

Helsinki, June 2008

B. Spangenberg

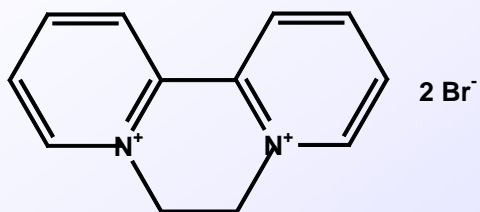
A new method for the quantification of Paraquat, Diquat, Difenzoquat, Mepiquat and Chlormequat in water by Thin-Layer Chromatography

Meltem Göcer, Kathrin Hoferer, Jürgen Zipfel and

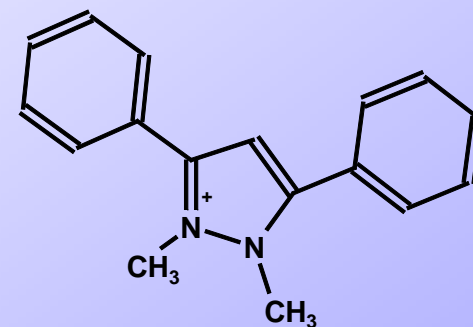
Bernd Spangenberg* (Offenburg, Germany)

Structures of Quats, investigated

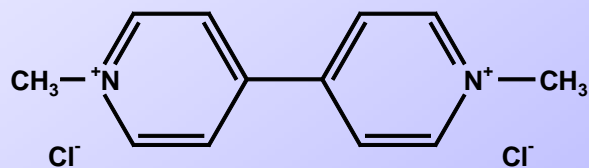
B. Spangenberg



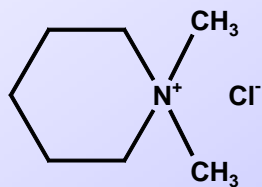
Diquat



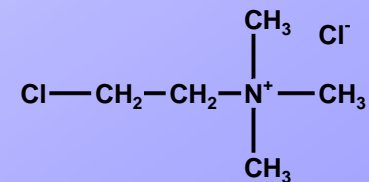
Difenzoquat



Paraquat



Mepiquat



Chlormequat

What are Quats ?

B. Spangenberg

Quats are used as quaternary ammonium herbicides.

All of these are easily reduced to the radical ion, which generates superoxide radicals that reacts with unsaturated membrane lipids.

Today Quats are among the most commonly used herbicides.

Quats and the European Union

B. Spangenberg

The European Union allowed Paraquat in 2004. Sweden, supported by Denmark, Austria, and Finland, brought the European Union Commission to court. On 11 July 2007 the court annulled the directive authorising Paraquat as an active plant-protection substance.

In the European Union, paraquat has been forbidden since 10th of July 2007.

What are Quats ?

B. Spangenberg

All Quats are potential ground water contaminants.

We actually don't know whether quats are a ground water problem or not.

We focussed on four challenges in quat analysis:

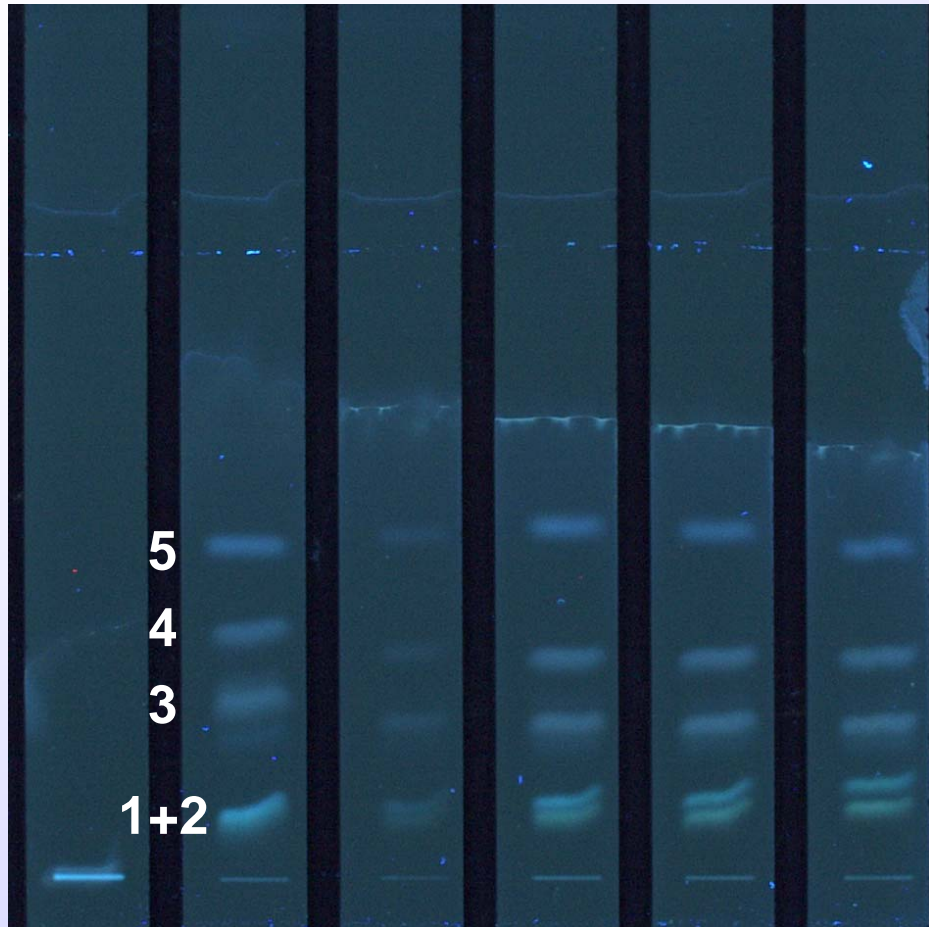
- 1.) optimisation of the separation system**
 - 2.) optimisation of the detection system**
 - 3.) improvements in the detection limits**
 - 4.) optimisation of sample pre-treatments**
-

We focussed on four challenges in quat analysis:

- 1.) optimisation of the separation system**
 - 2.) optimisation of the detection system**
 - 3.) improvements in the detection limits**
 - 4.) optimisation of sample pre-treatments**
-

Influence of salt content on the mobile phase

B. Spangenberg



Stationary phase:
LiChrospher®, Merck

Mobile phase:
1-propanol, methanol
and (A – F), (1+1+3, V/V)

A: water

B: 0.5 m NaCl

C: 1.0 m NaCl

D: 1.5 m NaCl

E: 2.0 m NaCl

F: 2.5 m NaCl

No. name

1: paraquat

2: diquat

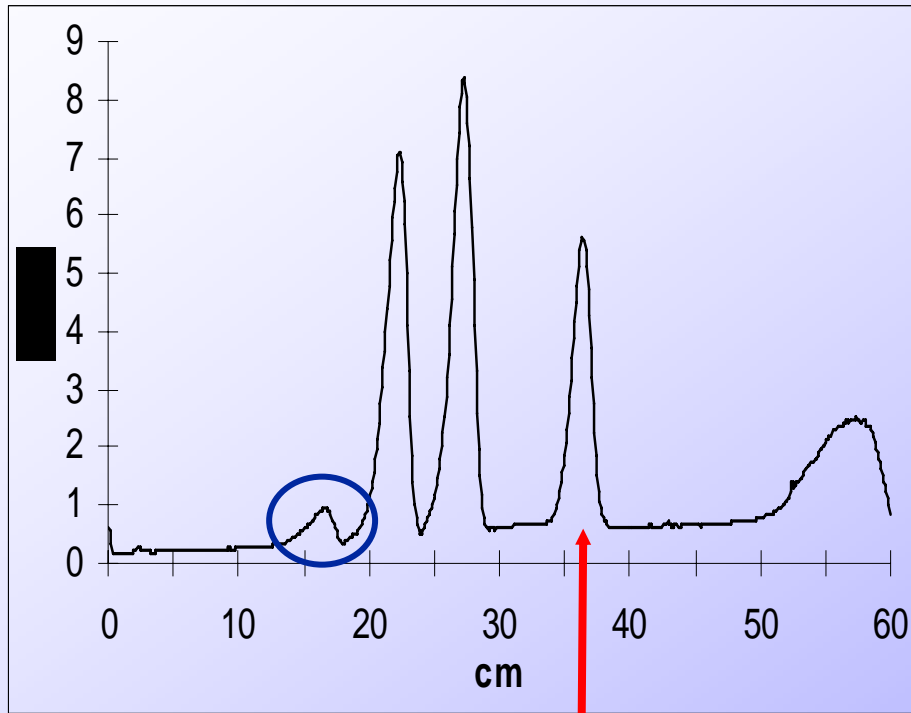
3: mepiquat

4: chlormequat

5: difenzoquat

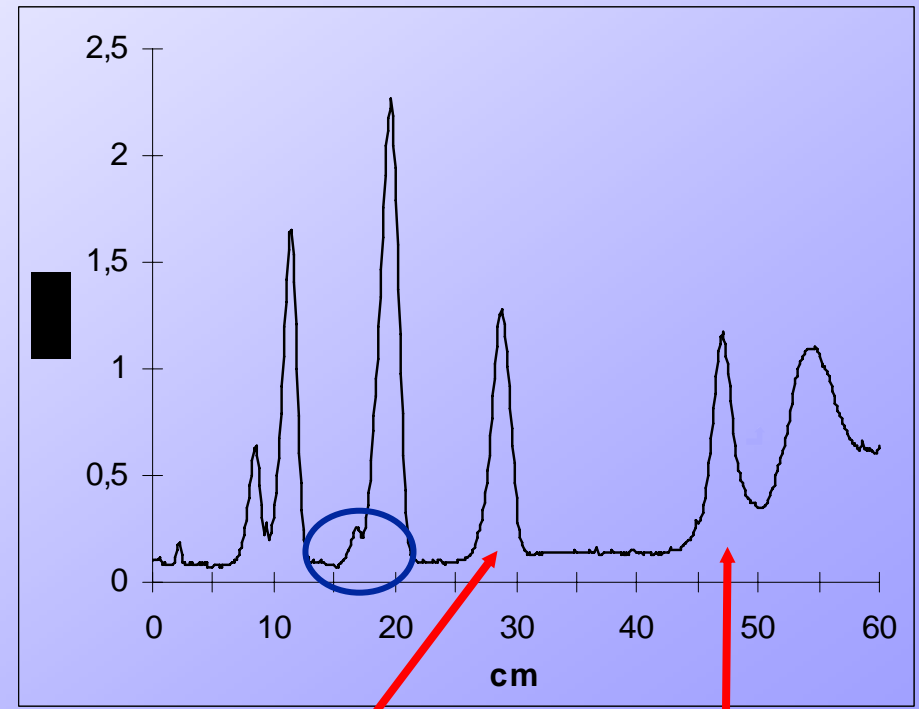
A

Methanol, 2-propanol, 2.5 n NaCl/H₂O (1+1+3)



chlormequat+difenzoquat

Methanol, 1-propanol, 2.5 n NaCl/H₂O (1+1+3)



chlormequat

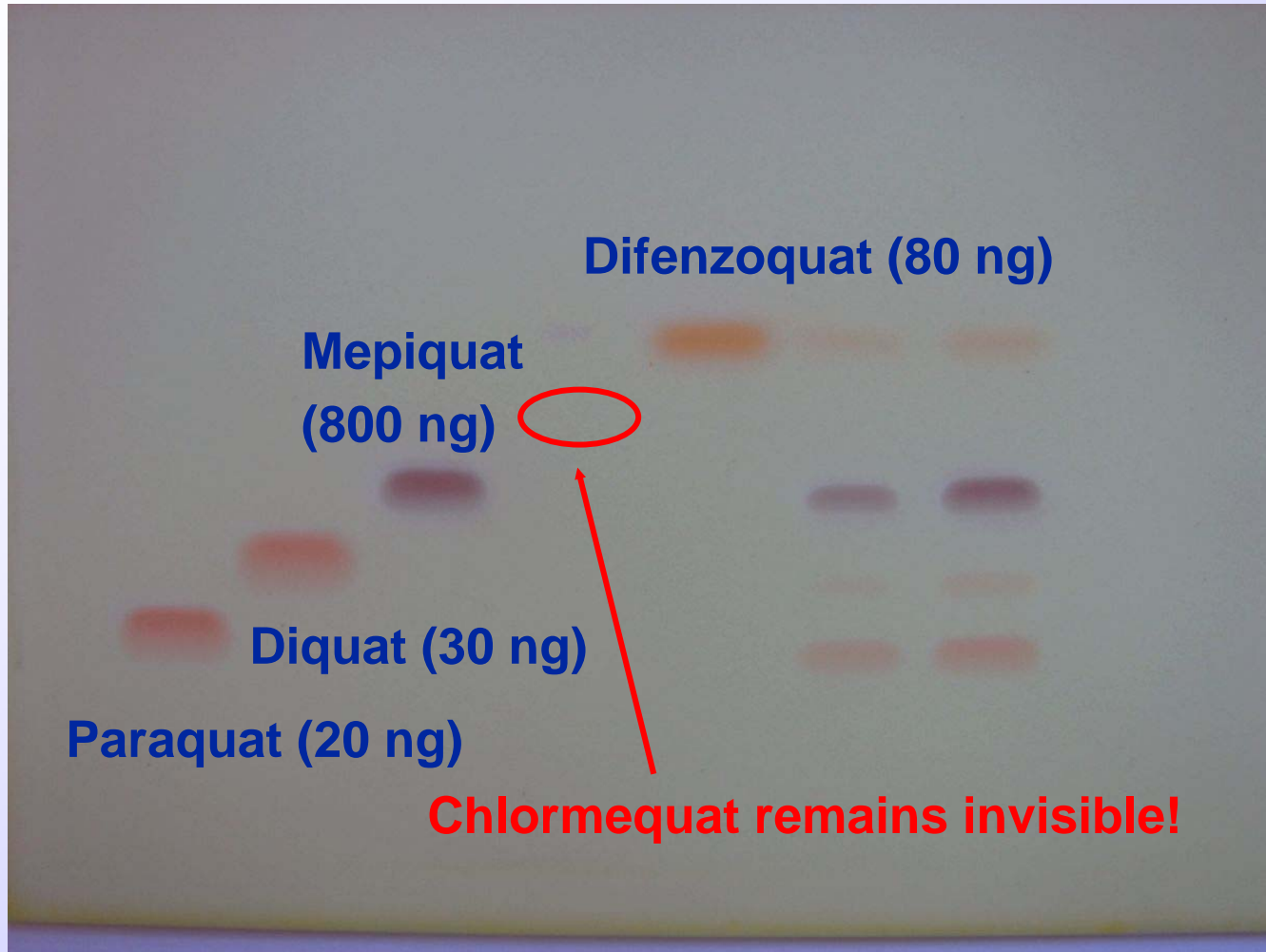
difenzoquat

We focussed on four challenges in quat analysis:

- 1.) optimisation of the separation system**
 - 2.) optimisation of the detection system**
 - 3.) improvements in the detection limits**
 - 4.) optimisation of sample pre-treatments**
-

Dragendorff-staining of quats (detection limits)

B. Spangenberg

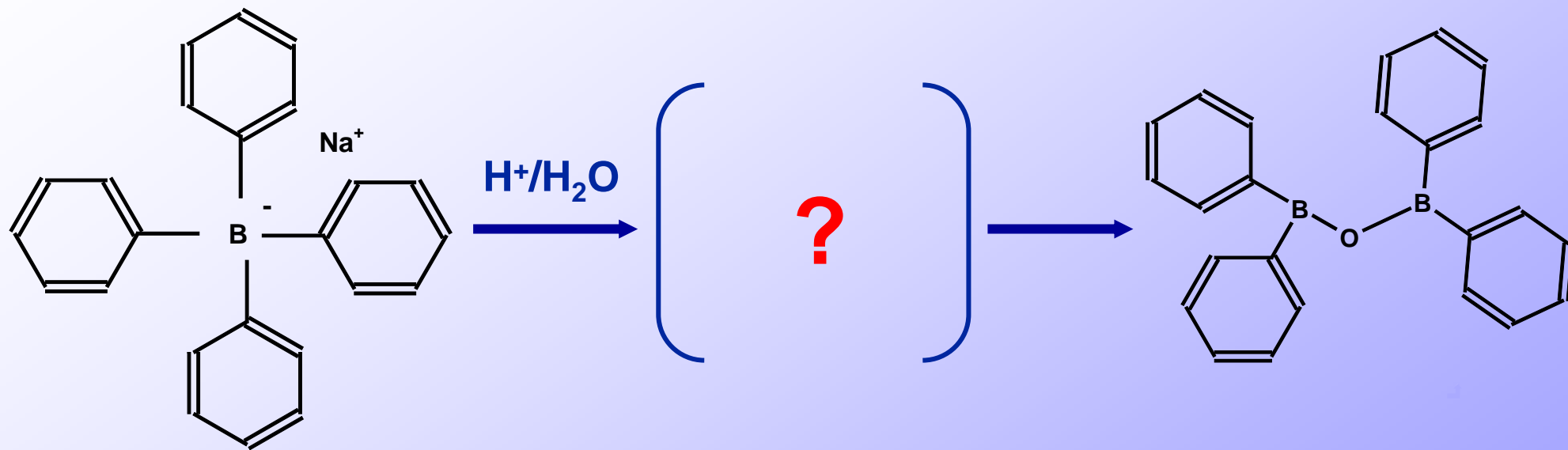


Dragendorff-staining

The amount of 0.85 g basic bismuth nitrate is dissolved in 10 mL acetic acid and 40 mL water. For solution b: 16g potassium iodide is dissolved in 40 mL water. The final reagent is mixed from 2 mL solution a and 2 mL b. Then 8 mL acetic acid are added and this mixture is topped up with water to 50 mL. Solution a and b remain stable for several weeks.

Formation of **tetraphenyl-diboroxyde**

B. Spangenberg



sodium tetraphenyl-borone

tetraphenyl-diboroxyde

To 50 mg sodium tetraphenyl-borone ($\text{Na}[\text{B}(\text{C}_6\text{H}_5)_4]$) in 50 mL water 50 μl HCl (32 %) were added.

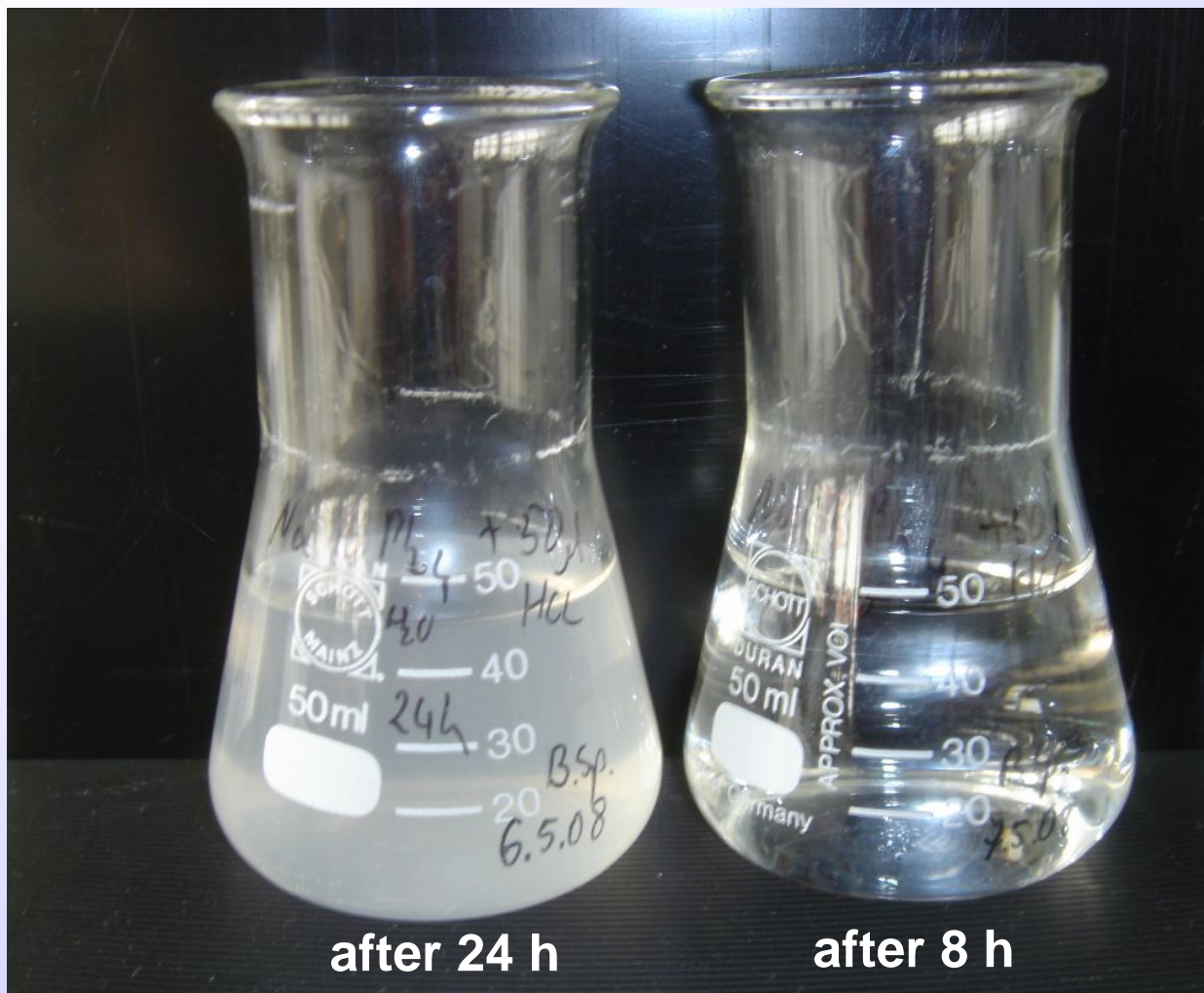
Formation of **tetraphenyl-diboroxyde**: R. Neu, Chem. Ber. 87 (1954), 802 - 805

Formation of **tetraphenyl-diboroxide**

B. Spangenberg

To 50 mg sodium tetraphenylborone ($\text{Na}[\text{B}(\text{C}_6\text{H}_5)_4]$) in 50 mL water 50 μl HCl (32 %) were added.

After 24 hours the solution turns turbid.



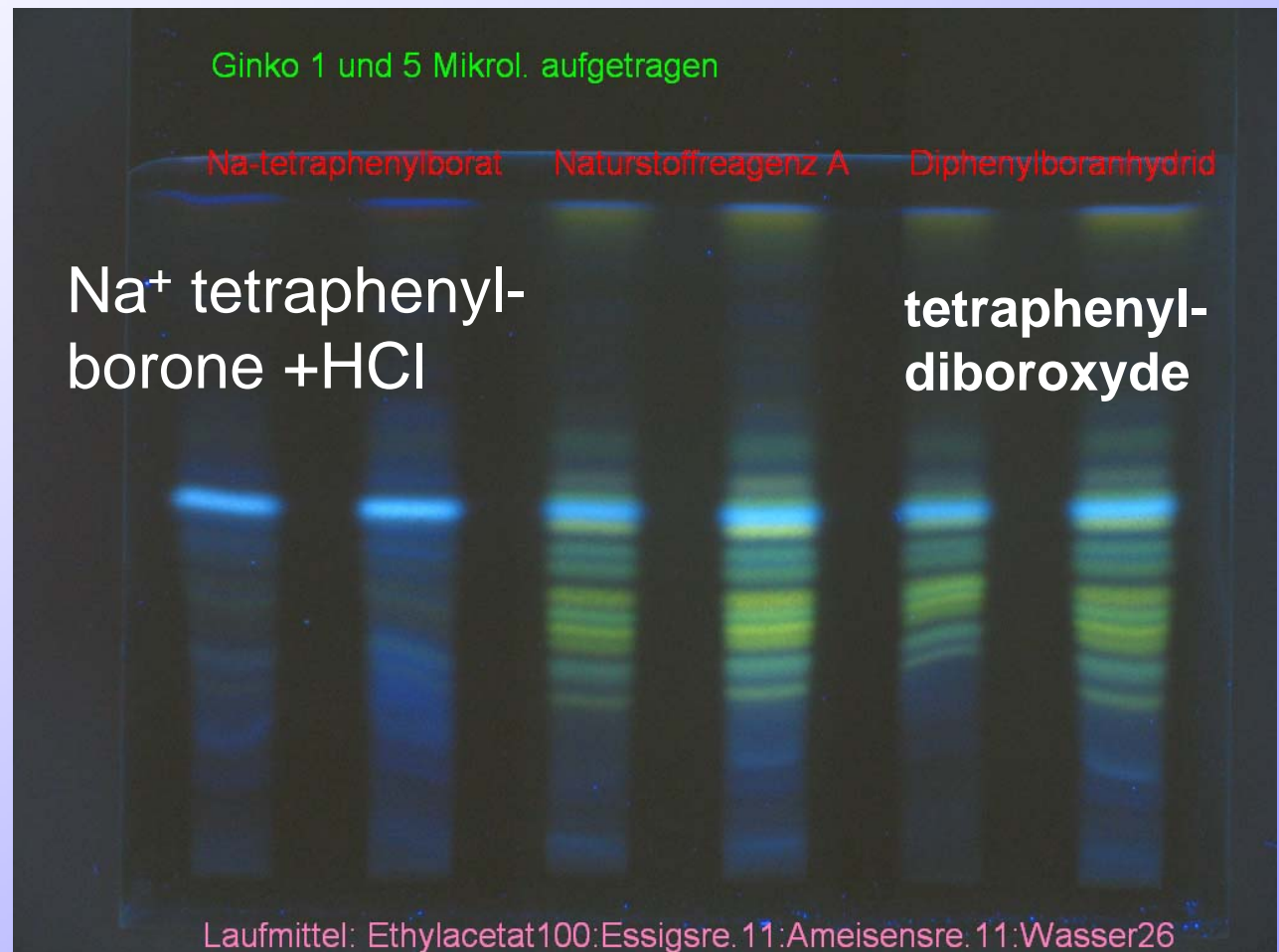
Formation of **tetraphenyl-diboroxide**: R. Neu, Chem. Ber. 87 (1954), 802 - 805

Staining reaction with *primulae flos*

B. Spangenberg

Na-tetraphenylborone,
dissolved in 50 mL
water + 50 μ l HCl
(32%).

This reagent stains
more specifically
than NEU-reagent or
**tetraphenyl-
diboroxide**.

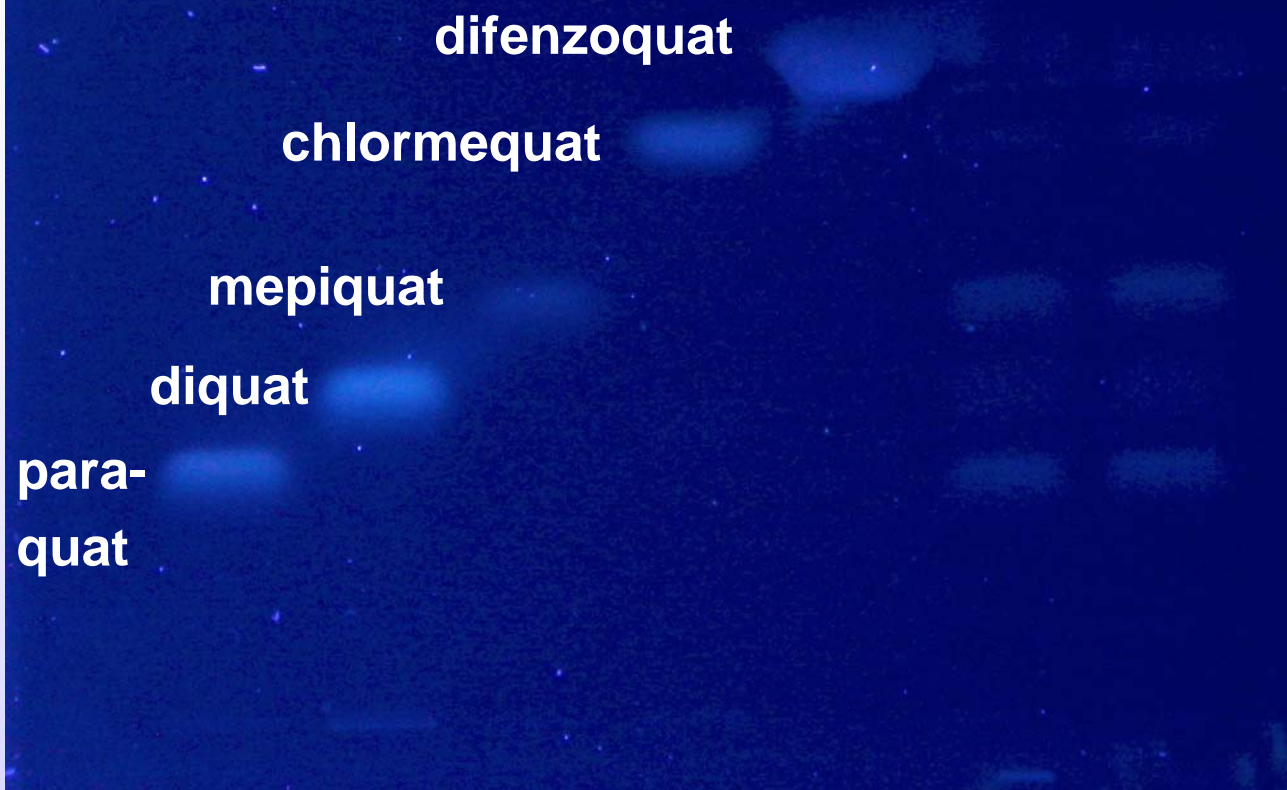


R. Neu, Z. anal. Chem. 143 (1954), 30 - 38

A new sodium tetraphenylborone staining

B. Spangenberg

Detection wavelengths : 470 – 530 nm



To 50 mg sodium tetraphenyl-borone ($\text{Na}[\text{B}(\text{C}_6\text{H}_5)_4]$ in 50 mL water 50 μl HCl (32 %) were added.

The wet plate is illuminated for 5 minutes by intense light of 254 nm. Spots of mepiquat, chlormequat and difenzoquat are converted into fluorescing zones. Paraquat and diquat spots were illuminated for 10 minutes with UV-light of 365 nm.

Idea from: R. Neu, Z. anal. Chem. 143 (1954), 30 - 38

We focussed on four challenges in quat analysis:

- 1.) optimisation of the separation system**
 - 2.) optimisation of the detection system**
 - 3.) improvements in the detection limits**
 - 4.) optimisation of sample pre-treatments**
-

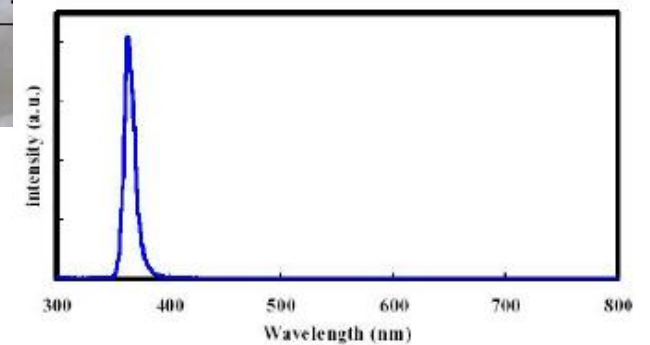
High intensity LED for TLC-fluorescence measurements

B. Spangenberg



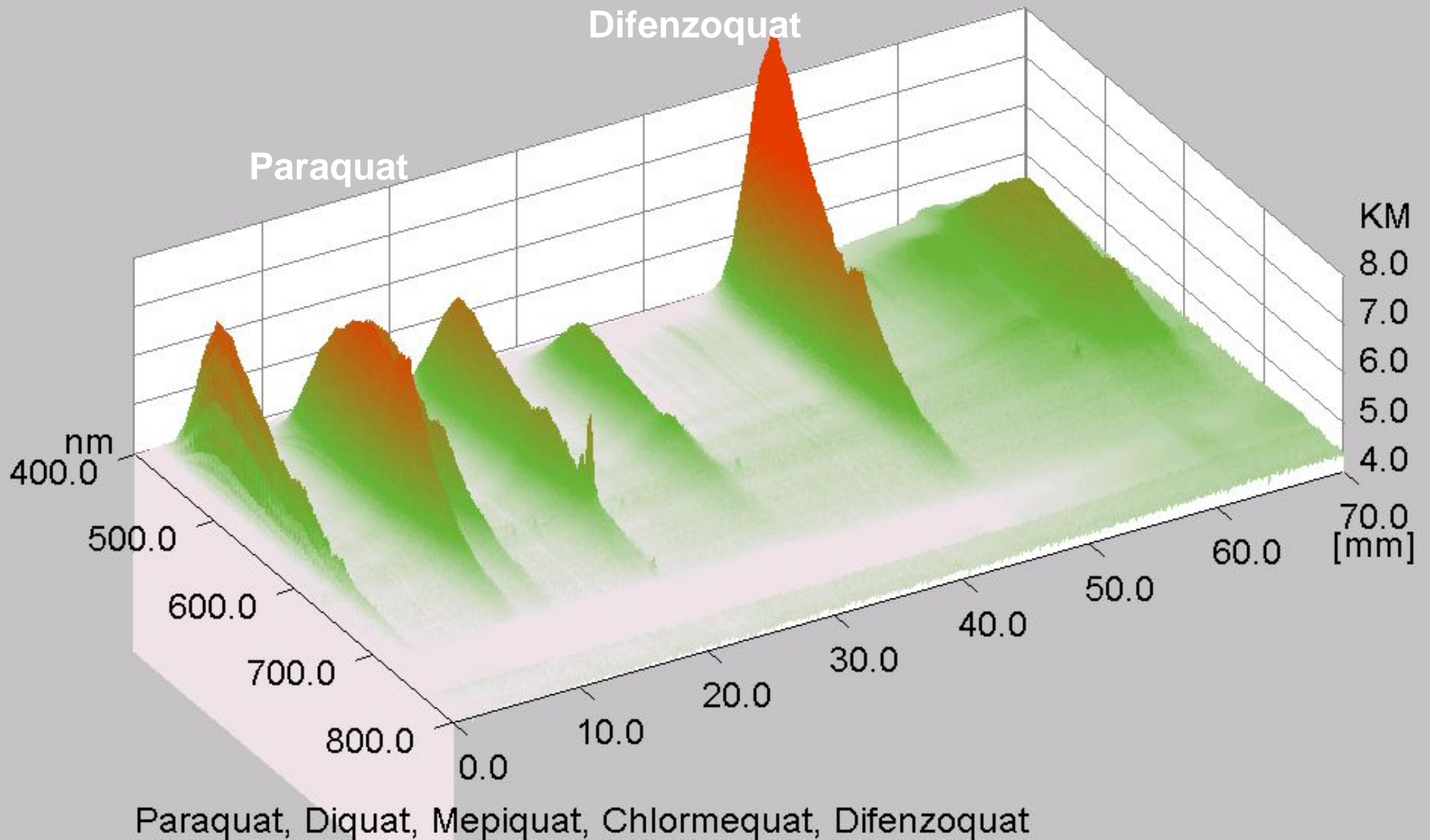
Light intensity: 100 mW !

365nm, ± 10 nm halbe Breite,



The diode shows absolutely constant light intensity!

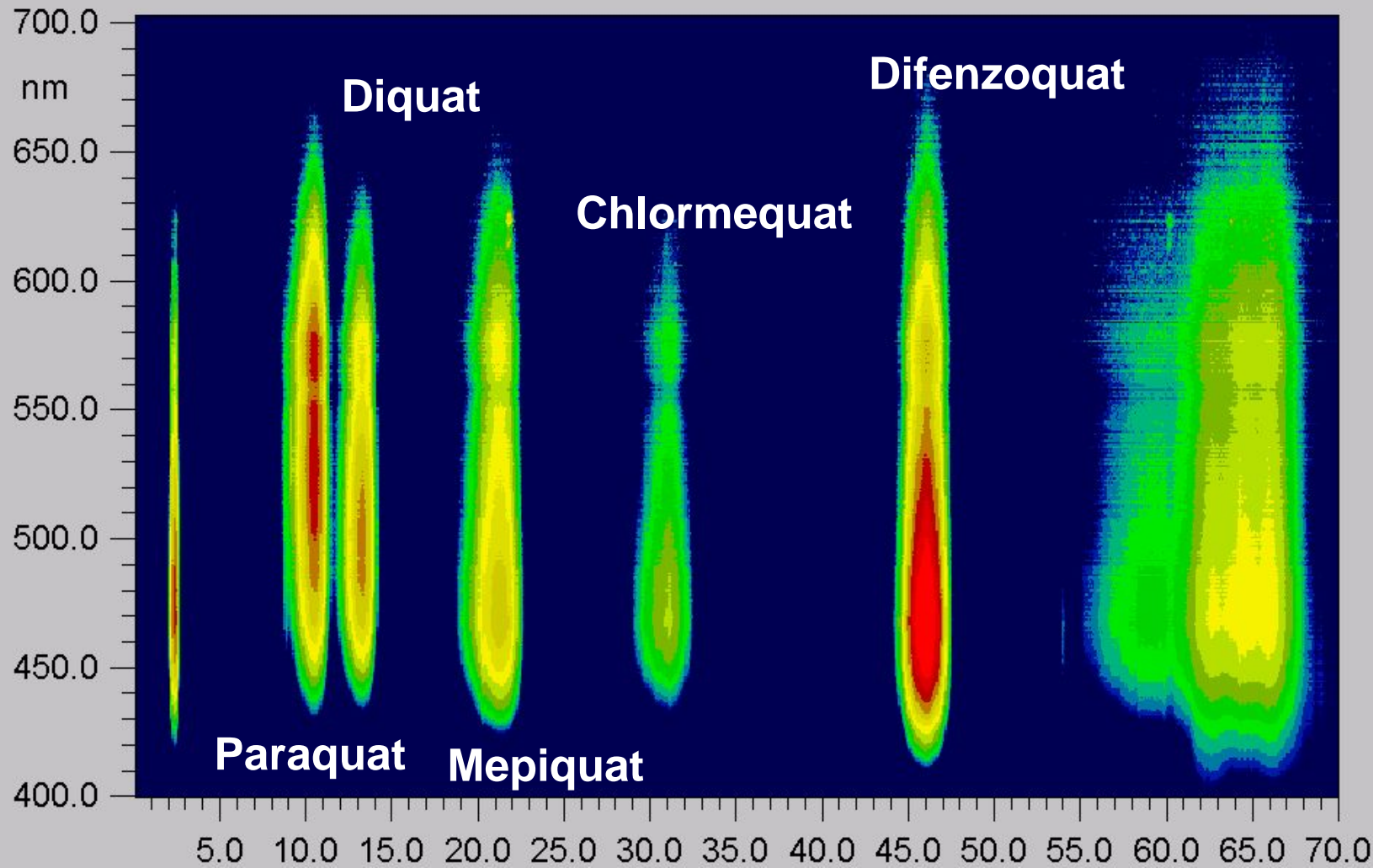
Fluorescence 3D plot of Quats



Fluorescence contour-plot of Quats

Front

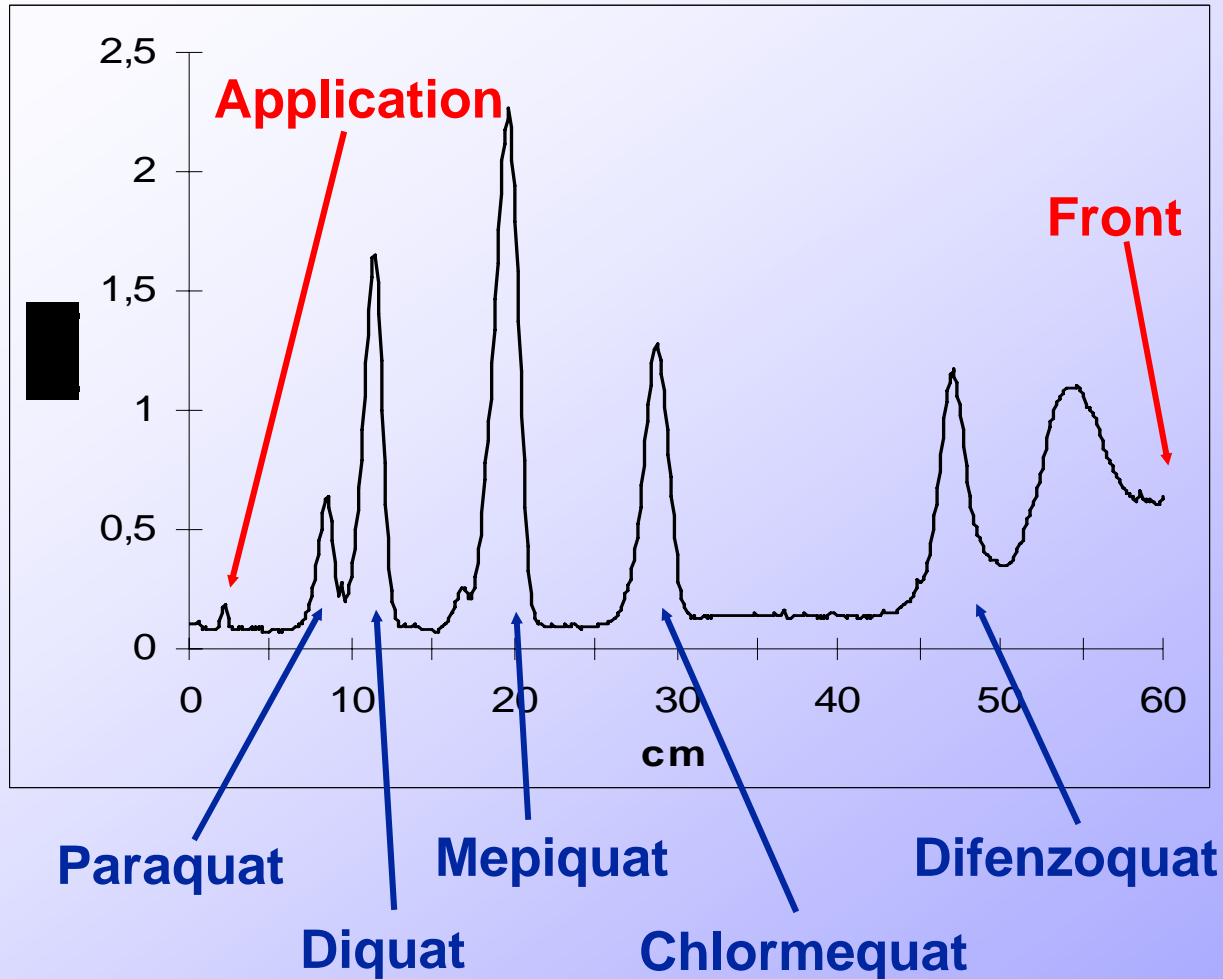
B. Spangenberg



Paraquat shows a green-yellow, diquat a green and all other quats a blue fluorescence.

Densitogram of Quats from a real water sample

B. Spangenberg



Stationary phase:

Silica gel „LiChrospher[®]“,
Merck company

Mobile phase:

Methanol, 1-propanol,
2.5 n NaCl/H₂O (1+1+3)

40 min developing time

Detection range of paraquat

B. Spangenberg

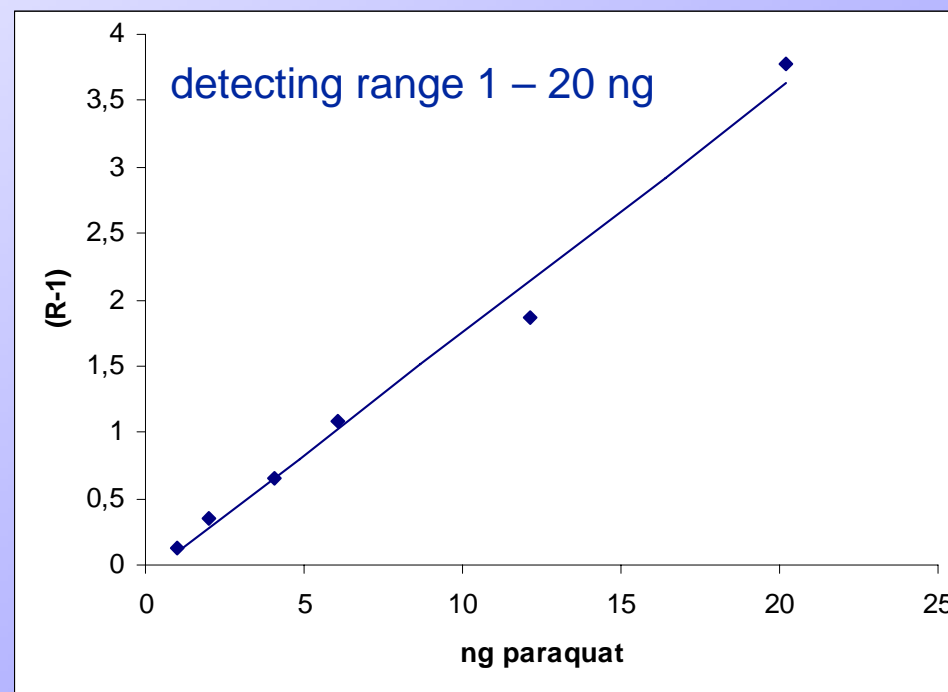
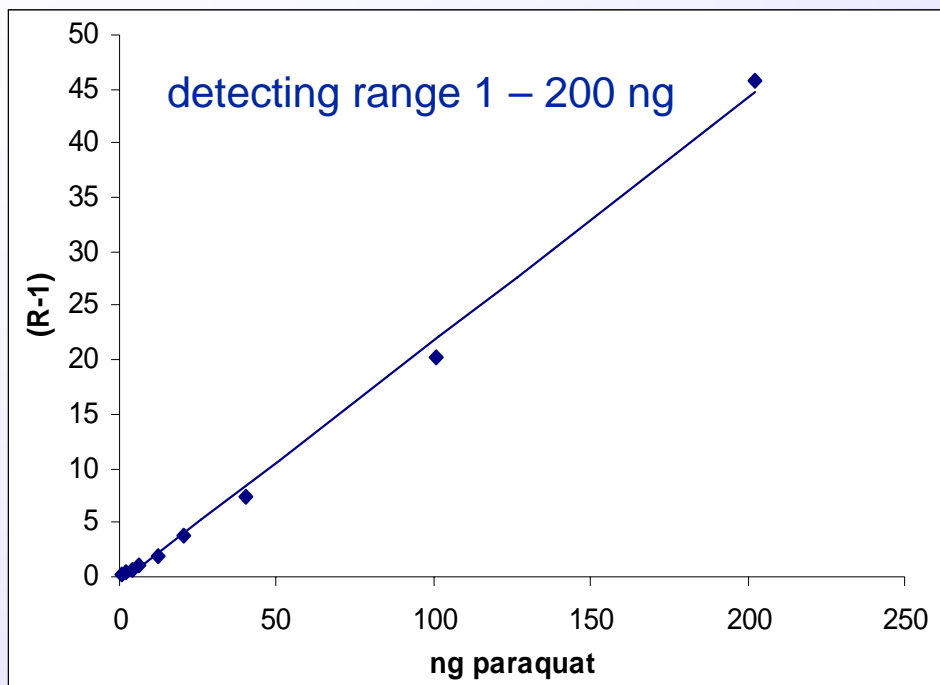
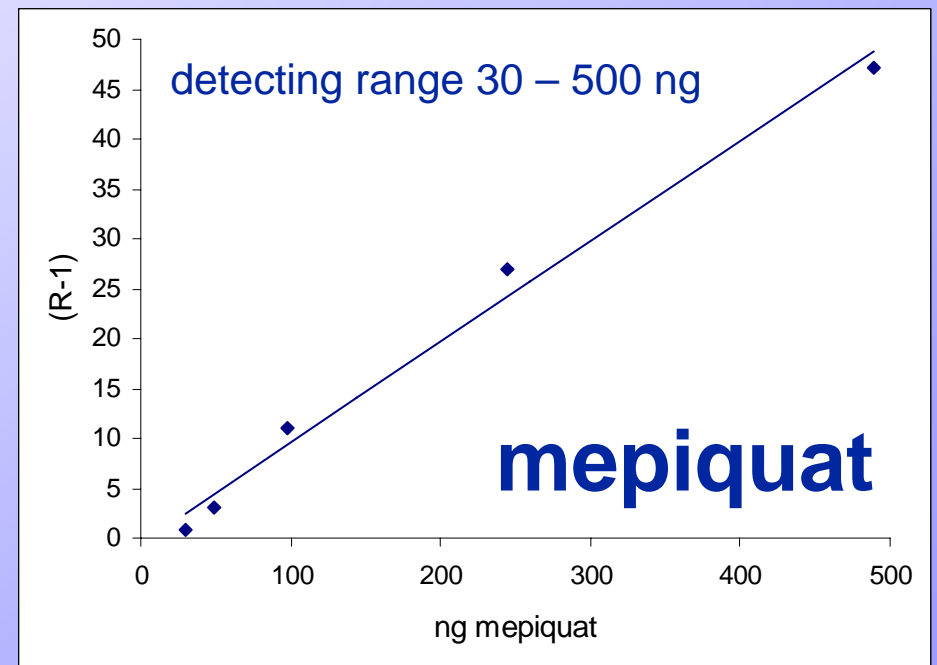
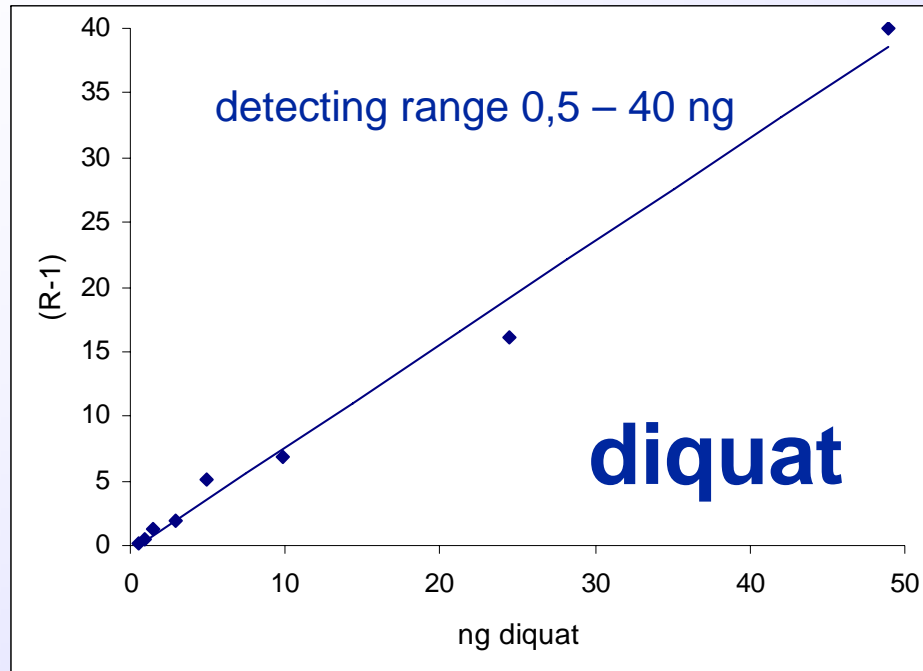


Plate was dipped in a solution of ethylene glycol/methanol 1+1, (V/V).

Linear regression and estimation of detection and quantification limits, according to W. Funk et. al. „Statistische Methoden in der Wasseranalytik“, VCH Weinheim 1985

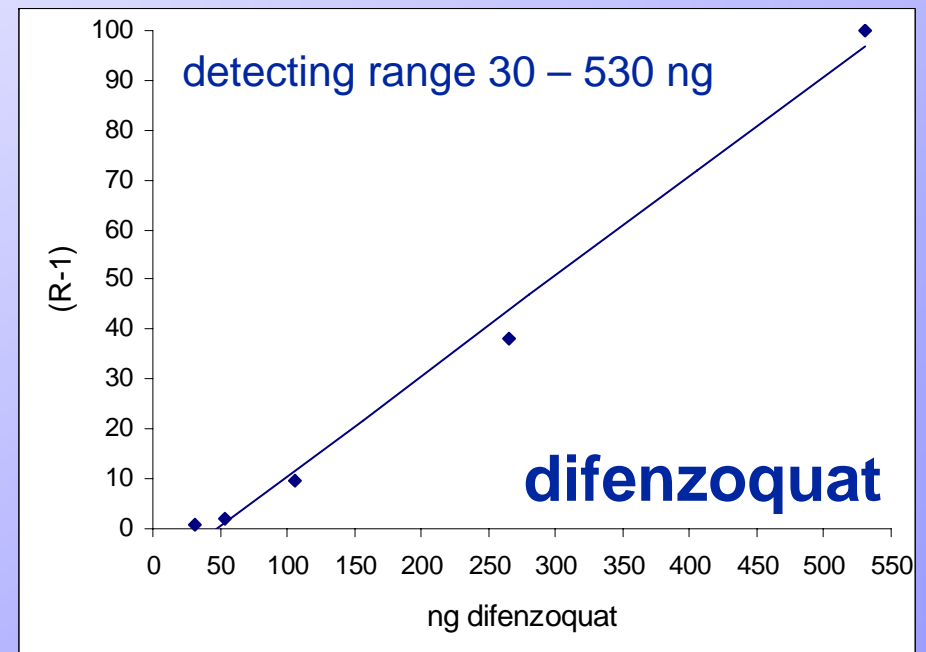
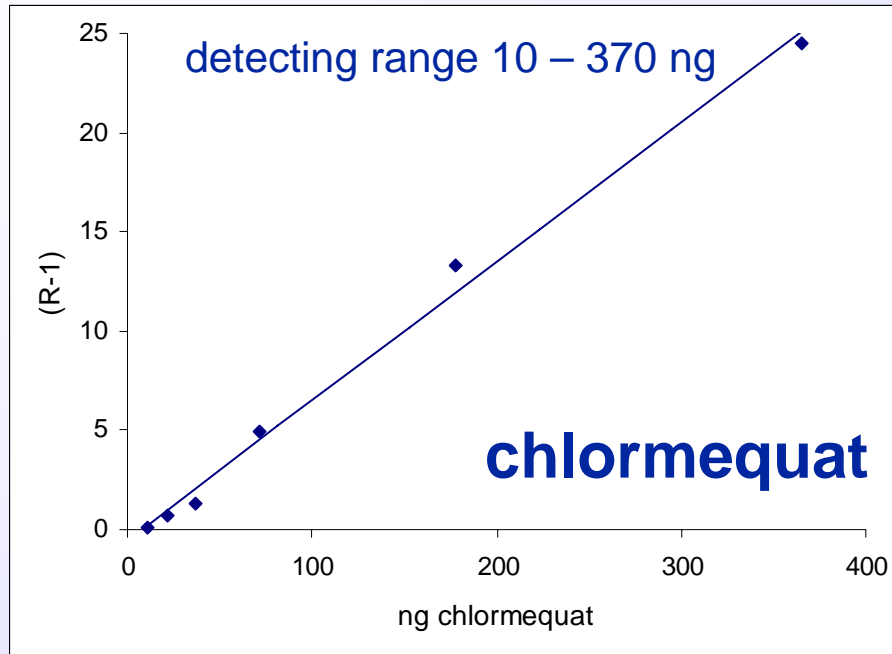
Detection range of diquat and mepiquat

B. Spangenberg



Detection range of chlormequat and difenzoquat

B. Spangenberg



We focussed on four challenges in quat analysis:

- 1.) optimisation of the separation system**
 - 2.) optimisation of the detection system**
 - 3.) improvements in the detection limits**
 - 4.) optimisation of sample pre-treatments**
-

Optimisation of sample pretreatments

B. Spangenberg



Water samples are enriched by use of MAC-3 c, kationic resin, standard grade (Dow Chemical Rheinmünster, Germany). The resin is packaged in a plastic cartridge (3 g), the volume 1000 mL sample is passed through the resin cartridge and eluated with 50 mL 0.1 m HCl.

The sample evaporation is done by use of a gentle stream of air over the eluate surface. The dry sample is topped up to 30 mL with methanol, filtered, evaporated to dryness and topped up with 500 μ l methanol.

The amount of 20 μ l sample is applied on plate.

Detection limits and quantification limits of quats

B. Spangenberg

name	detection limit	quantification limit (20 µL)*	
Paraquat	6.25 ng	9.0 ng	220 ng/L
Diquat	2.25 ng	3.25 ng	82 ng/L
Mepiquat	35 ng	50 ng	1.25 µg/L
Chlormequat	25 ng	30 ng	0.78 µg/L
Difenzoquat	90 ng	105 ng	2.60 µg/L

*: for 1 L water, extracted in 500 µl methanol and 20 µl applied on plate

Detection levels in drinking water (according to US EPA method 549.2):

Paraquat 680 ng/L and diquat 720 ng/L

Estimation of detection and quantification limits, according to W. Funk et. al.

„Statistische Methoden in der Wasseranalytik“, VCH Weinheim 1985

Recovery rate

B. Spangenberg

<u>Name</u>	<u>RF-value</u>	<u>recovery rate</u>	<u>rel.sdv.</u>
Paraquat	12.0	98.9 % (20.2 µg/L)	40.6 %
Diquat	16.3	26.7 % (4.9 µg/L)	28.0%
Mepiquat	28.4	107.4 % (49 µg/L)	12.9%
Chlormequat	43.9	63.8 % (36 µg/L)	18.8%
Difenzoquat	67.5	115.6 % (53 µg/L)	48.5%

Conclusion

B. Spangenberg

- **all five quats are quantitatively detectable**
 - **the detection limits are quite good**
 - **the sample preparation step (and the recovery rate) is insufficient**
-



Thank you very much
for your attention