REVIEW

Determination of Water- and Fat-Soluble Vitamins in Different Matrices Using High-Performance Liquid Chromatography

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High-performance liquid chromatographic methods are the most often used methods for the determination of water-soluble vitamins (WSV) and fat-soluble vitamins (FSV). General approaches in quantification, occurring forms of vitamins, influences, which can affect stability of vitamins, necessary precautions in sample handling, pre-run sample stabilization, extractions procedures, and HPLC quantifications are mentioned and compared. This paper provides basic guidance for using HPLC in analysis of WSV and FSV. Finally, some methods for the quantification of WSV and FSV in pharmaceutical preparations, food supplements, and biological samples are reviewed.

Abbreviations used in the text:
AA: Ascorbic acid, ACN: Acetonitrile, BHT: Butylated hydroxytoluene, CT: Column temperature, D_2: Ergocalciferol, D_3: Cholecalciferol, DHAA: Dehydroascorbic acid, DHIAA: Dehydroisoascorbic acid, ECD: Electrochemical detection, EmW: Emission wavelength, Et_2O: Diethyl ether, EtOH: Ethanol, ExcW: Excitation wavelength, FAD: Flavin adenine dinucleotide, FLD: Fluorescence detection, FMN: Flavin mononucleotide, FR: Flow rate, FSV: Fat-soluble vitamins, HPLC: High-performance liquid chromatography, IAA: Isoascorbic acid, IPOH: Isopropanol, IS: Internal standard, IV: Injection volume, LLE: Liquid-liquid extraction, LoD: Limit of detection, LoQ: Limit of quantification, MeOH: Methanol, MP: Mobile phase, NAD: Nicotinamide adenine dinucleotide, NADP: Nicotinamide adenine dinucleotide phosphate, RID: Refractometric detection, RP: Reversed phase, SAX: Strong anion exchanger, SFE: Supercritical fluid extraction, SPE: Solid-phase extraction, TCA: Trichloroacetic acid, TEA: Triethylamine, TFA: Trifluoroacetic acid, UVD: Ultraviolet detection, WSV: Water-soluble vitamins.

1. INTRODUCTION

As generally known, vitamins are essential substances, which are necessary for normal health and growth and in sufficient amounts should be supplied by food. If this intake is insufficient or if special dietary requirements exist, multivitamin preparation should be taken in order to prevent vitamin deficiency. Numerous such preparations, often formulated as film-coated dragee or effervescent, are available on the market.

Requirement on sufficient input of vitamins and hence, keeping health of individual, results in a need for accurate quantitative measurements of vitamins in food. Likewise the content of vitamins in pharmaceutical preparations needs to be checked in order to ensure correct intake and the accuracy of the label statements. Loss of vitamins in pharmaceutical preparations can be related to the specific formulation, technology of manufacturing, and storage. By analogy, technique and intensity of food processing and duration of food storage have influence on amounts of vitamins in food.

In contemporary time HPLC, connected with different detection techniques, is the leading analytical method for the quantification of vitamins as well as for most of other analytes, thanks to the possibility of rapid separation and quantification.

Present work summarizes basic and up-to-date information about vitamin quantification in different matrices. Attention is paid to the following WSV and FSV and their related compounds: AA, thiamine, riboflavin, nicotinamide, pantothenic acid, pyridoxine, folic acid, cyanocobalamin, retinol, ergocalciferol, cholecalciferol, tocopherol, and vitamin K.

2. THEORETICAL

Nowadays, there is a growing need for more rapid and specific methods for vitamin analysis. Individual vitamins can be chromatographed isocratically, as well as certain combinations of two or more vitamins; the simultaneous chromatography of more complicated mixtures may require a gradient elution. Determination can be carried out by normal-phase, ion-exchange or ion-pairing chromatography, RP chromatography being the most common method. A use of some newly developed RP stationary phases allows separation of polar vitamins without the necessity of ion-pair reagent addition. For example, a use of a stationary phase for basic compounds involving a ligand with amide groups (RP-AmideC_{16}) provides good separation for simultaneous determination of the B-group vitamins [1, 2]. Several detection techniques can be applied, however, UV detection is the most common.

Sample preparation and pre-run sample stabilization are the most important steps to ensure that subsequent HPLC analysis is effective. Sample preparation has to be carefully optimized especially for vitamins subject to degradation due to light, oxidizing reagents, pH, heat, and others. Especially for vitamins executing function of antioxidants, which makes them unstable by their nature, it is necessary to use suitable methods to acquire accurate results.

Considering sample matrix and evaluated vitamins, appropriate type of extraction (direct extraction, LLE, SPE, SFE, *etc.*) should be chosen. It is important to optimize such parameters as solvent used, sample/solvent ratio, particle size of sample (pulverization of sample), time of extraction, and temperature for sample preparation and if necessary to assure protection from light and O_2.

For simpler matrices direct extraction is mostly used. LLE is still frequently used today for FSV in complex matrices despite drawbacks, which include the use of toxic solvents, time-consuming procedures, problematical automation, multiple transfers, and need for solvent removal. In some cases repeating the extraction two or three times is adequate to achieve quantitative recovery. An alternative is SPE, which was proved to be an effective tool for simultaneous extraction, clean-up and concentration of WSV and FSV. This technique has gained much popularity in the last years.

Methods currently available for the determining physiological concentrations of vitamins in biological samples by HPLC require several preliminary steps of clean-up and, sometimes, of preconcentration because they do not possess sufficient sensitivity [3].

3. FORMS OF VITAMINS

Vitamins are usually present as chemical individuals in pharmaceutical preparations, but in food products individual vitamins occur in several forms and are present in significantly smaller amounts. The most incident forms of vitamins of interest are discussed in the text below.

Vitamin C occurs in the form of AA and DHAA. The *D*-isomer of AA, *i.e.* IAA, is not found in natural products but may be present in the so-called vitamin C-enriched products, where it is added as an antioxidant [4].

The most closely to thiamine related compounds are its mono- and pyrophosphate esters. However, nonphosphorylated structural analogues of vitamin B_1 activity, antagonists, metabolites, and compounds of similar organic structure (oxythiamine, 4-methyl-5-(2-hydroxyethyl)thiazole, *S*-benzoylthiamine, *etc.*) are also of primary importance for biochemical purposes [1].

The concentration of vitamin B_2 is usually determined as total riboflavin by converting FMN and FAD to riboflavin prior to quantification. Riboflavin occurs also as the glycoside or bound to amino acids histidine, cysteine, and tyrosine [5]. Besides already mentioned forms, there are also less known flavin derivatives present in nature. These are *e.g.* 10-hydroxyethylflavin, 10-formylmethylflavin, 7α-hydroxyriboflavin, 8α-hydroxyriboflavin, 8α-hydroxy-FMN and some forms of isomeric alloxazinic structure such as lumichrome, 7- and 8-carboxylumichromes [6].

Niacin is found in foods as nicotinic acid, nicotinamide, NAD and its reduced form NADH, NADP and its reduced form NADPH [5]. The amide form of nicotinic acid, which is normally used for enrichment, has the same biological activity as nicotinic acid [7].

In contrast to most of foods and tissues where pantothenate is generally present in bound coenzyme form (coenzyme A) or acyl carrier protein, free pantothenic acid dominates in human and bovine milk [8, 9]. Infant formulas and a lot of foodstuffs are supplemented by addition of free pantothenate, as calcium pantothenate, which is less hygroscopic than the acidic form.

Pyridoxine, pyridoxal, pyridoxamine, their corresponding 5′-phosphate esters and 4-pyridoxic acid are active forms of vitamin B_6 [10].

Folates are group of pteroic acid polyglutamate compounds with similar biological activity as folic acid. Folic acid is a synthetic pteroic acid monoglutamate, not naturally occurring [11, 12].

Naturally occurring vitamin B_{12} originates solely from synthesis by bacteria and other microorganisms growing in soil or water, in sewage, and in the rumen

and intestinal tract of animals [13]. Vitamin B_{12} includes not only cyanocobalamin, but also other cobalamins: hydroxocobalamin, methylcobalamin, and 5′-deoxyadenosylcobalamin [14]. Because of its stability, cyanocobalamin is the form that is typically used in vitamin supplements [14].

Vitamin A activity in food is related to the presence of both retinol and a large number of provitamin carotenoids. In milk and in meat, vitamin A occurs mainly as fatty acid retinyl esters [15]. Foods such as milk or infant formula are most commonly fortified with vitamin A in the form of retinyl acetate or retinyl palmitate, because these molecules are more stable and less susceptible to oxidation [16]. Some other retinoids (retinal, retinoic acid, *etc.*) can be formed from retinol in the body by action of enzymes [17].

Vitamin D can occur as D_3, derived from the action of sunlight on the 7-dehydrocholesterol, or D_2, derived from the action of sunlight on the ergosterol [5]. Vitamin D is metabolized to 25-hydroxyvitamin D in the liver and subsequently to 1,25-dihydroxyvitamin D or 24,25-dihydroxyvitamin D in the kidney. 1,25-Dihydroxyvitamin D is known to be an active form of vitamin D. Although 24,25-dihydroxyvitamin D was considered as an inactive form of vitamin D, several recent reports have demonstrated its physiological activity [18, 19]. Eggs, their yolks in particular, are considered to be one of the most important sources of vitamin D in the diet. Apart from cholecalciferol, yolks also contain the biologically more active 25-hydroxycholecalciferol [20].

Natural vitamin E is of eight chemical compounds: α-, β-, γ-, and δ-tocopherols and four corresponding tocotrienols. These different forms have different biological activities [21]. The biological activities of α-, β-, γ-, and δ-tocopherols and α-tocotrienol have a ratio of 10 : 4 : 1 : 0.1 : 3 [22]. The major form of vitamin E in milk is α-tocopherol [15].

In nature, there are two types of vitamin K: K_1 (phylloquinone) and K_2 (menaquinones). Vitamin K_1 is a single compound, but vitamin K_2 is comprised by a series of vitaminers with multiisoprene units at the position 3 of the naphthoquinone: MK1 to MK14 [23]. Menadione is a fat-soluble vitamin (K_3) that is present in synthetic feeds.

4. SAMPLE PREPARATION, STABILITY OF VITAMINS

As can be found in Tables 1 and 2, sample preparation is mostly simpler and less laborious when WSV are quantified – often it is direct extraction with buffers, diluted acids, diluted alkali or their mixtures with organic solvents. For more complicated matrices (presence of fats or proteins), sample preparation can be performed using various procedures, such as direct extraction, enzymatic hydrolysis or saponification followed by extraction with organic solvents. If necessary, precipitation of proteins is performed by lowering their solubility in aqueous medium by addition of a water-miscible organic solvent (acetonitrile, acetone, methanol, *etc.*) or by lowering pH of the solution (by TCA, *etc.*) [24, 25]. Direct injection (after dilution, centrifugation or filtration) is used for evaluation of WSV in juices and other beverages [26—28].

A somewhat different approach in quantification of FSV can be observed. Traditionally, fat-soluble vitamin analysis in complex matrices is performed by alkaline saponification of the entire sample, or of an isolated lipid fraction, followed by liquid extraction with organic solvents [16]. Saponification has often been used to remove the bulk of fat (triacylglycerides) and facilitate extraction by releasing carotenoids, retinoids, tocopherols, and vitamin D compounds from the sample matrix [15, 29]. Saponification is carried out by treatment of the sample in a strong alkali environment; this decreases the load of material that is extracted together with vitamins into organic phase. Saponification procedures are performed after addition of NaOH or KOH solution, at ambient or elevated temperature, in the presence of antioxidants and under inert atmosphere. Saponification is always followed by extraction with an organic solvent, such as Et_2O, diisopropyl ether, chloroform, hexane or their mixtures [22]. Saponification has inherent disadvantages including emulsion formation, analyte degradation (especially vitamin E and retinoids) and relatively complex sample manipulation, which represent a source of possible errors [30]. To avoid analyte decomposition, some studies perform direct analysis after only diluting the oil in a suitable organic solvent with subsequent chromatographic analysis [31].

Direct extraction of the liposoluble fraction is achieved by extraction with an organic solvent such as hexane, or hexane—IPOH for normal-phase systems, and with anhydrous EtOH, ACN—MeOH—IPOH mixtures, IPOH or THF for reversed-phase systems [22]. Direct extraction of analyte can be also performed using supercritical fluid extraction, as it is presented in paper [30]. Sometimes a use of SPE is suitable or necessary, because the analytes presented in trace amounts (vitamins K, D, *etc.*) must be concentrated to permit detection and interfering components in these samples must be removed. Forehanded and often used is enzyme treatment (lipase, takadiastase, *etc.*), which releases FSV, normally bound on different molecules. Stability of individual vitamins and extraction procedures are mentioned in the text below.

– The extraction process for the measurement of vitamin C must extract both AA and DHAA and prevent the oxidation of AA. Traditionally, metaphosphoric acid was proven to be a useful dissolving agent for the determination of ascorbic acid and for the preanalysis stabilization [32]. Other solvents that can be

Table 1. Summary of Selected Methods Developed for Quantification of WSV

Source	Sample matrix	Column used	Mobile phase, Elution	Other chromatographic conditions	Separated compounds
[64]	Multivitamin preparation	Nova-Pack C18, 4 μm, 150 × 3.9 mm	A = 0.05 M-CH_3COONH_4 B = MeOH Gradient program: 0 min: 92.5 % A, 7.5 % B 1.5 min: 92.5 % A, 7.5 % B 1.6 min: 84 % A, 16 % B 15 min: 70 % A, 30 % B	FR: 1 cm^3 min^{-1}; IV: 40 mm^3; UVD: λ/nm: 270, 362; CT: ambient	Pyridoxine: 2.5 min; Thiamine: 2.9 min; Nicotinamide: 3.2 min; Riboflavin phosphate: 10 min; Cyanocobalamin: 13 min
Extraction:	Dilution of the sample with water → application onto C18 cartridge (conditioned with MeOH and then with water) → elution of WSV from the C18 cartridge with water and then with mixture of MeOH—water (φ_r = 6 : 4). In the second step were eluted FSV (see section FSV).				
LoD:	Pyridoxine 1.37 mg dm^{-3}, thiamine: 3.18 mg dm^{-3}, nicotinamide: 9.92 mg dm^{-3}, riboflavin phosphate: 1.84 mg dm^{-3}, cyanocobalamin: 0.04 mg dm^{-3}.				
[58]	Multivitamin preparation	Nucleosil 100-5 C18, 5 μm, 250 × 4.6 mm	A = 0.1 M-$(NH_4)_2CO_3$ B = Water C = MeOH Isocratically: 5 % A, 15 % B, 80 % C	FR: 1.5 cm^3 min^{-1}; IV: 10 mm^3; UVD: λ/nm: 254; CT: ambient	Chlorpheniramine maleate (IS): 1.3 min; Pyridoxine: 1.8 min; Thiamine: 4.7 min
Extraction:	With MeOH.				
[40]	Multivitamin preparation	Phenylpropanol-amine bonded silica, 5 μm, 150 × 4.6 mm	A = 30 mM-phosphate buffer at pH 3.0 B = ACN Isocratically: 94 % A, 6 % B	FR: 1.0 cm^3 min^{-1}; IV: 20 mm^3; UVD: λ/nm: 361; CT: not stated	Cyanocobalamin: 3.5 min
Extraction:	With 50 % MeOH.				
LoQ:	Cyanocobalamin: 25 ng.				
[59]	Multivitamin preparation	LiChrospher 60 RP-select B, 250 × 4.6 mm	A = phosphate buffer (10.9 mM-KH_2PO_4—5 mM-hexanesulfonate—36 mM-TEA in water adjusted to pH 2.8 with H_3PO_4) B = MeOH Isocratically: 80 % A, 20 % B	FR: 1.0 cm^3 min^{-1}; Injection: 20 mm^3; UVD: λ/nm: 264, 280, 245; CT: 30 °C	Nicotinic acid: 4.1 min; Nicotinamide: 4.9 min; Riboflavin 5′-phosphate: 5.7 min; Pyridoxine: 7.5 min; Riboflavin: 13.5 min; Thiamine: 19.4 min; Folic acid: 15.3 min
Extraction:	With mixture of water—acetonitrile—acetic acid (φ_r = 94 : 5 : 1). Folic acid was extracted with 0.3 % NH_4OH.				
LoD:	Folic acid: 25 ng cm^{-3}, nicotinamide: 50 ng cm^{-3}, nicotinic acid: 50 ng cm^{-3}, pyridoxine: 50 ng cm^{-3}, riboflavin: 25 ng cm^{-3}, riboflavin 5′-phosphate: 50 ng cm^{-3}, thiamine: 100 ng cm^{-3}.				
[12]	Multivitamin / mineral preparation	Ultrasphere ODS, 5 μm, 250 × 4.6 mm	A = 0.03 M-KH_2PO_4 adjusted to pH 2.2 with H_3PO_4 B = MeOH Isocratically: 78 % A, 22 % B (12 min) Then elution of retained compounds with increasing amount of MeOH in MP.	FR: 1.0 cm^3 min^{-1}; IV: 100 mm^3; UVD: λ/nm: 280; CT: 22 °C	Folic acid: 11.5 min
Extraction:	Addition of extraction buffer (0.5 M-sodium acetate—5 % AA buffer with addition of Na_2EDTA (6 g dm^{-3}) and sulfanylethanol (0.7 cm^3 dm^{-3}) adjusted to pH 6.0 with NaOH) into the sample → autoclaving → cooling → dilution → centrifugation → filtration.				
LoQ:	Folic acid: 0.05 μg cm^{-3}.				
[13]	Multivitamin tablets, fermentation media	μBondapak C18, 10 μm, 300 × 3.9 mm	A = Water B = MeOH Isocratically: 70 % A, 30 % B	FR: 0.8 cm^3 min^{-1}; IV: 20 mm^3; FLD: λ/nm: 305/275 (EmW/ExcW); CT: ambient	Cyanocobalamin: 3.5 min
Extraction:	With 0.1 M-KH_2PO_4 buffer adjusted with KOH to pH 7.0.				
LoD:	Cyanocobalamin: 0.1 ng cm^{-3}.				

Table 1 (Continued)

Source	Sample matrix	Column used	Mobile phase, Elution	Other chromatographic conditions	Separated compounds
[35]	Athlete food, nutritional supplements, juices	Inertsil ODS, 3.5 μm, 150 × 4.6 mm	A = 0.02 M-monosodium L-glutamate adjusted with phosphoric acid to pH 2.1 Isocratically: 100 % A	FR: 0.8 cm^3 min^{-1}; IV: 5 mm^3; ECD: glassy carbon *vs.* Ag/AgCl electrode, applied potential 400 mV; CT: ambient	AA: 4.7 min
Extraction:	With MP.				
LoD:	AA: 0.1 ng.				
[28]	Drinks and fruit juice concentrates	Bio-Rad Aminex HPX-87H, 9 μm, 300 × 7.8 mm	A = 0.005 M-H_2SO_4 B = ACN Isocratically: 84 % A, 16 % B	FR: 0.5 cm^3 min^{-1}; IV: 20 mm^3; UVD: λ/nm: 215, 254, 280, RID; CT: 25 °C	DHAA: 9.8 min; AA: 10.5 min; Also separated furanic compounds and sugars.
Extraction:	Without extraction.				
LoD:	AA: 0.1 mg dm^{-3} (254 nm), DHAA: 1 mg dm^{-3} (230 nm).				
[55]	Fruit juices, beverages	μBondapak C18, 300 × 3.1 mm	A = Water B = MeOH C = Tetrabutylammonium phosphate Isocratically: 96.5 % A, 2.5 % B, and 1.0 % C	FR: 1.5 cm^3 min^{-1}; IV: 5 mm^3; UVD: λ/nm: 254; CT: not stated	AA
Extraction:	Mixed with 6 % metaphosphoric acid.				
[60]	Multivitamin preparation	Supelcosil LC-8-DB, 5 μm, 250 × 4.6 mm	A = Sodium hexanesulfonate and triethanolamine in water at pH 2.8 B = MeOH Isocratically: 85 % A, 15 % B	FR: 2 cm^3 min^{-1}; IV: 10 mm^3; UVD: λ/nm: 280; CT: ambient	AA: 1.7 min; Nicotinamide: 3.1 min; Pyridoxine: 5.5 min; Phenol (IS): 10 min; Thiamine: 15 min; Riboflavin: 21 min
			A = Sodium hexanesulfonate and triethanolamine in water at pH 2.8 B = MeOH Gradient program: 0 min: 92 % A, 8 % B 10 min: 82.8 % A, 17.2 % B		Nicotinamide: 4.1 min; PABA: 7.2 min; Pyridoxine: 10 min; Phenol (IS): 13 min; Thiamine: 24 min; Riboflavin: 27 min
Extraction:	With 0.01 M-HCl.				
LoQ:	Thiamine: 0.06 mg cm^{-3}, riboflavin: 0.025 mg cm^{-3}, nicotinamide: 0.05 mg cm^{-3}, pyridoxine: 0.02 mg cm^{-3}, AA: 0.01 mg cm^{-3}, PABA: 0.02 mg cm^{-3}.				
[1]	Pharmaceuticals (tablets, capsules)	Spherisorb ODS-2, 5 μm, 150 × 4.0 mm and guard column	A = 1 % acetic acid and 0.1 % sodium pentanesulfonate B = 1 % acetic acid and 0.1 % sodium pentanesulfonate and 50 % ACN Gradient program: 0 min: 88 % of A and 12 % of B 7 min: 88 % of A and 12 % of B 8 min: 40 % of A and 60 % of B 13 min: 40 % of A and 60 % of B 14 min: 88 % of A and 12 % of B Equilibration for 15 min	FR: 1.0 cm^3 min^{-1}; IV: 50 mm^3; UVD: λ/nm: 230, 250, 264; CT: ambient	Thiamine monophosphate: 5.4 min; 4-methyl-5-(2-hydroxyethyl)thiazole: 11.4 min; *S*-benzoylthiamine: 12.1 min; Thiamine: 14.2 min

Table 1 (Continued)

Source	Sample matrix	Column used	Mobile phase, Elution	Other chromatographic conditions	Separated compounds
[1]	Pharmaceuticals (tablets, capsules)	RP-AmideC16, 5 μm, 150 × 4.0 mm and guard column	A = 25 mM-KH_2PO_4 at pH 7 B = ACN Gradient program: 0 min: 100 % A 10 min: 100 % A and 10 % of B 20 min: 100 % A and 10 % B 21 min: 100 % A Equilibration for 15 min	FR: 1.0 cm^3 min^{-1}; IV: 50 mm^3; UVD: λ/nm: 230, 250, 264; CT: ambient	Thiamine pyrophosphate: 5.2 min; Thiamine monophosphate: 5.7 min; Oxythiamine: 6.1 min; Thiamine: 6.9 min; 4-methyl-5-(2-hydroxyethyl)thiazole: 11 min; Amprolium: 12 min; *S*-benzoylthiamine: 17 min
Extraction: With water. LoQ: Thiamine pyrophosphate: 0.02 μg cm^{-1}, thiamine monophosphate: 0.2 μg cm^{-1}, oxythiamine: 0.02 μg cm^{-1}, thiamine: 0.03 μg cm^{-1}, 4-methyl-5-(2-hydroxyethyl)thiazole: 0.08 μg cm^{-1}, amprolium: 0.2 μg cm^{-1}, *S*-benzoylthiamine: 0.03 μg cm^{-1}.					
[25]	Infant milk	Tracer Spherisorb ODS2 C18, 5 μm, 250 × 4.6 mm	A = Acetic buffer (5 mM-octanesulfonic acid—0.5 % TEA—2.4 % glacial acetic acid and 15 % MeOH adjusted to pH 3.6 ± 0.1) Isocratically: 100 % A	FR: 1.0 cm^3 min^{-1}; IV: 20 mm^3; UVD: λ/nm: 261, 287, 290, 282, 268, 361, 246; CT: ambient	Nicotinamide: 5 min; Pyridoxal: 7 min; Pyridoxine: 10 min; Pyridoxamine: 12 min; Folic acid: 14 min; Riboflavin: 17 min; Cyanocobalamin: 23 min; Thiamine: 48 min
Extraction: Addition of TCA into the sample (precipitation of proteins) → shaking → centrifugation → supernatant was separated and residue extracted again → centrifugation → supernatants were combined and diluted. LoQ: < 0.05 mg cm^{-3} for nicotinamide, pyridoxal, pyridoxine, pyridoxamine, folic acid, and riboflavin, < 0.1 mg cm^{-3} for thiamine and < 0.3 mg cm^{-3} for cyanocobalamin.					
[32]	Juices, milks, nutrients	Inertsil ODS-3, 5 μm, 150 × 4.6 mm	A = 100 mM-KH_2PO_4 containing 1 mM-EDTA.2Na adjusted with phosphoric acid to pH 3 Isocratically: 100 % A	FR: 0.6 cm^3 min^{-1}; IV: 20 mm^3; ECD: applied potential at 400 mV *vs.* an Ag/AgCl electrode; CT: ambient	AA: 5.5 min
Extraction: With MP. LoD: AA: 0.5 ng.					
[4]	Food, plasma, multivitamin tablets	Phenomenex Jupiter C18, 5 μm, 250 × 4.6 mm	A = 2.3 mM-dodecyltrimethylammonium chloride and 2.5 mM-EDTA.2Na in 66 mM-phosphate—20 mM-acetate buffer adjusted to pH 4.5 Isocratically: 100 % A	FR: 1.2 cm^3 min^{-1}; IV: 20 mm^3; UVD: λ/nm: 247; FLD: λ/nm: 430/350 (EmW/ExcW); CT: 20 °C; Post-column derivatization with 28 mM-*O*-phenyldiamine in 12 mM-trisodium citrate—55 mM-EDTA.2Na buffer adjusted to pH 3.7; Reagent delivery: 0.3 cm^3 min^{-1}; Reaction temperature: 55 °C	DHIAA: 3.8 min; DHAA: 4.6 min; AA: 7.8 min; IAA: 9.1 min
Extraction: Food: With a mixture of 1 % metaphosphoric acid and 0.5 % oxalic acid adjusted to pH 2 under CO_2 → centrifugation. Plasma: 10 % metaphosphoric acid and MeOH added into the sample → centrifugation → dilution with 0.4 M-acetate buffer of pH 3.9 → centrifugation. Multivitamin tablets: as food, without CO_2.					

Table 1 (Continued)

Source	Sample matrix	Column used	Mobile phase, Elution	Other chromatographic conditions	Separated compounds
[33]	Parenteral nutrition mixtures	Luna C18, 5 μm, 150 × 4 mm	A = MeOH B = 0.067 M-Na_2HPO_4 buffer with 0.05 M-alkyltrimethylammonium bromide of pH 7.8 Isocratically: 40 % A, 60 % B	Flow rate: 0.7 cm^3 min^{-1}; Injection: not stated; UVD: λ/nm: 278; CT: not stated	AA: 4.1 min
Extraction:	AA was analyzed directly, DHAA after conversion with dithiothreitol to AA. Amount of total DHAA was determined from the difference of total AA and AA in original sample. Sample manipulation under N_2.				
LoD:	DHAA: 1 μg cm^{-3}.				
[37]	Pork meat products	Spherisorb ODS C18, 5 μm, 250 × 4.0 mm	A = 0.01 M-H_2SO_4 Isocratically: 100 % A	FR: 1.0 cm^3 min^{-1}; IV: 20 mm^3; FLD: λ/nm: 395/290 (EmW/ExcW); CT: 30 °C	Pyridoxamine: 2.6 min; Pyridoxal: 5.4 min; Pyridoxine: 6.9 min; 4-deoxypyridoxine (IS): 11.2 min
Extraction:	Addition of 0.1 M-HCl and IS into the sample → shaking → heating in water bath at 100 °C for 30 min → cooling → adjustment of pH with 2 M-sodium acetate to the value 4.0—4.5 → addition of takadiastase → incubation for 3 h at 45 °C → addition of TCA → heating for 5 min at 95—100 °C → cooling → dilution → filtration.				
[9]	Milk and infant formulas	Luna C8, 5 μm, 250 × 4.6 mm hydrophobic shielded endcapped	A = 0.1 M-KH_2PO_4 adjusted to pH 2.25 with H_3PO_4 B = ACN Isocratically: 97 % A, 3 % B	FR: 1.4 cm^3 min^{-1} (at 18 min increased to 1.8 cm^3 min^{-1}); IV: 10 mm^3; UVD: λ/nm: 200, 205, 204; CT: not stated	Pantothenic acid: 14 min
Extraction:	Dilution of the sample with water → mixing → standing for 20 min → addition of acetic acid → mixing → standing for 20 min → dilution of sample → centrifugation → filtration.				
LoD:	0.3 mg/100 g of anhydrous sample.				
[26]	Wine, beer, and fruit juices	Hypersil C18, 5 μm, 200 × 2.1 mm	A = 0.05 M-NaH_2PO_4 adjusted with H_3PO_4 to pH 3.0 B = ACN Gradient program: 0 min: 95 % A, 5 % B 8 min: 75 % A, 25 % B 12 min: 95 % A, 5 % B Equilibration for 3 min	FR: 0.6 cm^3 min^{-1}; IV: 20 mm^3; FLD: λ/nm: 525/265 (EmW/ExcW); 500 nm cut-off filter was used; CT: not stated	FAD: 5.8 min; FNM: 6.2 min; Riboflavin: 7.3 min
Extraction:	Without extraction.				
[10]	Yeast cell-free culture media, baker's yeast extract, egg, milk	Phenosphere ODS2, 5 μm, 250 × 4.6 mm and guard column	A = 0.15 M-NaH_2PO_4 adjusted to pH 2.5 with 70 % perchloric acid Isocratically: 100 % A	FR: 1.0 cm^3 min^{-1}; IV: 20 mm^3; FLD: λ/nm: 389/290 (EmW/ExcW); Post-column derivatization with sodium bisulfite (1 g dm^{-3}); Reagent delivery: 0.1 cm^3 min^{-1}; CT: ambient	Pyridoxamine 5′-phosphate ester: 3.5 min; Pyridoxamine: 4.0 min; Pyridoxal 5′-phosphate ester: 6.0 min; Pyridoxine 5′-phosphate ester: 6.5 min; Pyridoxal: 9.5 min; Isopyridoxal (IS): 11 min; Pyridoxine: 13.5 min; 4-pyridoxic acid: 18 min
Extraction:	Baker's yeast extract: Addition of IS and 1 M-perchloric acid into the sample, mixing for a few seconds and again after 30 min → centrifugation → adjustment of pH of supernatant to the value 3—4 with 10 M-KOH → centrifugation → filtration. Egg yolk extract and milk extract: The same procedure, but after the 1st centrifugation was used solution from the clear middle part (to avoid protein at the bottom and fat on the top).				

Table 1 (Continued)

Source	Sample matrix	Column used	Mobile phase, Elution	Other chromatographic conditions	Separated compounds
[2]	Baby foods	Supelco RP-Amide C16, 5 μm and guard column	A = 10 mM-KH_2PO_4 at pH 6 B = ACN Gradient program: 0 min: 100 % A 13 min: 100 % A 14 min: 94 % A, 6 % B 20 min: 94 % A, 6 % B 21 min: 88 % A, 12 % B 31 min: 88 % A, 12 % B Equilibration for 15 min	FR: 1 cm^3 min^{-1}; IV: 100 mm^3; UVD: λ/nm: 249, 266, 326, 361; CT: not stated	Nicotinic acid: 3.2 min; Pyridoxal: 4.3 min; Pyridoxine: 5.2 min; Thiamine: 6.7 min; Nicotinamide: 7.3 min; Inosine: 13.1 min; Folic acid: 19.2 min; Cyanocobalamin: 26.7 min; Riboflavin: 28.4 min
Extraction:	Homogenization of sample with 0.1 M-HCl → heating for 30 min at 90 °C → cooling → adjustment of pH to the value 4 with 1 M-sodium acetate → addition of takadiastase → stirring for 2 h at 50 °C → addition of TCA → heating for 10 min at 90 °C → cooling → dilution with mobile phase → centrifugation → filtration.				
LoQ:	Nicotinic acid: 0.064 μg cm^{-3}, pyridoxal: 0.063 μg cm^{-3}, pyridoxine: 0.068 μg cm^{-3}, thiamine: 0.032 μg cm^{-3}, nicotinamide: 0.128 μg cm^{-3}, inosine: 0.029 μg cm^{-3}, folic acid: 0.015 μg cm^{-3}, cyanocobalamin: 0.032 μg cm^{-3}, riboflavin: 0.010 μg cm^{-3}.				
[65]	Fruit juice	Eurospher 100 C18, 5 μm, 250 × 4.6 mm	A = 5 mM-tetrabutylammonium hydrogen sulfate—25 mM-NaCl in water B = 5 mM-tetrabutylammonium hydrogen sulfate—25 mM-NaCl—1 mM-KH_2PO_4—65 % ACN in water Gradient program: 0 min: 90 % A, 10 % B 10 min: 90 % A, 10 % B 15 min: 64 % A, 36 % B 35 min: 50 % A, 50 % B 38 min: 50 % A, 50 % B Equilibration for 5 min	FR: 1.0 cm^3 min^{-1}; IV: 20 mm^3; UVD: λ/nm: 284; CT: 25 °C	Folic acid: 17 min
Extraction:	Centrifugation of sample → filtration → SAX cartridge was conditioned with hexane, MeOH, and water and dried → application of sample onto cartridge → rinsing of cartridge with water → drying → elution with 0.1 M-sodium acetate containing 10 % NaCl.				
LoQ:	Folic acid: 0.06 mg dm^{-3}.				
[61]	Fortified cereal products	Microsorb-MV C18, 3 μm, 100 × 4.6 mm with guard column	A = 0.0035 M-KH_2PO_4 and 0.0032 M-K_2HPO_4, pH 6.8, containing 0.005 M-tetrabutylammonium dihydrogenphosphate B = MeOH Isocratically: 75 % A, 25 % B	FR: 1 cm^3 min^{-1}; IV: 20 mm^3; UVD: λ/nm: 280; CT: ambient	Folic acid: 13 min
Extraction:	Homogenized sample was stirred for 1 h in 0.1 M-K_2HPO_4 (pH 8—9) containing 0.05 % ascorbate → adjustment of pH to the value 6.9 with phosphoric acid → addition of α-amylase → incubation 1h at 65 °C → enzyme inactivation by raising of temperature to 90 °C → cooling → centrifugation → filtration and if needed SPE was performed on SAX cartridges (cartridge was conditioned with hexane, MeOH, and 0.1 M-K_2HPO_4 (pH 8—9) containing 0.05 % AA → application of sample → rinsing with 0.02 M-buffer → elution of folic acid with 0.1 M-Na_2HPO_4 with 0.05 % AA).				
LoD:	Folic acid: 2 ng (for solution of reference substance).				
[27]	White wines	Hypersil ODS C18, 5 μm, 200 × 2.1 mm	A = 0.05 M-NaH_2PO_4 adjusted with H_3PO_4 to pH 3.0 B = ACN Gradient program: 0 min: 95 % A, 5 % B 8 min: 75 % A, 25 % B 12 min: 95 % A, 5 % B Equilibration for 3 min	Flow rate: 0.6 cm^3 min^{-1}; IV: 20 mm^3; FLD: λ/nm: 525/265 (EmW/ExcW); CT: ambient	Riboflavin: 8 min
Extraction:	Without extraction.				

Table 1 (Continued)

Source	Sample matrix	Column used	Mobile phase, Elution	Other chromatographic conditions	Separated compounds
[6]	Raw egg white and yolk, egg powder, milk, milk products and liver	Altech Alphabond C18, 300 × 4.6 mm and guard column	A = MeOH B = 0.05 M-ammonium acetate buffer at pH 6.0 Gradient program: 0 min: 30 % A, 70 % B 1 min: 30 % A, 70 % B 10 min: 70 % A, 30 % B	FR: 1 cm^3 min^{-1}; IV: not stated; FLD: λ/nm: 530/450, 430/380 (EmW/ExcW); CT: not stated	FAD: 6.5 min; 7α-hydroxyriboflavin: 6.5 min; 10-hydroxyethylflavin: 7.3 min; Riboflavin 4′,5′-cyclic phosphate: 8.4 min; FMN: 10.3 min; Riboflavin β-galactoside: 12.0 min; Riboflavin: 13.2 min; 10-formylmethylflavin: 15.4 min
Extraction:	Sample was suspended in mixture of MeOH—CH_2Cl_2 ($\varphi_r = 9:10$), shaking for 60 s → addition of 0.1 M-$(NH_4)HCO_3$ → shaking for 60 s → centrifugation at 4 °C → filtration. Semi-preparative HPLC of flavins from food on μBondapack column (10 μm, 100 × 25 mm at flow rate 3 cm^3 min using MeOH and 0.05 M-ammonium acetate buffer at pH 6.0 as mobile phase, gradient elution).				
LoD:	Riboflavin: ≈ 1 ng cm^{-3}, FAD: ≈ 6 ng cm^{-3}.				
[62]	Polymeric carrier	Phenomenex Luna C18, 5 μm, 150 × 2.0 mm	A = MeOH B = 0.01 M-phosphate buffer, pH 5.0 containing 4 mM-tetrabutylammonium hydrogensulfate Isocratically: 23 % A, 77 % B	FR: 0.3 cm^3 min^{-1}; Injection: 20 mm^3; UVD: λ/nm: 270; CT: not stated	Folic acid: 9.5 min; Benzoic acid (IS): 19 min
Extraction:	Micelles were dissolved in 10 % NH_4OH → dilution with mobile phase → addition of IS → SAX cartridges (strong anion exchanger) were conditioned before use with MeOH and 0.01 M-phosphate buffer, pH 9.0 → application of sample → rinsing of cartridge with demi water → elution with mixture of acetonitrile—0.1 M-NaH_2PO_4 buffer (pH 3.0) containing 0.2 M-potassium chlorate ($\varphi_r = 50:50$) or with 0.1 M-NaH_2PO_4 buffer (pH 9.0) containing 0.3 M-potassium chlorate.				
LoD:	Folic acid: 0.07 μg cm^{-3}.				
[63]	Blood	μBondapak C18, 100 × 8 mm	A = 0.1 M-sodium citrate buffer adjusted to pH 4.0 with orthophosphoric acid B = 1 % acetic acid C = MeOH Isocratically: 43 % A, 42 % B, 15 % C	FR: 3.0 cm^3 min^{-1}; IV: 200 mm^3; UVD *vs.* scintillation of collected eluate; IS: ^{3}H-folic acid; CT: not stated	Folic acid: 12 min
Extraction:	Blood was allowed to clot → centrifugation → addition of IS into serum → serum was deproteinated with 60 % perchloric acid → pH was adjusted to the value 7.0 with 6 M-KOH → sample solution was loaded onto C18 cartridge (conditioned with MeOH and then with citrate—phosphate buffer) → elution of retained folic acid with MeOH → evaporation under vacuum → residuum was redissolved in 0.5 % sodium ascorbate.				
LoQ:	Folic acid: 0.3 ng cm^{-3}.				
[56]	Plasma and serum	Spherisorb ODS C18, 5 μm, 250 × 4.0 mm	A = 5 mM-cetyltrimethylammonium bromide and 50 mM-KH_2PO_4 at pH 4.5 Isocratically: 100 % A	FR: 1 cm^3 min^{-1}; IV: 20 mm^3; UVD: λ/nm: 254; CT: ambient	AA: 8.9 min; 4-hydroxyacetanilide (IS): 14 min
Extraction:	Conversion of DHAA to AA with dithiothreitol at pH 7.0 → plasma/serum was diluted with 10 % metaphosphoric acid ($\varphi_r = 1:1$) → addition of IS → centrifugation. Amount of DHAA was determined from the difference of total AA and AA.				

Table 1 (Continued)

Source	Sample matrix	Column used	Mobile phase, Elution	Other chromatographic conditions	Separated compounds
[66]	Urine	μBondapak C18, 5 μm, 250 × 4.6 mm	A = 0.1 M-TFA B = 0.1 M-TFA and MeOH (φ_r = 10 : 90) Gradient program: 0 min: 99.5 % A and 0.5 % B 5 min.: 99.5 % A and 0.5 % B 12 min: 10 % A and 90 % B 15 min: 10 % A and 90 % B Equilibration	FR: 1 cm^3 min^{-1}; IV: 100 mm^3; UVD: λ/nm: 254; CT: ambient	AA: 3.1 min; 5-methylcytosine (IS): 4.2 min; B_6: 5.0 min; B_1: 6.2 min; B_2: 13 min
Extraction:	Sample was loaded onto C18 cartridge (conditioned with MeOH and acidified water, pH 4.2 (with HCl)) → elution from cartridge with acidified water (pH 4.2; with 0.005 M-HCl) and then with MeOH → evaporation → residuum was redissolved in acidified water, pH 3.7.				
LoD:	B_1: 1.80 μg cm^{-3}, B_2: 0.22 μg cm^{-3}, AA: 0.42 μg cm^{-3}, and B_6: 6.7 μg cm^{-3}.				
[54]	Plants	Asahipak GS-320 7E, 250 × 7.6 mm	A = 17 mM-acetic acid containing 0.5 mM-EDTA Isocratically: 100 % A	FR: 1.0 cm^3 min^{-1}; IV: not stated; Post-column derivatization with 0.02 M-benzamidine solution and 0.75 M-borate buffer containing 0.2 M-potassium sulfite, pH 10.5 (both 0.25 cm^3 min^{-1}); Reaction temperature: 100 °C; Reaction time: 1.5 min; FLD: λ/nm: 400/325 (EmW/ExcW)	DHAA: 11 min; AA: 13 min; Together separated AA-carbamylated derivatives.
Extraction:	Homogenization with 1 % metaphosphoric acid.				
LoD:	All quantified substances: < 0.5 μmol.				

used as stabilizing agents are 0.1 M-KH_2PO_4 at pH 3.0 with 0.001 M-EDTA.2Na or 0.01 M-phosphate buffer at pH 6.8 with 0.25 % cysteine and are discussed in the same paper. As another extraction solvent, authors [4] propose a combination of metaphosphoric acid and oxalic acid, pH 2. As declared, this buffer provides a high stabilization of AA and DHAA and leads to minimal interactions with the chromatographic system. In the presence of oxidizing reagents, AA is oxidized to DHAA. DHAA then hydrolyzes to 2,3-diketogulonic acid and this compound is degraded to threonic and oxalic acid [33, 34]. Influence of individual conditions during extraction (pH, light, temperature, *etc.*) on stability of AA is in detail discussed in [35]. AA is the least stable component in parenteral nutrition mixtures [33].

– The stability of thiamine depends on pH, temperature, ionic strength, and the presence of ions [1]. The compound is increasingly unstable as the pH rises and is decomposed by oxidizing or reducing agents [34]. Decomposition of the vitamin involves fragmentation into thiazole and pyrimidine derivatives [1]. Enzymatic treatment of sample matrix after acidic treatment appeared essential to achieve total extraction of thiamine and riboflavin from foods, while for the other vitamins of B-group the recovery after acidic hydrolysis was similar to recovery after acidic followed by enzymatic hydrolysis [2].

– Flavins are sensitive to light, alkaline or extremely acidic pH. Riboflavin decomposition in food is usually reported as a percentage of the initial amount present or as a determined lumichrome content, which is a photoproduct of riboflavin degradation in acid and neutral solutions. Riboflavin and its coenzymes under alkaline conditions are photodegraded to biologically inactive lumiflavin [6]. Riboflavin and FMN in neutral aqueous solutions are stable even when heated to 100 °C [36]. In diluted acids, however, the ester bond of flavin nucleotides is rapidly hydrolyzed [6]. Degradation of flavin mononucleotide and flavin adenine dinucleotide in wines is linked to the photogeneration of thiols and is responsible for "sunlight flavour" [26, 27].

– Nicotinamide is normally very stable [7]. Upon acid treatment, the NADH and NADPH are converted to the 6-cyclo compounds. Under alkaline conditions the NAD and NADP are degraded to nicotinaldehyde. Thus, it is not possible to measure all of the nicotinic

Table 2. Summary of Selected Methods Developed for Quantification of FSV

Source	Sample matrix	Column used	Mobile phase, Elution	Other chromatographic conditions	Retention times
[64]	Multivitamin preparation	Nova-Pack C18, 4 μm, 150 × 3.9 mm	A = MeOH B = ACN Isocratically: 95 % A, 5 % B	FR: 2 cm^3 min^{-1}; IV: 20 mm^3; UVD: λ/nm: 285; CT: ambient	Cholecalciferol: 2.1 min; Tocopherol acetate: 2.7 min; Retinyl palmitate: 7.3 min
Extraction:	The sample was diluted with water → application onto C18 cartridge (conditioned with MeOH and then with water), elution of WSV from the C18 cartridge with water and with mixture of MeOH—water (φ_r = 6 : 4). Elution of fat-soluble vitamins from the cartridge with $CHCl_3$.				
LoD:	Cholecalciferol: 0.05 mg dm^{-3}, tocopherol acetate: 3.09 mg dm^{-3}, retinyl palmitate: 5.00 mg dm^{-3}.				
[43]	Retinoid solutions	Phenomenex Luna C18, 3 μm, 150 × 4.6 mm	A = MeOH and 10 mM-ammonium acetate (φ_r = 75 : 25) B = MeOH and THF (φ_r = 84 : 16) Gradient program: 0 min: 100 % A, 0 % B 25 min: 100 % A, 0 % B 35 min: 0 % A, 100 % B 45 min: 0 % A, 100 % B 50 min: 100 % A, 0 % B	FR: 0.8 cm^3 min^{-1}; IV: 20 mm^3; UVD: λ/nm: 350; FLD: λ/nm: 520/350, 520/450 (EmW/ExcW), CT: ambient	13-*cis* retinoic acid: 27.5 min; 9-*cis* retinoic acid: 29.5 min; All-*trans* retinoic acid: 31.5 min; Retinol palmitate: 47 min; β-carotene: 49 min
Extraction:	Without extraction.				
LoD:	13-*cis* retinoic acid: 12 pmol (UVD), 7 pmol (FLD); 9-*cis* retinoic acid: 11 pmol (UVD), 7 pmol (FLD); all-*trans* retinoic acid: 11 pmol (UVD), 5 pmol (FLD); retinyl palmitate: 15 pmol (UVD), 1 pmol (FLD); β-carotene: 5 pmol (UVD), 4 pmol (FLD).				
[44]	Pharmaceutical preparations (capsules, gel, cream, solution)	Phenomenex Prodigy 5ODS3, 5 μm, 250 × 3.2 μm	A = ACN B = EtOH C = 1 % glacial acetic acid Isocratically: 68 % A, 8 % B, 24 % C	FR: 0.4 cm^3 min^{-1}; IV: 20 mm^3; FLD: λ/nm: 520/350 (EmW/ExcW); CT: 32 °C	13-*cis* retinoic acid: 28 min; 9-*cis* retinoic acid: 31 min; All-*trans* retinoic acid: 33 min
		Phenomenex Luna Phenyl—Hexyl, 5 μm, 250 × 4.6 mm	A = ACN B = MeOH C = 1 % glacial acetic acid Isocratically: 86 % A, 10 % B, 4 % C	FR: 0.8 cm^3 min^{-1}; IV: 20 mm^3; FLD: λ/nm: 520/350, 330/296, 520/450 (EmW/ExcW); CT: 32 °C	All-*trans* retinoic acid: 4.6 min; Tocopherol acetate: 8 min; Retinyl palmitate: 12 min; β-carotene: 14 min
Extraction:	Capsules/cream – 1st method: With mixture of ACN—EtOH—1 % acetic acid (φ_r = 70 : 20 : 10) by sonicating (5 min) → centrifugation → dilution. Capsules – 2nd method: With EtOH by sonicating (5 min) → centrifugation → dilution. Gel: Sonicating with MP (1 min) → centrifugation → dilution with MP. Solution – 1st method: Sample was diluted with ACN and loaded onto conditioned C18 cartridge. Elution with mixture of ACN—MeOH (φ_r = 1 : 1). Solution – 2nd method: Sample was diluted with mixture of ACN—EtOH—CH_2Cl_2 (φ_r = 60 : 30 : 10).				
LoD:	9-*cis* retinoic acid: 11 pmol (FLD λ/nm: 520/350 (EmW/ExcW)); β-carotene: 5 pmol (FLD λ/nm: 520/450 (EmW/ExcW)); retinyl palmitate: 0.8 pmol (FLD λ/nm: 520/350 (EmW/ExcW)); tocopherol acetate: 12 pmol (FLD λ/nm: 330/296 (EmW/ExcW)).				
[30]	Cosmetic creams and lotions	μBondapak C18, 10 μm, 300 × 3.9 mm and guard column	A = MeOH B = ACN Isocratically: 75 % A, 25 % B	FR: 1.5 cm^3 min^{-1}; IV: not stated; UVD: λ/nm: 280, 325; CT: ambient	Tocopherol acetate: 6 min; Retinyl palmitate: 10 min
Extraction:	By CO_2 at pressure 250 MPa and at 40 °C → evaporation → residue redissolved in mixture of THF—MeOH (φ_r = 4 : 1).				

Table 2 (Continued)

Source	Sample matrix	Column used	Mobile phase, Elution	Other chromatographic conditions	Retention times
[21]	None. (Optimization of separation for pure analytes)	Genesis silica, 5 μm, 250 × 4.6 mm	A = Hexane B = 1,4-dioxane Isocratically: 96 % A, 4 % B	FR: 1.5 cm^3 min^{-1}; IV: not stated; FLD: λ/nm: 326/294 (EmW/ExcW); CT: ambient	α-tocopherol: 6.5 min; α-tocotrienol: 7.6 min; β-tocopherol: 9.2 min; β-tocotrienol: 10.2 min; γ-tocopherol: 11.7 min; γ-tocotrienol: 12.7 min; δ-tocopherol: 15.2 min; δ-tocotrienol: 19 min
		HypersilAPS-2, 5 μm, 250 × 4.6 mm	A = Hexane B = 1,4-dioxane Isocratically: 95 % A, 5 % B	FR: 2.5 cm^3 min^{-1}; IV: not stated; FLD: λ/nm: 326/294 (EmW/ExcW); CT: ambient	α-tocopherol: 2.7 min; α-tocotrienol: 3.1 min; β-tocopherol: 5.3 min; β-tocotrienol: 6.1 min; γ-tocopherol: 6.9 min; γ-tocotrienol: 7.8 min; δ-tocopherol: 10.9 min; δ-tocotrienol: 14.7 min
Extraction: None.					
[69]	Powdered milk and local flour	LiChrosorb RP18, 5 μm, 125 × 4.5 mm	A = ACN Isocratically: 100 % A	FR: 0.8 cm^3 min^{-1}; IV: 20 mm^3; UVD: λ/nm: 292; CT: ambient	Retinol: 4 min; δ-tocopherol: 9 min; β+γ-tocopherol: 11 min; α-tocopherol: 13 min
Extraction: Heating of the sample with 50 % NaOH (w/v) for 3 min at 30 °C → addition of ethanol and hydroquinone → heating in water bath for 30 min at 80 °C → cooling → addition of water → extraction with dimethyl ether—petroleum ether (φ_r = 1 : 1) repeatedly → evaporation under vacuum at 40 °C → residue was redissolved in MeOH.					
[79]	Emulsified nutritional supplements	Inertsil ODS-2, 5 μm, 150 × 4.6 mm	A = MeOH B = EtOH Isocratically: 50 % A, 50 % B	FR: 0.4 cm^3 min^{-1}; IV: 100 mm^3; FLD: λ/nm: 295/325 (EmW/ExcW); CT: 40 °C	Tocopherol acetate: 11 min
Extraction: Sample was dissolved in 5 % aqueous sodium sulfate solution containing 1 mM-EDTA·2Na → application of sample onto conditioned cartridge (with MeOH and water) → rinsing with demi water, 5 %, 25 %, and 50 % ACN → elution with ACN → filtration. LoD: Tocopherol acetate: 0.1 ng.					
[68]	Infant milk formulae	Tracer Spherisorb ODS2 C18, 5 μm, 250 × 4.6 mm and guard column	A = Water B = ACN C = MeOH Isocratically: 4 % A, 1 % B, 95 % C	FR: not stated IV: 20 mm^3; UVD: λ/nm: 292, 323; CT: not stated	All-*trans* retinol: 6 min; α-tocopherol: 20 min
Extraction: Saponification by 60 % KOH solution in EtOH at ambient temperature with an addition of AA under N_2 overnight → extraction using hexane (3 times) → washing with water → addition of BHT → filtration → evaporation → residue was redissolved in MeOH. LoQ: All-*trans* retinol: 0.02 μg cm^{-3}, α-tocopherol: 0.04 μg cm^{-3}.					
[76]	Emulsified nutritional supplements	Inertsil ODS-2, 5 μm, 150 × 4.6 mm with guard column Hitachigel 3011-0, 5 μm, 100 × 4.6 mm	A = MeOH B = ACN Elution program and column switching: Injection into guard column, column washed for 4 min with 100 % eluent A at flow rate 0.8 cm^3 min^{-1} at ambient temperature. Then retained substances were flushed to the analytical column using 25 % of A and 75 % of B for 15 s and then isocratic elution with 100 % eluent A	FR: 0.6 cm^3 min^{-1}; IV: 200 mm^3; UVD: λ/nm: 265; CT: 40 °C	D_2: 12 min
Extraction: Sample was dissolved in brown volumetric flask in 0.2 M-K_2HPO_4 with 1 mM-EDTA.2Na → application onto C18 cartridge → rinsing with demi water and 10 % MeOH → elution with MeOH → evaporation to dryness → residue was redissolved in MeOH → filtration. LoD: D_2: 0.1 ng.					

Table 2 (Continued)

Source	Sample matrix	Column used	Mobile phase, Elution	Other chromatographic conditions	Retention times
[67]	Liquid preparations	LiChrospher 100 RP-18, 5 μm, 125 × 4.0 mm	A = MeOH Isocratically: 100 % A	FR: 1.5 cm^3 min^{-1}; IV: not stated; UVD: λ/nm: 300, 450; CT: ambient	(AA: 1.0 min); Retinyl acetate: 2.5 min; Retinyl propionate: 2.9 min; DL-α-tocopherol: 4.8 min; Retinyl palmitate: 15.5 min; β-carotene: 19 min
Extraction:	Mixing with $CHCl_3$ → dilution with MeOH.				
LoQ:	AA: 1.5 μg cm^{-3}, retinyl acetate: 0.2 μg cm^{-3}, retinyl propionate: 0.1 μg cm^{-3}, DL-α-tocopherol: 0.8 μg cm^{-3}, retinyl palmitate: 0.2 μg cm^{-3}, β-carotene: 0.04 μg cm^{-3}.				
[29]	Olive oil	Tracer Extrasil ODS-2, 5 μm, 150 × 4.0 mm	A = MeOH B = Water C = Butanol Gradient program 0 min: 92 % A, 3 % B, 5 % C 3 min: 92 % A, 3 % B, 5 % C 4 min: 92 % A, 8 % C 9 min: 92 % A, 8 % C Equilibration for 10 min	FR: 2.0 cm^3 min^{-1}; IV: 50 mm^3; UVD: λ/nm: 292, 450; CT: 45 °C	α-tocopherol: 2.7 min; β-carotene 8.0 min
Extraction:	Saponification by KOH solution in ethanol with an addition of AA under N_2 → addition of NaCl solution (25 g dm^{-3}) → extraction with mixture of hexane—ethyl acetate (φ_r = 85 : 15) → evaporation → residue was redissolved in MeOH.				
LoQ:	α-tocopherol: 23 ng, β-carotene: 31 ng.				
[31]	Vegetable oils	Tracer Extrasil ODS-2, 5 μm, 150 × 4.4 mm	A = MeOH B = Water Isocratically: 96 % A, 4 % B	FR: 2.0 cm^3 min^{-1}; IV: 50 mm^3; UVD: λ/nm: 292; CT: 45 °C	δ-tocopherol: 2.7 min; γ+β-tocopherol: 3.3 min; α-tocopherol: 3.9 min; α-tocopherol acetate: 5.5 min
Extraction:	Sample was diluted in hexane (φ_r = 1 : 9) → mixing with MeOH and ethanolic solution of IS (φ_r = 1 : 3 : 1) → centrifugation → filtration.				
LoQ:	α-tocopherol: 23 ng, δ-tocopherol: 25 ng.				
[70]	Milk	Hypersil C18 BDS, 3 μm, 150 × 0.3 mm	A = MeOH and water (φ_r = 99 : 1) B = MeOH and THF (φ_r = 70 : 30) Gradient program: 0 min: 100 % A, 0 % B 4 min: 100 % A, 0 % B 10 min: 0 % A, 100 % B 15 min: 0 % A, 100 % B 17 min: 100 % A, 0 % B Then equilibration	FR: 6 mm^3 min^{-1}; IV: 0.06 mm^3; UVD: λ/nm: 325, 264, 280; CT: 20 °C	Retinol: 3 min; Retinyl acetate: 4 min; D_2: 6.5 min; D_3: 7 min; Tocopherol: 7.5 min; Provitamin D_2: 9.5 min; Provitamin D_3: 10.5 min; Tocopherol acetate: 11 min; Phylloquinone: 11.5 min; Retinyl palmitate: 13 min
Extraction:	Addition of ethanol and 0.025 % BHT into the sample → sonicating for 2 min → repeated extraction with hexane → washing of hexane extract with mixture of MeOH—water (φ_r = 9 : 1) → filtration → evaporation under N_2 → residue was redissolved in MeOH.				
LoD:	Retinol: 1 ng cm^{-3}, retinyl acetate: 2 ng cm^{-3}, D_2: 46 ng cm^{-3}, D_3: 5 ng cm^{-3}, tocopherol: 32 ng cm^{-3}, tocopherol acetate: 38 ng cm^{-3}, provitamin D_2: 9 ng cm^{-3}, provitamin D_3: 7 ng cm^{-3}, phylloquinone: 16 ng cm^{-3}, retinyl palmitate: 7 ng cm^{-3}.				
[45]	Breast milk	Grom-Sil-CN-2PR, 3 μm, 250 × 4.6 mm	A = Hexane B = IPOH Isocratically: 98 % A, 2 % B	FR: 2.0 cm^3 min^{-1}; IV: not stated; FLD: λ/nm: 480/325 (EmW/ExcW); CT: not stated	Retinol: 2.7 min
Extraction:	Homogenization of milk by sonicating for 2 min → addition of EtOH with BHT and 12.5 M-KOH (subsequently protection against O_2 with N_2) → sample was kept in darkness for 25 min at 80 °C whilst slightly agitated → cooling to ambient temperature → extraction with mixture of hexane—toluene (φ_r = 1 : 1) → centrifugation.				
LoD:	Retinol: 30 nmol dm^{-3}.				

Table 2 (Continued)

Source	Sample matrix	Column used	Mobile phase, Elution	Other chromatographic conditions	Retention times
[71]	Cooked meals	Spherisorb ODS-2 C18, 5 μm, 250 × 4.6 mm	A = MeOH B = Water Isocratically: 96 % A, 4 % B	FR: not stated; IV: 20 mm^3; UVD: λ/nm: 265, 294, 325; CT: not stated	Retinol: 4.0 min; Retinyl acetate (IS): 6 min; D_2: 11.5 min; D_3: 12.4 min; α-tocopherol: 16.0 min
Extraction:	Sample was added into the mixture of absolute ethanol and 50 % KOH (φ_r = 4 : 1) → addition of AA → heating for 30 min at 80 °C under N_2 → repeated extraction with Et_2O → Et_2O fractions were combined and washed with demi water → drying with anhydrous Na_2SO_4 → evaporation → residue was redissolved in ethanolic solution of IS.				
LoD:	Retinol: 0.2 μg/100 g of food, α-tocopherol: 8.3 μg/100 g of food.				
[78]	Baby food products	Gynkotek ODS Hypersil, 5 μm, 250 × 4.6 mm, guard column, reduction column dry-filled with zinc powder 20 × 4.0 mm	A = CH_2Cl_2 B = MeOH C = Methanolic solution containing 1.37 g zinc chloride, 0.41 g sodium acetate and 0.30 g acetic acid Isocratically A : B : C (φ_r = 100 : 900 : 5)	FR: 1.0 cm^3 min^{-1}; IV: 100 mm^3; FLD: λ/nm: 243/430 (EmW/ExcW); CT: 40 °C	2′,3′-dihydrophylloquinone (IS); Phylloquinone: not stated
Extraction:	Extraction with mixture of CH_2Cl_2—MeOH (φ_r = 2 : 1) → filtration through dehydrated sodium sulfate → dilution with MeOH → addition of IS → evaporation → residue was redissolved in hexane → purification as follows: mixing with mixture of MeOH—water (φ_r = 9 : 1) → centrifugation → evaporation of hexane layer at 40 °C → residue was redissolved in MP.				
LoD:	Phylloquinone: 0.04 ng cm^{-3}.				
[77]	Oils and margarines	Vydac 201 TP54, 5 μm, 250 × 4.6 mm	A = 0.05 M-sodium acetate at pH 3 B = MeOH Isocratically: 5 % A, 95 % B	FR: 1.0 cm^3 min^{-1}; IV: 30 mm^3; Dual electrode ECD: in redox mode upstream electrode: −1.1 V, downstream electrode: 0 V; CT: not stated	Menaquinone-4 (IS): 6.7 min; Phylloquinone: 10.8 min; 2′,3′-dihydrovitamin K_1: 13.0 min
Extraction:	Dilution of the sample with hexane → addition of IS → shaking → standing for 30 min → evaporation of aliquot → residue was redissolved in hexane → filtration → purification of sample using semi-preparative straight-phase HPLC (column μPorasil, 5 μm, 300 × 3.9 mm) with hexane containing 1 % Et_2O as mobile phase at flow rate 1.5 cm^3/min. Collection time: 2 min before and 1.5 min after elution of vitamins. Collected fraction was evaporated and redissolved in 0.5 cm^3 of MP for analytical HPLC.				
[73]	Emulsified nutritional supplements	Inertsil ODS 80A, 5 μm, 150 × 3.0 mm	A = MeOH B = EtOH Isocratically: 50 % A, 50 % B	FR: 0.4 cm^3 min^{-1}; IV: 100 μl; FLD: λ/nm: 480/350 (EmW/ExcW); CT: 40 °C	Retinyl palmitate: 7.5 min
Extraction:	C2 cartridges were conditioned with MeOH and demi water prior to use → sample was dissolved in 20 mM-monosodium L-glutamate → application onto cartridge → rinsing with demi water → elution with EtOH.				
LoD:	Retinyl palmitate: 0.1 pg dm^{-3}.				
[16]	Infant formulas	Tracer Spherisorb ODS-2 C18, 5 μm, 250 × 4.6 mm and guard column	A = MeOH Isocratically: 100 % A	FR: 1.0 cm^3 min^{-1}, IV: not stated; UVD: λ/nm: 292, 325; CT: 50 °C	Retinol: 3.2 min; Retinyl acetate: 4.2 min; δ-tocopherol: 5.4 min; γ-tocopherol: 6.0 min; α-tocopherol: 6.8 min; α-tocopherol acetate: 8.8 min; Retinyl palmitate: 19.5 min
Extraction:	1st method: The sample was stirred with CH_2Cl_2—EtOH (φ_r = 2 : 1) under N_2 for 30 min at ambient temperature → filtration → addition of water → shaking → layers were allowed to separate → filtration of CH_2Cl_2 phase through anhydrous Na_2SO_4 → evaporation → residue was redissolved in Et_2O → filtration (to eliminate nonlipid substances) → evaporation → lipid residuum was redissolved in EtOH → addition of hexane → stirring → centrifugation → filtration of top organic layer. 2nd method: The sample was dissolved in absolute EtOH → stirring → addition of hexane → stirring → centrifugation → filtration of clear organic top layer.				
LoQ:	Retinyl acetate: 0.4 ng, δ-tocopherol: 25.3 ng, γ-tocopherol: 37.9 ng, α-tocopherol: 36.8 ng, α-tocopherol acetate: 6.3 ng.				

Table 2 (Continued)

Source	Sample matrix	Column used	Mobile phase, Elution	Other chromatographic conditions	Retention times
[80]	Animal feeds	Novapak C18, 150 × 3.9 mm	A = MeOH Isocratically: 100 % A	FR: 1.5 cm^3 min^{-1}; IV: 50 mm^3; UVD: λ/nm: 290; CT: not stated	All-*trans* retinol: 2.3 min; Cholecalciferol: 4.6 min; α-tocopherol: 5.7 min; Ergosterol: 7.7 min
Extraction:	Ground sample was extracted with mixture of acetone—chloroform (φ_r = 30 : 70) in extraction tube flushed with N_2 → shaking on a vortex mixer (1 min) → standing for 5 min → shaking again (1 min) → centrifugation → evaporation under N_2 → residue was redissolved in butanol.				
LoD:	All four quantified vitamins: ≈ 10 ng/g of feed sample.				
[20]	Egg yolk	Vydac 201 TP 54	A = MeOH B = Water Isocratically: 83 % A, 17 % B	1 cm^3 min^{-1}; IV: 25 mm^3; UVD: λ/nm: 264; CT: 25 °C	25-(OH)-D_3: 13.5 min; 25-(OH)-D_2 (IS): 15.5 min
Extraction:	Addition of IS into the sample → saponification → extraction → filtration → SPE purification on silica cartridge conditioned with hexane: application of sample → rinsing with hexane → elution of D_2 and D_3 with 0.5 % IPOH in hexane → elution of 25-(OH)-D_2 and 25-(OH)-D_3 with 6 % IPOH in hexane. 25-(OH)-D fraction was evaporated and clean-up was performed using semi-preparative HPLC using 10 μm (300 × 3.9 mm) μPorasil column and mixture of hexane and IPOH (φ_r = 97 : 3) as MP. Flow rate 1 cm^3/min, UVD at 264 nm, collection time: 1.5 min before 25-OH-D_2, 2 min after 25-OH-D_3.				
[50]	Yoghurt	OD-224 RP18, 5 μm, 220 × 4.6 mm and guard column RP18, 7 μm, 15 × 3.2 mm	A = 2.5 mM-acetic acid—sodium acetate in MeOH and water (φ_r = 99 : 1) Isocratically: 100 % A	FR: 1.0 cm^3 min^{-1}; IV: 10 mm^3; ECD: glassy carbon electrode at +1300 mV *vs.* Ag/AgCl electrode; UVD: λ/nm: 280 nm; CT: not stated	All-*trans* retinol: 4 min; Cholecalciferol: 8 min; α-tocopherol: 10 min
Extraction:	1st method (without hydrolysis): Sample was mixed with hexane and chloroform (φ_r = 2 : 1) at 1000 turns/min for 2 h and protected from light → centrifugation → separation of organic phase → evaporation at 50 °C → residue was redissolved in MeOH → clean-up on C18 cartridges → sample diluted with MeOH. 2nd method (with alkaline hydrolysis and without clean-up): sample was saponified overnight at ambient temperature with mixture of ethanol and 80 % aqueous KOH (φ_r = 50 : 15) with addition of AA → extraction with hexane → evaporation → residue was redissolved in MeOH → filtration.				
[52]	Emulsified nutritional supplements	Inertsil ODS-2, 5 μm, 150 × 4.6 mm and column RC-10 with platinum oxide catalyst 30 × 4 mm	A = MeOH B = EtOH Isocratically: 50 % A, 50 % B	FR: 0.6 cm^3 min^{-1}; IV: 100 mm^3; FLD: λ/nm: 430/320 (EmW/ExcW); CT: 40 °C; Post-column reduction on column RC-10	Phylloquinone: 8 min
Extraction:	The sample was dissolved in 5 % aqueous Na_2SO_4 solution containing 1 mM-EDTA.2Na → application onto conditioned C18 cartridge (with MeOH and H_2O) → rinsing with demi water and 10 % EtOH → elution with EtOH.				
LoD:	Phylloquinone: ≈ 0.1 pg.				
[72]	Emulsified nutritional supplements	Inertsil ODS 80A, 5 μm, 150 × 4.6 mm	A = MeOH B = EtOH Isocratically: 50 % A and 50 % B	FR: 0.6 cm^3 min^{-1}; IV: 100 mm^3; FLD: λ/nm: 480/350 (EmW/ExcW); CT: 40 °C	Retinyl acetate: 10 min
			A = ACN B = EtOH Isocratically: 70 % A, 30 % B	FR: 1.6 cm^3 min^{-1}; IV: 200 mm^3; UVD: λ/nm: 450; CT: 40 °C	β-carotene: 5.4 min
Extraction:	The sample was dissolved in 5 % aqueous sodium sulfate solution containing 1 mM-EDTA.2Na → application onto C18 cartridge conditioned with MeOH and demi water → rinsing with demi water and 10 % aqueous EtOH → elution with EtOH.				
LoD:	Both vitamins: ≈ 0.1 ng.				

Table 2 (Continued)

Source	Sample matrix	Column used	Mobile phase, Elution	Other chromatographic conditions	Retention times
[51]	Human plasma	Kromasil C1, 5 μm, 100 × 4.6 mm	A = MeOH B = ACN C = Water Isocratically: 50 % A, 35 % B, 15 % C	FR: 1.5 cm^3 min^{-1}; IV: 100 mm^3; UVD: λ/nm: 292; CT: ambient	α-tocopherol: 4.5 min
Extraction: Plasma was mixed with tungstate—magnesium chloride → addition of MeOH → mixing → centrifugation.					
[19]	Human plasma	J'sphere ODS-H80, 4 μm, 150 × 4.6 mm	A = ACN B = Water Isocratically: 70 % A, 30 % B	FR: 1 cm^3 min^{-1}; IV: not stated; UVD: λ/nm: 265; CT: 40 °C	25-(OH)-D_3: 17.3 min; 25-(OH)-ergosterol (IS): 23.5 min
Extraction: Addition of EtOH into plasma → mixing → centrifugation → addition of IS (ethanolic solution) and 0.2 M-KOH into supernatant → extraction with Et_2O → washing with 25 % MeOH → evaporation of organic layer in vacuum → residuum was redissolved in hexane—IPOH (φ_r = 98.5 : 1.5) and passed through silica gel column → rinsing with hexane—IPOH (φ_r = 98.5 : 1.5) → elution with hexane—IPOH (φ_r = 84 : 16) → evaporation to dryness → residue was redissolved in MeOH. LoD: 25-(OH)-D_3: 5 ng cm^{-3}.					
[18]	Plasma	Nucleosil C18, 5 μm, 300 × 7.5 mm	A = MeOH with 0.025 M-$HClO_4$ B = ACN with 0.025 M-$HClO_4$ Isocratically: 5 % A, 95 % B	FR: 1.2 cm^3 min^{-1}; IV: 50 mm^3 (fraction 25-(OH)-D), IV: 180 mm^3 (fraction 24,25-$(OH)_2$-D), Coulometric ECD: guard cell: + 0.65 V, analytical cell 1: + 0.20 V, analytical cell 2: + 0.60 V CT: not stated	24,25-$(OH)_2$-D_3: 18.5 min; 24,25-$(OH)_2$-D_2: 18.7 min; 25-(OH)-D_3: 23.4 min; 25-(OH)-D_2: 25.2 min
Extraction: Plasma was diluted with water and extracted with mixture of MeOH—CH_2Cl_2 → evaporation → residue was redissolved in 5.5 % IPOH in hexane and subjected to preparative HPLC: column Zorbax SIL (250 × 4.6 mm), mobile phase: 5.5 % IPOH in hexane, flow rate 1.5 cm^3/min. Collected fractions 25-(OH)-D and 24,25-$(OH)_2$-D were evaporated under vacuum and redissolved in 5 % MeOH in ACN. LoD: Both 24,25-$(OH)_2$-D_3 and 25-(OH)-D_3: ≈ 50 pg cm^{-3}.					
[81]	Plasma	Altech C18, 5 μm, 250 × 4.6 mm	A = MeOH with 0.1 % TEA B = EtOH with 0.1 % TEA Isocratically: 85 % A, 15 % B	FR: 1 cm^3 min^{-1}; IV: 20 mm^3; UVD: λ/nm: 245; CT: not stated	Retinoic acid: 1.9 min; Menadione: 3.2 min; Retinol: 4.2 min; Retinal: 5.8 min; D_2: 8.0 min; D_3: 8.6 min; K_2: 10.0 min; Phylloquinone: 16.4 min
Extraction: Plasma was mixed with 1 M-HCl → addition of hexane or CH_2Cl_2 or $CHCl_3$ (eventually addition of sodium dodecylsulfate or Triton X-100) → mixing → centrifugation. LoD: Retinoic acid: 1.5 ng, retinol: 1.0 ng, retinal: 1.0 ng, D_2: 5.0 ng, D_3: 5.0 ng, phylloquinone: 5.0 ng, K_2: 4.0 ng, menadione: 0.4 ng.					
[3]	Plasma	LiChrospher 100 RP-18, 5 μm, 125 × 4 mm and guard column	A = MEOH B = MEOH and water (φ_r = 99 : 1) C = MeOH and THF (φ_r = 7 : 3) Isocratically: 100 % A	FR: 1.5 cm^3 min^{-1} (LiChrospher) or 0.2 cm^3 min^{-1} (Spherisorb); IV: 5 mm^3; UVD: λ/nm: 250, 265, 284, 328; CT: 20 °C	D_3: 2.1 min; Tocopherol: 3.6 min; Phylloquinone: 5.4 min; Retinyl palmitate: 10 min
		Spherisorb ODS-2, 3 μm, 100 × 2.1 mm and guard column	Gradient program: 0 min: 100 % B 2 min: 100 % B 5.5 min: 95 % C 10 min: 100 % C Equilibration for 2 min		D_3: 4.0 min; Tocopherol: 5.3 min; Phylloquinone: 6.4 min; Retinyl palmitate: 8.0 min

Table 2 (Continued)

Source	Sample matrix	Column used	Mobile phase, Elution	Other chromatographic conditions	Retention times
Extraction:	EtOH was added into the sample → mixing → addition of hexane → mixing → organic layer was removed and extraction was repeated → extracts were combined → washing with mixture MeOH—water ($\varphi_r = 9 : 1$) → centrifugation → upper organic layer was separated and filtered → evaporation under N_2 → sample was redissolved in EtOH.				
LoD:	Normal-bore column: D_3: 19.8 ng, tocopherol: 106.3 ng, phylloquinone: 6.4 ng, retinyl palmitate: 11.8 ng. Narrow-bore column: D_3: 0.4 ng, tocopherol: 2.8 ng, phylloquinone: 0.4 ng, retinyl palmitate: 0.5 ng.				
[23]	Human serum	Shodex C18, 250 × 4.6 mm followed by column packed with platinum catalyst (100 × 4.6 mm), where VKs were reduced	A = EtOH containing 0.025 M-sodium perchlorate B = MeOH containing 0.025 M-sodium perchlorate Isocratically: 50 % A, 50 % B	Flow rate: 0.6 cm^3 min^{-1}; IV: 100 mm^3; ECD: glassy carbon electrode at + 0.6 V *vs.* Ag/AgCl electrode; CT: 40 °C	Vitamin K analogues: MK-4: 10 min; MK-5: 16 min; Phylloquinone: 17 min; MK-6: 20 min; MK-7: 27 min; MK-8: 38 min; MK-9: 50 min; MK-10: 70 min
Extraction:	Blood was centrifuged → mixture of EtOH and hexane ($\varphi_r = 1 : 4$) was added into the serum → mixing for 20 min → centrifugation → hexane layer was evaporated under N_2 at 45 °C → residue was redissolved in EtOH and loaded to Accubond ODS cartridge previously conditioned with water and MeOH → cartridge was rinsed with MeOH → vitamins K were eluted with mixture of MeOH—EtOH ($\varphi_r = 2 : 3$) → eluent was evaporated to dryness under N_2 at 45 °C and redissolved in MP.				
LoD:	All quantified vitamins in the range: 2—10 pg.				
[74]	Human plasma	Merck LiChrospher 100 RP-18, 5 μm, 250 × 4.0 mm	A = ACN B = MeOH C = Water Gradient program: 0 min: 90 % A, 4 % B, 6 % C 5 min: 90 % A, 4 % B, 6 % C 11 min: 40 % A, 60 % B 15 min: 40 % A, 60 % B Equilibration for 2 min.	FR: 1.5 cm^3 min^{-1}; IV: 100 mm^3; UVD: λ/nm: 267, 292; CT: 40 °C	Retinol: 4.0 min; 25-(OH)-D_3: 4.9 min; 25-(OH)-D_2: 5.3 min; 1α-hydroxyvitamin D_3 (IS): 7.4 min; α-tocopherol: 13.6 min; Tocopherol acetate: 14.8 min
Extraction:	Addition of IS and EtOH into the sample → mixing → extraction with hexane—CH_2Cl_2 ($\varphi_r = 90 : 10$) → centrifugation → hexane—CH_2Cl_2 layer was evaporated under N_2 and redissolved in ACN.				
LoD:	All quantified vitamins: ≤ 1.5 μg dm^{-3}.				
[75]	Human plasma	Two EC Nucleosil, 5 μm, 250 × 4.6 mm were coupled in series	A = Hexane B = IPOH Isocratically: A and B (659.34 : 0.786, w/w)	FR: 1.0 cm^3 min^{-1}; IV: 60 mm^3; UVD: λ/nm: 297; CT: ambient	α-tocopherol: 40.8 min; d_6-α-tocopherol: 42.5 min
Extraction:	Addition of 1.006 g cm^3 NaBr solution into the plasma → centrifugation → upper phase containing triacylglycerols was collected → addition of demi water, EtOH, deferoxamine mesylate and hexane → mixing → centrifugation → hexane phase was collected and water phase extracted again with mixture of hexane and BHT → hexane layers were combined → evaporation under N_2 → the residue was redissolved in mixture of hexane and BHT.				
LoQ:	d_6-α-tocopherol: 83 pmol cm^{-3}.				
[17]	Human serum	Inertsil Silica 100-5, 5 μm, 250 × 4.6 mm	A = hexane : IPOH : acetic acid ($\varphi_r = 1000 : 43 : 0.675$) B = hexane : IPOH : acetic acid ($\varphi_r = 1000 : 17.5 : 0.675$) Gradient program: 0 min: 100 % A, 0 % B 15 min: 100 % A, 0 % B 25 min: 0 % A, 100 % B 35 min: 0 % A, 100 % B 45 min: 100 % A, 0 % B 59 min: 100 % A, 0 % B	FR: 1.0 cm^3 min^{-1}; IV: 50 mm^3; UVD: λ/nm: 350; CT: 25 °C	13-*cis* retinal: 7 min; 9-*cis* retinal: 8.5 min; All-*trans* retinal: 10 min; 13-*cis* retinoic acid: 12.5 min; 9-*cis* retinoic acid: 14 min; All-*trans* retinoic acid: 15 min; Carotenoid (IS): 29 min; 13-*cis* retinol: 32.5 min; All-*trans* retinol: 37 min; All-*trans*-4-oxo-retinoic acid: 38.5 min; 9-*cis*-4-oxo-retinoic acid: 41 min; 13-*cis*-4-oxo-retinoic acid: 43.5 min
Extraction:	Addition of EtOH, 2 M-NaOH, IS, and hexane into the serum → shaking → centrifugation → upper organic layer (fraction A) and lower layer were collected separately → addition of 2 M-HCl and hexane into water layer → shaking → centrifugation → upper layer (fraction B) was collected → collected fractions A and B were evaporated under N_2 → residue was redissolved in hexane.				

acid-containing species by a single extraction procedure [5].

– As pantothenic acid is sensitive to high and low pH, acidic and alkaline hydrolyses are not useful for liberation of pantothenic acid from foods. This liberation is performed by enzyme treatment. A number of enzymes have been proposed: takadiastase, papain, mylase, clarase, alkaline phosphatase, pantetheinase and they are used alone or in association, for the determination of pantothenic acid in food [8]. The liberation of pantothenic acid from bound forms is always required for the total quantification of vitamin B_5 in foods by various techniques [8]. In the paper [9] an addition of acetic acid was found to be effective to achieve a protein and fat-free fraction suitable for direct injection and vitamin quantification.

– Pyridoxine is normally stable. Extraction can be performed using mineral acids or enzyme treatment [10, 37]. According to the paper [38] acidic hydrolysis at 121 °C for the duration of 30 min followed by enzymatic hydrolysis with acidic phosphatase and β-glucuronidase liberates the main forms of vitamin B_6. Possibilities of liberation of bonded vitamin B_6 from food are discussed also in paper [39].

– As it was already mentioned cyanocobalamin for its stability is used for food supplementation [14]. Cyanocobalamin was observed to be stable in a parenteral mixture for 4 d at 2—8 °C [34]. For extraction, for example 50 % methanol [40], or buffers [13] can be used.

– The presence of inorganic ions in vitamins tablets, particularly copper and iron salts, may cause oxidation or complex binding of folic acid into complex during extraction. Stability of folic acid in extracts from multivitamin/mineral preparations was significantly higher when a chelating agent (EDTA) was present [12]. The addition of EDTA to extraction buffer excludes the possibility of using a microbiological detection of folic acid [12] as a comparative method. Riboflavin is known to sensitize the photodegradation of folic acid resulting in the deactivation of the vitamin [41]. UV light converts folic acid into fluorescent material. When folic acid is irradiated with UV light it is first converted to pterine-6-carboxaldehyde and *p*-aminobenzoyl-L-glutamic acid. On further irradiation the aldehyde is converted to the corresponding pterine-6-carboxylic acid, which is fluorescent and finally to the decarboxylated 2-amino-4-hydroxypteridine. Daylight, pH, and heat have the most destructive effects on the solution of folic acid. At pH 7.6 folic acid solutions exhibit optimum stability [42]. Due to the nature of folic acid, SPE can be easily performed on cartridges with strong anion exchanger.

– Retinoids are thermolabile, photosensitive, and easily attacked by oxidants. This is mainly due to electron-rich polyene chain [24, 43, 44]. The electron-rich polyene chain in retinoids makes them extremely sensitive to light below 500 nm, oxygen, trace metals, strong acids, and excessive heat. If retinoids are exposed to daylight, extensive isomerization will occur within a short time. All sample treatment should therefore preferably be performed in amber containers under red or yellow light [24]. They are also labile towards strong acids. Anhydrous solvents containing even traces of acid cause structural changes of retinoids. Thus, use of strong acid should be avoided. Alkali usually is not harmful to retinoids. Vitamin A, which is strongly bound in the fat globules of human milk, is present primarily as retinyl esters [45]. As these fat globules are very stable, extreme conditions of saponification must be applied to assure the complete release of vitamin A from the linkage. In this process, the retinyl esters are hydrolyzed so that vitamin A is quantified as retinol using HPLC after extraction [45]. Purging the sample with argon gas [24] and the use of antioxidants is one of the most common strategies to prevent oxidation during the extraction and sample treatment, especially when the samples are saponified to obtain free carotenoids. Ethoxyquin, pyrogallol, ascorbic acid, and sodium ascorbate are examples of antioxidants used, but BHT is the most extensively used antioxidant. Normally BHT is used at 0.01 % or 0.1 % in the extraction solution [46, 24]. Care should be taken since BHT can interfere with some chromatographic systems. Samples to be analyzed the same day can be kept at 4 °C, otherwise they should be stored at −20 °C or lower temperatures [24].

– Individual studies have provided quite contradictory data about stability of vitamin D and it appears that the stability of this vitamin is strongly dependent on the processing technique used [47]. Losses of vitamin D during household cooking were < 10 % and hence, household cooking would not seem to lessen the intake of vitamin D from foods [47]. According to information of paper [48] general assumption is that the stability of vitamin D is high. Source [49] provides information that vitamin D is sensitive to alkaline pH, light, and heat. Saponification was proved to be necessary for extraction of vitamin D from yoghurt [50], while good recoveries were obtained for tested vitamins A and E (nearly comparable with results after saponification) using method with direct extraction.

– Vitamin E is photolabile and sensitive to heat and oxidation [30]. Also in this case, if saponification is necessary, a usage of antioxidants is recommended. According to source [5], both vitamins A and E in food could not be measured simultaneously, because the longer time required for saponification of retinyl palmitate destroys some of the α-tocopherol. Authors of [51] developed a method utilizing magnesium chloride and tungstate to avoid saponification and high temperatures, which normally affect stability of vitamin E in the presence of oxygen. Another approach of sample preparation for vitamins A and E is a usage of SFE as described in paper [30] for cosmetics. SFE

is emerging as a valuable alternative to conventional liquid extraction for the isolation of organic analytes from solid and semi-solid matrices.

– Vitamin K_1 is known to be unstable in the presence of metal ions. Addition of EDTA into sample solution increases stability of the vitamin [52]. Paper [53] reported that stability studies on the vitamin K_1 content of vegetable oils demonstrated that the vitamin was stable to heat and processing but it was rapidly destroyed by both fluorescence and sunlight.

5. HPLC QUANTIFICATION

RP HPLC *vs.* normal phase offers certain practical advantages, such as better column stability, reproducibility of retention times, and faster equilibration [29] and therefore it is preferably used.

Buffers, mostly KH_2PO_4 in the pH range 2—3 [32, 35], diluted acids [28, 54] or buffers with an addition of ion-pair reagent, namely alkylammonium salts or hydroxides [4, 33, 55, 56], sometimes with an addition of organic solvent, are used as mobile phases for evaluation of vitamin C. C_{18} sorbent is mainly used. It would be mentioned that AA isomers can be separated on silica-based aminopropyl columns, but the AA is not stable on these columns [5]. In contrast to AA, DHAA has a weak UV absorption and no response to electrochemical detection [4]. In order to increase the sensitivity for dehydroascorbic acid, derivatization prior to or after the chromatographic separation is necessary. Prior to HPLC, dehydroascorbic acid may be reduced to ascorbic acid by homocysteine, L-cysteine or dithiothreitol or derivatized with *o*-phenyldiamine to form the fluorophore [56, 57, 4]. Usually, DHAA is determined as the difference between the total AA, after DHAA reduction, and AA content of the original sample [57].

It is usually possible to chromatograph vitamins of B-group simultaneously, if those are present in sufficient concentrations (in particular cyanocobalamin). Some of B vitamin compounds well suit to ion-pair or ion-exchange chromatography (see below) due to their ionic nature. Consequently their retention can be increased by addition of ion-pair reagent. In contrary, riboflavin and cyanocobalamin are sufficiently retained on C_{18} sorbents. Mixtures of phosphate or acetate buffers with an addition of ion-pair reagent (salts of alkanesulfonic acids for B_1, B_3, B_6 and alkylammonium salts or hydroxides for FA) and of organic solvents like MeOH or ACN are used as mobile phases. Either isocratic [9, 10, 12, 13, 25, 37, 58—63, *etc.*] or gradient elution [1, 2, 6, 26, 27, 60, 64—66, *etc.*] is used. Usage of new stationary phases for basic compounds involving a ligand with amide groups (such as RP-AmideC_{16}) enables determination of B-group vitamins without necessity of addition of ion-pair reagent [1, 2]. UV detection is most common for vitamin B compounds. Cyanocobalamin is detected usually at $\lambda \approx 360$ nm, pantothenic acid, which has no significant chromophore, is detected at nonselective wavelength ≈ 200 nm with increasing risk of interferences from most organic substances. Due to weak retention of pantothenic acid, multiwavelength UV detection could be chosen for the unambiguous evaluation of putative pantothenate peak identity under chromatographic conditions used. Common wavelength about 270—280 nm is often used for determination of the rest of B vitamin compounds. The use of FLD increases the specificity and the sensitivity of the detection, but except pyridoxine, riboflavin [25], and cyanocobalamin [13], which show a natural fluorescence response, a post-column derivatization is necessary. The application of ion-pairing RP chromatography to determination of niacin in food products often requires complex clean-up procedures, like cartridge extraction and column switching [7].

MeOH, ACN, EtOH, THF, butanol or their mixtures (sometimes with small addition of water) in reversed-phase chromatography or hexane with addition of IPOH and/or acetic acid or 1,4-dioxane in normal-phase chromatography are used as mobile phases for quantification of vitamins A, D, E, and K.

Because of weak retention of carotenes on silica phases, RP chromatography with UVD is mainly applied for carotenoids [29—31, 43, 44, 64, 67—74, *etc.*]. On the other hand, normal-phase chromatography is required for retinoid analysis especially if *cis*- and *trans*-isomers of retinol have to be separated [15, 17, 75]. Retinoids have multiple carbon—carbon bonds in conjugation and therefore absorb UV light in the range $\lambda = 300$—400 nm. UVD of β-carotene is often at $\lambda \approx 450$ nm. Retinol and its esters display a pale green fluorescence when excited with near-UV light. The wavelength of excitation maxima is in the range $\lambda = 325$—335 nm and that of emission maxima in the range $\lambda = 470$—490 nm is only to some degree dependent on the solvent while the quantum yield is highly affected [24].

For the detection of vitamin D compounds either UV detection at $\lambda \approx 265$ nm [20, 76, *etc.*] or electrochemical detection is used [18, 50]; RP chromatography is mainly used. In case of vitamin D compounds, RP chromatography with UVD allows the use of vitamin D_2 as an internal standard for vitamin D_3 [15, 20]. Vitamin D_3 can be quantified at the same time after the fraction containing vitamins D_2 and D_3 is separated for example in the SPE step.

Even though normal-phase HPLC is not so robust as reversed-phase chromatography and it is not always sufficiently versatile for routine analysis, most reversed-phase systems are not able to separate the β- and γ-isomers of tocopherols and tocotrienols [21]. All four tocopherols and tocotrienols can be separated and quantified by normal-phase chromatography with silica stationary phases and FLD [15, 21]. Normal-phase systems are only suitable for the direct analy-

sis of cooking oils and fats, since apolar normal-phase eluents are good solvents for these samples [22]. UVD at $\lambda \approx 280$—290 nm or FLD is used for detection of tocopherols.

ECD of vitamin K can be performed after reduction on platinum catalyst [23] or using dual electrochemical detector [77], fluorescence detection after reduction on platinum catalyst [52] or zinc catalyst [78]. UVD is not frequently used; wavelength 280 nm is used in paper [70] for evaluation of vitamin K_1 in milk. Paper [23] recommends preferably to use ECD due to its higher sensitivity and selectivity in comparison with FLD or UVD.

Further information about HPLC quantification and also sample preparation can be found in Tables 1 and 2, which provides brief view of developed and published methods.

6. CONCLUSION

This paper shows that analytical evaluation of vitamins is continually living topic. Although there is on-going progress in analytical chemistry, the chromatographic determination of vitamins in biosamples still belongs among the most challenging tasks due to the complexity of sample matrices, low concentrations of vitamins, and low stability of some of them. There is not universal method for determination of WSV or FSV in all sample matrices; for each sample or group of samples a new or at least slightly modified current method should be developed, to be selective, robust, and accurate for given case. Careful optimization of extraction conditions and separation, and selection of suitable detection are basics of success.

For simultaneous quantification of several WSV in multivitamin (pharmaceutical) preparations methods described in papers [59, 60] can be recommended, because they reach ≈ 100 % recovery for all quantified vitamins. Simultaneous quantification of vitamins A and E can be successfully performed using methods [44] or [64], because they also demonstrated recoveries ≈ 100 %. Paper [64] deals also with vitamin D_3 determination, however, the recovery for this vitamin was found about 78 %. On the other hand, it should be mentioned that quantitative vitamin D analysis can represent a complex problem.

In case of more complex matrices, authors in their work are usually interested in quantification of one or a small number of vitamins in order to develop an accurate method. As an example of accurate method, where several WSV in complex matrices are quantified, can serve paper [2]. Reached recoveries of individual vitamins were higher than 95 %. Other works, for example [66, 25], which also simultaneously quantify number of WSV showed lower recoveries. Recoveries of methods [70] and [3], for simultaneous FSV quantification, were about 89—107 % and 89—100 %, respectively.

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