



**Suggestions for DSC, GVS, and
XRD methods for quantitating
residual amorphous content in
crystalline drug substance.
Workshop**

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Purpose

- To provide GSK perspective on when and how the residual amorphous component of crystalline drug substance should be monitored and controlled. This will be covered within the Amorphous, Activated, and Nanomaterials Session.
- For this workshop, this presentation will address recommendations for DSC, GVS, and XRD methods development to quantitate residual amorphous content in crystalline drug substance,

Internal (GSK) guidance document provides

- Short primer – what is amorphous and do you really have amorphous materials in your sample
- Amorphous Strategy – Decision Tree
- Choice of Technique
- Links to Technique-Specific Details (to be covered in the workshop)
- Quantitation Strategy and Role of Standards
- Method Validation
- Consideration of Risk

DSC, GVS, XRD – primary methods considered suitable for routine QC test.

- DSC considered method by first intent.
- All methods should consider reporting results based solely on instrument response rather than using a calibration curve.
- In other words, do not report % w/w amorphous. This will be discussed further at the Amorphous session within the symposium.
- The use of amorphous standards best used to help develop the method and determine approximate LOD, LOQ rather than enable a % wt/wt determination.

DSC (Differential Scanning Calorimetry)

Basic method – reporting out the enthalpy of crystallisation

This method solely records the instrument response to a sample and allows for sample to sample comparison. It is a suitable amorphous method to implement for characterisation of micronised of an API in early development as it does not require the use of an amorphous standard.

Methodology:

Ball mill a sample “hard” to obtain a material that is predominantly amorphous (e.g. 5 mins at 70 amp on a Retch mill). Assess the amorphous nature using XRPD.

DSC continued

Run a sample on using a DSC method with a temperature range appropriate for the material using the following parameters...

10°C/min

Large crimped pans

The largest quantity of material appropriate for the pan type.

If crystallisation is present, determine the integration limits and record the enthalpy of the event. The integration limits will cover the whole area of the event but not include any separate events (the derivative may be useful in determining the limits).

If no crystallisation event is observed, ball mill again with a higher energy.

DSC Continued

Next, induce a lower amount of amorphous disorder in another sample using a lower energy (e.g. ball mill for a shorter time or use pestle & mortar). This technique may be more representative of the level of amorphous observed in typical micronised material. Confirm the amorphous nature using XRPD.

Run the sample using the DSC method described above. Again integrate any crystallisation event – if no crystallisation event is observed, try again with a higher energy. This second sample acts as a crude linearity check in that the enthalpy of crystallisation should be less than the ball milled sample (and roughly in proportion to the observed level of disorder seen by XRPD).

DSC continued

The reported result will be the enthalpy of the crystallisation event. It is not necessary to have an amorphous standard at this stage, so a % amorphous will not be reported.

Note: When (and if) an appropriate amorphous standard is manufactured, it is possible (if the method remains the same) to retrospectively calculate the % amorphous for a sample for which the enthalpy of crystallisation has been reported.

DSC continued

Enhancements to the method:

Options for optimising the method include...

Modulated DSC – particularly if there are overlapping events. Some sensitivity is lost due to the slow scanning rates.

High speed DSC – Running at higher heating rates may improve sensitivity.

DSC continued

Limit of Detection (in the absence of amorphous standard)

A limit of detection can then be determined using confirmed crystalline material.

Run defined method in 6 replicates and calculate the standard deviation of the response.

LOD = Based on 3 x SD of the measured values of the blanks

Calibrating against an amorphous standard

An amorphous standard is required for this method. To produce a standard optimise the amount of ball milling on the sample. Ball mill the sample “hard” as described above and run the sample using the “qualitative method”. Repeat using even more aggressive ball milling conditions. If the enthalpy of the instrument response does not get any bigger, it can be concluded that the sample is as close as possible to 100% amorphous. If the instrument response does not plateau, increase the ball milling once again and repeat until a plateau is reached. Care must be taken not to start heating or degrading the sample.

Check amorphous content as accurately as possible using an appropriate quantitative orthogonal technique (e.g. ssNMR or XRPD).

DSC continued

Results from SSNMR or XRPD may be used to determine % w/w amorphous of ball milled material.

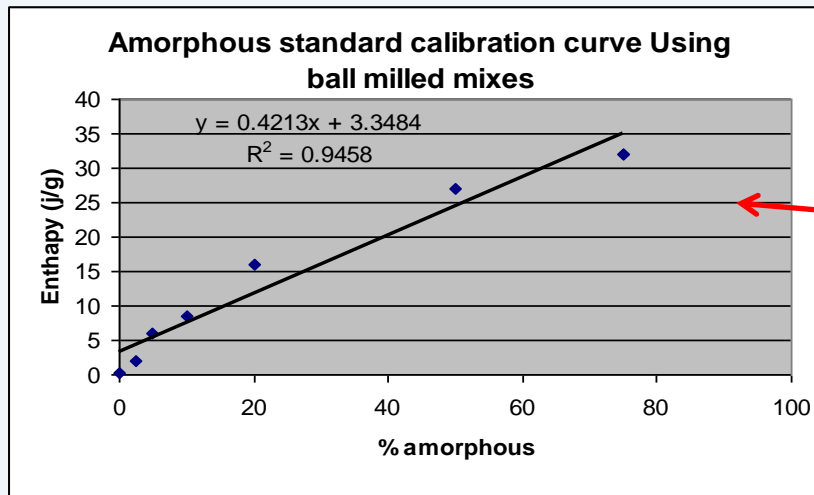
An amorphous quantification calibration graph may then be built by plotting physical mixes of confirmed crystalline material and quantified ball milled material. This would be done by weighing quantities of the material two materials directly into the DSC pan.

These mixes can be calibrated by weight against the 'actual' amorphous content of the ball milled standard. Some examples are given below which show what mixes can be achieved depending on the strength of the amorphous content. **It may be desirable to use a standard that has a moderate amorphous content, because it would make the preparation of low level amorphous mixes easier.**

DSC continued

	% Amorphous : Crystalline content		
Actual amorphous content of "100% standard"	50 : 50 mix	25 : 75 mix	10 : 90 mix
50%	25 : 75	12.5 : 87.5	5 : 95
20%	10 : 80	5 : 95	2 : 98
10%	5 : 95	2.5 : 97.5	1 : 99

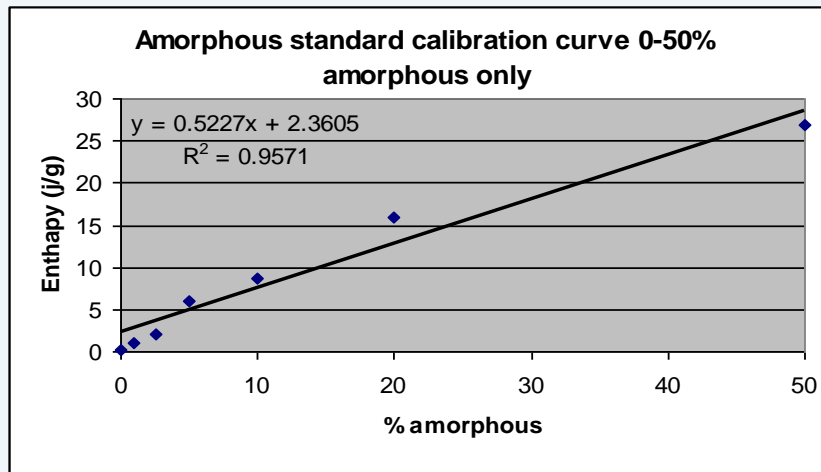
These 'mixes' are weighed directly in to the DSC pan, not physically mixed prior to weighing.



Range wider than likely range for amorphous content.

DSC continued

The relevant area of the calibration graph can then be used to generate a formula for quantifying amorphous material. For example, if it is unlikely to ever see more than 20% amorphous, it may be more appropriate to use the 0-50% or 0-10% region of the graph, rather than the whole region 0-100%.



The formula for the quantification of amorphous content may then be calculated from the trend line data. The LOD can either be calculated from 3x the SD of blank measurements or from 3x the SD of the trend line (whichever is greater). The LOQ will be calculated from 10x the SD of the trend line.

GVS (Gravimetric Vapor Sorption)

The use of Gravimetric Vapour Sorption instruments for the determination of amorphous phase is recommended for situations where the crystalline phase is desired; i.e. where a low level of amorphous is suspected to be present within a predominately crystalline phase. Where the intent is to generate an amorphous phase for example by spray drying, it is recommended that the lack of crystallinity is investigated by alternative techniques such as X-ray Powder Diffraction (XRPD) as these can offer a more straightforward solution compared with GVS.

GVS continued

See DSC comments for preparation of amorphous material.

Typically, GVS analysis is carried out in relative humidities of 0 – 90% at 25°C.

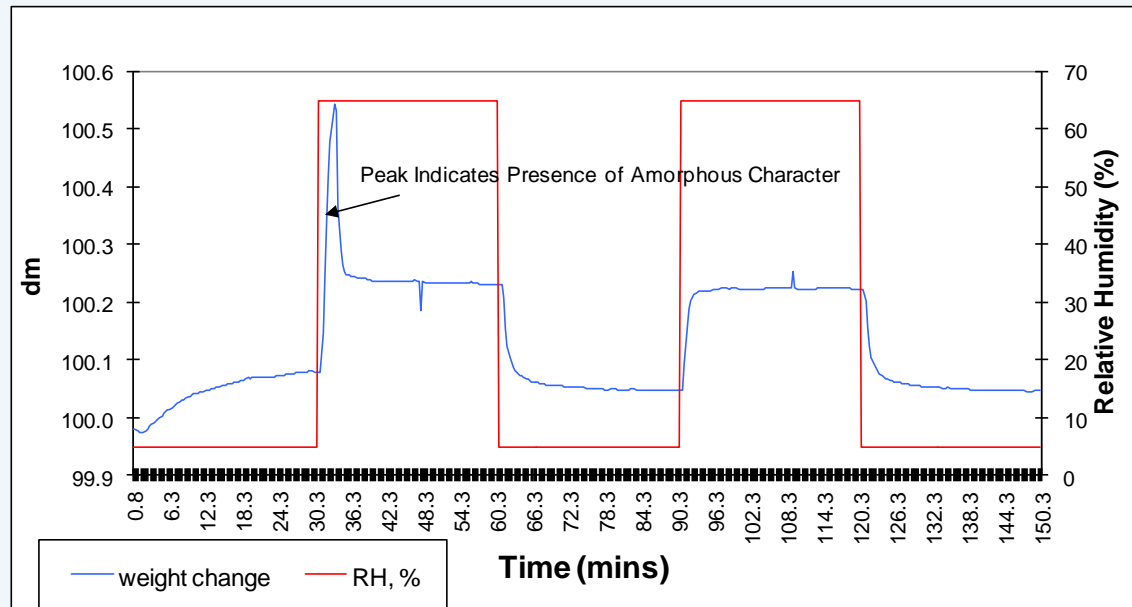
While water is not always suitable for ensuring amorphous re-crystallisation, and the use of organic solvents may be required, **it is nevertheless recommended that water be used in the first instance.**

The method relies on water lowering the glass transition to the point where the molecules can reorder into a more stable crystalline phase.

When evaluating or selecting an organic solvent, typically, solvents in which the material is highly soluble are a good starting point. The safety implications of using organic solvents must be fully evaluated and documented prior to commencing any work with organic solvents.

GVS continued

GVS response for a micronized drug substance.



GVS continued

There are a number of instrument parameters that should be evaluated in order to maximise the re-crystallisation event and provide a robust method. Consideration should be given to the following;

Experimental profile. It is recommended a pulse method be used by first intent for the characterisation of the amorphous phase. Alternatively, a ramp method, could be investigated if a pulse method proves non robust. A ramp method involves changing between low and high %RH at a constant rate, i.e. 0-90%RH over 90 minutes. As with the pulse method, a second ramp is performed following the first one and the profiles of the 2 cycles compared in order to determine the amorphous content.

Sample weight. There should be sufficient sample present to be representative of the bulk and to maximise the re-crystallisation event. However, too much material may result in caking across the surface of sample which could hinder complete crystallisation. During method optimisation and validation, once other instrument parameters have been fixed, a range of samples weights should be investigated in order to assess the impact on the re-crystallisation event. It is recommended a sample size between 20 - 40mg be used as a starting point.

Pan type. Mesh pans will increase the surface area of the sample which is exposed to the solvent and increase the re-crystallisation kinetics. It is recommended mesh pans be used as first intent. It is also recommended the sample be dispersed across the pan rather than heaped in the centre in order to further increase the surface area of the sample exposed to the solvent.

GVS continued

RH range. Typically, for pulse methods, 30 and 70% RH are used as the low and high RH values. It is recommended these values be used as first intent but this may require further investigation depending on the behaviour of the compound under investigation.

Gas flow rate. Typically this would be set to maximum in order to maximise the kinetic event.

Temperature. It is recommended that 25°C be used for all experimental work, however, changing the temperature will have an effect on the re-crystallisation kinetics and when the %RH at which the re-crystallisation event occurs. A higher temperature will speed up the event, a lower one will suppress it.

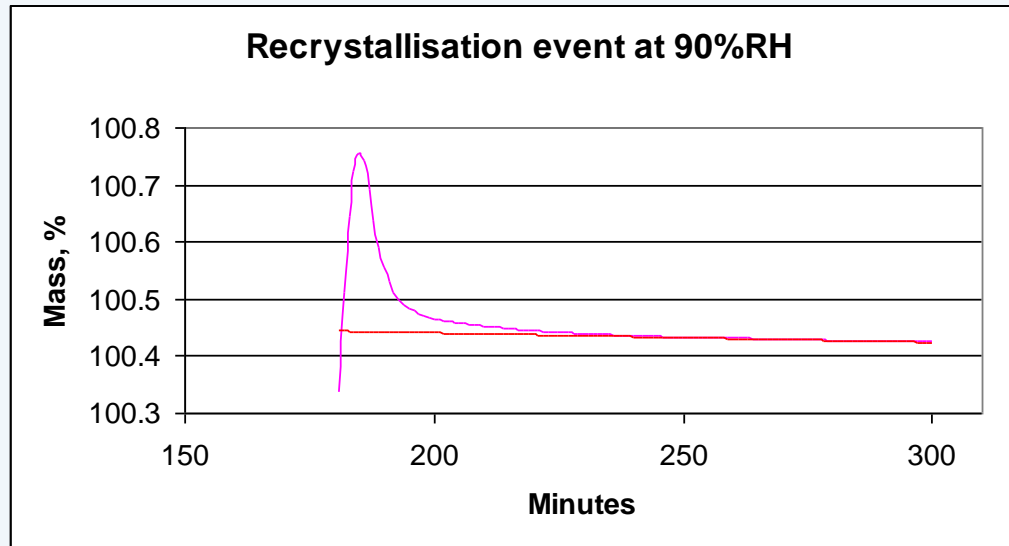
GVS continued

There are several methods of interpreting the data in order to quantitatively characterise the re-crystallisation event. Typically, data from the first cycle and data from the second cycle are compared, the first cycle includes a response due to amorphous material, the second cycle does not. By comparing the cycles, the amount of amorphous material present can be determined. There are several ways of interpreting the data in order to achieve this.

Suggest determination of peak area rather than peak height of the amorphous re-crystallisation event unless crystallization kinetics are suitably precise.

The use of the % difference between maximum peak signal (weight) and minimum peak signal may also be used but will also be affected by crystallization kinetics.

Example of the Determination of Peak Area or Peak Height Using Microsoft Excel

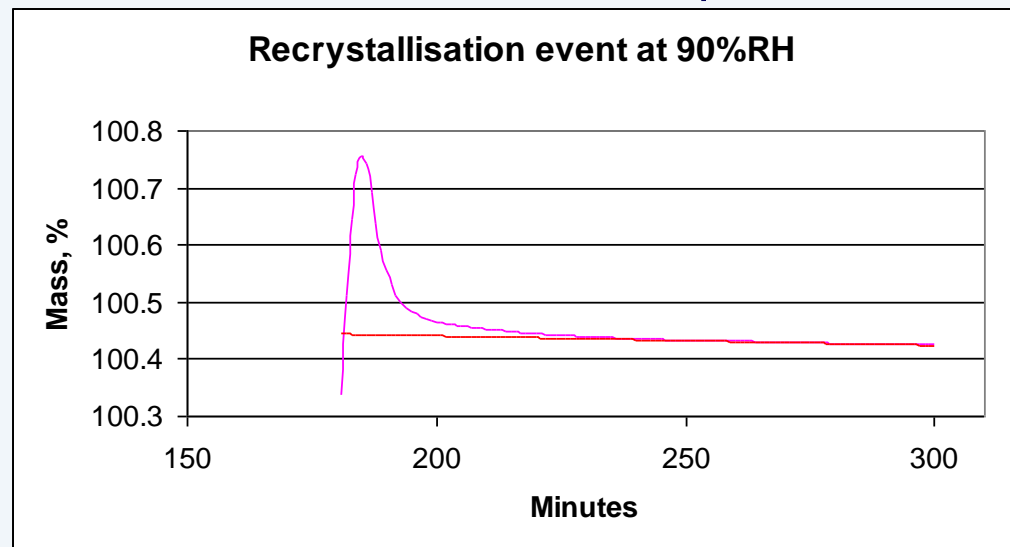


A tangent is fitted to the tail of the peak by means of linear regression. The peak area is calculated by summing the relative mass difference between the peak and the tangent with respect to time in minutes.

GVS continued

To establish a true LoD / LoQ, a standard of known amorphous content would be required as measured by orthogonal techniques, typically ssNMR. This sample can then be used to create a range of standards of differing amorphous concentration via dilution with crystalline material. These mixtures can then be plotted and analysed statistically to determine the LoD / LoQ.

Amorphous content may be reported solely on instrument response without a calibration curve – such as area of peak in mass %.



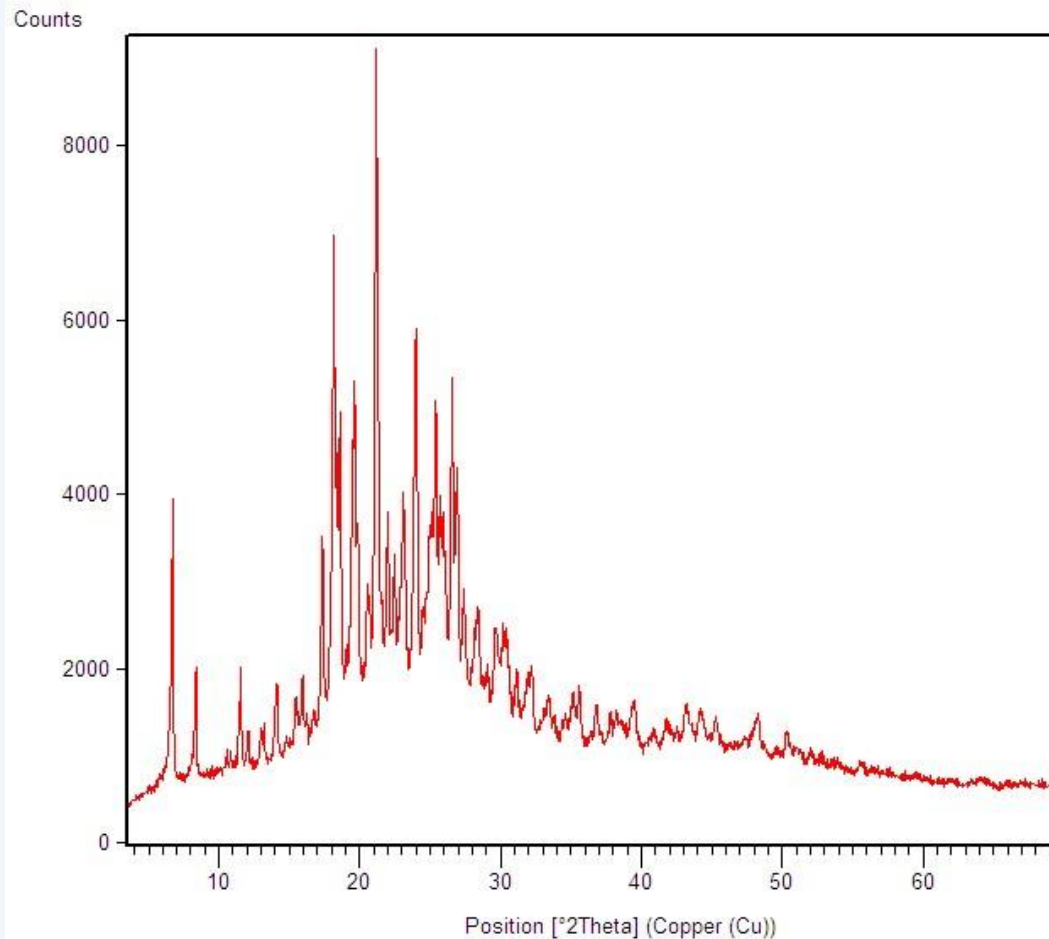
Powder X-ray Diffraction (PPXRD)

PXRD is recommended as a tool for characterizing amorphous standards to be used by other techniques with lower LOD/LOQ – such as DSC or GVS.

PXRD can also be used to determine amorphous content for samples with relatively large amounts of amorphous (e.g 50%) that may be observed in early process development.

In general, quantitation of amorphous content below 20% is difficult and will require careful inspection of the XRPD scan to ensure consistency of integration before and after the removal of the amorphous component. Detection of amorphous is limited to 10% in usual cases and quantitation below 20% is usually not reproducible.

PXRD continued

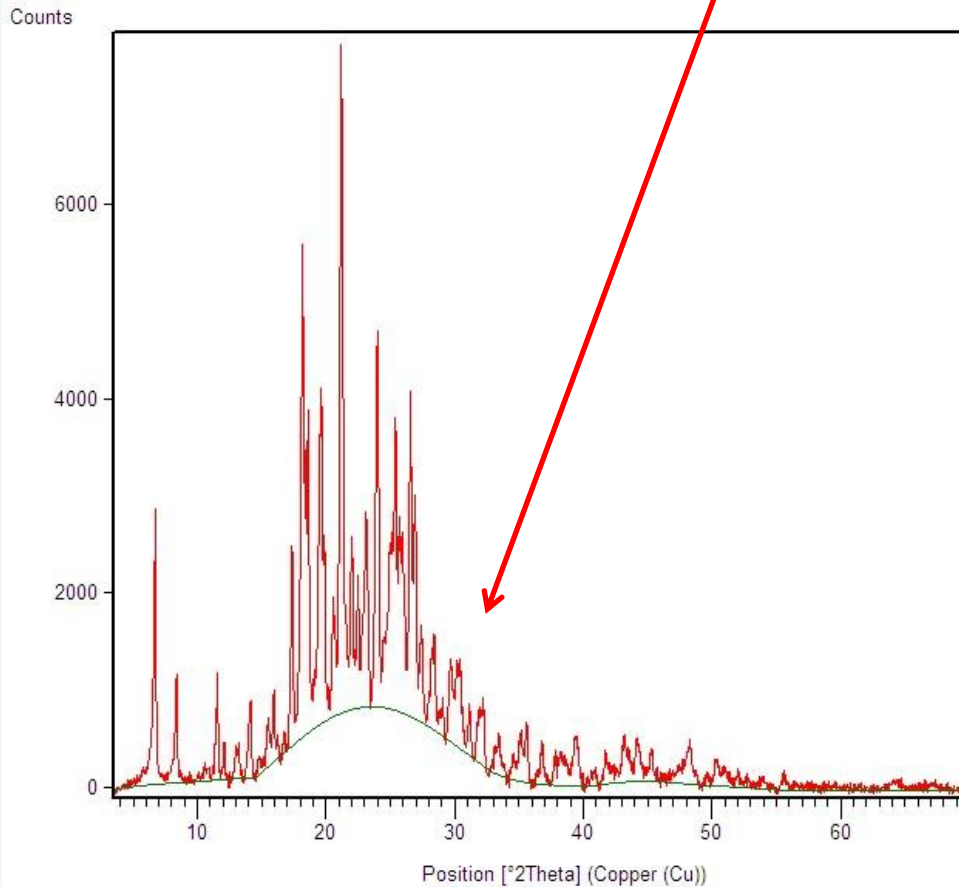


Determine baseline for total diffraction signal without including instrumental background.

For cavity mount preparations, the use of a very crystalline reference material (e.g. NaCl) may help in establishing the true baseline.

PXRD continued

Caution: This approach only suitable when micro/nanocrystalline material not present.



After removal of instrumental background, integrated total signal, including amorphous “halo” followed by integrating crystalline-only component as shown to the left.

% amorphous = $100 - \left[\frac{\text{area due to crystalline component}}{\text{total diffraction area}} \times 100 \right]$

Solid state nuclear magnetic resonance (SSNMR) is a “nuclei-counting” technique that can provide % w/w amorphous (exclusive of residual solvents) without the use of amorphous and crystalline standards.

Comparison of % amorphous results by PXRD vs results from solid state NMR can help guide integration limits other appropriate integration parameters, sample preparation, etc.

Typically % amorphous by SSNMR and % amorphous by PXRD using the % area/area approach will be similar for samples with % amorphous between 20 to 80%.

If SSNMR amorphous and crystalline responses overlap, uncertainty in the deconvolution of the responses would be factor as it would with any other spectroscopic technique.



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