Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(10):473-479

Review Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

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.[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 I: Analysis of single component Statins by UV-spectroscopy methods

Sr. No.	Drug	Method	Description	Ref. No.
1	Estimation of **Pravastatin** by spectrophotometric method	Ultraviolet Spectroscopy	**Detection wavelength:** 737nm **Linearity range:** 5-25µg/ml **Co-relation Coefficient:** 0.999.	5
2	Determination of **Fluvastatin** Sodium in Bulk and Pharmaceutical Formulations	Ultraviolet Spectroscopy	**Detection wavelength:** 304 nm in sodium hydroxide **Linearity range:** 5-25 µg mL-1 **Co-relationCoefficient:** 0.999. **LOD:** 0.0811 µg mL-1 **LOQ:** 0.2460 µg mL-1 **Relative standard deviations:** ≤ 0.937% **%Recovery:** 98.60% -101.70%.	6
3	Determination of **Pravastatin Sodium** in bulk and tablet formulations	Ultraviolet Spectroscopy	**Detection wavelength:** 240 nm in water **Linearity range:** 2-18µg/ml. **Co-relationCoefficient :** 0.9999 **%Recovery:** 97.6-100.1	7
4	Estimation of **Atorvastatin Calcium** Form Tablet Dosage Forms	Ultraviolet Spectroscopy	**Detection wavelength:** 240 nm in 2.0 M urea solution **Linearity range:** 5-45 µg/ml **Co-relationCoefficient**: 0.999. **LOD:** 0.1025 **LOQ:** 0.3789	8
5	Determination of **Rosuvastatin calcium** in marketed formulation	Spectrophotometric Method	**Detection wavelength:** 1. **At absorption maxima:** 252 nm 2. **Area under curve:** 247-257 nm 3. **First order derivative maxima and minima:** 238 nm and 205 nm **Solvent :** Methanol **Linearity range:** 5-35 µg/ml for all 3 methods. **Co-relationCoefficient :** 1. 0.974 2. 0.982 3. 0.982	9
6	Estimation of **Atorvastatin Calcium** in Tablet Dosage Form	Spectrophotometric Method	**Detection wavelength:** 246nm in methanol **Linearity range:** 5-25 µg/ml **Co-relationCoefficient**: 0.999. **Absorption maxima:** 10 µg/ml **Recovery studies:** 99.96%-100.03%	10

7	Development and Validation of a UV-Spectrophotometric Method for Quantification of **Atorvastatin** in Tablets	UV-spectrophotometric method	**Detection wavelength:**248nm **Solvent** : methanol: water (50:50) **Linearity range:** 5-15 µg/ml **Absorption maxima** : 10 µg/ml **Recovery studies :**98.78%-100.36%	11
8	**Simvastatin** in bulk and tablet dosage form	Stability indicating UV-spectrophotometric method	**Detection wavelength :** 237 nm **Linearity range**: 3-18 µg/ml **Correlation coefficient** 0.9998 **Limit of Detection:** 0.73 µg/ml **Limit of Quantification:** 2.07 µg/ml	12
9	Validated Simple UV Spectrophotometric Method for the Estimation of **Pitavastatin** in bulk and Pharmaceutical Dosage Form	UV-spectrophotometric method	**Detection wavelength:** 249.5nm **Solvent :** 0.1N HCL **Linearity range:** 2-12µg/ml. **Co-relationCoefficient**: 0.9996 **%Recovery :** 99.83 + 0.39 % **LOD:** 0.122 µg/mL **LOQ**: 0.371 µg/mL	13
10	Simple UV Spectrophotometric Determination of **Rosuvastatin Calcium** in Pure Form and in Pharmaceutical Formulations	UV Spectrophotometric Determination	**Detection wavelength:** 244 nm **Solvent :** methanol **Linearity range:** 2-18 µg/mL. **Co-relationCoefficient:** 0.9978 **Molar absorptivity:** 7.2646 x 104 L/mol.cm	14
11	Simultaneous Estimation of **Ezetimibe** and **Lovastatin** by Derivative Spectroscopy	Derivative Spectroscopy	**Detection wavelength:** **Ezetimibe :** 265.20nm **lovastatin :** 245.4 nm **Linearity range:** **Ezetimibe** : 1- 40 µg/ml **Lovastatin** : 1-40 µg/ml **LOD:** **Ezetimibe :** 0.39 µg/ml **Lovastatin :** 0.12 µg/ml **LOQ:** **Ezetimibe :** 1.3 µg/ml **Lovastatin :** 0.41 µg/ml	15
12	**Simvastatin** and **Metformin** hydrochloride in bulk and solid dosage form	UV spectrophotometric method	**Detection wavelength :** **Simvastatin :** 247 nm **Metformin hydrochloride :** 232.2 nm **Linearity range**: **Simvastatin :** 5-15 µg/ml **Metformin hydrochloride :** 2-16 µg/ml **Regression Coefficient (r2**) : **Simvastatin :** 0.9982 **Metformin hydrochloride :** 0.9977 **Limit of Detection:** **Simvastatin :** 0.333 **Metformin hydrochloride :** 0.201 **Limit of Quantification:** **Simvastatin :** 1.009 **Metformin hydrochloride :** 0.609	16
13	Simultaneous uv spectrophotometric estimation of **rosuvastatin** and **ezetimibe** in their combined dosage forms	Simultaneous UV-spectrophotometric method	**Q-absorption Ratio method** using two wavelengths, **Detection wavelength :** **Ezetimibe** = 232.4 nm **Isoabsorptive point** = 237 nm **Linearity range**: **Rosuvastatin :** 1-10µg/ml **Fenofibrate :** 2-20µg/ml **Co-relationCoefficient** **Rosuvastatin :** 0.998 **Fenofibrate :** 0.999	17
14	UV Spectrophotometric estimation of **Rosuvastatin Calcium** and **Fenofibrate** in bulk Drug and Dosage Form using Simultaneous Equation Method	UV Spectrophotometric	**Detection wavelength :** **Rosuvastatin :** 244nm **Fenofibrate :** 286.7nm **Solvent :** methanol **Linearity range**: **Rosuvastatin :** 1-10µg/ml **Fenofibrate:** 2-20µg/ml **Co-relationCoefficient** **Rosuvastatin :** 0.998 **Fenofibrate :** 0.999	18
15	Simultaneous Spectrophotometric Estimation of **Fluvastatin** and **Fenofibrate** in	Spectrophotometric Estimation	**Detection wavelength :** **Fluvastatin :** 304 nm	19

	Bulk Drug and Dosage Form by using Simultaneous Equation Method		**Fenofibrate :** 288 nm **Solvent :** methanol **Linearity range**: **Fluvastatin :** 8-24 µg/ml and **Fenofibrate :** 2-16 µg/ml **Co-relationCoefficient** 0.999 for both	
16	Development and Validation of Spectrophotometric Method For Simultaneous Estimation of **Rosuvastatin Calcium** and **Aspirin** In Bulk and Pharmaceutical Dosage Form	Spectrophotometric Method	**Detection wavelength :** **Q-absorption** **Iso-absorptive point :** 257nm as **λmax of Rosuvastatin calcium :** 244nm **Linearity range**: **Rosuvastatin calcium :** 10-50 µg/ml **Aspirin :** 40-120 µg/ml **Percentage purity :** 98-102% **Accuracy :** 98-102% **Co-relationCoefficient** **Rosuvastatin :** 0.997 at 244 and 0.9966 at 257nm. **Aspirin:** at 244 0.9994 and at 257 nm is 0.998. **LOD :** **Rosuvastatin calcium:**1.6729µg/ml **Aspirin :**7.4277 µg/ml **LOQ** **Rosuvastatin calcium:** 5.0696 **Aspirin :** 22.5083	20
17	Validated spectrophotometric methods for the simultaneous determination of **Telmisartan and Atorvastatin** in bulk and tablets	Spectrophotometric methods	**First-order derivative spectroscopy**. **Detection wavelength :** 1. zero crossing of TELM 223 nm 2. Zero crossing of ATV 272 nm. **Linearity range**: **Atorvastatin :**4-32 µg/ml **Telmisartan :** 5–40 µg/ml **Co-relationCoefficient** **Telmisartan:** 0.997 **Atorvastatin :**0.9939 **Limit of Detection:** **Atorvastatin :** 0.37 **Telmisartan :** 0.40 **Limit of Quantification:** **Atorvastatin :** 2.17 **Telmisartan :** 2.12	21
18	Stability-indicating uv-vis spectrophotometric method for estimation of **Atorvastatin calcium and Fenofibrate** in tablet dosage form	UV-vis spectrophotometric method	**Detection wavelength :** **Atorvastatin :**-247 nm **Fenofibrate :** 287 nm **Linearity range**: **Atorvastatin :** 6-16 µg/ml **Fenofibrate :** 2-12 µg/ml **Co-relationCoefficient** 0.999 for both **Limit of Detection:** **Atorvastatin :** 0.2695 µg/ml **Fenofibrate :** 0.0222 µg/ml **Limit of Quantification:** **Atorvastatin :** 0.8780 µg/ml **Fenofibrate :** 0.222 µg/ml	22
19	Development and validation of uv spectrophotometric methods for simultaneous estimation of **Rosuvastatin and Telmisartan** in tablet dosage form	UV-spectrophotometric methods	**Detection wavelength :** Dual Wavelength (method 1) **Telmisartan:** difference between 237.7 nm & 250 nm. **Rosuvastatin:** e difference between 246 nm & 255 nm **Ratio derivative method** Concentration of **Telmisartan** : 8 µg/ml as divisor for **Rosuvastatin** concentration of **Rosuvastatin :** 2 µg/mL as divisor for **Telmisartan** **First derivative of ratio** **Telmisartan** : 237.2 nm for RSV **Rosuvastatin** : 229 nm for TEL. **Linearity range**: **Telmisartan** : 8-24 µg/mL **Rosuvastatin** : 2-6 µg/mL **Co-relationCoefficient** 0.999 for both drug and both method **% Recovery :** 98% - 102% for both the drugs.	23

20	**Simvastatin** under hydrolytic condition	Liquid chromatography	**Mobile phase:** **Acetonitrile**–28 mM phosphate buffer solution, pH 4 (65 + 35) **Stationary phase:** C18 column Column temperature : 251°C **Flow rate**: 1.0 ml/min	24
21	Estimation of **Pitavastatin calcium** in tablet dosage forms by column liquid chromatography	Liquid chromatography	**Mobile phase** Isocratic mode **Acetonitrile: water : triethylamine** in ratio of 80 : 19.8 : 0.2 (v/v/v) pH 3.5 0.05 with orthophosphoric acid **Flow rate:** 1.5 mL/min. **UV detection:** 220nm wavelengths. **Retention time:** 5.70 min.	25
22	**Simvastatin** in microemulsion formulation	RP-HPLC Method	**Detection wavelength:** 238 nm **Mobile phase:** 0.1% Triethylamine buffer (pH 7.5): Acetonitrile (20:80v/v) **Stationary phase:** (Phenomenex Luna C18 5µm 4.6×250mm (i.d) column **Retention time:** 8.6 minute. **Flow rate**: 1.0 ml/min **Linearity range**: 200- 600 ng/ml **Correlation coefficient:** 0.999. **Limit of Detection:** 5ng/ml **Limit of Quantification :** 40ng/ml	26
23	**Simvastatin** in Bulk and Pharmaceutical Formulation	HPLC Method	**Detection wavelength:** 238 nm **Mobile phase:** Mixture of methanol and 0.1% ortho phosphoric acid in water (10:90) **Stationary phase:** C18 column (150x4.6 mm, 2.7 µm) **Retention time:** 3.106 min	27
24	Development and validation of a reverse-phase liquid chromatographic method for determination of related substances of pitavastatin (FOR 2 AND 4 MG TABLETS)	Liquid chromatographic	**Mobile phase:** **Mobile Phase A** Acetonitrile: Buffer in proportion of 10:90 (v/v) **Mobile Phase B** acetonitrile : water in the ratio of 90:10(v/v) **Flow rate:** 1.0ml/min **UV detection**: 250nm wavelengths. **Run time**: 30 minutes.	28
25	Stability indicating LC-MS/MS method for estimation of **Lovastatin** in human plasma:	LC-MS/MS method	**Mobile phase:** Acetonitrile and 2 mM ammonium acetate buffer ratio of 90:10, v/v. pH 3.6 **Linearity range:** 0.121–35.637 ng/mL **run time** of 4.5 min	29
26	**Simvastatin** and **Ezetimibe** in Tablets	HPLC Method	**Detection wavelength :** 240 nm **Mobile phase:** 0.01 M ammonium acetate buffer and acetonitrile (35:65 v/v) **Stationary phase:** C18 Supelcosil column (250 mm x 4.6 mm; 5µ) **Retention time:**. **Simvastatin** = 8.5 min **Ezetimibe** = 5.9 min **Flow rate**: 1.0 ml/min	30
27	**Simvastatin** and **Niacin** in binary Combination	HPTLC Method	**Detection wavelength :** 236 nm **Mobile phase:** Methanol: Water: Acetic acid (80:20:0.1) **Stationary phase:** RP18 plate **concentration range** **Niacin** = 12.5-37.5 µg/spot **Simvastatin** = 0.25-0.75 µg/spot **Correlation coefficient**: greater than 0.999.	31
28	**Sitagliptin a**nd **Simvastatin** in Tablets	RP-HPLC Method	**Detection wavelength:** 253 nm **Mobile phase:** methanol and water (70:30, *v/v*) with 0.2 %	32

			of n-heptane sulfonic acid adjusted to pH 3.0 with *ortho* phosphoric acid **Stationary phase:** C_8 (Qualisil BDS, 250×4.6 mm, 5 µ) **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	33
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 x 4.6 mm i.d.). **Retention time:**. **Simvastatin** = 8.5 min **Niacin** = 1.8 min **Flow rate**: 1.0 ml/min	34

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 withTelmisartan. 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.

Acknowledgement
The authors are thankful to Dr. K. Pundarikakshudu, Director of L. J. Institute of Pharmacy, Ahmedabad, India for providing all the facilities and encouragement to carry out the work.

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