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A microcrystalline cellulose/metal-organic framework hybrid for enhanced ritonavir dispersive solid phase microextraction from human plasma

Fotouh R. Mansour [a,*], Reda M. Abdelhameed [b], Sherin F. Hammad [a], Inas A. Abdallah [c], Alaa Bedair [c], Marcello Locatelli [d,*]

[a] Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Tanta University, Tanta 31111, Egypt
[b] Applied Organic Chemistry Department, Chemical Industries Research Institute, National Research Centre, Giza 12622, Egypt
[c] Department of Analytical Chemistry, Faculty of Pharmacy, University of Sadat City, Elgeish Street, Tanta, Sadat City, Monufia 32897, Egypt
[d] Department of Pharmacy, University "G. d'Annunzio" of Chieti-Pescara, Via dei Vestini 31, Chieti 66100, Italy

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ABSTRACT

A hybrid of microcrystalline cellulose (MCC) with metal organic frame (MOF) was developed as an efficient adsorbent for dispersive solid phase microextraction (dSPME) of ritonavir from spiked human plasma. The developed sorbent was characterized by a high-resolution scanning electron microscope (HR-SEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR). The MCC was immersed in a solution of $CaCl_2$, stirred with soda water and 1,3,5-benzenetricarboxylic acid, resulting in white MCC/MOF composites, which were separated, washed, and dried. The MCC/MOF hybrids were examined under the HR-SEM and showed morphological features different form the MCC and Ca-1,3,5-benzenetricarboxylic acid (Ca-BTC) MOF. The diffraction patterns of Ca-BTC/MCC composites clearly displayed the characteristic Ca-BTC MOF diffraction bands, indicating that MCC was successfully incorporated in the formation of crystalline MOF hybrids. The FTIR spectra exhibited the bands of MCC, as well as the bands of Ca-BTC MOFs. The prepared Ca-BTC/MCC MOF was applied for dSPME of ritonavir from human plasma, before the determination by high performance liquid chromatography with UV detection. After method optimization, the best extraction efficiency was achieved by using 12.50 mg of Ca-BTC/MCC MOF as a sorbent and 250 μL of acetonitrile as an eluent. This novel sorbent (Ca-BTC/MCC) merges the advantages of the high surface areas of MOFs 820 $m^2\ g^{-1}$ with the biodegradability and sustainability of MCC. Besides, Ca-BTC/MCC exhibited higher extraction efficiency compared with unmodified Ca-BTC MOF. To the best of our knowledge, this work applies the Ca-BTC/MCC sorbents for dSPME of ritonavir in human plasma, for the first time. These results open the door to more applications of these composites in sample preparation for biomedical analysis.

1. Introduction

Sample preparation is one of the most significant and critical stages in chemical analysis. Several techniques, such as liquid-liquid extraction (LLE) (Hammad et al., 2022), protein precipitation (Hammad et al., 2021), and solid-phase extraction (SPE) (Bagheri et al., 2012), have been developed and extensively used (Hamed et al., 2023; Nerín, 2007; Zhao et al., 2012) SPE is more versatile and efficient than LLE in terms of simplicity, low organic solvent usage, adsorbent flexibility, and high enrichment factor (Jamali et al., 2013). The particle size and surface area of the sorbent in SPE plays a critical role in SPE efficiency (Fritz & Macka, 2000). In 1989, Pawliszyn developed solid phase microextraction (SPME) as a miniaturized sample preparation process (Arthur & Pawliszyn, 1990; Belardi & Pawliszyn, 1989). In SPME, an appropriate sorbent is coated on/in a fiber during sample treatment (Nawała et al., 2018). Compared with conventional extraction, micro-extraction is a non-exhaustive extraction technique that only requires a few milligrams of the sorbent or of the extractant (Mansour & Danielson, 2017; Mansour & Khairy, 2017). In the last three decades, SPME has been widely employed as a sample preparation method for the extraction of organic and inorganic analytes from complicated real-world samples (Bagheri et al., 2012).

Despite all of the benefits and applications of this technology, the classic SPME has some limitations related to thickness and homogeneity

* Corresponding authors.
E-mail addresses: fotouhrashed@pharm.tanta.edu.eg (F.R. Mansour), marcello.locatelli@unich.it (M. Locatelli).

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of the sorbent coating, and short SPME fiber's lifespan. Dispersive solid phase microextraction (dSPME) is a non-fiber SPME technology that was designed to overcome classic SPME's intrinsic limitations. The sorbent is not deposited on the fiber core in this technique, but is instead poured directly into the sample solution to extract the analyte. The sorbent is then disseminated to enhance the sorbent's contact surface with the sample solution (for analyte extraction) or acceptor phase (for analyte desorption into an organic solvent) and drastically shorten extraction and desorption time (Abdallah et al., 2023; Tsai et al., 2009).

The development of novel material with tiny particle size and large surface area is an ongoing research trend (Hashemi et al., 2018; Mabrouk et al., 2023). Porous materials have numerous applications in pharmaceutical and biomedical analysis. These porous materials include zeolites, activated carbon, conjugated microporous polymers, hyper-cross-linked polymers (Khodabandeh et al., 2017; Mansour et al., 2021), layered double hydroxides (Abdallah et al., 2023) and metal organic frameworks (MOFs) (Hammad et al., 2024). MOFs are an attractive class of porous materials composed of metal clusters and organic linkers. (Duan et al., 2020). MOFs are built by coordinating an assembly of metal ions known as secondary building units with organic cross linkers (Xuan et al., 2012).

Microcrystalline cellulose (MCC) could be prepared from cellulose, the most abundant renewable polymer in nature with several uses. MCC has been employed as a sorbent phase in various SPME techniques due to its numerous benefits, including high concentration of hydroxyl groups, and the fact that it is renewable, recyclable, and biodegradable (Bhasney et al., 2020). Because of these benefits and qualities, MCC is one of the finest alternatives for use as a sorbent phase in microextraction procedures (Ghaemi & Amiri, 2020; López-García et al., 2018; Norrrahim et al., 2018). On the other hand, a hybrid material with unique features is desired to considerably improve the properties of the sorbent in the dSPME process (Liu et al., 2013; Zhang et al., 2014). In this work, a new MCC/MOF hybrid was prepared and applied for the first time to extract ritonavir from human plasma.

Ritonavir (Fig. 1) is a peptidomimetic HIV protease inhibitor that combines efficacy, selectivity, and oral bioavailability in an unfamiliar way. (Marsh et al., 1997). This antiviral agent has potent activity against HIV-1 laboratory and clinical strains (Marsh et al., 1997). The reported C_{max} and C_{min} of ritonavir are 11.2 ± 3.6 and 3.7 ± 2.6 μg/mL, respectively, at 600 mg twice daily according to European medicine agency (Agency, 1985). Different analytical techniques were used to determine ritonavir in biological fluids including LC-MS/MS (Damaramadugu et al., 2010; DiFrancesco et al., 2007; Gajula et al., 2012; C. Liu et al., 2022; Myasein et al., 2009; Notari et al., 2012; Rouzes et al., 2004), HPLC/UV (Frappier et al., 1998; Hoetelmans et al., 1998; Marsh et al., 1997; Sarasa-Nacenta et al., 2001), TLC (Imam et al., 2023) and spectrophotometry (Jarusintanakorn et al., 2013). LC-MS/MS is the most common technique to determine ritonavir in biological fluids, owing to its high sensitivity and great selectivity. However, LC-MS/MS is sophisticated technique, and it requires well-trained personnel (Abdallah et al., 2022).

In a previous work, we prepared a composite of MCC and Ca-1,3,5-benzenetricarboxylic acid (Ca-BTC) MOF for the first time and compared its performance as a sorbent to the unmodified Ca-BTC MOF (Abdelhameed et al., 2023). The results showed that the Ca-BTC/MCC MOF hybrid exhibited better extraction recoveries than Ca-BTC MOF with analytes that were rich in hydrogen bond donating groups or have relatively high molecular weights. In this work, we apply the Ca-BTC/MCC MOF hybrid to enrich ritonavir plasma samples by dSPME before HPLC-UV. This simple procedure of sample preparation helps to pre-concentrate the analyte using the minimal amounts of sorbents and solvents, which is congruent with the current interest in green analytical chemistry and the sustained development goals.

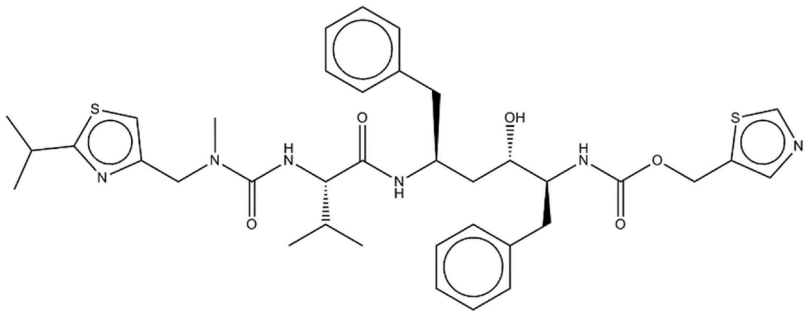
2. Experimental

2.1. Materials

Ritonavir (99.90 % purity grade) was provided by Global Napi Pharmaceuticals, located in the 6th of October City (Egypt). Gliclazide (99.90 %) was made available from Amoun (Al Qalyubia, Egypt). Acetonitrile (ACN), phosphoric acid, methanol (MeOH), potassium dihydrogen phosphate, ethanol (EtOH), $CaCl_2$, 1,3,5-benzenetricarboxylic acid (BTC), sodium hydroxide, ethanol, and microcrystalline cellulose were purchased from Merck (Darmstadt, Germany). Trichloroacetic acid was purchased from Fine Chem Limited (Mumbai, Maharashtra 400,030, India).

2.2. Instrumentation

SEM images were obtained by an HRSEM Quanta FEG 250 with field emission gun to analyze the morphological properties of MOFs. A Malvern Panalytical X'PertPRO PANalytical diffractometer (K X-ray at 45 kV, 40 mA, = 1.5406) was used to characterize both crystallinity and phase purity of the prepared MOFs. The Fourier transforms infrared spectra of MOFs were measured using a JASCO FT/IR 6100 spectrometer. BET measurements were carried out using Autosorb-01 (Quantachrome TouchWin™). Separations were carried out using a Dionex UltiMate 3000 HPLC (Thermo ScientificTM, DionexTM, Sunnyvale, CA, USA). A WPS-3000TSL autosampler, an LPG-3400SD quaternary pump, a VWD-3000 variable wavelength detector, and a TCC-3000SD column thermostat comprise the instrument. Chromeleon 7 software was used for data processing and collecting. A Cyan-CL008 Centrifuge (Medilabs Kampala, Uganda) was employed for phase separation while pH adjustment was performed using a Jenway® 3510 pH-meter (Staffordshire, UK).



Ritonavir

Fig. 1. Chemical structure of ritonavir.

2.3. Chromatographic conditions

At 40 °C, chromatographic separation was carried out on a Thermo Hypersil ODS C8 column (250×4.6 mm, 5 μm). A mixture of phosphate buffer (50 mM, pH 2.5): acetonitrile (40:60, *v:v*) was used as the mobile phase, in an isocratic mode, the flow rate was 1 mL/min, and the injection volume was 20 μL. The response of the pharmaceuticals (Ritonavir and the internal standard) were monitored using 220 nm UV detection.

2.4. Preparation of Ca-BTC MOF

To obtain a clear solution of Ca-BTC, 0.42 g of 1,3,5-benzenetricarboxylic acid was dissolved in 100 mL of 1 N NaOH solution. Concomitantly, $CaCl_2$ was dissolved in 10 mL of pure water (0.316 g). At 25 °C, the two solutions were mixed and stirred for 20 min. The reaction mixture was kept in the oven for 24 h at 90 °C. After this period, Ca-BTC was collected and rinsed with 99.9 % EtOH before filtering through a Whatmann filter paper.

2.5. Preparation of Ca-BTC/MCC MOF composites

To prepare the Ca-BTC/MCC MOF composites, 0.5 g MCCs were individually immersed in a 50 mL water solution containing 0.316 g $CaCl_2$ and stirred for 1 h at room temperature. Then, 50 mL of soda water containing 0.42 g of 1,3,5-benzenetricarboxylic acid was stirred continuously into the mixture. The MCC turned white after 8 h. The composites were centrifuged from the mixture, washed twice with ethanol, and then vacuum-dried for 12 h at 60 °C.

2.6. Preparation of standard and working solutions

Separate stock standard solutions of ritonavir and gliclazide were produced in methanol at 1 mg/mL and kept at 4 °C until use. By diluting the methanolic stock solution with deionized water, the working solution was freshly constructed. All working solutions had a concentration of 20 μg/mL.

2.7. Extraction procedures

The sample treatment starts with deproteinization using trichloroacetic acid (TCA). Accordingly, 2 mL of human plasma sample, spiked with ritonavir and gliclazide, was mixed with 75 μL of TCA, and vortexed for 1 min for efficient mixing. The tube was then centrifuged for 5 min at 6000 rpm for protein precipitation. After that, 12.5 mg of MOF was added to 1 mL of supernatant and vortexed for 5 mins. Afterwards, the tube was centrifuged for 5 mins, and the supernatant was discarded, 250 μL of ACN was added to the sorbent to elute the analytes. Finally, the tube was vortexed and centrifuged again for 5 mins each, before the supernatant was filtrated by syringe filter (0.22 μm) and transferred to the HPLC instrument for analysis. The procedures of dSPME are summarized in Fig. 2.

2.8. Method optimization

To achieve the best extraction efficiency, one variable at a time (OVAT) approach was used. Different experimental variables of dSPME were studied to attain maximum enrichment. These variables included the type of adsorbent, amount of adsorbent, and type and volume of eluent solvents. Ca/BTC MOF and the MCC/MOF hybrid were investigated as sorbent types. The sorbent amount was investigated in the range of 2.5–15 mg. Different solvents, including water, methanol, acetonitrile, acetone, and ethanol, were tested to select the best eluent. Additionally, the eluent volume was optimized in the range of 150–1500 μL. The experimental conditions that yielded the highest peak intensities were deemed optimum and selected for application.

2.9. Method validation

The validation of HPLC method was carried out according to the

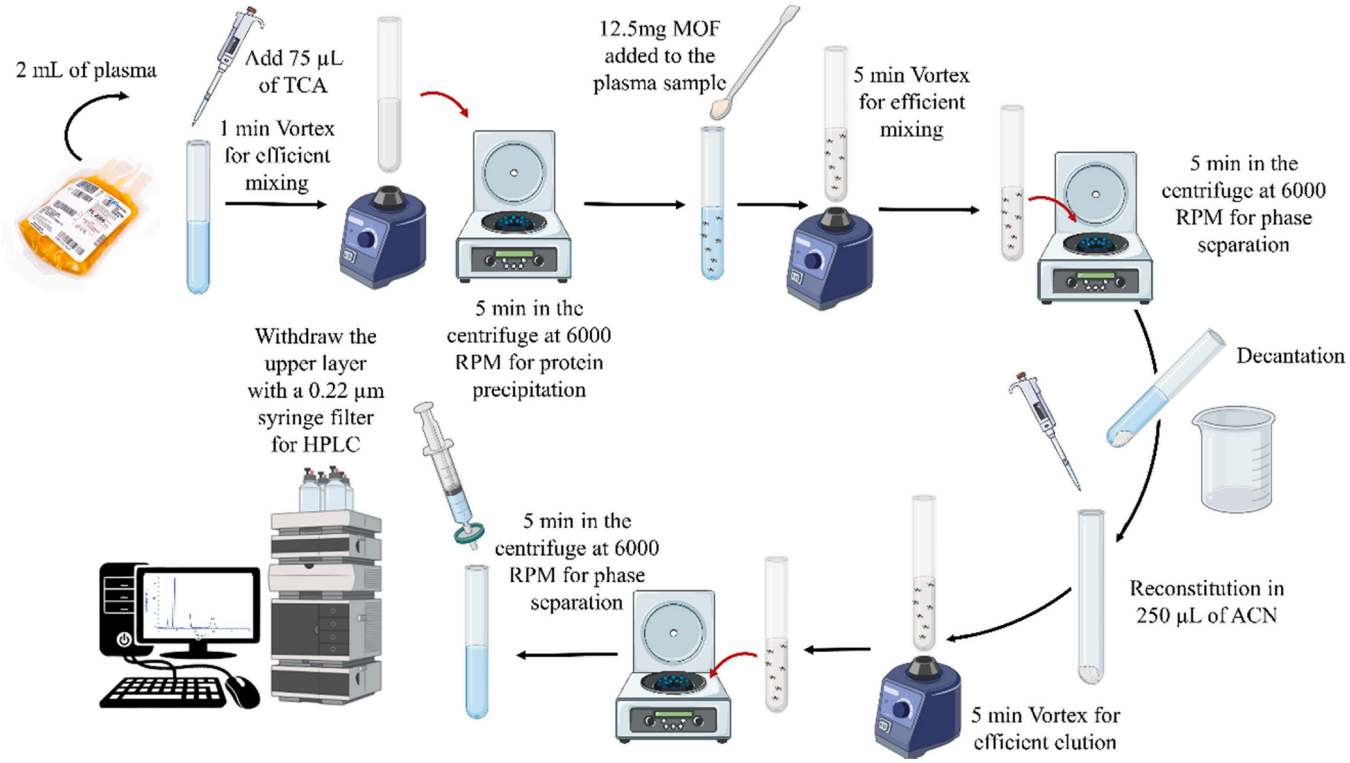


Fig. 2. Schematic presentation of the dSPME procedures of the determination of ritonavir in human plasma.

guidelines of the US Food and Drug Administration for bioanalytical methods (FDA, 2018) with respect to selectivity, linearity and range, lower limit of quantitation (LLOQ), trueness, and precision.

2.9.1. Selectivity

The method's selectivity was assessed by evaluating human plasma from six distinct lots to look for possible interferences with ritonavir peak. Blank plasma samples were prepared (without analytes) and chromatographically compared to another set of standard samples spiked with ritonavir at their respective LLOQ values.

2.9.2. Linearity and range

The calibration curve was created by plotting the nominal standard concentration against the peak area ratio of ritonavir to gliclazide. The selected concentrations of ritonavir were 1, 2, 5, 10, 15, 20 μg/mL.

2.9.3. Precision and trueness

The trueness and the intra-day precision were assessed by analyzing six replicates containing ritonavir at four quality control (QC) levels: LLOQ, low QC (LQC), medium QC (MQC), and high QC (HQC), which were 1, 3, 10, 18 μg/mL for. Inter-day trueness and precision were determined by assessing six replicates containing ritonavir at four QC samples on three separate runs. The proposed method's trueness was evaluated as a percentage recovery. The FDA guidelines indicated that the recovery (%) should not exceed 15% for all QC levels except the LLOQ, which is allowed to be 20% or less of the nominal values. The relative standard deviation RSD(%) was used to assess precision. The acceptable standards for RSD (%) are 15% across the QC samples except that 20% at the LLOQ is allowed.

3. Results and discussion

3.1. Preparation and characterization of sorbents

Both the MOF and the MCC/MOF hybrid were prepared using the conventional solution method, following the procedures summarized in Section 2.4 and 2.5. Scheme 1 illustrates the synthesis of the Ca-BTC/MCC MOF composite sorbent. Ca-BTC was prepared *in situ* in the presence of MCC. The structures of Ca-BTC and MCC underwent changes during the formation of the Ca-BTC/MCC MOF composite sorbent, and these changes in chemical bonds and interactions were confirmed using FTIR.

Fig. 3a shows the diffraction pattern of MCC. The broad peaks at 12.10°, 20.10°, 22.30°, and 34.60°, which were typical for cellulose II crystals, could be seen in the MCC crystal structures. Fig. 3b shows the diffraction pattern of Ca-BTC. The unit cell parameters for the PXRD peaks are as follows: $a = 10.94$, $b = 6.73$, $c = 18.58$, and $\alpha = \beta = \gamma = 90.00°$ (orthorhombic). The PXRD peaks have been indexed with space group Pnm a 63. Fig. 3c presents the diffraction patterns of Ca-BTC/MCC. The compounds clearly display the Ca-BTC diffraction bands,

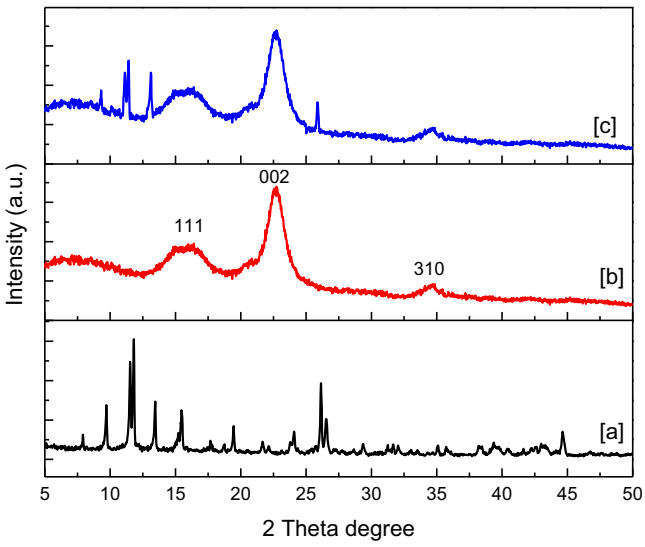


Fig. 3. PXRD pattern of [a] MCC, [b] Ca-BTC, and [c] Ca-BTC/MCC.

indicating that MCC has been successfully incorporated into the formation of crystalline Ca-BTC.

Fig. 4a shows the FTIR spectrum of MCC. The band at 1024 cm^{-1} is related to C—O, while the CH_2—CH group appears at 2900 cm^{-1}, and the

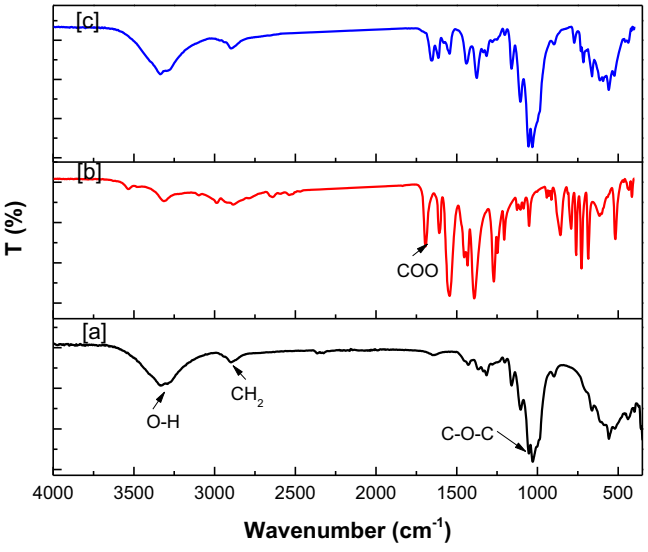
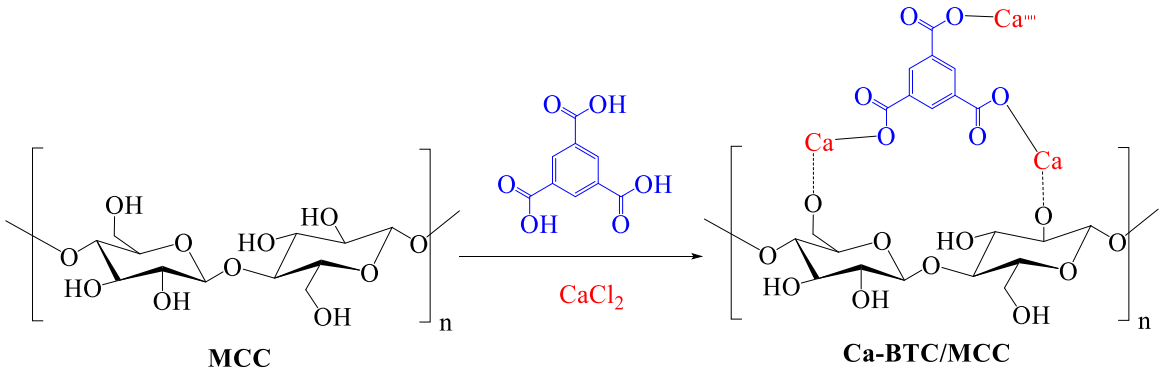


Fig. 4. FTIR spectrum of [a] MCC, [b] Ca-BTC, [c] Ca-BTC/MCC.



Scheme 1. The formation mechanism of Ca-BTC/MCC MOF.

O—H group appears at 3347 cm^{-1}. Fig. 4b shows the Ca-BTC FT-IR spectrum, in which the O–H expansion across hydrogen bonding with water and CaO are indicated by the peaks at 3306 cm^{-1}, 1149 cm^{-1}, and 1021 cm^{-1}, respectively. The isotope of the bending mode of unbound water is seen at 1632.01 cm^{-1}. Asymmetric and symmetric O—C-O stretching of the carboxyl groups appear in the range from 1700 to 1300 cm^{-1}. Peaks at 1574.50 cm^{-1}, 1556 cm^{-1}, and 1510 cm^{-1} are associated with asymmetric O—C-O stretching, while those at 1435.50 cm^{-1} and 1392 cm^{-1} are due to symmetric O—C-O stretching. Fig. 4c reports the interaction between Ca-BTC and MCC in the FTIR spectrum. In addition to the MCC uptake bands, the composite reveals characteristic Ca-BTC uptake bands.

The morphology of MCC was investigated for the sake of comparison. The 3D lattice structure with evenly distributed particles was evident, with large particle agglomerations, as shown in the SEM images in Fig. 5a and b. The normal crystal shape of Ca-BTC was shown in the FE-SEM images in Fig. 5c and d. The morphological characteristics of the Ca-BTC/MCC MOF were examined using an electron microscope, and the results are shown in Fig. 5e and f. The crystal size of Ca-BTC/MCC under a microscope was measured, and it was found to be 12.10 × 2.20 mm. The features of the composite shape were completely different from those of MCC and Ca-BTC, confirming the successful encapsulation of both compounds. The measured BET surface areas from N_2 adsorption-desorption for MCC, Ca-BTC, and Ca-BTC/MCC were 10.60, 560.00, and 820.00 m^2 g^{-1}, respectively.

3.2. Chromatographic method development

The pH of the mobile phase and the ratio of the organic modifier to the aqueous buffer were studied in order to create the chromatographic separation technique of ritonavir. According to the drug bank, ritonavir has pKa values of 1.8 (basic) and 13.68 (acidic). Consequently, the mobile phase buffer was chosen to have pH values between 2.5 and 6.10 in order to ensure that both functional groups are mostly in the unionized state. Utilizing phosphate buffer (50 mM, pH 2.5): ACN (40:60, *v:v*), an acceptable resolution was achieved. Increasing the ACN ratio not only shortened the run duration without overlapping with the MOF or plasma peaks, but it also enhanced the symmetry of the peaks. To achieve acceptable trueness and precision, the use of internal standard is very valuable in order to normalize the overall analyte response. Different internal standards were investigated including daclatasvir, favipiravir, molnupiravir, curcumin, and gliclazide. The daclatasvir peak was overlapped with ritonavir peak, while favipiravir and molnupiravir peaks overlapped with MOF peak. Huge peak tailing was observed in case of curcumin. Between the cited possible internal standards, gliclazide was finally selected as internal standard, showing a good separation. Additionally, good peak symmetry for both ritonavir and gliclazide were achieved as indicated in Fig. 6. The huge peak at the beginning of the chromatogram is due to plasma and excess MOF hybrid.

3.3. Method optimization

Different experimental variables of dSPME were studied to achieve the maximum enrichment. These variables included the type of adsorbent, amount of adsorbent, type and volume of eluent solvents. Optimization of these variables was implemented via the OVAT approach, by monitoring the peak areas at each condition. In preliminary investigations two types of adsorbents were investigated including Ca-BTC MOF and Ca-BTC/MCC MOF, the results showed that Ca-BTC/MCC MOF achieved superior extraction efficiency in comparison with Ca-BTC

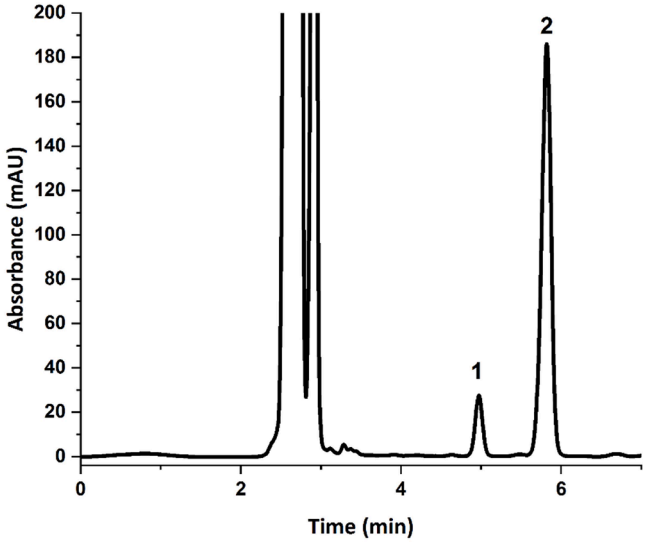


Fig. 6. Chromatographic separation of gliclazide (1, Internal standard IS) and ritonavir (2) (20 μg/mL each).

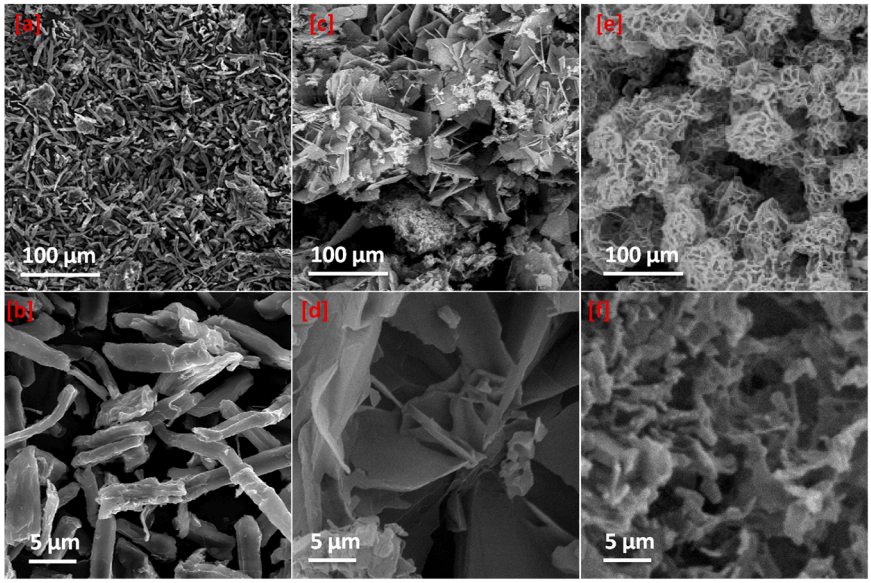


Fig. 5. SEM images of [a, b] MCC, [c,d]Ca-BTC, and [e,f] Ca-BTC/MCC.

MOF. To compare the extraction efficiency between Ca-BTC MOF and Ca-BTC/MCC MOF, the ritonavir peak area was used, the results indicated that Ca-BTC/MCC MOF showed better extraction efficiency than Ca-BTC MOF .This could be explained by the high surface area of Ca-BTC/MCC MOF (820 m^2 g^{-1}), and its superior performance in high molecular weight compounds.

3.3.1. MCC/MOF amount optimization

The MCC/MOF hybrid amount was studied in the range 2.5–15 mg. Different amounts of MCC/MOF hybrid were added to a screw cap tube containing 1 mL of aqueous sample, the tube was vortexed for 5 mins, the tube was centrifuged for 5 mins, the supernatant was decanted, 1 mL of MeOH was added to elute the analytes. Finally, the tube was vortexed again for 5 mins, and then centrifuged. The supernatant was filtrated by syringe filter (0.22 μm) before HPLC analysis. As reported in Fig. 7a,

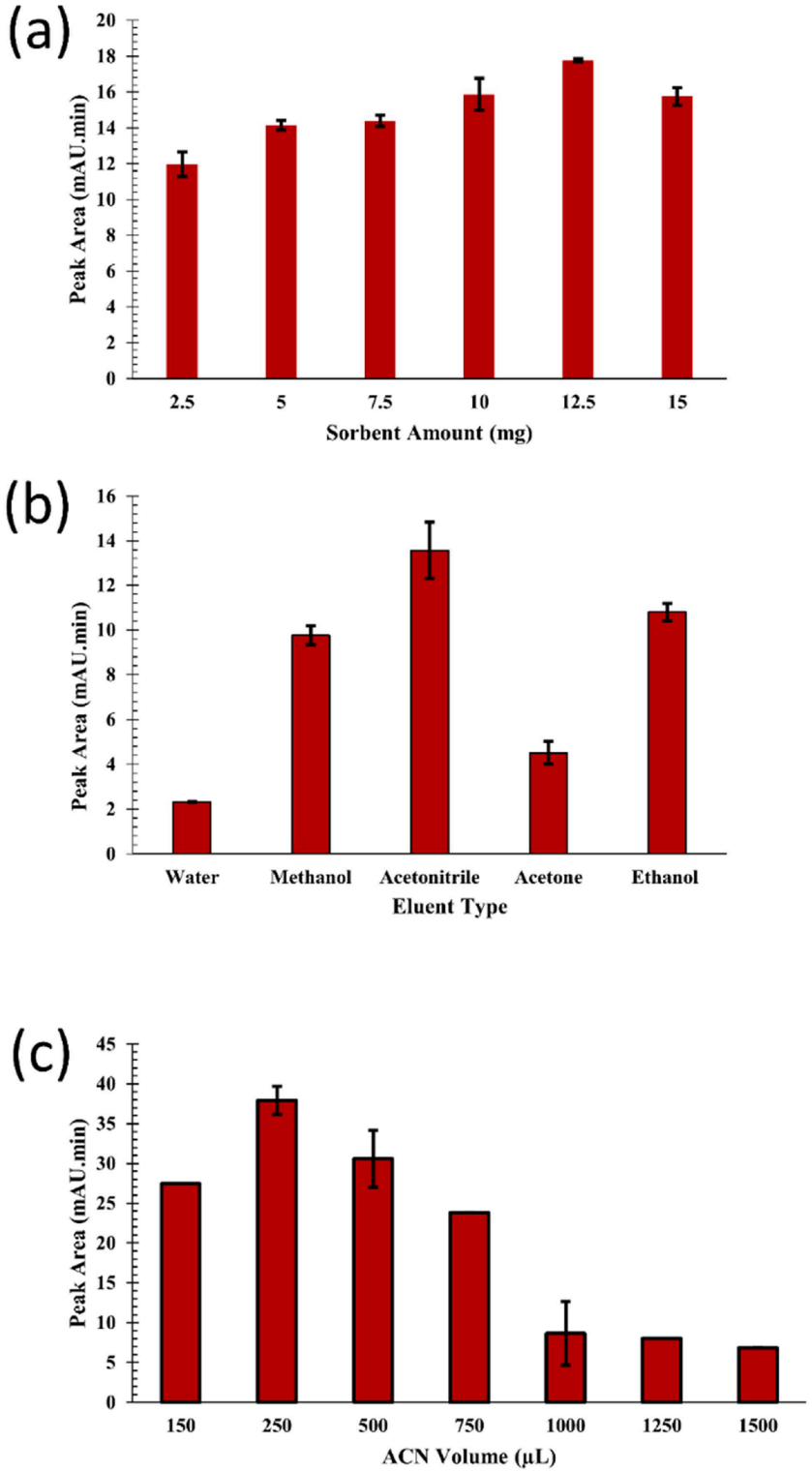


Fig. 7. Effect of (a) MOF amount (in mg), (b) Eluent type and (b) Eluent volume (in μL) on the peak area of ritonavir.

increasing the sorbent amount from 2.5 to 12.5 mg was associated with a corresponding increase in peak area, which could be due to the increase in active sites available for adsorption with the increase in MCC/MOF hybrid amount. No further increase in peak area was observed beyond 12.5 mg, thus this amount was used in the following procedures.

3.3.2. Eluent solvent optimization

One of the most critical factors in dSPME is that eluent type**,** which should have high elution strength to elute the target analyte with the least possible amount. Different eluents were studied including water, methanol, acetonitrile, acetone and ethanol. 12.5 mg of the MCC/MOF hybrid was added to a screw cap tube containing 1 mL of aqueous sample, the tube was vortexed for 5 min, the tube was centrifuged for 5 min, the supernatant was discarded, 1000 μL of the different eluting solvents were added to elute the analytes. The other procedures were repeated as in Section 3.3.1. As illustrated in Fig. 7b, ACN achieved the best extraction efficiency which could be due to the preferential solubility of ritonavir in ACN compared with short chain alcohols (methanol and ethanol) and ketones (acetone). Accordingly, ACN was selected as an eluting solvent in this application.

3.3.3. Eluent volume optimization

The volume of the eluent is one of the most important factors in dSPME, it should high enough to achieve complete elution of ritonavir, and not too much to avoid diluting the analyte. To study the eluent volume effect, different volumes of ACN were studied in the range of 150–1500 μL, under the same experimental conditions in Section 3.3.2. The results shown in Fig. 7c indicated that increasing the volume of ACN from 150 to 250 μL substantially increased the peak area of ritonavir. However, additional volumes of ACN above 250 μL decrease in peak area due to the dilution effect. Based on these results, the optimum conditions were attained using 12.5 mg of the MCC/MOF hybrid as a sorbent, and 250 μL as an eluent.

3.4. Method validation

3.4.1. Selectivity

The selectivity of the proposed method was studied by analyzing six individual human blank plasma, the chromatograms of each blank plasma was compared with the spiked human plasma with ritonavir and gliclazide to test for the presence of any interferences. The chromatograms showed no interfering peaks at the retention times of ritonavir nor gliclazide as indicated in Supplementary Material **Fig. S1**, which indicates the selectivity of the developed method**.**

3.4.2. Linearity, range and lower limit of quantitation

The calibration curve was created by graphing peak area ratios (ritonavir to gliclazide) versus ritonavir concentrations. The linearity range was determined to be 1–20 μg/mL for ritonavir with a correlation coefficient of 0.9969, indicating good linearity. The quantitation lower limit was established by identifying the lowest concentration of ritonavir in human plasma that can be quantified reliably and precisely. The regression analysis data and the lower limit of quantitation are summarized in Supplementary Material **Table S1**.

3.4.3. Trueness and precision

Trueness and precision were assessed at four concentrations of QC samples, including, HQC, LQC, MQC, and LLOQ, each of which was tested in six replicates. The trueness was assessed using the recovery (%), while precision was assessed using the RSDs (%) within-day and between-days. The recovery (%), indicated in Table 1, ranged between 93 and 103 %, while the RSD (%) was found to be $\leq$ 9.9 %. These results demonstrate that the developed dSPME HPLC/UV method using the prepared MCC/MOF hybrid had acceptable trueness and precision, according to the FDA guidelines of bioanalysis.

4. Application to human plasma samples

For application in human plasma, 75 μL of 100 % freshly prepared trichloroacetic acid was used to precipitate 2 mL of spiked human plasma with ritonavir and gliclazide in screw cap tube. After this step, the tube was vortexed for 1 min and centrifuged for 5 mins at 6000 rpm. 1 mL of plasma was withdrawn in another screw cap tube, 12.5 mg of MOF was added to the tube and vortexed for 5 mins. The tube was centrifuged for 5 mins, the supernatant was discarded, 250 μL of ACN was added to elute the analytes. Finally, the tube was vortexed for 5 mins, centrifuged, and the supernatant was filtrated by syringe filter (0.22 μm) before HPLC analysis. As reported in Table 2,% recovery was ranged between 91.11–104.6 % which is accepted according FDA guidelines. Therefore, the developed method could be applied in real samples applications.

5. Comparison with other reported methods

The developed method was compared with a reported method based on homogeneous liquid-liquid microextraction (HLLME) coupled with HPLC/DAD for the determination of ritonavir in human plasma (Abdallah et al., 2023). The reference HLLME HPLC/DAD method and the developed dSPME HPLC/DAD method were repeated six times, and the results were compared using the Student *t*-test and the F-test at a 95 % confidence level (α= 0.05). The statistical analysis revealed no

Table 2
Application of the developed HPLC method for determination of ritonavir in different plasma samples.

Drug	Sample	Added Concentration (μg/mL)	Found Concentration (μg/mL)	%
Ritonavir	Plasma 1	3.00	2.86	95.33
		9.00	9.41	104.6
		18.00	16.40	91.11
	Plasma 2	3.00	2.83	94.33
		9.00	8.89	98.77
		18.00	16.97	94.27
	Plasma 3	3.00	2.98	99.30
		9.00	8.94	99.33
		18.00	17.60	97.77

Table 1
Trueness and precision of the developed HPLC method for determination of ritonavir in spiked plasma samples.

Drug	Intraday			Interday		
	Added Concentration (μg/mL)	Found Concentration (μg/mL)	% ± RSD	Added Concentration (μg/mL)	Found Concentration (μg/mL)	% ± RSD
Ritonavir	1.00	0.93	93.00 ± 8.07	1.00	0.96	96.0 ± 8.7
	3.00	2.89	96.3 ± 4.2	3.001	3.09	103.00 ±8.99
	10.00	9.98	99.8 ± 9.9	10.00	9.97	99.7 ± 8.7
	18.00	18.33	101.8 ± 8.9	18.00	18.30	101.7 ± 7.2
Mean			97.74			99.59
%RSD			7.80			8.40

significant differences between the two methods in terms of accuracy and precision, as indicated in Supplementary Material **Table S2**.

The developed method was also compared with the reported SPE methods for the determination of ritonavir. Due to the low plasma concentrations of ritonavir, most analytical methods used UPLC-MS/MS and HPLC-MS/MS for ritonavir determination after sample preparation using SPE and SPME, as indicated in Supplementary Material **Table S3**. The developed method relied on using dSPME as a sample preparation, which has been proven a simple, cheap, fast, and environmentally friendly sample preparation method. The high surface area of the MOF enhanced the performance, and the biodegradability of MCC supported the method's sustainability. Moreover, the developed method was capable of determining low concentrations of ritonavir by HPLC-DAD without the need for sophisticated detection techniques like HPLC-MS/MS and UPLC-MS/MS.

6. Conclusion

This work introduces the synthesis and characterization of reusable, biodegradable, stable, ecofriendly and efficient adsorbent for dSPME of ritonavir from spiked human plasma. The adsorbent was characterized by FTIR, HRSEM and PXRD. The dSPME-based analytical approach was developed for the selective determination of ritonavir in plasma with good extraction efficiency and sensitivity. Furthermore, the approach is quick and inexpensive, with a high recovery, making it a valuable tool for clinical and forensic laboratories. The herein validated method evades the method transferability drawbacks due to running in an isocratic mode, highlighting its suitability in biomedical analysis. This work paves the way for more applications of the Ca-BTC/MCC MOF hybrid in the determination of pharmaceuticals and xenobiotics in biological fluids.

CRediT authorship contribution statement

Fotouh R. Mansour: Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Reda M. Abdelhameed:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sherin F. Hammad:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Inas A. Abdallah:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Alaa Bedair:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marcello Locatelli:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.carpta.2024.100453.

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