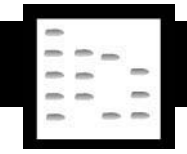
VITAMINS



Fat-Soluble: Thin-Layer (Planar) Chromatography

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Introduction

Thin-layer chromatography (TLC) is a very widely used chromatographic technique allowing the separation of simple mixtures followed by a qualitative identification or a semiquantitative visual analysis of the samples. All this can be performed in an inexpensive and simple way without requiring highly sophisticated instrumentation.

On the other hand, high performance thin-layer chromatography (HPTLC) is a highly instrumental technique allowing fast and very efficient separations with quantitative results of accuracy and precision rivalling those obtained by the far more popular techniques such as high performance liquid chromatography (HPLC) and gas chromatography (GC). The small particle size (5 μm) and the more uniform layer of the stationary phase of the commercially precoated HPTLC plates are responsible for this increased efficiency and sensitivity.

This article focuses on the specific separation of fat-soluble vitamins by TLC. Strategies from sample preparation, stationary phases, mobile phases and detection modes will be discussed for each vitamin separately. However, from the recent reviews published biennially in *Analytical Chemistry* it can be seen that the number of new applications of TLC to the analysis of fat-soluble vitamins is diminishing all the time.

The chemistry (stability) of the different compounds, will be treated because of its importance in TLC analyses.

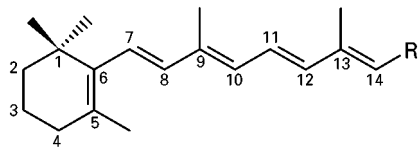


Figure 1 Structure of vitamin A. Related compounds include retinol (R=CH_2OH), retinal (R=CHO), retinoic acid (R=COOH) and retinyl palmitate (R=$CH_2OCO(CH_2)_{14}CH_3$).

Vitamin A

The structures of vitamin A and of some related compounds are presented in **Figure 1**. The parent compound, all-*trans*-retinol or vitamin A, is an isoprenoid structure with five conjugated double bonds resulting in an absorption maximum at 325 nm (in *n*-hexane or ethanol), in a high molar extinction coefficient and in a sensitivity of the compound towards isomer formation and/or oxidation. The formation of isomers is catalysed by light and iodine while the relative amount of the isomers depends on the wavelength and on the solvent used. The four exocyclic double bonds can theoretically result in the formation of 16 isomers. All have been characterized. An increase in the number of *cis* bonds generally results in a lower absorption maximum as well as a decrease of the molar extinction coefficient relative to the all-*trans* isomer. Vitamin A and the Vitamin A-related compounds are also sensitive towards oxidation and peroxidation by contact with air. The presence of transition group metals is known to catalyse this reaction.

To prevent degradation it is imperative to take special precautions when working with vitamin A-related compounds, for example, storing the samples at very low temperature, working under subdued light, avoiding drastic reagents and contact with air or peroxide-containing organic solvents.

The lability of these compounds makes research in the vitamin A field a real analytical challenge. Especially during the TLC process, special precautions are necessary, as will be described below.

Chromatographic Conditions

TLC on polar inorganic nonmodified sorbents such as alumina and silica remains very popular. Silica plates can be activated by heating at 120°C for 1 h in an attempt to enhance resolution, while spraying the plates with a solution of an antioxidant has been reported to prevent degradation of the compounds on the plates.

As with what is known from liquid chromatography, chromatographic systems based on silica plates with eluents of hexane, petroleum ether or cyclohexane, with a variable amount of a more polar solvent such as 8% diethyl ether, 50% diethyl ether or 20% ethyl acetate, offer the best separation of the geometric isomers of vitamin A compounds. In a similar way, high performance silica gel thin-layer

Table 1 Representative R_F values of geometric isomers of vitamin A compounds

Compound	*a*	*b*	*c*	*d*
Retinol				
All-*trans*	0.09	0.14	0.21	
9-*cis*		0.17	0.23	
13-*cis*		0.23	0.28	
11-*cis*	0.12	0.28	0.28	
Retinal				
All-*trans*	0.27	0.47	0.46	
9-*cis*		0.52	0.50	
11-*cis*	0.47	0.58	0.53	
13-*cis*		0.60	0.55	
Retinoic acid				
All-*trans*				0.34
13-*cis*				0.39

a, Silica gel: hexane–ether (92:8, by vol.); *b*, silica gel: hexane–ether (50:50, by vol.); *c*, silica gel: cyclohexane–toluene–ethylacetate (50:30:20, by vol.); *d*, silica gel: diethyl ether–cyclohexane–acetone–glacial acetic acid (40 : 60 : 2 : 1, by vol.).

plates eluted with diethyl ether–cyclohexane–acetone–glacial acetic acid allows the separation of all-*trans*- and 13-*cis*-retinoic acid in gel formulations (**Table 1**). Separation of vitamin A from the lipophilic vitamins is also possible on silica plates eluted with mixtures of benzene–petroleum ether–acetic acid. Under these conditions the water-soluble vitamins remain at the origin. Very often, classical TLC on silica plates serves as a kind of clean-up step before offline quantification, e.g. for the quantification of vitamin A in fruits and vegetables. With the introduction of the smaller HPTLC plates, more efficient separations together with shorter development times are possible. This has allowed the quantitative determination of retinol and of α-tocopherol in plasma with tocopheryl acetate as an internal standard.

In isolated cases kieselguhr plates or talc, starch or cellulose thin layers have been impregnated with 10% paraffin oil in cyclohexane. This was applied to a study of the hydrophobicity of a number of vitamin A-related compounds and for a separation of vitamin A-acetate from vitamin A-palmitate. For these studies the impregnated plates were eluted with mixtures of methanol–water (95 : 5, by vol.) and of acetone–concentrated acetic acid (30 : 20, by vol.).

Both the elution order of the compounds under investigation and the composition of the elution solvents clearly demonstrate a reversed-phase type of retention under these conditions.

Reversed-phase stationary phases such as RP-2 or C_{18} are also used in the analysis of vitamin A-related compounds. The separation on the RP-2 phase, however, is less efficient than that obtained on paraffin oil-impregnated kieselguhr. Separation on a C_{18} phase and on a silica phase (on one single plate) has been used in a two-phase two-dimensional TLC determination of all-*trans*- and 13-*cis*-retinoic acid in cream samples. The reversed-phase step served to separate the retinoic acid isomers from the cream excipients, while the silica sorbent was ideally suited for the separation of the two isomers from each other. Generally, on reversed-phase TLC plates, methanol or acetonitrile can separate retinol from retinyl acetate while dichloroethane with acetonitrile can separate the long chain retinyl esters.

Detection

Quenching the fluorescence of the indicator fixed on the thin-layer plate itself (F_{254}) is a very common and nondestructive way to localize spots on a TLC plate. Of course, this can also be applied to vitamin A compounds. Retinol and retinyl esters on the other hand can be identified by the yellow-green fluorescence they exhibit under 366 nm UV light. Other techniques to visualize vitamin A compounds include absorption of iodine vapours (with the formation of brown spots) or destructive procedures such as spraying with sulfuric acid.

Other spray reagents include $SbCl_3$ or $SbCl_5$ solutions in chloroform, a 5% solution of phosphomolybdic acid in ethanol and a mixture of equal volumes of a 1% aqueous solution of potassium permanganate and a 5% aqueous solution of sodium carbonate. After heating the plate coloured spots appear for vitamin A. The same reagents are often applied to visualize vitamins D and E.

As an alternative a large array of dyes has been evaluated as visualizing agents for fat-soluble vitamins, including vitamin A. The different dyes (aniline blue, alkaline blue, brilliant green, neutral red, bromocresol green, bromothymol blue, thymol blue, phenol red, helasol green, brilliant cresyl blue and bromophenol blue) are used as a solution of 50 mg of the dye either in 100 mL of water or in 100 mL of a 2% aqueous sodium hydroxide solution. Evaluation of the plates is then performed 20 min after spraying or after acceleration of the reaction by heating the plates at 110°C for 15 min.

For quantitative measurements, densitometric evaluation can be applied to vitamin A compounds. In this way, absorbance can be measured by diffuse reflectance at 290 nm using a mercury lamp, while UV spectra can be recorded between 200 and 400 nm with a deuterium lamp. Detection limits for retinol using this technique are around 160 ng mL^{-1} using 200 μL plasma. By using tocopheryl acetate as an

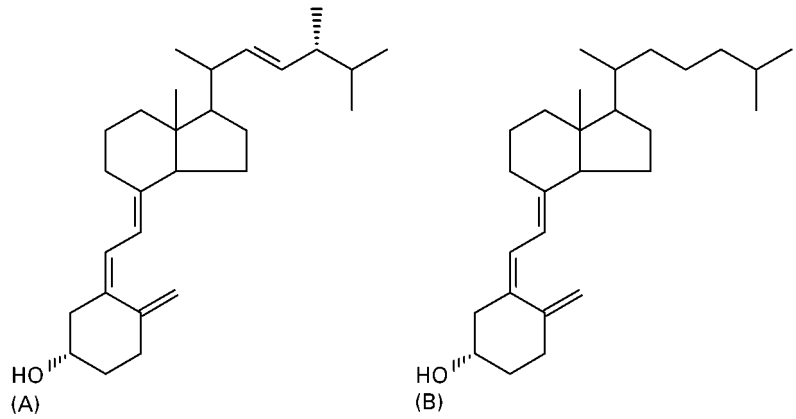


Figure 2 Structure of (A) vitamin D_2 (ergocalciferol) and (B) vitamin D_3 (cholecalciferol).

internal standard, the coefficient of variance (inter plate and intra plate) can be kept below 12.5%.

Using the dyes as a visualizing agent, the highest sensitivity on a silica plate can be obtained with bromophenol blue (in 2% aqueous sodium hydroxide) without heating the plate. Under these conditions 3 μg can be visualized. The same reagent applied on a partition-type TLC plate (silica gel impregnated with a 5% solution of paraffin oil in chloroform) resulted in a fivefold decrease in sensitivity towards vitamin A.

Vitamin D

Vitamin D and its structural analogues are a group of 9,10-seco-steroids: their basic structures are shown in **Figure 2**. The D_2 series (ergocalciferol) is of vegetable origin and has a side chain derived from ergosterol containing an additional C_{22-23} double bond and a C_{24} methyl group, whereas vitamin D_3 (cholecalciferol) is formed in the skin of humans and animals and has a side chain derived from cholesterol. The conjugated system of three bonds results in a molar extinction coefficient of 18 300 L mol^{-1} cm^{-1} with a λ_{max} at 264 nm. Both analogues are derived photochemically from their respective precursor (provitamin D). Indeed, irradiation of this provitamin D results in various photolysis products such as tachysterol, lumisterol and pre-vitamin D. Pre-vitamin D then undergoes spontaneous rearrangement to vitamin D. In the human body vitamin D_3 is extensively metabolized. The liver converts it to 25-hydroxy vitamin D_3 while further hydroxylation in the kidney yields, among others, 1,25-dihydroxy vitamin D_3. As with vitamin A, protection from light and from air is of great importance for the analysis of vitamin D by TLC.

TLC and, recently, HPTLC have found several applications in vitamin D analysis, including differentiation of vitamin D analogues, separation of vitamin D from other lipids (e.g. sterols, other fat-soluble vitamins), determination of the purity of radiolabelled vitamin D derivatives and analysis of vitamin D metabolites as a part of radioligand assays.

Chromatographic Conditions

Polar inorganic sorbents such as silica gel or, occasionally, alumina have been applied to the separation of vitamin D analogues. In this way, provitamin D_3, tachysterol$_3$, lumisterol$_3$ and pre-vitamin D_3 were separated on silica gel and the eluting order of the compounds could be correlated with the increasing planarity of the compounds. Vitamins D_2 and D_3 can be separated on their basis of their hydrophobicity; the double bond in the hydrocarbon chain of vitamin D_2 results in lower hydrophobicity compared with vitamin D_3. This was proven by comparing the R_F values of the two compounds both in an adsorption system (silica gel eluted with benzene–methanol, 9 : 1) and in a partition system. For the latter experiment, kieselguhr plates impregnated with a 10% solution of paraffin oil in benzene were eluted with different mixtures of methanol–water or acetonitrile–water. In spite of the extra methyl function in the side chain of D_2 the double bond makes this compound more polar than D_3, as demonstrated by their R_F values in the latter system (**Table 2**).

For the separation of vitamin D from other lipids, e.g. in foods, in most cases silica gel plates are applied. In cases where vitamin D has to be separated from sterols (cholesterol, β-sitosterol, stigmasterol or lanosterol), however, alumina may be the preferred stationary phase because silica gel can show too high an adsorption strength towards free sterols.

In particular cases, multiple development of the plates may be necessary, e.g. when vitamin D has to be determined in cod liver oil. Despite the availability of column chromatographic procedures or, more

Table 2 Representative R_F values of vitamins D_2 and D_3[a]

Eluent	*Compound*	
	D_2	D_3
Methanol–water (v/v)		
100 : 0	0.78	0.74
95 : 5	0.56	0.48
90 : 10	0.36	0.33
85 : 15	0.18	0.15
80 : 20	0.05	0.04
Acetonitrile–water (v/v)		
100 : 0	0.60	0.55
95 : 5	0.50	0.41
90 : 10	0.38	0.29
85 : 15	0.29	0.25
80 : 20	0.23	0.17
75 : 25	0.12	0.09

[a]Kieselguhr impregnated with 10% paraffin oil in benzene.

recently, of HPLC, TLC is still used in the clean-up of extracts of lipid-rich matrices such as foods, tissues, oils or multivitamin preparations. The same even holds true for applications of TLC in the sample preparation step for separation of the different metabolites of vitamin D. Of course, in biological matrices the content of the vitamin D metabolites is too low to allow visualization by spray reagents. Typically, the areas corresponding to the compounds of interest are then scraped off and wetted with a small volume of solvent to make it directly amenable to a quantitative radioligand determination.

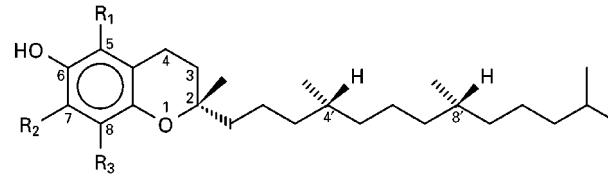
Silica gel and silica gel impregnated with silver nitrate have also been used to monitor the purity of radiolabelled vitamin D. The advantage of the application of TLC for this type of study is based on the fact that TLC offers a total picture of all impurities, whereas in liquid chromatography it can never be totally excluded that some impurities are not eluted.

Polar organic sorbents (e.g. cellulose) or nonpolar bonded phases are infrequently used in vitamin D analysis by TLC. However, separation on kieselguhr plates impregnated with paraffin oil, described above, clearly demonstrates that nonpolar bonded phases are worth evaluation for the separation of D_2 from D_3.

Detection

Although not very sensitive, UV absorbance or fluorescence quenching (the latter on plates containing a fluorescence indicator) are universal procedures that are valid for the detection of vitamin D.

Iodine vapours, 0.005% aqueous solution of fuchsin or a 0.05% aqueous solution of bromocresol green, can also be used to visualize vitamin D-related compounds.



Compound	R_1	R_2	R_3
α-Tocopherol	CH_3	CH_3	CH_3
β-Tocopherol	CH_3	H	CH_3
γ-Tocopherol	H	CH_3	CH_3
δ-Tocopherol	H	H	CH_3

Figure 3 Structure of tocopherols.

As already mentioned, for quantification purposes of vitamin D-related compounds, TLC is often incorporated as a clean-up step before offline measurement either by gas chromatography–mass spectrometry (GC-MS) or radioimmunoassay (RIA). This clean-up function offers a certain future for TLC and HPTLC, especially for laboratories specializing in RIA and lacking HPLC equipment.

Vitamin E

Vitamin E is a collective term for tocopherols and tocotrienols, a series of potent antioxidants derived from 6-chromanol by substitution with a saturated (tocopherols) or partially unsaturated (tocotrienols) isoprenoid side chain and one to three methyl functions (**Figure 3**). The principal form is α-tocopherol (5,7,8-trimethyltocol) which in nature occurs in the 2*R*, 4′*R*, 8′*R* configuration. Tocol can be regarded as the unsubstituted parent molecule, while α-, β- and γ- and δ-tocopherol form a homologue series of tri-, di- and monosubstituted tocols, respectively. The dimethyltocols (β- and γ-tocopherol) are positional isomers.

All vitamin E derivatives have strong reducing properties, with α-tocopherol being the most biologically active homologue. By scavenging free radicals and other oxidative species, α-tocopherol is known to protect membrane lipids from peroxidation. Other functions described for vitamin E remain more controversial. In the absence of air, vitamin E derivatives are quite stable to heat and alkali. However, in the presence of air they are rapidly oxidized by alkali and metal ions. Vitamin E derivatives absorb light in the UV region (λ_{max} 292–295 nm; ε 3530 L mol^{-1} cm^{-1}) and they are natively fluorescent (λ_{ex} 205 and 295 nm; λ_{em} 330 nm).

Table 3 TLC conditions for vitamin E-related compounds[a]

Compounds	*Mobile phase*	*Visualization*	*Comments*
α-Tocopherol in rat liver	1D: Benzene–ethanol (99 : 1, v/v) 2D: Hexane–ethanol (9 : 1, v/v)	20 h at 110–120°C	
α-, γ-, δ-Tocopherol in feeds, oils	Petr.ether–diethyl ether–acetic acid (90 : 10 : 1, v/v)	0.004% 2,7-dichlorofluorescein	β-Tocopherol and γ-tocopherol co-migrate
α-Tocopherol in pig organs	1D: Benzene–ethanol (99 : 1, v/v) 2D: Hexane–ethanol (9 : 1, v/v)	Ethanolic bathophenanthroline-$FeCl_3$	
α-, β-, δ-Tocopherol and α-$Tocopherol_3$ in algal lipids	Hexane–isopropylether (85 : 15, v/v)	15 min at 100°C 10% copper(II) sulfate phosphoric acid 10 min at 190°C	β-Tocopherol and γ-tocopherol co-migrate
α-, β-, γ-, δ-Tocopherol and α-, β-, γ-, δ-$tocopherol_3$ in cereals and plant oils	1D: Chloroform 2D: Hexane–isopropylether (80 : 20, v/v)		γ-Tocopherol and β-$tocopherol_3$ co-migrate

[a]All separations were done on silica plates.

Chromatographic Conditions

For TLC separation of vitamin E derivatives, silica gel plates have been widely used. Within the group of tocopherols migration is correlated with the degree of ring methylation. However, for the separation of β- from γ-tocopherol (two dimethyl tocols), often two-dimensional TLC is necessary with an eluent based on petroleum ether and diisopropyl ether for the second TLC run (**Table 3**).

Resolution of the naturally occurring tocopherols and tocotrienols also requires two-dimensional TLC. The separation between β-tocotrienol and γ-tocopherol, in particular, remains an analytical challenge. Both capillary GC and HPLC have now replaced TLC approaches, but the solvents used in HPLC often rely on solvent systems applied in earlier TLC separations.

Traditionally, TLC on silica gel or on alumina has also played an important role in the clean-up of extracts of biological materials for the spectrophotometric analysis of tocopherols/tocotrienols in the presence of a large excess of interfering lipids. The whole procedure, however, often included saponification, extraction, column chromatography and two successive TLC runs before the final spectrophotometric measurement.

Both silica gel and alumina lend themselves to separation of tocopherols from their decomposition products (α-tocopherylquinone, α-tocopherylhydroquinone) from other fat-soluble vitamins or from other lipophilic antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole, ethoxyquin, gallate esters and ascorbyl palmitate.

More recently, reversed-phase chromatographic conditions have been evaluated for the separation of α-, β-, γ- and δ-tocopherol. Kieselguhr G plates impregnated with a 10% solution of paraffin oil in benzene and eluted with methanol–water (9 : 1, by volume) offer the best separation. Of the four tocopherols considered, the difference between the R_F values of β- and γ-tocopherol was small.

Alternatively, reversed-phase C_{18} plates have also been applied to the separation of α-tocopherol from other antioxidants or from the other tocopherols. A new and interesting trend consists of the separation of D and L enantiomers of tocopherol on chiral plates (stationary phase, chiral plate solvent: propanol–water–methanol (8.5 : 1.0 : 0.5, by volume) activated by heating at 100°C for 15 min). Because of the different biological activities of both enantiomers, this type of separation should be further investigated.

Detection

The commonest mode of detecting tocopherols and tocotrienols on TLC plates is based on quenching the fluorescence of supports impregnated with a fluorescent indicator. Alternatively, tocopherols and tocotrienols can be visualized by nonspecific procedures such as charring preceded by spraying with sulfuric acid, perchloric acid, nitric acid or 10% copper(II) sulfate in 8% phosphoric acid. More specific visualization procedures are based on the reducing properties of the vitamin E-related compounds. In this way, ferric ions are reduced to ferrous ions which react with α, α′-dipyridine or bathophenanthroline to form a red-coloured complex (Emmerie–Engel reaction). Phosphomolybdic acid and a 20% antimony pentachloride solution in chloroform both produce characteristic colour reactions allowing β-tocopherol to be distinguished from γ-tocopherol, or all four tocopherols from each other. Quantification of vitamin E-related compounds after TLC separation can be performed either off-plate or on-plate. Off-plate methods include scraping the areas of interest from the plate and eluting the compounds with an organic solvent, followed either by a colorimetric measurement or

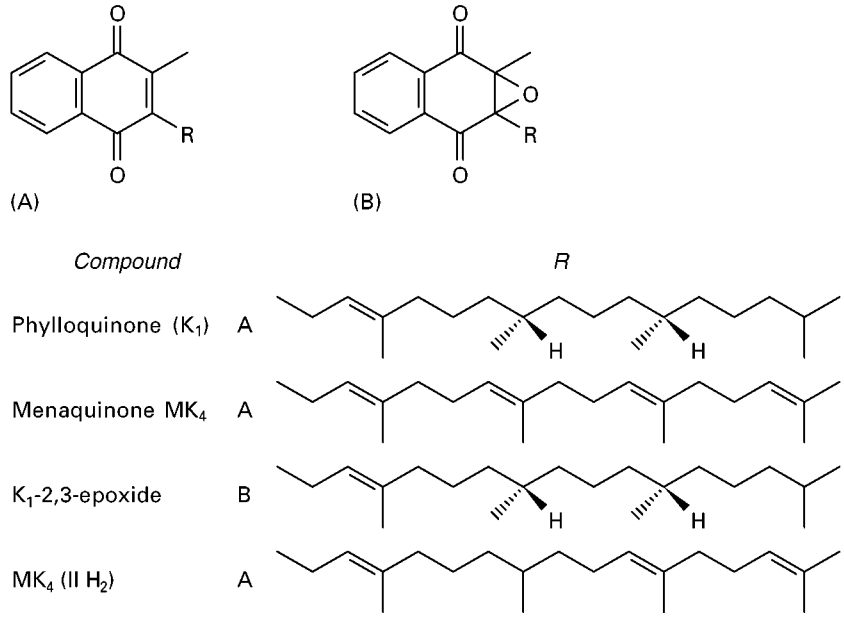


Figure 4 Structure of vitamin K and related compounds.

by GC determination. On-plate quantification is based on densitometry of the coloured spots obtained with chromogenic spray reagents, on the native UV absorbance or on the native fluorescence properties of the compounds of interest.

Vitamin K

All K-vitamers are derivatives of the same 2-methyl-1,4-napthoquinone nucleus. The number of isoprene units or the number of carbon atoms in the side chain can be used to characterize the molecules. Accordingly, MK-4 contains four isoprene units, or $K_{1(20)}$ has 20 carbon atoms in the side chain (**Figure 4**). Three molecules, each representative of a particular group of K-vitamers, are of special importance:

1. Phylloquinone (Vitamin $K_{1(20)}$) is synthesized by green plants and is found in chloroplasts of photosynthetic plants. Epoxidation of the double bond between carbons 2 and 3 of the naphthoquinone nucleus results in $K_{1(20)}$-epoxide
2. Menaquinone-*n*, also called MK-*n*, is characterized by a propenyl side chain often containing a large number of isoprene units (up to 13), with *n* indicating the number of units. Menaquinones (ranging from MK-4 to MK-13) are synthesized by bacteria (e.g. *Escherichia coli* and *Staphylococcus aureus*)
3. Synthetic vitamin K_3 (menadione or MK-0) does not occur in nature. In the body, menadione exhibits vitamin K activity by virtue of its *in vivo* conversion to menaquinones, chiefly MK-4, by microorganisms or by alkylating enzymes.

Phylloquinone and the other K-vitamers are destroyed in alkaline media and are sensitive to daylight (isomer formation). The K-vitamers are easily reduced but are fairly stable towards oxidizing conditions and heat. Both vitamin $K_{1(20)}$ and MK-*n* show a characteristic UV absorption spectrum with maxima at 244, 249, 263, 270 and 331 nm (in methanol). Their molar extinction coefficient at 249 nm is of the order of 20 000 L mol^{-1} cm^{-1}.

Chromatographic Conditions

TLC procedures for vitamin K can be divided into three main types:

1. adsorption chromatography on silica plates for the separation of *cis-trans* isomers
2. argentation chromatography (also on silica layers) to separate saturated and unsaturated homologues of vitamin K
3. reversed-phase chromatography for the separation of methylated and demethylated K-vitamers

These three systems are complementary and will be treated below.

One major advantage of TLC on silica gel is that silica gel has little or no tendency to catalyse the degradation of vitamin K. This is in contrast to alumina-based separations. Separations on silica are mainly based on differences in polarity, which makes the procedure the method of choice for the isolation

of vitamin K from other lipids. In this way, TLC on silica plates developed with light petroleum ether–diethyl ether (85 : 15, by volume) is included in the sample preparation for the determination of vitamin K in lipid-rich animal tissues. Although no recent publications have been found, TLC on silica plates is especially suited for the separation of geometric isomers (*cis-trans* isomers).

Silica plates have been impregnated with 5–20% silver nitrate. Under these conditions lipids containing unconjugated double bonds in their side chain form complexes with the silver ions and show a higher retention than the saturated counterparts. Consequently, separation between saturated ($K_{1(20)}$), partly saturated [MK-*n* (H_n)] and fully unsaturated homologues (MK-*n*) becomes possible. On the other hand, in argentation chromatography the resolution between *cis* and *trans* isomers is completely lost. Silver ions are not destructive for vitamin K, so samples can be eluted from the silica afterwards. However, for high molecular weight menaquinones, irreversible adsorption to argentation TLC plates has been reported.

Unlike in argentation TLC, where retention is correlated to the degree of unsaturation, in reversed-phase TLC the retention is based on the length of the side chain. Both techniques are thus perfectly complementary for the separation of menaquinones.

In addition to silica plates and argentation TLC, reversed-phase TLC has been applied to vitamin K-related compounds. Typical eluents consist of water and an organic solvent such as methanol, acetonitrile or tetrahydrofuran. However, because of wettability problems with aqueous solvents, often nonaqueous reversed-phase conditions are used with dichloromethane and methanol (70 : 30, by vol.) as eluting solvent.

Detection

As with the other fat-soluble vitamins, fluorescence quenching can be applied to localize the position of vitamin K-related compounds on a TLC plate. More sensitive but often destructive for the compounds of interest include spray reagents such as 70% perchloric acid (5–10 min at 105°C), a 0.05% solution of rhodamine B in ethanol, a 0.2% anilinonaphthalene sulfonic acid solution in methanol and a 10% solution of phosphomolybdic acid in ethanol.

Again densitometry (based on reflectance, transmission) has completely replaced visual inspection as well as the offline quantification after elution of the bands. Densitometry allows internal standardization and results in a higher degree of sensitivity and speed of analysis.

General Conclusions

From the above overview it should be clear that TLC is no longer the method of choice for the analysis of fat-soluble vitamins. The major reason for this lies in the great progress made in HPLC. Newer trends such as HPTLC and densitometric scanning may give TLC a new momentum but never to the extent that it will again supersede HPLC as a routine technique for the determination of fat-soluble vitamins in foods or biological materials. Undoubtedly, however, modern instrumental TLC can offer automation, improved repeatability and more accurate quantification compared to classical TLC.

See also: **II/Chromatography: Thin-Layer (Planar):** Spray Reagents. **III/Vitamins:** Liquid Chromatography.

Further Reading

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Liquid Chromatography

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Introduction

Vitamins are a group of organic compounds essential to life in very low concentrations. They are either