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RESEARCH ARTICLE

A rapid MCM-41 dispersive micro-solid phase extraction coupled with LC/MS/MS for quantification of ketoconazole and voriconazole in biological fluids

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Abstract

A rapid dispersive micro-solid phase extraction (D-μ-SPE) combined with LC/MS/MS method was developed and validated for the determination of ketoconazole and voriconazole in human urine and plasma samples. Synthesized mesoporous silica MCM-41 was used as sorbent in D-μ-SPE of the azole compounds from biological fluids. Important D-μ-SPE parameters, namely type desorption solvent, extraction time, sample pH, salt addition, desorption time, amount of sorbent and sample volume were optimized. Liquid chromatographic separations were carried out on a Zorbax SB-C_{18} column (2.1 × 100 mm, 3.5 μm), using a mobile phase of acetonitrile–0.05% formic acid in 5 mM ammonium acetate buffer (70:30, *v*/v). A triple quadrupole mass spectrometer with positive ionization mode was used for the determination of target analytes. Under the optimized conditions, the calibration curves showed good linearity in the range of 0.1–10,000 μg/L with satisfactory limit of detection (≤0.06 μg/L) and limit of quantitation (≤0.3 μg/L). The proposed method also showed acceptable intra- and inter-day precisions for ketoconazole and voriconazole from urine and human plasma with RSD ≤16.5% and good relative recoveries in the range 84.3–114.8%. The MCM-41-D-μ-SPE method proved to be rapid and simple and requires a small volume of organic solvent (200 μL); thus it is advantageous for routine drug analysis.

KEYWORDS

dispersive micro-solid phase extraction, ketoconazole, LC/MS/MS, MCM-41, voriconazole

1 | INTRODUCTION

Ketoconazole and voriconazole are systemic azole pharmaceuticals that can be taken orally or intravenously. Ketoconazole, a synthetic imidazole antifungal, is effective for superficial fungal infections, genital candidosis and chronic mucocutaneous candidosis, and has been widely used in immunocompromised patients and advanced prostatic carcinoma (Parfit, 1999; Levine, 1982; Danesmend and Warnock, 1988; Borelli *et al*., 1979). Meanwhile, voriconazole, a triazole antifungal agent, has been approved for the treatment of invasive fungal infection with a broad spectrum, including *Aspergillus* Cryptococcus and *Candida* species (Wang, Huang, Sun, Xiao, & Wang, 2015). Voriconazole is used in the treatment of invasive pulmonary aspergillosis and respiratory disorders as an antifungal agent (Steinbach and Stevens, 2003; Walsh *et al*., 2008). Clinical studies have indicated that a high incidence of adverse reactions, such as liver dysfunction, visual disturbance and neurological toxicity, may occur during the treatment with voriconazole (Dolton *et al*., 2012; Luong *et al*., 2012; Somchit *et al*., 2012; Tan, Brayshaw, Tomaszweski, Troke, & Wood, 2006; Ueda *et al*., 2009). Azoles may also participate in interactions with many drugs in the event of substantial amounts of residue in the human body, thus posing a possible threat to human health (Gordien *et al*., 2009; Xia, Zhi, Wang, Chen, & Cheng, 2012). It is, therefore, crucial to perform therapeutic drug monitoring of azoles in biological matrices, for which, rapid, simple, accurate and sensitive methods are imperative.

Biological materials are complicated and may contain acids, bases, salts, proteins and other organic compounds with similar properties to the target analytes, which often exist at minute concentration levels in samples (Kataoka, 2010). Regardless of the development of highly efficient analytical instrumentations for end-point determination of analytes

in biological samples, such as UV–vis spectrophotometry, fluorimetry, gas and liquid chromatography, capillary electrophoresis and immunoassay methods, most of them cannot handle the matrix directly (Farhadi, Hatami, & Matin, 2012). Therefore, a sample preparation procedure is necessary to remove major interferences and to extract and concentrate analytes from complex matrices prior to instrumental analysis.

Classical sample preparation techniques such as liquid–liquid extraction (LLE) (Araujo, Conrado, Palma, & Costa, 2007; Decosterd *et al*., 2010; Baietto *et al*., 2012; Verweij-van Wissen *et al*., 2012; Pauwels, Vermeersch, Eldere, & Desmet, 2012), supercritical fluid extraction (Chou, Chang, Liu, Yang, & Wu, 2007) and solid-phase extraction (SPE; Gordien *et al*., 2009; Huang, Yu, Tang, & Peng, 2010; Beste, Burkhardt, & Kaever, 2012; Chitescu, Oosterink, de Jong, & (Linda) Stolker, 2012) have been developed for the isolation and enrichment of azole antifungal compounds. LLE is the most preferable extraction method; however, it is laborious, time-consuming, consumes large amounts of toxic organic solvents and shows poor analyte enrichment. Although SPE uses smaller amounts of solvent than LLE, a specific sorbent of SPE is relatively expensive. Supercritical fluid extraction with carbon dioxide as extraction must be performed at above the critical temperature of 31°C and critical pressure of 74 bar, which requires a highly efficient delivery extraction system that adds to higher operating costs.

In recent years, much effort has been devoted to the development of microextraction methods for azole antifungals compounds such as liquid-phase microextraction (Moradi *et al*., 2010), solidification of floating organic droplet microextraction (Adlnasab, Ebrahimzadeh, Yamini, & Mirzajani, 2010), solid-phase microextraction (Bordagaray, García-Arronaa, & Millán, 2013), ultrasound-enhanced surfactant-assisted dispersive liquid–liquid microextraction (Xia *et al*., 2012) and solid-phase membrane tip extraction (Yahaya *et al*., 2014). These microextraction methods not only minimize the extraction phase to a microliter scale, but also contribute to rapid, high-sensitivity, inexpensive and environmentally friendly analysis.

A recently introduced microextraction method known as dispersive micro-solid phase extraction (D-μ-SPE) overcomes most of the shortcomings in conventional extraction methods. D-μ-SPE is performed by adding the sorbent into the sample to interact directly with the analytes and the analytes are then desorbed to a small volume of appropriate desorption solvent after discarding most of liquid and drying. D-μ-SPE avoids channeling or blocking of the pores, which easily occurs in conventional SPE columns or disks, by allowing the analytes in aqueous sample to interact equally with all sorbent particles to achieve greater capacity per mass of sorbent used (Tsai, Huang, Huang, Hsue, & Chuang, 2009; Fu, Tzing, Chen, Wang, & Ding, 2012). D-μ-SPE satisfies most of the criteria of good sample preparation method, including low consumption of sorbent and organic solvents, simplicity and being relatively fast.

This work describes a rapid, simple, environmentally friendly and highly sensitive D-μ-SPE incorporated with mesoporous silica, MCM-41 (MCM-41-D-μ-SPE) approach to the determination of ketoconazole and voriconazole in human urine and plasma with good accuracy using liquid chromatography tandem mass spectrometry (LC/MS/MS). Hexagonally ordered mesoporous silica MCM-41 has distinct characteristics of large surface area, uniform pore structure and huge pore volume with unique mechanical, chemical and thermal stability (Hou, Ma, Du, Deng, & Gao, 2004). Owing to its distinct properties, MCM-41 has been successfully used as a extraction sorbent and drug delivery support material for nonsteroidal anti-inflammatory drugs (Kamaruzaman, Sanagi, Endud, Wan Ibrahim, & Yahaya, 2013; Günaydin and Yilmaz, 2015).

The proposed method offers rapid analysis with an analysis time of <10 min with simple sample preparation steps of extraction using simple apparatus. The method was optimized and validated and applied to the spiked human urine and plasma samples.

2 | EXPERIMENTAL

2.1 | Chemicals materials

Voriconazole (VRZ) and ketoconazole (KTZ) were obtained from Clearsynth (Mumbai, India). The chemical structures and pK_a of the selected azole antifungals are shown in Figure 1. Tetraethyl orthosilicate and cetyltrimethylammonium bromide were purchased from Sigma-Aldrich (St Louis, MO, USA). Ammonia solution (NH_4OH) (28%) and hydrochloric acid (36%) were obtained from QRëC Asia (Selangor, Malaysia). Ammonium acetate, sodium hydroxide and formic acid were purchased from Merck (Darmstadt, Germany). All chemicals were of analytical-reagent grade. LC-grade acetonitrile, isopropanol and methanol were obtained from J.T. Baker (Pennsylvania, USA). Stock standard solutions (1000 μg/mL) of the azole drugs were prepared in methanol and stored in the freezer at about −4°C. The stock solutions prepared were stable for at least a month. Double-distilled deionized water of 18.2 MΩ was purified using Nano ultrapure water system (Barnstead, USA). Analytical-grade sodium chloride (NaCl) was purchased from Bendosen (Bendosen, Norway).

2.2 | LC/MS/MS conditions

Liquid chromatography was performed on an Agilent 1200 series ultra-high-performance liquid chromatograph (Santa Clara, CA, USA) coupled to an Agilent 6460 triple quadrupole mass spectrometer with electrospray ionization in positive mode (LC/MS/MS). The LC/MS–MS comprised an Agilent Zorbax SB-C_{18} column (100 × 2.1 mm, 3.5 μm particle size; Santa Clara, CA, USA). The isocratic mobile phase used was 5 mM ammonium acetate buffer with 0.05% (*v*/v) formic acid and acetonitrile (30:70, v/v). The flow rate was set at 0.2 mL/min. The overall run time was 5.0 min. The detection was operated in the multiple reaction monitoring (MRM) mode under unit mass resolution in the mass analyzers. The MRM transitions were *m/z* 531.2 → 82.1 and *m/z* 349.31 → 127.0 for

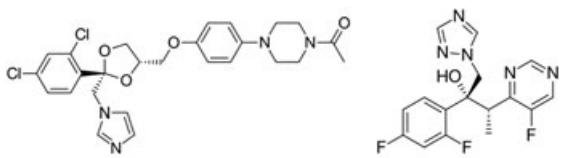

FIGURE 1 The chemical structures and pK_a of the selected azole antifungals. [a] Adlnasab *et al*. (2010). [b] http://www.drugbank.ca (accessed 12 May 2016)

KTZ and VRZ, respectively. The optimized MS–MS conditions were as follows: collision gas, nitrogen; flow rate, 6 L/min; temperature, 300°C; nebulizer pressure (N_2), 15 psi; capillary potential, 400 V.

2.3 | Sample pretreatment for human urine and plasma

Urine samples were collected from a healthy volunteer with no recent history of drug taking and the sample was diluted to a ratio of 1:1 (v/v) with distilled water. A spiked urine sample was prepared by diluting the standard mixture in 15 mL of diluted urine sample and subsequently the sample pH was adjusted to 4.0. Drug-free human plasma sample was obtained from Penang General Hospital (Penang, Malaysia) and stored at −4°C prior to analysis. Exactly 3 mL of plasma sample was spiked with mixed standard solutions and adjusted to pH 4.0 using 1 M of hydrochloric acid followed by vortexing to ensure complete mixing of the content. The plasma sample was then mixed with 2 mL of acetonitrile and centrifuged at 4185 ***g*** force for 10 min to precipitate the protein. The resulting supernatant solution (~5 mL) was transferred to a sample vial and diluted to 15 mL with water.

2.4 | D-μ-SPE

MCM-41 without any further modification was synthesized and characterized based on a previously reported work (Taib, Endud, & Katun, 2011). Firstly, the diluted sample (15 mL) was placed in a 50 mL centrifuge tube containing 20 mg of MCM-41 sorbent. Then, the resulting mixture was vortexed (VELP Scientifica, Italy) at maximum speed (2400 rpm) for 1.5 min to facilitate the dispersion of sorbent in the sample. After the extraction, the sorbent was collected on a filter paper (Whatman, UK) and transferred to a 500 μL safe-lock tube. The tube was then added with 200 μL of methanol and sonicated for 5 min to desorb the analytes. The solution was filtered through a 0.2 μm nylon syringe filter (Membrane Solutions, China) prior to LC/MS/MS analysis.

2.5 | Validation of the method

Validation of MCM-41-D-μ-SPE was carried out in terms of linearity, limits of detection (LODs), limits of quantification (LOQs), precisions and recovery. LODs were obtained as three times the signal-to-noise ratio and LOQs measured as 10 times the signal-to-noise ratio. Intra- and inter-day precisions were determined at low, medium and high concentrations (0.5, 5, 100 and 5000 μg/L) with triplicate analyses on the same day ($n = 3$) and over three different days ($n = 9$) for both samples, respectively. Relative recovery was calculated as the percentage of mean concentration of target analytes found after extraction (derived from the plotted matrix-matched calibration curve) against the concentration spiked in the sample.

3 | RESULTS AND DISCUSSION

3.1 | MCM-41-D-μ-SPE optimization

The Fourier transform infrared spectroscopy (FTIR) (Figure 2a) and X-ray Diffraction (XRD) (Figure 2b) analyses were in fairly good agreement with previously reported works, which confirmed that the material was MCM-41 (Taib *et al*., 2011; Shen *et al*., 2014). From the FTIR result, the broad peak at 3453 cm^{-1} was due to O–H stretching of water; the band at 1641 cm^{-1} corresponded to bending mode of O–H. Two obvious peaks observed at around 1086 and 800 cm^{-1} were attributed to the asymmetric and symmetric stretching mode of Si–O–Si group, respectively. The peak at 968 cm^{-1} could be assigned to the lattice defect of MCM-41 framework. Finally, the adsorption band at 459 cm^{-1} corresponded to the bending vibration of Si–O–Si. An XRD diffractogram of MCM-41 exhibited an intense signal at $2\theta = 2.2°$ owing to the (1 0 0) plane and weak signals between 4.0° and 6.0° owing to (1 1 0), (2 0 0) and (2 1 0) planes. These are characteristics of the long ordered hexagonal mesoporous phase of MCM-41 material. More than three peaks were resolved in the diffractogram, indicating a highly ordered structure. From the BET analysis, the specific surface area of MCM-41 was found to be 1046 m^2/g. Several important parameters affecting MCM-41-D-μ-SPE were optimized to enhance the extraction efficiency of target KTZ and VRZ from urine and plasma samples. Details of optimized parameters are discussed herein.

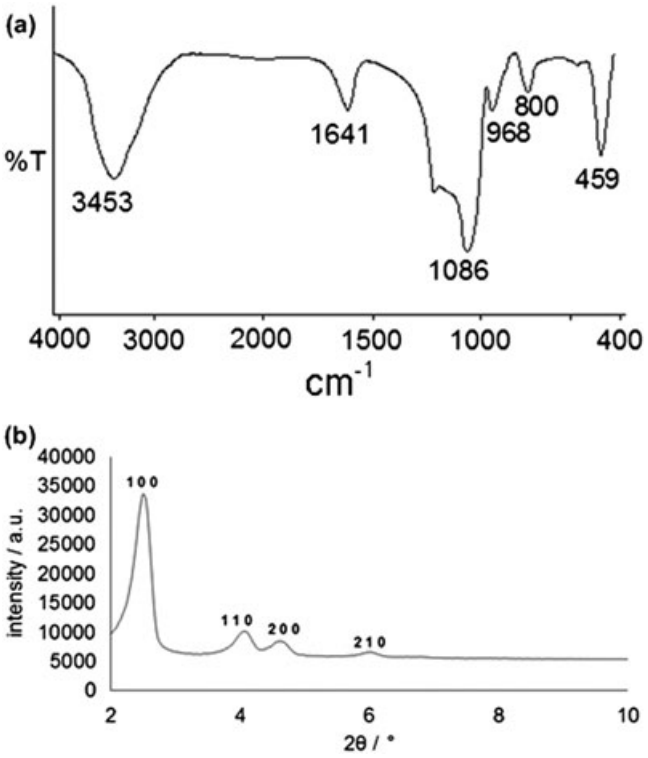

FIGURE 2 FTIR (a) and XRD (b) results of synthesized MCM-41

3.2 | Effect of desorption solvent

The choice of the desorption solvents relies on its compatibility with the chromatographic system. Common organic solvents, namely methanol, acetonitrile and isopropanol, which are compatible with LC/MS/MS analysis, were evaluated as desorption solvents for azole antifungal drugs in this study. Under identical conditions, methanol showed the highest peak areas for most of the analytes followed by isopropanol and acetonitrile (Figure 3a). This observation can be explained by high solubility of KTZ and VRZ in methanol (both stock standard solutions

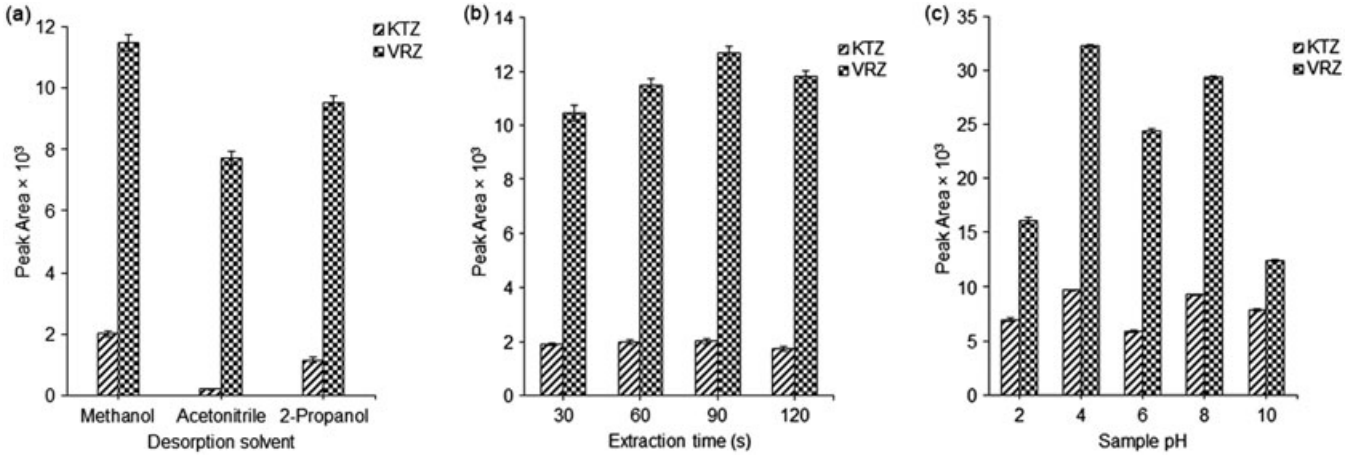

FIGURE 3 Effect of type of desorption solvent (a), extraction time (b) and sample pH (c) on MCM-41-dispersive micro-solid phase extraction (D-μ-SPE) from spiked water ($n = 3$ in each case). Error bars represent the standard deviations

were diluted in methanol). Therefore, methanol was selected as the desorption solvent and used in subsequent analyses.

3.3 | Effect of extraction time

Mass transfer in D-μ-SPE is a time-dependent process. Thus, the effects of different extraction times ranging from 20 to 120 s were investigated in this study. The results (Figure 3b) indicated that extraction time did not significantly affect the extraction efficiency. The reason for this could be that the dispersive mode in D-μ-SPE allowed for the direct contact between the sorbent and the sample solution, which expedite rapid mass transfer toward system equilibrium. It was found that 90 s is adequate to achieve maximum extraction of azole drugs by MCM-41. Beyond a 90 s extraction time, no significant increase was observed for both target analytes. Therefore, 90 s was adopted as the optimum extraction time and used in subsequent experiments.

3.4 | Effect of sample solution pH

Sample solution pH is a very effective parameter to improve the extraction efficiency of acidic and basic compounds. The extraction efficiency in MCM-41-D-μ-SPE is enhanced by converting the analytes into molecular forms. Since KTZ and VRZ are weak base drugs with pK_a values in the range of 2.27–12.71 (see Figure 1), they are likely to exist in their protonated or cationic forms at pHs lower than their pK_a values and molecular forms at pHs higher than their pK_a. Thus, the effect of sample solution pH was investigated from pH 2 to 10. The results (Figure 3c) reveal that the best extraction efficiencies for KTZ and VRZ were obtained at sample pH 4. These indicated that the adsorption mechanisms in MCM-41-D-μ-SPE were contributed by hydrophobic interactions and hydrogen bondings (Qin, Ma, & Liu, 2007). Thus, pH 4 was selected as the optimum sample pH in this study. Similar pH conditions were also reported previously for the analysis of KTZ in various environmental matrices (Huang *et al*., 2010; Chen *et al*., 2012).

3.5 | Effect of salt addition

The effect of salt addition to the sample solution was examined by adding NaCl to the sample solution at different concentrations (0, 5, 10, 15 and 20%, *w*/*v*). It was found that the presence of NaCl in the microextraction system did not improve the extraction efficiency of KTZ and VRZ. It has been reported that salt may interact with the targeted drugs in the sample solution, through electrostatic or ion-pairing interactions, thus reducing the mass transfer of the drugs to the sorbent (Chaves, Silva, Queiroz, Lanças, & Queiroz, 2007). Therefore, no salt was added to the sample solution in subsequent experiments.

3.6 | Effect of desorption time

Different desorption times (2, 5 and 10 min) were investigated. The extraction efficiency was highest at desorption time of 5 min (Figure 4a). No further improvement in the amount of KTZ and VRZ present in the concentrated solvent was observed when desorption time was prolonged to 10 min. Thus, desorption time of 5 min was fixed for the following experiments.

3.7 | Effect of amount of MCM-41

To evaluate the effect of the amount of sorbent on the extraction efficiency, different amounts of MCM-41 (10, 20 and 30 mg) were tested. The results (Figure 4b) clearly showed that the optimum extraction of KTZ and VRZ was achieved using 20 mg of MCM-41 as sorbent. Further addition of MCM-41 (30 mg) did not result in significant increases in extraction efficiency. Hence, subsequent experiments were carried out using 20 mg of MCM-41 as sorbent.

3.8 | Effects of sample volume

In order to further enhance the partitioning of KTZ and VRZ into the sorbent, the effect of sample volume was examined using different sample volumes (10, 20 and 30 mL). As shown in Figure 4(c), the chromatographic signals for KTZ and VRZ increased with sample volume and reached their maximum levels at 10–15 mL. Nonetheless, the extraction efficiency decreases at a sample volume of 20 mL. This phenomenon

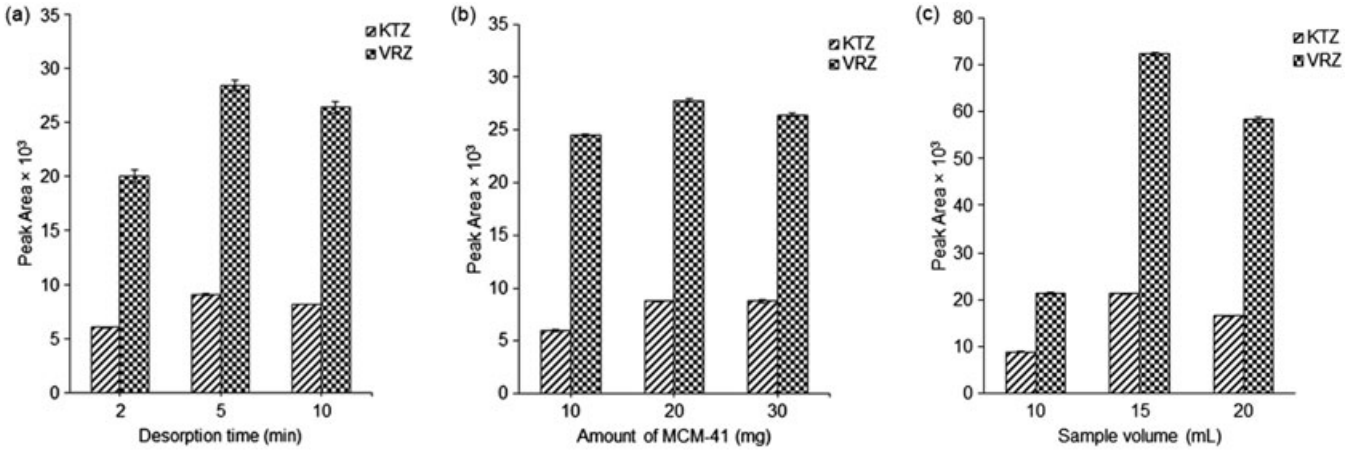

FIGURE 4 Effect of desorption time (a), amount of MCM-41 (b) and sample volume (c) on MCM-41-D-μ-SPE from spiked water ($n = 3$ in each case). Error bars represent the standard deviations

could be due to the saturation of MCM-41 adsorption sites for a larger sample volume (See, Sanagi, Wan Ibrahim, & Naim, 2010). Therefore, a sample volume of 15 mL was adopted in subsequent experiments.

3.9 | Validation of the methods

KTZ and VRZ were spiked into blank urine and plasma samples at different concentrations for the purpose of method validation. Under the optimized extraction conditions, a series of experiments were carried out to determine the linearity, LODs and LOQs of the proposed method. It is clear that the proposed MCM-41-D-μ-SPE method showed good linearity in the concentration range of 0.1–10,000 and 0.3–10,000 μg/L, with coefficient of determination, r^2, of ≤0.9937. Under the optimized conditions, the proposed method showed excellent detection limits (0.008–0.03 μg/L) and acceptable precisions (RSD ≤ 12.34%; Table 1). Intra-day and inter-day precisions were assessed by performing three replicate ($n = 3$) analyses of spiked urine and plasma samples at different concentration levels (0.5, 5, 100 and 5000 μg/L) on the same day and over three different days ($n = 9$). The proposed method showed satisfactory intra- and inter-day precisions, indicated by the RSDs of ≤7.3 and ≤16.5%, respectively (Table 2). The method also showed good

TABLE 1 MCM-41-D-μ-SPE-LC/MS/MS validation data for spiked urine and plasma samples

Matrix/analytes	Linearity range (μg/L)	Coefficient of determination (r^2)	LOD[a] (μg/L)	LOQ[b] (μg/L)	Precision, RSD (%, $n = 3$)
Urine					
KTZ	0.1–10,000	0.9990	0.008	0.025	0.70–12.34
VRZ	0.1–10,000	0.9999	0.01	0.03	0.93–6.48
Plasma					
KTZ	0.1–10,000	0.9937	0.03	0.08	0.72–4.53
VRZ	0.3–10,000	0.9990	0.06	0.3	0.43–12.30

[a]Calculated from signal-to-noise =3.

[b]Calculated from signal-to-noise =10.

TABLE 2 Relative recoveries (%) and precisions (% RSD) of MCM-41-D-μ-SPE-LC/MS/MS of spiked urine and plasma samples

Concentration level (μg/L)	Urine		Plasma	
	KTZ	VRZ	KTZ	VRZ
Intra-day recoveries (RSD, %, n = 3)				
0.5	108.3 (4.5)	89.4 (6.8)	109.1 (8.7)	93.5 (6.0)
5	93.4 (4.1)	94.5 (2.7)	100.9 (8.0)	98.7 (14.1)
100	98.8 (1.9)	101.6 (1.7)	101.8 (7.2)	104.5 (6.2)
5000	114.8 (1.9)	102.8 (2.8)	114.4 (7.2)	89.0 (8.9)
Inter-day recoveries (RSD, %, n = 9)				
0.5	104.2 (7.1)	84.3 (2.5)	94.2 (14.7)	85.4 (16.5)
5	109.5 (5.2)	100.0 (1.4)	113.7 (14.2)	86.4 (15.2)
100	113.1 (2.2)	107.2 (1.5)	107.4 (4.9)	104.3 (3.6)
5000	111.7 (0.7)	114.3 (1.3)	106.0 (5.9)	102.0 (6.7)

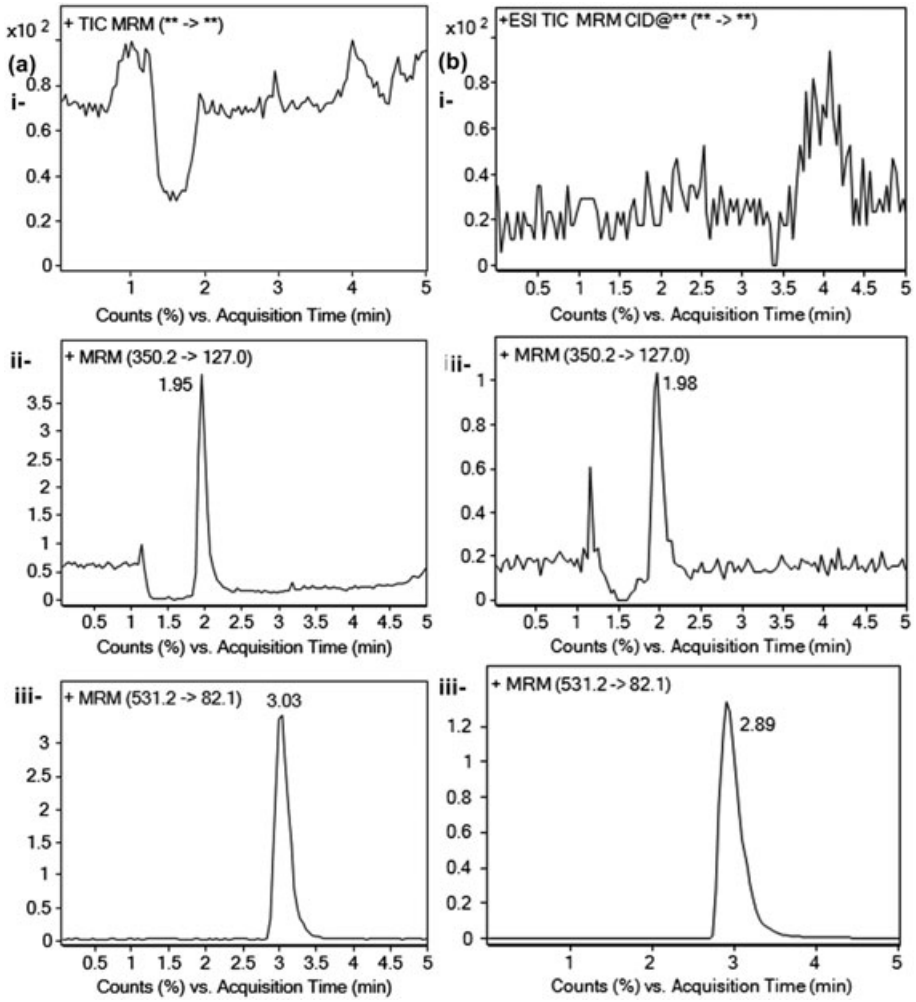

FIGURE 5 Total ion chromatogram for: (a) i, blank urine, ii, spiked urine at 5 μg/L of VRZ, iii, spiked urine at 5 μg/L of KTZ; and (b) i, blank plasma, ii, spiked plasma at 5 μg/L of VRZ, iii, spiked plasma at 5 μg/L of KTZ after being subjected to MCM-41-D-μ-SPE-LC/MS/MS under optimum conditions

relative recoveries in the range of 84.3–114.8% for KTZ and VRZ from urine and plasma samples. The recovery is certainly acceptable, and the results demonstrate that the extraction efficiencies are stable indicated by good RSD values. Figure 5 shows a total ion chromatogram for spiked (a) urine and (b) plasma samples at 5 μg/L of VRZ and KTZ after they were subjected to MCM-41-D-μ-SPE-LC/MS/MS under optimum conditions. Clean chromatograms of KTZ and VRZ were derived by using MCM-41-D-μ-SPE combined with LC/MS/MS method, which indicates an efficient sample clean-up.

TABLE 3 Comparison of analytical characteristics of MCM-41-D-μ-SPE of azole antifungal drugs with other reported methods

Matrices	Method	Analyte(s)	Extraction time (min)	LOD/LLOQ (μg/L)	Precision, RSD (%)	Recovery (%)	Reference
Human urine and plasma	MCM-41-D-μ-SPE-LC/MS/MS	KTZ VRZ	10	0.008–0.06	0.7–16.5 (n = 9)	84.3–114.3	This work
Human plasma	Protein precipitation–UPLC-MS/MS	VRZ	11	2	6.24–9.47 (n = 6)	84.11–88.33	Wang *et al*., 2015
Human plasma	SPE (HLB)-HPLC-MS/MS	VRZ	10	2	3.69–8.12 (n = 15)	98.8–102.7	Beste *et al*., 2012
Human blood	UESA-DLLME-HPLC-DAD	KTZ	3	1.1	3.9–6.2 (n = 3)	80–89	Xia *et al*., 2012
Plasma and urine	SFODME-HPLC-PDA	KTZ	40	0.014	4.7–8.6 (n = 5)	93.6–98.15	Adlnasab *et al*., 2010
Human plasma	SPE-HPLC-UV	KTZ, VRZ	—	20	0.012–1.008 (n = 24)	87.6–98.8	Gordien *et al*., 2009

D-μ-SPE, dispersive micro-solid phase extraction; HLB, hydrophilic and lipophilic balance; KTZ, ketoconazole; SPE, solid-phase extraction; UESA-DLLME, ultrasound-enhanced surfactant-assisted dispersive liquid–liquid microextraction; VRZ, voriconazole.

3.10 | Comparison of MCM-41-D-μ-SPE with other published methods

Analytical performance of MCM-41-D-μ-SPE combined with LC/MS/MS was compared with other reported methods (Table 3). In general, each method has its advantages and disadvantages. SPE produced satisfactory sensitivity, precision and recoveries, yet it is relatively expensive owing to the selective sorbent of hydrophilic and lipophilic balance towards azole compounds (Gordien *et al*., 2009; Beste *et al*., 2012). Solidification of floating organic droplet microextraction (SFODME) showed excellent sensitivity; however it requires longer extraction time to reach equilibrium (>30 min; Adlnasab *et al*., 2010). Ultrasound-enhanced surfactant-assisted dispersive liquid–liquid microextraction (UESA-DLLME) achieved good sensitivity in shorter extraction time, but the use of chlorinated extraction solvent is toxic to human health (Xia *et al*., 2012). Recently, a simplified protein precipitation method was developed for the extraction of VRZ from human plasma. This method was rapid and met the requirements of high sample throughput in bioanalysis. However, the detection limit was not satisfactory (Wang *et al*., 2015). MCM-41-D-μ-SPE utilized only 20 mg of mesoporous silica which allowed maximum interaction between the analytes and the sorbent particles, resulting in shorter extraction time and higher sensitivity compared with other methods. Moreover, MCM-41-D-μ-SPE requires simple apparatus and ultrasonication system. Hence, the proposed method can be a useful alternative approach for 'green' microextraction of azole antifungals in biological matrices.

4 | CONCLUSIONS

The development and validation of MCM-41-D-μ-SPE and LC/MS/MS methods for the quantification of KTZ and VRZ in urine and plasma matrices have been described. This validated microextraction and chromatographic separation method covers a wide range of linearity for KTZ and VRZ (0.1–10,000 μg/L) with high sensitivity of detections and is suitable for the estimation of KTZ and VRZ at different therapeutic dose levels for pharmacokinetics studies, as well as for therapeutic drug monitoring. The proposed MCM-41-D-μ-SPE procedure is simple, easy, economical and only requires 20 mg of MCM-41 and 200 μL of organic solvent for each analysis. The simplicity and small volume can be advantageous for routine drugs analyses.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

ABBREVIATIONS USED

D-μ-SPE	dispersive micro-solid phase extraction
KTZ	ketoconazole
LLE	liquid–liquid extraction
MRM	multiple reaction monitoring
SPE	solid-phase extraction
VRZ	voriconazole

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