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Chromatography–mass spectrometry methods for the quantitation of statins in biological samples

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Abstract

The 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitors, more commonly known as 'statins', are a novel class of drugs widely used for the treatment of hypercholesterolaemia in patients with established cardiovascular disease as well as those at high risk of developing atherosclerosis. Published chromatographic–mass spectrometric methods for the quantification of presently available seven statins, atorvastatin, simvastatin, lovastatin, pravastatin, fluvastatin, rosuvastatin and pitavastatin are reviewed. High performance liquid chromatography (HPLC) in combination with tandem mass spectrometry (MS/MS) is the analytical technique of choice for the quantification of statins in biological samples. This review envisages that most of the methods used for quantification of statins are in plasma and they are suitable for therapeutic drug monitoring of these drugs.

Keywords: Statins; LC–MS/MS; Mass spectrometry; Chromatography

1. Introduction

The advent of the inhibitors of 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase (HMGR) also known as "statins": Lovastatin, Simvastatin, Pravastatin, Fluvastatin, Atorvastatin, Cerivastatin, Rosuvastatin and Pitavastatin (NK-104), for the treatment of lipoprotein metabolism disorders, constitutes a milestone in the history of prevention and therapy of atherosclerosis-related disorders [1,2]. Data arising from both primary and secondary prevention trials in which statins have been used (4S, WOSCOPS, CARE, LIPID, AFCAPS, HPS, ASCOT-LLA) [3–9] have been consistent in showing beneficial effects on total and cardiovascular mortality. The overall clinical benefits observed with statin therapy, however, appear to be greater than that might be expected from changes in lipid profile alone, suggesting that the beneficial effects of statins may extend beyond their effects on serum cholesterol levels. Recent experimental and clinical evidence indicates that some of the statins involve improving or restoring endothelial function, enhancing the stability of atherosclerotic plaques, decreasing oxidative stress and inflammation, inhibiting the thrombogenic response in the vascular wall, inhibiting platelet aggregation, immunomodulation and stimulation of bone formation and inhibition of growth of tumor cells [10–22].

In this review, we will focus on chromatography–mass spectrometry methods that might be useful for therapeutic plasma level monitoring of statins.

2. Chemistry and pharmacokinetics of statins

2.1. Chemistry and functional properties

Statins can be grouped into naturally derived and chemically synthesized [23–26]. Statins derived from fungal fermentation include lovastatin, simvastatin, pravastatin and mevastatin, whereas fluvastatin, atorvastatin, cerivastatin, rosuvastatin and pitavastatin (NK–104) are synthetic compounds. Mevastatin (compactin) is the first statin identified, which is not in clinical use [27]. Currently commercially available statins are lovastatin (Mevacor, Merck Frosst) [28], pravastatin (Pravachol, Bristol-Meyers Squibb) [29], simvastatin (Zocor, Merck Frosst) [30], fluvastatin (Lescol, Novartis) [31], atorvastatin (Lipitor, Parke-Davis) [32] and rosuvastatin (Crestor, Astra-Zeneca) [33].

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Cerivastatin (Baycol/Lipobay, Bayer) [34] was voluntarily withdrawn from the market in 2001 after reports of rhabdomyolysis [35–37]. Pitavastatin is a new highly effective statin already available for use in Japan and is currently under-going Phase III trials in Europe and US [38,39].

Lovastatin and simvastatin are prodrugs and are converted into their active forms (β-hydroxy acid) in the liver, whereas the others are active in their parent forms [24]. All statins function similarly by binding to the active site of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGR) and thus inhibiting the enzyme. However, structural differences in statins may partially account for differences in potency of enzyme inhibition [40].

Statins are competitive inhibitors of HMGR [41]. All statins share a structural component that is very similar to the HMG portion of HMG-CoA, and all differ from HMG-CoA in being more bulky and more hydrophobic (Fig. 1). The statins also differ from each other in the rigid, hydrophobic structures covalently linked to the HMG like moiety. The naturally derived statins contain a substituted decalin ring structure. Fully synthetic statins with larger flurophenyl groups are linked to the HMG like moiety. These additional groups range in character from very hydrophobic (e.g. cerivastatin) to partly hydrophobic (e.g. rosuvastatin). While all the statins inhibit hepatic HMGR at varying degrees, important structural differences exist among the statins that distinguish their lipophilicity, half-life and potency [23]. For example, lovastatin and simvastatin were shown to cross the blood brain and placental barriers but pravastatin and fluvastatin do not [42]. In addition, one of the more potent newer statins, rosuvastatin, is relatively hydrophilic and has a greater number of bonding interactions with the catalytic site of HMGR compared with mevastatin, fluvastatin, simvastain, cerivastatin

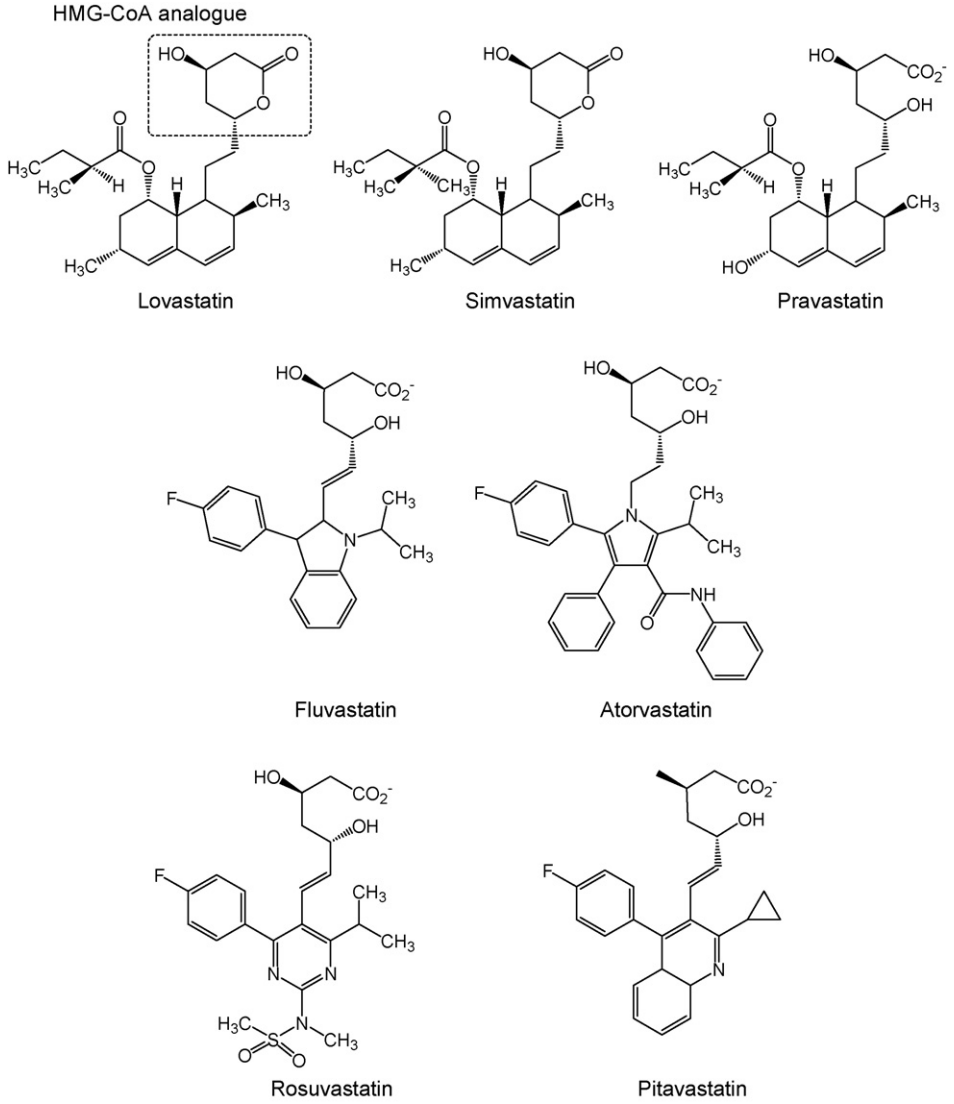

Fig. 1. Chemical structures of the statins. Among all statins, lovastatin, simvastatin and pravastatin are derivatives of fungal products while other newly developed statins are completely synthetic. The fungal products lovastatin, simvastatin and pravastatin are structurally related and they have a hydronaphthalene ring in common. Lovastatin and simvastatin are orally administered as inactive prodrugs in the lactone forms while pravastatin is given in the active open acid form. Other totally synthetic statins have different structures although they also have an open acid HMG-like moiety between the 4-fluorophenyl- and isopropyl- (or cyclopropyl-) groups. The structural difference may account for their solubility differences in water.

and atorvastatin [43–47]. Pitavastatin, like rosuvastatin, is a fully optimized compound, with respect to size and shape [39].

2.2. Pharmacokinetic properties of statins

There is considerable variation in the pharmacokinetic properties of the various statins after oral administration. With the exception of lovastatin and simvastain (which are administered as lactone prodrugs and must be hydrolyzed in the body to the corresponding β-hydroxy acid to achieve pharmacological activity) [48], all statins are administered as the active β-hydroxy acid. The extent of absorption of statins varies considerably from 30% to 98% [49–57]. All statins are absorbed rapidly following oral administration, with time to reach peak concentrations (T_{max}) of within 4 h. Food has no effect on bioavailability of these agents, except for lovastatin where it is increased [58]. With the exception of pravastatin, all statins are highly bound to plasma proteins [49,50,54,56,58,59]. As a result of extensive protein binding, the extent of systemic exposure to unbound pharmacologically active drug therefore remains extremely low. Moreover, statins are highly extracted by the liver and drug displacement interactions are of limited importance [49,58,60]. In addition, statins have a slow onset of effect [59] and are, therefore insensitive to temporary changes in unbound plasma drug concentration.

After absorption, the liver biotransforms all statins causing their low systemic bioavailability. In this respect, pitavastatin is different as it undergoes only moderate first-pass metabolism [39]. The apparent total body clearance of most statins [49,53,54,58,60] is very high due to an important hepatic first-pass effect [61]. The cytochrome P450 (CYP) enzyme system is responsible for the metabolism and elimination of many drugs, including all statins other than pravastatin [50]. The CYP system is mainly located in the hepatocyte and has various different isoforms, which appears to be involved in the metabolism of different drugs [62]. CYP3A4 iso-enzymes are the most abundant in liver microsomes and in gut wall [63], and account for approximately 30% in liver and 80% in small intestinal mucosa [64]. In addition to CYP3A4, three distinct cytochromes, CYP2C8, CYP2C9 and CYP2D6 play an important role in the metabolism of statins. Rosuvastatin is glucorinated for excretion, while simvastatin, lovastatin and atorvastatin are metabolized by CYP3A4 [65–67]. Cerivastatin is metabolized by CYP3A4 [68] and CYP2C8 [69] and fluvastatin is metabolized by CYP2C9 [70,71]. Pravastatin is unique amongst statins in not being primarily metabolized by the CYP system. Several different reactions are involved in pravastatin metabolism including isomerization, sulfonation, glutathione conjugation and oxidation [72–74]. Unlike most other statins, both pitavastatin and rosuvastatin are similar to pravastatin in that they are minimally metabolized by the liver and in humans are hardly metabolized by the CYP P450 mediated pathways [39]. Lactonisation is the major metabolic pathway of pitavastatin in humans and the lactone form can be converted non-enzymically back into the parent drug. In healthy human volunteers, pitavastatin has longer terminal elimination half-life of 11 h, probably due to the enterohepatic circulation [39]. The amount of the administered dose of statin that is excreted in urine varies from negligible amounts for atorvastatin [56] to 20% and 30%, respectively, for pravastatin and cerivastatin [75,76]. In particular, pravastatin differs from the other statins in that it shows a dual role of elimination. Caution must be exercised with the concurrent administration of drugs that interfere with the CYP system in the presence of statins.

Treatment with statin is mainly considered for long-term use and often constitutes part of a multiple-drug regime, which commonly leads to drug interactions. It is now recognized that the statins metabolized by the CYP450 system are more likely to generate muscle toxicity because of the risk of drug interactions with many drugs that inhibit CYP450, notably the CYP3A4 isoform [77,78], drug interactions may increase plasma levels of statins, with a consequent increased risk of toxic effects. Besides the common adverse effects all statins harbor the risk of myopathy and fatal rhabdomyolysis. Usually the frequency of myopathy is low but the incidence increases when statins are used in combination with agents that share common metabolic pathways. As statins do not differ in their pharmacodynamic property, the difference in their pharmacokinetic profiles constitutes the rationale for choosing a specific statin suitable for combination therapy [79].

3. Bioanalytical methods description and discussion

The determination of drug(s) in biological samples is an essential part of drug discovery and drug development providing the pharmacokinetic information that defines safety margins and treatment regimens. Currently, high performance liquid chromatography (HPLC) in combination with tandem mass spectrometry (MS/MS) is the analytical technique of choice for the quantification of drugs in biological samples. In this review, we focused on chromatography–mass spectrometry methods for the quantitation of statins in biological samples. Several bioanalytical methods have been developed for the quantitation of statins. Refer the recent review [80] for the quantification of statins using ultra violet (UV) or fluorescence detection methods in biological samples. The chromatography–mass spectrometry methods for the quantification of statins in biological samples are listed in Table 1.

3.1. Atorvastatin

Atorvastatin is administered in its active acid form and undergoes extensive first-pass metabolism [81] that results in very low plasma concentrations (ng/mL levels). Liver metabolism produces two active hydroxy metabolites, *ortho*-hydroxyatorvastatin and *para*-hydroxyatorvastatin, and three corresponding inactive lactone metabolites [82]. The actual plasma concentrations of both parent compound and metabolites are of major interest in pharmacokinetic studies. About 70% of the total plasma HMG-CoA reductase inhibitory activity is accounted for by active metabolites of atorvastatin and the plasma concentration of these active metabolites is very

Table 1
Chromatography–mass spectrometry methods for the quantification of statins and their metabolites in biological matrix

Drug, Metabolites	Matrix	Column	Mobile phase (v/v)	Sample preparation	GC or LC	IS	LOQ (ng/mL)	Ref.
Atorvastatin								
AV, 2-OH-AV, 4-OH-AV	Plasma	Waters symmetry C_{18}	0.03% Formic acid/acetonitrile (30:70)	LLE	LC	RV	0.1	[88]
AV, 2-OH-AV, 4-OH-AV	Plasma	YMC J'Sphere H80, C_{18}	Acetonitrile/0.1% acetic acid (70:30)	LLE	LC	d_5-AV; d_5-4-OH-AV	0.25	[84]
AV, 2-OH-AV, 4-OH-AV, AV-LC	Serum	YMC Basic, C_{18}	Gradient (A) water/methanol/formic acid (95:5:0.0043) (B) acetonitrile/methanol/formic acid (95:5:0.0043)	LLE	LC	A deuterium labeled analog	0.5	[86]
AV, 2-OH-AV	Plasma	Atlantis dC_{18}	Acetonitrile/0.1% acetic acid (70:30)	LLE	LC	Clindamycin	0.1	[90]
AV, 2-OH-AV, 4-OH-AV	Plasma	Omnisphere C_{18}	Gradient (A) acetonitrile/formic acid (1 mM; 30:70) (B) acetonitrile/formic acid (1 mM; 70:30)	SPE	LC	Methaqualone	0.2 for AV, 2-OH-AV; 0.5 for 4-OH-AV	[91]
AV	Plasma	–	Acetonitrile/0.1% acetic acid (70:30)	-	–	Losartan	0.4	[89]
Simvastatin								
SVA, SV-LC, LVA, LV-LC, PV	Plasma	Ultra 2 methyl, 5% phenyl	–	SPE	GC	SVA, SV-LC, LVA, LV-LC	0.2	[93]
SV, SVA	Plasma	DB-1 fused-silica	–	SPE	GC	LV	0.1	[92]
SV, SVA	Plasma	Phenomenex Synergi Max-RP	Acetonitrile/methyl ammonium acetate (1 mM, pH 4.5) (80:20)	SPE	LC	Stable isotope labeled	0.05	[101]
SV, SVA	Plasma	Supelco Discovery C_{18}	Acetonitrile/methanol/0.1 M ammonium acetate (62:10:28)	SPE	LC	LV, LVA	0.1	[102]
SV, SVA	Plasma	Kromasil C_{18}	Acetonitrile/ammonium acetate (1 mM, pH 4.5) (75:25)	LLCE/LSE	LC	Stable isotope labeled	0.05	[97]
SV, SVA	Plasma	Waters Symmetry C_{18}	Acetonitrile/aqueous 3 mM formic acid (75:25)	SPE	LC	LV, LVA	0.5	[98]
SV, SVA	Plasma	Kromasil C_{18}	Acetonitrile/ammonium acetate (1 mM, pH 4.5) (75:25)	LLE	LC	Stable isotope labeled	0.05	[124]
SV	Plasma	Shim-pack C_{18}	Methanol/water (9:1)	LLE	LC	LV	0.1	[94]
SV	Plasma	Alltech C_{18}	Acetonitrile/aqueous formic acid 10 mM (90:10)	LLE	LC	LA	0.1	[125]
SVA	Plasma	Genesis C_{18}	Gradient (A) 2.28 mM ammonium hydroxide solution (B) 2.28 mM ammonium hydroxide solution in acetonitrile	LLE	LC	LVA	0.1	[125]
Lovastatin								
LVA, LV-LC, SVA, SV-LC, PV	Plasma	Ultra 2 methyl, 5% phenyl	–	SPE	GC	SVA, SV-LC, LVA, LV-LC	0.2	[93]
LV, LVA	Plasma	Kromasil C_{18}	Gradient (A) 1 mM ammonium acetate (pH 4); (B) acetonitrile	SPE	LC	SV, SVA	0.5	[104]
LV	Plasma	–	–	LLE	GC	SV	360	[126]
Pravastatin								
SVA, SV-LC, LVA, LV-LC, PV	Plasma	Ultra 2 methyl, 5% phenyl	–	SPE	GC	SVA, SV-LC, LVA, LV-LC	0.2	[93]
PV, R-416	Plasma	Inertsil ODS-3 C_{18}	Acetonitrile/water/ammonium acetate/formic acid/triethylamine (400:600:0.77:0.2:0.6)	SPE	LC	R-122798 Pravastatin analog	0.1	[113]
PV	Plasma	–	–	SPE	LC	LVA	0.4	[127]
PV	Plasma	Zorbax C_8	Acetonitrile/ammonium formate (1 mM, pH 3.3) (66:34)	SPE	LC	LVA	0.25	[110]
PV, SQ-31906,	Plasma/Serum	Method A: Oasis; Method B: Waters Symmetry C_{18}	Gradient Method A—20 mM formic acid and acetonitrile; Method B—1 mM formic acid and acetonitrile	B: SEC	LC	PV-d_3	Method A: 1; Method B: 0.5	[108]

PV, R-416	Plasma	Inertsil ODS-2	Acetonitrile/10 mM ammonium acetate (60:40) containing 0.02% acetic acid and 0.06% triethylamine	SPE	LC	R-1437	0.625	[112]
PV, PV-d_5, PV-LC, SQ-31906, SQ-31906-d_5	Serum	Keystone Betasil ODS	Gradient: eluent A—ammonium acetate (pH 5.5; 5 mM); eluent B—acetonitrile/methanol (1:1)	SPE	LC	PV-d_3 for PV, PV-d_5, SQ-31906, SQ-31906-d_5; PV-LC-d_3 for PV-LC	0.5	[111]
Fluvastatin								
FV	Plasma	BPX5 fused-silica capillary	–	LLE	GC	^{18}O-FV	2	[115]
FV	Plasma	Chromolith speed ROD RP-18e	10 mM Ammonium acetate/methanol (2:98)	SPE	LC	RV	2	[116]
FV	Plasma	Chiralcel OD-R	Acetonitrile/methanol/water (24:36:40)	LLE	LC	Warfarin	1.5	[117]
Rosuvastatin								
RV	Plasma	Luna C18(2)	Methanol/0.2% formic acid (70:30)	SPE	LC	D_6-RV	0.1	[118]
N-desmethyl RV	Plasma	Luna C18(2)	Methanol/0.2% formic acid (65:35)	SPE	LC	D_6-RV	0.5	[119]
RV	Plasma	Waters Atlantis C_{18}	0.2% Formic acid/methanol (30:70)	LLE	LC	Cilostazol	0.2	[121]
RV	Plasma	X-Terra MS C_{18}	0.05 M Formic acid/acetonitrile (45:55)	LLE	LC	Carbamazepine	1	[120]
RV	Plasma	Lund C_{18} microbore	Methanol/0.2% formic acid (7:3)	SPE	LC	D_6-RV	0.1	[122]

AV: atorvastatin, AVA: atorvastatin acid, AV-LC: atorvastatin lactone, SV: simvastatin, SVA: simvastatin acid, SV-LC: simvastatin lactone, LV: lovastatin, LVA: lovastatin acid, LV-LC: lovastatin lactone, PV: pravastatin, PV-LC: pravastatin lactone, FLU: fluvastatin, RV: rosuvastatin. LLE: liquid–liquid extraction, SPE: solid-phase extraction, SEC: sample extraction, LLCE: liquid–liquid cartridge extraction. LC: liquid chromatography, GC: gas chromatography. MS: mass spectrometric, MS/MS: tandem mass spectrometric, ESI: electrospray ionization, API: atmospheric pressure ionization, APCI: atmospheric pressure chemical ionization, NICI: negative ion chemical ionization, EII: electron impact ionization. LOQ: limit of quantitation, IS: internal standard.

low (pg/mL levels). Several liquid chromatography–tandem mass spectrometry (LC–MS/MS) methods were reported for the quantitation of atorvastatin and its metabolites in biological samples. The GC–MS method [83] was specific for atorvastatin, though very sensitive needs two derivatization steps. The Bullen et al. [84] reported first LC–MS/MS method to quantitate atorvastatin and its two active metabolites in human, dog and rat plasma. The method consisted of washing plasma samples at high pH with diethyl ether and subsequently extracting the analytes and two internal standards [d_5]-atorvastatin and [d_5]-*ortho*-hydroxyatorvastatin from acidified plasma by using diethyl ether. Van Pelt et al. [85] published an unusual but easy method for determination of atorvastatin and five of its metabolites using deuterated internal standards by a four-column parallel chromatography system with MS–MS detection. Jemal et al. [86] reported an LC–MS/MS method for the simultaneous quantitative determination of both the acid and lactone forms of atorvastatin, 2-hydroxyatorvastatin and 4-hydroxyatorvastatin (a total of six analytes) in human serum using corresponding deuterated internal standards. Stable isotope labeled compounds are ideal internal standards in LC–MS/MS assay for the quantification of drugs and metabolites in biological matrices. The stable isotope labeled compounds have the same solubility, extraction and chromatographic behavior as their non-labeled counterparts, their difference in molecular weights make them distinguishable in LC–MS/MS from the non-labeled counterparts. Moreover, the use of stable labeled compound as an internal standard for mass spectral quantitative assays offers significant improvements in the accuracy and precision of the assay over the use of a structural analog as internal standard. d_5-Atorvastatin and d_5-atorvastatin lactone were prepared from d_5-aniline whereas their corresponding hydroxy metabolites were synthesized using d_5-benzaldehyde [87]. As these deuterated compounds are seldom commercially available, several LC–MS/MS methods were developed and validated using a structural analogs as internal standard. Recently, Nirogi et al. [88] reported a sensitive LC–MS/MS method for the quantitation of atorvastatin and its two active metabolites in human plasma using rosuvastatin as internal standard. Even losartan, clindamycin and methaqualone were also used as internal standards in recently published LC–MS/MS methods [89–91].

Highly sensitive LC–MS/MS methods were published for quantifying atorvastatin and its active metabolites at concentrations down to 100 pg/mL after administration of atorvastatin at the lowest registered dose (10 mg) in clinical studies. In all reported methods mass spectrometry was conducted in positive ion electrospray mode. Compared with other statins, such as lovastatin, simvastatin and pravastatin, the formation of the $[M+H]^+$ ion is expected to be more facile with atorvastatin since this compound contains two nitrogen atoms [86]. For all atorvastatin and its biotransformation products, the major product ion is formed by the neutral loss of the phenylamino group or phenylaminocarbonyl group. The former route is favored with lactone compounds whereas the latter route is favored with the acid compounds.

3.2. Simvastatin

Following oral administration simvastatin, an inactive lactone, is hydrolyzed *in vivo* rapidly to its corresponding β-hydroxy acid, simvastatin acid. The latter is a potent inhibitor of HMG-CoA reductase. GC–MS methods are highly sensitive and selective enough to determine the therapeutic plasma levels of both simvastatin and simvastatin acid [92,93], but the operation and clean up procedure prior to analysis is complicated. Yang et al. [94] have presented an LC–ESI-MS method for the determination of simvastatin with a simple liquid–liquid extraction procedure, however, metabolite of simvastatin was not taken into consideration. The simultaneous determination of simvastatin and simvastatin acid in biological samples which could save a considerable amount of sample preparation and analytical run time, was considered to be difficult initially owing to the different polarities of the two analytes. It is known that simvastatin undergoes hydrolysis under both acidic and basic conditions while simvastatin acid is subject to lactonization at low pH. These issues have brought considerable challenges such as assay specificity, sensitivity and stability to the quantification of simvastatin and simvastatin acid in plasma. LC–MS/MS methods for simultaneous determination of simvastatin and simvastatin acid in human plasma were reported using various extraction procedures including solid-phase extraction [95], liquid–liquid extraction [96] and solid-supported liquid–liquid extraction [97]. A method using direct-injection electrospray LC–MS/MS to assess simvastatin and simvastatin acid concentration was developed with a run time of only 2.5 min for each sample [98]. This method does not involve sample preparation except for adding the internal standard solution to the plasma samples prior to analysis by direct-injection LC–MS/MS. However, the interconversion rate (simvastatin ↔ simvastatin acid; 1.0%) and limit of quantification (0.5 ng/mL) value were not acceptable to profile pharmacokinetics of simvastatin and simvastatin acid in human samples. Among the off-line extraction methods, the solid-supported liquid–liquid extraction on Chem Elut® cartridges has been extensively used: it was sensitive (LLOQ was 0.05 ng/mL), reproducible and showed excellent extraction efficiency with no or negligible interconversion between simvastatin and simvastatin acid. However, the automated version of the solid-supported liquid–liquid extraction [99,100] was not straightforward. The solvent evaporation step was very tedious due to the fact that a large volume of elution solvent is required to achieve optimum recovery. Therefore, Yang et al. [101] utilized a novel extraction method in the LC–MS/MS method which requires no solvent evaporation and reconstitution and greatly reduces sample preparation time and improves assay efficiency. Recently, Barrett et al., [102] reported a highly sensitive and selective isocratic HPLC method for the quantitative determination of simvastatin and simvastatin acid. Detection was performed on an electrospray ionization triple quadrupole mass spectrometer equipped with an ESI interface operated in positive and negative ionization mode. The linearity for the calibration curve in the concentration range of 0.1–16 ng/mL for both simvastatin and simvastatin acid was reported.

3.3. Lovastatin

Simultaneous determination of lovastatin, simvastatin and pravastatin in plasma using GC with chemical ionization mass spectrometry has been assayed by derivatization with pentafluorobenzyl bromide [93]. In this assay the analytes are isolated from plasma by a solid-phase extraction procedure to separate the lactone and acid forms of the drugs. Then the lactone was converted to the acid form, which was subsequently derivatized by pentafluorobenzyl bromide. Korfmacher et al. [103] has developed an LC–APCI-MS/MS method for the determination of lovastatin and its hydroxy acid in dog plasma. Wu et al. [104] reported a LC–MS/MS method to quantitate lovastatin and its hydroxy acid metabolite in mouse and rat plasma. In this method a simple solid-phase extraction procedure was employed to isolate lovastatin and hydroxy acid metabolite from the biological matrices (0.1 mL of mouse or rat plasma) and there is no need for a time-consuming derivatization step. Simvastatin and simvastatin hydroxy acid were used as internal standards for lovastatin and lovastatin hydroxy acid, respectively. The assay has a LLOQ of 0.5 ng/mL in mouse and rat plasma for both lovastatin and its hydroxy acid based on 0.1 mL aliquots of plasma.

3.4. Pravastatin

Several sensitive methods [93,105–107] using gas chromatography with electron impact or negative ion chemical ionization-mass spectrometry (GC–EI-MS or GC–NICI-MS) were reported for the determination of pravastatin and R-416, the main metabolite of pravastatin in human plasma. More recently, methods using LC–ESI-MS/MS for the analysis of pravastatin, R-416 and pravastatin lactone in human plasma using solid-phase extraction or on-line purification have been reported [108–111]. Kawabata et al. [112] reported an assay for plasma concentrations of pravastatin and R-416 by LC–APCI-MS and compared the assay performance between LC–APCI-MS and GC–APCI-MS in analyzing the plasma samples collected from healthy volunteers after single oral administration of pravastatin. The LLOQ of these assay methods was in a range of 0.5–0.625 ng/mL and the plasma concentrations of many patients were below the LLOQ at 12 and 24 h following oral administration of pravastatin. Since pravastatin is to be given to the patients once daily, monitoring of the plasma concentrations over a period of 24 h is necessary. Recently Kawabata et al. [113] reported a highly sensitive assay method based on an LC–APCI-MS/MS for the determination of pravastatin and R-416 in human plasma with an LLOQ of 0.1 ng/mL. Selective reaction monitoring technique employed by this method was able to effectively eliminate background chemical interference arising from the complex plasma matrix. The analytes were extracted from plasma samples by a solid phase extraction method using a Bond Elut® C_8. Almeida et al. [114] determined pravastatin concentrations by LC–MS/MS using a solid-phase extraction procedure (LLOQ of 0.4 ng/mL) using lovastatin hydroxyacid as internal standard.

3.5. Fluvastatin

The analysis of fluvastatin from human plasma is of major interest in pharmaceutical research. Recently several procedures were described in the literature taking the advantage of the benefits of mass spectrometric detection. Leis et al. [115] reported a GC–MS method for the quantitative determination of fluvastatin in human plasma using [^{18}O]-fluvastatin as an internal standard. GC–MS under negative ion chemical ionization conditions was used for quantitative measurement of the drug and was isolated from plasma by extractive alkylation with pentafluorobenzyl bromide and further derivatized to the *bis*-trimethylsilyl derivative. In this method the LLOQ was 2 ng/mL. Though GC–MS methods are sensitive but these are time consuming due to multiple sample preparation/derivatization/extraction procedures. Nirogi et al. [116] reported for the first time an LC–MS/MS method for the quantification of fluvastatin in human plasma with an LLOQ of 2 ng/mL using structural analog rosuvastatin as internal standard. This method offers significant advantages over those reported, in terms of improved sensitivity and selectivity, faster run time (1.5 min), rapid solid-phase extraction and lower sample volume requirements. The enantioselective analysis of fluvastatin using LC–MS/MS was developed and applied to the investigation of enantioselectivity in the kinetic disposition of fluvastatin administered in a single dose to a patient with primary hypertension and hypercholesterolemia [117]. The enantiomers of fluvastatin were extracted from plasma with diisopropyl ether at pH 5.0 and LLOQ was 1.5 ng/mL for both enantiomers.

3.6. Rosuvastatin

Several methods to determine rosuvastatin concentrations in plasma have recently been developed. Hull et al. [118] reported for the first time an LC–MS/MS method for the quantitative determination of rosuvastatin in human plasma using solid-phase extraction. Recently, Hull et al. [119] developed and validated a assay in human plasma for the quantification of the *N*-desmethyl metabolite of rosuvastatin employing automated SPE followed by HPLC with positive ion electrospray tandem MS. Deuterated [d_6]rosuvastatin was used as internal standard in both methods published by Hull et al. [118,119]. However, these methods require a relatively large sample volume and a complicated and time-consuming sample preparation procedure including five-step SPE process. Trivedi et al. [120] developed a relatively rapid LC–MS/MS method using a commercially available carbamazepine as an internal standard. The assay procedure involved a simple one-step liquid–liquid extraction of drugs from plasma into ethyl acetate. This method was developed and validated for the simultaneous determination of rosuvastatin and fenofibric acid in human plasma. Recently, Xu et al. [121] also reported a rapid LC–MS/MS method using a commercially available compound cilostazol as internal standard. Ether was used as extraction solvent to determine rosuvastatin in human plasma by a simple one-step liquid–liquid extraction. This method offers relatively higher sensitivity (0.2 ng/mL) as compared with existing methods and requires a relatively small sample volume (0.25 mL). Oudhoff et al. [122] investigated the potential of microbore HPLC in combination with MS–MS for the sensitive detection of rosuvastatin in human plasma. This microbore HPLC–MS/MS method enables the analysis of small sample aliquots of plasma and allow pharmacokinetic profiles to be generated from single rats or mice.

3.7. Pitavastatin

The growing number of trials that have highlighted the benefit of intensive lowering of total- and low-density lipoprotein cholesterol levels especially with statins has created a need for more efficacious agents. Pitavastatin (NK-104) is a new synthetic HMG-CoA reductase inhibitor which was developed and has been available in Japan since July 2003 [39]. It is reported to be more effective in LDL-cholesterol reduction than pravastatin, simvastatin or atorvastatin with a longer duration of action and similar or reduced potential for drug interactions [39]. In the literature only one HPLC method with UV detection using a column-switching technique was reported for the simultaneous determination of pitavastatin and its lactone in plasma [123]. There is a need to develop a LC–MS/MS method for the quantification of pitavastatin and pitavastatin lactone in human plasma, as HPLC methods are often time-consuming and offers minimal selectivity.

4. Conclusions and perspectives

The current state-of-the art of chromatography–mass spectrometry methods for quantification of statins have been presented. The literature compilation has revealed that a variety of methods are available for atorvastatin, simvastatin and pravastatin. For rosuvastatin only a limited number of methods were reported while no methods for pitavastatin. This is because of the fact that rosuvastatin was a new statin approved in 2003 and pitavastatin is undergoing phase III clinical trials. Our analysis of the published data revealed that the LC–MS was extensively used for quantification of statins in biological matrices. It is evident that LC–MS/MS is the technique of choice for the quantification of statins in biological matrices. The particular advantage of LC–MS/MS methods when compared to other techniques is the sensitivity, selectivity and simplicity of the technique. Most of the methods have used the reverse phase chromatography and in sample preparations solid-phase extraction is preferred. In most of the reported methods mass spectrometry was conducted in electrospray ionization mode and typical quantification limits are in pg/mL range. In this review, we have discussed the present state-of the bioanalytical methods for quantification of all the statins. There is a great scope for development of newer analytical methods for latest drugs such as pitavastatin. Since some of these statins are recently introduced into the market, we have undertaken a comprehensive program to develop and validate new bioanalytical methods using LC–MS/MS.

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