Stability-indicating HPLC-DAD Method for Simultaneous Determination of Atorvastatin, Irbesartan, and Amlodipine in Bulk and Pharmaceutical Preparations

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The present study describes a convenient stability-indicating HPLC-DAD method for the separation and simultaneous determination of the drugs atorvastatin, irbesartan, and amlodipine in bulk and formulations. The method proposed demonstrated good separation between atorvastatin, irbesartan, amlodipine and their main degradation products. Separation was executed on a Waters XBrigde C18 column (5 μm, 25 × 0.46 cm) using a gradient mobile phase system consisting of acetonitrile and orthophosphoric acid buffer (pH 2.2) at a flow rate of 1 mL/min and UV detection at 240 nm. The drugs were subjected to acidic and basic hydrolysis, oxidation, exposure to UV light, and exposure to temperature (dry heat) to apply stress conditions. Linearity of the method was evaluated in the range 5–30 μg/mL for each drug with correlation coefficient values (r^2) of 0.9982, 0.9973, and 0.9986 for atorvastatin, irbesartan, and amlodipine, respectively. The limits of detection were 0.05, 0.06, and 0.08 μg/mL for the three compounds, respectively. No interference was observed with the detection of the compounds in presence of degradable products; hence, it is confirmed that the analysis is stability-indicating.

Keywords: Degradation studies, Atorvastatin, Irbesartan, Amlodipine HPLC

Introduction

Complications due to cardiovascular diseases (CVDs) represent some of the most common causes for morbidity and mortality in humans.[1,2] Management of CVDs is achieved through a combination of different drugs acting at different levels.[3] Atorvastatin (Figure 1(a)) is a member of the statins class and is used to control low-density lipoprotein (LDL)-cholesterol and for the treatment of hyperlipidemias as a whole.[4] Irbesartan (Figure 1(b)) competes with angiotensin II for binding to the type-1 angiotensin II receptor (AT1) used as an antihypertensive.[5] Amlodipine besylate (Figure 1(c)) is a long-acting calcium channel blocker used as an antihypertensive and to lower anginal chest pain.[6,7] Many dosage forms of atorvastatin, irbesartan, and amlodipine as single drugs or in combination with others are available for effective therapy. The first oral dosage form, Caduet, was developed containing a combination of amlodipine besylate and atorvastatin for the treatment of high blood pressure and high cholesterol levels simultaneously.[3] Aimix is a combination of tablets LD/HD of irbesartan with amlodipine 100 mg with 5 and 10 mg, respectively; in Japan, this is the first fixed-dose combination available so far.[7] Patent has also been granted in Korea for first irbesartan and atorvastatin combination, which is under Phase III clinical trials and getting ready for commercialization.[8]

The present study involves the development and validation of a method to simultaneously study the degradation products of atorvastatin, irbesartan, and amlodipine using HPLC. No method has been reported so far on the combined degradation products of amlodipine with irbesartaan, or irbesartan with atorvastatin. Stress conditions involve acidic and basic hydrolysis, oxidation, exposure to UV light, and exposure to temperature (dry heat). However, from a literature survey it was evident that many stability-indicating HPLC methods are designed for the determination of atorvastatin, irbesartan, and amlodipine, separately.[3,9–13] Interestingly, one ultrahigh performance liquid chromatography (UPLC) method was reported on the combined determination of atorvastatin, amlodipine, and irbesartan with hydrochlorothiazide, but the authors worked with single dose of each compound and the method lacked the stability-indicating nature.[5]

To the best of our knowledge, there is no reported analytical method that estimates all above drugs simultaneously. Hence, there is a need for the development of a single analytical method that allows the simultaneous quantification of all the analytes and their degradation products, thus obviating the need for a separate method for each drug. These drugs are manufactured in single or in combined dosage forms by many pharmaceutical industries, and most pharmaceutical industries use methods that are time consuming and requiring the use of different solvents for the determination of different dosage forms. However, with the proposed method, these disadvantages are removed, as the analysis time is short and cost is saved by using a simple mobile phase composition for the estimation of these individual drugs and their combinations. This method is useful for the analysis of combination formulations as well as

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for individual formulations in a single run within a run time of less than 15 min. The present method was developed with the aim of keeping the pH below 3 in order to minimize the tailing of any basic drug, since silinol becomes protonated at acidic pH.[9] Separation of compounds containing amine groups is critical, and the proposed method is able to separate these compounds without using any basic modifiers such as triethylamine.

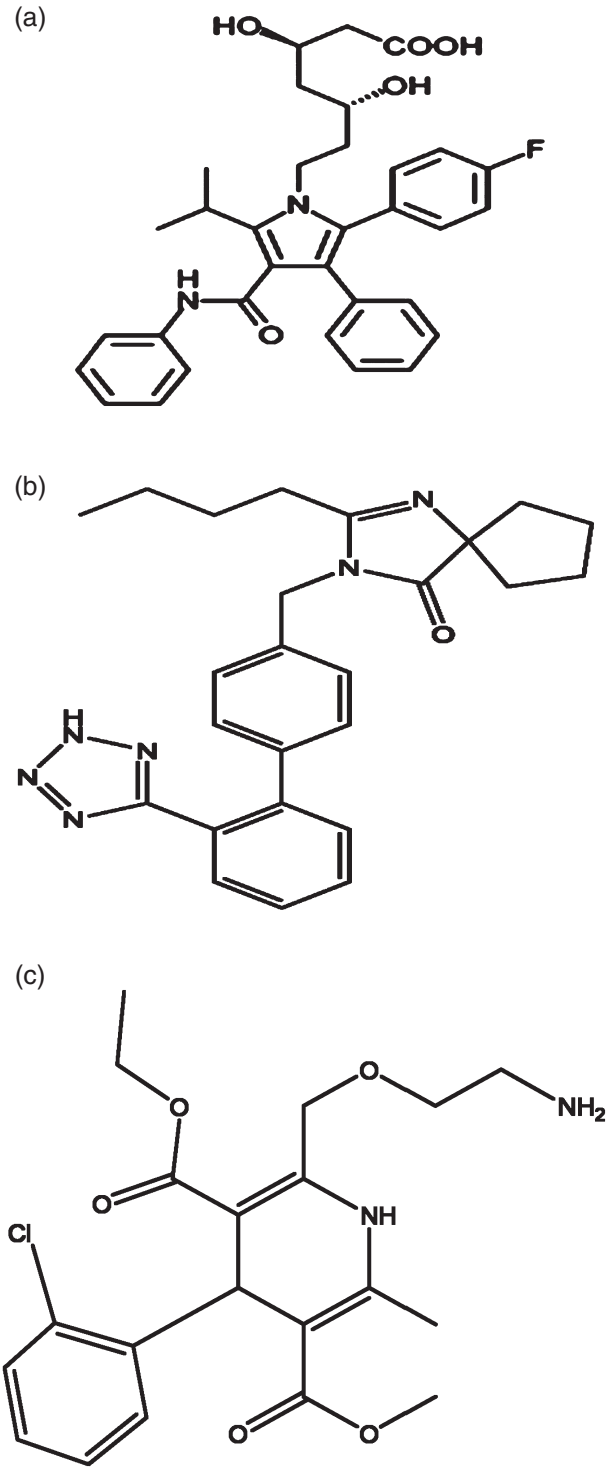

Figure 1. (a) Atorvastatin, (b) irbesartan, and (c) amlodipine.

Experimental

Atorvastatin, irbesartan, and amlodipine reference standards were a kind gift from Jamjoom Pharma, Jeddah, KSA. Lipitor (atorvastatin 40 mg) from Parke-Davis, Arena (irbesartan 150 mg) from Jamjoom Pharma, Jeddah, KSA, and Norvasc (amlodipine 10 mg) from Pfizer Laboratories were purchased from a local pharmacy. HPLC-grade acetonitrile, methanol, and phosphoric acid of (Merck, Darmstadt, Germany) and freshly prepared deionized water were used throughout the experiments. A Waters HPLC system with Waters 2998 PDA (photodiode array detector) and Waters e2695 separation modules pump with auto-injector was used along with a Waters XBrigde C18 column (5 μm, 25 × 0.46 cm). Empower 2 software (Waters Corporation, Milford, MA, USA) was used for data acquisition.

Standard and Sample Preparation. Stock standard solutions of atorvastatin, irbesartan, and amlodipine (100 μg/mL each) were prepared by accurately weighing 10 mg of each, transferring to 100 mL volumetric flasks, and making up with acetonitrile. These stock solutions were further diluted to obtain the working concentration ranges (5–30 μg/mL).

Procedure for Commercial Tablets. Homogenized mixture of 20 tablets of each drug was obtained by weighing, powdering, and triturating them. The active substance (100 μg/mL concentration) was dissolved in acetonitrile to prepare the sample solutions and sonicated for 10 min. These solutions were then filtered through a 0.45 μm filter with appropriate dilution to yield a linearity concentration range of each drug, which were then chromatographed.

Analysis of Percentage Recoveries. Aliquots of atorvastatin, irbesartan, and amlodipine standard solutions were transferred into a series sample solutions in 10 mL volumetric flasks. The procedure described above during sample preparation was then used. The corresponding regression equations were used to calculate the percentage recoveries.

Chromatographic Conditions. A gradient mobile phase system consisting of acetonitrile/phosphoric acid buffer (pH 2.2) employing the gradient program shown in Table 1 was used. At a flow rate of 1.0 mL/min, all analytes were detected at 240 nm. A representative chromatogram is shown in Figure 2. At room temperature, separation was carried out on a C18 XBrigde column.

Forced Degradation and Stability-Indicating Study

Acidic and Basic Conditions. Atorvastatin, irbesartan, and amlodipine solutions were treated with 5 mL of 1 M hydrochloric acid solution or 1 M sodium hydroxide solution. Solutions were kept for 2 h in a water bath, which had already been maintained at 90 °C. After specified time intervals,

Table 1. Gradient program.

S. No.	Time (min)	Aqueous phase %	Organic phase %
1	5	62	38
2	5.50	30	70
3	15	30	70

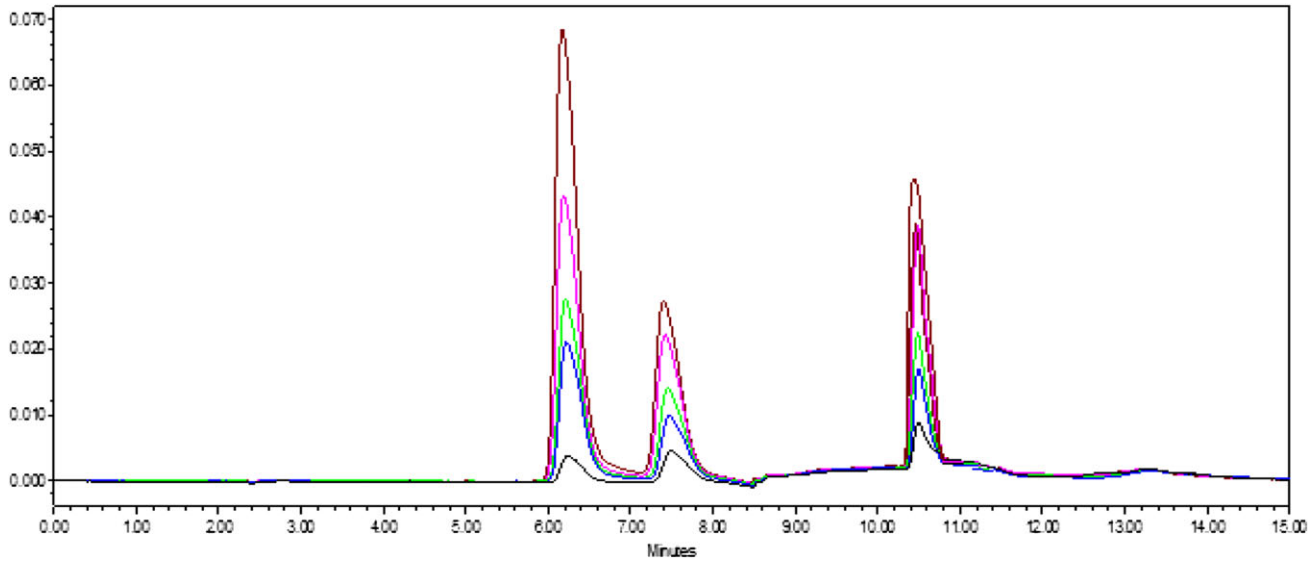

Figure 2. Representative chromatograms of linearity study of irbesartan at retention time of 6.18 min, amlodipine at 7.42 min, and atorvastatin at 10.46 min.

neutralization of all solutions at pH 7.0 was carried out and the volume adjusted with acetonitrile.

Oxidation with Hydrogen Peroxide. Atorvastatin, irbesartan, and amlodipine solutions were treated with 5 mL of 3% H_2O_2 solution and were placed in a water bath at 80 °C for 30 min. After specified time intervals, the volumes of the solutions were adjusted with acetonitrile to obtain a final concentration.

Photolytic Degradation. The powder of each drug, equivalent to 10 mg, was kept under UV irradiation for 24 h at 254 nm. After specified times, the powder of each drug was dissolved in acetonitrile, aliquots from these stock solutions were transferred to separate flasks, and the volume was made up to the mark with acetonitrile.

Dry Heat Degradation. Ten milligrams of the powder of each drug was kept for 3 h in an oven at 90 °C. After a specified time, each powder was transferred to a flask and dissolved in acetonitrile to obtain the final concentrations.

Results

The developed method was validated to establish the linearity, specificity, precision and accuracy, limits of detection and quantification, and percent recovery according to ICH guidelines.[14]

Linearity. Linearity was determined by the calibration curves obtained by analysis of the standard solution of atorvastatin, irbesartan, and amlodipine. Slope and the other statistics of the calibration curves were calculated by linear regression (Table 2). The correlation coefficients obtained were 0.9982, 0.9973, and 0.9986 for atorvastatin, irbesartan, and amlodipine, respectively.

Specificity. The specificity of the method was demonstrated by the successful resolution of the intact drugs from their forced degradation products.

Table 2. Regression statistics: LOD and LOQ

Parameters	Atorvastatin	Irbesartan	Amlodipine
Linearity range (μg/mL)	5–30	5–30	5–30
LOD (μg/mL)	0.05	0.06	0.08
LOQ (μg/mL)	0.41	0.55	0.78
Slope	18 853	48 798	18 644
Intercept	13 301	14 769	3417
Coefficient of determination (r^2)	0.9982	0.9973	0.9986

Table 3. Accuracy of the proposed method.

Analyte	Spiked concentration (μg/mL)	Accuracy (%)
Atorvastatin	10	102.40
	20	100.52
	30	102.14
Irbesartan	10	100.99
	20	98.36
	30	98.29
Amlodipine	10	101.66
	20	102.72
	30	102.39

Accuracy and Precision. The results of the accuracy and precision of the method at three different concentration levels are demonstrated in Tables 3 and 4. The percentage RSD values in each case did not exceed more than 2%, which clearly indicated the good precision of the analytical methods at low levels for all drugs. The results are shown in Table 4.

Limits of detection and quantitation (LOD and LOQ). The sensitivity of the method was investigated by means of the limit of detection and quantitation for atorvastatin, irbesartan,

and amlodipine at a signal-to-noise ratio of 3 and 10, respectively, according to ICH guidelines.[14] The LOD values were found to be 0.05, 0.06, and 0.08 μg/mL, while the LOQ values were 0.41, 0.55, and 0.78 μg/mL for the three drugs, respectively (Table 2).

Relative recovery. The mean recoveries of the drugs were calculated by the standard addition method. Solutions were prepared with a standard powder (known amount) of each drug added to powdered samples of the tablets to yield the desired concentrations. The assay was repeated, and the observed concentrations of the drugs are presented in Table 5. The corresponding percentage recoveries values did not exceed 101.90% for all three drugs.

Degradation studies. Forced degradation experiments were carried out on the three standard drugs by subjecting them to acid and alkali hydrolysis, chemical oxidation, dry heat degradation, and exposure to UV light. The drugs were successfully separated from all the degradation products in all these forced degradation experiments. The purity of the drug peaks was confirmed, and no co-elution from any of the degradation products was detected.

The chromatograms of acid-degraded sample of atorvastatin showed a degradation product peak at the retention time of 11.40 min (Figure 3) and was 37% decomposed. No strong signs of degradation were observed in basic condition even after heating at 90 °C for 2 h, and an almost identical peak appeared at its specific retention time. These results were quite similar to those in other reported studies.[10] Irbesartan was found to be less stable to acid and alkaline hydrolysis (Figure 4), resulting in 55% and 45% decomposition, respectively. It was also reported that irbesartan hydrolyzed by the breakage of the amide link in 1,3-diazaspiro [4.4] non-1-en-4-one, which was identified as ((1-(1-((2 (*E*-(1*H*-tetrazol-5-yl) biphenyl-4-yl) methyl amino) pentylidene amino)cyclopentane carboxylic acid)).[13,15] Acidic hydrolysis results of amlodipine showed no change; therefore it can be concluded that it is stable in this condition. However, under basic conditions, amlodipine was found to be unstable (Figure 5), and 63% decomposed. This degradation under acidic and basic conditions of amlodipine is due to the presence of the acetyl group.[3] Amlodipine showed less stability under peroxide degradation conditions and decomposed up to 72%, as was evident from the chromatogram (Figure 6).[11] It has been reported that compounds containing "1, 4-dihydropyridine" are prone to photolytic decomposition,[16–19] and amlodipine was found to be no exception to this, as it oxidized to its pyridine analog when exposed to light (Figure 7) resulting in 14% decomposition. No differences in the peak areas were observed in case of atorvastatin and irbesartan powders when irradiated with UV light for 24 h (Figure 7). Under thermal (dry heat) degradation conditions, all the three drugs were found to be stable. The peak areas were found to be comparable to those of the standards of the same concentrations (Figure 8). Oxidative stress conditions were found not to affect atorvastatin and irbesartan (Figure 6, Table 6). These findings are in agreement with those of previous studies.[3,11] The peaks of all degradation products were found to resolve well from the drug peaks.

Table 4. Precision of the proposed method.

Analyte	Concentration used (μg/mL)	%RSD
Atorvastatin	100	1.26
Irbesartan	100	0.28
Amlodipine	100	0.28

Table 5. Percentage recoveries of the proposed method.

Analyte	Spiked concentration (μg/mL)	Recovery (%)
Atorvastatin	5	100.41
	10	101.00
	15	99.94
Irbesartan	5	101.90
	10	100.11
	15	99.98
Amlodipine	5	100.21
	10	99.79
	15	100.89

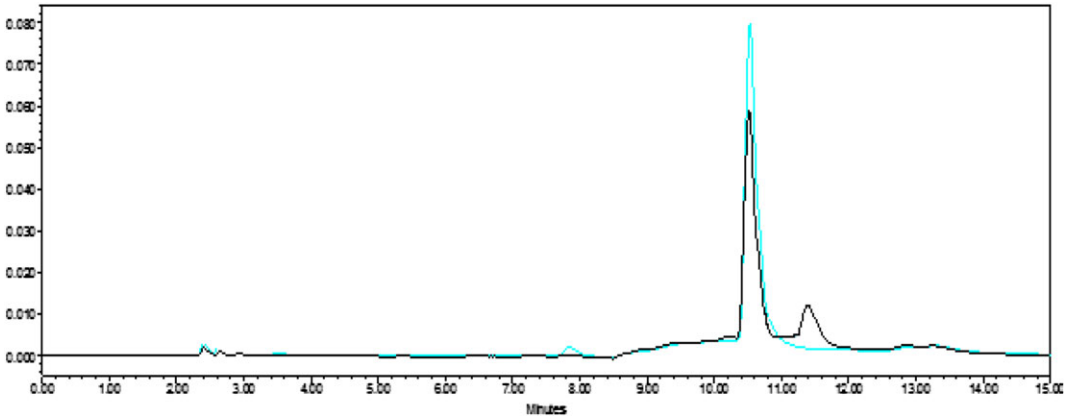

Figure 3. Chromatograms of acidic (black) and basic hydrolysis products (green) of atorvastatin.

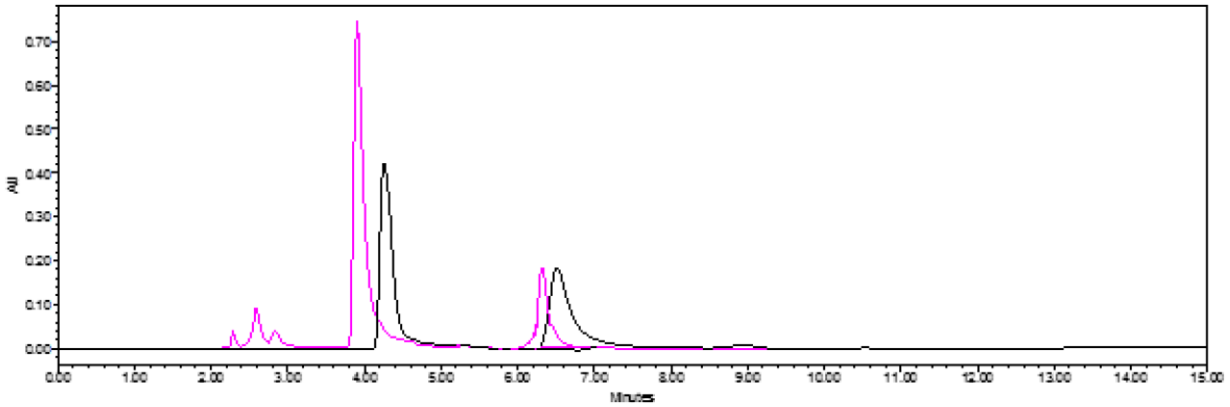

Figure 4. Chromatograms of acidic and basic hydrolysis products of irbesartan.

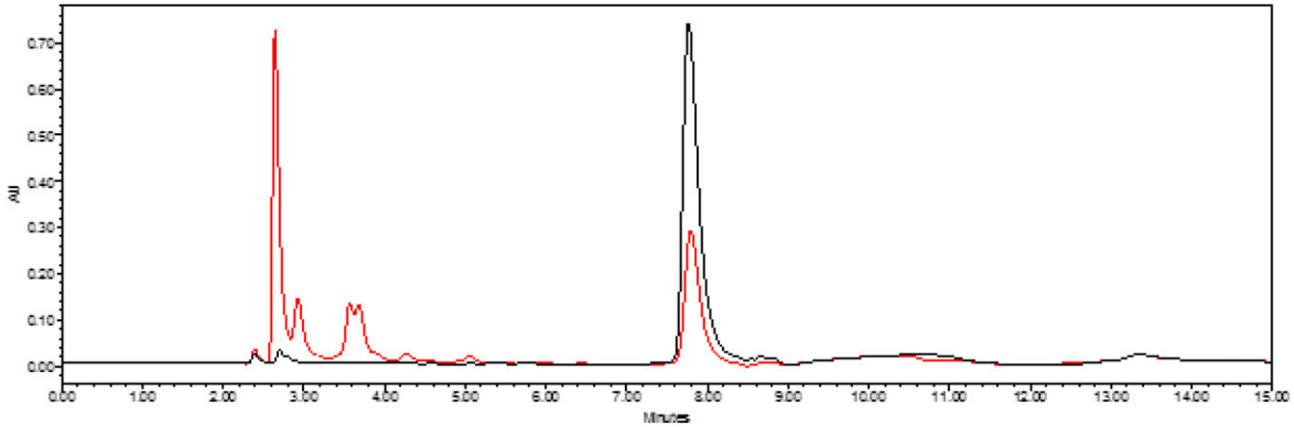

Figure 5. Chromatograms of acidic and basic hydrolysis products of amlodipine.

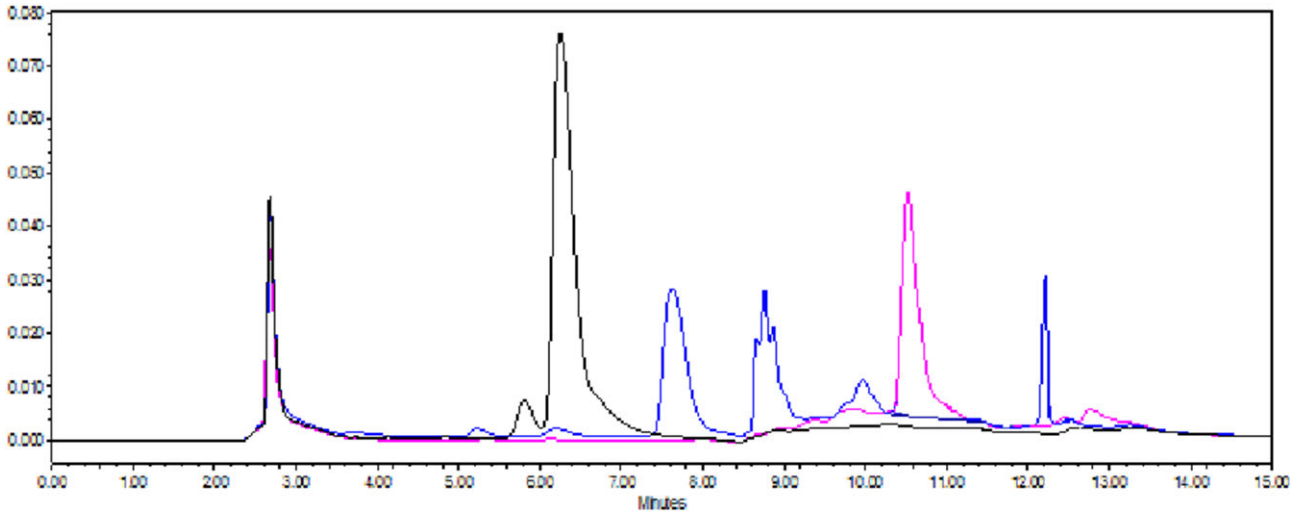

Figure 6. Chromatograms of oxidative hydrolysis products of irbesartan (black), amlodipine (blue), atorvastatin (pink).

Discussion

By looking at the solubility profile, solutions of all the analytes were prepared in acetonitrile since atorvastatin was only very slightly soluble in distilled water, acetonitrile, and phosphate buffer (pH 7.4).[20] Irbesartan is practically insoluble in water but slightly soluble in alcohol and methylene chloride,[21] while amlodipine besylate is sparingly soluble in ethanol and slightly soluble in water.[22] In the case of atorvastatin, methanol was used as a co-solvent. All the molecules are sufficiently

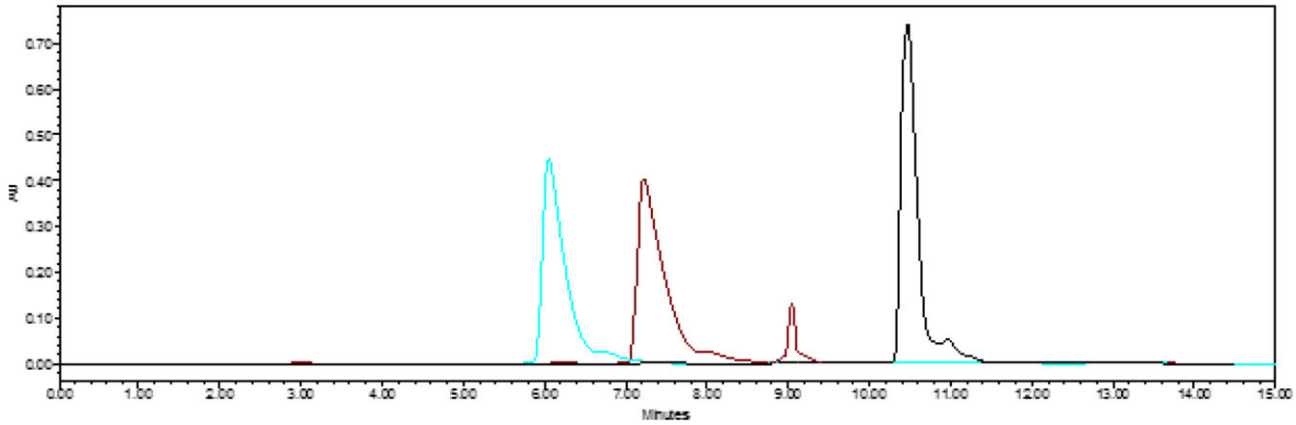

Figure 7. Chromatograms of UV-light hydrolysis products of irbesartan (green) amlodipine (brown), and atorvastatin (black).

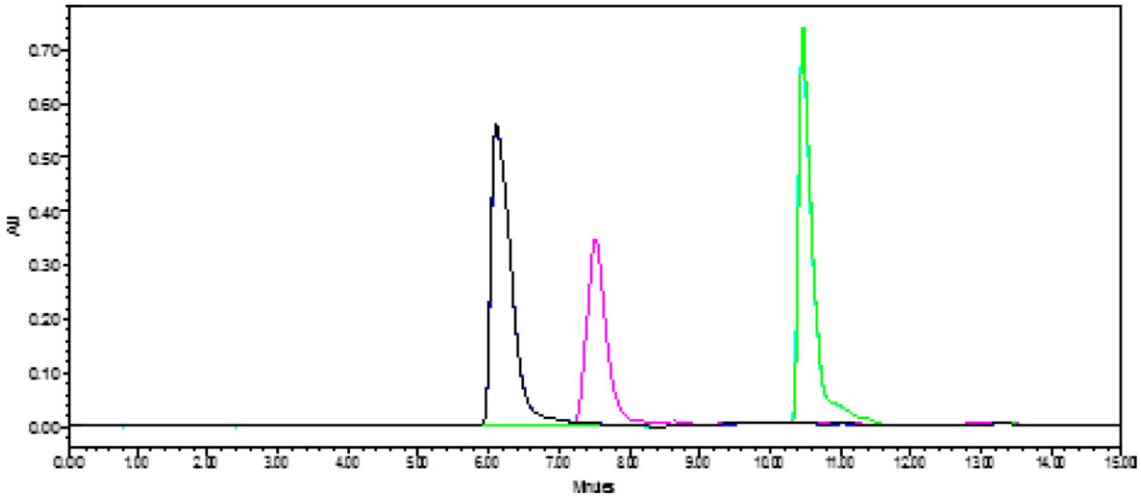

Figure 8. Chromatograms of dry-heat hydrolysis products of irbesartan (black), amlodipine (pink), atorvastatin (green).

Table 6. Comparing peak areas of normal and degraded conditions.

	Area under curve (AUC)		
Conditions	Atorvastatin	Irbesartan	Amlodipine
Standards	1 322 866	557 912	712 255
Acid hydrolysis	841 499	250 148	698 977
Basic hydrolysis	1 300 949	310 246	261 032
Oxidative hydrolysis	1 299 149	541 979	201 025
Photolytic degradation	1 315 986	549 956	610 985
Dry heat degradation	1 291 986	550 894	701 321

nonpolar so that they are well retained on C18-bonded phases and co-eluted with each other. Atorvastatin has a dissociation constant (pK_a) of 4.46 and a partition coefficient [log *P* (octanol/water)] of 6.36.[20] Irbesartan is a nonpolar compound and at pH 7.4 has a partition coefficient of 10.1 (pK_a 4.2),[21] while amlodipine has a partition coefficient of 2.66 in octanol/water and pK_a of 8.73.[23–25]

Optimum conditions were achieved by overcoming various hurdles systematically. The main problem encountered during these investigations was the lack of resolution between amlodipine and irbesartan. In order to overcome these hurdles, several mobile phases were evaluated using various proportions of different aqueous and organic phases. Optimization of sensitivity, speed, and peak shape was achieved by attempting different combinations of organic solvents (acetonitrile and methanol) with buffer (phosphate). Acetonitrile showed better response than methanol. Initially, acetonitrile with the different ratios of the aqueous phase was used as mobile phase with pH 3.5–4 but the separation of amlodipine and irbesartan was not achieved. In these first attempts, poor separation was frequently observed between amlodipine and irbesartan, eluted approximately with same retention times, and some chromatograms showed broad peaks. Acetonitrile with the phosphate buffer at pH adjusted to 2.2 gave good results.

At lower pH values, silanols on a reversed-phase HPLC column are protonated and basic compounds become positively charged, so that the peak-tailing problem of the basic drug will be eliminated.[9] In this work, the separation of drugs was achieved on a Waters XBrigde C18 column, which can tolerate low pH (up to 2). The phosphate buffer of pH 2.2 was finally employed, and separation was achieved by employing

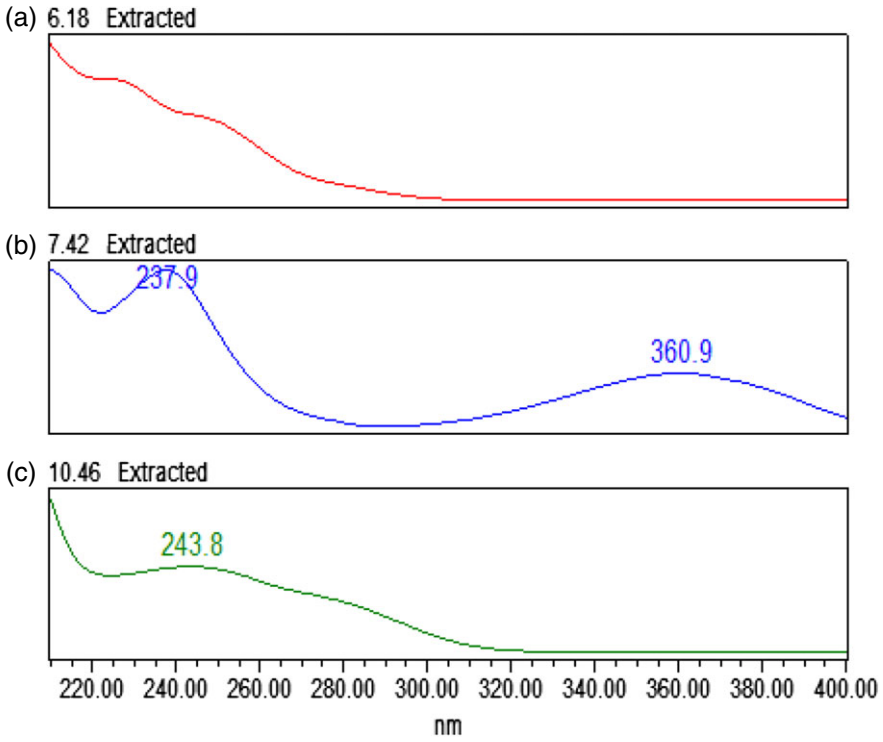

Figure 9. Spectra obtained from UV/DAD: (a) irbesartan, (b) amlodipine, and (c) atorvastatin.

the gradient program shown in Table 1. It was possible to detect the UV spectral peaks of the drugs with the help of the UV-PDA detector, and the isosbestic points for all drugs were selected (*i.e.*, 240 nm) as seen in Figure 9. The flow rate was kept constant at 1 mL/min throughout the run, and the applied gradient program produced a stable baseline without any drift or deformation. The separation was performed at room temperature. The method we have developed is more economical, consuming smaller amounts of the organic phase.

Conclusion

An accurate and precise stability-indicating HPLC analytical method meeting the ICH guidelines has been developed and validated for the simultaneous analysis of atorvastatin, irbesartan, and amlodipine in active and tablet dosage forms, which was found to be convenient and could be used for all purposes. None of the reported analytical methods describes a stability-indicating scheme for the determination of atorvastatin, irbesartan, and amlodipine from their potential impurities and forced degradation products or possible co-administered drugs. To the best of our knowledge, this is the first reported method indicating the stability for the determination of atorvastatin, irbesartan, and amlodipine simultaneously in active and dosage forms. This present paper described an accurate, user-friendly, economical, and quick gradient-based reversed-phase stability-indicating HPLC method for the simultaneous determination of atorvastatin, irbesartan, and amlodipine.

Conflicts of interest. The authors report no potential conflicts of interest in this work.

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