Application Note
No. 720051H-E

Analysis of Vitamin K using Reduction Catalyst Column

Vitamin K is essential for the synthesis of several proteins involved in blood clotting and calcium-binding. The vitamin K-dependent proteins include the bone proteins such as osteocalcin which has a function of bone metabolism, and coagulation/anticoagulation proteins which control blood coagulation. However, its physiological and physiological roles and behavior in vivo are still unclear. To clarify these, a simple and highly sensitive measurement method is needed.

As of today, the electrochemical reduction method, post-column derivatization method by sodium tetraborate solution and reduction method by platinum column are used for highly sensitive analysis of vitamin K, using reduction of the K-quinone to the fluorescent K-hydroquinone.

In this report, vitamin K, reduced by a reduction column set behind a separation column, is detected by the fluorescence detector with high selectivity and sensitivity. Fig. 1 shows the system configuration, and Fig. 2 shows the measurement principle. Chromatogram of standard samples are shown in Fig. 3.

Conditions:

Column:	CrestPak C18S (4.6 mm I.D. x 150 mm)
Eluent:	CH_3OH/C_2H_5OH (70/30)
Flow rate:	1.0 mL/min
Column temperature:	40 degree celsius
Reduction catalyst column:	CATALYSISPAK PT (4.6 mm I.D. x 10 mm)
Reaction temperature:	40 degree celsius
Wavelength:	Ex 320 nm, Em 430 nm, Gain x100
Sample:	STD mixture (Vitamin K_1, K_2, K_3) (1.0 mg/L each)
Injection volume:	10 µl

Keywords: 1. Vitamin K_1, 2. Vitamin K_2, 3. Vitamin K_3, 4. CrestPak C18S, 5. CATALYSISPAK PT

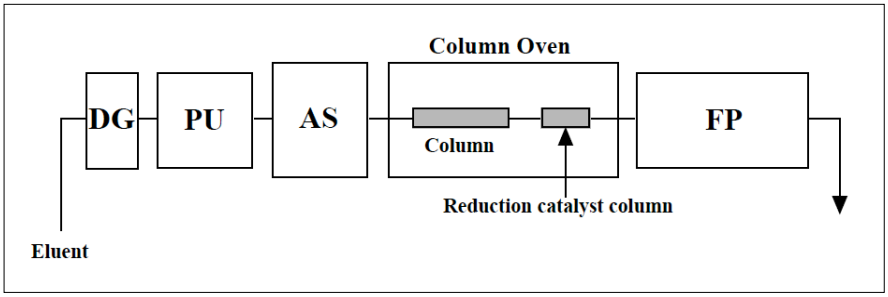

Fig. 1 Flow diagram

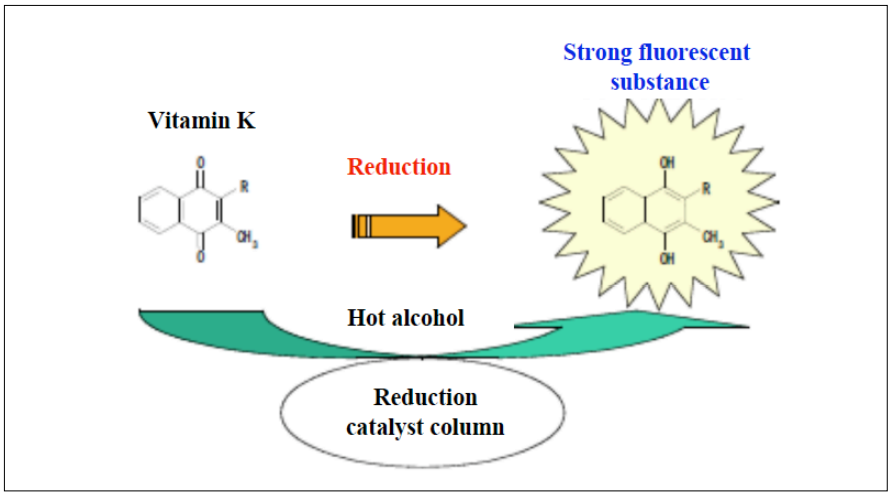

Fig. 2 Measurement principle

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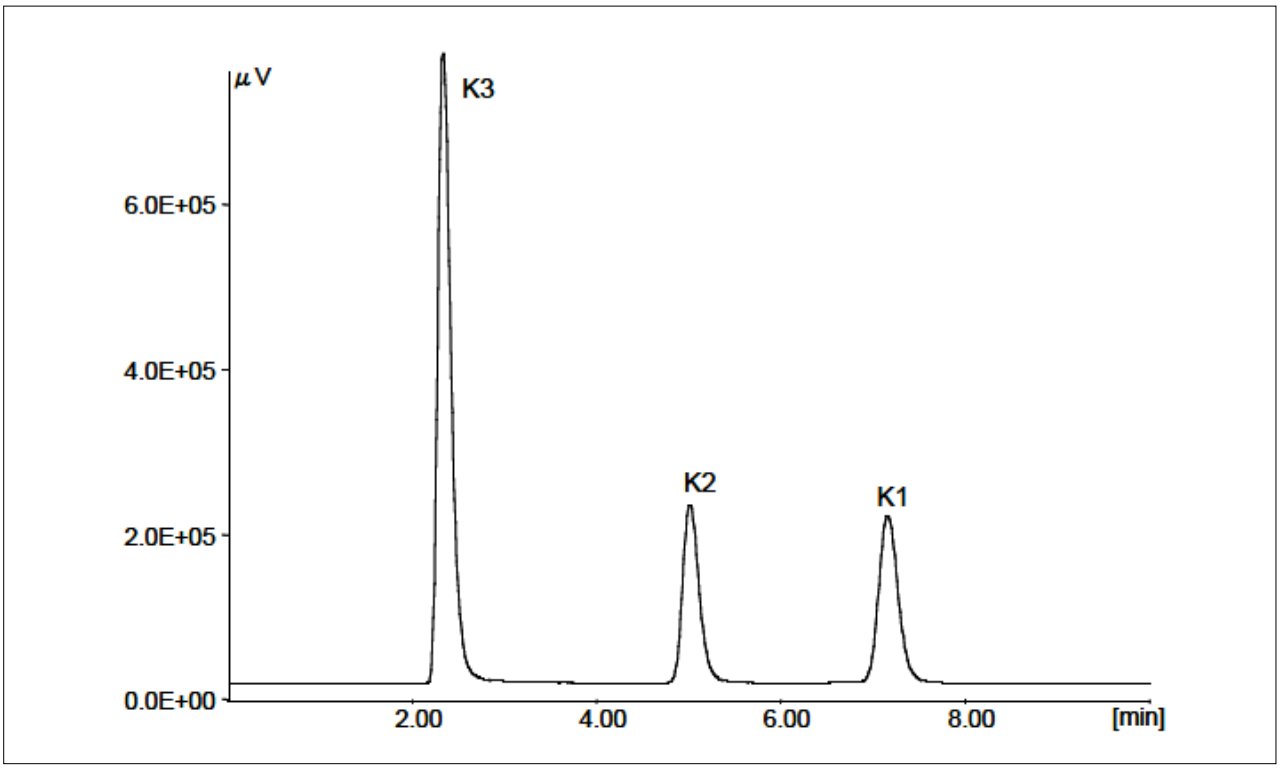

Fig. 3 Chromatogram of viamin Ks

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