Determination of Aliskiren in Tablet Dosage Forms by a Validated Stability-indicating RP-LC Method

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Abstract

A reversed-phase liquid chromatography (RP-LC) method is validated for the determination of aliskiren in tablet dosage form. The LC method is carried out on a Waters XBridge C_{18} column (150 × 4.6 mm i.d.), maintained at 25°C. The mobile phase consisted of acetonitrile:water (95:5, v/v)/phosphoric acid (25 mM, pH 3.0) (40:60, v/v), run at a flow rate of 1.0 mL/min, with photodiode array detector set at 229 nm. The chromatographic separation is obtained with aliskiren retention time of 3.68 min, and it is linear in the range of 10–300 µg/mL (r = 0.9999). The limits of detection and quantitation are 2.38 and 7.93 µg/mL, respectively. The specificity and stability-indicating capability of the method are proven through degradation studies, which also showed that there is no interference of the formulation excipients, showing that peak is free from any coeluting peak. The method showed adequate precision, with a relative standard deviation (RSD) values lower than 0.92%. Good values of accuracy were also obtained, with a mean value of 99.55%. Experimental design is used during validation to calculate method robustness. The proposed method is applied for the analysis of the tablet dosage forms, contributing to improve the quality control and to assure the therapeutic efficacy.

Introduction

Hypertension is one of the most common and powerful risk factors for cardiovascular diseases. The control of the hypertension and also serum cholesterol and smoking has been widely promoted in order to reduce the risk of cardiovascular diseases and their complications (1,2). Overall, heart failure in either form is a major global health problem contributing to a significant morbidity and mortality and requires a significant portion of health care spending (3).

The renin angiotensin system plays an important role in the regulation of cardiovascular and renal function and in maintaining the electrolyte balance of the body and has long been recognized as a desirable target for antihypertensive therapy (4–9). More recent research has lead to the discovery of several classes of nonpeptidic renin inhibitors. Aliskiren (2(*S*),4(*S*),5(*S*),7(*S*)-*N*-(2-carbamoyl-2-methylpropyl)-5-amino-4-hydroxy-2,7-diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy)phenyl]-octanamide hemifumarate) (Figure 1), is the first representative of a new class of nonpeptide, low molecular weight, orally active transition-state renin inhibitors (10–13). Its high potency against human renin compensates for its relatively low absolute bioavailability (2–3%). Its long half-life ($t_{1/2}$ 30–40 h) makes it suitable for once daily administration and the plasma steady-state levels are achieved after 5–8 days of treatment (14–16). Therefore, aliskiren is used as a new effective treatment for hypertension (8,17).

An automated reversed-phase liquid chromatography (RP-LC) method with fluorescence detection for the determination of the renin inhibitor CGP 60536 (aliskiren) in animal and human plasma and urine has been developed and validated (18). A validated liquid chromatography-tandem mass spectrometry (LC–MS–MS) method employing positive ion electrospray ionization (ESI^+) was applied for the evaluation of the pharmacokinetic profile of aliskiren in healthy volunteers and patients with hepatic impairment (16,19).

The aliskiren pharmaceutical product is commercially available, but at the moment, there is no published method validated for the quantitative analysis of the drug in pharmaceutical formulations. Therefore, the aim of the present article was to develop and validate a RP-LC method for the quantitative analysis of aliskiren in tablet dosage forms, contributing to improve the quality control and assuring the therapeutic efficacy.

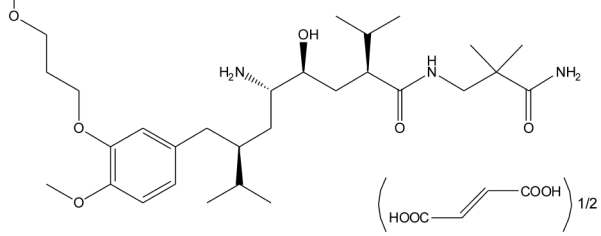

Figure 1. Chemical structure of aliskiren hemifumarate.

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Experimental

Extraction procedure of aliskiren reference substance

Due to the lack of aliskiren reference substance, the active pharmaceutical ingredient was extracted from commercial tablets (Rasilez 300 mg). Twenty tablets were reduced to a fine powder and the aliskiren was extracted with ultrapurified water (pH 7), filtered and then submitted to a SpeedVac concentrator (Model SPD 1010, Thermo Electron Corporation, Milford, MA) until dryness. The obtained powder was analyzed for purity by the proposed RP-LC method, MS, differential scanning calorimetry (DSC), and by melting point. To confirm the identity, additional techniques such as diffuse reflectance infrared Fourier transform spectroscopy (DRIFT) and nuclear magnetic resonance (NMR) were carried out (data not shown). Nonaqueous titration of weak bases was also performed to determine the degree of purity of the drug (20).

Chemical and reagents

A total of six batches of Rasilez (Novartis, São Paulo, Brazil) tablets, containing 150 and 300 mg of aliskiren base were obtained from commercial sources, and were identified by arabic numbers from 1–6. LC-grade acetonitrile and phosphoric acid were purchased from Tedia (Fairfield, OH). For all of the analyses, ultrapure water was used (Labconco, Kansas City, MO). Aliskiren dosage form was labeled to contain the following excipients: magnesiun stearate, macrogol, crospovidone, povidone, microcrystalline cellulose, talc, hypromellose, anhydrous colloidal silica, E171 titanium dioxide, E172 red iron oxide, and E172 black iron oxide.

Apparatus and analytical conditions

The RP-LC method was performed on a Shimadzu LC system (Shimadzu, Kyoto, Japan) equipped with an SCL-10A_{VP} system controller, a LC-10 AD_{VP} pump, a DGU-14A degasser, a SIL-10AD_{VP} autosampler and an SPD-M10A_{VP} photodiode array (PDA) detector. The peak areas were integrated automatically by computer using a Shimadzu Class VP (V 6.14) software program. The experiments were performed on a reversed phase Waters (Milford, MA) XBridge C_{18} column (150 × 4.6 mm i.d., with a particle size of 5-µm, 135 Å). A guard column was used to protect the analytical column. The Shimadzu LC system was operated isocratically at ambient controlled temperature (25°C) using a mobile phase of acetonitrile–water (95:5, v/v)–phosphoric acid (25 mM, pH 3.0) (40:60, v/v), run at a flow rate of 1.0 mL/min, and using PDA detection at 229 nm. The injection volume was 30 µL. Other columns tested: Waters (Dublin, Ireland) XTerra MS C_{18} (150 mm × 3.9 mm i.d., 5 µm, 132 Å); Phenomenex (Torrance, CA) Synergi Fusion C_{18} (150 mm × 4.6 mm i.d., 4 µm, 80 Å); Phenomenex Luna C_{18} (150 × 4.6 mm i.d., 5 µm, 100 Å); Phenomenex Gemini C_{18} (250 × 4.6 mm i.d., 5 µm, 110 Å); Phenomenex Synergi Max-RP C_{12} (150 × 4.6 mm i.d., 4 µm, 80 Å); Shimadzu (Kyoto, Japan) Shim-pack CLC-ODS C_{18} (150 × 4.6 mm i.d., 4 µm, 100 Å).

Preparation of aliskiren reference solution

The stock reference solution was prepared by weighing accurately, 11.23 mg of aliskiren hemifumarate (purity: 98.32%), transferred to 10 mL volumetric flask and diluted to volume with methanol, obtaining a concentration of 1 mg/mL of aliskiren base. The stock solution was stored at 2–8°C, protected from light and diluted daily to an appropriate concentration in water.

Preparation of sample solutions

To prepare the sample solutions, tablets containing 150 and 300 mg of aliskiren were accurately weighed and crushed to a fine powder. An appropriated amount was transferred into an individual 50 mL volumetric flask. After addition of 30 mL water, the flasks were vortex mixed for 1 min. Then the samples were made up to volume with water, transferred to appropriate tubes and centrifuged at 2000 × *g* for 10 min. Aliquots of the clear supernatant liquid at final concentration of 100 µg/mL of the aliskiren base were stored at 2–8°C, protected from light, and daily filtered through a 0.45-µm membrane filter (Millipore, Bedford, MA).

Validation of the RP-LC method

Analytical method development and validation play a major role in the discovery, development, and manufacture of pharmaceuticals (21). The International Conference on Harmonization (ICH) (22) requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance. A stability-indicating method is the one that quantifies the drug and also resolves its degradation products (23–26). The present method was validated using samples of tablet dosage forms with the label claim of 300 mg by determination of the following parameters: specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), robustness, and system suitability test.

Specificity and forced degradation studies

In order to determine the specificity of the method, a placebo solution (in-house mixture of all the tablet excipients) was prepared in accordance with the method prescribed in the Handbook of Pharmaceutical Excipients (27) and was analyzed to evaluate the absence of interference from the formulation excipients on the aliskiren peak. Moreover, the stability-indicating capability of the method was determined by subjecting a reference sample solution (200 µg/mL) to accelerated degradation by acidic, basic, neutral, oxidative, and photolytic conditions to evaluate the interference in the quantitation of aliskiren. A sample solution prepared in 2 M hydrochloric acid was used for the acidic hydrolysis, and a sample solution in 2 M sodium hydroxide for the basic hydrolysis evaluation. Both solutions were maintained at ambient temperature for 5 h and neutralized with acid or base, as necessary. For study in neutral condition, drug dissolved in water was heated at 50°C for 96 h. The oxidative degradation was induced by storing the samples solutions in 10% hydrogen peroxide, at ambient temperature for 30 h, protected from light. Photodegradation was induced by exposing the sample in quartz cuvette to 200 watt h/square meter of near ultraviolet light (UV-C) at 25 cm of distance for 1 h. After the procedures, the samples were diluted in water to a final concentration of 100 µg/mL. Then, the specificity and the stability-indicating capability of the method were established by determining the peak purity of the samples using a PDA detector (22).

Linearity and range

The linearity was determined by constructing three independent analytical curves, each one with six concentrations of reference solution, in the range of 10–300 µg/mL (10, 30, 50, 100, 200, and 300 µg/mL), prepared in water. Before injection of the solutions, the column was equilibrated for at least 20 min with the mobile phase flowing through the system. Three replicate of 30 µL injections of the reference solutions were made to verify the repeatability of the detector response. The peak areas of the chromatograms were plotted against the respective concentrations of aliskiren to obtain the analytical curve.

Precision and accuracy

The precision of the method was determined by the repeatability and the intermediate precision. Repeatability was examined by nine evaluations of the same concentration sample of aliskiren, on the same day, and under the same experimental conditions. The intermediate precision of the method was assessed by carrying out the analysis on three different days (inter-days), and also by other analysts performing the analysis in the same laboratory (between-analysts). The accuracy was evaluated by applying the proposed method to the analysis of an in-house mixture of the excipients with known amounts of the drug, to obtain solutions at concentrations of 80, 100, and 120 µg/mL, equivalent to 80%, 100%, and 120% of the nominal analytical concentration, respectively. The accuracy was calculated as the percentage of the drug recovered from the formulation matrix.

LOD and LOQ

The LOQ was taken as the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy, and the LOD was taken as the lowest absolute concentration of analyte in a sample that can be detected but not necessarily quantified. The LOD and LOQ were calculated from the slope and the standard deviation of the intercept of the mean of three calibration curves, determined by a linear regression model, as defined by ICH guideline (22).

Robustness

The robustness was determined by analyzing the same samples under a variety of conditions of the method parameters, such as: flow rate, column temperature, changing the mobile phase composition, and pH. The response surface method design was applied to evaluate the relationships between one or more measured responses using Design-Expert software v. 7.1 (Stat-Ease Inc., Minneapolis, MN). Besides, the D-optimal criteria was used to select design points to minimize the variance associated to the estimates of specified model coefficients, with a low number of experiments. Moreover, the stability of sample solutions in mobile phase was assessed after the storage of the samples for 48 h at 2–8°C, and also placed into the autosampler, at room temperature for 15 h.

System suitability test

The system suitability test was also carried out to evaluate the resolution and reproducibility of the system for the analysis to be performed, using five replicates injections of a reference solution containing 100 µg/mL of aliskiren. The parameters measured were peak area, retention time, theoretical plates, and tailing factor (peak symmetry).

Analysis of aliskiren in pharmaceutical formulations

For the quantitation of aliskiren in the tablet dosage forms, the respective stock solutions were diluted to appropriate concentration (100 µg/mL) with water, filtered, injected in triplicate, and the percentage recoveries of the drug calculated against the reference substance.

Results and Discussion

Optimization of the chromatographic conditions

To obtain the best chromatographic conditions, the mobile phase was optimized to provide sufficient selectivity and sensitivity in a short separation time of aliskiren base (p*K*a = 9.49). The retention factor observed in all analysis was higher than 1, in accordance to Thompson and LoBrutto (2007) (28). Phosphoric

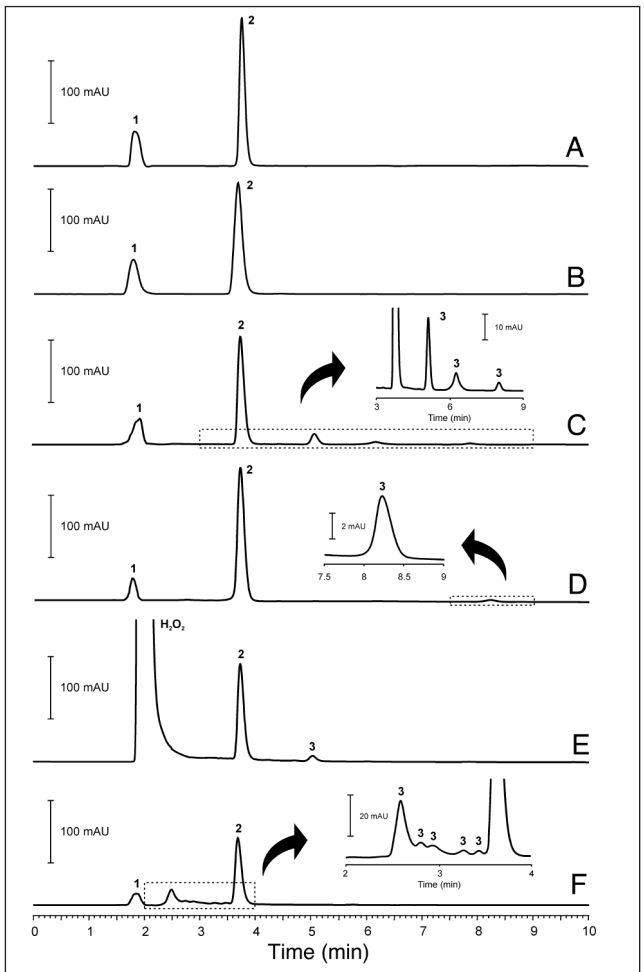

Figure 2. RP-LC chromatograms of aliskiren (100 µg/mL). (A) Aliskiren reference solution, and after: (B) neutral heated hydrolysis; (C) basic hydrolysis; (D) acidic hydrolysis; (E) oxidation; and (F) exposition to UV light. Peak 1: fumarate; 2: aliskiren; 3: degraded products. Chromatographic conditions: Waters XBridge C18 column (150 x 4.6 mm i.d., 5 µm), 25°C; mobile phase: acetonitrile–water (95:5, v/v)–phosphoric acid (25 mM, pH 3.0) (40:60, v/v); flow rate: 1.0 mL/min; detection: 229 nm.

acid, ammonium acetate, and phosphate solutions were evaluated using the pH range of 3 and 7. The suitable mobile-phase should control the selectivity and to achieve reproducible separations with acceptable peak shape. Thus, the precise pH control is essential as well as the high buffer capacity. The buffer capacity of the mobile-phase was suitable and the aliskiren molecule was fully protonated and properly eluted in the column. Phosphoric acid has a p*K*a of 2.1 and its buffer range (p*K*a ± 1 unit) is 1.1 and 3.1. Therefore, the pH value of 3.0 was chosen due to better peak symmetry and high sensitivity, and phosphoric acid solution had adequate buffering capacity to maintain the chosen pH. The use of acetonitrile resulted in better sensitivity, shorter analysis time, and improving the peak symmetry (~1.15). Columns from different sources were evaluated, and the Waters XBridge C_{18} analytical column was selected as it provides the best chromatographic performance and acceptable peak characteristics, including tailing factor and number of theoretical plates. Moreover, the best chromatographic separation of degradation products was achieved on the column used. The optimized conditions of the RP-LC method were validated for the analysis of aliskiren in tablet dosage forms, and a typical chromatogram obtained by the proposed method, demonstrating the resolution of the symmetrical peak corresponding to aliskiren, is shown in Figure 2A. The retention time observed (3.68 min) allows a fast determination of the drug, which is suitable for the quality control laboratories.

Method validation

Specificity and forced degradation studies

A stability-indicating method is defined as an analytical method that accurately quantifies the active ingredients without interference from degradation products, process impurities, excipients, or other potential impurities (23,29). Forced degradation studies should be the first step in method development. The presence of degradants and impurities in pharmaceutical formulations can result in changes in their chemical, pharmacological and toxicological properties affecting their efficacy and safety. Therefore, the adoption of stability-indicating methods is always required to control the quality of pharmaceuticals during and after the production. This greatly contributes to the possibility of improving drug safety (24,30,31).

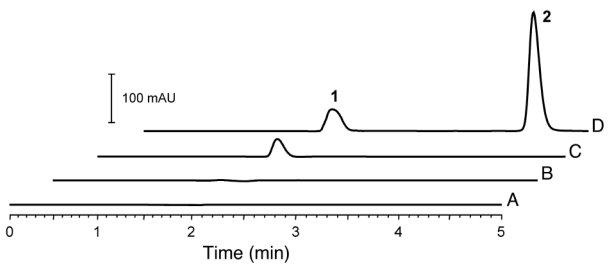

Figure 3. RP-LC chromatograms of (A) blank; (B) in-house mixture of the tablet excipients; (C) fumaric acid solution; and (D) aliskiren in pharmaceutical formulation solution (100 µg/mL). Peak 1: fumarate; 2: aliskiren. Chromatographic conditions: Waters XBridge C18 column (150 x 4.6 mm i.d., 5 µm), 25°C; mobile phase: acetonitrile–water (95:5, v/v)–phosphoric acid (25 mM, pH 3.0) (40:60, v/v); flow rate: 1.0 mL/min; detection: 229 nm.

During the forced degradations, the neutral heated hydrolysis resulted in significant decrease of the area without any additional peak, indicating that the probable degradation products were not detected by UV. Under the acidic hydrolysis, aliskiren content exhibited a decrease of the area (7.94%) at 5 h in 2 M hydrochloric acid solution and one additional peak detected at 8.21 min. Just one additional peak was also detected in the oxidative condition at 5.05 min with significant decrease of aliskiren peak. Under the basic hydrolysis, nearly 69% of the aliskiren was degraded in 2 M sodium hydroxide solution for 5 h, and three additional peaks were identified at 5.05, 6.15, and 7.87 min. For the photolytic condition, 59.76% of the aliskiren was degraded at 1 h, and five additional peaks were detected between 2.5 and 3.5 min, but these peaks did not interfere in the aliskiren peak purity. The chromatograms of the forced degradation studies are shown in Figure 2. No interference from formulation excipients and fumaric acid solution was found, as showing that the peak was free from any coeluting peak. Typical chromatograms of the blank, excipients and fumaric acid solution obtained by the proposed RP-LC method are shown in Figure 3. The studies with the PDA detector showed that the aliskiren peak was free from any coeluting peak, with values of peak purity index higher than 0.9999, thus demonstrating that the proposed method is specific for the analysis of aliskiren.

Linearity

The analytical curves constructed for aliskiren were found to be linear in the 10–300 µg/mL range. The value of the correlation coefficient calculated (r = 0.9999, y = (21312.18 ± 222.20)

Table I. Inter-day and Between-Analysts Precision Data of RP-LC for Aliskiren in Samples of Tablet Dosage Form

	Inter-day			Between-analysts		
Sample	Day	Percentage* (%)	RSD† (%)	Analysts	Percentage* (%)	RSD† (%)
1	1	100.03	0.47	A	101.02	0.27
	2	100.01		B	100.61	
	3	100.84		C	101.13	
2	1	101.80	0.37	A	100.89	0.39
	2	101.18		B	100.11	
	3	101.12		C	100.60	

* Mean of three replicates.
† RSD = Relative standard deviation.

Table II. Accuracy of RP-LC for Aliskiren in Samples of Tablet Dosage Form

Nominal conc. (µg/mL)	Mean conc. found* (µg/mL)	RSD† (%)	Accuracy (%)
80	79.05	0.24	98.81
100	98.72	0.17	98.72
120	121.34	1.21	101.11

* Mean of three replicates.
† RSD = Relative standard deviation.

x – (47960.33 ± 16906.58), where, x is concentration and y is the peak absolute area) indicated the linearity of the analytical curve for the method. The validity of the assay was also verified by means of ANOVA, which demonstrated significant linear regression and nonsignificant linearity deviation ($P < 0.05$).

Precision

The precision evaluated as the repeatability resulted in a relative standard deviation (RSD) value of 0.92% ($n = 9$). The intermediate precision was assessed by analyzing two samples of the pharmaceutical formulation on three different days (inter-day); the mean values obtained were 100.29% and 101.36% with RSD 0.47% and 0.37%, respectively. The between analysts precision was determined by calculating the mean values and the RSD for the analysis of two samples of the pharmaceutical formulation by three analysts; the values were found to be 100.92% and 100.53% with RSD 0.27% and 0.39%, respectively. The results are shown in Table I.

Accuracy

The accuracy was assessed from three replicate determinations of three different solutions containing 80, 100, and 120 µg/mL. The absolute means obtained for aliskiren are shown in Table II with a mean value of 99.55% and RSDs lower than 1.21%, demonstrating that the method is accurate within the desired range.

LOD and LOQ

For calculating of the LOD and LOQ, a calibration equation, $y = 21312.18x – 47960.33$, was generated by using the mean values of the three independent analytical curves. The LOD and LOQ were obtained by using the mean of the slope, 21312.18 ± 222.20, and the standard deviation of the intercept of the independent curves, determined by a linear regression line as 16906.58. The LOD and LOQ calculated were 2.38 and 7.93 µg/mL, respectively.

Robustness

Two approaches are possible to evaluate robustness, either a one variable at a time (OVAT) procedure or an experimental design procedure. The OVAT procedure varies the levels of one factor while keeping the other factors at nominal levels, to evaluate the effect of this former factor on the method response(s). When applying an experimental design, the effect of a given factor is calculated at several level combinations of the other factors. Thus, in an experimental design, a reported factor effect is an average value for the whole domain, and it represents more globally what is happening around the nominal situation (32–34).

To evaluate the robustness of an analytical method usually the OVAT approach is applied, however it is not recommended. The most important reason is that when the factors are examined in given intervals, the effects are estimated for a smaller domain around the nominal levels with the OVAT than with the experimental design approach. Moreover, the OVAT approach requires more (too many) experiments, especially when the number of examined factors becomes larger, and secondly, the importance of factor interactions cannot be taken into account (23,34). The experimental ranges of the selected variables evaluated are given in Table III. The analysis of variance ANOVA was performed and the model terms (variables) were not significant ($P < 0.05$). Moreover, the sample solutions were stable during 15 h into the autosampler and during 48 h when maintained at 2–8°C, showing nonsignificant change (< 2.0%) relative to freshly prepared samples, as suggested (35).

System suitability test

The RSD values calculated in the system suitability test for the parameters tested were within the acceptable range (RSD < 2.0%), as shown in Table IV, indicating that the system is suitable for the analysis intended.

Table III. Chromatographic Conditions and Range Investigated During Robustness Testing

	Factors					Responses*		
Experimental	Phosphoric acid (mM)	Aqueous (%)	Flow (mL/min)	pH	Temp (°C)	RSD† (%)	Assay (%)	Peak Symmetry
1	23	59	0.9	2.9	27	0.56	102.86	1.15
2	27	61	1.1	3.1	27	0.49	102.26	1.15
3	27	59	1.1	2.9	27	0.19	103.84	1.15
4	23	61	0.9	2.9	22	0.31	103.94	1.17
5	23	59	1.1	2.9	23	0.06	103.72	1.16
6	27	61	0.9	2.9	27	0.17	102.30	1.16
7	27	61	0.9	3.1	23	0.06	103.65	1.15
8	23	61	1.1	3.1	23	0.63	103.19	1.15
9	23	59	1.1	3.1	27	0.10	103.93	1.16
10	27	61	1.1	2.9	23	0.06	103.10	1.14
11	27	59	1.1	3.1	23	0.05	103.36	1.14
12	23	61	1.1	2.9	27	0.78	102.34	1.18
13	23	61	0.9	3.1	27	0.18	102.86	1.15
14	25	60	1	3	25	0.49	103.14	1.16
15	27	59	0.9	3.1	27	0.57	102.04	1.17
16	27	59	0.9	2.9	23	0.06	103.43	1.17
17	23	59	0.9	3.1	23	0.03	103.33	1.16

* Mean of three replicates.
† RSD = Relative standard deviation.

Table IV. Results of the System Suitability Test

	Aliskiren*			
Parameter	Minimum	Maximum	RSD† (%)	Status
Theoretical plates	4775	4932	1.14	Passed
Retention time	3.65	3.66	0.10	Passed
Peak area	2072495	2109455	0.68	Passed
Tailing factor	1.15	1.17	0.19	Passed

* Mean of five replicates.
† RSD = Relative standard deviation.

Table V. Determination of Aliskiren in Tablet Dosage Forms by the RP-LC Method

Theoretical amount		Experimental amount*		
Sample	mg per tablet	mg	Recovery (%)	RSD† (%)
1	150	153.02	102.01	0.26
2	150	153.36	102.24	0.22
3	150	153.75	102.50	0.05
4	300	304.08	101.36	0.14
5	300	297.48	99.16	0.15
6	300	307.74	102.58	0.44

* Mean of three replicates.
† RSD = Relative standard deviation.

Method application

The proposed RP-LC method was applied for the determination of aliskiren in tablet dosage forms, as shown in Table V. The results demonstrated the quality of the pharmaceutical samples and the applicability of the method for quality control analysis.

Conclusion

The results of the validation studies show that the RP-LC method is specific, stability-indicating, sensitive, accurate, and possesses significant linearity and precision characteristics without any interference from the excipients and degradation products. The advantages of the chromatographic technique are very well established for the quality control of most of the pharmaceuticals due to its efficiency, high resolution, significant precision, and accuracy. Therefore, the proposed method was successfully applied and suggested for the quantitative analysis of aliskiren in tablet dosage forms, contributing to improve the quality control and to assure the therapeutic efficacy.

Acknowledgements

The authors wish to thank CNPq for the financial support.

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Manuscript received September 17, 2009;
revision received February 16, 2010.