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A review on liquid chromatographic methods for the bioanalysis of atorvastatin

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Abstract

Background: The unsatisfied clinical need has encouraged the development and validation of bioanalytical procedures for the quantification of drugs in biological samples because the monitoring of drug concentrations helps in personalizing the patient's pharmacotherapy, assessing the adherence to therapy, and is also extensively useful for pharmacokinetics and drug-drug interactions studies.

Main Body: The present review aimed to provide insightful information about the various liquid chromatographic methods developed till 2019 for the analysis and quantification of atorvastatin, its metabolites, and co-administered drugs in the various biological matrices like the serum, plasma, and urine with special emphasis on sample preparation techniques applied before chromatographic analysis along with different chromatographic conditions and their validation data. A total of 88 published papers that have used liquid chromatography techniques to quantify atorvastatin in biological fluids are included in the study. Out of the total reported liquid chromatographic methods, 34% used UV spectrophotometer as a detector, and 55% used MS/MS as a detector. Whereas 38% of them used protein precipitation procedure, 33% applied liquid-liquid extraction approach, and 12% employed solid-phase extraction technique for sample preparation.

Conclusion: In the last decade, numerous bioanalytical procedures have been developed for the quantification of atorvastatin in different biological samples using liquid chromatographic techniques. Moreover, advancement in technology developed several new and advanced sample preparation approaches like dispersive liquid-liquid extraction, microextraction by packed sorbent, which have high recovery rates than conventional procedures. Thus, the summarized review may be consulted as an informative tool to support the optimization of new bioanalytical methods for the quantification of atorvastatin.

Keywords: Atorvastatin, Biological fluids, Bioanalytical techniques, Liquid chromatography, Sample preparation

Background

Statins (HMG CoA reductase inhibitors) are considered as the drug of choice for the treatment of hypercholesterolemia because of their extremely beneficial effect in reducing total cholesterol and low-density lipoprotein cholesterol levels in the human body [1]. Statins competitively inhibit the HMG-CoA reductase which further depletes the intracellular supply of cholesterol [2]. Atorvastatin (AT) (Fig. 1) is one of the oldest and major drug under the class of statins, which is chemically (3R,5R)-7-2-(4-Fluorophenyl)-3-phenyl-4-(phenyl arbamoyl)-5-propan-2-ylpyrrol-1-yl-3,5-dihydroxyheptanoic acid [3] and shows the highest low-density lipoprotein-cholesterol lowering efficacy in human at a maximum daily dose of 80 mg. With a molecular formula of $C_{33}H_{15}FN_2O_5$ and a molecular weight of 558.064 g mol^{-1}, AT exists in two different forms, one is open-structured hydroxyl acid while another one is ring-structural lactone which degrades in both high alkaline and low acidic conditions [4]. AT has a significantly longer plasma half-life of 18–24 h compared to other statins [5]. AT is administered in its active acid form which afterward metabolized in its two active hydroxyl metabolites, i.e., ortho-hydroxy

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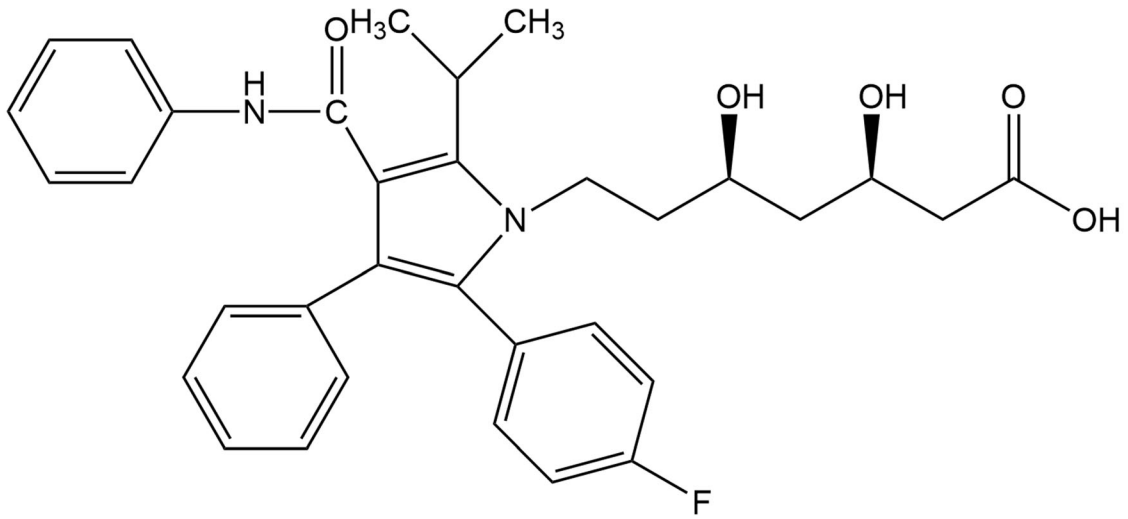

(3*R*,5*R*)-7-(2-(4-fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1*H*-pyrrol-1-yl)-3,5-dihydroxyheptanoic acid

Chemical Formula: $C_{33}H_{35}FN_2O_5$

Molecular Weight: 558.64

Fig. 1 Chemical structure of AT

atorvastatin (o-OH AT) and para-hydroxy atorvastatin (p-OH AT) and three inactive lactone metabolites [6]. Runny nose, sneezing, and coughing are the most common side effects of AT whereas muscle problems, liver problems, loss of appetite, and upper stomach pain are certain serious side effects of AT [7]. AT is also clinically used to reduce the risk of stroke [8]; myocardial infarction [9], which further reduces the risk for angina [10]; and revascularization procedures [11].

Although plasma drug monitoring is not crucial for the AT, an increasing number of patients taking AT have drawn the attention of healthcare professionals for mandatory monitoring of plasma concentration in various conditions. Moreover, monitoring the plasma concentrations of AT helps personalize the patient's pharmacotherapy and to assess the adherence to therapy. Since AT is also co-administered with many other drugs as polypharmacy therapy, the bioanalytical assays are also extensively used for its pharmacokinetics and drug-drug interactions' studies. Thus, the unsatisfied clinical need has encouraged the development and validation of several liquid chromatography methods as bioanalytical procedures for the determination of AT in body fluids with high accuracy, precision, and better reproducibility. The present review encapsulates all reported liquid chromatography-based bioanalytical procedures available in the literature that are used for the quantification of AT alone, with its metabolites and with co-administered drugs in different biological samples. Also, a succinct tabular outline of the bioanalytical procedures has been included in the review which consists of chromatographic conditions, sample preparation strategies, validation data, etc.

Main text

Indeed, the best approach for the accurate analysis of drug concentration in biological samples is the use of high-resolution liquid chromatographic techniques such as HPLC and UPLC coupled with UV spectrophotometer or MS/MS detectors. Liquid chromatography techniques are not only considered as an important tool to support the therapeutic drug monitoring of drugs in the biological fluids but also during quality control of pharmaceutical formulations as well as during non-clinical and clinical drug development. From 1998 to 2019, different liquid chromatographic procedures to analyze AT in biological fluids were developed, validated, and applied in pharmacokinetic studies and therapeutic drug monitoring. Out of the total reported liquid chromatographic methods, 34% used UV spectrophotometer as a detector, and 55% used MS/MS as a detector.

Analysis using HPLC

HPLC is the preferable method for the analysis of drugs and currently the widest accepted method for separation technique. Table 1 [12–51] enlist the various HPLC methods coupled with UV, DAD, and PDA detectors used to detect AT and co-administered drugs in biological fluids. Usually, the reverse phase HPLC method

Table 1 Reported analytical method for the estimation of AT and co-administered drugs in biological fluids using the HPLC technique

Analyte(s)	Biological matrix	Sample preparation	Mode of elution; mobile phase	Stationary phase	Flow rate (mL min^{-1})	Detection	R_t (min)	Calibration range	LOD	LOQ	% Recovery	Ref.
AT and curcumin	Mouse plasma	PP	Isocratic elution; ACN:MeOH:2% (v/v) AcA (37.5:2.5:60, v/v/v)	Purospher STAR RP18 endcapped column (55 × 4 mm, 3 μm)	1.0	DAD247 nm	9.4	600–50,000 ng mL^{-1} r^2 = 0.9992	66.70 ng mL^{-1}	----	91.28%	[12]
	Lung homogenate supernatant							600–50,000ng mL^{-1} r^2 = 0.9992	85.70 ng mL^{-1}	----	99.83%	
	Brain homogenate supernatant							100–50,000 ng mL^{-1} r^2 = 0.9978	0.80 ng mL^{-1}	----	101.57%	
	Liver homogenate supernatant							100–15,000 ng mL^{-1} r^2 = 0.9979	25.00 ng mL^{-1}	----	89.82%	
	Spleen homogenate supernatant							600–50,000 ng mL^{-1} r^2 = 0.9970	300.00 ng mL^{-1}	----	89.08%	
Rosuvastatin, gemfibrozil, and AT	Human plasma	MDμ-SPE-SSME	Isocratic elution; ACN:sodium phosphate buffer (pH 3; 0.05 M) (59:41, v/v)	C18 column (250 × 4.6 mm, 5 μm)	1.0	UV230 nm	≈9.0	30–2000 ng mL^{-1} r^2 = 0.9930	10.00 ng mL^{-1}	9.70 ng mL^{-1}	98.75%	[13]
	Human Urine							25–2000 ng mL^{-1} r^2 = 0.9910	8.00 ng mL^{-1}	8.70 ng mL^{-1}	99.55%	
AT and clopidogrel	Human plasma	PP	Isocratic elution; KH_2PO_4 buffer (pH 2.5): ACN (50:50, v/v)	C8 column (250 × 4.6 mm, 5 μm)	1.3	PDA243 nm	10.3	10–50 μg mL^{-1} r^2 = 0.9974	----	10.40 μg mL^{-1}	97.80%	[14]
AT, clopidogrel and Aspirin	Rat plasma	PP	Gradient elution; A = Dibasic phosphate buffer (pH 3.0;10 mM), B = ACN	Beta-basic C18 column (250 × 4.6 mm, 5 μm).	1.0	UV232 nm	16.2	10–10,000 ng mL^{-1} r^2 = 0.9990	----	10.00 ng mL^{-1}	92.85%	[15]
AT	Human plasma	DLLME-SFO	Isocratic elution; MeOH:water (70:30,v/v) (pH[a]3.0 with Sodium phosphate buffer)	Shim-pack CLC-C18 column (150 × 4.6 mm, 5 μm)	1.5	UV	≈1.7	0.2–6000 μg mL^{-1} r^2 = 0.9950	0.07 μg mL^{-1}	----	98.80–113.80%	[16]
AT and pioglitazone	Rat plasma	LLE	Isocratic elution; MeOH:ACN:AF (pH 3.5; 1 mM) (48:19:33 v/v/v)	Gemini C18 column (250 × 4.6 mm, 5 μm)	1.0	DAD260 nm	11.3	6–300 ng mL^{-1} r^2 = 0.9967	3.00 ng mL^{-1}	6.00 ng mL^{-1}	74.03%	[17]
AT	Human serum	SALLE	Isocratic elution, AA buffer:ACN (50:50, v/v) (pH[a]3.0 with o-phosphoric acid)	Symmetric C18 column (250 × 4.6 mm, 5 μm)	1.0	UV246 nm	----	1–10,000 ng mL^{-1} r^2 = 0.9980	0.50 ng mL^{-1}	----	99.50%	[18]
AT micro-emulsions	Rat plasma	PP	Isocratic elution; MeOH:water (70:30, v/v) (0.05% glacial AcA)	C18 column (250 × 4.6 mm, 5 μm)	1.0	UV248 nm	5.6	100–50,000 ng mL^{-1} r^2 ≥ 0.9854	20.00 ng mL^{-1}	50.00 ng mL^{-1}	91.16%	[19]

Table 1 Reported analytical method for the estimation of AT and co-administered drugs in biological fluids using the HPLC technique *(Continued)*

Analyte(s)	Biological matrix	Sample preparation	Mode of elution; mobile phase	Stationary phase	Flow rate (mL min^{-1})	Detection	R_t (min)	Calibration range	LOD	LOQ	% Recovery	Ref.
AT, rosuvastatin	Rat plasma	LLE	Isocratic elution; MeOH:water (68:32, v/v) (pH^a 3.0)	BDS Hypersil C18 column (250 × 4.6 mm, 5 μm)	1.0	UV241 nm	11.3	20–200 ng mL^{-1} r^2 = 0.9920	1.30 ng mL^{-1}	10.30 ng mL^{-1}	96.48%	[20]
AT	Human serum	LLE	Isocratic elution; 10% MeOH in sodium phosphate buffer (pH 3.5; 0.05 M with o-phosphoric acid): MeOH (43:57, v/v)	Ascentis C18 column (250 × 4.6 mm, 5 μm)	1.2	UV247 nm	19.8	3–150 ng mL^{-1} r^2 = 0.9990	1.20 ng mL^{-1}	3.00 ng mL^{-1}	85.10%	[21]
Pioglitazone, gliquidone, and AT	Human serum	PP	Isocratic elution; MeOH:water (90:10, v/v) (pH^a3.50 with o-phosphoric acid)	Purospher Star RP18 endcapped column (250 × 4.6 mm, 5 μm),	1.0	UV235 nm	3.6	5–50 μg mL^{-1} r^2 = 0.9989	0.60 μg mL^{-1}	1.90 μg mL^{-1}	98.80–104.3%	[22]
AT, Losartan, atenolol, and aspirin	Plasma	PP	Isocratic elution; ACN:KH_2PO_4 (pH 3.4; 0.02 M) (70:30, v/v)	HiQ Sil C18HS column (250 × 4.6 mm, 5 μm)	1.0	UV236 nm	5.2	25–150 ng mL^{-1} r^2 = 0.9997	----	25.00 ng mL^{-1}	99.46%	[23]
AT and simvastatin	Human plasma	SPE, DLLME	Isocratic elution; AA solution (pH 3.5; 0.02 M): ACN (25:75, v/v)	Luna C18 column (150 × 4.6 mm, 5 μm)	0.7	DAD254 nm	----	45–900 ng mL^{-1} r^2 = 0.9990	25.00 ng mL^{-1}	75.00 ng mL^{-1}	90.00%, 83.00%	[24]
AT	Human plasma	PP	Isocratic elution; MeOH:ACN:Sodium phosphate buffer (pH 4.5; 0.01 M) (40:30:30,v/v/v)	Shim-Pak C18 column (250 × 4.6 mm, 5 μm).	1.0	UV247 nm	----	5–160 ng mL^{-1}	7.80 ng mL^{-1}	22.90 ng mL^{-1}	98.70%	[25]
AT and fenofibrate	Rabbit plasma	PP	Isocratic elution; KH_2PO_4:ACN (28:72, v/v) (pH^a 4.1)	Capcell Pak C8 DDS5 column (250 × 4.6 mm, 5 mm)	1.0	UV260 nm	4.2	1–40 μg mL^{-1} r^2 = 0.9993	0.05 μg mL^{-1}	0.20 g mL^{-1}	97.04%	[26]
AT and rosuvastatin	Human serum	LLE	Isocratic elution; MeOH:water (68:3,v/v) (pH^a 3.0)	Brownlee analytical C18 column (150 × 4.6 mm, 5 μm)	1.5	UV241 nm	6	3–384 ng mL^{-1} r^2 = 0.9990	1.00 ng mL^{-1}	3.00 ng mL^{-1}	98.20%	[27]
Lisinopril, AT, pravastatin, and rosuvastatin	Human Serum	PP	Isocratic elution; MeOH:water: ACN (80:17.5:2.5, v/v/v) (pH^a3.0)	Purospher STAR C18 column (250 × 4.6 mm, 5 μm)	1.0	UV225 nm	8.3	625–25,000 ng mL^{-1} r^2 = 0.9994	1.30 ng mL^{-1}	4.10 ng mL^{-1}	100.86%	[28]
Fluvastatin, pravastatin, and AT	Human plasma	PP	Isocratic elution; ACN:KH_2PO_4 (60:40, v/v) (pH^a 3.5 by o-phosphoric acid)	ZORBAX Extend-C18 column (150 × 4.6 mm, 5 μm)	1	UV210 nm	2.4	5–40 μg mL^{-1} r^2 = 0.9990	2.5 μg mL^{-1}	4.00 μg mL^{-1}	99.80%	[29]
AT and gemfibrozil	Human plasma	PP	Isocratic elution; AA buffer (pH 5.0; 0.1 M):ACN (45:55, v/v)	C18 column (250 × 4.6 mm, 5 μm)	1.0	PDA240 nm	3.4	1–20 μg mL^{-1} r^2 = 0.9997	30.00 ng mL^{-1}	0.10 μg mL^{-1}	93.54%	[30]
AT	Human plasma	LLLME	Isocratic elution; ACN:0.1% AcA (70:30, v/v)	ODS-3 column (150 × 4.6 mm, 5 μm)	1.0	UV246 nm	2.7	1–500 ng mL^{-1} r^2 = 0.9960	0.40 ng mL^{-1}	1.00 ng mL^{-1}	22.90%	[31]
AT	Beagle dog plasma	PP	Isocratic elution; ACN:AA buffer (pH 4; 0.1 M) (65:35, v/v)	Kromasil C8 column (150 × 4.6 mm, 5 mm)	1.0	UV270 nm	6.6	50–2500 ng mL^{-1} r^2 = 0.9995	8.00 ng mL^{-1}	25.00 ng mL^{-1}	91.30%	[32]

Table 1 Reported analytical method for the estimation of AT and co-administered drugs in biological fluids using the HPLC technique *(Continued)*

Analyte(s)	Biological matrix	Sample preparation	Mode of elution; mobile phase	Stationary phase	Flow rate (mL min^{-1})	Detection	R_t (min)	Calibration range	LOD	LOQ	% Recovery	Ref.
AT	Human plasma	PP	Isocratic elution; NaH_2PO_4 (0.01 M):ACN (60:40, v/v) (pH[a]5.5)	Nucleosil-100 C8 column (125 × 4 mm, 5 μm)	1.5	UV245 nm	3.6	20–800 ng mL^{-1} r^2 = 0.9998	1.00 ng mL^{-1}	----	100.20%	[33]
AT	Human serum	LLE	Isocratic elution; sodium phosphate buffer (pH 4; 0.05 M):MeOH (33:67, v/v)	Shim-pack CLC- C18 column (100 × 4 mm, 5 μm)	2.5	UV247 nm	3.4	4–256 ng mL^{-1} r^2 ≥ 0.9965	1.00 ng mL^{-1}	4.00 ng mL^{-1}	95.00 ± 4.00%	[34]
Metformin, amlodipine, glibenclamide, and AT	Human plasma	PP	Gradient elution; A = 0.1% o-phosphoric acid (pH 3.0), B = ACN	Novapack Phenyl column (150 × 4.6 mm, 5 μm)	----	UV 227 nm	11.8	100–5000 ng mL^{-1}	----	1.00 ng mL^{-1}	91.19%	[35]
AT	HumanPlasma	PP	Isocratic elution; ACN:MeOH: water (45: 45:10, v/v/v)	RP C18 column (150 × 4.6 mm, 5 mm)	1.0	UV240 nm	1.9	500–86,000 ng mL^{-1} r^2 = 0.9980	8.40 ng mL^{-1}	17.90 ng mL^{-1}	98.90%	[36]
AT	Human plasma	MSPE	Isocratic elution; ACN:o-phosphoric acid (pH 6;10 mM) (75:25, v/v)	C18 column (250 × 4.6 mm, 5 μm)	1.0	Fluorimetric282 nm, 400 nm	4.4	30–120 ng mL^{-1} r^2 = 0.9996	10.00 ng mL^{-1}	30.00 ng mL^{-1}	101.35%	[37]
AT and valsartan	Human serum	Solid based DLLE	Isocratic elution; sodium acetate buffer (pH 4.0; 0.02 M):ACN: MeOH (60:20:20, v/v/v)	Spherisorb C18 column (25 × 4.6 mm, 5 μm)	0.7	DAD210 nm	8.0	10–5000 ng mL^{-1} r^2 = 0.9970	2.60 ng mL^{-1}	8.20 ng mL^{-1}	81.00%	[38]
AT	Rat plasma	PP	Isocratic elution; ACN:water (pH 3.3): MeOH (50:40:10, v/v/v)	Knalier Vertex Plus C18 (250 × 4.6 mm, 5 μm)	0.9	UV240 nm	9.1	10–12,000 ng mL^{-1} r^2 = 0.9998	0.70 ng mL^{-1}	2.20 ng mL^{-1}	102.48%	[39]
	Intestinal perfusionsolution							10–12,000 ng mL^{-1} r^2 = 0.9988	1.00 ng mL^{-1}	2.90 ng mL^{-1}	102.02%	
AT and amlodipine	Rat plasma	PP	Isocratic elution; dibasic phosphate buffer(pH 3.0): ACN (55:45, v/v)	Beta-basic C18 column (100 × 4.6 mm, 5 μm)	1.0	UV240 nm	12.1	0.05–10 μg mL^{-1} r^2 = 0.9990	----	0.30 μg mL^{-1}	92.80%	[40]
Clopidogrel, metabolite, and AT	Human plasma	PP	Gradient elution; A = sodium phosphate buffer (pH 2.6; 10 mM), B = ACN, C = MeOH	BDS Hypersil C18 column (250 × 4.6 mm, 5 μm)	1.0	PDA220 nm	10.9	5–2500 ng mL^{-1} r^2 = 0.9994	2.00 ng mL^{-1}	5.00 ng mL^{-1}	94.50%	[41]
AT and ezetimibe	Human plasma	PP	Isocratic elution, KH_2PO_4 (pH 3.5; 0.05 M adjusted with o-Phosphoric acid): ACN (40:60, v/v)	X-Terra C8 column (150 × 4.6 mm, 3.5 μm)	1.2	PDA235 nm	3.5	5–25 μg mL^{-1} r^2 = 0.9940	----	1.30 μg mL^{-1}	100.42%	[42]
AT	Human plasma	Dμ- SPE	Isocratic elution; MeOH:ACN:water (76: 13:11, v/v/v)	Knauer C18 column (250 × 4.6 mm, 5 μm)	1.0	UV253 nm	----	0.3–2000 ng mL^{-1} r^2 = 0.9996	0.06 ng mL^{-1}	0.20 ng mL^{-1}	101.80%	[43]
AT	Human plasma	Ultrasound-assisted MSPE	Isocratic elution; ammonium dihydrogen phosphate buffer (pH 3.0): ACN (50:50, v/v)	Teknokroma C18 column (250 × 4.6 mm, 5 μm)	1.5	UV246 nm	5.0	0.4–500 ng mL^{-1} r^2 ≥ 0.9990	0.10 ng mL^{-1}	----	94.58%	[44]

Table 1 Reported analytical method for the estimation of AT and co-administered drugs in biological fluids using the HPLC technique *(Continued)*

Analyte(s)	Biological matrix	Sample preparation	Mode of elution; mobile phase	Stationary phase	Flow rate (mL min^{-1})	Detection	R_t (min)	Calibration range	LOD	LOQ	% Recovery	Ref.
AT and glimepiride	Human serum	LLE	Isocratic elution; ACN: water (containing 1% v/v triethylamine) (55:45, v/v/v) (pH[a] 5.6 with o-phosphoric acid)	Inertsil C18 column (250 × 4.6 mm, 5 μm)	1.0	UV230 nm	6.9	1–1600 ng mL^{-1} r^2 = 0.9960	----	1.00 ng mL^{-1}	94.83%	[45]
AT and Lercanidipine	Rat plasma	PP	Isocratic elution; ACN:AA buffer (pH 3.5; 0.1 M) (50:50, v/v)	Wakosil II C18 column (250 × 4.6 mm, 5 μm)	1.2	UV235 nm	10.2	0.05–40 μg mL^{-1} r^2 = 0.9990	----	----	89.00-94.00%	[46]
AT and valsartan	Human serum	MDμ- SPE	Isocratic elution; MeOH:ACN: Sodium phosphate buffer (pH 3.0) (40:25:35, v/v/v)	Licrosorb RP-18 column (100 × 4.6 mm, 3.5 μm)	1.0	UV240 nm	----	10–2000 ng mL^{-1} r^2 = 0.9982	2.40 ng mL^{-1}	8.00 ng mL^{-1}	92.83%	[47]
AT	Rat plasma	PP	Isocratic elution; MeOH:water (70:30, v/v/v) (pH[a] 5.5)	C18 column (250 mm × 4.6 mm, 5 μm)	----	UV	4.9	50–1000 ng mL^{-1} r^2 = 0.9973	10.0 ng mL^{-1}	15.0 ng mL^{-1}	93.55%	[48]
AT	Human serum	MD- SPE	Isocratic elution; ACN:water (45:55, v/v)	Licrosorb RP 18 column (100 × 4.6 mm, 3.5 μm).	1.0	DAD	----	1–1000 ng mL^{-1} r^2 = 0.9973	0.10 ng mL^{-1}	0.40 ng mL^{-1}	96.03%	[49]
Captopril, rosuvastatin, simvastatin, and AT	Human serum	PP	Isocratic elution; ACN:water (60:40, v/v) (pH[a] 2.9 with phosphoric acid)	Purospher STAR C18 column (250 × 4.6 mm, 5 mm)	1.5	UV230 nm	≈ 3.9	625–25,000 ng mL^{-1} r^2 = 0.9995	5.87 ng mL^{-1}	17.80 ng mL^{-1}	99.76%	[50]
Rosuvastatin, gemfibrozil, and AT	Human plasma	TAALLME-SFO	Isocratic elution; ACN:Sodium phosphate buffer (pH 3; 0.05 M) (61:39, v/v)	ODS III column (250 × 4.6 mm, 5 mm)	1.0	UV230 nm	----	2.5–3000 ng mL^{-1} r^2 = 0.9950	0.80 ng mL^{-1}	----	101.00%	[51]

[a]The pH of the mixed solvent system

has been used to quantify AT using C18 analytical columns because of the presence of pyrrole and phenyl as aromatic functional groups that make analysis suitable by the reverse phase method [52]. However, some published work has used the C8 column as the stationary phase. The mobile phase that has been used in the quantification of AT in biological matrices, primarily comprised of acetonitrile (ACN), methanol (MeOH), water, and different buffer solutions that have either isocratic or gradient elution with a flow rate of 1 mL min^{-1}. Retention of analyte in the analytical column hinge upon the pH of elution, so in reported bioanalytical assays, pH of the mobile phase has been maintained between 2 and 4 to make better retention of analyte in the analytical column and enhanced the resolution of the peak because these pH values are lesser than pKa value of AT and AT remain in unionized form for a longer time and interact more with the stationary phase [52]. The retention time (R_t) of AT reported in Table 1 variegates from 1.7 to 19.8 min, but in most of the developed HPLC methods, R_t has ranged 2 to 5 min. The wavelength of UV detector used to detect AT has ranged from 210 to 270 nm, but commonly, 247 nm has been used as detection wavelength. Conversely, Tekkeli et al. quantify AT in human plasma using fluorimetric detection at excitation and an emission wavelength of 282 and 400 nm, respectively [37]. For the accuracy in quantification, diclofenac [20, 26, 31, 34] and ibuprofen [29, 39] have been often employed as internal standards in various developed HPLC methods to determine AT. Considering the validation of developed methods, all the developed methods have been validated as per ICH Q2 (R1) guidelines for system suitability, sensitivity, selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), and inter-day and intra-day accuracy and precision. LOD of the AT has been determined based using signal intensity to baseline noise ratio (S/N) of 3:1 by comparing test results from samples of a known concentration of analyte with the blank sample while LOQ determination has been based on signal intensity ten times more than baseline noise, i.e., S/N = 10. Least LOQ and LOD value of 0.2033 ng mL^{-1} and 0.0608 ng mL^{-1} respectively were observed in the method proposed by Dastkhhon et al. [43]. whereas the method developed by Gholami and Ahmadi was highly sensitive with LOQ value of 0.38 ng mL^{-1} and LOD value of 0.10 ng mL^{-1} with linearity in concentration calibration range of 1 to 1000 ng mL^{-1} and %RSD less than 4.2% [49].

Analysis using LC-MS/MS and UPLC-MS/MS

The liquid chromatography-based bioanalytical procedures using MS/MS detectors are listed in Table 2 [53–100]. On the other hand, few studies used UPLC MS/MS method for the estimation of AT in the biological fluids and are also indexed in Table 2 [55, 56, 65, 71, 74, 81, 87, 91, 94, 95, 100]. Similar to HPLV-UV methods, C18 analytical columns have been extensively used in LC-MS/MS assay as a stationary phase because their long alkyl chain provides more efficient separation of nonpolar compounds in MS/MS detection [101], whereas a column packed with bridge ethyl hybrid (BEH) has been used in UPLC method as a stationary phase [71, 81, 87, 95] because of their high mechanical resistance [102]. Although MeOH, ACN, and water are used as mobile phases for quantification in LC-MS/MS, certain additives like formic acid (FA), ammonium acetate (AA), and acetic acid (AcA) have been added in the mobile phase to enhance ionization which further increases the sensitivity of the method. Isocratic elution has been predominantly preferred over gradient elution in the analysis of AT with an elution rate of 0.2–0.5 mL min^{-1}. R_t has found to be quite small, ranged between 0.66 and 6.3 min. AT and its metabolites can be detected in both positive and negative ionization modes of MS, yet most of the published work has performed LC-MS/MS analyses of AT in positive ionization mode using electrospray ionization (ESI), but few reported studies used negative ions [54, 56, 58, 67, 80, 94] of AT for the analysis even though, polarity switching within run is required in ESI^- mode [89]. On the other hand, the atomic pressure chemical ionization (APCI) technique has been used by Tahboub for ionization of AT in positive mode [64]. Figures 2 and 3 represent the product ion spectra of AT and metabolites in both ESI^+ and ESI^- modes respectively. Interestingly, a study by Partani et al. concluded that analysis in ESI^- mode gives quite low LOQ value because the negative ions enhance the selective detection and also improve the sensitivity of the method [67]. Principally, quantification of AT has been carried out using multiple reaction monitoring (MRM) transition with the precursor ion $M+H^+$ at m/z 559 Da and product ion at m/z 440 Da, while SRM transition has also been employed in numerous ESI^+ methods with transitions at m/z 559 → 440 [52, 65, 70, 81, 88, 89]. Importantly, Jang et al. utilized the MRM transition of m/z 559.3 → 250.2 for the detection of AT in the human urine samples [92]. Negative ions of AT have been analyzed at m/z 557.4 → 278.1 in the MRM transition [56, 58, 67] but in some ESI^- mode quantification of AT has been carried out with product ion at m/z 397 [56, 60, 80] while AT was detected using MRM transition 557.0 → 453.0 in the bioanalytical method proposed by Xia et al. [94]. Interestingly, Varghese and Kochupappy Ravi [53] and Ma et al. [84] performed the analysis of AT using LC–ESI–MS in selected ion monitoring (SIM) mode at m/z 559. In most of the LC-MS/MS assays, either deuterium-labeled analogs of AT (d_5-AT) or structural analogs of AT such as rosuvastatin [53, 55, 58, 66, 86, 94], pitavastatin [66, 84], and pravastatin [70, 80] have been selected as internal standards to get most accurate results.

Table 2 Reported analytical method for the estimation of AT, its metabolites, and co-administered drugs in biological fluids using LC-MS/MS technique

Analyte(s)	Biological matrix	Sample preparation	Mode of elution; mobile phase	Stationary phase	Flow rate (mL min^{-1})	Detection	R_t (min)	Calibration range	LOD	LOQ	% Recovery	Ref.
AT and telmisartan	Human plasma	LLE	Isocratic elution; AA (pH 4;10 mM): MeOH (20:80, v/v)	Luna C18 (150 × 4.6 mm, 5 μm)	0.4	MS ESI^+SIM559	3.8	1–35 ng mL^{-1} r^2 = 0.9930	----	1.00 ng mL^{-1}	81.48%	[53]
AT, fluvastatin, simvastatin, pravastatin, rosuvastatin, lovastatin, pitavastatin, and metabolites	Human plasma	LLE	Gradient elution; A = water containing 1% ACN, 0.1% FA,2 mM AF, B = ACN containing 1% water, 0.1% FA, 2 mM AF	Hypersil Gold C18 column (100 × 2.1 mm, 1.9 μm)	0.7	HRMS/MS$HESI^+$/$HESI^-$ SRM559 → 440	4.5	1–100 ng mL^{-1}	----	1.00 ng mL^{-1}	----	[54]
AT	Human plasma	LLE	Gradient elution; A = 0.2 %FA, B = ACN	Acquity UPLC BEH C18 column (100 × 2.1 mm, 1.7 μm)	0.3	MS/MS ESI^+MRM559.05 → 440	----	0.2–100 ng mL^{-1} r^2 = 0.9998	----	----	----	[55]
AT, simvastatin, fluvastatin, lovastatin, pitavastatin, rosuvastatin, pravastatin, and metabolites	Human serum	PP-MEPS	Gradient elution; A = ACN, B = AA (0.5 mM)	Acquity UPLC BEH C18 column (50 × 2.1 mm, 1.7 μm)	0.3	MS/MS ESI^-SRM557.2 → 278.2	2.5	5–1000 ng mL^{-1} r^2 = 0.999	----	10.00 ng mL^{-1}	----	[56]
					0.5	MS/MS ESI^-SRM557.2 → 278.2	4.4	0.5–500 ng mL^{-1} r^2 = 0.9984	----	1.00 ng mL^{-1}	----	
					0.5	MS/MS ESI^-SRM557.2 → 397	4.4	0.1–100 ng mL^{-1} r^2 = 0.9993	----	1.00 ng mL^{-1}	----	
AT, o-OH AT, p-OH AT, and AT Lactone	Rat plasma	SPE	Gradient elution; A = ACN, B = 0.1% AcA	ZORBAX Eclipse C18 Analytical column (100 × 4.6 mm, 3.5 μm)	0.4	MS/MS ESI^+SRM559.47 → 440.03	4.5	0.1–20 ng mL^{-1} r^2 = 0.9982	0.05ng mL^{-1}	----	----	[57]
AT, o-OH AT, and p-OH AT	Human plasma	LLE	Isocratic elution; 0.3% FA in water: 0.3% FA in ACN (50:50, v/v)	Purospher STAR RP 18 endcapped column (55 × 2 mm, 3 μm)	0.5	MS/MS ESI^-557.4 → 278.1	2.4	1.99–80.52 ng mL^{-1} r^2 = 0.9954	----	2.04 ng mL^{-1}	82.87%	[58]
AT and amlodipine	Human plasma	LLE	Isocratic elution; ACN:AA buffer (pH 3.0;10 mM) (70:30, v/v)	ZORBAX XDB-C18 column (30 × 2.1 mm, 3.5 μm)	0.15	MS/MS ESI^+MRM559.3 → 440.2	3.8	0.2–20 ng mL^{-1} r^2 = 0.9990	0.10ng mL^{-1}	0.20 ng mL^{-1}	94.56%	[59]
AT, o-OH AT, and p-OH AT	Human plasma	LLE	Isocratic elution; ACN:0.20% FA (65:35, v/v)	Luna C18 column, 100 × 4.6 mm, 5 μm)	0.6	MS/MS ESI^-MRM557.3 → 397.1	3.7	0.2–202 ng mL^{-1} r^2 ≥ 0.9969	----	**----**	76.14%	[60]
Simvastatin, lovastatin, AT, and metabolites	Human plasma	PP	Gradient elution; A = Water, B = ACN (0.2% FA and 2 mM AF)	ZORBAX Extend C18 column (50 × 2.1 mm, 3.5 μm)	0.4	MS/MS ESI^+SRM559.2 → 440.2	8.4	0.1–100 nMr^2 ≥ 0.9950	----	0.10 nM	102.08%	[61]
AT, o-OH AT, and p-OH AT	Human plasma	SALLE	Gradient elution; A = water (1%FA), B = ACN	Kinetex XB C18 column (50 × 2.1 mm, 2.6 μm)	0.8	MS/MS ESI^+MRM559.3 → 440.2	2.2	0.02–15 ng mL^{-1} r^2 = 0.9986	----	0.02 ng mL^{-1}	87.93%	[62]
AT and olmesartan	Human	LLE	Isocratic elution; ACN:MeOH:0.1% FA	Eclipse Plus C18	0.5	MS/MS	2.0	2–80 ng	0.19ng	0.60	80.26%	[63]

Table 2 Reported analytical method for the estimation of AT, its metabolites, and co-administered drugs in biological fluids using LC-MS/MS technique *(Continued)*

Analyte(s)	Biological matrix	Sample preparation	Mode of elution; mobile phase	Stationary phase	Flow rate (mL min^{-1})	Detection	R_t (min)	Calibration range	LOD	LOQ	% Recovery	Ref.
	plasma		(80:10:10, v/v/v)	column (75 × 4.6 mm, 3.5 μm)		ESI^+MRM559.1 → 440.2		$mL^{-1}r^2$ = 0.9950	mL^{-1}	ng mL^{-1}		
AT and other cardio vascular drug	Human plasma	PP	Gradient elution; A = 0.1% (v/v) aqueous FA with 1.0 mM AF), B = 0.1 % (v/v) FA with 1.0 mM AF in ACN	C18 column (50 × 4.6 mm, 5 μm)	0.6	MS/MS $APCI^+$MRM559.6 → 440.3	5.5	----	----	7.20 ng mL^{-1}	90.50%	[64]
AT, metoprolol, amlodipine, pravastatin, rosuvastatin, and metabolites	Human plasma	PP	Gradient elution; A = water with 0.1% FA, B = ACN with 0.1% FA	Acquity UPLC CSH column (50 × 2.1 mm, 1.7 μm)	0.5	MS/MS ESI^+SRM559 → 440	2.5	0.2–500 ng $mL^{-1}r^2$ ≥ 0.9900	0.30 ng mL^{-1}	----	80.00–103.00%	[65]
AT and ezetimibe	Human plasma	LLE	Isocratic elution; 0.2% FA in water:ACN (30:70, v/v)	Eclipse-plus C18 column (100 × 4.6 mm, 3.5 μm)	0.6	MS/MS ESI^+ MRM559 → 440	2.7	20–3000 ng $mL^{-1}r^2$ = 0.9998	----	----	76.32%	[66]
AT, o-OH AT, and p-OH AT	Human plasma	SPE	Isocratic elution; 0.005% FA in water: ACN:MeOH (35:25:40, v/v/v)	Ascentis Express C18 column (75 × 4.6 mm, 2.7 μm)	06	MS/MS ESI^- MRM557.4 → 278.1	4.1	0.05–100.138 ng $mL^{-1}r^2$ = 0.9991	----	0.05 ng mL^{-1}	77.40%	[67]
Glimepiride, metformin, and AT	Human plasma	PP	Isocratic elution; ACN: AA (pH 3.0;10 mM) (60:40, v/v)	Alltima HP C18 HL column (50 × 4.6 mm, 3 μm)	1.1	MS/MS ESI^+MRM559.5 → 440.4	1.0	0.5–150.53 ng $mL^{-1}r^2$ ≥ 0.9900	----	1.50 ng mL^{-1}	96.72%	[68]
Amlodipine, AT, o-OH AT, and p-OH AT	Human plasma	LLE	Isocratic elution; ACN: AA buffer (20 mM) (50:50, v/v) (0.3% FA mixed)	Cap- cellpak CR 1: 4 column (150 × 2 mm, 5 μm).	0.45	MS/MS ESI^+MRM559.42 → 440.25	5.4	0.035–25 ng $mL^{-1}r^2$ = 0.9966	----	0.04 ng mL^{-1}	80.26%	[69]
Amlodipine, AT, o-OH AT, and p-OH AT	Human plasma	PP	Isocratic elution; water:MeOH (14:86, v/v) (pH^a 3.2 with TCA)	Synergi 4 μm polar-RP 80Å column (150 × 4.6 mm, 4 μm)	0.5	MS/MS ESI^+SRM559.09 → 440.21	5.5	1.5–150 ng $mL^{-1}r^2$ = 0.9999	----	1.50 ng mL^{-1}	103.47%	[70]
AT and ezetimibe	Human plasma	LLE	Gradient elution; A = 0.1% FA in water, B = ACN	Acquity UPLC BEH C18 column (50 × 2.1 mm, 1.7 μm)	0.7	MS/MS ESI^+MRM559.5 → 440.4	1.0	0.1–20 ng $mL^{-1}r^2$ = 0.9990	----	0.10 ng mL^{-1}	95.30%	[71]
AT and aspirin	Human plasma	LLE	Isocratic elution; 0.2% AcA buffer: MeOH:ACN (20:16:64, v/v/v)	ZORBAXXDB Phenyl column (75 × 4.6 mm, 3.5 μm)	0.8	MS/MS ESI^+MRM559.2 → 440	1.3	0.2–151 ng $mL^{-1}r^2$ = 0.9900	----	0.60 ng mL^{-1}	82.30%	[72]
AT and glimepiride	Human plasma	LLE	Isocratic elution; 0.1% FA:ACN (30:70, v/v)	ACE 5 C18 column (50 × 4.6 mm, 5 μm)	0.5	MS/MS ESI^+MRM559.5 → 440.1	1.9	0.2–30 ng $mL^{-1}r^2$ ≥ 0.9950	----	0.20 ng mL^{-1}	80.34%	[73]
AT and metabolites	Plasma	SPE, MEPS	Gradient elution; A = ACN, B = AA (pH 4;0.5 mM)	Acquity UPLC BEH C18 column (100 × 2.1 mm, 1.7 μm)	0.25	MS/MS ESI^+SRM559.5 → 440.4	3.0	0.5–100 nMr^2 = 0.9993	0.03 Nm	0.08 nM	----	[74]
AT and metabolites	Human	PP	Gradient elution; A = 0.1% v/v glacial	ZORBAX-SB	0.35	MS/MS	3.9	0.05–100 ng	----	0.05	88.60–	[75]

Table 2 Reported analytical method for the estimation of AT, its metabolites, and co-administered drugs in biological fluids using LC-MS/MS technique *(Continued)*

Analyte(s)	Biological matrix	Sample preparation	Mode of elution; mobile phase	Stationary phase	Flow rate (mL min^{-1})	Detection	R_t (min)	Calibration range	LOD	LOQ	% Recovery	Ref.
	plasma		AcA in 10% v/v MeOH in water, B = 40% v/v MeOH in ACN	Phenyl column (100 × 2.1 mm, 3.5 µm)		ESI^+MRM559.2 → 440.2		$mL^{-1}r^2 \geq$ 0.9975		ng mL^{-1}	111.00%	
AT and metabolites	Plasma	PP	Gradient elution; A = water, B = MeOH (Both with 0.10% FA and 2 mM AA)	Raptor Biphenyl column (30 × 3 mm, 5 mm)	0.75–1.2	MS/MS ESI^+SRM 559.3 → 440.2	----	----	----	----	----	[76]
AT and amlodipine	Human plasma	PP	Gradient elution; A = 0.1% of FA in water, B = 0.1% of FA in ACN	Eclipse XDB-C18 column (100 × 2.1 mm, 3.5 µm)	0.4	MS/MS ESI^+MRM559.3 → 440.2	----	0.46–1000 ng $mL^{-1}r^2$ = 0.9965	----	0.46 ng mL^{-1}	96.23%	[77]
Ramipril, AT, benazepril, and amlodipine	Human plasma	LLE	Isocratic elution; 0.1% FA:ACN (15:85, v/v)	ZORBAX-SB C18 column (50 × 4.6 mm, 5 µm)	1.0	MS/MS ESI^+MRM559 → 440.2	0.6	0.05–20.5 ng $mL^{-1}r^2$ = 0.9900	----	0.26 ng mL^{-1}	67.10%	[78]
AT and metabolites	Human plasma	SPE	Isocratic elution, ACN:0.60% (v/v) AcA (70:30, v/v)	SymmetryC18 column (75 × 4.6 mm, 3.5 µm)	0.5	MS/MS ESI^+559.2 → 440.2	2.67	0.05–252.92 ng $mL^{-1}r^2$ = 0.9900	----	0.05 ng mL^{-1}	66.18%	[79]
AT and metabolites	Human plasma	SPE	Isocratic elution; ACN:MeOH:0.1% FA in water (50:30:20, v/v/v)	Cyno analytical column (125 × 4 mm, 5 µm)	0.5	MS/MS ESI^-MRM557.31 → 397.16	2.4	0.2–40 ng $mL^{-1}r^2 \geq$ 0.9900	0.06ng mL^{-1}	----	50.00–68.00%	[80]
Simvastatin and AT	Human serum	SPE	Gradient elution; A = ACN, B = AA(pH 4.0; 0.5 mM)	Acquity UPLC BEH C18 column (100 × 2.1 mm, 1.7 µm)	0.25	MS/MS ESI^+, SRM559 → 440	2.51	0.1–100 nMr^2 = 0.9999	0.05 nM	0.15 nM	85.66%	[81]
AT and o-OH AT	Human plasma	LLE	Isocratic elution; ACN:water (95:5, v/v)(0.2 % FA mixed)	Sunfire C18 column (100 × 2.1 mm, 3.5 µm)	0.5	MS/MS ESI^+MRM559.4 → 440.5	2.66	0.1–10 ng $mL^{-1}r^2$ = 0.9970	----	0.10 ng mL^{-1}	85.73%	[82]
AT and p-OH AT	Human plasma	SPE	Isocratic elution; 0.1% AcA in water: ACN (40:60, v/v)	Genesis C18 column (50 × 2.1 mm, 4 µm)	0.2	MS/MS ESI^+MRM559.3 → 440.1	1.70	0.229–91.6 ng $mL^{-1}r^2$ = 0.9960	----	0.23 ng mL^{-1}	53.06%	[83]
AT	Human plasma	LLE	Isocratic elution; AA (5 mM): MeOH: Methanoic acid (30:70:0.1, v/v/v)	Hypersil C18 column (150 × 4.6 mm, 5 µm)	1.0	MS ESI^+SIM559.25	4.45	0.25–20 ng $mL^{-1}r^2$ = 0.9996	----	0.25 ng mL^{-1}	58.13%	[84]
AT and o-OH AT	Human plasma	LLE	Isocratic elution, ACN:0.1% AcA (70:30, v/v)	Atlantis d-C18 column (10 × 3 mm, 3 µm)	0.3	MS/MS ESI^+MRM mode559 → 440	3.1	0.10–40 ng $mL^{-1}r^2$ = 0.9990	0.02 ng mL^{-1}	0.10 ng mL^{-1}	91.50%	[85]
AT and metabolites	Human plasma	LLE	Iscocartic elution; 0.03% FA:ACN (30:70, v/v)	Symmetry C18 column (100 × 4.6 mm, 5 µm)	1.0	MS/MS ESI^+ MRM559 → 440	1.8	0.1–20 ng mL^{-1}	----	0.10 ng mL^{-1}	54.20%	[86]
Rosuvastatin, simvastatin, AT, and metabolites	Human plasma	LLE	Gradient elution; A = 10 mM AF and 0.04% FA, B = ACN.	Acquity UPLC BEH C18 column (100 × 2.1 mm, 1.7 µm)	0.4	MS/MS ESI^+559.2 → 440.3	3.2	0.25–100 ng $mL^{-1}r^2$ = 0.9910	----	0.25 ng mL^{-1}	97.20%	[87]

Table 2 Reported analytical method for the estimation of AT, its metabolites, and co-administered drugs in biological fluids using LC-MS/MS technique *(Continued)*

Analyte(s)	Biological matrix	Sample preparation	Mode of elution; mobile phase	Stationary phase	Flow rate (mL min^{-1})	Detection	R_t (min)	Calibration range	LOD	LOQ	% Recovery	Ref.
AT and metabolites	Human plasma	SPE	Gradient elution; A = ACN:FA (1 mM) (30:70, v/v), B = ACN: FA (1 mM) (60: 40, v/v)	Omnisphere C18 column (30 × 2 mm, 3 μm)	0. 2	MS/MS ESI^+ SRM559.0 → 440.2	6.2	0.2–30 ng mL^{-1} r^2 = 0.9910	0.06 ng mL^{-1}	----	53.00–78.00%	[88]
AT and metabolites	Human serum	LLE	Gradient elution; A = 950 mL of water +50 mL of MeOH+ 43 mL of 88% FA, B = 950 mL of ACN + 50 mL of MeOH+43 mL of 88% FA.	YMC Basic column (50 × 2 mm, 5 mm)	0.3	MS/MS ESI^+SRM 559.2 → 440.2	2.78	0.5–200 ng mL^{-1}	----	0.50 ng mL^{-1}	60.00–100.00%	[89]
AT and metabolites	Human plasma	LLE	Isocratic elution; ACN:0.1% AcA (70:30, v/v)	YMC J'Sphere H80 C18 column (150 × 3.2 mm, 4 mm)	0.2	MS/MS ESI^+MRM559.3 → 440.2	4.3	0.250–25 g mL^{-1} r^2 = 0.9989	----	0.25 ng mL^{-1}	98.40%	[90]
	Dog plasma						4.8	0.250–25 ng mL^{-1} r^2 = 0.9994			98.90%	
	Rat plasma						3.5	0.250–25 ng mL^{-1} r^2 = 0.9995			98.00%	
AT and metabolites	Human plasma	PP	Isocratic elution; 0.2% FA:ACN (55:45, v/v)	Acquity UPLC BEH C18 column (50 × 2.1 mm, 1.7 μm)	0.4	MS/MS ESI^+SRM 559.2 → 440.2	2.4	0.025–200 ng mL^{-1} r^2 = 0.988	----	0.02 ng mL^{-1}	97.37%	[91]
AT, mevastatin, pravastatin, rosuvastatin, pitavastatin, fluvastatin, simvastatin, and lovastatin	Human urine	Shoot and dilute method	Gradient elution; A= 0.1% AcA, B = ACN	Kinetex C18 column (50 × 3 mm, 2.6 μm)	0.35	MS/MS ESI^+MRM559.3 → 250.2	6.4	1–500 ng mL^{-1} r^2 = 0.9946	----	----	92%	[92]
Bisoprolol, clopidogrel, and AT	Human plasma	PP	Gradient elution; A = water, B = ACN (0.1% FA)	Halo C18 column (50 × 2.1 mm, 2.7 μm)	0.5	MS/MS ESI^+ MRM559.3 → 440.3	3.5	0.5–125 ng mL^{-1} r^2 = 0.9950	----	0.50 ng mL^{-1}	102.40%	[93]
AT	Human plasma	PP	Gradient elution; A = 0.2% (v/v) FA in water, B = ACN	Leapsil C18 column (100 × 2.1 mm, 2.7 μm)	0.3	MS/MS ESI^-MRM557.0 → 453.0	2.0	0.01–200 ng mL^{-1} r^2 = 0.9992	----	0.05 ng mL^{-1}	85.63–92.51%	[94]
AT and amlodipine	Human plasma	LLE	Isocratic elution; ACN: AF (pH 4.5;10 mM) (70:30, v/v)	Acquity UPLC BEH C18 column (50 × 2.1 mm, 1.7 μm)	0.25	MS/MS ESI^+MRM557 → 440.2	0.66	0.05–50 ng mL^{-1} r^2 = 0.9996	----	0.05 ng mL^{-1}	91.30–94.00%	[95]
AT and metabolites	Human plasma	SPE	Gradient elution; A = 0.1% AcA in water, B = ACN	Genesis C18 column (50 × 2.1 mm, 4 μm)	----	MS/MS ESI^+ MRM559.4 → 440.3	----	$r^2 \geq 0.9900$	----	----	94.31%	[96]
AT	Human Plasma	μ-SPE	Gradient elution; A = AA (10 Mm), B = ACN	C18 column (100 × 3 mm, 5 μm)	0.6	MS/MS ESI^+ MRM559.2 → 440.1	4.5	0.2–80 ng mL^{-1} r^2 = 0.9965	0.05 ng mL^{-1}	0.20 ng mL^{-1}	94.60%	[97]
AT and niacin	Human plasma	LLE	Isocratic elution; ACN: 0.1% FA (80:20, v/v)	Hypurity Advance column (50 × 4.6 mm, 5 mm)	0.8	MS/MS ESI^+MRM559.0 → 440.3	1.1	0.1–30 ng mL^{-1} $r^2 \geq$ 0.9900	----	----	77.83%	[98]

Table 2 Reported analytical method for the estimation of AT, its metabolites, and co-administered drugs in biological fluids using LC-MS/MS technique *(Continued)*

Analyte(s)	Biological matrix	Sample preparation	Mode of elution; mobile phase	Stationary phase	Flow rate (mL min^{-1})	Detection	R_t (min)	Calibration range	LOD	LOQ	% Recovery	Ref.
AT and olmesartan	Rat plasma	PP	Isocratic elution; ACN**:** 0.04% glacial AcA (80**:**20, v/v)	Aquasil C18column (50 × 2.1 mm, 5 µm)	0.25	MS/MS ESI^+MRM559.0 → 440.0	1.68	1–1000ng mL^{-1} r^2 = 0.9998	0.15 ng mL^{-1}	1.00 ng mL^{-1}	85.72%	[99]
AT and metabolites	Human plasma	LLE	Isocratic elution; ACN**:** Water(75**:**25, v/v) (0.05 % v/v FA mixed)	Acquity UPLC HSS T3 column (100 × 3 mm, 1.8 µm)	0.3	MS/MS ESI^+MRM559.4 → 440.1	2.30	0.2–40 ng mL^{-1}	----	0.20 ng mL^{-1}	48.53%	[100]

[a]The pH of the mixed solvent system

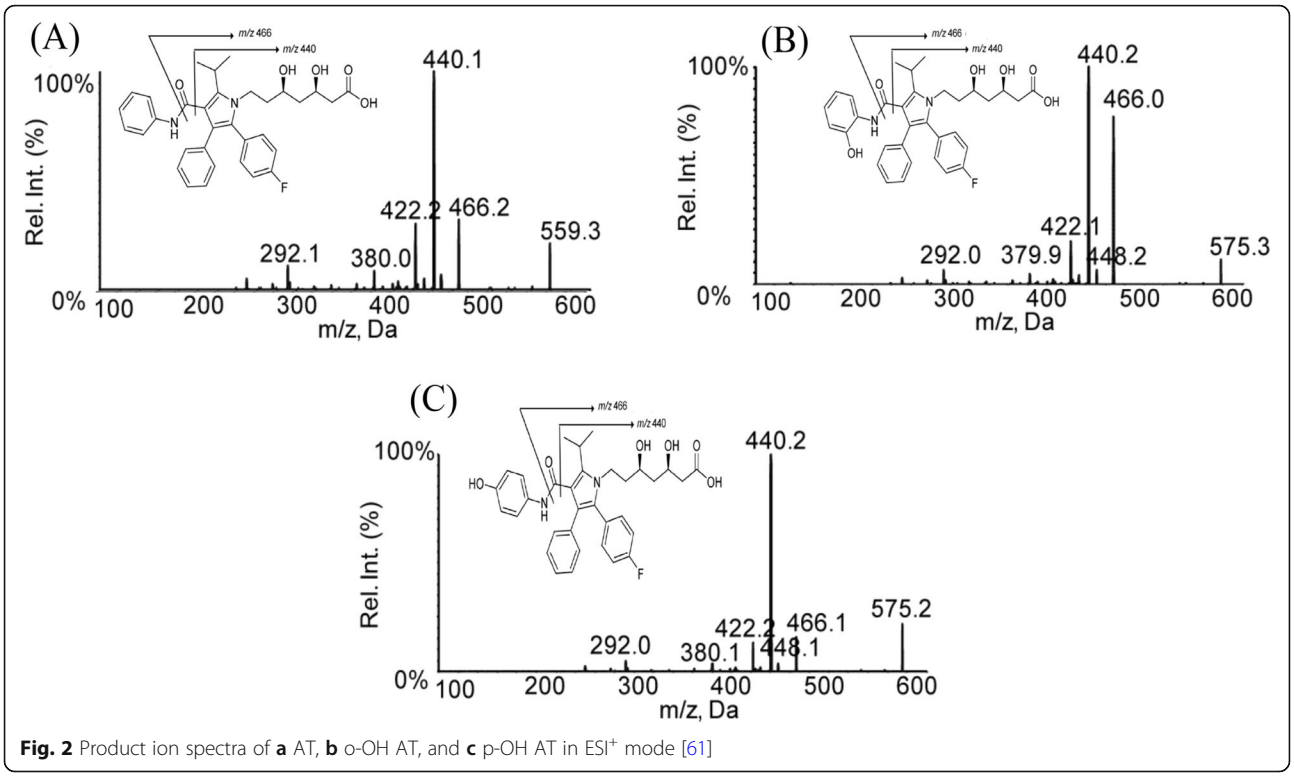

Fig. 2 Product ion spectra of **a** AT, **b** o-OH AT, and **c** p-OH AT in ESI^+ mode [61]

LOD and LOQ in the reported methods have been calculated based on their signal to noise ratio, as discussed earlier; nevertheless, in some developed methods, lowest concentration of their linear calibration curve has been chosen as LOQ for analysis. The bioanalytical method developed by Zhou et al. is most sensitive than all other reported LC-MS/MS methods with the lowest LOQ of 0.036 ng mL^{-1} and the calibration curve in the range of 0.03–25 ng mL^{-1} along with intra-day and inter-day RSD of 4.02% and 5.23% respectively [69]. In more than 50% of total reported LC-MS/MS methods, LOQ is equal to or less than 0.5 ng mL^{-1} which indicates the developed LC-MS/MS methods are highly sensitive. Importantly, Novakova and co-workers have demonstrated the role of the two variables, i.e., concentration and pH of buffer solution in the mobile phase that affects the signal to noise ratio of MS/MS detector, and observed that an increase in a concentration above 5 mM significantly reduces the response of mass spectrometer and the concentrations lower than 0.5 mM were not enough to keep buffering capacity and thus had a negative impact to the response of the MS/MS detector; thus, the best response of AT and simvastatin was observed at 0.5 mM concentration of buffers. Also, AA gave better peak shapes as compared to ammonium formate (AF), and finally, AA buffer (pH 4.0; 0.5 mM) was selected as an optimized buffer for mobile phase composition [81].

Analysis of metabolites and co-administered drugs

Most of the references cited in Tables 1 and 2 also describe the simultaneous determination of AT with certain other statins [13, 20, 24, 27–29, 50, 51, 54, 56, 61, 65, 81, 87] and other co-administered drugs such as amlodipine [35, 40, 59, 64, 65, 69, 70, 77, 78, 95], ezetimibe [42, 66, 71], gemfibrozil [13, 30, 51], clopidogrel [14, 15, 41, 93], fenofibrate [26], aspirin [15, 23, 72], ramipril [64, 78], pioglitazone [17, 22], glimepiride [64, 68, 73], olmesartan [62, 98], and many other. Various methods have also been developed and validated using LC-MS/MS to quantify the metabolites of AT and are enlisted in Table 2 [54, 56–58, 60–62, 65, 67, 69, 70, 74–76, 79, 80, 82, 83, 85–91, 96, 100].

In 1999, Bullen et al. were the first to propose an LC-MS/MS method for the determination of AT and 2 of its metabolites, o-OH AT and p-OH AT in rat, dog and human plasma using a C18 column with isocratic elution of ACN and AcA (70:30, v/v) and detection in ESI^+ MRM mode with transitions 559.3 → 440.2 for AT, 575.3 → 440.2 for p-OH AT, and 575.3 → 440.2 for o-OH AT [90]. Correspondingly, both the acid and lactone form of AT were simultaneously analyzed by Jemal et al. along with their 4 metabolites, i.e., o-OH AT, p-OH AT, o-OH AT-lactone, and p-OH AT-lactone in human serum in ESI^+ mode with eight SRM channels using a highly sensitive and selective LC-MS/MS method [89]. However, MacWan et al. also proposed a method for quantification of AT and its 5 metabolites in human

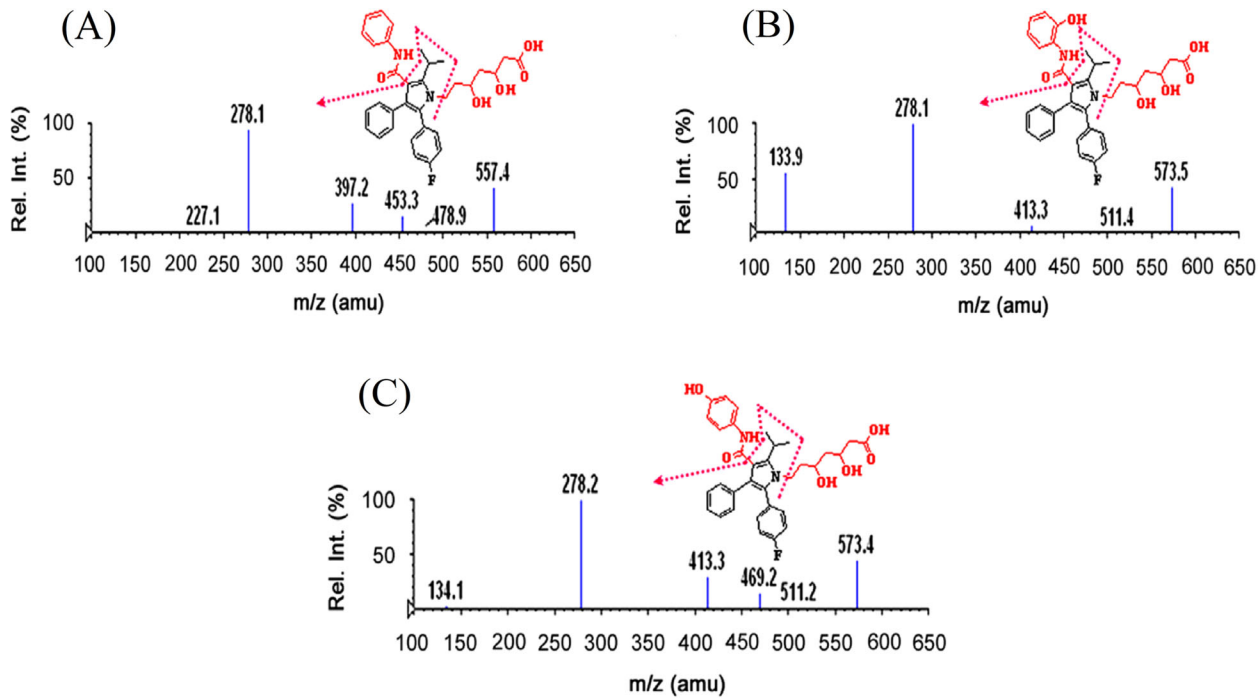

Fig. 3 Product ion spectra of **a** AT, **b** o-OH AT, and **c** p-OH AT in ESI^- mode [66]

plasma using phenyl column [75]. Interestingly, Van pelt et al. modified the conventional LC-MS system by incorporating three valves and four columns into it for the analysis of AT and acid and lactone metabolites in human plasma to reduce the overall run time from 4.5 to 1.65 min [96].

Courlet et al. developed a UHPLC-MS/MS method for the simultaneous quantification of amlodipine, metoprolol, pravastatin, rosuvastatin, AT, o-OH AT, and p-OH AT in human plasma using their stable isotopically labeled analogs as IS. All the compounds were analyzed using ESI^+ mode, except for pravastatin which was detected using ESI^- mode [65]. Vlčková et al. optimized a UHPLC–MS/MS method for selective quantification of seven statins along with their inter-converted products and metabolites in biological samples by comparing the three MS/MS detector with different specifications [55]. Likewise, Wagmann et al. proposed a highly selective method to detect seven statins and their metabolites in human blood plasma using LC-high-resolution MS/MS and applied in the therapeutic drug monitoring of these statins on 14 human plasma samples [53]. Also in 2014, eight different cardiovascular drugs were simultaneously quantified in human plasma using an APCI LC-MS/MS method by Tahboub [64]. However, an RP-HPLC-UV method was designed by Bhatia et al. for the simultaneous estimation of four cardiovascular drugs, i.e., AT, aspirin, atenolol, and losartan in tablet dosage form and plasma [23].

Analysis in biological fluids other than blood

Instead of analysis in common biological fluids such as plasma and serum from humans or animals, certain researchers have successfully developed liquid chromatography-based bioanalytical procedures for the quantification of AT in various other biological fluids. Silva et al. determined AT and curcumin in mouse plasma and also in the lung, brain, liver, and spleen homogenate supernatants using an HPLC-DAD assay [12]. Identically, AlKhani et al. designed a precise HPLC-UV method to quantify AT in both rat plasma and rat intestinal perfusion solution for its further application in pharmacokinetic studies [39]. The development of analytical methods for the detection of drugs and its metabolites in the urine samples generally helps to understand in vivo metabolism and clearance process, so, various methods have been developed to analyze AT in the urine samples [13, 43, 44, 47, 92]. In the year 2017, Beydokhti et al. proposed a method to simultaneously determine AT, rosuvastatin, and gemfibrozil in deionized water, wastewater, human plasma, and urine using HPLC-UV method and urine samples were prepared for analysis by diluting with 10 mL of deionized water [13]. Likewise, eight statins were simultaneous determined in human urine using a highly selective and sensitive LC-MS/MS method developed by Jang et al. in which dilute and shot approach was used for urine sample preparation [92].

Sample preparation or extraction of drug from the biological matrix

Traditionally, sample preparation is carried out by diverse techniques such as precipitation of the plasma proteins (PP), liquid-liquid extraction (LLE), and solid-phase extraction (SPE), before the final bioanalysis using liquid chromatography interfaced with UV or MS or MS/MS or capillary gas chromatography to prevent the interference of endogenous substances such as lipids, proteins, salts, acids, bases, and cells present in biological fluid with the chromatographic detection. Out of the total methods cited in Tables 1 and 2, 38% used PP for sample preparation, 33% applied LLE techniques, and 12% employed SPE technique.

Protein precipitation (PP) is the most commonly applied sample preparation technique used for the extraction of AT from biological matrix because of its rapidity, simplicity, and suitability to both hydrophilic and lipophilic analytes. PP process generally involves the addition of ACN or MeOH as precipitating agents followed by subsequent vortex and centrifugation. The % mean recoveries obtain from PP are quite significant. Acidification of the solvent has been generally employed in many PP methodologies to extract AT because the addition of acids such as trichloroacetic acid (TCA) or formic acid (FA) or AcA in the organic solvent enhanced the precipitation process due to changes in the pH [15, 25, 33, 35, 40, 65, 75, 77, 93]. Also, ice-cold extraction solvents have been used in many procedures to prevent degradation and achieve high purification of samples [15, 25, 34, 40, 46, 99]. Mathur and Devi used 10% perchloric acid as a precipitating agent along with a mixture of K_2CO_3 (2.5 M) and KOH (6 M) to maintain pH between 6.0 and 6.5 [48].

Various bioanalytical methods used LLE strategy for the sample pre-treatment of AT before the final analysis by using ethyl acetate, methyl-tertiary butyl ether, dichloromethane, or diethyl ether as organic extraction solvents. Instead of the single solvent procedure, various methods have also used a combination of different solvents [17, 27, 53, 54, 69, 85, 86] or various buffering agents such as FA [17, 60]. AA [20] and phosphate buffer [33, 70–72, 85] for the extraction of AT using LLE method. Also, the addition of buffering agents to the extraction solvent has improved the recovery of analyte from biological matrices. Bullen et al. used NaOH as a buffering agent to boost the extraction of AT from the rat, dog, and human plasma [90]. Despite its few disadvantages such as time-consuming, use of toxic, expensive, and environmentally unfriendly organic solvents [18] and less selectivity toward hydrophilic drugs and metabolites [103], LLE is still extensively employed as a sample preparation technique.

Because of its laborious multistep procedure including column conditioning, sample loading, washing, and elution, followed by evaporation of the eluent, only 12% of the total reported methods adopted SPE approaches for the extraction of AT from biological matrices. Different types of reverse phase SPE cartridges or columns such as HLB or C18 SPE cartridges have been used in SPE as a stationary phase. Moreover, ACN and MeOH have been commonly employed as a solvent for column conditioning, washing, and elution along with some acid, base, or buffers to facilitate elution. SPE isolates analyte more effectively than LLE but not widely employed. Apart from its tedious procedures, cartridges used in SPE are also too expensive, and the plugging of the cartridge is also a major concern in this extraction procedure. Interestingly, Partani et al. carried out their whole SPE sample preparation process in an ice-cold water bath (excluding vortex mixing, SPE, and drying) and under low light conditions to prevent the degradation of the analyte by the temperature and light [67].

Besides conventional sample preparation approaches, many researchers used modified and novel extraction procedures to isolate AT from biological matrices. Both Hassan et al. and Yang et al. employed a novel Salting-out assisted liquid-liquid extraction (SALLE) technique for the extraction of AT from human serum and human plasma respectively using ACN salted with AA and magnesium chloride as a water-miscible solvent [18, 62]. Interestingly, this fast sample pre-treatment procedure shortens the chromatographic run time of AT. Martins et al. utilized dispersive liquid-liquid micro-extraction (DLLME) as a novel extraction method for the isolation of AT from biological matrices and compare it with the conventional SPE method [24]. A further modification in the DLLME technique with solidification in the prepared floating organic droplets has been made by Taheri et al. using a low-density solvent (Undecanol), which floats and solidifies at the top of the extraction vial during the extracted phase and separates the analyte easily [16]. Furthermore, Farajzadeh et al. used sugar cubes as a disperser base instead of a disperser solvent in the DLLME procedure to extract AT from human serum [38]. Notably, Beydokiti et al. resolved the problem of lack of sample clean-up by using tandem air-agitated LLE based on solidification of floating organic droplets (TAALLME-SFO) technique for the extraction of AT from a biological matrix. However, the use of chlorinated solvents is also a major drawback of this method [50]. Interestingly, instead of two-phase liquid microextraction, in 2009, Farahani et al. designed a novel three-phase liquid microextraction process (LLLME) in droplet-based mode to separate the analyte from complex biological matrix before HPLC assay [31].

Rukthong et al. utilized a simple, sensitive, and rapid solid-phase microextraction (SPME) technique to extract AT from human plasma using an RP-C18 SPE sorbent material which is packed in a 100-μL pipette tip to eliminates the clogging problem [97]. Because of comparative high sorption capacity and high selectivity for analytes, many researchers employed magnetic nanoparticles (MNPs) as sorbents instead of conventional SPE sorbent in the extraction of AT from biological matrices and the extraction technique involving MNP is termed as magnetic solid-phase extraction (MSPE). Tekkeli et al. used graphene nanosheets decorated with Fe_3O_4 nanoparticles as a magnetic sorbent for the extraction of AT from human plasma along with ACN as an extraction solvent [37]. Similarly in another study, Khoshhesab et al. used an ultrasound-assisted MSPE technique using sodium dodecyl sulfate-coated Fe_3O^4 MNP as a sorbent for the extraction and found it comparatively easy and fast while comparing with conventional LLE and LLLME methods [44]. Dastkhoon et al. in 2017 developed dispersive micro solid-phase extraction (Dμ-SPE) approach as a novel modification in the SPE technique for extraction of AT from human plasma and urine using Cu@SnS/SnO_2 nanoparticles loaded on activated carbon as a sorbent for separation of AT from matrices [43]. Interestingly, a combined magnetic-dispersive SPE (MD-SPE) approach was designed by Gholami and Ahmadi for the extraction of AT from human serum using polypyrrole-Fe_3O_4 MNP as sorbent [49]. Notably, Azadi and Ahmadi further used magnetic dispersive micro solid-phase extraction (MDμ-SPE) technique for the extraction of AT and valsartan from human serum and urine using CMC-coated Fe_3O_4 MNP [47]. In the same way, Beydokhti et al. developed a novel extraction technique by the combining magnetic dispersive micro SPE process and supra-molecular solvent-based microextraction process (Mdμ-SPE–SSME) to extract AT, rosuvastatin, and gemfibrozil from human plasma and urine sample with the help of layered double hydroxide-coated Fe_3O_4. Also, in comparison with the conventional SPE technique, Mdμ-SPE–SSME reduces the overall extraction time and eliminates the elution step [13]. Interestingly, Vlčková et al. developed microextraction by packed sorbent (MEPS) technique to extract AT and its metabolites from biological samples using a gas-tight syringe as an extraction device [56, 74]. Briefly, MEPS is a novel and miniature version SPE technique, and in comparison with the conventional SPE methods, the MEPS technique is quite simpler and time-saving and requires a lesser amount of sample. Moreover, the MEPS technique has good recovery in a short time with a lesser volume of samples.

Conclusion

Analysis of AT in the biological fluid is quite useful for pharmacokinetic studies, therapeutic drug monitoring, and drug-drug interaction studies. Numerous developed bio-analytical methods that are useful for quantitation of AT either alone or with its metabolites or co-administered drugs in plasma, serum, or urine are herein summarized and discussed. Due to advancement in technology, many new LC methods have been developed in the last decade with high sensitivity and accuracy. However, LC-MS/MS technique is more sensitive, selective, and precise as compared to the HPLC-UV technique for the quantification of AT. Also, various extraction techniques used to extract AT from biological matrices are discussed herein. Indeed, PP is the most commonly applied technique for sample preparation, followed by LLE and SPE, yet numerous advance methods have been developed such as SALLE, DLLME, and MEPS for the extraction of AT from biological fluids, to get high recovery. Thus, this review can be used as an informative tool to develop and optimize certain new LC methods for quantification of AT in biological matrices.

Abbreviations

AA: Ammonium acetate; AcA: Acetic acid; ACN: Acetonitrile; AF: Ammonium formate; APCI: Atmospheric pressure chemical ionization; AT: Atorvastatin; Dμ-SPE: Dispersive micro solid-phase extraction; DAD: Diode array detector; DLLME: Dispersive liquid-liquid micro-extraction; ESI^-: Negative electrospray ionization; ESI^+: Positive electrospray ionization; FA: Formic acid; LLE: Liquid-liquid extraction; LLLME: Liquid-liquid-liquid microextraction; LLME: Liquid-liquid microextraction; LOD: Limit of detection; LOQ: Limit of quantification; MSPE: Magnetic solid-phase extraction; MDμ-SPE: Magnetic dispersive micro solid-phase extraction; MeOH: Methanol; MEPS: Microextraction by packed sorbent; MRM: Multiple reaction monitoring; o-OH AT: Ortho-hydroxy Atorvastatin; PDA: Photodiode array detector; p-OH AT: Para-hydroxy Atorvastatin; PP: Protein precipitation; R_t: Retention time; r^2: Regression coefficient; SALLE: Salting-out assisted liquid-liquid extraction; SIM: Selected ion monitoring; SPE: Solid-phase extraction; SPME: Solid-phase microextraction; SRM: Selected reaction monitoring; TCA: Trichloroacetic acid; UPLC: Ultra performance liquid chromatography

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Authors' contributions

KW: Investigation, Methodology, Writing-Original Draft, Conceptualization. ACR: Validation, Supervision. All authors have read and approved the manuscript.

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