# Simultaneous HPLC determination of Metronidazole, Lidocaine and Miconazole in a combined intravaginal semi solid dosage form

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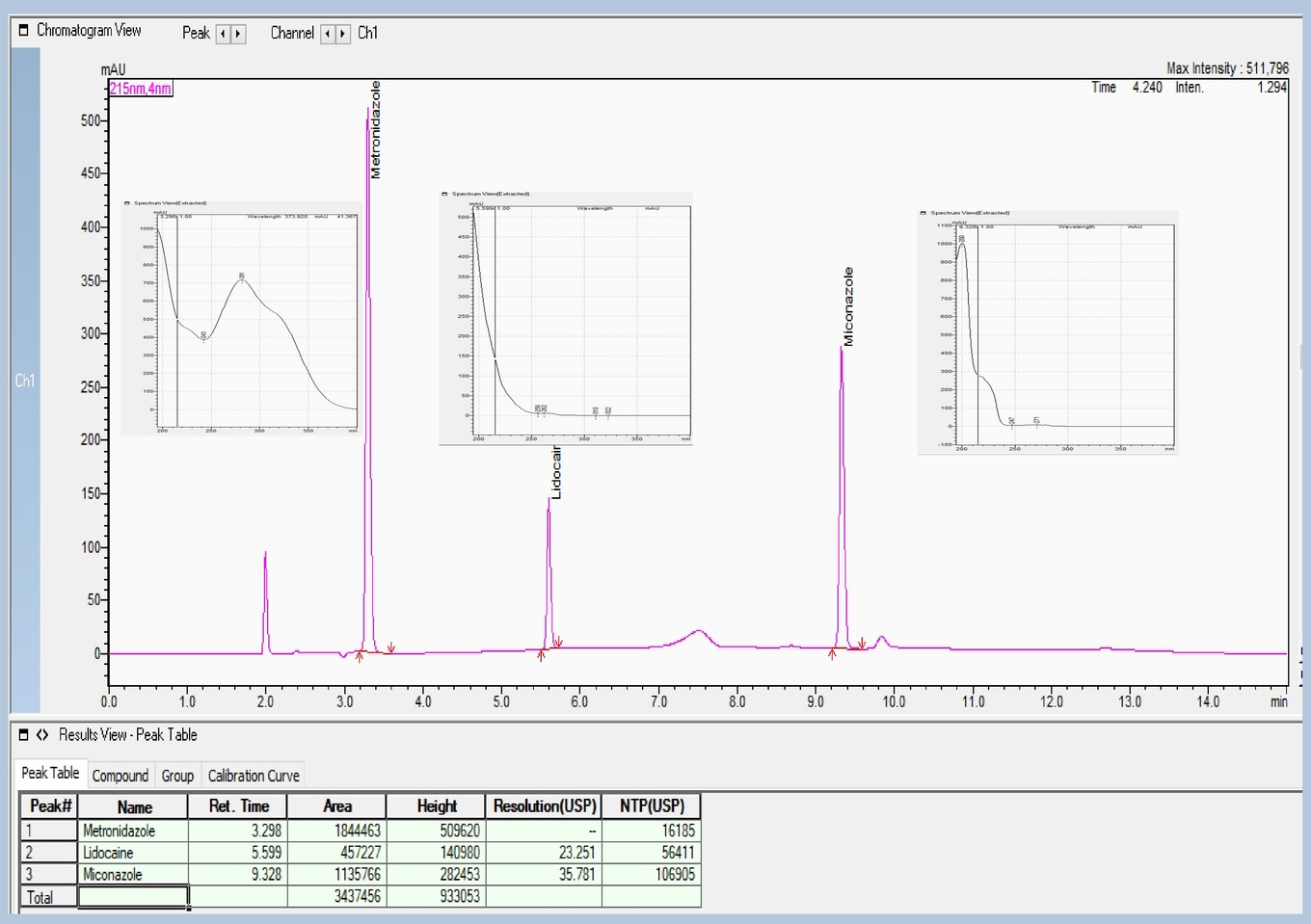
## Introduction

There are number of pharmacopoeial (Ph. Eur., BP, USP) and non-pharmacopoeial methods available for determination of metronidazole, miconazole and lidocaine (Figure 1), individually, but only few non-pharmacopoeial methods for their simultaneous determination in some combined pharmaceutical dosage forms (Akay et al., 2002; Belal and Haggag, 2012). The pharmaceutical formulation of interest that was subject of this research, was combined semi solid preparation for intravaginal use, containing metronidazole, miconazole and lidocaine as active substances.

The aim of our work was to develop and validate a method for simultaneous quantification of these three active substances in a combined pharmaceutical formulation. For that purpose, simple and selective high performance liquid chromatography (HPLC) method was developed and validated.




| Peak# | Name | Ret. Time | Area | Height | Resolution(USP) | NTP(USP) |
|---|---|---|---|---|---|---|
| 1 | Metronidazole | 3.298 | 1844463 | 509620 | -- | 16185 |
| 2 | Lidocaine | 5.599 | 457227 | 140980 | 23.251 | 56411 |
| 3 | Miconazole | 9.328 | 1135766 | 282453 | 35.781 | 106905 |
| Total | | | 3437456 | 933053 | | |

Figure 2 – Chromatogram with peaks from lidocaine, metronidazole and miconazole obtained using the novel method on a sample from a semisolid dosage form

## Method

The separation was carried out on Zorbax RX-C8 250 mm x 4.6 mm, 5 µm column with mobile phase A (MFA) consisting of 0.1% (V/V) o-phosphoric acid with 0.1% (V/V) of perchloric acid and mobile phase B (MFB) consisting of acetonitrile. Flow rate of 1.2 mL/min, injection volume of 5 µL, the column temperature of 35ºC and detector at wavelength of 215 nm were set as other parameters of the method. The gradient is initiated with 80% (V/V) MFA and 20% (V/V) MFB, continued with linear variation to 6th minute when MFA drops to 30% (V/V) and MFB increases up to 70% (V/V). From 6 to 10 minutes the ratio between mobile phases remains the same as previous. The MFA returns to 80% (V/V) and MFB to 20% (V/V) accordingly in 1 minute and the column is re-equilibrated for 4 minutes.

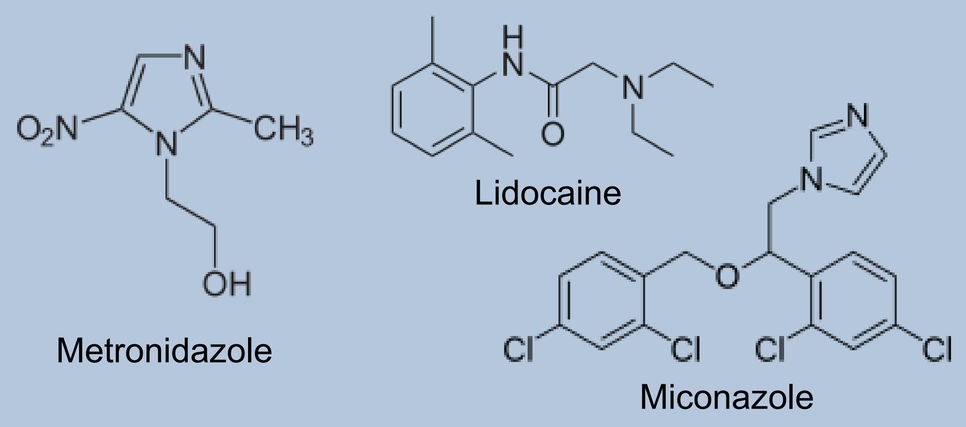



Figure 1 – Chemical structures of the active pharmaceutical ingredients in the tested semisolid dosage form

## Materials

**Reagents:**
- 85% o-phosphoric acid (Merck, Germany)
- 70-72% perchloric acid (Merck, Germany)
- Acetonitrile (Sigma Aldrich, USA)
- Deionized water prepared in Replek Farm by use of Simplicity UV System, with conductivity of 0.05 µS/cm.
- Reference standards of Metronidazole CRM, Lidocaine CRM and Miconazole CRM (Sigma Aldrich, USA)
- Vaginal suppositories containing the three active substances from Replek Farm Ltd., Skopje, N. Macedonia.

**Instruments:**
- Analytical balance AG285 (Mettler Toledo)
- US bath TP690/H
- Optical shaker KS 260 basic
- HPLC system Shimadzu Nexera XR UPLC system with LPG quaternary pump with degasser, auto sampler, column oven, PDA detector and controller
- Data acquisition, analysis and reporting software Lab solution version 5.97
- Zorbax RX C8 column (Agilent Technologies, Santa Clara, United States)

## Results and conclusion

The gradient elution of the analytes was achieved in 15 minutes, with retention time of metronidazole, lidocaine hydrochloride and miconazole nitrate of about 3.2 minutes, 5.5 minutes and 9.3 minutes, respectively (Firgure 2).

*Method validation results*
- *Linearity - correlation coefficient > 0.9990; RSD of the response factors for each concentration level < 2 %, in all cases*
- *Method and system precision - relative standard deviation of the response was less or equal to 2 %, in both cases, for each substance*
- *Method accuracy - the obtained recovery values were within the range of 100 ± 2 %, for each substance*

The developed reverse phase HPLC method provides simple, specific, accurate, precise and reproducible simultaneous quantitative analysis of metronidazole, lidocaine and miconazole in combined semi solid pharmaceutical formulations. The established method was validated and proved as suitable for its intended use, in accordance to ICH guideline Q2(R1).

**References**

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