

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

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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.



LCMS-8050 Triple Quadrupole Mass Spectrometer

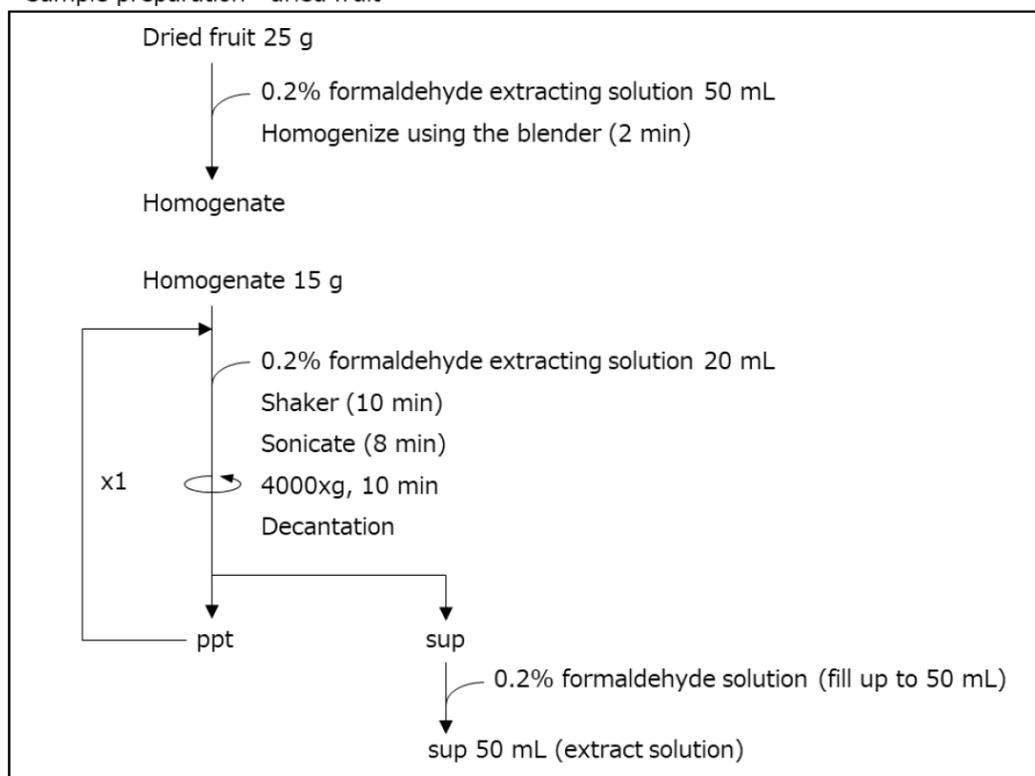
## 2. Methods and Materials

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 4000xg 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.

Sample preparation - dried fruit

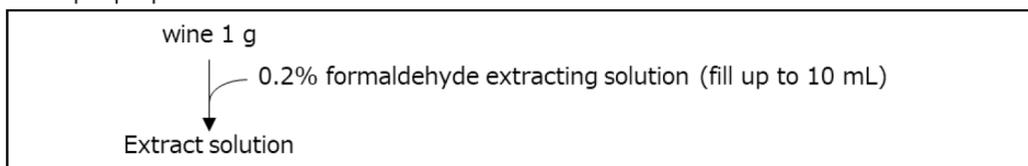


## 2. Methods and Materials

### 2-2. Sample preparation of wine

Wine sample (1 g) was diluted to 10 mL with 0.2% formaldehyde solution.

Sample preparation - wine

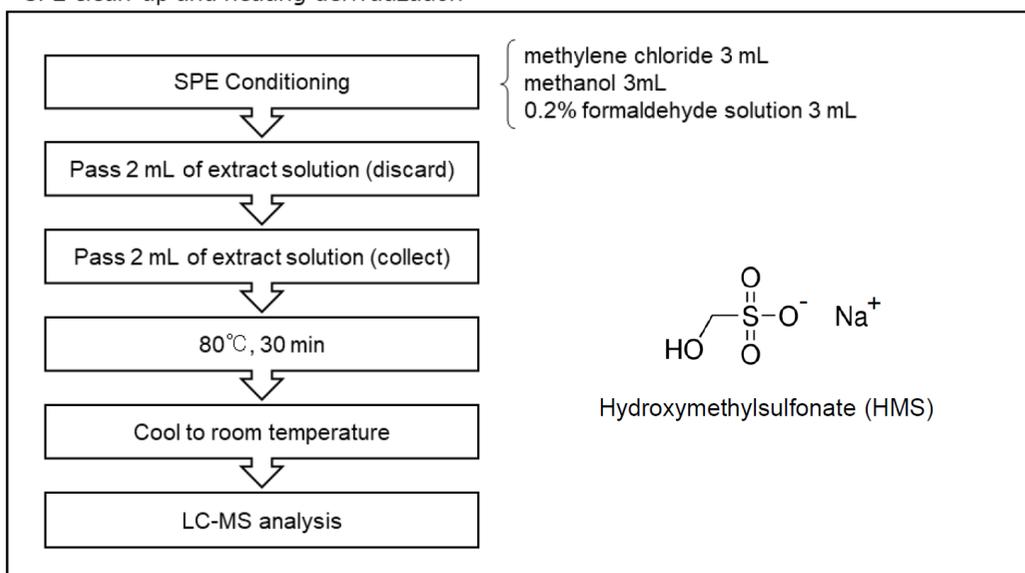


### 2-3. SPE clean up and heating derivatization

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°C for 30 minutes and then cooled to room temperature.

SPE clean-up and heating derivatization



## 2. Methods and Materials

### 2-4. LC-MS/MS analysis

100 µL of the cooled eluate was mixed with 50 µL of the 5 ppm of Na<sub>2</sub><sup>34</sup>SO<sub>3</sub> standard solution and 350 µL 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 ( <sup>34</sup> S)	113.00>83.00	100	13.0	Quantitation
	113.00>82.00	10	27.0	Reference

#### Analytical condition

<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 °C	Injection vol.	: 2 µL
<LCMS-8050>			
Ionization	: ESI, Negative mode	DL temp.	: 150 °C
Interface temp.	: 200 °C	Heat block temp.	: 500 °C
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  $\mu$ L.

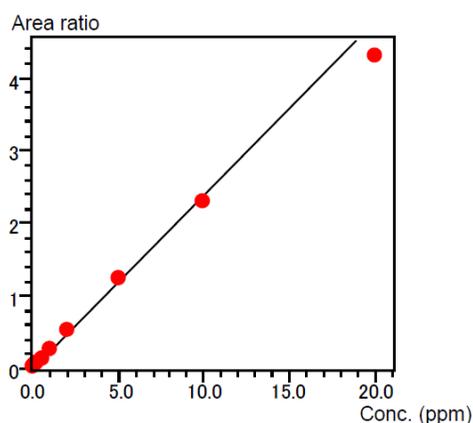


Figure 1. Calibration curve of HMS

### 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  $\mu$ g or 50  $\mu$ g of SO<sub>2</sub> 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<sub>2</sub> concentration and recovery rate. For SO<sub>2</sub> spiking concentration of 5  $\mu$ g/g food, the recovery rates were ranged from 84.9 to 99.2%. For SO<sub>2</sub> spiking concentration of 10  $\mu$ g/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<sub>2</sub> 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 <sub>2</sub> ( $\mu$ g/g food)			Recovery rate (%)	
	0 $\mu$ g/g	5 $\mu$ g/g	10 $\mu$ g/g	5 $\mu$ g/g	10 $\mu$ g/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				

### 3. Results and Discussion

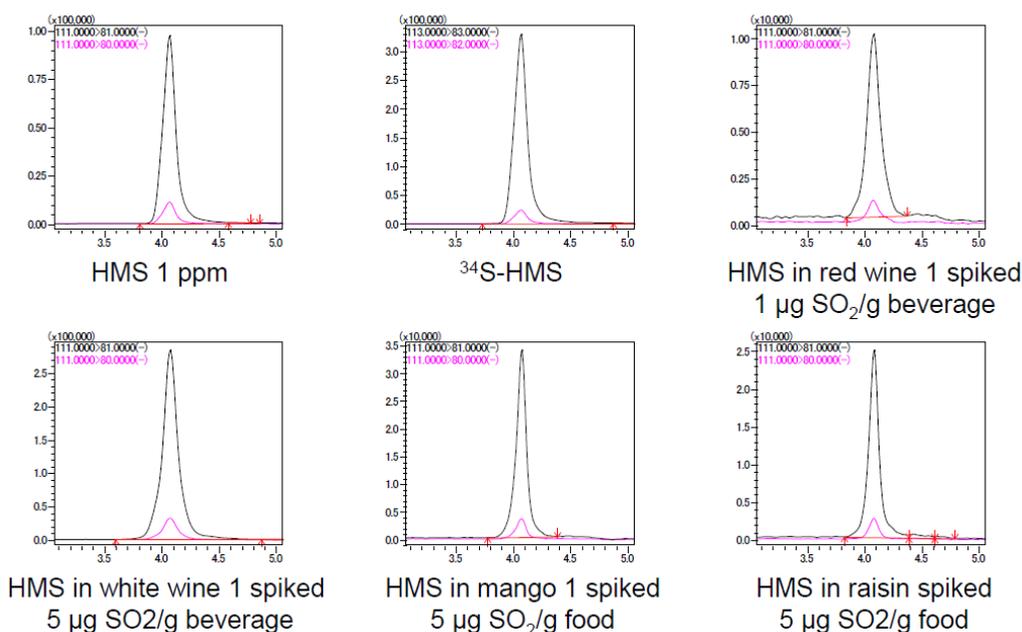
#### 3-2. Recovery rate of sulfite

<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<sub>2</sub> 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<sub>2</sub> concentration and recovery rate. For red wine, good results were obtained with a recovery rate of 99.7% even with SO<sub>2</sub> 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 <sub>2</sub> (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						



**Figure 2. MS chromatograms of HMS and internal standard.**

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

### 4. Conclusion

- 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)

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