Estimation of Gemfibrozil in Tablet Dosage Form by HPTLC Method

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ABSTRACT

A simple, precise, accurate and rapid high-performance thin-layer chromatographic method has been developed for the estimation of Gemfibrozil in tablet dosage forms. The stationary phase used was pre-coated silica gel 60 F254. The mobile phase used was a mixture of Toluene; Hexane; Ethyl Acetate; Glacial acetic acid \[6:2:2:0.1 \text{ v/v/v/v}\]. The detection of spots was carried out at 276 nm. The calibration curve was found to be linear between 1000ng/spot to 5000ng/spot for Gemfibrozil. The limit of detection and the limit of quantification for Gemfibrozil were found to be 176.40ng/spot and 534.54ng/spot. The method was found to be accurate with 99.71-100.09 % recovery and precise with %RSD 0.26-0.53 for intra-day \([n=3]\) and % RSD 0.20 – 0.44 for inter-day \([n=3]\) for Gemfibrozil. The proposed method can be successfully used to determine the drug content of marketed formulation. The developed method of HPTLC for Gemfibrozil was also validated by performing different validation parameters. The result demonstrated that the procedure is accurate, precise and reproducible, suitably applied for the determination of Gemfibrozil in different dosage forms.

Keywords: Gemfibrozil [GEM], U.V. Spectrophotometry, HPTLC, Validation, Tablet.

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INTRODUCTION

Gemfibrozil [GEM] is 5-[2, 5-dimethylphenoxy]-2, 2-dimethylpentanoic acid. The drug is used for the treatment of hyperlipidemia. Gemfibrozil is a lipid regulating agent which decreases serum triglycerides and very low density lipoprotein [VLDL] cholesterol, and increases high density lipoprotein [HDL] cholesterol. While modest decreases in total and low density lipoprotein [LDL] cholesterol may be observed with drug therapy, treatment of patients with elevated triglycerides due to Type IV hyperlipoproteinemia often results in a rise in LDL cholesterol. LDL-cholesterol levels in Type IIb patients with elevations of both serum LDL-cholesterol and triglycerides are, in general, minimally affected by drug treatment; however, Gemfibrozil usually raises HDL-cholesterol significantly in this group. Gemfibrozil increases levels of high density lipoprotein [HDL] subfractions HDL2 and HDL3, as well as apolipoproteins AI and AII. It also increases activity of Peroxisome proliferators-activated receptor-alpha [PPARα] ‘transcription factor ligand’, a receptor that is involved in metabolism of carbohydrates and fats, as well as adipose tissue differentiation. This increase in the synthesis of lipoprotein lipase thereby increases the clearance of triglycerides. A literature survey regarding quantitative analysis of these drug revealed that attempts were made to develop analytical methods for Gemfibrozil and its metabolite in plasma using Gas chromatography [6], RP-HPLC [7, 8], LC-MS [9-11] and also developed method with combination of Gemfibrozil and rosiglitazone in human plasma using spectrofluorimetric and RP-HPLC and also developed method for Gemfibrozil in pharmaceutical dosage form using spectrofluorimetric method. This Aim of the work was to develop an accurate, specific and reproducible HPTLC method for the determination of Gemfibrozil in dosage form. Also the proposed method is shown to be useful in determination of drug in tablet formulation in routine Analysis.

MATERIALS AND METHODS

**Instruments:**

- High Performance Thin Layer Chromatography [HPTLC]
- Camag Linomat V: Semi automatic application, band application by spray on technique [2-500 μL]
- Camag twin trough glass chamber [10×10 & 20×20]
- Camag TLC Scanner III: Scan speed up to 100 mm/s, spectral range 190-800 nm
- Camag TLC Reprostar III with digital camera for 254 nm, 366 nm and with light.
- Camag UV Cabinet with dual wavelength UV lamp: Dual wavelength 254/366nm
- Stationary phase: Silica Gel 60 G F$^{254}$ coated on aluminium sheet
- Hamilton 100μL HPTLC syringe
- Sonicator
  - Model: TEC-4
  - Roop Telesonic Ultrasonix
  - Compact Ultrasonic Cleaner
Analytical Balance

- Model: BP211D
- Make: Sartorious Gottingen AG, Germany
- Maximum: 210 gm.

Reagents and Chemicals:

Analytically pure Gemfibrozil was procured as gift samples from Cadila Pharmaceuticals Ltd [Dholka, Gujarat, India], Methanol [A. R. Grade], Hexane [A. R. Grade], Toluene A. R. Grade, Ethyl Acetate[A. R. Grade]: E-Merck [India] Ltd., Mumbai, were used for preparation of solutions. Tablet formulation [Lopid 600mg, Pfizer] was procured from the local market with the labeled amounts of 600mg Gemfibrozil.

Selection of chromatographic conditions:

Proper selection of the HPTLC method depends upon the nature of the sample [ionic or ionizable or neutral molecule], its molecular weight and solubility. To optimize the chromatographic conditions, the effect of chromatographic variables such as mobile phase composition and solvent ratio were studied. The resulting chromatograms were recorded and the chromatographic parameters such as retention factor and resolution were calculated. The conditions that gave the best resolution, symmetry and capacity factor were selected for estimation.

- Stationary phase: Pre-coated Silica Gel G60 F254 Aluminum sheet, 10×10 cm [E.Merck, Germany], thickness layer 0.2 mm. Plate was prewashed using methanol and allowed to dry in oven at 50°C for 15 min. and allow to come to room temperature and used immediately.

- Mobile phase: Toluene; Hexane; Ethyl Acetate; Glacial acetic acid [6:2:2:0.1 v/v/v/v].

- Optimized condition:
  - Chamber saturation time: 20mins.
  - Distance run: 70 mm.
  - Temperature: 27°C
  - Wavelength: 276 nm.
  - Slit dimension: 6 mm
  - Scanning speed: 20 mm/s.
  - Spotting parameter:
    - Band width: 6 mm
    - Space Between bands: 11.6 mm
    - Syringe capacity: 100μL.
• Procedure:

  ➢ Preparation of mobile phase:

    The mixture of 6 ml of Toluene, 2ml of Ethyl acetate and 2 ml Hexane and 0.1ml of Glacial Acetic Acid previously filtered through 0.45 μm filter paper used as mobile phase.

  ➢ Preparation of standard stock solution [2000 μg/ml]:

    ➢ Gemfibrozil [GEM] standard stock solution [500 μg/ml]:

      GEM [50mg] standard was accurately weighed and transferred to a 100 ml volumetric flask and dissolved in Methanol [50ml]. The flask was shaken and volume was made up to the mark with Methanol to give a solution containing 500 μg/ml GEM.

  ➢ Calibration curve for ROS: [1000 to 5000ng/spot]:

    Stock solution was filled in the syringe and under nitrogen stream by a semiautomatic sample applicator; it was apply in form of band of drug on a plate having concentration of 1000 to 5000ng/spot of GEM. Plate was developed using Toluene; Hexane; Ethyl Acetate; Glacial acetic acid [6:2:0.1 v/v/v/v] at 25±1°C and dried in air. Developed plate was allowed to dry and subjected to densitometric measurement in absorbance mode at wavelength 276 nm using Camag TLC scanner III. Spectra of the compounds were recorded in the range of 1000 –5000 nm and peak purity of the chromatographic peak was checked by scanning individual peak at 3 different positions [peak start, peak apex, peak end]. The graph of peak area v/s concentration for the drugs was plotted.

  ➢ Estimation of GEM in Tablet dosage form:

    Twenty tablets were weighed accurately, the average weight was found and finally powdered .A quantity equivalent to 25 mg of GEM was transferred to a 50 ml volumetric flask containing Methanol [5ml]. The flask was ultra-sonicated for a 10mins to dissolve the drug. The volume was adjusted to the mark with Methanol to give a solution containing 500μg/ml. Allow to stand for five minutes. The aliquot was filtered through whatman filter paper [No. 42]. 8μL of the prepared sample solution was applied on pre washed TLC plate, developed, dried in air and photometrical analyzed as described above. From the peak area obtained in the chromatogram, the amount of the drug was calculated.

Validation of the Developed Method:

The method was validated for Accuracy and Repeatability by the following procedures [13]:

July – September 2011 RJPBCS Volume 2 Issue 3 Page No. 892
1. **Accuracy:**

   Accuracy is the closeness of the test results obtained by the method to the true value. The accuracy of the method was determined by calculating recovery study of GEM by the method of standard addition of known amounts of GEM [0, 1000, 2000, 3000ng/ spot] was added to a pre-quantified sample solution. The recovery was verified by estimation of drugs in triplicate preparations at each specified concentration level.

2. **Repeatability:**

   Standard solutions of ROS [1000, 2000, 3000, 4000 and 5000ng/ spot] were prepared and chromatograms were recorded. Area was measured of the same concentration solution was measured six times and RSD was calculated.

3. **Precision:**

   The Precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It provides an indication of random error results and was expressed as coefficient of variation [CV].

4. **Intra and Inter day Precision:**

   Variations of results within the same day [inter-day], variation of results between days [inter-day] were analyzed. Intraday precision was determined by analyzing GEM for three times in the same day. Inter-day precision was determined by analyzing the drug daily for three days.

5. **Linearity and Range:**

   The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity.

6. **Specificity and selectivity:**

   Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix. While selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix.
Commonly used excipients in the tablet [Lopid] preparation were spiked in a pre weighted quantity of drug and then absorbance was measured and calculation done to determine the quality of the drug.

7. Robustness:

The solution were prepared and then analyzed with change in the analytical conditions like different laboratory, different analyst, and different instrument.

RESULT & DISCUSSION

The mobile phase containing, Toluene; ethyl acetate; hexane; glacial acetic acid [6:2:2:0.1v/v/v/v] was found to be satisfactory and gave well-resolved peaks for GEM [Fig-1]. The Rf value for GEM was 0.61. The UV scanning spectra of GEM revealed that at 276nm posse’s significant absorbance [Fig-1].The calibration curve for GEM [Fig-3] was obtained by plotting the peak area of GEM versus the concentration of GEM over the range of 1000- 5000ng/spot, and it was found to linear with R²=0.9991. The regression analysis of the calibration curves is shown in [Table-1]. The limit of detection for ROS was 176.40ng /spot.  

![Chromatogram of standard solution containing 2000ng/spot of GEM using mobile phase as Toluene; ethyl acetate; hexane; glacial acetic acid (6:2:2:0.1v/v/v/v).](image)

The linear range, correlation coefficient, detection limit and standard deviation for GEM by HPTLC method shown in [Table-2]. Accuracy was determined by calculating recovery. The method was found to be accurate with recovery 99.71-100.09% for GEM [Table-7]. The label claim Percentage of GEM 100.50 Which was satisfactory [Table-3]. The method was found to be precise with CV 0.26 – 0.53 for intraday [n=3] and CV 0.20 – 0.44 for inter day [n=3] [Table-4]. The method was found to be reproducible and specific as no interference observed when drug
was estimated in presence of excipients. The method was also rugged as there was no change in area up to 48 hours of preparation of solution in Methanol [Table-5].

![Photograph of developed HPTLC plate of GEM Tablet.](image)

**Fig. 2:** Photograph of developed HPTLC plate of GEM Tablet.

![Calibration curve for GEM.](image)

**Fig. 3:** Calibration curve for GEM.

The validation parameters are summarized in [Table-6]. The proposed HPTLC method was applied to the detection of GEM and its dosage forms [Table]. The results obtained for GEM was comparable with the corresponding label claim percentage [Table-3].

<table>
<thead>
<tr>
<th>Concentration ng/spot</th>
<th>Area Mean ± S.D (n=5)</th>
<th>Coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>1221.96 ± 6.23</td>
<td>0.51</td>
</tr>
<tr>
<td>2000</td>
<td>2416.12 ± 7.17</td>
<td>0.30</td>
</tr>
<tr>
<td>3000</td>
<td>3432.6 ± 10.94</td>
<td>0.32</td>
</tr>
<tr>
<td>4000</td>
<td>4709.28 ± 13.65</td>
<td>0.29</td>
</tr>
<tr>
<td>5000</td>
<td>5813.48 ± 24.75</td>
<td>0.43</td>
</tr>
</tbody>
</table>

**Table 1: calibration readings for GEM by HPTLC method**
### Table 2: Statistical data for GEM by HPTLC method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear Range (ng/spot)</td>
<td>1000-5000</td>
</tr>
<tr>
<td>Slope</td>
<td>1.14</td>
</tr>
<tr>
<td>Intercept</td>
<td>75.82</td>
</tr>
<tr>
<td>Standard Deviation of Slope</td>
<td>0.16</td>
</tr>
<tr>
<td>Standard Deviation of Intercept</td>
<td>0.5</td>
</tr>
<tr>
<td>Limit of Detection (ng/spot)</td>
<td>176.40</td>
</tr>
<tr>
<td>Limit of Quantification (ng/spot)</td>
<td>534.54</td>
</tr>
</tbody>
</table>

### Table 3: Results of HPTLC Assay

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Actual concentration (ng/spot)</th>
<th>% GEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lopid (Tablet)</td>
<td>2000</td>
<td>100.09</td>
</tr>
</tbody>
</table>

*n=5 determinations*

### Table 4: Precision data for GEM by HPTLC method

<table>
<thead>
<tr>
<th>Concentration ng/spot</th>
<th>Intra-day (n=3)</th>
<th>CV</th>
<th>Inter-day (n=3)</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>1224.8 ± 6.55</td>
<td>0.53</td>
<td>1219.73 ± 5.36</td>
<td>0.44</td>
</tr>
<tr>
<td>2000</td>
<td>2412.36 ± 6.38</td>
<td>0.26</td>
<td>2418.23 ± 7.28</td>
<td>0.3</td>
</tr>
<tr>
<td>3000</td>
<td>3431.5 ± 14.24</td>
<td>0.41</td>
<td>3435.86 ± 7.11</td>
<td>0.2</td>
</tr>
</tbody>
</table>

### Table 5: Solvent suitability study for GEM by HPTLC method

<table>
<thead>
<tr>
<th>Time</th>
<th>Area GEM (2000ng/spot)</th>
<th>Result % GEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr.</td>
<td>2418.2</td>
<td>100.08</td>
</tr>
<tr>
<td>4.0 hr</td>
<td>2428.4</td>
<td>100.5</td>
</tr>
<tr>
<td>8.0 hr</td>
<td>2414.6</td>
<td>99.93</td>
</tr>
<tr>
<td>24.0 hr</td>
<td>2412.4</td>
<td>99.84</td>
</tr>
<tr>
<td>48.0 hr</td>
<td>2416.4</td>
<td>100.01</td>
</tr>
</tbody>
</table>
Table 6: Summary of Validation Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>GEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery (%)</td>
<td>99.71 – 100.09</td>
</tr>
<tr>
<td>Repeatability (RSD, n=5)</td>
<td>0.51</td>
</tr>
<tr>
<td>Precision (CV)</td>
<td></td>
</tr>
<tr>
<td>Intra-day (n=3)</td>
<td>0.26 – 0.53</td>
</tr>
<tr>
<td>Inter day (n=3)</td>
<td>0.20 – 0.44</td>
</tr>
<tr>
<td>Specificity</td>
<td>Specific</td>
</tr>
<tr>
<td>Solvent suitability</td>
<td>Suitable for 48 Hrs</td>
</tr>
</tbody>
</table>

Table 7: Determination of Accuracy

<table>
<thead>
<tr>
<th>Amt. of sample GEM ng/spot</th>
<th>Amt. of drug added GEM ng/spot</th>
<th>Amt. recovered GEM ng/spot</th>
<th>% Recovery GEM %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>0</td>
<td>1998.40</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>1000</td>
<td>3002.80</td>
<td>100.09</td>
</tr>
<tr>
<td>2000</td>
<td>2000</td>
<td>3994.26</td>
<td>99.85</td>
</tr>
<tr>
<td>2000</td>
<td>3000</td>
<td>4985.50</td>
<td>99.71</td>
</tr>
</tbody>
</table>

CONCLUSION

The method that was developed for the determination of GEM is based on different analytical techniques; proposed method was validated and found to be simple, sensitive, accurate, and precise. Statistical comparison of the assay results obtained for GEM in tablet formulations by using this method indicated no significant difference. Hence, the method can be used successfully for routine analysis of tablet dosage forms of GEM.

ACKNOWLEDGMENT

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REFERENCES