

Validation of an Isocratic HPLC Assay of Losartan Potassium in Pharmaceutical Formulations and Stress Test for Stability Evaluation of Drug Substance

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SUMMARY. This paper describes the validation of an isocratic HPLC method for the assay of losartan potassium tablets and capsules and the evaluation of the stability of drug substance after stress tests by photodiode array detection. The method employ a Shimadzu CLC-C8 column with triethylamine solution (0.5%) pH 2.4 and acetonitrile 60:40 (v/v) as the mobile phase and ultraviolet (UV) detection at 225 nm. A linear response ($r > 0.999$) was observed over the concentration range of 15-45 $\mu\text{g/ml}$. The results showed good recoveries, ranging from 98.77 to 101.45% and the relative standard deviation (R.S.D.) intra-day and inter-day were $\leq 0.80\%$. The peak purity was determined by PDA detector. As the method could effectively separate the drug from its degradation products, it can be used in stability studies for drug substance.

RESUMEN. "Validación de un método isocrático de valoración de losartano potásico en tabletas y cápsulas mediante HPLC y ensayos de stress para la evaluación de la estabilidad de la materia prima". El presente trabajo describe la validación de un método isocrático de HPLC destinado a la valoración cuantitativa de losartano potásico en tabletas y cápsulas. Fue también evaluada la estabilidad de la materia prima después de ensayos de degradación forzada por detector de PDA. La fase móvil consiste en una mezcla de solución de trietilamina 0,5% (pH 2,4) y acetonitrilo (60/40) (v/v). Se utilizó columna Shimadzu CLC-C8 150 x 4,6 mm (5 μm) y la longitud de onda de trabajo fue de 225 nm. Se comprobó que este método fue lineal ($r > 0,999$) para un intervalo de 15-45 $\mu\text{g/ml}$. Los porcentajes de recuperación variaron entre 98,77 y 101,45%; los coeficientes de variación obtenidos en los estudios de precisión fueron $\leq 0,80\%$. El método permite separar el fármaco de los productos de degradación, por lo que se le puede emplear en estudios de estabilidad.

INTRODUCTION

Losartan potassium, 2-butyl-4-chloro-1-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1*H*-imidazole-5-methanol monopotassium salt (Fig. 1), is the first member of a new class of non-peptide angiotensin II receptor antagonist^{1,2}. It reduces effectively hypertension by suppressing the effects of angiotensin II at its receptors, thereby blocking the renin-angiotensin system^{3,4}. Losartan has been demonstrated to be superior to previous peptide receptor antagonists and angiotensin converting enzyme (ACE) inhibitors because of its enhanced specificity, selectivity, and tolerability⁵. Currently, losartan potassium is marketed alone or combined with hydrochlorothiazide⁶.

The literature reports many analytical methods for the quantitation of losartan in tablets using HPLC⁷⁻¹¹. These methods employ mobile

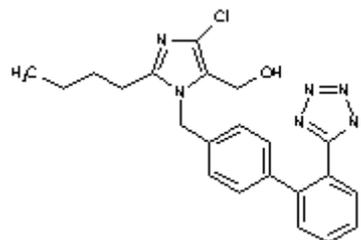


Figure 1. Chemical structure of losartan potassium.

phases with buffer solutions and the separation of losartan degradants was not achieved with isocratic methods.

The purpose of this study was the development of a simple isocratic HPLC method with ultraviolet detection for losartan potassium assay in tablets and capsules and evaluate the stability of drug substance after stress tests by photodiode array detection.

PALABRAS CLAVE: Estabilidad, HPLC, Losartano en tabletas y cápsulas, PDA, Validación.

KEY WORDS: HPLC, Losartan tablets and capsules, PDA, Stability, Validation.

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EXPERIMENTAL

Materials

HPLC grade acetonitrile, reagent grade hydrochloric acid 37%, sodium hydroxide, phosphoric acid 85% and triethylamine were purchased from Merck (Darmstadt, Germany). Reagent grade hydrogen peroxide 30% was purchased from Isopap (Rio de Janeiro, Brazil). Distilled water ($>18 \mu\Omega$), purified using a Millipore MilliQ Plus water system (Billerica, USA), was used to prepare the mobile phase and the sample and standard solutions.

Losartan potassium reference substance (assigned purity 99.2%) used was donated by Aché Laboratórios Farmacêuticos (Brazil). The analytical samples were: losartan potassium 50 mg tablets, available commercially and losartan potassium 50 mg capsules, manufactured in a local compounding pharmacy.

Chromatographic equipment and conditions

The development and validation of the assay was performed on a Shimadzu HPLC system (Kyoto, Japan) consisting of a LC-10AD pump, a Rheodyne injector, an SPD10AV_{vp} UV-Visible detector, a DGU-14A degas, and an SCL-10A VP controller with CLASS-VP Software. The analytical column used to achieve chromatographic separation was Shimadzu CLC-C8 column (150 x 4.6 mm I. D., 5 μ m particle size). The peak purity was determined on a SPD-M 10A VP photodiode array detector (PDA).

The mobile phase consisted of a triethylamine solution (0.5%) pH 2.4 and acetonitrile 60:40 (v/v). The pH of the solution was adjusted to 2.4 ± 0.05 with 85% phosphoric acid prior to mixing with acetonitrile. Injections were carried out using a 20 μ l loop at room temperature (20 ± 2 °C) and the flow rate was 1.0 ml/min. Detection was performed at 225 nm and the identification of losartan potassium was made comparing its retention time and their UV spectra using the PDA detector.

Standard and sample solutions

A 30.0 mg amount of losartan potassium reference substance was accurately weighed, dissolved in mobile phase and diluted to volume in a 100 ml volumetric flask. Standard solution was obtained by diluting the above solution with mobile phase to a concentration of 30 μ g/ml.

A composite of 20 tablets was prepared by grinding them to a fine, uniform size powder,

using mortar and pestle. After calculating the average tablet weight (152.5 mg), an amount of material, corresponding to 30 mg of losartan potassium, was accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately 30 ml mobile phase was added and the solution was shaken for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 30 μ g/ml.

After calculating the average capsule weight (164.2 mg), a portion of combined contents of 20 capsules, corresponding to 30 mg of losartan potassium, was accurately weighed and quantitatively transferred into a 100 ml volumetric flask. Approximately 30 ml mobile phase were added and the solution was shaken for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 30 μ g/ml.

Method validation

Method validation was performed following ICH specifications for specificity, range of linearity, accuracy, precision and robustness¹².

Stress tests and peak purity evaluation

Accelerated degradation studies were performed to provide an indication of the stability of the drug and specificity of the method. Losartan potassium reference substance was stressed under conditions that usually cause degradation, included the following: dry heat (80 °C), acidic media with 0.1 M HCl, basic media with 0.1 M NaOH and oxidating media with 30% H₂O₂.

After the exposure to the conditions, mentioned above, the solutions were allowed to cool to room temperature and neutralized with acid or base (when necessary). Single wavelength data at 225 nm was collected following the method. Additional PDA detector data was collected for the purposes of the peak purity evaluation.

RESULTS AND DISCUSSION

Validation

System Suitability

Having optimised the efficiency of a chromatographic separation the quality of the chromatography was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each

validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2000¹³. In all cases, the relative standard deviation (R.S.D) for the analyte peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented in Fig. 2.

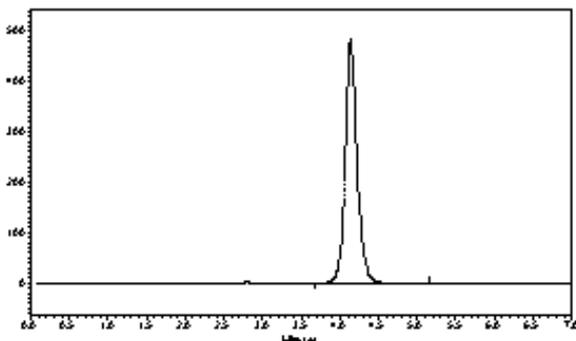


Figure 2. Chromatogram of losartan potassium reference substance.

Specificity

The excipients in the tablets used in this study contained the following inactive ingredients: microcrystalline cellulose, lactose, corn starch, magnesium stearate, hydroxypropyl cellulose, hydroxypropyl methylcellulose, titanium dioxide. The capsules tested contained lactose, corn starch, magnesium stearate and colloidal silicon dioxide as excipients. Injections of placebo tablet and placebo capsule solutions showed no interference with the main peak (Fig. 3 e 4).

The three point peak purity of the losartan potassium peak obtained from reference substance, tablet and capsule solutions were determined by PDA detector. The value for each one was 0.999956, 0.999946 and 0.999972, respectively. Spectra taken during the upslope, apex and downslope indicated that no excipients or impurities interfered with losartan potassium peak.

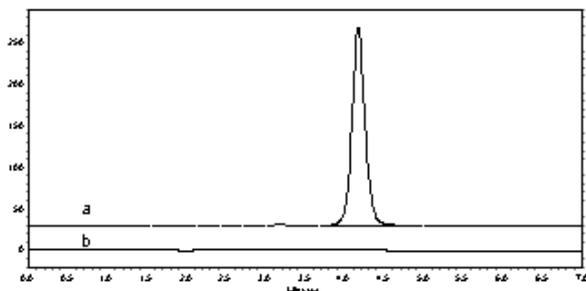


Figure 3. Chromatogram of losartan potassium tablets (a) and placebo (b).

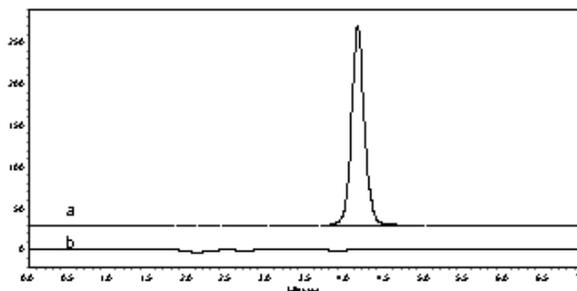


Figure 4. Chromatogram of losartan potassium capsules (a) and placebo (b).

Range of linearity

Standard curves were constructed daily, for three consecutive days, using seven standard concentrations in a range of 15 to 45 µg/ml for losartan potassium. This concentration range corresponds to levels of 50-150% w/w of the nominal analytical concentration. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was $y = -1299 + 89210x$ ($r = 0.9999$). The R.S.D. values of the slope were 0.36% ($n=3$) and the R.S.D. of y-intercept was 0.87% ($n=3$).

Precision

Method precision was demonstrated by the assay of a serie of six samples, prepared as described above, on three consecutive days. The inter- and intra-day means and relative standard deviation (R.S.D.) were calculated (Table 1). The assay method precision acceptance criteria set in the validation were a R.S.D. ≤ 2.0% for

Tablets				
Day	Intra-day precision		Inter-day precision	
	Mean	%R.S.D.	Mean	%R.S.D.
1	99.76	0.49	99.71	0.45
2	99.97	0.44		
3	99.40	0.24		
Capsules				
Day	Intra-day precision		Inter-day precision	
	Mean	%R.S.D.	Mean	%R.S.D.
1	91.84	0.48	92.49	0.80
2	92.75	0.79		
3	92.87	0.65		

Table 1. Intra-day (n=6) and inter-day (n=18) precision results for tablets and capsules assay on three consecutive days.

	% of target					
	75	100	125	75	100	125
	Recovery from tablets (%)			Recovery from capsules (%)		
Day 1	100.17	100.50	101.45	98.77	100.10	99.88
Day 2	101.15	100.45	100.37	100.25	99.79	100.06
Day 3	100.24	100.54	100.43	98.92	99.64	100.67
Mean (n=3)	100.52	100.50	100.75	99.31	99.85	100.2
% R.S.D.	0.54	0.04	0.60	0.82	0.24	0.41
Grand mean (n=9)		100.59			99.79	
% R.S.D		0.42			0.61	

Table 2. Accuracy of method determined by recovery of losartan potassium from tablets and capsules solutions spiked with standard solution.

each data set (1 day) and for all the data combined (3 days). The data of Table 2 meet these acceptance criteria.

Accuracy

The accuracy of the method was evaluated by determination of the recovery of losartan potassium on three days at three levels concentration. Tablets and capsules sample solutions were spiked with losartan potassium standard solution, corresponding to 75 to 125% of the nominal analytical concentration (30 µg/ml). The results showed good recoveries ranging from 98.77 to 101.45%. The mean recovery data obtained for each level as well as for all levels combined (Table 2) were within 2.0% of the label claim for the active substance with an R.S.D. < 2.0%, which satisfied the acceptance criteria set for the study.

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chro-

matographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above.

a) Variations in mobile phase

The effect of variations in percent acetonitrile (ACN) from 40 to 35 and to 45% in mobile phase was evaluated. While the tailing factor, number of theoretical plates and resolution showed a little change with acetonitrile ratio variations, the retention time and, consequently, the capacity factor were significantly altered with these changes (Table 3). Considering that the capacity factor obtained in this method is already close to the minimum value acceptable, that is 2.0¹³, an increase in ACN concentration is not recommended, although the other parameters were still fine.

The pH variations tested were higher values than the value set to this method because extremes pH can cause damage to the chromatographic column. Table 3 shows the effect of in-

Variations	Chromatographic parameters					
	Retention time	Peak area	Capacity factor	Resolution*	Tailing factor	Theoretical plates
35% ACN	5.40	2725125	3.15	3.31	1.10	3866
45% ACN	3.49	2734451	1.68	3.58	1.20	3350
pH 2.8	4.40	2714631	2.39	2.99	1.12	3669
pH 3.2	4.38	2717941	2.36	3.54	1.14	3643
Different column	4.16	2699212	2.17	3.51	1.26	4043
Without variations	4.17	2721154	2.21	4.42	1.03	4176

Table 3. Chromatographic parameters of robustness evaluation. * In relation to the nearest peak.

creasing the mobile phase pH by 0.4 and 0.8 units. The chromatographic parameters of losartan potassium showed only minor fluctuations with mobile phase pH changes.

b) Different column

A LiChrospher 100 RP-8 column (150 x 4 mm I. D., 5 µm particle size) purchased from Merck (Darmstadt, Germany) was used with the original mobile phase and the chromatographic parameters were monitored. As with pH changes, the chromatographic parameters of losartan potassium show only minor fluctuations with different column employed (Table 3).

c) Stability of analytical solution

Also as part of evaluation of robustness, solution stability was evaluated by monitoring the peak area response. Standard solutions were analysed right after its preparation and 1, 2, 4, 6, 8 and 24 h after. The change in losartan potassium peak area response over 24 h was 0.49% and there was no change in chromatographic parameters of drug peak.

The results from the robustness studies showed that all chromatographic parameters met the acceptance criteria established for system suitability, except the capacity factor when the percentage of ACN was increased to 45%.

Stress tests and peak purity evaluation

Temperature stress

No degradation (<0.1%) of losartan potassium was observed for reference substance stressed in a drying chamber at 80 °C for 24 h (Fig. 5). The three point peak purity was 0.999987.

Acid stress

Two degradation products were observed in losartan potassium reference substance after refluxing for 2 h in 1.0 M hydrochloric acid (HCl). These products showed relative retention time (RRT) of 3.42 and 4.60 (Fig. 6). Spectroscopic analysis will be necessary to confirm if these degradation products obtained are the dimers identified by ZHAO and coworkers¹⁴ for an acid degraded sample. The three point peak purity for losartan potassium peak was 0.999983.

Base stress

No degradation (<0.1%) of losartan potassium was observed for reference substance after refluxing for 2 h in 1.0 M sodium hydroxide (Fig. 7). The three point peak purity was 0.999985.

Peroxide stress

As demonstrated in Fig. 5, no degradation (<0.1%) of losartan potassium was observed for

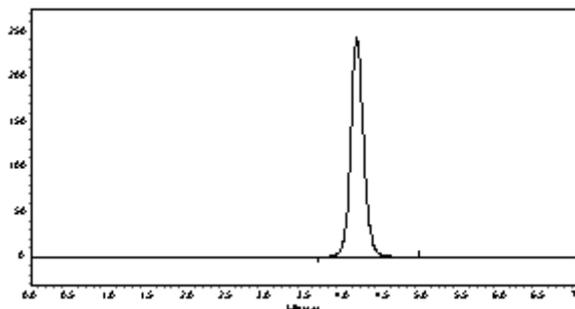


Figure 5. Chromatogram of losartan potassium after temperature stress (24 h at 80 °C).

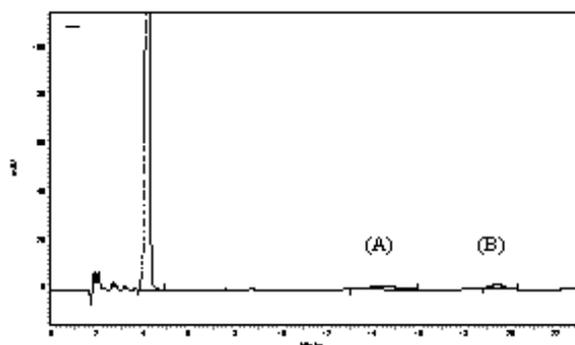


Figure 6. Chromatogram of losartan potassium after acid stress (2 h refluxing 1.0 M HCl). Degradation products (A) and (B).

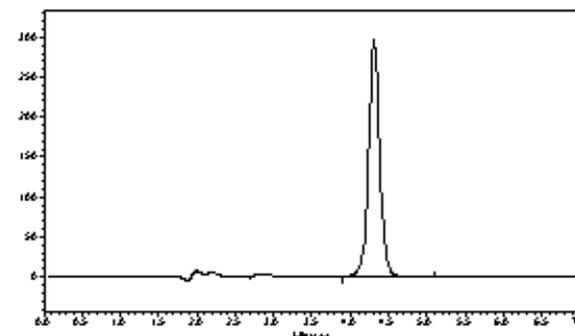


Figure 7. Chromatogram of losartan potassium after base stress (2 h refluxing in 1.0 M NaOH).

reference substance stressed in 30% hydrogen peroxide for 30 min. The chromatograms showed an extra peak at 2 min from hydrogen peroxide (Fig. 8). The three point peak purity for losartan potassium peak was 0.999944.

In all cases the resolution between losartan peak and its nearest peak was > 2.0. Photodiode array detection peak homogeneity tests showed that no peak interfered with the losartan peak after accelerated degradation.

CONCLUSION

The proposed method for the assay of losar-

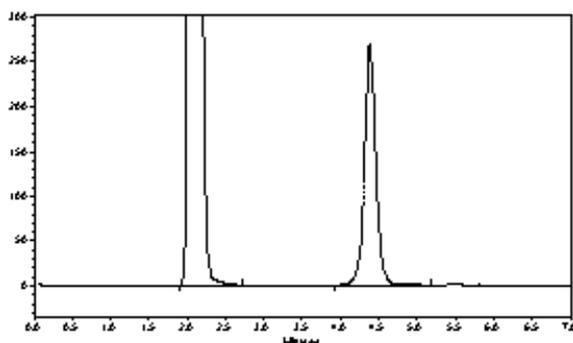


Figure 8. Chromatogram of losartan potassium after hydrogen peroxide 30% stress (30 min).

tan potassium in tablets or capsules is very simple and rapid. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could effectively separate the drug from its degradation products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulations.

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