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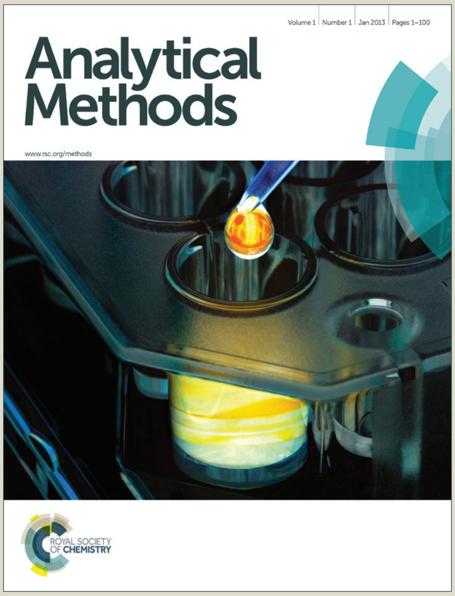

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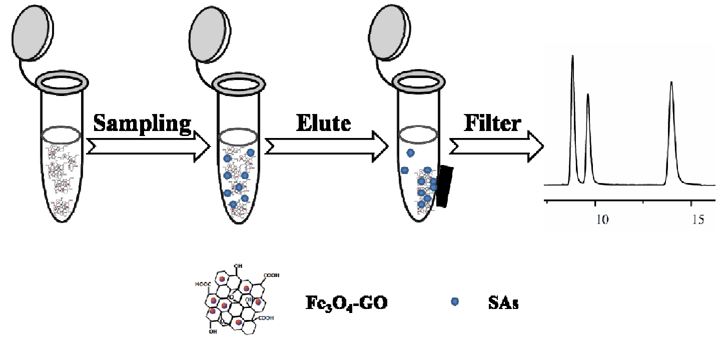

Magnetite-graphene oxide composites were used for magnetic solid-phase extraction to preconcentrate the trace sulfonamides in water samples before determination by high-performance liquid chromatography.

Magnetite-graphene oxide composites as a magnetic solid-phase extraction adsorbent for the determination of trace sulfonamides in water samples

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Abstract: A facile approach was used to prepare Fe_3O_4-graphene oxide (Fe_3O_4-GO) nanocomposites, and the application of these nanocomposites was investigated in the magnetic solid-phase extraction (MSPE) coupled with high-performance liquid chromatography with diode-array detection (HPLC-DAD) of three sulfonamides (SAs) from water samples. The Fe_3O_4-GO nanocomposites possess the advantages of both GO and Fe_3O_4. The Fe_3O_4-GO nanocomposites were characterized by scanning electron microscopy and vibrating-sample magnetometry. Sulfadiazine (SDZ), sulfadimidine (SDD) and sulfathiazole (STZ) were selected as target analytes to validate the extraction performance of the Fe_3O_4-GO nanocomposites as a MSPE adsorbent. The results indicated that the limits of detection of these sulfonamides were in the range of 0.05–0.10 μg/mL, that the recoveries ranged between 67.4% and 119.9%, and that the linearity ($R^2 > 0.9900$) and precisions (between 0.04% and 9.03%) were good. The developed method can be used to determine and monitor SA residues in environmental water samples.

1 Introduction

Sulfonamides (SAs), a class of antibacterial drugs, have been commonly used in medical and veterinary applications to treat numerous human and animal infectious diseases [1]. Because of the persistence and relatively high mobility of SAs in the environment, they can enter the groundwater and be carried into aquifers and surface waters [2]; therefore, the establishment of an effective method for the determination of trace SAs is necessary.

In the development of sample pretreatment techniques, solid-phase extraction (SPE) [3-6], liquid-liquid extraction (LLE) [7], dispersive liquid-liquid microextraction (DLLME) [8-10], molecularly imprinted polymers (MIPs) [11], and magnetic solid-phase extraction (MSPE) [12] have been used to preconcentrate SAs from environmental water samples and other real samples. MSPE was developed as a novel mode of SPE; its adsorbents include magnetic and magnetizable materials. This method has significant advantages in separation science, such as straightforward separation of solids and liquids, which saves time and facilitates operation. Sun *et al.* [12] used alumina-coated magnetite nanoparticles as an MSPE adsorbent to preconcentrate seven SAs in soil samples and demonstrated the strong potential of the MSPE method in the pretreatment of complicated samples. Gao *et al.* [13] used synthesized microspheres to determine eleven SAs in milk samples, and their results indicated that the extraction can be carried out quickly.

Graphene, as a new allotrope of carbon, has a high profile in the scientific and engineering communities [14]. Graphene oxide (GO) is the synthetic precursor of graphene. It possesses a large surface area and exhibits fast carrier mobility and excellent optical transparency, which makes it a novel SPE adsorbent. The graphene-based SPE and MSPE methods have been used to enrich various compounds for environmental analysis, food-safety analysis and bioanalysis. Recent reports have demonstrated that graphene and graphene-based materials are outstanding SPE materials that provide high extraction efficiencies and enrichment factors, and low cost and low consumption can be attained [15]. A recent study indicated that graphene and graphene-based materials exhibit higher adsorption capacities for analytes with benzene-ring structures because of π-π stacking interactions [16]. Tabani *et al.* [17] used graphene oxide-SPE combined with electromembrane extraction to ultra-preconcentrate chlorophenoxy acid herbicides in environmental samples and obtained high enrichment factors. Luo *et al.* [18] employed magnetic graphene as the adsorbent for the extraction of SA antibiotics from environmental water samples to expand the applications of graphene in analytical chemistry. Han *et al.* [19] developed a Fe_3O_4/GO-MSPE method and used it to preconcentrate several polycyclic aromatic hydrocarbons in environmental water samples.

Because of the advantages of magnetic materials and the adsorption capacity of GO, we synthesized Fe_3O_4-GO and used it as an MSPE adsorbent, coupled with high-performance liquid chromatography (HPLC), to enrich and determine trace sulfadiazine (SDZ), sulfadimidine (SDD) and sulfathiazole (STZ) in water samples.

As shown below, the developed method can be used to determine SAs in environmental water samples.

2 Experimental

2.1 Reagents and Samples

Analytical standards of SDZ and SDD were obtained from the National Institutes for Food and Drug Control (Beijing, China). STZ and acetonitrile (HPLC grade) were purchased from Sigma-Aldrich (Shanghai, China). The structures of the three analytes are shown in **Fig. 1**. Graphene oxide (GO) was supplied by Nanjing XFNano Material Tech Co. (Nanjing, China). The diameter of the GO was in the range of 500 nm to 5 μm, and its thickness was in the range of 0.8 to 1.2 nm. Iron chloride hexahydrate ($FeCl_3$•$6H_2O$) was provided by Xilong Chemical Co., Ltd. (Guangdong, China). Iron (II) chloride tetrahydrate ($FeCl_2$•$4H_2O$) was obtained from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Methanol (HPLC grade) was purchased from Merck (Darmstadt, Germany). Acetone (analytical grade) was supplied by Beijing Chemical Works (Beijing, China). The experimental water was purified with a Milli-Q apparatus (Millipore, USA).

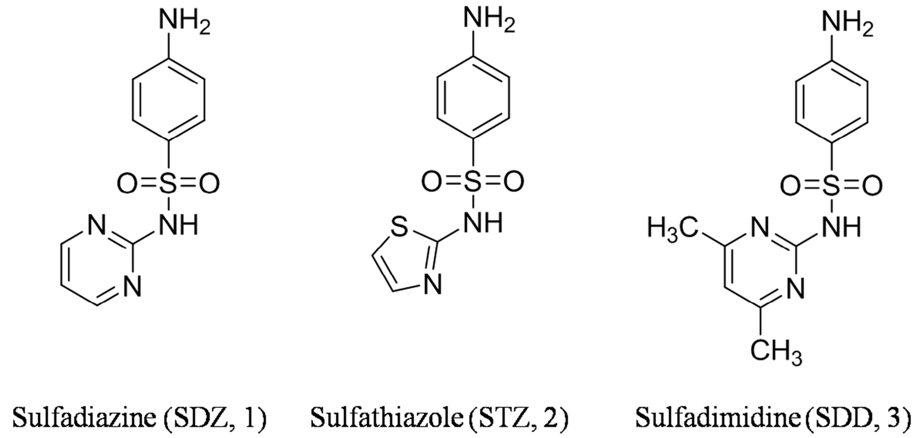


Fig. 1 The structures of the three SAs. *(1-SDZ; 2-STZ; 3-SDD)*

Individual stock solutions of the three analytes were prepared in methanol at a concentration of 1.0 mg/mL and were stored in the dark at 4 °C. Working solutions containing all three of analytes were prepared by appropriate dilution of the stock solutions.

Tap-water samples were collected from a water tap in our laboratory (Beijing, China) and river-water samples were collected from the Juma River (Beijing, China). The river- and tap-water samples were filtered through a 0.45 μm filter membrane immediately after being sampled and were subsequently stored at 4 °C.

2.2 Apparatus

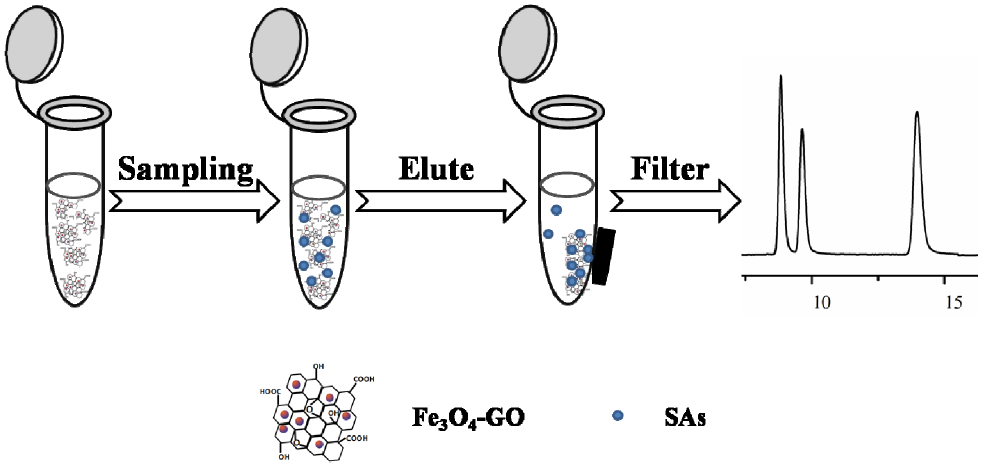
Scanning electron microscopy images were obtained using an S-4800 scanning electron microscope (Hitachi, Japan). The magnetization curves were recorded using a 7410 vibrating-sample magnetometer (Lake Shore, USA). The HPLC-DAD analysis was performed on an Agilent 1200 system (Agilent, USA). The three SAs were separated on an analytical reversed-phase C_{18} column (YMC-Triart C_{18}, 5 µm particle diameter, 4.6 mm i.d. × 25 cm) (YMC, Japan) at 25 °C. An isocratic gradient mobile phase of acetonitrile-water (85:15, v/v) at a flow rate of 1.0 mL/min was used for the chromatographic separation of the three SAs. The detection wavelength was set at 270 nm, and the injection volume was 10 µL.

2.3 Synthesis of Fe_3O_4-GO nanocomposites

A facile method based on a previously reported method was used to synthesize the Fe_3O_4-GO nanocomposites. The overall synthetic procedure is as follows. First, 0.65 g of $FeCl_3$•$6H_2O$ and 0.25 g of $FeCl_2$•$4H_2O$ were dissolved in 50 mL of ultrapure water, and 0.05 g of GO was dispersed into the aqueous solution to obtain a homogeneous suspension by ultrasonication. Second, NH_3•H_2O was added to adjust the pH of the final mixture to 9-10, and the nanocomposites were synthesized by ultrasonication for 1 h. Finally, the synthesized Fe_3O_4-GO nanocomposites were washed with water and methanol several times and then dried at 80 °C. The Fe_3O_4-GO nanocomposites were ground into powders for the subsequent experiments.

2.4 Magnetic solid-phase extraction (MSPE) procedure

Fe_3O_4-GO nanocomposites were used as the MSPE adsorbent; the extraction procedure is shown in **Scheme 1**. First, 5.0 mg of Fe_3O_4-GO nanocomposites were accurately weighed and dispersed into a 1.0 mL of an aqueous sample solution by being vortexed at 2,000 rpm for 20 min to form a homogeneous suspension. Second, after adsorption equilibrium was reached, the adsorbent was isolated from the sample solution using a strong magnet, and the solution was removed. Then, 1.0 mL of methanol was used to elute the analytes adsorbed onto the adsorbent by vortexing at 2000 rpm for 10 min; the eluent was collected and filtered through a 0.22 μm organic membrane. Finally, the collected elution was evaporated to dryness under nitrogen at 40 °C. The residuum was redissolved in 0.2 mL of methanol/water (1:1, v/v) for HPLC analysis.



Scheme 1 The proposed MSPE procedure.

3 Results and Discussion

3.1 Characterization of Fe$_3$O$_4$-GO nanocomposites

The morphology of the prepared nanocomposites was characterized by scanning electron microscopy (SEM). As shown in **Fig. 2a**, the GO sheet exhibited an irregular shape with some wrinkles and maintained a large surface area, which provided a large number of adsorption sites. Moreover, we observed that the pristine Fe$_3$O$_4$

nanoparticles were aggregated (**Fig. 2b**), whereas the Fe_3O_4 nanoparticles were homogeneously anchored onto the surface of the GO sheets (**Fig. 2c**).

The magnetic properties of the Fe_3O_4 nanoparticles and the Fe_3O_4-GO nanocomposites were determined at room temperature using a vibrating-sample magnetometer by cycling the field between -20,000 and 20,000 Oe (**Fig. 2d**). None of the curves exhibited magnetization hysteresis loops, which indicated that the Fe_3O_4 nanoparticles and Fe_3O_4-GO nanocomposites were superparamagnetic. The saturation magnetization value was 28.1 emu/g for the Fe_3O_4-GO nanocomposites, which ensured complete magnetic separation from aqueous solutions under an external magnetic field.

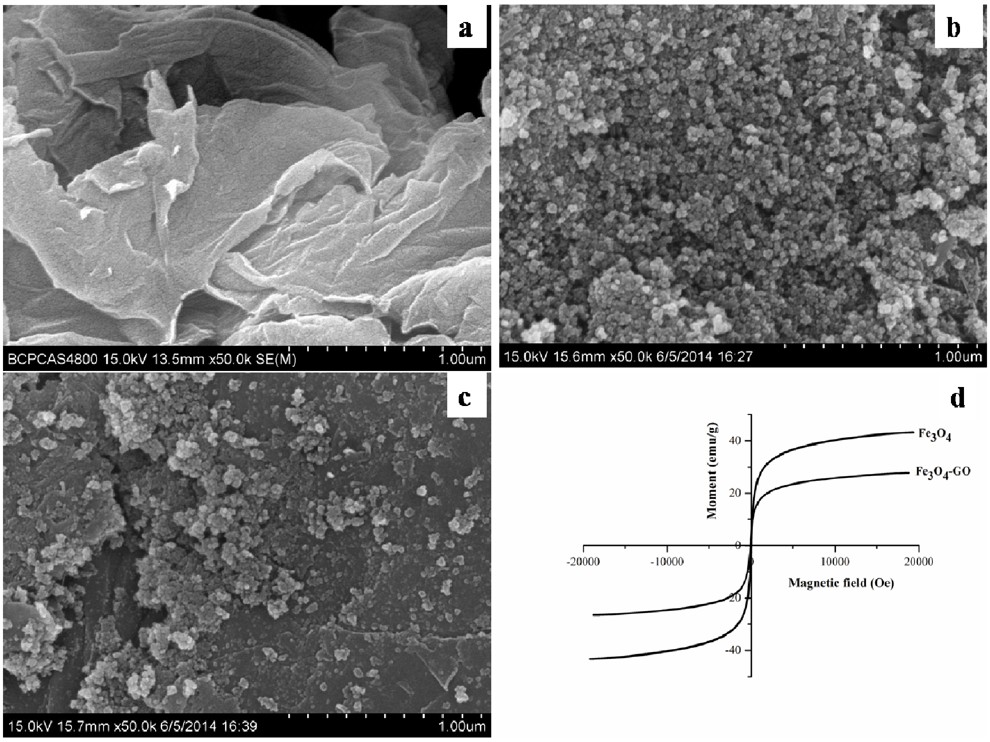


Fig. 2 SEM images of GO (a), Fe_3O_4 (b) and Fe_3O_4-GO nanocomposites (c), and the results of VSM measurements of Fe_3O_4 and Fe_3O_4-GO nanocomposites (d).

3.2 Optimization of the MSPE procedure

To obtain the optimal effect for the three SAs, 1.0 mL of ultrapure water spiked with 20 μg/mL of each of the three SAs was employed to investigate the MSPE performance. The elution solvents, elution time and extraction time were optimized. In addition, we investigated the amount of Fe_3O_4-GO nanocomposites adsorbed.

The selection of an effective solvent for the elution of the SAs adsorbed onto the adsorbent is important. In this study, three different organic solvents with a volume of 1.0 mL were used to elute the SAs from Fe_3O_4-GO nanocomposites. The obtained peak areas are shown in **Fig. 3a**. Methanol was observed to exhibit a greater desorption capacity than acetonitrile or acetone.

The extraction time is also a significant parameter in MSPE. A short extraction time leads to incomplete adsorption of the target substance in the solution onto the adsorbent; however, a long extraction time makes the MSPE process unnecessarily lengthy. Therefore, different extraction times were investigated. As shown in **Fig. 3b**, the peak area reached a maximum for the three SAs at 20 min. We concluded that the adsorption equilibrium between the adsorbent and the sample solution was achieved at 20 min. As a result, an extraction time of 20 min was selected for subsequent experiments.

When methanol was used for MSPE elution, the elution time was further optimized to achieve the optimal elution effect. As shown in **Fig. 3c**, no significant differences were observed between elution times of 5 min and 40 min. To save time, 10 min was chosen for the elution time.

In addition, we investigated the adsorption capacity of the synthesized Fe_3O_4-GO nanocomposites; the results are shown in **Fig. 3d**. Ten milligrams of Fe_3O_4-GO nanocomposites were used to preconcentrate the SAs under the optimized conditions. We concluded that the adsorption capacities of the synthesized Fe_3O_4-GO nanocomposites were 10 μg/mg, 8 μg/mg and 6 μg/mg for SDZ, STZ and SDD, respectively.

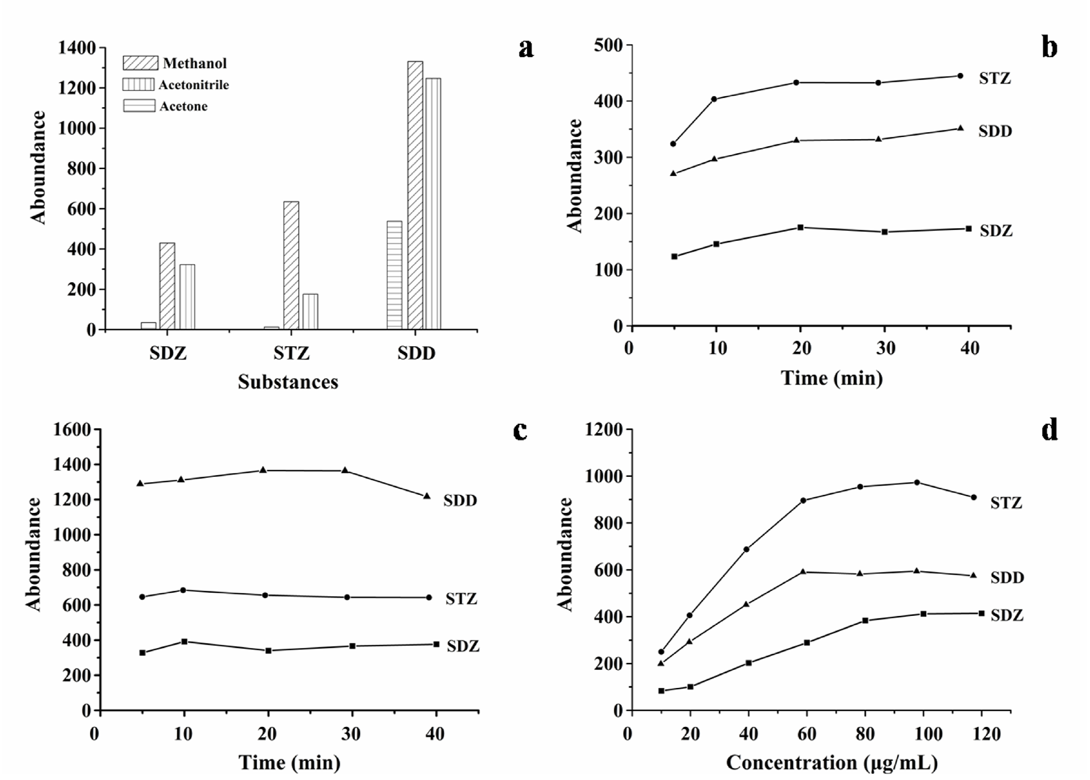


Fig. 3 The effect of elution solvent (a), extraction time (b), and elution time (c) for the MSPE procedure, and the adsorption capacity of Fe_3O_4-GO nanocomposites for SDZ, STZ and SDD (d).

3.3 Validation of the developed method

The analytical performance obtained by the proposed Fe_3O_4-GO-based MSPE method is presented in **Table 1**. Good linear relationships were obtained for all three of the SAs (R^2 = 0.9900-0.9998) in the concentration range of 0.20-20 μg/mL, and the limits of detection (LODs) varied from 0.05-0.10 μg/mg. In addition, the precision of the developed method was assessed by performing intra-day and inter-day assays. The intra-day precisions were measured for six parallel procedures in one day and the RSDs of the peak areas were in the range of 0.05-9.03% (*n* = 6). The inter-day precisions were calculated on three consecutive days and the RSDs of SAs peak areas were in the range of 0.30-5.48% (*n* = 9), respectively. Thus, the proposed method exhibited excellent precision.

Table 1 Analytical parameters of the Fe_3O_4-GO MSPE method

Analyte	Linear range (μg/mL)	Regression equation	R^2	LODs (μg/mL)	Intra-day precision (*n* = 6)		Inter-day precision (*n* = 9)	
					Retention time	Peak area	Retention time	Peak area
SDZ	0.2-20	y=67.01x+45.92	0.9930	0.05	0.05%	9.03%	0.30%	5.48%
STZ	0.2-20	y=57.70x+0.44	0.9998	0.10	0.04%	5.38%	0.41%	5.07%
SDD	0.2-20	y=85.93x+48.94	0.9900	0.05	0.08%	5.74%	0.32%	3.19%

3.4 Application of the Fe_3O_4-GO-based MSPE method to real water samples

The developed method was used to determine three SAs in real environmental water samples of river and tap water. The filtered samples were spiked with SAs at concentrations of 2.0 and 5.0 μg/mL and were subsequently analyzed using the proposed method (*n* = 3). The recoveries, which ranged from 80.2-119.9% for the river-water samples and 67.4-90.1% for the tap-water samples, are listed in **Table 2**. The typical chromatograms obtained from unspiked water, water spiked with 2.0 μg/mL of SAs (without treatment) and water spiked with 2.0 μg/mL of SAs (with treatment) were shown in **Fig. 4**. The satisfactory recoveries indicated the absence of obvious effects from the matrix composition of the water samples.

Table 2 Recoveries from environmental water samples (tap water and river water, *n* = 3)

Analyte	River water		Tap water	
	Concentration added (μg/mL)	Average recovery (%)	Concentration added (μg/mL)	Average recovery (%)
SDZ	2.0	118.6	2.0	67.4
	5.0	119.9	5.0	72.1
STZ	2.0	84.0	2.0	76.7
	5.0	99.4	5.0	75.6
SDD	2.0	80.2	2.0	72.0
	5.0	104.7	5.0	90.1

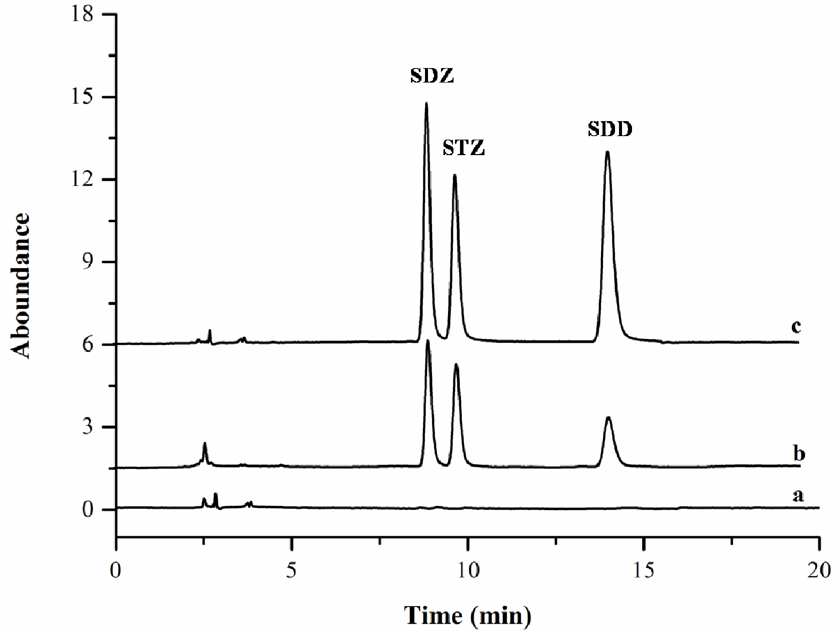


Fig. 4 A typical chromatogram of unspiked water (a), water spiked with 2.0 µg/mL of SAs (without treatment) (b) and water spiked with 2.0 µg/mL of SAs (with treatment) (c).

3.5 Comparison with other methods

A comparison of the developed method with other methods for the extraction of SAs is presented in **Table 3**. The results revealed significant advantages for the proposed method in the extraction and determination of SAs from environmental water samples. Lower LODs, satisfactory recoveries and favorable precision were obtained with the proposed MSPE method compared to other methods. The results indicated that the Fe_3O_4-GO nanocomposites exhibited excellent adsorption capacity. Thus, Fe_3O_4-GO nanocomposites-based MSPE could be used in a novel and simple method for the effective extraction of analytes from complicated matrices.

Table 3 Comparison of proposed method with other methods applied for SAs

Sample preparation	Method	Samples	Linear range (μg/mL)	LODs	Recovery (%)	RSDs (%)	Refs.
LLE	LC-MS	biogas plants	0.025-10	0.01-0.08 mg/kg	70-130	1.9-16.9	[7]
SPE	CZE	meat	0.5-50	0.028-0.063 mg/kg	60.9-111.4	2.5-3.4	[3]
DLLME	HPLC	milk	2.01×10^3-2.5×10^5	0.60-1.21 μg/L	90.8-104.7	2.9-9.7	[8]
MSPE	HPLC	environmental water	0.0005-0.1	0.09-0.16 ng/mL	74.2-104.1	3.3-10.7	[18]
GCE	HPLC	milk	0.06-10.5	1.2-6.0 ng/mL	96-104	3.5-7.8	[6]
MSPE	HPLC	environmental water	0.2-20	0.05-0.1 μg/mL	67.4-119.9	0.04-9.0	Proposed method

4 Conclusions

In this work, Fe_3O_4-GO nanocomposites were prepared using a simple method and were subsequently employed as adsorbents for the enrichment of trace amounts of three SAs in environmental water samples because of the convenience with which the materials are manipulated and their high adsorption capacity. The results obtained using the described methodology were superior to those obtained using classical preparation procedures, such as SPE and DLLME; the nanocomposites adsorbent provided lower LODs, wider linear ranges, satisfactory recoveries and favorable precisions. The experimental results were consistent with previous results reported in the literature [16], and the large delocalized π-electron system of the graphene can form strong π-π stacking interactions with aromatic rings. We demonstrated that Fe_3O_4-GO nanocomposites-based MSPE is effective for sample pretreatment. Most importantly, the results of this work expand the applications of GO.

Acknowledgments

This work was supported by National Natural Science Foundation of China (Grant No. 21005050) and Beijing Natural Science Foundation (Grant No. 2133061).

All experiments were performed in compliance with relevant laws.

The authors declare that there are no conflicts of interest.

Notes and references

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References

1 J. D. Li, Y. Q. Cai, Y. L. Shi, S. F. Mou and G. B. Jiang. *J. Chromatogr. A*, 2007, **1139**, 178-184.

2 L. Sun, L. G. Chen, X. Sun, X. B. Du, Y. S. Yue, D. Q. He, H. Y. Xu, Q. L. Zeng, H. Wang and L. Ding. *Chemospere*, 2009, **77**, 1306-1312.

3 N. S. Ye, P. Z. Shi, Q. Wang and J. Li. *Chromatographia*, 2013, **76**, 553-557.

4 N. Sun, Y. H. Han, H. Y. Yan and Y. X. Song. *Anal. Chim. Acta*, 2014, **810**, 25-31.

5 M. U. Farooq, P. Su and Y. Yang. *Chromatographia*, 2009, **69**, 1107-1111

6 A. M. Bueno, A. M. Content and A. Rios. *J. Sep. Sci.*, 2014, **37**, 382-389.

7 A. Spielmeyer, J. Ahlborn and G. Hamscher. *Anal. Bioanal. Chem.*, 2014, **406**, 2513-2524.

8 N. Arroyo-Manzanares, L. Gamiz-Gracia and A. M. Gracia-Cammpana. *Food Chem.*2014, **143**, 459-464.

9 X. Xu, R. Su, X. Zhao, Z. Liu, Y. P. Zhang, D. Li, X. Y. Li, H. Q. Zhang and Z. M. Wang. *Anal. Chim. Acta*, 2011, **707**, 92-99.

10 Y. Y. Wen, J. H. Li, W. W. Zhang and L. X. Chen. *Electrophoresis*, 2011, **32**, 2131-2138.

11 X. Z. Shi, Y. Meng, J. H. Liu, A. L. Sun, D. X. Li, C. X. Yao, Y. Lu and J. Chen. *J. Chromatogr. B*, 2011, **879**, 1071-1076.

12 L. Sun, X. Sun, X. B. Du, Y. S. Yue, L. G. Chen, X. Y. Xu, Q. L. Zeng, H. Wang and L. Ding. *Anal. Chim. Acta*, 2010, **665**, 185-192.

13 Q. Gao, D. Luo, J. Ding and Y. Q. Feng. *J. Chromatogr. A*, 2010, **1217**, 5602-5609.

14 Q. Liu, J. B. Shi and G. B. Jiang. *TrAC-Trend Anal. Chem.*, 2012, **37**, 1-11.

15 N. S. Ye and P. Z. Shi. *Sep. Purif. Rev.*, 2015, **44**, 183-198.

16 S. Wu, X. Q. Lan, L. J. Cui, L. H. Zhang, S. Y. Tao, H. N. Wang, M. Han, Z. G. Liu and C. G. Meng. *Anal. Chim. Acta*, 2011, **699**, 170-176.

17 H. Tabani, A. R. Fakhari, A. Shahsavani, M. Behbahani, M. Salarian, A. Bagheri and S. Nojavan. *J. Chromatogr. A*, 2013, **1300**, 227-235.

18 Y. B. Luo, Z. G. Shi, Q. A. Gao and Y. Q. Feng. *J. Chromatogr. A*, 2011, **1218**, 1353-1358.

19 Q. Han, Z. H. Wang, J. F. Xia, S. Chen, X. Q. Zhang and M. Y. Ding. *Talanta*, 2012, **101**, 388-395.