Determination of Ketoconazole in Shampoo by High Performance Liquid Chromatography

Inara STAUB & Ana Maria BERGOLD*

Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, UFRGS,
Av. Ipiranga 2752, 90610-000. Porto Alegre, Brasil.

SUMMARY. A high performance liquid chromatography isocratic procedure was developed for the assay of ketoconazole in shampoo. Two solutions, (A) monoisopropylamine-methanol (2:500, v/v) and (B) ammonium acetate-water (1:200, w/v) were prepared. The mobile phase is a mixture of A and B (7:3, v/v), with pH adjusted to 5.5 with acetic acid. The chromatographic system consists of a LiChrospher® 100 RP-8 column (150 x 46 mm, 5 µm), flow rate of 1.0 mL/min and UV detector at 225 nm. The method validation yielded good results, Relative standard deviation (RSD) of 0.42% and accuracy of 97.2% were obtained. Calibration curve was linear between 60.0 and 480.0 µg/mL, with correlation coefficient of 0.9999.

INTRODUCTION

Ketoconazole (Fig. 1), an imidazole derivative, chemically 1-acetyl-4-[4-[[2RS,4SR]-2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]perazine 1, is an antifungal agent with topical and systemic action and can be incorporated into several pharmaceutical forms. As an example it could be mentioned a ketoconazole shampoo, which is effective against seborrhoeic dermatitis, as well as, Pityriasis versicolor 2, 3.

The main effect of imidazoles is the inhibition of the sterol-14α-desmethylase, an enzymatic system dependent upon cytochrome P450, with a consequent inhibition of fungal development 4.

Several different analytical procedures have been described for the determination of ketoconazole in pharmaceutical formulations: potentiometric 5, spectrophotometric 6, 7, chromatographic 7, 8 and microbiological 8 methods.

Even though this is not a new drug, there are no official methodologies for ketoconazole determination in shampoo 1, 9. Heyden et al. 10 described an HPLC system for simultaneous determination of ketoconazole and formaldehyde, in a formulation that contains imidazolidinylurea as a formaldehyde releasing preservative. Keto-
Ketoconazole was determined using a UV detector at 254 nm and formaldehyde was measured at 345 nm, after derivatisation with a 2,4-dinitrophenylhydrazine solution.

Most of the proposed methods are either not sensitive enough, or require complicated and expensive instruments, or are time consuming. Therefore, there is still a need for a sensitive and simple method for the determination of ketoconazole in shampoo.

HPLC method is widely used for drug analysis, since it offers high sensitivity and selectivity. The aim of this study was to develop and validate an easy, fast and accurate high performance liquid chromatography method for the quantitative determination of ketoconazole contained in a shampoo formulation.

**MATERIAL AND METHODS**

**Samples**
Ketoconazole was obtained from Galena (São Paulo). A shampoo formulation was prepared with ketoconazole 2%, pH 5.5. Ketoconazole was dissolved in chloridric acid N prior to shampoo addition. Sodium lauryl ether sulfate, coconut fatty acids diethanolamine, methyl paraben, sodium chloride and distilled water were used as excipients for shampoo preparation. The sample was packed into an amber glass recipient.

**Reagents and solutions**
The mobile phase was prepared with methanol HPLC, water purified by Millipore® system, monoisopropylamine and ammonium acetate with analytical grade. A solution of monoisopropylamine-methanol (2:500, v/v) (A) and a solution of ammonium acetate in water (1:200, w/v) (B) were prepared. Solutions A and B were then mixed (A:B) (7:3, v/v). Final solution pH was adjusted to 5.5 with acetic acid.

**Chromatographic conditions**
Ketoconazole concentration was determined with a liquid chromatograph Shimadzu (LC-10AD pump, UV-VisSPD-10AV detector) and the following parameters: 1 mL/min flow, UV detector at λ 225 nm, LiChrospher® 100 column RP-8, 5 µm (150 mm x 46 mm). The system was operated at room temperature (20 ± 1 °C).

**Validation**

**Linearity**
Ketoconazole (reference substance) stock solution (0.6 mg/mL) was prepared in a 25 mL amber volumetric flask by dissolving 15.0 mg of reference substance in 1 mL HCl N and completing the volume with mobile phase. Appropriate amounts of stock solution were diluted in mobile phase in order to obtain ketoconazole concentrations of 60.0, 120.0, 240.0, 360.0 e 480.0 µg/mL. Linearity was determined intra- and inter-day, being evaluated in three days.

Preparation of standard solution
Ketoconazole (reference substance) was prepared by weighing 15.0 mg of substance into a 50 mL amber volumetric flask; 2 mL of HCl N were added and the volume was completed with mobile phase. Ketoconazole concentration was 300.0 µg/mL.

Ketoconazole assay
In order to obtain a concentration of 320.0 µg/mL for ketoconazole assay, 0.4 g of shampoo were weighed into a 25 mL volumetric flask and the volume was completed with mobile phase. Six different samples were analysed, by injecting three times each sample solution. The analyses were carried out during three sequential days.

**Accuracy test**
To confirm the accuracy of the proposed method, the recovery test was performed, following the recomendations of USP 25. Recovery was determined by adding known amounts of ketoconazole standard solution to the sample at the beginning of the process (220.0 µg/mL). The concentrations obtained were 264.0, 308.0, 352.0 µg/mL, respectively. Analyses were conducted in three days and each sample was injected three times.

**RESULTS AND DISCUSSION**
Validation of an analytical method is the process in which the method is established, in order to demonstrate that it is suitable for required analyses. Analytical characteristics used in validation of a method are: accuracy, precision, specificity, detection limit, quantification limit and linearity. In the present work we have not conducted tests on detection and quantification limits due to the concentration employed which was 320.0 µg/mL. In such concentration, ketoconazole peak was greater than base line noise. Moreover, according to ICH, it is not necessary to determine quantification and detection limits for the final product.

Linearity of an analytical procedure is to obtain results directly proportional to substance
concentration in the sample. The calibration curve of ketoconazole was developed by plotting peak area versus concentration (Fig. 2). The curve was linear with a linear regression coefficient of 0.9999 and the linear regression equation was $y = 183697 + 56671x$.

System suitability tests for HPLC was evaluated through chromatographic behavior of the method. Parameters such as number of theoretical plates (N) and precision of injection were determined. The number of theoretical plates, which measure the column efficiency, was determined (N = 1800). Precision of injection was evaluated through RSD; values in a range from 0.4 to 1.5% were obtained.

The method showed to have good reproducibility in both intra-and inter-day analyses. The precision of the method was expressed by the similarity of a series of measurements obtained from several samples. The average obtained between days was 102.6% and RSD was 0.42% (Table 1).

Retention times obtained during precision studies were excellent in all samples. After chromatographic analysis (Fig. 3) the retention time of ketoconazole was found to be 4.0 min and the peak observed in 1.8 min in the sample was attributed to formulation constituents, since the shampoo formulation itself was separately injected and showed the same peak, proving that the excipients did not interfere in the method.

The results show a suitable ketoconazole recovering in the samples (97.2%) with an RSD of 1.22% (Table 2).

It is remarkable that ketoconazole is rapidly altered in shampoo and solution dosage forms.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Declared amount (mg/mL)</th>
<th>Found amount (mg/mL)</th>
<th>Purity (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham 1</td>
<td>20.00</td>
<td>20.44</td>
<td>102.2</td>
<td></td>
</tr>
<tr>
<td>Sham 2</td>
<td>20.00</td>
<td>20.58</td>
<td>102.9</td>
<td></td>
</tr>
<tr>
<td>Sham 3</td>
<td>20.00</td>
<td>20.58</td>
<td>102.9</td>
<td>0.42</td>
</tr>
<tr>
<td>Sham 4</td>
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<td>20.48</td>
<td>102.4</td>
<td></td>
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<tr>
<td>Sham 5</td>
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<td>20.61</td>
<td>103.1</td>
<td></td>
</tr>
<tr>
<td>Sham 6</td>
<td>20.00</td>
<td>20.40</td>
<td>102.0</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1.** Results of ketoconazole determination in shampoo by HPLC method.

*Each value is the mean of nine analysis.*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added (mg/mL)</th>
<th>Found a (mg/mL)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham 1</td>
<td>0.0440</td>
<td>0.0433</td>
<td>98.42</td>
</tr>
<tr>
<td>Sham 2</td>
<td>0.0880</td>
<td>0.0855</td>
<td>97.14</td>
</tr>
<tr>
<td>Sham 3</td>
<td>0.1320</td>
<td>0.1268</td>
<td>96.04</td>
</tr>
</tbody>
</table>

**Table 2.** Recovery of a standard solution added to shampoo samples and analysed by HPLC.

*Each value is the mean of nine analysis.*
since its color turns into red. The proposed method is able to determine secondary peaks formed when dosage forms acquire a strong red color, being an useful tool to detect possible degradation products (Fig. 4). Degradation of ketoconazole will be the subject of another paper.

CONCLUSIONS

The proposed high performance liquid chromatographic method for ketoconazole determination in shampoo showed linearity in the studied concentrations. The method is simple, fast, precise and accurate. The preparation of samples is easy, the excipients did not interfere in the method and it can be used in quality control routine analysis.

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REFERENCES