SHIMADZU

LC-MS Application Data Sheet No. 036

Analysis of vitamin K in food using LC-MS

Conventionally, HPLC has been used to analyze vitamin K in foods. HPLC achieves highly sensitive detection and quantification, but complex pretreatment is required to eliminate interfering components from the sample. This data sheet reports on using LC-MS for detection and quantification of vitamin K in foods, which allows simpler pretreatment than the typical pretreatment methods for HPLC and is unaffected by interfering components.

Fig.1 shows the structures of phylloquinone (vitamin K_1) and menaquinone-4 (vitamin K_2) and their mass spectra obtained using atmospheric pressure chemical ionization (APCI). Fig. 2 shows a mass chromatogram of phylloquinone and menaquinone-4 standards. Fig. 3 shows the calibration curves for phylloquinone and menaquinone-4, created in the range 1 to 100 ppb. These exhibit excellent linearity with r^2 values of 0.99969 and 0.99947, respectively.

8mL hexane and 1mL water were added to 0.1g of the homogenized food sample. This was then shaken and centrifuged. The residue recovered from the hexane layer under reduced pressure was dissolved in 200μL ethanol, centrifuged again, and the supernatant taken as the analysis sample. Fig.4 shows the HPLC chromatograms and SIM chromatograms of samples of margarine and Japanese natto (fermented soya beans).

The results indicate a phylloquinone concentration of 9.06ppb and menaquinone-4 concentration of 9.38ppb in natto and a phylloquinone concentration of 58.03ppb in margarine. No menaquinone-4 was detected in the margarine.

The pretreatment method used for this analysis resulted in almost 100% recovery. Calculations indicate that 0.1g natto contains 2.90ng phylloquinone and 3.00ng menaquinone-4 and 0.1g margarine contains 18.56ng phylloquinone.

These results indicate that LC-MS permits the detection and quantification of vitamin K in food samples with simple pretreatment and shorter work times.

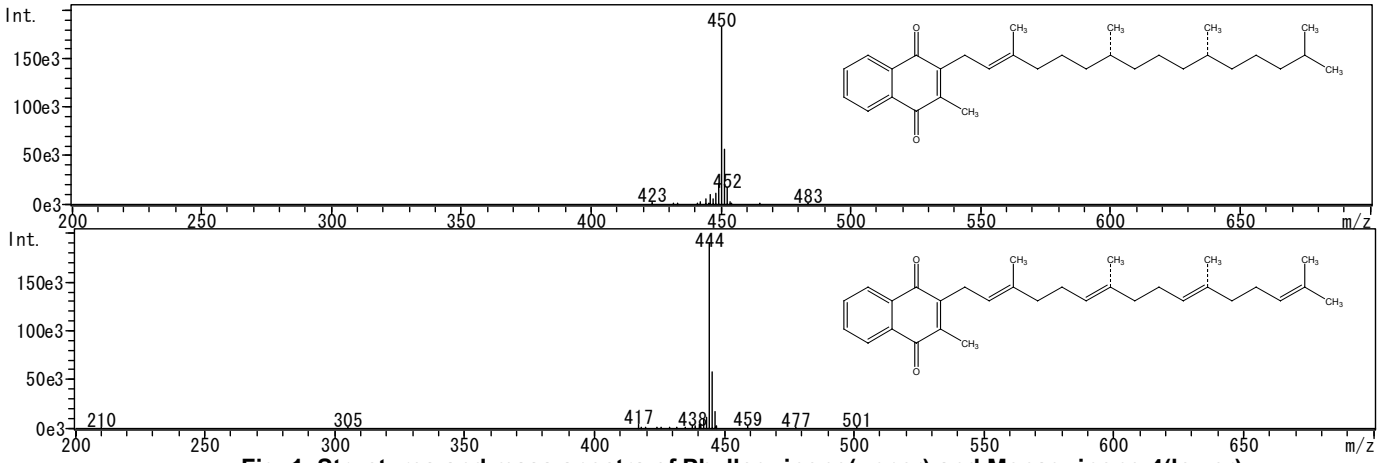

Fig. 1 Structures and mass spectra of Phylloquinone(upper) and Menaquinone-4(lower)

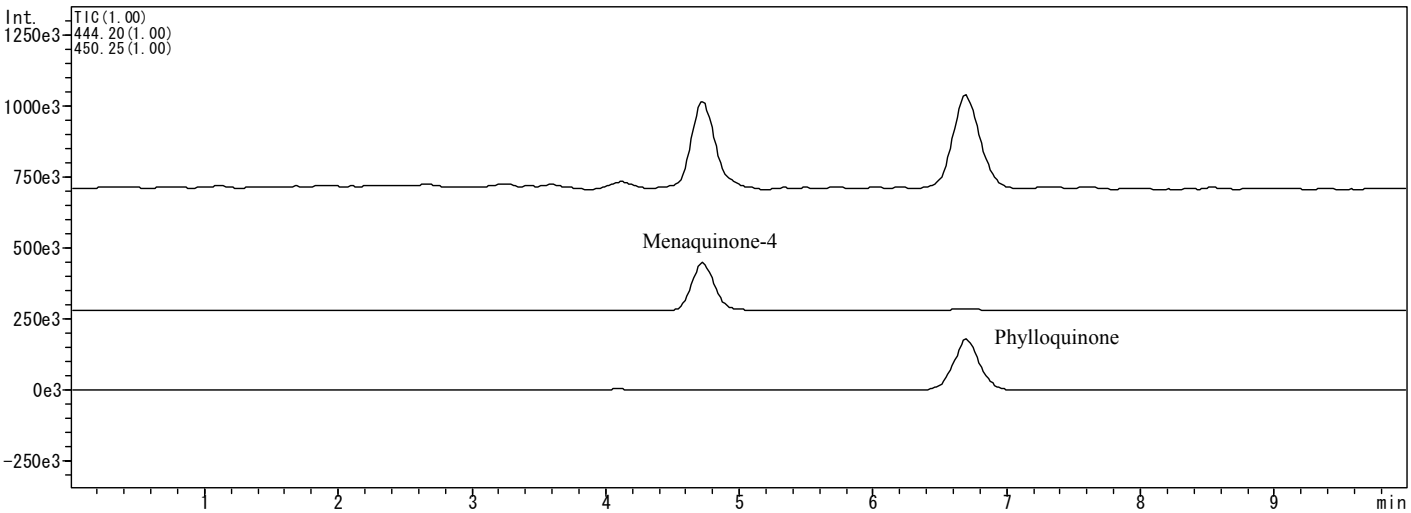

Fig. 2 Mass chromatograms of Phylloquinone and Menaquinone-4

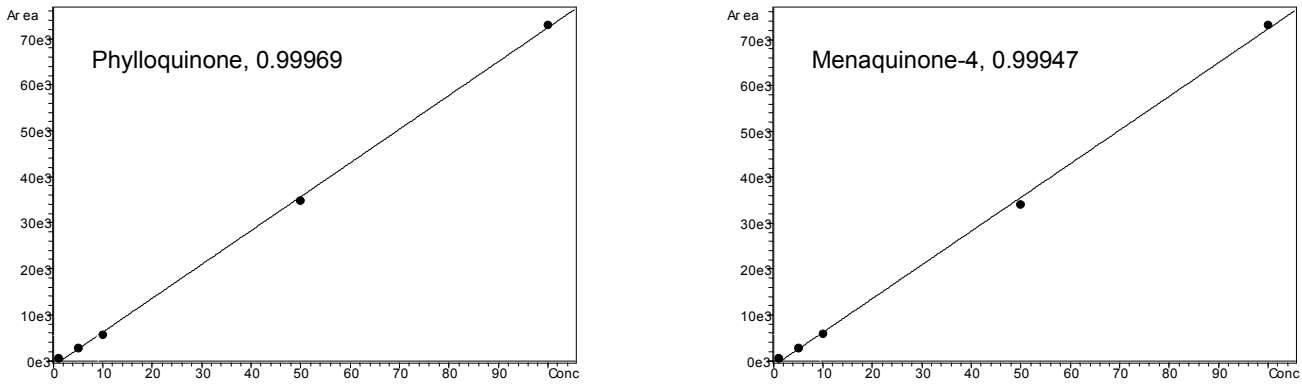

Fig. 3 Calibration curves of Phylloquinone and Menaquinone-4 (Range: 1 to 100 ppb)

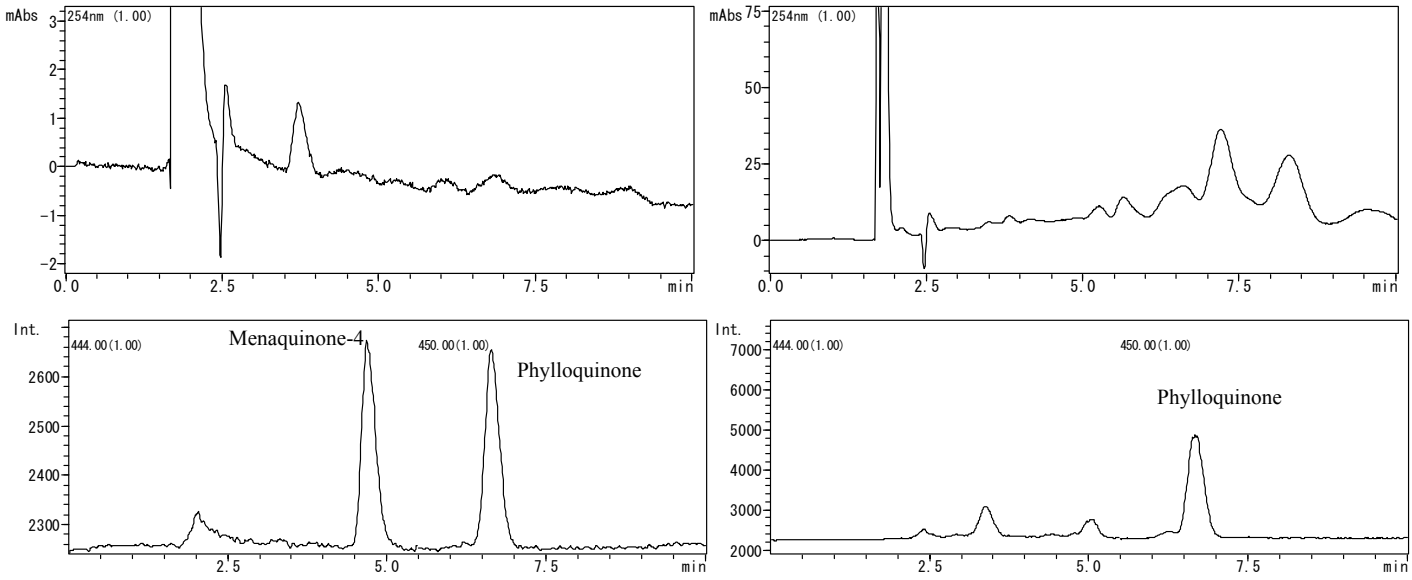

Fig. 4 UV chromatograms (upper) and SIM chromatograms (lower) of margarine (left) and natto (right)

Table 1 Analysis conditions

Column	: Shim-pack VP-ODS (2.0 mmI.D. x 150 mm)		
Mobile phase	: 25% 2-propanol - methanol		
Flow rate	: 0.2 mL/min		
Injection volume	: 10uL		
Column temperature	: 40 °C		
Probe voltage	: -4.0 kV (APCI-Negative mode)		
CDL temperature	: 250°C	**Block heater temperature**: 200°C	
Nebulizing gas flow	: 2.5 L/min		
CDL voltage	: 0V		
Q-array DC voltage	: -45V	**Q-array RF**	:150

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