PAUL SCHERRER INSTITUT

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Structural Solutions

Mathilde Reinle-Schmitt :: Paul Scherrer Institut
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Challenges of absolute quantification of pharmaceuticals by the internal standard methods

Quantitative Phase Analysis by XRPD Workshop, PPXRD 14, Fort Myers, 6th June 2016

This document was presented at PPXRD - Pharmaceutical Powder X-ray Diffraction Symposium

Sponsored by The International Centre for Diffraction Data

PPXRD Website – www.icdd.com/ppxrd

ICDD Website - www.icdd.com

Introduction

- QPA: high degree of accuracy and precision
- Focus on the internal standard method
- Appropriate choices of primary importance

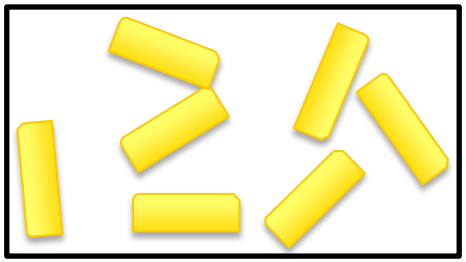
Why an internal standard?
Recipe to choose it?
Case study

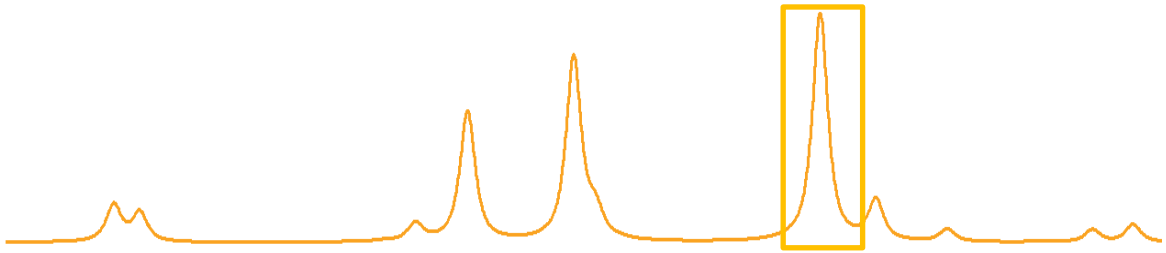
http://chemistry.fas.nyu.edu/object/chem.sif.xdf.gadds.app1

An **internal standard** for pharmaceuticals

Phase α, μ_α

$$I_{(hkl),\alpha} = \frac{K_{1,\alpha}}{\mu_\alpha}$$





An **internal standard** for pharmaceuticals

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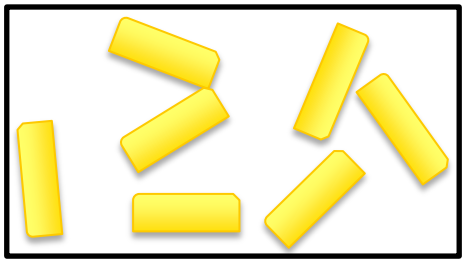
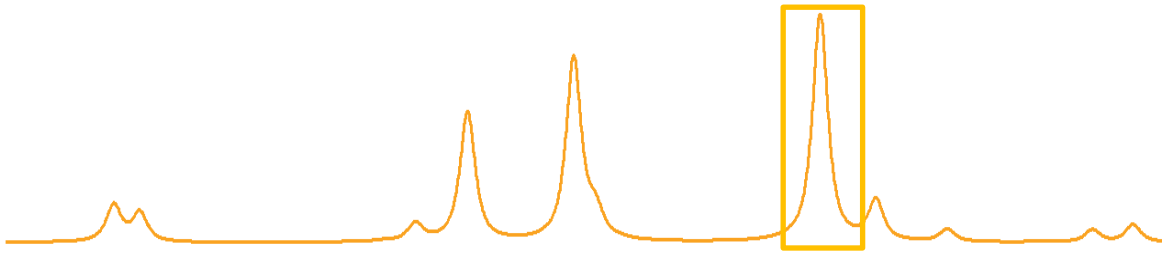
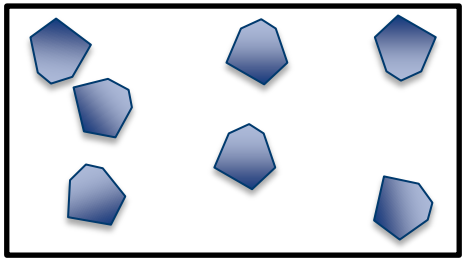
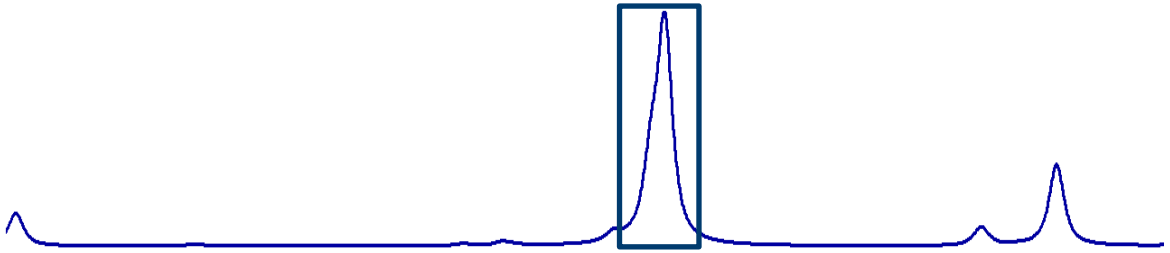


$$I_{(hkl),\alpha} = \frac{K_{1,\alpha}}{\mu_\alpha}$$



Phase β, μ_β





$$I_{(hkl),\beta} = \frac{K_{1,\beta}}{\mu_\beta}$$

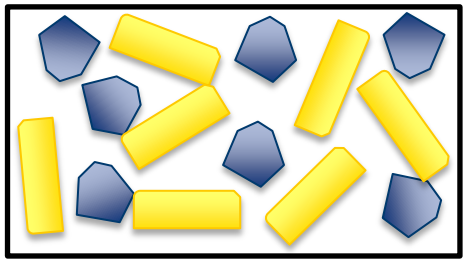
An **internal standard** for pharmaceuticals

- Mixture

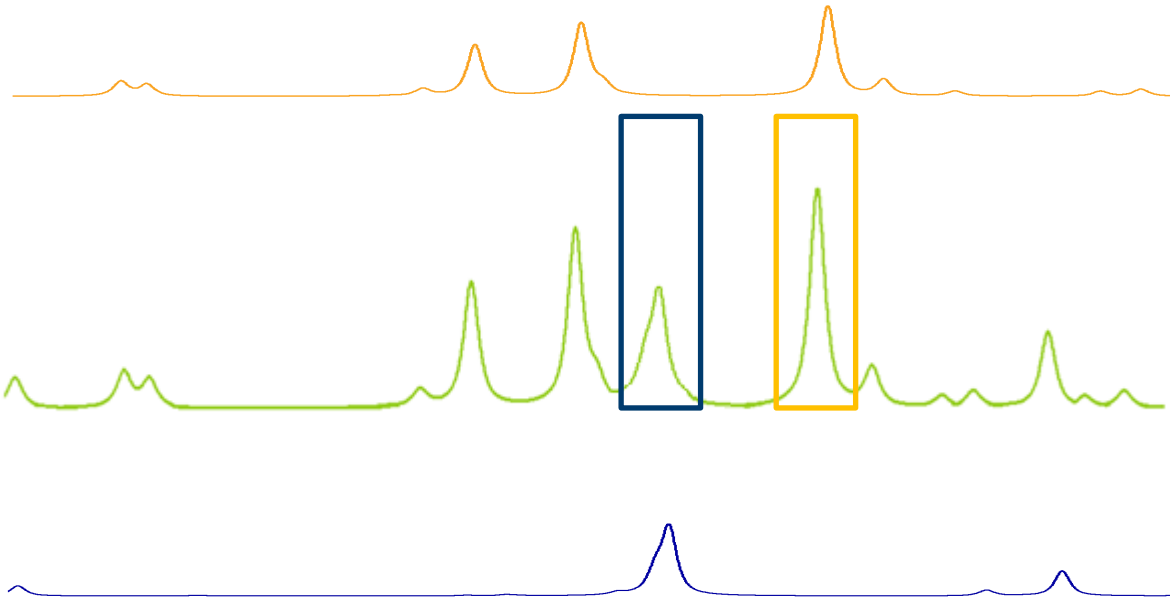
$$I_{(hkl),\alpha} = \frac{K_{1,\alpha}}{\mu_m} c_\propto$$

$\mu_\alpha, c_\alpha, w_\alpha$

μ_m



$\mu_\beta, c_\beta, w_\beta$



$$I_{(hkl),\beta} = \frac{K_{1,\beta}}{\mu_m} c_\beta$$

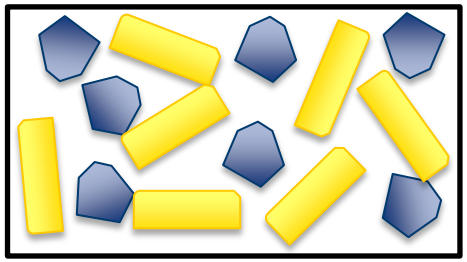
An **internal standard** for pharmaceuticals

- Mixture

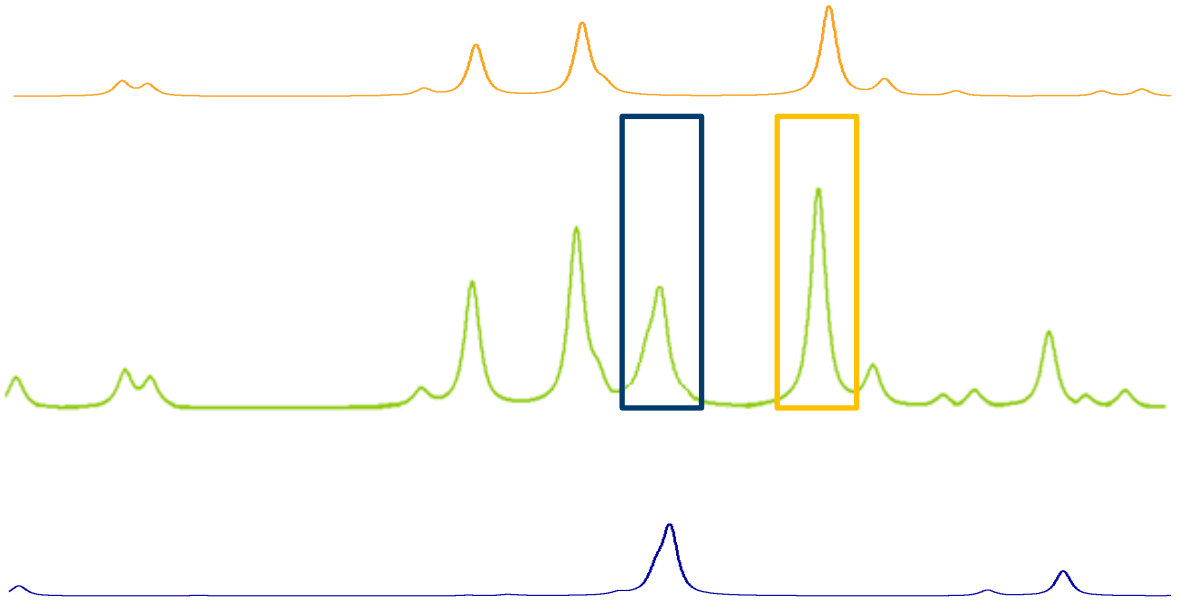
$$I_{(hkl),\alpha} = \frac{K_{1,\alpha} w_\alpha}{\rho_\alpha \mu_m^*}$$

$\mu_\alpha, c_\alpha, w_\alpha$

μ_m



$\mu_\beta, c_\beta, w_\beta$



$$I_{(hkl),\beta} = \frac{K_{1,\beta} w_\beta}{\rho_\beta \mu_m^*}$$

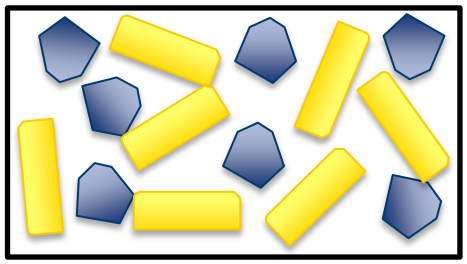
An **internal standard** for pharmaceuticals

- Mixture

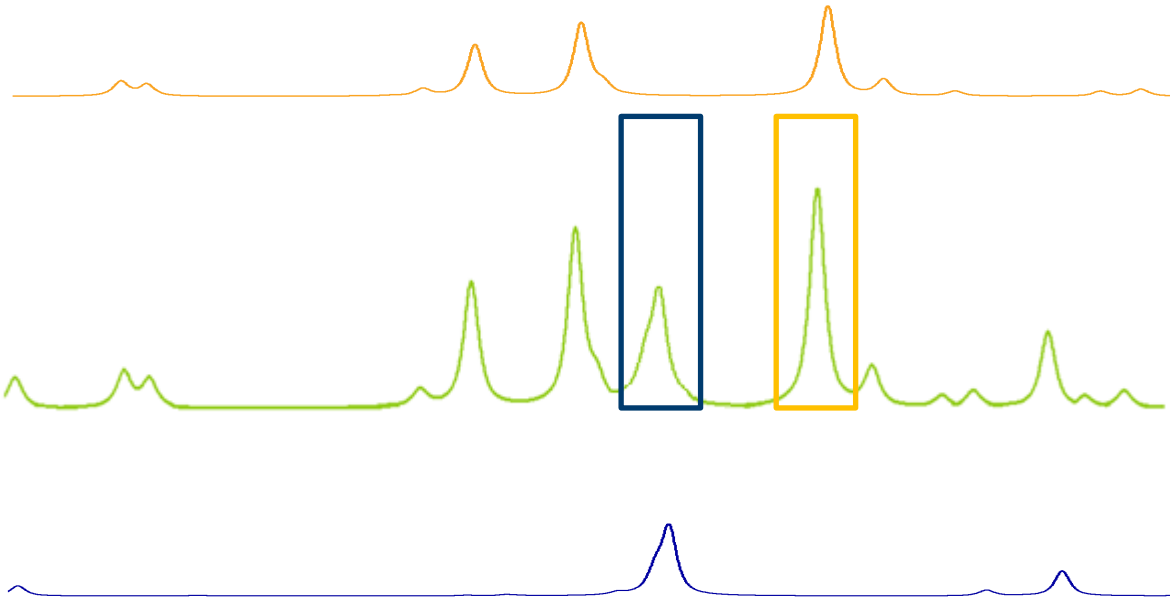
known unknown

$$w_\alpha = I_{(hkl),\alpha} \frac{\rho_\alpha \mu^*_m}{K_{1,\alpha}}$$

$\mu_\alpha, c_\alpha, w_\alpha$



μ_m



$\mu_\beta, c_\beta, w_\beta$

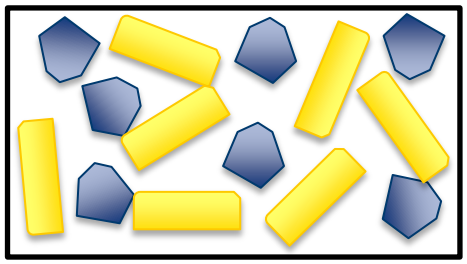
$$w_\beta = I_{(hkl),\beta} \frac{\rho_\beta \mu^*_m}{K_{1,\beta}}$$

An **internal standard** for pharmaceuticals

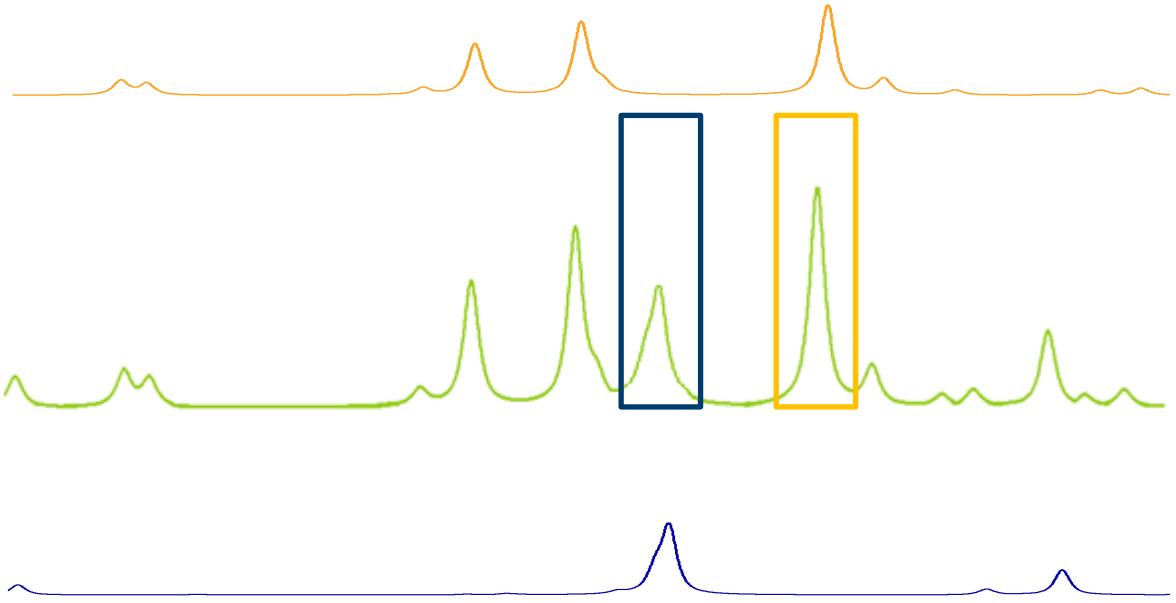
- Ratio

$$w_\alpha = I_{(hkl),\alpha} \frac{\rho_\alpha \mu^*_m}{K_{1,\alpha}}$$

$\mu_\alpha, c_\alpha, w_\alpha$



μ_m



$\mu_\beta, c_\beta, w_\beta$

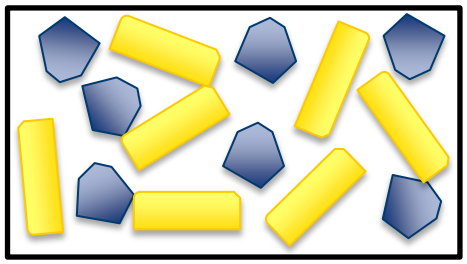
$$w_\beta = I_{(hkl),\beta} \frac{\rho_\beta \mu^*_m}{K_{1,\beta}}$$

An **internal standard** for pharmaceuticals

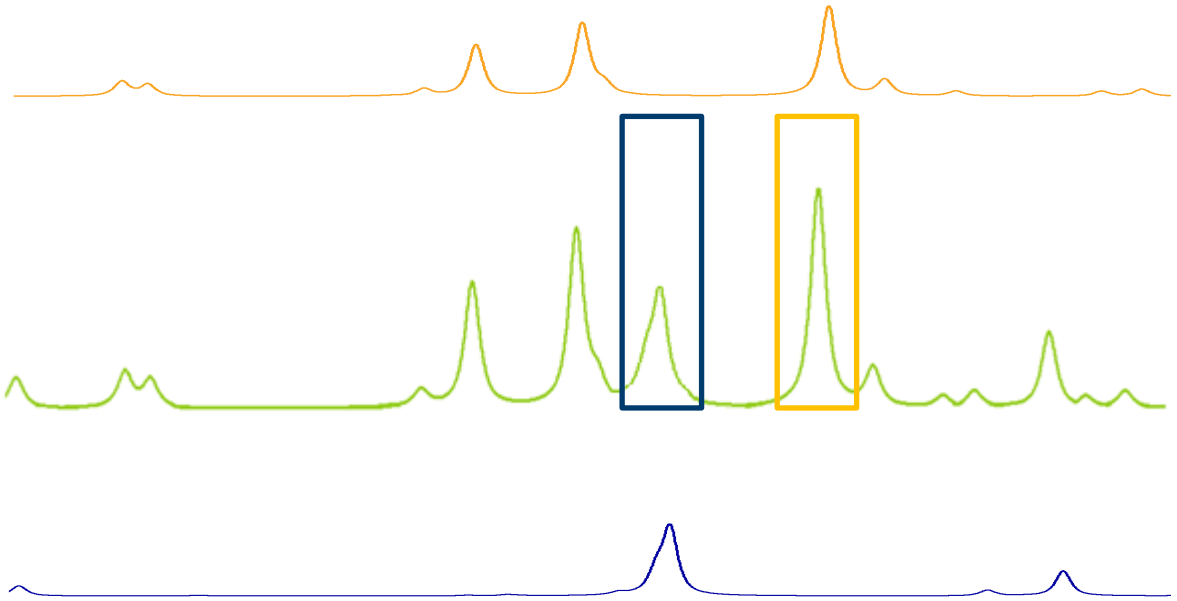
- Ratio

$\mu_\alpha, c_\alpha, w_\alpha$

μ_m



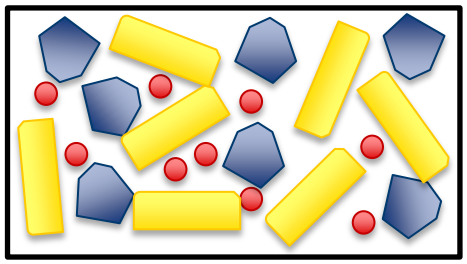
$\mu_\beta, c_\beta, w_\beta$

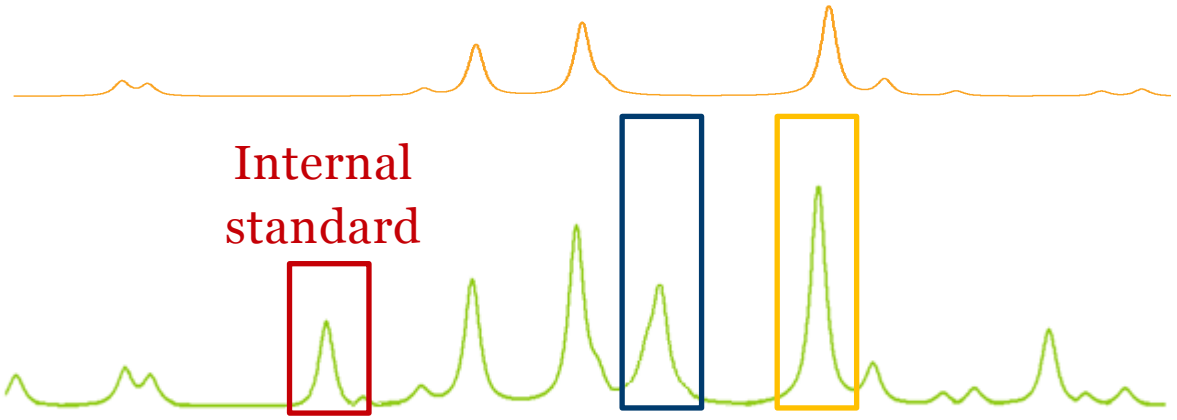


$$\frac{w'_\alpha}{w_\beta} = \frac{I_{(hkl),\alpha}\frac{\rho_\alpha}{K_{1,\alpha}}}{I_{(hkl),\beta}\frac{\rho_\beta}{K_{1,\beta}}}$$

An **internal standard** for pharmaceuticals

- Add the internal standard

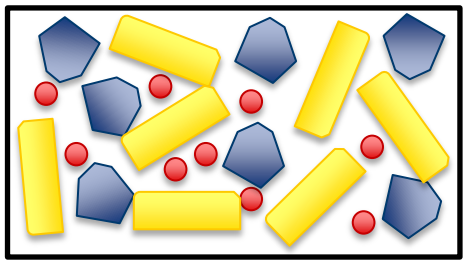


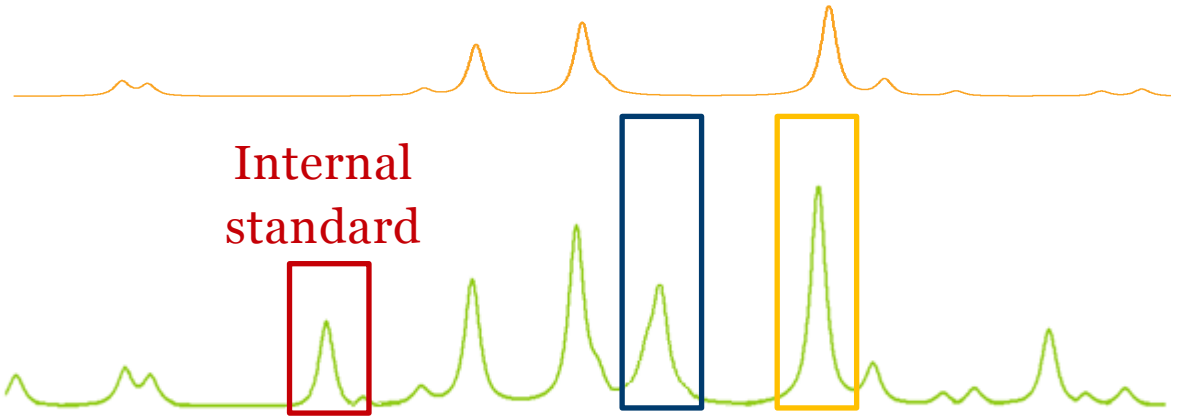


$$\frac{w'_{\alpha}}{w_{\beta}} = \frac{I_{(hkl),\alpha}\frac{\rho_{\alpha}}{K_{1,\alpha}}}{I_{(hkl),\beta}\frac{\rho_{b}}{K_{1,\beta}}}$$

An **internal standard** for pharmaceuticals

- Add the internal standard

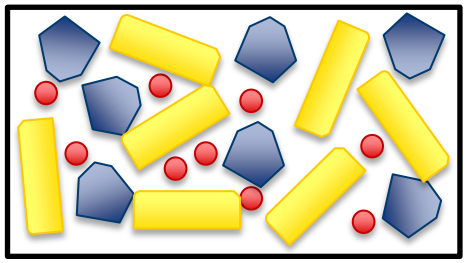


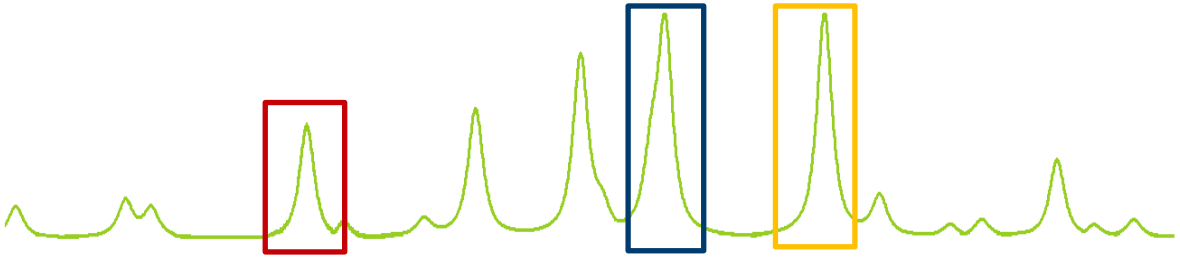


$$\frac{w'_{\alpha}}{w_S} = \frac{I_{(hkl),\alpha}\dfrac{\rho_{\alpha}}{K_{1,\alpha}}}{I_{(hkl),S}\dfrac{\rho_S}{K_{1,S}}}$$

An **internal standard** for pharmaceuticals

- Add the internal standard

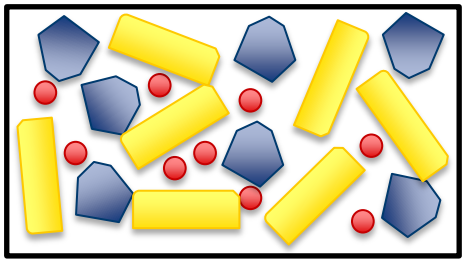




$$w_\alpha{}' = \frac{I_{(hkl),\alpha}}{I_{(hkl),S}} \frac{K_{1,S}}{K_{1,\alpha}} \cdot \frac{\rho_\alpha}{\rho_S} w_S$$

Calibration constant from ad-hoc mixtures with known w_S and w'_α:

$$\frac{K_{1,S}}{K_{1,\alpha}} \frac{\rho_\alpha}{\rho_S} = \frac{I_{(hkl),S}}{I_{(hkl),\alpha}} \frac{w_S}{w'_\alpha}$$



An **internal standard** for pharmaceuticals

- Single peak method

$$w_\alpha = I_{(hkl),\alpha} \frac{\rho_\alpha \mu^*_m}{K_{1,\alpha}} \qquad w'_\alpha = \frac{I_{(hkl),\alpha}\, K_{1,S}}{I_{(hkl),S}\, K_{1,\alpha}} . \frac{\rho_\alpha}{\rho_S} . w_S$$

- Whole pattern method

$$w_\alpha = S_\alpha \frac{(ZMV)_\alpha \mu^*_m}{K} \qquad w_\alpha{}' = \frac{S_\alpha (ZMV)_\alpha}{S_S (ZMV)_S} . w_S$$

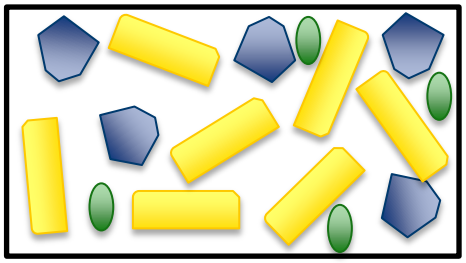
- Absolute scale

An **internal standard** for pharmaceuticals

- Unknown compounds
- Rietveld: only crystalline phases

$$\sum_{i=1}^{n} w_i = 1$$

Without Internal Standard



Amorphous, 20 w%/w ⇨ **not detected**

Crystalline, 30 %w/w

Crystalline, 50 %w/w

100 %w/w

37,5 w%/w

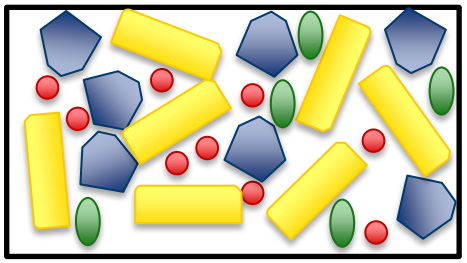
62,5 w%/w

WRONG!

An **internal standard** for pharmaceuticals

- Unknown compounds
- Amorphous quantification, Absolute scale

With Internal Standard



New weight ratios!

100 %w/w

Amorphous, 15 w%/w ⇨ **not detected**

Crystalline, 22,5 %w/w — 26,47 %w/w

Crystalline, 37,5 %w/w — 44,11 %w/w

Crystalline, 25 %w/w — 29,41 %w/w

C=0.85

(Correction factor: $0{,}25 = 0{,}2941 \cdot C$)

⇨ 100-(22,5+37,5+25) = 15 %w/w

⇨ 22,5 %w/w

⇨ 37,5 %w/w

⇨ 25 %w/w

100 %w/w

⇨ 20 w%/w

⇨ 30 %w/w

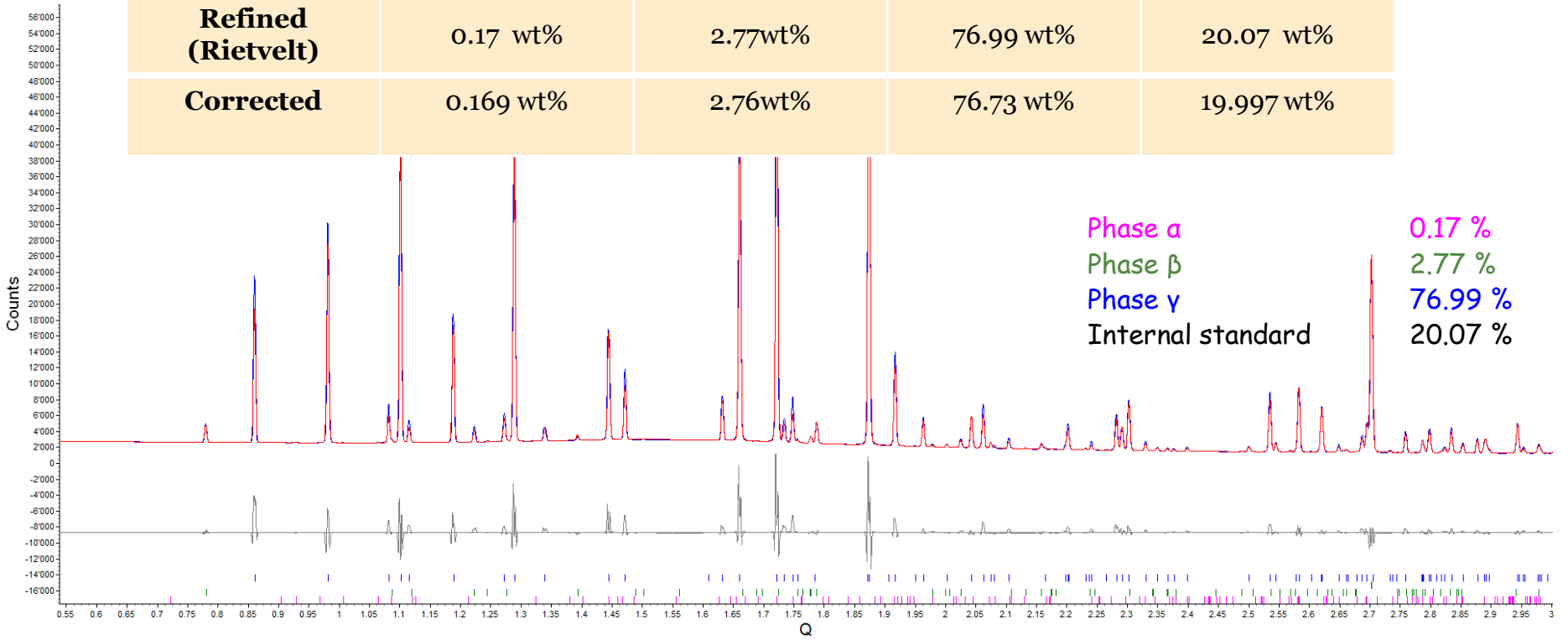
⇨ 50 %w/w

CORRECT!

An **internal standard** for pharmaceuticals

- Example: absolute QPA of the following mixture:

	Phase α (minority)	Phase β	Phase γ (majority)	Internal standard
Weighted	0.09 wt%	2.38 wt%	77.527 wt%	19.997 wt%
Refined (Rietvelt)	0.17 wt%	2.77wt%	76.99 wt%	20.07 wt%
Corrected	0.169 wt%	2.76wt%	76.73 wt%	19.997 wt%



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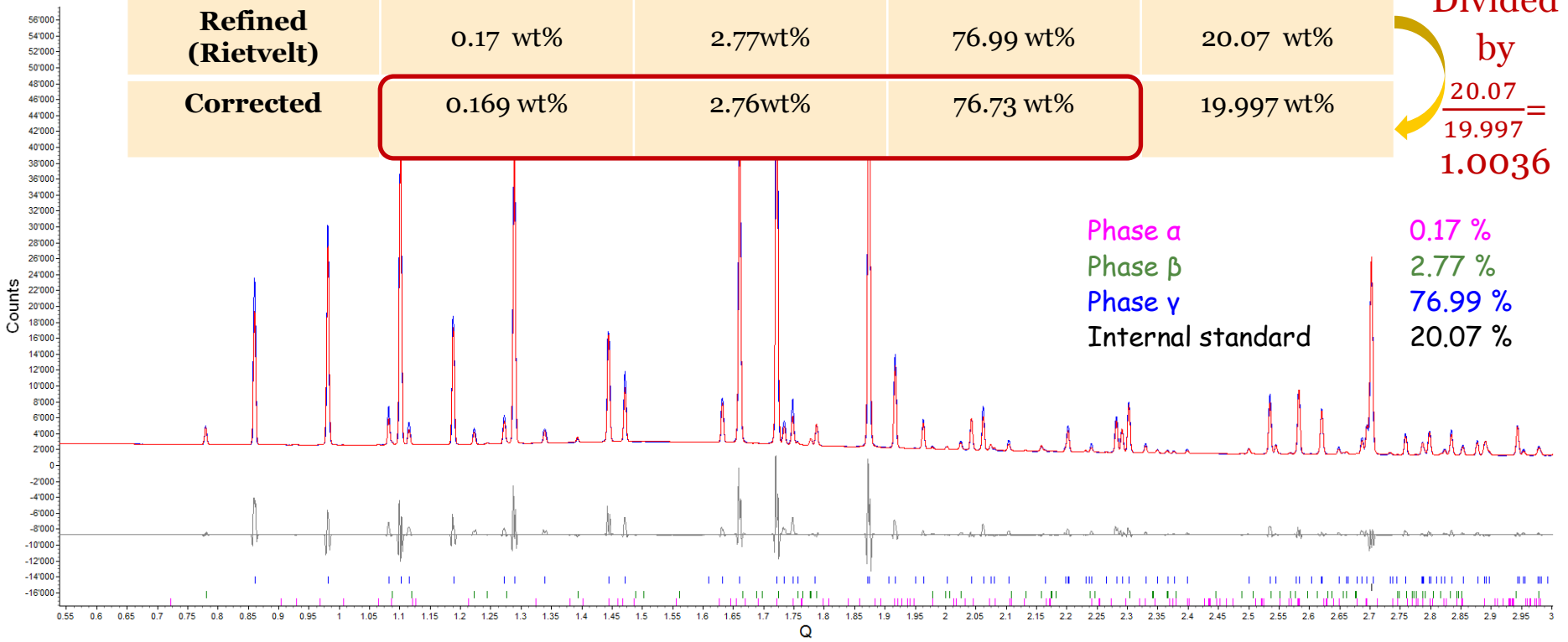
Divided by $\frac{20.07}{19.997} = 1.0036$



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Divided by $\frac{20.07}{19.997} = 1.0036$

⇨ Amorphous=100-0.169-2.76-76.73-19.997=0.34 wt%

Phase α: `corrected_weight_percent` 0.169

Phase β: `corrected_weight_percent` 2.76

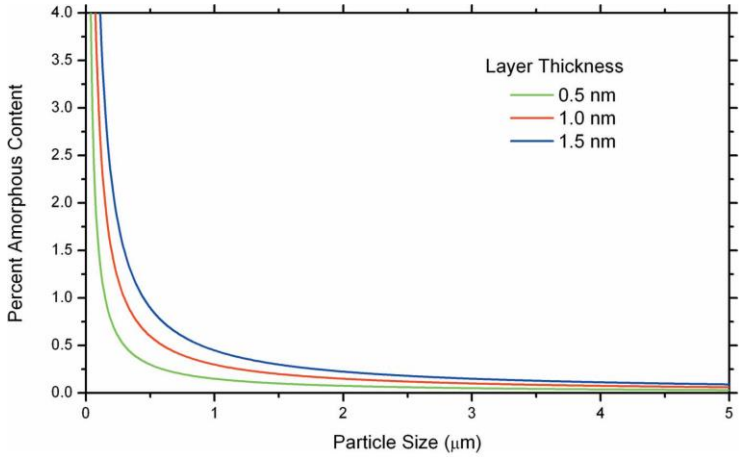
Phase γ: `corrected_weight_percent` 76.73

Internal standard: `spiked_phase_measured_weight_percent` 20

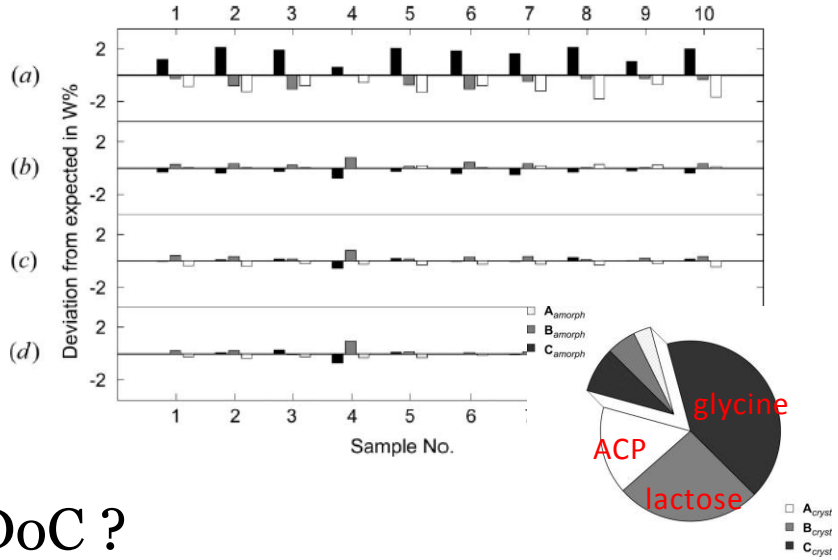
Amorphous: `weight_percent_amorphous` 0.34

Amorphous problem

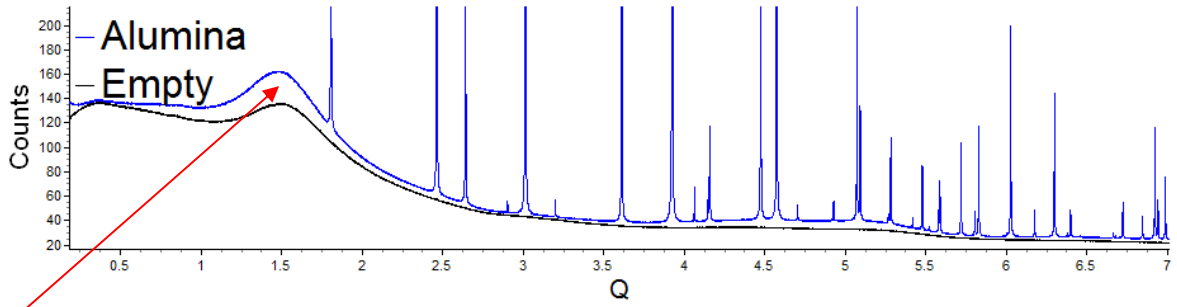
- Minimum amorphous content



- Not negligible



- Amorphous/crystalline ratio, DoC ?



Difference > expected 0.8%

Cline, J. P., Von Dreele, R. B., Winburn, R., Stephens, P. W. & Filliben, J. J. (2011). Acta Cryst. A67, 357-367.

Schreyer, M. et al., Journal of applied crystallography, 44, 1, 17 (2011).

An **internal standard** for pharmaceuticals

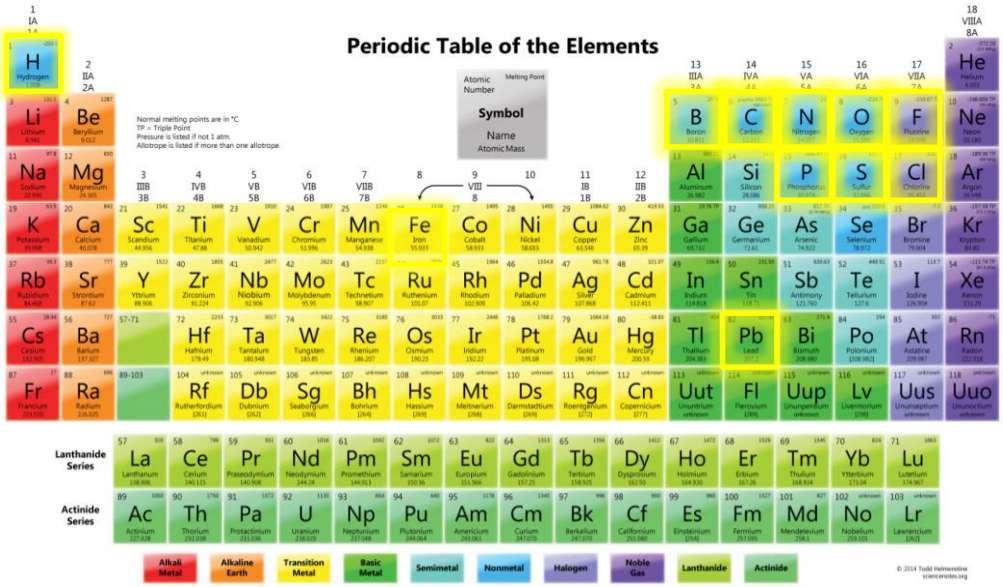
- Unknown compounds
- Amorphous quantification, Absolute scale
- Direct correction for instrumental effects
- Comparable matrix effects
- Universal

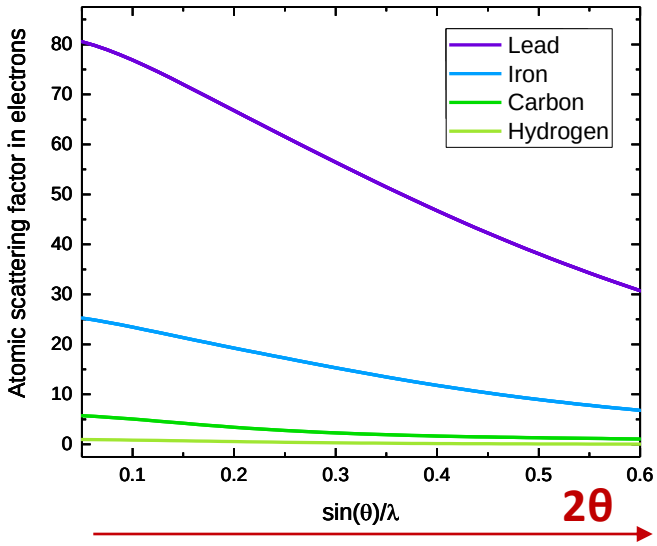
- Internal standard tailored to analyte
- Time consuming powder processing
- Powder samples only
- Destructive sample preparation

What about the sample?

An internal standard for **pharmaceuticals**

- Poor scattering power

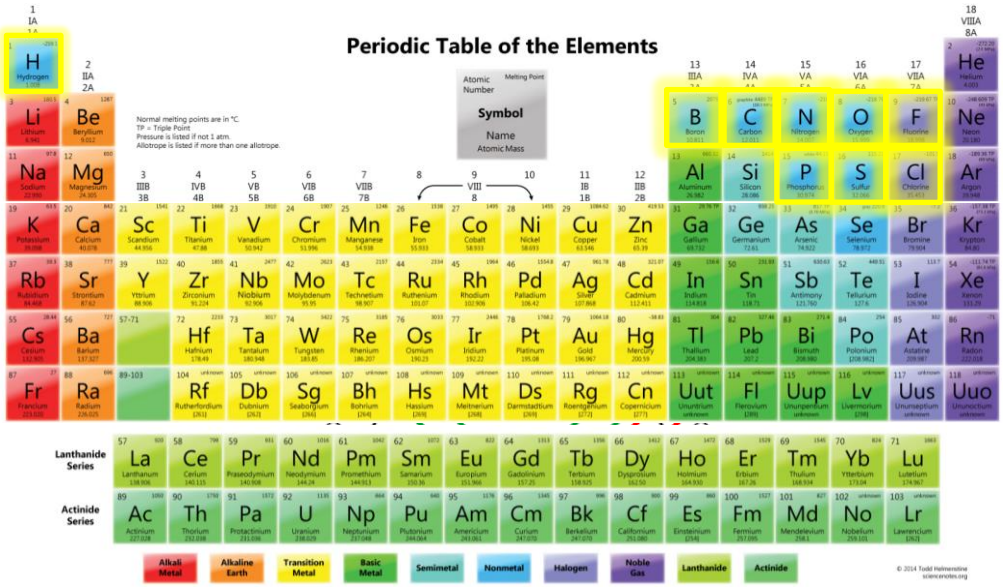


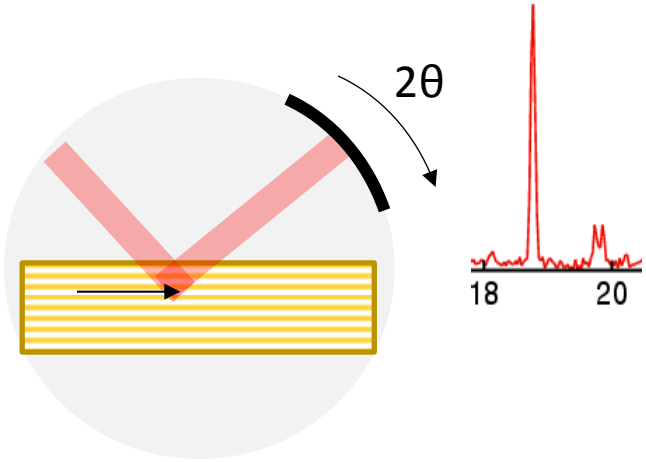


- Longer acquisition time
- Improved signal/noise and signal/background ratios

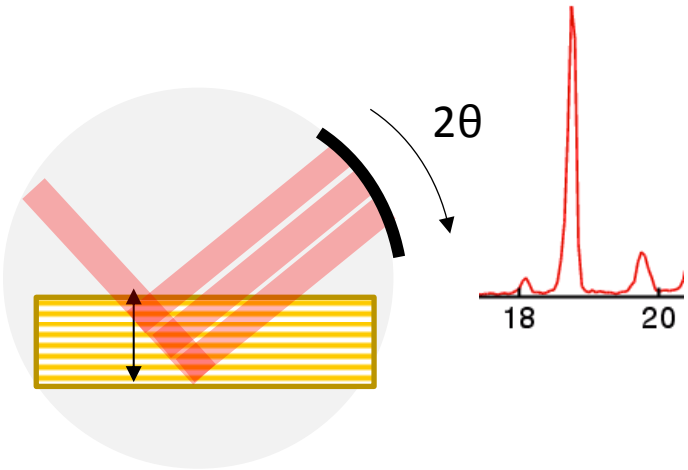
An internal standard for **pharmaceuticals**

- Low absorption





- Geometry of the experiment?



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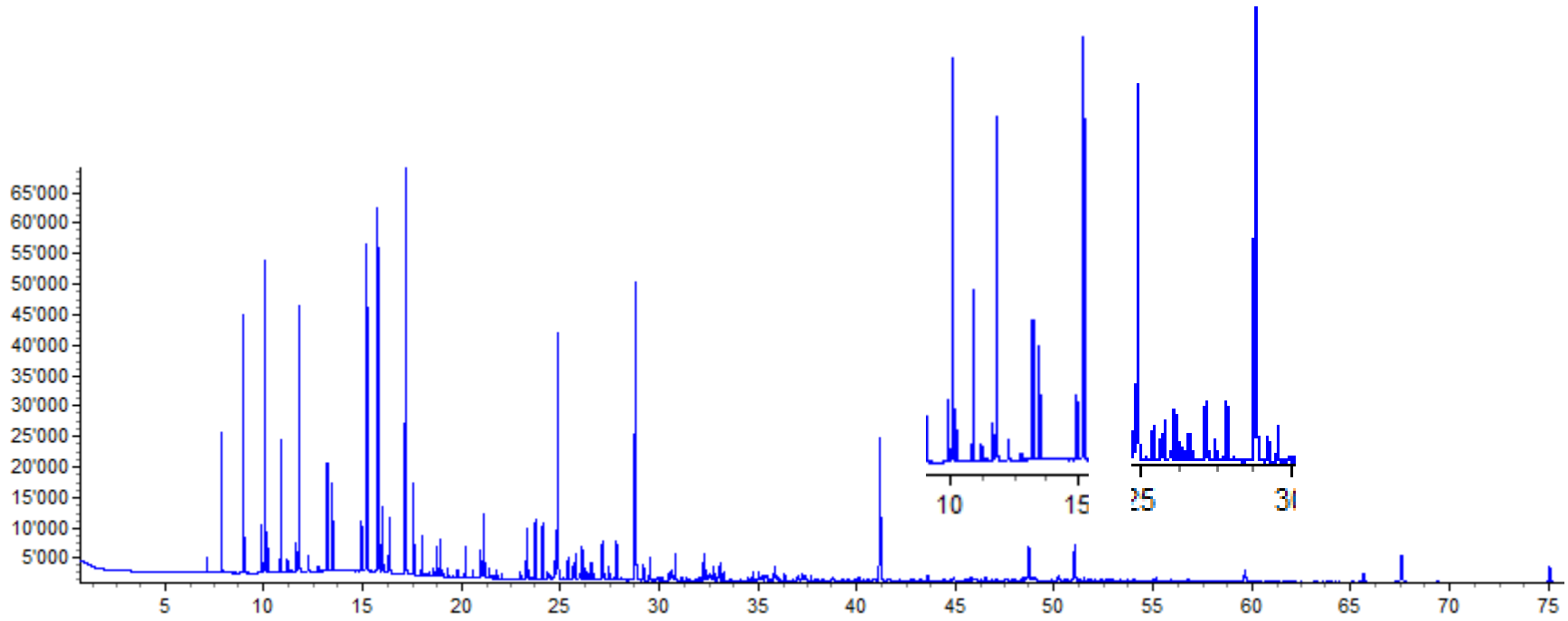
An internal standard for **pharmaceuticals**

- 95% of organics crystallize in 5 space groups:
  - $P2_1/c$
  - $P1$
  - $P2_12_12_1$
  - $P2_1$
  - $C2/c$ …
- Low symmetry

Higher symmetry

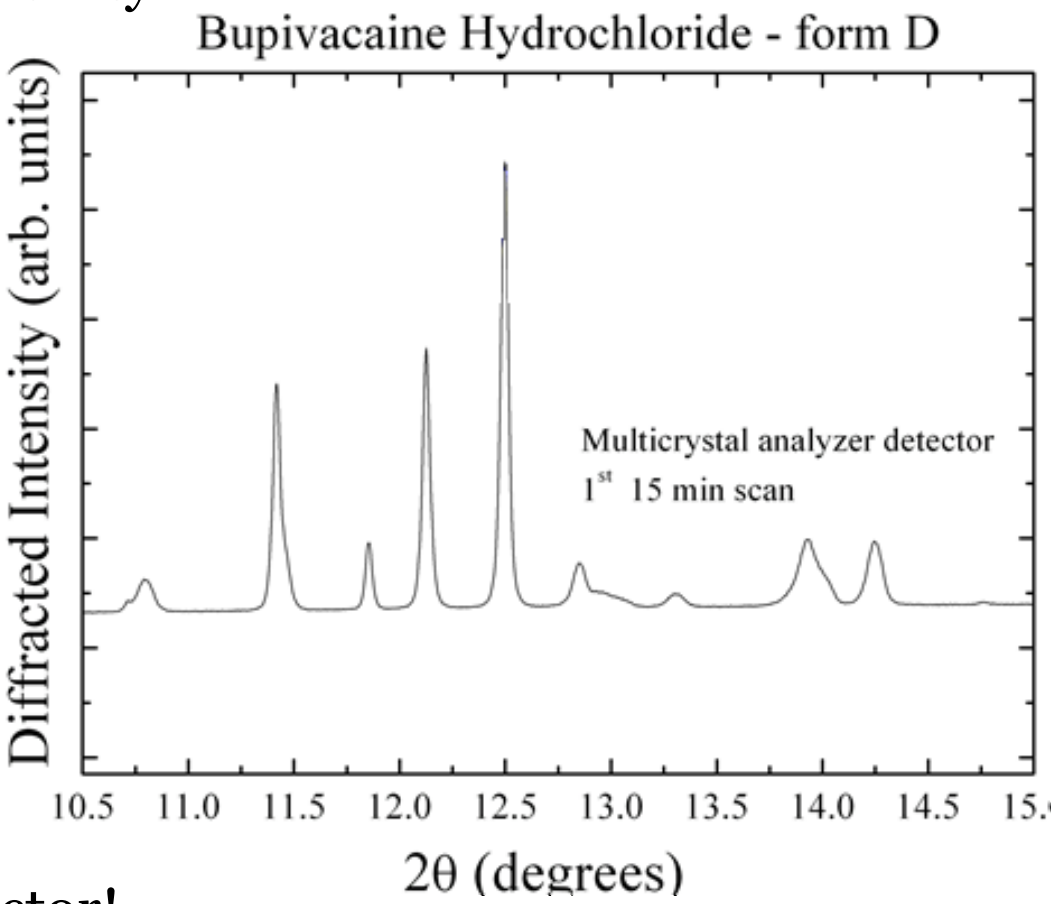
An internal standard for **pharmaceuticals**

- Increasing reflection density with increasing angle, where intensity drops
- Large cell size



An internal standard for **pharmaceuticals**

- Radiation sensitivity



- Adapt the detector!

What about the instrument?

An internal standard for pharmaceuticals with synchrotron radiation

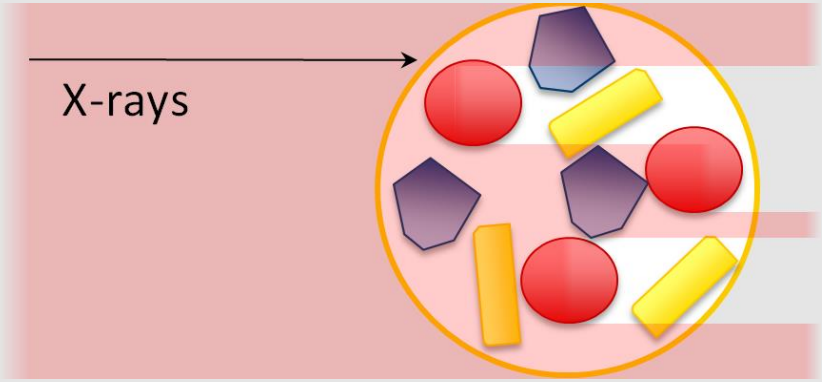
- Synchrotron radiation, transmission, capillaries (angular resolution/counting statistics)

- High brilliance and angular resolution
- Tunability of the wavelength
- Mythen detector (also in laboratory)

- Very small sample volume, particle statistics problem
- Larger number of positions measured, automatization

Microabsorption

- Little absorption contrast required



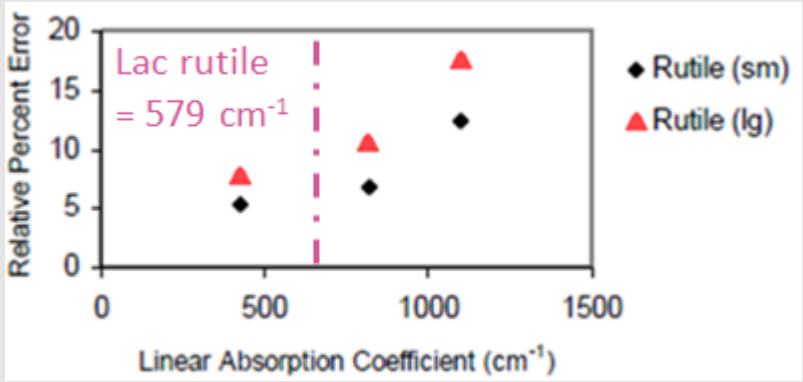
High absorbing
internal standard

Low absorbing
Analyte mixture

- Underestimated weight ratio for high absorber
- Brindley correction complicated to apply
- Rather avoid than correct:
  - Similar linear absorption coefficient
  - Tuning the wavelength
  - Particle sizes

Microabsorption

- Experience with Alumina, which amount?
- Effect of particle size on QPA accuracy



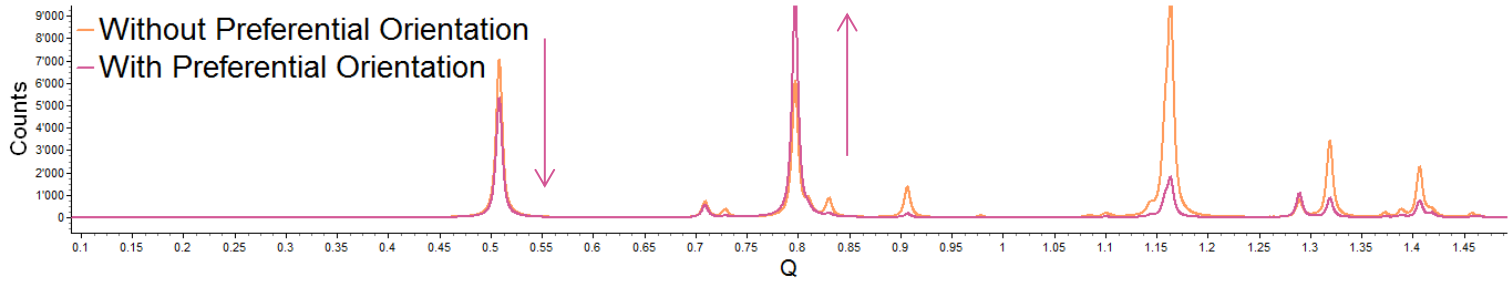
- Upper limit to the particle size

	Density (g.cm-3)	Attenuation length (CXRO, in microns)
Paracetamol	1.26	5120
Salicylic Acid	1.44	4000
Carbamazepine	1.29	5880
Corundum	**4.02**	**299**

Adapted from: Pederson et al., Advances in x-ray analysis, 46, 2003.
Pederson et al., Advances in x-ray analysis, 47, 2004.

Microstructure: Particle size

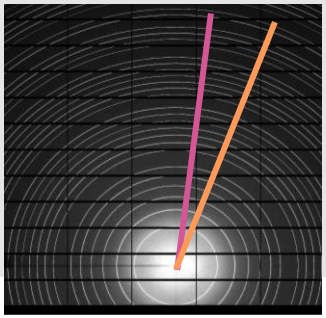
- Precision of QPA: homogeneity / particle statistics
- Closest particle size, similar density and particle shape
- No extinction effects
- Preferential orientation: isometric particles

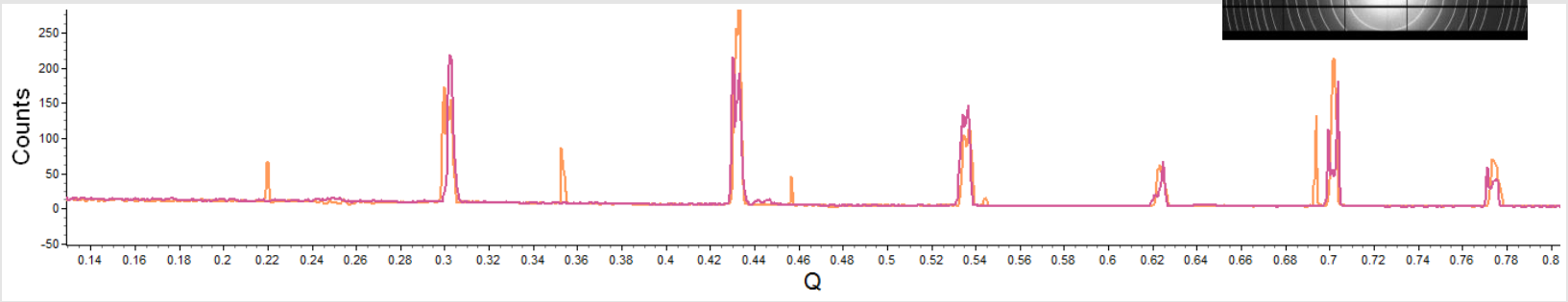


- Preparation step mandatory
  - Grinding
  - Ball milling…

Microstructure: Crystallite size

- Avoid fluctuations along Debye-Scherrer rings





- Avoid fluctuations between replicate samples

Crystallite diameter (μm)	*40*	*10*	*1*
Crystallites (20 mm^3)	5.97×10^5	3.82×10^7	3.82×10^{10}
Number diffracting	12	760	38 000
σ_{PS}	0.289	0.036	0.005

$$\sigma_{PS} = \sqrt{N_{\text{diff}}} \Big/ N_{\text{diff}}$$

Robert E. Dinnebier and Simon J. L. Billinge, Print ISBN: 978-0-85404-231-9, DOI:10.1039/9781847558237
adapted from: D. K. Smith, Adv. X-Ray Anal., 1992, 35, 1-15

PAUL SCHERRER INSTITUT

PSI

Excelsus
Structural Solutions

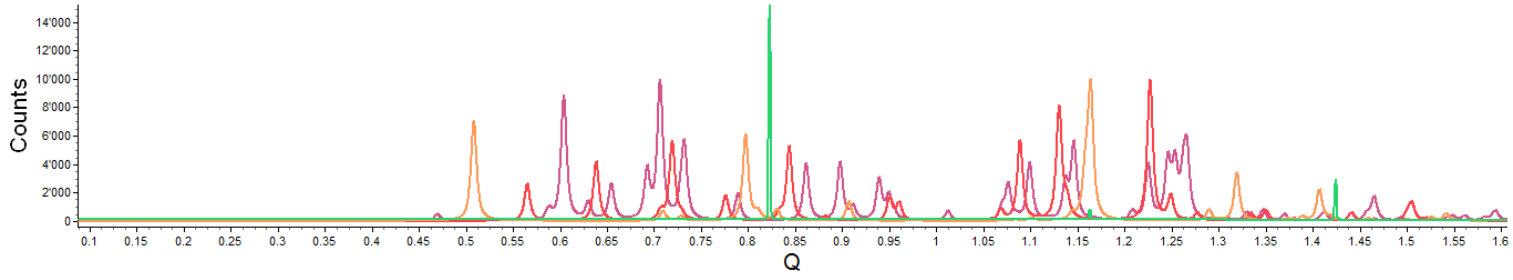
Symmetry

- Limit peak overlap
- High symmetry
- High crystallinity

https://crystalsymmetry.wordpress.com/2014/08/15/the-space-group-list-project-as-a-poster/

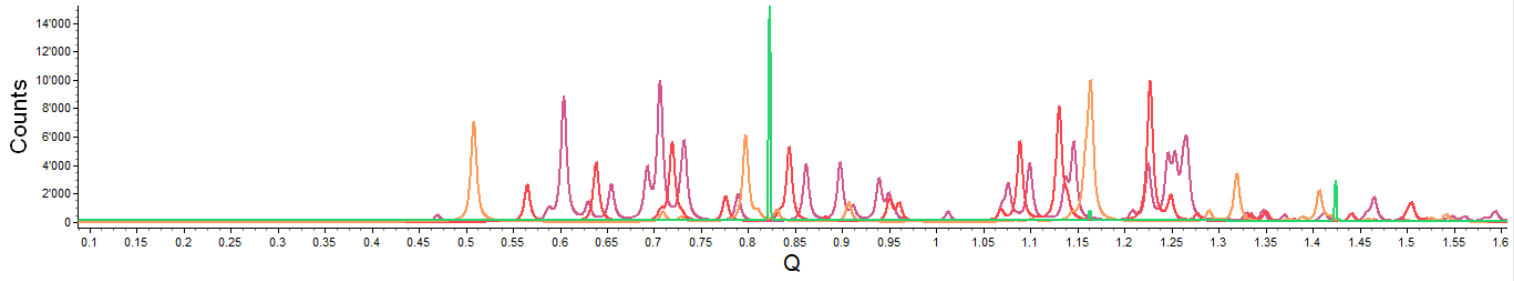
Symmetry

- Limit peak overlap
- High symmetry
- High crystallinity

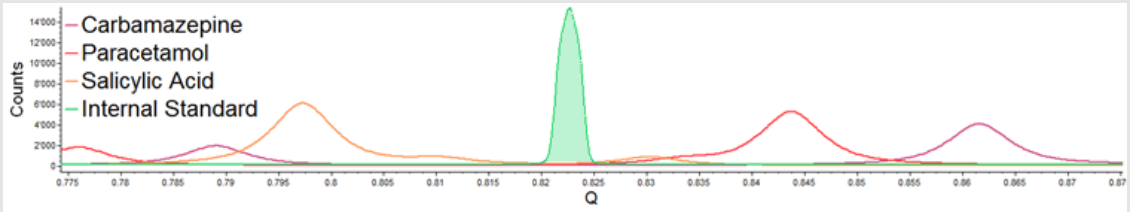


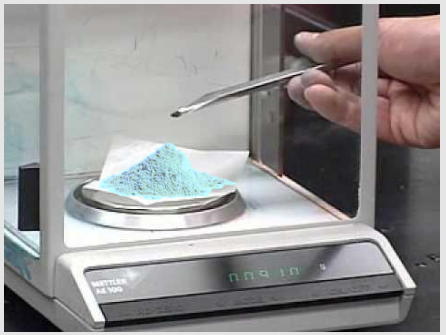
Symmetry

- Limit peak overlap
- High symmetry
- High crystallinity



- Angular range
- Well-known structure
- Known crystallinity





Additional constraints

- Stability
  - during preliminary powder preparation/mixing
  - in mixture
  - to x-rays
  - in time during storage

- Safe to handle
- Cheap, easily available
- Not present in the analyte mixture !

Formulated drugs

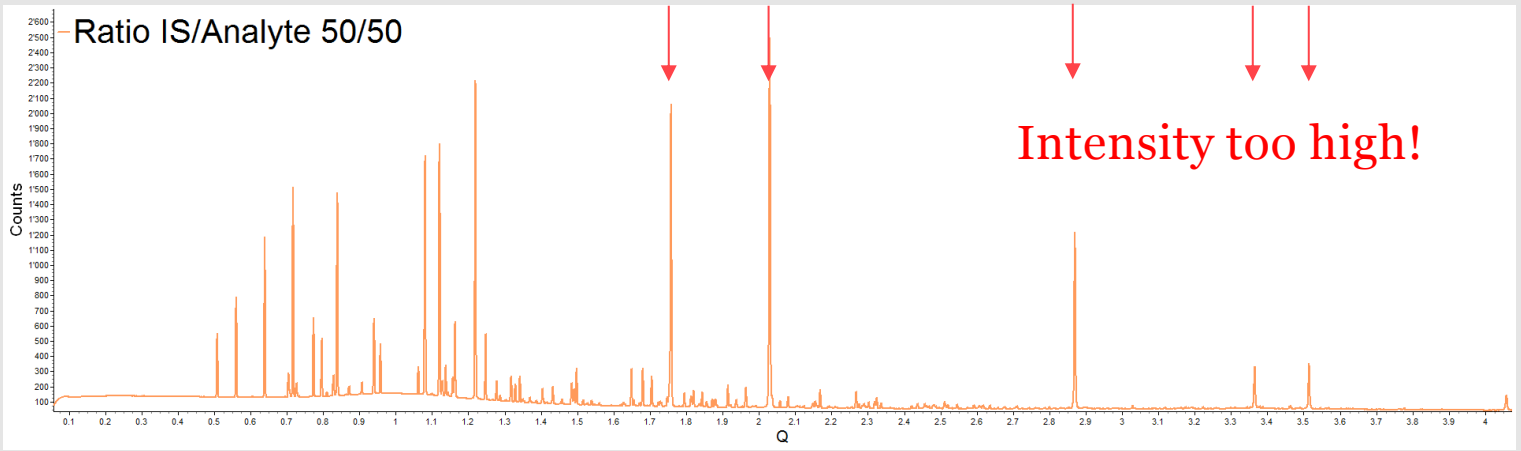
besides internal standard addition
analyte mixture should stay **identical!**

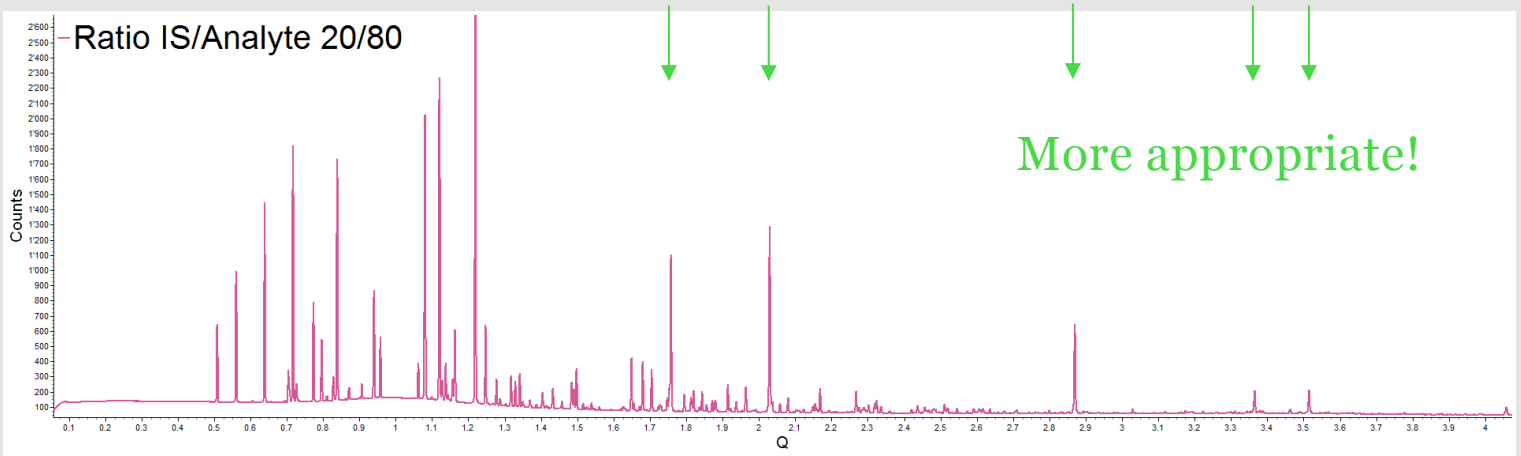
Bruni G. et al., J. Pharm. Biomed. Anal., 2011 Apr 5;54(5):1196-9

Amount of internal standard

- Comparable diffracted intensities

ALWAYS TEST FIRST !





Case study

- QPA on absolute scale of traces in organic mixture with synchrotron radiation
- Constraints due to:
  - ✓ Internal standard method
  - ✓ Use of SR-XRPD
  - ✓ Application on pharmaceuticals

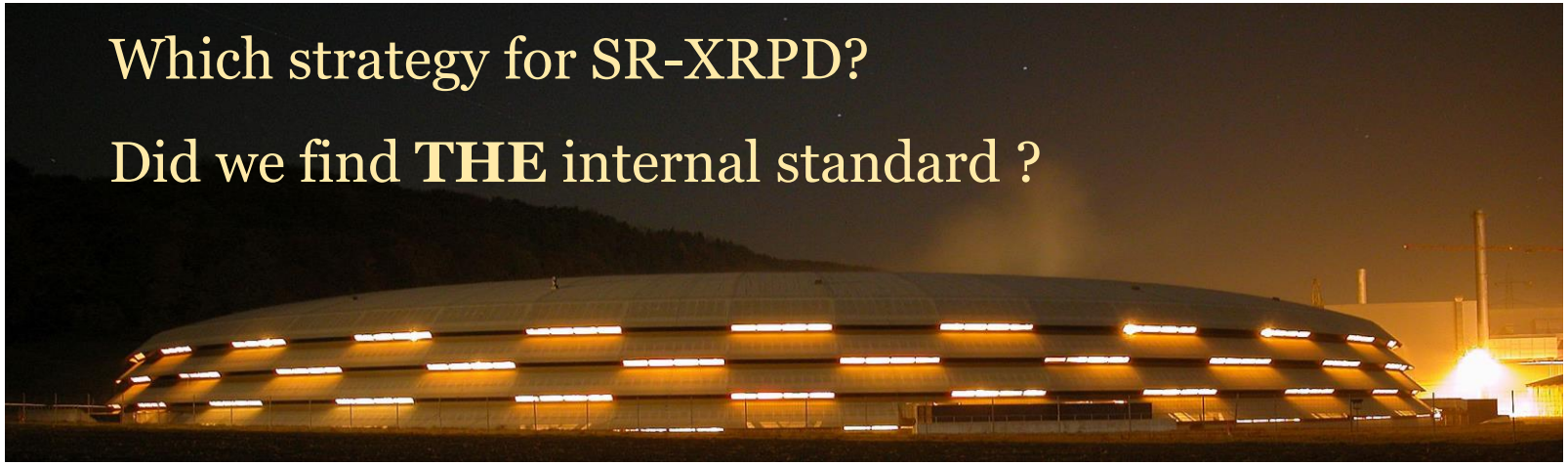
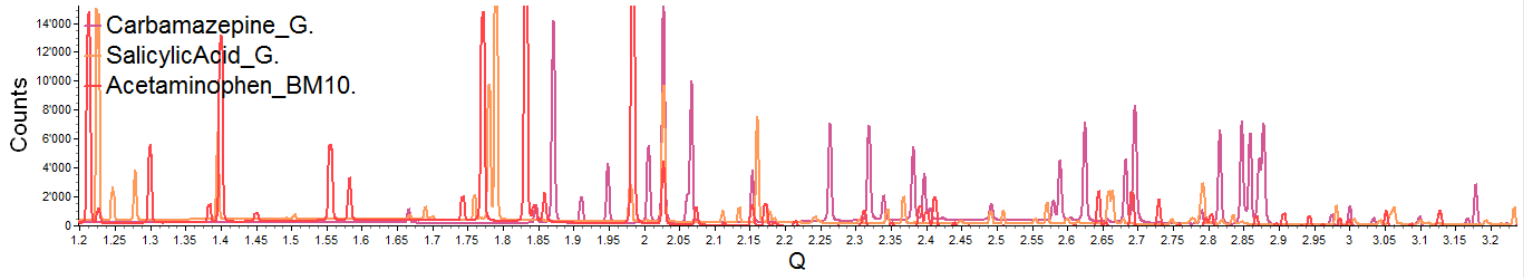


Photo: Paul Scherrer Institute

Case study: Analyte mixture

- QPA of a ternary organic mixture with several candidates
- Negligible impact of intrinsic properties on the refinement
- APIs well-known structure:
  - Majority phase: Acetaminophen (Ball milled) – 75 to 96 %w/w
  - Medium phase: Salicylic Acid (Ground) – 3 to 20 %w/w
  - Minority phase: Carbamazepine (Ground) - 0.1 to 5 %w/w

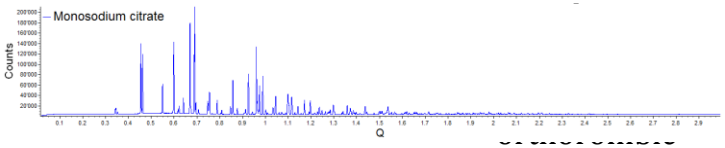
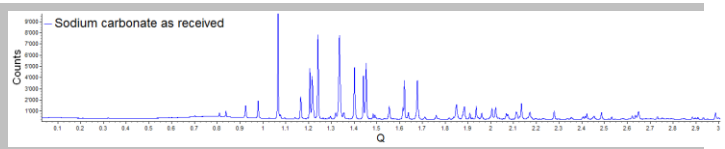


- Adapt particle and crystallite size

Case study: Internal standard candidates

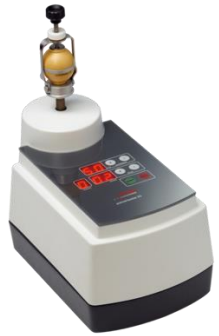
Internal Standard	Chemical formula	Density ($g.cm^{-3}$)	Crystal structure
Hexamethylene-tetramine	$(CH_2)_6N_4$	1,33	Cubic
Diamond	C	3,51	Cubic
Lithium fluoride (precipitated, 99,995%, Sigma Aldrich)	LiF	2,635	Cubic
Monosodium citrate (Jungbunzlauer)	$NaH_2C_6H_5O_7$	1,5	Two known polymorphs: monoclinic & orthorombic
Sodium carbonate (anhydrous, ≥99,9999%, Sigma Aldrich)	Na_2Co_3	2,54	monoclinic or orthorombic
Zeolite (Faujasite)	$[Na_{28.8}Ca_{14.4}(H_2O)_{263}]$ $[Si_{134.4}Al_{57.6}O_{384}]$	1,93	Cubic
Corundum (as a reference)	Al_2O_3	4,02	Trigonal-hexagonal

Case study: Internal standard candidates

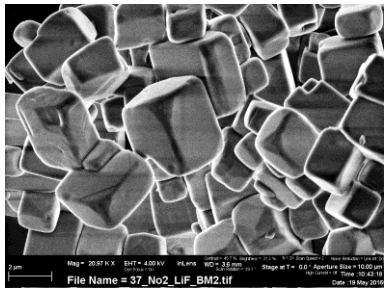
Internal Standard	Chemical formula	Density ($g.cm^{-3}$)	Crystal structure	
Hexamethylene-tetramine	$(CH_2)_6N_4$	1,33	Cubic	Selected
Diamond	C	3,51	Cubic	Very hard
Lithium fluoride (precipitated, 99,995%, Sigma Aldrich)	LiF	2,635	Cubic	Selected
Monosodium citrate (Jungbunzlauer)				Low symmetry
Sodium carbonate (anhydrous, ≥99,9999%, Sigma Aldrich)				Low symmetry
Zeolite (Faujasite)	$[Na_{28.8}Ca_{14.4}(H_2O)_{263}][Si_{134.4}Al_{57.6}O_{384}]$	1,93	Cubic	Later stage
Corundum (as a reference)	Al_2O_3	4,02	Trigonal-hexagonal	Selected

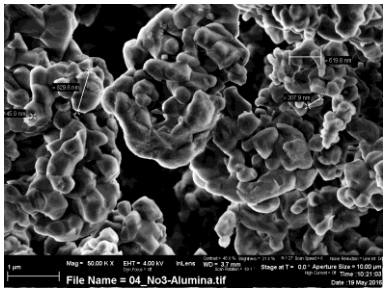
Case study: Sample preparation

- Target particle size distribution: 1-5 microns
- Microsieving 5 to 20 microns:
  - As received, ground and ball-milled HMTA and LiF
  - agglomeration problem / too large psd



- Ball-milling
- Characterization techniques:
  - Laser granulometry
  - Optical microscopy
  - Scanning electron microscopy





Source: fritsch-international.com

Case study: Sample preparation

APIs

Internal standard

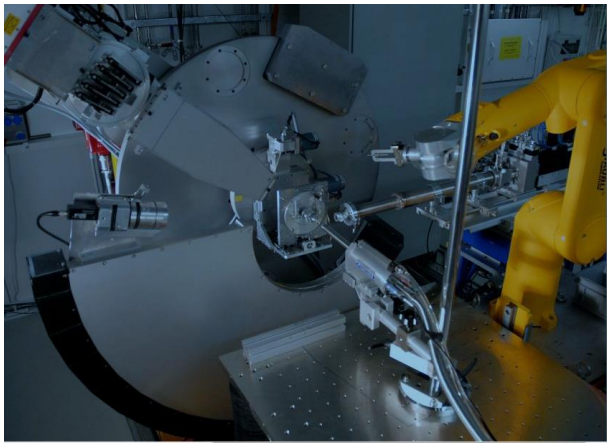
Amorphization ?

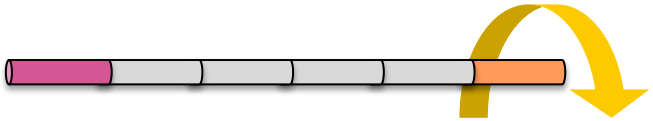
Electrostaticity ?
Agglomeration ?
Water uptake ?

CONSISTENT SAMPLE PREPARATION !

Case study: SR-XRPD

- Avoid transparency effect: transmission geometry
- Achieve sufficient statistics in small volumes:
  - Trade of between capillary diameter and achieved resolution
  - Capillary spinning
  - Data on multiple cap. volumes

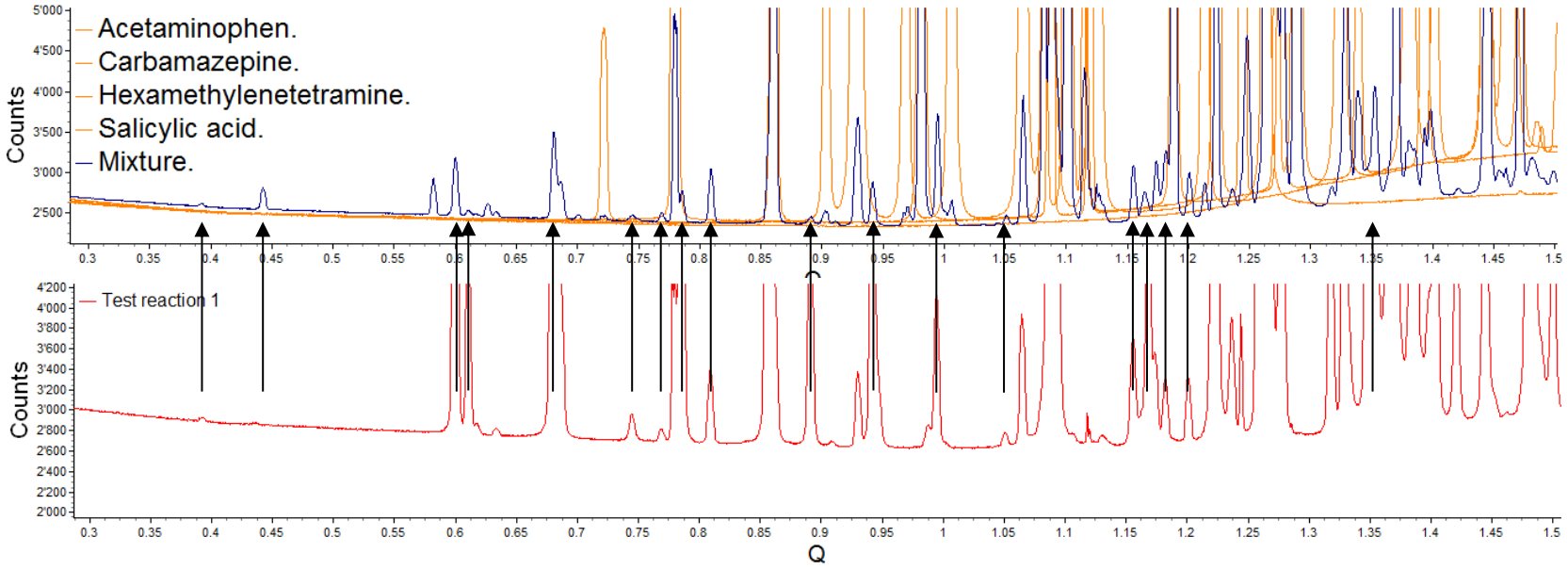




Case study: HMTA

Crystal structure	PO	Reactivity	Agglomerates	Particle/ crystallite size	Homogeneity	Micro-absorption	QPA analysis
✔	no	⚠	no	no	no	no	?

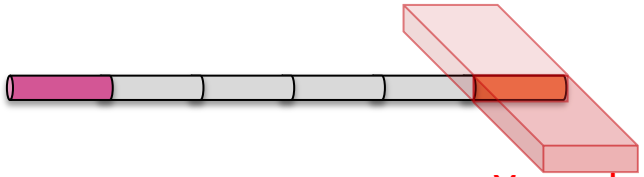
- HMTA: not stable in mixture !

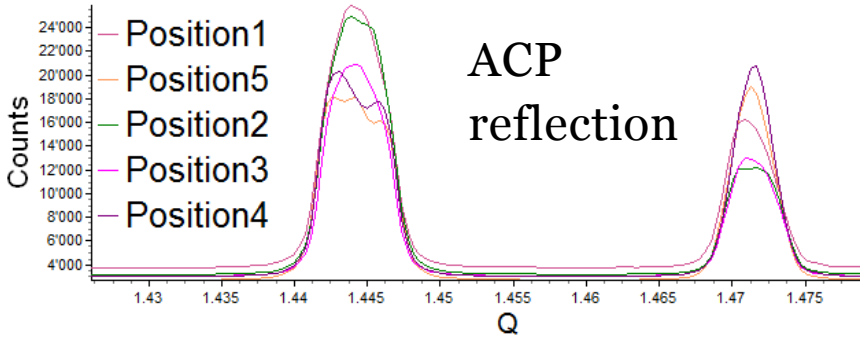


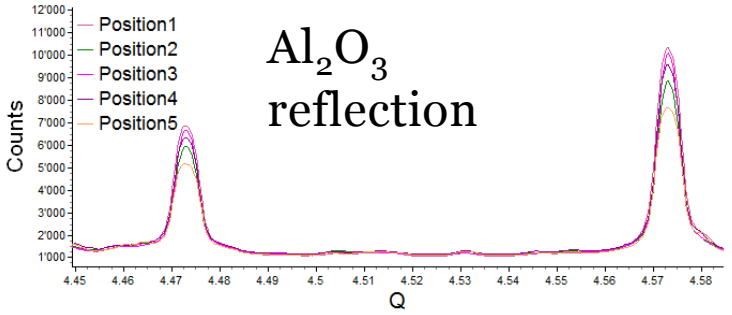
Case study: Al_2O_3

Crystal structure	PO	Reactivity	Agglomerates	Particle/ crystallite size	Homogeneity	Micro-absorption	QPA analysis
✔	no	✔	no	✔		no	Al_2O_3 systematically overestimated

- Inhomogeneity along capillary





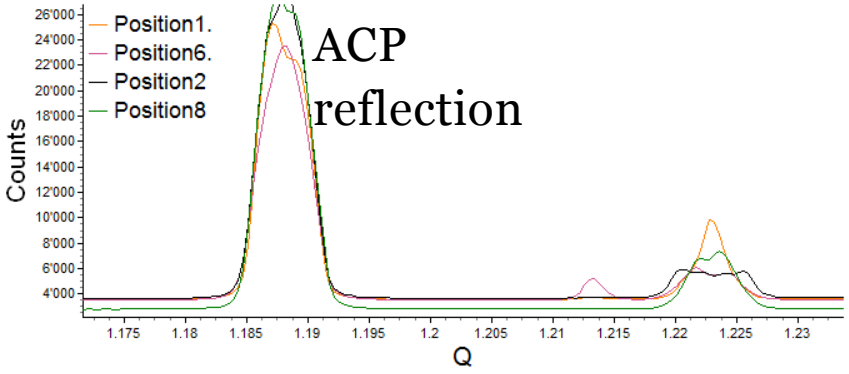


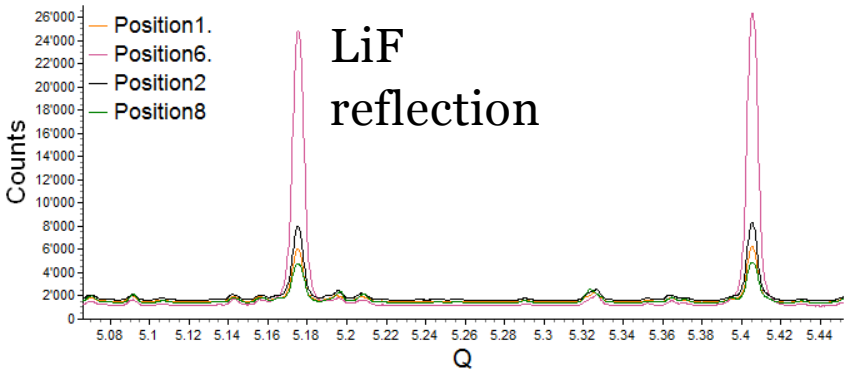
- High density

Case study: LiF

Crystal structure	PO	Reactivity	Agglomerates	Particle/ crystallite size	Homogeneity	Micro-absorption	QPA analysis
✔	no	✔	⚠	✔	⚠	no	LiF systematically overestimated

- Inhomogeneous distribution in spite of milling and careful mixing

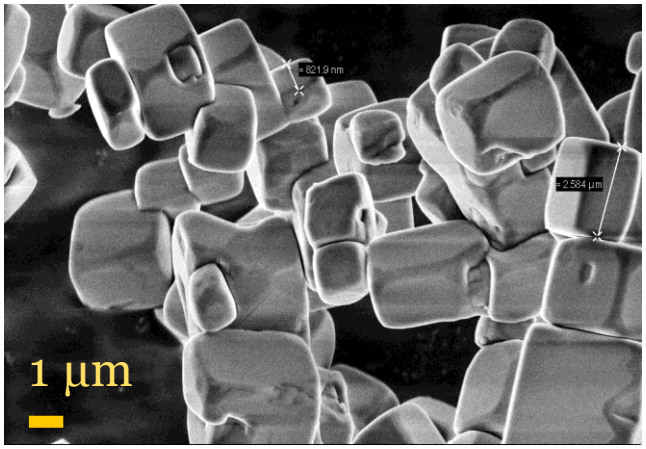


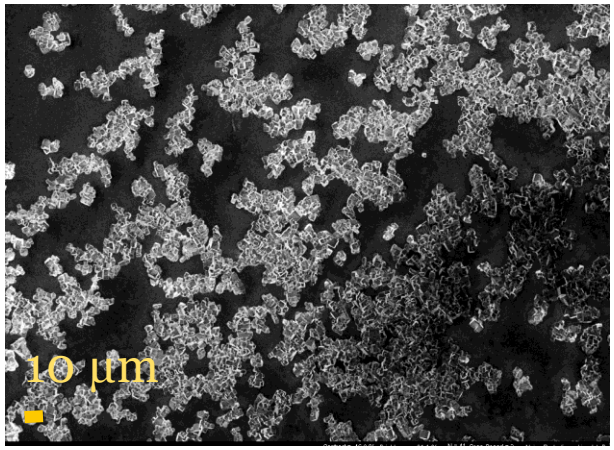


Case study: LiF

Crystal structure	PO	Reactivity	Agglomerates	Particle/ crystallite size	Homogeneity	Micro-absorption	QPA analysis
✔	no	✔	⚠	✔	⚠	no	LiF systematically overestimated

- Inhomogeneous distribution in spite of milling and careful mixing
- Strong agglomeration, hygroscopic





Case study: Preliminary results

Alternative?

	Acetaminophen	Salicylic Acid	Carbamazepine
Crystal structure	✗	✗	✓
PO	yes	yes	no
Reactivity	✓	✓	✓
Agglomerates	no	no	no
Particle/ Crystallite size	✗	✗	✓
Homogeneous distribution	✗	✗	no
Micro-absorption	no	no	no
QPA satisfying	no always underestimated	no always overestimated	no always overestimated

Conclusion

Constraints must be taken into account due to

- Internal standard's role in itself
- Analyte mixture
- Instrument used

  ⇨ Adapt internal standard to analyte and instrument

Step-by-step approach, preliminary tests on important points

Know your pure phases the best you can (amorphous content)

Consistency in sample preparation

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X-Ray Diffraction Procedures: For Polycrystalline and Amorphous Materials, 2nd Edition, 1974
Harold P. Klug, Leroy E. Alexander, ISBN: 978-0-471-49369-3

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Thank you for your attention!

Contact: m.reinle-schmitt@excels.us