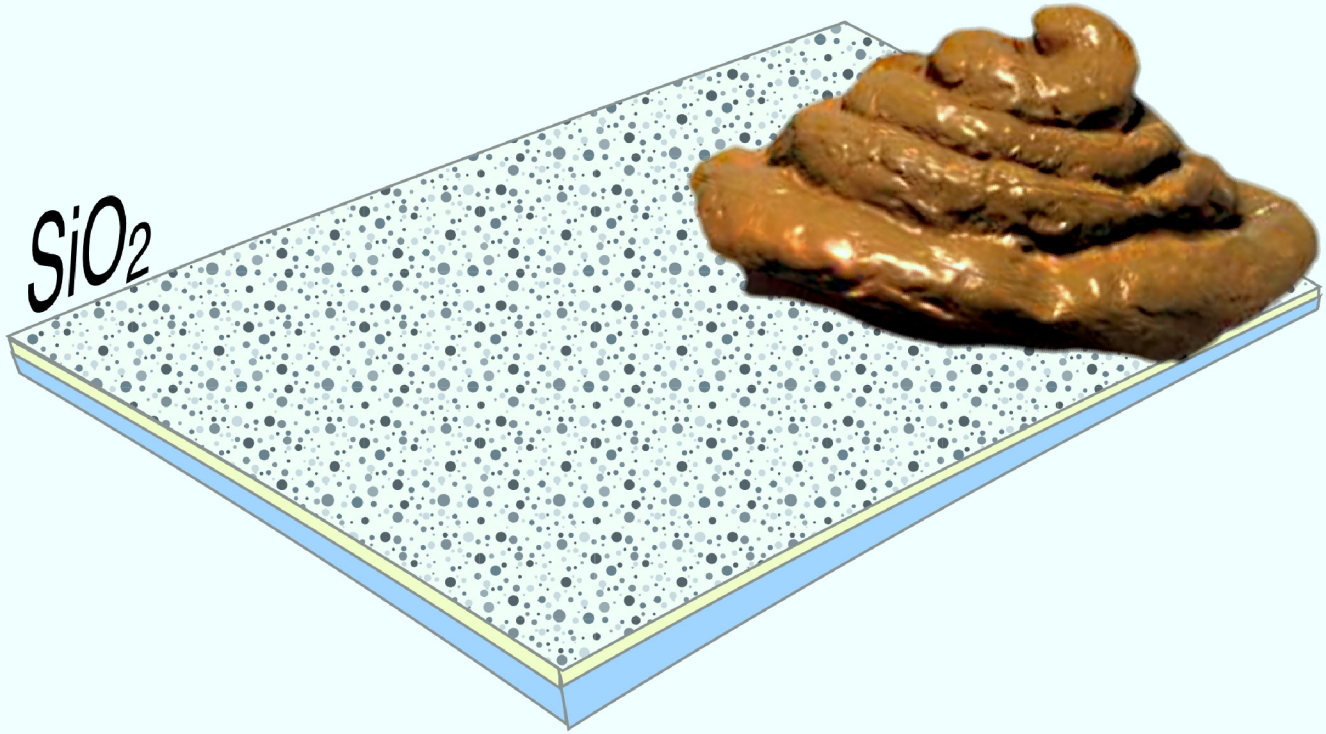
Klaus Burger, Dog's muck, Lyon 2003

A dog's muck ...

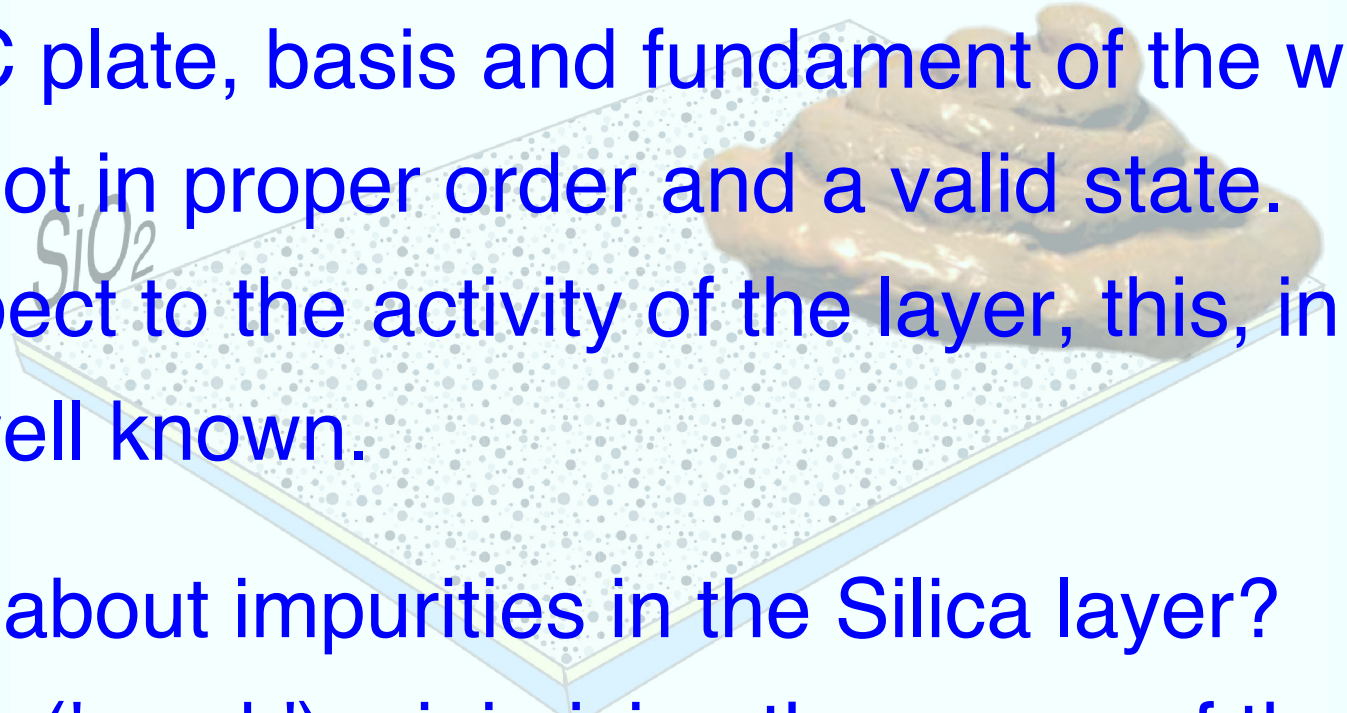


and how to prevent it!

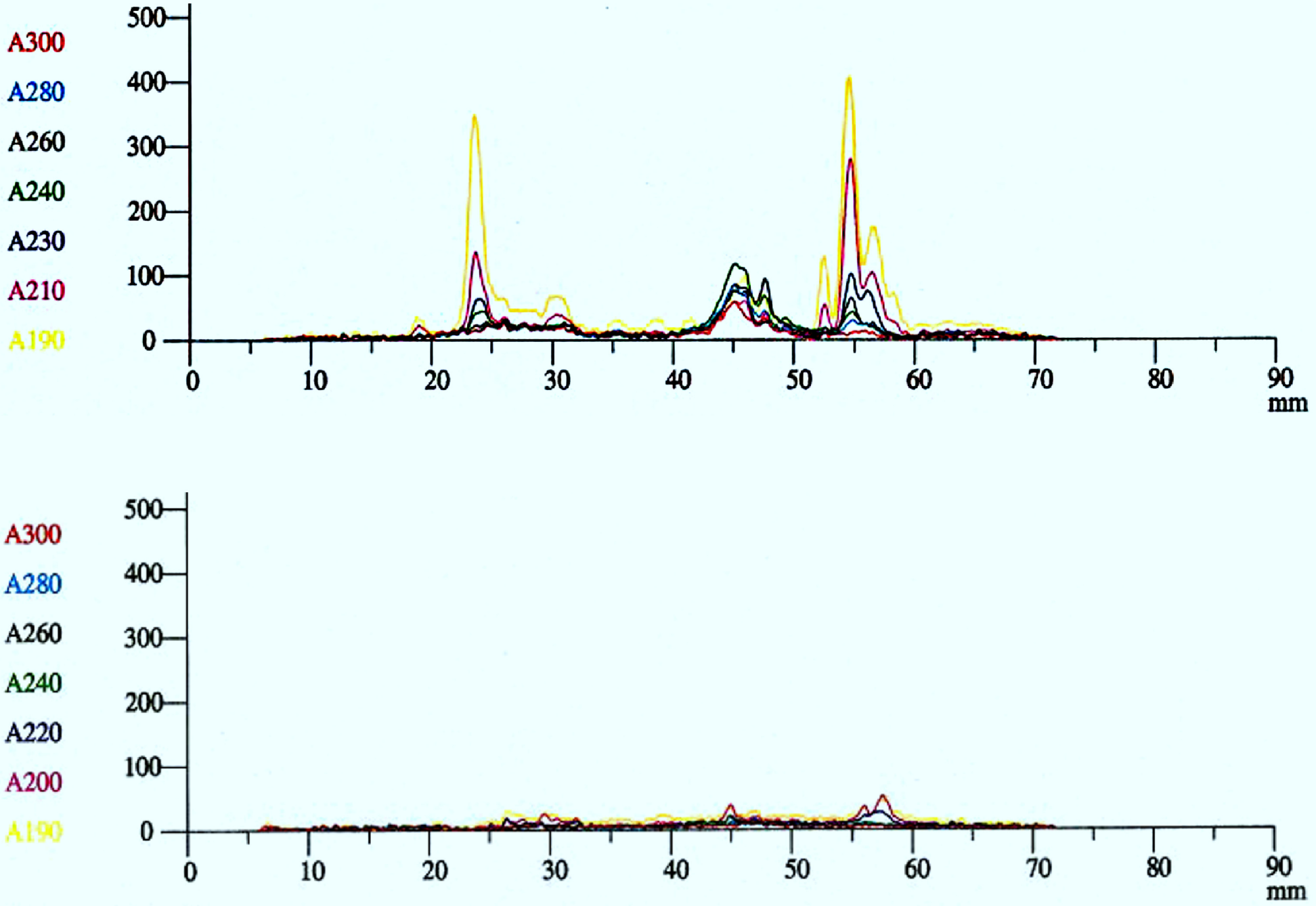
Not an appetizer

Klaus Burger

The best chromatographic equipment with autosampler, separation system, sophisticated biological detection, scanner and computer aided evaluation etc. is of no use, if the TLC plate, basis and fundament of the whole process, is not in proper order and a valid state.
With respect to the activity of the layer, this, in the meantime, is well known.

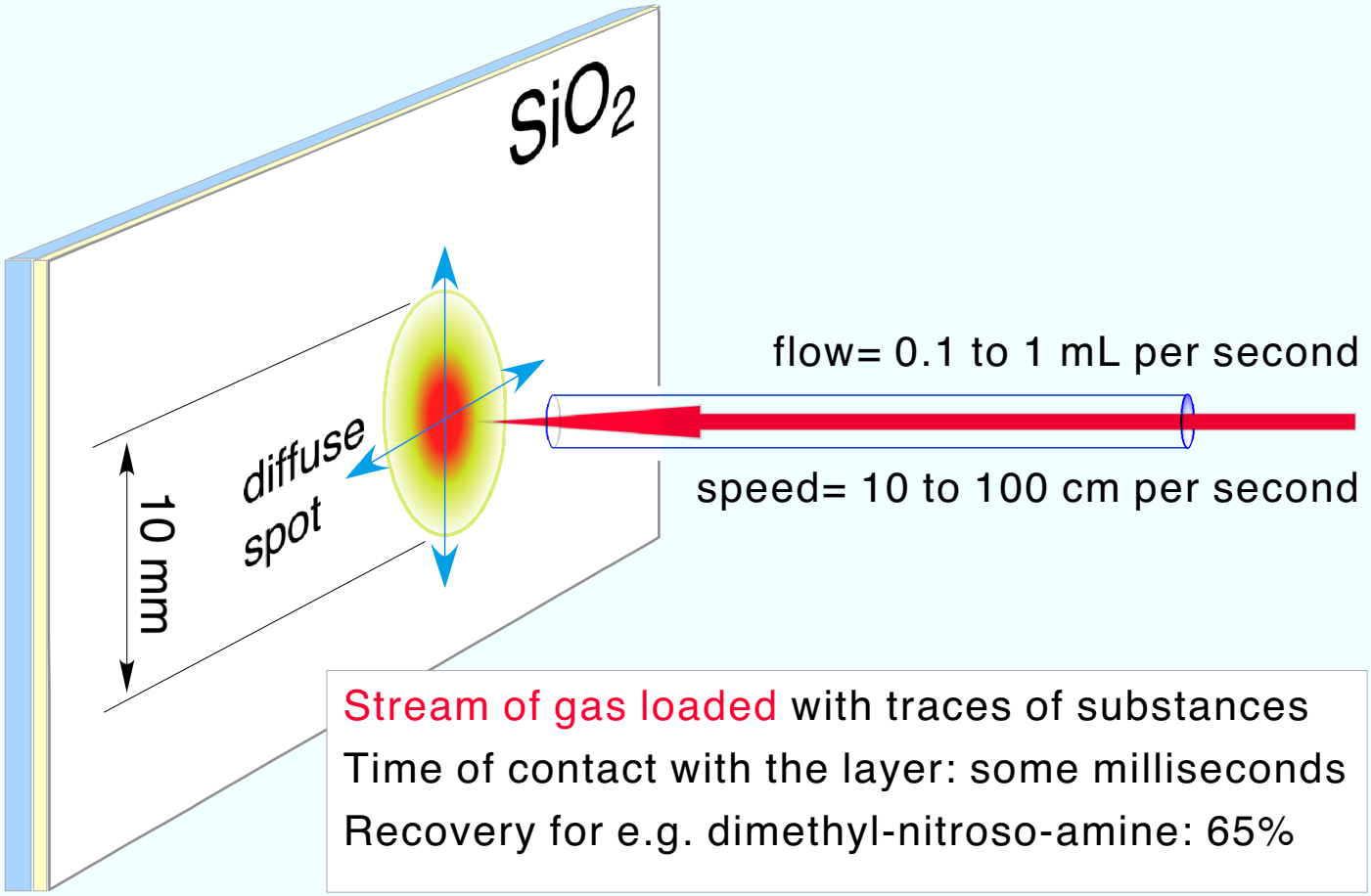


But what about impurities in the Silica layer?
Impurities ('muck') minimizing the power of the method, preventing proper work, should no longer be ignored.



TLC plate before and after cleaning

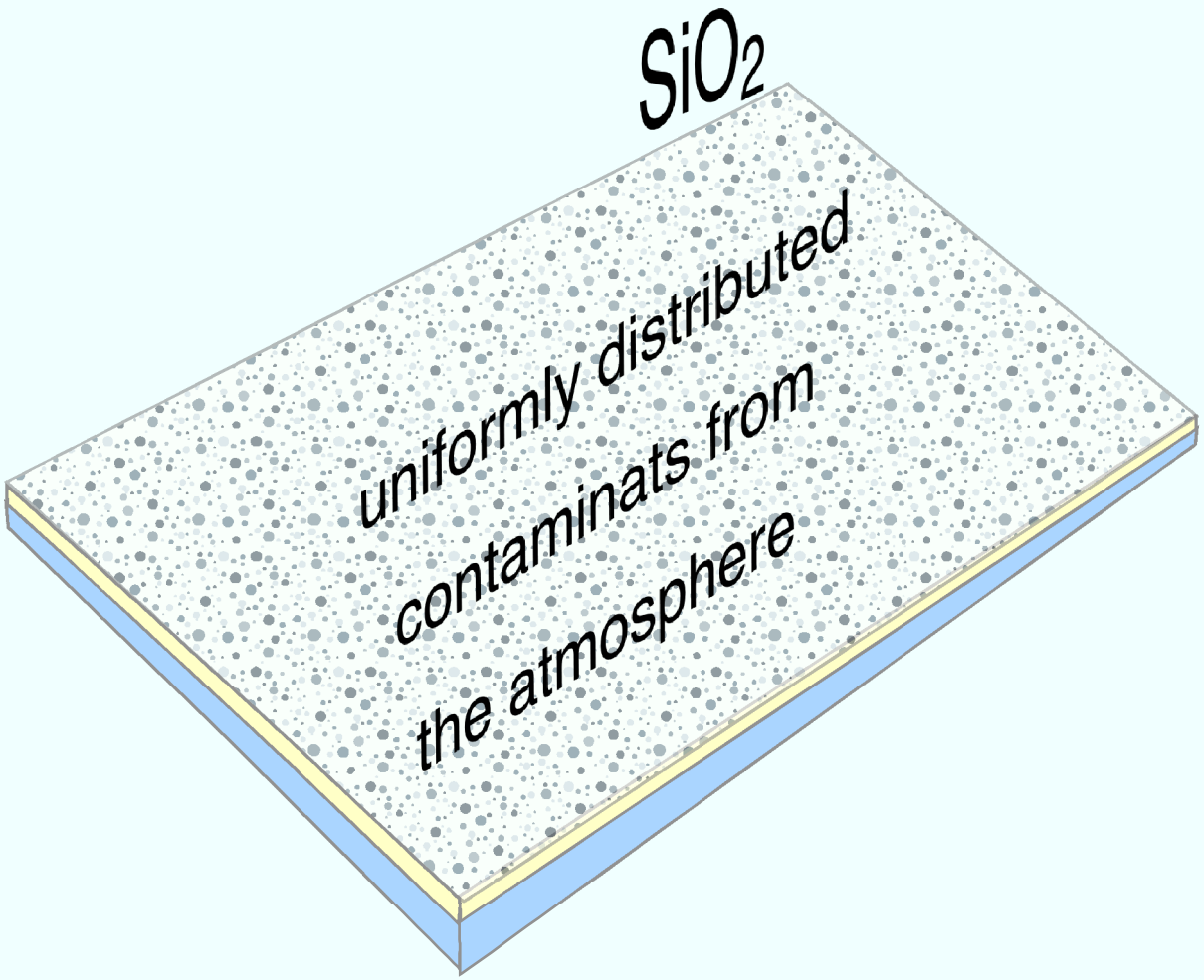
Klaus Burger



GAS is useful to determine traces of the analyte (ppb to ppt) by evaporation of a solution of the sample.
Separation from the matrix and enrichment in the layer by a factor of about 10^6 takes place in one simple single step.
Limit: the vapor pressure of the analyte should be at least 1 mBar at 100 °C.

GAS (Gas stream Application of Substances)

Klaus Burger

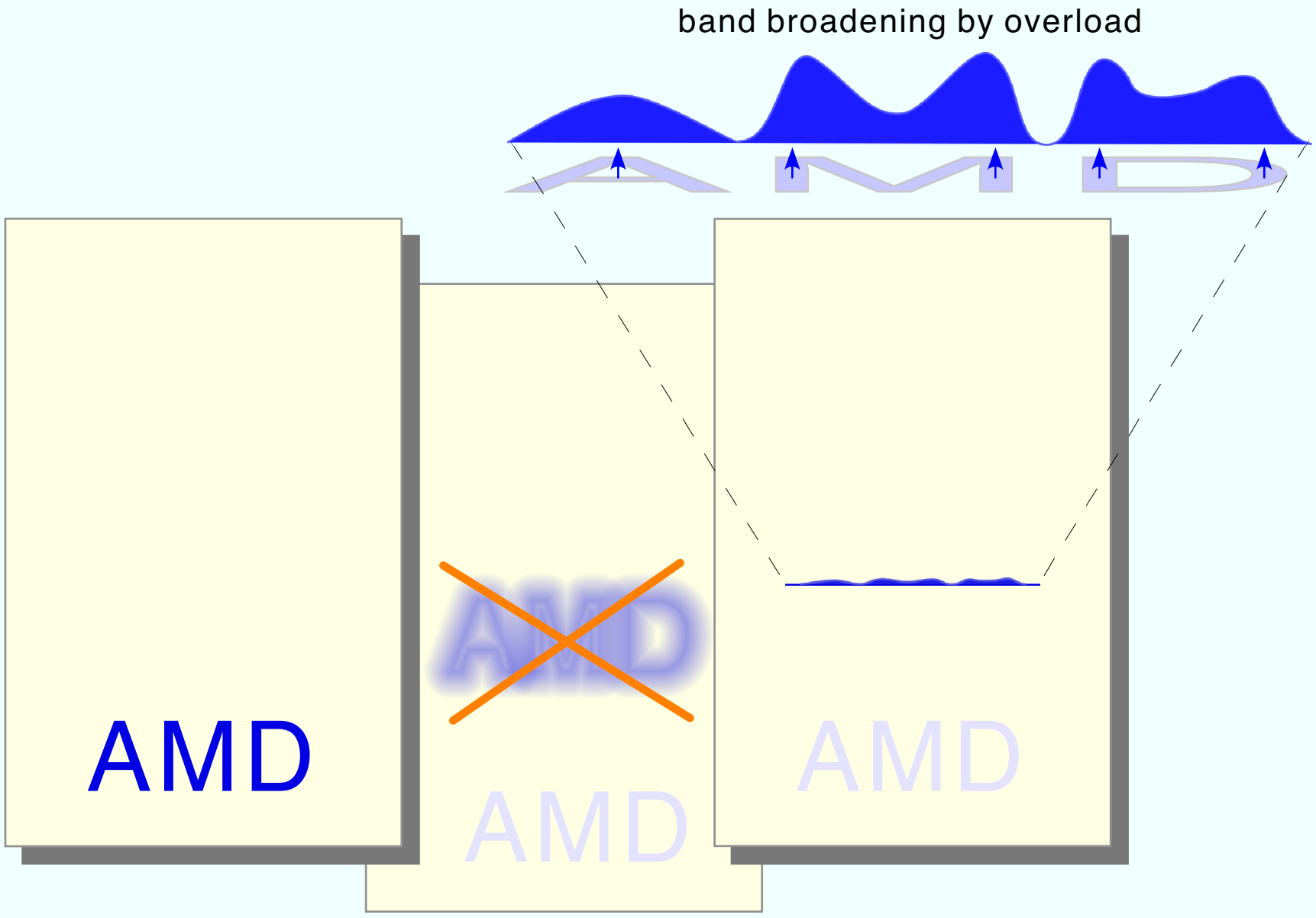


Silica layer of a TLC plate contaminated via atmosphere

Klaus
Burger

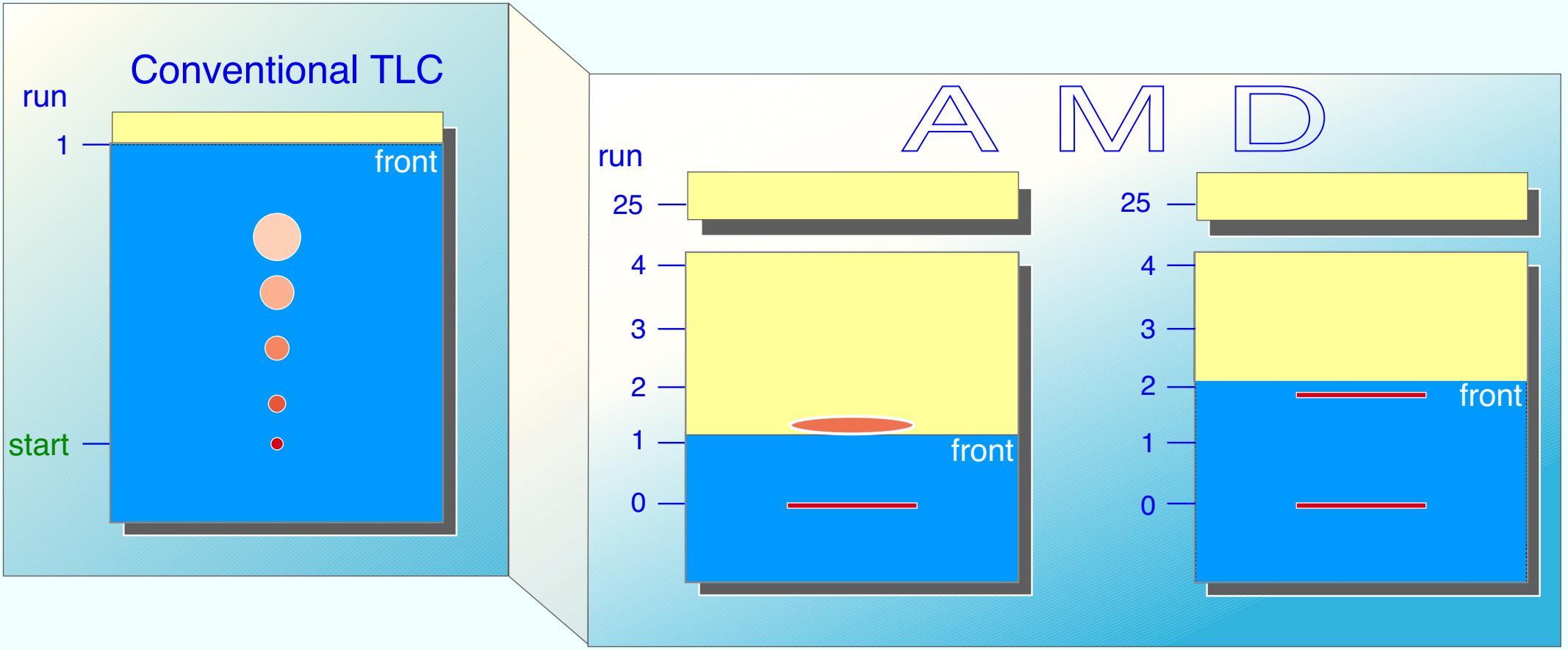
TLC migration of a dye from different starting positions

Klaus Burger



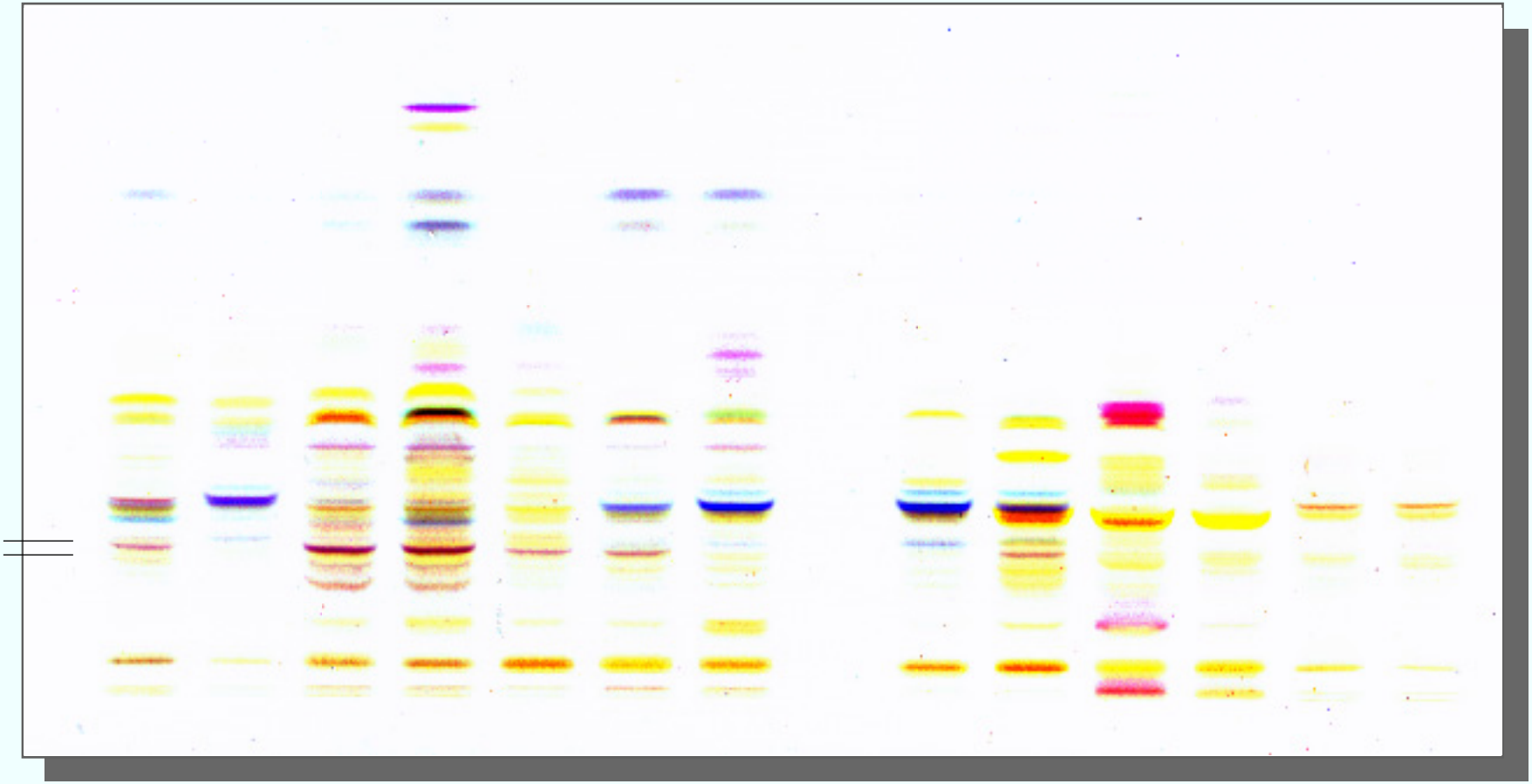
AMD migration of a dye from different starting positions

Klaus Burger



The focussing effect of AMD

Klaus Burger



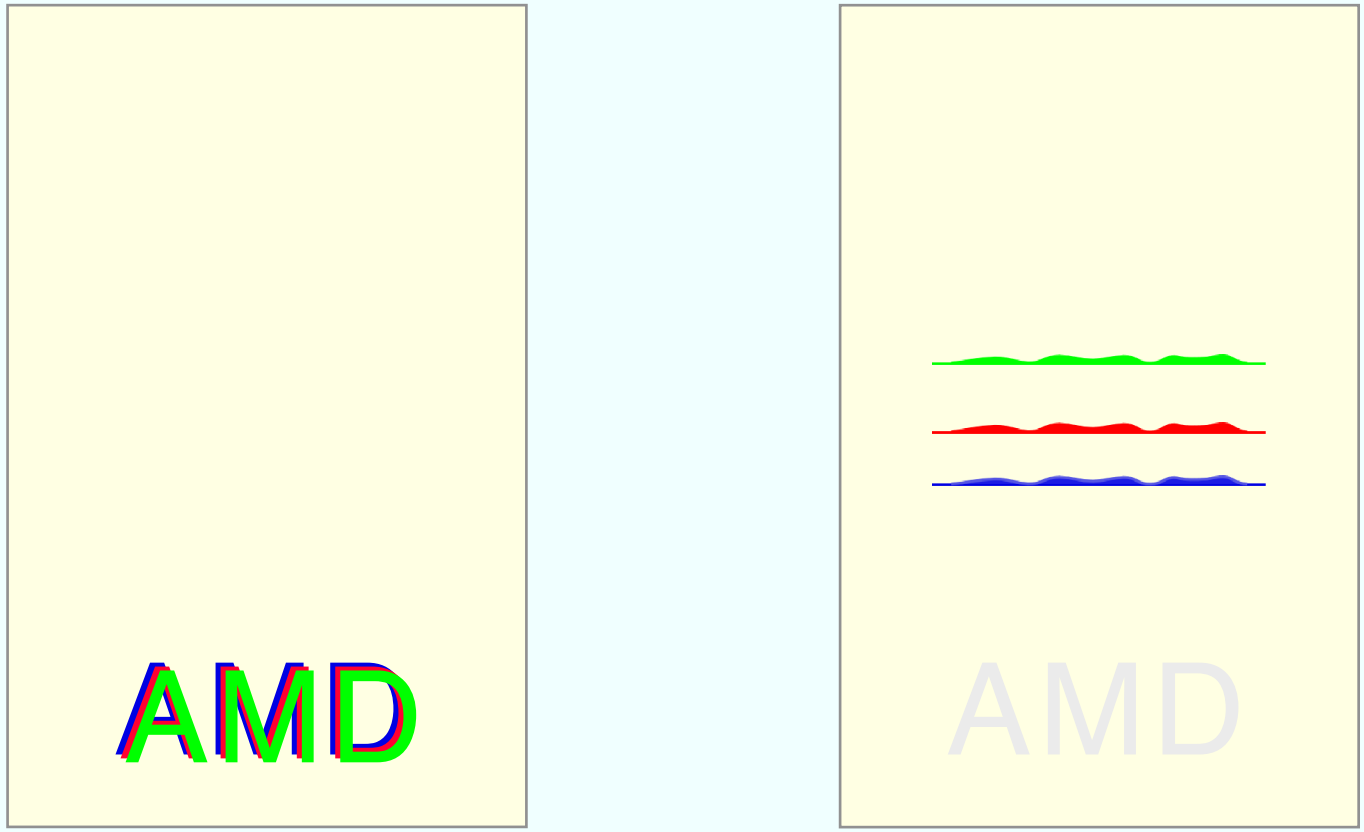
For optimal conditions a peakwith at half height of 0.1 mm is obtained
Real life samples show 20 to 40 baseline separations

Dyes in complex matrix

Klaus Burger

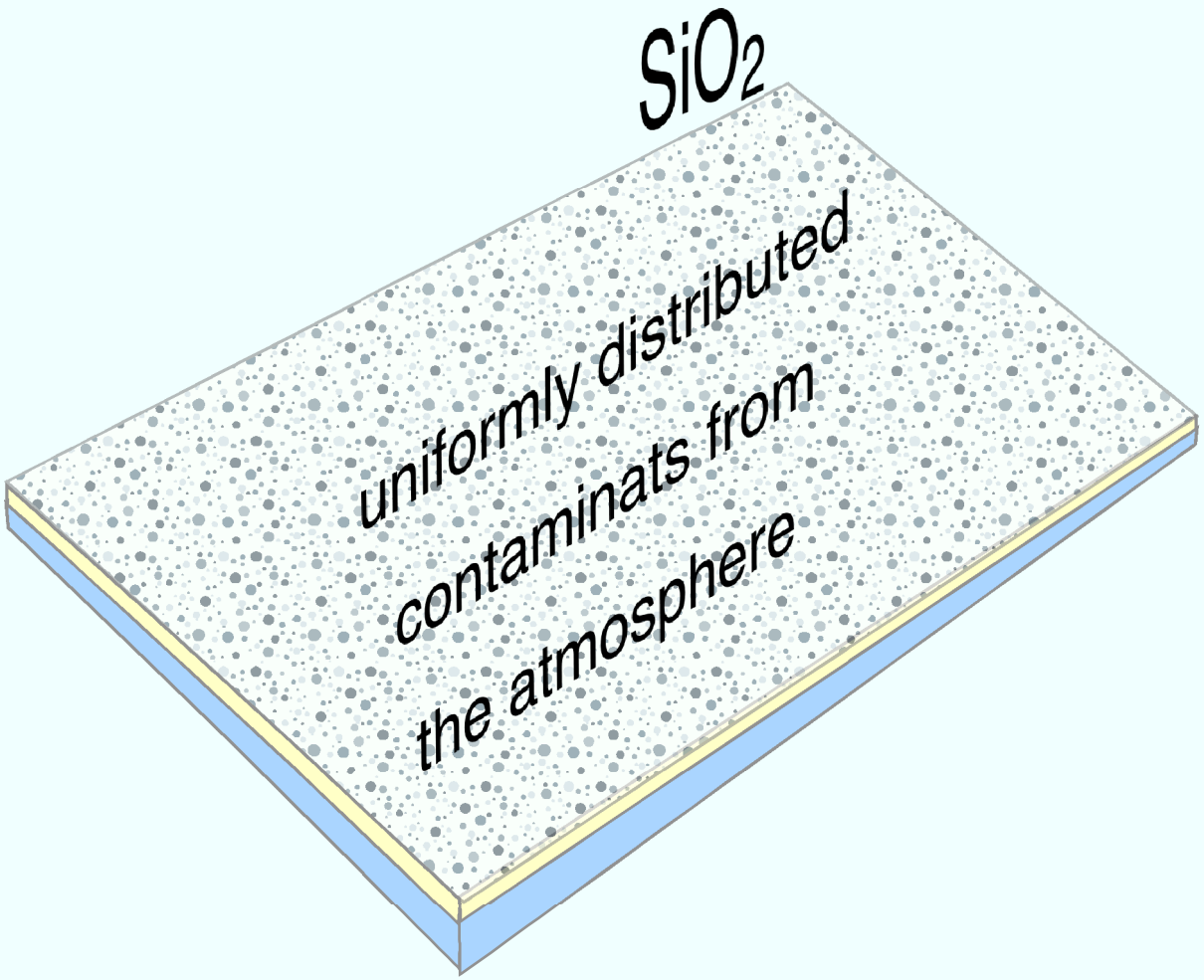
TLC migration of a three dyes from different starting positions

Klaus Burger



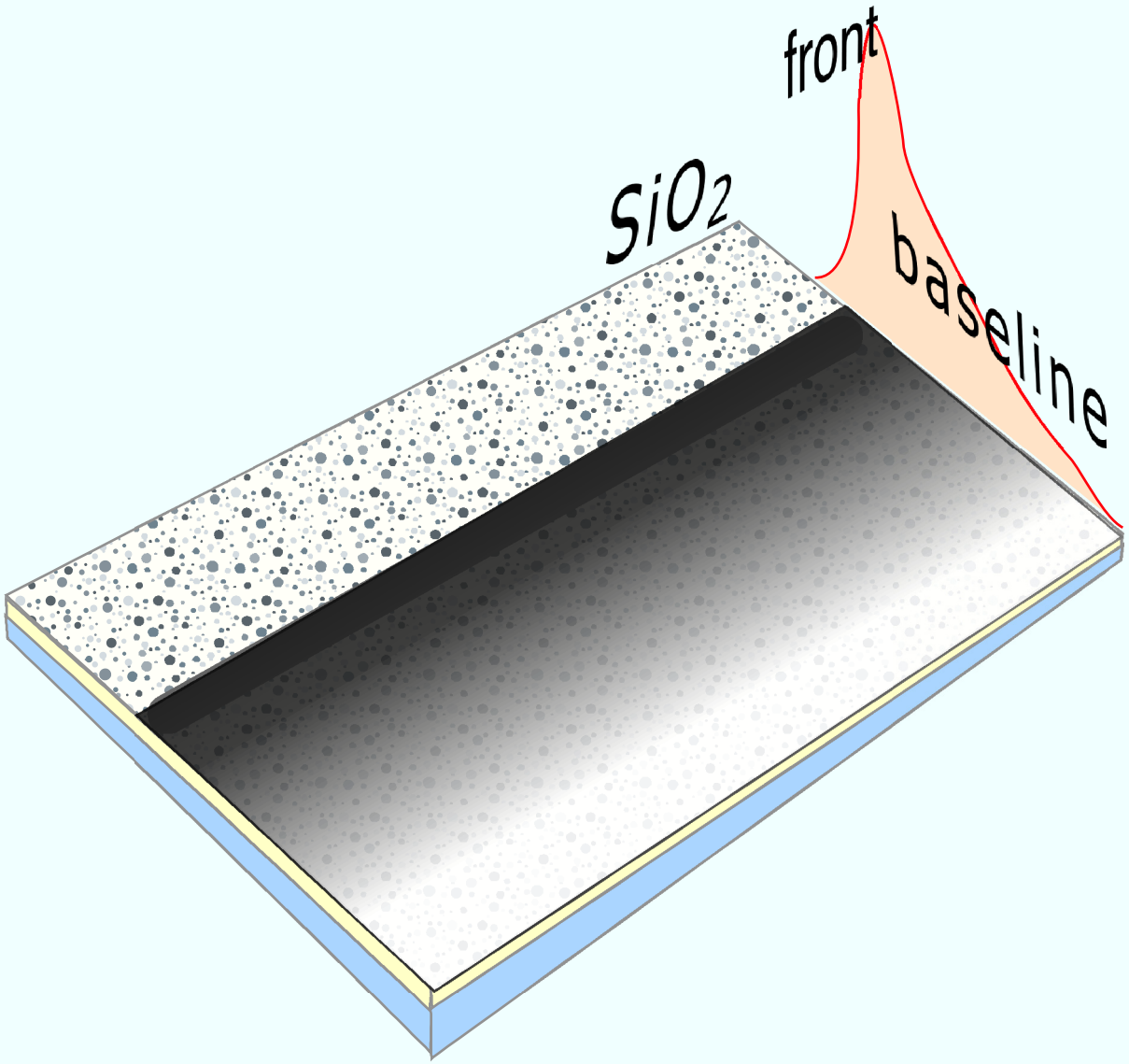
AMD migration of a three dyes from different starting positions

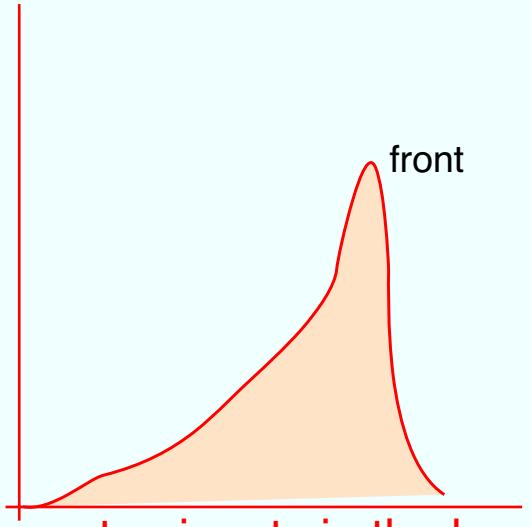
Klaus Burger



Silica layer of a TLC plate contaminated via atmosphere

Klaus
Burger





contaminants in the layer

preventing

successful coupling with e.g.

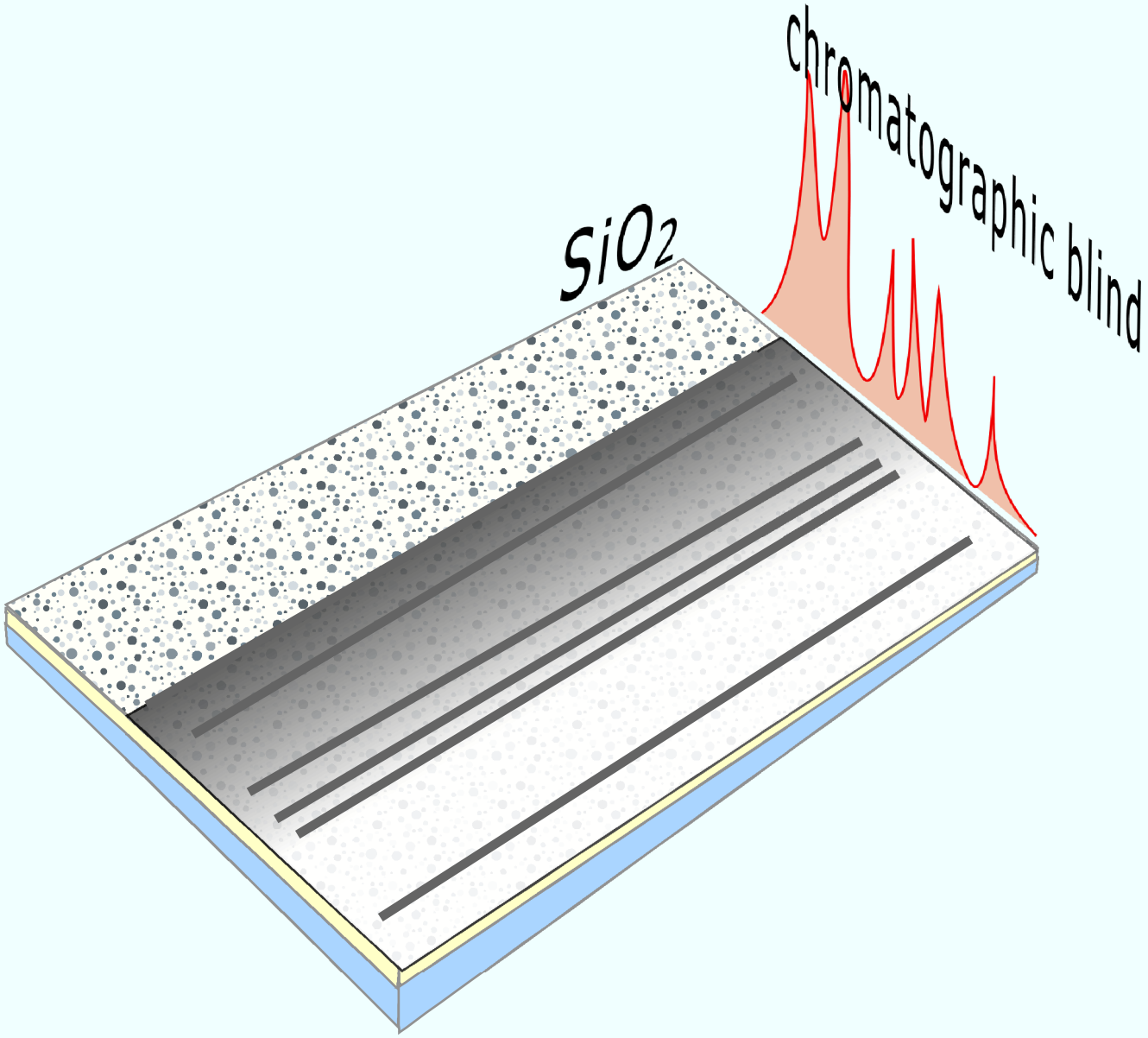
MS, RAMAN (SERS),

Bioactivity and even UV.

Acid or alkaline contaminants can extremely influence the migration behavior of corres-ponding analytes

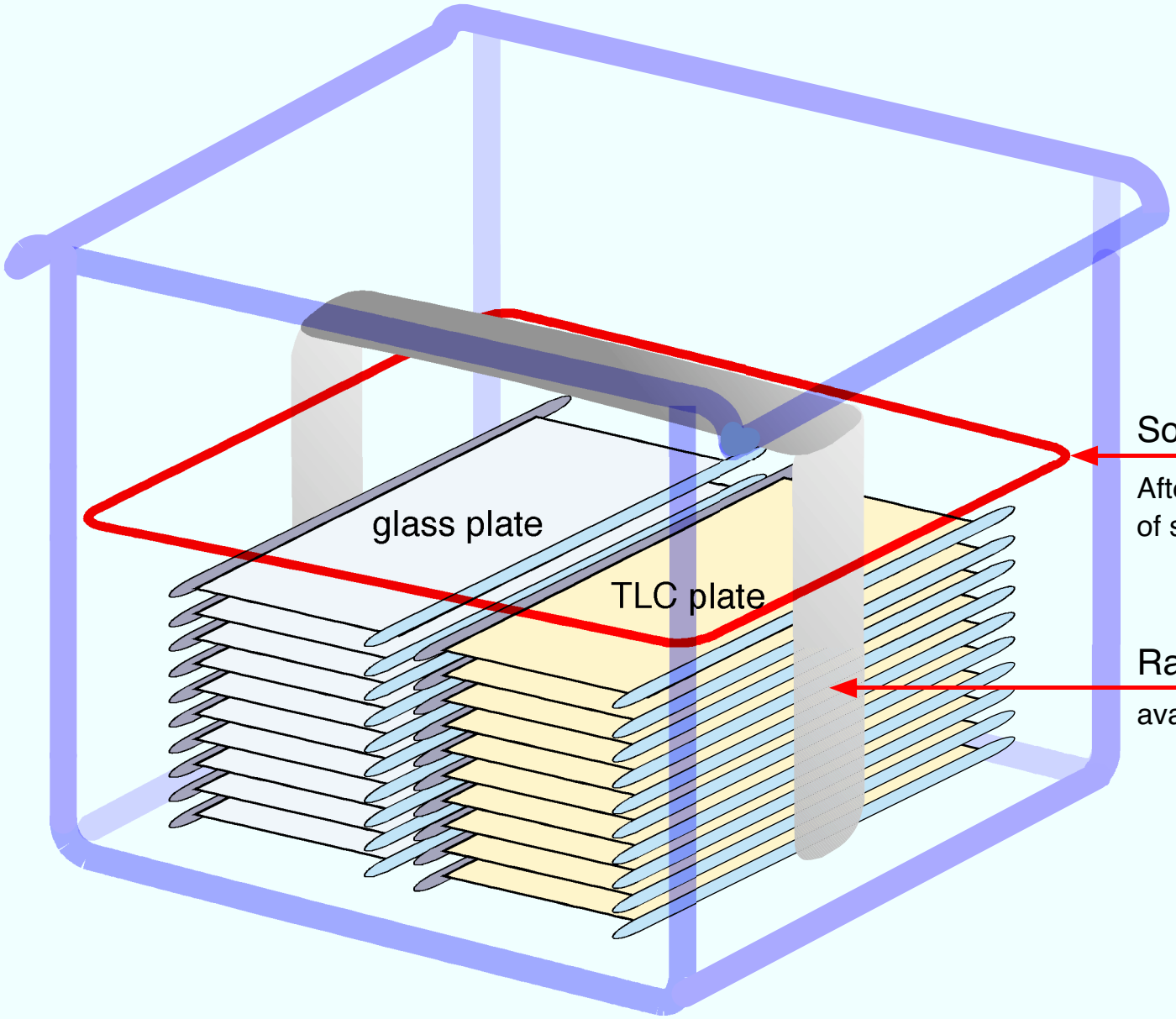
TLC migration of the contaminants in the layer

Klaus Burger



AMD migration of the contaminants in the layer

Klaus Burger



Solvent level

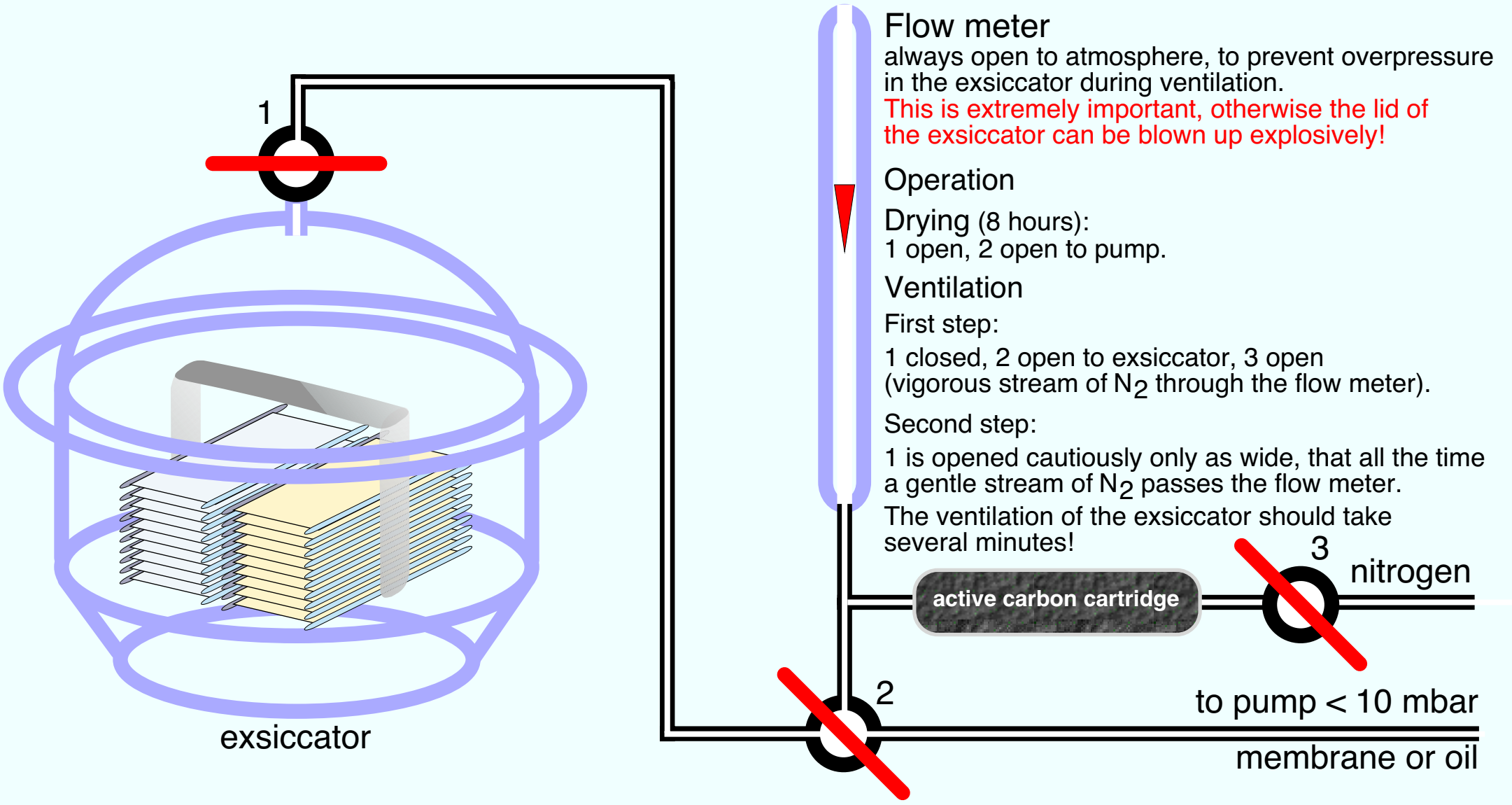
After cleaning of 6 TLC plates per liter of solvent, the solvent is discharged

Rag for 2 x 10 plates

available from e.g. DESAGA

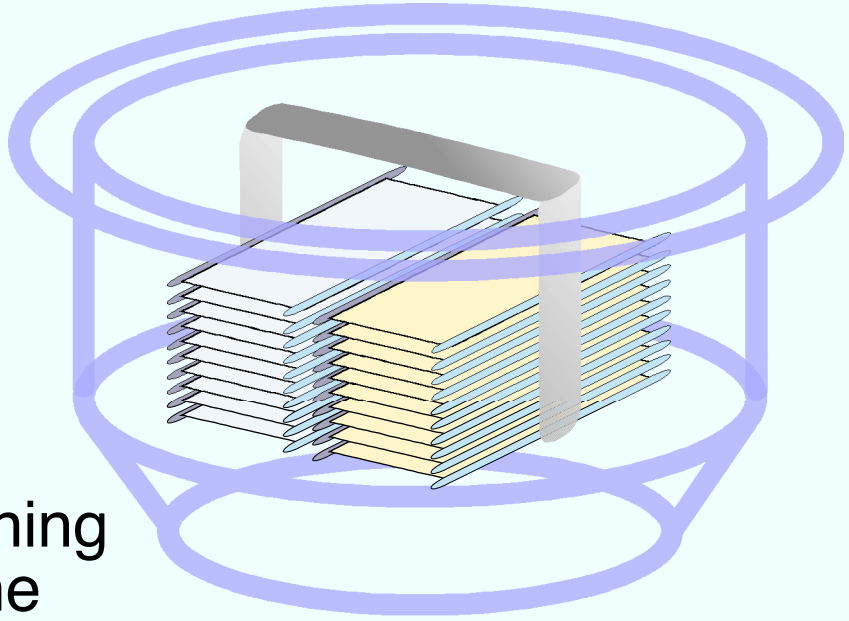
Cleaning TLC plates by diving

Klaus Burger



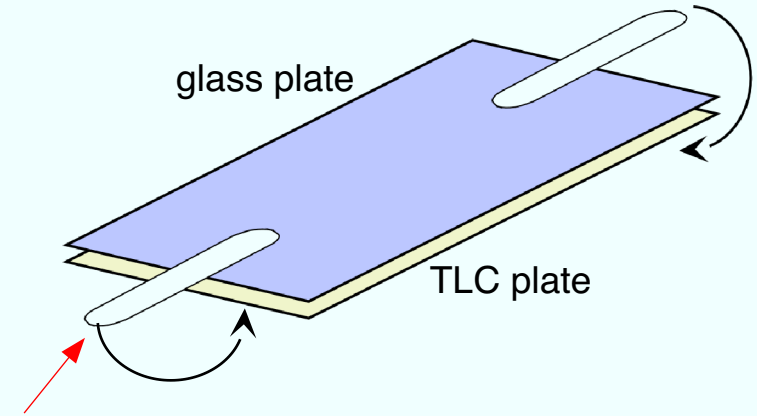
Drying TLC plates by vacuum

Klaus Burger

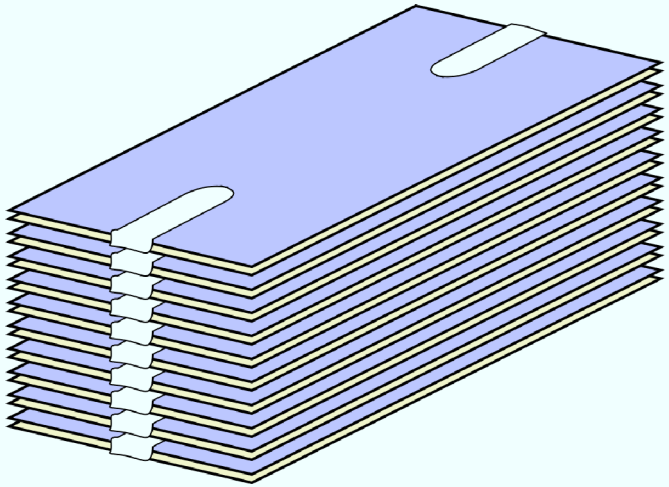


opening
of the
ventilated exsiccator

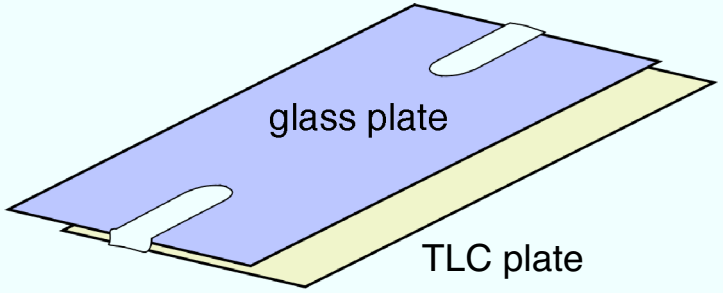
protection of the layer



PTFE sealing band, to fix the glass on the TLC plate
(without glue and additives causing blinds again!)



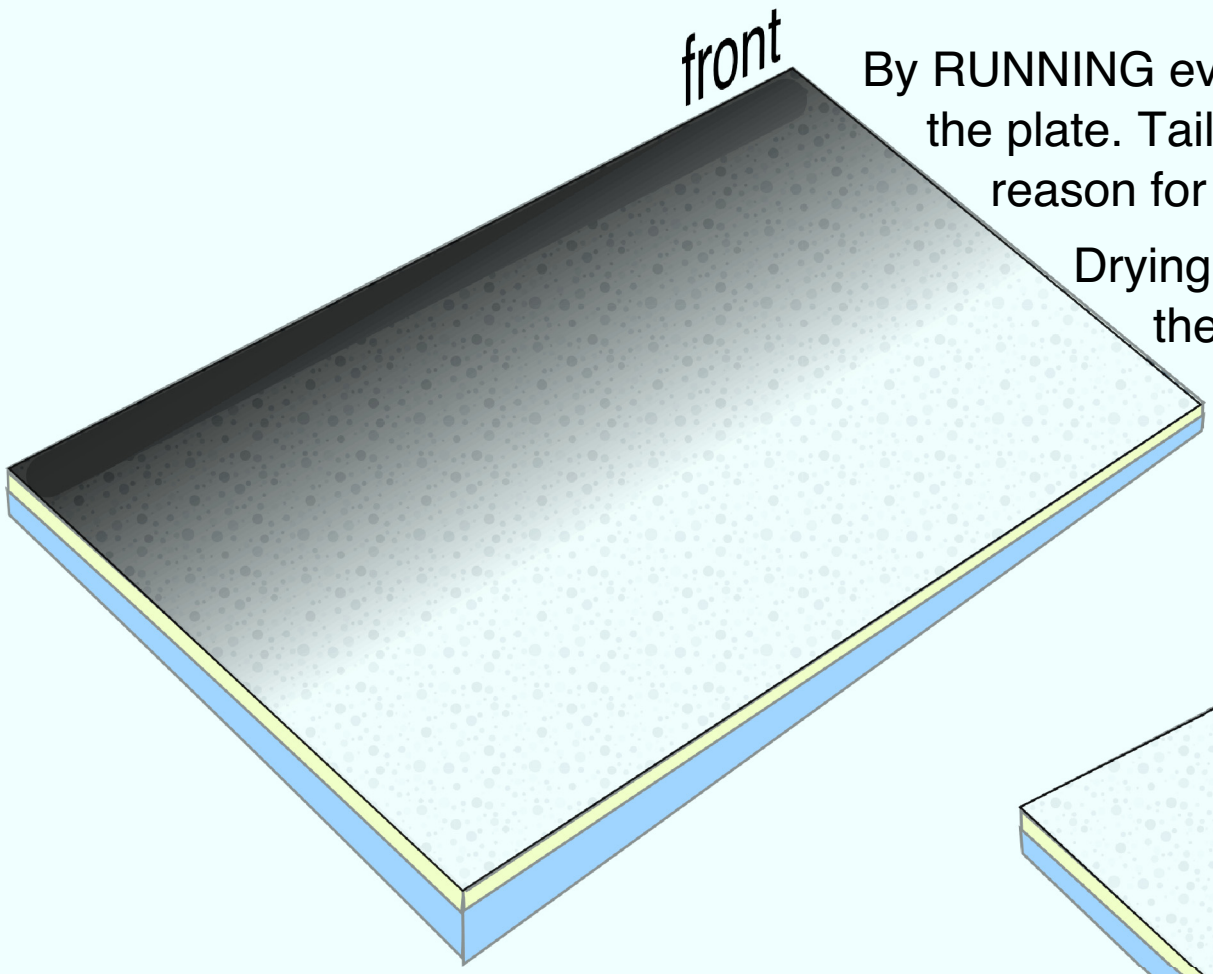
storage of the TLC plates



application of samples

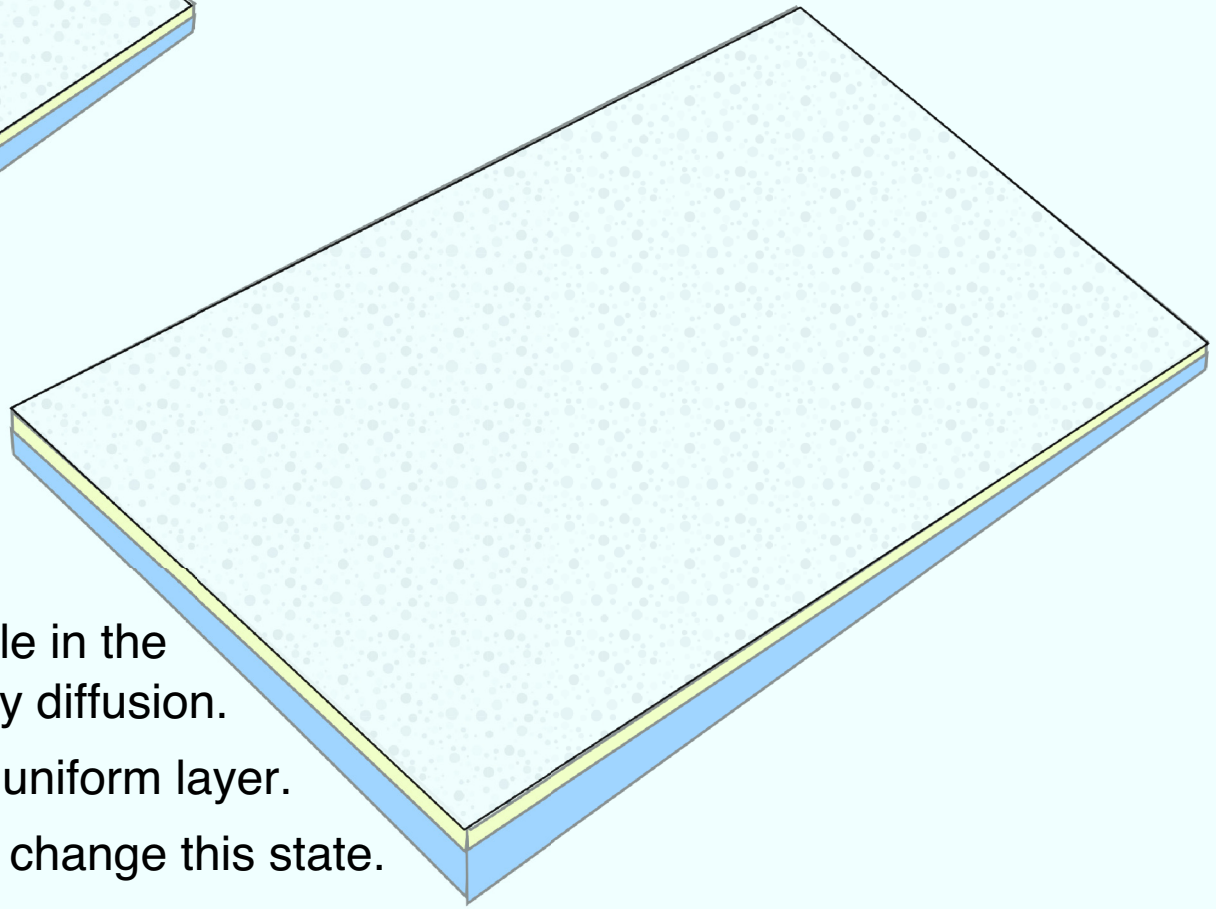
All these steps MUST be performed in a CLEAN BENCH

Klaus
Burger



By RUNNING every impurity remains in the upper part of the plate. Tailing components in the layer are the reason for a 'gradient' baseline.

Drying by heat can cause changes in the silica of the layer and the binder molecules.



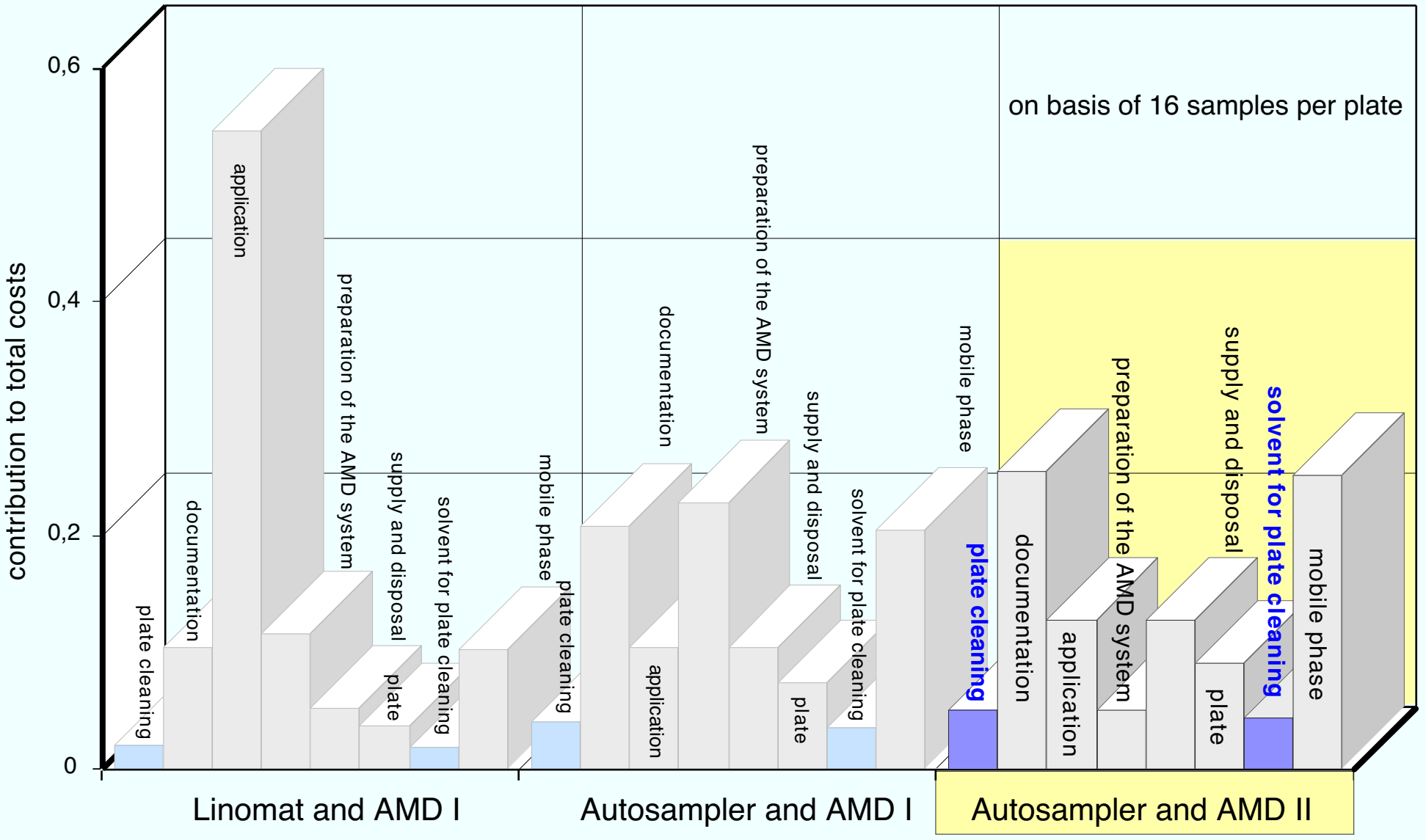
By DIVING every impurity, mobile in the cleaning solvent, is removed by diffusion.

The result is an uniform layer.

Drying by vacuum doesn't change this state.

Two cleaning methods

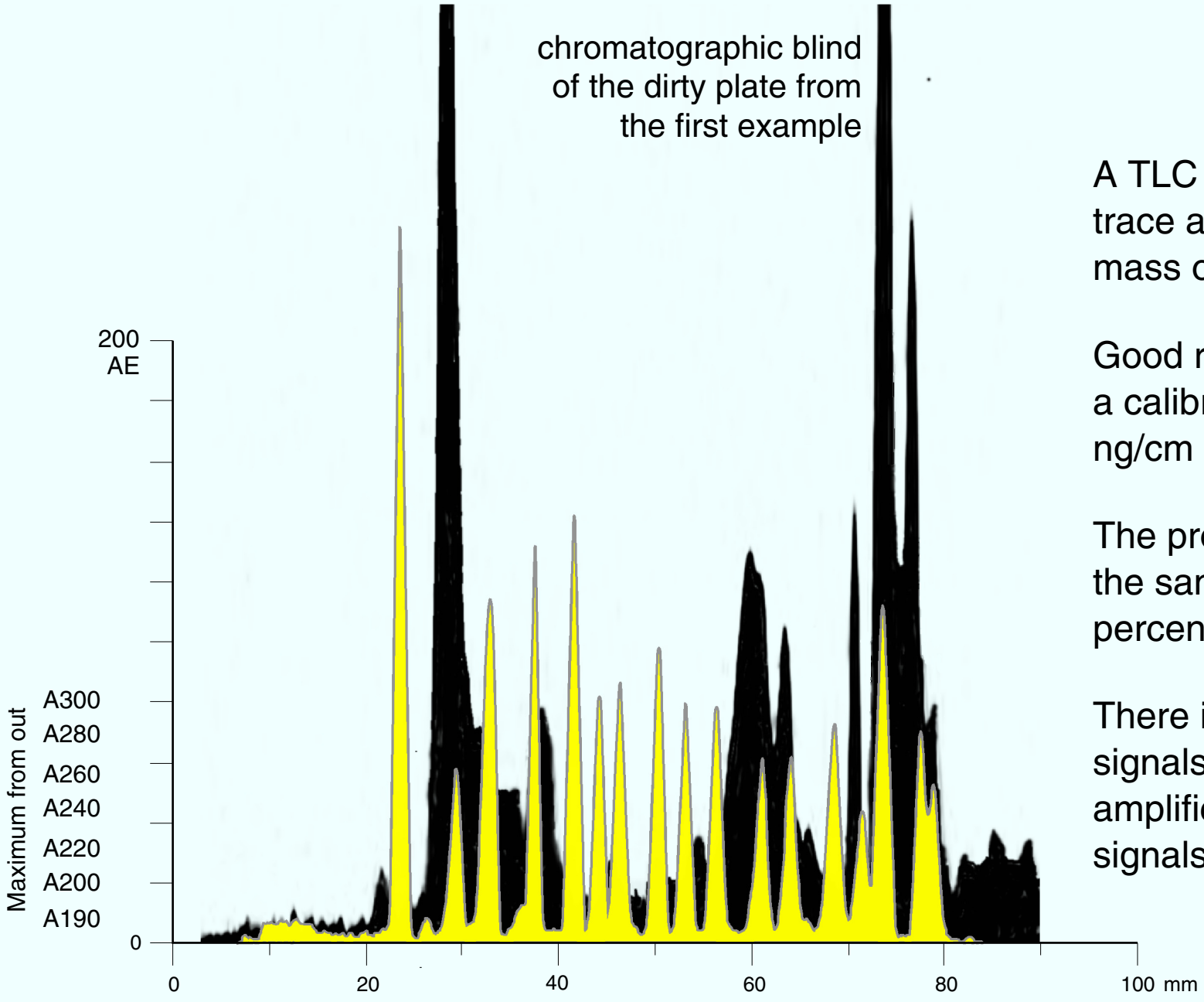
Klaus Burger



The expense for plate cleaning is between 2 and 4% of the total costs of an AMD analysis

Costs of an AMD analysis

Klaus Burger



A TLC determination always is a trace analysis with respect to the mass of the analyt in the layer.

Good results will be obtained for a calibration between 3 and 300 ng/cm (application as band).

The procedure therefor is always the same for the ppb's up to the percentage range!

There is no way to suppress the signals of the blind by weak amplification of the measured signals.

Blind of a dirty plate compared to a calibration on a clean plate with 100 ng per component

Klaus Burger

After controlling the airborne blind

and ordering AMD suited solvents

(to block the second source of blinds)

life is very comfortable.

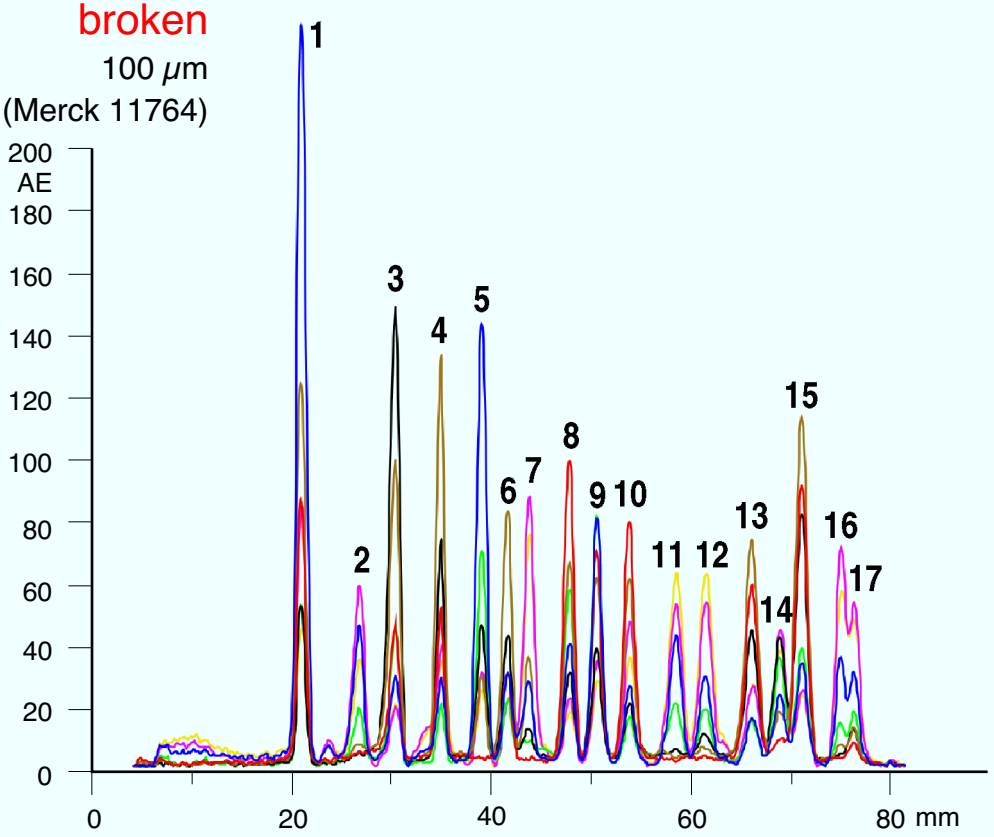
He now can start to work…

HPTLC plate silica

broken

100 µm

(Merck 11764)



A190 A200 A220 A240 A260 A280 A300

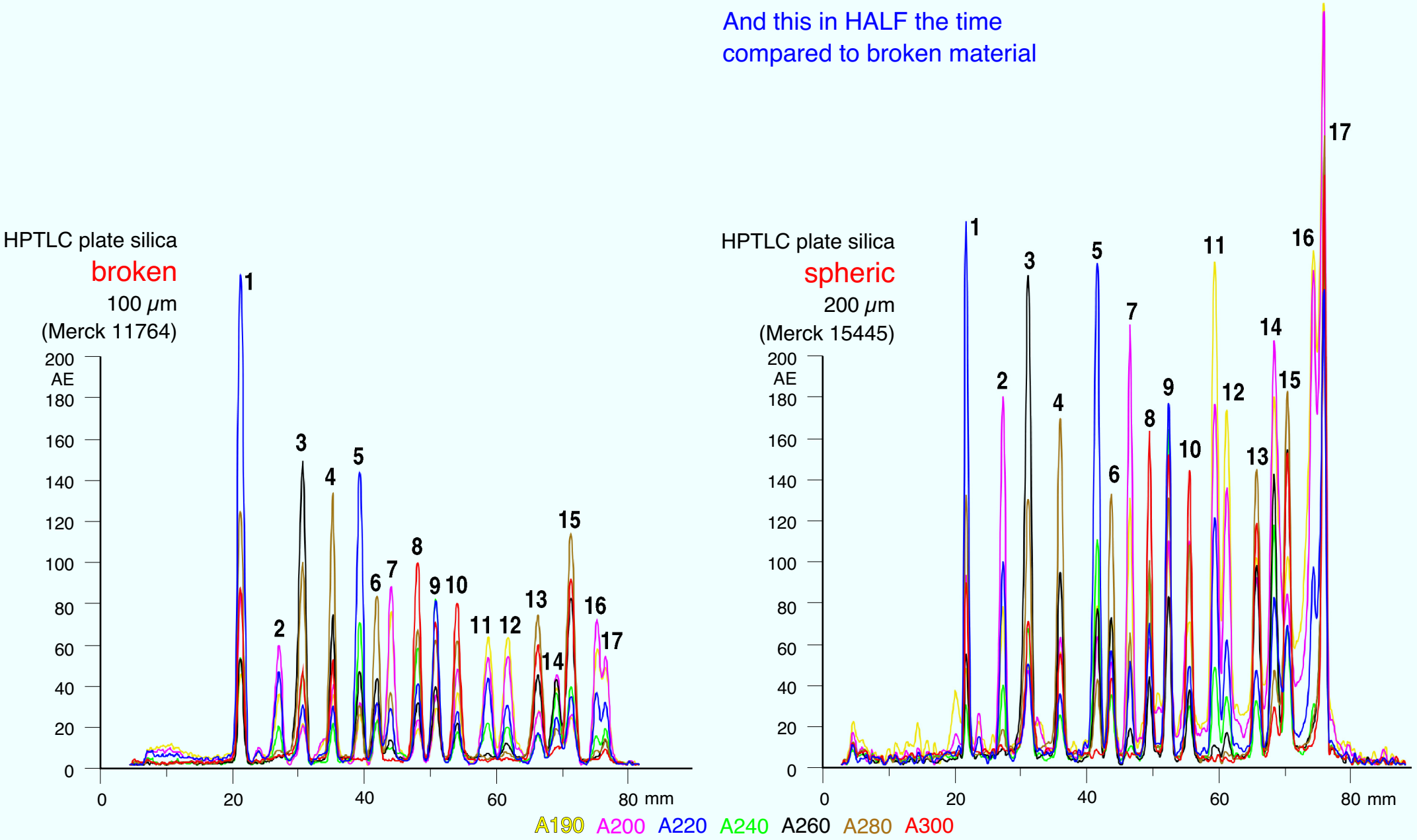
1= Naphtalin-1-sulfonic-acid
2= Procloraz
3= Imidachloprid
4= Ethidimuron
5= Simazine
6= Bromacil
7= Carbofuran
8= Metribuzin
9= Azinphos Methyl
10= Coumaphos
11= Prosulfocarb
12= Dichlorfluanid
13= Parathion Ethyl
14= Fenthion
15= Dinosep
16= Tokuthion
17= front

AMD separation of some standards

100ng per component

Klaus Burger

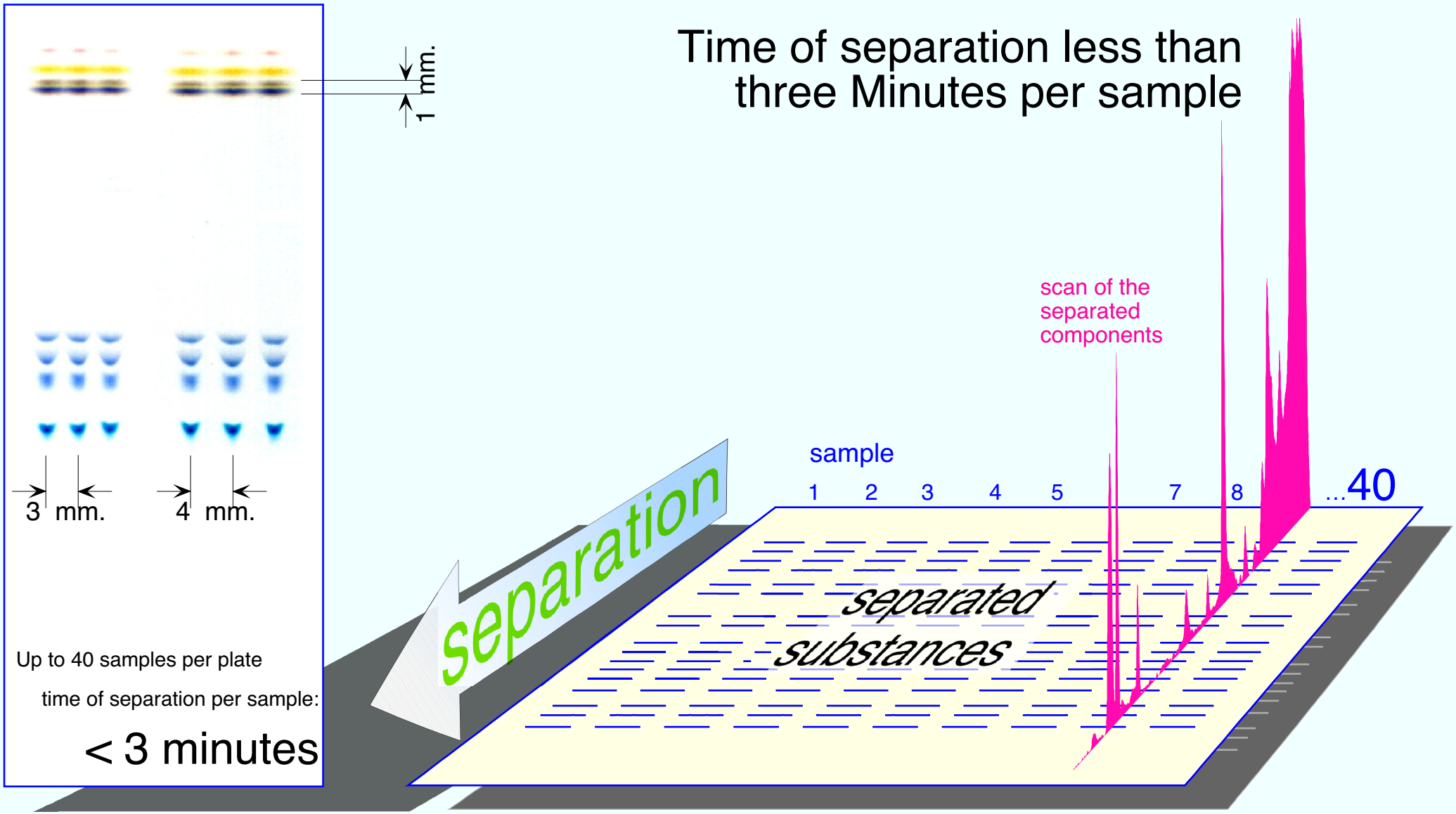
And this in HALF the time
compared to broken material



AMD separation of some standards

100ng per component, separated on different HPTLC plates

Klaus Burger



AMD is parallel

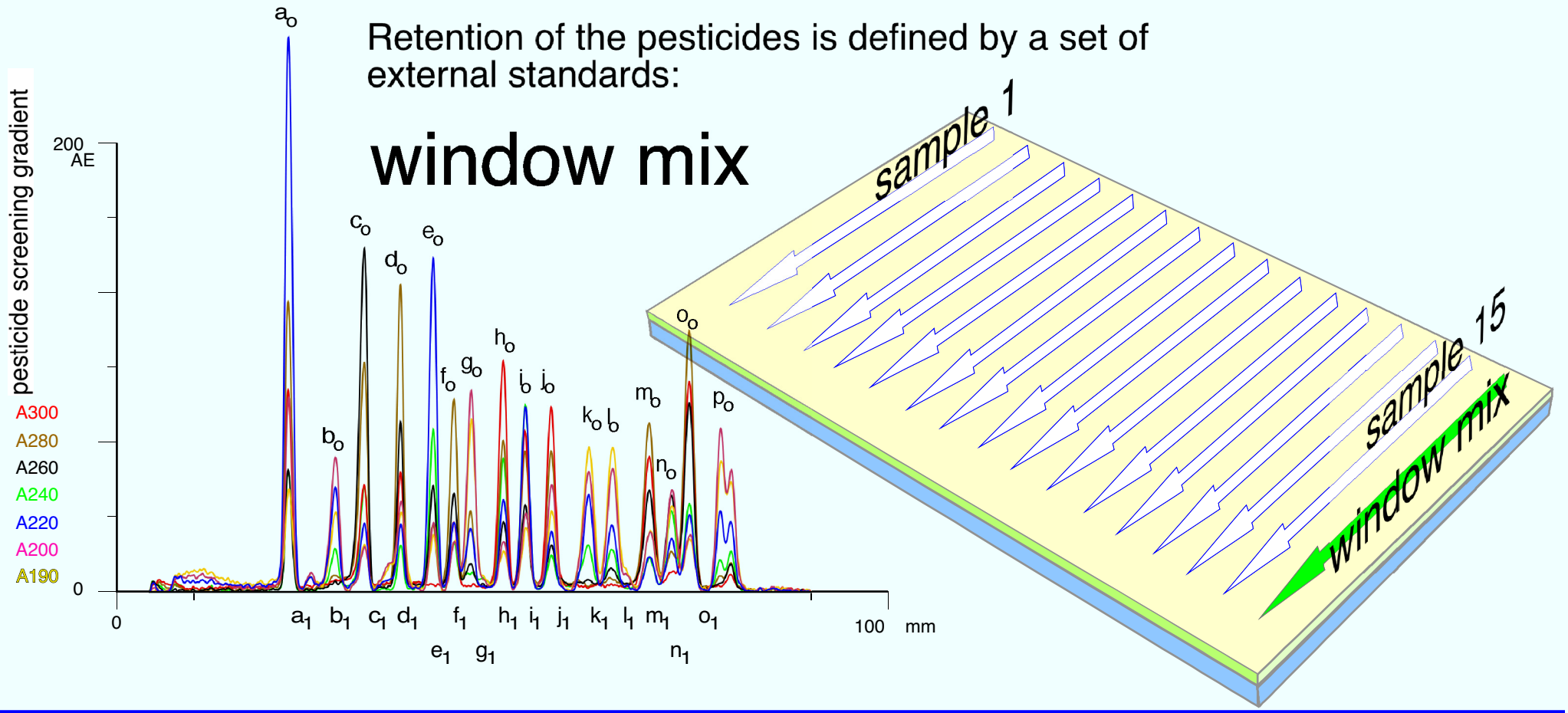
Klaus
Burger

Step 1: Screening of 15 samples for 200 components in the 'pesticide screening gradient'.

Step 2: Pesticides in the samples are characterized by 31 retention windows a_o through p_o and their UV multidetection, both documented in a database.

Step 3: Positive findings are validated by chromatography with a second selectivity.

Result: ≥1500 determinations per plate.

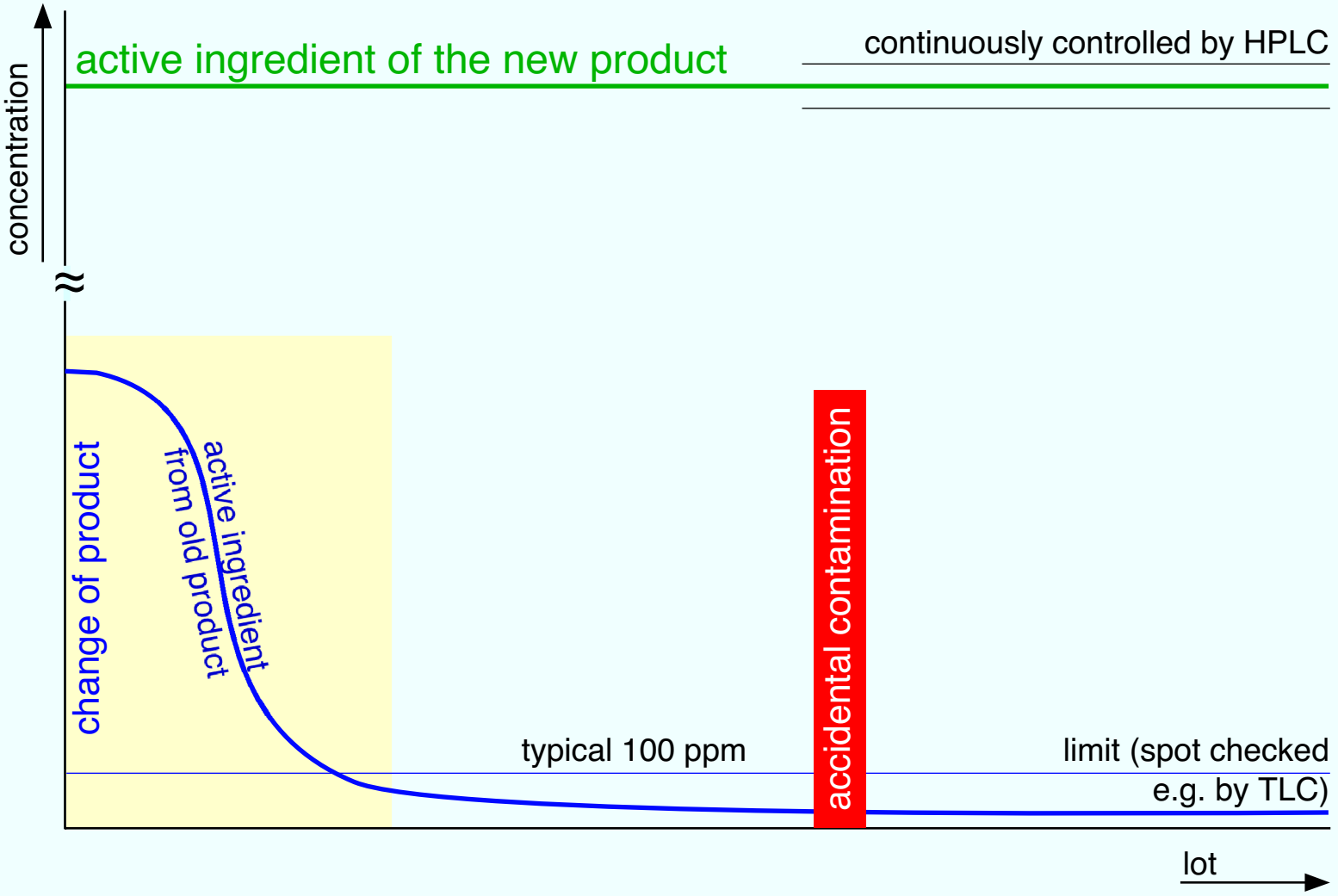


Pesticides in soil and Water:

Screening for 200 components parallel in 15 samples

Klaus Burger

PRODUCT SCREENING



Usual analytical strategy to control mixing processes

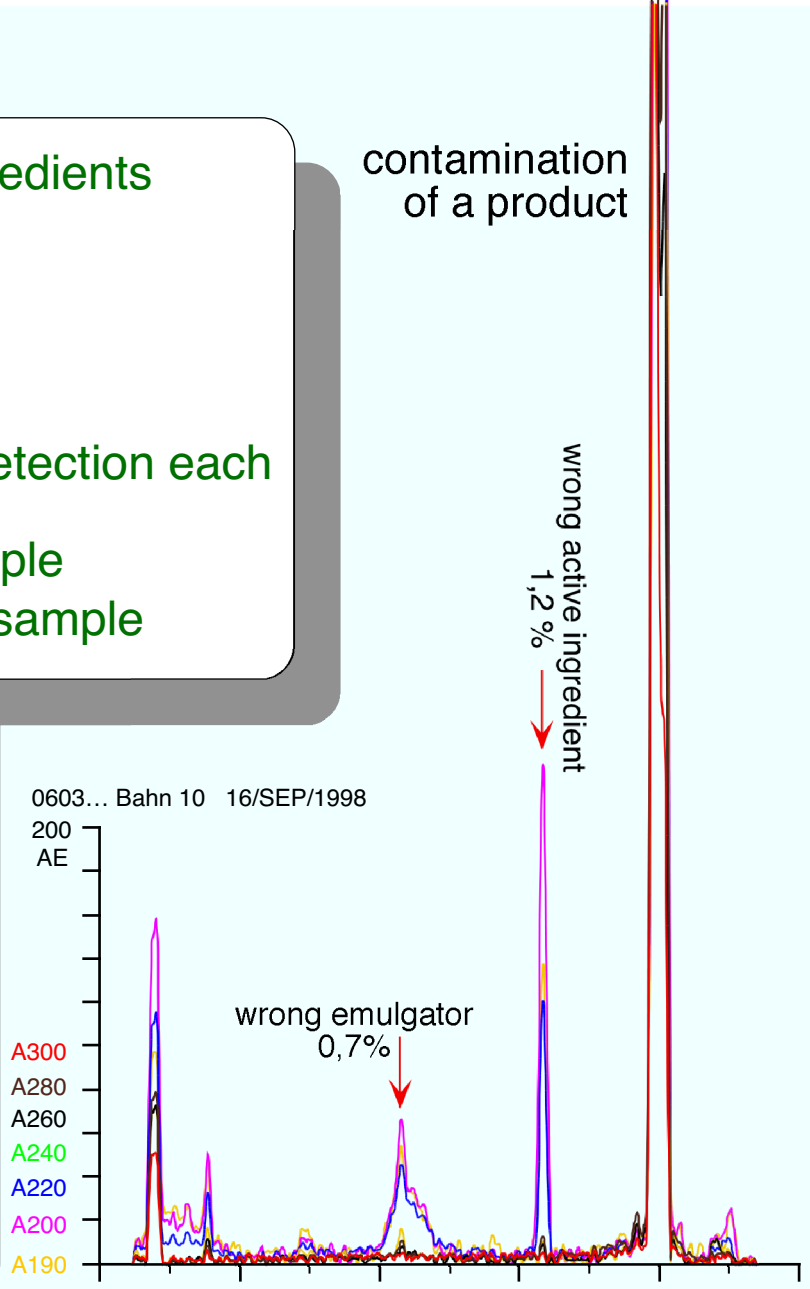
Klaus Burger

products per year	300 with 63 active ingredients
samples per month	300
strategy	each lot is analyzed
separation and measuring	2 separations with 2 detection each
data collection	parameters of the sample 4 chromatograms per sample
evaluation	each lot

procedure

- setting up of reference scans for each product
- comparison of samples and reference
- deviations from the intended composition are documented and tested for contamination

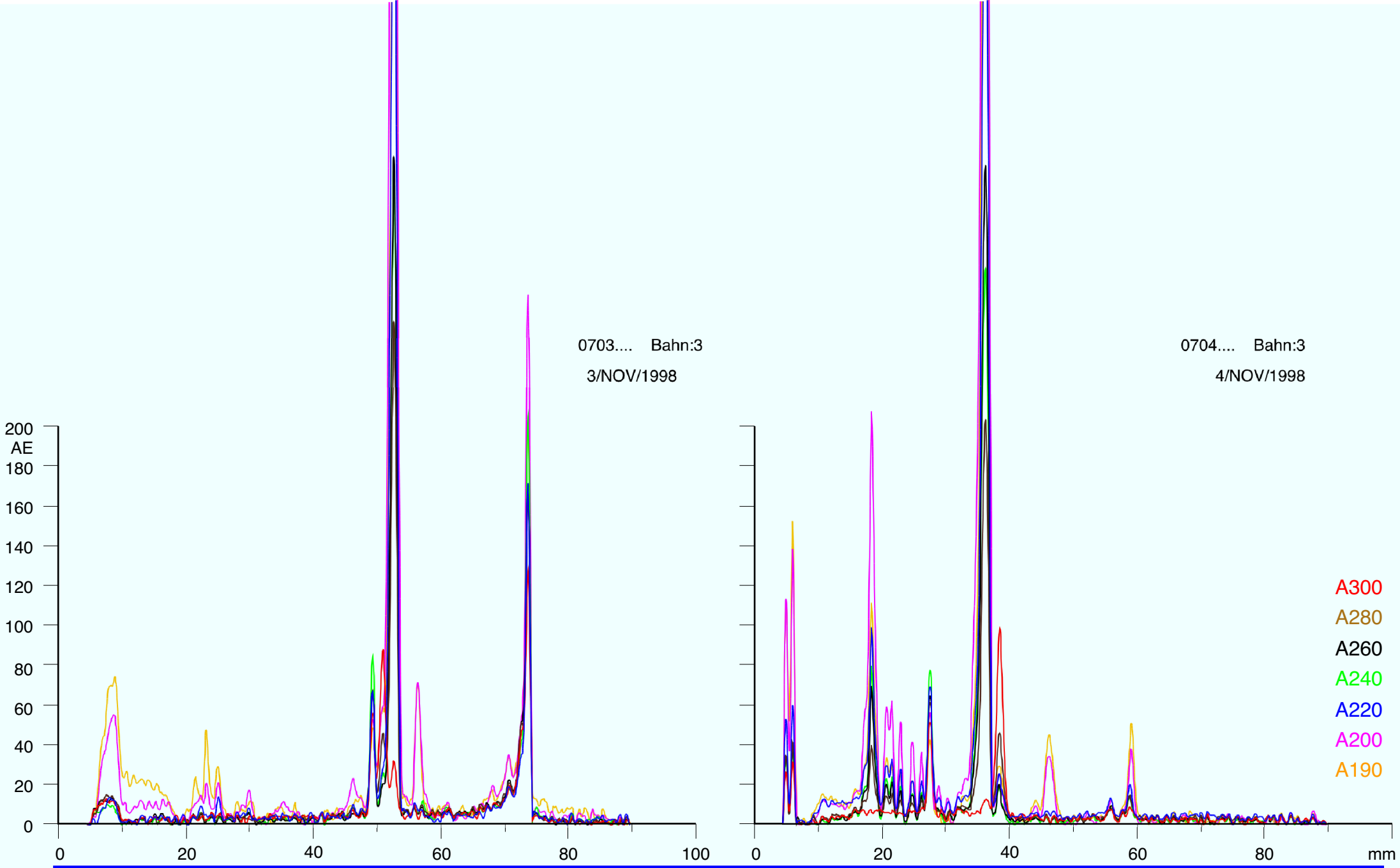
contamination of a product



PRODUCT SCREENING

Screening of the complete production of a formulating plant

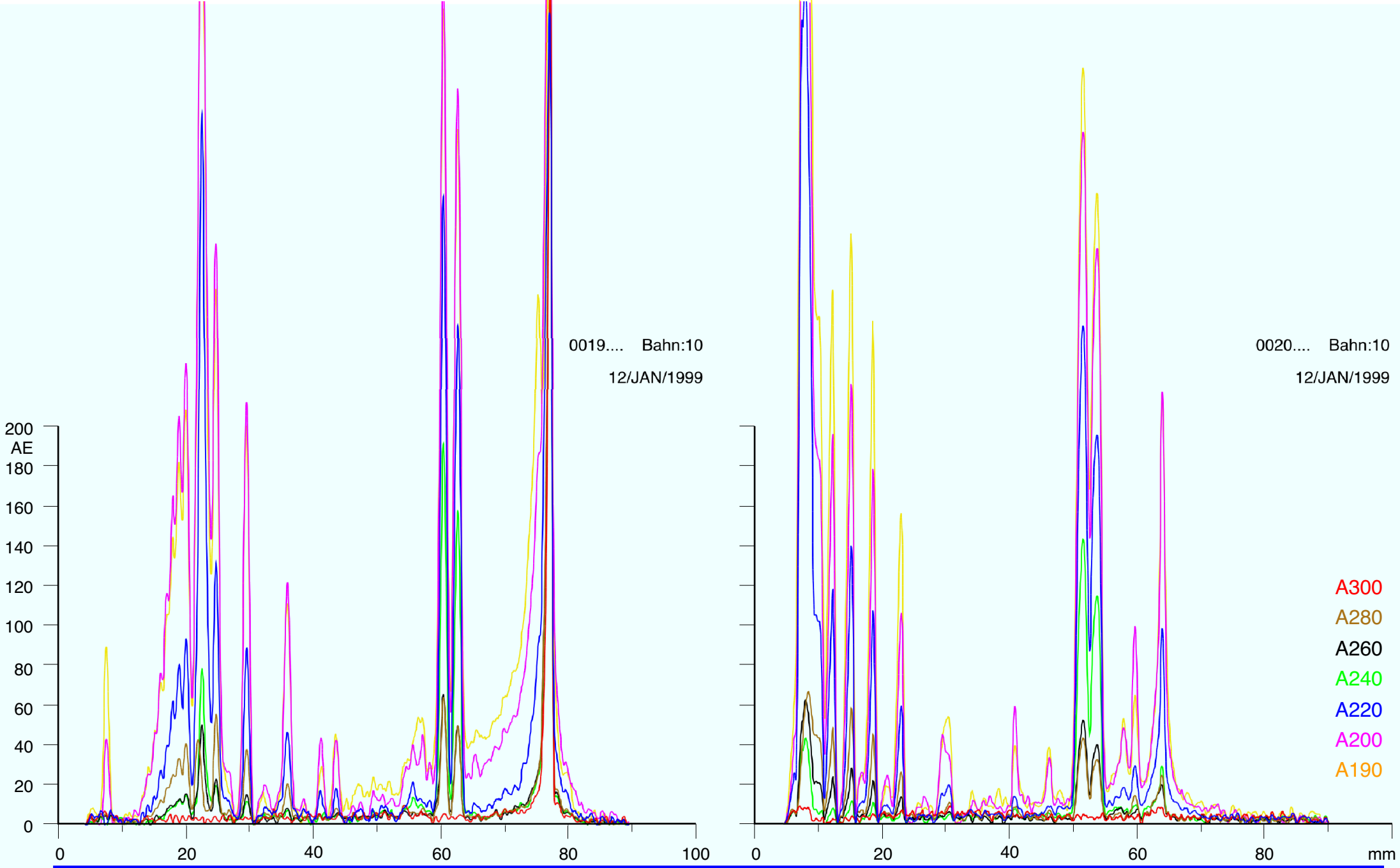
Klaus Burger



Screening: Product 2

Confirmation: Product 2

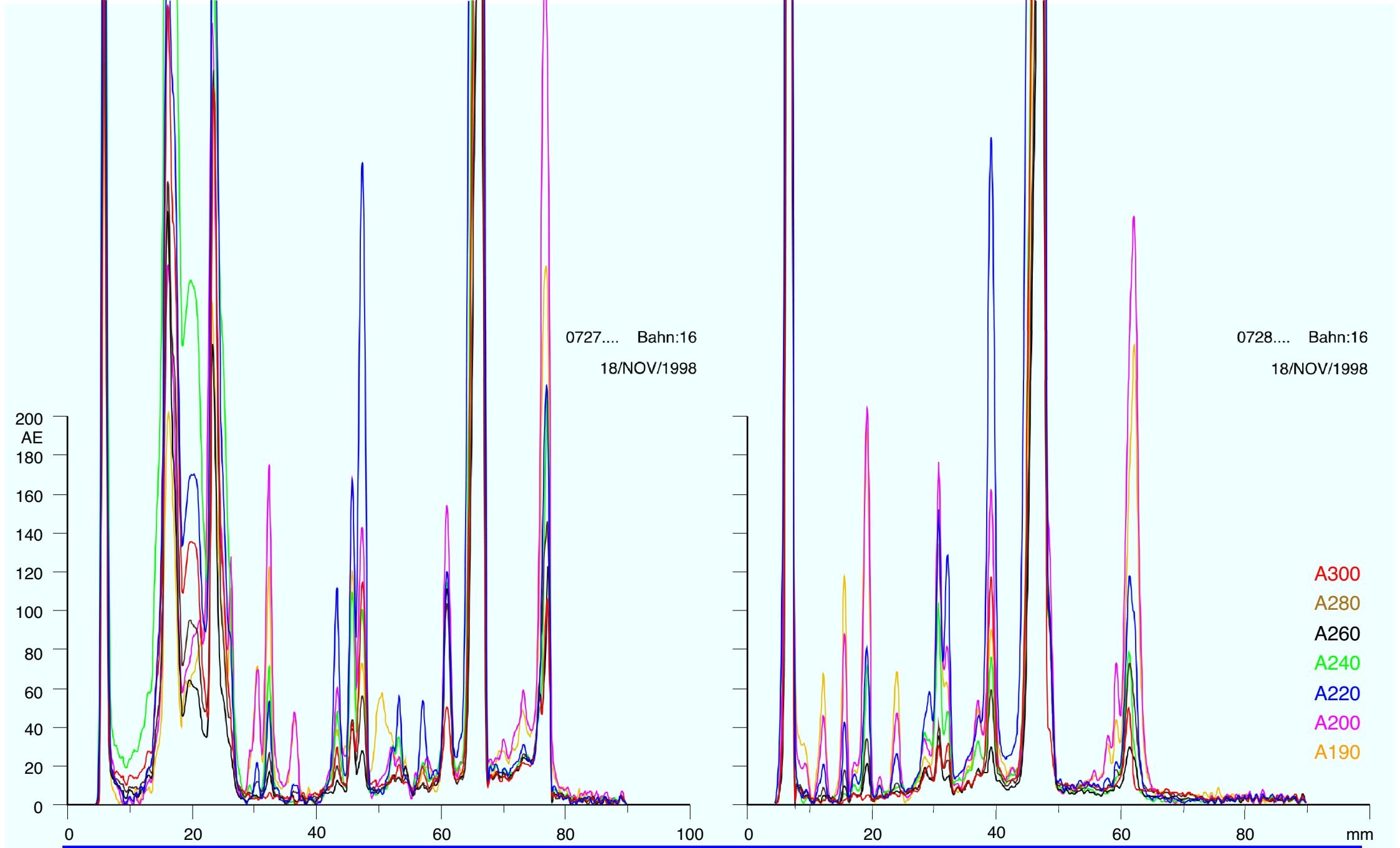
Klaus Burger



Screening: Product 6

Confirmation: Product 6

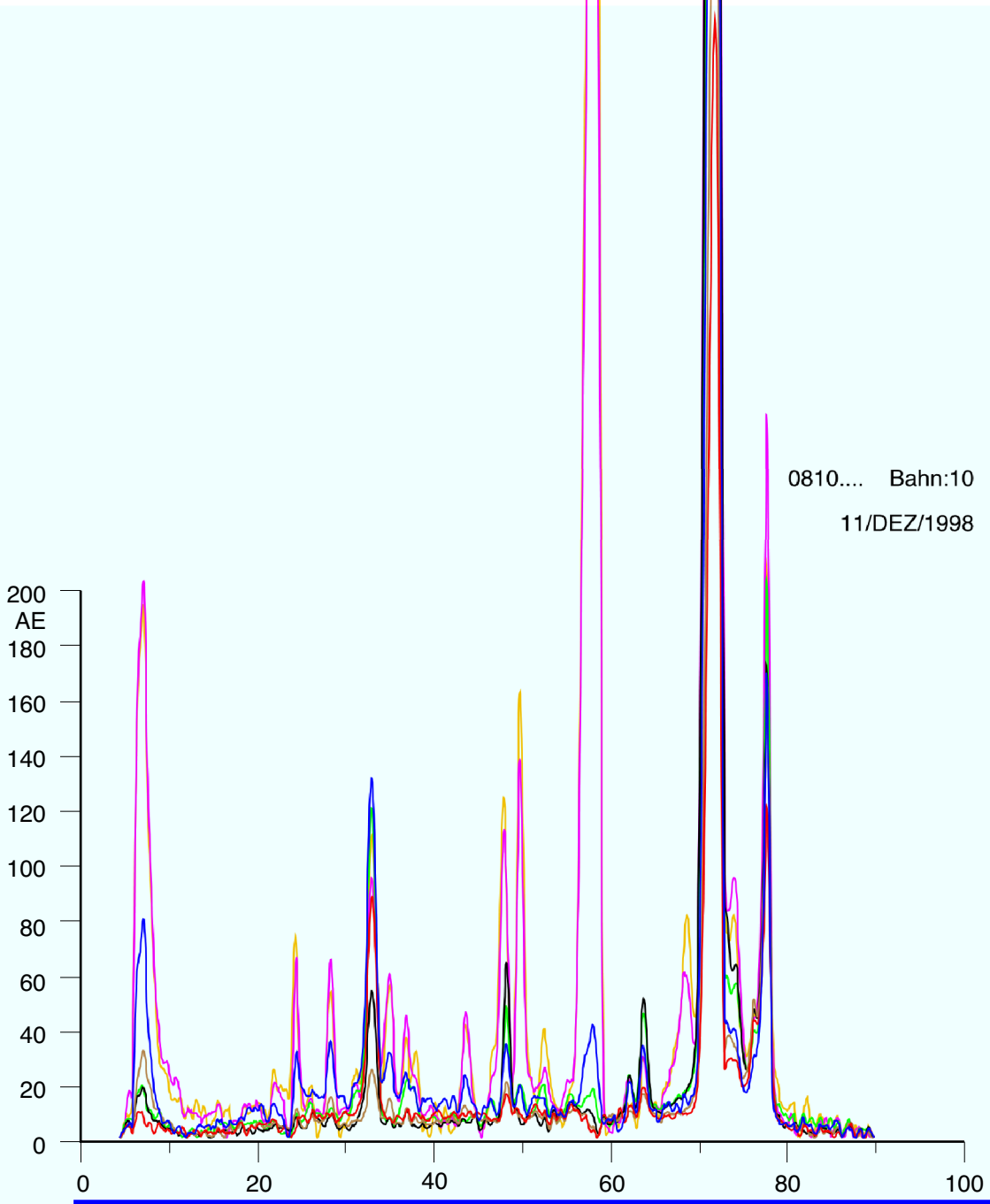
Klaus Burger

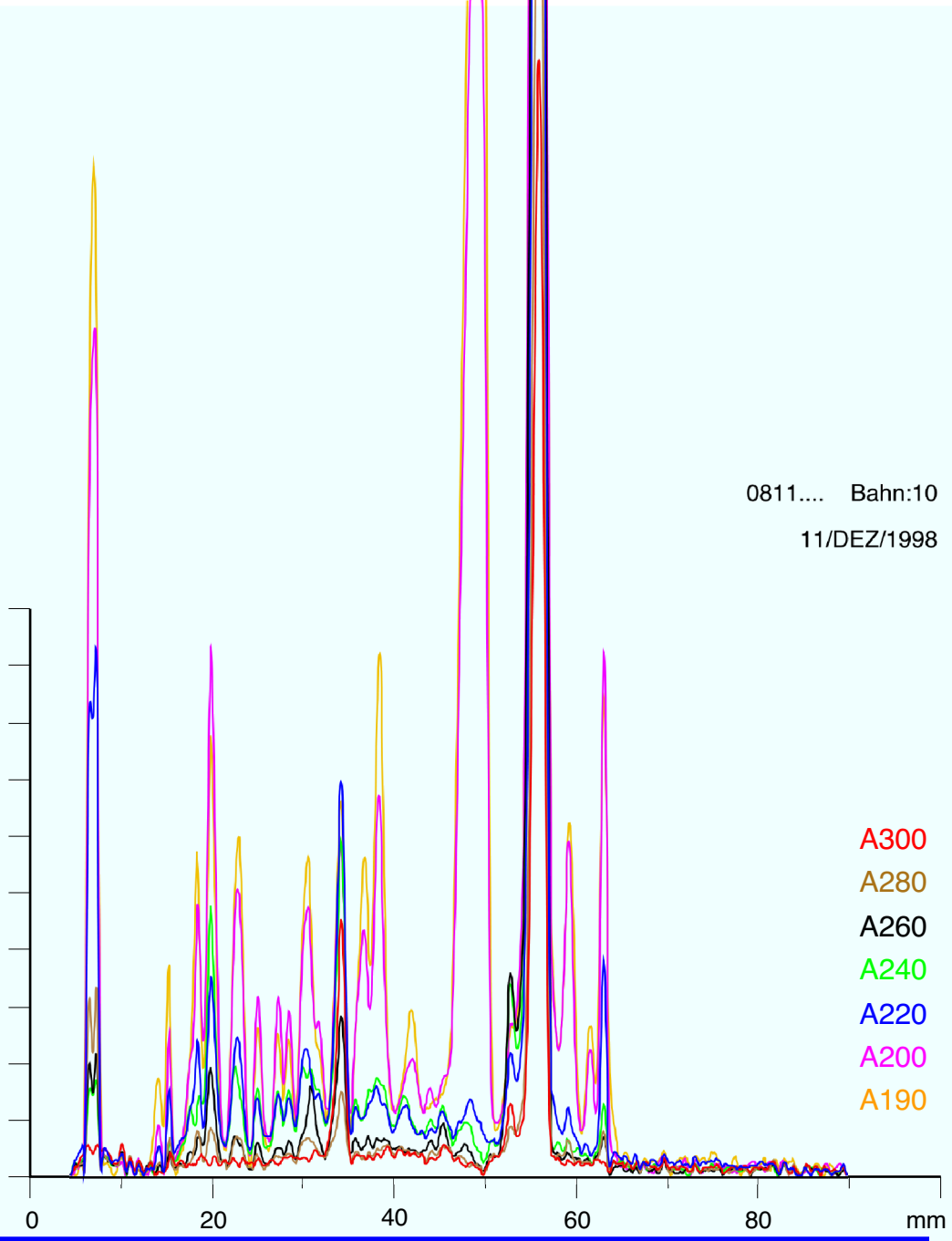


Screening: Product 11

Confirmation: Product 11

Klaus Burger

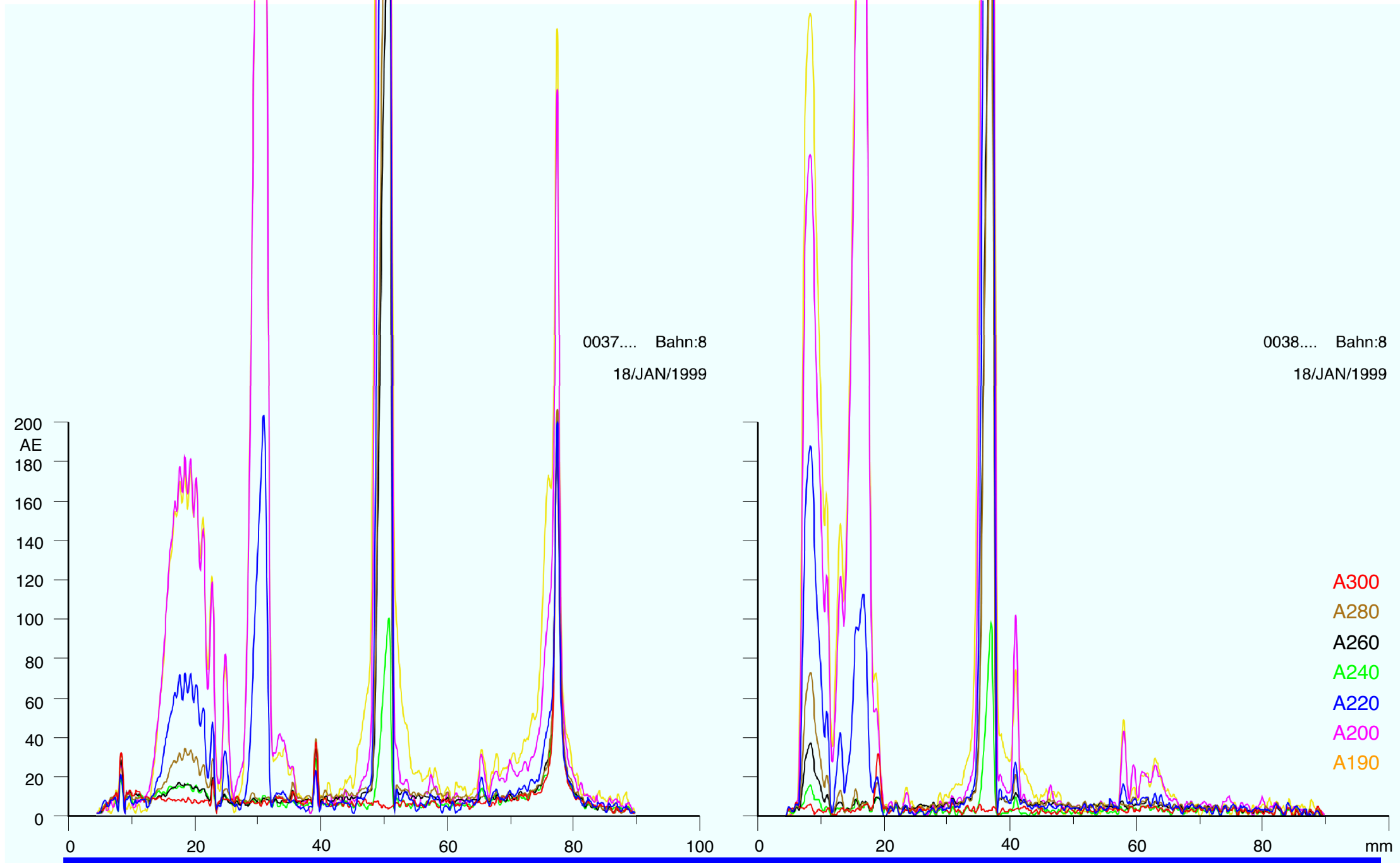




Screening: Product 12

Confirmation: Product 12

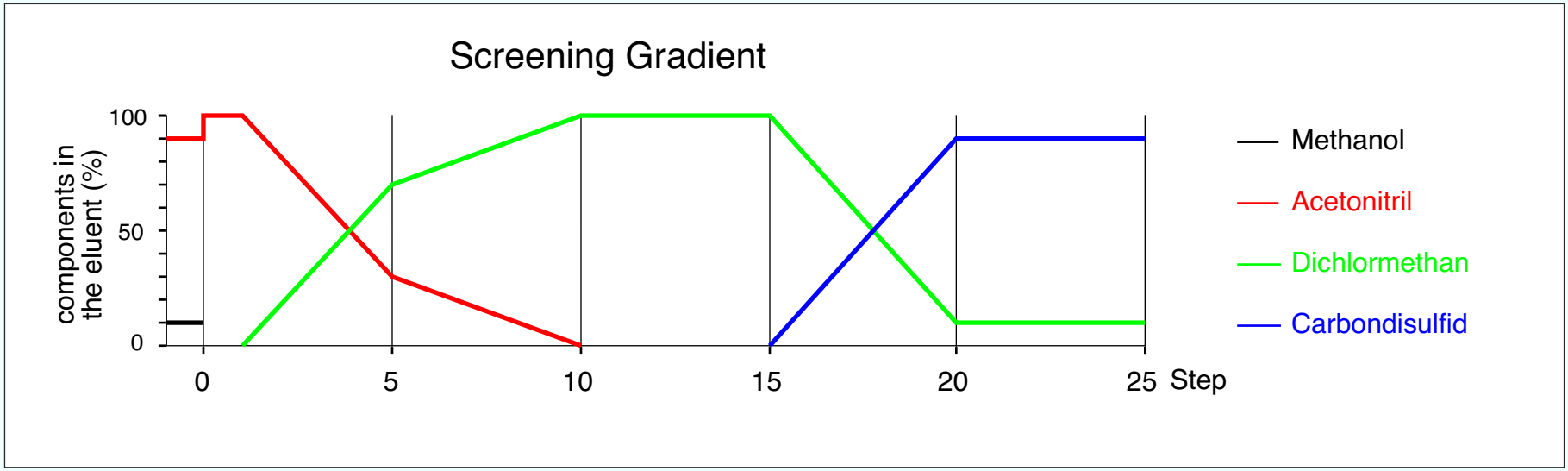
Klaus Burger

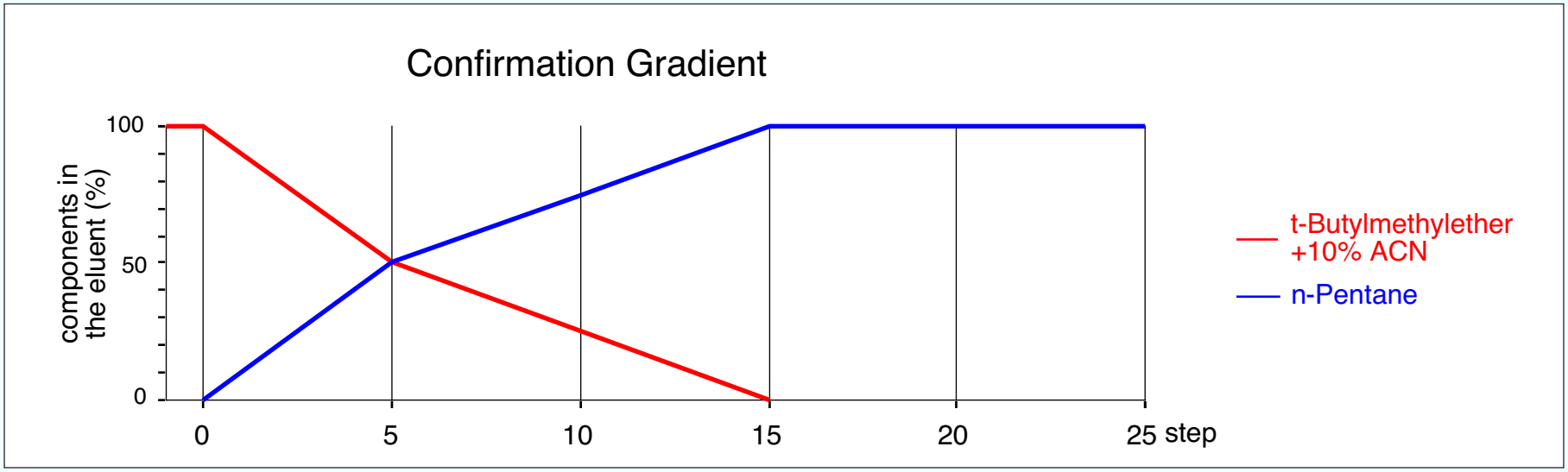


Screening: Product 18

Confirmation: Product 18

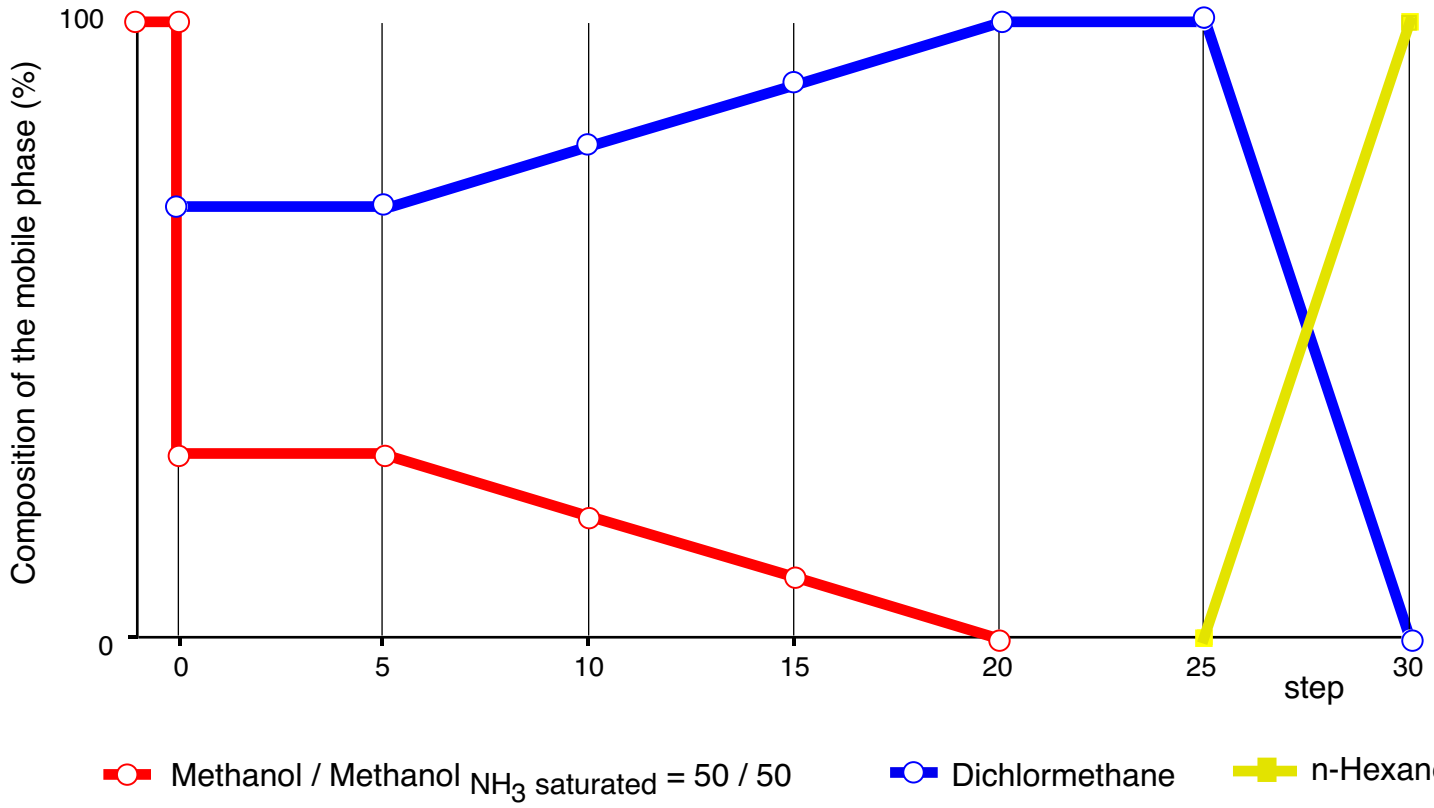
Klaus Burger





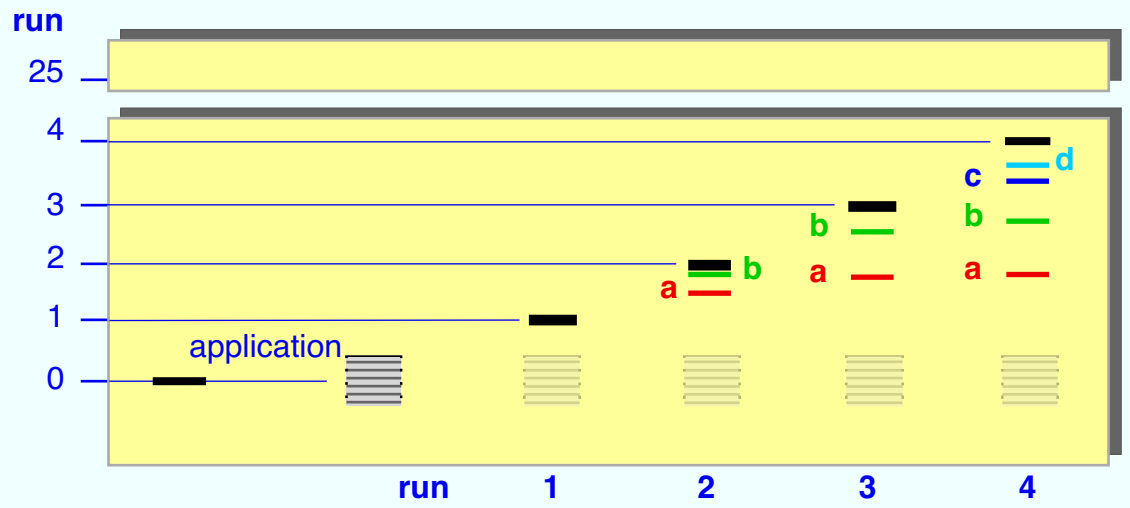
An AMD elution gradient always starts polar and ends unpolar. Each gradient can distinguish at least 25 components.
By combination of this both gradients with pronounced different selectivity, the separation power of both is multiplied.

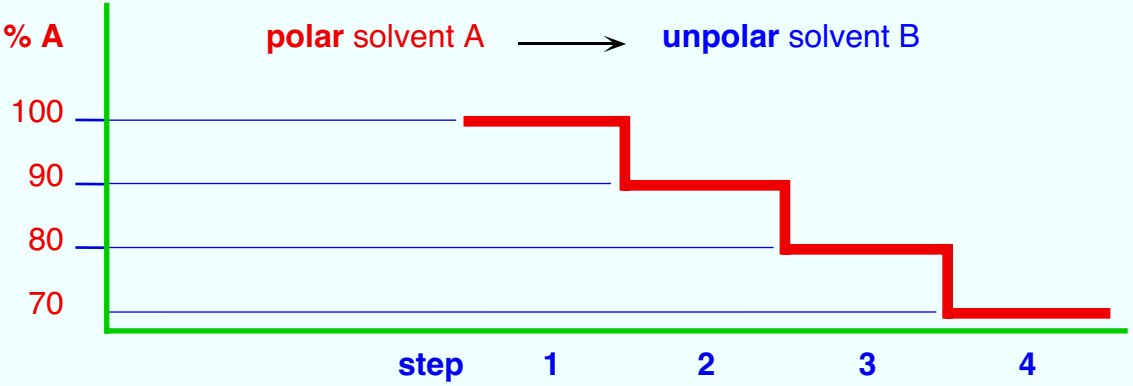
This means: 25 x 25 > 500 components can be distinguished by AMD chromatography.



'Universal Gradient' - gradient elution on silica

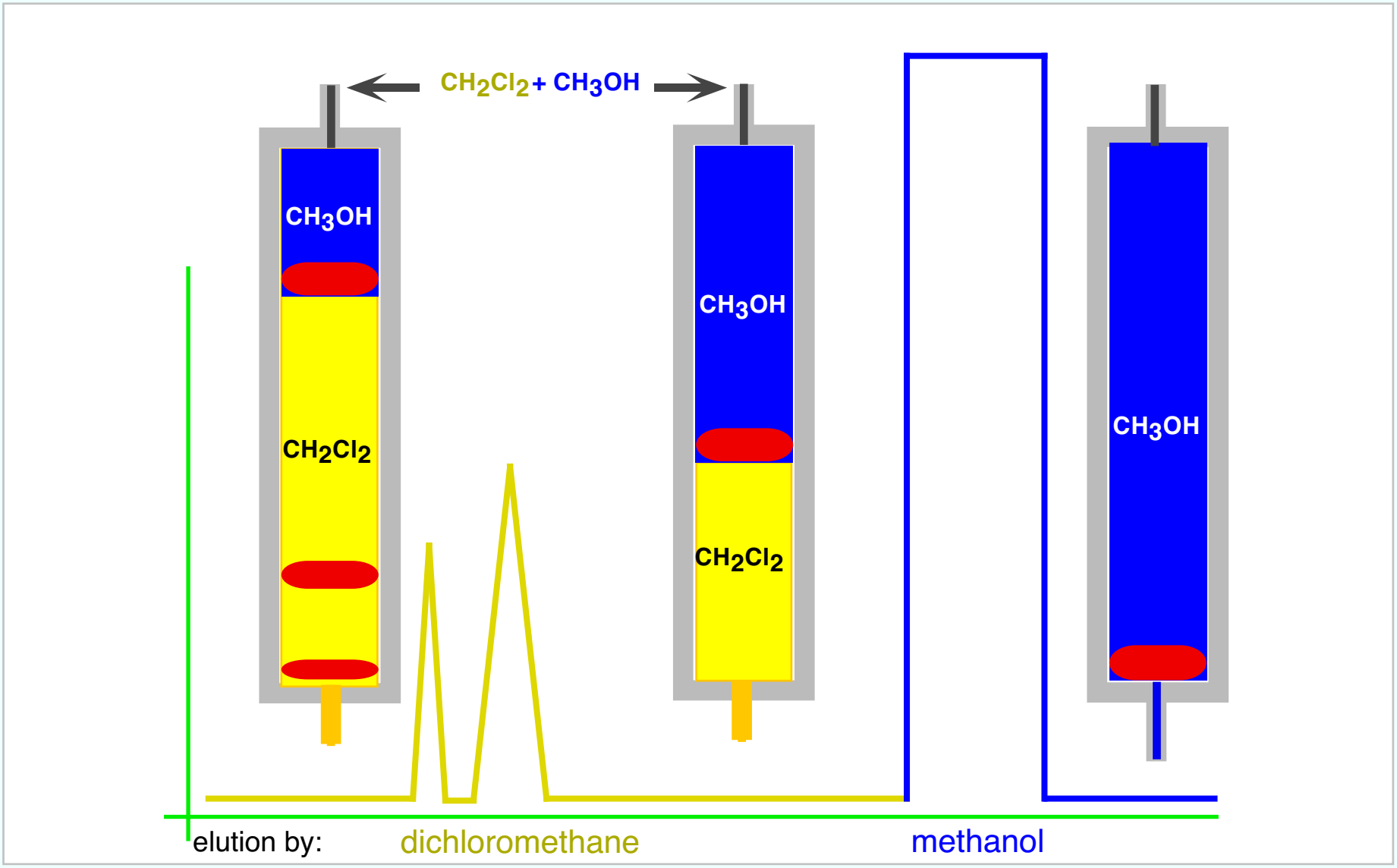
Klaus Burger





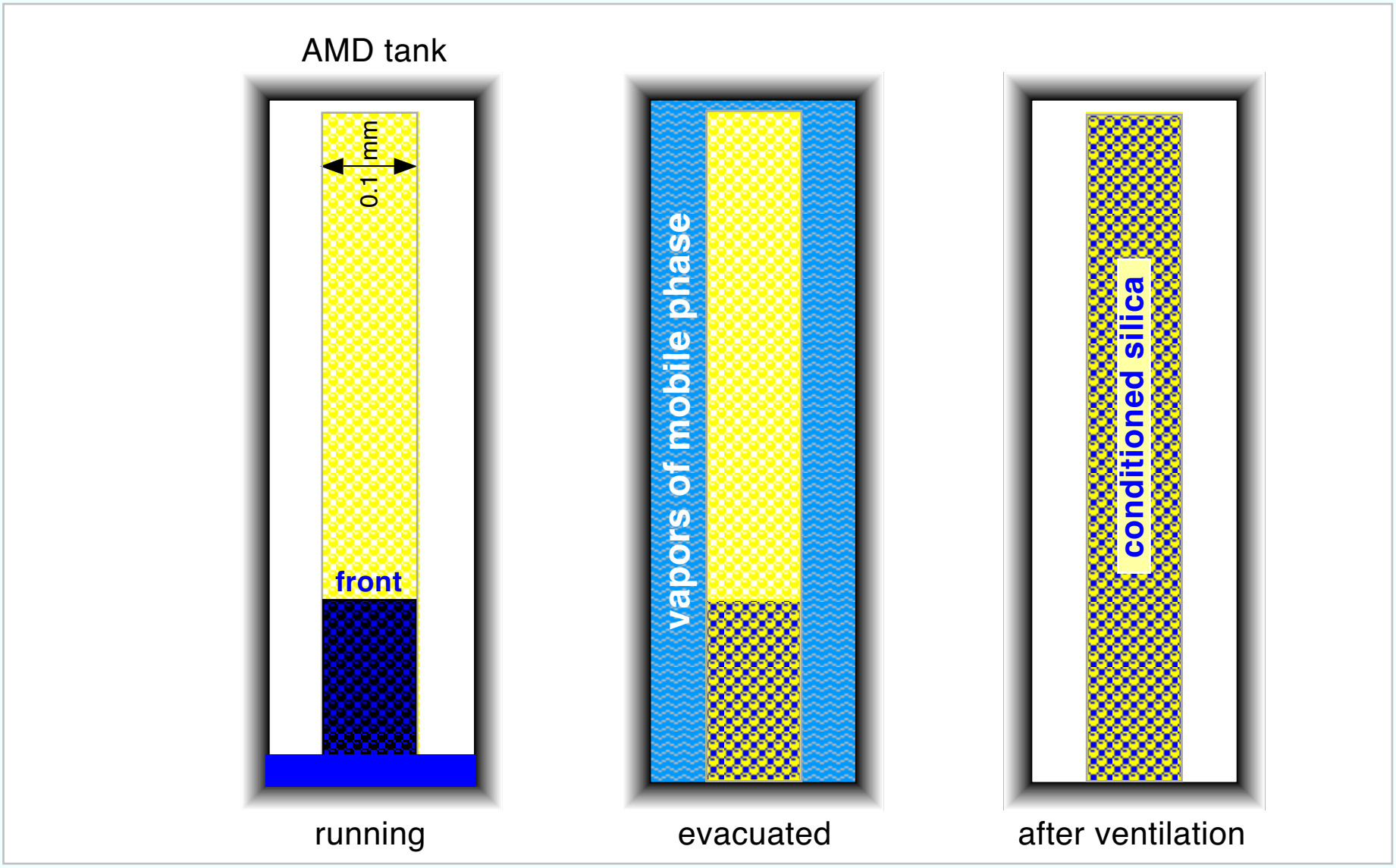
Gradient elution on silica with AMD

Klaus Burger



Gradient Elution on Silica in a Column

Klaus Burger



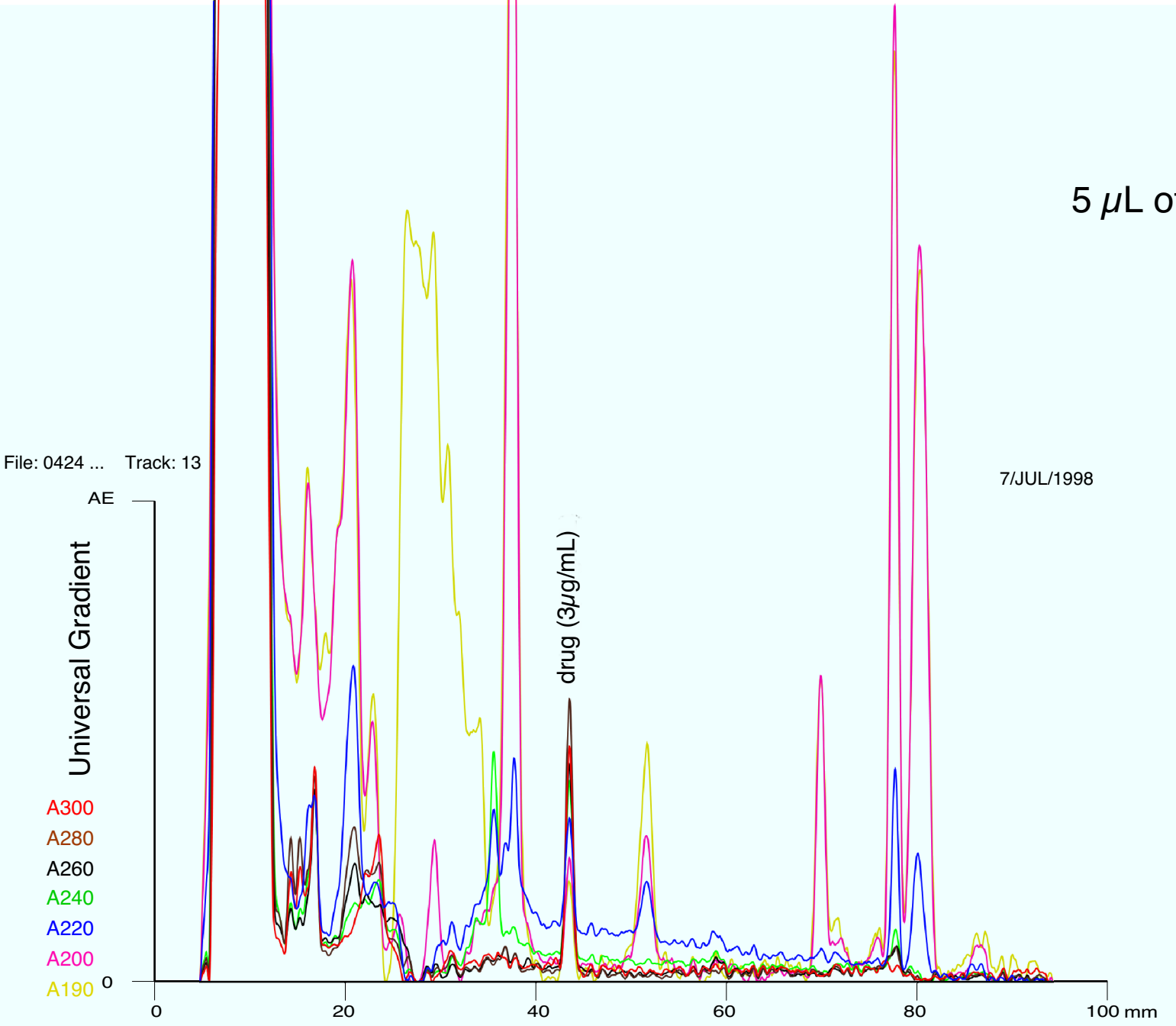
Conditioning of the layer by drying with vacuum

Klaus Burger

Due to gradient elution on silica AMD is an extremely universal liquid chromatographic separation technique.

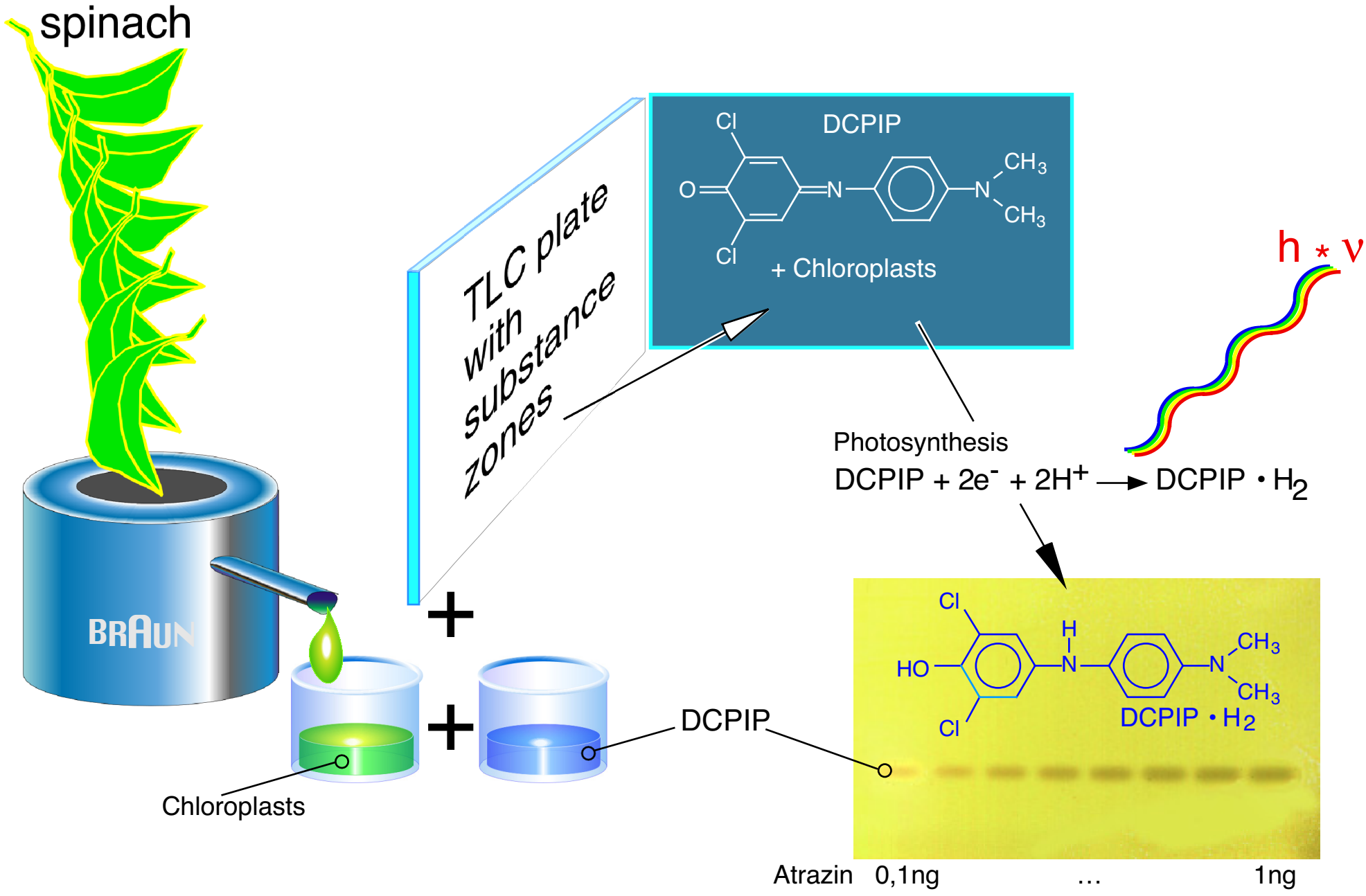
Acids, bases, neutral, hydrophilic and lipophilic compounds even inorganics can mostly be separated in the same system without any optimization.

5 μL of serum



Universal screening for drugs and metabolites in serum or full blood

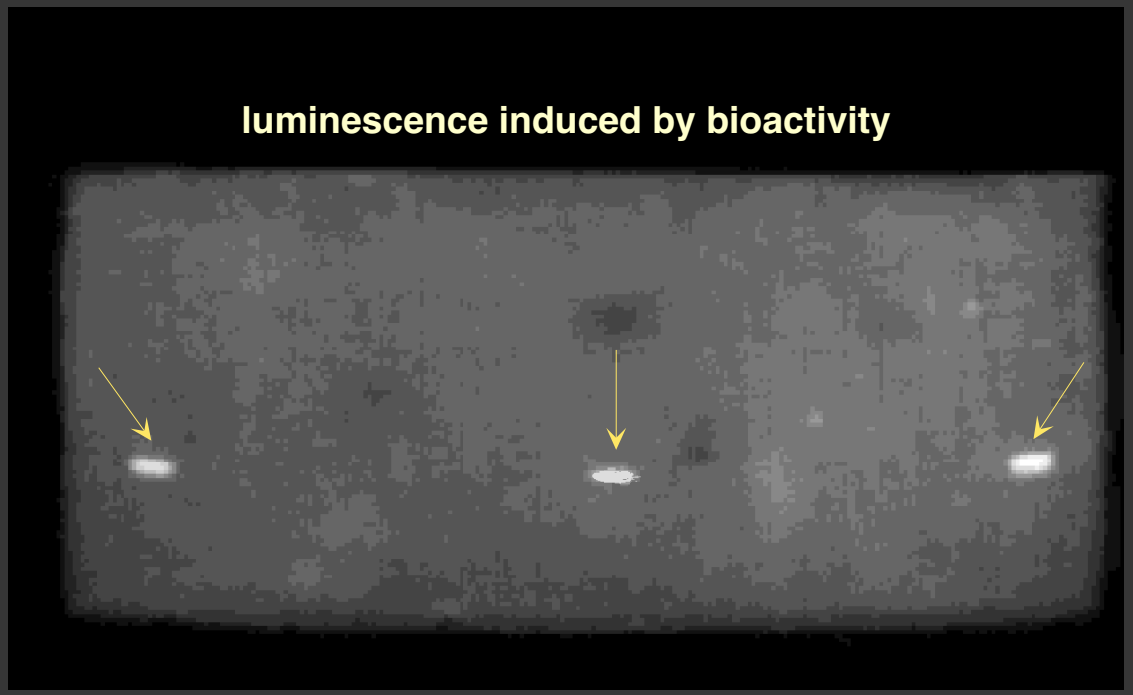
Klaus Burger



Bioactivity Screening for Photosynthesis Blocker

Klaus Burger

Universal Gradient as a must for a successful screening



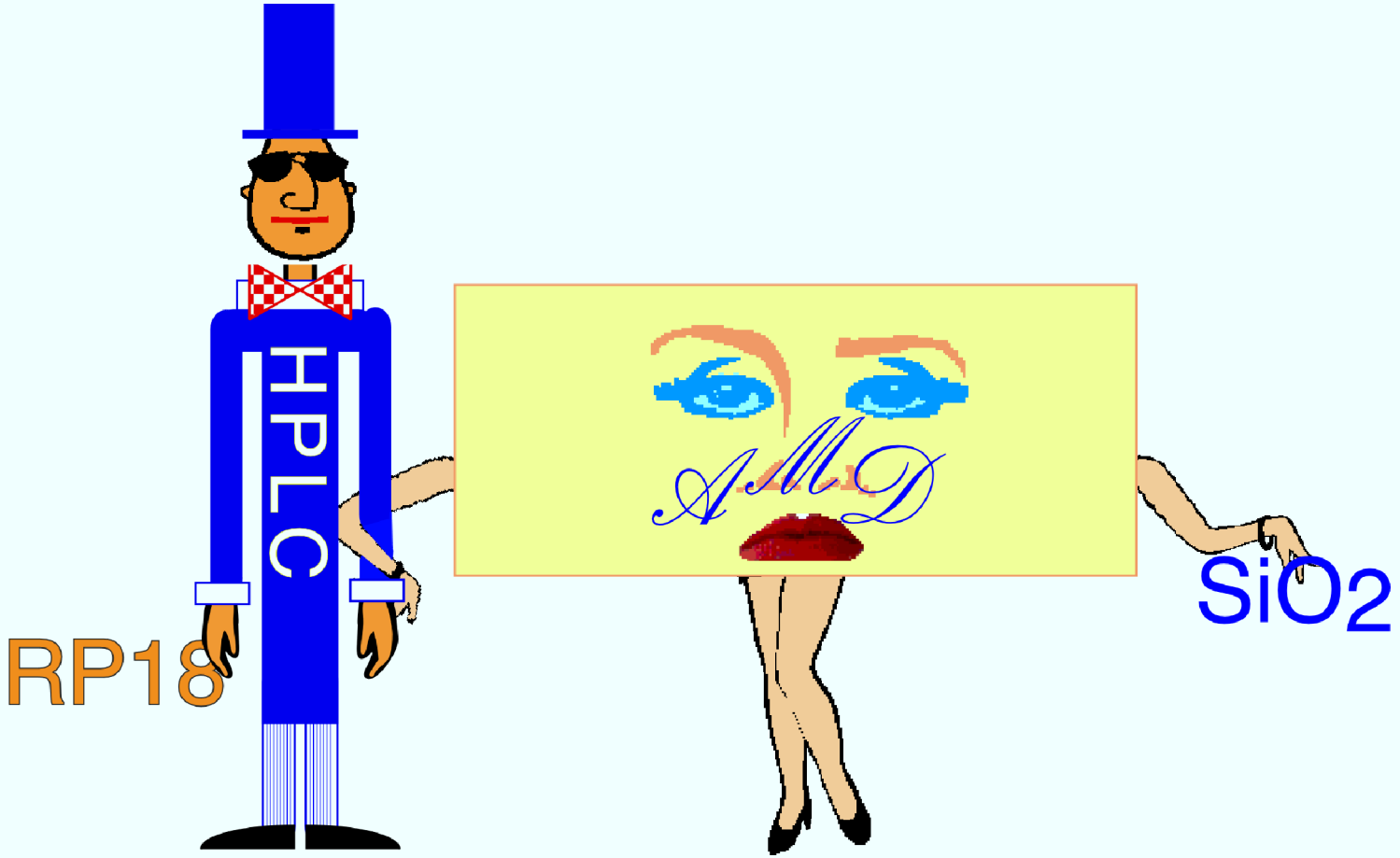
Screening for antibiotics in extracts from biological material.
Detector: Taylor made luminescent bacteria

Klaus Burger

As an universal, open and clean! separation system, AMD can easily be coupled to other techniques.

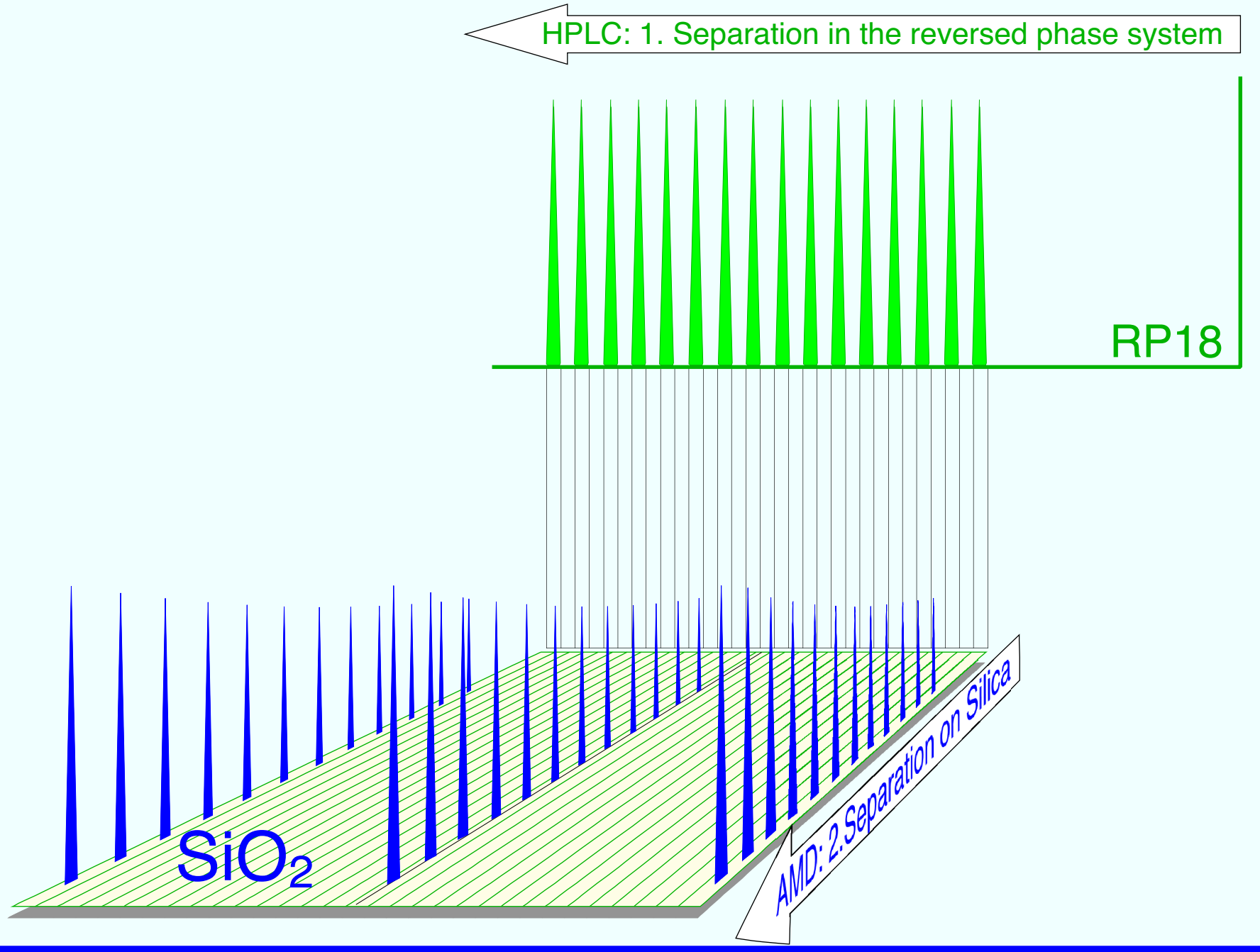
Example

HPLC online coupled with AMD



A powerful couple

Klaus Burger



Online Coupling HPLC-AMD: Principle

Klaus Burger

It's comfortable paradise.

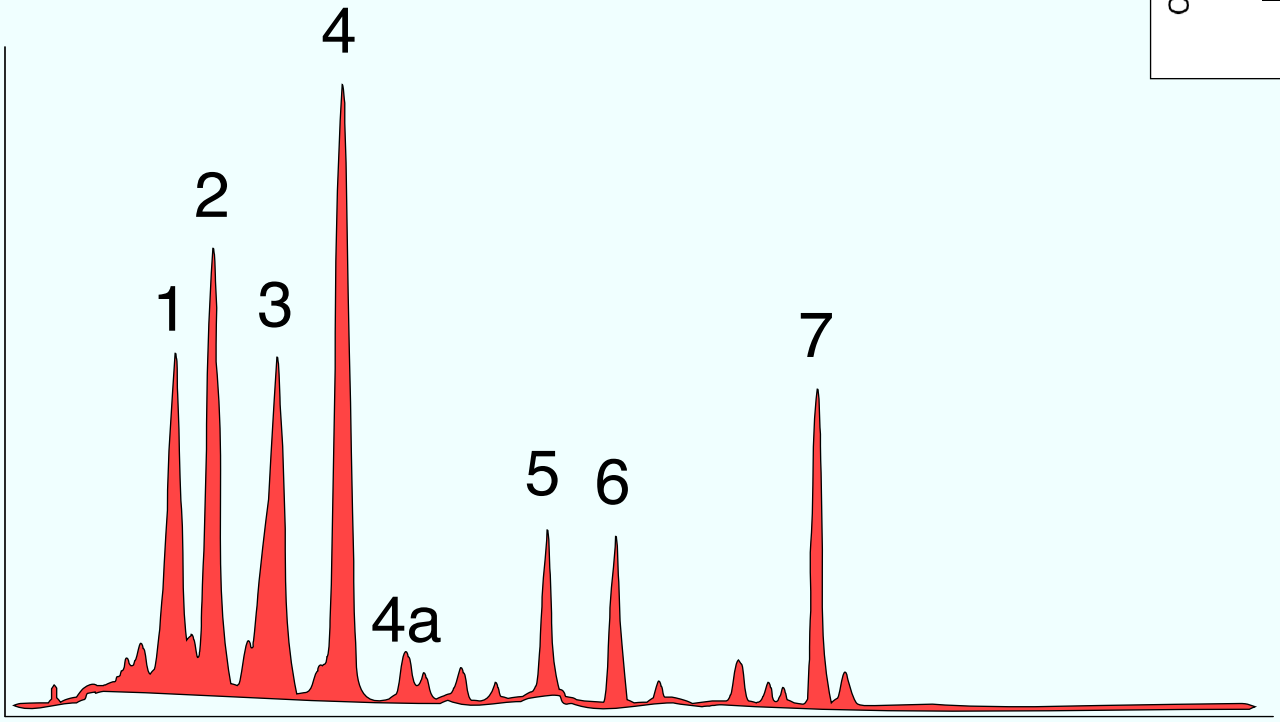
Where are the separation problems?

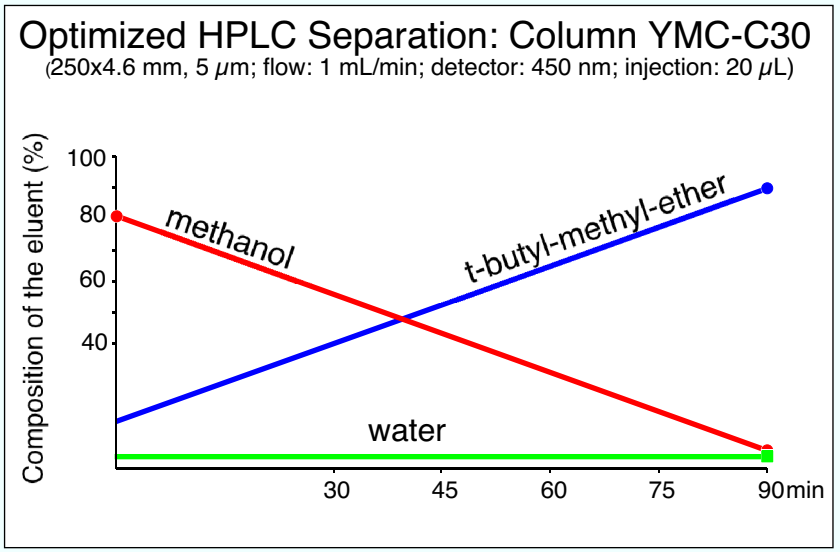
He now can answer questions some people don't dare to ask…

For the effective genetic manipulation of pepper it was necessary to determine the content of carotenoids in the fruits.

About 200 naturally occurring carotinoids are known.

The analytical method has to distinguish between these carotenoids AND all components from the matrix

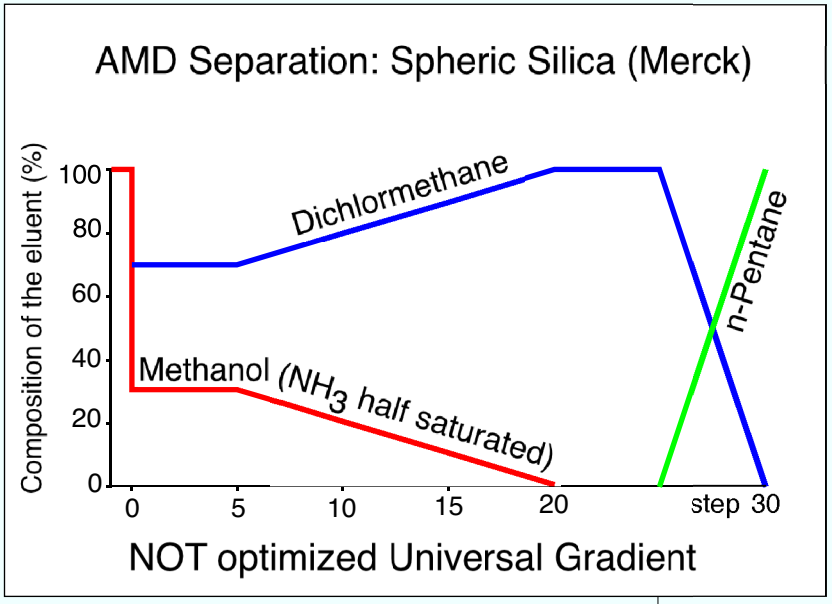


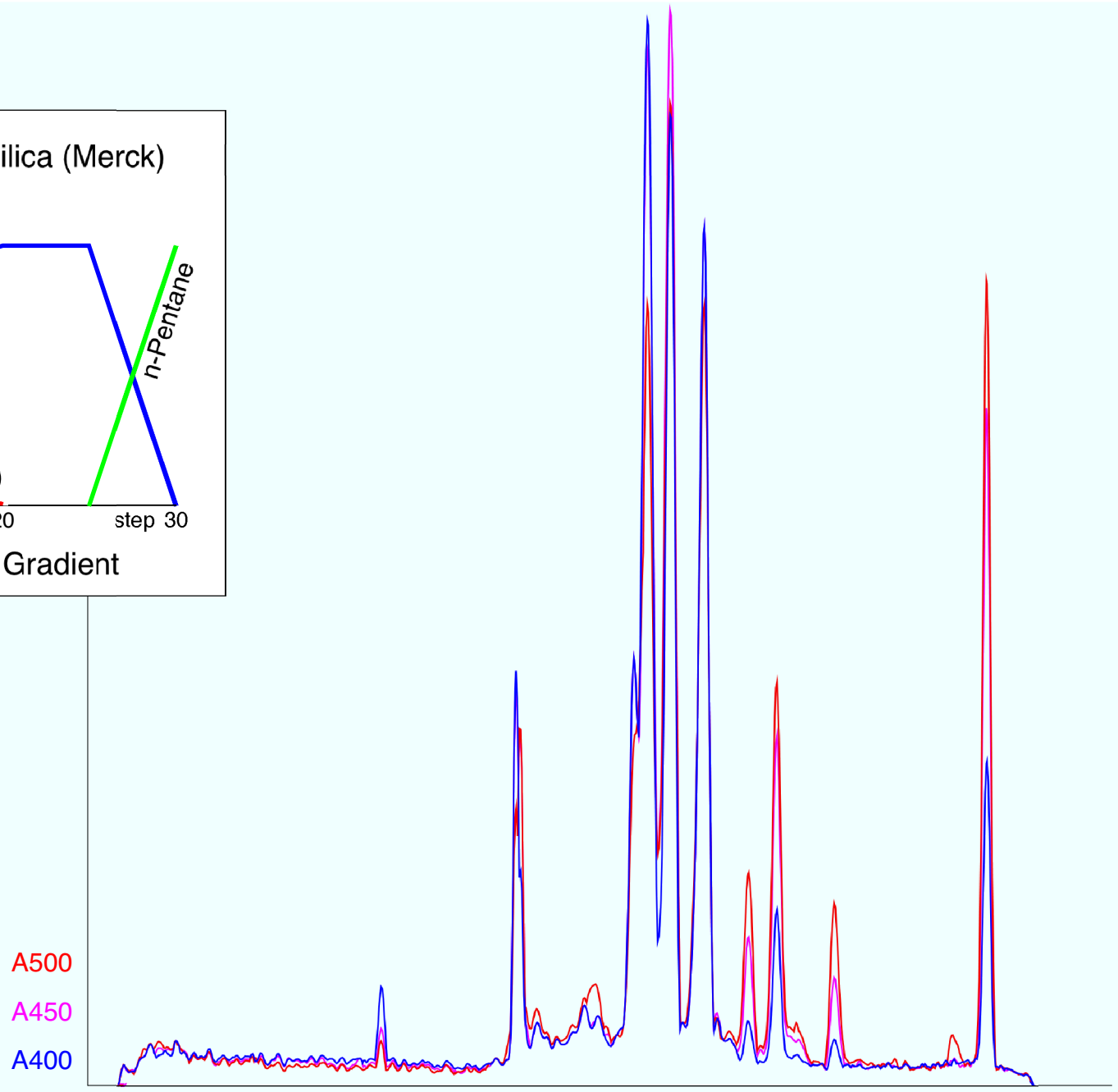


1 = Capsorubine
2 = Violaxanthine
3 = ?
4 = Capsanthine (main carotenoid of red pepper)
4a = Zeaxanthin
5 = ß-Cryptoxanthine
6 = ?
7 = all-trans-ß-Carotene

Carotenoids: HPLC separation of an extract from pepper, VIS-detection

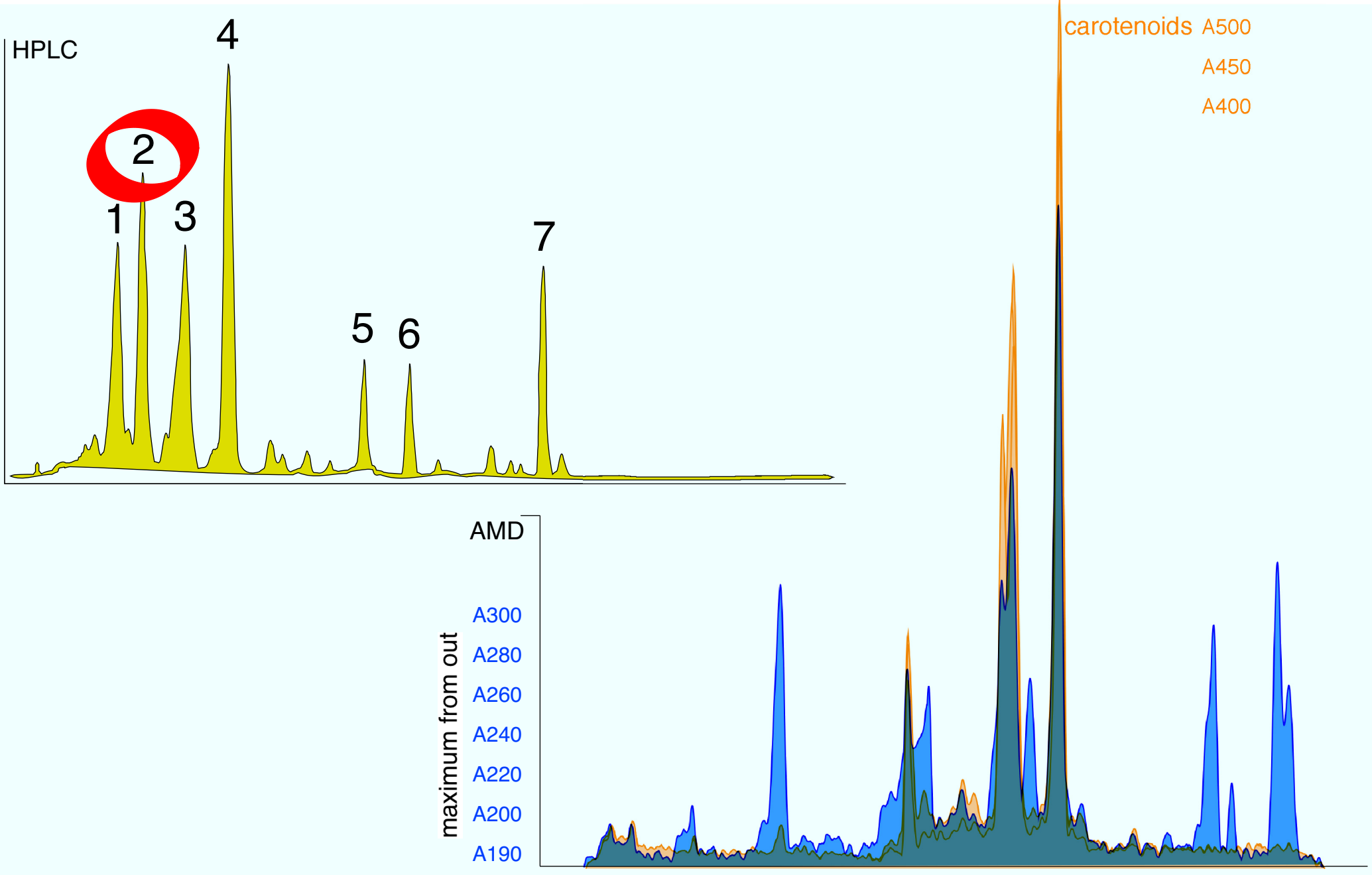
Klaus Burger





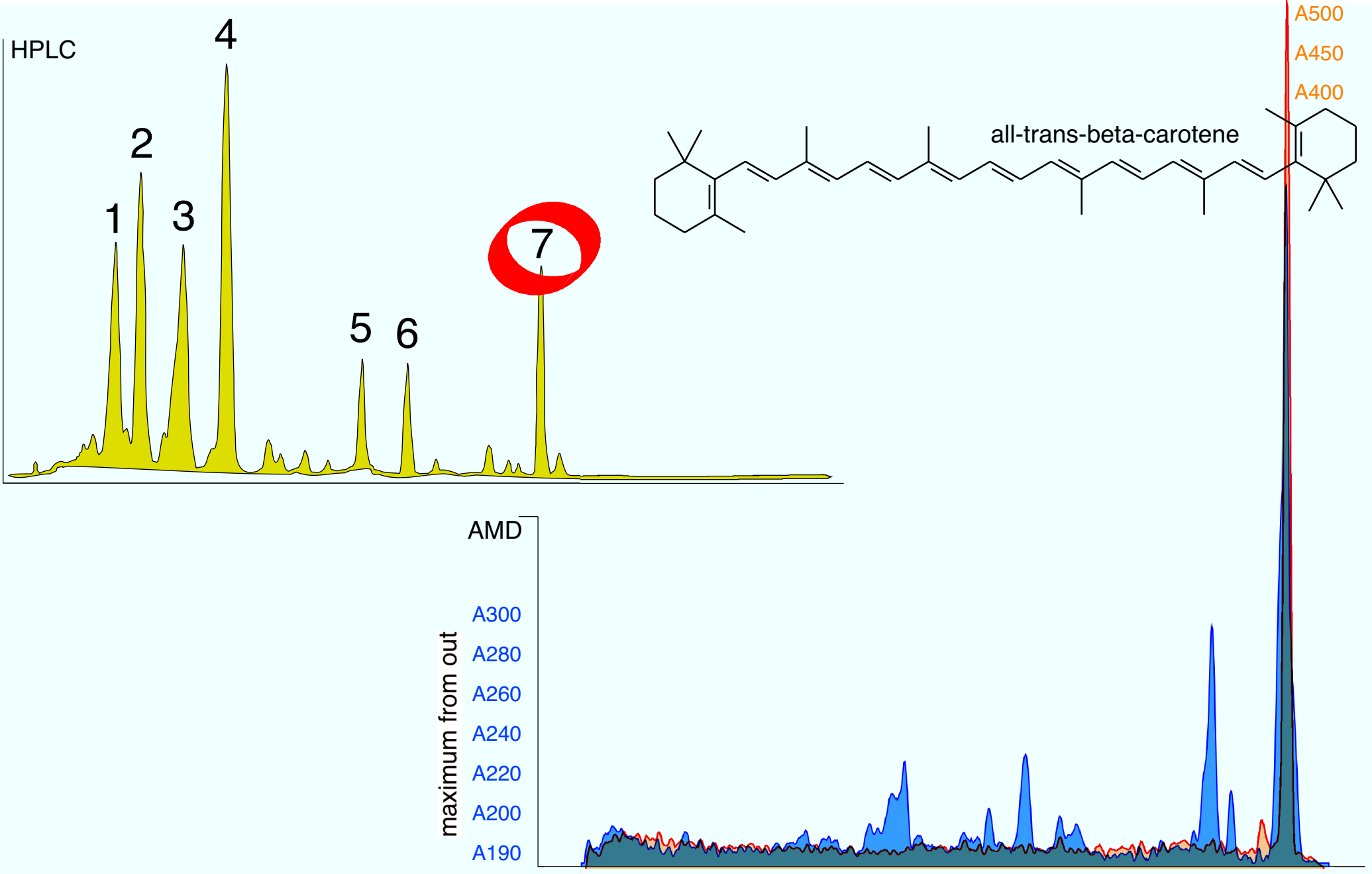
Carotenoids: AMD separation of an extract from pepper, VIS-detection

Klaus Burger



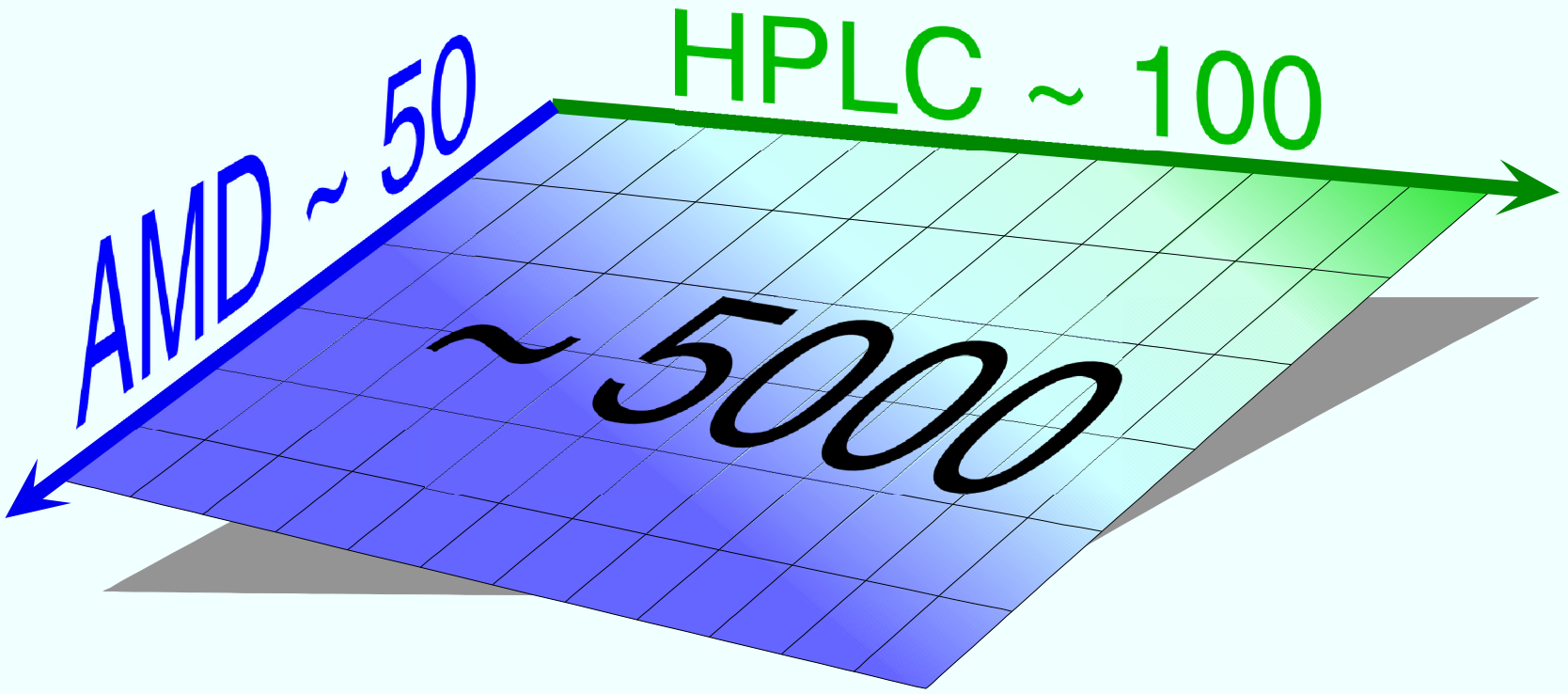
Carotenoids: Fraction 2 from HPLC separated by AMD

Klaus Burger



Carotenoids: Fraction 7 from HPLC separated by AMD

Klaus Burger



> 1000 components can be separated

and beware of the dogs …

… et dona ferentes!

Klaus Burger