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ARTICLE

Preparation of a restricted access material – macroporous hybrid monolithic column for on-line solid-phase extraction of the sulfonamides residues from honey

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A novel restricted access material - hybrid monolithic column (RAM-HMC) was prepared by the sol-gel method in a stainless-steel column and in situ modification method in through-hole of the HMC. The hydrophobic HMC was prepared by acid-catalytic reaction of methyltrimethoxysilane and tetraethoxysilane. The hydrophilic structures were formed on the through-hole surface of the HMC by grafting 3-(2, 3-epoxypropoxy) propytrimethoxysilane. The synthetic conditions were optimized for obtaining the uniform microchannel and the stable skeleton structure. The chemical group, morphology, dynamic adsorption capacity, swelling, hydrophilic and hydrophobic characteristic of the monolithic column were characterized by IR, SEM, dynamic binding, solvent adsorption, chromatographic analysis of serum proteins and benzene series, respectively. When the RAM-HMC was used as precolumn for on-line extraction of sulfonamide residues from the honey samples, an enrichment factor of 16.9 and a better sample clean-up effect were obtained under the optimized conditions. The average recoveries of three sulfonamides antibiotics spiked honey at 0.05, 0.1 and 0.2 mg kg^{-1} were in the range of 73.50-105.80% with the precision of 2.25-5.32%. The limits of detection and quantitation of the proposed method were in a range of 6.9-14.6 μg kg^{-1} and 23.0-48.2 μg kg^{-1}, respectively. The proposed method was successfully applied to on-line extraction and determination of sulfonamides antibiotics in the honey sample.

Introduction

Sulfonamides (SAs) are a group of antibiotic drugs widely used in veterinary for treating diseases and promoting growth of livestock. These cases raise the possibility that antibiotic residues may remain in edible animal tissues and milk intended for human consumption, and in some cases, these can cause a serious threat to human health, such as allergies, toxic effects and bacterial resistances. [1, 2] In order to ensure the safety and quality of foodstuffs, FAO/WHO, European Union (EU) and Chinese Ministry of Agriculture have established a maximum sulfonamide residue limit (MRL) of 0.1 mg kg^{-1} in foodstuffs of animal origin, including milk. [3-5] These limits require the development of sensitive and selective methods for antibiotic residues in food. A number of detection methods, such as HPLC, [6, 7] LC–MS and LC-MS/MS, [8-10] have been developed for the analysis of sulfonamides residues in food. However, in order to remove the matrix components, multiple sample clean-up processes are generally used which have lower efficiency and result in lower recovery. Thus finding an efficient sample purification method is necessary for the determination of sulfonamides residue in food.

In order to approach this matter, a restricted access material (RAM) - hybrid monolithic column (HMC) was prepared by in situ synthesis and modification method in a stainless-steel column for on-line sample clean-up and preconcentration of the sulfonamides residues from honey. RAM was introduced in 1991 by Desilets et al.[11] RAM sorbents represent a special class of materials that are able to fractionate a biological sample into a protein matrix and analyte fraction. The inner layer is accessible only to small molecules and has the ability for their retention and separation. The outer surface employs both size exclusion and hydrophilic interactions to prevent large biomolecules from accessing the inner surface. The application of RAM to on-line extraction of drugs from biological fluids has become well established.[12-15]

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RAM was used as pre-column packing material for on-line extraction, which was easy to operate and demonstrated to be efficient for both extraction and separation of drugs and biomacromolecules.[14-15] However, packing a high-efficiency column needs skill as well as packing materials with suitable properties. Columns having one-piece network structures are thought to be desirable, because it is sometimes hard to pack a high-efficiency column with particles having new surface modifications. As an alternative, with the advantages of facile preparation, fast mass transfer and low backpressure, monolithic columns have been numerous reports on polymer- and silica-based monolithic columns for HPLC.[16-18] Recently, the hybrid organic–inorganic monolithic columns have obtained great development, which possess the merits of organic polymer- and silica-based monolith, and do not require aging and drying steps at high temperature, avoiding the cracking and shrinking of the silica skeleton.[19-23] For the outstanding advantage at easy modification, it is possible that the RAM-HMC was prepared and applied to on-line extraction of drugs from food samples.

In this work, an internal surface reversed-phase monolithic column (also called the RAM-HMC) was prepared by in situ modification of hybrid monolithic columns (HMC) in stainless steel column. The HMC was synthesized via the co-condensation of Methyltrimethoxysilane (MTMS) and tetraethoxysilane (TEOS). The hydrophilic layer was formed on the microchannel surface of HMC through the grafting reaction between the surface Si-OH and 3 - (2, 3-epoxypropoxy) propytrimethoxysilane (EPTS), and then the ring opening reaction. The synthetic conditions were optimized for obtaining the uniform microchannel and the stable skeleton structure. The chemical group, morphology, thermal stability, dynamic adsorption characteristic and swelling of the monolithic column were characterized by IR, SEM, dynamic binding test and swelling test, respectively. To the best of our knowledge, it is the first report on the preparation and on-line application of the RAM-HMC with the hydrophobic inner surface and the hydrophilic outside surface in through-hole. The RAM-HMC was used as pre-column coupling with HPLC for the determination of sulfonamides antibiotics in honey.

Experimental

Chemicals and materials

Sulfadiazine (SD), sulfamethazine (SM2), sulfamethoxazole (SMO) and were purchased from Fluka (Buchs, Switzerland) and their structures are shown in Fig. 1. The stock solutions of all standards were prepared by dissolving each compound in methanol at a concentration of 0.5 g L^{-1} and were stored in the dark at 4 ^{o}C. The working standard solution was diluted to the desire concentration for experiment. Bovine serum albumin (BSA) and lysozyme (LZM) were obtained from Sigma Chemical Co. (St Louis, MO, USA). Methyltrimethoxysilane (MTMS), 3 - (2, 3-epoxypropoxy) propytrimethoxysilane (EPTS) and tetraethoxysilane (TEOS) were obtained from Nanjing Lianye Chemical Co. Ltd. (Shanghai, China). All other chemicals were of analytical or HPLC grade and were used without further treatment. Double deionized water (DDW) was used all the experiment. All sample solutions for HPLC were filtered through a 0.22 μm filter to remove large polymers and particles.

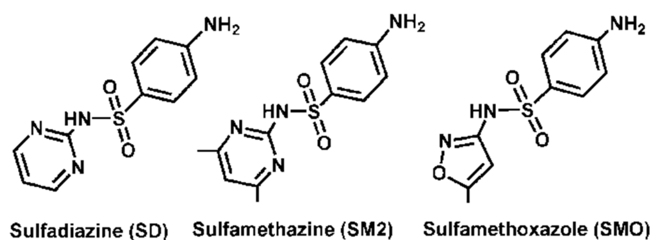

Fig. 1 Chemical structures of sulfadiazine, sulfamethazine and sulfamethoxazole.

Instruments and analytical conditions

A Shimadzu HPLC system (Kyoto, Japan) consisting of pump (LC-20AT), UV detector (SPD-20A), and LC solution chromatographic workstation was used for all analyses. The chromatographic separation was carried out on a Venusil XBP C18 column (5 μm, 250 mm × 4.6 mm, Agela, China) with the isocratic mobile phase (acetonitrile (ACN) -0.1 M K_2HPO_4 solution (15:85, v/v)) at a flow rate of 0.8 mL min^{-1}. An aliquot of 10 μL sample was injected for HPLC analysis, and the signals were detected at a wavelength of 265 nm. The scanning electron micrographic (SEM) micrographs of the monolithic were obtained by a KYKY-2800B scanning electron microscope (Beijing, China) at 25 kV.

Preparation of RAM-HMC

Preparation of the HMC. The macroporous structure of monolithic silica-based capillary columns were prepared as described by Laschober at el.[19] Based on this preparation concept, a macroporous hybrid monolithic column was directly prepared in a stainless steel chromatographic column (50 mm × 4.6 mm). 2 mL different ratio of MTMS and TEOS, 0.75 mL of MeOH and 0.55 mL of 1.0 mol L^{-1} nitric acid were mixed in Erlenmeyer flask. The mixture was sonicated for approximate 2 min and then poured into a stainless steel column with sealed at the bottom and top. Then, the stainless steel column was vertically placed in water bath at 40 ^{o}C for 12 h. The monolithic column was eluted with MeOH, then suffused with DMF and dried under vacuum at 40 ^{o}C for chemical modification in the next-step.

In situ modification of the HMC with EPTS. To modify the epoxy group, the hybrid monolithic column was first flushed by MeOH for 2 h, 0.1 M HCl solution for 2 h, water until the pH value of outlet solution to 7.0, MeOH for 30 min and nitrogen gas for removing residue solvents, respectively. 100 mL of EPTS in anhydrous MeOH (3:2, v/v) was continuously pumped through the monolithic column for 12 h with the LC pump at a constant flow rate of 0.05 mL min^{-1} and simultaneously kept at 40 ^{o}C water bath. The resulting modified

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HMC was sequentially rinsed with MeOH and DMF extensively to remove the residues, respectively, and dried under vacuum at 40 °C for overnight.

For the synthesis of alkyl diol hybrid monolithic column, the ring opening reaction of epoxy groups were carried out. 100 mL of perchloric acid solution (10%, v/v) was continuously pumped through the epoxy-modified hybrid monolithic column for 12 h with the LC pump at room temperature. The monolithic column was sequentially rinsed with water and MeOH for 2 h, respectively, and the obtained RAM-HMC was stored in MeOH for the further research and application.

Determination of the content of hydroxyl group on the monolithic column

The average loading of hydroxyl groups on the monolithic columns were determined by titration of the excess of acetic acid after acetylation of the monolithic column with acetic anhydride. [20] Each of the monolithic material was weighed 0.15 g and placed in a tube, along with 1.0mL of acetic anhydride and 5.0 mL of pyridina. Then the tube was put into a thermostat-controlled water-bath at 60 °C for 12 h. After that, 10 mL of water was added into the tube to make the excess acetic anhydride change to acetic acid. The solution was then titrated with 1.0 mol L^{-1} NaOH solution at the temperature of 25 °C. Phenolphthalein was used as the indicator. Blank assay was operated at the same way. The loading of hydroxyl groups on the hybrid monolithic column was taken as the average of three parallel experiments. The content of hydroxyl group on the monolithic column (X) was calculated by using the following equation (1):

$$X\% = [(V_0\text{-}V_1)\ CM\ /\ 1000W]\ 100\% \qquad (1)$$

Where V_0 is the volume of NaOH that was consumed in the blank assay (mL); V_1 is the volume of NaOH that was consumed in the titration of nominal sample (mL); C is concentration of standard solution (mol L^{-1}); M is the molar mass of OH; W is the quality of sample (g).

Solvent absorption test [23]

Solvent absorption experiments were performed in distilled water (DW), methanol (MeOH), acetonitrile (ACN) and toluene. Dry particles of monolithic materials were carefully weighed and mixed with 1 mL of each solvent before sealed with parafilm and shaken vigorously for 2 min. After equilibrated for 6 h in room tempeture, the particles were collected, wiped gently with filter paper to remove excess liquid on the surface, and immediately weighed (Ws). Then, the monolithic materials were dried to a constant weight (W_0) in a vacuum oven. The solvent uptake ratio (Sr) of monolithic materials was calculated from the following equation (2):

$$Sr\ (\%) = [(Ws\text{- } W_0)/\ W_0]\times 100 \qquad (2)$$

Where Ws is the mass of swollen polymer and W_0 is the mass of dry polymer.

Determination of dynamic binding capacity and the stability and durability test

Breakthrough curve was employed in this work to investigate the dynamic binding property of the monolithic column according to our previous work.[23] First of all, the monolithic column was connected to the UV-detector and thoroughly flushed with water-ACN (90:10, v/v) until a stable baseline was observed at 265 nm. Secondly, the monolithic column was shortly disconnected from HPLC system and the tube from the reservoir to the inlet of the whole monolithic column was filled with a solution of the SD (0.2 mg mL^{-1}) in water-ACN (90:10, v/v). Then, the monolithic column was again connected with the LC system, and the breakthrough curve was obtained by starting the pump (flow rate, 0.8 mL min^{-1}) and simultaneously recording the signal at 265 nm. The dynamic binding capacity was calculated in accordance with $q = (t_{50\%}\text{-}t_0)\ FC/m$, where q is the dynamic capacity at 50% of breakthrough, F is the volume flow rate; C is the adsorbate concentration in the feed, m is the mass of the RAM-HMC, t_0 is the dead time (the retention time of acetone), $t_{50\%}$ is the time of 50% breakthrough.

The stability and durability of the monolithic columns were characterized by back pressure under higher pressure during long-term use in an on-line coupling with HPLC system.

On-line SPE-HPLC procedures

The RAM-HMC was applied as a pre-column to evaluate the applicability for on-line SPE-HPLC determination SAs in honey, which was placed in the sample-loop position. A schematic diagram of the on-line SPE-HPLC system and specific experimental process was referred to our previous work.[23] A briefly describe, the pre-column was conditioned with 2 mL of water–ACN (75: 25, v/v) and 2 mL of water–ACN (90: 10, v/v), respectively, at a flow rate of 0.8 mL min^{-1}. When the injection valve was in the position of “load”, the standard solutions or spiked honey samples were loaded on to the pre-column, so that the analytes were selectively adsorbed and concentrated in the pre-column. The enrichment time was correlated with the loading volume, and the flow rate 0.8 mL min^{-1}. The pre-column was then washed with methanol–water (5: 95) solution to elute the interfering substances in the “wash” position. Subsequently, the analytes were eluted with the mobile phase in the “injection” position, the flow rate of 0.8 mL min^{-1} and the monitored by UV detection at 265 nm. Finally, the column was regenerated with water–ACN (90:10, v/v) to get a stable baseline at the flow rate of 0.8 mL min^{-1} before a new analysis. The determination of each analyte was carried out in triplicate.

Sample preparation

The honey samples were purchased from a local supermarket. 4.178 g of honey samples were accurately weighed and spiked with SM2, SMO and SD at three levels of 0.05, 0.1 and 0.2 mg kg^{-1}, and put into a 20 ml centrifuge tube, 10 mL 5% $HClO_4$ solution were added. The mixed samples were shaken for 1 min on the mixer and underwent ultrasound-assisted extraction for 10 min. After the mixed samples were centrifuged for 5 min at 4000 rpm, the supernatants were filtered through a 0.22 μm syringe filter. The experiments were repeated three times.

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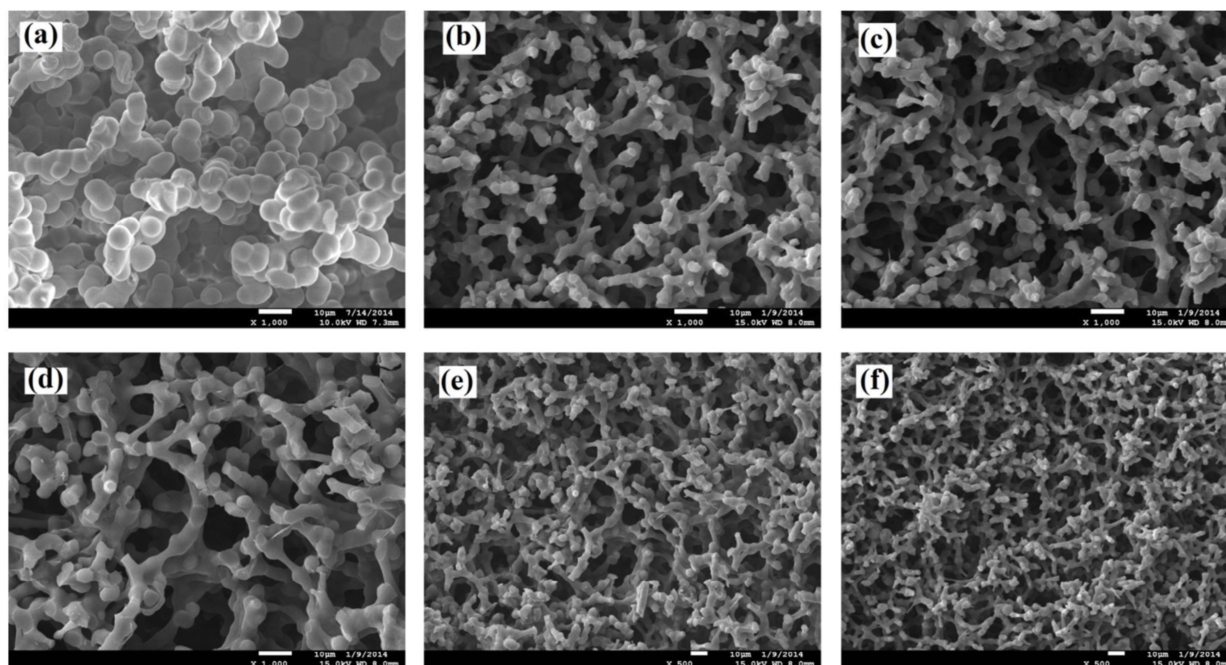

Fig. 2 SEM images of the monolithic column at the different ratio of MTMS and TEOS (see Table 1). (a) HMC1; (b) HMC; (c) HMC2; (d) HMC3; (e) HMC; (f) RAM-HMC.

Results and discussion

Optimization of preparation conditions and characteristic of RAM-HMC

Initially, we synthesized RAM-HMC in a stainless steel chromatographic column (50 mm × 4.6 mm), according to the method reported in the literature.[19] The result of this research showed that the stainless steel column was significantly different from the capillary column. When the monolithic column was prepared in a stainless steel chromatographic column, some questions were found to be shrinkage, swelling, large mass transfer resistance and poor reproducibility. The preparation method of the HMC was optimized by changing the proportion of MTMS and TEOS, while other factors, including the hydrolysis conditions of TEOS and aging/drying conditions, have the same trend as the experimental results in some literatures.[19, 21]

The influence of the ratio of MTMS/TEOS on the skeleton structure. The influence of dual precursor proportion on the material properties was studied. The ratio of MTMS and TEOS was adjusted to optimize the properties of the HMC. For the HMC, the organic precursor was provided hydrophobic groups and the inorganic precursor was supplied stability network structure. The SEM images of the monolithic column at different ratios of MTMS and TEOS were shown in Fig. 2. As you can see in Fig. 2a, the skeleton structure of the HMC1 without TEOS was particle accumulation. When the ratio of MTMS and TEOS were 1:0.5, 1:1 and 1:2 (Fig. 2b, c and d), the similar homogeneous and stable network skeleton was obtained and the collapse phenomenon of the structure disappeared. This indicates that TEOS can enhance the skeleton structure stability.

Table 1 Adsorption rate of solvent and titration experiments data from HMC and RAM-HMC

Monolith	V_{MTMS}:V_{TEOS}	Adsorption rate of solvent (%)			
		Water	Methanol	ACN	Toluene
HMC1	1:0	36.84	49.12	50.00	92.32
HMC	1:0.5	40.17	52.94	56.88	90.71
HMC2	1:1	67.63	71.83	72.10	82.3
HMC3	1:2	85.40	88.67	86.26	40.2
RAM-HMC	1:0.5	49.33	55.54	62.62	88.42

The influence of the ratio of MTMS/TEOS on the adsorption rate of solvent. The influence of the ratio of MTMS/TEOS on the adsorption rate of solvent on the monolithic materials was studied. As shown in Table 1, water, methanol, ACN and toluene were performed in solvent uptake experiments. When the proportion of TEOS increased, the solvent uptake ratio of water, methanol and ACN increased while that of nonpolar solvents (toluene) reduced, which is likely that the polar solvents diffuse into the pores and cavities of the silica monolithic materials. A lower content of TEOS is conducive to the adsorption analyte when the sample solution and the mobile phase were polarity. On the other hand, the rigidity of skeleton structure in the monolith increased with increasing the amount of TEOS, and it was found that the skeleton structure stability of the monolith increased with the increase of aging time. So, the ratio of MTMS: TEOS (1:0.5) was selected to endure the rigidity and stability of the monolith during aging and drying. The absorption rate of polar solvent on the RAM-HMC is slightly higher than the HMC, which is due to a small amount of solvent residue in hydrophilic outer layer.

The investigation of grafting EPTS on the HMC. To acquire a hydrophilic layer on the through-hole surface of the HMC, epoxy was introduced by chemical modifications with

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EPTS. The chemical character and surface profile of the monolithic columns were studied using SEM, FT-IR and titration. As shown in Fig. 2e and Fig. 2f, the structure and diameter of the RAM-HMC were just like the described in the literature.[19] Fig. 2e and Fig. 2f illustrate the microchannel diameter around 15 µm in the HMC while the diameter around 10 µm after the grafting EPTS. The average diameter of through-hole is larger than 10 µm, which provides low backpressure and enough pore spaces to much more chemical modifications. It not only showed the EPTS grafting successfully, but also the 10 µm macroporous still dominantly existed in the RAM-HMC. A good fast mass transfer efficiency could be obtained.

In order to validate the grafting rate of EPTS and the increase of hydrophilic groups, the HMC and RAM-HMC were taken for titration experiments according to the method reported in the literature.[20] It has been obtained that the content of hydroxyl group was 17.10% and 30.12% from the HMC and RAM-HMC, respectively. This showed that the hydrophilicity enhances using above epoxy modified method.

Back-pressure of the monolithic columns. The stability and durability of the monolithic columns were investigated by the back-pressure test for fast analysis and reutilization. The low back-pressure allows the RAM-HMC operation at higher flow rate. So, a series of pressure resistance experiments were carried out in different flow rates and materials. As shown in Fig. 3, the HMC1, HMC and RAM-HMC were used at flow rates of 1.0 mL min^{-1} (Fig. 3a) and 2.0 mL min^{-1} (Fig. 3b), respectively. The risk of collapse of the HMC1, HMC and RAM-HMC must be taken into account in order to ensure the skeleton stability to high back pressure, especially in long-term use. When the HMC1 was continuous used at flow rates of 1.0 mL min^{-1} for 6 h and 2.0 mL min^{-1} for 20 min, respectively, the columns had collapsed and the pressure rose suddenly. The HMC and RAM-HMC were continuous used over 12 h and showed an outstanding skeleton stability compared with HMC1. Under the optimal chromatographic conditions, the RAM-HMC is proven reusable more than 1000 times. So the RAM-HMC is more suitable for on-line SPE coupling with HPLC at high pressure, high flow rates and long-term reutilization.

IR spectrum of the monolithic columns. To ascertain the EPTS grafted in the HMC, FT-IR spectra were obtained from HMC (Fig. 4a) and RAM-HMC (Fig. 4b). As shown in Fig. 4a, the strong peaks near 1138 and 1044 cm^{-1} were assigned as the Si-O-Si stretching stretches. The bands around 778 and 437 cm^{-1} resulted from Si-O vibrations. O-H vibration was reflected at 3456 and 1636 cm^{-1}. The peaks at 2979 and 1279 cm^{-1} were corresponded to the C–H stretching vibration in $Si\text{-}CH_3$. Compared to HMC, there is no obvious difference with EPTS-modified hybrid monolithic column except that the peaks at 924 cm^{-1} and 1420 cm^{-1} for Si-O-H and C–H bond in methylene group, respectively. As shown in Fig. 4b, the peaks significantly enhanced at 1138 or 1044 cm^{-1} for Si-O-Si, 778 cm^{-1} or 437 cm^{-1} for Si-O. These results indicated that EPTS was bonded successfully.

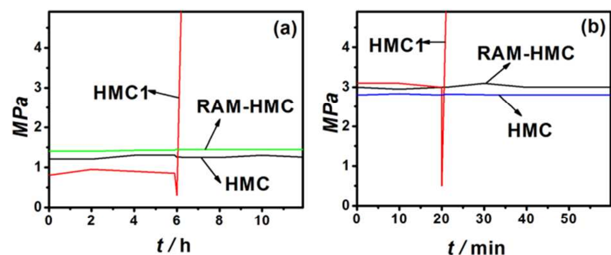

Fig. 3 Back-pressure of HMC1、HMC and RAM-HMC in the LC system, (a) flow rate: 1.0 mL min^{-1}; (b) flow rate: 2.0 mL min^{-1}. Mobile phase: acetonitrile (ACN) -0.1 M K_2HPO_4 solution (15:85, v/v).

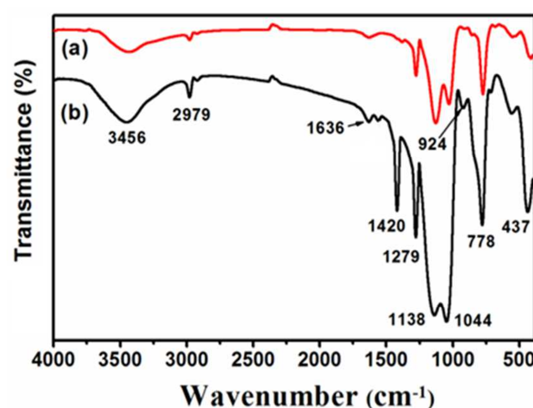

Fig. 4 FT-IR spectra of (a) HMC and (b) RAM-HMC.

Chromatographic evaluation

Dynamic binding capacity. As a typical example, some of the experimental breakthrough points of SD, SM2 and SMO on the RAM-HMC were obtained by the half height method from corresponding experimental breakthrough curves shown in Fig. 5. These points correspond to the amount of substrate analytes retained on the RAM-HMC. By comparing the positions of the breakthrough point RAM-HMC in Fig. 5, it can be found that the rebinding abilities are nearly same. The dynamic adsorption capacity of RAM-HMC for SD, SM2 and SMO were 4.15, 3.95 and 3.98 mg g^{-1}, respectively. The result revealed that RAM-

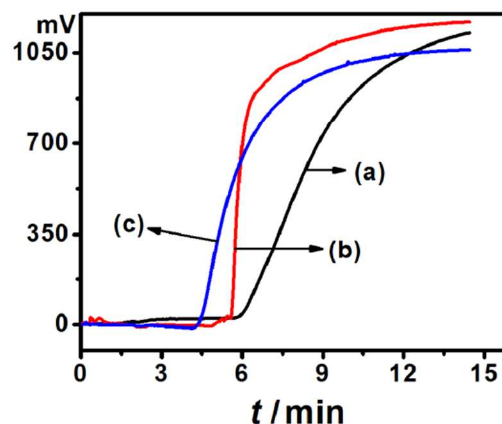

Fig. 5 Breakthrough curves of SAs on the RAM-HMC. (a) SD; (b) SM2; (c) SMO; mobile phase: 200 mg·L^{-1} of single standard solution; 0.8 mL·min^{-1}; detection: UV 265 nm.

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HMC has a good rebinding ability for SAs.

Hydrophobic and hydrophilic properties. The hydrophilicity of the RAM-HMC was evaluated by the recovery of serum proteins according to the procedure described previously.[14] A 20 μL of 1% (w/w) protein solution (BSA and LZM respectively dissolved in 0.1 M phosphate buffer, pH 6.8) was loaded onto the RAM-HMC using 100 mM phosphate buffer/acetonitrile = 80/20 (v/v) as the eluent at a flow rate of 0.8 mL min^{-1} and detected by UV detection at 280 nm. The protein solution was injected three times into each column (RAM-HMC and HMC) and the recovery from each injection was calculated from the absorbance ratio with and without the column. The average recovery of protein from RAM-HMC and HMC was 84.24% - 95.63% and 52.65 - 75.48%, respectively. The results have indicated that the RAM-HMC showed lower absorption of protein than the HMC. In other words, the hydrophilic external layer of the RAM-HMC avoids destructive protein deposition on the surfaces, while small molecule compounds can through the hydrophilic layer into the micropore surface.[14] Despite the difference in molecule weight of two proteins, their chromatographic retention time was 0.70 min and 0.73 min is roughly the same. This is further evidence that the hydrophilic groups on the external surface of the RAM-HMC hardly interact with denatured protein.

The hydrophobicity of the RAM-HMC was validated by the relative retention factors of various types of apolar solutes to anthracene in water/acetonitrile (60:40, v/v).[24] As it could be observed in Fig. 6, the order of the peak in HPLC curve confirmed reversed-phase chromatographic property of the RAM-HMC, which shows that the RAM-HMC can well adsorb small-molecule compounds.

Optimization of On-line extraction conditions

The ability of pre-enrichment and sample clean-up of the RAM-HMC were evaluated by on-line extraction procedures using the RAM-HMC as a pre-column in the loop position of the liquid-phase sample injection valve. The loading solution, washing solution and flow rate was optimized to enhance the selectivity of the RAM-HMC.

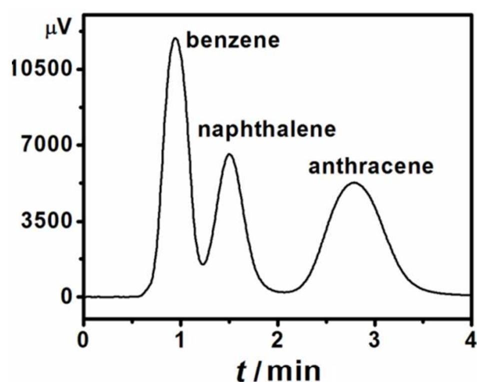

Fig. 6 The reverse phase characteristic chromatogram of RAM-HMC was obtained using benzene, naphthalene and anthracene. Mobile phase: water/acetonitrile (60/40, v/v); flow rate: 0.5 mL·min^{-1}; detection: UV 254 nm; injection volume: 10 μL.

The sample loading solution and flow rate were optimized according to the procedure described in dynamic binding experiment section. A series of 200 mg $L^{-1}$of SD in acetonitrile-water (5:95, 10:90, 50:50, 75:25, v/v) were continuously loaded onto the RAM-HMC, respectively. It was found that the adsorption capacity increased with decreasing the acetonitrile content, and some crystalline particles emerge in sulfadiazine solution when the acetonitrile content was less than 10%. The maximum adsorption capacity (4.15 mg g^{-1}) was obtained when acetonitrile-water (10:90, v/v) was used as sample loading solution.

The sample loading flow rate will affect the rebinding efficiency due to ideal time of interaction between analyte and adsorbent. The flow rate of the sample mobile phase over the range of 0.1-2.0 mL min^{-1} was investigated, and the result indicated that the breakthrough time differs widely but the breakthrough volume changes minimally and the separation factor decreases merely slightly as superficial bed velocity increases, which proved that the resistance to mass transport is substantially smaller in RAM-HMC with convection. When the loading flow rate was 0.8 mL min^{-1}, the adsorption capacity was better. Therefore, the loading flow rate of 0.8 mL min^{-1} was employed.

The washing step was optimized to enhance the selectivity and clean-up effect of the RAM-HMC for removing weakly retained impurities, and leaving analytes and strong retained materials. This requires washing adsorbent with solutions that are stronger than the sample matrix, but weaker than analytes. In this study, several solutions (methanol/water, 0: 100, 3: 97, 5: 95, 7: 93 and 10: 90, v/v) and different volumes (1-3 mL) were investigated. The result showed that matrix interference reduced gradually as the volume of washing solvent increased, but the loss of analytes increased accordingly. The same tendency appeared with the increase of the ratio of methanol. Finally, 1.5 mL of methanol/water (5: 95) was used in the washing step.

The elution solution can elute analytes, leaving the impurities is retained stronger than the analytes. The elution step has been optimized using acetonitrile and phosphate buffer as eluting solution due to its compatibility with the following chromatographic separation. The column-switching HPLC system was applied to separate SAs from a C18 column after the RAM-HMC extraction. To choose a better condition for protein exclusion and the analyte analysis, The influence of the elution mobile phase, 0.1 M K_2HPO_4 solution containing different concentrations of acetonitrile in the range of 10–40% (v/v), on the retention of three SAs and protein was investigated. The retention times of three SAs decreased with increasing acetonitrile content in the elution mobile phase. When acetonitrile content was higher than 20%, the peaks of protein and SAs were overlapping. So acetonitrile - 0.1 M K_2HPO_4 solution (15:85, v/v) at the flow rate of 0.8 mL min^{-1} was finally selected as the mobile phase for eluting monolithic column and chromatographic separation on a C18 column.

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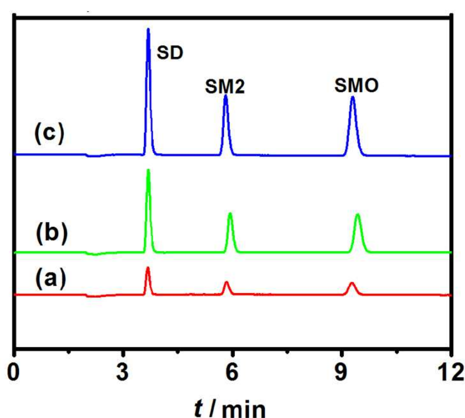

Fig. 7 Enrichment chromatograms were obtained from the on-line extraction of the standard solution. Injection volume: (a) 10 µL, (b) 20 µL and (c) 50 µL. The mixture standard solution (SM2, SMO and SD) concentration: 0.05 mg L^{-1}. Mobile phase: ACN -0.1 M K_2HPO_4 solution (15:85, v/v). Flow rate: 0.8 mL·min^{-1}.

On-line preconcentration and clean-up

A mixture standard solution (0.05 mg L^{-1}) of the sulfonamides antibiotics (SM2, SMO and SD) was used as the loading solution at the optimized flow rate to evaluate the enrichment ability of RAM-HMC. The enrichment factors (EF) were obtained by comparing the slopes of the liner portion of the calibration cures with and without on-line extraction. When it was 20 times of the conventional injection volume, the enrichment factors were 15.6, 15.3 and 16.9 for SM2, SMO and SD, respectively. The liner regression equations, correlation coefficient, limit of detection and enrichment factors of three sulfonamides were listed in Table 2. Different injection volume of chromatogram was showed in Fig. 7, the peak area increased and sensitivity improved with the increase of enrichment time and injection volume. These results showed the RAM-HMC not only enriched sulfonamides antibiotics but also had highly stable and repeatable capacity, so that could be expected the on-line preconcentration demands.

In order to investigate the purification performance of the RAM-HMC for food sample, spiked honey samples was determination by on-line extraction coupling with HPLC. Fig. 8 shows the Chromatograms of (a) blank honey sample (non-spiked), (b) spiked honey sample and (c) spiked honey sample with a clean-up of the RAM-HMC. As it can be observed in the chromatograms, after pretreatment, the noises in Fig. 8c was much less than those in Fig. 8a and Fig. 8b, which is helpful to improve the sensitivity of the method.

Determination of sulfonamides antibiotics by on-line SPE-HPLC

As shown in Table 3, some of parameters such as precision, accuracy, detection limit and quantitation limit were investigated to verify the method performance. The stock solutions of SM2, SD and SMO were spiked into the blank honey samples (n =3) at the concentrations of 0.05 mg kg^{-1}, 0.1 mg kg^{-1} and 0.2 mg kg^{-1}. Compared with the literature method previously reported,[7-10] the proposed method was more convenient and timesaving using the on-line SPE-HPLC. The average recoveries of SM2, SD and SMO in honey samples were 89.03-105.08%, 91.40-94.65% and 73.50-100.40%,

Table 2 The liner regression equations, correlation coefficient (r), limit of detection (LOD) and enrichment factors (EF) of three sulfonamides

	Analyte	Liner equation	r	Liner range (mg L^{-1})	EF	LOD (µg kg^{-1})
Standard solution	SM2	$Y=-148+6.18\times10^6$ C	0.9840	0.05 - 0.2		12.56
	SD	$Y=-153+1.02\times10^7$ C	0.9847			10.23
	SMO	$Y=-13+7.30\times10^6$ C	0.9916			13.77
On-line extraction	SM2	$Y=-1257+9.64\times10^7$ C	0.9636		15.6	1.08
	SD	$Y=-2320+1.57\times10^8$ C	0.9524		15.4	0.93
	SMO	$Y=-3395+1.23\times10^8$ C	0.9442		16.9	1.13

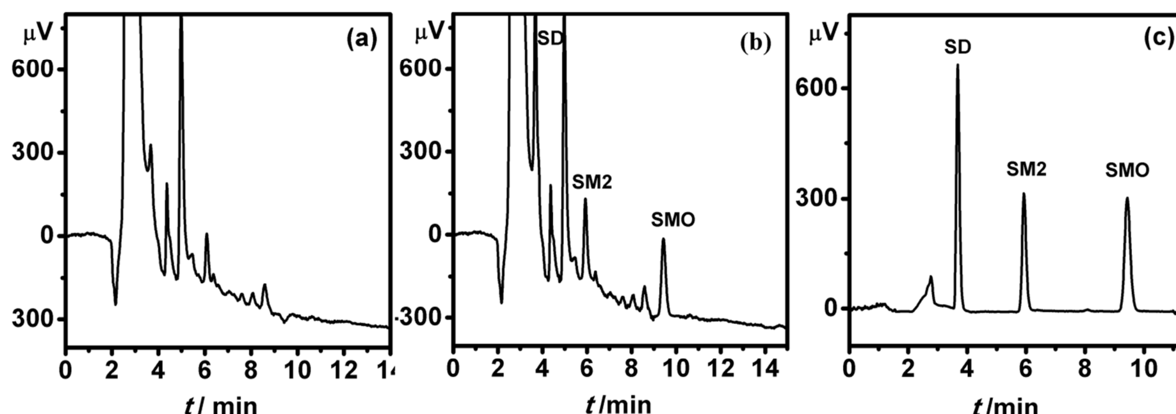

Fig. 8 Chromatograms were obtained by the on-line extraction of SD, SM2 and SMO from the honey samples. (a) blank honey (non-spiked); (b) spiked honey; (c) spiked honey with a clean-up of the RAM-HMC. Mobile phase: ACN -0.1 M K_2HPO_4 solution (15:85, v/v). Fow rate: 0.8 mL·min^{-1}. Samples spiked concentration: 0.05 mg L^{-1}. Injection volume: 10 µL.

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Table 3 Average recoveries (R) and relative standard deviation (RSD, *n*=3) of three sulfonamides obtained by on-line extraction of the spiked honey samples (*n*=3)

Analyte	Spiked (mg kg^{-1})	Detected (mg kg^{-1})	R (%)	RSD (%)	LOD[a] (μg kg^{-1})	LOQ[b] (μg kg^{-1})
	0.05	0.0459	89.03	3.04		
SM2	0.1	0.0940	94.00	4.88	6.9	23.0
	0.2	0.2116	105.08	2.25		
	0.05	0.0457	91.40	3.45		
SD	0.1	0.0919	91.90	5.04	14.1	46.9
	0.2	0.1893	94.65	5.32		
	0.05	0.0368	73.50	4.75		
SMO	0.1	0.0890	89.00	3.86	14.6	48.2
	0.2	0.2008	100.40	3.50		

[a] LOD calculated as 3 times the signal-to-noise ratio. [b] LOQ calculated as 10 times the signal-to-noise ratio.

respectively, with RSDs of 2.25-5.32% (Table 3). The limits of detection (LOD, S/N=3) and the limits of quantitation (LOQ, S/N=10) of the method were 6.9 and 23.0 μg kg^{-1} for SM2, 14.1 and 46.9 μg kg^{-1} for SD, 14.6 and 48.2 μg kg^{-1} for SMO, respectively. The limits of quantitation could meet the requirement of MRL detection. So the present sample preparation procedure is simple and could be effective for the analysis of environmental, food and biological samples.

Conclusions

The preparation feasibility of restricted access material- hybrid monolithic column with the hydrophobic inner surface and the hydrophilic outside surface in through-hole was demonstrated by characterization and application. The results showed that the RAM-HMC have a macroporous dictyo-skeleton structure which can solve collapsed, jam and reutilization effectively with a high flow speed. Experimental results also indicate that small variations of the synthetic parameters influence the morphological features of the HMC, which means that all of them are suited. So, more deep researches are needed to improve the preparation reproducibility of the HMC. The RAM-HMC was used as pre-column coupling with HPLC for the determination of sulfonamides antibiotics in honey, which not only provided a convenient, economical, fast and highly efficient separation, but it also can exclusion of biological macromolecules and enrichment purification analytes effectively. This methodological study and application in the separation, enrichment and purification of chemical contaminants in food will be an important field.

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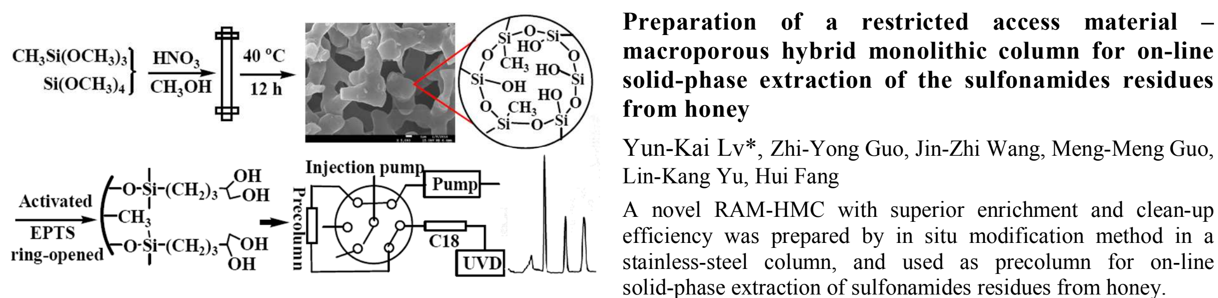
Graphical Abstract



Preparation of a restricted access material – macroporous hybrid monolithic column for on-line solid-phase extraction of the sulfonamides residues from honey

Yun-Kai Lv*, Zhi-Yong Guo, Jin-Zhi Wang, Meng-Meng Guo, Lin-Kang Yu, Hui Fang

A novel RAM-HMC with superior enrichment and clean-up efficiency was prepared by in situ modification method in a stainless-steel column, and used as precolumn for on-line solid-phase extraction of sulfonamides residues from honey.

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