PAPER ON EDUCATION

UV Spectrophotometry Method Validation for Quantification of Paracetamol in Tablet Formulations: A Proposal of Experimental Activity for Instrumental Analysis

Enock José Alves Goes Junior[a], Jakson Santos Roeder[a], Kaiky Borges Lima Oliveira[a], Lucas de Castro Gois[a], Mateus Pereira Ferreira[a], and Jonatas Gomes da Silva[b*]

[a]Universidade Católica de Brasília, QS 07 lote 01 EPCT, Águas Claras, 71.966-700, Taguatinga – DF, Brazil.
[b]Universidade Federal do Oeste da Bahia, Campus Reitor Edgard Santos, Rua Bertioga, 892, Morada Nobre I, 47.810-059, Barreiras-BA, Brazil.

Abstract:

In the present article it is described the validation of a simple, precise, accurate, rapid and low-cost UV spectrophotometric method for quantification of paracetamol in tablets. The method was linear in the range between 6.4 - 9.6 µg mL^{-1}, presenting a good correlation coefficient (r = 0.9984) and adequate limits of quantification (2.64 µg mL^{-1}) and detection (0.87 µg mL^{-1}). Precision and analysis showed low coefficient of variation (< 4.0 %) and a good average recovery percentual (99.22% - reference and 101.78% - generic) was obtained. The method was applied for paracetamol determination in two tablet formulations and these results are in good accordance with the declared values of manufacturer, at a 95% confidence level. The experimental activity is simple, showing it to be used in experimental activities on teaching laboratories improve student learning.

Keywords: paracetamol; teaching laboratories; UV spectrophotometry; validation studies

1. Introduction

Paracetamol (PAR) or acetaminophen (Figure 1), is widely used, without medical prescription, as antipyretic, analgesic and light anti-inflammatory, being an efficient agent and used to reduce the fever and e relief headaches, toothache, low back pain, muscle aches, arthritis and postoperative. The controlled and limited use of the PAR is secure, not showing prejudicial secondary effect. However, high dose or its prolonged use may cause different effects for human health due to the accumulation of toxic de metabolites, that can lead the serious and sometimes fatal hepatotoxicity and nephrotoxicity [1-3] Thus, is important to develop and valid simple, fast, sensible and accurate analytical methods for detection and quantification of PAR in pharmaceutical formulations, promoting reliable results in the quality control in pharmaceutical industry.
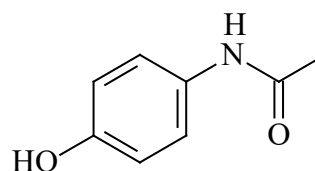

Figure 1. Chemical structure of paracetamol.

Different analytical methods such as spectrophotometric [4,5], spectrofluorimetric [6], chromatographic [7-9] and voltammetric [10,11] have been reported in literature for quantification of PAR in pharmaceutical formulations. The Brazilian Pharmacopeia describes the

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*Corresponding author. E-mail: ✉ gomes.jonatas@gmail.com

spectrophotometric method as the official method for the dosage of PAR in tablets [12]. In the method described by the Brazilian pharmacopoeia, the quantification of PAR in tablets is performed using the absorbance values of a standard solution (with concentration equal to that of the pharmaceutical formulation) and of a tablet solution, using 0.010 mol L^{-1} NaOH as the solvent. In the method proposed in this study, the quantification of paracetamol will be through an analytical curve, using as solvent 0.010 mol L^{-1} NaOH.

In the detection and quantification of drugs in pharmaceutical formulations, the spectrophotometric technique in the ultraviolet region (UV) is widely applied, being used especially in the quality control of pharmaceutical industries because it fulfills essential requirements (rapidness, low operational cost and reliable results) for routine analysis [13,14]. Some examples of the use of spectrophotometry in the UV region in the quantification of drugs in pharmaceutical formulations are found in the literature such as carvedilol [13], efavirenz [14], simvastatin [15], acyclovir [16], olanzapine [17], fluoxetine [18] and paracetamol [19].

The need to provide quality of analytical measurements, meaning the reliability of results, is becoming increasingly demanded and necessary [14]. Therefore, a new analytical method must be validated so that reliable results can be achieved. In this context, quality control became an important tool in the pharmaceutical industry, ensuring a safe and effective product [16]. The validation of an analytical method for the quantification of a chemical species must ensure, through experimental studies, that it fulfills the requirements of analytical applications, guaranteeing the reliability of the results. To validate the analytical method, the following parameters must be evaluated: selectivity; linearity; range, accuracy and detection limit; quantification limit; accuracy, and robustness [20].

In this context, this study aims to validate a method using spectrophotometry in the UV region for quantification of PAR in tablet formulations as a proposal of practical activity for the instrumental analysis subject in the pharmacy graduate course. It has been emphasized that the practical class is an important toll that favors the teaching-learning process in chemistry classes. Through the experimentation, it gets together practice and theory, making possible the manual and intellectual skills development, the argumentation, the problems resolution and the understanding of concepts, that can stimulate the student interest [21-23]. In literature have been reported some examples of practical activities proposals for the instrumental analysis subject of chemistry and pharmacy graduation courses [24-26]

2. Results and Discussion

In order to verify the possibility of development of a spectrophotometric method, for quantification of PAR in tablet formulations, the UV spectra from Figure 2 were obtained from a solution of 10 µg mL^{-1} PAR in 0.010 mol L^{-1} NaOH and one band of maximum absorption was observed in 258 nm. Based on the UV spectra obtained was decided to carry out the spectrophotometric measurements at the maximum absorption wavelength of the PAR.
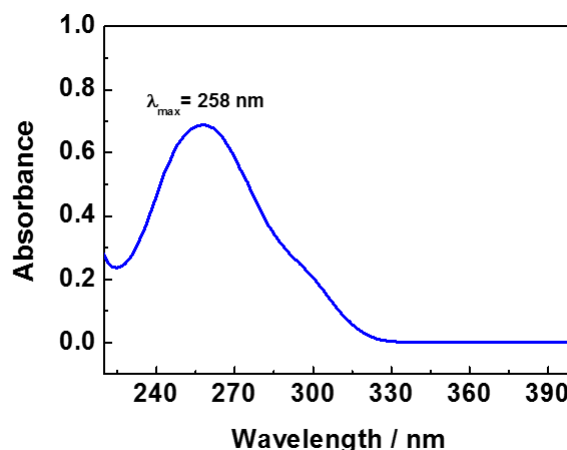

Figure 2. UV Spectra of 10 µg mL^{-1} PAR in 0.010 mol L^{-1} NaOH.

For the validation of the analytical method aiming to quantify the PAR in tablet formulations a selectivity study was carried out by analyzing the UV spectra (Figure 3) obtained for PAR and the excipients (stearic acid, starch, hypromellose, macrogol and povidone). In the UV spectra it is observed that the excipients do not present an absorption band in the studied wavelength range. Based on the spectrophotometric measurements performed the excipients are not a potential interferents in the analysis of pharmaceutical formulations.
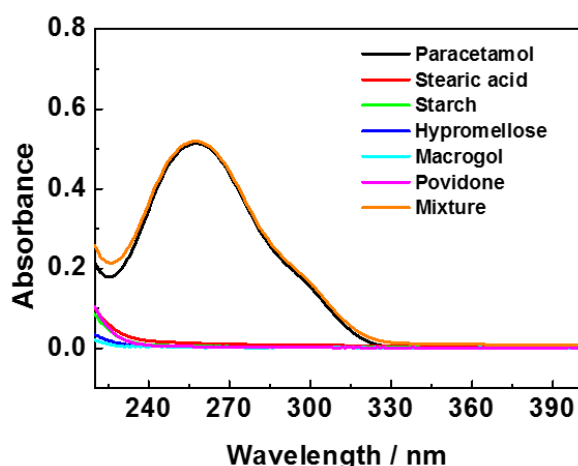
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Figure 3. UV Spectra of paracetamol and the excipients.

After the study of selectivity, the UV absorption spectra for PAR were obtained with concentration varying from 6.4 to 9.6 µg mL^{-1} (from 80 - 120% of the test's theoretical concentration) in 0.010 mol L^{-1} NaOH (Figure 4).

The analytical curve obtained through the method of least squares presented the equation y = 0.0685x + 0.004 and a correlation coefficient of 0.9984, indicating the linearity of the method within the concentration limits studied, besides being in accordance with the minimum acceptable criterion of the correlation coefficient (r = 0.990) described in the Guide for the Validation of Analytical Methods (ANVISA - Brazilian Health Regulatory Agency - Resolution n° 166, of July 24,

2017) [20]. Through the analysis of variance, the statistical significance of the adjusted curve and the linearity of the method (Table 1) were tested. In the curve fitting test, the obtained value of f (2.345) was lower than the f$_{critical}$ (3.710), proving that there is no lack of fit in the studied concentration range, at a 95% confidence level. Then, the validity of the regression (linearity) was analyzed by comparing the tabulated and calculated f values. The value of f (1677.1) is much larger than the value of f$_{critical}$ (4.670), which indicates that the model is linear, at a 95% confidence level.
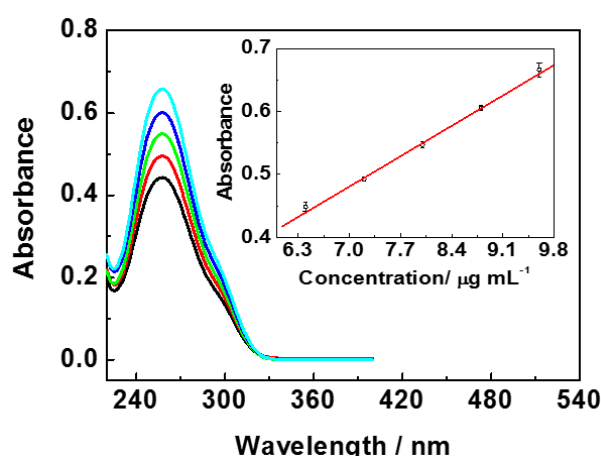

Figure. 4. UV Spectra of paracetamol obtained with concentration varying from 6.4 to 9.6 µg mL^{-1} in 0.010 mol L^{-1} NaOH. Insert: Analytical curve.

Table 1. Analysis of variance table for the fit of a curve obtained through the method of least squares.

Source	SQ	DF	MQ	F	F$_{critical}$
Regression	0.090091202	1	0.090091202	1677.1	4.670
Residual	0.000698357	13	5.371974 x 10^{-5}	Linear model	
Lack of fit	0.000288420	3	9.614005 x 10^{-5}	2.345	3.710
Pure error	0.000409936	10	4.099365 x 10^{-5}	There is no lack of fit	
Total	0.090806810	14	0.006486201		

SQ = Sum of Squares. DF = Degree of Freedom. MQ = Mean Square.

From the analytical curve, the detection limits of 0.87 µg mL^{-1} and the quantification of 2.64 µg mL^{-1} were calculated ensuring that the proposed method can detect and quantify a wide concentration range with safety, and can be applied in the detection and quantification of PAR in tablets.

Then, the repeatability and the intermediate precision were evaluated through spectrophotometric measurements of solutions containing PAR at three different concentration levels, in two days, and with different analysts. The results obtained for the repeatability and intermediate precision, presented in Table 2, show that the proposed method presents good precision with coefficient of variation (CV) values less than 4%.

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Table 2. Repeatability and intermediate precision results obtained through UV spectrophotometric measurements of PAR containing solutions at three different concentration levels (6.4, 8.0 and 9.6 µg mL^{-1}).

Day	Analyst	Concentration (µg mL^{-1})	CV (%)*	CV (%)**
1	1	6.4	2.73	-
		8.0	0.17	-
		9.6	1.56	-
2	2	6.4	3.26	2.88
		8.0	2.38	1.53
		9.6	1.01	1.50

CV = Coefficient of variation; * repeatability (n = 3); ** intermediate precision (n = 6).

As the precision of the proposed method was confirmed, assays were performed to determine the accuracy through the PAR standard addition, obtaining mean recoveries for three different levels of PAR (0.5, 1.0 and 1.5 µg mL^{-1}) in the reference and generic tablets equal to 99.22% ± 2.09 and 101.78% ± 3.33, respectively. The values of t (0.646 - reference; 0.926 - generic) are lower than the $t_{critical}$ (4.303), indicating that the recovery values found are statistically equal to 100%, at a 95% confidence level, confirming that there is no interference (excipients) in the samples of the pharmaceutical formulations analyzed (reference and generic) and that the proposed method shows good accuracy.

In order to evaluate the robustness, the ability of the proposed method to withstand small variations of the analytical parameters, the results were verified in relation to variable reagent brand used in the preparation of the 0.010 mol L^{-1} NaOH solution. The experimental results obtained for the robustness are presented in Table 3.

Table 3. Results related to the robustness of the proposed method.

Parameters		Average concentration (µg mL^{-1})	SD	CV (%)	
NaOH brand	Dinâmica	7.24	0.07	1.04	ANOVA
	Reagen	7.33	0.03	0.41	f_{value} = 1.05; $f_{critical}$ = 5.14
	Vetec	7.27	0.10	1.47	

n = 3. SD = Standard deviation. CV = Coefficient of variation.

The statistical analysis of the obtained data, varying the brand of the reagent used in the preparation of the solution of 0.010 mol L^{-1} NaOH performed through ANOVA demonstrated that changing the reagent's brand has not a significant influence on the results, at a 95% level of confidence. Therefore, the proposed method was robust in this parameter.

Table 4 presents the results obtained in the quantification of PAR in pharmaceutical formulations using the spectrophotometric method.

Table 4. Results obtained in the PAR analysis in pharmaceutical formulations using the proposed spectrophotometric method.

Samples	PAR (mg tablet^{-1})		Relative error (%)
	Label value	Spectrophotometric method	
Reference	750	743 ± 6	-0.93
Generic	750	780 ± 1	4.00

n = 3; Relative error = [(spectrophotometric method – label value)/ label value] x 100.

The t-test was applied to compare the average obtained using the spectrophotometric method with the values described on the labels of the pharmaceutical formulations analyzed. For the reference tablets, the value of t (2.021) was lower than the $t_{critical}$ (4.303), indicating that the average value obtained is statistically equal to 750 mg tablet^{-1} (p < 0.05). However, for the generic tablets, the value of t (51.96) was higher than the $t_{critical}$ (4.303), indicating that the average value obtained is statistically different to 750 mg tablet^{-1} (p < 0.05). The difference obtained for the generic tablets can be attributed to the sample contamination or systematic errors occurred

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during the generic tablets analysis made by students of the instrumental analysis subject.

3. Material and Methods

3.1. Instrumentations

The UV spectrophotometric measurements were performed in triplicate using the UV-Vis Cary 50 (Varian®) double-beam spectrophotometer with a 190 to 1100 nm detector and 1 cm quartz cuvettes, which was linked to a computer. All the mass measurements were made in a semi-micro analytical balance ($\sigma \leq 0,05$ mg) AUW220D (Shimadzu®).

3.2. Materials and Reagents

All chemicals used were of analytical reagent grade and the solutions were prepared in ultrapure water (Direct-Q® 8 UV Smart, Millipore, 18.2 Ω). PAR (99% purity) was purchased from Sigma-Aldrich (USA) and used without further purification. PAR standard stock solution (1 mg mL^{-1}) was prepared by dissolving 10 mg of PAR in ultrapure water in a 10 mL volumetric flask. The 0.010 mol L^{-1} sodium hydroxide (NaOH – 97%, Vetec, Brazil) solution was prepared by dissolving a suitable mass of this compound in ultrapure water. Tablet formulations containing PAR (750 mg) were purchased from a local drugstore.

3.3 Validation Procedure

This proposed method was validated according to the ANVISA Resolution nº 166 from 27/07/2017 [20], which regulates the validation of analytical and bioanalytical methods in Brazil, and also the recommendations from ICH (The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) [27]. The parameters of method validation evaluated were: selectivity; linearity, working range, precision, detection limit; quantification limit; accuracy; and robustness. The method was statistically compared with the declared values of manufacturer.

3.3.1. Selectivity

The method's selectivity was evaluated

through the UV spectrum analysis obtained for PAR and for the excipients stearic acid, starch, hypromellose, macrogol and povidone. The 1 mg mL^{-1} of stock solutions were prepared by dissolving 10 mg of excipients in a 0.010 mol L^{-1} NaOH in a 10 mL volumetric flask. The spectrophotometric measurements were carried out in solutions of 8 µg mL^{-1} excipients and a mixture (8 µg mL^{-1} PAR + 8 µg mL^{-1} excipients) prepared by diluting stock solutions. The profiles of the obtained spectra were compared with the spectrum of a solution of 8 µg mL^{-1} PAR.

3.3.2. Linearity and working range

The linearity was determined by obtaining three analytical curves in the PAR interval from 6.4 to 9.6 µg mL^{-1} (80 – 120% of the test's theoretical concentration) [20]. The linear equation (slope and intercept on the y-axis) was obtained using the method of least squares, and the correlation coefficient was calculated. The data of each level of concentration were evaluated through the analysis of variance (ANOVA) [28].

2.3.4. Limits of detection (LOD) and quantification (LOQ)

The limits of detection and quantification were calculated from the standard deviation of the intercept on the y-axis (σ) and the slope from the analytical curve (AC), Equations 1 and 2.

$$LOD = \frac{3,3\,\sigma}{AC} \qquad (1)$$

$$LOQ = \frac{10\,\sigma}{AC} \qquad (2)$$

The standard deviation of the intercept on the y-axis was calculated by the following Equations [29]:

$$S_{y/x} = \sqrt{\frac{\Sigma\,(y_i - \hat{y})^2}{n - 2}} \qquad (3)$$

$$SD_a = S_{y/x}\sqrt{\frac{\Sigma x_i^2}{n\Sigma(x_i - \bar{x})^2}} \qquad (4)$$

3.3.5. Precision

Repeatability (intra-day precision) was evaluated through spectrophotometric measurements of solutions containing PAR in three different concentrations (6.4, 8.0 and 9.6 µg mL^{-1}), with three replicates each, contemplating the linear interval of the method. Similarly, the intermediate precision (inter-day precision) was evaluated by different analysts performed the spectrophotometric measurements on two different days. The coefficient of variation (CV) was calculated using Equation 5, and the results were compared with the maximum acceptable value described in the Guide for Validation of Analytical Methods (ANVISA Resolution n° 166, of July 24, 2017) [20].

$$CV = \frac{SD}{DAC} \times 100 \qquad (5)$$

In which: CV is the coefficient of variation; SD is the standard deviation of spectrophotometric measurements and DAC is the determined average of PAR concentration.

3.3.6. Accuracy

The accuracy was evaluated through the PAR standard addition test on samples of pharmaceutical formulations (reference and generic) and their recovery on three different levels (0.5, 1.0 and 1.5 µg mL^{-1}). The recovery values (expressed in percentage) were obtained through the spectrophotometric measurements of the final solutions as a function of the theoretical amount of standard added. The PAR concentrations were obtained through the analytical curve.

3.3.7. Robustness

Robustness was evaluated through the spectrophotometric measurements varying the brand of NaOH (Vetec, Reagen and Dinâmica). The analysis was made in triplicate and the PAR concentrations were obtained through the analytical curve.

3.4. Quantification of PAR in pharmaceutical formulations using the spectrophotometric method

Ten tablets of each commercial sample had their content weighed, pulverized and homogenized. Powder equivalent to 0.100 g of PAR was transferred to a 100 mL volumetric flask and 50 mL of 0.010 mol L^{-1} NaOH was added to it and the volume was completed with ultrapure water. The solubilization of PAR in the commercial samples was achieved with the aid of Ultrasonic USC-1400 A (UNIQUE) machine for 15 minutes. After the filtration, 80 µL of the resulting solution was transferred to a 10 mL volumetric flask and the volume was completed with 0.010 mol L^{-1} NaOH. The analyses were performed in triplicate and the PAR concentrations were obtained through the analytical curve.

3.5. Statistical analysis

The statistical analysis of the data was performed through an analysis of variance (one-way ANOVA), tests t and f with a 95% of confidence level (probability less than 5% - $p < 0.05$). The statistical evaluation of the results was made using the Software MS Excel®.

4. Conclusions

The proposed practice for instrumental analysis subject for pharmacy graduation course, using the UV spectrophotometric for quantification of PAR in tablet formulations, it is shown simple, fast, secure and low cost. When applied in the teaching laboratory provided to the students the contact with the UV spectrophotometric technique, providing the discussion and understanding this technique, of the important concepts involving in the validation methods studies (selectivity; linearity, working range, precision, detection limit; quantification limit; accuracy; and robustness) and statistical tests application.

Acknowledgments

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