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Review Article

ANALYTICAL METHODS FOR THE QUANTITATION OF AMLODIPINE BESYLATE AND ATORVASTATIN CALCIUM IN PHARMACEUTICAL DOSAGE FORM AND BIOLOGICAL FLUIDS

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ABSTRACT

Amlodipine is the best-prescribed medication for cardiovascular disease major risk factor for hypertension and atorvastatin well known for diabetic. First discussed low cost ultraviolet-visible technique for the determination and quantitation of drugs in pharmaceuticals and biological fluids. Chromatographic techniques have an application with respect to trace analysis. Different types of chromatography such as high-performance liquid chromatography, high performance thin layer chromatography have most frequent applications in the field of pharmaceutical as well as biomedical analyses. Chromatography combined with mass spectrophotometry has the ability to collect molecular ion, followed to prepare a spectrum to assess molecular weight as well as structure. High-performance liquid chromatography coupled with mass spectrophotometry is a reliable and dynamic technique for the analysis of small and large drugs molecule. The advantages and disadvantages of all techniques are compared with each other with respect to sensitivity, reproducibility and other important parameters. The investigation also focused for the quantitation on both drugs in pharmaceutical preparations and plasma samples with the help of all available analytical techniques.

Keywords: Drugs, Amlodipine, Atorvastatin, HPLC, UPLC, TLC, HPTLC, CE, LCMS, UP-LCMS

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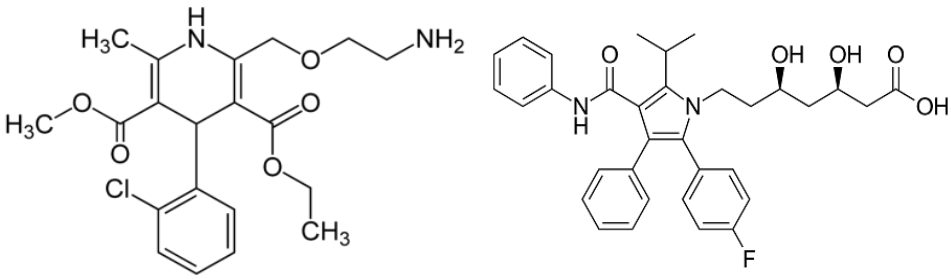
INTRODUCTION

Amlodipine besylate (fig. 1a) is scientifically known as (RS)-3-ethyl-5-methyl-2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate benzene sulfonate. It was first introduced and prescribed for coronary artery disease. It was also helpful for angina and peripheral artery disease [1]. Now days mostly targeted for the patient having hypertension. The European Society of Cardiology was conducted survey until the year 2000 and their report based on statistical analysis showed nine hundred seventy-two million people were in this category. The number will increase with time and expected by the year 2025, approximately 1.56 billion [2]. This drug is under the umbrella of calcium channel blocker. The mechanism of such blockers to control the transportation of calcium to coronary (mainly smooth muscle) and arteries, that reflects on muscles became relaxes, reduces peripheral resistance and ultimately lowering the blood pressure [3]. Atorvastatin (fig. 1b) is chemically known as (βR, δR)-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-lH-pyrrole-1-heptanoic acid.



(a) (b)

Fig. 1: Structure of amlodipine (a) and atorvastatin (b)

The synthetic drug, atorvastatin is generally under the class known statins. These reductase inhibitors are familiar as 3–hydroxyl–3–methylglutaryl–coenzyme A (HMG–CoA). All drugs are prescribed for cardiovascular disease and reduction of heart attack, as well as for clinical demand [4–10]. Active pharmaceutical ingredients (APIs) such as amlodipine are commonly manufactured as their acid addition salts to promote solubility and improve both stability and bioavailability. The objective of this review article was to research more about ultraviolet–visible and chromatographic technique's applications, specifically for the quantification of amlodipine and atorvastatin in bulk, pharmaceutical formulations, and biological fluids. Compared the result between all the developed method for amlodipine and atorvastatin about the limit of detection and quantitation value. Discussed briefly the importance of all analytical techniques with respect to cost, time of analysis, sensitivity and limitations.

Analytical Techniques

UV–visible spectrophotometer

Spectroscopic methods are mostly used for the determination of drugs in bulk as well as with pharmaceutical formulations. Ultraviolet (UV) and visible spectrophotometry are important and common techniques for the quantitative analysis of drugs. Because these are low-cost techniques, simple and no requirement of pretreatment as well as any elaborate preparatory step prior to assay. The visible spectrophotometer is depending on the redox and complex formation reaction. However, some deficiencies are also with the techniques with respect to the

presence of two or more drugs have similar UV characteristic. The detailed literature survey studies elaborate on the advantages and disadvantages among all developed method for both drugs using UV–visible spectrophotometer [11–38] are presented in table 1.

Table 1: Determination of amlodipine and atorvastatin using uv–visible spectrophotometer

Drug	Reagent/Solvent	λ_{max} (nm)	Application	Reference
Amlodipine	Chloramin-T	390	Bulk powder	[11]
Besylate	Methanol	239, 238	Bulk drug and tablets	[12]
	Ethanol	343	Rat plasma	[13]
	Water	238	Tablets	[14]
	Methanol, HCl	240	Tablets	[15]
	7,7,8,8-tetracyanoquinodimethane (TCNQ), tetracyanoethylene (TCNE)	745, 396	Pure and dosage form	[16]
	Phosphoric acid and water	366	Pure and dosage form	[17]
	Methanol	360	Pharmaceutical dosage forms	[18]
	HCl	239	Bulk and tablets	[19]
	Methanol	360	Bulk and tablet dosage form	[20]
	Methanol	200–350	Tablets	[21]
	Methanol	359	Bulk mixture and tablets	[22]
	Methanol	360	Tablets	[23]
	Methanol: Water (50:50, v/v)	241	Tablet	[24]
	Methanol and water	239, 245	Tablet dosage form	[25]
	Urea solution	243	Bulk and pharmaceutical dosage form	[26]
	Water	366, 239	Pure sample and tablets	[27]
	Methanol	239	Tablet dosage form	[28]
Atorvastatin	Methanol	247	Pure and tablets	[29]
Calcium	p-dimethylaminobenzaldehyde	540	Bulk and dosage form	[30]
	Pararosaniline HCl	547	Tablet	[31]
	Iodine	291, 360	Pure and pharmaceutical formulations	[32]
	ACN: Water =70:30,v/v	268, 245	Tablet dosage form	[33]
	Methanol	247	Bulk and pharmaceutical dosage form	[34]
	Methanol	244	API and tablet	[35]
	Methanol	246	Tablet dosage form	[36]
	Methanol: Water (50:50, v/v)	250	Tablet	[24]
	Urea, Ferric chloride and potassium ferricyanide	240, 787	Tablets and biological fluids	[37]
	Methanol	246	API and pharmaceutical formulations	[38]

High performance liquid chromatography

High-performance liquid chromatography (HPLC) technique is more accurate and based on the properties of the analyte with the existing mobile phase and stationary phase. Depending on the stationary and mobile phase, different types of chromatography techniques are developed. Recently high-performance liquid chromatography has achieved lots of attention in the field of pharmaceutical analysis in dosage forms and biological fluids because of its simplicity, sensitivity and high specificity. The conventional rule for the analysis of polar compounds by utilizing a non-polar stationary phase with the polar mobile phase and vice versa for the non-polar compounds. Stationary phase binding the analyte is directly proportional with the surface area of the non-polar segment of the analyte associate the ligand as well as with aqueous eluent. One of the main parameters found as evidence about the quality and efficacy of drug products and formulations is stability testing. The products nature is changing with humidity, temperature, light, retesting time, storage conditions, shelf life, so it is necessary to control the environmental factors. To develop method different types of the column (ODS C_{18}, BDS C_8, ODS C_8, BDS C_{18} and Discovery HS C_{18}) with a combination of different mobile phases containing buffers, organic modifier (acetonitrile, methanol) can be used. Amlodipine and atorvastatin were quantified using HPLC with ultraviolet in bulk and pharmaceutical dosage form [39–83] (table 2).

Ultra pressure liquid chromatography

The ultra-performance liquid chromatography (UPLC) is a new and modern technique for liquid chromatography. Worldwide HPLC was a predominant technique for the last 30 to 40 y for the drug analysis. Speed, sensitivity, and resolution are the main keywords for the drugs analysis with UPLC compare to HPLC. The particle size can play a significant role in this type of chromatography that governed by the well-known Van Deemter equation. For UPLC, the preferred size diameter is less than 2µm to get more sensitivity, short analysis time and improved resolution. The quality of the product analyzed by UPLC will give better with less time. However, the main disadvantage is related to the column life. The analysis requiring high pressure (100 M Pa) that damage the column efficiency. The novel and selective methods were developed for the determination of both drugs with a marketed formulation as single or combined dosage form [84–98] (table 3).

Thin layer chromatography

Thin-layer chromatography (TLC) is a simple separation technique typically for the mixture of nonvolatile compounds. The adsorbent material, specifically cellulose, silica gel, aluminum oxide is covered on plastic or glass sheet, known as the stationary phase. Aleppo bentonite with modified phenyl support can also be applied as the stationary phase, has the ability to separate amlodipine and atorvastatin with a mobile phase consisting of sodium phosphate buffer and acetonitrile (50:45, v/v) in pharmaceutical dosage form [99]. Methanol, toluene triethylamine combination and silica gel adsorbent distinct atorvastatin in pharmaceutical formulation with high resolution [100]. However, triethylamine can be replaced with chloroform and acetic acid in tablet dosage form on the aluminum plate [101]. However, propanol and water system (70:30, v/v) has the capability to separate amlodipine with a detection limit of 0.4 µg [102].

Table 2: Important parameters for the determination of drugs using HPLC

Drug	Mobile phase	Stationary phase	Flow rate (ml/min)	Detector (nm)	Application	Reference
Amlodipine Besylate	TEA buffer (pH 3) and ACN = 25:75, v/v	Stainless steel C_{18} (4.6×150 mm, 5 µm)	1.0	258	Pure and pharmaceutical dosage form	[39]
	0.1% OPA: Acetonitrile = 70:30, v/v	Phenomenex C_{18} (4.6×100 mm, 3.5µm)	0.7	230	Pharmaceutical dosage form	[40]
	Phosphate Buffer (pH 4.5): Acetonitrile = 55: 45,v/v	Phenomenex C_{18} (25×0.46 cm, 5µm)	1.0	224	Tablet	[41]
	Potassium dihydrogen phosphate buffer (pH 5.3): Acetonitrile = 55:45, v/v	Shodex RP C_{18} (250 ×4.6 mm, 5 µm)	1.0	237	Pure and dosage form	[42]
	Ammonium acetate (0.5 M, pH 6.8 at 0.5): Acetonitrile = 40:60, v/v	Eclipse XDB C_{18} (4.6×250 mm, 5 μm)	0.5	239	Bulk drug and excipient	[43]
	Potassium dihydrogen orthophosphate (0.05M, pH 3.5): Acetonitrile = 45:55, v/v	C_{18} ODS (250×4.6 mm, 5.0 µm)	1.5	230	Pharmaceutical formulation	[44]
	Phosphoric acid (0.025 M) and Acetonitrile	Zorbax SB-C_8 (4.6×250 mm, 5 µm)	1.0	225 238	Tablets	[45]
	Phosphate buffer: Methanol (73:27, v/v)	Zorbax Eclipse Plus (150x4.6 mm, 5µm)	1.1	270	Tablets	[46]
	Mobile phase A–Phosphate buffer, pH 2.8: Methanol=60:40, v/v Mobile phase B–Phosphate buffer, pH 2.8: Methanol: Acetonitrile =20:40:40, v/v/v	Inertsil ODS-3 C_{18} (150×4.6 mm, 3µm)	1.0	340	Tablet formulation	[47]
	Phosphate buffer (pH=3): Acetonitrile: Methanol = 25:45:30,v/v/v	Zorbax ODS (4.6x250 mm, 5 µm)	1.0	254	Tablets	[48]
	Acetonitrile=Methanol (70:30, v/v)	Hypersil C_{18} (250×4.60 mm, 5µm)	1.0	222	Bulk and pharmaceutical dosage form	[49]
	Potassium dihydrogen phosphate (pH 5.5, 0.03M)-Acetonitrile (65:35, v/v)	Phenomenex C_{18} (250x4.6 mm, 2.6µm)	1.2	240	Tablets	[50]
	Potassium dihydrogen orthophosphate buffer (50 mmol, pH 3.7): Acetonitrile = (56:44, v/v)	Kromasil KR 5 C_{18} (250×4.6 mm, 5 µm)	1.0	232	Pharmaceutical formulations	[51]
	Potassium dihydrogen orthophosphate buffer (pH 3.2):Acetonitrile=(60:40, v/v)	Hypersil gold C_{18} (250×4.6 mm, 5 µm)	1.0	237	Pharmaceutical dosage form	[52]
	Acetonitrile, water and potassium dihydrogen phosphate buffer pH 2.7 (45:35:20,v/v/v)	Phenomenex Luna (250×4.60 mm, 5 µm)	1.0	230	Tablet dosage form	[53]
	Acetonitrile-Phosphate buffer (0.05 M, pH 2.8):Acetonitrile (60:40, v/v)	Phenomenex Kinetex (150×4.6 mm)	0.8	227	Tablet and human plasma	[54]
	Phosphate buffer pH 4.0: Acetonitrile (40:60, v/v)	ODS C_{18} (250 mm×4.6 mm, 5µm)	1.0	247	Bulk and tablet dosage form	[55]
	Potassium dihydrogen phosphate buffer (pH 4.5): Methanol (25:75,v/v)	Prontosil C_{18} (250 × 4.6 mm, 5 µm)	1.4	240	Tablet formulation	[56]
	Acetonitrile and water (60:40, v/v)	HIQ SII C_{18} -10 (250x4.5 mm)	1.0	248	Tablet	[57]
Atorvastatin Calcium	Ammonium acetate buffer (10 mmol, pH 4): Acetonitrile (40:60, v/v)	Symmetry C_{18} (75×4.6 mm, 3.5 µm)	1.0, 2.0	220	Tablet dosage form	[58]
	Potassium dihydrogen orthophosphate buffer (pH 3.2):Acetonitrile=(52:48, v/v)	Kromasil C_{18} (150×4.6 mm, 5 µm)	1.0	210	Tablet dosage form	[59]
	Acetonitrile: dichloromethane: acetic acid (68.6:30.6:0.8, v/v/v)	Acclaim 120 C_{18} (250×4.6 mm, 5 µm)	1.0	246	Pharmaceutical formulations	[60]
	0.01N Sodium dihydrogen ortho phosphate buffer (pH 4): Methanol = 50:50, v/v	Kromasil C_{18} (150x4.6 mm, 5 µm)	1.0	240	Tablet dosage form	[61]
	Water (pH 4.5, adjusted with phosphoric acid): Acetonitrile = 15:85, v/v	Zorbax SB C_{18} (150×4.6 mm, 3.5 µm)	1.0	261	Tablet formulation	[62]
	Phosphate buffer (pH=3): Acetonitrile: Methanol = 25:45:30,v/v/v	Zorbax ODS (4.6x250 mm, 5 µm)	1.0	254	Tablets	[48]
	Potassium dihydrogen phosphate (10 Mm, pH 3): Acetonitrile = 59:41,v/v	Kromasil 100 C_{18} (250x4.6 mm, 5 µm)	1.0	245	Tablets	[63]
	Ammonium dihydrogen phosphate buffer (pH 5): Methanol = 60:40, v/v	Grace C_{18} (250x4.66 mm, 5µm)	1.0	240	Pharmaceutical dosage form	[64]
	Phosphate buffer (pH 3.3):	Agilent XDB C_{18} (150×4.6	1.0	280	Bulk and	[65]

Acetonitrile = 30:70,v/v	mm, 5µm)			pharmaceutical dosage form	
Buffer (pH 5):Acetonitrile=(60:40, v/v)	Grace C_{18} (150×4.66 mm, 5 µm)	1.0	240	Pharmaceutical dosage form	[66]
Orthophosphoric acid (0.1%):Acetonitrile =66:34, v/v	Kromasil 100 C_{18} (25 cmx4.6 mm, 5µm)	1.0	246	Bulk and tablets	[67]
Solvent A–10 mmol Phosphoric acid buffer (pH=2.6) Solvent B–Acetonitrile Solvent C–Methanol	BDS Hypersil C_{18} (250×4.6 mm, 5 μm)	1.0	220	Human plasma	[68]
Potassium dihydrogen phosphate (pH 4.5): Acetonitrile = 30:70,v/v	Inertsil C_{18} ODS (4.6x250 mm, 5 mm)	1.0	254	Tablet dosage form	[69]
Potassium dihydrogen phosphate (pH 5.5, 0.03M)-Acetonitrile = (65:35, v/v)	Phenomenex C_{18} (250x4.6 mm, 2.6µm)	1.2	240	Tablets	[50]
Acetic acid solution (0.1%, pH 3.8): Acetonitrile = 45:55 v/v	Cartridge–LiChroCARTR (250×4 mm) Column–LiChrospher 100 RP-18, 5 µm	0.8	246	Tablets	[70]
Potassium dihydrogen orthophosphate buffer (0.025 M, pH 5): Acetonitrile = 55:45 v/v	Eclipse XDB (4.6x150 mm, 5 µm)	1.5	246	Pharmaceutical formulations	[71]
Acetonitrile: Phosphate buffer (10 mmol, pH 3): Acetonitrile = 45:55 v/v	Altima C_{18} (25 cm× 4.6 mm, 5µm)	1.0	240	Bulk and dosage form	[72]
KH_2PO_4 (50 mmol, pH 4.1): Acetonitrile = 28:72 v/v	Capcell pak C_8 (4.6x250 mm, 5 µm)	1.0	260	Pharmaceutical dosage form	[73]
Ammonium acetate buffer (pH 3): Acetonitrile = 50:50, v/v	Phenomenex C_{18} (250×4.6 mm, 5 µm)	1.2	247	Pharmaceutical formulation	[74]
Potassium dihydrogen phosphate (0.02 M) buffer: Methanol: Acetonitrile=10:50:40, v/v/v	Zorbax SB C_{18} (250×4.6 mm, 5 µm)	1.1	236	API and formulations	[75]
Potassium dihydrogen phosphate (10 mmol, pH 3.0): Acetonitrile = 60:40, v/v	Waters C_{18} (250×4.6 mm, 5µm)	1.0	246	Pharmaceutical tablet formulations	[76]
Water (pH 3.2 with phosphoric acid): Methanol = 10:90, v/v	HiQ Sil C_8 (4.6×250 mm)	1.0	260	Tablet dosage form	[77]
Sodium phosphate buffer (0.05 M, pH 4.1): methanol = 30:70, v/v	LiChrosphera C_{18} (250x4.6 mm, 5 mm)	1.0	247	Bulk and tablet formulation	[78]
Acetate buffer (pH 3.7): methanol = 18:82, v/v	Luna C_{18} (250×4.6 mm, 5µm)	1.5	248	Tablet dosage form	[79]
Ammonium acetate buffer (0.01 M, pH 3): Acetonitrile = 50:50, v/v	Inertsil ODS (250 mm×4.6 mm, 5 µm)	1.0	254	Pharmaceutical formulations	[80]
Potassium dihydrogen phosphate buffer (0.02 M, pH 4): methanol = 20:80, v/v	Phenomenex C_{18} (250×4.6 mm, 5 µm)	1.0	240	Capsule dosage form	[81]
Water (pH 2 with phosphoric acid): Acetonitrile = 48:52, v/v	LiChrospher ODS, LiChrosorb ODS, Spherisorb ODS and Hypersil OS C_{18}	1.5	245	Pharmaceutical dosage forms	[82]
Ammonium acetate buffer (pH 4): Acetonitrile: THF = 70:25:5, v/v/v	Luna C_{18} (250×4.6 mm, 5µm)	1.0	248	Bulk drug and tablets	[83]

High-performance thin layer chromatography

High-performance thin layer chromatography (HPTLC) is an accelerated separation technique and flexible enough to determine the drug sample. The advantages associated with HPTLC are a short analysis time for the complex sample without pretreatment, independent construction of chromatogram with multiple samples, easy to transfer samples that will increase the confidence and reliability of the technique. It can be employed for qualitative and quantitative purposes. Several combinations of mobile phases with different size of plates have been successfully studied by HPTLC in pharmaceutical preparations [103–115] (table 4).

Capillary electrophoresis

Capillary electrophoresis (CE) is based on charge and size under electric field separate molecules. The capillary tube is made of glass loaded with an electrolyte solution. Electrophoretic mobility is an important parameter for the separation, chemical constituents as well as solvent viscosity. However, the limitation with gel electrophoresis with regards to applied voltage due to ohmic heating damage the gels and restrict the separation. Need to require large voltage often 10–20 thousand for experiments with CE. Different types of capillary electrophoresis are capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), micellar electrokinetic capillary chromatography (MEKC), capillary electro chromatography (CEC), capillary isoelectric focusing (CIEF) and capillary isotachophoresis (CITP).

Phosphate buffer (pH 6.5) and methanol mixture combined (80:20, v/v) used as background electrolyte with capillary fused silica column under 15 KV voltage at room temperature for the determination amlodipine and atorvastatin [116]. In tablet formulation no interference from present common excipients [117]. Phosphate buffer can separate both drugs in 5 min with high precision but in the presence of acidic products need to involve borate buffer [118–119]. It is likewise possible to quantify within 3 min in combined dosage form with high efficiency and resolution [120]. CZE method validated for solid dosage form in the presence of electrolyte as a methanol borate buffer [121]. MEKC succeeded by controlling surfactant, sodium dodecyl sulfate (SDS) concentration and acquired within 2 min [122] compared to CZE [117] 13 min migration time having comparable resolution.

Table 3: Determination of amlodipine and atorvastatin in bulk and pharmaceutical formulations using UPLC

Drug	Mobile phase	Stationary phase	Flow rate (ml/min)	Detector (nm)	Run time (min)	Application	Reference
Amlodipine Besylate	Mobile phase A–Trifluoro acetic acid in water (0.1%), Mobile phase B–Acetonitrile	BEH C_{18} (2.1×30 mm, 1.7µm)	0.6	246	2.5	API and pharmaceutical dosage forms	[84]
	Potassium dihydrogen ortho phosphate Buffer (pH 2.4): Acetonitrile = 40: 60,v/v	C_{18} (4.6x50 mm,3.5 µm)	0.5	240	10.0	Tablet dosage form	[85]
	Phosphate buffer (pH 3): Acetonitrile = 55:45, v/v	Kinetex C_{18} (4.6x150 mm,2.6µm)	1.0	230	6.0	Dosage form	[86]
	Glacial acetic acid (1%) buffer: Acetonitrile = 58:42, v/v	BEH C_{18} 2.1×100 mm,1.7 µm)	0.25	240	5.0	Tablet dosage form	[87]
	Potassium dihydrogen phosphate buffer (0.02 M): Acetonitrile = 45:55, v/v	BEH C_{18} (100×2.1 mm,1.7µm)	0.3	242	5.0	Tablet dosage form	[88]
	Triethyl amine buffer (0.1%, pH 3) and buffer; Gradient elution	Kromasil C_{18} (50×2.1 mm,3.5 µm)	0.8	240	2.2	Pharmaceutical dosage form	[89]
	Mobile phase A-Sodium perchlorate buffer (0.053 M, pH 3.2): Acetonitrile = 90:10, v/v Mobile phase B-Sodium perchlorate buffer (0.053 M, pH 3.2): Acetonitrile = 20:80, v/v Gradient programme	BEH RP_{18} (100x2.1 mm,1.7µm)	0.6	237	4.5	Tablets	[90]
	Mobile phase A: Ammonium acetate buffer (5 mmol, pH 4) Mobile phase B: Buffer: Acetonitrile (10: 90, v/v) Gradient programme	BEH C_{18} (50×2.1 mm, 1.7 µm)	0.613	230	3.3	Pharmaceutical dosage form	[91]
Atorvastatin Calcium	Ammonium dihydrogen phosphate buffer (10 mmol, pH 3): Acetonitrile = 50:50, v/v	Thermo C_{18} (50x 2.1 mm,1.9 µm)	0.3	255	2.5	Tablet	[92]
	O-phosphoric acid (1%): Acetonitrile = 55:45, v/v	Phenomenex C_{18} (100×4.6 mm, 2.6 µm)	1.0	254	10.0	Tablets	[93]
	Ammonium acetate buffer (0.01 M, pH 6.7) and Acetonitrile; Gradient programme	Kromasil C_{18} (2.1×50 mm, 2.5 µm)	0.2	245	5.0	Bulk drug and tablet	[94]
	Triethyl amine buffer (0.1%, pH 3) and buffer; Gradient programme	Acquity Kromasil C_{18} (50×2.1 mm, 3.5 µm)	0.8	240	2.2	Pharmaceutical dosage form	[89]
	Phosphoric acid (0.02 M) buffer and acetonitrile; Gradient programme	Zorbax C_{18} (50×3.0 mm,1.8 µm)	0.5	------	12.0	Bulk drug	[95]
	Orthophosphoric acid (0.1%) and acetonitrile = 55:45, v/v	BEH C_{18} (2.1×50 mm,1.7 µm)	0.35	230	4.0	Capsule dosage form	[96]
	Perchloric acid (0.1%, adjusted to pH 2.5) and Acetonitrile; Gradient programme	BEH C_{18} (100×2.1 mm,1.7 µm)	0.6	215	3.0	Tablet dosage form	[97]
	Buffer, ortho phosphoric acid (0.06%) with ion pair reagent sodium lauryl sulfate (0.045M) and Acetonitrile = 50:50, v/v	Zorbax C_{18} (4.6x50 mm,1.8 µm)	1.0	210	6.0	Capsule dosage form	[98]

High and ultra-pressure liquid chromatography combined with mass spectrophotometry

High pressure and ultra-performance liquid chromatography with the mass spectrometry (LC-MS, UPLC-MS) are analytical techniques that combine liquid chromatography with mass spectrophotometer. The aim to develop a fast and reliable analytical method for the determination of atorvastatin and amlodipine together with the presence of metabolites using the above techniques. The MS combined with LC has high selectivity and sensitivity. The techniques are commonly applied for bioavailability and pharmacokinetics investigation. Mostly quantification of parent drug, active and inactive metabolites are of interest for various studies with biological fluids. It is also required to verify the interaction between drug to drug and side effects as well as the toxicity of different metabolites after metabolism in the human body. The LCMS [123–136] (table 5) and UPLC-MS [137–142] (table 6) were used for the determination of amlodipine, atorvastatin and their metabolites in pharmaceutical dosage forms and biological fluids.

DISCUSSION

The UV-visible spectrophotometer does not require any pH adjustment and very close to the pharmacopeia method for both drugs. Recently HPTLC can be applied as an alternative option for traditional TLC method. It is easy to handle with software, which is not possible with TLC. HPTLC enhanced the capability to determine impurity with the help of the hydrophilic phase combined silica gel, an important parameter in all pharmacopoeias. One single run is enough to achieve two parameters such quantity and its impurity by HPLC, main pharmacopeias method to quantify the assay

percentage. The setting of parameters for the reaction is not easy as well as for characterization with CE that is why maybe not recommended in official method. UPLC is a high-cost instrument compare with other chromatographic methods, ability to study pharmacokinetics such as adsorption, metabolism. The limit of detection (LOD) and limit of quantitation (LOQ) value is very less with chromatographic method combined with mass spectro-photometry (table 7). Amlodipine and atorvastatin concentration in pharmaceutical formulations and biological fluids are based on different parameters and the calibration curve of the analyte (table 8). During method development and validation, accurately quantified the analyte as well provide brief information related to impurity profiling, a key equipment for the formulation process of the drug.

Table 4: Determination of drugs using HPTLC in pharmaceutical preparations

Drug	Mobile phase	Stationary phase	R_f value	Detector (nm)	Application	Reference
Amlodipine Besylate	Acetonitrile: Water: Toluene (60:30:10, v/v/v)	Aluminium plate coated with Silica Gel 60 F_{254}	0.57	270	Tablet formulation	[103]
	Ethyl acetate: Methanol: Ammonia (7.5:2:0.5, v/v/v)	Aluminium plate (20×10 cm, 0.2 mm) coated with Silica Gel 60 F_{254}	0.50	365	Dosage form	[104]
	Chloroform: Butan-1-ol: Ammonia (6: 4: 0.1, v/v/v)	Aluminium plate (10×10 cm, washed with methanol) coated with Silica Gel 60 F_{254}	0.27	254	Tablet dosage form	[105]
	Chloroform: Toluene: Methanol: Acetic acid (6: 2.5: 1.5: 0.5, v/v/v/v)	Aluminium plates (10×10 cm) precoated with silica gel 60 F_{254}	0.3	244	Synthetic mixture	[106]
	Methanol: Toluene: Ethyl acetate: 10% Ammonia solution (2:3.5:5:1, v/v/v)	Aluminium plates (10x10 cm, 0.2 mm) precoated with silica gel 60F_{254}	0.28	237	Pharmaceutical dosage form	[107]
	Toluene: Ethyl acetate: Methanol: Triethylamine (4:1:1:0.4, v/v/v)	Aluminium plates (10x10 cm, 0.2 mm) precoated with silica gel 60F_{254}	0.39	254	Bulk and tablets	[108]
	Methanol: n-butanol: 25% Ammonia solution (4:4:2, v/v/v)	Plates (10 mm, bottom and 10 mm, side edges, band length 6 mm) precoated with silica gel	0.80	218	Pharmaceutical dosage form	[109]
Atorvastatin Calcium	Chloroform: Methanol: Toluene: Ammonia (5:2:1:0.2, v/v/v/v)	Aluminium plates (20×10 cm) precoated with silica gel 60 F_{254}	0.17	289	Tablet dosage form	[110]
	Ethyl acetate: Methanol: Ammonia (7.5:2:0.5, v/v/v)	Aluminium plate (20×10 cm, 0.2 mm) coated with Silica Gel 60 F_{254}	0.26	365	Dosage form	[104]
	Toluene: Ethyl acetate: Methanol: Glacial Acetic Acid (7:2:1:0.1, v/v/v/v)	Precoated silica gel 60 F_{254} TLC plates (20×20 cm^2, 0.2 mm)	0.38	262	Pharmaceutical dosage form	[111]
	Toluene: Methanol (8:2, v/v)	Plate precoated with silica gel 60 F_{254} (20×10 cm, 0.2 mm)	0.15	260	Dosage form	[112]
	Toluene: Methanol: Ethyl acetate: Acetic acid (5: 1:1:0.3, v/v/v/v)	Plate coated with silica gel 60 F_{254} (10×10 cm, 250 mm)	0.63	279	Tablet formulations	[113]
	Acetonitrile: Methanol: Ethyl acetate: Glacial Acetic Acid (2:4:4:0.06, v/v/v/v)	Precoated silica gel 60 F_{254} (10×10 cm, 0.2 mm)	0.77	223	Pharmaceutical dosage form	[114]
	Toluene: Methanol: Ethyl Acetate: Glacial Acetic Acid (7: 1.5: 1: 0.5, v/v/v/v)	Aluminum backed silica gel 60 F_{254} plates (10×10 cm)	0.28	276	Capsules	[115]

Table 5: Determination of drugs in biological fluids using LCMS

Drug	Mobile phase	Stationary phase	Flow rate (ml/min)	Application	Reference
Amlodipine Besylate	Acetonitrile: Water (10 mmol ammoniumacetate, 0.5% ammonia Solution) = 95:5, v/v	Chiralcel OZ-RH (150×4.6 mm, 5 µm)	0.5	Rat plasma	[123]
	Ammonium acetate in 0.1% formic acid: Methanol: Acetonitrile=40:30:30, v/v/v	Zorbax SB C_{18} (50×4.6 mm,3.5 µm)	0.7	Human plasma	[124]
	Acetonitrile: Ammonium formate (2 mmol, pH 4) = 90:10, v/v	Chromolith RP_{18e} (100 mm×4.6 mm)	0.5–1.1	Human plasma	[125]
	Acetonitrile: Water (10 mmol CH_3COONH_4, pH 3.0) = 70:30, v/v	Zorbax XDB C_{18} (2.1×30 mm,3.5 µm)	0.15	Human plasma	[126]
	Acetonitrile: Formic acid (10 mmol) = 80:20, v/v	Atlantis dC_{18} (2.1x100 mm, 3 µm)	0.3	Human plasma	[127]
	Acetonitrile: Ammonium formate solution (5 mmol) = 80:20, v/v	Luna C_{18} 100A (150x4.6 mm, 5 µm)	0.8	Human plasma	[128]
	Acetonitrile: Water (0.1% formic acid) = 50:50, v/v	Aquasil C_{18} (50×2.1 mm, 5 µm)	0.2	Rat plasma	[129]
	Methanol and ammonium acetate (10 mmol); Gradient programme	Diamond C_{18} (150×4.6 mm, 5 µm)	0.5–0.8	Human plasma	[130]
Atorvastatin Calcium	Mobile phase A–Acetonitrile Mobile phase B–0.1% CH_3COOH Gradient programme	Zorbax C_{18} (4.6×100 mm, 3.5 µm)	0.4	Rat plasma	[131]
	Acetonitrile: Water (10 mmol CH_3COONH_4, pH 3.0) = 70:30, v/v	Zorbax C_{18} (2.1×30 mm, 3.5 µm)	0.15	Human plasma	[126]
	Water and methanol (modified with 2 mmol ammonium formate and 0.2% formic acid); Gradient programme	Zorbax C_{18} (50×2.1 mm, 3.5 µm)	0.4	Human plasma	[132]
	Formic acid (0.005%): Acetonitrile: Methanol = 35:25:40,	Ascentiss C_{18} (75×4.6	0.6	Human	[123]

	v/v/v	mm, 2.7 µm)		plasma	
	Formic acid (0.2%): Acetonitrile = 30:70, v/v	Agilent C_{18} (100×4.6 mm, 3.5 µm)	0.6	Human plasma	[124]
	Acetic acid buffer (0.2%): Methanol: Acetonitrile = 20:16:64, v/v/v	Zorbax Phenyl (75x4.6 mm, 3.5 µm)	0.8	Human plasma	[135]
	Acetonitrile: Methanol: 0.1% Formic acid (0.1%) = 50: 30: 20, v/v/v	Cyno (125×4 mm, 5µm)	0.5	Human plasma	[136]

Table 6: Determination of drugs in dosage form and biological sample using UPLC–MS

Drug	Mobile phase	Stationary phase	Flow rate (ml/min)	Run time (min)	Application	Reference
Amlodipine Besylate	Mobile phase A–0.1% formic acid in water Mobile phase B–0.1% formic acid in acetonitrile: Methanol = 90:10, v/v	BEH C_{18} (2.1×100 mm,1.7 µm)	0.3	5.5	Human plasma	[137]
	Solvent A–0.1% formic acid in water Solvent B–0.1% formic acid in acetonitrile: water= 95:5, v/v	Hypersil (50×2 mm, 1.9 µm)	0.25	5.0	Dosage form	[138]
	0.1% formic acid in ammonium acetate buffer (0.02 M, pH 3.5): Methanol = 25:75, v/v	BEH C_{18} (50×2.1 mm, 5 µm)	0.6	10.0	Human plasma	[139]
Atorvastatin Calcium	0.2% (v/v) formic acid in water and acetonitrile, gradient programme	Leapsil C_{18} 100×2.1 mm, 2.7 µm)	0.3	5.0	Human plasma	[140]
	0.05% (v/v) formic acid in water: Acetonitrile = 25: 75, v/v	Acquity T3 (3×100 mm, 1.8 µm)	0.3	4.0	Human plasma	[141]
	Solvent A–0.1% formic acid in water Solvent B–0.1% formic acid in acetonitrile: water = 95:5, v/v	Hypersil (50×2.0 mm, 1.9 µm)	0.25	5.0	Bulk and dosage form	[138]
	Acetonitrile and Ammonium acetate buffer (0.05 mmol, pH 4); Gradient programme	BEH C_{18} (100×2.1 mm, 1.7 µm)	0.25	5.25	Serum sample	[142]

Table 7: Comparison of LOD and LOQ values for amlodipine and atorvastatin with various analytical techniques

Drugs	Analytical technique	Sample/Application	LOD (µg/ml)	LOQ (µg/ml)	Reference
Amlodipine Besylate	UV–Visible	Pure and dosage form	0.291	0.963	[42]
	HPLC	Pure and dosage form	0.074	0.223	[42]
	UPLC	Tablet dosage form	0.02	0.062	[88]
	CE	Tablets	0.13	0.44	[121]
	LCMS	Human plasma	0.302 ng/ml	0.997 ng/ml	[129]
	UPLCMS	Dosage form	0.23 ng/ml	0.69 ng/ml	[139]
Atorvastatin Calcium	UV–Visible	Pharmaceutical formulation	0.31	0.93	[31]
	HPLC	Tablet dosage form	0.189	0.603	[58]
	UPLC	Tablet dosage form	0.026	0.078	[88]
	CE	Pharmaceutical formulations	0.27	0.89	[122]
	LCMS	Human plasma	0.05 ng/ml	0.165 ng/ml	[134]
	UPLCMS	Dosage form	0.56 ng/ml	1.7 ng/ml	[139]

Table 8: Advantages and disadvantages between all analytical techniques applied for amlodipine and atorvastatin

Analytical technique	Disadvantages	Advantages
UV–Visible	Defective equipment designs due to stray light, linearity range decreases; Quality of detector affected signal measurement and sensitivity reduced	Simple operation procedure, fast, cost effective, accurate in readings and used widely
TLC	Low reproducibility, inadequate separation length, unavailable automation, without scanning, less efficiency, only qualitative and open system	Low cost, easy separation, require small size, visible with UV light, non-volatile analyte
HPLC	Difficult with coelution and adsorbed analyte, necessary mobile phase filtration, degassing and pH 8 (basic) impossible for separation, need high skilled analyst	High resolution, repeatability, reliable data, develop method can modified with regards to quantification level
HPTLC	Glass plate with heavy weight, fragile, excessive cost; Aluminum plate loses shape above120°C, prewashing desired for plate, concentration dependent spectral shape	Applicable for matrix sample, not require filtration of mobile phase, can work with pH 8, no contamination, visual detection, high efficiency, no need high skilled analyst
UPLC	Involved high pressure, moderate column life, high maintenance cost	Highly sensitive, less solvent consumption, small flow rate, decrease particle size
CE	Low repeatability, high cost, problem during characterization of peak, effect of matrix and parameters optimization for analyte	Able to determine ion, charge, highly polar compound; different mode of separation, less volume and effluent
HPLCMS	Pretreatment of analyte before each analysis, high cost reagent, complex sample preparation	Rapid analysis, short run time, large no analyte batches, easy to quantify parent drug and its metabolite
UPLCMS	Multi reaction monitoring, equipment cost high and necessary derivatization	Robust, sensitive, capable of bioequivalence study, easy for biological fluids, low LOD and LOQ (table 7)

CONCLUSION

The main objective of this review is to provide sufficient information for the researcher, academic, scientist about the analysis of important calcium channel and statins receptor. After going through with the proposed review, people can understand the common method for the analysis of drugs. These medications are very significant due to its growing demands in our daily life that is why it was one of the fastest growing products in the pharmaceutical industry. The ultraviolet and visible spectrophotometer are low-cost instrument as well as easy to handle and available everywhere. It is more suitable for pure pharmaceutical formulations. The biological fluids and trace analysis will go for highly sensitive instrument combined with the mass spectrophotometer. Therefore, the present review will give an idea for determination and quantification of both drugs using UV–visible, HPLC, HPTLC, UPLC, LCMS and UPLC-MS in bulk, pharmaceutical formulations, and biological fluids.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

The authors report no conflicts of interest

REFERENCES

1. Triggle DJ. Calcium channel antagonists: clinical uses-past, present and future. Biochem Pharmacol 2007;74:1-9.
2. Chmiel K, Knapik KJ, Jurkiewicz K, Sawicki W, Jachowicz R, Paluch M, *et al.* A new method to identify physically stable concentration of amorphous solid dispersions (I): case of Flutamide+Kollidon VA64. Mol Pharm 2017;14:3370–80.
3. Kumar DB, Patel J, Chhatoi P, Begum S, Dey S. Analytical method development and validation of amlodipine and benazepril hydrochloride in combined dosage form by RP-HPLC. Int J Chem Pharm Sci 2011;2:26–30.
4. Sun S, Wang R, Fan J, Zhang GQ, Zhang H. Effects of danshen tablets on pharmaco kinetics of atorvastatin calcium in rats and its potential mechanism. Pharm Biol 2018;56:104-8.
5. Garza L, Dols J, Gillespie M. An initiative to improve primary prevention of cardiovascular disease in adults with type II diabetes based on the ACC/AHA(2013) and ADA(2016) guidelines. J Am Assoc Nurs Pract 2017;29:606–11.
6. Lampropoulos K, Megalou A, Bazoukis G, Tse G, Manolis A. Pre-loading therapy with statins in patients with angina and acute coronary syndromes undergoing PCI. J Interv Cardiol 2017;30:507–13.
7. Cruz Correa OF, Leon Cachon RB, Barrera Saldana HA, Soberon X. Prediction of atorvastatin plasmatic concentrations in healthy volunteers using integrated pharmacogenetics sequencing. Pharmacogenomics 2017;18:121–31.
8. Dennison TJ, Smith JC, Badhan RK, Mohammed AR. Fixed-dose combination orally disintegrating tablets to treat cardiovascular disease: formulation, *in vitro* characterization and physiologically based pharmacokinetic modeling to assess bioavailability. Drug Des Dev Ther 2017;11:811–26.
9. Eng H, Scialis RJ, Rotter CJ, Lin J, Lazzaro S, Varma MV, *et al*. The antimicrobial agent fusidic acid inhibits organic anion transporting polypeptide–mediated hepatic clearance and may potentiate statin-induced myopathy. Drug Metab Dispos 2016;44:692–9.
10. Virani P, Sojitra R, Raj H, Jain V. A review on irbesartan co administered with atorvastatin for the treatment of cardiac risk. J Crit Rev 2014;1:25-8.
11. Abdulsalam FO, Prema KR, Asha IT. Spectrophotometric study oxidation of amlodipine besylate by chloramine-T in acidic buffer pH (4.3) medium. Der Pharma Chem 2018;10:67–73.
12. Raskar MA, Ganesh RG, Ashok BC, Pritam DG. Validated simultaneous spectrophotometric estimation of telmisartan, hydrochlorthiazide and amlodipine besylate in combined tablet dosage form. Der Pharm Lett 2015;7:120–4.
13. Srinivasulu P, Gowthami BK, Kumar TNVG, Raju DSN, Vidyadhara S. Determination of amlodipine in rat plasma by uv spectroscopy. Indo Global J Pharma Sci 2015;5:205–9.
14. Naveed S, Hina Q, Wardha J, Urooj B. Simple UV spectrophotometric assay of amlodipine. Am J Chem Appl 2014;1:66–9.
15. Manisha M, Abhilasha M, Nandy BC. Spectrophotometric simultaneous estimation of amlodipine besylate and losartan potassium in tablet dosage forms. Asian J Pharm Clin Res 2014;7:61–3.
16. Nina A, Fatimeh H. Spectrophotometric method for the determination of amlodipine besylate in pure and dosage forms using 7,7,8,8-tetracyanoquinodimethane and tetracyanoethylene. Bull Faculty Pharm Cairo Univ 2014;52:109–14.
17. Rajesh BHV, Praveen KJ, Raveendra GB, Sri PLS, Praveen TK, Rao PS. UV spectroscopic method for estimation of amlodipine besylate in tablets. Int J Pharm Chem Bio Sci 2014;4:69–73.
18. Samixa PR, Patel CN. Development and validation of spectrophotometric method for determination of aliskiren, amlodipine and hydrochlorothiazide in combined pharmaceutical dosage forms. Asian J Pharm Anal 2014;4:166–71.
19. Pawar PY, Mane BY, Sumit MA, Vipul VT. Simultaneous estimation of amlodipine besylate and atenolol in combined dosage form by Vierodt's method using U. V. spectroscopy. Der Pharma Chem 2013;5:97-102.
20. Laxmileena DP, Sachin VG, Deepali DJ, Yogita AK, Sampada DD, Pramod LI. Development and validation of UV–spectrophotometric methods for simultaneous estimation of amlodipine besylate and clopidogrel bisulfate in bulk and tablet dosage form. Der Pharma Chem 2013;5:282–7.
21. Mahmoud RS, Parviz A, Maryam D. Simultaneous quantitative determination of amlodipine and atorvastatin in tablets using artificial neural networks. Math Com Modell 2013;58:1588–94.
22. Varsha RG, Baheti KG, Indraksha S, Dehghan MH. Estimation of amlodipine besylate, valsartan and hydrochlorothiazide in bulk mixture and tablet by UV spectrophotometry. Indian J Pharm Sci 2012;74:18-23.
23. Kondawar MS, Kamble KG, Raut KS, Maharshi KH. UV Spectrophotometric estimation of amlodipine besylate and telmisartan in bulk drug and dosage form by multi wavelength analysis. Int J Chem Tech Res 2011;3:1274–8.
24. Smita TK, Swapnil DJ, Neela MB, Manish SB. Development and validation of derivative spectrophotometric method for estimation of atorvastatin calcium and amlodipine besylate in tablet dosage form. Int J Pharm Pharm Sci 2011;3 Suppl 4:195–7.
25. Pandurang ND, Sunita RB. Simultaneous UV spectrophotometric methods for estimation of amlodipine besilate and olmesartan medoxomil in tablet dosage form. J Chem Pharm Res 2011;32:650–6.
26. Shyni B, Molly M, Senthilkumar KL. Spectrophotometric method of estimation of amlodipine besylate using hydrotropic solubilization. J Appl Pharm Sci 2011;1:177–80.
27. Gayathri S, Devi TSR, Gunasekaran S. Qualitative and quantitative analysis on some cardiovascular drugs. Asian J Chem 2010;22:5824–34.
28. Kardile DP, Kalyane NV, Thakkar TH, Patel MR, Moradiya RK. Simultaneous estimation of amlodipine besylate and olmesartan medoxomil drug formulations by HPLC and UV-spectrophotometric methods. J Pharm Sci Res 2010;2:599–614.
29. Bilal Y, Selcuk K. UV and first derivative spectrophotometric methods for the estimation of atorvastatin in pharmaceutical preparations. J Adv Pharma Res 2018;2:89–94.
30. Sobhy MAA, Lobna MAA, Maha AMM. Spectrophotometric determination of atorvastatin calcium and rosuvastatin calcium in bulk and dosage form using p-dimethylaminobenzaldehyde. J Appl Pharm 2017;9:233.
31. Alshabrawy A, Ahmed M, Nageh A. Sensitive spectrophotometric determination of atorvastatin in pharmaceutical formulation by ion pair complexation with pararosaniline hydrochloride. J Adv Pharma Res 2017;1:193–200.
32. Ramadan AA, Hasna M, Jenan S. Determination of atorvastatin calcium in pure and its pharmaceutical formulations using iodine in acetonitrile by uv-visible spectrophotometric method. Int J Pharm Pharm Sci 2015;7:427-33.
33. Jadhav NR, Ramesh SK, Sameer JN. Dual wavelength spectrophotometric method for simultaneous estimation of

atorvastatin calcium and felodipine from tablet dosage form. Adv Chem 2014;6. http://dx.doi.org/10.1155/2014/131974

34. Suresh JNK, Babu CN, Dhanalakshmi T, Florence K, Ramanjaneyulu MS, Sambasivanaik N. Development and validation of spectrophotometric method for estimation of atorvastatin calcium bulk and pharmaceutical formulations. World J Pharma Pharm Sci 2014;3:1305–13.
35. Safila N. Simple uv spectrophotometric assay of atorvastatin API formulation and their comparative study. Global J Med Res: B Pharm Drug Discovery Toxi Med 2014;14:34–8.
36. Kailash PP, Bhandari A. Spectroscopic method for estimation of atorvastatin calcium in tablet dosage form. Indo Global J Pharma Sci 2011;1:294–9.
37. Swapnil DJ, Manish SB, Shivaji LT, Sachin AP. Spectrophotometric methods for estimation of atorvastatin calcium form tablet dosage forms. Int J Pharm Tech Res 2010;2:1948-53.
38. Sandeep SS, Atul AS, Ravindra AF, Sanjay JS. Application of UV-spectrophotometry and RP-HPLC for simultaneous determination of atorvastatin calcium and ezetimibe in pharmaceutical dosage form. Eurasian J Anal Chem 2006;1:31–41.
39. Dhiraj K, Susanta KP, Sudhir KS. Development of stability indicating RP-HPLC method for simultaneous estimation of amlodipine and olmesartan in pure and pharmaceutical dosage form. Int J Pharm Sci Drug Res 2018;10:379–85.
40. Bhagyalaxmi C, Sahoo SK, Kavitha D, Nagamani M, Lagnajit M. Development and validation of new analytical RPHPLC method for the estimation of amlodipine and perindopril in tablet dosage form. Int J Pharma Res Health Sci 2018;6:2433–36.
41. Vidhi AM. RP-HPLC method development and validation for simultaneous estimation of irbesartan, amlodipine besylate and hydrochlorothiazide in tablet. World J Pharm Res 2018;7:690–701.
42. Abhi K, Manju M. Development and validation of RP-HPLC and UV-spectrophotometric methods for rapid simultaneous estimation of amlodipine and benazepril in pure and fixed dose combination. Arabian J Chem 2017;10:S3021–8.
43. Ariadne MB, Jerusa SG, Marcello GT. Development and validation of a chromatography method using tandem UV/charged aerosol detector for simultaneous determination of amlodipine besylate and olmesartan medoxomil: application to drug-excipient compatibility study. J Anal Methods Chem 2017;Vol 2017:8. https://doi.org/10.1155/2017/4878316
44. Osman RAM, Elbashir AA. Development and validation of stability indicating HPLC method for the simultaneous analysis of amlodipine, hydrochlorothiazide and valsartan in pharmaceutical formulation, J Anal Pharm Res 2017;6:00188.
45. Rasha AS, Tarek SB, Fawzy AEY, Sohila ME. Validated stability-indicating HPLC-DAD method of analysis for the antihypertensive triple mixture of amlodipine besylate, valsartan and hydrochlorothiazide in their tablets. Arabian J Chem 2017;10:S1381–94.
46. Gunasekar M. Simultaneous determination and estimation of amlodipine and perindopril in raw and tablet formulation by stability indicating RP-HPLC method. IOSR J Pharma Biol Sci 2016;11:24–9.
47. Chamarthi RPK, Mohan GVK. Development and validation of amlodipine impurities in amlodipine tablets using design space computer modeling. Am J Anal Chem 2016;7:918–26.
48. Hassan SA, Elzanfaly ES, El-Zeany SB, Salem MY. Development and validation of HPLC and CE methods for simultaneous determination of amlodipine and atorvastatin in the presence of their acidic degradation products in tablets. Acta Pharm 2016;66:479–90.
49. Mounika P, Rani PJ, Pranathi G, Haque MA, Sireesha D, Harshini S, *et al*. Development and validation of RP-HPLC method for the simultaneous estimation of amlodipine and metoprolol in bulk and pharmaceutical dosage form. Indo Am J Pharm Res 2015;5:3254–60.
50. Hafez HM, Elshanawany AA, Abdelaziz LM, Mohram MS. Development of a stability-indicating HPLC method for simultaneous determination of amlodipine besylate and atorvastatin calcium in tablets. Austin J Anal Pharm Chem 2014;1:11.
51. Manish S, Charmy K, Omkar S, Priti M. Concurrent estimation of amlodipine besylate, hydrochlorothiazide and valsartan by RP-HPLC, HPTLC and UV–spectrophotometry. J Chromatogr Sci 2014;52:27–35.
52. Bharti AR, Deshmane SV, Biyani KR. Stability indicating RP-HPLC method for development and validation for simultaneous estimation of amlodipine and hydrochlorothiazide in pharmaceutical dosage form. Int J Curr Pharm Res 2014;6:16-9.
53. Anandkumar RT, Gurupadayya BM, Soni N. Simultaneous estimation of hydrochlorothiazide, amlodipine, and losartan in tablet dosage form by RP-HPLC. Int J Chem Anal Sci 2013;4:33–8.
54. Samya MEG, Abdelmageed OH, Omar MA, Deryea SM, Abdel Megied AM. Development and validation of HPLC method for simultaneous determination of amlodipine, valsartan, hydrochlorothiazide in dosage form and spiked human plasma. Am J Anal Chem 2012;3:422–30.
55. Karunakar N, Puranik SB, Kumar GVS, Masood MD, Sridhar KA. Development and validation of RP-HPLC method for simultaneous estimation of amlodipine and indapamide in bulk and tablet dosage form. Asian J Biomed Pharm Sci 2012;2:55–9.
56. Kayal SD, Khan FA, Tated AG, Bakal RL, Chandewar AV. Method development and validation for the simultaneous determination of amlodipine besylate and telmisartan in tablet dosage form by RP-HPLC. Int J Pharm Res Dev 2011;3:144–53.
57. Pournima SP, More HN, Pishwikar SA. RPHPLC method for simultaneous estimation of amlodipine besylate and olmesartan medoxomil from tablet. Int J Pharm Pharm Sci 2011;3 Suppl 3:146–9.
58. Hassan AA, Alnami AM, Arishi MAA, Alameer RK, Bratty MA, Rehman ZU, *et al*. A fast and validated reversed-phase HPLC method for simultaneous determination of simvastatin, atorvastatin, telmisartan and irbesartan in bulk drugs and tablet formulations. Sci Pharm 2018;86:1.
59. Rao S, Balaswami B, Ramana PV, Sanjeeva P, Srenivasulareddy G. New stability-indicating RP-HPLC-PDA method for simultaneous estimation of triplicate mixture of ramipril, atorvastatin and clopidogrel in tablet dosage form. Int J Appl Pharm 2018;10:90-6.
60. Azmi SNH, Al-Mamari AK, Al-Hosni BS, Al-Fazari MR. High performance liquid chromatographic-UV method for determination of atorvastatin calcium in pharmaceutical formulations. J New Dev Chem 2017;1:38–50.
61. Yusuf SM, Samparna S, Yallareddy K, Pavani B, Sivakala T. Analytical method development and validation of atorvastatin and clopidogrel in tablet dosage form by RP-HPLC. Eur J Pharma Med Res 2017;4:553-8.
62. Jaiprakash NS, Mohammed A, Zahid Z, Ahmed RZ, Dehghan MHG, Gonjari I. Development and validation of RP-HPLC method for determination of atorvastatin calcium and nicotinic acid in combined tablet dosage form. J Saudi Chem Soc 2016;20:S328–33.
63. Rudwan EH, Mohammed ABWE, Saeed AEM. A new RP-HPLC method for quantitative analysis of atorvastatin calcium in bulk and pharmaceutical dosage form by using design of experiment technique optimization. Int Res J Pure Appl Chem 2016;13:10.
64. Patil PM, Bobade AS. Development and validation of stability indicating RP-HPLC for determination of atorvastatin calcium and ezetimibe in bulk and pharmaceutical dosage forms. Int J Pharm Pharm Sci 2016;8:38–42.
65. Khaleel N, Rahaman SA. Validated stability indicating RP-HPLC method for simultaneous determination of atorvastatin, fenofibrate and folic acid in bulk and pharmaceutical dosage form. Der Pharm Lett 2016;8:13–32.
66. Patil PM, Bobade AS. Development and validation of stability indicating RP-HPLC for determination of atorvastatin calcium and ezetimibe in bulk and pharmaceutical dosage forms. Int J Pharm Pharm Sci 2016;8:38-42.
67. Rudwan EH, Hussien ABWEM, Saeed AEM. Development and validation of stability indicating high performance liquid chromatography method for determination of atorvastatin calcium in the presence of its degradation products. Der Pharma Sin 2015;6:19–27.
68. Octavian C, Spiridon AM, Belu I, Ftiolics AT, Neamuu J. Development and validation of an HPLC method for simultaneous quantification of clopidogrel bisulfate, its

carboxylic acid metabolite, and atorvastatin in human plasma: application to a pharmacokinetic study. J Anal Methods Chem 2015;12. http://dx.doi.org/10.1155/2015/892470

69. Swamy GK, Rao JVLNS, Kumar JMR. New stability indicating validated RP-HPLC method for simultaneous estimation of irbesartan and atorvastatin in combined tablet dosage forms. Am J Pharm Tech Res 2015;5:380–9.
70. Simionato LD, Ferello L, Stamer SG, Repetto MF, Zubata PD, Segall AI. A validated reversed-phase HPLC method for the determination of atorvastatin calcium in tablets. Austin Chromatogr 2014;1:4.
71. Mallesh K, Sobahi TR, Helw AME, Abdelaal MY. Development and validation of a RP-HPLC method for assay of atorvastatin and its application in dissolution studies on thermosensitive hydrogel-based nanocrystals. Trop J Pharm Res 2014;13:1681–7.
72. Ramesh D, Habibuddin M, Dash RN, Humaira T. Simultaneous determination of atorvastatin calcium and losartan potassium in bulk and combined dosage forms by validated RP-HPLC with UV detection. Am J Pharm Tech Res 2013;31:675–85.
73. Bhinge SD, Malipatil SM, Jondhale A, Hirave R, Savali AS. A new approach to the RP-HPLC method for simultaneous estimation of atorvastatin calcium and fenofibrate in pharmaceutical dosage forms. E J Chem 2012;9:1223–9.
74. Ganesh M, Hemalatha P, Sakthimanigandan K, Mei PM, Lee SG. Simultaneous estimation of atorvastatin and ezetimibe in combined formulation by RP-HPLC. Asian J Chem 2012;24:1867–71.
75. Kumar P, Ghosh A, Chaudhary M. Stability indicating method development for simultaneous estimation of ezetimibe and atorvastatin in pharmaceutical formulations by RP-HPLC. Pharm Anal Acta 2012;3:164.
76. Charde MS, Gupta A, Chakole RD. Determination of atorvastatin calcium in pharmaceutical formulations by reverse phase-high performance liquid chromatography. Int J Adv Pharm Anal 2011;1:48–53.
77. Rupali H, Bendagude R, Kondawar M. RP-HPLC method for simultaneous estimation of atorvastatin calcium and fenofibrate in tablet dosage forms. J Pharma Res 2010;3:2400-1.
78. Mustafa G, Azeem A, Ahmad FJ, Khan ZI, Shakeel F, Talegaonkar S. Stability-indicating RP-HPLC method for analysis of atorvastatin in bulk drug, marketed tablet and nano emulsion formulation. J Chil Chem Soc 2010;55:184–8.
79. Jain N, Raghuwanshi R, Jain D. Development and validation of RP-HPLC method for simultaneous estimation of atorvastatin calcium and fenofibrate in tablet dosage forms. Indian J Pharm Sci 2008;70:263–5.
80. Seshachalam U, Kothapally CB. HPLC analysis for simultaneous determination of atorvastatin and ezetimibe in pharmaceutical formulations. J Liq Chromatogr Relat Tech 2008;31:714–21.
81. Shah DA, Bhatt KK, Mehta RS, Shankar MB, Baldania SL, Gandhi TR. Development and validation of a RP-HPLC method for determination of atorvastatin calcium and aspirin in a capsule dosage form. Indian J Pharm Sci 2007;69:546–9.
82. Stanisz B, Lukasz K. Validation of HPLC method for determination of atorvastatin in tablets and for monitoring stability in solid phase. Acta Poloniae Pharm Drug Res 2006;63:471–6.
83. Sidika E, Aktas ES, Ersoy L, Ficicioglu S. An HPLC method for the determination of atorvastatin and its impurities in bulk drug and tablets. J Pharm Biomed Anal 2003;33:1017–23.
84. Bharti T, Richa S. Development of short and robust analytical method on UPLC-MS for quantitative estimation of different API's in their formulated products–a green analytical chemistry approach. Eur J Biomed Pharm Sci 2017;4:405–17.
85. Balakrishnan A, Vijay AR, Senthilkumar N, Gangadevi N. Analytical method development and validation of amlodipine–an antihypertensive and chlorthalidone–a thiazide diuretic by RP-UPLC method. Int J Chem Pharm Sci 2016;7:12–7.
86. Hanan AM, Nesrin KR, Sherine SD, Azza AM. Development and validation of UPLC methods for simultaneous determination of selected antihypertensive drugs in their dosage forms. Int J Adv Sci Eng Tech 2015;3:45–50.
87. Patel KN, Patel NB, Sevak MR, Desai HT. Development and validation of RP-UPLC method for simultaneous estimation of amlodipine and indapamide in their combined tablet dosage form. J Pharm Sci Bio Sci Res 2015;5:101–9.
88. Waghmare AN, Muddukrishna BS, Vasantharaju SG. Analytical method development and validation of simultaneous estimation of amlodipine and atorvastatin by RP-UPLC. Mintage J Pharm Med Sci 2014;3 Suppl 2:22–5.
89. Mallikarjuna S, Ramalingam P, Sriram P, Garima J, Srinivas SK. Development and validation of stability indicating RP-UPLC method for simultaneous estimation of amlodipine besylate and atorvastatin calcium in pharmaceutical dosage forms. J Chromatogr Sep Tech 2013;4:187.
90. Santaji N, Vangala RR, Rao DD, Rao IK. Rapid simultaneous determination of telmisartan, amlodipine besylate and hydrochlorothiazide in a combined poly pill dosage form by stability-indicating ultra-performance liquid chromatography. Sci Pharm 2011;79:69–84.
91. Lakshmi NYS, Barhate VD. Development and validation of stability indicating UPLC method for the simultaneous determination of beta-blockers and diuretic drugs in pharmaceutical dosage forms. J Chem Metrol 2010;4:1–20.
92. Lakshmi KS, Seetharaman R. Design and study of reverse phase ultra-high performance liquid chromatographic method for simultaneous estimation of two antidiabetic drugs with an antihyperlipemic drug using statistical approach. Int J Pharm Pharm Sci 2014;6:257–62.
93. Darwish MK, Manal MF, Zaazaa HE, Razeq SAA, Zeinab AN. Formulation, optimization and simultaneous determination of atorvastatin calcium and losartan potassium in pure and bilayer tablets, J Global Trends Pharm Sci 2014;5:1756–68.
94. Goel A, Sanjula B, Jasjeet KS, Kona SS, Ravi SG, Gupta A, *et al*. Development and validation of stability-indicating assay method by UPLC for a fixed dose combination of atorvastatin and ezetimibe. J Chromatogr Sci 2013;51:222–8.
95. Kakumani KK, Rao CK, Lakshmi MV, Mukkanti K. A validated stability indicating RP-UPLC method for atrovastain calcium. Am J Anal Chem 2012;3:392–9.
96. Kaila HO, Ambasana MA, Shah AK. A simple and rapid ultra-performance liquid chromatographic assay method for the simultaneous determination of aspirin, clopidogrel bisulphate and atorvastatin calcium in capsule dosage form. Int J Chem Tech Res 2011;3:459–65.
97. Shetty SK, Surendranath KV, Radhakrishnanand P, Borkar RM, Devrukhakar PS, Johnson J, *et al*. Stress degradation behavior of a polypill and development of stability indicating UHPLC method for the simultaneous estimation of aspirin, atorvastatin, ramipril and metoprolol succinate. Am J Anal Chem 2011;2:401–10.
98. Seshadri RK, Desai MM, Raghavaraju TV, Krishnan D, Rao DV, Chakravarthy IE. Simultaneous quantitative determination of metoprolol, atorvastatin and ramipril in capsules by a validated stability indicating RP-UPLC method. Sci Pharm 2010;78:821-34.
99. Abdul AR, Hussen AA, Mohammad M. TLC simultaneous determination of amlodipine, atorvastatin, rosuvastatin and valsartan in pure form and in tablets using phenyl-modified aleppo bentonite. Int J Pharm Pharma Sci 2014;6:180–8.
100. Shirkhedkar A, Surana S. Simultaneous densitometric TLC analysis of atorvastatin calcium and fenofibrate in the bulk drug and in pharmaceutical formulations. J Plan Chromatogr Mod TLC 2009;22:355–8.
101. Nikalje AG, Choudhari VP. Validated TLC method for simultaneous quantitation of atorvastatin, ezetimibe, and fenofibrate in bulk drug and formulations. Acta Chromatogra 2011;23:267–80.
102. Logoyda L, Korobko D, Saprun S. Development of methods for identification of calcium channel blockers in medicines. Int J Res Ayurved Pharm 2016;7:88–91.
103. Sonia K, Manikandan K, Hamunyare N, Peddamadi BS, Lakshmi KS. Method development and validation of simultaneous estimation for amlodipine besylate and olmesartan medoxomil by HPTLC method. Int J Res Pharm Sci 2018;9:201–5.
104. Rao NM, Sankar DG. Development and validation of HPTLC method for the simultaneous estimation of amlodipine besylate and atorvastatin calcium in combined dosage form. Eurasian J Anal Chem 2016;11:155–68.
105. Marolia BP, Bodiwala KB, Shah SA, Prajapati PB, Satani BH, Desai SA. Development and validation of HPTLC method for simultaneous estimation of amlodipine besylate,

hydrochlorothiazide and telmisartan in their combined tablet dosage form. Pharm Methods 2016;7:48–53.
106. Shah DA, Patel DV, Mehta FA, Chhalotiya UK, Bhatt KK. High-performance thin-layer chromatography method for estimatingthe stability of a combination of irbesartan and amlodipine besylate. J Taibah Univ Sci 2015;9:177–86.
107. Sindhav JR, Chhalotiya UK, Shah DA, Mehta FA, Bhatt KK. Stability-indicating HPTLC method for simultaneous quantification of moxonidine and amlodipine besylate in their combined pharmaceutical dosage form. Austin Chromatogr 2015;2:1031, 7.
108. Jain PS, Patel MK, Surana SJ. Development and validation of HPTLC method for simultaneous determination of amlodipine besylate and metoprolol succinate in bulk and tablets. Indian J Pharm Sci 2012;74:152–6.
109. Kothapalli LP, Panchaware MS, Nanda RK, Thomas AB. Stability-indicating HPTLC method for simultaneous determination of lisinopril anhydrate and s-amlodipine besylate in pharmaceutical dosage form. Asian J Res Chem 2012;5:1061–6.
110. Deshmukh TB, Deo SS. Development and validation of novel HPTLC method for the simultaneous estimation of atorvastatin calcium and telmisartan in tablet dosage form. Int J Pharm Chem Biol Sci 2018;8:82–90.
111. Misari PK, Ezhava SB. Development and validation of HPTLC method for simultaneous estimation of atorvastatin calcium and vitamin d_3 in pharmaceutical dosage form. World J Pharm Sci 2014;2:1511–9.
112. Kolsure AK, Chavan BB, Chabukswar AR, Kuchekar BS. Development and validation of a HPTLC method for simultaneous estimation of atorvastatin calcium and losartan potassium in combined dosage form. J Med Pharm Innov 2014;1:21–5.
113. Kaliappan I, Kumar PSS. Development and validation of stability indicating HPTLC and HPLC methods for simultaneous determination of telmisartan and atorvastatin in their formulations. J Chem 2013. http://dx.doi.org/10.1155/2013/725385.
114. Ginoya CG, Thakkar DV. Development and validation of HPTLC method for simultaneous determination of metoprolol succinate and atorvastatin calcium in a pharmaceutical dosage form. Int Res J Pharma 2013;4:102–7.
115. Wankhede SB, Dixit NR, Chitlange SS. Stability indicating HPTLC method for quantitative determination of atorvastatin calcium and metoprolol succinate in capsules. Der Pharmacia Lett 2011;3:1–7.
116. Mohamed MH, Maha S, Haya AJ. Development of capillary electrophoresis technique for simultaneous measurement of amlodipine and atorvastatin from their combination drug formulations. J Liq Chromatogr Relat Technol 2009;32:2923–42.
117. Alshehri MM. A validated capillary electrophoresis method for simultaneous determination of ezetimibe and atorvastatin in pharmaceutical formulations. Saudi Pharm J 2012;20:143–8.
118. Eleonora M, Teodora B, Gabriel H, Valentin I, Anca C. Simultaneous determination of amlodipine and atorvastatin by capillary electrophoresis from fixed pharmaceutical formulations. Farmacia 2016;64:398–402.
119. Said AH, Eman SE, Maissa YS, Badr AEZ. Development and validation of HPLC and CE methods for simultaneous determination of amlodipine and atorvastatin in the presence of their acidic degradation products in tablets. Acta Pharm 2016;66:479–90.
120. Modroiu A, Hancu G, Vlad RA, Stacescu S, Soare R, Kelemen H. Simultaneous determination of amlodipine and telmisartan from pharmaceutical products by way of capillary electrophoresis. Curr Issues Pharm Med Sci 2016;29:42–5.
121. Mahesh A, Adbullah B, Hussain MB. Capillary electrophoresis method development for simultaneous determination of atorvastatin and ezetimibe from solid dosage form. J Young Pharm 2017;9:122–5.
122. Blanka SS, Gabriel H, Istvan SS, Bela K, Hajnal K. Simultaneous determination of atorvastatin and ezetimibe from combined pharmaceutical products by micellar electrokinetic capillary chromatography. Braz J Pharm Sci 2017;53:e16122.
123. Wanga L, Liua W, Zhanga Z, Tiana Y. Validated LC–MS/MS method for the determination of amlodipine enantiomers in rat plasma and its application to a stereo selective pharmacokinetic study. J Pharm Biomed Anal 2018;158:74–81.
124. Haque AM, Priya SDK, Mohanty D, Bakshi V, Boggula N. Analytical method development and validation of amlodipine in human plasma using liquid chromatography-mass spectrometry/mass spectrometry. Asian J Pharm Clin Res 2018;11:393–7.
125. Shah JV, Parekh JM, Shah PA, Shaha PV, Sanyal M, Shrivastav PS. Application of an LC–MS/MS method for the analysis of amlodipine, valsartan and hydrochlorothiazide in polypill for a bioequivalence study. J Pharm Anal 2017;7:309-16.
126. Hossein D, Hamidi M. Method validation of amlodipine and atorvastatin by liquid chromatography–mass spectrometry (LC–MS) method in human plasma. Cogent Med 2016;3:1129790.
127. Alvi SN, Hussein RF, Dgither SA, Hammami MM. Quantitation of amlodipine in human plasma by LCMS/MS assay. Int J Pharm Pharm Sci 2016;8:268-72.
128. Jangala H, Vats P, Khuroo AH, Monif T. Development and validation of a LC-MS/MS method for the simultaneous estimation of amlodipine and valsartan in human plasma: application to a bioequivalence study. Sci Pharm 2014;82:585–600.
129. Shankar GG, Deme P, Kuncha M, Sistla R. Simultaneous determination of amlodipine, valsartan and hydrochlorothiazide by LC-ESI-MS/MS and its application to pharmacokinetics in rats. J Pharm Anal 2014;4:399-406.
130. Chan ML, Wei CM, Bu FL, Chen R, Wang XL, Li R, *et al*. Determination of amlodipine in human plasma by LC-MS/MS and its bioequivalence study in healthy chinese subjects. Pharmacol Pharma 2013;4:191–200.
131. Sakac MC, Vujic Z, Vujcic Z, Markovic B, Vasiljevic D. LC–MS/MS method for quantification of atorvastatin, o-hydroxyatorvastatin, p-hydroxyatorvastatin, and atorvastatin lactone in rat plasma. Acta Chromatogr 2016;28:281-98.
132. Jiang W, Luzum JA, Phelpsa MA, Kitzmiller JP. Liquid chromatography–tandem mass spectrometry assay for the simultaneous quantification of simvastatin, lovastatin, atorvastatin, and their major metabolites in human plasma. J Chromatogr B 2015;983–984:18–25.
133. Partani P, Verma SM, Gurule S, Khuroo A, Monif T. Simultaneous quantitation of atorvastatin and its two active metabolites in human plasma by liquid chromatography/(–) electrospray tandem mass spectrometry. J Pharm Anal 2014;4:26–36.
134. Ramzia IEB, Elkady EF, Sherif ZAE, Kadry AM. LC–MS–MS simultaneous determination of atorvastatin and ezetimibe in human plasma. J Chromatogr Sci 2014;52:773–80.
135. Gajula R, Pilli NR, Ravi VB, Maddela R, Inamadugu JK, Polagani SR, Busa S. Simultaneous determination of atorvastatin and aspirin in human plasma by LC–MS/MS: its pharmacokinetic application. Sci Pharm 2012;80:923–40.
136. Ashutosh JJ, Dasandi B, Rathnam S, Mehta AA. Liquid chromatographic-MS/MS determination of atorvastatin and metabolites in human plasma. Eurasian J Anal Chem 2010;5:46–52.
137. Kalaiyarasi D, Jaganathan KS, Krishna MV. Bioanalytical method validation for perindopril and amlodipine in human plasma using UPLC/ESI-MS/MS. World J Pharm Res 2018;7:1567–80.
138. Ahmed M, Alshabrawy A, Nageh A. UPLC-MS/MS method for kinetic studies and simultaneous determination of amlodipine and atorvastatin in bulk, and their combined dosage form. Anal Chem Ind J 2016;16:102.
139. Walid ME, Elkady EF, Zaher AAE, Bagary RIE, Patonay G. Simultaneous determination of aliskiren hemifumarate, amlodipine besylate and hydrochlorothiazide in spiked human plasma using UPLC-MS/MS. J Chromatogr Sci 2015;53:1178–84.
140. Xia B, Li Y, Zhang Y, Xue M, Li X, Xu P, et al. UHPLC-MS/MS method for determination of atorvastatin calcium inhuman plasma: application to a pharmacokinetic study based on healthy volunteers with specific genotype. J Pharm Biomed Anal 2018;160:428–35.
141. Liyun C, Zhijie Z, Xipei W, Lan T, Liping M, Guodong H, et al. Simultaneous determination of atorvastatin and its metabolites in human plasma by UPLC-MS/MS. Anal Methods 2017;9:1038–45.
142. Lucie N, Hana V, Dalibor S, Petr S, Dagmar S, Milan B, *et al*. Ultrahigh performance liquid chromatography tandem mass spectrometric detection in clinical analysis of simvastatin and atorvastatin. J Chromatogr B 2009;877:2093–103.