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Fluorescence spectroscopy in planar chromatography

Bernd Spangenberg, Offenburg, Germany

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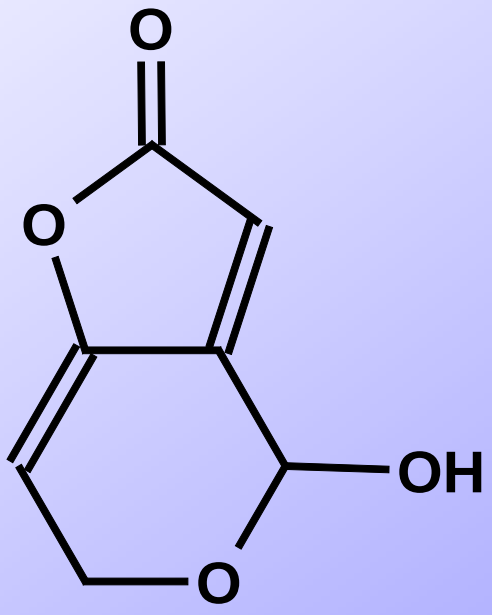
How to quantify patulin?



4-Hydroxy-4*H*-furo[3,2-c]pyran-2(6*H*)-one

What is patulin ?

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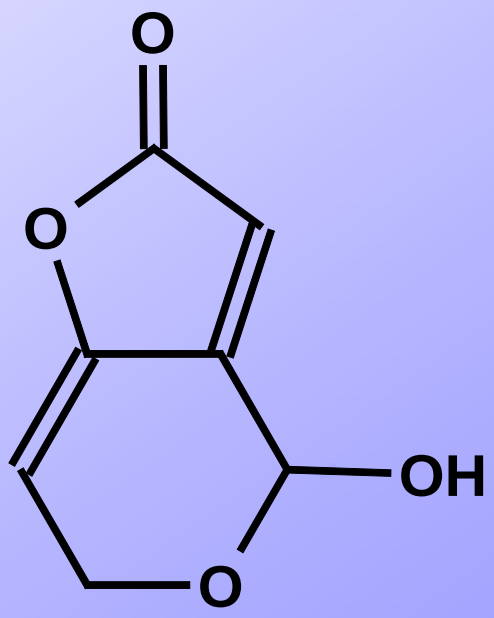
Patulin is a mycotoxin, highly toxic to animal cells and tissues.



Patulin has carcinogenic and mutagenic properties.

The main source of patulin in human diet is probably apple juice.

Patulin is a good quality indicator of fruits.

Quantification of patulin

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TLC methods predominated in the early seventies for the quatification of patulin.

They later gave way to those methods based on HPLC.

Four reasons were responsible for this:

Why not TLC?

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1. **TLC is tedious and time consuming**
2. **a confirmation of patulin is not given**
3. **TLC shows a lack of separating power**
4. **TLC is not sufficiently sensitive (20 µg/L).**

from: S. J. Kubacki, H. Goszcz, Pure&Appl. Chem. 60, 871-876, 1988

Allowable Patulin limits in the EU

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The European countries limit the allowable patulin content within food at 25 to 50 µg/L.

The patulin contamination of baby food has been limited to 10 µg/L.

Detection limits of patulin

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method	*application volume*	*detection limit*
TLC (UV):	50 µL	50 ng (20 µg/L)
TLC (MBTH):	50 µL	30 ng (12 µg/L)
HPLC (UV):	50 µL	5 ng (5 µg/L)
HPLC/MS:	50 µL	1 ng (1 µg/L)
GC/MS:		? (4 µg/L)
CE:	2 mL	7.6 ng (3.8 µg/L)
HPTLC:	10 µL	? ?

from: W. Worobo et. al., Comprehensive reviews in food science and food safety 1, 8-21 2005

A modern HPTLC scanner station

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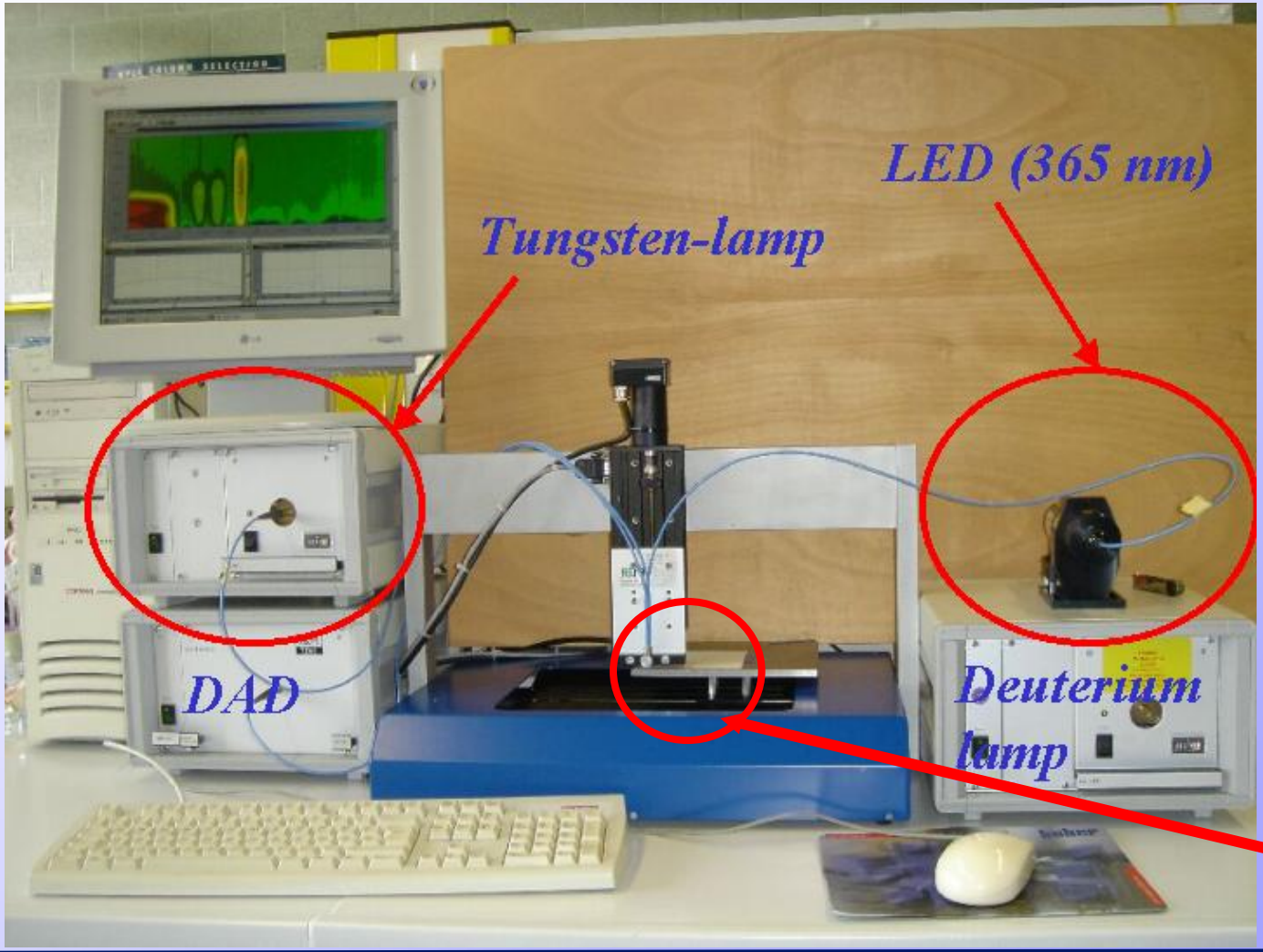

DAD-range:
190 – 1100 nm

Light intensity distribution of different lamps

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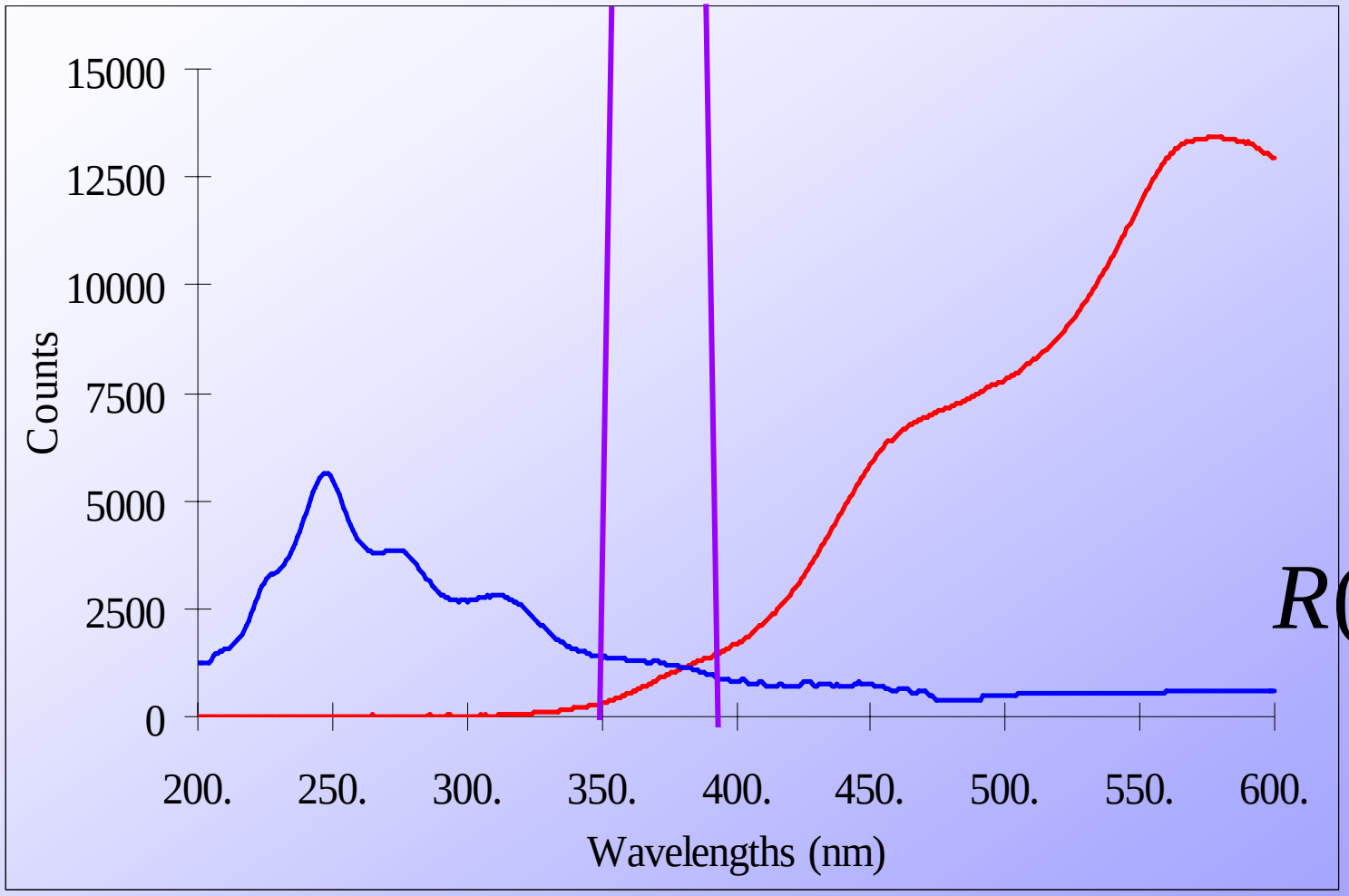

D_2-lamp (UV)

W-lamp (vis)

LED (fluorescence)

$$R(\lambda) = \frac{J(\lambda)}{J_0(\lambda)}$$

3D-plot of patulin

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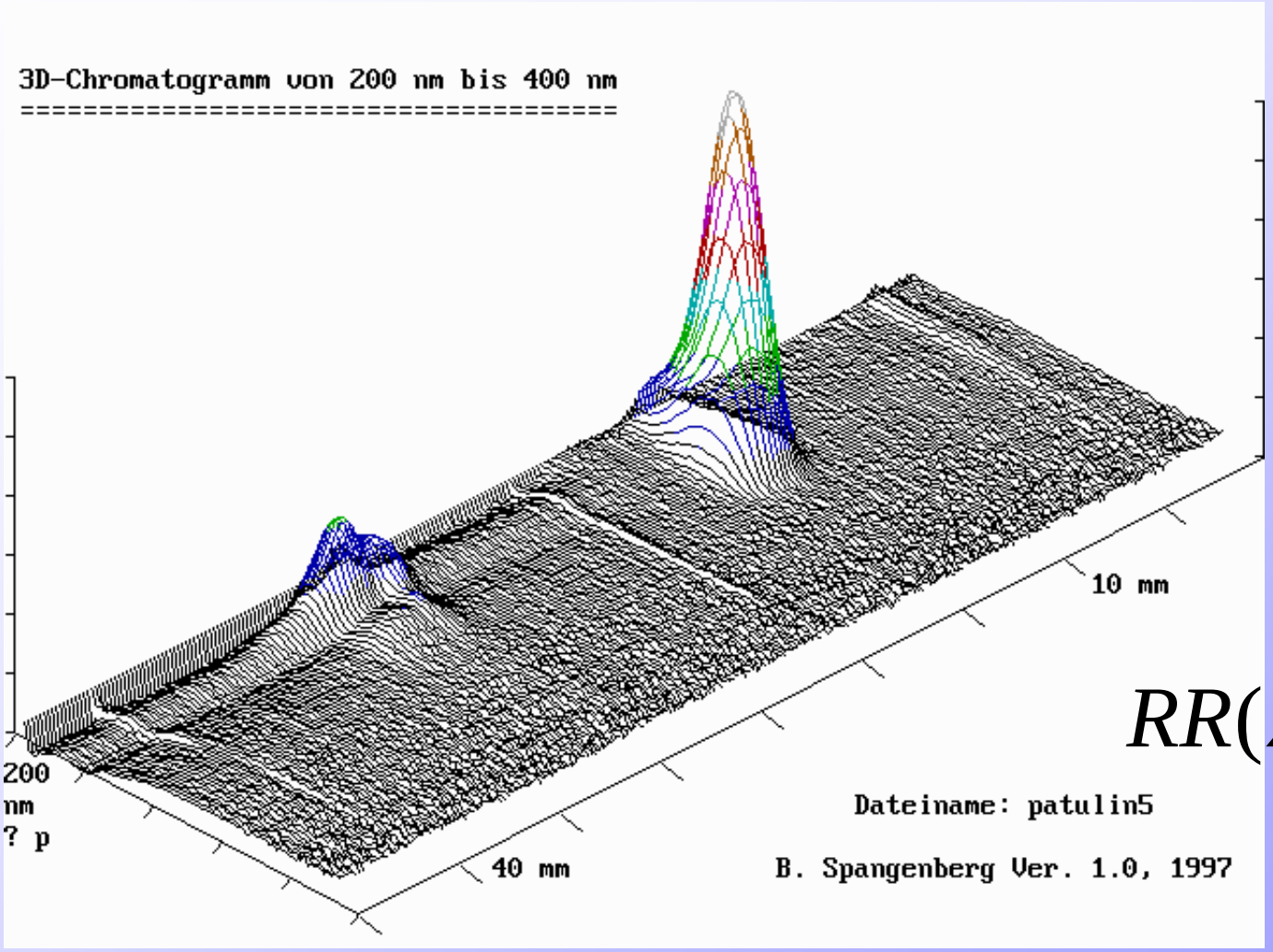

336 ng patulin on silica gel

toluene, dioxane (7+3)

$$RR(\lambda) = \left(\frac{1}{R(\lambda)} - 1 \right)$$

UV-detection of patulin

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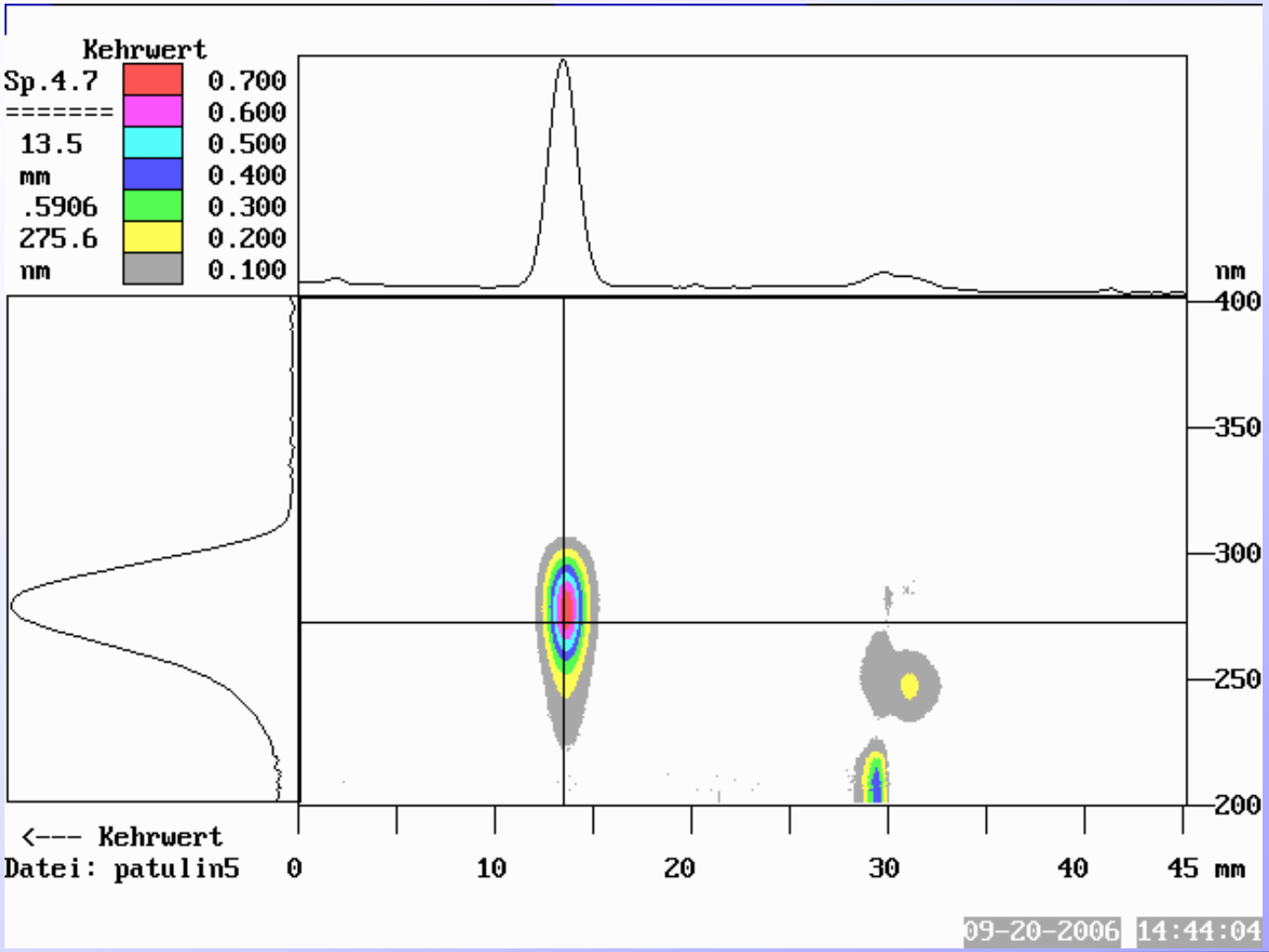

336 ng patulin on silica gel

toluene, dioxane (7+3)

detection time of a single spectrum: 0.5 sec.

shown are 450 single spectra

Lamp: D_2

vis-detection of patulin

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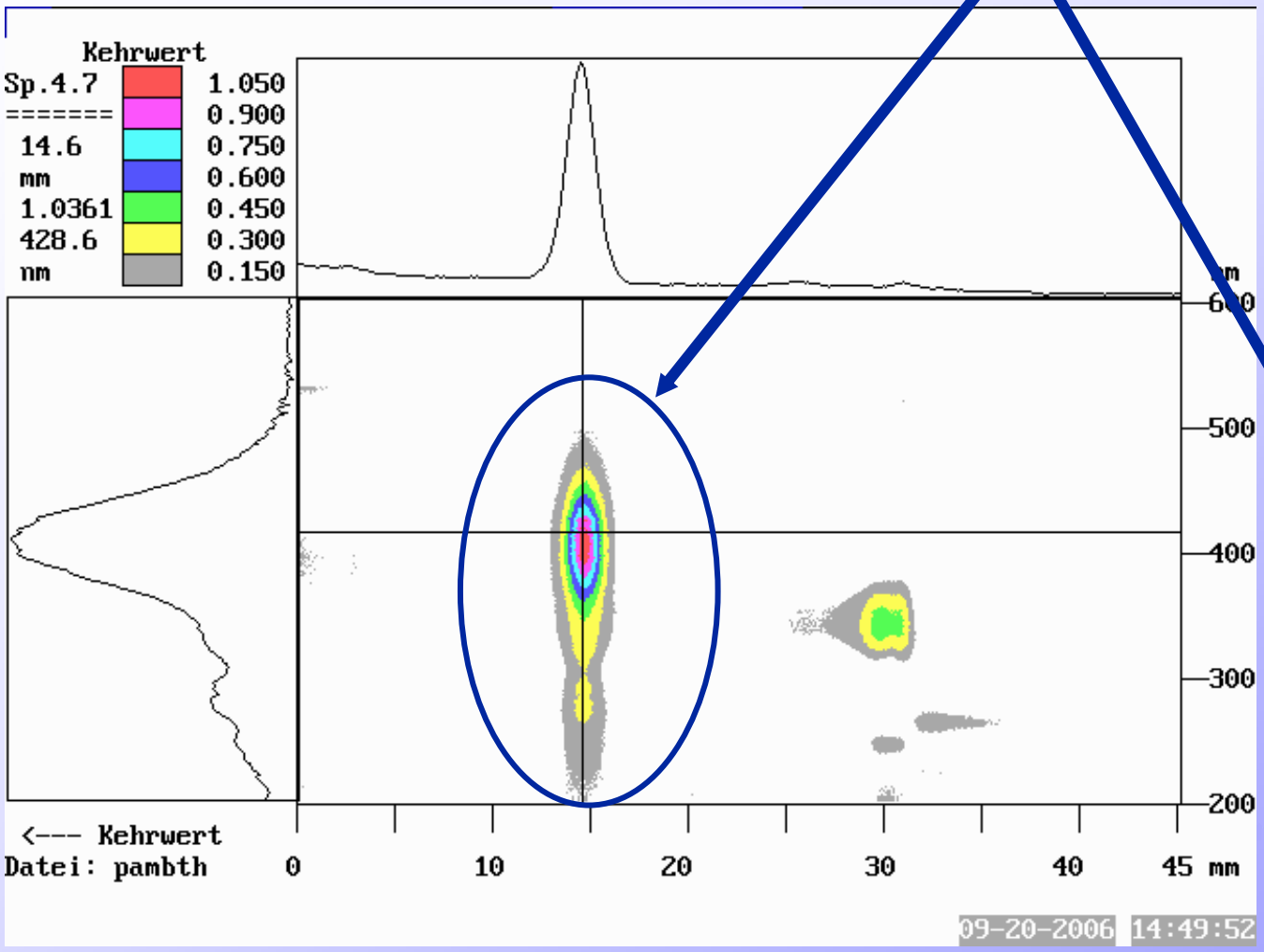

336 ng Patulin on silica gel, derivated by use of MBTH

Lamp: D_2+ W

High intensity LED

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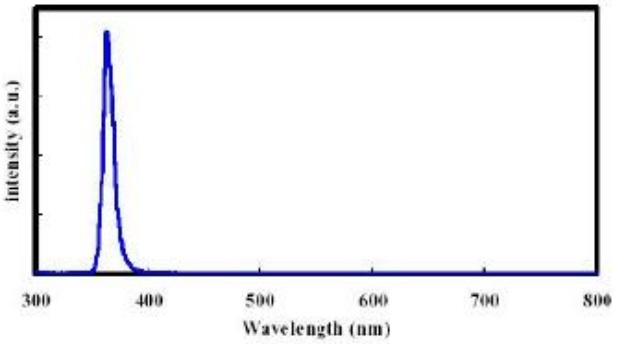
Light intensity: 100 mW !

365nm, ± 10nm halbe Breite,



The diode shows an absolutely constant light intensity!

Conditions for patulin quantification

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plate:	**silica gel (without fluorescence indicator)**
mobile phase:	**toluene, dioxane (7+3)**
dipping liquid:	**100 mg phenylhydrazine HCl in 100 mL methanol + 100 µl H_2SO_4**
dipping time:	**4 seconds**
reaction conditions:	**10 min at 100 °C, fluorescence**

Fluorescence contour plot of a patulin separation

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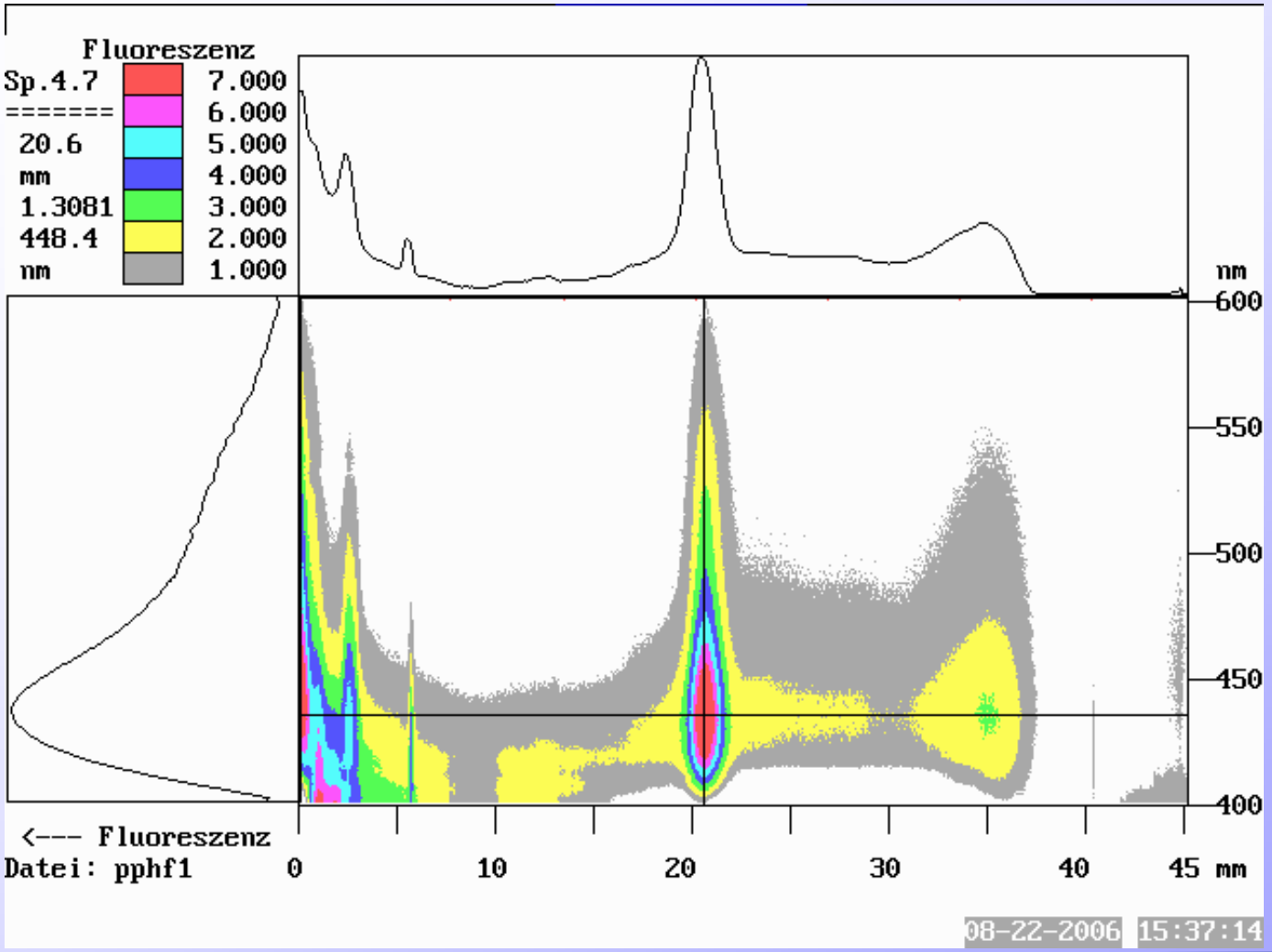

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673.6 ng patulin

on silica gel

derivatisation with phenyl-hydrazine

$$F(\lambda) = R(\lambda) - 1$$

Lamp: LED

Calibration curve and densitogram of patulin

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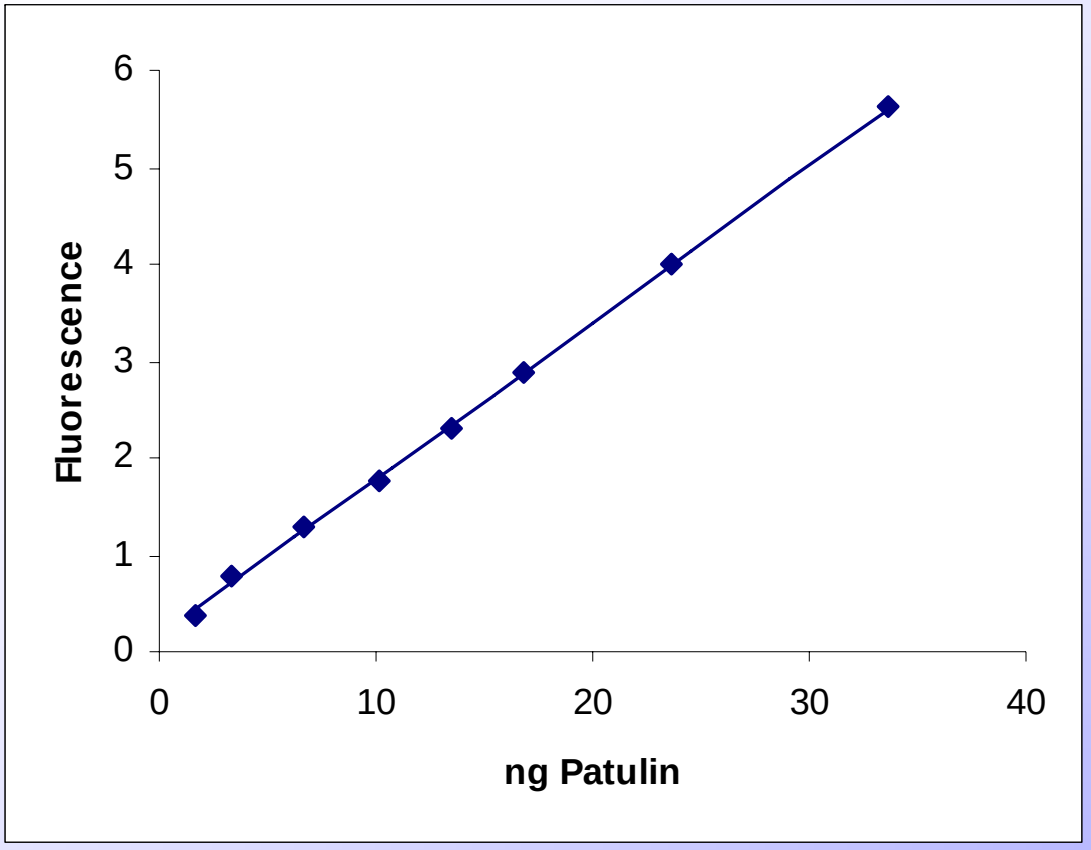
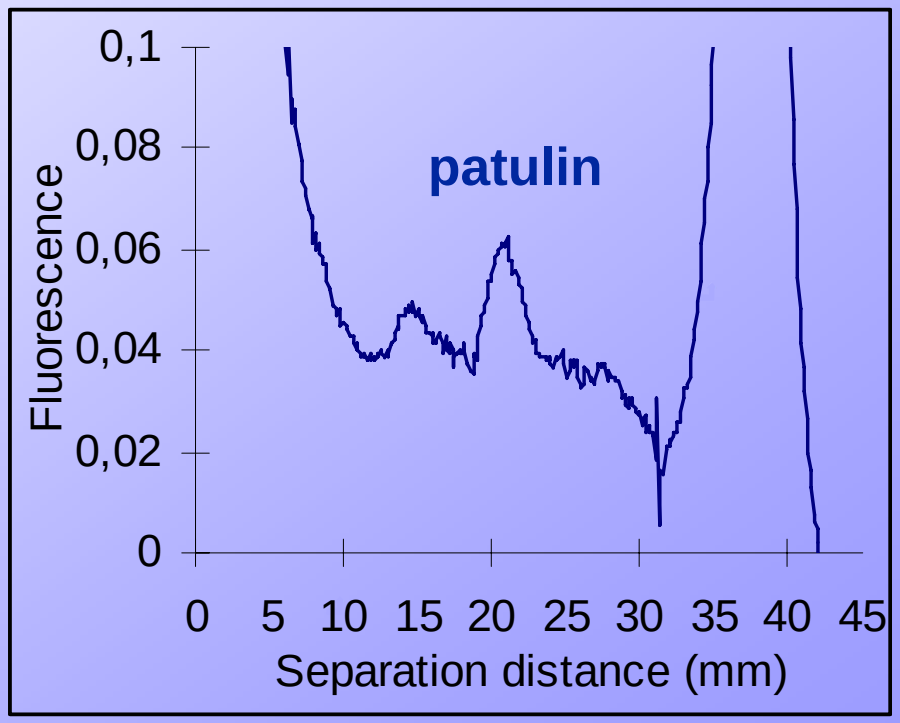


Densitogram of 3.4 ng patulin (detection limit).



Fluorescence detection limit of patulin in HPTLC: 3.4 ng.

Detection limits of patulin

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method	*application volume*	*detection limit*
TLC (UV):	50 µL	50 ng (20 µg/L)
TLC (MBTH):	50 µL	30 ng (12 µg/L)
HPLC (UV):	50 µL	5 ng (5 µg/L)
HPLC/MS:	50 µL	1 ng (1 µg/L)
GC/MS:		? (4 µg/L)
CE:	2 mL	7.6 ng (3.8 µg/L)
HPTLC:	**10 µL**	**3.4 ng (6.8 µg/L)**

from: W. Worobo et. al., Comprehensive reviews in food science and food safety 1, 8-21 2005

Conditions for patulin quantification

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plate:	**silica gel (without fluorescence indicator)**
overspotting liquid:	**50 mg phenylhydrazine HCl in 9 mL methanol + 1 mL DMSO + 100 µL H_2SO_4**
overspotting volume:	**2 µL**
reaction conditions:	**10 min at 100 °C**
mobile phase:	**methyl t-butyl-ether, heptane (8+2)**
detection:	**fluorescence**

Fluorescence contour plot of a patulin separation

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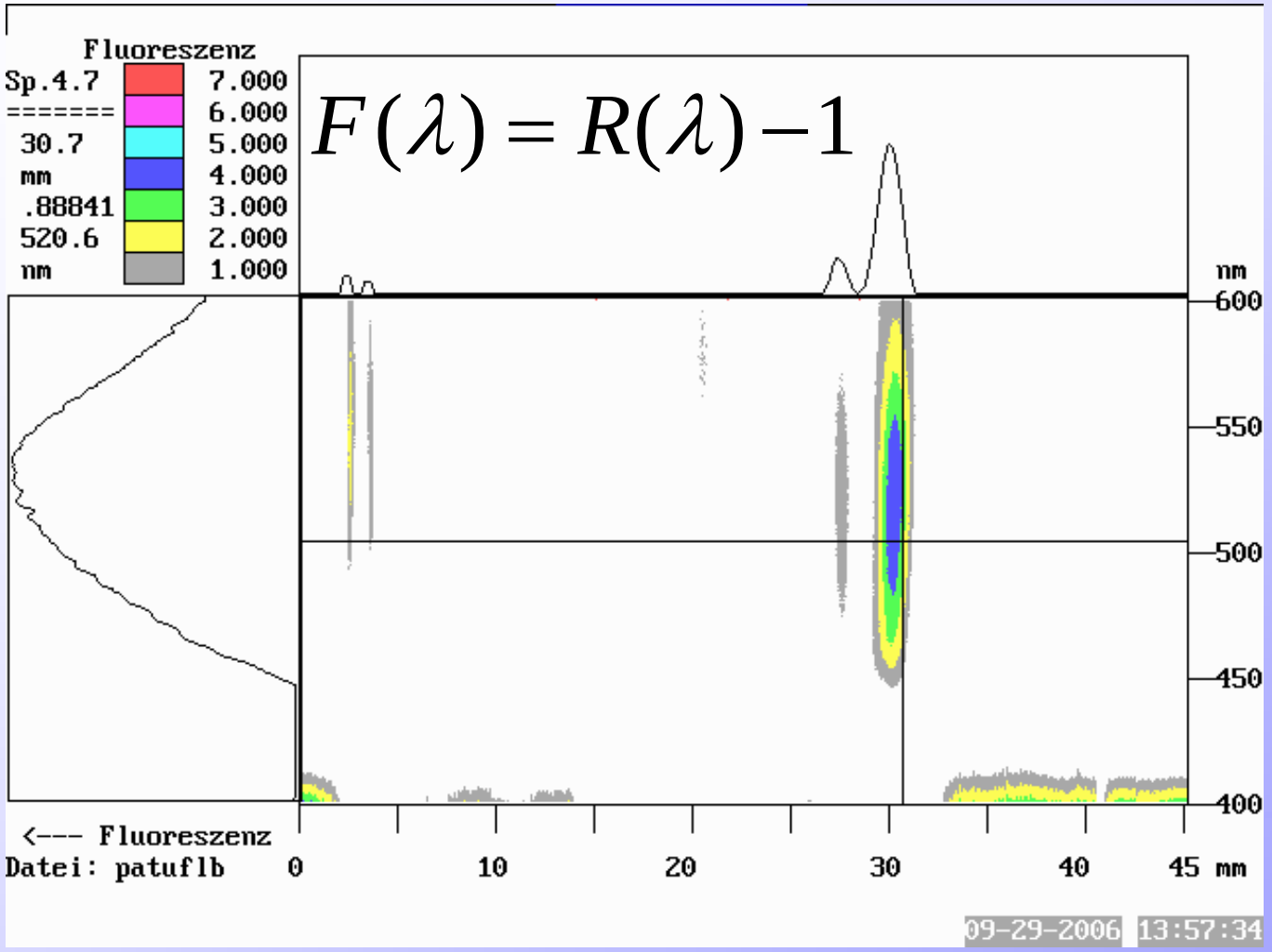

673.6 ng patulin on silica gel overspotted with 2 µL phenyl-hydrazine in CH_3OH, 100°C, 10 min,

Densitogram of patulin (5 ng)

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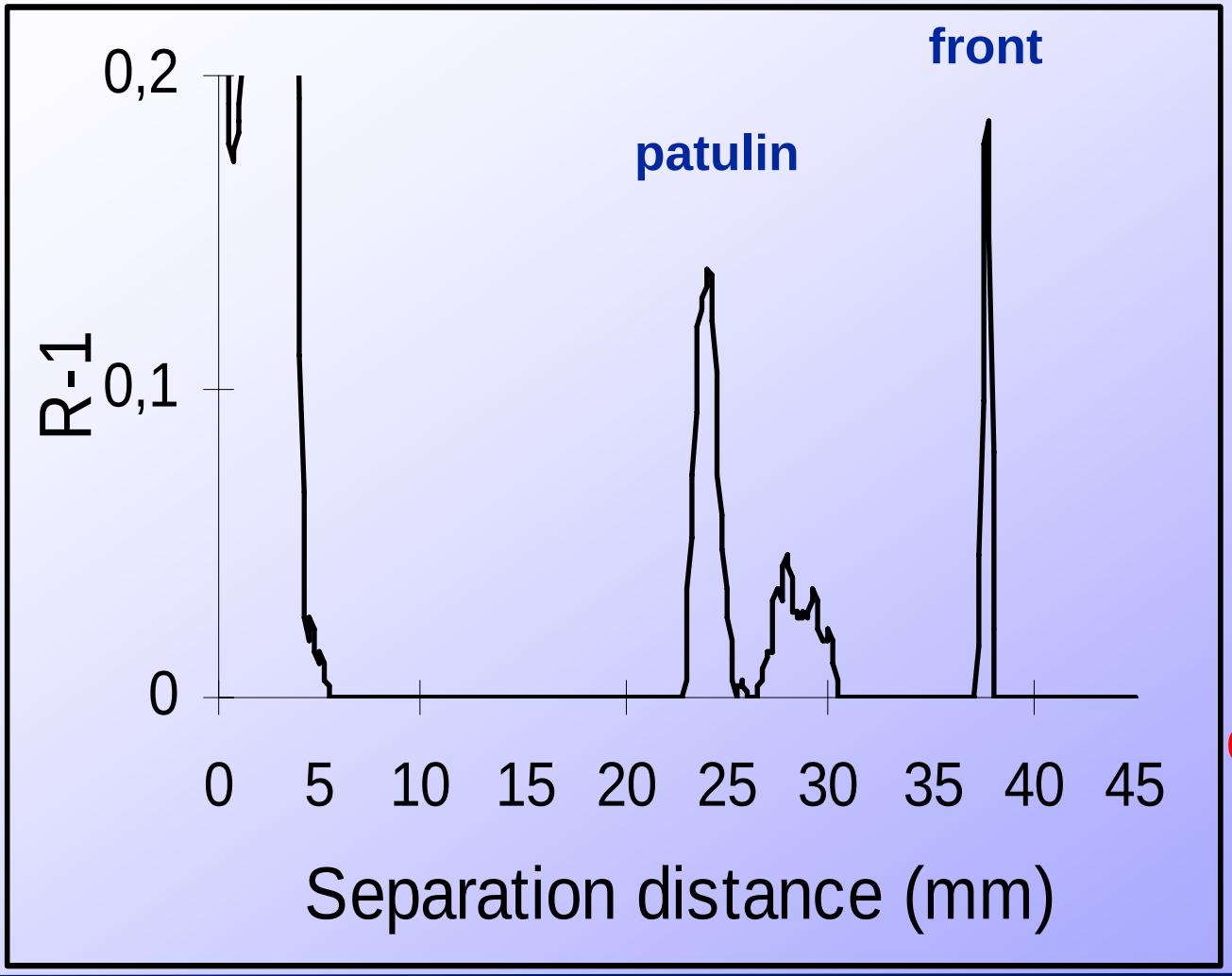

Densitogram of 5.05 ng patulin.

= 10 ppb!

detection limit: 1.7 ng

Conclusion

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1. **TLC is tedious and time consuming**
2. **a confirmation of patulin is given via fluorescence spectra.**
3. **HPTLC shows sufficient separating power**
4. **HPTLC is sufficiently sensitive (3.4 µg/L).**

Acknowledgment

I greatly appreciate all the laboratory help of

Regina Brämer* and *Andrea Seigel

and many thanks to *Dr. Jörg Stroka*, Geel, Belgium for helpfull discussions.

Location of Offenburg

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Fluorescence contour plot of a patulin separation

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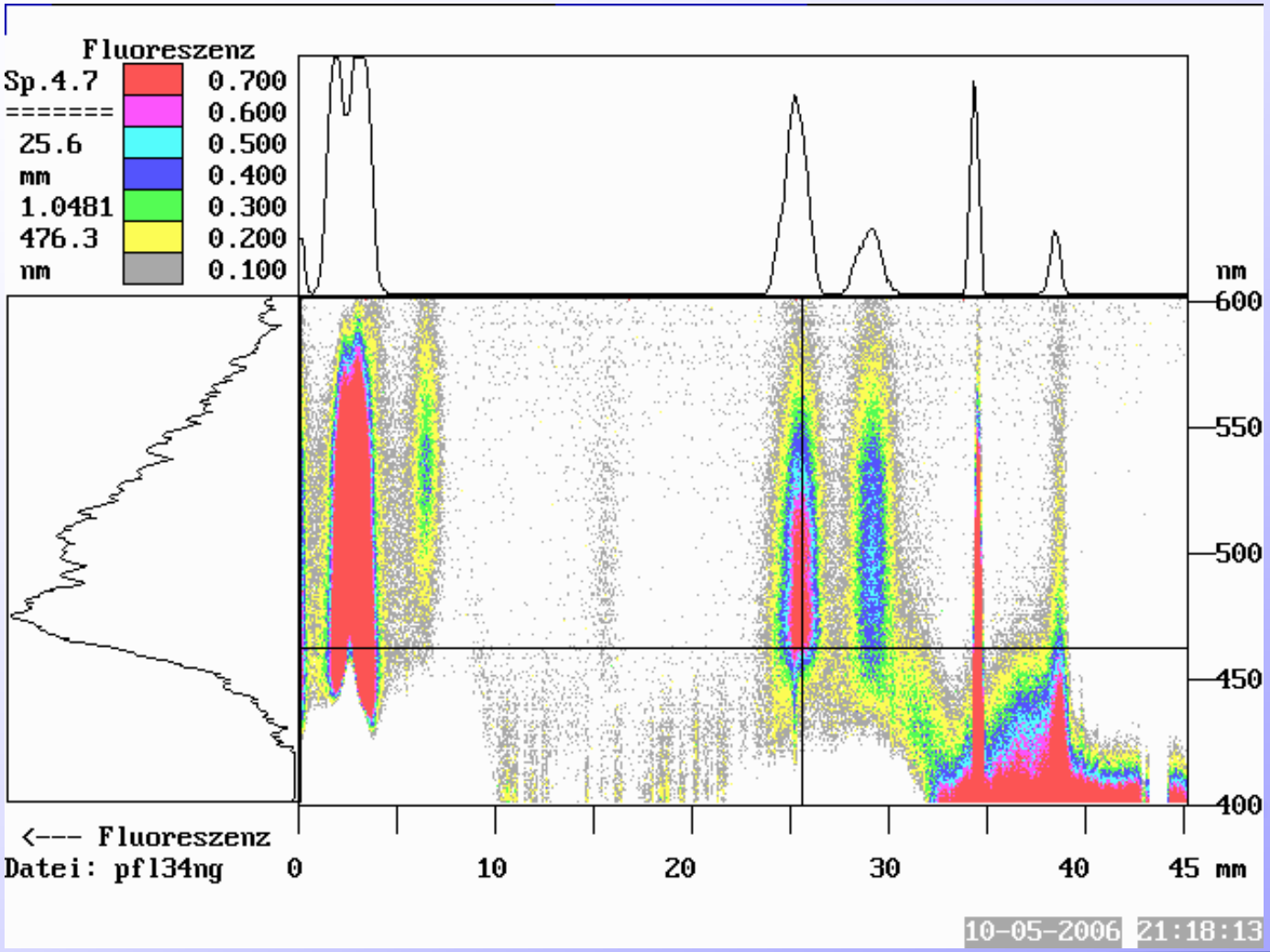

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33.58 ng patulin on silica gel overspotted with 2 µL phenyl-hydrazine in CH_3OH, 100°C, 10 min,