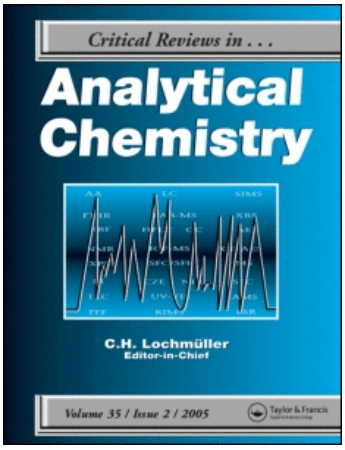

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H. M. Stahr; Daniel W. Armstrong

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PLANAR CHROMATOGRAPHY APPLICATIONS IN ANALYTICAL TOXICOLOGY

Author: **H. M. Stahr**
Veterinary Diagnostic Laboratory
Iowa State University
Ames, Iowa

Referee: Daniel W. Armstrong
Department of Chemistry
Texas Technological University
Lubbock, Texas

I. PLANAR CHROMATOGRAPHY

A. Recent Practice

Planar chromatography is a natural tool for analytical toxicology. This technique allows chemical analysis to be done on a surface in such manner that the analyst can observe and control the process to achieve the desired end. The goal of this analysis is to fully separate and identify an unknown chemical that caused a harmful (often fatal) effect, usually on a biological system or entity. The most sophisticated confirmation may be made by spectral analysis, or the simplest colorimetric detection may be made. On a preparative scale, isolation and full characterization may also be made.

Applied to samples that are unpredictable, planar chromatography allows development of adequate separation by the analyst even if published techniques are not available for a sample or sample matrix. It is a surface for doing analytical chemistry.

This paper will attempt to organize applications of planar chromatography by analyte. There are many fields of analytical toxicology where planar separations are employed, including forensic, veterinary, human, environmental, clinical, pharmaceutical, food and cosmetic, toxicology, and many more. For example, analyses for drugs of abuse are, depending on the purpose, pharmaceutical or pharmacological, forensic, environmental, clinical, and veterinary or human toxicology. The levels of significance are not likely to be the same for each application. For instance, etorphine may be used clinically as a tranquilizer (high doses) or as an illegal drug to induce stimulation in racing horses (at ppb).

Paper chromatography, the oldest planar technique, is still employed. Sherma and Fried[1] report paper chromatography has delcined markedly during 1982 to 1983. They quote Maugh,[2] as saying "TLC [thin layer chromatography] has increased during 1982 to 1983 to become as popular as HPLC [high performance liquid chromatography]." Macek[3] states that 27% of all chromatographic publications were devoted to TLC. In this project, a computer search was made for analytical toxicology application of planar chromatography. Over 700 publications were found during the last 5 years by this search. While numbers of publications do not always correlate with the use of the technique, TLC or planar chromatography PC, thin layer chromatography or planar chromatography, has become the standard technique in racing-animal testings, pharmaecutical, food, feed, forensic, and toxicological laboratories.

Recent developments in planar chromatography have caused the demise of paper chromatography. Acetylated (paper) cellulose was very useful in Benzo (A) pyrene analysis.[4] However, acetylated cellulose even as a coating on a solid plate is less effective than reverse phase (C^{18}) silica gel surfaces[5] for this analysis. All the problems which were solved by acetylate cellulose (irreversible absorption of analyte, rapid oxidation of analyte when solvent surfaces were exposed to UV, adequate recoveries of analyte for fluorimetric analysis, or even *in situ* analysis by densitometry) are all better achieved by C^{18} reverse phase TLC.[6]

The ability to resolve bands of very polar materials (proteins, sugars, acids, amines) by aqueous solvents is possible with reverse-phase C^{18} silica plates.[7]

The greatest achievement of planar chromatography for the quality control industry is the speed of analysis it provides. Kaiser[8] has reported that as many as 50 plates each having 20 analytical samples applied at each end of the plate or a total of 40 × 50 (2000) analyses have been done by overpressurized planar chromatography, all in less than 1 hr. With computerized control of densitometric analysis,[9] the planar chromatography technique becomes the most efficient of analysis tools.

While any technique is limited only by the imagination and innovative ability of analysts, paper chromatography suffers from serious physical limitations. The physical properties of cellulose necessary to make a sheet and the materials used in making the sheet cause an undefined surface area, and provides capillary porosity which can cause analyte loss. Coating a solid surface (glass, aluminum, plastic) with a uniform material provides a much more controllable analysis surface for planar chromatography.

Reverse phase (nonpolar) and normal phase (polar) surfaces are available from most manufacturers and even degrees of polarity are also available. Capillary-driven development may be vertical, angular, or horizontal. Programed multiple, overpressurized forced, gravity-driven, circular, and anticircular development have all found a place[9] in planar chromatography. Solvent optimization has been studied, and schemes to assist in individual applications have been made by Heilweil[10] and Snyder et al.[11] Armstrong et al.[12] have shown that colloidial mobile phases may be used for separations of high molecular weight polymers and as development fluids for analytes.[13] Stahr and Martin[14] have applied these systems to analysis of mycotoxins. Beesley and Armstrong have made coatings of cyclodextrins on planar surfaces and Stahr and Martin[14,14a] and Domoto have used these surfaces to separate mycotoxins.

Commercial densitometers are either manual or automatic and use recorder, integrator, or computer readouts. These devices may scan one channel at a time or the whole surface. The Kontes 800 Scanner® is the least expensive of all densitometers. Other well-accepted scanners are the Shimadzu and LKB Instruments. One system, the Ca Mag,[9] allows complete scanning of the plate before development and after to provide a three-dimensional, computer-drawn representation of the analytical separation to optimize the quantitative analysis. Another system developed by BioRad Corporation allows video presentation and operator interaction with a microcomputer driven software system for analysis of one channel the length of a plate. Then quantitative analysis and readout is made of the results with the system.[15]

Automatic applicators[16] apply single or multiple bands of analyte to planar surfaces. Manual devices allow a submicro direct application of an analyte portion. Such well-defined and reproducible applications are valuable for conventional TLC and absolutely essential for micro high performance instrumental planar chromatography.

Observation or detection of bands may be done by visual (manual) or instrumental devices. The Zeis system[17] allows direct spectral and fluorometric analysis of bands on planar surfaces. Laser detection of fluorescent bands[18,19] probably is the most sensitive of all spectral detection techniques.

Mass spectral[4,20] analysis of eluted bands automatically or by manual elution is probably the next most sensitive technique. Fluorescence or fluorescence quenching, colorimetric, and charring are relatively sensitive in that respective order. Automatic dipping[22] to enhance fluorescence or to provide a uniform application of a reagent is possible. Spraying with a reagent and heating by an oven, heat gun, or hot plate is most commonly done.

Autoradiographic (photographic) or instrumental radiometric[23] detection of radiolabeled substances is widely used. These are particularly useful in studies of analytic changes by biological systems and/or recoveries of analytes through analytical systems of analysis.

B. General Considerations

All analysis work requires sensitivity, speed, specificity, and efficiency. Analytical toxicology has these requirements as well as the need for analysis of extremely small concentrations of toxic substances in very complex matrices.

1. Cleanup of Samples

The cleanup techniques for extracts of very complex samples such as animal specimens, plant specimens, moldy feed and food specimens, and dead biological specimens are challenging. Analysis of complex analytes like toxaphene, PCBs, chlordane, petroleum, polymers, dyes, tars, paints, and pyrethoids in biological samples after chemical and biological transformation is an awe-inspiring task. All require selective partitioning and cleanup. The advent of rapid column cleanup devices[24,25] has allowed rapid partitioning of selected analytes into fractions more easily handled. Reverse phase silica (polymer) columns and combination of media (silica, charcoal, polymer) allow rapid sample cleanup and preparation for TLC. By using a nonpolar (reverse phase substrate) and polar mobile phase to isolate a fraction of similar compounds and then a more selective separation for a specific class of compounds by adsorption or size exclusion, samples can be cleaned up for TLC. Then, planar surface chromatogram can be used in more nearly optimum or at least repeatable fashion.[26]

2. Application of Analytes to Surfaces

Often the concentration of the extract in the separation process of the cleanup and preliminary separation leave large solvent volumes to handle. Concentration of extracts by vacuum, gas effusion, or heat vaporization of volatile solvent may be done. There are manual systems and automatic systems available for this purpose.[16] Concentration of extracts reduces the tedium of replicate applications to the TLC surface.[25]

3. Documentation

Since planar chromatography is uniquely open and provides a means to "see" the separation and visually manage and observe the separation, photographic recording[27,28] is an excellent means of documenting separations. Visible, UV or IR light can be used to produce visible or fluorescent images and may be used to make photographs to record separation. This is particularly important for legal samples since the qualitative information is readily provided for a photograph. Densitometer traces or photographs of display tubes are also excellent deocumentation for the record. Particularly densitometric representations provided by such instruments as the Ca Mag scanner with software which produced a three-dimensional image of the complex surface is an analytical resource of great value.[9]

An analytical toxicology documentation system including form pages and details are presented in the *ACVT Analytical Toxicology Methods Manual*.[29]

Radioautography[30] allows accountability for metabolized compounds even when they are not yet characterized. Radio-scanning devices[23] also provide a record of value to document and record the presence of daughter compounds separated by planar chromatography.

Standardization[31,32] of chromatograms are important in documentation of separations.

C. Applications

1. Analysis of Drug Samples

A drug is a chemical compound or chemical substance which produces an effect on a biological system or individual. Toxicologists in forensic, clinical (human and veterinary), cosmetic, and food areas are concerned about drugs and drug residues.

Psychotropic drugs, growth promotants, and antibiotics are all amenable to analysis by planar chromatography. Qualitative identification by systematic separations and selective visualizations are used[33-35] to identify drugs. The separated bands can be further characterized

by spectral means to identify or confirm[16,21] or may be quantitatively analyzed by instrumental means directly[17,18] or after removing from the planar surface.[20,25]

Feed and food residues of drugs fed to animals such as DES (diethylstilbesterol), MGA (melengesterol acetate), zearalenol (Ralgro), also rumensin and other ionophores are of concern.[36,37] Sulfas, drugs of abuse, antibiotics, and antimicrobials, are frequently analyzed by paper[37] or silica gel normal or reverse phase.[36,6] Table 10 shows the relative migration rates (Rfs) and methods of detection of some typical antimicrobial agents.

The use of the inhibition of microbial growth[38] by substances on the planar surface is a rapid, sensitive, and selective tool to detect minute quantities of such materials and to account for active reaction products of these substances.[39] Hair samples have been used to determine drug exposure history.[40]

2. Analysis of Rodenticides

Largely veterinary, food and feed, and environmental toxicologists are concerned with the analysis of samples for rodenticides. The use of reverse-phase and normal-phase surfaces[6] provides a tool to qualitatively separate, confirm, and quantitatively analyze for groups of compounds.

Anticoagulants or coumarin-like compounds such as warfarin, dicumarol, diphacinone, coumachlor, and other coumarins may be screened for by reverse phase thin layer chromatography (RPTLC), then confirmation and quantitation made. A useful devise is to use marker compounds like diphacinone and warfarin to "bracket" the rodenticides and then, if suspect bands are observed, to verify them by spectral means on or off the plate. Table 4 shows some typical anticoagulant rodenticides, planar chromatography Rfs, and detection means. One of the problems in the analysis for anticoagulants is that the effect of the drug is present weeks after the levels of the drug in the body has decreased several log cycles of concentration.[41]

Sodium fluoroacetate and fluorinated analogs may be analyzed by TLC and removed to verify its presence by spectral means or by fluoride production from the compound by alkaline reaction and its detection by very sensitive and selective ion electrodes.[42,43]

The use of Armstrong's and Stine's[13] cyclodextrin and colloidal separation should be valuable for such drug compounding ingredients as carbowaxes or polymeric aldehydes (Snarol®) or other high molecular weight materials of significance.

Forensic toxicologists become interested when rodenticides are used as human intoxicants. Strychnine is probably the most common of these substances, but "rat poisons" are used to kill quite frequently. It will be discussed under alkaloids.

3. Analysis of Insecticides

Nearly any chemical substance has been tried as an insecticide. In no other pesticide field has as much effort been made or success been achieved. From the fly swatter to natural and synthetic pyrethroids man has traveled and, in addition to killing insects, has cut a wide swath through his environment and possibly affected his own health. The question which is the greater evil, the insects or the insecticides, remains to be answered.

Analysis of pesticides is also amenable to planar chromatography, which screens for chlorinated hydrocarbons, organophosphorus compounds, and carbamates, as well as pyrethroids. Quantitative analysis for PCBs, chlorinated hydrocarbons, organophosphorus compounds, and pyrethroids have been published for paper,[42] normal phase TLC,[43,44] and reverse phase.[25] For small laboratory use in remote areas, planar chromatography is probably the only practical means to analyze for pesticides. The use of all the modern devices for instrumental PC described above make the analysis sophisticated and most useful.

For pesticide research where the specific lesion produced by the pesticide is being studied, PC is the choice for use with radio labeled compounds. Also, in studies of chemical changes

Table 1
Rfs OF COMMON CHLORINATED PESTICIDES RELATIVE TO ALDRIN

Pesticide	R_f
Chlordane	0.81[a]
op-DDT	0.79
op-DDE	0.79
op-DDD	0.50
Hexachlorobenzene	1.11
2,4,6-TCP	0.19
PCP	0.13
2,4D-Butylester	0.45
2,4D-Acid	0.00
BHC	0.31
Lindane	0.47
Aldrin	1.00
Dieldrin®	0.59
PBB	1.00
Arochlor-1254	1.00[a]
Toxaphene	0.75[a]
Heptachlor®	0.91
Endrin	0.66

[a] Gives a broad band.

in the pesticide by the biological systems of the species studied, radio labeled insecticide and PC are the method of choice.[45,46]

Herbicides, fungicides, and miscellaneous pesticide chemicals may also be analyzed by PC for toxicological purposes. The chlorophenols and similar compounds are more amenable to analysis by normal phase PC if they have been derivatized by adding an alkyl moiety[47] to reduce the polar nature of these polar groups. Methyl or ethyl esters or ethers may be made by methylation with diazomethane or diazoethane to allow normal phase PC to be done with developing solvents that are used to analyze for chlorinated hydrocabon (CH) pesticides. Other derivatives may be made to increase the specificity and/or sensitivity of the compounds. Trichlorobenzoquinoneimine (TCBI),[48] $AlCl_3$, or dansyl derivatives can be made. TCBI is selective for carbamates; dansyl reagent produces fluorescent derivatives of compounds with active hydrogen.

Using reverse-phase PC allows underivatized compounds containing polar groups to be analyzed.[25] Bases may be chromatographed as free bases in basic developing solvents or as charged ions in acidic development solvents.

Visualization of CHs is possible with $AgNO_3$ after irradiation with UV light. This calls for scrupulously clean surfaces and a lack of iron or transition element or reducing and/or oxidizing agents in the PC surface. Spraying a "developed" plate with H_2O_2 when free silver (dark brown) bands from chlorinated organic compounds are partially obscured by a general darkening, due to impurities, will allow redeveloping with UV irradiation. This allows more definite detection of the CH bands by removing interfering reducing compounds while still observing the compounds of interest. This technique was used as a screening test in CH poisoning. Levels of 0.2 μg can be detected by this technique. By spotting 1 g equivalent of brain extract, levels in excess of 1 μg were easily found to verify CH toxicity. Less than 0.2 ppm CH pesticide, the detection limit, is not an acutely toxic exposure level.[50] Table 1 shows the data for separations of typical CH by PC.

Combinations of anisealdehyde for sensitivity, TCBI for selectivity, and dansyl for extreme sensitivities allow detection of carbamates, qualitatively and quantitatively. Ultraviolet de-

Table 2
CARBAMATE PESTICIDES

Carbamates	Planar Rf NP	RP
Carbofuran	0.37	0.43
Carbaryl	0.45	0.40
Methomyl	0.09	0.00
Baygon	0.44	0.435
Temik	0.20	0.00
Temik sulfone	0.01	0.00
Landrin	0.47	0.32
	0.51	0.33

Note: NP = Normal phase high resolution Whatman (4/1) toluene ethyl acetate. RP = Reverse phase C^{18} Whatman (65/35/ + 0.5% NaCl) ethanol/water/acetic acid + 0.5 sodium chloride.

Table 3
Rfs OF ORGANOPHOSPHORUS PESTICIDES RELATIVE TO METHYL PARATHION

Pesticide	Rf
Counter	2.83
Thimet	2.67
Dyfonate	2.50
Diazinon	1.33
Malathion	0.67
Methyl parathion	1.00

tection of anisealdehyde has been used for poisoning levels 0.1 = 1 ppm of carbamates in stomach contents. A combination of normal-phase and reverse-phase PC with selective detection made this a powerful tool especially useful for labile compounds like carbamates. Table 2 is a listing of seven common carbamate pesticides used in Iowa and their PC analysis.

Organophosphorus (OPS) compounds may be detected by bromophthalein agents, anisealdehyde spray, or 4-aminopyridine reagents. A combination of all may be needed, as well as acetylcholinestrase detection for tissue levels of OPS.[51,52] Cleanups of tissue extracts are essential for preparation of samples for TLC. SepPak C^{18}, deactivated florisil, carbon and deactivated silica gel columns may be used to prepare samples for PC analysis. Table 3 shows the relative Rfs of organophosphorus pesticides encountered at the Veterinary Diagnostic Laboratory at Iowa State University.

4. Analysis of Biotoxins

a. Alkaloids

Alkaloids could have been discussed under drugs and/or rodenticides; also alkaloids such as biotoxins are analyzed well by PC.[54] Slaframine and swainsonine are readily separated by TLC (particularly C^{18} reverse phase [RP]) and detected by iodine.[53] Pyrrolizidine alkaloids[25] are readily detected by PC using normal phase (NP) and RP surfaces and iodine detection. The use of iodine can serve a double detection. The use of iodine can serve a double purpose, by converting the alkaloids into purple bands with dimethylaminobenzaldehyde (DMAB) spray after ring opening occurs in the heterocyclic ring.

Indole alkaloids, such as cyclopiazonic acid and ergot alkaloids, may be detected by their fluorescence, fluorescence quenching, or DMAB[50] detection[25] after conversion to purple bands by ring opening.[49]

When normal-phase silica plates are used with alkaloids and amines, the use of Ca ion chelating (releasing) agents such as ethylene diamine tetraacetic acid (EDTA) or oxalate, provides a development with more compact bands and less streaking, caused by interaction with Ca ions absortion on unsatisfied alkaline earth ions.[49]

Tremogenic compound such as penitrems, paspalitrems, or aflatrems are detected by

Table 4
Rfs OF RODENTICIDES (NORMAL PHASE)

Rodenticide	UV	Relative Rf	Rf
Raucumin	Blue	1.2	0.80
Chlorofacinone	Yellow	0.25	0.19
Brodifacoum	Blue	1.5	0.9
Fumarin	Pink	1.0	0.77
Pival	Gold	1.0	0.78
Warfarin	Blue	1.0	0.75
Dicoumarol	Blue	0.3	0.27
Diphacinone	Gold	0.3	0.28
Difenacoum	Blue	1.4	0.85
Coumachlor	Blue	1.0	0.8

Note: 3/2/1 solvent — toluene/ethyl — acetate/acetone.

Table 5
TYPICAL Rf VALUES OF SOME MYCOTOXINS ON SILICA GEL (ADSORBOSIL 5)

Mycotoxin	Tol.-EtAC-90% HCOOH (6:3:1)	C_6H_6-MeOH-AcOH (24:2:1)
Citrinin	0.16—0.48	0—0.20
Luteoskyrin	0—0.47	0—0.23
Nivalenol	0—0.02	0—0.01
Butenolide	0.10	0.03
Kojic acid	0.16	0.03
Aflatoxin G_2	0.17	0.13
Nivalenol acetate (fusarenone X)	0.19	0.09
Aflatoxin G_1	0.23	0.14
Aflatoxin B_2	0.26	0.20
Aflatoxin B_1	0.31	0.23
Diacetoxyscirpenol	0.33	0.24
Aspertoxin	0.35	0.13
T-2 toxin	0.36	0.28
Patulin	0.41	0.21
Penicillic acid	0.47	0.22
Gliotoxin	0.53	0.39
Ochratoxin A	0.55	0.35
Zearalenone	0.78	0.42
Sterigmatocystin	0.85	0.75

From Scott, P., *Advances in Thin Layer Chromatography*, Touchstone, J. C., Ed., John Wiley & Sons, New York, 1982. With permission.

iodine, fumes fluorescence, and fluorescence quenching after PC chromatography.[25,55]

The plant alkaloids perlolines have been detected by PC chromatography. Unfortunately, the lack of authentic standards has limited the work on these compounds. Mycotoxin spectra and TLC separations are given in two references under mycotoxin analysis.[57,58] These latter two are shown in Tables 5 and 6. Histamines have been analyzed by PC and detected by iodine and dansyl reagent.[25]

Table 6
MYCOTOXINS — COLLABORATIVE STUDIES — SPIKED SAMPLES

Mycotoxin	Foodstuff	Added level (μg/kg)	CV% Visual	CV% Visual or densitometric
Ochratoxin A	Barley	44.9		30.5
		89.7	36.3	23.3
Ochratoxin B	Barley	48.8	37.7	34.4
		97.6		54.4
Et Ochratoxin A	Barley	60.0		50.2
		120.0		33.2
Et Ochratoxin B	Barley	61.0		76.0
		122.0		36.8
Ochratoxin A	Green coffee	57.0	49.6	
		154.0	37.0	
		230.0	39.1	
Sterigmatocystin	Barley	100	16.2	
		200	8.2	
	Wheat	100	20.3	
		200	22.5	
Zearalenone	Corn	300	53.0	
		1000	38.2	
		2000	27.0	
Patulin	Apple juice	50	44.0	
		120	38.3	
		340	48.7	

b. Mycotoxins

Probably the most used PC analysis after drugs and pharmaceuticals is for mycotoxins. In developing countries when conditions are favorable for production of mycotoxins, PC is the practical method for analysis. Also when volume analyses are necessary (e.g., quality assurance [QA] or quality control [QC] in commercial companies), PC may be used to provide cost effectiveness. A summary chart of methods published for mycotoxins is shown in Tables 5 and 6. These demonstrate the range of applications and mycotoxins. $AlCl_3$ enhances fluorescence of vomitoxin, Zearalenol, and some rodenticides.

Tables 5 and 6 are from Scott's chapter in *Advances in Thin Layer Chromatography.* They are an excellent summary of TLC methods for mycotoxins and are not necessary to supplement. The references for the literature in the tables from Scott are listed under Mycotoxin References. Tables 8 and 9 are a listing of some mycotoxins and the methods used at the Veterinary Diagnostic Laboratory at Iowa State University.

5. Analysis of Polynuclear Hydrocarbons

Polynuclear hydrocarbons (PNH) are important analytes for the veterinary toxicologist in detecting or verifying clay pigeon poisoning or asphalt and crude oil poisonings. The spectra of bands define the composition of each according to source and help define exposure levels. NP or RP planar chromatography with hydrocarbon solvents for NP and water and alcohol for RP may be used. Normal-phase PC is particularly useful if the plate is "coated" or covered with a hydrocarbon oil. Uncoated NP plates, which have PNH separations on them, allow rapid oxidation of the bands to occur. The separated bands can be lost in hours. This coating makes the separations more permanent and the detection more sensitive.

Reverse phase (C^{18}) plates provide greater stability toward UV and oxygen and provide

Table 7
MULTIMYCOTOXIN METHODS

Method	Commodity	Aflatoxin	Ochratoxin A	Ochratoxin C	Zearalenone
Eppley[81]	Various	<32 (B_1)	<55		<500
	Corn	1—3 (B_1 or G_1)	50		200
Vorster[82]	Grains, peanuts	4 (B_1)	20	20	
Stoloff et al.[83] + acid	Grains	20 (B_1 or G_1)	45—90	50—100	200—500
Scott et al.[84]	Grains	+	+		
BF (Scott et al.)[84]	Grains	+	+		+
BF + $CuCO_3$ (Thomas et al.)[85]	Corn	2 (B_1)			100
Hagan and Tietjen[86]	Oils	+	+	+	+
Roberts and Patterson[87]	Feed	3 (B_1)	80		1000
Wilson et al.[88]	Corn	2 (B_1 or G_1)	20		
	Beans		20		200—300
	Peanuts		40		
Takeda et al.[89]	Grains	10 (each)	40		
Blazer et al.[90]	Corn	2 (B_1)	40	200	
Joseffson and Möller[91]	Cereals	5 (each)	10	35	
Yamamoto[92]	Flours	5 (B_1)			

Detection Limits (μg/kg)

Sterigmatocystin	Patulin	Penicilic acid	Citrinin	T-2 toxin	Diacetoxylscirpenol	Penitrem A
100						
60	400—1000	+				
		+	100		+	
+						
+						
330	600		+	+	4000	+
		300—400	100—200			
		300—500	400—500			
		1000	ND			
40						
10	50					
40						

Note: + = Toxin detectable but limit not determined.

From Scott, P., *Advances in Thin Layer Chromatography*, Touchstone, J. C., Ed., John Wiley & Sons, New York, 1983. With permission.

inverse order of separation for many PNH. Most PNH fluoresce, so UV may be used to provide selective and sensitive detection. Probably laser[18] fluorescence is the most sensitive technique for these compounds.

6. *Analysis of Metals*

Heavy metals may be detected by dithiazone derivitization and PC separation and detection. Randerath[59] also describes methods for virtually all metals using PC. While spectral chemical techniques are very well-developed, the PC approach gives greater selectivity than some colorimetric or complexometric detection systems without separation. Stahr et al.[60] has

Table 8
Rfs OF MISCELLANEOUS MYCOTOXINS ON NORMAL PHASE TLC

Toxin	Rf	Solvent	Visualization	Limit detection
Sterigmatocystin	0.85	6/3/1 Toluene/ethylacetate/formic acid	Aluminum chloride Yellow fluorescence[a]	0.1 μg
	0.75	24/2/1 Benzene/methanol/acetic acid		
Alternariol	0.6	90/10 Chloroform/acetone	Blue fluorescence[a]	0.5 μg
Alternariol methyl ether	0.7		Blue fluorescence[a]	
Penicillic acid	0.5	3/2/1/1 Tolues/ethylacetate/acetone/ acetic acid	Anisealdehyde Blue fluorescence[a]	0.5 μg
Patulin	0.4	90/10 Chloroform/acetone	10% Pyridine in methanol	0.1 ng
Moniliformin	0.6	3/2 Chloroform/methanol	Fluorescence quenching[a]	0.1 μg
Dicumarol	0.2	90/10 Chloroform/acetone	Ammonia Fluorescence[a]	0.5 μg

[a] Long wavelength UV light.

Table 9
Rfs OF MYCOTOXINS OF DIAGNOSTIC SIGNIFICANCE

Toxin	Rf Reverse phase	Rf NP	Visualization	Detection sensitivity
	65/31/1 + 0.5% Sodium chloride Ethanol/water/acetic acid	3/2/1/1 Toluene, ethylacetate/ acetone/acetic acid		
Citrinin	0.78	0.20	Long wavelength UV, yellow fluorescence (acid fumes)	0.2 μg
Ochratoxin A	0.68	0.64	Long wavelength UV, blue fluorescence	0.1 μg
Penitrem A	0.35 85/15/1 Ethanol/water/ammonium hydroxide	0.70 90/10 Chloroform/methanol	Blue color dimethylamino benzaldehyde spray	1—2 μg
Aflatrem	0.20	0.80	Blue color dimethylamino benzaldehyde spray, purple	1—2 μg
Ergotamine	0.50	0.20	Purple color dimethylamino benzaldehyde spray; blue fluorescence, long wavelength UV	0.1 μg
Slaframine	0.3 85/15/1	0.5 90/10 Chloroform/ methanol	Iodiometric	1.0 μg
Zearalenone	0.2	0.69	Long wavelength UV fluorescence and short wavelength UV fluorescence	0.1 μg
Zearalenol	0.3	0.60	Aluminum chloride, long wavelength UV fluorescence	0.1 μg
Rubratoxin	0.8	0.40	20% Sulfuric acid/methanol, long wavelength UV green fluorescence:	0.5 μg
			short wavelength UV fluorescence quenching	0.2 μg

Table 10
PLANAR CHROMATOGRAPHY SEPARATION OF ANTIMICROBIAL AGENTS

Compound	Rfs on C^{18} reverse phase 65/35/1[a]	Normal phase TLC 85/15[b]	Fluorescent	DMAB color	Anisealdehyde color
Chlorotetracycline	0.13	0.73	+		Orange yellow
Beta-tetracycline	0.17	0.73	+		Orange yellow
Tetracycline	0.23	0.82	+		Orange yellow
Oxytetracycline	0.55	0.95	+		Orange yellow
Chloroamphenicol	0.71	0.77			Yellow
Penicillin	0.78	0.88			Yellow
Lincomycin	0.82	0.95			Yellow
Sulfaquinoxalin	0.51	0.55		Yellow	
Arsanilic acid	1.00	1.00			
Hydroxy nitro Furazolidone	0.096	0.066		Yellow	Brown
Nitrofurazone	0.73	0.89		Yellow	Brown

[a] 65% ethanol/35% HOH/1% HAC.
[b] 85% ethanol/15% HOH/1% NH_4OH; EDTA treated silica.

described the use of TLC to analyze for Se. It serves as a low cost technique for developing countries to screen for Se levels in deficiencies or poisonings. The use of PC with normal phase and paraffin coating to enhance the fluorometric sensitivity was described by Funk et al.[22] Bruno et al. described the use of PC to analyze metallic dithiazones at subnanogram levels.[80]

7. PC for Verification of Evidence

A history of drug use may be determined by analysis of hair extract by TLC.[65] Arson residues[61] (gasoline or turpentine) may be analyzed by PC to determine the origin and help associate the combustion fluid with a particular source.

Analysis of lipsticks[62] or dyes[63] allows the association of a suspect to a stain for legal purposes. Analysis for succinylcholine[67] in tissues by TLC and confirmation by mass spectroscopy made possible diagnosis of one of the most difficult poisons ever. Table 11 shows applications, system separation details and references for some forensic applications of PC. Of particular note is the analysis for succinylcholine, once thought undetectable for the "perfect crime."

8. PC for Environmental Toxicology

Association of combustion products and air pollution can be done by PC analysis. Hazardous wastes can be checked for identity and sorted for disposal or storage.

Industrial hygiene monitoring of air pollution or environmental monitoring of air or water wastes can be done for organic chemicals such as chlorinated hydrocarbon pesticides or PCBs.

9. PC for Clinical Toxicology

Monitoring of body fluids for heavy metals may be done by PC.[59,64,65] Such complex molecules as cholera toxin[68] binding gangliosides can be analyzed by PC. Porphyrins[73] and bilirubin dyes may be analyzed by PC. Hypotensive drugs,[71] plasma lipids choleic acids, and chenocholic and cholic acid[69] have been analyzed by PC.

Prostaglandins are important smooth muscle controlling chemicals and can be monitored by PC. Lipids on skin may be monitored by PC to determine[77] patterns for diagnosis of disease.[79]

Table 11
USES OF PLANAR CHROMATOGRAPHY FOR FORENSIC TOXICOLOGY

Application	Chromatography system	Ref.
Analysis of cannabinoids	Chloroform/dichloroethane (15:10) Xylene/1,4 dioxane (19:1) Normal phase silica Iodine detection	93
Analysis of barbiturates	Chloroform/acetone (4:1) Isopropyl alcohol/chloroform/ammonia (9/9/2) Normal phase silica Detection carbazone, mercuric chloride	94
Succinylcholine	1st Development: Methanol: 0.1 HCl (80:20) 2nd Development: Acetone: 0.1 HCl (1:1) Iodoplatinate detection	95
Lipstick dyes	Benzene/nonyl alcohol/conc. HCl (65:35:5) Normal phase silica gel	96

10. General Applications

Other biological research such as nervous system moderators[70] and gangliosides may be monitored by PC. Nitrosoamines important in food, clinical, and environmental toxicology are analyzed by PC.[72,75,76,78] LSD[74] may be analyzed by PC. Even nonvolatile nitrosoamines may be analyzed by this technique.

Table 10 shows the Rfs of antimicrobials which can be analyzed by TLC. Residue chemists, feed and food technologists, and regulatory toxicologists have a need for analysis for antibiotics and antimicrobial agents.

The nature of PC is that it is simple, inexpensive, exact, and allows for nonvolatile and labile chemicals to be analyzed. It can be implemented by utilizing the most expensive and sophisticated instruments or the most common detector, the human eye and brain.

APPENDIX

Reviews and Summaries of Planar Chromatography

Biannual reviews on TLC and paper chromatography are made by J. Sherma and Bernard Fried for *Analytical Chemistry* and published in the annual reviews. Formerly Drs. Sherma and Gunter Zweig made the reviews. Each volume of *Journal of Chromatography* has tables of separations.

Reviews:

1. **Fenimore, D. C.,** High performance thin layer chromatography, *Anal. Chem.*, 53(2), 252A, 1981.
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J. Chromatogr.
J. Chromatogr. Res.
J. Liquid Chromatogr.
Anal. Chem.
J. High Resolution Chromatogr.
Analyst
Microchem. J.
Ther. Drug Mont.
J. of Anal. Toxicol.
J. Am. Chem. Soc.
J. Anal. Biochem.
Radio Chem.
Anal. Chimica Acta
JAOAC
J. Appl. Environ. Microbiol.
Appl. Spectrosc.
J. Environ. Toxicol.
Vet. Human Toxicol.
J. Oil Chem. Soc.
Cereal Chem.
Pesticide Sci.
J. Biochem.
Anal. Lett.
J. Forensic Sci.
J. Agric. Food Chem.
J. Pharm. Sci.
J. Chem. Ecol.
Z. Lebenism. Unter. Fursh.
Gig. Sanit.
Drug Metab. Dispos.
Biochem. Biophy. Acta
Soil Sci.
J. Hyg.
Forensic Sci. J.
Appl. Entomol. Zool.
Invest. Radiol.
Plant Dis.
Food Cosmet. Toxicol.
J. Anal. Chem.
Toxicol. Appl. Pharmacol.
Arch. Toxicol.
Cancer Res.
Talanta
J. Nucl. Med.
Bull. Environ. Toxicol.
Clin. Toxicol.
Can. J. Chem.
Clin. Chim. Acta
Arch. Pharm.
Toxicon
J. Clin. Pathol.
Ford Chem. Toxicol.
Clin. Biochem.
Can. J. Bot.
Nahung
Biochem. Biophys. Acta
Arch. Dermatol. Res.
Chem. Pharm. Bull.
J. Pharm. Sci.
Chromatographie
Am. J. Drug Alcohol Abuse
J. Econ. Entomol.
Bull. Narc.
J. Clin. Chem./Clin. Biochemistry
Xenobiotica
J. Labelled Comp. Radiophase
Environ. Res.
Mycopathologia
Mycologia
Phytopathology
J. Dairy Sci.
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J. Cosmet. Chem.
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