QPA instrumentation, sample and validation aspects

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Which method aspects to consider?

- Required LoD / LoQ
- Regions free of peak overlaps
- Possibility to create reliable standards (amount of standards)
- Sensitivity to process variations (changes in particle size etc.)
- Tendency to changing preferred orientation (particle shape) or crystallite size
- Are the crystal structures known (and how well?)
- Sensitivity to instrument variations (incl. tube aging)
- Reproducibility of amorphous content
- Possibility for internal standards (limitations: formulations,…)
- Aspects of method validation

The analytical problem often dictates the choice of quantitative method

The ideal X-ray powder sample for quantitative analysis

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- The ideal powder sample
  - Millions of grains in the measured area
  - Randomly oriented grains
  - Flat sample
  - Smooth surface
  - Densely packed
  - Homogeneous
  - Small grain size (less than 10 microns)
  - Infinitely thick (reflection geometry)

- **But the reality is different!!**

Preferred orientation (aka Texture): non-random orientation of crystallites

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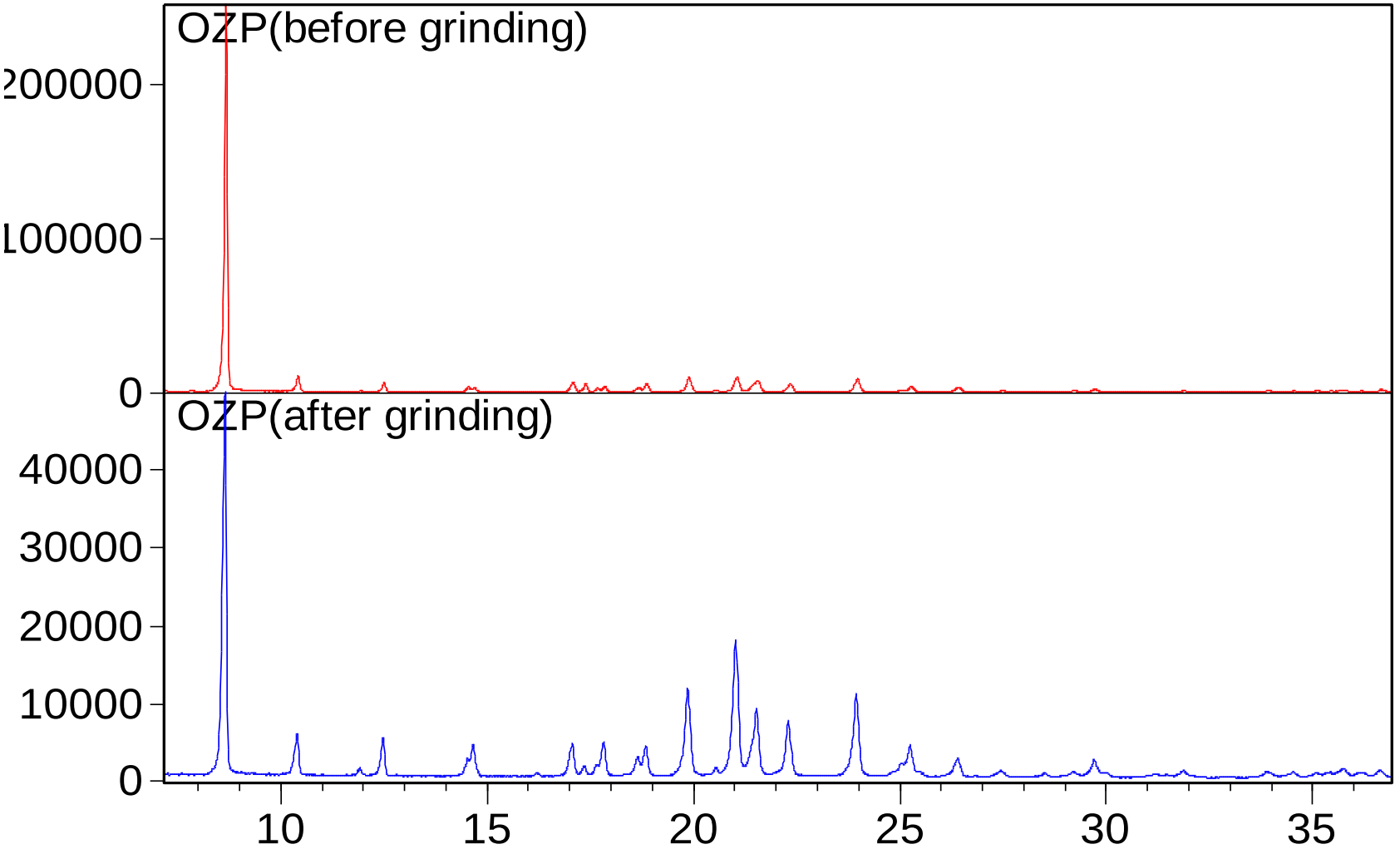
- If the crystallites in a powder sample have **plate** or **needle like** shapes it can be very difficult to get them to adopt random orientations
  - Top-loading, where you press the powder into a holder, usually causes problems with preferred orientation
  - Back-loading is generally preferred in these cases, but pharmaceutical samples remain challenging

- Preferred orientation causes a systematic deviation from the idealized calculated powder pattern (peak intensity errors)

Preferred orientation – effect of grinding

Improvement of the crystallite size distribution due to grinding



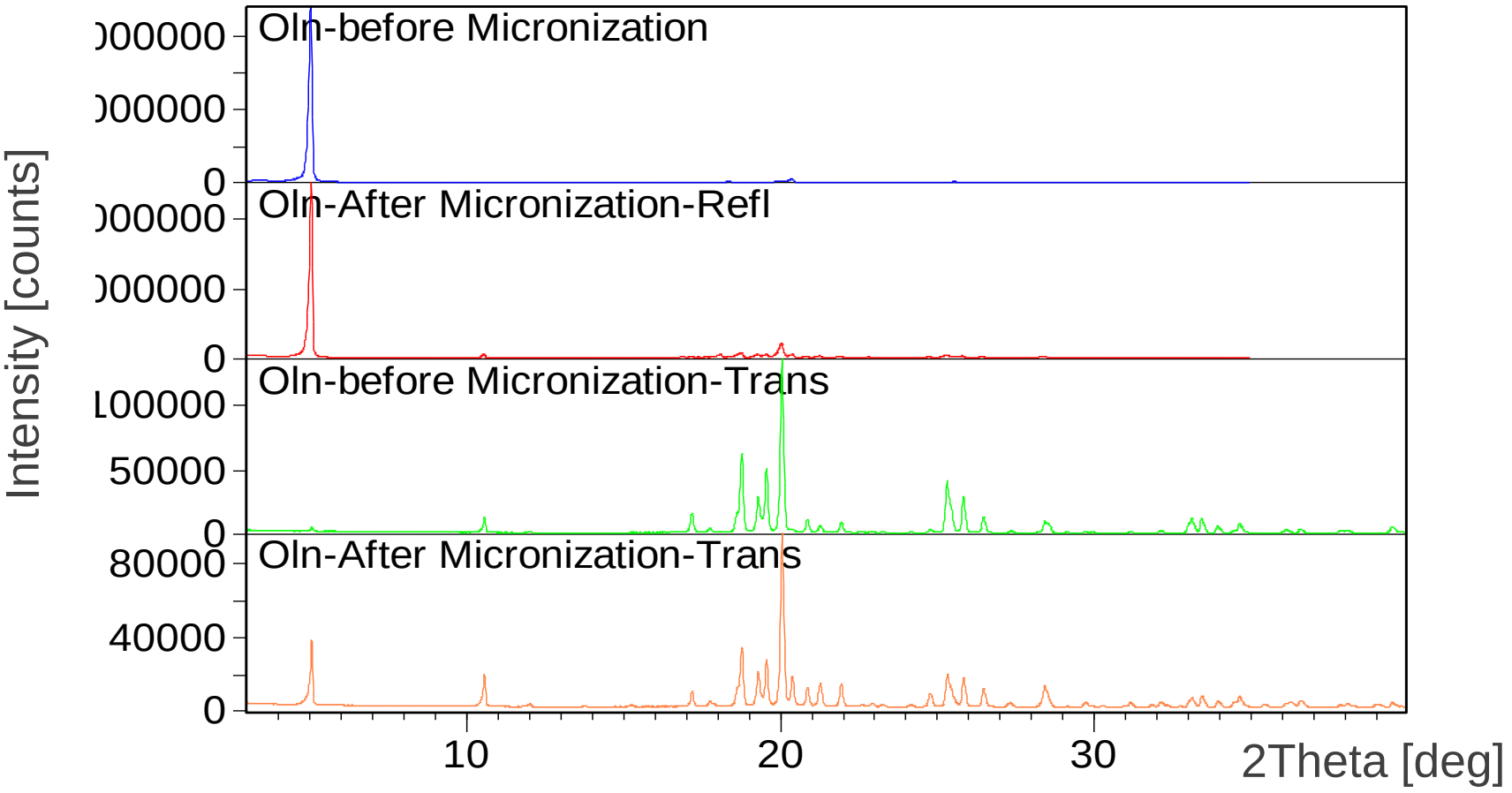
Preferred orientation – effect of grinding

- Organic material can easily be over-ground:
  - Danger of amorphization
  - Small crystallite sizes produce peak broadening
  - Potential phase transitions

- If the preferred orientation is reproducible, it can be taken into account in the method

- In some cases it helps to adapt the instrument geometry

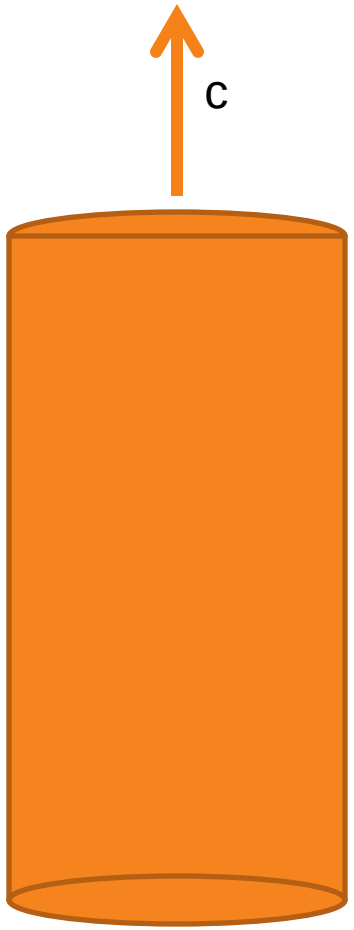
Preferred orientation – effect of XRD geometry



(Transmission geometry has other challenges in QPA – see later)

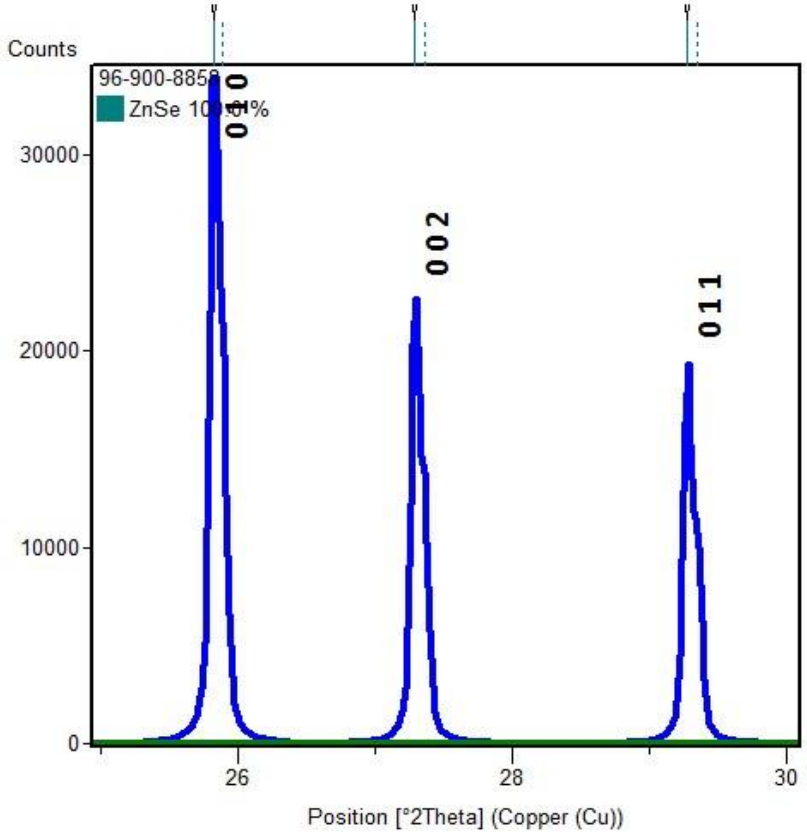
Anisotropic peak broadening

- Small crystallite sizes produce peak broadening
- If the (nano)crystals in a sample have an anisotropic shape, then different peaks will be broadened differently
  - Example: nanorod in which the axial direction of the rod corresponds to the c-axis of the crystal
    - The crystal dimension in the c direction is much larger than the direction in the a or b directions
    - The (00l) peaks, which correspond to planes stacked along the c-axis, will be sharper– corresponding to the larger dimension
    - The (h00), (0k0), and (hk0) peaks, which correspond to planes stacked along the diameter of the nanorod, will be broader– due to the smaller dimension.

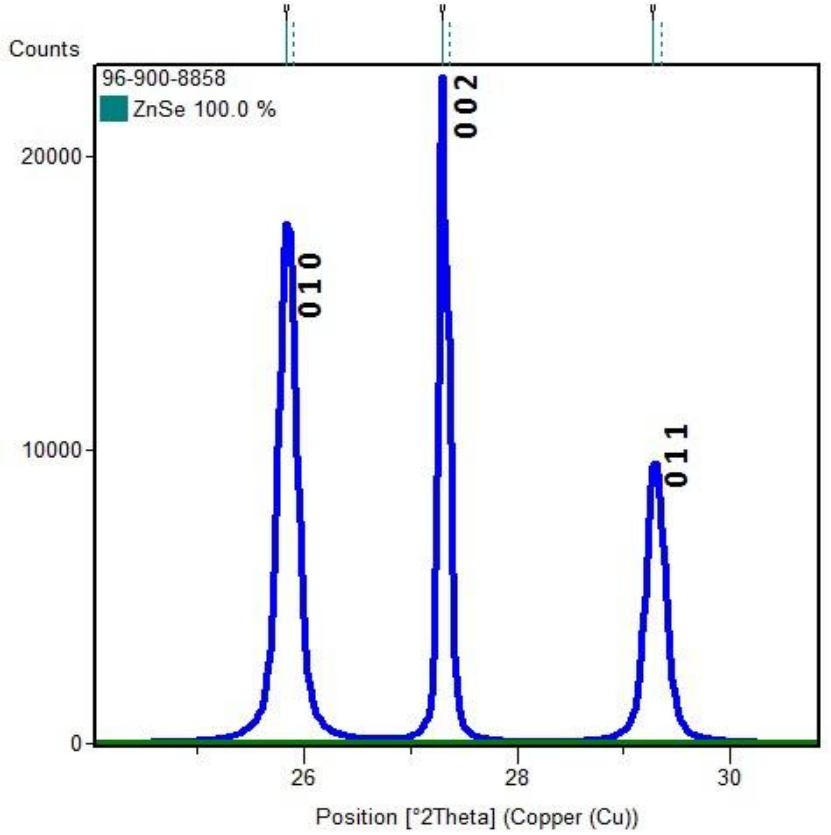


Anisotropic peak broadening

Anisotropic peak broadening can also change peak heights, giving the appearance of preferred orientation



Isotropic crystallite size



Anisotropic crystallite size
(nanorod along the c-axis)

Anisotropic broadening in organic material

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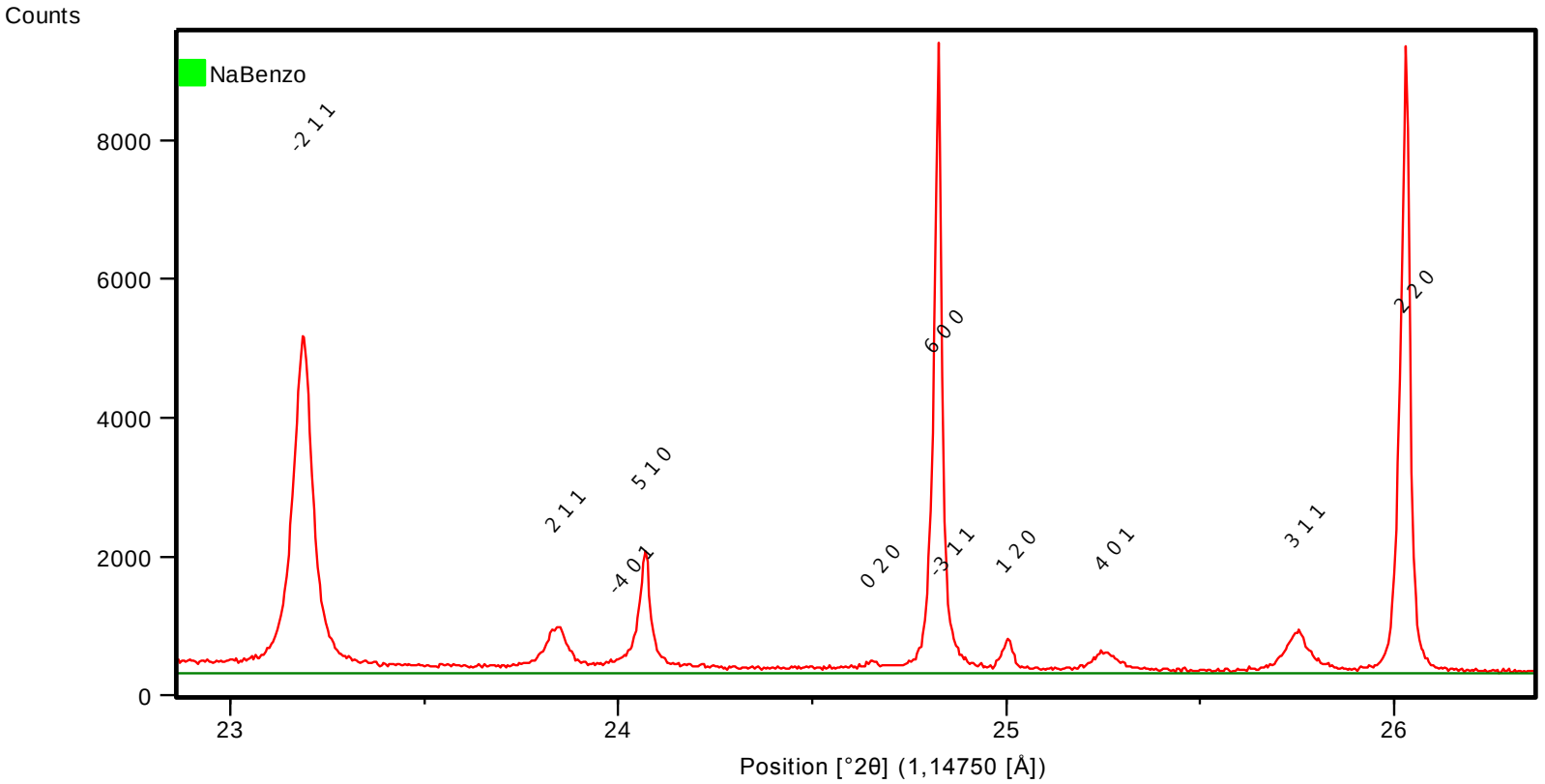
Sodium *para*-hydroxybenzoate

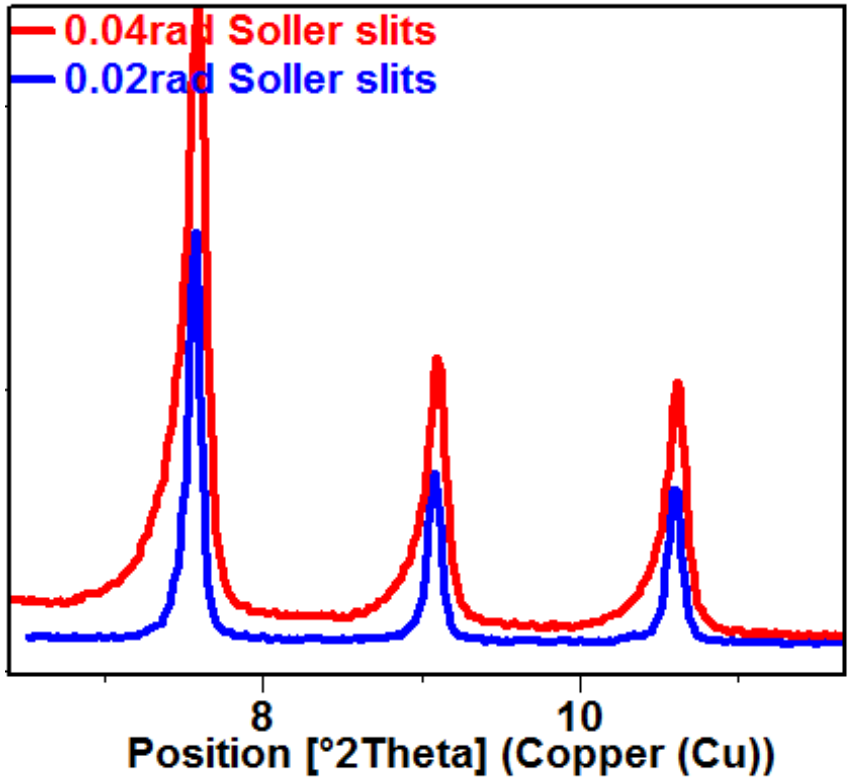


Source: GSAS example files

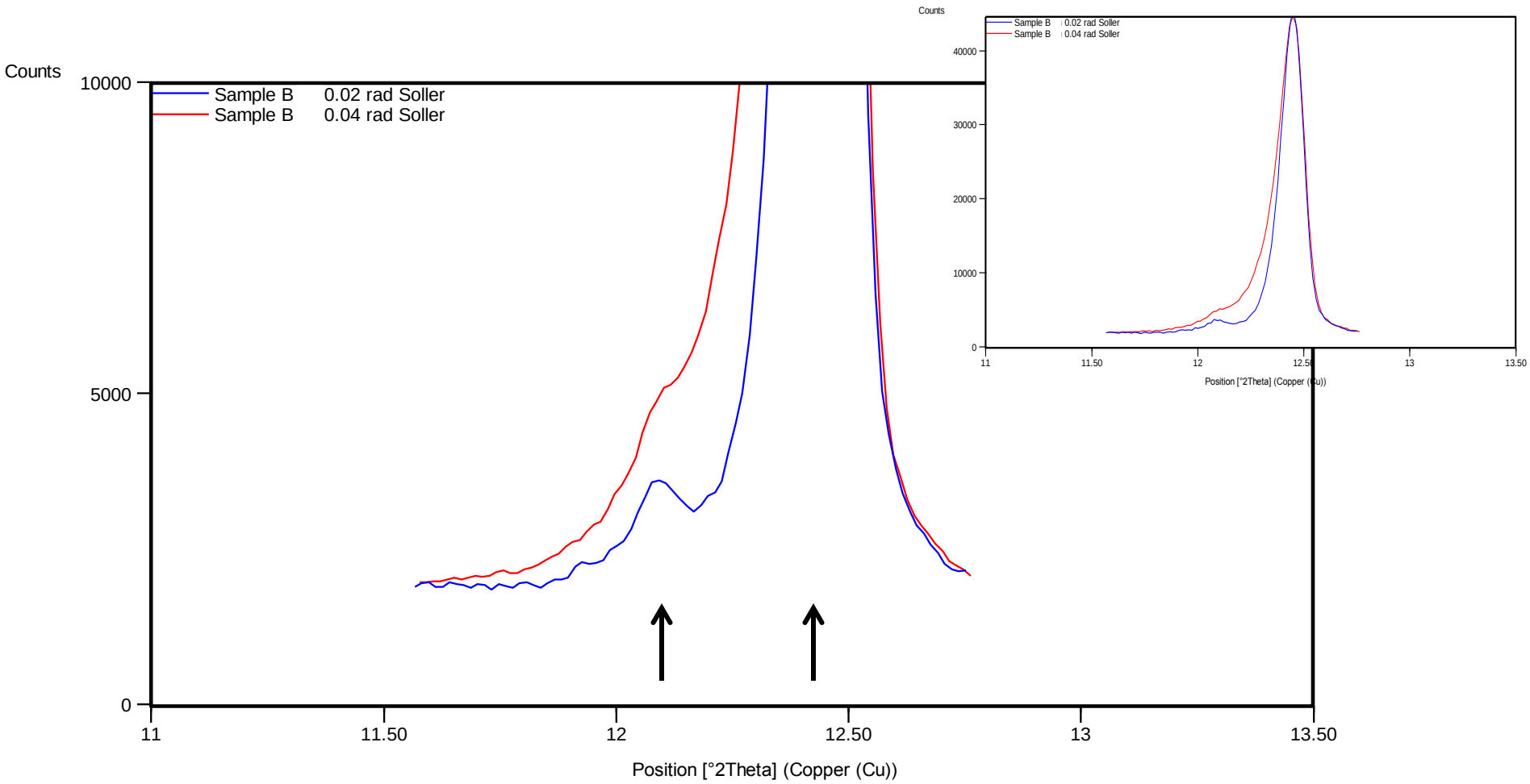
R. E. Dinnebier, R. Von Dreele, P. W. Stephens, S. Jelonek and J. Sieler, *J. Appl. Cryst.* (1999). 32, 761-769

Dealing with peak asymmetry

- Peak asymmetry is produced by:
  - Axial divergence
  - Sample transparency
- Axial divergence can be reduced by using Soller slits
- Sample transparency can be reduced by other sample preparation (not suitable for all methods)

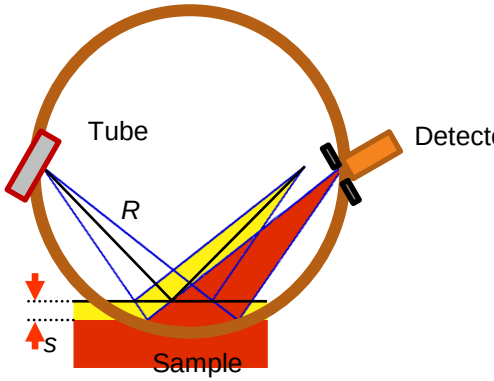


Reduced axial divergence



Sample transparency error

- X-rays penetrate into the sample
  - the depth of penetration depends on:
    - the mass absorption coefficient of the sample
    - the incident angle of the X-ray beam
- This produces errors because not all X-rays are diffracting from the same location
  - Angular errors and peak asymmetry
  - Largest for organic and low absorbing (low atomic number) samples
- Can be eliminated by using parallel-beam optics
- Can be reduced by using a thin sample



$$\Delta 2\theta = \frac{\sin 2\theta}{2\mu R}$$

μ is the linear mass absorption coefficient for a specific sample

Transparency of various materials

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$$I = I_0 e^{-\mu \rho d}$$

99% Absorption at 90°

Material	Density	Mass Absorption Coeff	Infinite Thickness mm	Mass Absorption Coeff	Infinite Thickness mm
		Cu KA 1.54A		Mo KA 0.7A	
H_2O	1	9.9	**4.6**	1.2	**37**
Carbon	2.62	4.3	**4.1**	0.62	**194**
SiO_2	2.32	32.2	**0.6**	3.7	**29**
Fe_2O_3	5.24	202	**0.04**	27	**8.8**
Fe	7.86	284	**0.02**	39	**9.4**
Pb	11.4	225	**0.02**	135	**3.9**

For $\theta < 25^o$
$t_p < 1.6$ mm

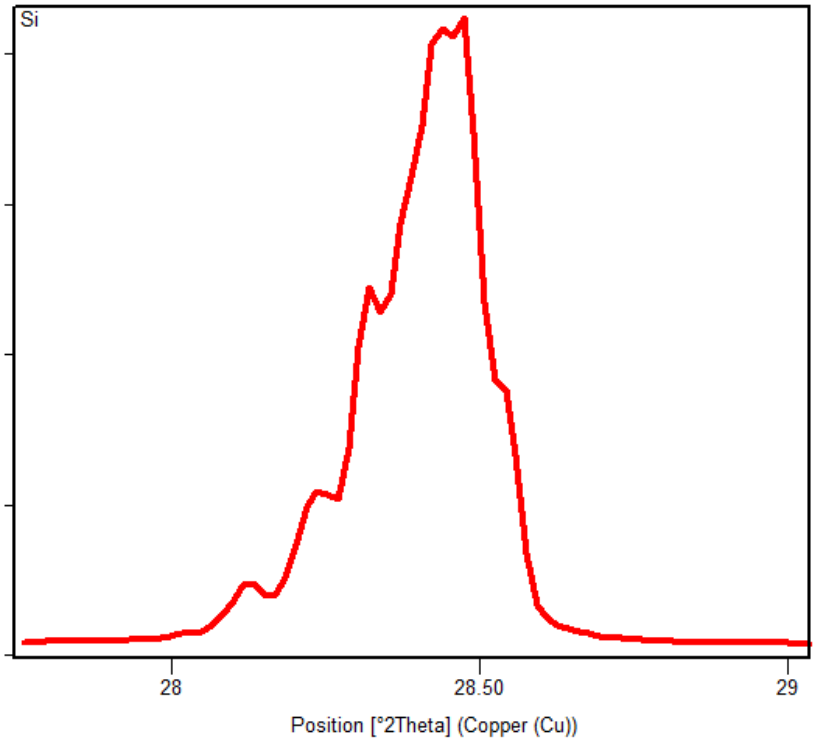
Rietveld quantification method assumes infinitely thick sample

Particle Statistics are determined by

- The number of crystallites that are irradiated
  - The irradiated volume
    - The irradiated area (width and length of the X-ray beam)
    - The depth of penetration of the X-rays
  - The average crystallite size
  - The particle packing factor (porosity)
- The fraction of irradiated crystallites that contribute to the diffraction peak
  - Divergence of the X-ray beam
  - Detector size and aperture (receiving slit)
  - Sample manipulation (spinning, wobbling,…)

Large grain sizes can create irregular peak shapes

- The Si powder in this sample was much too coarse
- This data is unusable for reliable refinement and QPA
- Better data is needed
  - Pulverize & grind the powder
  - Spin the sample
  - Oscillate the sample
  - Use a Wobble scan
  - Use a larger beam size
  - Use a larger detector



Spotty Debye diffraction rings from a coarse grained material

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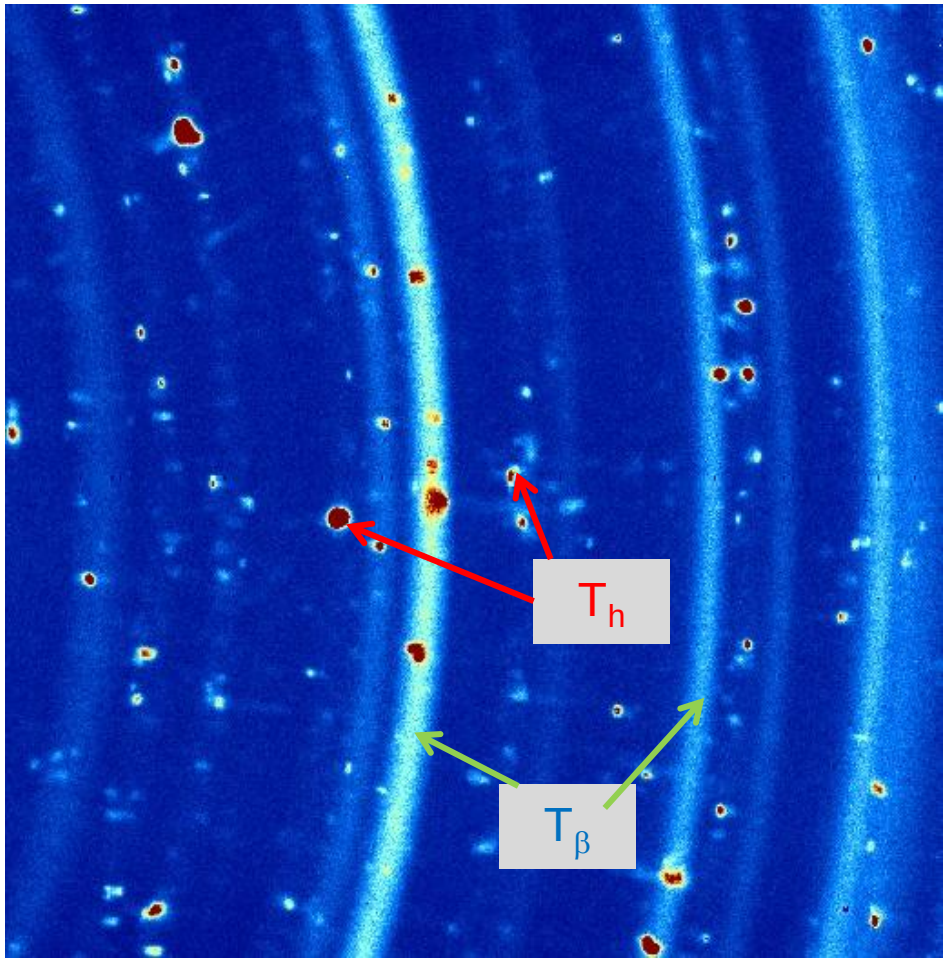


Mixture of fine and coarse grains compound

Mixture of Trehalose dihydrate T_h and crystallizing Trehalose anhydrate T_β (70 ºC, 40% rH)

Rietveld Method - preconditions

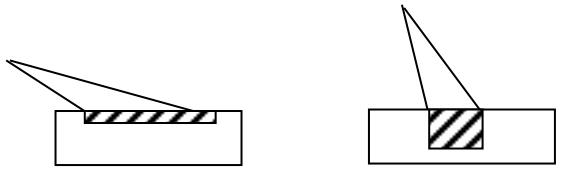
There are some clear requirements concerning the sample:

- Crystallites need to be randomly oriented
- Crystallite size should be < 10 µm and >120 nm
- The number of crystallites has to be “sufficiently” large
- **The sample has to be “Infinitely” thick (varies with wavelength)**
- **The sample has to be larger than the X-ray beam**

The Rietveld method assumes that the sample volume irradiated by the X-ray beam is constant over the entire range of the scan => The same number of crystallites contribute to the diffraction of all peaks.

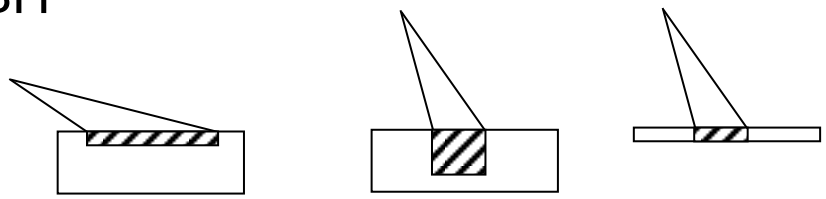
The length of the X-ray beam and the depth of penetration both change during a scan with Bragg-Brentano parafocusing optics (with fixed divergence slits):

- The length of the X-ray beam changes as $1/\sin\theta$
- The depth of penetration increases as $\sin\theta$
- These two factors result in a **constant irradiated volume**



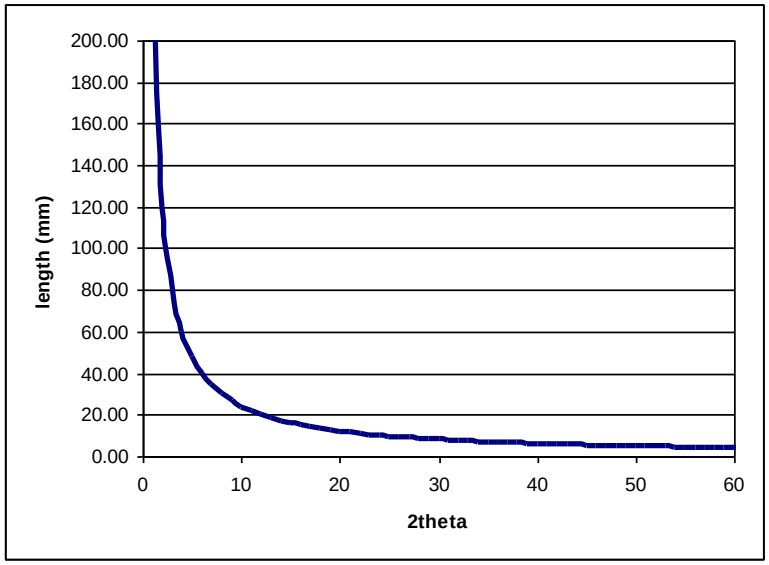
The constant volume assumption

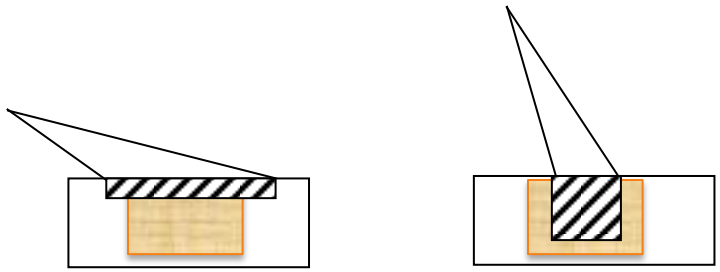
- In a polycrystalline sample of ‘infinite’ thickness, the change in the irradiated area (as the incident angle varies) is compensated by the change in the penetration depth
- These two factors result in a constant irradiated volume
  - (as area decreases, depth increase; and vice versa)
- This assumption is important for many aspects of XRPD
  - Matching intensities to those in the PDF reference database
  - Crystal structure refinements
  - Quantitative phase analysis
- This assumption is not (necessarily) valid for thin films or small quantities of sample on a ZBH



Varying length of X-ray beam

- At low angles, the beam might be wider than your sample
  - “Beam spill-off”
- Length approx: L = R x tanα /sinθ
  - R is goniometer radius
  - α is the divergence angle of the beam





Deviations from the constant volume assumption: beam overflow

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- Beam overflow (beam spill-off)
- At low angles, the X-ray beam might be larger than the sample
  - Example: a ½ deg divergence slit will produce a 48.5mm long X-ray beam at 5deg 2θ
    - This will be larger than your typical sample (which is e.g.) 10 mm x 10mm
- Corrections
  - Use a smaller divergence slit for low angle data
    - This will yield weaker peak intensities at high angles of 2theta
  - Use corrections in SW
  - Throw away (clip or exclude) low angle data where beam was larger than sample
  - Use automatic divergence slits

Dealing with thin samples

- Use of automatic divergence slits
  - Useful for very thin samples, when the penetration depth of the X-ray beam exceeds the sample thickness over the entire measurement range
  - Maintains a constant irradiated length, and the thinness of the sample enforces a constant penetration depth
    - consequently, the irradiated volume is constant

Problems encountered & possible solutions

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- (Varying) preferred orientation
  - Consider some other peak(s)
  - Grind / micronize
  - Change diffraction geometry
- Particle statistics
  - Grind, spin, wobble
- Sample preparation errors
  - Standardize & normalize
- Tube intensity decay
  - Normalize

QPA instrumentation (general aspects)

- Bragg-Brentano (reflection) geometry
  - Reproducible sample preparation
  - Samples can be “infinitely thick”

- Transmission geometry
  - Absorption is thickness and 2θ dependent (good sample preparation is more challenging – dedicated sample holders for transmission QPA required)
  - More possibilities to improve particle statistics (e.g. wobbling) – might improve LoD

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Validation of QPA methods

Method validation

- The validation of a quantitative method in the pharmaceutical industry needs to follow regulations (EP / USP / JP /…) and prescribed methodologies
- With the choice of the QPA method also validation requirements should be taken into account

ICH – Q2B Validation of Analytical Procedures

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Analytical Procedure	IDENTIFICATION	TESTING FOR IMPURITIES		METHOD
Characteristics		Quantitative	Limit(LoD)	
Accuracy	-	+	-	Known ref.
Precision				
Repeatability		+	-	Multiple replicates
Interm. Precision		+	-	Random variations (e.g. analyst)
Specificity	+	+	+	Spiking
Detection Limit	-	-	+	S/N; SD
Quantitation Limit	-	+	-	S/N; SD
Linearity	-	+	-	Regression
Range	-	+	-	Impurity e.g. reporting level to 120% of spec
Robustness	-	+	+	Process variations
System suitability test	+	+	+	Validation

Detection and quantification

- Detection – unambiguous identification of the presence of a given polymorph
- Quantification – concentration level determination with accuracy and precision

LoD & LoQ – ICH Guidelines

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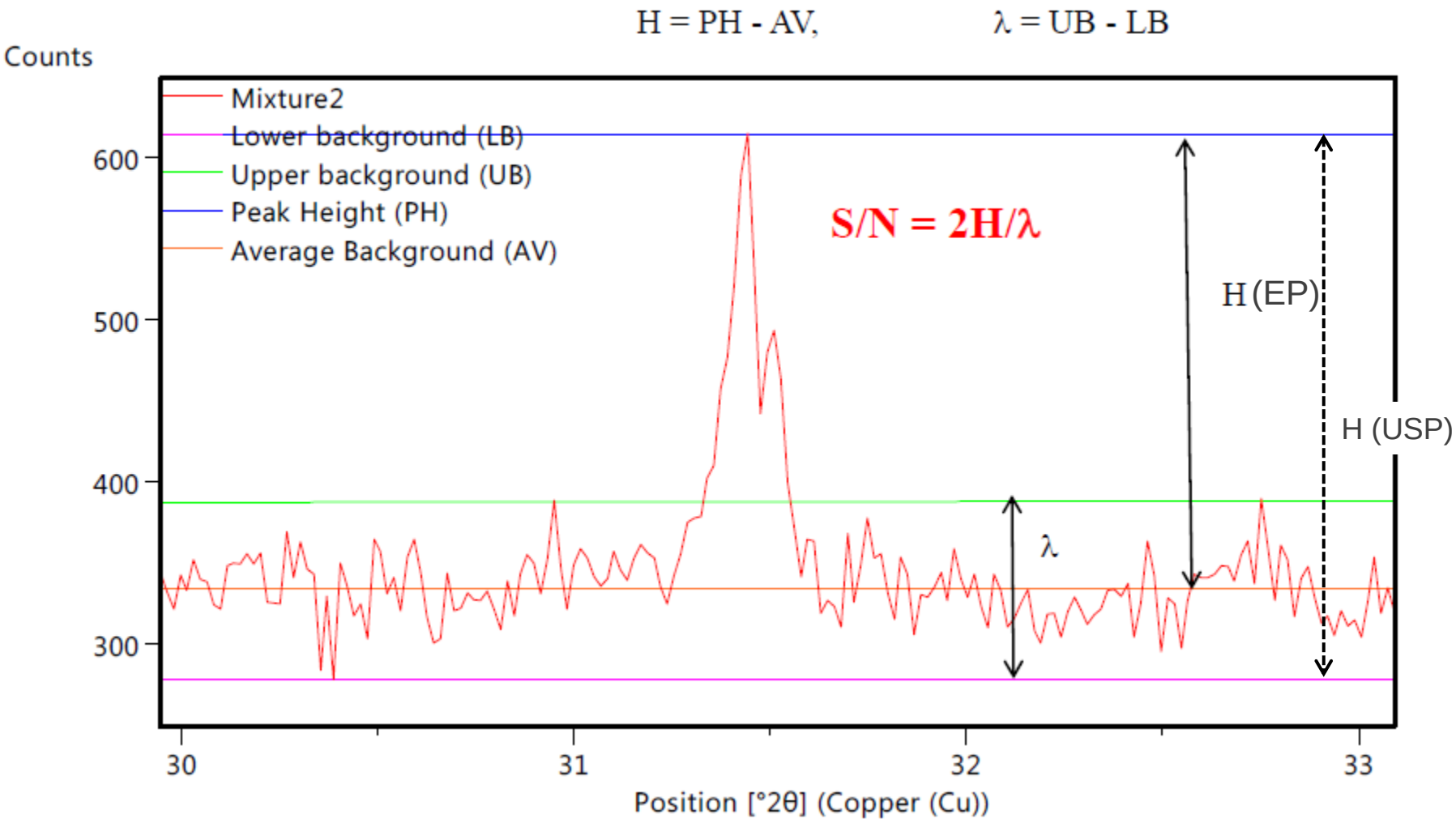
- From Signal-to-Noise ratio (S/N)
  - LoD: 2-3 : 1
  - LoQ: 10 : 1
- From standard Deviation (σ) of Response and the Slope(S)
  - LoD = 3.3 σ/S
  - LoQ = 10 σ/S

- For XRD it is advised to consider **both** the standard deviation of the blank (counting statistics) and the standard deviation of the regression curve for σ
- The definition of the signal-to-noise ratio (S/N) is not clearly, consistently defined between the different Pharmacopeia regulations (EP / USP)

Signal-to-Noise ratio



EP / USP

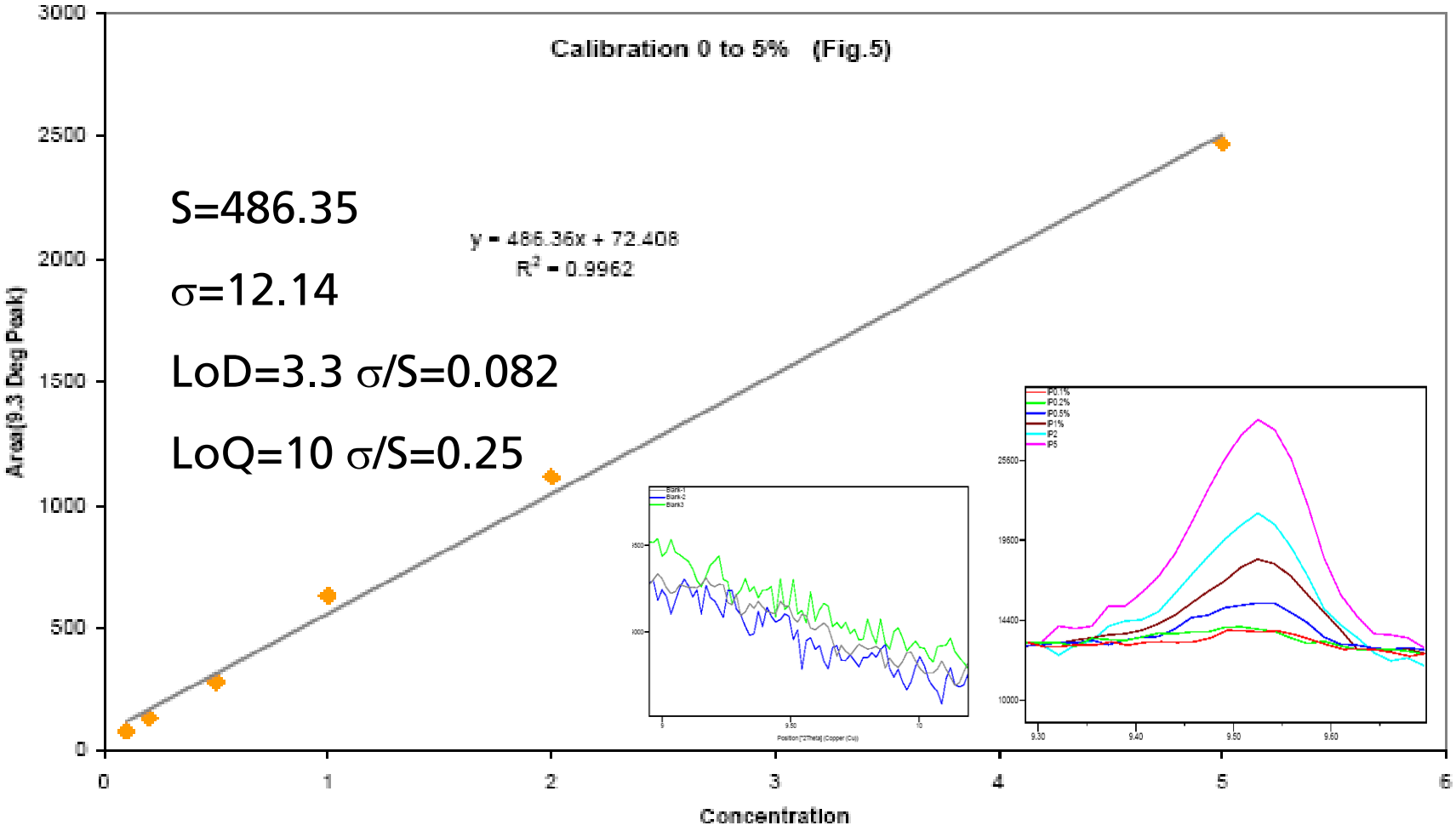
- The noise region for the EP S/N is specified as 20 times the width at half height (FWHM) and that of the USP S/N is specified as at least 5 times the width at half height.
- In XRD patterns the required angular range might not be free of peaks (if range is shortened it might not be representative for the noise level)

- Alternatively the noise can be calculated from the background:
  - In XRD, the noise follows Poisson distribution:

$$\sigma_{cs} = \text{sqrt}\ (I_b)$$

  - 99.7% confidence interval: Noise = $3\ \sigma_{cs} = 3\ \text{sqrt}\ (I_b)$

Linearity, LoD & LoQ from calibration and blank



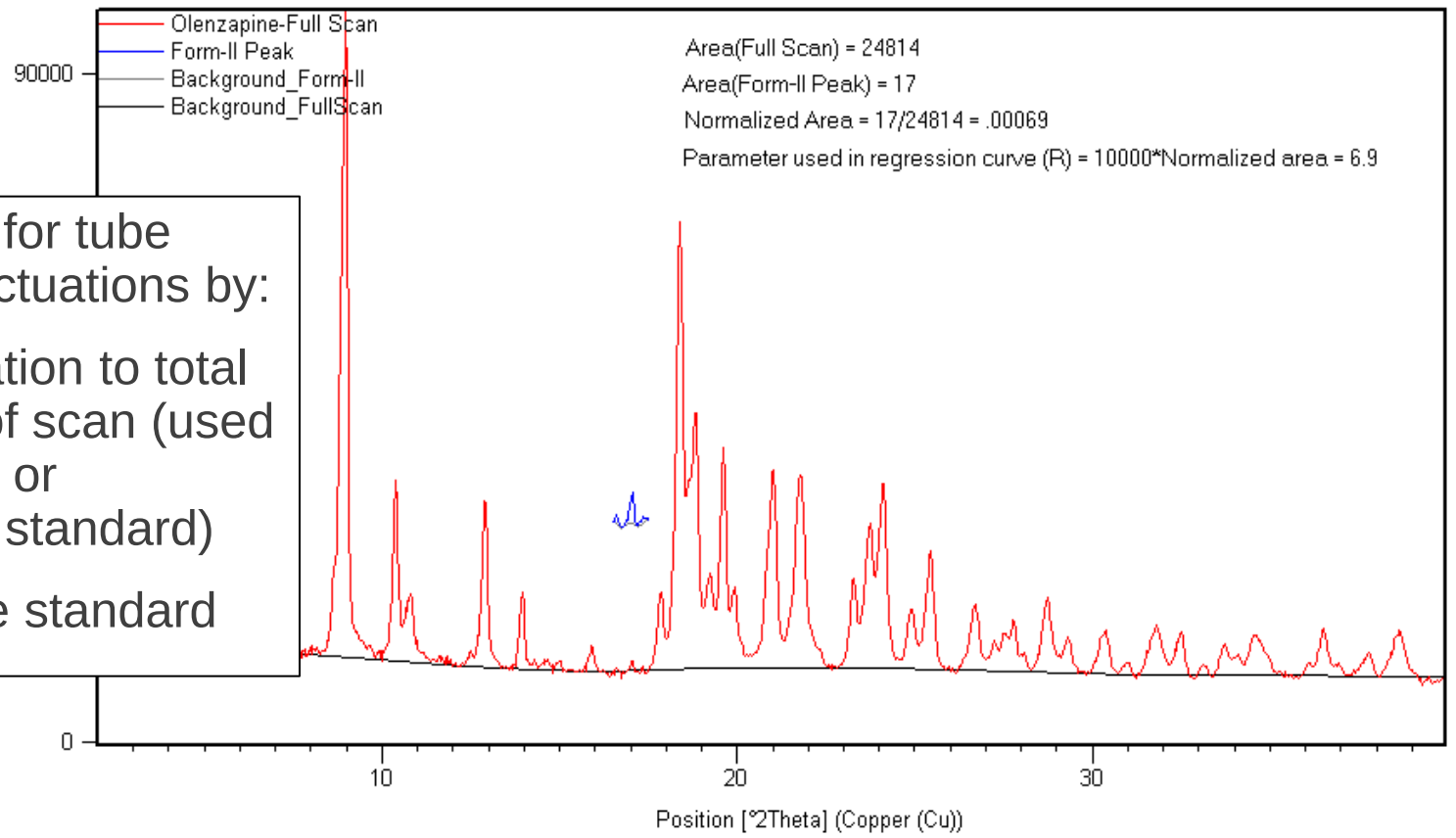
QPA method validation

- The method characteristics (incl. accuracy, precision, specificity, and linearity) need to be investigated for single peak as well as full pattern methods - e.g. by using:
  - Analysis of known references
  - Multiple sample replicates
  - Spiking sample with known amount of QPA phase
  - Calibration line with different concentrations

Method Robustness

- Things that can affect method robustness
  - Sample preparation
  - Tube intensity decay
  - Preferred orientation
  - Particle statistics

Normalization



Accounting for tube intensity fluctuations by:

- Normalization to total intensity of scan (used scan area or reference standard)
- Reference standard

Methods have to be defined wrt expected tube aging / tube variations (LoD / LoQ)

Sources of errors

- Single measurement
  - Counting Statistical Error (CSE)
- Repeatability (sample to sample)
  - Sample homogeneity
  - Preferred orientation
  - Particle statistics

⇒ **With good counting statistics the sample reproducibility error will become the limiting factor for the accuracy of the method**