Pharmaceutical Applications of TLC

Huba Kalász, Department of Pharmacology and Pharmacotherapy, Semmelweis University, Hungary,
Maria Báthori, Department of Pharmacognosy, University of Szeged, Hungary.

This article summarizes the application of thin-layer chromatography (TLC) to the separation (analysis) of pharmaceuticals. The vast majority of applied methods involve classic TLC; however, both forced-flow TLC and planar electrophoresis are gaining in importance.

The main practices of TLC, its associated detection methods and a selection of applications will be described.

Introduction

Employment of thin-layer chromatography (TLC) to pharmaceutical and medical/clinical/biological research comprises more then 50% of the technique's total application (1). There are several essential features of TLC, connected to its simplicity, which are important in the analysis of pharmaceutical preparations:

- The separation process is easy to follow; for example, the separation of coloured compounds (Figure 1) and the distortion of chromatographic zones.
- Several samples can be separated in parallel and two-dimensional separations are easy to perform.
- Specific and sensitive colour reagents can be used to detect separated spots.
- Contact detection allows radiolabelled compounds to be monitored and microbial activity in spots to be assessed.
- The TLC plates are disposable; therefore, neither regeneration nor essential clean-up are required.
- Development (separation by the progress of mobile phase through the stationary-phase bed) and detection are generally distinct processes in time. It is for this reason that a development in acetone-containing mobile phase (strong absorption at 254 nm) can be followed by detection at 254 nm.

Sample Application

Various techniques are used for sample application, including spotting with the help of disposable glass capillaries, micropipettes, syringes and capillary dispensers. Spotters can also be used to load a series of spots at the same time. Sample loading is generally performed as a distinct step before the start of development, although on-line sample application is also possible.

Developments

Classic TLC: This is a dipping method, meaning that the dry plate is immersed in the mobile phase, generally in a flat-bottom chamber. The chambers have a single compartment and can be covered with either a glass or stainless steel lid. Twin trough chambers offer several ways to improve the results of development, including low solvent consumption, reproducible pre-equilibration with the solvent vapour, and gas-phase equilibration with volatile reagents. Horizontal chambers are used in the development of HPTLC separations with the mobile phase moving from the edges to the middle of the plates.

Planar chromatography is easy to perform when development is based on capillary forces. A dry stationary phase is used, and the mobile phase is situated at the bottom of the chamber. There is a vapour phase over the mobile phase surface, and in front of the stationary phase, and the stationary phase is backed with a support plate. Equilibrium therefore involves evaporation–condensation between the mobile phase and the vapour phase, as well as between the stationary phase and the vapour phase. Depending on the degree of saturation of the vapour phase, saturated, unsaturated and sandwich chambers are differentiated. The saturated chamber makes a faster separation possible, and the results are less dependent on chromatographic conditions (e.g., temperature, the dimensions and

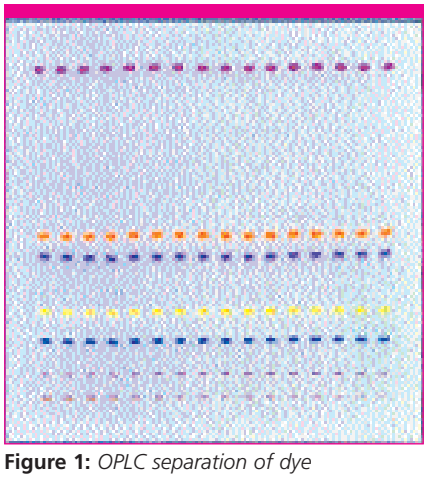

Figure 1: *OPLC separation of dye components.*

shape of chamber, etc.). It is mainly capillary forces that generate mobile phase movement through the stationary phase. As the stationary phase is dry (not wetted previously), the micro profile of the mobile phase is that of the advancing meniscus (i.e., concave). Moreover, there is a constantly decreasing flow velocity, as both the viscosity of the gross mobile phase and the weight of the gross mobile phase mass increases as the mobile phase progresses. These effects can be limited using a mobile phase mixture with limited viscosity; however, they are more obvious when an aqueous mobile phase is applied. Also, the application of stationary phases having fine particles (HPTLC plates) gives relatively fast flow-rates at the beginning of the development (up to 50 mm), but increased offset over 50 mm.

Forced-flow planar chromatography (FFTLC): The shortcomings of classic TLC can be minimized using forced-flow systems. This procedure ensures a constant and optimized flow velocity of the mobile phase, resulting in improved separations (Figures 2–4).

A partial solution came with the use of circular TLC in which centrifugal forces aid development. However, because of the geometrical arrangement of these systems, the flow velocity is continuously changing along the radius of the chromatogram (decreasing with circular and increasing with anticircular), and only the mean flow velocity can be optimized.

Further progress in TLC development arrived with the introduction of over pressured thin-layer chromatography (OPLC) (2). OPLC eliminates the vapour phase and also arranges a closed compartment for the whole development. In spite of eliminating the vapour phase, the system mirrors the characteristics of a super-unsaturated chamber. Moreover, OPLC efficiency is extremely high, and the very low plate height value remains throughout the development. The efficiency gains stem from an improvement in the flow profile as the concave shape of the advancing meniscus can be counteracted by the convex laminar flow (Figure 2). Especially good results are obtained by coupling OPLC with digital autoradiography, as published by Klebovich et al. (3).

The practical advantage of FFTLC is the fast progress of the mobile phase. Runs can be completed in under 1000 s with less than 5 mL of mobile phase.

Multiple developments: Multiple development has been widely used for planar chromatography of pharmaceuticals; for example, with conventional TLC (4), OPLC (5), as well as programmed multiple development (PMD) (6) and automated multiple development (AMD) (7). Multiple development improves separation efficiency, but its wide use is limited by the generation of artifact spots and also by irreversible adsorption (6).

Mode of Development

Elution-type development has been used in the majority of TLC analyses. However, an effective separation can be achieved using displacement chromatography; a method especially useful for the TLC of steroids (8) and other pharmaceuticals.

Monitoring

Pharmaceutical quality control is one of the most strictly validated procedures within chemical analysis. Monitoring can be performed using several methods, including UV/visible detection, radioactivity

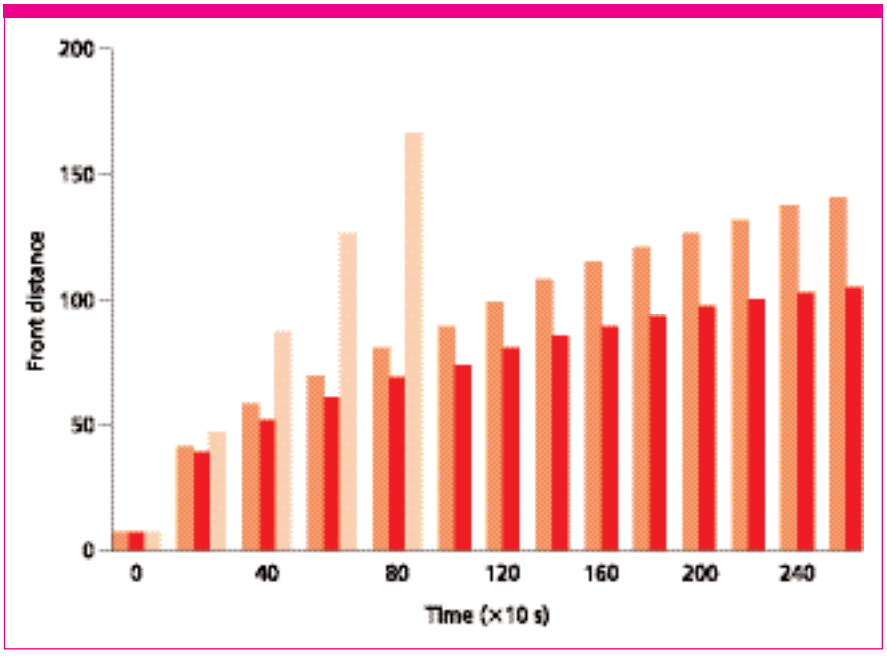

Figure 2: *Front distance versus time characteristics decline in both (■) unsaturated and (■) saturated chambers. (■) Forced-flow thin-layer chromatography provides constant mobile phase flow-rate. A silica gel stationary phase and chloroform mobile phase were used.*

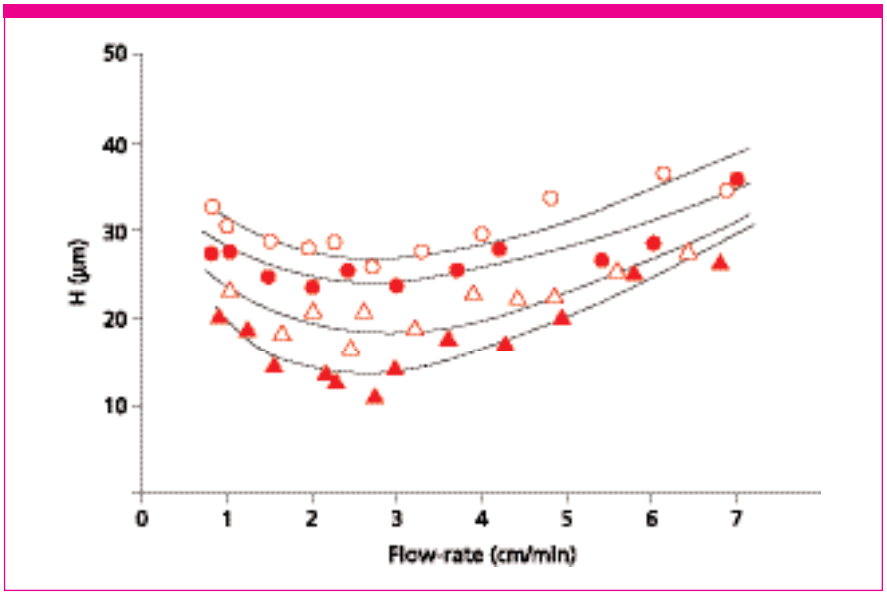

Figure 3: *A plot of theoretical plates versus mobile phase flow-rate illustrates the efficiency of forced-flow thin-layer chromtography. (○) on-line OPLC, (●) on-line sample application, off-line detection, (△) off-line sample application, on-line detection, (▲) off-line OPLC. Reproduced with permission from the Journal of Planar Chromatography.*

"A wide choice of spray reagents…is one of the advantages of planar chromatography."

detection etc. However, evaluation of TLC results is greatly facilitated by its on-line combination with mass spectrometry (9).

The simplest method involves the use of a stationary phase containing a fluorescent compound, with excitation at 254 nm. As a high percentage of pharmaceuticals contain an aromatic ring with the same characteristic, they can be detected on TLC plates as dark spots. A more sophisticated method involves monitoring the UV spectrum in situ on the plate. Consequently, physicochemical information is gained in addition to detection. When quantitative evaluation is performed, the reflection mode is generally used. The vast majority of quantitative evaluation is based on the calibration curve. Alternatively, for estimating the amounts of individual components, the Kubelka-Munk equation may be used.

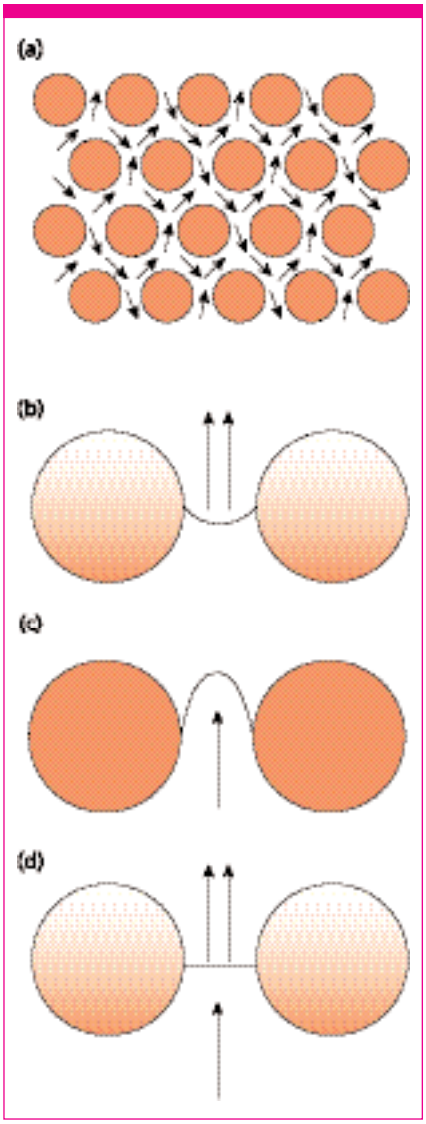

Figure 4: *(a) Micro flow paths of the mobile phase around particles of stationary phase. Flow profiles of (b) TLC, (c) HPLC and (d) forced-flow TLC. The figure segments of (b) and (c) indicate the form of the advancing meniscus and laminar flow, while (d) shows how the advancing menuiscus and the laminar flow may counteract each other.*

A wide choice of spray reagents are available, and this is one of the advantages of planar chromatography. Tests can be performed on specific individual groups of compounds using only a single spray reagent. This method has been used from the very beginning of TLC, with a similar procedure being used for postcolumn reactions in HPLC. Bathori (10) employed a combination of direct observation of the dark spots by UV detection at 254 nm (without using the spray reagent) and plate spraying with vanillin/sulfuric acid, and observation under both visible and 366 nm light. Using this "triple detection", an ecdysteroid-specific monitoring of the spots was possible. Another application of this sensitive detection technique involves the use of an intercalation dye to label PCR products when DNA specificity is required.

The use of contact detection methods also facilitate the diversity of TLC. Autobiography to detect antibiotics and X-ray methods to monitor radiolabelled compounds have been widely used. The latter has been applied to the sequencing of nucleic acids, as a simple and inexpensive procedure.

A more sophisticated method has been applied to the detection of metabolites of a radiolabelled drug by digital autoradiography, and their subsequent identification by FAB-MS or FAB-MS–MS (11).

Quantitative Analysis and Validation

Ebel (12) published an extensive review on the methodology of TLC and HPTLC quantification. He explained the principles of reflectance measurements, including the Kubelka-Munk equation, and the reason for inhomogeneity of photometric absorption. The possibilities and limits of calibration and evaluations were also detailed, including the sources of systematic and statistical errors.

Homogeneity Control and Stability Studies

Stability studies deal with the storage of drug preparations, and are generally required by various national and international drug administrations. They are subject to strict methodological control, each step of which must be validated. Certain advantages of TLC are useful in this process; for example, parallel analysis of several spots at the same time and the visualization of spots with poor or no migration through the chromatographic bed. A purity test for a Pausogest tablet is shown in Figure 5. In the vast majority of instances a one-dimensional run is used.

Another example involves investigating compound stability during the separation process. These problems can be addressed using two-dimensional TLC. In the instance of partial decomposition, doubled spots will result from the first dimensional run. However, only one of these spots will be doubled during the second dimensional development; that is, the unchanged compound will be doubled, but the degraded compound will not.

Analysis and Identification of Drugs in Body Fluids

Singh et al. (13) reviewed the use of TLC for drug screening and confirmation of drug presence in urine. Brzezinka et al. (14) published results for the identification of unknown compounds from various body fluids. They used a combination of TLC and MS. Eight-peak mass spectra and TLC behaviour (hR_F) in three different mobile phases for almost 500 different drugs were determined. The molecular ion of the EI mass spectra and elementary compositions are also given.

Romano et al. (15) reviewed the qualitative analysis of drugs and metabolites. They used principal component analysis to evaluate standardized R_F values of 443 drugs and their metabolites investigated in four different mobile phases.

Ojanpera et al. (16) outlined the possibilities of TLC application in forensic toxicology on the basis of their results during a three-year period. Urine samples were subjected to hydrolysis with β-glucuronidase and liver samples were treated with trypsin. To screen for the presence of acidic and neutral drugs, an octadecyl silica stationary phase was recommended. At the same time, both plain silica and octadecyl silica were suggested for the identification of basic, amphoteric and quaternary drugs. Sixty different drugs were found in the 618

instances of the medical examiner. Some of the drugs (acebutolol, diclofenac, diflunisal, hydrochlorothiazide, metoclopramine, naproxen, ranitidine, sotalol and tiaprofenic acid) were found exclusively in urine.

Certain other drugs (clomipramine, fluoxetine, perphenazine and verapamil) were detected solely in the liver.

Analysis and Identification of Drug Residues

Determination of drug residues in food is an important application of TLC. Beef, pork (17), fish, poultry, milk etc. can be subjected to

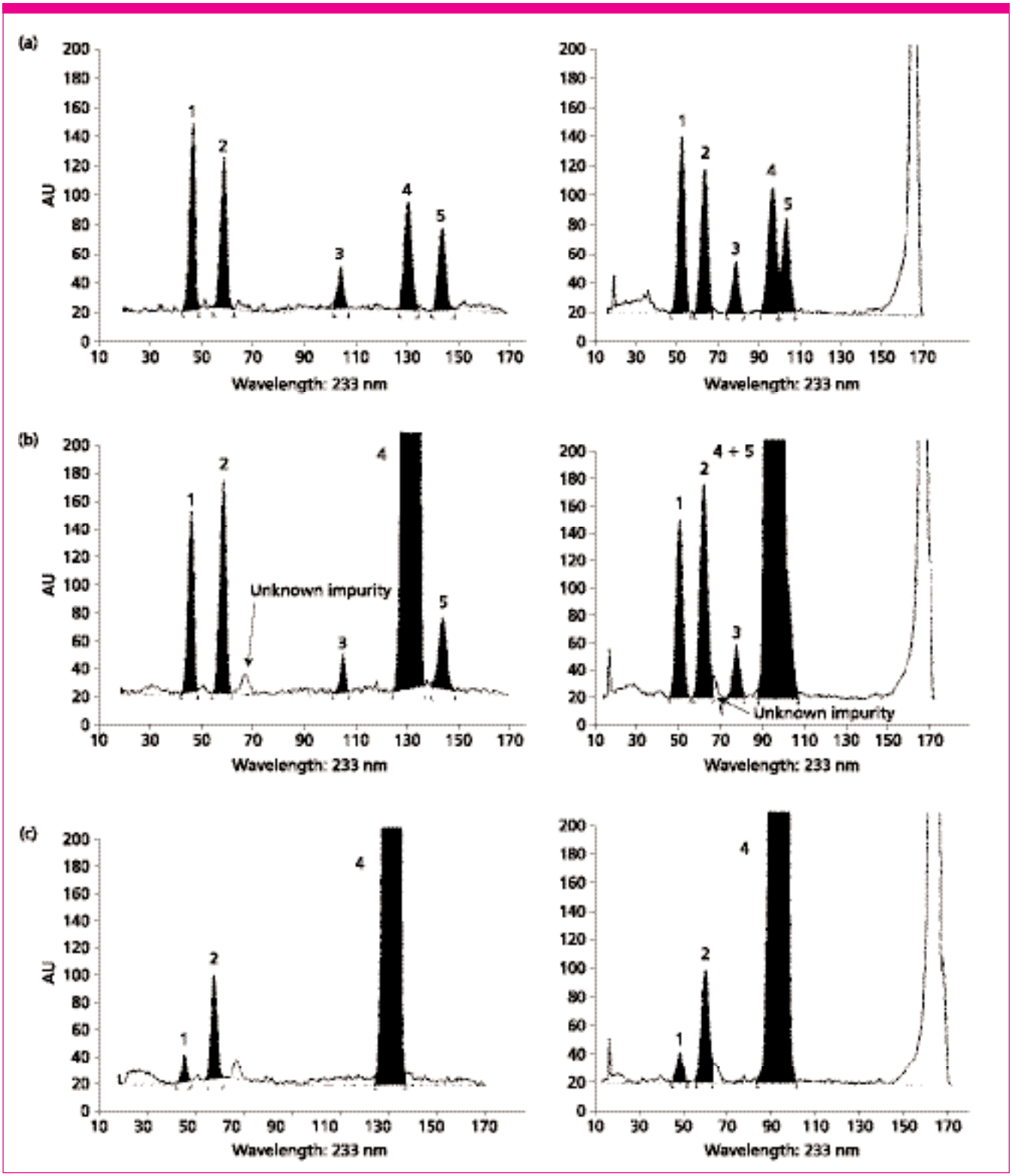

Figure 5: *A comparison of OPLC (left) and normal chamber TLC (right). (a) phtaloyl amlodipine and its impurities (0.5 µg each); (b) 50 µg phtaloyl amlodipine spiked with 0.5 µg impurities; (c) 50 µg phtaloyl amlodipine. Peaks: 1 = phtalimido ester of phtaloyl-amlodypine, 2 = diether of phtaloyl-amlodypine, 3 = dicrotonate of phtaloyl-amlodypine, 4 = phtaloyl-amlodypine, 5 = i-propyl ester of phtaloyl-amlodypine. Reproduced with permission from the Journal of Planar Chromatography.*

analysis of multiclass, multiresidue screening. Antibiotics were identified and semiquantitatively determined using bioautography by Gafner (18).

Metabolic Studies

Both autoradiography and digital autoradiography greatly facilitate determination of the metabolic profile of drugs (11). Either ^{3}H or ^{14}C labelling, or double labelling can be used, and the results of digital autoradiography may be verified by counting of the scraped spots.

Determination of Physicochemical Characteristics

Spectral characteristics are determined by off-line or on-line methods. The UV/visible determination of separated spots is frequently performed on-line. Similarly, there are publications describing determination of the mass spectra (19), nuclear magnetic resonance (NMR) spectra (20) and the infrared spectra off-line and occasionally on-line. HPTLC–FTIR can be performed on-line (21), however, physically removing material from the spot (including a portion of the stationary phase) enables rapid identification (11).

Drug Receptors and Binding Sites

Two major studies have been published in this area. The first involved the identification of receptor binding sites on calcium channels using photoaffinity labelling, and their subsequent isolation by solubilization, size-exclusion chromatography, enzymatic fragmentation and immunprecipitation. The size of the labelled (binding) protein, and that of the labelled fragment were determined by planar electrophoresis.

The second study involved the investigation of allelic forms of dopamine receptors. It is believed that dopamine receptors play an important role in the physiology of drug abuse. A screening programme was developed to compare the allele specificity of dopamine receptors in a control (normal) population with that found in a group of drug abusers. Planar electrophoresis on agarose gel enabled determination of the allele size after a non-invasive sample collection, PCR multiplication, and fragmentation (Boór, personal communication).

Comparison with HPLC

Renger recently published a comparison of HPTLC and HPLC analyses (9).

Use of Pharmaceuticals in Planar Chromatography

A special use of pharmaceuticals is in chromatographic media. Vancomycin can be used as a chiral discriminator in the separation of optical isomers of amino acids (22). The results of a validation process are given in Table 1.

Future Progress

A novel variant of OPLC has been developed and it is being successfully applied to pharmaceutical analysis. By monitoring a TLC separation with digital autoradiography, 2D photography etc, an improved and faster quatitative evaluation was possible. On-line combinations of spectroscopic methods with planar chromatography promises fast and reliable identification. Planar electrochromatography, displacement thin-layer chromatography, and their combination will be methods of choice in the future.

Table 1: *Results of Validation of Paraben using Thin-Layer Chromatography (27).*

Parameter		Methyl paraben	Propyl paraben	Butyl paraben
Specificity	R_F	0.32	0.40	0.52
	T	1	1	1
	R_s	1.5		2.0
Linearity	Range (ng spot^{-1})	360–1080	80–240	40–120
	Slope	1.2	2.0	2.1
	Intercept	370	28	3.4
	R	0.9992	0.9992	0.9994
Accuracy	S (%)	99.8	99.8	101.6
	RSD (%)	2.3	1.4	1.2
Precision-				
-repeatability of measurement	RSD (%)	0.7	0.5	1.0
-repeatability of sample preparation	RSD (%)	1.4	2.6	2.8
Least detectable concentration (LD)	Concentration (ng spot^{-1})	< 9	4	4

Drug Monitoring

Therapeutic and forensic drug monitoring is an important task. Books dedicating special chapter(s) to detail TLC of drugs (include Stahl (23), and Wagman and M.J. Weinstein (24). Important publications were devoted to providing an overview (e.g., 25–26).

An extensive report by Ferenci-Fodor et al. (28) described in situ densitometric purity testing of drug substances. Based on the International Conference on Harmonization, Validation of Analytical Procedures (29), several characteristics of drug substances were included in the analytical validation.

Antiepileptics: Aboul-Enein and Thiffault (30) determined primidone and its urinary metabolites using TLC on silica.

Analgesics: TLC is a preferred method for analysing the active components of analgesic tablets and caplets (31–38). Acetylsalicylic acid, salicylic acid, paracetamol, phenacetin, acetaminophen, caffeine, ibuprofen, ketoprofen, oxaprozin, etc. are routinely determined on silica gel and silica gel HPTLC plates containing a fluorescent indicator and using a wide variety of mobile phases. A highly sophisticated variation of TLC involves monitoring drugs and metabolites with MS or tandem MS (39).

Anxiolytics: A basic review covering the TLC of benzodiazepines was written by Klimes and Kastner (40). In it, the authors detail the separation and identification of benzodiazepine standards and benzodiazepines from pharmaceutical preparations, and evaluate benzodiazepines in biological materials. The review provides all the important separation parameters, such as the drugs tested, the stationary and mobile phases used, the mode of detection, together with a list of important references. Purity and metabolism testing of deramciclame were performed by OPLC (41).

Numerous benzodiazepines were characterized by Volf (42). Silica plates were used and a colour reaction was performed using Bratton-Marshall reagent (consecutive spraying with sodium nitrite in HCl followed by *N*-1-naphtylethylenediamine in ethanol) after thermal treatment of the separated spots. A mobile phase of chloroform–acetone (8:2) provided a good separation and the Bratton-Marshall reagent gave colour characteristics to individual benzodiazepines.

Abuse Drugs: Drug abuse is not restricted

to any single group of chemical compounds or pharmacological activity. Skalican et al. (43) used TLC to analyse psychotropic drugs, such as scopolamine, ephedrine, morphine, cocaine, physostigmine, ethylmorphine, codeine, phencyclidine and LSD. Forty different mobile phases were investigated, and the authors also checked the affect of atmospheric humidity, temperature, time of saturation of the vapour phase etc.

Two-dimensional TLC was used for the investigation of opiates by Novakova (44). The detection was performed using either Dragendorff's or Marqui's reagent, fast blue B or fast black K. Morphine was also detected in the form of its dansyl derivative.

Morphine, codeine and their derivatives were investigated by Kalász et al. (45), with the work including determination of R_F values, lipophilicity and behaviour using displacement chromatography.

Amphetamines were identified by Fater et al. (46). Unidimensional single development, unidimensional multiple development and two-dimensional development were used to separate the various amphetamine derivatives. Multiple detection (UV at 254 and 366 nm, and using postchromatographic derivatization with Marquis reagent) improved both sensitivity and specificity.

Morphine, caffeine and paracetamol were simultaneously determined in urine samples by Krishnamurthy et al. (47). A simple method on HPTLC silica plates was used for determination, and a control was provided by reversed-phase HPLC. The correlation coefficients for the concentration response analysis were 1.0, 0.99 and 0.94 for morphine, caffeine and paracetamol, respectively.

Antidepressant and antimania agents: Wiater et al. (48) optimized the TLC separations of several drugs, including opipramol, amitriptyline, imipramine, doxepin, chlorpromazine and promazine. Both plain and RP-18 silica were used as stationary phases, and visualization was achieved by spraying with concentrated sulfuric acid or Dragendorff reagent.

Lambroussi et al. (49) investigated the TLC behaviour of fluoxetine, norfluoxetine and promethazine using silica gel impregnated with paraffin and inclusion complexes with cyclodextrin.

Antiparkinsonian drugs: (–)-Deprenyl (*Jumex*®, *Eldepryl*®, *Movergan*®, etc.), its metabolites and several of its analogues were subjected to planar chromatography (50). Both straight-phase and reversed-phase TLC, and displacement TLC (8, 51) were used.

Antihistamines: Two-dimensional separations of ten different, but structurally related, H_1-antihistamines were described by Muller and Ebel (52). Computer-simulated, two-dimensional separations were used for method optimization incorporating a selection of five different mobile phases, together with silica gel, silica gel RP 18W and silica gel CN HPTLC plates.

Impregnation of silica gel with transition metal ions improves the separation of antihistamines (53). The Mn(II), Fe(II), Ni(II) and Cu(II) content of the silica gel increased the R_F value using a benzene–butanol–acetic acid–water mobile phase, and also improved the separation power of the system.

Drugs acting on the cardiovascular system: Schutz and Meister (54) separated 20 β-blockers on silica using several different mobile phases. The detection process involved spraying with either *o*-phthalaldehyde or ammonium vanadate, or a prechromatographic derivatization using dansyl chloride. The lipophilicities of some antiarrhythmic and antihypertensive compounds were determined by Malawska et al. (55), and the dependence of lipophilicity on pH was also investigated.

Verapamil hydrochloride, from various formulations, was determined using HPTLC by ElGhany et al. (56). The calcium channel blocker diltiazem was analysed both raw and from dosage forms (57). The separation was performed using HPTLC plates, and monitored at 240 nm. Tivert and Backman (58) used ZPG (*N*-benzoxycarbonyl-glycyl-L-proline) as a chiral discriminator to separate enantiomers of β-blockers with aminoalcohol structures. The analysis was performed using standard compounds as well as samples of controlled-release tablets. Szikszay et al. (59) compared quantitative purity tests performed by OPLC and classic TLC (Figure 5).

Non-steroidal anti-inflammatory drugs (NSAIDs): R_F values for 35 NSAIDs were determined using TLC (60). Normal-phase TLC was adequate for their separation; however, reversed-phase was shown to be a useful alternative to complete the

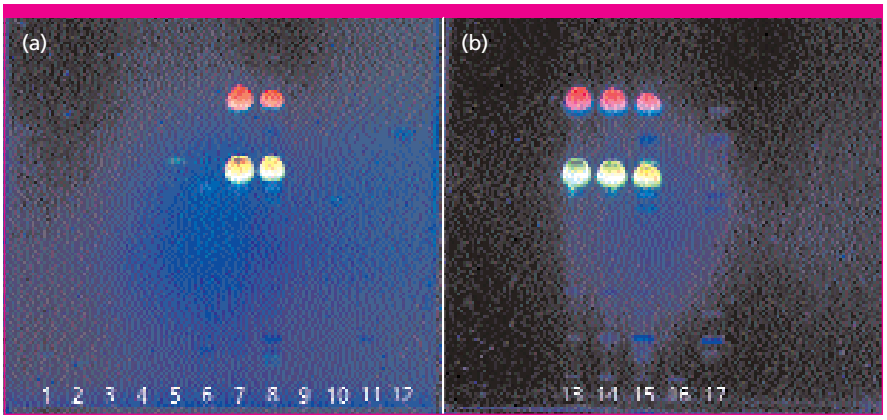

Figure 6: *(a) and (b) HPTLC silica plates with OPLC development. Mobile phase:* n*-hexane–butyl acetate–ethyl acetate–chloroform (60:15:15:20 v/v/v/v for OPLC or 30:15:15:20 v/v/v/v for development in a glass chamber). The experiments were performed using either a Personal OPLC (OPLC-NIT Ltd, Budapest, Hungary) with 5 MPa external pressure, or a saturated normal glass chamber (Desaga, Heidelberg, Germany). The developed plates were evaluated using a TLC Scanner 3 (Camag, Muttenz, Switzerland) at 233 nm with reflectance mode. 1 = 0.3 µg of 6-alpha-hydroxy-estradiol (degradation product), 2 = 0.3 µg of 6-beta-hydroxy-estradiol (degradation product), 3 = 0.3 µg of 6-keto-estradiol (degradation product), 4 = 0.3 µg of estrone (degradation product), 5 = 0.3 µg of 4-chloro-estradiol (impurity originated from the synthesis of the active ingredient), 6 = 0.3 µg of 9,11-dehydro-estradiol, 7 = Pausogest corresponding to 50 µg of norethisterone acetate and 100 µg of estradiol, 8 = Pausogest corresponding to 25 µg of norethisterone acetate and 50 µg of estradiol, spiked before sample preparation with impurities and degradation products, 9 = placebo, 10 = 0.3 µg of norethisterone (impurity and degradation product), 11 = 0.3 µg of 6-hydroxy-norethisterone acetate (degradation product), 12 = 0.3 µg of 6-keto-norethisterone acetate (degradation product), 13 = 50 µg of norethisterone acetate and 100 µg of estradiol used for production of Pausogest tablet, 14 = Pausogest corresponding to 50 µg of norethisterone acetate and 100 µg of estradiol, 15 = Pausogest corresponding to 25 µg of norethisterone acetate and 50 µg of estradiol, spiked before sample preparation with impurities and degradation products, 16 = placebo, 17 = mixture of impurities and degradation products (0.3 µg each). Reproduced from the material of presentation (91) by permission.*

separations reached by normal-phase TLC. Torok and Paal (61) determined novamidazofen using TLC on silica. Nimesulide was determined in commercial brands of Nimulid tablets suspension by Argekar and Sawant (36, 62). The assay also investigated the stability of tablets when exposed to stress conditions, such as heat at 80 °C, low pH (0.1 M HCl), high pH (0.1 M NaOH) and oxidation (30% H_2O_2). Forgacs et al. (63) determined the hydrophobicity parameters of 18 NSAIDs.

Vitamins: Vitamin B_1 and K_4 were analysed in pharmaceutical preparations by Hachula (57, 64). The method comprised TLC, followed by extraction and either spectrophotometry or fluorescence scanning after derivatization (64).

Medicines for the gastrointestinal tract: Lansoprazole is a proton pump inhibitor used to treat peptic ulceration. Quantitative analysis of lansoprazole was performed adequately on HPTLC silica plates with a mixture of ethyl acetate–methanol–ammonia. The method could determine the potential degradation products of lansoprazole (65). Determination of silymarin in Yiganling capsules was achieved on silica gel by Liu and Yang (66), who emphasized the advantages of TLC.

Anabolics compounds: An HPTLC method was described (67) for 46 anabolic compounds at injection sites, including their extraction, HPTLC separation and fluorescence detection. Some hormonal anabolic compounds have been detected at the ppb (parts per billion) level by TLC (68).

Antidiabetics: Some antidiabetic drugs and their degradation products have been separated using TLC (69) from dosage forms including gliclazide, glipizide and glibenclamide.

Cytostatic drugs: Metabolism of ifosfamide enantiomers, and six ifosfamide metabolites were separated on HPTLC silica by Blaschke and Widey (70); detection was by autoradiography.

Diuretics: Bernhard et al. (71) described an analytical method for monitoring hydrochlorothiazide in urine. This drug is used for the treatment of hypertonicity (often in combination with β-blockers) and also as a doping agent. TLC silica plates provided a reliable analysis. Quantitative determination was performed both with and without postchromatographic derivatization.

Potassium canrenoate, a diuretic steroid, was determined using reversed-phase ion-pair OPLC (85). The dependence of R_F values on the ratio of water to organic modifier (methanol) and on the concentration of ion-pairing agent (ethanolic cetrimide), and the dependence of theoretical plate height on the linear velocity of the mobile phase were also described.

Antimicrobial agents: Separation of sulfonamides has been a central project for TLC users as this group of drugs are widely used for treating both human and animal populations. In addition to the drug tablets (72, 73), urine (45) and tissue (liver and muscle) residue levels have been widely tested.

Quantifications of penicillins, cephalosporins and several other types of antibiotics were performed by Dhanesar (74, 75). These studies included determining the linear calibration curve and densitometer scan (spectrum) of antibiotic spots on hydrocarbon-impregnated HPTLC silica gel plates. Penicillins were also determined using hydrocarbon-impregnated HPTLC plates (76).

Quintens et al. (77) used an in-house silanized silica gel stationary phase to analyse 30 cephalosporins (40). Quantitative TLC and HPTLC analysis of neomycins A, B and C was performed by Funk et al. (78). The stationary phase was prewashed by complete immersion in 2-propanol for at least four hours, followed by drying at 110 °C for one hour. Ascending developments were performed at both 21 and 50 °C using methanol–25% ammonia–acetone–chloroform mobile phase. Postchromatographic derivatization using fluorescamine was performed, and quantification of the bright pale blue fluorescent zones was achieved with fluorimetric scanning (302 nm excitation, 400 nm emission).

Naidong et al. implemented a series of assay and purity controls for various derivatives of tetracyclines (79, 80), chlortetracycline and demeclocycline (bb), and a comparison was made with liquid (column) chromatography. The separation of doxycycline, tetracycline and oxytetracycline on sodium EDTA-treated silica was demonstrated by Xie (81).

Argekar and Powar (82) performed the simultaneous HPTLC determination of diloxanide furoate (antiamoebic drug) and tinidazole (antiprotozoal and antiamoebic drug) tablets. Silica gel 60 F_{254} and dichloromethane–methanol (9.6:0.25, v/v) were used as stationary and mobile phases, respectively. The resolution of these compounds was 2.6, and the relative standard deviations of the determinations ($n = 5$) were 1.1 and 1.3 for diloxanide furoate and tinidazole, respectively. Ciprofloxacin hydrochloride and tinidazole (83) were also determined in tablets.

Aminoglycoside antibiotics, such as amikacin, paromomycin, gentamycin, sisomicin and netilmicin were separated using HPTLC plates. Vega et al. (84) screened antibiotic residues in poultry meat, and described the extraction, clean-up and TLC for the quantitative determination of gentamycin, streptomycin, erythromycin, chloramphenicol, etc.

Steroids: A review of the thin-layer (and paper) chromatography of steroids has been published by Heftmann (86). The normal-phase TLC of steroids was investigated by Windhorst et al. (87). Also, 72 steroids were studied on HPTLC silica, using 15 different mobile phases.

Poole et al. (88) proposed TLC systems for the separation of pharmaceutically important oestrogens. Ferenczi-Fodor et al. (89) investigated the potency of irreversible adsorption during multiple development by TLC and OPLC of several steroids, including allylestrenol, levonorgestrel, estrone, estradiol etc. Ferenczi-Fodor et al. (90, 91) analysed the drug purity/impurity using OPLC. The purity test of a Pausogest tablet is shown in Figure 6.

Medicinal Plants and their Products

A wide variety of traditional medicinal plants and plant extracts have been investigated by TLC. These separations were mainly achieved using TLC silica gel and various mobile phases with monitoring at 254 nm, or following specific spray reagents. Many of these results have been published in Chinese; for example, in the *J. Chinese Trad. Patent Medicine* (Zhongchengyao), *Chinese J. Pharm. Anal.* (Yauwu Fenxi Zazhi), *J. Chinese Herb. Med.* (Zhongcayao), *J. Chin. Trad. Med.* (Zhongguo Zhongyao Zazhi), *J. Shanyang. Pharm. Univ.* (Shenyang Yaoke Daxue Xuebao), *J. West China Med.* (Zhongguo Zhongyao Zazhi) to name but a few.

Bathori et al. (92) separated a wide variety of plant ecdysteroids using both reversed-phase and normal-phase TLC. Identification was performed by developments in several different mobile phases, and employing triple detection.

Gyeresi et al. (93) produced a summary on the employment of chromatographic methods recommended by various pharmacopoeias. TLC was used for the analysis of several medicinal plants and products originating from medicinal plants. The components analysed were divided into several classes, including carbohydrate drugs, glycosides, fatty acids and other lipids, terpenes, balsams, resins and alkaloids. TLC was recommended for identification, purity testing and assay (92).

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