μ - PLC

to detect

falsified fruit preservation.

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µ-PLC has been developed

to detect product falsifications.

Its technique, tools and first applications have been e-published as a free book :

www.planar-chromatography-by-kaiser.com

What is the difference µ-PLC to HPTLC ?

The following is NOT available in HPTLC.

- Sample size: below 1 nl to over **1 ml.**
- 2 to 6 samples partially overlapped
  on 10x10 cm plates circular positioned.
- Strictly focussed in the plate centre as µ-circle.
- Completely dried (air / N_2 , 2 L/min, 20 degr C).
- Constant flow mobile phase .
- Gas phase reactions, if needed.
- Digital photo multi integration, if needed.

Specialities in µ-PLC versus HPTLC :

- Taking, giving, positioning and enriching liquid or solved samples in µ-PLC **exclusively by micro brushes**.
- **No mobile phase tank**. Replaced by a static gas phase layer of 1 mm thickness in precise horizontal position.
- **No beta phases** in µ-PLC.
- Mobile phase in constant flow from a closed 1 ml micro flasc through a wick into the HPTLC plate centre under **glass cover**.

Precision and accuracy conditions in µ-PLC :

- Phase inlet position + - 0.2 mm central.
- Plate positioning strictly vertical.
- Phase flow, drying and gas phase treatment at constant temperature and strictly symmetrical .

No errors exist in compare µ-PLC besides wrong sample or sampling

- No any quantitation needed if compared samples differ in their overlapped region, then quantity errors simply NOT possible.
- In THIS case we have 100 % save results with the statement: „the substances differ qualitatively for sure“
- This is the first time an analytical result „it differs“ is error free. But overlapping is mandatory which means: it is an analytical „sample-**within**-sample“ technique.

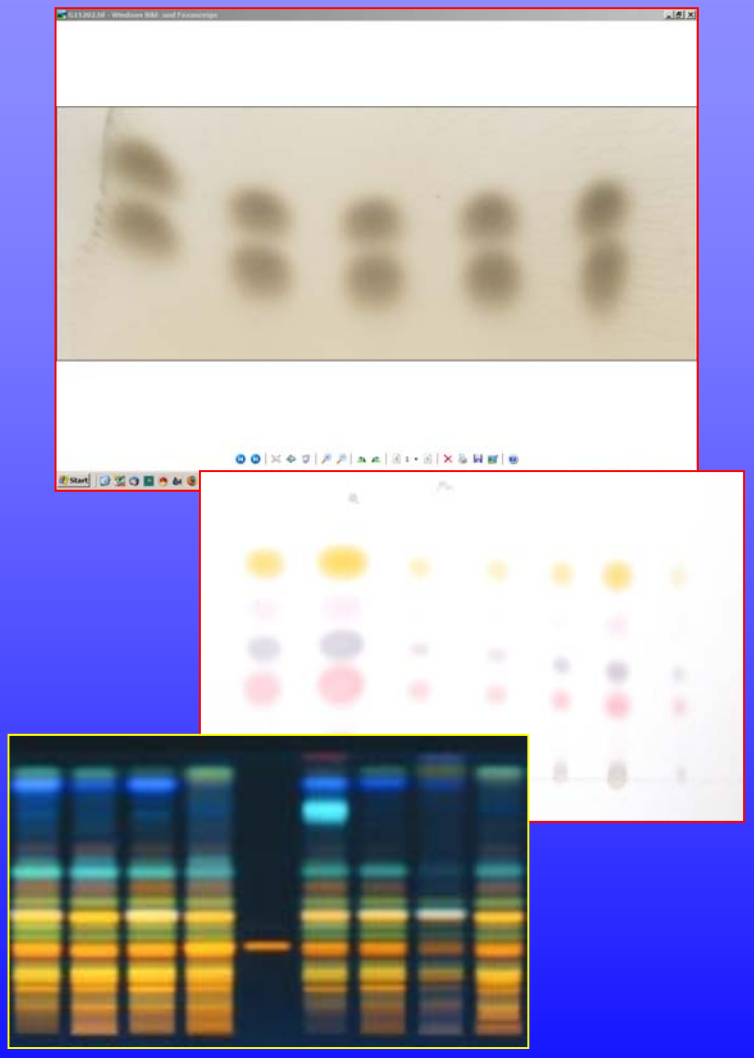
Possible quantitation quality in µ-PLC:

In case quantitative differences are detectable there ARE errors – of course, but:

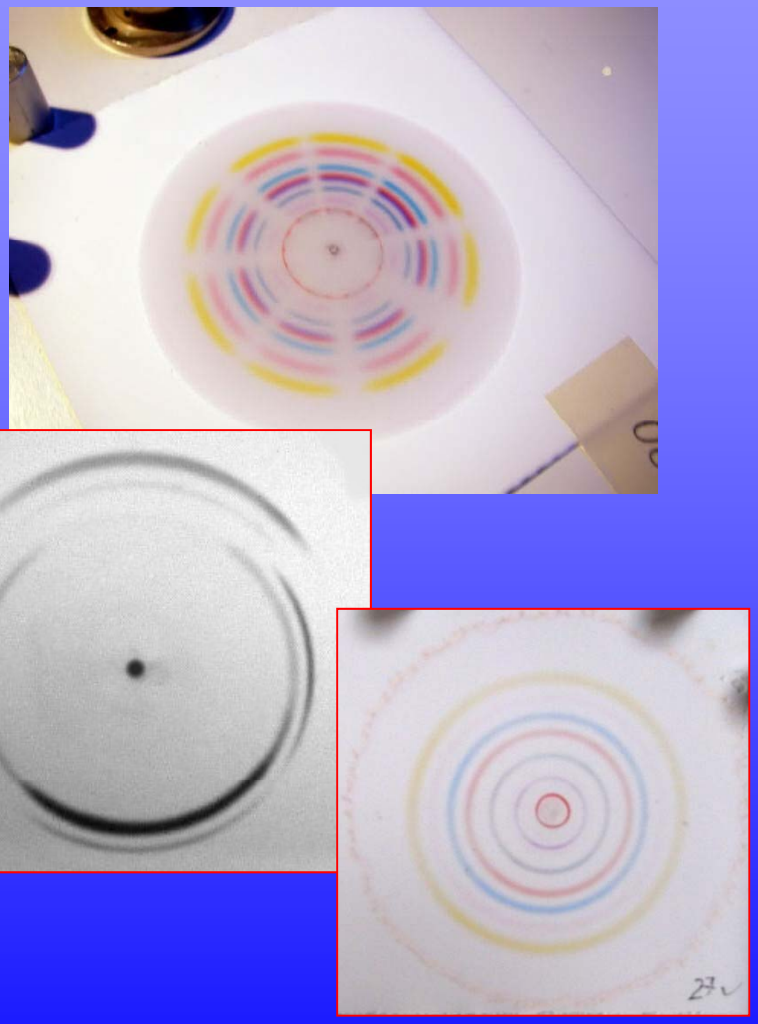
Quant. comparability data normally reach **+- 0.5 %** and could be improved to **+- 0.05 %** rel. standard deviation

by **multi integration**, which runs at N >= 4 , never at N < 4, like in most of the regulated analyses.

Classical PLC and HPTLC



µ-PLC is always circular



comparing samples:

Classical PLC/HPTLC analyses run samples

> parallel to each other.

All GC, HPLC a.s.o. analyse samples

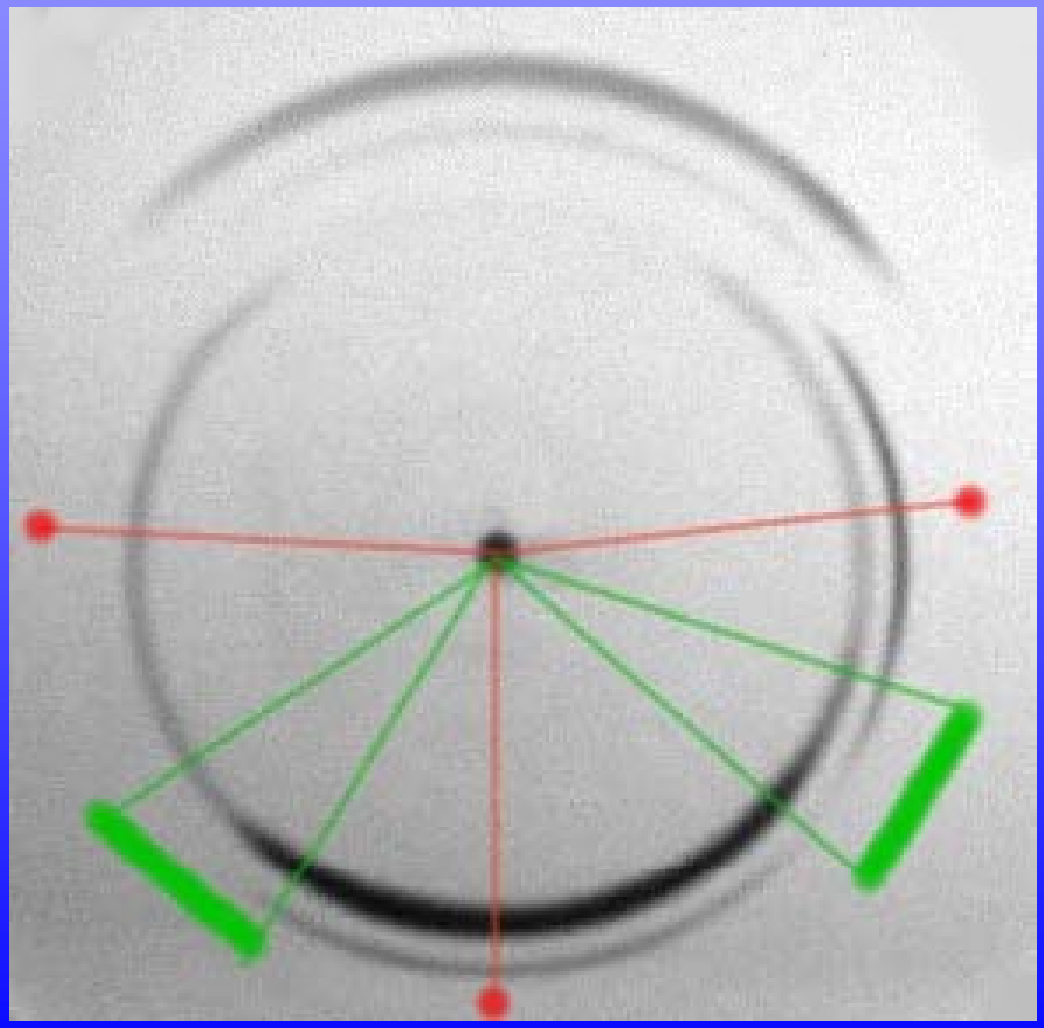
> next to each other.

This differs fundamentally from µ-PLC.

It analyses circular by partial overlapping

> within each other.

Only this new technique offers 100% „safety of results“, see the simple facts in the green triangles :

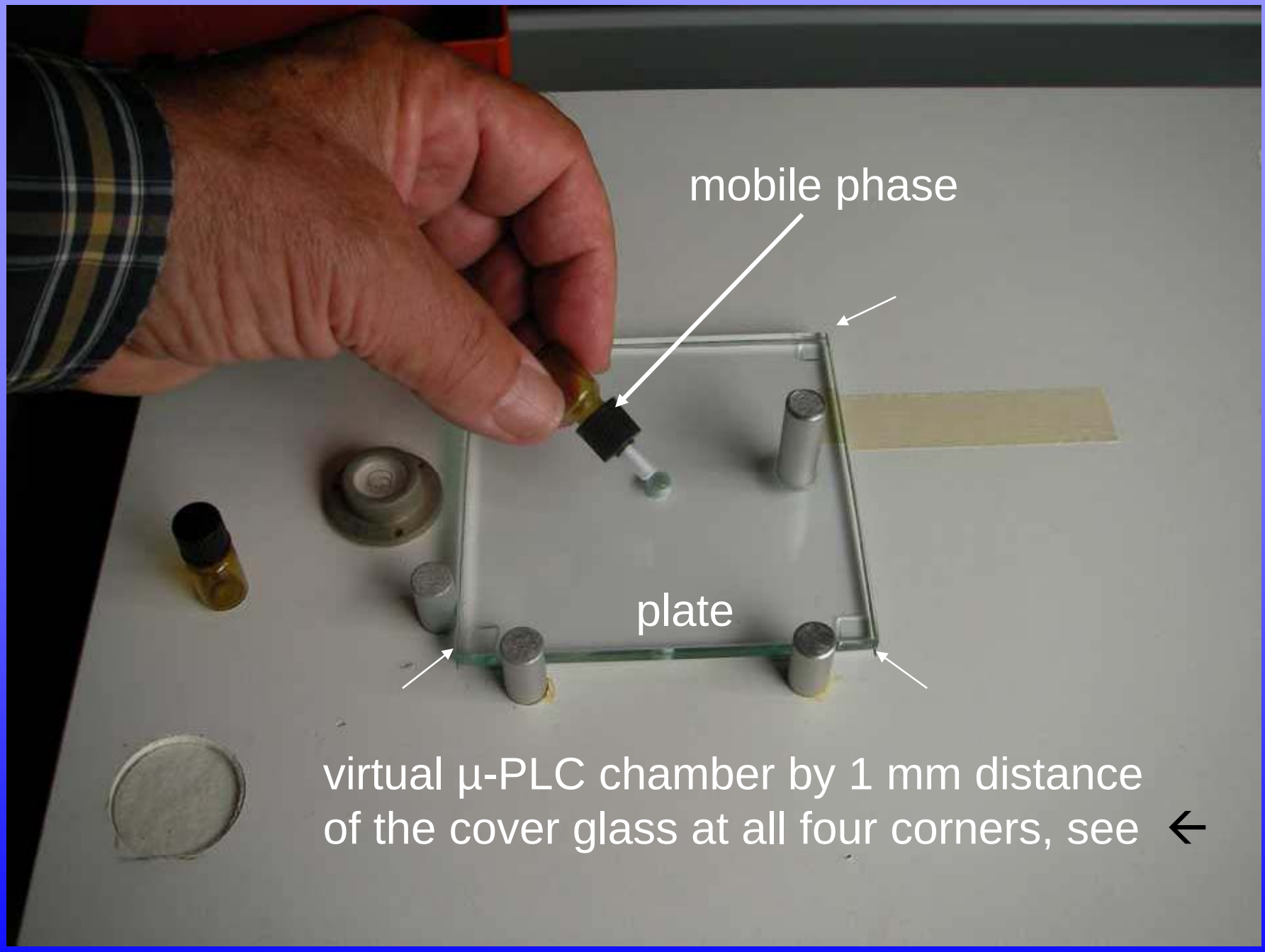


µ-PLC instrument is a complicated and / or expensive tool ??

- **Not at all.**
- **Read the free e-book**

www.planar-chromatography-by-kaiser.com

About 200 pages, 200 figures, click-connected.


mobile phase
plate
virtual µ-PLC chamber by 1 mm distance
of the cover glass at all four corners, see ←


CAMAG

Taking extract samples from a fruit surface by a wet µ-brush

- 1 cm^2 extr. by micro brush no.3 to 5 with CH_2Cl_2

Why does this work ?

- The capillary forces in a slightly wet brush tip are STRONGER than in a < 0.5 mm i.d. micro glass tube. Equal type liquids are very fast soaked from the tube into the slightly wet brush.
- But the capillary forces at the porous PLC layer surface are stronger than in a wet micro brush tip.
- Therefore: there is a perfect material transportation from a fruit surface into the brush tip and from here into the thin layer – nearly quantitative and fast.
- This is physics. Now the chemistry:

The fruit surface is covered by natural products solvable in CH_2Cl_2. They chemisorb on silicagel. Impregnated fruit surfaces add further material, which may be visible by planar chromatography, see the „BIO“-orange material below:

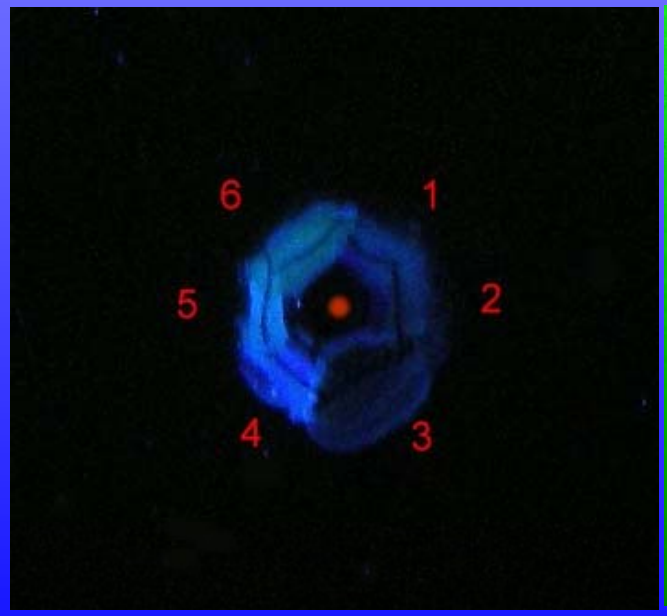
Sampling spot limitations

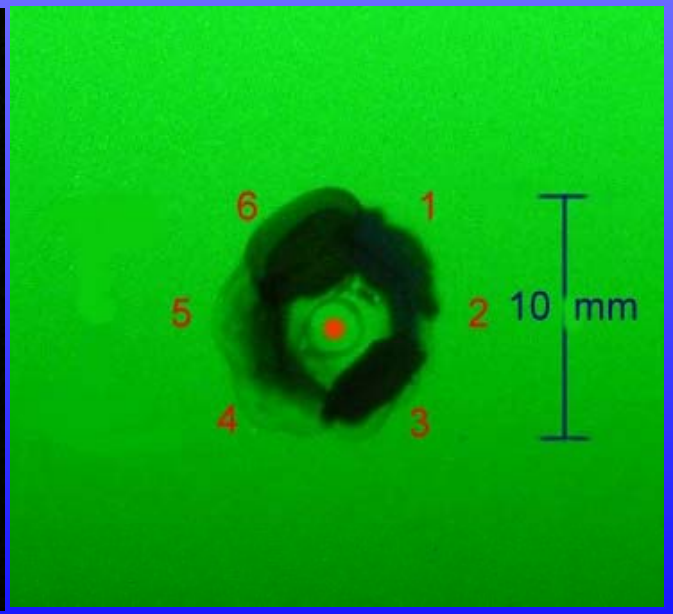
on 10 mm hexagon, 6 samples.

TH = Thiabendazol, IM = Imazalil, OP = ortho Phenylphenol

about 20 µl, 0.1 % solutions each.

Chemisorbing natural skin materials show PLC problems.





1 = Moro Orange

2 = Mandarin + IM

3 = Bio Lemon

4 = Banana

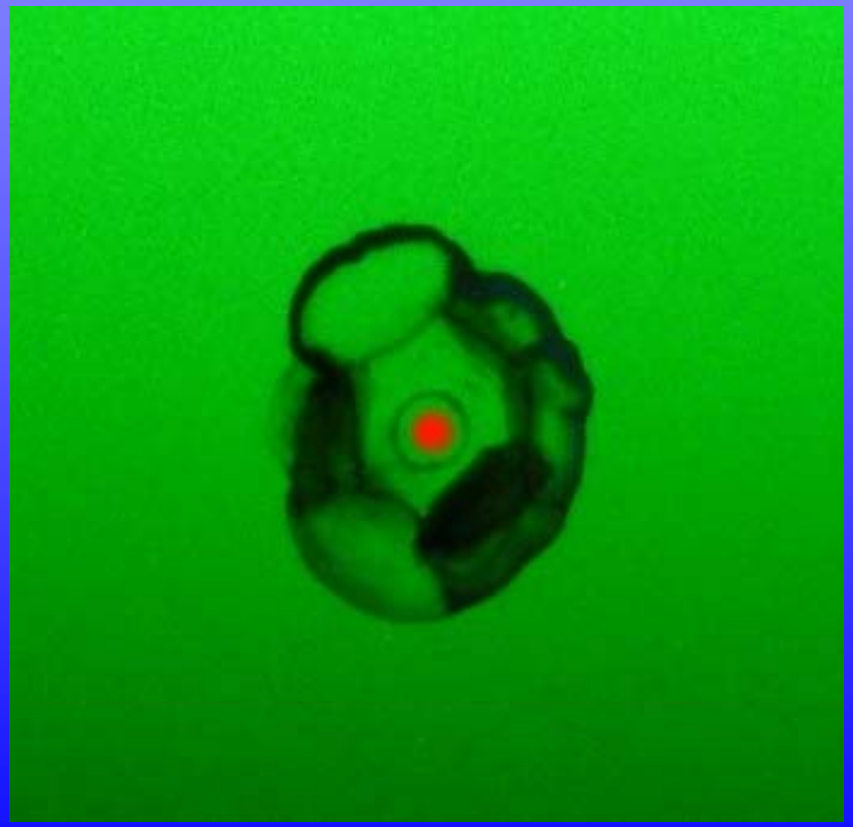
5 = Apple

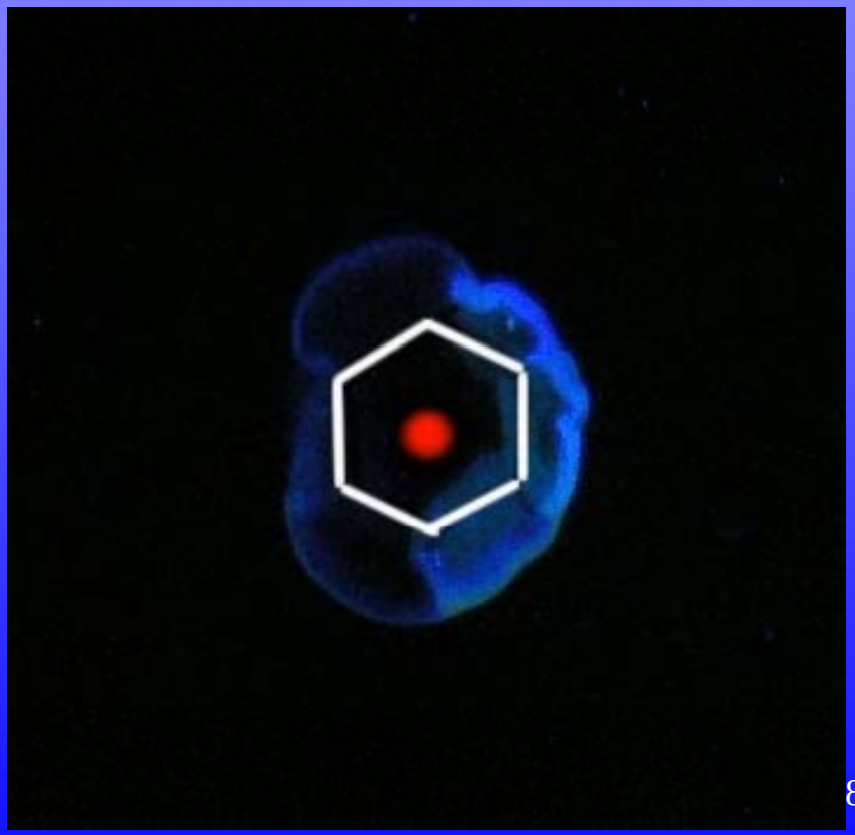
6 = TH + IM + OP

Also fokussing is at its limits

after the first 20 µl CH_3OH

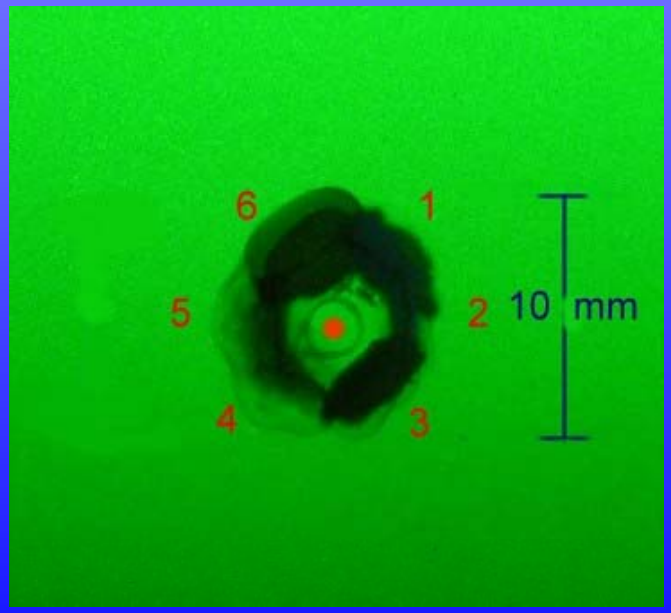
because of the chemisorbing natural skin substances





Despite of poor sampling spots and focussing at limits :

it is clearly seen : fruit ‚1' **is illegally** coated.
Fruits 2, 3 and 5 look like not truly ‚BIO'



1 = Moro Orange
2 = Mandarin + IM
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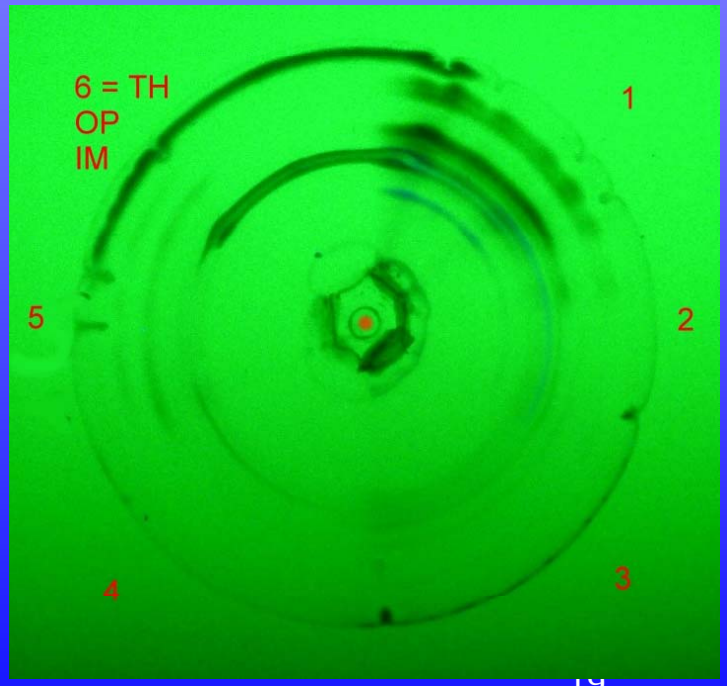
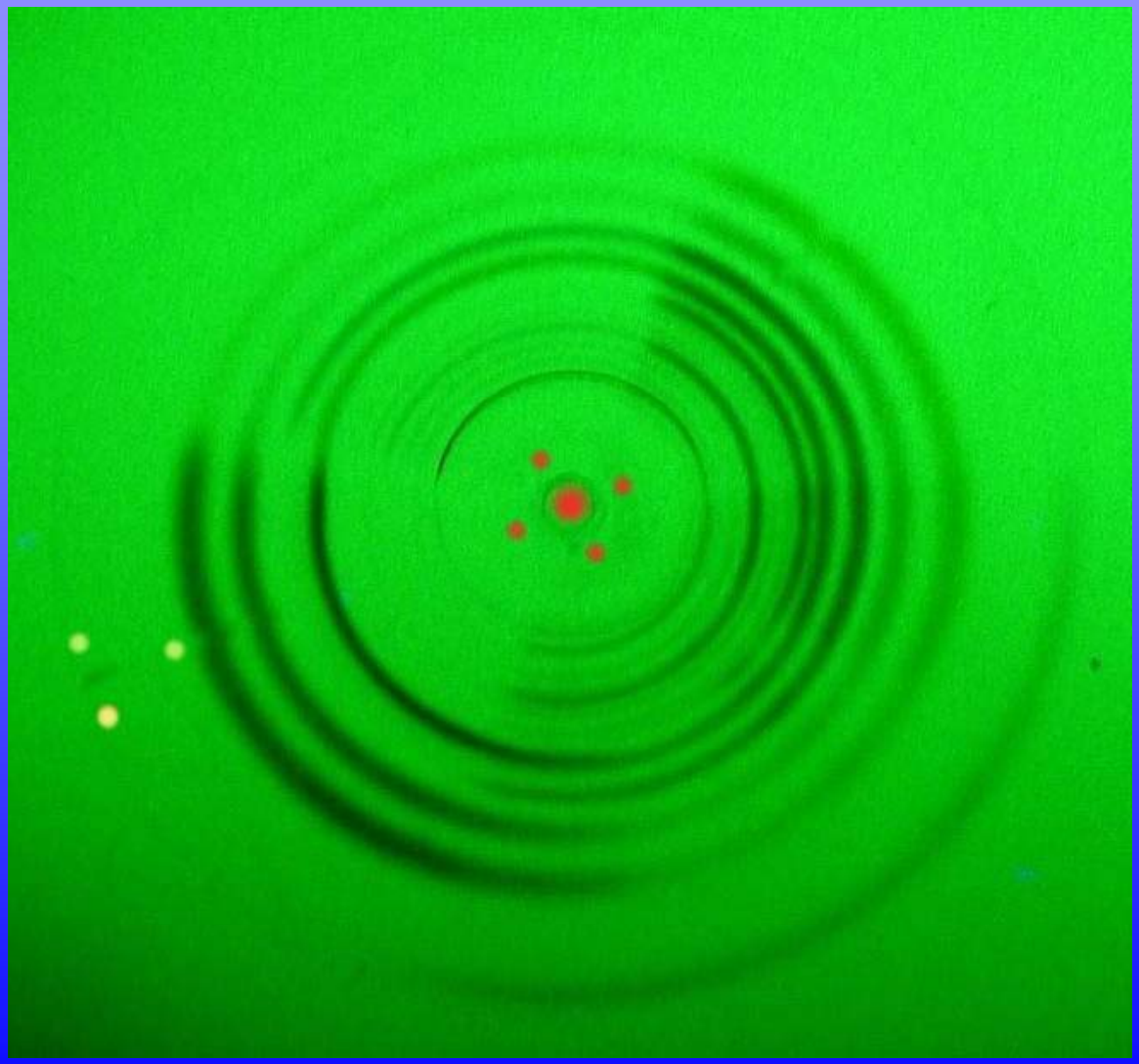


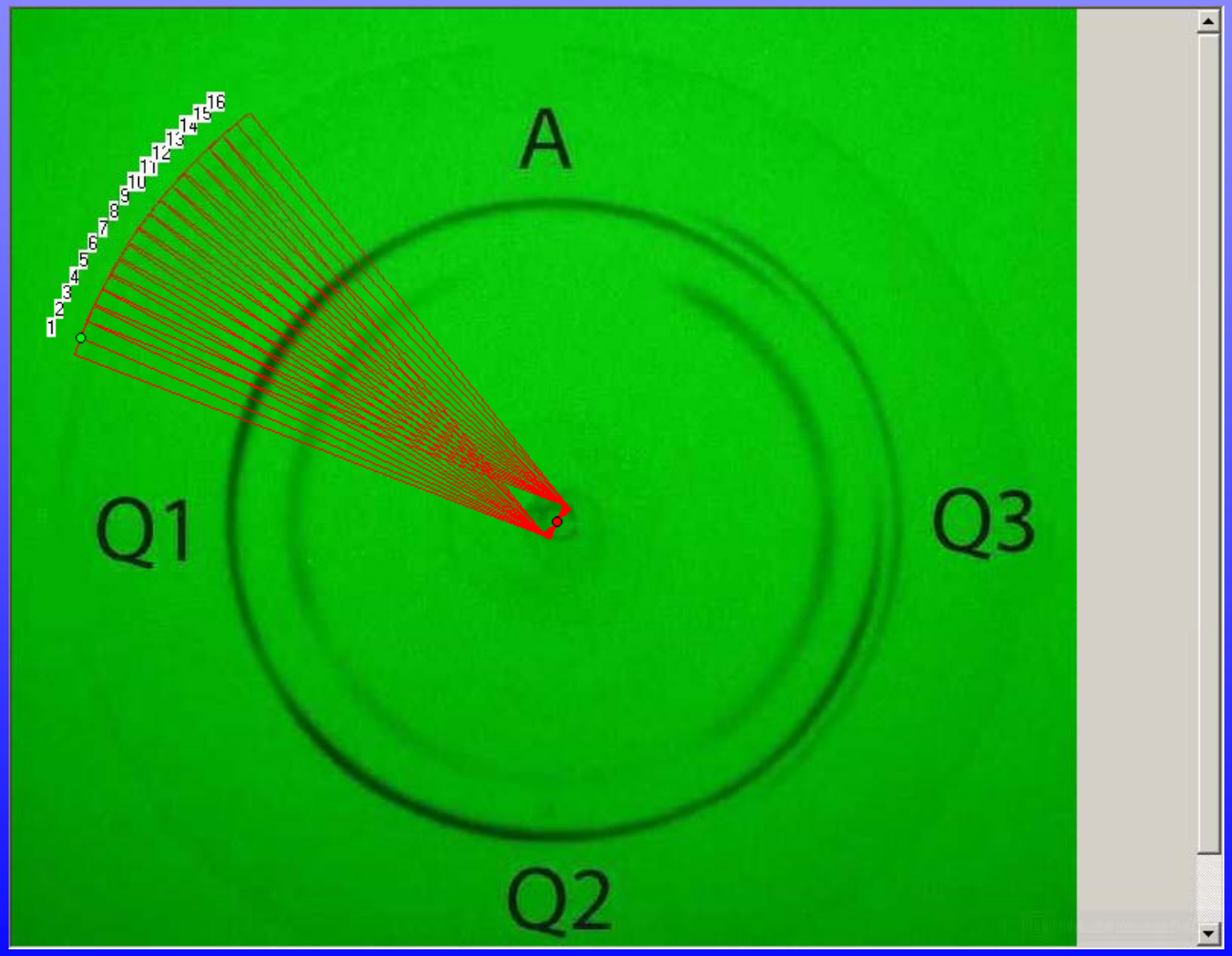
Under normal conditions in overlapped sampling focussing and separation works fine under µ-PLC



This way it is easy to identity falsified medical products – example VIAGRA :

only A is authentic. Q1, Q2, Q3 are faiked.

Quantitation if necessary with N up to 16 repetitions



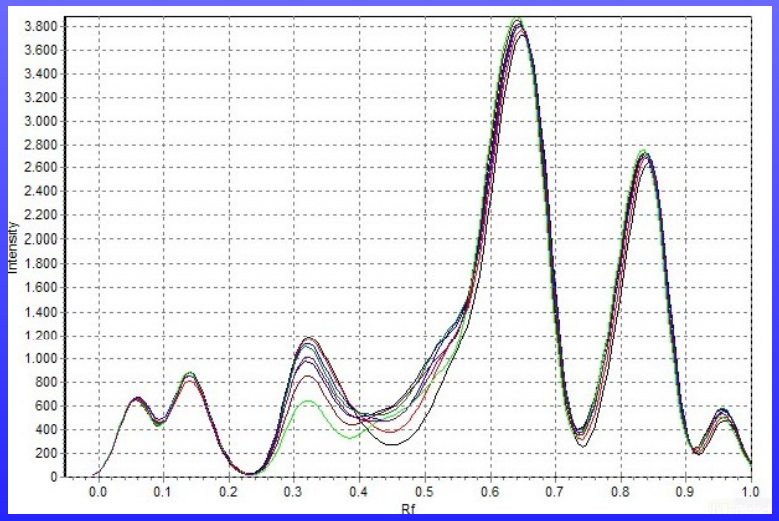
- Three of the clearly falsified VIAGRA‘s have been ordered through the Internet.

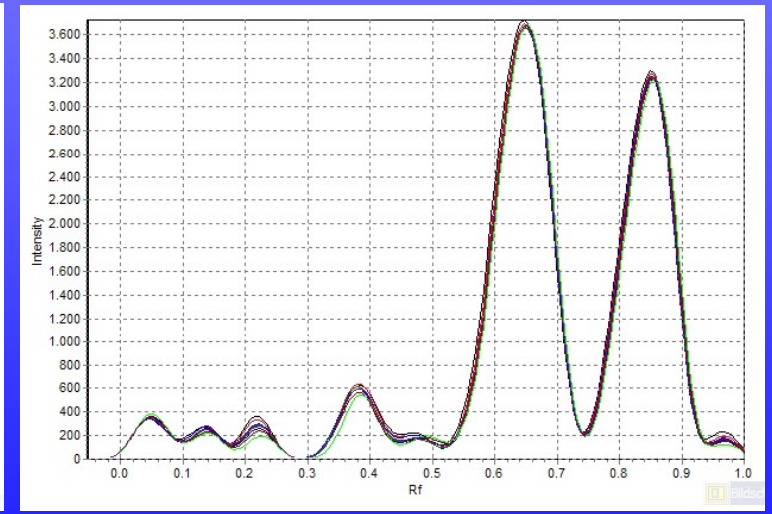
- I got these samples from my ‚Apotheker‘ who asked me for a critical compare analysis because of problems his customers had.

So only ONE pill, the authentic one, was OK

- A few days ago we got from a pharma company
  one original drug and its ‚remake' –
  as important anti cancer medicine.
  Remake and Original DIFFER strongly!

Remake 7 integr.chrom. each Original



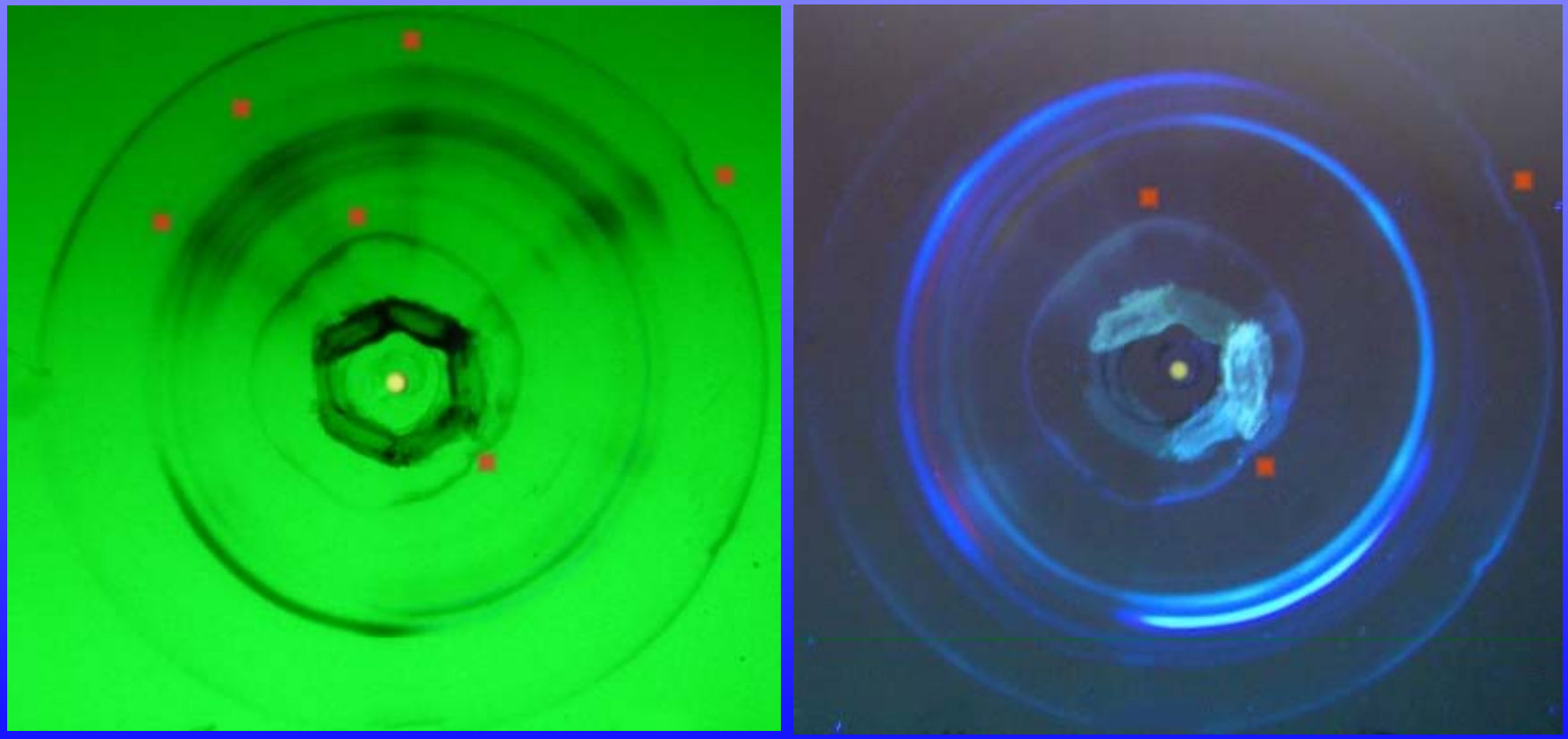


Back to chemisorbing plant material,

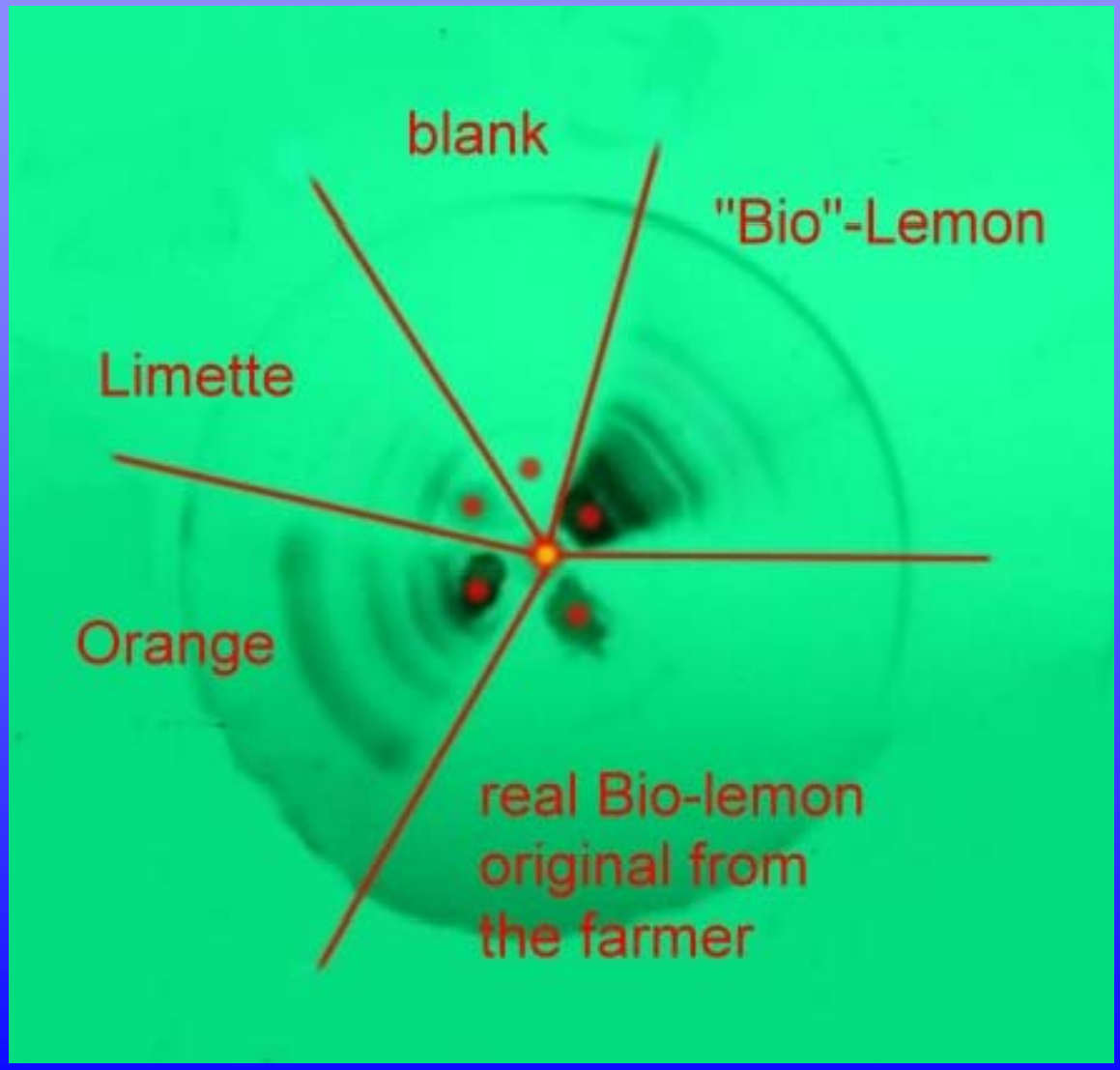
it complicates separation.

It reduces the phase flow locally irregular –
see the red pointed positions.

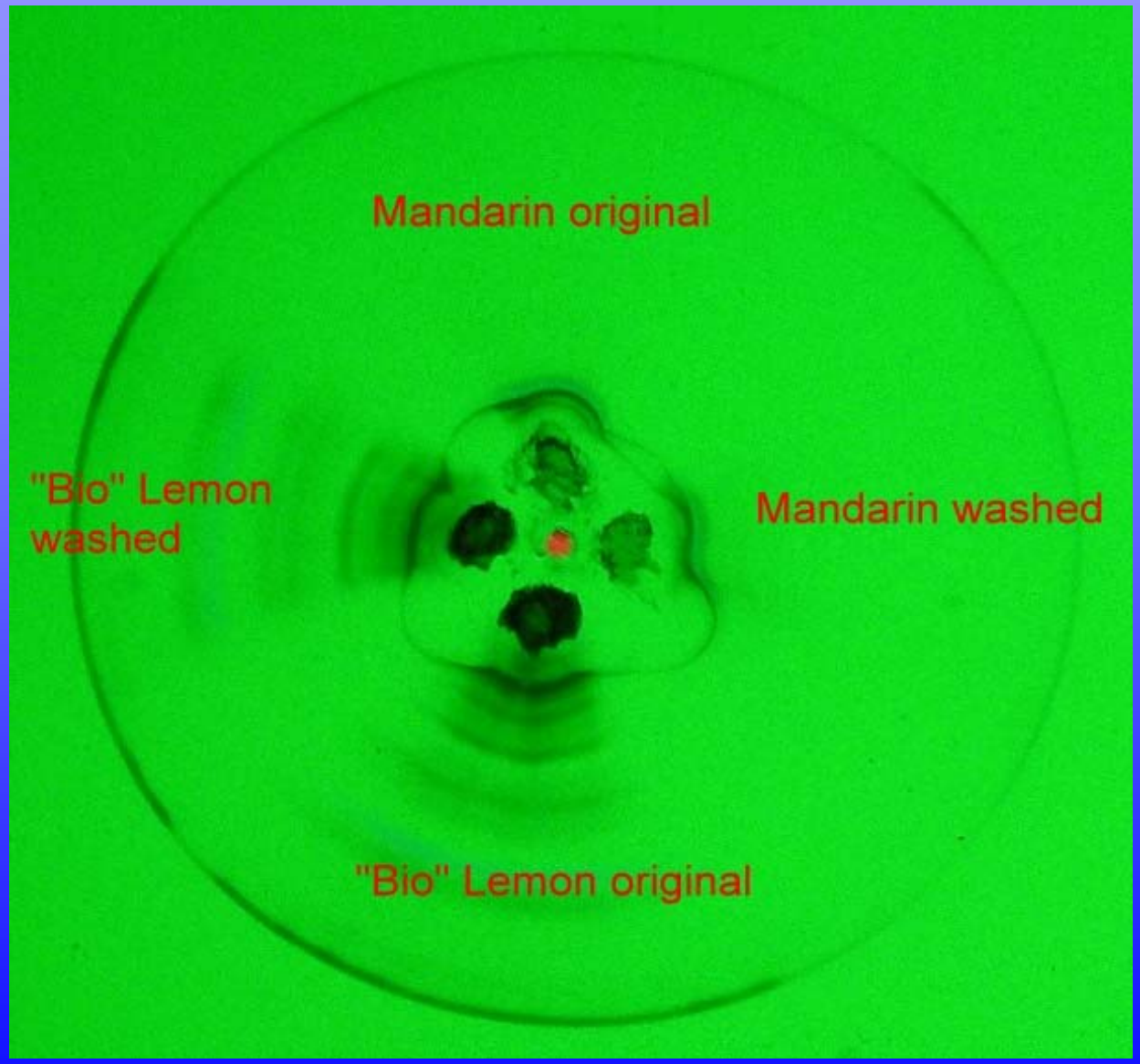
left: UV 254 right: FLU



„Bio“-Lemon versus a real Bio-lemon : Clear answer: „BIO“ is illegally treated .



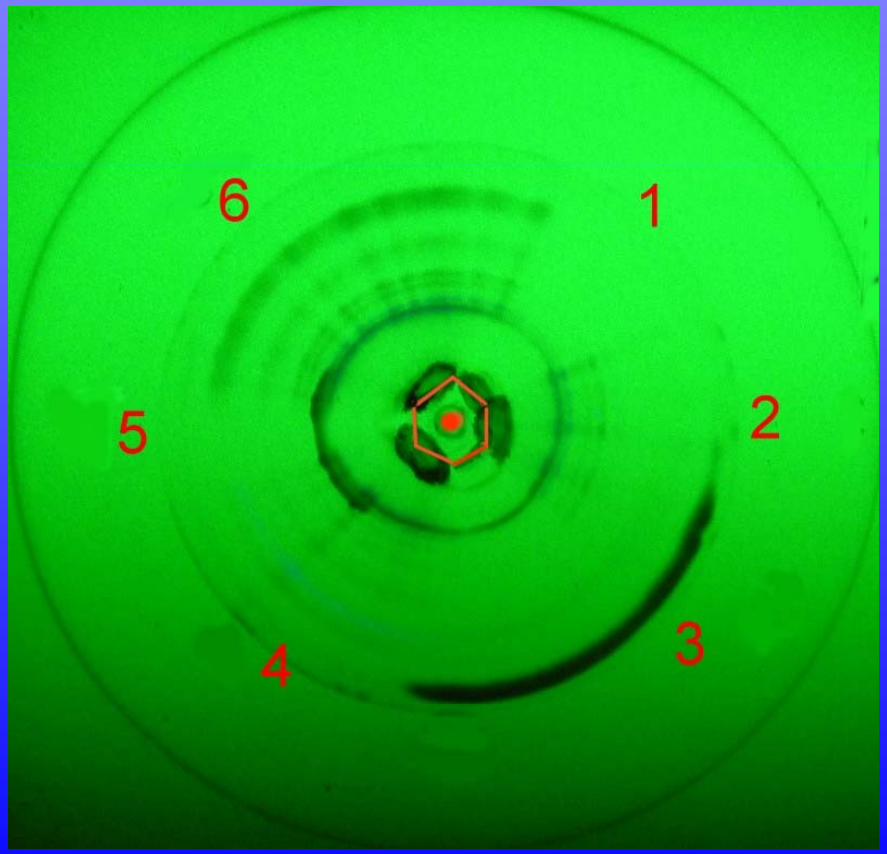
Chemicals: not removable by washing

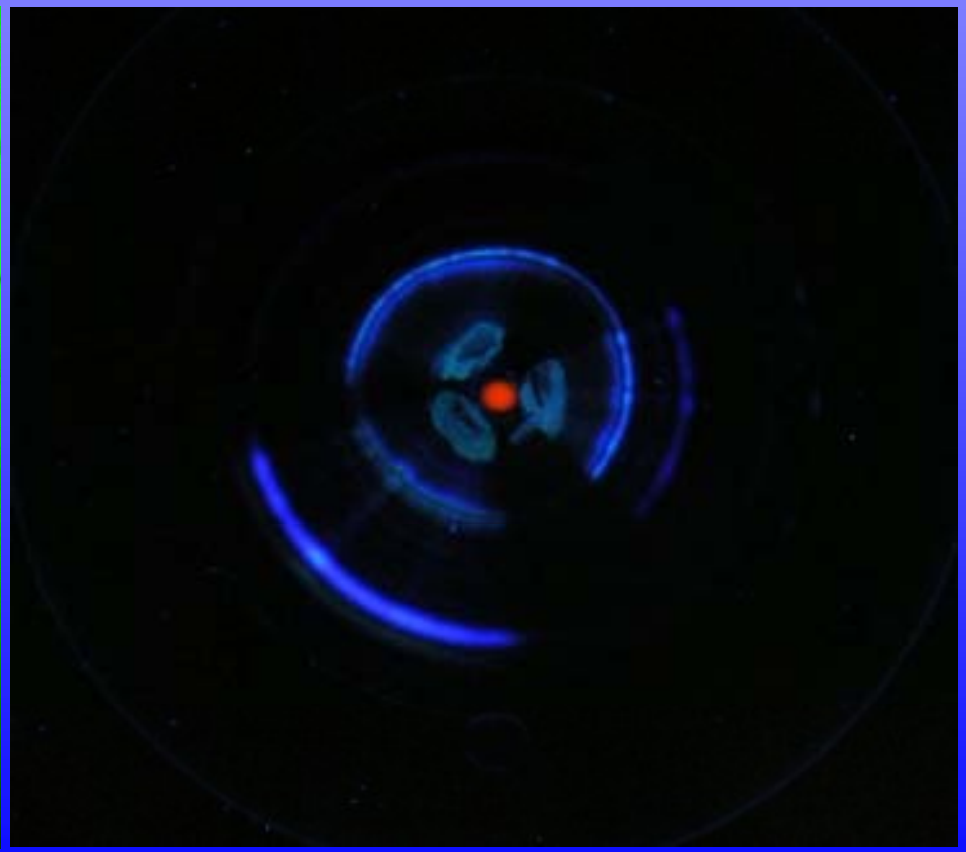


with hot water:

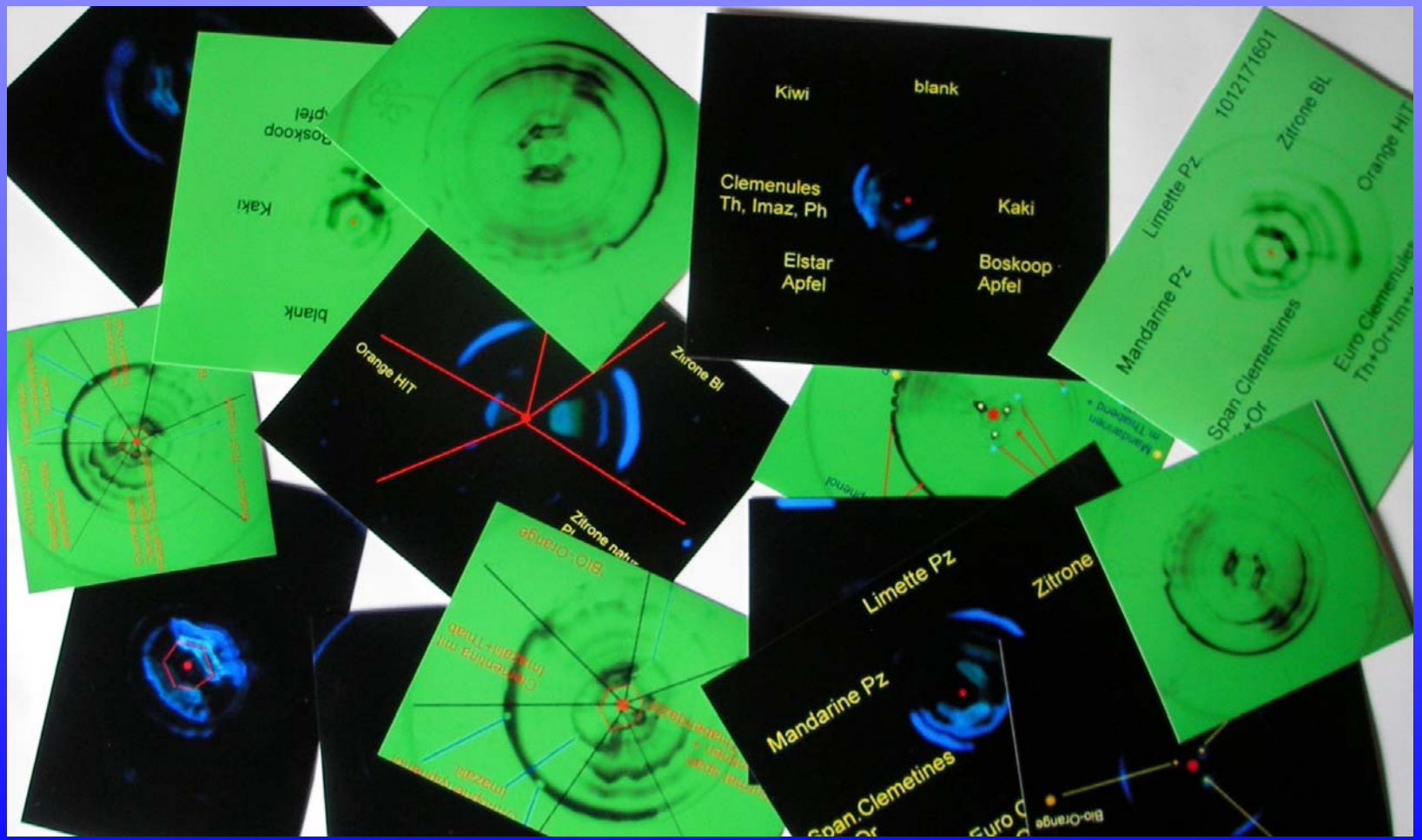
No effect also by add-ons like ascorbic acid or using warm olive oil.

1 = IM-mandarin1 [shop ‚A‘ (minimum sampling)] **OK**
2 = mandarin2 [shop ‚B‘ same town, same road,] **NOK**
3 = OP (ortho phenylphenol) test substance
4 = Bio-lemon [shop ‚B‘ (minimum sampling)] **NOK**
5 = TH (Thiabendazol)
6 = Bio-orange [shop ‚B‘ (minimum sampling)] **NOK**

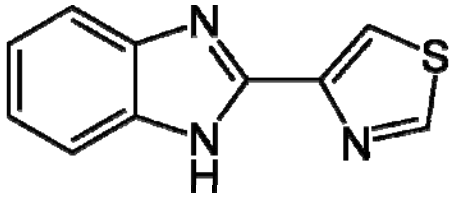




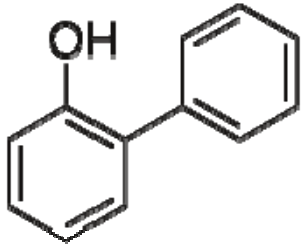
20 diff. plant surface analyses would to day show >10 not acceptable results: (as a mean)



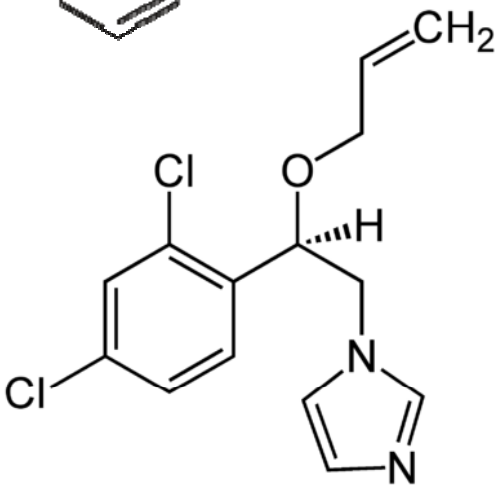
Still in 2011 accepted protection substances :



- Thiabendazol
- (TH)



- o-Phenylphenol
- (PH)



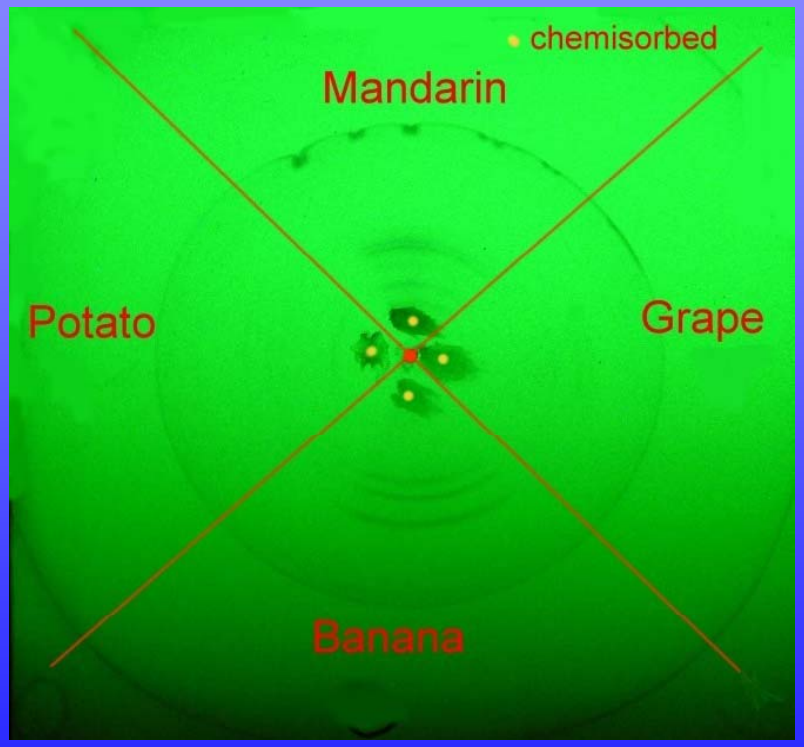
- Imazalil
- (IM)

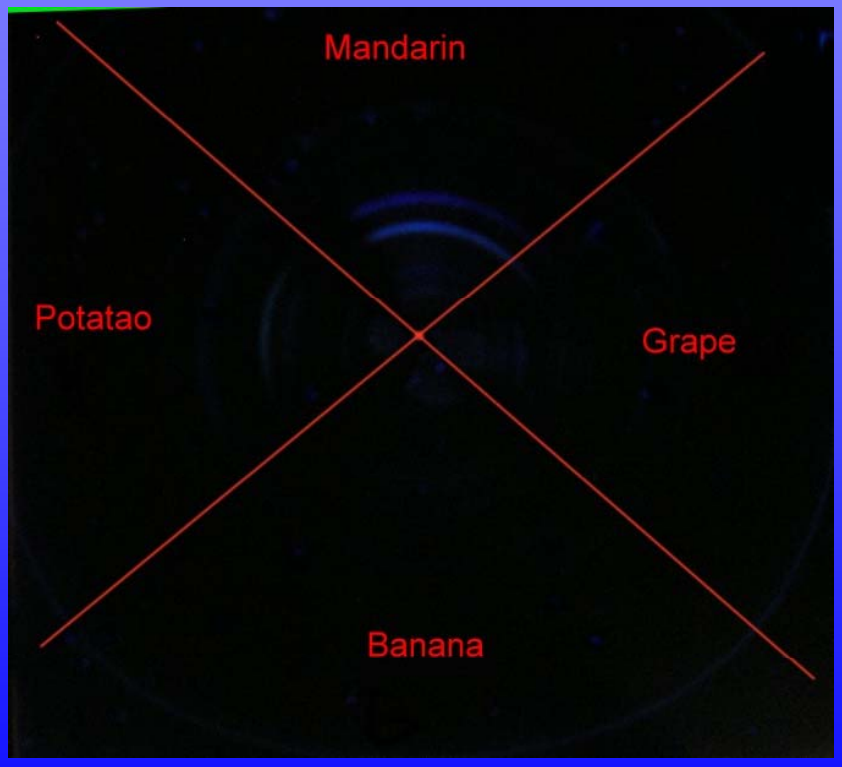
TH, **IM** and **PH** also used on potatoes, bananas, grain. Information about treated goods is often regulated by law. Critical concentrations.

- Lots of health warnings / strict regulation / time of future usage is by part already limited.
- http://de.wikipedia.org/wiki/Imazalil
- http://www.sciencelab.com/xMSDS-2_Phenylphenol-9926513
- http://www.private-health-organisation.de/
- About the chemistry and toxicity of falsified materials is by now NOTHING KNOWN.

Natural skin surface protection

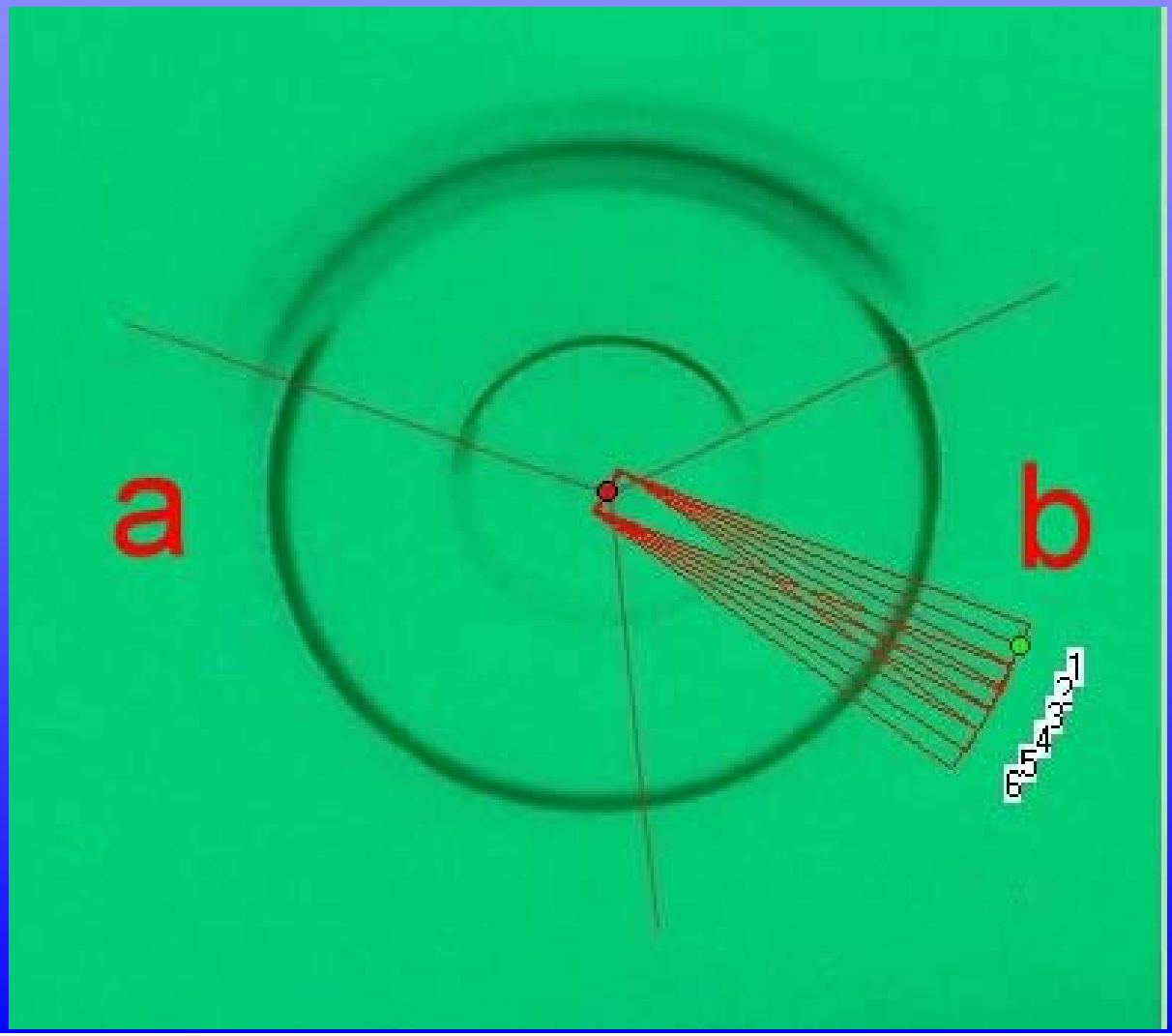
- Also on GRAPE, BANANA, POTATO :



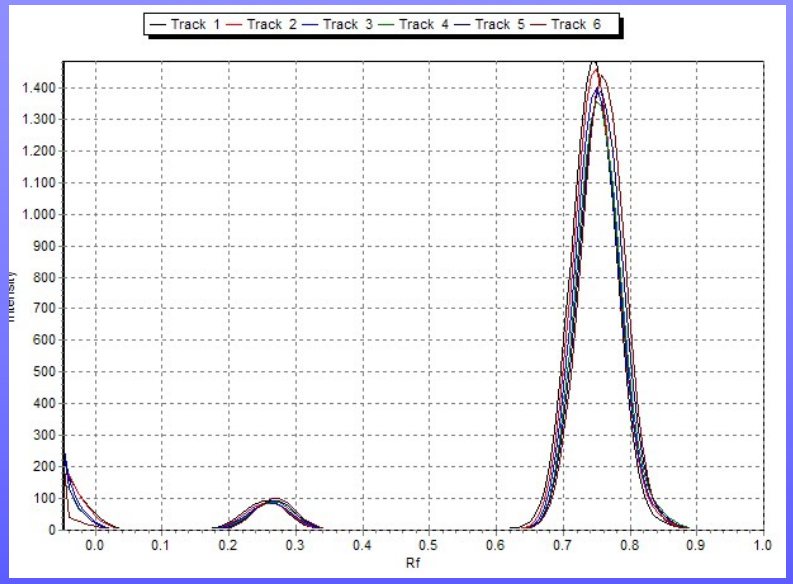


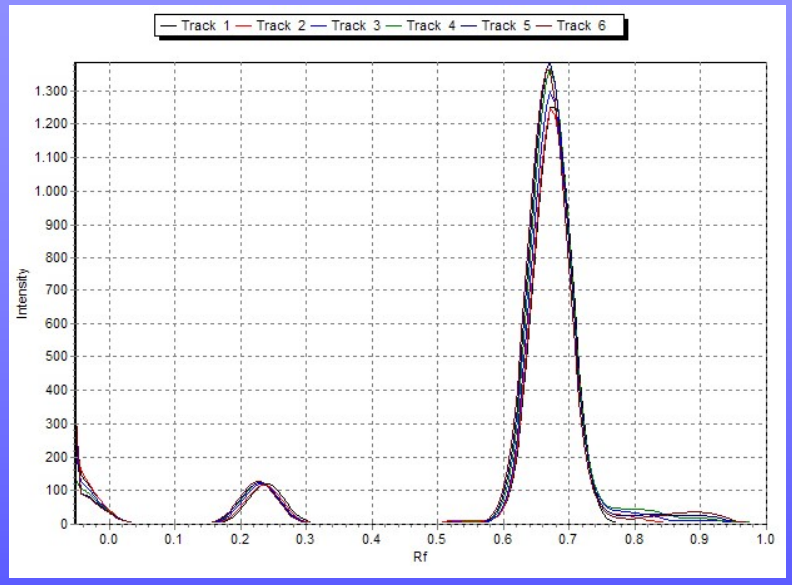
TEST compound quality is quantitatively checked:

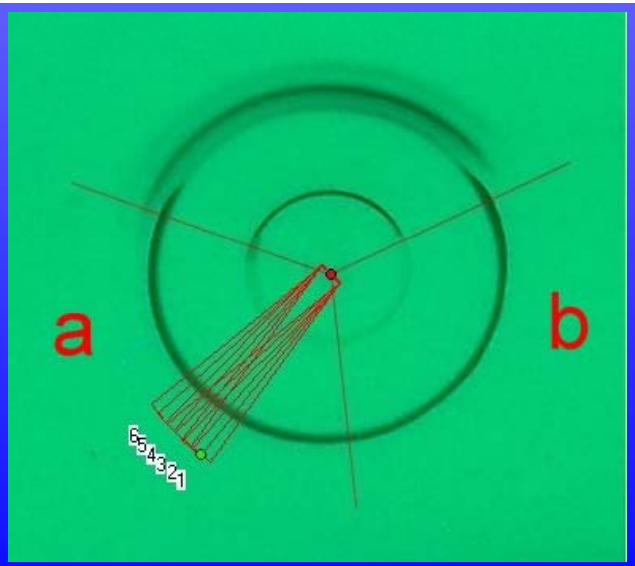
Quantitation by multi integration, N = 6 .

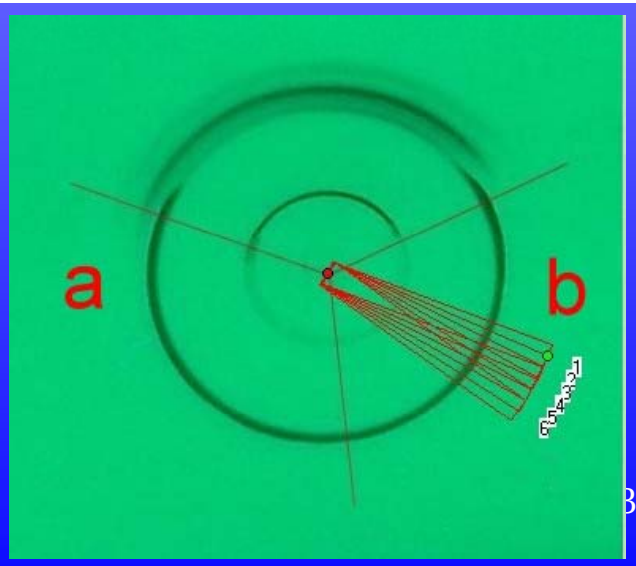


Comparing two „equal ??“ TEST compounds a & b quantitatively. N = 6









%-values impurity in TEST subst. a,b:

the purity difference ‚a' versus ‚b' is **highly signifikant**
TAU >t(99) because of a large enough N=6

- purity of test substance ‚a' = 97.8 +- 0.09 %
- purity of test substance ‚b' = 97.3 +- 0.11 %

Rp Table = Rf as % position in integration tracks

Rp-data	Peak 1	Peak 2
Track 1	0,26	0,74
Track 2	0,26	0,75
Track 3	0,27	0,75
Track 4	0,27	0,75
Track 5	0,27	0,76
Track 6	0,27	0,76

'a'

area Table

raw area	Peak 1	Peak 2	Rp x area1	Rp x area2	area 2 %
Track 1	979	15870	254,54	11743,80	97,879
Track 2	916	15554	238,16	11665,50	97,999
Track 3	893	14814	241,11	11110,50	97,876
Track 4	919	14444	248,13	10833,00	97,761
Track 5	942	14834	254,34	11273,84	97,794
Track 6	979	15226	264,33	11571,76	97,767
mean	938,0	15123,7			**97,846**
s					**0,091**
RSD %					**0,09**

Rp Table = Rf as % position in integration tracks

Rp data	Peak 1	Peak 2
Track 1	0,20	0,68
Track 2	0,20	0,68
Track 3	0,21	0,68
Track 4	0,21	0,69
Track 5	0,21	0,70
Track 6	0,22	0,70

'b'

area Table

raw area data	Peak 1	Peak 2	Rp x area1	Rp x area2	area 2 %
Track 1	1426	15848	285,20	10776,64	97,422
Track 2	1411	15401	282,20	10472,68	97,376
Track 3	1444	15841	303,24	10771,88	97,262
Track 4	1497	16577	314,37	11438,13	97,325
Track 5	1699	17396	356,79	12177,20	97,153
mean	1495,4	16212,6			**97,308**
s					**0,105**
RSD %					**0,11**

Quantitation by multi integration

For quantitation the µ-PLC formula is simple:

Total areaY – in % =

RpY x areaY x 100 / sum (RpY…n x areaY…n)

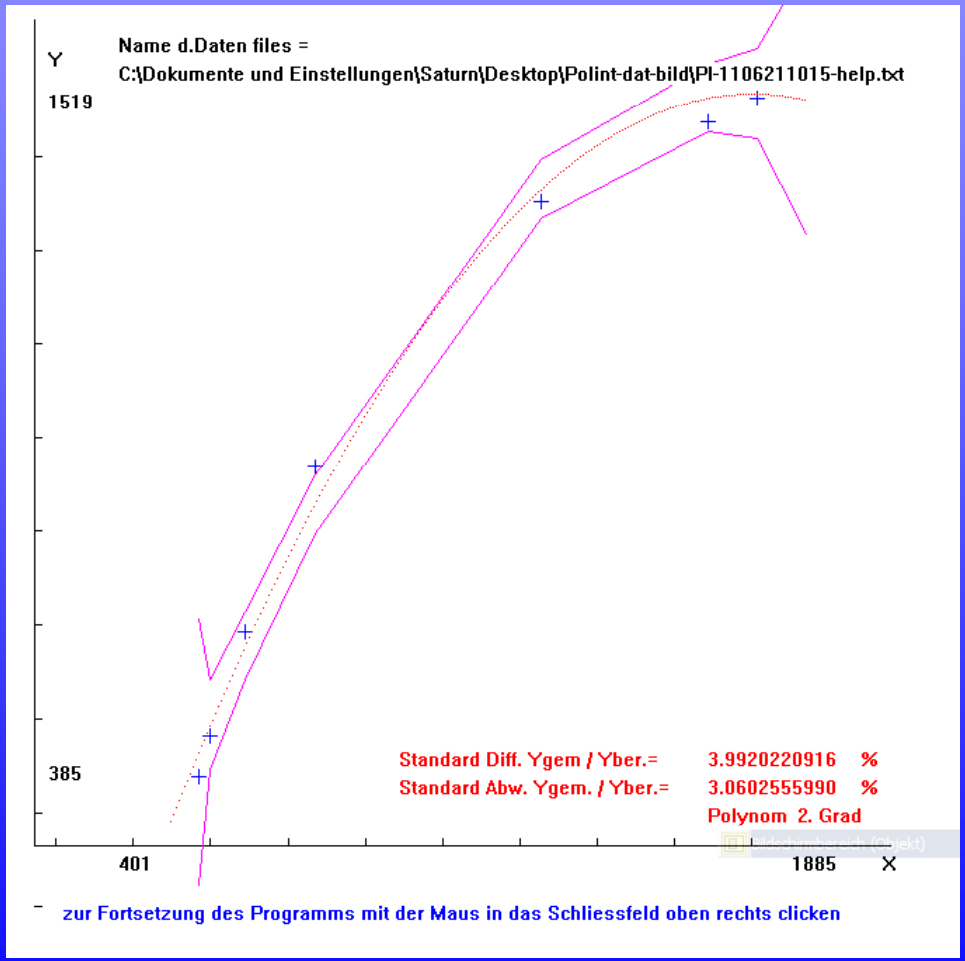
Reason:

circular PLC with multiple runs; N always > 4

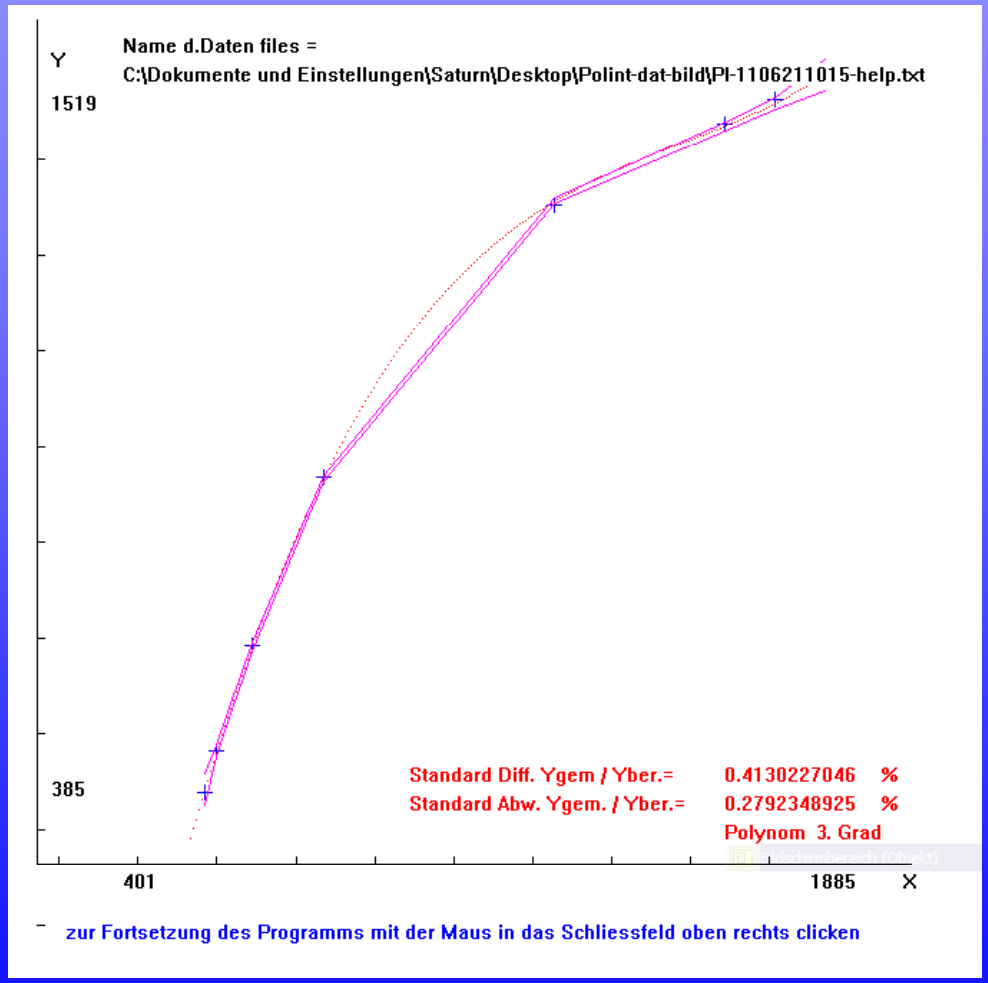
Rp is the relative bow position from centre to integration border. Here no Rf value acceptable.

Quantitation in PLC: by non linear calibration lines only !

The fundamental law of NON linearity in PLC calibration lines needs graphical statistics at best using „**Polynomial Interpolation**“ You have it ? (It exists as PI-rek-E11 program)



Sorry: this was a polynom 2nd degree. You better use a 3rd degree Polynom:



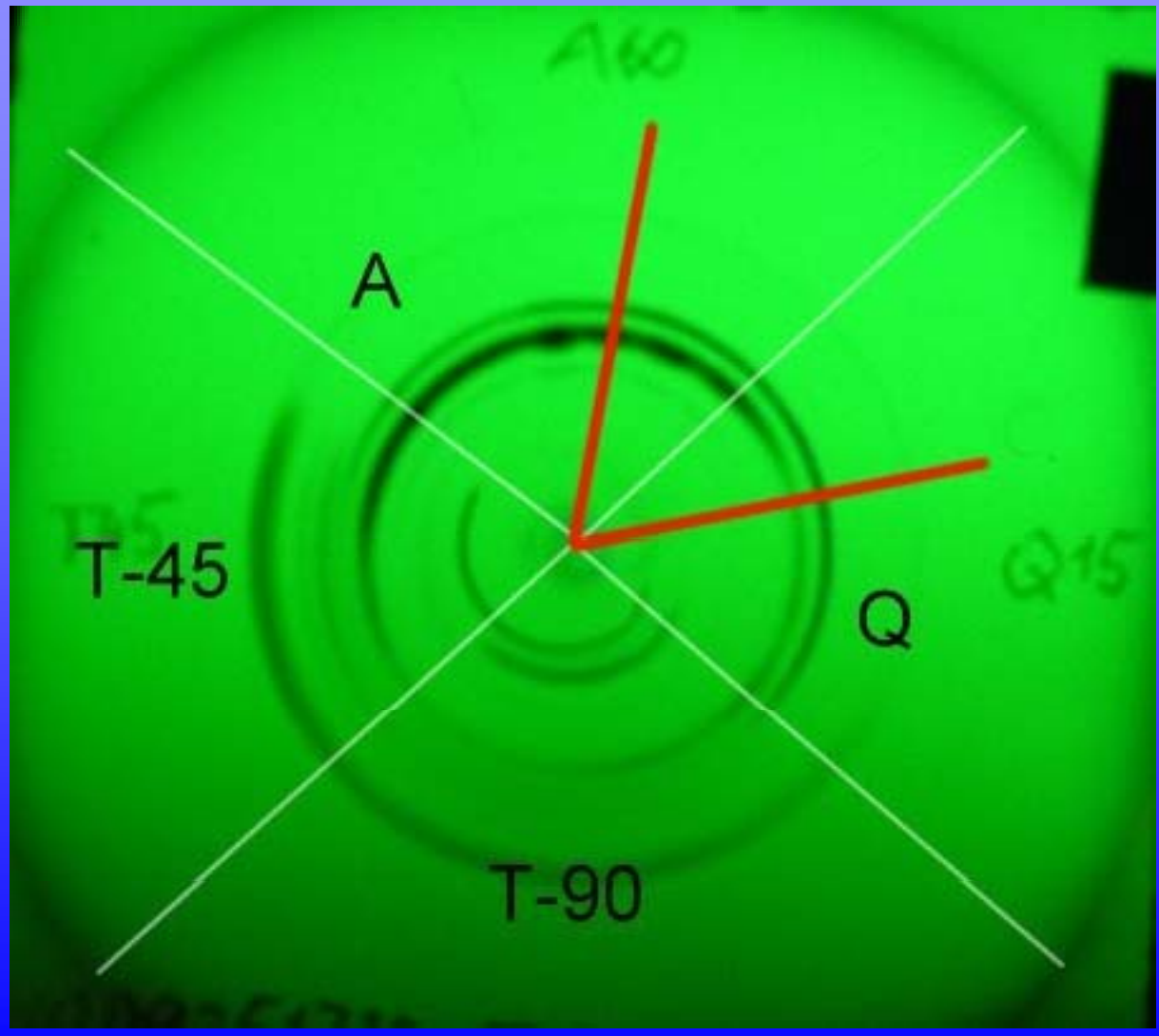
Thank You !

Baikal See in Ostsibirien

Foto by Dr. Olga Kaiser

During the μ-PLC film production we found drug falsification :

A is NOT Q

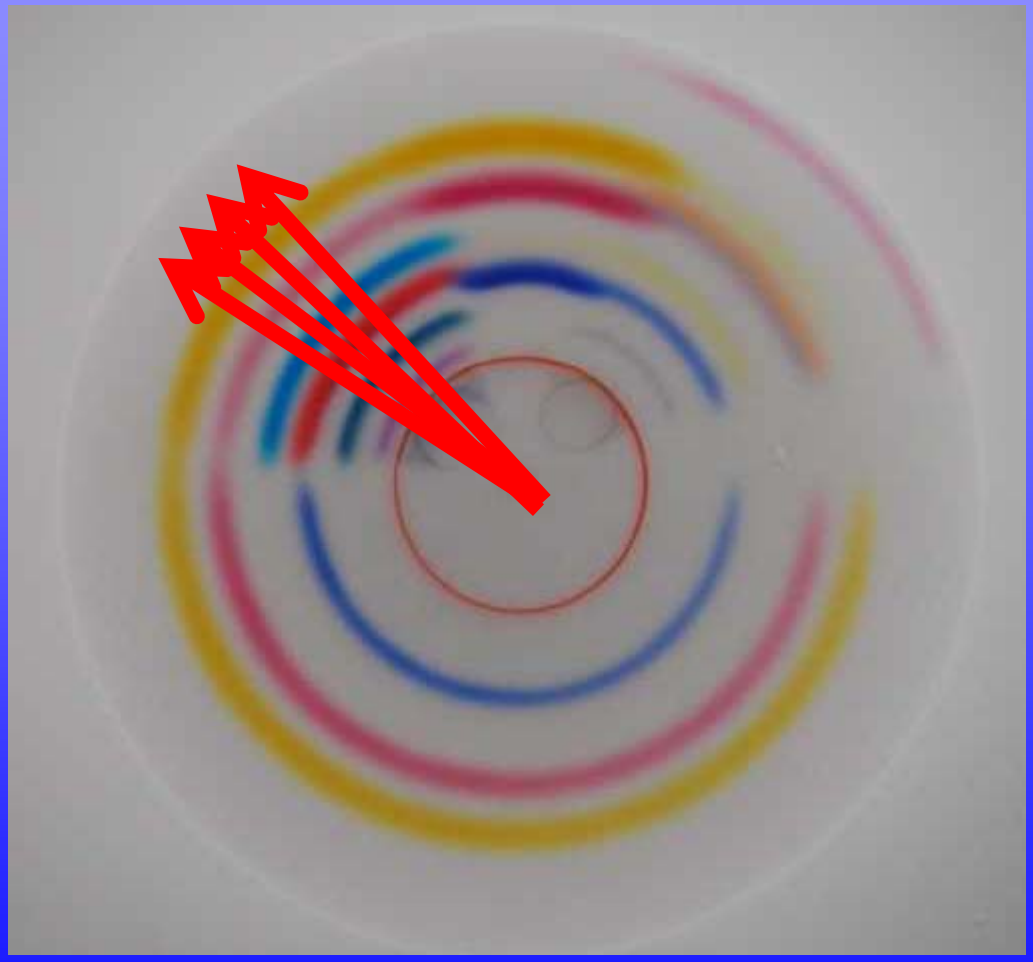


Method rules:

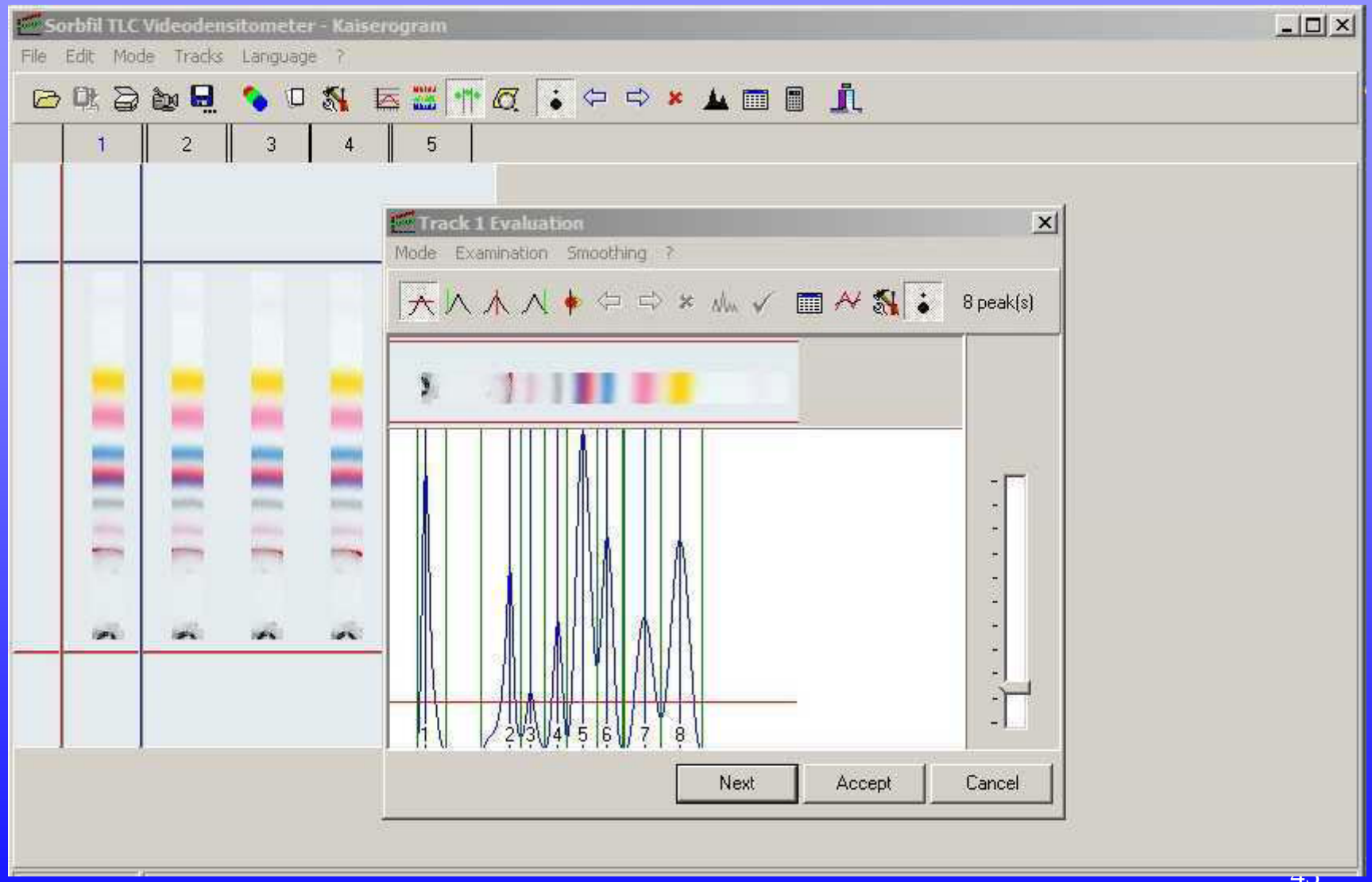
- Cleanest CH_2Cl_2 necessary (blank !!).
- Enrich extract samples only under UV to check blockage by chemisorbing substances early.
- Separation partially „in-each-other“ is MUST.
- ‚Hexagon sampling‘ is at its limits,
  only 3..4 samples are better ?
- > one run needed to correct signal symmetry.
- Place qualitative inner standards onto the focus border circle.
- Use always UV 254 at least together with 336 nm.

Multi PLC runs repair symmetry

Multi integration: 4 tracks, each switched by 1 degree angle.



Integration can be checked in single steps. This costs time but brings quality.



Digital values, 7 substances, N = 5

No	area	RSD%	height	RSD%
1	11154.8	0.937	2571.2	0.718
2	11003.8	0.525	1807.0	0.639
3	38429.8	0.600	4596.8	0.272
4	46302.4	0.350	4000.0	0.163
5	39413.0	0.520	3502.0	0.093
6	7733.8	0.695	978.8	0.606
7	24617.0	0.143	2052.8	0.156
S	178645	**0.164** %	19509	**0.091 %**

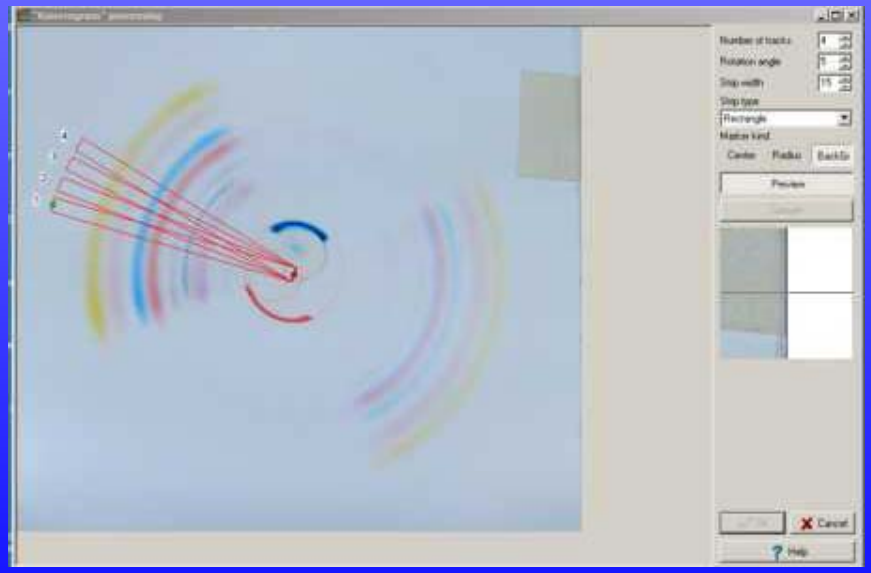
RSD = 100 x rel. standard deviation / mean

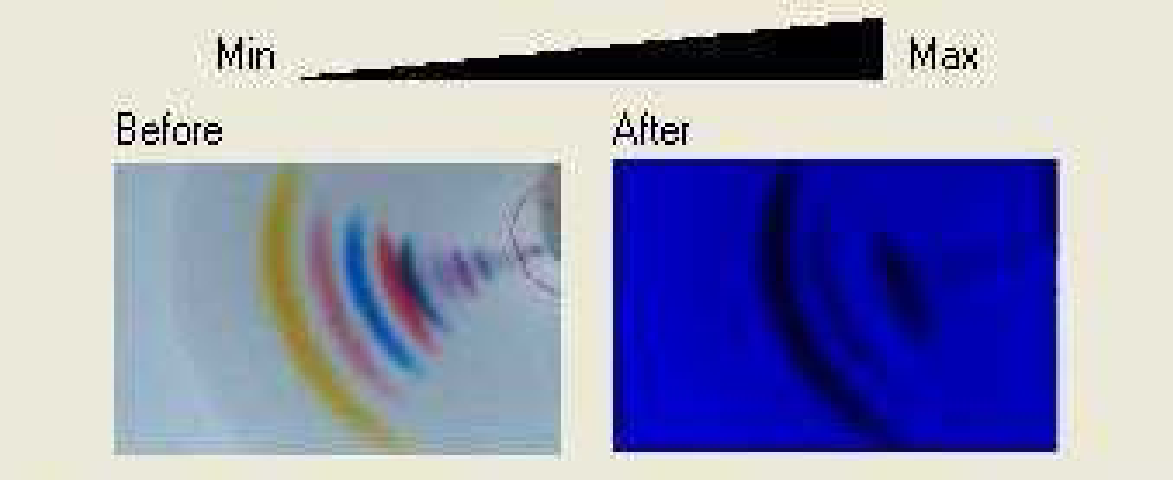
This is possible because of plate structure reduction.

Multiple scans show good comparability. This is necessary to reach +- 0.1 to +- 0.05 % comparability standard deviation.

NOTE: Comparability – not reproducibility.

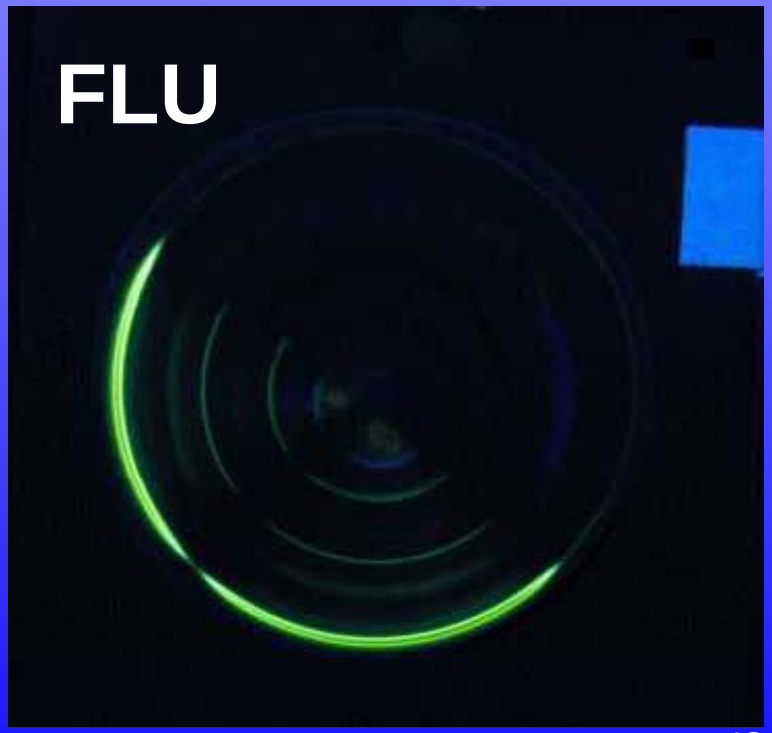
This is harder to find in standard analyses.





VIS

Light optmization is possible by multi integration software.



Four phases simultaneously