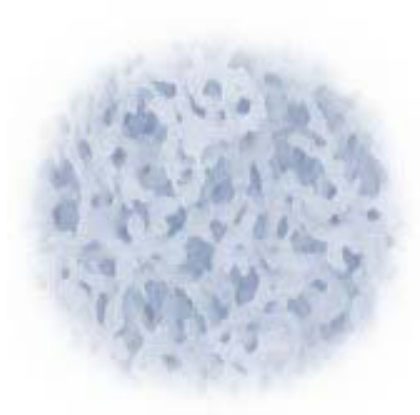
Traditionally Innovative – Committed to Thin-Layer Chromatography

HPTLC, Berlin October 6 – 11, Dr. Dagmar Leiss, Merck KGaA

Experience drives innovation

During 100 years Merck ...

- was the 1st manufacturer of chromatography products - just one year after the discovery of the chromatographic principle
- provided impulses & innovations for all areas of chromatography

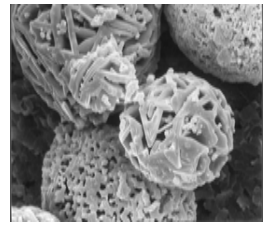


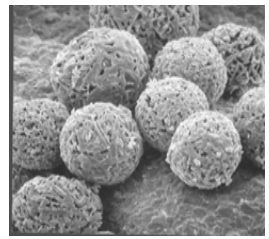
Today Merck ...

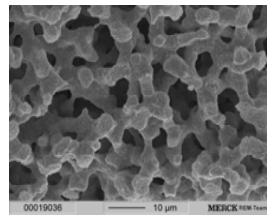
- is the largest supplier of chromatographic silica gels worldwide
- is the world leader in Thin-layer chromatography
- is the technology leader in HPLC with *Chromolith*® columns

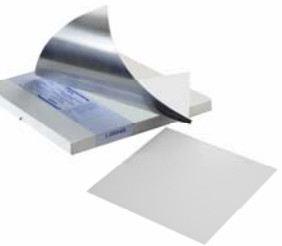
Merck means constant quality

From sorbent to plate - every single product comes from own production and quality controle

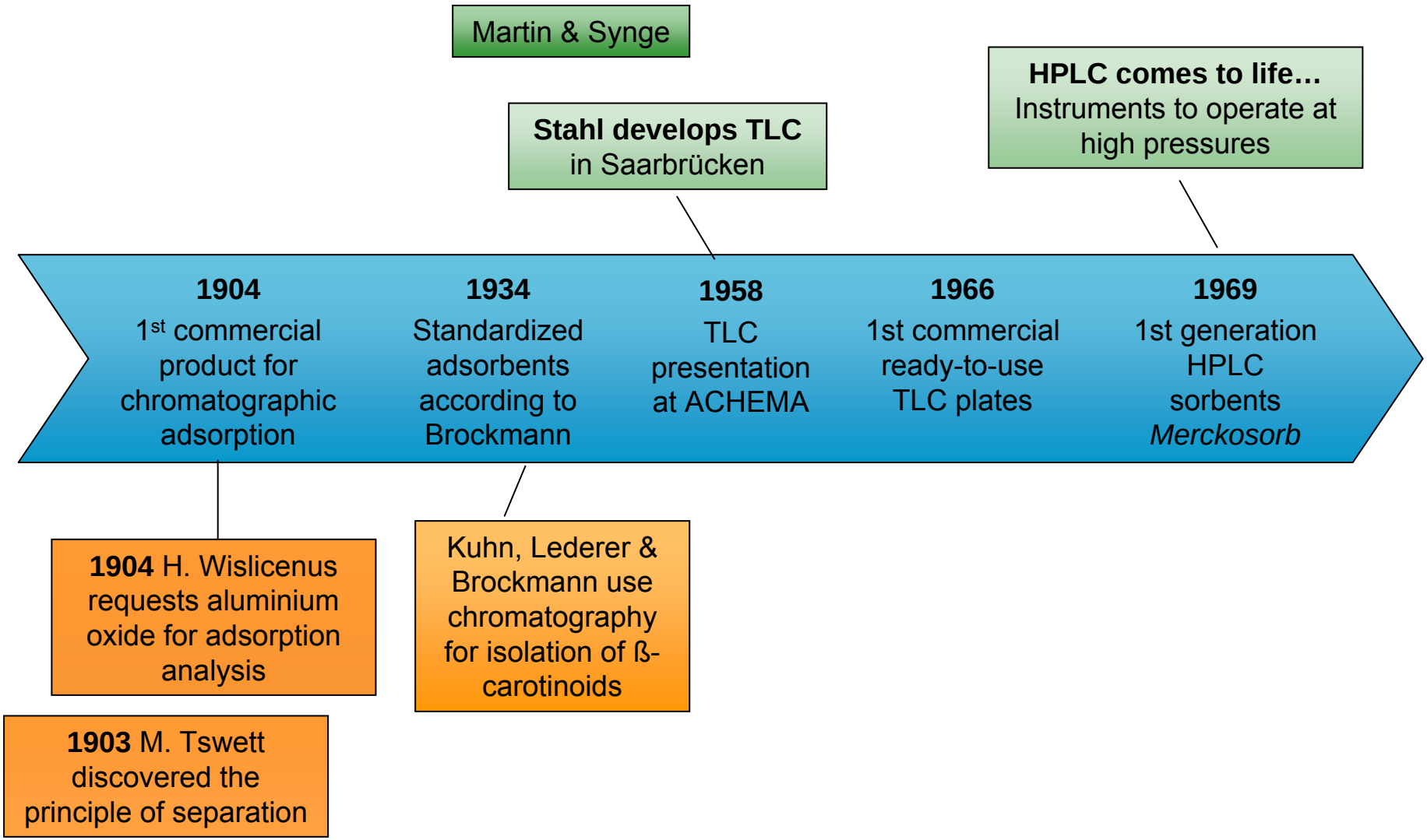








Milestones of chromatography



Milestones of chromatography

Increasing No. of basic pharmaceutical compounds: Need for enhanced materials

Combinatorial chemistry in drug discovery High throughput screening of thousands to millions compounds

The age of modern Life Sciences: From genomics to proteomics

1973 2nd generation spherical HPLC materials *LiChrospher*

1987 3rd generation HPLC materials *LiChrosorb RP-Select B*

1988 Tentacle media *Fractogel*

1993 new sorbents line *Purospher*

2000 monolithic silica columns *Chromolith*

2002 monolithic TLC plate *UTLC*

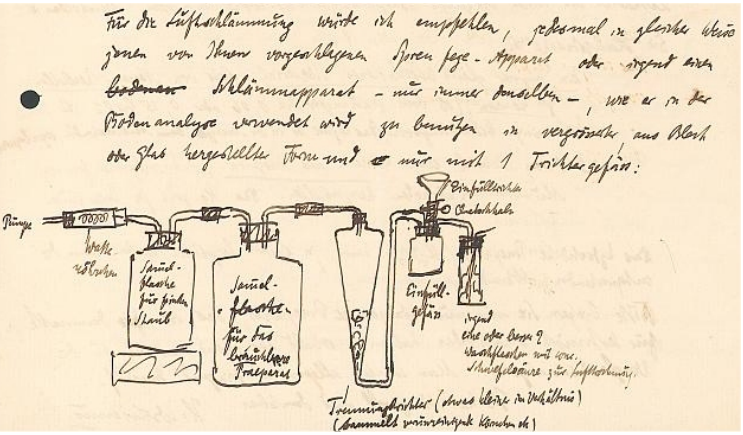
Emerging Biotech Industry. Need for higher-capacity ion exchangers for downstream processing of recombinant proteins

Need for higher productivity at lower cost in the pharmaceutical industry

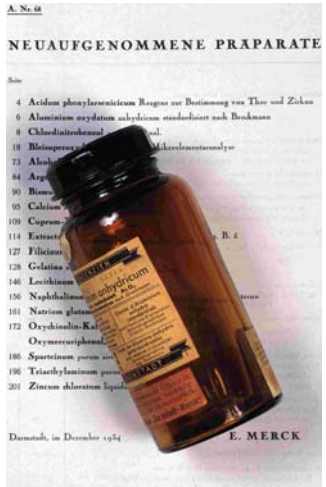
Upcoming Bio-processing: Efficient and economic recovery of complex bio-molecules for healthcare

TLC production in the past

1904



Scheme of a vacuum apparatus for the pro-duction of aluminium oxide (acc. to Wislicenus, 1904)



1967

Prodction of pre-coated TLC plates, Darmstadt

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)

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Drying in drying tunnel

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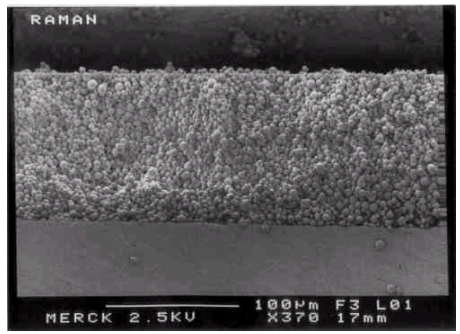
Sampling, in-process control

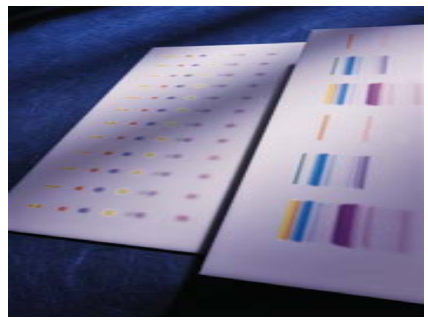
↓

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

↓

Packaging, final control

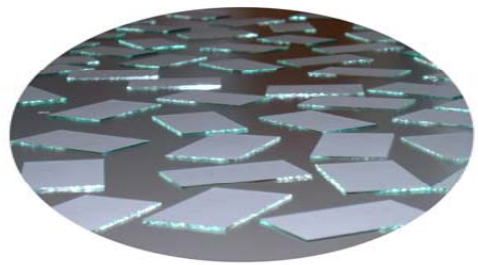




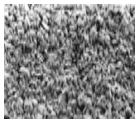
TLC production today

- 26 employees in production plant
- >6 million plates (glass, aluminium, plastic) per year
- Every single plate is visually inspected to ensure optimal quality
- More than 70 different products for thin-layer chromatography

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

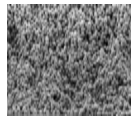
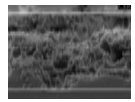


A comprehensive range of plates




- Types

  TLC, HPTLC, HPTLC LiChrospher®, UTLC, PLC, special plates

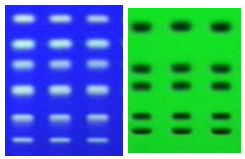



- Sorbens

  Silica 60 (40); Modifiied silica: RP2, RP8, RP18, NH_2, Diol, CN; Aluminium oxide, Cellulose, Kieselguhr;

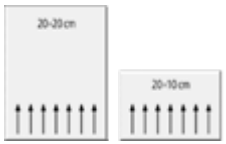
- Backings

  Glass, aluminium, plastic



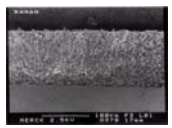
- Fluorescence indicators

  - with or without: F_{254}:green, F_{254s}: blue, F_{366}:blue



- Plate sizes (in cm)

  20 x 20, 10 x 20, 5 x 10 ….. 5 x 7,5, 2,5 x 7,5



- Plate thickness

  250 µm, 200 µm, 100 µm, 10 µm

Unique
HPTLC LiChrospher® with spherical particles

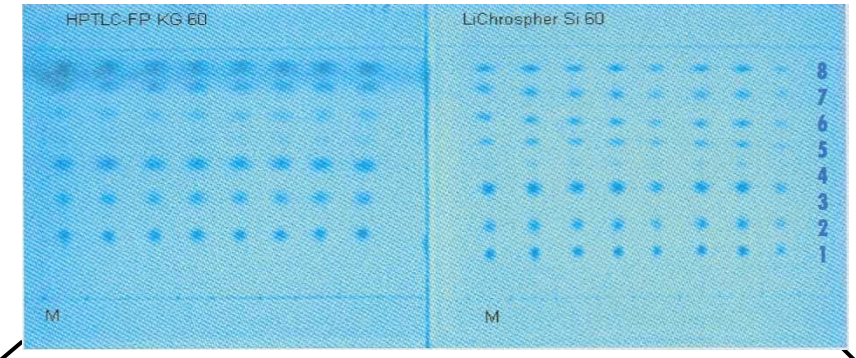
- Up to 20% reduced analysis time
- Lower detection limits (up to factor 2,5)
- Highly compact bands

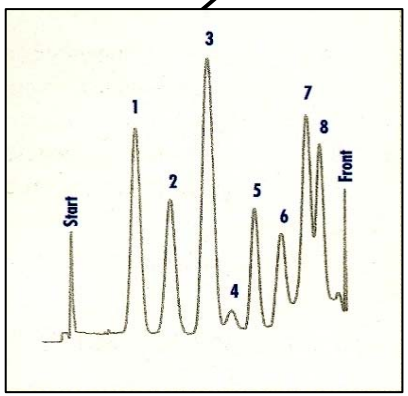
Substance	Visually		Spectrophotometrically	
	Silica gel 60 F_{254}	LiChrospher® Si 60 F_{254s}	Silica gel 60 F_{254}	LiChrospher® Si 60 F_{254s}
Ascorbic acid	100	100	100	25
Cortisone	50	25	25	10
Atrazine	50	25	10	5
Prometryne	25	10	10	5
Theophylline	50	25	25	10
o-Aminophenol	50	25	25	5
m-Aminophenol	10	5	10	5
p-Aminophenol	> 100	50	50	25

Comparison of detection limits on HPTLC Lichrospher® and classical HPTLC plates (UV 254 ng/spot)

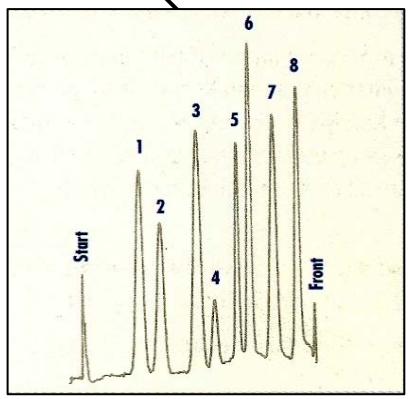
HPTLC LiChrospher®
Produces highly compact bands

Comparison of a mixture of pharmaceutical substances





1 Hexazinone
2 Metoxuron
3 Monuron
4 Aldicarb
5 Azinphosmehtyl
6 Prometryn
7 Pyridat
8Trifluralin



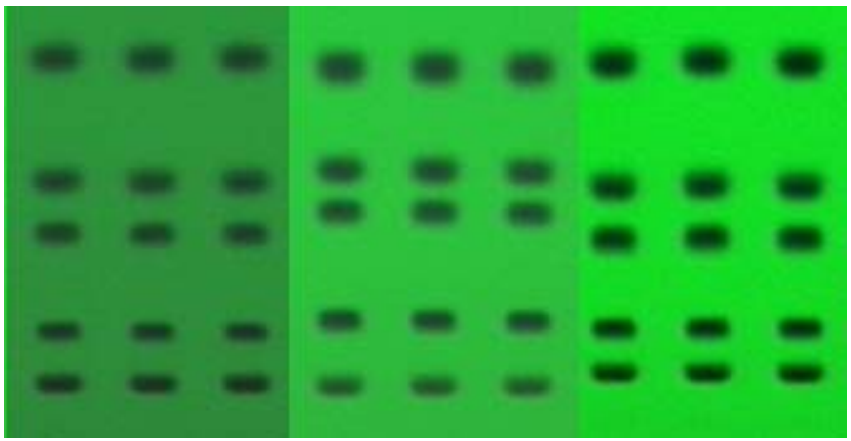
HPTLC silica gel 60

HPTLC LiChrospher® silica gel 60

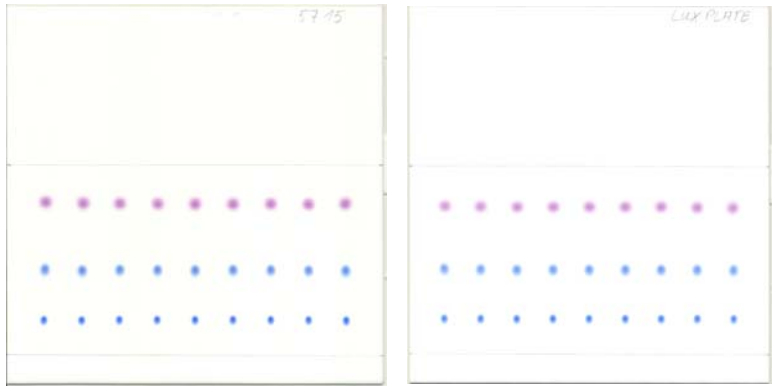
Special LuxPlate®

- 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®

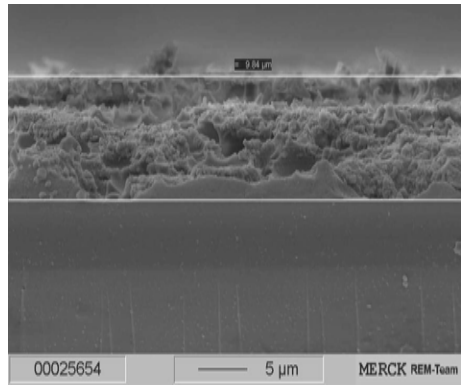


LuxPlate® | Classical silica 60 F_{254}



Unique
Ultra thin monolithic silica plate

- No binder
- Short migration distance
- Short migration time
- High sensitivity

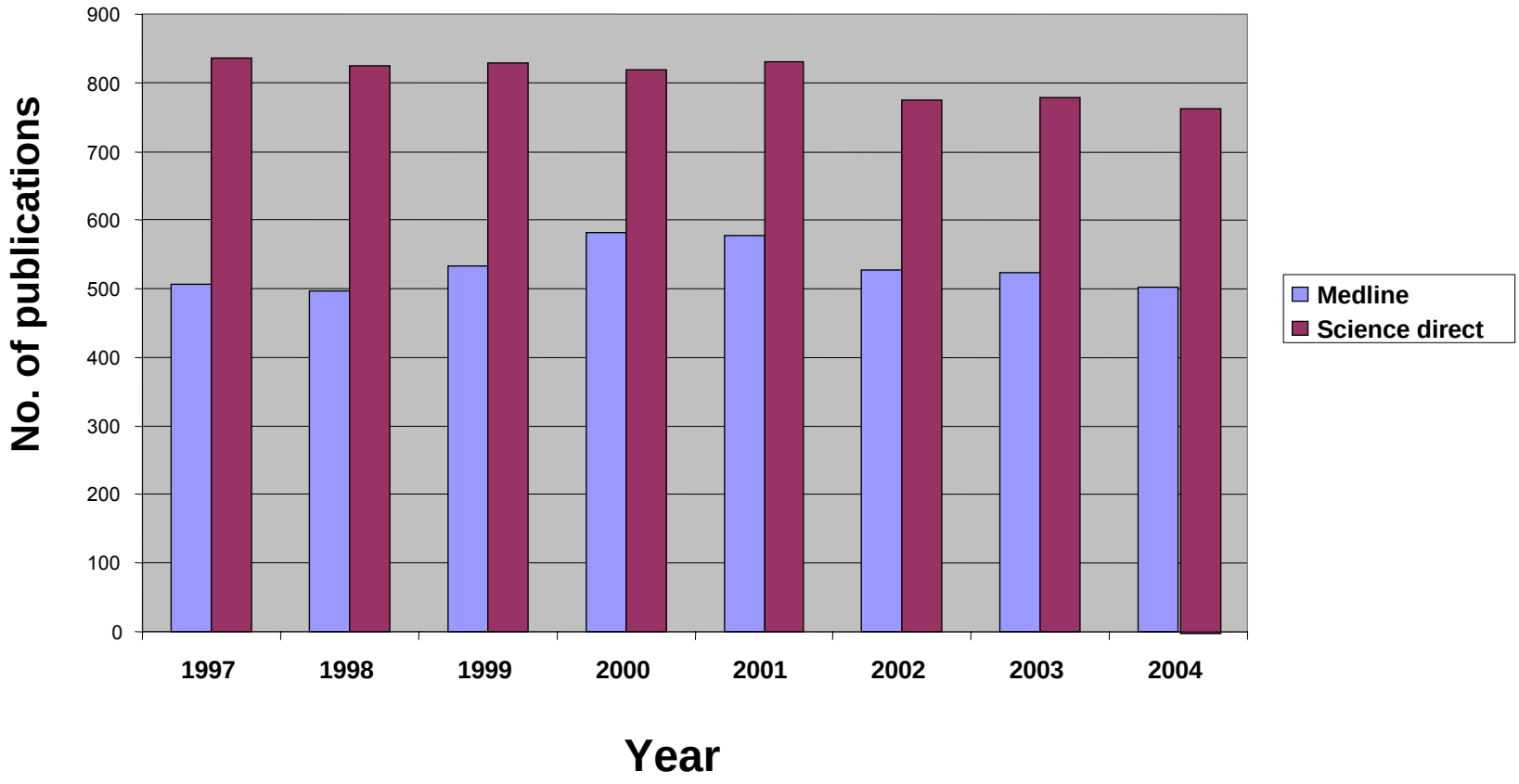


Scanning REM picture

Plate format:	60 x 36 mm
Layer thickness:	10 µm
Stationary phase	Silica SiO_2 monolithic
Additions:	No binder
Sample volume:	Spot wise: 5–20 nl
	Band wise: up to 100 nl
Detection limit:	10 pg
Migration distance:	1–3 cm
Analysis time:	1–6 min

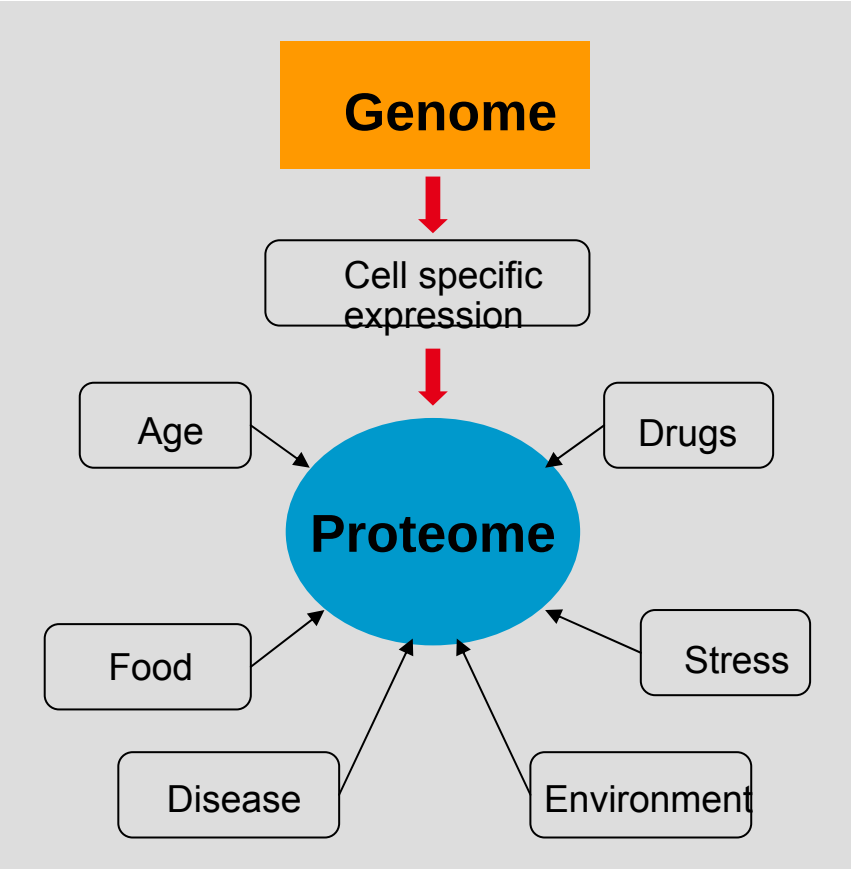
Is Thin-layer chromatography invisible?

Average number of new publications dealing with Thin-layer chromatography



New challenges & opportunities

Peptides and Proteins are future Biomarkers and/or Drug Targets and Drugs



Proteins are the primary intervention points for therapeutics

and thus tie in more directly to clinical decision points

Challenges in Proteomics
Call for new strategies

- **Sample Complexity**
  - Dynamic range & sensitivity
  - Post translational modifications (PTMs)
  - Membrane proteins
- **Quantification**
  - Differential analysis
  - Relative quantitation
- **Productivity**
  - Automation
  - Throughput
  - Multiplexing

Core technologies for proteome analysis

- **2DGE / MS**
  - Widely used, high resolution
  - Laborious, fragile, poor reproducibility
  - Not amendable for automatisation
  - Not suitable for peptides

- **2D LC / MS / MS**
  - Easy to automatise
  - Requires complex system set
  - Limited use for posttranslational modifications (PTMs)

Could HPTLC complement 1 or 2DGE, HPLC…?

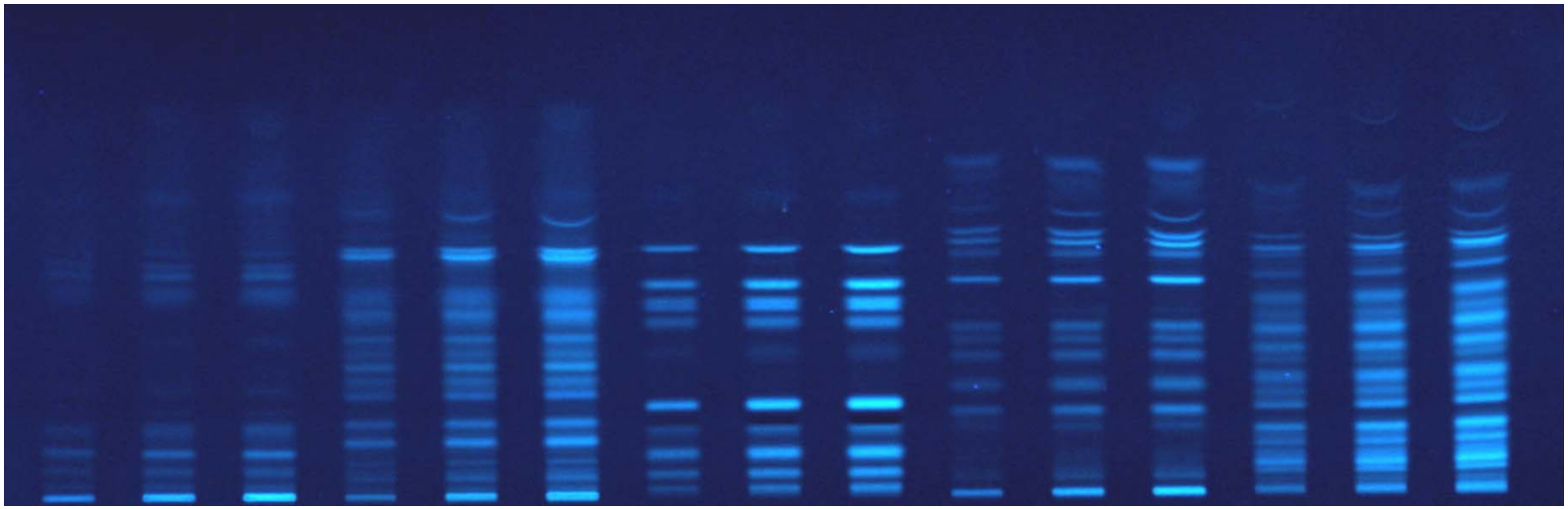
Why to analyze peptides instead of proteins?

- Analysis of PTMs e.g. phosphoprotein function requires identification of the different phosphorylation sites within a protein.

These sites can only be detected by dedicated **phosphopeptide** analysis, **phosphoprotein** analysis does not deliver this information.

1 D peptide separation on HPTLC Silica

Phosphitin Myoglobin Cytochrome C β-Casein BSA

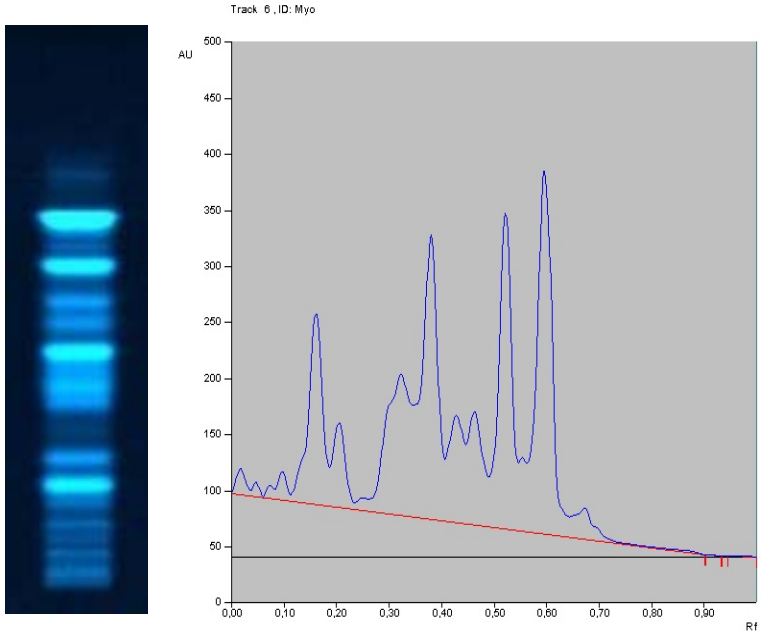


Sample volume: 1-, 1,5-, 2 µl
Mobile phase: 2-butanol/ammonia/pyridine/water
(39/10/34/26)

- Extra thin, extra smooth
- Highly stable in water
- Special optimized protocol

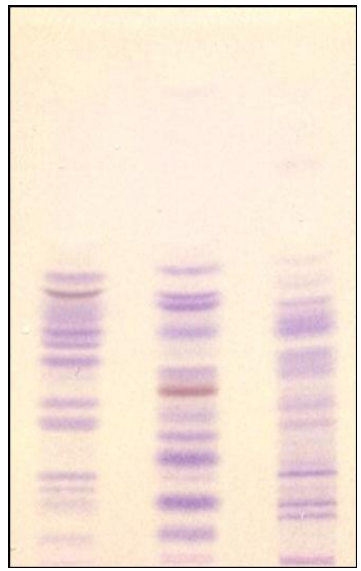
1 D peptide separation on ProteoChrom® HPTLC Silica

Fluram® staining



Sample:	Tryptic digest of myoglobin
Concentration:	4 µg / µl
Sample volume:	700 nl
Documentation:	UV 366 nm

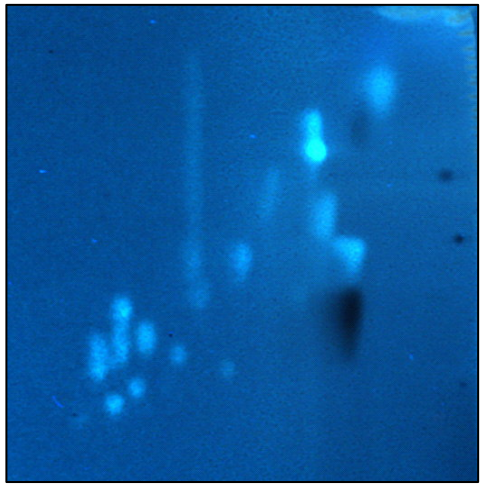
Ninhydrin staining



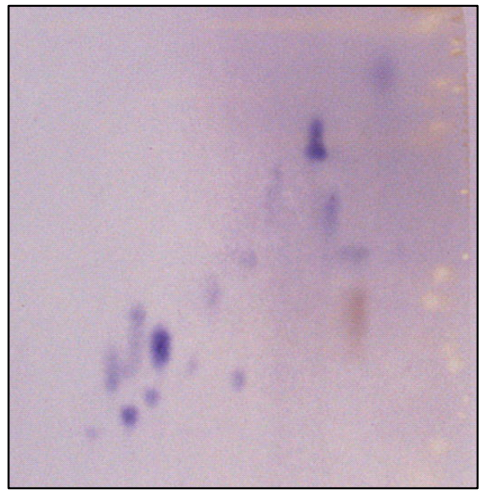
Sample:	Tryptic digest of 1 Myoglobin 2 Cytochrome C 3 BSA
Concentration:	2 ug / µl
Sample volume:	3,5 µl

ProteoChrom® HPLC cellulose for 2 D separation of peptides

Fluorescamin stain



Ninhydrin stain



Sample volume:	5 µl
Concentration:	2 mg/ml
Application system:	Linomat V (CAMAG)
Migration distance:	5 cm
Migration time:	1st D: 45 min 2nd D: 50 min
Mobile phase:	1st dimension: 2-butanol/acetic acid/pyridine/water (30/6/20/24) 2nd dimension 2-butanol/ammonia/pyridine/water (39/10/34/26)

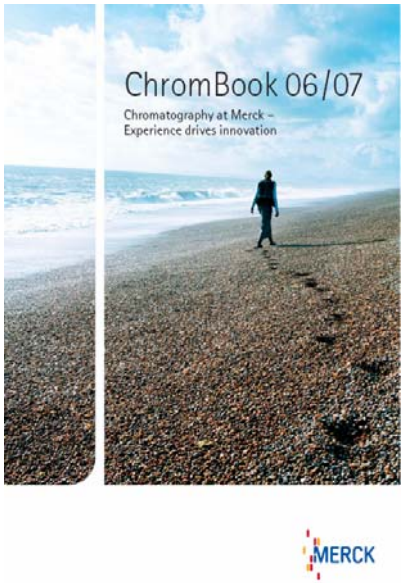
A glimps into the future

100 years old –
Thin-layer chromatography has the potential for many new and/or advanced applications

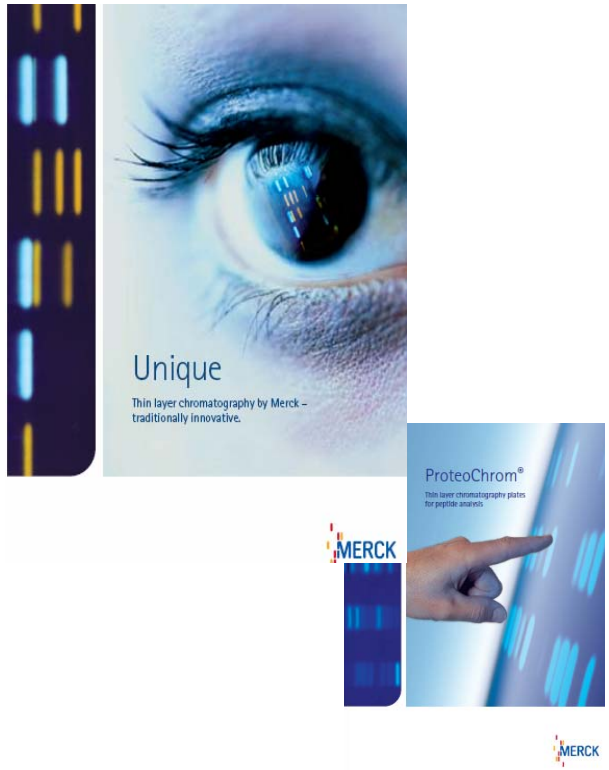
Merck is committed to advancing
Thin-layer chromatography!

More Information on Merck Chromatography?

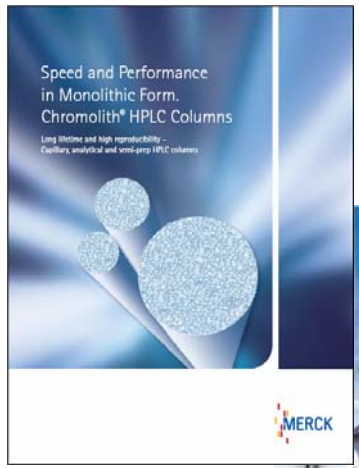
Chrombook 06/07



TLC



Chromolith® columns



Purospher® columns

Thank you !