



Sulfite Quantification in Foods and Beverages Using Single Quadrupole LC-MS

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1. Introduction

- Sulfites are one of the most common food additives used as antioxidants and bleaching agents, and they are added to various products such as dried fruit, vegetable, frozen shrimp, juice, or wine. Although sulfites are very useful food additives, it is known that consuming food or beverages containing them can cause reactions like allergies. Therefore, the U.S. Food and Drug Administration (FDA) requires the labeling of foods containing more than 10 mg/kg of sulfites, and they have also issued a quantitative analysis method for sulfites¹⁾.
- We have developed a clean preprocessing without the use of dichloromethane as specially regulated substance, and a quantitative analysis method for sulfites in foods and beverages using a UHPLC-based LC-MS with reference to this FDA method.

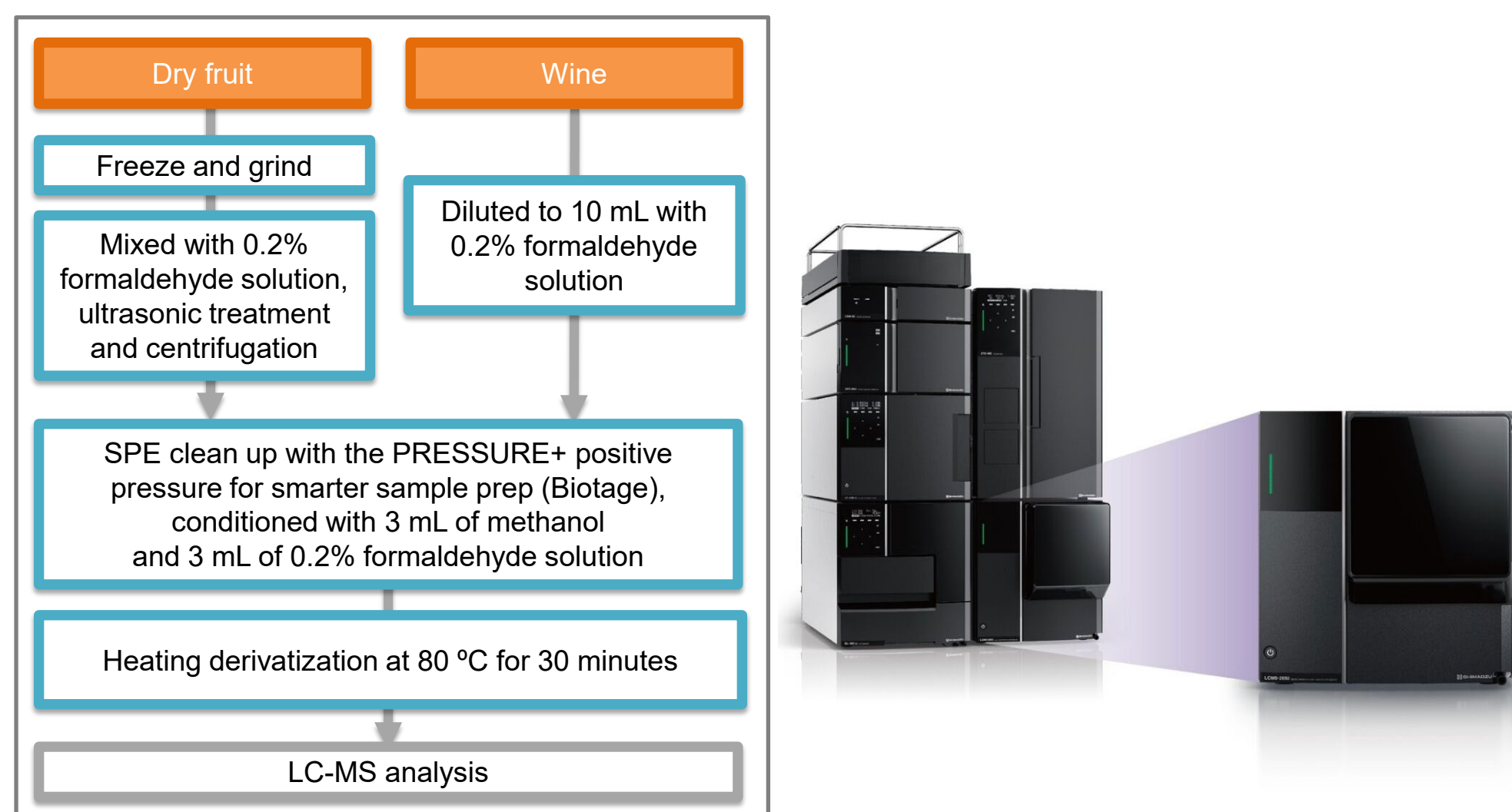


Fig. 1 Workflow of this research

Fig. 2 LCMS-2050

2. Methods

- Sulfites were mixed with 0.2% formaldehyde extraction solution and converted to hydroxymethylsulfonate (HMS) for detection. Sodium sulfite (Na_2SO_3) was dissolved in the extraction solution and converted to HMS for use as the standard sample for the calibration curve. Isotope-labeled $\text{Na}_2^{34}\text{SO}_3$ was used as the internal standard converted to HMS using the formaldehyde solution as well²⁾.

- Sample preparation for dried fruit: 5 g of frozen and pulverized dried fruit was mixed with 40 mL of 0.2% formaldehyde extraction solution and subjected to 8 minutes of ultrasonic treatment. After centrifugation at 4,000xg for 10 minutes, the supernatant was collected by decantation followed by adjusting to the final volume of 50 mL with the extraction solution.
- Sample preparation for wine: Each wine sample (1 g) was diluted to 10 mL with 0.2% formaldehyde solution.
- SPE clean up and heating derivatization: A C18 SPE cartridge (InertSep C18, 500 mg/6 mL, GL Sciences) were used to remove lipid components from the extracts of dried fruit and wine.
- Using the PRESSURE+ positive pressure for smarter sample prep (Biotage), the SPE cartridges were conditioned with 3 mL of methanol followed by 3 mL of 0.2% formaldehyde solution. By comparing the recovery rates with and without using dichloromethane during conditioning, the difference of recovery rate was -7% to 5%, so it was decided that purification was performed without dichloromethane.
- Next, the extracts of dried fruit or wine were passed through the SPE cartridges, with the initial 2 mL discarded and the next 2 mL collected. The collected eluate was heated at 80°C for 30 minutes followed by cooling down to room temperature.
- LC-MS analysis: 100 μL of the cooled eluate was mixed with 50 μL of a 5 $\mu\text{g}/\text{mL}$ internal standard solution and 350 μL of acetonitrile, and the filtrated solution was analyzed by LC-MS. The analysis conditions are shown in Table 1.

Table. 1 Analysis condition

[HPLC conditions] (Nexera™ X3)		[MS conditions] (LCMS-2050)	
Column	InertSustain AX-C18 (100 mm x 2.1 mm I.D., 3.0 μm)	Ionization	ESI/APCI (DUIS™) Negative mode
Mobile phase A	2 mM Ammonium formate-0.1% Formic acid-Water	Mode	SIM (m/z 111, 113)
Mobile phase B	0.1% Formic acid-Acetonitrile	Interface voltage	-0.5 kV
Gradient program	B conc. 30% (0-10 min) - 100% (10.01-15 min) - 30% (15.01-20 min)	Nebulizing gas flow	3.0 L/min.
Flow rate	0.2 mL/min	Drying gas flow	5.0 L/min.
Column temp.	30 °C	Heating gas flow	7.0 L/min.
Injection volume	2 μL	Desolvation temp.	250 °C
		DL temp.	150 °C
		Probe position	+2 mm

3. Results

- Calibration curve is shown in Fig. 3. Additionally, Fig. 4 shows the MS chromatograms of HMS in the standard and recovery test samples. A calibration curve with good linearity ($R^2 > 0.999$, $n = 3$) in the range of 0.02 to 2 ppm was obtained.

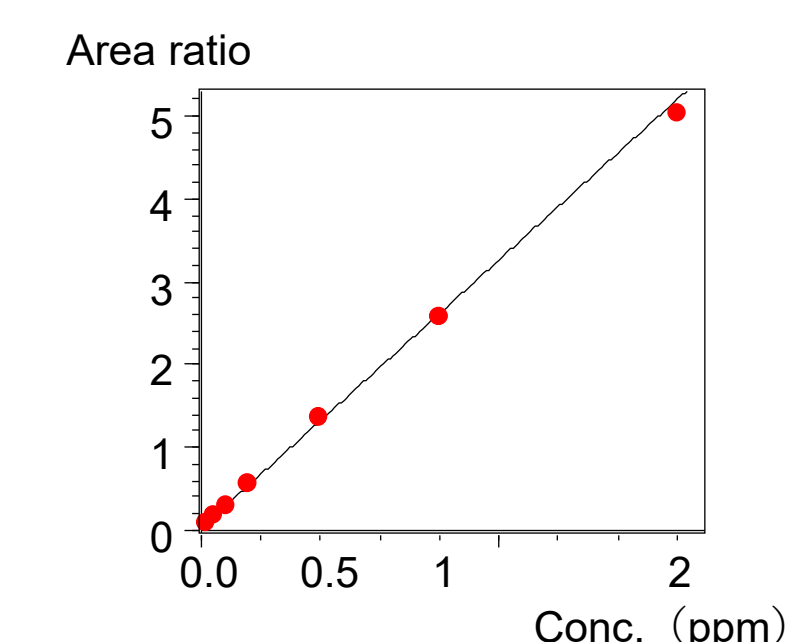


Fig. 3 Calibration curve of HMS

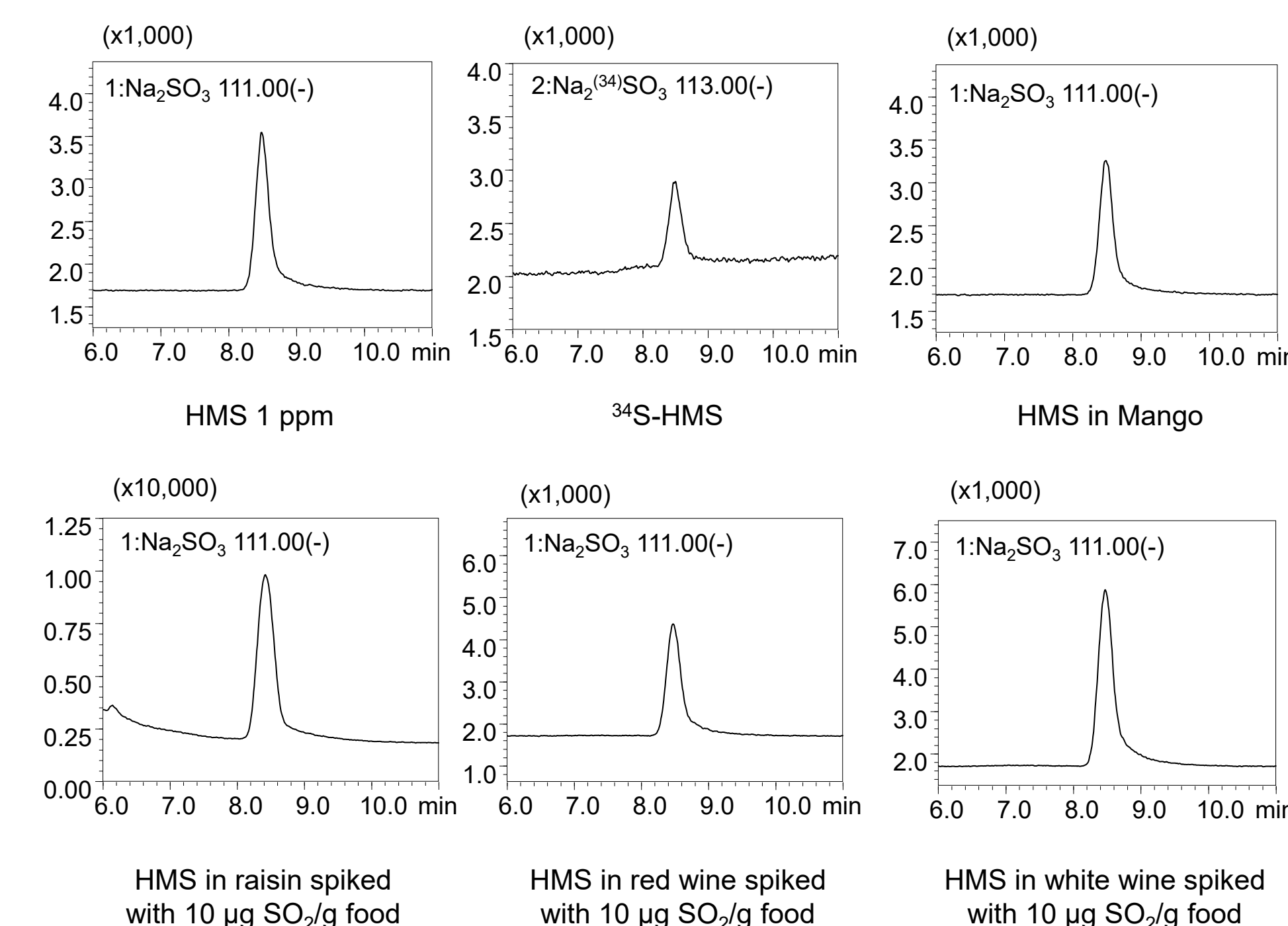


Fig. 4 MS chromatogram of HMS and internal standard

- Sulfites were quantified in dried fruits, including raisin, pineapple, and mango, as well as in Beverages, including red wine and white wine. The results are shown in Table 2. For pineapple and mango, the eluate from SPE cartridge was diluted 10-fold with 0.2% formaldehyde solution, mixed with internal standard and acetonitrile followed by analysis with an LC-MS. The other samples were analyzed without additional dilution.

Table. 2 Quantification of sulfites in dried fruit and wine

Food/Beverage	HMS Conc. (ppm)	SO ₂ converted value (mg/kg food)
Raisin	ND	(0.0)
Pineapple	14.8*	376.4*
Mango	10.4*	264.7*
Red wine	ND	(0.0)
White wine	0.2	6.1

*The calculations are based on the values of the diluted samples.

- Recovery tests were performed for four samples (raisin, red wine, white wine, and water) suspected to have no added sulfites or a low sulfite content. After the addition of Na_2SO_3 , extraction or dilution was performed, followed by cleanup using an SPE cartridge. The results are shown in Table 3. For all samples, good recovery rates were obtained at the required labeling level of 10 mg SO_2/kg food.

Table. 3 Recovery rate

Food/Beverage	Recovery rate (% , n=3)
Raisin	103.5
Red wine	95.4
White wine	98.4
Water	96.0

4. Conclusion

- This method provides the analysis of sulfites in dried fruits and wine. Good recovery rates were achieved at the mandatory labeling limit of 10 mg/kg.
- This analysis method enables quantitative analysis of sulfites in food products with labeling requirements by safer pretreatment using a single quadrupole mass spectrometer.

Reference

- 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.
- U.S. Food and 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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