Analytical methods for the determination of statin drugs used in Dyslipidemia and Hypercholesterolemia disease

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Abstract- Statins are the first-choice drugs for the treatment of dyslipidemia and hypercholesterolemia disease. Statins are Hydroxymethyl glutaryl coenzyme A (HMG-CoA) reductase inhibitors which is most widely prescribed drugs for the patients at risk of cardiovascular events. Cardiovascular disease (CVD) is ranked as the number one cause of mortality and a major cause of morbidity worldwide. All statins share a common mechanism of action and differ in their chemical structures, pharmacokinetic profiles and lipid-modifying efficacy. Abnormalities of serum lipids triglyceride, cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) are known risk factors for vascular disease. Some of the drugs used are rosuvastatin, atorvastatin, simvastatin and pravastatin. Clinical studies demonstrated that rosuvastatin is the most effective drug for reducing low-density lipoprotein cholesterol and triglycerides, followed by atorvastatin, simvastatin and pravastatin. A brief review of the analytical methods developed for the estimation of the above drugs was discussed in the present study.

Index Terms- statins, hypercholesterolemia, drugs, analytical methods

I. INTRODUCTION

Cardiovascular disease (CVD) is ranked as the number one cause of mortality and a major cause of morbidity worldwide. Reducing high blood cholesterol which is a risk factor for cardiovascular disease is an important goal of medical treatment. Statins are the first-choice agents because it reduces blood cholesterol. All cause of mortality, coronary heart disease and stroke events were reduced with the use of statins as was for revascularization.[1]

Years before the causal relationship between blood cholesterol level and coronary heart disease risk was widely accepted. In 1970, scientist in atherosclerosis research firmly convinced that cholesterol lowering would work and interest in pharmacological approaches began as early as 1950s and ultimately led to the discovery of the statins.[2] Clinical studies were starting to convey the contribution of cholesterol to atherosclerosis and led to the need of new drug development. In 1976, Japanese biochemist Akira Endo was able to isolate three compounds from the fungus species *Penicillium citrinum*, which were able to impede cholesterol synthesis in a mouse liver enzyme system by blocking the 3-hydroxy-3- methylglutaryl-CoA reductase (HMGCoA) enzyme.[3]

Statins are the treatment of choice for hypercholesterolaemia 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. All statins share a common mechanism of action and differ in their chemical structures, pharmacokinetic profiles and lipid-modifying efficacy. The chemical structures of statins govern their water solubility, which in turn influences their absorption, distribution, metabolism and excretion. All statins are selective for their effect in the liver because of first-pass metabolism and passive diffusion which is responsible for hepatic uptake of lipophilic statins, while hydrophilic agents are taken up by active carrier mediated processes.[4]

Recent clinical trials have indicated that statins significantly reduce stroke risk in patients with vascular disease. Several epidemiological studies have clearly shown that hypercholesterolemia is the most important risk factor for coronary heart disease in industrialized countries. 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) have been proven to decrease coronary events in primary and secondary prevention of coronary heart disease. Abnormalities of serum lipids like triglyceride, cholesterol, low-density lipoprotein and high-density lipoprotein are known risk factors for vascular disease. Cholesterol and low-density lipoprotein have a direct relationship with the incidence of coronary heart disease, while high-density lipoprotein has an inverse relationship.[5] Clinical studies also demonstrated that rosuvastatin is the most effective for reducing low-density lipoprotein cholesterol, followed by atorvastatin, simvastatin and pravastatin. Hydroxymethyl glutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are among the most widely prescribed drugs with benefits in patients at risk of cardiovascular events. Although statins are well tolerated by most patients.[6]

II. STATINS USED IN DYSLIPIDEMIA AND HYPERCHOLESTEROLEMIA DISEASE

Rosuvastatin ($C_{22}H_{28}FN_3O_6S$) was developed by Pharmaceutical Company Astra-Zeneca and in 2003 it was approved by the United States.[7] It has been developed for the treatment of dyslipidemia and hypercholesterolemia. It is a new generation HMG-CoA reductase inhibitor which inhibits the enzyme 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, the rate limiting enzyme that converts HMG-CoA to mevalonate a precursor of cholesterol and thereby stop the synthesis of cholesterol. [8-12] It is available with brand name Rosuvas. Act as Rosuvas, Rosulip-F etc. in tablet dosage form. Analytical

methods were developed for the determination of Rosuvastatin using spectrophotometry [8-13], HPLC [14-19] and LC-MS [20-23]. (Table 1)

Atorvastatin (**$C_{33}H_{35}FN_2O_5$**) was developed by Bruce Roth in 1985. In 1996 Pfizer received FDA approval for lipitor.[24] It is used to reduce LDL-cholesterol, apolipoprotein B, and triglycerides and to increase HDL-cholesterol in the treatment of hyperlipidaemias.[25] (HMG-CoA) reductase enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis. [26-29] It is available with brand name Atorva. Act as X'tor, Vasolip etc in tablet dosage form. Analytical methods were developed for the determination of Atorvastatin using spectrophotometry [30-34], HPLC [15,26,28,29,31], MS [24,35,36], GC/MS[37]. (Table 2)

Simvastatin (**$C_{25}H_{38}O_5$**) was discovered and developed at Merck in 1980. It was the first member of the statin class and a derivative of fermentation product of *Aspergillus terreus*. This prodrug is converted into β hydroxy acid of simvastatin which is a potent inhibitor of HMG CoA reductase, a key enzyme required for the synthesis of cholesterol in liver. It is used for the treatment of hypercholesterolemia. [38,39,40] It is available with brand name Zocor and act as Simvas 10, starstat 10 in tablet dosage form. Analytical methods were developed for the determination of Simvastatin using HPLC[39-42], spectrophotometry[40,43], LC-MS[44]. (Table 3)

Fluvastatin (**$C_{24}H_{26}FNO_4$**) is a white crystalline powder and a potent inhibitor of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase which converts HMG-CoA to mevalonate which is an early rate-limiting step in cholestrol biosyntesis.[45-48] It lowers the plasma lipoprotein and cholesterol levels to prevent cardiovascular disease.[49] It is available with brand name Lescol XL. Analytical methods were developed for the determination of fluvastatin using HPLC[45,46], capillary electrophoresis[47], spectrophotometry[48-51]. (Table 4)

Pravastatin (**$C_{23}H_{36}O_7$**) was identified by Sankyo Co., Ltd. (now Daiichi Sankyo) as an active compactin metabolite in canine urine during the compactin drug development phase.[53] It averts the cholesterol production in the liver by blocking the enzyme HMG-CoA reductase that produces cholesterol.[54-56] Pravastatin is indicated for the treatment of hyperlipidemia and familial hypercholesterolemia. It is available with brand name Pravator and act as prastatin and pravator in tablet dosage form. Analytical methods were developed for the determination of Pravastatin using HPLC[46,56,57,58,60], capillary electrophoresis[53], LC-UV[54], Spectrophotometry[55,56,59]. (Table 5)

Table 1: Review of analytical methods for Rosuvastatin

Method	Mobile phase/ reagent	Column	References
Spectropho -tometry	Methanol		8
Spectropho -tometry	Methanol		9
Spectropho -tometry (derivative)	Methanol		10
Spectropho -tometry	Acetonitrile Phosphate buffer (pH 9.8)		11
Spectropho -tometry	0.1 N sodium hydroxide		12
Spectropho -tometry	Methanol		13
HPLC	Methanol:triflu-oroacetic acid(50:50) Potassium dihydrogen phosphate	Waters acquity C 18	14
HPLC	Acetonitrile: water: methanol	C 18	15
HPLC	Phosphate buffer (pH 2.5): Methanol: Acetonitrile (45:33:22)	Agilent Zorbax CYANO C 18	16
HPLC	toluene: chloroform: n-butanol: formic acid(6:2:1.5:0.5)	C 18	17
HPLC	0.1% trifluoroacetic acid:MeOH, gradient elution	Acquity BEH C18	18

HPLC	ACN:water (40:60, v/v), pH 3.5 adjusted with phosphoric acid	YMC C-8	19
LC-MS	2% formic acid: MeOH (20:80)	Phenomene x Luna C18	20
LC-MS	0.2% formic acid: MeOH (30:70)	Atlantis C18	21
LC-MS	ACN:methanoic acid (0.1%) (60:40)	Diamonsil C18	22
LC-MS	ACN:10 mM ammonium acetate pH 3.1 (55:45)	C18	23

Table 2: Review of analytical methods for Atorvastatin

Method	Mobile phase / reagent	Column	References
Spectroph-otometry	Methanol		30
Spectroph-otometry	Methanol		31
FT-Raman spectrosco-py	Methanol		32
Spectroph-otometry	Methanol		33
Spectroph-otometry	Phosphate buffer (pH 3.5)		34
HPLC	Acetonitrile: water: methanol	C 18	15
HPLC	Acetonitrile: 80% ortho-phosphoric acid (48:52): methanol	C 18	26
HPLC	Acetonitrile: ammonium acetate buffer (pH 4.7; 0.01M)	UPLC BEH C18	28
HPLC	Sodium phosphate buffer (0.05 M, pH 4.0): methanol (33:67)	C 18	29
HPLC	Ammonium acetate buffer pH 5.0: Acetonitrile: Triethylamine (50:50:0.2)	Luna C 18	31
LC-Q-TOF-MS	ACN: aqueous 5mM ammonium formate solution	Zorbax Eclipse XDB-C18	35
LC-MS	Acetonitrile–0.025M: NaH_2PO_4: buffer pH 4.5 (55:45)	C 18	24
LC-MS	Water: methanol (14:86): trichloroacetic acid (TCA)	Synergi polar column RP80A	36
GC-MS	Hexane, methanol (1:50)	capillary column HP5-MS	37

Table 3: Review of analytical methods for Simvastatin

Method	Mobile phase/ reagent	Column	References
HPLC	Acetonitrile 28 mM: phosphate buffer solution, pH 4 (65:35)	C 18	39
HPLC	Methanol:acetonitrile:water (60:20:20)	LiChrospher C18	40
HPLC	Acetonitrile: water (60:40)	Phenomenex C18 Luna	41
HPLC	Methanol:water: acetonitrile	C 18	42
Spectropho-tometry	Methanol and water		40
Spectropho-tometry	Methanol		43
LC-MS	Acetonitrile: methanol: 0.1M ammonium acetate (62:10:28)	Discovery C 18	44

Table 4: Review of analytical methods for Fluvastatin

Method	Mobile phase/ reagent	Column	References
HPLC	Methanol: acetonitrile: water (60:20:20)	LiChrospher C18	45
HPLC	Methanol: acetonitrile: acetic acid (0.1 M) (250: 16: 100)	Nucleosil C18	46
Capillary Electrophor -esis	Phosphate buffer (pH 8)	fused-silica capillary	47
Spectrophot -ometry (difference method)	Methanol, 0.1N NaOH , 0.1N HCl		48
Spectrophot -ometry (derivative method)	Methanol		49
Spectrophot -ometry	Phosphate buffer 7.4		50
Spectrophot -ometry	NaOH		51
Spectrophot -ometry	Ethanol		55
Spectrophot -ometry (derivative method)	Ethanol		56
Spectrophot -ometry	Methanol and acetonitrile		59

Table 5: Review of analytical methods for Pravastatin

Method	Mobile phase/ reagent	Column	References
HPLC	Methanol: acetonitrile: water (60:20:20)	LiChrospher C18	46
HPLC	Acetonitrile(0.1%): diethyl amine (50:50)	HYPERSIL C 18	56
HPLC	Methanol: phosphate buffer (70:30%)	C 18	57
HPLC	Water: acetonitrile: acetic acid(40:59:1)	Phenomex C 18	58
HPLC	ACN: MeOH(0.08M): Orthophosphoric acid (23:20:57)	Hypersil ODS	60
Capillary Electropho -resis	borate buffer (pH 8.5): 10% acetonitrile	Fused silica capillary	53
LC-UV	Methanol: water(80:20): 85% *o*-phosphoric acid	Purospher Star C18	54

III. CONCLUSION

A comprehensive information of various drugs and their detailed information regarding the mechanism, analytical methods developed by various authors and the key features of the methods were given in the present review article.

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