# VALIDATION OF A CHROMATOGRAPHIC METHOD FOR MICONAZOLE ASSAY FROM ORAL SUSTAINED RELEASE MUCOADHESIVE TABLETS

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**Abstract**

In order to detect the Miconazole nitrate in oral mucoadhesive sustained-release tablets, HLPC compendium assay method with UV detection, used in European Pharmacopoeia, has been developed and used. The working conditions of HLPC separation were set using C18 column (100 mm x 4.6 mm) and the mobile phase: 0.6% ammonium acetate in a mixture of 370 parts of acetonitrile, 290 parts of methanol and 340 parts of water. Detection was performed in UV at $\lambda$ = 235 nm, where the miconazole nitrate reaches the maximum level of absorption. The method has been validated by determining the parameters: linearity in the chosen concentration field (0.10 – 0.70 mg/mL), correlation coefficient $r^2$ = 0.9998, the precision of the method with a RSD of 0.833%, the intermediate precision of the method (RSD = 0.386%) and the accuracy of the method with a 99.95% retrieval percentage.

A simple method of determining the antifungal has been validated, verifying the analytic performances of this method through the most important validation parameters.

The created HPLC method of assay the miconazole nitrate is simple, easy to apply, sensitive, linear, precise and accurate.

**Rezumat**

În vederea determinării nitratului de miconazol din preparate farmaceutice noi, comprimate mucoadezive orale cu cedare prelungită, s-a dezvoltat şi aplicat metoda compendială HPLC de dozare cu detecție în UV din Farmacopeea Europeană. Au fost stabilite condițiile de lucru de separare prin HPLC folosind o coloană C18 (100 mm x 4,6 mm) şi faza mobilă: 0,6% acetat de amoniu într-un amestec de 370 volume acetonitril, 290 volume metanol şi 340 volume apă. Detecția se efectuează în UV la $\lambda$ = 235 nm unde nitratul de miconazolul prezintă un maxim de absorbție. Metoda a fost validată prin determinarea parametrilor: liniaritatea în domeniul de concentraţie ales (0,10 – 0,70 mg/mL), coeficientul de corelație $r^2$ = 0,9998, precizia metodei cu un RSD de 0,833%, precizia intermediară a metodei (RSD = 0,386%) şi acurateţea metodei cu o regăsire procentuală de 99,95%.

A fost validată o metodă simplă de determinare a antimicoticului fiind verificate performanţele analitice ale metodei prin cei mai importanţi parametri de validare.

Metoda HPLC de dozare a nitratului de miconazol elaborată este simplă, uşor de aplicat, sensibilă, liniară, precisă şi exactă.

**Keywords:** Biomucoadhesive tablets, miconazole nitrate, HPLC, UV detection.

## Introduction

The relative limited therapeutic range of currently existing oral medicine determined us to perform some studies in order to develop oral mucoadhesive sustained-release tablets, containing miconazole nitrate as the active ingredient.

The main objective of the present study was to obtain an optimal formulation of oral mucoadhesive tablets with miconazole nitrate in order to treat the oral candida, which assures an optimal antifungal level in the oral cavity for a longer period of time.

Miconazole nitrate is used in a wide specter of fungal infections, especially in dermatophyte diseases and Candida, as well as Gram positive germs. The antifungal azoles act by inhibiting the cytochrome P450 and the Lanosterol 14-alpha demethylase, an enzyme with a major importance in the biosynthesis of ergosterol [1, 2, 3, 4].

The specialized literature indicates a series of methods for miconazole determination from pharmaceutical preparations or biological fluids such as reverse phase liquid chromatography, gas chromatography and others [3, 4, 5, 6, 7, 8, 9, 10].

Many of the methods described in specialized literature, used for determining the azoles, are very laborious, require many reagents and are very time consuming. That's why the HPLC compendium method with UV detection was developed and validated within the European Pharmacopoeia, for miconazole nitrate as the active ingredient [11]. The working conditions for determining the miconazole nitrate as active ingredient have been set, together with excipients from sustained-release oral mucoadhesive tablets containing miconazole nitrate 25 mg / tablet.

## Materials and Methods

*Chromatographic Conditions*

Merck Hitachi system high pressure liquid chromatograph equipped with: UV detector and an automatic system of sample injection of 0 – 100 µL; Column Kromasil C18, 100 x 4.6 mm, 5 µm, comprising the mobile phase: solution of 0.6% ammonium acetate in a mixture of 370 parts - acetonitrile, 290 parts - methanol and 340 parts water, at a flow rate of 1.9 mL/min and

an injected volume of 10 μL; UV detection at 235 nm; column temperature - 30°C.

*Materials*

Reference solution: miconazole nitrate, working standard - 99.0% and econazole nitrate, working standard - 99.0% and reagents: double distilled water, HPLC grade acetonitrile, tetrahydrofuran, HPLC methanol, ammonium acetate, the used solvent - methanol: tetrahydrofuran (1:1).

*Method*

The reference and resolution liquids and concentration sample of 0.5 mg/mL were injected, dissolved in solvent, and the chromatograms were recorded at 235 nm.

The compliance of the chromatographic system was achieved only if the resolution between the peak of econazole and the one of miconazole was at least 6.

Working solutions:

- solution E1 (80%): 20 mg of miconazole nitrate working standard were weighed and 200 mg mixture of excipients, transferred into a 25 mL flask with 20 mL solvent and well stirred for 10 minutes. The volume was completed with the same solvent. 5 mL from the obtained solution was filtered and diluted in a 10 mL flask with the same solvent.

- solution E2 (100%): 25 mg of miconazole nitrate working standard and 195 mg mixture of excipients were weighed and transferred into a 25 mL flask with 20 mL solvent and stirred for 10 minutes. The volume was completed with the same solvent. 5 mL from the obtained solution was filtered and diluted at 10mL flask with the same solvent.

- solution E3 (120%): 30 mg of miconazole nitrate working standard and 190 mg mixture of excipients were weighed and transferred into a 25 mL flask with 20 mL solvent and stirred for 10 minutes. The volume was completed with the same solvent. 5 mL of the obtained solution was filtered and diluted at 10 mL flask with the same solvent.

**In order to validate the method for the assay of** the active ingredient, the following parameters were assessed: linearity, specificity (selectivity), accuracy, precision, intermediate precision and stability in time of the solutions.

The **linearity** studies were comparatively performed on seven solutions of miconazole nitrate reference substance, with a content of miconazole nitrate reference substance ranging between 0.10 – 0.70 mg/mL. Each solution was injected 3 times, taking into account the average areas for each concentration. The resulted data were analyzed by linear regression

(the calibration curve equation for the concentrations domain, the correlation coefficient).

The **precision** of the miconazole nitrate assay method was verified by determining the repeatability of the results obtained on 6 injections of the sample solution having the same concentration of 0.5 mg/mL miconazole nitrate. The average value, the standard deviation S and the relative standard deviation RSD, which must not exceed 2% were calculated.

In order to determine the **intermediate precision,** the reproducibility of the results obtained on 6 injections of the sample solution with the same concentration of 0.5 mg/mL miconazole nitrate, performed by 2 analysts in different days. The results were reported calculating the retrieval %, relative standard deviation RSD and the RSD between the two analysts.

In order to confirm the **accuracy** of the proposed analytical method, it was determined the retrieval efficiency of the active ingredient from three reconstituted samples is determined, according to the manufacturing formula, with the concentration values at the lower, theoretical and upper limits of the eligibility interval (80%, 100% and 120%).

There were performed 3 injections for each concentration level.

The retrieval efficiency was calculated.

In order to determine the **specificity** of the analysis method, the interference degree with other substances (excipients from the matrix of the product) and the active ingredient was monitored. In order to determine the **specificity** parameter *a placebo* sample (containing all excipients, except for the active ingredients) was prepared and processed like the assay sample.

## Results and Discussion

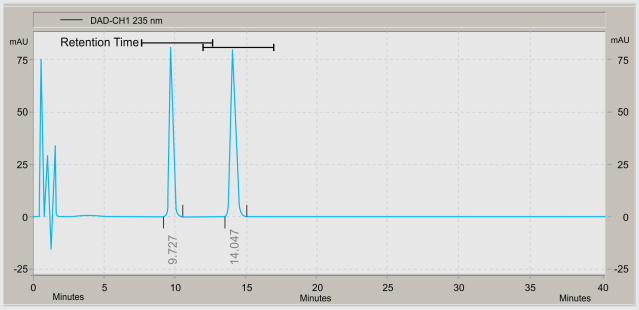
The statistical analysis of the acquired data for determining the linearity of the method is presented in Table I.

**Table I**

*Method linearity*

| Concentration % | Miconazole Nitrate Content (mg/mL) | Standard Solutions average (mg/mL) |
|---|---|---|
| 20 | 0.1032 | 1826038 |
| 30 | 0.2064 | 3538336 |
| 40 | 0.3096 | 5445812 |
| 60 | 0.4128 | 7225620 |
| 80 | 0.5160 | 8842050 |
| 100 | 0.6192 | 10718359 |
| 120 | 0.7224 | 12469449 |

In Figure 1 the HPLC chromatogram of the miconazole nitrate reference solution (of 0.5 mg/mL concentration) of the resolution liquid for the compliance of the chromatographic system is showed.




DAD-CH1
235 nm
Results

| Retention Time | Area | Area% | Height% | Height% | Name | Resolution (USP) |
|---|---|---|---|---|---|---|
| 9.727 | 6218480 | 42.53 | 324202 | 50.62 | Izoconazole Nitrate | 0.00000 |
| 14.047 | 8404336 | 57.47 | 316274 | 49.38 | Miconazole Nitrate | 7.04857 |
| Totals | 14622816 | 100.00 | 640476 | 100.00 | | |

**Figure 1**
HPLC Chromatogram of the reference solution

The obtained correlation coefficient R exceeds 0.9998 on standard solutions of miconazole nitrate reference substance and fits within the limits set by the validation protocol. The method is linear.

The experimental values obtained in determining the precision of the method are presented in Table II.

**Table II**

*Precision of HPLC determination method of miconazole nitrate*

| No. det. | Sample Solutions Areas | Miconazole Nitrate Content (mg/cpr) | Retrieval (%) |
|---|---|---|---|
| 1 | 8842050 | 25.000 | 100.00 |
| 2 | 8844220 | 25.006 | 100.02 |
| 3 | 8976441 | 25.380 | 101.52 |
| 4 | 9002306 | 25.450 | 101.80 |
| 5 | 8842896 | 25.000 | 100.00 |
| 6 | 8990336 | 25.420 | 101.68 |
| **Reference Solution Concentration (mg/mL)** | | 0.516 mg/mL | |
| **Reference Solutions Areas (mAU/S)** | | 8842050 | |
| **Retrieval average** | | **100.836** | |
| **S** | | **0.83** | |
| **RSD** | | **0.833** | |

The method is considered reproducible since RSD < 2%. The results obtained in determining the intermediate precision are presented in Table III.

**Table III**

*Intermediate precision of HPLC determination method of miconazole nitrate*

| **Analyst 1** | | | | **Analyst 2** | | | |
|---|---|---|---|---|---|---|---|
| No. | Sample Solutions Areas (assay) (mUA/S) | Miconazole Nitrate Content (mg/cpr) | Retrieval (%) | No. | Sample Solutions Areas (assay) (mUA/S) | Miconazole Nitrate Content (mg/cpr) | Retrieval (%) |
| **1** | 8842050 | 25.000 | 100.00 | **1** | 8883710 | 25.07 | 100.28 |
| **2** | 8844220 | 25.006 | 100.02 | **2** | 8883905 | 25.00 | 100.00 |
| **3** | 8976441 | 25.380 | 101.52 | **3** | 8880167 | 25.06 | 100.24 |
| **4** | 9002306 | 25.450 | 101.80 | **4** | 8837644 | 24.94 | 99.76 |
| **5** | 8842896 | 25.000 | 100.00 | **5** | 8869536 | 25.03 | 100.12 |
| **6** | 8990336 | 25.420 | 101.68 | **6** | 8855361 | 24.99 | 99.96 |
| Standard Solutions Concentration ( mg /mL) | | | 0.516 | 0.515 | | | |
| RSD(%) | | | 0.833 | RSD(%) | | | 0.153 |
| RSD 2/RSD 1 | | | **0.386** | | | | |

The results obtained in determining the accuracy of the method are presented in Table IV.

**Table IV**

*Accuracy of HPLC assay method for miconazole nitrate*

| **Sample** | | **Miconazole Nitrate** | | | |
|---|---|---|---|---|---|
| | | **Weighed Quantity (mg/cpr)** | **Resulted Concentration (mg/mL)** | **Resulted Quantity (mg/cpr)** | **Retrieval (%)** |
| E1 | 1 | 20.15 | 0.4028 | 20.14 | 99.95 |
| | 2 | 20.13 | 0.4025 | 20.125 | 99.97 |
| | 3 | 20.14 | 0.4027 | 20.13 | 99.95 |
| Average Value | | - | 0.4026 | 20.131 | 99.956 |
| **RSD%** | | **-** | **0.0273** | **0.0273** | **-** |
| E2 | 1 | 25.14 | 0.5027 | 25.135 | 99.98 |
| | 2 | 25.16 | 0.5030 | 25.15 | 99.96 |
| | 3 | 25.18 | 0.5034 | 25.17 | 99.96 |
| Average Value | | - | 0.5030 | 25.151 | 99.96 |
| **RSD%** | | **-** | **0.0485** | **0.0485** | **-** |
| E3 | 1 | 30.05 | 0.600 | 30.0 | 99.83 |
| | 2 | 30.07 | 0.6012 | 30.06 | 99.96 |
| | 3 | 30.06 | 0.6010 | 30.05 | 99.96 |
| Average Value | | - | 0.6007 | 30.036 | 99.91 |
| **RSD%** | | **-** | **0.0814** | **0.0812** | **-** |

The RSD values obtained in determinations of the three samples for the resulted quantities fit within the eligibility conditions of maximum 2%.

The retrieval average percentages also fit the eligibility interval.

The method was accurate.

In order to determine the specificity of the HPLC assay method of the miconazole nitrate in the oral mucoadhesive tablets, chromatograms for: solvent, mixed solution of *placebo*-excipients, reference solution "a", reference solution "b", sample solution have been recorded (Table V).

**Table V**

*Solutions Retention Times at 235 nm*

| Reference Solutions | Retention Times (minute) |
|---|---|
| Solvent Methanol Tetrahydrofuran (1:1) | No peak for miconazole nitrate retention time |
| Reference Solution "a" | retention time = 14.027 |
| Reference Solution "b" | retention time= 9.753 econazole nitrate<br>retention time=14.080 miconazole nitrate<br>Resolution =7.045 |
| Sample Solution | retention time = 14.047 |
| Mixed Solution of Placebo-Excipients | No peak for miconazole nitrate retention time |

As it can be seen in Table V, the excipients from the *placebo*-sample and the solvent do not interfere in the miconazole nitrate assay of the oral mucoadhesive tablets.

The results of the study on the influence of time variation on the stabilities of the used solutions in the dosing method of the miconazole nitrate are presented in Tables VI and VII.

**Table VI**

*Miconazole nitrate HPLC assay method*

| Time (hours) | Reference Solution Concentration (mg/mL) | Peak Area (mUA/S) | Retrieved Reference Solution Concentration (mg/mL) | Retrieval (%) |
|---|---|---|---|---|
| 0 | 0.515 | 8845020 | 0.5150 | 100.00 |
| 4 | 0.515 | 8976441 | 0.5226 | 100.30 |
| 8 | 0.515 | 9002306 | 0.5240 | 100.40 |
| **Standard Solution Concentration (mg/mL)** | | | 0.515 | |
| **Peak Area of miconazole nitrate from the standard solution (mUA/S)** | | | 8842050 | |

**Table VII**

***Stability*** *of solutions used for validation of HPLC assay method*

| Time (hours) | Standard Solution Concentration (mg/mL) | Peak Area (mUA/S) | Concentration (%) in miconazole nitrate (mg/cpr) | Retrieval (%) |
|---|---|---|---|---|
| 0 | ~ 0.50 | 8842896 | 25.04 | 102.10 |
| 4 | ~ 0.50 | 8990345 | 25.45 | 102.50 |
| 8 | ~ 0.50 | 9002314 | 25.50 | 103.15 |
| **Standard Solution Concentration (mg/mL)** | | | 0.515 | |
| **Peak Area of miconazole nitrate from the standard solution (mUA/S)** | | | 8842050 | |

The results in Table VII show that both the reference solution and the sample solution are stable and can be used for maximum 8 hours after their preparation.

## Conclusions

In the present study we present the results obtained for the validation of HLPC assay method of miconazole nitrate in sustained-release mucoadhesive oral tablets. The method validated for the following parameters: linearity on a concentration field of 0.10 - 0.70 mg/mL with a correlation coefficient of 0.9998, the precision of the method with a 0.833% RSD, the intermediary precision with a 0.386% RSD and the accuracy of the method with a 99.95% percentage retrieval.

We started from the analytic method presented by the European Pharmacopoeia in order to identify and dassay the active ingredient; this was optimized and we proved it can be used for the oral mucobioadhesive tablets with miconazole nitrate since the method has selectivity. The developed method is rapid, sensitive, reproducible, does not require the processing of laborious samples and is also not that expensive as other methods in literature which use more expensive and laborious techniques [11].

In conclusion, the HLPC assay method for miconazole nitrate is simple, easy to apply, linear, precise and accurate and can be successfully applied in quantitative assessment of this substance in the proposed pharmaceutical forms.

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