Topics

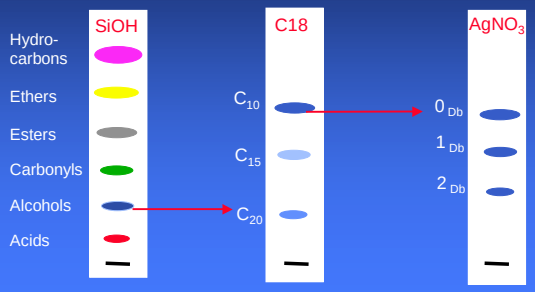
- Pretreatment of the plate, sample application
- Simple approach for solvent optimization: selectivity first
- How to control the chamber climate?
- AMD: a dream of a separation, but how to get it?

- How to visualize substances on a plate – benefits of multiple detection
- Chemical and biological detection: impressive tools of HPTLC
- Densitometric techniques: promising and limiting aspects
- Confirmation of results by mass spectrometry

- Method validation

Separation mechanisms

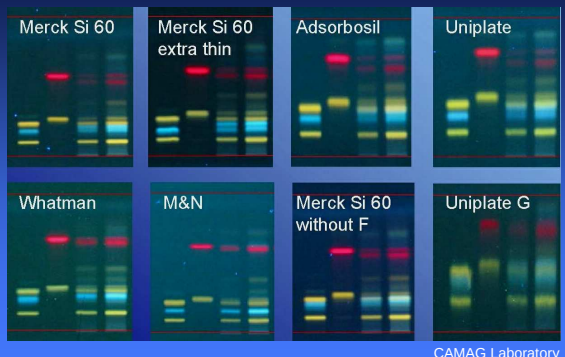
Adsorption	Partition	Complex
Functional groups polarity	Chain length lipophilicity	Complex stability e.g. double bondings



Guidelines for stationary phase selection

Silica gel	All classes of compounds
Aluminium oxide	Basic compounds (alkaloids, amines, etc.), steroids, terpenes, aromatic and aliphatic hydrocarbons
Amino phase	Sugars, carboxylic acids, sulfonic acids, phenols, purines, pyrimidines, nucleotides
Cyano phase	All classes of compounds, PHB esters
Diol phase	All classes of compounds, steroids, hormones
RP 2, 8, 18 phases	Polar substances, separation according to lipophilic properties and chain length, steroids, tetracyclins, phthalates, barbiturates, nucleo bases, aminophenols
Polyamide	Phenols, flavonoids, nitro compounds
Silica gel impregn.	PAHs (caffeine), number of diol groups (boric acid), number of isolated double bonds (silver nitrate)
Chiral phase	Enantiomers

Just silica gel...

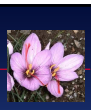


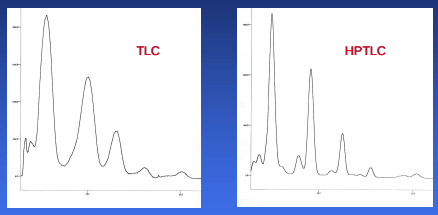
CAMAG Laboratory

TLC versus HPTLC versus UTLC

		TLC	HPTLC	UTLC
Silica gel		irregular particles	irr./glob. particles	monolithic without binders
Mesopores		60 Å = 6 nm	60 Å = 6 nm	30-40 Å = 3-4 nm
Mean particle size		10 - 15 µm	5 - 7 µm	1 - 2 µm macro pores
Particle distribution		wide	narrow	narrow
Layer thickness		200, 250 µm	100, 200 µm	10 µm
Number of samples		max. 12 20 x 10 cm	36 – 72 20 x 10 cm	10 6 x 3,6 cm
Migration distance		100 - 150 mm	30 - 70 mm	10 - 30 mm
Migration time		15 - 200 min	5 - 30 min	1 - 6 min
Solvent use		50 - 100 mL	5 - 20 mL	1 - 4 mL
Detection limit:	Abs	100 - 1000 ng	10 - 100 ng	1 - 10 ng
	Fluor	1 - 100 ng	0,1 - 10 ng	0,01 – 0,1 ng

TLC versus HPTLC





E. Hahn-Deinstrop

Zone diffusion according to van Deemter

$$\bar{H} = A + \frac{B}{v} + C \cdot v$$

B for HPTLC: $\bar{H}$ ~ 12 µm

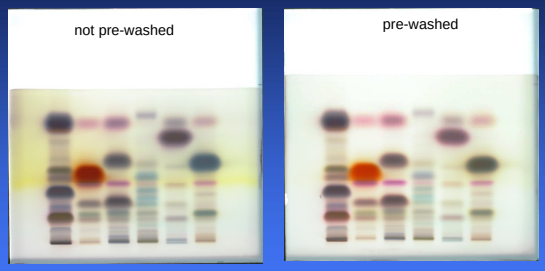
A, C for TLC: $\bar{H}$ ~ 30 µm

A	Layer quality, Eddy diffusion
B	Diffusion term, longitudinal diffusion
C	Retardation term, local non-equilibrium

$$\bar{H} = 2\,l\,dp + \frac{2\,\gamma\,D}{v} + \frac{w\,dp^2\,v}{D}$$

$\bar{H}$	Plate height, HETP
v	Velocity of solvent front
l	Function of layer packing
dp	Particle diameter
γ	Labyrinth factor
D	Diffusion coefficient
w	Factor of packing structure

Prewashing of old layers



Pre-chromatographed with methanol, see CBS 91

Prewashing of the layer

- ✓ to get rid of impurities (lab atmosphere, packing material, i.e. shrink wrapping foil etc.)
- ✓ to get rid of binder components which can be eluted by polar solvents
- ✓ to get a better baseline
- ✓ to improve LOD and LOQ
- ✓ to improve reproducibility

Important...
- for old layers
- for ultra trace level analysis
- if working range is starting from LOQ
- for quantitative HPTLC

Best way to do it?

According to Maxwell et al., JPC 12, 109-113 (1999)
- Two step cleaning method: with methanol first pre-development then immersion for 5 min, air-dry for 5 min, followed by heating at 80 °C for 15 min

According to Jork et al.
- immersion in iso-propanol over night or for at least 2 hours, followed by heating at 120°C for 30 min

According to CAMAG
- pre-development with methanol followed by heating at 120°C for 20 to 30 min

According to Dr. Burger

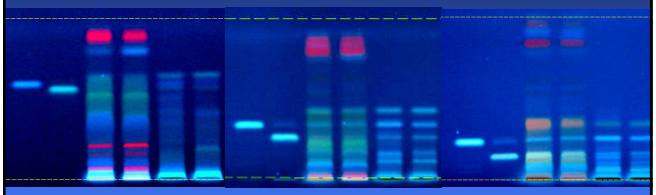
in a clean bench for at least 8 hours, followed by heating at 30 min at 50 - 100 °C
- neutral: with methanol
- acidic: formic acid – methanol 1:100, then methanol or
- basic (for acidic plates, e.g. Merck No. 15445): solution of 0,0001% sodium hydroxide (2 mL 0,1 M NaOH in 10 L methanol), then methanol

Best way to do it?

Note:
- Use very clean solvents for prewashing!
- Avoid any contamination again during drying!
- Cool down the active plate to room temperature in a dust and fume free environment (e.g. a large empty desiccator) and let it equilibrate with the relative humidity of the laboratory atmosphere!
- Take care of storage and declaration of prewashed plates!

Impregnation of the layer

No impregnation | Impregnation in a 4% solution of sodium acetate for 2 s | Impregnation in a 10% solution of sodium acetate for 20 s



Separation of ginkgolides with toluene - ethyl acetate – acetone - methanol 20:10:10:1.2
derivatization with acetic anhydride, see CBS 91

Impregnation of the layer

Formation of complexes with	Concentration of impregnation solution	Fields of application
EDTA	10%	Cephalosporins, tetracyclines, metal ions, phospholipids, phenols
Boric acid or borate	5%	Ascorbic acids derivatives, sugars, phosphatidylinositols, urethane derivatives, mono-/di-/triglycerides, stearic acid, lipids
Transition metals salts	5-20%	Amino acids, aromatic amines, sulfonamide, anilines, quinolines, phenol derivatives
Iron(III) salts	5-20%	Phenolic acids
Silver nitrate	3-20%	Interaction of Ag^+ with π-electrons of double/triple bounds. Fatty acids, diglyceride/triglyceride, phospholipids, glycolypids, steroids

Impregnation of the layer

Formation of charge transfer complexes

Caffeine	4 %	Polycyclic aromatic hydrocarbons (PAH)

Ion-pairing

Quaternary ammonium salts	0.05 M	Sulfa drugs, penicillins

Adjustment of pH-value

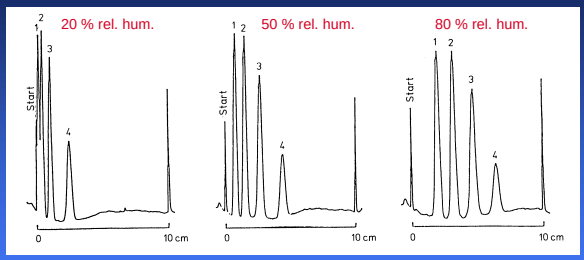
Inorganic acids	0.1-0.5 N	Phenols, acids, aromatic amines
Potassium/sodium hydroxide	0.1-0.5 N	Alkaloids, amines, basic compounds
Buffer salts		Curcumin derivatives, sugars, heavy metals, phloroglucinols

Impregnation of the layer

Modification of partition coefficient

Formamide		Local anesthetics, alkaloids, digitalis glycoside, nitrophenols
Ammonium sulfate		Lipids, phospholipids
Sodium nitrite		Phenols
Sodium bisulfite-citrate buffer		Sugars
Sodium sulfate	0.1 M	Sugars
Sodium acetate	4-10 %	Terpene lactones
Lithium/sodium/potassium salts		Metal ions, aromatic amines
Ammonium thiocyanate		Metal ions
Butylamine		Metal ions

Activity of the layer



Rabel, F. in Sherma, J., Fried, B.: Handbook of Thin-Layer Chromatography, Marcel Dekker, New York, 2003

‘Activation’ of the layer?

→ Silica gel: after 3 min 50 % of the max. water content is adsorbed
→ Aluminum oxide: after 12 min 80 % ...

↓

- Within a few minutes the humidity of the air is adsorbed
- Activation or storage in the desiccator - what happens during application?
- Breathing onto the layer can cause local de-activation

↓

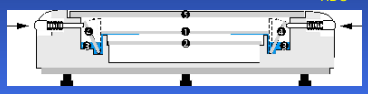
Reproducible humidity regulation by conditioning with definite dilutions of sulfuric acid or saturated salt solutions just before chromatography … or use mid- or unpolar stationary phases

Defined activity of the layer

mass % H_2SO_4	% rel. humidity		saturated salt solution	% rel. humidity
10	96		$Pb(NO_3)_2$	98
20	88		KBr	84
30	75		$NaNO_2$	66
40	56		$NaHSO_4 \cdot H_2O$	52
50	35		KF	31
60	16		HCOOK	21
70	3		$ZnCl_2 \cdot 1.5\ H_2O$	10

Humidity control

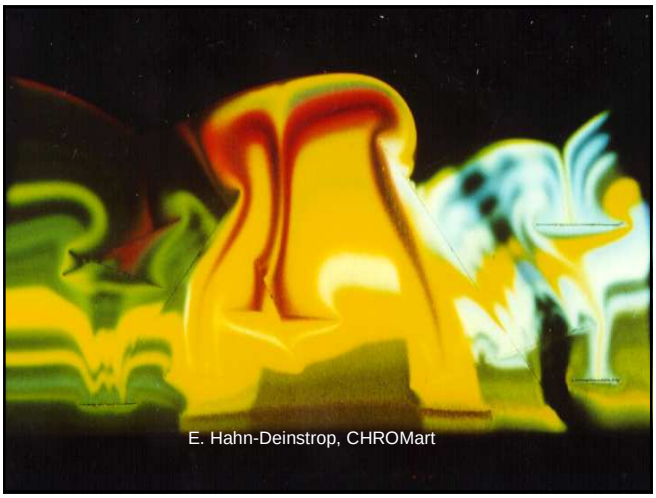
HDC



ADC2 (CAMAG)

Plates - pretreatment

- Separation mechanisms
- Guidelines for the selection
- Manufacturer/batch dependence
- TLC versus HPTLC versus UTLC
- Prewashing
- Impregnation
- Plate activity



Overview of sample application

- Critical step in the TLC procedure → GLP conform
  Instrument Validation, Operational Qualification

- How to do it best?
  - ✓ Advantages of automated application
  - ✓ Modes of application
  - ✓ Contact or spray-on technique? Bands or spots?
  - ✓ Advantages of bandwise application
  - ✓ Influence and properties of the application solvent

- Special cases
  - ✓ Overspotting
  - ✓ Application for preparative purposes

Application devices

Fully automatic devices
- CAMAG Automatic TLC Sampler (ATS4)
- DESAGA AS 30 TLC Applicator and Sampler
- Zinsser Analytic GmbH Lizzy-TLC
- Baron TLS 100

Half automatic devices
- CAMAG Linomat 5

Manual devices
- CAMAG Nanomat 4
- OM Laboratory SA-101 Multiple Sample Applicator
- DESAGA TLC Spotter PS 01
- Romer TLC AutoSpotter

Examples for application volumes

ATS 4

Syringe
10, 25, 100 µL

100 nL – 1 mL

Linomat 5

Syringe
100, 500 µL

1 µL – 500 µL

Nanomat 4

Capillary

0.5, 1, 2, 5 µL

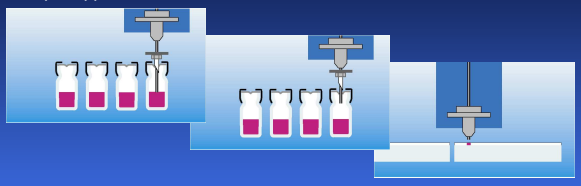
High sample volumes:
- Option with heated spray nozzle
- Spraying as rectangles/area

Advantages of automated application

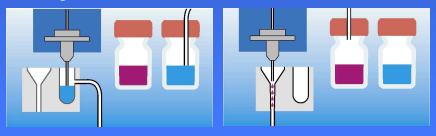
- ✓ GLP conform documentation
- ✓ Better reproducibility
  - → No damage of layer
  - → Exact volume applied
- ✓ Enables band application which improves separation
- ✓ Time-saving
- ✓ Standardized rinsing procedure (avoids cross over)
- ✓ Independent of personal variances
- ✓ A „must“ for quantitative HPTLC!

High performance mode of application

Sample application



Rinsing



Instrument qualification

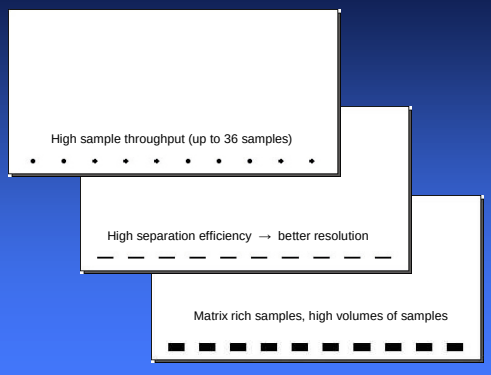
ATS 4 Instrument Validation

Positioning test	Target	Detected	?
Table backlash	<200µm	Xxx	OK
Table reproducibility	<25µm	Xxx	OK
Table leeway	Yes	Yes	OK
Tower backlash	<200µm	Xxx	OK
Tower reproducibility	<50µm	Xxx	OK
Tower leeway	Yes	Yes	OK
Rack backlash	<200µm	Xxx	OK
Rack reproducibility	<50µm	Xxx	OK
Rack leeway	Yes	Yes	OK
Syringe backlash	<100µm	Xxx	OK
Syringe reproducibility	<10µm	Xxx	OK
Syringe leeway	Yes	Yes	OK
Lift backlash	<200µm	Xxx	OK
Lift reproducibility	<50µm	Xxx	OK
Lift leeway	Yes	Yes	OK
Punch move adjustment	<200µm	Xxx	OK
Punch delay min.	>180ms	Xxx	OK
Punch delay max.	<300ms	Xxx	OK
Punch leeway	Yes	Yes	OK
Syringe test (Spray)	Target	Detected	?
Spray test	4-6	7	
Spray test (repeat)	4-6	5	OK
Leakage test 1	Yes	Yes	OK
Leakage test 2	Yes	Yes	OK
Rinsing	Yes	Yes	OK
Syringe test (Contact)	Target	Detected	?
Contact test	Yes	Yes	OK
Leakage test 1	Yes	Yes	OK
Leakage test 2	Yes	Yes	OK
Rinsing	Yes	Yes	OK

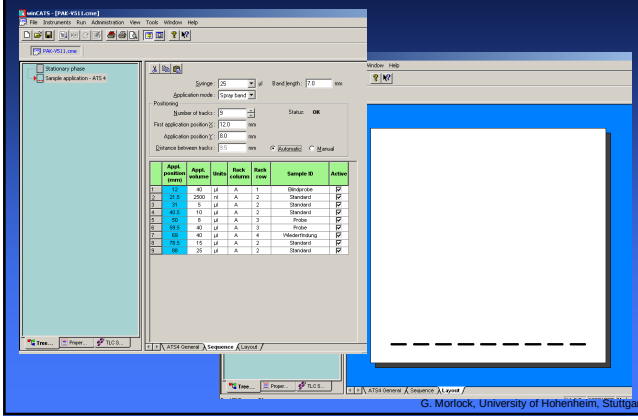
ATS 4 Operational Qualification

- ✓ Checksum of the installed software
- ✓ Cleanup of spray nozzle
- ✓ Check of state of the septum punch
- ✓ Manual confirmation of gas-tightness of the syringe and application pattern
- ✓ Reproducibility of phenacetin
  - by contact application:
    volume error is ≤ 1.5 %
    (or the total error is ≤ 2.1 %)
  - by spray application:
    volume error is ≤ 1.5 %
    (or the total error is ≤ 1.8 %)

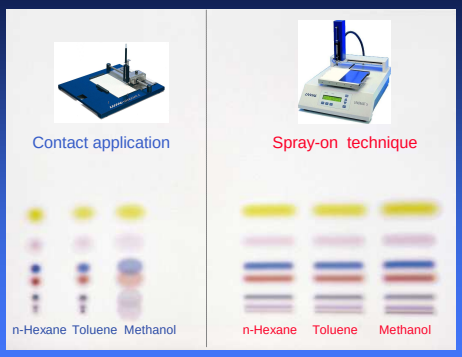
Modes of application



Sequence and layout

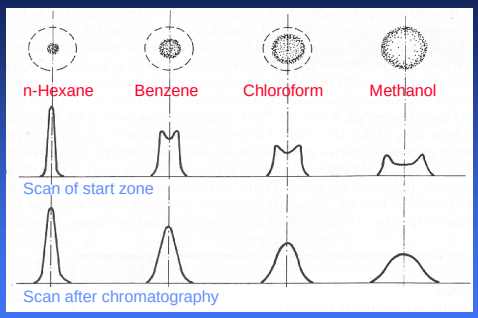


Contact or spray-on technique?



Note: Application solvent has great influence by contact application.
Band application improves separation!

Influence of the application solvent



Note: Application solvent has a great influence by contact application.
It should have as less elution power as possible!

Properties of the application solvent

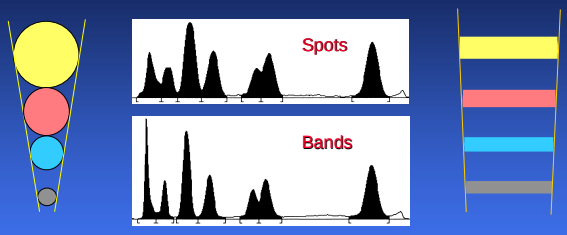
Volatility

- Volatility enables evaporation - if the application solvent is not completely evaporated it can influence chromatography.
- The more volatile, the faster the application rate can be.

Elution power

- Elution power should be as low as possible, however sample should sufficiently be dissolved.

Bands or spots?



Note: Band application improves separation
- especially by high sample volumes!

Advantages of bandwise application

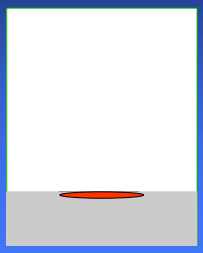
- ✓ Better resolution (about 32 % according to Touchstone and Levin, J. Liqu. Chromatogr. 3 (1980) 1853)
- ✓ Better S/N ratio because of evaluation of the homogeneous middle part
  ⇒ Better reproducibility, LOQ and LOD
- ✓ Enabling a multi-level calibration by application of different volumes of the same standard solution via spray-on technique
  ⇒ Less labor time and avoidance of dilution errors

How to get a focussed start zone?

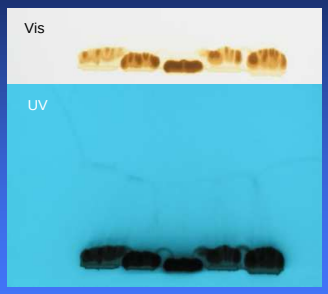
- ✓ Choose suitable solvent for application
- ✓ Decrease rate of application
- ✓ Dilute the sample and apply higher volumes
- ✓ Concentration of 0,001 % (10 ng/µL) to 0,1 % (1 µg/µL)
- ✓ Apply band-wise instead of spot-wise
- ✓ Focusing of high and matrix-rich volumes

Focusing

- ✓ High volumes of matrix-rich samples can be applied as areas followed by a focusing pre-run with a polar solvent, e.g. methanol, up to the upper edge of the start zone area

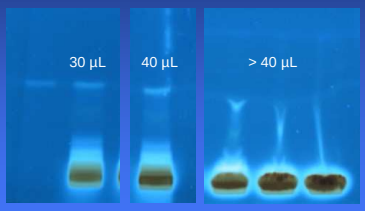


High matrix loading



Area application

Improves LOD of sucralose in matrix to 1 mg/kg

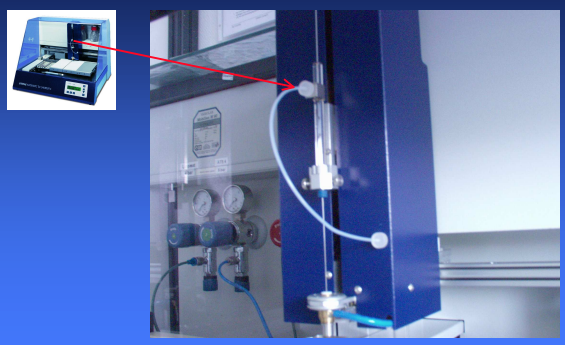


Application in trace analysis

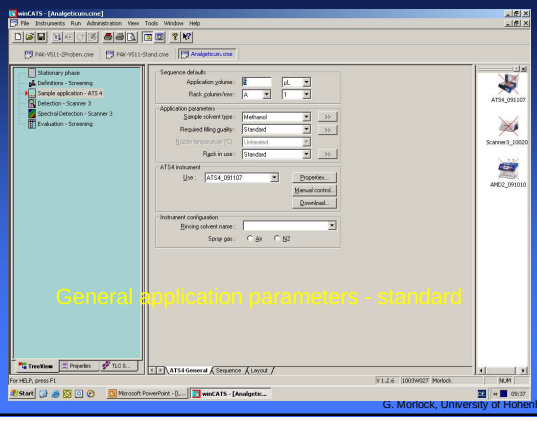
- ✓ Samples of high volumes (e. g. 100 µL)
- ✓ Matrix-rich samples (obstacle for migration of MP)
- ✓ Sample is valuably (completely applicated)
- ✓ Need for automated application (spray-on)

Application in trace analysis

Application in trace analysis

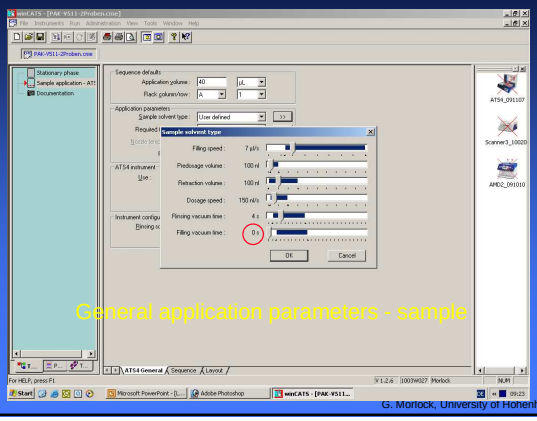


Application in trace analysis



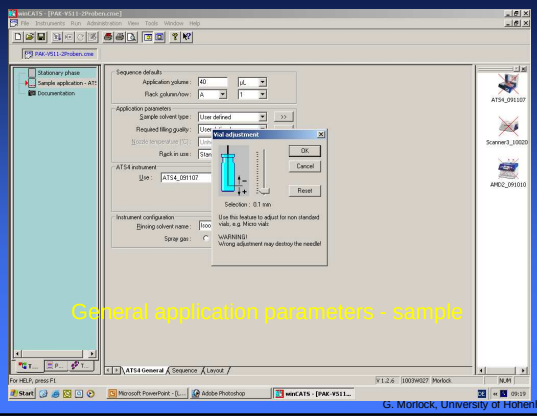
General application parameters - standard

Application in trace analysis



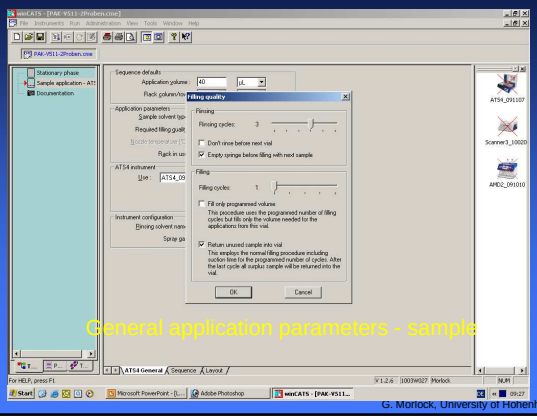
General application parameters - sample

Application in trace analysis



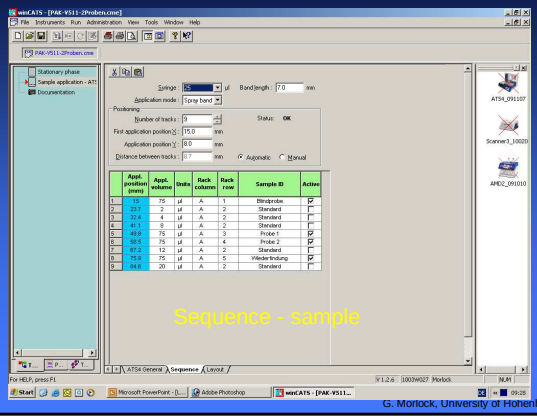
General application parameters - sample

Application in trace analysis



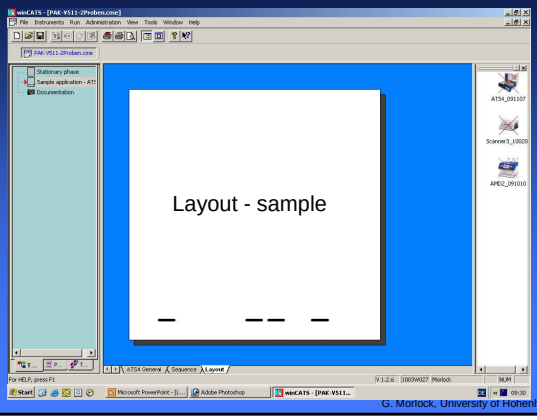
General application parameters - sample

Application in trace analysis

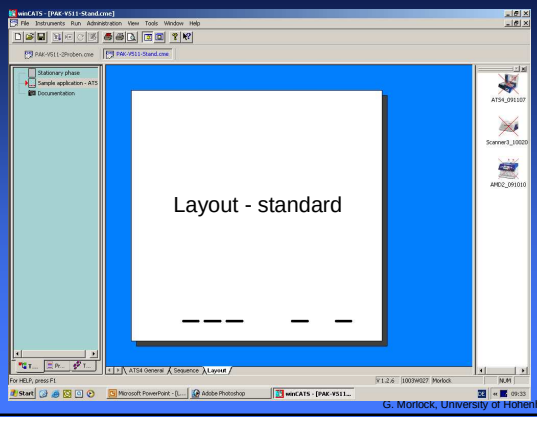


Sequence - sample

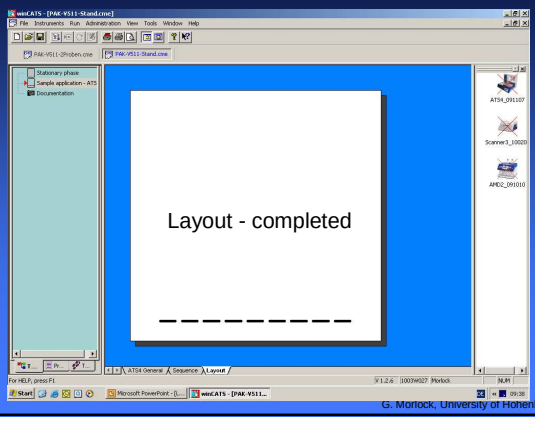
Application in trace analysis



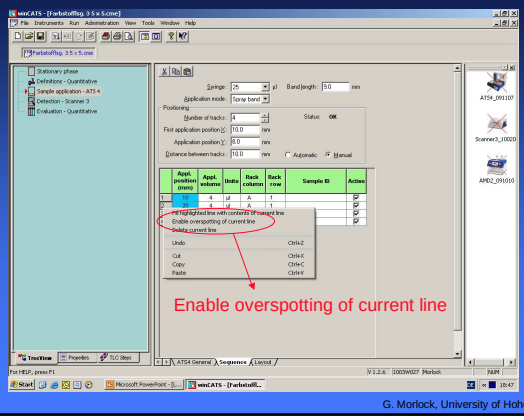
Application in trace analysis



Application in trace analysis



Overspotting of bands



Overspotting of bands

1. Compiling a standard mixture

Separation of 15 heterocyclic aromatic amines (HAA)

Häberle, S.: Diploma Thesis, University of Hohenheim, 2004

Overspotting of bands

2. Enabling pre-chromatographic derivatization in situ

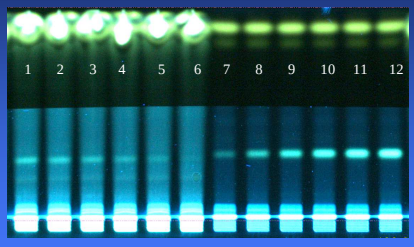
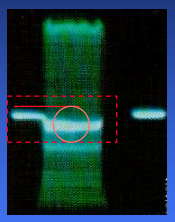


Plate image illuminated at UV 366/>400 nm

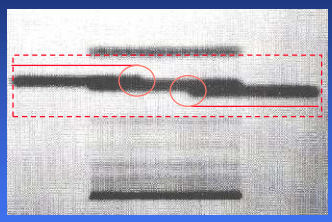
see D. Müller et al., Poster

Overspotting of bands

3. Confirmation of matrix interferences



Approval



Disapproval – not the same!

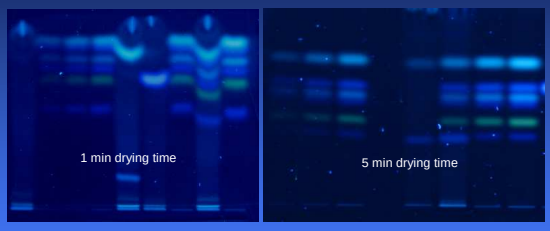
Hahn-Deinstrop, E.: Applied Thin-Layer Chromatography.
Best practice and avoidance of mistakes, 2006, Wiley-VCH, Weinheim.

Application for preparative purposes

- High volume of sample (e. g. 1 mL)
- Higher syringe volume (e. g. 500 µL)
- Application as streak (e. g. 18 cm band)
- Higher TLC layer thickness (> 500 µm)
- Special devices
  - ✓ CAMAG Linomat 5
  - ✓ Alltech TLC sample streaker
  - ✓ ATS4

To avoid this...

Drying of the starting zones



Overview of sample application

- Critical step in the TLC procedure → GLP conform
  Instrument Validation, Operational Qualification
- How to do it best?
  - ✓ Advantages of automated application
  - ✓ Modes of application
  - ✓ Contact or spray-on technique? Bands or spots?
  - ✓ Advantages of bandwise application
  - ✓ Influence and properties of the application solvent
- Special cases
  - ✓ Overspotting
  - ✓ Application for preparative purposes

Joseph, Maria and the Baby
E. Hahn-Deinstrop, CHROMart, GIT Special Separation 1 (2-3) 2000.

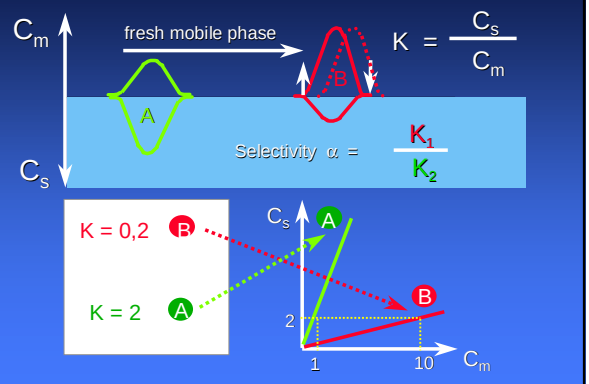
Overview of mobile phase

- ✓ Classification of solvents (Trappe , Snyder)
- ✓ Optimization scheme
- ✓ Isotherms, peak asymmetry
- ✓ Variations in temperature
- ✓ Stabilizers (manufacturer, batch)
- ✓ Viscosity (law of migration)
- ✓ Developing distance
- ✓ GLP recommendations

Chromatographic separation

Interactions	kJ/mol
Van der Waals forces	5 - 20
Dipole-induced dipole	8 - 25
Dipole-dipole	25 - 40
Hydrogen bonding	25 - 40
Ionic bonding	250 - 1050
~~Covalent bonding~~	~~670 - 3360~~

Partition and adsorption isotherms



Classification of solvents

According to Trappe

- ✓ eluotropic series listed according to increasing elution power
- ✓ elution power is defined as adsorption energy per unit surface area of sorbent
- ✓ dependent on the sorbent
- ✓ standardized on pentane

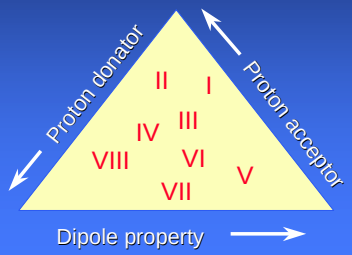
Eluotropic series of different sorbents

Silica gel	Aluminum oxide	Polyamide
n-Hexane	Pentane	Water
Pentane	n-Hexane	Methanol
Cyclohexane	Cyclohexane	Ethanol
Carbon tetrachloride	Carbon tetrachloride	Propanol
Toluene	Toluene	n-Butanol
Chloroform	Diethylether	Ethylmethylketone
Dichloromethane	Chloroform	Acetone
Diethylether	Dichlormethane	Acetonitrile
Ethyl acetate	Acetone	Formamide
Acetone	Ethyl acetate	Dimethyl formamide
Ethanol	Pyridine	Dil. sodium hydroxide
Methanol	Ethanol	
Pyridine	Methanol	
Water	Water	

Classification of solvents

According to Snyder

- solvent strength
- selectivity groups (selectivity triangle)



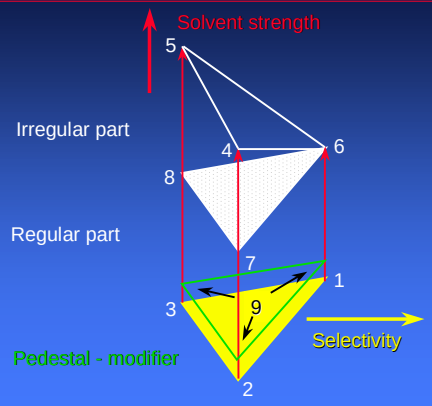
Normal phases

Group	Solvent	Solvent strength
Decrease	n-Hexane	0
I	n-Butylether	2,1
	Isopropylether	2,4
	Methyl-t-butylether	2,7
	Diethylether*	2,8
II	n-Butanol	3,9
	2-Propanol*	3,9
	1-Propanol	4,0
	Ethanol*	4,3
	Methanol	5,1
III	Tetrahydrofuran*	4,0
	Pyridine	5,3
	Methoxyethanol	5,5
	Dimethylformamide	6,4

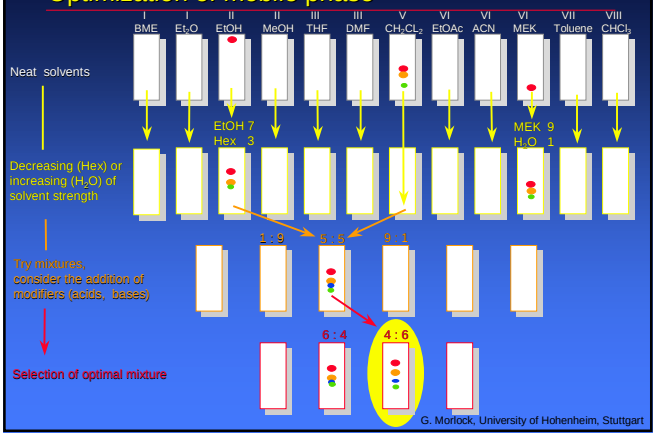
Reverse phases

Group	Solvent	Solvent strength
Decrease	Water	0
II	Methanol*	2,6
	Ethanol	3,9
	2-Propanol	4,2
III	Tetrahydrofuran	4,5
VI	Acetonitrile*	3,2

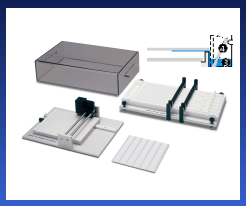
Prisma model



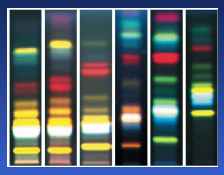
Optimization of mobile phase

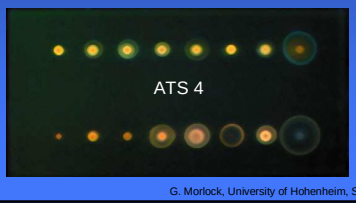


Neat solvents

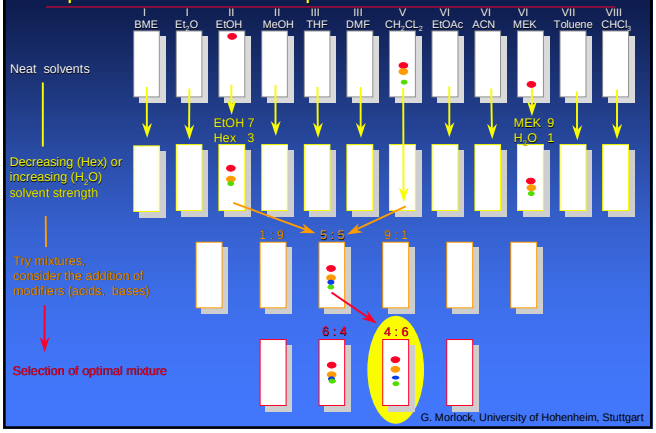


HPTLC Vario Chamber



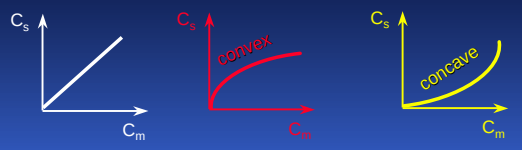


Optimization of mobile phase



Peak asymmetry

Partition isotherm



Concentration profile

Shape of the zone

Tailing

- Overloading of the layer with substance
  - ➢ reduce amount or (take plate with higher layer thickness)
- Retarded desorption due to active sorbent
  - ➢ use chamber saturation, preconditioning, modified layer
- Reaction between substance and sorbent
  - ➢ prewash, change or modify layer; mask interferences
- Local gradient by polar solvent rests from application
  - ➢ remove solvent rests
- Convex partition/adsorption isotherm
  - ➢ change system, reduce substance
- Dissociation of weak acids or bases
  - ➢ buffer layer or/and solvent, add acids or bases to solvent
- Chemical change of substance
  - ➢ modify layer, work in protected atmosphere

Heading

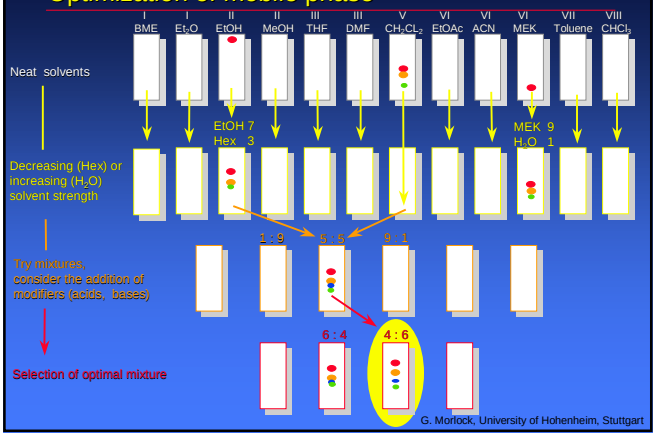
Wet start zone & weak mobile phase

- dry start zone, stronger mobile phase

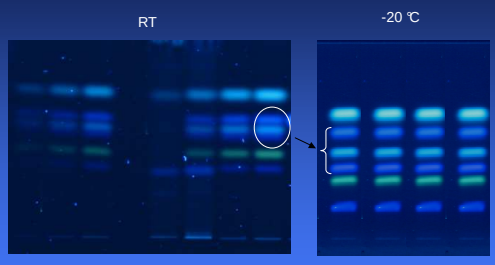
Concave partition/adsorption isotherm

- change system

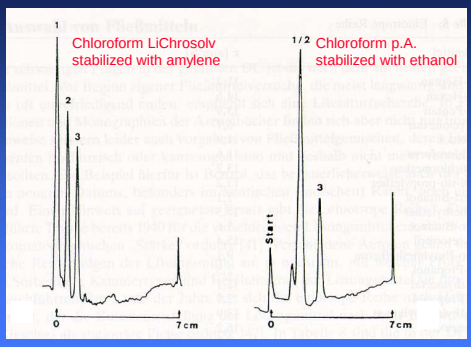
Optimization of mobile phase



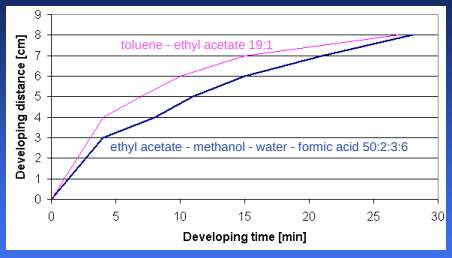
Temperature dependence of the separation



Stabilizers (manufacturer, purity grade)



Developing distance - velocity



Note: Do not exceed a developing distance of 6 cm on HPTLC plates.

The higher the developing distance, the lower the velocity of mobile phase, the more influence of diffusion effects!

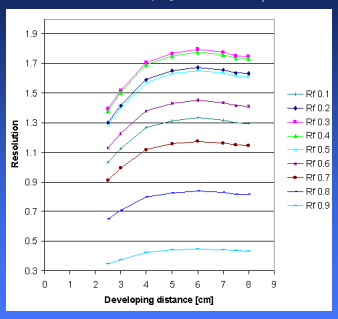
Law of migration

$$V_F = k \frac{\gamma}{\eta * 2 * z_F}$$

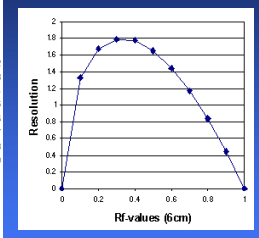
- V_F velocity of solvent front
- γ surface tension
- η viscosity
- z_F migration distance

Developing distance - resolution

Influence of the developing distance and R_F values



R_F values in detail at 6 cm



Note: Components of complex mixtures should be spread over the entire separation distance. The most critical substance pair should be maintained at R_F 0.3 for best separation.

Use of solvents

Multiple use of solvents

Chloroform – methanol – ammonia 56:14:1

Do´nt re-use solvents!

Preparation of solvents

Ethyle acetate – formic acid – acidic acid – water 100:11:11:27

Prepare solvents freshly!

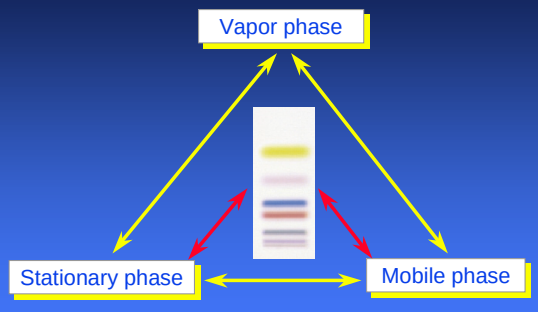
Hahn-Deinstrop, E.: Applied Thin-Layer Chromatography. Best practice and avoidance of mistakes, 2000, Wiley-VCH, Weinheim, ISBN 3527-298398.

Overview of mobile phase

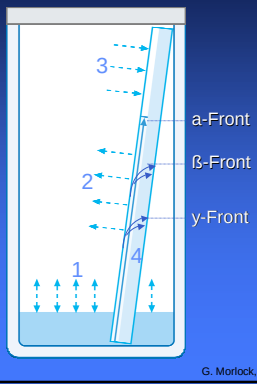
- ✓ Classification of solvents (Trappe , Snyder)
- ✓ Optimization scheme
- ✓ Isotherms, peak asymmetry
- ✓ Variations in temperature
- ✓ Stabilizers (manufacturer, batch)
- ✓ Viscosity (law of migration)
- ✓ Developing distance

Christiane´s Legs
E. Hahn-Deinstrop, CHROMart, GIT Special Separation 1 (2-3) 2000.

How to control the chamber climate?

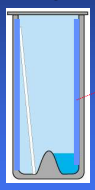


Mobile phase mixtures



Chamber climate under control

TTC



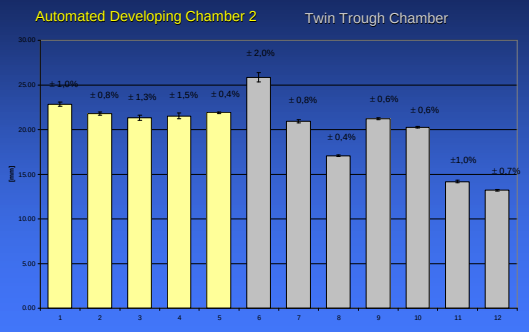
with wetted filter paper

HDC

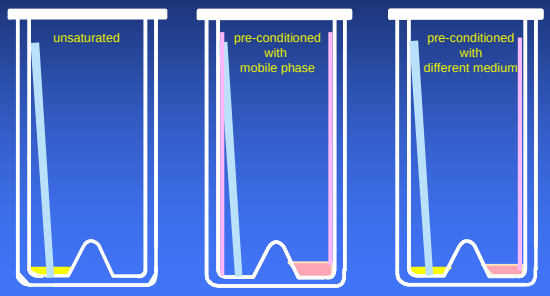
solvent at the bottom

ADC2 (CAMAG)

Migration distance under control

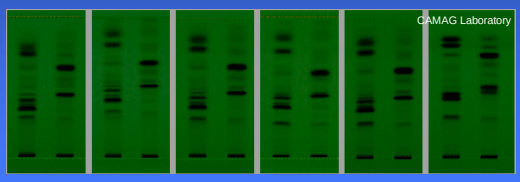


Modes of the Twin Trough Chamber



Developing chambers

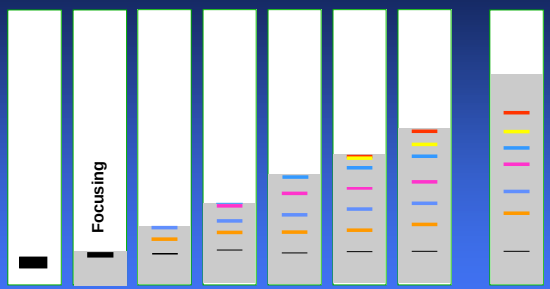
- ✓ Flat Bottom/Twin Trough Chamber
- ✓ Automatic Developing Chamber (ADC)
- ✓ Horizontal Developing Chamber (HDC)
- ✓ Automated Multiple Development (AMD)



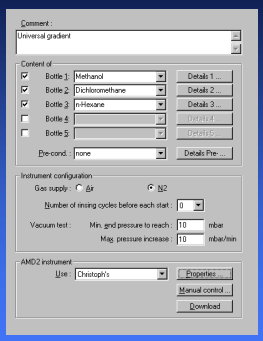
Automated Multiple Development (AMD)

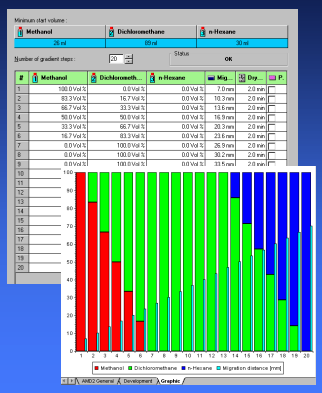
- ✓ Burger et al. (1984): polarity gradient by multiple de with different solvents
- ✓ Also possible: pH gradient
- ✓ Drying under vacuum improved precision and reliability
- ✓ Focusing to sharp zones
- ✓ Zone profile independent of migration distance
- ✓ Migration distance independent of matrix
- ✓ Automation
- ✓ Separation of substances differing in polarity to a high extent
- ✓ Separation number > 40 at a migration distance of 80 mm

Stepwise development

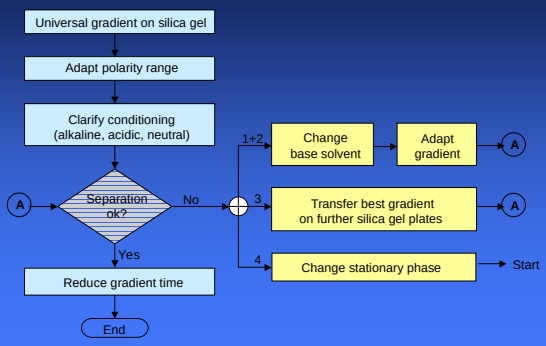


Step gradient





Gradient optimization

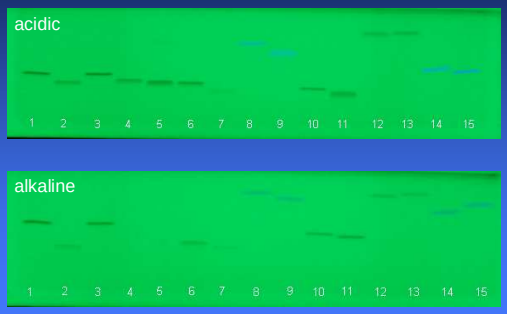


Gradient optimization

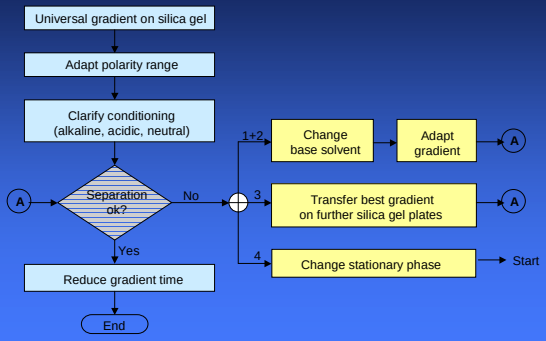
1. Start with a universal gradient

Increasing solvent	Base solvent	Decreasing solvent
methanol	dichloromethane	n-hexane
methanol	t-butyl methyl ether	n-hexane
acetonitrile	dichloromethane	n-hexane
methanol/water	acetonitrile	dichloromethane
methanol/water	t-butyl methyl ether	dichloromethane
various solvents	ethyl acetate	various solvents
acetone	various solvents	various solvents

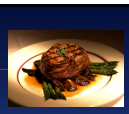
Check conditioning

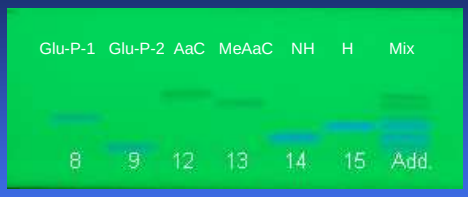


Gradient optimization



Separation of 6 apolar HAA





Rules

Polarity gradients gentler than those given in the table below cannot be recommended:

Polarity change over 10 steps	min. change of volume [%]
Methanol to dichloromethane	5
Acetonitrile to dichloromethane	10
T-butyl methyl ether to n-hexane	15
Dichloromethane to n-hexane	30

To avoid increasing diffusion of peaks 5-10 steps are sufficient for isocratic development.

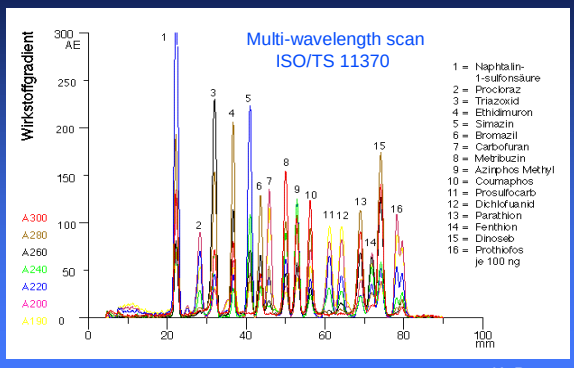
Hints

If the time of the gradient is too long (e.g. a gradient with 25-steps on a 200 µm plate with 3 mm increments takes about 4 h)

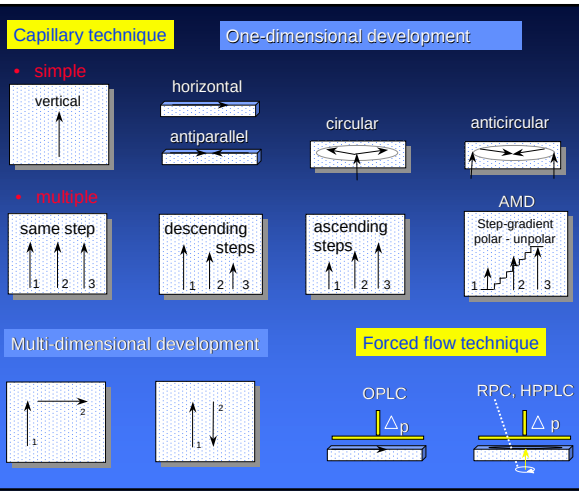
- Use 100 µm layers: shorter developing and drying times (about 2.5 h)
- Leave out parts not used: reduction to 20 steps (about 2.5 h)
- Use shorter drying times if possible
- Use spherical silica gel plates – reduces time, also drying time, to about 50 %

✓ 20 step gradient on spherical silica gel in 1.5 h for 18 samples, i.e. 5 min per sample.

Pesticides in drinking and surface water



K. Burger



General GLP recommendations

- ✓ Use multi component solvent systems only once - composition will change!
- ✓ Prepare solvents freshly!
- ✓ Don`t use the trough chamber as "shaker"!
- ✓ Consider volume contraction – measure separately!
- ✓ Pre-wash old layers!
- ✓ Don`t breathe onto the layer or blow fluffs off - you should condition in other modes!
- ✓ Note all relevant factors incl. humidity and temperature.
- ✓ For chamber saturation use a filter paper wetted with solvent and let the vapor phase equilibrate for at least 20 min.
- ✓ Use data pair method to reduce plate inhomogeneity.

Data pair method