

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]-1H-pyrrole-1-heptanoic acid.

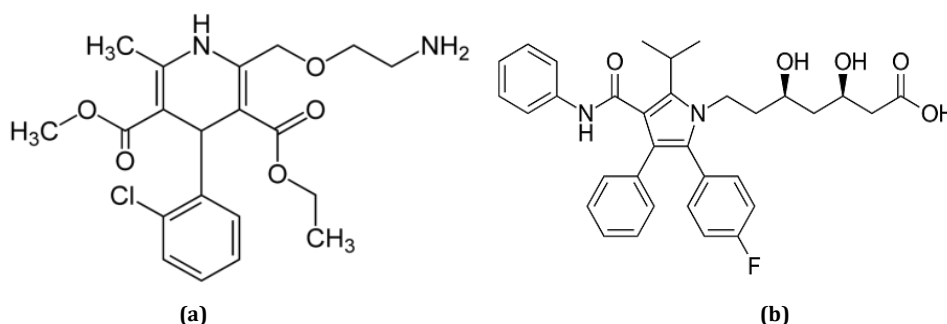


Fig. 1: Structure of amlodipine (a) and atorvastatin (b)

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 Besylate	Chloramin-T	390	Bulk powder	[11]
	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 Calcium	Methanol	247	Pure and tablets	[29]
	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 C₁₈, BDS C₈, ODS C₈, BDS C₁₈ and Discovery HS C₁₈) 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 2 μ m 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 μ g [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, 3.5 μm)	0.7	230	Pharmaceutical dosage form	[40]
	Phosphate Buffer (pH 4.5): Acetonitrile = 55: 45, v/v	Phenomenex C ₁₈ (25×0.46 cm, 5 μm)	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	Inertsil ODS-3 C ₁₈ (150×4.6 mm, 3μm)	1.0	340	Tablet formulation	[47]
	Mobile phase B-Phosphate buffer, pH 2.8: Methanol: Acetonitrile =20:40:40, v/v/v					
	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 cm×4.6 mm, 5µm)	1.0	246	Bulk and tablets	[67]
Solvent A-10 mmol Phosphoric acid buffer (pH=2.6)	BDS Hypersil C ₁₈ (250×4.6 mm, 5 µm)	1.0	220	Human plasma	[68]
Solvent B-Acetonitrile					
Solvent C-Methanol					
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 ₂ PO 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/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, Sphersorb 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 isotachopheresis (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	BEH RP ₁₈ (100x2.1 mm,1.7µm)	0.6	237	4.5	Tablets	[90]
	Mobile phase B-Sodium perchlorate buffer (0.053 M, pH 3.2): Acetonitrile = 20:80, v/v						
	Gradient programme						
	Mobile phase A: Ammonium acetate buffer (5 mmol, pH 4)	BEH C ₁₈ (50×2.1 mm, 1.7 µm)	0.613	230	3.3	Pharmaceutical dosage form	[91]
Mobile phase B: Buffer: Acetonitrile (10: 90, v/v)							
Gradient programme							
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;	Kromasil C ₁₈ (2.1×50 mm, 2.5 µm)	0.2	245	5.0	Bulk drug and tablet	[94]
	Gradient programme						
	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;	BEH C ₁₈ (100×2.1 mm,1.7 µm)	0.6	215	3.0	Tablet dosage form	[97]
Gradient programme							
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 pharmacopeias. 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 ₃ COONH 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]	
	Atorvastatin Calcium	Methanol and ammonium acetate (10 mmol); Gradient programme	Diamond C ₁₈ (150×4.6 mm, 5 μm)	0.5–0.8	Human plasma	[130]
		Mobile phase A–Acetonitrile Mobile phase B–0.1% CH ₃ COOH Gradient programme	Zorbax C ₁₈ (4.6×100 mm, 3.5 μm)	0.4	Rat plasma	[131]
		Acetonitrile: Water (10 mmol CH ₃ COONH 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 Formic acid (0.2%): Acetonitrile = 30:70, v/v	mm, 2.7 µm) Agilent C ₁₈ (100×4.6 mm, 3.5 µm)	0.6	plasma Human	[124]
Acetic acid buffer (0.2%): Methanol: Acetonitrile = 20:16:64, v/v/v	Zorbax Phenyl (75×4.6 mm, 3.5 µm)	0.8	plasma Human	[135]
Acetonitrile: Methanol: 0.1% Formic acid (0.1%) = 50: 30: 20, v/v/v	Cyno (125×4 mm, 5µm)	0.5	plasma Human	[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	BEH C ₁₈ (2.1×100 mm, 1.7 µm)	0.3	5.5	Human plasma	[137]
	Mobile phase B-0.1% formic acid in acetonitrile: Methanol = 90:10, v/v 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]
Atorvastatin Calcium	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]
	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 Acetonitrile and Ammonium acetate buffer (0.05 mmol, pH 4); Gradient programme	Hypersil (50×2.0 mm, 1.9 µm)	0.25	5.0	Bulk and dosage form	[138]
		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 above 120°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

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