LT035

GL Sciences Inc.

# Analysis of vitamin K in foods using HPLC with column-switching

Vitamin K is a fat-soluble vitamin. Vitamin K1 (phylloquinone) and vitamin K2 (menaquinones) are both naturally occurring, with vitamin K1 being abundant in green plants, and K2 abundant in cheese and fermented soybeans. K2 is menaquinone-n (MK-n); according to the number of isoprenes in its side chain. In general, MK-4 is abundant in fermented soybeans and MK-7 is abundant in fermented soybeans.

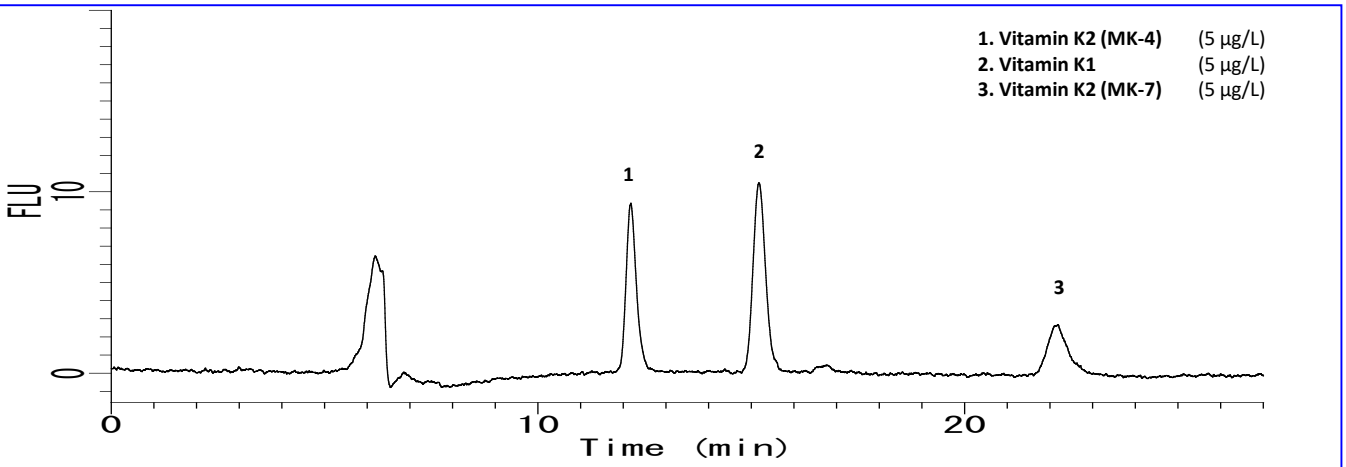
Vitamin K3 is an industrially synthesized compound that does not exist in nature.

Vitamin K was analyzed by solvent extraction and injection onto a solid-phase extraction-purified test solution into a column-switching HPLC system.

In the HPLC system, a switching valve is controlled to facilitate the concentration of target components and removal of contaminants.

## Examples: Analysis of vitamin K

(K.Suzuki)



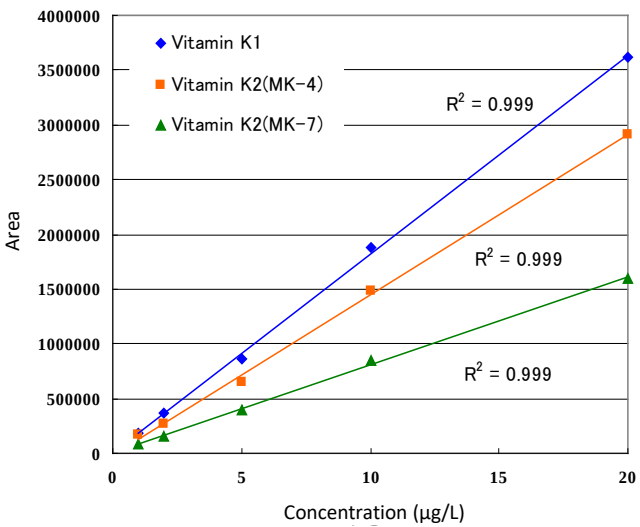

Chromatogram of the standard solution

**HPLC conditions**

| | |
|---|---|
| **Precolumn** | :Inertsil ODS-3 (5 µm, 50 x 4.6 mm I.D.) |
| **Analytical column** | :Inertsil ODS-3 (5 µm, 250 x 4.6 mm I.D.) |
| | Reduction column platinum black column |
| **Eluent** | : A) $C_2H_5OH$ B) $CH_3OH$ |
| (Pump A) | A/B = 5/95 v/v, 1 mL/min |
| **Eluent** | : A) $C_2H_5OH$ B) $CH_3OH$ |
| (Pump B) | A/B = 50/50 v/v, 1 mL/min |
| **Temperature** | :40 ˚C |
| **Detector** | : FL Ex 320 nm Em 430 nm |
| **Injection volum** | :50 µL |

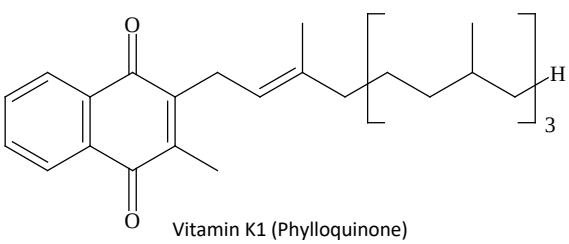
2.5 minutes after injection, the sample is introduced onto the analysis column from the precolumn

Return the valve 6 minutes after injection.

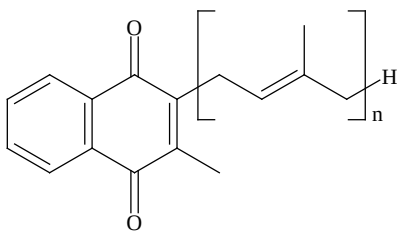



Calibration curve

## Structural formula




Vitamin K1 (Phylloquinone)




Vitamin K2 (Menaquinone-n:MK-n)

Structures are created using Chemistry 4-D Draw which is provided by ChemInnovation Software, Inc.

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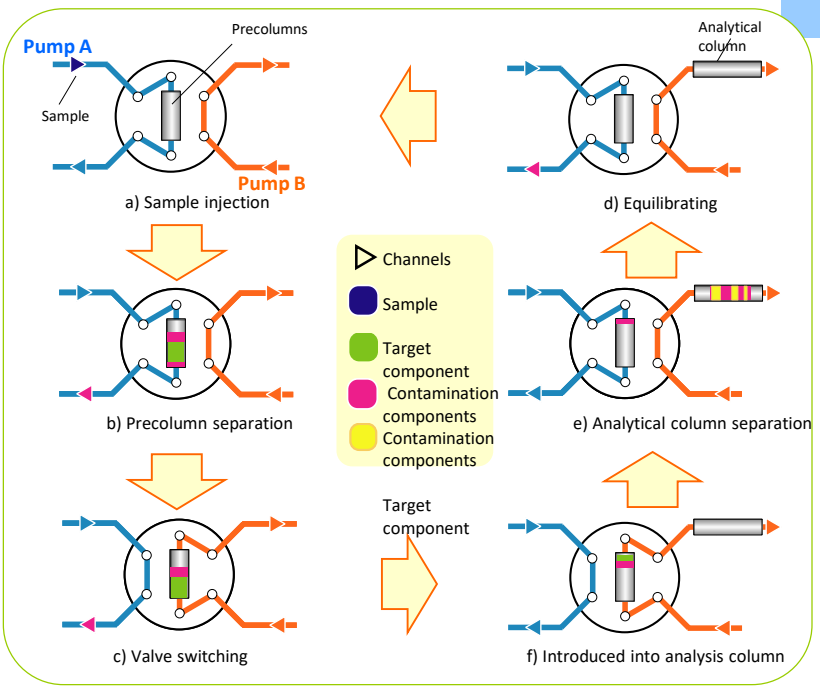
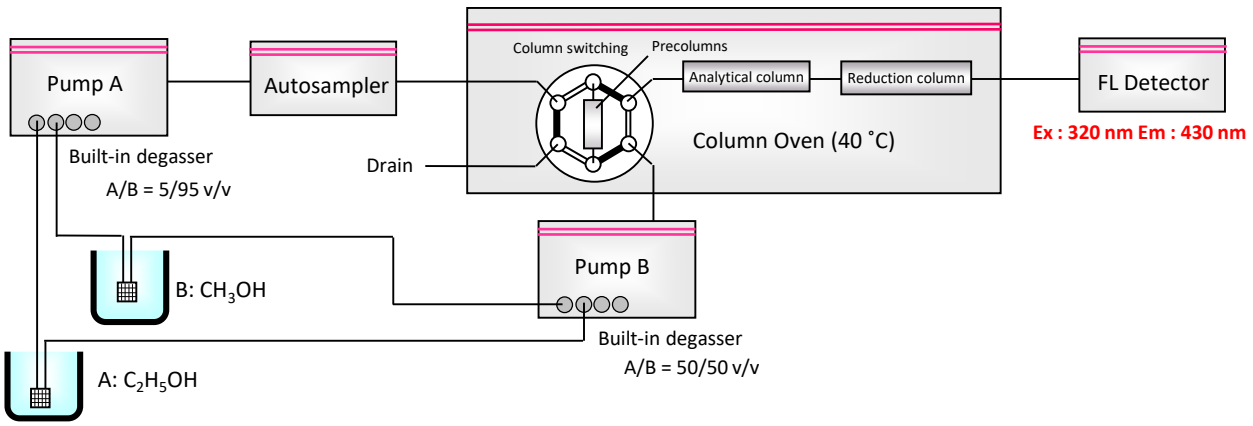
Column switching is frequently used to remove contaminants and to concentrate compounds. Manual pretreatment has the disadvantage of not controlling reproducibility, an automated column switching system is very effective because the timing is accurately controlled.

A diagram of the flow path of the column switching system is shown in the figure below, and the flow of sample injection is shown in the figure to the right. First, a sample injected by an autosampler is introduced onto a precolumn to remove contaminants and to concentrate the target components. Then, just before the target components are eluted from the pre-column, a six-way valve is switched and the pre-column is flushed with a solvent of different composition from the reverse direction. As a result, the target components retained on the precolumn are eluted and introduced onto the assay column. After the sample is introduced onto the analysis column, the valve switches again to wash and equilibrate the pre-column. This process enables sample concentration and removal of contaminants, allowing further separation of the target components on the analysis column.




## Analytical conditions and flow chart

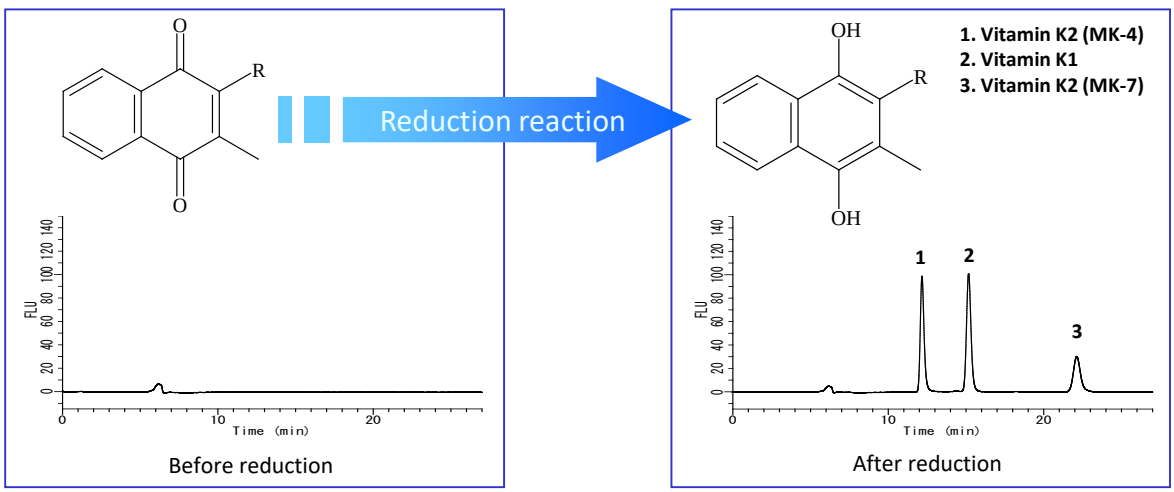



# Fluorescence of Vitamin K using a Reduction Column

When vitamin K (quinone) is reduced to the hydroquinone, it becomes fluorescescent.

Reduction can be made by contact with a catalyst such as platinum black or by using a reducing reagent. The latter method has the disadvantage of large reagent deactivation as well as the need for an additional pump.

In this paper, the former method was adopted, and platinum black was used in a packed column.



# Examples: Vitamin K pretreatment in foods

## Cereal and dry matter extractions

- **Sample**
  - 5g
- **Extraction**
  - 10 mL of n-hexane
  - Homogenize
  - 10 mL of *n*-hexane
  - Shake 5 min
  - Centrifugal separation 2500 rpm, 5 min
- **Preparative isolation**
  - 5 mL aliquot.
- **Solvent removal**
  - Below 40 ˚C under reduced pressure
- **Constant volume**
  - 2 mL of *n*-hexane

To purification

## Extraction of fats and oils and fats

- **Sample**
  - 0.1 g
- **Extraction**
  - Methanol 10 mL
  - Shake 10 min
  - Centrifugal separation 2500 rpm, 5 min
- **Preparative isolation**
  - Separate methanol layer
  - Purified water 1 mL
  - 8 mL of *n*-hexane
- **Extraction**
  - Shaking 5min
  - Centrifugal separation 2500 rpm, 5 min
  - Separate 5 mL *n*-hexane layer
- **Solvent removal**
  - Below 40 ˚C under reduced pressure
- **Constant volume**
  - 2 mL of *n*-hexane

To purification

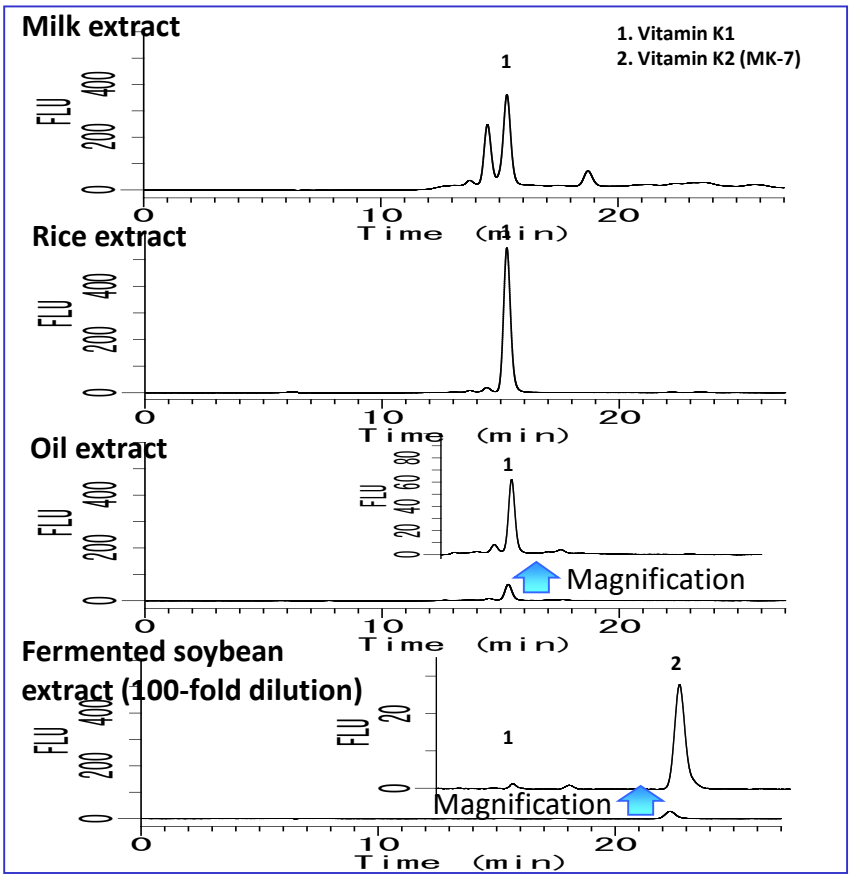
## Extraction of other general foods

- **Sample**
  - 1 g
- **Extraction**
  - Acetone 10 mL
  - Homogenize
  - 10 mL of *n*-hexane
  - Shake 5 min
  - Centrifugal separation 2500 rpm, 5 min
- **Preparative isolation**
  - 5 mL aliquot.
- **Solvent removal**
  - Below 40 ˚C under reduced pressure
- **Constant volume**
  - 2 mL of *n*-hexane

To purification

## Purification operation

- **Mega BondElut SI 1g / 6mL**
- **Sample through**
  - Load sample
- **Washing**
  - 5 mL of *n*-hexane
- **Elution**
  - Mixture of diethyl ether and *n*-hexane (4:96, v/v) 5mL
- **Solvent removal**
  - Below 40 ˚C under reduced pressure
- **Constant volume**
  - If necessary, perform thin-layer chromatography and further purify.
  - Ethanol 200 µL
- **Column switching HPLC-FL**



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