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C_{18}-coated stir bar sorptive extraction combined with high performance liquid chromatography–electrospray tandem mass spectrometry for the analysis of sulfonamides in milk and milk powder

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ABSTRACT

A simple, rapid, sensitive, inexpensive and less sample consuming method of C_{18}-stir bar sorptive extraction (SBSE)–high performance liquid chromatography (HPLC)–tandem mass spectrometry (MS/MS) was proposed for the determination of six sulfonamides in milk and milk powder samples. C_{18} silica particles coated stir bar was prepared by adhesion method, and two kinds of adhesive glue, polydimethylsiloxane (PDMS) sol and epoxy glue were tried. It was found that the C_{18}-coated stir bar prepared by PDMS sol as adhesive glue is more robust than that prepared by epoxy glue when liquid desorption was employed, in terms of both lifetime and organic solvent tolerance. The preparation of C_{18} stir bar was simple with good mechanic strength and the stir bar could be reused for more than 20 times. Granular coating has relatively high specific surface area and is propitious to sorptive extraction based process. Compared to conventional PDMS SBSE coating, C_{18} coating shows good affinity to the target polar/weak polar sulfonamides. To achieve optimum SBSE extraction performance, several parameters including extraction and desorption time, ionic strength, sample pH and stirring speed were investigated. The detection limits of the proposed method for six sulfonamides were in the range of 0.9–10.5 μg/L for milk and 2.7–31.5 μg/kg for milk powder. Good linearities were obtained for sulfonamides with the correlation coefficients (R) above 0.9922. Finally, the proposed method was successfully applied to the determination of sulfonamides in milk and milk powder samples and satisfied recoveries of spiked target compounds in real samples were obtained.

1. Introduction

Sulfonamides are N-substituted derivatives of sulfanilamide, a group of amide of paraaminobenzensulfonic acid, which are widely used in veterinary practice due to their broad spectrum of activity in preventing and treating bacterial infections, effectiveness in growth promoting and low cost. However, the residues of sulfonamides in food could cause hypersensitive allergic reactions and drug-resistant problems to human beings, and one or more members of this drug class even have a potential carcinogenic character [1,2]. To ensure food safety for consumers, both EU Commission and the U.S. Food and Drug Administration (FDA) have laid down the maximum residue limits (MRL) of 100 μg/L for sulfonamides as a total in target tissues (muscle, fat, liver, kidney) and milk from all food-producing species [3]. In China, the public notice NO. 235 of Agriculture Department has set the total limited level of sulfonamides as 100 μg/kg in all animal derived food [4].

As important nutritional materials, milk and milk products are of increasing concern for the detection of sulfonamide residues. The most commonly used method to detect sulfonamides residue in milk products is chromatography coupled to mass spectrometry (MS) or tandem MS (MS/MS) because of their high sensitivities and accuracy for compounds confirmation [5–7].

However, when high performance liquid chromatography (HPLC)–MS/MS is used for the analysis of sulfonamides in milk and milk powder products, an appropriate sample pretreatment step is often required for both cleaning up samples and enriching target analytes, due to the low level of analytes in real sample and being a very complicated matrix. Solid phase extraction (SPE) is one of the most commonly used sample pretreatment techniques for trace analysis of sulfonamides in samples with complex matrix, but generally, it is time-consuming and requires a large amount of solvent and sample [2,7]. Liquid phase microextraction (LPME) can provide a good preconcentration ability, but relatively low precision, which might be attributed to the manually handling of small amount extraction solvent [8]. Recently, a liquid–liquid–liquid microextraction for the determination of sulfonamides in water was proposed with detection limits at μg/L level [9]. Solid phase microextraction

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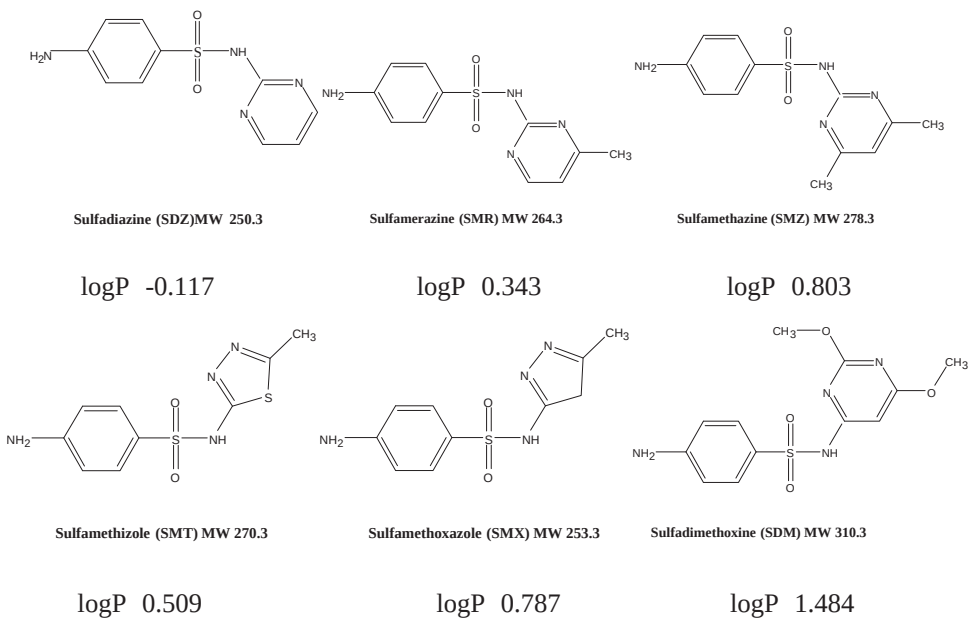

Fig. 1. Chemical structures and molecular weights of the target sulfonamides.

(SPME) developed from SPE is considered to provide better performance over SPE [10]. Firstly, the required time for SPME process is less than that needed in SPE. Secondly, SPME uses 100 times less organic solvent than SPE does. Thirdly, the required sample volume in SPME is much smaller than SPE (25 mL vs 500 mL). Stir bar sorptive extraction (SBSE), which was developed from SPME in 1999, is a relatively new sample pretreatment technique [11]. This sorptive extraction technique is based on the same extraction principles as SPME, but the coated amount of extraction phase is 50–250 times higher than that of SPME, implying a significant increase in recovery and extraction capacity [12]. In the past few years, SBSE has been developed rapidly and successfully applied to trace analysis of various target analytes in environmental and biological samples with extremely low detection limits. However, the overwhelming majority researches on SBSE are focused on the non-polar compounds because only one kind of stir bar coating (PDMS) is commercially available at present. To extend its application, different kinds of stir bar coatings were prepared in some laboratories [13–19]. However, there are only two studies on the SBSE extraction of polar/weak polar sulfonamides till now [20,21]. Li [20] and Yuan [21] proposed molecular imprinted polymer (MIP) and monolith material as SBSE coatings for sulfonamides, respectively. Nevertheless, it must be pointed out that the extraction equilibrium time is relatively long for MIP (60 min) and monolith (150 min) stir bars. Therefore, it is of great significance to prepare an appropriate SBSE coating for the rapid analysis of sulfonamides.

As a classical sorbent, silica based-C_{18} is widely used in SPE and SPME [13,22,23]. Lu et al. [24] employed C_{18} as matrix solid-phase dispersion (MSPD) sorbent on-line interfacing with HPLC–MS/MS for extraction and determination of 13 sulfonamide residues in grass carp tissues. However, to the best of our knowledge, there is no report using C_{18} silica particles as SBSE coating. In SPME, small particles were deposited [22] or adhered [13] onto the glass fibers, and the latter preparation method is once used to prepare a restricted access material-coating of stir bar for the extraction of polar caffeine and its metabolites by SBSE [23]. In this work, polydimethylsiloxane (PDMS)-sol and epoxy glue were used to prepare C_{18}-coated stir bars, respectively, and the performance of the stir bars prepared by these two kinds of adhesive glue for the extraction of sulfonamides was investigated and critically compared. The factors affecting on the extraction of sulfonamides were studied and the optimized extraction conditions were established. The prepared C_{18}-coated stir bars using PDMS-sol as adhesive glue was finally employed for HPLC–MS/MS analysis of sulfonamides in milk and milk powder samples for validation.

2. Experimental

2.1. Materials and reagents

C_{18}-silica (100 μm) was purchased from Qingdao Haiyang Chemical Co., Ltd. (Qingdao, Shandong, China). Hydroxyl-terminated polydimethylsiloxane (OH-PDMS) was purchased from Aldrich (Milwaukee, WI, USA). Methyltrimethoxysilane (MTMS), trifluoroacetic acid (TFA) and epoxy glue were obtained from China Medicine (group) Shanghai Chemical Reagent Corporation (Shanghai, China). Poly(methylhydrosiloxane) (PMHS) was obtained from the Chemical Plant of Wuhan University (Wuhan, China), and the capillary glass bars were obtained from Apparatus Factory of West China University of Medical Sciences (Chengdu, Sichuan, China). Sodium chloride and all solvents used in this study were of analytical grade. High purity water obtained by a Milli-Q water purification system (18.2 MΩ cm, Millipore, Bedford, MA, USA) was used throughout the whole experiments.

Sulfadiazine (SDZ, 99%), sulfamerazine (SMR, 99%) and sulfamethazine (SMZ, 98 + %) were purchased from Alfa Aesar (Ward Hill, MA, USA); sulfamethizole (SMT, 99%), sulfamethoxazole (SMX, 99%) and sulfadimethoxine (SDM) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The structures and log P values of the target sulfonamides are presented in Fig. 1. $^{13}C_3$-Caffeine as internal standard (IS) was purchased from ISOTEC^{TM} (Irvine, CA, USA). 0.1 mg/mL stock solutions of each target sulfonamide and $^{13}C_3$-caffeine were prepared in methanol.

2.2. HPLC–MS/MS conditions

The HPLC–MS/MS consists of an Agilent-1200 liquid chromatographic system equipped with vacuum degasser, a quaternary pump, a column oven and an autosampler (Agilent Technologies, Waldbronn, Germany), and a quadrupole-time of flight-mass

Table 1
Optimized HPLC–ESI-MS/MS conditions for the analysis of target sulfonamides.

Compounds	Precursor ion $[M+H]^+$	Product ions (m/z)	Optimal collision voltage (eV)	Retention time (min)
SDZ	251.1	156.0; 108.0	10	15.7
SMR	265.1	156.0; 110.1	5	16.8
SMZ	279.1	156.0; 186.0	7	18.7
SMT	271.0	156.0; 108.0	10	19.3
SMX	254.1	156.0; 108.0	10	21.7
SDM	311.1	156.0; 245.1	8	26.6
$^{13}C_3$-Caffeine	198.1	140.1	10	20.1

spectrometry (Q-TOF MS) with an electrospray ionization source (ESI) (Bruker Daltonics GmbH, Rheinstetten, Germany). The analytes were separated using a reversed phase C_{18} HPLC column (Zorbax ODS, 3.5 μm, 2.1 × 150 mm, Agilent, Santa Clara, USA) at 15 °C. Mobile phase was methanol in water and its gradient profile was as follows: t_{0min}, 2% (v/v) methanol; t_{2min}, 7% (v/v) methanol; t_{4min}, 10% (v/v) methanol; t_{20min}, 50% (v/v) methanol; t_{25min}, 2% (v/v) methanol; t_{30min}, 2% (v/v) methanol. The mobile phase flow rate was 0.2 mL/min and the sample injection volume was 70 μL. All analytes were analysed in positive ion mode. The optimum conditions for operating the mass spectrometer were: electrospray voltage 4.5 kV, electrospray pressure 1.6 bar, desolvation gas 8.0 L/min, desolvation temperature 180 °C, scan range 50–400 m/z. The optimal collision voltage, product ions and retention time of each analytes are presented in Table 1. The quantitative determination was assessed by summing the ion intensity of precursor ion and product ions.

2.3. Preparation and operation procedure of SBSE

"Dumbbell-shaped" bare stir bar was prepared and activated referring to Ref. [25]. PDMS sol solution for adhering of C_{18} consisted of 100 μL PDMS, 100 μL MTMS, 10 μL PMHS and 100 μL 95% aqueous TFA. The 100 μm C_{18}-silica was used in this work. Stir bar was coated with a thin film of PDMS-sol first and then covered with C_{18}-silica by rolling it in C_{18}-silica bed. After the surface was coated completely, the prepared stir bar was placed into a constant temperature drier for 24 h at 60 °C. Prior to use, C_{18}-coated stir bar should be cleaned and activated in methanol by ultrasonication for 10 min to remove impurities and improve the extraction ability. C_{18}-coated stir bars obtained by epoxy glue were prepared following the same process except epoxy glue instead of PDMS-sol was used. Besides, conventional PDMS stir bars were prepared according to Guan's method [26] for comparison: 1 g PDMS, 250 μL MTMS, 250 μL PMHS, 250 μL CH_2Cl_2 and 250 μL of 95% aqueous TFA were mixed in agitation.

The prepared stir bar was immersed into 4 mL aqueous sample solution and was stirring at 600 rpm for 10 min, and then transferred into a small test tube containing 90 μL methanol. After desorption by ultrasonication for 10 min, the stir bar was taken out to dry its surface carefully and was placed into 2 mL methanol for cleaning. 70 μL of the elution was injected into HPLC–MS/MS for subsequent analysis. The scheme of "dumbbell-shaped" C_{18}-coated SBSE is shown in Fig. 2.

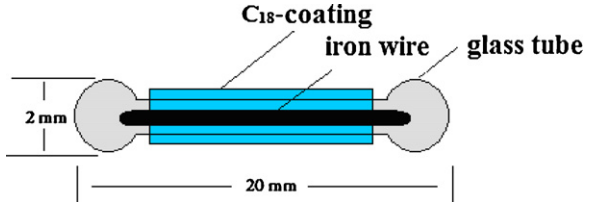

Fig. 2. Scheme of "dumbbell-shaped" stir bar.

2.4. Sample collection and preparation

Fresh milk and milk powder samples were purchased from the local market (Wuhan, China). 1.0 mL milk sample was spiked with 10 μL standard solution containing six sulfonamides (2 μg/mL for SDM and 10 μg/mL for the others) and 20 μL of 10 μg/mL $^{13}C_3$-caffeine as internal standard. The spiked milk sample was homogenized by agitation for 10 min, and then was diluted to 10 mL with hydrochloric acid solution (pH 2) containing 15% NaCl. After well shaking, the sample solution was centrifuged at 12 000 rpm (TGL-16GA centrifuge, Xinke scientific instrument Co. Ltd., Hunan, China) for 10 min to remove the proteins and fats of milk, and 4 mL of the upper clear liquid was used for extraction by SBSE and subsequent analysis by HPLC–MS/MS.

0.5 g milk powder was accurately weighed and was spiked with 5 μL standard solution containing six sulfonamides (2 μg/mL for SDM and 10 μg/mL for the others) and 20 μL of 10 μg/mL $^{13}C_3$-caffeine as internal standard. The spiked milk powder sample was placed for a whole night for the permeation of the target analytes, and then was diluted to 10 mL with hydrochloric acid solution (pH 2) containing 15% NaCl. After 10 min of centrifugation at 12 000 rpm, 4 mL of the upper clear liquid was taken for extraction by SBSE and subsequent analysis by HPLC–MS/MS.

Blank milk and milk powder samples with $^{13}C_3$-caffeine as internal standard were prepared according to the procedures above except the spiking step.

3. Results and discussion

3.1. Preparation and characterization of C_{18}-coated stir bars

The effect of the viscosity of PDMS sol on coating C_{18} particles onto the stir bar was evaluated by varying the concentration of CH_2Cl_2 in the sol and using 100 μm C_{18} particles as coating materials. It was found that the larger the amount of CH_2Cl_2 was added into the sol, the more easily C_{18} particles fell off from the stir bar. This phenomenon could be attributed to thinner PDMS glue on the stir bar. When larger amount of CH_2Cl_2 was added into the PDMS sol, the viscosity of PDMS sol was decreased. The lower viscosity of PDMS sol, thinner PDMS glue was coated on the bare glass stir bar. However, thinner PDMS glue could not stably adhere 100 μm C_{18} silica particles onto the glass stir bar, resulting in C_{18} silica particles falling off. Therefore, the optimized PDMS sol consists of 100 μL PDMS, 100 μL MTMS, 10 μL PMHS and 100 μL 95% TFA, without the addition of CH_2Cl_2. With above PDMS sol as adhesive glue, the thickness of the prepared PDMS coating on the stir bar was estimated to be about 30 μm.

Fig. 3 is the scanning electron micrographs of a "dumbbell-shaped" C_{18}-coated stir bar. As could be seen in Fig. 3a, the diameter of the glass bulb is larger than that of the bar coated with C_{18}-silica, which means the glass bulbs at two ends would help to hold up the stir bar, preventing the body of stir bar from direct contacting with the bottom of vial during the stirring process. Fig. 3b and c shows the surface topography of the C_{18}-coated stir bar. As could be

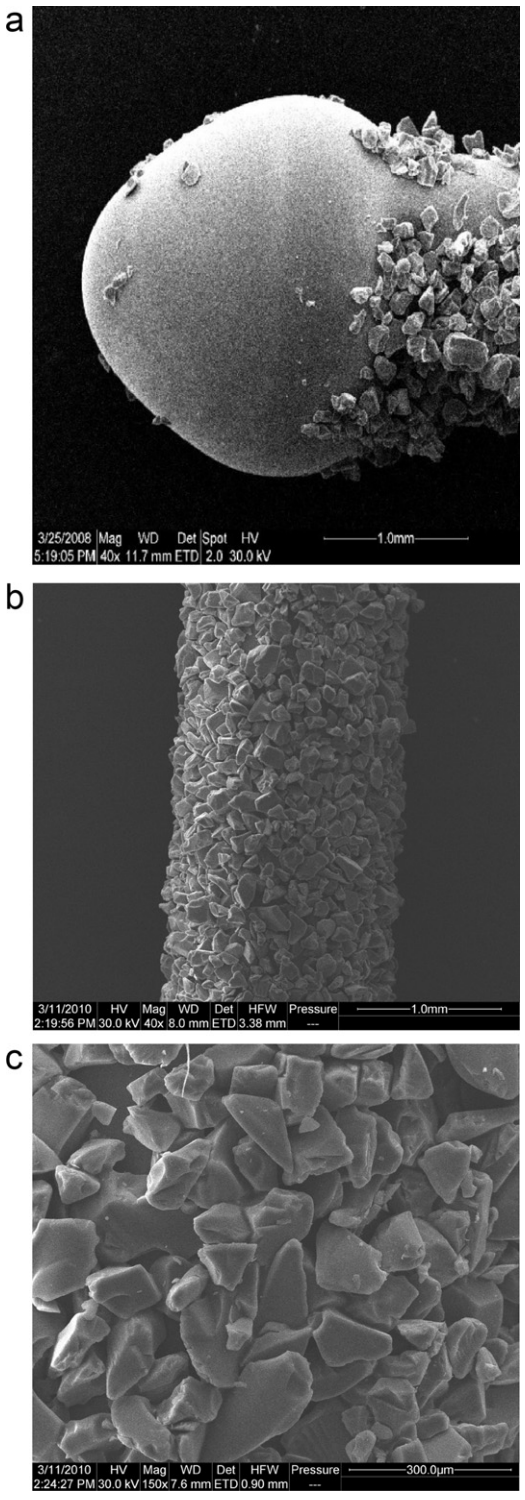

Fig. 3. Scanning electron micrographs of "dumbbell-shaped" C_{18}-coated stir bar prepared by PDMS-sol as adhesive glue.

seen, C_{18} silica particles were uniformly adhered onto the surface of glass bar. Granular coating has relatively high specific surface area and is propitious to sorptive extraction based process. Compared to conventional PDMS SBSE coating (as shown in Fig. 4), C_{18} coating indicated good affinity to the target polar/weak polar sulfonamides.

The preparation reproducibility for C_{18}-coated stir bars prepared by PDMS-sol was evaluated by using six bars to extract target analytes from aqueous solutions (extraction conditions: 10 min extraction time, 600 rpm stirring rate, 4 mL sample solution containing 15% NaCl, pH = 2). The data listed in Table 2 reveals an accepted reproducibility for preparation of C_{18}-coated stir bar with relative standard deviations (RSDs) ranging from 8.2% to 19.8%.

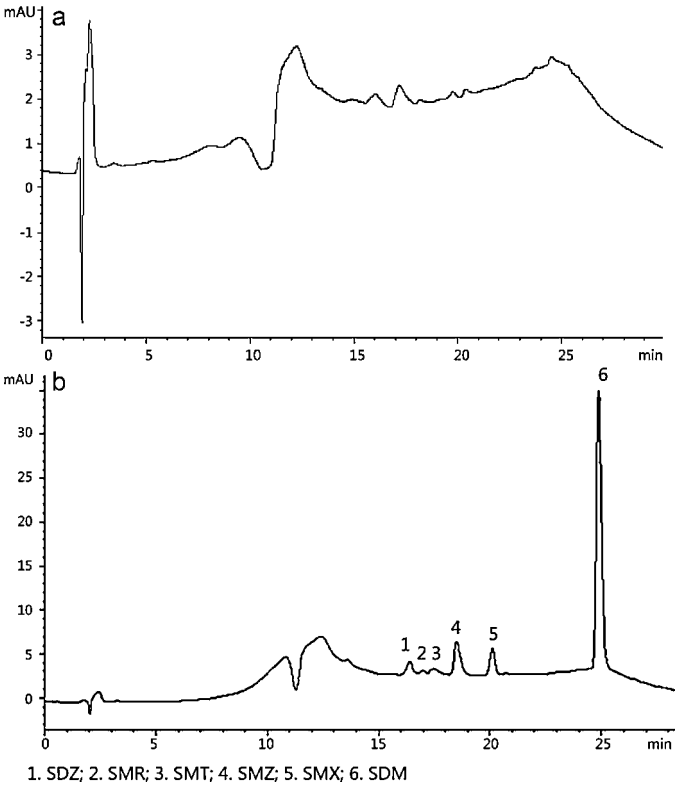

Fig. 4. HPLC–UV chromatograms of sulfonamides (each 200 μg/L) obtained by (a) conventional PDMS coated SBSE (b) C_{18}-coated SBSE in the standard aqueous solution. Extraction time: 30 min; desorption time: 10 min; pH = 2.

Table 2
Preparation reproducibility for C_{18}-coated stir bar prepared by using PDMS as adhesive glue.

Compounds	Bar-to-bar (%, $n = 6$)
SDZ	14.5
SMR	12.3
SMZ	19.8
SMT	17.2
SMX	9.0
SDM	8.2

Using 100 μg/L sulfonamide aqueous solutions as samples and the chromatographic peak areas as the signal responses, the lifetimes of C_{18}-coated stir bars prepared by PDMS-sol and epoxy glue were investigated, respectively, and the experimental results were shown in Fig. 5. As can be seen, the C_{18}-coated stir bar prepared by PDMS-sol can be used more than 20 times (the RSDs for 20 extractions ranged from 4.3% to 19.6%, see Table 3), while the

Table 3
Relative standard deviations (RSDs) for 20 extractions by C_{18}-coated stir bar with PDMS as adhesive glue.

Compounds	RSD (%)
SDZ	19.6
SMR	17.5
SMZ	16.9
SMT	16.4
SMX	13.2
SDM	4.3

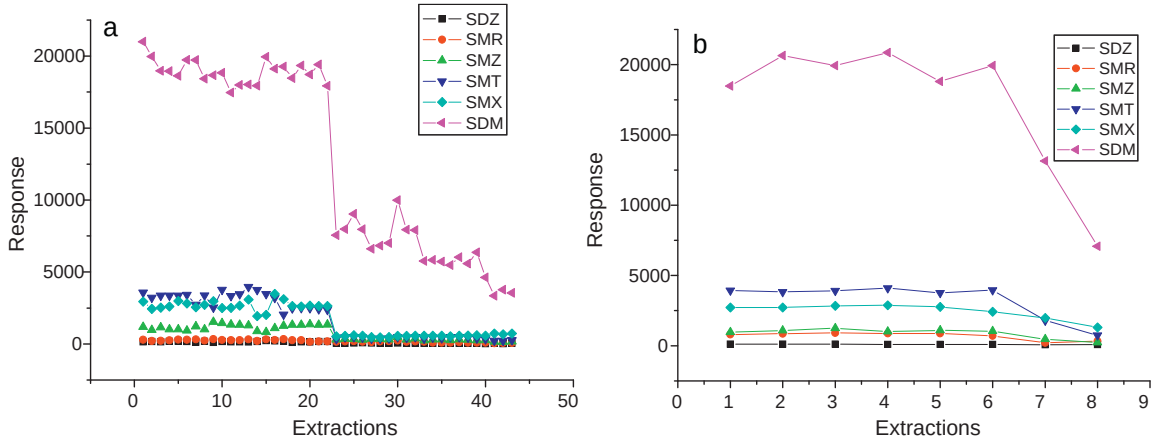

Fig. 5. Lifetimes of C_{18}-coated stir bar obtained by using (a) PMDS-sol and (b) epoxy as adhesive glue. 100 μg/L for each sulfonamide in an aqueous solution.

C_{18}-coated stir bar prepared by epoxy glue can be only used for 6 times and then the extraction efficiency of sulfonamides decreased obviously. To test their robustness to organic solvent, both stir bars were immersed in methanol under ultrasonication. The experimental results demonstrated that the C_{18}-coated stir bar prepared by PDMS-sol was stable even after ultrasonication for 30 min, whereas the C_{18}-coated stir bar prepared by epoxy glue began to peel off after 20 min ultrasonication. All these facts indicated that the C_{18}-coated stir bar prepared by PDMS-sol glue was more robust than the C_{18}-coated stir bar prepared by epoxy glue, at least in the liquid desorption mode. Then, C_{18}-coated stir bar prepared by PDMS-sol glue was employed in the following experiments.

3.2. Optimization of SBSE parameters

In SBSE, parameters such as extraction time, desorption time, ionic strength, sample pH and stirring speed will greatly influence the extraction efficiencies of target analytes. For extraction of the target sulfonamides by C_{18}-coated stir bar, these influencing factors were investigated and optimized by using aqueous solution containing six sulfonamides, each at 100 μg/L.

The effect of extraction time on the extraction efficiency of target sulfonamides was investigated with extraction time in the range of 5–25 min and the results were shown in Fig. 6. As could be seen, the extraction equilibrium almost reached after 10 min extraction, so 10 min was selected as the extraction time. It was much faster than the monolithic material stir bar sorptive extraction equilibrium time of 150 min [21] and MIP coated stir bar of 60 min [20]. The effect of desorption time on the extraction efficiency of target sulfonamides was studied by ultrasonicating the stir bar in methanol for 5, 10, 15, 20 and 25 min, respectively, and desorption volume of methanol was 90 μL, which was the smallest volume to submerge the stir bar in the applied desorption tube. The experimental results showed that desorption equilibrium was achieved after 10 min desorption, and 10 min was selected as desorption time in subsequent experiments.

The extraction efficiencies of target analytes depend to a great extent on the pH value of sample solution, especially for the rather strong polar sulfonamides. Considering pKa values of the target sulfonamides (SDZ, pKa = 6.5; SMR, pKa = 8.0; SMZ, pKa = 7.4; SMT, pKa = 5.51; SMX, pKa = 5.7; SDM, pKa = 6.21 [27,28]), the effect of pH in the range of 2–5 on the extraction of the target sulfonamides by C_{18}-SBSE was studied. For this purpose, a series of aqueous solutions containing 100 μg/L target sulfonamides were adjusted to pH 2, 2.5, 3, 4 and 5 with hydrochloric acid, respectively, and subjected to the extraction. As could be seen from Fig. 7, the best extraction efficiencies were obtained in the pH range of 2–2.5 for all target analytes. Thus pH 2 was selected as the sample pH for the extraction of target sulfonamides by C_{18}-SBSE in this study.

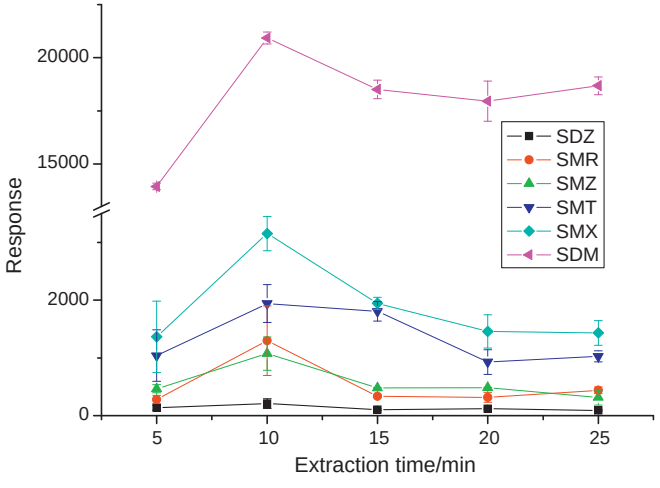

Fig. 6. The effect of extraction time on the extraction of the target sulfonamides by C_{18}-coated SBSE prepared by PDMS-sol as adhesive glue. 100 μg/L for each sulfonamide in an aqueous solution.

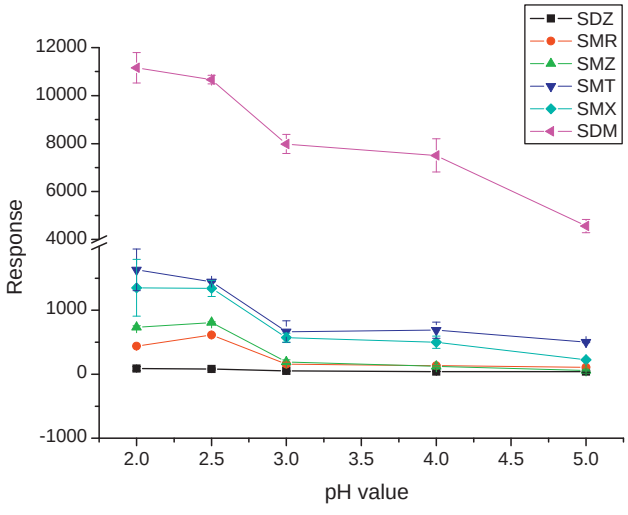

Fig. 7. Effect of sample pH on the extraction of target sulfonamides by C_{18}-coated SBSE prepared by PDMS-sol as adhesive glue. 100 μg/L for each sulfonamide in an aqueous solution.

Table 4
Analytical performance of C_{18}-coated SBSE–HPLC–MS/MS.

Compounds	LOD (μg/L)	Linear range (μg/L)	Correlation coefficient (R)	RSD (%, n = 10, c = 100 μg/L)
SDZ	0.97	5–500	0.9944	14.8
SMR	0.74	5–500	0.9947	16.7
SMZ	0.63	2–500	0.9966	14.3
SMT	0.57	2–500	0.9947	11.1
SMX	0.40	2–500	0.9981	7.3
SDM	0.04	0.2–200	0.9922	9.5[a]

[a] c = 20 μg/L.

In SBSE, the increase of ionic strength of sample solution favors the extraction of organic analytes into the stir bar coating [29,30]. To evaluate the impact of ionic strength on the extraction of target sulfonamides by C_{18}-SBSE, extractions were performed for the aqueous solution samples with NaCl concentrations varying from 0% to 20% (w/v). The experimental results indicated that extraction efficiencies of target sulfonamides were increased with increasing NaCl concentration in aqueous solution sample from 0% to 10%. For all target sulfonamides except SDM, extraction efficiency was kept almost unchanged after NaCl concentration exceeded 10%. While for SDM, the highest extraction efficiency was obtained in the solution containing 15% NaCl. Based on these results, 15% (w/v) NaCl was added into sample solution for the extraction of target sulfonamides by C_{18}-SBSE in this work.

The effect of stirring speed on the extraction was studied with stirring speed ranging from 600 to 1000 rpm. No obvious effect of stirring speed on the extraction efficiency of target sulfonamides was observed. In practical operation, 600 rpm was applied to reduce the physical deterioration of stir bar coatings.

In summary, the optimal conditions for the extraction of sulfonamides by C_{18}-coated SBSE are as follows: 4 mL sample solution containing 15% NaCl (w/v) was adjusted to pH 2 and stirred at 600 rpm for 10 min. After extraction, the stir bar was desorbed in 90 μL methanol by ultrasonication for 10 min.

3.3. Analytical performance

Under optimized conditions, the analytical performance of C_{18}-coated SBSE–HPLC–MS/MS for the analysis of six target sulfonamides was evaluated and results are listed in Table 4. The linear range was studied by preparing a calibration curve over a concentration range of 0.1–2000 μg/L for each sulfonamide, and a good linear relationship with correlation coefficients (R) between 0.9922 and 0.9981 was achieved for six sulfonamides in their respective linear range as described in Table 4. The relative standard deviations (RSDs) were found to be in the range of 7.3–16.7%, based on 10 parallel analyses of 4 mL aqueous solutions containing sulfonamides (20 μg/L for SDM and 100 μg/L for the other five analytes). The limits of detection (LODs) were calculated as the concentration of the target analyte that produced a signal-to-noise ratio (S/N) of 3. The LODs for six sulfonamides obtained by the proposed method were in the range of 0.04 μg/L (SDM) to 0.97 μg/L (SDZ). Additionally, the extraction capability for target sulfonamides by the prepared C_{18}-coated stir bar is in accordance with the retention capability for target sulfonamides by C_{18} HPLC column. In other words, the longer retention time for the analyte, the better affinity the C_{18}-coated stir bar will exhibit towards it and vice versa. Therefore, it can be predicted that the prepared C_{18}-coated stir bar favors the extraction of both polar and nonpolar compounds which can be retained by C_{18} HPLC column.

For comparison, the LODs and extraction time for target sulfonamides obtained by other methods involving SPE, SPME and SBSE in recent years are listed in Table 5. Apparently, Refs. [31,32] applying SPE as the pretreatment technique provided the lowest LODs for target sulfonamides, probably due to much more amount of extraction phase (200 mg), larger sample volume (500 mL vs 25 mL or 4 mL) and more effective sorbents applied in SPE than that in SPME and SBSE. Nevertheless, a shorter time was required (10 min) to achieve the extraction equilibrium for C_{18}-coated SBSE, with much less organic solvent/sample consumption (90 μL methanol; 4 mL sample solution) than that in SPE. Moreover, the eluent volume for the proposed SBSE method matched the injection volume of the subsequent chromatographic system, whereas the eluent of SPE has to be concentrated before sample introduction into the subsequent chromatographic system in most cases. Compared to the methods involving SPME [32–34], the LODs for the target analytes obtained by the proposed method are lower, especially for SDM, whose LOD was lowered by 2 orders of magnitude. Besides, stir bar is more robust than SPME fibre, and easier to be preserved. In addition, the LODs of this work are comparable or better than those

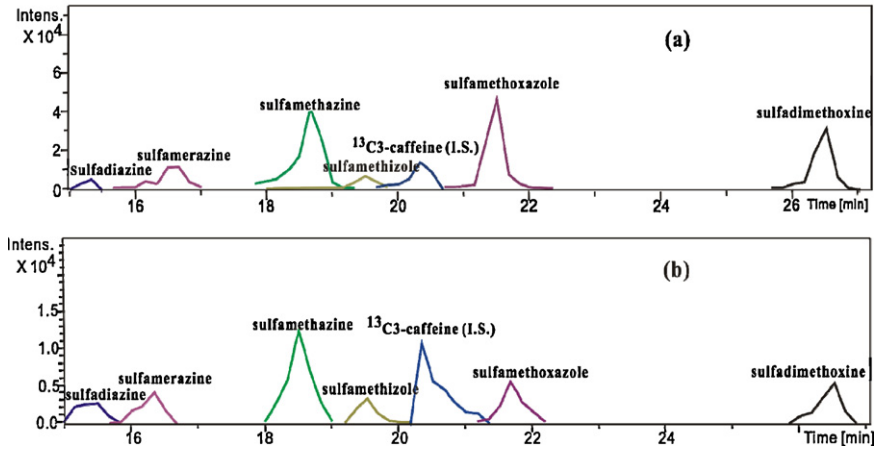

Fig. 8. The extracted MRM chromatograms of the six sulfonamides and I.S. with C_{18}-coated SBSE prepared by PDMS-sol as adhesive glue for (a) spiked milk sample and (b) spiked milk powder sample.

Table 5
Comparison of the LODs (μg/L) and extraction time (ET, min) obtained by this work with some SPE, SPME and SBSE methods reported in the literatures for determination of sulfonamides.

Analytes	HLB-SPE–HPLC–MS/MS [31]		MCX-SPE–HPLC–MS/MS [32]		CW/TPR-SPME–HPLC–MS/MS[a] [32]		CW/TPR-SPME–HPLC–MS/MS[a,b] [35]		PDMS/DVB-SPME–HPLC–MS[c,d] [33]		In-tube SPME–HPLC–UV[e] [34]		MIP-SBSE–HPLC–UV [20]		Monolith-SBSE–HPLC–DAD [21]		This work	
	LODs	ET	LODs	ET	LODs	ET	LODs	ET	LODs	ET	LODs	ET	LODs	ET	LODs	ET	LODs	ET
SDZ	–	100	3.36×10^{-3}	20	9.04	20	–	30	36	40	2.0	250	0.30	60	1.30	150	0.97	10
SMR	1.0×10^{-3}		2.88×10^{-3}		55.3		–		32		–		0.41		–		0.74	
SMZ	1.0×10^{-3}		3.66×10^{-3}		16.2		0.061/0.038[b]		16		2.8		0.20		1.29		0.63	
SMT	1.0×10^{-3}		–		–		–		–		–		0.61		–		0.57	
SMX	–		9.00×10^{-3}		14.0		0.41/0.077		20		1.7		0.66		1.85		0.40	
SDM	1.0×10^{-3}		6.72×10^{-3}		12.4		0.027/0.013		25		–		–		–		0.04	

[a] CW/TPR, carbowax-templated resin.
[b] Influent/effluent.
[c] PDMS/DVB, polydimethylsiloxane/divinylbenzene.
[d] μg/kg.
[e] In-tube SPME using poly(methacrylic acid-ethylene glycol dimethacrylate) (MAA-EGDMA) monolithic capillary column as the extraction medium.

Table 6
LODs and recoveries of target sulfonamides in real samples by C_{18}-coated SBSE–HPLC–MS/MS.

Compounds	Milk[a]		Milk powder[b]	
	LOD (ng/mL)	Recovery (%)	LOD (ng/g)	Recovery (%)
SDZ	10.5	87	29.4	99
SMR	9.9	99	25.2	80
SMZ	10.5	94	15.3	115
SMT	8.7	98	31.5	71
SMX	7.5	120	24.0	68
SDM	0.9	93	2.7	105

[a] Spiked at 100 ng/mL target SAs (20 ng/mL for sulfadimethoxine).
[b] Spiked at 100 ng/g target SAs (20 ng/g for sulfadimethoxine).

MIP [20] and monolith SBSE [21], and the extraction time is much shorter.

3.4. Sample analysis

The milk and milk powder samples were analysed by C_{18}-coated SBSE–HPLC–MS/MS with an external calibration curve and 20 μg/L of $^{13}C_3$-caffeine as internal standard (I.S.), and no target sulfonamides were found in the milk and milk powder samples. Therefore, the milk and milk powder samples were spiked (see Section 2.4) and the spiked samples were analysed. Table 6 is the recovery obtained for the spiked samples. As could be seen, the recoveries of the target sulfonamides except SMX were ranged from 87% to 99% and from 71% to 115% for the spiked milk sample and the spiked milk powder sample, respectively. However, the recovery of 68% and 120% were obtained for SMX in the spiked milk and milk powder samples due to the matrix interference. The LODs of six sulfonamides in real samples were nearly one order of magnitude higher than that obtained for the analytical performance evaluation in Section 3.3. Fig. 8 is the extracted MRM chromatograms for the spiked milk and milk powder samples obtained by C_{18}-coated SBSE–HPLC–MS/MS. The MRM transitions of m/z 251.1 → 156.0 (SDZ), 265.1 → 265.1 (SMR), 279.1 → 279.1 (SMZ), 271.0 → 156.0 (SMT), 254.1 → 156.0 (SMX), 311.1 → 311.1 (SDM) and 198.1 → 140.1 ($^{13}C_3$-caffeine) were monitored.

4. Conclusions

To improve the extraction capability of SBSE for polar compounds, C_{18}-coated stir bar was prepared by using PDMS-sol and epoxy glue as adhesive glue, respectively. The extraction performance of these two kinds of C_{18}-coated stir bar for the extraction of six target sulfonamides has been investigated and critically compared, and the results showed that C_{18}-coated stir bar prepared by using PDMS-sol as adhesive glue provides better extraction performance than the C_{18}-coated stir bar prepared by using epoxy glue, in terms of both lifetime and organic solvent tolerance. A novel method of C_{18}-coated SBSE combining with HPLC–MS/MS was established to determine six sulfonamides in milk and milk powder samples. The proposed method is sensitive, accurate, simple, rapid and low-cost, and provides a reasonable alternative for trace sulfonamides analysis in milk and milk powder samples. What is more, the prepared C_{18}-coated stir bar allows the extraction of polar compounds by SBSE, and great potentiality is expected for the application of SBSE to the analysis of compounds with different polarity due to a good extraction capability of C_{18} to a variety of compounds.

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