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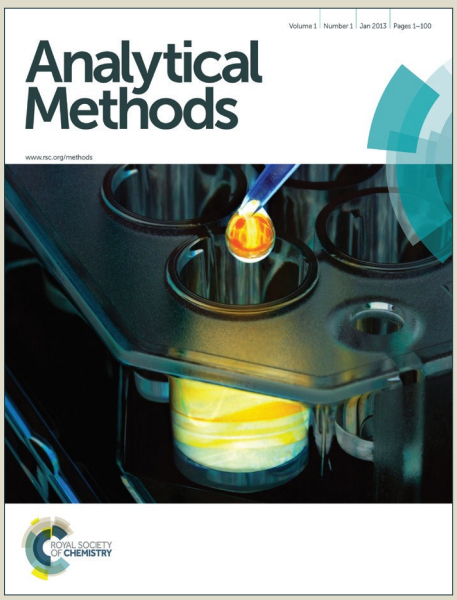

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A simple, fast and green Nanofibers Mat-based disk solid-phase extraction technique for chrysoidine analysis in soybean products

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Abstract

A simple, fast and green sample preparation method followed by HPLC-DAD was developed for the determination of chrysoidine in soybean products. Polypyrrole (PPy) functionalized nanofibers mat (PPy-NFs mat) was positioned in a home-made filter assembled to Nanofibers mat-based disk solid-phase extraction (NFsM-disk SPE) device for sample cleanup and preconcentration. After a simple extraction with alkaline ethanol-water solution, the sample extractants were diluted and directly purified by the technique. Then, eluent was analyzed forthrightly by HPLC without evaporation-concentration step. Compared to present methods, it was a faster sample preparation (10 min/sample) method with remarkably reduced organic solvent (15.7 mL). The developed method was validated by the analysis of six different kinds of spiked soybean products, resulting in satisfied recoveries (78.2-106.5%; RSD ≤ 9.7%),

and limit of quantitation (0.05 mg/kg), which demonstrates the feasibility of the proposed method. In addition, PPy-NFs mat could be reused for three times excluding matrix interference.

Keywords

Nanofibers mat; Disk SPE; Polypyrrole; Chrysoidine; Soybean produces

1. Introduction

Chrysoidine is a widely used industrial azoic dye for coloring leather, wood, paper, bamboo and so on. Due to its acute and chronic toxicity to mammals when in taken by oral or skin route, chrysoidine is strictly forbidden to be applied on foodstuffs as food additive[1, 2]. Nevertheless, illegal use of chrysoidine in soybean products has been reported from time to time[1, 3, 4], because the dye makes soybean products aesthetically and psychologically more attractive to consumers, and it is cheaper and more stable than natural food colors.

Soybean products are a kind of popular consumed foodstuffs in Asian countries like China, Japan and Korea and they are increasingly popular with people in America and Europe. For the assurance of food safety, some analytical methods have been reported for the determination of illegally used chrysoidine in soybean products[2-8]. Unfortunately, most of these methods are time-consuming and need a large volume of organic solvents leading to lower productivity and environmental contamination. The Direct GB/T 23496-2009, a unique official method is published by Chinese government in 2009 for the routine detection and monitoring of chrysoidine in foods[4]. It is based on liquid-liquid extraction (LLE) for sample preparation, which was a long process (>2.3 h/sample) consuming large amounts of organic solvents (55mL). Among these methods on sample preconcentration and cleanup, solid-phase extraction (SPE) is another most often used method. However, SPE has lower accuracy and consumes longer time owing to additional procedures, such as evaporation and reconstitution.

It is known that sample preparation is the time-determining step in the whole analytical procedure, accounting for approximately two-thirds of total analysis time. Therefore, selecting and optimizing an appropriate sample preparation scheme is a key factor in the final success of the analysis[9]. Consequently, major scientific

challenges for chrysoidine analysis is to detect rapidly and sensitively, meanwhile, to decrease the organic solvent consumption. Thus, a better sample preparation technique to determinate chrysoidine in foods is welcomed.

Nanofibers mat-based disk solid-phase extraction (NFsM-disk SPE) shows great application potential as a new type of sample preparation technique. We have applied the NFsM-disk SPE technique for trace pollutants analysis in food, e.g., electrospun Nylon 6 nanofibers membrane as SPE adsorbent for the enrichment of five phthalates esters in edible oil[10] and bisphenol A in plastic bottled drinking water[11]. The results suggest the superiority of NFsM-disk SPE which is identified by other researchers. Compared with micro-sized particles commonly used for solid-phase extraction (SPE), the NFsM-disk sorbent bed has extremely strong mutual penetration with target analytes due to the 3D structure as a result of the intertwining nature of the nanofibers, which improved sample-sorbent contact greatly, so it shortens the time of SPE procedure greatly. An additional advantage of NFsM-disk SPE is nanofibers' large specific surface area allowing the use of a few milligrams sorbent bed that achieves high extraction efficiency[12], and meanwhile, the reduction of solvents is a distinctive feature of the NFsM-disk SPE technique[13]. Besides, electrospun nanofibers are easily modified the surface functionality to improve the extraction efficiency for polar compounds and ionic species[14]. Our previous research using polypyrrole (PPy) functionalized nanofibers mat (PPy-NFs mat) as disks SPE sorbent has demonstrated highly effective extraction for sulfonated azo dyes in environmental water samples[15]. Therefore, the NFsM-disk SPE opened up possibilities for a simple, fast and efficient sample prepararion procedure for determination of chrysoidine in soybean products.

In this work, we described for the first time NFsM-disk SPE for food analysis. A novel SPE sorbent baed on PPy-NFs mat was prepared and evaluated for the determination of chrysoidine in soybean products by HPLC-DAD. Six different kinds of soybean products were chosen for the study of the method applicability.

2. Materials and methods

2.1. Chemicals and Reagents

Chrysoidine (>98% purity) was purchased from Adamas Reagent Co., Ltd. (Shanghai, China). The HPLC-grade methanol was obtained from Tedia Company,

Inc. (Ohio, USA). Other chemicals, i.e. anhydrous ethanol, aqueous ammonia (25% w/v), hydrochloric acid (HCl), sodium hydrochloric (NaOH), ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) and pyrrole monomer, were of analytical grade and purchased from Chemical Reagent Factory (Shanghai, China). Nylon 6 (PA6) was purchased from Debiochem (Nanjing, China). Water from Milli-Q purification system (Millipore, MA, USA) was used for the preparation of the aqueous mobile phase as well as for the preparation of the standard solutions.

The standard stock solution of chrysoidine (1000 mg/L) was prepared in ultrapure water and stored in a refrigerator at 4°C for 3 months. The standard working solutions were daily prepared by diluting the stock solution with ultrapure water.

2.2. Apparatus

All analysis was performed on a Shimadzu HPLC system (Shimadzu, Kyoto, Japan) equipped with LC-20AD pumps and a diode array detector SPD-M20. A vortex mixer (Qite, Shanghai, China), a thermostatic ultrasonic bath (Xinzhi, Ningbo, China), and a high speed refrigerated centrifuge (Xiangya, Hunan, China) were used for the pretreatment of soybean products samples.

A home-made NFsM-disk SPE device was designed as shown in Fig. 1, which was used in the purification and enrichment steps. Its core component is a filter filled with one piece of round PPy-NFs mat with a diameter of approximately 10.0 mm. Both ends of the filter can be screwed down, with a sample tube on the top and a collecting tube on the bottom. 12 filters were connected to a Supelco SPE system (Sigma-Aldrich, Shanghai, China). Thus, 12 samples were simultaneously prepared within a single sample preparation test.

2.3. Preparation of PPy-NFs mat

PPy-NFs mat was fabricated according to a method reported by our group previously [15]. Briefly, the PA6 nanofibers mat was firstly fabricated as templet using electrospinning. The electrospun PA6 nanofibers mat with 100 μm thick was cut into pieces of 10 cm × 10 cm size, then immersed in a 0.1mol/L anhydrous ethanol solution of pyrrole for 1h at room temperature. The oxidative polymerization of pyrrole monomer and the deposition of PPy on the PA6 nanofibers were initiated by

the addition of the same volume of 0.23 mol/L anhydrous ethanol solution of ferric chloride for 24 h. The PA6 nanofibers mat changed appearance from white to black as the result of the coating of the black PPy during polymerization process. The synthesized mat was washed by anhydrous ethanol and water in several times until the solution turned colorless, and then dried naturally. Before it's used, PPy-NFs mats were pre-activated with 200 μL of water and 200 μL of methanol and following with 200 μL of ammonia-water solution (5:95, v/v).

2.4. Sample preparation

All of soybean product samples, including yuba oil tofu skin, dried beancurd, soybean milk, tofu pudding and bean sauce, were obtained from local markets. After each raw sample was pulverized to powder, 1.0 g powder was homogenised in 1.0 mL of anhydrous ethanol with vortex mixer for 30 s to get tested sample.

The 1.0 g of pre-homogenised sample was extracted with 10 mL of extract solvent in an ultrasonic bath at 40 ℃ for 30 min; then centrifuged at 10000 rpm for 5 min. The extraction super-extract was collected. The extraction procedure was repeated once. 2 mL of the pooled super-extracts was dilutied with water to 20 mL as the loading solution of NFsM-disk SPE.

After pH adjusted, the loading solution was normally passed through the activated NFsM disk at appropriate flow rate. After rinsing with 200 μL of water, the retained target analyte was eluted. Then, 20 μL of eluent was undergone to HPLC analysis directly.

2.5. HPLC-DAD analysis

The Dikma C_{18} column (250 mm×4.6 mm, 5μm) was used for analysis at 30°C. The HPLC separation was performed by methanol and water (containing 0.1% (v/v) formic acid) as the mobile phase (methanol/water = 55/45, v/v). The flow rate was 1.0 mL/min and the injection volume was 20 μL. The wavelength of maximum absorption for chrysoidine at 458 nm was used for quantification.

2.6. Optimization of sample preparation conditions and method validation

In this study, three extractants including anhydrous ethanol, anhydrous ethanol-water (70:30, v/v) and anhydrous ethanol-3% ammonia in water (70:30, v/v) were tested for the optimum extraction of chrysoidine in the spiked samples.

And parameters affecting NFsM-disk SPE efficiency was optimized by 2 mL of 0.1 μg/mL standard solution such as sample pH, sorbent amount and type and volume of eluent. The reusability of PPy-NFs mat was investigated by utilizing the simulated sample that was prepared as follows. 1.0 g of chrysoidine-free yuba sample was extracted by 20 mL of anhydrous ethanol-3% ammonia in water (70:30, v/v), and the 10-fold dilution of the extract solution was used as solvent to prepare simulated sample containing chrysoidine at concentration of 0.1 μg/mL. The simulated samples was also used to optimize sample loading volume considering the effect of the sample matrix on breakthrough volume.

All the optimization tests mentioned above were achieved by varying one parameter at a time and keeping other parameters constant (six replications for each parameter). The average recoveries, as well as relative standard deviation (RSD %), was used to evaluate the optimization results.

Under the optimum extraction and NFsM-disk SPE conditions, the analytical performance of the proposed method was evaluated by the linearity, sensitivity, as well as accuracy and precision in terms of the recovery and relative standard deviation (RSD %) using spiked samples.

2.7. Matrix effect evaluation

To identify the influence of the matrix, matrix effect percentages (% ME) was calculated according the following equation[16]:

$$\% \text{ ME} = 100 \times (R_2 - R_B)/R_1$$

where R_2 and R_B stand for the peak area of the spiked sample and non-spiked sample after extraction, respectively; R_1 is response for the corresponding chrysoidine spiked in 10-fold dilution of the extract solution.

3. Results and discussion

3.1. Optimization of sample extraction

An efficient extraction procedure is one of primary steps for accurate determination of target analytes in samples. A feasible extraction method could acquire the maximum amounts of target analytes from sample matrix with minimum interference. For the development of an efficient extraction process, in this work, three extractants used for chrysoidine extraction from soybean products were initially adapted from the Direct GB/T 23496-2009. As shown in Table 1, anhydrous ethanol-3% ammonia in water (70:30, v/v) yielded the highest extraction efficiency and the most stable recoveries for chrysoidine compared to anhydrous ethanol (used in the Direct GB/T 23496-2009) and anhydrous ethanol-water (70:30, v/v). Moreover, most of interference present in soybean products were precipitated and were efficiently removed by the subsequent centrifugation due to the alkaline condition. Thus, 20 mL of anhydrous ethanol-3% ammonia in water (70:30, v/v) was chosen as extract solvent.

3.2. Evaluation of the NFsM-disk SPE sorbent

To evaluation the effectiveness of PPy-NFs mat for the adsorption and desorption of chrysoidine, some important parameters were investigated. Firstly, the influence of pH was tested. 2.5 mg of pre-activated PPy-NFs mat was used to load through 2 mL of 0.1 μg/mL standard solutions at different pH values (3, 5, 7, 9 and 11). The sample pHs were adjusted by 1.0 mol/L HCl or NaOH solution. The results shown in Fig. 2 indicated that the recovery increased with an increase in pH values, the highest being at basic condition, where the recovery was 92.2% at pH 9, and the adsorption capacity reduced extremely with the recovery of 35.0% at pH 3. When pH increased from 9 to 11, there is no significant variation on the recoveries of chrysoidine. From the results, we speculated that the sorption mechanism of chrysoidine by PPy-NFs mat was π–π interaction. Since, in solutions at pH lower than 5, the positively charged protonated species is the prevalent form of chrysoidine, because the pKa value for chrysoidine is 5.41[17]. Also, PPy surface is positively charged at pHs below 10[18]. Consequently, the lower sorption at pH 3 can be rationalized taking into account the electrostatic repulsion exerted by PPy-NFs mat surface towards chrysoidine, both being positively

charged at this pH. When pH is higher than 7, the neutral species of chrysoidine is predominant, so that the extraction efficiency of chrysoidine increased significantly with the increase of pH because PPy containning a conjugated π structure can efficiently capture aromatic compounds easily through π–π interaction. Hence the optimal sample pH was considered as alkaline condition (pH≥9). In order to obtain stable and reliable recoveries, we chose pH 10 for the subsequent analysis.

To ensure that target analyte was adsorbed completely with sufficient sorbents, the PPy-NFs mat amounts in the range of 2.0-4.0 mg (0.5 mg increase at each step) were examined with 2 mL of 0.1 μg/mL standard sample solution (pH=10). As the results shown, no observable change of the recoveries for target analyte was found when the sorbent dosage was increased from 2.0 to 4.0 mg. It was found that 2.0 mg of mat can still achieve satisfied recoveries, but such mat was so thin and fragile that it was easy to be damaged while dealing with large volume water samples (>10mL). That is, 2.5 mg of PPy-NFs mat was used to achieve effective pretreatment.

To save analytical time and achieve satisfactory extraction performance, the flow rate of the loading solution for SPE operation was investigated. It was found that 2.0 mL/min flow rate of sample was best, since it was not only without any loss of recoveries but also ensured optimal analysis time. Therefore, such a flow rate of sample loading was chosen in the further experiment.

The selection of eluents is one of the crucial factors in the SPE procedure. In this experiment, four kinds of eluents including anhydrous ethanol, methanol, ammonia-methanol (5:95, v/v) and acetic acid-methanol (5:95, v/v) were compared. The results indicated that methanol was better with the recovery of 93.5 %±5.8%. It was found that 500 μL of eluent was sufficient to desorb the trapped analyte. Any further reduce in elution volume decreased desportion efficiency. Thus, the lowest volume providing the best recovery was 500μL of methanol.

In order to obtain better enrichment coefficient that improve analytical sensitivity, it was necessary to determinate the breakthrough volume. Different volumes (10, 20, 25, 30, 40 mL) of simulated sample at pH 10 were performed the preconcentration step. The trapped analyte on PPy-NFs mat was eluted with 500 μL methanol. Good recoveries (>80%) were obtained for analyte even loading 25 mL sample volume (see Fig. 3).

3.3. Validation of the NFsM-disk SPE method

3.3.1. Analytical performance

Linearity was studied by analyzing spiked chrysoidine-free yuba. Satisfactory linearities (r^2=0.998) were obtained within the range of 0.05-50.0 mg/kg. Limit of quantification (LOQ) (estimated at S/N ratio of 10) was 0.05 mg/kg.

3.3.2. Precision and accuracy

The precision of the method was verified by measuring recoveries from spiked blank sample matrices of six different soybean products (yuba, oil tofu skin, dried beancurd, soybean milk, tofu pudding, bean sauce samples) at three spiking levels (0.1, 1.0 and 5.0 mg/kg) for chrysoidine. Mean recovery data and RSDs are given in Table 2, which expressed the repeatability of the proposed method. For all investigated sample matrices, the results obtained were satisfactory, with recoveries of 78.2-106.5%. The RSDs were calculated in terms of intra-day repeatability and inter-day reproducibility (three alternative days). Intra-day RSDs were below 9.7%, while inter-day RSDs were lower than 10.5%. The overall precision and accuracy of the method were sufficient for the quantification of chrysoidine in soybean products.

3.3.3. Reusability of NFsM-disk SPE sorbent

To evaluate the reusability of PPy-NFs mat, six pieces of 2.5mg PPy-NFs mat were studied and each mat was successively used to clean up 20 mL simulated sample for several times. After every SPE process, 500 μL of methanol and 500 μL of water were used to pass through each PPy-NFs mat respectively in order to get rid of the possible residue of target analyte. Then, the PPy-NFs mat was re-applied in the next SPE extraction cycle. It was found that the average recovery of chrysoidine was 87.5±6.2% at its third cycle used. However, the higher back pressure resulted in an unstable loading flow rate. So the result was confirmed that PPy-NFs mat could be used for 3 times at least on the premise of satisfied extraction efficiency and analysis time. The NFsM-disk SPE has shown competitive advantage compared to the commercially available SPE columns which can be used only once.

3.4. Method comparison

3.4.1. Comparison with the Direct GB/T 23496-2009

According to the GB/T 23496-2009, anhydrous ethanol was used for the extraction of chrysoidine in soybean products. Four extraction steps were utilized. Firstly, to 20 g of sample, 5 g of sodium sulphate and 25 mL of anhydrous ethanol were added and sonificated for 30 min at RT. The mixture was centrifuged (10 min, 3000 rpm), and the super-extract was transferred. Secondly, the solid residue was extracted again with 30 mL of anhydrous ethanol in three times, with each sonification lasting for 30min at RT. Then the combined sample extracts were centrifuged (10 min, 3000 rpm) and collected. Thirdly, the pooled super-extracts were concentrated under reduced pressure to dryness at 40 ℃. The evaporation residue was redissolved in some anhydrous ethanol and the solution was transferred in a tube. Finally, the obtained solution was evaporated under a gentle nitrogen stream at 50 ℃. The residue was reconstituted in 2 mL of the mobile phase, i.e. methanol-20 mM ammonium acetate solution (65:35, v/v), and filtered using a 0.45 μm filter followed by HPLC-UV analysis.

Despite it being seemingly straightforward method, tediously time-consuming process (>2.3 h/sample) and large amounts of organic solvents (55 mL) are evident. It is well known that vaporization steps are time-consuming and not easy to control. In our study, we did find that concentration by nitrogen blowing system resulted in a low recovery of chrysoidine (<50%).

The newly proposed method used NFsM-disk SPE to achieve purification-concentration procedure without the step of vaporization-concentration. It overcomes the above problems and made the sample preparation simple. The diluted extract solution with the volume of ethanol below 7% was used to perform NFsM-disk SPE directly following the steps described in Section 2.4. The proposed NFsM-disk SPE method was not only simple in handling sample preparation, but also saved considerable time (1.5-2 h for whole analysis). Moreover, the NFsM-disk filters are very easy to be incorporated into commercial SPE system. So 12 samples were simultaneously prepared within a single sample preparation. This implies that sample preparation time requied per sample is less than 10 min. Clearly, the NFsM-disk SPE method extremely promote the analysis efficiency.

We further compared the analytical performance of our proposed method with the Direct GB/T 23496-2009. The analytical results obtained using the proposed method were compared with those obtained using GB/T 23496-2009[4]. It was confirmed that the concentrations of chrysoidine in soybean products at different spiked levels (1.0, 10.0 and 20.0 mg/kg) determined by the proposed method were equivalent with those determined by using the Direct GB/T 23496-2009 method (r^2> 0.98) (see Fig. 4). It was reported that chrysoidine content in illicitly dyed soybean products ranged from 1.2 to 38.6 mg/kg[4]. Although the LOQ of the proposed method (0.05 mg/kg) is slightly higher than 0.03 mg/kg from GB/T 23496-2009, it can satisfy the practical requirement for detection. It was concluded reasonably from the results that the proposed NFsM-disk SPE method is not only simple and fast, but also has trustworthy analysis results.

3.4.2. Comparison with other existing SPE-HPLC method

To overcome the limitations of LLE, SPE techniques have been reported as an alternative for the clean-up and enrichment of chrysoidine in soybean products. It was known that the extraction efficiency of SPE depends on the types of sorbent. The frequently used SPE sorbents in literatures are non-polar reversed-phase sorbents with silica base (C_{18})[19], hydrophilic polymeric sorbents (HLB)[5] and ion-exchange sorbents (Strata-X-AW)[2]. Besides that, molecularly imprinted polymer packed in tube or column has been performed for the extraction of chrysoidine, but much lower recoveries were obtained (68.43-80.25%)[3, 7, 8] (see Table 3). Clearly, we can see the following advantages of the proposed NFsM-disk SPE compared to other SPE-HPLC methods: (1) conspicuous reduced SPE sorbent (2.5 mg); (2) lower consumption of organic solvent (15.7 mL); and (3) elution simplicity with a little eluent (500 μL).

In addition, all of these sorbents mentioned above are packed in SPE column or tube, where high back pressure and clogging of the sorbent during loading can occur resulting in an unstable loading flow rate that not only affects the extraction efficiency, but also needs considerable SPE time. Compared with these SPE columns, disk-SPE shows low back pressure because of its larger media cross-sectional area, which allows processing of large volume sample[20]. And the 3D structure of nanofibers mat are helpful to improve extraction efficiency even using less sorbents due to increased interface between sample and sorbent bed. The whole sample analysis process was

completed in less than 2 h and 12 samples were prepared at the same time. So 10 min was required for a sample. Therefore, the proposed NFsM-disk SPE improved SPE efficiency.

PPy has been used for the extraction of polar or ionic compounds due to its inherent and unusual multifunctional properties[21-23]. However, to our knowledge, except that PPy prepared as nanoparticles or nanowires for the extraction of pesticide residues in beverage and estrogens in milk indicating good extraction efficiency[24, 25], no reports about disk SPE using PPy nanofibers as sorbent for food analysis has appeared up to now. In this work, PPy-NFs mat combined with the unique advantages of nanofibers and PPy showed obvious advantages as SPE sorbent for fast purification and concentration of chrysoidine in bean products.

3.5. Matrix effect and real sample analysis

The bean products was fortified with 0.1 mg/kg of chrysoidine. %ME were 99.1%, 98.5%, 92.4%, 94.3%, 99.2% and 92.8% for yuba, soybean milk, dried beancurd, tofu pudding, oil tofu skin and bean sauce, respectively. The results showed that the determination of chrysoidine was slightly affected by the sample matrix.

To evaluate the applicability of the developed method for real samples, a variety of soybean products, including yuba (6 samples), soybean milk (12 samples), dried beancurd (5 samples), tofu (8 samples), tofu pudding (15 samples), oil tofu skin (20 samples) and bean sauce (10 samples) purchased from local markets were analysed by the proposed method (three replications for each sample). Except for one of oil tofu skin samples (0.14 mg/kg of chrysoidine), no chrysoidine were detected in any other matrices. The representative HPLC–DAD chromatograms for the positive oil tofu skin sample were shown in Fig. 5. The results indicated the quality safety of most of bean products since chrysoidine has been included in routine monitoring program for the detection of forbidden pigments in foodstuffs.

4. Conclusions

In this work, we developed a novel NFsM-disk SPE method coupled with HPLC analysis for quantitation of chrysoidine in some soybean products. The method has satisfactory analytical performance, such as high recoveries, low LODs and good

precision. Compared with National Standard Method and other SPE-HPLC methods, NFsM-disk SPE has superiority in the field of minimizing the organic solvent consumption and total analysis time. These results indicated that NFsM-disk SPE is a simple, rapid and green SPE method for the extraction of target material in food samples.

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Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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Table 1. Effect of extraction solvents on the average recoveries (n=3).

Extraction solvent	Recovery (%)	RSD (%)
Anhydrous ethanol	88.6	8.0
Anhydrous ethanol-water (70:30, v/v)	81.5	6.5
Anhydrous ethanol-3% ammonia in water(70:30, v/v)	95.2	5.5

Table 2. Recoveries and variations obtained for chrysoidine spiked in different soybean products matrixs analyzed with HPLC-DAD (n=6).

Sample	Spiked level (mg/kg)	Average recovery (%)	RSD (%)
yuba	0.1	93.5	6.4
	1.0	90.0	6.8
	5.0	106.5	5.3
oil tofu skin	0.1	92.9	7.8
	1.0	91.4	5.5
	5.0	95.3	5.2
dried beancurd	0.1	78.2	9.7
	1.0	79.5	6.3
	5.0	83.9	7.6
soybean milk	0.1	95.5	2.3
	1.0	98.5	4.7
	5.0	93.9	3.2
tofu pudding	0.1	101.2	5.4
	1.0	98.7	3.8
	0.5	96.4	5.0
Bean sauce	0.1	81.2	9.4
	1.0	87.7	5.9
	5.0	92.4	7.2

Table 3. Comparison of SPE-HPLC methods for the determination of chrysoidine in bean products.

Sample	Determination technique	Sample preparation	Sorbent amount (mg)	Organic solvent consumption (mL)	Eluent volume and procedure	Analytical total time (h/sample)	Recovery (%)	LOQ	Reference
Bean products	HPLC-DAD	A simple procedure using ammonia-water-anhydrous ethanol and NFsM-disk SPE cleanup	2.5	15.7	500μL without evaporation and reconstitution	10 min	78.2-106.5	0.05 mg/kg	This work
Bean products	HPLC-MS/MS	Extraction with acetonitrile/ammonia-water (9:1, v/v) and SPE cleanup using Strata-X-AW cartridge	60	59.7	18 mL with evaporation and reconstitution	-	81-126	0.002-0.006 mg/kg	[2]
Oil bean curd	HPLC-UV	A doubly deionized water extraction followed by MIP-based SPE cleanup	50	-	2.5 mL without evaporation and reconstitution	-	89.3-97.6	0.04 μg/L	[3]
Dried beancurd, bean sauce, yuba	LC-MS	A extraction procedure using acetonitrile and SPE cleanup using HLB caatridge	60	8.5	2 mL with evaporation and reconstitution	-	78.3-101.8	0.002 mg/kg	[5]
Yuba, stewed tofu	HPLC-UV	Extraction with ethanol and MIP-based SPE cleanup	300	42	6 mL without evaporation and reconstitution	-	68.43-80.25	-	[8]

- : Not given.

Figure captions

Figure 1. Schematic representation of NFsM-disk SPE device.

Figure 2. Effect of sample pH on the recoveries (n=6). Conditions: sample loading volume, 2 mL; concentration of chrysoidine: 0.1 μg/mL; eluent, 500μL methanol.

Figure 3. Effect of sample loading volume on SPE efficiency (n=6). Conditions: sample pH, 10; eluent, 500μL methanol.

Figure 4. Comparison of the analytical results between the proposed method and the Direct GB/T 23496-2009. Each point is the average of individual sextuple determinations. The dotted line corresponds to a perfect correlation (y = x).

Figure 5. HPLC chromatograms of (a) 100 μg/L standard solution and (b) the positive oil tofu skin sample. Conditions: HPLC conditions were described in section 2.5.; Detection wavelength, 484nm.

Figure 1.

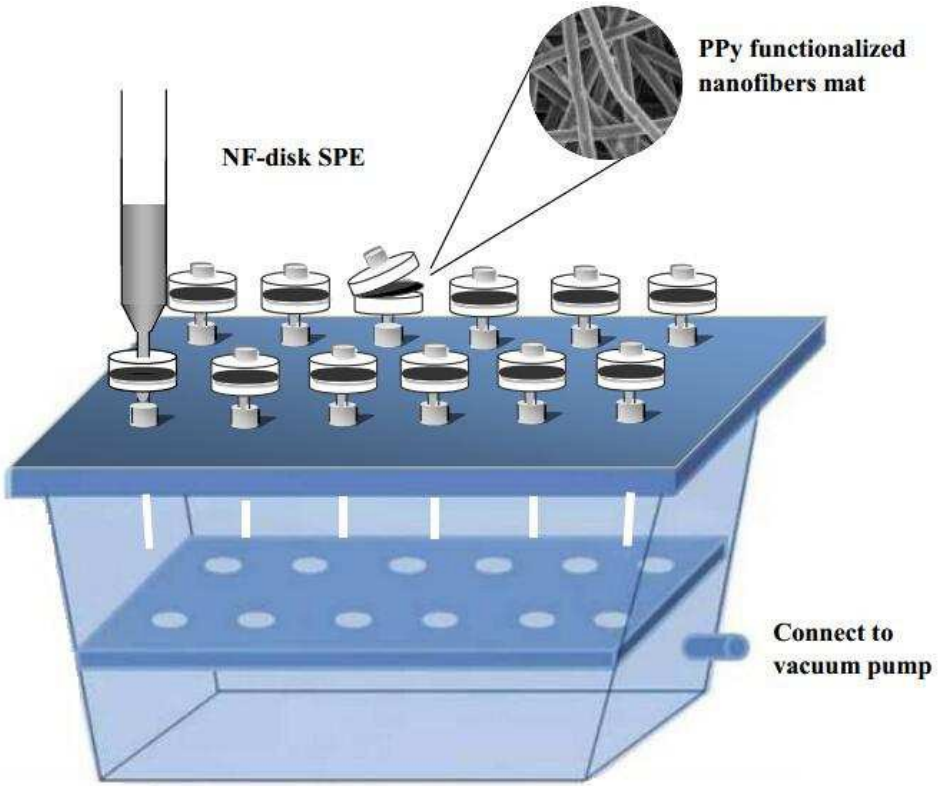


Figure 2.

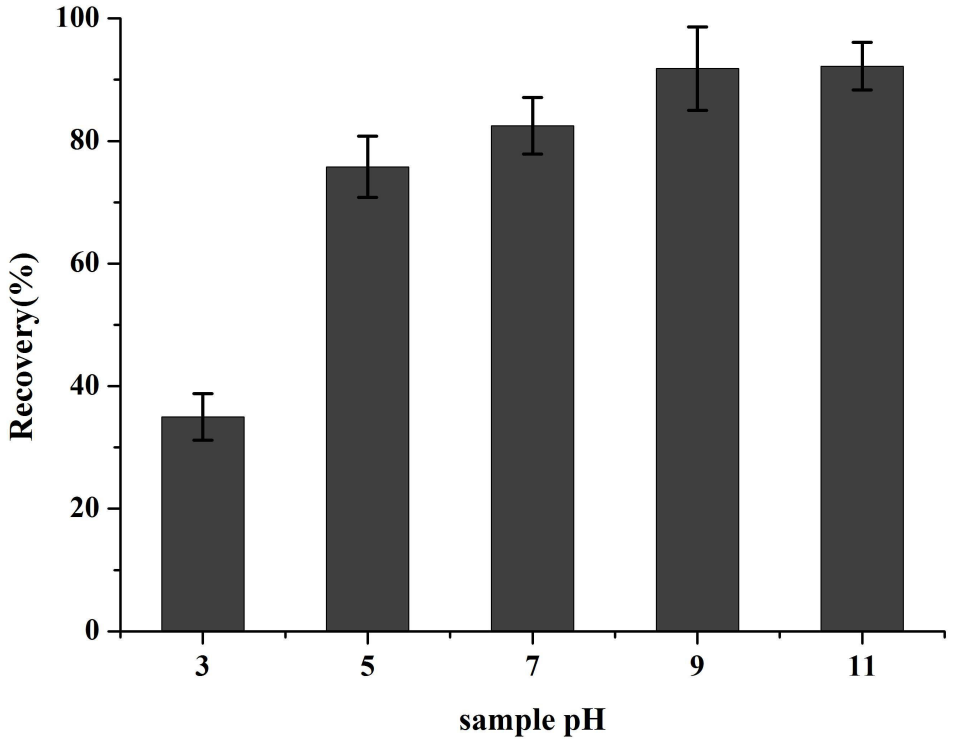


Figure 3.

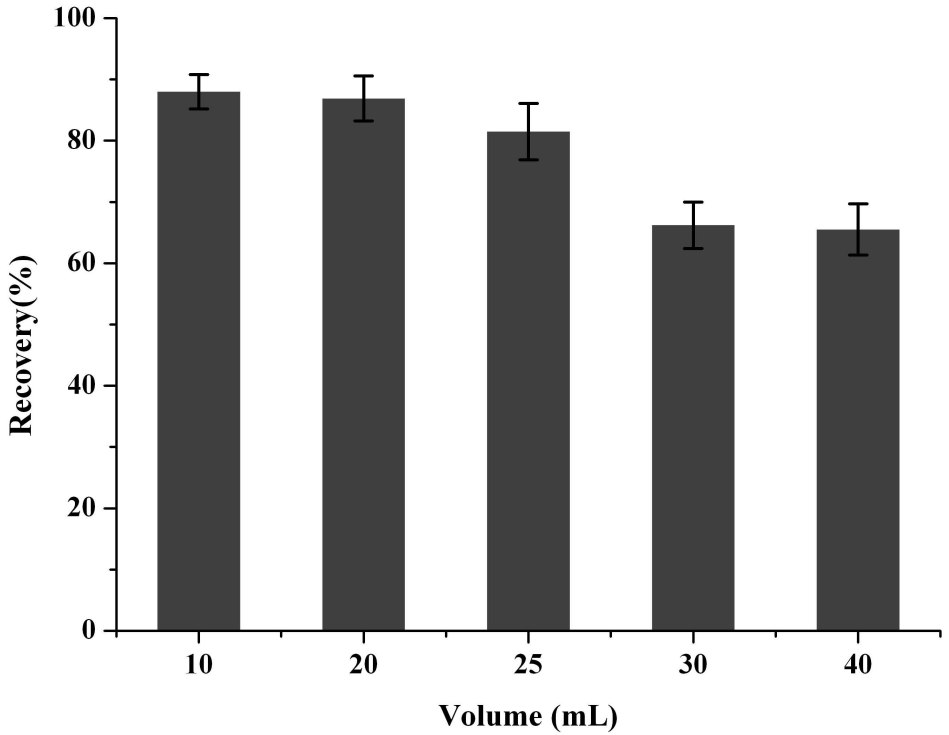


Figure 4.

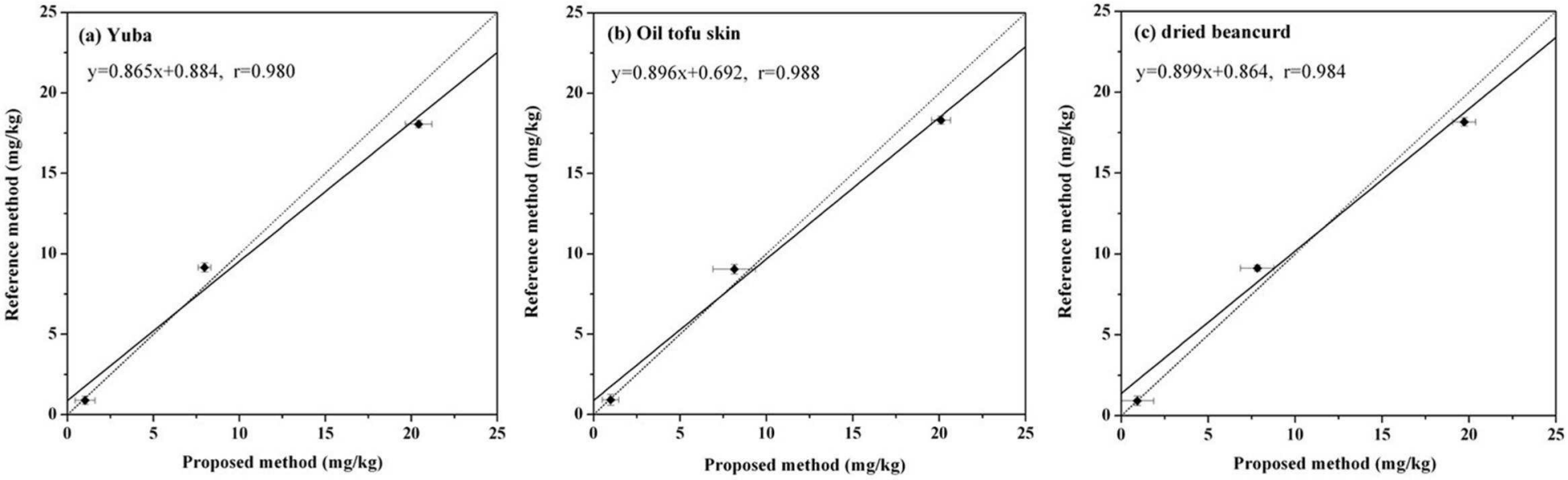
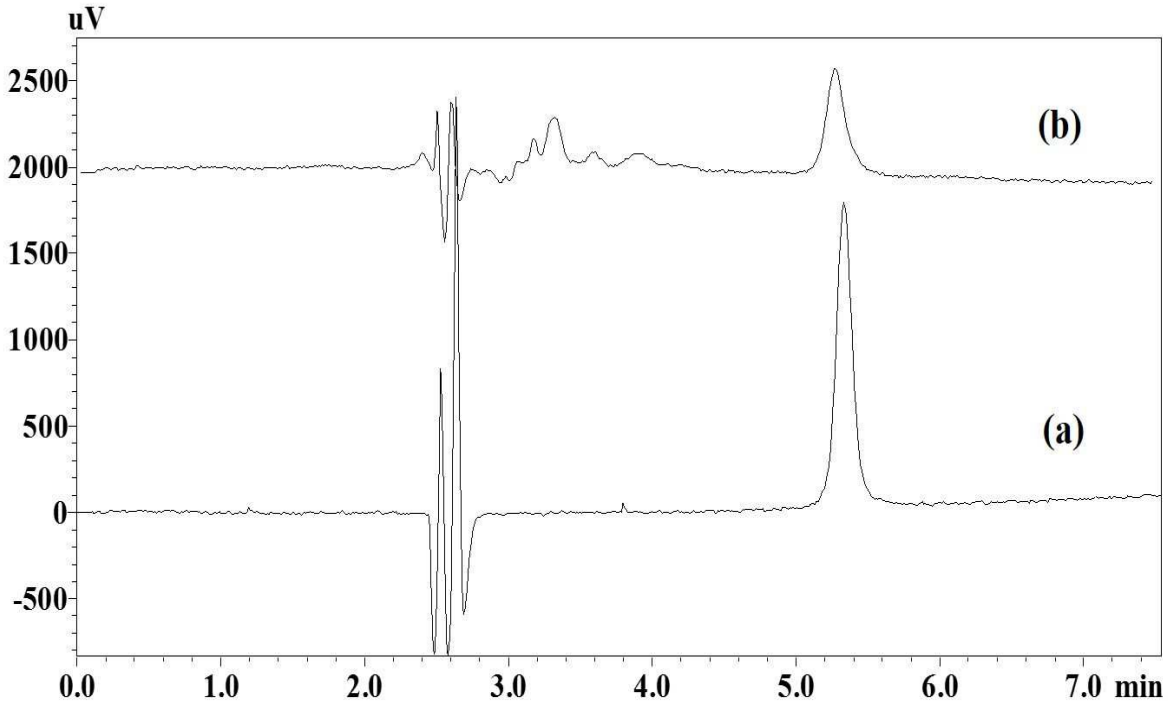


Figure 5.



We proposed a simple and fast method for the determination of chrysoidine in soybean products.