Immobilization of a Natural Deep Eutectic Solvent on PVA Nanofibers without Morphological Disruption: Toward Green Pharmaceutical Analysis

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ABSTRACT

In this research, a green and reproducible Quick Easy Cheap Effective Rugged Safe (QuEChERS) method based on syringe filter based micro-solid phase extraction (SF-μSPE) coupled with HPLC-UV using a green sorbent was developed and optimized for the extraction of five anti-diabetic drugs from wastewater, serum, and plasma real samples. A novel green sorbent composed of a liquid mixture of thymol: menthol ([Thy]:[Men], 1:1) hydrophobic natural deep eutectic solvent (HNADES) and curcumin (Cur) immobilized into the non-toxic and biodegradable polyvinyl alcohol (PVA) electrospun nanofibers' mat was synthesized simply via cheap equipment. Cur was added to enhance the hydrophobicity and functionality of the sorbent. The immobilization process was performed by soaking the mat in the liquid mixture for a specific duration. The correct synthesis and experimental molar ratio of the HNADES components were confirmed by NMR (^{1}H and ^{13}C) and ATR-FTIR spectroscopy. The prepared green sorbent (Cur-HNADES/PVA) was characterized using ATR-FTIR, FE-SEM, EDX/EDX mapping analysis, and water contact angle (WCA) measurement, and it exhibited satisfactory adsorption capacity for the target analytes.

Under optimal conditions (pH = 6.0, adsorption cycle = 3, sample volume = 5.0 mL, desorption cycle = 1, type and volume of elution = 80:20 %v/v MeOH/ACN and 500.0 μL), the method was validated in terms of specificity, linear dynamic ranges (LDRs = 0.1-2000.0 μg L^{-1} and 0.1-1800.0 μg L^{-1}), limits of detection (LODs = 0.03-0.09 μg L^{-1}), and precision (within-day RSDs% = 0.32-1.45% and between-day RSDs% = 0.59-2.03%). Evaluation of the greenness aspects of the proposed method was accomplished using the Green Analytical Procedure Index (GAPI) and Analytical GREEnness (AGREE) approaches. It is noteworthy that the conducted research represents the first report on the synthesis and application of this novel and green sorbent for the determination of anti-diabetic drugs in the mentioned real samples.

Keywords: **Immobilization, Green QuEChERS, Hydrophobic natural deep eutectic solvent, Anti-diabetic drugs, Syringe filter based micro-solid phase extraction**

1. INTRODUCTION

A chronic metabolic disorder marked by insufficient insulin secretion and impaired action on target cells, non-insulin-dependent diabetes mellitus (NIDDM), or Type 2 diabetes mellitus (T2DM), leads to defective glucose uptake. As the most widespread form of diabetes, T2DM is expected to affect over 400 million individuals globally by 2030 [1].

For the pharmacologic management of T2DM, both oral and injectable medicines are available. There exists a variety of oral medications, such as empagliflozin (EMP), metformin (MET), sitagliptin (SIT), gliclazide (GLC), and repaglinide (RPG), with each medication having a different function to keep the blood glucose level balanced. Several of these functions encompass augmentation of insulin secretion, enhancement of glucose utilization, and reduction in glucose production [2]. Fortunately, these drugs are effective in the management of blood glucose levels; however, they can cause side effects like gastric discomfort, diarrhoea, blurred vision, cholestasis, and coma [3]. Dosage adjustment is a noteworthy approach that can be considered to mitigate these side effects while optimizing the drug's therapeutic effect on the patient's body [1,4]. In addition to body fluids, wastewater from pharmaceutical companies, through which these drugs are released into the ecosystem, must be the subject of detection and measurement to reduce environmental risks caused by the referred drugs [5]. Due to the complicated matrix along with the low concentration of analytes in the aforementioned samples, direct analysis of them is unlikely [6]. Therefore, a green, effective, quick, and sustainable method of sample preparation is an indispensable step prior to analytical procedures [7,8]. Various cleaning and extraction procedures followed by high-performance liquid chromatography with ultraviolet detector (HPLC-UV), such as solid phase extraction (SPE) [9–11], dispersive solid phase extraction (DSPE) [12], and dispersive liquid-liquid microextraction (DLLME) [13–16] have been previously used as sample preparation techniques. Utilizing a high quantity of harmful reagents and solvents, alongside prolonged extraction time or multi-step processes, makes the aforesaid methods inconsistent with the principles of Green chemistry [17,18].

Accordingly, in this study, a green Quick Easy Cheap Effective Rugged Safe (QuEChERS) method based on syringe filter based micro-solid phase extraction (SF-μSPE) was developed pursuant to sustainable and Green chemistry, which means it employs low toxicity or safe solvents and reagents, generates less waste, and has a lower energy demand [19]. In this regard, among eco-friendly solvents, deep eutectic solvent (DES), which is a combination of proper hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA) as initiating materials in specific molar ratios, gives rise to a homogeneous eutectic liquid at room temperature (mostly owing to the formation of hydrogen-bonding), was introduced [20,21]. The most popular and promising type of DESs, which are solely mixtures of biocompatible and naturally derived components such as organic acids, carbohydrates, alcohols, and sugars, have been termed natural deep eutectic solvents (NADESs) [22]. NADESs have superior features, including non-toxicity, easy adjustability for particular purposes, cost-effectiveness, straightforward synthesis without the need for additional purification steps, and trivial volatility [23,24]. The mobility of DESs, due to the fact that most of them are in liquid form at ambient temperature, is the primary hindrance to their employment as beneficial extractive media [25,26]. However, to immobilize the liquid DESs and prevail the aforementioned limitation, various solutions exist. These include the polymerization of DES [27] and its incorporation into the matrix of electrospun nanofibers via soaking the nanofibers' mat in DES [28]. These procedures integrate beneficial features of DESs and polymers and elevate their analytical performance as a consequence of the synergistic effect within such systems [29,30].

In the current research, the thymol: menthol ([Thy]:[Men], 1:1) hydrophobic natural DES (HNADES) was prepared using a one-step process. In order to improve the stability of the green sorbent in aqueous media and increase extraction efficiency, the optimal quantity of curcumin (Cur) was added to the synthesized liquid HNADES (Cur-HNADES) [31,32]. Curcumin is a natural phenolic compound found in turmeric rhizomes. The existence of hydroxyl and methoxy moieties, along with aromatic rings in its structure, facilitates potent interactions through hydrogen -bonding and π-π stacking with other components of the sorbent and target analytes. These interactions prove curcumin's multifunctional nature and its superior potential to enhance the sorbent's functionality, ultimately leading to improved extraction proficiency [33]. According to the previous statements, nanofibers of polyvinyl alcohol (PVA) were utilized for the immobilization of liquid Cur-HNADES. The nanofibers were fabricated via an electrospinning procedure, which is an effortless, affordable, relatively fast, and promising technique for producing nanofibers [34,35]. PVA is a biocompatible, eco-friendly, highly spinnable, and chemically stable polymer that can strongly interact with Cur-HNADES, primarily through hydroxyl moiety on its backbone [36]. Eventually, in this study, the novel, green, hydrophobic, and entirely biodegradable Cur-HNADES/PVA sorbent was synthesized for the first time and utilized in a green QuEChERS SF-μSPE/HPLC-UV method for the simultaneous determination of specified anti-diabetic drug residues in real samples, to the best of our knowledge. The greenness characteristics of the proposed method were assessed using GAPI and AGREE approaches.

2. EXPERIMENTAL

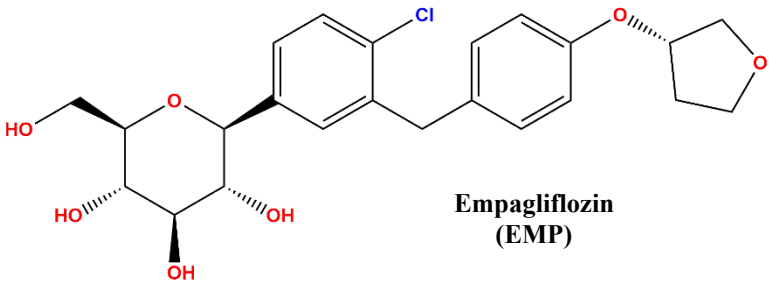
2.1. Materials and Reagents

Thymol (Thy, ≥ 99%) and DL-menthol (Men, natural, ≥ 99%) were bought from Sigma-Aldrich (St. Louis, USA). Polyvinyl alcohol (PVA, M_w ~ 75000 g mol^{-1}), curcumin powder (Cur, for synthesis), deuterated chloroform ($CDCl_3$, deuteration degree 99.95%, for NMR spectroscopy), potassium dihydrogen phosphate (KH_2PO_4), phosphoric acid (H_3PO_4), trifluoroacetic acid (TFA), hydrochloric acid (HCl, 37% w/w), and sodium hydroxide (NaOH, pellets) were acquired from Merck Company (Darmstadt, Germany). Methanol (MeOH) and acetonitrile (ACN) with HPLC grade were provided from Duksan Pure Chemicals Co., Ltd. (Kyungkido, Ansan, South Korea). Water with HPLC grade (deionized, ultra-pure, and with a resistance of 18.3 MΩ.cm) was prepared by the Milli-Q system (Millipore, Bedford, USA). Five anti-diabetic drugs in the form of active pharmaceutical ingredients (APIs), comprising empagliflozin (EMP, pKa = 12.57), metformin (MET, pKa = 2.8 & 11.5), sitagliptin (SIT, pKa = 7.7), gliclazide (GLC, pKa = 5.8), and repaglinide (RPG, pKa = 4.19 & 5.78), were graciously obtained from Alborz Zagros Pharmaceutical Corporation (ACTOVER Group, Karaj, Iran). Their chemical structures were drawn by ChemDraw software (version 20.1.1) and are shown in Fig. 1. Stock standard solutions (1000.0 mg L^{-1}) were individually prepared in HPLC grade MeOH and stored at 4ºC. Working standard solutions (1.0 mg L^{-1}), which were mixtures of the mentioned drugs, were freshly provided every day by adequately diluting stock solutions in HPLC grade water.

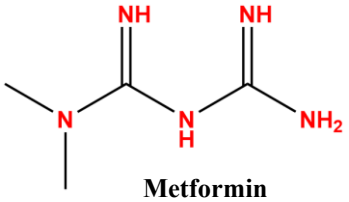
2.2. Instrumentation and HPLC Conditions

An ATR spectrometer (Thermo Nicolet Nexus 470, Massachusetts, USA) was utilized for taking attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectra in the region of 600 to 4000 cm^{-1} (mid-IR). In order to assess morphology and surface structure, field emission-scanning electron microscopy (FE-SEM, FEI ESEM QUANTA 200, USA) was employed. Energy-dispersive X-ray spectroscopy (EDX, EDAX Silicon Drift 2017, USA) and EDX mapping were accomplished to analyze and map the distribution of elements on the surface of the sorbent. Additionally, the hydrophobicity of the synthesized sorbent was studied by measuring the water contact angle (WCA) using the sessile drop method (contact angle analyzer, JIKAN, CAG-20 SE). The fabrication of PVA nanofibers was carried out through an electrospinning system comprising a syringe pump model SP1000HPM and a high voltage power supply model HVSOPOV (Fanavaran Nano Meghias, Tehran, Iran). The pH of sample solutions was tuned aided by a digital pH meter (Metrohm model 827, Switzerland). A magnetic stirrer (Heidolph, Germany) and centrifuge device (EBA 20 Hettich, Germany) were used for stirring and phase separation, respectively.

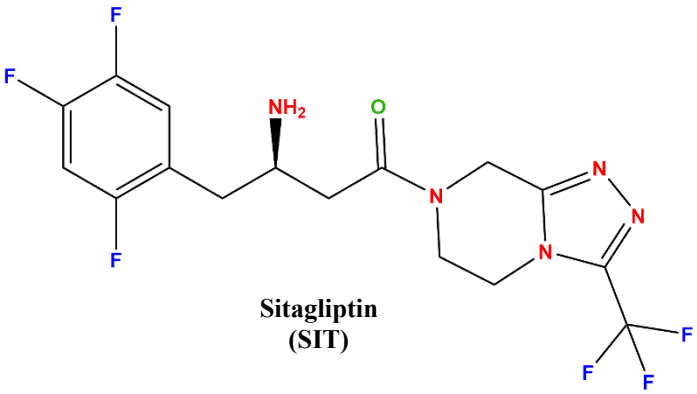
Separation and analysis of the selected analytes were done using an Agilent HPLC system model 1200 (Santa Clara, USA), which comprised of a quaternary pump, manual injection port, sample loop with a volume of 20 µL, UV-detector, and a C_{18} HPLC column (Knauer, Germany, ODS-H, 250 mm × 4.6 mm, 5 µm), along with a C_{18} pre-column. The mobile phase of (A) phosphate buffer (1.45 g KH_2PO_4 in 1000 mL HPLC grade water, yielding approximately 10 mM) adjusted to pH = 3.4 by phosphoric acid, and (B) a mixture of MeOH/ACN (with a ratio of 10/1) was employed as isocratic elution (78:22, %v/v) at a flow rate of 0.8 mL min^{-1}. The wavelength of the UV-detector was adjusted to 230 nm (for EMP, MET, and GLC) and 210 nm (for SIT and RPG). Under the mentioned conditions, the total run time was 16.0 min, and the order of peaks along with their retention times (t_R) was as follows: I) EMP (t_R= 3.75 min), II) MET (t_R= 5.92 min), III) SIT (t_R= 8.63 min), IV) GLC (t_R= 11.13 min), and V) RPG (t_R= 13.59 min). The integration of peaks and data collection was accomplished with Agilent Chemstation software.



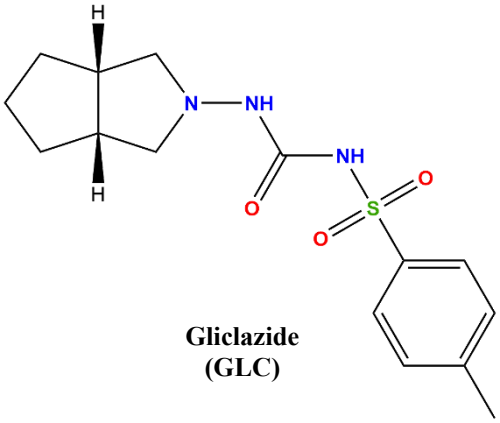
Empagliflozin
(EMP)



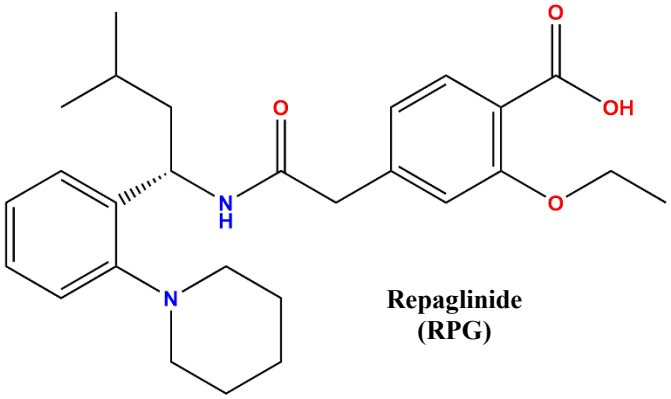
Metformin
(MET)



Sitagliptin
(SIT)



Gliclazide
(GLC)



Repaglinide
(RPG)

Fig. 1: *The chemical structure of the target anti-diabetic drugs.*

2.3. Pretreatment of Real Samples

In this study, plasma, serum, and wastewater were investigated. Plasma-containing drug and drug-free serum samples were acquired from Alavi Pathology Laboratory (Tehran, Iran) and were kept in a refrigerator (at 4ºC) in advance of use. The plasma belongs to a patient undergoing empagliflozin treatment. The examined wastewater originated from the effluent of the APIs production department at Parsian Pharmaceutical Company (Karaj, Iran). Before applying pretreatment and the developed extraction procedure, the real samples were spiked with working standard solutions containing suitable concentrations of the target analytes (0.0, 25.0, 50.0, and 75.0 mg L^{-1}). The specified amounts of HCl (100.0 µL) and TFA (100.0 µL) were poured into the spiked plasma sample (2.0 mL) to denature the proteins. The mixture was then centrifuged (3500 rpm, 5.0 min) to facilitate complete sedimentation of the denatured proteins, and the supernatant was gently transferred to a vial [37]. The resulting solution was diluted at the ratio of 1:3 with HPLC grade water. Removal of proteins from the spiked serum sample was performed by adding an equivalent amount of ACN, followed by centrifugation at 3000 rpm for 5.0 min [38]. The upper solution was gathered and then diluted with HPLC grade water (at the ratio of 1:3). In order to diminish the matrix of the wastewater, it was diluted (at the ratio of 1:2) with HPLC grade water. The pH of all samples was set to 6.0 prior to extraction.

2.4. Synthesis of Hydrophobic Natural Deep Eutectic Solvent (HNADES)

Thymol, with the IUPAC name of 2-isopropyl-5-methylphenol, is a natural phenolic terpenoid and one of the main constituents of the Thymus vulgaris species. Menthol, or 5-methyl-2-(propane-2-yl) cyclohexan-1-ol, is also a naturally occurring terpenoid found in mint plants (i.e., peppermint). Thanks to their low-cost and poor solubility in water, these natural terpenoids are ideal choices for producing cost-effective and stable HNADES [39].The [Thy]:[Men] HNADES was synthesized in a one-step procedure using the heating and stirring method. In this regard, 1.50 g of Thy as HBD and 1.56 g of Men as HBA (in a molar ratio of 1:1) were weighed, poured, and mixed into a PYREX screw cap tube containing a magnetic stir bar. Afterwards, the tube was tightly capped and placed on the magnetic stirrer inside a 60ºC water bath. The heating and stirring were continued up to a clear, slightly yellowish liquid, free from solid particles of constituents, was achieved (around 15 min) [22,40]. The resulting volume with the mentioned values is about 2.0 mL. At ambient temperature, the clarity and liquid state of the synthesized [Thy]:[Men] HNADES remained stable for a long time (more than one month).

2.5. Synthesis of Cur-HNADES and PVA Nanofibers

In the next step, for the preparation of Cur-HNADES, pursuant to the reported literature [41], about 4.8 mg of Cur was gently added to 1.0 mL of [Thy]:[Men] HNADES while being stirred at 40ºC. After the complete addition of Cur, the solution was continuously stirred for 1 hour, and ultimately, a homogeneous orange solution was attained.

Concurrently, 0.4 g of PVA was gradually transferred into 5.0 mL of double distilled water at the temperature of 80ºC and stirred (at 500 rpm) at a fixed temperature for 2 hours [42]. Next, the clear PVA solution was poured into the 5.0 mL plastic syringe containing a needle with an internal diameter of 0.26 mm. Electrospinning was done under the optimal conditions as follows: applied voltage = 20.5 kV, flow rate= 1.2 mL h^{-1}, and the needle tip-collector interval was tuned to 10 cm. After completing the electrospinning procedure, the residual solvent was allowed to evaporate for 24 hours at room temperature. Subsequently, the nanofibers' mat was peeled off from the aluminum foil (used as a collector).

2.6. Synthesis of the Green Sorbent (Cur-HNADES/PVA)

As mentioned earlier, to elevate the extraction proficiency of [Thy]:[Men] HNADES, it was immobilized with the assistance of a PVA nanofibers' mat. For this purpose, a circular piece of the mat with a diameter of 1.3 cm was cut and soaked in the Cur-HNADES solution in a glass Petri dish, and then sealed tightly. After 24 hours, it can be seen that the mat color has changed from white to orange, and the Cur-

HNADES solution was almost totally absorbed. It is noteworthy that approximately 2.0 mL of Cur-HNADES solution is required for a complete soak of the mat with the indicated dimensions. In the current approach, in accordance with literature studies, cross-linking occurs because of dipole-dipole interactions, especially hydrogen-bonding and π-π stacking between the functional groups of Cur-HNADES and the PVA nanofibers' mat [28]. Besides, in the study conducted by Mahmud et al., it was reported that the cross-linking in PVA nanofibers loaded with Cur was also facilitated by heat [43]. So, as the final step of the synthesis, to enhance the interaction and cross-linking between Cur and PVA, the Cur-HNADES/PVA sorbent was placed in a vacuum oven at a temperature of 60ºC for about 2 hours. Regarding the WCA result (which will be discussed later) and the extractions performed in aqueous media, the prepared green sorbent is hydrophobic and substantially more efficient than liquid Cur-HNADES.

2.7. QuEChERS Sample Preparation based on SF-µSPE Procedure

Firstly, the configuration of the QuEChERS extraction procedure based on SF-µSPE was established using a syringe filter holder setup (Re-usable Polycarbonate Syringe Filter Holder, 13 mm). In this setup, the Cur-HNADES/PVA sorbent (cut into a circular piece with a diameter of 1.3 cm, 2.0 mg) was placed in the middle of two pieces of filter paper. Then, it was connected to the tip of a plastic syringe (with a volume of 5.0 mL) [44]. For conditioning and moistening the sorbent, a mixture of HPLC grade water and MeOH (50:50, %v/v, and 3.0 mL) was utilized. Next, pursuant to the optimized values of parameters associated with the extraction procedure, 5.0 mL of a sample solution containing the target analytes (1.0 mg L^{-1}, pH= 6.0) was poured into the syringe and passed over the sorbent by pushing the syringe plunger. This procedure was iterated three times for the promotion of extraction efficacy. Thereafter, rinsing the sorbent was done with 2.0 mL of HPLC grade water to eliminate any residual sample matrix. The adsorbed target analytes were eluted by 500.0 µL of MeOH/ACN (80:20, %v/v) as an appropriate desorption solvent. The total elapsed time for the entire procedure was 3.0 min. Furthermore, it is necessary to rinse the setup between extraction processes to ameliorate reproducibility and maintain carry-over at an acceptable level [45]. Finally, 20.0 µL of the enriched desorption solvent attained through green QuEChERS extraction was injected into HPLC-UV for chromatographic analysis.

3. RESULT AND DISCUSSION

3.1. Characterization of Sorbent (Cur-HNADES/PVA Mat)

3.1.1. ATR-FTIR Spectroscopy

The FTIR spectrum of Cur (orange spectrum in Fig. 2) encompasses significant peaks at approximately 1030 cm^{-1} (C—O—C stretching), 1275 cm^{-1} (aromatic C—O stretching), 1433 cm^{-1} (olefinic C—H bending), 1506 cm^{-1} (C=O and C=C vibrations), 1627 cm^{-1} (stretching of benzene ring), and 3510 cm^{-1} (phenolic O—H stretching) [46]. The spectrum of Cur-HNADES is also shown in Fig. 2 (pink spectrum). Upon comparing this spectrum with the spectrum of pure HNADES, it was observed that the characteristic peaks of Cur did not exist in the aforesaid spectrum. The possible reason for the absence of Cur peaks can be due to overlapping with peaks related to the HNADES structure, which were more intense because of the higher concentration of HNADES components compared to Cur concentration in the Cur-HNADES mixture [47]. On the other hand, the existence of Cur in HNADES was verified through the transformation of the solution's color into a homogenous orange after the dissolution of Cur, and it can be perceived with the unaided eye.
In the spectrum of PVA nanofibers (purple spectrum in Fig. 2), the peaks at approximately 2931, 1243, 3326, and 1370 cm^{-1} are ascribed to stretching and bending vibrations of the methylene (CH_2) group, and stretching and bending vibrations of the O—H group, respectively. Moreover, the intense peaks at 1085 and 1724 cm^{-1} demonstrate stretching vibrations of C—O and C=O (non-hydrolyzed acetyl group available on the backbone of the PVA structure), respectively [28].

The green spectrum in Fig. 2, corresponding to the Cur-HNADES/PVA sorbent, includes the peaks of the main functional groups of both Cur-HNADES and PVA nanofibers that are present in their individual spectra. However, the characteristic peak of Cur at about 3510 cm^{-1} is not observed in the spectrum of the Cur-HNADES/PVA sorbent, which is owing to the interaction between Cur and PVA nanofibers through hydrogen-bonding [47]. As a result, it is authenticated that the Cur-HNADES is absorbed into the PVA nanofibers' mat primarily through hydrogen-bonding as well as other interactions.

3.1.2. FE-SEM Analysis

For the investigation of morphological and microstructural changes in PVA electrospun nanofibers' mat before and after treatment with Cur-HNADES, FE-SEM analysis was applied, and the obtained results are displayed in Fig. 3. In the FE-SEM images of the neat PVA nanofibers' mat (a and b), continuous, smooth, and bead-free nanofibers with a narrow diameter distribution (ranging from 68.7 to 70.0 nm) are visible. Also, these images show an absence of beads in the structure of the mat, which can be assigned to the optimization of electrospinning conditions as previously explained in the "Synthesis of Cur-HNADES and PVA nanofibers" section.

As evident in the FE-SEM images c and d, which belong to the Cur-HNADES/PVA nanofibers' mat, it can be understood that soaking the pure PVA nanofibers' mat in the Cur-HNADES liquid mixture and its permeation into the mat did not lead to the loss of nanofibers' porous structure and had a slight impact on their morphology. Furthermore, in the aforesaid FE-SEM images, compared to the images of the neat PVA nanofibers' mat, there has been a noticeable increase in the diameters of the nanofibers, which was also due to the absorption procedure of Cur-HNADES into the mat [48]. This process happened through interactions among the constituents of Cur-HNADES and functional groups, mainly hydroxyl groups, exist in the PVA nanofibers' structure.

3.1.3. Water Contact Angle (WCA) Measurement

The effect of immobilizing the Cur-HNADES mixture on the wetting features of the PVA nanofibers' mat was monitored by measuring the static water contact angle (static WCA). This analysis was done by placing two drops of distilled water (each drop's volume = 10.0 microliters) on two spots of the same Cur-HNADES/PVA mat surface, resulting in an average WCA of 99.7º, as shown in Fig. 4. The WCA was determined after about 10 seconds. Given that materials with a WCA ≥ 90º are considered hydrophobic [49], it can be concluded that the Cur-HNADES/PVA sorbent exhibits good hydrophobicity. This property is owed to the presence of the Cur-HNADES mixture within the mat. In conclusion, a sorbent suitable for extraction in aqueous media has been successfully prepared.

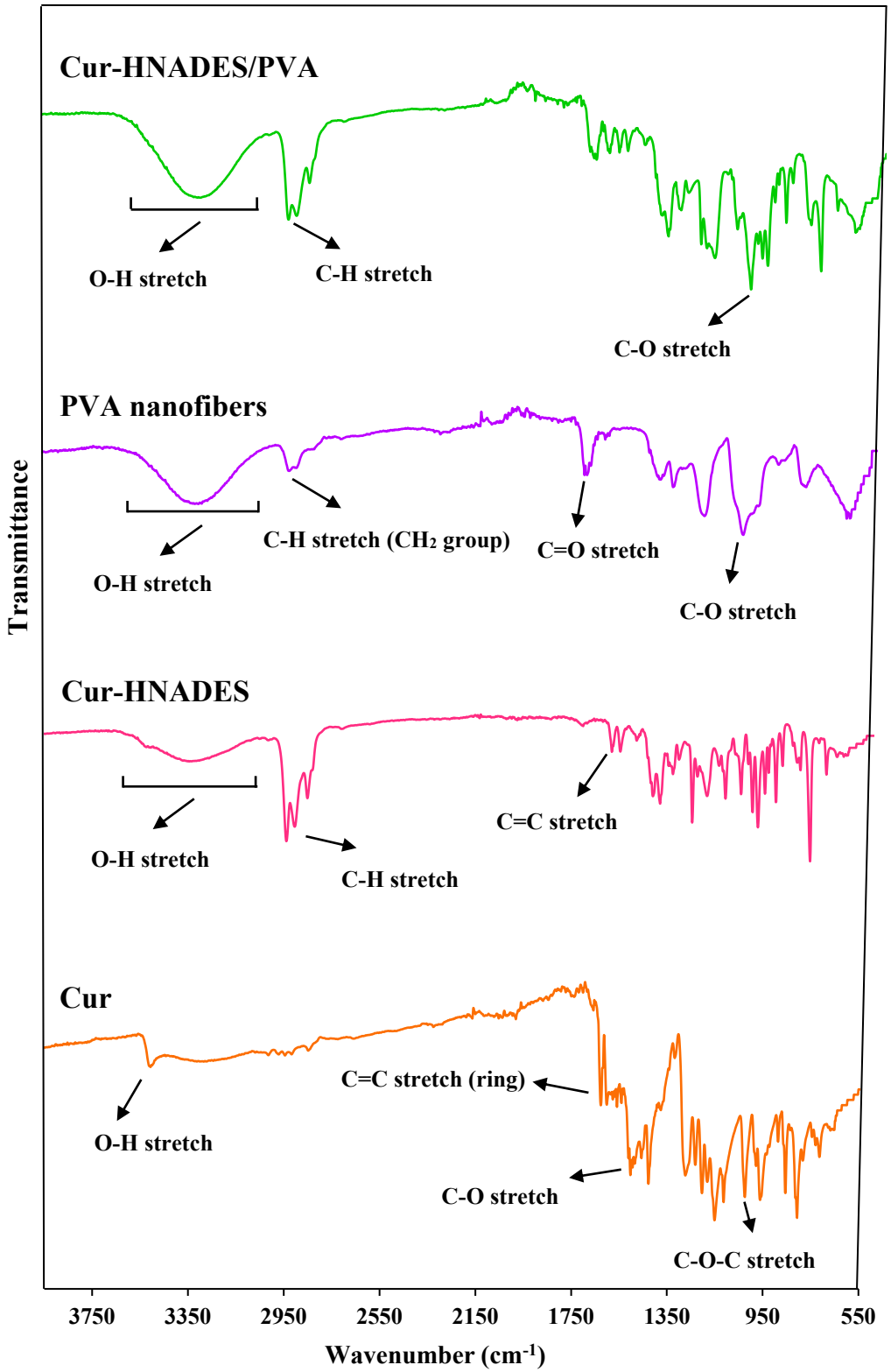


Fig. 2: *ATR-FTIR spectra of Cur, Cur-HNADES, PVA nanofibers' mat, and Cur-HNADES/PVA nanofibers' mat (final sorbent).*

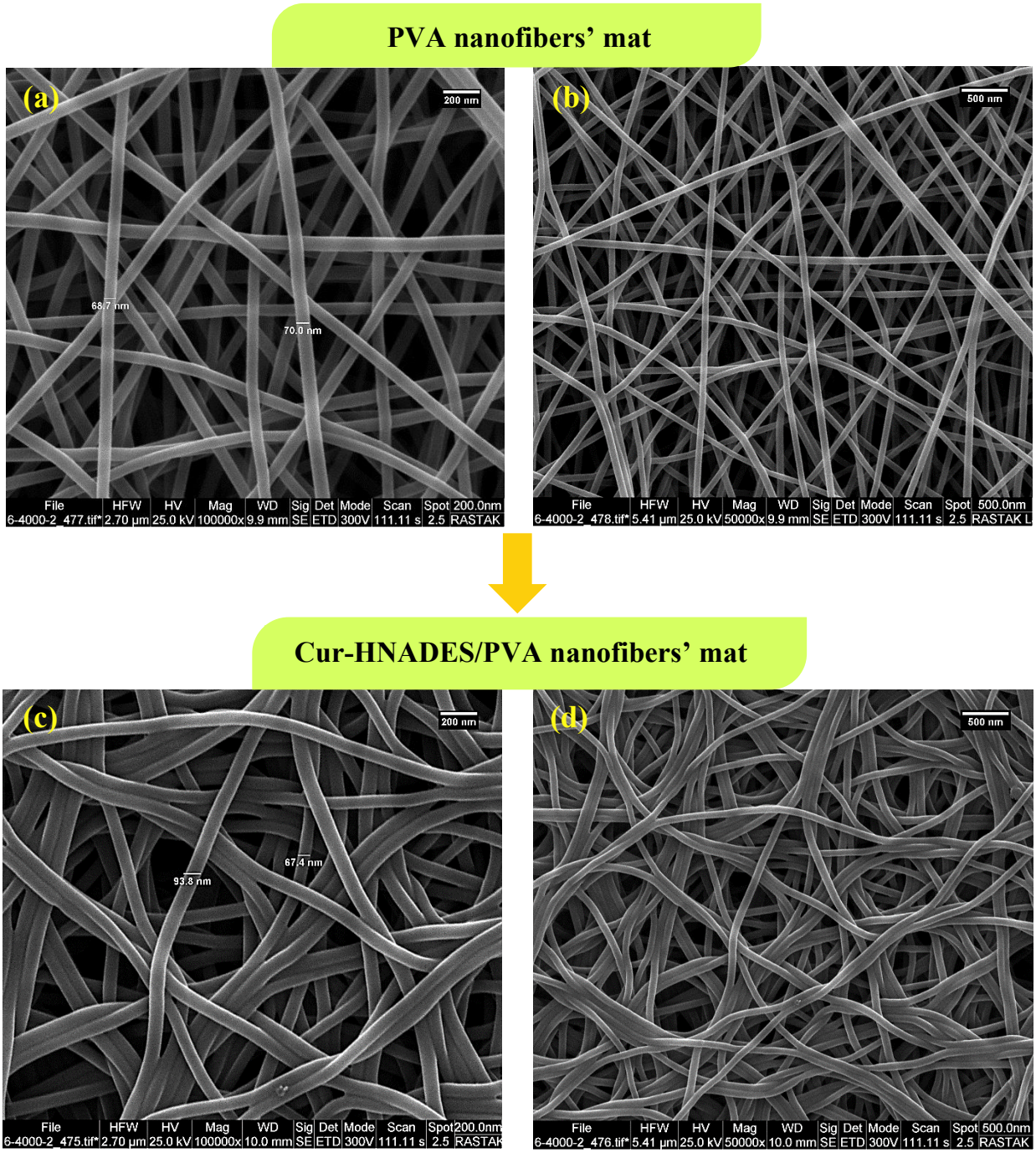


Fig. 3: *FE-SEM images of PVA nanofibers' mat (a & b) and Cur-HNADES/PVA sorbent (c & d) at two magnifications of 100000× and 50000×.*

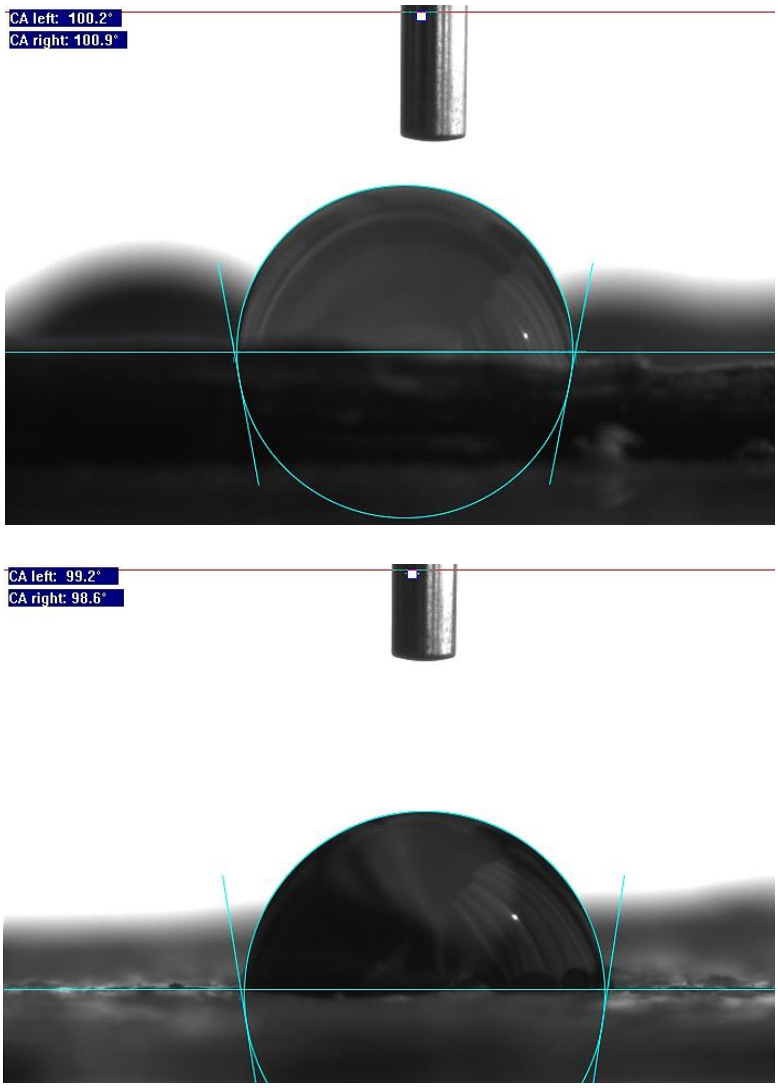


Fig. 4: *Water contact angle images (WCA) of the prepared Cur-HNADES/PVA sorbent.*

3.2. Optimization of Green QuEChERS SF-µSPE Parameters

3.2.1. Effect of Cur in the Prepared Sorbent

Curcumin (Cur) is a non-toxic and natural polyphenolic pigment found in *turmeric.* The existence of phenolic groups in the structure of Cur contributes to its very low solubility in aqueous media [50]. The incorporation of Cur into the sorbent ameliorates its hydrophobicity and creates appropriate functional groups. Indeed, π-π stacking and hydrogen-bonding, facilitated by these functional groups, constitute the predominant interactions between the sorbent and the intended analytes [32]. Furthermore, as reported by Sekharan et al., [Thy]:[Men] HNADES was one of the three HNADES in which Cur was readily dissolved and exhibited high solubility [41]. As a result, two distinct sorbents, namely Cur-HNADES/PVA and HNADES/PVA, were prepared using an identical procedure, and their extraction capabilities towards the target analytes were

compared under the same conditions. As indicated in Fig. 5(a), the peak areas of the analytes desorbed from the Cur-HNADES/PVA sorbent are significantly higher than those from the HNADES/PVA sorbent. Thus, the Cur-HNADES/PVA sorbent was employed in the following extraction procedures.

3.2.2. pH of Sample Solution

Assessing the pH of the sample solution is indispensable to prevent the hydrolysis of the analytes and preserve their stability in aqueous media. Therefore, several pH values (in the range of 3.0-10.0) were examined by adding 0.1M HCl or NaOH solutions drop by drop, and the results are illustrated in Fig. 5(b). It was realized that the highest peak areas were attained at a pH of 6.0. This result, considering the pKa values of EMP (12.57), MET (2.8 & 11.5), SIT (7.7), GLC (5.8), and RPG (4.19 & 5.78), illustrates that the majority of the analytes at the specified pH exist in their neutral forms. As a result, they interact more easily and efficiently with the sorbent, which is also neutral at pH = 6.0 (it is noteworthy that the pH of [Thy]:[Men] HNADES is around 6.8, and Cur is in its neutral form within a pH range of 1-7, as detailed in references [41,51]). It should be noted that EMP and MET are in a monovalent cationic form at this pH, meaning that they have a partial positive charge. Finally, the subsequent extraction procedures were carried out at the optimum pH of 6.0.

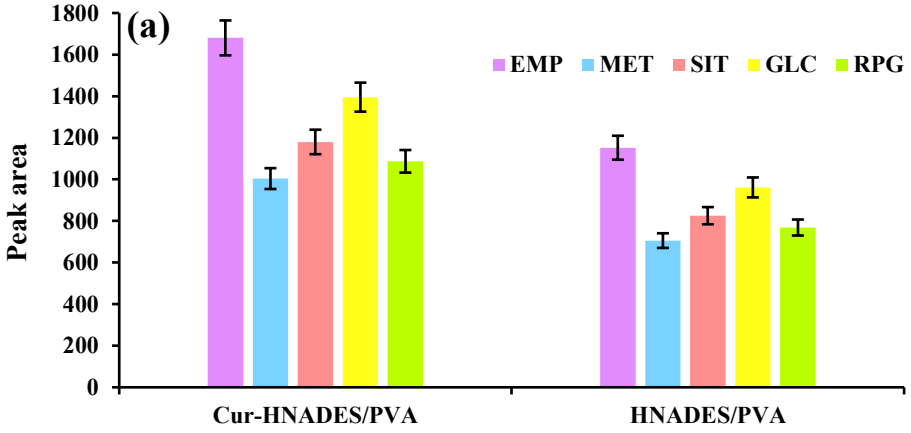
3.2.3. Adsorption Cycle

In order to reach equilibrium in the adsorption process, the solution containing the analytes must be in contact with the sorbent for an adequate duration. For this purpose, as well as saving time, the number of adsorption cycles (ranging from 1 to 6 cycles) was investigated. As it is clear in Fig. 5(c), the peak areas demonstrate enhancement up to the third cycle of adsorption; thereafter, no discernible changes were seen. Hence, three cycles were selected as the optimal value for the adsorption cycle.

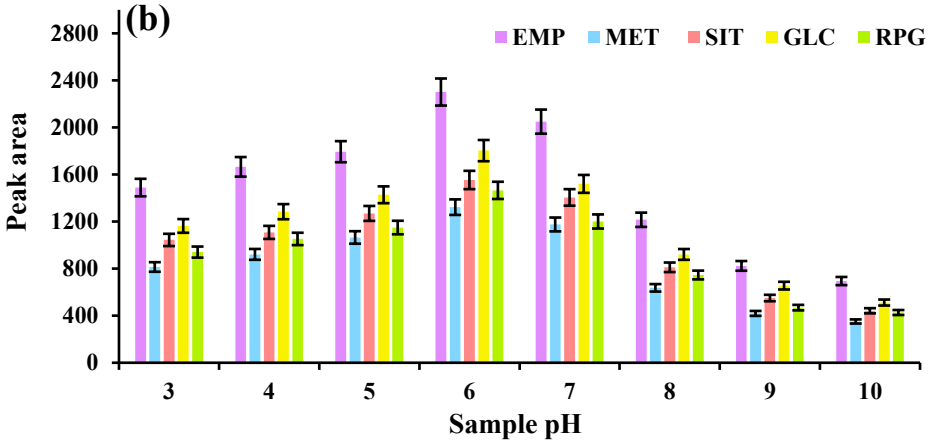
3.2.4. Type and Volume of Elution Solvent

One of the most outstanding factors affecting the efficacy of quantitative extraction is the type of elution solvent. It should be capable of disrupting the interactions between the target analytes and the sorbent, without destruction of the sorbent's structure, and it should also be adaptable with an HPLC device [52]. On the other hand, most of the published literature in the field of determining the intended anti-diabetic drugs has employed MeOH or ACN as the elution solvent. In this regard, to specify the appropriate eluent, mixtures of MeOH [53] and ACN [54] with different ratios were tested. From the presented results in Fig. 6(a), it can be understood that the 80:20 %v/v MeOH/ACN mixture is the best elution solvent.

The eluent volume was also studied in the range of 200.0-800.0 µL, considering its substantial impact on the preconcentration factor (PF) and the aim to avoid the excessive use of organic solvents. As can be perceived from Fig. 6(b), the utilization of 500.0 µL of the elution solvent yielded the most favorable results. Diminishing the peak areas in eluent volumes less and greater than 500.0 µL is the result of its shortage for complete elution of the adsorbed analytes from the sorbent surface, and the dilution effect (which leads to PF decreasing), respectively. So, 500.0 µL of the 80:20 %v/v MeOH/ACN mixture was allocated for the next steps.



Effect of Curcumin presence in the sorbent



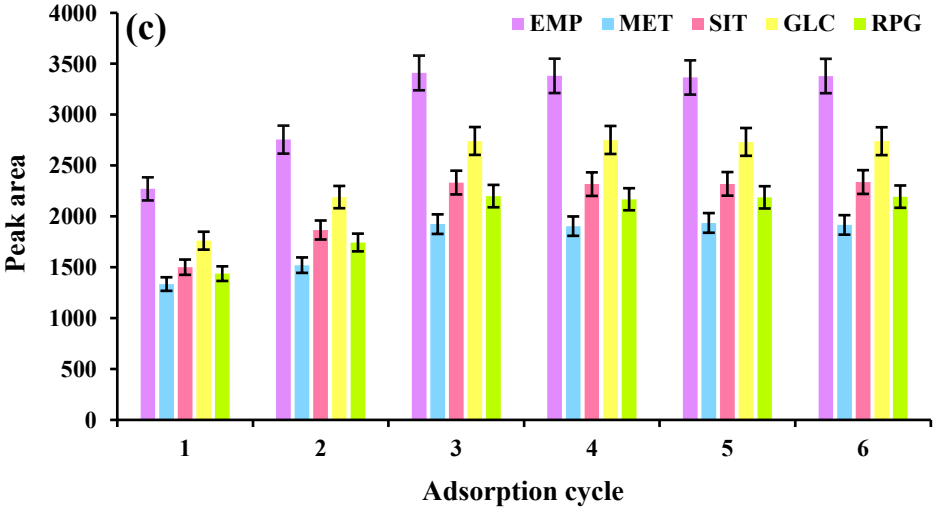


Fig. 5: *The effect of (a) Curcumin presence in the prepared sorbent, (b) pH of the sample solution, and (c) adsorption cycle on the extraction of intended analytes.*

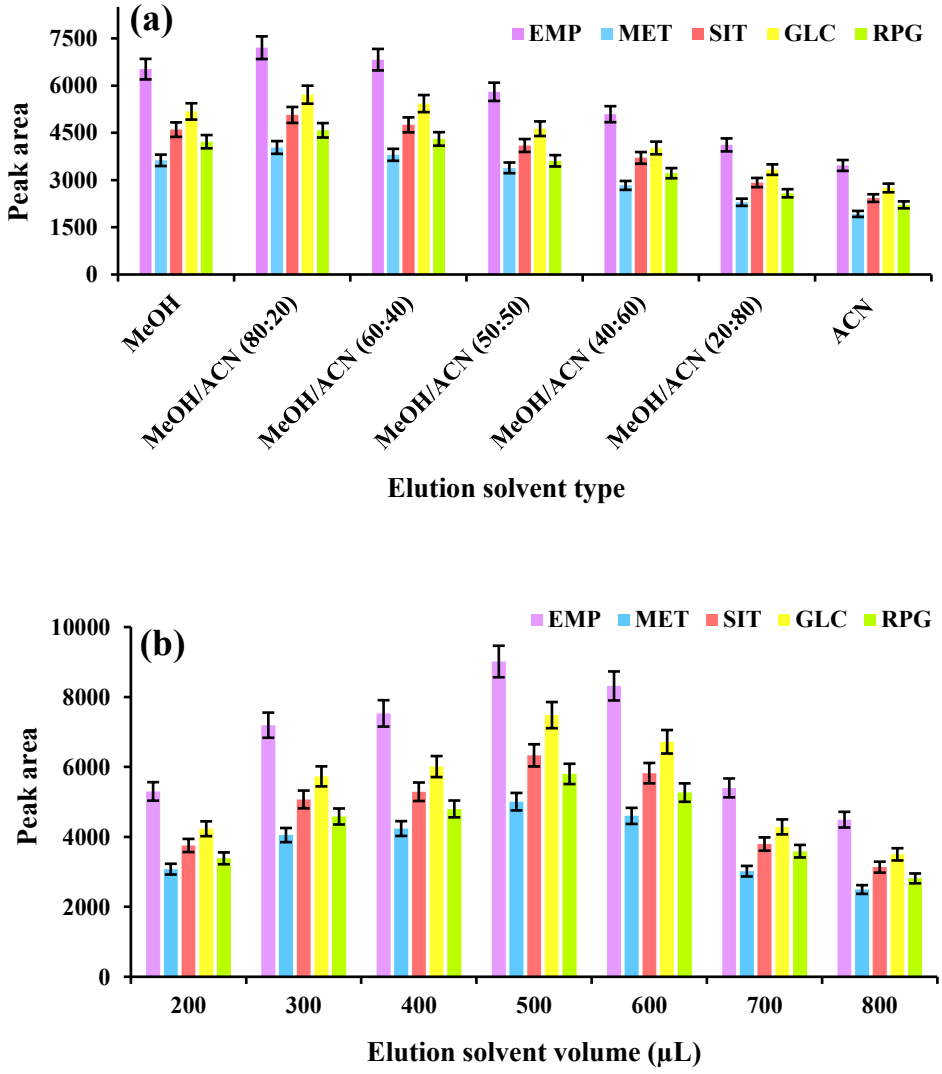


Fig. 6: The effect of (a) type, and (b) volume of elution solvent on the extraction of the intended analytes.

3.3. Validation of the Method

3.3.1. Linear Dynamic Range (LDR), LOD, and LOQ

The major validation parameters were determined using the calibration curve, which was constructed via plotting the peak area of each target analyte in the chromatogram against its corresponding concentrations in the working standard solutions. In this regard, a total of seventeen working standard solutions were meticulously prepared, spanning a wide concentration range from 0.1 to 2000.0 µg L^{-1}. Each point on the curves

represents the mean of three iterations. It can be noticed from the resulting curves that the developed method demonstrated satisfactory LDRs in the ranges of 0.25-2000.0 µg L^{-1} for EMP and GLC, 0.1-2000.0 µg L^{-1} for MET and RPG, and 0.1-1800.0 µg L^{-1} for SIT, with admissible correlation coefficients (R^2) ranging between 0.9950 and 0.9983. The LOD values, ascertained based on a signal-to-noise ratio of 3 (S/N = 3), ranged from 0.03 to 0.09 µg L^{-1}, whereas the LOQ values were selected as the lowest concentrations within the LDRs (0.1-0.25 µg L^{-1}).

3.3.2. Precision

The within-day precision of the method was estimated by calculating the RSD% of repeatability. This was done by performing the green QuEChERS method on spiked sample solutions with various concentrations (250.0, 750.0, and 1200.0 µg L^{-1}). Each spiked sample at a specific concentration was analyzed five times (n = 5) on the same day, and the RSD% values were found to be in the span of 0.32-1.45%. The sample solutions were provided with the aforesaid concentrations, and the analysis was replicated over three successive days to estimate inter-day precision based on reproducibility RSD%, which ranged from 0.59 to 2.04%.

3.3.3. Enrichment Factor (EF), Preconcentration Factor (PF), and Extraction Recovery Percentage (ER%)

The EF, described as the ratio of the extraction calibration slope to the direct calibration slope, was calculated to be between 9.74 and 9.93. Also, the PF, representing the ratio of the volume of the sample solution to the volume of the elution solvent, was achieved to be 10.0 in this method. The slight difference between the EF and PF values (less than 3%) suggests the quantitative nature of the proposed QuEChERS extraction procedure [55]. The extraction efficiency was found out in terms of ER% using Eq. (1):

$$ER\% = \left(EF \times \frac{V_{eluent}}{V_{sample}}\right) \times 100 \quad (1)$$

Where V_{eluent} and V_{sample} are the elution solvent and sample volumes, respectively. In this regard, the ER% values for the target analytes were found to be in the span of 97.44-99.33%. The summary of the reported data is compiled in Table 1.

Table 1. *Validation data for QuEChERS SF-µSPE of intended anti-diabetic drugs using Cur-HNADES/PVA sorbent under optimal conditions.*

Analyte	LOD (µg L^{-1})	LOQ (µg L^{-1})	LDR (µg L^{-1})	Regression equation	R^2	EF	ER (%)	RSD (%) Within-day			RSD (%) Between-day		
								250	750	1200	250	750	1200
EMP	0.09	0.25	0.25-2000	y = 7757.1 x + 197.73	0.9958	9.74	97.44	1.45	0.72	0.41	2.03	1.28	0.76
MET	0.03	0.10	0.10-2000	y = 4691.7 x + 53.01	0.9983	9.86	98.64	1.15	0.68	0.50	1.82	1.09	0.66
SIT	0.04	0.10	0.10-1800	y = 5953.5 x + 57.08	0.9974	9.93	99.26	1.31	0.54	0.32	1.98	1.15	0.72
GLC	0.06	0.25	0.25-2000	y = 6467.3 x + 132.42	0.9950	9.93	99.33	0.93	0.62	0.52	1.52	0.93	0.59
RPG	0.04	0.10	0.10-2000	y = 5242.6 x + 83.98	0.9963	9.91	99.08	1.44	0.58	0.44	1.79	1.22	0.75

3.3.4. Real Sample Analysis and Accuracy Appraisal

To investigate the feasibility and accuracy of the proposed QuEChERS SF-μSPE/HPLC-UV method utilizing the green Cur-HNADES/PVA sorbent, intended anti-diabetic drug residues were quantified in various real samples, comprising wastewater, serum, and plasma samples. The spiking and pretreatment procedures for real samples were accomplished as detailed in Section 2.3. Each analysis was replicated three times under optimized conditions, and the corresponding results are listed in Table 2.
The accuracy (or trueness) of the developed method was checked through measuring the concentration of target analytes in both unspiked and spiked real samples, based on the calculation of RR% by Eq. (2):

$$RR\% = \left(\frac{C_{found} - C_{real}}{C_{added}}\right) \times 100 \quad (2)$$

In the above equation, C_{found} and C_{real} refer to the measured concentration of analytes in the spiked and unspiked (or initial) real samples, respectively, while C_{added} represents the certain concentration of the standard solution added to the samples. In this study, the attainment of acceptable RR% values in the span of 88.84-96.63%, with RSDs% of ≤ 4.1%, suggests the high accuracy of the proposed method. Accordingly, this validation substantiates the method's outstanding capability to accurately determine intended anti-diabetic drug residues in complicated samples. The chromatograms displayed in Fig. 7 are associated with unspiked and spiked states of wastewater, serum, and plasma real samples.

Table 2. Determination of intended anti-diabetic drugs in real samples with QuEChERS SF-μSPE/HPLC-UV method.

Sample	Analyte	C_{added} (μg L^{-1})	C_{found} (μg L^{-1})	RR[a] (%)	RSD (%) (n=3)	ME[b] (%)
Wastewater	EMP	-	17.32	-	3.19	-
		25.0	40.18	91.44	2.04	94.20
		50.0	63.37	92.10	1.36	94.62
		75.0	88.13	94.41	0.63	95.29
	MET	-	-	-	-	-
		25.0	23.25	93.00	2.85	92.19
		50.0	47.36	94.72	1.60	93.51
		75.0	71.62	95.49	0.88	95.09
	SIT	-	-	-	-	-
		25.0	23.41	93.63	2.68	92.77
		50.0	47.55	95.10	1.24	94.68
		75.0	72.26	96.35	0.57	95.33
	GLC	-	21.78	-	2.67	-
		25.0	45.18	93.60	1.25	94.86
		50.0	69.12	94.68	0.78	95.19
		75.0	94.09	96.41	0.51	96.14
		-	-	-	-	-
		25.0	23.52	94.10	1.70	93.79

	RPG	50.0	48.04	96.08	1.13	94.29
		75.0	72.47	96.63	0.86	96.41
Serum	EMP	-	-	-	-	-
		25.0	22.85	91.40	3.31	91.84
		50.0	46.67	93.34	2.68	92.07
		75.0	70.93	94.57	1.82	93.45
	MET	-	-	-	-	-
		25.0	22.73	90.92	3.82	90.76
		50.0	46.35	92.70	1.57	92.82
		75.0	69.85	93.13	1.25	94.27
	SIT	-	-	-	-	-
		25.0	22.56	90.24	2.38	91.76
		50.0	46.09	92.18	1.41	92.55
		75.0	70.35	93.80	0.65	94.81
	GLC	-	-	-	-	-
		25.0	22.89	91.56	2.35	92.45
		50.0	46.13	92.26	1.40	93.67
		75.0	70.31	93.75	1.23	94.11
	RPG	-	-	-	-	-
		25.0	22.92	91.68	3.04	91.88
		50.0	46.32	92.64	1.25	93.45
		75.0	70.15	93.53	0.70	94.79
Plasma	EMP	-	43.90	-	4.10	-
		25.0	66.11	88.84	2.29	91.36
		50.0	89.34	90.88	1.40	92.71
		75.0	113.07	92.21	0.96	93.55
	MET	-	-	-	-	-
		25.0	22.53	90.12	2.67	90.51
		50.0	46.15	92.30	1.62	91.37
		75.0	69.72	92.96	1.04	93.75
	SIT	-	-	-	-	-
		25.0	22.39	89.56	4.09	91.39
		50.0	45.29	90.58	1.43	92.07
		75.0	69.22	92.29	1.24	93.53
		-	-	-	-	-

		25.0	22.59	90.36	3.36	90.41
	GLC	50.0	45.96	91.92	1.40	91.84
		75.0	69.51	92.68	0.65	93.15
		-	-	-	-	-
		25.0	22.41	89.64	2.59	90.73
	RPG	50.0	45.52	91.04	1.69	92.54
		75.0	70.06	93.41	1.23	93.80

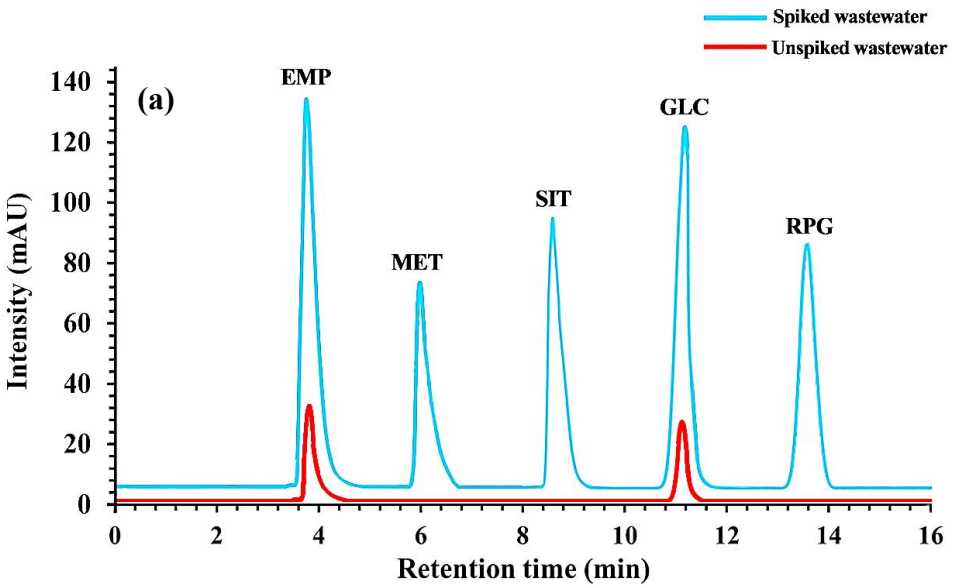
3.5. Adsorption Capacity

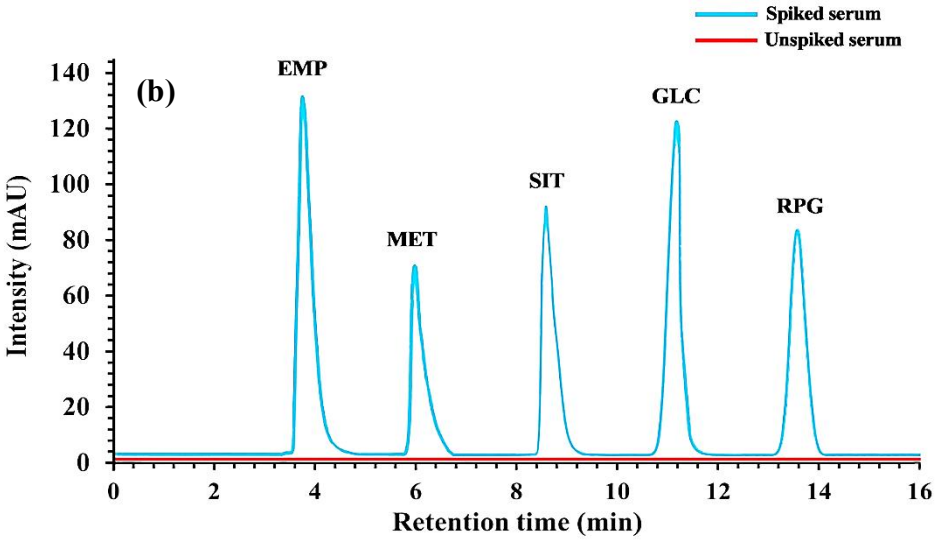
The meticulous examination of the adsorption capacity exhibited by sorbents incorporating nanofibers is a pivotal and indispensable aspect of scientific inquiry. Therefore, Eq (4) is employed to calculate it:

$$Capacity = Q_e = \frac{(C_i - C_e) \times V}{m} \quad (4)$$

Where C_i (mg L^{-1}) and C_e (mg L^{-1}) illustrate the initial and equilibrium concentrations of each target analyte in the sample solution, respectively, V (L) is the volume of the sample solution, m (g) represents the weight of the dry sorbent, and Q_e (mg g^{-1}) signifies the maximum capacity per gram of sorbent at equilibrium.

To calculate the maximum adsorption capacity of the Cur-HNADES/PVA nanofibers' mat, a mass of 2.0 mg of the sorbent was introduced into a 5.0 mL mixture of analytes, encompassing a range of initial concentrations from 5.0 to 250.0 mg L^{-1}. The process was carried out under optimal conditions, and the system was allowed to equilibrate for 2 hours. Subsequently, the sorbent was carefully removed from the solution. The results are revealed that the adsorption capacity for the target anti-diabetic drugs ranged from 72.52 to 77.77 mg g^{-1}. This narrow range manifests the reliable and efficient adsorption performance of the synthesized sorbent towards the selected analytes. The findings suggest that the sorbent possesses a significant affinity for the target analytes, making it a promising candidate for applications in anti-diabetic drugs purification and separation procedures.


Spiked wastewater
Unspiked wastewater
(a)
EMP
MET
SIT
GLC
RPG
Intensity (mAU)
Retention time (min)


Spiked serum
Unspiked serum
(b)
EMP
MET
SIT
GLC
RPG
Intensity (mAU)
Retention time (min)

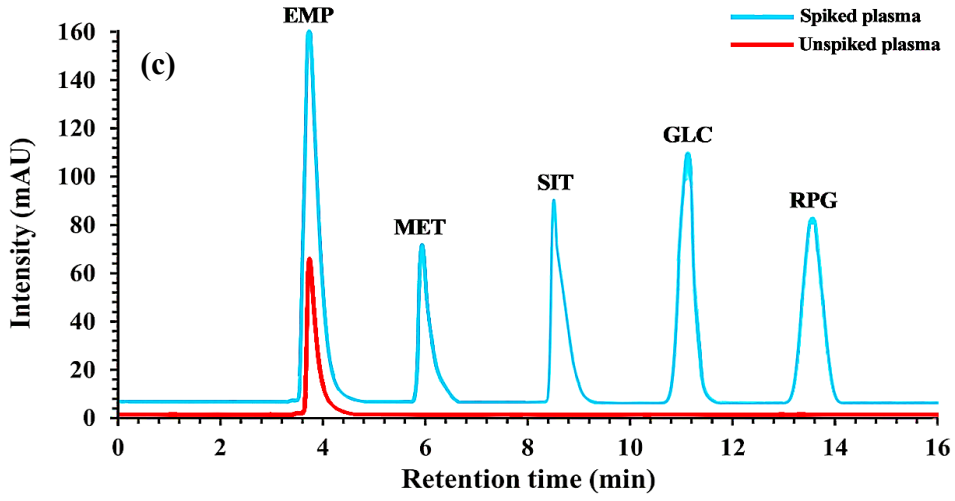

Spiked plasma
Unspiked plasma
(c)
EMP
MET
SIT
GLC
RPG
Intensity (mAU)
Retention time (min)

Fig. 7: *The HPLC-UV chromatograms acquired through the implementation of the proposed method for the analysis of the intended anti-diabetic drugs in (a) unspiked and spiked (50.0 µg L^{-1}) wastewater, (b) unspiked and spiked (75.0 µg L^{-1}) blank serum, and (c) unspiked and spiked (50.0 µg L^{-1}) plasma real samples under optimal conditions.*

3.6. Reusability of the Green Cur-HNADES/PVA Sorbent

From a Green chemistry perspective, high reusability is one of the prominent features of a green sorbent, which assists in saving materials and reducing time consumption for sorbent regeneration [56]. To ascertain the reusability of the prepared sorbent, several extraction-elution cycles were conducted using the same piece of sorbent with a sample solution containing 1.0 µg L^{-1} of each analyte, all under optimal conditions. The findings (Fig. 8) reveal that the green Cur-HNADES/PVA sorbent could be reused up to 115 successive cycles without a substantial decline in peak areas of analytes, and fortunately, it has the potential to be applied in the long-term.

3.7. Greenness Appraisal for the suggested method

Two different approaches, GAPI [57] and AGREE [58], were implemented to appraise and ascertain the green characteristics of the QuEChERS SF-µSPE/HPLC-UV procedure based on the green Cur-HNADES/PVA sorbent.

I) The GAPI method. The semi-quantitative GAPI method offers sufficient data, from sample collection, transport, and storage to instrumental analysis, enabling the evaluation of the greenness of the entire developed method. The schematic representation of this approach comprises five pentagrams, symbolizing five main criteria and their respective subsets. Furthermore, the environmental impacts of each criterion are visualized through the use of green, yellow, and red colors, signifying high, medium, and low degrees of greenness, respectively. Fig. 9 demonstrates the GAPI schematic of the suggested procedure.

II) The AGREE method. The AGREE approach is founded on the twelve principles of Green chemistry, in which each principle is assigned a score in the range of 0.0 to 1.0 (with 1.0 indicating perfect greenness). These scores are represented using a color spectrum from red to green. The resulting AGREE graph, which shows an attained score of 0.61 for the suggested procedure, is depicted in Fig. 10. The redness of principle 3 (score = 0.0) was owing to the off-line measurement device, while the amount of toxic reagents produced in the entire method was about 10 mL per sample analysis (this amount was calculated by considering the 0.8 mL min^{-1} flow rate and 16.0 min analysis time), leading to principle 11 having an orange color (score = 0.2). Since the QuEChERS SF-µSPE/HPLC-UV method using the green Cur-HNADES/PVA sorbent managed to reach a score of 0.61, it can be concluded that it has an acceptable greenness for extraction of intended anti-diabetic drugs in various real samples.

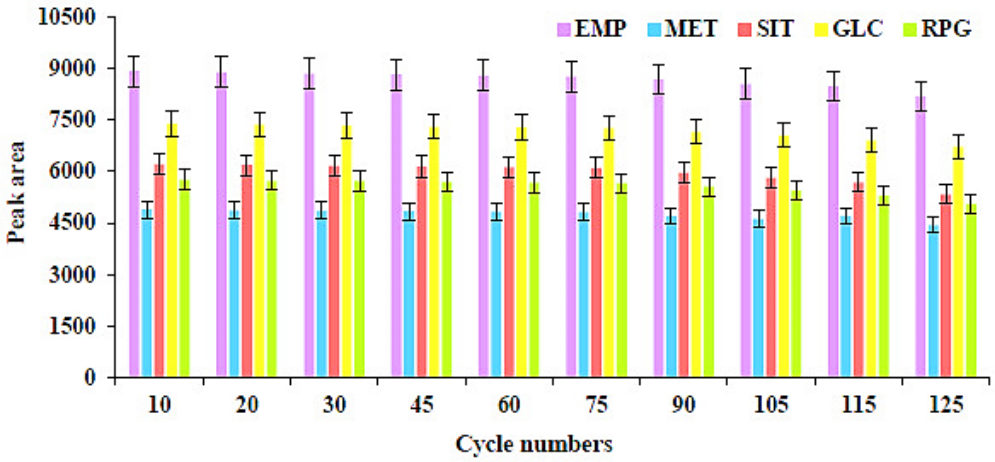


Fig. 8: *Reusability of the Cur-HNADES/PVA sorbent for determination of the intended anti-diabetic drugs.*

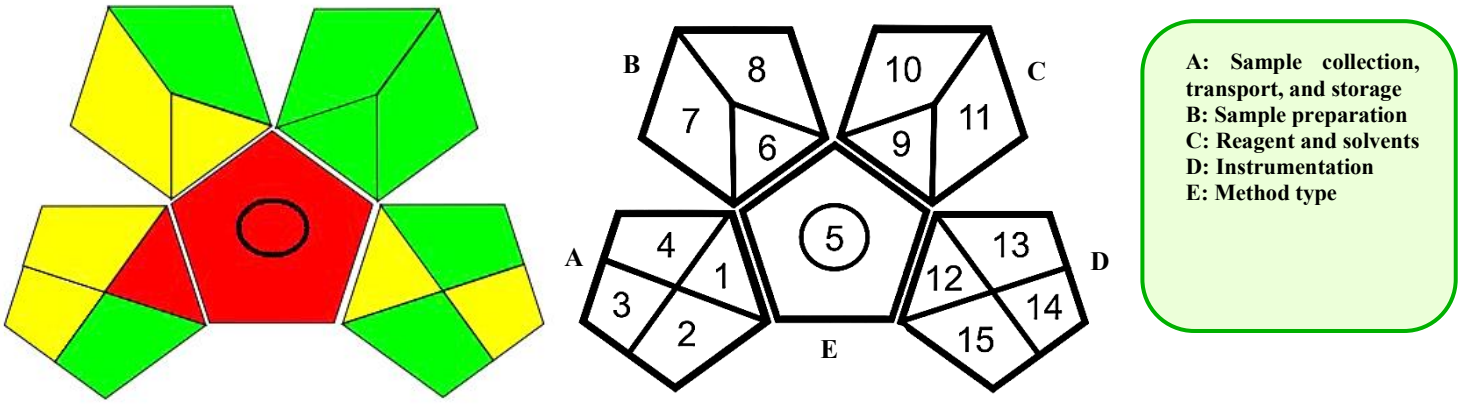


Fig. 9: *Greenness evaluation of the proposed QuEChERS SF-μSPE/HPLC-UV method for determination of the intended anti-diabetic drugs in wastewater, serum, and plasma samples using the GAPI approach.*

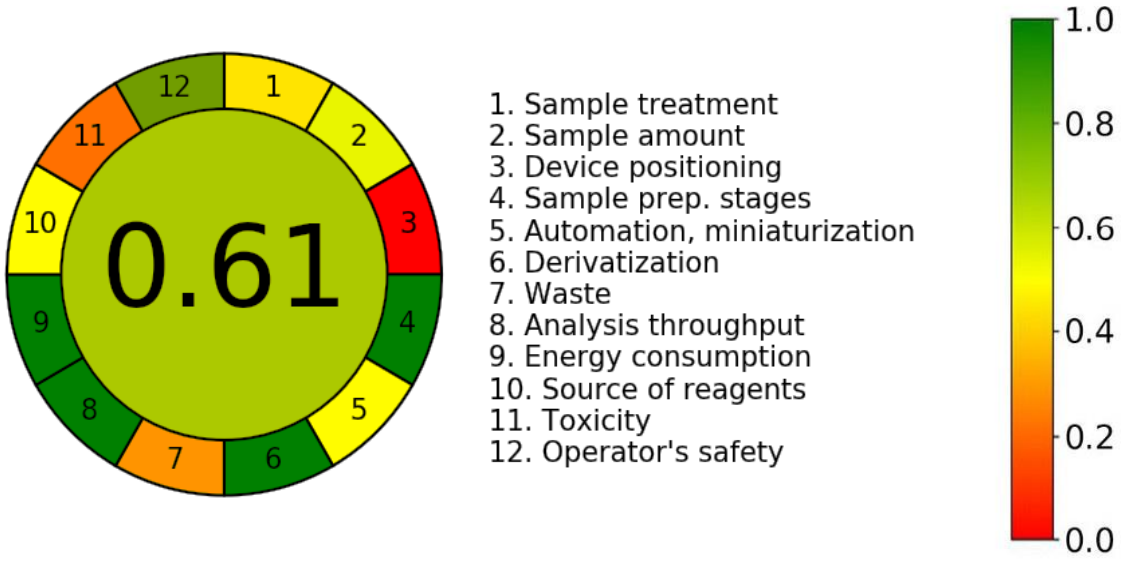


Fig. 10: *Results of the AGREE approach for the QuEChERS SF-µSPE/HPLC-UV method (left) and the corresponding color scale for reference (right).*

3.8. Comparison with Other Methods

In order to highlight the supremacy of the green QuEChERS SF-µSPE/HPLC-UV method, its analytical characteristics were compared with those of other formerly reported procedures [9–16] for measuring intended anti-diabetic drugs in various real samples. The compared parameters and their corresponding results are listed in Table 3. As it is obvious, the proposed method has a fast extraction procedure and demands a considerably small amount of sorbent. Other distinguished features of this procedure are its simplicity, wide linearity (LDR, especially in the range of low concentrations), very low LOD, and superb precision. On the other hand, although the RRs% of the method are very close to those of other methods in the table, the number of analytes studied in this work (5) is more than nearly all others. Besides, only in the suggested method a green sorbent based on HNADES was prepared and applied for the extraction procedure. This comparison verifies that the green QuEChERS SF-µSPE/HPLC-UV method is accurate, easy, quick, environmentally friendly, and has excellent proficiency for extracting and determining of referred anti-diabetic drug residues in different complex samples.

Table 3. *Comparison of the QuEChERS SF-μSPE/HPLC-UV method based on green Cur-HNADES/PVA sorbent with reported methods for determination of intended anti-diabetic drugs.*

Extraction method	Extraction sorbent/ solvent	Analysis method (flow rate, time)	Analyte	Sample matrix	Extraction time (min)	LDR (μg L^{-1})	LOD (μg L^{-1})	RR (%)	RSD (%)	Ref.
SPE	MIP (50 mg)	HPLC-UV (1.2 mL min^{-1}, 9.0 min)	GLC	Plasma	-	1000.0-10000.0	330.0	95.40-98.80	3.70-13.60	9
SPE	Commercial cartridge	HPLC-UV (0.5 mL min^{-1}, 20 min)	GLC RPG	Plasma	-	50.0-2000.0	-	79.90-84.50 80.80-84.50	5.90-13.90 4.90-14.20	10
SPE	MIP (50 mg)	HPLC-UV (0.4 mL min^{-1}, 9 min)	SIT	Rat's plasma and urine	10	0.10-100.0	0.03	98.94	1.24-11.08	11
DSPE	NGO (25 mg)	HPLC-UV (0.6 mL min^{-1}, 12 min)	MET	Plasma	15	10.0-2000.0	3.0	94.33-95.03	0.80-7.90	12
VA-DLLME	1-octanol (30 μL)	HPLC-UV (1.0 mL min^{-1}, 10 min)	RPG	Environmental water	6	1.0-100.0	0.40	98.48-100.81	0.13-1.20	13
UA-DLLME	1-dodecanol (100 μL)	HPLC-UV (1.0 mL min^{-1}, 7.5 min)	EMP	Plasma	4	2.0-2500.0	0.67	94.80-113.90	7.50-9.03	14
DLLME	Dichloromethane (100 μL)	HPLC-UV (1.0 mL min^{-1}, 8 min)	GLC	Serum	4	120.0-2800.0	45.0	93.0-109.0	3.10-10.80	15
VALLME-BE[g]	1-octanol (150 μL)	HPLC-UV (1.5 mL min^{-1}, 8 min)	MET	Plasma	4	20.0-2000.0	1.40	94.80-108.0	3.30-10.80	16
QuEChERS SF-μSPE	Cur-HNADES /PVA (2 mg)	HPLC-UV (0.8 mL min^{-1}, 16 min)	EMP	Wastewater	3	0.25-2000.0	0.09	88.84-94.57	0.41-2.03	This study
			MET			0.10-2000.0	0.03	90.12-95.49	0.50-1.82	
			SIT	Serum		0.10-1800.0	0.04	89.56-96.35	0.32-1.98	
			GLC	Plasma		0.25-2000.0	0.06	90.36-96.41	0.52-1.52	
			RPG			0.10-2000.0	0.04	89.64-96.63	0.44-1.79	

4. CONCLUSIONS

In the current study, a green analytical QuEChERS SF-μSPE/HPLC-UV procedure for the determination of the referred anti-diabetic drugs was suggested. This procedure utilizes a novel, green, completely biodegradable, and hydrophobic Cur-HNADES/PVA sorbent, which is synthesized readily via low-cost and low-energy demanding equipment. A substantial innovation of this study lies in the preparation of a new material constructed from Cur-HNADES, which is immobilized within a matrix of PVA electrospun nanofibers' mat. This material exhibits outstanding functionality and is notably easy to work with compared to the conventional Cur-HNADES liquid mixture. The adsorption capacity of the prepared green sorbent for the specified anti-diabetic drugs, ranging from 72.52 to 77.77 mg g^{-1}, signifies a pronounced affinity of the sorbent towards the intended analytes. Alongside the noted privileges, the sorbent demonstrated remarkable reusability (up to 115 cycles). Through method validation, its superior potential for the efficient and relatively rapid simultaneous extraction of the target analytes from various real samples was authenticated. Specificity, high precision (RSDs% ≤ 4.1%), low matrix effect, and satisfactory accuracy (RRs% = 88.84-96.63%) were

observed in the analysis of the target anti-diabetic drug residues in wastewater, serum, and plasma samples. Finally, the greenness characteristics of the proposed procedure were appraised through the GAPI and AGREE approaches, revealing that the green QuEChERS SF-μSPE/HPLC-UV method based on the Cur-HNADES/PVA sorbent is considered an acceptable green analysis.

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