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Research article

Development and validation of a simple method for the determination of Atorvastatin calcium in pure and pharmaceutical formulations using spectrofluorimetry

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ABSTRACT

A simple, accurate, precise, sensitive and selective spectrofluorimetric method was developed and validated for the determination of Atorvastatin calcium (ATV), an HMG-CoA reductase inhibitor, in its pure and tablet dosage form. The proposed method was based on direct measurement of the native fluorescence of ATV. Fluorescence analysis was accomplished by using an emission wavelength 385 nm after excitation at the wavelength of 270 nm in acetonitrile, without difficult preparation steps of the sample solution such as separation, extraction, pH adjustment or derivatization. All variables affecting the fluorescence intensity such as measurement time, temperature, and diluting solvent were investigated and optimized. Under the typical conditions, a validation study for linearity, range, accuracy, precision, selectivity and robustness of the proposed method was implemented according to ICH guidelines. The fluorescence intensity was linear over concentration range of (0.4–12) μg/ml ($r = 0.9999$), and the lower limits of detection and quantification were 0.079 and 0.24 μg/ml, respectively. Good accuracy and precision results were obtained through using the presented method with excellent mean recovery value 100.08 ± 0.32 which was in the acceptable range (98.0–102.0%), and RSD <2%, proving the precision of the developed method. Specificity was proved in the presence of excipients and Amlodipine besylate (AML) which encountered usually as combined drug with ATV. The developed method was successfully applied to the analysis of pharmaceuticals containing the mentioned drug with no interference from other drugs or dosage form additives, and the recoveries were in the range of 99.11 ± 0.75 to 100.89 ± 0.70. Furthermore, the obtained results were compared with reported HPLC method. Then, the *t*- and *F*- values were calculated and compared with the theoretical ones, which indicate good precision and high accuracy of the proposed method. Therefore, this method is valuable, reliable, and very suitable to be applied in routine quality control laboratories.

1. Introduction

The emission of light from electronically excited substances is known as luminescence [1,2]. Every molecule-in general-has a series of energy levels, and by absorbing a distinct quantum of light that is proportional in energy to the variance between both of the energy states, it can move from a lower energy level to a higher one. On the other hand, only a few molecules are able to interact with light and exhibit luminescence [3].

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Luminescence can be divided into a number of different categories according to the mode of energy required to excite the luminescent molecule. Photoluminescence considered to be the most encountered type, and it is formally classified, relying on the nature of the excited states into two groups, fluorescence and phosphorescence [1,4]. Fluorescence occurs, after the absorption of light, by emitting a photon from the lowest vibrational energy level of a singlet excited state to a lower energy level of the singlet ground state as a result to the energy lost (e.g. vibrational relaxation) [4]. It usually takes place in liquid solutions at a moderate temperature, and effected by several factors, could be structure related factors (change of pH values, substituents, solvent polarity, and molecular rigidity), the presence of quenching substances, or inner-filter effect (the effect of concentration) [5].

According to the fact that the intensity of the emitted light depends upon the concentration of a fluorescent substance, therefore, fluorescence has been significantly used for the quantification of materials by measuring the emission intensity under suitable excitation wavelength. Therefore, spectrofluorimetry is convenient for analytical purposes, by the usage of spectrofluorimeter apparatus [5].

Atorvastatin calcium is a synthetic statin that was approved in 1997, chemically described as Calcium (βR, δR) -2- (p-fluorophenyl) -β, δ-dihydroxy-5-isopropyl-3-phenyl-4- (phenylcarbamoyl) pyrrole-1-heptanoic acid (1:2) trihydrate. The empirical formula is $C_{66}H_{68}F_2N_4O_{10}.3H_2O$, with a molecular weight 1209.4 g/mol [6]. It is white to off-white crystalline powder, freely soluble in methanol, slightly soluble in ethanol, insoluble to very slightly soluble in distilled water, pH 7.4 phosphate buffer and in acetonitrile. It is also insoluble in the acidic aqueous solutions which have pH value equal to 4 or below [7].

ATV is a selective competitive inhibitor of 3-hydroxy-3-methylglutaryl-Co-enzymeA reductase (HMG-CoA reductase) which catalysis an early rate-limiting step in cholesterol biosynthesis [8,9], as is feature a fluorophenyl group capable of forming bound at the active site of this enzyme [10], and is used for the treatment of hypercholesterolaemia, combined (mixed) hyperlipidaemia (Fredrickson Type IIa or Type IIb), hypertriglycerideamia (Type IV) and dysbetalipoproteinaemia (Type III). It reduces LDL-C, apolipoprotein B, TG, and increases HDL-C [6]. It is administrated as the calcium salt of the active hydroxyl acid, and has half-life of about 20 h, it is safe for patients with renal dysfunction as it does not require dose adjustment, and it is prescribed for children 11 years and older [8]. It is believed that of all statins, ATV is the only one with a metabolite that has an equivalent ability for the inhibition of HMG-CoA reductase compared with the parent drug [10].

The pharmacological importance of ATV has promoted several analytical methods for the control of its concentration, and evaluate the compliance of this substance with pharmacopeial specifications.

Literature survey revealed different analytical approaches described for the determination of ATV individually or in combination with other substances in laboratory prepared mixtures, pharmaceutical preparations, and biological fluids. The most commonly used methods for this purpose include chromatographic methods with different detectors [11–21], spectrophotometric methods [22–28], and spectrofluorimetric methods [11,29–31].

Most of the reported methods suffer from many disadvantages compared with spectrofluorimetry. Chromatographic methods require expensive equipment and chemicals; in addition, long run time is taken for each experiment. While spectrophotometric methods are not such sensitive and usually need for tedious control of experimental conditions especially in multistep reactions. Recently, spectrofluorimetry is considered to be the technique of choice in quality control laboratories owing to its remarkable features that is simplicity, rapidity of the sample preparation and analysis, inherent sensitivity, high throughput, and low detection limits compared with spectrophotometric and chromatographic methods [32].

Therefore, the aim of this study, is to develop a simple, accurate, sensitive, selective, and economic spectrofluorimetric method for the determination of ATV in pure and tablet dosage forms with no interference with excipients and combined drugs like amlodipine, also without difficult preparation steps of the sample solution such as prior separation steps, extraction, pH adjustment or dye complex formation to adjust excitation and emission wavelengths, which makes this method not time consuming and very suitable in academic institutions and pharmaceutical industries.

2. Experimental

2.1. Chemicals and reagents

The working standard of ATV was kindly supplied by Ibn-Alhaytham pharmaceutical industries, Aleppo-Syria. According to the certificate of analysis, the percentage purity was reported to be 99.14%. The pharmaceutical formulation samples were: Atorvex® tablets (Asia Pharmaceutical Industries, Aleppo-Syria), Atorvast® tablets (Avenzor Pharmaceutical Industries, Damascus-Syria), Atorvatin® tablets (Alpha-Aleppo Pharmaceutical Industries, Aleppo-Syria) labeled to contain 10, 20, 40 mg of ATV per tablet. Low-lip® tablets (AL Fares Pharmaceutical company, Damascus-Syria) labeled to contain 10, 40 mg of ATV per tablet. Atoraz® tablets (Razi Pharmaceutical Industries, Aleppo-Syria) labeled to contain 10, 20 mg of ATV per tablet. Stati-Health® tablets (Human Pharma, Tartous-Syria) labeled to contain 10 mg of ATV per tablet. Amlostin® tablets (Nawras Pharmaceutical Industries, Hama-Syria) labeled to contain 5/10 mg of Amlodipine besylate and ATV per tablet, respectively. Cardef® tablets (Hama Pharma for Pharmaceutical Industries, Hama-Syria) labeled to contain 10/10 mg of Amlodipine besylate and ATV per tablet, respectively. All samples were purchased at random from the local pharmacies and were stored at ambient temperature and humidity. They were analyzed within the expiry dates.

All the reagents used in the study were of analytical grade: methanol 99.7% was supplied from (Scharlau- Spain), acetonitrile (Biosolve- France), acetone (Panreac-Spain), dimethylformamide and ethanol (Chem Lab-Belgium), isopropanol and acetic acid glacial 99.51% (Surechem Products LTD- England), double distilled water has been used in all experiments.

2.2. Instrumentation

In this study, Fluorescence spectrophotometer (Hitachi F-2700, Japan) equipped with a xenon lamp (150 W) and 1 cm quartz cells was used for all measurements. Excitation and emission wavelength were set at 270 nm and 385 nm, respectively. The wavelength band pass of excitation and emission monochromators were both set at 10 nm. All the assays were achieved at room temperature. pH of acetic acid 5% was adjusted using a pH meter from (GLP21, Crison- Spain). Solubility of the compound was improved using the sonication with an ultrasonic processor (Power sonic 405, Hwashin- Korea). The Megafuge 2.0 R centrifuge (Heraeus Instruments, Germany) was employed for the centrifugation process. An analytical balance ±0.1 mg furnished by (ED224S, Sartorius- Germany). All glass ware was cleaned with double distilled water and dried whenever required.

Chromatographic analysis was carried out with HPLC system, which consisted of (Agilent 1260 infinity, Germany) equipped with UV detector, vacuum degasser, and auto injector. The separation was performed on an InertSustain C18 column (250*4.6 mm id, 5 μm particle size). The flow rate was monitored at 1.5 ml/min. The detection was carried out at 244 nm, with a selected injection volume of 5 μl. The temperature of the column oven was preserved at 40 °C.

2.3. Preparation of standard stock solution

From the standard (authentic) ATV powder, an accurately weighed amount (100 mg) of the studied drug was transferred into a 100 ml volumetric flask, dissolved in 60 ml methanol, and was sonicated for 15 min. The solution was then diluted to the mark with the same solvent to obtain the following working standard solution of 1 mg/ml of ATV. This solution was kept at ambient temperature in light protected place and remained stable for at least 3 weeks. The starting standard solution was diluted quantitively to obtain the required concentrations for assay.

2.4. Determination procedure

2.4.1. Procedure for the determination of ATV by the proposed method

Accurately measured aliquots of ATV working standard solution equivalent to 0.4–12 μg/ml were transferred from its stock solution into series of 10 ml volumetric flasks. Then it was completed to mark with acetonitrile and mixed thoroughly. The fluorescence intensity of ATV solutions was measured at λem 385 nm by using an excitation wavelength of 270 nm against reagent blank solution of acetonitrile that had been treated similarly. The fluorescence intensity F was plotted against concentration C. The calibration curve was organized and the regression parameters were calculated, then, the calibration curve was utilized to estimate the amount of ATV in tablets.

2.4.2. Procedure for the determination of ATV by HPLC method

Different volumes of 1 mg/ml ATV standard solutions equivalent to 0.3125–0.5 mg/ml ATV were pipetted into 10 ml standard volumetric flasks and diluted up to the mark with methanol. HPLC column was passed with the mobile phase that consisted of (10^{-3} M cetrimide, acetonitrile in the ratio of 35: 65 v/v, pH 10) for base line correction. The elution was monitored at 244 nm, keeping an injection volume of 5 μl ATV solution into the sample part, and a flow rate of 1.5 ml/min for the mobile phase. After that, the chromatogram of the sample was recorded while temperature of the column oven was kept at 40 °C. The calibration graph was constructed by plotting the peak area of the chromatograms against concentrations of ATV, and the linear regression equation was computed to estimate ATV in commercial tablets.

2.4.3. Procedure for pharmaceutical samples by the proposed method

Ten individual tablets of each formulation were weighed and pulverized carefully in a mortar. After that, a quantity of these mixed powders, equivalent to 10 mg of ATV, was transferred into a 10 ml volumetric flask and was extracted with 10 ml of methanol. The content was twirled and sonicated for 10 min till the active component was dissolved. Portion of this solution was centrifuged at 2500 rpm for 10 min, then suitable dilution with acetonitrile was made to get a final concentration in the range of linearity (C = 6 μg/ml). After that, the analysis took place following the proposed procedure (*2.4.1*). The actual content of the tablets was calculated using the corresponding regression equation from the previously plotted calibration graph. Three replicates were acquired.

2.4.4. Procedure for pharmaceutical samples by the HPLC method

Three commercially available preparations of ATV were selected for the HPLC analysis. All the brands labeled to contain 10 mg ATV. Ten tablets of each preparation were precisely weighed and finely shrivelled to powder. An amount equivalent to 10 mg of active ingredient was weighed and dissolved in methanol using a 10 ml volumetric flask. Then, the solution was sonicated for 10 min and an appropriate dilution with the same solvent was prepared to get a final concentration (C = 0.25 mg/ml) of the linearity range. After that, the drug solution was passed through syringe filter 0.45 μm Millipore membrane for ultra-purification. Then, the amount of ATV in commercial tablets was determined by following recommended procedure as prescribed abovementioned (*2.4.2*).

3. Results and discussion

3.1. Fluorescence spectra

Atorvastatin calcium has native fluorescence in acetonitrile (Fig. 1). illustrates the excitation (A) and emission (B) spectra of 6 μg/ml of the studied drug. The excitation spectra were obtained showing a maximum excitation wavelength at 270 nm, then the emission spectra were scanned from 300 to 500 nm, showing an emission wavelength maximum at 385 nm. The emission spectra of ATV standard solutions, and that of the pharmaceutical formulations, were scanned against acetonitrile as blank. It was found that the fluorescence intensity of the drug was directly proportional with its concentration in a certain range.

3.2. Optimization of fluorescence conditions

All the factors impacting the fluorescence intensity were optimized by changing one variable and maintaining the others constant by using 6 μg/ml of the studied drug.

3.2.1. Effect of measurement time

The effect of measurement time on fluorescence intensity of ATV was studied. Emission spectra measurements were performed using acetonitrile at seven time intervals (0, 2, 5, 10, 20, 30, and at 60 min). As shown in (Fig. 2), A steady and maximum intensity was noticed when sample solution was measured within 2 min. Therefore, all samples were measured immediately to obtain the maximum intensity of the emission spectrum.

3.2.2. Effect of temperature

In this study, the effect of temperature on fluorescence intensity was examined. To do that, measurements were achieved at five different temperature values (25 °C, 35 °C, 45 °C, 55 °C, and 65 °C). According to the practical study illustrated in (Fig. 3), high and steady intensity was obtained at 25 °C. It was noticed that fluorescence intensity was reduced as the temperature increases due to the decrease in the fluorescence quantum yield since the non-radiative processes associated with thermal agitation (collisions with solvent molecules, intramolecular vibrations and rotations) are more effective at high temperatures [4]. Thus, lab temperature (25 °C) was selected as the optimum value to carry out further experiments.

3.2.3. Effect of diluting solvent

In order to select the most appropriate solvent for dilution, the effect of solvents on the native fluorescence of ATV was investigated. Different solvents with different polarities were examined: water, methanol, ethanol, isopropanol, acetonitrile, acetone, dimethylformamide (DMF), NaOH 0.1 M, HCl 0.1 M and acetic acid 5% (pH 2.5). Then, results were illustrated in (Fig. 4). Interestingly, the fluorescence intensity of the studied drug was completely quenched in acetone, while it was found to be unaltered with ethanol and isopropanol. A relatively lower and less stable fluorescence intensity was observed in methanol. However, the highest fluorescence intensity was observed in acetonitrile; therefore, it was selected for further investigations as it afforded maximum sensitivity of measurement and stability of readings.

3.3. Method validation

The aforementioned method was validated pursuant to International Conference on Harmonization guidelines (ICH) on the validation of analytical procedure, in terms of linearity, range, limit of detection, limit of quantitation, accuracy, precision, selectivity, and robustness [33].

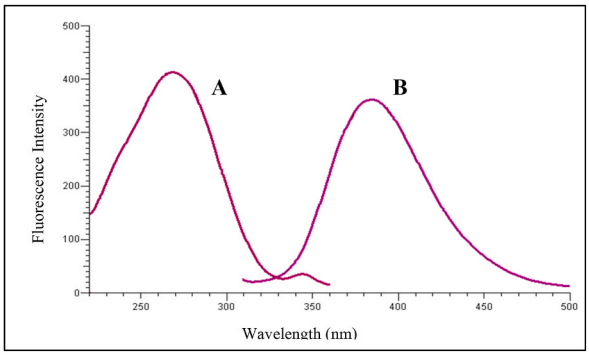

Fig. 1. Excitation (A) and emission (B) spectra of 6 μg/ml ATV in acetonitrile at 385 nm and 270 nm, respectively.

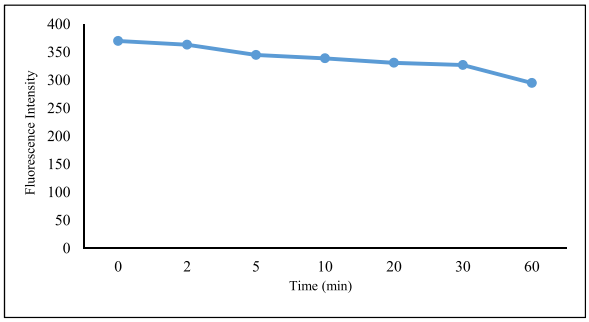

Fig. 2. Effect of measurement time on fluorescence intensity of 6 μg/ml ATV.

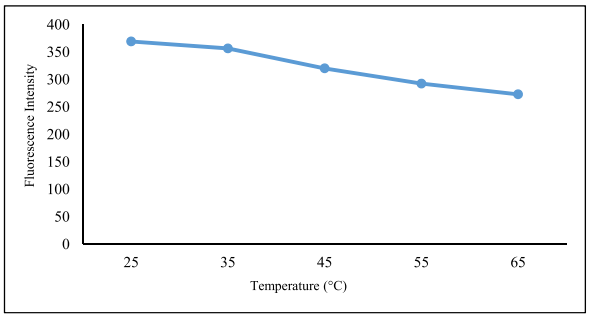

Fig. 3. Effect of temperature on fluorescence intensity of 6 μg/ml ATV.

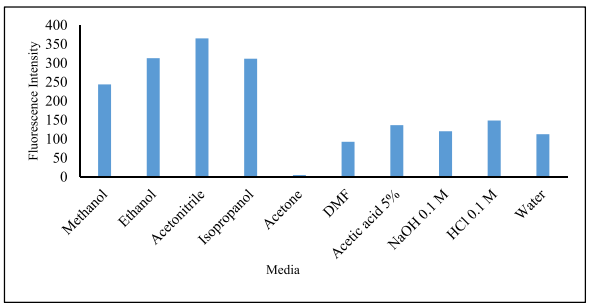

Fig. 4. Effect of different diluting solvents on fluorescence intensity of 6 μg/ml ATV.

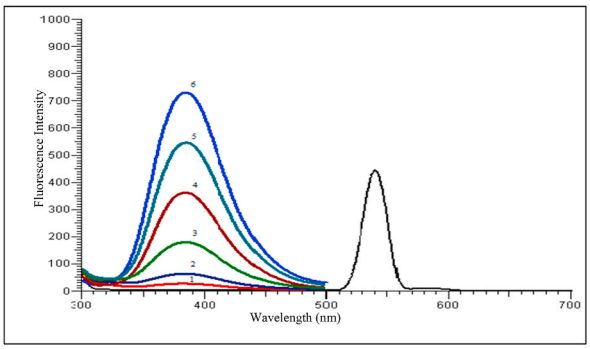

Fig. 5. Fluorescence spectra of standard solutions of ATV at concentrations as the follows: 1–0.4, 2–1, 3-3, 4–6, 5–9, 6–12 μg/ml against acetonitrile as blank.

3.3.1. Linearity and range

Linearity is known as the ability of an analytical method to obtain test results, that are proportional with the analyte concentration in the sample within a specified range. Under the optimum described experimental conditions, linearity was evaluated by the analysis of working standard solution of ATV at six different concentration levels within the working range according to the general procedure previously established for the proposed method (Fig. 5).

All measurements were performed in triplicates. Standard calibration graph was constructed through plotting the fluorescence intensity versus ATV concentration, then regression equation was computed (Fig. 6).

The correlation coefficient was 0.9999 indicating good linear relationship between measured values of fluorescence intensity and concentration of ATV over a concentration range of 0.4–12 μg/ml. Detection and quantification limits were obtained to evaluate sensitivity of this approach. They were assessed via slope method (calculation method) depending on the standard deviation of the intercept of the calibration curve and the slope of regression line, using the following equations:Where σ is the standard deviation of y-intercept, and S is the slope of the calibration graph. The intercept, slope and other statistics parameters were listed in Table 1.

3.3.2. Accuracy

Accuracy of the proposed method was studied by analysing the percentage recoveries of ATV standard solution at three different concentrations within its linearity range, each in triplicates. Recovery values were obtained by comparing the actual amount with the nominal one. The results obtained -including the mean of the recoveries, standard deviation, and relative standard deviation-are displayed in Table 2.

According to the obtained results, the method recovery of standard drug was proved to be accurate. The mean recovery value (100.08) % was in the acceptance range of accuracy (98–102) %, and the RSD did not exceed 2%. Therefore, the recovery of the proposed method was confirmed and accepted.

In addition, the accuracy of the proposed technique was checked by using standard addition method for tablets. A fixed weight of tablet equivalent to 10 mg of the drug was used, then (80%, 100%, and 120% of the fixed concentration were added separately using pure drug. The proposed method was used to reanalyse these mixtures for three times and the percentage recovery was estimated. The excellent level of recovery is satisfactory for quality control examination of ATV in their pharmaceutical tablets by the suggested method, and the decrease in the standard deviation indicates the realistic accuracy of the presented method (Table 3).

3.3.3. Precision

Repeatability of the proposed method was estimated by measuring six replicates of fixed concentration (6 μg/ml) of ATV prepared in one laboratory on the same day. Intra-day precision was evaluated by preparing samples of drug standard solution at three concentration levels, covering 50, 100, and 150% of the concentration (6 μg/ml), and analyzing them in triplicates on the same day using the submitted method, while inter-day precision was determined by analyzing the same samples on two progressive days. The relative standard deviation values (RSD%) were calculated and acceptable results were obtained. The values of RSD% of repeatability were listed in Table 4. The results of intraday and interday precision for the drug were summarized in Table 5.

RSD% values were found to be less than 1% for repeatability and intra-day studies, and <2% for interday precision which reflects good precision of the proposed method at the two levels of repeatability and intermediate precision.

3.3.4. Selectivity

In the present study, selectivity was evaluated by studying the effect of some excipients that are frequently encountered in pharmaceutical preparations. Sample solutions were prepared through blending known amount of ATV (10 mg) with different quantities of each excipient. Then, these samples had been analyzed by the proposed method. The spectra of these samples were checked for the appearance of any new spectrum of each added excipient. As shown in (Fig. 7), none of the studied excipients such as calcium carbonate (Fig. 7A), magnesium stearate (Fig. 7B), and PVP-K30 (Fig. 7C) has given a significant fluorescence intensity or quenching activity, besides, the spectrum of the drug in the sample solutions was identical to the spectrum received by the standard solution at the wavelength applied.

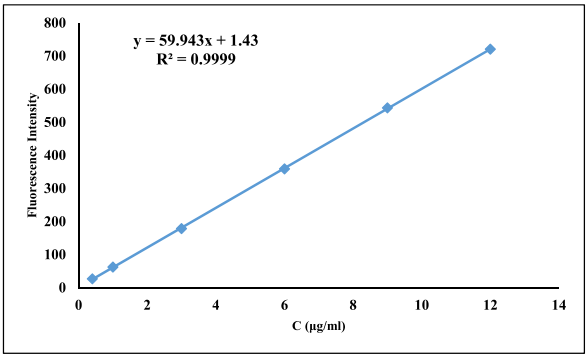

Fig. 6. Calibration curve of ATV concentrations versus fluorescence intensity in acetonitrile at λex 270 nm.

Table 1
Analytical and regression parameters for the analysis of ATV by the proposed spectrofluorimetric method.

Parameter	Proposed method
λex/λem (nm)	270/385
Linearity range (μg/ml)	0.4–12
Regression equation	y = 59.943x + 1.43
N	6
Correlation coefficient (r)	0.9999
Slope	59.943
SD of slope	0.214
Intercept	1.43
SD of intercept	1.439
Limit of detection LOD (μg/ml)	0.079
Limit of quantification LOQ (μg/ml)	0.24

Table 2
Accuracy results for determination of ATV in pure form by the developed spectrofluorimetric method.

C Taken (μg/ml)	F.I[a]	C Found (μg/ml)	Mean[b] ± SD	Recovery%	Mean recovery% ± SD	RSD%
3	179.2	2.96	2.99 ± 0.019	99.66	100.08 ± 0.32	0.32
	181.2	2.99				
	181.9	3.01				
6	361.5	6	6.01 ± 0.033	100.16		
	364.8	6.06				
	360.3	5.98				
9	542.5	9.02	9.04 ± 0.014	100.44		
	544	9.05				
	544.5	9.05				

[a] Fluorescence Intensity.
[b] Average of three determinations (n = 3).

Table 3
Accuracy results for determination of ATV in pure form by the developed spectrofluorimetric method using standard additions technique.

Dosage form	C taken (μg/ml)	C added %	C found[a]	Recovery%[a] ±SD	Mean recovery% ± SD
Atorvex ® tablets	4	80	7.18	99.89 ± 1.255	99.89 ± 0.053
		100	7.98	99.83 ± 1.014	
		120	8.79	99.96 ± 0.326	

[a] The value is the Average of three determinations.

Table 4
Repeatability study for the determination of ATV by the developed spectrofluorimetric method.

Sample No	Theoretical concentration (μg/ml)	F.I[a]	Mean[b] ±SD	RSD%
1	6	361.7	362.58 ± 1.744	0.48
2		360.2		
3		364.9		
4		361.2		
5		362.8		
6		364.7		

[a] Fluorescence Intensity.
[b] Average of six determinations (n = 6).

In addition, a possible effect of AML, which is commonly combined with ATV in tablets, was thoroughly studied with no interference observed at the selected emission and excitation wavelengths for the determination of ATV (Fig. 8A). illustrates emission spectrum of ATV standard solution. In addition, fluorescence spectrum of standard solution of ATV in presence of AML, and sample solution of commercial tablets of ATV were illustrated in (Fig. 8B), and (Fig. 8C), respectively. Recovery results in presence of excipients and AML were displayed in Table 6 and Table 7, respectively.

Results in Table 6 reveal that recoveries of the drug were within the acceptance limits, and ranging from 99.47 ± 0.35 to 101.31 ± 0.18, which demonstrate the absence of interference from other materials in the pharmaceutical formulations. In addition, the data in Table 7 confirm that no interference was observed from AML. These results indicate that the presented method was highly selective for the analysis of ATV with no interference with excipients or the combined drug AML. Thus, the proposed method can be considered

Table 5
Intermediate precision study for the determination of ATV by the developed spectrofluorimetric method.

Proposed method	Concentration (μg/ml)		RSD%	SE[b]	C.L[c]
	Taken	Found± SD[a]			
Intra-day precision	3	2.98 ± 0.016	0.56	0.009	0.039
	6	5.99 ± 0.047	0.78	0.027	0.116
	9	8.98 ± 0.053	0.59	0.03	0.131
Inter-day precision	3	2.98 ± 0.025	0.84	0.01	0.026
	6	5.98 ± 0.065	1.1	0.026	0.068
	9	8.99 ± 0.061	0.68	0.024	0.064

[a] Standard deviation of three and six independent determinations for intra-day and inter-day precision, respectively.
[b] SE standard error.
[c] C.L confidence limit at 95% confidence level and two degrees of freedom (t = 4.303) for intra-day; and five degrees of freedom (t = 2.571) for inter-day precision.

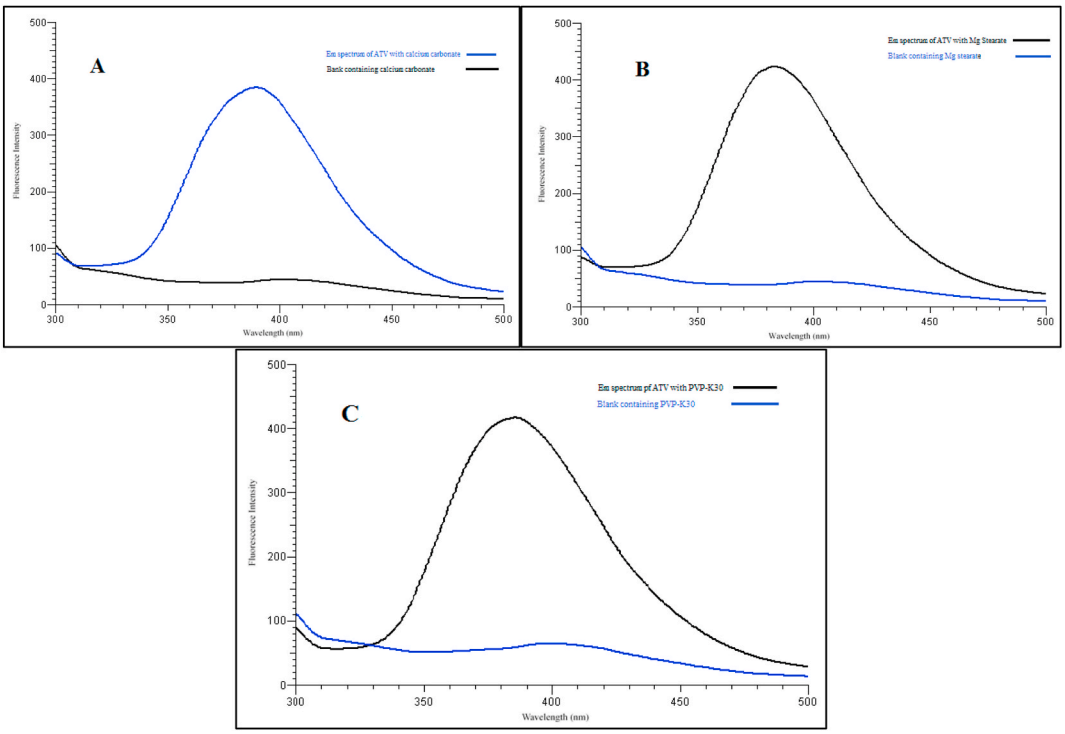

Fig. 7. Fluorescence spectra of standard solutions of 6 μg/ml ATV in presence of some excipients: (A) calcium carbonate, (B) Mg stearate, and (C) PVP-K30 against (acetonitrile-excipient) as blank.

selective, and can be suitable for the routine analysis of ATV in its dosage forms.

3.3.5. Robustness

The ability of the proposed method to stay unaffected by small variations in the experimental parameters mirrors the robustness of the method. Robustness was well checked by investigating the effect of deliberate variations in the following experimental variables: time for measurement (+1, +2 min), the temperature of measurement (±5 °C), and the excitation wavelength (±1 nm). For this purpose, percentage recovery was determined for each experiment, through changing one parameter and keeping the others constant. The acquired information recorded at Table 8 demonstrated that The percentage recovery ranged from 99.04 ± 0.2 to 101.03 ± 0.18, and all calculated RSD% values did not exceed 2%. Thus, these little variations that may happen during the analytical work did not have any significant effect on fluorescence intensity of the drug, indicating that the proposed method is considered to be robust and can be applied easily in quality control laboratories with no regards to minor changes.

3.4. Application to pharmaceutical formulations for quality control

Six pharmaceuticals containing ATV, and two pharmaceuticals containing ATV/AML combination were analyzed using the

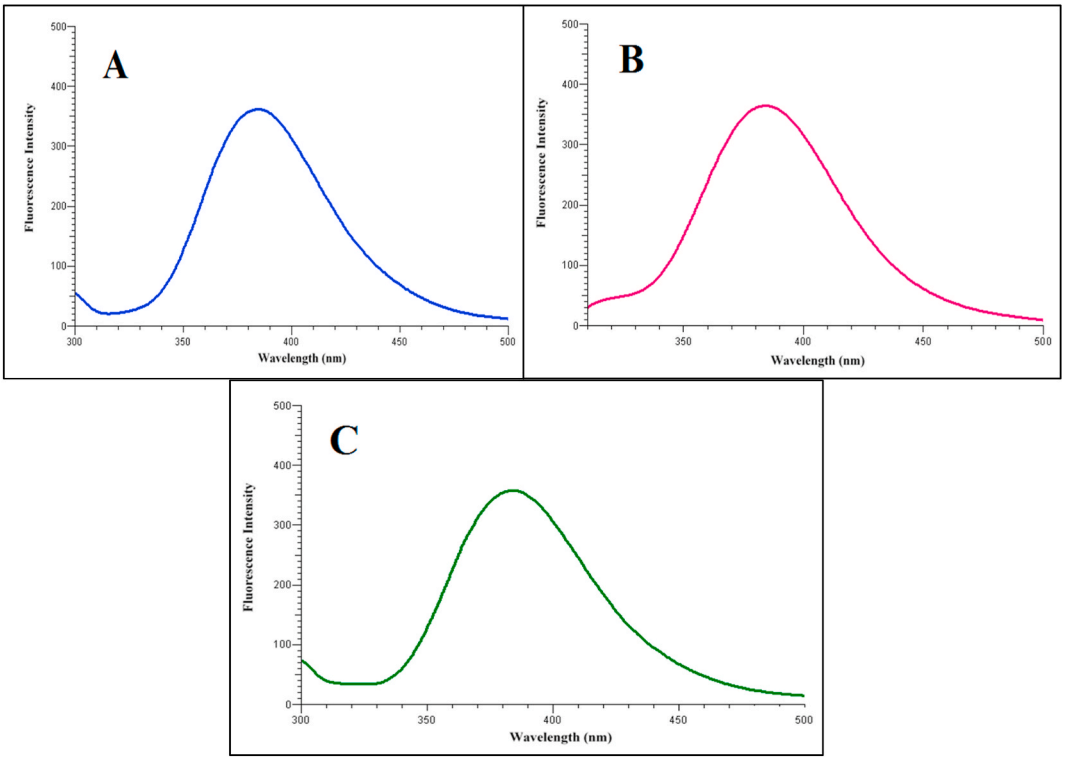

Fig. 8. Fluorescence spectra of (A): standard solutions of 6 μg/ml ATV, (B): standard solutions of 6 μg/ml ATV in presence of 3 μg/ml of AML, and (C): sample solution of 6 μg/ml ATV in commercial tablets.

Table 6
Selectivity of the proposed method for the analysis of ATV in presence of common excipients.

Excipients	Amount added[a] (mg)	Recovery%[b]	RSD%
Calcium carbonate	20	100.87	0.21
Lactose monohydrate	20	100.38	1.10
Microcrystalline cellulose	20	101.31	0.18
PVP.K30	5	99.47	0.35
Talc	10	99.67	1.30
Magnesium stearate	0.6	100.29	1.43

[a] The amount added of each excipient to 10 mg of ATV.
[b] The value is the Average of three determinations.

Table 7
Selectivity of the proposed method for the analysis of ATV in presence of AML.

Amount of ATV (mg)	Amount added of AML (mg)	Recovery%[a]	RSD%
10	5	99.10	0.53
10	10	100.18	1.25

[a] Average of three determinations.

described method. Quantification was carried out in triplicates and the mean recoveries were calculated. The obtained data of commercial tablets contents were reported in Table 9 and Table 10.

The acceptable range mentioned in USP pharmacopeia for ATV tablets is 94.5–105% of the labeled amount [34]. The actual content of ATV was in the range of (99.11%–100.89%) of the labeled claim and RSD% values were less than 2% for its individual dosage forms, whereas the actual content of ATV in its combination with AML were (99.06% and 99.90%) of the labeled claims as shown in Table 10, with RSD% values of 0.19 and 0.30 for Amlostin® and Cardef®, respectively, which were identical to the pharmacopeial acceptance criteria. Therefore, the proposed method considered to be very applicable for the determination of ATV in pharmaceuticals. In addition, the method was successfully applied for the estimation of ATV in presence of AML, which allows a rapid, selective, and easy analysis of ATV content in quality control experiments.

Table 8
Robustness of the proposed spectrofluorimetric method through the analysis of ATV (6 μg/ml).

Method parameters		Recovery%[a]	RSD%
Measurement time (min)	0	100.85	0.42
	1	99.78	0.35
	2	99.36	0.26
Temperature (°C)	20	99.28	0.17
	25	99.61	0.13
	30	99.41	0.68
Excitation wavelength (nm)	269	101.03	0.18
	270	100.30	0.54
	271	99.04	0.2

[a] The value is the Average of three determinations.

Table 9
Results obtained by application the developed spectrofluorimetric method for the determination of ATV in pharmaceuticals.

Dosage forms	Manufacture name	Labeled content (mg)	Batch. No	C Found (μg/ml)	Mean[a]±SD	Recovery%[ab]	RSD%
Atorvast® tablets	Avenzor	10	251430	5.89 6.03 5.94	5.95 ± 0.057	99.32	0.92
		20	241195	5.97 5.89 6.05	5.97 ± 0.065	99.55	1.09
		40	251433	6.02 6.08 6.04	6.04 ± 0.024	100.85	0.46
Atorvex® tablets	Asia	10	111427	6.03 6.01 5.98	6.00 ± 0.020	100.20	0.34
		20	12763	5.93 5.90 6.00	5.94 ± 0.041	99.11	0.75
		40	13093	6.00 5.92 5.94	5.95 ± 0.033	99.27	0.61
Atorvatin® tablets	Alpha	10	201116B	6.05 6.1 6.00	6.05 ± 0.040	100.89	0.70
		20	201115A	5.99 5.92 6.02	5.97 ± 0.041	99.70	0.72
		40	201114A	5.90 5.92 6.04	5.95 ± 0.061	99.27	1.03
Atoraz® tablets	Razi	10	T61	6.08 6.01 5.92	6.00 ± 0.065	100.15	1.09
		20	T30	5.94 5.93 5.96	5.94 ± 0.012	99.12	0.16
Stati-Health® tablets	Human	10	011	5.90 5.97 5.97	5.94 ± 0.032	99.19	0.57
Low-Lip® tablets	Al Fares	10	164	6.07 6.00 6.03	6.03 ± 0.028	100.59	0.48
		40	104	6.05 6.05 6.03	6.04 ± 0.009	100.80	0.19

[a] Average of three determinations.
[b] Recovery values were calculated considering the theoretical concentration was taken (6 μg/ml).

3.5. Statistical analysis

The results acquired from applying the proposed method for quality control of ATV in its tablets were obtained and statistically compared with the reference method [16] with respect to *t*- and *F*-tests at 95% confidence level as shown in Table 11. The average of recovery values was found to be at the range of 100.2 ± 0.111 to 100.7 ± 0.181. The calculated *t*- and *F*-values were less than the

Table 10
Results obtained by application the developed spectrofluorimetric method for the determination of ATV in its combined dosage forms with amlodipine besylate.

Dosage forms	Manufacture name	Labeled content AML/ATV (mg)	Batch. No	C Found (μg/ml)	Mean[a] ±SD	Recovery %[ab]	RSD%
Amlostin®	Nawras	5/10	03	5.93 5.93 5.96	5.94 ± 0.014	99.06	0.19
Cardef®	Hama Pharma	10/10	F_2 12	5.99 6.01 5.97	5.99 ± 0.016	99.90	0.30

[a] Average of three determinations.
[b] Recovery values were calculated considering the theoretical concentration was taken of ATV (6 μg/ml).

Table 11
Statistical comparison between the results obtained by the proposed spectrofluorimetric method and reference method for the determination of ATV dosage forms.

Dosage form	Labeled content (mg)	Recovery%[a] ±SD		*t*-value[b]	*F*-value[b]
		Proposed method	Reference method [16]		
Atorvex® tablets	10	100.20 ± 0.111	100.45 ± 0.122	2.613	1.209
Atorvast® tablets	20	100.70 ± 0.181	101.20 ± 0.470	1.730	6.731
Atorvatin® tablets	40	100.52 ± 0.559	100.81 ± 1.346	0.340	5.785

[a] Average of three determinations; were calculated considering the labeled amount reported by the manufacturing.
[b] Tabulated values at 95% confidence limit are $t = 2.776$, $F = 19.000$.

tabulated ones indicating that there is no significant difference between the proposed method and the reported one with respect to accuracy and precision, which mirrors the suitability of the proposed method for the examination of ATV in dosage forms.

3.6. Comparison with announced spectrofluorimetric methods

Performance characteristics such as linearity range, accuracy, precision, and selectivity of the proposed method were compared with previous announced spectrofluorimetric methods that were reported for the analysis of ATV. Required data were obtained and listed in Table 12.

It can be observed from the table that our presented approach shows a better result in terms of many performance measures. A higher correlation coefficient was found in the present work compared with the other methods, indicating a very good linear relationship between fluorescence intensity and concentration of ATV. The proposed method had a wider linearity range (0.4–12 μg/ml) than the methods of M. M. K. Sharaf Al-Din et al. [29] which were based on the same principle of analysis, where the first method was

Table 12
Comparative study between some previously published spectrofluorimetric studies and the presented study.

Fluorescence principle	Application	analyte	λex/λem[a] (nm)	Performance[b]					Reference
				Linearity (r)	Range (μg/ml)	Accuracy (Recovery %)	Precision (RSD%)	Selectivity (RSD%)	
Derivatization with NBD-Cl	Pure and tablets	ATV/ BEZ*	470/520	0.9992	0.05–0.5	100.05	0.71	1.20	[31]
Synchronous fluorescence	Pure and combined tablet dosage form	ATV/ EZE	272/$\Delta\lambda$ = 100 nm for ATV	0.9995	0.4–8	100.64	0.87	0.5	[30]
Vierodt's method			273/380	0.9995	0.4–8	101.7	0.39	1.05	
Direct measurement with acetate buffer pH 3.4	Pure and tablets	ATV	276/389	0.9996	1.5–4	99.95	0.6	–	[29]
Direct measurement with 5% acetic acid				0.9998	1–4	100.20	0.38	–	
Direct measurement with methanol				0.9995	0.5–3	99.99	0.65	–	
Direct measurement with methanol	Pure and binary mixtures	ATV/ AML	274/369	0.9991	0.5–10	99.59	1.04	–	[11]
Direct measurement with acetonitrile	Pure and tablets	ATV	270/385	0.9999	0.4–12	100.08	0.87	0.76	**Present study**

[a, b] The excitation/emission wavelengths and the performance parameters of ATV, respectively. * Bezafibrate.

linear over a range of 1.5–4 μg/ml in presence of acetate buffer, while using acetic acid 5% as diluent solvent made the method more sensitive with a range of 1–4 μg/ml, and 0.5–3 μg/ml was the range of the last approach using methanol. Moreover, the proposed method has more precise results with RSD value of 0.87% compared with the method reported by B. A. Moussa et al. [11]. The present work has good results of accuracy and precision that is identically close with those obtained by applying synchronous fluorescence which prescribed by M. F. Ayad and N. Magdy [30]. Selectivity was only studied and evaluated in our study compared with the other methods [11,29] with RSD value 0.76% which is less than the reported values [30,31], except for the synchronous fluorescence technique, RSD found to be 0.5%. Therefore, the developed method is considered to be more precise, accurate, selective, and applicable for the quantitative and qualitative analyzing of ATV than the reported method, and could be practically suitable in quality control laboratory.

4. Conclusion

The present study describes a simple, fast and selective spectrofluorimetric method, which has been successfully developed and validated for the determination of Atorvastatin calcium in its pure form and pharmaceutical formulations depending on the measurement of the native fluorescence of ATV in acetonitrile. Different variables, which influencing the development of the analytical procedure, were deliberately changed and the conditions of the experiments were optimized. The proposed approach proved to be specific, precise, and accurate with a comparable low detection limit value, and was applied for the assurance of ATV in its tablets without interference from basic excipients or additives drugs.

Distinct advantages of the present method were observed, including the simplicity and rapidity of sample preparation and fluorescence spectra and the requirement of only common instruments with no need for sophisticated expensive apparatus. Based on all the remarkable advantages, the method is considered very suitable and applicable for the routine procedures of the assay of ATV in academic institutions and pharmaceutical industries.

Author contribution statement

Nisreen Ahmad: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Yaser Bitar: Contributed reagents, materials, analysis tools or data.
Saleh Trefi: Conceived and designed the experiments.

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Declaration of interest's statement

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