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Rapid and simple HPLC analysis of vitamin K in food, tissues and blood

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Abstract

The lack of precise data in food composition tables and also in human and animal plasma concentrations of phylloquinone and other vitamers demands intensive investigation of both; appropriate analytical methods are necessary. Sensitive and selective detection, simplicity and rapidity of the method, and precision and reliability of the data are recommended. Based on a RP-HPLC assay using post-column derivatization and fluorescence detection, combined with a liquid–liquid sample clean-up, a simple and rapid assay is presented. The application to food, human plasma and animal tissues is shown. The samples cover a wide range of matrices, from those of fairly low, e.g. human plasma and milk, to those of high phylloquinone content, e.g. broccoli. For verification purposes mass-spectroscopy (for human plasma) or different fluorescence wavelengths (for the food items) were applicated. Coefficients of variation did not exceed 10% for any matrix and there were no remarkable losses over the whole analytical process. The lower limit of detection for plasma samples was estimated as 0.04 ng/ml.

Keywords: Vitamin K; Phylloquinone; HPLC; Food; Biological samples

1. Introduction

The role vitamin K plays as a cofactor in the synthesis of gamma-carboxyglutamic acid concerning on one hand the blood coagulation factors and on the other hand osteocalcin is generally accepted (Suttie, 1992; Vermeer, Hamulak, Knapen & Jie, 1993).

For the further understanding of vitamin K's nutritional role there is a large demand for reliable data of vitamin K content either in human and animal blood or in food and feed stuff. First steps are done in offering a provisional table taking into account how the data were obtained (Booth, Sadowski, Weihrauch & Ferland, 1993).

Due to its importance we focus on the vitamer phylloquinone or also called vitamin K_1, which is synthesised in plants. The so-called menquinones are found in microorganisms and their role is still uncertain.

The best choice for the analytical method nowadays is HPLC pushing back cumbersome bioassays or thin-layer chromatography. Electrochemical or fluorescence detection (after reduction to the hydroquinone form) offers sensitivity and selectivity needed for the small amounts of the analyte.

Another important point is the sample clean-up. Sometimes an additional normal-phase HPLC is used as it is a solid-phase extraction step (Booth, Davidson & Sadowski, 1994; Shearer, 1986). The method presented here offers a rapid and simple sample preparation using a liquid–liquid extraction purification step (Cham, Roeser & Kamst, 1989) followed by a reversed-phase HPLC and fluorescence detection combined with a post-column reduction of the quinones (Haroon, Bacon & Sadowski, 1987; Booth et al., 1994).

2. Materials and methods

2.1. Sample preparation and HPLC

The HPLC apparatus used consisted of a conventional pump and a fluorescence detector. The column was a reversed-phase C-18 material. It was followed by a short column dry filled with zinc powder. A non-aqueous

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solvent system — methanol and dichloromethane — containing zinc chloride, sodium acetate and acetic acid, served as the mobile phase. The sample preparation tubes were solvent-tight and their caps contained Teflon inlets. All preparation steps were done under subdued daylight. Depending on the samples matrix appropriate extraction solvents were used. The hexane-extract, if necessary after evaporation of non-hexane solvents, was then washed with a mixture of methanol and water. The upper hexane layer was removed and evaporated under vacuum. Finally the residue was dissolved in the eluent and injected into the HPLC. 2′, 3′-Dihydrophylloquinone as ethanolic solution was used as internal standard. The methods were reported in detail earlier (Jakob & Elmadfa, 1995, 1996).

2.2. Verification and test of performance

For verification purposes, the peak purity for food items was tested by comparing the peak ratios at several excitation wavelengths (243, 272 and 330 nm) to those obtained from standard solutions. The plasma was tested by investigating a pooled fraction sample by means of mass-spectrometry (PD-MS). All items were tested for the absence of the internal standard.

To obtain the precision for this method, all items were analysed in replicate (within-run, for the plasma day-to-day too). For recovery experiments, known amounts of phylloquinone were added to the sample prior to extraction.

Table 1
Phylloquinone content of some food items

Food item	*n*	Phylloquinone content, μg/100 g	Min	Max
Sunflower oil	1	0.97		
Corn oil	1	1.63		
Olive oil	1	16.5		
Pumpkin seed oil	1	112		
Potato	5	1.62	0.49	3.41
Carrot	6	5.94	3.12	11.8
Cauliflower	4	12.0	6.4	11.8
Brocoli	7	195	137	247
Oatmeal	4	4.07	3.72	4.53
Milk	7	0.36	0.30	0.5
Yoghurt	4	0.34	0.20	0.36
Emmentaler cheese	5	2.39	2.29	2.47
Eggs	11	1.85	0.48	3.51

3. Results and discussion

3.1. Phylloquinone contents in foods

The phylloquinone content obtained from 13 food samples is given as an overview on Table 1. There were included both items of low content, such as eggs or milk,- and those of high phylloquinone content, such as broccoli or pumpkin seed oil. The results are in accordance with those reported by other groups (Booth et al., 1994; Ferland & Sadowski, 1992; Langenberg, Tjaden, De Vogel & Langerak, 1986). Concerning the edible oils differences could be caused by storage degradation of the phylloquinone from being on the shelf. Except for the pumpkin seed oil, all vegetable oils were presented in clear glass bottles, which did not protect from light exposure.

3.2. Phylloquinone contents in human plasma and trout liver tissue

Application of the method to plasma collected from 298 Austrian school children (7–19 years old, from different provinces and different school types) lead to the following results: the plasma phylloquinone concentration ranged from 0.09 to 2.22 nmol/l, with a mean of 0.6 nmol/l and a median at 0.49 nmol/l; the 25th percentile was at 0.33 nmol/l (Jakob & Elmadfa, 1995).

Table 2
Repeatablility and recovery

Sample	*n*	Phylloquinone content	rsd, %	Recovery, %
Human plasma	11 (Within-day)	0.34 ± 0.02 ng/ml	5.6	92 ... 104
	8 (Day-to-day)	0.34 ± 0.02 ng/ml	7.1	
Trout liver	6	10.3 ± 0.4 ng/ml	4.3	97
Sunflower oil	5	0.97 ± 0.08 μg/100 g	8.4	
Pumpkin seed oil	5	112 ± 1.9 μg/100 g	1.7	
Potato	10	2.23 ± 0.14 μg/100 g	6.5	101
Cauliflower	9	14.8 ± 0.4μg/100 g	3.0	
Broccoli	8	250 ± 19 μg/100 g	7.6	97
Oatmeal	5	4.10 ± 0.2 μg/100 g	4.9	
Milk, 3.6% fat	10	0.32 ± 0.01 μg/100 g	4.4	99
Eggs	10	3.06 ± 0.15 μg/100 g	4.8	101

The trout were fed a feed supplemented with tocopherol and menadion. Two main groups were one without and another with menadion supplementation. Within both groups three levels of tocopherol were supplemented, respectively. The amounts of phylloquinone in the liver ranged from 7.73 to 13.3 μg/100 g; the higher values were found at the highest tocopherol supplementation level (2000 mg tocopherol/kg feedstuff). In addition: the trout plasma showed a contrary effect (Jakob, Dierenfeld, Hoppe & Elmadfa, 1994).

3.3. General aspects of performance and reliability

Concerning the chromatograms, the phylloquinone peak and that of the internal standard were well separated from matrix signals both in food items and in plasma or liver tissue. The identity of the analyte was confirmed by the tests mentioned above. Within-run and day-to-day repeatability were adequate as well as the recovery testing (Table 2). For the plasma a lower limit of detection of 0.04 ng/ml (0.09 nmol/l) was found for samples of 1 ml plasma. Only a small sample size was required: amounts of 0.1 g for the foods or 0.5–1 ml for the plasma were sufficient; a careful homogenization for the food items is, therefore, recommended. The method allowed analysis of up to 10 and more plasma samples during a day. The method shows also the versatility by working as well with foods as it does with human plasma or trout liver tissue.

4. Conclusion

Overcoming laborious and time consuming methods for the determination of vitamin K_1 (phylloquinone), a simple, rapid and versatile HPLC assay was presented. It is to be emphasized that widely available laboratory equipment and inexpensive chemicals were used. Materials of different origin were investigated and the method was found adequate for these purposes. In the future the method will serve as an instrument for both studies of nutritional status in humans or animals and the draw-up of current food composition and nutrition tables. So we will find an easy way for further studies concerning the phylloquinone.

References

Booth, S. L., Sadowski, J. A., Weihrauch, J. L., & Ferland, G. (1993). Vitamin K_1 (phylloquinone) content of food: a provisional table. *J. Food Comp. Anal., 6*, 109–120.

Booth, S. L., Davidson, K. W., & Sadowski, J. A. (1994). Evaluation of an HPLC method for the determination of phylloquinone (vitamin K_1) in various food matrices. *Journal of Agricultural and Food Chemistry, 42*, 295–300.

Cham, B. E., Roeser, H. P., & Kamst, T. W. (1989). Simultaneous liquid-chromatographic determination of vitamin K_1 and vitamin E in serum. *Clinical Chemistry, 35*, 2285–2289.

Ferland, G., & Sadowski, J. A. (1992). Vitamin K_1 (phylloquinone) content of edible oils: effects of heating and light exposure. *Journal of Agricultural and Food Chemistry, 40*, 1869–1873.

Haroon, Y., Bacon, D. S., & Sadowski, J. A. (1987). Chemical reduction system for the detection of phylloquinone (vitamin K_1) and menaquinones (vitamin K_2). *Journal of Chromatography, 384*, 383–389.

Jakob, E., & Elmadfa, I. (1995). Rapid HPLC assay for the assessment of vitamin K_1, A, E and beta-carotene status in children (7–19 years). *Intern. J. Vit. Nutr. Res., 65*, 31–35.

Jakob, E., & Elmadfa, I. (1996). Application of a simplified HPLC assay for the determination of phylloquinone (vitamin K_1) in animal and plant food items. *Food Chemistry, 56*, 87–91.

Jakob, E., Dierenfeld, E. S., Hoppe, P. P., & Elmadfa, I. (1994). Interactions of the supplementation of the vitamins E and K in regards to their plasma concentration in trout. *Fat Sci. Technol., 96*, 411.

Langenberg, J. P., Tjaden, U. R., De Vogel, E. M., & Langerak, D. I. (1986). Determination of phylloquinone (vitamin K_1) in raw and processed vegetables using reversed phase HPLC with electrofluorimetric detection. *Acta Aliment., 15*, 187–198.

Shearer, M. J. (1986). Assay of K vitamins in tissues by high-performance liquid chromatography with special reference to ultraviolet detection. In F. Chytil, & D. M. McCormick, *Methods in enzymology, Vol. 123* (pp. 235–251). Orlando: Academic Press Inc.

Suttie, J. W. (1992). Vitamin K and human nutrition. *J. Am. Diet. Assoc., 92*, 585–590.

Vermeer, C., Hamulyák, K., Knapen, M. H. J., & Jie, K.-S. G. (1993). Extrahepatic functions of vitamin K in the Human. *Intern. J. Vit. Nutr. Res., 63*, 325.