Intrinsic Stability analysis and Assay Method for Determination of Fenofibrate in formulation by HPLC

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Abstract:

A stability-indicating LC assay method was developed for the quantitative determination of Fenofibrate (FENO) in pharmaceutical dosage forms. Chromatographic separation was achieved by use of Phenomenex ODS 5µ C18 column (250 X 4.6 mm), SPD – 10 UV detector and LC 10 ADVP Pumps, Rheodyne injector with 20µL capacity. The mobile phase was prepared by mixing Acetonitrile and Water (90:10v/v) and pH was adjusted to 5 with 0.1 N HCl. FENO degraded in acidic and alkaline conditions, while it was more stable in neutral, oxidative, thermal and photolytic conditions. The described method was linear over a range of 10-60 μg/ml for determination of FENO (r= 0.9999). The precision was demonstrated by relative standard deviation. %RSD of intra-day and inter-day studies was found to be 0.1565 and 1.01 respectively. The mean recovery was found to be 99.96%. The method was found to be suitable for analysis of in presence of its degradation products.
INTRODUCTION

Fenofibrate, 1-Methylethyl 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoate. It is official in B.P.-2009, U.S.P.-2005. Fenofibrate (Fig. 1) is a drug of the fibrate class, i.e. fibric acid derivative. Fenofibric acid is the active metabolite of Fenofibrate, produces reductions in total cholesterol.\(^1\)\(^2\) Hence used in the cardiac therapies. According to the literature survey it was found that few methods such as HPLC, NMR, UV are reported for determination of Fenofibrate . Pauline M. Lacroix et al.\(^3\), 1998 developed HPLC method for assay and impurity profiling by NMR; Alaa El-Gindy et al.\(^4\), 2005 proposed HPLC and derivative spectrophotometric methods used to investigate the kinetics of acidic and alkaline hydrolytic processes of fenofibrate and vinpocetine; Krishna R. Gupta et al.\(^5\), 2010 proposed quantitative estimation of fenofibrate using zero order and first order derivative spectroscopy. The UV and HPLC methods are also reported in combined dosage form for stability indicating assay and dissolution (A.A. Kadav & D.N. Vora, 2008; Shahla Jamzad and Reza Fassihi, 2006).\(^6\)\(^7\) Hence, it was thought to be worthwhile to perform intrinsic stability analysis and assay of FENO in formulation by HPLC.

METHODS

The marketed formulations and standard drug of Fenofibrate were obtained as a gift sample from Ranbaxy CV, Cipla (Mumbai) and Zydus. Double distilled Water was used. Methanol used was of HPLC grade and procured from Qualigen fine chemicals, Mumbai. Other chemicals were of either AR grade or GR grade and Obtained from Rankem, Summer Chemicals, Loba Chemie etc.

Instrumentation

Chromatographic separation was performed on Shimadzu binary gradient system consisted of Phenomenex ODS 5µ C\(_{18}\) column (250 X 4.6 mm), SPD – 10 UV detector and LC 10 ADVP Pumps, Rheodyne injector with 20μL capacity. The mobile phase was prepared by mixing Acetonitrile and Water (90:10 v/v) and pH was adjusted to 5 with 0.1 N HCl. The mobile phase was filtered through a 0.45 µ membrane filter and sonicated for 15min. Analysis was performed at ambient temperature. The detection was monitored at 287 nm.

Optimized chromatographic conditions

<table>
<thead>
<tr>
<th>Column</th>
<th>Phenomenex ODS 5 µ C18 column (250 X 4.6mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile Phase</td>
<td>Acetonitrile and Water, pH 5 (90:10 v/v)</td>
</tr>
<tr>
<td>Detection Wavelength</td>
<td>287 nm</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.5 mL/min</td>
</tr>
<tr>
<td>Temperature: Ambient</td>
<td>28-30 C</td>
</tr>
<tr>
<td>Injection volume</td>
<td>20 µL</td>
</tr>
</tbody>
</table>

HPLC METHOD DEVELOPMENT

I) Preparation of solutions

- Preparation of 0.1 N HCL solution
  
Conc. HCL 8.5 mL was diluted to 1000 mL with distilled water.

- Preparation of Mobile phase
  
  The mobile phase was prepared by mixing Acetonitrile and water, pH-5 adjusted with 0.1 N HCL in the ratio of 90:10 v/v.

i. Fenofibrate stock solution (F):

An accurately weighed quantity of Fenofibrate (FENO) ~25.0 mg was transferred to a 25.0 mL volumetric flask, and volume was made up to the mark with methanol (1000 μg/ mL).

ii. Working standard solution (F1):

A 1.0 mL portion of the above solution was further diluted up to 10.0 mL with methanol (100 μg/ mL).

STRESS DEGRADATION STUDIES

A) Solution State Stability

1. Preparation of Standard Solution

Accurately weighed quantity of FENO (~25 mg) was transferred to 100.0 mL dry round bottom flask. To it 25.0 mL of reagent (Acid, Alkali, 3% hydrogen peroxide, water) was added and refluxed for three hours. The sample was withdrawn at the end of three hours. The solution was quantitatively transferred to 50.0 mL volumetric flask, 5.0 mL DMA was added to it and volume was made up to the mark with methanol. A 2.0 mL portion of this solution was further diluted up to 25.0 mL with methanol. A 20 µL volume of the solution was injected and chromatographed separately.
2. **Preparation of Sample solution (marketed formulation - Stressed condition)**
Accurately weighed quantities of powder equivalent to 25 mg of FENO were transferred to a series of 6 different 100.0 mL dry round bottom flasks (RBF) and designated as flask a to f. To it 25.0 mL of reagent (Acid, Alkali, 3% hydrogen peroxide, water) was added. The samples were refluxed for specified time as indicated, a (30min), b (60 min), c (90 min), d (120 min), e (150 min) and f (180 min). The content of the flasks were quantitatively transferred to 6 different 50.0 mL volumetric flasks, 5.0 mL DMA was added to each flask and volumes were adjusted up to the mark with methanol. The content in each flask was sonicated for 20 min. All the solutions were filtered separately through whatmann filter paper (no.41). A 2.0 mL portion of each filtrate was separately diluted upto 25.0 mL with methanol. All the samples were chromatographed using 20 µL volume.

3. **Preparation of Sample solution for UV spectrophotometry**
The standard and sample marketed solutions were prepared following the general procedure. Each solution was scanned in the spectrum mode in the wavelength range 400-200 nm in 1.0 cm cell using methanol as blank.

4. **Solid State Stability**

**General procedure of preparation of Standard & Marketed Formulation**

1. **Preparation of Standard Solution (Unexposed)**
An accurately weighed quantity (~25.0 mg) of FENO was withdrawn and transferred to 50.0 mL volumetric flask. To it 5.0 mL of DMA was added, shaken and volume was made up to the mark with methanol. The content was sonicated for 20 min. and filtered through Whatmann filter paper (no.41). A 2.0 mL portion of filtrate was further diluted to 25.0 mL with methanol so as to get the final concentration as 40µg/mL. A 20 µL volume of this solution was injected and chromatographed.

2. **Preparation of Standard Solution (Exposed)**
An accurately weighed quantity exposed powder of FENO equivalent 25.0 mg was withdrawn and transferred to 50.0 mL volumetric flask. To it 5.0 mL of DMA was added, shaken and volume was made up to the mark with methanol. The content was sonicated for 20 min & filtered through whatmann filter paper (no.41). A 2.0 mL portion of filtrate was further diluted to 25.0 mL with methanol so as to get the final concentration as 40µg/mL. A 20 µL volume of this solution was injected and chromatographed.

3. **Preparation of Sample Solution (Stressed condition)**
An accurately weighed quantity powder of marketed formulation equivalent 25.0 mg of FENO was transferred to 50.0 mL volumetric flask. To it 5.0 mL of DMA was added, shaken and volume was made up to the mark with methanol. The content was sonicated for 20 min & filtered through whatmann filter paper (no.41). A 2.0 mL portion of filtrate was further diluted to 25.0 mL with methanol so as to get the final concentration as 40µg/mL (on label claim basis). A 20 µL volume of this solution was injected and chromatographed.

4. **Preparation of Sample solution for UV spectroscopy**
The standard and sample marketed solutions were prepared following the general procedure described above. Each solution was scanned in the spectrum mode from the wavelength range 400-200 nm in 1.0 cm cell using methanol as blank.

**APPLICATION OF PROPOSED METHOD FOR ASSAY OF MARKETED FORMULATION**

**Preparation of sample**
Twenty tablets were weighed and average weight was calculated. The tablet were triturated thoroughly and mixed. An accurately weighed quantity of tablet powder equivalent to 25.0 mg of FENO was transferred to 50.0 mL volumetric flask. 5.0 mL of DMA was added to the flask, shaken and volume was made up to the mark with methanol. The content was sonicated for 20 minutes and was filtered through whatmann filter paper (no.41). A 5.0 mL portion of the filtrate was diluted to 25.0 mL with methanol. A 2.0 mL portion of this solution was further diluted to 10.0 mL with methanol. Five such samples were prepared. The 20 µL volume of the final diluted solution were injected separately, the representative chromatograms was recorded.

**RESULTS AND DISCUSSION**

**Selection of Detection Wavelength**
The UV spectrum of working standard solution was recorded against methanol as a blank shown in Fig. 2.
HPLC method development and optimization

The optimized chromatographic condition mentioned below was kept constant throughout the experimentation and mobile phase was allowed to equilibrate with stationary phase which was indicated by a steady line.

- Column: Phenomenex ODS 5 μ C18 column (250 X 4.6mm)
- Detection Wavelength: 287 nm
- Flow rate: 1.5 mL/min
- Temperature: Ambient 28-30°C
- Injection volume: 20 μL

A 20 μL solution of above mix standard was injected through manual injector and chromatogram was recorded using mobile phase containing Acetonitrile and Water, pH 5 (90:10 v/v). A standard chromatogram for Fenofibrate so recorded in shown in fig 3.

3.3 Study of system suitability parameters

1. After equilibration of column with mobile phase, five replicate injections of 20 μL solution of (F1) was injected through the manual injector and the chromatograms were recorded and the system suitability parameter were noted and values are shown in Table 1.

2. 3.4 Study of Linearity

The graphs of concentration of drug vs. area under curve were plotted and the correlation coefficient was found to be (r=0.998) for the drug.

3.5 Intrinsic stability analysis by HPLC

3.5.1 Solution State Stability

a. Alkaline hydrolysis (0.1N NaOH)

The study of chromatograms for alkaline hydrolysis reveals that (fig. 4a-c) gradual degradation of the drug was observed after refluxing for three hours.

The study of chromatogram (Fig. 4b) FENO – 1 reveals that the degradation of drugs under alkaline hydrolysis were out of promotion, two additional peaks were formed [Deg1 (t_r - 1.89 min), and Deg2 (t_r - 3.17 min)] as they appear in chromatogram after refluxing for 30 min. The Deg 1 was gradually increased & the Deg 2 was gradually decreased up to 180 min of reflux.

Also from the study of chromatogram (Fig. 4c) FENO - 2 reveals that the degradation of drugs under alkaline hydrolysis were out of promotion as three additional peaks were generated [Deg1 (t_r - 1.56 min), Deg2 (t_r – 3.43 min) and Deg3 (t_r - 4.11 min)] which gradually increased except Deg 3 as they appear in chromatogram after refluxing for 180 min. The Deg 1, Deg 2 and Deg 3 may be due to degradation of FENO, as three additional peaks (Deg 1, 2 and 3) were observed in standard FENO when subjected to similar conditions.

The drug was found to be degraded to around 80-85% with appearance of three degraded products it is classified as overdone (highly susceptible) to the condition.

Overlain UV spectra of all the sample solutions were compared with the UV spectrum of standard solution (fig 4d & 4e) which showed change in the spectral pattern along with changed λ_max at 286.4 nm indicating that drugs have undergone of degradation.

b. Acidic hydrolysis (0.1N HCL):

The study of chromatograms for acidic hydrolysis reveals (fig. 5a-c); gradual degradation of the drug was observed after refluxing for three hours.

From the study of chromatogram (Fig. 5b) FENO - 1, one additional peak was formed [Deg1 (t_r - 2.67 min)] as it appear in chromatogram after refluxing for 60 min. The Deg 1 was gradually increased up to 180 min of reflux. The degradation of FENO-1 was found to be overdone under acidic hydrolysis.

Also from the study of chromatogram (Fig. 5c) FENO – 2 reveals that degradation of drugs were out of promotion as one additional peak [Deg1 (t_r - 2.27 min)] generated which gradually increased as it appeared in chromatogram after refluxing for 180 min. The Deg 1 may be formed due to degradation of FENO, as same additional peak (Deg 1) was observed in standard FENO when subjected to similar conditions.
The drug was found to be degraded to around 30-60% with appearance of one degraded products it is classified as overdone (highly susceptible) to the condition. Overlain UV spectra of all the sample solutions were compared with the UV spectrum of standard solution (fig 5d & 5e) which showed change in the spectral pattern with changed $\lambda_{\text{max}}$ at 286.4 nm indicating that drugs have undergone degradation.

**c. Neutral hydrolysis (distilled water)**

The study of chromatograms for neutral hydrolysis reveals (fig. 6a-c) that % of label claim of FENO was found to be decreased up to refluxing for three hours.

From the study of chromatogram (Fig. 6b) FENO – 1 was found to be susceptible to neutral hydrolysis as no additional peaks were formed up to 180 min of reflux. Also from the study of chromatogram (Fig. 6c) FENO - 2 generated one additional peak [Deg1 (t1 - 1.12 min)] which gradually increased as it appeared in chromatogram after refluxing for 180 min so it is adequately susceptible to neutral hydrolysis. The Deg 1 may be formed due to degradation of FENO, as same additional peak (Deg 1) was observed in standard FENO when subjected to similar conditions.

The drug was found to be degraded to around 5-8% with appearance of one degraded products it is classified adequately susceptible to the condition. Overlain UV spectra of sample solution recorded on was compared with the UV spectrum of mixed standard solution (fig 6d-e) which showed no change in the spectral pattern indicating that drugs have undergone no degradation but shows hypochromic effect at the $\lambda_{\text{max}}$.

**d. Oxidative hydrolysis (3 % H2O2)**

The study of chromatograms (fig. 7a-c) reveals that the drug when subjected to oxidative hydrolysis, % of label claim of FENO was found to be decreased on refluxing for three hours. No additional peak was observed in Standard and samples and the degradation was found to be around 15% it is categorised as falsely susceptible to the condition. Overlain UV spectra of sample solution recorded on was compared with the UV spectrum of mixed standard solution (fig 7d-e) which showed no change in the spectral pattern indicating that drugs have undergone false degradation but exhibits hypochromic effect at the $\lambda_{\text{max}}$.

### 3.5.2 Solid State Stability Studies

#### a) Humidity Study (40ºC/75% RH)

**i. Marketed formulation (In powder form)**

The humidity study was carried out on the standard drug and the marketed formulations by exposing it to 40ºC/75% RH for a period of 90 days.

The study of the chromatogram (Fig. 8b) reveals that no additional peak was observed on exposure to 40ºC/75% RH of FENO-1 powdered sample of FENO 1 for a period of 90 days, there was no significant change in the area under curve for the drug up to 21st day. The area under the curve was decreased on 28th day but no additional peaks were seen in chromatogram. The results indicated that, on 90th day FENO-1 was degraded up to 38% i.e. degradation was found to be overdone.

The study of the chromatogram (Fig. 8c) reveals that there was no additional peak observed on exposure to 40ºC/75% RH to powdered sample of FENO 2 for a period of 90 days, there was no significant change in the area under curve for the drug up to 45th day. The area under the curve was decreased on 60th day but no additional peaks were seen in chromatogram. The results indicated that, on 90th day FENO was degraded up to 28% i.e. degradation was found to be overdone.

Also when the standard drug was exposed to the similar condition, the chromatogram (fig. 8a) showed that there was no additional peak generation & no significant change in the area under curve.

Overlain UV spectra of sample solution recorded was compared with the UV spectrum of standard solution (fig 8 d-f) which showed no change in the spectral pattern indicating that drugs have undergone no degradation but exhibit hypochromic effect at the $\lambda_{\text{max}}$.

#### b) Photo stability studies

**i. UV Light**

When standard drug & samples were exposed to UV light at 254 nm for a period of 7 days, the study of chromatogram (Fig.9b) reveals that there was no additional peak & no significant change in the area under curve for FENO-1 up to 7th day i.e. FENO-1 was found to be soft susceptible to degradation by UV light.
While the study of the chromatograms (Fig. 9a-c) reveals that the area under the curve was decreased to large extent on 7th day with no additional peak generation seen in the chromatogram in case of Standard exposed & FENO-2 respectively i.e. FENO-2 was found to be overdone (highly susceptible) to UV light.

The results indicated that, on 7th day, Std expo, FENO-1 & FENO-2 were degraded up to 17.46, 9.48 % & 19.99 % respectively.

ii. Visible light

The study of the chromatogram (Fig. 10b) reveals that the area under the curve was decreased to large extent on 7th day with no additional peaks were observed in the chromatogram in case of FENO-1 i.e. sample was found to be false susceptible to degradation by visible light.

While the study of chromatograms (Fig. 10a-c) reveals that there was no additional peak & no significant change in the area under curve for Std expo & FENO-2 up to 7th day which reveals that FENO-2 was found to be soft susceptible to degradation by visible light.

The results indicated that, on 7th day standard exposed, FENO-1 & FENO-2 were degraded around 05-20 %.

C. Thermal studies (50°C)

When powdered sample was exposed to dry temperature at 50°C for a period of 15 days, there was no significant change in the area under curve for samples, no additional peaks were observed in the chromatogram (Fig. 11a-c). The results indicated that, on 15th day FENO was degraded up to the extent of 10% i.e. sample was found to be false susceptible to thermal degradation.

Summary of intrinsic stability studies are shown in Table 2.

APPLICATION OF PROPOSED METHOD FOR ASSAY OF MARKETED FORMULATION

The 20 µL volume of the final diluted solution were injected separately, the representative chromatograms was recorded and shown in fig. 12a. The UV spectrum was also recorded against methanol as a blank and was shown in fig. 12b.

Method Validation

The method was validated as per the guidelines in terms of parameters like, precision, accuracy (recovery studies), system suitability parameters, linearity and range etc.

i. Accuracy

Accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition method. The mean % recovery was found to be 99.96. The % RSD was found to be 0.647. Results of recovery study are summarized in Table 4.

ii. Precision

Precision of proposed method was ascertained by replicate analysis of homogeneous samples. Precision of any analytical method is expressed as SD and RSD of series of measurements. The mean percent label claim was found to be 99.384. The % RSD was found to be 0.628.

Results shown in Table 3.

iii. Ruggedness

Intermediate precision (Intraday and Interday) shows the % Label claim values within limits (% RSD not more than 2). The method was found to be précise. The ruggedness studies were carried out using different analyst variation. The results of intermediate precision parameter are shown in Table 5.

iv. Linearity and range

Accurately weighed quantities of tablet powder equivalent to 80, 90, 100, 110 and 120% of label claim (FENO) were taken and dilutions were made as described under marketed formulation. Then, each solution was injected and chromatograms were recorded.

The correlation coefficient was found to be 0.999 of FENO.

v. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The system suitability parameter was evaluated for each varied condition. The amount of FENO was calculated from sample solution in each varied condition. Results are shown in Table 6. The results of above study shown that the method were robust under varied conditions.
CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of Fenofibrate from pure and its dosage forms and was capable of giving idea about the intrinsic stability of the drug. The mobile phase is simple to prepare and economical. The sample recovery in a formulation was in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of Fenofibrate in pure form and its dosage forms.

Table 1: Observation of system suitability parameters

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Standard Weight Taken</th>
<th>A.U.C of FENO (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>~25.0 mg</td>
<td>453.339</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>453.736</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>453.576</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>452.987</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>452.767</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>453.281</td>
</tr>
<tr>
<td>±S.D.</td>
<td></td>
<td>0.402</td>
</tr>
<tr>
<td>%RSD</td>
<td></td>
<td>0.088</td>
</tr>
<tr>
<td>Theoretical plate/column</td>
<td></td>
<td>7797</td>
</tr>
<tr>
<td>Retention time</td>
<td></td>
<td>4.127</td>
</tr>
<tr>
<td>Asymmetry</td>
<td></td>
<td>1.259</td>
</tr>
<tr>
<td>Resolution</td>
<td></td>
<td>9.426</td>
</tr>
</tbody>
</table>

Table 2: Summary of intrinsic stability for FENO

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Stressed condition</th>
<th>% undegraded drug Exposed Std.</th>
<th>FENO-1</th>
<th>FENO-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaline reflux (180 min)</td>
<td>22.49</td>
<td>21.85</td>
<td>15.99</td>
</tr>
<tr>
<td>2</td>
<td>Acidic reflux (180 min)</td>
<td>92.42</td>
<td>71.90</td>
<td>40.66</td>
</tr>
<tr>
<td>3</td>
<td>Neutral reflux (180 min)</td>
<td>76.13</td>
<td>92.32</td>
<td>95.52</td>
</tr>
<tr>
<td>4</td>
<td>Oxidation reflux (180 min)</td>
<td>83.52</td>
<td>87.92</td>
<td>86.24</td>
</tr>
<tr>
<td>5</td>
<td>Humidity studies (90 days)</td>
<td>68.21</td>
<td>62.49</td>
<td>73.15</td>
</tr>
<tr>
<td>6</td>
<td>UV light (7 days)</td>
<td>82.54</td>
<td>91.52</td>
<td>80.01</td>
</tr>
<tr>
<td>7</td>
<td>Visible light (7 days)</td>
<td>95.38</td>
<td>81.41</td>
<td>91.49</td>
</tr>
<tr>
<td>8</td>
<td>Thermal studies (15 days)</td>
<td>94.83</td>
<td>93.10</td>
<td>91.94</td>
</tr>
</tbody>
</table>

Table 3: Results of estimation in marketed formulation

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>AUC Standard of (mV) AUC of Sample (mV)</th>
<th>Amt. Estimated in Avg. wt. of Tab(mg)</th>
<th>% Label claim</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>451.797</td>
<td>427.486</td>
<td>144.40</td>
</tr>
<tr>
<td>2</td>
<td>451.797</td>
<td>431.730</td>
<td>143.76</td>
</tr>
<tr>
<td>3</td>
<td>451.797</td>
<td>426.163</td>
<td>143.04</td>
</tr>
<tr>
<td>4</td>
<td>451.797</td>
<td>429.939</td>
<td>143.84</td>
</tr>
<tr>
<td>5</td>
<td>451.797</td>
<td>435.513</td>
<td>145.47</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>99.384</td>
</tr>
<tr>
<td>±SD</td>
<td></td>
<td></td>
<td>0.628</td>
</tr>
<tr>
<td>%RSD</td>
<td></td>
<td></td>
<td>0.628</td>
</tr>
</tbody>
</table>
Table 4: Results of Accuracy Study

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Amt. Contributed by sample (mg)</th>
<th>Amt. of drug (mg)</th>
<th>Total Amt. of Drug Estimated (mg)</th>
<th>Amount Recovered (mg)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24.89</td>
<td>4.8</td>
<td>29.65</td>
<td>4.76</td>
<td>99.16</td>
</tr>
<tr>
<td>2</td>
<td>24.81</td>
<td>10.6</td>
<td>35.47</td>
<td>10.66</td>
<td>100.57</td>
</tr>
<tr>
<td>3</td>
<td>24.89</td>
<td>15.9</td>
<td>40.78</td>
<td>15.89</td>
<td>99.93</td>
</tr>
<tr>
<td>4</td>
<td>24.75</td>
<td>21.7</td>
<td>46.59</td>
<td>21.84</td>
<td>100.64</td>
</tr>
<tr>
<td>5</td>
<td>24.83</td>
<td>26.1</td>
<td>50.81</td>
<td>25.98</td>
<td>99.52</td>
</tr>
</tbody>
</table>

Mean ± SD: 99.96 ± 0.647
% RSD: 0.647

Table 5: Results of Intermediate precision

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean % label claim ± S.D. of FENO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Different Analyst (n=3)</td>
<td>99.99 ± 0.85</td>
</tr>
<tr>
<td>Intraday Variation (n=4)</td>
<td>100.38 ± 0.15</td>
</tr>
<tr>
<td>Interday Variation (n=3)</td>
<td>98.71 ± 0.99</td>
</tr>
</tbody>
</table>

Table No. 6: Observation and result of Robustness study

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Deliberate Changes</th>
<th>R.T.</th>
<th>Asymmetry</th>
<th>% Label Claim</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard Condition</td>
<td>4.143</td>
<td>1.385</td>
<td>100.57</td>
</tr>
<tr>
<td>2</td>
<td>Change in flow rate (1.2 ml)</td>
<td>5.163</td>
<td>1.433</td>
<td>101.47</td>
</tr>
<tr>
<td>3</td>
<td>Change in flow rate (1.8ml)</td>
<td>3.460</td>
<td>1.409</td>
<td>101.55</td>
</tr>
<tr>
<td>4</td>
<td>Change in Wavelength (282nm)</td>
<td>4.147</td>
<td>1.346</td>
<td>98.77</td>
</tr>
<tr>
<td>5</td>
<td>Change in Wavelength (292nm)</td>
<td>4.140</td>
<td>1.346</td>
<td>99.12</td>
</tr>
<tr>
<td>6</td>
<td>Change in pH (4.8)</td>
<td>4.253</td>
<td>1.385</td>
<td>100.53</td>
</tr>
<tr>
<td>7</td>
<td>Change in pH (5.2)</td>
<td>4.237</td>
<td>1.385</td>
<td>98.86</td>
</tr>
<tr>
<td>SD</td>
<td>0.497</td>
<td>0.031</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Overall SD</td>
<td>0.576</td>
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</tr>
</tbody>
</table>

Fig 1: Structure of Finofibrate
Fig. 2: UV spectra of standard drug unexposed

Fig. 3: Chromatogram of standard

Fig. 4a: Chromatogram of standard drug under Alkali hydrolysis

Fig. 4b: Overlain chromatograms of sample under alkali hydrolysis (FENO-1)

Fig. 4c: Overlain chromatograms of sample under alkali hydrolysis (FENO-2)
Fig. 4d: Overlain UV spectra of sample under Alkali hydrolysis (Feno-1)

Fig. 4e: Overlain UV spectra of sample under Alkali hydrolysis (Feno-2)

Fig. 5a: Chromatogram of standard drug under Acid hydrolysis

Fig. 5b: Overlain chromatograms of sample under acid hydrolysis (Feno-1)
Fig. 5c: Overlaid chromatograms of sample under acid hydrolysis (Feno-2)

Fig. 5d: Overlaid UV spectra of sample under Acid hydrolysis (Feno-1)

Fig. 5e: Overlaid UV spectra of sample under Acid hydrolysis (Feno-2)

Fig. 6a: Chromatogram of standard drug under Neutral hydrolysis

Fig. 6b: Overlaid chromatograms of sample under Neutral hydrolysis (Feno-1)
Fig. 6.5c: Overlain chromatograms of sample under Neutral hydrolysis. (Feno-2)

Fig. 6d: Overlain UV spectra of sample under Neutral hydrolysis (Feno-1)

Fig. 6e: Overlain UV spectra of sample under Neutral hydrolysis (Feno-2)

Fig. 7a: Chromatogram of standard drug under Oxidative hydrolysis

Fig. 7b: Overlain chromatograms of sample under Oxidative hydrolysis (Feno-1)

Fig. 7c: Overlain chromatograms of sample under Oxidative hydrolysis (Feno-2)
Fig. 7d Overlain UV spectra of sample under Oxidative hydrolysis (Feno-1)

Fig. 7e Overlain UV spectra of sample under Oxidative hydrolysis (Feno-2)

Fig. 8a: The overlain chromatograms of standard under Humidity studies

Fig. 8b: The overlain chromatograms of sample under Humidity studies. (Feno-1)

Fig. 8c: The overlain chromatograms of sample under Humidity studies. (Feno-2)
Fig. 8d: The overlain UV spectra of standard under Humidity studies.

Fig. 8e: The overlain UV spectra of sample under Humidity studies. (Feno-1)

Fig. 8f: The overlain UV spectra of sample under Humidity studies. (Feno-2)

Fig. 8g: The overlain chromatograms of sample under Humidity studies. (Feno-1)

Fig. 8h: The overlain chromatograms of sample under Humidity studies. (Feno-2)
Fig. 8i: The overlain UV spectra of sample under Humidity studies. (Feno-1)

Fig. 8j: The overlain UV spectra of sample under Humidity studies. (Feno-2)

Fig. 9a: The overlain chromatograms of standard under UV light

Fig. 9b: The overlain chromatograms of sample under UV light. (Feno-1)

Fig. 9c: The overlain chromatograms of sample under UV light. (Feno-2)
Fig. 9d: The overlain UV spectra of standard under UV light

Fig. 9e: The overlain UV spectra of sample under UV light (FENO-1)

Fig. 9f: The overlain UV spectra of sample under UV light (FENO-2)
Fig. 10a: The overlain chromatogram of standard under Visible light

Fig. 10b: The overlain chromatogram of sample under Visible light (FENO-1)

Fig. 10c: The overlain chromatogram of sample under Visible light (FENO-2)
Fig. 10d: The overlain UV spectra of standard under visible light

Fig. 10e: The overlain UV spectra of sample under Visible light. (Feno-1)

Fig. 10f: The overlain UV spectra of sample under Visible light. (Feno-2)

Fig. 11a: The overlain chromatograms of standard under Thermal study (Exposed)
Fig. 11b: The overlain chromatograms of sample under Thermal study. (Feno-1)

Fig. 11c: The overlain chromatograms of sample under Thermal study. (Feno-2)

Fig. 11d: The overlain UV spectra of standard under Thermal study (Expo)
ABBREVIATIONS AND SYMBOLS

FENO : Fenofibrate
Feno-1: Stanlip Tablet
Feno-2: Trichek Capsule
DMA : Dimethylacetamide

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REFERENCES