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Paper and Thin-Layer Chromatography

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This review covers the literature on paper and thin-layer chromatography (PC and TLC) cited in *Chemical Abstracts* for the period December 8, 1975 to December 12, 1977. Also searched were the bibliography sections of the *Journal of Chromatography*, *Analytical Abstracts*, and about one dozen other journals devoted to analytical chemistry and chromatography.

During the past two years it became apparent that there was a significant reduction in the literature devoted to PC compared to that covering TLC. Also, a number of papers include results both from PC and TLC. For these reasons, we have decided that from now on the sections formerly covering specifically PC and TLC will be combined.

Because of space limitations imposed by the Editors of ANALYTICAL CHEMISTRY, we restricted our review to select papers representing, in our opinion, the most significant advances in PC and TLC. For this reason, many papers appearing in the following journals had to be omitted from this review: *Analytical Biochemistry*, *Planta Medica*, *Helvetica Chimica Acta*, *Zeitschrift fuer Physiologische Chemie*, *European Journal of Biochemistry*, *Journal of Steroid Biochemistry*, *Journal of Biological Chemistry*, *Biochimica et Biophysica Acta*, *Clinica Chimica Acta*, *Journal of Agricultural and Food Chemistry*, *Journal of Antibiotics*, *Steroids*, *Biochemical Medicine*, and *Journal of Clinical Chemistry and Clinical Biochemistry*. We are hoping, however, that most of these significant papers on applications of PC and TLC to specific classes of compounds will be cited in the *Analytical Chemistry* Applied Reviews, published in alternate years.

During the past two years, increasing use has been made of in situ densitometry for the quantitative analysis of a variety of organic compounds; these methods will be covered in both the General and Applications Sections.

High Performance TLC (HPTLC) has been recently reviewed (*192A*) and is expected to find wider acceptance, once commercial equipment becomes available. HPTLC is usually performed on commercial glass plates coated with very fine silica gel particles of uniform size (5 to 10 μm). Resultant chromatograms are very tight, well separated bands and spots, and the detectability is in the nanogram to picogram range. Samples must be applied in volumes less than one microliter for full realization of the increased efficiency of this technique. Solvent development is carried out radially with commercial equipment or in a small glass tank (N-Tank). If larger volumes are required to be applied, as in the case of residues and biological extracts, HPTLC can best be accomplished by programmed multiple development (Apparatus available from Regis Chemical). Alternatively, a two-zoned commercial plate is available from Merck and contains a microfine silica gel for HPTLC and a nonadsorptive synthetic silica gel, effecting the desired band narrowing at the interphase. Chemically bonded C_2, C_8, and C_{18}-HPTLC plates are also commercially available for reversed-phase HPTLC. With the advent of HPTLC, separation efficiency by TLC is now comparable to that achieved by gas chromatography or High Performance Liquid Chromatography (HPLC).

The following symposia on paper- and thin-layer chromatography have been published: the FACSS Second National Meeting (*J. Chromatogr. Sci.* **1975**, 13 (12) and **1976**, 14 (1)); International Camag Symposium on TLC (*J. Chromatogr.* **1976**, 123 (1)), and the 1977 Meeting on Advances in Chromatography (*J. Chromatogr.* **1977**, 142 (11)).

Short-courses on TLC continue to be offered at various times and locations by the American Chemical Society, Center for Professional Advancement, and Kontes, Inc. The ACS course is available in audioform (*132A*) on tape cassettes with a 75-page manual; this course has been reviewed in ANAL. CHEM. **1976**, 48(9), 778A.

Kontes, Inc. publishes a quarterly newsletter on quantitative TLC, which is available from the company at no cost. *A Dictionary of Chromatography*, authored by R. C. Denney, contains entries related to PC and TLC (J. Wiley, Publ.: New York, N.Y., **1976**); a review of this dictionary may be found in *J. Assoc. Offic. Anal. Chemists* **1976**, 59 (6) 1426. The 20th Harvey W. Wiley Award was given to Gunter Zweig "for his outstanding achievement in the development of chromatographic techniques for pesticide analytical methodology" (Oct. 1977).

For nomenclature used in PC and TLC, the reader is referred to the following article: *Chromatographia* **1968**, 1, 338; for HPTLC, ANAL. CHEM. **1977**, 49(12), 1867.

GENERAL CONSIDERATIONS

Books and Reviews. The second edition of Randerath's *Thin-Layer Chromatography* was published in German (*127A*); the bibliography of paper- and thin-layer chromatography covering the period 1970 to 1973 was prepared by Macek, Mais, and co-workers (*95A*); and Heftmann completed his *Chromatography of Steroids* in 1976 (*58A*).

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Gunter Zweig is Chief, Chemistry Branch, Criteria and Evaluation Div., Office of Pesticide Programs, EPA. Previously, he was senior scientist and director of the Life Sciences Div., Syracuse University Research Corp., 1965–73. Dr. Zweig received his B.S. in chemistry and Ph.D. in biochemistry from the University of Maryland in 1944 and 1952, respectively. From 1951 to 1953, he was associated with R. J. Block at the Boyce Thompson Institute, Yonkers, N.Y., and he co-authored with Block and R. Le Strange, one of the first books on paper chromatography, "Paper Chromatography, A Laboratory Manual" (Academic Press, 1952). In subsequent years, Dr. Zweig, R. J. Block, and E. L. Durrum published two editions of "Paper Chromatography and Paper Electrophoresis". A book on paper chromatography was published in 1971 by Academic Press and coauthored with Joseph Sherma. From 1953–57, Dr. Zweig worked as biochemist at the C. F. Kettering Foundation, Yellow Springs, Ohio, on problems related to photosynthesis and developed techniques of autoradiography and paper chromatography. In 1957 he moved to the University of California where he founded the Pesticide Residue Research Laboratory (later Environmental Toxicology Department) on the Davis Campus. From 1963–64 Dr. Zweig was awarded a Rothschild Fellowship and spent the year at the Weizmann Institute in Israel. In 1977, he was awarded the Wiley Medal of the AOAC for his contribution to the chromatographic analyses of pesticides. During the past 20 years, he has been active in the fields of analytical techniques and mode-of-action studies of pesticides, particularly herbicides, and has been editor of a nine-volume treatise on "Analytical Methods and Pesticides, Plant Growth Regulators, and Food Additives" (1963–77, Academic Press). Vol. VI to IX appeared in 1972/77 with Joseph Sherma as coauthor. Dr. Zweig has published over 70 articles in his specialty fields and is on the Editorial Board of "Handbook of Analytical Toxicology". He is co-editor of the "Handbook of Chromatography." Dr. Zweig is a member of ACS, AAAS (Fellow), New York Academy of Sciences, Sigma Xi, and the American Institute of Chemists.

Joseph Sherma received a B.S. in chemistry from Upsala College, East Orange, N.J., in 1955 and a Ph.D. in analytical chemistry from Rutgers University in 1958. His thesis research in ion exchange chromatography was under the direction of the late William Rieman III. Dr. Sherma joined the faculty of Lafayette College in 1958 and is presently full professor in charge of two courses in analytical chemistry. At Lafayette, he has continued research in chromatography and has additionally worked 11 summers in this field with Harold Strain (Argonne National Laboratory), James Fritz (Iowa State University), Joseph Touchstone (University of Pennsylvania Medical School), Gunter Zweig (SURC), and Brian Bidlingmeyer (Waters Associates). More than 160 lectures and publications concerned with column, paper, and thin-layer chromatography of metal ions, plant pigments, and other organic compounds; the electrophoresis of metal ions and organic compounds; and the chromatographic analysis of pesticides have resulted from this research. Dr. Sherma and Gunter Zweig have co-authored seven books on chromatography and pesticide analysis, and they have prepared the 1970–74 biennial reviews of liquid chromatography and the 1976 review of paper and thin-layer chromatography for *Analytical Chemistry*. Dr. Sherma spent six months in 1972 on sabbatical leave at the EPA Perrine Primate Laboratory, Perrine, Fla., and the summers of 1975 and 1976 at the USDA in Beltsville, Md., doing research in pesticide analysis with T. M. Shafik and M. E. Getz, respectively. He wrote an analytical quality control manual for pesticide analysis in 1975 and is currently updating this and another pesticide analytical manual yearly with Morton Beroza of the AOAC under contract with the EPA. Dr. Sherma has received two awards for superior teaching at Lafayette. He is a member of the ACS, Sigma Xi, Phi Lambda Upsilon, SAS, and the American Institute of Chemists.

Several important reviews were published during this period: techniques of paper chromatography (*94A*), modern techniques in TLC (*77A*), recent developments in TLC (*68A*), techniques of TLC (*166A*), instrumentation for TLC (*93A*), and new methods for quantitative TLC (*97A*). A book on the subject of quantitative TLC by Touchstone and Sherma will be published shortly and was, therefore, not available for this review. A book on general aspects of TLC was published by Touchstone and Dobbins in 1977 (both books by Wiley).

The subject of HPTLC was covered in a book published in German (*62A*). Programmed multiple development for HPTLC was reviewed (*72A*). Specific aspects of HPTLC have been discussed in a special edition of the *Journal of Chromatography Library* (*192A*) as, for example, the ring-developing technique (*15A*), performance and characteristics of precoated HPTLC silica gel plates (*53A*), quantitative analysis by HPTLC (*61A, 131A*).

Various applications of PC and TLC have been reviewed as, for example, PC and TLC in clinical biochemistry (*185A*), TLC of carbohydrates (*40A*), TLC of phospho- and glycolipids (*129A*), TLC of bile alcohols and acids (*37A*), TLC of steroids and sterols (*92A*), chromatographic analysis of hormone residues in food (*143A*), synthetic dyes—PC and paper electrophoresis (*164A*) and TLC (*147A*), PC of antibiotics (*11A*), polymer separation and characterization of polymers (*5A, 67A*).

The analysis of environmental pollutants by PC and TLC has been covered by several authors as, for example, air pollutants by PC (22A) and TLC (*8A*), paper chromatographic analysis in soil chemistry (*23A*), TLC in water pollution (*4A*), PC in waste water analysis (*65A*) and TLC (*101A*).

Theory and Fundamental Studies. Equations have been derived for resolution by continuous TLC to predict the feasibility of separations (*45A*). A dynamic equation for distribution coefficient is proposed that accounts for solute-phase interaction and can be applied to TLC (*148A*). A thermodynamic model of a TLC process takes into account all of the components of the system leading to optimization of the process (*133A*).

Different techniques of chromatography have been compared by a unified theoretical treatment: comparison of adsorption mechanism in TLC and high-speed liquid chromatography (LC) (*47A*), development of a procedure to scale up TLC experiments to HPLC (*54A*), an attempt to compare results from column LC to those from TLC using silica particles of similar size (*152A*), data transfer from HPTLC to HPLC (*75A*), development of a graphical method to extrapolate TLC data to liquid–solid LC (*159A*), standardization of results from PC and TLC with dimethylformamide (DMF) as stationary phase (*24A*). TLC and LC data of aromatic nitro compounds have been compared (*157A*).

The role of the adsorbent in TLC has been the subject of a number of studies: solvent–adsorbent interaction in HPTLC (*71A*), the adsorption of polymers (polystyrene) on H silica gels (*6A*), the activation of TL-adsorbents does not appear useful for solvents with a high affinity for water (*181A*), a study of the properties of synthetic Zeolites A and X (*162A*), comparison of HPLC and TLC with various types of silica (*158A*).

The effect of the orthogonal temperature gradient used in an S-chamber on TLC with silica gel was studied (*44A*).

Different methods have been recommended to apply correction factors to R_f values from TLC (*30A*) or to obtain true R_f values (*99A*). A number of studies on R_M values ($R_M = \log(1/R_M) - 1$) have been carried out: relationship between R_M from PC and partition coefficients of dissociable compounds (*81A*), relationship between R_M and composition of mixed nonideal solvent systems in adsorption TLC (*134A–137A, 141A*) and nonpolar and electron donor components (*138A*); R_M from two adsorbents, silica gel G and Al_2O_3G (*139A*), R_M values related to TLC-adsorbents of different porosity (*140A*), group identification of organic compounds based on ΔR_f and ΔR_M obtained from two different solvent systems in TLC (*155A*), correlation between TLC migration and chemical structure of pharmaceutical amines (*180A*).

Other fundamental studies of solvent systems in TLC have been reported: the thickness of the mobile phase in TLC (*25A*), a PC method to prove that the correlation between stationary phases in gas chromatography can be used for liquid–liquid partition (*96A*), the adsorption of binary solvents in TLC (*182A*), relationship between composition of binary solvents and solute retention in TL adsorption chromatography (*100A*), salt effects in reversed-phase TLC on silica gel (*175A*).

A number of significant, fundamental studies have covered the separation of different classes of organic compounds as, for example, a new two-dimensional solvent system for TLC of amino acids (*106A, 107A*), the mechanism of separation of phenolic acids, flavones, and hydrophilic amino acids on polyamide thin-layers (*174A*), the molecular mechanism of adsorption of phenols on alumia TL's (*187A*), nucleic acid constituents and phenols on chitosan-TL's (*90A*), a simple molecular model of adsorption chromatography using aromatic nitro compounds on magnesium silicate thin-layers (*160A*),

effect of the mobile phase in TLC of steroidal pharmaceuticals (*55A*), systematic study of TLC for the qualitative identification of sulfonamides (*28A*), systematization of TLC methods for low-volatile organic substances of toxicological relevance (*41A*).

Other compounds which have been systematically studied by PC and TLC include the following: quinoline and acridine derivatives by PC on Whatman No. 4 paper impregnated with formamide (*125A*), the determination of the number of hydroxyl groups in polyols by micro-TLC (*7A*), a method to distinguish between mono- and dibasic acids on the basis of pH–R_f curves from TLC (*12A*), mobility of aromatic acids as a function of water or formic acid in the vapor phase during TLC development (*51A*), TLC behavior of 1,8-dihydroxyanthraquinone and its methyl ethers (*21A*), paper partition chromatography of 5-pyrazolone derivatives (*13A*), heterocyclic bases and corresponding *N*-oxides by TLC (*14A*), and the separation of deuterated imipramine from the unlabeled compound by TLC (*57A*).

Several papers on the theoretical treatment of quantitative TLC are cited in this review: experimental and theoretical calibration curves in fluorescence analysis of TLC plates (*66A*), confirmation of Kubelka–Munk theory for densitometry by the application of a flying-spot densitometer (*63A*, *64A*), the design of a double-beam flying-spot apparatus and speculation about the future use of lasers (*120A*).

Chromatographic Systems (Solvents, Adsorbents, etc.). Cellulose, modified by chelate-forming groups (sodium imino diacetate or methyl dibromopropionate), was made into chromatographic paper for improved separation of inorganic ions (*80A*). Whatman No. 4, paper, impregnated with paraffin oil, was used to separate quinoline bases (*3A*). Whatman No. 5 paper, impregnated with $H_6(SiW_{10}Nb_2O_{40})$, was used as ion exchanger to separate Cs^+, Zr^{4+}, Ru^{3+}, UO_2^{2+}, and Co^{2+} (*98A*).

A number of new or modified adsorbents have been described to improve separations by TLC or to resolve specific mixtures or compounds. Among these developments are the following.

A method was developed to determine the adherence of silica gel to glass (*111A*). The preparation and characterization of chemically bonded TLC plates for reversed-phase chromatography were described (*42A*).

Separation efficiency has been improved by the use of 10-μm Silica Gel R (*46A*). (See also description of HPTLC in Introduction, above.) Similar experiments were reported with the use of microparticular silica gel (Partisil 5 or Lichrosorb) without binding agent. Improved separation of chlorophylls a and b was achieved (*153A*). Micro-polyamide thin-layers on passivated Al foil emit less background fluorescence than corresponding TLC-Ready Polyamide sheets, thus increasing the sensitivity of fluorescent spots (*191A*).

The preparation of permanent adsorbent layers (PAL) by means of fused glass powder has been described (*178A*). Similar techniques have been applied to a variety of adsorbents, e.g., fluorescent sintered plates (*115A*) and dextran gels for TL gel filtration (*113A*); the thermal stability of sintered thin-layers was studied (*114A*).

The preparation of TLC plates with cellulose as adsorbent was described (*173A*). For gradient TLC it was found that best results were achieved by impregnating the silica gel layers with formamide or DMF from the gas phase, resulting in 70% impregnation (*168A*).

Steroids have been resolved on thin-layers impregnated with phosphomolybdic acid (*154A*); silica gel impregnated with tetraalkyl ammonium salts has been found useful for the chromatography of chlorinated pesticides and substituted acetanilides (*9A*); quinine chloride has been chromatographed and eluted from thin-layers of acid-soluble $CaSO_4$–$CaCO_3$ adsorbent for quantitative analysis (*1A*).

Techniques and Apparatus. A thin-layer chromatographic device consisting of 2 plates at different temperatures and forming a sandwich-type chamber, is especially useful for the separation of macromolecules (*43A*). A novel twin-trough developing tank makes it possible to reduce the volume of developing solvent in TLC, and automatically adjusts the solvent development (*117A*). A compact apparatus has been patented for developing circular TLC (*81A*).

Several techniques have been developed to follow the path of the solvent front during development: one is a fluorescence-quenching optical device (*145A*), and the other consists of a mixture of dyes (*183A*).

Another sandwich-type apparatus is described for continuous, quasi-column TLC (*156A*). A simple apparatus has been constructed from Perspex to accommodate 16 to 20 samples for TL-gel filtration (*76A*). An inexpensive, rapid method for preparing TLC plates has been adapted for use in classrooms (*163A*). Two simplified techniques for scoring (lining) of TLC plates for chromatographing multiple samples have been described (*103A*, *188A*).

Rod-TLC is a new method by which the substances are separated on a commercially available Chromatorod consisting of a rod with bonded sintered partition medium. The separated solutes, e.g., drugs, are detected by means of FID (flame ionization detection) (*2A*, *190A*).

Solvent-free extraction of samples with supercritical gases (CO_2 or N_2O at 40 °C and 70 to 400 bar) is coupled with a receiving apparatus for subsequent TLC (*170A*, *171A*). A flow of inert gas by means of a bridge-like applicator holder avoids contact with oxygen and rapid evaporation of solvent from samples applied to paper or thin-layers prior to chromatography (*105A*). Several other novel techniques for applying samples for PC and TLC are the following: special applicators for automated operations (*89A*, *104A*), a simple, disposable streaking sample applicator for TLC (*172A*).

Paper chromatography of amino acids from biological sources, including urine, has been carried out on paper fitted with horizontally lined-up trapezoidal slots near the lower edge, thus preventing mingling of samples (*50A*). Primary amines are derivatized with fluorescamine in situ at the origin and chromatographed as fluorescent derivatives (*108A*).

Programmed multiple development (PMD) is especially suitable for HPTLC and transfer of data from TLC to HPLC (*73A*, *177A*); detailed methods for PMD have been described (*74A*).

Thermomicro Transfer Separation and Application (TAS) procedures have been pioneered by Stahl and reviewed by him (*167A*). In the isothermal mode of TAS, the sample is heated and the vapor steam directed to the origin on the TL-plate for subsequent chromatographic development (*169A*). Further refinement of this method is thermofractography in which the sample is heated from 50 to 450 °C, and the fractionated volatile components are collected on a slowly moving TL-plate which is then developed normally, producing a thermofractogram (*165A*). An apparatus designed for this technique has been patented (*39A*). Food additives have been analyzed by TAS (*88A*). A simple hot-plate method is similar to TAS by heating the sample in a depressed Al plate and transfering the volatilized component(s) by condensation directly to the TL-plate (*179A*); amino acids have been determined by this technique (*87A*).

A cryogenic TLC method, termed cryochromatography, utilizes ultracold temperatures (−20 to −70 °C) to separate phosphoglycerides without diluting or destroying the sample (*60A*).

Three elution methods have been selected for this review: the first involves the direct transfer of the excised spot from TLC to the injection port of the GLC, where it is volatilized and chromatographed (*128A*); the second is a device for the direct transfer of TLC zones into standard size scintillation vials for radioactive counting (*124A*); the third is a combination TLC chromatograph collector and elution apparatus (*3A*).

Two methods for fixing and preserving TLC's have been described, one uses perchlorovinyl resin (*126A*) and the other a series of transparent plastic polymers (*16A*).

Detection and Identification of Separated Zones. Direct elution of 2,4-dinitrophenylhydrazone derivatives of keto-steroids from developed TLC's gave the best results for quantitative analysis (*52A*).

Several fluorescence and chemiluminescence detection techniques for PC and TLC have been reported: use of gaseous electrical discharge to induce fluorescence in a variety of organic compounds (*150A*), photographic documentation of fluorescent spots by the use of special UV-cutoff filters for the camera lens (*142A*), fluorescamine as a dipping reagent for the fluorescent visualization of compounds with primary amino groups (*109A*), chemiluminescence by spraying dansyl derivatives with 2,4,6-trichlorophenyloxalate and H_2O_2 (*26A*); the chemiluminescence is measured with a rapid-scanning

spectrometer fitted with a detector surface divided into 500 channels (*27A*); luminescence induced by cooling the TL-plate to 77 K for the detection of tetrahydrocarbazole, carbazole, and 1,4-benzodiazepine (*29A*).

By spraying Stripmix on TLC's containing tritiated compounds, thin-layers could be stripped for radioactivity measurements (*20A*). A bioautographic technique for the visualization of antimicrobials has been perfected (*56A*). Photographic documentation for TLC has been discussed (*59A*).

TLC coupled with spectrometry and polarography has made great strides during this reporting period. Direct elution of substances from TL-plates and transfer to a mass spectrometer has been found to be feasible (*78A*, *84A*, *116A*), specifically for benzo[*a*]pyrene (*70A*). In situ infrared identification of the chlorinated insecticide endrin, separated by TLC, was effected by IR/Fourier transformation (*48A*). Polarographic titration of eluted zones from TL-plastic foils was made possible by the addition of EDTA to eliminate interferences from metal ions (*112A*).

TLC and flameless atomic absorption have been combined for the determination of inorganic ions and organometallic complexes (*69A*). Electrical conductivity detection for drugs, separated on PC, appears to be simpler and more suitable than conventional detection reagents (*18A*).

Several specific detection reagents or improvements of older detection methods have been developed during the past 2 years: 4,4′-tetramethyldiaminodiphenylmethane (TDM) for peptides (*184A*), increasing the elution efficiency for aromatic acids by the use of toluene as elutrient (*149A*), hydroxylamine–$FeCl_3$ was found to be a stereospecific reagent for mandelonitrile glycoside (*146A*), OsO_4 applied as vapor to TLC for the detection of olefins, quinones, other reducing compounds, amines, and amino acids (*83A*), 4,4′-bis(dimethylamino)diphenylcarbinol for the detection of thiols and carboxylic acids (*19A*).

Quantitative Analysis. A number of studies were designed to identify the requirements to achieve optimum results for quantitative analysis of developed chromatograms. For example, baseline noise and variation in optical, in situ densitometry of TLC's are attributed to particle distribution and layer thickness variations within the TLC media (*91A*). The detection limit as a function of layer thickness and zone spreading is discussed as it relates to single-beam fluorimetry or double-beam densitometry (*121A*). The most significant background noise in UV-transmission densitometry arises from the particular nature of the adsorbent and its irregularities (*49A*). Optical transmittance measurements on the non-illuminated side of the TLC are nearly independent of the depth distribution of the analyte (*122A*). Non-equilibrium chromatographic development in an open system produced almost ideal spot configuration for optical scanning of sulfa drugs (*38A*). A device for uniform spraying of TL-plates has been described, to achieve more uniform quantitative in situ densitometry (*82A*).

A variety of optical devices and techniques for densitometry have been described in the literature, as for example: dual-wavelength zig-zag scanning (*32A*, *189A*), a linear spectrodensitometer (*176A*), an experimental high-performance photodensitometer (*17A*, *123A*), an automatic scanning device with analog (*33A*) and digital (*35A*) equipment, an instrument for the automatic control of a TLC spectrophotometer which can also be operated in the manual mode (*119A*), a photometric detector and apparatus for continuous-flow TLC (*144A*).

Flame ionization detection (FID) has been adapted to rod-TLC (*190A*) and regular TLC for quantitative analysis (*79A*). A twin-beam densitometer has been used for the quantitative estimation of unresolved substances (*31A*). Calibration factors for UV-reflectance spectrometric measurement are transferable to in situ fluorescence measurements (*10A*).

Picogram quantities of hydrocortisone, aflatoxins, and Rhodamine B were determined quantitatively after HPTLC (*130A*). Lecithin separated by PC and TLC was exposed to I_2 vapors and quantitated by densitometric scanning (*110A*). A commercial elution apparatus, ELUQUICK, is used for quantitative analysis by TLC (*85A*). *p*-Aminobenzoic acid in urine has been determined quantitatively by densitometry and serves as an excellent student laboratory experiment (*151A*). Salicylic acid has been determined quantitatively by in situ fluorimetry by a 2-point method (*34A*).

Preparative Methods and Automation. In continuous or intermittent TLC, a moving heated zone causes thermal migration of the sorbate, leaving sorbent, essentially free from sorbate, behind (*118A*). Feed throughput in preparative, steady-state 2-dimensional chromatography can be improved by injecting feed along a line that is slanted, while solvent is continuously fed to the bottom of the apparatus (*186A*). A patented apparatus for preparative TLC is described (*102A*). The layer of adsorbent is applied to a base plate, and the sample is applied in a narrow strip in the adsorbent layer. The base plate is immersed in solvent for chromatography. The sample is applied in solid form and is dispensed in a groove in the adsorbent layer which is then compressed or compacted.

A spectrodensitometer driven by stepping motors is controlled by a programmable desk calculator. Each spot on the TLC is positioned in the center of a light beam; software for finding the position of the spots is included in the directions (*36A*). This represents computer-controlled automatic evaluation of thin-layer chromatograms.

APPLICATIONS

Acids. Unsaturated higher aliphatic acids were separated by PC with 98% acetic acid–85% formic acid–water (75:25:2.5) solvent and detected as brown spots with 0.1% $HMnO_4$ spray reagent (*51B*). Azo derivatives of chromotropic acid were separated on paper by development with various proportions of pyridine–28% NH_3–1-pentanol (*187B*). PC and TLC were used to detect *o*-hydroxyhippuric acid (salicyluric acid) in the urine of sick children (*124B*).

The difficult separation of abscisic acid and six gibberellins by TLC was studied (*247B*). Diaminodicarboxylic acid stereoisomers were separated on thin layers of cellulose by development with methanol–water–acetic acid (40:10:2) and their didansyl derivatives on silica gel with benzene–pyridine–acetic acid (40:10:1) mobile phase (*76B*). The determination of vanillinmandelic acid in urine in order to study stress reactions was carried out by TLC separation, detection with a diazonium salt, and quantitation at 515 nm (*195B*). Quantitation of *o*-hydroxyphenylacetic acid in urine was accomplished by cellulose TLC with butanol–ethanol–NH_3–water (14:5:1:6) solvent, visualization with 2,6-dichloroquinone-4-chlorimide reagent, elution with $Na_2B_4O_7$ solution, and measurement of color at 580 nm (*259B*). Aromatic sulfonic acids were detected on paper or thin-layer chromatograms by spraying with hydroxylamine followed by drying and spraying with cupric acetate solution; densitometry at 480 nm was used for quantitation (*130B*). Two-dimensional TLC of 15 lichen depsides was studied (*88B*). R_F values were reported for the TLC of 31 phenolic acids (*123B*) and 29 miscellaneous organic acids (*162B*).

Alcohols. Epimeric pairs of alcohols of quinolizidine derivatives were separated on silica gel layers with chloroform–ethanol (3:2) as mobile phase (*422B*). The chromatographic behavior of 3–6 carbon alditols on silica gel layers with various developing solvents and detection by 0.1% sodium periodate reagent was studied (*286B*). 4-*N*,*N*-dimethylaminoazobenzene-4′-isothiocyanate was used as a color reagent to distinguish amino alcohols (blue color) and amino acids (red color) on a polyamide thin layer (*71B*).

Alkaloids. The following alkaloids were separated and R_F values reported on silica gel layers: 26 steroidal alkaloids with 8 different solvent systems and detection in daylight or 366 nm UV light after spraying with 50% aqueous sulfuric acid and heating to 80 or 300 °C (*185B*); Cinchona alkaloids one-dimensionally with 22 solvent systems (*77B*) and two-dimensionally with chloroform–methanol–17% NH_3 (24:6:0.05) followed by ethyl ether–diethylamine (17:1) (*415B*); quaternary and tertiary alkaloids and alkaloid *N*-oxides with methanol–0.2 M ammonium nitrate (3:2) and methanol–2 M NH_3–1 M NH_4NO_3 (7:2:1) solvents (*398B*). The mechanism of alkaloid separations on unmodified silica gel in very polar, neutral, or acidic media was studied (*277B*).

Alkaloids were chromatographed on titanium arsenate papers in aqueous and mixed solvent systems (*301B*). The influence of pH on the chromatographic behavior of 48 alkaloids on Bio-Rad AG 1-X4 and Cellex D ion-exchange layers and microcrystalline cellulose was studied (*250B*). Separations of the same alkaloids in mixed solvents on alginic acid, Rexyn

102, and Dowex 50-X4 ion-exchange layers were carried out and retention mechanisms determined (*251B*).

The π-acceptor reagent 7,7,8,8-tetracyanoquinodimethane detected 0.5-10 μg/mm² of alkaloids with various colors on silica gel layers (*322B*). LSD was isolated and identified in urine by a combination of HPLC and TLC; the solvent for the latter was acetone–chloroform–methanol (15:4:1), and the detection reagent was *p*-dimethylaminobenzaldehyde (*83B*). A general procedure for isolation and identification of 18 hydromorphone alkaloids and 6α- and 6β-hydroxy metabolites was worked out (*84B*). Addition of $Li(OH)_2$ increased the release of alkaloids from the oven in the TAS-method (*78B*).

Morphine was quantitated in the urine of drug addicts (0.25–0.50 μg/mL) by a combination of 2D silica gel TLC and spectrofluorometry after elution of the spot. Solvents were methanol–chloroform–NH_3 (85:15:0.7) and ethyl ether (water saturated)–acetone–diethylamine (85:8:7) (*399B*). In situ densitometry was used to determine natural β-pyridinic antibiotics detected with Dragendorff reagent (*24B*), ergotamine tartrate and caffeine in the nanogram range (fluorescence and quench modes) (*7B*), and ergot alkaloids [formamide-impregnated silica gel developed with THF–diisopropyl ether–toluene–diethylamine (20:70:10:0.5), fluorescence mode] (*299B*).

Amino Acids. A simple clinical method for separating amino acids involved adding 0.5 mL of a 24-h urine sample to filter paper, drying, cutting into pieces, and eluting with 2-propanol. The diluted eluate was developed two-dimensionally with acetone–butanol–acetic acid–water (34:34:10:22) followed by 0.4 M ninhydrin in acetone–butanol (50:50) (*2B*). A clinical screening test for genetic defects of metabolism was carried out in a similar manner and applied to 27 000 newborns. Eighty μL of capillary blood was taken on filter paper, the alcohol eluate was separated on silica gel with the first solvent above, and detection was by ninhydrin spray (*3B*).

The chromatography of halogenated amino acids on polystyrene, paraffin, and cellulose anion and cation exchangers was studied (*248B*). Binary and ternary separations of amino acids were carried out on titanium arsenate papers with butanol, acetic acid, butanol–acetic acid, and methanol–HCl developers (*300B*). 3-Iodotyrosine, 3,5-diiodotyrosine, 3,3′,5-triiodotyrosine, thyroxine, monoiodohistidine, diiodohistidine, and iodide were separated on silica gel with benzyl alcohol–acetone–1.1 N NH_3 as the solvent (*144B*). Optical isomers of tryptophan were separated on cellulose layers (*278B*) and several amino acid racemates on cellulose–alumina layers (*17B*). *o*-Phthalaldehyde detects amino acids and peptides on silica gel or cellulose chromatograms as sensitively (50–100 pmol) as fluorescamine and is cheaper (*255B*).

Lysine and arginine were quantitated in wheat and tissue by colorimetry at 510 nm after TLC separation, detection with ninhydrin, and elution of the spots with copper sulfate in ethanol (*21B*). Densitometry was used to quantitate amino acids after separation on strong acid cation-exchange layers and ninhydrin detection (*42B*). Optimal conditions for densitometry after detection with fluorescamine were elaborated (*382B*). The vidicon tube of an electronically controlled TV camera was used to scan amino acid chromatograms (*98B*).

Basic and acidic amino acids in plants were separated and identified by combined double electrophoresis and TLC (*104B*). TLC of amino acids was applied to the study of urea clearance and amino acid excretion in urine (*169B*) and the structural sequence of collagen and other proteins (*168B*). The accuracy of ^{15}N determination in amino acids and amides on chromatograms was improved by preheating the silica at 560 °C for 3 h and extraction of the amides with 200 μL of 50% ethanol followed by evaporation at 40 °C in a discharge tube for emission spectrometry (*191B*). Amino acids in gastric juice were semiquantitatively determined by 2D-TLC (*105B*).

The following derivatives were separated: dansyl amino acids on polyamide layers with water–pyridine–formic acid (93:3.5:3.5) and benzene–acetic acid (4.5:1) as solvents (*413B*); 4-*N*,*N*-dimethylaminoazobenzene-4′-sulfonyl amino acids by 2D development on polyamide (*69B*); 21 *p*-phenylazophenyl thiohydantoins on silica gel (*94B*); 24 colored thiohydantoins (*70B*); 23 methylthiohydantoin acids by 2D-TLC on double-faced polyamide layers (*232B*); phenyl- and methylthiohydantoins of arginine, cysteic acid, and histidine on polyamide (*231B*); DNP-amino acids by 2D chromatography on paper (*263B*); and Dns-amino acids using a new second solvent [benzene–acetic acid (4.5:1)] in the traditional 2D Woods–Wang method (*244B*).

Antibiotics. Chephalosporin antibiotics were detected on Avicel, alumina, and silica gel layers with ethanol–butanol–chloroform–10% HCl (10:10:10:1) developing solvent and I_2, $SnCl_2$, and *p*-dimethylaminobenzaldehyde color reagents (*411B*). Fourteen different antibiotics were detected in milk by TLC at 0.1–15 μg/mL levels (*52B*). Classification of antitumor antibiotics was based on mobility in a solvent using a certain adsorbent rather than on R_F values (*188B*).

Densitometry was utilized in the following determinations: impurities in tetracycline, kieselguhr layer impregnated with ethylene glycol–water–acetone–ethyl acetate (2:2:15:15), fluorescence mode (*416B*); degradation products of tetracycline, fluorescence mode (*272B*); cladinose and methylcladinoside, silica gel layer (*240B*); gramicidin, high performance silica gel layer, acetic acid–butyl acetate–butanol–methanol–water (20:40:7.5:2.5:12) solvent, automatic spraying of 4-dimethylaminobenzaldehyde detection reagent, simultaneous transmittance–reflectance scanning at 570 nm (*228B*); monensin in feeds, vanillin detection reagent, reflectance scanning (*224B*); siomycins A, B, and D_1, sintered silica gel plate, H_2SO_4 detection reagent, fluorescence mode (*172B*); and patulin in fruits and vegetables, reflectance scanning at 273 nm (*295B*).

Bases and Amines. Noradrenaline and its major metabolites were separated by PC in butanol–ethyl acetate–acetic acid–7% SO_2 solution–90% formic acid–10 M HCl (200:170:90:140:5:10) with detection by Folin–Cioucalteau color reagent (*267B*). Polyamines were separated on silica gel developed in pyridine–*tert*-butanol–concd NH_3 (1:1:1) (*414B*). Closely related aliphatic amines were separated by TLC on calcium oxalate-impregnated silica gel by developing with ethanol–NH_3 (80:20) with detection by spraying with 50% CS_2 in methanol followed by 1% silver nitrate solution (*351B*). Closely related aromatic amines (e.g., *o*-, *m*-, *p*-isomers) were separated on the same impregnated layers with benzene–ethyl acetate (90:10) or carbon tetrachloride–ethyl acetate (90:10) as mobile phase and detection by exposure to N oxides (*350B*). Paper chromatography separated secondary aromatic amines according to their substitution pattern (paper impregnated with a chlorinated paraffin, isopropanol solvent) or according to the alkyl substituent (dimethyl phthalate-impregnated paper, heptane solvent); detection was with nitric acid and diazo reagent (*337B*). The chromatography of sympathomimetric amines was studied on silica gel layers and fiber sheets (detection by NO_2) (*334B*), sulfinylamines on silica gel layers (*92B*), and methylolmelamines on cellulose layers [butanol–ethanol–water (4:1:2) mobile phase, $AgNO_3$ detection reagent] (*53B*).

Papers impregnated with stannic molybdate were used for the chromatographic separation of various amine hydrochlorides in different concentrations of aqueous sodium nitrate and hydrochloric acid solutions, and migration was studied theoretically (*312B*). Sodium borate-impregnated silica gel was used for the separation of 3-*O*-methyl catecholamines from their corresponding catecholamines (*167B*). The migration of aliphatic amines and aminophenols on silica gel in *o*-chlorophenol was studied as a function of the humidity of the adsorbent (*152B*). Quaternary ammonium compounds were separated by ion-pair TLC on silica gel developed with chloroform–methanol mixtures in which excess sodium bromide or iodide was dissolved; alternatively, methanolic solutions of the bromide or iodide counterions were sprayed onto the plate prior to chromatography (*100B*).

Pyridine and its 1-, 2-, or 6-unsubstituted derivatives (5–10 μg) were detected as violet spots on paper or layers upon spraying with a solution of *trans*-aconitic acid (*236B*). Aromatic amines (5–7 μg) were detected in the presence of aliphatic amines by spraying with 1% sodium periodate after development on silica gel G with chloroform–methanol (8:2) (*43B*).

Direct densitometry with a dual-wavelength, zigzag scanner was applied to the quantitation of ephedrine diastereomers (*163B*) and α- and β-naphthylamine and benzidene (*165B*) after separation on silica gel. Ethylenediamine and its reaction products with urea were determined by densitometry after separation on silica gel by double development and detection with furfurol and 1 N hydrochloric acid (*225B*).

TLC was used for the chromatography of: 12 guanidino compounds [2D development on cellulose with 2-propanol–acetic acid–water (60:45:15) followed by 2-propanol–DMF–water (40:20:20)] (*402B*); nuclear acetamides on silica gel, acetone–toluene–cyclohexane (3:1:1) solvent, detection with H_2SO_4 (*1B*); dansylated products of serotonin, dopamine, noradrenaline, and adrenaline on kieselguhr, 2D development with ethyl acetate–cyclohexane (3:2) and benzene–triethylamine (5:1), determination by fluorometry after spot elution (*30B*); and aromatic isocyanates on silica gel, cyclohexane–acetone (2:1) solvent, detection with cerium sulfate (isocyanates were distinguished from corresponding amines) (*226B*). Paper chromatography separated the heterocyclic sulfonamides neoxazol and sulfametin [butanol–acetic acid–water (50:10:40) solvent, detection by diazotization and coupling with 8-hydroxyquinoline-5-sulfonic acid] (*27B*) and biological pyridines [2D development in butanol–acetic acid–water (250:60:250) and 95% ethanol–NH_3 (95:5)] (*13B*).

Carbohydrates. Mono- and oligosaccharides were separated on cellulose layers by double development with ethyl acetate–pyridine–water–acetic acid–propionic acid (50:50:10:5:5); heating after spraying with diphenylamine–aniline–H_3PO_4 reagent provided spot visualization (*409B*). Isomeric pentoses and hexoses were separated on silica gel (no impregnation with borate or phosphate) by continuous flow development with chloroform–methanol–water (65:35:4) for pentoses and acetone–water (15:1) for hexoses ($NaIO_4$–$AgNO_3$ detection) (*22B*), and continuous flow TLC of oligosaccharides was also carried out (*86B*). Maltosaccharides were separated by gel permeation chromatography on polyacrylamide layers (*202B*). Sugars were determined in 1 μL of urine of patients being screened for meliturias after 2D-TLC on cellulose in the systems boric acid–butanol–propanol–water (10:3:5:2) and acetonitrile–water (85:15) (*178B*). PC estimation of fructose and myoinositol in 1 μL of semen allowed evaluation of vesicular or prostatic function (*252B*). Desalting of urine could be omitted in the routine determination of sugars on layers prepared from Filter-Cel–calcium sulfate–0.02 M sodium acetate (15 g:2 g:60 mL), development with ethyl acetate–pyridine–water (60:25:20), detection by anisaldehyde reagent (*425B*). In situ acid and enzyme hydrolysis was carried out on silica gel layers in studies of the composition and structure of oligosaccharides from leaves (*257B*).

New detection reagents described for carbohydrates include sodium hydroxide and ethanol for paper chromatograms (*87B*); dansyl hydrazine for reducing sugars prior to separation, spot elution, and spectrofluorometry (*14B*); vanadium pentoxide (general reagent) (*160B*); *p*-aminohippuric acid–phosphoric acid–ethanol (with or without thiobarbituric acid) for polysaccharides (*180B*); and *o*-aminobiphenyl for selective detection of mono- and oligosaccharides when used in sequence with other sprays (*256B*).

Densitometry was used to quantitate reducing sugars detected with triphenyl tetrazolium chloride reagent (silica gel layer scanned at 490 nm) (*33B*). Gluconic and lactobionic sugar acids were determined in pharmaceutical preparations by a fluorescence densitometry method based on glycol cleavage with lead acetate followed by treatment with dichlofluorescein (*150B*). Densitometry was compared to colorimetry for quantitating starch hydrolysis products separated by TLC (*85B*).

Carbonyls. R_F values were reported for 25 *trans*- and 12 *cis*-configurated 2-halovinyl ketones for three solvent systems on silica gel; detection was by spraying with methanolic solutions of pyridine and potassium hydroxide (0.06-μg detection limit) or an acetone solution of 4-(4-nitrobenzyl)pyridine (0.02 μg) (*125B*). Monocarbonyls were determined in stored whole milk powder by spotting 2,4-dinitrophenylhydrazones, formed on a reaction column from the extracted compounds, on an inert strip of kieselguhr G (to avoid decomposition) incorporated below an analytical Seasorb 43–Celite (5:2) thin layer (*209B*). Various oxo and thio compounds (ketones, acids, aldehydes) were detected (20-μg limit) with 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl-2*H*-tetrazolium chloride solution in methanol after separation on silica gel or alumina with 2-propanol–isooctane (1:3), acetonitrile–water (9:1), or hexane–isooctane (1:1) (*318B*). Eight dinitrophenylhydrazones of quinones, 2 azobenzenes, and 6 nitrophenols were separated on Sephadex LH-20 layers (*316B*). A comparative study indicated silica gel layers with benzene mobile phase and Rhodamine B detection reagent were optimal for the TLC of anthraquinones (*377B*). Picomole levels of aromatic and aliphatic aldehydes were detected by overspotting the origins of the silica gel plates with 5 μg of aniline to form *N*-phenylaldimine, developing with hexane–ethyl ether–dichloromethane (10:3:2), exposure to UV irradiation to regenerate aniline by photolysis of the imine, and viewing the yellow fluorescence under longwave UV light after spraying with fluorescamine reagent (*429B*). 2,4-Dinitrophenylhydrazones of 6 aldehydes and 5 ketones (10 ng/spot) were quantitated on silica gel layers (carbon tetrachloride–2-picoline solvent) by scanning reflection and transmission simultaneously at 370 nm (*222B*).

Dyes and Pigments. TLC data were reported for the following dye classes: cyanine dyes on silica gel in methanol– and propanol–formic acid (80:20) systems (*230B*); 27 disperse dyes on silica gel (*109B*); and Sudan dyes on silica gel in benzene–chloroform (*270B*). A scheme for the identification of 29 food and cosmetic dyes involved development on a cellulose layer with 2-propanol–water–carbon tetrachloride (60:25:15), followed by scraping, elution, and rechromatography of dye zones with different solvents (*330B*). Programmed multiple development on cellulose and silica gel layers was used for purity control of food colorings (*90B*). Rhodamine B in corn kernels and flour was isolated and evaluated by TLC-densitometry (*32B*).

Porphyrin mixtures were separated on silica gel layers using benzene–ethyl acetate–methanol (85:13.5:2) solvent (*203B*). Spectrophotometry of fluorescent porphyrins eluted from scraped chromatographic zones (*365B*) was suitable for clinical monitoring of the urine of patients with porphyria (*404B*). Chlorophylls and their derivatives were separated and identified on cellulose layers without alteration in the solvent systems petroleum ether–pyridine (9:1) (for chlorophylls, pheophytins, and pheophorbides) and heptane–pyridine (7:3) (for pheophorbides, chlorin, rhodin, and their esters) (*342B*).

Enzymes, Peptides, and Proteins. Treatment with formaldehyde, formaldehyde–ozone, or formaldehyde–hydrochloric acid produced fluorescence in tryptophan-containing peptides on paper or silica gel (*238B*). Dinitrophenyl derivatives of neutral dipeptides were separated on silica gel sheets with benzene–pyridine–acetic acid (80:20:2) or chloroform–methanol–acetic acid (95:5:1) (*271B*). Diastereomers of protected dipeptides were resolved by TLC (*9B*). Dansyl–peptide 2D silica gel TLC maps were determined using methyl acetate–2-propanol–NH_3 (9:7:1.5) followed by chloroform–benzyl alchol–ethyl acetate–acetic acid (12:8:10:5) (*204B*). A linear relation exists between log molecular weight and relative distance of migration for peptides and glycopeptides ranging from 1700–10 500 and from 1500–3300, respectively, on Sephadex G-50 layers developed with 6 M guanidine–hydrochloric acid (*184B*). Chemical reactions of peptides forming derivatives or removing certain groups can be carried out directly on silica gel thin layers, and the product and any unreacted material can then be separated and detected (*117B*). An improved procedure was devised for transfer of serum proteins from a Sephadex G-200 thin layer to a strip of paper for further separation by electrophoresis (*223B*). Gel filtration TLC was used to assess protein–protein coupling reactions; α-amylase was assayed in overlaid agarose gel by staining a paper replica with Coomassie Brilliant Blue R 250 (*210B*).

Fats and Lipids. The chromatography of phospholipids and glycolipids on Whatman SG-81 silica gel-loaded paper was reviewed (*421B*). A modified bromthymol blue staining method (sodium hydroxide replaced by sodium carbonate) detected 0.1–1.0 μg of phospholipids on silica gel as stable blue spots on a pale blue background (*423B*). One–10 nanomole amounts of phospholipids were detected under shortwave UV light by spraying with 0.1% ethanolic *N*-phenyl-1-naphthylamine fluorogenic reagent (*41B*). A stable universal molybdate reagent for phospholipids was proposed (*395B*). Aqueous ammonium 8-anilino-1-naphthalenesulfonate was better than 2′,7′-dichlorofluorescein for detecting neutral lipids and better than PMA for phospholipids (*401B*). Phosphatides and glycolipids were analyzed by paper chromatography of their partial hydrolysis or alcoholysis products (*95B*).

Acetylenic fatty acids were separated on silica gel using petroleum ether–diethyl ether (23:3) as the mobile phase with PMA detection (*253B*). Radioactively labeled C_{3-9} fatty acids

were separated on silanized silica gel with methanol–water solvent (*317B*). Recovery of free and esterified fatty acids (>90%) from silica gel plates depended upon conversion of silica gel to potassium silicate by use of KOH, followed by acidification and extraction with solvent (*118B*). The TLC of neutral glycerides was reviewed, including argentation, preparative, quantitation, and radiometric techniques (*265B*). An international study was made for factors involved in the determination of triglycerides in serum (*66B*), and HPTLC with the solvent methyl acetate–2-propanol–2.5 mg/mL aqueous potassium chloride (45:30:20) was used to determine ganglioside mixtures from brains of patients with Sanfilippo disease (*432B*). Glyceryl ethers were chromatographed on Whatman SG-81 silica gel paper with isooctane–isopropyl acetate–isopropanol (250:10:25) solvent (*157B*). Fats, oils, and food products were analyzed by thermofractography, whereby lipids were volatilized and deposited by a carrier gas onto the origin of a layer (*355B*).

^{14}C-labeled lipids were separated by three successive chromatographic runs on juxtaposed thin layers. The lipid extract was applied to alkalinized silica gel, while the second layer was regular silica gel G. The three one-dimensional migrations were carried out with diethyl ether–hexane–benzene (55:30:15), hexane–benzene (80:20), and diethyl ether–benzene–acetic acid (62:20:18), after which lipid fractions were scraped and counted by liquid scintillation procedures (*67B*). A clinical method for quantitation of glycosphingolipids in human plasma consisted of extraction, acetylation of total lipids, separation of acetylated glycosphingolipids on a Florisil column, deacetylation, dialysis in water, separation of single fractions by silica gel TLC, and quantitation of each lipid by sulfuric acid–orcinol reagent (*12B*). Blood serum phospholipids were quantitated by spectrophotometry after silica gel TLC with chloroform–methanol–NH_3 (6.5:2.5:0.5), scraping and elution of zones, digestion with perchloric acid, and color reaction with ammonium molybdate and malachite green (*148B*). Lipids in tissue samples were separated by TLC, the zones were scraped and collected, and the lipids were charred without elution from the adsorbent (*201B*).

Direct densitometry was used for the following lipid quantitations: fluorometric scanning of lipids on silica gel sprayed with 2′,7′-dichlorofluorescein (*186B*); total lipid, free and esterified cholesterol, free fatty acid, and triglycerides on ammonium sulfate impregnated silica gel G chromatograms (*176B*); ethanolamine- and serine-containing phosphoglyceride DNP derivatives (*170B*); phospholipids in capillary blood from newborns after PMA detection (*93B*); triglycerides after argentation TLC (*79B*); and neutral and phospholipids in 0.2 mL of serum and whole blood (*15B*). Correction factors for quantitating lipids by charring based on the polyunsaturated fatty acid content were derived (*119B*). Mathematical treatment of known densitometric data indicated that the fatty acid carbon content of the various lipids may be related to pathological conditions (*137B*). A procedure was described for treatment (halogenation and esterification) of serum lipids on thin-layer chromatograms so that they could be better stained with basic fuchsine in acetone buffer for densitometry (*280B*).

Flavonoids. The particular red fruit used in the manufacture of a food product could be identified by the anthocyanin pattern produced by paper or cellulose layer chromatography of cleaned-up extracts; the solvent for PC was butanol–acetic acid–water (6:1:2) and for TLC hydrochloric acid–formic acid–water (14:1:8) (*241B*). Flavonoid C-glycosides were separated and identified by TLC (*73B*). Thermofractography was used to aid characterization of flavonoids based on volatilization and fragmentation patterns of components deposited on silica gel plates (*354B*). Structural characteristics of flavonoids separated on cellulose + polyamide layers were determined by in situ measurement of reflectance spectra (*349B*). Robinin, a flavonoid glycoside drug, was quantitatively determined in flower extracts by densitometry of the yellow spot on silica gel chromatograms developed with butanol–acetic acid–water (4:1:1) and sprayed with 1% alcoholic aluminum chloride (*433B*).

Hormones and Steroids. Steroids, along with lipids, pesticides, drugs, amino acids, and metal ions, are the classes in which the most important and numerous advances have been made in applications of PC and TLC. The effect of amide group density of nylon on the polyamide TLC of estrogens (*181B*), and R_M correlations of cholesterol esters, ethers, carbonic acid esters, and halogen derivatives were reported (*366B*).

The following steroid separations were carried out: α- and β-ecdysone on Quantum preadsorbent silica gel, chloroform–methanol (4:1) solvent, UV detection (*323B*); 6 major androgens and estrogens simultaneously on preadsorbent silica gel layers developed with chloroform–ethyl acetate (80:20) at 32–72% humidity (*324B*); thromboxanes from prostaglandins by one- and two-dimensional TLC in 9 different solvent systems (*23B*); 17-ketosteroid 2,4-dinitrophenyl hydrozones on silica gel using the solvent dichloromethane–ethyl acetate (186:14) (*31B*); estradiol, testosterone, and 5α-dihydrotestosterone in the same plasma sample by overflow PC development (*65B*); isomers and epimers of the pregnane series by multiple development on silica gel or neutral or basic alumina (*114B*); 8 corticosteroids from their respective 17-oxo oxidation products on silica gel using dichloromethane–dioxane–water (100:50:50 and 120:30:50), UV detection (*237B*); 11 androgens with benzene–ethyl acetate (2:1) and benzene–acetone (7:1) (2D) and 5 estrogens with benzene–acetone (8:1) on silica gel (*291B*); corticosteroids on polyamide layers with dichloromethane–methanol (98:2) solvent (recommended as a substitute for LH-20 column chromatography) (*329B*); steroids extracted from urine by 2D-PC (double development in both direction) with dichloroethane–ethanol (92.5:7.5) and diisopropyl ether–ethanol (95:5), detection with tetrazolium reagent (*346B*); steroidal ketones, oximes, and lactams on silica gel, ceric sulfate or iodine detection (*343B*); 10 steroidal lactams, 19 steroidal tetrazoles, 17 basic azasteroids, and 9 quaternary azasteroids on silica gel (*344B*); prostaglandins E_2 and F_{2a} from their pulmonary metabolites on silica gel plates impregnated with both silver nitrate and boric acid (*345B*); and steryl acetates on silver nitrate-impregnated silica gel, petroleum ether–diethyl ether (99:1) solvent, ceric ammonium nitrate detection reagent (*215B*). Various chromatographic techniques were compared for determination of urinary neutral steroid profiles (*147B*).

The following detection reagents were described: sodium periodate for A-ring-hydroxylated estrogens and their methyl ethers on ascorbic acid-impregnated paper and thin-layer chromatograms, 2.5–5 μg sensitivity (*139B*); arsenic trichloride–acetic acid or chloroform (1:1) for steroids (5 μg) separated on silica gel G in benzene–ethyl acetate (9:1) (*216B*); stannous chloride–chloroacetic acid–chloroform (5 g:5 g:90 mL) for steroids separated on silica gel G in benzene–methanol (19:1), grey to light violet spots on a light blue background, 0.5-μg limit (*219B*); 7% As_2O_5 in formic acid for steroids separated on silica gel G in hexane–ethyl acetate (5:1) (*220B*); *p*-toluenesulfonic acid for corticosteroids, distinctive colors in daylight and under UV light (*264B*); and two molybdovanadophosphoric acids, with heating and 280 nm UV irradiation, characteristic colors at 10^{-7}–10^{-2} g levels (*332B*).

TLC was applied to bile acids as follows: separation of conjugated and unconjugated bile acids from biological fluids by double development on silica gel with propionic acid–isoamyl acetate–water–propanol (3:4:1:2) for 10 cm and then isooctane–isopropyl ether–acetic acid–2-propanol (2:1:1:1) to the top of the plate (*37B*); determination of bile acids in human blood serum, duodenal contents, and bile, after anion-exchange chromatography, on silica gel developed with isooctane–diisopropyl ether–acetic acid–isopropanol–ethylene chloride–water (2:1:1:1:1:0.26), detection with PMA (*47B*); separation of conjugated and free bile acids on silica gel in isopropanol–acetic acid (93:7) and hexane–methyl ethyl ketone–acetic acid (56:36:8) (*72B*); and separation of free and methylated fecal bile acids, first from fatty acids and then each other, in two silica gel systems (*348B*).

The steroid glycoside digitoxin and its metabolites were assayed in human serum by fluorescence densitometry at the therapeutic range of 10–50 ng/nL (*122B*). Digitoxin and cardioactive metabolites in human blood and urine were separated and determined on formamide-impregnated silica gel (*360B*). HPTLC on a formamide-impregnated silica gel plate with xylene–methyl ethyl ketone (7:10.5) or chloroform–THF–formamide (5:11:0.5) solvents separated 3–30 ng of aglycones and 5–50 ng of tetraosides from a digitalis cardenolide mixture (*205B*).

The following steroid quantitations were performed: 12 bile acids and bile salts after sulfuric acid charring and scraping of zones (*212B*); pregnanediol and pregnanetriol in urine, extract separated on silica gel in chloroform–acetone (9:1) or chloroform–acetone–methanol (75:15:10), zones scraped, charred, and absorbance read at 430 nm (*16B*, *174B*); free and bound cholesterol in human serum and plasma, zones separated by TLC were scraped, extracted with acetic acid, and measured colorimetrically using ferric chloride–sulfuric acid reagent (*110B*); estrogens and antithyroid drugs (10–200 ng) separated by HPTLC prior to densitometry (*193B*); and digoxin and dihydrodigoxin by scanning of fluorescence produced in TLC or PC with ascorbic acid reagent (*325B*).

Hydrocarbons. Polychlorinated naphthalenes were chromatographed on normal and reversed phase layers, and the results were compared to HPLC (*55B*). Six isomers of hexachlorocyclohexane were separated on silica gel G by developing with cyclohexane–chloroform (8:20) or heptane 2-propanol (10:0.5) and detected by spraying with 0.1% diphenyl in acetone followed by UV exposure (*376B*).

Polycyclic aromatic hydrocarbons were separated on polyamide layers developed with toluene–methanol (4:1) (*50B*). Polycyclic aromatic hydrocarbons were quantitated by PC in suspended matter (*56B*); in cigarette smoke (*214B*); by TLC-fluorometry (*341B*); by channel TLC (*435B*); by HPTLC-fluorodensitometry (ng amounts) (*333B*); in smoked food products by fluorodensitometry on cellulose acetate layers (*347B*); and by fluorodensitometry on acetylated cellulose (*419B*). Comparisons between excitation and emission fluorescence spectra on acetyl cellulose–alumina plates (1:2) and in solution were made for 66 polycyclic aromatic hydrocarbons (*10B*).

Monomers and Polymers. Hydrolysis products of nylon 66 and Perlon L were separated and identified by PC with bromocresol green detection reagent (*374B*). Acrylonitrile–styrene copolymers were separated into fractions differing in acrylonitrile content (35–42%) in an apparatus consisting of a silica gel TLC plate with a temperature gradient (30–40 °C) and a counter plate (2 mm separation from the layer) at 30 °C; the mobile phase was toluene with 5–20% acetone (*143B*). These polymers were fractionated according to composition in the 0–70 mol % acrylonitrile range by gradient elution on silica gel with benzene or toluene to which acetone is gradually added (*142B*). The feasibility of TLC separation of oligmers according to molecular weight and the separation mechanism was found to depend on the difference in adsorption activity of the terminal and central units of the macromolecules (*39B*, *136B*).

Organometallics. Various organic and inorganic mercury compounds were separated by silica gel TLC (*132B*, *260B*). Structure–R_F correlations and separation mechanism for Co, Cu, Ni, Fe, and Cr acetylacetonates on silica gel (*283B*) and for Co, Zn, Hg, Pd, Pt, and Rh acetylthioacetanilides and thiobenzoylmethane chelates (*262B*) were established. A series of papers reported on the separation of inorganic isomers by silica gel TLC, e.g., geometric isomers of borazines, carboranes, phosphazenes, silazanes, and siloxazanes (*207B*). Organic arsenic compounds were separated on silica gel with methanol–chloroform (10:90) and ethanol–ethyl acetate (50:50) solvents (*412B*). Trimethyltin chloride was detected in the presence of monomethyltin chlorides by TLC on alumina with benzene–ethanol (9:1) developing solvent and detection by 1-(2-pyridylazo)-2-naphthol followed by iodine vapor (*396B*). Organotin compounds were quantitated in foods on silica gel chromatograms by densitometry at 580 nm after staining with pyrocatechol violet (*418B*).

Pesticides. The in situ fluorescence densitometry of pesticides on paper and thin-layer chromatograms was reviewed (*268B*). A systematic identification scheme for pesticides by TLC on silica gel based on R_F values in different solvents and various detection reagents is being devised; part 10 of the series covering 89 insecticides, fungicides, and herbicides of different chemical types was published (*279B*). The fluorometric detection of pesticides on alumina layers with and without heat treatment was investigated and compared to silica gel layers (*60B*). A new method was devised for pattern analysis of pesticide formulations; solutions or extracts were spotted on silica gel layers with 254 nm phosphor, and the plate was developed 5 times with 3 solvents, namely, hexane–benzene (7:3) (twice), acetone–benzene (1:39), and hexane–dioxane (3:1) (twice); the plate was irradiated with UV each time for recording the chromatogram (*275B*).

The detection of chlorinated pesticides by dipping into silver nitrate was carefully studied (*261B*). The interference of fatty acids in the TLC estimation of chlorinated insecticides in butter was eliminated by spraying the silica gel layer with monoethanolamine and heating before spraying with silver nitrate reagent (*196B*). The densitometric determination of chlorinated insecticides such as endrin and γ-BHC in toxicological analysis was studied (*372B*, *373B*). The separation of chlorinated insecticides on silica gel papers and layers was compared, and the densitometry of DDT from water on the silica gel paper impregnated with silver nitrate was demonstrated (*335B*). Chlorophenoxy acid herbicides were densitometrically determined in water after TLC separation on silica gel layers impregnated with silver nitrate (*336B*). Kieselguhr layers coated with paraffin oil were best for separations of eleven PCBs with development by acetonitrile–methanol–water solvent (*54B*).

TLC was applied to organophosphorus (OP) pesticides as follows: 25 solvent systems and several reagents were evaluated for detection of 12 OP insecticides in tissues (*369B*); 32 insecticides were separated on silica gel in benzene–acetic acid (90:10), hexane–methanol (80:20), and hexane–acetone (60:40) (*367B*); ng amounts of parathion were detected as paraoxon by cholinesterase inhibition with *p*-nitrobenzenediazonium fluoborate as the chromogenic reagent (*233B*); zinc carbonate layers with petroleum ether–acetone (95:5) solvent were used for separation, and packed columns of zinc carbonate for cleanup (*313B*); dimethoate distribution in an autopsy tissue sample was determined on silica gel with cyclohexane–chloroform (70:30) as the solvent and palladous chloride detection reagent (*371B*); disyston and its sulfoxide and sulfone were determined in the foam layer of red pine on silica gel [acetone–cyclohexane (1:4) solvent] or alumina (chloroform solvent) layers with detection by palladous chloride–calcein reagent (*58B*); pirimiphos-ethyl and -methyl were determined by spectrophotometry at 248 nm of bands extracted from silica gel chromatograms developed with hexane–acetone (17:3) (*430B*); methyl parathion and metabolites were determined in animal material and feeds by visual estimation of blue spots obtained by reduction of $-NO_2$ groups to $-NH_2$, diazotization, and coupling with Bratton–Marshall reagent (*213B*); phosphamidon was determined in human tissue by silica gel TLC [chloroform–acetone (80:20) solvent], silver nitrate detection reagent, and visual comparison of spots (*370B*); dimethoate was estimated in feed extracts by silica gel TLC, palladous chloride detection, DMF elution of scraped zones, and absorbance measurement at 435 nm (*68B*); fluorescence densitometry of dansylated phenols obtained by hydrolysis of OP pesticides (*239B*); densitometry of dichlorvos, malathion, and parathion after silica gel TLC separation and silver nitrate detection (*356B*); densitometry of the heat-induced fluorescence of coumaphos, related compounds, and metabolites from water (*403B*); and fluorescence densitometry of fenitrothion, its breakdown products, and other amine-generating compounds after reduction of $-NO_2$ to $-NH_2$ and reaction with fluorescamine (*431B*).

A student laboratory experiment on the determination of carbaryl on plant leaves was published (*45B*). Carbofuran and its hydroxy and keto metabolites were estimated in grain and soil by silica gel TLC (ethyl ether solvent), *p*-nitrobenzenediazonium fluoborate detection reagent, and visual comparison of spot sizes (*154B*). Carbaryl and baygon were base-hydrolyzed, chromatographed on silica gel using hexane–acetone (8:2) as solvent and Tollen's reagent for detection (*199B*). Polyamide 11 with water–methanol–methyl ethyl ketone (4:4:2) was the best of many polyamide systems tested for carbamate and phenyl urea separations (*315B*). Benthiocarb and related compounds were separated using methanol–ethyl-acetate (1:4) solvent system (*112B*). Semiquantitative TLC determined byproducts in metoxuron (*327B*) and chlorotoluron (*171B*) formulations in collaborative studies. Chlordimeform residues in cucumber and apple extracts were determined directly on silica gel with benzene–acetone (9:1) solvent and detection by Dragendorff reagent; alternatively, the pesticide was converted to 4-chloro-*o*-toluidine, which was developed with benzene–chloroform–ethyl acetate (40:40:20) and detected with nitrite-α-naphthol (*149B*). The carbamate

insecticide thiofanox and its metabolites were separated on silica gel (*363B*).

Bifenox and related compounds were separated by TLC (*111B*), and warfarin sensitivity to air and light during TLC was studied (*40B*). Dithiocarbamate fungicides were detected as blue spots by congo red spray, followed by exposure to bromine and a second spraying (*307B*) Carboxin residues in wheat were extracted, cleaned-up, chromatographed on silica gel in hexane–acetone (4:1), and detected as a yellow spot with an absorption maximum of 306 nm by spraying with palladous chloride (*200B*). Residues of benomyl and its decomposition product MBC on fruits were determined by a visual bioautography technique on cellulose layers (sensitivity 0.01 μg/g) (*211B*); benomyl residues in extracts of 7 crops were converted to MBC and visually determined on silica gel as white spots on a green background by spraying with a spore suspension and incubating at 24 °C for 48 h (*99B*); benomyl residues in the soil and leaves and fruit of tomato plants were visually determined by extraction, cleanup, silica gel TLC [benzene–acetic acid–ethanol (14:0.7:0.3) mobile phase], and PMA detection (*36B*). *s*-Triazine herbicides were separated on Polyamide 6.6 with petroleum ether–chloroform (49:1) or water–methanol–acetic acid (14:4:1) solvents (*314B*), and silica gel systems and detection reagents for 1,3,5- and 6-methylthio-1,3,5-triazine separations were studied (*391B*). Fluorescence densitometry was applied to the water and soil analysis of herbicides containing, or hydrolyzable into compounds with, $-NH_2$ or $-OH$ groups; the herbicides were separated on silica gel plates treated with dansyl chloride [benzene–acetone (96:4) solvent] (*298B*). In situ fluorometry was also used for the determination of quinomethionate residues in crops (*131B*).

Pharmaceuticals and Drugs. TLC screening programs were reviewed (*46B*), and standardization of solvents and color reagents in toxicology was proposed (*19B*). Determination of partition coefficients of 1,4-benzodiazepines by HPLC and TLC was compared, and R_F values given for 11 compounds on oleyl alcohol-impregnated kieselguhr G (*183B*). Volatilizing substance, chromatographic solvent, method of visualization, and R_F value were listed for 5 single and 6 combination pharmaceuticals studied by the TAS procedure (*151B*).

The following separations were reported: methadone and its salts on silica gel with isoamyl alcohol–chloroform–acetone–water (3:5:5:1) solvent system (*80B*); optical isomers of amphetamine and methamphetamine on silica gel after spotting *N*-trifluoroacetyl-L-proline chloride or *N*-benzyloxycarbonyl-L-proline chloride directly on the initial spot before development (*120B*); cannabinoids and their monooxygenated derivatives by silica gel TLC (R_F values for 33 compounds given) (*128B*); cannabinoids by sequential TLC with one neutral and nonpolar, another basic, and a third acidic solvent (*258B*); azapropazone and related benzotriazines by double development with chloroform and then chloroform–methanol (90:10) on silica gel (*319B*); the α-adrenergic blocker *S*-2-(5-aminopentylamino)ethylphosphorothioic acid and its degradation products on silica gel using water as solvent and ninhydrin as visualizing reagent (*321B*); 14 isomeric thiazolybenzothiazole antiinflammatory compounds on silica gel G using benzene–acetone–ethyl acetate (80:20:5) as solvent and potassium permanganate as detecting reagent (*328B*); thiazide diuretics and antihypertensive drugs by silica gel TLC (*359B*); 14 homopyrimidazole derivatives on silica gel, alumina, and NH_3-saturated silica gel (mechanism and theory discussed) (*364B*); and phenothiazine derivatives on silica gel (6 solvent systems and 7 visualization reagents given) (*394B*).

Identifications were facilitated by chromatography in the following instances: 26 phenothiazine drugs by reversed-phase TLC in solvents of differing pH (*182B*); 11 coumarin and 8 phenylindanedione anticoagulant drugs on silica gel in 10 of 43 solvents tested (*285B*); barbiturates by combined TLC and melting point determination after scraping, elution, and sublimation (*294B*); 29 basic psychotropic drugs in urine by silica gel TLC (*379B*); erythromycin base, stearate, estolate, and ethylsuccinate in formulations using silica gel plates, chloroform–methanol–acetic acid (90:10:1 and 5:90:5) solvents, and potassium dichromate–sulfuric detection reagent (*400B*); antihypertensive drugs in urine by silica gel TLC using butanol–water–acetic acid (40:20:10) or chloroform–acetic acid–methanol (80:20:15) and a variety of chromogenic or fluorogenic reagents (*410B*); and 6 analgesics by silica gel TLC in acetone solvent (*59B*).

The following detection reagents were reported: Dragendorff reagent followed by silver nitrate for narcotic principles and metabolites (*62B*); diazotization and coupling with Bratton–Marshall reagent for drugs which give a primary amine group on acid hydrolysis (*113B*); 1-nitro-2-naphthol reagent gave blue to grey-blue spots which became violet after standing for cannabis components (*310B*); Dragendorff reagent gave orange-red spots changing color upon sodium nitrite treatment for polyethylene glycols and their esters during drug screening (*173B*); aqueous cobalt thiocyanate for lipophilic drugs (*146B*); and anisaldehyde for cannabinoids and their methyl ethers (*116B*).

An assay for the determination of carbamazepine in blood serum based on TLC fluorescence spectroscopy was successfully tested in two international quality control programs (*156B*). Analgesic, antipyretic, and barbituric mixtures were resolved on silica gel, eluted by borate buffer, and determined spectrophotometrically at 240 nm (*89B*). Drugs have been widely determined by in situ TLC spectrodensitometry: the analgesic-antiinflammatory drug mepirizole and its *N*-methyl isomer in tablets, solutions, and suppositories by fluorometry (*4B*); active ingredients from ointments and suppositories by remission scanning at 248–325 nm on silica gel F-254 plates (*6B*); glycosides in drug extracts by reflectance at 225 nm (*29B*); quinidine and salicylic acid in capillary blood by fluorescence scanning (*82B*); boldine in extracts and drug preparations after spraying with magnesium acetate and exposure to 365 nm UV light (*127B*); sulfonamides in pharmaceutical preparations (*140B*); anisylbutamide and carbutamine (photodensitometry compared to NMR) (*135B*); piprozlin and its main metabolite (*141B*); ng amounts of aromatic drugs after nitration, reduction, diazotization, and coupling (codeine by fluoroescence) (*158B*); swertiamarin, the main bitter principle of Swertiae Herba, with a dual wavelength scanner (*166B*); isoniazid and *p*-aminosalicylate in a rectal ointment (*289B*); the antiarrhythmic drug Falirytmin in blood by reflectance scanning at 578 nm after detection with ammonium molybdate reagent (*293B*); celnovocaine after detection with β-naphthol (*296B*); routine analysis of pharmaceutical specialties, automatic spotting of solutions (*297B*); 8-methoxypsoralen in human serum by fluorescence quenching (*357B*); acebutolol and its major metabolite in serum by fluorometry (*358B*); simultaneous determination of carbamazepine, diphenylhydantoin, mephenytoin, phenobarbital, and primidone in serum by fluorometry (*405B*); diazepam and its metabolite (*406B*); and phenothiazine in honey after detection with silver nitrate reagent (*424B*).

Phenols. A variety of phenolic compounds were well resolved on Whatman 1 paper developed with acetonitrile–0.1 M ammonium acetate (7:3 or 6:4) buffered to pH 7 or 4.5 (*229B*). Fifty-eight phenols were chromatographed on thin layers of AG 1-X4 and BD-cellulose anion exchangers with aqueous, organic, and mixed solutions (*249B*). Different adsorption layers and solvents were evaluated for separation of chlorophenols (*378B*). Phenols were separated on papers impregnated with stannic molybdate by developing with 1.0 M sodium nitrate at pH 6 (*311B*) and on anilinium chloride-impregnated silica gel layers developed with benzene–ethyl acetate (1:1, 3:1, or 9:1) (*75B*). Detection of 2 μg of phenols was obtained by spraying with 5% $NaNO_2$ solution in the latter case. Comparison of 5 layer materials and 11 solvents indicated chlorinated cresols were best separated on silica gel with dichloromethane solvent (*326B*). A TLC identification system for 126 phenols was described (*102B*).

Phenols and indole derivatives were detected specifically (1–10 μg) as brown spots by spraying paper or thin layers with 5% $(NH_4)_2Ce(NO_3)_6$ in acetone and 5% $NH_2OH{\cdot}HCl$ in 80% acetone (*81B*). Color reactions of 14 aromatic amines and 21 *o*-diphenolic or vicinal triphenolic compounds were tabulated (*25B*). Phenols were quantitated by channel TLC [linear relation between spot length and concentration between 1–8 μg/spot using chloroform–ethyl acetate (65:35) as solvent] (*145B*); unsaturated constituents of phenolic lipids were analyzed by TLC-MS (*387B*); and 2-phenylphenol was determined in urine, after separation from interferences on silica gel using chloroform–acetone (1:1), by reaction with 4-aminoantipyrene and chloramine-T followed by colorimetry at 520 nm (*26B*).

Purines, Pyrimidines, and Nucleic Acids. Alkylated and brominated thiouracils were separated by TLC with acetone–benzene–methanol–NH_3 (6:2:2:1) solvent (*426B*). Various pyrimidine derivatives were separated on silica gel using chloroform–ethyl acetate–methyl ethyl ketone (90:10:10) and chloroform–methanol–methyl ethyl ketone (92:8:10) mobile phases and potassium permanganate detection reagent (*353B*). Cellulose thin layers were used to separate purine and pyrimidine bases and deoxyribonucleoside analogues (6 solvent systems) (*64B*). An analytical system comprising one PC and three TLC steps was detailed for study of the components of the purine reutilization pathway; TLC was carried out on PEI-cellulose, ECTEOLA-cellulose, and unmodified cellulose (*91B*). Other uses of ion-exchange layers were to separate purine 3′,5′-cyclic nucleotides and nucleosides [PEI-cellulose, 1 M ammonium acetate (pH 9)–95% ethanol (7:13) solvent] (*383B*); pyridine and adenine nucleotides (PEI-cellulose, solvent containing lithium chloride or sodium formate buffer) (*34B*); nucleoside monophosphates (2D separation on PEI-cellulose) (*155B*); 5′-mono-, di-, and triphosphates of 1-β-D-arabinofuranosylcytosine and of 1-β-D-arabinofuranosyluracil (PEI- and DEAE-cellulose) (*227B*); and nucleic acid bases, nucleosides, and nucleotides (2D development on a strong acid cation exchange layer in the H^+ form in one direction and NH_4^+ in the second) (*380B*). Comparison of batch anion-exchange and chromatographic assays of cyclic nucleotide phosphodiesterases showed underestimation of enzyme activity by the batch method; R_F values of 17 compounds were given (*153B*).

Terpenes. Two spray reagents for terpene derivatives were described. Stannous chloride–monochloroacetic acid–chloroform (5 g:5 g:90 mL) or arsenic trichloride–chloroform (1:1) detected ≥ 0.5 μg of many terpene derivatives on silica gel plates developed with benzene–ethyl acetate (9:1). Steroids and flavonoids also reacted with the former (*217B, 218B*).

Toxins. Diterpene ester inflammatory toxins were identified by TLC-MS using 6 silica gel or alumina and 3 diethylene glycol systems (*121B*). Thirty-seven mycotoxins and other fungal metabolites were systematically analyzed by TLC R_F values in eight solvent systems, various color reactions, and UV detection (*106B*). Mycotoxins were separated on silica gel layers (*57B*) and detected bioautographically (*107B*).

A screening method for detection of aflatoxin, ochratoxin, zearalenone, penicillic acid, and citrinin in corn and dried beans was described (*417B*). Aflatoxin M_1 in a commodity extract was identified by reacting an origin spot with trifluoroacetic acid, developing with chloroform–acetone–2-propanol (85:10:7), and comparing to authentic M_1 similarly treated (*385B*). Aflatoxin B_1 and the feed antioxidant ethoxyquin were distinguished by in situ generation of fluorescence spectra of the respective spots after development on silica gel with chloroform–acetone–water (88:12:1.5) (*190B*). A 2D-TLC procedure was superior to the cellulose chromatographic method of the AOAC for analysis of aflatoxin M_1 in milk (*48B*). The Eluchrom automatic elution system removed aflatoxins from TLC plates with as little as 0.2 mL acetone following direct prewetting of the spot with 1-5 μL of water (*189B*). The TLC determination of zearalenone in corn was collaboratively studied, and two collaborators successfully used fluorescence densitometry (*340B*).

Vitamins. B vitamins were separated on cellulose layers with butanol–water–acetic acid (40:55:5), iodoplatinic acid, ninhydrin, UV light, and potassium permanganate being used for detection (*18B*). Cobyrinic acid, an intermediate in vitamin B_{12} biosynthesis, was separated from its precursors on CM-cellulose ion exchange paper developed with 2% acetic acid (*246B*). Seven B_6 vitamins were separated by 2D-TLC on silica gel developed with isoamyl alcohol–acetone–water–diethylamine (24:18:6:8) and then isobutyl alcohol–ethanol–1.5 N NH_3–isoamyl alcohol–diethylamine (20:6:7:2:2). Spots were located with diazotized *p*-nitroaniline or fluorometrically, and labeled compounds were scraped off and counted by liquid scintillation methods (*266B*). Vitamin D_3 in premixes and mixed feeds was determined after 2D separation on silica gel [petroleum ether–ethyl ether (1:3) and chloroform–acetone (9:1) solvents] and molybdic acid detection (*38B*). Fat soluble vitamins were separated on starch, cellulose, and talc thin layers impregnated with paraffin oil (*290B*).

Miscellaneous Organic Compounds. Seven solvent systems were given for separation of sterigmatocystin and its 5-methoxy- and *o*-methyl analogues on silica gel G; iodine vapor was the most sensitive detection reagent (limit 32 ng) (*362B*). Six solvent systems and eight detection methods were reported for the silica gel TLC of coumarin derivatives (*384B*).

Nineteen triphenodioxazines were examined in four nonaqueous TLC developing systems with detection by concentrated sulfuric acid (*192B*). Imidazole compounds from brain were separated by a 2D cellulose TLC method combined with a multiple spray procedure involving ninhydrin, Pauly's reagent, and *o*-phthalaldehyde (*245B*). Aromatic *N*-heterocyclic compounds separated by TLC were detected by the fluorescence of their cupric iodide complexes and fluorescence thermochromism (*408B*).

Ten *N*-aryl-*N*′-(2-benzimidazolyl)thiocarbamides, which are potential antithyroid agents, were separated by TLC on silica gel G using acetone–benzene–butanol (2:5:3) as solvent and butanolic ferric chloride or basic lead acetate as detecting agents (*390B*). Nine C_{14} sultones and chlorosultones were estimated in sulfonates, sulfates, and formulated products by 1D or 2D development on silica gel and detection by vapor phase charring (420B). Sodium alkanesulfonates and alkyl benzenesulfonates were separated without derivatization on polyamide layers with aqueous NH_3–pyridine and detected by spraying with pinacryptol yellow reagent and viewing under 254 nm UV light (*368B*). α-Olefinsulfonates were detected as yellow spots on a blue background by spraying silica gel chromatograms with bromothymol blue followed by $NaHCO_3$ solution (*281B*). Sulfonyl chlorides, sulfenyl chlorides, and α-dichloro-β-disulfones were detected as amber spots on silica gel chromatograms upon spraying with 10% sodium iodide in acetone (*194B*).

The detection of 24 *N*-nitrosamines by TLC using fluorescamine was studied; UV irradiation yielded amines, of which the primary ones gave fluorescent spots and the secondary ones dark spots (*427B*). Reversed phase paper and TLC were used to estimate *N*-nitrosamines in food products such as cured meat (*397B*). *N*-Aryl-*N*-nitrosamines were detected on thin layer plates with 2,4-dinitrophenylhydrazine and PMA; detections of aromatic amines and carbonyl compounds were also tabulated (*428B*).

Trichloromethyl, phenyl, and pentafluorophenyl derivatives of P were separated by ascending PC with different solvents and detection reagents (*49B*). An improved enzyme reagent for visualization of phosphate esters was obtained by deglobulinization and purification of horse serum (*5B*). Ferrocene derivatives and other cyclopentadienyl organometallics were separated by cellulose TLC (*164B*). Ortho-, pyro-, and tripolyphosphate were separated by development with 2 M lithium chloride on PEI–cellulose and detected by spraying with ammonium molybdate followed by ascorbic acid reducing agent and heating (*331B*). [^{3}H]inositol and [^{3}H]inositol monophosphates were separated on silica gel glass fiber sheets (*177B*).

Separations were made of piperazine and its carcinogenic *N*-nitroso derivatives on silica gel with neutral solvent systems (*309B*) and of some hydroxychalcones and their derivatives on nitrobenzene-treated silica gel G plates [benzene–ethyl acetate (48:2) solvent, sulfuric acid and ferric chloride detection reagents] (*74B*). Twenty-one new anils were separated on silica gel, spots were scraped, eluted with acetone, and identified by IR and UV spectrometry (*388B*). Sterigmatocystin was estimated in grains by extraction, partition cleanup, TLC separation with benzene–acetic acid (9:1), and enhancement of fluorescence by an aluminum chloride spray reagent (*11B*). To test their applicability as quantitative redox indicators, tetrazolinium salts and their formazans were examined by TLC and spectrometry (*407B*). The Van Urk–Salkowski chromogenic reagent was found to be sensitive to 25–50 ng and selective for indoles (*115B*). Furan compounds were separated on alumina thin layers using chloroform–methanol, benzene–methanol, benzene–ethyl acetate, and benzene–ether mixtures (*159B*).

Densitometry was applied to: the forensic examination of saw dusts from 15 kinds of wood by flying spot scanning (*320B*); determination of pyran coumarins in *Peucedanum arenarium* extracts (*381B*); analysis of fruits and vegetables for caffeic acid esters and catechins (*161B*); determination of alkyl ethoxylate mixtures separated by programmed multiple development (*126B*); determination of coumarins in bergamot essential oil by fluorometry (*61B*); determination of free

serotonin from the urine of patients with abnormal metabolic conditions after detection with *o*-phthaldialdehyde fluorogenic reagent (*138B*); and analysis of fresh or canned tuna for histamine by scanning at 570 nm after detection with ninhydrin (*254B*).

Inorganics. Chromatographic studies of the following separations were reported (on silica gel layers unless otherwise noted): lanthanides complexed with anils (*389B*); titanium from many other ions developed with methanol–50% nitric acid (9:1) solvent (R_F values for 46 metal ions listed) (*393B*); nitrate, nitrite, and ammonium ions (*375B*); metal xanthates (*308B*); 47 metal ions with 1.0 M NaCl solution in 30% acetone solvent (*302B*); octahedral geometric isomers of transition metals (*206B*); tropolone chelates of metals (*198B*); transition metal complexes of *S*-methyl-*N*-2-(-pyridyl)methylenedithiocarbazate (*133B*); selenium, tellurium, and gold by paper chromatography (*134B*); 13 rare earths in one chromatogram using the solvents: THF–paraldehyde–nitric acid (20:70:10); triphenylphosphine oxide (0.3 M in paraldehyde)–nitric acid (90:10); and diisopropyl ether–THF–nitric acid (100:60:10) (*175B*); mixed cobalt(III) complexes on paper with butanol–acid–water solvent mixtures (*242B*); α-dioximine complexes of transition metals by PC and TLC with methanol–butanol–0.1 N hydrochloric acid solvents in different proportions (*392B*); phosphite and phosphate by circular PC (*361B*); and ethylthiourea complexes of Cu, Ag, and Au by PC (*292B*).

The following chromatographic systems were applied to studies of metal ion separations: stannic antimonate for the TLC of 40 metal ions in 31 aqueous and mixed solvent systems containing DMSO (*305B*); stannic arsenate for the TLC of 42 metal ions with arsenic acid, hydrochloric acid, nitric acid, and tartaric acid developers (*303B*); ECTEOLA–cellulose in hydrochloric acid media with or without an organic solvent (*282B*); silica gel impregnated with tributyl phosphate and aqueous solutions or organic complexing agents (e.g., EDTA, tartaric acid) for 24 metal ions (*276B*); cellulose layers developed with tartrate–water–ethanol solvents for 60 ions (*20B*); paper impregnated with 5,5′-methylenedisalicylic acid for 30 cations (*63B*); thorium phosphate papers developed with aqueous acids with and without organic solvents (*96B*); silica gel impregnated with Aliquot-33$^+$ NO_2^- and developed with 1 M $NaNO_2$ for reversed phase TLC (*97B*); silica gel with acetone–phosphoric acid solvent (*129B*); DEAE–cellulose layers in aqueous thiosulfate media for 47 metal ions (*234B*); TEAE–cellulose layers in hydrochloric acid media with and without methanol for 41 ions (*235B*); zirconium antimonate paper developed with acetic acid for alkali metals (*273B*); stannic tungstate papers in acetone–nitric acid–water systems for V, Fe, Ti, Zr, and Th (*304B*); chelating paper incorporating α(β)-alanine-*N*,*N*-diacetic acid groups for 10 ions (*221B*); paper impregnated with 5% ascorbic acid and developed with butanol–hydrochloric acid (1–10 N) (1:1) containing 5% ascorbic acid for six metals (spotted behind the solvent front) (*243B*); cellulose layers impregnated with monothiothenoyltrifluoroacetone in pyridine solvent at pH 5 for Cu and Fe (*179B*); papers impregnated with liquid paraffin, zirconium phosphate, and zirconium tungstate with solvents containing DMSO for Mn, Fe, Co, Ni, Cu, and Zn (*108B*); EDTA impregnated silica gel and cellulose layers for 15 metals (*352B*); cellulose phosphate (*339B*) and carboxymethylcellulose (*338B*) layers in sulfuric acid and ammonium sulfate media for 58 ions; and silica gel plates developed with THF–paraldehyde–nitric acid (20:70:10) for all rare earths (*306B*). Monovalent anions were separated on Sephadex LH-20 layers (*103B*).

Detection reagents described include 3-mercapto-4-amino-5-methyl-1,2,4-triazole for Ru, Rh, Pd, Pt, and Au separated on silica gel and for evaluation by ring colorimetry (*197B*); potassium thiocarbonate for metals of the qualitative analysis groups 1–4 and some platinum metals separated on silica gel (*208B*); *o*-vanillin oxime for metals separated on paper (4–10 μg sensitivity under UV light) (*101B*); and aluminum(III)–morin fluorescent complex for 25 anions which caused quenching (*284B*).

Ni, Co, Pd, Ti, Y, and Zr were determined by TLC on cellulose and alumina combined with ring colorimetry (diphenylthiovioluric acid detection reagent) (*274B*); cobalt isolated from biological materials was separated from other metals by PC with butanol–acetone–hydrochloric acid–water (76:36:44:14) and detected with rubeanic acid (*269B*). Toxic metals in biological samples were identified by TLC of their dithizonates (*35B*). Segmented CM-cellulose/cellulose layers were used for the "slip chromatography" of Pb in ceramics, a method whereby the length of the Pb zone is proportional to its concentration (*8B*). Free sulfur in chemical reagents was determined by visual comparison of extracts and standards on silica gel plates developed with heptane using iodine and sodium azide reagents for detection (*28B*). The TAS method was applied to the separation of inorganic mercury compounds (*434B*).

Quantitation was achieved for Cd and Te in CdTe by peak chromatography on Bismuthol II-impregnated paper (*287B*); for uranyl ions by densitometry after detection on paper chromatograms with 2.0% potassium ferrocyanide (*44B*); for free elemental sulfur by densitometry on a phosphor-containing layer (*288B*); and by densitometry for stannous ions converted to a fluorescent chloro complex on a cellulose layer by using hydrochloric acid plus potassium or sodium chloride (*386B*).

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GENERAL CONSIDERATIONS

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