

# Application News

## No. C133

### Liquid Chromatography Mass Spectrometry

## Simultaneous Analysis of 16 Sweeteners Using Triple Quadrupole LC/MS/MS [LCMS-8050]

Artificial sweeteners such as aspartame, sucralose, and acesulfame potassium fall under the category of designated additives according to Japan's Food Sanitation Act, and prescribed standards are in place for their use in some foods and quantities.

Cyclamate and other artificial sweeteners used in some regions outside Japan are included among undesignated additives in Japan, and inspection is required in specific imported foods.

Consequently, quantitation for large numbers of sweeteners, including not only permitted in Japan but also undesignated, are needed.

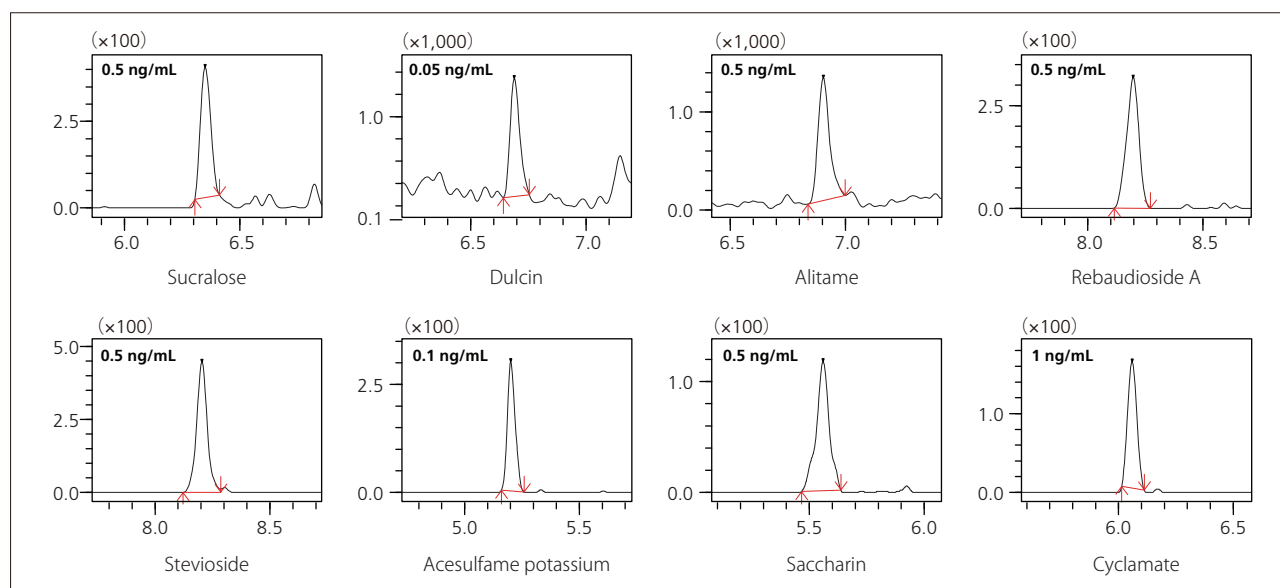
Application News C121 described the simultaneous analysis of nine artificial sweeteners including both designated and undesignated additives using an LCMS-8040 triple quadrupole LC/MS/MS system. In this article, we introduce an example of simultaneous analysis of 16 sweeteners using an LCMS-8050.

### Standard Mixture Analysis

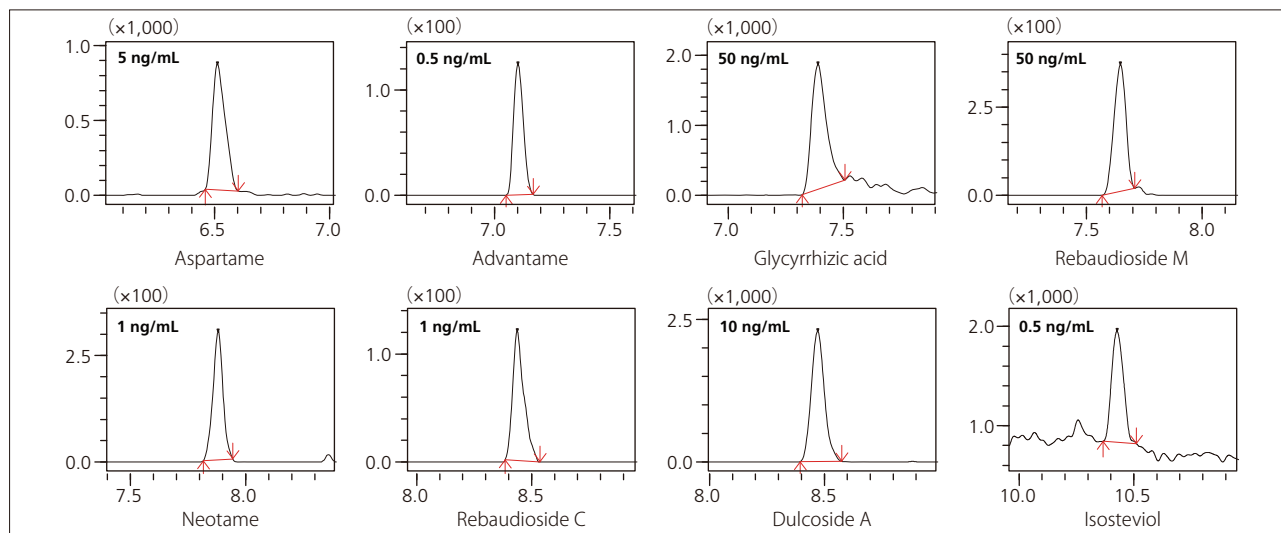
MRM analysis was performed on 16 sweeteners using the analytical conditions shown in Table 1. Chromatograms of each compound near their lower limit of quantitation are shown in Fig. 1, with calibration curve ranges and correlation coefficients shown in Table 2. Results that met an accuracy of 100 % ±20 % and area repeatability (%RSD) of within 20 % were used for calibration point. Good linearity was obtained for all compounds, with correlation coefficients of 0.997 or higher.

**Table 1 Analytical Conditions**

Column	: Unison UK-C18 (150 mm L. × 3.0 mm I.D., 3.0 μm)	Injection Volume	: 1 μL
Mobile Phases	: A 5 mmol/L Ammonium formate - Water : B 5 mmol/L Ammonium formate - Methanol	Probe Voltage	: + 4.0 kV (ESI-positive mode) / -3.0 kV (ESI-negative mode)
Gradient	: B.Conc. 0 % (0.0-2.0 min) → 70 % (4.5 min) → 90 % (8.0-12.0 min) → 0 % (12.01-15.0 min)	Nebulizing Gas Flow	: 3 L/min
Flowrate	: 0.4 mL/min	Heating Gas Flow	: 10 L/min
Column Temperature	: 40 °C	Interface Temperature	: 300 °C
		DL Temperature	: 150 °C
		Block Heater Temperature	: 250 °C
		Drying Gas Flow	: 10 L/min



**Fig. 1-1 Chromatograms of 16 Sweeteners**



**Fig. 1-2 Chromatograms of 16 Sweeteners (continued)**

**Table 2 Linearity of 16 Sweeteners**

Compound Name	Polarity	Transition	Holding Time (min)	Calibration Curve Range (ng/mL)	Correlation Coefficient
Sucralose	+	414.00>199.10	6.36	0.5 - 100	0.999
Dulcin	+	181.20>108.10	6.70	0.05 - 10	0.999
Alitame	+	332.20>129.00	6.92	0.5 - 100	0.999
Rebaudioside A	+	984.50>325.10	8.21	0.5 - 100	0.999
Stevioside	+	822.00>319.30	8.23	0.5 - 100	0.999
Acesulfame potassium	-	161.90>82.00	5.23	0.1 - 10	0.999
Saccharin	-	181.90>42.00	5.58	0.5 - 50	0.997
Cyclamate	-	178.00>80.00	6.08	1 - 100	0.999
Aspartame	-	293.40>261.10	6.53	5 - 100	0.999
Advantame	-	457.30>200.30	7.12	0.5 - 100	0.999
Glycyrrhizic acid	-	821.20>351.10	7.41	50 - 1000	0.999
Rebaudioside M	-	1289.60>802.90	7.66	50 - 1000	0.999
Neotame	-	377.30>200.00	7.90	1 - 100	0.999
Rebaudioside C	-	949.50>787.20	8.46	1 - 100	0.999
Dulcoside A	-	787.50>625.20	8.50	10 - 1000	0.999
Isosteviol	-	317.30>317.30	10.46	0.5 - 1000	0.999

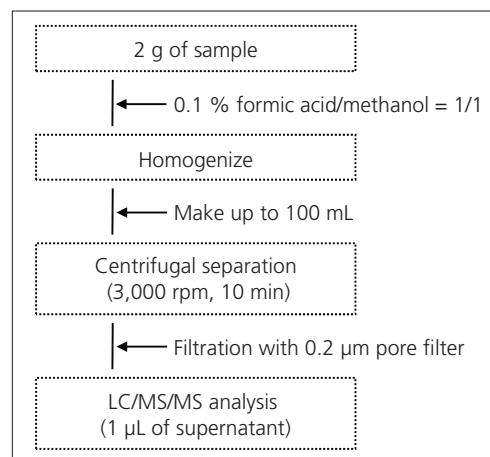
**Recovery from Real World Samples**

Sweeteners were added to sample solutions prepared according to the procedure shown in Fig. 2, and recovery of these additives was verified by measuring the samples after 100-fold or 1000-fold dilution. The results are shown in Table 3.

Dialysis and solid phase extraction are common methods used in sample pretreatment for sweetener analysis, but these operations have the drawback of being complex, time-consuming, and laborious. Pretreatment by solvent extraction requires no special equipment, and can be performed quickly and simply.

**Table 3 Recovery**

Compound Name	Additive Concentration	Real World Sample	Dilution Ratio	Recovery (%)
Glycyrrhizic acid	100 µg/mL	Soy sauce	100	85.20
Acesulfame potassium	10 µg/mL	Powdered soft drink	1000	81.21
Aspartame	10 µg/mL	(café au lait)	1000	104.2
Neotame	10 µg/mL	Ketchup	100	108.5



**Fig. 2 Pretreatment Workflow**

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