ISSN: 2357-0547 (Print)
ISSN: 2357-0539 (Online)

Research Article / JAPR
Ibrahim et al., 2019, 3 (4), 194-199

Journal of Advanced Pharmacy Research

Development of a Method for the Determination of Miconazole in Water Samples Using Gas Chromatography Mass Spectrometry

Mohamed W. Ibrahim[1], Ahmad A. Mohamad[2], Ahmed M. Ahmed[3] *

[1]*Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt*
[2]*Pharmaceutical of Analytical Chemistry Department, Faculty of Pharmacy, Heliopolis University, Cairo, Egypt*
[3]*Research Laboratories, Ministry of Health, Giza, Egypt*

***Corresponding author:** *Ahmed M. Ahmed, Research Laboratories, Ministry of Health, Giza, Egypt*
Tel. +201119538119
Email address: ahmedabdrabou31@yahoo.com

Submitted on: 05-08-2019; Revised on: 27-08-2019; Accepted on: 05-09-2019

To cite this article: Ibrahim, M. W.; Mohamed, A. A.; Ahmed, A. M. Development of a Method for the Determination of Miconazole in Water Samples Using Gas Chromatography Mass Spectrometry. *J. Adv. Pharm. Res.* **2019**, *3* (4), 194-199. DOI: 10.21608/aprh.2019.15686.1089

ABSTRACT

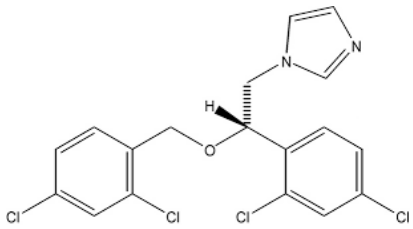
This paper describes an enhanced gas chromatography mass spectrometry (GC-MS) strategy for the analysis of Miconazole in water samples. In this study, determination of Miconazole has been carried out according to standard method for water and waste water analysis. Samples of collected water were agriculture stream water, River Nile water and Hospital waste water samples from El-Gharbia governorate in Egypt. Miconazole was extracted by solid - liquid extraction and analyzed by GC-MS. The chromatographic separation was performed using a ZB5 column (30 m × 0.53 mm, 1.50 µm), and helium as a carrier gas. The limit of detection and limit of quantification for Miconazole were 0.75and 2.50 ng/mL respectively. The intra- and inter-day precisions were lower than 0.85% while the accuracy ranged from 98.55% to 101.53. Finally, solid phase extraction (SPE) in combination with GC-MS is a sensitive and effective method for the determination of Miconazole in water samples.

Keywords: *Determination; Gas chromatography Mass Spectrometry; Miconazole; Water samples*

INTRODUCTION

Miconazole[1-(2,4-Dichloro-beta-((2,4-dichloro benzyl)oxy)phenethyl)imidazole] is an anti-fungal medication related to fluconazole, ketoconazole, itraconazole, and clotrimazole. It is used to treat fungal infections. Miconazole was approved by the FDA in 1974 and it is official in European pharmacopoeia (9th edition). This medication is effective only for infections caused by fungal organisms. It will not work for bacterial or viral infections.[1] Miconazole comes as a cream, lotion, powder, spray liquid, and spray powder to be applied to the skin. It also comes as a cream and suppository to be inserted into the vagina. [2,3] Structural formula of Miconazole is shown in **Figure 1**. Determination of Miconazole in environmental water samples usually requires the application of sample preparation procedures to extract the analyte from the aqueous solution and bring it to a suitable concentration level prior to final GC analysis. Liquid–liquid extraction (LLE) and solid phase extraction (SPE) are commonly used for the extraction of Miconazole. Miconazole was determined in pharmaceutical preparation such as tablets,[4] powder sample,[5] and water samples by using different methods such as Gas Chromatography and Flame Ionization Detector

(GC-FID),[6] Spectrophotometric Method,[7] liquid chromatography–electrospray tandem mass spectrometric,[8] and gas chromatography-ion trap-tandem mass spectrometry.[9] The objective of this study was to develop a SPE-GC-MS method for determination of Miconazole in water samples. Then the developed method was validated for linearity, precision, accuracy, limit of detection and limit of quantification.



M.WT. = 416.127

Figure 1. Structural formula of Miconazole

MATERIAL AND METHODS

Chemicals and Solvents

Miconazole nitrate 99.5% was kindly provided by Egyptian international center for import. Methanol (99.9%), acetonitrile (99.9%), acetone (99.9%) and Ammonium acetate all these chemicals were of analytical grade and all were purchased from Sigma-Aldrich (Steinheim, Germany).

Instrumentation

Gas chromatography (GC)

The chromatographic analysis was performed by the GC "Perkin Elmer" Clarus 500 model equipped with a mass detector ("Perkin Elmer" Technologies, America), and a column compartment. The chromatographic separation was achieved on a Zebron ZB5 column (30m, 0.53 mm, 1.50 µm, Phenomenex, USA). The column was operated by a flow rate of 1 mL min^{-1} and an injection volume of 2 µL. The carrier gas was helium. The control of the GC system and data processing were performed using "Perkin Elmer" TurboMass™ GC/MS software.

Analytical method

Preparation of standard stock solution

Standard stock solution of Miconazole was prepared in methanol at a concentration of 20 µg mL^{-1}. Miconazole working solutions in the desired concentration range was prepared by appropriate dilution of standard stock solution with methanol. The stock solution was prepared once a month, kept at 2–8 °C in a refrigerator and brought to room temperature before use.[10]

Calibration Curve

A series of working standard drug solutions equivalent to 10–200 ng mL^{-1} for Miconazole was prepared by diluting the stock standard solution with the methanol. To construct the calibration curve six replicates (2 µL) of each standard solution were injected immediately after preparation into the column and the peak area of the chromatograms were measured. Then, the mean peak area was plotted against the corresponding concentration of Miconazole to obtain the calibration graph as shown in **Figure 3**.

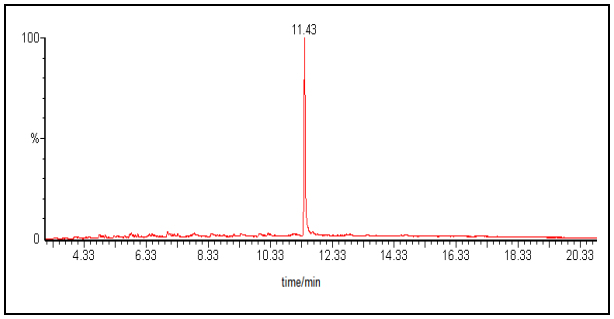


Figure 2. GC-MS chromatogram of Miconazole 100 ng mL^{-1}.

Collection of water samples

A total of 60 water samples were collected in amber glass bottles in April 2018. Samples of collected water were agriculture stream water, River Nile water and Hospital wastewater samples from El-gharbia governorate: (Before the outlet of Tala stream, Tala stream, River Nile Complex Companies, Basion region, Kafr El Dawar Village, Residential block in Zifta, Zifta General Hospital, Before the outlet of Meet El Nasara, Meet El Nasara stream, Al - Qassed Canal in Dafra, Residential block in Al Etwa elqablia, Tenth of Ramadan region, Residential block in Shakarf, Kafr El Zayat General Hospital, Al Sunta, Meet El Mokhles, biltaj, kafar hjazy, Manshawy General Hospital in Tanta and Samannoud General Hospital) (3 samples each). Coordinates of sampling locations were shown in **Table 1.**

Sampling

Water samples 2.5 Liter were collected in glass bottles at 50 cm below water level. Waters were collected by qualified personnel using standard sampling field protocol. The bottles were covered with screw caps and the samples were then stored at 4 °C until extraction and analysis. [11]

Extraction of Miconazole from water samples

Before extraction of Miconazole, water sample was filtrated. Extraction of Miconazole from water samples was performed using solid phase extraction

Table 1. Coordinates of sampling locations*

NO	Sampling locations	North	East
1	Before the outlet of Tala stream	30.79226	30.79649
2	Tala stream	30.827569	30.804606
3	River Nile Complex Companies kafr el zayat	30.823600	30.81137
4	Basion region	30.938260	30.782150
5	Kafr El Dawar Village	31.020441	30.7216948
6	Residential block in Zifta	30.724708	31.252347
7	Zifta General Hospital	30.712162	31.250128
8	Before the outlet of Meet El nasara	30.936420	30.252370
9	Meet El nasara stream	30.941090	31.245058
10	Al - Qassed Canal in Dafra	31.028206	30.730023
11	Al etwa elqablia	31.002042	30.934112
12	Tenth of Ramadan region	30.790235	30.972120
13	Shakarf	30.885279	30.912863
14	Kafr El Zayat General Hospital	30.836151	30.818528
15	Al Sunta	30.746967	31.133272
16	Meet El Mokhles	30.7844885	31.1583957
17	Biltaj	30.4971510	31.0034770
18	kafar hjazy	30.947139	31.162244
19	El-Menshawy General Hospital in Tanta	30.789354	31.001314
20	Samannoud General Hospital	30.965763	31.243411

**These numbers indicate the locations and coordinates of the sampling locations by determining the location from the north and east using GPS.*

Table 2. Analytical parameters and linear regression data of miconazole

Parameter	
Linearity range (ng mL^{-1})	50-200
LOD (ng mL^{-1})	0.75
LOQ (ng mL^{-1})	2.50
Regression equation*	
Correlation coefficient	0.999
Slope (b)	53330
Intercept (a)	6990

**Y= a + bC, where Y is the peak area and C is the concentration in ng mL^{-1}.*

procedure. The optimised procedure used for the analysis of Miconazole in water samples was as follows: Oasis HLB cartridges were conditioned with 10 mL of a mixture of ACN/dichloromethane (1:1 v/v), followed by 5 mL of methanol and 3 mL of bidistillated water, without allowing the cartridge to dry out. Then, 2.5 mL of methanol were added to 250 mL of water and the samples were passed through the conditioned cartridges at a flow rate of approximately 4 mL/min under vacuum. The cartridges were dried for 2 h under vacuum and afterwards the analytes were eluted from the solid phase with 3 mL of acetone, 3 mL of n-hexane/acetone (1:1 v/v), and finally with 3 mL of n-hexane. The extract was evaporated to dryness under a stream of nitrogen. The absolute recovery of the target compound from the water samples was 99.03%. 2µL of each extracted solution was injected into the gas chromatography.

Method Validation

The analytical method for quantification of Miconazole has been validated for linearity, precision, accuracy, and robustness following appropriate recommendations of the ICH Q2 (R1) regulatory guidelines recommendations.[12]

Linearity

Six working standard solutions of Miconazole in the concentration of 10-200 ng mL^{-1} was prepared in triplicate and injected. Calibration graphs were plotted between concentration and mean peak area.

Limit of detection and limit of quantification

The limit of detection (LOD) is minimum amount of analyte in sample detectable and larger than uncertainty associated with it and the limit of quantification (LOQ) is amount quantitatively measured with suitable precision and accuracy. LOD and LOQ were calculated using the following equations: [12]

$$LOD = c_s \frac{3}{S/N}$$

$$LOQ = c_s \frac{10}{S/N}$$

Where S/N is the average signal to noise ratio and C_s is the concentration of the injected analyte.

Accuracy and Precision

The accuracy and precision were determined at three different concentration levels (50, 100 and 200 ng mL^{-1}) in terms of both intra and inter-day precision. For intra-day precision three distinct concentrations of Miconazole in the linearity range was prepared in triplicate and was analyzed on the same day. For inter-day precision the same concentrations were analyzed on three consecutive days and RSD values were calculated. Instrument precision was analyzed by injection repeatability. The accuracy and precision were calculated and expressed in terms of percent recovery and standard deviation, respectively.

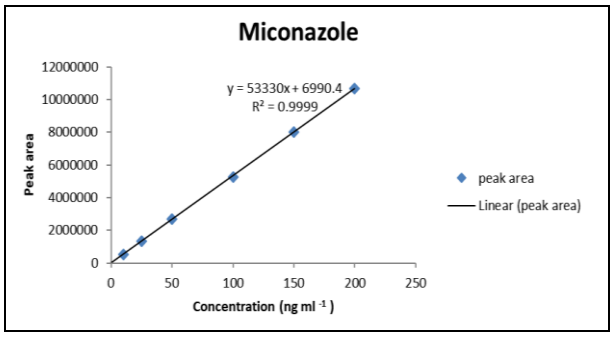


Figure 3. Calibration curve of Miconazole.

RESULTS AND DISCUSSION

Optimization of chromatographic condition

The oven temperature program was as follows: initial temperature maintained at 100 °C for 1 min, raised to 175 °C at a rate of 25 °C/min, hold 1 min, then raised to 280 °C at a rate of 5 °C/min, hold 3 min, and the injector temperature was maintained at 250 °C. Methanol was used as diluent. The Chromatograms obtained in these operating conditions for diluent, standard solution of Miconazole was shown in **Figure 2.**

Validation

Calibration graphs were constructed between the peak areas versus their corresponding concentrations as shown in Fig.3. Good linearity was obtained in the concentration of 10-200 ng/mL for miconazole and the results are shown in **Table 2**. The precision of the method was calculated and relative standard deviation (RSD) values were evaluated according to ICH. The RSD% values ranging from 0.26% to 0.58% for intra-day precision and from 0.18% to 0.85% for inter-day precision studies, respectively, confirmed that the method was sufficiently precise. Low RSD values indicated satisfactory precision for the drug. Good recoveries were obtained and were found to be between 98.55-101.53% for Miconazole; the results are given in the **Table 3**. LOD and LOQ were determined using signal to noise ratio method. For Miconazole it was 0.75 and 2.50µg/L. The estimated limits were verified by analyzing a suitable number of samples containing the analyte at the corresponding concentrations. There was no considerable change in the peak areas and RT. This method was rapid, simple and sensitive to the determination of Miconazole in water using a precision device such as GC.

Table 3. Intra –day and Inter –day precision

Drug	Concentration (ng/ml)	The concentration found (ng/mL) ± SD; RSD			
		Intraday precision	Accuracy	Interday precision	Accuracy
Miconazole	50	50.37 ± 0.29; 0.58	100.74	50.77 ± 0.43; 0.85	101.53
	100	98.92 ± 0.43; 0.43	98.92	98.55 ± 0.18; 0.18	98.55
	200	200.64 ± 0.52; 0.26	100.32	200.40 ± 0.53; 0.26	100.20

Table 4. Mean ± SD of miconazole residue levels (ng/mL) in water samples

NO	Sampling locations	Found concentration (mean ± SD)
1	Before the outlet of Tala stream	not detected
2	Tala stream	11.98±0.73
3	River Nile Complex Companies kafr el zayat	not detected
4	Basion region	not detected
5	Kafr El Dawar Village	not detected
6	Residential block in Zifta	not detected
7	Zifta General Hospital	14.93±0.27
8	Before the outlet of Meet El nasara	not detected
9	Meet El nasara stream	not detected
10	Al - Qassed Canal in Dafra	not detected
11	Al etwa elqablia	not detected
12	Tenth of Ramadan region	not detected
13	Shakarf	not detected
14	Kafr El Zayat General Hospital	13.14±0.82
15	Al Sunta	not detected
16	Meet El Mokhles	not detected
17	Biltaj	not detected
18	kafar hjazy	not detected
19	El-Menshawy General Hospital in Tanta	21.99±0.38
20	Samannoud General Hospital	20.35±0.62

Miconazole residues

In this study, a method was developed to determine Miconazole in agriculture stream water, River Nile water and Hospital waste water samples using the chromatographic technique in El-Gharbia governorate. **Table 4** contains a summary of Miconazole concentrations in samples collected during the study. These results indicated that, Miconazole was not observed in River Nile water in all collected samples. However, it was observed in some agricultural streams and Hospital waste water samples.

CONCLUSION

A validated, sensitive and accurate GC-MS analytical method was developed for the analysis of Miconazole in water samples. The method was fully validated according to the ICH guidelines and presented

good linearity, accuracy, precision and robustness. The LOD and LOQ values were established by using signal to noise ratio method. Miconazole was observed in some agricultural streams and Hospital wastewater samples of El-Gharbia governorate hospitals. However, it was not observed in River Nile water in all sampling locations. The proposed method can be successfully applied for determination of Miconazole in water samples using SPE- GC-MS method.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgements

The author acknowledges to staff members Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy Al-Azhar University, Cairo, Egypt for their co-operation throughout the whole work. Also acknowledgment goes to Research Laboratories, Ministry of health, Giza, Egypt for providing the necessary facilities and support in completing this work.

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