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# Rapid turbidimetric assay for quantification of fusidic acid in a dermatological cream



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## ABSTRACT

Fusidic acid is an antibiotic steroid widely used for the treatment of serious infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) strains. Microbiological methods are indispensable to determine the mean percentage of antimicrobial in medicaments during manufacturing and quality control processes. The aim of this study was to develop and validate a microbiological method for the quantification of fusidic acid in dermatological cream by turbidimetry, using *Staphylococcus epidermidis* (ATCC 12228) and casoy broth as the culture medium. The validation parameters were in accordance with ICH specifications and demonstrated accuracy, precision, selectivity, and robustness, with linear ranges from 0.25 to 2.25  $\mu\text{g mL}^{-1}$ . This method is an alternative to the diffusion agar assay currently employed to quantify fusidic acid in dermatological cream, since it is sensitive, fast, and more economical.

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## 1. Introduction

Fusidic acid (FA) is an antibiotic produced from the fungus *Fusidium coccineum* and belongs to the class of steroids [1] (Fig. 1); however, it does not exert effects associated with steroids [2]. It has been widely used to treat infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) strains [3,4], and it is also effective against other species of Gram-positive bacteria [5,6].

Several physicochemical methods have been described in the literature for the analysis of FA in different matrices, such as spectrophotometry [7–9], electrophoresis [10], electrospray ionization tandem mass spectrometry (ESI-MS) [11], atomic absorption spectroscopy [12], thin-layer chromatography [13–15], high-performance liquid chromatography [13,14,16–27], and surfactant–dye binding degree method [28]. These methods are widely used to evaluate antimicrobial agents, and although precise, cannot represent the true biological activity of a drug.

For this reason, microbiological methods, which are more time consuming, are essential and indispensable for determining the mean percentage of antimicrobial in medicaments during manufacturing and quality control processes, as shown by studies conducted by our research group. In addition, microbiological assays do not require specialized equipment and no toxic solvents are involved [29,30].

Official compendia generally describe two methods for assessing the mean percentage of antibiotics: turbidimetric method and agar diffusion method [31,32]. Some studies have determined FA in different matrices using agar diffusion [27,33–35]. However, the use of the turbidimetric assay method for this purpose has not been reported. Our research group has shown that the turbidimetric assay presents higher sensitivity and rapidity than traditional agar diffusion assays. The first advantage of using turbidimetric assay methods is related to the use of liquid medium, in that the solution under analysis diffuses completely, and therefore responds to lower concentrations of the antimicrobial agent. Moreover, the duration of this type of analysis is short owing to incubation period—this method requires only 4 h of incubation, whereas diffusion in agar requires 21 h of incubation [31]. The aim of this study was to validate a turbidimetric microbiological method to evaluate the mean percentage of FA in a dermatological cream.

Moreover, the reversed phase-liquid chromatography (RP-LC) method, developed and validated previously by our study group, was chosen as a comparative method in the determination of this drug.

## 2. Experimental

### 2.1. Chemical and reagents

The fusidic acid reference standard (FA-RS), with a stated purity

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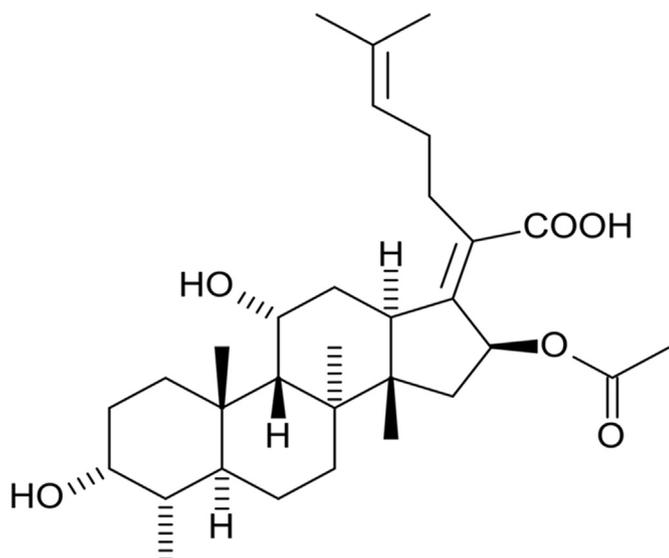


Fig. 1. Chemical structure of fusidic acid (CAS: 6990-06-3).

of 100%, was purchased commercially from Sigma-Aldrich (Brazil), and a sample of dermatological cream (Verutex™) containing FA, with  $20 \text{ mg g}^{-1}$  of the active ingredient, was supplied by LEO Pharma Laboratory (Itapevi-SP, Brazil). All adjuvants used in the production of the formulation without the active ingredient (placebo) were of analytical grade: hydrochloric acid (Lab Synth), cetyl alcohol (Audaz), butylated hydroxyanisole (Sigma-Aldrich), glycerol (Ely Martins), white petrolatum (Tec-Lab), liquid petrolatum (Columbia), polysorbate 60 (LabSynth), and potassium sorbate (Rica Nata). The culture medium casoy broth (Oxoid, England), brain heart infusion (BHI, Merck, Germany) broth, and Mueller-Hinton (Acumedia, USA) were used. Analytical-grade formaldehyde (Qhemis, Brazil) was used to interrupt the growth of microorganisms. The solutions were prepared with ethylic alcohol (LabSynth) and ultrapure water (Milli-Q™).

## 2.2. Apparatus and microbiological conditions

For the turbidimetric assay, the culture media were weighed using a semi-analytical balance model B160 (Miconal™, SP, Brazil) and were sterilized before use in a vertical autoclave AV model (Phoenix Lufanco™, SP, Brazil). The microorganisms were incubated in a Shaker incubator MA420 model (Marconi™, SP, Brazil) and an oven (ECB Digital 1.2; (Odontobrás™, SP, Brazil). A spectrophotometer DU 530 (Beckman Coulter™, CA, USA) was used to determine absorbance. Calibration curves were constructed using Microsoft Excel (2010). Other apparatuses used were an ultrasonic bath (Unique™) and an analytical balance model H10-Class I (Mettler Toledo, Switzerland) to weigh the samples. Microbiological test was performed using FA of concentrations  $0.25$ ,  $0.75$ , and  $2.25 \mu\text{g mL}^{-1}$  and 6% of the microorganism *S. epidermidis* (ATCC 12228) in broth culture medium casoy.

## 2.3. Preparation of the standard solution of FA

The stock solution was prepared by weighing  $12.5 \text{ mg}$  of FA-RS accurately, transferring to a  $50\text{-mL}$  volumetric flask, and diluting with ethanol to obtain a concentration of  $250 \mu\text{g mL}^{-1}$ . This solution was further diluted with ethanol in a  $50\text{-mL}$  volumetric flask to obtain a concentration of  $25 \mu\text{g mL}^{-1}$ . Aliquots of  $250$ ,  $750$ , and  $2250 \mu\text{L}$  of this solution were transferred to  $25\text{-mL}$  volumetric flasks, and the volume was made up with ultrapure water to

obtain working solutions of  $0.25$ ,  $0.75$ , and  $2.25 \mu\text{g mL}^{-1}$ , which were designated  $S_1$ ,  $S_2$ , and  $S_3$ , respectively, in the bioassay.

## 2.4. Preparation of sample solution of dermatological cream

To prepare the sample stock solution,  $0.625 \text{ g}$  of cream was accurately weighed, added to ethanol, and subjected to ultrasound. After  $15 \text{ min}$ , this solution was transferred to a  $50\text{-mL}$  volumetric flask, and the volume was made up with ethanol to obtain a solution with a concentration of  $250 \mu\text{g mL}^{-1}$ . This solution was diluted with ethanol in  $50\text{-mL}$  volumetric flask to obtain a concentration of  $25 \mu\text{g mL}^{-1}$ . Aliquots of  $250$ ,  $750$ , and  $2250 \mu\text{L}$  were transferred to  $25\text{-mL}$  volumetric flasks, and the volumes were made up with ultrapure water to obtain working solutions with concentrations of  $0.25$ ,  $0.75$ , and  $2.25 \mu\text{g mL}^{-1}$ , which were designated  $T_1$ ,  $T_2$  and  $T_3$ , respectively, in the assay.

## 2.5. Preparation of placebo solutions

In accordance with Aulton [36] principle, a formulation with no active ingredient (placebo) was developed. The stock placebo solution was prepared in the same manner as the sample (Section 2.4), but without the active ingredient, FA ( $12.5 \text{ mg}$ ). Aliquots of  $2250 \mu\text{L}$  of this solution were transferred to  $25\text{-mL}$  volumetric flasks, and the volume was made up with ultrapure water to simulate the highest concentration used in the assay ( $2.25 \mu\text{g mL}^{-1}$ ).

## 2.6. Preparation of culture medium

Culture media were prepared in accordance with the manufacturers' guidelines: dissolved in water under heating, distributed in test tubes ( $10 \text{ mL}$ ), and autoclaved at  $121 \text{ }^\circ\text{C}$  for  $15 \text{ min}$ . After autoclaving, the tubes were cooled and immediately used in the bioassay.

## 2.7. Turbidimetric assay

The turbidimetric assay was performed in accordance with the official compendia [31,32]. For the preparation and standardization of inoculum, the *S. epidermidis* strain (ATCC 12228) was cultivated and maintained in tryptic soy agar medium in a freezer. The strain was inoculated with a platinum loop in casoy broth and maintained in an oven at  $35 \pm 2 \text{ }^\circ\text{C}$  for  $24 \text{ h}$  before the assay, for the growth of test microorganism.

Thereafter, the procedure of standardization of the inoculum was performed: *S. epidermidis* previously incubated in casoy broth were diluted with pure casoy broth to achieve a suspension turbidity of  $25 \pm 2\%$  (transmittance), using a spectrophotometer at  $580 \text{ nm}$  with a  $10\text{-mm}$  absorption cell, against casoy broth as a blank. The bioassay was performed using the  $3 \times 3$  parallel line assay design (three doses of the standard and three doses of the sample). Six-hundred milliliters of the standardized *S. epidermidis* (ATCC 12228) suspension was added to six test tubes containing  $10 \text{ mL}$  of casoy broth. In three of these tubes ( $S_1$ ,  $S_2$ , and  $S_3$ ),  $200 \mu\text{L}$  of the standard working solutions was added (at concentrations of  $0.25$ ,  $0.75$ , and  $2.25 \mu\text{g mL}^{-1}$ , respectively), and in the other three ( $T_1$ ,  $T_2$ , and  $T_3$ ), the same was performed with the working sample solutions. The process was performed in triplicate. Then, the test tubes were incubated in a shaker, in a water bath, at  $35 \pm 2 \text{ }^\circ\text{C}$  for  $4 \text{ h}$ . After the incubation period, the growth of microorganisms was interrupted by adding  $500 \mu\text{L}$  of 12% formaldehyde solution to each tube. Then, the spectrophotometer was reset with the test tube containing the negative control ( $10 \text{ mL}$  of casoy broth containing  $500 \mu\text{L}$  of the formaldehyde solution), and the absorbance was determined at  $530 \text{ nm}$  using a spectrophotometer.

## 2.8. Method development

Preliminary tests were conducted to standardize the conditions of the microbiological turbidimetric assay, in which parameters such as solvents (ethanol, purified water, buffer solutions pH 6.0 and pH 8.0), culture medium (casoy broth, BHI, Mueller-Hinton), microorganism (*Bacillus subtilis* ATCC 9372, *Kocuria rhizophila* ATCC 9341, *S. epidermidis* ATCC 12228, *S. aureus* ATCC 6538), concentrations of the inoculum (1%, 3%, 4%, 5%, 6%, 7%, and 8%) and drug (0.25, 0.30, 0.40, 0.50, 0.75, 0.90, 1.00, 1.20, 1.50, 2.00, 2.25, 2.70, 3.00, 3.60, 4.0, 8.00, 16.00, 32.00, and 64.00  $\mu\text{g mL}^{-1}$ ) were evaluated.

## 2.9. Method validation and mean percentage calculation

The method was validated in accordance with the International Conference on Harmonisation (ICH) guidelines [37]. The mean percentage of FA in the dermatologic cream was calculated using the Hewitt equation [38].

### 2.9.1. Linearity

Linearity was determined by the analytical curve, plotted using the logarithm of the concentration versus the mean of the absorbance values, obtained on three different days for each concentration of FA (0.25, 0.75, and 2.25  $\mu\text{g mL}^{-1}$ ). The results were analyzed by least squares, and linearity and parallelism were analyzed by analysis of variance (ANOVA).

### 2.9.2. Precision

Precision was assessed by repeatability and intermediate precision. Repeatability was verified by analyzing nine determinations covering the linear range of the method, i.e., three concentrations: low (0.25  $\mu\text{g mL}^{-1}$ ), medium (0.75  $\mu\text{g mL}^{-1}$ ), and high (2.25  $\mu\text{g mL}^{-1}$ ), obtained from the calibration curve. This procedure was performed in triplicate, over a short period, on the same day and under the same experimental conditions in the same laboratory and by the same analyst. Intermediate precision, in turn, was evaluated by determining the mean percentage of FA, on three different days (inter-day) by different analysts, but under the same experimental conditions and in the same laboratory in triplicate. Both results were evaluated based on the relative standard deviation (RSD) values between the determinations.

### 2.9.3. Accuracy

Accuracy was determined by the recovery assay, in which known quantities of FA-RS were added to a known quantity of the sample dermatological cream. The accuracy of the method was evaluated in triplicate at three concentrations ( $R_1=80$ ,  $R_2=100$ , and  $R_3=120\%$ ) [37], and the percentage recoveries were calculated as determined by the Association of Official Analytical Chemists [39] (Eq. (1))

$$R(\%) = \frac{C_f - C_u}{C_a} \times 100 \quad (1)$$

where  $C_f$  is the total drug concentration measured after addition of the standard,  $C_u$  is the total drug concentration in the formulation, and  $C_a$  is the standard concentration added to the formulation.

### 2.9.4. Selectivity

Selectivity turbidimetric microbiological method was performed to verify if the adjuvants present in the dermatological cream formulation had inhibitory effect that interfered with the analysis, even after neutralization of potassium sorbate preservative and of the ethanol used as a solvent. ANOVA was

performed to analyze the absorbances of the solution of the free analyte matrix–placebo (Section 2.5) and the absorbances of the positive control (10 mL of casoy broth containing 600  $\mu\text{L}$  inoculum –*S. epidermidis* 6%).

### 2.9.5. Robustness

To evaluate the robustness of the method, four parameters were varied independently: wavelength (528 nm, 530 nm, and 532 nm), volume of the culture medium in the test tubes (9.9 mL, 10.0 mL, and 10.1 mL), incubation duration of the inoculum (3 h 55 min, 4 h 00 min, and 4 h 05 min), and the brand of the culture medium, casoy broth (Oxoid and Acumedia). For this purpose, mean percentage of FA in the dermatological cream was determined under the different conditions proposed. The obtained responses were evaluated according to the RSD calculated among the dosages.

## 2.10. RP-LC method

RP-LC was previously developed and validated by our study group for the determination of FA in dermatological cream and was selected as the comparative method. The procedure was performed in the isocratic mode and the mobile phase consisted of acetonitrile and water (72:28, v/v), pH 3.5 adjusted with acetic acid. Chromatographic separation was conducted on an analytical column Agilent™ Zorbax Eclipse XDB C<sub>18</sub> column (250 × 4.6 mm, 5  $\mu\text{m}$ ), combined with a pre-column HPLC Metaguard 4.6 mm Polaris C<sub>18</sub> PTC 5U–Agilent™ at room temperature (25 °C), at a flow rate of 1.0 mL min<sup>-1</sup> and ultraviolet (UV) detection at 210 nm.

## 2.11. Comparison of methods

The results of the microbiological assay were statistically compared with those obtained with the RP-LC method, by using the analysis of variance (ANOVA) to evaluate the difference between the two methods at a significance level of 5%.

Chromatographic separations were conducted using a Waters™ HPLC system, equipped with a Waters 1525 binary gradient chromatography pump, Rheodyne Breeze 7725i manual injector, with a 20- $\mu\text{L}$  loop, Waters 2487 UV–vis detector, and Empower™ software, and an Agilent™ Zorbax Eclipse XDB C<sub>18</sub> column (250 × 4.6 mm, 5  $\mu\text{m}$ ) combined with a pre-column HPLC Metaguard 4.6 mm Polaris C<sub>18</sub> PTC 5U Agilent™ at room temperature (25 °C). The mobile phase consisted of acetonitrile and water (72:28, v/v) adjusted to pH 3.5 with acetic acid. The flow rate was 1.0 mL min<sup>-1</sup>, detection wavelength was 210 nm, injection volume was 20  $\mu\text{L}$ , and retention time of fusidic acid was approximately 8 min, as shown in Fig. 2.

## 3. Results and discussion

### 3.1. Method development

In a microbiological assay, the drug should demonstrate a significant response against the tested microorganism [13,31,32]. In this sense, different microorganisms were tested in combination at different microbiological conditions to find a suitable linear range for the quantification of FA in a dermatological cream.

In the preliminary tests, FA did not show antimicrobial activity against *B. subtilis* at high drug concentrations (64  $\mu\text{g mL}^{-1}$ ) and low inoculum concentrations (1%). However, *K. rhizophila* did not show significant growth in any of the three culture media.

Strains of *S. aureus* showed linear responses, with appropriate correlation between FA concentration and its inhibitory action on

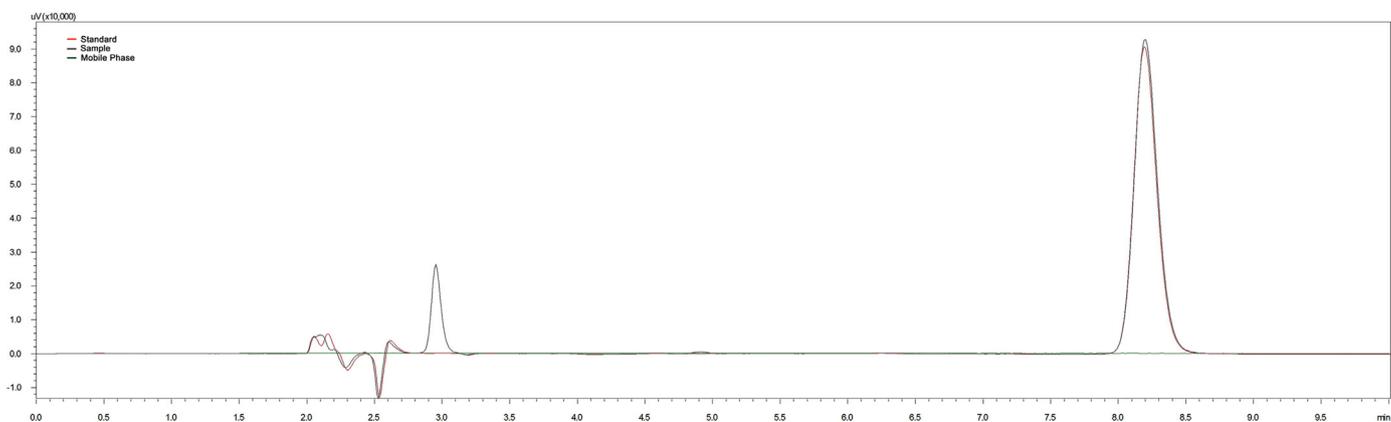


Fig. 2. Overlay of chromatograms of fusidic acid reference standard (standard), dermatological cream (sample), and mobile phase.

Table 1

Absorbance values determined by the microbiological turbidimetric assay for construction of the analytical curve of fusidic acid.

Absorbance (mean of three determinations)						
	FA-RS <sub>1</sub> (0.25 µg mL <sup>-1</sup> )	FA-RS <sub>2</sub> (0.75 µg mL <sup>-1</sup> )	FA-RS <sub>3</sub> (2.25 µg mL <sup>-1</sup> )	FP <sub>1</sub> (0.25 µg mL <sup>-1</sup> )	FP <sub>2</sub> (0.75 µg mL <sup>-1</sup> )	FP <sub>3</sub> (2.25 µg mL <sup>-1</sup> )
Analysis 1	0.427	0.331	0.234	0.424	0.325	0.231
Analysis 2	0.439	0.333	0.236	0.431	0.329	0.234
Analysis 3	0.415	0.328	0.240	0.411	0.322	0.242
Mean	0.427	0.331	0.236	0.422	0.325	0.235
RSD (%)	2.73	0.81	1.37	2.46	1.03	2.40

FA-RS: Fusidic acid reference standard; RSD: relative standard deviation; FP: finished product.

the microorganism. However, we opted for the microorganism *S. epidermidis*, since it showed high sensitivity to the drug, excellent reproducibility, and adequate linearity, besides being a microorganism of low pathogenicity. Among the various inoculum concentrations tested, the concentration of 6% showed the best results, with desirable growth of microorganism, and the concentrations of 0.25, 0.75, and 2.25 µg mL<sup>-1</sup> of FA showed good linearity and accuracy.

Buffer solutions were not suitable for the extraction of FA from dermatological cream; thus, ethanol was used for the preparation of stock solutions and ultrapure water was used to prepare working solutions. Regarding the use of ethanol, it is known that this solvent promotes the inhibition of bacterial growth; however, this inhibition was neutralized by dilution, similar to the potassium sorbate preservative present in the formulation of the finished product [31]. The BHI broth and casoy culture medium promoted satisfactory and similar growth for all microorganisms, and the casoy broth was selected because its cost was lower than that of other media.

### 3.2. Method validation

#### 3.2.1. Linearity

The drug showed linear response at concentrations between 0.25 and 2.25 µg mL<sup>-1</sup>. The representative equation and correlation coefficients ( $r$ ) for FA-RS and the sample of the dermatologic cream were  $y = -0.087\ln(x) + 0.3065$  ( $r = 1.0$ ) and  $y = -0.085\ln(x) + 0.3031$  ( $r = 0.9997$ ), respectively. The values of  $r$  were considered highly significant for the method. In the parallel-line model, two curves were constructed, one for FA-RS and the other for the sample of dermatological cream. The results were analyzed by analysis of variance (ANOVA), at significance level of 5%. The method showed linearity and did not deviate from parallelism, as shown in Tables 1 and 2.

Table 2

Statistical analysis (ANOVA) of the mean values of the absorbance determined by the microbiological turbidimetric assay to construct the analytical curve of fusidic acid.

Sources of variation	df	SS	QM	F <sub>calculated</sub>	F <sub>critical</sub>
Preparation	1	0.0001	0.0001	1.54	4.96
Regression	1	0.1066	0.1066	2408.93 <sup>*</sup>	4.96
Parallelism	1	0.0000	0.0000	0.26	4.96
Quadratic	1	0.0000	0.0000	0.44	4.96
Squared difference	1	0.0000	0.0000	0.18	4.96
Between doses	5	0.11	0.02	482.27 <sup>*</sup>	3.33
Between tubes	2	0.00	0.00	1.88	4.10
In (error)	10	0.00	0.00	–	–
Total	17	0.11	–	–	–

df: Degrees of freedom; QM: Quadratic means; SS: Sum of squares.

<sup>\*</sup>  $p < 0.05$ .

#### 3.2.2. Precision

In the test for precision by repeatability, the RSD values were 2.46%, 1.03%, and 2.40% for 0.25, 0.75, and 2.25 µg mL<sup>-1</sup> of FA in the dermatological cream, respectively. In the intermediate precision, the RSD value calculated from the mean percentage of FA, on different days and by different analysts was 1.52%. The method showed good precision, since all RSD values were lower than 5%, as recommended by the literature [37]. Thus, the lower values confirmed that the proposed method has the capacity to generate, for the same sample, reproducible results with low variation between independent assays.

#### 3.2.3. Accuracy

As shown in Table 3, the percentage recoveries confirm that the method can determine with accuracy, the content of FA in dermatological cream, since the percentage of the average recovery was close to 100%.

**Table 3**

Accuracy of the microbiological turbidimetric assay for the quantification of fusidic acid in dermatological cream.

	FA-RS added ( $\mu\text{g mL}^{-1}$ )	FA-RS found <sup>a</sup> ( $\mu\text{g mL}^{-1}$ )	Recovery (%)	Mean (%)	SD <sup>b</sup>
R <sub>1</sub>	0.60	0.63	102.54	101.95	0.90
R <sub>2</sub>	0.75	0.79	103.84		
R <sub>3</sub>	0.90	0.94	99.47		

<sup>a</sup> Mean of three determinations.

<sup>b</sup> Standard deviation.

**Table 4**

Robustness of the microbiological turbidimetric assay for the quantification of fusidic acid in dermatological cream.

Variable	Parameters	Mean percentage (%)	Mean	RSD <sup>a</sup> (%)
Wavelength (nm)	528	103.05	103.93	0.77
	530	104.13		
	532	104.61		
Volume of culture medium (mL)	9.9	99.73	103.50	3.47
	10.0	103.89		
	10.1	106.88		
Incubation time for <i>Staphylococcus epidermidis</i>	3 h 55 min	99.86	103.20	2.96
	4 h 00 min	103.84		
	4 h 05 min	105.88		
Brand of casoy broth	Oxoid	103.91	104.74	1.13
	Acumedia	105.58		

<sup>a</sup> Relative standard deviation.

### 3.2.4. Selectivity

The adjuvants in the formulation cream did not interfere with the quantification of FA, since the mean absorbance of the placebo solution (0.512) did not differ significantly (significance level of 5%) from the mean absorbance of the positive control (0.523), where the *p*-value was 0.06468, indicating the selectivity of the method.

### 3.2.5. Robustness

The results of the robustness of the method are presented in Table 4. The RSD values obtained were less than 5%, highlighting the robustness of the turbidimetric assay for the analysis of FA in the sample.

### 3.3. Comparison of the methods

The comparative analysis of methods is an artifice used to verify whether the procedures can be interchangeable. In this sense, the results should be sufficient to prove their equivalence at a certain range [32]. To compare the proposed microbiological method and physicochemical method of RP-LC, the mean percentage and average contents of FA in the dermatological cream, obtained by both methods were analyzed, respectively. The percentages of FA content determined by both methods were 104.00% (RP-LC) and 104.60% (microbiological method). Statistical analysis indicated that the values did not differ significantly, because the *p*-value (0.2995) was higher than 0.05% at significance level of 5%.

Thus, the results obtained in this study were satisfactory, and the amount of FA calculated by both methods was within the range of 90–110% specified in the British Pharmacopoeia [13]. Despite the statistical analysis indicating that the methods are equivalent and suitable for the quantification of FA in dermatological cream, it is necessary to highlight that there are differences between these methods.

The RP-LC method is widely used by pharmaceutical companies, since it is practical and can accurately determine degradation

products and impurities in the matrix. However, it requires costly equipment, analytical columns, and organic solvents as constituents of the mobile phase; therefore, maintenance of the system is costly and leads to environmental contamination. In addition, a physical–chemical method may not indicate the true biological activity of the antibacterial. It is known that the results obtained in microbiological tests and those obtained in physical–chemical methods may be different. Accordingly, for the analysis of antibiotics, such as AF, it is recommended that physical–chemical methods are performed in parallel with microbiological assays to ensure reliable results regarding their therapeutic efficacy.

Furthermore, the turbidimetric assay is a technique that does not use organic solvents and thereby does not generate chemical waste to the environment. However, this method requires a longer runtime than the RP-LC method, but the analysis time required for the microbiological turbidimetric assay is shorter than that for the agar diffusion assay currently used for the analysis of FA.

## 4. Conclusion

Taken together, the proposed method has met all the requirements set out in the international guideline, proving to be linear, precise, selective, accurate, and robust. Furthermore, statistical analysis (ANOVA) showed that the proposed turbidimetric assay and the RP-LC method developed and validated by our study group did not differ significantly. At the same time, the turbidimetric assay allows determination of the true biological activity of FA, when compared with physical–chemical methods, such as LC.

Further, this method involves simple and rapid procedures of sample preparation, without generating toxic waste to the environment. In addition, it is safe for the operator, since the microorganism used is not considered pathogenic. In this sense, the validated method can quantify FA in dermatological cream in a reliable and secure way for quality control; therefore, it is an alternative method to the diffusion agar assay.

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