

Contents

1	History of Planar Chromatography	1
1.1	History of Paper Chromatography (PC)	1
1.2	History of Thin-Layer Chromatography	7
1.3	The History of Quantitative Planar Chromatography	8
	References	10
2	Theoretical Basis of Thin Layer Chromatography (TLC)	13
2.1	Planar and Column Chromatography	13
2.2	TLC Capillary Flow	15
2.3	TLC Distribution Equilibrium	18
2.3.1	Adsorption Chromatography	18
2.3.2	Partition Chromatography	20
2.4	The Retardation Factor (R_f)	22
2.4.1	The Empirical R_f Factor	22
2.4.2	The Thermodynamic R'_f Factor	24
2.5	Mobile Phase Composition	25
2.6	Transfer of TLC Separations to Columns	27
2.7	The R_m Value	28
2.8	Temperature Dependence of TLC Separations	29
2.9	Advanced Theoretical Considerations	30
2.10	Indices Characterizing Separation and Resolution	37
2.11	Zone Broadening in Planar Chromatography	40
2.11.1	The A term	40
2.11.2	The B term	41
2.11.3	The C term	42
2.11.4	Local Plate Height H	42
2.11.5	The van Deemter Equation	43
2.12	Optimum Separation Conditions in TLC	45
2.13	Separation Number	47
2.14	Real Plate Height	50
	References	51

3	The Stationary Phase in Thin-Layer Chromatography	53
3.1	Activating and Deactivating Stationary Phases	54
3.2	Snyder's Adsorption Model	55
3.3	Layer Characteristics	57
3.3.1	Layer Thickness (d_f)	59
3.3.2	Average Particle Size (d_p)	60
3.3.3	Particle Size Distribution	60
3.3.4	Specific Surface Area (O_s)	60
3.3.5	Pore Volume (V_p)	61
3.3.6	Average Pore diameter (P_d)	61
3.4	The Most Important Stationary Phases in TLC	62
3.4.1	Aluminium Oxide	62
3.4.2	Magnesium Silicate	62
3.4.3	Silica Gel	63
3.4.4	Chemically Bonded Silica Gel Layers	66
3.4.5	Kieselguhr	67
3.4.6	Cellulose	68
3.4.7	Polyamides	70
3.4.8	Ion Exchange Resins	71
3.4.9	Chiral Phases	72
3.4.10	Layers with Fluorescent Indicators	73
3.4.11	Making Your Own Plates	73
3.5	Light Absorption on Plate Surfaces	74
	References	77
4	The Mobile Phase in Adsorption and Partition Chromatography	81
4.1	Solvent Characteristics	81
4.2	Solvent Theory for Adsorption Chromatography	
	(According to Snyder)	83
4.2.1	Solvent Strength (ϵ^0)	85
4.2.2	Solvent Strength of Binary Mixtures	86
4.3	Solvent Theory in Partition Chromatography	88
4.3.1	Solvent Theory (According to Snyder)	89
4.3.2	Other Methods for Characterizing Solvents	92
4.4	Optimizing Solvent Composition	93
4.5	The PRISMA Model (According to Nyiredy)	97
4.6	Solvent Additives	99
4.7	Appendix: Solvent Properties	102
	References	103
5	Preparing and Applying Samples	105
5.1	Sample Preparation	105
5.1.1	The QuEChERS Approach	105
5.1.2	Solid-Phase Extraction	106
5.1.3	Stir Bar Sorptive Extraction	108

5.2	The Dosage Quality	108
5.3	Choice of Application Position	112
5.4	Practical Application Methods	113
5.4.1	Sample Application via Plate Contact	113
5.4.2	Sample Application Without Plate Contact	114
5.4.3	Sample Application via Contact Spotting	114
5.4.4	Plate Overloading and Incomplete Drying	116
	References	117
6	Basis for TLC Development Techniques	119
6.1	Influence of the Vapour Phase	119
6.2	Chamber Types for Linear Development	123
6.2.1	N-Chambers ("Trough Chambers")	123
6.2.2	S-Chamber ("Small Chamber")	124
6.2.3	Vario-KS-Chamber	125
6.2.4	The H-Chamber ("Horizontal Chamber")	125
6.3	Controlling Separations via the Vapour Phase	126
6.3.1	Solvent Composition During Separation	126
6.3.2	Plate Pre-loading via the Vapour Phase	130
6.4	Circular Separations	133
6.5	Solvent Gradients	134
6.5.1	Theory of Solvent Gradients	134
6.5.2	Evaporation-Controlled Gradient Elution	140
6.5.3	Multiple Development in TLC	142
6.5.4	Automated Multiple Development (AMD)	143
6.6	Normal Phase Separations with Water-Containing Solvents	145
6.7	Plate Development with Forced Flow	147
6.7.1	Rotation Planar Chromatography (RPC)	147
6.7.2	Over-pressure Layer Chromatography (OPLC)	147
6.8	Two Dimensional TLC (2D TLC)	148
6.8.1	Development in Orthogonal Directions	148
6.8.2	Grafted TLC	149
6.8.3	Stability Test and SRS Technique	151
6.9	Drying the Plate	153
	References	153
7	Specific Staining Reactions	155
7.1	Chemical Reactions Prior to Separation (Pre-chromatographic Derivatization)	157
7.1.1	Sample Enrichment by Pre-chromatographic Derivatization	157
7.1.2	Pre-chromatographic In Situ Derivatization	159
7.1.3	Pre-chromatographic Staining	163
7.1.4	Reagents in the Mobile Phase	167

7.2 Post-chromatographic Reactions (Derivatization After Development)	168
7.2.1 Fluorescence Enhancer	170
7.2.2 pH and Redox Indicators	171
7.2.3 Universal Reagents (Charring Reagents)	172
7.2.4 Aldehyde Reagents	173
7.2.5 CH- and NH-Reacting Reagents	176
7.2.6 Boron-Containing Reagents	180
7.2.7 Alkaline Reagents	182
7.2.8 Chloramine-T Reagent	183
7.2.9 Diazotization Reactions	184
7.2.10 Iodine-Starch and <i>Wursters</i> Reagents	185
7.2.11 Reactions with Metal Reagents	187
7.2.12 Reagents for Metal Cations	190
7.3 Reactions via the Gas Phase	191
7.3.1 Ammonium Bicarbonate Reagent	192
7.3.2 Tin(IV) Chloride Reagent	192
7.3.3 Formic Acid Reagent	193
7.3.4 Hydrogen Chloride Reagent	193
7.3.5 Trichloroacetic Acid Reagent	193
7.3.6 Nitric Acid Reagent	194
7.4 Thermal Treatment of TLC Plates	194
7.5 Activity Analysis Using Chemical Reagents	194
7.5.1 <i>Folin-Ciocalteu</i> Reagent	195
7.5.2 Checking for Free Radical Scavenger Activity Using DPPH Reagent	195
7.5.3 Nucleophilic Reaction Ability	196
References	197
8 Bioeffective-Linked Analysis in Modern HPTLC	201
8.1 Principle of the Method	202
8.1.1 Contaminant Analysis in the Environment and Food and the Principle of Bioactivity-Based Analysis	202
8.1.2 Aims and Fundamental Aspects of Bioeffective-Linked Analysis by Thin-Layer Chromatography	203
8.1.3 HPTLC as a Method for Bioeffective-Linked Analysis	203
8.2 General Rules for the Analysis of Bioeffective Compounds	205
8.3 Enzyme Tests	206
8.3.1 Urease-Inhibition Test for Heavy Metals	206
8.3.2 Analysis Using Redox Enzymes	207
8.3.3 The Detection of Cholinesterase Inhibitors	208
8.4 Inhibition of Photosynthesis by Herbicides	216
8.4.1 Reagent Preparation	216
8.4.2 Hill Reaction	217
8.4.3 Detection Using Algae	217

8.5	Detecting Bioeffective Compounds with Photobacteria	218
8.5.1	Practical Use of Photobacteria	218
8.5.2	Reaction Time Optimization	220
8.5.3	Applications of Photobacteria	221
8.6	Detection of Fungicidal- and Antibiotical-Active Substances in Environmental Samples	222
8.6.1	Determining Fungicides	222
8.6.2	Screening of Pesticides in Food and Surface Water	222
8.6.3	Detection of Compounds by Antibiotic Activity	223
8.7	Yeast Estrogen Screen	225
	References	227
9	Planar Chromatography Detectors	231
9.1	Transmittance Measurements in Thin-Layer Chromatography	231
9.1.1	The Lambert-Beer Law	232
9.2	Reflectance Measurements in TLC and HPTLC	233
9.2.1	The Kubelka-Munk Equation	234
9.2.2	Reflectance Measurements with a Diode-Array Scanner	237
9.2.3	Spatial Resolution on the Plate	239
9.2.4	Spectral Distribution on HPTLC Plates	240
9.2.5	Spectral Evaluation Algorithm	242
9.2.6	Video-Densitometric Measurements	245
9.3	Infrared and Raman Detection in Thin-Layer Chromatography	247
9.3.1	Analysis of Thin-Layer Chromatograms by Diffuse Reflectance Infrared Fourier Transformation	247
9.3.2	Analysis of Thin-Layer Chromatograms by Near-Infrared FT-Raman Spectroscopy	248
9.3.3	Analysis of Thin-Layer Chromatograms by Surface-Enhanced Raman Scattering Spectrometry	249
9.4	Mass Spectrometric Detection in TLC	250
9.4.1	Direct Plate Extraction (SSSP)	250
9.4.2	MALDI Techniques (MALDI-MS)	252
9.4.3	Atmospheric Pressure Mass Spectrometry	252
9.5	Thin-Layer Radiochromatography (TL-RC)	254
9.5.1	Direct Radioactivity Measurements on TLC Plates	254
9.5.2	Phosphor Imaging	255
	References	257
10	Diffuse Reflectance from TLC Layers	261
10.1	The <i>Lambert</i> Cosine Law	261
10.2	Theory of Diffuse Reflectance	263
10.2.1	Special Case a: The Reversal Reflectance Formula	267
10.2.2	Special Case b: The Fluorescence Formula	267
10.2.3	Special Case c: The Kubelka-Munk Expression	268

10.3	Mass-Dependent Reflection	270
10.4	Simplifying the Expression	274
	References	274
11	Fluorescence in TLC Layers	277
11.1	Theory of Fluorescence and Phosphorescence	277
11.2	Fluorescence Enhancement	280
11.3	Quantification in TLC by Fluorescence	282
11.3.1	Low Sample Concentration Fluorescence in Light Scattering Media	283
11.3.2	High Sample Concentration Fluorescence in Light Scattering Media	284
11.4	Contour Plots for Fluorescence Evaluation	285
11.5	TLC Plates Containing a Fluorescent Dye	285
	References	288
12	Chemometrics in HPTLC	289
12.1	Calculation of R_F Values	289
12.2	Compound Identification Using UV–Visible and Fluorescence Spectra	291
12.3	Correlation Spectroscopy	293
12.3.1	Theory of Correlation Spectroscopy	293
12.3.2	Combination of R_F and UV–Visible Spectral Library Search	296
12.3.3	Zone Purity Check	296
12.4	Selection of the Measurement Wavelength	298
12.5	Statistical Photometric Error (Detector Variance)	301
12.5.1	Reciprocal Model	301
12.5.2	Absorbance Model	302
12.5.3	Kubelka–Munk Model	303
12.5.4	Fluorescence model	304
12.5.5	Minimizing the Statistical Photometric Error	305
12.6	Diode Bundling and Data Smoothing	305
12.7	Signal Integration: Area or Height Evaluation?	307
12.8	Deconvolution of Overlapping Peaks	308
12.9	New Visualization Methods for Plots	310
	References	313
13	Statistics for Quantitative TLC	315
13.1	The Mean Value	315
13.2	Variance and Precision	316
13.2.1	Definition of Variance	316
13.2.2	Relative Variance	318
13.2.3	Quantification of Relative Variance	319

13.3	Trueness, Precision, and Accuracy	320
13.4	The Gauss Distribution	321
13.4.1	Area of the Gauss Distribution	322
13.4.2	Quantiles of the Gauss Distribution	322
13.4.3	Test for a Normal Distribution	324
13.5	Student's Distribution (t Distribution)	325
13.6	Error Propagation	326
13.7	Calibration Methods	328
13.7.1	The Linear Calibration Function	329
13.7.2	Estimating $\bar{X}_C$	330
13.7.3	Estimating a and $\bar{Y}_C$	331
13.7.4	Estimating the variances of a and $\bar{Y}_C$	333
13.8	The F -Test	334
13.9	The Linear Regression Variance	334
13.10	The Analytical Function of Linear Regression	335
13.11	Quantitative Analysis Using External Standards	338
13.12	Second-Order Calibration Function	339
13.13	Analytical Function of the Second-Order Calibration	343
13.14	Risk of Systematic Error	345
13.14.1	Constant Systematic Error	345
13.14.2	Proportional Systematic Error	345
13.15	Use of Internal Standards	346
13.16	Standard Addition Method	347
13.17	Mean t Test	350
	References	351
14	Planning an Analysis and Validation in TLC	353
14.1	Terms Used in Validation	353
14.2	Method Validation	354
14.2.1	Testing Specificity	354
14.2.2	Quantifying Analytes	357
14.2.3	General Aspects of Calibration	358
14.2.4	Linearity and Working Range	359
14.2.5	Choosing a Calibration Function	360
14.2.6	External Standard Calculation	362
14.2.7	Optimized Calibration Methods	362
14.2.8	Precision	363
14.2.9	Accuracy	364
14.2.10	Confidence Interval	367
14.2.11	Limit of Detection and Limit of Quantification	367
14.2.12	Robustness	370
14.3	Control Charts as Quality Indicators in Routine Analysis	372
	References	373
Index	375	