

## SPECTROSCOPIC AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY METHODS FOR DETERMINATION OF STATINS

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**Abstract.** The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, more commonly known as ‘statins’, are widely used for the treatment of hypercholesterolaemia in patients with established cardiovascular disease as well as those at high risk of developing atherosclerosis. The great importance of this drugs requires development of effective analytical methods involving high sensitivity and resolution. The spectroscopy and the liquid chromatography are well established methods in the field of pharmaceutical analysis. Various spectroscopic and high-performance liquid chromatographic methods for determination of statins, their related impurities and co-administered drugs in the bulk drug forms and pharmaceutical formulations are reviewed.

**Key Words:** statin, liquid chromatography, spectroscopy, pharmaceutical dosage form

### Introduction

Statins are class of agents that specifically inhibits 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a rate-limiting enzyme in cholesterol biosynthesis. They are highly effective in reducing total cholesterol and the low-density lipoprotein (LDL) cholesterol levels in the human body. High-plasma LDL cholesterol is a risk factor of cardiovascular disease, such as atherosclerosis, which is characterized by deposition of cholesterol on the arterial wall. In addition to lipid-lowering activity, statins improve endothelial function, maintain plaque stability, prevent thrombus formation and reduced oxidative stress [1-6].

The seven HMG-CoA reductase inhibitors presently used are Lovastatin (LOV), Simvastatin (SMV), Pravastatin (PRV), Fluvastatin (FLV), Atorvastatin (ATV), Rosuvastatin (ROS) and Pitavastatin (PTV).

Statins can be grouped into fermentation-derived and chemically synthesized. LOV, SMV and PRV are structurally similar. LOV is a natural product which is derived from the fungus *Aspergillus terreus*. SMV and PRV are produced by semi-synthetic processes from LOV and mevastatin respectively. FLV, ATV, ROS and PTV are completely synthetic compounds and have structures distinct from the other statins [1, 7]. Chemical structures, nomen-

clatures and molecular weights of these drugs are shown in **Table 1**.

Statins are often co-administered with other drugs (acetylsalicylic acid, antihypertensive medicines - ACE inhibitors, calcium channel blockers) in therapy of cardiovascular disease, and also in combined therapy of multiple disorders, with antidiabetics, diuretics, nonsteroidal anti-inflammatory drugs and other analgetics, antibiotics etc.

Statins are often manufactured in combined pharmaceutical formulations together with ramipril, acetylsalicylic acid, enalapril, metoprolol, atenolol, hydrochlorothiazide, amlodipine, ezetimibe etc.

The application of statins 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.

Spectroscopic and high-performance liquid chromatography (HPLC) techniques are important groups of methods which play a significant role in the field of pharmaceutical analysis. The common availability of the instrumentation, the simplicity of procedures,

**Table 1.** Chemical structures of the statins

Drug (abbrev.)	Chemical structure	Chemical name	Molecular mass
Atorvastatin (ATV)		(3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid	558.64
Fluvastatin (FLV)		(3R,5S,6E)-7-[3-(4-fluorophenyl)-1-(propan-2-yl)-1H-indol-2-yl]-3,5-dihydroxyhept-6-enoic acid	411.46
Lovastatin (LOV)		(1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (2S)-2-methylbutanoate	404.54
Pitavastatin (PTV)		(3R,5S,6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)quinolin-3-yl]-3,5-dihydroxyhept-6-enoic acid	421.46
Pravastatin (PRV)		(3R,5R)-3,5-Dihydroxy-7-[(1S,2S,6S,8S,8aR)-6-hydroxy-2-methyl-8-[[2-(2S)-2-methylbutanoyl]oxy]-1,2,6,7,8,8a-hexahydro-1-naphthalenyl]heptanoic acid	424.53
Rosuvastatin (ROS)		(3R,5S,6E)-7-[4-(4-fluorophenyl)-2-(N-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid	481.54
Simvastatin (SMV)		(1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxotetrahydro-2H-pyran-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl 2,2-dimethylbutanoate	418.57

economy, speed, precision and accuracy of the technique make spectroscopic methods attractive. However, direct methods are not suitable for simultaneous determination of drugs with spectral overlapping. On the other hand the specificity of the HPLC method is excellent and simultaneously sufficient precision and accuracy are also achievable. The wide varieties of chromatographic columns available and detectors make possible the analysis of virtually all pharma-

ceutical compounds. However HPLC requires more complicated equipment, which is more expensive.

The aim of this work is to summarize the recent spectroscopic and HPLC methods for analysis of statins. Various spectroscopic and high-performance liquid chromatographic methods for determination of statins, their related impurities and co-administered drugs in the bulk drug forms and pharmaceutical formulations are reviewed.

## Analytical methods

### Spectroscopic methods

Direct, derivative and chemometric spectroscopic methods have been used for the analysis of different statins in their pure and pharmaceutical dosage forms.

#### *Ultraviolet spectrophotometric methods*

Simple spectrophotometric methods have been developed for determination of ATV in bulk and tablets formulations using methanol [8] or methanol:water (50:50) as solvent [9]. The absorbance maximum of ATV has been found at 244 nm and 248 nm respectively. Similar methods have been elaborated for determination of SMV. The estimation of SMV has been carried out using different solvents – methanol (method I) at 236 nm, 2-propanol (method II) at 230 nm and conc.  $H_2SO_4$  (method III) at 415 nm [10]. A simple spectrophotometric method for the assay of ROS in pharmaceutical formulations [11] has been developed using water as solvent. The absorbance maximum of ROS has been observed at 244 nm. Derivative spectrophotometric and absorbance ratio methods have been developed for the estimation of ATV in tablets [12] and combination of ATV and Ezetimibe in binary mixtures, bulk powder and pharmaceutical dosage forms [13 – 15]. FLV and Zofenopril have been determined simultaneously in two-component mixtures and in pharmaceutical preparations using the first, second and third derivatives of the zero-order spectra. FLV has been determined at wavelengths 339.03, 252.57 and 258.50 nm respectively [16]. A pH independent spectrophotometric method has been developed for the determination of PRV in pharmaceutical formulations. The method is based on the measurement of absorbance at isosbestic point. Isosbestic point of PRV has been determined by zero-order spectrophotometric method and difference spectrophotometric method and has been observed at 249 nm [17]. PRV and Fenofibrate in combined preparations have been simultaneously determined using the second-order derivative response at 237.6 and 295.1 nm for PRV and Fenofibrate respectively [18]. Derivative spectrophotometric methods have been used for the assay of PRV in presence of its degradation products [19]. These methods are based on the use of the third derivative spectrophotometry at 266.4 nm, first derivative of the ratio spectrum at 250.7 nm and first derivative of pH-induced difference spectrophotometry at 255.4 nm. The same paper describes

methods for determination of SMV and Ezetimibe in binary mixtures by first derivative of the ratio spectrum at 249.6 nm and 265.2 nm, and first-derivative spectrophotometry at 266.4 nm. Four spectrophotometric methods without any preliminary separation have been developed for simultaneous determination of SMV and Ezetimibe in laboratory prepared mixtures and tablets. The methods based on manipulating ratio spectra are: extended ratio subtraction, simultaneous ratio subtraction, ratio difference and absorption factor [20]. A simple, spectrophotometric method has been developed for the determination of ROS in pure form and its pharmaceutical formulations in acetonitrile. The method is based on the oxidation of ROS by iodine and formation of triiodide ( $I_3^-$ ) complex. The formed complex has been measured at 291 and 360 nm [21]. ROS and Aspirine have been estimated in bulk and dosage forms by first derivative spectrophotometric method and absorption maxima have been found to be at 259 nm for ROS and 238 nm for Aspirin [22]. A Q-absorption method has been described in which wavelengths selected were 257 nm as iso-absorptive point and 244 nm as  $\lambda_{max}$  of ROS [23]. Recently a review devoted to determination of ROS and Fenofibrate in combined dosage forms describes various spectrophotometric methods [24]. A method comprised of quantitative determinations of Clopidogrel in presence of HMG Co-A reductase inhibitors (ATV, PRV, SMV and ROS) in buffers of pH-1 and pH-4 (simulating gastric environments), pH-7.4 (simulating blood pH) and pH-9 (simulating intestinal pH) at body temperature (37°C) has been developed. Derivative spectroscopy coupled with zero-crossing measurements has been used for separating the interfering wavelengths of Clopidogrel and statins [25]. Chemometric assisted spectrophotometric methods based on classical least squares (CLS), principle component regression (PCR) and partial least squares (PLS) have been developed for simultaneous estimation of ATV and Ramipril [26] and SMV and Ezetimibe [27] in pharmaceutical preparations. The application of chemometric techniques allows using only zero-order spectra for quantification of the drugs.

#### *Visible spectrophotometric methods*

Simple spectrophotometric methods have been developed for the estimation of ATV, FLV and PRV, based on oxidative coupling reaction with 3-methyl-2-benzothiazolinone hydrazone hydrochloride

monohydrate in the presence of Ce (IV) in an acidic medium to form colored products with  $\lambda_{\max}$  at 566, 615 and 664 nm, respectively [28]. Ce (IV) has also been used for determination of ATV, ROS, Ciprofloxacin and Pantoprazole Sodium Sesquihydrate in pharmaceuticals. The methods are based on the oxidation of the drug by a known excess of Ceric Ammonium Sulphate in sulphuric acid medium and subsequent determination of unreacted Ce (IV) using Amaranth dye at 523 nm [29]. On the other hand two methods have been elaborated for estimation of ROS in bulk and pharmaceutical dosage forms [30]. First method is based on the oxidative coupling of ROS with 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) in presence of Ce (IV) as oxidant. The  $\lambda_{\max}$  of the colored species has been found to be 658 nm. Second method is based on the formation of complex between ROS and cobalt thiocyanate. The blue colored complex formed has been extracted into nitrobenzene, which possesses characteristic absorption maximum 626 nm. Charge-transfer complexes of ROS with various  $\pi$ -acceptors [31] (tetracyanoethylene, p-chloranilic acid, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, 2,3,5,6-tetrabromo-1,4-benzoquinone, 1,3,5-trinitrobenzene, 2,3,5,6-tetrachloro-1,4-benzoquinone, 7,7,8,8-tetracyanoquinodimethane, and 2,4,7-trinitro-9-fluorenone) have been studied. The formation of the colored complexes has been utilized in the development of spectrophotometric methods for the determination of ROS in tablets. The oxidation of PTV by ferric chloride in presence of o-phenanthroline (method A) or 2, 2' bipyridyl (method B) or potassium ferricyanide (method C) has been used for elaboration of methods for determination of PTV in bulk drugs and in pharmaceutical formulations. The colored complex formed has been measured at 510, 530 and 755 nm for method A, B and C respectively [32]. On the other hand potassium permanganate in acidic medium is also used for oxidation of PTV and the unreacted oxidant has been measured at 550 nm. [33]. An extractive spectrophotometric method has been developed for the assay of SMV, PRV and ATV in pure form and in tablets. The method comprises the formation of colored ion-pairs between the drugs and the Mo(V)-thiocyanate binary complex followed by their extraction with 1,2-dichloroethane and quantitative determination at 470 nm [34]. Spectrophotometric methods have been developed for the determination of five 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA)

reductase inhibitors, namely ATV, FLV, PTV, ROS and SMV, in pharmaceutical preparations. The methods are based on the reaction of drugs as n-electron donors with 7,7,8,8-tetracyanoquinodimethane as  $\pi$ -acceptor to give highly colored complex species. The resulting solutions have been measured at 843 nm [35].

#### *Spectrofluorimetric methods*

Spectrofluorimetric procedure for determination of ATV in pharmaceutical formulations has been developed. In this method, the native fluorescence characteristics of ATV have been studied in both acidic and basic media. High sensitivity has been obtained with 5% acetic acid and the fluorescence intensity has been measured at  $\lambda_{\text{ex}}$  276 nm and  $\lambda_{\text{em}}$  389 nm [36]. On the other hand, spectrofluorimetric methods have been elaborated for the determination of ROS, Ezetimibe and PTV in pharmaceutical preparations. The first method is based on measuring the fluorescence of the drugs at their optimum excitation and emission wavelengths. The fluorescence intensity has been measured at  $\lambda_{\text{ex}}$  315 nm, 260 nm, and 245 nm, and at  $\lambda_{\text{em}}$  362 nm, 309 nm, and 373 nm for ROS, Ezetimibe, and PTV respectively. The second method has been developed for simultaneous determination of ROS and Ezetimibe. The fluorescence has been measured at  $\lambda_{\text{em}}$  309 nm for Ezetimibe and 432 nm for ROS upon excitation at  $\lambda_{\text{ex}}$  260 nm for both [37].

#### *Kinetic spectrophotometric methods*

Kinetic spectrophotometric methods for determination of ATV [38] and PRV [39] in pure and pharmaceutical dosage forms have been described. The first method consist of the oxidative coupling reaction of ATV with 3-methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate (MBTH) in the presence of Ce (IV) in an acidic medium to form colored product with  $\lambda_{\max}$  at 566 nm. The reaction was followed spectrophotometrically by measuring the increase in absorbance at 566 nm as a function of time. On the other hand, the method for PRV determination is based on the formation of colored product between PRV and 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole in acetone medium. The reaction was followed spectrophotometrically by measuring the increase in absorbance at 462 nm as a function of time. The initial rate and fixed time methods have been used in both procedures.

From the mentioned above it can be seen that, most of the reported spectroscopic methods are based

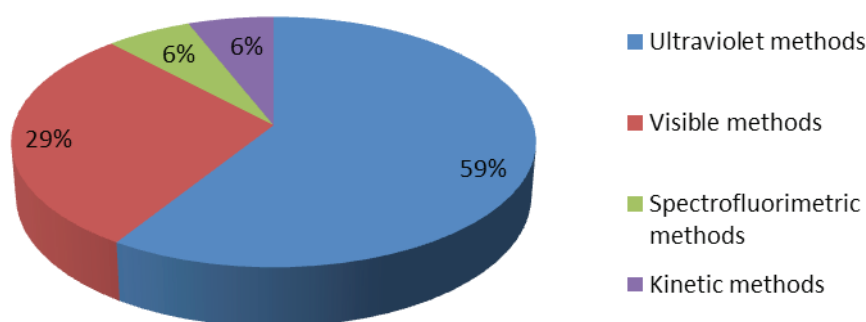


Figure 1. Reported spectroscopic methods

on ultraviolet spectrophotometric methods, as shown in Figure 1.

### High-performance liquid chromatography

There is an impressive increase in the use of high-performance liquid chromatography for determination of statins. HPLC has been used frequently in all fields of statins research. In the available literature there is a recent review, describing chromatographic and electrophoretic analytical methods for determination of statins, published in 2012 [1]. That's why in this paper we are going to focus on articles, published after 2012 or older ones, which we found that, have not been included in the mentioned review. There are many reported HPLC methods for separation and quantitative determination of statins in pure and in pharmaceutical dosage forms (alone or in combination with other drugs). These methods, based on different stationary phases (C8, C18), different mobile systems

and using UV or diode array (DAD) detectors are summarised in **Table 2** [18, 26, 40-84].

### Conclusion

The article describes different spectroscopic and HPLC methods for analysis of statins (Atorvastatin, Fluvastatin, Lovastatin, Pitavastatin, Pravastatin, Rosuvastatin and Simvastatin) in pure forms, in different pharmaceutical dosage forms and in multicomponent mixtures. Ultraviolet, visible, spectrofluorimetric and kinetic spectrophotometric methods are presented. These methods are fast and suitable for the analysis of simple matrices without overlapping spectra. For more complex matrices, they require prolonged sample pretreatment and data processing. On the other hand HPLC methods are preferable for separation and quantification of drugs with overlapping spectra in multicomponent mixtures.

Table 2. HPLC methods for determination of statins

Drug/formulation	Application	Detector / Column	Chromatographic conditions	Ref
<b>ATV</b>				
ATV	Drug substance	HPLC-DAD SunFire C18 ODS (250x4mm, 5 $\mu$ m)	acetonitrile: phosphoric acid 0.1% (65:35) flow rate=1.5 ml/min; $\lambda_{\max}$ =238 nm	[40]
ATV	Tablets	HPLC-UV LiChrospherR 100 RP-18 (250x4mm, 5 $\mu$ m)	0.1% acetic acid: acetonitrile (45:55), pH-3.8 flow rate=0.8 ml/min; $\lambda_{\max}$ =246 nm	[41]
ATV and impurities	Drug substance	HPLC-UV C18 (250x4.6mm, 3.5 $\mu$ m)	A: phosphate buffer pH-5.4 B: acetonitrile: tetrahydrofuran: (90:10) gradient elution mode flow rate=1.5 ml/min; $\lambda_{\max}$ =220 nm	[42]

ATV and FLV, PRV	Drug substance	HPLC-DAD ODS-AQ YMC (50x4.6mm, 3 $\mu$ m)	ethanol: formic acid (pH-2.5, 25 mM) (50:50) flow rate=1 ml/min; $\lambda_{max}$ =238 nm	[43]
ATV and PRV, SMV	Drug substance Dosage form	HPLC-UV Poroshell 120 SB C18 (150x4.6mm, 2.7 $\mu$ m)	0.1% <i>o</i> -phosphoric acid: methanol gradient elution mode flow rate=1 ml/min; $\lambda_{max}$ =238 nm	[44]
ATV and ROS, SMV, Captopril	Drug substance Dosage form	HPLC-UV Purospher Star, C18 (250x4.6mm, 5 $\mu$ m)	acetonitrile: water (60:40) pH-2.9 flow rate=1,5 ml/min; $\lambda_{max}$ =230 nm	[45]
ATV and ROS, SMV, Diltiazem	Drug substance Dosage form	HPLC-UV Purospher Star, C18 (250x4.6mm, 5 $\mu$ m)	acetonitrile: water (85:15), pH-2.6 flow rate=1 ml/min; $\lambda_{max}$ =230 nm	[46]
ATV and ROS, SMV, Enalapril	Drug substance Dosage form	HPLC-UV Purospher Star, C18 (250x4.6mm, 5 $\mu$ m)	acetonitrile: water (60:40), pH-2.8 flow rate=1.8 ml/min; $\lambda_{max}$ =230 nm	[47]
ATV and ROS, Fenofibrate, Fenofibric acid	Drug substance Dosage form	HPLC-UV C18 (250x4.6 mm, 10 $\mu$ m)	acetonitrile: water (82:18) flow rate=1.5 ml/min; $\lambda_{max}$ =254 nm	[48]
ATV and ROS, Ezetimibe, Telmisartan	Drug substance Dosage form	HPLC-UV Phenomenex C18 (150x4.6mm, 5 $\mu$ m)	methanol: acetonitrile: dipotassium hydrogen phosphate buffer (pH-3.0), flow rate=2.0 ml/min; $\lambda_{max}$ =239 nm	[49]
ATV and Aspirine	Capsules	HPLC-UV Phenomenex Gemini C18 (250x4.6mm, 5 $\mu$ m)	potassium dihydrogen phosphate: methanol (20:80), pH-4 flow rate=1.0 ml/min; $\lambda_{max}$ =240 nm	[50]
ATV and Fenofibrate	Drug substance Dosage form	HPLC-DAD Thermohypersil BDS C18 (100x4.6mm, 5 $\mu$ m)	methanol: water (40:60), pH-2 flow rate=1.0 ml/min; $\lambda_{max}$ =274 nm	[51]
ATV and Metformine, Sitagliptin	Tablets	HPLC-DAD HyperSil COLD (150x4.6mm, 4 $\mu$ m)	methanol: buffer (1% conc. HNO <sub>3</sub> , 2% conc. ammonia solution) (70:30), pH-8 flow rate=1.0 ml/min; $\lambda_{max}$ =254 nm	[52]
ATV and Nicotinic acid	Tablets	HPLC-UV ZORBAX SB-C18 (150x4.6mm, 3.5 $\mu$ m)	acetonitrile: water (85:15) pH-4.5 flow rate=1 ml/min; $\lambda_{max}$ =261 nm	[53]
ATV and Ramipril	Drug substance Dosage form	HPLC-UV Phenomenex-Luna RP C18 (250x4.6mm, 5 $\mu$ m)	acetonitrile: 0.1M sodium perchlorate pH-2.5 (70: 30) flow rate=1.5 ml/min; $\lambda_{max}$ =210 nm	[26]
ATV and Aspirin, Atenolol, Clopidogrel, Ezetimibe, Fenofibrate, Glimepiride, Losartan, Metformin, Telmisartan	Drug substance Dosage form	HPLC-DAD Phenyl column (250x4.6mm, 5 $\mu$ m)	acetonitrile: triethylamine: acetate buffer (pH-5.0) gradient elution mode flow rate=1.0 ml/min; $\lambda_{max}$ =230 nm	[54]

<b>FLV</b>				
FLV and ATV, PRV	Drug substance	HPLC-DAD ODS-AQ YMC (50x4.6mm, 3 $\mu$ m)	ethanol: formic acid (pH-2.5, 25 mM) (50:50) flow rate=1 ml/min; $\lambda_{\max}$ =238 nm	[43]
<b>LOV</b>				
LOV and SMV	Tablets	HPLC- DAD LiChrosorb C18 (250x4.6mm, 5 $\mu$ m)	acetonitrile: water (30:70) flow rate=1.5 ml/min; $\lambda_{\max}$ =240 nm	[55]
LOV and PRV, SMV	Drug substance Dosage form	HPLC-DAD LiChrospher C8 (250x4mm, 5 $\mu$ m)	acetonitrile: 0.1% phosphoric acid (65:35) flow rate=1.5 ml/min; $\lambda_{\max}$ =238 nm	[56]
<b>PTV</b>				
PTV	Tablets	HPLC-UV Agilent Eclipse XDB C18 (150x4.6mm, 5 $\mu$ m)	phosphate buffer (pH-3.4): acetonitrile (65:35) flow rate=0.9 ml/min; $\lambda_{\max}$ =244 nm	[57]
PTV and related substances	Tablets	HPLC-DAD Phenomenex Kinetex C18 (75x4.6mm, 2.6 $\mu$ m)	A: acetate buffer (pH-3.8): acetonitrile (90:10) B: acetonitrile: water (90:10) gradient elution mode flow rate=1 ml/min; $\lambda_{\max}$ =250 nm	[58]
PTV and related substances	Drug substance Dosage form	UHPLC-DAD Poroshell 120 SB-C18 (100x4.6mm, 2.7 $\mu$ m)	A: sodium formate: acetonitrile: formic acid (75:25:0.2); B: acetonitrile: sodium formate: formic acid (95:5:0.05) gradient elution mode flow rate=2 ml/min; $\lambda_{\max}$ =250 nm	[59]
PTV and Ezetimibe	Dosage form	HPLC-DAD Phenomenex Luna C18 (250x4.6mm, 5 $\mu$ m)	0.1% <i>o</i> -phosphoric acid: acetonitrile: triethylamine (19.8:80:0.2); pH-3 $\pm$ 0.05 flow rate=1.4 ml/min; $\lambda_{\max}$ =235 nm	[60]
<b>PRV</b>				
PRV	Tablets	HPLC-UV Phenomenex Luna C18 (150x4.6mm, 5 $\mu$ m)	acetonitrile: potassium dihydrogen phosphate (30:70), pH-3 flow rate=1.5 ml/min; $\lambda_{\max}$ =240 nm	[61]
PRV	Tablets	HPLC-DAD LiChrospher C18 (125x4mm, 5 $\mu$ m)	methanol: water: trimethylamine: glacial acetic acid (455:545:2:1.2) flow rate=2 ml/min; $\lambda_{\max}$ =238 nm	[62]
PRV	Dosage form	HPLC-UV Tracer Extracil C8 (250x4.6mm, 5 $\mu$ m)	ammonium acetate: methanol: trimethylamine (40:60:0.17) flow rate=1 ml/min; $\lambda_{\max}$ =239 nm	[63]
PRV and ATV, FLV	Drug substance	HPLC-DAD ODS-AQ YMC (50x4.6mm, 3 $\mu$ m)	ethanol: formic acid (pH-2.5, 25mM) (50:50) flow rate=1 ml/min; $\lambda_{\max}$ =238 nm	[43]
PRV and ATV, SMV	Drug substance Dosage form	HPLC-UV Poroshell 120 SB C18 (150x4.6mm, 2.7 $\mu$ m)	0.1% <i>o</i> -phosphoric acid: methanol gradient elution mode flow rate=1 ml/min; $\lambda_{\max}$ =238 nm	[44]

PRV and LOV, SMV	Drug substance Dosage form	HPLC-DAD LiChrospher C8 (250x4mm, 5 $\mu$ m)	acetonitrile: 0.1% phosphoric acid (65:35) flow rate=1.5 ml/min; $\lambda_{max}$ =238 nm	[56]
PRV and Clopidogrel	Tablets	HPLC-UV Phenomenex Synergi Hydro-RP (50x4.6mm, 4 $\mu$ m)	water: methanol: trifluoroacetic acid (45:55:0.025) flow rate=1 ml/min; $\lambda_{max}$ =238 nm	[64]
PRV and Fenofibrate	Dosage form	HPLC-UV phenyl Hypersil C18 (125x4.6 mm, 5 $\mu$ m)	acetonitrile: 0.1% diethyl amine (50:50), pH-4.5 flow rate=1.0 ml/min; $\lambda_{max}$ =240 nm	[18]
PRV and Diltiazem, Meloxicam Naproxen sodium	Drug substance Dosage form	HPLC-UV Purospher Star C18 (250x4.6mm, 5 $\mu$ m)	methanol: water (80:20), pH-3.4 flow rate=1 ml/min; $\lambda_{max}$ =220 nm	[65]
<b>ROS</b>				
ROS	Drug substance Dosage form	HPLC-DAD Nucleodur C8 (250x4.6mm, 5 $\mu$ m)	0.1M formic acid: methanol (25:75) flow rate=1,0 ml/min; $\lambda_{max}$ =280 nm	[66]
ROS	Tablets	HPLC-UV Zorbax SB C18 (250x4.6mm, 5 $\mu$ m)	sodium phosphate buffer (0.05 M, pH-4.0): methanol: acetonitrile (5:35:60) flow rate=0.9 ml/min; $\lambda_{max}$ =244 nm	[67]
ROS and ATV, SMV Captopril	Drug substance Dosage form	HPLC-UV Purospher Star C18 (250x4.6mm, 5 $\mu$ m)	acetonitrile: water (60:40), pH-2.9 flow rate=1,5 ml/min; $\lambda_{max}$ =230 nm	[45]
ROS and ATV, SMV, Diltiazem	Drug substance Dosage form	HPLC-UV Purospher Star C18 (250x4.6mm, 5 $\mu$ m)	acetonitrile: water (85:15), pH-2.6 flow rate=1 ml/min; $\lambda_{max}$ =230 nm	[46]
ROS and ATV, SMV Enalapril	Drug substance Dosage form	HPLC-UV Purospher Star C18 (250x4.6mm, 5 $\mu$ m)	acetonitrile: water (60:40), pH-2.8 flow rate=1.8 ml/min; $\lambda_{max}$ =230 nm	[47]
ROS and ATV, Fenofibrate Fenofibric acid	Dosage form	HPLC-UV C18 (250x4.6mm, 10 $\mu$ m)	acetonitrile: water (82:18) flow rate=1.5 ml/min; $\lambda_{max}$ =254 nm	[48]
ROS and ATV, Ezetimibe Telmisartan	Dosage form	HPLC-UV Phenomenex C18 (150x4.6 mm, 5 $\mu$ m)	acetonitrile: methanol: dipotassium hydrogen phosphate buffer (pH-3), (34.27:20:45.73) flow rate=2.0 ml/min; $\lambda_{max}$ =239 nm	[49]
ROS and Amlodipine	Dosage form	HPLC-DAD Luna C18 (250x4.6mm, 5 $\mu$ m)	phosphate buffer (pH-2.5): acetonitrile gradient elution mode flow rate=1.5 ml/min; $\lambda_{max}$ =240 nm	[68]
ROS and Aspirin	Capsules	HPLC-UV HyperChrom ODS-BP C18 (200x4.6mm, 5 $\mu$ m)	acetonitrile: 0.05M phosphate buffer (55:45), pH-3 flow rate=1 ml/min; $\lambda_{max}$ =241 nm	[69]
ROS and Ciprofloxacin	Drug substance Dosage form	HPLC-UV Purospher Star, C18 (250x4.6mm, 5 $\mu$ m)	methanol: water (90:10), pH-3 flow rate=1 ml/min; $\lambda_{max}$ =255nm	[70]
ROS and Ezetimibe	Dosage form	HPLC-DAD C18 (250x4.6mm, 5 $\mu$ m)	sodium acetate buffer (pH-4): acetonitrile (30:70) flow rate=1.2 ml/min; $\lambda_{max}$ =254nm	[71]

ROS and Ezetimibe	Dosage form	HPLC-UV Phenomenex Luna C18 (250x4.6mm, 5 $\mu$ m)	ammonium acetate buffer: methanol: acetonitrile: (30:40:30), pH-7.2 flow rate=1,5 ml/min; $\lambda_{\max}$ =230 nm	[72]
ROS and Ezetimibe	Tablets	HPLC-DAD Waters C18 (250x4.6mm, 5 $\mu$ m)	acetonitrile: water: phosphate buffer pH-8 (40:10:50) flow rate=1 ml/min; $\lambda_{\max}$ =230 nm	[73]
ROS and Gemifloxacin	Drug substance Dosage form	HPLC-UV Purospher Star C18 (250x4.6mm, 5 $\mu$ m)	methanol: water (90:10), pH-3 flow rate=1 ml/min; $\lambda_{\max}$ =263nm	[74]
ROS and Hydrochlorthiazide	Drug substance Dosage form	HPLC-UV ACE C18 AR (250x4.6mm, 5 $\mu$ m)	sodium perchlorate buffer (pH-3): acetonitrile (60:40) flow rate=1 ml/min; $\lambda_{\max}$ =280 nm	[75]
ROS and Metformin	Drug substance Dosage form	HPLC-DAD Phenomenex C18 (250x4.6mm, 5 $\mu$ m)	acetonitrile: phosphate buffer (35:65), pH-3.8 flow rate=1 ml/min; $\lambda_{\max}$ =252 nm	[76]
ROS and Captopril, Enalapril Lisinopril	Drug substance Dosage form	HPLC-UV Purospher Star C18 (250x4.6mm, 5 $\mu$ m)	methanol: water (75:25), pH-3 flow rate=1 ml/min; $\lambda_{\max}$ =214 nm	[77]
ROS and Glimepiride, Gliquidone, Metformin	Drug substance Dosage form	HPLC-UV Purospher Star C18 (250x4.6mm, 5 $\mu$ m)	methanol: water (90:10), pH-3 flow rate=1 ml/min; $\lambda_{\max}$ =231 nm	[78]
ROS and Ibuprofen, Mefenamic acid, Meloxicam	Drug substance Dosage form	HPLC-UV Purospher Star, C18 (250x4.6mm, 5 $\mu$ m)	methanol: water: acetonitrile (80:17.5:2.5), pH-3.0 flow rate=1 ml/min; $\lambda_{\max}$ =230 nm	[79]
<b>SMV</b>				
SMV and LOV	Tablets	HPLC-DAD LiChrosorb C18 (250x4.6mm, 5 $\mu$ m)	acetonitrile: water (30:70) flow rate=1.5 ml/min; $\lambda_{\max}$ =240 nm	[55]
SMV and ATV, PRV	Drug substance Dosage form	HPLC-UV Poroshell 120 SB C18 (150x4.6mm, 2.7 $\mu$ m)	0.1% <i>o</i> -phosphoric acid-methanol gradient elution mode flow rate=1 ml/min; $\lambda_{\max}$ =238 nm	[44]
SMV and LOV, PRV	Drug substance Dosage form	HPLC-DAD LiChrospher C8 (250x4mm, 5 $\mu$ m)	acetonitrile: 0.1% phosphoric acid (65:35) flow rate=1.5 ml/min; $\lambda_{\max}$ =238 nm	[56]
SMV and ATV, ROS Captopril	Drug substance Dosage form	HPLC-UV Purospher Star, C18 (250x4.6mm, 5 $\mu$ m)	acetonitrile: water (60:40) pH-2.9 flow rate=1,5 ml/min; $\lambda_{\max}$ =230 nm	[45]
SMV and ATV, ROS, Diltiazem	Drug substance Dosage form	HPLC-UV Purospher Star, C18 (250x4.6mm, 5 $\mu$ m)	acetonitrile: water (85:15), pH-2.6 flow rate=1 ml/min; $\lambda_{\max}$ =230 nm	[46]
SMV and ATV, ROS Enalapril	Drug substance Dosage form	HPLC-UV Purospher Star C18 (250x4.6mm, 5 $\mu$ m)	acetonitrile: water (60:40), pH-2.8 flow rate=1.8 ml/min; $\lambda_{\max}$ =230 nm	[47]

SMV and Gemfibrozil	Dosage form	HPLC-UV C18 (250x4.6mm, 5µm)	ammonium acetate pH-5: acetonitrile (15: 85) flow rate=1.0 ml/min; $\lambda_{max}$ =237 nm	[80]
SMV and Niacin	Tablets	HPLC-UV C18 (150x4.6 mm).	methanol: water (triethylamine 0.05%) (85:15) pH-4 flow rate=1 ml/min; $\lambda_{max}$ =250 nm	[81]
SMV and Atenolol, Aspirin, Hydrochlorothiazide, Ramipril	Dosage form	HPLC-UV X-Terra C18 (150x4.6mm, 5µm)	sodium perchlorate buffer: acetonitrile gradient elution mode flow rate=0.8 ml/min; $\lambda_{max}$ =220 nm	[82]
SMV and Aspirin, Ramipril	Dosage form	HPLC-UV LiCrosphere 100 RP-18 (250x4.6mm, 5µm)	acetonitrile: 0.1% <i>o</i> -phosphoric acid (70:30); pH-2.5 flow rate=1 ml/min; $\lambda_{max}$ =225 nm	[83]
SMV and Sitagliptin	Drug substance Tablets	HPLC-UV Welchrom C18 (250x4.6mm, 5µm)	phosphate buffer: acetonitrile: methanol in (45:35:20) flow rate=1 ml/min; $\lambda_{max}$ =255 nm	[84]

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