

# Simultaneous Determination of Amlodipine and Aliskiren in Tablets by High-Performance Liquid Chromatography

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**A new, simple, rapid and specific reversed-phase high-performance liquid chromatography (HPLC) method was developed and validated for the simultaneous determination of amlodipine besylate and aliskiren hemifumarate. The HPLC separation was achieved on an RP-18 column (250 × 4.6 mm) using a mobile phase of triethylamine–orthophosphoric acid buffer (50 mM, pH 3.0), acetonitrile and methanol (50:40:10, v/v/v) at a flow rate of 1 mL/min. The method was validated for specificity, linearity, precision, accuracy and robustness. The degree of linearity of the calibration curves, the percent recovery values of amlodipine and aliskiren and the limits of detection (LOD) and quantification (LOQ) for the HPLC method were determined. The linearity of the method was found to be in the concentration range of 5.0–50.0 µg/mL for aliskiren hemifumarate and 2.65–26.50 µg/mL for amlodipine besylate, LOD and LOQ values were 0.51, 0.95, 1.70 and 3.18 µg/mL for amlodipine besylate and aliskiren hemifumarate. The proposed method was successfully applied to amlodipine besylate and aliskiren hemifumarate in pharmaceutical dosage mixtures without any interference from the excipients. The method was found to be precise, accurate, reproducible and robust. The results agreed with those obtained using the developed reference method.**

## Introduction

A fixed-dose combination of antihypertensive drugs can simplify dosing regimens, improve hypertension control, decrease dose-dependent side-effects and reduce cost as the first-line treatment for hypertension (1). These potential advantages recommend it for the combination of antihypertensive therapy to be used as an initial treatment (2).

Amlodipine besylate (Figure 1) is listed in Extra-Pharmacopoeia and European Pharmacopoeia (3, 4) and is chemically (4R,S)-3-ethyl-5-methyl-2-(2-amino-ethoxy-methyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl pyridine-3,5-dicarboxylate monobenzene sulfonate. It is approved for the treatment of variant and stable angina and hypertension. It is a relatively new long-acting calcium channel blocker with a slow onset of vasodilatory action (5).

Different methods have been reported for the quantification of amlodipine besylate, which include high-performance liquid chromatography (HPLC) (6–18), high-performance thin-layer chromatography (19, 20), gas chromatography (21), ultraviolet–visible (UV-VIS) spectrophotometry, derivative spectroscopy (22–35), fluorimetry (36, 37), capillary electrophoresis (38) and electroanalytical methods (39–42).

Aliskiren hemifumarate (Figure 2), (2(S),4(S),5(S),7(S)-N-(2-carbamyl-2-methyl propyl)-5-amino-4-hydroxy-2,7-diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy) phenyl]-octanamide hemifumarate, is the first oral direct renin inhibitor approved for clinical use that exhibits a novel and advantageous pharmacokinetic and pharmacodynamic profile for the long-term treatment of hypertension. Aliskiren blocks the renin system at its rate-limiting step by directly inhibiting the catalytic activity of renin, thereby reducing the generation of angiotensin I and angiotensin II (42, 43).

A few methods have been reported for the quantification of aliskiren hemifumarate, which include HPLC (44–46) and UV-VIS spectrophotometry (47).

The simultaneous quantification of aliskiren and amlodipine has not been reported, although there is also a new formulation (Tekamlo). Therefore, the focus of the present study was to develop and validate a reversed-phase (RP) HPLC method for the simultaneous determination of amlodipine and aliskiren in pharmaceutical formulations.

## Experimental

### Chemicals

Amlodipine besylate and aliskiren hemifumarate were supplied by Mustafa Nevzat and Novartis (Istanbul, Turkey). Their pharmaceutical preparations, Monovas tablet (10.0 mg of amlodipine besylate, Istanbul, Turkey) and Tekturna tablet (165.75 mg of aliskiren hemifumarate, Chicago, IL) were purchased from drug stores. All other chemicals and solvents were of analytical or HPLC grade (Merck, Istanbul, Turkey).

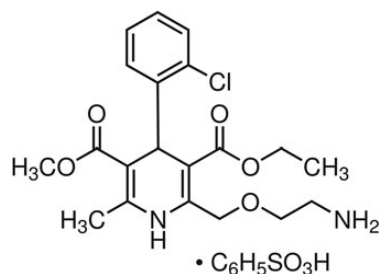
### Instrumentation

HPLC analyses were performed on a Thermo Separation Products (TSP)-1100 system controller with a TSP-P4000 pump, a TSP-AS autosampler with a 100 µL loop and a TSP-UV 3000 UV detector. Compounds were separated on a Purospher STAR RP-18 endcapped LiChroCART RP-18 (Merck, Darmstadt, Germany) analytical column (250 × 4.6 mm, 5 µm) and guard column (4 × 3 mm, 5 µm; Hichrom, Kromasil). The flow rate of the mobile phase was maintained at 1.0 mL/min.

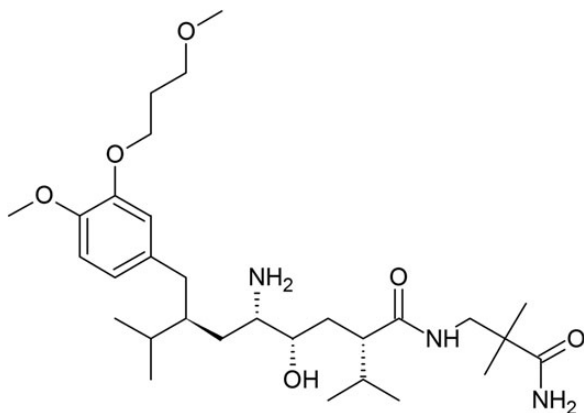
## Methods

### Preparation of solution

A stock solution of amlodipine besylate (0.106 mg/mL) and aliskiren hemifumarate (0.20 mg/mL) was prepared in water.



**Figure 1.** Chemical structure of amlodipine besylate.



**Figure 2.** Chemical structure of aliskiren.

#### Mixture of solution

Volumes of 0.25–2.50 mL were taken from the stock solutions of aliskiren hemifumarate and amlodipine besylate and each separately completed to 10 mL with water.

#### Analysis of tablet mixture

Twenty tablets were weighed (amlodipine besylate and aliskiren hemifumarate) and powdered portions equivalent to 150.0 mg of aliskiren hemifumarate and 10.0 mg of amlodipine besylate were transferred to a 25 mL calibrated flask; 20 mL of water was added and the solution was sonicated for 30 min. The solution was completed to volume with water, mixed well and filtered. The prepared solution was quantitatively diluted with methanol to obtain a suitable concentration for the analysis.

#### Method validation

The method was validated in accordance with International Conference on Harmonization (ICH) guidelines (48) for the validation of analytical procedures.

#### Linearity

The calibration curves of aliskiren hemifumarate and amlodipine besylate were constructed by linear regression. The plots of peak areas versus concentrations of the associated compound were employed.

#### Limits of Detection and Quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the drugs according to the proposed method were determined using calibration standards. LOD and LOQ were calculated as  $3.3 \sigma/S$  and  $10 \sigma/S$ , respectively, where  $S$  is the slope of the calibration curve and  $\sigma$  is the standard deviation of the intercept of the regression equation.

#### Precision and accuracy

The precision and accuracy of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). The intra-day precision was calculated as the relative standard deviation (RSD) of the results from three standard samples during the same day and the inter-day precision was studied by comparing the assays on three different days. The accuracy of the method was expressed by relative mean error (RME).

#### Recovery

The recovery of amlodipine besylate and aliskiren hemifumarate was calculated from the mixtures of tablets.

#### Robustness

The robustness of the method was evaluated by introducing small variations in the optimum conditions. These variations included mobile phase, flow rate and column oven temperature.

#### Specificity

The specificity of the method was investigated by observing any interference encountered from the excipients of the tablets, which did not interfere with the proposed methods.

#### System suitability

To ascertain the resolution and reproducibility of the HPLC method, system suitability tests were performed by using the working standard solution of amlodipine besylate and aliskiren hemifumarate. Resolution ( $R_s$ ), theoretical plate number ( $N$ ) and tailing factor ( $T$ ) were measured as the criteria for system suitability testing.

## Results

#### HPLC method development

Several parameters were examined to optimize the HPLC analysis of amlodipine and aliskiren. The first attempt was to determine the consistency of the mobile phase (pH 3.0). Different mixtures of acetonitrile, methanol and triethylamine–ortho phosphoric acid buffer (50 mM) were tried as the mobile phase, from 40:30:30, 40:20:40, 40:15:45, 50:20:30 and 40:10:50 (v/v/v). The most suitable peaks were appeared when a solvent system of 40:10:50 (v/v/v) was utilized with a flow rate of 1.0 mL/min. The elution order was aliskiren [retention time ( $t_R$ ) = 4.12 min] and amlodipine ( $t_R$  = 5.22 min). Typical chromatograms of aliskiren and amlodipine are shown in Figure 3.

## Method validation

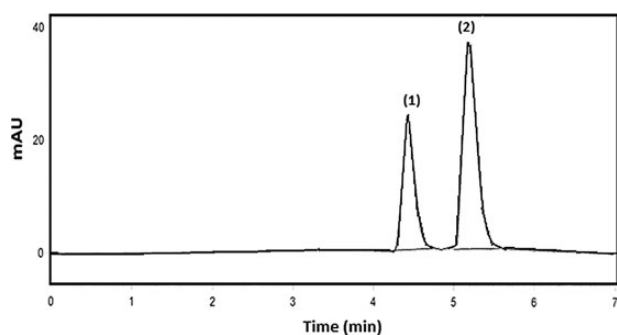
### Linearity

The analytical curves were obtained from six concentrations of reference solutions in the ranges of 5.0–50.0 µg/mL for aliskiren hemifumarate and 2.65–26.50 µg/mL for amlodipine besylate. Each solution was prepared in triplicate. The linearity was evaluated by linear regression analysis by the least-squares regression method, which was used to calculate the concentration coefficient, *Y*-intercept and slope of the regression line.

The calibration curves for aliskiren and amlodipine were linear for HPLC method over the concentration ranges of 5.0–50.0 and 2.65–26.5 µg/mL, respectively; linear regression equations and correlation coefficient ( $r^2$ ) are as follows:  $Y_{ALS} = 7.921 + 6.433x$ , ( $r^2 = 0.9960$ ) and  $Y_{AML} = 29.67x - 18.51$ , ( $r^2 = 0.9990$ ).

### LOD and LOQ

The LODs for amlodipine besylate and aliskiren hemifumarate were found to be 0.51 and 0.95 µg/mL, respectively. Under the



**Figure 3.** HPLC chromatogram of amlodipine and aliskiren: 26.50 µg/mL of aliskiren (peak 1); 1.60 µg/mL of amlodipine (peak 2).

**Table I**

Validation Summary

Validation parameters (system suitability)	Results	
	Amlodipine	Aliskiren
<i>N</i>	4,250	3,861
Linearity range (µg/mL)	2.65–26.50	5.0–50.0
<i>T</i>	1.71	1.61
<i>R<sub>s</sub></i>	2.1	
<i>R</i>	0.9990	0.9960
<i>t<sub>R</sub></i>	5.22	4.12
LOD (µg/mL)	0.51	0.95
LOQ (µg/mL)	1.70	3.18

**Table II**

Intra-Day and Inter-Day Precision and Accuracy of Amlodipine and Aliskiren Assay (*n* = 3)

Drug	Intra-Day				Inter-Day			
	Nominal concentration (µg/mL)	Measured concentration (µg/mL); mean ± SD	Precision (RSD; %)	Accuracy (RME; %)	Nominal concentration (µg/mL)	Measured concentration (µg/mL); mean ± SD	Precision (RSD; %)	Accuracy (RME; %)
Amlodipine	3.25	3.27 ± 0.03	0.92	0.61	3.25	3.26 ± 0.02	0.64	0.31
	9.50	9.30 ± 0.04	0.43	-2.15	9.50	9.32 ± 0.06	0.64	-1.93
	20.50	20.52 ± 0.03	0.15	0.09	20.50	20.51 ± 0.02	0.10	0.05
Aliskiren	17.50	17.52 ± 0.10	0.59	0.11	17.50	17.35 ± 0.04	0.23	-2.04
	26.25	26.38 ± 0.05	0.20	0.49	26.25	27.38 ± 0.06	0.23	0.47x
	43.75	43.66 ± 0.06	0.14	-0.21	43.75	43.65 ± 0.12	0.27	-0.23

developed HPLC conditions, the LOQs were 1.70 and 3.18 µg/mL for amlodipine besylate and aliskiren hemifumarate (Table I).

### Precision and accuracy

The intra-day and inter-day reproducibility values, expressed as RSD, were 0.15–0.92, 0.1–0.64% and 0.14–0.59, 0.23–0.27% for amlodipine besylate and aliskiren hemifumarate, respectively; therefore, it is obvious that the method is remarkably accurate, which ensures that reliable results are obtained (Table II).

### Recovery

The results (Table III) showed that the mean recovery values of amlodipine besylate and aliskiren hemifumarate were in the ranges of 97.89–100.62% and 101.04–103.59%.

### Robustness

The robustness is a measurement of the method's capacity to remain unaffected by small but deliberate variations in method parameters; this was studied by testing the influences of small changes in the composition of the mobile phase and the column oven. All critical separations were achieved with the indicated minimum baseline resolution.

### System suitability

System suitability was tested on the basis of results obtained from several representative chromatograms. According to ICH guidelines, the system is suitable when  $R_s > 2$ ,  $N > 2,000$  and  $T < 2$ . The values obtained for this method were within the acceptable ranges (Table I).

### Specificity

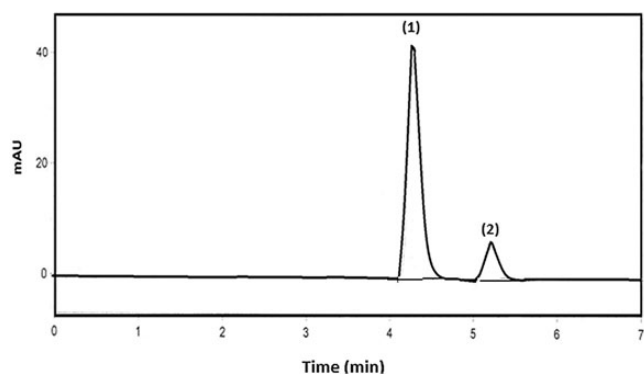
The specificity analysis revealed the HPLC method did not suffer interferences from the formulation excipients, because there were no other peaks in the retention times of amlodipine and aliskiren.

### Comparison method

There is no comparison method in the literature, so a new UV-VIS spectrophotometric method was developed in this study. The absorbance of standard and sample solutions was measured at 365.0 nm for amlodipine and at 279.5 nm for aliskiren. Amlodipine besylate and aliskiren hemifumarate were found to be linear in the ranges of 5.0–66.0 µg/mL and 20.0–272.0 µg/mL, respectively. The slope, intercept and correlation coefficient values were also calculated. The correlation

**Table III**Recovery Results of Amlodipine and Aliskiren ( $n = 3$ )

Drug	Nominal concentration ( $\mu\text{g/mL}$ )	Measured concentration; mean $\pm$ SD ( $\mu\text{g/mL}$ )	Recovery (%)	RSD (%)
Amlodipine	3.25	$3.27 \pm 0.03$	100.62	0.92
	9.50	$9.30 \pm 0.04$	97.89	0.43
	20.50	$20.52 \pm 0.03$	100.10	0.15
Aliskiren	16.70	$17.30 \pm 0.24$	103.59	1.37
	33.60	$33.95 \pm 0.27$	101.04	0.78
	50.45	$51.84 \pm 0.28$	102.76	0.54

**Figure 4.** HPLC chromatogram of amlodipine and aliskiren in tablet mixtures: 23.98  $\mu\text{g/mL}$  of aliskiren (peak 1); 1.15  $\mu\text{g/mL}$  of amlodipine (peak 2).**Table IV**

Determination of Pharmaceuticals by the Proposed HPLC and UV-VIS Spectrophotometric Methods (Monovas 10 mg; Tekturna 150 mg)\*

	Amlodipine		Aliskiren	
	HPLC	UV-VIS	HPLC	UV-VIS
Recovery $\pm$ SD (%)	$101.00 \pm 0.07$	$101.0 \pm 0.16$	$101.1 \pm 0.62$	$100.9 \pm 0.36$
$t$	0.62		1.67	
$F$	0.60		2.92	

\* $n = 5$ ; tabulated values of  $t$  and  $F$  at 95% confidence limit are 2.23 and 5.05, respectively.

coefficients of amlodipine and aliskiren were 0.9981 and 0.9924, respectively.

### Application

The proposed method was applied to the determination of aliskiren hemifumarate and amlodipine besylate in tablets (Figure 4). A comparative determination of the same samples was also investigated by using the developed method. A statistical comparison of the results by Student's  $t$ -test and the variance ratio  $F$ -test at 95% confidence level revealed no significant differences between the accuracy and precision of the two methods (Table IV).

### Discussion

The simultaneous quantification of aliskiren and amlodipine has not been reported, although there is now a new formulation (Tekamlo). Therefore, a new, simple, rapid and specific HPLC

method was developed and validated for the simultaneous determination of amlodipine besylate and aliskiren hemifumarate. The HPLC separation was achieved on an RP-18 column by using a mobile phase of triethylamine–orthophosphoric acid buffer (pH 3.0), acetonitrile and methanol (50:40:10, v/v/v) at a flow rate of 1 mL/min. The linearity of the method was found to be in the concentration ranges of 5.0–50.0  $\mu\text{g/mL}$  for aliskiren hemifumarate and 2.65–26.50  $\mu\text{g/mL}$  for amlodipine besylate. LOD and LOQ values were 0.51, 0.95, 1.70 and 3.18  $\mu\text{g/mL}$  for amlodipine besylate and aliskiren hemifumarate. The proposed method was successfully applied to determine amlodipine besylate and aliskiren hemifumarate in pharmaceutical dosage mixtures without any interference with the excipients. The method was found to be precise, accurate, reproducible and robust. The results agreed with those obtained by using the developed reference method.

### Conclusions

This method proposes, for the first time, the development of a sensitive, simple and quick HPLC method for the simultaneous determination of amlodipine and aliskiren in pharmaceutical combined dosage forms. The short analytical run time of 5.3 min and the relatively low flow rate lead to an environmentally friendly chromatographic procedure that allows the analysis many samples in a short period of time and with less mobile phase. All statistical values were within the acceptable limits. Therefore, this HPLC method can be used for routine drug analysis.

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