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Application News

High Performance Liquid Chromatograph Mass Spectrometer LCMS-8050

Sulfite Quantification in Foods and Beverages Using Triple Quadrupole LC-MS/MS

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User Benefits

- Rapid analysis of sulfites, which are added to various foods and beverages as antioxidants.
- Wide quantification range reduces the need for re-analysis of samples with high sulfite content.
- Good quantification was achieved with a method based on a new U.S. FDA method (C-004.03).

■ 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 in some cases. Therefore, the U.S. Food and Drug Administration (FDA) requests the labeling of foods containing more than 10 mg/kg of sulfites and have also published an analytical method for quantifying sulfites[1,2,3]. We have developed a quantitative analysis method for sulfites in food and beverages with a short analytical time using a UHPLC-based LC-MS/MS with reference to this new FDA method.

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 a Nexera™ UHPLC. For dried fruits (raisins, mangoes), red wine, and white wine, good recovery results were obtained at 10 mg/kg or lower, meeting the level required for labeling in most countries.

■ Methods and Materials

0.2% formaldehyde solution: 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.

Sample preparation for dried fruit: Each dried fruit 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, the stirring, sonication and centrifugation steps were repeated. The supernatants were mixed and filled up to 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: The 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 each of dichloromethane, methanol, and 0.2% formaldehyde solution in turn, 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 was collected. The eluate was heated at 80°C for 30 minutes and then cooled to room temperature.

LC-MS/MS analysis : 100 µL of the cooled eluate was mixed with 50 µL of the 5 µg/mL of $Na_2{}^{34}SO_3$ internal standard solution and 350 µL of acetonitrile. If precipitation occurred, the solution was filtered with a 0.2 µm PTFE filter. LC-MS/MS analysis conditions and MRM conditions are shown in Tables 1 and 2.

Table 1 LC-MS/MS conditions

HPLC conditions (Nexera X3)	
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
Gradient 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.	: 40°C
Injection volume	: 2 µL
MS conditions (LCMS-8050)	
Ionization	: ESI, negative mode
Nebulizing gas	: 2.5 L/min
Drying gas	: 10 L/min
Heating gas	: 10 L/min
DL temp.	: 150°C
Interface temp.	: 200°C
Heat block temp.	: 500°C
Probe position	: +4 mm

Table 2 MRM conditions

Compound	MRM transition	Collision (V)	Purpose
HMS	111.00>81.00	13.0	Quantification
	111.00>80.00	27.0	Reference
HMS (^{34}S)	113.00>83.00	13.0	Quantification
	113.00>82.00	27.0	Reference

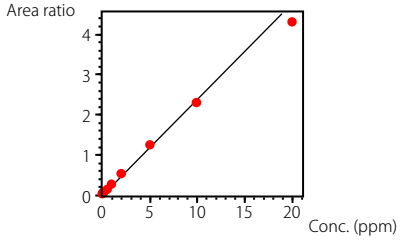

Fig. 1 Calibration curve of HMS

■ Calibration curve and MS chromatograms

Moving the probe position further away allows the upper limit of quantification to be raised to 20 ppm while maintaining linearity. This saves labor involved in diluting samples. The calibration curve is shown in Fig. 1. Also, we were able to reduce the analysis time from 24 minutes to 12 minutes by adjusting the time program.

■ Quantification and recovery test

Sulfites were quantified in raisin, apricot, two kinds of mango, two kinds of red wine, and two kinds of white wine. The results are shown in Table 3. For apricot, after diluting the SPE cartridge eluate 10-fold with 0.2% formaldehyde extract, the solution was mixed with internal standard and acetonitrile for LC-MS/MS analysis. Other samples were analyzed by LC-MS/MS without additional dilution.

Four samples with no or low levels of sulfites were used for recovery tests. After addition of Na_2SO_3, extraction and SPE cartridge cleanup were performed. Results are shown in Table 4. A good recovery rate was obtained for all samples when the additive amount was 10 µg SO_2/g or less. We achieved excellent recovery for red wine even at 1 µg SO_2/g.

■ Summary

In this report, we introduced sulfite analysis in dried fruit and wine. A good recovery rate was obtained at 10 mg/kg or less, the level which is mandatory for labeling. In addition, since this analytical method has a wide quantification range, dilution work can be reduced for samples with high sulfite concentrations.

Table 3 Quantification of sulfites in dried fruit and wine

Food/ Beverage	HMS conc. (ppm)	SO_2 converted value (µg/g food)
Raisin	(0.000)	(0.00)
Mango 1	(0.004)	(0.11)
Mango 2	16.27	413
Apricot	40.97	1041
Red wine 1	(0.001)	(0.02)
Red wine 2	1.40	36
White wine 1	1.51	38
White wine 2	3.96	101

Table 4 Recovery rate (%)

Food/ Beverage	Spiking conc. (µg SO_2/g food)		
	1	5	10
Raisin		99.2	96.3
Mango 1		84.9	91.2
Red wine 1	99.7	104.0	103.0
White wine 1		95.2	100.5

References
1) U.S. Food and Drug Administration (2016). 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 and Drug Administration (2021). Method number: C-004.03, Determination of Sulfites in Food using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

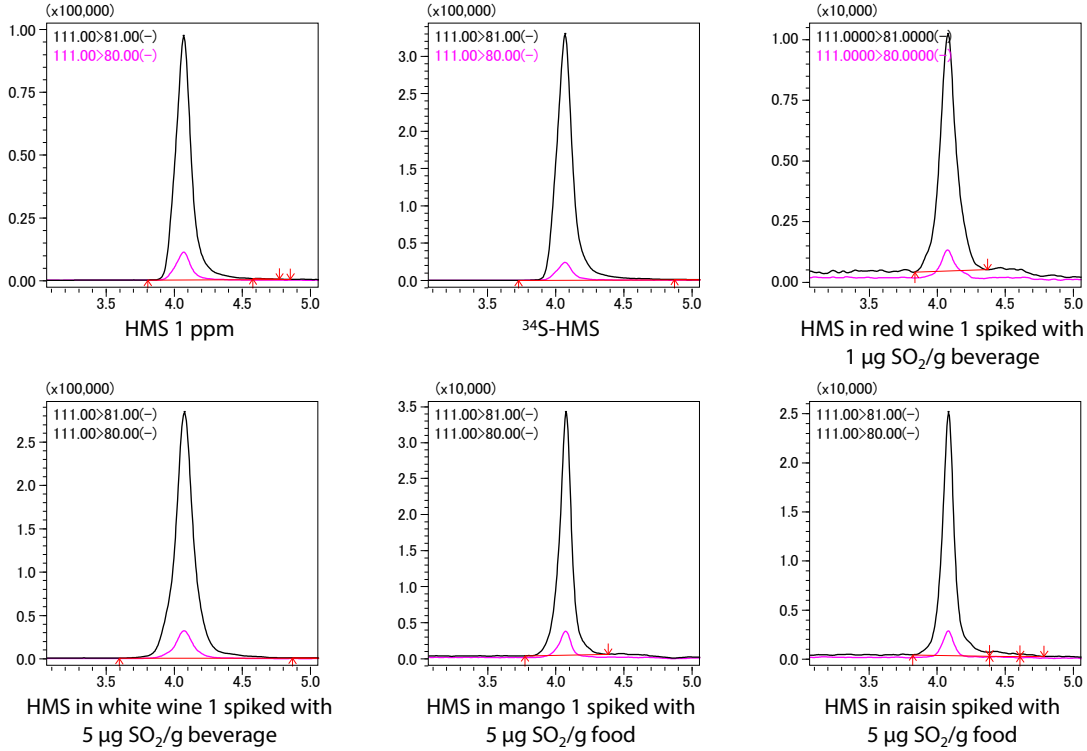

Fig. 2 MS chromatograms of HMS and internal standard

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