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Various analytical methods for analysis of atorvastatin: A review

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ABSTRACT

Hyperlipidemia is produced due to abnormal elevated level of lipids in the blood and is a major risk factor for many heart diseases such as atherosclerosis and stroke. Lipids have been implicated in the development of atherosclerosis in humans. In hyperlipidemia there are increased levels of both LDL and triglycerides. Treatment of hyperlipidemia with statins has become an integral part of management of vascular diseases. Statins are the first line therapy for lowering lipid levels. Among statins atorvastatin is the most effective and currently available antihyperlipidemic drug. An enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase play an important for endogenous cholesterol synthesis. Atorvastatin is HMG-CoA (3-hydroxy-3-methyl-glutaryl-CoA) reductase inhibitor which significantly reduces the lipid levels (low-density lipoprotein, triglycerides, very low-density lipoprotein) and also increases the HDL (high-density lipoprotein) levels. Various analytical methods such as reverse phase high-performance liquid chromatography (RP-HPLC), high performance thin layer chromatography (HPTLC), thin layer chromatography (TLC), ultra-performance liquid chromatography (UPLC), liquid chromatography tendam mass spectroscopy, near infrared spectroscopy, capillary electrophoresis (CE), spectrophotometric methods for determination of atorvastatin as single and in combination with other drugs have been reported. In this review an attempt has been made to covers all the recent analytical methods which has been used for analysis of atorvastatin.

Keywords: Atorvastatin, Analytical Methods, HPLC, HPTLC.

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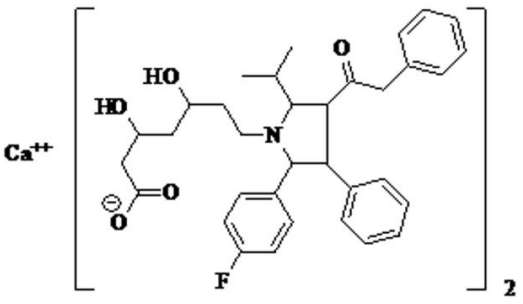
INTRODUCTION

Atorvastatin is chemically (3*R*,5*R*)-7-[2-(4-Fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid (Figure1)[1,2]. Atorvastatin is antilipidemic agent and it is a member of the drug class known as statins.[3] It is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzymeA (HMG-CoA) reductase enzyme that participates in the endogenous cholesterol synthesis.[4] The drug is odorless white crystalline powder and practically insoluble in water, soluble in methanol, slightly soluble in methylene chloride. [5] Atorvastatin is more potent and appears to have the highest LDL-CH lowering efficacy at maximal daily dose of 80 mg. At this dose a greater reduction in TGs is noted if the same was raised at baseline. Atorvastatin has a much longer plasma half-life of 18-24 hr. than other statins and has additional antioxidant property.[6] The *ortho* and *para* hydroxylated products are the main metabolite of Atorvastatin.[7] The most common side effects of Atorvastatin are runny nose, sneezing and coughing and gas. Serious side effects include muscles problems, liver problems, loss of appetite; upper stomach pain.[8]Atorvastatin is contraindicated during pregnancy and in nursing mothers. It should not be used in children or teenagers.[9] Ketoconazole, cyclosporine, erythromycin: enhanced effect and risk of myopathy with statins; niacin, gemfibrozil enhance reduction in LDL levels but increase risk of myopathy with statins.[10]

Dosage

Adults: Dose: 10-40 mg/ day (max. 80 mg).[11]



Combinations of Atorvastatin[12,13]:

1. Atorvastatin + Losartan
2. Atorvastatin + Irbesartan and other angiotensin receptor blocker
3. Atorvastatin + Ezetimibe
4. Atorvastatin + Ramipril
5. Atorvastatin + Glimepiride and another Antidiabetic agent
6. Atorvastatin + Fenofibrate
7. Atorvastatin + Amlodipine
8. Atorvastatin + B Group vitamin
9. Atorvastatin +Nicotinic Acid
10. Atorvastatin + Aspirin, Clopidogrel
11. Atorvastatin + Atenolol
12. Atorvastatin+ Hydrochlorothiazide

ANALYTICAL METHODS

For the estimation of atorvastatin in pharmaceutical dosage form and in human plasma various analytical methods such as HPLC, HPTLC, TLC, Spectrophotometric methods, Capillary electrophoresis, UPLC, LC/MS methods have been reported, among these methods HPLC is most important analytical method which have been widely used for the quantitative analysis of atorvastatin. In this review an attempt has been made to compiled all the analytical methods which have been recently used for the analysis of atorvastatin.

1. HPLC Chromatographic methods:

Several high-pressure liquid chromatographic (HPLC) methods have been reported for determination of atorvastatin in single and combination with others drug. HPLC methods are widely used chromatographic method for analysis of atorvastatin in plasma and pharmaceutical dosage form. RP-HPLC method was developed by Bangaruthalli et al (2019) for simultaneous estimation of telmisartan and atorvastatin calcium. The separation was obtained on ODS C18 column with flow rate 1.0ml/min. Mobile Phase consists of methanol: acetonitrile: buffer in ratio of 35:25:40 and the detection was carried out at 235nm.The instrument used was SHIMADZU HPLC auto sampler. The retention time of atorvastatin calcium and telmisartan was found to be 2.350 and 3.490 minutes respectively. The correlation coefficient (r^2) was found to be 0.997 and 0.999 for telmisartan and atorvastatin calcium respectively and the % mean recovery was found to be 100.943% and 100.576% for telmisartan and atorvastatin calcium respectively. [14] In another study Chaudhari et al. (2018) worked on RP-HPLC method development and validation for simultaneous estimation of atorvastatin, aspirin, ramipril and metaprolol succinate in tablet dosage form. All the drugs were separated on a (250 cm.×4.6 mm,) C18 column with a 5µm particle size. The mobile phase, which optimized through an experimental design, was a 90:10 (v/v) mixture of phosphate buffer (pH 4) an acetonitrile, pumped at a flow rate of 1ml/min. UV detection was performed at 210 nm. The retention time of atorvastatin, aspirin, ramipril and metoprolol succinate was found to be 8.013 min, 4.497 min, 7.240 min and 3.403 min respectively. The method was validated with concentration ranges of 3-9µg/ml for atorvastatin, 22.5 9µg/ml-67.59µg/ml for aspirin, and 1.54.59µg/ml for ramipril and 15-45µg/ml for metoprolol succinate. The LOD values were 0.3489µg/ml, 2.6739µg/ml, 0.2369µg/ml and 1.6279µg/ml while the LOQ values were 1.056µg/ml, 8.100µg/ml, 0.716 µg/ml and 4.931 µg/ml for atorvastatin, aspirin, and ramipril and metoprolol succinate.[15] In 2018 Pratiksha et al. reported stability indicating RP-HPLC method for estimation of atorvastatin and clopidogrel in pharmaceutical dosage form. The chromatographic separation was achieved on a C18 column using a mixture of phosphate buffer (pH= 3.0): acetonitrile (40:60) as a mobile phase and UV detection of analyte was carried out at 242nm. Atorvastatin and clopidogrel eluted at retention time 6.90 and 10.05 respectively. Current method was validated with respect to precision, accuracy, linearity, robustness according to ICH guidelines. Atorvastatin, clopidogrel and their combination drug product was subjected to stress conditions (acid, base, oxidation, thermal, humidity and photolysis). Major degradation was found under thermal and humidity stress conditions while only a minor degradation was observed during photolysis. [16] A fast and validated RP-HPLC method was carried out by Hassan et al (2018) for simultaneous determination of simvastatin, atorvastatin, telmisartan and irbesartan in bulk drugs and tablet formulations. In this study the chromatographic separation was accomplished by using symmetry C18 column (75 mm × 4.6 mm; 3.5 µm) with a mobile phase consisting of ammonium acetate buffer (10 mM; pH 4.0) and acetonitrile in a ratio 40:60 v/v. Flow rate was maintained at 1 ml/min up to 3.5 min, and then suddenly changed to 2ml/min till the end of the run (7.5min). The data was acquired using ultraviolet detector monitored at 220 nm. The method was validated for linearity, precision, accuracy and specificity. The developed method has shown excellent linearity ($r^2 > 0.999$) over the concentration range of 1–16 µg/ml. The limits of detection (LODs) and limits of quantification (LOQ) were in the range of 0.189–0.190 and 0.603–0.630 µg/ml, respectively. [17.] Tomlesh et al. (2018) worked on development and method validation of atorvastatin calcium and telmisartan in tablet dosage form by RP-HPLC method. The HPLC separation was achieved on Chemsil C18 column (150 mm x 4.6 mm id, 5 µ particle size) with isocratic condition at ambient temperature using mobile phase as a buffer (0.02 M ammonium acetate buffer pH 4.0 by glacial acetic acid): acetonitrile: tetrahydrofuran in the ratio (400:400:14 v/v/v). The analysis was performed at flow rate 1.5 ml/min. Quantification was achieved with UV detection at 246 nm. Retention time of atorvastatin calcium and telmisartan were found to be 5.70 ± 0.20 minute and 6.72 ± 0.20 minute respectively. The linearity was studied in the concentration range 10-60 µg/ml and 402-40 µg/ml for atorvastatin calcium and telmisartan respectively.[18] Determination of atorvastatin in tablet by RP-HPLC was carried out by Yugatama et al. (2017) in this study the experiment was performed on Cosmosil C18 (150 cm x 4.6 mm, 5µm) column as the stationary reverse phase chromatography, a mixture of methanol-water at pH 3 (80:20 v/v) used as the mobile phase, flow rate was maintained 1 ml/min and UV detection was carried out at wavelength of 245 nm. LOD and LOQ were found 0.2 and 0.7 ng/ml and the developed method showed good linearity in the concentration range 20 - 120 ng/ml. [19] In another study Porwal et al. (2017) reported validated HPLC-UV method for simultaneous determination of metformin, amlodipine, glibenclamide and atorvastatin in human plasma and application to protein binding studies. Optimum separation conditions were obtained with Water's Novapack Phenyl (150 mm×4.6 mm, i.d., 5.0 µm) column with mobile phase consisting of 0.1% Phosphoric acid (pH 3.0) and acetonitrile (ACN) in gradient mode with column oven temperature maintained at 30°C and elution monitored

by a UV detector at 227 nm. Protein precipitation was employed to extract the selected analyte form human plasma. The recoveries were found more than 90% for all analytes in cold aqueous 10% trichloroacetic acid (TCA) and acetonitrile. The accuracy of samples for six replicate measurements at LLOQ (Lower limit of quantification) level was within limit.[20] HPLC and capillary electrophoresis (CE) study for simultaneous determination of amlodipine and atorvastatin in the presence of their acidic degradation products in tablets was carried out by Hassan et al. (2016). In this proposed work two methods were developed for separation and quantitation of amlodipine (AML) and atorvastatin (ATV) in the presence of their acidic degradation products. The first method was a simple isocratic RP-HPLC method while the second was capillary electrophoresis (CE). For HPLC separation used Agilent zorbax® ODS column (5 µm, 4.6 x 250 mm) and flow rate was maintained 1.0 ml/ min. UV detection were performed at 254.0 nm. An Agilent 7100 CE system, with a diode array detector (Agilent Technologies) was controlled by chemstation software. The mobile phase was prepared by mixing acetonitrile: methanol: phosphate buffer pH = 3.0 (45:30:25, V/V/V); pH was adjusted to 2.5 ± 0.1 with orthophosphoric acid. CE separation was carried out at 18 ± 2 °C using a voltage of 15 kV (positive polarity) and detection was carried out at 200 nm. Peak purity was assessed using DAD (diode array detector). Bare fused silica capillaries (Agilent Technologies) of 50 µm id, 325 µm od, with a total/effective length of 48.5/40.0 cm was used during analysis. Preconditioning of the capillary before each run was carried out by flushing with 0.1 mol/l NaOH, MilliQ water and finally with a background electrolyte (BGE) for 3 min each. Post-conditioning was carried out by flushing with MilliQ water for 5 min. Samples were injected hydrodynamically at 5 kPa for 5 s and 50 mmol/l borate buffer at pH 8.0 was used as the BGE. TLC plates 20 x 20 cm (Sigma-Aldrich, Germany) were coated with a 0.2-mm silica gel 60 F254 layer. The sample was applied to the plates using micro-droppers and a UV lamp at 254 nm was used for visualization of the spots. The linearity of the proposed methods was established over the ranges 1–50 µg m/l for AML and ATV in the HPLC method and in the range of 3–50 and 4–50 µg/ml AML and ATV, respectively, in the CE method. [21] Sangshetti et al (2016) worked on development and validation of RP-HPLC method for determination of atorvastatin calcium and nicotinic acid in combined tablet dosage form. The analysis has been performed by using Agilent ZORBAX SB-C18 (150×4.6 mm, 3.5um) and mobile phase containing acetonitrile: distilled water (85:15) at pH 4.5 (adjusted with phosphoric acid). The detection was carried out at 261 nm with a flow rate of 1.0 ml/min. The retention times of atorvastatin calcium and nicotinic acid were found 6.092 and 3.125 min, respectively. The linearity for atorvastatin calcium and nicotinic acid were in the range of 2–12 and 10–80 µg/ml respectively. The recoveries of atorvastatin calcium and nicotinic acid were found to be in the range of 99.031% and 99.744% respectively.[22] In 2016 Cansel et al. conducted development of a suitable dissolution method for the combined tablet formulation of atorvastatin and ezetimibe by RP-LC method. In this proposed method, the effects of pH and surfactant on the dissolution of poorly water-soluble combined drug therapy with a different pKa values in an *in vitro* environment was investigated. The optimized test conditions achieved under sink conditions with USP apparatus 2 at a paddle rotation speed of 75 rpm and 900 ml in 0.01 M acetate buffer (pH= 6.8) containing 0.45% SDS as a dissolution medium. Quantification of dissolution samples were analyzed with a new fully validated RP-LC method with UV detection at 242 nm.[23] Bkhaitan et al. (2015) carried out stability-indicating HPLC-DAD method for simultaneous determination of atorvastatin, irbesartan, and amlodipine in bulk and pharmaceutical preparations. Separation was executed on a Waters XBrigde C18 column (5 µm, 25 × 0.46 cm) using a gradient mobile phase system consisting of acetonitrile and orthophosphoric acid buffer (pH 2.2) at a flow rate of 1 ml/min with UV detection at 240 nm. The drugs were subjected to acidic and basic hydrolysis, oxidation, exposure to UV light, and exposure to temperature (dry heat) to apply stress conditions. Linearity of the method was evaluated in the range 5–30 µg/ml for each drug with correlation coefficient values (r^2) of 0.9982, 0.9973, and 0.9986 for atorvastatin, irbesartan, and amlodipine, respectively. The limits of detection were 0.05, 0.06, and 0.08 µg/ml for the three compounds, respectively. [24.] In 2014 Kumar et al. carried out new validated RP-HPLC analytical method for simultaneous estimation of atorvastatin and ezetimibe in bulk samples as well in tablet dosage forms by using PDA detector The chromatographic separation was performed on an X Terra C8 (4.6 x 250 mm; 5 µm), with phosphate buffer [pH 3.5] and acetonitrile in the ratio of 40:60 (v/v) as mobile phase. The detection was carried out at 240 nm. The accuracy was found to be 99.59% and 98.98% for atorvastatin and ezetimibe respectively. The linearity was 5-25 µg/ml for both the drugs. The intra-day RSD was 0.57% and inter-day RSD was 0.13% for atorvastatin calcium and intra-day RSD was 0.56% and inter-day RSD was 0.09% for ezetimibe.[25] In another study Kurakula et al. (2014) carried out development and validation of a RP-HPLC method for assay of atorvastatin and its application in dissolution studies on thermosensitive hydrogel-based nanocrystals. Chromatographic identification was achieved on C18 (5 µm) column using acetonitrile and 0.025 M potassium dihydrogen ortho-phosphate buffer pH 5 (45:55 v/v) as mobile phase, at a flow rate of 1.5 ml/min. Detection was carried out with photo diode array detector (PDA) at 246 nm. The developed HPLC method was validated according to International Conference on Harmonisation (ICH) Q2(R1) guidelines and applied to dissolution studies on atorvastatin thermosensitive hydrogel-based nanocrystal formulation, using Lipitor® as standard. The retention time of atorvastatin was 4.5 min and drug response were linear in the range of 0.1 - 0.5 µg/ml with a correlation coefficient of 0.9995. Precision was determined to be between 0.16 - 0.61 percent relative standard deviation (% RSD) for the analysed samples. The limit of detection and of quantification was 35.6 and 71.2 ng/ml. The assay of atorvastatin nanocrystal and Lipitor® gave 99.37 and 99.12 % recovery, respectively. Dissolution studies showed atorvastatin release of 40 and 65 % at 40 min from thermosensitive hydrogel nanocrystal formulation and Lipitor®, respectively indicating sustained release.[26] Oliveira et al. (2013) studied degradation kinetics of atorvastatin under stress conditions and chemical analysis by HPLC. The chromatographic separation was achieved on C18 column (ODS, 250 × 4 mm, 5 µm, SunFire) with mobile phase: acetonitrile/phosphoric acid 0.1% v/v (65:35). The flow rate was maintained 1.5 ml/min during study. The injection volume was 10 µl and detection was carried out with UV-DAD at λ max 238 nm at 303 K. ATV has been found to degraded under acid and basic conditions, including a first order kinetic degradation under acid conditions, as compared to a zero-order kinetic degradation under basic conditions, which tends to be less stable when studied within acid mediums. The linear correlation coefficient (r^2) was found greater than 0.99 in concentration range of 14 to 26 µg/ml. A detection limit was found 0.45 µg/ml and a quantification limit was found 1.36 µg/ml.[27] Simultaneous

estimation and validation of atorvastatin calcium (AST) and aspirin (ASP) in combined capsule dosage form by RP HPLC was carried out by Suma et al. (2012). The chromatographic separation was achieved on a 5 – micron C 18 column (250 x 4.6mm) using a mobile phase consisting of a mixture of acetonitrile: ammonium acetate buffer 0.02M (68:32) pH 4.5. The flow rate was maintained at 0.8 ml / min. The detection of the constituents was done using UV detector at 245 nm for AST and ASP. The retention time of AST and ASP were found be 4.5915 ± 0.0031min and 3.282 ±0.0024 min respectively. [28] In 2012 Bhinge et al. reported a new approach to the RP-HPLC method for simultaneous estimation of atorvastatin calcium and fenofibrate in pharmaceutical dosage forms. Atorvastatin calcium, fenofibrate and diclofenac (internal standard) were well separated using a reversed phase column and mobile phase consisting of acetonitrile: potassium di hydrogen phosphate (50 mm) (72:28 v/v) (pH 4.1). The mobile phase was pumped at 1.0 ml/min flow rate and atorvastatin calcium and fenofibrate were detected by uv-vis detection at 260 nm. The retention time for atorvastatin calcium, internal standard and fenofibrate were 4.34, 5.35 and 12.05 min, respectively. The LOD and LOQ was found to be 1.95 and 4.80 µg/ml for atorvastatin calcium whereas for fenofibrate it was found to be 1.73 and 3.98 µg/ml in mobile phase.[29] Talluri et al. (2012) worked on synchronized separation of atorvastatin—an antihyperlipidemic drug with antihypertensive, antidiabetic, antithrombotic drugs by RP-LC for determination in combined formulations. The chromatographic separation was carried out by gradient elution mode with acetonitrile as organic modifier and 0.1% triethylamine acetate (TEAA) buffer pH 5 at a flow rate of 1 ml/min and a diode array detector at wavelength 230 nm was employed for detection of the analytes. Calibration curves were linear in the range of 5–150 mg/ml for all the drugs with correlation coefficients of determination (r2 values) 0.999. Limits of detection (LODs) and Limits of quantification (LOQs) ranged from 0.1 to 0.27 mg/ml and 0.3 to 0.89 mg/ml respectively. Intra-day and inter-day precision were studied at three concentration levels (20, 60 and 100 mg/ml). The intra-day and inter-day RSD for all compounds was less than 2.0%. The accuracy for all compounds was found to be between 98% and 102%.[30] Nagaraju et al. (2011) developed and validated RP-HPLC method for the estimation of atorvastatin calcium in bulk and pharmaceutical formulations. The separation was achieved on Phenomenex C18 (250 × 4.6 mm, 5µ) column using a mobile phase containing of acetonitrile: ortho-phosphoric acid (0.1%): tetrahydrofuran (48:0.04:52). The eluent was monitored at 244 nm. The results have been validated statistically and recovery studies confirmed the accuracy of proposed method.[31] Simultaneous estimation of atorvastatin calcium and fenofibrate in tablet dosage form was carried out by Hirave et al. (2010) by RP-HPLC. The best separation was achieved on HiQ sil C8 (4.6x250mm) column. Detection of the drugs was carried out at 260 nm by using a mobile phase having composition methanol: water pH 3.2 (90:10 v/v) at a flow rate of 1 ml/min. [32] In 2010 Jena et al. reported an analytical method for the simultaneous determination of atorvastatin calcium and amlodipine besylate in tablet dosage by RP-HPLC. The chromatographic resolution of drugs was achieved in mobile phase having composition of a phosphate buffer (1 ml ortho phosphoric acid in 1000 ml of water) acetonitrile and methanol in the ratio 53:43:4 v/v by using Grace Smart RP C18 column (250 x 4.6, 5µm). The samples were eluted at 1 ml/min and UV detection was carried out at 246 nm using photo dine array detector.[33] Kumar et al. (2010) proposed a new RP-HPLC method for simultaneous estimation of atorvastatin calcium and telmisartan in tablet dosage form. Chromatographic separation of selected drugs was achieved on (Waters symmetry C18, 250mm x 4.6mm, 5µ) column. Composition of mobile phase was maintaining ammonium acetate (0.02M, pH 4.0 adjusted with glacial acetic acid) and acetonitrile in ratio 40:60 v/v. The detection wavelength was 254 nm and flow rate were maintained 1.0 ml/min.[34] Shetty et al. (2010) worked on quantitative application to a polypill by the development of stability indicating LC method for the simultaneous estimation of aspirin, atorvastatin, atenolol and losartan potassium. Efficient chromatographic separation was achieved on a C18 stationary phase with simple mobile phase combination of buffer and acetonitrile: buffer consists of 0.1% orthophosphoric acid (pH 2.9), delivered in a gradient mode and quantitation was carried out using ultraviolet detection at 230 nm with a flow rate of 1.0 ml/min. The retention times of atenolol, aspirin, losartan potassium, and atorvastatin were 3.3, 7.6, 10.7 and 12.9 min respectively. The combination drug product was exposed to thermal, acid/base hydrolytic, humidity and oxidative stress conditions, and the stressed samples were analysed by proposed method. The method was validated with respect to linearity; the method was linear in the range of 37.5 to 150.0 µg/ml for ASP (aspirin), 5.0 to 20.0 µg/ml for ATV (atorvastatin) and 25.0 to 100.0 µg/ml for ATL (atenolol) and LST (losartan potassium). Acceptable precision and accuracy were obtained for concentrations over the standard curve ranges. The validated method was successfully applied to the analysis of star pill tablets constituting all the four drugs; the percentage recoveries obtained were 99.60% for ASP, 99.30% for ATV, 99.41% for ATL and 99.62% for LST.[35] Stability indicating RP-HPLC method for simultaneous determination of atorvastatin (ATR) and nicotinic Acid (NTA) was proposed by Gupta et al. (2009). The proposed RP-HPLC method utilizes a Phenomenex® C18, 5µm, 250mm X 4.6mm i.d. column, at ambient temperature, optimum mobile phase consisted of acetonitrile and 50mM potassium dihydrogen phosphate buffer (68:32, v/v), pH adjusted to 4.5±0.1 with phosphoric acid solution, effluent flow rate monitored at 0.8 ml/min, and UV detection at 247 nm. The combination drug product was exposed to thermal, acid/base hydrolytic, humidity and oxidative stress conditions, and the stressed samples were analysed by proposed method. The described method was linear over the range of 2-10 µg/ml and 20-100 µg/ml for ATR and NTA, respectively. The mean recoveries were 100.99 and 102.65% for ATR and NTA, respectively. The limit of detection for ATR and NTA was found to be 0.16 and 0.12 µg/ml, respectively.[36] Zaheer et al. (2008) worked on stability-indicating high-performance liquid chromatographic determination of atorvastatin calcium in pharmaceutical dosage form. The chromatographic conditions comprised of a reversed-phase C18 column (250 x 4.6 mm), 5 µ with a mobile phase consisting of a mixture of methanol: acetonitrile: phosphate buffer solution in the ratio (45:45:10). Flow rate was 1 ml/ min. Detection was carried out at 246 nm. The retention time of atorvastatin was 6.98 min. Atorvastatin calcium was subjected to acid and alkali hydrolysis, oxidation, photochemical degradation and thermal degradation. The linear regression analysis data for the calibration plots showed good linear relationship in the concentration range 52.20 to 156.60 µg/ml. The value of correlation coefficient, slope and intercept were, 0.9999, 36.02 and 26.45, respectively. The drug showed degradation under acidic, basic, photochemical and thermal degradation conditions. All the peaks of degraded product were resolved from the active pharmaceutical ingredient with significantly different retention time. Another RP-HPLC method for simultaneous determination of atorvastatin calcium and

ezetimibe in pharmaceutical formulations was reported by Qutab et al. (2007), best separation of selected drugs was achieved on a 250 x 4.6 mm, 5µ Hypersil phenyl-2 column. Eluent was monitored at 242 nm using a solvent system of 0.1 M ammonium acetate (pH 6.5) and acetonitrile in the ratio of 28:72 (v/v).[38] Shah et al. (2007) developed and reported a RP-HPLC method for determination of atorvastatin calcium and nicotinic acid in combined tablet dosage form. Chromatographic separation was achieved on Phenomenex Luna C18, 5 mm column having 250 x 4.6mm i.d. Isocratic separation was achieved with mobile phase containing 0.02 M potassium dihydrogen phosphate: methanol: acetonitrile (20:40:40, pH 4). Samples were eluted with flow rate 1.0 ml/min and effluents were monitored at 240 nm.[39] Shah et al. (2007) worked on stability indicating RP-HPLC estimation of atorvastatin calcium and amlodipine besylate in pharmaceutical formulations. Best separation was found on Phenomenex Gemini C18 (250 × 4.6 mm i.d., 5µm) column with isocratic mode. The mobile phase consisted of 0.02 M potassium dihydrogen phosphate: MeOH (20: 80, pH 4.0). The flow rate was maintained 1.0 ml/min and drugs were monitored at 240 nm.[40] In 2007 Mohammadi et al. carried out stability-indicating high-performance liquid chromatographic (HPLC) assay for the simultaneous determination of atorvastatin (AT) and amlodipine (AM) in commercial tablets. The separation was achieved on a Perfectsil Target ODS-3 (250 × 4.6 mm i.d., 5 µm) column using a mobile phase consisting of acetonitrile-0.025 M NaH_2PO_4 buffer (55:45, v/v, pH 4.5), flow rate was maintained 1 ml/min and UV detection was carried out at 237 nm. The drugs were subjected to oxidation, hydrolysis, photolysis and heat to apply stress conditions. Degradation products produced as a result of stress studies did not interfere with the detection of AT and AM and the assay can thus be considered stability-indicating.[41] Alla (2007) has reported a stability-Indicating LC method for the simultaneous determination of metoprolol (ME), atorvastatin (AT) and ramipril (RA) in combined pharmaceutical dosage form. A chromatographic separation of the three drugs was achieved with a Hypersil C8, 15-cm analytical column using buffer-acetonitrile (55:45 v/v). The buffer used in mobile phase contains 0.02 M sodium perchlorate in double distilled water. The flow rate was maintained 1.0 ml/min and detection was carried out at wavelength 210 nm for ME, AT and RA using as ultra violet detector. Methanol was used as diluent. The resolution among ME, AT and RA were found to be more than 2.0. Theoretical plates for ME, AT and RA were >2500.[42] Stability-indicating reversed-phase liquid chromatographic methods for simultaneous determination of atorvastatin and ezetimibe from their combination drug products was carried out by Chaudhari et al. (2007). Separation of atorvastatin (ATV) and ezetimibe (EZE) was obtained on Li Chrospher 100 C18, 5-micron, 250 cm x 4.0 mm id column at ambient temperature with mobile phase consists of acetonitrile-water-methanol (45+ 40 + 15, v/v/v) having pH 4.0 ± 0.1. The flow rate was maintained 1.0 ml/min and UV detection was carried out at 250 nm. ATV, EZE, and their combination drug product were exposed to thermal, photolytic, hydrolytic, and oxidative stress conditions, and the stressed samples were analysed by the proposed method. The response was found linear over the concentration range of 1-80 µg/ml for ATV and EZE. The mean recoveries were found 99.27 and 98.5% for ATV and EZE, respectively.[43] Raja et al. (2006) have developed a RP-HPLC method for the simultaneous determination of atorvastatin and amlodipine in tablet dosage form. The mobile phase used was a mixture of acetonitrile and 0.03 M phosphate buffer (55:45 v/v, pH 2.9). The detection of atorvastatin and amlodipine was carried out on dual γ absorbance detector at 240 nm and 362 nm, respectively. [44]. Stanisz et al. (2006) carried out validation of HPLC method for determination of atorvastatin in tablets and for monitoring stability in solid phase. Separation of atorvastatin was successfully achieved on a C-18 column utilizing water and acetonitrile at the volumetric ratio of 48:52, adjusted to pH 2.0 with 80% ortho-phosphoric acid. The detection wavelength was 245 nm. The method was validated and the response was found to be linear in the drug concentration range of 0.04 mg/ml and 0.4 mg/ml. The mean values ± RSD of the slope and the correlation coefficient were 8.192 ± 0.260 and 0.999, respectively. The RSD values for intra- and inter day precision were < 1.00% and 0.90%, respectively. The degradation kinetic of atorvastatin at 363 K in a relative humidity of 76.4% was observed to be autocatalytic first order reaction. The kinetic parameters were as follows: k (where k represents the velocity constant; s^{-1}) = (1.42 ± 0.19) 10-6; t0.5 (where t0.5 represents the time needed for a 50% decay of atorvastatin; days) = 32.82 ± 0.9; t0.1(where t0.1 represents the time needed for a 10% decay of atorvastatin; days) = 13.86 ± 0.8.[45] Pasha et al. (2006) carried out analysis of five HMG-CoA reductase inhibitors-atorvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin: pharmacological, pharmacokinetic and analytical overview and development of a new method for use in pharmaceutical formulations analysis and *in vitro* metabolism studies. For separation of drugs ternary gradient elution at a flow rate of 1 ml/min was employed on an Intertisl ODS 3V column (4.6 x 250 mm, 5 microm) at ambient temperature. The mobile phase consisted of 0.01 m ammonium acetate (pH 5.0), acetonitrile and methanol. Theophylline was used as an internal standard (IS). The HMG-CoA reductase inhibitors and their metabolites were monitored at a wavelength of 237 nm. Drugs were found to be 89.6-105.6% of their label's claim in the pharmaceutical formulations. For *in vitro* metabolism studies the reaction mixtures were extracted with simple liquid-liquid extraction using ethyl acetate. Baseline separation of statins and their metabolites along with IS free from endogenous interferences was achieved. Nominal retention times of IS, atorvastatin, lovastatin, pravastatin, rosuvastatin and simvastatin were 7.5, 17.2, 21.6, 28.5, 33.5 and 35.5 min, respectively.[46] Determination of atorvastatin in human serum by reversed-phase high performance liquid chromatography with UV detection was carried out by Bahrami et al. (2005). Chromatographic separation was accomplished using C18 analytical column with a mobile phase consisting of sodium phosphate buffer (0.05 M, pH 4.0) and methanol (33:67, v/v). Atorvastatin and the internal standard were detected by UV absorbance at 247 nm. The average recoveries of the drug and internal standard were 95 and 80 %, respectively. The analytical performance was studied and the method was applied in a randomized cross-over bioequivalence study of two different atorvastatin preparations in 12 healthy volunteers.[47] In 2004 Manoj et al. reported RP HPLC method for simultaneous estimation of atorvastatin and aspirin in capsule formulation. The maximum resolution was achieved by mobile phase acetonitrile: 0.05 M potassium dihydrogen phosphate buffer: methanol in the ratio (50:30:20, v/v/v) at pH 3.0. This mixture was found to be appropriate allowing good separation of both the components at a flow rate of 1.5 ml/min and detection wavelength 240 nm. In these condition atorvastatin calcium and aspirin were eluted at the 4.7 and 2.2 min respectively. The concentration of atorvastatin calcium and aspirin estimation in the capsule was found to be in the range 99.33-101.43 % and 99.71-101.60 %.[48] Determination of atorvastatin and its impurities

in bulk drug and tablet by gradient RP-HPLC assay with UV detection was carried out by Erturk et al. (2003). Best resolution was obtained on a Luna C18 column with acetonitrile ammonium acetate buffer pH 4-THF as a mobile phase. Samples were eluted with the mobile phase at flow rate 1.0 ml/ min. Detected was carried out at 248 nm.[49]

2. U.V. Spectroscopic Methods

Various U.V. spectroscopic methods for simultaneous determination of atorvastatin and its combination with other drugs have been reported. Among various analytical methods for the estimation of atorvastatin U.V. spectroscopic methods play an important role in the field of analysis and in the quantitative determination. Bernard et al. (2018) carried out new spectrophotometric method for the estimation of atorvastatin calcium (ATR) and aspirin (ASP) using urea as hydrotropic solubilizing agent. The developed method used the simultaneous equation method (method-A) using 243 nm and 233 nm as absorbance maxima for ATR and ASP respectively and Q-absorbance ratio method (method-B), which is based on the measurement of absorptivity at iso-absorptive point 239 nm and 243 nm (absorption maximum of atorvastatin). The calibration curves for both drugs were found to be linear in the concentration range of 10-50µg/ml. The mean recovery of the drugs from the combination tablets was found to be 98.83 % for atorvastatin and 97.77 % for aspirin for method-A and 98.09 % and 98.06 % for method-B respectively.[50] In 2018 Tomlesh et al. carried out simultaneous estimation of atorvastatin calcium and telmisartan in tablet dosage form by spectrophotometry. The absorption maxima at 246 nm and 298 nm were used for the estimation of atorvastatin calcium and telmisartan respectively. Both the drugs obey Beer-Lambert's law within the range of 01-06 µg/ml for atorvastatin calcium and 04-24 µg/ml for telmisartan with a correlation coefficient (r^2= 0.9998) and (r^2= 0.9999) respectively. The recovery study was carried out by standard addition method. The average percent recovery was found to be 99.67 for atorvastatin calcium and 100.20 for telmisartan. The method was validated according to international conference on harmonization (ICH) guidelines with respect to linearity, recovery, precision, LOD, and LOQ[51]. In another study Alshabrawy et al. (2017) worked on sensitive spectrophotometric determination of atorvastatin in pharmaceutical formulation by ion pair complexation with pararosaniline hydrochloride. In this study different factors affecting the formation of the ion pair and its stability were studied and optimized in order to obtain the best conditions for the experiment. Method validation was done over a concentration range of 1 to 8 µg/ml. The method was proven to be sensitive with limit for quantitation of 0.93 µg/ml and limit of detection of 0.31 µg/ml. The red colored ion pair is easily extractable in organic solvent and showed maximum absorption at 547 nm. [52] Al-Adl et al. (2017) studied spectrophotometric determination of atorvastatin calcium and rosuvastatin calcium in bulk and dosage form using p-dimethylaminobenzaldehyde. This method depended on the formation of colored chromogen between atorvastatin calcium and rosuvastatin calcium and p-dimethylaminobenzaldehyde (PDMAB) in acidic conditions. The reaction mixture exhibited maximum absorbance at λ max 540 and 570 nm for atorvastatin calcium and rosuvastatin calcium, respectively. The method was linear over the concentration range of 20-160 µg/ml and 2-16 µg/ml for atorvastatin calcium and rosuvastatin calcium, respectively.[53] Ramadan et al. (2015) worked on determination of atorvastatin calcium in pure and its pharmaceutical formulations using iodine in acetonitrile by UV-Visible spectrophotometric method. The method was based on the oxidation of atorvastatin calcium by iodine and formation triiodide (I_3^-) complex. The formed complex was measured at 291 and 360 nm against the reagent blank prepared in the same manner. The developed method obeys beer's law within a concentration range of 0.5586-11.173 µg/ml. The relative standard deviation did not exceed 3.0% and regression analysis showed a good correlation coefficient (r^2= 0.9995). The limit of detection (LOD) and the limit of quantification (LOQ) were to be 0.056 and 0.17 µg/ml, respectively. The developed method can be applied for the determination of atorvastatin in pure and its commercial tablets without any interference from excipients (at λ max =291 & 360 nm), ezetimibe (EZE), fenofibrate (FEN) and aspirin (ASP) at λ max =360 nm with average recovery of 99.45 to 102.4%.[54] Virani et al. (2015) have discussed simultaneous estimation of irbesartan and atorvastatin by first order derivative spectroscopic method in their synthetic mixture. The derivative spectrophotometric method was based on the determination of both the drugs at their respective zero crossing point (ZCP). The first order derivative spectra were obtained in methanol and the determinations were made at 225.20 nm (ZCP of atorvastatin) for irbesartan and 308.15 nm (ZCP of irbesartan) for atorvastatin. The linearity was obtained in the concentration range of succinate 5-30 µg/ml for irbesartan and 5- 30 µg/ml for atorvastatin succinate. The mean recovery was 99.25 and 99.65% for irbesartan and atorvastatin succinate, respectively.[55] A new simple spectroscopic method for estimation of atorvastatin tablets using hydrotropic solubilization technique was carried out by Rani et al (2015). For the estimation of atorvastatin (ATV) in tablet dosage form used 0.5 M sodium benzoate aqueous solution as a hydrotropic agent. Atorvastatin (ATV) showed maximum absorbance at a wavelength of 268 nm and beer's law was obeyed in the concentration range of 0.1-0.6 µg/ml. The method was validated according to ICH guidelines. [56] Naveed et al. (2014) carried out simple UV spectrophotometric assay of atorvastatin API formulation and their comparative study. The assay was based on the ultraviolet UV absorbance maxima at about 244 nm wavelength of atorvastatin using methanol as solvent. A sample of drug was dissolved in methanol to produce a solution containing atorvastatin. Similarly, a sample of ground tablets of different brand were extracted with methanol and diluted with the same methanol. The absorbance of sample preparation was measured at 244 nm against the solvent blank and the assay was determined by comparing with the absorbance of available brand.[57] Ashour et al. (2013) carried out new kinetic spectrophotometric method for determination of atorvastatin in pure and pharmaceutical dosage forms. The method involved the oxidative coupling reaction of atorvastatin (AVS) with 3-methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate (MBTH) in the presence of Ce (IV) in an acidic medium to form colored product with λ max at 566 nm. The reaction was followed spectrophotometrically by measuring the increase in absorbance at 566 nm as a function of time. The initial rate and fixed time methods were adopted for constructing the calibration curves. The linearity range was found to be 2-20 µg/ml for initial rate and fixed time methods. The limit of detection for initial rate and fixed time methods is 0.093 and 0.064 µg/ml, respectively. Molar absorptivity for the method was found to be 3.36×104 l/molcm.[58] Kaliappanlango et al (2012) carried out validated spectrophotometric methods for the simultaneous determination of telmisartan (TETM) and atorvastatin (ATV) in bulk and tablets. The first method was based on first-order derivative spectroscopy. The sampling wavelengths

were 223 nm (zero crossing of TETM) where ATV showed considerable absorbance and 272 nm (zero crossing of ATV) where TETM showed considerable absorbance. The second method Q-analysis (absorbance ratio), involved formation of Q-absorbance equation using respective absorptivity values at 280.9 nm (isosbestic point) and 296 nm (λ max of TETM). The third method involved determination using multicomponent mode method; sampling wavelengths selected were 296.0 and 246.9 nm. TETM and ATV followed linearity in the concentration range of 5-40 and 4-32 µg/ml for method I, 5-30 µg/ml and 2-24 µg/ml for method II and III, respectively. [59] Spectrophotometric estimation of atorvastatin calcium & fenofibrate in tablet dosage form was carried out by Hirave et al (2013). UV spectrophotometric method was developed for the estimation of atorvastatin calcium & fenofibrate in tablet dosage form by using simultaneous equation method. The drug obeyed beer's law & showed good correlation near to 0.999. Absorption maxima of atorvastatin calcium & fenofibrate were found to be at 246 and 286 nm respectively. Beer's law was obeyed in concentration rang of 1-10 µg/ml for atorvastatin calcium & 2-20 µg/ml for fenofibrate. The method has been validated for linearity, accuracy & precision. The recovery was more than 99%.[60] In another study estimation of aspirin and atorvastatin calcium in combined dosage form by spectrophotometric method was carried out by Patel et al. (2010). Methanol was used as a solvent in this study. During analysis second order derivative spectroscopy method was used to eliminate spectral interference, with 266.78 nm and 237.35 nm as zero crossing points for aspirin and atorvastatin calcium respectively.[61] Simultaneous spectrophotometric estimation of atorvastatin calcium and amlodipine besylate in combined tablet dosage form by area under curve method was carried out by Jani et al (2010). In this study methanol was used as a solvent. The proposed area under curve method involved the measurement of area at selected analytical wavelength ranges and performing the analysis using "Cramer's Rule" and "Matrix Method". Two analytical wavelength ranges selected were 256-238.5 nm and 368-352 nm for the estimation of ATR (atorvastatin) and AML (amlodipine). The linearity of the proposed method was investigated in the range of 5-50 µg/ml (r^2 = 0.9998) for ATR and 5-50 µg/mL (r^2= 0.9997) for AML, respectively. The percentage mean recovery was found to be 99.83% for ATR and 99.60% for AML. The method was statistically validated for its linearity, accuracy and precision. Both inter-day and intra-day variation was found to be showing less % RSD value indicating high grade of precision of the method.[62] In 2011 Kumbhar et al. carried out development and validation of derivative spectrophotometric method for estimation of atorvastatin calcium (AT) and amlodipine besylate (AM) in tablet dosage form. The AT and AM stock solutions were prepared in (50:50 V/V) methanol: water. The method of analysis was derivative spectroscopy to eliminate spectral interference by measuring absorbances at 241nm and 250 nm for AM and AT respectively. The AT and AM were linear in concentration range of 0-14 µg/ml and 0-7 µg/ml respectively. The limit of detection (LOD) and limit of quantitation (LOQ) of AM was 0.29 and 0.75 µg respectively. The limit of detection (LOD) and limit of quantitation (LOQ) of AT was 0.21 and 0.60 µg.[63] Simultaneous spectroscopic estimation of atorvastatin calcium and aspirin in pharmaceutical dosage form was successfully carried out by Patel et al. (2012). In this study method-1 simultaneous equations and method-2 Q-absorbance ratio method was used. λ max was found 240 nm and 230 nm for atorvastatin calcium and aspirin respectively and 290.5 nm (isoabsorptive point). A 0.1N NaOH was used as solvent. Linearity was observed in the concentration range of 2-26 µg/ml for atorvastatin calcium and 5-25 µg/ml for aspirin respectively.[64] Havele et al. (2011) carried out simultaneous determination of atorvastatin calcium and pioglitazone hydrochloride in its multicomponent dosage forms by UV spectrophotometry. A simple, accurate and precise spectrophotometric method was developed for simultaneous estimation of atorvastatin & pioglitazone in bulk and tablet showed maximum absorbance at 210 nm for atorvastatin while showed maximum absorbance for pioglitazone at 225 nm. The overlain spectra showed maximum absorbance at 242 nm.[65] Spectrophotometry and liquid chromatography methods for estimation of atorvastatin calcium and fenofibrate in tablets was reported by Nakarani et al. (2007). Two simple and accurate methods to determine atorvastatin calcium (ATO) and fenofibrate (FEN) in combined dosage forms were developed using second-derivative spectrophotometry and reversed-phase liquid chromatography (LC). ATO and FEN in combined preparations (tablets) were quantitated using the second-derivative responses at 245.64 nm for ATO and 289.56 nm for FEN in spectra of their solution in methanol. The calibration curves showed linearity [(r^2) = 0.9992 for ATO and 0.9995 for FEN] in the concentration range of 3-15 µg/ml for ATO and FEN. In the LC method, analysis was performed on a Hypersil ODS-C18 column (250 mm x 4.6 mm id, 5µ particle size) in the isocratic mode using the mobile phase methanol-water (90: 10, v/v), adjusted to pH 5.5 with orthophosphoric acid, at a flow rate of 1 ml/min.[66] A simple UV-spectrophotometric method for simultaneous estimation of telmisartan and atorvastatin calcium in bulk and tablet dosage form was carried out by Chaudhari et al (2010). In this study an UV spectrophotometric method using simultaneous equation was developed for the simultaneous determination of telmisartan and atorvastatin calcium in a binary mixture. In the proposed method, the signals were measured at 296.0 nm and 247.0 nm corresponding to absorbance maxima of telmisartan and atorvastatin calcium in methanol respectively. Linearity range was observed in the concentration range of 5-30 µg/ml for both the drugs. Concentration of each drug was obtained by using the absorptivity values calculated for both drugs at two wavelengths, 296.0 nm and 247.0 nm and solving the simultaneous equation.[67] In 2010 Vijayalakshmi et al. carried out simultaneous UV spectrophotometric determination of atorvastatin calcium and telmisartan in tablet dosage form. Two accurate, precise, sensitive and economical procedures for simultaneous estimation of atorvastatin calcium and telmisartan in tablet dosage forms have been developed. In this study first method employed formation and solving of simultaneous equations using 219 nm and 257nm as two analytical wavelengths for both drugs in 0.1 N NaOH. The second method was Q- analysis based on measurement of absorptivity at 240 nm (as isobestic point) and 257nm (λ max of ATV). Atorvastatin calcium and telmisartan at their respective λ max 257 nm and 219 nm and at 240 (isosbestic point) showed linearity in a concentration range of 50-150 µg/ml and 10-50 µg/ml respectively.[68] Spectroscopic study for estimation of atorvastatin calcium in tablet dosage form was reported by Prajapati et al (2011). Standard stock solution was prepared in methanol and λ max was found 246nm. The proposed method obeys beer's law in the range of 5-25 µg/ml. Absorption maxima was determined with 10 µg/ml by scanning in the range of 200-400 nm. % Recovery studies was found in the range of 99.96%-100.03%.[69] In another UV spectrophotometric study the simultaneous estimation of atorvastatin calcium and pioglitazone hydrochloride was carried out by Sasikala et al (2013). Atorvastatin calcium and pioglitazone hydrochloride obeyed beer's law in

concentration ranges of 2-16 µg/ml with correlation coefficient of 0.9992 for atorvastatin calcium and correlation coefficient of 0.9967 for pioglitazone hydrochloride respectively. The zero-crossing point for atorvastatin calcium and pioglitazone hydrochloride was found 226 and 278 nm respectively in water. The LOD values were found to be 0.594 µg/ml and 0.105 µg/ml for atorvastatin calcium and pioglitazone hydrochloride respectively. The LOQ values were found to be 1.800 µg/ml and 0.318 µg/ml for atorvastatin calcium and pioglitazone hydrochloride respectively. The % RSD for intraday and interday precision were found to be less than 2 %. Recovery by this method was found to be 98 % - 101 % for both the analytes.[70] Jadhav et al (2014) carried out dual wavelength spectrophotometric method for simultaneous estimation of atorvastatin calcium and felodipine from tablet dosage form. The calibration curves determination for both drugs has been carried out in 0.1 N HCl, phosphate buffer pH 6.8, and acetonitrile (ACN)-water (70: 30 V/V). Linearity range was observed in the concentration range of 2 to 12 µg/ml for felodipine (FEL) and 20 to 100 µg/ml for atorvastatin (ATR). Percent concentration estimated for ATR and FEL was 100.12 ± 1.03 and 99.98 ± 0.98, respectively.[71] In 2014 Ibrahim et al. reported simultaneous determination of amlodipine besylate and atorvastatin calcium by using spectrophotometric method with multivariate calibration and hplc method. The method was based on the spectrophotometric measurements of the drugs in the range of 200-400 nm together with multivariate calibration methods. Resolution of the binary mixture under investigation has been accomplished mainly by using partial least squares (PLS) and principal component regression (PCR). The RP-HPLC method utilized a YMC-pack pro C18 ODS-A (25 cm x 4.6 mm, 5 µm) column, at room temperature, optimum mobile phase consisted of methanol and 0.01 M sodium dihydrogen phosphate buffer (75:25, v/v), pH adjusted to 3.5 with orthophosphoric acid solution. The flow rate was monitored at 1.2 ml/min, and UV detection at 239 nm.[72] Spectrophotometric estimation of atorvastatin calcium in tablet dosage forms was carried out by Jadhav et al. (2010). This method utilized 2.0 M urea solution as, hydrotropic solubilizing agent. In the urea solution atorvastatin calcium showed maximum absorbance at 240 nm. The 2.0 M urea solution did not show any interference with the sampling wavelength. Another method was formation of green color complex between the drug atorvastatin calcium and 0.3 % w/v ferric chloride and 0.02 % w/v potassium ferricyanide. The green colored complex showed the maximum absorbance at 787 nm. The hydrotropic agent and additives used in the manufacture of tablets did not interfere in the analysis. The results of tablet analysis were found be in range of 99.26 to 100.12% with standard error values of 0.2728 and 0.2082 by hydrotropy and colorimetry respectively. The results of analysis of both methods were validated statistically following ICH Q2A (R1) guidelines. Both methods were found to be useful for accurate, sensitive, selective, precise and robust analysis of atorvastatin from marketed formulations.[73] Patil et al. (2009) reported simultaneous determination of atorvastatin calcium and telmisartan in tablet dosage form by spectrophotometry. In this study three accurate, precise, sensitive and economical procedures for simultaneous estimation of atorvastatin calcium and telmisartan in tablet dosage form have been developed. The methods employed were absorbance correction method (I), first order derivative spectroscopic method (II) and duel wavelength method (III). The first method employed wavelength 328 nm for direct estimation of telmisartan where atorvastatin calcium shows nil absorbance. Estimation of atorvastatin calcium was carried out after correction for absorbance of telmisartan at 241 nm. The second method was based on first order derivative spectroscopy. Wavelengths 297 nm and 241.8 nm were selected for the estimation of the atorvastatin calcium and telmisartan, respectively. In the third method, atorvastatin calcium was determined by plotting the difference in absorbance at 258 and 291 nm (difference is zero for telmisartan) against the concentration of atorvastatin calcium. Similarly, for the determination of telmisartan, the difference in absorbance at 225 and 252 nm (difference is zero for atorvastatin calcium) was plotted against the concentration of telmisartan. Both the drugs obey beer's law in the concentration range 5-30 µg/ml.[74] Simultaneous estimation of atorvastatin calcium and amlodipine besylate in tablet dosage forms by spectrophotometric method was proposed by Chaudhari et al (2010). The method was based on property of additivity of absorbances. The two wavelengths on amlodipine besylate curve were found out where it showed same absorbance, which were 257.4 and 360.0 nm. At 360.0 nm, amlodipine besylate showed some absorbance while atorvastatin calcium showed zero absorbance. Both the drugs gave absorbance at 257.4 nm. The method involved solving of an equation based on measurement of absorbances at two wavelengths 257.4 and 360.0 nm.[75] Bernard et al (2013) studied simultaneous estimation of atorvastatin calcium and amlodipine besylate by UV spectrophotometric method using hydrotropic solubilization. The developed method used the absorption ratio or Q-value which was based on the measurement of absorptivity at 293 nm (iso-absorptive point, both the drugs were found to have same absorbance at this wave length) and 247 nm (absorption maximum of one of the drugs, atorvastatin). Both the drugs were insoluble in water and require corrosive organic solvents for solubilization. Therefore, used 2M urea by hydrotropic solubilization method. The calibration curves for both drugs were found to be linear in a concentration range of 10-60µg/ml. No significant interference was observed from the tablet excipients and 2M urea used for solubilization. The mean recovery of the drugs from the combination tablets was 100.65% for atorvastatin and 101.42% for amlodipine respectively.[76]

3. Other Analytical Method

UPLC-MS/MS method was developed by Rezk et al. (2018) for quantification of amlodipine and atorvastatin in human plasma. In this study eplerenone was used as an internal standard (IS). Multiple-reaction monitoring in positive electrospray ionization mode was utilized in Xevo TQD LC-MS/MS. Double extraction was used in sample preparation using diethyl ether and ethyl acetate. The prepared samples were analyzed using an Acquity UPLC BEH C18 (50 cm × 2.1 mm, 1.7 µm) column. Ammonium formate and acetonitrile, pumped isocraticaly at a flow rate of 0.25 ml/min. Method validation was done as per the US Food and Drug Administration guidelines. Linearity was achieved in the range of 0.1-10 ng/ml for amlodipine (AML) and 0.05-50 ng/ml for atorvastatin (ATO). Intra-day and inter-day accuracy and precision were calculated and found to be within the acceptable range.[77] Simultaneous determination of acetylsalicylic acid, hydrochlorothiazide, enalapril and atorvastatin in a polypill-based quaternary mixture by TLC was carried out by Maslanka et al. (2018). Chromatographic separation was performed using TLC silica gel 60 plates with fluorescent indicator F_{254} as the stationary phase and a mixture of *n*-hexane–ethyl acetate–methanol–water–acetic acid (8.4 + 8 + 3 + 0.4 + 0.2, v/v/v/v/v) as the mobile phase. Densitometric measurements were carried out at λ max= 210 nm when determining enalapril (ENA) and at λ max =

265 nm in the case of the other drugs. Peaks of examined substances were well separated in the recorded chromatograms, enabling the evaluation of the results in terms of both qualitative and quantitative analysis. The method was specific for the analyzed components and was characterized by high sensitivity. The LOD was between 0.043 and 0.331 µg/spot, and LOQ was between 0.100 and 0.942 µg/spot. Recovery was in the range of 97.02–101.34%. The linearity range was broad and ranged from 0.600 to 6.0 µg/spot for acetylsalicylic acid, from 0.058 to 1.102 µg/spot for hydrochlorothiazide, from 0.505 to 6.56 µg/spot for enalapril, and from 0.100 to 1.000 µg/spot for atorvastatin. The method was characterized by good precision, with RSD values that ranged from 0.10 to 2.26%.[78] Szentmiklosi et al. (2017) reported micellar electro kinetic capillary chromatography method for simultaneous determination of atorvastatin and ezetimibe in combined pharmaceutical products. The best results were obtained when using fused silica capillary (48 cm length X 50 µm ID) and 25 mM borate buffer electrolyte at pH 9.3 containing 25 mM sodium dodecyl sulfate (SDS), + 30 kV applied voltage, 20 ºC system temperature. The separation was achieved in approximately 2 minutes, with a resolution of 7.02, the order of migration being atorvastatin followed by ezetimibe.[79] Liquid chromatography–mass spectrometry (LC–MS) method for estimation of amlodipine and atorvastatin in human plasma was carried out by Danafar et al (2016). Analytes were extracted from plasma by simple liquid–liquid extraction technique using ethyl acetate. The reconstituted samples were chromatographed on C18 column by pumping acetonitrile–water (10 mM CH_3COONH_4, pH 3.0) = 70:30 (v/v) at a flow rate of 0.15 ml/min. The standard curves were assigned to be linear in the range of 0.2–20 ng/ml for atorvastatin and 0.1–10 ng/ml for amlodipine with mean correlation coefficient of ≥0.999 for each analyte. The intra-day and inter-day precision and accuracy results were well within the acceptable limits.[80] In 2015 Yang et al. reported high-throughput salting-out-assisted liquid–liquid extraction for the simultaneous determination of atorvastatin, *ortho*-hydroxy atorvastatin, and *para*-hydroxy atorvastatin in human plasma using ultrafast liquid chromatography with tandem mass spectrometry. A high-throughput, specific, and rapid liquid chromatography with tandem mass spectrometry method was established and validated for the simultaneous determination of atorvastatin and its two major metabolites, *ortho*-hydroxy atorvastatin and *para*-hydroxy atorvastatin, in human plasma. Chromatographic separation was performed on a Kinetex XB C18 column utilizing a gradient elution starting with a 60% of water solution (1% formic acid), followed by increasing percentages of acetonitrile. Analytes were detected on a tandem mass spectrometer equipped with an electrospray ionization source that was operated in the positive mode, using the transitions of *m/z* 559.3 → *m/z* 440.2 for atorvastatin, and *m/z* 575.3 → *m/z* 440.2 for both *ortho*- and *para*-hydroxy atorvastatin. Deuterium-labeled compounds were used as the internal standards. The method was validated over the concentration ranges of 0.0200–15.0 ng/ml for atorvastatin and *ortho*-hydroxy atorvastatin, and 0.0100–2.00 ng/ml for *para*-hydroxy atorvastatin with acceptable accuracy and precision.[81] In another study, Gligor et al (2015) discussed direct and simultaneous quantification of atorvastatin and amlodipine in tablets by NIR Spectroscopy. Near infrared spectroscopy (NIRS) is a technique widely used for direct and non-destructive analysis of solid samples. In this study NIRS method for the simultaneous quantification of atorvastatin and amlodipine in fixed-dose combination tablets was developed and fully validated. The partial least squares (PLS) calibration model was developed based on the 26 samples prepared according to a D-optimal experimental design with 2 factors and 5 levels. The best predictive model for atorvastatin was developed using standard normal variate pre-processing method, 7 PLS factors; the best predictive model for amlodipine was developed using first derivate followed by standard normal variate pre-processing method and 7 PLS factors. The method was validated in terms of linearity, trueness, precision and accuracy. Furthermore, comparative data obtained on independent samples showed no statistical difference ($p>0.05$) between the results predicted by the NIRS method and the values obtained using HPLC reference method.[82] LC-ESI-MS/MS method for simultaneous estimation of atorvastatin and its active metabolites in human plasma was carried out by Jadhav et al (2014). The aim of the proposed research work was to develop and validate a simple, selective and sensitive method for the analysis of atorvastatin (ATR), ortho-hydroxy atorvastatin (o-HATR) and para- hydroxy atorvastatin (o-HATR) in human plasma using liquid chromatography-tandem mass spectrometry (LC-ESI-MS/MS). All three analytes and corresponding deuterium (d5)-labeled internal standards were extracted from 500 µl of human plasma by liquid-liquid extraction (LLE) technique. An isocratic mode is used to well separate analytes and interference peaks using a CHIRALPAK IC-3 (150 x 4.6 mm, 3 µm) column using a mobile phase consisting of acetonitrile: 0.1% acetic acid (50:50, v/v). The m/z of ATR, o- HATR and p-HATR are 559.20/440.00, 575.30/440.20 and 575.30/440.00 respectively. Mass spectrometry detection was carried out in positive electrospray ionization mode, with multiple-reaction monitoring scan. Linearity ranges are 0.5 to 160 ng/ml for ATR, 0.5 to 50 ng/ml o-HATR and 0.2 to 10 ng/ml p-HATR respectively.[83] Ramadan et al (2014) carried out TLC for simultaneous determination of amlodipine (AMD), atorvastatin (AT), rosuvastatin (RSV) and valsartan (VAL) in pure form and in tablets using phenyl-modified aleppo bentonite. Study was carried out using acetonitrile: buffer (0.025M of $NaH_2PO_4.2H_2O$ in water) 45:55, v/v as mobile phase having pH 6.0. The particles of Aleppo Bentonite which have diameter less than 45 µm were treated by concentrated hydrochloric acid (BA), after that grafted firstly by dimethyldichlorosilane, then secondly by grignard reagent (phenyl magnesium bromide). The surface properties of phenyl-modified bentonite were studied by nitrogen adsorption at 77K. The retardation factors (Rf) of AMD, AT, RSV and VAL were 0.27, 0.41, 0.62 and 0.78, respectively. Linearity for determination of AMD, AT, RSV and VAL was in the range 0.50-10.00 for AMD and 1.00-20.00 µg/spot AT, RSV and VAL. The minimum determined concentration was 0.5 µg/spot for AMD and 1.0 µg/spot for AT, RSV and VAL with percent relative standard deviation (RSD%) 4.0%, 3.8%, 3.2% and 4.2%, respectively. The limits of detection (LOD) and the limits of quantification (LOQ) were found to be 0.063 and 0.19, 0.125 and 0.38, 0.106 and 0.32, 0.142 and 0.43 µg/spot for AMD, AT, RSV and VAL, respectively.[84] Baghdady (2013) reported spectrophotometric and TLC-densitometric methods for the simultaneous determination of ezetimibe and atorvastatin calcium. Three sensitive methods were developed for simultaneous determination of ezetimibe (EZB) and atorvastatin calcium (ATVC) in binary mixtures. First derivative (D1) spectrophotometry was employed for simultaneous determination of EZB (223.8 nm) and ATVC (233.0 nm) with a mean percentage recovery of 100.23 ± 1.62 and 99.58 ± 0.84, respectively. Linearity ranges were 10.00-30.00 µg/ml and 10.00-35.00 µg/ml, respectively. Isosbestic point (IS) spectrophotometry, in conjunction with second derivative (D2) spectrophotometry was employed for analysis of the

same mixture. Total concentration was determined at IS, 224.6 nm and 238.6 nm over a concentration range of 10.00–35.00 µg/ml and 5.00–30.00 µg/ml, respectively. ATVC concentration was determined using D2 at 313.0 nm (10.00–35.00 lg/ml) with a mean recovery percentage of 99.72 ± 1.36, while EZB was determined mathematically at 224.6 nm (99.75 ± 1.43).[85] Gajula et al. (2012) worked on simultaneous determination of atorvastatin and aspirin in human plasma by LC–MS/MS: Its Pharmacokinetic. The analytes were extracted from human plasma by the liquid–liquid extraction technique using methyl *tert*-butyl ether. The reconstituted samples were chromatographed on a Zorbax XDB Phenyl column by using a mixture of 0.2% acetic acid buffer, methanol, and acetonitrile (20:16:64, v/v/v) as the mobile phase at a flow rate of 0.8 ml/min. Proguanil and furosemide were used as the internal standards for the quantification of atorvastatin and aspirin, respectively. Prior to detection, atorvastatin and aspirin were ionized using an ESI source in the multiple reaction monitoring (MRM) mode. The ions were monitored at the positive *m/z* 559.2→440.0 transition for atorvastatin and the negative *m/z* 179.0→136.6 transition for aspirin. The calibration curve obtained was linear ($r^2 \geq 0.99$) over the concentration range of 0.20–151 ng/ml for atorvastatin and 15.0–3000 ng/ml for aspirin. A run time of 3.0 min for each sample made it possible to analyze more than 300 human plasma samples per day.[86] Simultaneous determination of atorvastatin, aspirin, and their degradation products in capsules with ultra HPLC was carried out by Vora et al. (2008). Separation was achieved on acquity UPLC TM BEH C18 column (2.1 × 50 mm, 1.7 µm). The mobile phase used was a mixture of acetonitrile and phosphate buffer (0.01 M, pH 2.0) and separation was achieved with flow rate 0.6 ml/min. Quantification was carried out on UV detector at 247 nm.[87] In 2008 Kadav et al. reported stability indicating UPLC method for simultaneous determination of atorvastatin, fenofibrate and their degradation products in tablets. The chromatographic separation was performed on acquity UPLC BEH C18 column (100 × 2.1 mm, 1.7 µm) using gradient elution of acetonitrile and ammonium acetate buffer (0.01 M, pH 4.7) at flow rate of 0.5 ml/min. UV detection was performed at 247 nm. Total run time was 3 min within which main compounds and six other known and major unknown impurities were separated.[88] HPTLC determination of atorvastatin in plasma was reported by Jamshidi et al. (2007). In this study, 2-step isocratic chromatography on silica gel 60F254 HPTLC layer and densitometric quantitation at λ = 280 nm was developed for the separation of atorvastatin from plasma constituencies and sodium diclofenac as peak tracer. The established HPTLC method was validated in terms of LOD/LOQ, linearity, recovery, and repeatability. The calibration function of the analyte was linear in the range 101-353.5 ng/zone and the correlation coefficient was 0.9969. The limits of detection and quantitation were 30.3 and 101 ng/zone. The recovery and relative standard deviation obtained from between-days analysis were 97.5-103.0 and 1.7-3.4%.[89] In another study, Chaudhri et al. (2006) reported HPTLC method for the simultaneous estimation of atorvastatin calcium and ezetimibe. The stationary phase used was precoated silica gel 60F254. The mobile phase used was a mixture of chloroform: benzene: methanol: acetic acid (6.0:3.0:1.0:0.1, v/v/v/v). The detection of spots was carried out at 250 nm. The method was validated in terms of linearity, accuracy, precision, and specificity. The calibration curve was found to be linear between 0.8 and 4.0 µg/spot for atorvastatin calcium and 0.1 and 1.0 µg/spot for ezetimibe. The proposed method can be successfully used for the determination of drug content of marketed formulation. [90] Later Yadav et al (2005) gave a simple and sensitive HPTLC method for the determination of content uniformity of atorvastatin calcium tablets. Precoated silica gel 60 F254 was used as stationary phase. The mobile phase used was a mixture of benzene: methanol, (7:3 v/v). Combination of benzene: methanol offered optimum migration (RF = 0.46 ± 0.02). Detection of the spots was carried out at 281 nm. The method was validated in terms of linearity (200-600 ng/spots), precision (intra-day variation: 0.25 to 1.01%, interday variation: 0.21 to 0.88%), accuracy (99.2 ± 0.48) and specificity. The proposed HPTLC method can analyze ten or a formulation unit simultaneously on a single plate and provided a faster and cost-effective quality control tool for routine analysis of atorvastatin calcium tablet formulation. [91] Farahani et al (2009) carried out quantitation of atorvastatin in human plasma using directly suspended acceptor droplet in liquid-liquid-liquid microextraction and high-performance liquid chromatography-ultraviolet detection. The methodology was based on liquid-liquid-liquid microextraction (LLLME) followed by high-performance liquid chromatography-ultraviolet detection (HPLC-UV). Atorvastatin (AT) was first extracted from 4.5 ml acidic aqueous sample (diluted plasma, donor phase, pH 1) at temperature 45 ° C through 400 µl octanol for 4.5 min, while being agitated by a stirring bar at 1250 rpm. Then, a 5.5 µl free suspended basic aqueous droplet (acceptor phase, pH 10) was delivered to the top-center position of the organic membrane. The mixture was stirred at 650 rpm for 7.5 min and the analyte was back-extracted into the droplet. Finally, the acceptor phase was taken into a micro syringe and injected directly into the HPLC. An enrichment factor of 187 along with substantial sample clean-up was obtained under the optimized conditions. The calibration curve showed linearity in the range of 1-500 ng/ml with regression coefficient corresponding to 0.996. Limits of detection and quantification were 0.4 and 1 ng/ml, respectively. A reasonable relative recovery (91%) and satisfactory intra-assay (4.4-7.0%, n=6) and inter-assay (4.9-7.7%, n=8) precision was found during analytical procedure.[92] Ashour et al. (2011) carried out a novel use of oxidative coupling reactions for determination of some statins (cholesterol-lowering drugs) in pharmaceutical formulations. All methods involved the oxidative coupling reaction of Atorvastatin (AVS), Fluvastatin (FVS) and Pravastatin (PVS) with 3-methyl-2-benzothiazolinone hydrazone hydrochloride monohydrate (MBTH) in the presence of Ce (IV) in an acidic medium to form colored products with λ(max) at 566, 615 and 664 nm, respectively. Beer's law was obeyed in the ranges of 2.0-20.0, 4.9-35.4 and 7.0-30.0 µg /ml for AVS-MBTH, FVS-MBTH and PVS-MBTH, respectively. Molar absorptivities for the above three methods were found to be 3.24×10(4), 1.05×10(4) and 0.68×10(4) l/mol/ cm respectively.[93] Al-Shehri et al. (2012) proposed an alternative capillary electrophoresis method for simultaneous determination of ezetimibe and atorvastatin in pharmaceutical formulations. Separation of both ezetimibe and atorvastatin was achieved utilizing fused silica capillary (58 cm × 75 µm ID) and background electrolyte solution that consisted of phosphate buffer (2.5 mM, pH 6.7): methanol (70:30 v/v). The method showed linearity over the range 2.5-50 µg/ml for ezetimibe ($r^2 = 0.9992$) and 1-100 µg/ml for atorvastatin ($r^2 = 0.9999$). Within-day and between-day RSD for ezetimibe and atorvastatin were found ⩽5.6% and ⩽2.9%, respectively. The detection limit was found 0.07 µg/ml for ezetimibe and 0.06 µg/ml for atorvastatin. The percentage recoveries of the two drugs from their tablets were 99.80 ± 1.76 and 100.19 ± 1.83, respectively.[94] Ali (2018) carried out high sensitivity determination of atorvastatin calcium in pharmaceuticals and biological fluids

using adsorptive anodic stripping voltammetry onto surface of ultra-trace graphite electrode. The developed adsorptive anodic stripping voltammetric method was applied for the determination of atorvastatin (ATOR) using phosphate buffer at pH 3. The low value of LOD and LOQ confirmed the sensitivity of the adsorptive anodic stripping voltammetric method.[95] LC–MS/MS study for determination of atorvastatin and aspirin in human plasma was carried out by Gajula et al. (2012). The analytes were extracted from human plasma by the liquid–liquid extraction technique using methyl *tert*-butyl ether. The reconstituted samples were chromatographed on a Zorbax XDB Phenyl column by using a mixture of 0.2% acetic acid buffer, methanol, and acetonitrile (20:16:64, v/v) as the mobile phase at a flow rate of 0.8 ml/min. Prior to detection, atorvastatin and aspirin were ionized using an ESI source in the multiple reaction monitoring (MRM) mode. The ions were monitored at the positive m/z 559.2→440.0 transition for atorvastatin and the negative m/z 179.0→136.6 transition for aspirin. The calibration curve obtained was linear ($r^2 \geq 0.99$) over the concentration range of 0.20–151 ng/ml for atorvastatin and 15.0–3000 ng/ml for aspirin.[96] Soni et al. (2014) carried out simultaneous determination of atorvastatin calcium and olmesartan medoxomil in a pharmaceutical formulation by reversed phase high-performance liquid chromatography, high-performance thin-layer chromatography (HPTLC), and UV spectrophotometric methods. The RP-HPLC separation was achieved on a Kromasil C18 column (250 x 4.6 mm, 5-micron particle size) using 0.01 M potassium dihydrogen o-phosphate (pH 4 adjusted with o-phosphoric acid)-acetonitrile (50: 50, v/v) as the mobile phase at a flow rate of 1.5 ml/min. Quantification was achieved by UV detection at 276 nm. The HPTLC separation was achieved on precoated silica gel 60F254 plates using chloroform-methanol-acetonitrile (4 + 2+ 4, v/v/v) mobile phase. Quantification was achieved with UV detection at 276 nm. The UV-Vis spectrophotometric method was based on the simultaneous equation method that involved measurement of absorbance at two wavelengths, i.e., 255 nm (lambda max of OLM) and 246.2 nm (lambda max of ATV) in methanol.[97] Simultaneous determination of atorvastatin and ezetimibe in human plasma by LC–MS–MS was carried out by El-Bagary et al. (2014). In this study pitavastatin was used as an internal standard. Liquid–liquid extraction was used for the purification and preconcentration of analytes from human plasma matrix. The chromatographic separation was achieved within 3.0 min by an isocratic mobile phase consisting of 0.2% formic acid in water-acetonitrile (30:70, v/v), flowing through Agilent Eclipse-plus C18, 100 3 4.6 mm, 3.5 mm analytical column, at a flow rate of 0.6 ml /min. Multiple reaction monitoring transitions were measured in the positive ion mode for atorvastatin and internal standard, while ezetimibe was measured in negative ion mode the standard curves were found to be linear in the range of 0.2–30.0 ng/m1 with a mean correlation coefficient >0.999 for both drugs. In human plasma, atorvastatin and ezetimibe were stable for at least 36 days at −70 ± 5 °C for 6 h at ambient temperature. After extraction from plasma, the reconstituted samples of atorvastatin and ezetimibe were found stable in an autosampler at ambient temperature for 6 h.[98] Quantification of atorvastatin, o-hydroxyatorvastatin, p-hydroxyatorvastatin, and atorvastatin lactone in rat plasma was studied by Sakac (2016) with LC–MS/MS method. The solid-phase extraction was used for preparation of samples. Rosuvastatin was chosen as an internal standard. Chromatographic separation was achieved on ZORBAX Eclipse C18 analytical, 4.6 × 100 mm (3.5 µm) column with a gradient mobile phase composed of acetonitrile and 0.1% acetic acid, at a flow rate of 400 µl/ min. The column was kept at constant temperature (25 °C), and autosampler tray temperature was set at 4 °C. The selected reaction monitoring (SRM) transitions were selected, (m/z, Q1 → Q3, collision energy) atorvastatin (559.47 → 440.03, 22 eV), atorvastatin lactone (541.36 → 448.02, 19 eV), ortho-hydroxy atorvastatin (575.20 → 440.18, 20 eV), para-hydroxy atorvastatin (575.54 → 440.18, 20 eV), and rosuvastatin (482.25 with selected combination of two fragments 257.77, 31 eV, and 299.81, 35 eV) in positive ion mode. The method was validated over a concentration range of 0.5–20 ng/ ml for ortho-hydroxy atorvastatin and para-hydroxy atorvastatin and 0.1–20 ng /ml for atorvastatin and atorvastatin lactone with excellent linearity (r2 ≥ 0.99). The detection limits were 0.1 and 0.13 ng/ ml for ortho-hydroxy atorvastatin and para-hydroxy atorvastatin, respectively, and 0.05 ng /ml for atorvastatin and atorvastatin lactone.[99] Rakibe et al. (2017) carried out UPLC, HR-MS and *in-silico* tools for simultaneous separation, characterization, and *in-silico* toxicity prediction of degradation products of atorvastatin (AT) and olmesartan (OM). AT showed labile behavior in acidic, basic, neutral, and oxidative stress and led to the formation of two degraded products, while OM degraded under acidic, basic and neutral and resulted in the formation of four degraded products (DPs). All the stressed samples of AT and OM were resolved on a C-18 column in single run on a gradient liquid chromatographic (LC) mode. A complete mass fragmentation pathway of both the drugs was established with the help of tandem mass spectrometry (MS/MS) studies. Then, the stressed samples were analyzed by LC–MS/MS to get the fragmentation patterns of DPs. LC–MS/MS data helped to propose chemical structure of all the DPs. The developed method has shown excellent linearity over the range of 10 to 150 µg/ml of OM and AT. The correlation coefficient (r^2) for OM and AT is 0.999 and 0.998, respectively. The main recovery value of OM and AT ranged from 99.97% to 100.54%, while the limit of detection (LOD) for OM and AT was 0.018 and 0.021 µg/ml, and limit of quantitation (LOQ) was found to be 0.051 and 0.063 µg/ml. Finally, the *in-silico* carcinogenicity, mutagenicity, and hepatotoxicity predictions of AT, OM, and all the DPs were performed by using toxicity prediction software's, viz., TOPKAT, LAZAR, and Discovery Studio ADMET, respectively.[100] Simultaneous determination of atorvastatin and its metabolites in human plasma by UPLC-MS/MS was carried out by Liyun et al. (2017). Atorvastatin, its metabolites, and the internal standard (IS) were isolated from human plasma by liquid–liquid extraction with ethyl acetate and then separated on an Acquity UPLC HSS T3 column (3.0 mm × 100 mm, 1.8 µm) using 0.05% (v/v) formic acid in water/acetonitrile (25: 75, v/v) as the mobile phase. Atorvastatin and all five metabolites were eluted within 4 min. Quantification was performed through positive ion electrospray ionization (ESI). The responses of atorvastatin and its metabolites *ortho*-hydroxy atorvastatin, *para*-hydroxy atorvastatin, atorvastatin lactone, *ortho*-hydroxy atorvastatin lactone, and *para*-hydroxy atorvastatin lactone were optimized at the m/z 559.4 → 440.1, m/z 575.4 → 466.2, m/z 575.5 → 440.5, m/z 541.3 → 448.3, m/z 557.3 → 448.3, and m/z 557.3 → 448.3 transitions, respectively. The assay was validated in the linear range of 0.2–40 ng/ml for atorvastatin and its metabolites. The intra- and inter-day precision variations were between 3.3% and 13.9%. The matrix effects of plasma were in the range of 102.7–105.5% for atorvastatin and 90.3–96.6% for atorvastatin lactone.[101] High performance liquid chromatography (HPLC) and high-performance thin layer chromatography (HPTLC) estimation of atorvastatin calcium and ramipril in capsule dosage forms was carried out by Panchal et al. (2010). The HPLC separation was achieved on a Phenomenex Luna C18 column

(250 x 4.6 mm id, 5 µm) in the isocratic mode using 0.1% phosphoric acid-acetonitrile (38: 62, v/v), pH 3.5 +/- 0.05, mobile phase at a flow rate of 1 ml/min. The retention times were found 6.42 and 2.86 min for atorvastatin calcium and ramipril, respectively. Quantification was achieved with a photodiode array detector set at 210 nm over the concentration range of 0.5-5 µ/ml for each, with mean recoveries (at three concentration levels) of 100.06 ± 0.49% and 99.95 ±0.63% relative standard deviation (RSD) for atorvastatin calcium and ramipril, respectively. The HPTLC separation was achieved on silica gel 60 F254 HPTLC plates using methanol-benzene-glacial acetic acid (19.6 + 80.0 + 0.4, v/v/v) as the mobile phase. The Rf values were 0.40 and 0.20 for atorvastatin calcium and ramipril, respectively. Quantification was achieved with UV densitometry at 210 nm over the concentration range of 50-500 ng/spot for each, with mean recoveries (at three concentration levels) of 99.98 ± 0.75% and 99.87 ± 0.83% RSD for atorvastatin calcium and ramipril, respectively. The mean assay percentages for atorvastatin calcium and ramipril were 99.90 and 99.55% for HPLC and 99.91 and 99.47% for HPTLC, respectively. [102]

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