Thin - layer chromatography in cleaning validation

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Why “cleaning validation”?

- Pharmaceutical manufacturers have to verify that the cleaning procedures for multiple use equipment will remove residues of previous products to acceptable level.
- Such residues might have a significant impact on the quality of a pharmaceutical product subsequently produced in the same equipment.
- "Guide to Inspection of Validation and Cleaning Processes" issued by the US Food and Drug Administration (FDA) in 1993 has increased the attention to the concepts of cleaning validation in pharmaceutical industry.
- The so-called “cleaning validation” is also required by Good Manufacturing Practice (GMP) and performed according to the cleaning validation protocol.

Protocol for cleaning validation

- **Not** a protocol for the validation of analytical procedure.
- Protocol for cleaning validation should be written for each equipment class.
- The protocol for sampling should exactly define the size, the number and the site (position) of the pharmaceutical equipment surfaces to be cleaned. The sampling sites should be marked on the equipment diagrams.
- The most important part of the protocol for cleaning validation is a **validated analytical procedure**, which describes the quantitative transfer of drug residue to the solution and its final determination.

Techniques used

- HPLC
- Planar chromatography
- Spectrophotometry
- Total organic carbon = TOC (sensitive but not selective; for water-soluble samples)

Development of analytical methods using planar chromatography

- As simple as possible.
- Quantitative transfer of drug residue to the solution and its final determination.
- Low detection and quantitation limit, specific, accurate and rugged.
- Possibility of visual estimation of chromatograms desirable (**high throughput screening of the analysed samples).**

How clean is clean enough?

- Acceptance limit in the next product (ppm or µg/g)
- Acceptance limit of surface contamination (mg/m^2 or $\mu g/cm^2$)

Starting points

- **Acceptance limit of surface contamination** (1, 5, 10 mg/m^2)
- **Surface areas**: fixed or variable (e.g. 1 to 10 dm^2)

Example

- **Acceptance limit of surface contamination (1, 5, 10 mg/m^2)**
- **Surface areas: 1-10 dm^2**
- **Acceptance limit: 1 mg/m^2 = 0.1 µg/cm^2**

  1 mg/m^2 = 0.1 mg/10 dm^2 = 0.01 mg/1 dm^2

  0.01 to 0.1 mg/50 ml =

  = 10 to 100 µg/50 ml = (µg/ml=ng/µl)

  = **1 to 10 ng/5 µl** =

  = **2 to 20 ng/10 µl**
- **Acceptance limit:10 mg/m^2: 2 to 20 ng/1 µl**
- **Working range with standards**: from LOD to about 10 times LOQ (linearity range)

Preparation of sample test solution

- Swabbing
- Extraction and filtration

Swabbing

- Swabbing material:
  - cotton, filter paper, glass-fibre filter paper
  - size (e.g. 0.5 g cotton, about 6x6 cm)
  - purification (sometimes needed)

- Solvent for swabbing and further extraction (**can differ**):
  - **swabbing** material is wetted by 2 to 3 ml of non-toxic solvent (e.g. water, ethanol, sometimes with additives) capable to dissolve the analyte

- Swabbing technique for quantitative transfer

Extraction

- **Solvent:**

  - solubility and stability of the analyte

  - volatility (sample test solution application!)

- **Technique:**

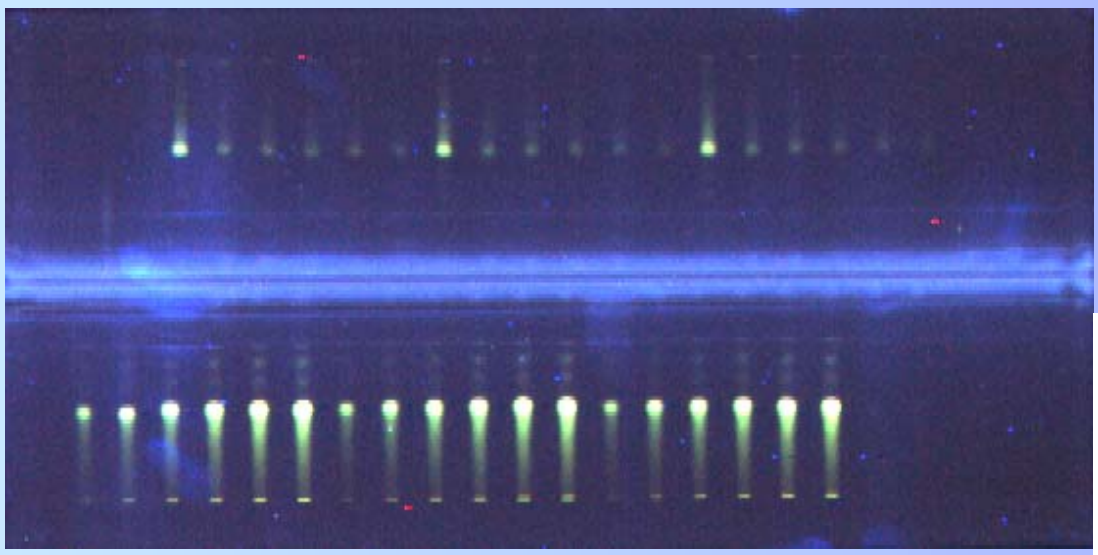
  - ultrasonication, shaking

- **Time** (ca. 15 min)
- **Temperature** (room temperature)
- **Filtration or centrifugation** (prevents troubles with cotton particles at application)

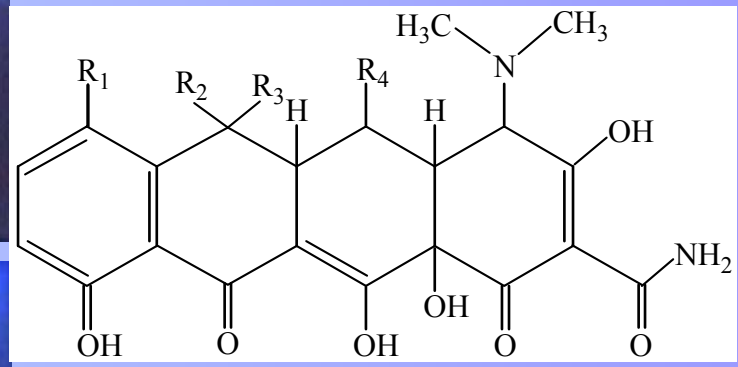
Planar chromatography

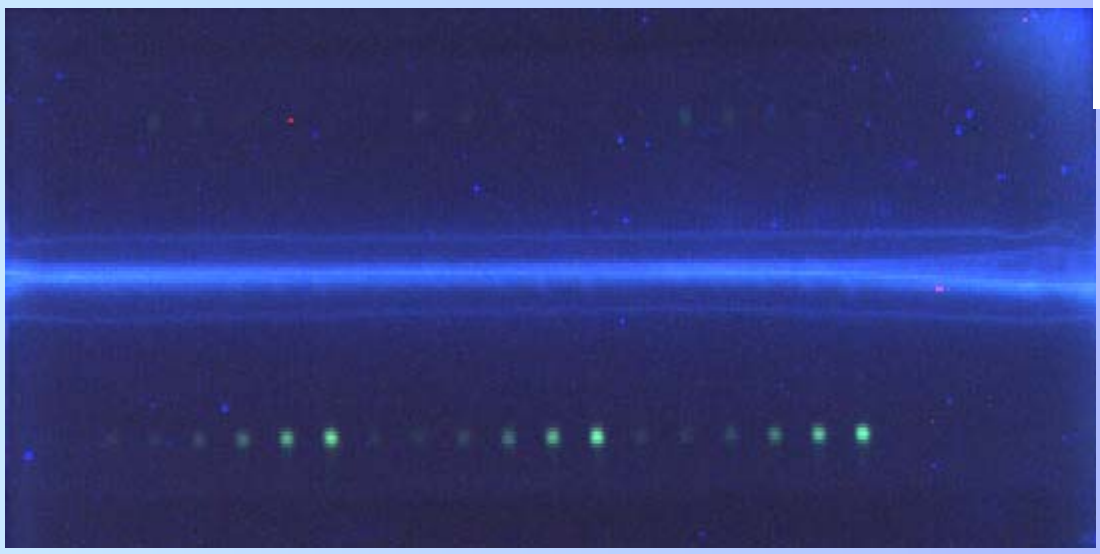
- Sorbent (silica gel with or without F_{254})
- Prewashing (predevelopment) of the plate
- Impregnation (sometimes needed)
- Applicator (Linomat IV, Linomat V, ATS4; usually 1 to 10 µl; contamination)
- Developing solvent (Vario-KS chamber)
- Developing chamber (horizontal 20x10 cm)
- Developing mode (simultaneously from both sides)
- Postchromatographic derivatisation (if needed!)
- Detection (absorption (UV, VIS), fluorescence)
- Quantitation and documentation (densitometer or image-analysing system)

TLC or HPTLC plate



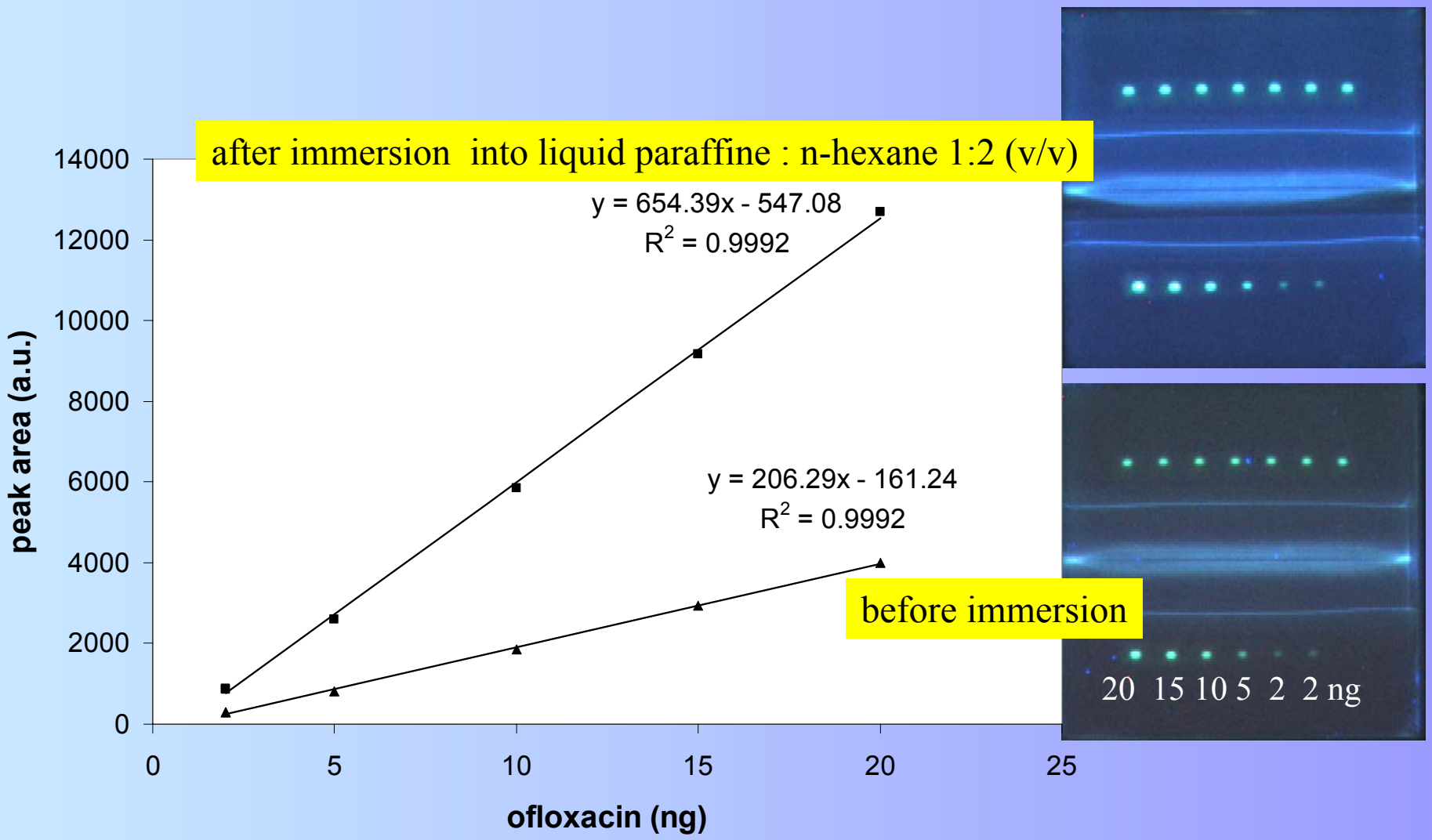
HPTLC plate





TLC plate

Enhanced fluorescence (ofloxacin)

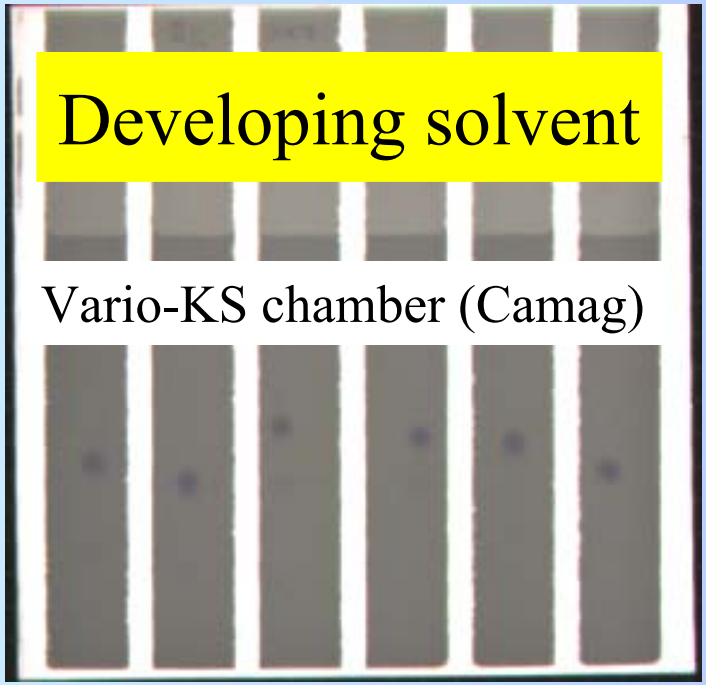


Postchromatographic derivatisation

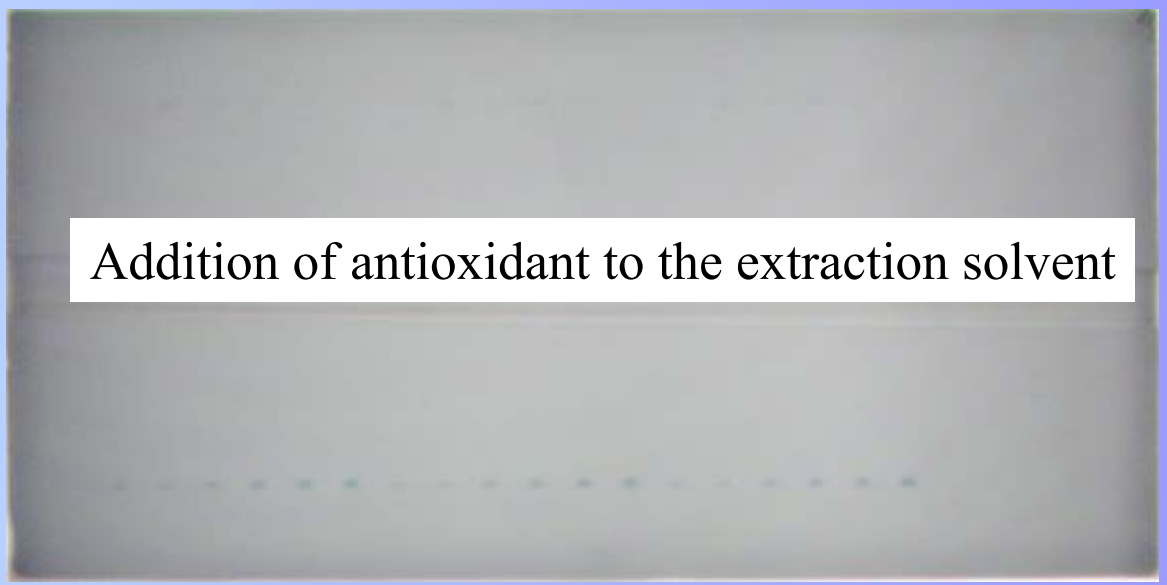
- Choice of derivatisation reagent (low LOD, not too toxic, stable, cheap)
- Controlled immersion (device, time)
- Controlled heating if necessary (temperature, time)

Postchromatographic derivatisation

Developing solvent



Vario-KS chamber (Camag)



Addition of antioxidant to the extraction solvent

Analytical procedure

- Acceptance limit of surface contamination (mg/m^2)
- Surface area to swab (fixed or variable)
- Volume of the extraction solvent (min. 25 ml)
- Application volumes
- Number and amount of standards (6 standards on each side of the plate)
- Number of repetitions of each sample test solution and blank

  (usually 2; data-pair technique)
- Evaluation: - screening (before or after the development)

  - quantitative determination

Evaluation

- **Semiquantitative:** screening (visual under lamp)
- **Quantitative:** - slit-scanning densitometers (absorption maximum)
  - image analysing systems (CCD cameras and flatbed scanners)

- Each type of data acquisition procedure has some advantages and some disadvantages.

Validation of analytical procedure

- **Written protocol**: choice of analytical parameters and criteria in accordance with specific requirements
- **Experimental work** (traceability, documentation)
- **Validation report**

Validation parameters and criteria for quantitative residue determination

- **Repeatability of the chromatographic system**
  (RSD < 15%, n=10, at LOQ; RSD < 10%, n=10, for chosen bigger amount ca. 10x LOQ)
- **Recovery of simulated samples**: 85% to 115%, n=6
- **Repeatability of the chromatographic method** (RSD < 15%, n=6)
- **LOD, LOQ**
- **Specificity** (blank, placeba, ...)
- **Linearity** (6 standards: 2 applications of the 1st calibration standard (LOQ); the highest calibration standard usually about 10x LOQ)
- **Robustness** (different stabilities: on the plate before and after development, stability of analytes in solutions)

Preparation of simulated samples

- Calculated amount of dissolved pharmaceutical is spread over the defined surface (e.g. stainless steel) and the solvent left to evaporate.
- **Sample test solutions:** prepared as described before
- **Example:**

  acceptance limit: 10 mg/m^2

  defined surface: 10 dm^2

  100% of the acceptance limit: 1 mg

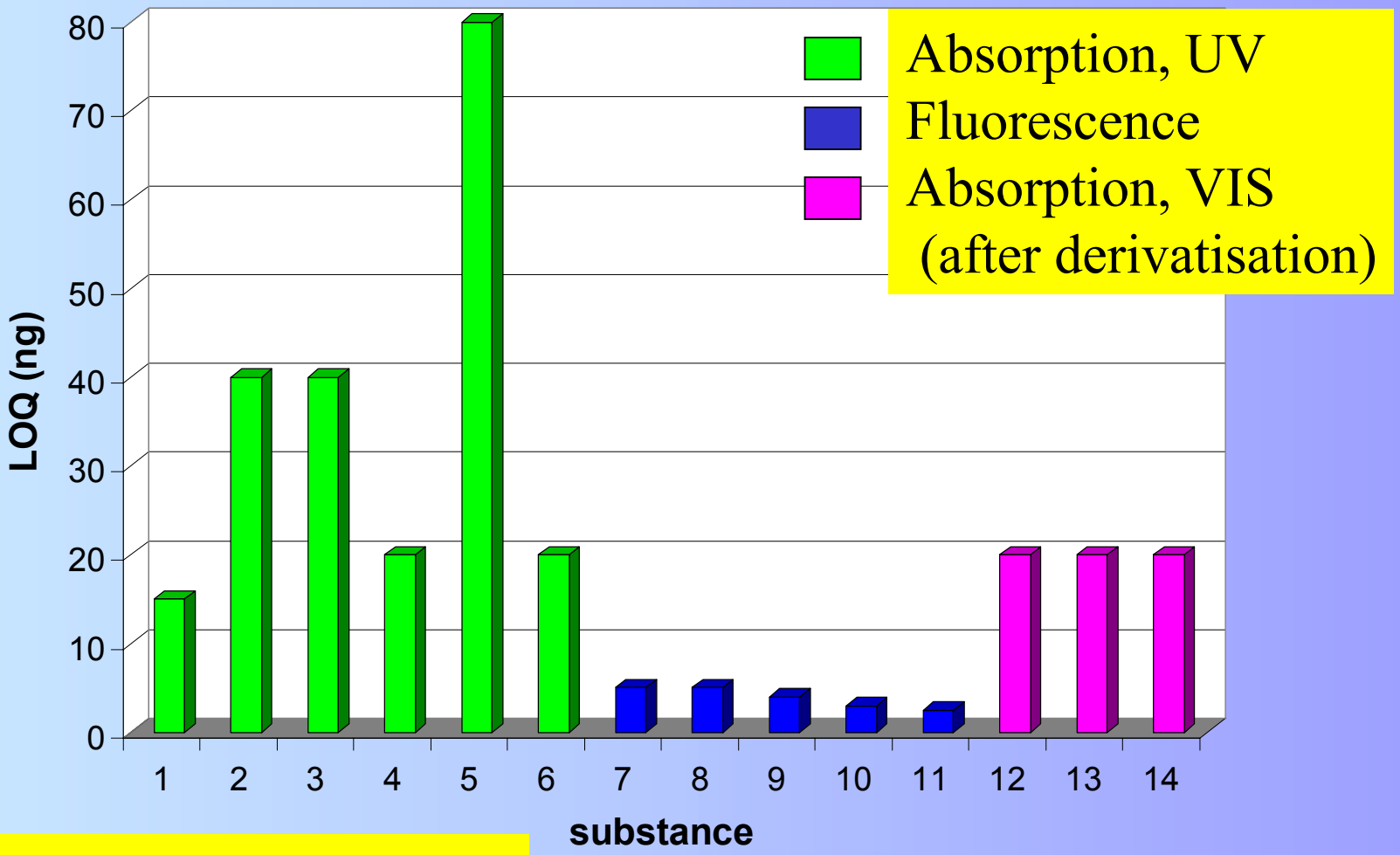
  50% of the acceptance limit: 0.5 mg

  120% of the acceptance limit: 1.2 mg

10 mg/m^2 at swabbing 10 dm^2

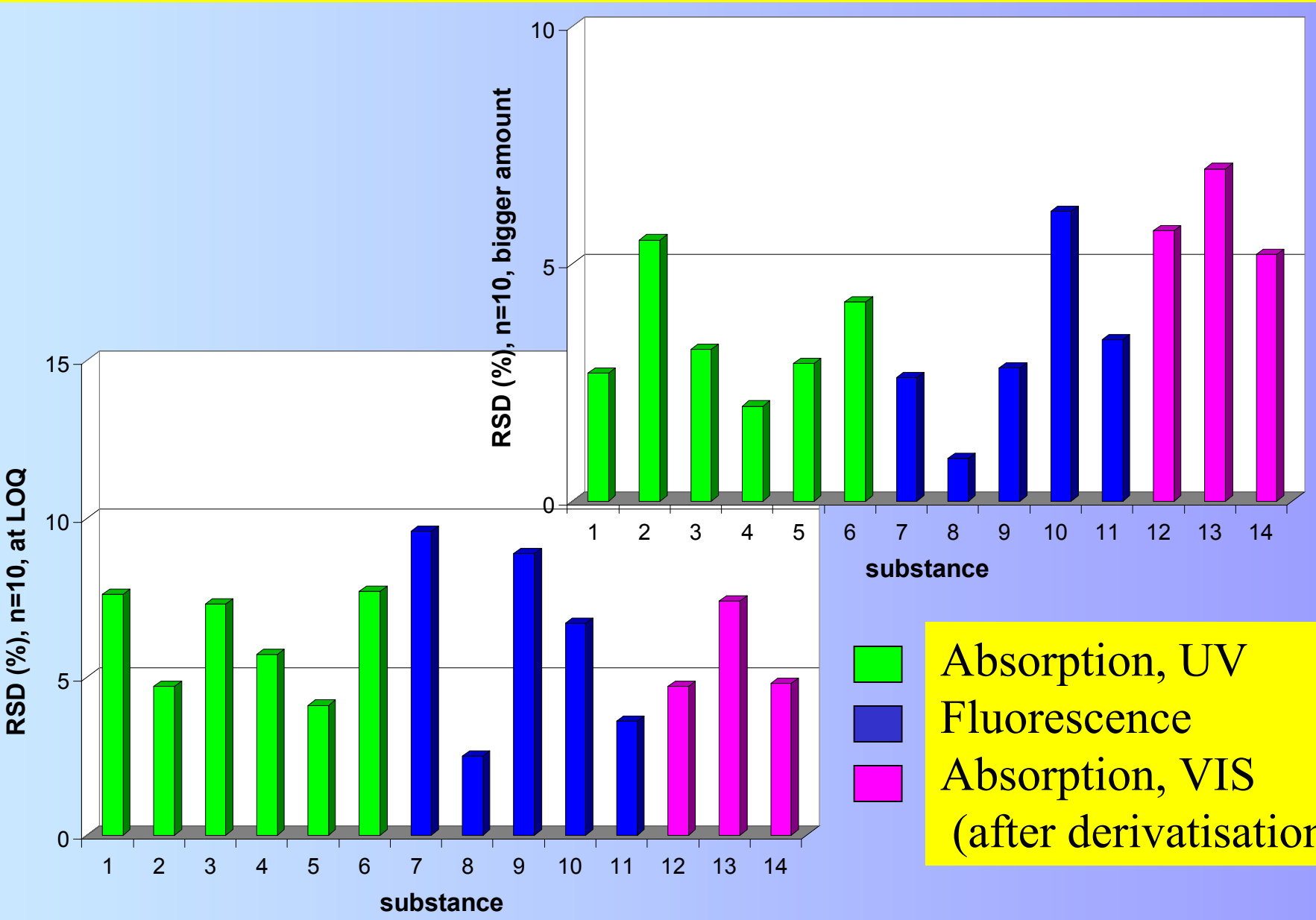
- 100% of the acceptance limit 10 mg/m^2 means
  1 mg at swabbing 10 dm^2
- At validation: 6 swabbings, 2 applications 5 µl from each solution
  1 mg/50 ml = 0.02 mg/ml = 20 µg/ml = 20 ng/µl = **100 ng/5 µl**
- **LOD = 5 ng, LOQ = 10 ng**
- Calibration standards: **10, 10, 20, 60, 100, 120 ng**
- Acceptable mean value from 6 sample test solutions on the plate:
  100 ng ± 15 ng, RSD 15%

LOQ of 14 analytes

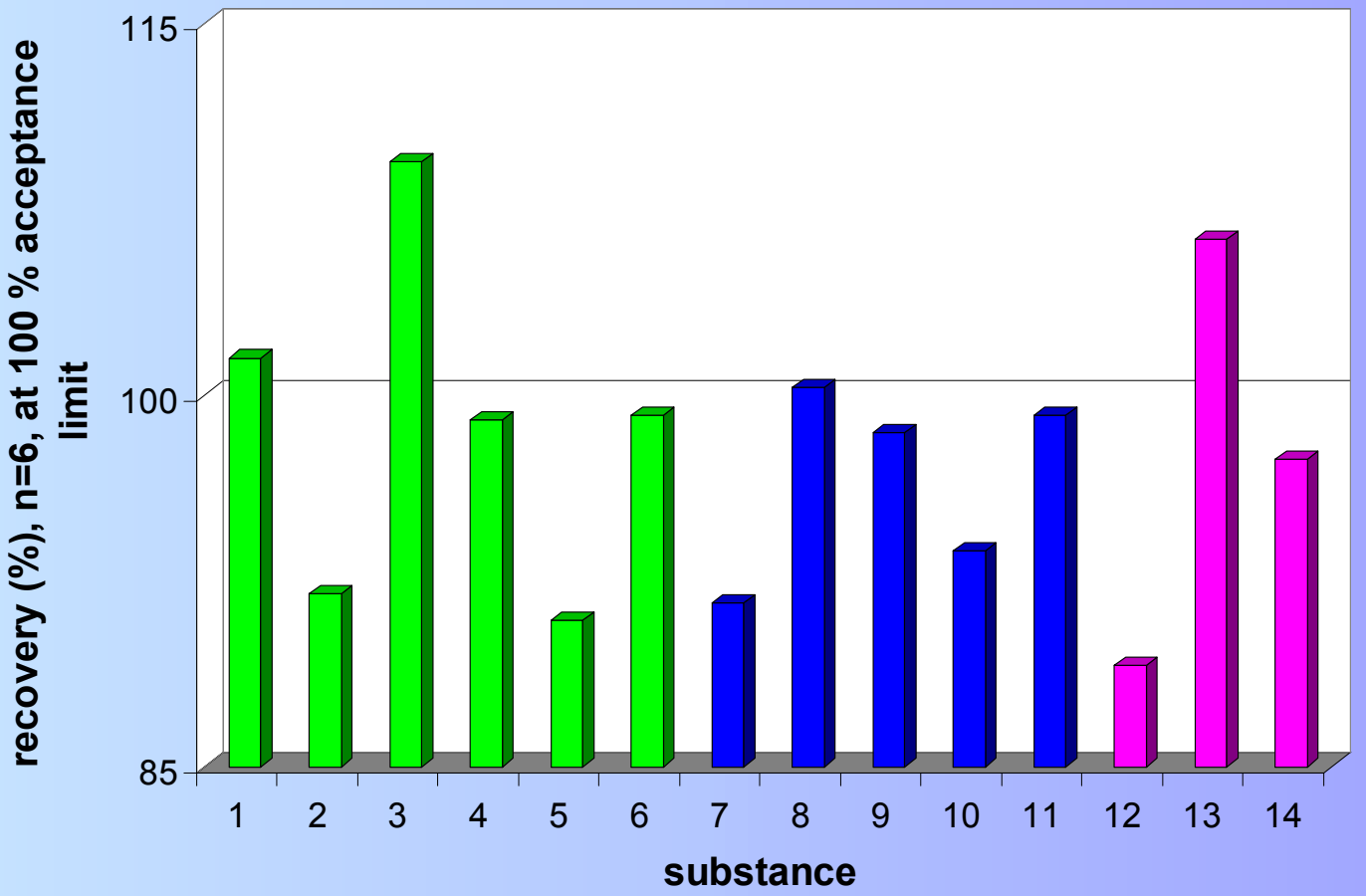


LOD: 2-3x lower than LOQ

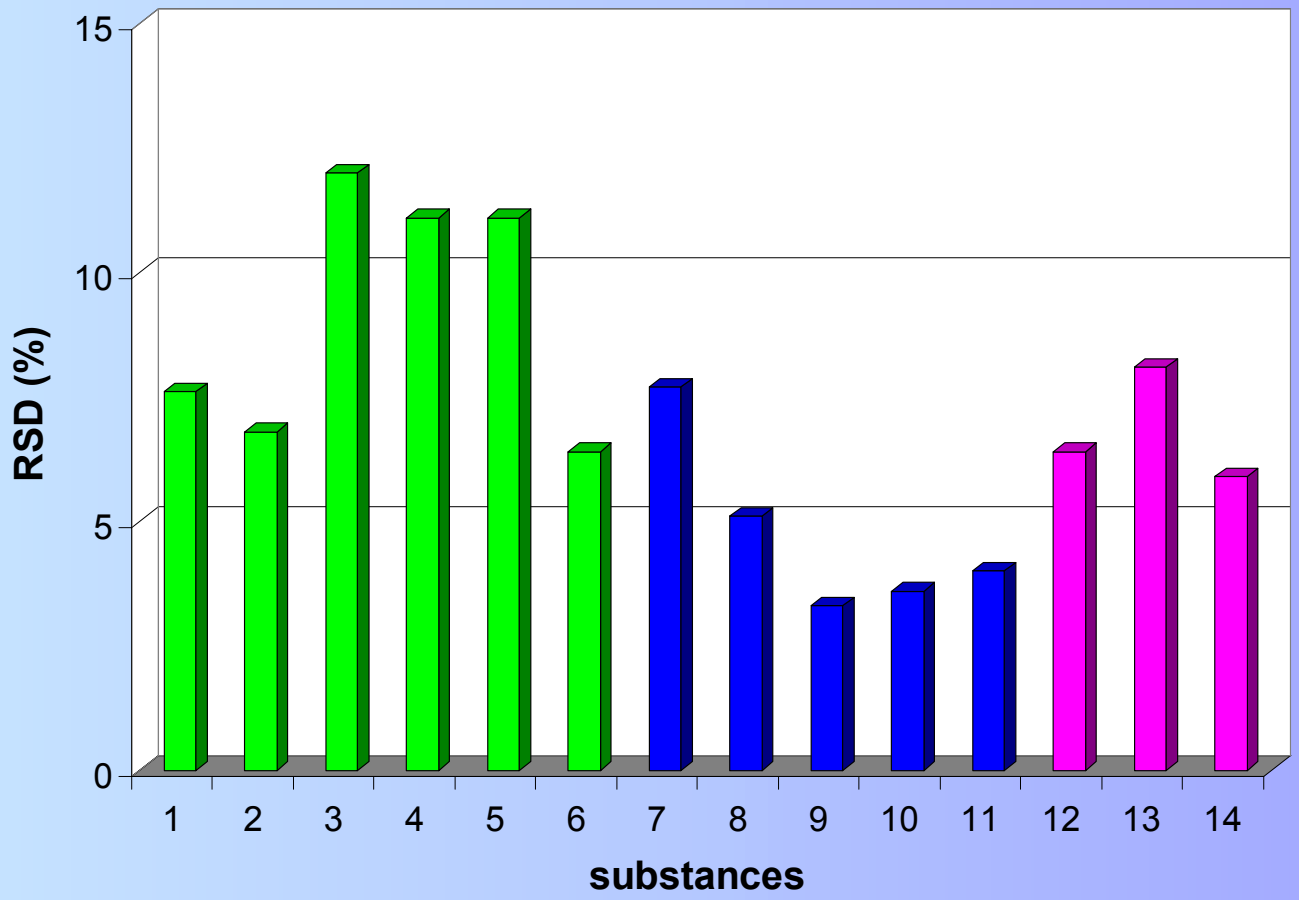
Repeatability of the chromatographic system



Mean recovery of 6 simulated samples

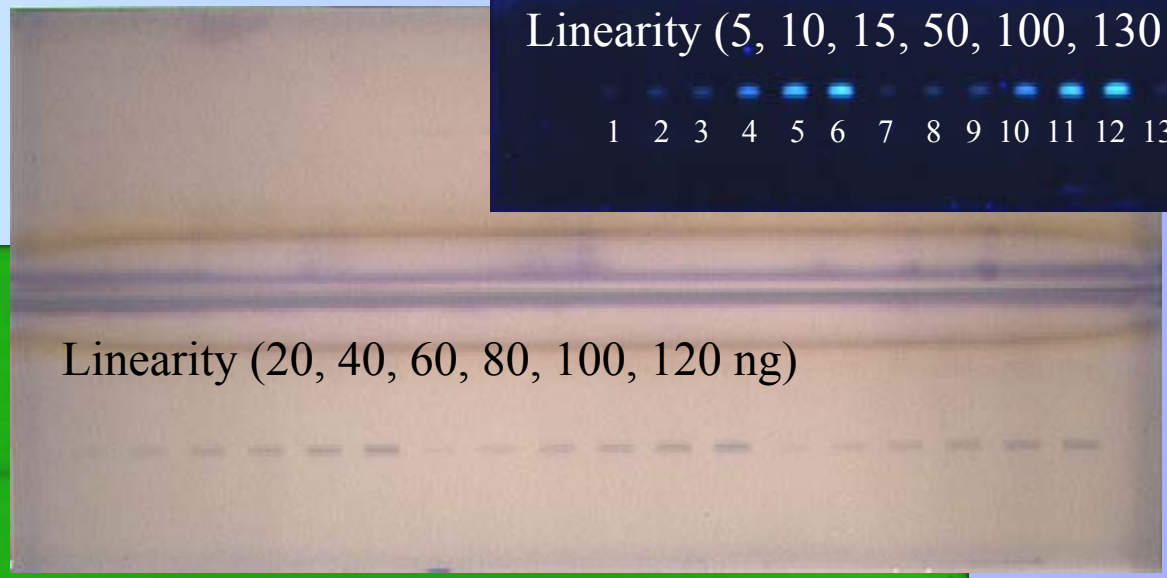


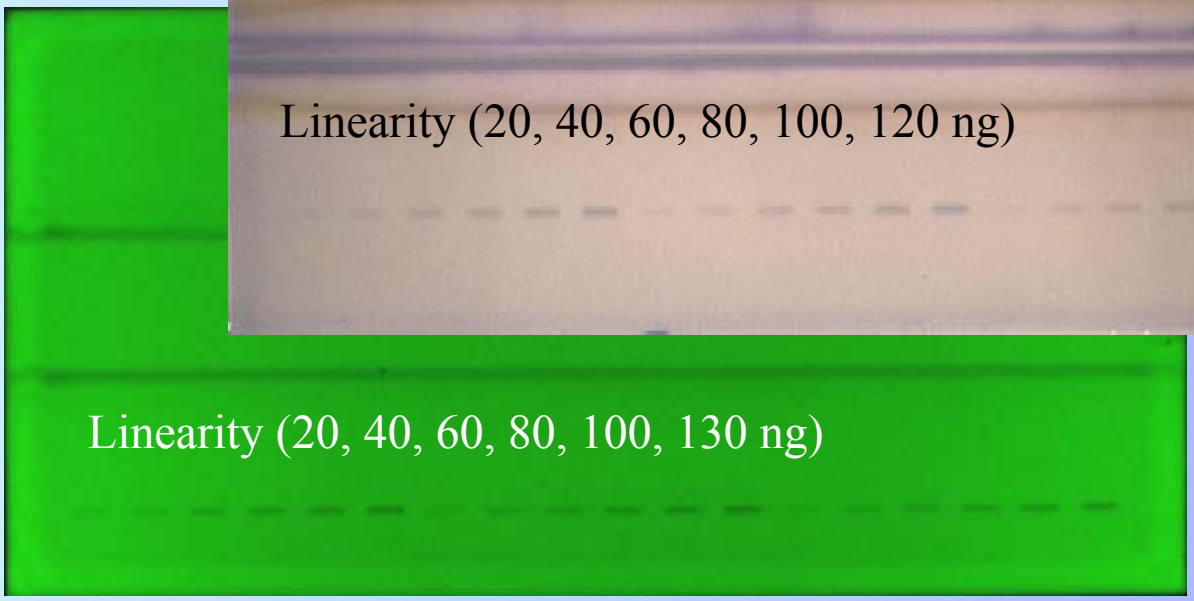
Repeatability of the chromatographic method (n=6) at 100 % of acceptance limit



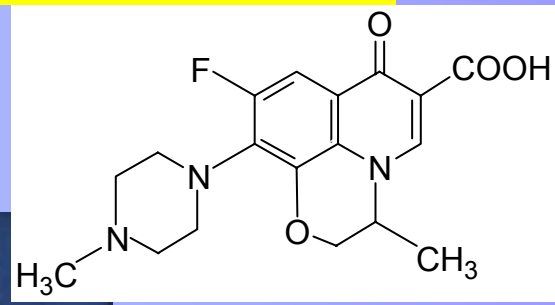
Examples

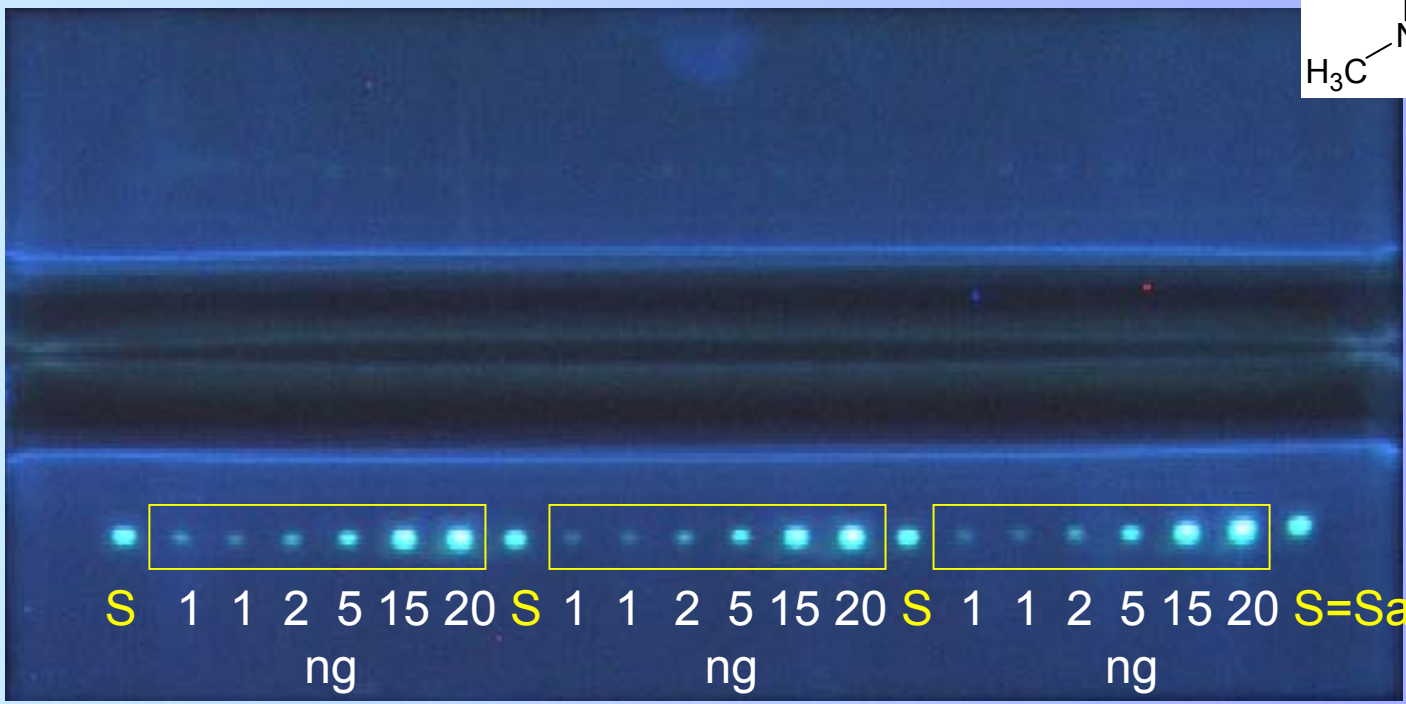

Detection limit
Selectivity
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Linearity (5, 10, 15, 50, 100, 130 ng)
Selectivity
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20


Linearity (20, 40, 60, 80, 100, 120 ng)


Linearity (20, 40, 60, 80, 100, 130 ng)

Ofloxacin


O
F
COOH
N
N
N
O
H_3C
CH_3


S 1 1 2 5 15 20 S 1 1 2 5 15 20 S 1 1 2 5 15 20 S=Sample
ng
ng
ng

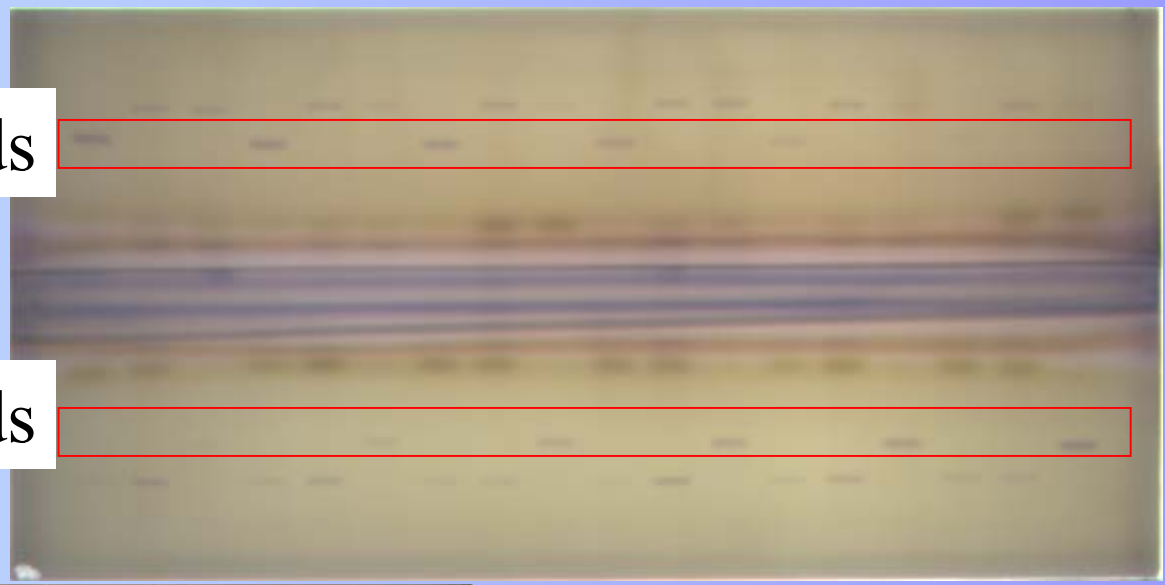
Screening

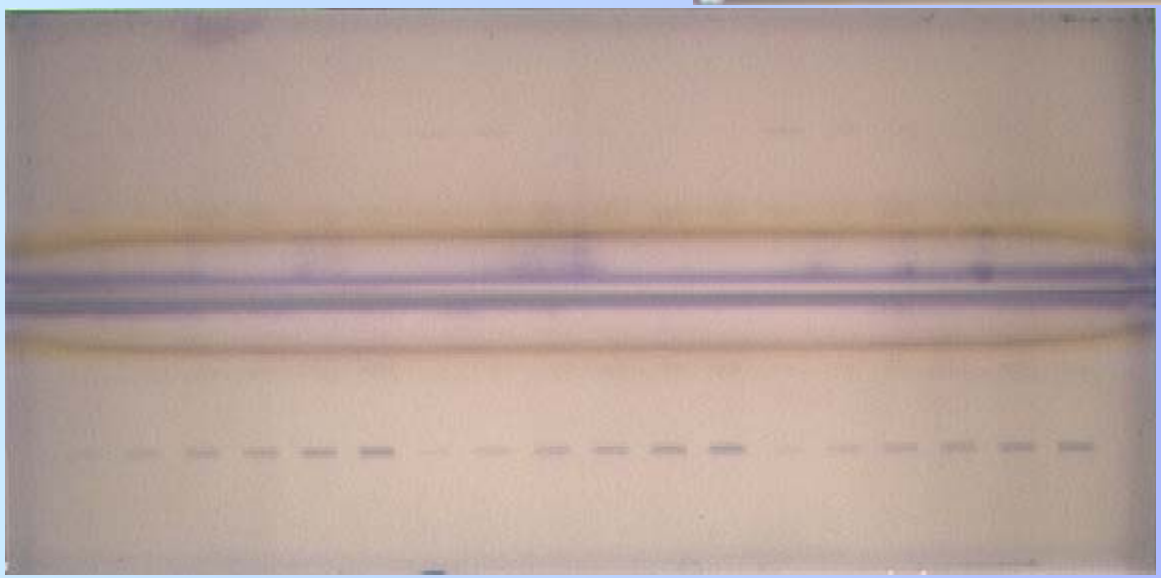
- **Semiquantitative evaluation** (sometimes possible before chromatographic development)
- **Applications:** standards (2 LOD, 2 LOQ), appropriate aliquots of sample test solutions
- At acceptance limit 10 mg/m^2 acceptable limit for screening is 7 mg/m^2

Analysis of real samples

Standards

Standards

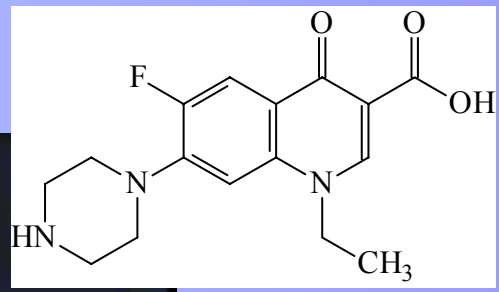




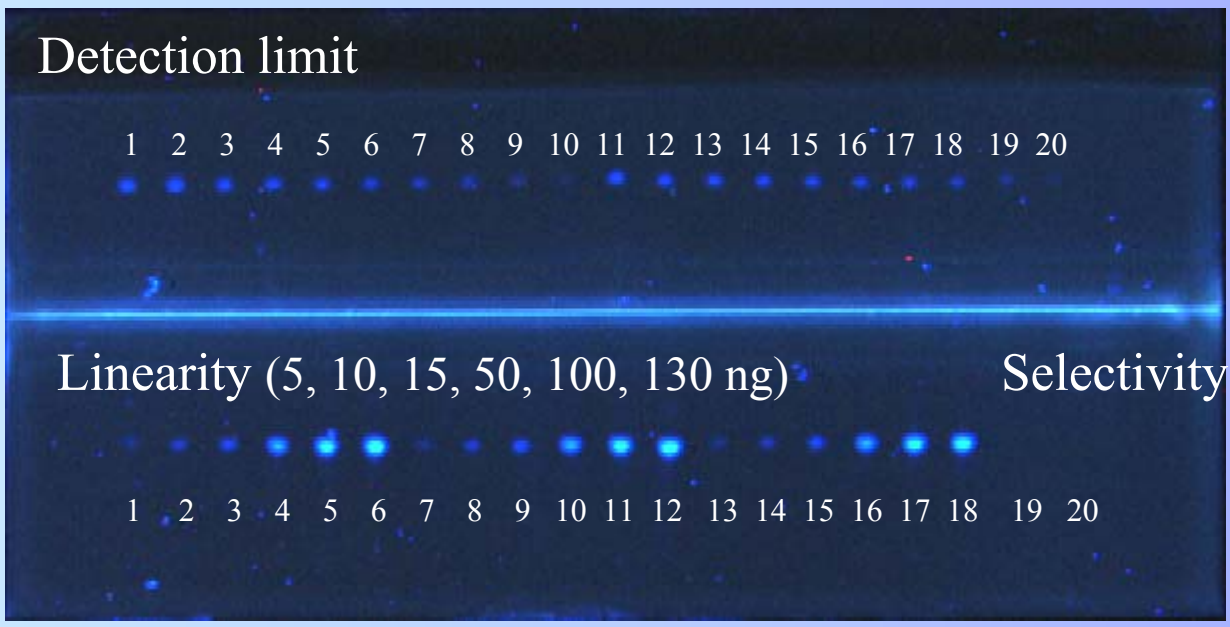
Conclusions

- Our experience shows that planar chromatography is a fit for purpose technique for cleaning validation in pharmaceutical industry.

Norfloxacin



B. Simonovska, S. Andrenšek, I. Vovk, M. Prošek, *J. Chromatogr. A,* **862** (1999) 209-215.


Detection limit
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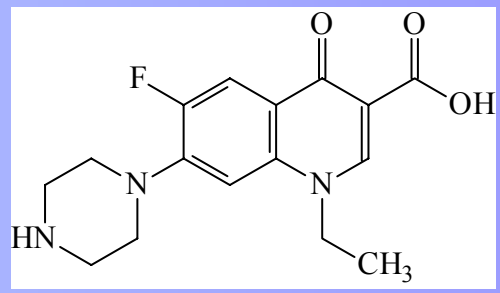
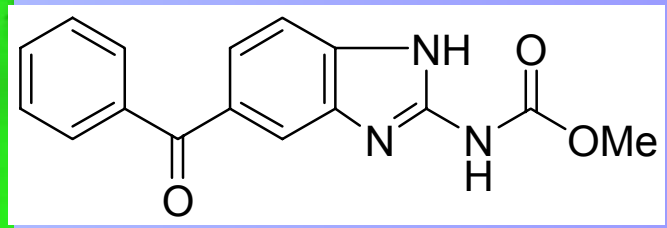
Linearity (5, 10, 15, 50, 100, 130 ng)
Selectivity
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

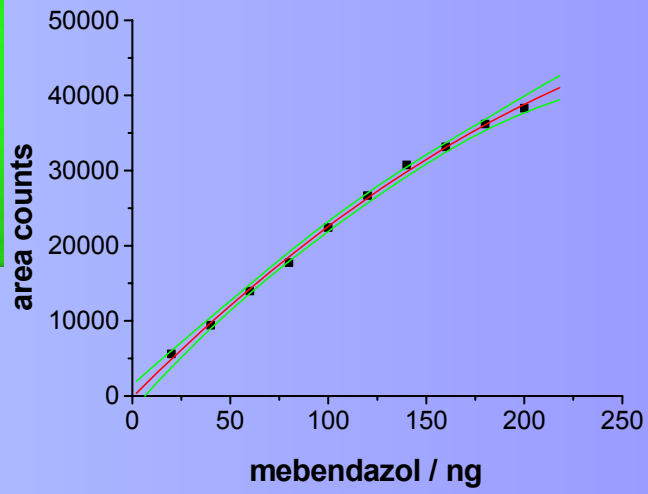

O
O
F
OH
N
N
HN
CH3

Norfloxacin: comparison of HPTLC and HPLC methods

	mean recovery [%] (n=5)	repeatability, RSD [%] (n=5)
HPLC	104.3	3.8
HPTLC (densitometer)	105.4	6.2
HPTLC (CCD camera)	104.9	3.5

Mebendazole – limit test

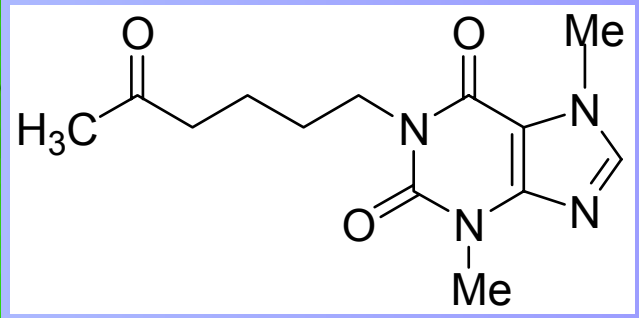


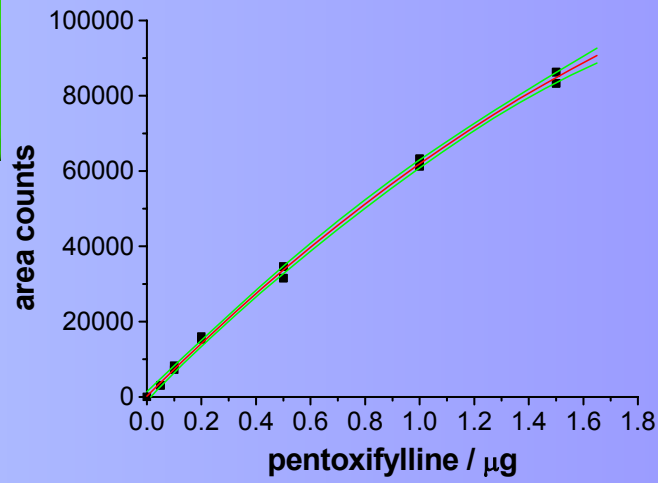


- **1 mg/m²**
- **HPTLC plate**
- **Standards:** 100; 120 and 140 ng
- **Developing solvent:** chloroform-methanol-formic acid 90:5:5 (v/v)
- **Detection**: observing under UV light at 254 nm
- **Quantification by Camag TLC scanner II**: 290 nm

I. Vovk, B. Simonovska, *J. AOAC Int.*, 84 (2001) 1258-1264.

Pentoxifylline – limit test





- **10 mg/m²**
- **HPTLC plate**
- **Standards:** 0.5; 1.0 and 1.5 µg
- **Developing solvent:** ethylacetate-methanol 85:15
- **Detection**: observing under UV light at 254 nm
- **Quantification by Camag TLC scanner II**: 254 nm

I. Vovk, B. Simonovska, *J. AOAC Int.*, 84 (2001) 1258-1264.