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Validated chromatographic methods for concurrent determination of atorvastatin and perindopril

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Abstract: Two simple, selective, precise, and accurate chromatographic methods, namely reversed-phase high-performance liquid chromatography (RP-HPLC) method and High-performance thin-layer chromatography (HPTLC), were developed and validated for the simultaneous determination of Atorvastatin (ATO) and Perindopril (PER). The developed HPTLC method was used for the separation and quantitation of the studied drugs on silica gel 60F254 TLC plates. Dichloromethane: Methanol: Ethyl acetate: Glacial acetic acid (6:2:2:0.1, v/v/v/v) was used as a developing system and the separated bands were UV-scanned at 221 nm. Linear relationships were obtained in the range of 800 – 4800 ng/band for Atorvastatin and 200 – 1200 ng/band for Perindopril with a regression coefficient greater than 0.999. The Rf value of the drug was found to be 0.70 ± 0.02 and 0.39 ± 0.03 for Atorvastatin and Perindopril respectively. The developed RP-HPLC depended on chromatographic separation of the studied drugs on a C18 column using Acetonitrile: Methanol: Potassium dihydrogen orthophosphate (pH 3 adjusted with orthophosphoric acid) ((40:10:50), v/v/v) as a mobile phase delivered at a constant flow rate of 1 mL/min with UV detection at 240 nm. The calibration curves were linear (r2 > 0.999) over the concentration range 20-100 µg/mL for Atorvastatin and 10-50µg/mL for Perindopril. The average retention times for Atorvastatin and Perindopril were 3.42 and 8.92 min, respectively. Factors affecting the developed methods have been studied and optimized. Further, methods validation has been carried out according to ICH guidelines. The proposed methods were successfully applied for the determination of the studied drug simultaneously in bulk and synthetic mixture qualitatively and quantitatively. Statistical analysis by the F test showed no significant difference between the results obtained by the two methods. The uncertainty measurement was also carried out for the quantification of both components. The proposed HPTLC method proved to be more sensitive, while the HPLC gave more reproducible results and was time-saving.

Keywords: Atorvastatin; perindopril; HPLC; HPTLC.

1. Introduction

Atorvastatin, chemically [R-(R*, R*)]-2-(4-Fluorophenyl)-β, δ-dihydroxy-5-(1-methyl ethyl)-3-phenyl-4-[(phenylamino)-carboxyl]-1H-pyrrol-1-heptanoic acid calcium salt, (Figure 1) is a second generation synthetic 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor which decreases de novo cholesterol synthesis. ATO decreases the amount of LDL-cholesterol in the blood, reduces blood levels of triglycerides, and slightly increases levels of HDL-cholesterol [1].

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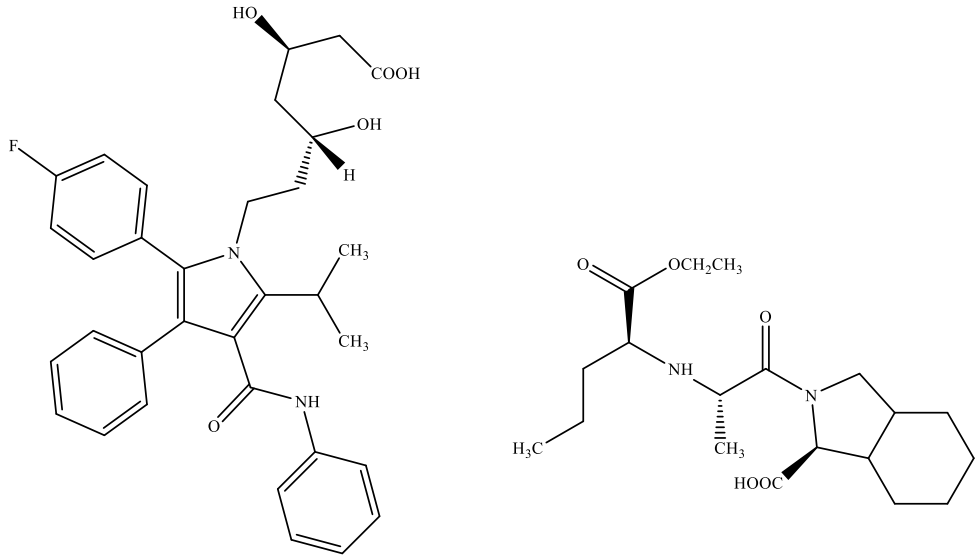

1 **2**

Figure 1. Chemical structure of Atorvastatin (**1**) and Perindopril (**2**)

Perindopril erbumine is an active composition ingredient that is used to reduce high blood pressure. The chemical name of Perindopril erbumine is (2S,3aS,7aS)-1-[(2S)-2-[[(2S)-1-ethoxy-1-oxopentan-2-yl] amino] propanoyl]-2,3,3a,4,5,6,7,7a-octahydroindole-2-carboxylic acid; 2-methylpropan-2-amine (Figure 1). The active composition of Perindopril erbumine competes with AT-I by binding to angiotensin-converting enzyme (ACE) and inhibits enzymatic proteolysis of AT-I to AT-II. Blood pressure decreases by inhibiting angiotensin – II and hence decreases the concentration of angiotensin – II levels in the blood. The disease conditions are heart attacks, strokes, and kidney problems can be overcome by Perindopril erbumine [2].

A combination of atorvastatin and perindopril is used in the treatment of decreased systolic blood pressure and LDL cholesterol level respectively in patients having hypertension and dyslipidemia. Drug combination for Tablet dosage form is currently in phase 3 clinical trial by servier international research institutes. The dose ratio is Perindopril 10 mg and Atorvastatin 40 mg. Since it is a fixed drug combination, when it will be available in the market as a formulation, our methods will be useful for the estimation of it [3]

Various methods have been reported for quantitative analysis of Atorvastatin and Perindopril either individually or in combination with other drugs. Most of the reported methods are high-performance liquid chromatography (HPLC) [4-22] and HPTLC methods [23-52] to quantify Atorvastatin and Perindopril in bulk, biological fluids, and pharmaceutical formulation. However, to the best of our knowledge, no HPLC and HPTLC methods have been reported yet for determining the quantity of Atorvastatin and Perindopril simultaneously in a synthetic mixture. This article aims to develop two chromatographic methods for the determination of ATO and PER in a synthetic mixture. This method was validated as per the ICH guidelines [53].

2. Experimental

2.1. Pure Samples

ATO and PER were supplied by Globela Pharmaceutical Pvt. Ltd., Gujarat, India. Their purity was reported to be 99.5% according to the company certificates.

2.2. Synthetic Mixture

A synthetic mixture was prepared after refereeing the patents [54, 55]. Common excipients and ratios were selected for the mixture. A total of 300 mg of the mixture was prepared and it was used. The proposed composition of the synthetic mixture is mentioned in Table 1.

Table 1. Composition of synthetic mixture

Sr. No	Ingredient	Quantity (mg)
1	Atorvastatin	40
2	Perindopril	10
3	Microcrystalline cellulose	40
4	Starch	15
5	Magnesium Stearate	5
6	Lactose	190
	Total	300

2.3. Chemicals and Solvents

All solvents and reagents were of HPLC and analytical grade, respectively. Methanol, Butanol Acetonitrile and n-Hexene were procured from SRL Diagnostic Pvt. Ltd., Mumbai, and Astron Chemicals Pvt. Ltd. microcrystalline cellulose and calcium phosphate are of analytical grades and purchased from Chiti Chem Corporation Pvt. Ltd., Vadodara, India. From Chemdyes Corporation Pvt. Ltd. in Vadodara, India, we obtain Cross povidone, Magnesium stearate, Hydroxypropyl Methylcellulose K100M, and Cross Carmellose Sodium. Whatman filter paper 42 and a nylon-66 membrane filter were procured from Merck KGaA, Germany.

2.4. Instruments

2.4.1. HPTLC Method

On pre-coated silica gel aluminum plate 60 F254 with a 10 cm × 10 cm and 0.2 mm thickness, provided by E. Merck, Germany and a Hamilton of 100 µL sample syringe was used for sample introduction. This plate was applied with the Camag Linomat 5 sample applicator (CAMAG, Switzerland). The densitometric scanning with the HPTLC system was carried out using a Camag TLC scanner, which was operated by Camag winCATS software, Version 1.4.8. A calibrated Sartorius CP124S (Sartorius Corporation, United States) was used to measure the weight of all three drugs and excipients.

2.4.2. HPLC Method

Shimadzu P series integrated HPLC was equipped with a quaternary gradient unit, an LC-20 AD solvent delivery unit, DGU-20AR degassing unit, detector, a CTO-10ASVP column oven, SPD-M40 PDA detector, and a SIL-20AC programmable autosampler controlled by Lab Solution software. The shim pack ODS C18 column 25 cm (4.6 mm x 250mm, 5 um) was used as a stationary phase. For filtration of a solution, a nylon-66 membrane filter was used.

2.5. Preparation of Solutions

2.5.1. Buffer Solution

0.68 g KH_2PO_4 (potassium dihydrogen phosphate) and 1 ml triethylamine were transferred in 500 ml distilled water and adjusted to pH 3 with 1% OPA (orthophosphoric acid). Then, the prepared buffer solution was filtered through a nylon-66 membrane filter.

2.5.2. Stock Standard Solutions

Weigh accurately 40 mg of ATO and 10 mg of PER and transfer them into two different 10 mL of a volumetric flask. So, the concentration would be 4000 µg/mL for ATO and 1000 µg/mL. Then shake it to dissolve and the volume was adjusted up to the mark with methanol: water (50:50 v/v), for each volumetric flask.

2.6. Method Validation

Both TLC method validation was performed according to the International Council for Harmonization of technical guidelines Q2 (R1).

2.6.1. Linearity and Range

TLC-densitometric method: For ATO and PER, calibration curves at six different concentrations were performed in a range of 800-4800 ng/band and 200-1200 ng/band, respectively six times on TLC aluminum plates (20 × 10 cm), prewashed with methanol and preactivated at 100 ºC for 15 min. Samples were applied as bands using the Camag TLC sampler. The band length was 8 mm and the bands were applied to 9 mm from the bottom of the plate. Ascending development was performed in a chromatographic tank previously saturated for an hour with dichloromethane: methanol: ethyl acetate: glacial Acetic Acid (6:2:2:0.1, v/v/v/v). The migration distance was 80 mm from the lower edge and the developed plates were then air dried. ATO and PER bands were scanned at 221 nm, and the calibration curves were constructed by plotting the mean integrated peak area versus the corresponding concentrations from which the regression equations were computed.

HPLC method: linearity was determined by analyzing five independent levels of the calibration curve in the range of 20-100µg/mL and 10-50 µg/mL for ATO and PER respectively (n=6). 20 µL injections were made for each concentration having the flow rate at 1 mL/min and the effluent was UV-scanned at 240 nm. The chromatographic separation was performed on a C_{18} column using acetonitrile: methanol: potassium dihydrogen orthophosphate (pH 3 adjusted with orthophosphoric acid) ((40:10:50), v/v/v) as a mobile phase. The peak areas were recorded and the calibration curves relating the obtained integrated peak areas to the corresponding concentrations were constructed.

2.6.2. Accuracy

TLC-densitometric method and HPLC methods: For accuracy, % recovery of ATO and PER was calculated by standard spiking method at three different levels 50, 100, and 150 %. The concentrations of ATO and PER were calculated each from its corresponding regression equation and then the mean recoveries and SD values for each component were calculated. Moreover, a standard addition technique has been performed to confirm the accuracy of the developed method

2.6.3. Precision

Repeatability was evaluated by analyzing middle concentrations of ATO (2400 ng/band) and PER (600 ng/band) for the HPTLC method and ATO (60 µg/mL) and PER (30 µg/mL) for HPLC seven times. For Intraday precision, analyzing the solution of ATO and PER was done at three different levels covering lower, medium, and higher concentrations of the calibration curve. It is performed in triplicate on the same day. Similarly, Intermediate precision was evaluated by assaying the three chosen concentrations of ATO and PER in triplicates on three consecutive days using the procedure stated under chromatographic conditions. The mean recoveries and SD values were then calculated.

2.6.4. Specificity

TLC-densitometric method: The specificity of the suggested TLC-densitometric method was evaluated by its application for analysis of synthetic mixtures containing ATO and PER. The chromatographic peaks of ATO and PER were confirmed by comparing Rf values and densitogram of synthetic mixture with standards.

HPLC methods: The specificity was ascertained by application of the developed method to laboratory-prepared mixtures of ATO and PER. Further, specificity was confirmed by calculating system suitability testing parameters such as capacity factor, resolution, and selectivity factor for the separated chromatographic peaks.

2.6.5. Sensitivity

LOQ and LOD are the sensitivity of the proposed method. By using a linear regression model, these parameters were calculated from a standard deviation of the intercept of calibration curves and the mean slope values. A series of concentrations of drug solution and its impurities were injected; LOD and LOQ were established by the slope method as mentioned below.

LOQ = 3:3 × Standard deviation of the response / Slope of mean
LOQ = 10 × Standard deviations of the response / Slope of mean

2.6.6. Robustness

TLC-densitometric method: Small but deliberate changes in different chromatographic conditions like the chamber saturation time and wavelength of the proposed developed method were determined at the middle concentration. 2400 ng/band was selected for ATO and 600 ng/band for PER. The mean Rf values and %RSD was calculated.

HPLC methods: The small deliberate change in HPLC conditions were used for determines robustness. In this method, two changes were measured for both ATO 40 µg/mL and PER 10 µg/mL. Effect of flow rate 0.9 ml/min, 1.1 ml/min, the effect of change in detection wavelength 223 nm and 227 nm was observed.

2.6.7. System Suitability Testing Parameters

TLC-densitometric method and HPLC methods: An overall system suitability testing was done to determine if the operating system was performed properly. Parameters such as resolution, tailing factor, and theoretical plates were determined.

2.7. Analysis of Synthetic Mixture

Weigh 40 mg of ATO and 10 mg of PER and transfer it into a 10 mL volumetric flask containing 5.0 mL of Methanol: Water (50:50). Then it was sonicated for 15 min for improving the solubility of the drug. Whatman filter paper No.42 was used to filter the solution and collect the filtrate in another 10 mL volumetric flask. From the above volumetric flask, pipetted out 1.0 mL aliquot, transferred it to another 10 mL volumetric flask, and volume was adjusted up to the mark with the same solvent to obtain a final concentration of 400 µg/mL for ATO and 100 µg/mL for PER, respectively.

For HPTLC, by using an HPTLC Hamilton 100 microliter syringe, 4 µl of the sample was applied on a TLC plate which gave 1600 ng/band concentration of ATO and 400 ng/band concentration of PER. For HPLC, take 1ml from the above solution and transferred it into a 10ml volumetric flask and make the volume up to mark with methanol to give solution strength (40,10µg/mL). Construction of calibration curves for each method was then followed and the concentrations of ATO and PER were calculated from the corresponding regression equation.

3. Results and Discussion

3.1. Method Developments and Optimization

3.1.1. TLC Densitometric Method

The selection of the mobile phase was carried out based on the literature review through the trial-and-error method. Different ratios of solvents were tried for optimizing the mobile phase like methanol: toluene, methanol: acetonitrile, dichloromethane: methanol, methanol: ethyl acetate, chloroform: methanol: ammonia, ethyl acetate: methanol: ammonia, dichloromethane: methanol: toluene: glacial acetic acid in different ratios. From different trials, dichloromethane: methanol: ethyl acetate: glacial acetic acid (6:2:2:0.1 v/v/v/v) showed good peak symmetry with well-resolved peaks of ATO and PER, respectively. Chamber saturation time was 30 minutes and diffusion of the analyte bands occurred because of solvent migration distances greater than 80 mm.

3.1.2. RP-HPLC Method

A validated isocratic RP-HPLC method with a PDA detector has been developed for the simultaneous quantitation of ATO and PER. It depends on the chromatographic separation of the three components using a C18 column and a mixture of Acetonitrile: Methanol: Buffer (40:10:50) (pH 3) as a mobile phase with UV detection at 240 nm.

All of the experimental conditions affecting the method performance were investigated. According to the literature review, all the reported methods depended on using a C18 column as a stationary phase. Hence, we started our trials on a C18 column with a developing system consisting of acetonitrile, methanol, and water in different ratios. However, Peak splitting of the drugs was observed. Then acetonitrile: methanol: buffer was used as a mobile phase in different ratios. Furthermore, phosphate buffer with different pH values was tested (pH 3 – 4.5) where the optimum separation with symmetric untailed peaks was obtained when using phosphate buffer, pH 3.

The effect of the mobile phase flow rate (1, 1.5, and 2 mL/min) on the separation was also tested, where the use of flow rate of 1 mL/min gave the optimum chromatographic separation within reasonable analysis time. Scanning wavelength was tested to improve the sensitivity of the developed method, where scanning at 240 nm gave the best signal-to-noise ratio.

Finally, a satisfactory separation was obtained by using the mixture of acetonitrile: methanol: buffer (40:10:50) (pH 3) as a mobile phase, maintaining the flow rate at 1 mL/min with UV detection at 240 nm, where ATO was separated after 3.42 min, PER was separated after 8.92 min.

3.2. Method Validation

3.2.1. Linearity and Range

The linearity of the proposed methods was in the range of 800-4800 ng/band for ATO and 200-1200 ng/band for PER in the HPTLC method and the range of 20-100 µg/mL for ATO and 10-50 µg/mL for PER for the RP-HPLC method. Regression and analytical parameters are shown in Table 2. Figure 2 shows a 3D overlay of the HPTLC Densitogram of the calibration concentration ranges of ATO and PER at 221 nm. Figure 3 shows overlay HPLC chromatogram of ATO (20-100 µg/mL) and PER (10-50 µg/mL).

Table 2. Regression data and analytical parameters of the applied methods

Parameter	HPTLC		HPLC	
	ATO	**PER**	**ATO**	**PER**
Linearity range	800-4800 (ng/band)	200-1200 (ng/band)	20-100 (ng/band)	10-50 (ng/band)
Regression coefficient (r^2)	0.9993	0.9993	0.9992	0.9991
Limit of detection (LOD)	134.72 (ng/band)	12.81 (ng/band)	0.68 (µg/mL)	1.06 (µg/mL)
Limit of quantification (LOQ)	408.26 (ng/band)	38.84 (ng/band)	2.05 (µg/mL)	3.21 (µg/mL)
Accuracy (%)	100.5-101.1	99.7-101.1	98.9-100.5	99.2-100.4
Repeatability (%RSD)	1.47	1.11	1.02	1.19
Intraday precision (%RSD)	0.45-1.24	0.62-1.34	0.17-1.36	0.23-1.23
Interday precision (%RSD)	0.68-1.24	0.98-1.65	0.44-1.78	1.09-1.44

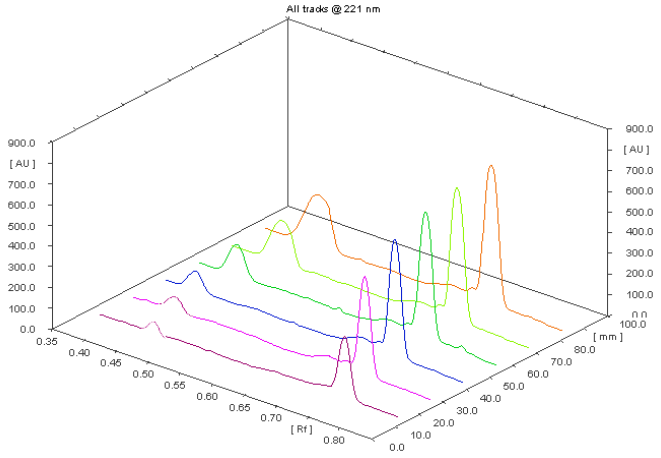

Figure 2. Three-dimensional overlay densitogram of Atorvastatin (800 – 4800 ng/band), Perindopril (200 -1200 ng/band) using Dichloromethane: Methanol: Ethyl Acetate: glacial acetic acid (6:2:2:0.1 v/v/v/v) at 221 nm

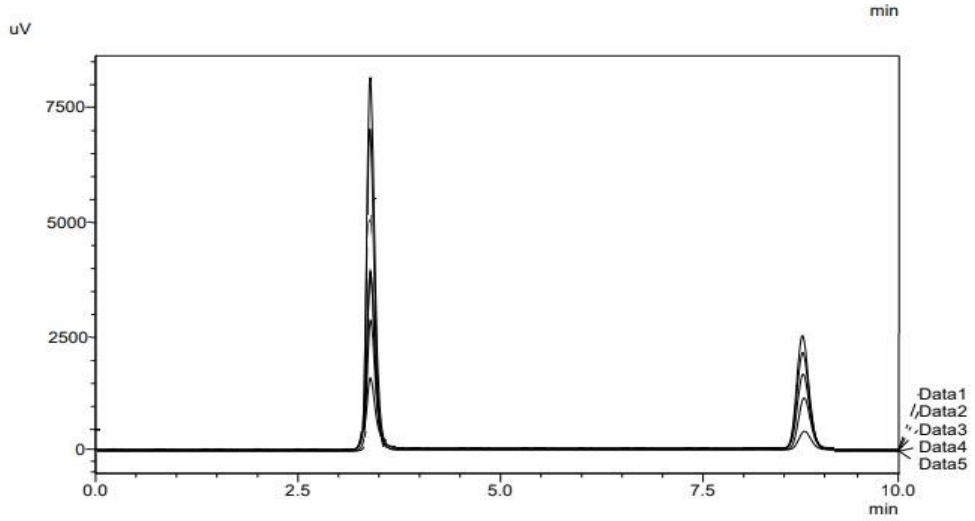

Figure 3. Overlays chromatogram of ATO (20-100 µg/mL) and PER (10-50 µg/mL)

3.2.2. Accuracy

The accuracy of the proposed methods was calculated from the corresponding regression equations. Good percentage recoveries were obtained and are shown in Table 2. Accuracy was further assessed by applying the standard addition technique to a synthetic mixture, where good recoveries were obtained, revealing the good accuracy of the proposed methods and proving that excipients did not interfere.

3.2.3. Precision

The proposed method provides acceptable intra- and Interday variation, indicating the good precision of the method and revealing that it is suitable for the quality control of the suggested components. % RSD was less than 2.0% which represents the proposed method is precise. Table 2 confirms the repeatability and good intermediate precision of the developed methods.

3.2.4. Specificity

The selectivity of the proposed methods was evident from the HPTLC and HPLC chromatograms shown in Figures S5 and S6 in supporting information, respectively.

3.2.5. Sensitivity

Low values of LOD and LOQ shown in Table 2 proved high sensitivity after the developed methods and revealed that the developed methods met specifications for detection limits.

3.2.6. Robustness

Robustness was performed for different chromatographic conditions and the Rf value of the band was considered for the evaluation purpose. The result of the proposed robustness studies is shown in Tables 3 and 4.

Table 3. Robustness study of proposed HPTLC method

Parameter	Change in condition	Mean Peak Area ± S.D (n=3)		% R.S.D.	
		ATO	PER	ATO	PER
Change in detection wavelength	219nm	15804.23±101.71	2074.8±41.37	0.64	1.99
	223nm	15552.27±102.49	2284.76±29.16	0.65	1.27
Change in saturation Time	25 min	15247.63±92.10	2203.06±11.89	0.60	0.53
	35 min	15145±240.88	2492.36±9.46	1.59	0.37

Table 4. Robustness study of proposed HPLC method

Parameter	Change in condition	Mean Peak Area ± S.D (n=3)		% R.S.D.	
		ATO	PER	ATO	PER
Change in detection wavelength	238 nm	30130±153.29	12914.33±77.79	0.50	0.60
	242 nm	33121.33±100.60	13866±86.43	0.30	0.62
Change flow rate	0.9 mL/min	30411±298.13	11777.3±200.13	0.98	0.50
	1.1mL/min	33349 ±168.27	13115.3±80.69	1.69	0.61

3.2.7. System Suitability Testing Parameters

System suitability testing parameters for HPTLC and RP-HPLC were determined. Results for all the studied parameters are shown in Tables 5 and 6.

Table 5. System suitability testing parameters of the developed HPTLC method

Parameters	ATO	PER
Peak resolution	0.74	-
Asymmetric factor	0.33	0.39
Peak purity	0.9991	0.9993

Table 6. System suitability testing parameters of the developed HPLC method

Parameters	ATO	PER
Retention Time	3.42 ± 0.0564	8.92 ± 0.01027
Theoretical Plate	3597 ± 9.36	5468 ± 74.32
Tailing Factor	1.457± 0.0756	1.365 ± 0.9861
Resolution	-	7.328 ± 0.04020

3.3. Analysis of Synthetic Mixture

The developed HPTLC and HPLC method is applied to determine ATO and PER in the synthetic mixture. The % purity of both drugs was found and it is more than 98%. The % assay values are given in Table 7.

Table 7. Assay of synthetic mixture

Parameters	**HPTLC**		**HPLC**	
	ATO	**PER**	**ATO**	**PER**
% Assay ± SD	101.6 ± 30.15	100.42 ± 45.34	100.98 ± 200.31	100.00 ± 60.53
%R.S.D.	1.21	1.76	0.60	0.46

3.4. Uncertainty Assessment

In all analytical methods, it is vital to calculate the measurement uncertainty of different components, which is affecting the measured result and reliability of the quantified result. The uncertainty sources are sampling, preparation of solutions, experimental conditions, instrumentation, calibration, and many more according to the type of sample. All these sources may add uncertainty to the final result and indicates how large these errors could be. Therefore, it is mandatory to incorporate uncertainty together with the reported results. The main uncertainty contributions of the current methods were considered as the standard preparation, slope of the calibration curve, recovery of the sample, and repeatability. They were calculated according to the EURACHEM guide, GUM documents, and reported methods [56-61]. The combined uncertainty was calculated using equation (1).

$$\text{Combined uncertainty (U)} = \sqrt{(\text{ustd})^2 + (\text{ucal})^2 + (\text{urec})^2 + (\text{urep})^2} \quad \textbf{(1)}$$

3.4.1. Uncertainty in the Standard Sample (u_{std})

Purity of Atorvastatin; In the supplier's certificate it is given as 99.5 % pure. The quoted uncertainty is taken as a rectangular (uniform) distribution, so the relative standard uncertainty u_{std} is equal to $\frac{100-99.5}{\sqrt{3}}$ = 0.289%

Purity of Perindopril; In the supplier's certificate it is given as 99.7 % pure. The quoted uncertainty is taken as a rectangular (uniform) distribution, so the relative standard uncertainty u_{std} is equal to $\frac{100-99.7}{\sqrt{3}}$ = 0.173%

3.4.2. Uncertainty in the Slope of Calibration Plot (u_{cal})

A calibration curve was plotted using a spreadsheet and slope and standard error was derived. The data is given in Table 8. Uncertainty in the calibration curve was calculated using the following equation.

$$u_{cal} = \frac{\text{standard error of slope x 100}}{\text{slope}}$$

Table 8. Data of slope of calibration curve

	ATO (HPLC)	PER (HPLC)	ATO (HPTLC)	PER (HPTLC)
Slope	605.691	1069.633	861.993	342.185
Standard Error	1.034	1.771	2.890	1.754
$\mathbf{u_{cal}}$	0.170	0.165	0.335	0.512

3.4.3 Uncertainty in Recovery of Sample (u_{rec})

The mean recovery from the accuracy data was taken into account for calculating the uncertainty in the recovery of the sample. The recovery data is given in Table 9.

Table 9. Data of sample recovery

	ATO (HPLC)	PER (HPLC)	ATO (HPTLC)	PER (HPTLC)
Mean Recovery	99.606	100.6	100.806	100.476
$\mathbf{u_{rec}}$	0.394	0.600	0.806	0.476

3.4.4 Uncertainty Associated with Repeatability (u_{rep})

The relative standard deviation of the reputability data of atorvastatin and perindopril was considered as the uncertainty of repeatability. The data is given in Table 2.

3.4.5 Expanded Uncertainty

According to equation 1, the combined uncertainty was calculated. Then, the expanded uncertainty was calculated at a 95% confidence level by multiplying the combined uncertainty with the coverage factor (k=2). The combined and expanded uncertainty value is given the Table 10.

Table 10. Data of combined and expanded uncertainty

HPLC	$\mathbf{u_{std}}$	$\mathbf{u_{cal}}$	$\mathbf{u_{rec}}$	$\mathbf{u_{rep}}$	Combined uncertainty	Expanded uncertainty
ATO	0.289	0.170	0.394	1.020	1.143	2.287
PER	0.173	0.165	0.600	1.190	1.353	2.707
HPTLC	$\mathbf{u_{std}}$	$\mathbf{u_{cal}}$	$\mathbf{u_{rec}}$	$\mathbf{u_{rep}}$	Combined uncertainty	Expanded uncertainty
ATO	0.289	0.335	0.806	1.470	1.733	3.467
PER	0.173	0.512	0.476	1.110	1.323	2.646

4. Conclusions

The presented HPLC and HPTLC methods provide highly selective methods for the quantitative determination of ATO and PER in a synthetic mixture. To the best of our knowledge, these were the first reported methods as per the literature survey to date. The HPLC method is the first developed one to analyze this binary mixture at a single wavelength in a short analysis time and has the advantage of being more reproducible. The HPTLC method has the advantage of high sensitivity and using HPTLC plates with smaller particle size and higher resolution ability besides using a small quantity of developing system. The major advantage of HPTLC is that multiple samples can be analyzed simultaneously using a small quantity of mobile phase, thus reducing the analysis time and cost per analysis. The developed HPTLC and HPLC techniques showed high selectivity, accuracy, and reproducibility. These merits

suggest the use of the proposed methods in routine and quality control analysis without the interference of commonly encountered pharmaceutical preparation additives.
The proposed analytical methods were compared using statistical analysis. The F test was applied and does not reveal a significant difference between the experimental values obtained in the sample analysis by the two methods. The calculated f-value was found to be less than the critical f-value at a 5% significance level.

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/ journal-of-chemical-metrology

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