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Cent. Eur. J. Chem. • 10(6) • 2012 • 1842-1849
DOI: 10.2478/s11532-012-0110-6

Central European Journal of **Chemistry**

A simple and precise conductometric method for the determination of losartan in pharmaceutical products

Research Article

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Received 18 May 2012; Accepted 1 August 2012

Abstract: Losartan is an antihypertensive agent that lost its patent protection in 2010, and, consequently, it has been available in generic form. The latter motivated the search for a rapid and precise alternative method. Here, a simple conductometric titration in aqueous medium is described for the losartan analysis in pharmaceutical formulations. The first step of the titration occurs with the protonation of losartan producing a white precipitate and resulting in a slow increase in conductivity. When the protonation stage is complete, a sharp increase in conductivity occurs which was determined to be due to the presence of excess of acid. The titrimetric method was applied to the determination of losartan in pharmaceutical products and the results are comparable with values obtained using a chromatographic method recommended by the United States Pharmacopoeia. The relative standard deviation for successive measurements of a 125 mg L^{-1} (2.71×10^{-4} mol L^{-1}) losartan solution was approximately 2%. Recovery study in tablet samples ranged between 99 and 102.4%. The procedure is fast, simple, and represents an attractive alternative for losartan quantification in routine analysis. In addition, it avoids organic solvents, minimizes the risk of exposure to the operator, and the waste treatment is easier compared to classical chromatographic methods.

Keywords: *Antihypertensive agent • Conductometric method • Losartan potassium • Pharmaceutical products • Titrimetric procedures*

1. Introduction

Losartan (2-butyl-4-chloro-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl] imidazole-5-methanol monopotassium salt) [1] is an angiotensin II receptor antagonist (ARA-II), which has been proposed as an alternative to more traditional angiotensin converting enzyme (ACE) inhibitors for the treatment of hypertension and heart failure: either alone, or combined with diuretics. By blocking the action of angiotensin, losartan dilates blood vessels thereby reducing blood pressure [2]. Moreover, it is a non-peptide drug which gradually develops long-lasting effect as an antihypertensive and represents a new alternative treatment for this increasingly frequent chronic disease [3]. Losartan is administered orally and is supplied in the pharmaceutical market in tablet dosage form. It is mainly metabolized in the liver to an active carboxylic acid metabolite, which is approximately 5-times more potent and has longer elimination half-life than losartan [4]. This acid metabolite is responsible for most of the angiotensin II receptor antagonism associated with losartan treatment [5]. Losartan structure is presented in Fig. 1.

Different methods have been described in the literature for the determination of losartan in pharmaceutical tablets. These methods employ techniques such as high performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC), capillary electrophoresis (CE), high performance thin layer chromatography (HPTLC), or spectrophotometry [6-11]. In biological fluids, the losartan and its metabolites, as well as other drugs such as hydrochlorothiazide, tranexamic acid and other antihypertensive agents, are mainly determined by HPLC, liquid chromatography/tandem mass spectrometry, and spectrofluorimetry [12-18]. Several analytical procedures have been proposed for the simultaneous quantification of losartan and other drugs in pharmaceutical formulations, including spectrophotometric, CE and HPLC procedures [19-29].

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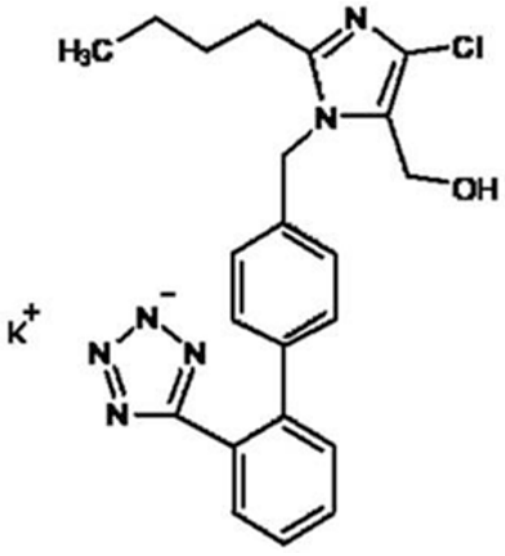

Figure 1. Structure of losartan.

However, many of these techniques are expensive, and/or require time-consuming derivatization steps.

Conductometric titration is a rapid, precise and reliable analytical technique which can be applied for routine analysis, and it requires very simple and low-cost instrumentation. Examples of the use of condutometric titrations for other phamaceutical products can be found in the literature [30-32]. Generally, titrimetric procedures have even been accepted by many modern pharmacopoeias as an official method such as the United States Pharmacopeia [1]. To the best of our knowledge, no information about the quantification of losartan in pharmaceutical preparations has ever been reported in the Brazilian, British or European Pharmacopoeias [33-35]. The development of alternative procedures for the determination of losartan in pharmaceutical formulations is now of great importance, especially for developing countries. Until last year, losartan was exclusively marketed by Merck & Co Inc. under the trade name Cozaar®. Since the end of the its patent period, the demand for analysis of losartan has significantly increased. Therefore, conductometric analysis can fullfil this requirement. In this paper, we present a simple and reliable alternative to existing losartan quantification methods. This procedure is based on conductometric titrations using hydrochloric acid as a titrant and it was applied to analyze pharmaceutical products containing losartan as the active principle. Although conductometric titrations are not very selective, in many pharmaceutical products, the majority of the constituents are neutral species which do not contribute to the conductivity and also do not react with the titrant agent.

2. Experimental procedure

2.1. Reagents and solutions

Standard losartan (potassium salt) was kindly donated by Sandoz-Pharmaceutical Industry (Paraná, Brazil) and was used without further purification. All other reagents were of analytical grade. Hydrochloric acid and sodium carbonate were obtained from Merck (Darmstadt, Germany). All solutions were prepared using ultrapure water, which was double filtered, passed through a reverse osmosis system, and finally purified in a Millipore Milli-Q system (resistivity ≥18.2 MΩ cm). Pharmaceutical products analyzed in this study were purchased in a local drugstore. A stock solution containing 500 mg L^{-1} losartan was prepared by dissolving an appropriate amount of this salt in Milli-Q water. Losartan standard solutions, in concentrations varying from 21 to 250 mg L^{-1}, were prepared by appropriate dilution of the losartan stock solution. A hydrochloric acid solution used as the titrant during the experiments was potentiometrically standardised using sodium carbonate as a primary standard throughout this study.

2.2. Sample preparation

For the determination of losartan in pharmaceutical products, 5 tablets of each sample were weighed and pulverized. A weighed portion of the powder which was equivalent to 50 mg of losartan, was transferred to a 100 mL volumetric flask and filled with Milli-Q water. Resulting losartan solutions were filtered through filter paper, and 15 mL aliquots were appropriately diluted in Milli-Q water into a titration cell. The resulting solutions were titrated with the HCl titrant solution (C_{HCl} = 0.00970 ± 0.00002 mol L^{-1}).

2.3. Conductometric titration

All experiments were performed using a conductivimeter (Digimed, model DM-3P) equipped with a conductivity cell (Digimed, model DMC-010M, k=1.0 cm^{-1}) consisting of two platinized platinum electrodes (each with a geometrical area of 1.08 cm^2), which were calibrated prior to analysis with a 1.0 mmol L^{-1} KCl solution (0.14 S cm^{-1}). All titrations were conducted in a 60 mL jacketed glass cell at 25±1°C (temperature controlled using a water bath, MLW, Germany). A 10 mL manual piston burette (Metrohm, model E-274, with 10 µL divisions) was used for all titrations. All experiments were performed under magnetic stirring (300 rpm). A 15 s gap between each addition of titrant was allowed in order to achieve stability of the conductivity signal. Obtained experimental conductivity values were corrected using Eq. 1:

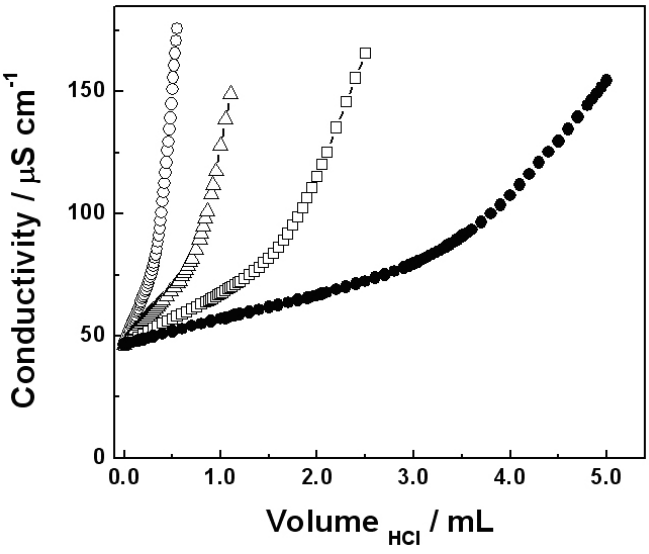

Figure 2. Conductometric curves obtained using a 250 mg L^{-1} losartan standard solution with different concentrations of HCl titrant solution: (●) 0.00970, (□) 0.0194, (△) 0.0485, and (○) 0.0970 mol L^{-1}.

$$\kappa_{cor} = \kappa_{exp} (V_i + V_a) / V_i \quad (1)$$

Where κ_{cor} is the corrected conductivity, κ_{exp} is the experimental conductivity, V_i is the initial volume, and V_a is the volume of added titrant. A graph of corrected conductivity *versus* volume of added titrant was constructed using a graphing program (Origin® from Micronal). The equivalence points of conductometric titrations correspond to the intersections between two straight-line segments fit using the experimental data.

2.4. HPLC analysis

Comparative HPLC measurements were performed for quantification of losartan in pharmaceutical preparations as recommended by the United States Pharmacopoeia [1], using a Shimadzu model SCL 10AVP HPLC equipped with a Phenomenex C18 chromatographic column (4.6×250 mm, 4 µm particle size) and UV-vis absorbance detector. 25 mg of losartan was transferred into a 100 mL volumetric flask and filled with the mobile phase (solution A: 0.1% phosphoric acid in water and solution B: acetonitrile) for analysis by gradient elution, using a flow rate of 1.0 mL min^{-1}. 10 µL of losartan solution was injected onto the chromatographic column, and the resulting chromatogram was recorded by detection at 254 nm.

3. Results and discussion

3.1. Preliminary studies

First, a 250 mg L^{-1} standard solution of losartan was titrated with different concentrations of HCl titrant, ranging from 0.00970 to 0.0970 mol L^{-1} (Fig. 2). Well defined conductometric curves were obtained at lower concentrations of HCl (curve -●-), with deviations up to -0.9% for theoretical concentration values of losartan. Furthermore, the use of a piston burette with 10 µL precision enabled proper fitting of titration equivalence points to the experimental data even when working at low titrant concentrations. The reason for the best results for titrations using the lower concentration of HCl is not very clear, but this is probably due to the fact that the volumes injected were 10 times larger than in the first trial.

To determine the lowest concentration of losartan that can be titrated with a 0.00970 mol L^{-1} HCl solution, a series of standard solutions (21 to 250 mg L^{-1}) were prepared and analyzed in duplicate. As shown in Table 1, results obtained using the proposed conductometric method are in agreement with theoretical values estimated for standard solutions of losartan, with a maximum deviation of -2.94% (for the 21 mg L^{-1} solution). For losartan concentrations lower than 21 mg L^{-1}, addition of titrant only produced small variations in conductivity, resulting in a rapid increase in the uncertainty of the results obtained.

Fig. 3A presents a typical conductometric curve, obtained for a 125 mg L^{-1} losartan solution. The conductivity measured before addition of the titrant is related to the K^+ and losartan$^-$ ions originating from the dissociation of the potassium salt. Until the equivalence point is reached, all H^+ ions injected in the conductometric cell are consumed in the losartan protonation process, resulting in the formation of a white precipitate. During this stage of the titration, a small increase in conductivity is observed because chloride

Table 1. Comparison between the expected and the experimentally obtained concentrations of losartan by conductometric titrations, at 25°C.

Expected concentration (mg L^{-1})	Experimental concentration ± SD[a] (mg L^{-1})	△[b] (%)
21	20.4 ± 1.0	-2.9
42	41.6 ± 1.9	-1.0
83	81.1 ± 1.9	-2.3
125	125.1 ± 2.5	+0.1
250	249.5 ± 3.9	-0.2

[a]*average ± standard deviation for two determinations*
[b]*relative difference between theoretical and experimental concentrations of losartan*

ions have greater mobility than the deprotonated losartan: for each protonated losartan ion, one chloride ion is liberated in the solution. After the equivalence point is reached, all of the losartan is protonated, and a sharp rise in conductivity occurs due to the presence of excess HCl. The "appearance" of free H_3O^+ cations after the equivalence point is associated with a sharp increase in the slope of the second branch of the titration curve. The corresponding chemical reaction is depicted in Fig. 3B.

In a repeatability study of the proposed conductometric titration method, a 125 mg L^{-1} losartan standard solution was titrated with a 0.00970 mol L^{-1} HCl solution (n = 5), resulting in an RSD of 2.3% (124.7 ± 2.8 mg L^{-1}) indicating good repeatability.

3.2. Determination of losartan in pharmaceutical products

Losartan containing pharmaceutical product samples were prepared and analyzed using the proposed conductometric titration method. Fig. 4 shows conductometric curves obtained for a 125 mg L^{-1} losartan standard solution and a losartan tablet sample (diluted to the same concentration as the standard solution), by titration with a 0.00970 mol L^{-1} HCl solution. Both curves displayed similar profiles during the titration experiments. However, the initial conductivity of the losartan tablet sample (curve-●-) was 45% greater than that found for the standard solution (curve -○-). This increase in the conductivity signal is attributed to the presence of other compounds in the tablet. Importantly, the difference in conductivity between the sample and standard solution at the end of the titration was only ~5%.

Results from determinations of 6 different commercial losartan pharmaceutical samples, obtained using the proposed method, were compared with results obtained using an HPLC based procedure described in the United States Pharmacopoeia [1]. Comparative results are summarized in Table 2. The composition of each pharmaceutical formulation is also included in order to show the other components present in each product. The third and fourth columns contain results obtained using the titrimetric and HPLC methods, as well as the respective standard deviations determined using two independent measurements for each sample. Finally, the relative differences (in percentages) between the results obtained by these two procedures versus labeled values are presented in the three last columns of Table 2.

As shown in Table 2, there is good agreement between the labeled values and results obtained by both the proposed conductometric titration method and the standard HPLC determination method. Conductometric titration showed deviations between -1.2 and +4.0%; while the corresponding HPLC method displayed greater deviations, from -2.2 to +6.8%. The reason for the greater deviation observed by HPLC is not clear, but probably was caused by the presence of interfering species. In any case, there is close agreement between the concentration of losartan determined by the proposed conductometric titration procedure and that obtained using the comparative chromatographic procedure for nearly all samples. With the exception of sample 6 (deviation +7.0%), a good deviation between experimental results and labeled values was obtained for all tablet samples which vary by approximately ± 4.0% (last column of Table 2). Sample 6 has a greater number of excipients, which may have contributed to the differences observed.
To evaluate the validity of the results obtained by the proposed conductometric method, these results were compared with the ones obtained by HPLC, adopting the null hypothesis. A paired t-test was applied to the values presented in Table 2, resulting in experimental t-value of 0.73. This result suggests that the two techniques presented no significant differences at the 95% confidence level, considering a *critical value of t* of 2.57 [36].

3.3. Interference study and recovery test

The effect of potential interfering species in the determination of losartan in pharmaceutical products

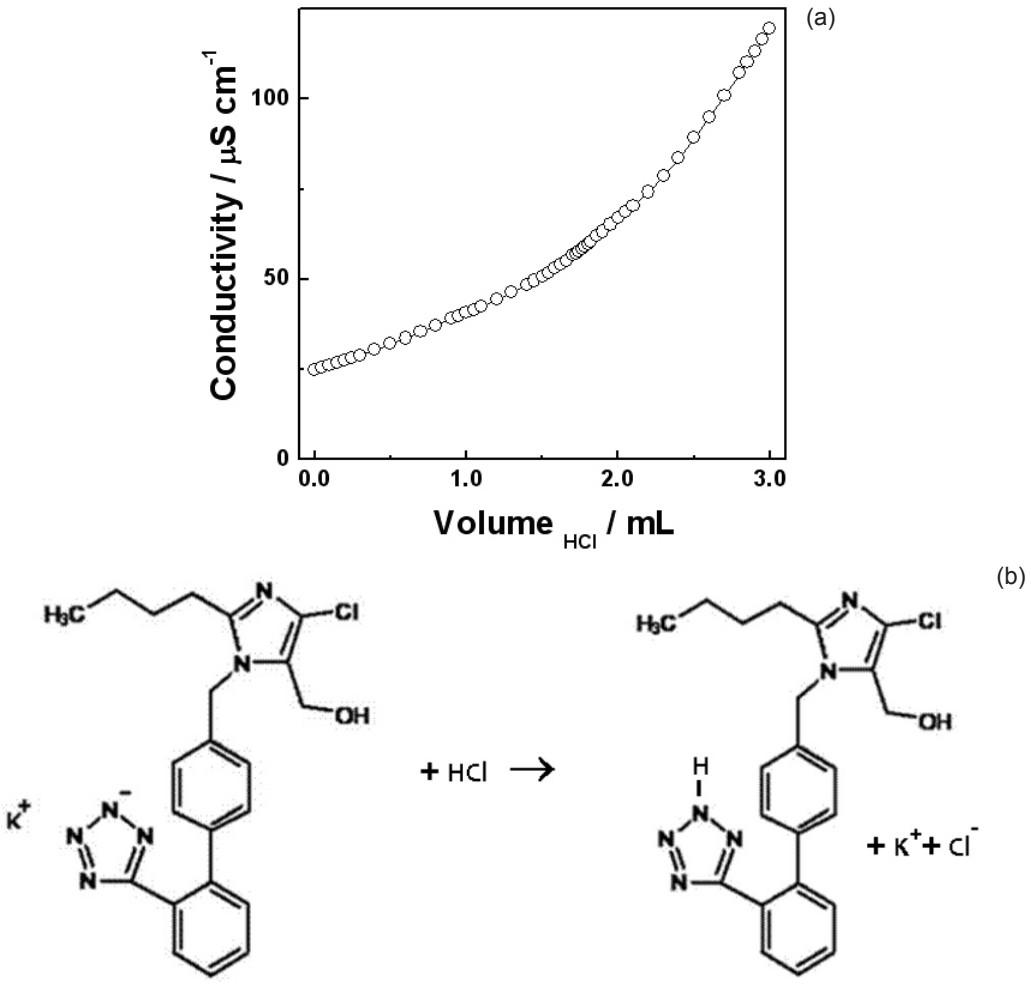

Figure 3. (A) Conductometric curve from a 125 mg L^{-1} losartan standard solution titrated with a 0.00970 mol L^{-1} HCl solution. (B) Representation of the reaction of losartan with the titrant.

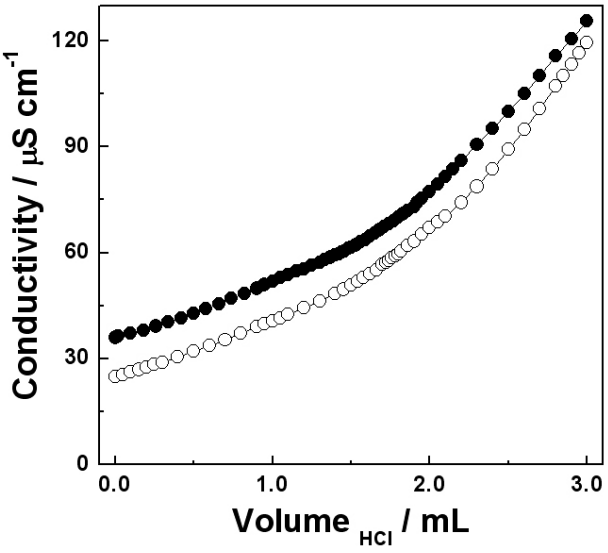

Figure 4. Conductometric curves from a (○) 125 mg L^{-1} losartan standard solution and (●) 125 mg L^{-1} losartan tablet sample.

Table 2. Results obtained after analysis of losartan in different tablet samples using conductometric titration and HPLC method recommended by the United States Pharmacopoeia [1].

Sample	Composition	HPLC ± SD[a]	Titration ± SD[a]	$\triangle_1$ (%)[b]	$\triangle_2$ (%)[c]	$\triangle_3$ (%)[d]
1	Losartan potassium, microcrystalline cellulose, lactose, starch, magnesium stearate, hydroxypropyl cellulose, hydroxypropylmethyl cellulose.	49.5 ± 0.8	49.4 ± 0.4	-1.0	-1.2	+0.2
2	Losartan potassium, microcrystalline cellulose, lactose, starch, magnesium stearate, titanium dioxide, polyethylene glycol.	51.6 ± 0.1	49.6 ± 0.8	+3.2	-0.8	+4.0
3	Losartan potassium, microcrystalline cellulose, lactose, starch, magnesium stearate, silicium dioxide.	48.9 ± 0.3	50.6 ± 0.6	-2.2	+1.2	-3.4
4	Losartan potassium, microcrystalline cellulose, lactose, starch, magnesium stearate, titanium dioxide.	51.4 ± 0.2	52.0 ± 0.6	+2.8	+4.0	-1.2
5	Losartan potassium, microcrystalline cellulose, lactose, starch, magnesium stearate, polyvinyl alcohol, titanium dioxide.	50.9 ± 0.2	50.9 ± 0.6	+1.8	+1.8	0
6	Losartan potassium, microcrystalline cellulose, lactose, starch, magnesium stearate, silicium dioxide, titanium dioxide, macrogol, polysorbate 80, hypromellose.	53.4 ± 0.6	49.9 ± 2.4	+6.8	-0.2	+7.0

[a]average ± standard deviation for two determinations; relative difference between:
[b]labeled value and HPLC method;
[c]labeled value and proposed method;
[d]the results obtained using HPLC and titrimetric methods.

Table 3. Study of addition and recovery of losartan in pharmaceutical products.

Sample	Losartan concentration (mg L^{-1}) added	determined ± SD[a]	Recovery (%)
3	25	25.0 ± 0.5	100
3	42	43.0 ± 1.9	102.4
3	83	83.2 ± 0.9	100.2
5	25	25.0 ± 1.5	100
5	42	41.6 ± 2.9	99
5	83	82.5 ± 1.0	99.4

[a]average ± standard deviation for two determinations

was evaluated for excipients commonly present in tablet samples (starch, lactose, polyethylene glycol, microcrystalline cellulose, magnesium stearate and polyvinyl alcohol). To evaluate the effect of these compounds, a standard solution of losartan (83 mg L^{-1}) was compared with similar solutions containing 25 mg L^{-1} of each interfering species. Starch was found to cause less than a 2% decrease in the equivalence point of the drug; while the presence of polyethylene glycol produced approximately 2% increase in the final equivalence point. No visible change in the results was observed when lactose was added. Note that many of the possible contaminants are insoluble and/or have low solubility (*e.g.* microcrystalline cellulose, magnesium stearate and polyvinyl alcohol) hindering the titration experiments even at low concentrations.

To evaluate the recovery of losartan, two of the six commercial samples were selected for these experiments. Both samples (3 and 5) were diluted to a final concentration of 125 mg L^{-1}. Three different concentration levels (25, 42 and 83 mg L^{-1}) were added to pharmaceutical samples; and for each composition, conductometric titrations were performed. The results were compared with those obtained for samples without addition of standard solution, and % recoveries were calculated for each sample. These results are presented in Table 3.

The average recovery of losartan ranged from 99 to 102.4% indicating that there is no significant interference from the matrix effect which further supports the accuracy of the proposed conductometric method.

4. Conclusions

We demonstrate that conductometric titration is a very effective alternative method for the determination of losartan in pharmaceutical products. Compared to other

analytical techniques, the procedure is simple, easily automated and provides accurate and precise results. In addition, it is independent of analyst subjectivity and does not require any complex pretreatment. Although several methods have been reported for the losartan determination in pharmaceutical formulations, they usually require time-consuming analysis and expensive instrumentation. Compared to official methodology from the United States Pharmacopoeia (high performance liquid chromatography), conductometric titration has some selectivity for losartan even in mixtures of substances commonly found in pharmaceutical samples. Furthermore, with the recent expiration of the patent on losartan, the development of new and simple method for losartan analysis is of great importance for poor and developing countries.

Acknowledgements

The authors are grateful for financial support from Brazilian Foundations FAPESP (fellowship 2010/50303-1), CNPq (process 480311-2009-9) and CAPES. We thank Sandoz (Brazil) and Prof. Dr. Fabio R.P. Rocha for donation of the losartan standard and tablet samples, respectively.

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