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Determination of Estrogens in Pork and Chicken Samples by Stir Bar Sorptive Extraction Combined with High-Performance Liquid Chromatography−Ultraviolet Detection

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ABSTRACT: A poly(dimethylsiloxane) (PDMS)/β-cyclodextrin (β-CD)/divinylbenzene (DVB)-coated stir bar was prepared by the sol−gel technique for the stir bar sorptive extraction (SBSE) of four estrogens from animal-derived foods, followed by liquid desorption (LD) and high-performance liquid chromatography−ultraviolet (HPLC−UV) detection. The influence of the coating composition on SBSE of target estrogens was investigated by an orthogonal experiment design, and the prepared PDMS/β-CD/DVB-coated stir bars show good reproducibility. Under the optimal experimental conditions, the limits of detection (S/N = 3) of the developed PDMS/β-CD/DVB SBSE−LD−HPLC−UV method were 0.21−1.6 μg/L for the target estrogens with enrichment factors of 19−51-fold, the dynamic linear range was 2−2000 μg/L, and the relative standard deviations of the method ranged from 6.0% to 9.7% (n = 8, c = 100 μg/L) and from 8.4% to 11.7% (n = 8, c = 10 μg/L). The developed method was simple, sensitive, and selective and was successfully applied to the analysis of estrogens in pork and chicken samples.

KEYWORDS: *stir bar sorptive extraction, poly(dimethylsiloxane)/β-cyclodextrin/divinylbenzene coating, estrogens, high-performance liquid chromatography, pork and chicken*

INTRODUCTION

With the improvement in living standards, the consumption of foods high in protein, such as meat and milk, is growing rapidly.[1] In the process of animal husbandry, hormone drugs are added into animal feeds illegally for economic purposes. Sex hormones as a group of hormone drugs include androgens and estrogens, while estrogens can be further divided into endogenous estrogens and exogenous estrogens. Endogenous estrogens which are synthesized by the ovaries are also known as natural estrogens, including estradiol, estriol, estrone, etc. Exogenous estrogens which mainly come from synthetic estrogens are also called environment estrogens, including dienestrol, hexestrol, etc.[2] Through the assimilation of protein, the added estrogens can improve the feed conversion rate, resulting in a rapid growth of the animals. However, estrogens enriched through the food chain could interfere with the mineral, fat, sugar, and protein metabolization in the human body[3] and even cause tumors such as breast cancer and prostate cancer.[4] Thus, development of highly sensitive and selective methodologies for the determination of estrogens is of great significance for food safety supervision.

Estrogens are usually determined by biological or chemical methods, such as enzyme-linked immunosorbent assay (ELISA), gas chromatography (GC), and high-performance liquid chromatography (HPLC). ELISA permits rapid screening, and a preconcentration step is sometimes unnecessary; however, nonspecific binding interactions may decrease its sensitivity.[1] GC is rapid and sensitive, but it often involves a derivatization step to convert the analytes into more volatile derivatives through silylation or acylation.[4] HPLC permits direct analysis of estrogens without derivatization and is the most commonly used technique for analysis of estrogens in different samples. However, in most cases, sample pretreatment steps are required before the analysis due to the very low levels of estrogens and the complicated matrix in the real-world samples. Classic sample pretreatment techniques such as liquid−liquid extraction (LLE)[5] and solid-phase extraction[6−8] have been applied for the extraction and preconcentration of estrogens. However, they suffer from the drawbacks of requiring large volumes of toxic organic solvents and being tedious and time-consuming. Modern trends of sample pretreatment techniques are toward the simplification, miniaturization, and minimization of the reagents (especially organic solvents) and sample amounts. This has led to the development of some environmentally friendly sample pretreatment techniques, such as liquid-phase microextraction (LPME)[9,10] solid-phase microextraction (SPME),[11−13] and stir bar soptive extraction (SBSE).[2,14−17]

SBSE was introduced as a novel sample preparation technique in 1999 by Baltussen and co-workers.[18] Similar to SPME, SBSE is an equilibrium technique, but the coating amount on the stir bar is 50−250 times higher than that on the SPME fiber, which results in a significant increase in recovery and extraction capacity.[19] Besides, SBSE has many other advantages, such as high sensitivity, good reproducibility, and being organic solvent free, and has been successfully applied to trace analysis in environmental, food, and biomedical samples. However, the PDMS coating was the only commercially available coating for the stir bar,[20] which greatly limited the application of SBSE. Almeida et al.[14] applied a commercial PDMS-coated stir bar to extract estrogens in water and urine

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matrixes, but the extraction time was too long (lasted for 2 h). Therefore, great efforts have been made to explore novel coatings for SBSE of estrogens from various samples in recent years. Huang et al. prepared novel monolithic stir bars for extraction of estrogens, such as poly(methacrylic acid stearyl ester−ethylene dimethacrylate)[2] and poly(vinylpyridine−ethylene dimethacrylate),[16] and the extraction times were obviously decreased (1 h and 20 min, respectively). Hu et al.[15] prepared a PDMS/β-cyclodextrin (PDMS/β-CD)-coated stir bar for determination of estrogens in a water matrix, and the addition of β-CD proved to increase the extraction efficiency of estrogens.

In this work, PDMS/β-CD/divinylbenzene (DVB)-coated stir bars were prepared by the sol−gel process, and a novel method of PDMS/β-CD/DVB SBSE−HPLC−UV was developed for the analysis of four target estrogens in animal-derived foods. The operation conditions affecting the extraction of the target estrogens by SBSE were optimized, and the analytical performance of the method was evaluated. To demonstrate the applicability, the developed method was applied to the analysis of four estrogens in real-world samples of pork and chicken.

MATERIALS AND METHODS

Reagents and Standards. Hydroxyl-terminated poly(dimethylsiloxane) (OH-PDMS) was purchased from Sigma-Aldrich (Milwaukee, WI). Methyltrimethoxysilane (MTMS) and poly(methylhydrosiloxane) (PMHS) were purchased from the Chemical Plant of Wuhan University (Wuhan, China). DVB was purchased from the Guangfu Fine Chemical Research Institute of Tianjin (Tianjin, China). β-CD, trifluoroacetic acid (TFA), dichlormethane, sodium chloride, sodium dihydrogen phosphate, sodium hydroxide, methanol, and ethanol were purchased from China Medicine (group) of Shanghai Chemical Reagent Corp. (Shanghai, China). The capillary glass bars were obtained from the Apparatus Factory of the West China University of Medical Sciences (Chengdu, China). Solid reagents and all solvents used in this study were of analytical grade. High-purity water obtained by a Milli-Q water purification system (18.25 MΩ cm, Millipore, Bedford, MA) was used throughout all the experiments.

17-β-Estradiol (E_2), dienestrol (DES), diethylstilbestrol (DIS), and hexestrol (HES) were purchased from J&K Acros Organics (Dr. Ehrenstorfer GmbH, Germany), and their structures and log $K_{O/W}$ and pK_a values are shown in Figure 1. Each standard solution of estrogens was prepared in methanol at a concentration of 1 mg/mL and stored at 4 °C in the refrigerator.

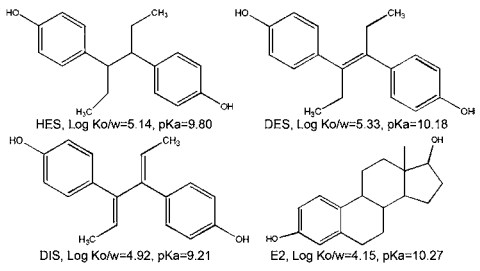

Figure 1. Structures of four estrogen compounds and their log $K_{O/W}$ and pK_a values.

Instrumentation. An Agilent 1100 series HPLC−UV system with a variable-wavelength detector was used for identification and quantification of estrogens. The separation was performed on a reversed-phase C_{18} HPLC column (Lichrospher ODS, 5 mm, 4.6 mm × 200 mm i.d., Hanbon, Jiangsu, China). Methanol/10 mmol/L NaH_2PO_4 (60/40, v/v, pH 3) was used as the mobile phase at a flow rate of 1 mL/min. The UV detector was set at 230 nm, and the injection volume was 50 μL.

An X-650 scanning electron microscope (Hitachi, Japan) with an acceleration voltage of 30 kV was used for characterization of the morphology and thickness of the PDMS/β-CD/DVB-coated stir bar. A 170SX Fourier transform infrared (FT-IR) spectrometer (Nicolet, Madison, WI) was used for characterization of the structure of the PDMS/β-CD/DVB stir bar coating.

Preparation of the Stir Bars. The PDMS/β-CD/DVB "dumbbell-shaped" stir bar was prepared on the basis of a procedure described previously.[21] First, a 20 mm capillary glass bar consisting of a 15 mm iron wire inside was prepared. Before coating, the glass bar was dipped in 1 mol/L NaOH solution for 24 h to expose the maximum number of silanol groups on the surface, then sequentially cleaned with water, 0.1 mol/L HCl solution to neutralize the excess NaOH, and water again, and dried at 60 °C for 3 h. Second, the PDMS/β-CD/DVB sol solution was prepared as follows: 100 mg of OH-PDMS, 50 mg of β-CD, 50 μL of DVB, 200 μL of CH_2Cl_2, 100 μL of MTMS, 20 μL of PMHS, and 100 μL of TFA were mixed by vortex. Finally, the treated bare bars were immersed into the sol solution for 5 min and then were taken out and placed into a constant-temperature drier for 24 h at 60 °C. Prior to use, the stir bars were cleaned in methanol by ultrasonication for 10 min to get rid of the organic contaminants in the coating.

Stir Bar Sorptive Extraction Procedure. Stirring extraction and liquid ultrasonication desorption modes were used in this work. A 10 mL volume of the aqueous sample solution was placed in a 25 mL glass vial. The stir bar was immersed into the sample solution, and the solution was stirred at 700 rpm for 20 min. After extraction, the stir bar was placed in a glass desorption tube containing 100 μL of methanol/10 mmol/L NaOH (60/40, v/v) to desorb the target estrogens in an ultrasonic bath for 20 min. A 50 μL volume of desorption solution was injected into the HPLC−UV instrument for subsequent analysis. The used stir bar was placed into 1 mL of methanol under ultrasonication for 5 min for cleaning and then taken out for the next use.

Sample Preparation. Pork and chicken were purchased from the local market (Wuhan, China). All animal-derived foods were freeze-dried, ground into powder, and stored in the refrigerator at −18 °C. Spiked samples were obtained by mixing a 0.5 g pork or chicken sample with estrogen standard solutions for the recovery test. The sample preparation procedure was identical to that reported in ref 11. Briefly, 10 mL of acetonitrile was added to 0.5 g pork or chicken samples (including the spiked samples), and the mixture was ultrasonicated for 30 min at room temperature. After ultrasonication, the samples were centrifuged for 30 min at 3600 rpm. The supernatant was filtered with a 0.45 μm PTFE membrane and diluted to 50 mL with a 20% NaCl (m/v) aqueous solution. A 10 mL volume of the prepared pork or chicken sample solution was subjected to the SBSE procedure, and a 50 μL volume of the desorption solution was injected into the HPLC−UV instrument for subsequent analysis.

RESULTS AND DISCUSSION

Preparation and Characterization of the PDMS/β-CD/DVB-Coated Stir Bars. In this work, the PDMS/β-CD/DVB-coated stir bars were prepared by the sol−gel technique. To get the best extraction performance for the target estrogens, the proportion of the functional components (including PDMS, β-CD, and DVB) in the sol solution was optimized by an $L_9(3^4)$ orthogonal experiment design (Table 1). It should be stressed that the amount of PDMS in the sol solution only varied from 100 to 200 mg in the orthogonal experiment design because our preliminary experiments indicated that the mechanical property of the prepared stir bar was poor and the coating would easily peel off under the ultrasonication if the amount of PDMS in the sol solution were too low (<100 mg). PDMS plays an important role in the formation and maintenance of the 3-D silicon network.[22,23] Accordingly, the proportion of

Table 1. Orthogonal Array $L_9(3^4)$ and Experimental Results

	factor			peak area of each analyte (n = 2)			
no.	mass of PDMS (mg)	mass of β-CD (mg)	vol of DVB (μL)	E_2	DES	DIS	HES
1	100	50	50	106.7	454.9	1374.7	602.1
2	100	100	100	77.2	413.3	988.85	497.8
3	100	150	150	68.3	361.6	1118.3	565.4
4	150	50	100	49.8	244.9	1163.8	362.6
5	150	100	150	64.6	339.7	1137.9	532.5
6	150	150	50	57.7	268.5	1005.9	436.1
7	200	50	150	54.9	275.9	873.4	440.9
8	200	100	50	62.7	303.7	1087.2	481.1
9	200	150	100	64.9	318.0	1017.0	491.9

PDMS in the sol indeed affected the signal intensities of the target estrogens.

β-CD is a cyclic oligosaccharide formed by seven glucopyranose units through glycosidic α-1,4-bonds which possesses a unique structure containing a "hydrophilic exterior and hydrophobic interior". β-CD has plenty of functional hydroxyl groups in the cyclic exterior, which is suitable for extraction of polar compounds. Additionally, β-CD has a hydrophobic cavity structure which can form inclusion complexes with estrogens. The special characteristics of β-CD result in an improved extraction efficiency, making β-CD a main functional component for the extraction of the target estrogens by SBSE.

DVB has two vinyl groups which usually act as cross-linkers in the polymerization, and the aromatic ring of DVB could interact with the analytes through $\pi-\pi$ conjugation. Therefore, the addition of DVB can increase the extraction efficiency of SBSE for the target estrogens.

The experimental results in Table 1 were analyzed by SPSS 17.0 software. It was found that PDMS had the most significant influence on the extraction efficiency of target analytes, DVB the second most, and β-CD the least. In the following research work, a sol solution consisting of 100 mg of OH-PDMS, 50 mg of β-CD, 50 μL of DVB, 100 μL of MTMS, 200 μL of CH_2Cl_2, 100 μL of TFA, and 20 μL of PMHS was employed.

The morphology and thickness of the PDMS/β-CD/DVB coating were investigated by scanning electron microscopy (SEM). Figure 2 shows the morphological structures of the PDMS coating (Figure 2A, 300×) and PDMS/β-CD/DVB coating (Figure 2B, 80×; Figure 2C,D, 300×) under different magnifications. As can be seen, compared to a smooth coating surface obtained for the PDMS-coated stir bar (Figure 2A), the surface of the PDMS/β-CD/DVB-coated stir bar was rough (Figure 2B,C), resulting in an enlargement of the specific surface area and improvement of the extraction efficiency for the target estrogens. From Figure 2 D, it can be estimated that the thickness of the PDMS/β-CD/DVB coating was ca. 122.5 μm.

Figure 3 shows the FT-IR spectrum of the PDMS/β-CD/DVB coating. As can be seen, the absorption peak at 3392.7 cm^{-1} is ascribed to the axial stretching vibrations of O−H of PDMS, the absorption peak at 2965.7 cm^{-1} is the stretching

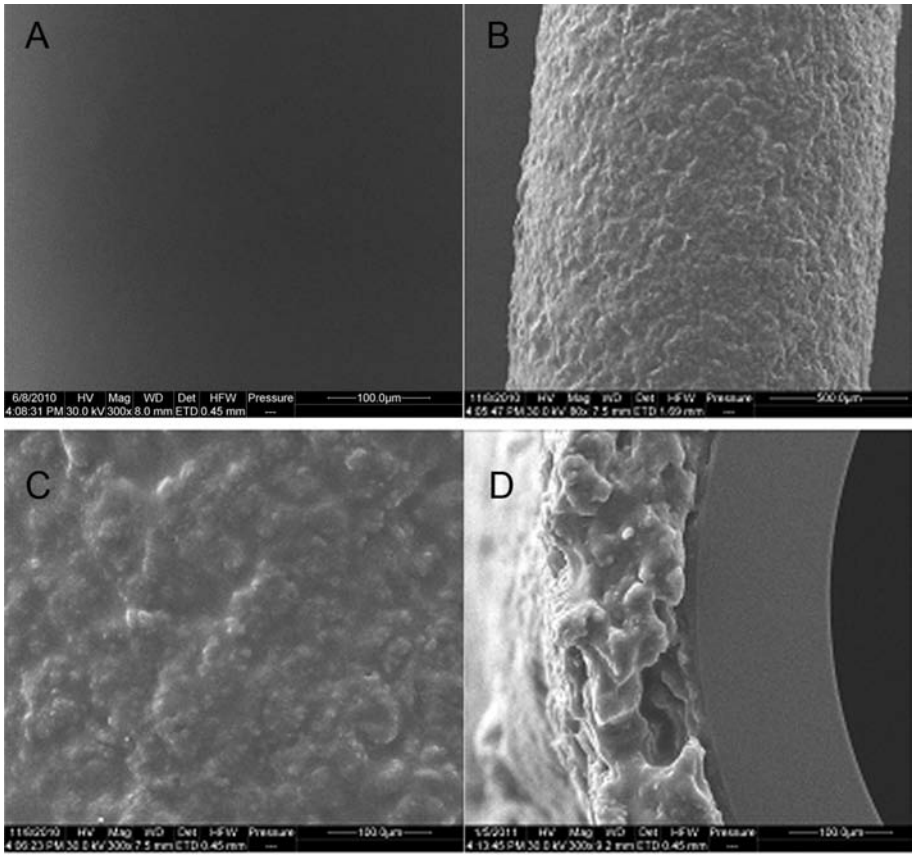

Figure 2. Scanning electron micrographs of (A) PDMS (300×), (B) PDMS/β-CD/DVB (80×), (C) PDMS/β-CD/DVB (300×), and (D) PDMS/β-CD/DVB (300×) stir bars.

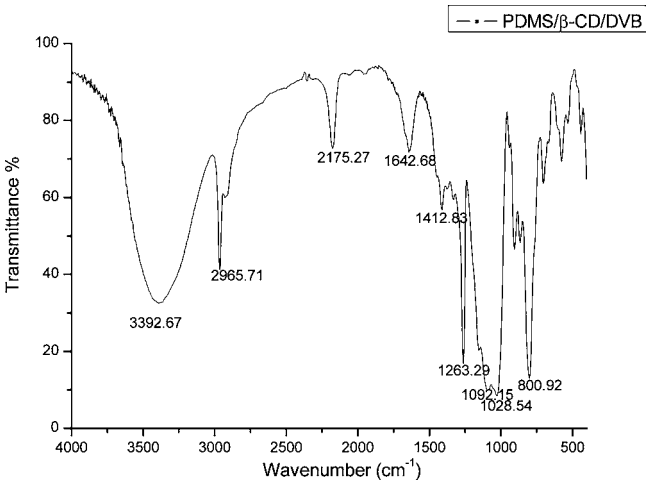

Figure 3. FT-IR spectrum of the PDMS/β-CD/DVB coating.

vibration of C−H of β-CD, and several strong peaks appearing in the range of 800.9−1263.3 cm^{-1} are the characteristic peaks of the benzene structure of DVB.

Optimization of the Extraction Conditions. To obtain a high extraction efficiency, several factors that influence the extraction of the four target estrogens by the PDMS/β-CD/DVB-coated stir bar, including the extraction time, stirring speed, desorption time, desorption solvent, pH value, and ionic strength, were investigated.

Effect of the Extraction Time and Stirring Speed. The extraction time is one of the most important factors that influence the extraction efficiency. The principle of SBSE is the same as that of SPME; they are based on equilibrium extraction, partitioning the solute between the sample matrix and the extraction phase.[24] The time to reach extraction equilibrium depends on the mass transfer rate and the property of the coating. In this work, the effect of the extraction time in the range of 10−30 min on the extraction of the target estrogens was investigated, and the experimental results indicated that the extraction equilibrium was almost reached after 20 min of extraction. Therefore, an extraction time of 20 min was selected for the subsequent experiments.

Stirring can accelerate molecular mass transfer and reduce the time to reach thermodynamic equilibrium. In this experiment, the effect of the stirring speed on the extraction of the four target estrogens was investigated by varying the stirring speed in the range of 500−900 rpm, and the experimental results demonstrated that extraction equilibrium was achieved at 600 rpm for DES, DIS, and HES, while the signal intensity of E_2 was increased slowly with the increase of the stirring speed from 500 to 900 rpm. However, a too high stirring speed may cause damage to the stir bar coating in the extraction process. Hence, a stirring speed of 700 rpm was employed in the following experiments.

Effect of the Desorption Solvent and Desorption Time. There are two desorption modes for SBSE, thermal desorption and liquid desorption. The thermal desorption mode is usually employed for GC analysis, but it is unsuitable for LC analysis. The ultrasonic-assisted liquid desorption (LD) mode was thus employed in this work for subsequent HPLC analysis. For this purpose, methanol, ethanol, acetone, acetonitrile, methanol/10 mmol/L NaH_2PO_4, and methanol/10 mmol/L NaOH were investigated as desorption solvents for the desorption of the target estrogens retained on the stir bar. It was found that the desorption solvent had an obvious effect on the subsequent HPLC separation. When pure organic solvents were used as the desorption solvent, the chromatographic peaks severely broadened, while the addition of aqueous solution into the organic solvent resulted in sharp and symmetrical peaks. This means that the mixed solvent matched the mobile phase much better than the pure organic solvent. The effect of the desorption solvent on the desorption efficiency of the target estrogens was studied, and it was found that the mixture of methanol/10 mmol/L NaOH was the best desorption solvent among the six desorption solvents tested in this work. The possible reason was that the four target estrogens containing phenolic hydroxyl groups were weak acidic compounds and the basic desorption solvent was favorable for their desorption from stir bar coatings. Then the effect of the ratio of methanol to 10 mmol/L NaOH on the desorption efficiency was investigated with the ratio varying in the range of 9/1 to 4/6. The experimental results revealed that the highest signal intensities were obtained for the target estrogens with the ratio of methanol to 10 mmol/L NaOH at 6/4 (v/v). Therefore, the mixture of methanol/10 mmol/L NaOH (6/4, v/v) was selected as the desorption solvent in this work.

In this work, the effect of the desorption time in the range of 5−25 min on the desorption was investigated by using 100 μL of methanol/10 mmol/L NaOH (6/4, v/v) as the desorption solvent. It was found that the desorption efficiencies of the four target estrogens increased with the increase of the desorption time from 5 to 15 min and then remained almost constant with the further increase of the desorption time from 15 to 25 min. Therefore, 20 min was selected as the desorption time in the following experiments.

Effect of the pH Value and Ionic Strength. Since estrogens are ionizable compounds, the pH value will affect the existence form of estrogens in aqueous solution and thus influence the extraction efficiency.[14] The effect of the pH value on the extraction of the target estrogens was investigated by changing the sample solution pH in the range of 3.0−8.0. The results showed that the signal intensities of the four target estrogens increased with increasing solution pH from 3.0 to 6.0 and then decreased with the further increase of the pH from 6.0 to 8.0. Hence, a pH of 6.0 was selected for the extraction of the four target estrogens from aqueous solution in this work. On the basis of this result, it can be deduced that not only the hydrophobic interaction but also the inclusion interaction was involved in the extraction of the target estrogens by the PDMS/β-CD/DVB-coated stir bar. When the pH value was lower than 6, the hydroxyl groups of estrogens and β-CD could be protonated by the excessive H^+, which increased the molecular polarity, decreased the hydrophobic interaction, and then weakened the inclusion interaction. When the pH value was around 6−7, estrogens and β-CD mainly existed as neutral molecules with low polarity; thus, the hydrophobic interaction between estrogens and β-CD became dominant, leading to enhancement of the inclusion interaction. With further increasing the pH value, estrogens mainly existed as the ion forms, which weakened the inclusion interaction again.

The ionic strength is another factor that influences the existence form of estrogens in aqueous solution. The effect of the ionic strength on the extraction efficiency of the target estrogens was investigated, and the experimental results demonstrated that the extraction efficiencies of the target estrogens obviously increased with increasing NaCl concentration from 0 to 20% (m/v) and then decreased with the

further increase of the NaCl concentration from 20% to 25% (m/v). Two processes which occurred simultaneously in the extraction could explain this phenomenon. Initially, the extraction efficiency for estrogens increases due to the "salt-out" effect, which drives more molecular estrogens into the SBSE coating; with the further increase of the salt concentration, the polar molecule may participate in an electrostatic interaction with the salt molecule, resulting in a decrease of the extraction efficiency.[25] Therefore, a NaCl concentration of 20% (m/v) in the aqueous sample solution was chosen in this work.

Analytical Performance. The preparation reproducibility and lifetime of PDMS/β-CD/DVB-coated stir bars were investigated by using aqueous sample solutions containing each target estrogen at 100 μg/L. As shown in Table 2, the relative standard deviations (RSDs) of the preparation reproducibility ranging from 5.8% to 9.6% (n = 9) in one batch and from 8.7% to 12.6% (n = 6) among different batches were obtained, indicating a good reproducibility for the preparation of PDMS/β-CD/DVB-coated stir bars. The recovery, calculated by the ratio of the averaged concentration for nine replicates (bar to bar) or for six replicates (batch to batch) obtained by external calibration to the spiked concentration (100 μg/L) ranged from 85.6% to 110.4% for bar to bar and from 99.5% to 119.1% for batch to batch, respectively. The lifetime of the PDMS/β-CD/DVB-coated stir bar was also evaluated, and the experimental results demonstrated that the PDMS/β-CD/DVB-coated stir bar could be used at least 50 times without a decrease of the extraction efficiency for the target estrogens.

Table 2. Preparation Reproducibility of PDMS/β-CD/DVB-Coated Stir Bars for the Determination of Estrogens

	bar to bar (n = 9)		batch to batch (n = 6)	
estrogen	RSD (%)	recovery (%)	RSD (%)	recovery (%)
E_2	9.6	101.1	11.5	108.6
DES	8.1	85.6	12.5	104.1
DIS	5.8	99.8	8.7	99.5
HES	8.6	110.4	12.7	119.1

Under the above optimized conditions, the analytical performance of the developed PDMS/β-CD/DVB SBSE–LD–HPLC–UV method was evaluated, and the results are summarized in Table 3. The limits of detection (LODs), based on a signal-to-noise ratio (S/N) of 3, ranged from 0.21 (DIS) to 1.6 (E_2) μg/L. A good linearity was obtained in the concentration range of 5–2000 μg/L for E_2 and 2–2000 μg/L for DES, DIS, and HES, with the correlation coefficient ranging from 0.9909 to 0.9986. The RSDs ranged from 6.0% to 9.1% at 100 μg/L and from 8.4% to 11.7% at 10 μg/L (n = 8), and the recovery ranged from 87.2% to 98.5% (c = 10 μg/L) and from 91.1% to 110.1% (c = 100 μg/L). The enrichment factors which were calculated by the ratio of the slopes of the calibration curves obtained with and without extraction ranged from 18.9- to 50.1-fold.

A comparison of LODs obtained by the developed method with those obtained by other methods, including SPE, SPME, and SBSE coupled to HPLC, are summarized in Table 4. As can be seen, the LODs of the developed method were higher than that of SPE–HPLC–MS,[7,8] similar to those of PDMS SBSE–HPLC–DAD (diode array detection),[14] monolithic SBSE–HPLC–DAD,[16] and MIP (molecularly imprinted polymer) SPME–HPLC–UV, [11] and lower than that of HF (hollow fiber) SPME–HPLC–UV.[12] On the basis of the experimental results obtained in this work along with those obtained for the PDMS/β-CD-coated stir bar as reported in ref 15, it can be seen that β-CD plays an important role in extraction of estrogens and the addition of DVB could increase the extraction efficiency through conjugation. In addition, the time to reach extraction equilibrium for the PDMS/β-CD/DVB-coated stir bar was much shorter (20 min) than those for the PDMS/β-CD-coated stir bar (40 min) and commercial PDMS-coated stir bar (2 h), which is another obvious advantage over the other SBSE methods reported in the literature.

Table 4. Comparison of the Limits of Detection (μg/L, μg/kg) with Those of Other Methods

method	E_2	DES	DIS	HES	ref
PDMS SBSE–HPLC–DAD[a]	1.0	0.6			14
monolithlic SBSE–HPLC–DAD[a]		0.21			16
MIP SPME–HPLC–UV[a]	0.98				11
HF SPME–HPLC–UV[a]		2.5	3.3	3.3	12
SPE–LC–MS[a]	0.1	0.05			7
SPE–LC–MS/MS[b]	0.005	0.006		0.004	8
SBSE–HPLC–UV[a]	1.6	0.57	0.21	0.27	this work

[a]Micrograms per liter. [b]Micrograms per kilogram.

Real Sample Analysis. The developed method was applied to the determination of four target estrogens in pork and chicken samples. Since acetonitrile was employed for the extraction of estrogens from real-world solid samples and 20% NaCl (m/v) was used to dilute the sample extraction solution in the sample pretreatment process, standard series prepared in acetonitrile and NaCl aqueous solution were employed for quantitative analysis of the real-world samples. The analytical results obtained by the external calibration method and recoveries for the spiked samples are listed in Table 5. As can be seen, the four target estrogens were not detected in the pork and chicken samples. The recoveries for the four target estrogens in spiked pork samples and spiked chicken samples

Table 3. Linear Ranges, Correlation Coefficients, Limits of Detection, Precisions, Recoveries, and Enrichment Factors for the Four Estrogens

compd	linear range (μg/L)	correlation coeff (r)	limit of detection (μg/L)	RSD (%, n = 8, c = 10 μg/L)	recovery (%, c = 10 μg/L)	RSD (%, n = 8, c = 100 μg/L)	recovery (%, c = 100 μg/L)	enrichment factor
E_2	5–2000	0.9986	1.6	11.0	91.5	9.1	94.9	19.1
DES	2–2000	0.9944	0.57	11.7	98.5	8.1	91.1	18.9
DIS	2–2000	0.9928	0.21	8.4	89.3	6.0	94.0	50.1
HES	2–2000	0.9909	0.27	9.4	87.2	9.7	110.1	38.5

Table 5. Analytical Results for the Pork and Chicken Samples (n = 3)

	pork			chicken		
compd	amt added (μg/g)	amt found (μg/g)	recovery (%)	amt added (μg/g)	amt found (μg/g)	recovery (%)
E_2	0	ND		0	ND	
	1	0.73 ± 0.07	72.8	1	0.98 ± 0.11	97.8
	5	4.23 ± 0.07	84.7	5	3.79 ± 0.50	75.9
	10	10.98 ± 0.84	109.8	10	11.08 ± 0.37	110.8
DES	0	ND		0	ND	
	1	0.69 ± 0.09	69.8	1	0.75 ± 0.11	74.5
	5	3.65 ± 0.10	73.1	5	5.81 ± 1.16	116.2
	10	8.57 ± 1.18	85.7	10	11.80 ± 1.01	118.9
DIS	0	ND		0	ND	
	1	0.96 ± 0.08	95.6	1	1.13 ± 0.03	113.2
	5	4.01 ± 0.22	80.2	5	4.32 ± 0.51	86.3
	10	7.100 ± 0.44	71.0	10	7.88 ± 0.33	78.8
HES	0	ND		0	ND	
	1	0.74 ± 0.13	73.7	1	1.05 ± 0.03	104.9
	5	3.84 ± 0.07	76.9	5	5.23 ± 0.87	104.6
	10	8.67 ± 1.11	86.7	10	9.58 ± 0.25	95.8

were in the range of 69.8−109.8% and 74.5−118.9%, respectively. Figure 4 shows the chromatograms of pork and spiked pork samples along with chicken and spiked chicken samples obtained by PDMS/β-CD/DVB SBSE−LD−HPLC−UV.

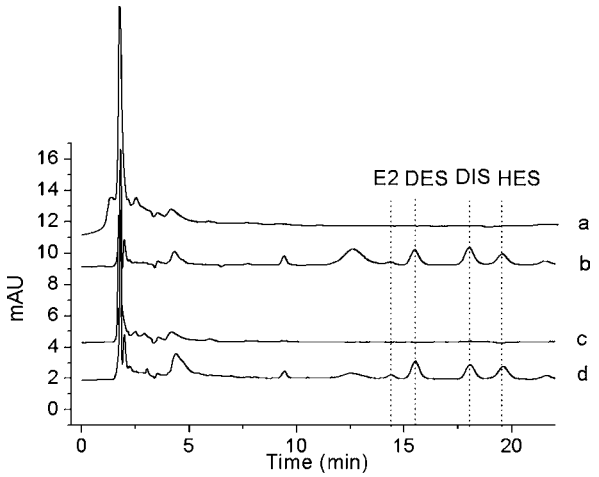

Figure 4. HPLC−UV chromatograms of estrogens obtained by PDMS/β-CD/DVB SBSE in (a) chicken, (b) spiked chicken (c = 5 μg/g), (c) pork, and (d) spiked pork (c = 5 μg/g).

In conclusion, a sol−gel PDMS/β-CD/DVB-coated stir bar was prepared and used for the extraction of four estrogens from animal-derived food samples in this work. The preparation of the PDMS/β-CD/DVB-coated stir bar by the sol−gel technique was simple and reproducible, and the PDMS/β-CD/DVB-coated stir bars possessed good chemical stability and mechanical stability. The developed method of PDMS/β-CD/DVB SBSE−LD−HPLC−UV was applied to the analysis of the four target estrogens in pork and chicken samples and exhibited some advantages, such as a short extraction time, a wide linear range, high sensitivity, and good reproducibility.

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Notes

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ABBREVIATIONS USED

SBSE, stir bar sorptive extraction; PDMS, poly(dimethylsiloxane); β-CD, β-cyclodextrin; DVB, divinylbenzene; LD, liquid desorption; LLE, liquid−liquid extraction; LPME, liquid-phase microextraction; SPE, solid-phase extraction; SPME, solid-phase microextraction; MTMS, methyltrimethoxysilane; PMHS, poly(methylhydrosiloxane); TFA, trifluoroacetic acid; E_2, 17-β-estradiol; DES, diethylstilbestrol; DIS, dienestrol; HES, hexestrol; HF, hollow fiber; MIP, molecularly imprinted polymer; LOD, limit of detection; RSD, relative standard deviation; ND, not detected

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