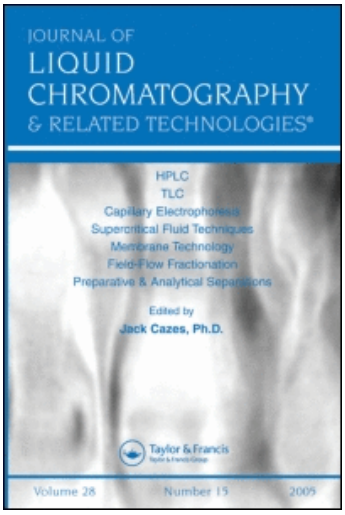

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Thin Layer Chromatography for the Analysis of Vitamins and Their Derivatives

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Thin Layer Chromatography for the Analysis of Vitamins and Their Derivatives

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Abstract: Vitamins are compounds with biological activity and they are essential and fundamental for normal health and organisms growth. Vitamins are available in diet and supplements and their deficiency can lead to some human diseases. The analysis of these compounds is indispensable for monitoring their content in pharmaceuticals and food in order to prevent some human diseases.

Thin layer chromatography (TLC) is generally regarded as a common analytical method for screening, separation, and preliminary identification of compounds. TLC plays an important role in the quality control of food and drugs in order to investigate the ingredients and to detect the impurities and also for checking the purity and the stability of preparations.

Keywords: Thin layer chromatography (TLC), Hydrophilic vitamins, Lipophilic vitamins

INTRODUCTION

Thin layer chromatography (TLC) is generally regarded as a common analytical method for screening, separation, and preliminary identification of compounds. TLC and also high performance thin layer chromatography (HPTLC) are chosen when many samples have to be compared, when flexibility is of importance, and when rapid qualitative, semi-quantitative and quantitative data are needed at low costs. Another advantage of TLC and HPTLC is the possibility of coupling them with more selective detection

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techniques such as Raman spectroscopy, infrared spectroscopy (IR), mass spectrometry (MS), etc.

TLC plays an important role in the quality control of food and drugs in order to investigate the ingredients and to detect the impurities and, also, for checking the purity and the stability of preparations.

Vitamins are compounds with biological activity and they are essential and fundamental for normal health and organisms growth. Vitamins are available in diet and supplements, and their deficiency can lead to some human diseases. Vitamins are ranked in two groups according to their solubility in water and in fats. One group is the hydrophilic vitamins, which are water-soluble, and the second group is the lipophilic vitamins, which are fat-soluble. Determination of vitamins is still an important problem in clinical and food analysis. TLC and HPTLC have been used alone, or in combination, with gas chromatography (GC) or high performance liquid chromatography (HPLC) as an assay for analysis of samples containing one or more vitamins.[1,2]

HYDROPHILIC VITAMINS

The group of hydrophilic vitamins consists of vitamin C, B complex vitamins (B_1, B_2, B_3, B_5, B_6, B_9, B_{12}), and vitamin H (Figure 1). An important characteristic of water-soluble vitamins is that they are not stored in organisms. They pass through the body by the bloodstream more quickly than the lipophilic vitamins, and are excreted in the urine. This behavior explains why the deficiency of these vitamins occurs rapidly. Excessive amounts of hydrophilic vitamins usually have no toxic effects because their excretion increases with the increasing of the ingested quantities. Therefore, a common method for analyzing all hydrophilic vitamins and their metabolites is required.

Different methods for the analysis of water-soluble vitamins and their metabolites are reported in literature.[3–6] Most of these methods are complicated and limited to the analysis of an individual vitamin and its degradation compounds. There are just a few papers that report the analysis in parallel of almost all hydrophilic vitamins.[7–9]

Vitamin C

Vitamin C, also known as ascorbic acid, L-ascorbic acid, dehydroascorbic acid, the antiscorbutic vitamin, L-xyloascorbic acid, and L-threo-hex-2-uronic acidy-lactone, is largely used to cure or to prevent many diseases. It cannot be produced by the body, and needs to be ingested. Good sources of vitamin C are green leafy vegetables, berries, citrus fruits, guavas, tomatoes, melons, papayas, etc. The body needs vitamin C for synthesis of collagen in connective tissue, neurotransmitters, steroid hormones, carnitine, conversion of cholesterol to bile acids, and enhancing iron bioavailability.

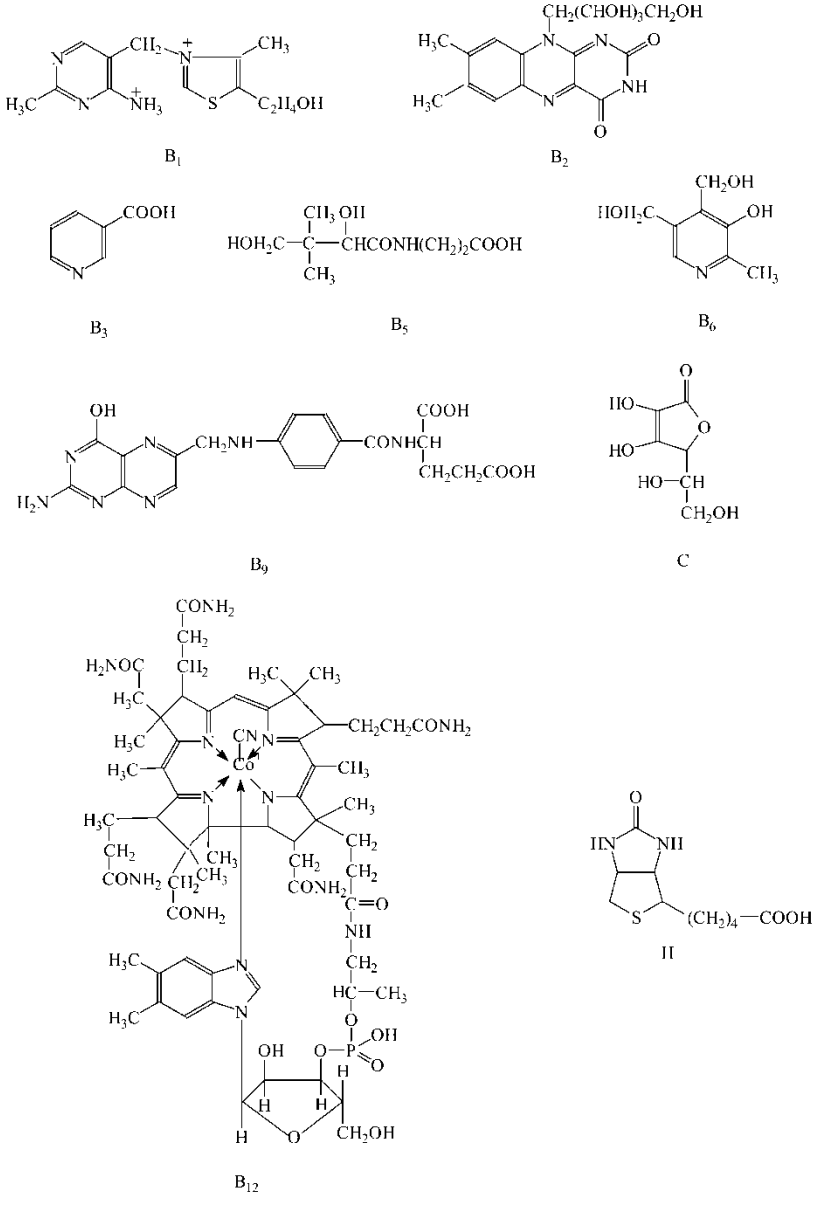


Figure 1. The structures of hydrophilic vitamins.

Ascorbic acid is a great antioxidant and helps protect the body against pollutants. Being a biological reducing agent, it is used for prevention of degenerative diseases, cardiovascular diseases, and cancers. Also, it enhances the immunity system. The deficiency of vitamin C causes various problems:

scurvy, "pinpoint" hemorrhages under the skin, poor wound healing, soft and spongy bleeding gums and loose teeth, edema, a lack of energy, poor digestion, painful joints, and bronchial infection and colds. To enhance the antioxidant properties, it is be best to take it with the other anti-oxidants, bioflavonoids, calcium, and magnesium. Vitamin C is destroyed or depleted in conditions such as: air, heat, water, prolonged storage, overcooking and processing, antacids, alcohol, antidepressants, birth control pills, and steroids.[10]

Vitamin B_1

Vitamin B_1, also called thiamin, is found in sunflower seeds, peanuts, wheat bran, beef liver, pork, seafood, egg-yolk, and beans. Vitamin B_1 enhances circulation, helps with blood formation and the metabolism of carbohydrates, helps growth in children, and assists in arthritis, infertility, depression, memory, and learning problems. It is used by the nervous system in the biosynthesis of neurotransmitter acetylcholine and gamma-amino butyric acid (GABA). It is also used in the manufacture of hydrochloric acid and, therefore, plays a role in digestion. Thiamin is used for treatment of beriberi disease. Because a very small amount of this vitamin is stored in the body, and depletion of this vitamin can happen within 14 days, a supplement may be necessary. A deficiency will result in beriberi, and minor deficiencies may be indicated by extreme fatigue, irritability, constipation, edema and an enlarged liver, forgetfulness, gastrointestinal disturbances, heart changes, irritability, labored breathing, and loss of appetite. Thiamin should be taken with the B group of vitamins and manganese. Thiamin is destroyed by cooking, and intake may be low if the diet is high in refined foods.

Vitamin B_2

Vitamin B_2 (riboflavin) is manufactured in the body by the intestinal flora and is easily absorbed, although very small quantities are stored, so there is a constant need for this vitamin. It can be found in organ meats, nuts, cheese, eggs, milk, lean meat, in green leafy vegetables, fish, legumes, whole grains, and yogurt. Vitamin B_2 is sensitive to light. In the body it is required in order to use oxygen in the metabolism of amino acids, fatty acids, and carbohydrates, to activate vitamin B_6 (pyridoxine), to create niacin, for red blood cell formation, antibody production, cell respiration, growth, and assists the adrenal gland. Vitamin B_2 also helps the absorption of iron and vitamin B_6 and may be helpful in the prevention and treatment of cataracts, and is most beneficial to the skin, hair, and nails. Deficiency manifests as cracks and sores at the corners of the mouth, eye disorders, inflammation of the mouth and tongue, skin lesions, hair loss, insomnia, light sensitivity, poor digestion, retarded growth, slow mental responses, and burning feet. Riboflavin is best taken with B group vitamins and vitamin C.

Vitamin B_3

Vitamin B_3 (niacin) can be manufactured by the body from two compounds, nicotinic acid and niacinamide. It can be found in liver, lean meat, poultry, fish, rabbit, nuts, peanut yeast, liver, cereals, vegetables, asparagus, seeds, milk, green leafy vegetables, fish, and coffee. Niacin is lost when food is cooked in water. Vitamin B_3 is required for cell respiration, in the release of energy, and for metabolism of carbohydrates, fats, and proteins, and for proper circulation and healthy skin. It is also useful in the nervous system, normal secretion of bile and stomach fluids, in the synthesis of sex hormones, for treating schizophrenia and other mental illnesses, and to improve the blood cholesterol profile. A deficiency may cause pellagra disease, canker sores, depression, diarrhea, dizziness, fatigue, halitosis, headaches, indigestion, insomnia, limb pains, loss of appetite, low blood sugar, muscular weakness, skin eruptions, and inflammation. Niacin is best taken with the B group vitamins and vitamin C. Large doses may produce hyperuricemia, hepatic abnormalities, flushing by dilating the blood vessels and hypopression, itching, elevated blood glucose, and peptic ulcers.

Vitamin B_5

Vitamin B_5, also known as pantothenic acid, can be manufactured in the body by the intestinal flora, and is found in beef, brewer's yeast, eggs, fresh vegetables, kidney, legumes, liver, mushrooms, nuts, pork, royal jelly, saltwater fish, torula yeast, whole rye flour, and whole wheat. Pantothenic acid can be lost during cooking by roasting or milling, by acids or alkali. Vitamin B_5 plays an important role in the secretion of hormones, such as cortisone, assisting the metabolism, helps to fight allergies, is beneficial in the maintenance of healthy skin, muscles, and nerves, is used in biosynthesis of lipids, neurotransmitters, steroid hormones, and hemoglobin. Vitamin B_5 deficiency manifests as fatigue, headaches, nausea, tingling in the hands, depression, personality changes, cardiac instability, frequent infection, abdominal pains, sleep disturbances and neurological disorders, muscle weakness and cramps. It is most effective when taken with the B group vitamins, vitamin A, vitamin C and vitamin E.

Vitamin B_6

Vitamin B_6, also known as pyridoxine, is found in brewer's yeast, eggs, chicken, carrots, fish, liver, kidneys, peas, wheat germ, and walnuts. Pyridoxine is sensitive to sunlight and cooking. Exercising may aid the production of the active form of vitamin B_6. Pyridoxine is important for the manufacture of vitamin B_3, in hormonal balance, good functioning of the immunity system,

for growth of new cells, in metabolism of proteins, fats and carbohydrates, controlling moods, assists learning difficulties, dandruff, eczema, and psoriasis. It is involved in the balancing of sodium and potassium, and promotes red blood cell production. It is important in the synthesis of nucleic acids RNA and DNA. Deficiency manifests as irritability, insomnia, weakness, skin lesions, promoting asthma, allergies, and kidney stones, and causes osteoporosis and arthritis. Vitamin B_6 should be taken together with the entire B group vitamins. Intake over 2 g per day may cause neurological damage.

Vitamin B_9

Vitamin B_9 (folic acid, folacin, folate, or pteroylglutamic acid) can be manufactured by the body and be stored in the liver, but is found in fresh green vegetables, such as spinach and broccoli, fruit, starchy vegetables, beans, whole grains, and liver. Light, heat, and storage for extended periods can destroy this vitamin. Folic acid is required for DNA synthesis and cell growth, for red blood cell formation, energy production, forming of amino acids, assisting in digestion and the nervous system, and works at improving mental as well as emotional health. A deficiency of folic acid on an unborn baby may increase the risk of developing spina bifida and other serious defects of the nervous system. The deficiency manifests as fatigue, acne, a sore tongue, keillitis, and long term deficiency may result in anemia and later in osteoporosis, and promotes cancer of the bowel. The action of vitamin B_9 is more effective if is taken with vitamin B_{12}, B_6, and C, and is mandatory in pregnant women. Vitamin B_9 influences the activity of antiepileptic drugs. Too much folic acid may mask vitamin B_{12} efficiency. Regular high intake of folic acid may cause digestive upsets, energy loss, and insomnia.

Vitamin B_{12}

Vitamin B_{12}, known as cyanocobalamin or cobolamin, is present in liver, organ meat, fresh milk, muscle meat, shellfish, eggs, cheese, fish, and can be manufactured in the body. It can be destroyed by excessive and chronic alcohol intake. Unlike other water-soluble vitamins that were absorbed nearly immediately, B_{12} needs about 3 hours. Cobolamin is needed in the manufacture and maintenance of red blood cells, to stimulate appetite, promoting growth, release of energy, and assisting in the metabolism of fats, proteins, and carbohydrates. It helps in prevention of mental deterioration and provides protection against allergies and cancer. Symptoms of a deficiency include a sore tongue, weakness, fatigue, weight loss, back pain, apathy, decreased reflexes, and neurological signs. The deficiencies may lead to Alzheimer disease,

pernicious anemia, and promoting digestive and head and neck cancers. Vitamin B_{12} is best to be used with iron, calcium, sodium, potassium, and vitamin C. A therapeutic dose is indicated in pernicious anemia, alcoholism, after surgery for bowel or stomach disease, in older people, and vegetarian diets.

Vitamin H

Vitamin H (biotin) is present in cheese, beef liver, cauliflower, eggs, mushrooms, chicken breasts, salmon, spinach, brewer's yeast, nuts, and can be manufactured in the body. Biotin is not easily destroyed, but avidin from raw eggs binds biotin, making its absorption impossible. Vitamin H is used in cell growth, in the production of fatty acids, in the metabolism of fats, and proteins, plays a role in the Krebs cycle, and is involved in maintenance of a steady blood sugar level, a good health of and skin, sweat glands, nerve tissue, and bone marrow, and assisting with muscle pain. Deficiency occurs very rarely and may result in dry scaly skin, fatigue, loss of appetite, nausea and vomiting, mental depression, tongue inflammation, and high cholesterol. Biotin should be taken with group vitamins B and vitamin C. The dose can be increased in long term users of antibiotics, who may also have to look at their biotin levels.

TLC and HPTLC, using different stationary and mobile phases, were successfully applied in the analysis of water-soluble vitamins and their metabolites from standards, and different pharmaceutical and biological samples (Table 1). Because hydrophilic vitamins have different chemical characteristics, the most difficult problem in the analysis, in parallel, of all these compounds is to find an optimum mobile phase. Moreover, in order to ensure accurate identification of water-soluble vitamins and their degradation compounds from standards and biological samples, selective spray reagents should be used after chromatographic separation.

LIPOPHILIC VITAMINS

Lipophilic vitamins are vitamins A, E, D, and K and they are soluble in fats. Lipophilic vitamins posses important biological properties and for this reason they have been the subject of wide investigation. Lipophilic vitamins can be stored in the body, especially in the liver. When required by a particular part of the body, the liver releases some fat-soluble vitamins, which are carried by the blood and delivered to the target cells and tissues.

The main techniques used for the analysis are the TLC, HPLC, and GC.[1] The application of TLC could solve many problems regarding the identification and determination of vitamins, related compounds, and their metabolites in various samples.[27]

Table 1. The chromatographic conditions for the separation of hydrophilic vitamins

Compounds	Stationary phase	Mobile phase	Detection	Sample	Ref.
Vitamin B_1, B_2, B_3, B_5 (as calcium salt), B_6, B_9, B_{12}, C, H	OPLC silica gel	n-Butanol-pyridine-water 50:35:15, v/v/v; rate-0.25 mL/min	UV light - vitamin B_1, B_2, B_3, B_6, B_9, C ninhydrin reagent – vitamin B_5 4-Dimethylaminocinnam aldehyde- vitamin B_{12}, H	Multivitamin pharmaceutical preparations	[11]
Vitamin B_1, B_6, B_{12}, C	Silica gel	Water	UV light	"Kombucha" drink	[12]
VitaminB_1, B_2, B_6, B_9, B_{12}	Silica gel impregnated with Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+}, or Mg^{2+} (0.1%,0.2%, 0.3%, 0.4% of each ion)	n-Propanol-n-butanol – water-ammonia 7:5:1:2, v/v/v/v n-propanol-n-butanol-water-ammonia 7:5:1:1.5, v/v/v/v n-propanol-n-butanol-water-ammonia 7:5:0.75:2, v/v/v/v	Iodine vapours	Standards	[13]
Vitamin B_1, B_2, B_3, B_5 (as calcium salt), B_6, B_9, B_{12}, C	Silica gel $60F_{254}$ HPTLC	Methanol-benzene programmed multiple development	UV light, ninhydrin reagent – vitamin B_5 Raman spectroscopy-vitamin B_3, B_6	Standards	[8]
Vitamin B_1, B_2, B_3, B_5 (as calcium salt), B_6, B_9, B_{12}, C	Fluorescent silica gel	Benzene-methanol-acetic acid-acetone 14:4:1:1, v/v/v/v	UV light ninhydrin reagent – vitamin B_5	Standards	[9]

Vitamin B_1, B_2, B_3, B_6, B_9, B_{12}, C	Silica gel 60 HPTLC with pre-adsorbent sample application zone (Whatman) silica gel 60 CF_{254} HPTLC with pre-adsorbent sample application zone (Merck)	1-Butanol-chloroform-acetic acid-ammonia-water 7:4:5:1:1, v/v/v/v/v Benzene-methanol-acetone-acetic acid 70:20:5:5, v/v/v/v Toluene-methanol-acetone-acetic acid 70:20:5:5, v/v/v/v Chloroform-ethanol-acetone-ammonia 2:2:2:1, v/v/v/v	UV-VIS light 0.5% solution in ether of iodine-Dragendorff reagent – vitamin B_1 1% methanolic solution of 1-chloro-2,4-dinitrofenzene and 3M NaOH solution – vitamin B_3, B_6 0.4% solution of 2,6-dichloroquinone-4-chloroimide – vitamin B_6	*H. trivolvis* snails	[14]
	Amino-bonded silica gel $60CF_{254}$ HPTLC (Merck)	Acetonitrile-water 70:30, v/v			
	Chemically bonded RPTLC with pre-adsorbent zone (Whatman) chemically bonded reversed phase RP-$18F_{254}$s HPTLC with pre-adsorbent zone (Merck)	25%methanol-borate buffer(5 mM, pH 7.0)-acetonitrile 14:2:2, v/v/v			
Vitamin B_1, B_6, B_{12}	Silica gel	Methanol-water 19:1, v/v	VIS	Pharmaceuticals	[5]
Vitamin B_1(thiochrome), B_2, B_3 (labeled with fluoresceinamine)	HPTLC silica gel	Methanol-water 70:30, v/v	Fiber-optic fluorimetry	Food	[15]

(*continued*)

Table 1. Continued

Compounds	Stationary phase	Mobile phase	Detection	Sample	Ref.
Vitamin B_1	HPTLC silica gel	Methanol-NH_3 (25%)-acetic acid-chloroform 18:2:1:1, v/v/v/v	Tert-butyl hypochlorite or $K_3[Fe(CN)_6]$-NaOH, after fluorescent enhancement dipping the plate in chloroform-liquid paraffin-triethanolamine 6:1:1, v/v/v	Pharmaceutical products	[16]
Vitamin B_2, FAD, FMN, riboflavin-β-galactoside, 7α-hydroxyriboflavin, 10-hydroxyethylflavin, 10-formylmethylflavin	Silica gel	1-Butanol-glacial acetic acid-water 2:1:1, v/v/v 1-Butanol-acetic acid-water 5:2:3, v/v/v Chloroform-methanol-ethyl acetate 5:5:2, v/v/v 1-Butanol-benzyl alcohol-glacial acetic acid 8:4:3, v/v/v 1-Butanol-ethanol-water 10:3:7, v/v/v Isoamyl alcohol-ethyl methyl ketone-glacial acetic acid-water 40:40:7:13, v/v/v/v 1-Butanol: isopropanol:water:glacial acetic acid 30:50:10:2, v/v/v/v Ethyl methyl ketone:acetic acid:methanol 3:1:1, v/v/v	UV light	Plain yogurt	[3]

Vitamin B_2, FAD, FMN, 4′, 5′-FMN	Silicagel	1-Butanol-glacial acetic acid-water 2:1:1, v/v/v 1-Butanol-acetic acid water 5:2:3, v/v/v 5% $NaHPO_4 \cdot 12H_2O$ 1-Butanol-formic acid-water-diethyl ether 77:10:13:15, v/v/v/v Chloroform-methanol-ethyl acetate 5:5:2, v/v/v 1-Butanol-benzyl alcohol-glacial acetic acid 8:4:3, v/v/v 1-Butanol-ethanol-water 10:3:7, v/v/v	UV light	Raw egg white, egg powder	[3]
	Cellulose	1-Butanol-glacial acetic acid-water 2:1:1, v/v/v 1-butanol-acetic acid-water 5:2:3, v/v/v collidine-water 3:1, v/v			
Nicotinic acid, nicotinamide, $NADP^+$, NAD^+, nicotinic acidmononucleotide, nicotinic acid adenine dinucleotide, nicotinamide mononucleotide	MN 300G cellulose	1M Ammonium acetate-95% ethanol 3:7, v/v (pH 5.0) 2-butyric acid-ammonia-water 66:1.7:33, v/v/v 600 g ammonium sulfate in 0.1M sodium phosphate	UV light	Urine	[1]
	Silica gel $60F_{254}$	2-Propanol-conc. HCl-water 70:15:15, v/v/v			

(*continued*)

Table 1. Continued

Compounds	Stationary phase	Mobile phase	Detection	Sample	Ref.
Vitamin B_3, niacinamide	Silica gel	Methanol-benzene-acetone-glacial acetic acid 20:7:5:5, v/v/v/v water	UV light	Pharmaceutical preparation	[17], [18]
Vitamin B_5, panthenol	Silica gel 60	2-Propanol-water 85:15, v/v	Ninhydrin reagent, spectrodensitometry	Pharmaceutical preparation	[19]
Vitamin B_6	Silica gel 60	Chloroform-methanol 3:1, v/v	*Saccharomyces carlsbergensis* ATCC 9080	reaction mixture (biosynthetic pathway of vitamin B_6 in *Rhizobium meliloti*)	[20]
Vitamin B_6, pyridoxal, pyridoxamine, pyridoxal ethil acetal, 4-pyridoxic acid, 4-pyridoxic acid lactone, pyridoxol phosphate, pyridoxal phosphate, pyridoxamine phosphate, pyridoxal phosphate phenylhydrazone	Silica gel HF_{254}	0.2% NH_4OH in water (1:139, v/v conc NH_4OH-H_2O) chloroform-methanol 75:25, v/v ethyl methyl ketone-ethanol-conc. NH_4OH-water 15:5:5:5, v/v/v/v	UV light Giggs reagent diazotized p-nitroaniline	Standards	[21]

	Silica gel HF_{254} treated with H_3BO_3	Chloroform-methanol 75:25, v/v			
	Cellulose MN 300G	1-Butanol saturated with 1 N HCl, upper layer 1-butanol-conc. HCl-water 25:5:10, v/v/v 1-butanol-acetic acid-water 15:10:10, v/v/v			
	Cellulose	1-Butanol saturated with 1 N HCl, upper layer Methanol-1-Butanol-benzene-water-triethylamine 20:10:10:10:5, v/v/v/v/v			
Vitamin B_{12}, degradation compounds of vitamin B_{12}	Silica gel	1-Butanol-2-propanol-water 10:7:10, v/v/v 2-Propanol-30% NH_3-water 7:1:2, v/v/v	UV light	food	[4]
Vitamin B_{12}, vitamin B_{12} derivatives (OH-B_{12}, CN-B_{12}, Ado-B_{12}, Me-B_{12})	Silica gel	1-butanol-2-propanol-water 10:7:10, v/v/v	UV light	Food	[22]
Vitamin C	Silica gel	Benzene-acetone-pyridine 80:12:8, v/v/v	UV light	Food	[23]

(*continued*)

Table 1. Continued

Compounds	Stationary phase	Mobile phase	Detection	Sample	Ref.
Vitamin C isomers, dehydroascorbic acid isomers	Silica gel, silica gel impregnated by spraying with 3% sodium borate solution cellulose, cellulose impregnated by spraying with 3% sodium borate solution silica gel, cellulose, borate plates, impregnated with silicon oil (5% solution in diethyl ether) (RP)	Acetonitrile-acetone-water-acetic acid 80:5:15:2, v/v/v/v Acetonitrile-butylnitrile-water-acetic acid 66:33:15:2, v/v/v/v	UV light	Food products, pharmaceutical preparation, fluids, biological tissues	[24]
Vitamin H, vitamin H metabolites	Microcellulose	1-Butanol-acetic acid-water 4:1:1, v/v/v 1-Butanol	UV light	Urine	[25]
Vitamin H	Silica gel	Ethyl acetate-ethanol 3:2, v/v	1% $K_4[Fe(CN)_6]$ solution, iodine vapours	Standard	[26]

Vitamin A

The dietary form of vitamin A is a yellow fat-soluble antioxidant vitamin, which is important in vision, particularly night vision, normal bone and tooth growth, reproduction, and the health of skin and mocous membranes. Vitamin A acts in the body as an antioxidant and may reduce the risk of cancers. The physiological forms of vitamin A include chemical compounds known as retinoids and their esters: retinol (vitamin A_1), 3-dehydroretinol (vitamin A_2), retinal (vitamin A aldehyde), 3-dehydroretinal, retinoic acid, neovitamin A, and neo-b-vitamin A. Also, carotenoids such as α-,β-, and γ-carotene, neo-β-carotene B, cryptoxanthine, myxoxanthine, aphanicin, echinenone, and torularhodin, are active analogs compound known as vitamin A.[28] The structures of vitamin A and analogous compounds are presented in Figure 2. The *cis* isomers are less stable and can readily convert to the all-*trans* configuration.

Vitamin A is ingested in a precursor form, also known as provitamins, namely retinyl esters in animal sources (milk, eggs, butter, cheese, liver from cod, shark, beef, pork, chicken, turkey) and carotenoids in plant sources (carrots, spinach, pumpkin, sweet peppers, apricots, mango, cantaloupe melon, sweet potatoes). Many carotenoids, around 500 known compounds, can be converted to vitamin A, but the most well known is β-carotene (Figure 2).

The deficiency of vitamin A leads to night blindness, keratomalacia, pale and dry skin. Vitamin A deficiency also diminishes the ability to fight infections. The symptoms of vitamin A excess are liver toxicity, hair loss, osteoporosis, and teratological effects. Moreover, vitamin A is susceptible to oxidation and degradation. Taking into account these characteristics, the

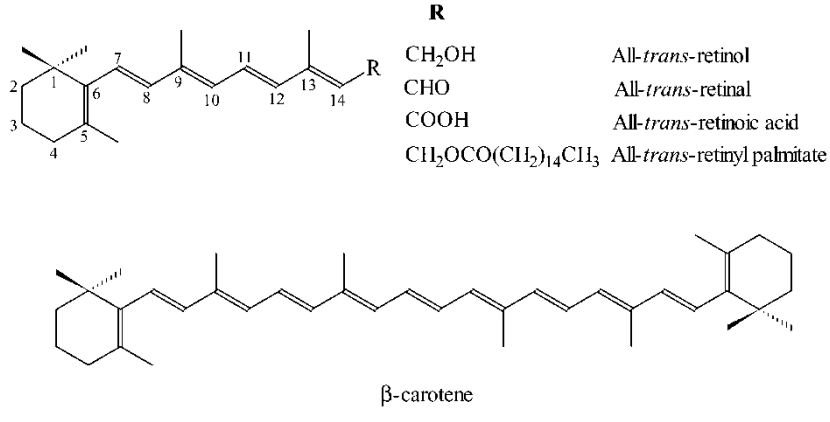


Figure 2. The structures of vitamin A, related compounds and β-carotene.

control of the vitamin A level is very important and recommended. Therefore, many applications of TLC in the analysis of vitamin A and related compounds in various samples are reported (Table 2).

Vitamin E

Vitamin E is a fat-soluble vitamin with important antioxidant properties. Vitamin E exists in nature in eight different forms or isomers, four tocopherols (Figure 3) and four tocotrienols. Tocopherols have important medical, biological, and physicochemical properties.[29] Each tocopherol form has its own activity, the measure of potency or functional use in the body, but α-tocopherol is traditionally recognized as the most active form of vitamin E in humans. Moreover, the antioxidant activity of tocopherols decreases in the order α-,β-,δ-,γ-tocopherol. The sources of vitamin E are vegetable oils (palm oil, sunflower, canola, corn, soybean, and olive oil), vegetables (nuts, sunflower seeds, seabuckthorn berries, grains), and wheat germ.[30] Vitamin E deficiency is usually characterized by neurological problems due to poor nerve conduction. Vitamin E prevents the formation of blood clots that could lead to a heart attack and prevent or delay coronary disease by limiting the oxidation of LDL-cholesterol. Vitamin E also may block the formation of nitrosamines, the carcinogens formed in the stomach from nitrites consumed in the diet. The overall wisdom of vitamin E supplementation has been increasingly questioned in recent years, and the analysis found that the vitamin E excess was associated with an all cause mortality risk.[31]

The separation of vitamin E isomers and the related compounds is treated in many papers and reviews.[29,32] It can be seen from these papers that, TLC still remains a useful separation technique. TLC was used for basic investigation of vitamin E and its analogs, for the separation of vitamin E and its derivatives from biological materials, even if is single or in mixtures with other vitamins (Table 2).

Vitamin D

The active compounds known as D vitamins are vitamin D_2 (calciferol, ergocalciferol), vitamin D_3 (cholecalciferol), vitamin D_4 (22,23-dihydroergosterol), vitamin D_5 (7-dehydrositosterol), vitamin D_6 (2-dehydrostigmasterol). The vitamin D_2, vitamin D_3, 25-hydroxycholecalciferol [$25(OH)D_3$], and 1,25-dihydroxycholecalciferol [$1,25(OH)_2D_3$] are the physiological forms (Figure 4). The most active form of the vitamin is 1,25 dihydroxy vitamin D_3 (calcitriol), a potent hormone. The most important role of vitamin D is maintaining blood levels of calcium and phosphorus, which it accomplishes by increasing absorption of these elements from food and reducing urinary

Table 2. The chromatographic conditions for the separation of lipophilic vitamins

Compounds	Stationary phase	Mobile phase	Detection	Sample	Ref.
All-*trans* and 13-*cis* retinoic acids	HPTLC silica gel	Diethyl ether-cyclohexane-acetone-glacial acetic acid 40:60:2:1, v/v	Concentrated H_2SO_4 (blue spots)	Gel and cream formulation	[39]
syn and *anti* isomers of all-*trans*, 9-*cis* and 11-*cis*-retinaloximes	TLC silica gel	Cyclohexane-benzene-ethyl acetate 5:3:2, v/v	UV light	Standards	[40]
Retinyl acetate oxidation products	TLC silica gel HR	Hexane-diethyl ether 95:5, v/v to 10:90, v/v	UV light	Various oxidized samples	[41]
All-*trans* retinoic acid, all-*trans* retinal, vitamin A acetate, vitamin A palmitate	Kieselguhr F_{254} impregnated with 10% paraffin oil in cyclohexane	Methanol-water 19:1, v/v	UV light	Standards	[42]
β-Carotene, cantaxanthin, lutein, violaxanthin, neoxanthin	TLC on chromarods	Light petroleum-chloroform-acetone 89.5:10:0.5	Flame ionization detection (FID)	Standards	[43]
Vitamin A palmitate and vitamin A acetate	TLSC on mixture of silica gel and microcrystalline cellulose	Benzene	Antimony(V) chlorides	Preparations containing crude drugs	[44]
β-Carotene, pheophytin a, chlorophyll a and b, lutein, violaxanthin, neoxanthin	HPTLC CN-coated plates	Chloroform-hexane-methanol 5:14:1, v/v	VIS light	Barley leaves	[45]

(*continued*)

Table 2. Continued

Compounds	Stationary phase	Mobile phase	Detection	Sample	Ref.
13-*cis*-, all-*trans*-, 9-*cis*- and 7-*cis*-β-carotene	Calcium hydroxide	1.2% Acetone in petroleum ether	VIS light	Spring cabbage, Italian spinach, cowpea leaves	[46]
β-Carotene, neoxanthin, violaxanthin, lutein, chlorophyll a and b, pheophytin a	C-18	Petroleum ether-aceto-nitrile-methanol 2:4:4, v/v	VIS light	Spinach leaves	[47]
β-Carotene, lycopene, rubixanthin, β-chryptox-anthin, zeaxanthin, lutein	Silica gel	15% v/v Acetone in petroleum ether	VIS light	*Rosa canina* fruit	[48]
(all-E,3R)-β-chryptox-anthin and (all-E,3R)-rubixanthin	Silica gel	n-Hexane-ethyl acetate 3:2 + 0.2% triethylamine	VIS light	Guava (*Psidium guajava* L.)	[49]
24 Carotenoids	Silica gel	Methanol-benzene-ethyl acetate 1:15:4, v/v	VIS light	Papikra	[50]
β-Carotene, lutein	Silica gel	Petrol ether-acetone-diethylamine 10:4:1,v/v	VIS light	Oak	[51]
Provitamin A	Aluminium oxide	Petroleum ether-ether 98:2, v/v	VIS light	Fruits and vegetables	[52]

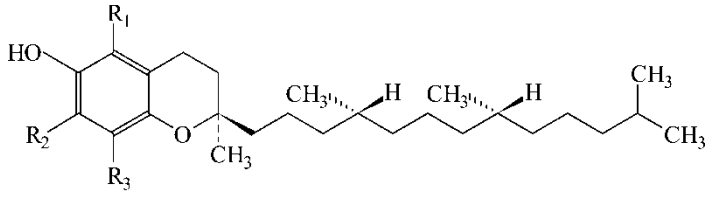
Carotenes, xanthophylls, chlorophylls	RP-18	Methanol-acetonitrile-dichloromethane-hexane 15:80:2.5:2.5, v/v	VIS light	Green vegetables	[53]
α-,β-carotene, β-chryptoxanthin	Cadmium hydroxide	Propanol-hexane 5:95, v/v	VIS light	Pharmaceutical preparations	[54]
β-Carotene, zeaxanthine, capsanthin, capsorubin	OPLC silica gel	Petroleum ether-benzene-acetone-acetic acid 40:10:2:2.5, v/v	VIS light	Ground red pepper	[55]
Retinol, α-tocopherol, tocopheryl acetate	HPTLC silica gel F_{254}	Chloroform-cyclohexane 55:45, v/v	UV light	Human plasma	[56]
DL-α-tocopherol	Chiralplates	2-propanol-water-methanol 17:2:1, v/v	UV light	Standards	[57]
α-,β-,γ-,δ-tocopherol	RP-18-HPTLC	Ethanol-water 10:0; 9.5:0.5; 9.0:1, v/v	Dipyridyliron reagent	Various biological samples	[58]
α-,γ-,δ-tocopherol, α-tocotrienol, tocol	Silica gel GF	Hexane-isopropyl ether 17:3, v/v	UV light	Standards	[59]
α-,β-,γ-,δ-tocopherol, tocopherol acetate, cholesterol	Whatman LK5 silica gel plates impregnated with boric acid	Chloroform-ethanol-water-triethylamine 35; 30:7:35, v/v containing 0.10 g/L ascorbic acid and 0.15 g/L butylated hydroxytoluene	UV light after spraying with primuline	Human platelets and human cultured endothelial cells	[60]

(*continued*)

Table 2. Continued

Compounds	Stationary phase	Mobile phase	Detection	Sample	Ref.
α-Tocopherol	TLC silica gel	Benzene-ethanol 99:1, v/v	Bromophenol blue	Vitamin E capsules, soybean oil	[61]
α-,β-,γ-,δ-tocopherol	HPTLC RP-18	Ethanol-water 19:1, v/v or n-propanol-water 9:1, v/v	UV light	Standards	[62]
α-,γ-,δ-Tocopherol	TLC silica gel	Chloroform	0.5% Dipyridyl and 0.2% iron(III) chloride in methanol	Standards	[63]
Tocopherol, tocopherol acetate	HPTLC silica gel	Iso-propanol-hexane 3:97, v/v	UV light	Standards	[64]
Vitamins E, D_2	Paraffin oil-impregnated corn starch	Acetone-conc. acetic acid 30:20, v/v	1:4 $SbCl_5$ in chloroform	Apricot-hazelnut and apricot-almond pulp	[65]
Vitamins E, E-acetate, D_3 and A-acetate	RP-18	Acetonitrile-benzene-chloroform 10:10:1, v/v	10% Antimony chloride	Standards	[66]
Vitamins D_2 and D_3	Silica gel $60F_{254}$	Benzene-methanol 9:1, v/v	0.005% Aqueous fuchsine solution	Standards	[67]
vitamin D_2	Silica gel	Benzene-acetone 95:5, v/v	UV light	Shiitake mushrooms	[68]
Vitamin D_3 metabolites and calcitriol	HPTLC silica gel 60	Chloroform-ethanol-water 183:16:1, v/v	UV light	Plasma	[69]

Vitamin D_3	HPTLC silica gel	Hexane (5 cm) and cyclohexane-diethyl ether 1:1, v/v (7 cm)	UV light	Cod liver oil	[70]
Vitamin D_3 metabolites	HPTLC silica gel	a) dichlormethane-isopropanol 9:1,v/v b) hexane-iso-propanol 85:15,v/v c) chloroform-ethyl acetate 5:5, v/v	UV light	Standards	[71]
Vitamins D_3 and K_1	Silica gel	Dichlormethane-methanol-acetic acid 45:4:1, v/v or chloroform-methanol 97:3, v/v	UV light	Standards	[72]
Vitamin K_4	Silica gel	Benzene-acetone 9:1, v/v	UV light	Drugs	[73]
Vitamin K_1 (phylloquinone, I)	Silica gel H F_{254}	Benzene-ethyl acetate 97:3, v/v	UV light	Tablets and injection solution	[74]
Vitamin K_3 (menaphthone, II)	Silica gel H F_{254}	Cyclohexane-chloroform-methanol-acetic acid 2:15:3:1, v/v	UV light	Tablets and injection solution	[75]
Vitamin K_4 (acetomenaphthone, III)	silica gel H F_{254}	Benzene-acetone 9:1, v/v	UV light	Tablets and injection solution	[74]
Vitamin K_3	Silica gel	Methanol-benzene 1:4, v/v	UV light	Drugs	[75]
Phylloquinone (K_1) and menaquinone-4 (MK-4)	Silica gel	85% petroleum ether-15% ethyl ether	UV light	Rats liver	[76]
Vitamin K	Alkyl-bonded silica	Dichlormethane-methanol 7:3, v/v	UV light	Bovine liver	[77]



R_1	R_2	R_3	
$-CH_3$	$-CH_3$	$-CH_3$	DL-a-Tocopherol
$-CH_3$	H	$-CH_3$	DL-b-Tocopherol
H	$-CH_3$	$-CH_3$	DL-g-Tocopherol
H	H	$-CH_3$	DL-d-Tocopherol

Figure 3. The chemical structures of tocopherols.

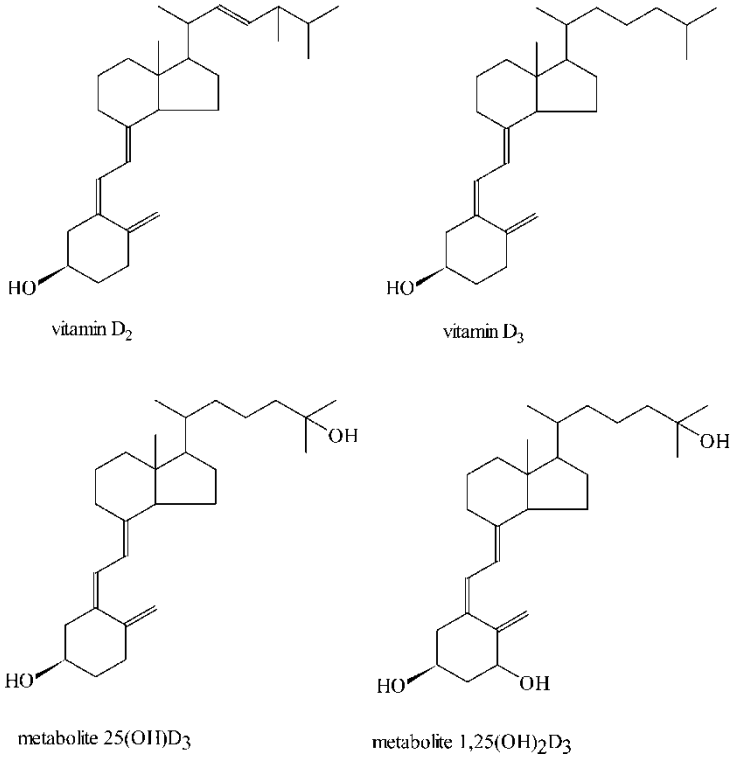


Figure 4. Structure of vitamins D_2, D_3 and vitamin D_3 metabolites.

calcium and phosphorus loss. Vitamin D also plays a role in immunity and blood cell formation, may protect people from multiple sclerosis, autoimmune arthritis, and juvenile diabetes, and is needed for an adequate blood level of insulin.[33] Vitamin D_2 is of vegetable origin, whereas vitamin D_3 is created in the body during a chemical reaction, which starts with the exposure of the skin to sunlight. Vitamin D_2 does not naturally occur in the human body unless it added by supplementation, and its biological activity in humans is less than that of vitamin D_3. Strictly speaking, vitamin D is not a true vitamin, because it can be manufactured by the body. However, vitamin D should not be excluded from the diet, especially for people who do not receive sufficient exposure to UVB sunlight. A dietary source of vitamin D is fortified foods, because only a few foods naturally contain vitamin D (shiitake mushrooms, fish liver oil, fatty fish, egg yolks, and butter). Vitamin D deficiency may result in osteomalacia, osteoporosis, abnormal bone formation, and it is common in vegetarians, dark-skinned people, alcoholics, and people with liver or kidney diseases. Too much vitamin D may lead to headaches, weight loss, and kidney stones.

Vitamin D_3 is converted into active metabolites by oxidation in the liver, when it passed into [25(OH)D_3], and then in the kidney where the [25(OH)D_3] is converted in the [1,25$(OH)_2$D3].[34–36] These compounds posses significant biological activities, and for these reasons, analytical methods were developed for their investigation in body fluids. The TLC is an analytical technique used in several applications, even though it was superseded by HPLC. TLC is used in the investigation of vitamins D_2 and D_3 and their metabolites in various

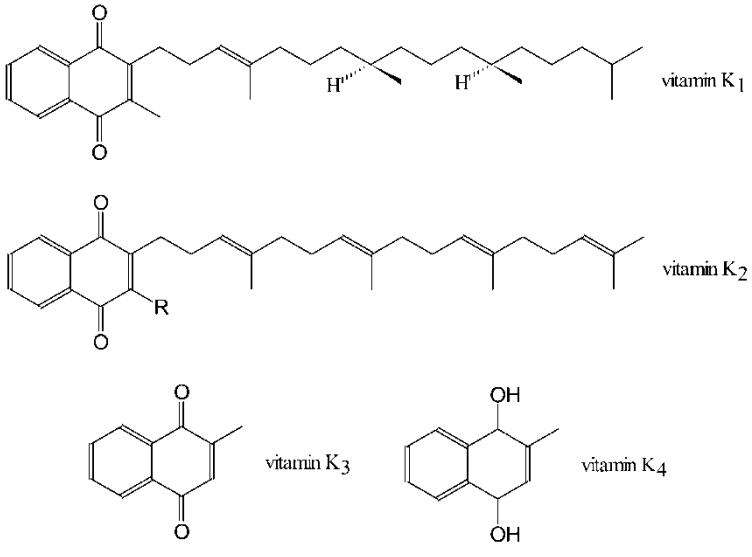


Figure 5. The structures of vitamins K.

natural samples, the separation of vitamin D from other compounds (lipids, sterols, other lipophilic vitamins), the analysis of metabolites of vitamin D in human fluids (Table 2).

Vitamin K

The forms of vitamin K are vitamin K_1 (phylloquinone, phytonadione), vitamin K_2 (menaquinone-4), vitamin K_3 (menadione), and vitamin K_4 (menadiol) (Figure 5). Other compounds known as K vitamins are menaquinone-*n* (MK-*n*), ubiquinone (Q-*n*), and plastoquinone (PQ-*n*).[28] Vitamins K_1 and K_2 are natural and they can be found in leafy green vegetables (spinach, kale, collards and broccoli) and in soybean oil, olive oil, cottonseed oil, and canola oil.[37] Vitamin K is needed for proper bone formation and blood clotting, and it is used in medicine to prevent hemorrhage due to its contribution to the formation and regulation of prothrombin and its conversion to thrombin. A vitamin K deficiency is rare, except for hospitalized patients who had poor food intake and were receiving antibiotics.[38] Regarding to the analysis of vitamin K, TLC procedures are outlined for the separation, isolation, and characterization of vitamin K from various kinds of samples (Table 2).

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