



Article

Development of a Novel, Fast, Simple HPLC Method for Determination of Atorvastatin and Its Impurities in Tablets

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Abstract: Our main target and concept was to develop a method for the determination of the most prescribed antilipemic drug, atorvastatin, together with its related substances, with a single sample preparation and during a single chromatographic run, in the shortest possible period of time, with the lowest possible mobile phase consumption. A new rapid, simple chromatographic method for the determination of atorvastatin and its main specified impurities was developed, using different chromatographic columns. With this new concept of a mobile phase and a powerful core-shell, or a superficially porous silica-based column, satisfactory results for targeted parameters, such as critical peak resolution, run time length, and column backpressure, were achieved. The analysis is performed within a run duration of less than 15 minutes, which is about six times shorter than the official European Pharmacopoeia method. The chromatogram performances suggests that the method limit of quantification (LOQ) can be about 7 times lower, and the limit of detection (LOD) about 20 times lower, using an injection volume of only 2 µl. This was confirmed by the performed method validation in accordance with the International Conference on Harmonization (ICH) guideline for the validation of analytical procedures Q2(R1), where the selectivity, linearity, accuracy, precision, limit of quantification, and limit of detection were tested and confirmed.

Keywords: atorvastatin; impurities; liquid chromatography; method optimization; tablets

1. Introduction

Statins are members of the anti-atherosclerotic drugs intended to treat and prevent the main cause of hypertension and coronary heart diseases. They are widely used in clinical practice with high recommendation and high efficiency. Atorvastatin, (3R,5R)-7-[2-(4-Fluorophenyl)-5-isopropyl-3-phenyl-4-(phenyl-carbamoyl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid calcium salt, belongs to a group of drugs called 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase inhibitors, or "statins". Atorvastatin is used together with diet to lower blood levels of "bad" cholesterol (low-density lipoprotein, or LDL), to increase levels of "good" cholesterol (high-density lipoprotein, or

HDL), and to lower triglycerides (a type of fat in the blood). Atorvastatin is used to treat high cholesterol and to lower the risk of stroke, heart attack, or other heart complications in people with type 2 diabetes, coronary heart disease, or other risk factors [1–3]. An intensive search to develop, create, and improve a new, faster, simpler, and less toxic, i.e., “greener”, analytical method for its quantification is needed. Atorvastatin is a naturally highly hydrophobic molecule, requesting the use of high percentages of hydrophobic organic solvents in the mobile phase for reversed-phase chromatographic analysis, which increases the harmfulness, toxicity, environmental hazard, and costs of quantitative analysis. Creating a method with a possible simultaneous usage for the quantification of atorvastatin as an active pharmaceutical ingredient (API) and its related compounds in tablets and raw materials, with a single sample preparation and chromatographic run, in the shortest possible time with lower mobile phase consumption, was our main target and concept. There are a few high-performance liquid chromatography (HPLC) analytical methods for simultaneous determination of atorvastatin with other active substances [4–13], for quantification of atorvastatin alone, or together with its impurities [14–21]. Also, there is one method reported for the simultaneous determination of atorvastatin and antihypertensive, antidiabetic, and antithrombotic drugs, with HPLC [22]. The performance of this method described allows its use in the quantification of atorvastatin along with the nine most commonly prescribed drugs available in the market as atorvastatin-combined dosage forms.

As a continuation of our previous efforts [21], the aim of this study was to check the applicability of a modern solid phase particle (SPP)-based 2.2 μm column in the development of a new simple, economical, and faster HPLC method for the determination of atorvastatin and its impurities, using less toxic and more UV-transparent components in the mobile phase composition. The present study describes a new method intended for routine use in quality control laboratories, using less sophisticated equipment at a low cost.

2. Results

2.1. HPLC Method from European Pharmacopoeia Monograph

The official and most commonly used method for the determination of impurities of atorvastatin is the method for related substances testing prescribed by the European Pharmacopoeia monograph for atorvastatin calcium. The monograph of atorvastatin from European Pharmacopoeia (EP) prescribes the HPLC method for impurities testing of atorvastatin active substance under the following conditions: octylsilyl C8 (L7) column with dimensions 250 mm x 4.6 mm and 5 μm particles (recommended Zorbax C8 Rx), combined isocratic and linear gradient mode of elution, with mobile phase composed of acetonitrile, tetrahydrofuran, and ammonium acetate buffer adjusted to pH 5.0 with glacial acetic acid, at flow rate 1.5 mL/min, UV signal monitoring at 244 nm and a run time of 85–90 min. We performed the EP method. A chromatogram obtained by the use of this method is presented in Figure 1.

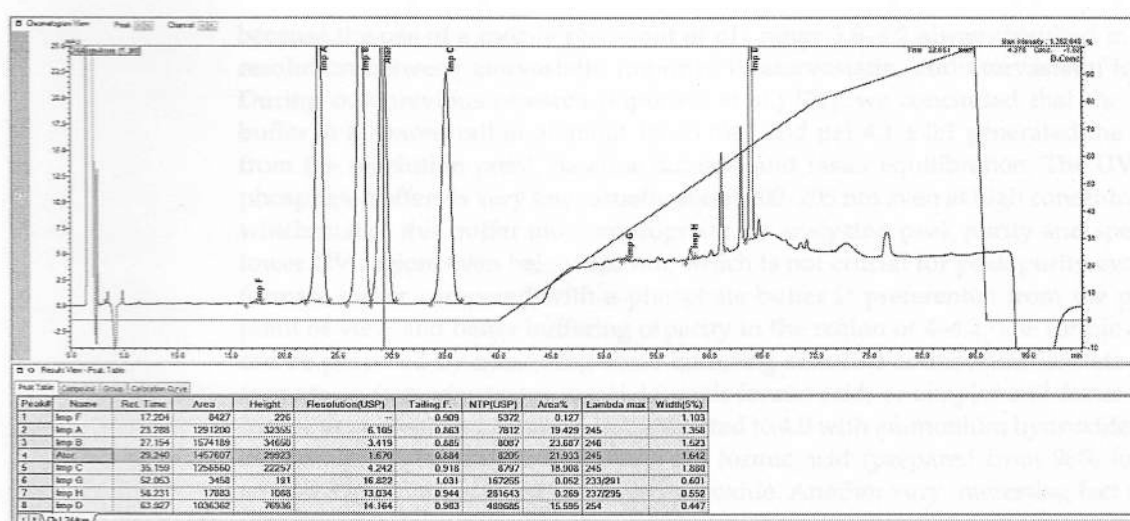


Figure 1. Chromatogram obtained using the method prescribed in European Pharmacopoeia (EP) 10 illustrating the elution order of atorvastatin impurities, obtained in our laboratory.

In spite of the pharmacopoeial method, there are also a few published HPLC analytical methods for the quantification of atorvastatin in combination with other active substance [4–13], or for the determination of atorvastatin alone, or together with its impurities [14–21], with UV detection. We found the articles published by Petkovska et al. [18] and Vakkum et al. [20] as the most applicative and useful for testing atorvastatin impurities. The method published by Petkovska et al. [18] prescribes the use of a short high efficiency column, Zorbax Eclipse XDB C18 Rapid Resolution HT 50 mm × 2.1 mm, 1.8 µm, mobile phase composed of an acetonitrile, tetrahydrofuran, and phosphate buffer with pH 3.5. Use of this method is limited only on UPLC systems with very low system dispersion, extra-column volume, because the prescribed state of the art column with 1.8 µm particles cannot be used with high dwell volume or high dispersion standard HPLC systems with lower performance characteristics. Additionally, these columns are very powerful and more complicated for less skilled analysts, with a higher probability of column clogging. This method uses tetrahydrofuran as a part of the mobile phase, which we intended to avoid because of its toxicity and instability. The method published by Vakkum et al. [20] prescribes the use of simple gradient elution with 0.1% *v/v* trifluoroacetic acid and acetonitrile, and column Zorbax Bonus RP. Very interesting and attractive separations were achieved. The achieved resolution between critical pairs was even 2, even though EP requires a minimum of 1.5. However, these high resolutions were achieved with peaks of impurities present at low concentrations, at their detection limits. At these low concentrations, the peaks are always narrow and easier to separate. The low pH value of the 0.1% *v/v* trifluoroacetic acid used as a part of the mobile phase is also critical, usually little less than 2, which is out of the recommended pH range between 2 and 9 for the used column, Zorbax Bonus RP. This will shorten the column life and use.

2.2. HPLC Method Development

During the method development, we focused on choosing a selective and simple mobile phase and appropriate chromatographic in order to achieve successful, effective, rapid, and reproducible separations [23]. At the beginning, we tried to replace the acetate buffer with another less UV-absorbing inorganic part of the mobile phase, that would enable better UV-spectral analysis. We also tried to exclude the toxic, expensive, and unstable peroxide-forming tetrahydrofuran, as a part of the mobile phase. The 0.05% *v/v* formic acid adjusted to pH 4.0 with ammonium hydroxide, which has a UV cut-off value of about 220 nm, was tested and compared to acetate buffer with a cut-off value of about 235–245 nm, depending on the buffer concentration. We chose this pH of the mobile phase

because the use of a mobile phase out of pH range 3.8–4.2 always resulted in decreased resolution between atorvastatin impurity B, atorvastatin, and atorvastatin impurity C. During our previous research (Piponski et al.) [21], we concluded that the phosphate buffer at a concentration of about 10–15 mM and pH 4.1 ± 0.1 generated the best result from the resolution point, baseline flatness, and faster equilibration. The UV cut-off of phosphate buffers is very low, usually about 200–205 nm even at high concentrations [24], which makes this buffer more appropriate for analyzing peak purity and spectrum at a lower UV region, even below 220 nm, which is not crucial for peak purity evaluation. A formate buffer compared with a phosphate buffer is preferential from the preparation point of view and better buffering capacity in the region of 4–4.2. The formic acid buffer can be prepared by measuring some buffering salts like ammonium formate or sodium formate and an adjustment to pH 4.0 with formic acid, or simpler and faster by using a formic acid diluted in water and pH adjusted to 4.0 with ammonium hydroxide or sodium hydroxide. We chose using a 0.05% *v/v* formic acid (prepared from 98% formic acid) adjusted to pH 4.0 with ammonium hydroxide. Another very interesting fact we noticed was the identical peak elution sequential pattern, i.e., the order of appearance of peaks with the EP described, even though two totally different mobile phases were used: the original EP method prescribes a mobile phase composed of acetonitrile, tetrahydrofuran, and acetate buffer with pH 5.0 with gradient elution (Figure 1), while this new developed method uses a mobile phase composed of 0.05% *v/v* formic acid adjusted to pH 4.0 with ammonium hydroxide and acetonitrile in single stepwise gradient elution mode (Figure 2). Comparison of the relative retention times of the specified impurities of atorvastatin obtained with these two methods is presented in Table 1. The identity of the peaks was confirmed by DAD UV spectral analysis and the spiking of samples and standards with standard substances of each specified impurity, separately. The PDA-derived chromatogram of atorvastatin and its seven impurities with their specific UV absorption spectra are presented in Figure 3 for easier identification of each.

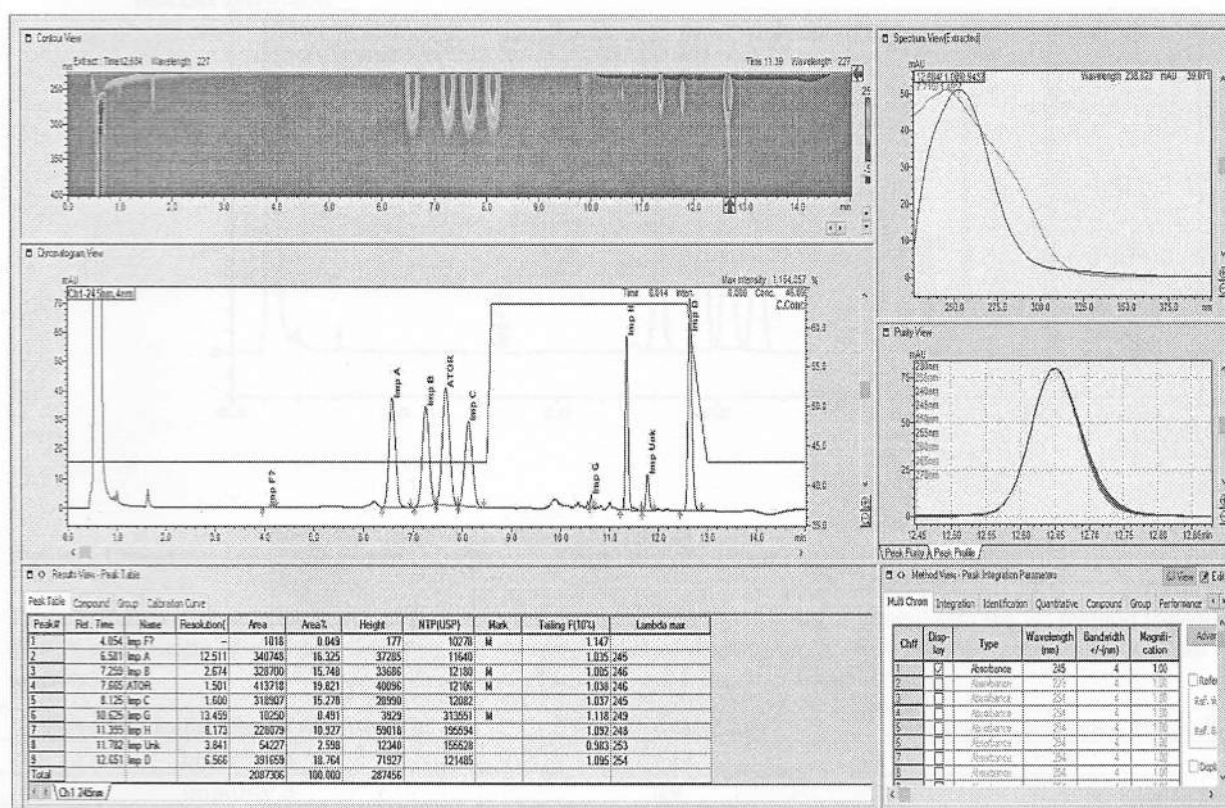


Figure 2. Chromatogram of the combined mixture of atorvastatin impurities, with plotted gradient shape and separate contour diagram at the top, extracted measuring channel at 245 nm, with a table of system suitability and peak purity parameters (obtained on Nexera-i 2040c-3D in a 15-min run).

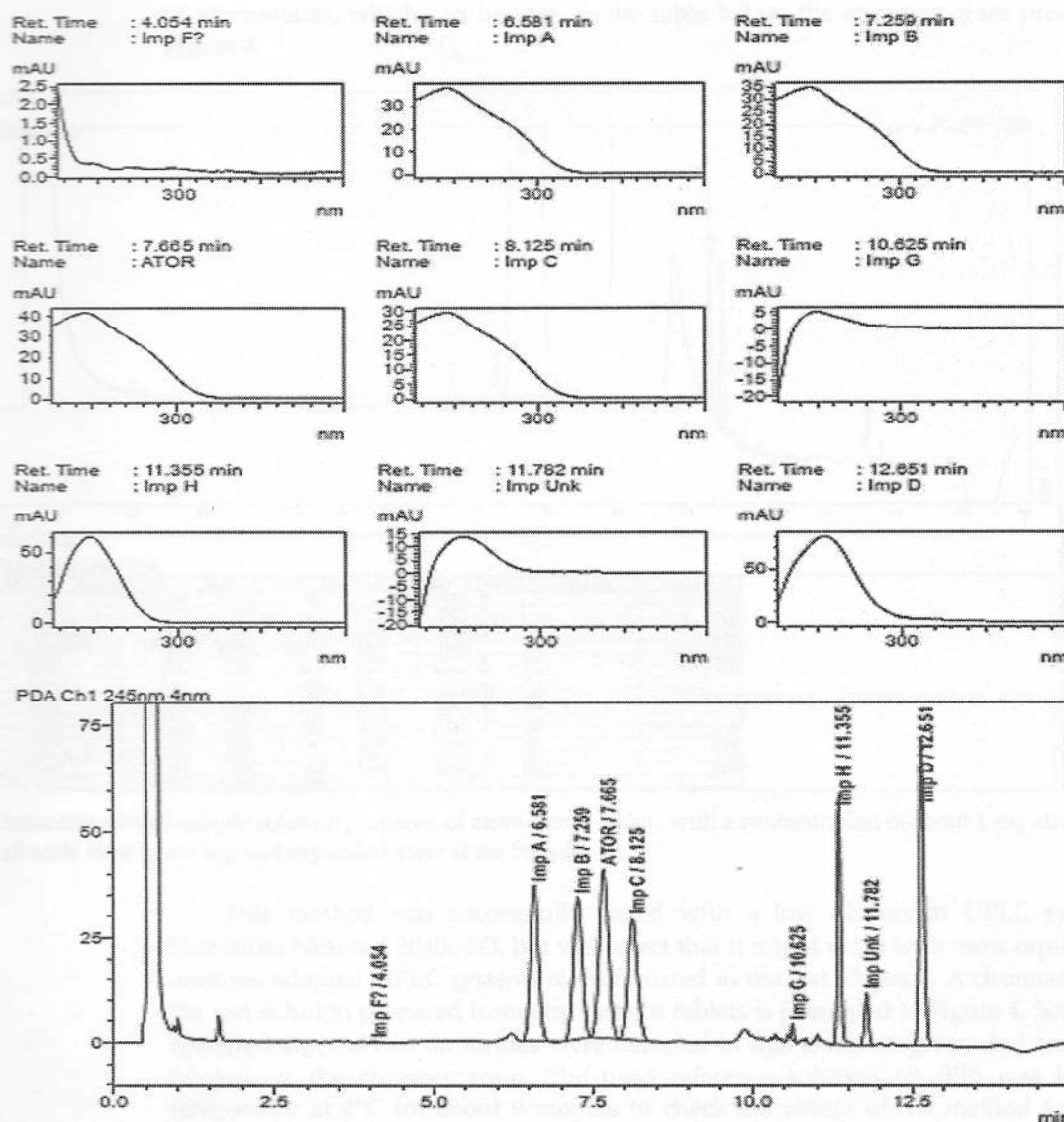


Figure 3. Chromatogram of atorvastatin and its impurities, presenting their characteristic UV absorption spectra.

Table 1. Relative retention times (RRTs) of specified impurities of atorvastatin obtained with the EP method (retention time of atorvastatin of about 33 minutes) and this new developed method (retention time of atorvastatin of about 7.5 minutes).

Specified Impurities of Atorvastatin	RRT (EP Method)	RRT (New Developed Method)
Impurity A	0.8	0.86
Impurity B	0.9	0.95
Impurity C	1.2	1.06
Impurity D	2.1	1.65

With use of the column Shim-pack XR II C18 75 mm x 3 mm, 2.2 μ m, perfect results were obtained, from the aspect of successful, rapid, and reproducible separation of atorvastatin and its impurities, yielding a number of theoretical plates (NTP) of about 12106 regarding the main peak of atorvastatin, as can be seen from the chromatograms on Figure 2. The theoretically calculated number of theoretical plates using the equation $N = L/2dp$ [24] corresponded to the software calculated number of about 11,835 for the peak of atorvastatin, which can be seen in the table below the chromatogram presented in Figure 4.

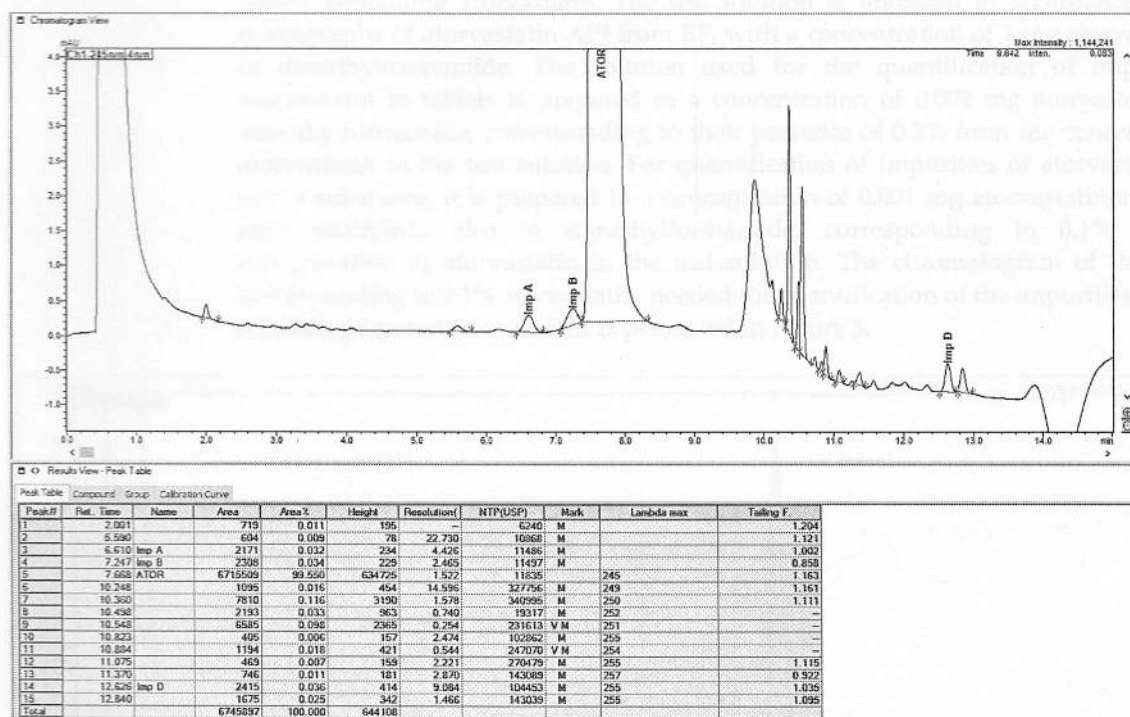


Figure 4. Chromatogram of sample solution prepared of atorvastatin tablets with a concentration of about 1 mg atorvastatin/mL, full scale view at the top and expanded view at the bottom.

This method was successfully tested with a low dispersion UPLC system, as Shimadzu Nexera-i 2040c-3D, but we expect that it might work with most capillary-connections adapted HPLC systems manufactured in the last 10 years. A chromatogram of the test solution prepared from atorvastatin tablets is presented in Figure 4. Some of the specified atorvastatin impurities were detected in this chromatogram, and are suitably labeled on the chromatogram. The used reference solution (c) (EP) was kept in a refrigerator at 4°C for about 9 months to check the ability of the method to separate additional formed degradation products, despite the specified impurities F, A, B, C, G, H, and D. The identity of the peaks detected in the test solution was confirmed with comparison of their UV spectral characteristics with the UV spectral characteristics of the peaks with the same retention time (tR), of the specified impurities, from the atorvastatin reference solution (c) (prepared in accordance to EP 10). The other detected, but not identified, peaks in the chromatogram of the test solution originate from other unspecified impurities.

The column recommended by European Pharmacopoeia for testing the impurities of the atorvastatin active substance, Zorbax Rx C8 250 mm x 4.6 mm, 5 μ m, was also tested under the conditions of this new developed method. The optimal percentage of acetonitrile in the isocratic part of the elution was 44% *v/v* in this case. The obtained resolution factors of critical pair were satisfactory, i.e., all had a value of minimum 1.5 (EP) or minimum 1.4 (USP).

The USP method for testing the impurities of atorvastatin is quite different than the EP method, using (L1) C18 column with mobile phase composed of tetrahydrofuran, acetonitrile, and combined phosphate-acetate buffer, with gradient mode of elution, with variable mobile phase flow rate, during the period of 65 min, for separation of 11 impurities.

The new method which we developed not only meets the system suitability requirements, but also easily achieves the required sensitivity for analysis of impurities that are present in a very low percentage in atorvastatin as an active substance, or in tablets containing atorvastatin. The test solution is prepared in accordance with the monographs of atorvastatin API from EP, with a concentration of 1 mg atorvastatin/mL in dimethylformamide. The solution used for the quantification of impurities of atorvastatin in tablets is prepared in a concentration of 0.002 mg atorvastatin/mL in dimethylformamide, corresponding to their presence of 0.2% from the concentration of atorvastatin in the test solution. For quantification of impurities of atorvastatin as an active substance, it is prepared in a concentration of 0.001 mg atorvastatin/mL or 1 µg atorvastatin/mL, also in dimethylformamide, corresponding to 0.1% from the concentration of atorvastatin in the test solution. The chromatogram of the solution corresponding to 0.1% atorvastatin, needed for quantification of the impurities in the test solution prepared from tablets, is presented in Figure 5.

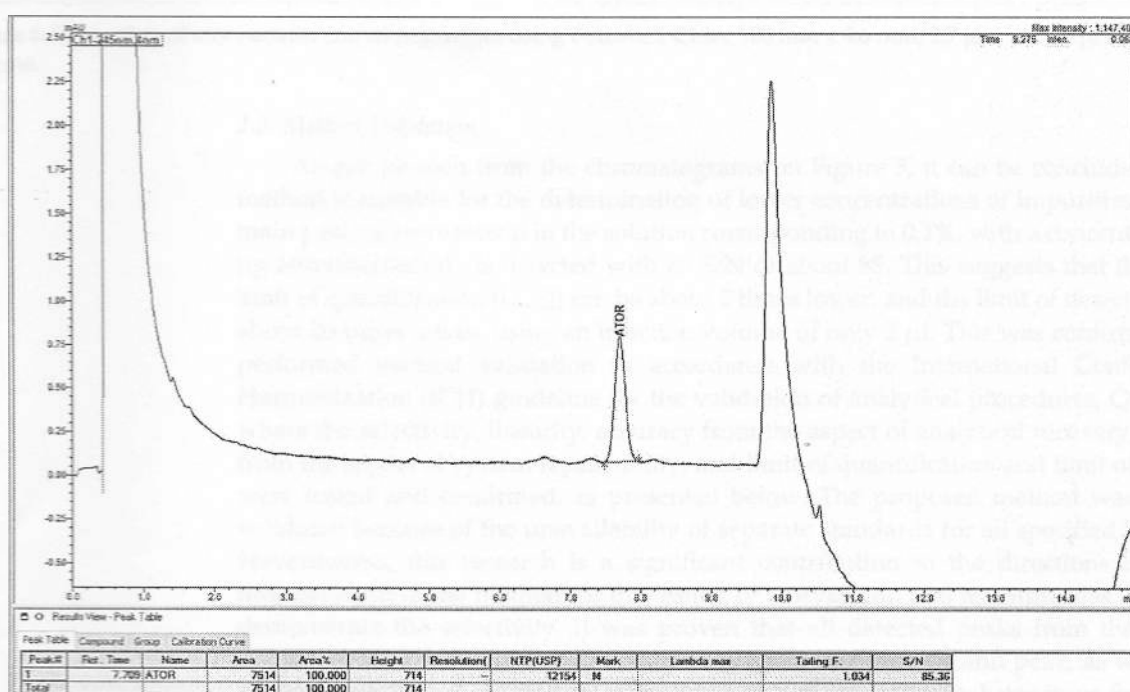


Figure 5. Chromatogram of the diluted test solution of atorvastatin corresponding to 0.1% of its concentration in the test solution prepared as for substance analysis criteria, with system suitability parameters.

Besides the selected Shim-Pack XR-ODS II 75 mm x 3 mm, 2.2 µm column, one other column, Agilent Poroshell C18ec 100 mm x 4.6 mm, 2.7 µm, also showed very good results in the separation of atorvastatin and its impurities, as can be seen in Figure 6. As can be noticed from Figure 6, this column also yields very good chromatogram separations which accomplish the required system suitability parameters, resolution, and peak symmetries. This column can be considered as an alternative to the first proposed Shim-Pack XR-ODS II because the sensitivity that offers the 3 mm internal diameter is superior in comparison to a 4.6 mm internal diameter. The method run time in this case is increased by about 20%–25% due to the bigger column length and volume.

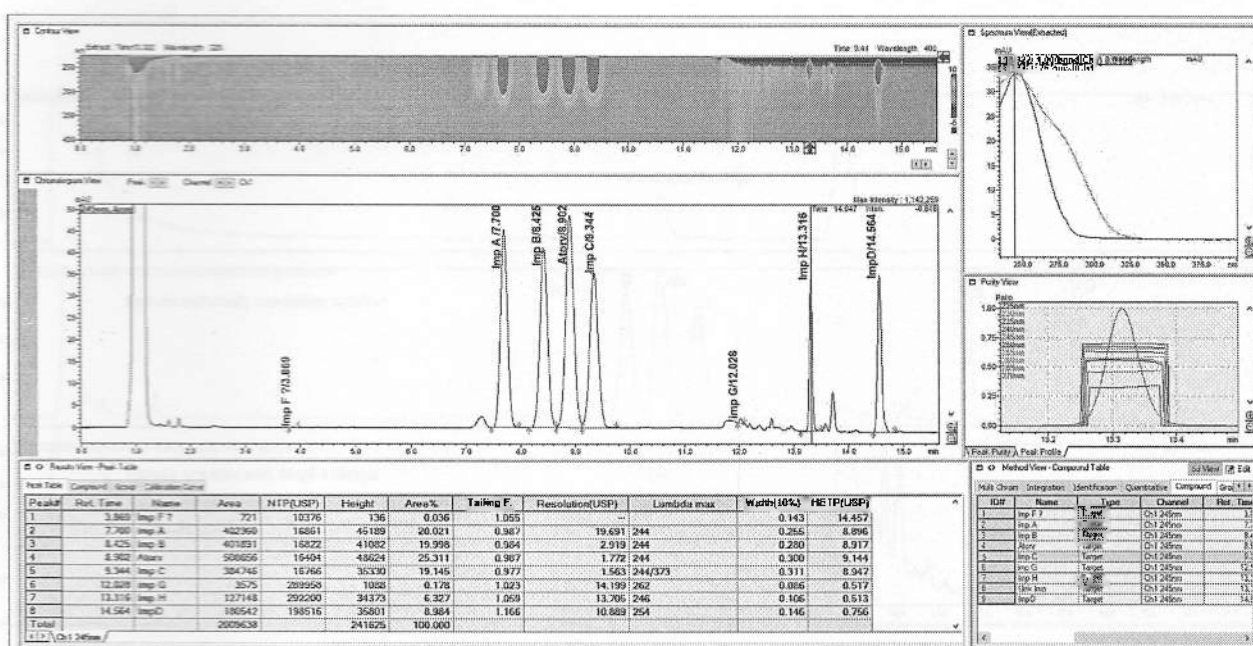


Figure 6. Separation of atorvastatin and its impurities using Poroshell C18ec 100 mm x 4.6 mm, 2.7 µm, with 3 µl injection volume.

2.3. Method Validation

As can be seen from the chromatograms on Figure 5, it can be concluded that the method is suitable for the determination of lower concentrations of impurities, since the main peak of atorvastatin in the solution corresponding to 0.1%, with a concentration of 1 µg atorvastatin/mL, is detected with an S/N of about 85. This suggests that the method limit of quantification (LOQ) can be about 7 times lower, and the limit of detection (LOD) about 20 times lower, using an injection volume of only 2 µl. This was confirmed by the performed method validation in accordance with the International Conference on Harmonization (ICH) guideline for the validation of analytical procedures, Q2(R1) [25], where the selectivity, linearity, accuracy from the aspect of analytical recovery, precision from the aspect of system repeatability, and limit of quantification and limit of detection were tested and confirmed, as presented below. The proposed method was not fully validated because of the unavailability of separate standards for all specified impurities. Nevertheless, this research is a significant contribution to the directions for further improvement of the method for the testing of atorvastatin and its impurities. In order to demonstrate the selectivity, it was proven that all detected peaks from the specified impurities of atorvastatin are well separated from the atorvastatin peak, as well as that placebo peaks do not interfere with the peaks of the active substance or its specified impurities (Figure 7).

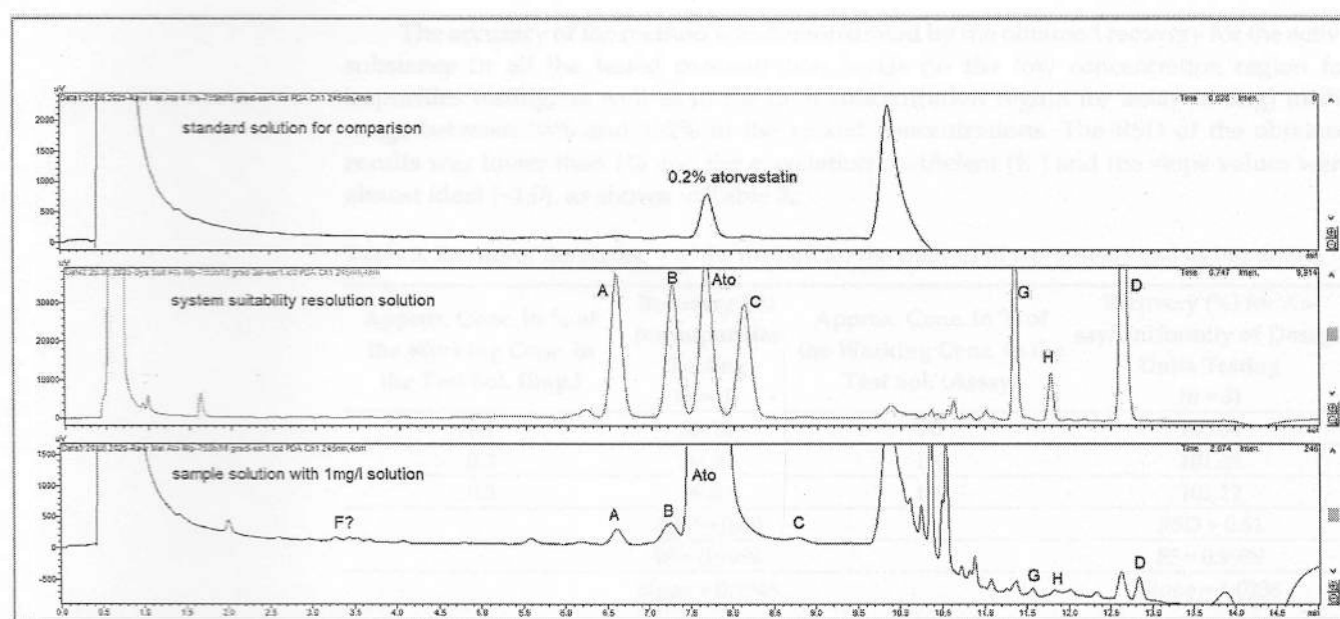


Figure 7. Summary overview of the selectivity and sensitivity of the method in 3 chromatograms illustrating: sample solution prepared from atorvastatin tablet (bottom), system suitability solution (middle), and diluted test solution corresponding to 0.2% atorvastatin (top).

Excellent linearity of the method for the analysis of impurities of atorvastatin was confirmed by the RSD of the obtained response factors that was lower than 3% and the obtained correlation coefficient that was almost ideal (Table 2).

Table 2. Results from linearity testing of the method for analysis of atorvastatin and its impurities.

Linearity Parameters	Impurities	Assay
Atorvastatin concentration range (% of working conc. of test sol.)	0.05%–0.3%	70%–130%
Linear regression equation	$y = 3014.8108x - 70.2973$	$y = 2832.6906x - 19216.7744$
RSD of response factors, %	2.47	0.93
Correlation coefficient, R^2	0.9998	0.9993

Excellent linearity of the method for assay testing of atorvastatin was also confirmed in the higher concentration range, by the RSD of the obtained response factors that was lower than 1% and the obtained correlation coefficient that was also almost ideal (Table 2). The limit of detection (LOD) of the method for the determination of atorvastatin and its impurities was determined to be 0.05 µg atorvastatin/mL, corresponding to ~ 0.005% of its concentration in the test solution. The limit of quantification of the method for determination of atorvastatin and its impurities was determined to be 0.14 µg atorvastatin/mL, corresponding to ~ 0.014% of its concentration in the test solution.

These differences between the results obtained from linearity testing in high and low concentration ranges are not significant, since the purpose of their use is clearly different: the proven linearity in high concentration range clearly proves that the method is suitable to be used for the quantification of atorvastatin as an active substance and in pharmaceutical dosage forms, whereas the proven linearity in the low concentration range clearly proves that the method is suitable to be used for the quantification of related substances of atorvastatin (specified and unspecified) that are present in the active substance or pharmaceutical dosage forms that contain this active substance in a very low percentage.

The accuracy of the method was demonstrated by the obtained recovery for the active substance in all the tested concentration levels (in the low concentration region for impurities testing, as well as in the high concentration region for assay testing) in the range between 98% and 102% of the spiked concentrations. The RSD of the obtained results was lower than 1% and the correlation coefficient (R^2) and the slope values were almost ideal (~ 1.0), as shown in Table 3.

Table 3. Results of the accuracy of the method for the analysis of atorvastatin and its impurities.

Approx. Conc. in % of the Working Conc. in the Test Sol. (Imp.)	Recovery (%) for Impurities Testing ($n = 3$)	Approx. Conc. in % of the Working Conc. in the Test Sol. (Assay)	Recovery (%) for Assay/Uniformity of Dosage Units Testing ($n = 3$)
0.1	98.12	70	100.04
0.2	98.37	100	101.38
0.3	99.03	130	101.12
	RSD = 0.60		RSD = 0.61
	$R^2 = 0.9999$		$R^2 = 0.9999$
	Slope = 0.9948		Slope = 1.0238

The precision of the method was confirmed by the testing of system repeatability, by performing 6 replicate injections of the diluted test solution corresponding to 0.2%, used for quantification of impurities, and a standard solution for assay testing prepared with a concentration of ~ 1 mg atorvastatin/mL. The obtained RSD values of the peak areas in both cases was lower than 1% (obtained RSD values: 0.73% for impurities testing and 0.18% for assay testing).

3. Discussion

The official and most commonly used method for the determination of impurities of atorvastatin is the method for related substances testing prescribed by the European Pharmacopoeia monograph for atorvastatin calcium. This pharmacopoeial method has a few drawbacks. The acetate buffer used as a part of the mobile phase is highly absorbing at the prescribed monitoring wavelength of 245 nm. Tetrahydrofuran is highly toxic and expensive, requires longer column equilibration, and is restrictive to types of components and salts that can be used in the mobile phase composition. Another important disadvantage of tetrahydrofuran used in chromatographic separation, especially in the gradient mode, is the obligate use of its non-stabilized form with butylated hydroxytoluene (BHT), which is highly inappropriate for use as a part of the mobile phase in methods with UV detection. A well-known fact is the native instability of tetrahydrofuran above 6 months' storage, resulting in the generation of explosive peroxides, which is usually prevented by the addition of about 0.2% BHT. This substance has a high absorbance in the UV region and creates high positive gradient baseline shifts and frequently releasing ghost peaks. Additionally, the European Pharmacopoeia method is long lasting, with a total duration time of the gradient elution of 90 min.

The impurities of APIs might originate from the synthesis pathway of the active substance, as by-products, or might be a product of the degradation pathway of the active substances that form under various environmental factors during the shelf-life of the active substances and/or the pharmaceutical products that contain them. In this case, the European Pharmacopoeia monograph for atorvastatin API specifies four main impurities of active substance atorvastatin: impurity A, impurity B, impurity C, and impurity D. Additionally, three more impurities of atorvastatin are present in this monograph: impurity F, impurity G, and impurity H, but their identification is not obligatory, according to the monograph. There are also unspecified impurities that might appear in the active substance or in the pharmaceutical dosage form that contains this active substance, but their identification is not obligatory according to European Pharmacopoeia monograph, since

they are limited by the general acceptance criterion for other/unspecified impurities and/or by the general European Pharmacopoeia monograph *Substances for pharmaceutical use* (2034). According to the European Pharmacopoeia monograph for atorvastatin as an active substance, the following limits are prescribed for its impurities: max. 0.3% for impurity A and impurity B; max. 0.15% for impurity C and impurity D; and max. 0.1% for other unspecified impurities. The limitation for other unspecified impurities for pharmaceutical dosage forms that contain atorvastatin will be increased from 0.1% to 0.2%, in accordance with ICH guidelines. Thus, the concentration range that was tested during our research and proven to be suitable for the intended use is in accordance with these requirements.

The new method we developed is proven to be selective for all of the specified impurities of atorvastatin (impurity A, impurity B, impurity C, impurity D, impurity F, impurity G, and impurity H) and can be used as a cost-effective and time-effective alternative for the European Pharmacopoeia method for the related substance testing of atorvastatin. Additionally, this method offers the possibility of using the same method for the quantification of API in the same run analysis.

During the method development, we focused on choosing a selective and simple mobile phase and appropriate chromatographic in order to achieve successful, effective, rapid, and reproducible separations. The column Shim-pack XR II C18 75 mm x 3 mm, 2.2 μ m achieved the best separation with the highest values for critical pair resolutions, during the shortest run time analysis, with about 10% higher backpressure compared with other columns with identical dimensions and particle size. The theoretically calculated number of theoretical plates using the equation $N = L/2dp$ corresponded to the software calculated number of about 11,835 for the peak of atorvastatin, which can be seen in the table below the chromatogram presented in Figure 4.

At the beginning, a mobile phase composed of 43% *v/v* acetonitrile and 57% *v/v* formate at pH 4.1 was used, followed by a steep 0.1 min increment for 22% of acetonitrile (total 65%) at 1 min behind the 4th peak of atorvastatin impurity C. Under these conditions, the lowest value of critical pair resolution obtained ranged from 1.96 to 2.11 between atorvastatin and atorvastatin impurity B. This column showed skewed peak shapes and worsened resolution when an increased concentration of formate buffer up to 0.1% *v/v* was used. This was accomplished with a mobile phase flow rate of 0.7 mL/min, a detection wavelength at 245 nm, and a column temperature of 30 °C.

Due to the long retention times and high column capacity factors, higher than 9, for the first four peaks that elute in the initial isocratic part of elution, a change of acetonitrile percentage in this isocratic elution mode for only 1% (absolute) results in a significant increase of retention times. The critical pair resolution diminishes in both cases, higher and lower percentage of acetonitrile, than the recommended 43% *v/v*. Optimal separation and retention of all peaks of interest were obtained by a stepwise increment of the percentage of acetonitrile in the mobile phase after 1 min after the baseline touch of the 4th eluted peak in the chromatogram, followed by a return to initial percentage at 2–5 min (depending on type and column dimensions) after the last peak of atorvastatin impurity D. From the above presented figures, it can be seen that critical pairs of peaks are the following: atorvastatin impurity B/atorvastatin, atorvastatin/atorvastatin impurity C, or atorvastatin impurity A/atorvastatin impurity B. This could be expected due to the very similar chemical structure of the first four eluting peaks, atorvastatin and its three closest peaks of impurity A, impurity B, and impurity C, that can be seen from their almost identical spectral characteristics, presented in Figure 3. Through experimental trials, the optimal percentage of acetonitrile in the mobile phase can be estimated for each HPLC column. A further change in the percentage of acetonitrile in the mobile phase with the aim to improve the resolution between two targeted peaks diminishes the resolution between the other neighboring peaks.

The developed method was validated in accordance with the ICH guideline for validation of analytical procedures Q2(R1), where the selectivity, linearity, accuracy from the

aspect of analytical recovery, precision from the aspect of system repeatability, and limit of quantification and limit of detection were tested and confirmed.

Beside the selected Shim-Pack XR-ODS II 75 mm x 3 mm, 2.2 μ m column, one other column, Agilent Poroshell C18ec 100 mm x 4.6 mm, 2.7 μ m, also showed very good results in the separation of atorvastatin and its impurities (Figure 6). This column can be considered as an alternative to the first proposed Shim-Pack XR-ODS II because the sensitivity that offers the 3 mm internal diameter is superior in comparison to 4.6 mm internal diameter.

This new method we developed appears to be incomparably “greener” than the pharmacopoeial and all the other previously published methods for the testing of atorvastatin impurities, since it is one of the shortest and most applicable for routine analyses in quality control laboratories in pharmaceutical companies.

Additionally, the HPLC method can be simply readapted and used in cases where faster quantification of atorvastatin is needed, for example, for the determination of the average API content in pharmaceutical dosage forms, content uniformity, and dissolution tests for tablets. This can be accomplished in an even simpler and shorter way, without switching gradients and waiting for re-equilibration.

4. Materials and Methods

4.1. Chemicals and Reagents

Atorvastatin calcium (purity 99.1%, as determined by HPLC) was purchased from Sigma-Aldrich (Switzerland). Atorvastatin 10 mg tablets were purchased from a local pharmacy. Standards of active substance atorvastatin and its four specified impurities: atorvastatin impurity A, atorvastatin impurity B, atorvastatin impurity C, and atorvastatin impurity D (containing also atorvastatin impurity F, atorvastatin impurity G, and atorvastatin impurity H) were supplied from EDQM and Sigma Aldrich (Merck, Germany).

The used chemicals: 98% formic acid, 30–35% ammonia, acetonitrile, and dimethylformamide were gradient grade, purchased from Merck, Darmstadt, Germany. The demineralized water used for analyses was an in-house product of Stilmas (Milan, Italy) with a conductivity of less than 0.05 μ S/cm.

4.2. Instrumental and Conditions

The following HPLC columns were tested: Agilent Poroshell C18ec 100 mm x 4.6 mm, 2.7 μ m; Agilent Poroshell C8ec 100 mm x 4.6 mm, 2.7 μ m; Agilent Poroshell C18ec 150 mm x 4.6 mm, 2.7 μ m; Agilent Poroshell C8ec 150 mm x 4.6 mm, 2.7 μ m; Zorbax C18 SB 150 mm x 4.6 mm, 3.5 μ m; Zorbax C8 Rx 150 mm x 4.6 mm, 3.5 μ m; (Agilent Technologies, USA; Waters Cortecs C18 100 mm x 4.6 mm, 2.7 μ m; Waters Cortecs C8 100 mm x 4.6 mm, 2.7 μ m; Waters Symmetry C18 150 mm x 4.6 mm, 3.5 μ m, (Waters Corporation, USA), and Shim-Pack XR-ODS II 75 mm x 3 mm, 2.2 μ m, (Shimadzu Corporation, Japan).

In this research, LC system UPLC Shimadzu LC 2040c-i 3D controlled by Lab Solutions version 5.97 was used.

The following additional instrumental equipment was used: analytical balance Mettler Toledo), MPC227 (Mettler, USA) pH meter Metrohm 827, US bath Branson 3510, and IKA orbital shaker KS4000i (Germany). The regenerated cellulose (RC) 0.45 μ m syringe filters were purchased from Agilent Technologies.

4.3. Sample Preparation

Sample preparation was performed as described in a monograph of atorvastatin active substance of European Pharmacopoeia 10, with samples of atorvastatin tablets, or active substance, dissolved and extracted in dimethylformamide. Atorvastatin impurity F, atorvastatin impurity G, and atorvastatin impurity H cannot be purchased from EDQM,

but their presence in the chromatograms is suitably marked on the figures presented in this article, based on assumption considering the European Pharmacopoeia (EP) referent chromatogram for atorvastatin calcium trihydrate and spectral characteristics of the obtained peaks.

4.4. Method Validation

Validation was performed by evaluation of the following parameters of the method: selectivity, linearity, accuracy from the aspect of analytical recovery, precision from the aspect of system repeatability, limit of quantification, and limit of detection. In order to demonstrate the selectivity of the developed method, chromatograms of placebo, diluted test solution corresponding to 0.2% used for quantification of impurities, reference solution (c) (EP), containing the specified impurities of atorvastatin: impurity F, A, B, C, G, H, and D and test solution, all prepared in accordance with the EP monograph for atorvastatin API, were analyzed and it was proven that all detected peaks from the specified impurities of atorvastatin are well separated from the atorvastatin peak, as well as that placebo peaks do not interfere with the peaks of the API or its specified impurities. The linearity of the method for analysis of impurities of atorvastatin was demonstrated in five concentration levels in the range between 0.05% and 0.3% of the working concentration of atorvastatin in the test solution (tested interval: 0.0005–0.003 mg atorvastatin/mL). The linearity of the method for assay testing of atorvastatin was also tested in five concentration levels in the range between 70% and 130% of the working concentration of atorvastatin in the test solution (tested interval: 0.7–1.3 mg atorvastatin/mL). The values for limit of detection and limit of quantification were calculated based on the data obtained during linearity testing in the low concentration range between 0.1% and 0.3% of the working concentration of atorvastatin in the test solution, using the following formulas: $LD = 3.3 \cdot s/Slope$ and $LQ = 10 \cdot s/Slope$. The accuracy of the method was tested using nine determinations over three concentration levels in the interval between 0.1% and 0.3% of the working concentration of atorvastatin in the test solution, for analysis of impurities, and in the interval between 70% and 130% of the working concentration of atorvastatin in the test solution, for analysis of the assay of atorvastatin. The analytical procedure was applied to synthetic mixtures of the drug product components with a known added amount of the active substance, corresponding to these concentration levels. The precision of the method was confirmed by testing system repeatability. The system repeatability was tested by performing six replicate injections of the diluted test solution corresponding to 0.2%, used for quantification of impurities, and a standard solution for assay testing prepared with concentration ~1 mg atorvastatin/mL.

5. Conclusions

A new simple, cost-effective, rapid, reproducible, and sensitive method for the determination of atorvastatin and its impurities was developed, through a different systematic approach of mobile phase composition, successfully applied on a properly selected adequate chromatographic column Shim-Pack XR-ODS II 75 mm x 3 mm, 2.2 μ m. This method is significantly “greener” than most of the published chromatographic methods and the pharmacopoeial methods for the determination of the impurities of atorvastatin. A very important advantage of the developed method is that during one single run, simultaneous determination of the quantity of atorvastatin and its impurities can be performed. The low injection volume of not more than 2 μ l offers the needed sensitivity for quantification of impurities present in low quantities, and still offers almost perfect peak symmetry not only of small peaks of impurities, but also of the peak of atorvastatin, enabling their simultaneous quantification. This was accomplished with appropriate mobile phase composition, and the use of a chromatographic silica column with a high quality bonded phase matrix. It successfully separates all the specified and detected unspecified

impurities of atorvastatin, achieving all the needed system suitability requirements during an almost six times shorter run time than the pharmacopoeial method.

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