2328863
3	2381114
4	2344043
5	2394859
6	2373903
Average	2366738
SD	24969
RSD	1.1
Wt of Std in mg	10.2
Purity in %	99.78

Test Solution			
Injection	**Wt of sample**	**Area**	**Assay**
Test prep.1	10.2	2370439	99.94
Test prep.2	10.1	2368278	100.83
Test prep.3	10.2	2368216	99.84
Test prep.4	10.2	2364270	99.68
Test prep.5	10.2	2363972	99.66
Test prep.6	10.1	2369809	100.9
Average		2367498	100.14
SD		2755	0.5702
RSD		0.1	**0.0057**

Intermediate Precision observations:
Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.
For Assay Analyze the sample of single batch six times by different analysts on different days using different instrument and where applicable use different column.
Intermediate precision checked by injecting 0.1 mg/ml (100 ppm) sample solution in six different preparations area standard RSD found was 1.1. Assay RSD of six different preparation of same Batch sample is 0.0032 % so Method is Intermediate presious.

Table 3: Intermediate precision observations:

Standarad Solution	
Injection	**Area**
1	2377644
2	2328863
3	2381114
4	2344043
5	2394859

6	2373903
Average	2366738
SD	24969
RSD	1.1
Wt of Std in mg	10.2
Purity in %	99.78

Test Solution			
Injection	**Wt of sample**	**Area**	**Assay**
Test prep.1	10.1	2327692	99.11
Test prep.2	10.0	2323275	99.91
Test prep.3	10.2	2369441	99.89
Test prep.4	10.0	2324434	99.96
Test prep.5	10.2	2367558	99.81
Test prep.6	10.2	2369173	99.88
Average		2346929	99.76
SD		23928	0.3221
RSD		1.0	**0.0032**

Linearity and Range:

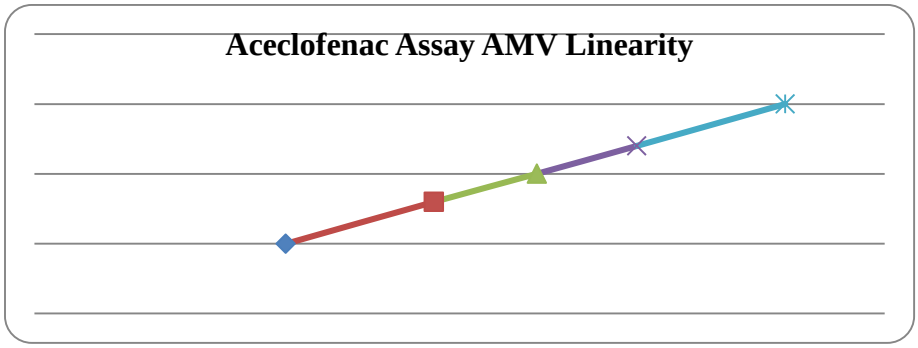
Linearity: The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Linearity should be evaluated by plot of signals as a function of analyte concentration or content.

Range: The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyst in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity

This developed method is linear over 0.05 mg/ml (50 ppm) to 0.15 mg/ml (150 ppm)

Sr. No	**linearity**	
	Aceclofenac (in ppm)	**Average area Response**
1	50	1183517
2	80	1879954
3	100	2364795
4	120	2834407
5	150	3532466
Correlation coefficient		**1.000**

Figure 3: Linearity plot



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Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness. Recovery Obtained is in between 98.2 % to 98.8 %. And overall mean Recovery obtained is 98.5 %

Accuracy Levels	Weight of Placebo (mg)	Area	Amount in µg		% Recovery	Mean % Recovery	Std. Dev.	% RSD	Overall % Recovery	Overall % RSD
			Amount added	Amount recovered						
Level I (50%)	NA	1184325	5168.6040	5092.8903	98.5	98.5	0.06	0.06	98.5	0.19
	NA	1163194	5078.8020	5002.0245	98.5					
	NA	1164577	5088.7800	5007.9683	98.4					
Level II (100%)	NA	2323555	10177.5600	9991.8652	98.2	98.5	0.31	0.31		
	NA	2325375	10157.6040	9999.6881	98.4					
	NA	2363459	10287.3180	10163.4598	98.8					
Level III (150%)	NA	3578891.71	15595.6140	15390.1236	98.7	98.5	0.20	0.20		
	NA	3568970.77	15575.6580	15347.4610	98.5					
	NA	3567914.63	15615.5700	15342.9194	98.3					

Robustness: Robustness of an analytical procedure as a measure of its capacity to remain unaffected by small but deliberate variations in procedural parameters listed in the documentation, providing an indication of the method's or procedure's suitability and reliability during normal uses. Robustness proved by changing two parameter Flow rate 1.5 ml/min and Column temperature 25ºC from its original value to ±2. No any adverse effect occurred on their estimation due to these normal changes % Difference between Standard and Test preparation is 0.5 and 1.20 respectively.

Solution Stability:

Stability study of analytical solution is performed for to check how much time solution is stable. Aceclofnac Test and standard solution is stable for 24.0 Hrs.

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