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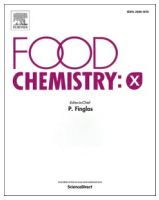

Pipette-tip kapok fiber-based solid-phase extraction/in-situ derivatization for the rapid and green analysis of furfural compounds

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ABSTRACT

Furfural compounds, including 5-hydroxymethylfurfural, furfural, and 5-methylfurfural, are common in foods and pose health risks. This study presents a pipette-tip solid-phase extraction with in-situ derivatization (PT-KF-SPE/ISD) method for rapid analysis of furfural compounds in various food matrices. Utilizing natural kapok fiber as an efficient adsorbent, this method integrates extraction and derivatization into a single step via a simple pull-push operation. Derivatization with 2,4-dinitrophenylhydrazine increases the hydrophobicity and ultraviolet absorption of furfural compounds, enabling sensitive liquid chromatography-ultraviolet detection. The method shows good linearity, sensitivity, and reproducibility, with limits of detection in ranges of 3.9–6.0 ng/mL. Real sample analysis confirms its applicability in detecting furfural compounds in beverages and herbal products, offering a reliable and eco-friendly solution for food safety and quality control. Five greenness assessment metrics demonstrate the method's excellent environmental friendliness. This approach highlights the advantages of combining natural adsorbents with in-situ derivatization for efficient food analysis.

1. Introduction

Furfural compounds, such as 5-hydroxymethylfurfural (HMF), furfural (F), and 5-methylfurfural (MF), are commonly found in foods such as fruit juices, and dairy products. These compounds form through the Maillard reaction during food storage and heat processing, with their levels influenced by factors like temperature, pH, and storage duration (Meng et al., 2024). Furfural compounds serve as markers for the Maillard reaction, which impacts food quality and safety. Although this reaction enhances flavor and color, compounds like HMF pose health risks due to their cytotoxic, mutagenic, genotoxic, and carcinogenic properties (Choudhary et al., 2021). Many organizations such as Codex Alimentarius and the EU regulate the maximum allowable levels of these compounds in foods (Godoy et al., 2022). Therefore, it is essential to develop precise methods for quantifying furfural compounds to ensure food safety and guide processing practices.

At present, many detection techniques have been successfully developed for analyzing furfural compounds, including spectrophotometry, gas chromatography, liquid chromatography (LC), mass spectrometry, electrochemical methods, enzyme-linked immunosorbent assays, and sensor-based methods (Gan et al., 2023). Among these techniques, LC is the preferred and most widely used method due to its satisfactory sensitivity, selectivity, availability, robustness, moderate cost, and ability to simultaneously detect multiple compounds (Kanu, 2021; Peris-Vicente et al., 2022).

However, due to the weak ultraviolet (UV) absorption of furfural compounds, detection is usually at low wavelengths, which can result in poor selectivity and specificity when analyzing complex samples.

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Additionally, the concentrations of these compounds are relatively low. To address these challenges, a derivatization step before LC analysis can improve their hydrophobicity and UV absorption, thereby enhancing selectivity and sensitivity (Zhang et al., 2018). Among the various derivatization reagents, 2,4-dinitrophenylhydrazine (DNPH) is the most widely used due to its ability to rapidly react with the aldehyde groups in furfural compounds under mild conditions (El-Maghrabey et al., 2021). The concentrations of furfural compounds in samples can vary greatly, with some samples containing relatively low levels. Therefore, purification and enrichment steps before LC analysis are often necessary (Ling et al., 2024; Yao et al., 2024). Various extraction methods, such as field-assisted liquid-liquid microextraction (Feng et al., 2017; Wu et al., 2018), dispersive liquid-liquid microextraction (Xu et al., 2018), and solid-phase extraction techniques (Pico et al., 2018; Vesely et al., 2003), have been developed. Combining derivatization and extraction yields better analytical selectivity and sensitivity for detecting furfural compounds in complex samples, and several such methods have been successfully developed (Chen et al., 2021; Wang, Chen, et al., 2024).

In addition to the pursuit of analytical selectivity and sensitivity, the growing emphasis on green analytical methods and sustainable development has garnered significant research interest in natural adsorbents (Bi et al., 2024). Pollen grains (Lu et al., 2014; Lu et al., 2015), cotton fibers (Han et al., 2021; Xu et al., 2023), and kapok fibers (Gan et al., 2024) have been applied as natural adsorbents in sample preparation. Specifically, our previous research has demonstrated that kapok fiber is an excellent adsorbent for hydrophobic compounds such as antidepressants from biological fluids (Gan et al., 2024).

This study aims to enhance this method by incorporating derivatization, creating a streamlined approach for analyzing furfural compounds. An innovative pipette-tip kapok fiber-solid phase extraction/in-situ derivatization (PT-KF-SPE/ISD) method that efficiently combines extraction and derivatization in a single, streamlined process was proposed. The DNPH solution, sample solution, and desorption solvent are successively drawn in/out to facilitate the loading of DNPH onto the KF, enabling simultaneous extraction/in-situ derivatization, and allowing the desorption of DNPH-furfural derivatives. The desorption solution requires no drying or re-dissolution steps and is directly transferred for high performance liquid chromatography (HPLC)-UV analysis, aiming to offer a rapid and effective approach for determining furfural compounds. The PT-KF-SPE/ISD method is rigorously optimized by focusing on the critical factors impacting extraction efficiency. Its applicability is evaluated by analyzing furfural compounds in various real samples, including fruit juices, tea beverages and herbal oral liquids. A detailed comparison with existing techniques highlights the unique advantages and innovations of the proposed PT-KF-SPE/ISD approach.

2. Materials and methods

2.1. Chemicals and reagents

5-Hydroxymethylfurfural (HMF) (99 %), furfural (F) (99 %), and 2,4-dinitrophenylhydrazine (DNPH) (≥98 %) were purchased from Aladdin Co., Ltd. (Shanghai, China). 5-Methyl furfural (MF) (99 %) was obtained from Bide Pharmatech Co., Ltd. (Shanghai, China). Chromatographic grade acetonitrile (ACN), ethyl alcohol (EtOH), methanol (MeOH), and acetone (AC) were acquired from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). Natural kapok fiber was locally sourced from a market in Zhengzhou (Henan, China) and was utilized directly without any additional pretreatment. Disposable syringes were purchased from Shuguang Medical Equipment Co., Ltd. (Henan, China). Ultrapure water (H_2O) was produced by a Millipore Milli-Q system (Bedford, MA, USA).

The mixed standard stock solutions of HMF, F, and MF were prepared in H_2O at a concentration of 10 mg/mL. These stock solutions were further diluted with H_2O to achieve the desired concentrations for various experiments. For the preparation of the DNPH solution, considering DNPH is sparingly soluble in water, a water solution containing 65 % ACN (*v*/v) was chosen as the solvent to increase the concentration of the derivatization reagent. An appropriate amount of DNPH was dissolved in the 65 % ACN (v/v) solvent until saturation was reached. The solution was then sonicated for 15 min, followed by centrifugation at 1500*g* for 10 min. The supernatant, namely the saturated DNPH solution prepared in 65 % ACN (v/v), was collected for future use. All these solutions were freshly prepared each week.

2.2. The PT KF-SPE/ISD procedure

The configuration of the device and the entire PT-KF-SPE/ISD procedure are depicted in Fig. 1. Similar to our previously reported method (Gan et al., 2024), the device was constructed as follows: approximately 5 mm of the top part of a 200 μL pipette tip was removed, ensuring that the remaining part could be securely attached to the lower port of a 2.5 mL syringe. Next, 15 mg of KF was loaded into the modified pipette tip, leaving approximately 3 mm from the tip. Finally, the pipette tip packed with KF was affixed to a 2.5 mL disposable syringe for later use.

The PT-KF-SPE/ISD procedure was conducted by pulling and pushing the syringe plunger to draw in and expel out the solution. The overall process entailed five stages: (1) Activation of the KF with 1 mL PBS buffer solution (pH 1.0, 20 mM) through four pull-push cycles; (2) Loading the derivatization reagent onto the KF with 1 mL saturated DNPH solution prepared in 65 % ACN (*v*/v) through two pull-push cycles; (3) Performing extraction/in-situ derivatization of furfural compounds with 1 mL of sample solution (pre-added with 10 μL FA) through six pull-push cycles.; (4) Washing the KF with 0.2 mL PBS buffer solution (pH 1.0, 20 mM) through three pull-push cycles; (5) Desorbing the DNPH-furfural derivatives with 150 μL of ACN through four pull-push cycles. The resultant desorption solution was then directly analyzed using HPLC-UV.

2.3. In-solution derivatization procedure

The in-solution derivatization procedure was conducted by carrying out the derivatization reaction directly in the solution, referring to previously reported studies (Chen, Bu, et al., 2023; Chen, Wang, et al., 2023) with some modifications, and performed as follows: 20 μL of a saturated solution of DNPH prepared in 65 % ACN (*v*/v) and 10 μL FA were directly added to 1 mL of sample solution. The mixture was then incubated for 20 min at room temperature (~25 °C).

2.4. HPLC-UV analysis

All HPLC-UV analyses were performed on an Agilent 1100 HPLC system, which consists of a degasser, an autosampler, a quaternary pump, an ultraviolet detector, and a column oven. Chromatographic separation was achieved using an Agilent TC-C18 (250 × 4.6 mm, 5 μm). The mobile phases consisted of a mixture of H_2O (A) and ACN (B) at a ratio of 30–70 (*v*/v). The flow rate was set at 1.0 mL/min, and the detection wavelength was 360 nm. The sample injection volume was 20 μL.

3. Results and discussion

3.1. Feasibility study of PT-KF-SPE/ISD method

Furfural compounds are highly polar and exhibit weak UV absorption (Gan et al., 2023). To address this, DNPH was used as a derivatization reagent to convert furfural compounds into hydrophobic derivatives, facilitating their extraction from polar aqueous samples (Guan et al., 2023). This also enhances their UV absorption, improving detection sensitivity and selectivity. To streamline the procedure, the PT-KF-SPE/ISD method was proposed.

KF was used as the SPE adsorbent, loaded into a modified pipette tip,

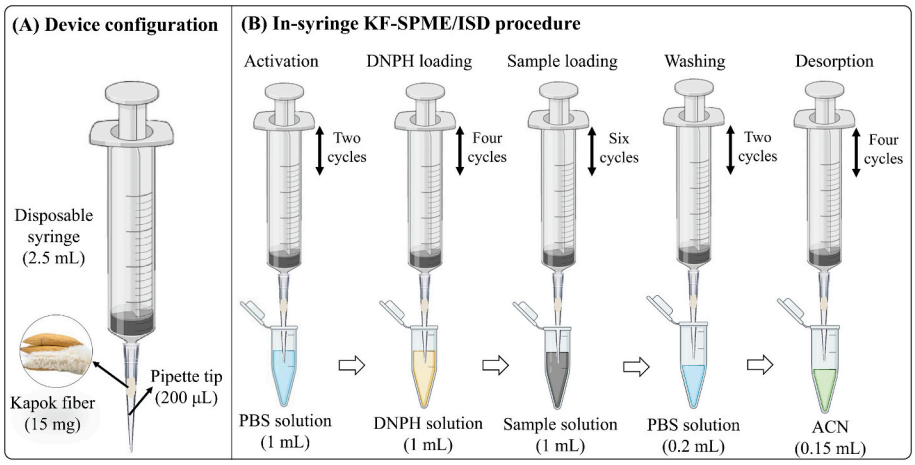

Fig. 1. Schematic illustration of (A) the device configuration and (B) the in-syringe KF-SPME/ISD procedure.

and connected with a syringe to construct the extraction device. The procedure began with the activation of KF using a phosphate buffer solution to ensure it was thoroughly wetted, facilitating subsequent adsorption. Following this, a saturated DNPH solution (prepared in 65 % ACN, v/v) was drawn in, allowing DNPH to be adsorbed onto the KF surface through hydrophobic interactions. Subsequently, the acidified sample solution was drawn in, where furfural compounds in the solution rapidly underwent in-situ derivatization with the DNPH adsorbed on the KF. The resulting hydrophobic derivatives remained adsorbed on the KF during the washing step, which removed residual sample solution. Finally, acidified ACN was used for desorption, releasing the derivatives from the KF into the desorption solution by disrupting the hydrophobic interactions between the DNPH-furfural compounds. The desorbed solution was then directly analyzed using HPLC-UV. Fig. 2 shows the chromatograms of a standard solution of furfural compounds (50 μg/mL) treated with the in-solution reaction procedure, a standard solution of furfural compounds (20 μg/mL) treated with the PT-KF-SPE/SD approach, and a blank solution treated with the PT-KF-SPE/ISD approach. These results demonstrate the effectiveness of the proposed method for the simultaneous derivatization and extraction of furfural compounds without interfering with the detection results.

3.2. Optimization of pretreatment conditions

To establish the most suitable pretreatment conditions, several factors potentially impacting the extraction/in-situ derivatization efficiency were investigated. These factors included the ACN content in DNPH solution, the pH of sample solution, the volume of DNPH solution,

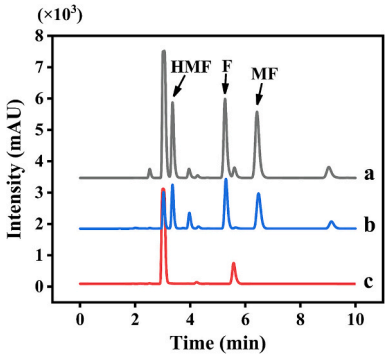

Fig. 2. Chromatograms of (a) a standard solution of furfural compounds (50 μg/mL) treated with the in-solution reaction procedure, (b) a standard solution of furfural compounds (20 μg/mL) treated with the PT-KF-SPE/SD approach, and (c) a blank solution treated with the PT-KF-SPE/ISD approach.

the type and volume of desorption solvent, the amount of KF, and the number of pushing/pulling cycles for DNPH solution, sample solution and desorption solution. Each of these variables was investigated separately, and each experiment was performed in triplicate ($n = 3$).

3.2.1. The ACN content in DNPH solution

DNPH is slightly soluble in water, but readily soluble in various organic solvents such as ACN. However, the interaction between DNPH and organic solvents is strong, making it challenging to transfer and adsorb DNPH to KF. In addition, KF, being an efficient oil-absorbing material, can absorb a large quantity of oily solvents, introducing a variety of impurities. Hence, an aqueous solution was chosen to dissolve DNPH in this study. To enhance the amount of DNPH dissolved in the solvent, ACN aqueous solutions with varying volume ratios from 0 to 90 % were selected for optimization. As displayed in Fig. 3A, the signal intensity of the analyte-DNPH derivatives gradually increases from 0 % to 65 %, peaking at 65 %, and then decreases as the ACN proportion increases further. This may be due to the increasing solvent force on DNPH with the rise in ACN proportion, which counteracts part of the adsorption force of KF, resulting in a decrease in the amount of DNPH adsorbed on the fiber. Given the small volume of the syringe (only 2.5 mL), using a high-concentration DNPH solution can reduce the volume of reagents required. Consequently, a 65 % ACN aqueous solution was selected as the optimal condition in the experiments.

3.2.2. The pH of sample solution

Most research indicates that the nucleophilic addition reactions between aldehydes and DNPH necessitate an acidic environment (Wang et al., 2021). To ascertain the optimal pH value, the influence of the pH of the sample solution was thoroughly investigated within a pH range of 1.0–10.0. The results presented in Fig. 3B showed that the signal intensity of derivatives reached a maximum at pH 1, and subsequently decreases as the pH of the sample solution increases. This may be due to the acidic conditions promoting the bonding of hydrogen atoms with oxygen in the carbonyl group, leading to oxygen protonation. This process facilitates the acquisition of π electrons, enhances the positivity of carbon, and makes it more susceptible to nucleophilic attack. Consequently, PBS with a pH of 1 was used to dilute the sample solution, and PBS with a pH of 1 was also chosen to activate KF in the experiments.

3.2.3. The volume of DNPH solution

The quantity of the derivatization reagent is a critical factor that influences both the reaction rate and efficiency. To minimize the sample processing time while ensuring sufficient derivative reactions of furfural compounds, the effect of the derivatization reagent volume within the

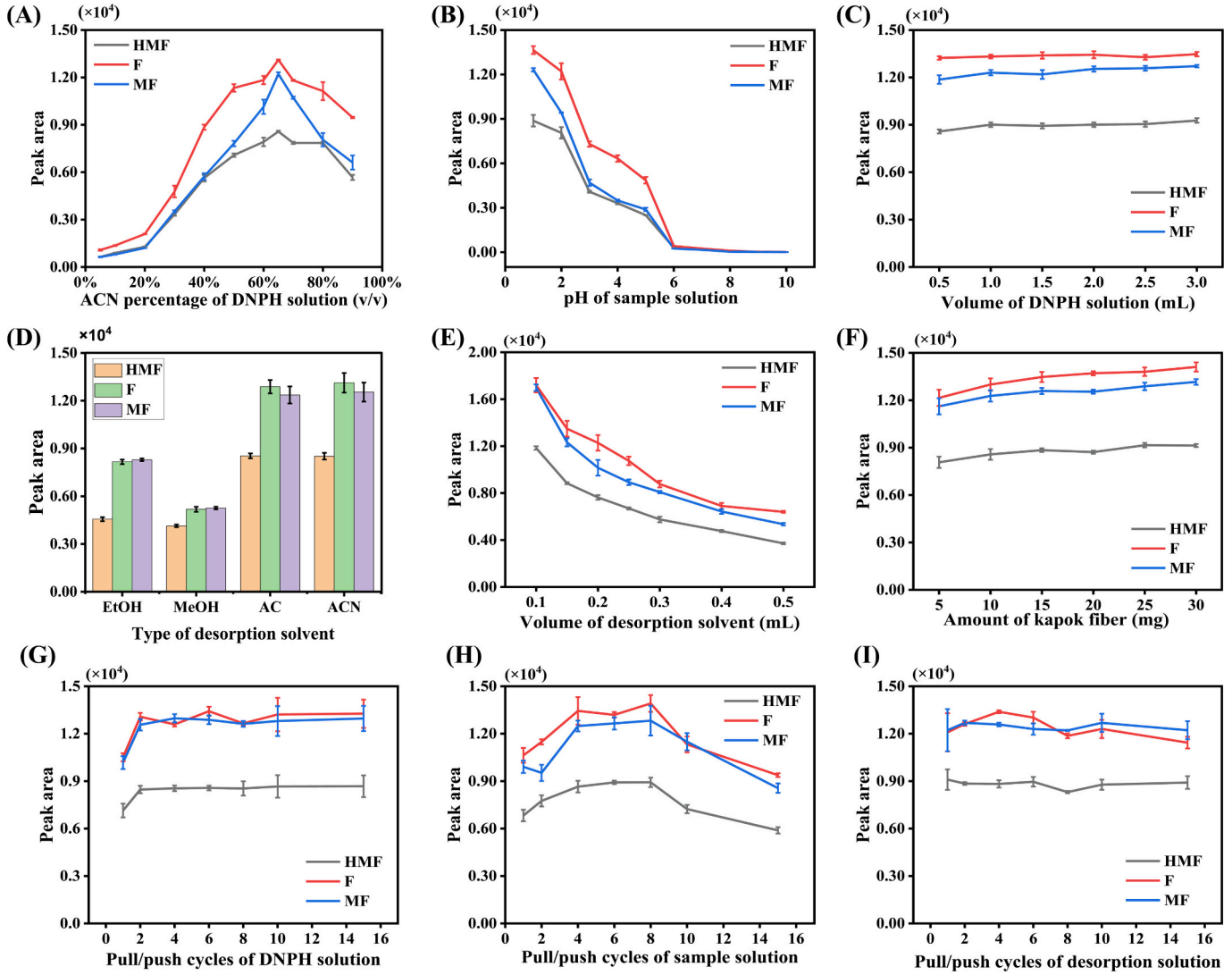

Fig. 3. Optimization of PT-KF-SPE/ISD conditions: (A) the ACN content in DNPH solution, (B) the pH of sample solution, (C) the volume of DNPH solution, (D) the type of desorption solvent, (E) the volume of desorption solvent, (F) the amount of KF, (G) pull-push cycles of DNPH solution, (H) pull-push cycles of sample solution and (I) pull-push cycles of desorption solvent.

range of 0.5–3 mL, was examined. As depicted in Fig. 3C, due to the high solubility of DNPH in an aqueous solution containing 65 % ACN, only 1 mL of the derivatization reagent is necessary to derive the furfural compounds in the sample solution. Consequently, to reduce reagent consumption and prevent excessive derivatization reagents from occupying the extraction site, which may affect subsequent experiments, a derivatization reagent volume of 1 mL was used in the experiments.

3.2.4. *The type and volume of desorption solvent*

The desorption solvent should be miscible with the organic solvent to elute the organic solvent containing aldehyde-DNPH derivatives from the KF, making the analyte-DNPH derivatives to dissolve well and be compatible with subsequent HPLC detection. The effects of four commonly used solvents (acetonitrile, ethanol, methanol and acetone) as desorption solvents were evaluated. The results exhibited in Fig. 3D demonstrated that the overall extraction efficiency of ACN for the three furfural compounds significantly surpassed that of methanol and ethanol, and is slightly higher than that of acetone. Given consideration of cost and toxicity, ACN was ultimately selected as the desorption solvent.

For simplicity, the desorption solution was directly transferred for HPLC-UV analysis. Given that the HPLC-UV is a concentration sensitive detector, the concentration of the analyte-DNPH derivatives in the desorption solution should be considered to obtain a more sensitive analysis. A small volume of desorption solution and high concentration of analyte-DNPH derivatives are beneficial to improve the sensitivity of analysis. Therefore, the impact of varying volumes of ACN desorption solvent (100, 150, 200, 250, 300, 400, and 500 μL) was further explored. The results shown in Fig. 3E indicated that the signal intensity gradually diminished as the volume of the extraction solvent increased. It was determined that 100–150 μL of ACN was sufficient to elute the majority of the target analytes. Due to the pre-concentration effect, the analyte concentration in the desorption solution is higher when using a smaller volume of desorption solvent, thereby effectively enhancing the signal intensity of the chromatographic peak. To obtain a sufficient volume of desorption solution post ACN desorption and to minimize the quantity and cost of ACN, the optimal volume of the desorption solvent was established at 150 μL.

3.2.5. *The amount of KF*

The appropriate amount of adsorbent is vital to achieve the maximum extraction efficiency of the analyte. Based on the previously optimized conditions, the impact of varying amounts of KF (5, 10, 15, 20, 25 and 30 mg) on signal intensity was further evaluated. As depicted

in Fig. 3F, the signal intensity significantly increased from 5 mg to 15 mg. However, beyond 15 mg, the signal intensity didn't show a notable increase with further increase in fiber mass. This suggests that 15 mg of KF is sufficient to effectively extract furfural compounds from the sample solution. Given that an increase in the mass of KF would elevate the suction/release resistance of the solution, we opted for a KF mass of 15 mg in the experiments.

3.2.6. Push-pull cycles of DNPH solution, sample solution and desorption solvent

To ensure a seamless process of extraction and in-situ derivation, it's necessary for the KF to adsorb sufficient derivatization reagent to undergo the derivatization reaction with furfural compounds, and to be completely desorbed. This experiment optimized three steps involving the number of push-pull cycles that could potentially impact the extraction efficiency. The influence of the number of push-pull cycles on the extraction efficiency was rigorously assessed within the range of 1–15 push-pull cycles (approximately 8 s per cycle). As shown in Fig. 3G, Fig. 3H and Fig. 3I, the optimal number of push-pull cycles for the derivatization reagent, sample solution and desorption reagent were 4, 6 and 4 cycles, respectively.

3.3. Reproducibility evaluation across different batches of KF

Considering that variations in fiber density, quality, thickness and surface characteristics could potentially influence the extraction efficiency, three distinct batches of KF were selected for the evaluation of extraction reproducibility. The results in Fig. S1 showed excellent reproducibility of parallel results within a single batch and minimal differences in signal intensity between batches. This suggested that the source of KF didn't significantly impact the extraction efficiency. These findings affirmed the performance consistency and excellent reproducibility of the proposed KF-SPE method, thereby validating its reliability for analytical applications.

3.4. Method validation

The presence of co-existing interferences can potentially impact the extraction/derivatization efficiency, thereby affecting the accuracy and reliability of the analytical results (Wang, Xu, et al., 2024; Xu et al., 2023). Generally, a matrix effect greater than 1.1 indicates a matrix enhancement effect, while a matrix effect less than 0.9 suggests a matrix inhibition effect. If the matrix effect falls within the range of 0.9–1.1, the influence of matrix effect can be considered negligible. In this study, the matrix effect of the method was evaluated by comparing the relationship between the peak area (excluding the blank) of furfural compounds in the sample solution with the peak area of the standard solution under optimized conditions. The results revealed that the matrix effect varied from 96.4 to 103.7 %. This indicated that the influence of coexisting complex sample matrices on the KF-SPE/ISD-HPLC-UV analysis was minimal. With this in mind, the following calibration curves were verified in standard solutions for convenience.

HMF, F and MF were prepared using pure water into a series of solutions with concentrations ranging from 20 to 2000 ng/mL. These solutions were subsequently analyzed using the method developed in this study. Three parallel groups were tested for each concentration, and the mean peak area was used to perform a linear regression for the analyte concentration (He et al., 2022). The limits of detection (LOD) and limits of quantitation (LOQ) were calculated based on signal-to-noise ratios of 3:1 and 10:1, respectively (Zhai et al., 2023). As shown in Table 1, the LODs for HMF, F and MF were 6.0, 3.9 and 4.2 ng/mL, respectively, while the LOQs were 19.8, 12.9 and 13.9 ng/mL, respectively. The calibration curves obtained for HMF, F and MF within the concentration range of 20–2000 ng/mL all showed a good linear relationship (correlation coefficient $\geq$ 0.995).

Furthermore, the precision and accuracy of the method were validated by evaluating intra-day and inter-day relative recovery and relative standard deviations (RSDs) at three concentrations of low, medium and high (25, 200, 1500 ng/mL) (Fan et al., 2023). For these three concentrations, three parallel sets of samples were prepared in a single day to calculate intra-day RSDs and relative recovery. Over the course of three consecutive days, three parallel samples were prepared each day, and the average values of these samples were used to calculate inter-day RSDs. The data summarized in Table 2. showed satisfactory relative recovery, ranging from 96.9 to 106.3 %, with RSD values below 5.0 %. These results demonstrated that the precision and accuracy of the developed method were suitable for quantitative analysis of furfural compounds.

Table 1
Calibration data for furfural compound analysis, including linear range, linear equations, determination coefficients, LODs, and LOQs.

Analyte	Linear range (ng/mL)	Linear equations	Determination coefficient (R^2)	LODs (ng/mL)	LOQs (ng/mL)
HMF	20–2000	y = 0.40424x + 5.60232	0.995	6.0	19.8
F	20–2000	y = 0.60562x + 6.65465	0.999	3.9	12.9
MF	20–2000	y = 0.57706x + 6.38656	0.999	4.2	13.9

3.5. Real sample analysis

The validated quantitative method was successfully applied to measure furfural compounds in various real samples, including tea beverages, fruit juices, and herbal oral liquids (Table S1). Notably, HMF was present in all samples, with the highest concentration in fruit juice 2 at 3.345 ± 0.122 μg/mL, highlighting its widespread occurrence due to processing and storage conditions. F was detected in certain samples, such as tea beverage 2, at 0.105 ± 0.004 μg/mL, indicating its potential formation during prolonged storage. MF, however, was not detected, suggesting it may be present in minimal quantities or below the detection limit. This method demonstrates good sensitivity and applicability across different sample types, providing valuable insights into the prevalence of furfural compounds and emphasizing the importance of controlling processing parameters to ensure food safety and quality.

It is important to note that this study aims to develop an affordable, readily available, and cost-effective method by using HPLC-UV for detection. However, a limitation of this approach is that it relies solely on retention time for qualitative and quantitative analysis. The selectivity, accuracy, and sensitivity are suitable for routine screening, but for more precise analysis, HPLC-MS is recommended due to its higher

Table 2
Intra-day and inter-day recovery and precision data for furfural compounds determination.

Analyte	Added (ng/mL)	Intra-day (n = 3)		Inter-day (n = 3)	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
HMF	25	103.9	1.2	103.2	1.3
	200	100.9	1.5	99.9	2.3
	1500	99.5	2.7	99.6	3.2
F	25	99.5	1.5	103	1.9
	200	99.7	0.4	102.8	4.2
	1500	98.7	2.7	96.9	5.0
MF	25	98.5	3.7	97.2	3.7
	200	98.5	2.7	99.2	3.0
	1500	106.3	4.5	99.7	2.2

selectivity, accuracy, and sensitivity (Cao et al., 2024; Tian et al., 2024).

3.6. Evaluation of method's greenness

To comprehensively assess the environmental footprint of the proposed analytical method, five distinguished greenness assessment tools, each focusing on a unique aspect of the method's greenness, were utilized (Cetinkaya et al., 2023; Shi et al., 2023). The combined use of these tools provides a holistic evaluation of the method's sustainability and adherence to the principles of green analytical chemistry.

The Green Analytical Procedure Index (GAPI) evaluates the green character of an entire analytical methodology, including sampling, transport, storage, sample preparation, and final determination (Płotka-Wasylka, 2018). A pictogram with a color scale represents the greenness of each stage of the analytical process. According to the GAPI results illustrated in Fig. 4A, the pictogram contains seven green, five yellow, and three red areas, reflecting the method's overall excellent greenness.

The Analytical GREEnness (AGREE) calculator is a metric system for assessing the greenness of analytical procedures based on the 12 principles of green analytical chemistry and allows for the weighting of different criteria (Ni et al., 2023). The clock-like graph produced by the AGREE tool (Fig. 4B) displayed a central score of 0.63, surpassing the 0.6 threshold, which signifies that the method is considered green.

The AGREE metric specifically for sample preparation (AGREEprep) gives prominence to ten categories impacting sample preparation (Wojnowski et al., 2022). Fig. 4C shows the colorful round pictogram representing the AGREEprep assessment of the established method. The inner circle is green, and the overall score is 0.63, indicating a strong green performance in sample preparation.

As an additional tool, the Sample Preparation Metric of Sustainability (SPMS) excludes aspects of the initial sampling and final detection technique and assesses the greenness of sample preparation techniques (Martínez-Pérez-Cejuela et al., 2024). The clock diagram in Fig. 4D uses colors to signify performance levels, ranging from green for "successful" to red for "inadequate." The central large square in the diagram indicates that the global numeric score for the sample preparation method is 6.63.

The blue applicability grade index (BAGI) is a complement to the greenness index and is mainly used to evaluate the practical aspects of the methods (Manousi et al., 2023). The total score > 60 in the asteroid pictogram (Fig. 4E) indicates the method's excellent applicability and is considered green.

In summary, the evaluations by these six greenness assessment tools collectively indicate that the method employed in this study exhibits excellent greenness.

3.7. Method advantages

This study introduces a rapid, convenient and effective method, named PT-KF-SPE/ISD, for detecting HMF, MF, and F. The method leverages the natural adsorbent KF to adsorb the derivatization reagent DNPH firstly, and then effectively proceed extraction and in-situ derivatization of furfural compounds simultaneously.

Compared to the reported methods for furfural compound analysis, the KF-SPE/ISD-HPLC-UV approach offers several key advantages. Firstly, it simplifies the extraction and derivatization processes, allowing for rapid and convenient operation without the cumbersome procedures often associated with conventional SPE methods. The method efficiently purifies and enriches analytes with minimal effort, reducing the sample preparation time to under three minutes, thereby enhancing overall efficiency. Secondly, the use of KF, a renewable and biodegradable material, not only highlights the method's environmental friendliness but also provides a cost-effective alternative to synthetic adsorbents. The KF material is abundant, recyclable, and has low environmental impact,

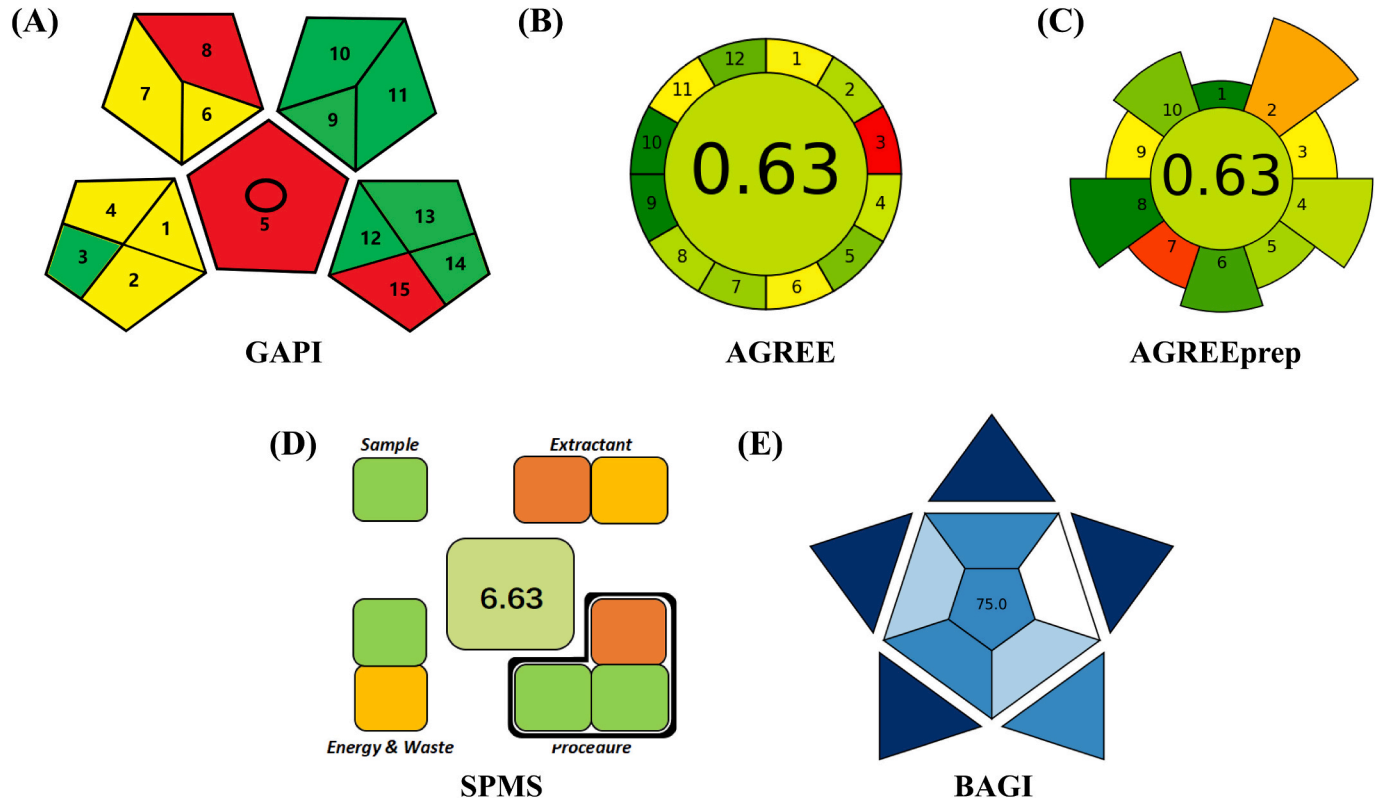

Fig. 4. Greenness assessment of the proposed analytical method using five different tools: (A) Green Analytical Procedure Index (GAPI), (B) Analytical GREEnness (AGREE), (C) AGREE for sample preparation (AGREEprep), (D) Sample Preparation Metric of Sustainability (SPMS), and (E) Blue Applicability Grade Index (BAGI). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

which aligns with the principles of green chemistry. Additionally, this method shows promise for high-throughput and automated extraction of multiple furfural compounds, offering a practical and economical solution for routine screening in food and pharmaceutical applications. Overall, the KF-SPE/ISD-HPLC-UV method stands out for its speed, simplicity, environmental sustainability, and potential for broader applications in quality control and safety assessments.

4. Conclusion

The developed PT-KF-SPE/ISD method offers a rapid, convenient, and efficient approach for the analysis of furfural compounds. By combining SPE and derivatization into a single streamlined process, this method significantly enhances the selectivity and sensitivity of furfural compound detection compared to traditional techniques while maintaining overall simplicity. The use of natural KF as an adsorbent further underscores the method's green credentials and contributes to its cost-effectiveness. The method demonstrated excellent reproducibility and accuracy in various real samples, including tea beverages, fruit juices, and herbal oral liquids, highlighting its applicability in routine food safety and quality assessments. The PT-KF-SPE/ISD method provides a practical solution for the simultaneous extraction and analysis of furfural compounds in complex matrices.

CRediT authorship contribution statement

Xiangyu Li: Writing – original draft, Methodology. **Qian Qin:** Writing – original draft, Methodology. **Yanbo Luo:** Methodology, Funding acquisition. **Yongqiang Pang:** Writing – review & editing. **Jinchao Wei:** Writing – review & editing. **Xingyi Jiang:** Writing – review & editing. **Di Chen:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

Declaration of competing 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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101795.

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