Factors Affecting Vitamin K Analysis in Serum, Food and Dietary Supplements

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BACKGROUND

Vitamin K (VK) is an essential vitamin that is necessary for normal blood coagulation. It functions in the carboxylation of glutamate to d-carboxyglutamic acid. VK also functions in bone mineralization, vascular demineralization and cell growth.

There are three major forms of vitamin K: K1, phylloquinone is primarily from vegetables; K2, menaquinones are from microbial and bacterial sources; and K3, menadione is a synthetic compound. VK has relatively weak UV absorbance near 250, 270, and 325nm. VK lacks endogenous fluorescence (FL) but is highly FL when reduced from the quinone to the semi-quinone. They also cover a broad range of polarity that may be difficult to separate from matrix components.

The concentrations vary several orders of magnitude from blood (low ng/mL), foods (ng-μg/g), and supplements (mg/g). The use of UV absorbance for HPLC detection lacks sensitivity and selectivity for most samples. These diverse samples require unique modes of sample preparation.

OBJECTIVES

- To compare the separation of vitamin K by Normal-Phase (NP) and Reversed-Phase (RP) with various HPLC columns.
- To compare various modes of detection for sensitivity, selectivity and specificity.
- To examine the influence of sample preparation of serum, food and dietary supplements on measured vitamin K.

METHODS

- Serum or plasma (500uL) was mixed with 1mL alcohol then extracted 3x with 2mL hexane. Samples were extracted w/wo K2-spike to determine recovery. The combined extract was dried in a SpeedVac and redissolved in 1mL hexane. The hexane was applied to preconditioned 500mg Si SPE columns. Lipids were washed through with 6mL hexane and vitamin K's were eluted with 6mL 4% ether/hexane. Eluate was dried by SpeedVac and reconstituted in 120μL ethanol. 40μL was injected for HPLC analysis.
- Dietary supplements (DS) and foods were extracted with DMSO then transferred to hexane. Alternatively, DS were extracted with CHCl3/CH3OH. Aliquots were dried by SpeedVac then dissolved in ethanol. 15μL was injected for HPLC analysis.

HPLC Conditions

Column: Spherisorb ODS2, 150 x 4.6mm, 3um or Luna C18, 150 x 4.6, 5um
Detection: UV 248, 270nm or Zn-reduction with FL Ex 248nm/Em 430nm
Mobile phase: 85% MeOH/9% IPA/5% ACN/1% Zn solution
(Zn solution = 10mM $ZnCl_2$, 5mM Na Acetate, 5mM Acetic Acid in MeOH)
Flow rate: 1.0 mL/min
Column temperature: ambient

Figure 1

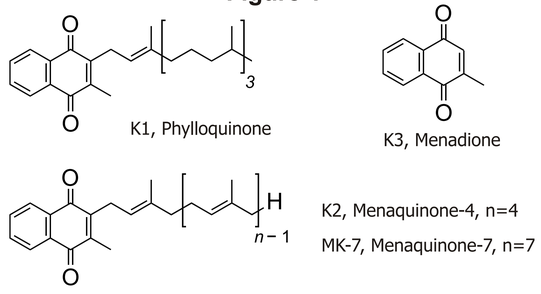

Figure 1 Illustrates the structures of K1 (phylloquinone), K2 (menaquinone-4 and MK-7), and K3 (Menadione).

Figure 2, below, illustrates Normal-Phase HPLC separations of K1, K2, K3, and MK-7 at 248nm. Columns tested were Silica, Cyanopropyl, Diol, and Diamine using hexane ± isopropanol.

Figure 2

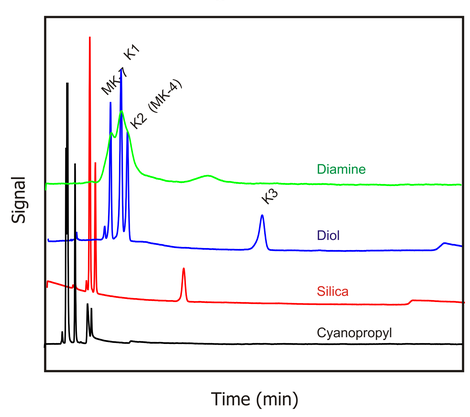

Figure 3

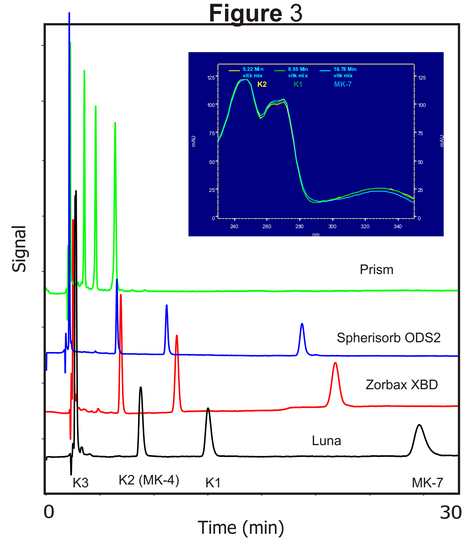

Figure 3, above, illustrates the separation of VK standards using Zorbax XBD, Luna C18, Spherisorb ODS2, and Prism reversed-phase HPLC columns.

Inset illustrates the UV spectrum of K1 (yellow), K2 (green), and MK-7 (blue). Absorbance maximum is ~248nm in mobile phase.

Figure 4, below, represents the separation of a serum, natto, and two dietary supplement extracts using the method described.

Figure 4

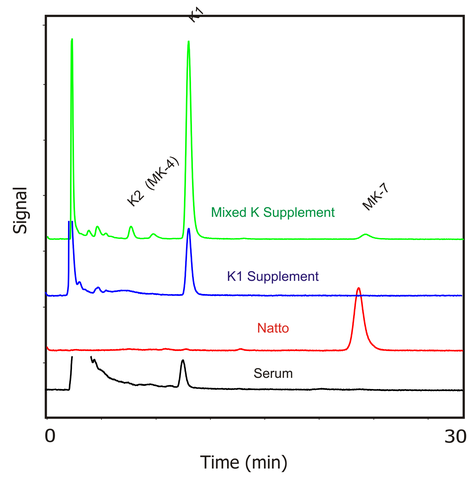

Table 1. Detector Response

Ultraviolet			Fluorescence		
Wavelength	LOD	Selectivity	Post-column Reduction	LOD	Selectivity
243nm	130pg	average	Coulometric	ineffective	no data
248nm	90pg	average	Pt	19pg	good
270nm	160pg	average	Zn	15pg	good

Limit of Detection (LOD) = mass on column to produce signal 3x noise

Table 2. Extraction Efficiency Food/Supplement

	% Spike Recovery	Endogenous MK-7 Relative to Folch
DMSO	99	106
Folch	97	100

Table 3. Performance in KEQAS Serum

	A	B
Batch 43	Within Target	Within Target
Batch 44	Within Target	Within Target
Batch 45	At Target	At Target

KEQAS - K External Quality Assurance Scheme

CONCLUSIONS

- Both NP and RP separations were tested (Fig 2 & 3). Most NP columns offered poor retention and peak shape of VKs. Reversed phase provided better separation of all VKs.
- Isocratically, K3 eluted in or near the solvent front (Fig 3) .
- Several C18 columns provided good selectivity for VKs (Fig 3). The Spherisorb ODS2 column provided the best combination of efficiency (15,200 plates), retention, symmetry (A=1.14) and tailing (T=1.09), for MK-7.
- Table 1. Both UV and post-column reduction with FL detection were tested. Electrochemical reduction (-200 to 1200mV) failed to produce a response while Zn yielded a slightly more efficient reduction than Pt.
- Table 2. For most Food/DS, DMSO extraction was equivalent and easier than Folch extraction.
- Recovery of K2 spiked into serum and DS was nearly 100% (88-99%).
- Table 3. The method has performed well for serum analysis in the KEQAS program.

REFERENCES

1 Shea MK, and Booth SL. Update on the role of vitamin K in skeletal health. Nutr Rev 2008, 66:549–557.
2 Wallin R, Schurgers L, and Wajih N. Effects of the blood coagulation vitamin K as an inhibitor of arterial calcification. Thromb Res,. 2008, 122:411-417.
3 Booth SL, Davidson KW, Sadowski JA. Evaluation of an HPLC method for the determination of phylloquinone (vitamin K1) in various food matrixes. J Agric Food Chem, 1994, 42:295–300.
4 Koivu-Tikkanen TJ, Ollilainen V, and Piironen VI. Determination of Phylloquinone and Menaquinones in Animal Products with Fluorescence Detection after Postcolumn Reduction with Metallic Zinc. J Agric Food Chem, 2000, 48:6325–6331.
5 Davidson KW and Sadowski JA. Determination of vitamin K compounds in plasma or serum by high-performance liquid chromatography using postcolumn chemical reduction and fluorimetric detection. Methods Enzymol, 1997, 282:408-421.