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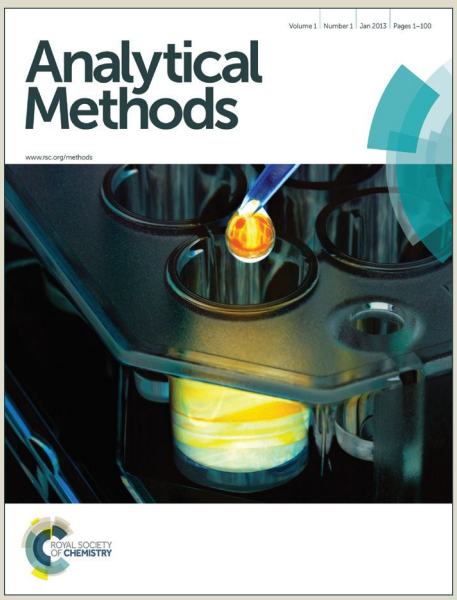

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ARTICLE

Determination of Trace Sulfonamides in Foodstuffs by HPLC Using a Novel Mixed-Mode Functionalized Ferrocene Sorbent for Solid-Phase Extraction Cleanup

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In this paper, a rapid and effective HPLC method, using a new 4-chloro-6-pyrimidinylferrocene-modified silica gel (NFcSi) as solid-phase extraction (SPE) sorbent, was developed for the purification and determination of trace sulfonamides in foodstuffs. The main influence factors of SPE including amount of NFcSi sorbent, sample flow rate, species and volume of eluant were investigated and evaluated in the sample pretreatment step. The optimized purification effect was achieved at the sample flow rate of 2 mL/min with 100 mg of NFcSi and 5 mL of washing solution (water, 100%). The HPLC analysis was carried out on a Shim-Pack VP-ODS column (150 × 4.6 mm i.d., 5 μm) with a mobile phase of MeOH/0.1% acetic acid-water (30:70, v/v). With a detection wavelength of 270 nm, the good linearities of sulfadimidine (SM_2), sulfamethozazole (SMZ) and sulfadimethoxine (SDM) were obtained in the concentration ranged from 10 to 5000 ng/mL ($R^2 \geqslant 0.9998$). The RSD% values (n = 6) of retention times and peak areas in intra- and inter-day assays for three sulfonamides were all below 0.55%, 1.80%, 0.20% and 0.78%, which showed good precision. In addition, overall recoveries of SM_2, SMZ and SDM through the extraction and purification steps were in range of 71.1-86.7%, 70.6-93.6% and 72.4-90.7%. Compared with the commercial SPE sorbents, NFcSi featured excellent selectivity to retain polar and nonpolar interferences in the sample matrices. The improved method was simple, rapid, accurate and promising for the determination of trace sulfonamides in foodstuffs with complex matrices.

Introduction

Sulfonamides are a group of synthetic antibiotics which play an important role in animal husbandry.[1] The extensive use of sulfonamides in animal production is due to their wide antimicrobial spectrum activity and their high activity against protozoa and fungi.[2-10] Irrespective of their use, residues of these substances can remain in animal food products and consequently endanger consumer health.[11] To ensure food safety for consumers, the European Union and other countries, including China, have established a maximum residue limit (MRL) of 100 μg/kg for sulfonamides in foods of animal origin such as meat, milk and eggs.[12] In past years, a number of quantitative methods have been developed to determine sulfonamides such as capillary electrophoresis, gas chromatography (GC) and high-performance liquid chromatography with ultraviolet detection (HPLC-UV) [13-15] or fluoroscene detection (HPLC-FLD) [16]. Nowadays, most analytical methods are based on LC-MS [17] and LC-MS/MS [18] which appear to be acknowledged as the most useful and authoritative methods for the quantification of sulfonamides in complex matrices. However, both LC-MS and LC-MS/MS are high-cost and cannot be easily adopted by nonspecialized laboratories. On the contrary, HPLC with UV detection possesses the advantages of simplicity, lower cost, and strong maneuverability compared to MS techniques. Meanwhile, it is noteworthy that the matrix effect is a serious problem in the trace analysis of sulfonamides in complex matrices such as pork, which make quantification and identification difficult. Therefore, removal of the matrix effect and sample pretreatment are of great importance in trace analysis.

Due to the complex nature of some foodstuffs such as pork, samples are often pretreated to remove protein, fat and reduce potential interference from the sample matrix for the cleanup of sulfonamides. The most common treatments are liquid-liquid extraction (LLE) and solid-phase extraction (SPE).[19] In comparison with LLE, solid phase extraction (SPE) is an important sample pretreatment technique, which has been popular in analytical field due to its merits such as low cost, high enrichment factor, rapidness, easy automation and wide scope.[20-26] As sulfonamides are small, polar and hydrophilic molecules, a large amount of matrix interferences tends to be extracted simultaneously with sulfonamides, which may interfere with the determination of the target compounds. Thus, additional time and pretreatment steps are required to remove the interfering compounds. To improve recovery, large quantities of organic solvents are used, which may be harmful to the environment. For conventional sorbents, these difficulties are magnified when the analytes are present at low concentrations. So, it is necessary to design a novel SPE

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sorbent with a multiple retention mechanism for the cleanup of sulfonamides from complex sample matrices.

Since Delville and his coworkers[27-30] reported the application of ferrocene as the stationary phase, it has attracted extensive attention in liquid chromatography[31] due to its varieties of separation mechanisms, including hydrophobic, hydrogen bonding, π-π, dipole-dipole and charge-transfer interactions. Complexing properties of ferrocene receptors depend mainly on the type and arrangement of binding site. Our group has been comitted to the research of ferrocene stationary phases and several novel multi-interaction and mixed-mode stationary phase based on ferrocene complexes modified silica gel has been synthesized in our laboratory.[32-33] It was noteworthy that the aromatic derivatives, which own polar groups such as carbonyl, NH_2, OH and NO_2, might tune the separation selectivity and tended to obtain better results on ferrocene-bonded phases by contrast. Recently, we prepared a novel functionalized ferrocene modified silica (NFcS) (Figure.1). In contrast with presented ferrocene in which the metallocenes are linked with carbonyl, one goal of this work was mainly to evaluate the recognition performance after the introduction of nitrogen heterocyclic in the ferrocene molecule. It could be predicated that multiple nitrogen in the new pyrimidine unit made the new ferrocene have stronger hydrogen bonding interaction than the previous ones. Thus it would let us explore the practical applications of the new ferrocene functionalized silica gel (NFcS). Our following work [34] indicated that the NFcS stationary phase exhibited high selectivity toward various compounds under different conditions with different mechanisms including hydrophobic, π-π, hydrogen-bonding, charge-transfer, acid-base equilibrium and anion-exchange interactions. A number of compounds, including polycyclic aromtic hydrocarbons, mono-substituted benzenes, aromatic amines, phenols, quinoline compounds, plant growth regulators, nucleoside and inorganic anions, have been well separated on the NFcS stationary phase. Because of the existence of multi-interaction, it is of great application value for purification or concentration trace analyte in a complex matrix.

In this work, a novel multiple retention mechanism sorbent (NFcSi) (Fig.1) was used with the aim of developing a rapid and reliable sample preparation procedure for the determination of sulfonamides in food samples. All of the main factors were optimized, and the results obtained by using the developed HPLC method based on NFcSi SPE indicated that it is more suitable for the determination of sulfonamides in the complex matrices.

Experimental

Reagents, Materials and Chemicals

4-Chloro-6-pyrimidinylferrocene was synthesized according to the reported procedure [35-36]. Silica gel (particle size of 40-60 μm and specific surface area of 500 m^2/g) was purchased from Aladdin Chemistry Co. Ltd (Shanghai, China). 3-Aminopropyltriethoxysilane was purchased from Jingchun Chemical Reagent Co., Ltd. (Shanghai, China). The standards of sulfadimidine (SM_2), sulfamethozazole (SMZ), sulfadimethoxine (SDM) were obtained from Animal Husbandry Bureau (Zhengzhou, China). HPLC grade methanol (MeOH), n-hexane and acetonitrile were provided by Fisher (Fair Lawn, NJ, USA). Deionized water was purified by a Milli-Q system from Millipore (Bedford, MA, USA). The polypropylene column tube and 20 μm PTFE sieve plates used for SPE were bought from DIKMA (Beijing, China). Commercially available MCX, MAX, HLB, NH and C18 cartridges (100 mg/3 mL) were obtained from Waters (Milford, MA, USA).

Standard sulfonamides were dissolved in methanol at a concentration of 10 μg/mL and stored at 4 °C as stock solutions. Six standard solutions at different concentration (10, 25, 50, 100, 500 and 5000 ng/mL) were prepared by diluting the standard stock solution with the matrix of blank pork.

Instruments and Measurement

The HPLC analysis was carried out on a Waters system (Milford, MA, USA) equipped with a 1525EF binary HPLC pump, a heated column compartment, a G1397A degasser (Agilent, Palo Alto, CA, USA) and a Shimadzu SPD-10AVP ultraviolet detector (Shimadzu, Japan). A Flash EA 1112 elemental analyzer and a Bruker Vector 22 IR spectrograph were used for characterization of the NFcSi SPE sorbent. A centrifuge (Zhongda Instrument Plant, Jiangsu, China) was used for centrifugal separation. A Vortex mixer (Sigma 3-30k, Shanghai, China) was used for mixing solutions. A rotary evaporator was used for enrichment (Yarong, Shanghai, China). The HPLC separations were performed on a Shim-Pack VP-ODS column (150×4.6 mm i.d., 5 μm) with a mobile phase of MeOH/0.1% acetic acid-water (30:70, v/v) at the flow rate of 1 mL/min. The wavelength was 270 nm, the injection volume was 10 μL, and the oven temperature was 30 °C. Under these chromatographic conditions, sulfonamides and the food components in the tested samples were all baseline separated and eluted. Each separation and/or determination was performed in triplicate.

Fig. 1.

Synthesis of NFcSi SPE Sorbent

As illustrated in Fig. 2, NFcSi sorbent was prepared by a two-step modification process as previously described.[34] First, aminopropyltriethoxyl-bonded silica gel (ABS) was obtained. In the second step, ABS was reacted with an excess of 4-chloro-6-pyrimidinylferrocene in tetrahydrofuran (THF) at 60 °C under nitrogen atmosphere. Different from the previous work, the spherical silica had a particle diameter of 40-60 μm instead of the previous 5 μm. The surface area is 500 m^2/g.

Fig. 2.

Retention Behaviors of Sulfonamides on the NFcS Stationary Phase

The retention behaviors were investigated on the previously studied NFcS column (150×4.6 mm, 5 μm) and Shim-Pack VP-ODS (150×4.6 mm, 5 μm) to evaluate the interactions between the NFcS stationary phase and sulfonamides.

Sample Preparation

Homogenization, Extraction, and Enrichment Process. All samples (pork, chicken, beef, egg, milk and honey) were obtained from Animal Husbandry Bureau (Zhengzhou, China). 2.5 Grams of homogenized sample was weighed and put into 50 mL centrifuge tubes in triplicate. To each centrifuge tube was added 10 mL of acetonitrile and 2.5 g of anhydrous sodium sulfate followed by 10 s of vortexing to extract the target sulfonamides. All of the centrifuge tubes were centrifuged for 3 min at 4000 rpm. Then the clear supernatant was transferred into a glass tube, this process was repeated, and the two clear supernatants were combined. About 20 mL of extraction solution was rotary evaporated to nearly dry and 1 mL of methanol was added.

SPE cleanup

In this paper, a new purification procedure based on a novel homemade NFcSi SPE sorbent is proposed for the analysis of three sulfonamides. The procedure consists of an acetonitrile extraction, centrifugation, and cleanup using homemade SPE cartridges. This method was compared with five different commercial SPE cartridges (C18, HLB, NH_2, MAX and MCX) with different interaction mechanisms to establish the best conditions for the determination of three sulfonamides in food products.

To investigate the availability of the NFcSi SPE for the cleanup of sulfonamides in complex matrix, 100 mg of NFcSi SPE sorbent was packed into a 3 mL SPE cartridge. The concentrated extracting solution (Sample Preparation) was passed through the six kinds of SPE cartridges at the rate of 2 mL/min, which had been preconditioned with 4 mL of MeOH and 4 mL of water. The cartridges were washed with 3 mL acetonitrile and eluted with 5 mL of water, and then collected eluant into a glass tube, making the final volume approximately 5 mL. The 5 mL of collected aqueous solution was filtered through a 0.22 μm nylon filter (Agilent, USA) prior to HPLC analysis. All tests were performed in triplicate. This process illustrated in Fig. 3.

Fig. 3.

Recovery Test

The extraction and purification were validated by recovery investigation. We spiked test samples at three concentration levels of 50, 500 and 5000 ng/g by adding sulfonamides to real samples. Then, the recoveries were determined by HPLC method after the spiked test samples were treated by extraction and SPE cleanup procedures.

Results and discussion

Characterization of NFcSi SPE Sorbent

In IR spectra of NFcSi (Fig. S1), the bands at 2937 and 2887 cm^{-1} are assigned to the stretching vibration of -CH_2- groups. The absorption bands of carbon-carbon double bond and carbon-nitrogen double bonds in pyrimidine ring appearing at 1449, 1525 and 1608 cm^{-1} confirmed that the pyrimidinyl ligand was successfully immobilized on silica gel. Elemental analysis showed (Tab. 1) the bonding amount of 4-chloro-6-pyrimidinylferrocene on NFcSi sorbent was about 256 μmol/g.

Tab. 1.

HPLC operating conditions

Taking into consideration the potential for harm and good separation under an isocratic methanol-based HPLC method, the chromatographic separation of sulfadimidine (SM2), sulfamethozazole (SMZ), sulfadimethoxine (SDM) was performed using NFcS stationary phase and Shim-Pack VP-ODS column. The latter provided better symmetry and possessed higher theoretical plates (Fig. S3). Therefore, Shim-Pack VP-ODS (150×4.6 mm, 5 μm) was selected as the optimal column with a mobile phase of MeOH /0.1% acetic acid-water (30:70, v/v) at the flow rate of 1.0 mL/min for the separation and determination of sulfonamides in the real samples. However, it is noteworthy that the retention of sulfonamides on the NFcS column was stronger (Fig. S2) than those on ODS column. Obviously, nitrogen heterocyclics on the NFcS stationary phase played an important role in sulfonamides' retention. This result again displays that hydrogen bonding interaction exists between sulfonamides and NFcS.

SPE Optimization for Trace sulfonamides with the NFcSi Sorbent

In this section, the main influence factors (amount of sorbent, flow rate of sample, species and volume of washing solution) on the SPE recoveries (n=3) of three sulfonamides are evaluated in detail to obtain the optimal purification conditions.

The amount of sorbent in SPE cartridge is closely correlated with the purification effect. In this part, the influence of NFcSi quantity on the sulfonamides' extraction was evaluated with the amount of 50, 100, 150, 200 and 250 mg, respectively. As shown in Fig. 4, the recovery of SMZ increased as the dosages of sorbent increased from 50 to 100 mg, while it steadily increased from 100 to 250 mg. However, the recovery efficiencies of SM_2 and SDM showed no significant difference. It is easily found that 100 mg of NFcSi sorbent was enough to achieve satisfactory extraction and purification efficiency compared with other dosages. Thus, the optimal amount of sorbent was 100 mg.

Fig. 4.

As is known, the flow rate of sample is another critical factor that not only affects the purification and enrichment of analytes but also controls the sample pretreatment time. In this section, flow rates ranging from 0.5 to 2.5 mL/min were investigated, and it was found that 2 mL/min was satisfactory to provide higher extraction recoveries for the sulfonamides (Fig. 5).

Fig. 5.

A proper washing solvent is of great importance to reduce interfering substances and improve the recovery. Thus, the influence of different elution solvents on the purification of trace sulfonamides (1 mL of working aqueous solution, 50 ng/mL) was studied including methanol, acetonitrile, ethanol, acetone, water and isopropyl alcohol. It was found that most of the solvents were ineffective other than water and acetone

(Fig. 6). Water appeared to be the best among the common solvents and the highest recoveries for the sulfonamides were obtained. Therefore, water was selected as the elution solvent in NFcSi SPE Sorbent system.

Fig. 6.

The volume of eluant is closely correlated with the purification effect. Obviously, the affinity between analytes and sorbent is strong and analytes need more solvent for complete desorption. As displayed in Fig. 7, 5 mL of water was able to achieve satisfactory recoveries when the volume of washing solution changed in the range of 2-8 mL. Hence, 5 mL of water was optimized for the subsequent experiments.

Fig. 7.

Comparison of SPE Cleanup by NFcSi Sorbent and Commercial Sorbents (C18, HLB, MAX, NH_2 and MCX)

Firstly, we compared the matrix removing effect on NFcSi SPE sorbent. Concentrated extracting solution of 2.5 g of blank pork (Sample Preparation) was passed through the homemade NFcSi SPE cartridges according to the SPE cleanup procedures as previous described, while another blank pork sample was with no SPE purification. About 5 mL of purged aqueous solution was collected in two glass tubes, respectively. Then the former liquid was analyzed by HPLC. To further evaluate the purification capacity of NFcSi SPE sorbent, the above two purged liquors were defatted with hexane as described in the literature.[37] The defatting procedures are described as follows: 15 mL of n-hexane was added to the 5 mL of purged liquor, then vortex for 30 s, allow to stand and remove the upper hexane to get rid of lipid. The remaining liquids were clear, and 10 μL of liquid was injected for HPLC separation. As were showed in Fig. 8, the purged liquor from NFcSi SPE sorbent can minimize the matrix interference. It was also proved that the homemade NFcSi sorbent could omit the defatting step and reduce the loss of sulfonamides during the purification process.

Fig. 8.

To further demonstrate the suitability of NFcSi SPE for real samples, comparison of SPE purification efficiency between NFcSi SPE and commercially available C18, HLB, MAX, NH_2 and MCX SPE was carried out. The pork sample spiked at 500 ng/mL sulfonamides were performed under the extraction and SPE cleanup procedures. The 10 μL of purged liquor from NFcSi SPE and defatted liquor from the other five SPE sorbents was injected to perform HPLC separations. Their chromatograms are shown in Fig. 9. The target peak of sulfonamides by homemade NFcSi SPE cleanup showed more symmetry and higher UV response than those of the other commercially SPE columns, which makes the determination of trace sulfonamides in complex matrix more accurate and sensitive. Although the purged liquor from NH_2 can minimize the matrix removing effect, the lower enrichment effect for SM_2 was observed than NFcSi sorbent. The NFcSi sorbent exhibited high selectivity toward various interference and coprecipitation compounds with different mechanisms including hydrophobic, π-π, hydrogen-bonding, charge-transfer, acid-base equilibrium and anion-exchange interactions. The proteins were retained on NFcSi depending on hydrogen-bonding interactions, whereas fat and nonpolar matrices were held on the basis of hydrophobic interactions. For sulfonamides, there is a weak hydrogen-bonding interaction with NFcSi, which leads to its being weakly retained on NFcSi. It proved that NFcSi sorbent is more suitable for the purification and enrichment of sulfonamides in pork.

Fig. 9.

Method validation

Linear Regression Equation and LOD of the Method

In this study, method validation such as recovery, linearity, and limits of detection (LODs) were measured. Calibration curves were constructed by plotting peak area (y) versus the corresponding concentration of sulfonamides (x, ng/mL). As showed in Tab. 2, wide linear ranges were 10-5000 ng/mL for three sulfonamides and all correlation coefficients (R^2) were bigger than 0.9998. The LODs of target sulfonamides were estimated by analyzing spiked samples at low concentrations and calculated on the basis of a peak-peak signal-to-noise (S/N) value that was S/N = 3. The LODs were 2-5 ng/g at a detection wavelength of 270 nm, which were low compared to those of published works by HPLC-MS[8], HPLC-PAD[10] and HPLC-FLD[38]. The intraday and interday RSDs (n = 5) of retention time (500 ng/mL) for three sulfonamides in blank pork were0.40%, 0.52%, 0.55% and 1.62%, 1.59%, 1.80%. The intraday and interday RSDs (n = 5) of peak areas (500 ng/mL) for three sulfonamides in blank pork were 0.16%, 0.10%, 0.20% and 0.60%, 0.49%, 0.78%, respectively.

Tab. 2.

Recovery and Application to Real Samples

The recovery investigations were carried out in accordance with the previous description. The standard sulfonamide solutions at three levels (50, 500 and 5000 ng/g) were added to real samples including pork, chicken, beef, egg, milk and honey. Then, the original samples with spiked samples were extracted, cleaned up by NFcSi SPE sorbent, and analyzed by HPLC. Typical chromatograms of pork are shown in Fig. 10. The contents and recoveries of sulfonamides were determined and are shown in Tab. 3. It can be seen from Tab. 3 that their recoveries ranged from 70.6% to 93.6%, indicating that the developed HPLC method based on NFcSi SPE cleanup possessed high precision. The contents of three sulfonamides in these foodstuffs ranged from N. D. to 98 ng/g. These values in these foods were lower than the maximum residue limits (MRLs) recommended by the European Union and other countries.[12] From the results above, it is concluded that the present SPE method based on NFcSi sorbent is more suitable for the determination of sulfonamides in samples with a complex matrix.

Fig. 10.

Tab. 3.

Conclusions

In conclusion, a HPLC method based on the new NFcSi-SPE cleanup has been successfully employed for the purification and determination of sulfonamides in pork, chicken, beef, egg, milk and honey samples with complex matrices. The NFcSi-SPE method enables a more efficient purification of sulfonamides from a complex matrix due to its multi-interaction ability. The multi-interaction ability can selectively keep the main interferences (such as proteins and fat) from passing through NFcSi while allowing sulfonamides pass without any obstacle. The SPE procedure is simple and easy to operate. Furthermore, NFcSi-SPE offers higher accuracy for the trace determination of sulfonamides in complex matrices. The proposed HPLC method based on NFcSi-SPE is promising for use in the pretreatment and determination of sulfonamides in foodstuffs.

Acknowledgements

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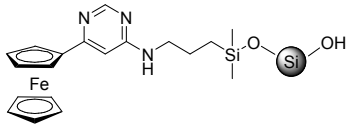

Fig. 1. Chemical structure of the 4-chloro-6-pyrimidinylferrocene bonded silica stationary phase (NFcS).

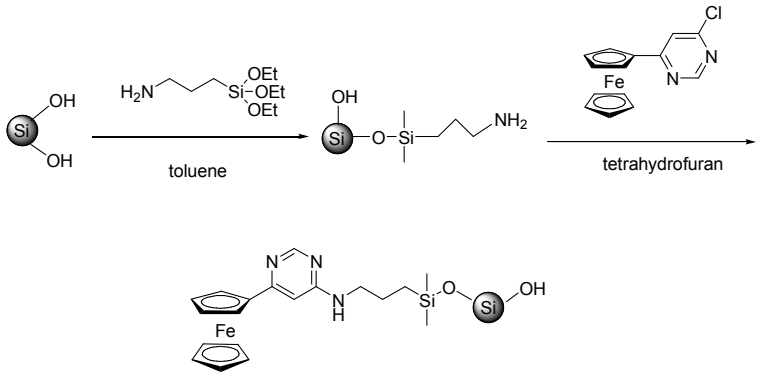

Fig. 2. Synthesis of 4-chloro-6-pyrimidinylferrocene bonded silica (NFcSi) sorbent.

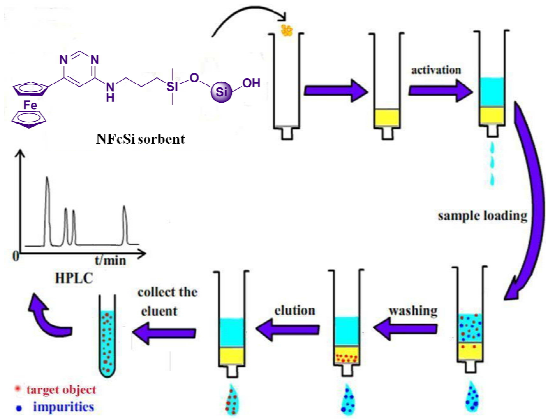

Fig. 3. Scheme of SPE process

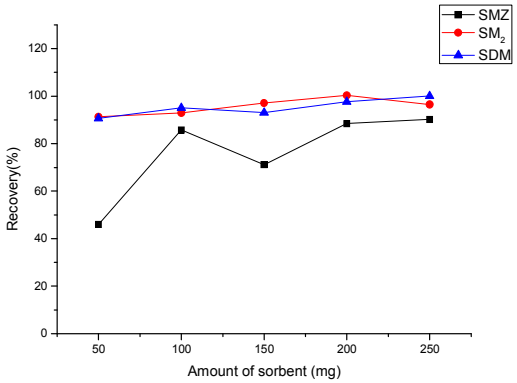

Fig. 4. Recovery efficiency with different amounts of NFcSi sorbent.

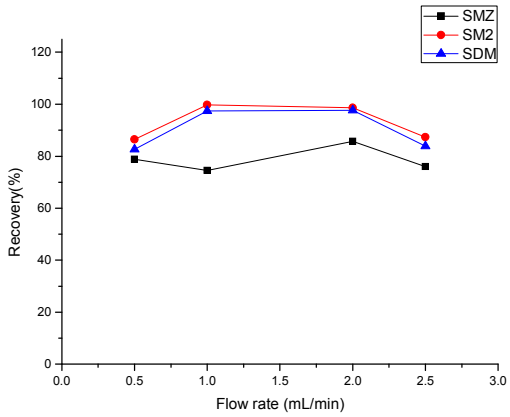

Fig. 5. Recovery efficiency with different flow rates of sample.

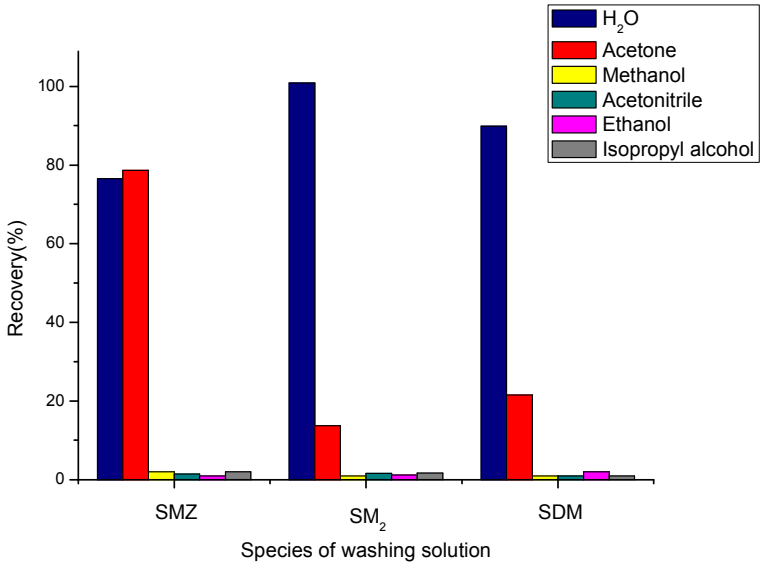

Fig. 6. Recovery efficiency with different elution solvents.

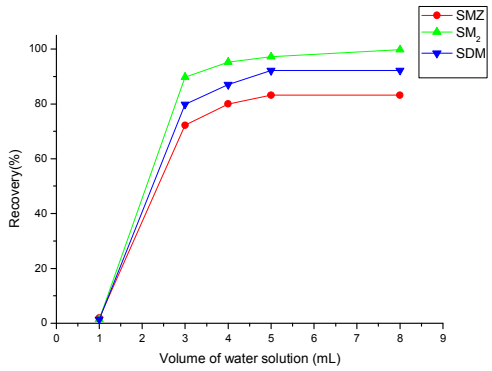

Fig. 7. Recovery efficiency with different volume of water solution.

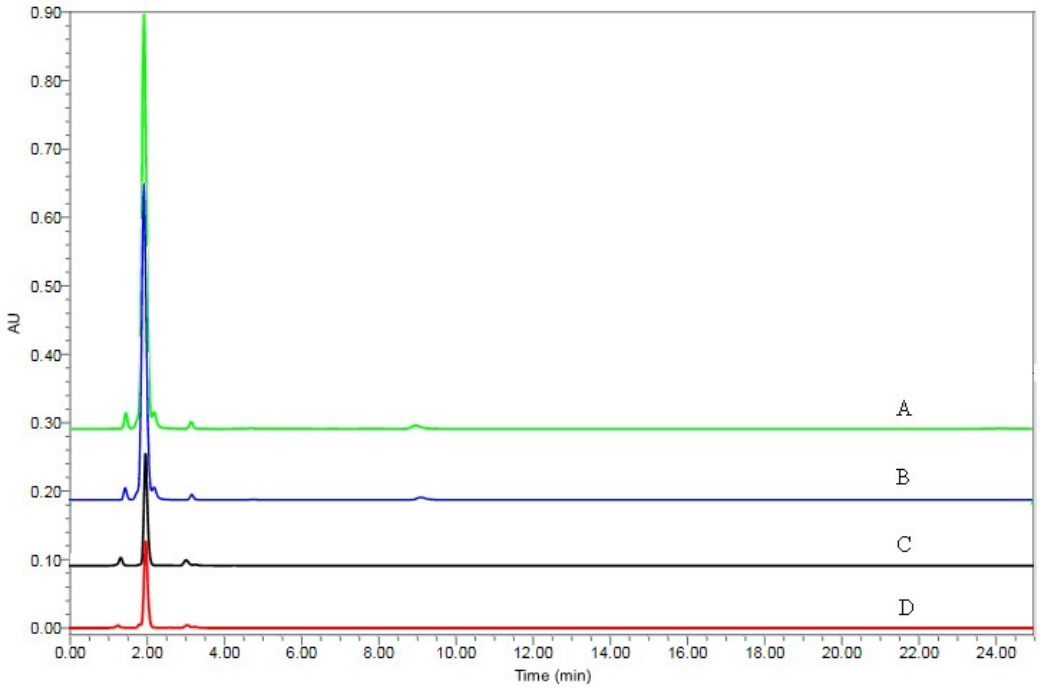

Fig. 8. Chromatograms of purged liquors from NFcSi with and without defatting steps in blank porks. Conditions: column, Shim-Pack VP-ODS (150 × 4.6 mm, 5 μm); mobile phase, MeOH/0.1% acetic acid-water (30:70, v/v); flow rate, 1.0 mL/ min; detection wavelength, 270 nm; column temperature, 30 °C.; injection volume, 10 μL. Chromatograms: (A) No SPE without defatted; (B) No SPE defatted; (C) NFcSi without defatted; (D) NFcSi defatted.

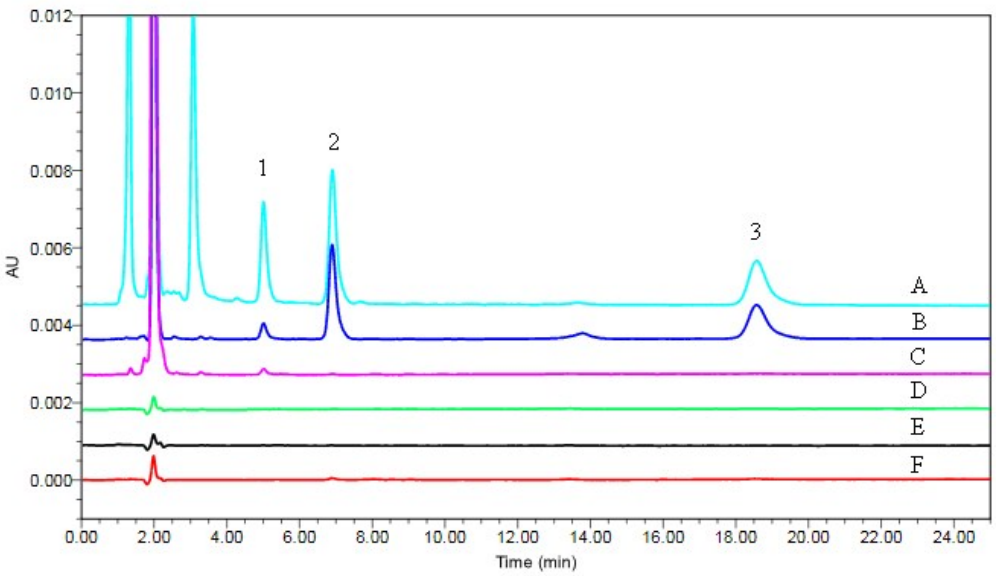

Fig. 9. Chromatograms comparison of purged liquors in blank pork spiked at 500 ng/mL sulfonamides from (A) NFcSi without defatting step and from (B) NH_2, (C) MCX, (D) C18, (E) HLB, (F) MAX with the defatting steps. Conditions were as for Figure 8. Peak: 1, SM_2; 2, SMZ; 3, SDM. The recoveries of three sulfonamides from each SPE cartridges were as follows. (A) NFcSi without defatting step, SM_2, 83.5%; SMZ, 90.5%; SDM, 80.2%; (B) NH_2, SM_2, 23.6%; SMZ, 88.7%; SDM, 80.1%; (C) MCX, SM_2, 15.4%; (D) C18, not detected; (E) HLB, not detected; (F) MAX, SMZ, 10.3%.

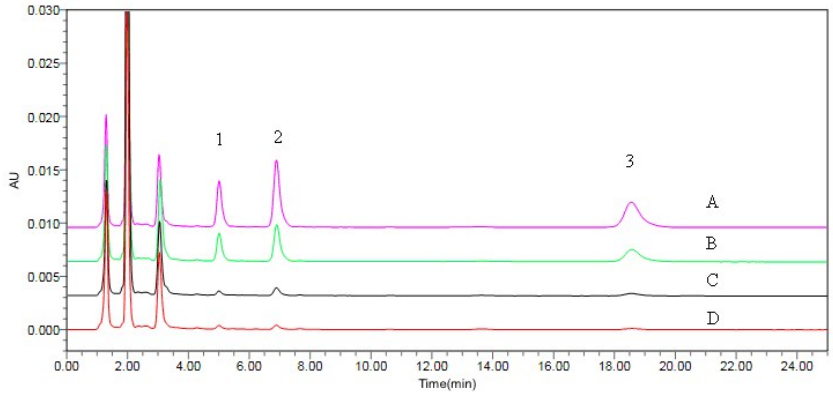

Fig. 10. Chromatograms of pork and spiked pork. Chromatograms: (A) pork spiked at 5000 ng/g;(B) pork spiked at 500 ng /g; (C) pork spiked at 50 ng /g; (D) pork. Conditions were as for Figure 8. Peak: 1, SM_2; 2, SMZ; 3, SDM.

Tab. 1. NFcS Stationary Phase and NFcSi SPE Sorbent.

	parameters of the silica gel		element analysis			
	particle size (μm)	specific surface area(m^2/g)	C (%)	H (%)	N (%)	bonded amount [a] (μmol/g)
ABS	5	300	4.08	1.08	1.35	/
NFcS stationary phase	5	300	9.12	1.61	1.45	300
ABS	40-60	500	7.09	1.70	2.42	/
NFcSi SPE sorbent	40-60	500	11.4	2.05	3.45	256

[a] Calculated from the carbon content.

Tab. 2. Linear Regression Equations and LODs of Sulfonamides.

sulfonamide	Linear Regression Equation	R^2	LOD（ng/g)
SM_2	$y = 43930\ x - 1351.3$	0.9998	4
SMZ	$y = 46946\ x - 1637.3$	0.9998	2
SDM	$y = 39563\ x - 933.43$	0.9998	5

Tab. 3. Analysis of Sulfonamide in Various Foodstuffs.

sample	sulfonamide	concentration (ng/g)	spiking 50 ng/g		spiking 500 ng/g		spiking 5000 ng/g	
			recovery (%)	RSD% (n=3)	recovery (%)	RSD% (n=3)	recovery (%)	RSD% (n=3)
pork	SM_2	82	73.4	3.62	75.6	1.29	72.3	4.29
	SMZ	98	83.5	2.55	90.2	2.67	85.6	4.16
	SDM	58	80.5	2.22	87.5	0.86	80.2	1.28
chicken	SM_2	16	72.5	3.68	78.0	1.25	78.2	3.22
	SMZ	58	82.3	2.28	89.6	0.92	86.5	1.76
	SDM	22	82.1	2.66	88.7	1.58	83.8	2.59
beef	SM_2	35	75.4	3.16	76.6	2.02	78.2	1.76
	SMZ	32	82.5	3.55	90.5	0.98	87.6	3.92
	SDM	28	83.6	4.32	80.6	1.68	81.3	4.02
egg	SM_2	95	71.1	4.27	80.8	2.51	84.0	1.83
	SMZ	92	70.6	2.36	70.8	1.57	72.0	3.52
	SDM	78	87.6	1.72	83.7	2.07	78.4	1.80
milk	SM_2	54	80.3	0.89	78.5	1.43	85.6	2.88
	SMZ	74	72.8	3.77	75.8	0.76	70.7	2.86
	SDM	N. D.[a]	84.3	1.39	84.9	2.45	90.7	0.94
honey	SM_2	N. D.[a]	75.9	2.51	86.7	0.78	75.5	3.24
	SMZ	92	85.4	0.86	91.2	1.59	93.6	2.54
	SDM	N. D.[a]	72.4	1.69	75.2	4.67	72.8	2.98

[a] N.D.: not detected.