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Application of HPLC method in determination of miconazole nitrate in environmental samples from el-gharbia governorate in Egypt

Abstract

This paper describes an enhanced High-performance liquid chromatography (HPLC) method 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 wastewater analysis. Samples of collected water were agriculture stream water, River Nile (Surface water samples) water and Hospital wastewater samples from El-gharbia governorate in Egypt. Miconazole was extracted by liquid-liquid extraction and analyzed by HPLC. The chromatographic separation was performed using a Phenomenex C8 column, flow rate of 0.8mL/min, and UV detection at 220nm. The optimized HPLC system was achieved using mobile phase composition containing methanol: water (85:15v/v). The intra-day and inter-day precisions were lower than 0.58 while the accuracy ranged from 99.06% to 101.53%. Finally, liquid-liquid phase extraction in combination with HPLC is a sensitive and effective method for the determination of Miconazole Nitrate in water samples. Miconazole was observed in some agricultural streams and waste water samples of El-gharbia governorate hospitals.

Keywords: determination, miconazole nitrate, water samples, HPLC chromatography.

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Abbreviations: HPLC, high-performance liquid chromatography; LLE, liquid–liquid extraction; SPE, solid phase extraction; GC, gas chromatography; FID, flame ionization detector; MS, mass spectrometry

Introduction

Miconazole]1-(2,4-Dichloro-beta-((2,4-dichlorobenzyl)oxy) phenethyl)imidazole [is an anti-fungal medication related to fluconazole, ketoconazole, itraconazole, and clotrimazole. It is used either on the skin or in the vagina for fungal infections. Miconazole was approved by the FDA in 1974. Miconazole prevents fungal organisms from producing vital substances required for growth and function. 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] Miconazole has a molecular formula of $C_{18}H_{14}C_{l4}N_2O$ and molecular weight of 416.127g/mol.[3] 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 HPLC or GC analysis. Liquid–liquid extraction (LLE) and solid phase extraction (SPE) are commonly used for the extraction of miconazole. Literature survey revealed a validation of a chromatographic method for miconazole assay from oral sustained release muco-adhesive tablets.[4]

Development of high performance liquid chromatography method for miconazole analysis in powder sample,[5] simultaneous determination of Miconazole Nitrate and Metronidazole in different pharmaceutical dosage forms by Gas Chromatography and Flame Ionization Detector (GC-FID),[6] development and validation of an extractive spectro-photometric method for miconazole nitrate assay in pharmaceutical formulations.[7] Validation of a solid-phase extraction and liquid chromatography–electro spray tandem mass spectrometric method for the determination of nine basic pharmaceuticals in wastewater and surface water samples,[8] determination of drugs of abuse in water by solid-phase extraction, derivatisation, gas chromatography-ion trap-tandem mass spectrometry[9] and application of GC-MS in determination of malathion in environmental samples.[10] The objective of this study was to develop a HPLC method for determination of miconazole in water samples. Then the developed method was validated for linearity, precision, accuracy and robustness.

Materials and methods

Chemicals and Solvents

Miconazole Nitrate 99.5% was kindly provided by Egyptian international center for import. Structural formula of miconazole is shown in Figure 1. Organic solvents methanol (HPLC gradient grade) all these chemicals were of analytical grade and all were purchased from Sigma-Aldrich (Steinheim, Germany). Deionized water (WP 4100 reagent grade water purifier-SMEG) was used for standard and sample preparations.

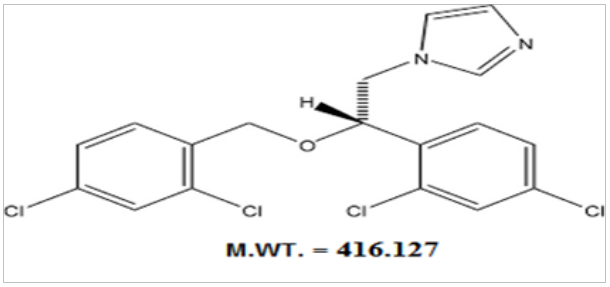

Figure 1 Structural formula of miconazole.

Instrumentation

HPLC Instrument: The HPLC system used was Hitachi L-2000 series (Japan), equipped with a Model L-2130 pump, an L injection loop and a UV-Vis detector L2420. The separation was carried out in a

Phenomenex C8 column Luna 10µ (150x4.6mm). The on-line solvent vacuum degasser, an auto sampler with 5 mobile phase consisted of methanol: water (85:15v/v). The system was operated isocratically at flow rate 0.8min/mL and UV wavelength 220nm.[5]

Analytical method

Preparation of standard stock solution: Standard stock solution of Miconazole was prepared by dissolving 5mg standard Miconazole in 10mL of methanol. Miconazole working solutions in the desired concentration range was prepared by appropriate dilution of standard stock solution with mobile phase. The prepared stock solution was kept at 4ºC until use.

Calibration curve: A series of working standard drug solutions equivalent to 10–100µg mL^{-1} for Miconazole was prepared by diluting the stock standard solution with the methanol. Standard solutions were found to be stable during the analysis time. To construct the calibration curve six replicates 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. Calibration curve of miconazole was shown in Figure 2.

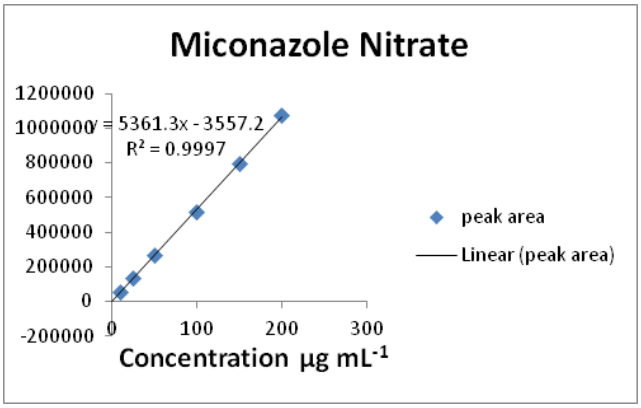

Figure 2 Calibration curve of miconazole.

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 2.5L were collected in glass bottles at 50cm 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 4ºC until extraction and analysis.

Extraction of miconazole from water samples: Extraction of Miconazole from water samples was performed using liquid-liquid phase extraction procedure. A measured volume of 1L of the sample was transferred in a 2L separation funnel and was extracted twice with 60mL of 15% methylene chloride in hexane. The samples were shaken vigorously; the organic layer was taken and dried over anhydrous sodium sulfate and evaporated in a rotating evaporator. The volume of solution was attained to 10mL with mobile phase. 3µl of each extracted solution was injected into the HPLC.[11] Table 2 contains a summary of Miconazole concentrations in samples collected during the study.

Table 1 Coordinates of sampling locations

NO	Sampling locations	N	E
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	30.8236	30.81137
4	Basion region	30.93826	30.78215
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	30.93642	30.25237
9	Meet El nasara stream	30.94109	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	30.97212
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	30.497151	31.003477
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

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Table 2 Contains a summary of miconazole concentrations in samples collected during the study

No.	Sampling locations	Found concentration (Mean±SD)
1	Before the outlet of Tala stream	not detected
2	Tala stream	12.07±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	15.03±0.25
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	22.23±0.54
20	Samannoud General Hospital	20.19±0.47

Results

Optimization of chromatographic condition

Methanol-water (85:15v/v), flow rate 0.8mL/min, and UV detector wavelength 220nm have been chosen as the optimized HPLC condition for determination of Miconazole Nitrate in different water samples because it gave the best baseline of miconazole peak (base to base), standard solution of Miconazole was shown in Figure 3.

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 10-100µg mL^{-1} was prepared in triplicate and injected. Calibration graphs were plotted between concentration and mean peak area. Analytical parameters and linear regression data of miconazole were shown in Table 3.

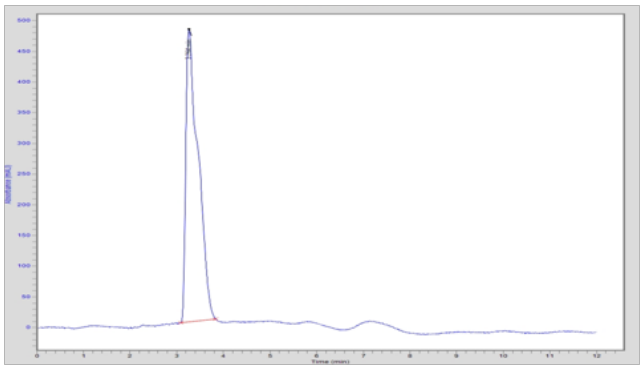

Figure 3 GC-MS chromatogram of miconazole 100µg mL^{-1}.

Table 3 Analytical parameters and linear regression data of miconazole

Parameter	
Linearity range (µg mL^{-1})	10-200
LOD (µg mL^{-1})	7.46
LOQ (µg mL^{-1})	22.61
Regression equation*	
Correlation coefficient	0.999
Slope (b)	5316
Intercept (a)	3557

Accuracy and precision

The accuracy and precision were determined at three different concentration levels (10, 50 and 100µg 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 and shown in Table 4.

Table 4 Intra-day and Inter-day precision

Drug	Concentration (µg/ml)	The concentration found (µg/L)±SD; RSD			
		intraday precision	accuracy	interday precision	accuracy
Miconazole	10	9.97±0.81 ; 0.82	99.73	9.91±1.27 ; 1.29	99.06
	50	50.37±0.57 ; 0.58	100.74	50.77±0.84 ; 0.85	101.53
	100	99.97±0.57 ; 0.58	99.97	99.11±0.68 ; 0.69	99.11

Robustness

Robustness is an important aspect of method validation for chromatographic methods. The influence of small deliberate changes

in the operations (variations) of the analytical procedure is evaluated from measured or calculated responses. This is to verify that the method performance is not affected by typical changes in normal experiments. Robustness was evaluated by changing the flow rate (0.8±0.1ml min^{-1}). The measured response variances were the % RSDs and shown in Table 5. The effect of different UV detector wavelength was also investigated in this study. UV detection was varied in three different wavelengths 210, 220 and 230nm, UV detector wavelength 220nm has been chosen as the optimized wavelength.

Table 5 Robustness data

Parameter	Validation	%RSD of the area miconazole
Flow rate	0.7ml/min (low)	0.47
	0.9ml/min (high)	0.54

Discussion

This study aims to detect miconazole nitrate in environmental samples using the HPLC method. Our new concept is to use a simplified method to determine concentration of Miconazole nitrate in the environment and follow-up development. To achieve accurate, rapid and effective separation of miconazole in a limited time, simple mobile phase was used for evaluation. Optimal conditions for the separation of miconazole and matrix peaks established were as follows: mobile phase that consisted of methanol: water (85:15v/v), isocratic elution at a flow rate of 0.8mL/min and UV detection 220. According to the requirements of ICH (2005), these conditions were found to be most suitable for separation and quantification of miconazole. The results of that analyzes showed that all water samples collected and analyzed were completely free of miconazole. However, it was observed in some agricultural streams and Hospital waste water samples. These results are consistent with the results of other studies conducted by some colleagues in the field of work. These results are expected as a result of the frequent and poor use of miconazole in hospitals or from some patients, whether in the treatment of skin or oral diseases and thus caused the presence in these places at these rates mentioned.

The results of this study are identical to the results of another research I have prepared with some colleagues but using GC-MS device where it was found that the two methods are valid for the detection of miconazole nitrate in environmental samples (Development of a method for the determination of Miconazole in Water Samples using Gas Chromatography Mass Spectrometry) is a research that has not yet been published but has been prepared in the current period under review. Considering the conclusions of the study presented shows that the presence of ratios of miconazole in some samples, which causes a lot of health and environmental risks in the case of accumulation of these substances and misuse and this is a new understanding of this problem (misuse negatively and exacerbates the problem and does not give good solutions). Therefore, I suggest trying to limit the use of these substances that may affect public health and the environment while improving the medical care of patients and full supervision of their treatment and determine the quantities and method of optimal use in order to avoid such risks. The next step is the detection of miconazole nitrate in environmental samples in Egypt, taking into account the detection of miconazole degradation products so that we can detect early the risk of degradation products and reduce these risks. I believe that over time, Dispose of miconazole nitrate in the wrong way or misuse and accumulation of miconazole in water will lead to major problems.

Miconazole residues

In this study, a method was developed to determine Miconazole in agriculture stream water, River Nile water and Hospital wastewater samples using the chromatography technique in El-gharbia governorate. 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 wastewater samples (Tala stream, Zifta general hospital, Kafr El Zayat General Hospital, El-Menshawy General Hospital in Tanta, Samannoud General Hospital). Table 2 Mean±SD of miconazole residue levels (µg/L) in water samples. The results of this research are exactly the same as the results I have developed using the GC-MS method (A search I've prepared and this search is under review now).

Limitations of the study

The most important limitations faced by this study are that the samples were withdrawn from a few places. We should have carried out the examination and analysis and extracted samples from more places and large areas covering a large part of the Egypt (From more hospitals) to obtain more comprehensive and credible results, but that would require a lot of money and Research is not funded by any organization but is a personal effort. Lack of time is also one of the important reasons and obstacles to research.

Conclusion

A validated, sensitive and accurate HPLC 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. Miconazole was observed in some agricultural streams and Hospital wastewater samples of El-gharbia governorate hospitals. However, it was not observed in River Nile (surface water) water in all sampling locations. The proposed method can be successfully applied for determination of Miconazole in water samples using liquid-liquid extraction HPLC method.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Data availability

The readers can access the data represented in this research article by contacting with the author on this email: mailto:ahmedabdrabou31@yahoo.com as the data presented is his own personal experiment and work.

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