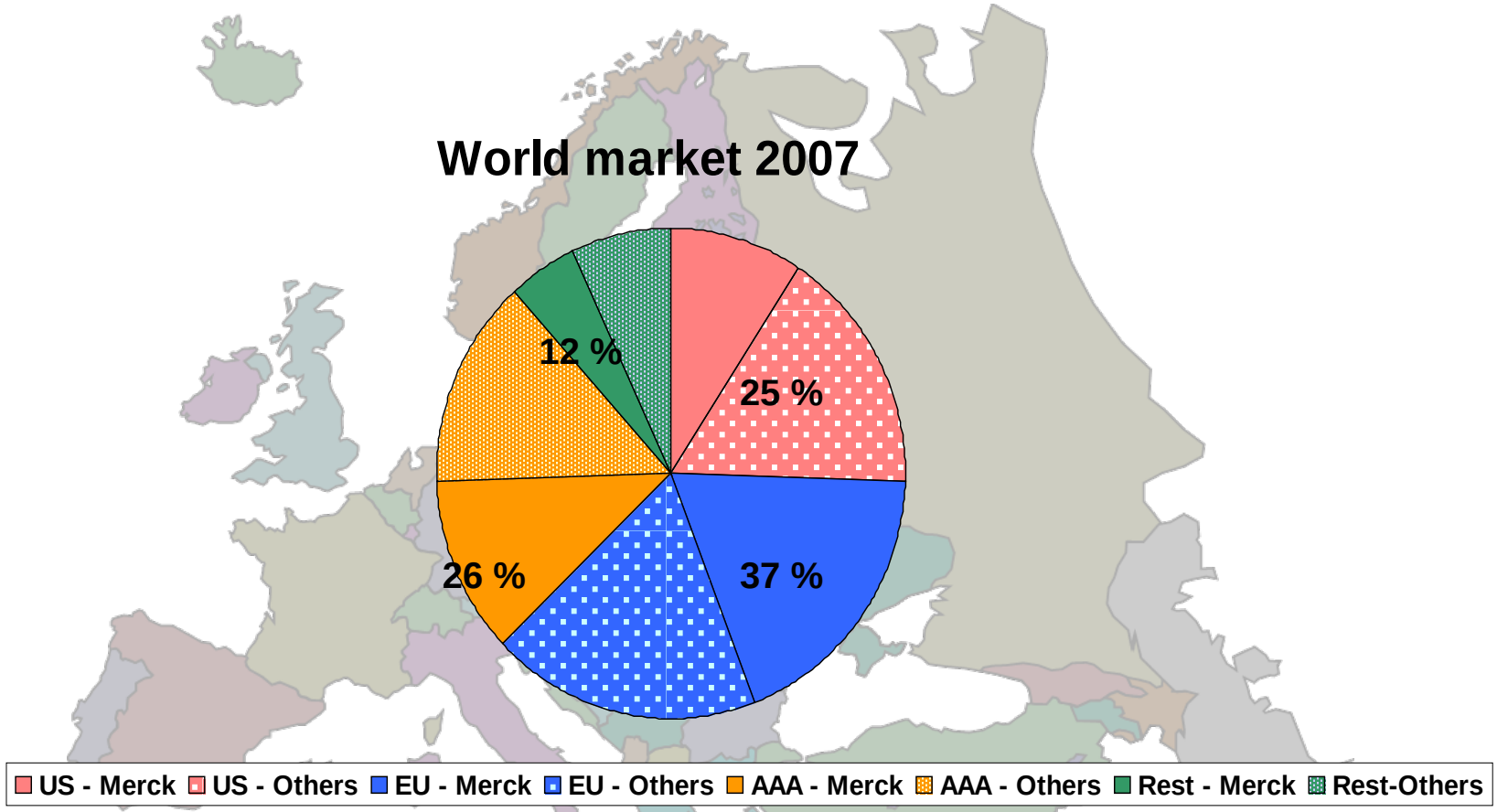
Latest development of Thin - Layer Chromatography at Merck

Dr. Mehmet Dogan, Merck Millipore /Lab Essentials/LC

CCM Meeting in LYON, Nov. 2011

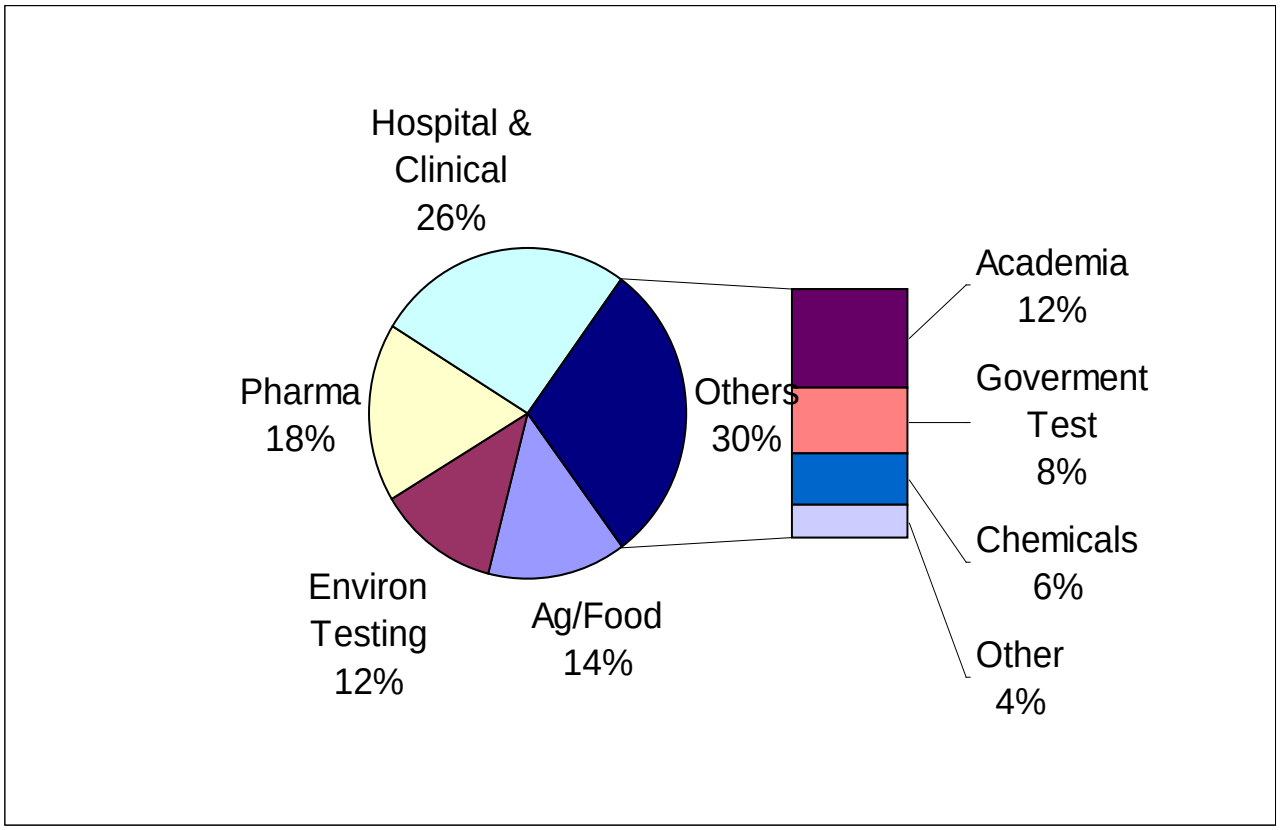
Market Thin Layer Chromatography



We are by far the market leader in Thin layer chromatography!

Market Thin Layer Chromatography

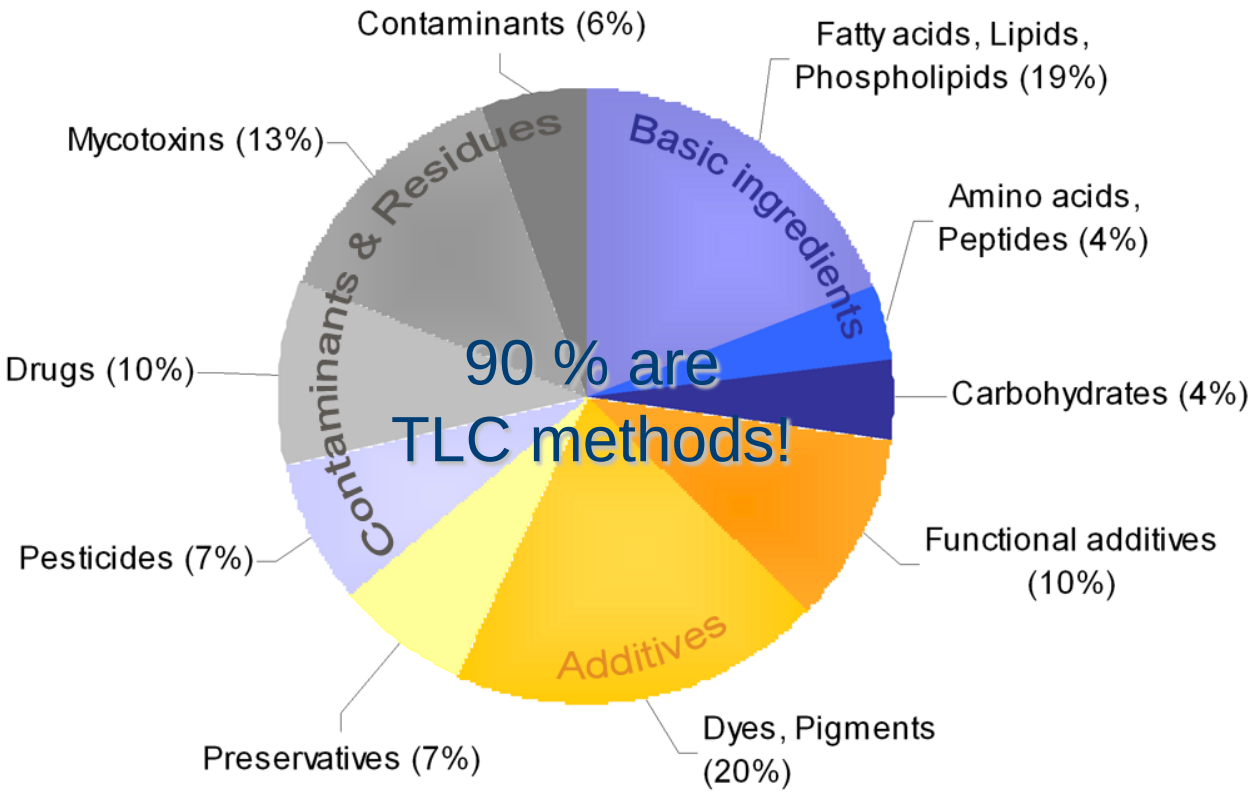
Total 40 – 50 Mio EUR
AGR: 2 - 4%



SDi Global Assessment Report 9th Edition, LCGC Oct.08

Planar Chromatography

Food analysis 1987-2007



G. Morlock, W. Schwack, J Planar Chromatogr 20 (2007) 399-407

Merck
Pioneered Thin Layer Chromatography

1938	Al_2O_3 layers (Izmailov and Shraiber)
1951	Silica gel layers with calcium sulphate (Kirchner)
1950	Egon Stahl is founder of thin layer Chrom. and standardized silica gels *(*Higher sensitivity more and universal scope of applications)
1958	Merck launched TLC during Achema exhibition
1966	Pre-coated TLC plates
1975	Pre-coated HPTLC plates
1978	Modified sorbents for TLC and HPTLC
1995	Spherical sorbents for HPTLC (LiChrospher®)
2002	Ultra thin monolithic silica plates (UTLC)
2003	LuxPlate®
2006	ProteoChrom® Plates
2011	G- Plates

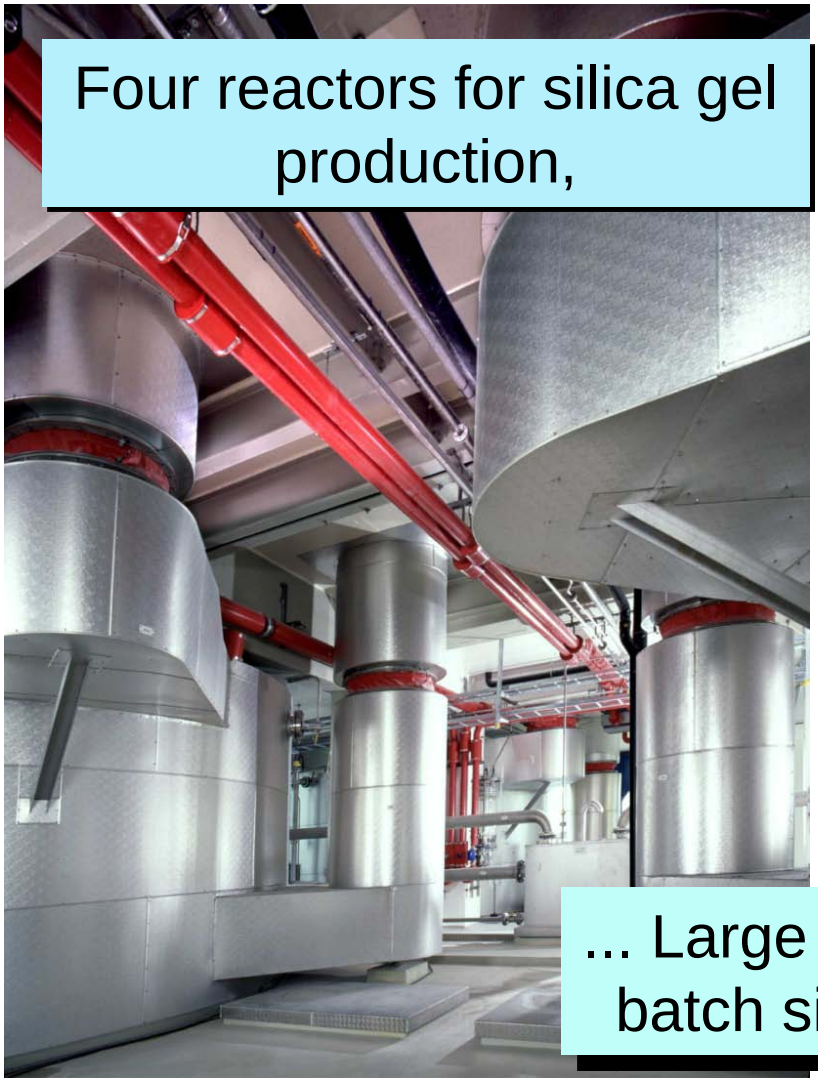
First presentation of pre-coated plates, Achema 1958

Silica and Aluminium oxide production facilities, Gernsheim

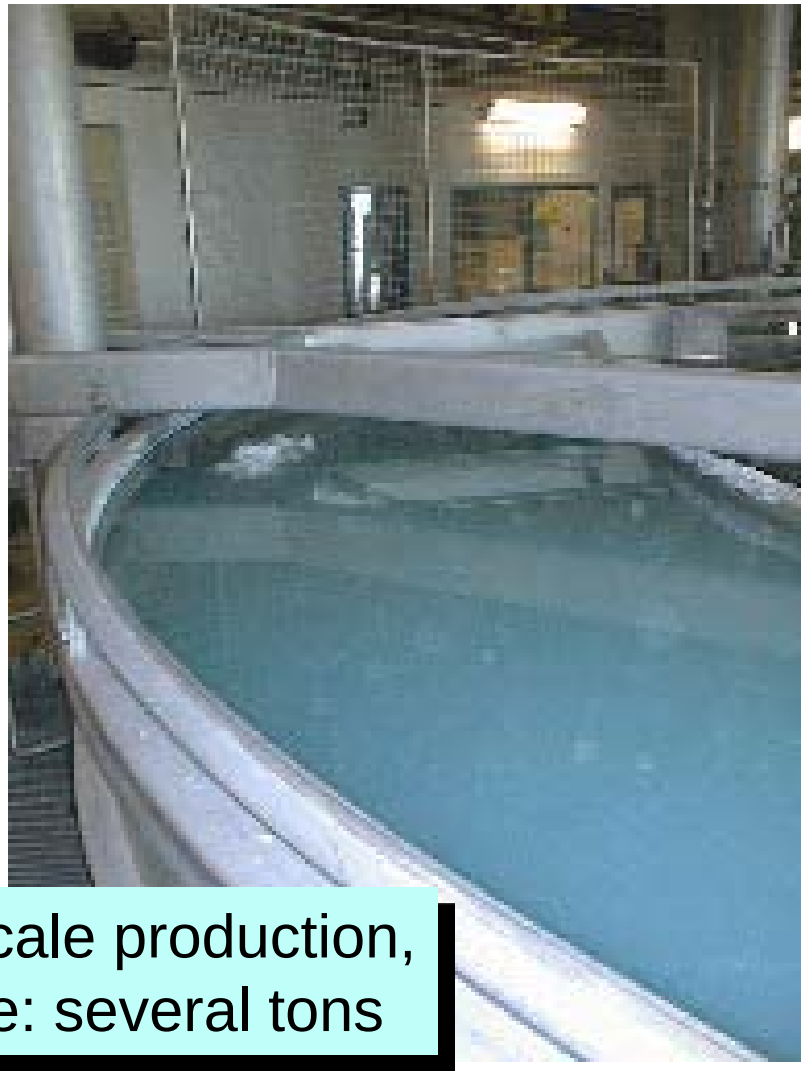
Production Plant

... the biggest chromatographic silica gel plant in the world,

Production Plant

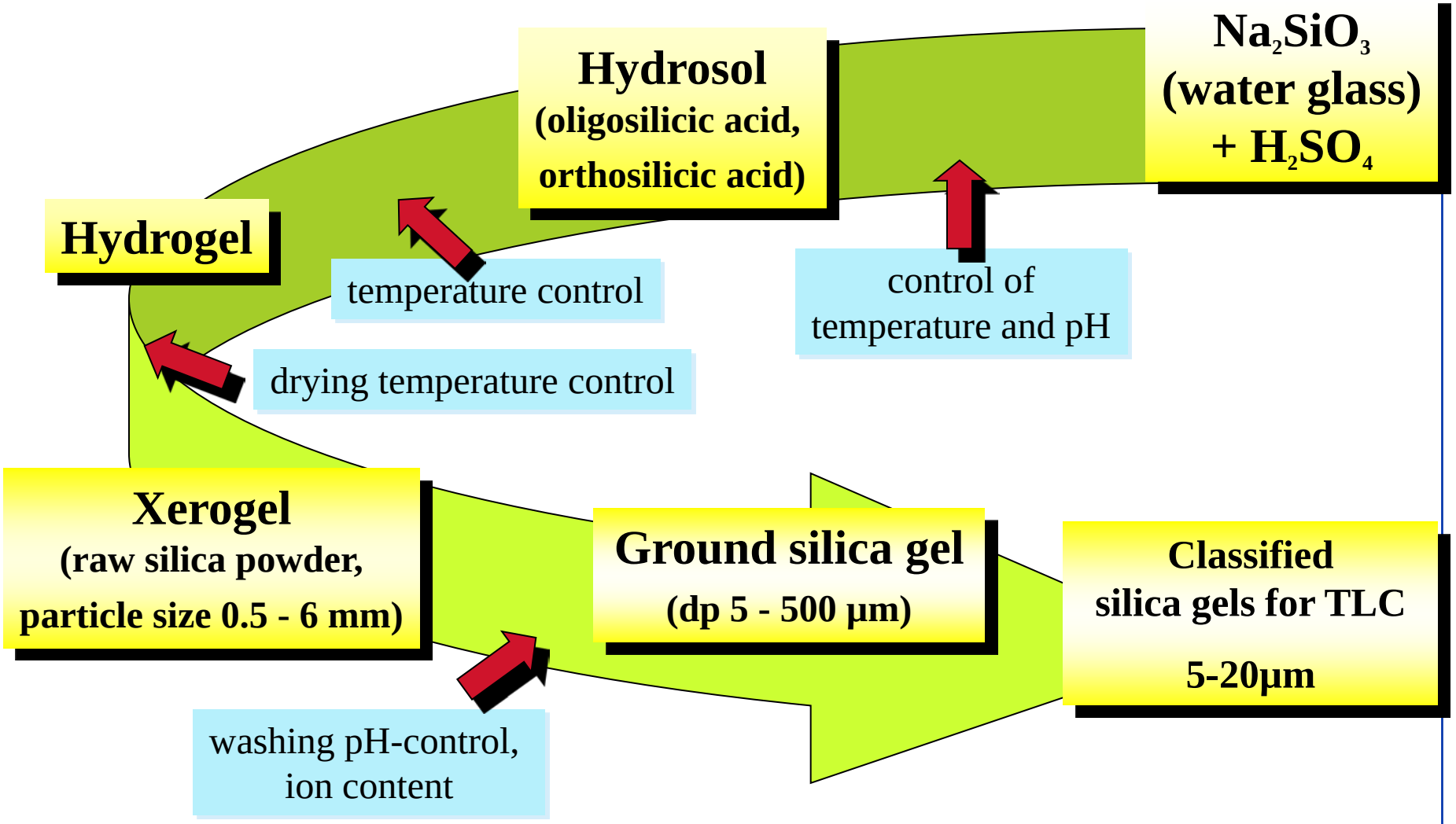


Four reactors for silica gel production,



... Large scale production, batch size: several tons

Silica Production Process



Production Process of TLC Plates

Preparation of suspension of silica gel in water
(eventually with fluorescence indicator)

↓

Coating of plates or sheets
(glass, aluminum, plastic)

↓

Drying in drying tunnel

↓

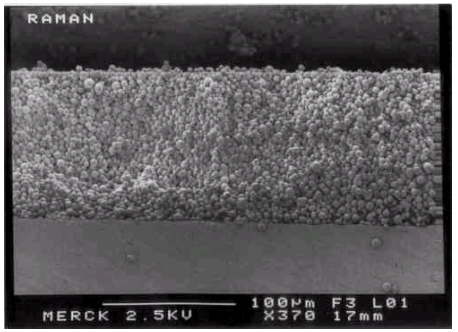
Sampling, in-process control

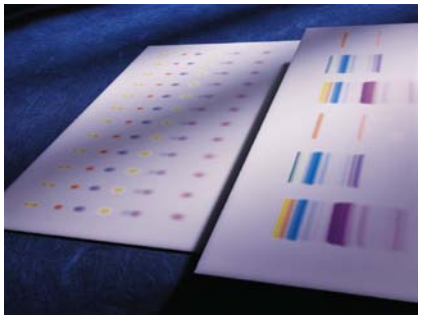
↓

Cutting (for formats smaller than 20 x 20 cm),

↓

Packaging, final control

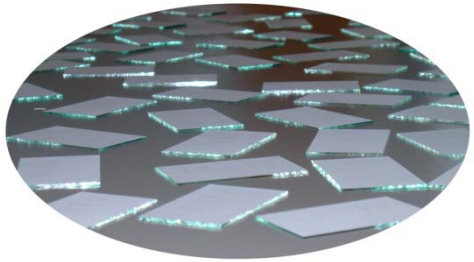




TLC Production Today

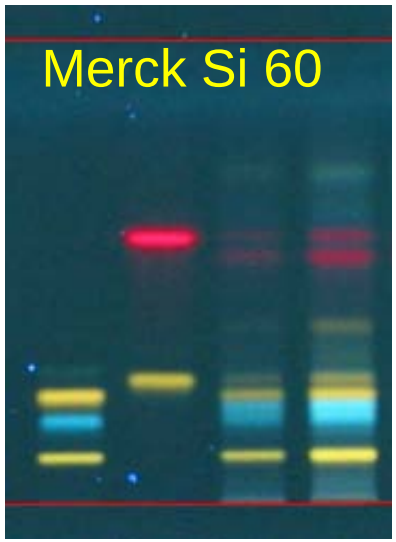
- 23 employes in production plant
- > 7 million plates per year
- Every single plate is visually inspected
- More than 60 different products

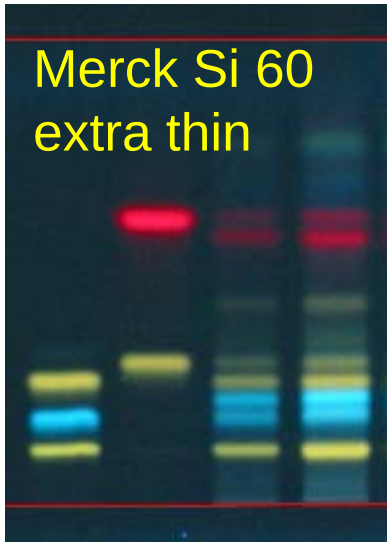
On these plates 45 million analyses a carried out each year!

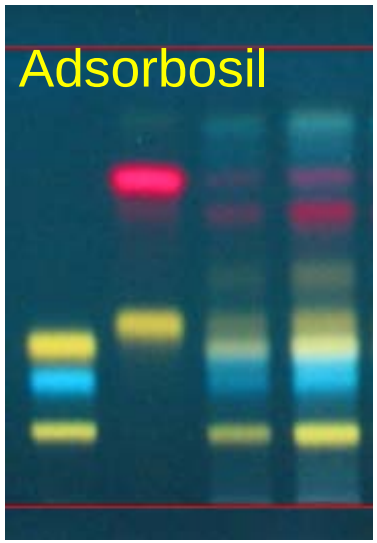


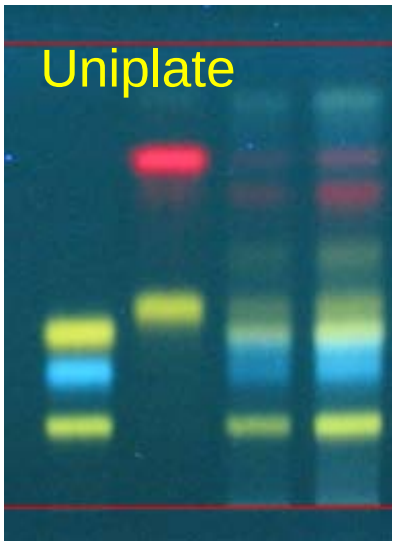
Comparison of Silica gel plates

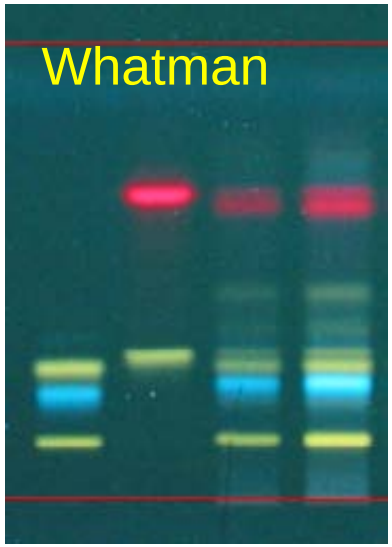
Hypericum extract : (Quercetin, quercitrin, hyperoside, rutin, (-)-epicatechin, 3,5-dihydroxy-1-methoxy-xanthone, 3,4-O-isopropylidenyl shikimic acid, shikimic acid)

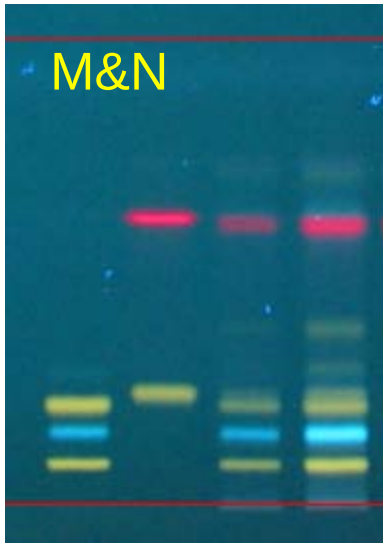


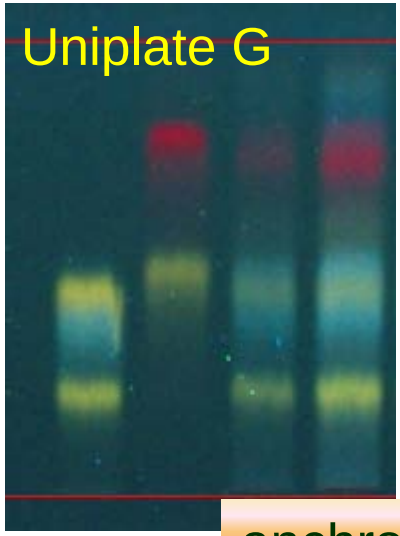






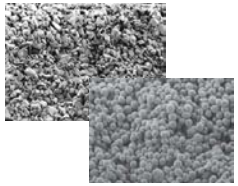






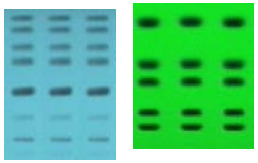
anchrom

TLC Range at a Glance

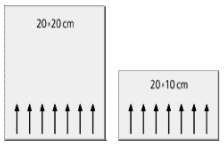


- Sorbents types
  - **Silica 60**
  - **Modified silica: RP2, RP8, RP18, NH_2, Diol, CN; Aluminium oxide, Cellulose**

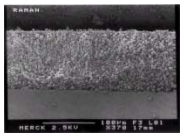
- Backings
  - **Glass,**
  - **Aluminium & Plastic**



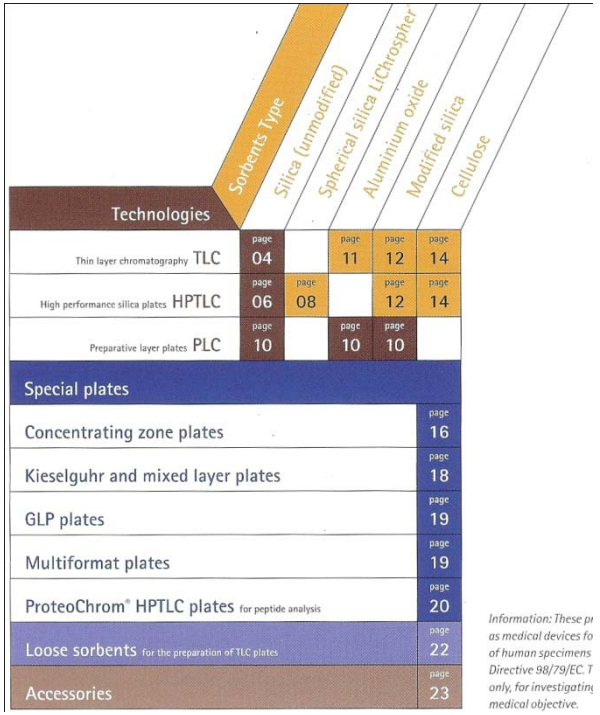
- Detection UV 254nm
  - **with fluorescence indicator F_{254}:green, F_{254s}: blue**



- Plate sizes
  - **2.5x7.5 / 5x5 ... to 20x20 / 20x10 cm**



- Plate thickness
  - **100 µm – 2 mm (UTLC 10 µm)**

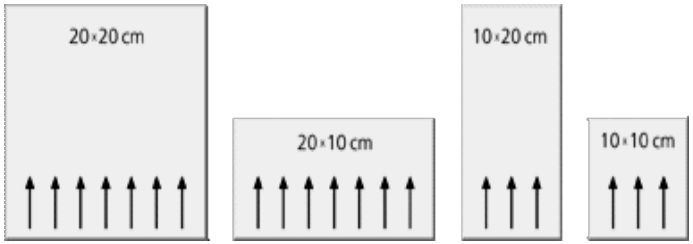


Glass, aluminium or plastic?

Support	Advantage
Glass	▪ no bending best for instrumental HPTLC ▪ inert material ▪ temperature stable
Aluminium Plastic	▪ 20% lower priced then glass ▪ simple to cut with scissors allowing for different formats

Plate Sizes Fitting the Application

Backing	classical TLC	HPTLC	PLC
Glass	20 x 20 cm 10 x 20 cm 5 x 20 cm 5 x 10 cm 5 x 7,5 cm	20 x 10 cm 10 x 10 cm 5 x 10 cm 5 x 5 cm	20 x 20
Aluminium	20 x 20 cm 10 x 20 cm 5 x 20 cm 5 x 10 cm 5 x 7,5 cm	20 x 20 cm 5 x 7,5 cm	
Plastic	20 x 20 cm 500 x 20 cm 4 x 8 cm		

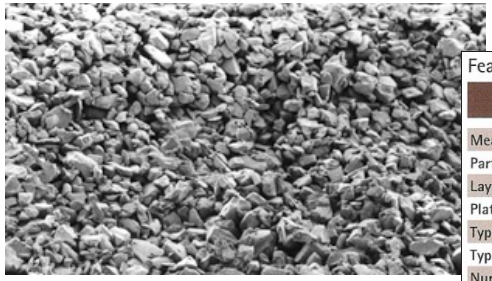


Warning to the correct use : size & direction !

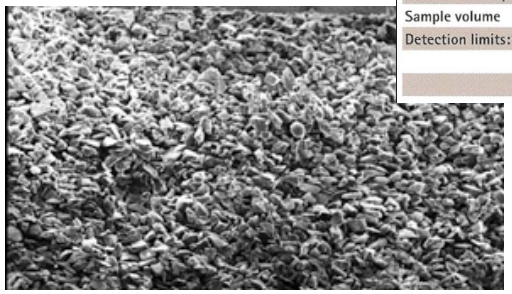
Quality Grades

Particle size distribution:

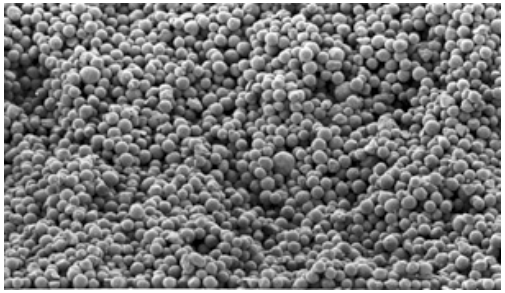
- Classical TLC
  5 - 20 µm



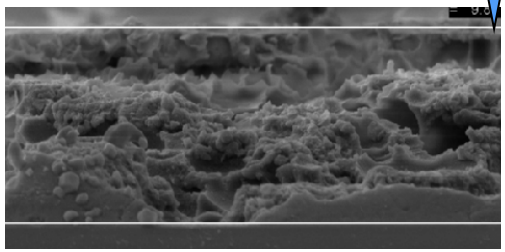
- HPTLC
  4 - 8 µm



- Spherical particles HPTLC
  4 - 8 µm



- Monolithic layer UTLC



Features of HPTLC versus classical TLC

	HPTLC	Classical TLC
Mean particle size	5 - 6 µm	10 - 12 µm
Particle size distribution	4 - 8 µm	5 - 20 µm
Layer thickness	200 µm (100 µm)	250 µm
Plate height	12 µm	30 µm
Typical migration distance	3 - 6 cm	10 - 15 cm
Typical separation time	3 - 20 min	20 - 200 min
Number of samples per plate	< 36 (72)	< 10
Sample volume	0.1 - 0.5 µl	1 - 5 µl
Detection limits:		
absorption	100 - 500 pg	1 - 5 ng
fluorescence	5 - 10 pg	50 - 100 pg

Quality

Not comparable

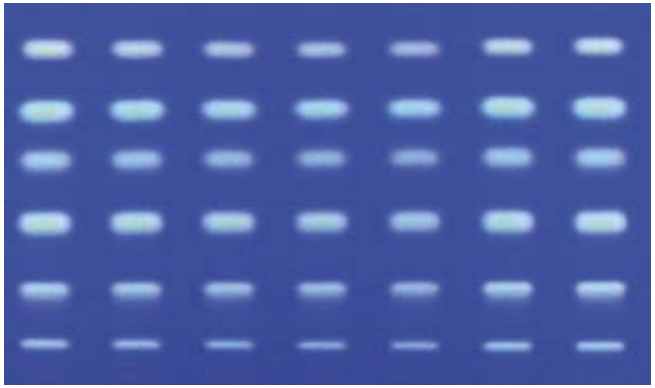
Sorbents Types

TLC	HPTLC	PLC
Silica gel 60	**Silica gel 60**	**Silica gel 60**
$Al2O_3$ 60/150	Al_2O_3 60/150	
Cellulose	Cellulose	
(Kieselguhr)		
RP-2	RP-2	
RP-8	RP-8	
RP-18	RP-18	RP18
	RP-18W	
NH_2	NH_2	
	CN	
	DIOL	

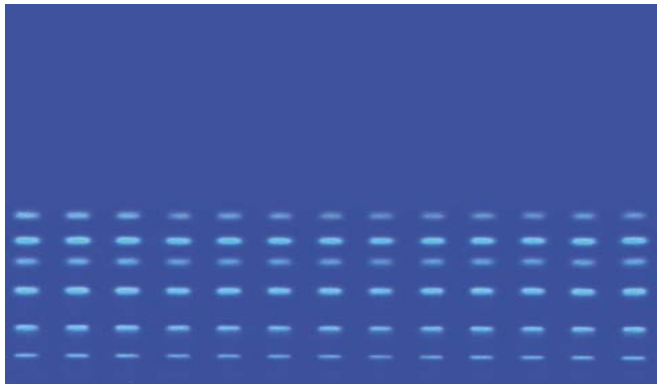
HPTLC versus TLC

- 5 – 10 fold increased sensitivity than classical TLC
- Faster analysis (only 15 min compared to 45 min)
- Gold standard for automated use with instrument

Classical TLC silica gel 60 plate



HPTLC silica gel 60 plate



Sample: Separation of dansyl amino acids

Compounds:	1. N-alpha-dansyl-L-asparagine
	2. alpha-dansyl-L-arginine
	3. Dansyl-L-cysteic acid
	4. N-Dansyl-L-serine
	5. Dansyl-glycine
	6. N-N-Didansyl-L-tyrosine
Sample volume:	TLC 4 µl; HPTLC 0.3 µl
Mobile phase:	Ethyl acetat/methanol/propionic acid (22/10/3)
Migration distance:	TLC 10 cm; HPTLC 5 cm
Analysis time:	TLC 42 min; HPTLC 13 min 45 sec
Detection:	UV 366

TLC Range at a Glance

- Sorbents types : **Silica 60**

HPTLC LiChrospher® unmodified silica gel 60

Packing Material	Format [cm]	Content	Backing	Ord. No.
HPTLC LiChrospher® silica gel 60 F_{254s}	20 x 10	25 plates	glass	1.15445.0001
HPTLC LiChrospher® silica gel 60 F_{254s}	20 x 20	25 plates	aluminium	1.05586.0001
HPTLC LiChrospher® silica gel 60 WR F_{254s} AMD extra thin*	20 x 10	25 plates	glass	1.05647.0001

Layer thickness: 200 µm | * Layer thickness: 100 µm | WR: wettable with water and pure silica gel 60

HPTLC LiChrospher® RP-modified silica gel 60

Packing Material	Format [cm]	Content	Backing	Ord. No.
HPTLC LiChrospher® silica gel 60 RP-18 W F_{254s}	20 x 10	25 plates	glass	1.05646.0001

Layer thickness: 200 µm | W: wettable with water

TLC Range at a Glance

- Sorbents types : **Silica 60**

HPTLC unmodified silica gel 60

Packing Material	Format [cm]	Content	Backing	Ord. No.
HPTLC silica gel 60	20 x 10	50 plates	glass	1.05641.0001
	10 x 10	25 plates	glass	1.05631.0001
	10 x 10	100 plates	glass	1.05633.0001
HPTLC silica gel 60 F_{254s}	20 x 10	25 plates	glass	1.15696.0001
HPTLC silica gel 60 F_{254}	20 x 10	50 plates	glass	1.05642.0001
	10 x 10	25 plates	glass	1.05628.0001
	10 x 10	100 plates	glass	1.05629.0001
	5 x 10	25 plates	glass	1.05616.0001
HPTLC silica gel 60	20 x 20	25 plates	aluminium	1.05547.0001
HPTLC silica gel 60 F_{254}	20 x 20	25 plates	aluminium	1.05548.0001
	5 x 7.5	20 plates	aluminium	1.05556.0001
HPTLC silica gel 60 WR F_{254s}	20 x 10	25 plates	glass	1.15552.0001
HPTLC silica gel 60 F_{254} AMD, extra thin*	20 x 10	25 plates	glass	1.11764.0001
HPTLC silica gel 60 WR F_{254s} AMD, extra thin*	20 x 10	25 plates	glass	1.12363.0001
HPTLC silica gel 60 F_{254} premium purity plate	20 x 20	25 plates	glass	1.05648.0001

Layer thickness: 200 µm | * Layer thickness: 100 µm | WR: wettable with water and pure silica gel 60

TLC Range at a Glance

RP-modified silica plates (TLC and HPTLC)

Packing Material	Format [cm]	Content	Backing	Ord. No.
Silica gel 60 RP-2 (silanized)*	20 x 20	25 plates	glass	1.05746.0001
Silica gel 60 RP-2 F_{254} (silanized)*	20 x 20	25 plates	glass	1.05747.0001
Silica gel 60 RP-8 F_{254s}*	20 x 20	25 plates	glass	1.15388.0001
	10 x 20	50 plates	glass	1.15424.0001
	5 x 20	50 plates	glass	1.15682.0001
	5 x 10	25 plates	glass	1.15684.0001
Silica gel 60 RP-18 F_{254s}*	20 x 20	25 plates	glass	1.15389.0001
	10 x 20	50 plates	glass	1.15423.0001
	5 x 20	50 plates	glass	1.15683.0001
	5 x 10	25 plates	glass	1.15685.0001
Silica gel 60 RP-18 F_{254s}	20 x 20	20 plates	aluminium	1.05559.0001
	5 x 7.5	20 plates	aluminium	1.05560.0001
HPTLC silica gel 60 RP-2 F_{254s}	10 x 10	25 plates	glass	1.13726.0001
HPTLC silica gel 60 RP-8 F_{254s}	10 x 10	25 plates	glass	1.13725.0001
HPTLC silica gel 60 RP-18	20 x 10	25 plates	glass	1.05914.0001
HPTLC silica gel 60 RP-18 W	20 x 10	25 plates	glass	1.14296.0001
HPTLC silica gel 60 RP-18 F_{254s}	10 x 10	25 plates	glass	1.13724.0001
HPTLC silica gel 60 RP-18 W F_{254s}	10 x 10	25 plates	glass	1.13124.0001

Layer thickness: 200 µm | * Layer thickness: 250 µm | W: fully wettable with water

CN, Diol, NH_2 modified silica plates (TLC and HPTLC)

Packing Material	Format [cm]	Content	Backing	Ord. No.
Silica gel 60 NH_2 F_{254s}	20 x 20	20 plates	aluminium	1.05533.0001
HPTLC silica gel 60 CN F_{254s}	10 x 10	25 plates	glass	1.16464.0001
HPTLC silica gel 60 Diol F_{254s}	10 x 10	25 plates	glass	1.12668.0001
HPTLC silica gel 60 Diol F_{254s}	20 x 10	25 plates	glass	1.05636.0001
HPTLC silica gel 60 NH_2	20 x 10	25 plates	glass	1.12572.0001
HPTLC silica gel 60 NH_2 F_{254s}	20 x 10	25 plates	glass	1.13192.0001
HPTLC silica gel 60 NH_2 F_{254s}	10 x 10	25 plates	glass	1.15647.0001

Layer thickness: 200 µm

- Sorbents types : modified **Silica 60**

TLC Range at a Glance

- Sorbents types : modified **Silica 60**

HPTLC concentrating zone plates

Packing Material	Format [cm]	Content	Backing	Ord. No.
HPTLC silica gel 60 concentrating zone 2.5 x 20 cm	20 x 10	50 plates	glass	1.13749.0001
HPTLC silica gel 60 concentrating zone 2.5 x 10 cm	10 x 10	25 plates	glass	1.13748.0001
HPTLC silica gel 60 F_{254} concentrating zone 2.5 x 20 cm	20 x 10	50 plates	glass	1.13728.0001
HPTLC silica gel 60 F_{254} concentrating zone 2.5 x 10 cm	10 x 10	25 plates	glass	1.13727.0001
HPTLC silica gel 60 F_{254} concentrating zone 2.5 x 5 cm	5 x 10	25 plates	glass	1.13187.0001
HPTLC silica gel 60 RP-18 PAH concentrating zone 2.5 x 20 cm	20 x 10	25 plates	glass	1.15037.0001
HPTLC silica gel 60 RP-18 F_{254s} concentrating zone 2.5 x 20 cm	20 x 10	25 plates	glass	1.15498.0001

Layer thickness: 200 µm

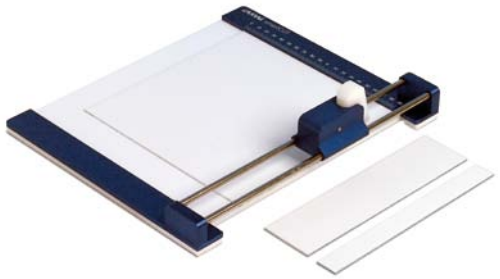
TLC Range at a Glance

- for the chemist : reaction follow-up with the right size

Multiformat plates

Packing Material	Scored [cm]	Content of one package [20 x 20 cm]	No. of plates possible	Ord. No.
Multiformat silica gel 60 F_{254} 20 x 20	5 x 10	25 plates	200	1.05620.0001
Multiformat silica gel 60 F_{254} 20 x 20	5 x 20	20 plates	80	1.05608.0001
HPTLC Multiformat silica gel 60 F_{254} 10 x 10	5 x 5	25 plates	100	1.05635.0001
HPTLC Multiformat silica gel 60 10 x 10	5 x 5	100 plates	400	1.05644.0001

- ... or tailor made plates sizes in an economic way with a SmartCut tool



TLC Range at a Glance

- Simple purification without starting a column : **the no-loss solution**

PLC silica gel 60

Packing Material	Format [cm]	Layer thickness	Content	Backing	Ord. No.
PLC silica gel 60	20 x 20	0.5 mm	20 plates	glass	1.13894.0001
	20 x 20	2 mm	12 plates	glass	1.05745.0001
PLC silica gel 60 F_{254}	20 x 20	0.5 mm	20 plates	glass	1.05744.0001
	20 x 20	1 mm	15 plates	glass	1.13895.0001
	20 x 20	2 mm	12 plates	glass	1.05717.0001
PLC silica gel 60 $F_{254+366}$	20 x 20	2 mm	20 plates	glass	1.05637.0001
PLC silica RP-18 F_{254s}	20 x 20	1 mm	15 plates	glass	1.05434.0001

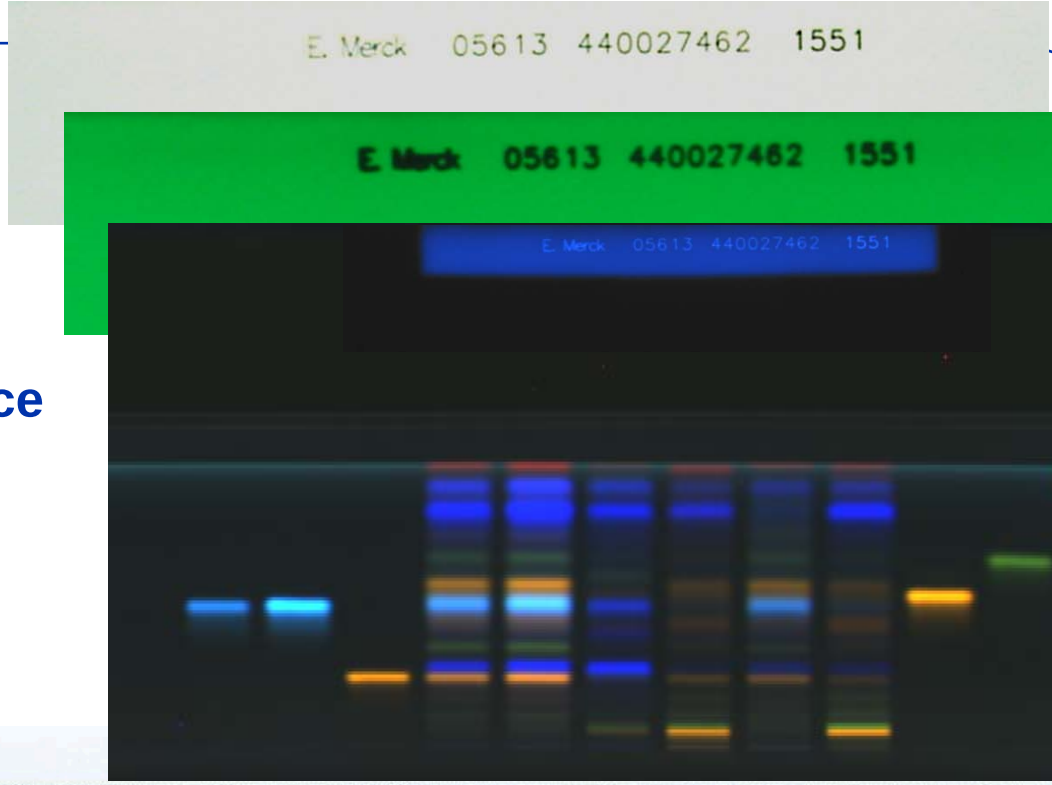
PLC aluminium oxide 60 and 150

Packing Material	Format [cm]	Layer thickness	Content	Backing	Ord. No.
PLC aluminium oxides 60 F_{254}	20 x 20	1.5 mm	12 plates	glass	1.05788.0001
PLC aluminium oxides 150 F_{254}	20 x 20	1.5 mm	12 plates	glass	1.05726.0001

PLC concentrating zone plates

Packing Material	Format [cm]	Layer thickness	Content	Backing	Ord. No.
Silica gel 60 F_{254} concentrating zone 4 x 20 cm	20 x 20	0.5 mm	20 plates	glass	1.13794.0001
	20 x 20	1 mm	15 plates	glass	1.13792.0001
	20 x 20	2 mm	12 plates	glass	1.13793.0001

TLC Range at a Glance



- for the **Quality Assurance** a reliable follow-up on documentation with individual numbers on plates

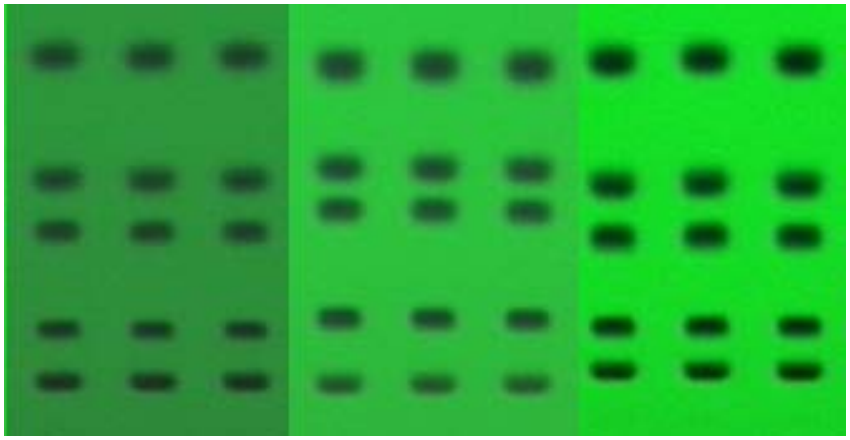
GLP plates

Packing Material	Format [cm]	Content	Backing	Ord. No.
TLC GLP silica gel 60 F_{254}	20 x 20	25 plates	glass	1.05566.0001
	10 x 20	25 plates	glass	1.05702.0001
HPTLC GLP silica gel 60	10 x 20	25 plates	glass	1.13326.0001
HPTLC GLP silica gel 60 F_{254}	10 x 20	25 plates	glass	1.05613.0001
	10 x 10	25 plates	glass	1.05564.0001

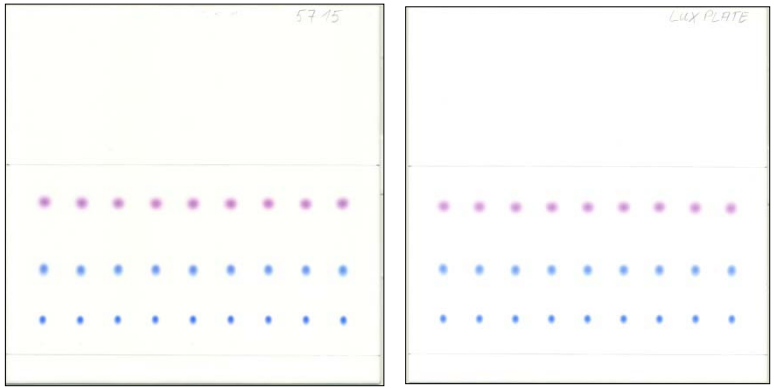
Special Product - LuxPlate®

- Higher content of fluorescent indicator for better contrast against background
- Highly robust, due to higher content of binder
- Comparable retention behaviour

Cassical silica 60 F_{254} | Competitor | LuxPlate®

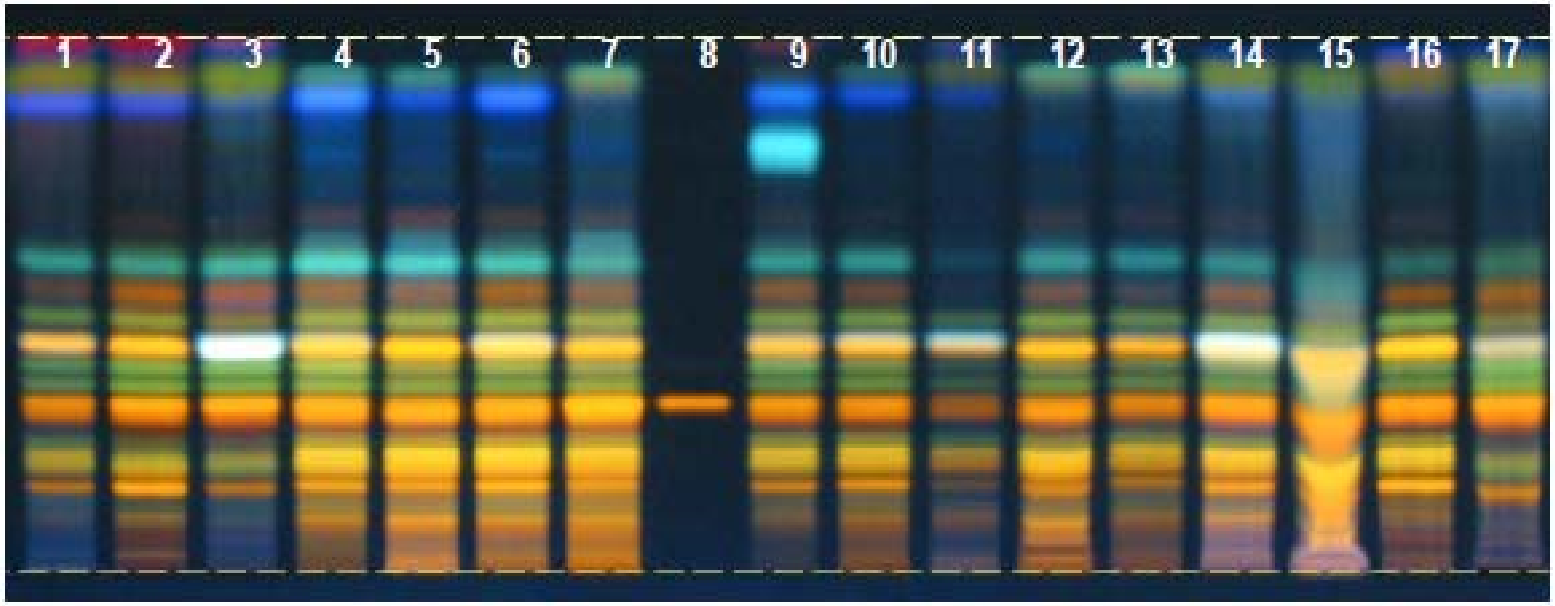


LuxPlate® | Classical silica 60 F_{254}



HPTLC Applications – Herbals

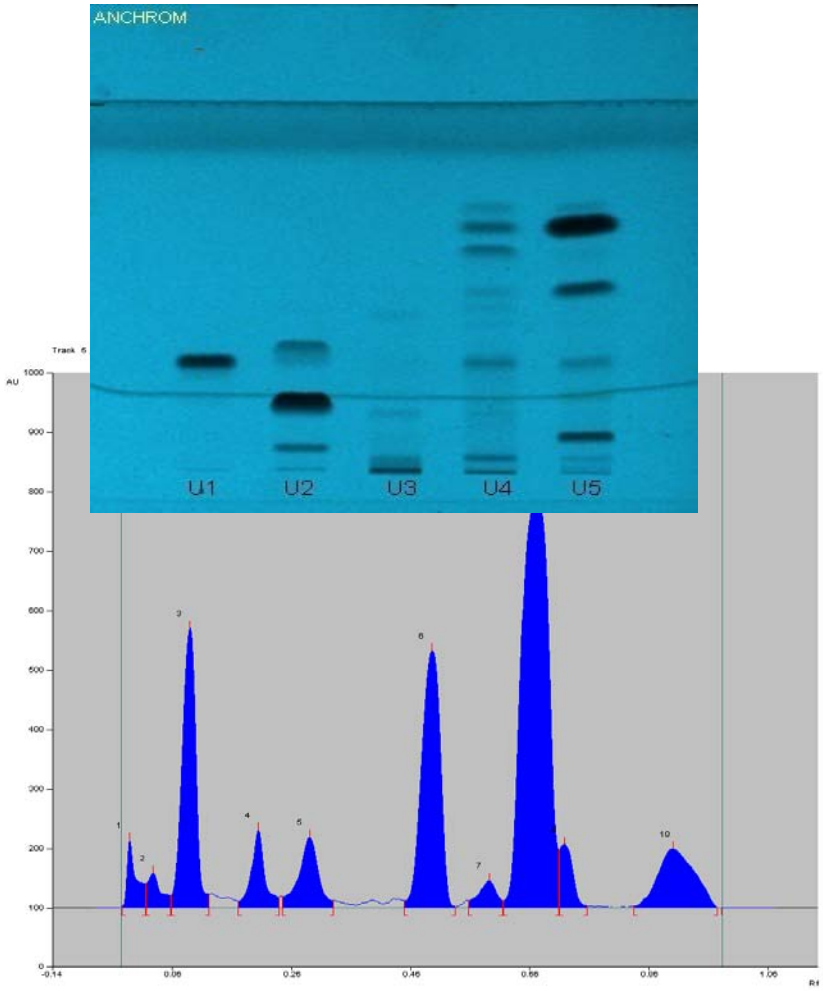
Example: Identification of Gingko



c) UV 366nm, after derivatization with natural products reagent/PEG

1, 2: Ginkgo leaf, 3: Ginkgo leaf capsule (freeze dried; 1.2-1.8% flavonoids; US), 4: Ginkgo leaf extract powder (Italy), 5: Ginkgo leaf extract powder (China), 6: Ginkgo leaf extract powder (France), 7: Ginkgo leaf extract powder (China), 8: Rutin, 9: Ginkgo leaf extract capsule (60 mg) w/gotu kola (US), 10: Ginkgo leaf extract capsule (60 mg; US), 11: Ginkgo leaf extract tablet (yielding 9 mg flavone glycosides; Switzerland), 12: Ginkgo leaf extract tablet (120 mg; US), 13: Ginkgo leaf extract tablet (120 mg; US), 14: Ginkgo tincture (1:5 dry leaf; US), 15: Ginkgo tincture (1:1 fresh leaf; US), 16: Ginkgo tincture (1:10 fresh leaf, Switzerland: current batch), 17: Ginkgo tincture (1:10 fresh leaf, Switzerland: 2 years past expiration date)

Identification of narcotics



Chromatogram at 254nm

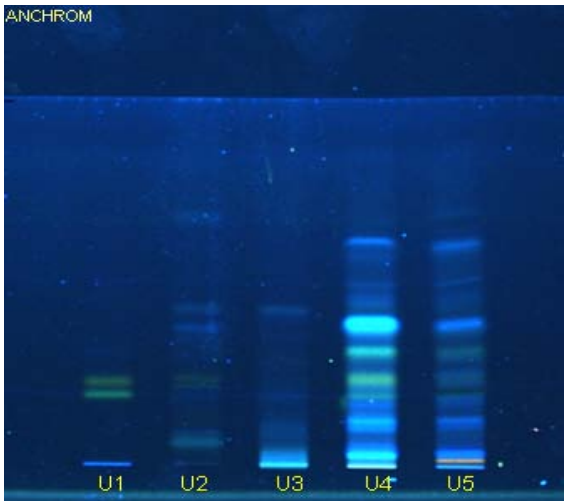


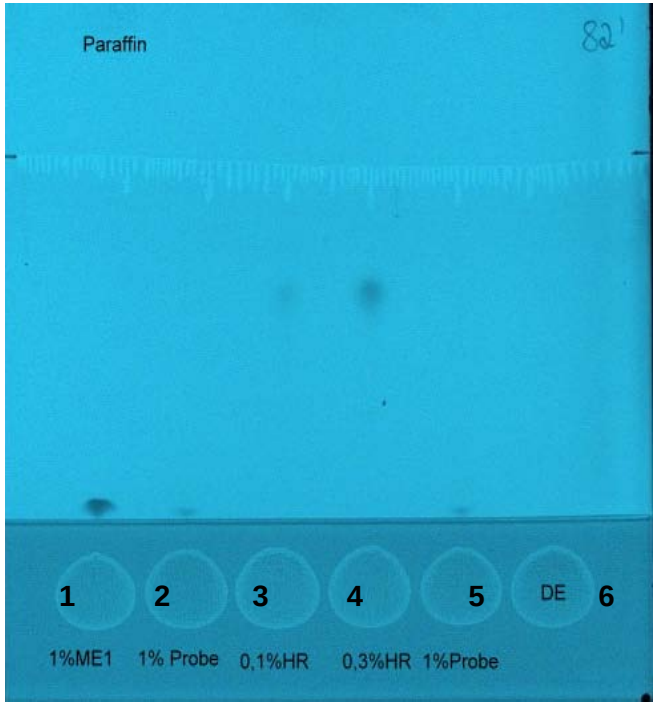
Image (366nm)

U1: Cocaine
U2: Phenargan
U3: Ganja
U4: Opium
U5: Heroin

Application - Cosmetics
Stability testing of cosmetic ingredients

HPTLC for analysing in difficult matrices such as oils or fat

Is the ingredient X stable as paraffin formulation?



Sample: Ingredient (ester, di-ester)
Formulation: Paraffin
Solvent: Dichlormethan
Application: Linomat V (CAMAG)
Plate: HPTLC Silica gel 60 RP18 F254s Conz.
Mobile phase: Ethanol/Wasser 80:20
Drying time: 60 min
Migration distance: 5,0 cm
Migration time: 82 min
Samples: 2 µl (in Dichlormethan)

1 Pure ingredient (ME1) in Paraffin oil 1% (positive controle)
2 Sample in paraffin foil 01:01
3 HR in paraffin oil 0,10% (expected degradation product)
4 HR in paraffin oil 0,30% (expected degradation product)
5 Sample in paraffin oil 01:01
6 Pure ingredient (DE) in paraffin oil 1,00% (positive control but not visible under UV)

Data kindly provided by Merck Pigments & Cosmetics

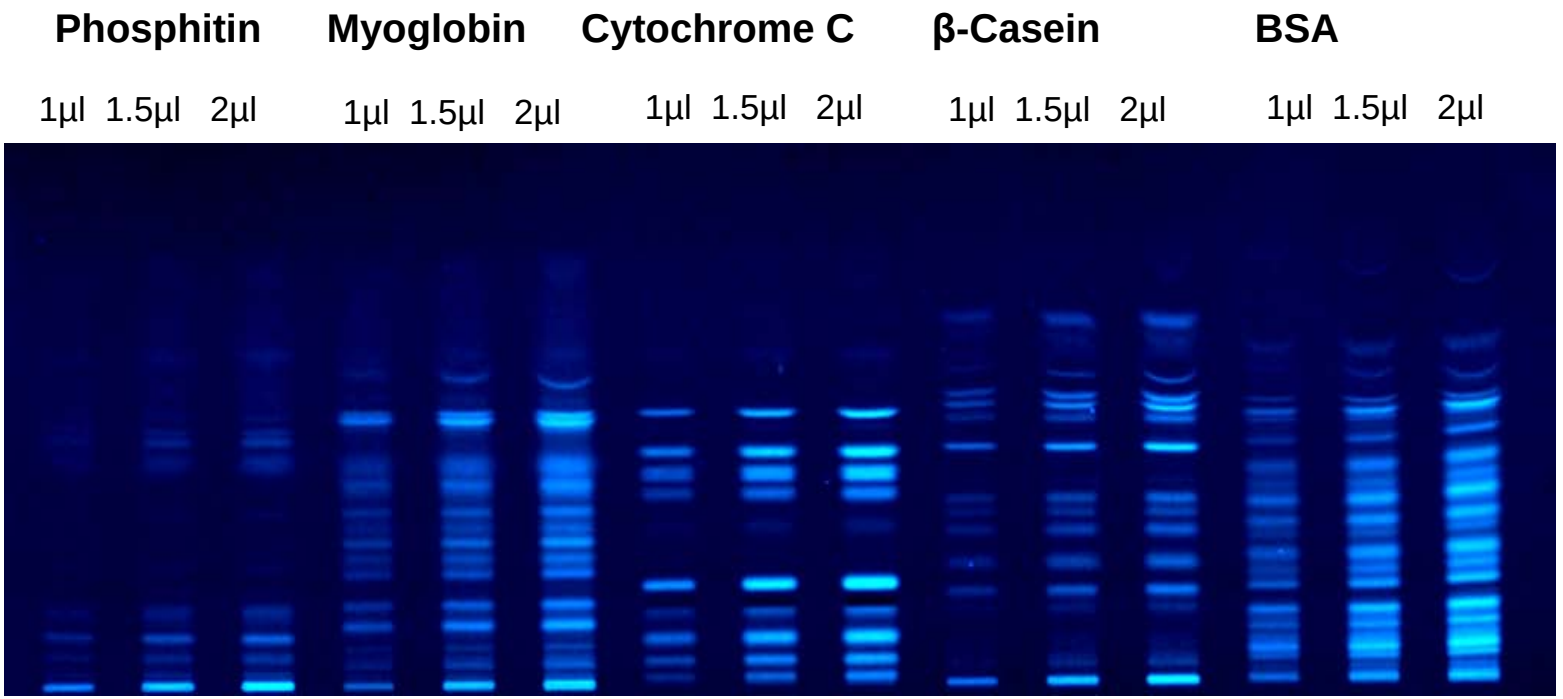
HPTLC Plates for Peptide Analysis

ProteoChrom®	Sorbent	Size	Layer	Backing	Special
1.05650 HPTLC Silica gel F_{254s}	High Performance Silica gel	20 x 10	100 µm	glass	Special binder
1.05651 HPTLC Cellulose	High performance Cellulose	10 x 10	100 µm	aluminium	High density layer

Packing Material	Format [cm]	Content	Backing	Ord. No.
ProteoChrom® HPTLC silica gel 60 F_{254}	20 x 10	25 plates	glass	1.05650.0001
ProteoChrom® HPTLC Cellulose	10 x 10	25 sheets	aluminium	1.05651.0001

Each ProteoChrom® package includes an insert sheet with detailed instructions for solvent systems, running conditions and staining solution, enabling straightforward experiments without time-consuming optimization work.

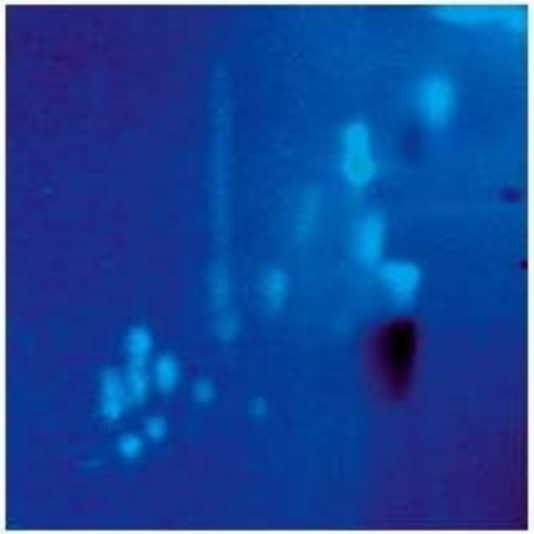
ProteoChrom® Features



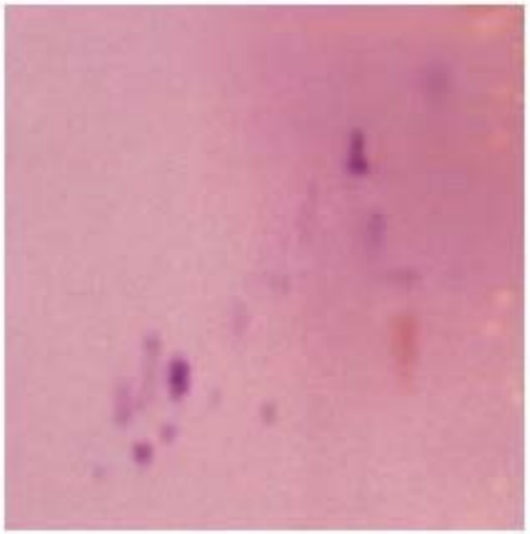
- Extra thin, extra smooth
- Robust, highly stable in water
- Include easy to follow, optimized protocols

ProteoChrom® HPTLC Cellulose 2 D separation of peptides

Fluorescamin



Ninhydrin



Sample volume:5 µl

Concentration: 2 mg/ml

Application: Linomat V (CAMAG)

Migration distance: 5 cm

Migration time: 1st D: 45 min

2nd D: 50 min

- Fast, just 4 h from protein digest to result
- Validated for peptide separation

TLC Range at a Glance

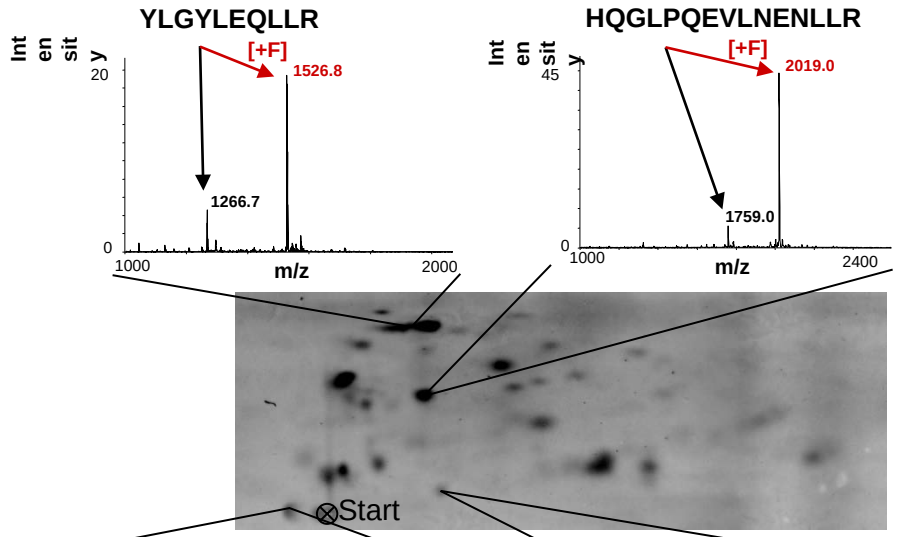
- **CLASSICAL DETECTION BY STAINING**

Ready-to-use spray solutions

Product	Solvent	Package	Contents of one package	Ord. No.
Dragendorff-Reagent	Acetic acid / ethyl acetate / water	Glass	100 ml	1.02035.0100
Molybdatophosphoric acid	2-propanol	Glass	100 ml	1.00480.0100
Ninhydrin	2-propanol	Glass	100 ml	1.06705.0100

Mass Spectrometry directly from the Plate

- **MODERN DETECTION BY COUPLING**



FDA Applications with modern TLC

Impurity and stability applications for synthetic drugs

Fingerprinting of plant extracts

Mycotoxins in foods

Natural and synthetic food colors

Vitamins

Summary

Single use of stationary phase (TLC and HPTLC) minimizes sample preparation

Parallel separations enhances sample throughput

Ease of postchromatographic derivatization

Can perform several screenings simultaneously for different analytes

Direct use of biological detection possible

Fast and low cost screening TLC- procedure used to identify samples that should be investigated further

We use same raw material for TLC, HPLC and Prep HPLC, which makes easy to transfer method from TLC to HPLC

Last but not least : **a nice tool for a better understanding of samples, and of the chromatographic process ! = a real comprehensive chromatography**

... Thanks to the patient chromatographic teachers, and of course THANK YOU FOR YOUR ATTENTION, too !...