Development and validation of high performance liquid chromatographic and derivative spectrophotometric methods for the determination of candesartan cilexetil in pharmaceutical forms

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Desarrollo y validación de métodos por cromatografía líquida de alta eficiencia y espectrofotométrico derivativo para la determinación de candesartán cilexetilo en las formas farmacéuticas
Desenvolupament i validació de mètodes per cromatografia líquida d'alta eficiència i espectrofotomètric derivatiu per a la determinació de candesartan cilexetilo en les formes farmacèutiques
Recibido : 15 de junio de 2011 ; aceptado : 24 de septiembre de 2011

RESUMEN

En este trabajo se desarrollaron dos métodos simples y rápidos para la determinación de candesartán cilexetil en formas farmacéuticas que contienen este fármaco como monodroga. Candesartán cilexetil es una profármaco que se hidroliza a candesartán durante el proceso de absorción gastrointestinal. Candesartán es un antagonista selectivo (AT1) de los receptores de angiotensina II usado para el tratamiento de la hipertensión arterial.
El método HPLC utiliza una columna Chromolith RP-18e y una fase móvil compuesta de acetonitrilo – solución acuosa de ácido trifluoroacético 0,1% en una proporción al 50,0: 50,0 (v/v), bajo un flujo isocrático.
El método espectrofotométrico UV-derivativo se basa en la relación lineal existente entre la concentración de fármaco y la medición de la derivada de la señal espectrofotométrica. Soluciones acuosas alcalinas de (NaOH 0,1 M) de candesartán cilexetil presentan un máximo a 246 nm y un mínimo a 263 nm ($1D_{246-263}$). La suma de estos dos valores absolutos es utilizada en el rango de concentración entre 6,34 mg L^{-1} y 25,34 mg L^{-1}. La exactitud medida como porcentaje de recuperación es de un 98,9%, con una desviación estándar de 0,76%.
Ambos métodos fueron validados de acuerdo a parámetros establecidos para especificidad, linelidad, precisión, exactitud, estabilidad y límites de cuantificación y detección. Estos métodos fueron aplicados para el ensayo de uniformidad de contenido de dos presentaciones comerciales de comprimidos.
Palabras clave: Candesartán cilexetil, métodos analíticos, comprimidos.

SUMMARY

In this work, two simple and fast methods for the determination of candesartan cilexetil in pharmaceutical forms, having it as a sole drug, were developed and validated. Candesartan cilexetil is a prodrug hydrolyzed to candesartan during absorption from the gastrointestinal tract. Candesartan is a selective AT1 subtype angiotensin II receptor antagonist used in the management of hypertension.
The HPLC method uses a Chromolith RP-18e column. The mobile phase is acetonitrile - 0.1% trifluoroacetic acid aqueous solution in ratio 50.0: 50.0 (v/v) in an isocratic elution at a flow rate of 1.5 mL min^{-1}. The diode array detector is operated at 251 nm, and column temperature is set to 20° C.
The UV-derivative spectrophotometric method is based in the linear relation between drug concentration and first-order derivative spectrophotometric measurement. Alkaline aqueous solutions (0.1 M NaOH) of candesartan cilexetil exhibit a maximum at 246 nm and a minimum at 263 nm ($1D_{246-263}$). The sum of these two absolute values is the signal used on the range concentration 6.34 mg L^{-1} to 25.34 mg L^{-1}. The accuracy of the method, as mean recovery percent, is 98.9 % and the relative standard deviation, 0.76 %.
Both methods were validated according to parameters established for specificity, linearity, precision, accuracy, stability and limits of quantification and detection. The limits of detection and quantification, chromatographic parameters and selectivity obtained are better than other published methods. These methods were applied for the content uniformity of solid dosage pharmaceutical forms of two commercial brands.
Keywords: Candesartan cilexetil, analytic methods, tablets

RESUM

En aquest treball es van desenvolupar i validat dos mètodes senzills i ràpids per a la determinació de candesartan cilexetil en formes farmacèutiques que contenen aquest fàrmac com monodrogues.
Candesartan cilexetil és un profàrmac hidrolitzat a candesartan durant l'absorció en el tracte gastrointestinal.

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Candesartan és antagonista selectiu (AT1) dels receptors d'angiotensina II utilitzats per al tractament de la hipertensió arterial.
El mètode HPLC utilitza una columna Chromolith RP-18e i una fase mòbil composta de acetonitril - solució aquosa d'àcid trifluoroacètic 0,1% en una proporció al 50,0: 50,0 (v / v) sota un flux isocràtic.
El mètode espectrofotomètric UV-derivatiu es basa en la relació lineal existent entre la concentració de fàrmac i la mesura de la derivada del senyal espectrofotomètrica. Solucions aquoses alcalines de (NaOH 0,1 M) de candesartan cilexetil presenten un màxim a 246 nm i un mínim a 263 nm ($1D_{246-263}$). La suma d'aquests dos valors absoluts és utilitzada en el rang de concentració entre 6,34 mg L^{-1} i 25,34 mg L^{-1}. L'exactitud mesurada com a percentatge de recuperació, és del 98,9% amb una desviació estàndard de 0,76%.
Ambdós mètodes van ser validats d'acord amb paràmetres establerts per especificitat, linelidad, precisió, exactitud, estabilitat i límts de quantificació i detecció. Aquests van ser aplicats per a lassaig d'uniformitat de contingut de dues presentacions comercials de comprimits.
Mots clau: candesartan cilexetil, mètodes analítics, comprimits.

1. INTRODUCTION

Candesartan cilexetil is a prodrug of the active compound candesartan, which is generated upon hydrolysis during absorption, and later on metabolized to the inactive MII (CV-15959) [1]. Candesartan is a selective angiotensin II type I receptor blocker [2] indicated in patients with chronic arterial hypertension [3, 4].
The chemical structure of candesartan cilexetil is (±)-1-(cyclohexylxicarbonyloxy) ethyl 2 – ethoxy-1 –((2' –(1H-tetrazol-5-ilo)biphenyl-4-yl)methyl)-1H-benzimidazole-7-carboxilate (Figure 1) and this compound is a white crystalline powder with melting point 163 ºC. It is insoluble in water and slightly soluble in ethanol [5].

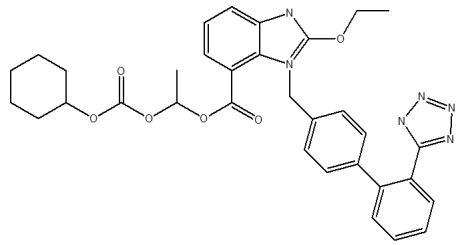

Fig. 1. Structure of candesartan cilexetil

A literature survey reveals several methods for the determination of candesartan cilexetil in pharmaceutical preparations or in biological fluids. The techniques employed include spectrofluorimetry [6], liquid chromatography [7-8], HPLC with fluorometric detection [9-10], micellar electrokinetic chromatography [11] and mass spectrometry [12, 13]. A derivative spectrophotometric method was development by Charoo et al. using methanol-0.35% polysorbate 20 in pH 6.5 phosphate buffer (1:9 v/v) as solvent [14]. Also, Erk reported two methods for the simultaneous determination of candesartan cilexetil and hydrochlorothiazide in pharmaceutical preparations. One, using HPLC with photodiode array detector [15] and another, based on derivative spectrophotometry [16]. However, these latter spectrophotometric methods have serious inconveniences originated in solubility issues and/or in complexity of candesartan cilexetil solutions preparation. For this reason, and considering the wide use of spectrophotometers and HPLC in pharmaceutical control laboratories, derivative spectrophotometric and HPLC methods were developed in this work to determine candesartan cilexetil - in an adequate and simple medium - having in mind its application to the content uniformity test.
The development and validation of novel and simple analytical methods is an important issue for the pharmaceutical industry [17]. The validation provides a high degree of confidence in the quality control and production processes of the dosage forms developed [18]. It is a mandatory practice in every laboratory of analysis in order to guarantee the quality of the products manufactured by the pharmaceutical industry [19].
The analytical techniques developed in this work were validated for its use in routine analysis of the solid pharmaceutical products containing candesartan cilexetil. All the parameters involved in the validation of the analytical methodology [20], including the United State Pharmacopeia (USP 2010)[21] and International Conference on Harmonization (ICH 2005) [22] guidelines were established for this drug.

2. EXPERIMENTAL

2.1. Instrumentation

The HPLC system consisted of a Waters 600 controller, helium degasser, column thermostat, quaternary pump and a Waters 996 photodiode array detector.
UV-derivative spectrophotometric analysis was achieved on UV- Vis, Unicam UV 2 - 100 Spectrophotometer (Cambridge, U.K.), equipped with 1 cm quartz cells and Vision software provided by the manufacturer. The samples were stirred in Ultrasonic bath, Transonic Digital Elma (Singen, Germany) and centrifuged in Centrifuge Heraeus Labofuge 400 (Hanau, Germany).
Measurements of pH were made with a pHmeter WTW Model pH 537 from Welheim, Germany.

2.2. Reagents and materials

Candesartan cilexetil standard 98.7% purity and excipients mixture (lactose, hydroxypropyl cellulose, polyethylene glycol, cornstarch, croscarmellose sodium, red iron oxide and magnesium stearate, USP grade) were obtained from Saval laboratories S.A. (Santiago, Chile) and used as received. Analytical grade sodium hydroxide reagent in pellets and HPLC gradient grade acetonitrile (99.9%) were purchased from Merck. Trifluoroacetic acid (99%) was supplied from Aldrich. Water was purified and bidistilled from quartz apparatus and passed through a Milli-Q system from Millipore.
Acid and alkaline solutions were prepared with hydrochloridric acid and sodium hydroxide from Merck.

2.3. Preparation of standard and stock solutions

Chromatographic method

Stock solutions of candesartan cilexetil for HPLC analysis were prepared by dissolving 50 mg of this compound in 25 mL of acetonitrile to obtain a final concentration of 2 mg mL^{-1}. All working solutions in acetonitrile were prepared daily by dilution of appropriate aliquots of the standard stock solution. These solutions were stored at 5º C and showed to be stable for at least 1 month.

Candesartan cilexetil standard solutions plus excipients were prepared in acetonitrile. 8 (or 16) mg de candesartan cilexetil standard and 324 (or 648) mg excipients were weighed and transferred into a 25 mL volumetric flask with acetonitrile as solvent.

Derivative spectrophotometric method

Candesartan cilexetil stock solutions (400 mg L^{-1} and 800 mg L^{-1}) in 0.1 M NaOH with or without excipient mixture were prepared from alkaline hydrolysis (0.1 M NaOH) of standard candesartan cilexetil. These solutions were prepared by dissolving appropriate amounts of candesartan cilexetil standard in 30 mL of 0.1 M NaOH solution, transferred to a 100 mL volumetric flask and the volume completed with the same solvent.

Excipients solution: 1630 mg excipient mixture for candesartan cilexetil tablets was transferred to a 100 mL volumetric flask and completed to volume with 0.1 M NaOH.

All solutions were stirred in an ultrasonic bath for 10 minutes, light protected, prepared daily and stored in a refrigerator in amber glass vessels.

2.4. Sample fortified preparation

Solutions of candesartan cilexetil standard solution plus excipients were prepared for HPLC and spectrophotometric analysis. All solutions were prepared in a quadruplicate assay.

Chromatographic method

Mixture for 8 mg dose tablets: Aliquots of 10 mL of candesartan cilexetil standard solution plus excipients for HPLC analysis were transferred to 25 mL volumetric flasks with acetonitrile as solvent. After centrifugation at 3500 rev min^{-1} for 5 minutes, aliquots were taken from supernatant to prepare solutions of 8.00, 11.52, 14.09, 16.01, 20.49 and 23.05 mg L^{-1} in acetonitrile.

Mixture for 16 mg dose tablets: Solutions of 7.97, 15.94 and 22.95 mg L^{-1} were prepared with the same procedure previously described.

Derivative spectrophotometric method

Mixture for 8 mg dose tablets: Aliquots of 20 mL of candesartan cilexetil solution plus excipients for spectrophotometric analysis were transferred to 50 mL volumetric flasks and the volumes completed with 0.1 M NaOH solution. After centrifugation at 3500 rev min^{-1} for 5 minutes, using supernatant, aliquots were taken for preparing solutions of 220.37, 300.47, 381.24, 454.05, 530.90 and 611.50 mg L^{-1} in 0.1 M NaOH.

Mixture for 16 mg dose tablets: Solutions of 150.50, 350.20 and 530.00 mg L^{-1} were prepared with the same procedure described.

2.5. Analysis and measurements

Chromatographic method

Aliquots of 20 µL of the standard solutions and samples were injected into the HPLC. Chromolith RP-18e (2 mm macropore, 10 cm × 4.6 mm i.d.) monolithic column from Merck was used as a stationary phase. Standard solution injections were interleaved during injection. The analysis was performed by setting the detector at the maximum of absorption, 251 nm. All experiments were carried out at column temperature of 20ºC. The mobile phase was a mixture of (A) aqueous solution of 0.1% trifluoroacetic acid and (B) acetonitrile (50: 50 v/v) in an isocratic elution mode was found to give optimal results. The mobile phase was degassed and filtered by passing through a 0.45 µm pore size membrane filter (Millipore) prior to use. The flow rate of mobile phase was 1.5 mL min^{-1}. The total time of analysis was 12 min. The diode array detector was set to monitor at a wavelength of 251 nm.

Derivative spectrophotometric method

Zero-order absorption spectrum of candesartan cilexetil in 0.1 M NaOH solution was obtained in the UV range 220-320 nm at a scan speed of 1200 nm min^{-1}, data interval 1.0 nm and bandwidth 2 nm. The first derivative spectra were obtained by instrumental electronic differentiation (Savitzky-Golay algorithm on Vision software). In the present method, we employed the absolute difference between the maximum at 246 nm and the minimum at 263 nm ($1D_{246-263}$) expressed in arbitrary units (Figure 3). In the zero-order absorption spectrum, a rather small and unresolved band was observed centered at 251 nm, being unsuitable for analytical use. However, in the first-order derivative absorption spectrum, very clear bands are displayed at 246 nm and 263 nm, appropriate as spectrophotometric signal for a quantitation method of candesartan cilexetil.

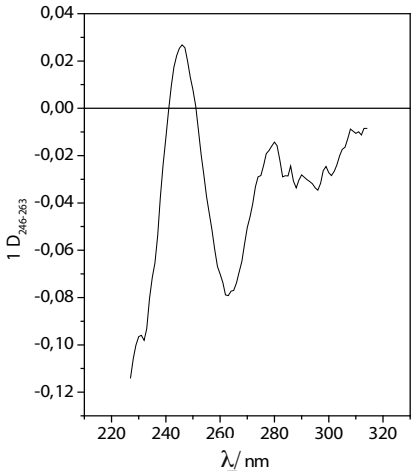

Fig. 2. First derivative spectrum of candesartan cilexetil in 0.1 M NaOH solution (15.84 mg L^{-1})

2.6. Assay of pharmaceutical forms for content uniformity test

Content uniformity was determined on tablets of unitary dose of 8 and 16 mg from two commercial brands. Ten pharmaceutical forms of each unitary dose and commercial brand were treated and analyzed.

Chromatographic method

Tablet nominal dose 8 mg (or 16 mg) was dissolved in 15 mL of acetonitrile in an ultrasonic bath for 15 minutes at 25ºC and adjusted to 25 mL with the same solvent in a volumetric flask. The former solution was centrifuged at 3500 rev min^{-1} for 5 minutes. The supernatant was filtered through a 0.45 µm membrane filter. Finally, 20 µL were injected in triplicate into the HPLC system for the assessment of drug content uniformity in each tablet.

Derivative spectrophotometric method

A commercially available tablet of candesartan cilexetil 8 mg (16 mg) was transferred to a 50 mL (100 mL) volumetric flask and 20 mL 0.1 M NaOH solution was added. It was shaked in ultrasonic bath for 10 minutes at 25 ºC and then the same solvent was added to complete the volume. After shaking, a portion of the content from the flask was centrifuged at 3500 rev min^{-1} for 5 minutes. A 5 mL aliquot of the supernatant was transferred to a 50 mL volumetric flask and volume completed with 0.1 M NaOH. The $1D_{246-263}$ was measured.

3. RESULTS AND DISCUSSION

3.1. Chromatographic analysis

Several columns were tested and satisfactory results were obtained when separation was performed on Chromolith RP-18e (2 mm macropore, 10 cm × 4.6 mm i.d.) column from Merck.

A series of mobile phases with different composition (nature and volume fraction of the organic eluent modifier, effect of pH and buffer concentration) at different temperatures (15 - 45° C) were also tested. The effect of flow rate on the chromatographic resolution was performed in the range between 0.5 – 2.0 mL min^{-1}; the optimal conditions were obtained with 1.5 mL min^{-1} using isocratic conditions. The best results were obtained using a mobile phase consisting of acetonitrile - 0.1% trifluoroacetic acid 50.0 / 50.0 (v/v). The asymmetry of candesartan cilexetil peak increased with acetic acid or orthophosphoric acid at the same concentration was used and the retention time increased when mobile includes a buffer pH was increased from pH 3 to 7.

The column temperature was set at 20°C because the results obtained showed that temperature does not affect significantly the chromatographic efficiency.

The optimal detection condition was obtained using a photodiode array detector set at 251 nm, corresponding to the maximum absorption wavelength. An inadequate reproducibility of peak area and poor linearity were achieved monitoring at 210 or 271 nm.

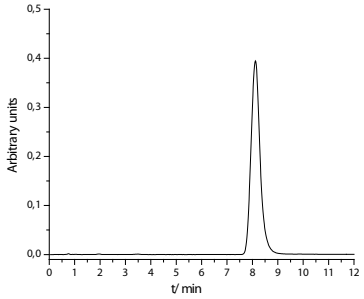

Fig.3. Chromatogram of candesartan cilexetil in acetonitrile monitored at 251 nm

Under these conditions, the retention time of candesartan cilexetil is 8.21 minutes. Figure 3 shows a typical HPLC chromatogram obtained with these optimal conditions.

Chromatographic performance data as retention factor (k') and tailing were calculated. Retention factor (k') was defined as $(t_R - t_0) / t_0$, where t_R is retention time of peak (min) and t_0 is void time (min). In the present method, the void time was 0.83 min. Retention factor in the range of $0.5 < k` < 20.0$ is desired to clearly separate the first peak from void time and to avoid a higher retention time for the last band. Retention factor of 8.89 was found for candesartan cilexetil, indicating a satisfactory separation. Tailing is defined as $W_{0.05} / 2\ t_W$, where $W_{0.05}$ is peak width at 5% of peak height (min) and t_W is distance between peak front and peak retention measured at 5% of the peak height (min). The tailing factor for candesartan cilexetil peak was 1.22.

3.2. Selectivity, alkali and acid degradations

Selectivity is the capacity of the method to accurately measure the analyte response in the presence of potentially interfering sample components (excipients or degradation products) or by studying the absence of any interference in same chromatographic run. In this work selectivity was evaluated by exposing of the amount of candesartan cilexetil to stress conditions, such as HCl (0.1 and 1.0 mol L^{-1}) and NaOH (0.1 and.1.0 mol L^{-1}) in aqueous solutions for 5, 10, 30 and 60 minutes at room temperature. The theoretical final concentration was 8 mg L^{-1}.

All acid solutions prepared were turbid, which accounts for the insolubility of candesartan cilexetil in this medium. Moreover, all alkali solutions were soluble and have a similar absorption spectrum with minimal variation of the measured signal. However, the chromatogram of alkaline solutions shows the total disappearance of the peak corresponding to candesartan cilexetil and the appearance of two peaks of degradation products (Figure 4). This result shows the determination by derivative spectrophotometric method of alkaline hydrolysis product of candesartan cilexetil.

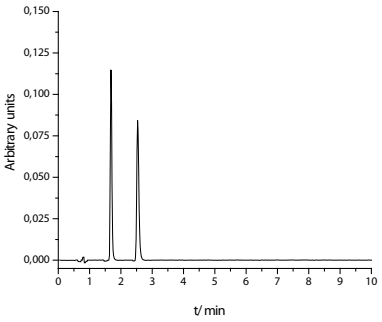

Fig.4. Chromatogram of alkaline hydrolysis products of candesartan cilexetil in 0.1 M NaOH monitored at 251 nm

3.3. Linearity and range

The HPLC and derivative spectrophotometric methods were validated using the following performance criteria: linearity and linear range, sensitivity, intra-assay and inter-assay precision, accuracy, limit of detection (LOD) and limit of quantitation (LOQ).

The linearity, linear range and sensitivity were established through the calibration curve obtained by triplicate analysis of candesartan cilexetil at nine concentration levels in acetonitrile solutions. Linearity was demonstrated over the concentration range of 152.4 - 750 mg L^{-1}. The linear equation was obtained by least-squares linear regression analysis of the peak area of analyte standard in arbitrary units versus concentration (mg L^{-1}):

Area candesartan cilexetil (A.U.) = 21441 x conc. candesartan cilexetil (mg L^{-1}) + 112049

(1)

The correlation coefficient of the linear standard curve was 0.9997. The sensitivity is the slope of the calibration graph (21441 ± 7838 A.U. L mg^{-1}).

The linearity of the spectrophotometric response for candesartan cilexetil solutions in 0.1 M NaOH was tested in nine concentrations and $1D_{246-263}$ was measured for each solution. The results showed good linearity in the range of 1.12 to 25.34 mg L^{-1} with a correlation coefficient equal to 0.9994. The standard deviation of slope is 3.05×10^{-5} and standard deviation of intercept is 4.60×10^{-4}.

Linear regression equation is:

$1D_{246-263}$ (A.U.) = 6.52×10^{-3} conc. candesartan cilexetil (mg L^{-1}) + 3.58×10^{-3}

(2)

3.4. Precision

The intra and inter day precision expressed as the relative standard deviation (RSD) of peak area and retention time were evaluated for HPLC method. The analyses were performed using ten solutions. Each solution was analyzed in triplicate. Table 1 shows typically determined values obtained for a formulation with 217 mg L^{-1} of candesartan cilexetil, the mean retention time, area response and the corresponding relative standard deviations. Relative standard deviations of retention times and area response for intra and inter day assay are 0.7 %, indicating a good instrument reproducibility for this method.

Table 1. *Reproducibility of retention times and peak areas of the candesartan cilexetil (217 mg L^{-1})*

Sample	Retention time (min)	Area response (A.U)
1	8.16	4760723
2	8.19	4795876
3	8.27	4763792
4	8.17	4783541
5	8.21	4770487
6	8.27	4720875
7	8.31	4787393
8	8.17	4711103
9	8.15	4738902
10	8.23	4701753
Mean value	8.21	4753445
RSD (%)	0.7	0.7

For the UV derivative method, the precision was determined in solutions of 16 mg L^{-1}. These solutions were prepared from supernatant solutions obtained of candesartan standard solution plus excipients. Relative standard deviation was 0.76 % (n=10) while the precision of the spectrophotometric measurement was 0.19 %.

3.5. Accuracy

The accuracy of the methods was evaluated from recovery assays. Thus, known aliquots of standard candesartan cilexetil solutions were spiked into their corresponding excipients mixture (candesartan cilexetil solution plus excipients). Tables 2 and 3, summarizes the results from accuracy experiments for 8 and 16 mg dose tablets, respectively.

The values obtained by the proposed HPLC method for 8 mg at six different concentrations levels show a recovery between 99.7 and 99.8% with <1.0 % R.S.D. For 16 mg, percentage recovery was 99.3, 100.1 and 100.0.0% for 150.5, 350.2 and 530.0 mg L^{-1}, respectively.

Accuracy for spectrophotometric method, evaluated at different concentrations gave mean recovery percentage of 98.9 % (Table 2 and Table 3) which agrees with the criteria previously mentioned. The slope and the intercept of the linear regression equation of actual concentration (x) versus found concentration (y) were 0.9829 and 0.093, respectively, with a correlation coefficient 0.9996. Parameters, slope and intercept do not significantly differ from 1 and 0, respectively, at the 95 % confidence interval, indicating that systematic errors were not observed.[14] Therefore the excipients used in the preparation of the pharmaceutical form (lactose, hydroxypropyl cellulose, polyethylene glycol, cornstarch, croscarmellose sodium, iron oxide and magnesium stearate) do not interfere in the determination.

Table 2*. Recovery results of candesartan cilexetil in mixture for 8 mg dose tablets*

Actual mg L^{-1}	Found[a] mg L^{-1}	RSD	Recovery[a] %	RSD
HPLC method				
220.37	220.11 ± 1.96	0.9	99.8 ± 0.9	0.9
300.40	299.04 ± 2.65	0.9	99.7 ± 0.9	0.9
381.24	380.32 ± 2.43	0.6	99.8 ± 0.6	0.6
454.05	452.97 ± 3.72	0.8	99.8 ± 0.8	0.8
530.90	529.34 ± 3.93	0.7	99.7 ± 0.7	0.7
611.50	609.95 ± 4.92	0.8	99.7 ± 0.8	0.8
Derivative spectrophotometric method				
8.00	7.93 ± 0.16	2.0	99.0 ± 2.0	2.0
11.52	11.43 ± 0.18	1.6	99.2 ± 1.6	1.6
14.09	13.90 ± 0.08	0.6	98.6 ± 0.6	0.6
16.01	16.02 ± 0.03	0.2	100.1 ± 0.2	0.2
20.49	19.96 ± 0.39	2.0	97.4 ± 2.0	2.0
23.05	22.89 ± 0.34	1.5	99.5 ± 1.5	1.5

[a] mean ± standard deviation of four determinations

Table 3*. Recovery results of candesartan cilexetil in mixture for 16 mg dose tablets*

Actual mg L^{-1}	Found[a] mg L^{-1}	RSD	Recovery[a] %	RSD
HPLC method				
150.50	149.34 ± 0.89	0.7	99.3 ± 0.7	0.7
350.20	348.29 ± 1.73	0.5	99.5 ± 0.5	0.5
530.00	530.09 ± 1.29	0.2	100.0 ± 0.2	0.2
Derivative spectrophotometric method				
7.97	7.94 ± 0.11	1.4	99.5 ± 1.3	1.4
15.94	15.98 ± 0.17	1.1	100.2 ± 1.1	1.1
22.95	23.27 ± 0.20	0.9	101.4 ± 0.9	0.9

[a] mean ± standard deviation of four determinations

3.6. Limits of detection and quantification

The limit of detection (LOD) and limit of quantitation (LOQ) were separately determined at the signal-to-noise ratios of 3 and 10, respectively, measured at the approximate retention time of the corresponding to candesartan cilexetil peak. Using HPLC method, a detection limit of 0.45 mg L^{-1} was observed and 1.34 mg L^{-1} was determined for quantitation limit. Moreover, limit of quantitation was estimated for the spectrophotometric method, obtaining value of 1.12 mg L^{-1}.

3.7. Content uniformity

Content uniformity was determined tablets of unitary dose of 8 and 16 mg from two commercial brands in accordance with the requirements of USP for tablets. Ten pharmaceutical forms of each unitary dose and commercial brand were analyzed by both methods described above. The acceptance limit for drug content uniformity set by USP is 85%-115% and RSD less than 6%.

The amount of candesartan cilexetil was obtained by interpolation on calibration curve. However for derivative spectrophotometric method is necessary to consider the dilution factor.

mg candesartan cilexetil / dosage form =

(3)

Slope : calibration curve 1D $_{246-263}$ versus Candesartan cilexetil concentration (mg L^{-1})
Intercept : calibration curve 1D $_{246-263}$ versus Candesartan cilexetil concentration (mg L^{-1})
Dilution factor:
d = 0.5 for 8 mg dose tablet
d = 1.0 for 16 mg dose tablet
Results from assay of candesartan cilexetil tablets are shown in Table 4. The amounts found of this drug from 8 mg tablets (tablet A) by HPLC and derivative methods were 7.95 ± 0.10 and 7.81 ± 0.10 mg, respectively. Also, the values found for 16mg tablets (tablet C) are satisfactory.
In summary, the results obtained by both methods are relatively similar.

Table 4. Content uniformity in tablets of two commercial brands (A and B) for two nominal dose Tablets 8 mg (A and B) and tablets 16 mg (A)

Tablet	mg / tablet Declared	Tablet A mg/ tablet Found	Tablet B mg/ tablet Found	mg / tablet Declared	Tablet C mg/ tablet Found
HPLC method					
1	8	8.03	7.78	16	8.00
2	8	7.98	7.85	16	8.01
3	8	7.95	7.87	16	7.87
4	8	8.01	7.93	16	7.93
5	8	7.79	7.78	16	7.78
6	8	8.00	7.86	16	7.86
7	8	8.02	7.90	16	7.90
8	8	7.75	7.79	16	7.79
9	8	7.91	7.92	16	7.92
10	8	8.02	7.85	16	8.03
mean		7.95 ± 0.10	7.85 ± 0.09		16.00 ± 0.10
RSD		1.3	1.1		0.6
Derivative spectrophotometric method					
1	8	7.65	7.80	16	16.37
2	8	7.83	7.84	16	16.40
3	8	8.01	7.55	16	15.65
4	8	7.87	7.51	16	16.59
5	8	7.67	7.80	16	15.66
6	8	7.62	7.74	16	15.97
7	8	7.78	7.61	16	15.95
8	8	7.84	7.56	16	15.79
9	8	7.96	7.65	16	16.50
10	8	7.90	7.64	16	15.55
mean		7.81 ± 0.13	7.67 ± 0.13		16.04 ± 0.39
RSD		1.7	1.5		2.4

4. CONCLUSIONS

Two simple and fast methods were developed and validated for determining candesartan cilexetil in pharmaceutical forms, where it present as a single active. The methods described in this work yield a linear, reproducible, sensitive, selective and precise response by using instrumentation currently available in control laboratories.
The HPLC method used an isocratic mobile phase and was operated with diode array detector at 251 nm.
The derivative spectrophotometric method proposed is rapid, clean, efficient and used 0.1 M NaOH, solution where candesartan cilexetil is completely soluble.
These methods present an optimal recovery and can be used to be applied for the content uniformity test in accordance with the analytical quality parameters established by The United States Pharmacopoeia (USP) and International Conference on Harmonization (ICH) guidelines.

BIBLIOGRAPHY

1. E. Melian; B. Jarvis. Drugs, 62, 787-816, 2002.
2. H. Siragy. The American Journal of Cardiology, 84, 3-8, 1999.
3. E. Michelson; S. Oparil; J. Levine; C. Zuschke; A. Gradman; E. Ripley; D. Jones; J. Hardison; D. Cushing; R. Prasad. The American Journal of Cardiology, 84, 289-293, 1999.
4. H. Gavras. American Journal of Hypertension, 13, S25-S30, 2000.
5. A. Al Sou'od; A. Al Omaria; M. Al Omarib; A. Adnan; A. Badwand. Journal of Pharmaceutical and Biomedical Analysis, 54, 503-509, 2011.
6. A. Sakur; H. Fael. International Journal of Pharmaceutical Sciences Review and Research, 4, 60-63, 2010.
7. U. Khan; S. Qutab; S. Razzaq; M. Ashfaq; Z. Shuja. Acta Chromatographica, 19, 119-129, 2007.
8. A. Peepliwal; C. Bonde1; K. Mohanraj. Acta Pharmaceutica Sciencia, 52, 247-253, 2010.
9. H. Stenhoff; P. Lagerström; C. Andersen. Journal of Chromatography B: Biomedical Sciences and Applications, 731, 411-417, 1999.
10. L. Gonzalez; J. Lopez; R. Alonso; R. Jimenez. Journal of Chromatography A, 949, 49-60, 2002.
11. S. Hillaert; W. Van den Bossche. Journal of Chromatography A, 979, 323-333, 2002.
12. (A. Pruvost; G. Wuerznerc; E. Ezana; M. Levi. Journal of Chromatography B, 877, 919-926, 2009.
13. S. Singh; S. Mehta; R. Shah; R. Priyadarshi. Journal of Pharmaceutical and Biomedical Analysis, 52, 345-354, 2010.
14. N. Charoo; M. Bashira; E. Abdallaa; K. Haj Alia. Analytical Letters, 42, 2232-2243, 2009.
15. N. Erk. Journal of Liquid Chromatography & Related Technologies, 26, 2581-2591, 2003.
16. N. Erk. Pharmazie, 58, 796-800, 2003.
17. G. Clarke. Journal of Pharmaceutical and Biomedical Analysis, 12, 643-652, 1994.
18. E.Van Bockstaele; I. Taverniers; M. De Loose. Trends in Analytical Chemistry, 23, 535-552, 2004.
19. (P. Hubert; E. Rozet; A. Ceccato; C. Hubert; E. Ziemons; R. Oprean; S. Rudaz; B. Boulanger. Journal of Chromatography A, 1158, 111-125, 2007.
20. G. Shabir. Journal of Chromatography A, 987, 57- 66, 2003.
21. The United State Pharmacopeia, USP 33-NF 28. US Convention, INC, Twinbrook Parkway, Rockville, MD, Uniformity of dosage units, 905, 2010.
22. Guidelines prepared within the International Conference on Harmonization (ICH) of technical requirements for the registration of pharmaceuticals for human use. Validation of analytical procedure: Text and methodology Q2 (R1), 1-13, 2005.