Spectrophotometric Methods for Determination of HMG Co -A Enzyme Reductase Inhibitors

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Abstract

Statins are lipid lowering medications used in the primary and secondary prevention of coronary heart disease by clocking HMG Co-A reductase, the rate limiting enzyme in cholesterol biosynthesis. There has been significant research on wide range of analytical techniques that could be useful in the estimation of inhibitors in formulations and biological matrices. Spectrophotometry includes the estimation of the substance concentration with the absorbance of visible or UV light by the substance and comparing it with standard values, measured under identical Conditions. This method is widely used for industrial and research purposes, since it is relatively inexpensive, easy to carry out and can be done in a routine manner. This review is intended to give an insight into some of the Spectrophotometric methods reported for the determination of Lovastatin, Simvastatin, Pravastatin, Atorvastatin, Rosuvastatin, Pitavastatin in pure forms, in different pharmaceutical dosage forms.
Keyword: Lovastatin, Simvastatin, Pravastatin, Atorvastatin, Rosuvastatin, Spectrophotometric methods.

I. INTRODUCTION: [1-6]

Cholesterol is vital requirement in a healthy human heart which is produced by cells to perform its function in the body. Hypercholesterolemia can occur with additional dietary intake of cholesterol along with other factors i.e. age, gender and physical inactivity. Hypercholesterolemia is one of the main risk factors of cardiovascular disease, causing high blood pressure and atherosclerosis.
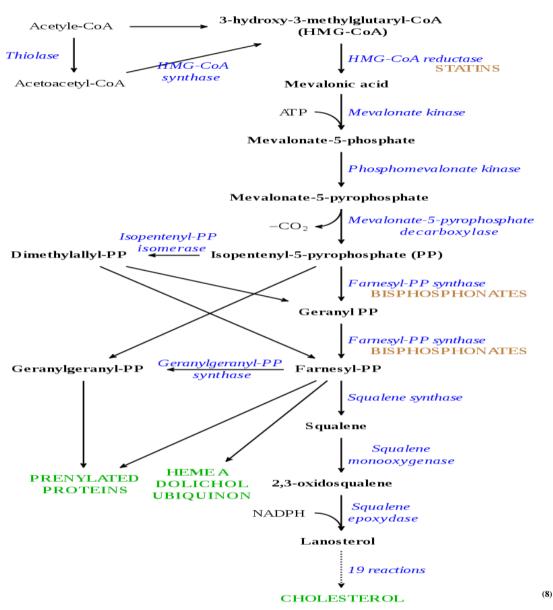
To prevent this, drugs or substances have been developed that may reduce cholesterol absorption from the digestive tract or destroy cholesterol by enzymatic means.

Statins are class of agents that specifically inhibits 3-hydroxy-3-methylglutaril coenzyme A (HMG-CoA) reductase, a rate-limiting enzyme in cholesterol biosynthesis. They are effective in reducing the risk of cardiovascular disease by reducing total cholesterol and the low-densitylipoprotein (LDL) cholesterol levels in the human body and are recommended in clinical guidelines as a first line of prevention for this condition. The HMG-CoA reductase inhibitors currently in use are Lovastatin (LOV), Simvastatin (SMV), Pravastatin (PRV), Atorvastatin (ATR), Rosuvastatin (ROS), Pitavastatin (PIT).Statins are often co-administered with other drugs such as acetylsalicylic acid, antihypertensive drugs for therapy of cardiovascular disease, and also in combined therapy of multiple disorders, with antidiabetics, diuretics, nonsteroidal anti-inflammatory drugs and other analgesics, antibiotics. The application of satins alone and in combination with antihypertensive, antipyretic, analgesic, anti-inflammatory and antithrombotic and antidiabetic drugs for long-term therapy requires development of high effective methods for monitoring of their potential impurities and degradation products. Identification and determination of drug-related substances is an important analytical task because impurities and degradation products of drugs are often responsible for side effects.

Mechanism of action of HMG-COA reductase inhibitors [7]

In synthesis of cholesterol, HMG-CoA reductase enzyme converts HMG-CoA to mevalonate which is rate limiting step. As statins are structural analogues of HMG-CoA reductase, they competitively inhibit activity of the enzyme. Owing to this, amount of cholesterol decreases which triggers DNA, resulting in increased synthesis of LDL receptor through mRNA. As the amount of LDL receptor increases, the circulating LDL in blood binds to LDL receptor to form complex leading to clearance of LDL from blood. This decreased amount ofLDL leads to decrease in cholesterol.

Pharmacokinetics

(8)

Statins differ in their potency and maximal efficacy in reducing LDL-CH levels. Lovastatin, simvastatin and pravastatin are low to moderate efficacy and potency (20-45% LDL reduction) while atorvastatin, rosuvastatin and pitavastatin are high efficacy (UPTO 55% LDL reduction). As HMG-CoA reductase activity is maximum at midnight, all statins are administered at bed time to obtain maximum effectiveness .Summary of different routes of administration, bioavailability, metabolism, plasma protein binding and excretion between the individual compounds is depicted in Table 1. The different physicochemical properties of HMG-co A reductase inhibitors are represented in Table 2.

Table 1: Pharmacokinetic properties of HMG-CoA reductase inhibitors

Pharmacokinetic parameters	LOV	SMV	PRV	ATR	ROS	PIT
Administration route	Oral	Oral	Oral	Oral	Oral	Oral
Bioavailability	<5	5	17	14	20	60
Metabolism	CYP3A4	CYP3A4	CYP3A4	CYP3A4	CYP2C9	CYP2C9
Elimination half-life ,h	13.37	1-3	1-3	14	19	11
Plasma protein binding, %	>95	95	60	98	90	99

Excretion	Urine	Urine	Urine	Hepatic& urine	Urine	Urine

Table 2: Physicochemical properties of studied HMG-CoA reductase inhibitors.

Drug	P ka	Solubility	Λ max, nm
LOV	13.49	Sparingly soluble in ethanol ,methanol and Acetonitrile insoluble in water	238
SMV	14.91	Freely soluble in chloroform , Methanol and ethanol practically insoluble in water	248
PRV	4.2	Soluble in methanol and water slightly soluble in isopropanol practically insoluble in acetone,acetonitrile, chloroform and ether	238
ATR	4.54	Freely soluble in methanol ,slightly soluble in ethanol, very slightly soluble in distilled water, acetonitrile	246
ROS	4	Slightly soluble in ethanol, sparingly soluble in water and methanol	252
PIT	4.3	Freely soluble in chloroform and THF, slightly soluble in methanol very slightly soluble in water and ethanol	238

The most commonly used methods for determination of statins are being high performance liquid chromatography (HPLC) and solid phase extraction processes. Though they are accurate, they have several disadvantages such as being time consuming, expensive and require extensive training to operate. Since the pharmaceutical industry is operated in a continuous manner, the routine analysis using these technologies is quite an obstacle for the drug manufacturing companies. Hence, there is a need to develop simple, quick and inexpensive methods that can be used in a routine manner. Spectrophotometry is widely used for industrial and research purposes since it is relatively inexpensive, easy to carry out and can be done in a routine manner. The purpose of this work is to summarize the recent spectrophotometric methods for analysis of statins.

SPECTROPHOTOMETRY

Spectrophotometry is a standard and inexpensive technique to measure light absorption or the amount of chemicals in a solution. It uses a light beam which passes through the sample, and each compound in the solution absorbs or transmits light over a certain wavelength. Spectrometry is measured by a spectrophotometer; an instrument that is made up of two instruments – a spectrometer and a photometer. The spectrometer produces the light of the wavelength and the photometer measures the intensity of light by measuring the amount of light that passes through the sample.

In addition to those two components, spectrophotometers consist of a light source, a monochromator, a sample chamber containing a cuvette, a detector (such as a photomultiplier tube or photodiode) to detect the transmitted light, a digital display and a data analysis

Reported spectrophotometric methods [9]

Ultraviolet spectrophotometric methods such as Simultaneous equation, absorption ratio, area under Curve, dual wavelength have been reported for determination of different HMG-CoA inhibitors in bulk and pharmaceutical dosage forms. Simple UV spectrophotometric and area under curve methods were developed for the determination of LOV In pharmaceutical dosage form. First order and Second order derivative methods were used for determination of LOV in combination with niacin as well As ascorbic acid (AA), quercetin (QC) and gallic acid (GA).Simple UV spectrophotometric, absorption ratio, first and second order derivative methods were developed for determination of SMV in pharmaceutical dosage form. Derivative spectrophotometry, simultaneous equation, dual wavelength and absorption ratio methods were used for determination of SMV in combination with Ezetimibe(EZE), metformin (MET), sitagliptin (SITP), gefitinib(GEF), losartan potassium (LOSA) labetalol (LTL).

Simple UV spectrophotometric method was developed for determination of PRV in pharmaceutical dosage form. Simple UV spectrophotometric and bioanalytical methods were developed for determination of (ATR) in pharmaceutical dosage form. Derivative spectrophotometry, simultaneous equation and standard Addition method were developed for ATR in combination with telmisartan (TEL), amlodipine, fenofibrate (FEN)and metformin (MET). A simple, precise and accurate UV spectrophotometric method for simultaneous estimation of Rosuvastatin calcium and Fenofibrate in binary mixture was developed. It involves absorbance measurement at 243 nm (λ max of Rosuvastatin calcium) and 287 nm (λ max of Fenofibrate) in methanol. The linearity was obtained in the range of 1-6 µg/ml and 4-28 µg/ml for Rosuvastatin calcium and Fenofibrate respectively. The recovery values were obtained within the limits of 98.41 – 99.17 % and 100.02 – 100.18 % for Rosuvastatin and Fenofibrate respectively. The % RSDs for Rosuvastatin Calcium and Fenofibrate were reported as less than 2% for both the drugs. The method has been successfully applied to pharmaceutical

formulation and was validated according to ICH guidelines.Two simple UV-spectrophotometric methods were developed for simultaneous determination of Rosuvastatin calcium and Fenofibrate in pharmaceutical formulations. Methanol AR grade was used as solvent. Method-I (Q-Absorbance ratio method) involves formation of Q-Absorbance equation at two wavelengths i.e., 255.99 nm (iso absorptive point) and 286 nm (λ max of Fenofibrate). The accuracy studies showed 100.56 % recovery for Rosuvastatin and 100.45 % recovery for Fenofibrate. The inter and intra-day precision obtained for Rosuvastatin Calcium and Fenofibrate were reported as 0.26-1.27 % (inter-day) ; 0.07-0.64 % (intra-day) and 0.27-1.20 % (inter-day) ; 0.06-0.70 % (intra-day) respectively. Method-II (Multicomponent Mode of Analysis) involves the measurement of absorbance at two wavelengths i.e., 243 nm (λ max of Rosuvastatin calcium)and 286 nm (λ max of Fenofibrate). The accuracy studies showed 100.87 % recovery for Rosuvastatin and 101.57 % recovery for Fenofibrate. The inter and intra-day precision obtained for Rosuvastatin Calcium and Fenofibrate were reported as 0.11-1.68 % (inter-day) ; 0.09-0.64 % (intra-day) and 0.18-1.98 % (inter-day) ; 0.13-0.87 % (intra-day) respectively. In both methods, Rosuvastatin calcium and Fenofibrate followed same linearity at the concentration range of 03-18 µg/ml at their respective λ max values. Both these methods were found to be accurate, precise and rugged as indicated by low values of %RSDs. Both these methods were also found to be rapid, economical and can successfully be applied for the routine analysis of bulk and combined tablet dosage forms.

Table 3: Spectrophotometric methods for determination of HMG-co A reductase inhibitors. [9-15]

S l. No	Drugs(s)	Method	Λ max, nm	Solvent
1	LOV	Absorbance maxima	248	Water
2	LOV	Absorbance maxima	233	Sodium acetate
3	LOV	Absorbance maxima	238	0.1 N NaOH
4	LOV	AUC	238	Water
5	LOV with SMV	Absorbance maxima	240	Ethanol
6	LOV with niacin	First and second derivative	261,273,245,249	Methanol
7	LOV with AA,QC,GA	Zero crossing and algorithm bivariate calibration derivative	238.4	Methanol:sodium thio sulphate (50:50,v/v)
8	SMV	Absorbance ratio	238	Ethanol: water (40:60,v/v)
9	SMV	Absorbance maxima	238	Methanol
10	SMV	Absorbance maxima	239	0.1N NaoH
11	SMV	Absorbance maxima	237	Methanol
12	SMV	Absorbance maxima	237.8	Ethanol
13	SMV	Absorbance maxima	235.8,238	Methanol
14	SMV	First and second derivative	238	Methanol
15	SMV	Second derivative	243	Aceto nitrile:0.025M phosphate buffer(pH 4.0)(65:35,v/v)
16	SMV with EZE	First derivative	219,265	Methanol
17	SMV with EZE	Simultaneous equation	223,254.5	Methanol: phosphate buffer (7:3,v/v)
18	SMV with EZE	Simultaneous equation	244,248	0.1 N NaOH
19	SMV with EZE	Simultaneous equation	235,266	Methanol
20	SMV with SITP	Simultaneous equation	232.2,247	Methanol :water(1:1,v/v)
21	SMV with SITP	Simultaneous equation	238,267	Methanol: water(90:10,v/v)
22	SMV with SITP	Simultaneous equation Simultaneous equation Q- Absorbance method	238,267,230,275, 254,274	Methanol Water Water
23	SMV with SITP	Simultaneous equation	232,246	Methanol: water(40:60,v/v)
24	SMV with GEF	Simultaneous equation	237.2,348	Methanol
25	SMV with LOSA	Second derivative	220.2,234.9	Methanol

26	SMV with LTL	Simultaneous equation	222.4,239	0.25N NaOH
27	PRV	Absorbance maxima	240	Distilled water
28	ATR	Bioanalytical method	241	Methanol
29	ATR	Absorbance maxima	245	Methanol: water(90:10,v/v)
30	ATR with TEL	First derivative Absorbance ratio	245,223	Methanol
31	ATR with FEN	Standard addition method	229,225	Methanol
32	ATR with amlodipine	Simultaneous equation	245,363	Methanol
33	ATR with FEL	Simultaneous equation	245,268	Aceto nitrile: double distilled water (70:30)
34	ATR with MET	Absorbance maxima	233,245	Methanol
35	ROS	Absorbance maxima	244	Methanol
36	ROS	Absorbance maxima	240	0.1N NaoH
37	ROS	Absorbance maxima	242	Methanol
38	ROS	Absorbance maxima Area under curve First order derivative	252,247,257	Methanol
39	ROS with FEN	First derivative	243,224	Methanol
40	ROS with TEN	First derivative	230,222	Methanol
41	ROS with PLH	Vierodts and Absorbance ratio	243,289	Methanol
42	PIT	Colours spectroscopy	510,530,755	Ferric chloride,2,2bipyridyl or potassium ferricyanide

II. CONCLUSION :

The article describes the various spectrophotometric reported for the determination of currently used HMG CoA reductase inhibitors viz. Lovastatin, Simvastatin, Pravastatin, Atorvastatin, Rosuvastatin, Pitavastatin in Pure forms, in different pharmaceutical dosage forms. Spectrophotometric methods can be used for industrial and research purposes, since they are relatively inexpensive, easy to carry out and can be done in a routine manner.

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