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Application News

GC-MS GCMS-TQ™ Series and Smart Metabolites Database™

Analysis of Sugars in Foods Using Smart Metabolites Database

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User Benefits

- 24 types of sugars in foods can be detected from a single peak with no formation of geometric isomers by derivatization.
- Approximate concentrations of 24 sugars in food can be calculated simply by pretreating samples with the addition of an internal standard.
- The same extraction method and analytical system from the simultaneous analysis of metabolites for metabolomic analysis can be used.

■ Introduction

The term "sugar" refers collectively to all monosaccharides such as glucose, and all disaccharides such as sucrose that are not sugar alcohols. Plants and animals contain a variety of such sugars, which serve as a source of energy.

For metabolomics using a GC-MS system, methoximation-trimethylsilylation (MeOx-TMS) is typically used to derivatize metabolites for simultaneous analysis. However, due to the especially reductive characteristic of sugars, the methoximation process can form chain structures that result in the detection of two types of geometric isomers. This makes quantitative analysis difficult due to inadequate separation between the sugar isomers sorbose and fructose and between the sugar isomers mannose and glucose.

Furthermore, plants and animals can contain a large number of sugars with a wide range of concentrations, from a low of 0.1 µg/mg to a high of several hundred µg/mg. Consequently, quantitative analysis of detected sugars can require the preparation of calibration curves with a concentration range that varies depending on the target compound, or consideration of the recovery rates from pretreatment and other factors, which can be very time-consuming. This has led to the need to determine approximate concentration levels.

To meet such needs, a semi-quantitative analysis method for sugars is now included in Smart Metabolites Database Ver. 2.

This article describes the process of obtaining semi-quantitative values from beef and tomato samples using the sugar analysis method, as well as the results of comparing these semi-quantitative values to quantitative values obtained using the standard addition method.

■ Smart Metabolites Database Ver. 2

Smart Metabolites Database Ver. 2 contains analytical condition settings and SIM and MRM transition settings optimized for the metabolites. The number of components registered for the simultaneous analysis of metabolites in biomarker search applications has been increased to 627 for SIM and 540 for MRM. In addition to compound-specific quantitative analysis methods for fatty acid methyl esters (FAMEs) and amino acids, compound-specific quantitative analysis methods have been newly added for analyzing sugars (Table 1).

Table 1 Overview of Smart Metabolites Database Ver. 2

	Derivatization Method	Analysis Method	Number of Registered Components
Simultaneous Analysis of Metabolites	TMS	SIM	627
		MRM	540
Fatty Acid Methyl Esters	Methylation	SIM MRM	50
Sugars	Acetylation	SIM MRM	24
Amino Acids	EZ:faast	Scan	33

■ Method for the Quantitative Analysis of Sugars

The method for sugar quantitation includes an optimized acetylation-based derivatization process that does not form geometric isomers and is not likely to derivatize other metabolite components.

In addition, calibration curve information with corrected dilution factors and recovery rates is registered by optimizing and fixing the pretreatment method.

The method can be used to calculate semi-quantitative values for 24 types of sugars in samples simply by adding the specified quantity of ribitol as an internal standard.

The pretreatment protocol is based on the Bligh-Dyer method for the simultaneous analysis of metabolites using a database, which enables measurements by the derivatization of sugars in a portion of the extract solution.

■ Experiment

Commercially marketed beef and tomato products were used as samples. 10 mg of each of the homogenized and freeze-dried samples was taken and pretreated based on the Bligh-Dyer method.

100 µL of each extract solution was then derivatized by acetylation. Semi-quantitative evaluation using the database was repeated 3 times per sample type. For the standard addition method, each sample was measured in advance to determine which sugars were detected before preparing calibration curves for ribose, xylose, fructose, mannose, glucose, and *myo*-inositol. Calibration curves were prepared for the standard addition method at concentrations ranging from 0.1 to 5 µg/mg (dry weight) for ribose, xylose, and *myo*-inositol, and 5 to 500 µg/mg for fructose, mannose, and glucose, which were detected at high concentrations. The analytical conditions are indicated in Table 2.

Table 2 System Configuration and Analytical Conditions

GC-MS:	GCMS-TQ8040 NX
Autoinjector:	AOC-30i/20s U
Database:	Smart Metabolites Database Ver. 2
Column:	BPX-5 (30 m, 0.25 mm I.D., 0.25 µm)
[GC]	
Injection Temp.:	280 °C
Column Oven Temp.:	150 °C (5 min) → (3 °C /min) → 220 °C → (10 °C /min) → 320 °C (3 min)
Injection Mode:	Split
Split Ratio:	15
Carrier Gas:	He
Carrier Gas Control:	34.0 cm/sec
Injection Volume:	1 µL
[MS]	
Ion Source Temp.:	230 °C
Interface Temp:	280 ° C
Data Acquisition Mode:	MRM

■ Analysis Results

Representative MRM chromatograms for the beef samples are shown in Fig. 1. Results from the comparison of the semi-quantitative values obtained from the database for beef and tomato samples to quantitative values obtained by the standard addition method are shown in Table 3.

Most of the sugars detected in the beef were at concentrations of 1 µg/mg or less, but fructose, glucose, and sucrose at concentrations of about 100 µg/mg or higher were detected in the tomato samples, thereby differing from other sugars by a factor of several hundred to several thousand.

With the semi-quantitative values obtained from the database, the average of three successive values was used for the comparison to the quantitative values obtained using the standard addition method. All measurement results were in the 48 to 191 % range. Although the calculated values tended to be too low for concentrations of 1 µg/mg or less, this was presumably due to the calibration curve information registered for such a wide range of concentrations.

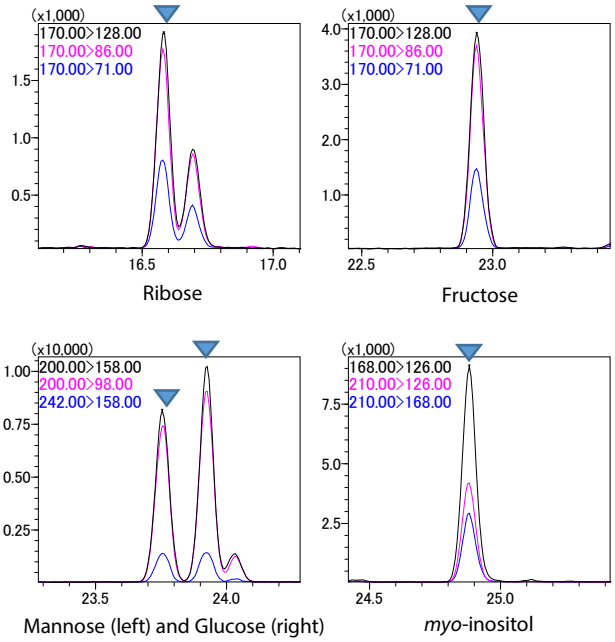

Fig. 1 MRM Chromatograms of Representative Sugars Detected in Beef

Table 3 Comparison of the Semi-Quantitative Values from the Beef and Tomato Samples to the Quantitative Results Obtained with the Standard Addition Method

Units: µg/mg

Beef	Quantitative Values Calculated with the Database						
Detected Compound	Beef 1	Beef 2	Beef 3	Avg	%RSD	Quantitative Values from the Standard Addition Method	Ratio (%) (DB semi-quant/std. add)
Ribose	0.14	0.14	0.14	0.14	2.9	0.29	48.5
Fructose	1.26	0.95	0.82	1.01	22.5	0.53	191.0
Mannose	1.00	0.84	0.69	0.84	18.6	0.60	139.6
Glucose	1.24	1.00	0.81	1.01	21.1	1.01	100.3
myo-inositol	0.12	0.10	0.08	0.10	21.1	0.10	101.8

Tomatoes	Quantitative Values Calculated with the Database						
	Tomato 1	Tomato 2	Tomato 3	Avg	%RSD	Quantitative Values from the Standard Addition Method	Ratio (%) (DB semi-quant/std. add)
Xylose	0.14	0.13	0.15	0.14	9.0	0.31	45.2
Ribose	0.07	0.06	0.07	0.07	8.9	0.11	62.9
Fructose	563	438	577	526	14.6	304	172.9
Mannose	104	91	113	103	11.0	91	112.5
Glucose	131	116	142	130	10.4	99	131.7
myo-inositol	0.92	0.76	1.01	0.90	14.0	0.99	90.5

■ Conclusion

When the pretreatment recovery rates and other factors are considered, the standard addition method appears to be the most reasonable method for the accurate quantitation of sugars in food samples. However, the pretreatment and analysis processes required to acquire calibration point data over a wide range of concentrations can be very tedious and time-consuming. At the same time, semi-quantitative analysis using the method for sugar quantitation can be used to determine the approximate concentrations of sugars in samples. When outputting accurate quantitative values, these semi-quantitative values are useful for narrowing down calibration points in order to implement the standard addition method.

Note: Semi-quantitative results can deviate significantly from true values depending on the type of sample and pretreatment method used. If accurate quantitative results are required, use standard samples to perform quantitative testing.

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