

Application News

No. C121

Liquid Chromatography Mass Spectrometry

Simultaneous Analysis of Nine Sweeteners Using Triple Quadrupole LC/MS/MS (LCMS-8040)

Artificial sweeteners such as saccharin sodium, aspartame, sucralose and acesulfame potassium fall under the category of specified additives in Japan's Food Sanitation Act, for which each specified criteria exist for their use in terms of eligible foods and amounts used.

Cyclamate, an artificial sweetener used in some regions of the world outside Japan, is an unspecified additive within Japan, for which inspection is required on specific imported foods.

In light of these situations, there is a demand for analyses of various different sweeteners, not only the quantitative testing of permitted sweeteners but also the testing of unspecified sweetener additives.

This article presents a simultaneous analysis of nine sweeteners including both specified additives and unspecified additives, using the LCMS-8040 high-performance liquid chromatograph-triple quadrupole mass spectrometer.

■ Analysis of a Standard Mixture

Fig. 1 shows chromatograms measured from a 5 μ L injected sample of a 10 ng/mL standard mixture of nine sweeteners, analyzed with the analytical conditions shown in Table 1. Chromatograms at around the lower limit of quantitation (LLOQ) are shown in Fig. 2. The retention time, calibration curve range, and correlation coefficient for each compound are shown in Table 2. A calibration point accuracy of within 100 ± 20 % and a percentage of area repeatability (%RSD) of within 20 % were employed. Good linearity was obtained for all compounds with a correlation coefficient of 0.997 or higher.

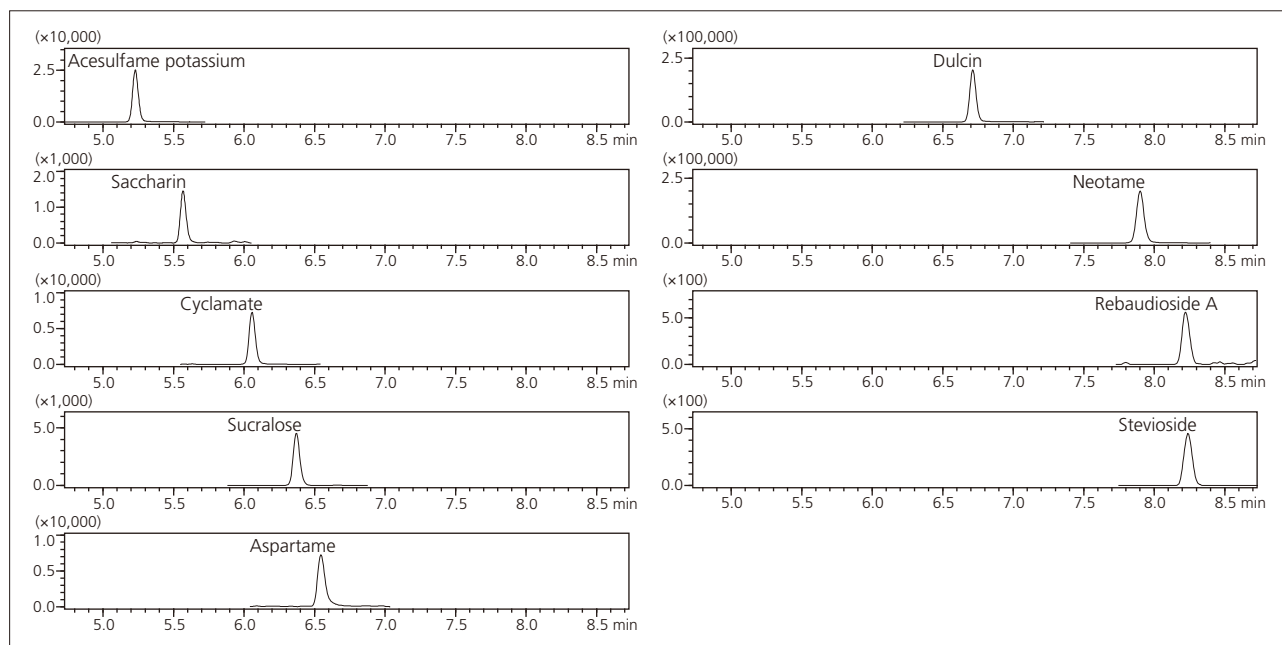


Fig. 1 Chromatograms from a 10 ng/mL Standard Mixture of Nine Sweeteners

Table 1 Analytical Conditions

Column	: Unison UK-C18 (150 mm L. \times 3.0 mm I.D., 3.0 μ m)
Mobile Phases	: A 5 mmol/L Ammonium Formate - Water : B Methanol
Gradient	: B Conc. 0 % (0.0 - 2.0 min) \rightarrow 70 % (4.5 min) \rightarrow 90 % (8.0 - 12.0 min) \rightarrow 0 % (12.01-15.0 min)
Flowrate	: 0.2 mL/min
Column Temperature	: 40 $^{\circ}$ C
Injection Volume	: 5 μ L
Probe Voltage	: + 4.5 kV (ESI-positive mode) / -3.5 kV (ESI-negative mode)
DL Temperature	: 300 $^{\circ}$ C
Block Heater Temperature	: 500 $^{\circ}$ C
Nebulizing Gas Flow	: 3 L/min
Drying Gas Flow	: 15 L/min

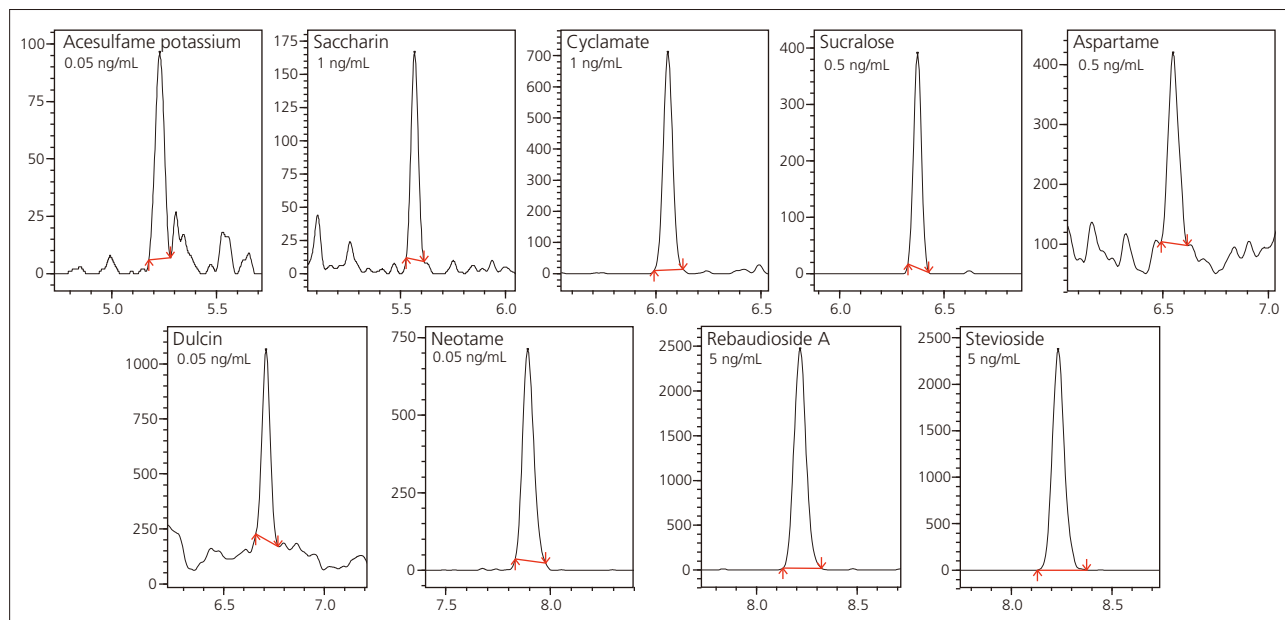


Fig. 2 Chromatograms of Nine Sweeteners at Around LLOQ

Table 2 Linearity of Nine Sweeteners

Compound Name	Polarity	Transition	Retention Time (min)	Calibration Curve Range (ng/mL)	Correlation Coefficient
Acesulfame potassium	-	162.00 > 82.10	5.228	0.05 – 100	0.997
Saccharin	-	182.00 > 42.00	5.561	1 – 100	0.999
Cyclamate	-	178.00 > 80.00	6.057	1 – 100	0.998
Sucralose	+	413.90 > 199.00	6.370	0.5 – 500	0.999
Aspartame	-	293.10 > 261.10	6.543	0.5 – 1000	0.999
Dulcin	+	181.20 > 108.10	6.712	0.05 – 10	0.999
Neotame	+	379.10 > 172.20	7.898	0.05 – 1000	0.999
Rebaudioside A	-	965.30 > 803.40	8.220	5 – 1000	0.999
Stevioside	+	822.30 > 319.20	8.238	5 – 1000	0.999

Recovery from Actual Samples

Seven sweeteners were added to foods (curry paste, rice cake flavored with mugwort, and sponge cake) pretreated by dialysis (Fig. 3), and the matrix effect was evaluated. The recovery of each added sweetener is shown in Table 3. Dulcin was the only sweetener for

which the recovery was calculated based on a 1000-fold dilution of the solution after dialysis treatment, while the recovery of all other sweetener samples was calculated based on 100-fold dilution. The recovery was good with all samples, ranging from 85 to 125 %.

Table 3 Recovery of Seven Added Sweeteners

Compound Name	Added Concentration	Recovery (%)		
		Curry Paste	Rice Cake Flavored with Mugwort	Chocolate Sponge Cake
Acesulfame potassium	5 µg/mL	100.8	94.2	93.7
Saccharin		97.0	87.7	88.3
Cyclamate		99.6	89.3	92.0
Sucralose		96.2	89.6	82.6
Aspartame		94.0	89.4	87.2
Dulcin		110.2	99.5	99.5
Neotame		122.5	106.9	110.0

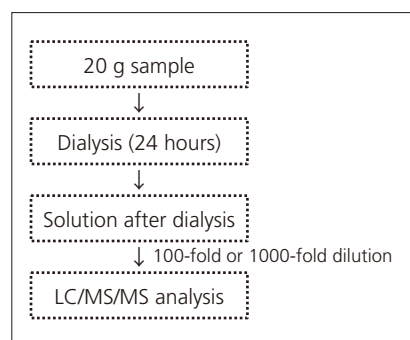


Fig. 3 Workflow of Pretreatment

This Application News was prepared with the cooperation of Tokyo Food Sanitation Association, who provided samples and guidance.