Published in final edited form as:

J Pharm Biomed Anal. 2019 May 30; 169: 133–141. doi:10.1016/j.jpba.2019.03.006.

A concise review of quantification methods for determination of vitamin K in various biological matrices

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Abstract

Vitamin K is an essential nutrient in the body and involved in numerous physiological and pathophysiological functions. Both the lack and surplus of vitamin K can put human health at risk. Therefore, it becomes necessary to monitor vitamin K concentrations in different biomatrices through establishing sensitive and specific analytical methods. This review collectively describes an updated overview of the sample pretreatment methodologies and methods for quantitative determination of vitamin K that have been used in last two decades. High Performance Liquid Chromatography (HPLC) is commonly utilized as a standard for separation of vitamin K in combination with different detection including spectroscopic, spectrometric, fluorometric and mass spectroscopy. Recent progress in sample pretreatment technologies and quantitation methodologies have enhanced the ability to identify and quantitate vitamin K in biomatrices to further advance our understanding of the role of this vitamin in human health and disease.

Keywords

Chromatography; High performance liquid chromatography; Biological samples; Vitamin K; Phylloquinone; Menaquinones

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Declarations of interest: none

Introduction

Vitamin K, a member of the fat-soluble vitamins, contains a common functional 2-methyl-1,4-naphthoquinone ring with different side chain at the 3 position (1). Vitamin K₁ (phyloquinone, PK, VK₁) and vitamin K₂ (menaquinones, MK_n, VK₂) are the two main natural forms of vitamin K whereas vitamin K₃ (menadione, MD) is a synthetic form of vitamin K, containing the same basic structure of PK and MK (2). PK is known as the major dietary form of vitamin K and is obtained by consuming leafy green vegetables and vegetable oils in the diet of most Western countries (3,4). MK_n, where n (from 4 to 13) represents the difference in the number of isoprenoid residues in the side chains (5), is thought to be primarily synthesized by intestinal bacteria (6), and found in fermented food such as fermented food, natto (7) and animal products like liver, egg yolk and meat (8) (9). However, MK-4 is unique in that it is not of bacterial origin, it thought to be the alkylation product of MD in animal feeds or tissue conversion from dietary PK (10). MD is an important intermediate in the metabolic conversion of vitamin K (11), which is widely studied in a variety of disease states, including human cancers (12-18).

Vitamin K has been extensively studied in various disease states because of its important nutritional role and its involvement in a variety of physiological processes. Vitamin K is an important cofactor in the synthesis of human blood coagulation proteins by catalyzing the carboxylation of glutamic acid (Glu) to gamma carboxyl glutamic acid (Gla) (19). The relationship between vitamin K intake with bone mineral density and fracture risks has been extensively studied in observational studies and randomized control trials in the last decades (2), with studies suggesting a protective effect of vitamin K supplementation in bone metabolism and vascular calcification (4). .. Moreover, additional properties of vitamin K like regulating energy metabolism and inflammation have also been investigated in recent studies (20).

Pharmacokinetic and pharmacodynamic studies of vitamin K in various biological matrices by different quantification methods have been developed during the last decades in order to examine the relationship between vitamin K concentrations and disease progression. However, establishing a valuable quantification method with high sensitivity and specificity in biological matrices is challenging due to the non-polar characteristics of vitamin K, low circulating concentrations of the vitamin, and the interference of endogenous lipids in quantitation. Moreover, vitamin K exhibits the largest intra: inter-individual variation ratios for diet and fasting plasma concentration among all the fat-soluble vitamins (10, 21-23)). Determination of vitamin K status can be achieved by indirect functional and direct quantification methods. However, indirect functional tests, such as testing surrogate markers like prothrombin time or undercarboxylated proteins, are not sensitive enough to detect subclinical vitamin K deficiency (4). The levels of undercarboxylated proteins only represent recent vitamin K intake but not long-term vitamin K status (24). The indirect methods have been largely replaced by chromatographic methods, the direct quantification of vitamin K in different biological matrices.

To our best knowledge, this is the first review of chromatographic methods for quantification of vitamin K in biological matrices. The aim of this review is to summarize chromatographic

methods used for the determination of vitamin K homologues or metabolites during the last 20 years and the identification of sensitive and specific methods currently utilized for assessment of vitamin K. This review summarizes the complete process of vitamin K quantitation involving the complexity of the sample preparation, parameters of method validation, calibration approaches, and different techniques used to quantitate vitamin K in various biological matrices.

1. Sample Pretreatment Methods

Sample extraction and purification is enormously important to significantly improve analytical performance in terms of selectivity, sensitivity and accuracy. The aim of sample pre-treatment is to remove interfering matrix components, to extract and concentrate the analytes of interest, and to reduce or alleviate potential damage to the columns from the unpurified biological samples. Protein precipitation (PP), liquid-liquid extraction (LLE), and solid-phase extraction (SPE) are the three most common techniques used for vitamin K purification process in biological matrix to achieve efficient analyte recovery by reducing ion suppression and enhancement of analyte signal. Other approaches such as performing derivatization reactions (25-28), emulsification microextraction (29), and sonication (30) have also been reported in sample preparations. PP is a rapid, generic and relative inexpensive sample preparation method. Precipitation can be achieved by addition of organic solvent (methanol, acetonitrile, or ethanol), salts (ammonium sulfate), metal ions, and concentrated acids (31). However, this technique is not sufficient by itself to remove salts and endogenous compounds in the bio-matrix. Therefore, it is typically used in combination with other sample preparation techniques such as LLE or SPE to obtain the desired extract sample purity. LLE method has been widely used in vitamin K sample preparation due to the lipophilic characteristic of vitamin K. Extraction solvents such as hexane, cyclohexane, isooctane, and chloroform have generally been used for vitamin K sample extraction. LLE provides an efficient extraction method with good selectivity and specificity to provide satisfactory sample clean-up (32), however additional steps may be required to fully eliminate matrix effects. Therefore, this method is time consuming and an environmental unfriendly procedure due to the need of large volume of toxic solvents. SPE is a fast, cost-effective, reliable and green method for trace analysis (33). It is considered as an effective and extensive clean-up process to remove endogenous compounds to alleviate interfering matrix effects but also concentrate the target analytes (34, 35). This technique is commonly adapted in analyzing urine or plasma samples, with a majority of sample components adsorbed into a particular sorbent via hydrophobic, electrostatic, or size exclusion interaction.

2. Methods for Vitamin K Quantitation

Developing practical and high-throughput analytical method for vitamin K homologue quantification is imperative because of increased interests of determination vitamin K concentrations in clinical and nutritional studies to better understand the role of vitamin K in health and disease. Chromatographic separation with various detection methods have typically been used for vitamin K quantification in biological matrices.

2.1. Chromatographic analysis

Supplementary Table S1 lists the chromatographic conditions adapted for target analytes of vitamin K and their applications. Reverse-phase HPLC and normal phase HPLC have been used to separate, identify and quantitate analytes of interests in samples. The most frequently reported column for separation of vitamin K homologues in bio-matrices was a C18 column. Other columns reported in the literature includes C8 (36), C30 (37-39), shield RP18 (25) and phenyl columns (40). Mobile phase mixtures typically contain polar and non-polar solvents for analyte separation and elution by isocratic or gradient elution profile and to decrease potential interference in the complex bio-matrices. Two most common polar solvents used as mobile phase were methanol and water, whereas isopropanol and acetonitrile were the two common non-polar solvents used as the mobile phase. Some of the studies showed acidic mobile phase pH can increase peak height and optimize peak shape (26), therefore, formic acid and acetic acid have been commonly added in the solvents. Gas chromatography separation has also been reported (28,41,42). Helium was typically used as carrier gas in these methods.

2.2. Detection Methods

In the last 20 years, several detection methods were reported for the determination of vitamin K in biological samples including fluorescence detection (FD), ultraviolet detection (UV), electrochemical detection (ECD), photodiode array (PDA), chemiluminescence (CL), and mass spectrometric detection (MS or MS/MS). HPLC-FD after pre- or post- column reduction reaction has been used for the direct quantification of vitamin K, however this approach requires complicated sample pre-purification to decrease possible chromatographic interferences from endogenous compounds. The use of LC-MS/MS bioanalytical methods to determine trace level of vitamin K in bio-fluids has dramatically increased in the last decade because of its distinctive advantages in improved sensitivity, specificity and throughput (40, 43). MS detection method overcomes the need of complex sample preparation procedure but still has its own limitations including relative expensive equipment and possible matrix effects interference in biological samples (44, 45). UV detection method is cost effective, but it has been largely replaced by LC-MS/MS recently due to the requirement of high analyte concentration in limited source of biological samples (31). ECD method has been reported as a valuable method with better sensitivity and selectivity compared to UV and FD methods while performing trace determination of electroactive compounds (46). However, the complexity of sample preparation and prolonged analysis time (80 min, >30 min respectively) limits this approach (47, 48). PDA detection has been applied to fat-soluble vitamin analysis, the drawbacks of this method have been documented in previous studies and includes reduced sensitivity compared to LC-MS/MS and a larger sample volume requirement (49, 50). Chemiluminescence directly or after UV irradiation has only rarely been used in vitamin K quantification (51-53).

3. Calibration Approaches for quantitation of Vitamin K

The availability of a suitable blank matrix for quantitation of endogenous compounds always remains a major challenge while developing chromatographic methods. A variety of approaches are utilized by researchers to combat the problems related to blank matrices

including, background subtraction (26, 76), standard addition (77), and by utilizing surrogate matrices (artificial matrices, stripped matrices and surrogate analytes) (40, 78, 79). In background subtraction, the same matrix is used for the calibration curve construction as will be analyzed as sample, so the recovery and matrix effect are the same for both samples and calibration curves. In the standard addition method, exactly the same matrix is used of study sample for the construction of calibration curve. In surrogate matrices, several other matrices are used in place of actual matrix of study samples including mobile-phase solvents. Other methods involving surrogate matrix include the use of artificial matrices, stripped matrices and surrogate analyte. An artificial matrix can be prepared, if the matrix composition is known as cerebrospinal fluid (CSF), tears, vaginal fluid, and sputum. Biological matrices (plasma, serum) can be stripped of particular endogenous components to generate analyte-free surrogate matrices for the construction of calibration curve by using treatment with activated charcoal or UV light. Stable-isotope-labeled standards as a surrogate analyte can be also used for the direct and sensitive quantification of analytes. These analytes are identical or have minimal differences in terms of extraction recovery, chromatographic retention, and signal intensity. Literature search reveals the use of above mentioned calibration curve approaches for vitamin K quantitation. Ahmed et al quantified vitamin K homologues in human plasma through HPLC-fluorescence detection method by utilizing background subtraction (26). HPLC-APCI-MS method has been also reported by Song et al. in human plasma for VK₁ quantitation through background subtraction calibration approach (80). Stripped matrices approach has also been taken in important consideration for vitamin K quantitation (54, 65). Liu & coworkers used UV light to prepare stripped plasma matrix for menadione quantitation in human plasma through LC-MS approach (27). Klapkova et al. also used UV light stripped method for determination of vitamin K and its analogues in postmenopausal women's serum by HPLC-FD detection (9). In one study, Riphagen et al. performed VK₁ (phyloquinone) and VK₂ (menaquinones-4 and -7) quantitation by the stable isotope dilution method and for quantitation of each vitamin, a deuterated analog of the analyte was selected as an internal standard (40).

4. Vitamin K quantitation in different biological matrices

Because of the increased interest of studying the relationship between vitamin K level and chronic diseases, developing various chromatographic methods for vitamin K quantification in the bio-matrices have been dramatically increased in the last decade, especially LC-MS/MS methods. Most studies addressed the role of vitamin K in human bone health, coagulation system, and other disease states such as cystic fibrosis and chronic kidney disease, which could be easily applied to pharmacokinetic and pharmacodynamic studies of vitamin K. This review summarizes methods developed in last two decades for vitamin K quantification in different biological matrices and their applications.

4.1 Human Serum, Plasma and Blood

A large variety of quantification studies for vitamin K homologues have been performed in human serum, plasma and blood samples because of their clinical relevance. LC-MS methods are the most common chromatographic method for vitamin K quantification used in blood samples. Table 1 summarizes the available research on the quantification of vitamin K

analogs in human serum, plasma and blood by various chromatographic methods. Hu et al. has successfully developed a LC-MS/MS method for the quantification of endogenous VK₁ and vitamin K₁ 2,3-epoxide (VK₁O) in human plasma to reflect the status of vitamin K-epoxide cycle. The developed method was successfully applied to pharmacodynamic and pharmacogenetic studies of warfarin (54). Yuan et al. established a novel cysteamine-derivatization based UPLC-MS/MS method for detection of free menadione instead of metabolized menadione from human plasma and urine samples for the very first time. It largely promotes the understanding of vitamin K metabolism *in vivo* (25). In another study, Riphagen & group provided a HPLC-MS/MS method for simultaneous quantification of VK₁, MK-4, and MK-7 to identify vitamin K deficiency in chronic kidney disease patients (40). Pediatric patients with cystic fibrosis suffering from fat soluble vitamin deficiency is very common in clinical settings (81). A LC-MS method has been developed to quantify fat-soluble vitamins in a lower required serum volume (0.2 mL) for children with cystic fibrosis, which helps to guide the diagnosis and treatment of the disease (55). In another study by Ducros & group, a HPLC-MS/MS method was developed to selectively quantify vitamin K₁ level in healthy human subjects and cystic fibrosis patients with a linearity up to 5400 ng/L and the limit of detection of 14 ng/L (57). Liu et al. quantified menadione by a novel LC-MS/MS method after derivatization with 3-mercaptopropionic acid and found 33-fold better lower limit of quantification than the underivatized compound. This highly sensitive method could be applied to future research on menadione and its prodrug (27). A method utilizing sample freezing and lipid precipitation was developed by Gentili & group while determining VK₁, MK-4 and VK₁O concentrations by HPLC-MS/MS. A limit of detection of 0.052 µg/L of VK₁, 0.065 µg/L of VK₁O, 0.124 µg/L of MK-4 were achieved by eliminating interference problems from isobaric lipids. (56). While comparing a HPLC-MS/MS method with HPLD-PDA method, Kopec & group found that MS/MS exclusively allowed the quantification of VK₁ in chylomicron-rich human plasma samples. MS/MS detection was found to be preferable for trace analysis in biological matrix, since lower sample requirement reduced the risk and discomfort of human subjects while sampling (49) Fu et al. developed a LC-MS method to measure deuterium labeled and unlabeled VK₁ in fasting, non-fasting human plasma samples and in lipid plasma fractions. They found the very low density lipoprotein (VLDL) was the major carrier of VK₁ in plasma and their method was suitable for VK₁ bioavailability studies (38). Suhara et al. used stable isotope ¹⁸O labeled internal standard to determine VK₁, MK-4, and MK-7 simultaneously in human plasma for postmenopausal women, elderly, and osteoporotic patients. The method achieved satisfactory sensitivity but require long run times (80 minutes) for separation of all analytes (58). Song et al. developed an assay for determination of VK₁ in human plasma by LC-MS to study vitamin K₁ pharmacokinetics (36). A high-throughput LC-MS/MS method was developed by Midttun & group to measure six fat soluble vitamins. Only 50 µL of serum or plasma was needed for sample preparation and total analysis time was 4.5 min for each sample. This automated assay can be applied in epidemiological studies for analyzing nutritional status, lifestyle, and diseases (59).

HPLC-FD method has also been developed to selectively quantify VK₁ or to determine VK₁, MK-4, and MK-7 simultaneously in human plasma or serum samples. All studies done by HPLC-FD methods require post column reduction reaction with zinc or platinum.

Klapkova et al. established a method for the routine measurement of VK₁, MK-4, and MK-7 in serum for postmenopausal women with or without osteoporosis with limit of detection of 0.03 ng/mL for VK₁ and MK-7, 0.04 ng/mL for MK-4 (9). In another study, Ahmed & group developed a simple HPLC-FD method for simultaneously quantify VK₁, MK-4, and MK-7 in human plasma, however, it was not as sensitive as LC-MS methods with limit of detection of 0.1 ng/mL for VK₁ and MK-4, 0.17 ng/mL for MK-7 (26). Simultaneous determination of VK₁ and MK-4 in human plasma by HPLC-FD method has been developed by Marinova and group via a highly sensitive method (10). Kamao et al. used various synthetic vitamin K analogs as internal standards to validate a HPLC-FD method to quantify VK₁, MK-4, and MK-7 in human plasma or serum. The modified HPLC method may be a useful tool for clinical and nutritional studies (60). Methods for selective quantification of VK₁ in human plasma by HPLC-FD have been developed in several studies. Meineke et al. described a convenient and robust method for determination of VK₁ with a low required plasma sample volume (100 µL). This method has been successfully applied to clinical pharmacological studies (61). To obtain the pharmacokinetic profile of VK₁ supplementation, Ohno and co-workers established a rapid UPLC-method with total analysis time of 6 minutes per sample (62). A study was carried out to determine VK₁ to identify vitamin K deficiencies in order to reduce the risk of blood clot and bone mineralization disorders (63). The influence of plasma triglyceride and acute-phase response on the VK₁ circulating concentrations in plasma has been studied by the validated HPLC-FD method (64). A sensitive and reproducible HPLC-FD method has been developed by Wang & group. The assay was successfully applied to study bone health in elderly people by selectively analyzing VK₁ concentrations in plasma or serum (65). Jakob et al. provided an efficient method of VK₁ quantification in human plasma, trout liver tissue, and foods in order to evaluate nutritional status and food nutritional composition (66).

LC-UV methods were usually used to simultaneously quantify fat soluble vitamins or selectively determine vitamin K₁ in relative larger required biological sample volume (2mL) compared to other methods. Wang et al. developed a sensitive HPLC-UV method to determine all vitamin K homologues in human and bovine serum with limit of detection of 0.33 ng/mL, 0.01 ng/mL, 0.01 ng/mL for VK₁, VK₂ and MD, respectively.. This rapid assay (total analysis time=12 min) can be applied to study pharmacokinetic and bioavailability profiles of vitamin K and other fat soluble vitamins (68). Ortiz Boyer group adapted a continuous clean-up preconcentration procedure to successfully shorten the sample treatment process and to reduce the interference of endogenous compounds. This method provided an useful tool for screening fat soluble vitamin concentrations (69). Selective quantification of VK₁ in human plasma, leafy vegetables, and oil was also achieved by HPLC-UV method provided by Otles & group (67).

A study was conducted to detect isotope ratios in VK₁ by new extraction method with LLE, enzyme hydrolysis and SPE using GC-MS method, which can be applied to future work on the measurement of kinetics and absorption of VK₁ (28). Dolnikowski et al. developed a GC-MS method to distinguish exogenous intake of VK₁ from endogenous pools to study vitamin K₁ absorption and metabolism in humans (42). Raith group has provided the GC-MS method used to quantify VK₁ levels in neonatal plasma with smaller sample volume

(200 µg). They developed a direct measurement method of VK₁ plasma concentrations after intravenous administration in neonates (41).

A highly sensitive and selective HPLC-ECD trace analysis method has been established to quantify multiple vitamin K analogs (VK₁, MK 4-10) in human serum. The limits of detection were 2-10 pg for all VKs with long analysis time (80 min). This method has been successfully applied to study the physiological and pathophysiologic roles of VKs in the bone health (47).

Chatzimichalakis et al. developed a HPLC-PDA method used to determine eight fat soluble vitamins in human plasma and urine. All vitamins achieved separation within 14 minutes requiring small sample volumes (100 µL of blood and urine). However, the method is not suitable to determine vitamin K levels in clinical samples due to the high limit of detection. (50).

Ahmed & group developed a HPLC with post-column peroxyoxalate chemiluminescence (PO-CL) detection method to quantify VK₁, MK-4, and MK-7 in human plasma for clinical and nutritional applications (52).

4.2 Human Urine

Urine sampling poses several advantages includes: non-invasive procedure, sufficient specimen volume, and easy collection process. However, successfully performing urine samples analyzation is difficult since there are a large variety of interfering components in the urine, including neutral, acidic, basic and amphoteric compounds (82). Developing analytical methods for vitamin K and its metabolites in the urine samples helps understanding vitamin K metabolism and clearance process *in vivo*. Table 2 summarizes the representative examples of vitamin K and its metabolites quantified in human urine by different chromatographic methods. Quantification of menadione in human urine samples is challenging since it has low molecular weight and is unlikely to produce stable multiple reaction monitoring (MRM)-suitable fragments. Kamao et al. overcame these obstacles while determining menadione in urine, rat plasma, cultured cell and media by using pseudo MRM based LC-MS/MS method (70). HPLC-FD method has been developed by Al Rajebi group to measure urinary manadione to illuminate the role of menadione in MK-4 formation process (39). There are two major urinary metabolites of vitamin K identified in human urine. Harrington et al. found that measurement of urinary metabolites could be potential noninvasive markers while assessing patient's total vitamin K status (48).

4.3 Rat Serum, Plasma, Blood and Tissues

Rat blood and tissues are common biological matrix used for method development and vitamin K quantification. Table 3 summarizes the representative examples published in the last 20 years. Rat plasma is easy to obtain and the most common tissue reported for vitamin K quantification is liver. Karl et al. developed a sensitive HPLC-APCI-MS method for simultaneous quantification of 11 vitamin K vitamers in feces, rat serum, and food. Separation of all vitamers can be achieved within 20 minutes and this method can be applied to human and animal studies to understand the role of vitamin K in health and diseases (30). By quantifying VK₁ and MK-4 in mice plasma and cerebra by LC-MS/MS method, Okano

et al. successfully identified two routes of MK-4 accumulation in cerebrum. (23). A LC-FD method with C₃₀ column was developed to measure *cis*- and *trans*- vitamin K₁ isomers selectively in rat liver tissues and plasma. They observed the *cis* component accumulation in tissues may potentiate an overestimation of biologically active form of VK₁ while using conventional C₁₈ chromatography (37). Gershkovich and co-workers developed a HPLC-UV method which can be applied to monitor adverse effects of vitamin misbalance of new drug candidates (71). Menadione and its thioether conjugates were identified by a HPLC-CL method. Successfully quantified menadione and thiol-quinones can further help to investigate pharmacokinetic profiles of menadione, and analytical, toxicological chemistry of quinones in the future (53).

4.4 Bacteria or cell culture media

It is generally presumed that the sources of menaquinones are derived from intestinal bacteria, fermented food and liver. Quantification of MK-n and microbial respiratory ubiquinones in bacteria and cell culture media can facilitate the understanding of the mechanism of bacterial respiratory chain (83), but also could be applied to future environmental and microbial studies. Table 4 consists of research work carried out for quantification like method development and validation in certain bacteria, cell line and cell culture media. Wei et al. established an efficient method for separation and extraction of MK-4, MK-5 and MK-6 from wet biomass of *Flavobacterium* without the need of drying the biomass (72). *Escherichia coli* is a representative of facultative anaerobic bacteria and it is a common matrix used for investigating respiratory chain enzymes. Gao and coworkers developed a LC-MS method to quantify MK-8, ubiquinone-7, and ubiquinone-8 in 20-30 mg of *E.coli* dry cells (73). Geyer et al. compared atmospheric pressure photoionization (APPI) source with atmospheric pressure chemical ionization (APCI) source while quantifying 11 ubiquinones and MK_n by tandem mass spectrometry. They found APPI-MS/MS method proved to be at least three times sensitive than APCI-MS/MS method, which can be applied to future environmental and cell culture studies (74).

4.5 Miscellaneous (pharmaceutical preparations and human breast milk)

Table 5 shows the methods used for quantification vitamin K in pharmaceutical preparation and human breast milk. Methadone component in pharmaceutical preparations was identified by a repeatable and reproducible HPLC-UV method. The total extraction time can be reduced to only 2 minutes by using dissolved carbon dioxide flotation after emulsification microextraction method (29).

Successful quantification of fat soluble vitamins in breast milk exerts important implications for the promotion of breast-feeding. VK₁, MK-4 and MK-7 were simultaneously quantified by a sensitive LC-MS/MS method in 3 mL of human breast milk. This method can be adapted to large scale studies and Dietary Reference Intakes of fat soluble vitamins (75).

Conclusion

Vitamin K has been recently considered as a topic of research as it plays a fundamental role in maintenance of bone health. A variety of methods for determination of vitamin K in

various biomatrices have been proposed in last few decades. The currently proposed analytical approaches for the vitamin K detection in diverse biomatrices is mainly based on HPLC coupled with different detection modes including UV, electrochemical, fluorescence, and mass spectrometry. Therefore, HPLC technique is anticipated to have the most obvious development in the future. Despite this, there is pronounced interest in the cost effective and rapid screening methods based on microbiological, immunoassays and biosensors. Development of newer methods incorporating appropriate clean up procedures, separation using UPLC chromatography combined with sensitive and selective MS/MS detection will further enhance the quantitation of vitamin K and analogues and help facilitate clinical studies evaluating the role of vitamin K in health and disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors contributions to manuscript are as follow: DM and YC designed the approach for the review; YZ performed the literature search, review and summarize. YZ, VB, ZM wrote the manuscript. DM and YC critically reviewed the article and made the final decision for publication. All authors read and approved the final manuscript.

Financial support: This work was supported in part by the Fred & Pamela Buffett Cancer Center Support Grant from the National Cancer Institute under award P30 CA036727 and the National Institutes of Health P50CA127297. YZ is thankful to University of Nebraska Medical Center, College of Pharmacy fellowship award.

Abbreviations:

(HPLC)	High performance liquid chromatography
(PP)	protein precipitation
(LLE)	liquid-liquid extraction
(SPE)	solid-phase extraction
(FD)	Fluorescence Detection
(MS or MS/MS)	Mass Spectrometric
(UV)	Ultraviolet Detection
(ECD)	Electrochemical Detection
(LC)	Liquid Chromatography
(UPLC)	Ultra Performance Liquid Chromatography

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Highlights

- Summary of vitamin K analysis in various biological matrices.
- This review collectively describes an updated overview of the sample pretreatment methodologies and methods for quantitative determination of vitamin K that have been used in last two decades.
- LC-MS/MS detection of vitamin K will further help to evaluate the role of vitamin K in health and disease.

Summarizes representative examples of Vitamin K quantified in Human Serum, Plasma and Blood by chromatographic methods

Table 1.

Analytes	Quantification method	Bio-matrix	Bio-matrix volume	Internal standard	Linearity	Limit of detection	References
Vitamin K ₁ , vitamin K ₁ 2,3 epoxide	LC-APCI-MS/MS	Human plasma	0.2 mL	Vitamin k ₁ -d7	100-10,000 pg/mL	30 pg/mL	(54)
Vitamin K ₃ (Menadione)	UPLC-ESI-MS/MS	Human plasma and urine	1.0 mL	MD-d8	0.05-50.0 ng/mL	LOQ: 0.03 ng/mL for plasma 0.02 ng/mL for urine	(25)
Vitamin K ₁ , MK-4, MK-7	HPLC-APCI-MS/MS	Human plasma	350 µL	PK-d7 MK-4-d7 MK-7-d7	Up to 15 nmol/L	LLOQ:PK & MK-4: 0.14 nmol/L MK-7: 4.4 nmol/L	(40)
Vitamin K ₁ , A, E and active vitamin D metabolites	LC-ESI-MS	Human serum	200 µL	Retinol acetate	Vitamin K ₁ : 1-500 ng/mL	Vitamin K ₁ : 0.1 ng/mL	(55)
Vitamin K ₃ (Menadione)	LC-MS/MS	Human plasma	1.0 mL	Plumbagin	0.03-50 ng/mL	LLOQ: 0.03 ng/mL	(27)
Vitamin K ₁ , MK-4, vitamin K ₁ 2,3 epoxide	HPLC-APCI-MS/MS	Human serum and plasma	0.5 mL	K ₁ -d7 MK-4-d7 K ₁ O-d7	0.1 µg/L to 10 µg/L	PK: 0.052 pg ^L MK-4:0.124 µg ^L K ₁ O: 0.065 µg/L	(56)
Vitamin K ₁ , carotenoids, retinyl esters, α-tocopherol	HPLC/photodiode array (HPLC-PDA) and HPLC-MS/MS method comparison	Human plasma	2.5 mL	d8-b-carotene	N/A	PK: N/A by HPLC-PDA PK:0.003 pmol by HPLC-MS/MS	(49)
Vitamin K ₁	HPLC-APCI-MS/MS	Human plasma	0.1-0.5 mL	Ring-D4-labeled vitamin K1	Up to 5400 ng/L	14 ng/L	(57)
Vitamin K ₁	HPLC-APCI-MS	Human plasma	0.5 mL	Vitamin K ₁ (25)	0.5-32nmol/L	Unlabel ed PK: 0.2 nmol/L Labeled PK: 0.5 nmol/L	(38)
Vitamin K ₁ , MK-4, MK-7	HPLC-APCI-MS/MS	Human plasma	0.5 mL	PK- ¹⁸ O MK-4- ¹⁸ O MK-7- ¹⁸ O	12.5-200 ng/mL	LOQ: PK: 40 pg/mL MK-4: 50 pg/mL MK-7: 80 pg/mL	(58)
Vitamin K ₁	HPLC-APCI-MS	Human plasma	0.5 mL	Teprenone	0.3-1000 ng/mL	LLOQ: 0.3 ng/mL	(36)
Vitamin K ₁ , 6 fat-soluble, 26 water-soluble vitamins	LC-APCI-MS/MS	Human plasma or serum	50 µL	² H ₄ -phyloquinone	0.33-33 nM	0.33 nM	(59)
Vitamin K ₁ , MK-4, MK-7	HPLC-FD	Human serum	500 µL	Vitamin K derivative (Immundiagnostik AG, Germany)	0.1-15 ng/mL	LOQ: PK: 0.03 ng/mL MK-4: 0.04 ng/mL MK-7: 0.03 ng/mL	(9)
Vitamin K ₁ , MK-4, MK-7	HPLC-FD	Human plasma	500 µL	2-Methyl-3-pentadecyl-1,4-naphthoquinone	0.3-100 ng/mL	PK:0.1 ng/mL MK-4: 0.1 ng/mL MK-7: 0.17 ng/mL	(26)
Vitamin K ₁ , MK-4, MK-7	HPLC-FD	Human plasma or serum	0.5 mL	Vitamin K analogs with various length of the alkyl side-chain	12.5-200 ng/mL	PK: 2 pg per injection MK-4 & MK-7: 4 pg per injection	(60)
Vitamin K ₁ , MK-4	HPLC-FD	Human serum	0.5 mL	Vitamin K1(25)	0.0625-20 ng per absolute injection of 40 µl	0.015 ng/mL	(10)

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Analytes	Quantification method	Bio-matrix	Bio-matrix volume	Internal standard	Linearity	Limit of detection	References
Vitamin K ₁	HPLC-FD	Human plasma	100 µL	Vitamin K ₂	2-500 ng/mL	30 pg/50 µL or 30 pg per injection	(61)
Vitamin K ₁	UPLC-FD	Human plasma	0.4 mL	MK-8	0.1-25 ng/mL	0.025 ng/mL	(62)
Vitamin K ₁	HPLC-FD	Human plasma	0.25 mL	Vitamin K derivative	Up to 71.04 nmol/L	20 fmol/50 µL or 20 fmol per injection	(63)
Vitamin K ₁	HPLC-FD	Human plasma	0.5 mL	Docosyl naphthoate	0.14-44.8 nmol/L	0.08 nmol/L	(64)
Vitamin K ₁	HPLC-FD	Human plasma or serum	0.1-0.5 mL	Vitamin K derivative (Immundiagnostik AG, Germany) and MK-6	Up to at least 100 nmol/L	2 pg (4 fmol) per injection (45 µL)	(65)
Vitamin K ₁	HPLC-FD	Food, trout liver tissue, human plasma	1 mL of human plasma	2,3-dihydrophyllquin one	N/A	0.04 ng/mL (0.09 nmol/L) in plasma	(66)
Vitamin K ₁	HPLC-UV	Human plasma Olive oil Chard	2 mL of serum	2',3'-dihydrophyllquin one	N/A	N/A	(67)
Vitamin K ₁ ,K ₂ ,K ₃ , A,D,E	HPLC-UV	Human and bovine serum	2 mL	N/A	0.5-20.0 µg/mL	K ₁ :0.33 ng/mL K ₂ :0.01 ng/mL K ₃ :0.01 ng/mL	(68)
Vitamin K ₁ ,K ₃ , A,D ₂ ,D ₃ ,E, and metabolite s of Vitamin D ₃	LC-UV	Human plasma	2 mL	N/A	Vitamin K ₁ & K ₃ : 0.1-100 ng/mL	N/A	(69)
Vitamin K ₁	GC-MS	Human plasma	2 mL	Methyl- ¹³ C vitamin K ₁ Ring-D ₄ vitamin K ₁	N/A	LOQ: 6 pg/µL	(28)
Vitamin K ₁	HPLC and GC/MS	Human serum	0.25 mL of plasma-HPLC 0.5 mL of serum-GC/MS	K ₁₍₂₅₎ -HPLC N/A-GC/MS	N/A	10 pg per 150 µL injection-HPLC 5 pg-GC/MS	(42)
Vitamin K ₁	GC-MS	Neonates plasma	0.2mL	² H ₃ labeled vitamin K ₁₍₂₀₎	4.0-4000 pg per sample	1.0 pg per 10 µL injection	(41)
Vitamin K ₁ , MK 4-10	HPLC-ECD	Human serum	0.5 mL	Authentic VK analogs	Up to 5000 ng	2-10 pg	(47)
Vitamin K ₁ ,K ₂ ,K ₃ ,A,D ₃ ,E,E-acetate,e-tocopherol	HPLC-PDA	Human serum an durine	0.1 mL	Xanthophyll	Up to 25 ng/µL	PK:0.03 ng/µL MK and vitamin K ₃ : 0.33 ng/µL	(50)
Vitamin K ₁ , MK-4, MK-7	HPLC-PO-CL	Human plasma	1 mL	2-methyl-3-pentadecyl-1,4-naphthoquinone	PK and MK-4: 0.01-10µM MK-7: 0.02-10 µM	PK: 32 fmol MK-4: 38 fmol MK-7: 85 fmol	(52)

Table 2.

Bioanalytical methods for the quantification of vitamin K in Human Urine

Analytes	Quantification method	Bio-matrix	Bio-matrix volume	Internal standard	Linearity	Limit of detection	References
Vitamin K ₃ (Menadione)	UPLC-ESI-MS/MS	Human plasma and urine	1.0 mL	MD-d8	0.05-50.0 ng/mL	0.03 ng/mL for plasma 0.02 ng/mL for urine	(25)
Vitamin K ₃ (Menadione)	LC-APCI-MS/MS	Human urine, rat plasma, human culture d cell and media	Urine: 0.5 mL Plasma: 0.5 mL Cultured cell and media: 1-5 mL	MD-d8	Up to 50 ng/40 µL or 50 ng per injection	MD: 40 pg/40 µL MD-d8: 2 pg/40 µL	(70)
Vitamin K ₃ (Menadione)	HPLC-FD	Urine	0.5 mL	MK-2	0-3.64 pmole	LLOQ: 0.3 pmole/ml	(39)
Urinary metabolites of vitamin K	HPLC-ECD	Urine	Unsupplemented: 0.5 mL Supplemented: 0.05 mL	2-methyl-3-(7'-carboxyheptyl)-1,4-naphthoquin one	N/A	<3.5 fmol (<1 pg)	(48)
Vitamin K ₁ ,K ₂ ,K ₃ ,A,D ₃ ,E,E-acetate,e-tocopherol	HPLC-PDA	Human serum and urine	0.1 mL	Xanthophyll	Up to 25 ng/µL	0.03 ng/µL- VK1 0.33 ng/µL-VK2 0.33 ng/µL-VK3	(50)

Table 3.

Representative examples of chromatographic methods for quantitation of Vitamin K in Rat Serum, Plasma, Blood, and Tissues

Analytes	Quantification method	Bio-matrix	Bio matrix volume	Internal standard	Linearity	Limit of detection	References
Vitamin K ₃ (Menadione)	LC-APCI-MS/MS	rat plasma, human	Plasma: 0.5 mL	MD-d8	Up to 50 ng/40 µL	MD: 40 pg/40 µL MD-d3 and MD-d8: 2 pg/40 µL	(70)
Vitamin K ₁ , MK 4-13	HPLC-APCI-MS	Rat chow Rat Serum Rat feces	Rats data not available	Deuterium-labeled PK	7.8125 nm to 500 nM	Human feces: 1 pmol/g to 30 pmol/g	(30)
Vitamin K ₁ , MK-4	LC-MS/MS	Mice tissue and plasma	Tissue wet weight: 1-2 g	PK- ¹⁸ O, MK-4- ¹⁸ O	N/A	N/A	(8)
Vitamin K ₁ isomers	LC-FD	Rat liver and rat plasma	0.5-1.0 g liver tissue Plasma: 100 µL	N/A	N/A	0.057 ng/mL	(37)
Vitamin K ₁	HPLC-FD	liver tissue,	N/A	2',3'-Dihydrophyloquin one	N/A	0.04 ng/mL (0.09 nmol/L)-plasma	(66)
Vitamin K ₁ ,D ₃	HPLC-UV	Rat plasma	160 µL	Probucol	200 to 5000 ng/mL	LOQ: PK:40 ng/mL D ₃ :20 ng/mL	(71)
Menadione, its thioether conjugates	HPLC-CL	Rat plasma	0.1 mL	N/A	10-240 nM	128 fmol	(53)

Table 4.

Representative examples of chromatographic methods for the quantitation of Vitamin K in Cell Line or Cell Culture Media

Analytes	Quantification method	Bio-matrix	Bio matrix volume	Internal standard	Linearity	Limit of detection	References
MK-4, MK-5, MK-6	HPLC-UV LC-ESI-MS	Flavobacterium	25 mL fermentation liquor	MK-4	N/A	N/A	(72)
Vitamin K ₃ (Menadione)	LC-APCI-MS/MS	Human urine, rat plasma, human cultured cell and media	Urine: 0.5 mL Plasma: 0.5 mL Cultured cell and media: 1-5 mL	MD-d8	Up to 50 ng/40 µL	MD: 40 pg/40 µL MD-d3 and MD-d8: 2 pg/40 µL	(70)
MK-8, ubiquinone-7, ubiquinone-8	LC-APCI-MS	<i>Escherichia coli</i>	20-30 mg of dry cells	Vitamin K ₁ , ubiquinone-6, ubiquinone-10	N/A	N/A	(73)
Ubiquinones (UQ ₆) Menaquinones (MK-4)	APCI-MS/MS APPI-MS/MS	<i>Escherichia coli</i>	N/A	Isotopes of UQ ₆₋₁₀ MK-4	MK-4: <3 to 8999 fmol/µL for APPI <9.0 to 8999 fmol/µL for APCI	MK-4: 2.2fmol/µL UQ ₆ : 1.7 fmol/µL	(74)

Table 5.

Methods for the quantitation of vitamin K in Pharmaceutical Preparations and Human Breast Milk

Analytes	Quantification method	Bio-matrix	Bio matrix volume	Internal standard	Linearity	Limit of detection	References
Vitamin K ₃ , A, D ₃ , E	HPLC-UV	Pharmaceutical preparations	N/A	N/A	N/A	K ₃ : 0.35 µg/L	(29)
Vitamin K ₁ , MK-4, MK-7, A, D, E	LC-MS/MS	Human breast milk	3 mL for vitamin K	[¹⁸ O ₂]-PK, [¹⁸ O ₂]-MK-4 and [¹⁸ O ₂]-MK-7	PK, MK- 4, MK-7: up to 2500 ng/mL	1-250 pg/50 µL	(75)