SHIMADZU

LC-MS/MS method development for sulfites in food and beverage

ThP 151

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1. Introduction

Sulfites are one of the most common food additives used as antioxidants and bleaching agents in a variety of foods. Sulfites are added to a wide range of products, including dried fruits and vegetables, frozen shrimp, juices, and wine. Although they are very useful food additives, ingestion of products containing sulfites is known to cause allergy-like reactions. Therefore, the U.S. Food and Drug Administration (FDA) requests the labeling of foods containing more than 10 mg/kg for sulfites and they also investigated the analytical method [1,2,3]. We developed the quantitative analysis of sulfites in short analytical time for food and beverage by an UHPLC based LC-MS/MS with reference to this new FDA method.

2. Methods and Materials

A 2% formaldehyde containing 50 mM ammonium acetate (adjusted to pH 4.5 with acetic acid) diluted 10-fold with water was used as extraction solution. In this method, unstable free sulfite was detected as hydroxymethylsulfonate (HMS) converted with 0.2% formaldehyde solution. The quantitative analysis was performed with a triple quadrupole mass spectrometer LCMS-8050 equipped with Nexera UHPLC (Shimadzu).

2-1. Sample preparation of dried fruit
The sample (25 g) was mixed with 50 mL of 0.2% formaldehyde solution followed by crushing with a blender for 2 minutes. 20 mL of the 0.2% formaldehyde solution was added to 15 g of homogenate, stirred with a shaker for 10 minutes, and sonicated for 8 minutes. After centrifugation at 4000x*g* for 10 min, the supernatant was transferred to a new centrifuge tube by decantation. After adding another 20 mL of the extraction solution to the precipitate, stirring, sonication and centrifugation were repeated. The supernatants were mixed and filled up to 50 mL with the extraction solution.

2-2. Sample preparation of wine
Wine sample (1 g) was diluted to 10 mL with 0.2% formaldehyde solution.

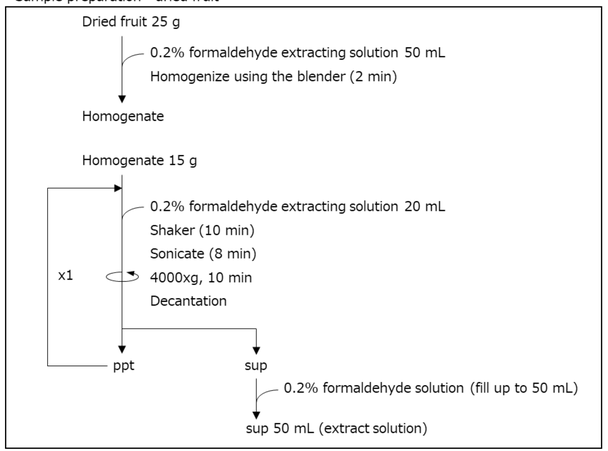
2-3. SPE clean up and heating derivatization
Sample extract was cleaned up with a C18 SPE cartridge to remove all lipophilic matrix components, and the eluent was heated to convert all sulfite-carbonyl adduct to the HMS adduct.
A C18 SPE cartridge (InertSep C18, 500 mg/6 mL, GL Sciences) was rinsed with 3mL of dichloromethane, methanol, and 0.2% formaldehyde solution, respectively, using the SPE vacuum manifold. The first 2 mL of sample extract that passed through the cartridge was discarded and the next 2 mL of sample extract that passed through the cartridge was collected. The eluate was heated at 80℃ for 30 minutes and then cooled to room temperature.

2-4. LC-MS/MS analysis
100 uL of the cooled eluate was mixed with 50 uL of the 5 ppm of $Na_2{}^{34}SO_3$ standard solution and 350 uL of acetonitrile. If precipitation occurred, it was filtered with a 0.2 µm PTFE filter.
The measurement was performed by MRM by the electrospray ionization method. The separation was performed by hydrophilic interaction chromatography (HILIC).

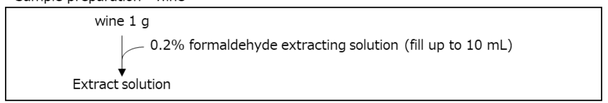
Table 1. MRM transition of hydroxymethylsulfonate (HMS) and internal standard.

Compound	MRM transition	Dwell time (msec)	Collision (V)	Role
HMS	111.00>81.00	100	13.0	Quantitation
	111.00>80.00	100	27.0	Reference
HMS (^{34}S)	113.00>83.00	100	13.0	Quantitation
	113.00>82.00	10	27.0	Reference

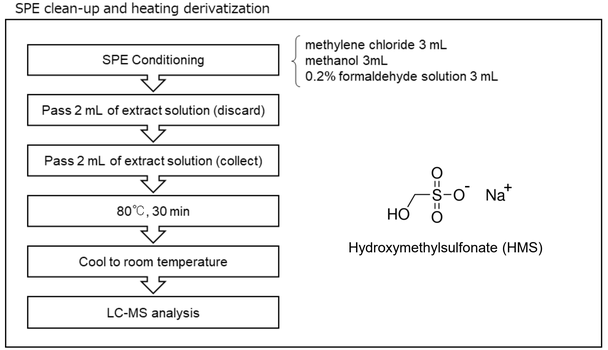
Sample preparation - dried fruit



Sample preparation - wine



SPE clean-up and heating derivatization



Hydroxymethylsulfonate (HMS)

<Nexera 40 X3 system>
Column : SeQuant ZIC HILIC (150 mm x 2.1 mm I.D., 5 µm)
Mobile phase A : 10 mM ammonium acetate / 90% Acetonitrile / Water
Mobile phase B : 10 mM ammonium acetate / 50% Acetonitrile / Water
Flow rate : 0.3 mL/min
Time program : B conc. 30% (0-1 min) → 70% (3-5.5 min) → 100% (5.51-7.75 min) → 30% (8-12 min)
The flow was loaded into the mass spectrometer between 3 to 5.5 min using a flow switching valve.
Column temp. : 30 ºC Injection vol. : 2 µL

<LCMS-8050>
Ionization : ESI, Negative mode DL temp. : 150 ºC
Interface temp. : 200 ºC Heat block temp. : 500 ºC
Nebulizer gas : 2.5 L/min Heating gas : 10 L/min
Drying gas : 10 L/min Probe position : 4 mm

3. Results and Discussion

3-1. Optimization of LC-MS/MS analysis method
First, we optimized the LC-MS analysis method. The FDA method created a calibration curve for HMS in the range of 0.01 to 4.5 ppm. Due to moving the probe position away, the upper limit of quantitation can now be raised to 20 ppm with a linearity, this will save the effort of the dilution of the sample. The calibration curve is shown in Figure 1. Also, we were able to reduce the analysis time from 24 minutes to 12 minutes by adjusting the time program. The injection volume was measurable at 2 µL.

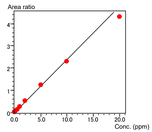


Figure 1. Calibration curve of HMS

3-2. Recovery rate of sulfite
<Dried fruits>
Sulfite was analyzed for raisin, apricot, and two kinds of mango. Dried fruits were purchased at the grocery store. These samples were filtered before LC-MS analysis because precipitation occurred when samples were mixed with IS and acetonitrile. Since raisin and one of mango contained almost no sulfite, the recovery rate was confirmed using these samples. The spike and recovery test was validated by spiking 25 µg or 50 µg of SO_2 to 15 g of homogenate, extraction and SPE clean up were performed. The recovery rate was calculated by converting from the HMS concentration.
Table 2 shows the results of SO_2 concentration and recovery rate. For SO_2 spiking concentration of 5 µg/g food, the recovery rates were ranged from 84.9 to 99.2%. For SO_2 spiking concentration of 10 µg/g food, the recovery rates were ranged from 91.2 to 96.3%. All the recovery test achieved a good result. Most countries require that foods with SO_2 concentrations exceeding 10 mg/kg be labeled as having added sulfites. It was confirmed that it has good analytical ability at the required concentration level.

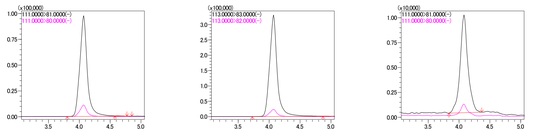
Table 2. The sulfite concentration and recovery rate of dried fruits

Food	SO_2 (µg/g food)			Recovery rate (%)	
	0 µg/g	5 µg/g	10 µg/g	5 µg/g	10 µg/g
Raisin	0.00	4.96	9.63	99.2	96.3
Mango 1	0.11	4.35	9.22	84.9	91.2
Mango 2	438.06				
Apricot	907.00				

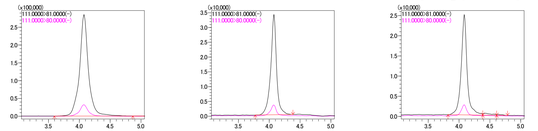
<Beverages>
Sulfite was analyzed for two kinds of red wine and two kinds of white wine. They were bought at the food store. Since almost no sulfurous acid was detected in one of red wine (red wine 1), a recovery test was conducted. Spiking concentration of sulfite was 1, 5, and 10 µg/g. In addition, SO_2 was added to one of white wine (white wine 1) so that it would be 5 or 10 µg/g, and an addition recovery test was conducted. Table 3 shows the results of SO_2 concentration and recovery rate. For red wine, good results were obtained with a recovery rate of 99.7% even with SO_2 spiked 1 µg/g. For both red wine and white wine, a good recovery rate of 95.2 to 104.0% was obtained.

Table 3. The sulfite concentration and recovery rate in beverages

Beverage	SO_2 (µg/g food)				Recovery rate (%)		
	0 µg/g	1 µg/g	5 µg/g	10 µg/g	1 µg/g	5 µg/g	10 µg/g
Red wine 1	0.02	1.02	5.22	10.32	99.7	104.0	103.0
Red wine 2	35.52						
White wine 1	38.23		42.99	48.28		95.2	100.5
White wine 2	100.64						



HMS 1 ppm | ^{34}S-HMS | HMS in red wine 1 spiked 1 µg SO_2/g beverage



HMS in white wine 1 spiked 5 µg SO2/g beverage | HMS in mango 1 spiked 5 µg SO_2/g food | HMS in raisin spiked 5 µg SO2/g food

Figure 2. MS chromatograms of HMS and internal standard.

4. Conclusions

- Created an LC-MS method that can be measured in 12 minutes.
- Success of calibration curve with wide concentration range.
- For dried fruits (raisins, mangoes), red wine, and white wine, good recovery results were obtained at 10 mg/kg or lower, which is required for labeling in most countries.

References
[1] U.S. Food Drug Administration (2018). Code of federal regulations: Part 101.100(a)(4), Title 21. Washington DC: Office of the Federal Register.
[2] Carlos, K. S., & de Jager, L. S. (2017). Determination of sulfite in food by liquid chromatography tandem mass spectrometry: Collaborative study. Journal of AOAC International, 100(6), 1785–1794.
[3] U.S. Food Drug Administration (2021). Method number: C-004.03, Determination of Sulfites in Food using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)