A REVIEW ON CHROMATOGRAPHIC AND SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF EZETIMIBE AND FLUVASTATIN

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ABSTRACT

Ezetimibe is selective cholesterol absorption inhibitor. It is an anti-hyperlipidemic agent. It is used in treatment of Hypercholesterolemia. Fluvastatin is a drug which comes under class of statin. It is used to reduce cholesterol levels and prevent cardiovascular disease. It decreases low density lipoprotein (LDL) cholesterol. Ezetimibe plus Fluvastatin was proved to be effective at a dose of 10mg and 80mg in management of hypercholesterolemia compare to Ezetimibe and Fluvastatin Monotherapy. Cholesterol absorption inhibitor plus statin showed the effective results in relief of the Hypercholesterolemia. This article narrate different chromatographic (HPLC, HPTLC) and different spectrophotometric method (UV) for determination of the Ezetimibe and Fluvastatin.

KEYWORDS: Ezetimibe, Fluvastatin, UV- Spectroscopy, HPLC (High Performance Liquid Chromatography), HPTLC (High Performance Thin Layer Chromatography).

INTRODUCTION[1-3]

Ezetimibe is selective cholesterol absorption inhibitor. It is an anti-hyperlipidemic agent. It is used in treatment of Hypercholesterolemia. It inhibits the absorption of cholesterol and decreasing delivery of intestinal cholesterol to the liver. It is metabolized into its glucuronide in liver and small intestine which prevent absorption of cholesterol.

Fluvastatin is HMG-CoA reductase inhibitor.3-Hydroxy 3-methyl glutaryl coenzyme A (HMG-COA) reductase is responsible for converting of HMG-CoA to mevalonate, the rate –
limiting step in cholesterol biosynthesis. It is used to reduce cholesterol levels and prevent cardiovascular disease. It decreases low density lipoprotein (LDL) cholesterol.

Combination of Cholesterol absorption inhibitor and statin found to be effective in reducing Low density lipoprotein cholesterol (LDL-C) and total cholesterol levels. Combination of Ezetimibe and Fluvastatin showed effective result in relief of Hypercholesterolemia.

Reported methods are categorized depending on the following considerations:
1. Single component analyzed by UV-spectroscopy methods and chromatographic method.
2. Analysis of Ezetimibe and Fluvastatin in combination with other drugs by UV-spectroscopy methods and chromatographic method.

Table I: Reported Analytical Method of Ezetimibe

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Drug</th>
<th>Method</th>
<th>Description</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ezetimibe in tablet dosage form</td>
<td>UV-Spectroscopic</td>
<td>Detection: 252nm&lt;br&gt;Correlation Co-efficient: 0.998&lt;br&gt;Linearity Range: 2-20μg/ml&lt;br&gt;% Recovery: 100.9 to 102.32%&lt;br&gt;LOD: 0.10μg/ml&lt;br&gt;LOQ: 0.30μg/ml</td>
<td>[4]</td>
</tr>
<tr>
<td>2</td>
<td>Ezetimibe in tablet dosage form</td>
<td>UV-Spectrophotometric</td>
<td>Detection: 232nm&lt;br&gt;Linearity range: 5-30μg/ml&lt;br&gt;Correlation Co-efficient: 0.999</td>
<td>[5]</td>
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<tr>
<td>3</td>
<td>Ezetimibe in tablet formulation</td>
<td>Spectrophotometric</td>
<td>Detection: 745nm&lt;br&gt;Linearity Range: 100-260μg/ml&lt;br&gt;Correlation Co-efficient: 0.995</td>
<td>[6]</td>
</tr>
<tr>
<td>4</td>
<td>Ezetimibe in Pharmaceutical formulations</td>
<td>Spectrophotometric</td>
<td>Detection: 234nm&lt;br&gt;Linearity Range: 5-20μg/ml&lt;br&gt;Correlation Co-efficient: 0.999&lt;br&gt;% Recovery: 96-98%</td>
<td>[7]</td>
</tr>
<tr>
<td>5</td>
<td>Ezetimibe in tablet dosage form</td>
<td>RP-HPLC</td>
<td>Stationary Phase: Phenomenex Luna C_{18} column&lt;br&gt;Mobile Phase: Acetonitrile: 0.02M&lt;br&gt;Phosphate buffer: Methanol (70:20:10v/v)&lt;br&gt;Detection: 235nm&lt;br&gt;Linearity Range: 10-100μg/ml&lt;br&gt;Flow Rate: 1ml/min&lt;br&gt;Retention time: 3.537 min&lt;br&gt;LOD: 1μg/ml&lt;br&gt;LOQ: 3.2μg/ml&lt;br&gt;% Recovery: 99.6-101%</td>
<td>[8]</td>
</tr>
<tr>
<td>6</td>
<td>Ezetimibe in Human serum</td>
<td>RP-HPLC</td>
<td>Stationary Phase: C_{18} Symmetry Shield column&lt;br&gt;Mobile Phase: Acetonitrile and 0.1 M Ammonium acetate aqueous solution 55:45 (v/v)&lt;br&gt;Linearity Range: 10-800ng/ml&lt;br&gt;Detection: 232nm&lt;br&gt;Flow Rate: 0.75ml/min</td>
<td>[9]</td>
</tr>
<tr>
<td>Step</td>
<td>Description</td>
<td>Method</td>
<td>LOD</td>
<td>LOQ</td>
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<tr>
<td>7</td>
<td>Ezetimibe in bulk and pharmaceutical formulation</td>
<td>RP-HPLC</td>
<td>4.60ng/ml</td>
<td>13.94ng/ml</td>
</tr>
</tbody>
</table>
|      |             |        | **Stationary Phase**: Kromasil C₈ column  
Mobile Phase: Acetonitrile: 0.02 M Potassium dihydrogen orthophosphate buffer (72:28 v/v)  
Detection: 232nm  
Flow Rate: 1ml/min  
Linearity Range: 10-45μg/ml  
Retention time: 4.24 min  
Correlation Co-efficient: 0.999  
% Recovery: 99.66%  
LOD: 0.0413μg/ml  
LOQ: 0.1253μg/ml | | [10] |
| 8    | Ezetimibe in tablet dosage form | Stability indicating RP-HPLC |  | |
|      |             |        | **Stationary Phase**: Zorbax SB C₁₈ column  
Mobile Phase: 0.02N Ortho phosphoric acid: Acetonitrile (20:80 v/v)  
Detection: 232nm  
Linearity Range: 1-10μg/ml | | [11] |
| 9    | Ezetimibe in pharmaceutical dosage form | RP-HPLC |  | |
|      |             |        | **Stationary Phase**: Betasil C₁₈ column  
Mobile Phase: Acetonitrile: 10Mm potassium dihydrogen phosphate (55:45 v/v)  
Linearity Range: 4-24μg/ml  
Retention time: 4.91 min  
Flow Rate: 1ml/min  
Detection: 233nm  
LOD: 286.77μg/ml  
LOQ: 869.01μg/ml | | [12] |
| 10   | Ezetimibe in pharmaceutical formulation tablets | HPLC |  | |
|      |             |        | **Stationary Phase**: C₁₈ column  
Mobile Phase: Acetonitrile: Ammonium acetate (10mM, pH 3.0), (75:25v/v)  
Flow Rate: 1ml/min  
Detection: 240nm  
Linearity Range: 10-60μg/ml  
% Recovery: 95.3%  
LOD: 5μg/ml  
LOQ: 10μg/ml | | [13] |
| 11   | Ezetimibe in bulk drug and formulation | RP-HPLC |  | |
|      |             |        | **Stationary Phase**: Agilent XDB C₁₈ column  
Mobile Phase: Di sodium hydrogen ortho phosphate buffer: Methanol (32:68v/v)  
Detection: 234nm  
Linearity Range: 20-100μg/ml  
Retention time: 5.7 min  
Correlation Co-efficient: 0.999 | | [14] |
| 12   | Ezetimibe in tablet dosage form | RP-HPLC |  | |
|      |             |        | **Stationary Phase**: ODS-3V Column  
Mobile Phase: Ammonium acetate buffer: Acetonitrile (45:55v/v)  
Flow Rate: 1.5ml/min  
Detection: 230nm  
% Recovery: 98-99% | | [15] |
Table II: Reported method of Ezetimibe in combination with other drugs. [16-29]

<table>
<thead>
<tr>
<th>Sr. No</th>
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<th>Description</th>
<th>Ref No.</th>
</tr>
</thead>
</table>
| 13     | Simvastatin and ezetimibe in bulk drug | Spectrophotometric | Detection: 223 and 258 nm  
Linearity Range: 1-25μg/ml  
Mean recovery: 99%-99.6%  
Correlation Co-efficient: 0.999 | [16]    |
| 14     | Ezetimibe and Carvedilol    | UV spectroscopic  | Detection: Ezetimibe- 232nm  
Carvedilol-238nm  
Linearity Range: Ezetimibe- 2-50μg/ml  
Carvedilol- 2-20μg/ml  
LOD: Ezetimibe- 0.4μg/ml  
Carvedilol- 1.3μg/ml  
LOQ: Ezetimibe- 0.7μg/ml  
Carvedilol- 2.1μg/ml | [17]    |
| 15     | Ezetimibe and Fenofibrate  | UV spectroscopic  | Detection: 286nm and 232nm  
Linearity Range: 2-20μg/ml  
% Recovery: 98%  
Correlation Co-efficient: 0.999 | [18]    |
| 16     | Valsartan and Ezetimibe    | Spectrophotometric | Detection: Valsartan- 425m  
Ezetimibe- 428nm  
Linearity Range: Valsartan- 5-40 μg/ml  
Ezetimibe- 1-50 μg/ml  
% Recovery: Valsartan- 99.3%  
Ezetimibe- 100.3%  
Correlation Co-efficient: Valsartan- 0.995  
Ezetimibe- 0.999 | [19]    |
| 17     | Simvastatin and Ezetimibe  | UV- Spectrophotometric | Detection: 235nm and 266nm  
Linearity Range: 2-20μg/ml  
% Recovery: 91-101%  
Correlation Co-efficient: 0.999 | [20]    |
| 18     | Rosuvastatin calcium and Ezetimibe in tablet dosage form | RP-HPLC | Stationary Phase: Licrosphere C18 column  
Mobile Phase: Methanol: Acetonitrile: Phosphate buffer, pH 3.5 (60:20:20 v/v)  
Detection: 279nm  
Flow Rate: 1ml/min  
Mean recovery: 99.01% - 100.64%  
Linearity Range: 2-5μg/ml  
LOD: Rosuvastatin-0.01μg/ml  
Ezetimibe - 0.004μg/ml | [21]    |
<table>
<thead>
<tr>
<th>No.</th>
<th>Method Description</th>
<th>Method Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>Atorvastatin calcium and ezetimibe as the bulk drug and in tablet dosage forms</td>
<td>LOQ: Rosuvastatin -0.03μg/ml Ezetimibe - 0.01μg/ml&lt;br&gt;Stationary Phase: Silica gel 60 F254 Mobile Phase: Toluene–Methanol 8:2 (v/v) Detection: 240nm Linearity Range: 0.4–2.4μg/ml Correlation Co-efficient: 0.999</td>
</tr>
<tr>
<td>20</td>
<td>Rosuvastatin calcium and Ezetimibe in Pharmaceutical dosage form</td>
<td>Stationary Phase: C18 Column Mobile phase: Acetonitrile: Water (75:25 % v/v) Detection: 252 nm. Flow rate: 0.6 ml/min Linearity Range: 5–40μg/ml Retention time: Rosuvastatin-2.91 min Ezetimibe-6.53 min LOD: Rosuvastatin-0.76 Ezetimibe-0.91 LOQ: Rosuvastatin-2.3 Ezetimibe-2.7</td>
</tr>
<tr>
<td>21</td>
<td>Atorvastatin calcium and ezetimibe</td>
<td>Stationary Phase: Hypersil BDS C18 column Mobile phase: Phosphate buffer pH-4.5 :Acetonitrile (35:65 v/v) Detection: 228 nm Flow rate: 1ml/min Linearity Range: 12.5-75μg/ml</td>
</tr>
<tr>
<td>22</td>
<td>Simvastatin and Ezetimibe</td>
<td>Stationary Phase: C18 ODS Hypersil column Mobile phase: Acetonitrile: Phosphate buffer (pH 4.5, 0.01M) (65:35 v/v) Detection: 232nm Flow rate: 1ml/min</td>
</tr>
<tr>
<td>23</td>
<td>Atorvastatin and Fenofibrate and Ezetimibe</td>
<td>Stationary Phase: C18 column Mobile phase: Methanol: Acetonitrile: Water (80:10:10) Linearity Range: Atorvastatin- 3-7μg/ml Fenofibrate- 48-112μg/ml Ezetimibe- 3-7μg/ml LOD: Atorvastatin- 0.05μg/ml Ezetimibe- 1.58μg/ml</td>
</tr>
<tr>
<td>Sr. No</td>
<td>Drug</td>
<td>Method</td>
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</tbody>
</table>
| 1     | Fluvastatin in Bulk and Pharmaceutical Formulations | UV Spectrophotometric | Detection: 304 nm  
Linearity Range: 5-25 μg/ml  
Correlation Co-efficient: 0.999  
% Recovery: 98%-101%  
LOD: 0.081 μg/ml  
LOQ: 0.246 μg/ml | [30] |
| 2     | Fluvastatin in Pharmaceutical Preparations | Spectrophotometric      | Detection: 462 nm  
Linearity Range: 15.0–50.0 and 10.0–90.0 μg/ml  
LOD: 0.017 and 0.134 μg/ml | [31] |

Table III: Reported Analytical Method of Fluvastatin. [30-36]
<table>
<thead>
<tr>
<th></th>
<th>Description</th>
<th>Column/Phase</th>
<th>Mobile/Stand Phase</th>
<th>Detection</th>
<th>Linearity Range</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Fluvastatin in human plasma</td>
<td>HPLC</td>
<td>Stationary Phase: C\textsubscript{18} column Mobile phase: Methanol:13 mM tetrabutylammonium fluoride (3:2 v/v) Detection: 305 and 380nm</td>
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<tr>
<td>4</td>
<td>Fluvastatin Sodium in Bulk and Dosage Form</td>
<td>Stability Indicating RP-HPLC</td>
<td>Stationary Phase: Phenomenex Luna C\textsubscript{18} column Mobile phase: Acetonitrile and 0.02M potassium phosphate buffer (50: 50, v/v, pH 5) Flowrate: 1ml/min Detection: 235nm Linearity Range: 5-40μg/ml Retention time: 4.50 min LOD: 1.1μg/ml LOQ: 3.3μg/ml</td>
<td></td>
<td></td>
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<tr>
<td>5</td>
<td>Fluvastatin sodium in Bulk and its dosage form</td>
<td>RP-HPLC</td>
<td>Stationary Phase: Hypersil ODS C\textsubscript{18} column Mobile phase: Methanol: 20mM Phosphate buffer (pH 3.0 adjusted with Phosphoric acid): acetonitrile (5:3: 2 v/v) Flowrate: 1.2ml/min Detection: 235nm Linearity Range: 1-6μg/ml Retention time: 7.65 min LOD: 0.0194μg/ml LOQ: 0.0588μg/ml</td>
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<tr>
<td>6</td>
<td>Fluvastatin sodium in bulk and Pharmaceutical dosage form</td>
<td>HPTLC</td>
<td>Stationary Phase: Silica gel 60 F\textsubscript{254} Mobile phase: Methanol: Ethyl acetate: Toluene: Glacial acetic acid (3:5:1.8:0.2 v/v) Detection: 235 nm Linearity Range: 100-600ng/band LOD: 26.74ng/band LOQ: 81.05ng/band</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>7</td>
<td>Fluvastatin sodium in Bulk drug and dosage form</td>
<td>HPTLC</td>
<td>Stationary Phase: Silica gel 60 F\textsubscript{254} Mobile phase: Chloroform: Toluene: Methanol (6:2:2) Detection: 305nm Linearity Range: 300-800ng/spot LOD: 65ng/spot LOQ: 200ng/spot</td>
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</tbody>
</table>
Table IV: Reported method of Fluvastatin in combination with other drugs.\[37-39\]

<table>
<thead>
<tr>
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<th>Method</th>
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<th>Ref No.</th>
</tr>
</thead>
</table>
| 8      | Fluvastatin and Fenofibrate in bulk drug and dosage form | UV Spectrophotometric   | **Detection:** Fluvastatin-304nm  
Fenofibrate-288nm  
**Linearity Range:** Fluvastatin- 8-24μg/ml  
Fenofibrate- 2-16μg/ml  
**Correlation Co-efficient:** 0.999  
% Recovery: 98% | [37]  |
| 9      | Fluvastatin+ Valsartan                     | RP-HPLC                 | **Stationary Phase:** X-Terra C\textsubscript{18} column  
**Mobile phase:** Acetonitrile: Potassium dihydrogen ortho phosphate buffer (pH 5, 60:40 v/v)  
**Flowrate:** 0.7ml/min  
**Detection:** 237 nm  
**Linearity Range:** Fluvastatin -20-60μg/ml  
Valsartan-40-120μg/ml  
**Retention time:** Fluvastatin- 2.5 min  
Valsartan- 3.5 min  
**LOD:** Fluvastatin-3.01μg/ml  
Valsartan-2.99μg/ml  
**LOQ:** Fluvastatin-10μg/ml  
Valsartan-9.99μg/ml | [38]  |
| 10     | Pravastatin+ Fluvastatin+ Atorvastatin +Rosuvastatin | Stability Indicating RP-HPLC | **Stationary Phase:** C\textsubscript{18} column  
**Mobile phase:** Methanol–Water (60:40 v/v) and (70:30 v/v )  
**Flowrate:** 1ml/min  
**Correlation Co-efficient:** 0.999  
**LOD:** Pravastatin-1.22 μg/ml  
Fluvastatin-2.02 μg/ml  
Atorvastatin-0.44μg/ml  
Rosuvastatin-1.55 μg/ml  
**LOQ:** Pravastatin-3.08 μg/ml  
Fluvastatin-6.12 μg/ml  
Atorvastatin -1.34 μg/ml  
Rosuvastatin -4.70 μg/ml | [39]  |
CONCLUSION
This review depicts the reported Spectroscopic and Chromatographic methods developed and validated for estimation of Ezetimibe and Fluvastatin. According to this review it was concluded that for Ezetimibe and Fluvastatin different Spectroscopic and Chromatographic methods are available for single and combination. The mobile phase containing Acetonitrile, Methanol, and Phosphate buffer were common for most of the chromatographic method to provide more resolution. For chromatographic method flow rate is observed in the range 0.6-1.5 ml/min to get good resolution time. For most of the Spectroscopic methods common solvent is Methanol. Hence this all methods found to be simple, accurate, economic, precise and reproducible in nature. Most of Methods were of RP-HPLC and UV absorbance detection because these methods provided with best available reliability, repeatability, analysis time and sensitivity.

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REFERENCES


