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High Performance Liquid Chromatograph Mass Spectrometer LCMS-2050

Application News

Analysis of Sulfites in Food and Beverages Using Single Quadrupole Mass Spectrometer

Saho Yoshioka, Nozomi Maeshima

User Benefits

- The development method provides rapid analysis of sulfites, which are added to various foods and beverages as antioxidants.
- Good quantification was achieved with the method based on the U.S. FDA method (C-004.03).
- The safety of pre-treatment protocols can be offered without the use of dichloromethane as a specified chemical substances.

■ 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 sulfites[1].

In the application news No. 01-00450-EN, we introduced an example of quantitative analysis of sulfites in food and beverages using a triple quadrupole mass spectrometer, referring to the analysis method proposed by the FDA. Meanwhile, the new method is introduced in this application, which can offer preprocessing without the use of dichloromethane as specially regulated substance and simplified quantification analysis of sulfites using LCMS-2050.

Fig. 1 Nexera™ and LCMS-2050

■ Sample Preparation and Analysis Conditions

Preparation of 0.2% formaldehyde extraction solution: 2% formaldehyde solution containing 50 mM ammonium acetate (adjusted to pH 4.5 with acetic acid) was prepared. This solution was diluted 10-fold with water and used as 0.2% formaldehyde extraction solution.

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 $Na_2{}^{34}SO_3$ was used as the internal standard converted to HMS using the formaldehyde solution as well[2].

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 4,000*xg* 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 µg/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 LC-MS conditions

[HPLC conditions] (Nexera™ X3)		
Column	:	InertSustain AX-C18*1 (100 mm x 2.1 mm I.D., 3 µm)
Mobile phase A	:	2 mM ammonium formate / 0.1% formic acid / water
Mobile phase B	:	0.1% formic acid / Acetonitrile
Flow rate	:	0.2 mL/min
Gradient program	:	B conc. 30% (0-10 min) – 100% (10.01-15 min)– 30% (15.01-20 min)
Column temp.	:	30°C
Injection volume	:	2 µL
[MS conditions] (LCMS-2050)		
Ionization	:	ESI/APCI (DUIS™), Negative mode
Mode	:	SIM (*m/z* 111, 113)
Interface voltage	:	-0.5 kV
Nebulizing gas flow	:	3.0 L/min
Drying gas flow	:	5.0 L/min
Heating gas flow	:	7.0 L/min
Desolvation temp.	:	250°C
DL temp.	:	150°C
Probe position	:	+2 mm

*1 P/N：5020-91038

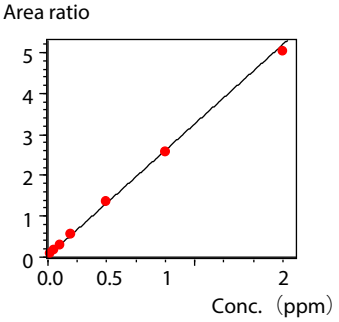

Fig. 2 Calibration curve of HMS

■ Calibration curve and MS chromatograms

Calibration curve is shown in Fig. 2. Additionally, Fig. 3 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.

■ Quantification and recovery test

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.

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.

■ Conclusion

This method provides the analysis of sulfites in dried fruits and wine. Good recovery rates were obtained below 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.

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.

Table 3 Recovery rate

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

<References>

1) 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.
2) 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)

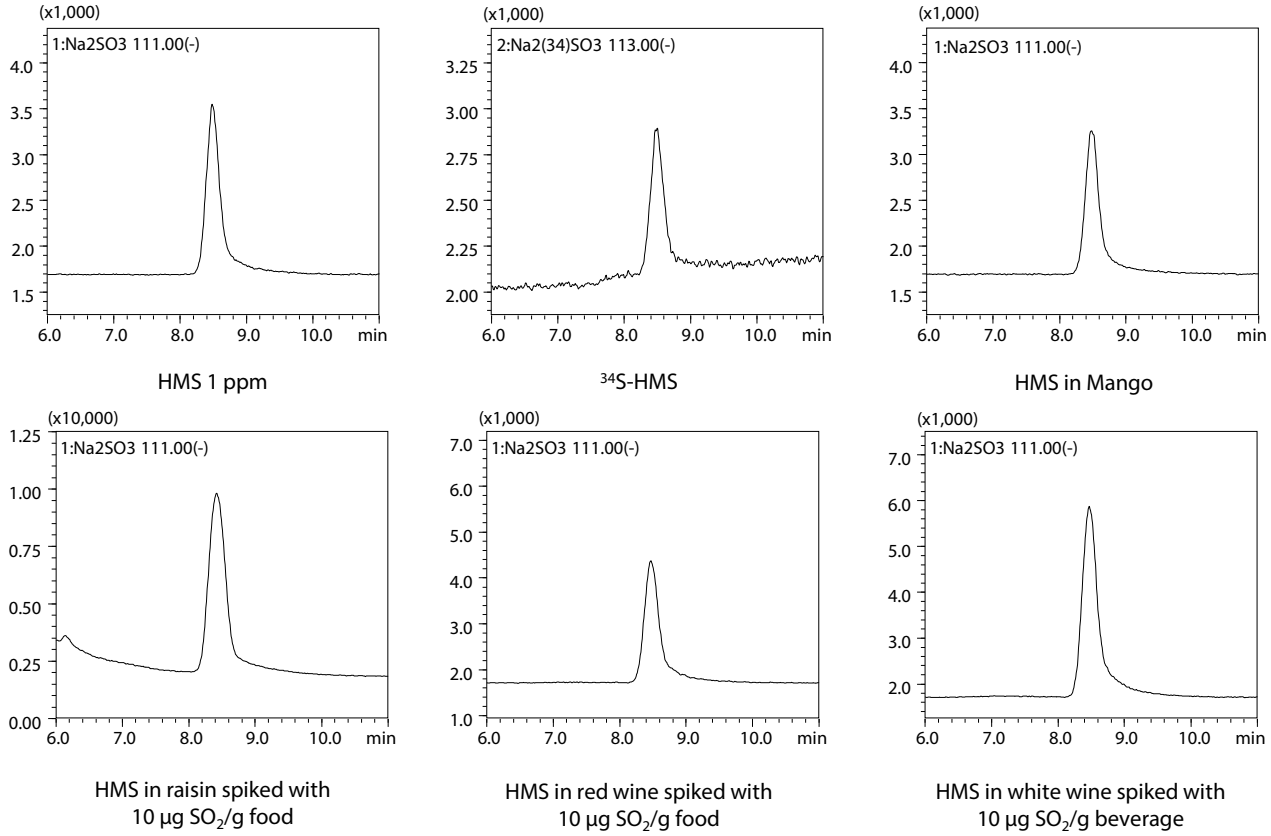

Fig. 3 MS chromatograms of HMS and internal standard

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