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ARTIGO

Excellent green analysis for quantification of gatifloxacin in eye drops by Eco-Scale Assessment

Excelente análise verde para quantificação de gatifloxacino em colírios pela Eco-Scale Assessment

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ABSTRACT: gatifloxacin (GAT), a fourth-generation fluoroquinolone, is currently marketed primarily in the form of eye drops. The literature shows physical-chemical methods with a predominance of the use of toxic organic solvents, which promote the generation of potentially harmful residues both for the environment and for the operator. The objective was to develop and validate an excellent green method in the visible region for quantification of GAT in eye drops. Purified water and NaOH 0.1 M, as a diluent, and phenolphthalein 1 %, for reaction, at 552 nm were used. The method was linear in the range of 7-12 µg/mL (0.9996), precise (relative standard deviation < 5 %), selective through sample adjuvants, exact (mean recovery of 100.88 %) and robust against changes in wavelength and microplate usage. The content obtained with the proposed method was 98.21 % and is in agreement with the official compendium and other methods described in the literature. The method is a green proposal to quantify GAT in eye drops according to the Eco-Scale Assessment tool.

Keywords: Eco-Scale Assessment. Eye Drops. Gatifloxacin. Green Analytical Chemistry. Spectrophotometry in Visible Region.

RESUMO: o gatifloxacino (GAT), uma fluoroquinolona de quarta geração, é atualmente comercializada principalmente na forma de colírio. A literatura mostra métodos físico-químicos com predominância do uso de solventes orgânicos tóxicos, que promovem a geração de resíduos potencialmente nocivos tanto para o ambiente quanto para o operador. O objetivo do trabalho foi desenvolver e validar um excelente método verde na região do visível para quantificação de GAT em colírios. Foram utilizados água purificada e NaOH 0,1 M, como diluente, e fenolftaleína 1 % para reação, a 552 nm. O método foi linear na faixa de 7-12 µg/mL (0,9996), preciso (desvio padrão relativo < 5 %), seletivo através dos adjuvantes da amostra, exato (recuperação média de 100,88 %) e robusto perante mudanças no comprimento de onda e uso de microplacas. O teor obtido com o método proposto foi de 98,21 % e está de acordo com o compêndio oficial e outros métodos descritos na literatura. O método é uma proposta verde para quantificar GAT em colírios de acordo com a ferramenta *Eco-Scale Assessment*.

Palavras-chave: Colírio. *Eco-Scale Assessment.* Espectrofotometria na Região do Visível. Gatifloxacino. Química Analítica Verde.

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INTRODUCTION

Gatifloxacin (GAT, Figure 1A), a fourth-generation fluoroquinolone, is currently marketed primarily in eye drop form for the treatment of eye infections, bacterial conjunctivitis, bacterial irritation and inflammation. More than 50 % of the physical-chemical methods found in the literature to assess GAT use acetonitrile and/or methanol. Among the methods by spectrophotometry in the ultraviolet-visible region (UV-Vis), for evaluating the final product eye drops, there are still opportunities for improvement to contemplate the green analytical chemistry (GAC)[1-9]. Some opportunities involve not using or reducing the use of toxic organic reagents and/or solvents, which impacts on improving the analyst's quality of life and productivity. Additionally, costs can be reduced by faster methods and by choosing less toxic options, as they will not require extensive and more costly waste treatments. In addition, waste generation can also be reduced. The benefits are multidimensional and involve both the analyst, the laboratory and the patient/client[10].

The Eco-Scale Assessment (ESA) is a tool that brings objective evidence about the greenness of analytical methods. It facilitates decision-making on the choice by analysts of the most appropriate and clean procedures for routine chemical-pharmaceutical analysis[11-18]. The ESA analysis is based on penalties for the use of chemical products, as well as their quantities used and their respective risk classifications assigned through warning words based on the Globally Harmonized System (GHS). In addition, high energy consumption, occupational risks and waste generation are also parameters evaluated. Thus, the assessment is carried out by subtracting penalties from a 100-point scale. Thus, the closer to 100, the more sustainable an analysis is[19,20].

The proposal of this work was to develop and validate an excellent green method by eco-scale assessment using spectrophotometry in the visible region for the evaluation of gat in eye drops.

EXPERIMENTAL PART

Materials and reagents

GAT standard (declared content of 99.59 %) and GAT sample (eye drops 0.3 % in 5 mL) were used.

Reagents used were purified water (Elga Purelab Option Q®), NaOH (LabMaster Ltda®) and phenolphthalein (Quemis®).

EQUIPMENT

UV-Vis spectrophotometer model 840-297300 (Thermo Scientific®), quartz cuvette with 700 µL capacity, analytical balance model AUW220D (Shimadzu®), ultrasound model somUSC-2800 (Unique®) were used.

STOCK AND SAMPLE SOLUTION PREPARATION

An amount of 2.5 mg of GAT standard was weighted and transferred to a 50 mL volumetric flask with purified water, which was subjected to 5 minutes of ultrasound and then the volume was completed with purified water and 500 µL NaOH 0.1 M, in order to obtain a stock solution of 50 µg/mL. So, aliquots were taken and transferred to a 10 mL volumetric flask and completed with purified water.

The contents of 10 eye drops were measured, combined and from this pool, 833 µL (equivalent to 2.5 mg of GAT) were transferred to a 50 mL volumetric flask with purified water, which was subjected to 5 minutes of ultrasound and then the volume was completed with purified water and 500 µL NaOH 0.1 M, in order to obtain a stock solution of 50 µg/mL. So, aliquots were taken and transferred to a 10 mL volumetric flask and completed with purified water.

METHOD DEVELOPMENT

Based on data in the literature, some reagents were tested for the assessment of GAT in eye drops by spectrophotometry in the visible region, such as chloranilic acid 0.1 %, phenolphthalein 1 %, iron chloride 0.5 %, acetic acid 50 % and potassium permanganate. The most suitable results were obtained using phenolphthalein 1 %.

VALIDATION PARAMETERS

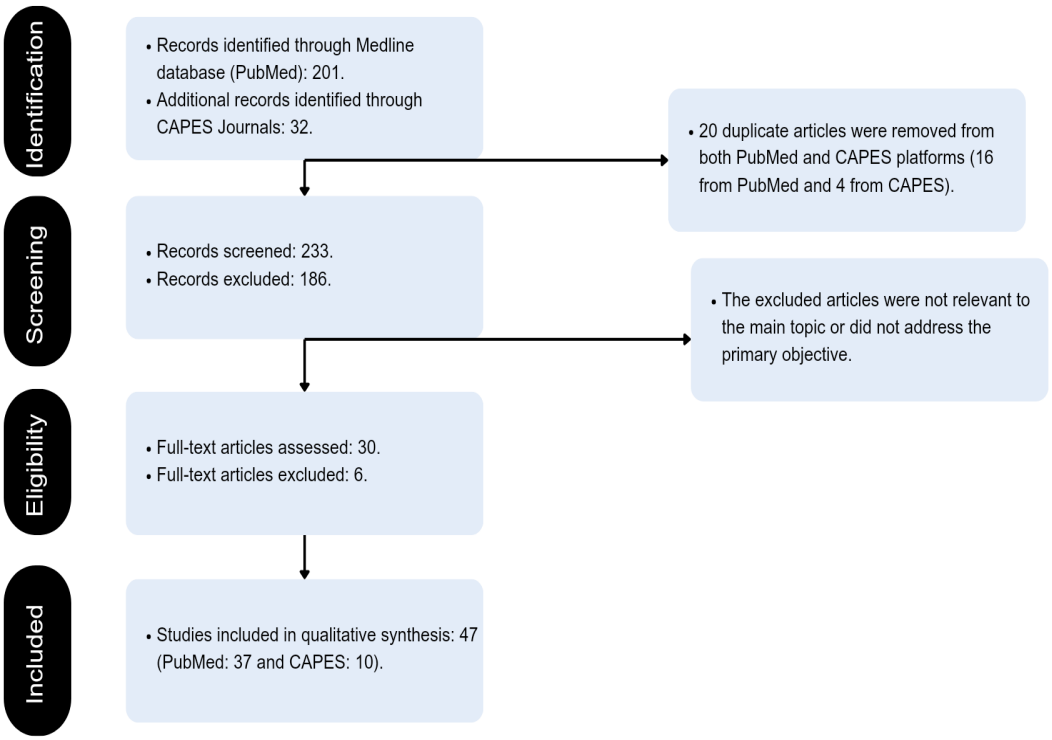
Method validation was performed according to the International Conference of Harmonization[21] for linearity, precision, selectivity, accuracy, robustness and limits of detection and quantification.

METHOD VALIDATION

Linearity

Aliquots were taken, from the stock solution, to prepare the solutions of 7, 8, 9, 10, 11 and 12 µg/mL. Linearity was performed in triplicate and different days. The absorbance was measured at 552 nm. Data obtained were evaluated by correlation coefficient,

regression analysis, analysis of variance (ANOVA) and residue graph.



Flowchart 1. Inclusion and exclusion criteria for articles.

PRECISION

Precision was evaluated in intraday, interday and interanalyst levels. Intraday level was performed in the same day, same analyst and same conditions, using 6 replicates at a concentration of 10 µg/mL. Interday level was performed in different days, same analyst and same conditions, using 12 replicates (6 replicates at 10 µg/mL concentration prepared and analyzed on one day and 6 replicates at 10 µg/mL concentration prepared and analyzed on another day). Interanalyst level was performed in the same day, same conditions and different analysts, using 12 replicates (6 replicates at 10 µg/mL concentration prepared and analyzed by one analyst and 6 replicates at 10 µg/mL concentration prepared and analyzed by another analyst). The results were analyzed by RSD (%).

SELECTIVITY

Selectivity was determined by comparing the response obtained for GAT standard and sample, using solutions at 10 µg/mL.

ACCURACY

Accuracy was evaluated by the standard recovery test using 3 levels (80, 100 and 120 %) in triplicate, considering 10 µg/mL 100 %. Standard and sample solutions were prepared in the concentration of 50 µg/mL. From the stock solution, aliquots of 0.2, 0.6 and 1.0 mL were transferred to 10 mL volumetric flasks containing 1.4 mL of sample aliquot, in order to obtain a final concentration of 8, 10 and 12 µg/mL, respectively. Recovered standard (%) and RSD (%) were determined.

ROBUSTNESS

Robustness was analyzed by small modifications in the method and evaluated by F-test and t-test. The modifications were: wavelength (552 nm, normal; 550 nm, modified), quartz cuvette (700 µL capacity, normal; 4 mL capacity, modified), NaOH 0.1 M volume (500 µL, normal; 480 µL, modified), phenolphthalein 1 % volume (300 µL, normal; 280 µL, modified), ultrasound time (use, normal; no use, modified) and microplate use (cuvette, normal; microplate, modified).

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

The limits of detection and quantification were obtained from the calibration curves, done during the linearity, using the Equations 1 and 2, respectively:

$$LOD = 3\,x\,\frac{SD}{a} \quad \text{Equation 1}$$

$$LOQ = 10\,x\,\frac{SD}{a} \quad \text{Equation 2}$$

SD: standard deviation; a: average slope

CONTENT ANALYSIS

Standard and sample solutions at a concentration of 10 µg/mL were prepared in triplicate on 3 different days. The absorbance values were compared and the GAT content in eye drops was calculated. The content was analyzed according to the results of other works present in the literature and the ANOVA was calculated.

ECO-SCALE ASSESSMENT

The penalty points (PP) were calculated, according to the Equation 3[11-12, 22].

ESA=100-[(chemical reagents pictogram x quantity of reagents x signal words)+ energy + occupational hazard + (waste amount x waste characteristic)] Equation 3

RESULTS AND DISCUSSION

Linearity

ANOVA data (Table 1) showed significant linear regression (Fcalculated>Fcritical) and no significant lack of fit (Fcalculated<Fcritical). Furthermore, the plot of residuals (Figure 1B) shows dispersion of the data with no trend. Thus, the method can be considered linear.

Table 1. ANOVA results for evaluating the linearity of the method

Parameters	552 nm
Linearity range (µg/mL)	7-12
Slope	0.0839
Intercept	0.2994
Correlation coefficient (r)	0.9996
Regression	345.74* (4.75)
Lack of fit	0.15 (3.26)

Legend: *Significant for p<0.05.

PRECISION

RSD (%) from the method precision analysis results are shown in Table 2 and they were smaller than 5.0 %, which proves the precision[21, 23-24].

Table 2. Results for evaluating the precision of the method.

Wave-length	Level	Absorbance						RDS (%)
		1	2	3	4	5	6	
552 nm	Intraday	0.539	0.566	0.548	0.547	0.559	0.551	1.73
	Interday	0.539	0.566	0.548	0.547	0.559	0.551	1.81
		0.530	0.558	0.545	0.561	0.554	0.550	
	Interanalyst	0.539	0.566	0.548	0.547	0.559	0.551	2.56
		0.525	0.527	0.526	0.530	0.540	0.528	

SELECTIVITY

The method is able to identify the GAT in eye drops, since the response of the standard solutions and the sample of GAT at a concentration of 10 µg/mL were overlapping. Furthermore, the fact that the eye drops contain benzalkonium chloride, disodium edetate, sodium chloride, hydrochloric acid/sodium hydroxide in the composition of the product, they do not interfere with the proposed method (Figure 1C).

ACCURACY

Standard recovery test results are shown in Table 3. The method can be considered exact, since the average recovery was 100.88 %, within the specification for pharmaceutical analyzes of 98 to 102 %[23, 25].

Table 3. Results for evaluating the accuracy of the method

	GAT standard added (mL)	GAT standard recovered (mL)	Recovery* (%)	Mean recovery (%)	RSD (%)
R1	8.0	7.99	99.87		
R2	10.0	10.11	101.08	100.88	0.92
R3	12.0	12.20	101.69		

Legend: *Average of 3 determinations in triplicate.

ROBUSTNESS

Changes in wavelength (2.54<2.78) and microplate usage (0.74<2.78) do not affect the method, since the $t_{calculated}$ was smaller than the $t_{tabulated}$ for a significance level of 5 %. The robustness when using the microplate allows (i) the analyzes to be miniaturized, generating less waste and material expenditure, (ii) the results are read simultaneously, generating faster results release and (iii) due to the method using the visible region, the microplates

can be made of polystyrene, which helps to reduce the cost of materials and, consequently, of analyses. However, changes in quartz cuvette (3.33<2.78), NaOH volume (21.32<2.78), phenolphthalein volume (12.89<2.78) and use of ultrasound (7.34<2.78) affect the method (Figure 2). Therefore, these changes must be avoided and information about these impacts must be included in the method.

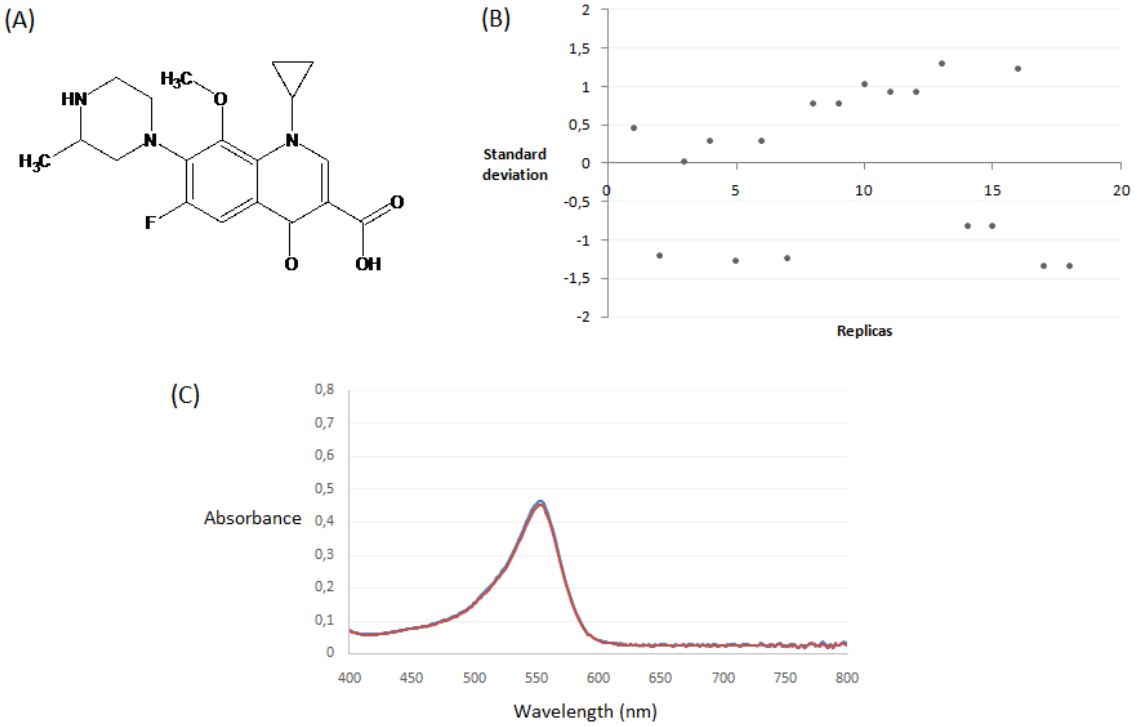


Figure 1. (A) Chemical structure of gatifloxacin (CAS 112811-59-3), (B) residue graphical and (C) superposition of spectra of standard (blue) and sample (red) gatifloxacin solutions at a concentration of 10 µg/mL at 552 nm

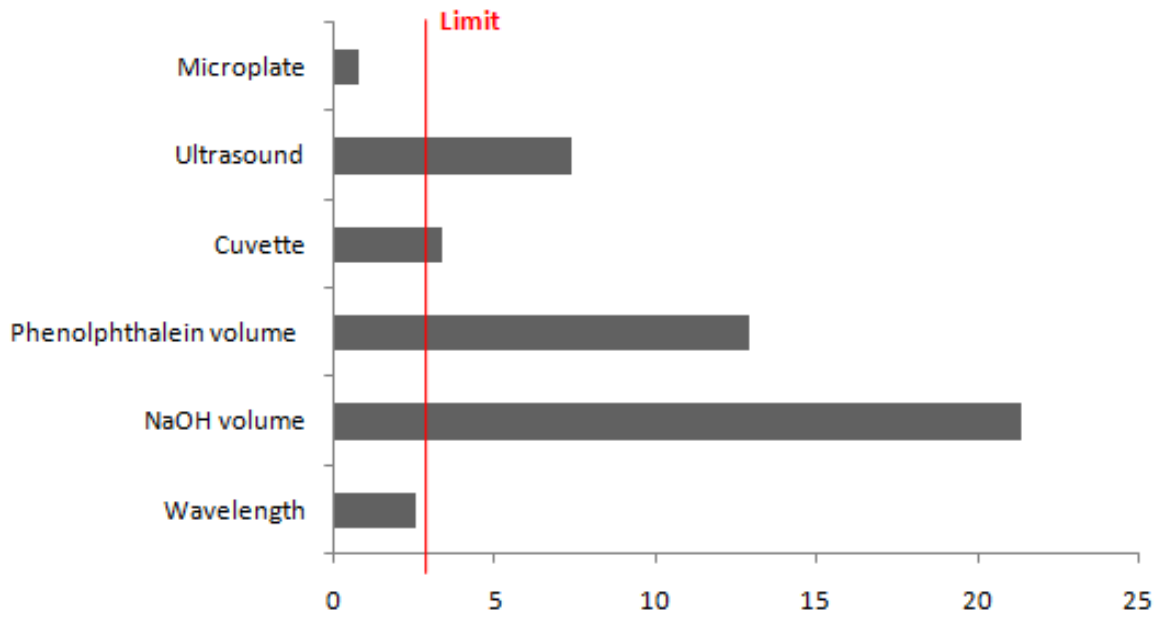


Figure 2. Effect of the modifications studied on the robustness of the method

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

Theoretical LOD and LOQ were, respectively, 0.49 and 1.47 μg/mL, showing the sensibility of the method.

CONTENT ANALYSIS

GAT content in eye drops was 98.21 % (Table 4) and, according to the Japanese Pharmacopoeia[8], which presents a specification for this product of 95 to 107 %, the batch would be approved. Furthermore, if the content result obtained with the proposed method is compared with other results found in the literature[26-27] for the same type of product, GAT in eye drops, they do not show a statistically significant difference (Fcalculated 740 < 7.71 Ftabulated).

Table 4. Content analysis of gatifloxacin in eye drops using the proposed method

Day	Average Content* (%)	Final content (%)	RSD (%)
1	96.81		
2	98.21	98.21	1.16
3	99.07		

Legend: *Average of 3 determinations in triplicate.

The robustness results (item 3.5), analyzed by F-test and t-test and demonstrated by Pareto, in addition to the content analysis (item 3.7) using commercial eye drops (5 mL bottles at 0.3%) can prove the practical applicability.

ECO-SCALE ASSESSMENT

According to Eco-Scale Assessment, the greenness of an analytical process can represent an: (i) excellent green analysis (> 75 points); acceptable green analysis (> 50 points); inadequate green analysis (< 50 points). The proposed method is considered an excellent green analysis, since the greenness of the analytical process was 80[11-12, 20, 22].

Despite the advantages of the method developed, it is important to raise possible limitations so that the choice of method by the analyst is more appropriate for the intended use. Therefore, verification of the solubility of the active ingredient, presence of chromophore groups, impact of adjuvants present in the sample, research on impurities and degradation products should be discussed.

A notable advantage of the spectrophotometric method developed in the Vis region is that it can be performed using 96-well microplates (proven use in the robustness parameter) with simultaneous results, which makes the method optimized because it is faster, miniaturized because it uses volumes around μL, which impacts on lower costs, generating less waste[18, 28-29].

This is a sustainable and green proposal for the evaluation of the final product of gatifloxacin. However, the analytical mindset and awareness present in the development of this method can be used in other methods, active ingredients and pharmaceutical forms, as demonstrated in the literature for rifaximin, cefadroxil, marbofloxacin, azithromycin and tinidazole, for example[16-18, 30-32].

CONCLUSION

Chemical-pharmaceutical laboratories, scientific literature and sustainability advance with the proposed method for the evaluation of gatifloxacin in eye drops. It is an excellent green method by the Eco-Scale Assessment using spectrophotometry in the visible region. It uses purified water, as a diluent, and phenolphthalein 1 %, for reaction, at 552 nm. The method was linear (7-12 μg/mL, r = 0.9996), selective, precise (RSD < 5 %) and exact (100.88 %).

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AUTHORS' CONTRIBUTIONS

Rafael Lima Freire contributed to the conception and design of the work, acquisition, analysis and interpretation of the research data, writing, critical review and final approval of the version for publication.
Aline Sinzervinch contributed to the writing, critical review and final approval of the version for publication.
Ricardo Neves Marreto contributed to the final approval of the version for publication.
Ana Carolina Kogawa contributed to the conception and design of the work, acquisition, analysis and interpretation of the research data, writing, critical review and final approval of the version for publication.

CONFLICT OF INTEREST

We declare no conflict of interest.

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