

Stability-indicating RP-LC method for quantification of fusidic acid in cream

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Fusidic acid is an antibiotic steroid indicated for the treatment of infections caused by the genus *Staphylococcus*, including methicillin resistant *Staphylococcus aureus* strains, and other Gram-positive bacteria. In the present study, a stability-indicating reversed-phase liquid chromatography (RP-LC) method was developed and validated for the determination of fusidic acid in dermatological cream as an alternative to existing methods. Analyses were performed using a C₁₈ column and guard column at room temperature, eluting with an isocratic mobile phase of acetonitrile and water (72:28, v/v), adjusted to pH 3.5 with acetic acid, pumped at a flow rate of 1.0 mL min⁻¹, detection at 210 nm and 20 µL of injection volume. The forced degradation study was conducted under acidic, alkaline, neutral, photolytic, and oxidative stress conditions. The method was validated according to ICH and FDA guidelines; it was linear, precise, accurate, selective, and robust over concentrations of 5–95 µg mL⁻¹, with detection and quantification limits of 0.43 and 1.31 µg mL⁻¹, respectively. Therefore, we conclude that this method is suitable for quantifying fusidic acid in pharmaceutical dermatological creams and determining its stability, representing a more economical and practical alternative for routine analysis in quality control.

Uniterms: Fusidic acid/stability-indicating/quality control/validation. Chromatography/reversed-phase.

INTRODUCTION

Fusidic acid (FA), an antibiotic steroid (Godtfredsen, Vangedal, 1962) (Figure 1), has been used widely in the topical and systemic treatment of infections caused by Gram-positive microorganisms since 1962 (Jones *et al*, 2011; Spelman, 1999). These include strains resistant to penicillin and other antibiotics (França, 2010), providing an alternative for the treatment of diseases caused by methicillin resistant *Staphylococcus aureus* strains, abbreviated as MRSA (Anderson, 1980; Falagas, Kopterides, 2007). Topical FA is prescribed by dermatologists for the treatment of boils, carbuncles, erythema, cellulitis, folliculitis, acne, paronychia, hidradenitis, infections resulting from burns and impetigo (George, Rubin, 2003; França, 2010; Smeenk, Sebens, Houwing, 1999; Spelman, 1999; Wilkinson, 1998), atopic dermatitis, neurodermatitis, prurigo, and contact dermatitis (Long, 2008).

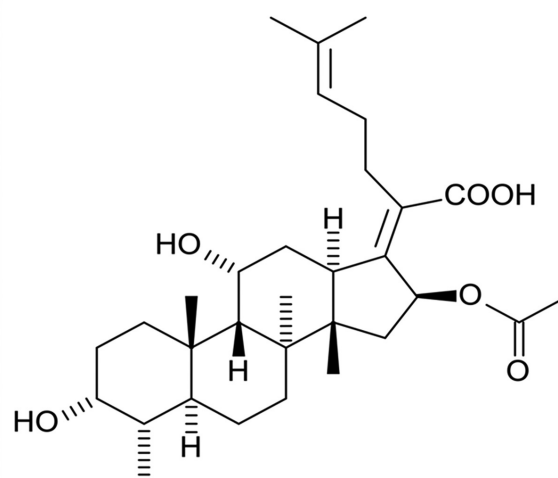


FIGURE 1 - Chemical structure of fusidic acid (CAS 6990-06-3).

Considering its importance in medical practice, the development of optimized analytical methods for the quality control of FA is essential for analyzing its quality, therapeutic efficacy, safety, and benefits for the pharmaceutical industry. Reversed phase liquid chromatography (RP-LC) is the technique of choice and

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occupies a prominent place among modern methods of analysis, due to its ease in effecting the separation, identification, and quantification of chemical substances and their degradation products (Snyder, Kirkland, Dolan, 2010).

In the literature there are some articles that describe this method for the quantification of FA in biological fluids (Hikal, 1983; Nawaz *et al.*, 2014; Rahman, Hoffman, 1988; Sewell, Palmer, 1991; Sorensen, 1988) and in pharmaceutical products (Ankam *et al.*, 2010; British Pharmacopeia, 2010; Byrne, Velasco-Torrijos, Reinhardt, 2014, 2015; Goswami, Gupta, Jogia, 2013; Hikal, Shibl, El-Hoofy, 1982; Hong, Rong, 2012; Peh, Tan, 2000; Nawaz *et al.*, 2014; Nianfen, Yandong, Mooling, 2009; Shaikh *et al.*, 2009). However, some of these methods are time consuming, have complex mobile phases, or require buffer solution, while others require long running times, high injection volumes and flow rates, or high FA concentrations in the sample.

Chromatographic methods prioritizing shorter analyses that are economically viable, reduce the quantities of waste to the environment, and are safer for the analyst, are of great importance and have gained prominence (Bonfilio *et al.*, 2009; Lopes, Salgado, 2009; Moreno, Salgado, 2008; Pedroso, Salgado, 2014; Rugani, Salgado, 2014; Silva, Salgado, 2012; Tótolí, Salgado, 2014; Vieira, Salgado, 2011).

Research improving the quality control of pharmaceutical products through analytical methods, besides benefiting the pharmaceutical industry, ensures higher product quality and increased consumer safety. In this sense, the aim of this study was to develop and validate a simple, rapid, sensitive, economic, and stability indicating method to quantify FA in pharmaceutical forms of dermatological cream, as a better alternative to existing literature methods.

MATERIALS AND METHODS

Chemical and reagents

HPLC grade acetonitrile, methanol, ethanol, and acetic acid (J. T. Baker, USA), along with deionized Milli-Q water (Millipore, Bedford, USA) were used to prepare the mobile phase. Sample VerutexTM, containing 20 mg g⁻¹ of the active ingredient, was supplied by LEO Pharma Laboratory (Itapevi-SP, Brazil). Fusidic acid reference standard (FA-RS), declared to be 100% pure, was purchased commercially from Sigma-Aldrich (Brazil). All adjuvants used in production of the formulation without the active ingredient (placebo) were of analytical grade:

hydrochloric acid (LabSynth), cetyl alcohol (Audaz), butylated hydroxyanisole (Sigma-Aldrich, Brazil), glycerol (Ely Martins), white petrolatum (Tec-Lab), liquid petrolatum (Columbia), polysorbate 60 (LabSynth), and potassium sorbate (Rica Nata).

Instrumentation and analytical conditions

Chromatographic separations were carried out using a WatersTM HPLC system, equipped with a Waters 1525 binary gradient chromatography pump, Rheodyne Breeze 7725i manual injector, with a 20 µL loop, Waters 2487 UV-Vis detector, and EmpowerTM software. The peak purity was determined using a ShimadzuTM HPLC system equipped with two LC-10AD pumps, SIL-10AF automatic injector, DAD detector SPD-M10A-VP, communication CBM-20^a module, and LC Solution software. A digital pH meter, model B474 (MiconalTM), ultrasonic bath (UniqueTM), analytical balance model DV215CD - Class I (OHAUSTM), vacuum filtration system (MilliporeTM, Bedford, USA), nylon membranes (0.45 µm pore size, SartoriusTM), membrane filters (0.45 µm pore size and 13 mm diameter, MinisartTM - RC/SRP/NY, Sartorius Stedim Biotech), and filter paper (QualyTM, J. Prolab) were also used.

Chromatographic analysis was performed using an AgilentTM Zorbax Eclipse XDB C₁₈ column (250 × 4.6 mm, 5 µm) combined with a pre-column HPLC Metaguard 4.6 mm Polaris C₁₈ PTC 5U AgilentTM at room temperature (25 °C). The mobile phase was composed of acetonitrile and water (72:28, v/v) adjusted to pH 3.5 with acetic acid. The flow rate was 1.0 mL min⁻¹, the detection wavelength was 210 nm, and the injection volume was 20 µL.

Preparation of the solutions

Preparation of standard solutions

The stock standard solution was prepared at a concentration of 100 µg mL⁻¹ and used to prepare working solutions by diluting to the appropriate concentration levels using the mobile phase as diluent. These solutions were filtered through 13 mm membranes with pore size of 0.45 µm after preparation.

Preparation of sample solutions

To an amount of the cream, equivalent to a concentration of 100 µg mL⁻¹ weighed into a beaker, was added acetonitrile and placed into ultrasound for 10 minutes. Afterwards, the resultant solution was transferred to a volumetric flask, diluted to the necessary volume with acetonitrile, and then filtered through filter paper. Working solutions were prepared by diluting aliquots of the stock

solution to the appropriate concentration, using the mobile phase as the diluent. These solutions were filtered through 13 mm membranes with pore size of 0.45 μm .

Preparation of placebo solutions

The stock placebo solution was prepared in the same manner as the sample, but without FA. Working solutions were prepared by diluting aliquots of the stock solution to the appropriate concentration, using the mobile phase as the diluent. These solutions were filtered through membrane filters after preparation.

Development and optimization of the chromatographic method

Various chromatographic parameters were evaluated during the development and optimization of the RP-LC method, as shown in Table I.

System suitability testing

System suitability test was performed by injecting seven replicates of the FA-RS solution at a concentration of 50 $\mu\text{g mL}^{-1}$. The number of plates, retention factor (k'), and asymmetry were evaluated according to FDA specifications (1994). In addition, the relative standard deviation (RSD) of the retention time and the chromatographic peak areas of FA were also analyzed.

Method validation

Method validation was performed following FDA (1994) and ICH (2005) specifications for linearity, precision, accuracy, selectivity, robustness, and detection and quantitation limits.

Linearity

The linearity was analyzed in triplicate, based on the results obtained from the analytical curve. The analytical curve was generated by plotting the peak area at each concentration level ($n=3$) versus drug concentrations of 5, 20, 35, 50, 65, 80, and 95 $\mu\text{g mL}^{-1}$. Statistical evaluation was performed by linear least squares regression mean (ANOVA) at a significance level of 5%, wherein the analysis of residues was also conducted.

Precision

Precision was assessed by repeatability (intra-assay) and intermediate precision (inter-assay). The precision by repeatability was verified by analysis of nine determinations covering the linear range of the method, i.e., three concentrations: low concentration (5 $\mu\text{g mL}^{-1}$), medium (50 $\mu\text{g mL}^{-1}$) and high (95 $\mu\text{g mL}^{-1}$) obtained from the calibration curve. This procedure was performed in triplicate on the same day, under the same experimental conditions, in the same laboratory, and by the same analyst. Intermediate precision was also checked by the analysis of nine determinations covering the linear range of the method. However, this procedure was performed by different analysts on different days. In both analyses, the results were evaluated based on the RSD.

Accuracy

Accuracy was determined by recovery tests, also called recovery factors (ICH, 2005), in which known quantities of FA-RS were added to known amounts of the sample. This parameter was analyzed in triplicate at three levels of recovery from the median of the concentration curve (50 $\mu\text{g mL}^{-1}$, $R_2 = 100\%$), wherein, R_1 consisted of 80% (40 $\mu\text{g mL}^{-1}$) and R_3 of 120% (60 $\mu\text{g mL}^{-1}$), as

TABLE I - Chromatographic parameters tested for development in the RP-LC method

Parameters	Description
Mobile phase	Ethanol and water (different ratios and pH) Methanol and water (different ratios and pH) Acetonitrile and water (different ratios and pH)
Column	Zorbax Eclipse XDB C ₁₈ – Agilent™ (4.6 x 250 mm, 5 μm)
Guard column	Metaguard Polaris 4.6 mm 5U C ₁₈ PTC – Agilent™
Wavelength	Determined by scanning by spectrophotometry absorption in the UV region
Flow rate	0.5 mL min ⁻¹ ; 1.0 mL min ⁻¹ ; 1.3 mL min ⁻¹
Injection volume	20 μL
Elution mode	Isocratic
Temperature	25 \pm 1 °C

established by the FDA (1994). The recovery percentage of the drug was calculated using Equation 1, determined by the *Association of Official Analytical Chemists* (2002).

$$R(\%) = \left[\frac{C_f - C_u}{C_a} \right] \times 100 \quad \text{Equation 1}$$

where C_f is the total drug concentration measured after standard addition; C_u is the total drug concentration in the formulation; C_a is the standard concentration added to the formulation.

Selectivity and studies of forced degradation

The selectivity of the method was evaluated by observing the chromatograms of FA-RS, free analyte in the matrix (placebo), array added analyte (sample) at the working concentration of $50 \mu\text{g mL}^{-1}$, and the mobile phase (acetonitrile and water, 72:28, v/v, pH 3.5). The purity of the chromatographic peak of FA was analyzed using a diode array detector (DAD).

In order to assess the selectivity of the method in relation to degradation products and prove that the method was stability indicating, a forced degradation study was performed using FA solution at a concentration of $50 \mu\text{g mL}^{-1}$. This study was conducted under acidic (0.01 mol L^{-1} HCl neutralized with 0.01 mol L^{-1} NaOH), alkaline (0.001 mol L^{-1} NaOH neutralized with 0.001 mol L^{-1} HCl), neutral (ultrapure water), oxidative ($3\% \text{ H}_2\text{O}_2$), and photolytic (UV 254 nm, 20 W) stress conditions. The first four reactions were carried out at 60°C , while photolytic stress, in which the solution was exposed to UV light, was conducted at room temperature. Degradation was stopped when approximately 5-20% of the FA had degraded (Alsante *et al.*, 2007). The degradation conditions promoted the appearance of other chromatographic peaks and their resolution was assessed.

Robustness

The robustness of the method was evaluated by Youden test through a dosage performed assay using a concentration of $50 \mu\text{g mL}^{-1}$. The Youden test evaluates seven variables, combined in eight experiments, in which the nominal conditions (normal) are represented by uppercase letters and the changed conditions, lower case. The variables must be consistent with practical reality and levels should reflect generally observed variation (Youden, Steiner, 1975). For this reason, changes were evaluated both with "upper" and "lower" chromatographic parameters (Table II). The results were obtained through

averaging the subtraction of the values of normal conditions from the values of changed conditions. For the method to be considered robust, the differences in the values should be smaller than twice the SD of all means in the test (Youden, Steiner, 1975).

TABLE II - Chromatographic parameters and their changes "lower" and "upper" used to assess the robustness of the RP-LC method

Variables	Changes "lower" and "upper"
Lot Column Agilent™ (250 x 4.6 mm, 5 μm)	A: B08125 a: B09180
pH	B: 3.5 b: 3.4 and 3.6
Flow rate (mL min ⁻¹)	C: 1.0 c: 0.9 and 1.1
Acetonitrile brand	D: J. T. Baker d: Tedia
Proportion of mobile phase (acetonitrile/water)	E: 72:28 e: 70:30 and 74:26
Wavelength (nm)	F: 210 f: 209 and 211
Temperature (°C)	G: 25 g: 23 and 27

Detection limit (LD) and quantitation limit (LQ)

The LD and LQ of the method were obtained from Equations 2 and 3, respectively:

$$LD = 3 (SD/a) \quad \text{Equation 2}$$

$$LQ = 10 (SD/a) \quad \text{Equation 3}$$

where SD is the standard deviation of the intersection and a is the mean slope, obtained from calibration curves of the linearity study.

RESULTS AND DISCUSSION

Development and optimization of the chromatographic method

In this study we used octadecylsilane (C_{18}) as the stationary phase because of its easy accessibility in laboratories and ability to separate solutes of different polarities, molecular weights, and chemical features. As the sample analyzed was composed of a complex matrix, a guard column was used to remove particles that would

have strong interactions with the stationary phase (Snyder, Kirkland, Dolan, 2010). This promoted an increased lifetime for the chromatographic column.

Regarding the analyzed mobile phases, that consisting of acetonitrile and water (72:28, v/v) adjusted to pH 3.5 with acid acetic acid exhibited better performance, better peak definition, higher efficiency, higher sensitivity, and shorter analysis time. In addition, acetonitrile is a lower viscosity solvent than ethanol and methanol, which allows the pressure in the analysis to be very low, considerably increasing the lifetime of the column and the chromatographic system. Determination of the mobile phase pH was a parameter that required caution because a very acidic pH could compromise the integrity of the chromatographic system (Snyder, Kirkland, Dolan, 2010). Furthermore, potential ionization of the analyte should be considered when choosing pH, so that no chromatographic peak has a low efficiency and/or peak tails. Accordingly, in mobile phases of $\text{pH} \geq 4.0$, we observed a considerable decrease in chromatographic efficiency.

For the flow rate, it was observed that to obtain a satisfactory retention factor, defined by the FDA (1994) as ≥ 2 , the flow rate should be below 1.3 mL min^{-1} . On the other hand, a flow rate of 0.5 mL min^{-1} promoted peak broadening, which consequently reduced chromatographic efficiency. The broadening of a chromatographic peak is a result of the processes of diffusion and mass transfer. The diffusion may either be turbulent (Eddy diffusion) or longitudinal (Snyder, Kirkland, Dolan, 2010).

Therefore, the method developed, besides being indicative of stability, had a retention time (Ankam *et al.*, 2010; Byrne, Velasco-Torrijos, Reinhardt, 2014; Goswami, Gupta, Jogia, 2013), flow rate (Ankam *et al.*, 2010; British Pharmacopeia, 2010; European Pharmacopeia, 2010), and injection volume (Ankam *et al.*, 2010) lower than other methods described in the literature for the quantification of FA in dermatological cream. This results in a reduced consumption of organic solvents, thus lowering both the cost of the method and the amount of toxic waste generated to the environment.

This method also has advantages in mobile phase composition when compared with other methods (Ankam *et al.*, 2010; Hong, Rong, 2012; Shaikh *et al.*, 2009), because, as well as being simple, the use of buffer was avoided. Using buffer solutions is disadvantageous for the pharmaceutical industry because they are expensive, require preparation prior to analysis because storage can cause fungus growth even when refrigerated, and are generally responsible for increased pressure in the chromatographic system by causing clogging that impairs the functionality of the equipment and damages the

columns, thus decreasing their useful lifetime (Snyder, Kirkland, Dolan, 2010; Watson, 2005).

System suitability testing

Tests that assess conformity of the system (system suitability) should be performed before the validation procedure or even before routine analysis. The compliance test is defined as a set of experiments designed to ensure that the equipment and the method that is being developed is able to generate results with satisfactory accuracy and precision (Paschoal *et al.*, 2008).

According to the FDA (1994), for the system to be considered reliable and reproducible, it is necessary to perform at least two of the following tests: repeatability, resolution, asymmetry, number of plates, and retention factor. Asymmetry is a very important parameter because it prevents broadening of the chromatographic peak, which can, in turn, produce errors in measuring analytes of interest (Snyder, Kirkland, Dolan, 2010).

The number of plates can be affected by several factors, including analysis conditions, amount of sample or solute, and, in particular, column length. The retention factor is intended to ensure that the proposed method is able to produce a suitable chromatographic separation. This parameter is closely related to the retention time of the analyte. A low retention factor, provided that it is within acceptable limits, promotes lower costs, a shorter analysis, and, consequently, decreased waste production (Snyder, Kirkland, Dolan, 2010).

System suitability testing showed that the chromatographic parameters in the development of this method were able to provide accurate and reliable results, since the average value calculated for asymmetry was less than 2.0, and the average plate number and retention factor were greater than 2000 and 2.0, respectively, as specified by the FDA (1994). Furthermore, adequate precision was observed for the values of retention time (about 8 minutes) and chromatographic peak areas of FA, which had an RSD below 2%, which is recommended for conformance testing (FDA, 1994).

Method validation

Linearity

The linearity parameter was confirmed in the concentration range $5\text{--}95 \mu\text{g mL}^{-1}$, wherein the calibration equation and correlation coefficient (r) were $y = 19080x + 7004.5$ and 0.9999, respectively. In the statistical analysis, results showed that the method had satisfactory linear regression because the p -value was 4.97604×10^{-11} .

In addition, in the analysis of the waste (Figure 2), the points randomly distributed, which is also indicative of good regression.

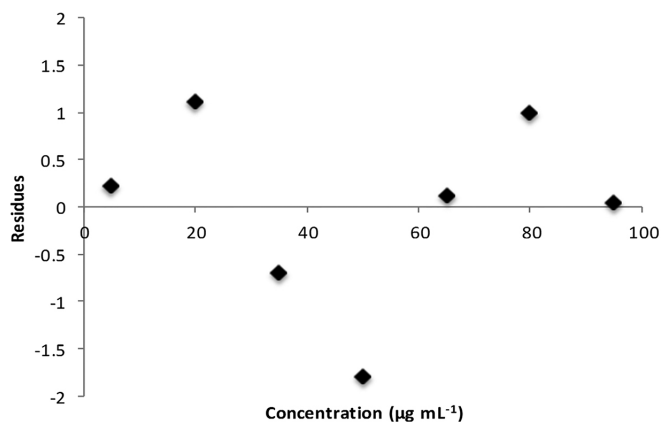


FIGURE 2 - Residue analysis for fusidic acid obtained by the RP-LC method.

Precision

In analyzing the method by repeatability and intermediate precision (Tables III and IV, respectively), the RSDs of areas obtained in all concentrations analyzed were no more than 2%, indicating good precision of the chromatographic method.

Accuracy

The recovery test showed that the method had satisfactory accuracy to measure FA in dermatological cream, since the percentage value of the average recovery was close to 100%, as shown in Table V.

Selectivity and studies of forced degradation

Analysis of the selectivity parameter (Figure 3) demonstrated that the adjuvants and mobile phase do not cause any interference in the peak of FA. The purity of the chromatographic peak of FA was calculated as 100% using a DAD.

TABLE III - Precision by repeatability for fusidic acid by RP-LC method

Concentration (µg mL ⁻¹)	Area	Mean area ± SD*	RSD† (%)
5	108930	108223.67 ± 1072.95	0.99
	108752		
	106989		
50	977933	977529.67 ± 8086.55	0.83
	985407		
	969249		
95	1861386	1880208.33 ± 16818.26	0.89
	1885479		
	1893760		

*SD = standard deviation; †RSD = relative standard deviation.

TABLE IV - Intermediate precision for fusidic acid by RP-LC method

Concentration (µg mL ⁻¹)	ANALYST ¹			ANALYST ²		
	Area	Mean area ± SD*	RSD† (%)	Area	Mean area ± SD*	RSD† (%)
5	108930	108223.67 ± 1072.95	0.99	108812	107025.33 ± 1554.46	1.45
	108752					
	106989					
50	977933	977529.67 ± 8086.55	0.83	975625	983593.33 ± 7499.59	0.76
	985407					
	969249					
95	1861386	1880208.33 ± 16818.26	0.89	1889630	1872195.00 ± 17738.94	0.95
	1885479					
	1893760					

*SD = standard deviation; †RSD = relative standard deviation.

TABLE V - Accuracy for fusidic acid by RP-LC method

	FA-RS added ($\mu\text{g mL}^{-1}$)	FA-RS found* ($\mu\text{g mL}^{-1}$)	Recovery (%)	Mean recovery (%)	SD†
R ₁	40	40.68	100.63	101.22	0.52
R ₂	50	50.88	101.38		
R ₃	60	61.16	101.64		

*Mean of three determinations; †SD = standard deviation.

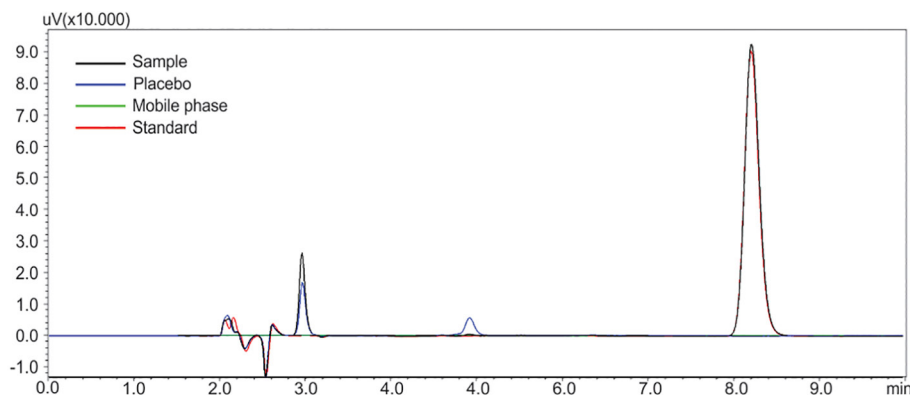
**FIGURE 3** - Chromatogram showing the selectivity of the RP-LC method in the quantification of fusidic acid.

Table VI shows the results obtained under degradation conditions, namely acidic, alkaline, neutral, photolytic, and oxidative stress. Additional degradation peaks were observed under neutral, acidic, and photolytic conditions (Figure 4), in which the chromatographic resolutions were satisfactory (2.49, 11.36, and 12.70, respectively). Resolution is a measure of the separation between two adjacent peaks and must produce values of ≥ 2.0 , to be judged as adequate (FDA, 1994). Under all the stress conditions there was a decrease in the peak area of FA. The drug was most stable under photolytic condition, while maximum degradation was observed under alkaline condition. All of these results combined to demonstrate that the method, as well as being selective, is indicative of stability, because it was able to differentiate FA from the other degradation products observed.

Robustness

In the analysis of robustness using the Youden Test, no significant effect was observed in the method when subjected to the proposed changes, because all “lower” changes had effects lower than 1.94 and all “upper” changes had effects lower than 1.10 (Table VII). These values corresponded with the values of twice the SD of all the means of the respective assays (0.97 and 0.55, respectively).

This test not only assessed whether the method is

TABLE VI - Forced degradation study of fusidic acid

Stress condition	Time	Degradation (%)
Acid hydrolysis (0.01 mol L ⁻¹ HCl)	0 h	3.23
	1 h	4.32
	5 h	7.78
	9 h	14.48
	24 h	30.10
Alkaline hydrolysis (0.001 mol L ⁻¹ NaOH)	0 h	17.97
	1 h	35.20
Neutral (ultrapure water)	0 h	0.50
	1 h	0.75
	5 h	1.00
	9 h	3.78
	24 h	7.38
	48 h	14.26
	72 h	27.70
Photolytic (UV 254 nm)	0 h	0.35
	1 h	0.63
	5 h	1.08
	9 h	4.36
	24 h	3.92
	48 h	6.25
	6 days	6.33
10 days	13.93	
Oxidation (3% H₂O₂)	0 h	5.01
	1 h	14.33
	5 h	95.84

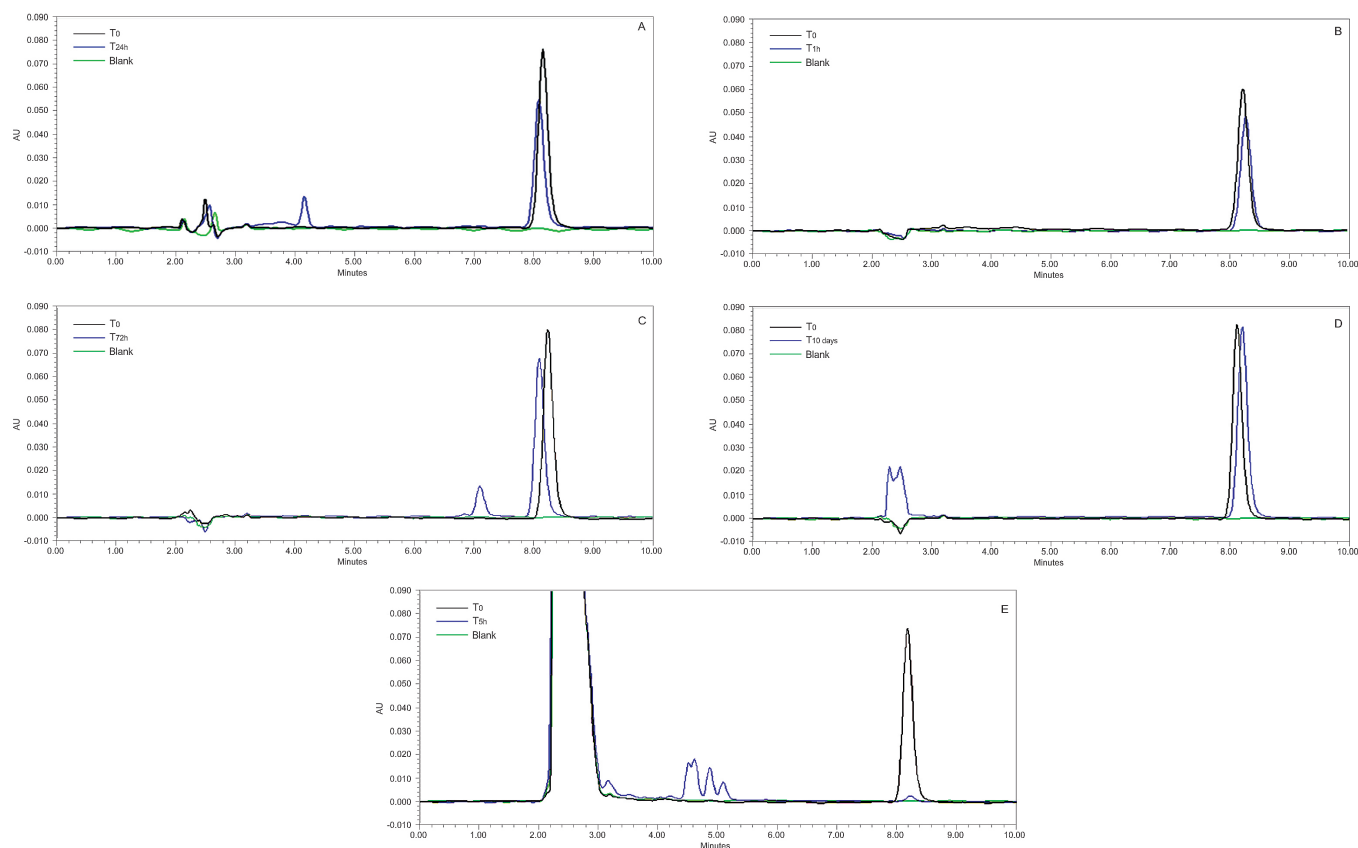


FIGURE 4 - Chromatograms obtained in the forced degradation study of fusidic acid. Degradation conditions: **A**: Acid hydrolysis ($0.01 \text{ mol L}^{-1} \text{ HCl}$), **B**: Alkaline hydrolysis ($0.001 \text{ mol L}^{-1} \text{ NaOH}$), **C**: Neutral (ultrapure water), **D**: Photolytic (UV 254 nm), and **E**: Oxidative stress ($3\% \text{ H}_2\text{O}_2$).

TABLE VII - Effects observed in the robustness evaluation of the RP-LC method

Variable	Effects	
	Changes “lower”	Changes “upper”
Lot Column Agilent™ (250 x 4.6 mm, 5 μm)	0.37	-0.20
pH	-0.51	0.68
Flow rate (mL min^{-1})	0.32	0.29
Acetonitrile brand	0.58	0.33
Proportion of mobile phase (acetonitrile and water)	-1.35	-0.45
Wavelength (nm)	0.22	-0.39
Temperature ($^{\circ}\text{C}$)	-0.77	-0.05

robust, but also indicates the influence of each parameter on the analytical results. The premise of the test is to introduce many changes at once in order to determine the effect of individual changes (Youden, Steiner, 1975). In

this instance, the effects obtained from variation of the different parameters were not significant and therefore the method was considered robust.

Detection limit (LD) and quantitation limit (LQ)

The values calculated for the LD and LQ were $0.43 \mu\text{g mL}^{-1}$ and $1.31 \mu\text{g mL}^{-1}$, respectively. These values indicated the ability of the method to reliably detect and quantify FA dermatological cream formulations established in the linear range.

CONCLUSION

The proposed RP-LC method for the quantification of FA in the form of pharmaceutical dermatological cream is fast, simple, efficient, economical, and sensitive compared to other methods described in the literature, and is stability indicating. All parameters were in accordance with the acceptance criteria for the validation of methods established by the ICH and FDA. Therefore, the validated method could easily be applied to the routine analysis of FA in the pharmaceutical industry.

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REFERENCES

- ALSANTE, K. M.; ANDO, A.; BROWN, L.; ENSING, J.; HATAJIK, T. D.; KONG, W.; TSUDA, Y. The role of degradant profiling in active pharmaceutical ingredients and drug products. *Adv. Drug Deliv. Rev.*, v. 59, n. 1, p. 29–37, 2007.
- ANDERSON, J. D. Fusidic acid: new opportunities with an old antibiotic. *Can. Med. Assoc. J.*, v. 122, n. 7, p. 765–769, 1980.
- ANKAM, R.; MUKKANTI, K.; DURGAPRASAD, S.; NAIDU, P.V.L. Simultaneous determination of halobetasol propionate and fusidic acid related substances by reversed phase high performance liquid chromatographic method. *Asian J. Chem.*, v.22, n.5, p.3369-3375, 2010.
- ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. AOAC. Official methods of analysis: appendix D: guidelines for collaborative study procedures to validate characteristics of a method of analysis. 18. ed. Gaithersburg: AOAC, Gaithersburg, MD, 2002.
- BONFILIO, R.; TARLEY, C.R.T.; PEREIRA, G.R.; SALGADO, H.R.N.; ARAÚJO, M.B. Multivariate optimization and validation of an analytical methodology by RP-HPLC for the determination of losartan potassium in capsules. *Talanta*, v.80, n.1, p.236-241, 2009.
- BRITISH PHARMACOPEIA. London: The Stationary Office, 2010. v.3, p.2724-2727
- BYRNE, J.; VELASCO-TORRIJOS, T.; REINHARDT, R. An RP-HPLC method for the stability-indicating analysis of impurities of both fusidic acid and betamethasone-17-valerate in a semi-solid pharmaceutical dosage form. *J. Chromatogr. Sci.*, v.53, n.9, p.1498-1503, 2015.
- BYRNE, J.; VELASCO-TORRIJOS, T.; REINHARDT, R. Development and validation of a novel stability-indicating HPLC method for the simultaneous assay of betamethasone-17-valerate, fusidic acid, potassium sorbate, methylparaben and propylparaben in a topical cream preparation. *J. Pharm. Biomed. Anal.*, v.96, p.111-117, 2014.
- EUROPEAN PHARMACOPEIA. 7. ed. Strasbourg: Council of Europe, 2010. v.2, p.2079-2080.
- FALAGAS, M.E.; KOPTERIDES, P. Old antibiotics for infections in critically ill patients. *Curr. Opin. Crit. Care*, v.13, n.5, p.592-597, 2007.
- FOOD AND DRUG ADMINISTRATION. FDA. *Validation of chromatographic methods*. Washington: Center for Drug Evaluation and Research, 1994.
- FRANÇA, F.F.A.C. *Andrejus Korolkovas Dicionário terapêutico Guanabara*. Edição 2009/2010, Rio de Janeiro: Guanabara Koogan, 2010.
- GEORGE, A.; RUBIN, G. A systematic review and meta-analysis of treatments for impetigo. *Br. J. Gen. Pract.*, v.53, n.491, p.480-487, 2003.
- GODTFREDSSEN, W.O.; VANGEDAL, S. The structure of fusidic acid. *Tetrahedron*, v.18, n.9, p.1029-1048, 1962.
- GOSWAMI, N.; GUPTA, V.R.M.; JOGIA, A.H. Development and validation of a novel stability-indicating RP-HPLC method for the simultaneous determination of halometasone, fusidic acid, methylparaben, and propylparaben in topical pharmaceutical formulation. *Sci. Pharm.*, v.81, n.2, p.505-518, 2013.
- HIKAL, A.H. Assay of fusidic acid in plasma by high-performance liquid chromatography. *Int. J. Pharm.*, v.13, n.3, p. 297-301, 1983.
- HIKAL, A.H.; SHIBL, A.; EL-HOOFY, S. Determinations of sodium fusidate and fusidic acid in dosage forms by high-performance liquid chromatography and a microbiological method. *J. Pharm. Sci.*, v.71, n.11, p.1297-1298, 1982.
- HONG, C.; RONG, Z. Determination of dexamethasone acetate and fusidic acid in compound dexamethasone and fusidic acid cream. *Chin. J. Antibiotics*, v.12, p.924-926, 2012.

- INTERNATIONAL CONFERENCE ON HARMONIZATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE. ICH. *Validation of analytical procedure: Text and methodology Q2(R1)*. Switzerland: ICH Steering Committee, 2005. Available at: <http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf>. Accessed on: 09 November 2015.
- JONES, R.N.; MENDES, R.E.; SADER, H.S.; CASTANHEIRA, M. In vitro antimicrobial findings for fusidic acid tested against contemporary (2008-2009) Gram-positive organisms collected in the United States. *Clin. Infect. Dis.*, v.52, supl.7, p.S477-S486, 2011.
- LONG, B.H. Fusidic acid in skin and soft-tissue infections. *Acta Derm. Venereol.*, supl.216, p.14-20, 2008.
- LOPES, C.C.G.O.; SALGADO, H.R.N. Development of a validated stability-indicating LC assay and stress degradation studies of linezolid in tablets. *Chromatogr.*, v.69, supl., p.S129-S135, 2009.
- MORENO, A.H.; SALGADO, H.R.N. Microbiological assay for ceftazidime injection. *J. AOAC Int.*, v.90, n.5, p.1379-1382, 2007.
- NAWAZ, M.; ARAYNE, M.S.; SULTANA, N.; HAIDER, A.; HISAINDEE, S. Simultaneous determination of fusidic acid and steroids from bulk drugs and human plasma by reversed phase HPLC. *Acta Chromatogr.*, v.26, n.1, p.57-66, 2014.
- NIANFEN, C.; YANDONG, W.; MOOLING, C. Determination of fusidic acid ophthalmic emulsion by HPLC. *China Med. Herald*, v. 22, p. 72-73, 2009.
- PASCHOAL, J.A.R.; RATH, S.; AIROLDI, F.P.S.A.; REYES, F.G.R. Validação de métodos cromatográficos para a determinação de resíduos de medicamentos veterinários em alimentos. *Química Nova*, v.31, n.5, p.1190-1198, 2008.
- PEDROSO, T.M.; SALGADO, H.R.N. Validation of analytical methodology for quantification of cefazolin sodium pharmaceutical dosage form by high performance liquid chromatography to be applied for quality control in pharmaceutical industry. *Braz. J. Pharm. Sci.*, v.50, n.1, p.213-223, 2014.
- PEH, K.-K.; TAN, Y.T. Simple high-performance liquid chromatographic method for simultaneous determination of fusidic acid and betamethasone dipropionate in a cream formulation. *Int. J. Pharm. Compd.*, v.4, n.3, p.229-231, 2000.
- RAHMAN, A.; HOFFMAN, N.E. High-performance liquid chromatographic determination of fusidic acid in plasma. *J. Chromatogr.*, v.433, p.159-166, 1988.
- RUGANI, K.S.; SALGADO, H.R.N. Stability-indicating LC method for the determination of cephalothin in lyophilized powder for injection. *Anal. Meth.*, v.6, p.4437-4445, 2014.
- SEWELL, G.J.; PALMER, A.J. The chemical and physical stability of three intravenous infusions subjected to frozen storage and microwave thawing. *Int. J. Pharm.*, v. 2, n.1, p.57-63, 1991.
- SHAIKH, S.; MUNEERA, M.S.; THUSLEEM, O.A.; TAHIR, M.; KONDAGULI, A.V. A simple RP-HPLC method for the simultaneous quantitation of chlorocresol, mometasonefuroate, and fusidic acid in creams. *J. Chromatogr. Sci.*, v.47, n.2, p.178-183, 2009.
- SILVA, L.M.; SALGADO, H.R.N. Validation of a stability-indicating RP-LC method for the determination of tigecycline in lyophilized powder. *J. Chromatogr. Sci.*, v.51, n.2, p.192-199, 2012.
- SMEENK, G.; SEBENS, F.W.; HOUWING, R.H. Use and adverse reactions of local antibiotics and disinfectants on the skin. *Ned. Tijdschr. Geneesk.*, v.143, n.22, p.1140-1143, 1999.
- SNYDER, L.R.; KIRKLAND, J.J.; DOLAN, J.W. *Introduction to modern liquid chromatography*. 3. ed. New York: John Wiley & Sons, 2010.no. de páginas ??
- SORENSE, N.H. Liquid chromatographic determination of fusidic acid in serum. *J. Chromatograph.*, v.430, n.2, p.400-405, 1988.
- SPELMAN, D. Fusidic acid in skin and soft tissue infections. *Int. J. Antimicrob. Agents*, v.12, supl., p.S59-S66, 1999.
- TÓTOLI, E.G.; SALGADO, H.R.N. Development and validation of an economic, environmental friendly and stability-indicating analytical method for determination of ampicillin sodium for injection by RP-HPLC. *World J. Pharm. Sci.*, v.3, n.6, p.1928-1943, 2014.

- VIEIRA, D.C.M.; SALGADO, H.R.N. Comparison of HPLC and UV spectrophotometric methods for the determination of cefuroxime sodium in pharmaceutical products. *J. Chromatogr. Sci.*, v.49, n.7, p.508-511, 2011.
- WATSON, D.G. *Pharmaceutical analysis: a textbook for pharmacy students and pharmaceutical chemists*. London: Elsevier, 2005. No. de páginas
- WILKINSON, J.D. Fusidic acid in dermatology. *Br. J. Dermatol.*, v.139, supl.53, p.37-40, 1998.
- YOU DEN, W.J.; STEINER, E.H. *Statistical Manual of AOAC: Association of Official Analytical Chemistry*. Washington: AOAC, 1975. 88p.

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