ISSN: 1813-4890

Determination of four sulfonamides in pork by graphene oxide solid phase extraction with LC-MS/MS

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Abstract

Graphene oxide was used as solid phase extraction material in the determination of sulfonamides residues in pork by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The samples were extracted by acetonitrile, degreased with n-hexane and evaporated to dry by rotation, dissolved with 3 mL acetonitrile-aqueous solution (10+90, volume ratio), adjusted pH to 6, purified by graphene oxide solid phase extraction column, and tested by LC-MS/MS. The samples were separated by 3.0×100 mm, 2.7 μm column (Poroshell 120 EC-C_{18}), detected in multi-reaction monitoring mode, and quantified by external standard method.The results showed that the four sulfonamides showed a good linear relationship in the concentration range of 10-500 μg/L, the correlation coefficient r=0.9979-0.9996, the recovery ratewas 72%-86% and the limit of quantitation was 10 μg/kg.

Keywords

Graphene oxide; Liquid chromatography-tandem mass spectrometry; Pork; Sulfonamides.

1. Introduction

Sulfonamides (SAS) has been widely used in the breeding process due to its advantages of broad spectrum antibacterial, cheap and convenient. The overuse of sulfonamides will cause residual, which will bring health threat to the user. China stipulates that the total maximum residue limit of sulfonamides in animal-derived foods is 100 μg/kg [1]. At present, there have been many reports on the detection of sulfonamides residues [2-5]. The more widely used method in the pre-treatment process is solid phase extraction [6-8], and the main fillers involved are carbon nanotubes, alumina, PSA, C18 and GCB.

Graphene oxide is a kind of two-dimensional structural nanomaterial prepared by natural graphite [9]. It not only has a large specific surface area, but also has carboxyl, carbonyl and other active groups, which has a good performance in adsorption properties. In this paper, graphene oxide solid phase extraction combined with liquid chromatography-tandem mass spectrometry was used to establish to determine the content of sulfadiazine (SDZ), sulfamethoxazole (SMZ), sulfadimethoxine (SDM), and sulfaquinoxaline (SQX) residues in pork.

2. Experimental Part

2.1. Instruments and reagents

1290-6460 liquid chromatography-tandem mass spectrometer with electro spray ion source (Agilent Company, USA); GM200 high speed homogenizer (IKA company, Germany); MMV-1000W oscillator(Tokyo Riken company, Japan); CR22GⅢ high speed refrigerated centrifuge (Hitachi company, Japan); R215 rotary evaporation instrument(Buchi company, Switzerland); MS3 vortex mixer(IKA company, Germany).

ISSN: 1813-4890

Sulfadiazine standard products: sulfadiazine, sulfamethoxazole, sulfadimethazine, sulfaquinoxaline, all purchased from Dr. Company of German, the purity is above 98%. The water used in the experiment was ultra-pure water; Methanol, acetonitrile and acetone were chromatographically pure. Anhydrous sodium sulfate and ammonia were analytically pure. Before use, the anhydrous sodium sulfate should be burned at 650℃ for 4 hours and placed in a dryer for use.

2.2. Instrument Conditions

Column: Poroshell 120 EC-C_{18}, 3.0×100 mm, 2.7 μm; Column temperature:30℃; Injection volume: 5 μL; Mobile phase: A was 0.1% formic acid water, B was methanol; The gradient elution conditions were: 0-1 min, 10-30%B; 1-3 min, 30-80%B; 3-7 min, 80%B; 7-9 min, 80-10%B; 9-10 min, 10%B. Flow rate: 0.3 mL/min.

Ion source: electro spray ion source(with ion focusing); Scanning mode: positive ion; Detection mode: MRM (multi-reaction monitoring); Dry temperature: 350℃; Dry gas flow rate: 10 L/min; Atomizing gas pressure: 45 psi; Sheath temperature: 350℃; Sheath gas flow rate: 11 L/min; Capillary voltage: 4000V; Collision gas: high purity nitrogen; The dwell time of each ion pair is 60 ms. The mass spectrum parameters of the four sulfonamides are shown in Table 1.

Table 1. Mass spectrum parameters of four sulfonamides

Compound Names	Qualitative ion pair (m/z)	Quantitative ion pair (m/z)	Breakage voltage (V)	Impact energy (eV)
Sulfadiazine, SDZ	251.0/156.0	251.0/156.0	100	15
	251.0/92.0		100	40
Sulfamethoxazole, SMZ	254.0/156.0	254.0/156.0	100	12
	254.0/92.2		100	25
Sulfadimethoxil, SDM	311.1/156.0	311.1/156.0	100	14
	311.1/108.0		100	21
Sulfaquinoxaline, SQX	301.1/156.0	301.1/156.0	80	10
	301.1/92.0		80	30

2.3. Sample Handling

Extraction: Weigh 5 g (±0.01 g) sampleinto50 mL centrifuge tube, add 15 mL acetonitrile, homogenize for 1 min, then add 10g anhydrous sodium sulfate, shake and extract for 1min, centrifuge for 8 min at 9000 r/min, repeat the operation, combine the extraction solution, add 10 mL acetonitrile-saturated n-hexane, shake and stand for delamination. The acetonitrile layer was transferred to a pear-shaped bottle, and concentrated to dry by rotary evaporation in a water bath at 40℃. The residue is dissolved in 3 mL acetonitrile-water solution (10+90, volume ratio), pH adjusted to 6, to be purified.

Purification: 3 mL methanol and 3 mL water were used to activate the prepared graphene solid phase extraction column, and then the sample solution was passed through the column at the rate of 1 mL/min, and finally elution was carried out with 5 mL ammoniacinitrile. The eluent was collected into a 15 mL centrifuge tube, and then dissolved with 1mL mobile phase after blowing dry with nitrogen, after through 0.22 μm organic phase filter membrane, the solution was used for machine detection.

3. Results and discussion

3.1. Optimization of graphite oxide dosage

Too little graphene oxide will lead to the loss of the target compound, and too much will adsorb the targetcompound, resulting in reduced recovery. In this study, the effects of 3, 5, 8, 10, 15 and 20 mg of grapheme oxide on the extraction efficiency were investigated. The experimental

ISSN: 1813-4890

results showed that the peak area of each compound increased when the amount of graphene oxide increased from 3 mg to 15 mg. The amount of graphene oxide on the extraction efficiency is shown in Fi. 1. When the amount of graphene oxide reached 15 mg, the peak area of each compound reached the highest value. After that, the peak area of each compound decreased when the amount of graphene oxide continued to increase. Therefore, the amount of graphene oxide was set at 15 mg.

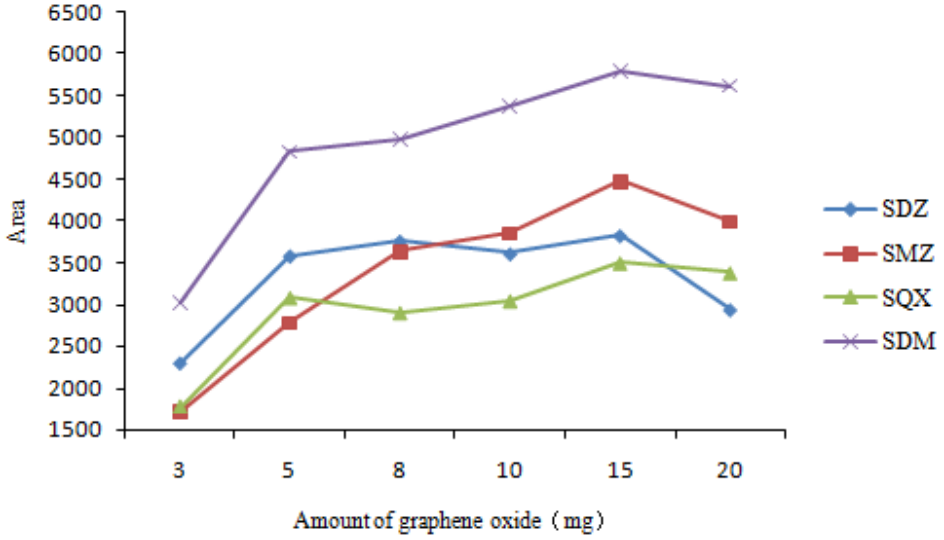

Fig. 1 The amount of graphene oxide

3.2. Effect of solution pH

The pH of the solution can affect the properties of the dispersed solid phase extractant and the target compound and the way they are combined. In this experiment, the effect of pH on the extraction efficiency was investigated when pH varied in the range of 4~10. The target substance fluctuated with pH, which may be related to the amphoteric properties of sulfonamides. According to the experimental results, and in order to facilitate the adjustment of pH, the pH value of the sample solution was adjusted to 6 in actual operation. The effect of pH on the extraction efficiency is shown in Fig. 2.

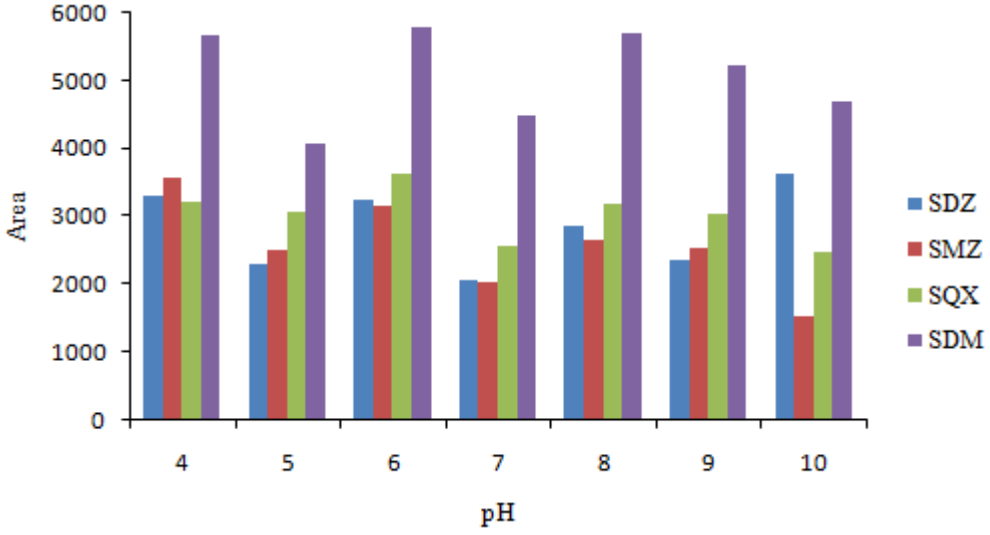

Fig. 2 The effect of pH on the extraction efficiency

3.3. Influence of ionic strength

Adding salt to the solution can reduce the solubility of the object to be tested, and then affect the distribution coefficient between the adsorbent and the sample solution, but the high concentration of salt will produce viscous resistance, thereby reducing the extraction efficiency.

ISSN: 1813-4890

In this experiment, sodium chloride was added to the solution to adjust the ionic strength, and its influence on the extraction efficiency was investigated. The concentration of sodium chloride in the sample solution was adjusted to 0, 5, 10, 15, 20 and 25% respectively. The results showed that there was no significant change in the peak area of the substance to be measured. Therefore, the ionic strength was not adjusted in this study.

3.4. Choice of eluent

The elution effect of methanol, ammoniated methanol, acetonitrile, ammoniated acetonitrile, acetone and ammoniated acetone were investigated respectively. The results showed that the elution effect of methanol and acetone was similar and superior to that of acetonitrile. The elution effect of methanol and acetonitrile with ammonia was improved obviously, and the elution efficiency of acetonitrile with ammonia was the highest, while the efficiency of acetone with ammonia was reduced. Therefore,ammoniated acetonitrile was selected as the elution solvent in this study.

3.5. Optimization of elution volume

After solid phase extraction under the same experimental conditions, elution was carried out with 1, 2, 3, 4, 5 and 6 mL eluent respectively. The peak area of each compound increased with the increase of elution volume in the range of 1~5 mL, reaching the maximum value at 5 mL, and then increasing the elution volume had no effect on the peak area of the target compound. Therefore, the elution volume in this study was set at 5 mL.

3.6. Optimization of instrument conditions

The four standard sulfonamide solutions were injected to test the separation effect under different combination conditions of formic acid water, ammonium acetate, methanol and acetonitrile, and the proportion and flow rate of mobile phase were adjusted. It was found that the peak shape and separation effect of the four sulfonamides were all good under the condition of gradient elution. At this time, the mobile phase used was methanol and formic acid water (0.1%). The flow rate of the mobile phase was 0.3 mL/min.

According to the structural characteristics of sulfonamides, ESI+ was selected as the ionization mode. Firstly, the primary mass spectrometry of each sulfonamide was performed to obtain the molecular ion peak of each compound, and the corresponding parent ion peak was selected to optimize its fragmentation voltage, and then the secondary mass spectrometry was performed to obtain the fragment ion, collision energy and other information. The optimized parent ion, daughter ion, fragmentation voltage and collision energy were used to establish multiple reaction monitoring (MRM) parameters for sulfanilamide detection. The MRM diagram of four sulfonamides is shown in Fig. 3.

3.7. Linear range, limit of quantitation and recovery rate

In order to avoid the influence of matrix effect, the standard solution was prepared by matrix addition method and the concentrations were 10, 20, 50, 100, 200 and 500 μg/L, respectively. The experimental results showed that in the concentration range of 10~500 μg/L, the linear relationship between the foursulfonamides was good, and the correlation coefficient was 0.9979~0.9996. The linear equation and correlation coefficient of the four sulfonamides were shown in Table 2. The limits of quantitation of sulfanilamide were determined by 10 times signal-to-noise ratio (SNR), all of which were 10 μg/kg.

5 g (accurate to 0.01 g) samples were accurately weighed, and four mixed standard sulfanilamide solution at three levels of 50 μL, 100 μL and 150 μL 1.00 mg/L were accurately added, which were pre-treated and determined on the machine under optimized experimental conditions. Each level was measured in parallel for 6 times, and the recovery rate was calculated. The average recovery rate was 72%-86%.

ISSN: 1813-4890

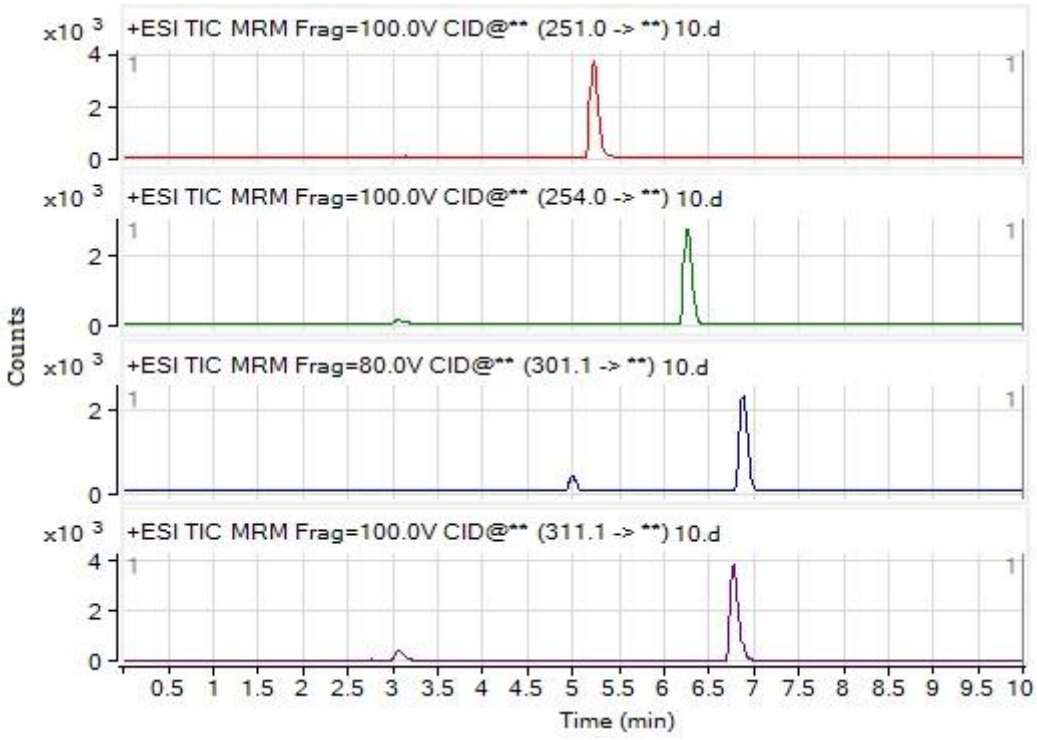

Fig. 3 MRM diagram of four sulfonamides

Table 2 Linear equation, correlation coefficient and limit of quantification of four sulfonamides (n=6)

Compound Name	Linear equation	Correlation coefficient	Limit of quantification (μg/kg)
SQX	Y=7324*X +12535	0.9987	10
SDZ	Y=15354*X+40825	0.9982	10
SDM	Y=17628*X -45086	0.9996	10
SMZ	Y=6915*X +15711	0.9979	10

4. Conclusion

Using graphene oxide as solid phase extraction material, a method was established for the determination of residues of four sulfonamides in pork by using solid phase extraction combined with liquid chromatography-tandem mass spectrometry. By optimizing the conditions of solid phase extraction, mass spectrometry and liquid chromatography, the purification effect is obvious, the matrix interference is reduced, the recovery rate is high, and the detection efficiency is improved. The established method is fast, efficient and practical.

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