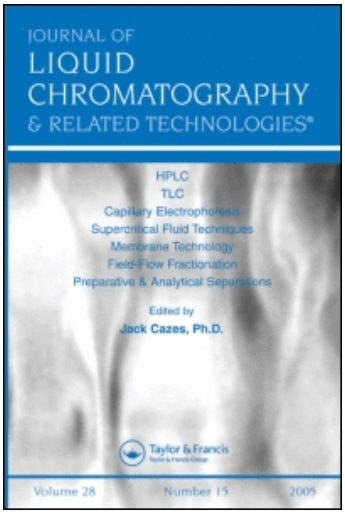

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Thin Layer Chromatographic Analysis of Biological Samples. A Review

Joseph Sherma[a]; Bernard Fried[b]
[a] Department of Chemistry, Lafayette College, Easton, Pennsylvania, USA [b] Department of Biology, Lafayette College, Easton, Pennsylvania, USA

To cite this Article Sherma, Joseph and Fried, Bernard(2005) 'Thin Layer Chromatographic Analysis of Biological Samples. A Review', Journal of Liquid Chromatography & Related Technologies, 28: 15, 2297 — 2314
To link to this Article: DOI: 10.1080/10826070500187491
URL: http://dx.doi.org/10.1080/10826070500187491

Journal of Liquid Chromatography & Related Technologies®, 28: 2297–2314, 2005

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070500187491

Thin Layer Chromatographic Analysis of Biological Samples. A Review

Joseph Sherma
Department of Chemistry, Lafayette College, Easton, Pennsylvania, USA

Bernard Fried
Department of Biology, Lafayette College, Easton, Pennsylvania, USA

Abstract: This article reviews the use of thin layer chromatography (TLC) and high performance thin layer chromatography for the analysis of biological samples of particular interest to biologists, biochemists, hematologists, immunologists, medical diagnosticians, and molecular biologists. Determinations of amino acids, drugs, carbohydrates, lipids, toxins, vitamins, indoles, antibiotics, peptides, pigments, phenols, bile acids, and coumarins in sample matrices such as blood, urine, feces, saliva, cerebrospinal fluid, body tissues, and other biologics are considered. The review discusses the advantages of using modern TLC for biological applications and summarizes important information on stationary and mobile phases and methods used for application of standards and samples, plate development, and zone detection, identification, and quantification.

Keywords: Thin layer chromatography, Biological applications, Amino acids, Carbohydrates, Drugs, Lipids, Pigments

INTRODUCTION

Thin layer chromatography (TLC) enables reliable separation and analysis of compounds from a wide variety of classes in many types of biological samples. The analyses are important in the areas of biology, biochemistry, hematology, immunology, medical diagnosis, and molecular biology.

Address correspondence to Joseph Sherma, Department of Chemistry, Lafayette College, Easton, PA, USA. E-mail: shermaj@lafayette.edu

A great number of different sorbents, mobile phases, and development modes are available to provide high efficiency separations of polar and nonpolar compounds, and use of proper techniques and commercial instruments permit accurate and precise automated quantitative analysis. The off-line nature of TLC allows use of a broad range of selective and universal detection methods in sequence for confirmation of identity, and the ability to simultaneously separate many samples applied on the same plate with corresponding standards leads to high sample throughput and relatively rapid and inexpensive analyses.

Although the separation efficiency of TLC is usually considered to be poorer than high performance liquid chromatography (HPLC), it has been demonstrated[1] that this is not always true. The separation of four weakly-sorbed natural estrogens (estetrol, estriol, 17beta-estradiol, and estrone) at different temperatures was shown to be much superior by high performance TLC (HPTLC) on Merck RP-18W plates compared to octadecyl bonded silica gel (C-18) (HPLC), and that HPTLC can be valuable for separation, detection, and quantification of steroids in biosamples. The many additional advantages of TLC and HPTLC compared to HPLC and other analytical methods are listed in Chapter 1 of Ref. [2].

The sections below briefly describe the steps of biological sample analysis by TLC and HPTLC and offer examples of typical specific applications to a variety of analytes and sample matrices. More detailed information on the theory, practice, equipment, instrumentation, and applications of basic and advanced TLC analysis can be found in an introductory book designed to be especially useful for biologists;[2] a comprehensive handbook of TLC;[3] a book containing chapters on TLC in bacteriology, plant sciences, parasitology, entomology, clinical chemistry, vertebrate skin secretion analysis, forensic toxicology, and veterinary toxicology, among others;[4] and a book chapter.[5]

MATERIALS AND TECHNIQUES

TLC is a type of liquid chromatography in which the stationary phase is a thin, uniform layer of a fine-particle sorbent on a glass plate, aluminum foil, or plastic sheet. In the basic TLC procedure, a solution of the sample is applied to the lower part of the plate, and the plate is developed by placing it in a closed chamber whose base is covered with the mobile phase, which is usually a mixture of solvents. After development, the plate is removed from the tank and the mobile phase front is marked for calculation of R_f (= distance of travel of the zone divided by the distance of the mobile phase front). The zones are detected to produce the resulting chromatogram of the separated zones. Traditional qualitative or semiquantitative TLC is simple to use and requires only low-cost apparatus (i.e., a development chamber, glass

capillary applicator, precoated plate or sheet, and a detection reagent sprayer). Quantitative TLC can be carried out if a densitometer is available.

Sample Preparation

Because TLC plates are not reused, it may be possible to carry out less sample preparation compared to column analytical methods, such as HPLC, in which strongly adsorbed impurities from a sample can be eluted slowly and interfere with the analysis of a later sample. However, most biological samples are complex mixtures (e.g., blood, urine, feces, saliva, cerebrospinal fluid, gastric fluid, body tissues, and other biologics) and require sample preparation for purification and concentration prior to TLC analysis. Separation from biological samples and purification of analytes are usually carried out by techniques such as protein precipitation,[6] desalting,[7] dialysis, hydrolysis, lyophilization,[8] saponification, ultrafiltration, liquid-liquid extraction partitioning with immiscible solvents,[9] immunoaffinity chromatography,[10] adsorption column chromatography, and solid phase extraction (SPE).

One of the most used sample preparation methods is the solvent partitioning procedure that uses chloroform-methanol (2:1) followed by washing the organic phase with 0.88% KCl (the Folch procedure). Column chromatographic and SPE cleanup are carried out by passing sample extracts through a column or cartridge, respectively, packed with a sorbent such as silica gel, Florisil, C-18,[11] Amberlite XAD-2 resin,[12] or an ion exchanger.[7] A series of solvents is passed through the column or cartridge to elute various analytes in different fractions prior to their TLC analysis. Impurities are eluted in a separate fraction and discarded or remain on the column after elution of the analytes.

Stationary Phases

Normal- or straight-phase adsorption TLC or high performance TLC (HPTLC) on silica gel with a less polar mobile phase has been used in the majority of reported analyses of biological samples. An example is the determination of neutral lipids in snail conditioned water and feces of infected snails.[13] Alumina, Florisil, and other inorganic adsorbents have had limited use.[14]

C-18 layers developed with a more polar aqueous mobile phase have been used for the reversed phase TLC of analytes in biological samples, e.g., nicotine and its main metabolite in the urine of pregnant women using acetonitrile-water (88:12) mobile phase[15] and carotenoid pigments in snail tissue and blood.[16] Bonded C-2 and C-8 layers are also reversed phase, while the hydrophilic diol-, cyano (CN)-, and amino (NH_2)-modified layers can function in either the normal or reversed phase mode, depending on the properties of the mobile phase.[17]

Native or microcrystalline cellulose layers provide separations of relatively polar compounds based on the mechanism of normal phase liquid-liquid partitioning,[18] while polyamide layers separate on the basis of degree of hydrogen bonding between the analytes and the layer.[19] Layers containing sodium form sulfonic acid resin (Polygram Ionex-25) were used to separate ionized compounds, such as amino acids by the mechanism of ion exchange,[18] and PEI (polyethyleneimine)-cellulose ion exchange TLC was used to analyze DNA adducts.[20]

Preadsorbent TLC and HPTLC plates have a strip of poorly-adsorptive diatomaceous earth or wide pore silicon dioxide adjacent to the main analytical layer. Band-shaped zones are formed, large applied volumes of dilute samples are concentrated, and very strongly attracted impurities are retained in the preadsorbent (also termed a concentration zone).[21]

Impregnation of layers with buffers, chelating agents such as EDTA,[22] metal ions, or other compounds can increase selectivity for a particular separation. For example, resolution of sugars is improved by impregnation of silica gel with 0.1 M sodium bisulfite solution and pH 4.8 citrate buffer.[23]

Chiral TLC for separation of enantiomers in biological samples has been carried out using ligand exchange, inclusion compounds, charge transfer, ion-pairing, imprinted polymers, cellulose layers, protein phases, and macrocyclic antibiotic phases.[24]

Application of Standard and Sample Solutions

Samples and standard solutions can be manually applied as round spots using a capillary micropipette. It has been shown that initial zones in the form of bands, rather than round spots, allow higher volumes to be applied if the analyte detection limit is low, and bands produce tighter developed zones, higher resolution separations, and better quantitative results by densitometry. Bands are most conveniently and simply produced when samples are manually applied with a calibrated syringe, such as a 10 or 25 uL Drummond digital dispenser, as diffuse vertical streaks to plates containing a preadsorbent spotting strip below the analytical layer. Development with mobile phase automatically produces tight band-shaped initial zones at the sharply-defined preadsorbent-analytical sorbent interface. An automated instrument is often used to apply spots or bands in quantitative analyses by densitometry.[25]

Mobile Phases

The mobile phase (developing solvent) is generally selected empirically using prior personal experience and literature reports of similar separations as a guide. It usually consists of two, three, or four components that can include

water, organic solvents, an aqueous buffer, an acid, and/or a base. An example is the classic Mangold mobile phase for neutral lipid separations on silica gel, consisting of petroleum ether-diethyl ether-glacial acetic acid (80:20:1).[13] Another widely used mobile phase is butanol-acetic acid-water (3:1:1) for separation of amino acids on silica gel and cellulose layers.[18] A number of systematic mobile phase optimization schemes have been designed, e.g., the use of the PRISMA, window diagrams, or overlapping resolution maps methods, or LSChrom software incorporating the Snyder classification theory.[26] Because the mobile phase is evaporated after development and does not interfere with the subsequent detection process, the choice of solvents that can be used in TLC mobile phase mixtures to obtain the needed selectivity is greatly increased compared to HPLC with its continuous flow, dynamic detection in the presence of the mobile phase.

Ten standardized mobile phases have been designated for the separation of 1600 toxicologically relevant substances. Identification is based on a database of R_f values for these systems on silica gel in saturated and unsaturated chambers.[27]

Chromatogram Development

Isocratic, linear, ascending development has been used primarily for analysis of biological samples by TLC and HPTLC. In this method, the mobile phase is contained in a large volume, covered glass chamber or tank (normal chamber or N-chamber). The twin or double trough chamber (Camag) is a special N-chamber that is modified with an inverted V-shaped ridge on the bottom, dividing the tank into two sections. The N-chamber allows development with a very low volume of solvent and easy pre-equilibration of the layer with vapors of the mobile phase or another conditioning liquid (e.g., a sulfuric acid-water mixture to control humidity) or volatile reagent, and has been widely used for biological analyses.

For two-dimensional (2-D) development, the sample mixture is applied in one corner of the TLC plate. The plate is developed with the first mobile phase, dried, and developed with a second mobile phase in a perpendicular direction, causing the components to be resolved over the entire layer surface. Improved separations of complex mixtures, e.g., amino acids in human urine,[28] result because of the doubled separation distance and use of mobile phases with different selectivities in the two developments, especially those having diverse separation mechanisms (e.g., adsorption and partition).

Separations can also be improved by multiple developments of the layer in the same direction with the same mobile phase or different mobile phases, each run the same distance or different distances. For example, triple development with acetonitrile-water (85:15) or ethyl acetate-acetic acid-methanol-water (60:15:15:10) on silica gel HPTLC plates was used to analyze carbo-

hydrates in snails.[29] The Skipski dual solvent system has been used in biological analysis for the separation of neutral lipids when unequivocal separation of acylglycerols from free sterols is required; it consists of development of a 20 cm × 20 cm silica gel layer with isopropyl ether-acetic acid (96:4) for a distance of 11.5 cm, drying in a hood with nitrogen or cool air from a hair dryer, and redevelopment for 16.5 cm with petroleum ether-diethyl ether-acetic acid (90:10:1).[2]

Automated multiple development (AMD) is a method providing very high efficiency and sample capacity; it involves instrumental incremental gradient development over increasingly longer distances with the mobile phases becoming progressively weaker. AMD was applied to the analysis of lipids[30] and porphyrins.[31]

The methods described above involve capillary flow. Overpressured layer chromatography (OPLC) is an instrumental forced flow method in which the mobile phase is pumped through the layer. This leads to decreased development time, low band spreading, and increased zone resolution and detectability.[32] OPLC has been applied to the separation of amino acids in protein hydrolysates.[33]

Zone Visualization (Detection)

Compounds that are naturally colored, such as chloroplast and bile pigments,[16] are viewed directly in daylight or white light with the naked eye. Compounds with native fluorescence are viewed as bright, colored zones on a dark background under 366 nm or 254 nm ultraviolet (UV) light on layers without fluorescent indicator. Compounds that absorb 254 nm UV light, particularly those with aromatic rings and conjugated double bonds, can be detected on an "F-layer" containing a fluorescent indicator or phosphor. When irradiated with 254 nm UV light, absorbing compounds diminish (quench) the uniform layer fluorescence and can be viewed as dark violet spots on a green or pale-blue background.

Universal or selective chromogenic (dyeing) and fluorogenic post-chromatographic derivatization reagents are applied by spraying onto the layer, dipping the layer into the reagent, exposing the layer to reagent vapors, or incorporating the reagent in the mobile phase or layer. Examples of detection reagents used in biological analysis are 10% ethanolic phosphomolybdic acid (PMA) for neutral lipids;[34] 10% cupric sulfate in 8% phosphoric acid for phospholipids;[34] alpha-naphthol for sugars;[29] ninhydrin for amino acids;[18] and iodine-Dragendorff reagent, 1-chloro-2,4-dinitrobenzene, 2,6-dichloroquinone-4-chloroimide, and *p*-dimethylaminocinnamaldehyde for hydrophilic vitamins.[35] Heating without application of a reagent allows detection of certain compounds on NH_2 layers as fluorescent zones.[36]

Immunostaining is an important specific biological detection procedure for TLC related to bioautography.[32] As described earlier,[5] in bioautography, test

organisms are uniformly distributed in an agar or gelatin detector layer. The TLC separation is carried out, mobile phase residues are removed, and the thin layer plate and detector layer are connected, e.g., through a blotting material. After an incubation period of hours or days, solutes display their inhibiting or beneficial action on the test organism. To obtain a result from these actions, radiolabeled substances are used, or a suitable reagent is either added to the detection layer or sprayed onto it after incubation (immunostaining TLC). The inhibition spots then appear as light zones on a colored background, or the reverse. Glycolipids, glycosphingolipids,[37] and gangliosides[38] are among the compound classes that have been detected by immunostaining.

Identification of Zones

R_f values and colors produced by selective detection reagents in comparison with those of standards are used initially to identify unknown sample zones. Identity is more certain if several TLC systems governed by different separation mechanisms are used for these comparisons.

The identity of zones can be confirmed by off-line and on-line coupling of TLC with modern spectrometric methods such as UV/visible,[39] fluorescence,[40] Fourier transform-infrared (FTIR),[41] Raman,[42] and mass spectrometry (MS).[43,44] As examples of applications of these hyphenated methods,[45] drugs have been determined by HPTLC-FTIR,[46] nucleotides by TLC/MS,[47] and drugs by TLC/MS-MS.[48]

Quantitative Analysis

Semiquantitative analysis can be carried out directly by visual comparison of the size and intensity of standard zones against sample zones. Indirect quantification can be based on UV-visible or fluorescence spectrometry[19] after scraping and elution of corresponding standard and sample zones.

Direct quantification using a densitometer is the most common method currently used for quantitative TLC and HPTLC biological analysis. Slit scanning densitometers have been mostly used, but videodensitometers are applied successfully in some cases.[49] A tungsten or halogen lamp is used as the source for scanning colored zones (visible absorption), such as ninhydrin-detected amino acids at 490 nm,[33] and a deuterium lamp for scanning UV-absorbing zones directly or as quenched zones on F-layers. The detector is a photomultiplier tube or a diode array detector.[40] Plots of scan area versus standard weights are established under the same conditions as for the sample zones separated on the same plate.

Many biological quantitative analyses by densitometry have been validated by determining parameters such as accuracy, precision, linearity, and limit of detection and quantification.[50]

Thin Layer Radiochromatography

Location and quantification of separated radioisotope-labeled substances on a thin layer have been carried out by use of x-ray or photographic film autoradiography,[51] zonal analysis with scintillation counting,[52] digital autoradiography (DAR), and storage phosphor screen imaging analysis (also termed bioimaging or radioluminography).

Phosphor image detection has been applied to the analysis of rat serum dosed with ^{14}C-labeled test substance on silica gel 60F plates developed with chloroform-hexane-ethanol-conc. ammonia (75:15:9:1).[53] DAR has been shown to be especially valuable in metabolism, pharmacokinetic, pharmacological, and biochemical studies,[54] and its use was reported for the quantification of the major sebaceous neutral ^{14}C-lipids.[55]

EXAMPLES OF BIOLOGICAL SAMPLE ANALYSIS BY TLC AND HPTLC

This section includes specific applications of TLC and HPTLC to selected analytes and biological sample matrices using the procedures outlined above. In each, a single mobile phase development was carried out in the ascending, capillary-flow mode unless otherwise stated. Sample preparation information is not given, but an in-depth description of techniques for collection and preparation of biological samples will be found in Chapter 4 of Ref.[2].

Amino Acids

Analyte: Homocysteine.
Sample: Blood.
Layer: Cellulose.
Mobile phase: 2-Mercaptoethanol.
Detection: Ninhydrin reagent.
Reference: Ref.[56]

Drugs

Analyte: Pyrazinamide.
Sample: Human serum.
Layer: Silica gel F HPTLC.
Mobile phase: Benzene-ethanol (92.5:7.5).
Detection: Viewing under 254 nm UV light.
Quantification: Absorbance densitometry at 269 nm.

Validation: Calibration was linear over the 20–200 mg L^{-1} range, detection limit was 1 mg mL^{-1}, and average efficiency of extraction was 97%.
Reference: Ref.[57]

Analyte: Ofloxacin, ciprofloxacin, and sparfloxacin.
Sample: Urine and blood.
Layer: Polyamide sheets.
Mobile phase: Micelle solutions with optimum molar ratio of sodium dodecyl sulfate to ethylenediamine tetraacaetic acid (EDTA) of 0.01:0.1.
Detection and quantification: Fluorescence densitometry at 282 nm/ >400 nm.
Validation: Detection limits $1.5–2 \times 10^{-6}$ M, relative standard deviation (RSD) 1.12–5.82%, and recoveries 96.7–104.2%.
Reference: Ref.[58]

Analyte: Cocaethylene and cocaine.
Sample: Urine.
Layer: Silica gel.
Mobile phase: Hexane-toluene-diethylamine (65:20:5).
Detection: Iodoplatinate reagent.
Reference: Ref.[59]

Analyte: Amphetamine and its major metabolites.
Sample: Urine.
Layer: Silica gel.
Mobile phase: Toluene-acetone-25% ammonia-ethanol (45:45:7:3:)
Detection: Viewing under 283 nm UV light.
Identification: HPTLC-FTIR direct coupling.
Quantification: Densitometry at 283 nm or fluorodensitometry at 365 nm/ >450 nm.
Reference: Ref.[41]

Analyte: Acidic, neutral, basic, amphoteric, and quaternary drugs.
Sample: Urine and liver.
Layer: (A) Silica gel, (B) C–18.
Mobile phase: (A) Toluene-acetone-ethanol-conc. ammonia (45:45:7:3), (B) methanol-water (65:35) and methanol-water-conc. HCl (50:50:1).
Detection and identification: Fast Black K salt, iodoplatinate, Dragendorff, Marquis, and Salkowsky reagents and in situ densitometric UV spectral scanning.
Reference: Ref.[60]

Analyte: Deramciclane metabolites.
Sample: Various body fluids.
Layer: Silica gel.

Mobile phase: Butanol–acetic acid-water (4:1:1).
Detection: DAR
Quantification: MS-MS (offline coupling) with fast atom bombardment (FAB) ionization.
Reference: Ref.[61]

Analyte: Gentamycin.
Sample: Plasma and urine.
Layer: Silica gel.
Mobile phase: Chloroform-methanol-20% ammonia (24:22:15).
Detection: NBD-Cl (Fast Blue B salt) reagent.
Quantification: Fluorescence densitometry at 436 nm excitation.
Validation: Precision $<2.8\%$, recovery $92.9\% +/- 1.74\%$ ($n = 5$).
Reference: Ref.[62].

Analyte: Tinidazole.
Sample: Serum.
Layer: Silica gel.
Mobile phase: Chloroform-acetonitrile-acetic acid (60:40:2).
Quantification: UV densitometry at 320 nm, metronidazole internal standard.
Validation: Linearity 1–10 ng, precision 6%, reproducibility 5%, recovery 96%, detection limit 1 mg mL^{-1}.
Reference: Ref.[63]

Carbohydrates

Analyte: Neutral sugars.
Sample: Cell walls.
Layer: Silica gel impregnated with phosphate buffer (0.2 M, pH 6.8).
Mobile phase: Acetonitrile-amyl alcohol-water (3:1:1); three successive developments over 9, 12, and 15 cm.
Detection: *N*-(1-Naphthyl)ethylenediamine dihydrochloride reagent, ng sensitivity.
Quantification: Transmission densitometry at 580 nm.
Reference: Ref.[64]

Lipids

Analytes: Neutral lipids (NL) and phospholipids (PL).
Sample: Apple snail (*Pomacea bridgesii*) whole bodies, digestive gland-gonad complex (DGG), viscera, head-foot, shell, operculum, plasma, and hemocytes.
Layer: Preadsorbent, channeled HPTLC silica gel.

Mobile phase: Petroleum ether-diethyl ether-acetic acid (80:20:1) (Fig. 1) or hexane-petroleum ether-diethyl ether-acetic acid (50:25:5:1) (NL); chloroform-methanol-water (65:25:4) (PL) (Fig. 2).
Detection: 5% Ethanolic PMA (NL); 10% cupric sulfate (PL).
Quantification: Reflectance densitometry at 610 nm (NL) or 370 nm (PL).
Reference: Ref.[65]

Toxins

Analyte: Aflatoxin M1.
Sample: Human urine.
Layer: HPTLC silica gel.
Mobile phase: Chloroform-acetone-2-propanol (17:2:1).
Detection: Natural fluorescence under 366 nm UV light.
Quantification: Reflectance fluorescence densitometry at 366 nm/400 nm settings, 2X enhancement of fluorescence by immersion of the plate in a solution of paraffin in hexane.
Validation: Recoveries were 75–85% in the range 20–100 ng L^{-1}.
Reference: Ref.[66]

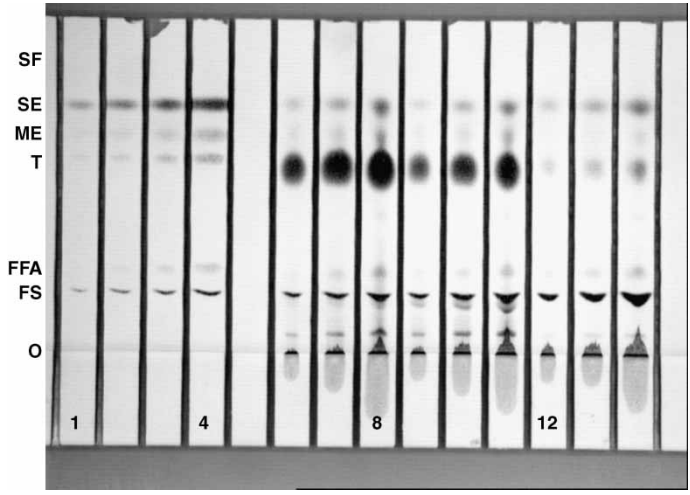

Figure 1. Photograph taken in white light with a Camag VideoStore documentation system of typical high performance thin layer chromatograms of the neutral lipid fractions of *Pomacea bridgesii* snails: 2, 4, 8, and 16 uL, respectively, of a 0.20 ug/uL mixed neutral lipid standard (lanes 1–4), and 2, 4, and 8 uL of a DGG extract (6–8), viscera extract (9–11), and head-foot region extract (12–14). Abbreviations: O, origin; FS, free sterols; FFA, free fatty acids; T, triacylglycerols; ME, methyl esters; SE, steryl esters; SF, mobile phase front. (Reproduced from Ref.[65] with permission of the Research Institute of Medicinal Plants, Budakalasz, Hungary.)

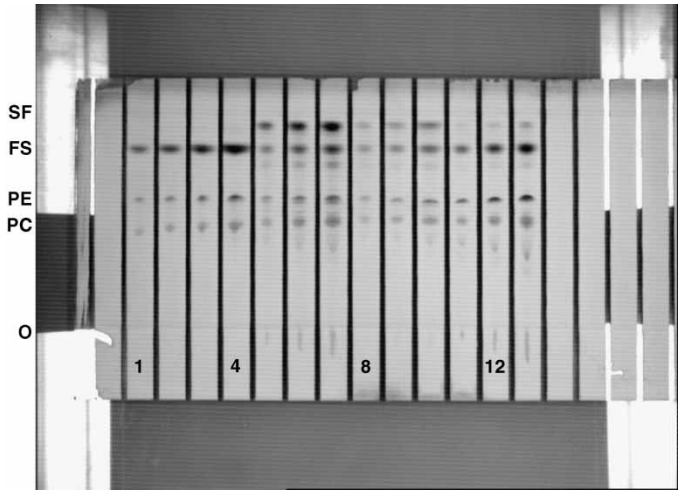

Figure 2. Photograph taken in white light with a Camag VideoStore documentation system of typical high performance thin layer chromatograms of the phospholipid fractions of *Pomacea bridgesii* snails: 2, 4, 8, and 16 uL, respectively, of a 0.25 ug/uL mixed polar lipid standard (lanes 1–4), and 1, 2, and 4 uL of a DGG extract (5–7), viscera extract (8–10), and head-foot region extract (11–13). Abbreviations: O, origin; PC, phosphatidylcholine; PE, phosphatidylethanolamine; FS, free sterols; SF, mobile phase front. (Reproduced from Ref.[65] with permission of the Research Institute of Medicinal Plants, Budakalasz, Hungary.)

Vitamins

Analyte: Vitamin B12 and its derivatives.
Sample: Culture fluids.
Layer: Alumina 60F (type E).
Mobile phase: 2-Methylpropan-1-ol-propan-2-ol-water (3:2:2).
Detection: Natural raspberry colored zones against a white background.
Quantification: Slit-scanning densitometry at 548 nm and videodensitometry.
Reference: Ref.[67]

Indoles

Analytes: Indole derivatives.
Sample: Bacterial culture broths.
Layer: Silica gel 60F.
Mobile Phase: Acetic acid-ethyl acetate-toluene-hexane (4:11:70:15), chosen as optimum by the PRISMA method.
Detection: Densitometry at 280 nm.
Reference: Ref.[68]

Antibiotics

Analyte: *trans*-Resveratrol.
Sample: Bacterial cell culture media.
Layer: Silica gel 60F.
Mobile phase: Chroroform-methanol (80:8) for OPLC.
Detection: Bioautography.
Reference: Ref.[69]

Peptides

Analyte: Amino acid enantiomers.
Sample: Peptides such as enkephalin and gramicidin.
Layer: LKC-18.
Mobile phase: Solvent mixtures containing the chiral selector beta-cyclodextrin (CD), such as methanol-0.2 M CD (3:2).
Detection: Fluorescence densitometry of dansyl chloride derivatives of amino acids from peptide hydrolyzates at 366 nm excitation.
Reference: Ref.[70]

Pigments

Analyte: Lutein and beta-carotene.
Sample: Snail whole bodies.
Layer: C-18 with concentration zone.
Mobile phase: Petroleum ether-acetonitrile-methanol (1:2:2).
Quantification: Densitometry of natural yellow zones at 448 and 455 nm, respectively.
Reference: Ref.[71]

Phenols

Analyte: Chlorophenols (metabolites of the insecticide lindane)
Sample: Urine (metabolism study).
Layer: Silica gel.
Mobile phase: Hexane-benzene-ethyl acetate (6:4:1).
Detection: Ammoniacal silver nitrate reagent and exposure to UV light.
Reference: Ref.[72]

Bile Acids

Analyte: Glycine and taurine bile acid conjugates.
Sample: Body fluids of patients with certain digestive diseases.
Layer: Silica gel 60F HPTLC.

Mobile phase: (A) chloroform-isopropanol-isobutanol-acetic acid water (30:20:10:2:1), (B) chloroform-*n*-propanol-isobutanol-acetic acid-water (30:20:10:2:1); developed once with a mixture of A and B (1:1) followed by three consecutive developments with B.
Detection: 3.5% Ethanolic PMA and densitometric scanning at 625 nm.
Reference: Ref.[73]

Furocoumarins

Analyte: 5- and 9-Methoxypsoralen and trimethyl psoralen.
Sample: Plasma and suction blister fluid.
Layer: C-18F.
Mobile phase: Methanol-water (8:2), optimization with the window diagram and overlapping resolution maps methods.
Detection: Viewing under 254 nm UV light.
Reference: Ref.[74]

REFERENCES

1. Zarzycki, P.; Kulhanek, K.M.; Smith, R.; Bartoszuk, M.A.; Lamparczyk, H. Planar chromatography versus column chromatography: a performance comparison. LCGC North America **2005**, *23*, 286, 288, 292, 294, 296, 299, 300.
2. Fried, B.; Sherma, J. *Thin Layer Chromatography*, 4th Ed.; Marcel Dekker, Inc.: New York, 1999.
3. *Handbook of Thin Layer Chromatography*, 3rd Ed.; Sherma, J., Fried, B., Eds.; Marcel Dekker, Inc.: New York, 2003.
4. *Practical Thin Layer Chromatography*; Fried, B., Sherma, J., Eds.; CRC Press: Boca Raton, FL, 1996.
5. Bladek, J.; Neffe, S. Application of thin layer chromatography in clinical chemistry. Separ. Purif. Rev. **2003**, *32*, 61–120.
6. Boddy, A.V.; Idle, J.R. Combined thin layer chromatography-photography-densitometry for the quantification of ifosfamide and its principal metabolites in urine, cerebrospinal fluid, and plasma. J. Chromatogr. **1992**, *575*, 137–142.
7. Negroni, E.; Chigorno, V.; Tettamanti, G.; Sonnino, S. Evaluation of the efficiency of an assay procedure for gangliosides in human serum. Glycoconj. J. **1996**, *13*, 347–352.
8. Wilson, J.F.; Williams, J.; Walker, P.A.; Toseland, B.L.; Smith, A.; Burnett, D. Performance of techniques used to detect drugs of abuse in urine: study based on external quality assessment. Clin. Chem. **1991**, *37*, 442–447.
9. Cenni, B.; Betschart, B. The determination of halofantrine in biological samples by high performance thin layer chromatography. J. Planar Chromatogr.—Mod. TLC **1994**, *7*, 294–296.
10. van Ginkel, L.A.; van Blitterswijk, H.; Zoontijes, P.W.; van den Bosch, D.; Stephany, R.W. Assay for trenbolone and its metabolite 17 alpha-trenbolone in bovine urine based on immunoaffinity chromatographic cleanup and off-line high performance liquid chromatography-thin layer chromatography. J. Chromatogr. **1988**, *445*, 385–392.

11. Kharat, V.R.; Verma, K.K.; Dhake, J.D. Determination of lacidipine from urine by HPTLC using off-line SPE. J. Pharm. Biomed. Anal. **2002**, *28*, 789–793.
12. Tyrpien, K.; Wielkoszynski, T.; Janoszka, B.; Dobosz, C.; Bodzek, D.; Steplewski, Z. Application of liquid separation techniques to the determination of the main urinary nicotine metabolites. J. Chromatogr. A **2000**, *870*, 29–38.
13. Schneck, J.L.; Fried, B.; Sherma, J. Thin layer chromatographic analysis of neutral lipids in snail conditioned water and feces of *Biomphalaria glabrata* infected with *Echinostoma caproni*. J. Liq. Chromatogr. & Relat. Technol. **2004**, *27*, 2039–2045.
14. Misztal, G.; Paw, B.; Skibinski, R.; Komsta, L.; Iwaniak, K. Analysis of some non-selective calcium channel blockers in normal phase chromatographic systems. J. Planar Chromatogr.—Mod. TLC **2002**, *15*, 458–462.
15. Tyrpien, K.; Bodzek, P.; and Manka, G. Application of planar chromatography to the determination of nicotine in urine of active and passive smoking pregnant women. Biomed. Chromatogr. **2001**, *15*, 50–55.
16. Eidam, P.M.; Schariter, J.A.; Fried, B.; Sherma, J. HPTLC analysis of tissue and blood of *Biomphalaria glabrata* snails to assess the effects of a diet of Romaine lettuce leaf versus midrib on the concentrations of lipids, pigments, and carbohydrates. J. Liq. Chromatogr. & Relat. Technol. **2001**, *24*, 1467–1478.
17. Cserhati, T. Retention behavior of some synthetic nucleosides on CN, diol, and NH_2 precoated high performance thin layer chromatographic plates. J. Chromatogr. Sci. **1991**, *29*, 210–216.
18. Pachuski, J.; Fried, B.; Sherma, J. HPTLC analysis of amino acids in *Biomphalaria glabrata* infected with *Schistosoma mansoni*. J. Liq. Chromatogr. & Relat. Technol. **2002**, *25*, 2345–2349.
19. Li, L.; Shen, G.; Jiang, J.; Cheng, X.; Zhou, X.; Zhang, Ch.; Zhao, G. Thin layer chromatographic determinations of the ratio of serum free branched chain amino acids to aromatic amino acids. Chinese, J. Med. **1985**, *68*, 298–301.
20. Steinberg, J.J.; Cajigas, A.; Brownlee, M. Enzymatic shotgun 5′-phosphorylation and 3′-sister phosphate exchange: a two-dimensional thin layer chromatographic technique to measure DNA deoxynucleotide modification. J. Chromatogr. **1992**, *574*, 41–55.
21. Abjean, J.-P.; Lahogne, V. Planar chromatography for quantitative determination of ampicillin residues in milk and muscle. J. AOAC Int. **1997**, *80*, 1171–1176.
22. Wang, P.-L.; Feng, Y.-L.; Chen, L. Simultaneous determination of norfloxacin, pefloxacin, and ciprofloxacin in body fluid by TLC-fluorescence spectrodensitometry. Microchem. J. **1997**, *56*, 229–235.
23. Anderton, C.A.; Fried, B.; Sherma, J. HPTLC determination of sugars in the hemolymph and digestive gland-gonad complex of *Biomphalaria glabrata* snails. J. Planar Chromatogr.—Mod. TLC **1993**, *6*, 51–54.
24. Bereznitski, Y.; Thompson, R.; O'Neill, E.; Grinberg, N. Thin layer chromatography-a useful techniques for the separation of enantiomers. J. AOAC Int. **2001**, *84*, 1242–1251.
25. Pandya, K.K.; Satia, M.C.; Modi, I.A.; Modi, R.I.; Chakravarthy, B.K.; Gandhi, T.P. High performance thin layer chromatography for the determination of nimesulide in human plasma, and its use in pharmacokinetic studies. J. Pharm. Pharmacol. **1997**, *49*, 773–776.
26. Palamarev, Ch.E.; Kandinska, M.I.; Palamareva, M.D. Automatic selection of mobile phases. IV. thin layer chromatography on silica of simple and complex organic compounds. J. Liq. Chromatogr. & Relat. Technol. **2004**, *27*, 2003–2018.

27. VCH: Weinheim, Germany, 1987 International Association of Forensic Toxicology. Report VII of the DFG Commission for Clinical-Toxicological Analysis (special issue of the TIAFT bulletin).
28. Hsieh, M.C.; Berry, H.K. Detection of metabolic diseases by thin layer chromatography. J. Planar Chromatogr.—Mod. TLC **1992**, *2*, 118–123.
29. Marsit, C.J.; Fried, B.; Sherma, J. Carbohydrate analysis, by high performance thin layer chromatography, of *Cerithidea californica* (Gastropoda: Prosobranchia). J. Liq. Chromatogr. & Relat. Technol. **2000**, *23*, 2413–2417.
30. Farwanah, H.; Neubert, R.; Zellmer, S.; Raith, K. Improved procedure for the separation of major stratum corneum lipids by means of automated multiple development. J. Chromatogr. B **2002**, *780*, 443–450.
31. Bladek, J.; Zdrojewski, A. Thin layer chromatography and solid phase extraction analysis of urinary porphyrins. Chem. Analit. (Warsaw) **2003**, *48*, 689–696.
32. Tyihak, E.; Botz, L.; Ott, P.; Nagy, S.; Kiraly-Veghely, Zs.; Mincsovics, E. The combination of overpressured layer chromatography and bioautography and its application to the analysis of molecules influencing cell proliferation. Chem. Analit. (Warsaw) **2003**, *48*, 543–553.
33. Tyihak, E.; Katy, Z.; Ostorics, Z.; Mincsovics, E. Analysis of amino acids by a personal OPLC system. 1. separation of the main protein amino acids in a double layer system. J. Planar Chromatogr.—Mod. TLC **1998**, *11*, 5–11.
34. Schariter, J.A.; Pachuski, J.; Fried, B.; Sherma, J. Determination of neutral lipids and phospholipids in the cercariae of *Schistosoma mansoni* by high performance thin layer chromatography. J. Liq. Chromatogr. & Relat. Technol. **2002**, *25*, 1615–1622.
35. Ponder, E.L.; Fried, B.; Sherma, J. Thin layer chromatographic analysis of hydrophilic vitamins in standards and from *Helisoma trivolvis* snails. Acta Chromatogr. **2004**, *14*, 70–81.
36. Klaus, R.; Fischer, W.; Hauck, H.E. Extension of the application of thermal in situ reaction on NH_2-layers to the detection of catecholamines in biological materials. Chromatographia **1993**, *37*, 133–143.
37. Muething, J.; Cacic, M. Glycosphingolipid expression in human skeletal and heart muscle assessed by immunostaining thin layer chromatography. Glycoconj. J. **1997**, *14*, 19–28.
38. Muething, J. High resolution thin layer chromatography of gangliosides. J. Chromatogr. **1996**, *720*, 3–25.
39. Ebel, S.; Kang, J.S. UV/Vis spectra and spectral libraries in TLC/HPTLC. 1. Background correction and normalization. J. Planar Chromatogr.—Mod. TLC **1990**, *3*, 42–46.
40. Spangenberg, B.; Post, P.; Ebel, S. Fiber optical scanning in TLC by use of a diode array detector-linearization models for absorption and fluorescence evaluation. J. Planar Chromatogr.—Mod. TLC **2002**, *15*, 88–93.
41. Pisternick, W.; Kovar, K.-A.; Ensslin, H. High performance thin layer chromatographic determination of *N*-ethyl-3,4-methylenedioxyamphetamine and its major metabolites in urine and comparison with high performance liquid chromatography. J. Chromatogr. B **1997**, *688*, 63–69.
42. Wagner, J.; Jork, H.; Koglin, E. HPTLC as a reference method in clinical chemistry: on-line coupling with spectroscopic methods. J. Planar Chromatogr.—Mod. TLC **1993**, *6*, 446–451.
43. Brzezinska, H.; Dallakian, P.; Budzikiewicz, H. Thin layer chromatography and mass spectrometry for screening of biological samples for drugs and metabolites. J. Planar Chromatogr.—Mod. TLC **1999**, *12*, 96–108.

44. Rudloff, S.; Pohlentz, G.; Diekmann, L.; Egge, H.; Kunz, C. Urinary excretion of lactose and oligosaccharides in pre-term infants fed human milk or infant formula. Acta Pediat. **1996**, *85*, 598–603.
45. Cserhati, T.; Forgacs, E. Hyphenated techniques in thin layer chromatography. J. AOAC Int. **1998**, *81*, 329–332.
46. Bauer, G.K.; Pfeifer, A.M.; Hauck, H.E.; Kovar, K.-A. Development of an optimized sorbent for direct HPTLC-FTIR on-line coupling. J. Planar Chromatogr.—Mod. TLC **1998**, *11*, 84–89.
47. Busch, K. Coupling thin layer chromatography with mass spectrometry. J. Planar Chromatogr.—Mod. TLC **1992**, *5*, 72–79.
48. Tames, F.; Watson, I.D.; Morden, W.E.; Wilson, I.D. Analysis of benzodiazepines by thin layer chromatography and tanden mass spectrometry. J. Planar Chromatogr.—Mod. TLC **2000**, *13*, 432–436.
49. Lekeyi, B.; Csanyi, J.; Nanasi, P. A rapid determination of sucrose and fructose in biological samples by videodensitometry. J. Liq. Chromatogr. **1986**, *9*, 1869–1875.
50. Anderton, S.M.; Layman, L.R.; Sherma, J. Determination of blood glucose by quantitative TLC on preadsorbent silica gel plates. J. Liq. Chromatogr & Relat. Technol. **1998**, *21*, 1045–1049.
51. De Maglio, D.C.; Svanberg, G.K. A thin layer chromatographic procedure for the separation of proline and hydroxyproline from biological samples. J. Liq. Chromatogr. & Relat. Technol. **1996**, *19*, 969–975.
52. Corley, R.A.; Swanson, S.P.; Buck, W.B. Glucuronide conjugates of T-2 toxin and metabolites in swine bile and urine. J. Agric. Food Chem. **1985**, *33*, 1085–1089.
53. Hazai, I. Use of multiple readings to increase the sensitivity of phosphor image detection in TLC. J. Planar Chromatogr.—Mod. TLC **2004**, *17*, 449–453.
54. Klebovich, I. Application of planar chromatography and digital autoradiography in metabolism research. In *Planar Chromatography*; Nyiredy, Sz., Ed.; Springer Scientific Publisher: Budapest, Hungary, 2001, 293–311.
55. Christelle, C.; Vingler, P.; Boyera, N.; Galey, I.; Bernard, B.A. Direct quantitative digital autoradiography TLC coupling for the analysis of neutral ^{14}C-lipids neosynthesized by the human sebaceous gland. J. Planar Chromatogr.—Mod. TLC **1997**, *10*, 243–250.
56. Ohtake, H.; Hase, Y.; Sakemoto, K.; Oura, T.; Wada, Y.; Kodama, H. A new method to detect homocysteine in dried blood spots using thin layer chromatography. Screening **1995**, *4*, 17–26.
57. Guermouche, M.-H. Assay of pyrazinamide in human serum by HPTLC. J. Planar Chromatogr.—Mod. TLC **1991**, *4*, 166–167.
58. Feng, Y.-L.; Dong, C. Simultaneous determination of trace ofloxacin, ciprofloxacin, and sparfloxacin by micelle TLC-fluorimetry. J. Chromatogr. Sci. **2004**, *42*, 474–477.
59. Bailey, D.N. Thin layer chromatographic detection of cocaethylene in human urine. Am. J. Clin. Path. **1994**, *101*, 342–345.
60. Ojanpera, I.; Ojansivu, R.-L.; Nokua, J.; Vuori, E. Comprehensive TLC drug screening in forensic toxicology: comparison of findings in urine and liver. J. Planar Chromatogr.—Mod. TLC **1999**, *12*, 38–41.
61. Ludanyi, K.; Gomory, A.; Klebovich, I.; Monostory, K.; Vereczkey, L.; Ujszaszy, K.; Vekey, K. Application of TLC-FAB mass spectrometry in metabolism research. J. Planar Chromatogr.—Mod. TLC **1997**, *10*, 90–96.

62. Bhogte, Ch.P.; Patravale, V.B.; Devarajan, P.V. Fluorodensitometric evaluation of gentamycin from plasma and urine by high performance thin layer chromatography. J. Chromatogr. B **1997**, *694*, 443–447.
63. Guermouche, M.H.; Habel, D.; Guermouche, S. Assay if tinidazole in human serum by high performance thin layer chromatography-comparison with high performance liquid chromatography. J. AOAC Int. **1999**, *82*, 244–247.
64. Batisse, C.; Daurade, M.-H.; Bounias, M. TLC separation and spectrodensitometric quantitation of cell wall neutral sugars. J. Planar Chromatogr.—Mod. TLC **1992**, *5*, 131–133.
65. Jarusiewicz, J.A.; Fried, B.; Sherma, J. High performance thin layer chromatographic analysis of neutral lipids and phospholipids in the apple snail *Pomacea bridgesii*. J. Planar Chromatogr.—Mod. TLC **2004**, *17*, 454–458.
66. Skarkova, J.; Ostry, V. An HPTLC method for confirmation of the presence of utlra-trace amounts of aflatoxin M1 in human urine. J. Planar Chromatogr.—Mod. TLC **2000**, *13*, 42–44.
67. Degtiar, W.G.; Tyaglov, B.V.; Degterev, E.V.; Krylov, V.M.; Malakhova, I.I.; Krasikov, V.D. Quantitative analysis of L-lysine, L-threonine, L-homoserine, and cobalamines in fermentation broth. J. Planar Chromatogr.—Mod. TLC **2000**, *13*, 217–220.
68. Yrjonen, T.; Haansuu, J.P.; Haahtela, K.; Vuorela, H.; Vuorela, P. Rapid screening of indole-3-acetic acid and other indole derivatives in bacterial culture broths by planar chromatography. J. Planar Chromatogr.—Mod. TLC **2001**, *14*, 47–52.
69. Tyihak, E.; Ott, P.; Moricz, A.M.; Katay, G.; Kiraly-Veghely, Z. Antibiosis, Antibiotics, and the formaldehyde cycle: the unique importance of planar chromatographic techniques to progress in these fields. J. Planar Chromatogr.—Mod. TLC **2004**, *17*, 84–88.
70. LeFevre, J.W.; Gublo, E.J.; Botting, C.; Wall, R.; Nigro, A.; Pham, M.T.; Ganci, G. Qualitative reversed phase thin layer chromatographic analysis of the stereochemistry of D- and L-alpha-amino acids in small peptides. J. Planar Chromatogr.—Mod. TLC **2000**, *13*, 160–165.
71. Kim, Y.; Fried, B.; Sherma, J. Thin layer chromatographic analysis of lutein and beta-carotene in *Biomphalaria glabrata* maintained on a high fat diet. Veliger **2002**, *45*, 256–258.
72. Balikova, M.; Novakova, E.; Kohlicek, J. Identification of polychlorinated phenols in urine by gas and thin layer chromatography. J. Chromatogr. **1988**, *431*, 431–437.
73. Lamperti, L.; Vega, M. Separation of hydroxylated conjugated bile acids by instrumental HPTLC. J. Planar Chromatogr.—Mod. TLC **1992**, *5*, 139–140.
74. Makki, S.; Thanh, T.T.; Chinkarenko, T.; Guinchard, C. Optimizing the one-dimensional planar chromatographic separation of the principal psoralens used in therapy. J. Planar Chromatogr.—Mod. TLC **1991**, *4*, 213–217.

Received March 24, 2005
Accepted April 11, 2005
Manuscript 6621