SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF METFORMIN HCl AND FENOFIBRATE IN THEIR SYNTHETIC MIXTURE

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ABSTRACT
Two simple spectrophotometric methods have been developed for simultaneous estimation of Metformin HCl and Fenofibrate from their synthetic mixture. Method-I involved simultaneous equation method and Method-II is the Q-absorbance method. For simultaneous equation method, the absorbances of the standard solutions were taken at two wavelengths 237 nm (λ-max of Metformin HCl) and 288 nm (λ-max of Fenofibrate). For Q-absorbance method, the absorbances of the standard solutions were taken at two wavelengths 237 nm (λ-max of Metformin HCl) and 249 nm (Isoabsorptive point), in methanol. Linearity range was found to be 3-20 μg/ml for Metformin HCl and Fenofibrate in both methods based on the ratio of the two drugs in combined dosage form. The accuracy and precision of the methods were determined and validated statistically. Both methods showed good reproducibility and recovery with RSD less than 2. Proposed methods were found to be rapid, specific, precise and accurate and can be successfully applied for the routine analysis of Metformin HCl and Fenofibrate in pharmaceutical dosage form.

Keywords: Metformin HCl, Fenofibrate, Simultaneous equation method, Q-absorbance method

INTRODUCTION
Metformin HCl (MET) chemically, Hydrochloride salt of N,N-dimethylimidodicarbonimidic diamide4. It is anti-diabetic drug, primarily acts by decreasing intestinal absorption of glucose reducing hepatic glucose production and increasing sensitivity thus enhancing peripheral uptake utilization of glucose5. Fenofibrate (FENO) chemically, 2-[4-(4-chlorobenzoyl)phenox]-2-methyl-propanoic acid, 1-methylethyl ester6. It is lipid lowering agent which lowers plasma triglyceride be enhancing lipoprotein lipase synthesis thus increasing very low density lipoprotein (VLDL) catabolish with consequent increase in high density lipoprotein7. The fixed dose combination increased patient convenience and improved compliance for patients already stabilized on two medications, FENO and MET which can control both the blood lipids and blood glucose of patients. MET is official in IP8, BP9, and USP10 while FENO is official in BP9. A deep Literature survey shows that combination of these two drugs is not official in any pharmacopoeia and no official or reported method is available for simultaneous estimation of MET and FENO in synthetic mixture. Various reported methods are available for estimation of MET alone such as UV3, HPLC11-13, LC/MS14-16, HPTLC17, Capillary Electrophoresis18, Voltametry19 and in combination with other drugs like UV20-24, HPLC20-35, HPTLC36, LC/MS37-39, Capillary Zone Electrophoresis40 while for estimation of FENO alone such as UV41, HPLC42-46, UPLC47, LC/MS/MS48-49 and in combination with other drugs like HPTLC50. In the present investigation an attempt has been made to develop simple, rapid, economic and accurate spectrophotometric method for simultaneous estimation of MET and FENO from their synthetic mixture.

MATERIALS AND METHODS
Instrumentation and Apparatus
A shimadzu model 1600 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 nm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software (UV Probe version 2.10). A Sartorius CP224S analytical balance (Gottingen, Germany), an ultrasonic bath (Frontline FS 4, Mumbai, India) was used in the study.

Reagents and Chemicals
Kindly gifted reference standards of MET (Torrent Pharmaceutical Ltd, Gujarat, India) and FENO (INTAS Pharmaceutical Ltd, Gujarat, India), Laboratory prepared synthetic mixture of MET and FENO, Methanol (AR Grade, S. D. Fine Chemicals Ltd., Mumbai, India) and Whatman filter paper no. 41 (Millipore, USA) were used in the study.

Preparation of solutions and mixture
Preparation of standard stock solutions: accurately weighed (10 mg) of a standard drugs were transferred in two different 100 mL volumetric flasks, dissolved and diluted in methanol to get 100 μg/mL standard stock solutions each of MET and FENO.

Preparation of Synthetic mixture of FENO & MET:
Placebo powder mixture was prepared by mixing 2 gm magnesium stearate, 2 gm starch, 10 gm talc and 6 gm lactose. Synthetic mixture was prepared by mixing 1 gm placebo powder mixture with 160 mg FENO and 500 mg MET.

Wavelength selection
The working standard solutions of MET and FENO, each of 10 μg/mL were scanned separately in the UV range of 200-400 nm. Spectrum data were recorded at an interval of 1 nm. From the absorption overlain spectra of the both drugs, different wavelengths i.e. λ1 = 237 nm (λmax of MET) and λ2 = 288 nm (λmax of FENO) for Method-I and λ1 = 237 nm (λmax of Met) and λ2 =249 nm (Isoabsorptive point) for Method-II were selected.

Preparation of calibration curve
From the standard stock solutions, 0.3, 0.5, 1.0, 1.5 and 2.0 mL aliquots of both drugs were transferred in two different series of 10 mL volumetric flasks and volumes were made up to mark with methanol to get working standards having concentration in the range of 3–20 μg/mL for both drugs.
Absorbances of each working standard solution of both drugs were measured at \( \lambda_1, \lambda_2 \) and \( \lambda_3 \). In Method-I, three Calibration curves of absorbance Vs concentration were constructed, of which one for MET at \( \lambda_1 \), while two for FENO at \( \lambda_4 \) and \( \lambda_5 \). At \( \lambda_2 \), MET have zero absorbance for any concentration.

In Method-II, three Calibration curves of absorbance Vs concentration were constructed of which two at \( \lambda_1 \) for MET and FENO, while one at \( \lambda_3 \) (i.e. isosbestic point of both drug). From respective calibration curve of both drugs absorptivity values were calculated and used in further calculation of the concentration of both drugs in the synthetic mixture.

### Table 1: Regression Analysis Data and Summary of Validation Parameter for the proposed Method-I & II.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method-I</th>
<th>Method-II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MET at 237 nm</td>
<td>FENO at 237 nm</td>
</tr>
<tr>
<td>Molar absorptivity (1 mole ( \cdot ) cm (^{-1} ) )</td>
<td>2.984</td>
<td>3.254</td>
</tr>
<tr>
<td>Regression equation ((y = mx + c))</td>
<td>0.0985</td>
<td>0.0237</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>0.0063</td>
<td>0.0239</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>0.9973</td>
<td>0.9977</td>
</tr>
<tr>
<td>Correlation coefficient ((r^2))</td>
<td>0.58</td>
<td>0.79</td>
</tr>
<tr>
<td>Robustness (CV, %)</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>LOD (µg/mL)</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>LOD (µg/mL)</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Repeatability (n=5) (CV, %)</td>
<td>0.59</td>
<td>1.43</td>
</tr>
</tbody>
</table>

### Table 2: Results of recovery study by proposed methods

<table>
<thead>
<tr>
<th>Drug</th>
<th>Level</th>
<th>Method I</th>
<th>Method II</th>
<th>Mean % Recovery ± S.D (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MET</td>
<td>I</td>
<td>500</td>
<td>506</td>
<td>99 ± 1.55 101.20±1.25</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>987</td>
<td>1026</td>
<td>98.7 ± 1.86 102.6±1.38</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>1492</td>
<td>1487</td>
<td>99.40 ± 1.32 99.13±1.55</td>
</tr>
<tr>
<td>FENO</td>
<td>I</td>
<td>80</td>
<td>78</td>
<td>97.50 ± 1.45 102±1.12</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>156</td>
<td>162</td>
<td>97.30 ± 1.18 101.2±1.24</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>240</td>
<td>236</td>
<td>101.6 ± 0.85 98.3±0.98</td>
</tr>
</tbody>
</table>

### Table 3: Intra-Day Precision data for analysis of MET and FENO by Proposed method \(n=5\)

<table>
<thead>
<tr>
<th>Conc. (µg/mL)</th>
<th>MET at 237 nm</th>
<th>FENO at 237 nm</th>
<th>FENO at 288 nm</th>
<th>MET &amp; FENO at 249 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.272 ± 0.005</td>
<td>0.022 ± 0.003</td>
<td>0.104 ± 0.002</td>
<td>0.38 ± 0.045</td>
</tr>
<tr>
<td>5</td>
<td>0.068 ± 0.006</td>
<td>0.074 ± 0.004</td>
<td>0.192 ± 0.006</td>
<td>0.68 ± 0.015</td>
</tr>
<tr>
<td>10</td>
<td>0.915 ± 0.004</td>
<td>0.148 ± 0.002</td>
<td>0.386 ± 0.003</td>
<td>1.15 ± 0.039</td>
</tr>
<tr>
<td>15</td>
<td>1.412 ± 0.004</td>
<td>0.376 ± 0.005</td>
<td>0.590 ± 0.005</td>
<td>1.49 ± 0.037</td>
</tr>
<tr>
<td>20</td>
<td>1.752 ± 0.002</td>
<td>0.512 ± 0.003</td>
<td>0.808 ± 0.004</td>
<td>1.58 ± 0.041</td>
</tr>
</tbody>
</table>

### Table 4: Inter-Day Precision data for analysis of MET and FENO by Proposed method \(n=5\)

<table>
<thead>
<tr>
<th>Conc. (µg/mL)</th>
<th>MET at 237 nm</th>
<th>FENO at 237 nm</th>
<th>FENO at 288 nm</th>
<th>MET &amp; FENO at 249 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.267 ± 0.003</td>
<td>0.020 ± 0.004</td>
<td>0.101 ± 0.005</td>
<td>0.58 ± 0.052</td>
</tr>
<tr>
<td>5</td>
<td>0.458 ± 0.005</td>
<td>0.072 ± 0.002</td>
<td>0.197 ± 0.005</td>
<td>0.77 ± 0.128</td>
</tr>
<tr>
<td>10</td>
<td>0.911 ± 0.006</td>
<td>0.146 ± 0.004</td>
<td>0.381 ± 0.002</td>
<td>1.45 ± 0.237</td>
</tr>
<tr>
<td>15</td>
<td>1.409 ± 0.005</td>
<td>0.376 ± 0.005</td>
<td>0.594 ± 0.006</td>
<td>1.29 ± 0.376</td>
</tr>
<tr>
<td>20</td>
<td>1.754 ± 0.003</td>
<td>0.517 ± 0.005</td>
<td>0.805 ± 0.003</td>
<td>1.63 ± 0.517</td>
</tr>
</tbody>
</table>
**RESULTS AND DISCUSSION**

**Method Development**

From the absorption overlay spectra of standard MET and FENO, the selected wavelengths are shown in Figure 1. The overlay spectra pattern of both drug suggests that at \( \lambda_{\text{MET}} \) have zero absorbance for any concentration there is sufficient distance between \( \lambda_{\text{MET}} \) of both drugs, the criteria run obtaining maximum precision by simultaneous equation method were calculated and found to be outside the range 0.1-2.0. The Isoabsorptive point was found to be 249 nm which was used in Q-Absorbance ratio method.

Calibration curves for MET at \( \lambda_1 \) while for FENO at \( \lambda_2 \) for Method-I, for Method-II at \( \lambda_1 \) and \( \lambda_2 \) for both drugs were constructed and Beer’s law range was found to be 3-20 \( \mu \)g/ml at all selected wavelengths for both Methods.

From the respective calibration curves and regression equation, the calculated absorptivity value were found to be \( a_x = 950, a_y = 240, a_z = 508 \) for Method-I and \( a_{x1} = 890, a_y = 270, a_z = 185 \) for Method –II.

The calculated absorptivity values at particular wavelength were substituted in the equations for Method-I and II and concentration of both drugs from synthetic mixture was found out.

**For Method-I:**

\[
C_X = \frac{(A_1 \ a_y - A_2 \ a_y)}{(a_x a_y - a_x a_y)} \quad (1)
\]

\[
C_Y = \frac{(a_x A_2 - a_x A_1)}{(a_x a_y - a_x a_y)} \quad (2)
\]

Where,

\( C_X \) and \( C_Y \) = the concentration of MET and FENO, respectively.

\( A_1 = \) absorbance of mixture at \( \lambda_1 \)

\( A_2 = \) absorbance of mixture at \( \lambda_2 \)

\( a_x = \) absorptivity of MET at \( \lambda_1 \)

\( a_y = \) absorptivity of FENO at \( \lambda_2 \)

\( a_z = \) absorptivity of the mixture at \( \lambda_2 \)

**For Method-II:**

\[
C_X = \frac{(Q_a - Q_d)}{A} \times (\frac{Q_x - Q_y}{x \ a_x}) \quad .... (3)
\]

\[
C_Y = (\frac{A}{a_x}) - C_X \quad (4)
\]

Where,

\( C_X \) and \( C_Y \) = the concentration of MET and FENO, respectively.

\( A = \) the absorbance of mixture at Isoabsorptive point (\( \lambda_3 \))

\( Q_x = \) (absorptivity at Isoabsorptive point, \( \lambda_3 \))/(absorptivity of MET at \( \lambda_2 \))

\( Q_y = \) (absorptivity at Isoabsorptive point, \( \lambda_3 \))/(absorptivity of FENO at \( \lambda_2 \))

\( Q_m = \) (absorptivity of the FENO at \( \lambda_2 \))/(absorptivity of the MET at \( \lambda_1 \))

**Validation of Method**

**Linearity:**

Both methods were found to be linear over the range of 3-20 \( \mu \)g/mL for both drugs at all selected wavelengths with the values of correlation coefficient (\( r^2 \)) > 99. The regression analysis data and optical parameters of both methods were shown in table 1.

**Accuracy:**

To check the accuracy of proposed methods, multilevel recovery study by standard addition method in placebo powder mixture was carried out. After mixing spiked standard drug at each level were ultrasonicated for 30 min and extracted in methanol. The solutions were filtered through Whatman filter paper No. 41. Suitable aliquots of filter at each level were diluted to get final solutions at each level. Absorbances of final solutions were measured at selected wavelengths for Method I & II and concentrations of both drugs were calculated by using respective regression equations. The results of multilevel recovery study for both methods were shown in Table 2. From the results it can be concluded that both developed methods were accurate.

**Method Precision (Repeatability):**

Repeatability of measurement of absorbance at selected wavelengths for both drugs using both method were evaluated using 5 replicates of the same concentration (10 \( \mu \)g/mL of MET and FENO). The calculated CV, % value for method precise was found to be within limit as shown in Table 1, suggested that both methods were repeatable.
Intermediate Precision: The intra-day and inter-day variation of both methods were evaluated at 5 different concentration levels (3, 5, 10, 15 and 20 μg/mL) for both drugs. The percentage Co-efficient of Variance (CV, %) values of within-day and day-to-day were calculated. The results of intra and inter-day variation are depicted in Table 3 and 4, respectively. The CV, % value for day to day and within day was found to be within limit suggested that both methods were sufficiently precise over the calibration range.

Limit of detection (LOD) and Limit of quantitation (LOQ): LOD was checked by visual method as per ICH guidelines. The values of LOD and LOQ were found to be 0.5 and 1.0 μg/mL (Table 1), for both drugs in both developed methods reveals that proposed method can be applied at low concentration level with sufficient sensitivity.

Robustness: The study was carried out by change in selected wavelengths for 0 ± 0.1, 0.2, 0.3, 0.4 and 0.5 nm and the CV, % values were calculated, which were found to be less than 2 %. The results suggested that method was robust under experimental conditions.

CONCLUSION
Both developed methods have linear response in the stated range for both drugs and are accurate, precise simple and rapid. The developed methods can be readily carried out at laboratory level and small scale industries using inexpensive instrument i.e. UV/Visible Spectrophotometer. In Future, both methods can be routinely used as qualitative test for MET and FENO from their combined dosage forms.

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