Matrix Effect and Application of 3D Analysis in LC/MS

Chromaster 5610 MS Detector is a mass spectrometer with new concept, designed for LC. By using CM5610 MS Detector Control and Analysis Software, the contour plots of the retention times, *m/z*, and ion intensities can be displayed. As a result, the differences between data can be visually analyzed. The matrix effect is known as a phenomenon in which the ionization of a target component changes due to the effect of contaminants. This time, the data obtained under the conditions susceptible to the matrix effect are compared by performing 3D analysis, and the example is introduced here.

5610 MS Detector

Comparison of Ion Intensities by Contour Plot

■ Analytical Conditions

Table 1 Conditions for MS Detector Setting

Ionization method	ESI
Ionization mode	Positive
Ionization voltage	2300 V
Measurement mode	Scan (*m/z* 200-600)

Table 2 Analytical Conditions for HPLC

Column	LaChromUltra II C18 (1.9 µm) 2.0 mm I.D. x 50 mm
Mobile phases	A = 0.1% HCOOH in CH_3CN (v/v), B = 0.1% HCOOH in H_2O (v/v), A:B = 70 / 30, 50 / 50, 30 / 70, in three separate runs.
Flow rate	0.3 mL/min (Split ratio = 1: 50)
Column temperature	40°C
Injection vol.	3 µL
Sample	10 mg/L Aspartame, 100 mg/L Caffeine mixture in CH_3OH

■ LC-MS Analysis

As an experimental sample, an aspartame and caffeine mixture solution was separated1 by three mobile phase which have different solvent mixture ratio, respectively, and the results are shown in Figure 1.
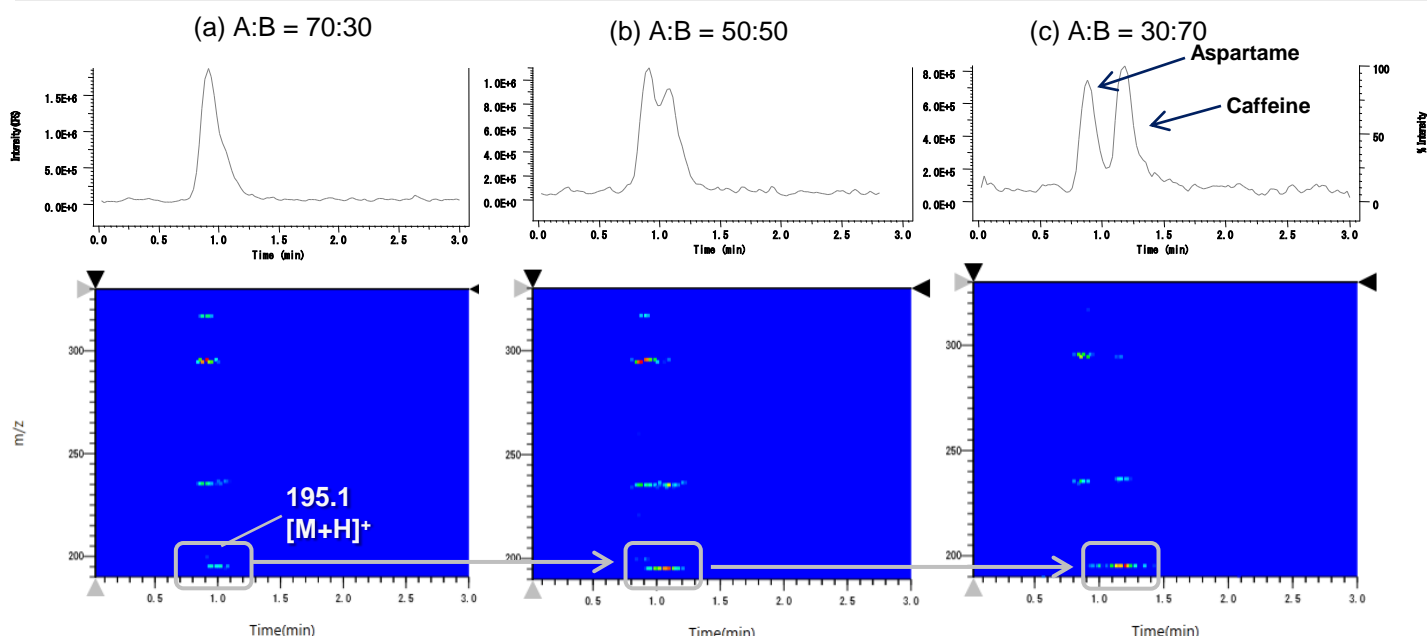

Figure 1 Total Ion Chromatograms (top) and Contour Plots (bottom) of Aspartame and Caffeine Mixture Solution

Compared to (a), obtained under the conditions of the insufficient LC separation, the intensity of the protonated caffeine ion, *m/z* 195.1, is increased under the conditions (b) and (c) for the improved LC separation. It is considered that there is the suppression of ions under the conditions of the insufficient LC separation (a). In the contour displays, the retentions time, *m/z*, and ion intensities can be viewed, and therefore, the differences in the data can be visually analyzed.

Confirmation of Matrix Effect by Comparison of Mixture Solution and Single Component Solution

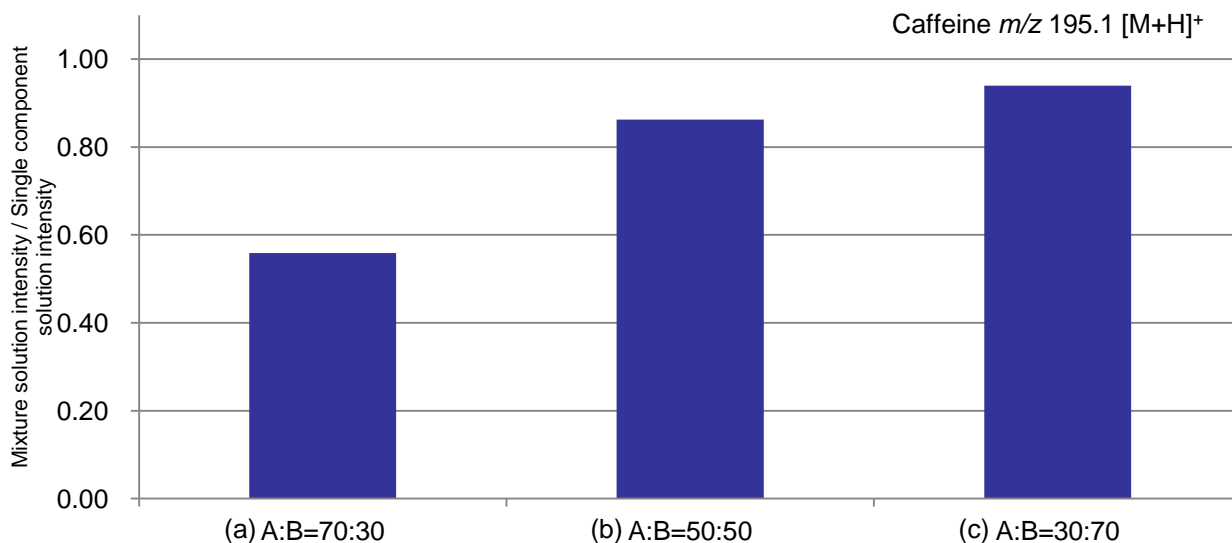■ Verification of Matrix Effect

Caffeine *m/z* 195.1 [M+H]$^+$

Figure 2 Verification of Matrix Effect

The technique to confirm the matrix effect by comparing the intensities of a mixture solution and single component solutions is known[1].

For the protonated caffeine ion, *m/z* 195.1, the intensity ratio between the single component solution and mixture solution was closer to 1 in the (b) and (c) obtained under the conditions for the improved LC separation. It was confirmed that there was the suppression of ions under the conditions in which the LC separation was insufficient.

1) Mochizuki, Naoki. (2011). Problems in LC-MS/MS Analysis to Ensure Food Safety. *YAKUGAKU ZASSHI,* 131(7) 1019-1025.

■ **Examples of Methods for LC Separation Improvement to Prevent Matrix Effect**

Method	Specific Method	Description
Change the system	Use of Low Diffusion Tubing Kit*1	The system volume is reduced and the peak broadening due to diffusion may be suppressed.
Change the analytical conditions	Change to a column with smaller particle size diameter	With the use of smaller particle size diameter, the surface area is increased compared to column with larger particle size diameter, and thus, the separation may be improved.
	Change to a core-shell column	It is known, the number of theoretical plates is higher for the fully porous column when the particle size diameter of core-shell column is same. As a result, the improved separation can be expected.
	Change the gradient ratio and analysis time	When using the gradient elution, by reducing the gradient ramp and increasing the analysis time, the separation may be improved.

In addition to the LC separation improvement, proper preparation method and quantitation method are also effective in order to prevent matrix effect.

*1 Please contact our sales department for the details.

<Main system configuration>

Chromaster 5110 Pump, Chromaster 5210 Autosampler, Chromaster 5310 Column Oven, 5610 MS Detector

NOTE: These data are an example of measurement; the individual values cannot be guaranteed.

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