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Packed-nanofiber solid phase extraction for daidzein, genistein, glycitein in human urine and their simultaneous detection†

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In this work, an integrated method based on the novel packed-fiber solid-phase extraction (PFSPE) combined with high-performance liquid chromatography-ultraviolet detector (HPLC-UV) detection system was proposed for the detection of daidzein, genistein and glycitein in human urine. To efficiently adsorb three kinds of isoflavones in biological fluid, polystyrene-based nanofibers were prepared and used as adsorbents in customized solid phase extraction devices. The composition of the nanofibers, the amount of different salt ions added, the type of eluent, and the interaction of eluent volume, the amounts of nanofibers added, and the extraction time were analyzed to find the optimal adsorption conditions, respectively. The method of optimal extraction conditions for the analysis of three soy isoflavones exhibited low limits of detection (LOD) and limits of quantitation (LOQ) of 1.68–2.74 $\mu\text{g L}^{-1}$ and 5.09–8.3 $\mu\text{g L}^{-1}$, respectively, and spiked recovery of 95.2–107.1%, and has been successfully applied to human urine samples. This study demonstrated the potential of PFSPE as methods for the adsorption and detection of daidzein, genistein, glycitein in biological fluid concurrently, providing a valuable tool for pharmacokinetic studies and investigations into the metabolism and bioavailability of isoflavones.

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Introduction

Soy isoflavones are a type of polyphenol compound structurally similar to estradiol,¹ enabling them to exert estrogen-like effects, and are natural selective estrogen receptor modulators.² Genistein (GEN), daidzein (DAI), and glycitein (GLY) are the principal soy isoflavones, their structural formula is shown in Fig. S1.†³ Numerous studies have demonstrated their potential health benefits,⁴ including lowering blood pressure,⁵ reducing the risk of lung cancer⁶ and cardiovascular disease,⁷ promoting brain development⁸ and so on. Furthermore, recent studies indicated that the urinary isoflavones levels are closely correlated with dietary soy intake, and can serve as a biomarker of soy intake.⁹ Compared to traditional dietary assessment methods, which have certain limitations, the precise analysis of isoflavones in urine samples provides a more accurate and objective means of quantifying soy food intake.¹⁰ It has practical

applications for exploring the intrinsic connection between diet and disease.

Given the health implications of isoflavones, it is crucial to develop an effective method for their purification, separation, identification, and quantification in biological fluids. However, the precise analysis of isoflavones in real samples still faces some challenges. On the one hand, after ingestion, isoflavone aglycone is metabolically transformed through coupling reactions, mainly to form glucosylated and sulfated derivatives, or further bioconverted into microbiome derived metabolites with different structures, including marphenol, dihydroisoflavones and dihydrodaidzein by intestinal microbiota. This phenomenon results in less than 1% of the aglycones being excreted in urine in human subjects, the low level of free isoflavones leads to experimental difficulties in studying the relationship between dose and pharmacological effects, thereby imposing stringent requirements on the sensitivity of the detection methods.¹¹ On the other hand, the complex matrix of human body fluids (*e.g.*, urea, inorganic salts, uric acid, creatinine) can interfere with target analyte detection.¹² Therefore, it is very important to select the appropriate pretreatment method for the enrichment and purification of the target material and enhance the detection signals. Up to now, the separation of isoflavones from human biological fluid usually depends on liquid–liquid extraction^{13,14} and solid phase extraction.¹⁵ Traditional pretreatment methods are often limited by inefficiencies

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in adsorption kinetics and the risk of secondary contamination by residual solvents or by-products. Meanwhile, these processes usually require a long processing time, rely on large amounts of harmful organic solvents, and are not economical and environmentally friendly.¹⁶ In addition, the use of reduced sample volumes and simplified preparation procedures is also essential for facilitating automated processing.¹⁷ Based on this, this study focuses on seeking an appropriate extraction media and sample pre-treatment methods to establish a new analytical method for the detection of free isoflavones in human urine to reduce the target transfer losses and minimize the time and labor requirements inherent in traditional sample preparation workflows.

Solid phase extraction (SPE) is an ideal choice for sample preparation due to its convenient operation, high selectivity, and environmental friendly resulting from the use of small amounts of organic solvents.¹⁸ The adsorption effect of solid phase extraction technology often depends on the choice of adsorbent materials. Traditional adsorption materials include magnetic nanoparticle,¹⁹ molecularly imprinted polymers,²⁰ graphene and composite materials,²¹ multiple walled carbon nanotubes,²² *etc.* However, the separation efficiency of these materials is usually limited, and secondary pollution may be generated during the extraction process. Therefore, developing of novel solid-phase adsorption materials remains a challenging yet prominent focus in analytical science. Electrospinning is a simple and efficient technology that uses high-voltage electrostatic force to directly and continuously process polymer solutions/melts into ultrafine (nano/micron) fibers. Since its emergence in the 20th century, electrospinning technology has gradually become a research hotspot in the field of nanofiber preparation due to its advantages such as strong controllability, simple process and wide adaptability of raw materials.²³ Nanofibers have the advantages of controllable fiber morphology and easy modification of function.²⁴ When combined with solid phase extraction, the enrichment efficiency of solid phase extraction can be improved.²⁵ In addition, the extraction process requires only minimal organic solvent consumption, and the extraction column can be reusable, They have been successfully applied to analyze human sample biomarkers,²⁶ chemical dyes,²⁷ veterinary drug residues,²⁸ antibiotic residues,²⁹ and mycotoxins.³⁰ The strong hydrophobic effect and π - π interaction generated by the abundant benzene ring structure in polystyrene molecules have a high affinity and selectivity for compounds containing aromatic rings, making polystyrene fibers able to effectively enrich soy isoflavones. Therefore, we selected nanofibers with polystyrene as the skeleton as the adsorption material for solid phase extraction of soy isoflavones.³¹

In this study, a new method for the determination of daidzein, genistein, glycitein in human samples by nanofiber-packed solid phase extraction and high-performance liquid chromatography was developed. Five kinds of nanofibers were prepared by electrospinning method. The effects of nanofiber type, salt ion type and concentration, eluent type and volume, nano-fiber dosage, extraction time and other factors on adsorption/desorption were investigated and optimized. The

proposed analytical method for the measure of the soybean isoflavones in human samples can achieve significant removal of interfering substances and enrichment of target analytes, thereby further enhancing the convenience and accuracy of the detection process. In addition, it has low requirements for urine sample volume, and can be expected to be a valuable tool for high-throughput detection in pharmacokinetic studies and the study of the relationship between isoflavone biomarkers and diseases.

Experimental section

Chemicals and instruments

Daidzein (purity $\geq 99.8\%$) and genistein (purity $\geq 99.5\%$) were purchased from Sangon Biotech Co., Ltd (Shanghai, China). Glycitein (purity 98%) and was purchased from Yuanye Biotech Co., Ltd (Shanghai, China). Polystyrene (PS, $M_w = 185\ 000$) was purchased from Department of Applied Chemistry, Nanjing University of Technology. Sodium chloride, sodium acetate, sodium dodecyl sulfate, carbon (C, purity $\geq 99.5\%$), cupric acetate (purity $\geq 98\%$) and montmorillonite (MMT, $M_w = 282.2$) were purchased from Aladdin Biotechnology Co., Ltd (Shanghai, China). Glacial acetic acid and the internal standard (IS) 4-hydroxy-benzophenone (4-HBPH) were purchased from Maclin Biochemical Technology Co., Ltd (Shanghai, China). Acetonitrile and methanol were purchased from TEDIA (USA). Sodium dodecyl sulfate, dimethylformamide (DMF), tetrahydrofuran (THF) and hydrochloric acid were purchased from Sinopharm Group (Shanghai, China).

The preparation of artificial urine refers to the method,³² with a slight modification. The detailed preparation plan can be found in the first section of the ESI.†

Instruments for electrospinning are syringe pump (Model HK-400, China) and a high voltage DC power supply (Tianjin, China). Instruments for nanofiber characterization are Hitachi Regulus 8100 Scanning Electron Microscope (Japan), and VERTEX 80v Fourier Transform Infrared Spectrometer (Bruker GMBH, Germany). Instruments sample testing is Waters 2695 high performance liquid chromatograph (Waters Corporation), Waters 2489 fluorescence detector (Waters Corporation), Inert Sustain C18 column (150 mm \times 4.6 mm, 5 μ m, Tsushima Corporation, Japan).

Preparation of nanofibers and SPE extraction device

The solution is configured as follows: 10 mL of THF and DMF (6 : 4, v/v) was mixed with 1 g of PS and 0.1 g of carbon and dissolved at an ambient temperature of 20 °C for 10 h to obtain a well-mixed electrospinning solution. Then, 10% (w/v) of PS solution was filled into a 10 mL disposable syringe with a 17-gauge stainless steel needle (inner diameter 1.05 mm). The 17 gauge steel pin was bound to the positive terminal of the power supply, while the aluminum foil plate serving as the receiving device was bound to the negative terminal opposite the needle tip. PS solution was sprayed to the receiving plate under the action of electric field force, during which the solvent rapidly evaporates, forming a continuous fiber deposition on the



receiving plate. In the same way, polystyrene-carbon (PS-C), polystyrene-montmorillonite (PS-MMT), polystyrene-silicon dioxide (PS-SiO₂) nanofibers were prepared respectively. The detailed preparation process of polystyrene-Cu nano-particles (PS-CuNPs) nanofibers is shown in the second part of the ESI.† Electrospraying parameters are set as follows: the receiving distance is 15 cm between the collection screen and the needle tip; a high pressure of 19 kV was applied, the flow rate of spinning fluid was 1.7 mL h⁻¹, the operating temperature was maintained between 20–25 °C, the relative humidity was kept below 35%. PFSPE columns are prepared by filling the bottom of an empty extraction column with nanofibers. The nanofibers in the column are lightly compacted with a 1 mm diameter wire to ensure efficient extraction. The optimization of spinning parameters is shown in Table S1.†

Sample pretreatment

We recruited 10 female volunteers aged 20–30 years (Jiangsu, China). Their morning urine samples were collected and stored at –20 °C. All volunteers participated in the trial voluntarily. The samples were thawed to room temperature before testing. After 30 min of ultrasound, the supernatant was put into the centrifuge, and the speed was set at 12 000 rpm for 15 min. The supernatant was stored for the subsequent nanofiber SPE procedure.

Nanofiber-packed solid phase extraction

The design of the pin-shaped extraction column with semi-automatic SPE processor can be used for automatic batch processing of sample liquid, as shown in Fig. 1. Fig. S2† shows an SPE array processor and an empty SPE column. Before extraction, fibers were washed in solid phase extraction columns with 150 μL methanol and 150 μL water, respectively. The 500 μL urine to be tested was then pour into the PFSPE column, where the column pressure was provided by a rotating pressure rod driven supercharger that pushes, the sample solution flows uniformly through the SPE column, and the flow rate is controlled to a drop every 5 to 6 seconds. As the solution passes through the SPE column, the target is captured by the filled adsorbent. After extraction, 300 μL of the eluent with

methanol : acetonitrile : acetic acid (v/v/v, 50 : 45 : 5) was used to drip wash the adsorbent obtain the eluant containing the target substance, and add IS to the eluent. Finally, the eluent was filtered with a nylon membrane of 0.22 μm, and then analyzed with HPLC-UV system.

HPLC analysis conditions

The mobile phase A is 0.5% formic acid aqueous solution, B is acetonitrile, and C is methanol. Flow rate: 0.8 mL min⁻¹; injection volume: 10 μL; column temperature: 35 °C; detection wavelength: 260 nm. The gradient elution procedure is shown in Table S2.†

Method validation

In the methodological investigation, the selectivity, linearity, LOD, LOQ, precision and recovery were explored under the optimal extraction conditions to verify the validity of the established analytical method.

The selectivity of the method was investigated by analyzing blank and labeled urine chromatograms of volunteers who had not consumed isoflavone products. The resulting chromatograms are compared to determine whether there are endogenous components that may interfere with the analyte during the retention time. If any interference peaks are found, optimize the elution gradient until a clean chromatogram is obtained.

Standard solutions of different concentrations of DAI, GEN and GLY were prepared by adding different concentrations of DAI, GEN and GLY to blank artificial urine. A calibration curve IS drawn from the peak area/IS area corresponding to the known concentration. The linear relationship was judged by the coefficient of determination *R*². LOD and LOQ, as the key indicators for evaluating sensitivity, correspond to 3 and 10 times the signal-to-noise ratio, respectively.

At low, medium and high concentrations, the labeled artificial urine samples were measured within one day and for five consecutive days. To investigate the inter-day accuracy and intra-day accuracy, each concentration was repeated 3 times independently. The intra-day precision was measured 6 times in a row, and the inter-day precision was measured for 5 consecutive days. The recovery rate was investigated by comparing the measured concentration of three isoflavones in the eluent after extraction with the added concentration before extraction.

Results and discussion

Characterization of nanofibers

The Fourier-transform infrared (FTIR) spectroscopy analysis of polystyrene (PS) nanofibers revealed distinct absorption bands corresponding to characteristic molecular vibrations, as shown in Fig. S3.† A prominent peak observed at 2922.07 cm⁻¹ is associated with the asymmetric C–H stretching vibration of methylene groups. The aromatic C–H stretching vibrations of the benzene ring are identified at 3024.30 cm⁻¹, while deformation vibrations within the aromatic structure generate a distinct absorption band at 1600.87 cm⁻¹. Additionally, a peak at 1028.03 cm⁻¹ arises from in-plane bending vibrations of C–H

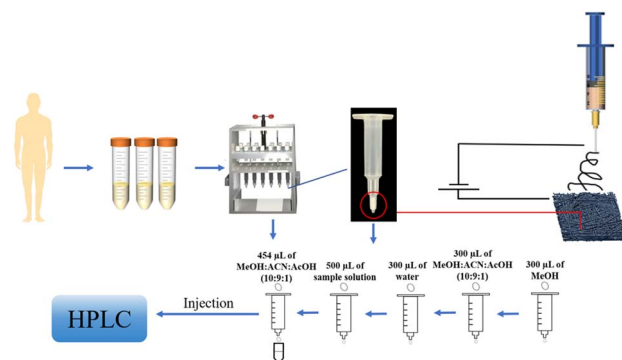


Fig. 1 Schematic of soybean isoflavone detection based on nanofiber solid phase extraction.



bonds in the benzene ring, further confirming the polymer's aromatic backbone. These spectral features align with the known chemical structure of polystyrene, validating the presence of its key functional groups.³³ The two bands 1452.36 cm^{-1} and 1492.86 cm^{-1} belong to the C=C vibration of the aromatic ring, and the characteristic group C=C of the nano carbon seem to overlap with this, which means that the composite fiber is successfully prepared.³⁴ The absorption peak at 466 cm^{-1} in PS-MMT and PS-SiO₂ nanofibers are attributed to the bending vibration of Si-O-Si.^{35,36}

The surface properties of nanofibers materials were studied by scanning electron microscopy (SEM). As shown in Fig. 2, the nanofibers showed a dense network structure and a rough structure on the surface of the fibers. The addition of nano carbon powder increased the adsorption site between the nanofibers and the target analytes, which was more conducive to the adsorption of the target analytes. The SEM images of the as-prepared PS-MMT nanofibers, PS-SiO₂ nanofibers and PS-CuNPs nanofibers are shown in Fig. S4.† After the load of different components, the structures of the PS nanofibers could be all maintained.

Optimization of solid phase extraction conditions

Optimization of nanofibers types. Different types of nanofibers have different functional groups and microstructure, which affect their interaction with soybean isoflavones and lead to different adsorption effects. The types of extractant electrospinning nanofibers in sample pretreatment were optimized. Set the peak area obtained by PS material to 1 so that it can be compared with other peak areas. As shown in Fig. 3, the eluent peak area of PS-C is relatively large, and the effect is the best. This may be due to the small diameter of the nano carbon powder, which increases the action site and leading to the improvement of adsorption efficiency. In addition, according to Fig. S5,† the water contact angle of PS nanofibers in air is 124.05° , indicating a low affinity for water. However, the water contact angle of PS-C decreased to 117.5° due to the addition of nano carbon powder, indicating that the introduction of nano carbon powder can improve the hydrophilicity of nanofibers to some extent. Since isoflavones have good solubility in polar solvents, the effective adsorption of nanofibers with good hydrophilicity will be more advantageous.

Optimization of salt ion type and concentration. The addition of salt can change the interaction between the nanofibers and the target under test, and can also change the ionic strength, thus affecting the solubility of the analyte and the

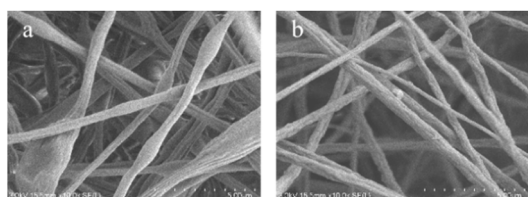


Fig. 2 SEM images of (a) PS nanofibers and (b) PS-C nanofibers.

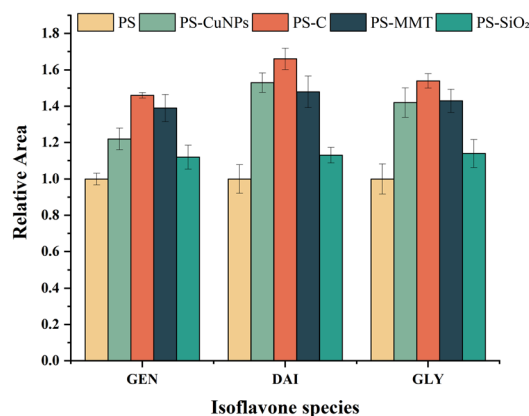


Fig. 3 Influence of nanofibers type on extraction effect. Error bars show the standard deviations ($n = 3$).

viscosity of the solution.³⁷ In this study, three salt ions (sodium acetate, sodium dodecyl sulfate and sodium chloride) were prepared with different concentrations to evaluate the effect of salt ions on extraction.

As shown in Fig. 4(a)–(c), the peak area of the target compound increases with the increase of the concentration of sodium dodecyl sulfate, reaching the maximum value at 1 ng mL^{-1} , however, the peak area decreased as the concentration increased. Sodium dodecyl sulfate binds to non-polar functional groups in PS, through hydrophobic interactions. This binding induces the conformational rearrangement of sodium dodecyl sulfate molecules to extend the hydrophilic sulfate head outward. The increased exposure of hydrophilic groups enhances the interaction between PS nanofibers and the polar target matrix, thus improving the adsorption efficiency.³⁸ At the same time, the carbon powder will be cleaned by sodium

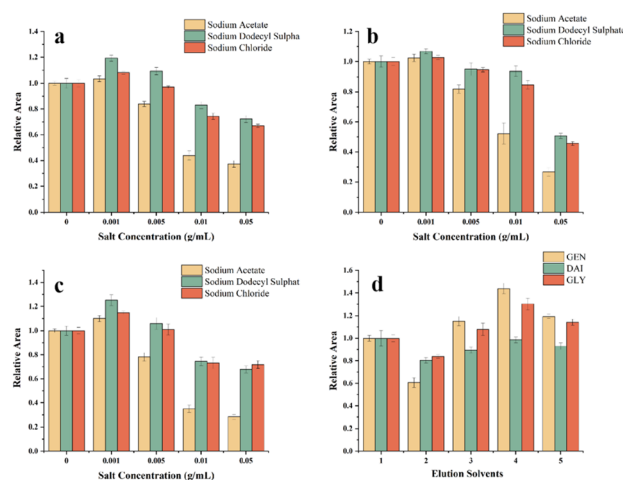


Fig. 4 Influence of salt ion type and concentration on the extraction effect of (a) daidzein (b) genistein (c) glycitein. (d) Influence of eluent type, and 1–5 were methanol, methanol : water (v/v, 6 : 4), acetonitrile, methanol : water : 0.1% phosphoric acid (v/v/v, 4 : 4 : 2), methanol : acetonitrile : acetic acid (v/v/v, 50 : 45 : 5). Error bars show the standard deviations ($n = 3$).



dodecyl sulfate, thereby increasing its adsorption capacity, and when enough salt is added, the extraction efficiency will gradually decrease due to the enhancement of sample viscosity.³⁹ Therefore, 0.001 g mL⁻¹ of sodium dodecyl sulfate was finally chosen.

Optimization of eluent type. The type of eluent is an important factor in the optimization of PFSPE, and it has a significant effect on the desorption efficiency of the target in nanofibers. Therefore, the type of eluent used for sample pretreatment was optimized, as shown in Fig. 4(d). Finally, methanol:acetonitrile:acetic acid (v/v/v, 50:45:5) was selected as the best eluent type.

Response surface optimization of critical factors. Based on the single factor experiment, the factors that have significant influence on the recovery rate of the target material and interact with each other were selected (nanofibers filling amount, elution volume, extraction time) to optimize the response surface. The results are shown in Fig. 5. According to the single factor test results, the fiber filling amount was set to 15–35 mg, the eluent volume was set to 300–500 μ L, and the extraction time was set to the range of 5–7 min. The data related to response surface analysis are shown in Tables S3–S5.† The *P* value less than 0.05 and 0.01 indicates that the item is statistically significant and highly statistically significant. Statistical analysis indicated that the lack-of-fit test yielded a not significant *p*-value, confirming the model's adequacy in describing the experimental data. Concurrently, the isoflavones recovery rate demonstrated significant regression terms ($p < 0.05$), validating the model's statistical relevance. These results collectively confirm the model's predictive validity for extrapolating outcomes within the studied parameter range. Therefore, the amount of fiber filling, eluent volume and extraction time had significant effects on the recovery of isoflavones. The response

surface optimization results showed that the optimal effect of solid phase extraction was obtained when nanofiber dosage was 32 mg, eluent volume was 454 μ L and extraction time was 6 min, respectively.

Method validation. A blank urine sample without analytes was used for analysis, there was no interference peak during the residence time of the analyte, and the signal response generated by the target object was lower than 20% of the peak area of the LOQ and 5% of the peak area of the internal standard, which verified the selectivity of the method to the target isoflavones.⁴⁰

The standard stock solution of soybean isoflavone was diluted with artificial urine, and the calibration curve was obtained in the concentration range of 0.005–10 μ g mL⁻¹. As shown in Table S6,† the standard curve of soybean isoflavone was linear in the specified concentration range ($R^2 \geq 0.999$), indicating excellent linearity, and the LOD and LOQ were 1.68–2.74 μ g L⁻¹ and 5.09–8.3 μ g L⁻¹, respectively.

The recovery rate was determined with three different concentrations (20, 50 and 100 ng mL⁻¹) of labeled blank artificial urine samples. As shown in Table S7,† the recoveries were 95.2–107.1%, which were all within the acceptable range. The method demonstrated high precision, with both intra-day and inter-day accuracies exhibiting relative standard deviations (RSDs) below 10%. The results confirmed that the developed assay is a sensitive and reliable analytical tool for the quantitative determination of the three isoflavones in human urine samples.

At present, various sample pretreatment methods have been used to extract and detect soy isoflavones in biological fluids. Table S8† compares the relevant parameters of the reported methods and this method. This method has good feasibility, small amount of organic solvent, low detection limit, reasonable recovery rate, and in the solid phase extraction process, the design of the semi-automatic extraction device can process multiple samples at the same time, greatly improving the processing efficiency.

Green characteristics and practical evaluation of the method. We evaluated the greenness, safety, and practicality of the validated method using the AGREEprep,⁴¹ ComplexMo-GAPI,⁴² and BAGI⁴³ metrics. The evaluation results are shown in Fig. S6 and Tables S9–S11.†

As shown in Fig. S6(a),† the AGREEprep analysis scored 0.67, highlighting the advantages of this method: online/*in situ* sample preparation (activation, extraction, elution), less waste (32 mg of nanofibers per sample), less use of organic solvents, and a semi-automated process using homemade extraction columns without heating/cooling, ensuring low energy consumption. As shown in Fig. S6(b),† the ComplexMo-GAPI evaluation scored 88 points, indicating that despite the use of methanol/acetonitrile, the environmental impact was reduced by extremely low solvent usage. As shown in Fig. S6(c),† the BAGI evaluation scored 67.5 points, showing its practical advantages: common reagents, simple instruments, and semi-automated operation. Despite the need for SPE preconcentration, this method can still maintain high throughput with small sample amounts.

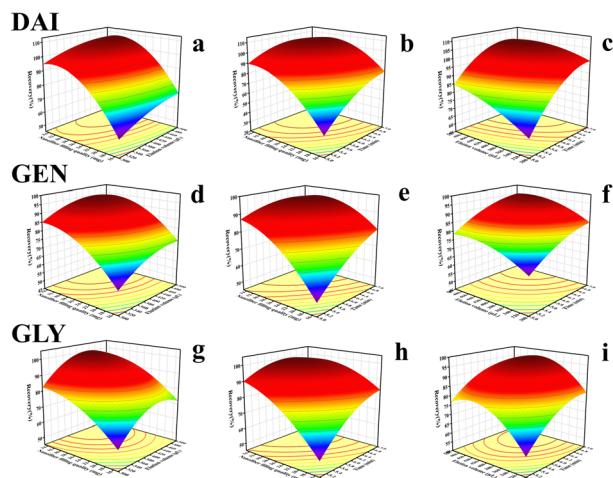


Fig. 5 The response surface plots illustrating the interactive effects of critical parameters on the absolute extraction recoveries of three isoflavones: (a), (d) and (g) fiber-filling quantity versus elution volume (extraction time fixed at 6 min); (b), (e) and (h) fiber-filling quantity versus extraction time (elution volume fixed at 400 μ L); (c), (f) and (i) elution volume versus extraction time (fiber-filling quantity fixed at 25 mg).



Actual sample analysis

Matrix effect evaluation. To investigate matrix effects in urine samples, standard solutions were diluted to 20 ng mL⁻¹, 50 ng mL⁻¹, and 100 ng mL⁻¹ with methanol and blank artificial urine, respectively. The matrix effect was evaluated by comparing the slope of the matrix calibration curve with the slope of the solvent calibration curve. The calculation formula is as follows:

$$ME = (\text{Slope}_1/\text{Slope}_2 - 1) \times 100\%$$

where Slope₁ was the slope of the matrix calibration curve, Slope₂ was the slope of the solvent calibration curve. As shown in Table S12,[†] the matrix effect of soy isoflavones in artificial urine samples was 3.7–8.9%, which were less than 15%, it showed that the influence of urinary matrix was effectively reduced.⁴⁴

Analysis of three types of isoflavones in actual human urine samples. Finally, the method was applied to the analysis of three types of isoflavones in actual human urine samples. Urine samples of 3.0 mL per serving were collected from 12 volunteers who had not consumed any soy products in the past 2 months, and no isoflavones were detected. Then the volunteers were given 15 g of dry fried beans and 500 mL of water mixed with 40 mg of soya-milk powder drink, and collected in the middle of their morning urine the next day. The concentration of each isoflavone in urine samples of 12 volunteers was determined with 3 technical replicates. As shown in Table S13,[†] the concentration range of DAI detected in the urine sample is 17.2–110.7 ng mL⁻¹, the concentration range of GEN detected is 9.3–62.1 ng mL⁻¹, and the concentration range of GLY detected is 5.0–19.2 ng mL⁻¹. In general, PS-C nanofiber can simultaneously adsorb three kinds of soybean isoflavones in human urine samples, and this method requires small sample size, convenient, and shows great potential in the pretreatment of a large number of human samples.

Conclusions

In summary, a packed-fiber solid phase extraction method coupled with high-performance liquid chromatography-ultraviolet detection system was developed for the quantitative analysis of genistein, daidzein, and glycitein in human urine. Electrospun nanofibers were engineered as adsorbents to achieve purification of target isoflavones from complex matrices. Five nanofibers were fabricated *via* electrospinning, and a customized extraction device was implemented to streamline the PFSPE workflow. After the systematic optimization of critical parameters including fiber filling, ionic strength (salt type/concentration), and elution solvent composition, the method exhibited preferable effect on the removal of impurities and the enrichment of target analytes. The method is simple to operate, requires a small urine sample volume, less organic solvent consumption, conforms to the principle of green analytical chemistry, and can be expected for the high throughput determination of genistein, daidzein, and glycitein in complex biological fluids. However, the current semi-automated method

has not yet fully achieved the integration of solid phase extraction and detection steps. It has certain limitations in the separation and analysis of large quantities of actual samples. In addition, it is also necessary to further optimize this method so that it can be applied to the analysis of more other targets. The following aspects can be further explored: developing portable detection device that can be integrated with this extraction device to achieve fully automated sample analysis, designing functional probes for the surface modification of nanofibers to improve the specific enrichment ability, constructing biosensing strategies based on different signal amplification technology to improve specific signal response. It is believed that the proposed assay has promising prospects in the precise analysis of actual samples.

Statement of informed consent

The authors state that the human urine related experiments were conformed to the ethical guidelines of the Declaration of Helsinki and approved by Nanjing Forestry University, China. Informed consent was obtained from all volunteers who contributed human urine to this work.

Data availability

The data supporting this article have been included as part of the ESI.[†]

Author contributions

Yuqi Dai: writing – original draft, writing – review & editing, visualization, investigation, formal analysis, data curation, methodology. Lanmei Zhang: writing – review & editing, supervision. Yuemeng Wang: writing – review & editing, supervision, methodology, funding acquisition. Anni Fu: writing – review & editing, resources, project administration, funding acquisition. Lanling Chu: writing – original draft, methodology, investigation, formal analysis, data curation, funding acquisition. Lei Zhao: writing – review & editing, validation, supervision, resources, project administration, methodology, funding acquisition, conceptualization.

Conflicts of interest

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

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