Development and validation of an UV derivative spectrophotometric determination of Losartan potassium in tablets

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Abstract

Development and validation of an analytical UV derivative spectrophotometric method to quantify Losartan potassium used as a single active principle in pharmaceutical forms were done. Pharmacopeias have not yet provided an official method for its quantification. A study was carried out of all the parameters established by USP XXIV to validate an analytical method for a solid pharmaceutical form, i.e. linearity, range, accuracy, precision and specificity. All these parameters were found in accordance with the acceptance criteria of Comité de Guías Oficiales de Validación de la Dirección General de Control de Insumos para la Salud de México. Based on the spectrophotometric characteristics of Losartan potassium, a signal at 234 nm of the first derivative spectrum ($1D_{234}$) was found adequate for quantification. The linearity between signal $1D_{234}$ and concentration of Losartan potassium in the range of 4.00–6.00 mg l^{-1} in aqueous solutions presents a square correlation coefficient (r^2) of 0.9938. The mean recovery percentage was $100.7 \pm 1.1\%$ and the precision expressed as relative standard deviation (R.S.D.) 0.88%. In addition, the proposed method is simple, easy to apply, low-cost, does not use polluting reagents and requires relatively inexpensive instruments. Then, it is a good alternative to existing methods for determining Losartan potassium in tablets provided that the pharmaceutical dosage form does not contain hydrochlorothiazide as second drug.

Keywords: Losartan potassium; Derivative spectrophotometric determination; Tablets

1. Introduction

Losartan 2-n-butyl-4-chloro-5-hydroxymethyl-1-((2′-(1H-tetrazol-5-yl)(biphenyl-4-yl)methyl) imidazole, potassium salt, is a strong antihypertensive agent, non-peptide, and exerts its action by specific blockade of angiotensin II receptors [1–3]. It develops a gradual and long-lasting effect as antihypertensive, becoming a new alternative to this frequent chronic disease treatment. Losartan potassium is a light yellow solid, molecular weight, 461; melting point, 183.5–184.5 °C; soluble in water (3.3 mg l^{-1} at pH 7.8); pKa value, 4.9 [4]. It is in the pharmaceutical market in the form tablets.

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United States Pharmacopeia (USP) XXIV, has not yet incorporated in an analytical monograph a method for Losartan quantification. However, several methods have been described for the determination of Losartan potassium drug substance in tablets. These methods employ techniques such as high performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC), capillary electrophoresis (CE) [4] and high performance thin layer chromatography (HPTLC) [5]. In biological fluids, the active principle as well as its metabolites have been determined by HPLC, UV detection [6], fluorescence detection [7], and liquid chromatography-electrospray ionization tandem mass spectrometry [8].

As an alternative to existing methods, we propose and validate a new procedure to determine Losartan potassium drug substance when it is as a single active principle in tablets based on UV derivative spectrophotometry.

An analytical method to control the quality of a pharmaceutical form should be under systematical evaluation to verify its usefulness in relation to the purposes of the design.

The aim of this work was to develop a method that could be used for the individual analysis of tablets and fulfilling the requirements of analytical quality necessary to be applied to the content uniformity tests indicated by The USP XXIV, for finished pharmaceutical products [9]. In this work acceptance criteria from the official validation guides of Dirección General de Control de Insumos para la Salud de Mexico (DGISM) [10] were adopted.

2. Experimental

2.1. Apparatus

a) UV–Vis, UNICAM UV 2-100, with 1 cm quartz cells (Cambridge, UK).
b) Ultrasonic Bath, Transonic Digital Elma (Singen, Germany).
c) Analytical balance, Precisa 40SM-200A (Zurich, Switzerland).
d) Micropipet, variable volume 200–1000 μl. Transferpette Brand (Werthein, Germany).
e) Centrifuge Heraeus Labofuge 400 (Hanau, Germany).

2.2. Reagents

a) Losartan potassium salt (99.61% purity).
b) Losartan potassium salt in tablets, commercially available.
c) Sodium hydroxide solution 1 M prepared from p.a. reagent in pellets (Merck).
d) Britton Robinson buffer, prepared from equal volumes of 0.1 M phosphoric acid, 0.1 M acetic acid and 0.1 M boric acid; p.a. reagents.
e) Excipients: lactose, talc, magnesium stearate and cellulose microcrystalline, USP grade.
f) Hydrochlorothiazide, USP grade.

2.3. Preparation of standard and sample solutions

2.3.1. Losartan potassium standard solution 500 mg l^{-1}

50.20 mg Losartan potassium standard (99.61% purity) was accurately weighed and transferred to a 100 ml volumetric flask and the volume completed with distilled water.

2.3.2. Sample tablet solution

A commercially available 50 mg Losartan potassium tablet was dropped into a 100 ml volumetric flask and some distilled water was added. It was treated in ultrasonic bath for 10 min at 25 °C and then distilled water was added to complete the volume. After shaking, part of the flask content was centrifuged at 3500 rev min^{-1} for 10 min. Some supernatant was used for the determination.

2.3.3. Losartan potassium standard solution plus excipients (500 mg l^{-1})

50.20 mg Losartan potassium standard and 106.5 mg of an excipient mixture (containing 1.7% magnesium stearate, 42.7% cellulose microcrystalline, 42.7% lactose, 12.8% talc) were transferred to a 100-ml volumetric flask and treated as indicated above (Section 2.3.2).

2.4. Spectrophotometric measurements

The absorbance of the solutions containing Losartan potassium at 5.00 mg l^{-1} was determined in the UV range 200–270 nm (Fig. 1) with a scan speed of 2400 nm min^{-1}, 2.0 nm data interval and 2 nm bandwidth. The first-derivative spectra were obtained by instrumental electronic differentiation (VISION software) in the range of 220–260 nm. The amplitude values obtained in the first derivative spectra were arbitrary units of the distance from the central zero base line to the negative peak obtained at 234 nm (Fig. 2).

2.5. Effect of pH on the spectrophotometric behavior of Losartan potassium solution

Eight 1000 μl aliquots of Losartan potassium aqueous solution (Section 2.3.1) were transferred to the respective100 ml volumetric flasks and volume was completed with 0.1 M Britton–Robinson buffer previously adjusted to the required value with a 1 M hydroxide sodium solution. A blank solution of the correspondent buffer was used in the measurement. The spectra of Losartan potassium between pH 2 and 9 were registered (Fig. 3).

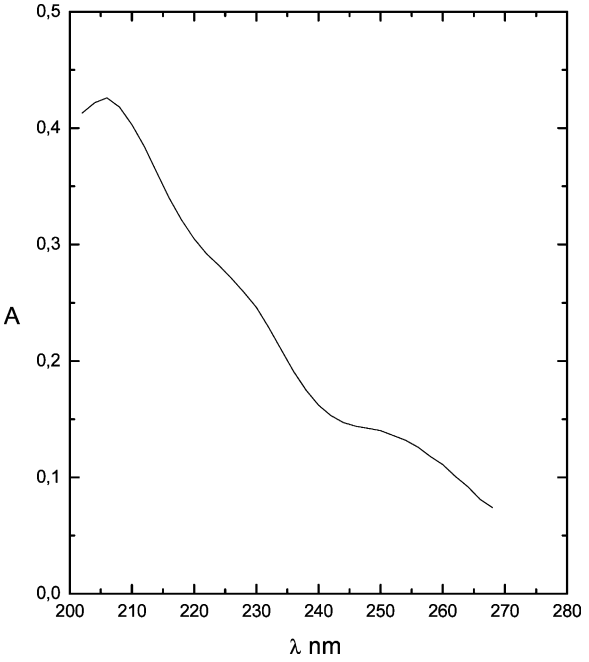

Fig. 1. Zero-order UV spectrum of Losartan potassium in aqueous solution (5.00 mg l^{-1}). The reference was water.

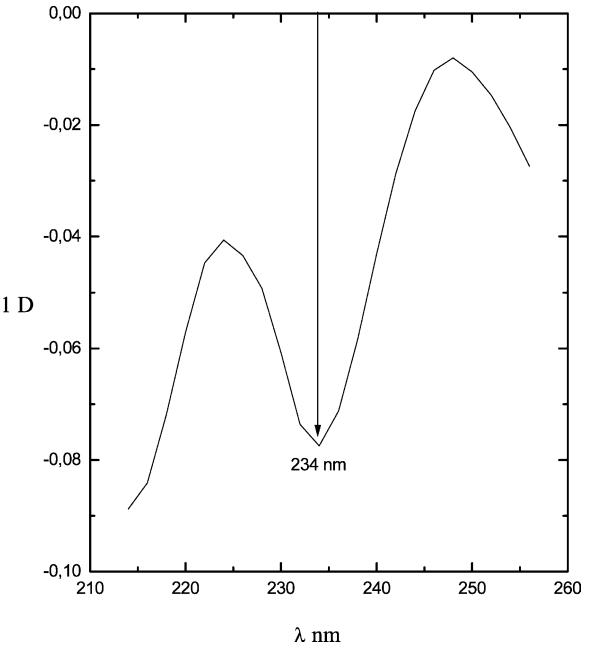

Fig. 2. First-derivative UV spectrum of Losartan potassium in aqueous solution (5.00 mg l^{-1}). The reference was water.

2.6. Determination of analytical performance parameters

2.6.1. Determination of linearity and range

Five aliquots of Losartan potassium aqueous solution (Section 2.3.1) were taken in triplicate and transferred to respective 100 ml volumetric flasks in such amounts as to obtain final concentrations of 4.00, 4.50, 5.00, 5.50 and 6.00 mg l^{-1} of Losartan potassium (ranging from 80 to 120% of a 50 mg nominal dose in tablets). Volume was completed with distilled water and each flask content was measured to determine $1D_{234}$ value (Fig. 4).

2.6.2. Determination of precision

Five 1000 μl aliquots of Losartan potassium standard solution plus excipients (Section 2.3.3) were transferred to respective 100-ml volumetric flasks and volume was completed with distilled

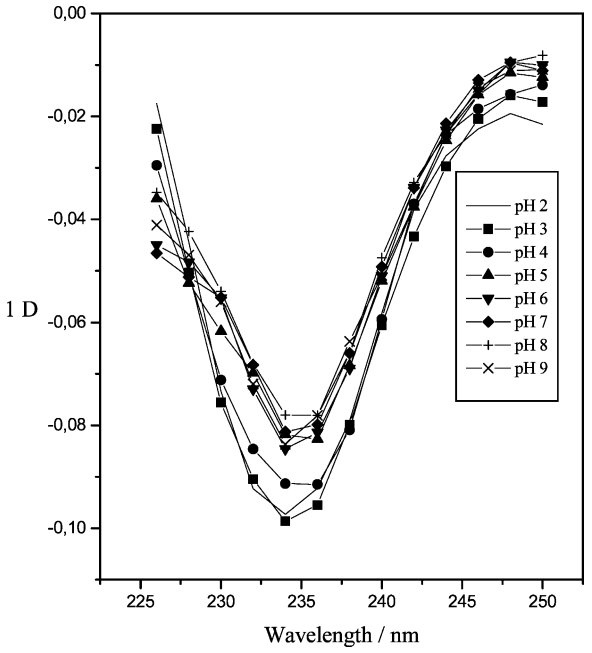

Fig. 3. First derivative spectra of 5.00 mg l^{-1} aqueous solutions of Losartan potassium adjusted at pH from 2 to 9 with Britton–Robinson buffer solution. A blank solution of the corresponding B-R buffer was placed in the reference beam of the spectrophotometer.

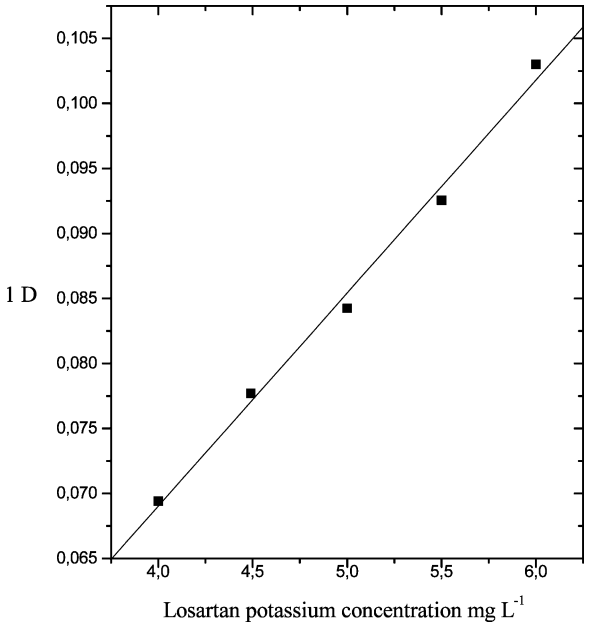

Fig. 4. Plotting of data of experimental calibration curve of Losartan potassium concentration vs. $1D_{234}$ values (Table 1).

water. A portion of each of these solutions was transferred to the sample cell of the spectrophotometer and the values $1D_{234}$ were measured and recorded.

2.6.3. Determination of accuracy

Aliquots of Losartan potassium standard solution plus excipients described in (Section 2.3.3) obtained after centrifugation were transferred to 100 ml volumetric flasks, in triplicate, for preparing solutions of 4.00, 4.50, 5.00, 5.50 and 6.00 mg l^{-1}. Spectrophotometric measurements were taken and values $1D_{234}$ for each solution were determined and recorded (Table 2).

Aliquots of 215, 295, 385, 480 and 585 μl of 18.00 mg l^{-1} Losartan solution prepared from stock solution (Section 2.3.1) were transferred into a spectrophotometric cell containing 2.0 ml of 2.50 mg l^{-1} Losartan prepared from (Section 2.3.1) to obtain 4.00, 4.50, 5.00, 5.50 and 6.00 mg l^{-1} Losartan solution. After shaking carefully, values $1D_{234}$ were measured. This operation was done in triplicate (Table 3).

2.7. Assay of pharmaceutical samples

A 50 mg Losartan potassium tablet was dropped into a 100 ml volumetric flask and procedure (Section 2.3.2) was followed. Then a 1:100 dilution was measured and the $1D_{234}$ was

Table 1
Experimental data of calibration curve $1D_{234}$ vs. Losartan potassium concentration

Concentration (mg l^{-1})	$1D_{234}$			$\overline{1D}_{234}$
4.00	0.0695	0.0693	0.0694	0.0694
4.49	0.0775	0.0779	0.0777	0.0777
5.00	0.0842	0.0836	0.0843	0.0840
5.50	0.0924	0.0924	0.0927	0.0925
6.00	0.1029	0.1033	0.1031	0.1031

The $1D_{234}$ are absolute numbers as the distance, in arbitrary units, from the zero line of the spectrum to the negative peak at 234 nm. The linear regression equation of the calibration curve is: $1D_{234} = 1.64 \times 10^{-2}$ Conc $\times 3.51 \times 10^{-3}$; standard deviation of slope 7.5×10^{-4}; standard deviation of intercept 3.8×10^{-3}; correlation coefficient 0.9969; regression standard deviation $S_{y/x} = 1.86 \times 10^{-4}$. A plotting of data in Fig. 4.

Table 2
Recovery results for Losartan potassium solution plus excipients

Added (mg l^{-1})	Found[a] (mg l^{-1})	Recovery (%)	R.S.D. (%)
4.00	4.09 ± 0.02	102.21	0.49
4.50	4.54 ± 0.01	100.89	0.22
5.00	5.05 ± 0.04	101.00	0.79
5.50	5.49 ± 0.02	99.82	0.36
6.00	5.96 ± 0.04	99.33	0.67

R.S.D., relative standard deviation.
[a] Mean ± standard deviation of three determinations.

Table 3
Recovery results for Losartan potassium standard solutions

Added (μg)	Found[a] (μg)	Recovery (%)	R.S.D. (%)
8.87	8.89 ± 0.01	100.2	0.15
10.31	10.38 ± 0.04	100.8	0.27
11.93	11.71 ± 0.06	98.2	0.46
13.64	13.46 ± 0.03	98.7	0.19
15.53	15.69 ± 0.03	101.0	0.20

R.S.D., relative standard deviation.
[a] Mean ± standard deviation of three determinations.

obtained. Losartan determination was performed by means of a calibration curve.

The final drug content was calculated by the formula:

$$\text{mg of Losartan content/dosage form} = \frac{(1D_{234} - a)}{b} \times 10$$

Table 4
Content uniformity of Losartan potassium tablets

Tablet	Declared (mg)	Found (mg)	Declared (%)
1	50	54.13	108.26
2	50	50.22	100.44
3	50	53.76	107.52
4	50	51.14	102.28
5	50	52.36	104.72
6	50	52.30	104.60
7	50	49.31	98.62
8	50	53.46	106.92
9	50	51.20	102.40
10	50	52.06	104.12

where $1D_{234}$ corresponds to the spectrophotometric measurement of the sample (U.D. $1D_{234}$) (U.D., derivative unit); a, the intercept of the calibration curve $1D_{234}$ versus conc Losartan potassium (mg l^{-1}) in U.D. $1D_{234}$; b, the slope of the calibration curve $1D_{234}$ versus conc Losartan potassium (mg l^{-1}) in U.D./(mg l^{-1}); 10 is the dilution factor.

2.8. Determination of content uniformity of tablets that contain only Losartan as active principle

Each one of the ten pharmaceutical forms (unitary doses of 50 mg) were treated as in the above paragraph. Results are shown in Table 4.

The acceptance criteria correspond to those established by USP XXIV [9].

3. Results and discussion

The zero order UV spectrum in aqueous solution of Losartan potassium shows a maximum close to 200 nm and an ill-defined shoulder extending from 220 to 240 nm (Fig. 1). This behavior precludes the analytical use of zero order absorbance if the aim is optimization of parameters of analytical quality. Otherwise, the first derivative spectrum shows an intense negative maximum at 234 nm with evidently useful characteristics from the analytical viewpoint (Fig. 2).

Variation in pH from 5 to 9 of aqueous Losartan potassium solution within the concentration range of 3.5–7.5 mg l^{-1} did not alter the spectral characteristics described above (Fig. 3). Thus, it is unnecessary buffering, since an aqueous solution is sufficient as is shown by the recovery assay in the accuracy determination.

The linearity of spectrophotometric measurement for Losartan potassium solutions within the concentration range 4.00–6.00 mg l^{-1}, equivalent to 80–120% of the nominal value of a tablet, was satisfactory. In the above-mentioned range, the linear regression equation, was $1D_{234}$ (U.D.) = $1.64 \times 10^{-2} \times$ Concentration (mg l^{-1}) + 3.51×10^{-3} with square correlation coefficient (r^2) of 0.9938 (Fig. 4). The 95% confidence limit levels for

the slope were $1.64 \times 10^{-2} \pm 2.39 \times 10^{-3}$ and $3.51 \times 10^{-3} \pm 1.21 \times 10^{-2}$ for the intercept [11].

The precision evaluated in a solution of 5.0 mg l^{-1} as relative standard deviation was 0.88 for n = 5. The accuracy studied by means of assays of recovery in Losartan potassium solutions and Losartan potassium plus excipients gave mean values of 99.78 and 100.65%, respectively. The results are shown in Tables 2 and 3. It can be seen that the excipients used in the preparation of the pharmaceutical form (magnesium stearate, cellulose microcrystalline, lactose and talc) do not interfere in the determination. So, this spectrophotometric method is appropriate to determine content uniformity of Losartan potassium when this is the only active principle in pharmaceutical forms.

It must be emphasized that Hydrochlorothiazide, active principle associated to the Losartan in some pharmaceutical forms, interferes in its determination. The UV first derivative spectrum of Hydrochlorothiazide in aqueous medium presents a strong and large absorption with a negative maximum at 230 nm that interferes in Losartan determination.

Finally, content uniformity test was performed in accordance with the requirements of USP XXIV with tablets (nominal dose 50 mg) of commercial brand. Ten tablets, individually processed as indicated in solution (Section 2.3.2), were found to contain between 98.6 and 108.3% of the nominal value, meeting therefore, with the requirements.

In relation to the other methods to determine Losartan potassium in tablets mentioned in literature [4,5], this derivative spectrophotometric method presents a similar accuracy and precision and a lower sensitivity, but enough for the proposed goals.

4. Conclusion

The developed method is an alternative to determine Losartan potassium in pharmaceutical dosage forms that contain it as unique active principle with quite satisfactory results for the specific purposes of its design. Its advantages over other existing methods are its simplicity, fastness, low-cost and non-polluting conditions.

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