FIRST DERIVATIVE UV-SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF FENTICONAZOLE NITRATE CREAM MATRIX

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ABSTRACT

The fenticonazole nitrate is an imidazole derivative and has a broad spectrum antimycotic activity. It has been commonly used for treatment of dermatologic and gynecologic fungal infections, mainly for those caused by Candida albicans. Although the importance of the drug, there are limited analytical methods developed for assuring its quality in complex pharmaceutical forms. Therefore, the objective of this work was to develop and validate such a matrix interference free analytical method for the fenticonazole nitrate assay in vaginal cream formulation. This paper presents a new, simple, inexpensive, efficient and matrix interference free by using derivative tools of UV spectrophotometric technique for determination of fenticonazole nitrate in vaginal creams. The method was validated as established in the International Conference on Harmonization (ICH) requirements and has shown selectivity, linearity, accuracy, and precision, besides low detection and quantitation limits. This procedure does not require complex or expansive equipment neither complicated separation method and uses simple reagents. It is an important tool for routine quality control of fenticonazole nitrate and may be useful for pharmaceutical industry quality control analysis of drug in complex matrixes as vaginal creams.

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INTRODUCTION

Fenticonazole is an effective and well-tolerated imidazole antifungal agent. It has a broad spectrum antifungal activity and is used for topical treatment of fungal infections of the skin and vulvovaginal tissues. It has long been known from *in vitro* and clinical studies that fenticonazole, as other imidazole derivatives, has high activity against a wide range of fungal pathogens, as well as gram-positive bacteria.[1]

*In vitro* studies suggest that fenticonazole may have a role in the treatment of mixed mycotic and bacterial infections, such as fungal skin infections superinfected with *Staphylococcus aureus*, coryneforms and streptococci. Fenticonazole has also demonstrated *in vitro* activity against bacteria that are commonly associated with vaginosis, such as *Gardnerella vaginalis*, *Mobiluncus* spp. and anaerobic, Gram-positive cocci.[2]

During recent years, the application of imidazole derivatives is increasing due to their efficacy in the treatment of mycoses, especially in patients with a decreased immunity (e.g. after organ transplantations, AIDS, etc.).[3] There are available some pharmaceutical formulations containing fenticonazole nitrate, such as ovules and creams for vaginal use. Some of those pharmaceutical formulations can pose an analytical challenge if a complex matrix such as creams.

Pharmaceutical quality control analyses has a goal of evaluate the quality attributes of pharmaceutical products, as an effort to guarantee the efficacy, safety and stability required for these products. The use of appropriate analytical methodologies is crucial. Quality control in the pharmaceutical industries requires reliable, simple, easy and fast analytical methods.[4] As UV-spectrophotometry meets these requirements, it is a widely used instrumental technique in industrial quality control laboratories.[5]

An analytical method free of matrix interference is a requirement for pharmaceutical analysis. For spectrophotometric methods, the evaluation of pharmaceutical matrix excipients impact on analytical results assists in the development of an interferer free analytical method.[4,6-9]

Derivative mathematical treatment applied to the spectrophotometric profile is a widely used technique for evaluating and distinguishing different analytical responses from a mixture of...
substances, which is a very useful tool for reducing the impact of the pharmaceutical matrix on analytical results.[6-8]

The European and British pharmacopoeias[10,11] officially recognize this antibiotic. Both recommend the potentiometric titrimetric to assay fenticonazole as raw material. Like other pharmacopoeias, the Brazilian pharmacopoeia does not include fenticonazole nitrate.[12] At the literature, on scientific database, paper addressing analytical methods for dosage assay of fenticonazole nitrate in cream matrix was not found. Therefore, the objective of this work was to develop and validate such a matrix interference-free analytical method for the fenticonazole nitrate assay in vaginal cream formulation. Also, contribute to the insertion of the monograph of fenticozole nitrate in the Brazilian pharmacopoeia.[12]

MATERIAL AND METHODS

Equipment
The equipment used were the following: Shimadzu® UV-1800 spectrophotometer (Kyoto, Japan); Mettler® H10 analytical balance (Zurich, Switzerland); Vetec® quantitative filter paper (Rio de Janeiro, Brazil) and Unique® ultrasonic bath model USC2800A (Indaiatuba, Brazil).

Reagents and chemicals
The fenticonazole nitrate chemical reference substance (CRS) (assigned purity 99.8%), the vaginal cream (pharmaceutical product) and placebo were kindly supplied by EMS® (Hortolândia, Brazil).

The placebo contains all the components of the pharmaceutical formulation in question except fenticonazole nitrate, namely: nonionic self-emulsifying wax, decyl oleate, propylene glycol, liquid petrolatum, disodium edetate dihydrate, simethicone, purified water. It was treated in the same manner as the commercial samples.

Synth® methanol and ethanol were used as solvents.

Spectrophotometric measurements
The UV spectra of reference and sample solutions were recorded using 1 cm quartz cells at a fast scan speed. The derivative spectra were determined at UV Probe program. Previously filtered methanol and ethanol using quantitative filter were used as a blank solution.
**Preparation of solutions**

*Stock and working standard solutions*

A stock standard solution containing fenticonazole nitrate was prepared by accurately weighing 5.0 mg of the fenticonazole nitrate CRS into a 100mL volumetric flask, diluted with methanol and submitted to ultrasonic bath for one hour. To get the working solution 7.5 mL of stock solution were transferred to a 25 mL volumetric flask and the volume was completed with methanol so the final solution had a 15 µg mL\(^{-1}\) concentration. It was filtered using quantitative filter before measurement.

*Sample solutions*

Ten fenticonazole nitrate tubes of the pharmaceutical product, containing 20 mg of fenticonazole per gram of cream, were used to make up a pool. The content of each tube were weighed and thoroughly mixed. The mass equivalent of 0.025 g was weighed into a 100 mL volumetric flask, diluted with methanol and submitted to ultrasonic bath for one hour. 7.5 mL of this solution was transferred to a 25 mL volumetric flask and the volume was completed with methanol so the final solution had a 15 µg mL\(^{-1}\) concentration. It was filtered using quantitative filter before measurement.

**Method validation**

Method validation was performed in accordance with International Conference of Harmonization (ICH) specifications\(^{[13]}\), which includes linearity, specificity, accuracy, precision, robustness, detection and quantitation limits as validation parameters.

*Specificity*

Specificity was evaluated by an analysis of the UV spectra of placebo solutions, fenticonazole nitrate standard and sample solutions at working concentration of 15 µg mL\(^{-1}\). Ethanol and methanol were tested as solvents. The aim of specificity test is to get an analytical method free of interferences of any excipient from its complex matrix and an extraction method able to extract completely the drug from the pharmaceutical product matrix.

*Linearity*

Linearity was evaluated by regression analysis of the fenticonazole nitrate standard solution measured in triplicate at seven different concentrations, ranging from 6.0 to 24 µg mL\(^{-1}\). The values are reported as the mean +/- relative standard deviation (S.D.) of the calibration
curves. The data were analyzed at a wavelength of 260 nm using the first derivative absorption UV spectrum. Evaluation parameters such as the correlation coefficient were calculated and presented. Moreover, the data were validated by an analysis of variance (ANOVA).

**Accuracy**

To perform the accuracy test the placebo fortified technique was applied. For that the placebo of the pharmaceutical product (vaginal cream) was fortified with known quantities of fenticonazole nitrate CRS. After that, seven solutions were prepared at the same concentration range used in linearity test, in triplicate. The percentage recovered of fenticonazole cream at each one concentration was calculated using its UV absorption and the equation curve obtained at linearity test.

**Precision**

The precision of the method was evaluated by repeatability and intermediate precision. Repeatability was carried out by the evaluation of seven different concentrations of the fenticonazole nitrate standard solution at the same day and under the same experimental conditions, following the concentration range used in specificity test. Intermediate precision was assessed by performing the analysis of three different concentrations (6, 15 and 24 µg mL⁻¹) by two analysts in different days at the same laboratory and under the same experimental conditions. The percentage of the relative standard deviation (R.S.D) of the analytical responses were calculated.

**Robustness**

Robustness testing is useful in order to prove that some variations at the procedure are negligible in the method outcome. It is usually studied by deliberately changing critical parameters and monitor possible alterations. A method is considered robust when these alterations produce no significant changes in its results.¹⁴

The robustness of the method was determined by reducing the time of sonication bath from 60 to 50 minutes and changing two nm in the analysis’s wavelength. The impact of these small and deliberate changes in method on drug dosage was evaluated.
**Limits of detection and quantitation**

The limit of detection (LOD) and the limit of quantitation (LOQ) of the method were inferred using the equations suggested by the ICH validation guideline. It considers the value of standard deviation of the curve intersection in y axis, and the average slope of the curve obtained from the three analytical curves of the linearity study.\(^{[13]}\)

**Assay of vaginal cream**

The validated derivative UV-spectrophotometric method was applied to quantify the fenticonazole nitrate in vaginal cream. The results were obtained by comparing the sample spectrophotometric measurements \((n = 3)\) with those obtained from the fenticonazole nitrate standard solutions \((n = 3)\) at the same concentration levels, the working concentration.

**RESULTS AND DISCUSSION**

**Method development**

The determination of drugs in complex matrix formulations such as the vaginal creams is a challenge for quality control laboratories. Usually, the placebo interferes at the sample measures, which requires a previous separation. This paper presents a new, simple, inexpensive, efficient and matrix interference-free UV derivative spectrophotometric method for determination of fenticonazole nitrate in vaginal creams.

To reach that, lot of tests were performed. Different solvents, as methanol and ethanol, were tested to find a completely extraction of the drug from the pharmaceutical product matrix. Besides the derivation of the UV absorption spectrum of fenticonazole nitrate were calculated and used to get no interferences of matrix excipients at the drug measurements.

There is usually an increased noise level with increasing derivation order therefore the order of derivative has to be carefully selected.\(^{[6,7]}\) In this research, good results were found using the first order derivation.

Methanolic extraction showed such better results than ethanolic extraction, since ethanol could not completely extract the drug from the cream matrix. Moreover, applying the wavelength of 260 nm and the first order derivative spectrum the matrix interference achieved was null. Therefore, these conditions were chosen as analytical parameters for fenticonazole nitrate analysis.
Method validation

After the method development, the analytical method was validated according to ICH recommendations.[13]

Linearity and range

The analytical curves obtained by plotting the mean absorbance at 260 nm using the first derivative UV absorption spectrum against the concentration were found to be linear at the 6 to 24 µg mL\(^{-1}\) range and yielded a correlation coefficient (r) of 0.9992. The data were also validated by an analysis of variance, which showed significant linear regression \(F_{\text{calculated}} (5613.57) > F_{\text{critical}} (5.94223E-25)\), \(p\) value = 0.05 and no significant deviation of linearity.

Selectivity

All selectivity tests results showed that the developed method has good selectivity to analyze and quantify the fenticonazole nitrate in vaginal cream matrix, being a matrix interference-free method. Development tests achieved are shown in Table 1 and Figures 1 to 3.

Table 1: Comparison of extraction and content of fenticonazole nitrate standard, sample and placebo using different extraction solvent and several wavelengths and derivative order of the UV spectrum.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Derivative Order</th>
<th>(\lambda) (nm)</th>
<th>Placebo interference</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Methanol</td>
<td>0</td>
<td>252</td>
<td>1.91</td>
<td>100.66</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>245</td>
<td>7.14</td>
<td>82.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>260</td>
<td>0</td>
<td>100.00</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0</td>
<td>252</td>
<td>2.65</td>
<td>3.38</td>
</tr>
</tbody>
</table>

The Figure 1 shows the inefficiency of ethanol in extracting the drug from its matrix, so that this solvent cannot be employed in the analytical assay.
Figure 1: Absorption spectra of fenticonazole nitrate SQR (A), product (B) and placebo (C) at a concentration of 15 µg mL\(^{-1}\) using ethanol as solvent.

Figure 2: Absorption spectra of fenticonazole nitrate SQR (A), product (B) and placebo (C) at a concentration of 15 µg mL\(^{-1}\) using methanol as solvent.
Significant interference from the cream excipients was detected in the region of fenticonazole nitrate absorption spectrum, which precludes the analytical use of zero-order spectrophotometry (Table 1, Figure 2). For this reason, the first-order derivative spectrophotometric method was considered ideal for solving the overlapping of excipients absorption over fenticonazole nitrate signal.

The zero crossing for placebo solutions appears at 260.0 (Figure 3). Therefore, the value were selected as optimum to determine fenticonazole in the presence of the vaginal cream pharmaceutical excipients.

Figure 3: First order derivative absorption spectra of fenticonazole nitrate SQR (A), product (B) and placebo (C) at a concentration of 15 µg mL⁻¹ using methanol as solvent.

Accuracy

The accuracy of the proposed method was assessed by determining the average recovery of drug using spiked placebo through all linear concentration range. Each concentration was tested in triplicate. As shown in Table 2, the mean percentage recovery of fenticonazole
nitrate was 98.81% and the R.S.D. was 1.169. The results indicate the suitability of the developed method in quantifying the concentration of fenticonazole nitrate in vaginal cream.

**Table 2: Recovery of fenticonazole nitrate in spiked placebo for accuracy determination.**

<table>
<thead>
<tr>
<th>Drug concentrations for spiked placebos (µg/mL)</th>
<th>Recovery (%)</th>
<th>Mean recovery (%)</th>
<th>R.S.D. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>99.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>98.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>98.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>100.01</td>
<td>98.81</td>
<td>1.169</td>
</tr>
<tr>
<td>18</td>
<td>98.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>98.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>98.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Precision**

The repeatability of analytical method was evaluated by calculating the % R.S.D. for seven concentrations (6, 9, 12, 15, 18, 21 and 24 µg mL\(^{-1}\)) of the standard solution performed on the same day and under the same experimental conditions. The intermediate precision was assessed by analyzing three concentrations (6, 15 and 24 µg mL\(^{-1}\)) by two different analysts. The R.S.D. value (%) for all results was 3.16%, confirming that the method is sufficiently precise.

**Robustness**

To evaluate the robustness of the proposed method low and deliberate changes were carried out on the time of ultrasonic bath and on the wavelength used in the measurement. Sample and standard solutions were evaluated under modified and standardized conditions. The interference of these changes over drug dosage test was monitored.

The changes in method parameters caused major changes in analytical results. The robustness test results show that the standardized parameters must be strictly adhered to, especially the chosen wavelength which causes a difference higher than 4% in drug content assay, as shown at Table 3.
Table 3: Robustness test results.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Changes</th>
<th>Content (%)</th>
<th>Difference on the content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>258 nm 260 nm* 262 nm</td>
<td>96.41 100.60* 100.60</td>
<td>4.19 - 0</td>
</tr>
<tr>
<td>Sonication time</td>
<td>50 min 60 min*</td>
<td>100.16 100.60*</td>
<td>0.44 -</td>
</tr>
</tbody>
</table>

*Standard procedure.

**Limits of detection and quantitation**

LOD and LOQ values were calculated to be 0.57 and 1.91 µg mL$^{-1}$, respectively.

**Assay of the pharmaceutical product**

The validated method was applied to quantify the fenticonazole nitrate in vaginal cream, a generic pharmaceutical product. The pool of ten tubes content of the pharmaceutical product were analyzed in triplicate. The drug content for the analyzed product was 99.7% with R.S.D. of 1.19% these results are expressed as the percentage drug related to the content label claim.

These results confirm the good extraction method used, as well as, the good precision of the validated method.

UV, UV-VIS and derivative spectrophotometry are broadly used techniques to quantify antibiotics and other drugs$^{[4-9]}$ because they produce reliable and selective results, in addition they are inexpensive, simple and do not require time-consuming sample preparation compared with others techniques.$^{[4-9,15]}$ The developed first derivative UV spectrophotometric method shows to be simple, reliable and suitable for fenticonazole vaginal cream assay. Moreover, UV-spectrophotometry produces very low amounts of residues and solvents, especially in comparison to liquid chromatographic techniques, which is an important ecological aspect currently discussed in routine laboratory analysis.$^{[16]}$

There is currently a growing emphasis on green chemistry in pharmaceutical research and manufacturing. Therefore, the use of simple, easy to perform, fast, accurate and environmentally friendly methods becomes increasingly interesting for quality control of the pharmaceutical industry. The investigation of alternative methods such as this one should be valued, as well as reflections on their multidisciplinarity, as it is a social, environmental and economic concern.$^{[17-19]}$
CONCLUSION
The new analytical method uses derivative tools to achieve a matrix interference free method for drug dosage assay of fenticonazole nitrate in vaginal cream applying UV spectrophotometric technique. This procedure does not require complex or expansive equipment neither complicated separation method and uses simple reagents. Moreover, this work presents a reliable, simple and suitable analytical method that met all validation parameters defined by ICH guideline.\textsuperscript{[13]} The validated method is an important tool for routine quality control of fenticonazole nitrate and may be useful for pharmaceutical industry quality control analysis.

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