DEVELOPMENT OF A RAPID AND REPRODUCIBLE TLC METHOD FOR PURITY ELUCIDATION AND RELATED SUBSTANCE IDENTIFICATION OF METRONIDAZOLE IN METRONIDAZOLE DOSAGE FORMS

Sylvia Atanasova-Stamova[1], Svetlana Georgieva[1], Maya Georgieva[2]

[1]Department of Pharmaceutical chemistry, Faculty of Pharmacy, Medical University of Varna
[2]Department of Pharmaceutical chemistry, Faculty of Pharmacy, Medical University of Sofia

ABSTRACT

INTRODUCTION: Thin-layer chromatography (TLC) is a quick, inexpensive microscale technique applicable for determination of the number of components in a mixture, monitoring the progress of an organic reaction, verification of substance identity and analysis of the fraction obtained from column chromatography.

AIM: The aim of our work is to develop a rapid TLC method for related substance identification, purity elucidation, and control of synthesis of metronidazole in dosage forms and during synthesis of derivatives.

MATERIALS AND METHODS: A rapid and reproducible TLC method for quality determination of metronidazole derivatives is described. The samples and standard were separated on silica gel 60 with fluorescent indicator UV_{254} plates with three different mobile phases: A) acetic acid-ammonia-acetone-methylene chloride cyclohexane (1:2:3:3:1), B) toluene-chloroform-methanol (2:7:1), C) toluene-chloroform-ethanol-acetic acid-ammonia (9:8:1:1:1). The visualization of spots was done at 254 nm.

RESULTS: As most appropriate was established to be the mobile phase consisting of toluene- chloroform-ethanol-acetic acid-ammonia (9:8:1:1:1). The identification and determination of metronidazole in analyzed drug formulations were proved by the correspondence between the Rf of drug samples and reference standard Rf-0.58.

CONCLUSION: The TLC method for related substance determination of metronidazole in drug formulations is an informative, rapid, and reproducible method. The method may be applied for further analysis and monitoring of synthesis of new metronidazole derivatives.

Keywords: *TLC method, quality, metronidazole, tablet dosage forms*

Address for correspondence:
Sylvia Atanasova-Stamova
Faculty of Pharmacy
Medical University of Varna
84 Tzar Osvoboditel Blvd
9002 Varna
e-mail: sylviastamova@yahoo.com

Received: October 18, 2020
Accepted: November 16, 2020

INTRODUCTION

Metronidazole is an antimicrobial drug. Its spectrum of activity is determined by the possibility of susceptibility microorganisms to activate the drug. The metronidazole structure is illustrated on Fig. 1.

The chemical structure of nitroimidazole derivatives, such as metronidazole, includes some ba-

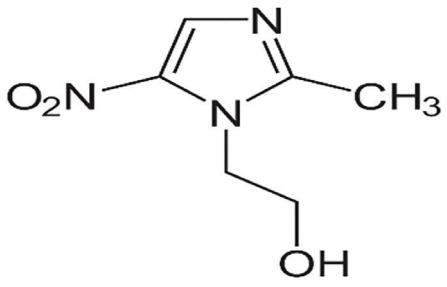

Fig. 1. Chemical structure of metronidazole

sic elements required for all of them. The imidazole heterocyclic ring constructs the main scaffold and the presence of a nitro group is essential for antimicrobial activity. However, they may be related to pro drugs, whose activation is based on reduction leading to formation of highly reactive products. The antibacterial and antiprotozoal activities are proportional to the concentration of the activated drug in the target cell. The mechanism of their activity involves four main steps. In the first two steps the drug enters into the cells by passive diffusion and an electron is transferred to the nitro group of metronidazole. The result of this step is obtaining of short-lived nitroso free radical with cytotoxic activity and possibility of interaction with cellular DNA. The third step is based on binding of the obtained nitroso free radical to the microorganism DNA and the subsequent damage of the DNA. The last step is a release of final products (1).

Metronidazole is applicable to the treatment of severe anaerobic infectious such as intraabdominal operation or other surgical procedures with different localization. Therefore, there are various pharmaceutical preparations containing metronidazole such as tablets, vaginal globules, and topical creams (2).

A lot of different methods for purity elucidation and related substance identification of metronidazole derivatives are described. The literature survey includes different types of analytical techniques for quality and quantity determination of metronidazole in body fluids and various drug forms, for example, HPLC (3), GC (4), TLC chromatographic methods (5), ultraviolet spectroscopy (6) and voltammetry (7).

Thin-layer chromatography (TLC) is a rapid and routine analytical method suitable for monitoring the progress of an organic reaction, determination of the number of components in a mixture, verification of substance's identity, and analysis of the fraction obtained from column chromatography (8). TLC is a highly selective method and it has proven to be as sensitive as HPLC in many analytical procedures. Other advantages are low-cost analyses; a small quantity of the mobile phase is enough for determination of a high number of samples, there is minimal sample preparation (9).

AIM

The aim of the present work is to develop a rapid TLC method for quality determination of metronidazole dosage forms, their purity elucidation and further application of this method for control of the synthesis of new metronidazole derivatives.

MATERIALS AND METHODS

Metronidazole standard (Fluorochem, Lot:FCC26022), toluene (anhydrous, 99.8%, Sigma-Aldrich), chloroform (HPLC grade, ≥99.9%, Sigma-Aldrich), methanol (HPLC, ≥99.9%, Sigma-Aldrich), acetic acid (glacial, Reagent Plus®, ≥99%, Sigma-Aldrich), ammonia puriss. (anhydrous, ≥99.95%, Sigma-Aldrich), acetone (HPLC, ≥99.9%, Sigma-Aldrich), dichloromethane (Sigma-Aldrich), cyclohexane (anhydrous, 99.5%, Sigma-Aldrich), ethanol (ethanol, ≥99.5%, for HPLC, Sigma-Aldrich) were used. Two different tablet dosage forms of metronidazole were kindly donated by the MedUniPharma pharmacy and were claimed to contain 500 mg metronidazole. The following equipment was used: sample applicator- 50 µL micro syringe (Hamilton, Bonaduz, Switzerland), TLC chamber (outer dimensions: 22 cm x 12 cm x 22 cm, CAMAG), pre-coated TLC sheets ALUGRAM® SIL G/UV_{254} (0.20 mm, silica gel 60 with fluorescent indicator UV_{254}, outer dimensions: 10 x 20 cm, MACHERY-NAGEL GmbH & Co. KG, Germany), Kern analytical balance, ABS 220-4N, AC/DC input 220 V AC, UK plug

EXPERIMENTAL PROCESS

A rapid and reproducible TLC determination of metronidazole drug forms is described. Based on literature data, we used three different mobile phases to determine the most sensitive and express among them for qualitative determination of metronidazole dosage form-tablets.

Sample Preparation

Standard solution of 50 mg metronidazole was dissolved in methanol and diluted with the same solvent to 10 mL.

Solutions from two different tablet dosage forms containing metronidazole were tested. To sample powder tablets equivalent to 50 mg metronidazole, we added 5 mL of methanol, diluted with methanol to 10 mL, and mixed to complete dilution.

The standard solution of metronidazole and the solutions of two tablet dosage formulations of metronidazole were freshly prepared on the day of analysis.

Mobile Phase

a. acetic acid-ammonia-acetone-methylene chloride-cyclohexane (1:2:3:3:1)
b. toluene-chloroform-methanol (2:7:1)
c. toluene-chloroform-ethanol-acetic acid-ammonia (9:8:1:1:1)

Chromatographic Procedure

Chromatographic analysis was achieved by using three different pre-coated silica gel plates SIL G/$UV_{254.}$ Five µL from each solution were spotted onto SIL G/UV_{254}plates, keeping 20 mm distance between spots. The plates were developed at room temperature in glass chromatographic chambers, previously pre-saturated for 90 minutes with mobile phases: A) acetic acid-ammonia-acetone-methylene chloride cyclohexane (1:2:3:3:1), B) toluene-chloroform-methanol (2:7:1), and C) toluene- chloroform-ethanol-acetic acid-ammonia (9:8:1:1:1). The solvents allowed to the formation of a path of 12 cm from the start line.

Detection

Plates were air dried and examined under screened ultraviolet light at 254 nm. The standards and samples appeared as violet spots. Relative ***Rf*** values were determined for each plates in each solvent system (A, B, C).

RESULTS AND DISCUSSION

The present study evaluates three different solvent systems as mobile phases for the most rapid and effective TLC determination of metronidazole dosage forms.

In the three obtained chromatograms are detected spots of standard substance and samples corresponding in size and color. We had tested various mobile phases in order to identify the optimal system for determination of metronidazole dosage for-

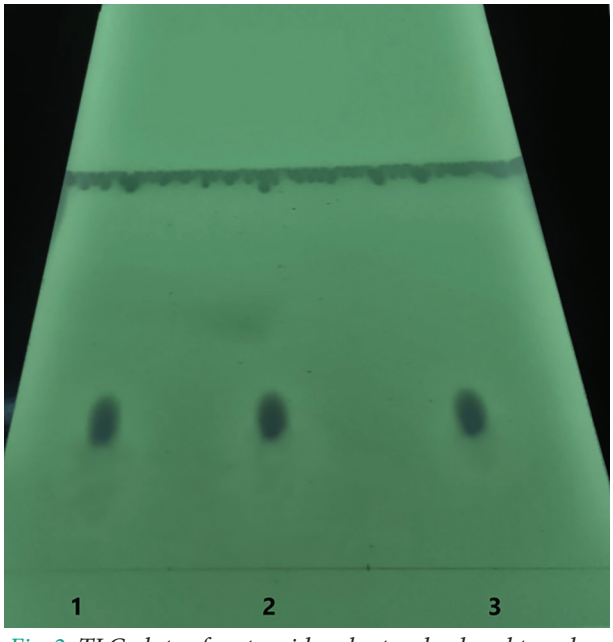

Fig. 2. TLC plate of metronidazole standard and two dosage formulations

Table 1. Chromatographic characteristics of metronidazole

Mobile Phase	***Rf*** Standard of Metronidazole	***Rf*** *Sample 1*	***Rf*** *Sample 2*	Detection in UV Light at 254 nm
acetic acid-ammonia-acetone-methylene chloride-cyclohexane (1:2:3:3:1)	0.72	0.72	0.72	violet
toluene-chloroform-methanol (2:7:1)	0.63	0.63	0.63	violet
toluene-chloroform-ethanol-acetic acid-ammonia (9:8:1:1:1)	0.58	0.58	0.58	violet

mulation by TLC (Fig. 2). To evaluate the precision of the experiment and repeatability, we did three replicate analyses per day for five consecutive days. The ***Rf*** values in the examined mobile phases are presented in Table 1.

We found the applied mobile phases to be sensitive enough for identification and determination of metronidazole-containing compounds. The most optimal ***Rf*** values were observed using the mobile phase: toluene- chloroform-ethanol-acetic acid-ammonia (9:8:1:1:1). The spots were clearly visible and similar, too. The identification and determination of metronidazole in analyzed drug formulations was proven by the correspondence between the ***Rf*** of the drug samples and reference standard ***Rf,*** both equal to 0.58. In a previous study we established that the excipients are part of tablet dosage forms and do not affect the sensitivity and specificity of metronidazole detection.

CONCLUSION

In conclusion, a fast, selective and reproducible TLC method for purity elucidation and related substance identification was developed. We found that all of the used mobile phases are sensitive enough for identification and determination of metronidazole containing compounds. The most optimal ***Rf*** values were observed using a mobile phase: toluene-chloroform-ethanol-acetic acid-ammonia (9:8:1:1:1). The method may be applied for further analysis of newly obtained metronidazole derivatives and for monitoring of the synthesis of new metronidazole derivatives.

Acknowledgments

This work was supported by the Medical University of Varna through Project No. 18019 of the Science Fund.

REFERENCES

1. Nagel JL, Aronoff DM. Metronidazole. In, Bennett JE, Dolin R, Blaser MJ, editors. Mandell, Douglas, and Bennett's principles and practice of infectious diseases.Vol.1. 8th ed. Saunders;2015, pp. 350-357.e2, doi:10.1016/B978-1-4557-4801-3.00028-X.
2. Melissa Johnson, Metronidazole: An overview. Available at: www.uptodate.com ©2020 UpToDate.
3. Tashtoush BM, Jacobson EL, Jacobson MK. Validation of a simple and rapid HPLC method for determination of metronidazole in dermatological formulations. Drug Dev Ind Pharm. 2008;34(8):840-4. doi: 10.1080/03639040801928598.
4. Wang JH. Determination of three nitroimidazole residues in poultry meat by gas chromatography with nitrogen-phosphorus detection. JChromatogr A. 2001; 918(2):435-8.doi: 10.1016/s0021-9673(01)00779-8.
5. Salem H, Riad SM, Rezk MR, Ahmed K. Simultaneous determination of metronidazole and diiodohydroxyquine in bulk powder and paramibe compound tablets by TLC-densitometry and HPLC. Pharmaceut Anal Acta. 2012; 3:10. doi: 10.4172/2153-2435.
6. Siddappa K, Mallikarjun M, Reddy PT, Tambe M. Spectrophotometric determination of metronidazole through Schiff's base system using vanillin and PDAB reagents in pharmaceutical preparations. Ecl Quím. 2008;33(4), doi:10.1590/S0100-46702008000400005.
7. Bartlett PN, Ghoneim E, El-Hefnawy G, El-Hallag I. Voltammetry and determination of metronidazole at a carbon fiber microdisk electrode. Talanta. 2005;66(4):869-74. doi: 10.1016/j.talanta.2004.12.048.
8. Bele AA, Khale A. An overview on thin layer chromatography. Int J Pharm Pharm Sci. 2011;2:256–67. doi:10.13040/IJPSR.0975-8232.2(2).256-67.
9. Sherma J. Thin-layer chromatography. In: Encyclopedia of analytical chemistry. Wiley & Sons. 2006. doi: 10.1002/9780470027318.a5918.