
J.R. Bhinge1,2, R.V. Kumar1,2, and V.R. Sinha1,*
1University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh-160014 and 2Center with Potential for Excellence in Biomedical Sciences, Panjab University, Chandigarh-160014

Abstract

The aim of the present study is to develop a stability-indicating assay method for the determination of aceclofenac after being subjected to different International Conference on Harmonization prescribed stress conditions, such as hydrolysis, oxidation, heat, and photolysis. Aceclofenac (2-[2-[2-(2,6-dichlorophenyl)aminophenyl]acetyl]oxyacetic acid) is decomposed under hydrolytic stress (neutral, acidic, and alkaline) and also on exposure to light (in solution form). The compound is stable to oxidative stress, heat, and photolytic stress (in solid form). The major degradation product is diclofenac, which is confirmed through comparison with the standard. Separation of the drug from major and minor degradation products is achieved on a C-18 column using methanol–0.02% of ortho phosphoric acid in a ratio of 70:30. The method is linear over the concentration range of 1–100 µg/mL ($r^2 = 0.9988$). The detection wavelength is 275 nm. The method is validated for linearity, range, precision, accuracy, specificity, and selectivity.

Introduction

Stress testing of the new drug substance is carried out according to stability test guideline Q1A (R2) issued by International Conference on Harmonization (ICH) for establishing its inherent stability characteristics and for supporting the suitability of the proposed analytical procedure (1,2). It is stated that the testing should include the effects of temperature, humidity where appropriate, oxidation, photolysis, and susceptibility to hydrolysis across a wide range of pH values (3,4). Aceclofenac is 2-{2-[2-(2,6-dichlorophenyl)aminophenyl]acetyl]oxyacetic acid. It is an NSAID (5) that exhibits a multifactor mechanism of action. Aceclofenac was developed in order to provide a highly effective pain relieving therapy with reduced side effects, especially GI events that are frequently experienced with NSAID therapy (6–10). Aceclofenac is a white or almost white crystalline powder, practically insoluble in water, freely soluble in acetone and in dimethylformamide, soluble in alcohol and methanol. It has molecular weight of 354.2 with a melting point of 149–150°C (5). It undergoes extensive degradation in alkaline conditions, yielding its main degradation product as diclofenac (11,12). The structural formulae of aceclofenac and diclofenac are as depicted in Figure 1. Various methods have been reported for its analysis, such as spectrophotometric (13), spectrofluorimetric (13), titrimetric (5), striping voltammetric (14), and high-performance liquid chromatography (HPLC) (11,12,15), along with a few others, for its determination in biological fluids (16,18). There is no published method on stress testing of the drug according to stability test guideline Q1A (R2) issued by ICH.

The aim of present study was to establish the inherent stability of aceclofenac through forced degradation studies according to ICH recommended test conditions, and to develop a simple and sensitive stability-indicating RP-HPLC assay method for its determination. The method can be used for the quantitation of aceclofenac in raw material and its formulations.

Experimental

All the solvents and reagents used were of analytical and HPLC grade. Methanol (HPLC grade) was procured from Merck (India, Ltd.), Sodium hydroxide (S.D. Fine-chem. Ltd. Mumbai, India), hydrochloric acid (Qualikems, New Delhi, India), and hydrogen peroxide (Qualigens Fine Chemicals, Mumbai, India) were of analytical reagent grade. The ultrapure water was prepared using ultrapure water purification setup (Sartorius, Göttingen, Germany), which was again filtered through a 0.22 µ filter. Aceclofenac was provided as a gift sample by Cadila Pharmaceut-

*Author to whom correspondence should be addressed: email vr_sinha@yahoo.com.
ticals (Ahmedabad, India) and was used without further purification. Diclofenac was provided as a gift sample by Panacea Biotech (Lalru, India).

**Instrumentation**

The HPLC system (Shimadzu, Kyoto, Japan) consisted of an LC-10AT pump, a SPD-10A UV-visible detector, and a DGU-14A degasser model. The separations were carried out on a C-18 reversed-phase column (Thermo Hypersil, 250 × 4.6 mm, 5 µ). The column was operated at a temperature of 30°C. The mobile phase was composed of methanol–0.02% orthophosphoric acid (70:30) at a flow rate of 1 mL/min. The wavelength of detection was 275 nm. The data were acquired and processed by the use of CLASS-VP software (Shimadzu, Kyoto, Japan). Specificity testing was done on another HPLC (Waters Delta 600) equipped with a Waters 600 controller pump, Waters 2996 PDA detector, and a degasser module (Waters, Milford, MA). Data acquisition and processing was performed by the use of Waters Empower 2 software. Forced degradation studies under acidic, alkaline, and neutral conditions were performed using a heating mantle with temperature control (Tempad, Mumbai, India). A dry air oven (NSW 143, New Delhi, India) was used to study the effect of dry heat. The photostability studies were carried out in a stability chamber (KBF 240, WTB Binder, Tuttlingen Germany) equipped with light sources as defined under option 2 of the ICH guideline Q1B. The light bank consisted of a combination of two blacklight OSRAM L73 lamps and four OSRAM L20 lamps. The blacklight lamp (L73) had a spectral distribution between 345 and 410 nm with a maximum at 365 nm. The output of white fluorescent lamps (L20) was similar to that specified in ISO 10977 (1993). Both UV and visible lamps were put on simultaneously. The study was performed by keeping the samples at a distance of 9 in from the light bank. The overall illumination at the point of placement was 5000 lux, which was tested using a calibrated lux meter (Escorp, New Delhi, India). The chamber was maintained at 40°C and 75% RH.

**Preparation of stock standard solution of aceclofenac**

A concentrated stock solution of aceclofenac was prepared at a concentration of 1 mg/mL in HPLC-grade methanol. Accurately weighed 100 mg of the drug were transferred to a 100-mL volumetric flask and 50 mL methanol was added. After shaking for 10 min, the volume was made up with methanol and the resulting solution was vortexed for 1 min.

**Stress studies**

**Hydrolytic studies**

Acidic hydrolysis of the drug was carried out in 0.1N HCl. The methanolic stock drug solution was diluted with acid to a concentration of 100 µg/mL. The solution was refluxed for a period of 8 h. Similarly, alkaline degradation studies were conducted in 0.01 N NaOH, where the methanolic stock drug solution was diluted with alkali to give a concentration of 100 µg/mL. The solution was then subjected to heating at 40°C for 8 h and 25°C for 2 h. Samples were suitably diluted before HPLC analysis. For studies in neutral conditions, the hydroalcoholic drug solution (100 µg/mL) was refluxed for 12 h. The samples were withdrawn and subjected to HPLC analysis after diluting to a suitable concentration.

**Oxidation**

Oxidative degradation studies were done in hydrogen peroxide solutions of different strengths at room temperature. The studies were done initially by exposing the drug to 1% H<sub>2</sub>O<sub>2</sub> for 30 min and then for 3 h. Studies were also performed in 3% H<sub>2</sub>O<sub>2</sub> solution initially for 6 h and continued for 24 h. Finally, the same concentration of the drug was also exposed to 10% H<sub>2</sub>O<sub>2</sub> for 24 h.

**Photostability**

Photolytic studies were performed on the hydroalcoholic drug solution (100 µg/mL) and
the powder drug sample. Each solution was exposed to light in a photostability chamber for 15 days. The samples of both solution and powder were kept in the dark for the same period. Samples were withdrawn at different time periods and analyzed.

Thermal stress studies
The drug was also subjected to dry heat at 70°C in a dry heat oven. The sample was analyzed after 15 days.

Method validation
Linearity
The linearity of the method was established by preparing a calibration curve in methanol. For this, a stock solution of the drug (1 mg/mL) in methanol was prepared. A range of concentrations (1–100 µg/mL) were then prepared after suitable dilution of the prepared stock with methanol. The solutions (20 µL) in the range of 1–100 µg/mL of drug were injected in triplicate into the HPLC column. Methanol–0.02% orthophosphoric acid (70:30) was used for elution. The linearity plots were constructed and data were treated using the linear regression analysis method.

Precision
To determine the intra-day and inter-day precision of the method, the drug solution at three different concentrations (2, 5, and 10 µg/mL) was injected in triplicate on the same day and also on six different days. The concentration was calculated from the areas obtained and the results were expressed as percentage relative standard deviation (%RSD).

Accuracy
Accuracy of the method was evaluated by spiking the drug at three different concentrations (10, 30, and 50 µg/mL) in samples of a marketed product. The percent recovery of the added drug was calculated from the linearity plots.

Specificity
The specificity of the method was established through determination of the drug in the presence of degradation products, as well as through determination of peak purity for the drug in the presence of degradation products using a PDA detector.

Assay of pharmaceutical formulation
The contents of ten indigenously developed delayed release aceclofenac tablets were thoroughly powdered and mixed, an amount of the powder equivalent to 100 mg of aceclofenac was accurately weighed and added to a 100-mL volumetric flask, 50 mL of methanol were added, and the volume was made with methanol. The resulting dispersion was sonicated for 15 min. The dispersion was kept as such for 30 min and again vortexed for 5 min and filtered through a 0.22 μ filter.

Results and Discussion
Degradation behavior of aceclofenac
The drug was decomposed under hydrolytic stress (neutral, acidic, and alkaline), and exposure to light (in solution form). The compound was stable in oxidative and photolytic stress and under dry heat in solid form. The major degradation product resembling to the structure of the drug was identified as diclofenac through comparison with the standard.

Acidic conditions
Aceclofenac underwent hydrolysis, on refluxing in 0.1N HCl for 8 h. The amount remaining was 16–17%. The drug degraded to four major degradation products with relative retention times (RRT) of 0.7, 1.16, 1.56, and 2.01 (Figure 2). The degradation product at RRT of 1.16 was found to be diclofenac, as the standard solution of diclofenac gave a peak at the same RT.

![Chromatograms showing the degradation behavior of aceclofenac in solid and solution states under photolytic conditions. The x-axis and y-axis of each chromatogram represent time (min) and voltage (V), respectively.](https://academic.oup.com/chromsci/article-abstract/46/5/440/372606)
Alkaline conditions

The results obtained on alkaline hydrolysis of aceclofenac were found to be analogous to those reported by Hasan et al with one additional peak at RRT of 0.69. The two degradation products were formed at RRT of 0.69 and 1.14, and again the major degradation product was found to be diclofenac. Studies carried out in 0.01N NaOH at 40ºC for 8 h showed complete degradation of the drug. In order to study the effect of milder conditions on the degradation behavior of aceclofenac, studies were performed in 0.01N NaOH at 25ºC for 2 h, but almost the entire drug was still degraded.

Neutral conditions

It was observed that approximately 36–37% of the drug degraded when refluxed in hydroalcoholic solution for 12 h. The amount of the drug left was found to be 62–63%. The major degradation products were found at RRT of 0.70, 0.94, 1.16, 1.56, and 2.01 (Figure 2).

Oxidative stress

The drug was found to be stable under the oxidative stress. No degradation of the drug was observed in 1% H2O2 on initially exposing for 30 min and then for 3 h. Even after exposing the drug to 3% H2O2 for 6 h and 24 h, no degradation was observed (Figure 3). Then the study was continued in 10% H2O2 for 24 h. Very little degradation was observed on exposing to 10% H2O2 for 24 h, giving a small degradation product peak at RRT of 0.63, 0.7, and 1.14. It was not possible to further increase the strength of the peroxide, because of the insolubility of the drug.

Photolytic studies

The drug solution was observed to be highly susceptible to light. Almost 24% of the drug degraded in the presence of light, giving clusters of minor degradation products along with seven major degradation products, which showed a sequential increase in the peak area and height (Figure 4). The solution kept in the dark also showed degradation, but to a lesser extent. The comparison of the amount of drug left in the solution after exposure to light and in dark is given in Table I.

The exposure of the drug in a solid state to light and dark showed far less degradation, with the formation of a single degradation product at RRT 1.98 (Figure 4).

Thermal stress

Aceclofenac was found to be stable to the dry heat, as negligible degradation was observed after exposing the drug to 70ºC for 15 days (Figure 5).

Method validation

Linearity and range

The data obtained from linearity studies was plotted against the concentration and the plots were treated with linear regression analysis. The data from the linearity curve showed that the response of the drug was strictly linear in the studied concentration range. A very high correlation of 0.9988 (±0.0013) was obtained with a slope of 41396. The %R.S.D. for slope was 0.40%.

Precision

The results of intra-day and inter-day precision studies, respectively, are shown in Table II. The results revealed that %RSD values for intra-day

<table>
<thead>
<tr>
<th>Time period of exposure</th>
<th>Light Percent drug remaining ± SD; R.S.D.</th>
<th>Dark Percent drug remaining ± SD; R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5th day</td>
<td>76.30 ± 0.44; 0.58</td>
<td>92.91 ± 1.24; 1.34</td>
</tr>
<tr>
<td>10th day</td>
<td>65.20 ± 0.84; 1.29</td>
<td>80.21 ± 1.50; 1.87</td>
</tr>
<tr>
<td>15th day</td>
<td>47.12 ± 0.45; 0.95</td>
<td>66.55 ± 0.90; 1.35</td>
</tr>
</tbody>
</table>

Table II. Precision Studies

<table>
<thead>
<tr>
<th>Actual concentration (µg/mL)</th>
<th>Measured concentration (µg/mL) ± S.D.; R.S.D. (%)</th>
<th>Repeatability (n=6)</th>
<th>Intermediate precision (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2.22 ± 0.20; 1.20</td>
<td>2.18 ± 0.04; 1.86</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4.88 ± 0.05; 1.11</td>
<td>5.04 ± 0.15; 3.16</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10.27 ± 0.07; 0.75</td>
<td>10.39 ± 0.07; 0.69</td>
<td></td>
</tr>
</tbody>
</table>

Figure 5. Chromatogram showing the degradation behavior of aceclofenac after a thermal stress study at 70ºC for 15 days. The x-axis and y-axis of each chromatogram represent time (min) and voltage (V), respectively.

Figure 6. Chromatogram depicting the separation of degradation products of aceclofenac in a mixture of reaction samples using methanol–orthophosphoric acid (70:30) as the mobile phase. The x-axis and y-axis of the chromatogram represent time (min) and voltage (V), respectively.
studies ranged between 0.75 and 1.20% and between 0.69 and 3.16% for inter-day precision.

**Accuracy**

Good recoveries were obtained in the range of 100.2 to 101.6%, which is indicative of high accuracy.

**Specificity**

Good resolution was obtained between the drug and the degradation products formed under different stress conditions, indicating good specificity of the method (Figure 6). Studies performed to determine the purity of the drug peak using the PDA detector showed a purity angle (PA) value of 0.101 and purity threshold (TH) value of 0.291. As the PA value was found to be less than TH, the method was found to be specific to the drug.

**Assay of pharmaceutical formulation**

The amount of aceclofenac in indigenously developed delayed release aceclofenac tablets was found to be 100.31 ± 1.58% (initially) and 97.24 ± 0.62% (three month 40ºC/75RH) with R.S.D. of 1.58% and 0.63%, respectively. The amount of aceclofenac in Hifenac tablets was found to be 99.50 ± 0.07% with R.S.D. of 0.63%. This showed that the method was simple and sensitive for the determination of aceclofenac in pharmaceutical formulations.

**Conclusion**

A simple and sensitive stability-indicating assay method for the determination of aceclofenac was established using ICH recommended forced degradation studies. The drug decomposed under hydrolytic stress (neutral, acidic, and alkaline) and also under exposure to light (in solution form). The compound was stable under oxidative stress, heat, and photolytic stress (in solid form). The major degradation product resembling to the drug was identified as diclofenac through a comparison with the drug peak using the PDA detector showing a purity angle (PA) value of 0.101 and purity threshold (TH) value of 0.291. As the PA value was found to be less than TH, the method was found to be specific to the drug.

**References**