Analysis of Methylxanthines by PDA Detector and Mass Detector

Chromaster 5610 MS detector is a new mass detector, designed for LC users, and it is different from conventional mass spectrometers. This time, the analysis examples of methylxanthines such as caffeine are introduced here. These components are often contained in coffee, cacao products, and supplement drinks. Methylxanthines have a common basic molecular structure. Therefore, UV spectral patterns are very similar and the differentiation based only on the UV spectra is difficult. However, a mass detector can provide the mass information unique to a component, and thus the identification accuracy is improved.

5610 MS Detector

LC-MS measurement of Methylxanthines series 4 compounds

■ Analytical Conditions

Table 1 MS Detector Setting Conditions

Ionization method	ESI
Ionization mode	Positive
Ionization voltage	2800 V
Measurement mode	Scan (*m/z* 100-300)

Table 2 HPLC Xonsiriona

Column	LaChrom Ⅱ C18 (3 μm), 4.6 mm I.D. x 100 mm
Mobile phase	A: 0.1%formic acid solution B: 0.1%formic acid solution - Methanol solution B10%(0min)→B50%(5min)→B10%(5.1-8min)
Flow rate	1.0 mL/min (250:1 sprit)
Column Temp.	50 ˚C
Detection w.l.	DAD 275 nm
Injection Volume	10 μL

■LC-MS Measurement

Each compound, Caffeine, Theobromine, Theophylline and 7-(2-hydroxyethyl) - Theophylline, is dissolved and diluted with methanol so as to prepare the sample at concentration of 10 μ g/mL. Due to the similar chemical structure, UV spectra obtained by DAD have a similarity. However, MS Detector permits to identify the compounds by the confirmation of [M + H]$^+$ ion.
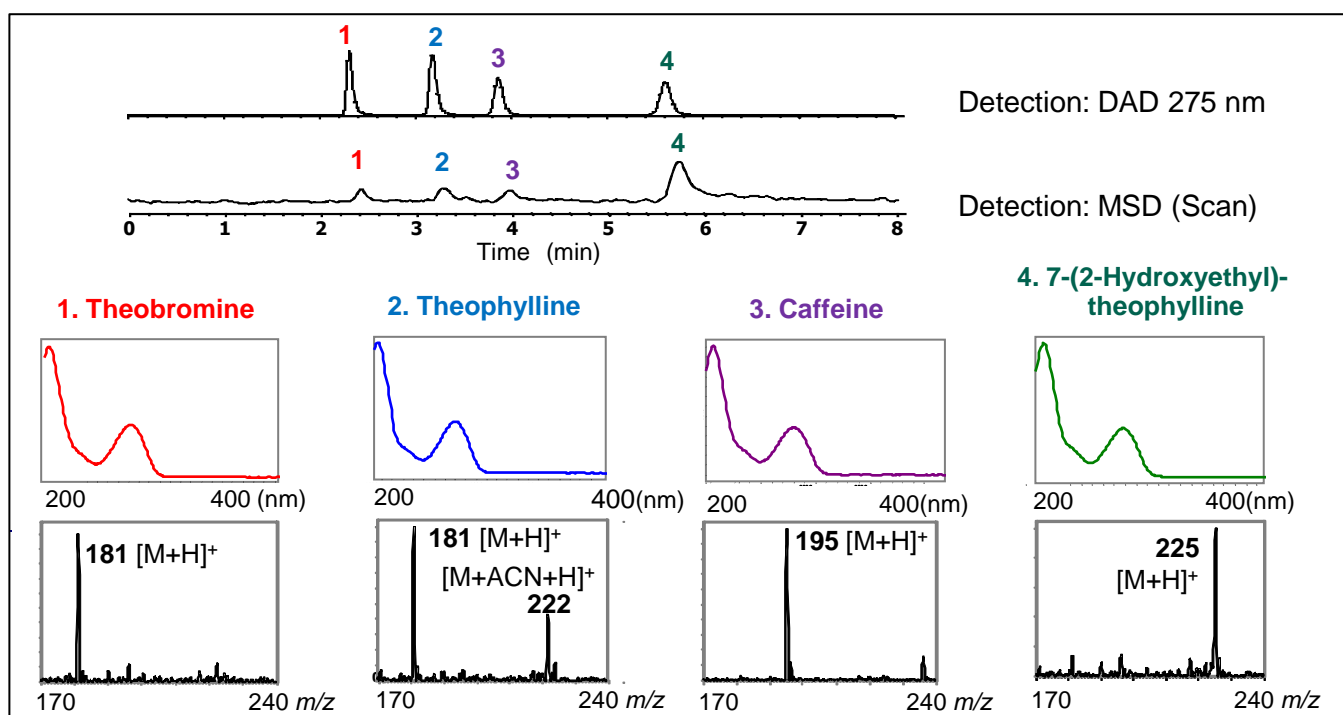

Detection: DAD 275 nm

Detection: MSD (Scan)

1. Theobromine   **2. Theophylline**   **3. Caffeine**   **4. 7-(2-Hydroxyethyl)-theophylline**

181 [M+H]$^+$

181 [M+H]$^+$ [M+ACN+H]$^+$ **222**

195 [M+H]$^+$

225 [M+H]$^+$

Fig. 1 Measurement result of DAD and MS Detector

Main system configuration: 5110 Pump, 5210 Autosampler, 5310 Column Oven, 5430 DAD, 5610 MS Detector

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

⊛ Hitachi High-Tech Science Corporation