Sucralose is a synthetic sweetener that has approximately 600 times the sweetness of sugar, zero calories and a flavor closely resembling sugar. Over 30 countries throughout the world have approved its use. Japan also approved its use as a food additive in 1999. In Japan, the permitted level of sucralose content in soft drinks is 0.40g/kg, and sucralose is quantified by titration. In the U.S., sucralose is quantified by HPLC. This Application News introduces an example of analyzing sucralose contained in soft drinks using a differential refractive index detector and an evaporative light scattering detector.

**Analytical Conditions**

Sucralose, also known as trichlorogalactosucrose, has a structure where sucrose's three hydroxyl groups have been replaced by three chlorine atoms (Fig. 1). Sucralose is generally analyzed by the combination of reversed-phase chromatography and a refractive index detector (RID). Here, an evaporative light scattering detector (ELSD) was serially connected with an RID for data comparison. Fig. 2 shows the result of analyzing 20µL of sucralose standard sample (400mg/L in purified water). The analytical conditions are shown in Table 1.

- **Column**: Shim-pack VP-ODS (150mm L×4.6mm I.D.)
- **Mobile Phase**: Water / Acetonitrile = 85 / 15 (v/v)
- **Flow Rate**: 1.0mL/min
- **Temperature**: 40˚C
- **Detection**:
  - RID-10A
  - ELSD-LT
    - Temperature : 35˚C
    - Gain : 7
    - Nebulize Gas: N2
    - Gas Pressure : 350kPa
- **Injection Volume**: 20µL

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**Table 1 Analytical Conditions**

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<tr>
<th>Column</th>
<th>Shim-pack VP-ODS (150mm L×4.6mm I.D.)</th>
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**Fig.1 Structure of Sucralose**

**Fig.2 Chromatogram of Standard Sucralose (400mg/L)**
Fig. 3 shows the chromatograms obtained by RID and ELSD when injecting 20µL of standard sucralose sample (40mg/L). The analytical conditions are the same as in Table 1. A more stable baseline is obtained with ELSD. However, ELSD’s detection response is not proportional to concentration. Therefore, the calibration curve must be plotted using double logarithmic coordinates.

**Figure 3 Chromatogram of Standard Sucralose (40mg/L)**

Fig. 4 shows the chromatograms obtained when injecting 20µL of soft drinks A and B, and canned coffee. The analytical conditions are the same as in Table 1. Beverage B and the canned coffee did not contain sucralose, so they were spiked in advance with sucralose to obtain a concentration of 400mg/L. Soft drinks A and B were filtered through a membrane filter before injection. For the canned coffee, the injection sample was obtained by centrifugal separation (12,000rpm × 15min) using an ultrafiltration membrane (M.W. 10,000).

**Figure 4 Chromatogram of Soft Drink A**

**Figure 5 Chromatogram of Soft Drink B (spiked 400mg/L)**

**Figure 6 Chromatogram of Canned Coffee Drink (spiked 400mg/L)**

**Analysis of Soft Drinks**

Two soft drinks A and B, and canned coffee were analyzed. The analytical conditions are the same as in Table 1. Beverage B and the canned coffee did not contain sucralose, so they were spiked in advance with sucralose to obtain a concentration of 400mg/L. Soft drinks A and B were filtered through a membrane filter before injection. For the canned coffee, the injection sample was obtained by centrifugal separation (12,000rpm × 15min) using an ultrafiltration membrane (M.W. 10,000).

Bibliography:
2) Food Chemicals Codex Fourth Edition