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ORIGINAL ARTICLE

Synchronized separation of atorvastatin—an antihyperlipidemic drug with antihypertensive, antidiabetic, antithrombotic drugs by RP-LC for determination in combined formulations

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Antithrombotic drug

Abstract A new rapid and sensitive high performance liquid chromatography (HPLC) method has been developed for the simultaneous determination of atorvastatin—an antihyperlipidemic drug along with most commonly prescribed drugs (antihyperlipidemic, antihypertensive, antidiabetic, antithrombotic) in bulk and marketed 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 µg/mL for all the drugs with correlation coefficients of determination (r^2 values) ≥ 0.999 . Limits of detection (LODs) and Limits of quantification (LOQs) ranged from 0.1 to 0.27 µg/mL and 0.3 to 0.89 µg/mL respectively. Intra-day and inter-day precision was studied at three concentration levels (20, 60 and 100 µg/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%. Thus, the performance of the method described allows its use in quantification of atorvastatin along with 9 most commonly prescribed drugs available in market as atorvastatin combined dosage forms.

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doi:10.1016/j.jpha.2012.02.006**1. Introduction**

Atheromatous disease is ubiquitous and underlies the commonest cause of death (myocardial infarction caused by thrombosis) and disability (stroke, heart failure) in industrial societies. Hyperlipidemia and hypertension are the important risk factors for atheroma, which are amenable to drug therapy. The statins are used in combination with several different types of cardiovascular drugs to treat the disease. Atorvastatin [2-(4-fluorophenyl)- β , δ -dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid], an antihyperlipidemic drug, is used along with thrombolytic drugs (aspirin, clopidogrel) in the treatment of



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myocardial infarction. It is also used with antihypertensive drugs (atenolol, telmisartan, losartan potassium) in the treatment of hypercholesteremia and in high blood pressure, with ezetimibe, fenofibrate (anti hyperlipidemic) to treat hypercholesteremia, with antidiabetics (glimeperide, metformin) in the case of diabetes and hypercholesteremia [1]. Therefore, atorvastatin is most frequently prescribed in combined formulations of different drugs.

A thorough literature survey reveals that determination of atorvastatin alone or in combination with other drugs viz., fenofibrate [2–4], ezetimibe [5,6], losartan [7], telmisartan [8], metformin and glimeperide [9], glimeperide [10], aspirin [11], clopidogrel and aspirin [12], losartan, aspirin and atenolol [13] was reported. The afore mentioned analytical methods have been developed in order to allow quantitative determination of a maximum of three combinations. In light of the increasing number of combinations, these separation procedures are extremely inefficient. It is obvious that a more convergent methodology is required in order to streamline and simplify these procedures. We have decided to develop a single method

to identify and quantify atorvastatin with most of the prescribed combinations available in the market.

Currently the most commonly used analytical method for determination and quantification of drugs is high performance liquid chromatography which offers greater versatility and robustness combined with different detection modes. The introduction of reliable photodiode-array detectors has dramatically improved the selectivity of HPLC and afforded a number of advantages previously attributed only to mass spectrometric detection [14]. Indeed, such a method would be useful for simultaneous determination of these drugs in different single or compounded formulations, therapeutic drug monitoring and toxicological screening in forensic samples. Here we report a rapid and sensitive method for the identification and quantitation of ten drugs (atorvastatin calcium, fenofibrate, ezetimibe, atenolol, losartan potassium, telmisartan, metformin hydrochloride, glimeperide, aspirin, clopidogrel bisulfate) using gradient reverse phase high-performance liquid chromatography.

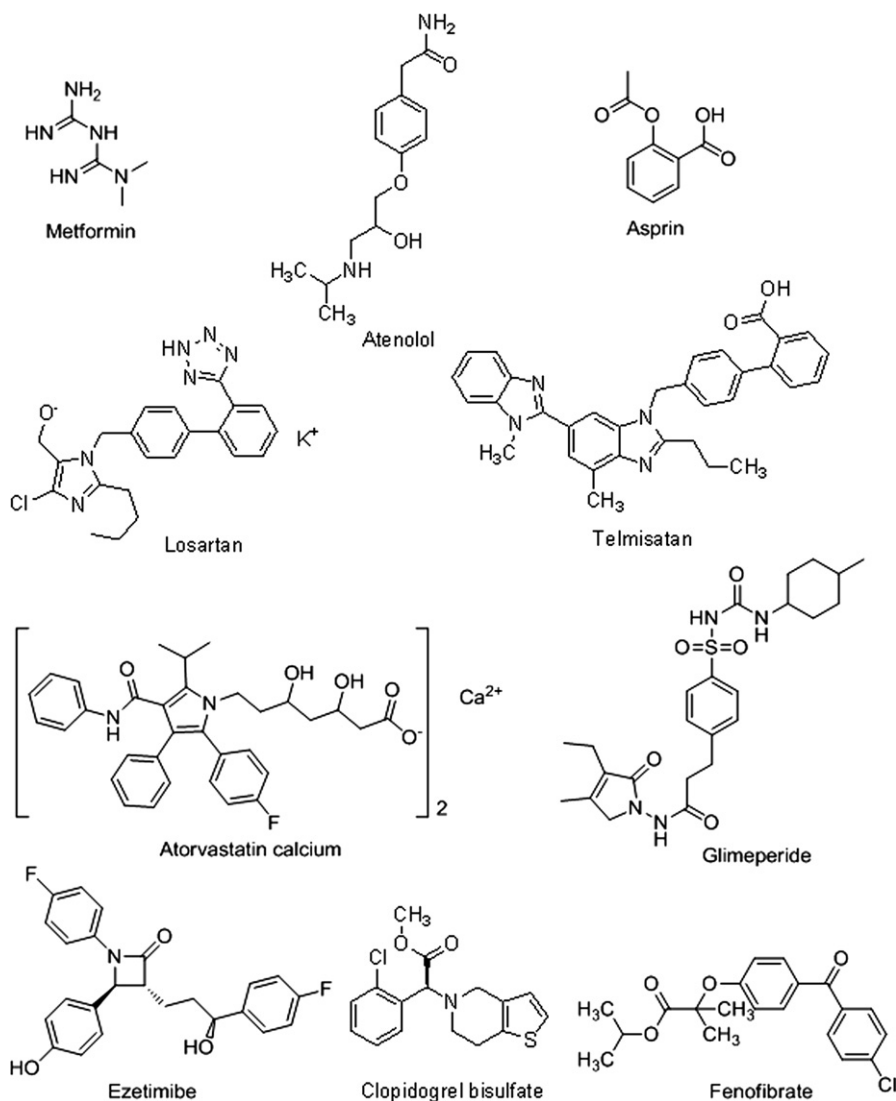


Figure 1 Chemical structures of the studied drugs.

2. Materials and methods

2.1. Chemicals and reagents

The active pharmaceutical ingredients were obtained as gift samples from the following local manufacturers: atorvastatin calcium (ATR), losartan potassium (LSN) and clopidogrel bisulfate (CLO) from Aurobindo pharma Ltd., aspirin (ASP) from Accord labs, ezetimibe (EZE) and telmisartan (TSN) from MSN Laboratories Ltd., metformin hydrochloride (MET) and glimepiride (GLI) from Dr. Reddy's Laboratories Ltd., atenolol (ATE) from Virupaksha Organics Ltd., fenofibrate (FEN) from Nivika Chemo-Pharma Ltd., Gujarat, India. HPLC grade acetonitrile and methanol were obtained from Merck India. Acetic acid, trifluoroacetic acid (TFA) and triethyl amine (TEA) (AR) were from S.D. Fine Chemicals Ltd., Mumbai. High purity water was prepared using Milli-Q gradient ultrapure water system (Billerica, MA 01821, USA). Triethylamine acetate (TEAA) buffer was prepared by neutralizing a 0.1% solution of triethylamine with glacial acetic acid until pH 5 was obtained. It was filtered through a 0.45 µm filter prior to use. Star pill (Cipla Ltd.), (ATR 10 mg, ATE 50 mg, LSN 50 mg, ASP 75 mg); Telsartan-atr (Dr. Reddy's Laboratories Ltd.), (ATR 10 mg, TSN 40 mg); Fibator-ez (Sun Pharmaceuticals Industries Ltd.), (ATR 10 mg, FEN 160 mg, EZE 10 mg); Deplatt-CV cap (Torrent Pharmaceuticals Ltd.), (ATR 10 mg, ASP 75 mg, CLO 75 mg); Stator GM2 (Abbott Healthcare pvt.Ltd.), (ATR 10 mg, GLI 2 mg, MET 500 mg) were purchased from local medical stores. Fig. 1 shows the chemical structures of the studied drugs.

2.2. Equipment and chromatographic conditions

The HPLC system consisting of two LC-20AD pumps, an SPD-M 20AUV/VIS detector, a rheodyne injector, an SPD-M20A diode array detector (PDA), a DGU-20A3 degasser and a CBM-20A communications bus module (all from Shimadzu, Kyoto, Japan) was used. The chromatographic and the integrated data were recorded using LC-solution

data acquiring software. The separation column was a 5 µm phenyl (250 mm × 4.6 mm i.d.) maintained at ambient temperature (27 °C). Elution was performed with gradient elution of acetonitrile and TEAA mobile phase. The acetonitrile content of the mobile phase was 5% initially and increased linearly from 5% to 50% during 5 min and further increased to 55% from 5 min to 12.5 min and kept constant until 17.5 min and increased to 100% up to 18.5 min. This was maintained up to 25 min. The mobile phase was used at a flow rate of 1 mL/min. The UV spectra of individual drugs were recorded in the wavelength range of 200–400 nm. The wavelength at 230 nm was considered for the detection of all drugs with adequate sensitivity. The pH measurements were carried out with pH meter (Eutech pH Tutor) equipped with a combined glass-calomel electrode, which was calibrated using standard buffer solutions of pH 4.0 and 7.0 before making measurements.

2.3. Preparation of stock and calibration solutions

Stock solutions of ATR, FEN, EZE, ATE, LSN, MET, ASP, CLO were prepared by dissolving 5 mg of each in 5 mL of methanol. GLI (5 mg) was dissolved in 5 mL of dioxane and sonicated for 5 min. TSN (5 mg) was dissolved in 5 mL of methanol and 200 µL of acetic acid added for enhancing the solubility. Using this stock solutions, serial dilutions were made to get 8 different concentrations (5, 20, 40, 60, 80, 100, 120, 150 µg/mL) to construct calibration curve. Responses were measured as peak areas and plotted against concentration.

2.4. Sample preparation for assay

Twenty tablets of different combinations of atorvastatin were weighed and grinded. Appropriate amount of powder was diluted with 10 mL of the diluent, sonicated for 15 min and filtered through a 0.45 µm whatmann filter paper, and further dilutions were made as discussed in Section 3.3.

Table 1 Chromatographic behavior of the drugs on different columns.

Sl. no.	Buffers	pH	C ₁₈ column	Phenyl column
1	0.05% Trifluoroacetic acid (TFA)	4	All drugs are separated, but TSN-broad peak, Tailing factor (T_f): MET (1.72), EZE (1.54), ATR (1.52), GLI (1.56), FEN (1.56)	MET retention increased to 4.29 ATR and TSN overlap, GLI—less Rs (1.76) Tailing factor (T_f): MET (2.13), LSN (1.68)
2	0.05% Trifluoroacetic acid (TFA)	5	ASP and ATE—overlap TSN and ATE—poor resolution ($R_s < 1$) EZE and TEL—less resolution ($R_s = 1.6$) Tailing factor (T_f): MET (2.15), LSN (1.57), CLO (1.55), FEN (1.56)	MET retention increased to 4.28 ATR and TSN, overlap Tailing factor (T_f): MET (2.1).
3	0.1 % Triethylamine acetate (TEAA)	4	ATR and TEL broad peaks, GLI and EZE—overlap, Tailing factor (T_f): MET (2.15), LSN (1.52), FEN (1.53)	MET retention increased to 3.61, GLI and EZE, overlap TSN and ATR—less Rs (1.78)
4	0.1 % Triethylamine acetate (TEAA)	5	ATR and TSN—less resolution ($R_s = 1.2$)—no base to base separation. Tailing factor (T_f): MET (1.54)	MET retention increased to 3.42, minimum resolution > 2.8 and tailing factor (T_f) < 1.2 for all the drugs.

3. Results and discussion

3.1. Method development and optimization

When methanol and acetonitrile were used in initial scouting, it was observed that acetonitrile was found to be better in terms of resolution and peak shapes as compared to methanol. Therefore, acetonitrile was used as an organic modifier for method development. We have avoided phosphate buffer as it is non-volatile and unamenable with an ELSD or a MS detector. Ammonium acetate buffer (10 mM) was chosen, but peak shapes and resolutions were found to be unacceptable for some of the analytes. ATR and TSN were less resolved and tailing was observed for ASP, EZE and GLI with broader peaks. The effect of different pH and mobile phase composition were also tried to improve the resolution and peak symmetry. The resolution was found to be improved at pH 4 and 5. Two buffers, TFA (acidic) and TEAA (basic) were chosen to work with different stationary phases for further improvement in selectivity. In addition to commonly using C₁₈ column, phenyl stationary phase was selected as majority of the analytes studied are aromatic. It is known that, phenyl stationary phases are π -basic (electron donating) and interact by π - π interactions through the phenyl ring with the compound, thereby improving the selectivity. In an attempt to improve peak symmetry and resolution on C₁₈ column, TFA (0.05%) was used. But severe tailing was observed for most of the analytes. In the case of TEAA buffer on C₁₈, ATR and TSN were less resolved with broader peaks and metformin was

eluted within 2.5 min. The experiment on phenyl column with TFA was unsuccessful as ATR and TSN were not fully resolved and tailing observed ($T_f > 2.0$) for MET. In the case of TEAA at pH 4.0, GLI was not separated from EZE and resolution between ATR and TSN was also poor. However, pH 5.0 was found to be suitable to overcome all the difficulties on phenyl column. The phenyl column was found to be more suitable because of less tailing with improved resolution for all the drugs and considerable increase in the retention time (3.5 min) of metformin (polar compound). The resolution was more than 2.8 min and tailing of less than 1.2 min. Therefore, phenyl column with TEAA buffer (pH 5) was selected for further studies. The chromatographic behavior of the all the drugs on different columns with different buffers is summarized in Table 1. Fig. 2 shows separation pattern of ATR and its combination drugs on phenyl and C₁₈ columns with both the buffers at pH 5.

3.1.1. Effect of buffer concentration

Of the various percentages (0.1–0.3%) of TEAA, 0.1% was found to be highly suitable as the chromatographic peaks of all the drugs were well separated from each other with symmetrical peak shapes.

3.1.2. Effect of pH

Further studies were carried out on the pH effect of the buffer (0.1% TEAA) on elution times, resolution and tailing factors. The symmetrical peaks were observed at pH 5 with improved

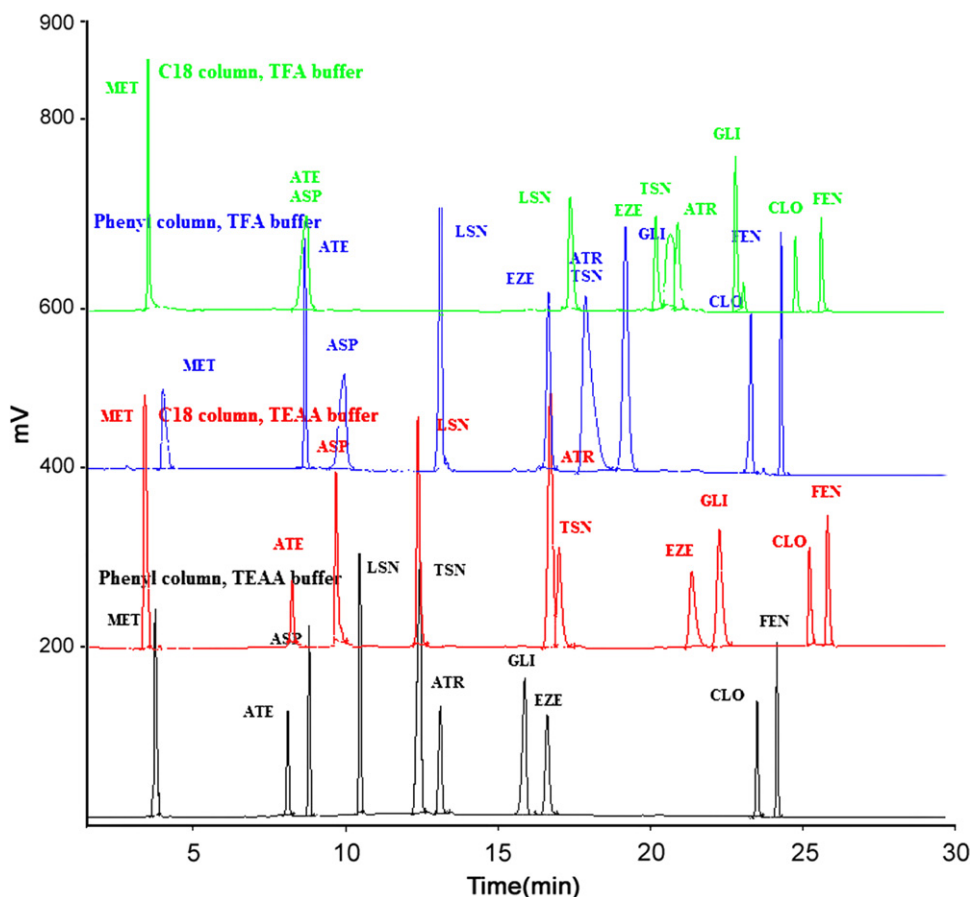


Figure 2 Separation pattern of ATN and its combinations on phenyl and C₁₈ columns (detector set at 230 nm, pH 5).

resolution and retention times, hence pH 5 was considered (Table 1). A typical chromatogram showing the separation of peaks of 10 drugs is depicted in Fig. 2.

3.2. Validation of the method

3.2.1. System suitability

The system suitability was checked by six replicate injections (100 µg/mL). The system is deemed to be suitable for use as the tailing factors and resolutions for all the drugs are less than 1.5 min and greater than 2 min respectively. The chromatographic resolution parameters for the drugs are reported in Table 2.

3.2.2. Linearity

The linearity of detector response to different concentrations of drugs was studied in the range of 5–150 µg/mL at 8 different concentrations (5, 20, 40, 60, 80, 100, 120, 150 µg/mL). The samples were analyzed in triplicates at all concentrations. Calibration curves were constructed and found that correlation coefficient values of all the studied drugs were observed to be ≥ 0.999 . The regression analysis data for calibration curves were calculated using the peak areas and the data are shown in Table 3.

Table 2 Chromatographic resolution parameters for the assayed drugs.

Drug	t_R	R_s	N	T_f
MET	4.1	–	11,258	1.20
ATE	8.1	26.0	48,313	1.26
ASP	8.5	2.3	53,511	1.04
LSN	10.6	15.1	74,720	1.08
TSN	12.8	10.4	39,690	1.13
ATR	13.6	3.2	55,789	1.03
GLI	15.9	13.4	45,443	1.17
EZE	17.2	3.6	59,358	1.03
CLO	23.8	31.0	462,506	1.20
FEN	24.4	4.3	573,068	1.16

t_R =retention time; R_s =resolution; N =number of theoretical plates; T_f =tailing factor.

3.2.3. Limit of detection (LOD) and limit of quantification (LOQ)

The LOD was determined based on signal to noise ratio using analytical response of three times of the background noise. The LOQ was determined as the lowest amount of analyte that was reproducibly quantified using analytical response of ten times of the background noise. The data are shown in Table 3.

3.2.4. Precision

Inter-day variabilities were determined by analysis of standard solutions at low (20 µg/mL), medium (60 µg/mL) and high concentration (100 µg/mL) analyzed in triplicate on each of three consecutive days. The intra-day precision was determined by a set of five samples of each concentration on a single day. The mean value of the concentration and relative standard deviation (R.S.D) are summarized in Table 4.

3.2.5. Accuracy

Accuracy of the method was determined by investigating the recovery of each drug at three levels 50 µg/mL, 100 µg/mL, and 150 µg/mL from placebo mixtures (lactose, maize starch, mannitol, calcium hydrogen phosphate, magnesium carbonate, gelatin, polyvidone microcrystalline cellulose, magnesium stearate, silicon dioxide, titanium dioxide) spiked with the API solution. Each concentration was analyzed in triplicate (Table 5).

3.2.6. Robustness

The robustness of the developed method was determined by analyzing the samples under a variety of conditions of the method parameters, such as flow rate, pH of the buffer and buffer concentration. Variation of the pH of the mobile phase (0.1% TEAA) by ± 0.2 and the % buffer concentration by $\pm 0.05\%$, flow rate by ± 0.2 mL/min did not have significant effect on chromatographic resolution in HPLC method.

3.3. Quantitative determination in pharmaceutical formulations

Twenty tablets each of Stator GM2 Bilayered-tab, Telsartan-atr, Star pill, Fibator-ez, Deplatt-CV cap were crushed and the powder equivalent to one tablet weight was weighed and diluted with dioxane: methanol (3:7, v/v) (or methanol) with 400 µL of

Table 3 Linearity, LOD, LOQ of the drugs studied.

Drug	Range (µg/mL)	Regression equation	Mean coefficient of correlation	LOD (µg/mL)	LOQ (µg/mL)
Metformin	5–150	$y = 101810x - 238772$	0.9996	0.15	0.49
Atenolol	5–150	$y = 22859x + 6874.9$	0.9994	0.13	0.43
Aspirin	5–150	$y = 1863.5x - 1441$	0.9996	0.22	0.72
Losartan	5–150	$y = 81221x - 102299$	0.9994	0.25	0.82
Telmisartan	5–150	$y = 131836x - 82655$	0.9991	0.10	0.30
Atorvastatin	5–150	$y = 43347x - 23320$	0.9996	0.12	0.40
Glimeperide	5–150	$y = 74923x - 117337$	0.9994	0.27	0.89
Ezetimibe	5–150	$y = 52750x + 740.27$	0.9994	0.18	0.59
Clopidogrel	5–150	$y = 29253x - 37989$	0.9995	0.21	0.69
Fenofibrate	5–150	$y = 35423x + 25578$	0.9996	0.23	0.75

Table 4 Inter-day and intra-day precision of drugs.

Drug	Concentration ($\mu\text{g}/\text{mL}$)	Inter-day precision ($n=9$)						Intra-day ($n=5$)	
		Day 0 ($n=3$)		Day 1 ($n=3$)		Day 2 ($n=3$)		Mean \pm SD ($\mu\text{g}/\text{mL}$)	RSD (%)
		Mean \pm SD ($\mu\text{g}/\text{mL}$)	RSD (%)	Mean \pm SD ($\mu\text{g}/\text{mL}$)	RSD (%)	Mean \pm SD ($\mu\text{g}/\text{mL}$)	RSD (%)		
Metformin	20	20.1 \pm 0.2	0.7	20.1 \pm 0.1	0.3	20.1 \pm 0.1	0.5	20.1 \pm 0.2	0.7
	60	60.1 \pm 0.1	0.2	60.5 \pm 0.3	0.5	60.6 \pm 0.5	0.8	60.7 \pm 0.5	0.8
	100	100.4 \pm 0.4	0.4	100.2 \pm 0.1	0.1	100.3 \pm 0.1	0.1	100.2 \pm 0.1	0.1
Atenolol	20	20.1 \pm 0.2	0.7	20.1 \pm 0.1	0.4	20.1 \pm 0.1	0.5	20.1 \pm 0.2	0.7
	60	60.2 \pm 0.1	0.2	60.5 \pm 0.3	0.4	60.7 \pm 0.2	0.3	60.9 \pm 0.1	0.2
	100	100.3 \pm 0.2	0.1	100.3 \pm 0.2	0.1	100.3 \pm 0.2	0.2	100.4 \pm 0.2	0.1
Aspirin	20	20.3 \pm 0.1	0.6	20.1 \pm 0.1	0.3	20.3 \pm 0.2	0.6	20.3 \pm 0.1	0.6
	60	60.2 \pm 0.1	0.2	60.4 \pm 0.2	0.3	60.7 \pm 0.2	0.3	61.0 \pm 0.1	0.2
	100	100.2 \pm 0.2	0.2	100.2 \pm 0.1	0.2	100.5 \pm 0.1	0.1	100.4 \pm 0.2	0.2
Losartan potassium	20	20.2 \pm 0.2	0.9	20.2 \pm 0.1	0.4	20.1 \pm 0.1	0.4	20.2 \pm 0.2	0.9
	60	60.5 \pm 0.3	0.4	60.5 \pm 0.4	0.7	60.7 \pm 0.2	0.3	60.7 \pm 0.6	0.9
	100	100.5 \pm 0.4	0.4	100.3 \pm 0.2	0.2	100.3 \pm 0.2	0.2	100.3 \pm 0.2	0.2
Telmisartan	20	20.3 \pm 0.1	0.3	20.1 \pm 0.6	0.3	20.1 \pm 0.2	0.9	20.3 \pm 0.1	0.3
	60	60.7 \pm 0.2	0.3	60.2 \pm 0.2	0.3	60.4 \pm 0.2	0.3	60.7 \pm 0.3	0.5
	100	100.3 \pm 0.1	0.1	100.4 \pm 0.2	0.2	100.3 \pm 0.3	0.2	100.4 \pm 0.2	0.2
Atorvastatin calcium	20	20.3 \pm 0.1	0.5	20.2 \pm 0.3	1.2	20.2 \pm 0.2	0.7	20.3 \pm 0.1	0.5
	60	60.4 \pm 0.1	0.2	60.5 \pm 0.2	0.3	60.8 \pm 0.1	0.2	60.7 \pm 0.3	0.4
	100	100.2 \pm 0.2	0.2	100.3 \pm 0.2	0.2	100.4 \pm 0.2	0.2	100.4 \pm 0.2	0.1
Glimepiride	20	20.3 \pm 0.1	0.2	20.2 \pm 0.2	0.9	20.3 \pm 0.2	0.6	20.3 \pm 0.1	0.2
	60	60.5 \pm 0.1	0.2	60.6 \pm 0.1	0.2	60.6 \pm 0.2	0.3	60.9 \pm 0.1	0.1
	100	100.2 \pm 0.2	0.1	100.1 \pm 0.2	0.1	100.3 \pm 0.2	0.1	100.2 \pm 0.2	0.2
Ezetimibe	20	20.2 \pm 0.1	0.5	20.2 \pm 0.2	0.6	20.2 \pm 0.1	0.4	20.2 \pm 0.1	0.5
	60	60.4 \pm 0.2	0.3	60.5 \pm 0.3	0.4	60.5 \pm 0.2	0.3	60.9 \pm 0.2	0.3
	100	100.2 \pm 0.1	0.1	100.4 \pm 0.2	0.2	100.4 \pm 0.2	0.2	100.3 \pm 0.2	0.2
Clopidogrel bisulfate	20	20.1 \pm 0.1	0.4	20.3 \pm 0.2	0.6	20.2 \pm 0.1	0.4	20.1 \pm 0.1	0.4
	60	60.3 \pm 0.2	0.3	60.5 \pm 0.1	0.2	60.6 \pm 0.2	0.3	60.9 \pm 0.1	0.2
	100	100.2 \pm 0.1	0.1	100.2 \pm 0.2	0.1	100.5 \pm 0.1	0.1	100.3 \pm 0.2	0.2
Fenofibrate	20	20.2 \pm 0.1	0.5	20.2 \pm 0.1	0.3	20.2 \pm 0.1	0.3	20.2 \pm 0.1	0.5
	60	60.5 \pm 0.2	0.3	60.7 \pm 0.2	0.3	60.7 \pm 0.2	0.3	60.9 \pm 0.1	0.2
	100	100.3 \pm 0.1	0.1	100.1 \pm 0.2	0.2	100.3 \pm 0.2	0.2	100.2 \pm 0.1	0.1

Table 5 Extraction recovery of the analytical method, determined by comparing peak area ratios of extracts with those obtained by direct injection of the same compound.

Drug	Recovery placebo+amount added (50 $\mu\text{g}/\text{mL}$)		Recovery placebo+amount added (100 $\mu\text{g}/\text{mL}$)		Recovery placebo+amount added (150 $\mu\text{g}/\text{mL}$)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Metformin	99.5	1.01	99.1	1.49	99.7	0.56
Atenolol	100.1	0.61	99.4	0.86	99.6	0.62
Aspirin	99.1	1.22	99.2	1.30	99.4	0.89
Losartan	99.0	1.41	99.4	0.76	100.4	0.87
Telmisartan	99.7	0.81	99.3	1.23	99.0	0.51
Atorvastatin	99.6	1.22	99.2	1.28	99.1	1.15
Glimeperide	99.7	1.10	98.6	0.68	99.0	1.12
Ezetimibe	99.3	1.42	98.8	1.12	99.7	0.59
Clopidogrel	98.9	1.24	98.9	1.01	100.1	0.95
Fenofibrate	99.1	1.43	99.0	1.17	99.8	0.74

Table 6 Quantitative determination in pharmaceutical formulations.

Tablet examined	Theoretical amount (mg/tablet)	Amount found (mean \pm SD) (mg)	Recovery (%)	RSD (%) ($n=6$)
Telsartan-atr	ATR-10 mg	10.1 \pm 0.1	101.0	1.5
	TSN-40 mg	39.8 \pm 0.4	99.5	1.1
Stator GM2	ATR-10 mg	9.9 \pm 0.1	99.3	1.5
	GLI-2 mg	2.0 \pm 0.04	100.5	1.8
	MET-500 mg	500.3 \pm 5.0	100.0	1.0
Deplatt-CV cap	ATR-10 mg	9.9 \pm 0.1	99.0	1.0
	ASP-75 mg	75.0 \pm 0.8	100.0	1.0
	CLO-75 mg	75.2 \pm 0.5	100.2	0.9
Fibator-ez	ATR-10 mg	10.1 \pm 0.1	101.7	1.1
	FEN-160 mg	160.2 \pm 0.8	100.1	0.5
	EZE-10 mg	9.9 \pm 0.2	99.8	1.5
Star pill	ATR-10 mg	9.9 \pm 0.1	99.4	1.0
	ASP-75 mg	75.1 \pm 0.8	100.1	1.0
	LSN-50 mg	49.5 \pm 0.5	99.0	1.0

acetic acid as an additive (or methanol) respectively and sonicated for 15 min and further dilutions were made with mobile phase to obtain concentrations within the linearity range (50, 20, 10, 50, 50, 75, 75, 40, 64 $\mu\text{g/mL}$ of MET, GLL, ATR, LSN, ATE, ASP, CLO, TSN, FEN respectively). All the samples were filtered through whatmann (GD/X 25, polypropylene, 0.45 μm) syringe filter, before injecting the samples into the HPLC instrument. The data are shown in Table 6.

4. Conclusion

The present method has demonstrated that drugs available in combinations with atorvastatin can be simultaneously assayed in bulk and dosage forms using RP-HPLC. The linearity of the proposed method was investigated in the range of 5–150 $\mu\text{g/mL}$. The limits of detection were in the range of 0.1–0.27 $\mu\text{g/mL}$ and the limits of quantification were in the range of 0.3–0.89 $\mu\text{g/mL}$ for the studied drugs. The method was found to be linear, accurate, precise and robust. The developed method was employed for simultaneous determination of the ten drugs in their combined dosage forms. This method may find wider applications in therapeutic drug monitoring and in forensic analysis.

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