Chromatographic and spectrophotometric method for estimation of statin class drugs in bulk and in different dosage forms

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ABSTRACT

Hydroxy Methyl Glutaryl CoA reductase (HMG CoA reductase) inhibitors also know as statins are the most efficacious and best tolerated Dyslipidemic drugs. Statins are the treatment of choice for the management of Dyslipidemia because of their proven efficacy and safety profile. They also have an increasing role in managing cardiovascular risk in patients with relatively normal levels of plasma cholesterol. They competitively inhibit conversion of 3-Hydroxy-3-Methyl glutaryl coenzyme A (HMG-co A) to mevalonate by the enzyme HMG-CoA reductase. This result in compensatory increase in Low Density Lipoprotein receptor expression on Liver cells which leads to increased receptor mediated uptake and catabolism of Intermediate density lipoprotein and Low density Lipoprotein. This article narrates different chromatographic (HPLC, HPTLC, UPLC, LC) & different spectrophotometric method (UV) for Statin class single drug as well as combination with other drug.

Key words: Hydroxy Methyl Glutaryl CoA reductase inhibitor (HMG CoA reductase inhibitor), UV- Spectroscopy, HPLC (High Performance Liquid Chromatography), HPTLC (High Performance Thin Layer Chromatography), LC (Liquid Chromatography)

INTRODUCTION

Due to the marked lowering of low-density lipoprotein cholesterol (LDL-C) Statins have become a cornerstone of treatment for Dyslipidaemia. Studies show that regardless of age, sex, prior history of coronary heart disease (CHD) or other co-morbid conditions, Statin treatment typically reduces relative risk of cardiovascular disease by 24-37%. There is also a growing body of evidence that effectivity of statins can be seen in people whose LDL-C is not considered elevated under current guidelines.[1]

The statins have the capacity to reduce cholesterol biosynthesis mainly in liver, where they are selectively distributed, as well as the modulation of lipid metabolism, derived from their effect of inhibition upon HMG-CoA reductase. Percent decrease in LDL cholesterol is positively correlate with antiatherosclerotic effects of the Statins. [2]

Statins act by blocking HMG-CoA reductase enzyme, which is useful in catalyzing the rate-limiting step in cholesterol synthesis. All statins are competitively inhibiting HMG-CoA reductase with respect to the binding of substrate, HMG-CoA, but not for that co-enzyme NADPH, suggesting that their HMG-CoA-like moieties bind to the HMG-CoA-binding portion of the enzyme active site.[3]

Statins competitively inhibit conversion of 3-Hydroxy-3-Methyl glutaryl coenzyme A (HMG-co A) to mevalonate by the enzyme HMG-CoA reductase. This result in compensatory increase in Low Density Lipoprotein receptor expression on Liver cells which leads to increased receptor mediated uptake and catabolism of Intermediate density lipoprotein and Low density Lipoprotein. Over long term, feedback induction of HMG-CoA reductase tends to
increase Cholesterol synthesis, but steady-state is finally attained with a dose-Dependent lowering of LDL-CH levels.\cite{4}

HMG CoA reductase inhibitors include Simvastatin, Mevastatin, Lovastatin, Atorvastatin, Fluvastatin, Rosuvastatin, Cerivastatin, Pravastatin, Pitavastatin. From that Simvastatin, Lovastatin, and Pravastatin are the statins which are derived from fungal metabolites and have elimination half-lives of 1–3 hr. While, Atorvastatin, Cerivastatin, Fluvastatin, Pitavastatin and Rosuvastatin are fully synthetic compounds.

This Review Article offers an overview of various analytical methods for estimation of HMG CoA reductase Inhibitors. Different methods have been developed for estimation of Statins like UV-Spectroscopy, Liquid Chromatography, HPTLC and RP-HPLC.

Reported methods are categorized depending on the following considerations:
1. Single component HMG CoA reductase Inhibitors analyzed by UV-Spectroscopy methods and Chromatographic method.
2. Analysis of HMG CoA reductase Inhibitors with combination with other class drugs by UV-Spectroscopy methods and Chromatographic method.

### TABLE 1: Analysis of single component HMG CoA reductase Inhibitors by UV-Spectroscopy methods

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Drug Description</th>
<th>Method</th>
<th>Detection wavelength</th>
<th>Linearity range</th>
<th>Co-relation Coefficient</th>
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<th>LOQ</th>
<th>% Recovery</th>
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<td>1</td>
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<td>Ultraviolet Spectroscopy</td>
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<td>5-25 µg/ml</td>
<td>0.999</td>
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<td>2</td>
<td>Determination of Fluvastatin Sodium in Bulk and Pharmaceutical Formulations</td>
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<td>304 nm in sodium hydroxide</td>
<td>5-25 µg/mL-1</td>
<td>0.999</td>
<td>0.0811 µg/mL-1</td>
<td>0.2460 µg/mL-1</td>
<td>≤0.937%</td>
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<td>97.6-100.1</td>
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<td>0.1025</td>
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<td>0.982</td>
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<td>10 µg/mL</td>
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<tr>
<td>No.</td>
<td>Method Description</td>
<td>Method Type</td>
<td>Detection Wavelength</td>
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<td>Linearity Range</td>
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<td>0.39 µg/ml</td>
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<td>0.998 µg/ml</td>
<td>0.20 µg/mL</td>
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<td>0.998 µg/ml</td>
<td>0.20 µg/mL</td>
<td></td>
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<td>Table</td>
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<td>Description</td>
<td>Method</td>
<td>Detection Wavelength</td>
<td>Linearity Range</td>
<td>Co-relation Coefficient</td>
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<td>UV-spectrophotometric methods</td>
<td></td>
<td>Rosuvastatin: e difference between 246 nm &amp; 255 nm</td>
<td>0.999 for both</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Generic Compounds Used**
- **Fluvastatin**: 8-24 µg/ml
- **Fenofibrate**: 2-16 µg/ml
- **Rosuvastatin calcium**: 10-50 µg/ml
- **Aspirin**: 40-120 µg/ml
- **Telmisartan**: 5-40 µg/ml
- **Atorvastatin**: 4-32 µg/ml
- **Fenofibrate**: 2-16 µg/ml
- **Rosuvastatin calcium**: 1.672 µg/ml
- **Aspirin**: 7.4277 µg/ml
- **Telmisartan**: 5.0696 µg/ml
- **Aspirin**: 22.5083 µg/ml

**Accuracy**
- **98-102%**

**% Recovery**
- **98% - 102% for both the drugs**
<table>
<thead>
<tr>
<th>Page</th>
<th>Description</th>
<th>Methodology</th>
<th>Mobile phase</th>
<th>Stationary phase</th>
<th>Temperature</th>
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<td>Liquid chromatography</td>
<td>Acetonitrile–28 mM phosphate buffer solution, pH 4 (65 + 35)</td>
<td>C18 column</td>
<td>251°C</td>
<td>1.0 ml/min</td>
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<td>Liquid chromatography</td>
<td>Acetonitrile–28 mM phosphate buffer solution, pH 4 (65 + 35)</td>
<td>C18 column</td>
<td>251°C</td>
<td>1.0 ml/min</td>
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<td>Simvastatin in microemulsion formulation</td>
<td>RP-HPLC Method</td>
<td>Acetonitrile: water : triethylamine in ratio of 80 : 19.8 : 0.2 (v/v/v)</td>
<td>Phenomenex Luna</td>
<td>251°C</td>
<td>1.0 ml/min</td>
<td>8.6 minute</td>
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<td>23</td>
<td>Simvastatin in Bulk and Pharmaceutical Formulation</td>
<td>HPLC Method</td>
<td>Mixture of methanol and 0.1% ortho phosphoric acid in water (10:90)</td>
<td>C18 column</td>
<td>251°C</td>
<td>1.0 ml/min</td>
<td>3.106 min</td>
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<td>Liquid chromatographic</td>
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<td>Phenomenex Luna</td>
<td>251°C</td>
<td>1.0 ml/min</td>
<td>3.0 minutes</td>
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<td>Stability indicating LC-MS/MS method for estimation of Lovastatin in human plasma:</td>
<td>LC-MS/MS method</td>
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<td>C18 column</td>
<td>251°C</td>
<td>1.0 ml/min</td>
<td>3.9 minutes</td>
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<td>HPLC Method</td>
<td>Acetonitrile: Water : triethylamine in ratio of 80 : 19.8 : 0.2 (v/v/v)</td>
<td>Phenomenex Luna</td>
<td>251°C</td>
<td>1.0 ml/min</td>
<td>3.5 minutes</td>
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<td>Simvastatin and Niacin in binary Combination</td>
<td>HPTLC Method</td>
<td>Methanol: Water: Acetic acid (80:20:0.1)</td>
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<td>251°C</td>
<td>1.0 ml/min</td>
<td>3.5 minutes</td>
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<td>Sitagliptin and Simvastatin in Tablets</td>
<td>RP-HPLC Method</td>
<td>Methanol and water (70:30, v/v) with 0.2 %</td>
<td>C18 column</td>
<td>251°C</td>
<td>1.0 ml/min</td>
<td>5.70 min</td>
</tr>
</tbody>
</table>
of n-heptane sulfonic acid adjusted to pH 3.0 with ortho phosphoric acid
Stationary phase: C\textsubscript{8} (Qualisil BDS, 250\times4.6 mm, 5 \(\mu\))
Retention time:
Simvastatin = 4.3 min
Sitagliptin = 30.4 min
Flow rate: 1.0 ml/min

#### 29 Simvastatin and Ezetimibe in Pure and Pharmaceutical Dosage Forms
HPTLC Method
Detection wavelength: 220 nm
Mobile phase: Ethyl acetate:chloroform (80:20)
Stationary phase: TLC plate precoated with silica gel 60F 254
Retention time:
Simvastatin = 0.76
Ezetimibe = 0.89
Flow rate: 1.0 ml/min

#### 30 Simvastatin and Niacin in tablet Dosage Form
RP-HPLC Method
Detection wavelength: 250 nm
Mobile phase: methanol:water in ratio 85:15 water consisting of Triethylamine (TEA) (0.05%v/v) v/v
Stationary phase: C18 column (phenomenx, 150 \times 4.6 mm i.d.).
Retention time:
Simvastatin = 8.5 min
Niacin = 1.8 min
Flow rate: 1.0 ml/min

### CONCLUSION
This review depict the reported Spectrophotometric and Chromatographic methods; developed and validated for estimation of HMG CoA reductase inhibitors. According to the literature review it was concluded that for HMG CoA reductase (Mevastatin, Lovastatin, Atorvastatin, Simvastatin, Fluvastatin, Rosuvastatin, Pravasatatin, Pitavastatin) different Spectroscopic &Chromatographic methods are available for Single component as well as for combination. 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.

Most common combination of HMG CoA reductase inhibitors were with Telmisartan. But there is no reported method for Simvastatin and Telmisartan in synthetic mixture. So there will be a great scope for development of highly Precise, Accurate, Simple as well as rapid analytical methods for latest drugs such as Simvastatin and Telmisartan.

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