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Review

Analytical methods for the quantitative determination of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors in biological samples

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Abstract

Published analytical methods for the quantitative determinations of presently available five 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (''statins''), lovastatin, simvastatin, pravastatin, fluvastatin and atorvastatin, are reviewed for therapeutic drug monitoring purpose in patients. Almost all assay reviewed are based on high-performance liquid chromatography or gas chromatography. Some purification steps (liquid–liquid extraction, solid-phase extraction, etc.) have been used before they are submitted to separation by chromatographic procedures and they are detected by various detection methods like UV, fluorescence and mass spectrometry. This review shows that most method may be used quantitative determination of statins in plasma and they are suitable for therapeutic drug monitoring purpose of these drugs.

Keywords: Reviews; 3-Hydroxy-3-methylglutaryl

Contents

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1. Introduction

Hiperlipidemia, the elevation of lipid concentration in plasma, is the manifestation of a disorder in the synthesis and degradation of plasma lipoproteins. The main lipids that are of relevance in hyperlipidemia are cholesterol and triglyceride. Cholesterol plays a crucial role in maintaining cell membrane integrity and physiological functions of the body; including steroid hormone synthesis. On the other hand, high levels of cholesterol concentration are associated with pathological conditions such as atheriosclerosis [1,2]. Central to the pathogenesis of atherosclerosis is the deposition of cholesterol in the arterial wall [3,4]. Atherosclerosis of the coronary and peripheral vasculature is the leading cause of death among men and women worldwide [5]. The most fetal effect of atherosclerosis is that it increases incidence of coronary heart disease (CHD) and mortality rate related to it [6,7]. The statistics show that 38 to 42% of all deaths are related to cardiovascular diseases in western and other developed countries [8–11].

Lowering cholestrol levels can arrest or reverse atherosclerosis in all vascular beds and can significantly decrease the morbidity and mortality associated with atherosclerosis. Each 10% reduction in cholesterol levels is associated with ~20–30% reduction in the incidence of coronary heart disease [12–19]. Thus, efforts have been made to reduce atherosclerosis and related disorders by reducing plasma cholesterol concentrations.

The main methods of treating hyperlipidemia (or hypercholesterolaemia) are dietary and lifestyle changes and administration of hypolipidemic drugs [19,20]. The principal groups of hypolipidemic agents described are bile acid binding resins, fibric acid derivatives, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (‘‘statins’’), derivatives of nicotinic acid, probucol and the omega-3 marine trigylicerides [12]. Statins are the most effective among all of these agents. These reductase inhibitors are proven to reduce coronary artery events and have less adverse effects than other hypolipidemic drugs [12,13,15,21].

Statins specifically inhibit HMG-CoA reductase by competition, the enzyme that catalyzes the conversion of HMG-CoA to mevolanate, which is an early rate-limiting step in cholesterol biosynthesis in the body [22]. These agents are highly effective in reducing total cholesterol and the low-density lipoprotein levels in several forms of hypercholesterolamia [23–27].

The five HMG-CoA reductase inhibitors presently available are lovastatin (LV), simvastatin (SV), pravastatin (PV), fluvastatin (FV) and atorvastatin (AV). LV, SV and PV are structurally similar. LV is a natural product which is derived from the fungus *Aspergillus terreus* [28,29]. SV and PV are produced by semi-synthetic processes from LV and mevastatin, respectively [30]. FV and AV are totally synthetic molecules and have structures distinct from the other statins. Chemical structures of statins are shown in Fig. 1.

In this review, analytical methods that might be useful for therapeutic plasma level monitoring of statins were focused and summarized.

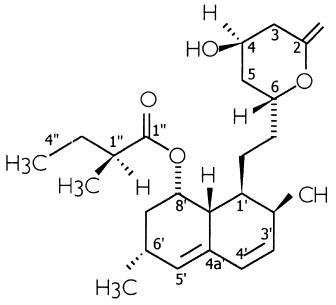
2. Metabolism and pharmacokinetics

PV, AV and FV are active in the administered form. These drugs are absorbed from the gastrointestinal tract and undergo extensive first-pass metabolism in the liver. FV and AV, more than 98%, PV approximately 50% are bound to plasma proteins. The drugs are excreted mainly in the feces via the bile, with a smaller proportion excreted in the urine [31–35].

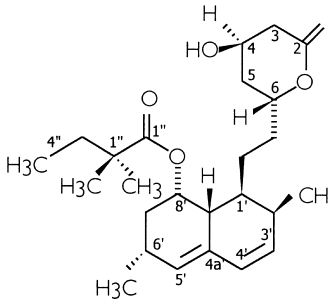
LV and SV are prodrugs that are administered as inactive lactone forms. Their lactone forms are absorbed from the gastrointestinal tract and hydrolysed to those active β-hydroxy acid form in the liver [36]. Both drugs and β-hydroxy acid metabolites are extensively (95%) bound to plasma proteins. The substances undergo extensive first-pass metabolism in the liver and are mainly excreted in the bile; about 85% of administered dose has been recovered from the feces as metabolite and about 10 to 15% from the urine, mainly as inactive forms [36,37].

The pharmacokinetics of HMG-CoA reductase inhibitors have been summarized in several reviews [38–46]. These drugs have a widely differing solubilities in water, thus, resulting in different pharmacokinetics characteristics [46,47].

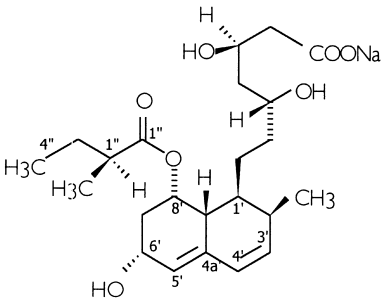
Statins are mainly considered for long-term use and often constitute part of a multiple-drug regime which commonly leads to drug interactions. Besides common adverse drug effects, such as nausea, ab-



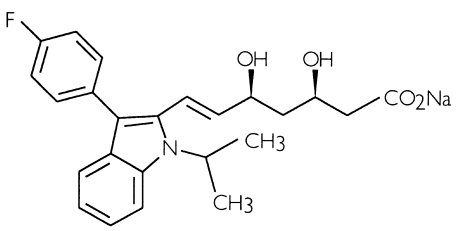
Lovastatin



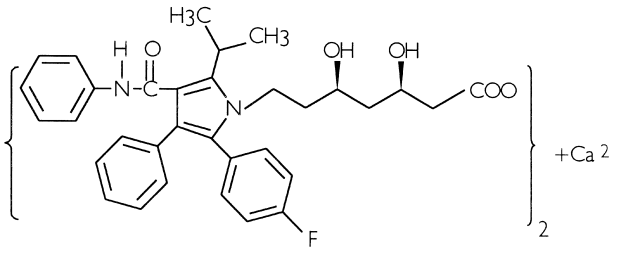
Simvastatin



Pravastatin



Fluvastatin



Atorvastatin

Fig. 1. Chemical structures of statins.

dominal discomfort and headache, all statins harbor the risk of myopathy and fatal rhabdomyolysis. Usually, the frequency of myopathy is low but the incidence increases during multiple-drug regime. All drug interactions have pharmacokinetic or pharmacodynamic basis and are predictable given an understanding of the pharmacology of the drugs involved. Statins do not differ in their pharmacodynamic property. Therefore, the differences in their pharmacokinetic profiles constitute the rationale for choosing a specific statin suitable for combination therapy [48,49]. The knowledge of their pharmacodynamic and pharmacokinetic properties can lead to a rational use and greater understanding of their

potential benefits [50]. Thus, analytical methods used for these purposes are important.

3. Analytical methods

Despite that the fact that HMG-CoA inhibitors seem to be structurally similar, analytical methods for their quantitative determination in blood have been developed individually for each drug because of their different solubility, stability and optic characteristics. Among all these studies, only one report published has included simultaneous analysis of three HMG-CoA inhibitors, SV, LV and PV [51]. Generally, hyperlipidemic patients are treated with multiple-drug regime and some patients may benefit from combination treatment of antihyperlipidemic agents. Since statins are not used with other statins simultaneously, there is no need for simultaneous analysis of statins.

Almost all assays used for the separation of statins are based on either high-performance liquid chromatography (HPLC) or gas chromatography (GC). HPLC is the most popular method of the two. All HPLC methods reviewed for statins are based on reverse phase separation. Most often a UV detector, rarely a fluorescence detector and a mass detector have been used. But, for analysis of atorvastatin, there are only HPLC–mass spectrometry (MS) assays which are not available for most laboratories because of financial reasons [52,53]. Some purification steps have been used before the samples are injected to chromatographic system as liquid–liquid extraction, solid-phase extraction, etc. Moreover, some derivatization procedures have been used to decrease sensitivity in some of the assay. Internal standards leading to more correct results in analytical methods have not been used in some of the assays. However, in most of the assays one of the statins has been chosen as internal standard owing to the similar structures of the substances.

Only one assay mentioned is based on thin-layer radio chromatography [54]. Although we have come across many Chinese or Korean originated papers in the abstract databases while reviewing analytical methods for statins, we have used only those that could be obtained on paper.

Finally, all methods allowing the analysis of each of the statins in human plasma or serum have been summarized and presented in the tables. Among these methods we have also reviewed the methods used for biological samples of animals as those could be considered to be applicable to biological samples of humans.

3.1. Lovastatin

Assay procedures for LV are listed in Table 1. Simultaneous determination of the LV, PV and SV in plasma using GC with chemical ionization mass spectrometry has been assayed by derivatization with pentafluorobenzyl bromide [51]. In this method the analytes are isolated from plasma by a solid-phase extraction procedure separating the lactone and acid forms of the drug. Then the lactone is converted to the acid form which is subsequently derivatized by pentafluorobenzyl bromide. GC–MS in the ion-monitoring mode has also been described for LV [55]. Wu et al. have developed an LC–MS–MS method for the assay of the LV and hydroxy-metabolite in mouse and rat plasma [56]. In this method, sample preparation procedure is simple and there is no need for a time-consuming derivatization step. Several HPLC methods have been introduced using UV detection [57–61] without derivatization.

3.2. Simvastatin

Assay procedures for SV are listed in Table 2. Two GC–MS methods have been introduced for SV and simvastatin acid (SVA). In one of these methods derivatization procedure for SV has not been used [62]. In another method, SV and SVA have been derivatized with ferroceneboranic acid [63]. This method is highly sensitive and selective enough to determine the therapeutic plasma levels of both SV and SVA although the operation and clean-up procedures prior to analyses seems to be complicated. Some simple HPLC procedures with UV detection have been described by Tan et al. [64], and Carlucci et al. [65]. The HPLC–UV methods, being the sample preparation comparatively less than arduous, still required another reaction step (protein precipitation, centrifugation, etc.) and the chromatographic run time is long. A highly sensitive and selective HPLC method using a fluorescent derivatization and column switching technique has been introduced by Ochiai et al. [66]. In this method, solid-phase

Table 1
Assay procedures for lovastatin and metabolites

Drug, metabolites	Medium[a] (volume in ml)	Extraction[b]	Derivatization	Separation[c] stationary phases	Detection[d]	Recovery (%)	Range (ng/ml)	LOQ/LOD (ng/ml)	IS	Ref.
SVA, SV-LC, LV, LV-LC PV	P	SPE	Yes	GC Ultra 2 Methyl, 5% phenyl,	MS	82.4 SV-LC 89.89 SVA 83.7 LV-LC	0.2–20 SV, SVA 1–50 PV	0.2 LOQ SVA, SV-LC LVA, LV-LC	SVA, SV-LC LVA, LV-LC	[51]
LV	P	LLE	No	GC	MS	>92	360–48000	100 LOD	SV	[55]
LV, LVA	Mouse, Rat P	SPE	No	HPLC Kromasil C_{18} (5 μm)	MS (API)	54 mouse 55 rat (LV), 100 mouse 67 rat (LV-OH)	0.5–100 LV, LVA	0.5 LOQ LV, LVA	SV SVA	[56]
LV, LVA	P	SPE	No	HPLC Supelco Discovery C_{18} (5 μm)	UV	79–105 LV, LVA	100–5000 LV, LVA	100 LOQ LV, LVA	Bay W 62 230	[57]
LV	P U	LLE	No	HPLC Novapak C_{18}	UV	73.4–82.9	500–10 000	0.5 LOQ	SV	[58]
LV	P	SPE	No	HPLC Novapak C_{18}	UV	92.43	5–500	5 LOD	–	[59]
LV	P	LLE	No	HPLC Hypersil BDS C_{18}	UV	101.70	2.5–80	1 LOD	–	[60]
LV, LVA	P	SPE	No	HLPC Sepralyte C_{18} (3 μm)	UV	85 LV 95 LVA	25–1000 LV, LVA	–	SV	[61]

LV=Lovastatin, LVA=Lovastatin acid, LV-LC=Lovastatin lactone, SV=Simvastatin, SVA=Simvastatin acid, SV-LC=Simvastatin lactone, PV=Pravastatin.
[a] P=Plasma, U=Urine.
[b] LLE=Liquid–liquid extraction, SPE=Solid-phase extraction.
[c] HPLC=High-performance liquid chromatography, GC=Gas chromatography.
[d] UV=Ultraviolet, MS=Mass spectrometric, API=Atmospheric pressure ionization, LOQ=Limit of quantitation, LOD=Limit of detection, I.S.=Internal standard.

Table 2
Assay procedures for simvastatin and metabolites

Drug, metabolites	Medium[a] (volume in ml)	Extraction[b]	Derivatization	Separation[c] Stationary phases	Detection[d]	Recovery (%)	Range (ng/ml)	LOQ/LOD (ng/ml)	IS	Ref.
SVA, SV-LC LV, LV-LC PV	P	SPE	Yes	GC Ultra 2 methyl, 5% phenyl, (0.11 μm)	MS	82.4 SV-LC 89.9 SVA 83.7 LV-LC 78.9 LVA	0.2–20 SV, SVA 1–50 PV,	0.2 LOQ SVA, SV-LC LVA, LV-LC	SVA, SV-LC LVA, LV-LC	[51]
SV	P	LLE	No	GC	MS	96–103	0.27–54	–	LV	[62]
SV, SVA	P	SPE	Yes	GC DB-1 fused-silica column	MS	54±5 SV 75±6.2 SVA	0.1–10 SV, SVA	0.1 LOQ SV, SVA	LV	[63]
SV	P	LLE	No	HPLC LiChrospher C_{18} (5 μm)	UV	>93.3	0.25–50	–	LV	[64]
SV, SVA	P	LLE	No	HPLC ODS Hypersil (5 μm)	UV	93.9–97.1 SV 91.4–96.2 SVA	20–1000 SV 25–1000 SVA	15 SV LOD 20 SVA	–	[65]
SV, SVA*	P	SPE	Yes	HPLC Capcell pak C_{18} (5 μm)	FLUO	90.9–117 SV, SVA	0.1–10 SV, SVA	0.1 LOQ SV, SVA	–	[66]
SV, SVA	P	SPE	No	HPLC Symmetry C_{18} (5 μm)	MS–MS (API)	≥75 SV ≥38 SVA	0.5–200 SV, SVA	0.5 LOQ SV, SVA	LV	[67]
SV, SVA	P	LLCE	No	HPLC Kromasil C_{18} (5 μm)	TMS	78 SV 87 SVA	0.05–50 SV, SVA	0.05 LOQ SV, SVA	Isotope-labeled SV and SVA	[68]
SV	P	LLE	No	HPLC ODS Shimpack (5 μm)	MS (ESI)	96.3–107.7	0.1–20	0.05 LOD	LV	[69]

SV=Simvastatin, SVA=Simvastatin acid, SV-LC=Simvastatin lactone, LV=Lovastatin, LVA=Lovastatin acid, LV-LC=Lovastatin lactone, PV=Pravastatin.
[a] P=Plasma.
[b] LLE=Liquid–liquid extraction, SPE=Solid-phase extraction, LLCE=Liquid–liquid cartridge extraction.
[c] HPLC=High-performance liquid chromatography, GC=Gas chromatography.
[d] UV=Ultraviolet, FLUO=Fluorescence, MS=Mass spectrometric, API=Atmospheric pressure ionization, TMS=Tandem mass spectrometric, ESI=Electrospray ionization, LOQ=Limit of quantitation, LOD=Limit of detection, I.S.=Internal standard. * Automated method with column-switching.

extraction (SPE) and derivatization procedures have been used, which are time-consuming. This method has adequate quantification limits (0.1 ng/ml) for both analytes, and is applied to the determination of SV and SVA in plasma after oral administration of SV to humans. Jemal et al. [67] have presented an LC–MS–MS procedure for determination of SV and SVA in plasma with a positive ion electrospray method after cleaning-up and the extraction of the sample with sample extraction column in line with the analytical column. In this method, a sample preparation procedure has not been used except the addition of the internal standard solution to the plasma samples prior to analysis by direct injection. The total run time per sample is only 2.5 min. Zhao et al. [68] have proposed an LC–MS–MS method with turbo ionspray interfaces after liquid–liquid cartridge extraction (LLCE). Yang et al. [69] have presented an LC–MS method with a simple liquid–liquid extraction procedure and less than a 5-min analysis time.

3.3. Pravastatin

Assay procedures for PV are listed in Table 3. Funke et al. [70] have presented a GC–MS procedure for PV and its metabolites in plasma and serum with chemical ionization (CI) mass spectrometry after purification of the extracts by C_{18} solid-phase extraction columns and derivatization with pentafluorobenzyl bromide (PFBB). A highly sensitive GC–MS method based on electron impact ionization (EII) using derivatization with diamethazone and bis(trimethylsilyl)trifluoroacetamide (BSTFA) has been described by Cai et al. [71]. Several HPLC methods using UV detection without derivatization have been introduced [72–77]. Dumousseaux et al. [78] have described a HPLC method with laser-induced fluorescence detection after derivatization with *n*-dansyl ethylenediamine. They have used immobilized antibody extraction and column switching technique to increase selectivity, specificity and simplicity. The limit of detection of the method is approximately 2 pg/injection. PV and its metabolites have been assayed in human plasma using HPLC methods with MS–MS detection by atmospheric pressure ionization (API) [79]. Unlike other methods, this method allows to determine PV, its metabolite; SQ-31906 (3-α-hydroxy isomeric compound) and PV-lactone simultaneously. Kawabata et al. [80] have described a HPLC method with MS detection by atmospheric pressure chemical ionization (APCI) without derivatization procedure. Jemal et al. [81] have presented two HPLC procedures based on MS–MS detection. In the first method one column (Oasis) has been used for analysis of single analyte (tetramethylbutyl amine salt of PV) in rat plasma. In the second method, a second column (C_{18}) has been added in-line with the Oasis column to achieve the needed chromatographic separation. The second method has been applied with slight modifications to the quantitative determination of PV and its isomeric biotransformation product in human, mouse, rat and monkey serum. The methods have been allowed direct injection of the plasma/serum samples. Zhu and Neirinck [82] have described a HPLC coupled with negative ion tandem mass spectrometric method. The method provides good specificity and sensitivity with a total run time at less than 2 min.

3.4. Fluvastatin

Assay procedures for FV are listed in Table 4. Kalafsky et al. [83], Toreson and Eriksson [84] and Lanchote et al. [85] have proposed three HPLC procedures with fluorimetric detection for the quantitative analyses of FV in human plasma. The method of Kalafsky et al. [83] can accurately detect 1 ng/ml FV using 1 ml of plasma. This simple and sensitive method with liquid–liquid extraction (LLE) from plasma with fluorescence detection has been successfully applied to the analysis of over 16 000 samples from bioavailability/pharmacokinetic studies without any evidence of interference from endogenous plasma compounds. The method of Toreson and Eriksson [84] has also determined FV enantiomers with LLE from plasma. In this method, the samples have been chromatographed on a C_{18} column as racemate and on a chiral OD-R column as enantiomers. In the achiral chromatographic system, post-column exposure to UV light has been applied, making the method 4–5-times more sensitive than it done with the native fluorescence of FV. The chiral system has enabled a simple separation of the enantiomers without derivatization of the sample. FV and enantiomers have also been determined by Lanchote et al. [85]. In this method an SPE column

Table 3
Assay procedures for pravastatin and metabolites

Drug, metabolites	Medium[a] (volume in ml)	Extraction[b]	Derivatization	Separation[c] stationary phases	Detection[d]	Recovery (%)	Range (ng/ml)	LOQ/LOD (ng/ml)	IS	Ref.
PV	P, U	LLE	No	TLRC LSS	–	88 for U	–	4.3 P LOD 300U LOD	Non-radioactive PVNa for TLRC Automatic external standard for LSS	[54]
SVA, SV-LC, LV, LV-LC PV	P	SPE	Yes	GC Ultra 2 methyl, 5% phenyl (0.11 μm)	MS	82.4 SV-LC 89.9 SVA 83.7 LV-LC 78.9 LVA	0.2–20 SV, SVA 1–50 PV	0.2 LOQ SVA, SV-LC LVA, LV-LC	SVA, SV-LC LVA, LV-LC	[51]
PV, 3-OH and triol metabolite	S P	SPE	Yes	GC Fused-silica Chrompak, CP SIL 19 CB column (0.12 μm)	MS (NICI)	>93 PV	0–80 PV, 3-OH metabolite 0–40 triol metabolite	0.3 LOD for three compounds	Tetrahidro-PV, Dihydro-triol metabolite	[70]
PV	P	LLE	Yes	GC	MS (EII)	90	1–60	0.015 LOD	–	[71]
PV	Rat liver	SPE	No	HPLC C_{18}	UV	80.8	–	13 LOD	–	[72]
PV	Rat muscle	SPE	No	HPLC C_{18}	UV	52–60	5–100 ng/g rat muscle	5 ng/g LOD Rat muscle	–	[73]
PV, 3-α-iso PV	S	SPE	No	HPLC Supelcosil LC_8 (5 μm)	UV	83.6±2.1 PV	–	2 LOD PV	Carbamazepine	[74]
PV	P	SPE	No	HPLC Purospher C_{18} (5 μm)	UV	69.2	2–200	2 LOQ	Triamcinolone acetonide	[75]
PV	P	SPE	No	HPLC LC_{18} (3 μm)	UV	75	5–200	2 LOD	Methonilic SV β-hydroxy acid	[76]
PV-Na, isomeric metabolite	U	SPE	No	HPLC Alkylphenyl column (5 μm)	UV	–	<1500 for two compounds	0.1 LOD for two compounds	9-fluoro-11β-hydroxy-16α, 17α-(1-methyl ethyldenedioxy) oxoandrosta-1,4-diene-17-carboxylic acid	[77]

Table 3. Continued

Drug, metabolites	Medium[a] (volume in ml)	Extraction[b]	Derivatization	Separation[c] stationary phases	Detection[d]	Recovery (%)	Range (ng/ml)	LOQ/LOD (ng/ml)	IS	Ref.
PV	P	IMAE	Yes	HPLC Column switching 300-C_4 column Cosmosil column A5 C_{18}-AR	LIFLUO	63	0.5–100	0.1 LOD	R-416	[78]
PV, PV-d_5 PV-LC SQ-31906 (3-α-iso PV) SQ-31906-d_5	S	SPE	No	HPLC Keystone Betasil ODS (5 μm)	MS–MS (API)	>90 for 5 analyte	0.5–100 for 5 analyte	0.5 LOQ for five analytes	PV-d_3 for PV,PV-d_5 SQ-31906, SQ-31906-d_5, PV-LC-d_3 for PV-LC	[79]
PV, main metabolite R-416 (3-α-iso PV)	P	SPE	No	HPLC Inertsil ODS-2 column, (5 μm)	MS (APCI)	–	0.625–80 PV, R-416	0.625 LOQ PV, R-416	R-1437	[80]
PV, SQ-31906 (3-α-iso PV)	Rat P Human S Mouse S Rat S Monkey S Mouse liver	Method B SEC	No	HPLC Method A Oasis column Method B (30 μm) Method B Waters Symmetry C_{18} (5 μm)	MS–MS (API)	Method A 80 Method B 50 PV 90 SQ-31906	Method A 1–1000 Method B 0.5–100	Method A 1 LOQ Method B 0.5 LOQ	PV-d_3	[81]
PV	P	SPE	No	HPLC Zorbax XDB C_8 (5 μm)	MS (NITMS)	90.5	0.25–300	0.25 LOQ	β-OH-LV	[82]

SV=Simvastatin, SVA=Simvastatin acid, SV-LC=Simvastatin lactone, LV=Lovastatin, LVA=Lovastatin acid, LV-LC=Lovastatin lactone, PV=Pravastatin., PV-LC=Pravastatin lactone.

[a] P=Plasma, U=Urine, S=Serum.

[b] LLE=Liquid–liquid extraction, SPE=Solid-phase extraction, SEC=Sample extraction, IMAE=Immobilized antibody extraction.

[c] HPLC=High-performance liquid chromatography, GC=Gas chromatography, TLRC=Thin-layer radio chromatography, LSS=Liquid scintillation spectrometry.

[d] UV=Ultraviolet, FLUO=Fluorescence, LIFLUO=Laser induced fluoresence, MS=Mass spectrometric, API=Atmospheric pressure ionization, APCI=Atmospheric pressure chemical ionization, NICI=Negative ion chemical ionization, EII=Electron impact ionization, NITMS=Negative ion tandem mass spectrometric, LOQ=Limit of quantitation, LOD=Limit of detection, I.S.=Internal standard.

Table 4
Assay procedures for fluvastatin and metabolites

Drug, metabolites	Medium[a] (volume in ml)	Extraction[b]	Derivatization	Separation[c] stationary phases	Detection[d]	Recovery (%)	Range (ng/ml)	LOD/LOQ (ng/ml)	IS	Ref.
FV-Na	P	LLE	No	HPLC Supercosil LC-18 (5 μm)	FLUO	–	1–1000	1 LOD	Sandoz compounds* 63–267	[83]
FV-Na, racemate enantiomers	P	LLE	No	HPLC Zorbax Rx-C_8 (5 μm) Chiralcel OD-R (10 μm)	FLUO	95 racemates 86 racemates	10–5000 nmol/l racemates 5–1200 nmol/l enantiomers	0.5 nmol/l (LOQ) racemates 5 nmol/l (LOQ) enantiomers	Sandoz compounds*	[84]
FV isomers [(−)-(3*S*,5*R*), (+)-(3*R*, 5*S*)]	P	SPE	No	HPLC Chiralcel OD-H	FLUO all compounds	>80 all compounds	0.75–625	0.75 (LOQ)		[85]
FV, metabolites (M-2, M-3, M-4, M-7)	P	SPE (system 1) LLE (system 2)	No	HPLC ODS (5 μm)	UV	92.7–102.5 M-2 94.4–142.3 M-3 94.2–10.5 M-5 67.8–102.8 M-4 67.8–102.8 M-7 89.4–97.3 FV	10–1000 all compounds	10 LOQ all compounds	Benzophenone (system 1) 9-cyanoanthracene (system 2)	[86]

FV=Fluvastatin, * Sandoz compound 63-267=[*R**,*S**,-(*E*)-](±)-7-[3-4-fluorophenyl)-1-(1-methylethyl)-1H-indol-2yl]-3,5-dihydroxy-6-methyl-6-heptenoic acid, monosodium salt.

[a] Plasma.

[b] LLE=Liquid–liquid extraction, SPE=Solid-phase extraction.

[c] HPLC=High-performance liquid chromatography.

[d] UV=Ultraviolet, FLUO=Fluorescence, LOQ=Limit of quantitation, LOD=Limit of detection, I.S.=Internal standard.

Table 5
Assay procedures for atorvastatin and metabolites

Drug, metabolites	Medium[a] (volume in ml)	Extraction[b]	Derivatization	Separation[c] stationary phases	Detection[d]	Recovery (%)	Range (ng/ml)	LOD/LOQ (ng/ml)	IS	Ref.
AVA, 2-OH-AV, 4-OH-AV AV-LC	S	LLE	No	HPLC YMC Basic, C_{18} (5 μm)	MS–MS (API)	60–100 for all compounds	0.5–200 for all compounds	0.5 LOQ for all compounds	A deuterium labeled analogs	[52]
AV, *o*-OH-AV, *p*-OH-AV	Human, dog, rat P	LLE	No	HPLC YMC J'Sphere H80, C_{18} (4 μm)	TMS	100–102 AV 70.6–104 *o*-OH-AV 47.6–85.6 *p*-OH-AV	0.25–25 for all compounds	0.25 LOQ for all compounds	$[d_5]$-AV $[d_5]$-*o*-OH-AV	[53]

AV=Atorvastatin, AVA=Atorvastatin acid, AV-LC=Atorvastatin lactone.
[a] S=Serum, P=Plasma.
[b] LLE=Liquid–liquid extraction.
[c] HPLC=High-performance liquid chromatography.
[d] MS=Mass spectrometric, API=Atmospheric pressure ionization, TMS=Tandem mass spectrometric, LOQ=Limit of quantitation, LOD=Limit of detection, I.S.=Internal standard.

has been used for plasma samples unlike any other method. In addition the method could be used allowed for studies on kinetic disposition of FV in combination with other drugs. Only one method based on UV detection has been reported by Nakashima et al. [86] for determination of FV and its five metabolites in human plasma. In this method, two different chromatographic systems have been used due to the rather wide range of hydrophilic characteristics of these compounds. A shorter run-time has been achieved with the two chromatographic system applied.

3.5. Atorvastatin

Assay procedures for AV are listed in Table 5. Only two HPLC procedures have been presented by Jemal et al. [52] and Bullen et al. [53] for the analyses of acid and lactone forms of AV and its active metabolites based on MS–MS (API) detection by using tandem mass spectrometry. Both of the methods are very sensitive, having low quantitation limits. The methods have been successfully applied to analysis of AV in human serum.

4. Conclusions

Analytical methodology is an essential component not only in the developmental phase of a drug substance but also in the continuing evaluation of clinical efficiency. This article is mainly designed to serve as a guide for analytical methods of statins in biological fluids to obtain evaluation of clinical efficiency. The published methods that might be useful for the analysis of each of the statins in human plasma or serum were summarized and presented.

In summary, almost all methods for the determination of statins have been published individually, mostly based on HPLC and GC. However, only one method has been published which allows the simultaneous determination of three statins [51]. Some purification steps (LLE, SPE, etc.) have been used before the samples are injected to chromatographic system. In addition, some derivatization procedures have been used some of the assay to decrease sensitivity. The drugs are detected by various detection methods like UV, fluorescence and mass spectrometry.

This review shows that most method allow quantitative determination of statins in plasma or serum, in the ng/ml range, therefore they are suitable for therapeutic drug monitoring purpose of this category drugs. But still there is neccessity to develop new easy and cheap analytical method for some of the statins.

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