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Determination of sulfonamides in food samples by membrane-protected micro-solid phase extraction coupled with high performance liquid chromatography

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ABSTRACT

In the present study, a simple and sensitive extraction method based on polypropylene membrane-protected micro-solid phase extraction (MP-μ-SPE) has been developed for analysis of sulfonamides in food samples. Poly (methacrylic acid-ethylene glycol dimethacrylate) (p-MAA-EDMA) was synthesized using orthogonal array experimental design, optimized with three factors at four levels and evaluated on yield, hydrophobic and cation-exchange properties. The optimized p-MAA-EDMA was then employed as the sorbent in the MP-μ-SPE for extraction of sulfonamides from milk and chicken muscle samples, followed by high performance liquid chromatographic analysis with ultraviolet detection. Under optimized extraction conditions, good linearities (0.010–1.0 μg mL^{-1} with $r^2 > 0.9900$), low limits of detection (0.38–0.62 ng mL^{-1}), and acceptable intra-day (2.7–13.7%) and inter-day (6.7–15.2%) relative standard deviations were obtained. It was demonstrated to be an effective approach to handle semi-solid/solid samples with good resistance to interference from "dirty" samples.

1. Introduction

In recent years, several serious food safety-related accidents occurred, which enhances the attention of the public and government to monitor and control contaminations in foods. Food analysis generally includes sampling, sample pretreatment, instrumental analysis and data handling. Since matrices of food samples are inevitably complex, appropriate sample pretreatment appears particularly crucial in these steps, which can reduce/eliminate interference of matrices, concentrate target analytes, and/or make them suitable for subsequent instrumental analysis [1,2].

Solid-phase extraction (SPE) is a common sample preparation method and still remains as a popular choice [3,4]. However, when applying SPE on food samples, additional steps, such as centrifugation, protein precipitation and filtration, are generally required to avoid blockage of the SPE columns. As an alternative, miniaturized sorbent phase-based extraction, e.g. solid phase microextraction (SPME), has found some applications in food analysis [5,6]. Compared to SPE, SPME simplifies and accelerates the procedure of analysis. However, it suffers from some defects, such as fragility of the fibers and carry-over between runs. Its resistance to the sample matrix interference may be poor and the extraction capacity may be unsatisfactory as a result of its limited sorbent phase on the silica fiber. To enhance the extraction sensitivity and reduce/eliminate matrix interference, other miniaturized sorbent phase-based extraction technologies, e.g. hollow fiber membrane-protected SPME [7], in-tube SPME [8], stir bar sorptive microextraction [9] and membrane-protected micro-solid phase extraction (MP-μ-SPE) [10], have been developed.

MP-μ-SPE is a simple and effective method to handle complex samples. In MP-μ-SPE, porous polypropylene membrane was folded into a small envelope and contained a small amount of sorbent. The open ends were sealed by heat. For extraction, the envelope containing sorbent was directly placed into sample solutions. Compared to conventional SPE, MP-μ-SPE has several advantages: (1) it avoids usage of frits to hold sorbents as seen in conventional SPE columns and thus simplifies packing of sorbents; (2) it is effective to extract analytes from suspension or semi-solid/solid samples, owing to the fact that the porous membrane can prevent particles from contaminating the sorbent phase. MP-μ-SPE can thus reduce sample matrix effect and avoid blockage which is generally encountered in SPE columns; (3) it is relatively inexpensive and consumes a small amount of solvents and sorbents without special auxiliary device for extraction; (4) it is easy-to-handle, which may indicate extra convenience for daily operation.

Applications of MP-μ-SPE are mainly concentrated on environmental and biological analysis. Basheer et al. applied this device to determine persistent organic pollutants in human ovarian tissue [11], aldehydes in rainwater [12], carbamate pesticides in soil

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[13], acidic drugs and organophosporous pesticides in wastewater [10,14]. Feng et al. reported the determination of phenols in environmental water samples [15]. Xu et al. described its application to the determination of polycyclic aromatic hydrocarbons in soil sample with microwave-assisted extraction [16]. Kanimozhi et al. adopted this device for the determination of estrogens in ovarian cyst fluid samples [17]. To our knowledge, applications of MP-μ-SPE to food samples have seldom been reported. Since MP-μ-SPE can reduce sample matrix interference and is applicable to semi-solid/solid samples, to fully embody its advantages and expand its applications, we aim to investigate its suitability to food analysis in the present study.

It is well known that the sorbent plays an important role in sorbent phase-based extraction. Hitherto, various sorbents, including multiwalled carbon nanotubes [10], HayeSep A/C_{18} [11], C_2 [12], C_{18} [13], molecularly imprinted polymer [15] and graphite fibers [16], have been used in MP-μ-SPE. Herein, poly (methacrylic acid-ethylene glycol dimethacrylate) (p-MAA-EDMA) was selected as the sorbent. This organic polymer exhibits hydrophobic and cation-exchange properties, has preferable pH stability, and possesses large surface area and good biocompatibility [18–22]. To obtain the suitable materials for extraction, synthesis of polymer was optimized by orthogonal array experimental design (OAD) based on three factors and four levels ($L_{16}(4^5)$). The produced materials were evaluated in terms of yield, hydrophobic and cation-exchange properties. The optimized material was employed as the sorbent in MP-μ-SPE for the extraction of sulfonamides in food samples.

2. Experimental

2.1. Reagents and materials

Four sulfonamides, sulfamerazine (SM), sulfamethazine (SMZ), sulfathiazole (STZ) and sulfadiazine (SD), were obtained from Alfa Aesar (Tianjin, China). Acetonitrile, methanol, acetic acid, sodium chloride, toluene, dodecanol, methyacrylic acid (MAA), azobisisobutyronitrile (AIBN) and ethylene glycol dimethacrylate (EDMA) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Ammonia and hydrochloric acid were bought from Kaifengdongda Chemical Reagent Co., Ltd (Henan, China). Sodium hydroxide was from Fengchuan Chemical Reagent Co., Ltd (Tianjin, China). Acetone was purchased from Tianli Chemical Reagent Co., Ltd (Tianjin, China). The solvents used were analytical grade for synthesis of materials and HPLC grade for high performance liquid chromatographic (HPLC) analysis. Ultrapure water was produced by a Heal Fore NW system (Shanghai, China). Accurel polypropylene sheet membrane (0.2-μm pore size and 200-μm thickness) was bought from Membrana (Wuppertal, Germany).

2.2. HPLC analysis

Determination of the sulfonamides was performed on a Hitachi (Tokyo, Japan) HPLC system, which consisted of a Model L-2130 pump, a Rheodyne 7725i valve (Cotati, CA, USA) and a L-2400 UV-vis spectrophotometric detector. Data were collected and processed by T3000P software (Hangzhou Hui Pu Technology Co., Ltd., Hangzhou, China). Chromatographic separations were achieved on a Belta ODS (3.9 mm × 150 mm, 5 μm) column (Waters, Milford, MA, USA) at a temperature of 22 °C. The mobile phase was methanol-1% acetic acid aqueous solution (14:86, v:v). The flow rate was 1.0 mL min^{-1} and the injection volume was 20 μL. The detection wavelength was set at 260 nm. All the experiments were performed at least in triplicate. The pH values were measured with a Mettler Toledo Delta 320 pH meter (Shanghai, China).

Table 1
Factors and level values in $L_{16}(4^5)$ matrix.

Levels	Factors		
	A EDMA (mmol)	B Acetonitrile/dodecanol (v:v)	C AIBN (mg)
1	9	1/6	18
2	12	1/7	36
3	15	1/8	54
4	18	1/9	72

2.3. Synthesis of p-MAA-EDMA materials

P-MAA-EDMA was synthesized using MAA, EDMA, AIBN and dodecanol as monomer, crosslinker, initiator and porogen, respectively. Optimization of the synthesis was designed by the OAD Assistant software. The experimental data were analyzed using the same software. As listed in Table 1, $L_{16}(4^5)$ matrix was used. Three factors, including the EDMA content, the volume ratio of acetonitrile to dodecanol and the weight of AIBN, and four levels for each factor were studied.

The general synthetic process was as follows. AIBN was dissolved completely in a certain volume of acetonitrile, and dodecanol was added according to the ratios listed in Table 1. Total volume of acetonitrile and dodecanol was fixed at 22.5 mL. The crosslinker EDMA and the monomer MAA (3 mmoL) were added in sequence. The reactant solution was agitated by sonication for 10 min, purged with nitrogen for another 10 min, sealed in a plastic tube and kept in an oven at 60 °C for 24 h. The polymerized products were white and monolithic. The solids were crushed and ground into powder, and washed with methanol in a Soxhlet extractor until no reactants were detected by UV analysis. The final products were dried at 45 °C.

2.4. Characterization of polymers

Hydrophobicity of the polymers was evaluated by toluene adsorption experiment. Each polymer powder (20 mg) was added in 4 mL aqueous solution containing toluene (30 μg mL^{-1}). The solution containing the polymer was sonicated for 20 min and kept still for another 20 h. After that, it was centrifuged at 7000 rpm for 5 min. The UV absorbance at 209 nm of the supernatant solution was measured, which reflected the concentration of residual toluene in the solution. The toluene adsorption capacity of the materials ($Q_{hydrophobicity}$) was calculated by the following Eq. (1):

$$Q_{hydrophobicity} = (C_0 - C') \times \frac{V}{W} \quad (1)$$

where C_0 and C' are the concentrations of toluene in aqueous solution before and after adsorption, and C′ is obtained from the calibration curve; V is the volume of the toluene solution, 4 mL herein; W is the weight of the material used for adsorption experiment, 20 mg herein.

The ion exchange capacity of the polymers was evaluated by acid-base reaction. Each polymer powder (50 mg) was added into a 10 mL sodium hydroxide solution with a pH of 12.0. The system was sonicated for 30 min and kept still for 24 h. pH change of supernatant solution was measured by a pH meter. The cation-exchange capacity (Q_{ie}) was calculated by the following Eq. (2):

$$Q_{ie} = (C_{OH} - C'_{OH}) \times \frac{V}{W} \quad (2)$$

where C_{OH} and C'_{OH} are the concentrations of sodium hydroxide solution before and after adsorption, which are calculated from pH of the corresponding solution; V is the volume of the sodium hydroxide solution, 10 mL herein; W is the weight of the material used for adsorption experiment, 50 mg herein.

SEM images were recorded on an SSX-550 (Shimadzu, Japan) instrument. Nitrogen sorption experiments were carried out on an ASAP 2010 instrument (Micrometrics, USA). The pore parameters, including pore size and pore volume, were evaluated from adsorption branches of the isotherms based on the BJH (Barrett–Joyner–Halenda) method. Surface area values were calculated according to BET (Brunauer–Emmett–Teller) method.

2.5. Sample preparation

Stock solutions of sulfonamides (0.5 mg mL^{-1} for SD and 1.0 mg mL^{-1} for the other analytes) were prepared separately in methanol and stored at 4 °C. Water samples were prepared by spiking ultrapure water with the analytes at a known concentration (0.5 μg mL^{-1}) to study the extraction performance under different conditions.

The milk samples were purchased from a local supermarket, which were checked to be free of target analytes. Thus, spiked milk samples were studied. Milk (0.5 g) was spiked with the stock solutions to a certain concentration and mixed thoroughly. One milliliter of acetonitrile and 8.5 mL of water were added. The pH of the milk dilution was adjusted to 4.0 with 1 mol L^{-1} hydrochloric acid. For the blank milk sample, it was subjected to the same pretreatment procedure but without spiking.

The chicken muscle was obtained from the local store, which was checked to be free of target analytes. Thus, spiked chicken muscle was studied. For spiking, minced chicken muscle was immersed in acetone containing desired concentration of analytes and mixed thoroughly. The mixture was kept in a dark environment until the solvent was evaporated completely. The muscle was stored at 4 °C. For each extraction, spiked chicken muscle (1.0 g) was added into 10 mL mixture of water and acetonitrile (9:1, v:v) with a pH adjusted to 4.0. For the blank chicken muscle sample, it was subjected to the same pretreatment procedure without spiking.To ensure the real samples are free of target analytes, a mixture of ethyl acetate, n-hexane, and isopropanol was used for the extraction of target analytes from real samples [23]. The extraction process was as follows. Ten milliliter of a mixture consisting of 9 ml n-hexane/ethyl acetate (6.25:3.75, v:v) and 1 mL isopropanol were added into the real samples (milk or minced chicken muscle) and sonicated for 20 min. The mixtures were then centrifuged at 9000 rpm for 10 min, and the supernatant was separated and evaporated to dryness under a nitrogen stream in a water bath. The dry residue was dissolved in 100 μL of methanol–1% acetic acid aqueous solution (2:3, v:v). Twenty microlitre of the reconstituted solution was injected into the HPLC system for analysis. No analytical signal was observed. The results demonstrated that the samples are free of target sulfonamides.

All the samples were freshly prepared daily. The MP-μ-SPE of two genuine samples was carried out under optimized extraction conditions.

2.6. Procedure of MP-μ-SPE using p-MAA-EDMA

The MP-μ-SPE device consisted of a polypropylene membrane envelope (1 cm length × 1 cm width), which was enclosed with 20 mg of p-MAA-EDMA powder. The open ends were heat-sealed as previously reported [10]. The extraction device is shown in Fig. 1.

The extraction was performed with six steps: (1) *Conditioning*. The device containing the polymeric materials was sonicated in acetonitrile and the corresponding extraction solution without the analytes for 10 min in order, and then dried with lint-free tissue. (2) *Extraction*. The conditioned device was immersed into 10 mL sample solution for a prescribed time with sonication. (3) *Washing*. After extraction, the device was taken out, washed with ultrapure water and dried with lint-free tissue. (4) *Desorption*. The device was put into 400 μL desorption solvent and sonicated for a prescribed time for desorbing target analytes. (5) *Concentration*. The device was removed from the desorption solvent, which was further dried with nitrogen. The residue was dissolved in 100 μL of methanol–1% acetic acid aqueous solution (2:3, v:v). (6) Twenty microlitre of the reconstituted solution was injected into the HPLC system for analysis. For each extraction process, the above six steps were repeated.

Fig. 1. The MP-μ-SPE device.

2.7. Method evaluation

Intra-day repeatability was studied for four replicate experiments and the inter-day repeatability was investigated for consecutive three days at optimized extraction conditions for acetonitrile-aqueous samples (1:9, v:v) containing 1.0 μg mL^{-1}, 0.1 μg mL^{-1} and 0.015 μg mL^{-1} of the four sulfonamides, respectively. The linearity was investigated over a concentration range of 0.010–10.0 μg mL^{-1} and was calculated by plotting corresponding HPLC peak areas (y) versus concentrations of the studied analytes (x, μg mL^{-1}). Limits of detection (LODs) were calculated at a signal-to-noise (S/N) of 3.

The relative recoveries were obtained by comparing the peak areas of the analytes extracted from spiked genuine samples to those from ultrapure water.

3. Results and discussion

3.1. Synthesis and characterization of p-MAA-EDMA

The p-MAA-EDMA materials were synthesized with the OAD optimization. The three factors and four level values are listed in Table 1. The sixteen synthetic trials were accomplished based on the $L_{16}(4^5)$ matrix. As shown in Table 2, the average values (r_1, r_2, r_3, r_4) of three factors at their levels were evaluated to reveal the influential factors of the yield, hydrophobicity and cation-exchange

Table 2
The analytical responses of experimental trials based on the $L_{16}(4^5)$ matrix.

No.	Factors					Assessment parameters		
	A	B	C	Dummy	Dummy	Weight (g)	$Q_{hydrophobicity}$ (μg/mg)	Q_{ie} (mmol/g)
1	1	1	1	1	1	0.785	4.116	1.580
2	1	2	2	2	2	1.661	4.926	1.520
3	1	3	3	3	3	1.947	5.185	1.450
4	1	4	4	4	4	1.992	4.905	1.410
5	2	1	2	3	4	2.115	5.041	1.186
6	2	2	1	4	3	0.7869	3.152	0.9980
7	2	3	4	1	2	2.172	4.929	1.086
8	2	4	3	2	1	2.611	5.652	1.086
9	3	1	3	4	2	2.531	5.695	1.064
10	3	2	4	3	1	3.080	5.434	0.9500
11	3	3	1	2	4	2.254	4.708	0.6780
12	3	4	2	1	3	3.140	5.245	0.9000
13	4	1	4	2	3	3.496	5.166	0.9000
14	4	2	3	1	4	3.695	5.625	0.8760
15	4	3	2	4	1	3.577	4.258	0.7660
16	4	4	1	3	2	0.4927	1.499	0.04560
r_1	1.596	2.232	1.080	2.448	2.513			
r_2	1.921	2.306	2.623	2.506	1.714			
r_3	2.752	2.487	2.696	1.909	2.343			
r_4	2.815	2.059	2.685	2.222	2.514			
r_{1a}	4.783	5.004	3.369	4.979	4.865			
r_{2a}	4.693	4.784	4.868	5.113	4.262			
r_{3a}	5.270	4.770	5.539	4.290	4.687			
r_{4a}	4.137	4.325	5.109	4.502	5.070			
r_{1b}	14.90	11.83	9.280	11.11	10.96			
r_{2b}	10.89	10.86	10.93	10.46	10.32			
r_{3b}	8.980	9.950	11.19	10.105	10.62			
r_{4b}	7.495	9.630	10.87	10.595	10.38			

Weight, the weight of the product; $r_1 \sim r_4$, the mean value of weight; $r_{1a} \sim r_{4a}$, the mean value of toluence adsorption capacity; $r_{1b} \sim r_{4b}$, the mean value of cation exchange capacity.

capacity of the polymer. The fourth and fifth columns in Table 2 represent dummy factors, which are called arbitrary levels of the dummy variance. With the direct observation analysis from the results, the optimal synthetic condition combinations for the highest yield, the strongest hydrophobicity and ion exchange capacity of the polymer were A_4–B_3–C_3, A_3–B_1–C_3 and A_1–B_1–C_3, respectively.

Furthermore, statistical analysis of variance (ANOVA) was utilized to assess the OAD results. The results of *F*-value for different variables were calculated according to the OAD Assistant software. Critical values were 29.500 ($p < 0.01$), 9.280 ($p < 0.05$) and 5.390 ($p < 0.1$). For weight of the final product, *F* values for factors A, B and C were 11.690, 1.000 and 20.058, respectively, which means factors A and C were statistically significant to the polymer yield at $p < 0.05$. For $Q_{hydrophobicity}$, *F* values for factors A, B and C were 2.661, 1.000 and 10.97, respectively, in which factor C was found to be significant to the hydrophobicity of the polymer at $p < 0.05$. For Q_{ie}, F values for factors A, B and C were 13.61, 1.292 and 1.000, respectively, which indicates that factor A was significant to the cation-exchange capacity of the polymer at $p < 0.05$.

Based on the above analysis, three synthetic combinations seemed optimal, A_4–B_3–C_3 (scheme A), A_3–B_1–C_3 (scheme B) and A_1–B_1–C_3 (scheme C). To obtain the best synthetic condition for the sorbent, these three schemes were tested experimentally. The products prepared from scheme A, B and C were named as Poly A, Poly B and Poly C, respectively. Fig. 2 shows the SEM images of the three polymeric materials and it is hard to tell the difference among them. Specific surface area (SA), average pore diameter (PD) and specific pore volume (PV) values of these materials are listed in Table 3. Poly B and C appeared to possess the comparative SA, PD and PV, while Poly A had obviously low values of these parameters. Larger SA, PD and PV parameters may be beneficial in applications. Considering it, Poly B and C was superior to Poly A. However, the yield of Poly C was almost one time lower than Poly A and B. The hydrophobicity of these three polymers was almost the same based on the toluene adsorption experiment, as listed in Table 3; anyway, they were stronger than those obtained in Table 2. The cation-exchange capacities increased from Poly A to Poly B and then to Poly C.

To further evaluate the extraction ability of these polymer materials, four sulfonamides were tested as target analytes. The extractions were performed with sample solutions at different pH values. Fig. 3(A) compared the analytical signals of individual analyte obtained on these polymer sorbents. To observe more directly, the total amount of sulfonamides extracted by the materials were depicted in Fig. 3(B). As seen from Fig. 3, Poly B resulted in highest HPLC responses for the four tested sulfonamides when extracting the sample solution at a pH of 4.0. As well, the extraction efficiency varied when pHs of samples changed. These observations may be due to the fact that Poly B had strong hydrophobicity, which played an important role in the extraction of sulfonamides; meanwhile, the material possessed cation-exchange ability, which may be an explanation for the change of extraction efficiency at different sample pHs. In addition, Poly B possessed favorable porous characteristics, which may be beneficial to extraction.

Based on the above evaluations, Poly B was selected for further studies in terms of yield, suitable hydrophobicity and cation exchange capacities, as well as favorable SA, PD and PV.

3.2. Optimization of MP-μ-SPE

The extraction depends on equilibrium between the extraction solution and the sorbent. Several parameters that influence the extraction were investigated, including the type and amount of organic solvent additive in the sample solution, the pH of sample solution, the category and pH of desorption solvent, desorption time, extraction time and the amount of salt in the sample solution.

Table 3
The porous parameters and BET surface areas of three kinds of poly (MAA-EDMA).

Polymers	SA (m^2/g)	PV (cm^3/g)	PD (nm)	Weight (g)	$Q_{hydrophobicity}$ (μg/mg)	Q_{ie} (mmol/g)
Poly A	85.9	0.35	4.6	3.721	10.26	0.584
Poly B	120.1	0.45	5.3	3.311	10.12	0.900
Poly C	160.3	0.59	4.8	1.943	9.858	1.616

SA, surface area; PV, pore volume; PD, pore diameter.

3.2.1. Preliminary extraction experiments

Both the polypropylene membrane envelope and p-MAA-EDMA materials are hydrophobic. As the analytes should penetrate the membrane to interact with the polymeric sorbent, wetting of the membrane with suitable organic solvent was necessary. As well, addition of organic solvent to the sample solution may reduce the interaction of analytes with the glass bottle, which may be conducive to enhance the extraction efficiency and reduce the possible errors. Three types of organic solvents, acetone, acetonitrile and methanol, were investigated, 10% of which was present in the sample solution respectively. Based on the experimental results, acetonitrile provided higher analytical signal than the other two (data not provided). Thus, acetonitrile was chosen as the solvent additive to the sample solution.

As mentioned above, ion-exchange mechanism was involved in the extraction. Thus, the pH of sample solution would play an important role in affecting the equilibrium between the analytes and sorbent. As shown in Fig. 3, regarding Poly B, the peak areas increased as the pH changed from 3.0 to 4.0, and then decreased sharply with the increasing pH. The reason could be that the analytes were protonated at pHs of between 3.0 and 4.0, hydrophobic interaction and ion exchange between the analytes and sorbent co-contributed to adsorption of target analytes. However, when the pH was further increased, the acid-base equilibrium could be interrupted, resulting in decreased peak areas. As the pH was approaching to 8.0, the peak areas increased again. The reason may be that the pK_a values of target analytes were ~6–7 [24,25]. At pH > 8.0, the analytes existed in neutral status. As a result, their cation exchange towards the sorbent decreased, and the hydrophobic interaction between the materials and analytes may be predominant. Thus, increased peak areas were observed. As the pH was further increased, the analytes existed mostly in neutral form, which is not beneficial for adsorption [18,26] and resulted in decreased analytical signal. Since the analytical signal was highest at a pH of 4.0, this pH was selected as the suitable extraction pH for further experiments.

3.2.2. Optimization of desorption conditions

To choose a suitable desorption solvent, three commonly used organic solvents, acetone, acetonitrile and methanol, were investigated. When these organic solvents containing 1% HAc were used as desorption solvents, the highest HPLC signals were found in the case of acetone (results not shown). Therefore, acetone was chosen as the desorption solvent for subsequent analysis.

Since cation-exchange mechanism contributed to the extraction, the pH of the desorption solvents should be highly related to the solvents desorption ability. In this study, both acidified and basified acetone were investigated. The peak areas obtained by elution with acidified and basified acetone were higher than those with pure acetone (results not shown). Acidic conditions were appropriate to deionize the sorbent, and basic conditions were suitable to deprotonate the analytes, both of which would decrease the cation exchange between the sorbent and analytes. It is found that basic desorption conditions (acetone containing 1% and 3% ammonia) brought slightly stronger analytical signals. Since the presence of 3% ammonia in acetone may cause the analytes to be unstable [27], acetone containing 1% ammonia was selected as the optimum desorption solvent.

Ultrasonication was used to desorb the analytes from the sorbents. The influence of desorption time on desorption was investigated in a range of 10–60 min. As depicted in Fig. 4, the analytical signal increased with the varying desorption time from 10 min to 30 min. However, the peak areas decreased significantly from 30 min to 60 min. The explanation of this observation may be that, as the desorption time increased, sonication may change the temperature of the water bath and thus the temperature of the sample solution. A new equilibrium between the analytes and the sorbent might be formed. Hence, 30 min was chosen as the optimal desorption time.

3.2.3. Effect of acetonitrile content in sample solution

The effect of acetonitrile content in the sample solution (5–25%, v/v) on the extraction was studied. The results are shown in Fig. 5. The maximum peak areas occurred when 10% acetonitrile was present in the sample solution. When the acetonitrile content in sample solution was low (e.g. 5%), hydrophobic envelope membrane cannot be easily penetrated by sample solutions and prevent the analytes from being in contact with the sorbent. As a result, the extraction efficiency was not high. On the other hand, when acetonitrile content increased significantly (e.g. 20% or more), analytes may tend to stay in the sample solution rather than to be adsorbed onto the sorbent, due to their relatively hydrophobic nature. Therefore, 10% acetonitrile in the sample solution was a compromise and chosen for subsequent experiments.

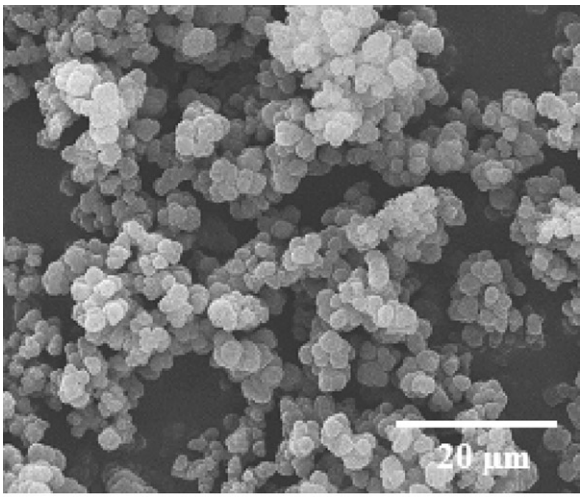
3.2.4. Effect of extraction time

The extraction process associated with MP-μ-SPE mainly depended on partitioning of analytes between aqueous solution and sorbent. The effect of extraction time was investigated in a range of 10–50 min. As shown in Fig. 6, the extraction efficiency increased obviously from 10 min to 30 min, and then slightly decreased. The reason could be that the extraction was a dynamic process, and the adsorption of analytes might be destroyed slightly when the extraction time was increased [29]. Therefore, 30 min was an acceptable extraction time for subsequent experiments.

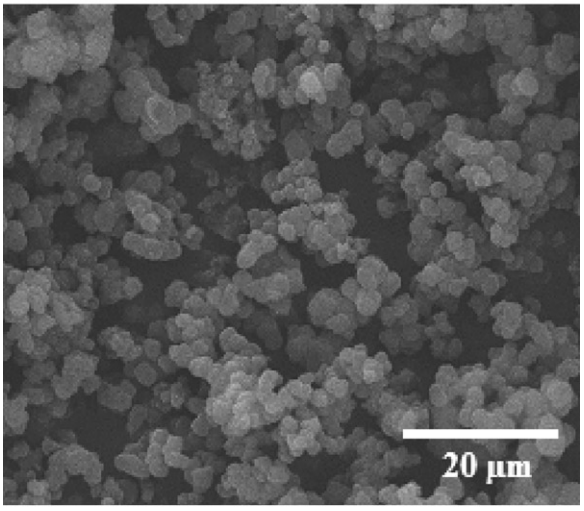
3.2.5. Effect of sodium chloride content in sample solution

The content of salt has played an important role in partitioning of analytes between sample solution and sorbent. Both the salting-out and salting-in effect have been observed in MP-μ-SPE [10,13,14,28]. In this study, the salt effect was investigated. As shown in Fig. 7, with 10 mg mL^{-1} to 160 mg mL^{-1} of sodium chloride in sample solution, the extraction efficiency was decreased. The presence of salt may increase the viscosity of solutions and reduce the adsorption ability of sorbent [14]. Considering the cation-exchange mechanism was involved in the extraction, the presence of salt may restrain the interaction between analytes and sorbent. Therefore, no salt addition was selected.

Based on the above optimization, the optimal extraction and desorption conditions were as follows. The sample will be extracted



Poly A



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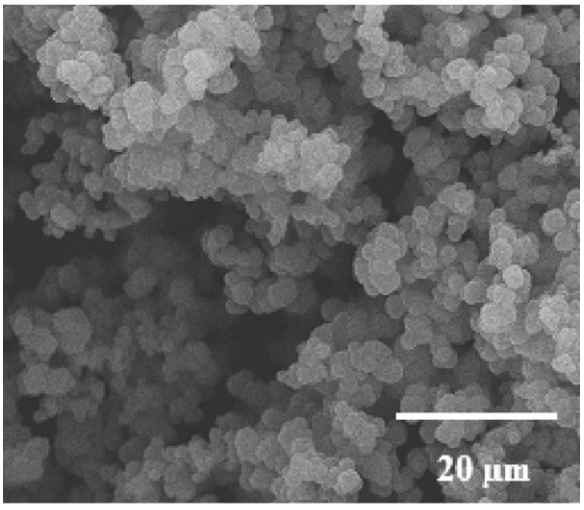
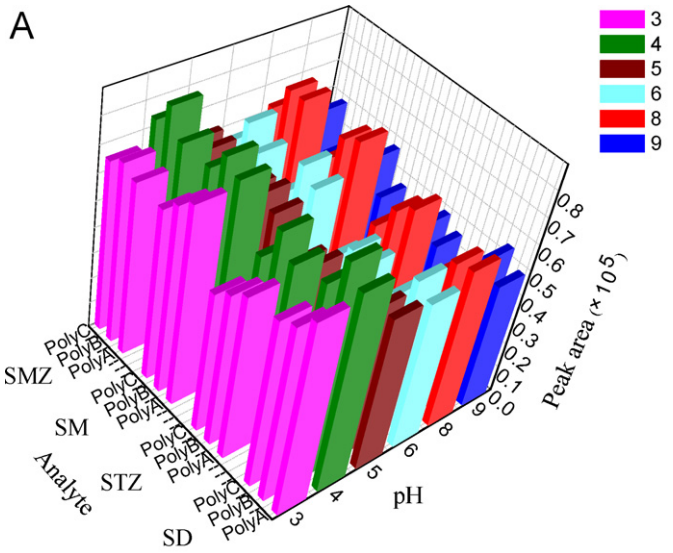


Poly C

Fig. 2. The SEM images of Poly A, Poly B and Poly C.



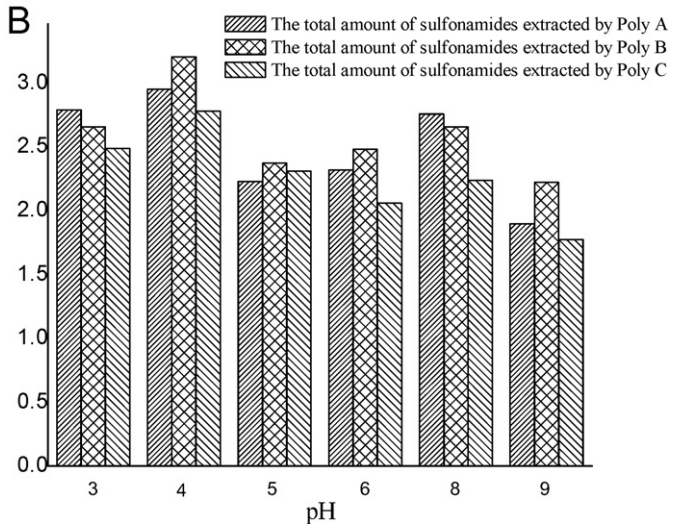


Fig. 3. (A) Comparison of the extraction efficiency of Poly A, Poly B and Poly C for the sulfonamides. Extraction conditions: 20 mg material, 10% acetonitrile in the sample solution at different pHs, extraction for 30 min with sonication, desorption for 30 min with sonication in 400 μL acetone containing 1% HAc. (B). The total amount of sulfonamides (in terms of peak areas) extracted by Poly A, B and C at different pHs.

in a solution of water and acetonitrile (9:1, v:v) at a pH of 4.0 for 30 min by sonication. The analytes will be desorbed in acetone containing 1% ammonia for another 30 min with sonication.

3.3. Method validation

All the validation data are presented in Table 4. The linearity was investigated over a concentration range of 0.010–1.0 μg mL^{-1}. Good linearities of the four analytes were obtained with $r^2 > 0.9900$. The repeatability studies were performed at the optimal extraction condition for samples containing analytes of three concentration levels respectively; each level was investigated at least in triplicate. Intra-day and inter-day relative standard deviations (RSDs) were in the range of 2.7–13.7% and 6.7–15.2%, respectively. Based on S/N of 3, the LODs were as low as 0.58, 0.54, 0.62 and 0.38 ng mL^{-1} for SD, STZ, SM and SMZ, respectively.

Table 4
Regression data and LODs of the sulfonamides in aqueous solutions.

Analytes	Linearity range ($\mu g\,mL^{-1}$)	Calibration equation	r^2	RSD (%, intra-day, $n=4$)			RSD (%, inter-day, $n=3$)			LOD ($ng\,mL^{-1}$)
				1.0 $\mu g\,mL^{-1}$	0.1 $\mu g\,mL^{-1}$	0.015 $\mu g\,mL^{-1}$	1.0 $\mu g\,mL^{-1}$	0.1 $\mu g\,mL^{-1}$	0.015 $\mu g\,mL^{-1}$	
SD	0.010–1.0	$Y=133406x+903.66$	0.9930	3.1	3.5	13.7	6.7	7.4	10.6	0.58
STZ	0.010–1.0	$Y=123955x+800.51$	0.9909	5.4	2.8	12.4	6.7	7.0	11.8	0.54
SM	0.010–1.0	$Y=134420x+868.16$	0.9967	6.6	2.7	11.9	8.0	8.8	15.2	0.62
SMZ	0.010–1.0	$Y=126927x+2245.1$	0.9929	5.4	6.4	10.1	7.1	9.3	13.4	0.38

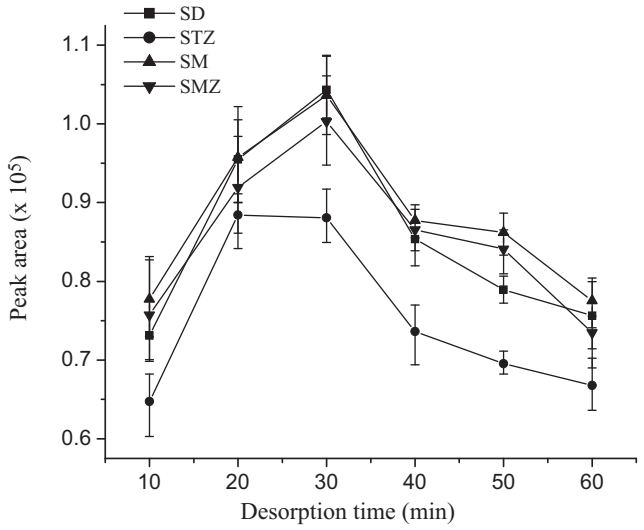


Fig. 4. The influence of desorption time. Extraction conditions: 20 mg Poly B, 10% acetonitrile additive in sample solution (pH = 4.0), desorption for different time in acetone containing 1% ammonia.

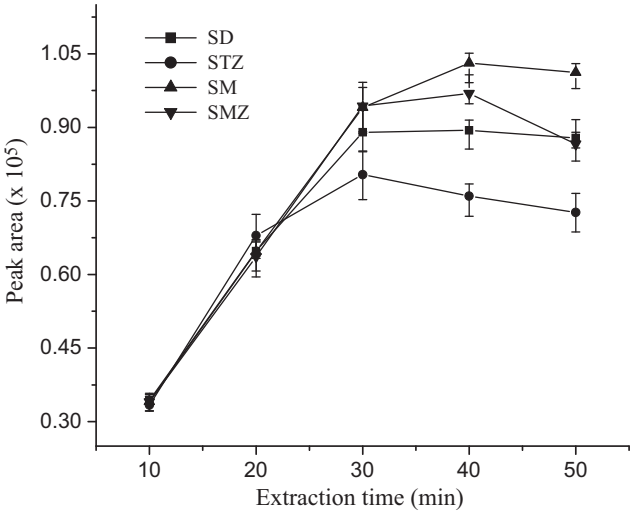


Fig. 6. The influence of extraction time on extraction efficiency. Extraction conditions: 20 mg Poly B, 10% acetonitrile additive in sample solution (pH = 4.0), extraction for different time with sonication, desorption for 30 min in acetone containing 1% ammonia.

4. Applications to the genuine food samples

There have been extensive studies about the determination of sulfonamides from diverse matrices. LLE is popular for solid/liquid sample matrices [26,29]; SPE is predominantly used for liquid samples, or combined with LLE for solid samples [30–32]; SPME is used for liquid and solid samples [27,33]. In this study, MP-μ-SPE was developed and the advantages were sufficiently shown for semi-solid/solid food samples. It prevents large particles from entering the sorbent phase by the porous membrane. This means that it may reduce excessive sample matrix effect and no extra pretreatment steps were required.

In this study, the applicability of this extraction method to genuine samples including milk and chicken muscle was investigated at the optimum extraction conditions. No target analyte was

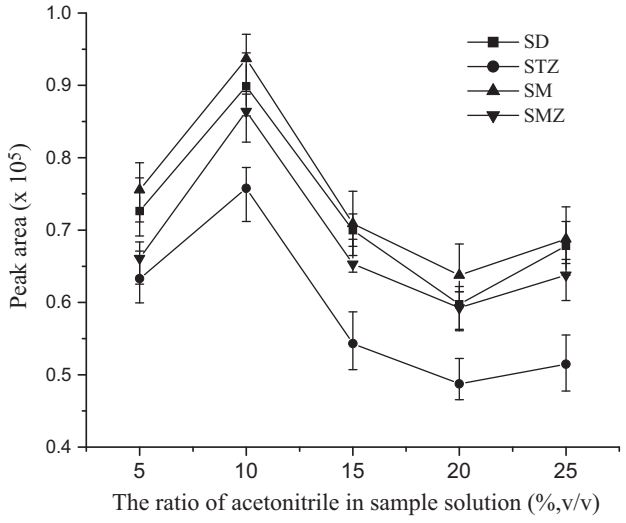


Fig. 5. The influence of acetonitrile content in sample solutions on extraction efficiency. Extraction conditions: 20 mg Poly B, different ratios of acetonitrile in the sample solution (pH 4.0), desorption for 30 min in acetone containing 1% ammonia.

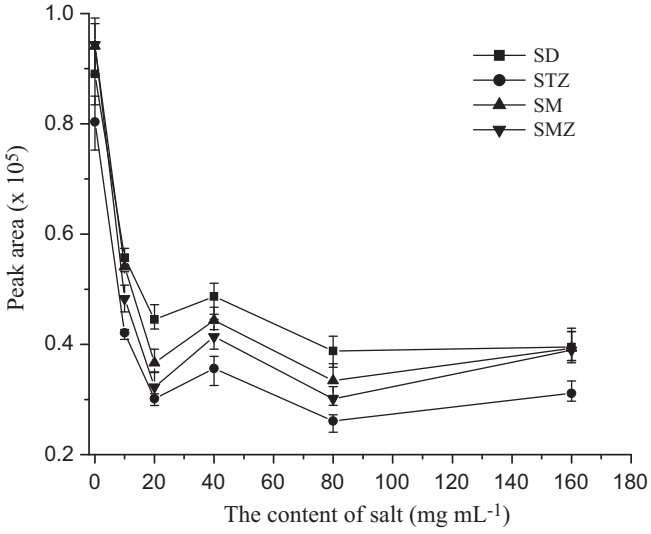


Fig. 7. The influence of salt concentrations in sample solutions on extraction efficiency. Extraction conditions: 20 mg Poly B, 10% of acetonitrile additive in sample solution (pH = 4.0) with the addition of different salt content, desorption for 30 min in acetone containing 1% ammonia.

Table 5
Regression data and LODs of the sulfonamides in genuine samples.

Analytes	Linearity range ($\mu g\,g^{-1}$)	Calibration equation	r^2	RSD %, intra-day, $n=4$			RSD %, inter-day, $n=3$			LOD ($ng\,g^{-1}$)	Relative recovery %
Spiked milk				20 $\mu g\,g^{-1}$	2 $\mu g\,g^{-1}$	0.3 $\mu g\,g^{-1}$	20 $\mu g\,g^{-1}$	2 $\mu g\,g^{-1}$	0.3 $\mu g\,g^{-1}$		
SD	0.2–20	$Y=99352x+8885$	0.9970	4.7	3.1	11.6	7.0	5.4	11.8	4.52	83.81
STZ	0.2–20	$Y=80926x+4476.2$	0.9959	5.1	7.7	12.3	8.8	7.1	12.9	9.92	71.82
SM	0.2–20	$Y=100340x+6591.6$	0.9944	6.4	6.0	7.4	6.6	9.9	10.2	6.64	81.40
SMZ	0.2–20	$Y=95870x+7399.3$	0.9969	8.8	6.0	13.1	9.2	9.4	13.9	10.63	83.29
Analytes	Linearity range ($\mu g\,g^{-1}$)	Calibration equation	r^2	RSD %, intra-day, $n=4$			RSD %, inter-day, $n=3$			LOD ($ng\,g^{-1}$)	Relative recovery %
Spike chicken muscle				10 $\mu g\,g^{-1}$	1 $\mu g\,g^{-1}$	0.15 $\mu g\,g^{-1}$	10 $\mu g\,g^{-1}$	1 $\mu g\,g^{-1}$	0.15 $\mu g\,g^{-1}$		
SD	0.1–10	$Y=55497x+1002.9$	0.9990	7.9	9.3	12.9	5.7	7.0	14.5	15.63	43.11
STZ	0.1–10	$Y=52919x+6757.8$	0.9931	9.6	8.8	11.7	8.5	7.1	16.0	10.23	48.31
SM	0.1–10	$Y=70290x+5193.3$	0.9956	9.2	8.6	14.4	8.7	8.4	16.8	7.72	55.63
SMZ	0.1–10	$Y=72575x+640.19$	0.9980	8.9	7.1	10.5	7.1	9.8	14.5	11.89	57.79

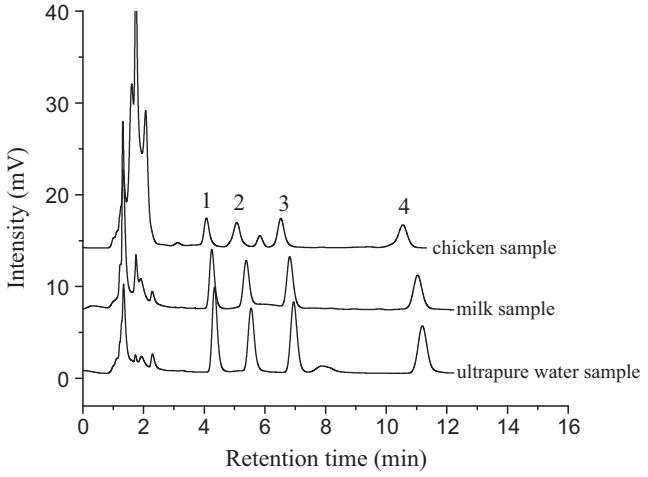


Fig. 8. Comparison of HPLC chromatograms among the ultrapure water solution (1 $\mu g\,mL^{-1}$), milk sample (20 $\mu g\,g^{-1}$) and chicken sample (10 $\mu g\,g^{-1}$) after extraction under optimum conditions.

detected in the samples. Hence, the spiked genuine samples were studied, as described in Section 2.5. As far as chicken muscle is concerned, the mixture of water and acetonitrile (9:1, v:v) was added as the transferring phase. The target analytes were supposed to transfer from the muscle to the transferring phase and then to the MP-μ-SPE sorbent with the aid of sonication.

As shown in Table 5, it can be found that all tested analytes exhibited good linearities with $r^2 > 0.9930$ in the range of 0.010–1.0 $\mu g\,mL^{-1}$. RSDs < 10% were obtained at high and medium concentrations. Slightly higher RSDs were observed at low concentrations but were still within acceptable range. The relative recoveries of analytes were studied. The results may indicate that matrices had slight effect on the extraction. The relative recoveries of analytes for milk sample were higher than the corresponding ones for chicken muscle sample. To reduce the systematic error, quantification could be made using matrix-matched standards. Anyway, the overall analysis was not affected. Good LODs, acceptable reproducibilities and clean chromatograms (as shown in Fig. 8) were obtained in the analysis of the complex spiked matrices. It could be complementary to existing methods for the determination of sulfonamides.

5. Conclusions

In this study, a three-factor four-level orthogonal array experimental design ($L_{16}(4^5)$) was adopted to optimize the synthesis of p-MAA-EDMA materials which exhibited suitable hydrophobic and cation-exchange properties with an acceptable yield. The material obtained under the optimal synthetic conditions was used as the sorbent for membrane protected-micro-solid phase extraction (MP-μ-SPE). Four sulfonamides were used as target analytes to evaluate the effectiveness of the MP-μ-SPE. The extraction and desorption conditions were systematically optimized. Good linearity, reasonable intra-day and inter-day RSDs, and acceptable LODs were obtained. The established method was successfully applied to genuine food samples of milk and chicken muscle. Since the sorbent was protected by porous polypropylene membrane, MP-μ-SPE reduced the interference of the "dirty" samples and could be directly used for semi-solid/solid samples. Meanwhile, it was a simple, convenient, and inexpensive extraction method. It combines cleanup and extraction in a single step, which is a promising alternative approach for food analysis.

Acknowledgments

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