Development and validation of a UV spectrophotometric method for biowaiver studies in pyrazinamide tablets

[Desarrollo y validación de un método espectrofotométrico UV para estudios de bioexención en tabletas de pirazinamida]

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Abstract

Context: For regulatory reasons, a bioequivalence character is as the substitution or exemption of bioavailability and/or bioequivalence research with in vitro dissolution tests, according to the Biopharmaceutical Classification System (BCS). These studies need validated quantification methods to give reliable results.

Aims: To develop and validate the UV spectrophotometric method for biowaiver studies in pyrazinamidine tablets.

Methods: The parameters for validation, such as specificity, linearity, range, accuracy, precision, stability of the analytical solution, filter test, limits of detection and quantification, were assessed according to the standards set by the guidelines of the International Conference on Harmonization and the United States Pharmacopeia. The dissolution operating conditions were 900 mL of dissolution media at pH 1.2, 4.5, and 6.8, with the paddle method at 75 rpm and sampling times of 5, 10, 15, 20, and 30 minutes.

Results: The wavelength of maximum absorbance was 268 nm for the three dissolution media. The method was linear in the range of 1.1112–13.3334 µg/mL (r²>0.998). The limits of detection ranged from 0.0157 to 0.0222 µg/mL, and the limits of quantification were between 0.0415 and 0.0546 µg/mL. The recovered values were between 99.4 and 103.0%. No interference from the placebo was observed. The relative standard deviation values were less than 2% for all conditions. For robustness, different conditions were evaluated as wavelengths, changes in concentration, and Whatman No. 40, 41, and 42 filters; they did not modify the method response. The samples were kept unchanged for a period of 24 hours at 25°C.

Conclusions: The UV spectrophotometric method was linear, accurate, specific, and precise in the concentration range studied and in the dissolution media at pH levels of 1.2, 4.5, and 6.8, being suitable for biowaiver studies in pyrazinamidine tablets.

Keywords: antitubercular drugs; bioequivalence; drug dissolution; solubility; spectrophotometry.

Resumen

Contexto: Por razones regulatorias, la bioequivalencia se caracteriza como la sustitución o exención de la investigación de biodisponibilidad y/o bioequivalencia con pruebas de disolución in vitro, según el Sistema de Clasificación Biofarmacéutica (SCB). Estos estudios necesitan métodos de cuantificación validados para dar confiabilidad a los resultados.

Objetivos: Desarrollar y validar el método espectrofotométrico UV para estudios de bioequivalencia en tabletas de pirazinamida.

Métodos: Los parámetros de validación, tales como especificidad, linealidad, rango, exactitud, precisión, estabilidad de la solución analítica, prueba de filtro, límites de detección y cuantificación se evaluaron según los estándares establecidos por los lineamientos de la International Conference on Harmonisation y la Farmacopeia de los Estados Unidos. Las condiciones operativas de disolución fueron: 900 mL de medios de disolución a pH 1.2; 4.5 y 6.8; con el método de paleta a 75 rpm, y tiempos de muestreo de 5, 10, 15, 20 y 30 minutos.

Resultados: La longitud de onda de máxima absorción fue 268 nm para los tres medios de disolución. El método fue lineal en el rango de 1,1112-13,3334 µg/mL (r²>0.998). Los límites de detección oscilaron entre 0.0157 y 0.0222 µg/mL, los límites de cuantificación estuvieron entre 0.0415 y 0.0546 µg/mL. Los valores recuperados se encontraron entre 99.4 y 103.0%, no se observó interferencia del placebo. Los valores de desviación estándar relativa fueron inferiores al 2% para la precisión. Para la robustez se evaluaron diferentes condiciones como diferentes longitudes de onda, cambios en la concentración y los filtros Whatman No. 40, 41 y 42; no modificaron la respuesta del método. Las muestras se conservaron sin cambios durante un periodo de 24 horas a 25°C.

Conclusiones: El método espectrofotométrico UV fue lineal, exacto, específico y preciso en el rango de concentración estudiado y en los medios de disolución a niveles de pH de 1.2; 4.5 y 6.8 siendo adecuado para los estudios de bioequivalencia en tabletas de pirazinamida.

Palabras Clave: bioequivalencia; fármaco disuelto; medicamentos antituberculosos; solubilidad; espectrofotometría.
INTRODUCTION

Biowaiver is characterized as the acceptance, for regulatory purposes (Arrunátegui et al., 2015; Ono et al., 2023), of the replacement or exemption of bioavailability and/or bioequivalence studies by in vitro dissolution tests (Silva et al., 2021), according on the Biopharmaceutical Classification System (BQS) (ICH, 2019; Khalid et al., 2020).

In 2005, the World Health Organization (WHO) proposed carrying out in vitro bioequivalence studies (biowaiver), a simplified method to ensure the bioequivalence of medicines, including antituberculosis drugs, a situation that ensures access to these medicines with the expected quality, for a greater number of patients (Strauch et al., 2011).

In vitro studies, developed to infer therapeutic equivalence, include dissolution tests whose results allow correlation with the biopharmaceutical behavior of a drug in immediate-release oral formulations of drugs from BCS class I and III (Amidon et al., 1995; Reppas et al., 2014; WHO, 2017).

Therefore, in order to get reliable results, it is important to develop and validate an adequate method to quantify the specific analyte in the dissolution medium of physiological pH (pH 1.2, 4.5, and 6.8) to demonstrate in vitro equivalence (biowaiver) between test and reference medicines (Friedel et al., 2018).

Pyrazinamide is an antituberculosis drug considered in the regimens for the treatment of tuberculosis (TB) in many countries, including Peru (MINSA, 2023).

In accordance with the BCS, pyrazinamide belongs to class III (high solubility, low permeability) and is available in single-dose solid formulations (Becker et al., 2008) or as fixed-dose combinations (FDC) with other antituberculosis drugs such as isoniazid, rifampicin and ethambutol (Agrawal and Panchagnula, 2004).

One of the methods used to evaluate drug delivery is the dissolution testing because the dissolution of the active pharmaceutical ingredient contained in solid pharmaceutical represents a good alternative to understand drug absorption processes in the gastrointestinal tract under physiological conditions (pH dissolution media from 1.2 to 6.8) (Agrawal and Panchagnula, 2004; Dévay, 2006).

The selection of an appropriate method that allows quantification of the dissolved drug is important to give reliable results in dissolution studies (USP, 2019b).

Official monographs describe dissolution methods for pyrazinamide tablets by using ultraviolet spectrophotometry at 268 nm (Ph. Bras, 2019; USP, 2019a). However, these methods are considered tools for quality control testing, while biowaiver studies require the validation of a method if a delivery drug is to be studied.

Analytical methods have been published for the quantification of pyrazinamide (assay) in tablets, such as ultraviolet spectrophotometry (Kushwaha et al., 2020; Tandel et al., 2012) or those reported for FDC that include ultraviolet (UV) spectrophotometry (Khan et al., 2017), and high-performance liquid chromatography (HPLC) (Chellini et al., 2015; Ramireddy et al., 2023).

In addition, for dissolution tests, high-performance liquid chromatography (HPLC) method for simultaneous determinations of pyrazinamide, isoniazid and rifampicin in drug combinations in FDC (3-FDC) (Mariappan et al., 2000; Singh et al., 2019) and near-infrared spectroscopy (NIRS) for simultaneous determinations of isoniazid, rifampicin, pyrazinamide and ethambutol in FDC (4-FDC) (de Oliveira et al., 2012) have also been reported.

However, for the purpose of the present study, it is necessary to validate the method using UV spectrophotometry, because it is very rapid, simple, low cost, and efficient in obtaining results in dissolution tests, and it also simulates physiological conditions in the pH ranges from 1.2 to 6.8. Therefore, this research aimed to develop and validate a safe and reliable method to guarantee the results of dissolution profiles that will be carried out later and allow us to infer equivalence in vitro (biowaiver) for the analysis of pyrazinamide tablets.

MATERIAL AND METHODS

Materials and reagents

The secondary reference standard of pyrazinamide was from Sigma-Aldrich (United States), lot LRAC1435, expiration 12/2023, and 99.96% purity.

Pharmaceutical product: pyrazinamide 500 mg tablets (lot HPR21008A and expiration 05/2025) manufactured by Nordic Pharmaceutical Company S.A.C. (India), which contained in its formulations corn starch, povidone K30, colloidal anhydrous silica, sodium starch glycolate type A, purified talc, and magnesium stearate.

The placebos were prepared with all the excipients in the appropriate proportions for pyrazinamide 500
mg tablets (povidone K30, colloidal anhydrous silica, corn starch, talc, sodium starch glycolate, and magnesium stearate) (Strauch et al., 2011).

Reagents: Monobasic potassium phosphate, sodium acetate trihydrate, 100% glacial acetic acid, and potassium chloride were acquired from Supelco (Germany); 37% fuming hydrochloric acid was acquired from J.T. Baker (United States), and 99.0% sodium hydroxide was purchased from Merck (Germany).

All reagents were of the American Chemical Society (ACS) grade. Additionally, ultrapurified water (MicroPure UV/UF, ThermoScientific, United States) was used to prepare all dissolution media.

UV analytical method

Spectrograms of the pyrazinamide standard solution (11.1112 µg/mL) were recorded on a Lambda 25 UV-VIS spectrophotometer (Perkin Elmer, United States) using a 10 mm path length quartz cell in the wavelength range from 220 to 300 nm to verify the absorption maxima of pyrazinamide in each of the dissolution media. 268 nm was the wavelength selected for all three dissolution media. The working temperature was 25°C.

Dissolution operating conditions

Dissolution tests were performed in the AT Xtend automated dissolver (Sotax, Switzerland), using 900 mL of dissolution medium with pH values of 1.2, 4.5, and 6.8; prepared according to United States Pharmacopeia (USP) 42 (2019) and maintained at 37°C, with paddle method (USP type II apparatus), and rotation speed of 75 rpm. Samples of 10.0 mL were withdrawn at 5, 10, 15, 20, and 30 min and then were filtered using the Whatman No. 41 filter with a 20-25 µm pore size before UV analysis. Six repetitions were considered for each dissolution media.

Validation development

The UV analytical method was validated following the recommendations of the International Conference on Harmonization (ICH) Q2 guideline (ICH, 2019) and the United States Pharmacopeia (USP, 2019c). The validation parameters studied were linearity and range, limits of detection and quantification, accuracy, precision, specificity, robustness, stability of the analytical solution, and filter test.

Linearity and range

A stock solution was prepared containing 1.0 mg/mL of standard pyrazinamide previously dissolved in distilled water.

Seven concentrations ranging from 1.1112 to 13.3334 µg/mL were prepared in triplicate in the three dissolution media. The readings (absorbance measurements) of the preparations were recorded in triplicate.

Limit of detection (LOD) and quantification (LOQ)

They were obtained by the method based on the extrapolation of the calibration curve to zero concentration. A new calibration curve was constructed whose concentrations were close to the LOQ (AEFI, 2001).

The LOD and LOQ were determined using equations [1] and [2].

\[
\text{LOD} = \frac{Y_{bl} + (3 \times S_{bl})}{b \times n}\]

\[
\text{LOQ} = \frac{Y_{bl} + (10 \times S_{bl})}{b \times n}\]

Where, \(Y_{bl}\): Independent term of the calibration curve, that was, the response of the method versus concentration of the analyte; \(S_{bl}\): Term independent of the calibration curve, that was, standard deviations of the method response versus analyte concentrations; \(b\): Slope of the calibration curve obtained by representing the response to the method versus analytic concentration; \(n\): number of data.

Accuracy

It was assessed by retrieving specified quantities of a reference standard incorporated into the placebo at the commencement of the tablet dissolution process at three concentration levels (lower, middle, and upper). Nine samples were prepared, three for each concentration level. This procedure was repeated for all three dissolution media. The analysis included evaluating recovery percentages within the range of 95 to 105%, relative standard deviation (RSD) (<2%), and t-test (p>0.05).

The recovery percentage was calculated using the equation [3].

\[
\text{Recovery (\%) = \frac{\text{Quantity of drug found}}{\text{Quantity of drug added}}} \times 100\]

Specificity

Specificity was determined by evaluating the placebo prepared similarly to the sample and dissolution media at pH 1.2, 4.5, and 6.8. The samples were scanned separately, and the spectrograms were recorded. Placebos or dissolution media should show no signal; if this was not possible, the interference should not be more than 2.0%.
Precision

Method repeatability

It was determined by analysing nine recovery samples (lower, middle, and upper). The procedure was repeated for all three media dissolution. The (RSD) of the nine samples must not be greater than 2.0%.

Intermediate precision

Six tablet samples were analyzed by two analysts on two different days and using different equipments: The UV-Vis spectrophotometer (Lambda 365 Model, Perkin Elmer, United States) and the UV-Vis spectrophotometer (Thermo Scientific, Orion Aqua-mate 8000, United States). The tests were carried out for all three dissolution media. Variability was evaluated by determining the RSD of the measurements.

Robustness

Three tablet samples were analyzed by introducing small changes in the method, such as a) wavelength and b) concentration of samples. The absolute difference between the variation conditions must not be more than 2.0%.

Stability of analytical solution

Six prepared tablet samples were analyzed at 0, 6, and 24 h (25°C). The tests were carried out for each dissolution media (pH 1.2, 4.5, and 6.8). The absolute difference between both conditions must not be greater than 2.0%.

Filter test

Samples centrifuged at 15°C (6000 rpm for 10 minutes) and filtered with different filters were compared: a) quantitative Whatman No. 40 filter paper, b) quantitative Whatman No. 41 filter paper, and c) quantitative Whatman No. 42 filter paper to determine the most suitable filter for the method under consideration. The absolute difference of the different filters must not be more than 2.0%. The experiment was made in triplicate.

Statistical analysis

The data collected from the validation parameters included were mean values, standard deviations, and RSD, which were analyzed according to the acceptance criteria described in the ICH Q2 guideline and the USP. The linearity parameter data were subjected to statistical analysis (one-way ANOVA, with a 95% confidence interval) and t-test for the accuracy parameter.

RESULTS AND DISCUSSION

Every analytical method must be subject to a validation study to ensure that the results obtained are those that correspond, that is, that they are suitable for the need (Araujo-Fernandez et al., 2022; EUROLAB, 2016).

This aspect is critical when researching medications, since their quality impacts the health coverage of countries, even more so when it comes to antituberculosis drugs such as pyrazinamide (Hauk et al., 2020; OMS, 2019). Spectrophotometric analytical methods are those chosen when working on dissolution profiles, given their ease of implementation and low cost (Friedel et al., 2018).

Pyrazinamide exhibits an ultraviolet radiation absorption capacity thanks to the presence of particular chromophore groups in its molecular composition that capture electromagnetic radiation at a specific wavelength (Kushwaha et al., 2020; Strauch et al., 2011). This property has allowed UV-Visible spectrophotometry to be one of the techniques used in pyrazinamide analysis (Kushwaha et al., 2020), being especially useful when there are many samples as collected in dissolution profile studies (Perez-Chauca et al., 2022).

The determination of the maximum absorbance length of pyrazinamide was carried out through exploratory scans of standard solutions in the UV region from 220 to 300 nm. Fig. 1 shows the absorption spectra of standard pyrazinamide solutions in different dissolution media. As can be seen, pyrazinamide showed an absorption maximum at 268 nm in the dissolution media at pH 1.2, 4.5, and 6.8. The selected wavelength served as the basis for the quantification of the drug under study. Once the optimal operational wavelength and the suitable concentration of pyrazinamide in various buffer solutions had been determined, the analytical method was validated in accordance with the provisions of the ICH guidelines Q2 (R1) and USP 42 (ICH, 2005; USP, 2019b; 2019c).

Linearity was evaluated by constructing calibration curves obtained by plotting the responses (absorbances) against drug concentrations in the concentration range of 1.1112 to 13.3334 µg/mL (Fig. 2), ensuring that the correlation between concentration and response remains linear within the defined intervals. The results of the regression analysis using the ANOVA test are shown in Table 1, which shows the coefficient of determination (r²) values greater than 0.9998 in the three dissolution media at pH 1.2, 4.5, and 6.8.

The parameter specificity was determined by comparing the spectra obtained from the standard
Figure 1. UV spectrogram of pyrazinamide (11.1112 μg/mL) in dissolution media at pH 1.2, 4.5, and 6.8.

Figure 2. Linearity curve of pyrazinamide in different dissolution media at pH 1.2, 4.5, and 6.8 determined by UV.

Figure 3. UV spectrogram of pyrazinamide ranging from 220 nm to 300 nm (A, B, C) in dissolution media at pH 1.2, 4.5, and 6.8.

solution, the sample solution prepared from tablets, the placebo, and the diluent (Fig. 3). Analysis of the different spectra showed that there was no significant interference from placebo or diluent at 268 nm wavelength observed in the standard and sample solutions, ensuring that only pyrazinamide was quantified.

The LOD and LOQ values obtained were in the ranges from 0.0157 to 0.0546 μg/mL in all three dissolution media, being below the range of the linearity curve (Table 1).
Table 1. Results of the study of linearity, range, and limits of detection and quantification using the UV spectrophotometric method.

<table>
<thead>
<tr>
<th>Acceptance criteria</th>
<th>pH 1.2</th>
<th>pH 4.5</th>
<th>pH 6.8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Range</strong></td>
<td>1.1112 to 13.3334 μg/mL</td>
<td>1.1112 to 13.3334 μg/mL</td>
<td>1.1112 to 13.3334 μg/mL</td>
</tr>
<tr>
<td><strong>Regression equation</strong></td>
<td>y = 0.0659x - 0.006</td>
<td>y = 0.0656x - 0.0062</td>
<td>y = 0.0655x - 0.0051</td>
</tr>
<tr>
<td><strong>Slope does not include zero</strong></td>
<td>0.0656 to 0.0660</td>
<td>0.0651 to 0.0660</td>
<td>0.0650 to 0.0660</td>
</tr>
<tr>
<td><strong>Intercept includes zero</strong></td>
<td>-0.0044 to 0.0075</td>
<td>-0.0026 to 0.0097</td>
<td>-0.0012 to 0.0097</td>
</tr>
<tr>
<td><strong>Determination coefficient (r²)</strong></td>
<td>0.9999</td>
<td>0.9999</td>
<td>0.9998</td>
</tr>
<tr>
<td><strong>p-value less than 0.05 (r)</strong></td>
<td>2.76 x 10⁻³⁸</td>
<td>4.68 x 10⁻³⁸</td>
<td>2.98 x 10⁻³⁷</td>
</tr>
<tr>
<td><strong>Response factor</strong></td>
<td>0.0644</td>
<td>0.0641</td>
<td>0.0643</td>
</tr>
<tr>
<td><strong>Limit of detection (LOD)</strong></td>
<td>0.0157 μg/mL</td>
<td>0.0222 μg/mL</td>
<td>0.0192 μg/mL</td>
</tr>
<tr>
<td><strong>Limit of quantification (LOQ)</strong></td>
<td>0.0415 μg/mL</td>
<td>0.0546 μg/mL</td>
<td>0.0449 μg/mL</td>
</tr>
</tbody>
</table>

*Based on three calibration curves.

Table 2. Results of the study of accuracy, repeatability, and intermediate precision.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Acceptance criteria</th>
<th>pH 1.2</th>
<th>pH 4.5</th>
<th>pH 6.8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Accuracy</strong></td>
<td>Recovery from 95% to 105%</td>
<td>2.2222 μg/mL</td>
<td>100.8 ± 1.05’</td>
<td>103.0 ± 0.23’</td>
</tr>
<tr>
<td></td>
<td>6.6666 μg/mL</td>
<td>99.7 ± 0.22’</td>
<td>101.5 ± 0.15’</td>
<td>99.6 ± 0.20’</td>
</tr>
<tr>
<td></td>
<td>13.3334 μg/mL</td>
<td>99.4 ± 0.04’</td>
<td>100.6 ± 0.06’</td>
<td>98.7 ± 0.11’</td>
</tr>
<tr>
<td>RSD no more than 2%</td>
<td>0.4%</td>
<td>0.1%</td>
<td>0.3%</td>
<td></td>
</tr>
<tr>
<td><strong>t-test for recovery percentage</strong></td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
<td>p&gt;0.05</td>
<td></td>
</tr>
<tr>
<td><strong>Repeatability</strong></td>
<td>RSD no more than 2%</td>
<td>0.4%</td>
<td>0.1%</td>
<td>0.3%</td>
</tr>
<tr>
<td><strong>Intermediate precision</strong></td>
<td>RSD no more than 2%</td>
<td>1.8%</td>
<td>1.4%</td>
<td>1.1%</td>
</tr>
<tr>
<td><strong>Dissolution profile variability</strong></td>
<td>RSD no more than 20%</td>
<td>5 min</td>
<td>4.6%</td>
<td>1.2%</td>
</tr>
<tr>
<td>RSD no more than 10%</td>
<td>10 min</td>
<td>1.9%</td>
<td>1.3%</td>
<td>1.4%</td>
</tr>
<tr>
<td>RSD no more than 10%</td>
<td>15 min</td>
<td>1.0%</td>
<td>1.6%</td>
<td>1.3%</td>
</tr>
<tr>
<td>RSD no more than 10%</td>
<td>20 min</td>
<td>1.4%</td>
<td>1.4%</td>
<td>1.4%</td>
</tr>
<tr>
<td>RSD no more than 10%</td>
<td>30 min</td>
<td>1.7%</td>
<td>1.7%</td>
<td>1.2%</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SD (n = 3).

Table 2 shows the results obtained for the accuracy and precision of the method, including repeatability and intermediate precision. The results of the evaluation of the accuracy of the method met the allowed recovery range from (95 to 105%). The percentage recovery of pyrazinamide for each level studied ranged between 99.4 and 103.0% in all three dissolution media, confirming the accuracy of the method. The results of repeatability and intermediate precision demonstrated that the method is precise within the concentration range evaluated. The relative standard deviation (RSD) values were less than 2%, in accordance with what is recommended by the ICH (ICH, 2005).

The RSD found in the evaluation of the repeatability of the method was less than 2% in the three dissolution media (0.10-0.4%). When evaluating the intermediate precision, the RSD values of the dissolved amounts of the drug at each of the sampling times (5, 10, 15, 20, and 30 min) were found to be below 1.75%. Furthermore, The World Health Organization (WHO), in its 51st report of 2017 on the guidelines for establishing interchangeability, indicates that an acceptable variability for the average temporal dissolution percentages is 20% (CV%) for the first time and at other time-points should be not more than 10% (WHO, 2017). Percentage coefficients of variation of less than 20% were found in the first sampling times (5 min) and at other time-points less than 10%, in the three dissolution media. Fig. 4 shows the dissolution profiles of pyrazinamide tablets in the three dissolution media, which complied with the WHO guidelines, which establish 85% as a requirement for very rapidly dissolving products. The drug must dissolve
in 15 minutes in the three buffer solutions (Ono et al., 2023; WHO, 2017). Singh et al. (2019) and Strauch et al. (2011) also reported similar results.

Table 3 presents the results of robustness, stability of the analytical solution and filter test. Regarding the robustness of the method, the changes introduced did not modify the response of the method. However, when using a wavelength of 266 nm, the results vary (RSD >2%). The stability of the analytical solution results was within acceptable limits. That is, the absolute difference values were less than 2%, suggesting that standard and sample solutions can be evaluated up to 24 h at room temperature after preparation without any significant loss. In the filter test, the results showed absolute difference values within acceptable limits, suggesting that Whatman No. 40, 41, and 42 filters can be used interchangeably to filter tablet samples in dissolution tests prior to UV analysis (USP, 2019b).

This study provides us with the opportunity to understand the fundamental importance of employing analytical methods that are appropriately developed and validated, in accordance with the analytical method validation guidelines established in the ICH and USP, in order to generate accurate measurements of the amounts of analyte (ICH, 2005; USP, 2019c).

Additionally, following the approach suggested here for developing and validating analytical methods will enhance the trustworthiness of the results in biowaiver studies in pyrazinamide tablets.

## Study limitations

The validation experiment design was developed for the pharmaceutical preparation of 500 mg of pyrazinamide uncoated tablets, which might not be suitable for pyrazinamide in film-coated tablets. Therefore, the authors suggest developing a validation study for this type of formulation.

## CONCLUSION

The UV spectrophotometric method proved to be linear, exact, specific, and precise in the concentration range studied and in the dissolution media at pH levels of 1.2, 4.5, and 6.8. In addition, the LOD and LOQ, robustness, and stability of the analytical solution and filter test were determined, and the results were in accordance with the acceptance criteria. Therefore, the UV spectrophotometric method is safe and reliable for the quantification of pyrazinamide.

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Table 3. Results of robustness study, stability of the analytical solution, and filter test.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Acceptance criteria</th>
<th>pH 1.2</th>
<th>pH 4.5</th>
<th>pH 6.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robustness</td>
<td>Absolute difference not more than 2%</td>
<td>C1 - C0</td>
<td>0.15%</td>
<td>0.43%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C2 - C0 (-2 nm)</td>
<td>6.50%</td>
<td>4.70%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C2 - C0 (+2 nm)</td>
<td>1.20%</td>
<td>0.60%</td>
</tr>
<tr>
<td>Stability of analytical solution</td>
<td>Absolute difference not more than 2%</td>
<td>24 h – 0 h</td>
<td>1.9%</td>
<td>1.9%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 h – 0 h</td>
<td>0.7%</td>
<td>0.4%</td>
</tr>
<tr>
<td>Filter Test</td>
<td></td>
<td>C5 – F1</td>
<td>0.4%</td>
<td>0.4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C5 – F2</td>
<td>0.8%</td>
<td>0.6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C5 – F3</td>
<td>0.5%</td>
<td>0.6%</td>
</tr>
</tbody>
</table>

C0: initial condition; C1: different concentration of samples; C2: different wavelength (± 2 nm); C5: centrifuged samples; F1: Whatman No. 40 filter paper; F2: Whatman No. 41 filter paper; F3: Whatman No. 42 filter paper.
and can, therefore, be applied in dissolution tests for in vitro equivalence studies (biowaiver).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

The authors wish to extend their thanks to the project: Tuberculosis, parasitosis, diabetes e hipertensión: Calidad fisicoquímica de medicamentos adquiridos por el estado peruano (PIC 06-2014, Peru) for providing financial support for this research.

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