

LC-MS Application Data Sheet No. 030

Analysis of Antioxidants in Medicines and Food

Lifestyle-related diseases (adult diseases) are diseases stemming from habits in everyday life, such as diet, smoking, and drinking alcohol. Strokes, cardiac infarction, diabetes and cancer are examples of lifestyle-related diseases. Due to the chronic nature and difficulty in treatment of these diseases, much importance is attached to preventing the onset of these diseases by improving habits in everyday life. A cause of these chronic diseases is the excessive generation of active oxygen within the body. Therefore, antioxidants contained in food are attracting attention as effective substances to prevent lifestyle-related diseases.

Examples of antioxidants contained in food include carotenoids (such as *B*-carotene, lycopene, capsaicin), flavonoids and vitamin E (refer to the LC-

MS Application Data Sheets No.014 and No.015 for examples of flavonoid analyses). Introduced here are examples of LC-MS analyses of vitamin E and *B*carotene, a typical carotenoid. Antioxidants in a commercially available health drink, vitamin E drug and pumpkin extract fraction were monitored in these analyses.

Fig.104 shows the structural formula of vitamin E related compounds and β -carotene related compounds. As shown in Fig.105, the peak of vitamin E appeares in 4.1 minutes and β -carotene in 11.9 minutes (analytical conditions are listed at the end of this report). Simple mass spectra where only the protonated molecules are given has been obtained for both compounds.





Fig. 105 Mass chromatograms of vitamin E, ß-carotene standard

Fig.106 shows the results (mass chromatograms) of analyzing a health drink, vitamin E drug and pumpkin extract fraction. For the health drink, in addition to vitamin E and β -carotene, components giving protonated molecules were also observed at m/z 327, 355 and 383 (at an interval of 28amu).

Components giving protonated molecules were also observed for the vitamin E drug (in addition to vitamin E) and for the pumpkin extract fraction (in addition to β -carotene) at m/z 599, 601, 603 and 605. As these components appear at an interval of 2amu,

it can be inferred that they are compounds containing many double bonds like a carotenoid, and that they are homologues differing in the number and position of the double bonds. In the pumpkin extract fraction, compounds of m/z 615 and 617 were also observed. The difference in the mass number (increases of 16amu) and the characteristics of the chromatogram show that the compounds at m/z 615 and 617 are oxidants of the compounds at m/z 599 and 601. As a result, it can be concluded that compounds at m/z 599, 601, 603, and 605 are also antioxidants.



Fig. 106 Mass chromatograms of healthy drink, drug containing vitamin E and THF extract of pumpkin

Table 34 Analytical conditions for LC-MS		
Column	: STR ODS-II (2.0 mml.D. x 150 r	nmL)
Mobile phase A	: methanol	Mobile phase B : ethanol
Gradient program	: 0%B(0min) ->80%B(15-20min)	Flow rate : 0.2 mL/min
Column temperature	: 40	
Probe voltage	: +4.5 kV (APCI-Positive mode)	
Probe temprature	: 400	Nebulizing gas flow : 2.5 L/min
CDL voltage	: -40 V	DEFs voltage : +50 V
Scan Range	: m/z 200-750 (1.0 sec/scan)	-

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