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Amino acid and vitamin determinations by TLC/HPTLC: review of the current state

Review Article

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Abstract: Several methods to determine amino acids and vitamins in biological and pharmaceutical samples have been reported. Thin layer chromatography (TLC) finds its place when the relatively costly equipment required by other methods is unavailable. This review covers the 1991-2010 literature on TLC/HPTLC (high performance thin layer chromatography) amino acid and vitamin determinations. It gives an overview of the special features as well as the problems in TLC/HPTLC determinations of amino acids and vitamins. Various chromatographic systems useful in amino acid and vitamin identification, separation and quantitation of are presented in tabular form. Future prospects of TLC/HPTLC for amino acid and vitamin determinations are also discussed.

Keywords: *Amino acid • Vitamin • Thin layer chromatography • Biological and pharmaceutical analysis*

1. Introduction

The unique separation reproducibility of commercially available precoated thin layer plates has generated renewed interest in the use of thin layer chromatography (TLC) as an analytical tool in the analysis of wide range of substances. Furthermore, the availability of adsorbents of different nature after surface modification has extended the versatility of TLC. High performance thin layer chromatography (HPTLC) involving the use of smaller average particle size of adsorbent in the preparation of TLC plates provides faster separations with reduced zone diffusion and enhanced sensitivity.

TLC advantages include: (a) it is convenient and simple; (b) being an off-line technique, each step can be performed independently; (c) there is a wide choice of reagents to give visual detection on the plate; (d) it consumes small amounts of solvent; (e) many samples can be spotted on a plate and separated simultaneously, and several plates can be analyzed in a tank containing the same mobile phase; (f) there is flexibility in choice of stationary and mobile phases; and (g) TLC plates are disposable [1]. A further advantage is the possibility of coupling to more selective detection techniques such as Raman spectroscopy, infrared spectroscopy (IR), mass spectrometry (MS), or gas chromatography (GC). In consequence, TLC/HPTLC methods have become indispensable tools of modern analytical chemistry [2].

TLC is classically used for routine separations, identification of the individual amino acids or vitamins, and their quantitative determinations. With the advent of the automated multiple development (AMD) technique all steps from application to mixing solvents, development, and drying can be automated. Although such automation is obviously expensive, it is a prerequisite for the wider use of HPTLC in the pharmaceutical industry.

There are several comprehensive books and reviews of application of TLC to amino acid and vitamin determinations [3-12]. This review summarizes work done during 1991-2010 on TLC and HPTLC of amino acids and vitamins. The information has been arranged in condensed form.

2. Chromatographic systems

The stationary and mobile phases together comprise the chromatographic system. Their selection controls

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how effective a separation is. The stationary and mobile phase combinations used are summarized in Tables 1 and 2.

2.1. Stationary phase

Adsorbent selection is based on: it should not react with the analyte or catalyze its decomposition, its color should not interfere with the chromatogram (preferably colorless), and it should be insoluble in the solvent. Its physical and chemical properties should remain unaltered under experimental conditions. The adsorbents used most frequently have been silica gel, cellulose, alumina, and kieselguhr *etc*. Other less commonly used layer materials include magnesium silicate, chitin, chitosan, ion exchangers, polyamide, starch, Sephadex™, and talc. Binder is often incorporated to hold the adsorbent firmly on the plate. Thin layers of silica gel G (gypsum binder) and silica gel S (starch binder) with or without fluorescent indicator have been used frequently. During the last decade, bonded stationary phases have led to renewed interest in reversed phase (RP)-TLC. These include C-18, C-12, C-8, C-2, aminopropyl, diphenyl, and cyanopropyl bonded stationary phases. RP-TLC represents a useful extension of TLC, especially for polar compounds.

Complex-forming stationary phases and chiral phases have also been introduced for separation of biological or environmental samples. Mixed layers (impregnated and non-impregnated) have been used for enhanced resolution. They are usually of medium activity compared to the separated phases. Mixed layers of up to three or four sorbents have been occasionally used for specific TLC applications.

2.2. Mobile phase

Mobile phase selection depends on the substances to be separated and the adsorbent. Separation of a complex mixture is greatly improved by the proper mobile phase. It should be as simple as possible and prepared from the purest solvent. The use of mobile phases containing more than four components should be avoided due problems in reproducible preparation. Because volatile mobile phases evaporate quickly from the sorbent, better reproducibility is achieved with mobile phases of lower volatility. Mobile phases used in the determination of amino acids and vitamins by TLC may be categorized as:

- *Inorganic solvents:* Solutions of mineral acids, bases, salts, and their mixtures.

-*Organic solvents:* Acids, bases, hydrocarbons, alcohols, amines, ketones, aldehydes, organophosphates and their mixtures.

- *Mixed solvents:* Organic solvents mixed with water, mineral acids, inorganic bases, buffers, or dimethyl sulfoxide.

- *Surfactant-mediated system:* Aqueous and hybrid solutions of cationic, anionic and nonionic surfactants.

3. Visualization

The process of detecting the spots after development is called visualization. For visualization of amino acids and vitamins on TLC plates the following physical, chemical and biological methods have been used:

(a) Physical methods such as autoradiography, X-ray fluorescence, UV, *etc*.

(b) Chemical methods requiring the spraying of plates with a suitable reagents (2,3-dichloro-1,4-naphthaquinone, 4-dimethylaminobenzaldehyde, hexamethyldisilazane, iodine–azide, isatin, molybdate, ninhydrin, and salicylaldehyde, *etc*.).

(c) Biological methods (bio-autography, enzyme inhibition and immunostaining, *etc*.) have been found useful for specific detection of compounds with physiological activity.

4. Applications

Numerous amino acid and vitamin separations by TLC are scattered in the literature. Below is shown the range of separations developed during 1991-2010.

4.1. Amino acids

The separation and identification of amino acids is important because of their increasing industrial, pharmaceutical, toxicological, and pesticidal applications. Almost all separations of amino acids are performed in normal phase (NP) systems. The four most common stationary phases are silica gel, cellulose, impregnated adsorbents, and ion exchangers, although impregnated silica gel, silanized, or octadecyl modified silica gels are used for some separations and metal ions are used as impregnating agents to modify silica surfaces.

With silica gel, two- or three-component solvent mixtures are usual. Acetone, methanol, water, acetic acid, and formic acid, sometimes with the addition of ammonia or pyridine, are commonly used as one of the components. The most widely used reagent for qualitative and quantitative detection of amino acids is ninhydrin.

Chiral HPTLC plates have been used for the resolution of amino acids (methionine, valine, leucine,

serine, and isoleucine) using methanol-water-acetonitrile (1:1:4). They were detected by plate immersion in 0.3% ninhydrin in acetone, followed by heating at 110°C for 10 min. A new topological index for predicting the resolution of D and L amino acids was developed [13]. Chiral plates in combination with acetonitrile-methanol-water were used in the quality control of L-tryptophan as well as for the separation of thyroxine enantiomers [14,15]. Amino acid racemates were separated on impregnated silica gel plates [16,17].

Compared to ß-Cyclohexdrin mobile phase additive, better separations of densyl amino acid enantiomers were realized with hydroxypropyl ß-cyclodextrin additive using reversed phase-TLC [18,19]. Similarly, RP-planar chromatography using bovine serum albumin as an impregnating agent has been utilized to resolve amino acid derivatives [20-25]. The bonded stationary phase consisting of a derivative of 4-hydroxyproline was found very useful to resolve important amino acid enantiomers [26]. Interestingly, a microcyclic antibiotic (vancomycin) has been found very useful chiral mobile phase additive for resolving densyl-DL-amino acids on RP-TLC plates [27].

The use of vancomycin and rifamycin –B thiostrepton as bonded stationary phase chiral selectors has resulted into good separations of complex mixtures of enantiomers compounds [28].

Erythromycin, another macrocyclic antibiotic, has been used as a chiral stationary phase to resolve various dansyl-DL-amino acids. Silica gel layers impregnated with 0.05% erythromycin and different combinations of 0.5 M aqueous NaCl-MeCN-MeOH resolved phenylalanine, valine, leucine, serine, glutamic acid, aspartic acid, norleucine, α-amino-n-butyric acid, methionine and tryptophan. All were detected at 254 nm as fluorescent green to greenish yellow spots [29]. Karakas and Yuksel prepared a new TLC adsorbent for amino acid separation by mixing $CaSO_4$ and Na_4SiO_4 [30]. R-Amino acids were separated on tin (IV) selenoarsenate layers with dimethyl sulfoxide (DMSO) [31].

The chromatographic behavior of dansyl derivatives of amino acids on silica with a series of single-solvent mobile phases was investigated by Nurok *et al*. [32]. Multiple linear regressions were used to construct a model to predict retention. The intercept and regression coefficients are solute-dependent, but the form of the predictive equation is the same for all solvents tested. Three new solvent systems, pyridine-benzene (2.5:20), methanol-carbon tetrachloride (1:20), and acetone-dichloromethane (0.3:8), provided improved resolution and identification of 18 PTH amino acids compared to previously reported systems [33]. Poole and Poole examined the influence of solvent entry position on PTH-amino acid resolution in one-dimensional multiple development TLC [34]. Zone separation distance and resolution are strongly influenced by the solvent entry position.

A computer-assisted simplex mixture design method has been developed for solvent optimization [35]. A TLC method has been developed for the separation of 12 PTH amino acids. The general simplex method used a two-factor selectivity rectangle and a special polynomial regression over nine TLC runs, with the R_F difference as the selection criterion. A TLC method has been developed for monitoring separation quality using a separation matrix [36].

Separations of 18 amino acids were compared on HPTLC silica gel, cellulose, and C-18 bonded silica gel layers. The ability to identify amino acids in the hemolymph and digestive gland-gonad complex of *Biomphalaria glabrata* snails was studied, and alanine and aspartic acid were quantified in hemolymph by scanning densitometry [37]. A new spray reagent, *p*-dichlorodicyanobenzoquinone, detected amino acids with 0.1-1 µg detection limit and produced varied colors that facilitate identification [38]. Dried blood spots from patients with homocystinuria were analyzed for homocysteine by cellulose TLC, while spots on Guthrie cards were directly transferred to TLC plates for neonatal screening for amino acid disorders [39,40].

Densitometry was used for quantitative analysis of amino acids on mixed natural zeolite-microcrystalline cellulose layers; determination of L-lysine, L-threonine, and L-homoserine, in fermentation broths; and quantitation of amino acids in protein extracted from cane sugar after hydrolysis, derivatization with dansyl chloride, and silica gel HPTLC with 5% EDTA-butanol-diethyl ether (5:10:35) [41-43]. Serva Blue W stain visualized cyclic peptides simply and effectively in studies of the stability of peptide inhibitors toward proteolytic degradation [44]. TLC on a silica plate with propanol-water (2.1:1) determined phosphotyrosine in tyrosine-phosphorylated protein without interference by contaminants from hydrozylates [45]. Changes in the amino acid composition of dehydrated orange juice during non-enzymatic browning were detected by separation on cellulose with 1-butanol–pyridine–water (2:3:1) and detection by spraying with 2,3,5-tripheny1-2*H*-tetrazolium chloride–NaOH reagent [46].

Surfactant-modified TLC is a promising method. A TLC system comprising silica gel impregnated with a micellar solution of cetrimide (5.0 mM) as stationary phase and 40% (w/v) aqueous dextrose as mobile phase was best for amino acid separation [47].

Sulfur-containing amino acids play an essential metabolic role [48]. Their phosphonic analogues were found to exhibit strong biological activity and find pharmacologic application. Replacement of the carboxylic group by the phosphonic group strongly affects their acidity and polarity, changing their chromatographic mobility. Kudzin and co-workers compared the mobility of the natural sulfur-containing amino acids (Cys, Hcys, Met) and their phosphonic analogues (Cys^P, $Hcys^P$, Met^P) in acidic, neutral and mildly basic solvent systems. In acidic systems aminophosphonic acids generally have lower R_F values than their carboxylic analogues. However, in a neutral or mildly basic development system the opposite occurs; aminophosphonic acids are retained less than the corresponding carboxylic amino acids [49].

Buhl and Galkowaska purposed a simple, precise, and sensitive TLC method for the determination of methionine in complex pharmaceutical preparations (Methiovit and Revalid). The system of silica gel plates and n-propanol–water–chloroform (5:2:1) enables good separation of DL-methionine from accompanying substances. Under these conditions, the R_F of DL-methionine was 0.56 while the R_F values of L-cystine, vitamins B_1, B_2, and B_6, vitamin PP, calcium pantothenate, and p-aminobenzoic acid were 0.02, 0.01, 0.72, 0.75, 0.79, 0.22, and 0.91, respectively [50]. Tryptophan and related metabolites were determined in foods by TLC on cellulose with chloroform–methanol–ammonia (12:7:1) and densitometry with a fiber optic fluorimeter (excitation 280 nm; emission > 347 nm) [51]. Taurine was determined in energy drinks by semi quantitative TLC based on the color formed with ninhydrin [52]. Hsieh and Berry used TLC for detection of abnormal amino acid metabolites [53]. The interesting work of Marklova applied two-dimensional TLC to the diagnosis of inherited metabolic diseases [54]. Lu *et al*. described comparative TLC of amino acids on silica with different solvent systems for validation of their method [55].

Zhao and co-workers studied the correlation between molecular connectivity index and retention [56]. Using a back-propagating artificial neural network Wang *et al*. studied the relationship between retention and molecular structures for a group of amino acids. The predicted results were in good agreement with those obtained experimentally [57]. Frost *et al*. suggested polynomial equations for the prediction of R_F of a series of p-nitrobenzyl esters of dansyl amino acids over a large retention range. The equations provide agreement between predicted and experimentally obtained R_F values within ≤ 0.05 [58].

A rapid HPTLC and TLC method for analysis of amino acids on silica was described [59]. Derivatization was by pressing a polymeric foam pad containing the solution onto the plate to obtain complete and homogeneous reagent distribution. Witkiewicz and co-workers used an instrument for overpressured layer chromatography in which the eluent is fed to the plate from below by a syringe pump. The velocity of the mobile phase was very stable and linearly dependent on the migration distance. Gas was used to apply overpressure. The separation resolution, rapidity and reproducibility were much improved [60].

Work on amino acid TLC/HPTLC not included in the text [61-184] is briefly summarized in Table 1.

4.2 Vitamins

Although not all vitamins are amines, they are organic compounds required by humans in small amounts for normal growth, metabolism and health. Human vitamin deficiencies lead to vitaminosis, gastrointestinal problems, cardiovascular and nervous disorders, and growth inhibition [185]. Therefore, the concentration of vitamins in biological objects, food products and pharmaceutical formulations should be controlled. Water and fat soluble vitamins differ in properties and structure. Vitamins can occur in trace or macroscopic amounts. Some occur in several bioactive forms; for example, vitamin B_6 can occur as pyridoxine, pyridoxal, and pyridoxamine.

Normal and reversed-phase TLC has been found useful for separation and identification of vitamins [186–190]. The mobile phases frequently used include mixed organic or aqueous–organic solvents [191,192]. Most studies have been performed using methanol, ethanol, butanol, acetone, chloroform, ammonia, pyridine, benzene, or toluene as one of the components of the mobile phase with adsorbents such as silica gel, cellulose, and surface-modified silica gel [193–198]. For reversed phase TLC of hydrophilic and lipophilic vitamins, RP18 and $RP18WF_{254}$ HPTLC plates have been used with mixed aqueous–organic mobile phases [190,199]. In addition to the use of conventional adsorbents, fat soluble vitamins have been separated on TLC plate coated with certain unconventional adsorbents such as corn starch, talc, rice starch etc. [104]. Various dyes have been used to detect D, A and E vitamins for on-plate identification after their separation by partition and adsorption TLC [200].

TLC has also been used to investigate potential interactions between vitamins A and D with frequently used therapeutics [201]. High sensitivity of the method allows the separation of pictogram amounts of vitamin D and its analogues on silica gel. A new method has been developed by Lobastova *et al.* [202] for the fast screening of synthetic products related to vitamins.

Table 1. Thin layer chromatographic studies of amino acids and their derivatives performed during 1991-2010.

Stationary phase	Mobile phase	Remarks	Ref.
RP (C-2 and C-18)	Acetonitrile – water and hydroxypropyl-β-cyclodextrin	Description of a method for the separation of enantiomers using TLC on RP phases with chiral mobile phase additive. Detection under UV 254 nm.	[61]
Chitin and its derivatives (chitosan)	Mixtures of methanol — buffer solution— propanol / acetonitrile in different ratios	Chitin and its derivatives (chitosan) are used as stationary phase for the separation of amino acids (threonine, glycine, serine, alanine, valine, leucine, methionine).	[62]
RP-18 Silica	Aqueous solution containing sodium chloride, urea, methanol, and 0.1M α- or β-cyclodextrin	Separation of optical isomers on reversed phase layers with mixed aqueous-organic-inorganic solutions containing α- or β-cyclodextrin. Detection under UV.	[63]
Cellulose	0.7 M Aqueous sodium sulfate	Simultaneous determination of phenylalanine, tryptophan and tyrosine by densitometry on cellulose layer using sodium sulfate solution as mobile phase.	[64]
Silica gel	Butanol — acetic acid — water (40:5:7)	A new reagent, acetyl acetone- formaldehyde is proposed for sensitive detection of separated amino acids under UV radiation.	[65]
RP HPTLC	Various binary aqueous solvents	Separation of α-amino acid enantiomers on reversed-phase HPTLC plates treated with copper acetate. Significant selectivity towards stationary phase was observed.	[66]
HPTLC silica gel 60	Different eluents	Separation of derivatised amino acids on HPTLC silica gel 60 plates. Comparison of visualization by spraying and by dipping. Detection limits were better by dipping.	[67]
Polyamide	Benzene — acetic acid (9:1) and ethyl acetate — methanol — acetic acid (20:1:1) for the first direction and formic acid (88%) — water (3:200) for the second.	Two-dimensional TLC of DNS-amino acids. Detection under UV 365 nm.	[68]
RP silica gel	Aqueous solutions of formic, acetic, propionic and perchloric acids	Discussion of salting-out and salting-in phenomena and the examination of influence of the polarity of the amino acids, their hydrophobicity and the strength of the acid in the eluent on the retention behavior of amino acid derivatives.	[69]
Silica gel	Chloroform — methanol — 17% ammonia (4:4:2) for the first direction and phenol — water (3:1) for the second.	Two dimensional TLC of amino acids on silica. Detection by spraying with 0.5% ninhydrin in acetone. Quantitation by spectrophotometry at 570 nm.	[70]
Antimony (V) phosphate-silica gel ´G´	Aqueous , non aqueous and mixed solvent systems	Quantitative separation of α-amino acids from two drugs (astymin-forte and santevini plus).	[71]
Cellulose	1 M Sodium chloride	Separation of D-and L-tryptophans and D- and L-methyltryptophans by adsorption TLC on microcrystalline and native cellulose layers. Much better separations were achieved on microcrystalline cellulose. Detection with ninhydrin.	[72]
RP	Mixtures of acetonitrile — water — methanol (4:2:1) for tryptophan, acetonitrile — water — propanol (3:4:2) for leucine and isoleucine and acetonitrile — water — propanol (3:1:1) for phenylalanine	TLC of eight amino acids on reverse phase layers, impregnated with a copper salt and an optically active amino acid.	[73]
Chiral plates and RP-18 silica	Ethanol — water — acetonitrile (1:1:4 and 5:5:3), methanol — water (1:8) and acetone — methanol — water (10:2:2)	TLC of α-methyl amino acids and certain other amino acids. Detection by spraying with 0.3% ninhydrin in acetone.	[74]
Cellulose	1 M sodium chloride, ethanol – pyridine – water (1:l:l), butanol – acetic acid – water (4:1:5). and ethanol – butanol – water (2:1:2, 1:1:1, 5:3:7, and 5:1:9)	TLC of substituted tryptophan enantiomers on microcrystalline cellulose layers with mixed aqueous and organic solvents. Visualization by iodine vapor.	[75]
Chitin and impregnated chitin layers	Binary and ternary mobile phases	Separation of optical isomers of amino acids on chitin and transition metal impregnated chitin layers. Good separation on copper impregnated chitin layer has been observed. The dependence of hR_F values on the composition of the mobile phases has been demonstrated.	[76]
RP-TLC	Aqueous lithium chloride, sodium chloride, potassium chloride, rubidium chloride, and cesium chloride solutions	Reversed-phase TLC and salting out effect on the mobility of 14 dansylated amino acid derivatives.	[77]
Imprinted (I) and Non-imprinted (0) Polymers	0 — 15% Acetic acid in acetonitrile	Molecular imprinting technique combined with TLC for separation of L- and D-phenylalanine anilides. Detection by spraying with fluorescamine (0.05% in acetone), and viewing under UV 366 nm.	[78]

Continued **Table 1.** Thin layer chromatographic studies of amino acids and their derivatives performed during 1991-2010.

Stationary phase	Mobile phase	Remarks	Ref.
Cellulose	Aqueous ß-cyclodextrin	Chiral separations on cellulose layer with aqueous ß-cyclodextrin.	[79]
RP-18	0.1 M Phosphate buffer containing 20% acetonitrile — 3% sodium chloride with the addition of various ß-cyclodextrins.	Chromatography of derivatized DL amino acids with ß-cyclodextrins modified mobile phases.	[80]
Silica gel impregnated with transition metal salts	Butanol — water — acetic acid (4:2:2)	Improved resolution of amino acids was realized as a result of complexation between amino acids and transition metals on silica gel impregnated with transition metal ions and their anions. Detection by spraying with ninhydrin solution and heating at 80-100°C for 5-10 min.	[81]
Silica gel	A series of 25 binary mobile phases containing a specified concentration of ethyl acetate as a common strong solvent	Prediction of retention of p-nitrobenzyl esters of dansyl amino acids. Both first- and second-order regression models were used to relate the R_F of individual solutes, the log k´ of individual solutes, or the average R_F of a mixture of solutes, to the properties of the weak solvent in each of a series of 25 binary mobile phases.	[82]
Silica	1-Butanol — acetic acid — water (75:19:19)	TLC of amino acids on silica layers developed with 1-butanol — acetic acid — water (75:19:19). Detection by 0.25% ninhydrin. Determination of alanine by densitometry at 496 nm.	[83]
DNS-amino acid — polyamide	1.5% Formic acid or benzene — acetic acid (9:1)	Analysis of leucine by two-dimensional TLC. Visualization under UV 365 nm	[84]
RP-18	Methanol — water (1:1, 1:3 and 1:5)	RP-TLC of amino acids that form colored compounds with ninhydrin. Quantitation by spectrophotometry.	[85]
Silica gel	1-Butanol — acetone — acetic acid — water (7:7:2:4)	Kinetic fluorescence detection of glycine and glutamine after TLC separation.	[86]
Cellulose	Butanol — acetic acid — water (12:3:5 and 20:3:5)	Preparative TLC of non-protein amino acids.	[87]
Silica	Butanol — acetic acid — water (4:1:1) and phenol — water (3:1)	Two-dimensional TLC of amino acids on silica layers. Detection by spraying with 0.5% ninhydrin in ethanol.	[88]
Silicic impregnated glass fibre sheets	Isopropanol — water (7:3)	TLC of 22 amino acids. Detection by spraying with 0.25% solution of ninhydrin in ethanol and heating at 105°C for 5 min.	[89]
Silica gel	n-Butanol — glacial acetic acid — water (3:1:1)	A rapid and reproducible TLC method for detection of amino acids (detection limit = 0.02 μg) on silica gel 'G' with n-butanol — glacial acetic acid — water containing mobile phase.	[90]
Silica	2-Propanol — 25% ammonia (7:3)	TLC of tryptophan. Detection by spraying or dipping with/into 4-(dimethylamino) benzaldehyde solution. Quantitation by densitometry at 625 or 276 nm.	[91]
Cellulose — silica (5:2)	Isopropanol — ethyl acetate — acetone — methanol — isopentanol — ammonia — water (9:3:3:1:1:3:3 and butanol — acetone — isopropanol — formic acid — water (18:8:8:3:6)	Two-dimensional TLC of 17 amino acids in Bos grunniens linnaeus horn hydrolysate. Detection by spraying with 0.5% ninhydrin in acetone.	[92]
HPTLC silica layers	Different concentrations of ethyl acetate in heptane and chloroform	Separation of derivatives of amino acids on HPTLC silica layers following multiple gradient development. Detection by densitometry at 485 nm.	[93]
Silica	Chloroform — methanol — formic acid (35:15:1)	TLC of amino acids. Detection by spraying with 0.2% ninhydrin in ethanol.	[94]
Aminopropylsilica-bonded layers	Benzene — acetone (11:9), benzene — methanol — chloroform (4:3:3) and petrol benzene — ethyl acetate (2:3)	TLC separation of derivatized DL-amino acids on chiral stationary phases. Visualization under UV 254 nm.	[95]
Silica gel	1-Propanol — 25% ammonia (11:9), 2-propanol — acetone — water — 25% ammonia (25:25:7:6) 2-propanol — ethyl acetate — 25% ammonia — water (40:40:3:50) and 2-propanol — 25% ammonia (7:3)	TLC separation of lysine, homoserine, threonine, and tryptophan from accompanying amino acids in culture liquids. Detection of tryptophan by dipping into 0.5% 4-dimethylaminobenzaldehyde (4-DMABA) in ethanol containing 5% conc. sulfuric acid and heating for 5-7 min at 110°C. Quantitation by densitometry.	[96]

Continued **Table 1.** Thin layer chromatographic studies of amino acids and their derivatives performed during 1991-2010.

Stationary phase	Mobile phase	Remarks	Ref.
Cellulose	α or β Cyclodextrin	Qualitative analysis of aromatic amino acids and aromatic amino alcohols on cellulose with highly concentrated solutions of α or β- cyclodextrin.	[97]
Silica gel	Acetate buffer (0.3 M, pH 6.0) — acetonitrile — n-butanol (12:5:10)	Utilization of Cu ion in TLC separation of amino acids on silica gel with acetate buffer — acetonitrile — n-butanol mobile phase and the comparison of TLC results with those obtained by RP-HPLC. Detection by spraying with 0.2% ninhydrin in acetone and heating at 60°C for 30 min.	[98]
Silica gel	Acetonitrile — water (2:5) containing 2-O-[(R)-2-hydroxypropyl]- β-cyclodextrin	TLC separation of enantiomers of six selected amino acids. Detection by spraying with a solution of 1.5 g salicylaldehyde in 100 mL toluene and heating at 50°C for 10 min.	[99]
Cellulose-silica biphasic layer	Isopropanol — ethyl acetate — acetone — methanol — para-amyl alcohol — ammonia — water (9:3:3:1:1:3:3) and butanol — isopropanol — acetone — formic acid — water (18:8:8:3:6)	Two-dimensional TLC for simultaneous separation of the metabolites of amino acids. Detection under UV 254 nm.	[100]
Silica gel	n-Butanol — acetic acid – water (4:1:1) and ethanol — water (70:3)	Application of TLC in combination with derivative spectroscopy for the determination of histidine, arginine, tryptophan and methionine in baby foods.	[101]
Chitin and chitosan	Ternary mobile phases	Qualitative analysis of seven DL- mixtures of amino acids on chitin, chitosan, copper impregnated chitin and chitosan layers.	[102]
Silica gel 60 F_{254} TLC plates and mixture (1:1 w/w) of microcrystalline cellulose (Merck) and natural tuff, mainly consisting of zeolite-clinoptilolite,	Phenol (saturated with water) — ethanol — acetic acid — water (12:4:1:4)	Quantitative TLC analysis of diffuse and distorted spots of amino acids obtained on non-homogeneous laboratory-prepared plates. Validation by comparison with image analysis and slit-scanning densitometry.	[103]
Corn starch , rice starch or talc layers and impregnated corn starch layers	2-Propanol — formic acid — water (20:1:5)	TLC of amino acids on unconventional layers. Detection with ninhydrin-lutidine reagent. Quantitation by densitometry at 254 or 366 nm.	[104]
Silica gel	Different combinations of acetonitrile, methanol and water	Resolution of enantiomers of amino acids and their dansyl derivatives on silica gel impregnated with (1R, 3R, 5R)-2-azabicyclo [3,3,0]octan-3-carboxylic acid using 0.5M aqueous sodium chloride and acetonitrile in different ratios as mobile phase. Visualization of dansyl amino acids under UV 254 nm and the detection of amino acids by spraying with ninhydrin in acetone.	[105]
Silica gel	Ethanol — ammonia — water (7:1:2)	Identification of amino acids by TLC. Detection by spraying with ninhydrin.	[106]
Silica gel	Butanol — acetonitrile — 0.005 M potassium dihydrogen phosphate — acetic acid (1:5:3:1)	Demonstration of the theoretical aspects of the long-distance development, the basic elements of the double layer cassette for personal OPLC instrument, and the first results in the field of amino acid analysis. Detection with ninhydrin at 490 nm.	[107]
Cellulose	n-Propanol — water (7:3)	HPTLC of 19 amino acids. After development detection with ninhydrin reagent and densitometry at 610 nm.	[108]
Silica gel	Ethanol — water (4:1, 8:1 and 2:1), propanol — acetic acid — water (3:1:1, 6:2:1, 1:1:1)	Determination of the TLC retention of amino acids on silica gel layers developed with different mobile phases. Visualization with ninhydrin. Densitometry at 460 nm. Correlation analysis was conducted and fifteen parameters are discussed. Experimental results were in good agreement with calculated values, which confirms the accuracy of the predictions.	[109]
Silica gel	Chloroform — methanol — ammonia conc. (2:2:1), Phenol — water (3:1)	Separation of amino acids by TLC. Detection under UV 254 nm. Identification of tryptophan and histidine by Fourier transform – surface – enhanced Raman scattering spectroscopy (FT-SERS). Detection limit 8 μg.	[110]

Continued **Table 1.** Thin layer chromatographic studies of amino acids and their derivatives performed during 1991-2010.

Stationary phase	Mobile phase	Remarks	Ref.
Chiral plate	Acetonitrile — methanol — water (4:1:1or 4:1:2) and acetonitrile — methanol — water — diisopropylethylamine (40:10:20:1)	TLC of the enantiomers of several unusual aromatic amino acids. Visualization with ninhydrin.	[111]
Chiral HPTLC plates	Acetonitrile — methanol — water (4:1:1)	TLC separation of enantiomers of amino acids (alanine, norvaline, norleucine, glutamic acid, phenylalanine, tyrosine, tryptophan and proline) using ninhydrin for visualization of resolved spots. Distinction between L and D isomers on the basis of proposed topological indexes.	[112]
Microcrystalline cellulose triacetate	2-Propanol or ethanol — water (4:1)	RPTLC of several chiral compounds (*e.g.* PTH- and MTH-amino acids, N- and C-substituted amino acids, 3, 5-dinitro-N-(1-phenylethyl)benzamide, N-benzoyl-leu-ßNA) on home-made microcrystalline cellulose triacetate plates. Detection by UV at 254 and 364. Quantitation by densitometry at 254 nm.	[113]
Soil	Water	The amendment of soils carried out by pesticides has shown variable influence on the mobility of amino acids.	[114]
Alumina	1% Cetyltrimethyl ammonium bromide in water — butanol (19:1)	TLC of 24 amino acids on alumina and lithium-impregnated alumina layers. L-Proline was selectively separated from other aliphatic and aromatic amino acids on plain alumina layers. Detection by spraying with ninhydrin and heating for 15-20 min at 90-100°C.	[115]
Silica gel	2-Propanol — 25% ammonia (7:3)	Identification of L-tryptophan by TLC. Detection by treatment with 4-dimethylaminobenzaldehyde and heating at 110°C for 7-10 min. Quantitation by densitometry at 625 nm. Rapid method for determination of L-tryptophan.	[116]
RP	Methanol — 0.2 M ß-cyclodextrin (2:3), acetonitrile — 0.2 M ß-cyclodextrin (35:65), and methanol — 0.2 M ß-cyclodextrin (3:7)	By using RP-TLC, eight DNS-DL-derivatives of phenylglycine, homophenylalanine, α-aminocaproic acid, allylglycine, *tert*-leucine, *N*-methylleucine, *O*-methylserine, and *O*-methyltyrosine have been successfully resolved.	[117]
Silica gel and RP-18	Toluene — pyridine — acetic acid (40:10:1) and aqueous solution of ß-cyclodextrin — acetonitrile or methanol	Purificaction of DNS-amino acids (DNS-D-arginine, DALDA, DNS-leucine, and DNS-Valine) on silica gel with toluene — pyridine — acetic acid (40:10:1). After extraction, stereochemical analysis on RP-18 with aqueous solution of ß-cyclodextrin containing mobile phase. Quantitation by densitometry at 366 nm.	[118]
RP-18	Ethanol — ammonia conc. — water (65:10:60)	TLC of phosphorylated amino acids (phospholysine, phosphoserine, phosphothreonine, phosphoarginine, phosphotyrosine, phosphohistidine). Detection with 0.2% ninhydrine in ethanol and heating at 37ºC for 30 min.	[119]
Silica gel	Acetonitrile — 0.5 M aqueous sodium chloride (5:2 and 14:3)	Separation of the enantiomers of dansyl-DL-amino acids on silica gel plates impregnated with vancomycin (0.34 mM) as chiral selector. Visualization under UV light at 254 nm.	[120]
Silica gel	Different combinations of acetonitrile — methanol — water	Direct enantiomeric resolution of DL-arginine, DL-histidine, DL-lysine, DL-valine and DL-leucine into their enantiomers was achieved by TLC on silica gel plates impregnated with optically pure (1R, 3R, 5R)-2-azabicyclo[3,3,0]octan-3-carbo-xylic acid (0.011 M) as a chiral selector.	[121]
Silica gel	Mixed organic solvents containing cyclohexane, hexane, heptanes or benzene in combination with ethyl formate or propyl formate or methyl acetate	4-Diethylaminodiazabenzene-4'-isothiocyanate (DEABITC) was synthesized and used to obtain thiohydantoin derivatives of α-amino acids. The colored derivatives were separated by TLC on silica gel using various solvent systems.	[122]
Silica gel	2-Propanol – 25% ammonia (11:9), 1-propanol – acetone – water – 25% ammonia (25:25:7:6), 2-propanol – ethyl acetate – 25% ammonia – water (40:40:3:50)	Qualitative analysis of L-lysine, L-threonine, and L-homoserine in fermentation broth. Visualization by dipping in 1% ninhydrin in acetone. Quantitation by densitometry at 500 nm.	[123]
Alumina	Oil-in-water microemulsion	Qualitative separation of aliphatic and aromatic amino acids on plain alumina and Li^+, Na^+, NH_4^+ impregnated alumina with oil-in-water microemulsion.	[124]
Silica gel	Microemulsion systems	TLC analysis of amino acids on silica gel layer with microemulsion systems as mobile phase.	[125]

Continued **Table 1.** Thin layer chromatographic studies of amino acids and their derivatives performed during 1991-2010.

Stationary phase	Mobile phase	Remarks	Ref.
Silica gel	1-Propanol — 25% ammonia (11:9), 2-propanol — acetone — water — 25% ammonia (25:25:7:6), 2-propanol — ethyl acetate — water — 25% ammonia (40:40:50:3) and 2-propanol — 25% ammonia (7:3)	TLC of lysine, threonine, homoserine, tryptophan and phenylalanine on silica layers. Detection of tryptophan by immersion of TLC plates for 40 s in a 0.5% ethanolic solution of p-dimethylamino-benzaldehyde containing 5% conc. sulfuric acid and heating at 110°C for 5-7 min. Quantitation by densitometry.	[126]
Silica gel	Butanol — chloroform — acetic acid (3:7:5, 6:8:4, and 10:1:4) and ethyl acetate — carbon tetra chloride— propionic acid (10.5:6.5:3.5)	TLC resolution of DL amino acids into their enantiomers on silica gel plates impregnated with (—)-quinine (0.1%) with detection limits ranging between 0.9 and 3.7 μg per spot. The hR_F values from racemic mixture for D and L isomers, given in parentheses were as methionine (25 and 50), alanine (7 and 16), threonine (5.5 and 11), valine (11.8 and 19.3), leucine (6.4 and 13.8) and isoleucine (6.4 and 12.9) respectively.	[127]
Silica gel	n-Propanol — water (7:3)	TLC of 22 amino acids. Detection by spraying with a modified ninhydrin reagent giving distinguishable colors with most amino acids, and with high sensitivity.	[128]
Silica gel	One component, two-component (butanol — acetic acid) and three-component (acetone — benzene — acetic acid) mobile phases in varying ratios.	The chromatographic behavior of 24 amino acids was studied on plain silica gel 'G' layers using different combinations of organic solvents.	[129]
RP-18	Methanol — water — acetonitrile (1:1:4)	TLC of nine amino acids (alanine, phenylalanine, valine, leucine, ileucine, tryptophan, tyrosine, asparatic acid, glutamic acid) on RP-18 impregnated with a proline derivative and copper(II) ions.	[130]
Alumina	0.01 M Aqueous sodium dodecyl sulfate plus 0.1 M aqueous Copper sulfate solution in volume ratio (9:1)	Separation of L-proline from other amino acids on alumina layers developed with 0.01 M aqueous sodium dodecyl sulfate plus 0.1 M aqueous copper sulfate in volume ratio (9:1).	[131]
Silica gel HPTLC	Acetonitrile — water — acetic acid (85:14:1)	HPTLC of arginine, asparatic acid, glutamic acid, serine, threonine and alanine on silica gel layers with acetonitrile — water — acetic acid (85:14:1) 3-fold horizontal development followed by heating at 80 °C for 10 min after each development. Detection of amino acids by derivatization with ninhydrin reagent, followed by heating at 120°C for 10 min.	[132]
Silica gel and cellulose	n-Butanol — acetic acid — water (3:1:1)	HPTLC of 19 amino acids on silica gel with preadsorbent sample application zone and on cellulose with n-butanol — acetic acid — water (3:1:1). Densitometry at 495 nm for histidine and 610 nm for all other amino acids.	[133]
Silica gel or cellulose	Chloroform — methanol (1:1), isopropanol — ammonia (7:3), ethanol — water (7:3), chloroform — methanol — ammonia (20:20:3), butanol — acetic acid — water (4:1:5)	Development of new selective and sensitive detection method (detection limits of amino acids were at the pmol/spot).	[134]
Talc, starch, silica gel, and alumina	Dimethylsulfoxide — 1.0 M hydrogen chloride (1:1)	TLC of 24 amino acids on untreated and triaryl phosphate-impregnated adsorbent layers. Detection with a 0.2% solution of ninhydrin in acetone.	[135]
Silica gel, RP-18, cellulose F and ion exchange sheets	n-Butanol — acetic acid — water (3:1:1) and citrate buffer (pH 3.3)	HPTLC of amino acids with four different TLC systems namely silica gel with preadsorbent zone, RP-18 with concentrating zone, cellulose F with n-butanol — acetic acid — water (3:1:1) and ion exchange sheets with citrate buffer (pH 3.3). Quantification by densitometry at 495 nm for histidine and 610 nm for tryptophan.	[136]
silica gel	Water-in-oil microemulsion	Silica gel impregnated with micellar copper sulfate solution as stationary phase and a water-in-oil microemulsion as mobile phase has been proposed for selective separation of DL-phenylalanine (R_F = 0.87) from other amino acids (range from 0.00-0.70).	[137]
Silica gel	Mixture of isopropanol — methanol — ammonia (0:1:1) to (9:1:0.5).	The isolation of amino acids with preliminary separation by thin layer chromatography.	[138]
Silica gel	Cetyltrimethylammoniumbromide — butanol — n-octane — water microemulsion	The chromatographic behavior of amino acids on the silica gel thin layers using micro-emulsion as a developer.	[139]

Continued **Table 1.** Thin layer chromatographic studies of amino acids and their derivatives performed during 1991-2010.

Stationary phase	Mobile phase	Remarks	Ref.
Silica gel, cellulose, strong acid cation exchange sheets and RP-18	Dichloromethane — methanol (1:1), propanol — 0.5 M sodium chloride (2:3) for RP phase, citrate buffer (pH 3.3) for the ion exchange layer and n-butanol — acetic acid — water (3:1:1) for the other two layers	HPTLC of 19 amino acids, detection by spraying heavily with ninhydrin reagent and the quantitation by densitometry at 495 nm (histidine) and 610 nm for all amino acids.	[140]
Silica gel ´H´	n-Butanol — acetic acid — water (4:1:5)	TLC performed for arginine derivatives in ginseng products after processing.	[141]
Silica gel	n-Propanol – water (70:30)	The proposed reagents (4-hydroxyacetophenone-isatin and 4-hydroxybenzaldehyde-isatin) formed distinguishable colors with most of the amino acids after final heating.	[142]
1:4 Stannic arsenate – cellulose (1:4) layers	n-Butanol — formic acid (7:3), isopropanol — acetic acid — water (8:1:1), and ethyl methyl ketone – ethyl acetate – formic acid – water (2:6:1:1)	Several important ternary separations have been achieved on the basis of different R_F values. Lysine (R_F = 0.11) and threonine (R_F = 0.53) were selectively separated from the mixture of amino acids present in common available drugs (Alamine Forte capsule; Albert David). Detection with ninhydrin and quantitative determination by spectrophotometry with hydrindantin-methyl cellosolve reagent.	[143]
RP-silica gel	n-Propanol — water mixture	RP-TLC on silicone fluid DC 200, triaryl phosphate (TAP) and tri-n-butylamine (TBA) impregnated silica gel-G layers with two component mobile phases (n-propanol-water) in varying ratios.	[144]
Silica gel	Multiple development with 5 mobile phases	Models of retention were used to predict R_F values and the values calculated were compared with those obtained experimentally. The spots widths were also predicted and compared with the actual widths.	[145]
Silica gel G	0.0001-0.01M Aqueous sodium bis (2-ethyl hexyl) sulfosuccinate (AOT), and 0.001M AOT — dimethylsulfoxide — methanol or ethanol or propanol (3:2:7)	TLC of amino acids on silica gel in combination with surfactant mediated eluents.	[146]
Silica gel	Toluene — dimethyl ether (8:2)	Racemic mixtures of dinitrobenzoyl amino acids can be resolved on impregnated silica layers and structure of the complexes was studied by X-ray. Enantiomeric amino acids yield complexes with different colors.	[147]
Silica gel HPTLC	Dichloromethane — methanol (1:1)	Determination of L-arginine hydrochloride in nutrition supplements using silica gel HPTLC plates, automated band-wise sample application, detection with ninhydrin and visible mode densitometry.	[148]
Silica HPTLC and TLC	Iodine-azide system	The iodine-azide system proved best to detect 1-60 p mol (HPTLC) and 3-100 p mol (TLC) per spot amino acids.	[149]
Silica gel	n-Butanol — ninhydrin — pyridine — water	Silica gel G thin layer plates developed with n-butanol — ninhydrin — pyridine — water solution used to analyze protein digests.	[150]
Silica gel	n-Propanol — water (7:3)	4-Hydroxyacetophenone/isatin-5-sulfonic acid (sodium salt) was used for identification of amino acids on TLC plates.	[151]
Sorbfil PTSKh-P-V and Kieselgel 60	Mixtures of 1-propanol, 2-propanol, ethanol, ethyl acetate, chloroform, acetone, acetic acid, 25% aqueous ammonia, and water	Proximate procedures for quantitative analysis of amino acids using high performance thin layer chromatography were developed.	[152]
Silica gel HPTLC	Mixed mobile phase systems such as 2-propanol — ethyl acetate/acetone — 25% ammonia — water, 2- or 1-propanol — 25% ammonia, 1-propanol — 25% ammonia — water, chloroform — 96% ethanol — acetic acid — water and t-amyl alcohol — 2-butanone — water	HPTLC of serine, threonine, phenylalanine, tryptophan, lysine, ornithine, arginine, valine and leucine on silica gel with mixed solvent systems. Quantitative determination by densitometry at 500 nm.	[153]
Cellulose	n-Butanol – acetone – acetic acid – water (35:35:10 :20) and n-butanol – acetic acid – water (40:10:10)	TLC with silica gel layer was used for separation and identification of the flavonoids and phenolic acids and TLC with cellulose layer was used for investigation of the amino acids.	[154]
Cellulose and silica gel	n-Butanol — acetone — glacial acetic acid — water (35:35:10:20)	Determination of amino acids in drugs by TLC. Samples of Althaeae radix contained identical amino acid pattern irrespective of their γ -irradiation treatment.	[155]

Continued **Table 1.** Thin layer chromatographic studies of amino acids and their derivatives performed during 1991-2010.

Stationary phase	Mobile phase	Remarks	Ref.
Cellulose and silica gel	n-Butanol — acetone — glacial acetic acid — water (35:35:10: 20 by vol) and ethyl acetate — methanol — citric acid/phosphate buffer (pH = 6) (30:30:40 by vol)	Analysis of free amino acids in Valerianae radix and its products by TLC.	[156]
Buffered soil (pH 2.06)	Water-in-oil-micro-emulsion	Examination of transportation behavior of amino acids through static flat bed of buffered soil as the stationary phase.	[157]
Silica gel	Butanol	Hexamethyldisilazane (1% solution in acetone) was used as spray reagent for detection of amino acids.	[158]
Silica gel	Ethanol — methanol — chloroform (1:1:2)	Prechromatographic derivatization with 2-propanol — benzyl isothiocyanate — triethylamine by putting the plate in a thermostat at 40°C for 30 min. Quantities in the range of 2-90 pmol of per spot were detected.	[159]
Silica gel	Mixed mobile phase systems containing sodium azide in combination of acetonitrile, methanol, tetrahydrofuron, 1,2-dioxane, acetone or ethanol in the presence of starch.	Separation of amino acids by normal-phase and reversed-phase TLC.	[160]
Soil	Aqueous solution of sodium salts of molybdate, borate, oxalate, carbonate, bicarbonate and dihydrogen phosphate anions.	The mobility of amino acids show the following order: glutamic acid > histidine > valine > leucine > serine > alanine. The mobility of the amino acids increased with the increasing concentration of anions up to a limit and thereafter declined except glutamic acid, which shows a fall in its movement throughout the entire range.	[161]
Cellulose and Zein-coated cellulose	Distilled water and aqueous solutions (0.5 -150 mM) of acetic acid, sodium acetate, sodium chloride, and magnesium chloride	Examination of interaction of arginine, histidine, lysine, tryptophan, and ornithine with the corn protein zein. The strength of interaction depends upon the pH and the salt concentration. Development of plate at ambient temperature in sandwich chamber and detection with ninhydrin.	[162]
Silica gel	Borate phosphate buffer, pH 2.3	TLC on silica layer impregnated with sodium dodecyl sulfate was performed for separation of L-arginine from L-serine and L-tyrosine.	[163]
Soil	Aqueous solutions of ammonium sulfate and urea at different concentration levels	Certain amino acids showed salting-out and/or salting-in effects over a limited ammonium sulfate concentration range. The effects of particle size, activation temperature, irradiation of the soil by γ-rays and the pH of the soil bed on the mobility sequence of the amino acids were also examined.	[164]
Silica gel	Water-in-oil microemulsion (sodium dodecyl sulfate — water — heptane — n-pentanol)	Selective separation of L-tryptophan from other amino acids by TLC.	[165]
Chiral TLC plates (RP)	Acetonitrile – methanol – water – aqueous phosphate buffer, 70:10:10:10 (%, v/v)	Amino acid enantiomers were separated on commercial chiral TLC plates in reversed phase mode.	[166]
Polar (NH , CN, diol) che-mically bonded stationary phases	Methanol — water, acetone — methanol — water — buffer, pH 9	The effect of impregnating agent on the mechanism of retention of the substances analysed by measurement of the retention of homologous groups of compounds chromatographed on both the modified and unmodified stationary phases have been investigated.	[167]
Cellulose	Tetrahydrofuron –water (85:15), acetonitrile – water (80:20), 2-propanol – water – acetic acid (89.5:9.5:1)	The study of retention behavior of amino acids on cellulose layers following multiple development technique (UMD and IMD).	[168]
Silica gel G	n-Propanol – water (70:30)	2,3-Dichloro-1,4-naphthaquinone and isatin were introduced as a new reagent for the detection of amino acids on silica gel 'G' TLC plates with very high sensitivity.	[169]
Silica gel	Triton X-100 (1.0×10^{-5} M) – sodium dodecyl sulfate (8.1×10^{-4} M) – acetone (1:1:5)	TLC of eight essential amino acids was performed on silica layers using mixed aqueous surfactant solutions as mobile phase. Lysine in Astymin (Forte) and Alamine (Forte) capsules was identified.	[170]
Cellulose	Butanol — glacial acetic acid — water (60:19:21)	Optimization of TLC separation of seven amino acids by use of the experimental design software packages.	[171]

Continued **Table 1.** Thin layer chromatographic studies of amino acids and their derivatives performed during 1991-2010.

Stationary phase	Mobile phase	Remarks	Ref.
Silica gel or RP-18	Phenol — water (3:1) or acetonitrile and triethylamine-phosphate buffer (50mM, pH 5.5)	TLC resolution of 17 DL amino acids derivatized with 1-fluoro-2,4 dinitrophenyl-5-Lvalinamide, 1-fluoro-2,4-dinitrophenyl-5-L-phenylalaninamide, or 1-fluoro-2,4-dinitrophenyl-5-L-valinamide.	[172]
Silica gel $60F_{254}$ HPTLC plates	Buffered zinc sulfate solution (pH 2.3)	Silica gel $60F_{254}$ HPTLC plates and buffered zinc sulfate solution (pH 2.3) was used for mutual separation of DL-Phe and L-Tyr.	[173]
Silica gel	Borate-phosphate buffer of pH 2.3	TLC Performed on sodium dodecyl sulfate impregnated silica layer to separate L-histidine from DL-tryptophan. DL-Tryptophan was also identified in clinical samples and drugs.	[174]
Silica gel	Mobile phases containing mixed micelles as well as modifying organic additives	Separation of three amino acids (L-lysine, L-histidine and L-tryptophan) from micrograms to milligrams level on silica gel stationary phase.	[175]
Cellulose	Organic–aqueous eluent systems modified with neutral and chaotropic salts	Selected amino acids were investigated on cellulose layers using organic aqueous eluent systems modified with neutral and chaotropic salts (chlorides, iodides, nitrates, thiocyanates, perchlorates and hexafluorophosphates) at low concentrations ranging from 10 upto 80 mM in whole mobile phase.	[176]
Ag^{+}-silica gel $60F_{254}$ HPTLC plates	Borate buffer pH 2.3	Silica gel $60F_{254}$ HPTLC plates impregnated with 5% Ag ion as stationary phase was used for the separation of tryptophan, alanine and phenylalanine or tyrosine.	[177]
Silica gel, alumina and cellulose	40% Aqueous solution of dextrose, fructose, maltose, lactose or sucrose	40% Dextrose-alumina and 40% dextrose-cellulose TLC systems were identified as most favorable for selective separation of glutamic acid and tryptophan from a mixture of amino acids.	[178]
Silica gel and RP-18	Methanol – 50% acetic acid (3:1) with silica gel and 5% acetic acid – methanol –acetonitrile (50:35:15) with RP-18	NP-TLC and RP-TLC methods have been used to study retention. A variety of mobile phase systems were evaluated for separation of L-arginine and its metabolites. The effects on selectivity of the mobile phase polar modifier of were also studied.	[179]
HPTLC cellulose and silica gel plates	2-Butanol – pyridine – glacial acetic acid –deionized water systems	Different thin layer chromatography (TLC) systems were evaluated for analysis of 21 biologically important essential and nonessential amino acids in complex mixtures such as biological tissues and fluids. R_F values were determined by slit scanning densitometry.	[180]
Sil G/UV254 and RP-18 W/UV254	Phenol — water (3:1)	Some non protein alpha- amino acids were derivatised with Marfey's reagent and its four variants. The resultant diastereomers were separated by normal and reversed phase TLC and RP-HPLC.	[181]
Silica gel HPTLC plates	n-Butanol — acetic acid — water (3:1:1)	A preliminary TLC followed by HPTLC analysis was used to determine the qualitative status of free amino acids in uninfected and infected snails.	[182]
Silica gel	Ethanol — water (1:1), butanol — ethanol — water (5:4:3) and butanol — water (1:1).	A DNA/PVA interpenetrating polymer network (IPN) formed by cross-linking polyvinyl alcohol (PVA) with glutaraldehyde (GA) and subsequent cross-linking DNA with a UV irradiation was used to coat the surface of the porous silica particles for TLC. Eight amino acid enantiomers were used to investigate their chromatographic behavior and high separation efficiency was observed.	[183]
Silica and kieselguhr	n-Butyl alcohol, ethyl acetate or ethylene glycol and their mixtures	From the point of view of chromatographic performance, a mixture of n-butyl alcohol — 70% aqueous ethylene glycol — ethyl acetate ratio 5:3:2 by volume proves to be more efficient than the individual components for separation of amino acids from their binary, ternary and quaternary mixtures. Application of the selected TLC system for the identification and separation of methionine present in Beplex forte (R_F = 0.48) and lysine in Bethadoxine-12 M (R_F = 0.03) have been performed.	[184]

Tocopherol (Vitamin E), a lipid soluble antioxidant is related to many physiological processes and protects polyunsaturated fatty acids in cell membranes from peroxidation [203,204].

Therefore, the separation of tocopherol isomers has been important. Sliwiok *et al.* developed a chromatographic method for the separation of tocopherol enantiomers/isomers and provided an interesting relationship between their R_f values and topological indexes [205].

TLC and HPTLC methods for the separation and densitometric quantification of vitamin K in bovine lever [206] and identification of retinoic acids, retinol and retinyl acetates in topical facial creams [207] have been developed. Isomers of ascorbic acid and dehydroascorbic acid in food products were separated and identified by TLC using different stationary phases [208]. Fibre-optic fluorodensitometry coupled with HPTLC was used to estimate thiamine, riboflavin and niacin at nanogram levels [209].

The effects of microwave heating on the loss of vitamin B12 in foods were studied by bonded silica and densitometry at 527 nm [210].

Work on TLC/HPTLC of vitamins not covered in the text [211-235] is presented in Table 2.

5. Conclusion

Tables 1 and 2 summarize suitable stationary and mobile phase combinations for amino acids and vitamin separation and identification in multi-component mixtures. More emphasis has been placed on amino acids than vitamins. For amino acids the stationary

Table 2. Thin layer chromatographic studies of vitamins performed during 1991-2010.

Stationary phase	Mobile phase	Remarks	Ref.
silica gel HPTLC plates	Butanol — pyridine — water (50:35:15)	Over pressure layer chromatographic procedure with photodensitometric detection for the simultaneous determination of water soluble vitamins in multivitamin pharmaceutical preparations.	[211]
Silica	Chloroform — cyclohexane (55:45)	Determination of retinol and α- tocopherol in human plasma by HPTLC method	[212]
Silica gel	Methanol, water and acetonitrile	Hydrophobicity of vitamins was determined using TLC	[213]
Silica plus GDX	Hexadecyltrimethylammonium bromide	Resolution of water soluble vitamins B_2, B_6 and B_{12} on silica mixed with a polymer packing material (GDX). Comparison of the results with those observed on alkyl-bonded silica layers.	[214]
Transition metal ion impregnated silica gel	Chloroform — n-butanol — acetic acid — ammonia (4:7:5:1or 3:5:0.5:5:0.5); benzene — butylacetate — n-propanol — acetic acid — ammonia (1:4:1:5:1); Carbon tetrachloride — butylacetate — propionic acid — NH_3 (3:7:9:3); carbon tetrachloride — butylacetate — methanol — ammonia (1.5:4.5:7:0.5); and carbon tetrachloride — butylacetate — propionic acid — methanol — water (1.5:4.5:7:0.5).	Identification of the best chromatographic systems for separation of vitamins of the ´B´ complex group. Better resolution, with disappearance of tailing in most of the cases with general decrease in hR_F values, was observed on impregnated plates compared to untreated ones in all the solvent systems.	[215]
Silica gel and cellulose	n-Butanol – glacial acetic acid – water (2:1:1); n-butanol – acetic acid–water (5:2:3); chloroform – methanol – ethyl acetate (5:5:2); n-butanol – benzyl alcohol – glacial acetic acid (8:4:3); collidine – water (3:1); 5% sodium hydrogen phosphate; n-butanol – formic acid –water – diethyl ether (77:10:13:15); n-butanol –ethanol – water (10:3:7); isoamyl alcohol – ethyl methyl ketone – glacial acetic acid – water (40:40:7:13); n-butanol – isopropanol–water–glacial acetic acid (30:50:10:2); ethyl methyl ketone–acetic acid – methanol (3:1:1).	TLC methods applied were useful for confirmation of presence or identification of some flavin derivatives. The unknown flavin derivatives were separated using preparative TLC and their retention times and/or R values were compared with that of synthetic flavins.	[216]
Silica gel G	Water	Determination of vitamin B_1 (thiamine) in the Kombucha drink. Atomic absorption spectrometry was also used for the determination of these vitamins	[217]
Silica gel	Water — methanol (95:5)	HPTLC method was developed for the determination of vitamin C in pharmaceutical tablets.	[218]
C18 RP-HPTLC plates	Ethanol or ethanol — water (9.5:0.5 or 9:1)	Separation of βα-, β-, γ-, and δ-tocopherols on C-18 RP-HPTLC plates.	[219]
Silica TLC and HPTLC plates	Binary and ternary mixtures	Determination of aflatoxin M_1 in milk and B_1 in eggs.	[220]
Silica gel	Several solvent systems	Chromatographic study of photolysis of aqueous cyanocobalamin solution containing vitamins B and C.	[221]

Continued **Table 2.** Thin layer chromatographic studies of vitamins performed during 1991-2010.

Stationary phase	Mobile phase	Remarks	Ref.
Silica gel	Mixtures of methanol and benzene	Separation of hydrophilic vitamins using fluorescent silica HPTLC plates.	[222]
Plastic-backed and glass-backed silica gel plates	Glacial acetic acid –acetone – methanol –benzene (3:1:4:14), butanol – formic acid –water (20:1:0.3) and water – glacial acetic acid – ethyl methyl ketone – ethyl acetate (1:2:2:5)	TLC separation of L-ascorbic acid and detection under UV at λ = 254 nm. Determination of L-ascorbic acid in complex pharmaceuticals and in pepper juice samples.	[223]
Silica gel Silica60F_{254} TLC plates	n-Hexane — ether (9:1) and benzene — chloroform (1: 1)	Determination of vitamins A, D_2 and E from mixtures on fluorescent TLC plates using n-hexane — ether (9:1) and benzene — chloroform (1:1) as mobile phases.	[224]
Silica gel, mixture of silica gel 60 and kieselguhr, aluminium oxide (neutral aluminium oxide 60F_{254}, and neutral aluminium oxide 150F_{254})	Toluene and methanol	TLC of lipophilic vitamins on silica gel (silica gel 60, Silica gel 60 F_{254}), silica gel plus kieselguhr and alumina with toluene, and on RP-18 with methanol. It was affirmed that in NP-TLC the R_F values of the investigated vitamins decrease in the following order: α- tocopherol acetate > α-tocopherol > cholecalciferol. The lipophilic vitamins have similar R_F values in the range from 0.45 to 0.49 in reversed phase thin layer chromatography (RP-TLC). Densitometry at UV 254 nm for quantitative estimation.	[225]
Silica gel 60 F_{254}, TLC plates RP-18WF_{254}	Acetone — n-hexane in various volume compositions dioxane — water and methanol — water in several volume compositions	Nicotinic acid and its derivatives were studied by normal phase- and reversed phase-HPTLC. Densitimetry was employed for quantitative estimation.	[226]
Silica gel, RP phase and cellulose		Separation and identification of hydrophilic vitamins (vitamin C and B complex) and lipophilic vitamins (vitamin A, E, D and K) in tablets, food and body fluids.	[227]
Silufol plates high-performance Sorbfil plates	Benzene (first mobile phase); 0.02 M aqueous micellar solution of sodium dodecyl sulfate (second mobile phase)	Fat soluble vitamins A and E and water soluble vitamins B (B_1, B_2, B_6 and B_{12}) can be separated by HPTLC using fractional elution. Benzene was used as the first mobile phase and a 0.02 M aq. micellar solution of sodium dodecyl sulfate was the second eluent.	[228]
silica gel 60F_{254} HPTLC plates	1,4-Dioxane, 1,4-dioxane –water (1:1, 1:2 or 2:1)	Thin layer chromatography on precoated silica gel 60F_{254} plates with dioxane – water (1:1) as mobile phase was used for separation of thiamine from B_2, B_3, B_5, B_6, B_{12}, and C. The spots were visualized under UV light. The R_F values of thiamine, B_2, B_3, B_5, B_6, B_{12}, and C were 0.03, 0.95, 0.93, 0.93, 0.84, 0.98, and 0.98 respectively. The proposed method is applicable for identification of thiamine in drug samples.	[229]
Silica gel 60F_{254} HPTLC plates	4% Aqueous sodium dodecyl sulfate — acetonitrile (1:2)	Resolved mixture of six hydrophilic therapeutic vitamins. Visualized under UV radiation (λ = 254 nm).	[230]
Silica gel and kieselguhr	Acetone — n-hexane in different volume ratio	Nicotinic acid and its derivatives were separated using adsorption TLC on aluminum plates precoated with a mixture of silica gel 60 and kieselguhr F_{254} non- impregnated and impregnated with 2.5% and 5% aqueous copper sulfate.	[231]
silica gel	Water	TLC system comprising a silica layer impregnated with nonionic surfactant (2% Tween 80) as stationary phase and double distilled water as mobile phase were used for identification and separation of cyanocobalamin (B_{12}), thiamine (B_1), and ascorbic acid (C) in drug formulations. The R_F values of the investigated vitamins found in the following order: C (0.97 – 0.99) > B_{12} (0.62-0.65) > B_1 (0.16 – 0.23).	[232]

Continued **Table 2.** Thin layer chromatographic studies of vitamins performed during 1991-2010.

Stationary phase	Mobile phase	Remarks	Ref.
Silica gel	Aqueous solution of sodium dodecyl sulfate (0.5%) —Ttriton X 100 (0.5%) and butanol	Mixed micelles (aqueous solution of anionic sodium dodecyl sulfate (0.5%) + nonionic Triton X 100 (0.5%) surfactants) and butanol were first used as mobile phase with silica gel layers for TLC of vitamins in identification and separation of vitamins B_1, B_{12} and C) present in drug samples (Becasule and Celin). The order of R_F values was vitamin C (0.80) > vitamin B_{12} (0.40) > vitamin B_1 (0.10).	[233]
RP8F$_{254S}$ and RP18F$_{254S}$ TLC plates	Methanol — water	Ergocalciferol, cholecalciferol, (±)-α tocopherol, tocopherol acetate, retinol, retinol acetate, retinol palmitate, menadione, and phytonadione have been investigated on RP8F$_{254S}$ and RP18F$_{254S}$ TLC plates with methanol — water in different volume proportions as mobile phases	[234]
Silica gel	Aqueous solution of Tween 80 at different concentration levels	A micellar TLC system comprising a silica layer impregnated with 0.01% sodium dodecyl sulfate as stationary phase and 0.1% aqueous solution of Tween 80 as mobile phase was identified as the most favorable system for the separation of folic acid (R_F = 0.99), pyridoxine (R_F = 0.70), riboflavin (R_F = 0.42), cyanocobalamin (R_F = 0.20), and thiamine (R_F = 0.05) from their mixture.	[235]

phases are preferred: silica gel > cellulose > RP > alumina ≈ biphasic sorbent > kieselguhr. For vitamins: silica gel > RP > cellulose > biphasic sorbent > kieselguhr. Mixed aqueous-organic or multi-component organic solvent systems have been the popular choice. It appears that the combination of individual solvents' properties provides a mixed system with improved chromatographic performance to obtain unique separations.

Silica gel as stationary phase and mixed multi-component organic solvents as mobile phase have been the most popular choice for TLC of both amino acids and vitamins. The use of alumina for vitamins and kieselguhr for both amino acids and vitamins as stationary phase and aqueous inorganic solvents as mobile phase has been limited. Starch, talc, polyamide, synthetic inorganic ion-exchangers, soil, aminoplast and impregnated sorbents have been little used.

6. Future Prospects

TLC coupling to other analytical techniques and its application to the analysis of real samples as well as purification will continue. The search for new layer materials, automation in quantitative analysis, and validation of TLC parameters will be the priority of chromatographers.

Responding to environmental issues, more emphasis should be on making TLC a "green" analytical technique. It is hoped that the emphasis will be on the use of green/less toxic eluents (ethyl acetate, ethylene glycol, polyoxyethylenes, isoamyl alcohol, ethyl lactate *etc.*). The possibilities of water as green eluent in combination with new layer materials should be explored.

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