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Article

Rapid Determination of Vitamins D_2 and D_3 in Dairy Products by High-Performance Liquid Chromatography after Solid Phase Extraction Based on Polystyrene/Polypyrrole Composite Nanofibers

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The detection of vitamin D in complex samples is challenging because of the instability of vitamin D in sample preparation. To resolve this problem, packed-fiber solid-phase extraction (PFSPE) has been successfully developed for the pre-treatment of samples containing vitamin D_2 (D_2) and vitamin D_3 (D_3) in dairy products. Based on PFSPE, vitamin D could avoid oxidation because of the shorter extraction time. The adsorbent materials, polystyrene/polypyrrole (PS/PPy) nanofibers, were prepared by electrospinning of PS and *in situ* polymerization of PPy on the surface of PS nanofibers. The linearity was within the range of 1-2000 ng mL^{-1} in dairy solution, and the recoveries were 91.9-109.1% with a relative standard deviation (RSD) < 13.6%. Therefore, the prepared method could be used for the detection of D_2 and D_3 in dairy products.

Keywords: nanofibers, solid-phase extraction, vitamin D_2, vitamin D_3, dairy products

Introduction

Vitamin D deficiency is associated with a number of mild chronic medical diseases, particularly bone disorders, and autoimmune and cardiovascular disease.[1,2] It can result in soft, thin and brittle bones, a common disease known as rickets in children.[3] Vitamin D has two physiologically significant forms: D_2 and D_3.[4] However, vitamin D is one of the few vitamins whose extensive intake can be toxic (hypercalcemia).[5]

Technologies for the determination of D_2 and D_3 can be categorized into immunological techniques (competitive protein binding assay (CPBA), enzyme immunoassay (EIA), and radio immunoassay (RIA)) and non-immunological techniques (high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS)).[6,7] Although the low cost for routine analyses is an advantage in immunological methodologies, the approaches suffer from problems such as cross-reactivity, inadequate sensitivity, recycling difficulty, and inability to achieve structural validation of the analyses.[8] Currently, the most popular analytical strategies are dependent on liquid chromatography coupled with ultraviolet detection or mass spectrometry.[9] In contrast to traditionally immunological techniques, chromatographic methods with the superiorities of sensitivity, flexibility and specificity have been favored.

Due to the low content of vitamin D in a complex matrix, the optimization of sample pre-treatment process plays an important role in enrichment and separation before instrumental analysis. The common sample preparation methods involve saponification,[10] derivatisation,[11] liquid-liquid extraction (LLE),[12] and solid phase extraction (SPE).[13] Saponification is commonly the first step used to remove neutral lipids in most reported methods including the official method.[14,15] Matrix fats would cause severe handling difficulties upon evaporation which is a key step for concentration of target compounds to achieve adequate sensitivity. It is predictable that saponification would result in the degradation of vitamin D, regardless of overnight cold saponification or hot saponification being chosen;[14]

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the evaporation step makes the process tedious, usually taking several hours, and would cause the degradation of vitamin D when exposed to light, air, and heat. The time taken before injection means that anti-oxygen agents such as butylated hydroxytoluene (BHT) must be added to avoid the oxidation of vitamin D.[12,13,16]

Some researchers have reduced the number of steps required in pre-treatment: Abernethy[11] developed a method based on the derivatization of D_3 to achieve the required sensitivity without evaporation. Moreover, a magnetic SPE technique based on polypyrrole-coated magnetic particles for vitamin D extraction in milk was developed, which reduces the extraction time and reduces organic solvent consumption.[17] Although these methods have improved sample preparation, they still require a saponification or evaporation step that often needs heating, which would result in extending sample preparation times and accelerating the degradation of vitamin D. Therefore, the establishment of rapid sample pre-treatment without heating is important when detecting vitamin D.

Recently, based on electrospinning and nanotechnology, the emergence and development of packed-fiber solid-phase extraction (PFSPE) has attracted greater attention.[18] Electrospun nanofibers with novel three-dimensional architectures, good stability, large surface area, and high porosity have been extensively applied in extraction fields, exhibiting characteristics of low-cost, circulating utilization, eco-friendliness, and fast adsorption and desorption.[19]

Among the emerged nanofibers made of polymers, polystyrene (PS) fibers are often employed as a substrate to offer support for other materials due to their special physico-chemical properties.[20] A polystyrene-polypyrrole (PS/PPy) composite electrospun fiber can be prepared by electrostatic spinning of polystyrene (PS) as a template: this is then coated with PPy via *in situ* oxidation polymerization on the surface of the PS fibers, with $FeCl_3$ serving as the oxidant, and Cl^- as a dopant before use as a sorbent to extract three water-soluble vitamins in urine.[21] The unique chemical structure and facile synthesis process endow PS/PPy composite electrospun fibers with abundant conjugated groups and polar atoms with surprising abilities to interact with a variety of molecules by the means of π-π stacking, hydrogen bonding, and Van der Waals and charge interactions.[21]

The aim of this work was to design a rapid sample treatment method without saponification hydrolysis and evaporation for the simultaneous determination of D_2 and D_3 by liquid chromatography. PS/PPy fiber-based SPE was developed for the first time for the treatment of D_2 and D_3 in dairy products (the structures of D_2 and D_3 are shown in Figure 1). Moreover, the key factors affecting the extraction efficiency of analytes, involving the amount of PS/PPy nanofibers, and the volume and flow rate of eluent were optimized. Finally, the method was employed for the detection of D_2 and D_3 in infant formula samples and milk samples.

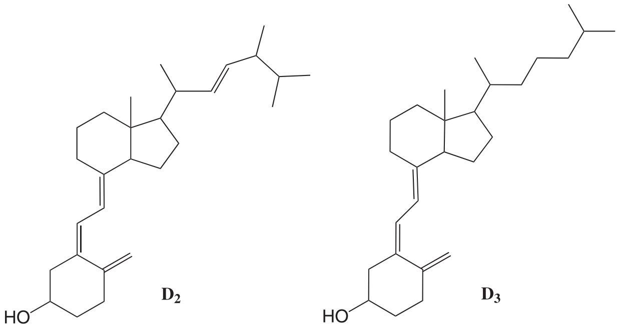

Figure 1. The structures of D_2 and D_3.

Experimental

Reagents and chemicals

Vitamins D_2 and D_3 and pyrene (internal standard, IS) were purchased from Shanghai Aladdin Biochemical Technology Company (Shanghai, China). Methanol (HPLC grade) was obtained from Nanjing Chemical Agents Company (Nanjing, China). Dimethylformamide (DMF), tetrahydrofuran (THF) and polystyrene (PS, retained molecular weight: 18500 Da) were purchased from Aladdin (Shanghai, China). Pyrrole (98%) as monomer and ferric chloride (98%) as oxidant were purchased from Alfa Aesar (Shanghai, China). C_{18} pre-treatment columns were bought from Dikma (Suzhou, China).

The standard stock solutions of vitamins D_2 and D_3 were prepared in methanol at 10 $\mu g\ mL^{-1}$, and stored at 4.0 °C in brown volumetric flasks. The internal standard pyrene was prepared at 1 $\mu g\ mL^{-1}$ in methanol in a brown volumetric flask and stored at 4 °C. Calibration standard solutions of vitamins D_2 and D_3 were prepared by diluting the standard stock solutions in 10% methanol-distilled water solution to obtain the serial concentrations in the range of 1-2000 ng mL^{-1}.

Instruments and apparatus

A high-voltage power supply (model DWP403-1AC, Tianjin, China) and a syringe pump were used for electrospinning. The nanofibers were characterized by using an Fourier transform infrared (FTIR, KBr disc) Spectrophotometer Nicolet 5700 (USA), Hitachi

S-3000N scanning electron microscope (SEM, Japan) at an accelerating voltage of 6.0 kV and a Tecnai G20 transmission electron microscope (TEM, FEI, USA) at an accelerating voltage of 200 kV. Brunauer-Emmett-Teller (BET) data was acquired by automatic specific surface area and pore analyzer (ASAP 2020M, USA). The HPLC system was a Shimadzu LC-20AD (Shimadzu Corporation, Japan) equipped with an analytical column (Kromasil C_{18}, 250 × 4.6 mm, 5 µm) attached to a photodiode array detector. The mobile phase consisted of methanol:distilled water (98:2), and the column temperature was set to 25 °C. The flow rate was kept constant at 1.0 mL min^{-1} and the wavelength for UV detection was set to 264 nm. The injection volume for HPLC analysis was 20 µL. The chromatographic method was chosen based on the previous research of Chen *et al*.[22]

Fabrication of PS/PPy nanofibers

The PS nanofibers were synthesized by loading 10 mL PS solution containing 1.2 g PS (12%, m/v), 4 mL DMF and 6 mL THF (4:6, v/v) into a glass syringe which was fitted to a steel needle with a filed-flat tip. A high-voltage generator was linked to the needle through a copper pin. A dense web of the white fibers was collected on tinfoil. The detailed explanation and procedure were described elsewhere.[23]

PPy was coated on electrospun PS fibers by *in situ* polymerization with $FeCl_3$ serving as the oxidant, together with Cl^- as a dopant. Firstly, 0.5 g PS nanofiber mat was immersed in 10 mL distilled water-ethanol solution (1:1, v/v). After washing, the mat was immersed in 100 mL 0.04 mol L^{-1} pyrrole solution. The polymerization of pyrrole and the deposition of PPy coatings on the surface of PS nanofibers were simultaneously activated by the addition of 0.084 mol L^{-1} $FeCl_3$ solution. Then, the mixture was stirred vigorously for 2 h. The synthetic route was based on previous studies.[24] The PS nanofibers changed from white to dark denoting the end of the reaction and indicating that the core/sheath PS/PPy nanofibers were successfully synthesized. After that, ultrasonication (40 kHz, output power 400 W) was used for washing PS/PPy composite fibers three times to remove redundant reactant containing pyrrole, Fe^{3+}, and Cl^-. Finally, the resulting PS/PPy nanofibers were oven-dried under vacuum at 50 °C before use.

Sample preparation

Dairy products including infant formulas and milks were purchased from local markets in Nanjing (China) and stored at 4 °C. 2 g of infant formulas were dissolved in distilled water in an ultrasonic water bath, then we set the volume to 10 mL to form the infant formula sample. We added 100 µL of 1 µg mL^{-1} pyrene, 0.5 mL acetonitrile, and 2.4 mL distilled water to a 5 mL centrifuge tube containing 1 mL liquid milk or prepared infant formula sample, and vigorously mixed them. Subsequently, the tube was centrifuged at 10,000 rpm for 10 min. The top layer of fat was discarded, and 1 mL of the intermediate layer of clear liquid was taken and left for PFSPE protocol based on a PS/PPy nanofiber column.

Extraction of D_2 and D_3 by home-made PFSPE column

The PFSPE column was filled with PS/PPy nanofibers, which was designed for the pre-treatment of dairy product samples. Some nanofibers, acting as an adsorbent, were packed into the column tip to a depth of approximately 10 mm (about 2 mg nanofiber). Before extraction, the PFSPE column was activated by 200 µL methanol and distilled water, successively. Then, 1 mL of sample solution was added to the activated PFSPE column and pushed through the column by air from a pressurized syringe. The flow rate of sample solution was controlled about 2 min mL^{-1}. After that, the column was rinsed using 100 µL distilled water, and D_2 and D_3 were desorbed with 200 µL ethanol. Finally, the eluent sample was filtered by 0.22 µm filter, and 20 µL eluent was analyzed by HPLC.

Results and Discussion

Characterization of nanofibers

As illustrated in Figure 2, Figures 2a and 2b are SEM images of PS and PS/PPy nanofibers, respectively. The average diameter of PS/PPy nanofibers is greater than that of PS nanofibers; moreover, PS/PPy nanofibers (Figure 2d) exhibit more alternate dark and bright spots, indicating the presence of more nanopores compared with PS nanofibers (Figure 2c). The textural properties, including the BET surface area and pore volume were listed in Table 1. Although the BET surface area of PS was larger than that of PS/PPy, the extraction recovery of PS/PPy for vitamin D was higher in comparison experiment (Figure 3a). The BET results showed that the specific surface area was not the only factor, and the special functional groups on PS/PPy fibers played a leading role for the extraction efficiency.

The structural description of the as-synthesized PS/PPy composite-fibers was further investigated by FTIR (Figure 4). In the PS/PPy fiber spectrogram, some absorption bands of PS weakened (as shown in the red frame) and some characteristic peaks of PPy appeared.

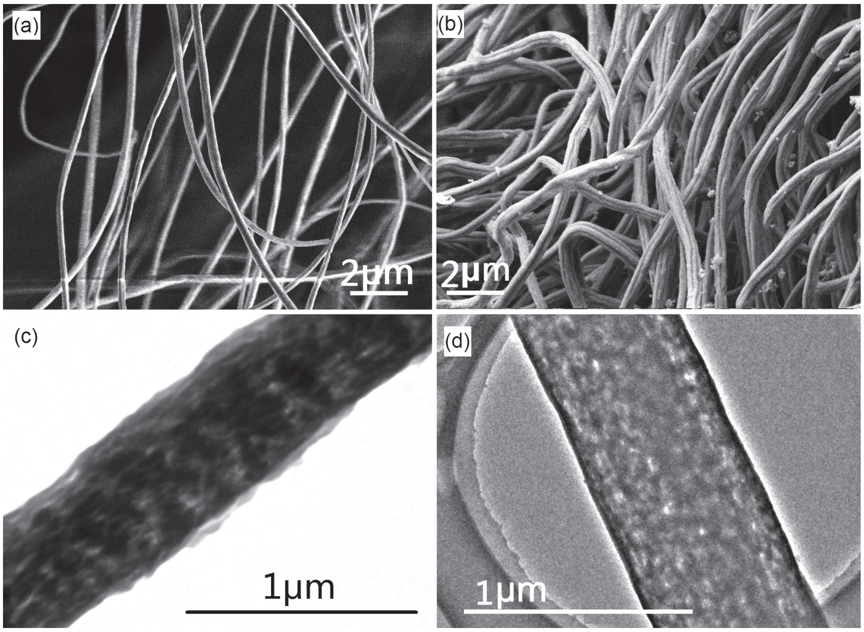

Figure 2. SEM images of (a) PS nanofibers; (b) PS/PPy nanofibers; TEM images of (c) PS nanofibers; (d) PS/PPy nanofibers.

Table 1. Textural properties of the tested materials

Material	BET surface area / (m^2 g^{-1})	Pore volume / (cm^3 g^{-1})
PS[a]	17.37	0.18
PS/PPy[b]	15.91	0.082

[a]PS: polystyrene, [b]PS/PPy: polystyrene/polypyrrole. BET: Brunauer-Emmett-Teller.

The peaks at 1454 and 3025 cm^{-1} are attributed to C–H and C=C stretching vibration of phenyl ring in PS and PS/PPy spectra.[25] The bands observed at 1548 and 1475 cm^{-1} are related to the fundamental vibrations of the pyrrole rings in PS/PPy spectrogram while they could not be observed in the PS spectrogram, which indicated that PPy was successfully coated onto the surface of the PS. In addition, the peaks arising from C–N stretching vibration and C–H in-plane vibration were respectively found at 1182 and 1042 cm^{-1}, and evidence of C–C out-of-plane ring deformation vibration was seen at 916 cm^{-1},[17] which further demonstrated that PS/PPy composite nanofibers had been successfully prepared.

Optimization of extraction conditions of the PFSPE

Comparison of extraction efficiency between PS and PS/PPy nanofibers

Vitamin D is a weak polar molecule which has double bonds and hydroxyl groups. The extraction efficiencies of two types of SPE columns filled separately by PS and PS/PPy nanofibers were compared. As shown in Figure 3a, the extraction efficiency of D_2 and D_3 on PS/PPy nanofibers is better than that on PS nanofibers. The reason for this is that the PPy polymer contains abundant conjugated groups, polar atoms, and doped ions, which could offer more binding sites for D_2 and D_3 by π-π stacking, hydrogen bonding, Van der Waals force, and charge interaction.[26]

Amount of packed nanofibers

To obtain better extraction efficiency and minimal dosage of nanofiber materials, the amount of sorbent was varied, and the results evaluated. Different amounts of nanofiber sorbent (0.5, 1, 2, 3 and 4 mg) were packed into the column tip to extract D_2 and D_3. 1 mL mixed working solution at 100 ng mL^{-1} was pre-treated by different dosages of PS/PPy nanofibers filled in the columns, and ethanol was employed as an elution solvent. As shown in Figure 3b, the amount of optimal recoveries for analytes could be observed at 2 mg; the extraction recoveries remained in balance when further increasing the amount of nanofibers, therefore, subsequent tests were conducted with 2 mg electrospun nanofibers.

Amount of eluent

The volume of eluent is one of the important parameters used in the quantitative detection of analytes. Too much eluent will reduce the detection sensitivity of the targets, too little eluent may reduce the elution efficiency. 1 mL of 100 ng mL^{-1} D_2 and D_3 working solution was pre-treated by the column including 2 mg PS/PPy nanofiber, and different volumes of ethanol were used as the elution solvent. As

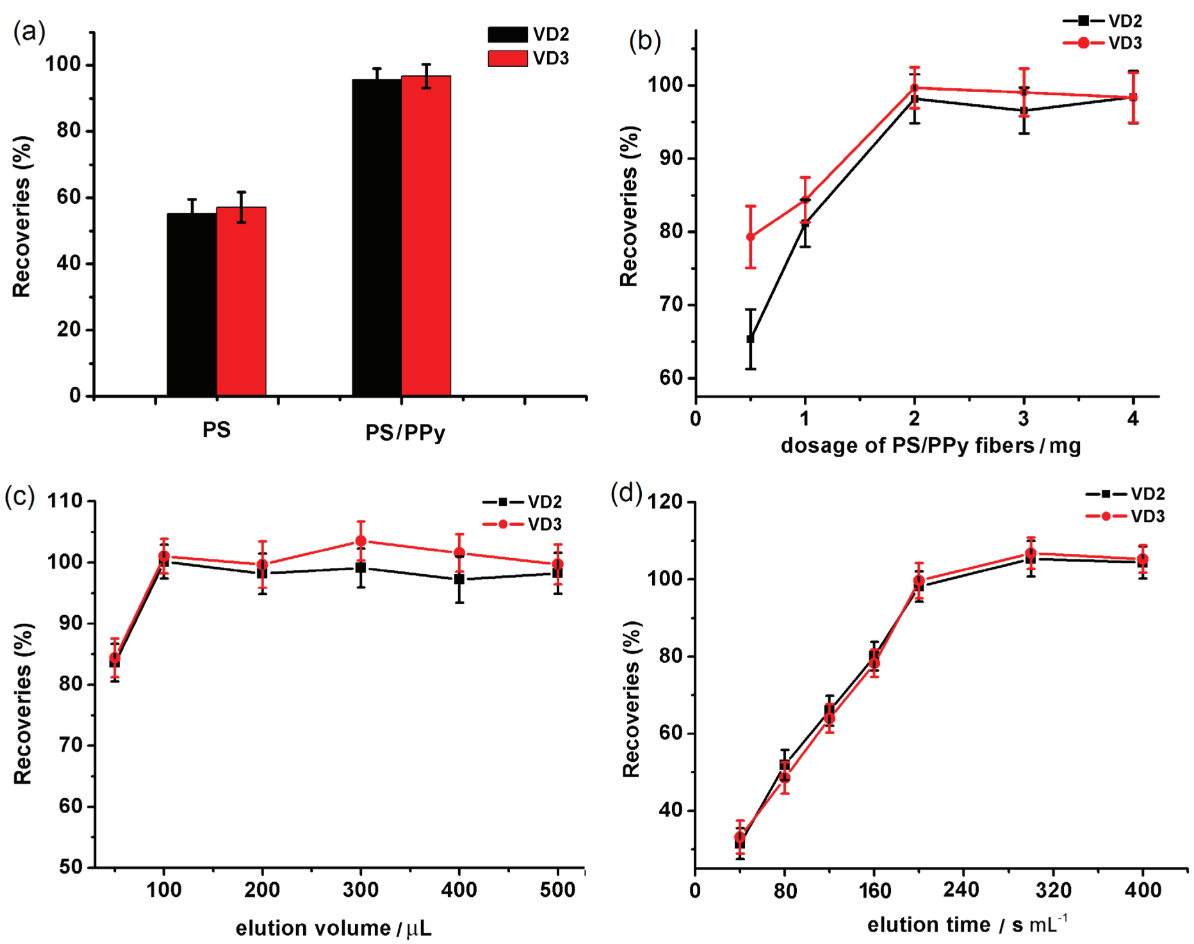

Figure 3. Optimization procedures: (a) comparison between PS and PS/PPy fibers for the extraction of D_2 and D_3; (b) the effect of amount of PS/PPy nanofibers on extraction recoveries; (c) the effect of eluent volume on extraction recoveries; (d) the effect of elution time on extraction recoveries. The working solutions of D_2 and D_3 used in the experiments were at a concentration of 100 ng mL^{-1}.

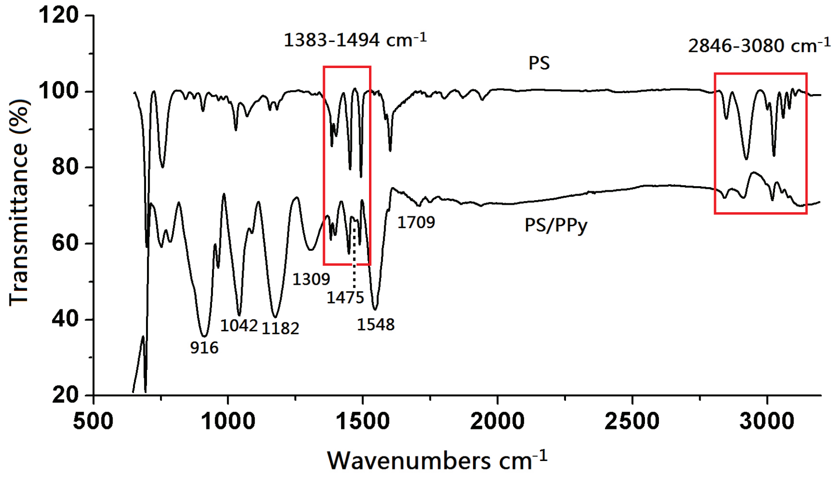

Figure 4. FTIR (KBr disc) spectra of PS and PS/PPy nanofibers.

shown in Figure 3c, the extraction recovery was improved when increasing the volume of elution solvent to 100 μL of ethanol. Considering the loss of eluent during filtration before sample injection, 200 μL was chosen as the optimum elution volume.

Elution time

The eluent time (in s mL^{-1}) is an essential factor affecting target-extraction. A high flow rate is conducive to rapid analysis, nevertheless, a short elution time may cause incomplete adsorption and desorption, which could result in low recoveries. In this work, 1 mL D_2 and D_3 mixed working solution at the concentration of 100 ng mL^{-1} was pre-treated by the PS/PPy fiber column, and different elution times from 40 to 400 s mL^{-1} were investigated. As shown in Figure 3d, the extraction recovery was improved by increasing the elution time to about 200 s mL^{-1}, indicating that the analytes were well desorbed from the fibers, thus, 200 s mL^{-1} was selected for the elution step in subsequent tests.

Method validation

Generally, an external standard method is used for the detection of vitamin D in most reported work. Sometimes, one of D_2 or D_3 can be used as an IS to quantify the other supplementary vitamin D during HPLC determination;[14] however, this is not the best approach when D_2 and D_3 occur in milk simultaneously. To correct the pre-treatment and measurement error, avoiding vitamin D mutual interference, the IS method was used for quantification in this work and pyrene was selected as the IS. The analytical performances of the established method are summarized in Table 2. Good linearities for D_2 and D_3 assay were achieved in the range 1-2000 ng mL^{-1} in dairy solution with correlation coefficients (r^2) reaching 0.99. The limits of detection (LOD) at a signal-to-noise ratio of 3 (S / N = 3) were 0.37 and 0.38 ng mL^{-1} in milk samples and 0.35 ng mL^{-1} in infant formula samples. The limits of quantification (LOQ) (S / N = 10) were 1.23 and 1.27 ng mL^{-1} for D_2 and D_3, respectively, in milk samples. The LOD and LOQ were 0.35 ng mL^{-1} (7.07 ng g^{-1}) and 1.17 ng mL^{-1} (23.6 ng g^{-1}), respectively, for D_3 in infant formula.

The accuracy expressed as recovery, and the precision expressed as the relative standard deviation (RSD), were calculated depending on the chromatographic peak area ratios of targets relative to the internal standard. As shown in Table 2, for milk, the recoveries of D_2 and D_3 were 91.9-100.5% and 92.5-109.1%, the intra-day precision 6.3-9.2% and 7.1-11.9%, and inter-day precisions 10.3 and 8.7%, respectively. For infant formula, the recoveries of D_3 were 95.4-103.4%, the intra-day precision 7.5-11.4%, and inter-day precision 9.8%.

Comparison of different methods

To verify the advantages of the present method, two types of comparisons were undertaken, including an experimental comparison with C_{18} SPE and a methodological comparison with the literature.

Firstly, a C_{18} column was used for the pre-treatment of vitamins D_2 and D_3 in infant formula samples. The operation process was in the same way as the proposed method in sample preparation and the extraction of D_2 and D_3 by home-made PFSPE column. However, the eluent could not be filtered smoothly through a 0.22-µm filter membrane, which might be attributed to the poor selectivity and loose structure of packed materials in C_{18} pre-column. In these cases, more impurities could not be filtered out and thus entered the eluent, resulting in their unsuccessful extraction. Secondly, the extraction recoveries of C_{18} column and PS/PPy column for vitamin D were explored at a concentration of 100 ng mL^{-1} standard solution. The extraction recovery data was listed in Table 3. The results showed that the extraction efficiency of D_2 and D_3 on C_{18} column is lower than that on PS/PPy column.

A comparative investigation of the proposed and other reported methods is summarized in Table 4. It can be concluded that the proposed method displays good analytical properties as well as outstanding advantages in simplifying operation steps, shortening processing

Table 2. Quality parameter of the proposed method for the determination of vitamins D_2 and D_3 in dairy products (n = 6)

Analyte	Linear range / (ng mL^{-1})	r^2	LOD / (ng mL^{-1})	LOQ / (ng mL^{-1})	Concentration / (ng mL^{-1})		Recovery / %	RSD / %	Precision / %	
					Spiked	Found			Intra-day	Inter-day
D_2 (in milk)	1.0-2000	0.9976	0.37	1.23	4.15	4.17	100.5	9.4	8.1	10.3
					83.1	76.4	91.9	6.3	9.2	
					207.7	198.76	95.7	7.1	6.3	
D_3 (in milk)	1.0-2000	0.9975	0.38	1.27	4.09	4.47	109.1	13.6	11.9	8.7
					81.9	75.7	92.5	8.4	9.5	
					204.7	218.5	106.7	14.2	7.1	
D_3 (in infant formula)	1.0-2000 (0.020-40.4 µg g^{-1})	0.9964	0.35 (7.07 ng g^{-1})	1.17 (23.6 ng g^{-1})	4.12	3.99	96.8	6.4	9.3	9.8
					82.3	78.5	95.4	8.7	11.4	
					208.2	215.3	103.4	11.5	7.5	

LOD: limit of detection; LOQ: limit of quantification; RSD: relative standard deviation; D_2: vitamin D_2; D_3: vitamin D_3; r^2: linear correlation coefficient.

Table 3. Extraction recovery for D_2 and D_3 based on C_{18} column and PS/PPy column (n = 6)

Extraction material	Analyte	Recovery / %	RSD / %
C_{18}	D_2	53.7	9.3
	D_3	59.5	7.5
PS/PPy	D_2	96.3	7.0
	D_3	98.5	5.5

RSD: relative standard deviation; D_2: vitamin D_2; D_3: vitamin D_3; PS/PPy: polystyrene/polypyrrole.

time, reducing the amount of sorbent, and reducing the assumption of organic solvent required.

Detection in real samples

To evaluate the applicability of the new method, infant formula and milk samples were analyzed using the method. As shown in Figure 5, Figure 5a is the chromatogram of internal standard pyrene, and Figure 5b is that of D_2 and D_3 standard substance, which were all pre-treated by PFSPE before sample injection. The signal of D_3 in the infant formula liquid samples was not detected without pre-treatment by PS/PPy nanofibers (Figure 5c), which might be attributed to the poor sensitivity and significant matrix interference; however, using pre-treatment with PS/PPy nanofibers, D_3 could be successfully detected in infant formula liquid samples (Figure 5d). The same result was also obtained in the chromatograms of milk samples without (Figure 5e) and with (Figure 5f) PFSPE. In brief, the chromatograms indicated that the PFSPE method assisted by PS/PPy nanofibers could reduce matrix interference and increase the signal strength of target compounds, showing good application prospects for the analysis of D_2 and D_3 in dairy product samples.

After optimization of the process parameters, the developed PFSPE method, coupled with HPLC-UV, was applied to the determination of vitamins D_2 and D_3 contents in dairy product samples (Table 5). The determined values for infant formula samples were between 101-125 ng g^{-1} for D_3, which were higher than the labelled value, and for milk samples they were between 8.1-23.3 ng mL^{-1} for D_2, and 7.9-25.7 ng mL^{-1} for D_3.

Conclusions

A more convenient technique for sample pre-treatment was proposed for the simultaneous extraction and

Table 4. Comparison of methods used for the analysis of vitamin D in dairy products

Analyte	Matrix	Method	Amount of sorbent / mg	Organic solvent consumption / mL	Saponification step	Evaporation step	Recovery / %	LOD / (ng mL^{-1})	LOQ / (ng mL^{-1})	Reference
D_2, D_3	milk	HPLC, LLE	–	> 100	yes	yes	–	0.82, 1.57	1.46, 5.23	12
D_2, D_3	milk, butter	HPLC, C18 cartridge SPE	–	32	–	yes	84-105	4.1, 0.3	–	13
D_3	bovine milk	LC-MS/MS, silica SPE	500	11	yes	yes	61-86	–	0.2	27
D_3	milk powder	HPLC, online RP18 SPE	–	–	yes	no	80-105	6.8	–	28
D_2, D_3	milk	HPLC, magnetic SPE	30	1.1	–	yes	72-90	0.02, 0.05	–	17
D_2	juice milk	HPLC, magnetic SPE	20	1	–	yes	65.2-69.5	0.9	–	29
D_2, D_3	milk	HPLC, magnetic SPE	15	1.1	–	yes	75.5-89.3	0.03, 0.08	–	30
D_3	infant formula	LC-MS, isopropanol extraction and no-heating saponification	–	10	–	yes	93.1-110.6	0.396 ng g^{-1}	1.68 ng g^{-1}	31
D_3	infant formula	HPLC, PFSPE	2	1.2	–	no	92.5-104.5	7.07 ng g^{-1}	23.5 ng g^{-1}	this work
D_2, D_3	milk	HPLC, PFSPE	2	1.2	–	no	95.7-100.5 92.5-106.7	0.37 0.38	1.23 1.27	this work

LOD: limit of detection; LOQ: limit of quantification; D_2: vitamin D_2; D_3: vitamin D_3; HPLC: high performance liquid chromatography; LLE: liquid-liquid extraction; –: not mentioned; SPE: solid-phase extraction; LC-MS: liquid chromatography-mass spectrometry; PFSPE: packed-fibers solid-phase extraction.

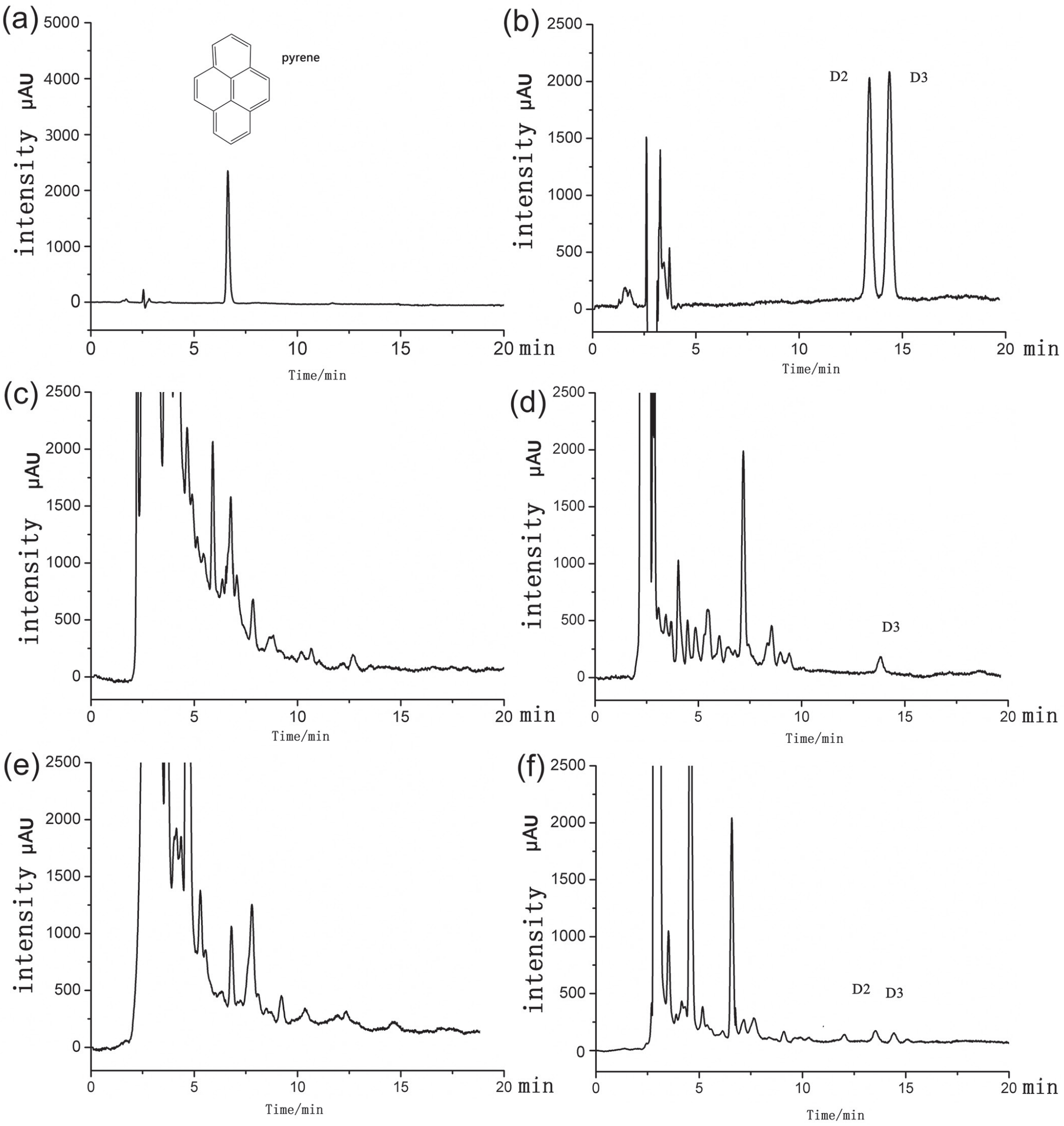

Figure 5. Chromatograms of (a) 100 ng mL^{-1} pyrene standard solution and (b) 100 ng mL^{-1} D_2 and D_3 mixed standard solution with pre-treatment by PFSPE; (c) infant formula liquid sample containing 100 ng mL^{-1} pyrene without pre-treatment by PFSPE; (d) infant formula liquid sample containing 100 ng mL^{-1} pyrene pre-treated by PFSPE; (e) milk sample containing 100 ng mL^{-1} pyrene without pre-treatment by PFSPE; (f) milk sample containing 100 ng mL^{-1} pyrene pre-treated by PFSPE.

Table 5. Amounts of vitamins D_2 and D_3 in dairy products collected from a local market in Nanjing

Dairy product (No. of samples analyzed)	Test variety	Vitamin D identified	Concentration		
			D_2 / (ng mL^{-1})	D_3 / (ng mL^{-1})	D_3 / (ng g^{-1})
Sample 1	infant formula	D_3	–		101
Sample 2	infant formula	D_3	–		125
Sample 3	infant formula	D_3	–		108
Sample 4	milk	D_2, D_3	8.1	11.8	
Sample 5	milk	D_2, D_3	9.4	12.6	
Sample 6	milk	D_2, D_3	14.1	7.9	
Sample 7	milk	D_2, D_3	23.3	25.7	
Sample 8	milk	D_2, D_3	18.5	19.3	

D_2: vitamin D_2; D_3: vitamin D_3.

enrichment of vitamins D_2 and D_3 by using a home-made PFSPE column. The proposed method, without saponification and evaporation steps, could prevent the degradation of D_2 and D_3. Meanwhile, the quick manipulation and efficient extraction could reduce organic solvent consumption and the dosage of sorbent materials, which made the method more economical and eco-friendlier, thus offering promising future applications in analyses of targets exhibiting sensitivity to air, light, and heat. The novel strategy based on PS/PPy composite fibers coupled with HPLC exhibited excellent analytical performances for D_2 and D_3 in dairy products.

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Author Contributions

Qing Han contributed with the formal analysis input and helped in the writing of the original draft of this article; Lanlan Wei contributed to establishing the methodology used in the research; Guozhe Deng, Ying Sun, and Jinhui Fan contributed to the data curation necessary for this work; Jianjun Deng and Zigang Tang contributed with the various resources to the work; Xia Wang contributed to the investigations described herein; Prof Xuejun Kang contributed to securing funding for the research, and the writing, review, and editing of this article.

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