Chapter – VI

Visible spectrophotometric and HPLC methods for the estimation of Lomitapide
Chapter VI

Lomitapide (LMP) marketed as Juxtapid and Lojuxta is a drug for the treatment of familial hypercholesterolemia, developed by Aegerion Pharmaceuticals.1-6

Juxtapid (Lomitapide) is an oral inhibitor of the microsomal triglyceride transport protein (MTP). The inhibition of MTP blocks the hepatic secretion of very low density lipoproteins and the intestinal secretion of chylomicrons.

Lomitapide is specifically indicated as an adjunct to a low-fat diet and other lipid-lowering treatments, including LDL apheresis where available, to reduce low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), apolipoprotein B (apo B), and non-high density lipoprotein cholesterol (non-HDL-C) in patients with homozygous familial hypercholesterolemia7-12.

The FDA approval of Juxtapid was based on a multinational, single-arm, open-label, 78-week trial in 29 adults with HoFH. After a six-week run-in period to stabilize lipid-lowering treatments, Juxtapid was initiated at 5 mg daily and titrated to daily doses of 10 mg, 20 mg, 40 mg, and 60 mg at weeks 2, 6, 10, and 14, respectively, based on tolerability and acceptable levels of transaminases. Subjects were instructed to maintain a low-fat diet (<20% calories from fat) and to take dietary supplements. After efficacy was assessed at Week 26, subjects remained on Juxtapid for an additional 52 weeks to assess long-term safety. During this safety phase, the dose of Juxtapid was not increased above each patient’s maximum tolerated dose established during the efficacy phase. The primary efficacy endpoint was percent change in LDL-C from baseline to Week 26. Twenty-three (79%) patients completed the efficacy endpoint at Week 26, all of whom went on to complete 78 weeks of treatment. The primary efficacy endpoint was percent change in LDL-C from baseline to Week 26. At Week 26, the mean and median percent changes in LDL-C from baseline were -40% (paired t-test p<0.001) and -50%, respectively, based on the intent-to-treat population13-26.
In conclusion, several new therapeutical options for the management of dyslipidemia are available. While the use is so far restricted to high risk patients with genetic hypercholesterolemia, data from the ongoing clinical studies will help to clarify whether a wider number of patients with dyslipidemia could be benefited from these therapies.

The literature reported only a few spectrophotometric methods, extractive spectrophotometry\textsuperscript{27-28}, and HPLC\textsuperscript{29-35} techniques for its estimation. However, the important functional groups of LMP are not fully exploited for designing suitable spectrophotometric methods for its determination\textsuperscript{36-55}.

Reactions of LMP with FeCl\textsubscript{3} and then with various reagents such as 1, 10-PTL, 2, 2'BPL, K\textsubscript{3}Fe (CN)\textsubscript{6}, BTB, MO, and SBT produce colored species. This reaction is explained and designed as suitable methods for the spectrophotometric determination of LMP in its bulk and pharmaceutical formulations.

Simplicity, cost effectiveness, sensitivity, selectivity, fair accuracy and precision has prompted the author to develop this spectroscopic and chromatographic methods for LMP determination.

\textbf{Table-6.1}

\textbf{Structural features of active functional groups of the Lomitapide}

<table>
<thead>
<tr>
<th>Official Name</th>
<th>Chemical Name</th>
<th>Structure</th>
<th>Functional groups present</th>
</tr>
</thead>
</table>
## Table-6.2

**Therapeutic importance and certain characteristics of the Lomitapide**

<table>
<thead>
<tr>
<th>Pharmocodynamic/Therapeutic Category</th>
<th>Characteristics</th>
<th>Therapeutic importance</th>
</tr>
</thead>
</table>
| Hypolipidermic or Lipid lowering drug belonging to the class of compounds called statins. | **Empirical formula:** C_{39}H_{37}F_{6}N_{3}O_{2}  
**Molecular weight:** 693.719 g/mol  
**Solubility:** It is insoluble in water and Soluble in methanol, ethanol and chloroform.  
**Appearance:** White hygroscopic crystalline powder  
**Half life:** 19 hours | Juxtapid (lomitapide) is an oral inhibitor of the microsomal triglyceride transport protein (MTP). |
### Table-6.3

Particulars of commercially available formulations of selected lipid lowering drug

<table>
<thead>
<tr>
<th>S. No</th>
<th>Pharmaceutical concern</th>
<th>Property name</th>
<th>Active ingredients</th>
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<tbody>
<tr>
<td>1</td>
<td>Lomitapide</td>
<td>Juxtapid</td>
<td>5 mg, 10 mg</td>
</tr>
<tr>
<td>2</td>
<td>Lomitapide</td>
<td>Juxtapid</td>
<td>5 mg, 10 mg, 20 mg</td>
</tr>
<tr>
<td>3</td>
<td>Lomitapide</td>
<td>Juxtapid</td>
<td>10 mg, 20 mg</td>
</tr>
<tr>
<td>4</td>
<td>Lomitapide</td>
<td>Lojuxta</td>
<td>5 mg, 10 mg, 20 mg</td>
</tr>
<tr>
<td>5</td>
<td>Lomitapide</td>
<td>Lojuxta</td>
<td>5 mg, 10 mg, 20 mg</td>
</tr>
</tbody>
</table>

### Table-6.4

Procedure for the assay of LMP in formulations

<table>
<thead>
<tr>
<th>Pharmaceutical formulation</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablets</td>
<td>An accurately weighed amount of tablet powder, equivalent to 100 mg was dissolved in 20 to 40 ml of methanol and the solution was made up to the mark in 100 ml volumetric flask with methanol. The concentration of the resulting solution was found to be 1 mg/ml. This solution was taken as the stock solution. The absorbance of the solution was measured at ( \lambda ) max of 240 nm against the corresponding reagent blank. The quantity of the drug was computed from the standard Beer-Lambert’s plot.</td>
</tr>
</tbody>
</table>
Fig. 6.1 Absorption spectrum of LMP (UV method)

Fig. 6.2 Beer’s law plot of LMP
Part – A

SPECTROPHOTOMETRIC METHOD

Experimental

Preparation of standard drug stock solution

The stock solution (1 mg/ml) of Lomitapide was prepared by dissolving 100 mg of the drug in 20 ml methanol in 100 ml volumetric flasks and made up to the volume with methanol to get a clear solution. 10 ml of this stock solution was taken into 100 ml volumetric flask and diluted up to the mark with methanol. The concentrations of this standard is 100 µg/ml, which are used for preparation of working standards in M1, M2, M3, M4, M5, and M6 methods.

Preparation of reagents for M1 to M6 methods

Chemicals and reagents used were of analytical grade and solutions

1. 1,10-PTL (0.01 M),
2. Ferric chloride (0.003 M)
3. Ortho phosphoric acid (0.02 M, 0.2 M)
4. 2, 2′-BPL (0.01 M),
5. Ferric chloride (0.5% w/v),
6. K₃Fe(CN)₆ (0.2% w/v)
7. HCl (1N)
8. BTB dye solution (0.2% w/v)
9. Solo chrome Black - T dye solution (0.2% w/v)
10. Methyl orange dye solution (0.1% w/v)

Developed and used procedure

Determination of LMP in bulk and pharmaceutical formulations was made by M1 to M6 methods which are developed on the basis of LMP reaction with FeCl₃ along with other color producing reagents.

Method M1 & M2

0.2 to 1.2 ml (1 ml =100 µg) solutions of LMP were taken into a series of 10 ml graduated test tubes. 1 ml of 1, 10- Phenanthraline solution, followed by 1 ml of ferric chloride solutions were added to the LMP solution taken in test tube. To another set of test tube containing LMP standard solutions, 2, 2′-Bi-pyridine and 1 ml ferric chloride reagents were added. These solutions were heated for 15 min at 100°C. 2 ml of ortho phosphoric acid was added to each one of them. This total volume was made
to 10 ml with distilled water. The absorbance maximum of this solutions were observed at 430 nm and 490 nm respectively. Linearity curve is plotted by using above prepared blue coloured working standards ranging from 1.0 – 12.0 µg/ml against the corresponding reagent blank. A good linearity was observed with correlation coefficient 0.9988 and 0.9983 for M₁ and M₂. The amount of LMP was computed from the Beer- Lambert’s plot. The schematic graph of absorbance maxima (λ_max) and linearity curve were shown in Fig. 6.3 to Fig. 6.6.

Fig. 6.3: Absorption spectrum of LMP with 1, 10 PTL/FeCl₃ system

Fig. 6.4: Beer’s law plot of LMP with 1, 10 PTL/FeCl₃ system
Fig. 6.5: Absorption spectrum of LMP with 2, 2’BPL/FeCl₃ system

Fig. 6.6: Beer’s law plot of LMP with 2, 2’BPL/FeCl₃ system

Method M₃

1.0 to 5.0 ml (1 ml=100 µg) LMP solutions were taken into a series of 10 ml graduated test tubes Ferric chloride (0.5%, 1 ml) and potassium ferri-cyanide (0.2%, 2 ml) were added and kept for 10 min. To these test tubes conc. HCl (1 N, 1 ml) was added A bluish green colored chromogen was formed. The absorbance maximum of
this solutions were observed at 680 nm. Linearity curve is plotted by using above 
prepared bluish green coloured working standards ranging from 10 – 60 µg/ml against 
the corresponding reagent blank. A good linearity was observed with correlation 
coefficient 0.999. The amount of LMP was computed from the Beer- Lambert’s plot. 
The schematic graph of absorbance maxima ($\lambda_{\text{max}}$) and linearity curve were shown in 
Fig. 6.7 to Fig. 6.8.

Fig. 6.7: Absorption spectrum of LMP with K$_3$Fe (CN)$_6$/FeCl$_3$ system

Fig. 6.8: Beer’s law plot of LMP with K$_3$Fe (CN)$_6$/FeCl$_3$ system
Method M₄

LMP (1 ml=100 µg) solution ranging from 1.0 to 6.0 ml were transferred into a series of 50 ml separating funnels. 2 ml of BTB (0.2%) was added and diluted to 10 ml with distilled water. 10 mL of chloroform was added to each funnel, and the contents were shaken for 2 minutes and allowed to stand result separate the aqueous and chloroform layers. The chloroform layer was separated and the layer was scanned by using Visible region. The absorbance maximum of this layer was found at 420 nm. A Linearity curve was plotted by using above prepared working standards ranging from 10 to 60 µg/mL. A good linearity was observed with correlation coefficient 0.9986. The schematic graph of absorbance maxima (λ_max) and linearity curve were shown in Fig. 6.9 and Fig.6.10.

![Absorbance vs Wavelength](image1)

**Fig. 6.9: Absorption spectrum of LMP with BTB/CHCl₃ system**

![Absorbance vs Concentration](image2)

**Fig. 6.10: Beer’s law plot of LMP with BTB/CHCl₃ system**
Method M₅

A series of 50 ml separating funnels were taken and LMP (1 ml=100 µg) solutions from 0.1 to 0.6 ml were introduced to them. 2 ml of MO (0.1%) was added to each of them and the total volume was made up to 10 ml with distilled water. 10 ml of chloroform was added and the contents were shaken for 2 min. The two phases were allowed to separate. The absorbance maximum of this chloroform layer was observed at 520 nm. A linearity curve was plotted by using above prepared LMP working standards against the corresponding reagent blank. A good correlation coefficient 0.9981. LMP present in the sample solution was computed from its calibration curve. The schematic graph of absorbance maxima (λ_max) and linearity curve were shown in Fig. 6.11 and Fig.6.12.

![Fig. 6.11: Absorption spectrum of LMP with MO/CHCl₃ system](image1)

![Fig. 6.12: Beer’s law plot of LMP with MO/CHCl₃ system](image2)
Method M₆

LMP (1 ml=100 µg) solution ranging from 0.1 – 0.6 ml were transferred into a series of 50 ml separating funnels, 2 ml of SBT (0.2%) was added, extracted with chloroform and the absorbance of violet color chromogen and the amount of LMP was calculated. The absorbance maxima of this extracted layer was observed at 440 nm and a good linear curve was observe from 1 – 6 µg/ml with correlation coefficient of 0.9975. The schematic graph of absorbance maxima (λ_max) and linearity curve were shown in Fig. 6.13 and Fig. 6.14.

![Absorption spectrum of LMP with SBT/CHCl₃ system](image1.png)

**Fig. 6.13:** Absorption spectrum of LMP with SBT/CHCl₃ system

![Beer’s law plot of LMP with SBT/CHCl₃ system](image2.png)

**Fig. 6.14:** Beer’s law plot of LMP with SBT/CHCl₃ system
Results and Discussion

Spectral Characteristics

Optimum conditions for the color development for the methods M₁ – M₆ were established by varying the parameters one at a time keeping the others fixed and observing the effect produced on the absorbance of colored species.

The optical characteristics such as absorption maximum, Beer’s law limits, molar absorptivity and sandell’s sensitivity are presented Table:6.5. The precision and accuracy were found by analyzing six replicate samples. Accuracy of these methods was ascertained by comparing the results obtained with the used and reference methods. The concentration Vs absorbance plots of the systems are illustrated. The optimum photometric range for LMP with each of the mentioned reagents were calculated.

Recovery experiments indicated the absence of interference from the commonly encountered pharmaceutical additives and excipients. Thus the proposed methods are simple and sensitive with reasonable precision and accuracy Table: 6.6 & Table 6.7. These methods can be used for routine determination of Lomitapide in quality control analysis.

Method M₁ & M₂

These methods were based on reduction of Fe⁺³ to Fe⁺² and subsequent orange colored complex formation by Fe⁺² ion with 1, 10-phenonthroline, 2, 2’–bypyridine in ferric chloride and ortho phosphoric acid medium.
Scheme 6.1: Reaction of LMP with 1, 10 phenanthroline

Fe^{2+} + \text{1, 10 Phenanthroline} \rightarrow \text{Colored complex}

Scheme 6.2: Reaction of LMP with 2, 2’Bipyridine

Fe^{2+} + \text{2,2’ Bipyridine} \rightarrow \text{Colored complex}
Method M₃

Reduction of ferric ion with LMP to ferrous ion followed by Fe⁺² reaction with potassium ferricyanide to produce an intense blue colored solution is the basis of this method.

Method (M₄ & M₅)

The drug LMP being basic in character reacts with acidic BTB and MO dyes and form orange colored ion association complex which are extractable with chloroform. The stoichiometric ratio of the drug to dye was determined by slope analysis method and found to be 1:2.

Scheme 6.3: Reaction of LMP with BTB
Scheme 6.4 Reaction of LMP with 1, 10-phenonthraline

Where $R = \text{X-} - \text{N}^{\equiv} \text{N-} - \text{N}^{\equiv} \text{N}$ and $X = \text{SO}_3^- \text{Na}$
Method M₆

The ion association complex formed between acidic SBT and basic drug has violet color which is extractable with chloroform. Its $\lambda_{\text{max}}$ in UV spectrum against reagent blank was found to be 440 nm.

Scheme: 6.5 Reaction of LMP with SBT

Recovery Studies

These were conducted by analyzing each pharmaceutical formulation in the LMP instance for the active ingredients by these new methods. Known amount of pure drug was added to each of the previously analyzed formulation and then total
amount of the drug was once again determined by these developed methods after bringing the active ingredient concentration within the Beer’s law limits.

Table-6.5

Optical and Regression Characteristics, Precision and Accuracy of the proposed methods for LMP

<table>
<thead>
<tr>
<th>Parameter</th>
<th>M₁</th>
<th>M₂</th>
<th>M₃</th>
<th>M₄</th>
<th>M₅</th>
<th>M₆</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ max (nm)</td>
<td>430</td>
<td>490</td>
<td>680</td>
<td>420</td>
<td>520</td>
<td>440</td>
</tr>
<tr>
<td>Beer’s law Limits (µg/ml)</td>
<td>2-12</td>
<td>2-12</td>
<td>10-60</td>
<td>10-60</td>
<td>1-6</td>
<td>1-6</td>
</tr>
<tr>
<td>Molar absorptivity (1 mole⁻¹ cm⁻¹)</td>
<td>5.753×10³</td>
<td>5.86×10⁴</td>
<td>1.0×10⁻³</td>
<td>7.2×10³</td>
<td>6.6×10³</td>
<td>5.85×10³</td>
</tr>
<tr>
<td>Sandell’s sensitivity (µg/cm²/0.001 absorbance unit)</td>
<td>0.00119</td>
<td>0.001216</td>
<td>0.0021075</td>
<td>0.0095</td>
<td>0.01397</td>
<td>0.01216</td>
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<tr>
<td>Slope (b)</td>
<td>0.0752</td>
<td>0.0748</td>
<td>0.0133</td>
<td>0.0130</td>
<td>0.1179</td>
<td>0.1186</td>
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<tr>
<td>Intercept(a)</td>
<td>0.0256</td>
<td>0.1246</td>
<td>0.0115</td>
<td>0.0606</td>
<td>0.1088</td>
<td>0.126</td>
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<tr>
<td>Correlation coefficient(r)</td>
<td>0.9988</td>
<td>0.9983</td>
<td>0.999</td>
<td>0.9986</td>
<td>0.9981</td>
<td>0.9975</td>
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<tr>
<td>Standard deviation</td>
<td>0.0049</td>
<td>0.0063</td>
<td>0.005</td>
<td>0.0045</td>
<td>0.0057</td>
<td>0.0060</td>
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<tr>
<td>% Relative standard deviation</td>
<td>1.42</td>
<td>0.73</td>
<td>0.62</td>
<td>1.82</td>
<td>1.3</td>
<td>1.62</td>
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<tr>
<td>0.05 level</td>
<td>±1.285</td>
<td>±1.226</td>
<td>±0.6520</td>
<td>±1.224</td>
<td>±1.445</td>
<td>±1.1706</td>
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<tr>
<td>0.01 level</td>
<td>±1.603</td>
<td>±1.756</td>
<td>±0.8312</td>
<td>±1.751</td>
<td>±2.140</td>
<td>±1.5413</td>
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</table>
### Table 6.6
Assay Results of LMP in dosage forms

<table>
<thead>
<tr>
<th>Method</th>
<th>Pharmaceutical Formulation</th>
<th>Labelled amount (mg)</th>
<th>Amount found (mg)</th>
<th>t-value</th>
<th>F-value</th>
<th>Found by reference method ±S.D</th>
<th>% recovery by proposed method ±S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>M₁</td>
<td>Lomitapide 10</td>
<td>10.04±0.018</td>
<td>1.412</td>
<td>1.762</td>
<td>9.93±0.064</td>
<td>100.4±0.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lomitapide 10</td>
<td>9.94±0.043</td>
<td>0.912</td>
<td>1.832</td>
<td>10.04±0.083</td>
<td>99.4±0.43</td>
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<tr>
<td></td>
<td>Lomitapide 10</td>
<td>10.03±0.042</td>
<td>0.417</td>
<td>1.224</td>
<td>9.93±0.017</td>
<td>100.3±0.42</td>
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<tr>
<td>M₂</td>
<td>Lomitapide 10</td>
<td>10.08±0.055</td>
<td>0.417</td>
<td>1.224</td>
<td>9.93±0.017</td>
<td>100.8±0.55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lomitapide 10</td>
<td>9.99±0.072</td>
<td>0.881</td>
<td>1.772</td>
<td>10.07±0.034</td>
<td>99.9±0.72</td>
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<tr>
<td></td>
<td>Lomitapide 10</td>
<td>9.92±0.033</td>
<td>1.264</td>
<td>2.661</td>
<td>10.02±0.046</td>
<td>99.2±0.33</td>
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<tr>
<td>M₃</td>
<td>Lomitapide 10</td>
<td>10.02±0.086</td>
<td>1.412</td>
<td>1.762</td>
<td>9.93±0.064</td>
<td>100.2±0.86</td>
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<tr>
<td></td>
<td>Lomitapide 10</td>
<td>9.91±0.036</td>
<td>0.818</td>
<td>2.212</td>
<td>10.01±0.032</td>
<td>99.1±0.36</td>
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<tr>
<td></td>
<td>Lomitapide 10</td>
<td>9.89±0.027</td>
<td>1.207</td>
<td>1.117</td>
<td>10.09±0.043</td>
<td>98.9±0.27</td>
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<tr>
<td>M₄</td>
<td>Lomitapide 20</td>
<td>19.80±0.0156</td>
<td>1.812</td>
<td>1.264</td>
<td>20.98±0.047</td>
<td>99.0±0.08</td>
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<tr>
<td></td>
<td>Lomitapide 20</td>
<td>20.06±0.096</td>
<td>1.312</td>
<td>2.574</td>
<td>19.96±0.083</td>
<td>100.3±0.48</td>
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<tr>
<td></td>
<td>Lomitapide 20</td>
<td>19.94±0.021</td>
<td>0.965</td>
<td>1.742</td>
<td>20.04±0.031</td>
<td>99.7±0.11</td>
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<tr>
<td>M₅</td>
<td>Lomitapide 20</td>
<td>20.03±0.031</td>
<td>1.525</td>
<td>1.475</td>
<td>19.95±0.075</td>
<td>100.2±0.16</td>
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<tr>
<td></td>
<td>Lomitapide 20</td>
<td>19.96±0.062</td>
<td>1.714</td>
<td>1.532</td>
<td>20.06±0.026</td>
<td>99.8±0.31</td>
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<tr>
<td></td>
<td>Lomitapide 20</td>
<td>20.01±0.018</td>
<td>0.733</td>
<td>1.536</td>
<td>19.91±0.033</td>
<td>100.1±0.09</td>
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<tr>
<td>M₆</td>
<td>Lomitapide 20</td>
<td>20.08±0.052</td>
<td>0.632</td>
<td>2.225</td>
<td>19.98±0.073</td>
<td>100.4±0.26</td>
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<tr>
<td></td>
<td>Lomitapide 20</td>
<td>19.94±0.023</td>
<td>0.964</td>
<td>1.432</td>
<td>20.04±0.031</td>
<td>99.7±0.12</td>
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<tr>
<td></td>
<td>Lomitapide 20</td>
<td>20.06±0.097</td>
<td>1.313</td>
<td>2.548</td>
<td>19.96±0.084</td>
<td>100.3±0.49</td>
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### Table-6.7
Recovery of LMP

<table>
<thead>
<tr>
<th>Amount of drug added(µg)</th>
<th>Mean (+S.D) amount found (µg)</th>
<th>Mean(+S.D) % of recovery (n=5)</th>
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<tbody>
<tr>
<td>10</td>
<td>9.75±0.14</td>
<td>97.5±1.4</td>
</tr>
<tr>
<td>20</td>
<td>19.65±0.19</td>
<td>98.3±0.95</td>
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Part – B

HPLC Method

Introduction

There are no reported HPLC methods in the literature for the determination of Lomitapidein biological fluids. The newer proposed HPLC method(M14) fulfilled the requirements of analytical parameters necessary to be applied to the routine analysis of Pharmaceutical formulations (existing as single active ingredient or combined dosage forms) using Shimadzu C18 column as stationary phase and acetonitrile + buffer (40:60) as mobile without internal standard.

Experimental

Preparation of standard drug solution for method M14

35 mg of LMP was dissolved in 20 ml of water taken in a 50 ml volumetric flask. The solution was made up to the mark with water to get standard solution (700 µg/ml)

Preparation of LMP drug solution from the Pharmaceutical formulations

LMP pharmaceutical formulations were pulverised and 1g of the powder which is equivalent to 17.5 mg of LMP was dissolved in 15 ml of mobile phases. The solution was sonicated for 10 min, and filtered through 0.45 µm membrane filter and final volume made up to 25ml with mobile phase get the stock solution (700 µg/ml). This solution was further diluted stepwise with mobile phase to get different required concentrations.

Reagents used

HPLC Grade Water, Acetonitrile (Qualigens) and Orthophosphoric acid buffer (Qualigens).
Table 6.8
Chromatographic conditions for LMP Method (M14)

<table>
<thead>
<tr>
<th>Stationary Phase</th>
<th>Mobile Phase</th>
<th>Flow rate (ml/min)</th>
<th>Run Time (minutes)</th>
<th>Column Temperature</th>
<th>Volume of injection</th>
<th>Detection Wavelength</th>
<th>Retention time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C18</td>
<td>Buffer: Acetonitrile 40:60</td>
<td>1.0</td>
<td>8.0</td>
<td>25°C</td>
<td>20</td>
<td>240</td>
<td>2.68</td>
</tr>
</tbody>
</table>

**Method M14**

Ten sets of the drug LMP solutions at a concentration of 5 to 25 µg/ml mobile phase were prepared. It was filtered before use through 0.45 µm membrane filter, degassed with a helium sparge for 15 min and pumped from the respective solvent reservoirs to the column at specific flow rate. Prior to injection of the drug, the mobile phase was pumped for about 30 min to saturate the column thereby to get the base line corrected, then 20 µl of each of the drug solution were injected for 5 times. Quantitative determinations were made by comparisons of the peak area from a standard injection. The amount of LMP present in the sample was calculated through the standard calibration curve.

**Fig. 6.15 Chromatogram for LMP (M14)**
Peak table

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Ret. Time</th>
<th>Area</th>
<th>Area%</th>
<th>Tailing factor (0%)</th>
<th>K'</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.68</td>
<td>11592</td>
<td>30.67</td>
<td>1.685</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Result and Discussion

The appropriate wavelength in UV region was selected for the measurement of active ingredients in each method. The method was validated by linear fit curve and all other parameters were calculated just like a visible spectrophotometric method and were discussed in the following lines.

Nature of the chromatograms

As LMP possesses different functional moieties and the mobile phase also contains different solvents. It is very difficult to predict their exact behaviour of separation in different columns. The author has made an attempt to indicate the nature of separation in the proposed methods for LMP (M_{14}). The polarities were changed by using different concentrations of organic solvents and water.

Parameter fixation

In order to ascertain and establish optimum conditions for good resolution rapid and accurate quantitative separation and estimation of LMP by the method (M_{14}), the author has performed control experiments by varying one variable at a time and fixing all other variables such as mobile phase composition, flow rate, nature of internal standards etc.

Detection characteristics

To test whether the LMP has been linearly eluted from the column in the method M_{14}, without the usage of internal standard, different amounts of LMP were taken and all solutions were analyzed by the mentioned procedures. Least square regression analysis for each method was carried out for the slope, intercept and correlation coefficient Table: 6.9.
Performance calculations

To ascertain the system suitability for the proposed method M14, a number of statistical values such as relative retention, theoretical plates HETP, and resolution peak asymmetry have been calculated with the observed reading.

Method validation

Precision

The precision of the method was ascertained from the peak area of LMP (M14) obtained by the determination of eight replicates of fixed amount of LMP. The percent relative standard deviation and percent range of errors (0.05 and 0.01 confidence limits) were calculated for LMP (M14).

Accuracy

To determine the accuracy of the proposed method, different amount of bulk samples of LMP in between the upper and lower linearity limits were taken and analyzed by the proposed method.
Table: 6.9

Performance calculations, detection characteristics, precision and accuracy of the proposed method for LMP

<table>
<thead>
<tr>
<th>Method</th>
<th>Parameter</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Retention time (t)(Min)</td>
<td>2.680</td>
</tr>
<tr>
<td></td>
<td>Theoretical plates (n)</td>
<td>91377</td>
</tr>
<tr>
<td></td>
<td>Plates per Meter (N)</td>
<td>22414</td>
</tr>
<tr>
<td></td>
<td>Height equivalent to theoretical plate(HETP)(mm)</td>
<td>0.0217</td>
</tr>
<tr>
<td></td>
<td>Peak asymmetry</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>Resolution Factor</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Linearity range (ng µl⁻¹)</td>
<td>1-7</td>
</tr>
<tr>
<td></td>
<td>Detection limits(ng µl⁻¹)</td>
<td>0.025474</td>
</tr>
<tr>
<td></td>
<td>Regression equation(Y=a+bc)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slope (b)</td>
<td>30.345</td>
</tr>
<tr>
<td></td>
<td>Standard deviation of intercept (sa)</td>
<td>4.4305</td>
</tr>
<tr>
<td></td>
<td>Standard error of estimation (se)</td>
<td>4.56×10⁻¹</td>
</tr>
<tr>
<td></td>
<td>Correlation coefficient (r)</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>Relative standard deviation (%)</td>
<td>0.326</td>
</tr>
<tr>
<td></td>
<td>Percentage range of errors(confidence limits)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05 level</td>
<td>0.0016</td>
</tr>
<tr>
<td></td>
<td>0.01 level</td>
<td>0.0021</td>
</tr>
<tr>
<td></td>
<td>% error in bulk samples</td>
<td>0.0061</td>
</tr>
</tbody>
</table>
Interference studies

The common excipients such as hydroxypropyl methylcellulose, lactose monohydrate, magnesium steric, microcrystalline cellulose, polyethylene glycol 3000 and sodium starch glycolate have been added to the sample solution and injected into HPLC to ascertain that effect in the formulations of LMP. It was found that they have not disturbed the elution of LMP ($M_{14}$). In fact many of them have no absorption in the UV spectrum.

Analysis of formulation

To find out the suitability of the proposed method for the assay of pharmaceutical formulations containing LMP without internal standard drug formulation was analyzed by the proposed and reference methods. The results obtained after statistical computation were satisfactory in agreement, not differed significantly in precision and accuracy of the proposed and reference method Table 6.10.

Table: 6.10
Assay Results of LMP in dosage forms

<table>
<thead>
<tr>
<th>Method</th>
<th>Pharmaceutical formulation</th>
<th>Labelled Amount (mg)</th>
<th>Proposed method</th>
<th>Found by reference method ±S.D</th>
<th>% Recovery by proposed methods± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Amount found ±S.D</td>
<td>t-value</td>
<td>F-value</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>M11</td>
<td>Tablet –I 10 10.012±0.023</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tablet-II 10 9.996±0.025</td>
<td>1.4</td>
<td>2.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tablet-III 10 9.994±0.024</td>
<td>1.06</td>
<td>1.62</td>
</tr>
</tbody>
</table>

*Average ±standard deviation of eight determinants the t and F values refer to comparison of the proposed method. Theoretical values at 95% confidence limits t=2.365 and F=4.88.
Recovery studies

They were conducted by analyzing each pharmaceutical formulation in the first instance for the active ingredient by the proposed method. Known amount of the pure drug was the added to each of the previously analyzed formulation, and then total amounts of the drug was once again determined by the proposed method after bringing active ingredient concentration within the linearity limits. The recovery values in the given Table 6.11.

Table: 6.11
Recovery of Lomitapide

<table>
<thead>
<tr>
<th>Amount of drug added(µg)</th>
<th>Mean (±S.D) amount found (µg)</th>
<th>Mean(±S.D) % of recovery (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>4.88±0.03</td>
<td>97.6±0.60</td>
</tr>
<tr>
<td>10</td>
<td>9.82±0.05</td>
<td>98.20±0.50</td>
</tr>
</tbody>
</table>
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