

# 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.

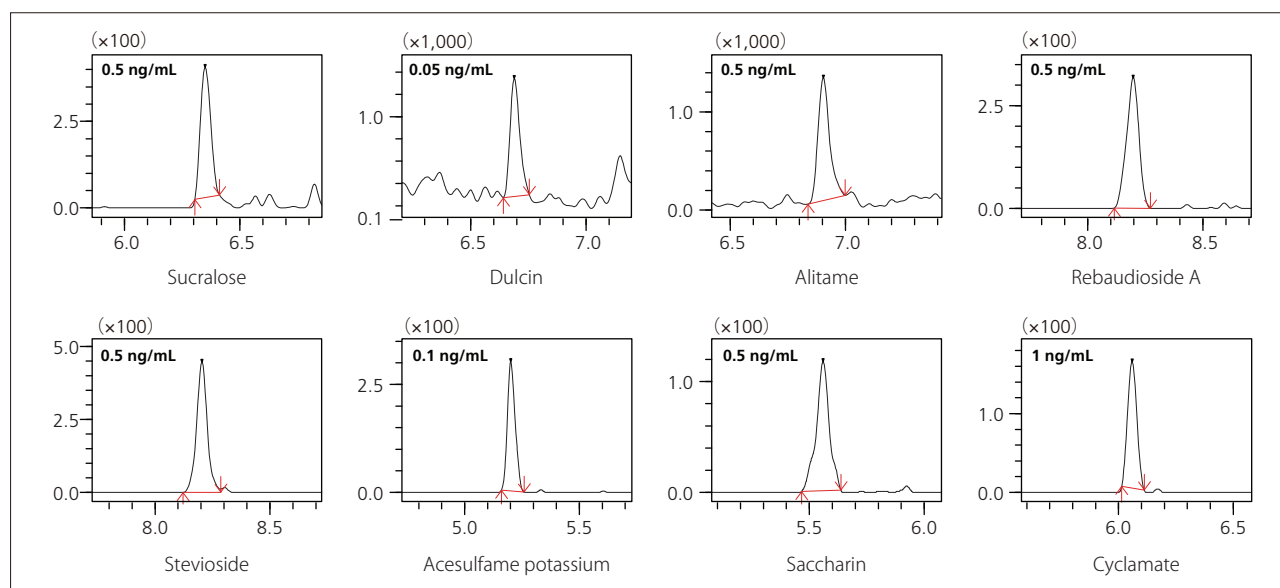
Application News C121 described the simultaneous analysis of nine artificial sweeteners including both designated and undesignated additives using an LCMS-8040 triple quadrupole LC/MS/MS system. In this article, we introduce an example of simultaneous analysis of 16 sweeteners using an LCMS-8050.

### Standard Mixture Analysis

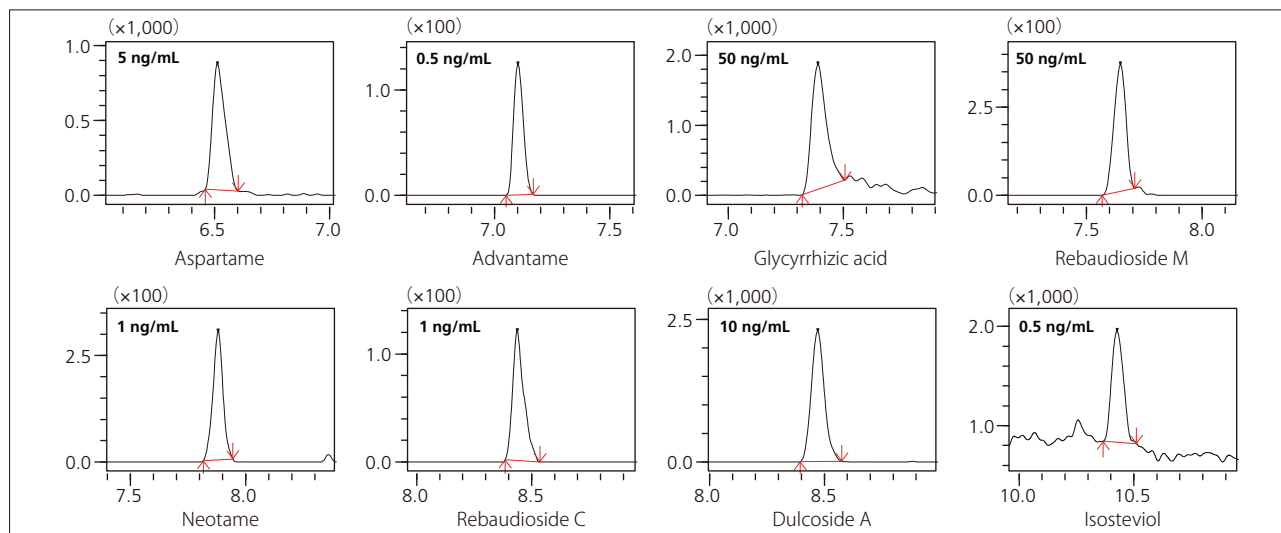
MRM analysis was performed on 16 sweeteners using the analytical conditions shown in Table 1. Chromatograms of each compound near their lower limit of quantitation are shown in Fig. 1, with calibration curve ranges and correlation coefficients shown in Table 2. Results that met an accuracy of 100 % ±20 % 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<br>(150 mm L. × 3.0 mm I.D., 3.0 μm)  | Injection Volume         | : 1 μL  |
| Mobile Phases      | : A 5 mmol/L Ammonium formate - Water<br>: B 5 mmol/L Ammonium formate - Methanol               | Probe Voltage            | : + 4.0 kV (ESI-positive mode) /<br>-3.0 kV (ESI-negative mode) |
| Gradient           | : B.Conc. 0 % (0.0-2.0 min)<br>→ 70 % (4.5 min) → 90 % (8.0-12.0 min)<br>→ 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                   |

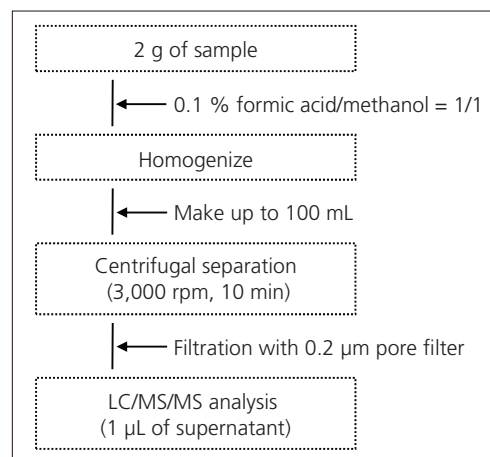
**Recovery from Real World Samples**

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**

This Application News was prepared with the cooperation of Japan Food Research Laboratories, who provided samples and guidance.

First Edition: Aug. 2016



**For Research Use Only. Not for use in diagnostic procedures.**

This publication may contain references to products that are not available in your country. Please contact us to check the availability of these products in your country.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu. Company names, product/service names and logos used in this publication are trademarks and trade names of Shimadzu Corporation or its affiliates, whether or not they are used with trademark symbol "TM" or "®". Third-party trademarks and trade names may be used in this publication to refer to either the entities or their products/services. Shimadzu disclaims any proprietary interest in trademarks and trade names other than its own.

The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.

Shimadzu Corporation

www.shimadzu.com/an/