

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, 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.

Lidocaine is local anesthetic of amide type. It interacts with voltage-gated Na⁺ channels in the nerve cell membrane and blocks the transient increase in permeability of excitable membranes to Na⁺. It is used to provide local anesthesia by nerve blockade at various site in the body and works by causing temporary numbness or loss of feeling in the skin and mucous membranes.

Metronidazole belongs to a class of medications called nitroimidazole antimicrobials. It is antibiotic and antiprotozoal medication and is used to treat infections by stopping the growth of certain bacteria and parasites. With wide range of antibacterial activities and antiparasitic properties this antimicrobial medication is set apart from other antibiotics to treat a wide variety of infections.

Miconazole nitrate is a synthetic imidazole derivative antifungal agent which has a wide spectrum of activity and is particularly effective against pathogenic fungi, including *Candida albicans*. Also it is effective against gram-positive bacteria. It shows effect during ergosterol

synthesis in the cytoplasmic membrane and changes permeability of the mycotic cell of *Candida* species and inhibits glucose utilization *in vitro*.

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, for simultaneous determination of lidocaine, metronidazole and miconazole in combined vaginal suppositories.

Materials and methods

Materials

The reagents that were used are 85% o-phosphoric acid, 70-72% perchloric acid and acetonitrile. The o-phosphoric acid and perchloric acid were purchased from Merck, Germany, while acetonitrile was purchased from Sigma Aldrich, USA. Deionized water that was used was prepared in Replek Farm by use of Simplicity UV System, with conductivity of 0.05 µS/cm. The reference standards such as Metronidazole CRM, Lidocaine CRM and Miconazole CRM were obtained from Sigma Aldrich, USA and the tested product, vaginal suppositories containing these three active substances were obtained from Replek Farm Ltd., Skopje, N. Macedonia.

The following instruments were used during the research: analytical balance AG285 from the producer Mettler Toledo, US bath TP690/H, optical shaker KS 260 basic and HPLC system Shimadzu Nexera XR UPLC

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system with LPG quaternary pump with degasser, auto sampler, column oven, PDA detector and controller. Data acquisition, analysis and reporting were performed by Lab solution version 5.97. The type of column used was Zorbax RX C8 purchased from Agilent Technologies, Santa Clara, United States.

Syringe filters Nylon 0.45 μm that have been used to filter the solutions before transferring into vial, were purchased from Agilent Technologist, United States.

Chromatographic 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 35°C 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.

Results and discussion

These three active substances are quite different in their hydrophobicity and solubility in water and in organic solvents. Therefore, it is impossible to achieve their separation by use of isocratic mode of elution, on reversed phase bonded matrix filled column.

Due to the significant difference in the physico-chemical properties of these three active substances, the positive outcome in their separation was achieved by using gradient elution method with a total run of 15 minutes where the ratio of MFA and MFB is constantly changing.

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. The three peaks were well separated from each other, with resolution for more than 20 between each of the neighboring peaks. The obtained values of theoretical plates for metronidazole, lidocaine and miconazole were 15641, 54165 and 106051, respectively.

The method was validated in accordance to ICH guideline Q2(R1). During selectivity testing, no interference from the formulation excipients was observed. The linearity of the method was proved in five

concentration levels, for each substance, and the following results were obtained by regression analysis: correlation coefficient > 0.9990 and relative standard deviation of the response factors for each concentration level $< 2\%$, in all cases. The precision of the system and of the method were also evaluated and the obtained relative standard deviation of the responses was less or equal to 2%, in both cases, for each substance. Accuracy of the method was studied by recovery investigation. The obtained recovery values were within the range of $100 \pm 2\%$, for each substance.

Conclusion

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). The proposed method, by use of simple sample preparation, low-cost reagents, and relatively short run time, provides reproducible simultaneous quantification of all active substances of interest and could be successfully used for routine analysis of intravaginal semi solid dosage formulations containing this combination of active substances, especially in pharmaceutical industry.

References

- Akay, C., Ozkan, S.A., Senturk, Z., Cevheroglu, S., 2002. Simultaneous Determination of Metronidazole and Miconazole in Pharmaceutical Dosage Forms by RP-HPLC. *II Farmaco*, 57(11), 953–957. [https://doi.org/10.1016/S0014-827X\(02\)01296-X](https://doi.org/10.1016/S0014-827X(02)01296-X).
- Belal, T.S., Haggag, R.S., 2012. Gradient HPLC-DAD Stability Indicating Determination of Miconazole Nitrate and Lidocaine Hydrochloride in their Combined Oral Gel Dosage Form. *J. Chrom. Sci.* 50, 401–409. <https://doi.org/10.1093/chromsci/bms019>.
- British Pharmacopoeia, 2022. British Pharmacopoeia Commission - The Stationery Office: London.
- European Pharmacopoeia, 10th ed., 2019-2020. European Directorate for the Quality of Medicines & Healthcare – Council of Europe: Strasbourg.
- International Conference on Harmonization, 2005. Validation of analytical procedures: Text and methodology Q2(R1). ICH, Geneva, Switzerland.
- The United States Pharmacopoeia / The National Formulary (USP 43-NF 38), 2020. The United States Pharmacopoeia Convention: Rockville.