PANalytical
get insight

Quantitative phase analysis by XRPD

Organizers and Instructors:

Arnt Kern, Burker AXS GmbH, Germany

Detlef Beckers, PANalytical B.V., The Netherlands

Fabia Gozzo, Excelsus Structural Solutions SPRL, Belgium

Robert Dinnebier, MPI für Festkörperforschung, Germany

Arnaud Grandeury, Novartis Pharma AG

Copyright : © 2015 PANalytical B.V., all rights reserved

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

Sponsored by The International Centre for Diffraction Data

This presentation is provided by the International Centre for Diffraction Data in cooperation with the authors and presenters of the PPXRD symposia for the express purpose of educating the scientific community.

All copyrights for the presentation are retained by the original authors.

The ICDD has received permission from the authors to post this material on our website and make the material available for viewing. Usage is restricted for the purposes of education and scientific research.

PPXRD Website – www.icdd.com/ppxrd

ICDD Website - www.icdd.com

Workshop program

Introduction and Overview of QPA methods Detlef Beckers, 30 min	**8.30**
QPA with diffraction methods Fabia Gozzo, 45min	**9.00**
Quantification of amorphous phases - theory Robert Dinnebier, 45min	**9.45**
Coffee break	***10.30***
QPA Instrumentation, validation and sample preparation Detlef Beckers, 45min	**11.00**
Quantification of traces: LoD & LoQ Fabia Gozzo (SR-XRPD), Detlef Beckers (lab-instrumentation), 30min+30min	**11.45**
Lunch	***12.45***
QPA as one piece of a bigger puzzle in pharmaceutical development Arnaud Grandeury, 45 min	**14.30**
Quantification of amorphous phases – practice part 1 Robert Dinnebier, 30 min	**15.15**
Coffee break	***15.45***
Quantification of amorphous phases – practice part 2 Robert Dinnebier, 1h	**16.15**

Copyright : © 2015 PANalytical B.V., all rights reserved

Overview of Quantitative Phase Analysis (QPA) methods

Detlef Beckers, PANalytical B.V.

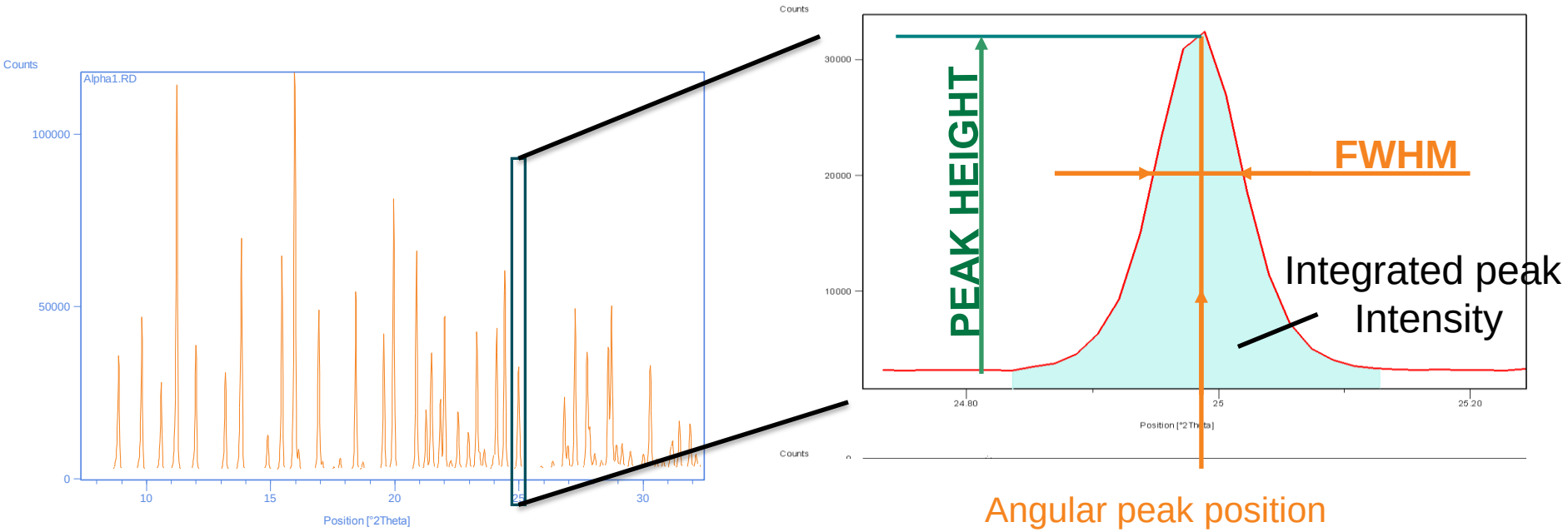
Copyright : © 2015 PANalytical B.V., all rights reserved

Introduction and background

A diffraction pattern is a fingerprint of a (crystalline phase)

Lattice structure ⇒ Peak position
Molecule arrangement ⇒ Relative intensities
Crystallite size / strain ⇒ FWHM

Mixture quantity ⇒ Intensity of pattern (net peak areas)



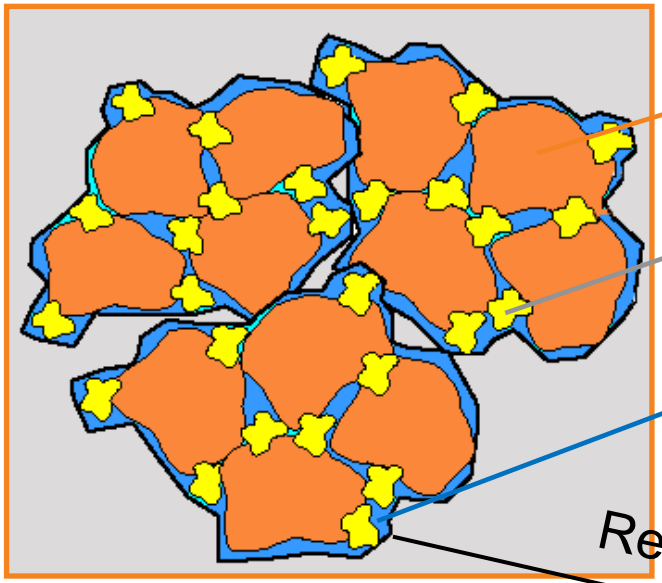
Angular peak position

Copyright : © 2015 PANalytical B.V., all rights reserved

Introduction and background

Each phase in the sample produces a characteristic pattern that is superimposed on those of the other phases

Multi-phase sample

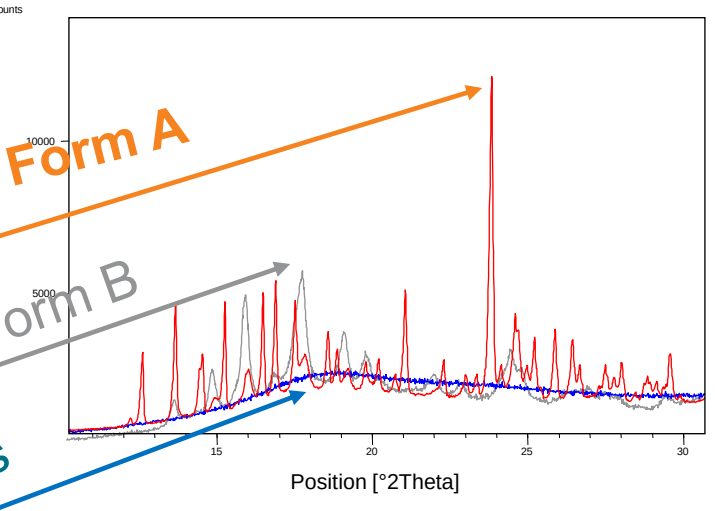


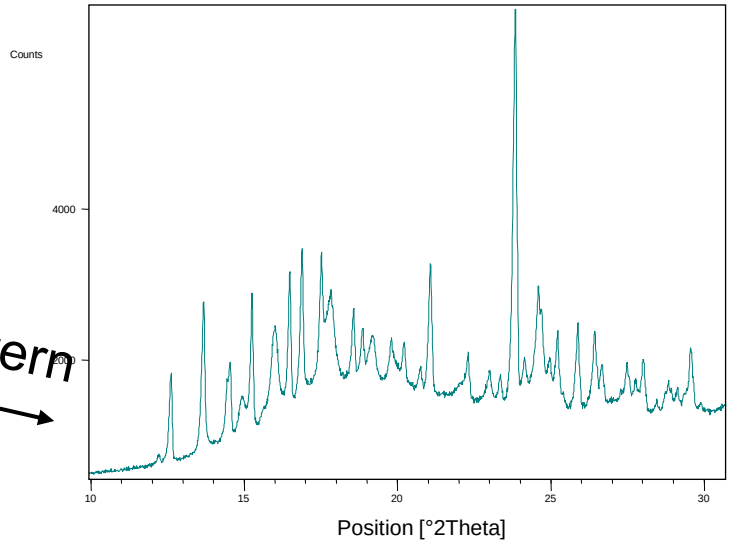
Crystalline Form A

Crystalline Form B

Amorphous

Resulting XRD pattern





Copyright : © 2015 PANalytical B.V., all rights reserved

Applications in the pharmaceutical industry

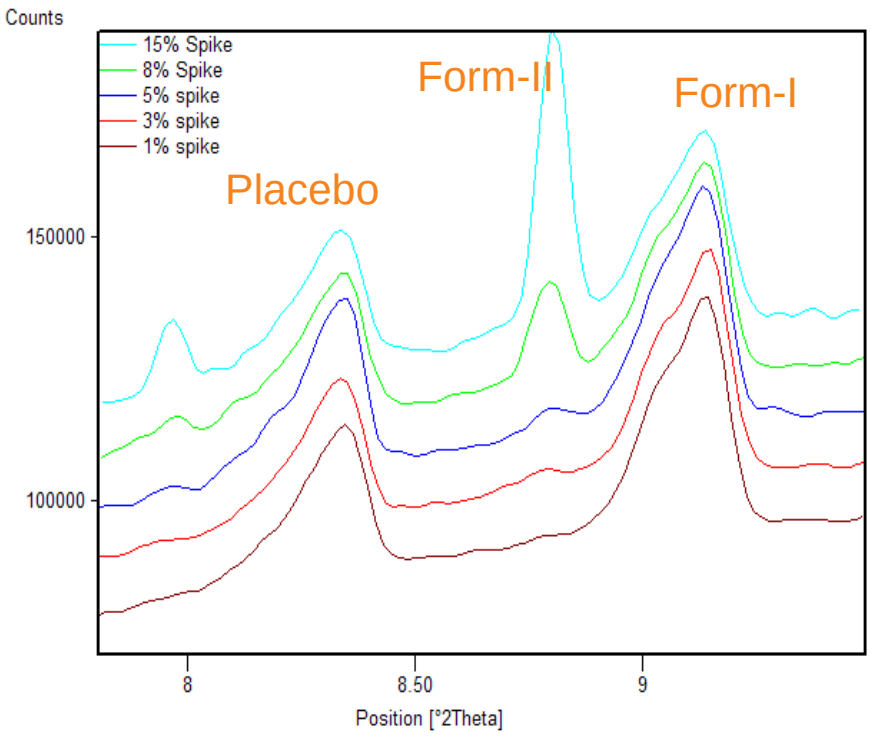
- Polymorphic purity: detect and quantify unwanted polymorphic forms in both drug substance and drug product
- Limit of Detection (LoD) and Quantification (LoQ)
- Assess the polymorphic composition in drug substance and product
- API / excipient concentration in formulation
- Degree of crystallinity in amorphous/crystalline mixtures (API / formulation)
- …

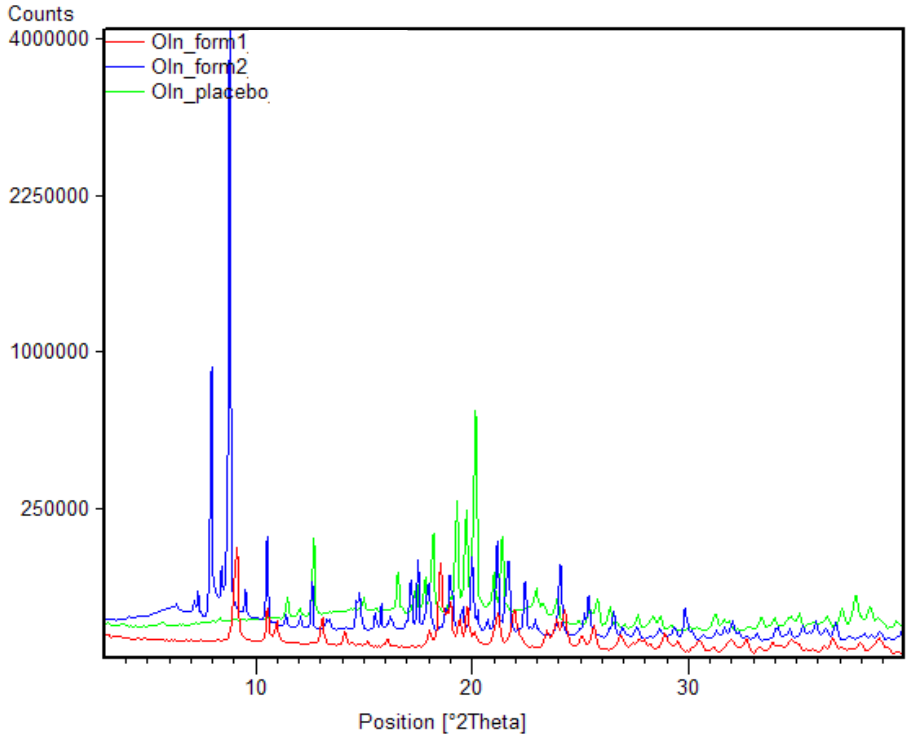
Copyright : © 2015 PANalytical B.V., all rights reserved

Quantification of crystalline phases

PANalytical
get insight

5% formulation of Olenzapine form-I (spiked with form-II)

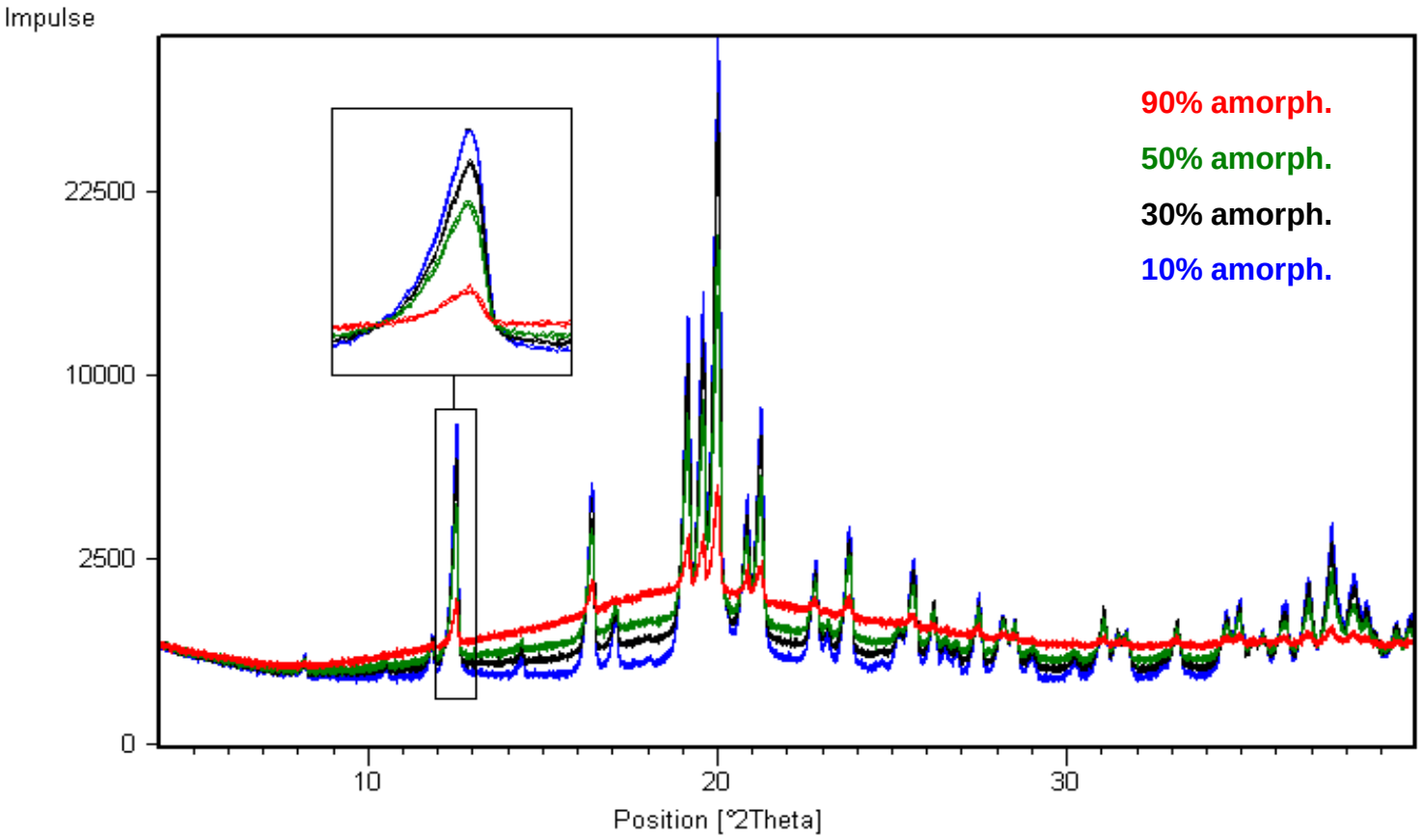




LoD < 3% of API impurity (< 0.15% of formulation) – measured in Bragg-Brentano geometry

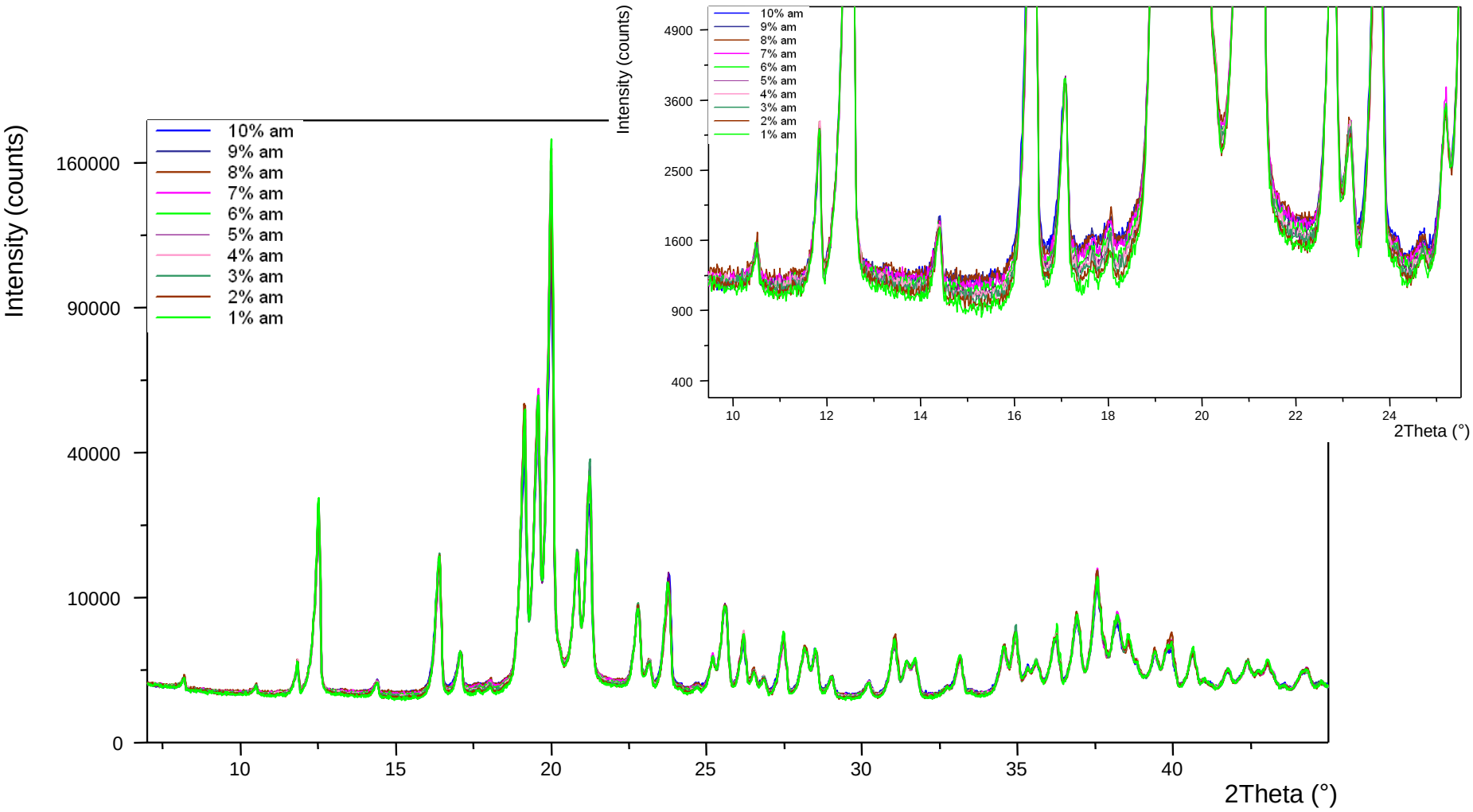
Copyright : © 2015 PANalytical B.V., all rights reserved

Quantification of amorphous phases



Copyright : © 2015 PANalytical B.V., all rights reserved

Quantification of amorphous phases



Copyright : © 2015 PANalytical B.V., all rights reserved

Overview of Quantitative Phase Analysis (QPA) methods

PANalytical
get insight

Single (few) peak method (small 2 Theta range): $I_{unknown} \leftrightarrow I_{known}$

- General model
- Straight line model (μ constant)
- Linear Multi-Variate Regression model
  (extension of straight line model with various independent reflections)
- Matrix flushing (sum of phases known and μ constant)
- Internal Standard model
- RIR method (uses ICDD data on relative intensities compared to corundum standard)
- Addition model (adding a known concentration of the compound of interest)
- Single line addition method (sample with just one crystalline and one amorphous phase)
- Dual line addition method (sample with a reference phase in high concentration preferably with non-overlapping peak)
- Thin layer with base plate correction (X-ray transparent sample, calculation of transmission factor for concentration determination)

Copyright : © 2015 PANalytical B.V., all rights reserved

Overview of Quantitative Phase Analysis (QPA) methods

Whole pattern methods (large 2 Theta range)

- Traditional Rietveld method
- FULLPAT / PONKCS method
- Degree of Crystallinity (1 reference) / Linear Calibration Model (multiple references)
- Internal Standard method (spiking to determine amorphous content)
- External Standard method (amorphous content determination)

- ***Partial Least Squares regression (PLSR) – not based on diffraction properties***

Copyright : © 2015 PANalytical B.V., all rights reserved

Which aspects to consider?

PANalytical
get insight

- 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)
- 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

Copyright : © 2015 PANalytical B.V., all rights reserved

Quantification by Partial Least Squares Regression - PLSR

PANalytical
get insight

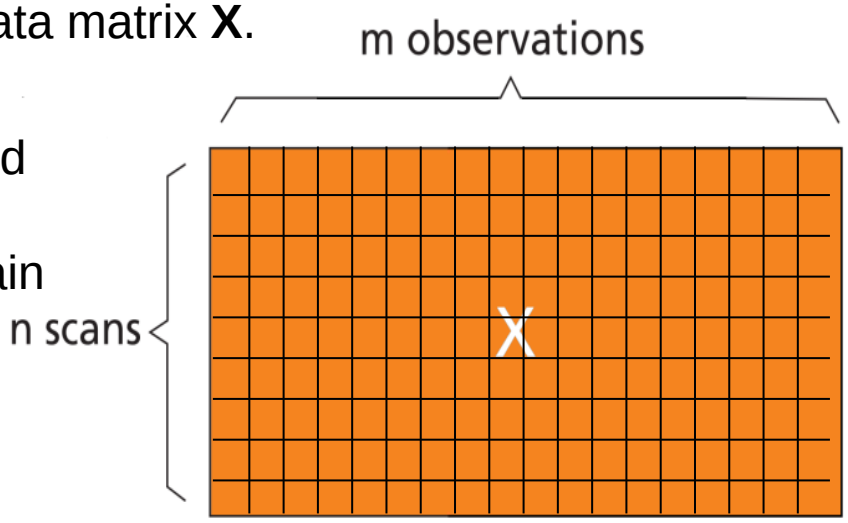
- Whereas traditional XRD quantification methods are analyzing certain pattern features (peak intensity / background /..) and make use of correlations with physical parameters (c ~ I, crystal structure ↔ I, …), PLSR is not using any physical property of an analytical result
- PLSR is a statistical evaluation that searches for correlations of a property parameter (c, crystallinity, T, pH, …) with the variation of a whole pattern or parts of a pattern (x-, y- coordinates)
- Therefore applicable to virtually any analytical technique
- PLSR is commonly used in other analytical techniques (NIR, DSC,…)
- In recent years also applied to XRD data. But not (yet) very popular.

Copyright : © 2015 PANalytical B.V., all rights reserved

Partial Least-Squares Regression (PLSR)

PLSR as developed by Herman Wold in 1960, is able to predict any defined property **Y** directly from the variability in a data matrix **X**.

In **XRPD** the **rows** of the matrix **X** are formed by the individual scans, the **columns** are formed by all measured intensities at a certain diffraction angle 2Θ.



XRPD data (matrix **X**) typically contains:

- Non-systematic variations (sample preparation, noise, …)
- Non-intended variations (impurities, differences in grain sizes, …)
- Systematic variations (different concentrations, …) => response vector **Y**

Projection based methods like **PCA** or **PLS** have the goal to extract a small number of scores/factors to optimally explain the (systematic) data variation in matrix **X**. The extracted scores/factors can then be used for regression analysis.

Copyright : © 2015 PANalytical B.V., all rights reserved

Comparison of analytical approaches

- Lactose used as model substance
  1. Amorphous lactose in crystalline matrix
  2. Crystalline lactose in amorphous matrix

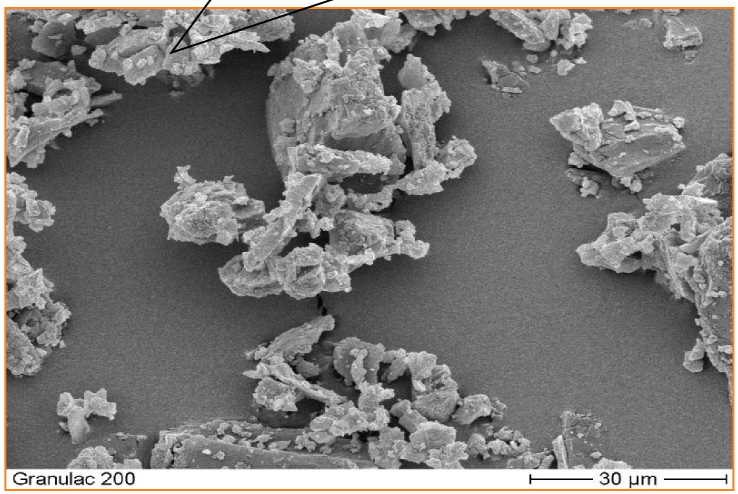
Copyright : © 2015 PANalytical B.V., all rights reserved

Example 1: Crystallinity - low amorphous content

PANalytical
get insight

Model substance: Alpha lactose monohydrate

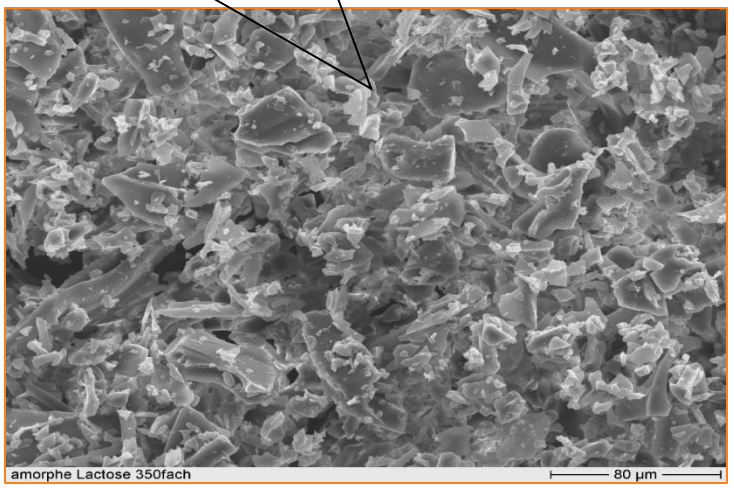
Crystalline lactose



Preparation:

- Storage at RH of 56% and 30°C to ensure complete recrystallisation

Amorphous lactose



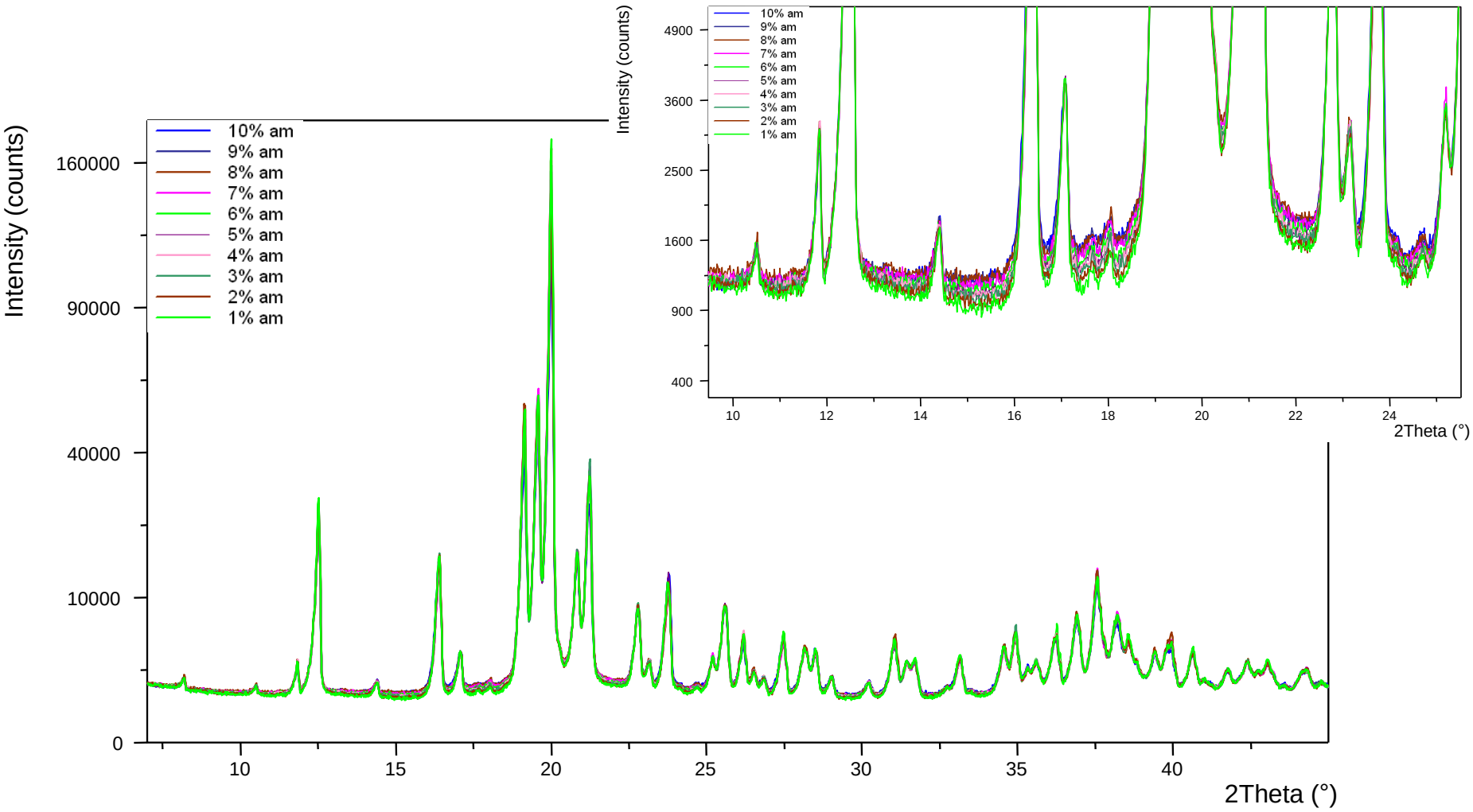
Preparation:

- Lyophilisation of saturated lactose solution

Preparation of binary mixtures: 0-10% amorphous content

Copyright : © 2015 PANalytical B.V., all rights reserved

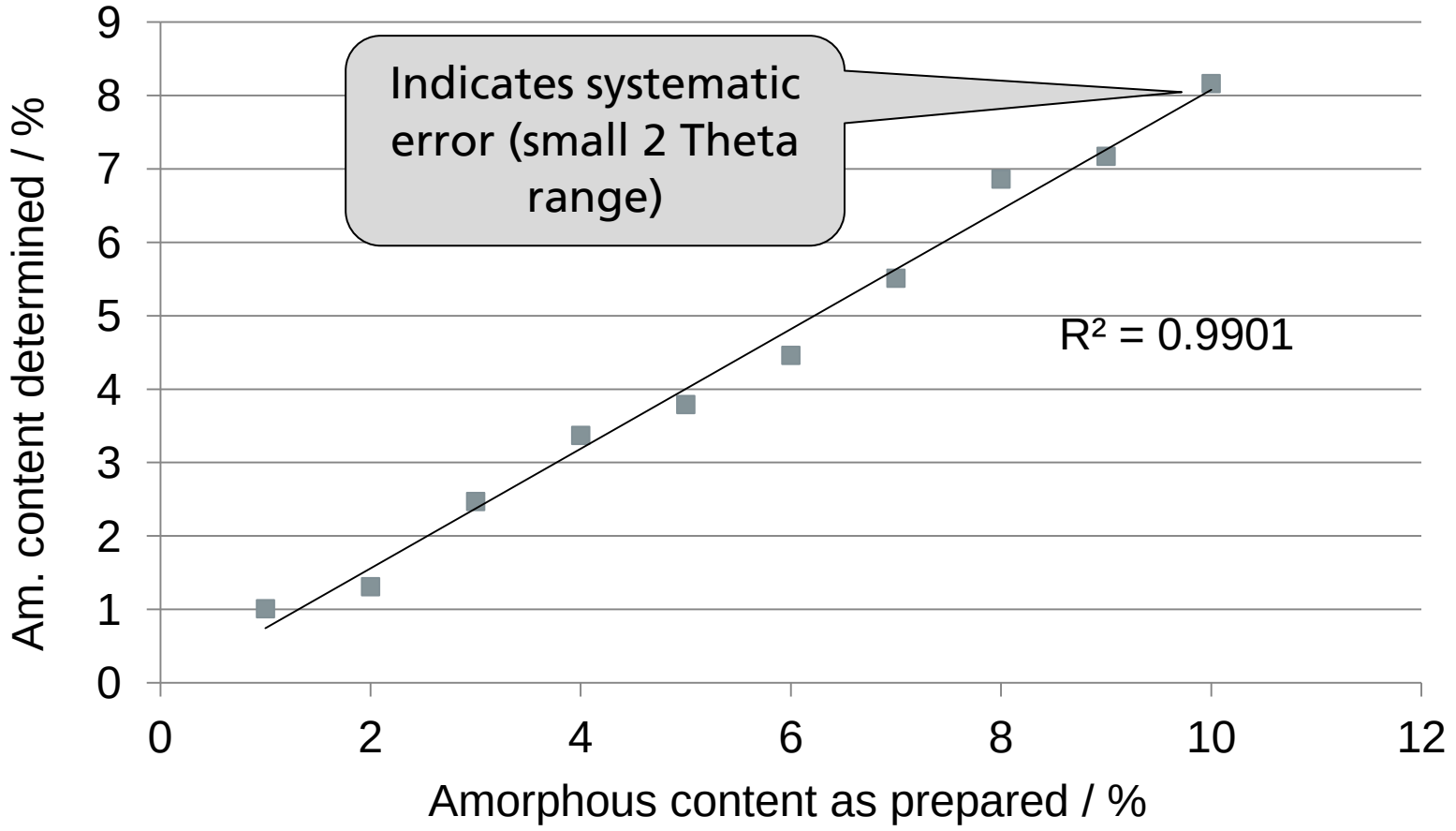
Example 1: Crystallinity - low amorphous content



Copyright : © 2015 PANalytical B.V., all rights reserved

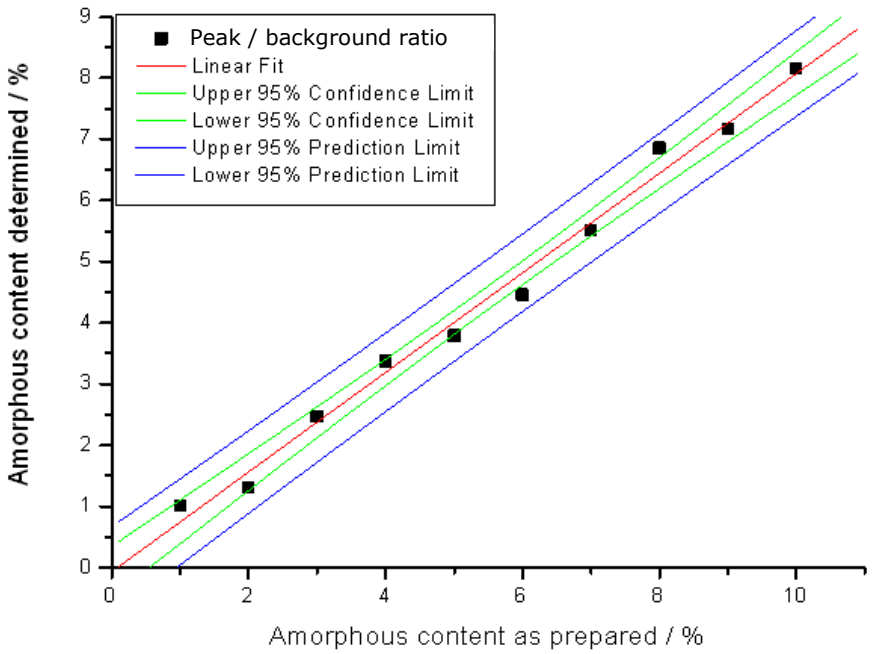
Example 1: Crystallinity - low amorphous content

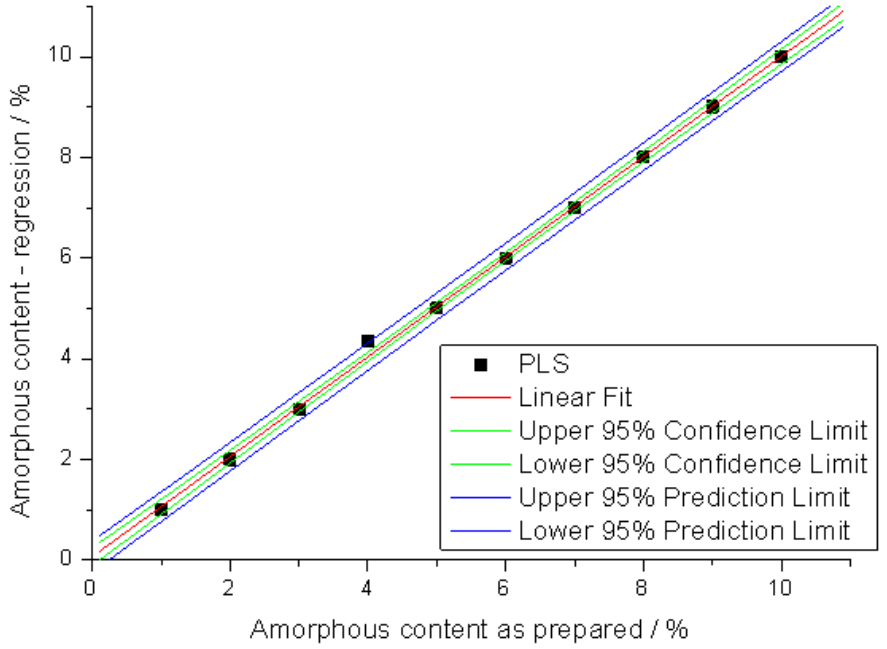
Traditional evaluation: integral peak / background intensity (range: 2θ = 11° - 34°)



Copyright : © 2015 PANalytical B.V., all rights reserved

Example 1 – Comparison of methods

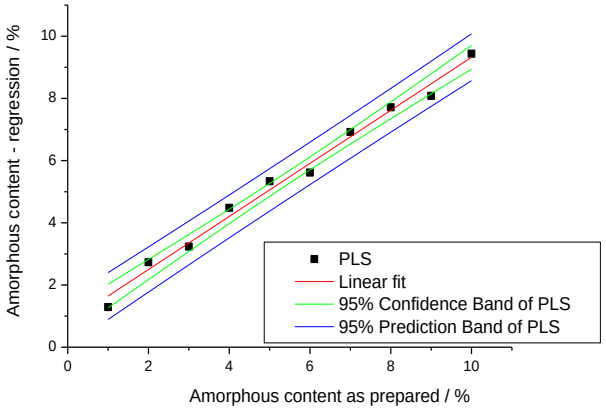




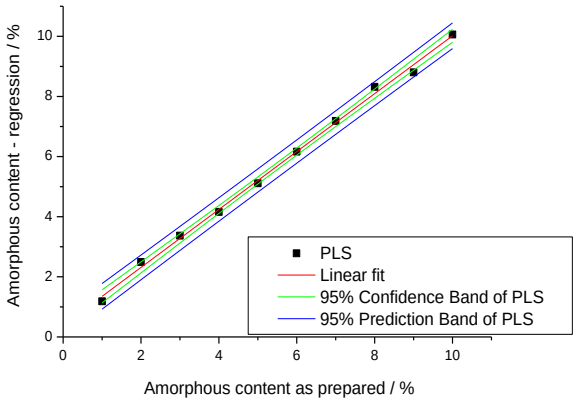
	Peak / background ratio	PLS – as modeled (3 factors, standardize)
R^2	0.9901	0.9999
RMSE(SD)	0.262	0.035
Error of intercept <ΔA>	0.179	0.024

Copyright : © 2015 PANalytical B.V., all rights reserved

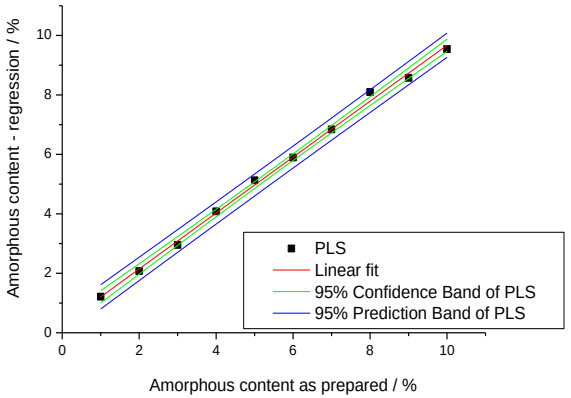
Example 1 – PLS



Cross validated



Data set 1



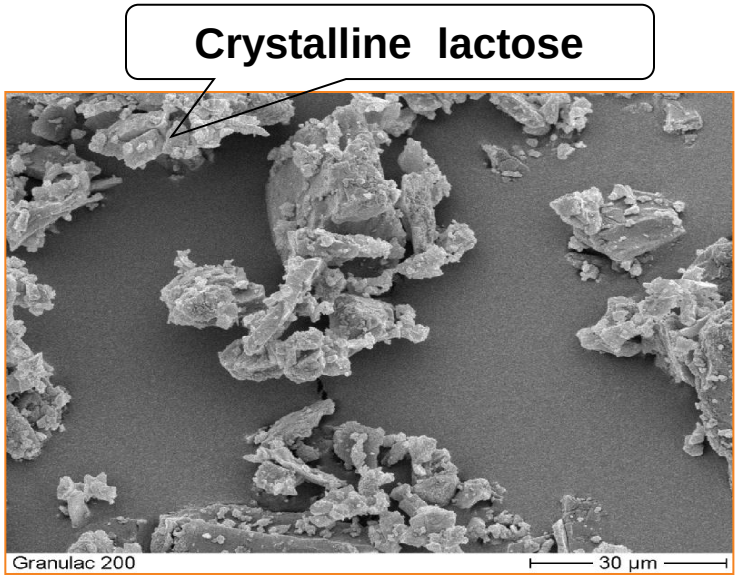
Data set 2

	PLS (as modeled – 3 factors, standardize)	PLS (cross validated 30% data removed)	PLS (on data set 1 – other step size)	PLS (on data set 2 shorter meas. time - scaled)
R^2	0.9999	0.9882	0.9970	0.9971
RMSE(SD)	0.035	0.282	0.160	0.152
Error of intercept <ΔA>	0.024	0.192	0.109	0.104

Copyright : © 2015 PANalytical B.V., all rights reserved

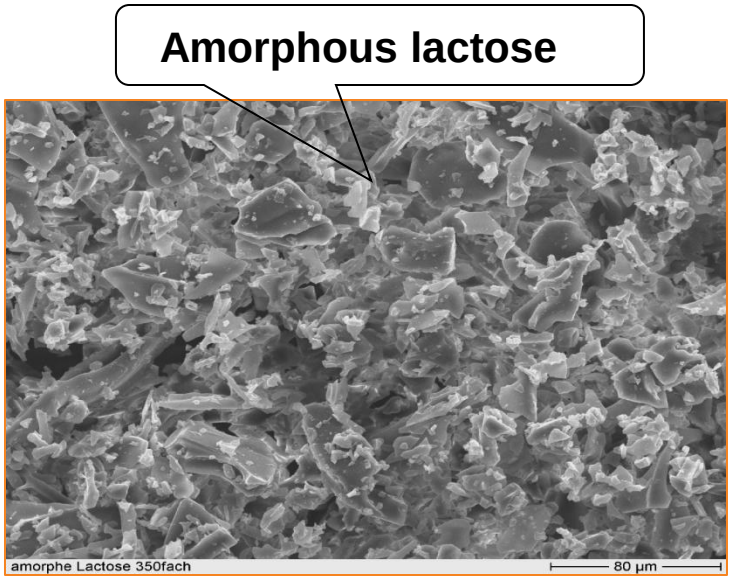
Example 2: Crystallinity – low crystalline content

Model substance: Alpha lactose monohydrate



Preparation:

- Storage at RH of 56% and 30°C to ensure complete recrystallisation



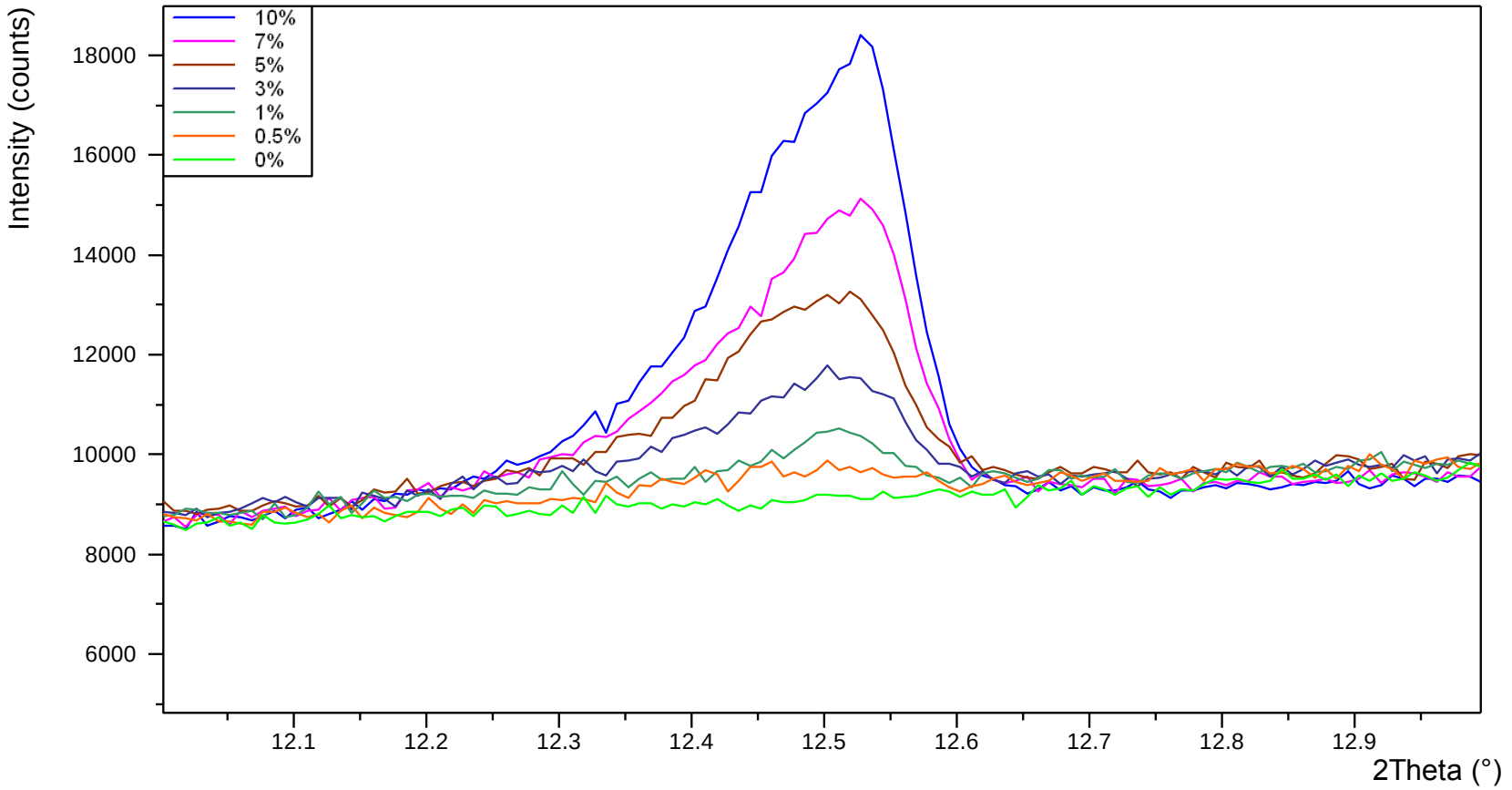
Preparation:

- Lyophilisation of saturated lactose solution

Preparation of binary mixtures: 0-10% crystallinity

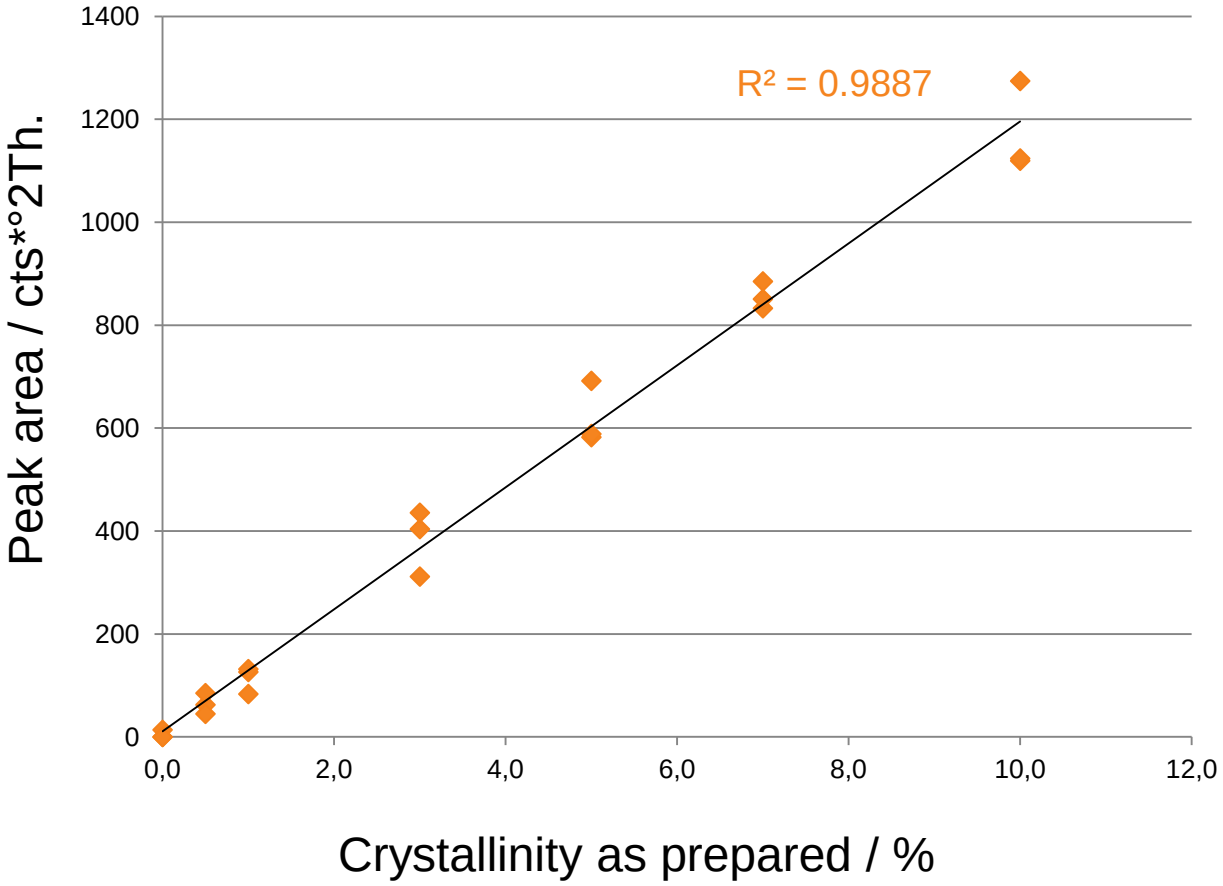
Copyright : © 2015 PANalytical B.V., all rights reserved

Example 2 – Crystallinity - low crystalline content



Copyright : © 2015 PANalytical B.V., all rights reserved

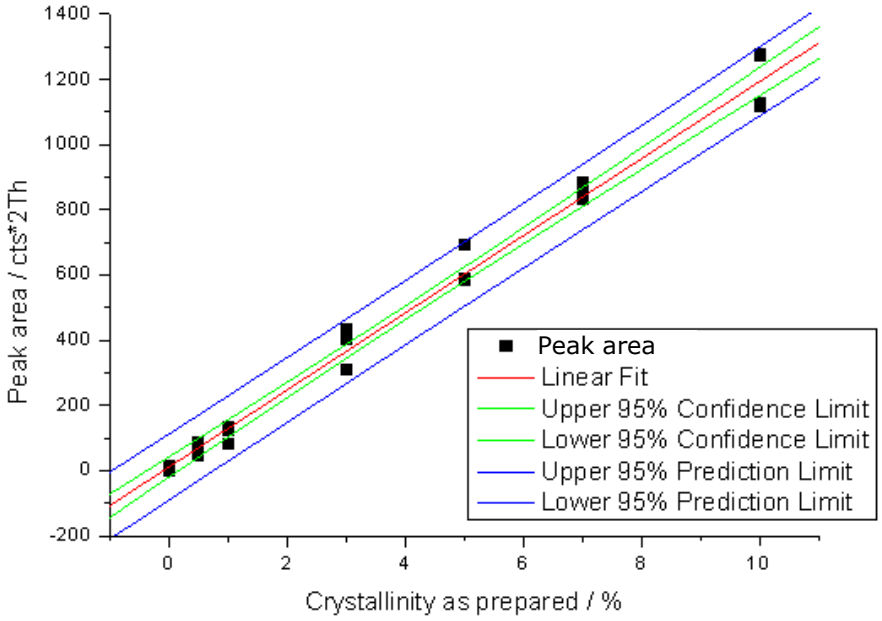
Example 2: Crystallinity - low crystalline content

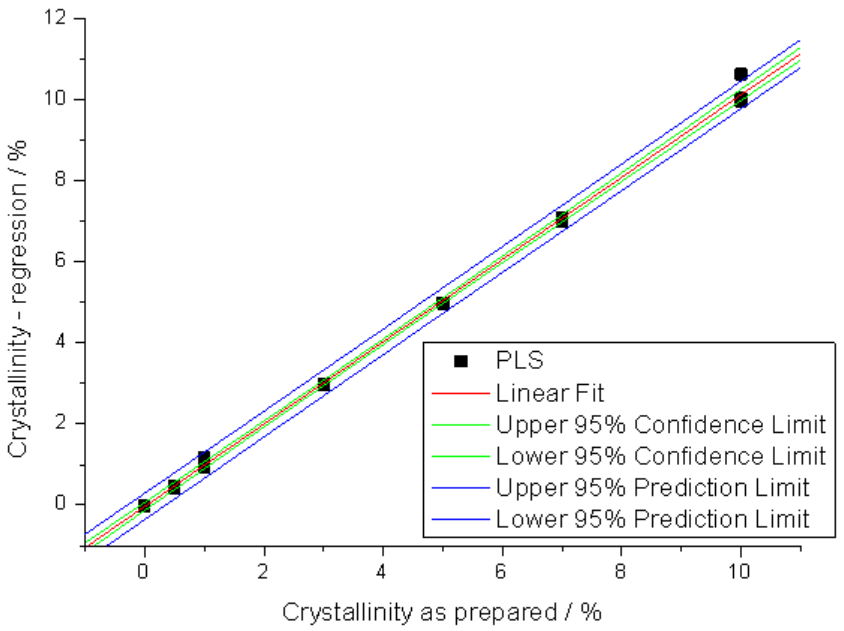


- Evaluation of net peak area (background fit, Pseudo-Voigt profile (FJC asymmetry))

Copyright : © 2015 PANalytical B.V., all rights reserved

Example 2 – Comparison of methods

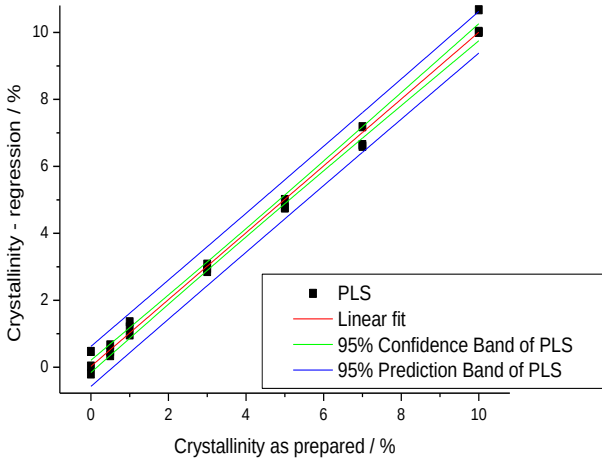




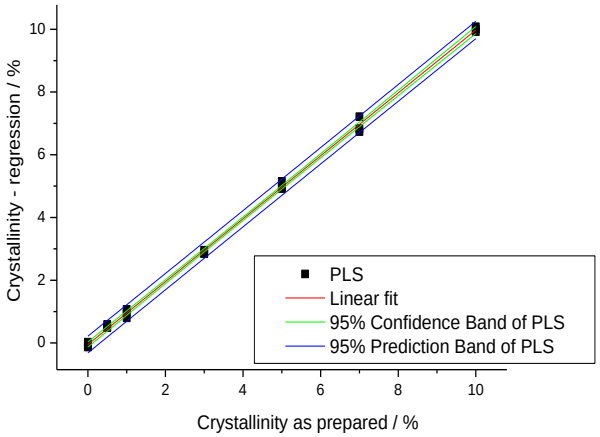
	Net Peak Area	PLS - as modeled (3 factors, center)
R^2	0.9887	0.9992
RMSE(SD)	0.389 (46.07)	0.101
Error of intercept <ΔA>	0.126 (14.89)	0.033

Copyright : © 2015 PANalytical B.V., all rights reserved

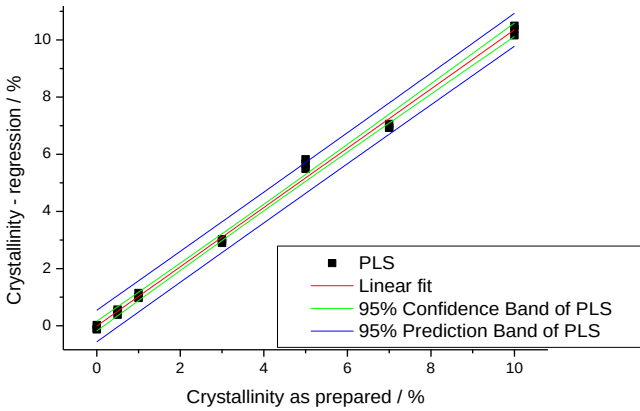
Example 2 – PLS



Cross validated



Data set 1



Data set 2

	PLS (as modeled – 3 factors, center)	PLS (cross validated 30% data removed)	PLS (on data set 1 – half meas. time, scaled)	PLS (on data set 2 same cond.- other samples)
R^2	0.9992	0.9942	0.9989	0.9954
RMSE(SD)	0.101	0.271	0.121	0.251
Error of intercept <ΔA>	0.033	0.088	0.039	0.081

Copyright : © 2015 PANalytical B.V., all rights reserved

Summary – PLS regression

PANalytical
get insight

- Requires (large) set of samples for calibration
- Calibration samples should cover all relevant sample variations (particle/crystallite sizes, operator dependencies)
- Possibility of over-fitting (limit number of factors, counting statistics)
- Factors not necessarily related to physical properties – validation (ICH) to check model applicability with all process parameter variations

+ Pure phases or crystal structures not required
+ Takes full pattern variation into account
+ Less sensitive to non-ideal sample preparation / measurement set-up
+ Can be more robust than traditional XRD methods
+ **User independent analysis**

Copyright : © 2015 PANalytical B.V., all rights reserved