



Validation of a UV spectrophotometric method to quantify losartan potassium in tablets from the dissolution test at pH 1.2, 4.5 and 6.8

[Validación de un método espectrofotométrico UV para cuantificar losartán potásico en tabletas a partir de la prueba de disolución a pH 1,2; 4,5 y 6,8]

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Abstract

Context: The validation of a method is synonymous with the quality of the obtained results. The dissolution test is an analytical technique to evaluate the quality and stability of drugs during their development.

Aims: To evaluate whether the UV spectrophotometric method for the quantification of losartan potassium in tablets from the dissolution test at pH 1.2, 4.5 and 6.8 meet with the validation parameters.

Methods: The determination and evaluation of validation parameters were carried out under the guidelines of the regulatory entities. Linearity and range, accuracy, precision, specificity, limits of detection and quantification, robustness, stability of the sample solution were evaluated, and the filter test was added. All data obtained were subject to an analysis of variance and t-student analysis with a confidence level of 95% ($\alpha = 0.05$).

Results: The UV spectrophotometric method meets the acceptance criteria for each validation parameter. Likewise, it was identified that the prepared solutions were stable at pH 6.8 for 24 hours; however, they were not stable at pH 1.2 and 4.5.

Conclusions: The method meets with the validation criteria and is suitable to be used for quantifying samples obtained from the losartan potassium tablet dissolution test.

Keywords: validation; losartan; spectrophotometry; dissolution test.

Resumen

Contexto: La validación de un método es sinónimo de calidad en los resultados obtenidos. La prueba de disolución es una técnica analítica para evaluar la calidad y estabilidad de los medicamentos durante su desarrollo.

Objetivos: Evaluar si el método espectrofotométrico UV para la cuantificación de losartán potásico en tabletas a partir de la prueba de disolución a pH 1,2; 4,5 y 6,8 cumple con los parámetros de validación.

Métodos: La determinación y evaluación de los parámetros de validación fueron realizados bajo las directrices de las entidades regulatorias. Se evaluaron linealidad y rango, exactitud, precisión, especificidad, límites de detección y cuantificación, robustez, estabilidad de la solución muestra y se adicionó la prueba de filtros. Todos los datos obtenidos fueron sujetos a un análisis de varianza y análisis t-student con un nivel de confianza del 95% ($\alpha = 0,05$).

Resultados: El método espectrofotométrico UV cumple con los criterios de aceptación planteados para cada parámetro de validación. Así mismo, se identificaron que las soluciones preparadas fueron estables a pH 6,8 por 24 horas; sin embargo, no fueron estables a pH 1,2 y 4,5.

Conclusiones: El método cumple con los criterios de validación y es apto para ser usado en la cuantificación de muestras obtenidas de la prueba de disolución en tabletas de losartán potásico.

Palabras Clave: validación; losartán; espectrofotometría; prueba de disolución.

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INTRODUCTION

The dissolution of the drug under physiological conditions is a critical step for its absorption and, therefore, its arrival at the site of action. Dissolution tests are performed on oral dosage forms to evaluate batch-by-batch quality, guide the development of new formulations, and ensure continuous product performance (Hasan et al., 2017). It has facilities, such as a control of the system, formulation, or adaptation to the necessary biological conditions (Hema Nagardurga, 2019). In addition, they reduce the cost, the number of trials and have a benefit in terms of ethics and pharmacological performance (Amidon et al. 1995, Abbirami et al., 2013).

The method of quantification of samples that are obtained from the dissolution test must be validated, reliable, robust, accurate, and precise before use for daily activities in the quality control environment. The validation process is carried out through numerous evaluations designed to verify that an analytical procedure is conducive to the intended reason and may be able to provide useful and legitimate analytical data (Rao, 2018; Lavanya Chowdary et al., 2020).

For the validation process of a method, the study parameters must be defined, including the acceptance limit. There is no correct sequence of parameters. Even some can be measured by combining experiments to minimize the number of control analyses and achieve short-term results. Due to the importance of the dissolution test, it is necessary to incorporate in the validation process of the method the filter test, to examine any interference by the filters used by the dissolution equipment and the stability test of the samples obtained to inform about any variation that may occur in it (Garcia et al., 2011).

Losartan potassium is a first-line drug for the treatment of hypertension (Al-Majed et al., 2015) and as such, is included in clinical guidelines for antihypertensive treatment in countries with high health surveillance (Whelton et al., 2018; NICE, 2019). It is also part of the model list of essential medicines of the World Health Organization (WHO) for its proven efficacy and safety (WHO, 2021). This condition leads to greater registration, commercialization and use, being necessary to implement measures that contribute to ensuring its biopharmaceutical quality, such as dissolution tests. On the other hand, different investigations have been reported on its biopharmaceutical classification, such as class I (high solubility and high permeability) (Ono et al., 2016), class II (low solubility and high permeability) (CDSCO, 2019), and class III (high solubility and low permeability) (Ramirez et al., 2010), generating uncertainty about its solubility and

the application of bioexention studies, therefore, it is necessary to validate low-cost methods, such as UV spectrophotometry, that ensure the quality and reliability of the quantitative results of the dissolution tests and that also simulate the physiological conditions, that is, in the dissolution media pH 1.2, 4.5 and 6.8 (Baena, 2008), since there is only a report of studies on the validation of UV spectrophotometric quantification for the dissolution test of losartan potassium at pH 6.8 (Gündoğan et al., 2008; Bonfilio et al., 2010). This study aimed to evaluate whether the UV spectrophotometric method for the quantification of losartan potassium in tablets from the dissolution test at pH 1.2, 4.5, and 6.8 meet with the validation parameters.

MATERIAL AND METHODS

Study material

Tablets of losartan potassium 50 mg of batch 9CL2097 from a pharmaceutical laboratory that markets in Peru, which contained in its formulation silicon dioxide, microcrystalline cellulose, lactose spray-dried (lactose monohydrate), croscramellose sodium, partially pregelatinized starch, and magnesium stearate. Its coating was made with white Opadry II (polyvinyl alcohol, titanium dioxide, talc, macrogol). The secondary standard was Sigma-Aldrich from lot LRAC0141 with a purity of 99.9%.

Dissolution test

Dissolution equipment (AT Xtend Model, Sotax, United States) was used, using the 2 USP apparatus (Paddle) at 50 rpm and 900 mL of dissolution medium, which were prepared according to the instructions of the United States Pharmacopoeia (USP 42) using the reagents potassium chloride (J.T. Baker, United States), hydrochloric acid 37% (Merck, United States), sodium acetate trihydrate 99.65% (J.T. Baker, United States), glacial acetic acid 99.7% (Merck, United States), monobasic potassium phosphate 99.63% (J.T. Baker, United States) and sodium hydroxide (Merck, United States). All reagents were ACS (American Chemical Society) quality. The equipment was programmed to sample 3 mL in times 45, 60 and 120 min for the pH media 6.8, 4.5 and 1.2, respectively.

Spectrophotometric conditions in the quantification of losartan potassium

After the dissolution process, an aliquot of 1 mL was taken and diluted with 3 mL of dissolution medium. It was read at 250 nm on a UV-Vis spectrophotometer (Lambda 365 Model, Perkin Elmer, United States) using Perkin Elmer UV WinLab software (ver-

sion 6.4.0.971), with a quartz cell of 10 mm optical pitch.

Validation parameters

All the acceptance criteria considered were taken based on the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use and the Spanish Association of Pharmacists in Industry (ICH, 2005; AEFI, 2001).

Linearity

Dilutions were performed from a 0.1 mg/mL solution of losartan potassium secondary standard to obtain the final concentrations in the range of 0.0051 and 0.0306 mg/mL. The linear regression line was calculated by the method of least squares. The response factor (<5%) and the statistical analysis of the results were evaluated by analysis of variance (ANOVA).

Accuracy

It was carried out by adding losartan potassium secondary standard to the dissolved tablet in each dissolution medium to obtain the concentrations corresponding to 20, 80 and 120%. Recovery percentages (95%-105%), percentage coefficient of variation (CV%), and relative error (<5%) were analyzed.

Intermediate precision

It was determined by analyzing six solutions sampled of losartan potassium tablets dissolved in each dissolution medium by two analysts on different computers and days. The UV-Vis spectrophotometer (Lambda 365 Model, Perkin Elmer, United States) and the UV-Vis spectrophotometer (Lambda 25 Model, Perkin Elmer, United States) were used. The values of CV% (<10%) were calculated between samples from the same group and the overall CV% (<10%).

Instrumental repeatability

A standard solution of concentration 0.0258 mg/mL was prepared and repeatedly analyzed 9 times. CV% values (<2%) were calculated.

Method repeatability

The same procedure of accuracy was followed, adding losartan potassium secondary standard to the tablet dissolved in each dissolution medium. Individual and mean intervals and CV% (<5%) were analyzed.

Limit of detection and quantification

They were determined by mathematical calculations using the method based on the extrapolation of the zero concentration calibration line, which was obtained from the linearity that was worked with the secondary standard of losartan potassium.

Robustness

Sample solutions of losartan potassium tablets dissolved in each dissolution medium were analyzed under different conditions of variation. Condition 1 (sample unchanged), condition 2 (spectrophotometric reading at a wavelength of 254 nm), and condition 3 (different dilution for sample solution reading). CV% (<10%) and absolute difference (<2%) were evaluated.

Stability of analytical solution

Sample solutions of losartan potassium tablets dissolved in each dissolution medium were analyzed at 0 and 24 hours. CV% (<10%) and absolute difference (<2%) were evaluated.

Filter test

Sample solutions of losartan potassium tablets dissolved in each dissolution medium were prepared and worked in two groups, unfiltered centrifuged samples, and samples filtered with different filters to select the appropriate filter for the proposed method.

Specificity

A spectral scan of a standard solution, sample solution dissolved in each dissolution medium, and dissolution media were performed in a range of 200 to 700 nm. The discriminatory capacity was confirmed by comparing the sweeps obtained and the absence of interference in the dissolution media.

Statistical analysis

The data obtained from the descriptive statistical parameters of validation (arithmetic means, standard deviations, and coefficient of percentage variation), were subject to analysis of variance and a t-student analysis with a confidence level of 95% ($\alpha = 0.05$) (Bolton and Bon, 2010).

RESULTS AND DISCUSSION

The coefficient of determination of the regression line (Fig. 1) in the three means of dissolution was greater than 0.99, which indicated linearity. This was

confirmed by determining the statistical significance of the regression by the ANOVA test, obtaining results within the specification criteria (Table 1). This shows that the method is linear within the established range (Moosavi and Ghassabian, 2018).

An adaptation was made for the evaluation of the accuracy parameter; known concentrations of the secondary standard of losartan potassium were added to the dissolved tablet in each dissolution medium. The dissolution time was different for each medium to ensure the complete dissolution of the drug in the dissolution medium. The results obtained were within the acceptance criterion (95%-105%), which ensures that the values are close to the true value (100%). Despite this, the CV% were not less than 2% (2 for pH 1.2, 3.7 for pH 4.5 and 2.6 for pH 6.8), but the relative error was less than 5% for all media (Table 1) (Lavanya Chowdary et al., 2020).

In the research, intermediate precision and repeatability were carried out, both instrumental and method, to determine the intra- and inter-daily variations. The estimation of intermediate precision is made with the calculation of the global CV%, which was lower than specified (<10%) for the three dissolution media, which indicates that there is no variation when changing the days of analysis, analyst or instrument. In addition, the CV% of each study group is mentioned to evaluate its variability where values lower than those specified (<10%) were obtained, even very low values, is the case of 2.75, which corresponds to Day 1 (Day 1) Equipment 1 (Equip 1) and Analyst 2 (Ana 2), which indicates similarity between the values obtained. Instrumental and method repeatability meets the requirements for each dissolution medium (Table 1). All these results indicate that the method possesses the precision required during routine use under the established analytical conditions (NATA, 2018).

For specificity analysis, sample and standard were used to evaluate the analyte in the absence of impurities. The spectrograms present in Figs. 2-4 were compared for the pH media 1.2, 4.5 and 6.8, respectively. There is no interference from the diluent since it does not present absorbance in the wavelength under study. The spectral scans of the standard and sample solution are similar as shown in the figures (Chikanbanjar et al., 2020).

The filter test was performed to determine if there is a significant difference between Whatman® No. 21 paper filters and fast-filtering black point filter paper discs; these samples were compared with centrifuged and unfiltered samples. As evidenced in Table 1, the results obtained with both types of filters do not show an absolute difference greater than 2%, so we chose to work with the most accessible filter. The United States Pharmacopoeia monographs on dissolution testing emphasize that the analysis of filtered samples should be performed because the sample may contain active substances and undissolved excipients at any time during the test. Therefore, filtration is considered a critical step before performing any subsequent analysis (USP, 2019; Smith et al., 2020).

Robustness analyzes the ability of the method to remain unchanged in the face of conditions of variation. Table 1 shows the results obtained when working at a different length present an absolute difference of less than 2%. This indicates that this condition of variation does not affect the ability of the method for the quantification of losartan potassium. When working with a different dilution at the time of analyzing the samples, the results are affected in the pH media 1.2 and 4.5, presenting an absolute difference of 6.95 and 17.78%, respectively (Vander Heyden et al., 2001).

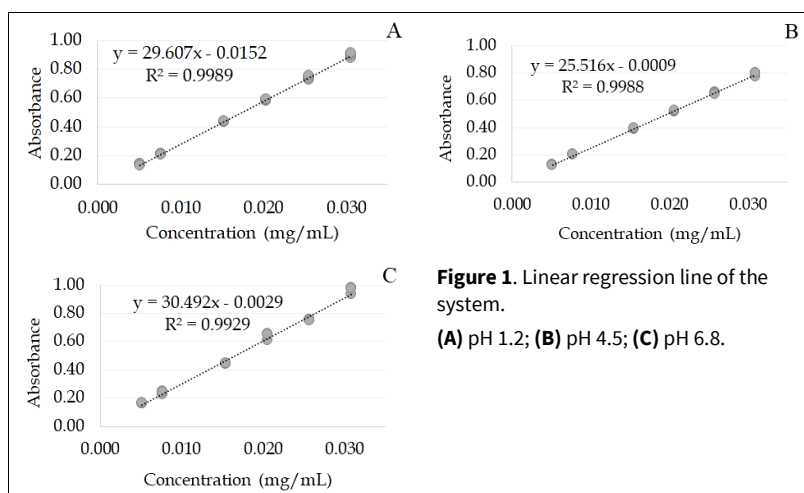


Table 1. Summary table of specifications for each validation parameter in the three losartan potassium dissolution media.

Parameters	Specifications	Results			
		pH 1.2	pH 4.5	pH 6.8	
	Range (mg/mL)	0.0051 – 0.0306	0.0051 – 0.0306	0.0051 – 0.0306	
Linearity	Coefficient of determination (r^2) not less than 0.99	0.9989	0.9988	0.99288	
	Slope of the line other than 0	29.084 – 30.131	25.043 – 25.988	29.124 – 31.860	
	Intercept equal to 0	-0.025 – 0.005	-0.010 – 0.008	-0.030 – 0.024	
	Probability of error: p value < 0.05	4.57941*10 ⁻²⁵	9.50431*10 ⁻²⁵	1.29689069*10 ⁻¹⁸	
	Response factor RSD: f(y/x) < 5%	3.255	2.909	4.360	
Accuracy	Recovery between 95% to 105%	Lower	99.97	97.18	99.10
		Half	101.07	102.24	104.47
		Higher	103.99	104.48	101.87
	CV less than 2%	2.0	3.7	2.6	
	Relative error less than 5%	Lower	0.03	2.82	0.90
		Half	1.07	2.24	4.47
Higher		3.99	4.48	1.87	
Intermediate precision	CV between samples of the same day less than 10%	Day 1, Equip 1, Ana 1	2.35	7.34	7.65
		Day 1, Equip 1, Ana 2	2.65	2.75	7.70
		Day 1, Equip 2, Ana 1	2.24	7.07	9.41
		Day 1, Equip 2, Ana 2	2.55	5.42	8.78
		Day 2, Equip 1, Ana 1	1.31	7.50	6.67
		Day 2, Equip 1, Ana 2	2.44	7.46	9.60
		Day 2, Equip 2, Ana 1	1.62	7.02	7.66
		Day 2, Equip 2, Ana 2	2.84	7.27	8.03
	Overall CV less than 10%	3.79	6.84	8.76	
	Instrumental repeatability	CV less than 2%	0.48	1.12	1.64
Method repeatability	Individual interval	100.7 ± 7.12	107.7 ± 18.09	102.8 ± 7.89	
	Average range	100.7 ± 1.03	107.7 ± 2.61	102.8 ± 1.14	
	CV less than 5%	3.09	3.9	3.42	
Detection limit (µg/mL)		0.182	0.066	0.248	
Quantification limit (µg/mL)		0.113	0.057	0.260	
Robustness	CV less than 10%	Condition 1	6.87	6.83	3.43
		Condition 2	9.52	7.21	2.72
		Condition 3	0.72	11.40	5.88
	Absolute difference less than 2%	Condition 2-1	1.10	1.06	1.04
		Condition 3-1	6.95	17.78	1.28
Filter test	CV less than 10%	Spin	7.66	20.34	8.91
		Filter 1	6.87	28.91	6.94
		Filter 2	3.08	21.83	5.67
	Absolute difference less than 2%	Spin-Filter 1	1.79	1.32	1.58
		Spin-Filter 2	0.30	0.77	1.39
	Stability of analytical solution	CV less than 10%	0 h	4.58	8.83
24 h			2.51	4.80	3.14
Absolute difference less than 2%		3.20	9.22	0.20	
Specificity	Interference must not exceed 2%	No interference	No interference	No interference	

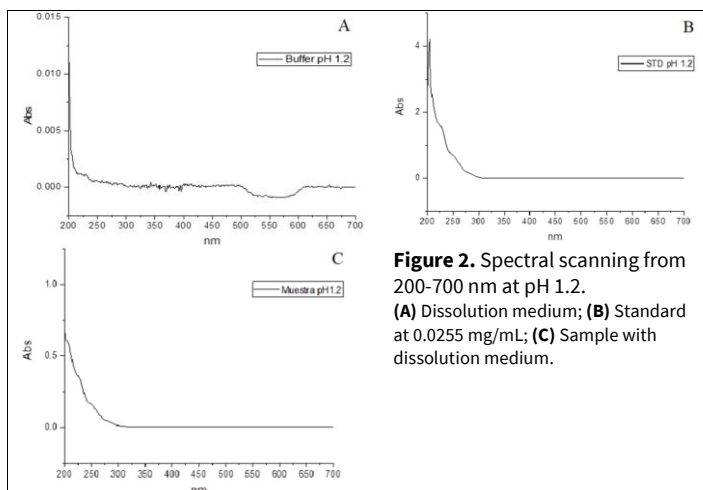


Figure 2. Spectral scanning from 200-700 nm at pH 1.2. (A) Dissolution medium; (B) Standard at 0.0255 mg/mL; (C) Sample with dissolution medium.

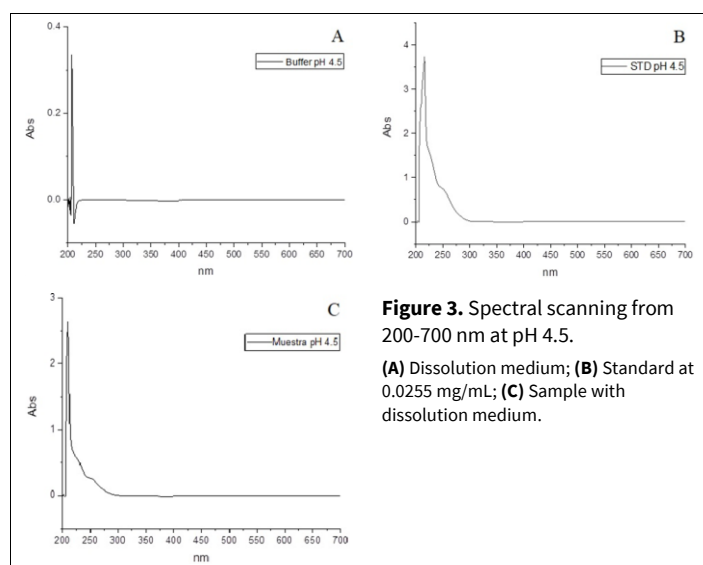


Figure 3. Spectral scanning from 200-700 nm at pH 4.5. (A) Dissolution medium; (B) Standard at 0.0255 mg/mL; (C) Sample with dissolution medium.

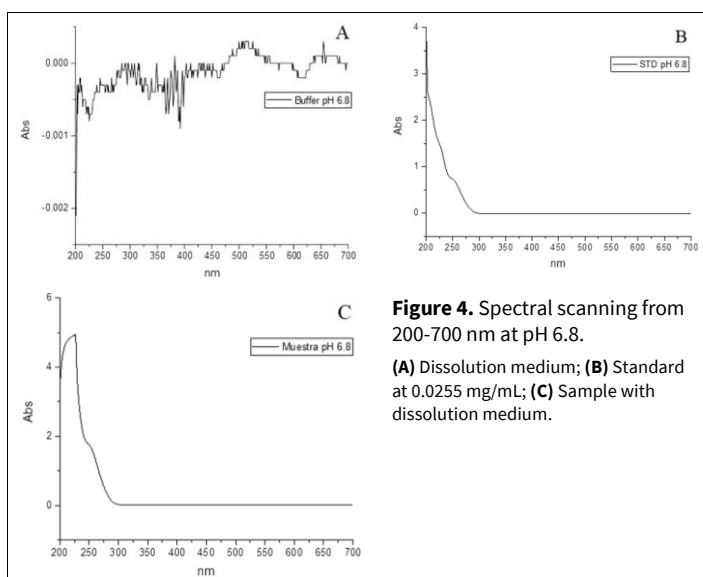


Figure 4. Spectral scanning from 200-700 nm at pH 6.8. (A) Dissolution medium; (B) Standard at 0.0255 mg/mL; (C) Sample with dissolution medium.

The stability of analytical solutions was performed in a period of 24 hours, as shown in Table 1. There was no significant difference in the dissolution medi-

um pH 6.8. The results in the other media show a high variability when preserved during this time compared to the samples read immediately, present-

ing an absolute difference of 3.20 and 9.22% for the pH media 1.2 and 4.5, respectively. This would indicate the need to analyze the samples immediately in these dissolution media (Chakraborty et al., 2018).

In this research, a better behavior of losartan was found at pH 6.8, which would mainly indicate the presence of anionic molecules in the sample. In turn, the lower solubility was observed at pH 4.5 due to the lack of charge in the molecule. At pH 1.2, losartan potassium has a moderate solubility; this leads us to infer that the availability of H⁺ ions provided by the medium can benefit the solubility of the molecule (de Souza et al., 2019).

CONCLUSION

The UV spectrophotometric analytical method for the quantification of the samples obtained from the losartan potassium dissolution test meets the acceptance criteria for each parameter under analysis and is therefore suitable for its usual use in the laboratory. A better dissolution and stability of analytical solutions were evidenced at pH 6.8. On the contrary, there was low stability at pH 1.2 and 4.5. It can be inferred that the method is linear, accurate, and precise in the specified set range. In addition, it is robust and stable for 24 h at pH 6.8.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Araujo-Fernandez AS	Uribe-Villarreal JC	Perez-Chauca E	Alva-Plasencia PM	Caballero-Aquiño OE	Ganoza-Yupanqui ML
Concepts or ideas				x	x	x
Design				x	x	x
Definition of intellectual content				x		x
Literature search	x	x	x		x	x
Experimental studies	x	x	x			
Data acquisition	x	x				
Data analysis	x		x	x		
Statistical analysis			x	x		
Manuscript preparation	x	x				x
Manuscript editing	x					x
Manuscript review	x	x	x	x	x	x

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