Workshop
Planar Chromatography
Part I

Gerda Morlock

Institute of Food Chemistry

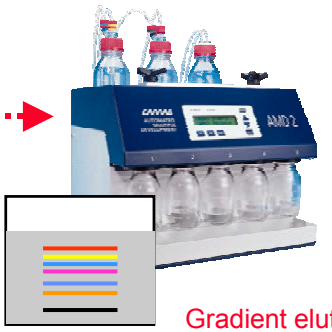
University of Hohenheim

Stuttgart, Germany

Planar Chromatography

Fully automatic application

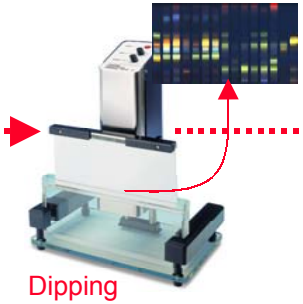
Half automatic application



Gradient elution

Fully automatic chromatography

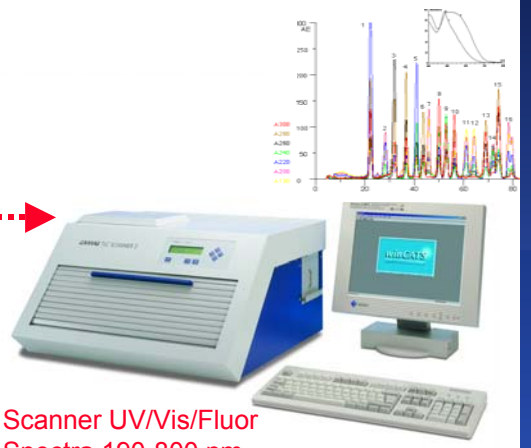
Horizontal development



Dipping

Spraying

Heating



Scanner UV/Vis/Fluor
Spectra 190-800 nm

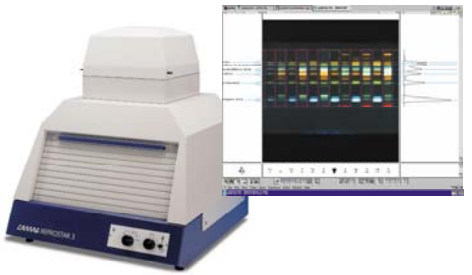


Image documentation
Video densitometry

Application Chromatography Derivatization Evaluation

today tomorow

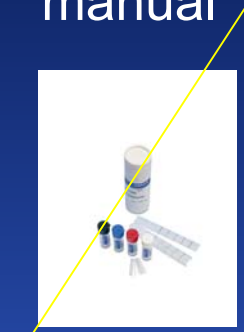
Overview of sample application

- Critical step in the TLC procedure
- 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
  - ✓ Devices and examples for application volumes
- Special cases
  - ✓ Overspotting
  - ✓ Application for preparative purposes
  - ✓ Application of effluent from HPLC
- GLP conform, Instrument Validation, Operational Qualification

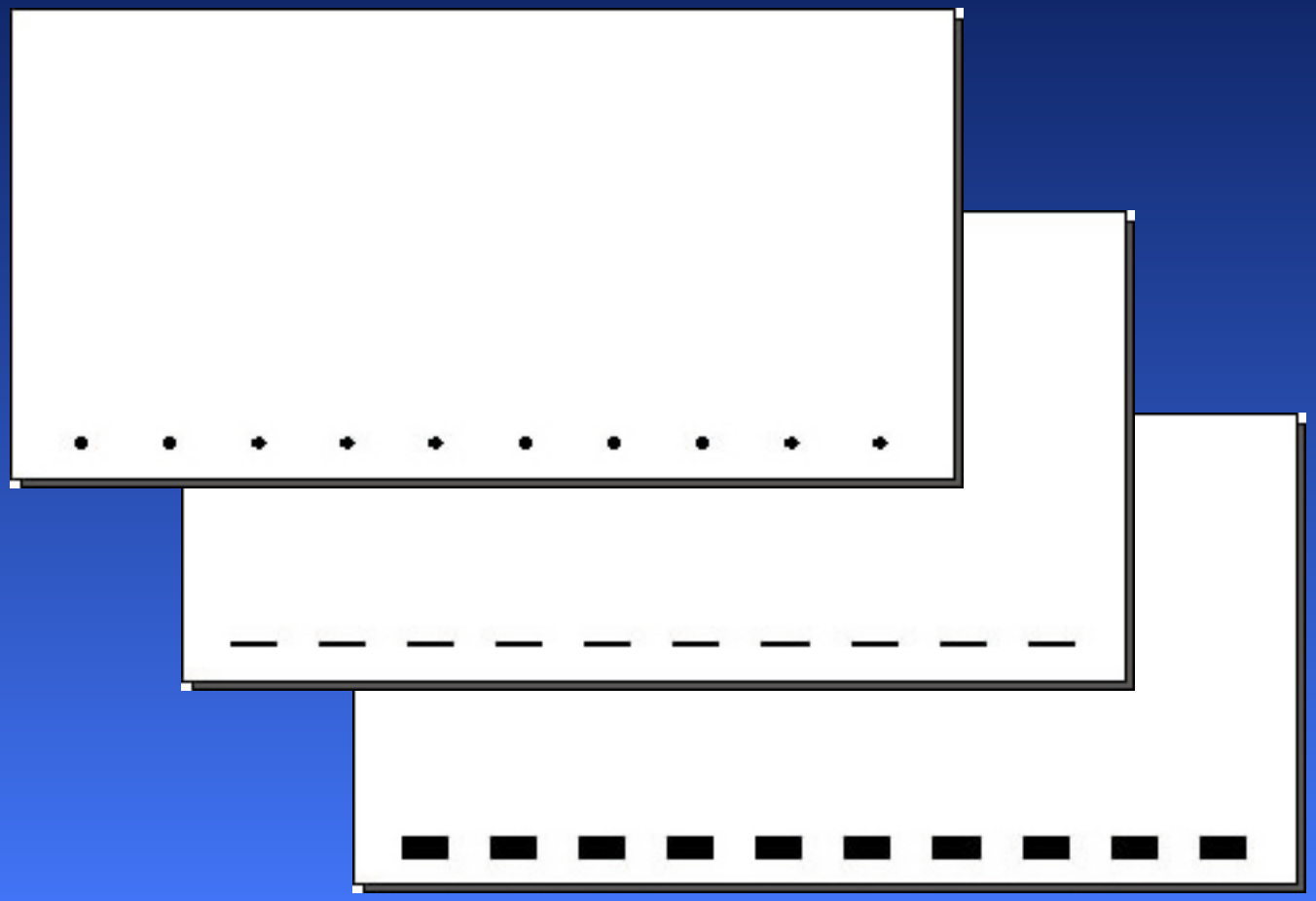
Advantages of automated application

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

manual



Modes of application



Contact or spray-on technique?

Nanomat
contact application

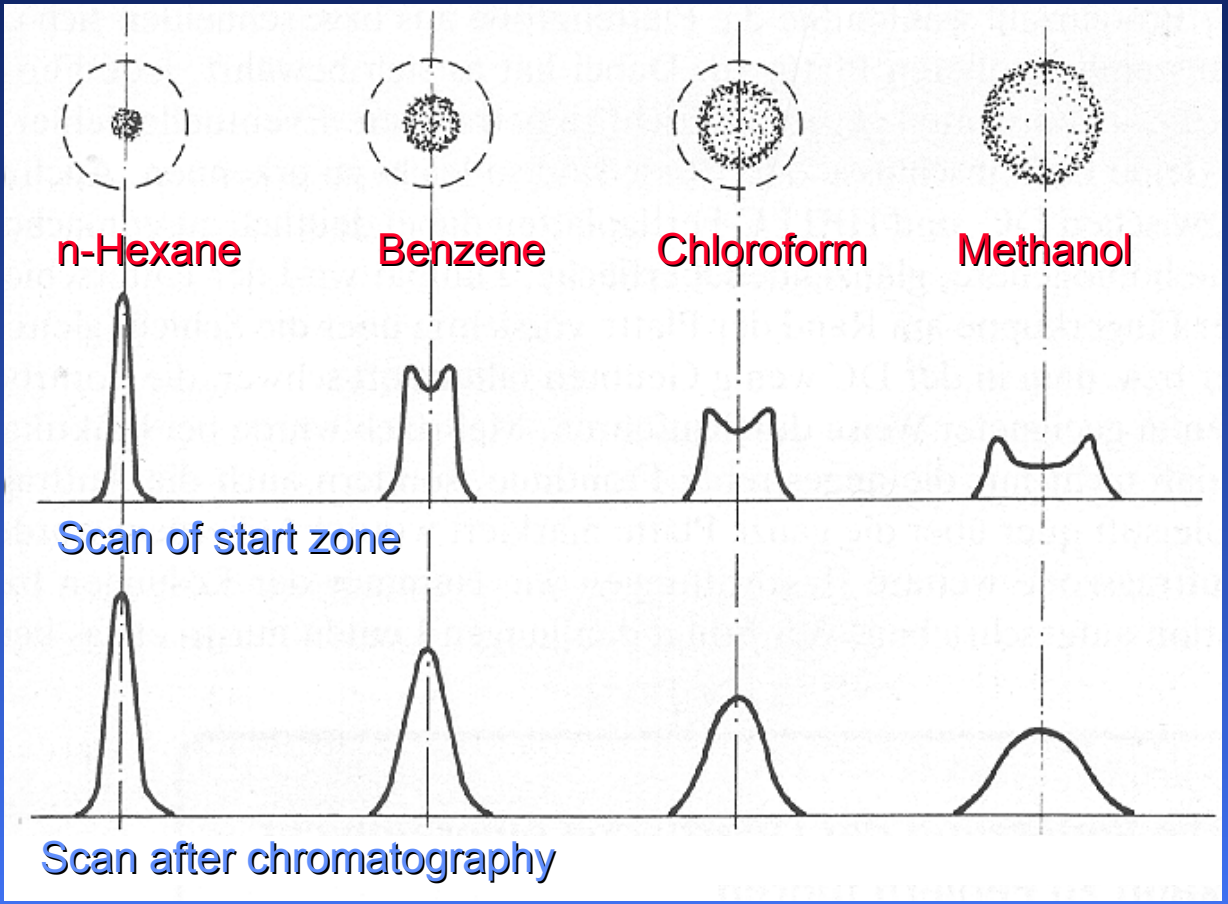
Linomat
spray-on technique

n-Hexane Toluene Methanol

n-Hexane Toluene Methanol

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

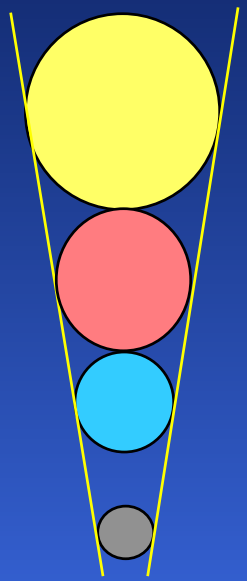
Volatility

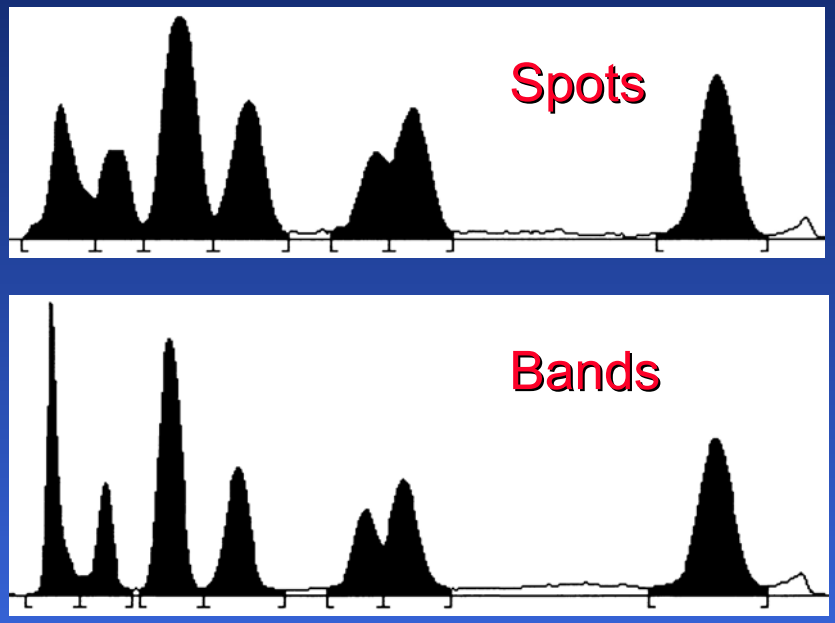
- Volatility enables evaporation - if the application solvent not completely evaporated it can influence chromatography (heading).
- The more volatile, the faster the application rate can be.

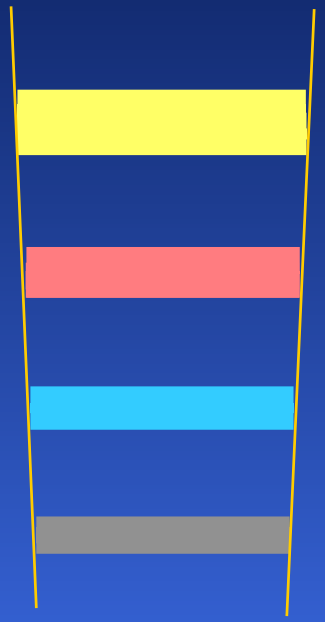
Elution power

- 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. Chromatog. 3 (1980) 1853)
- ✓ Better S/N ratio because of evaluation of the homogeneous middle part (consequently 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 bandwise instead of spotwise
- ✓ High volumes of matrix-rich samples can be applied as areas followed by a focussing pre-run with a polar solvent, e.g. methanol, upto the upper edge of the start zone area

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

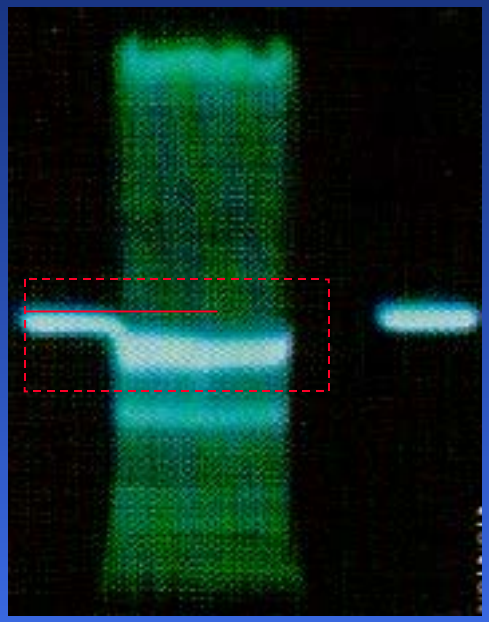
High sample volumes:
- Option with heated spray nozzle
- Spraying as rectangles/area

Note: The higher the application volume, the more volatile and unpolar the solvent for application should be - otherwise it should be applied slowly.

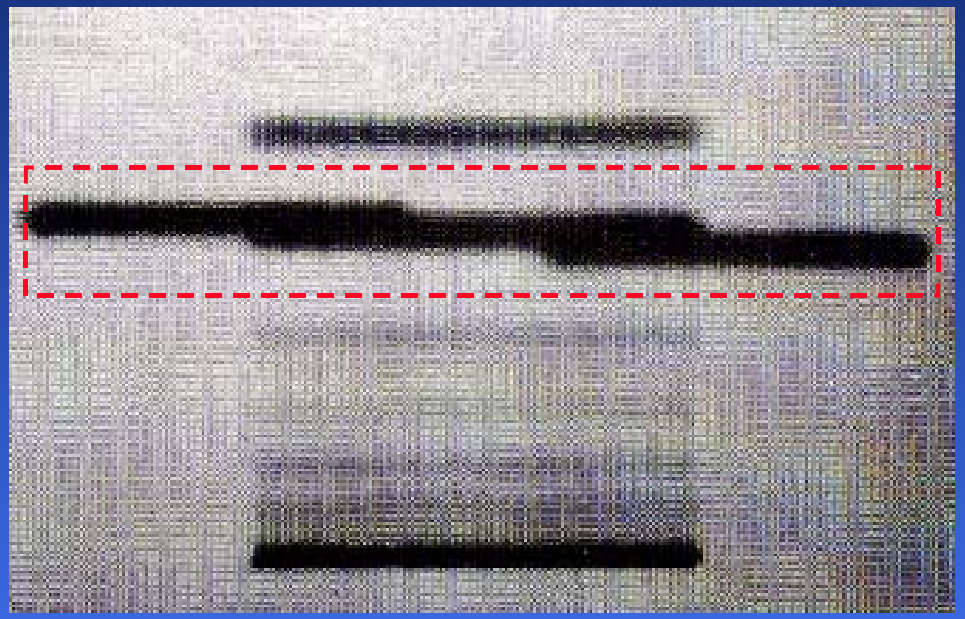
Overlapped application of bands

Shift of hR_F value due to matrix interference or different compounds?

Overlapped application of standard and sample gives the right answer!



Approval



Disapproval

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

Application for preparative purposes

- With 500 µL syringe
- High volume of sample applied as streak, e.g. 18 cm band
- TLC layer thickness > 500 µm
- Devices
  - ✓ CAMAG Linomat 5: Half automatic device, PC controlled
  - ✓ Alltech TLC sample streaker: Manual device

Application of effluent from HPLC

- Special application device called DuoChrom
- Flow rate 100 µL/min for methanol (40 µL/min for methanol - water 3:7)
- Average cut time 1-2 min, delay time 2 - 600 s
- Application as rectangles/area
- Spray-on technique with heated spray nozzle allows higher flow rates

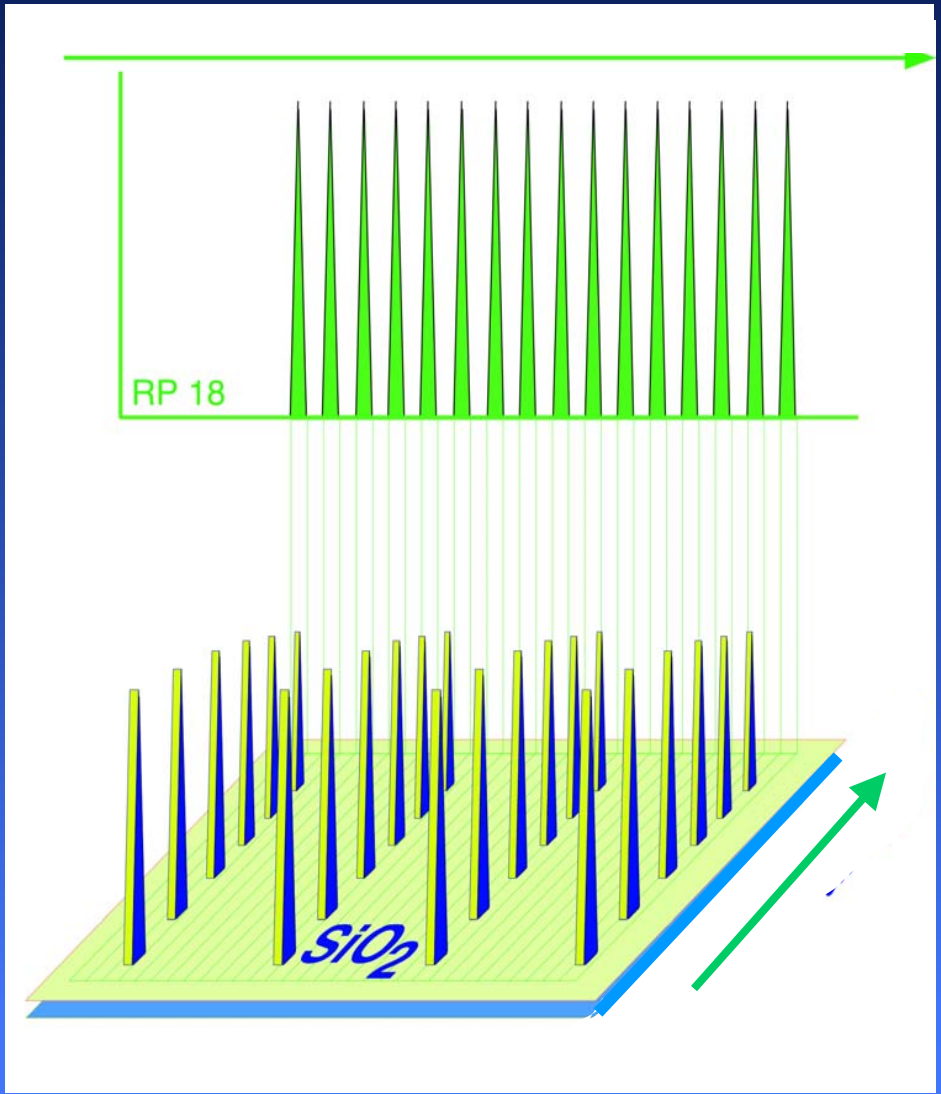
HPLC-AMD coupling

1. HPLC

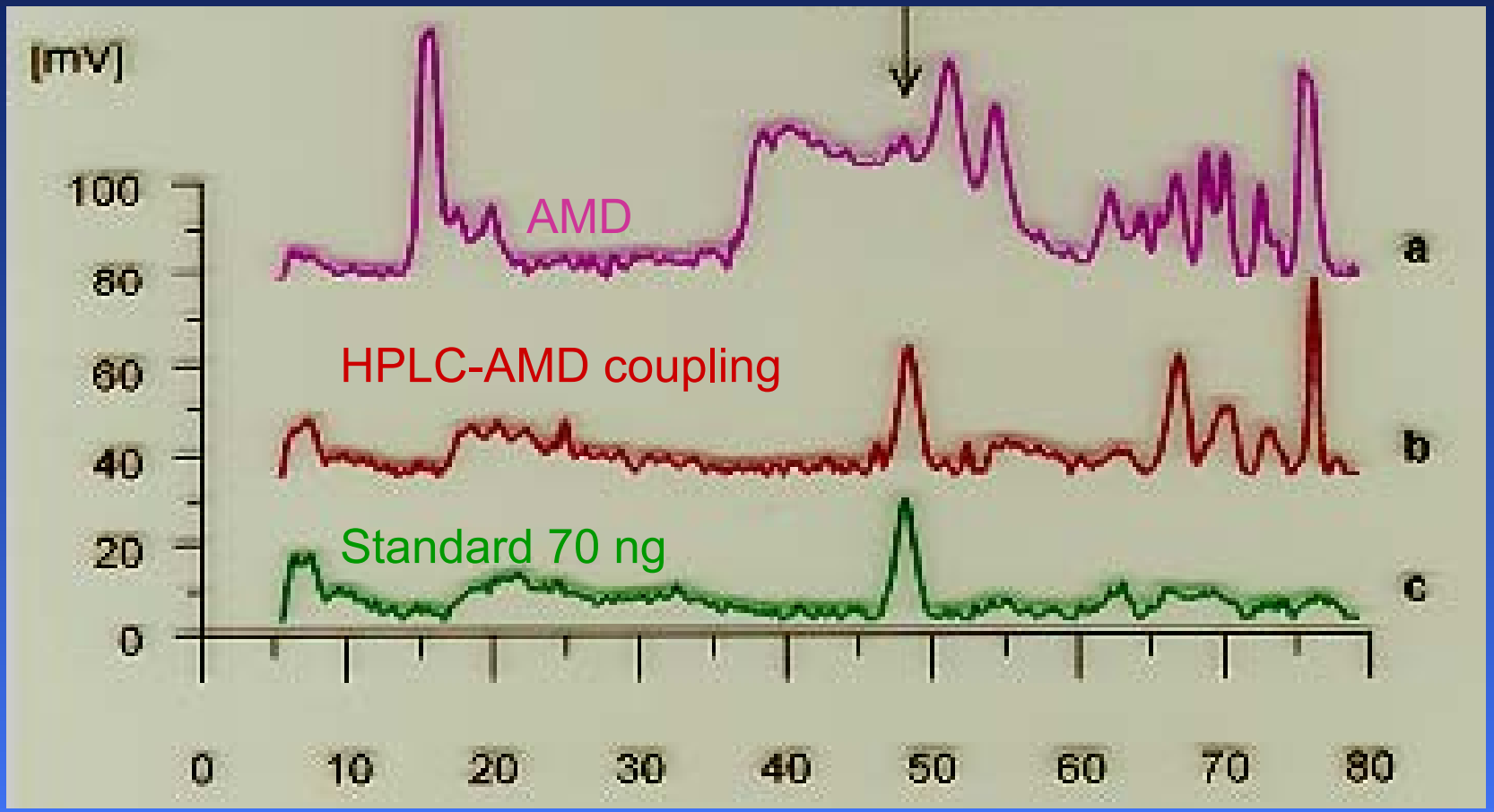
Reversed phase

2. HPTLC

Normal phase

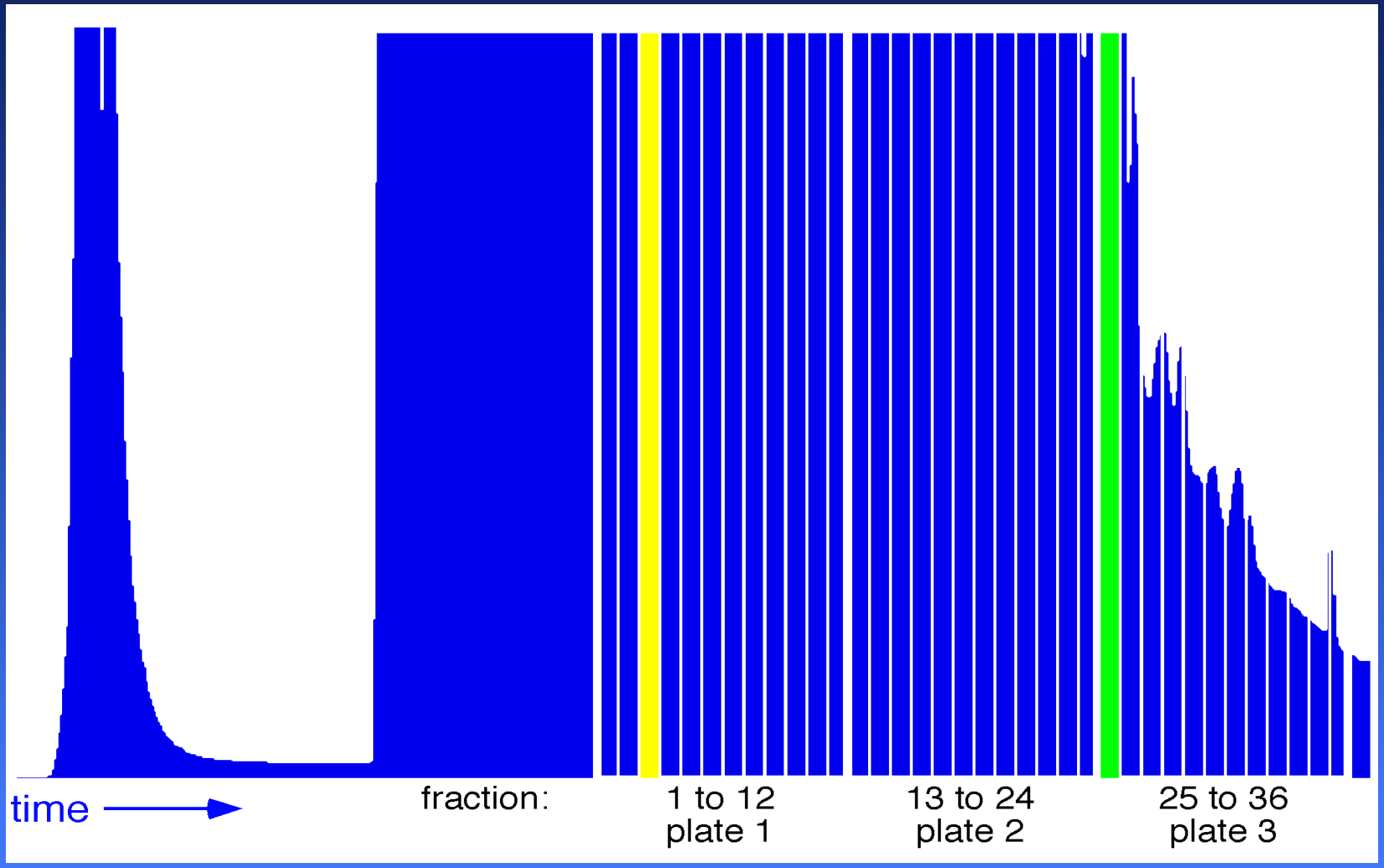


Iprodione in lettuce



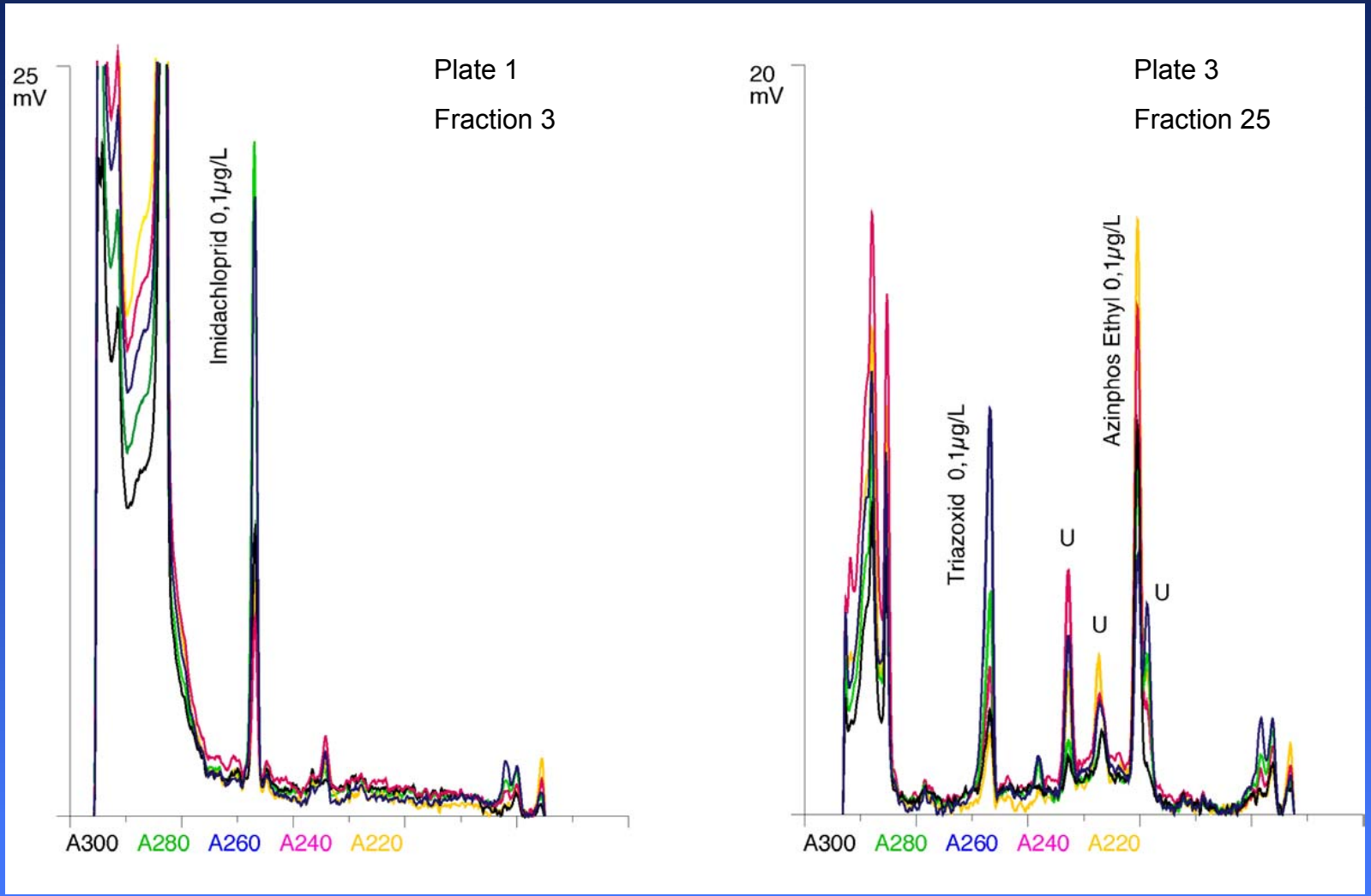
U. Wippo, H.-J. Stan,
Deutsche Lebensmittel-Rundschau 5, 144-148 (1997)

Surface water spiked with 50 pesticides



klaus.burger@mail.isis.de

Surface water spiked with 50 pesticides

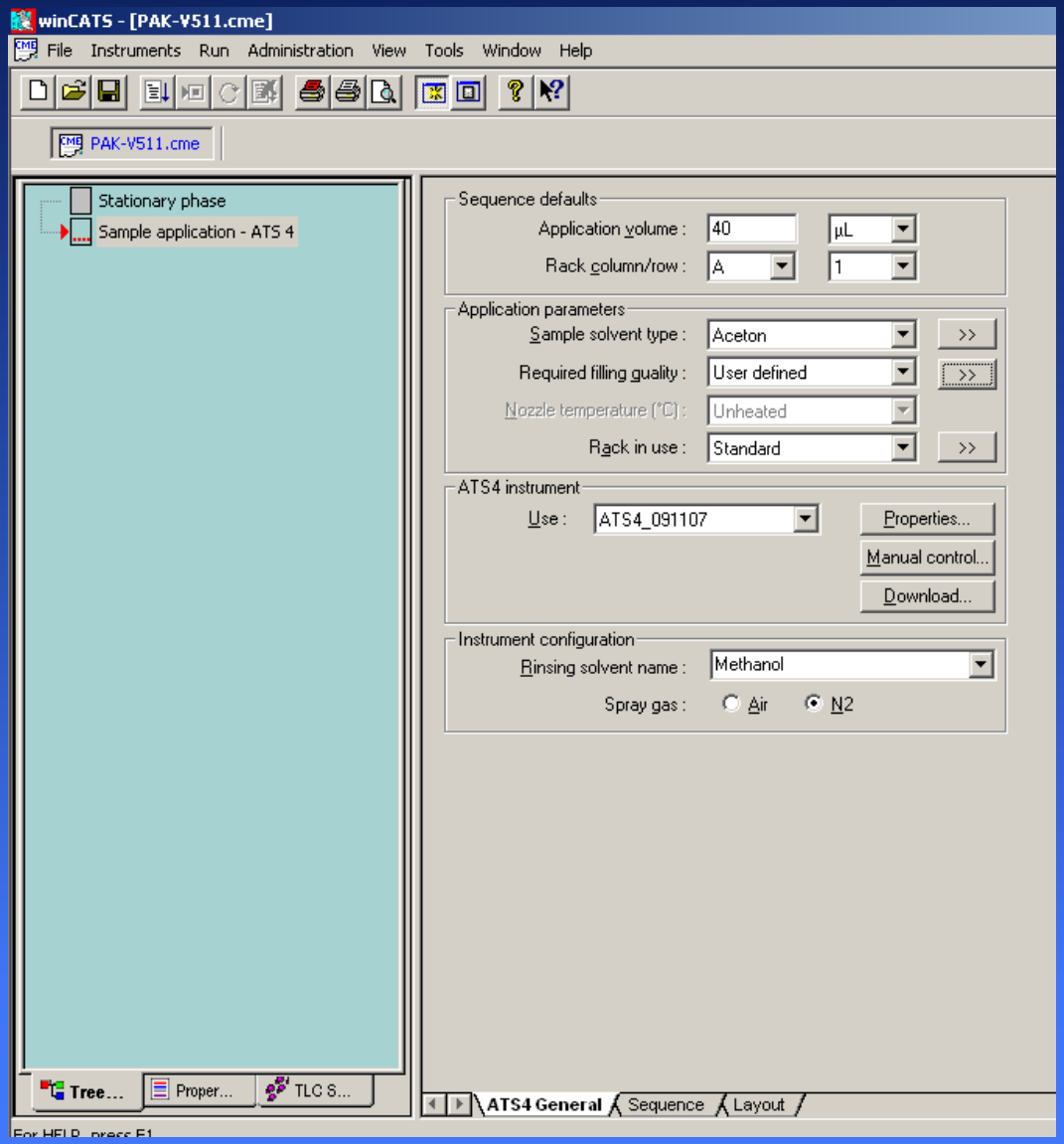


klaus.burger@mail.isis.de

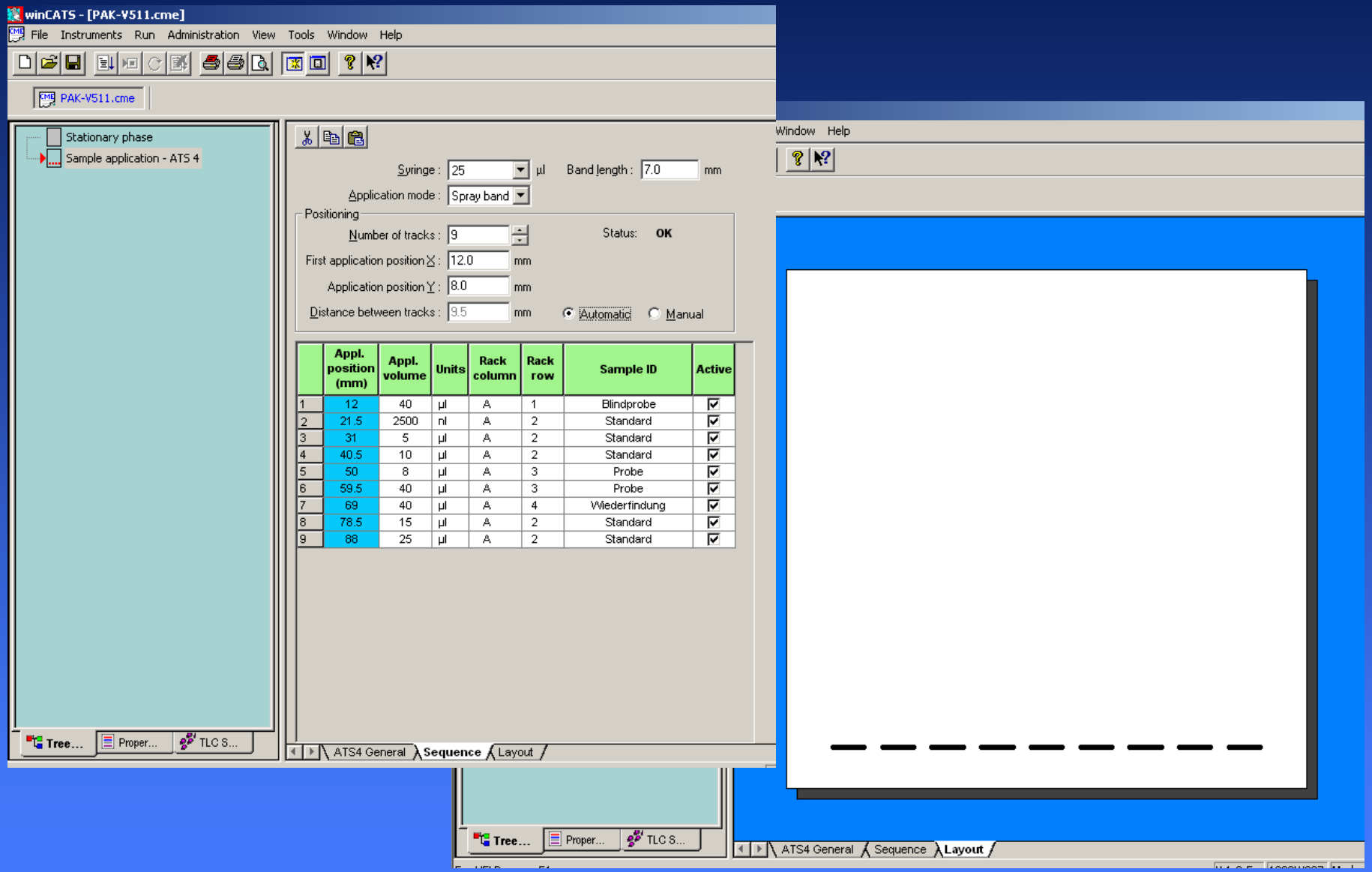
Benefits of HPLC-AMD

- ✓ Multi-method
- ✓ Enhanced separation power
- ✓ Peak purity tests
- ✓ Post-chromatographic derivatization
- ✓ Results by two independend methods
- ✓ Use as single devices
- ✓ Gain in flexibility and analytical quality

General application parameters

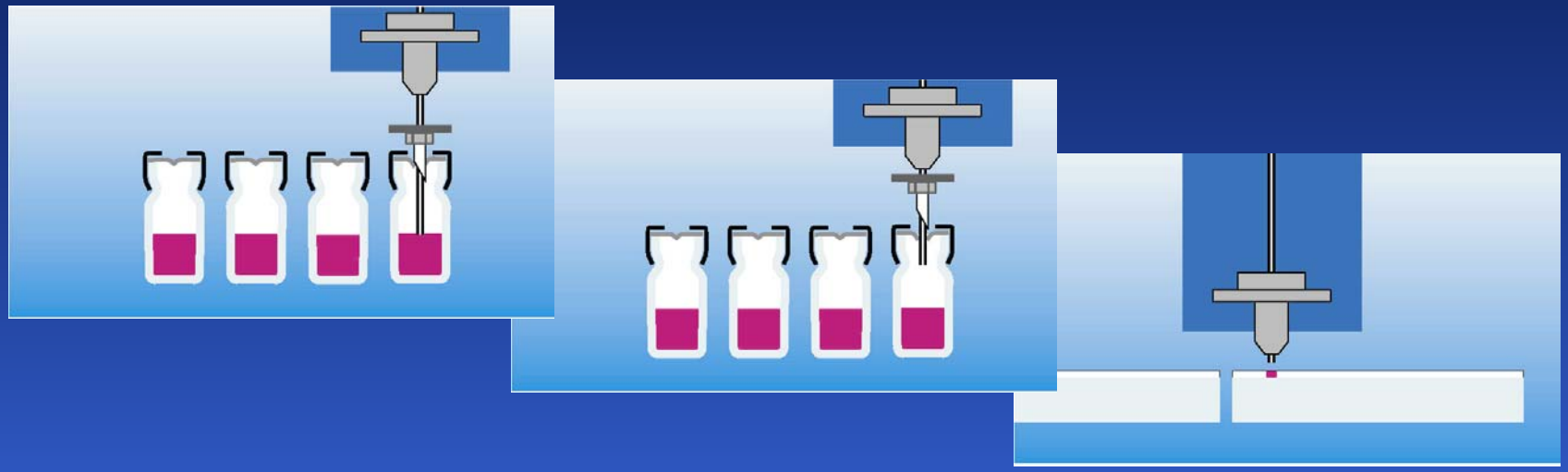


Sequence and layout

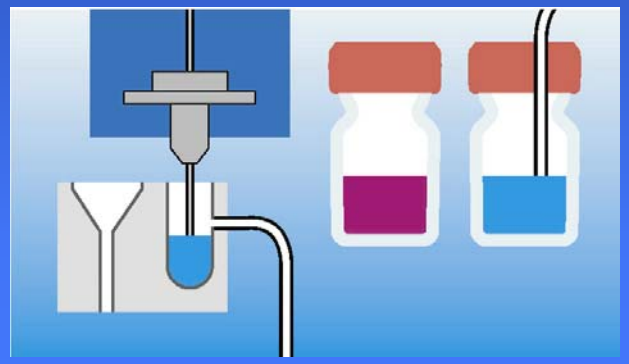


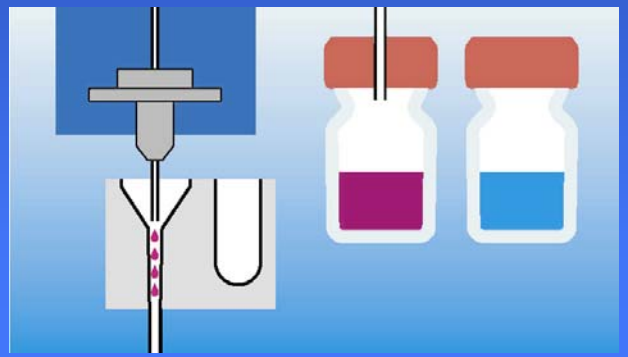
High performance mode of application

Sample application



Rinsing





Sample application

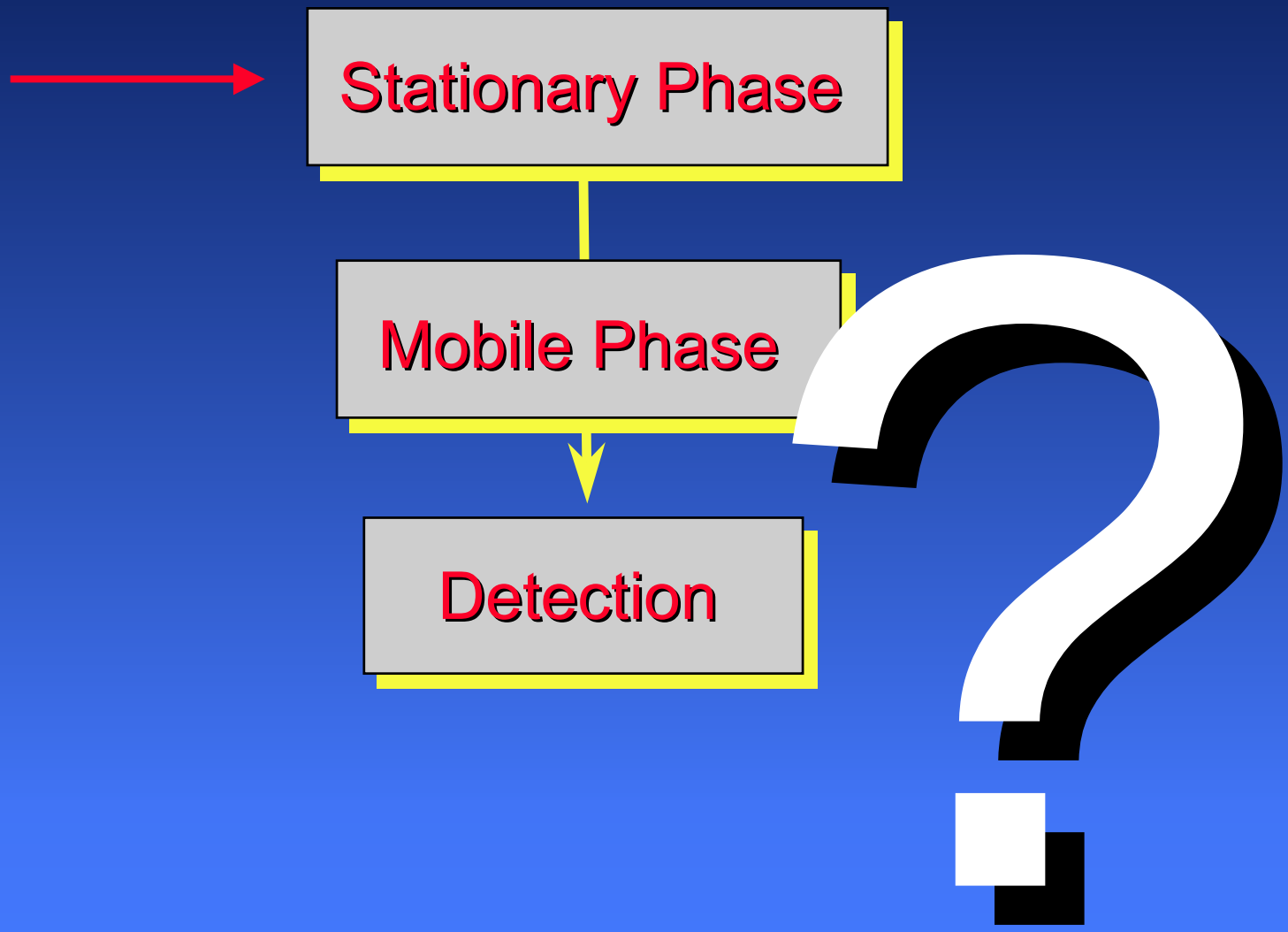
ATS 4 Instrument Validation

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
- ✓ Cleaning 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 %)

Which TLC system?



Overview of stationary phase

- Separation mechanisms
- Guidelines for the selection
- Impregnation
- Prewashing
- Activation
- TLC versus HPTLC versus UTLC
- Layer support and binder
- Fluorescence (= phosphorescence) indicator
- Manufacturer/batch dependence
- Declaration
- Detection reagents

Separation mechanisms

- ✓ Adsorption chromatography
- ✓ Partition chromatography
- ✓ Complex chromatography
- ✓ Ion exchange chromatography

Separation mechanisms

Adsorption

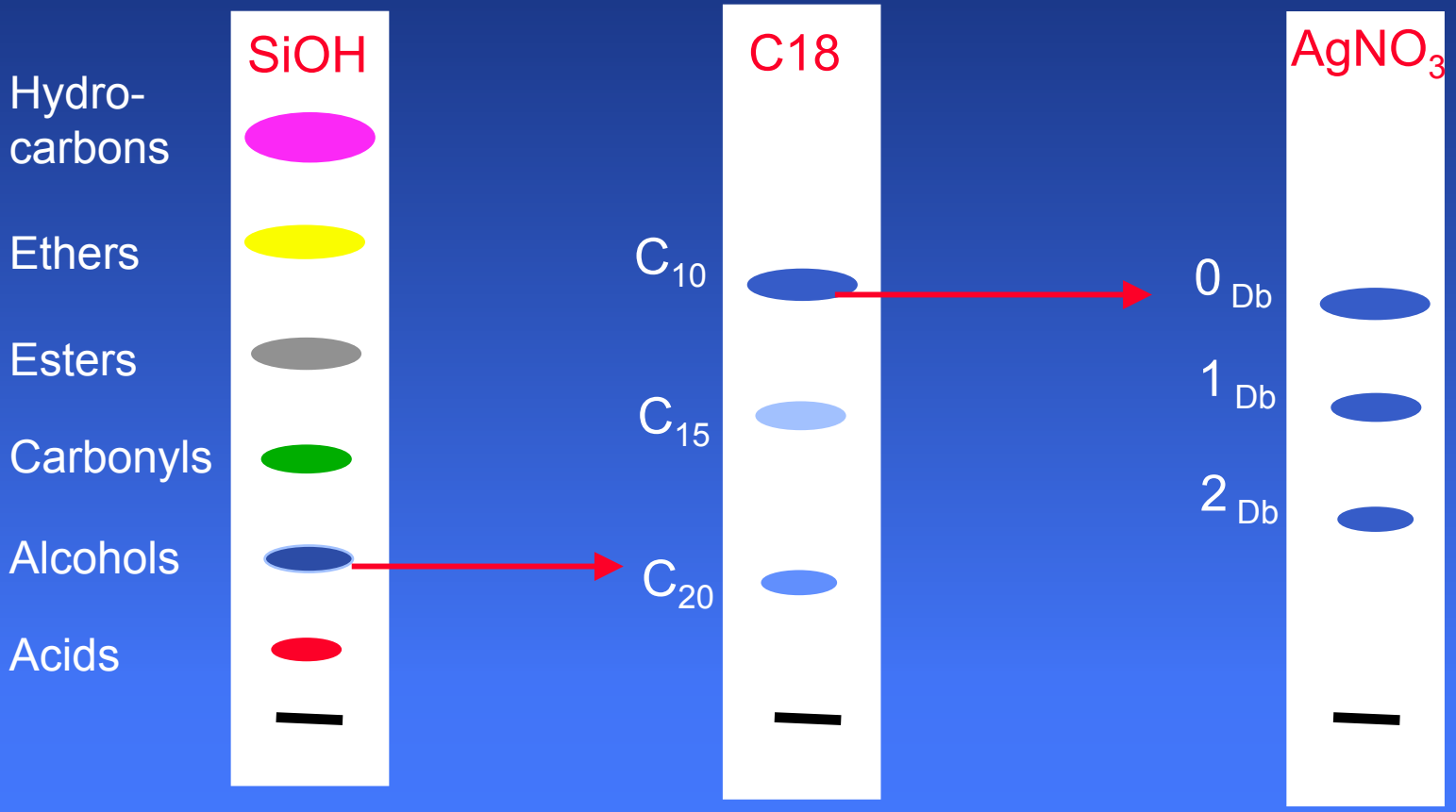
Functional groups
polarity

Partition

Chain length
lipophilicity

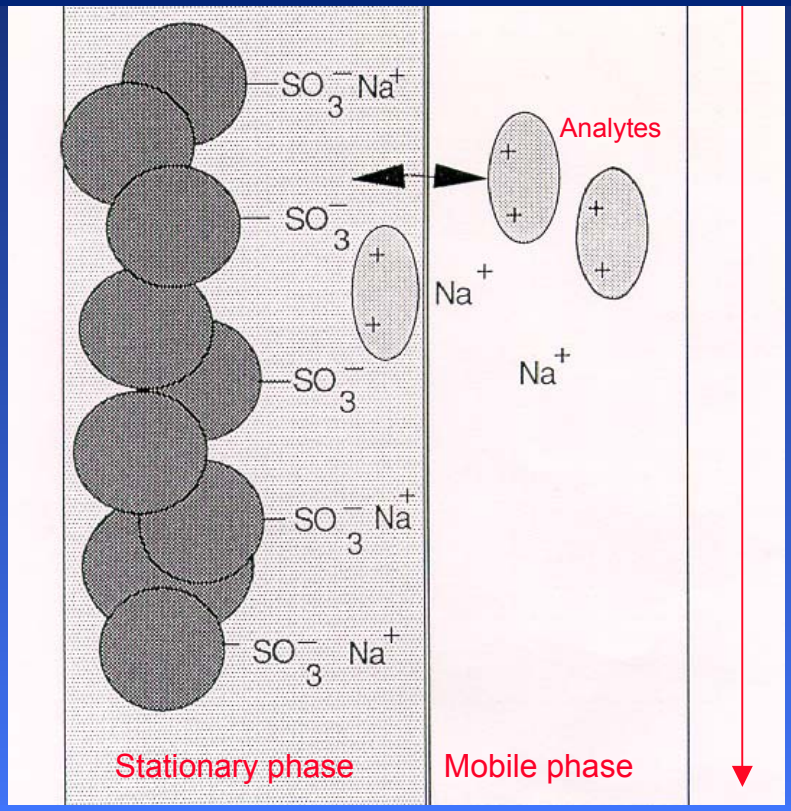
Complex

Complex stability
e.g. double bondings



Separation mechanisms

Ion exchange



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

Terminology and polarity

- Normal phase

  polar SP + non polar MP

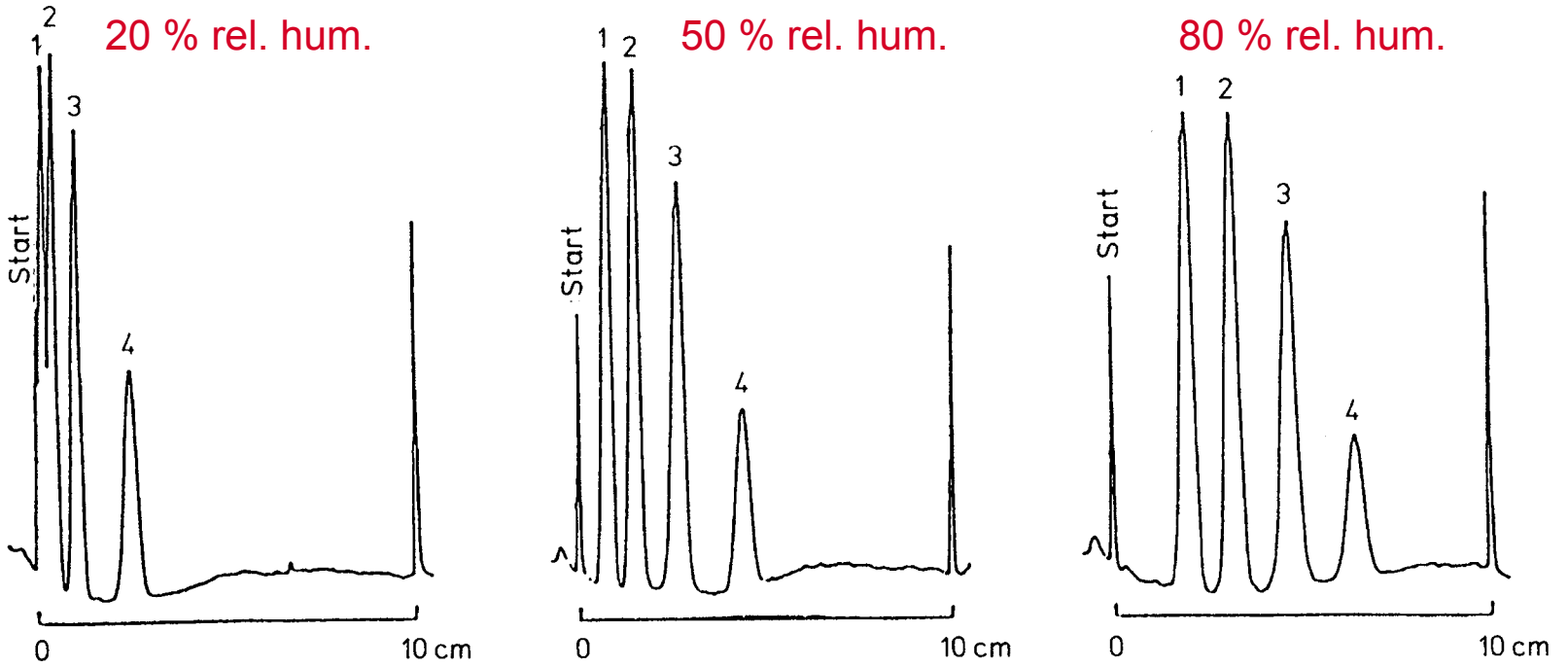
- Reverse phase

  non polar SP + polar MP

- Polarity of the layer

  Si > NH_2 > CN/Diol > RP-2 > RP-8 > RP-18

Impregnation of the layer



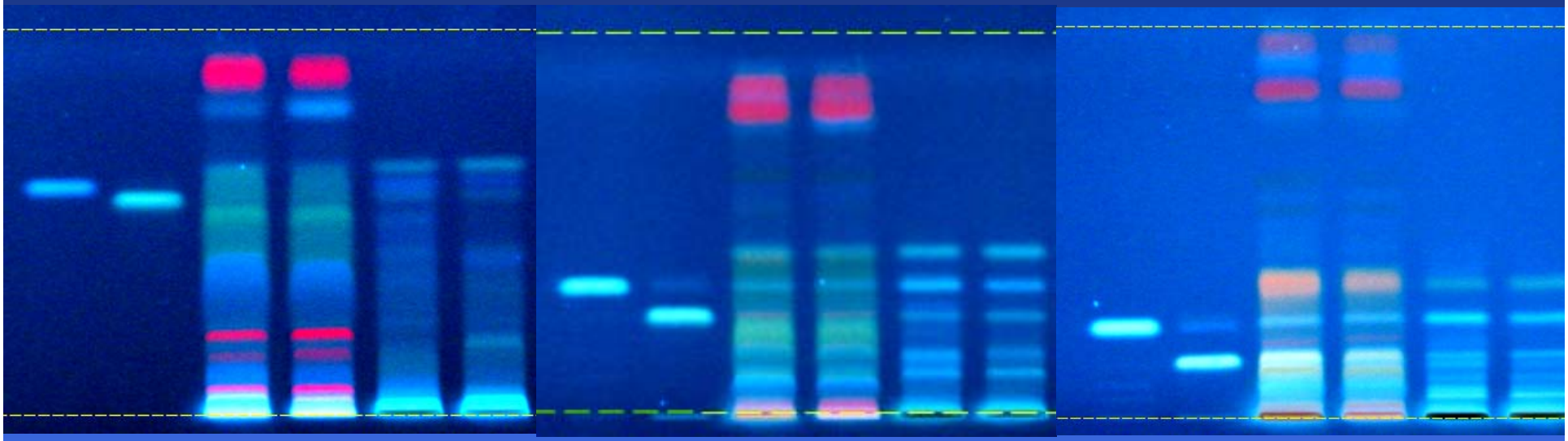
Rabel, F. in Sherma, J., Fried, B.: Handbook of Thin-Layer Chromatography, Marcel Dekker, New York, 2003, ISBN 0-8247-0895-4.

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

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 analysis (ppt range)
- if working range is near the LOD or LOQ

Prewashing of old layers

a

Pre-chromatographed with methanol, see CBS 91

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. (about 10 years old)

- immersion in iso-propanol over night or for at least 2 hours, followed by heating at 120°C for 30 min

According to CAMAG (current recommendation)

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

According to Dr. Burger (current recommendation)

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!
- Be care of storage and declaration of prewashed plates!

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 or use mid- or unpolar stationary phases

Activation 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

Activation of the layer

45% relative humidity

32% relative humidity

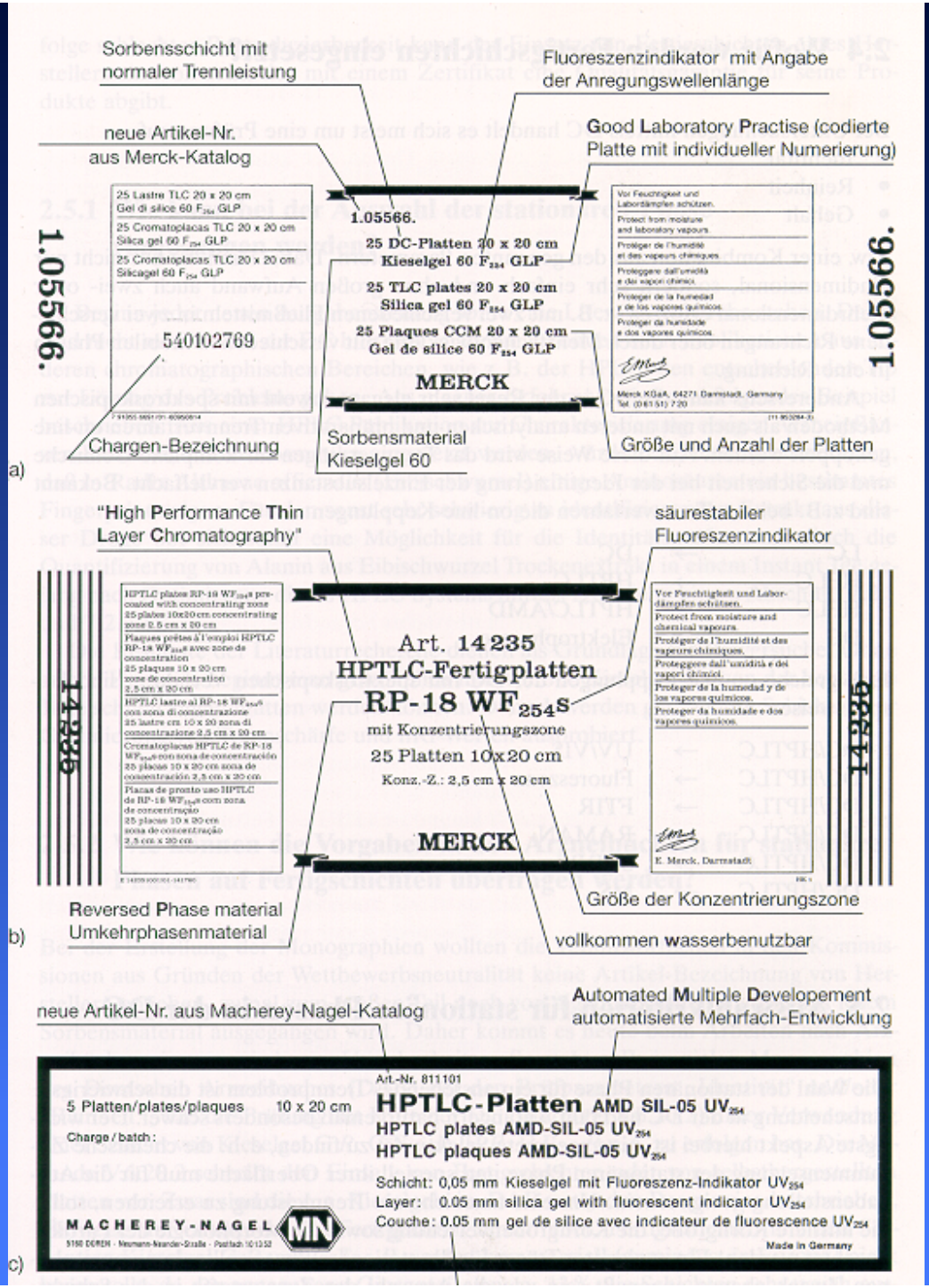
Fingerprint (alkylamides) of Echinacea purpurea with
toluene - ethyl acetate - cyclohexane - formic acid 24:6:3:0.9
derivatization with anisaldehyde, see CBS 91

TLC versus HPTLC versus UTLC

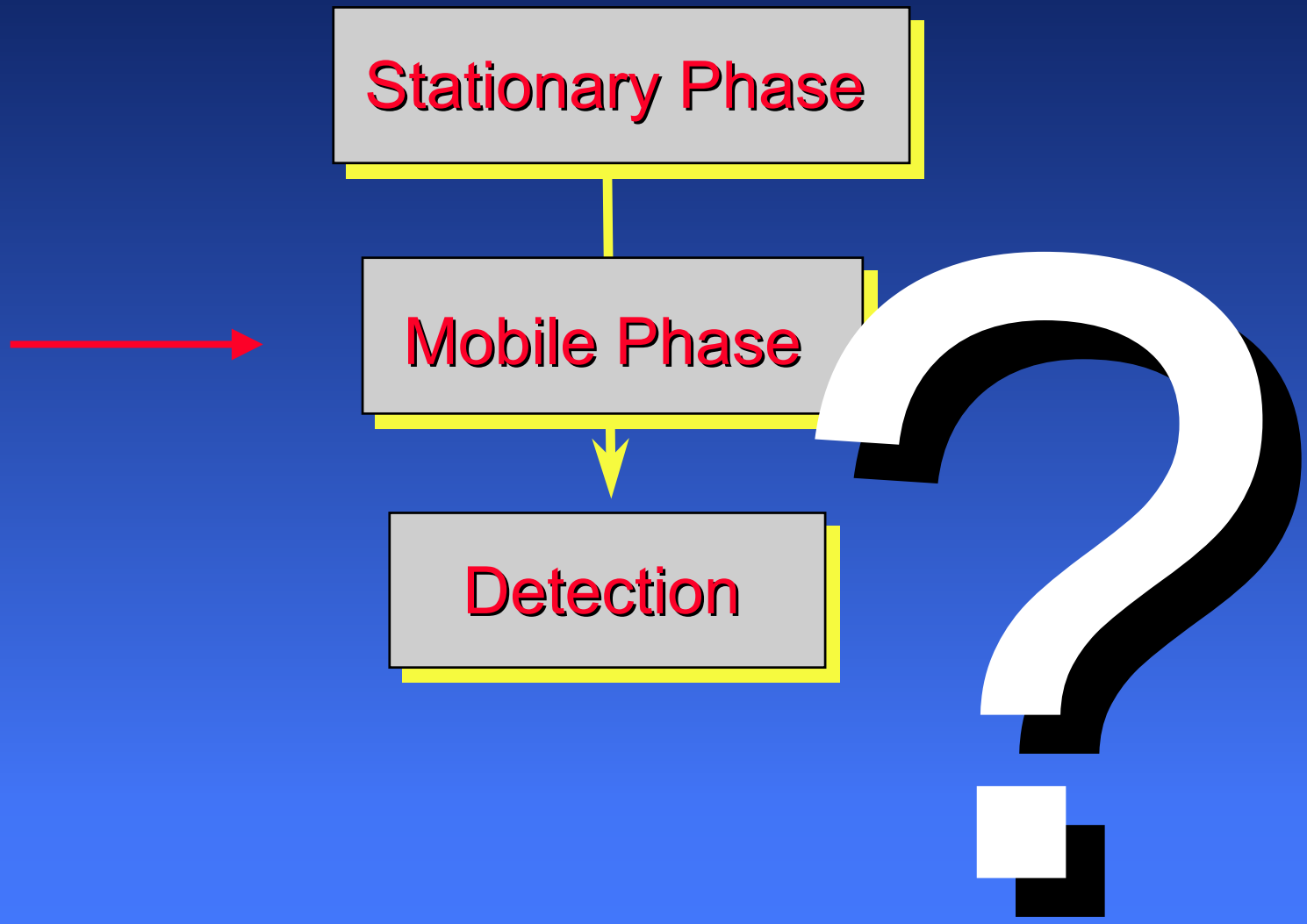
		TLC	HPTLC	UTLC
Silica gel		irregular particles	irr./glob. particles	monolithic without binders
Mesopores		60 Å = 6 nm	60 Å = 6 nm	30-40 Å = 3-4 nm
Mean particle size		10 - 15 µm	5 - 7 µm	1 - 2 µm macrospores
Particle distribution		wide	narrow	narrow
Layer thickness		200, 250 µm	100, 200 µm	ca. 10 µm
Number of samples		max. 12 20 x 10 cm	36 – 72 20 x 10 cm	12 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

Note abbreviations!

60	mean pore size in Angström (= 6 nm)
F	with fluorescent indicator
254	excitation wavelength of F
s	acid stabile fluorescent indicator (blue)
R	specially purified
RP 2, 8, 18	reversed phase with 2, 8, 18 hydrocarbon chain length
W	water-tolerant layer
PSC	preparative layer, thickness > 0,25 mm
(G	gypsum as binder)
(H	without foreign binders)


Sorbensschicht mit normaler Trennleistung
Fluoreszenzindikator mit Angabe der Anregungswellenlänge
neue Artikel-Nr. aus Merck-Katalog
Good Laboratory Practise (codierte Platte mit individueller Numerierung)
1.05566.
25 DC-Platten 20 x 20 cm
Kieselgel 60 F254 GLP
25 TLC plates 20 x 20 cm
Silica gel 60 F254 GLP
25 Plaques CCM 20 x 20 cm
Gel de silice 60 F254 GLP
MERCK
540102769
Chargen-Bezeichnung
Sorbensmaterial Kieselgel 60
Größe und Anzahl der Platten
a)
"High Performance Thin Layer Chromatography"
säurestabiler Fluoreszenzindikator
14236
Art. 14235
HPTLC-Fertigplatten
RP-18 WF254S
mit Konzentrierungszone
25 Platten 10x20 cm
Konz.-Z.: 2,5 cm x 20 cm
MERCK
Reversed Phase material
Umkehrphasenmaterial
Größe der Konzentrierungszone
vollkommen wasserbenutzbar
b)
neue Artikel-Nr. aus Macherey-Nagel-Katalog
Automated Multiple Developement
automatisierte Mehrfach-Entwicklung
Art.-Nr. 811101
5 Platten/plates/plaques 10 x 20 cm
HPTLC-Platten AMD SIL-05 UV254
HPTLC plates AMD-SIL-05 UV254
HPTLC plaques AMD-SIL-05 UV254
Charge / batch :
Schicht: 0,05 mm Kieselgel mit Fluoreszenz-Indikator UV254
Layer: 0.05 mm silica gel with fluorescent indicator UV254
Couche: 0.05 mm gel de silice avec indicateur de fluorescence UV254
MACHEREY-NAGEL
Made in Germany
c)

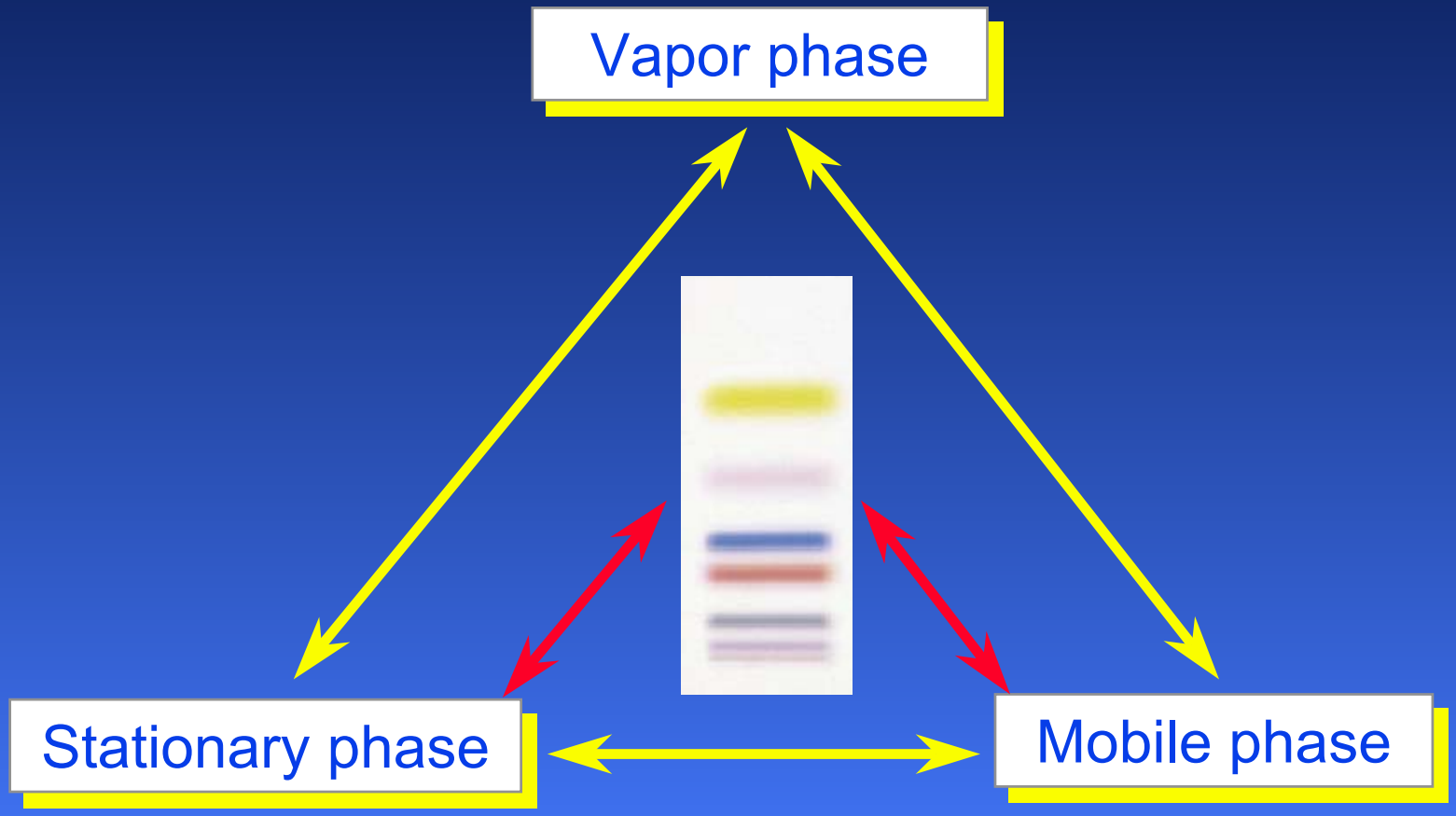
Which TLC system?



Overview of mobile phase

- ✓ Chromatographic separation
- ✓ Classification of solvents (Trappe , Snyder)
- ✓ Optimization scheme
- ✓ Isotherms, peak asymmetry
- ✓ Polarity differences in mobile phase mixtures
- ✓ Vapor pressure of solvents
- ✓ Variations in temperature
- ✓ Stabilizers (manufacturer, batch)
- ✓ Diffusion (van Deemter)
- ✓ Viscosity (law of migration)
- ✓ Developing distance
- ✓ GLP recommendations

Chromatographic separation



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~~

Chromatographic separation

- The mobile phase moves by capillary forces through the particle pores (6 – 10 nm).
- The substances are dissolved in the mobile phase and are transported over a certain migration distance.
- Different adsorption and/or partition equilibria cause different remaining times in the stationary phase.

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

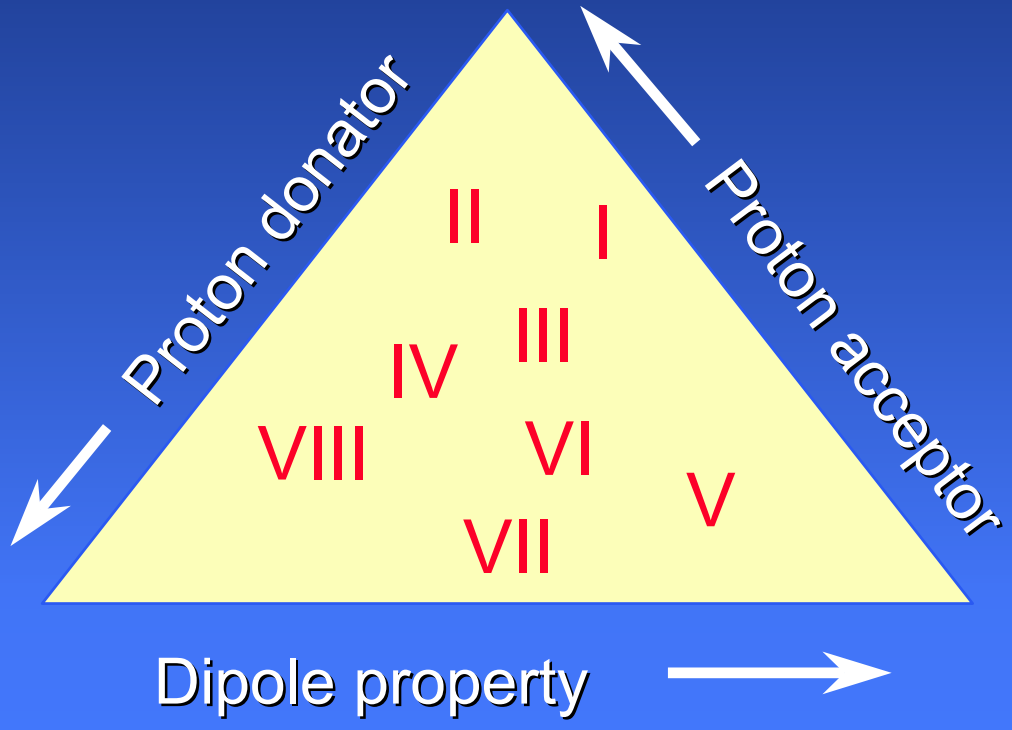
Eluotropic series of different sorbents

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)



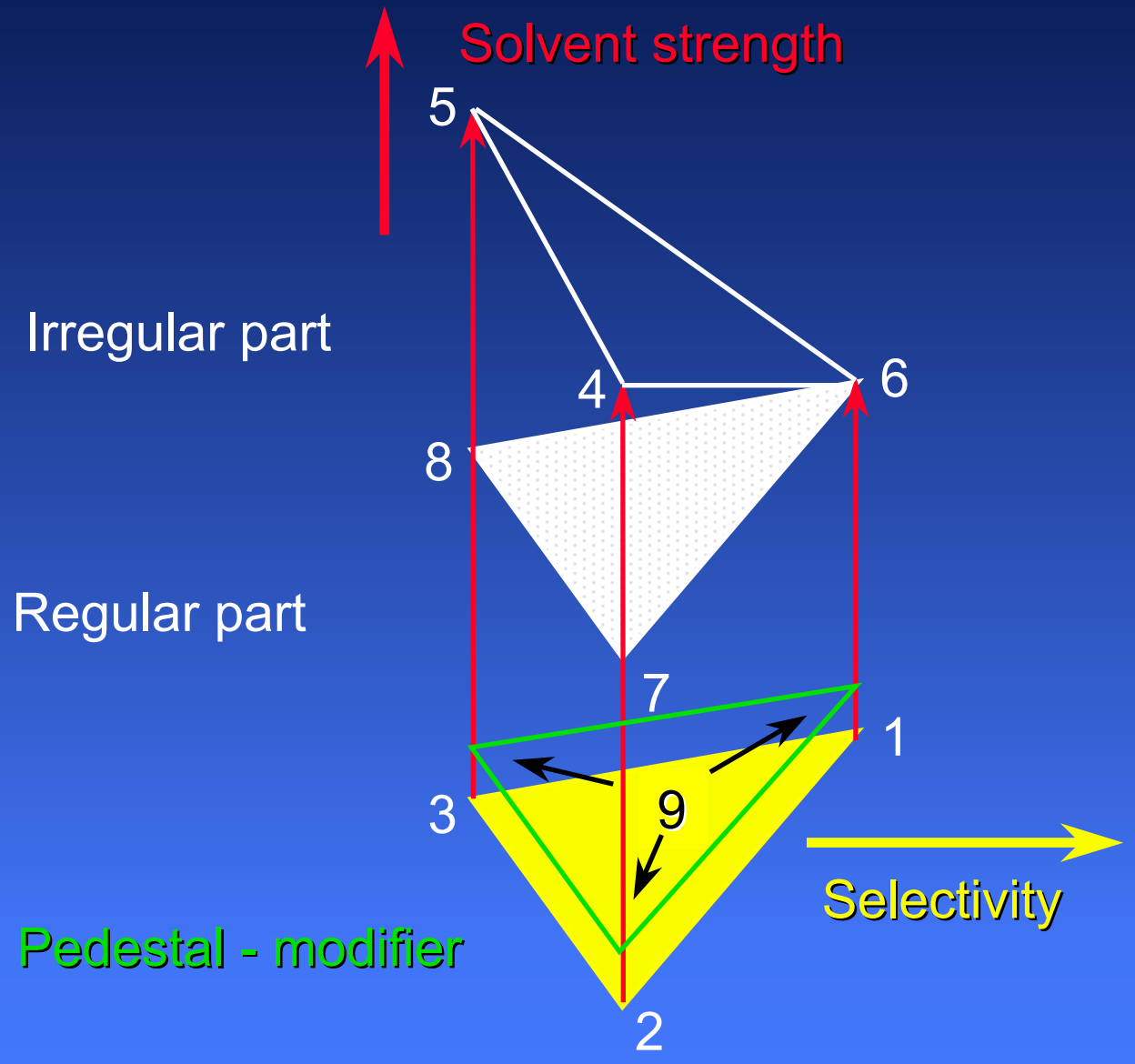
Normal phases

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

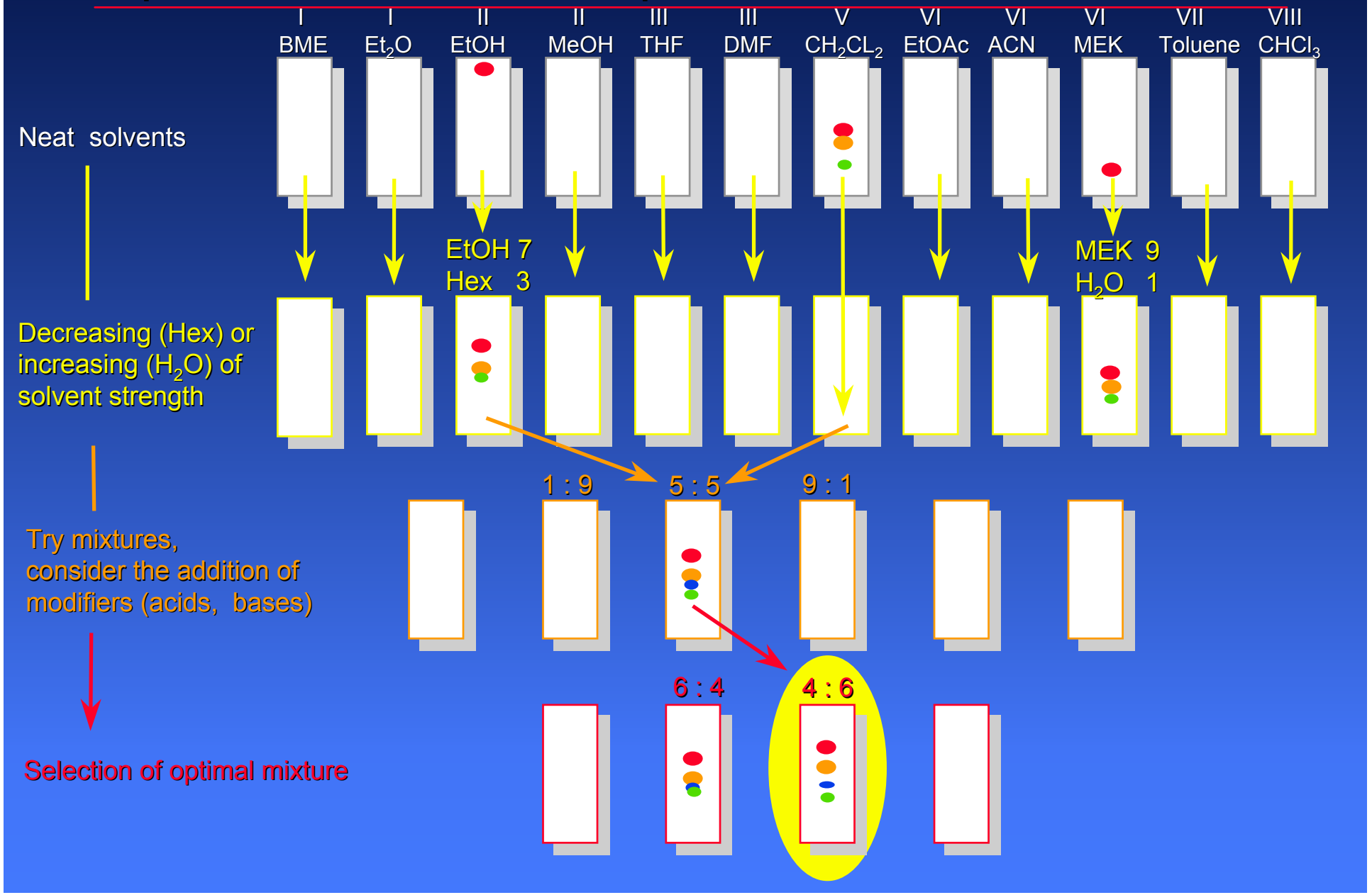
Reverse phases

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

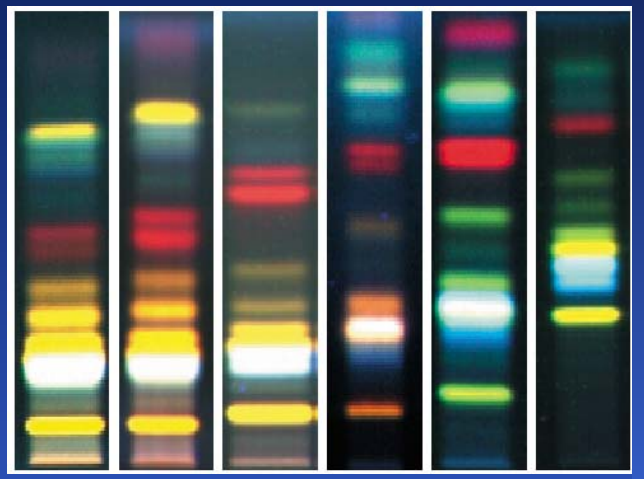


Optimization of mobile phase

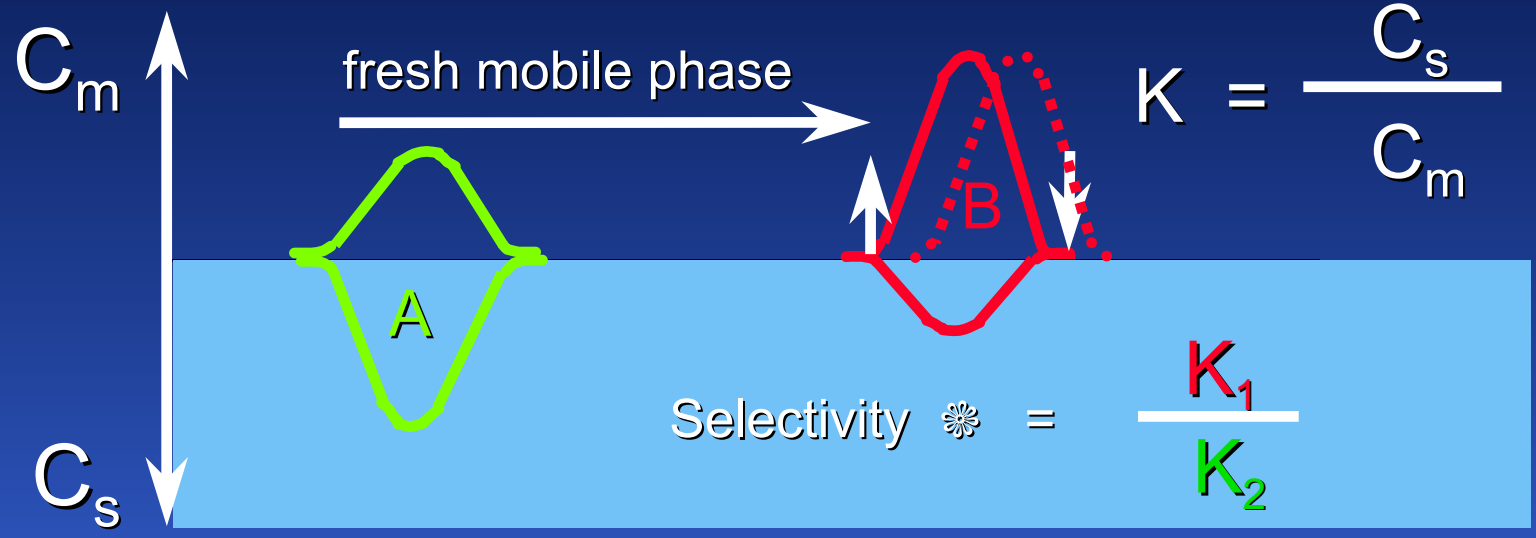


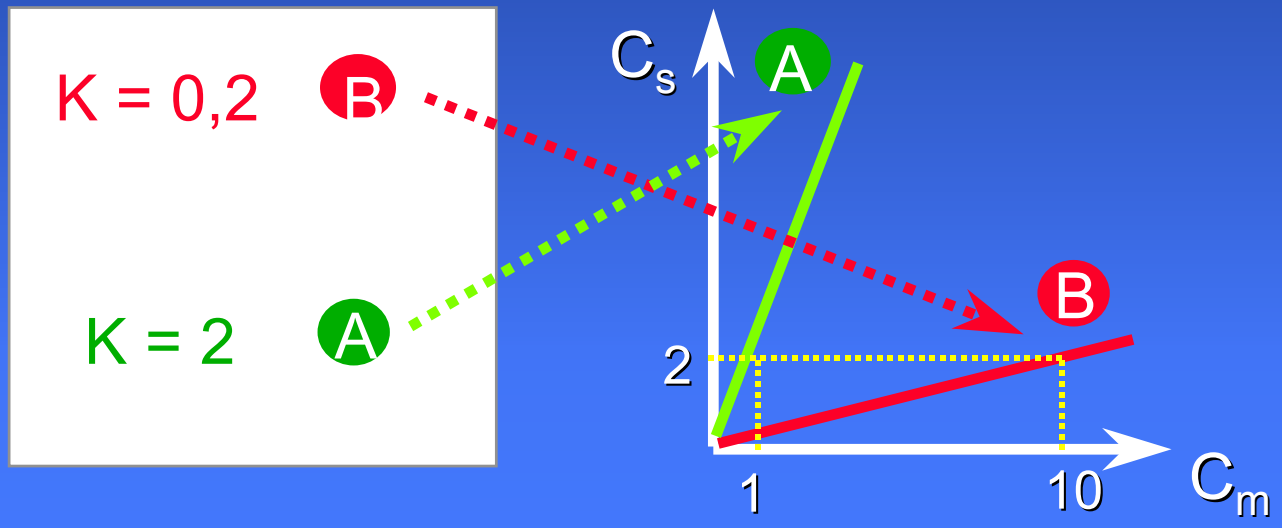
Neat solvents

HPTLC Vario Chamber



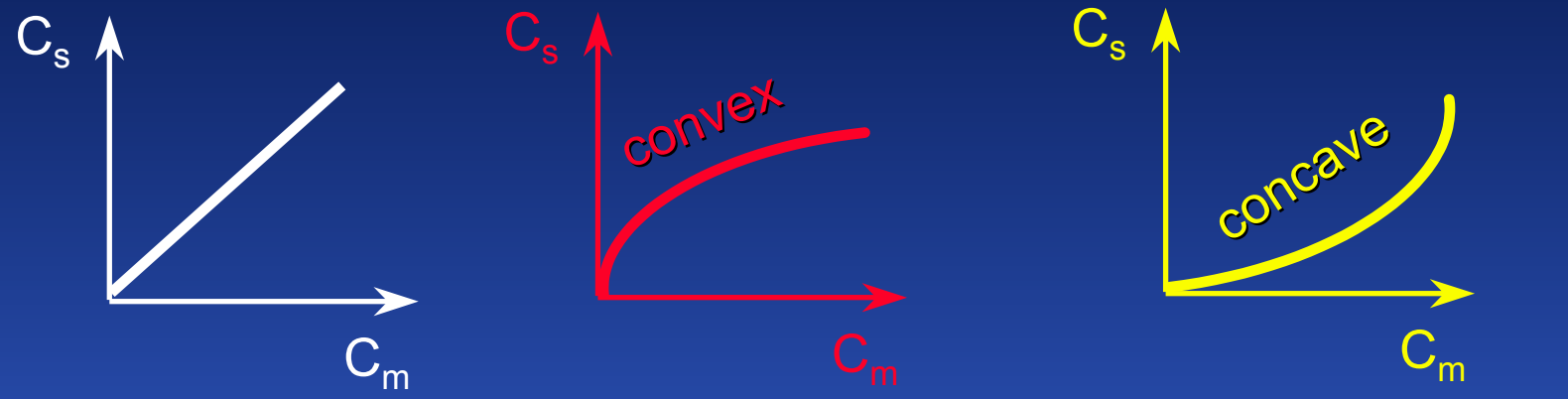
Partition and adsorption isotherms



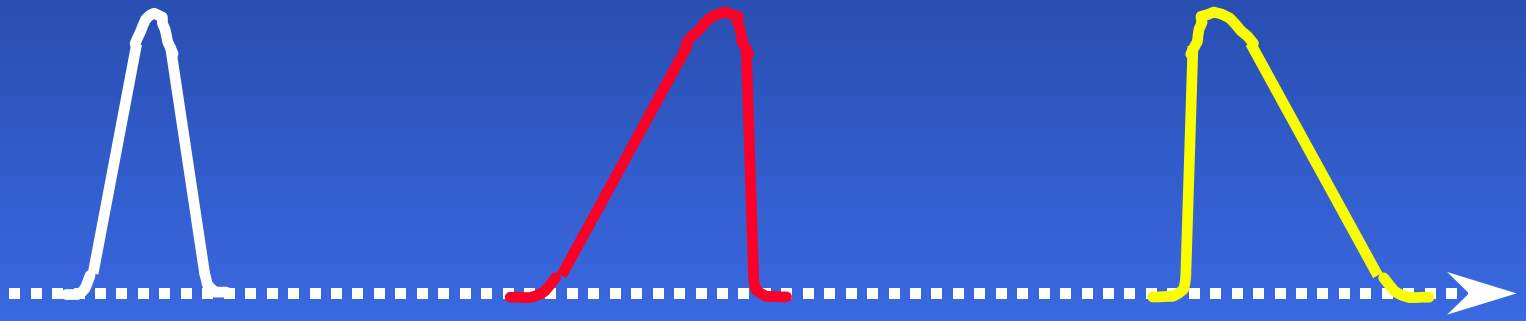


Peak asymmetry

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

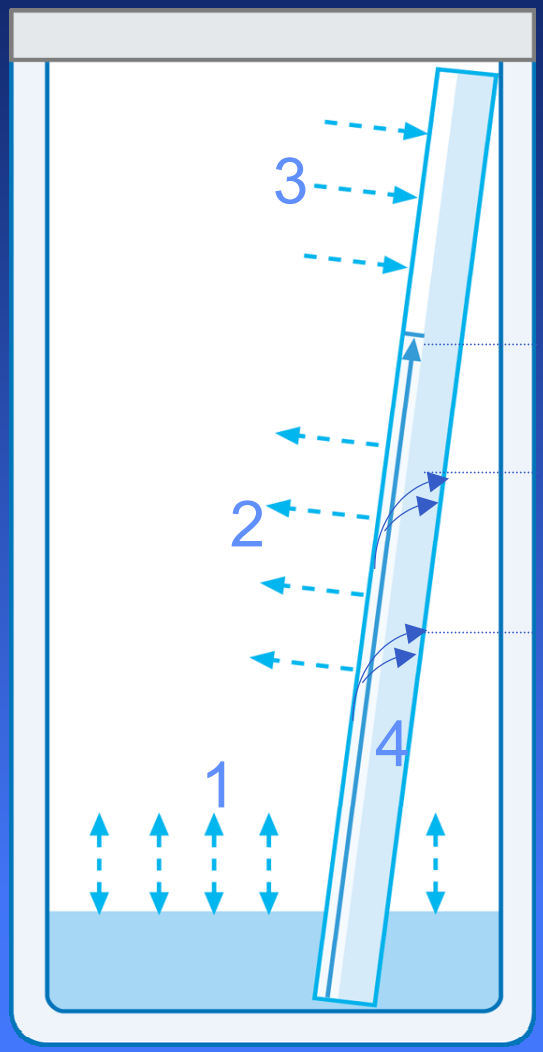
Concave partition/adsorption isotherm

- change system

Mobile phase mixtures

TLC - isocratic? No!

- Solvent composition changes
- Solvent migration rate changes



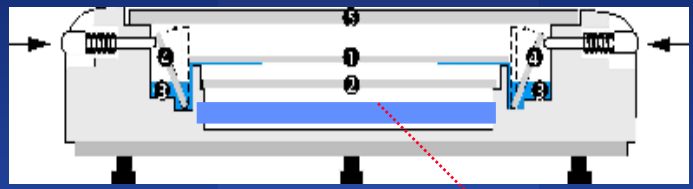
a-Front

ß-Front

y-Front

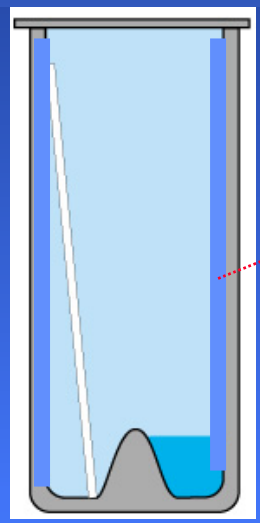
Chamber saturation

HDC



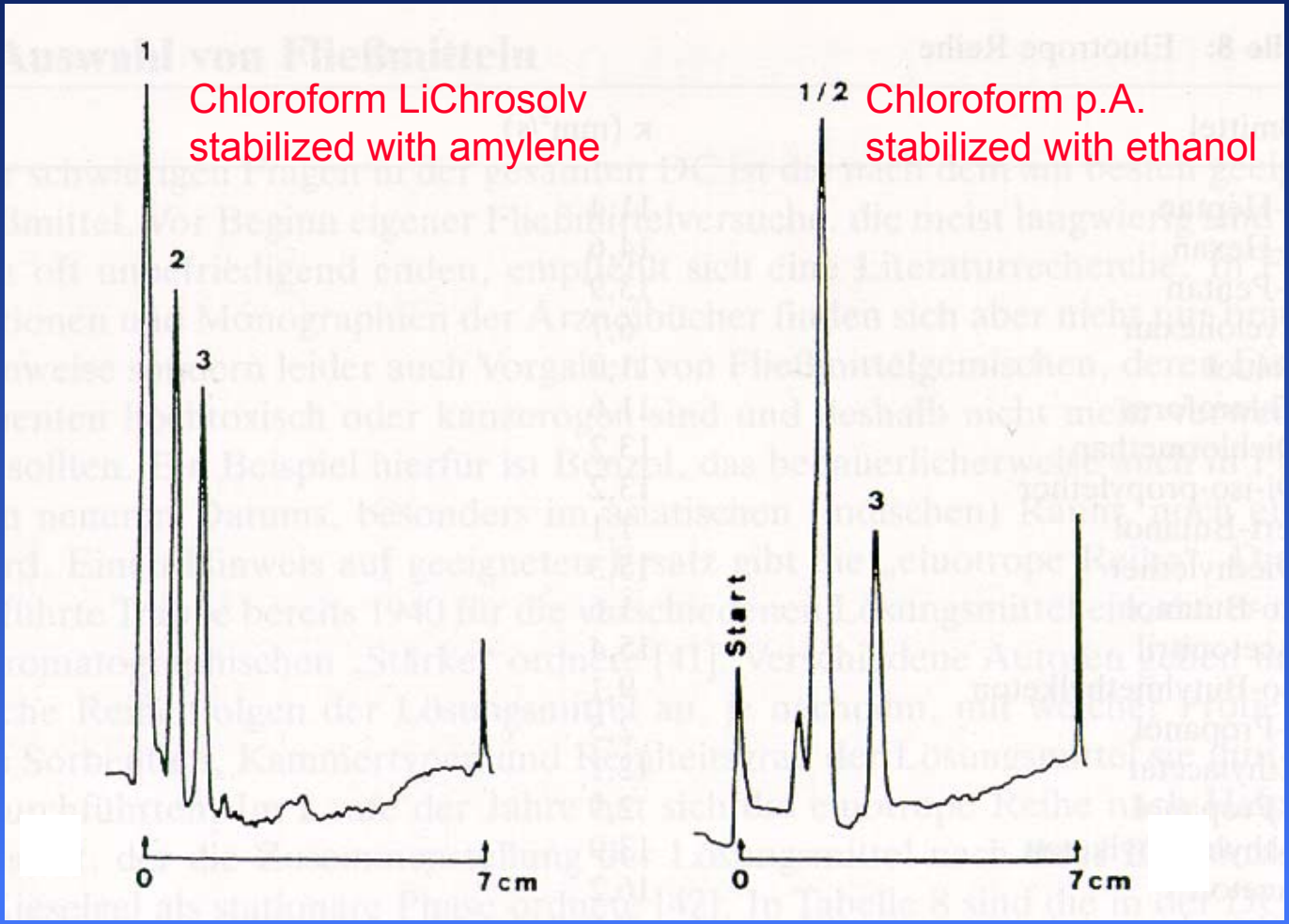
solvent at the bottom

TTC



with wetted filter paper

Stabilizers (manufacturer, purity grade)



Zone diffusion according to van Deemter

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

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

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

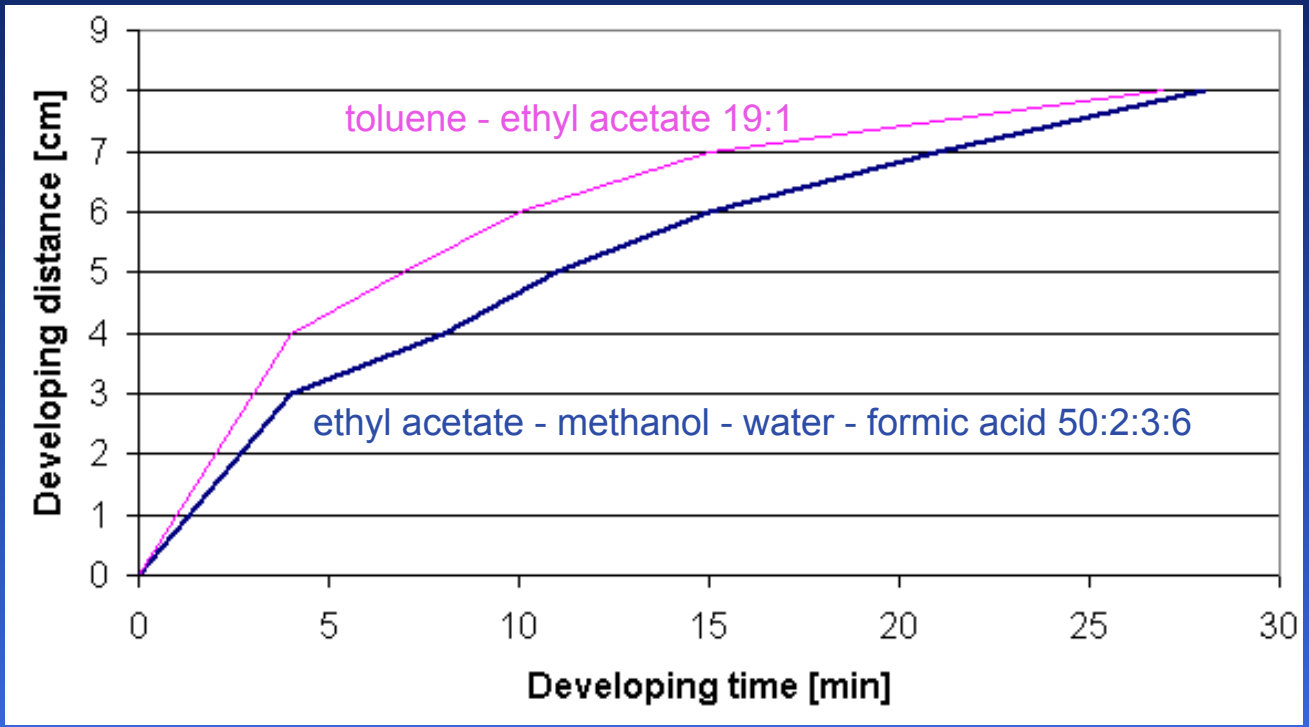
$\bar{H}$	Plate heigth
v	Velocity of solvent front
l	Function of layer packing
dp	Particle diameter
γ	Labyrinth factor
D	Diffusion coefficient
w	Factor of packing structure

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 - velocity

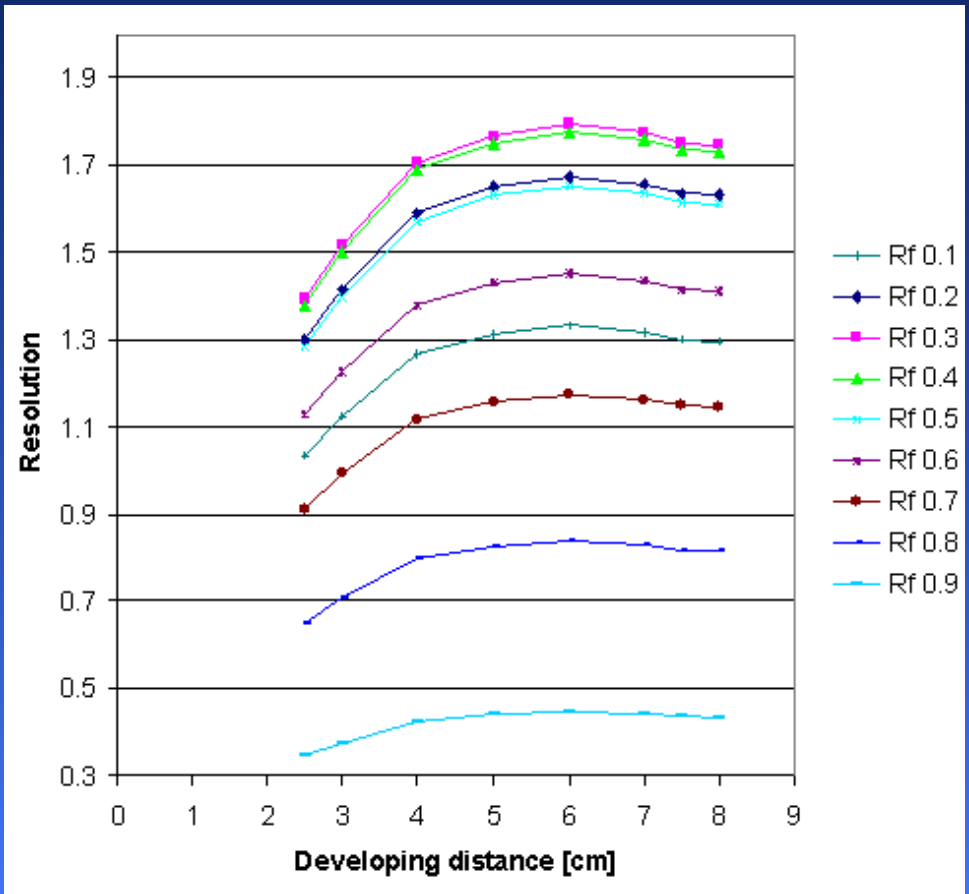


Note: Do not exceed a developing distance of 6 cm on HPTLC plates.

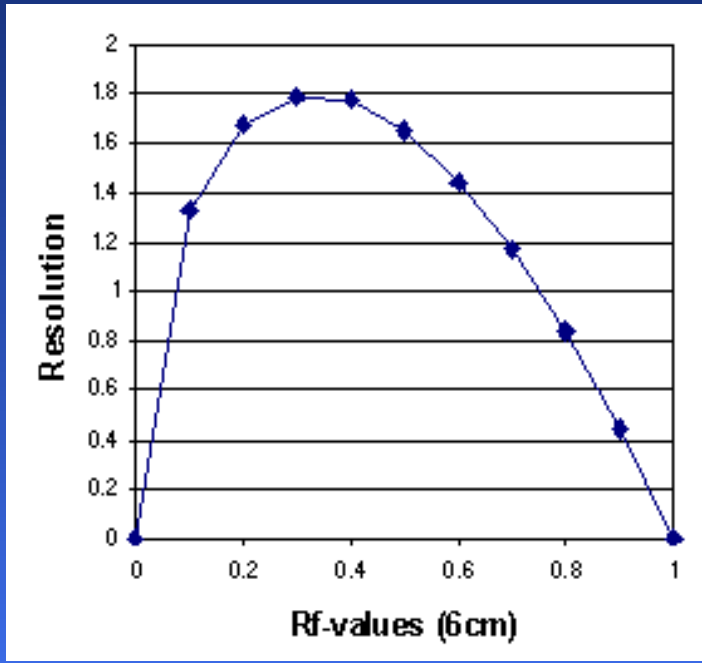
The higher the developing distance, the lower the velocity of mobile phase, the more influence of diffusion effects!

Developing distance - resolution

Influence of the developing distance and R_F values



R_F values in detail at 6 cm



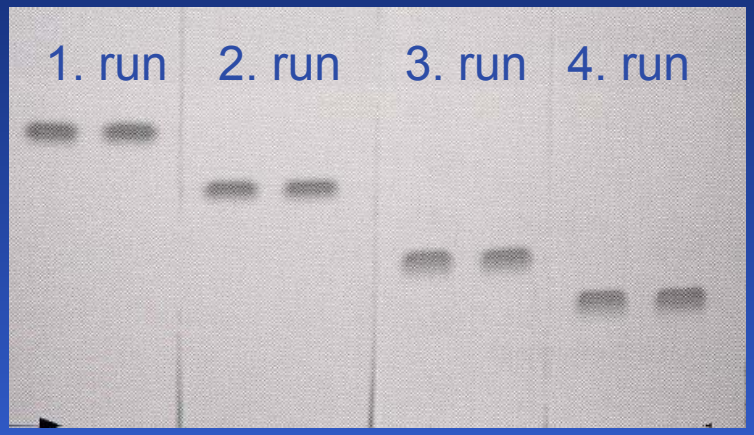
Note: Components of complex mixtures should be spread over the entire separation distance. The most critical substance pair should be maintained at R_F 0.3 for best separation.

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!
- ✓ Prewash 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 30 min
- ✓ Use data pair method to reduce plate inhomogeneity

Use of solvents

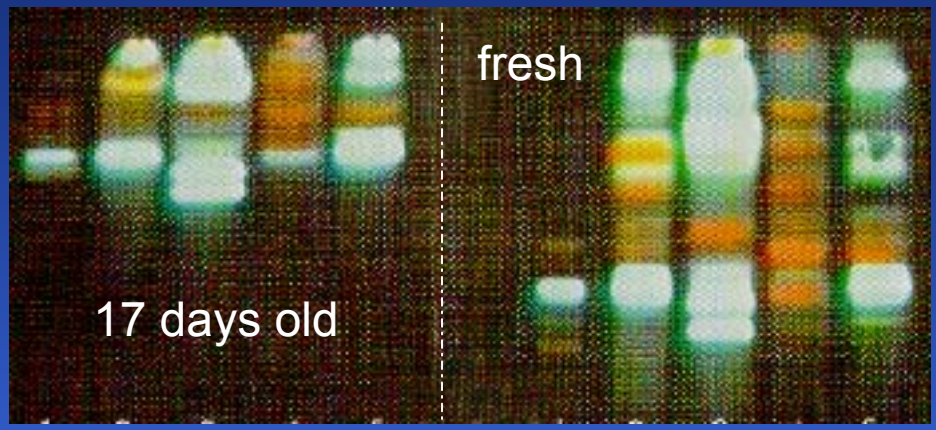
Multiple use of solvents



Chloroform – methanol – ammonia 56:14:1

Do´nt re-use solvents!

Preparation of solvents

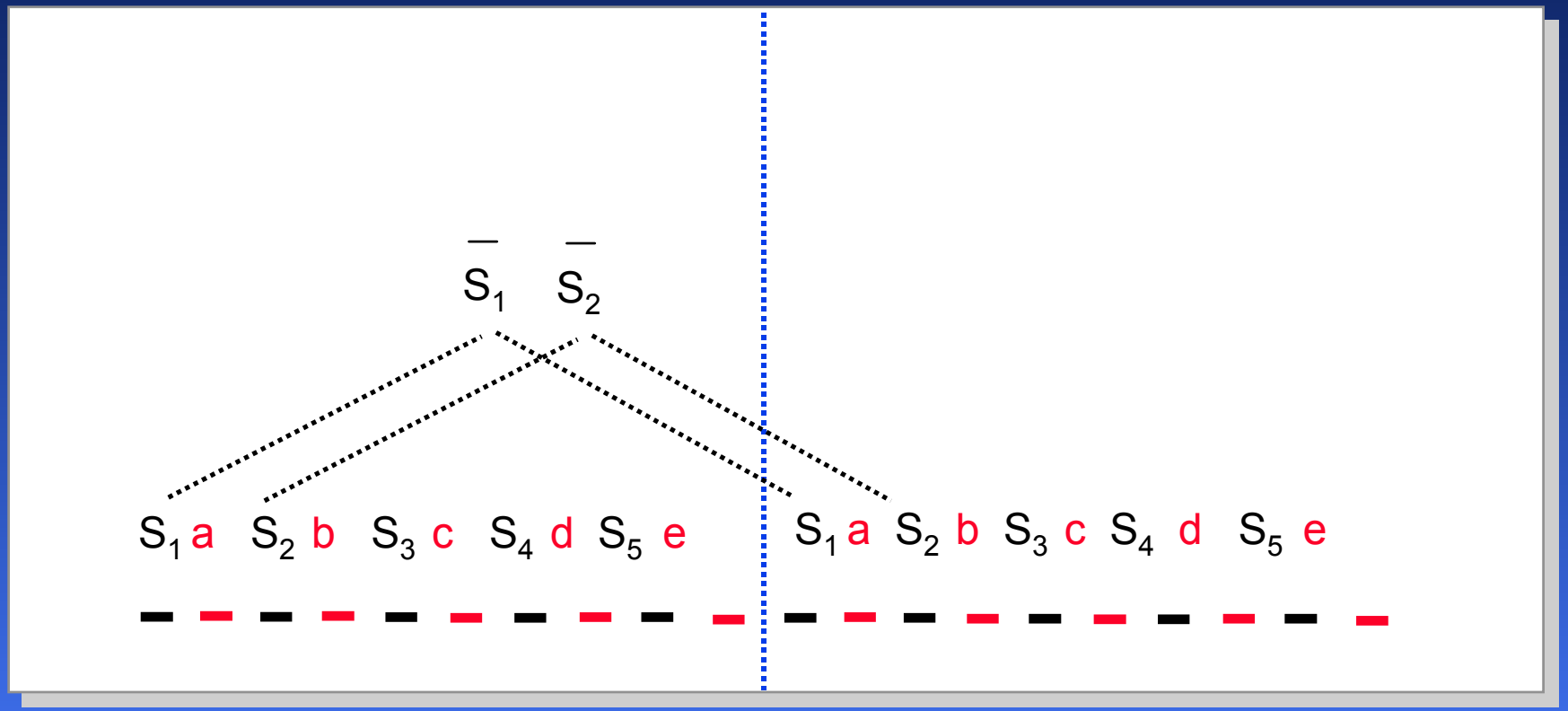


Ethyle acetate – formic acid – acidic acid – water 100:11:11:27

Prepare solvents freshly!

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

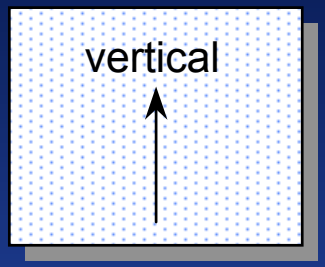
Data pair method

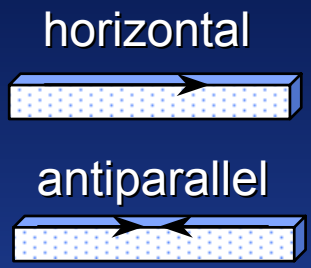


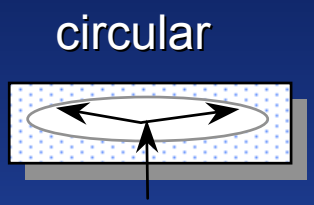
Capillary technique

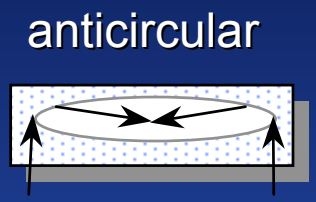
One-dimensional development

☛ simple

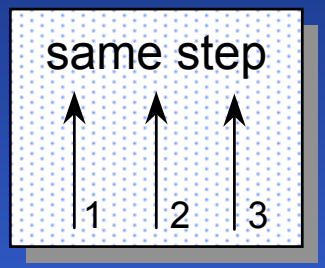


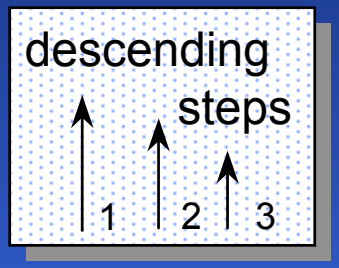


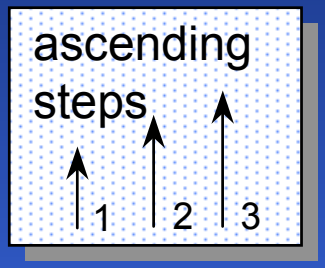


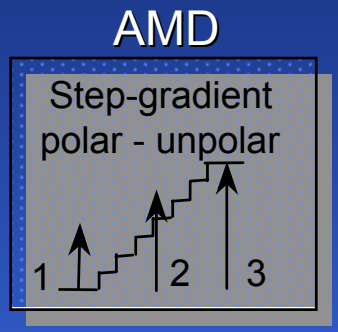


☛ multiple

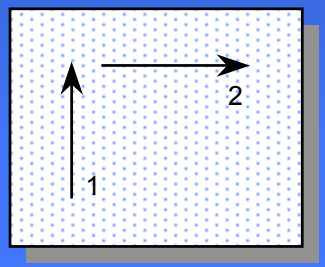


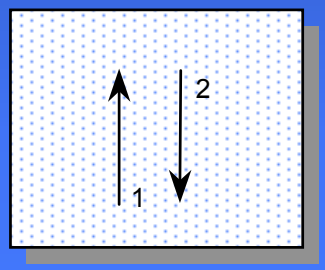




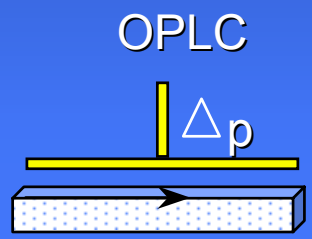


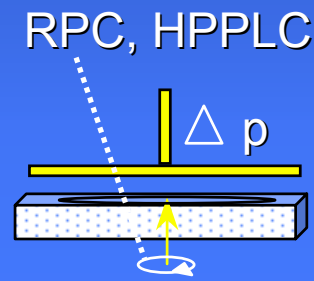
Multi-dimensional development





Forced flow technique

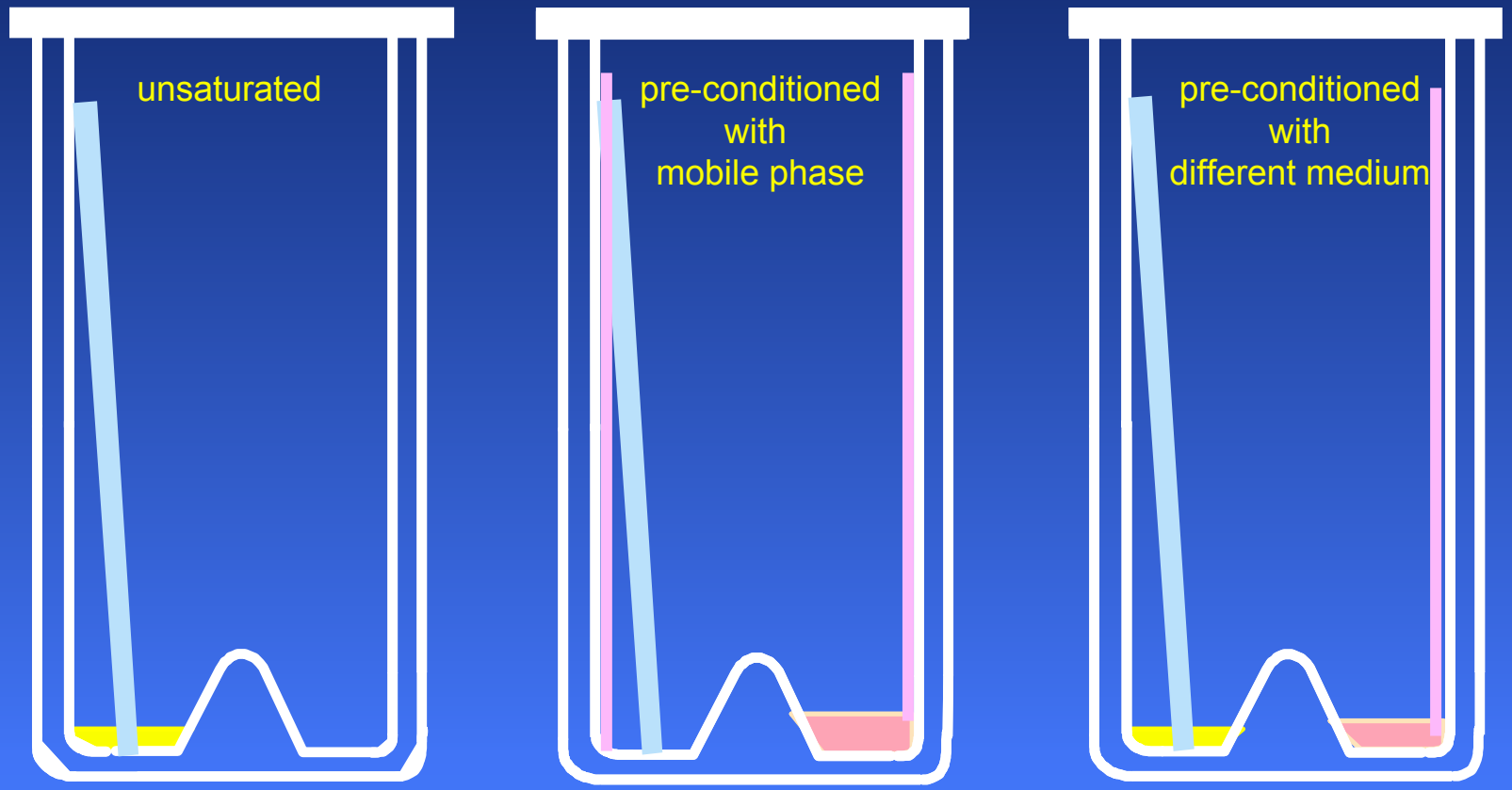




Examples of developing chambers

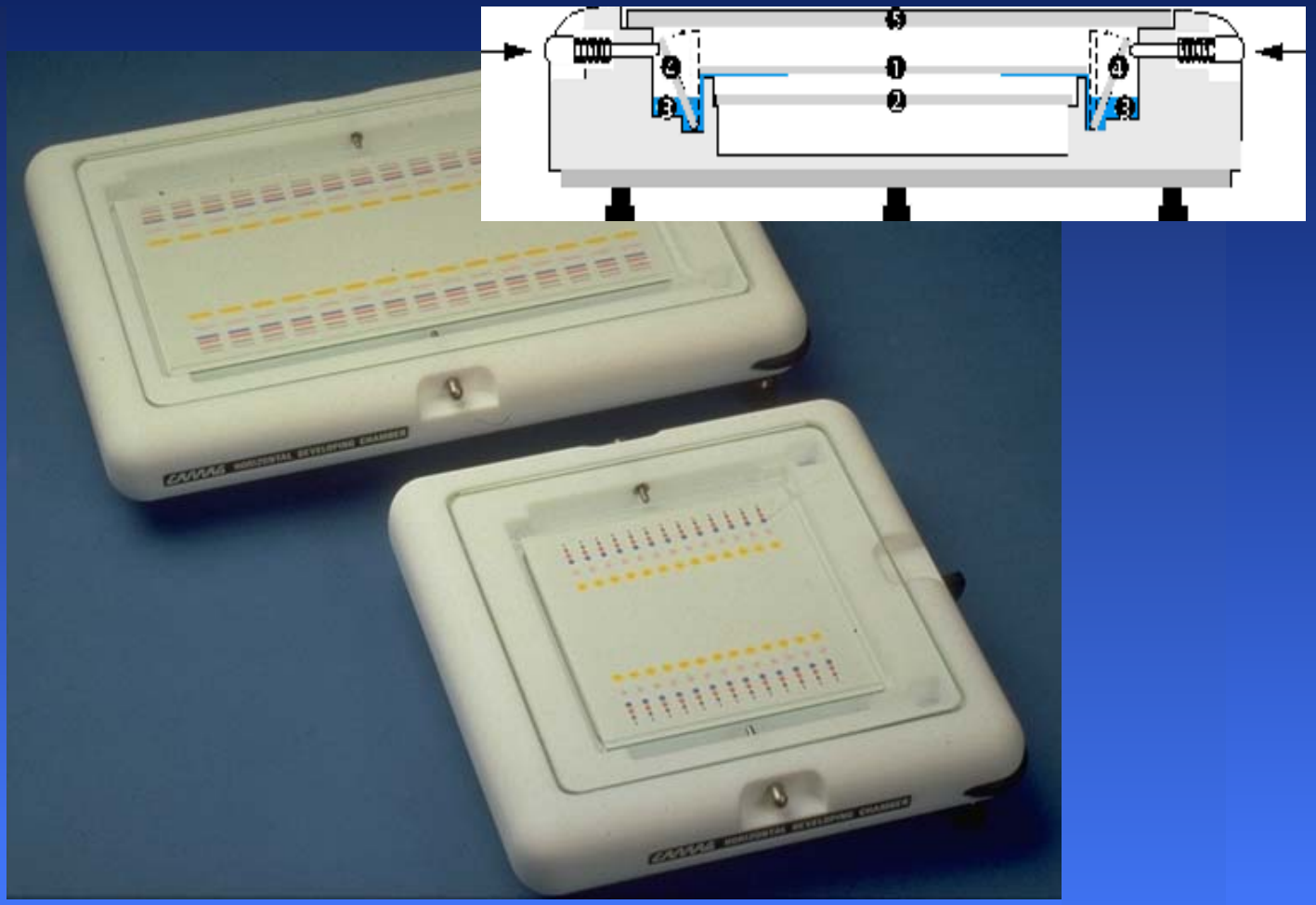
- ✓ Twin Trough Chamber
- ✓ Automatic Developing Chamber (ADC)
- ✓ Horizontal Developing Chamber (HDC)
- ✓ Automated Multiple Development (AMD)

Modes of the Twin Trough Chamber

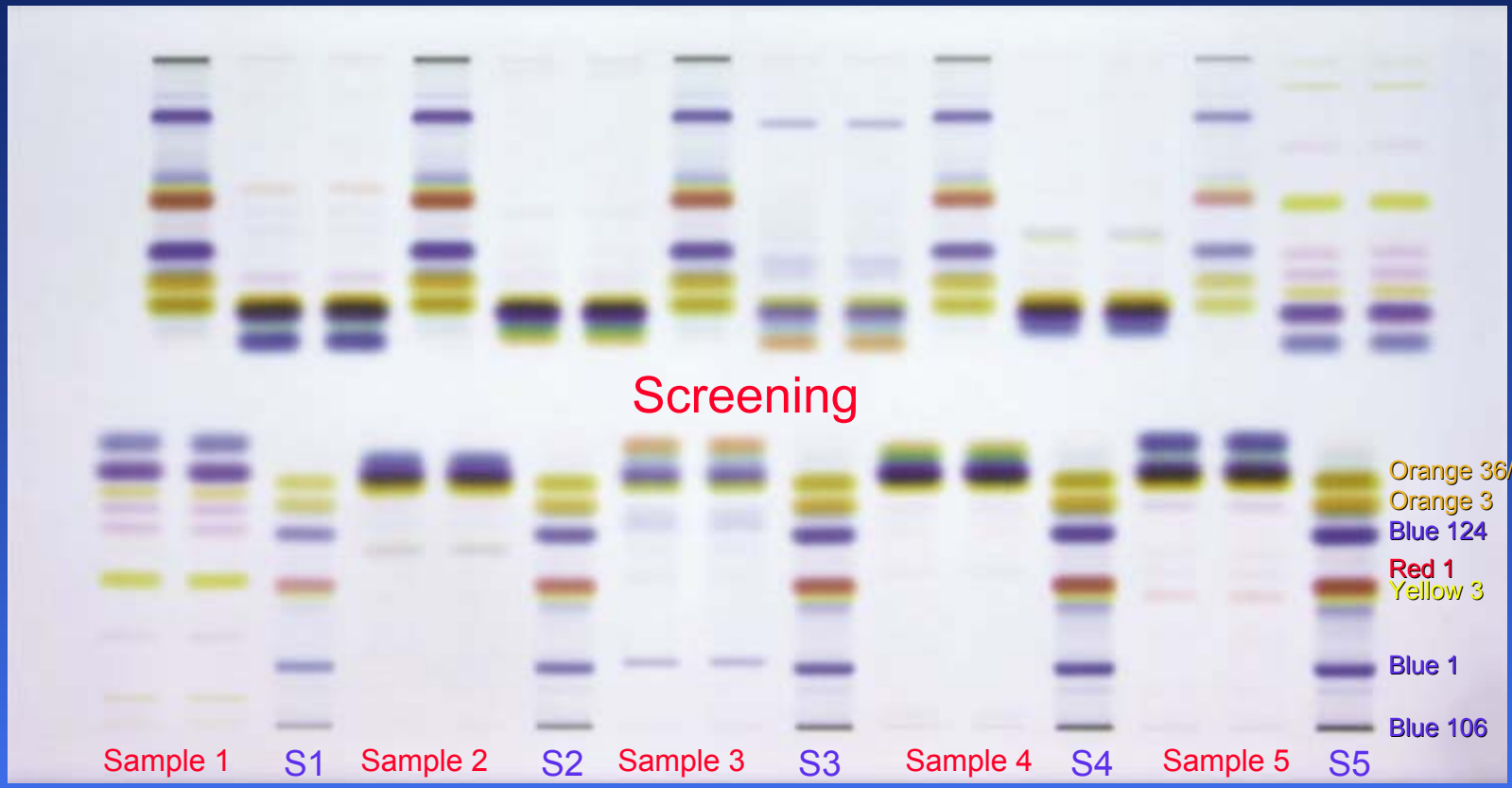


Automatic Developing Chamber (ADC)

Horizontal Developing Chamber (HDC)



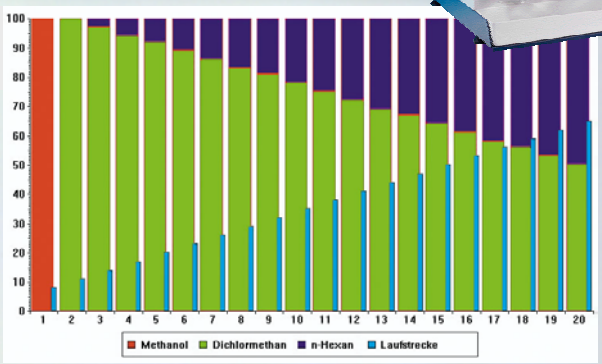
Allergenic disperse dyes in textiles



A. Bonhoff et al., STR Testing & Inspection AG, Steinach, Switzerland, optimized at CAMAG Lab, see CBS 82

Allergenic disperse dyes in textiles

Confirmation



mit Orange 3

ohne Orange 3

Orange 3
Orange 36/37
Blue 124
Red 1
Yellow 3
Blue 1
Blue 106

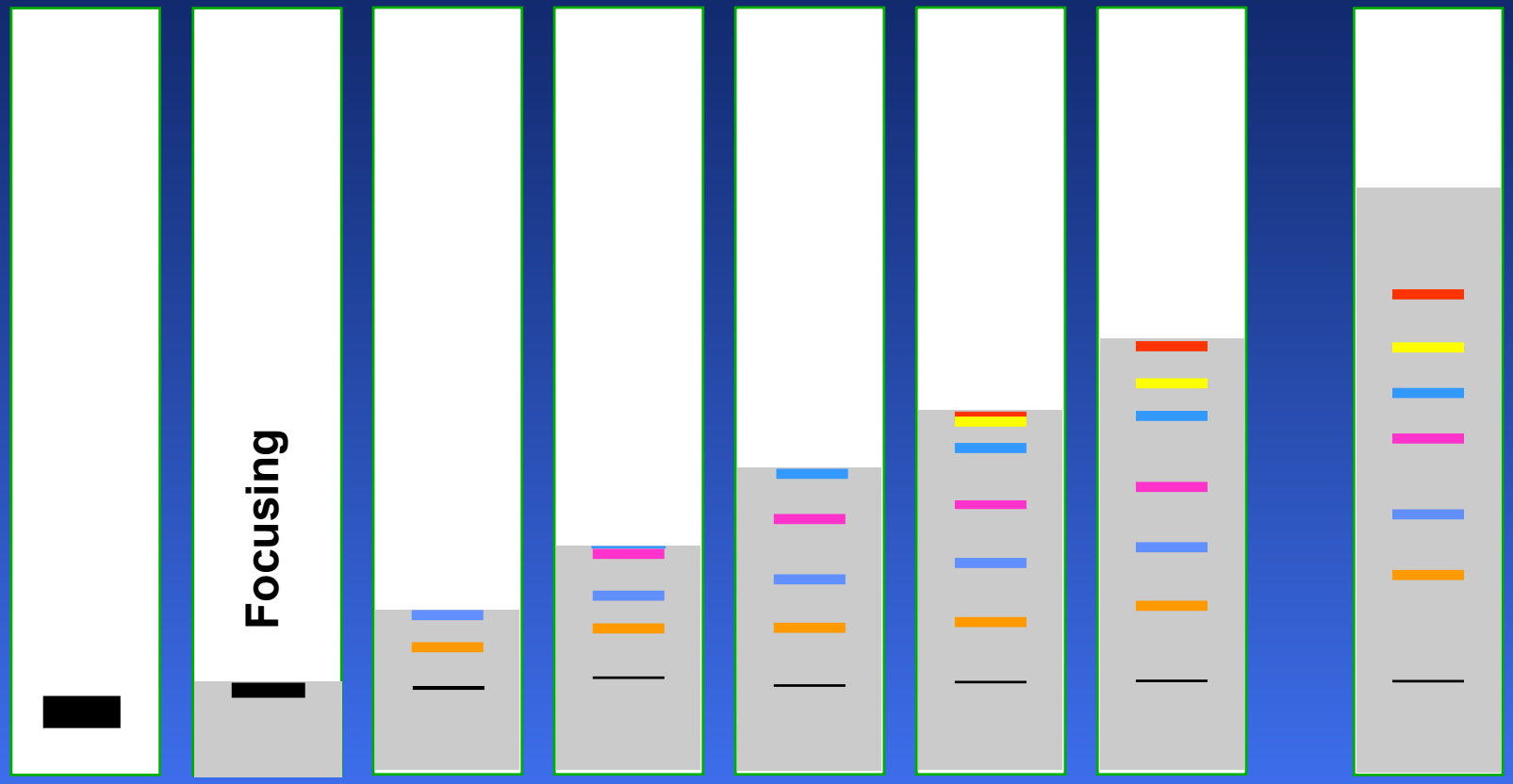
Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5

Automated Multiple Development (AMD)

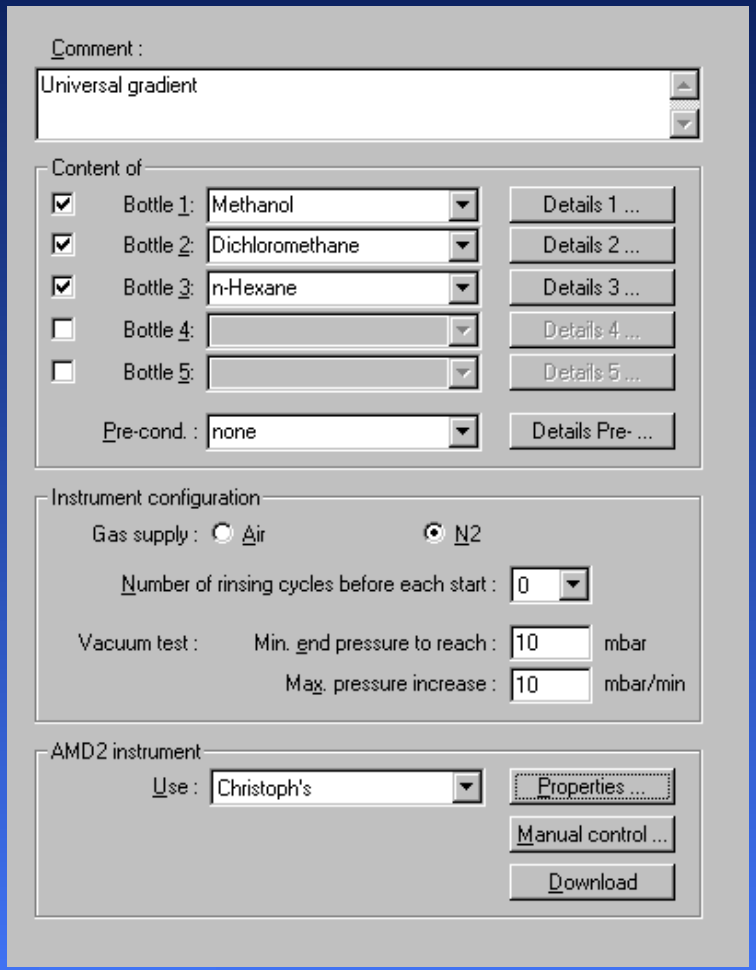
Automated Multiple Development (AMD)

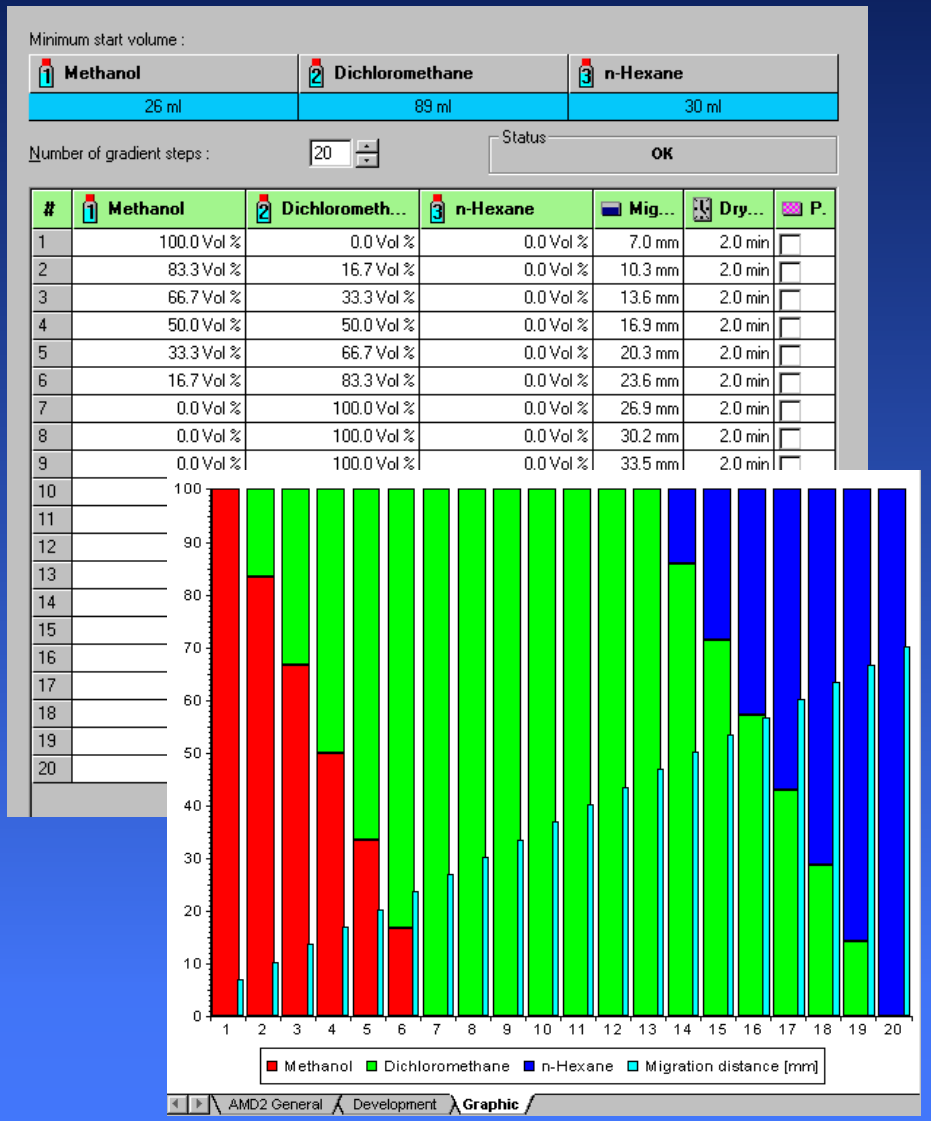
- ✓ Burger et al. (1984): polarity gradient by multiple development with different solvents
- ✓ Also possible: pH gradient
- ✓ Drying under vacuum improved precision and reliability
- ✓ Focusing to sharp zones
- ✓ Zone profile independent of migration distance
- ✓ Migration distance independent of matrix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

AMD - stepwise development

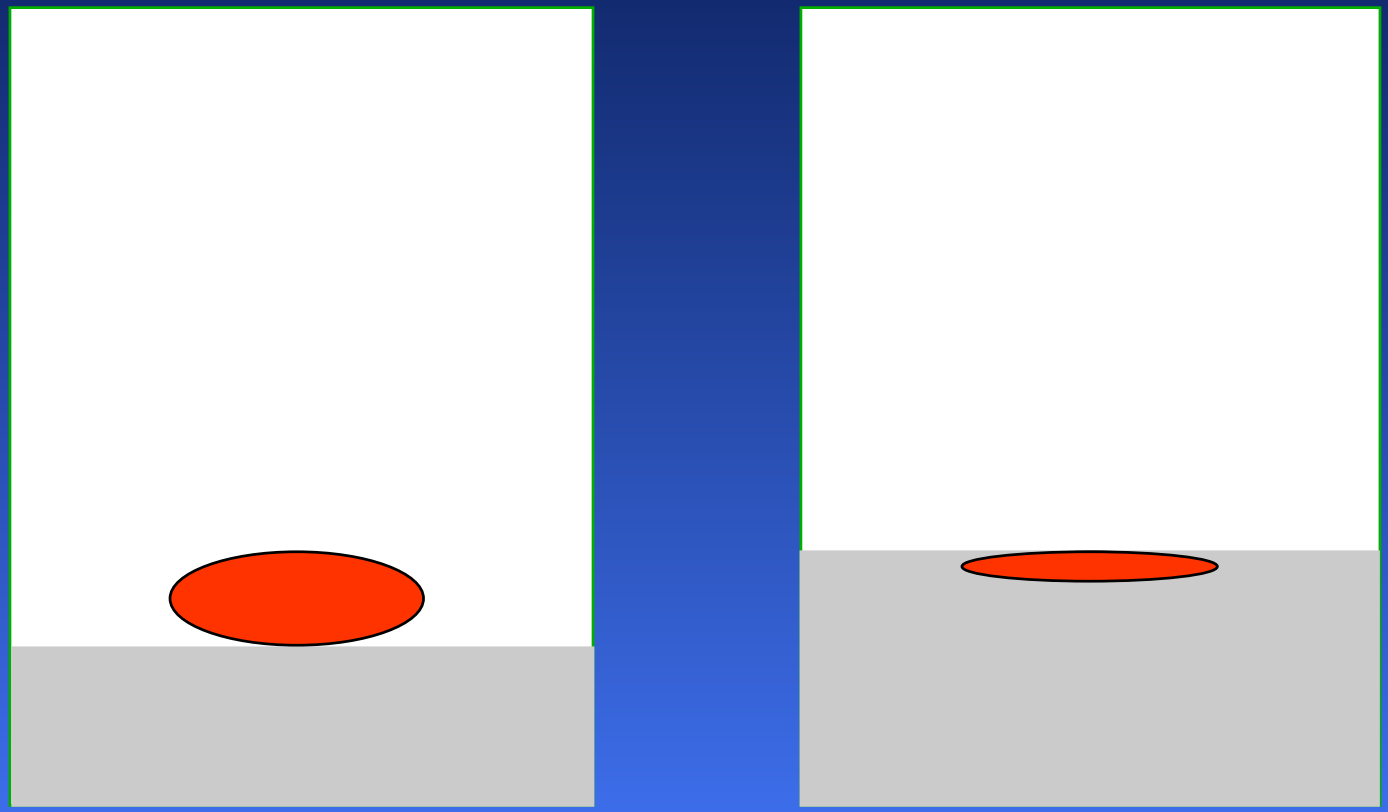


AMD – GLP conform





AMD - focusing effect



AMD - procedure for gradient optimization

1. Start with a universal gradient - examples:

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

AMD - procedure for gradient optimization

2. If necessary change pH of the universal gradient
   - Add small amounts (0.01-2 %) of NH_3, HCOOH, CH_3COOH etc. to the polar solvent
   - Fill the conditioning bottle with 0.1-4 N solution of acids or bases
3. Go on with the best universal gradient
   - leave out parts not used
   - spread parts where substances are close together
   - ✓ optimized shallower gradient results
4. If no sufficient separation was yield so far
   - take a base solvent of different selectivity, e.g. t-butyl methyl ether, acetonitrile etc.
   - change the stationary phase, e.g. diol, amino, cyano or RP18 W

AMD - hints

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.

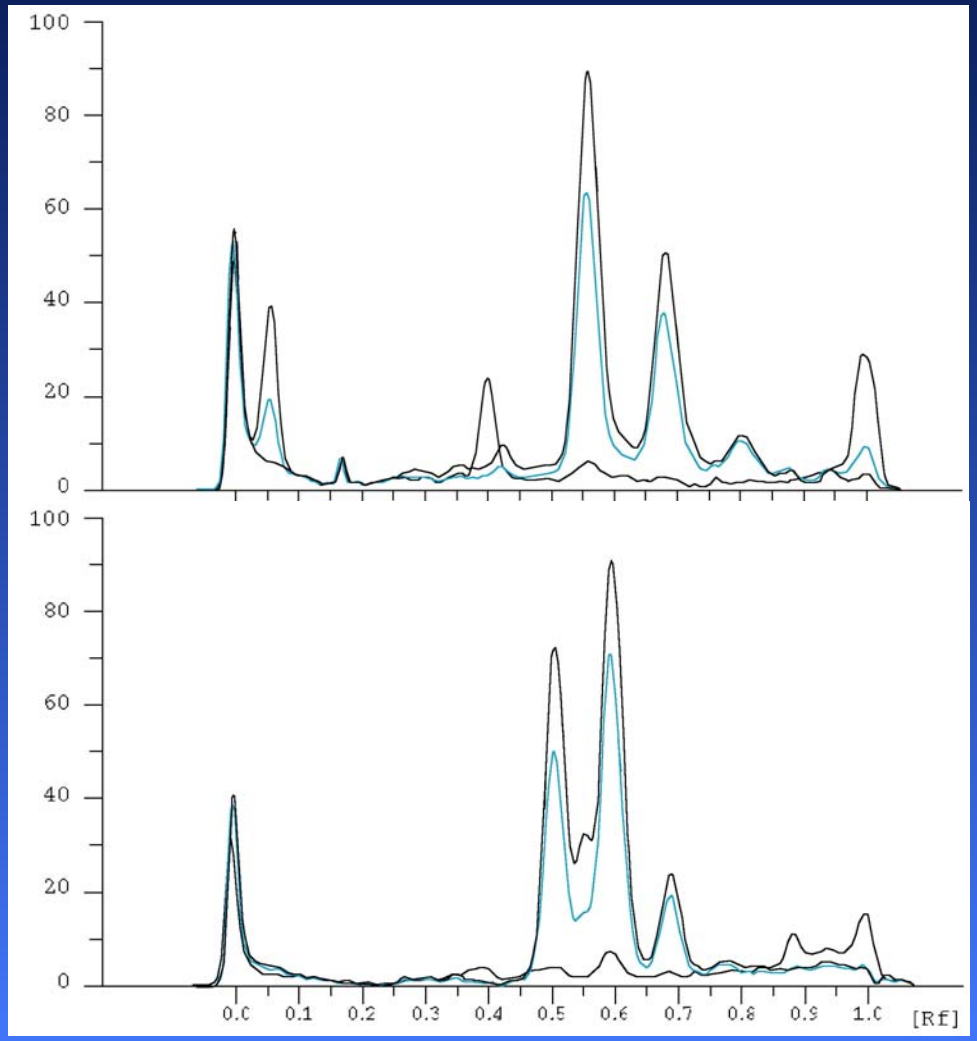
AMD - hints

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)

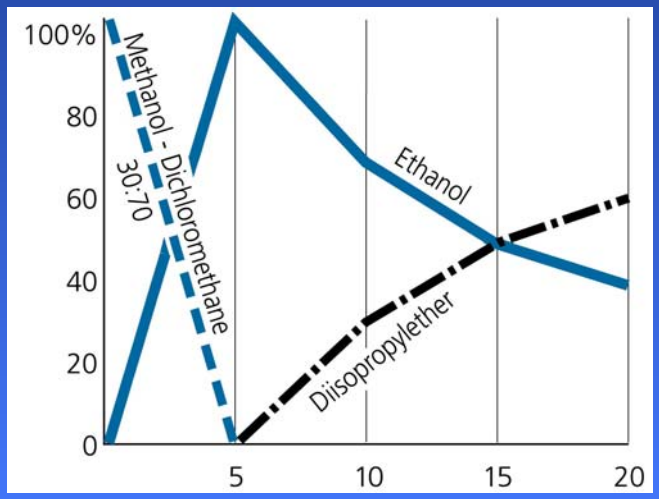
- Use 100 µm layers: shorter developing and drying times (about 2.5 h)
- Leave out parts not used: reduction to 20 steps (about 2.5 h)
- Use shorter drying times if possible
- Use spherical silica gel plates – reduces time, also drying time, to about 50 %

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

Identification of ball pen inks

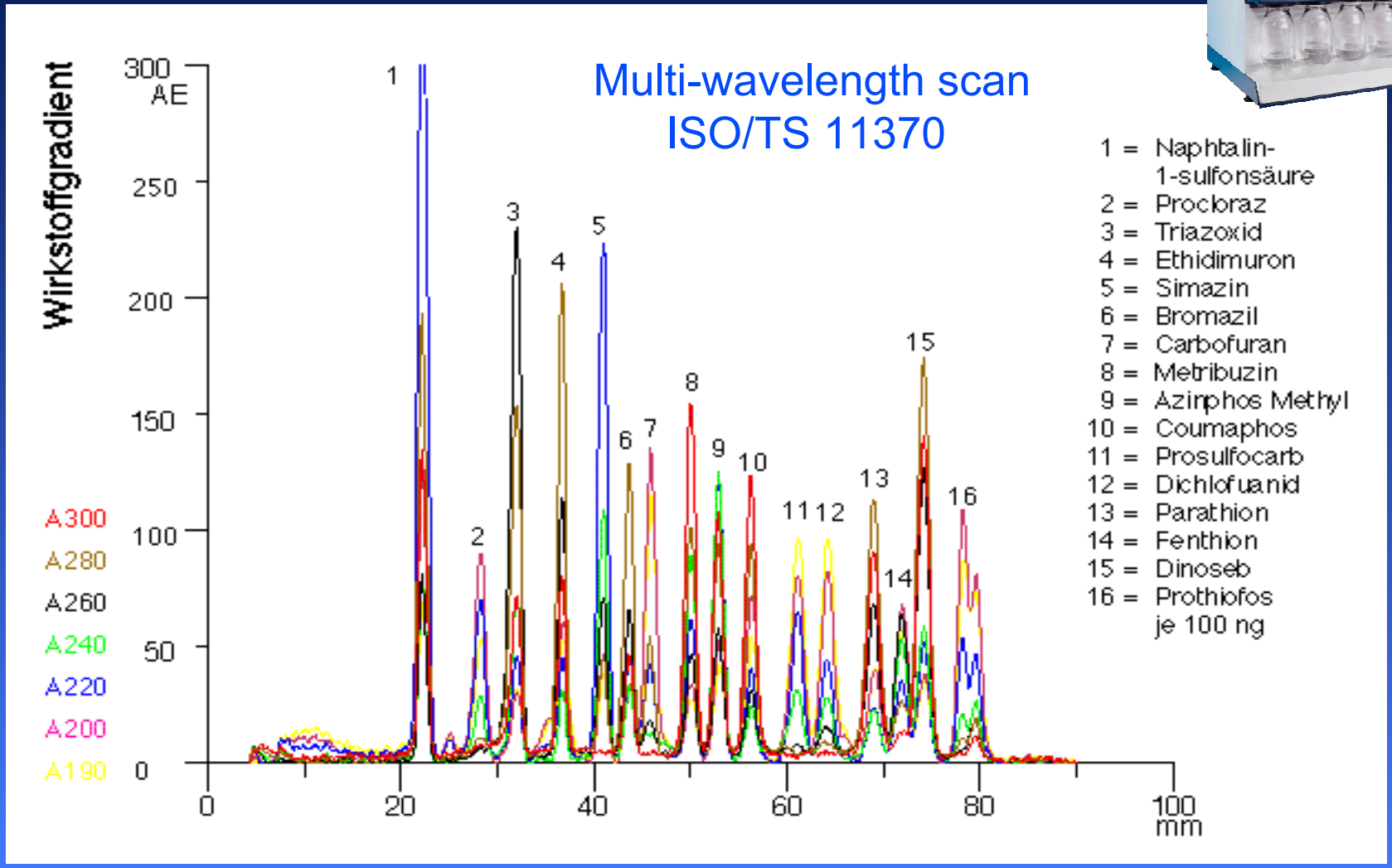


- Product classification
- Determination of document age



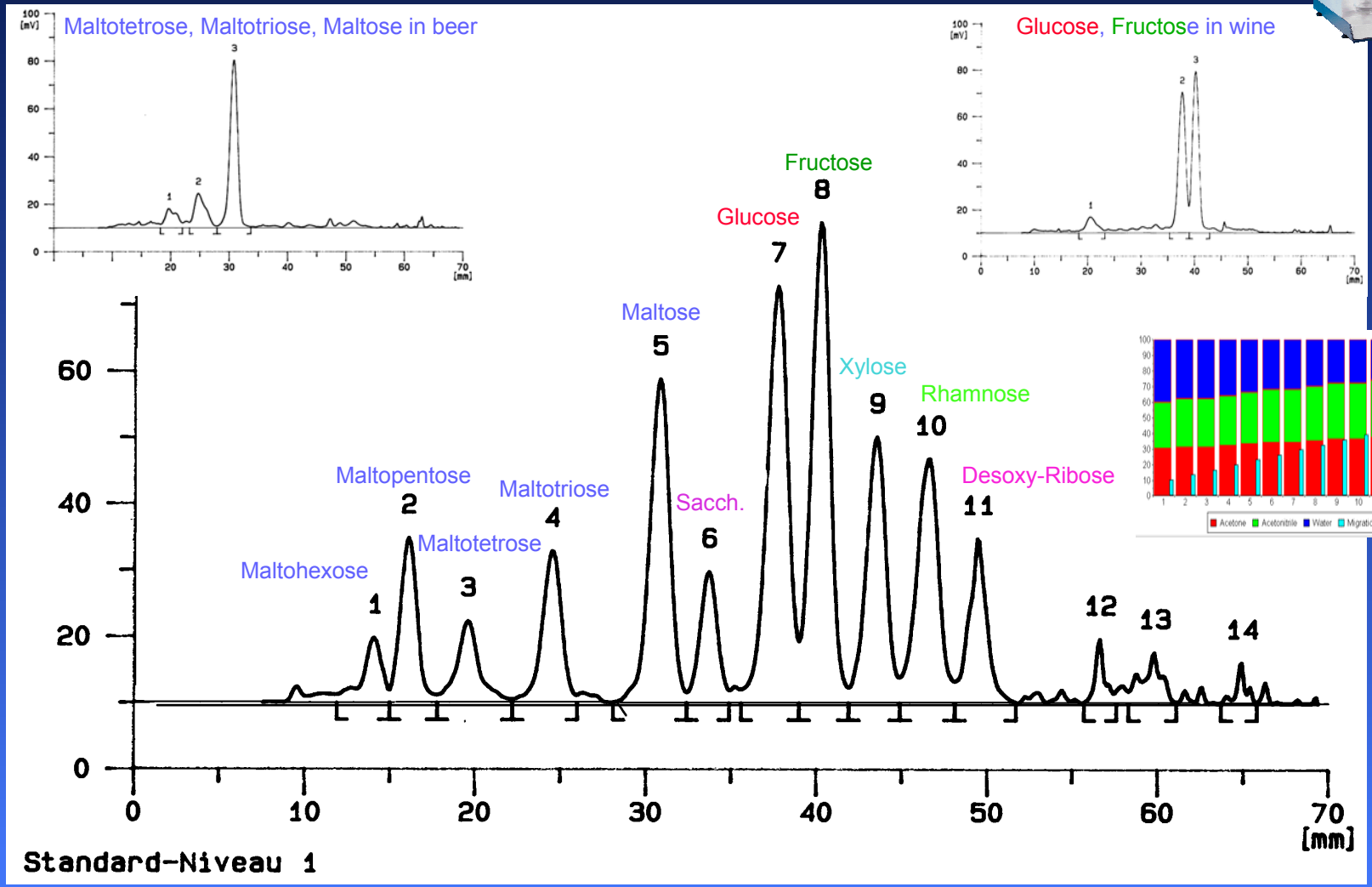
F. Köhler, P. Seiler, Bundeskriminalamt, Wiesbaden, see CBS 74

Pesticides in drinking and surface water



klaus.burger@mail.isis.de

Carbohydrates in beer and wine



G. Lodi et al., University of Ferrara, Italy, see CBS 69