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COMPARISON OF QUANTITATIVE METHODS FOR ANALYSIS OF POLYPHASIC PHARMACEUTICALS

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Adequate very sensitive quantification methods are needed for the development and are also now required for the monitoring of undesirable solid form(s) as routine tests. The pre-requisite for quantitation are selectivity, sensitivity and most important the purity of standards and their proper storage, what is a challenge for metastable forms.

Several analytical techniques are available such as X-ray diffraction, spectroscopy, thermal analysis and microcalorimetry. The different steps of the validation of the analytical methods and problems to be solved are discussed. Examples illustrate the different techniques and compare their possible advantages and limits. The relative standard deviation of measurements should allow for checking the homogenization procedure of mixtures for calibration. The validation should be carried out following ICH guidelines for validation of analytical methods. Comparison of different techniques in adequate concentration range add confidence in the analytical results.

Keywords: *amorphous content of drug substances, crystalline, drug products, FTIR, FT-Raman, microcalorimetry, phase changes, polymorphism, pseudopolymorphism, quantitative analysis, solution calorimetry, thermal analysis, X-ray diffraction*

Introduction

The chemical industrial development in pharmaceutical industry is faced with the acceleration of the development time of new medicines and with harmonization guidelines which are required by health authorities for worldwide registration. The existence of several crystalline and amorphous phases for a drug substance – called in this article 'polyphasic drug substance' – has been identified as key source of deficiency [1, 2]. In development of new entities the choice of the solid phase to be developed as drug substance and as drug product is done very early in order to avoid delays due to change of formulation. Bioequivalence bridging studies and upscale has to be taken into consideration since synthetic processes will be optimized from the first mg material to the production amount in tons range [3–9]. The reproducible production of organic crystals in the correct form (habit, solvate, polymorph) is a subject which causes much heart-ache to chemists, engineers, pharmacists and formulators. A great number of recent books, review articles deal with the thermodynamic, kinetic and structural aspects of 'polyphasic' drug substances [10–20].

Figure 1 summarizes the main steps of development. Salt screening and polymorphism screening belong to the design of the drug product formulation. Why does the analytical laboratory should monitor polymorphism? First, in order to fulfill the decision tree of ICH [3], secondly to develop robust processes and to guarantee the quality of the marketed product.

This article is focused on the analytical part of this area by comparing quantitative methods most commonly used in the industry.

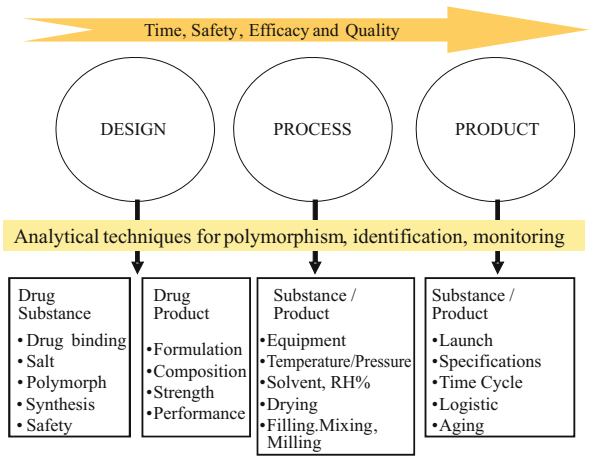

Fig. 1 Analytical quantitative methods and project development

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Experimental

Experiments described in this article were performed with the following instruments: Perkin Elmer DSC-7 with robot system, Mettler TA 851 with autosampler, X-ray diffractometer Scintag XDS 2000 or X1 with autosampler and Pelletier detector, X-ray diffractometer Bruker Advance D8 with autosampler and Vantec PDS detector, DVS from Dynamic Vapour System for the sorption-desorption curves. FTIR experiments were performed with Brucker FTIR Vertex 70, Raman spectra with Brucker RFS100/S with laser 1064 nm. Microcalorimetry and solution calorimetry were performed with the instruments Thermal Activity Monitor of Thermometric.

Standards of polymorphs have been analysed for purity, assay, solvents and are stored in controlled conditions.

Materials and analytical consideration

Following the polymorphism screening, the different phases have been identified and their thermodynamic relationships clarified [8, 9]. The objective of the analytical control depends on the product design, on the amount of manufactured samples of pure forms. In some cases a limit of quantitation can be sufficient when the drug substance and the drug product are manufactured with the thermodynamic stable form and when manufacture does not require the need of monitoring. Quantitative methods require generally standards of polymorphs what is often difficult for metastable solid phases. Samples have to be analyzed in order to have proper standards. The physical purity of the standard used is often the origin of different results when comparing different techniques or even the same technique with different standards. The difficulties are first the homogeneity of the sample if diluted in a matrix, the particle size or preferred orientation. For samples in transformation state, the problem inherent of in-homogenous sample is evident. It is a major drawback of the analysis of samples submitted to stress conditions. Therefore a great number of measurements should be necessary. For quantitative analysis, methods have to be validated according to ICH guideline Q2 [21] with linearity, accuracy, precision, intermediate precision, limit of quantitation, limit of detection. The instruments have to be qualified and calibrated according to cGMPs.

Analytical steps

Selection of analytical technique and instrumentation
Design of the method and the desired limit of detection

Preparation of the standard of polymorphs, analysis, storage and handling
Calibration

- Homogeneous known mixtures
- Number of concentrations and experiments
- Design calibration curve or mathematic model (two polymorphs or several forms, matrix effect)
- Define LOQ, LOD

Validation

- Precision, repeatability, intermediate precision
- Accuracy, recovery

Selection of the analytical technique

The selection of the analytical technique is driven by the selectivity and the limits of quantitation (LOQ) and detection (LOD) as well as by general considerations of the techniques. A system suitability for selectivity and LOD should be a part of the routine method. The limit of quantitation for the drug product can be deduced by multiplying the LOQ for the drug substance by the ratio drug substance to excipient. For example with a loading of 20% a LOQ of 1% for the drug substance would give a LOQ of the undesirable form in the desirable form of 5% if the excipients do not interfere. Review about analytical methods, see [12].

Standards, preparation, purity, storage and handling

The purity of the standards and their proper handling and storage are of most importance. They should be controlled as the usual crystalline form including chemical purity, residual solvents and assay. Several analytical methods such as X-ray diffraction, FTIR, FT-Raman, DSC, TG allow for comparing quality and specificity. If no pure standard is available, and especially if the standard is partially amorphous, the signal obtained is lower as it should be. The limit of detection measured is higher as the real situation. We observed a change of LOD from 20 to 10% by using a more crystalline standard.

The study of the behaviour under accelerated or stressed storage conditions (temperature, humidity) is necessary. We observed crystallization of amorphous samples or transformation of solvates in hydrated form or transformation of metastable polymorphs into the stable form when samples were not stored in very tight packaging in refrigerator or freezer. The control of standard within measurements for the establishment of calibration curve or within measurements is mandatory. System suitability test should be part of procedures, including LOQ, LOD.

Preparation of standard and mixtures

The observation of published examples show dispersion of results and the calibration lines do not suggest very robust and accurate methods of analysis. It is well accepted that samples should have very similar particle size. Grinding in a mortar or milling in small mills or sieving has been proposed for good homogenization of the mixtures. Niemczyk [74] proposed to add small portions stepwise, e.g. for a 1% mixture a dilution stepwise of 10% mixture with the major component. The procedure is very long. Okomura [75] proposed to mill compounds in an air jet mill to <10 μm and in addition to mix the compounds in vibration mill with rubber balls. From our experience milling drug substances in air jet mill <10 μm can give amorphization up to 10–20%. Therefore the behaviour of polymorphs under grinding should be known (XRPD or melting heat).

A check for homogenization can be done by the observation of the standard deviation of several measurements performed with the same mixture.

Methods

X-ray powder diffraction (XRPD) is extensively used for quantitative analysis of mixtures of crystalline forms and to a less extent the determination of the degree of crystallinity (100% minus amorphous content) [22–29]. Preferred orientation and crystallite statistics, particle size are minimized by preparation of analytes. Difference in mass absorption coefficient of the compound changing from anhydrous to hydrate should be taken into consideration in the calibration. The danger of amorphization while reducing the particle size is of concern and has to be verified along the development of the method. There are two primary methods for quantitation, either using individual peaks or the whole pattern. Partial least square (PLS) is a multivariate technique which requires as many sources of sample variations as possible. Several softwares use the Rietvield method. It was used successfully for carbamazepine [23]. In development the use of individual peaks are preferred since the matrix effects are not well known. The peaks with the best repeatability should be chosen [28]. Internal standard can be used or the ratio between peaks of two forms are calculated for the calibration line. For the determination of crystallinity the area under the amorphous background and the area of the individual peaks are calculated and the crystallinity as peaks ratio to the total area calculated. From our experience it is difficult to obtain accurate results for less than 5% amorphous content (95% crystallinity). However, for some compounds such as sucrose a LOQ of crystallinity was found 1.8% with a LOD of 0.9% [24].

The limit of detection is correlated with the detector type and the amount of sample submitted to the analysis. It depends on the method design (reflection or transmission, scanning rate, etc.). All modern instruments are highly automated and high-throughput precise analysis can be performed in very short time and limit of detection of crystalline forms down to 1% or even 0.1% are possible. This is a

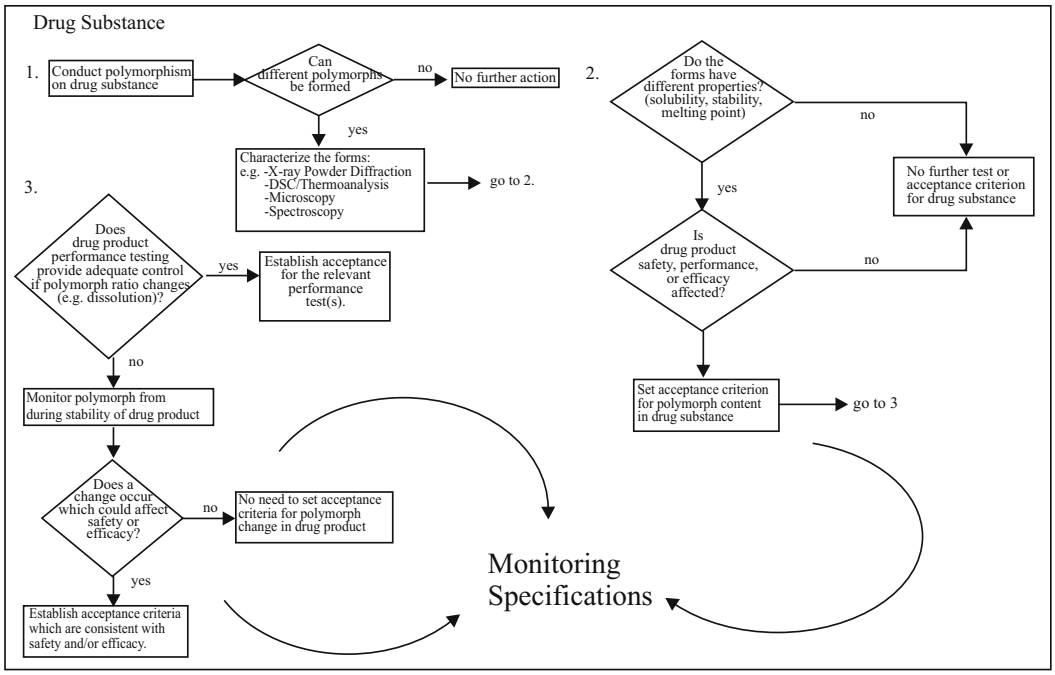

Fig. 2 Quantification of solid forms and ICH Q6 specification tree

considerable advantage for the analysis of drug products where the drug substance is diluted. Considering the transformation of metastable forms, an alerting problem of aging can be observed very early in the stability programs. Cooper *et al.* developed a quantitative XRPD method for a tablet and could monitor a polymorphic transformation of stability samples (See decision tree Fig. 2 for drug products). XRPD is described in USP [30]. It had become common practice to calculate powder diffraction patterns from single crystal structure to aid establishment of phase purity. This makes the X-ray diffraction technique as unique for this advantage. Figure 3 shows such a determination of phase purity [9].

In addition X-ray diffraction can be combined with DSC enabling to obtain X-ray diffraction of high or low temperature crystalline forms and with TG or sorption-desorption in different humidity [31, 32].

Crystal modeling allows to calculate single crystal structure from X-ray diffraction, to simulate mixture of polymorphs and to predict LOQ.

Infrared techniques have been extensively used [33]

Mid-FTIR (4000 to 400 cm^{-1}) is the method of choice for chemical identification of substances and is prescribed in monographs in pharmacopeias, generally in transmission. Samples should be diluted pressed in tablets KBr, NaCl, KCl with generally danger of phase transformations. We frequently observed transformation during tablet pressing. For polymorphic analysis, suspensions in Nujol mulls or dilutions in powdered KBr in diffuse reflectance (Reflection mode DRIFT) are preferred. Attenuated total reflectance (ATR) is now very performant for polymorphic analysis in reflectance mode. The effect of the pressure has to be controlled.

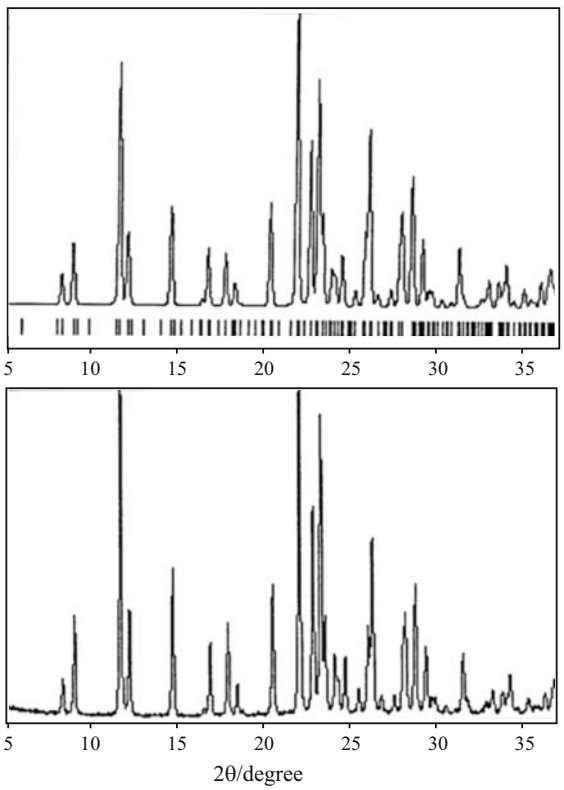

Fig. 3 Peak purity. Comparison of the top – calculated X-ray diffraction with the bottom – experimental diffractogram of a monohydrate [9]

Generally the peak ratio of the polymorph to a common peak of two forms are used for calibration [34–36]. First or second derivative of absorbance are often used. FTIR is described in pharmacopeia and calibration requirements described [37]. The instrument resolution and the number of scans measured are relevant for accurate results. Recent publications detailed validation results for Aprepiant [35] and Ranitidine [36].

Near-IR technique has been introduced in pharmacopeias [39, 40]. The method is used for quantification of drug substances in drug products. It has been successfully used for sulfathiazole and indomethacin with LOD in the range of 1% [42]. It requires a great number of calibration standards when multivariate chemometrics are used. Details for validation and required steps for quantification are described in USP29 [41].

Raman is increasingly being used and described in pharmacopeias with indication for calibration [43, 44]. Differences in spectra arises because of differing intermolecular interactions or molecular conformations. LOQ was found 1% in case of mannitol [45]. The use of Raman for quantitative analysis is described in USP 29 [43] with the steps to be done for quantitative analysis. Heating effects and fluorescence are strong limiting factor, (even with laser wavelength of 1064 nm) and the optimisation is a compromise between laser power, limit of detection, particle size and matrix effect. We found that particle size is also very important. This was also observed by [46].

A big advantage *vs.* IR is that the sample is not diluted and can be measured through glass.

Terahertz radiation lies between the IR and microwave regions of the electromagnetic spectrum and have a frequency between 0.1 and 2 THz corresponding to 3.3 to 100 cm^{-1}.

In the past, conventional far-infrared Fourier transform spectroscopy was used. Newly the radiation are obtained using ultra-short laser pulses in the femtosecond time scale. The method is called terahertz pulsed spectroscopy (TPS). First instrument of Terahertz Spectroscopy have been developed by

TeraView and recent results for polymorphism quantitation published [48].

Solid state NMR spectroscopy is an established technique [49–52]. It has been used for Neotame and formeterol [50, 51]. However, the method is at present not ready to be used as a fast routine analysis.

Thermal analysis, differential scanning calorimetry

The method is described in USP and Pharm.Eur. [53]. As the method detects also every small energetic changes, very sensitive quantitative analytical determinations can be expected. However due to kinetic effects [17] it should be used with great care. The endothermic transition of desolvatation or dehydration, or the enantiotrop transition can be used for crystalline forms [17]. The amorphous form can be determined by the exothermic recrystallization peak which follows the glass transition or – if no crystallisation occurs – the melting peak can be used for the measurement of crystallinity. Both methods have been used for the examples given below. Modulated DSC (MDSC) have been used with dynamic moisture absorption for detecting amorphous content up to 5–6% in a batch which showed marked stability differences [54]. The new high speed DSC [55] has been proposed for accurate determination of amorphous content in sucrose by the measurement of the glass transition heat capacity change.

Thermally Stimulated Current spectrometry appears to be capable of detecting amorphous phase as low as 1% [55].

Solution calorimetry and microcalorimetry

Solution calorimetry is described in USP for the determination of the amorphous form [56]. The heat of solution of amorphous and crystalline standards are measured. The values obtained allow for determining the crystallinity of analytes. This method supposes that no other crystalline form may interfere. The method can also be used for polymorphs [17, 57].

The decreases of the T_g is the principle of the microcalorimetric method of the determination of the amorphous part. The substance is subjected to vapors of water or organic solvent in an isothermal microcalorimeter. The heat flow of crystallization is measured and is proportional to the amorphous content. Depending of substances, LOD of amorphous content down to 0.5–1% have been obtained [58–64].

Most methods have been used for lactose. Table 1 summarizes techniques used with lactose monohydrate, lactose anhydrous and lactose amorphous as model substance and compares the limit of detection obtained [65–71]. Lehto *et al.* published very recently [69] results obtained with 7 different techniques to quantify the amorphous content of spray lactose: XRPD, DSC, StepScan DSC, isothermal microcalorimetry, solution calorimetry, Raman and gravimetric moisture sorption and suggested to employ a combination of methods.

Results and discussion

Example 1

This case deals with the determination of two forms A and C in a form B. IR and Raman were not selective enough. Since single peaks of diffractions for each form A and C were separated from B with LOD in the range 0.5–1%, a method XRPD was developed. The ratio [A/A°]/[A/A°+B/B°] and [C/C°]/[C/C°+B/B°] with A, B, C peak areas measured in mixtures and A°, B°, C° the peak areas measured of pure forms A, B and C. The calibration line would there permit a quantification method independent of standards. Therefore the linear regression should be of very good quality.

Figure 4 shows the XPRD of mixtures 0.5 to 5% form A in form B, the corresponding linearity and the

Table 1 Methods proposed for lactose

Method	LOD amorphous	LOD crystalline	Comment	Reference
Isothermal microcalorimetry	1%			[66]
Solution calorimetry	2%		Comparison with microcalorimetry	[67]
X-ray diffraction	1%	0.5%	DSC found not reliable	[71]
NIR	0.2%	0.5%		[71]
DMA	2%			[73]
DVS	0.7%			[68]
NMR	0.5%		Comparison with microcalorimetry	[70]

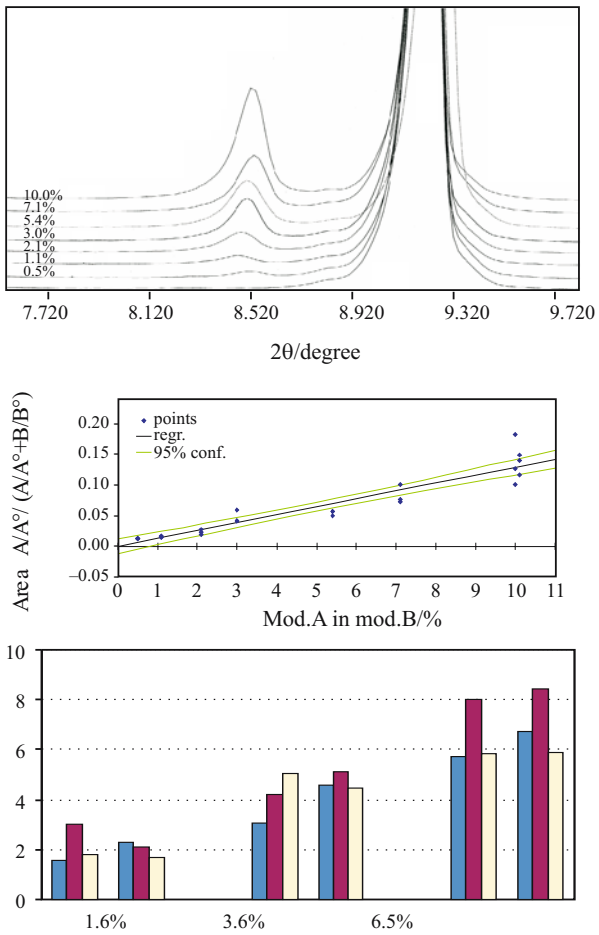

Fig. 4 Example 1. Quantitation A in B by X-ray diffraction. Top: X-ray diffractograms, middle: linear regression with r=0.944, bottom: intermediate precision

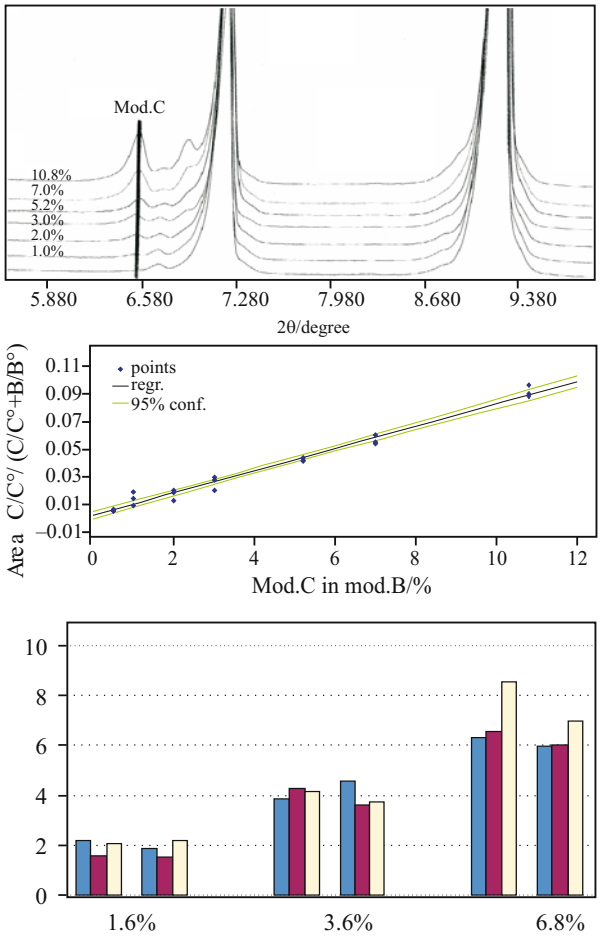

Fig. 5 Example 1. Quantitation C in B. Top: X-ray diffractograms, middle: linear regression with r=0.991, bottom: intermediate precision

intermediate precision. Figure 5 shows the same pictures for the determination of the form C in the form B. The same method of preparation: slight grinding in a pistil mortar was used. By comparing the two figures, the inhomogeneity of the mixtures A and B is evident. The correlation coefficient r is 0.944 for the mixture with form A and 0.991 for the mixture with form C. The intermediate precision is quite better with form C.

The observation of the samples of polymorphs A, B and C showed that the particle size are increasing in the order B, C, A. Therefore it was decided to use a better method to reduce the particle size in order to obtain good mixtures. Each mixture was measured 6 times and the relative standard deviation calculated. The best method should give the smaller standard deviation. For the method 1, the mixing time of the mixture was 30 s and in addition 2 min in a vibrator. For the method 2, form A was previously slightly ground 30 s. For the method 3, form A was ground 30 s and thereafter sieved in a 90 μm sieve. The mixture was treated like method 1.

Table 2 Example 1. Influence of the preparation method on the homogeneity of mixtures used for calibration. 6 measurements each mixture with methods 1 and 3

Form A	1	2	5	7	10	30
			Method 1			
S_{rel} area peak	17.1	21.6	10.4	14.1	13.8	14.5
S_{rel}/%	20.7	21.2	11.4	12.7	15.3	11.9
			Method 3			
S_{rel} area peak	4.4	9.1	9.1	4.7	3.3	4.3
S_{rel}/%	2.2	4.1	2.8	1.5	2.0	2.7

Table 2 allows for comparing the methods 1 and 3. The sieving of form A prior to mixing is very helpful. For a mixture 30% A, the relative standard deviation of the ratio is 11.9% with method 1 and 2.7% with method 3. From the 1 to 30% mixtures, the method 3 is considered as adequate since the relative standard deviations of each mixture measured 6 times are in the range 2–4%.

The calibration lines are different: $y=ax+b$ giving $0.0119x\pm0.0024$ for method 1 and $0.0093x\pm0.0009$ for method 3. The difference is 20% in the slope (Fig. 6).

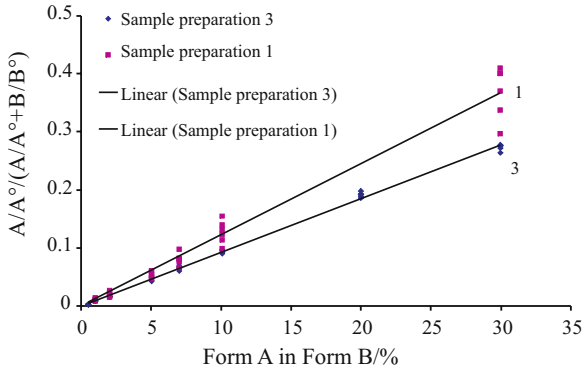

Fig. 6 Example 1. Quantitation A in B. Optimized method of preparation. Linearity. Method 1, r=0.988. Method 3: r=0.999

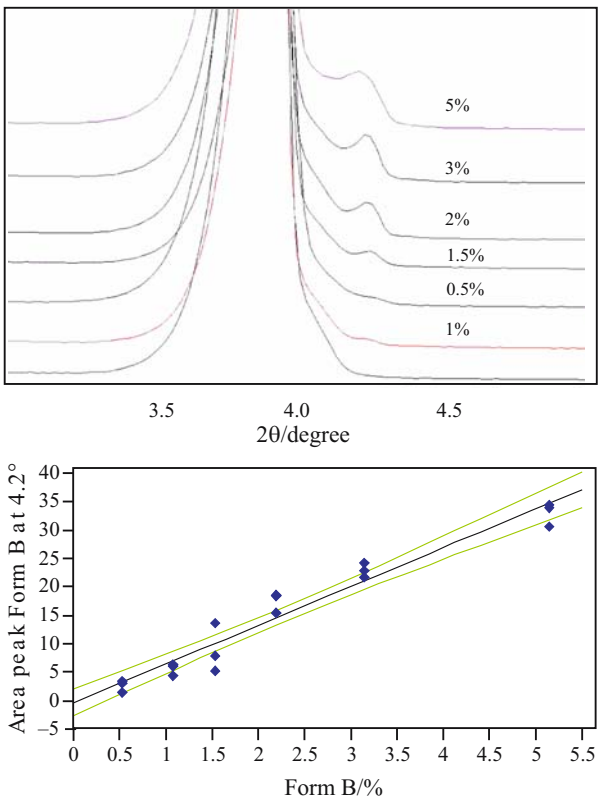

Fig. 7 Example 2. Forms A and B. Determination of form B by XRPD. Calibration curve in range 0.5–5.5%

Example 2

It is the case of iso-energetic polymorphs with nearly the same melting point and the same melting energy with a very slight difference of solubility. Form A was selected for development and XRPD, FTIR and Raman evaluated for a method of quantitation. Figures 7 to 10 correspond to these determinations. Figure 7 shows the X-ray diffraction region chosen for the peak of undesirable modification B and the linearity expressed as area of the peak at 4.2° 2θ in the range 0.5–5% with measurements in triplicate. The correlation coefficient r is 0.99. Figure 8 represents the linearity of two experiments in the range 0.5–50% and a zoom of the part 0–15%.

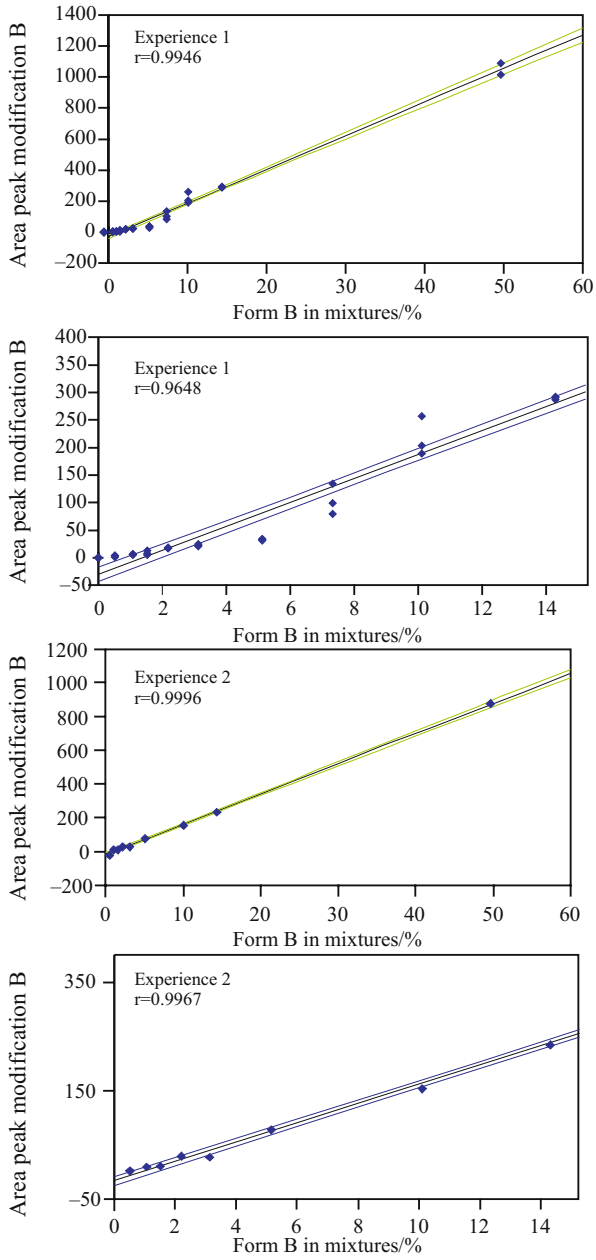

Fig. 8 Example 2. Comparison of quality of mixtures

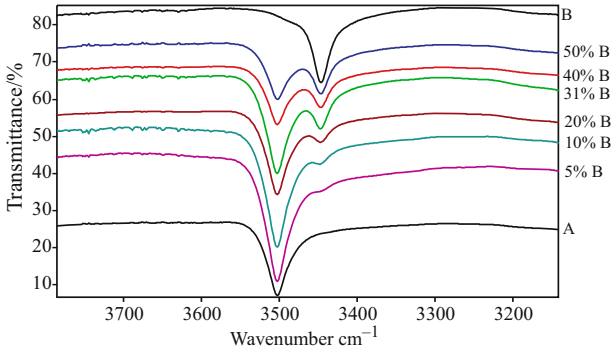

Fig. 9 Example 2. Determination of form B by FTIR in Nujol

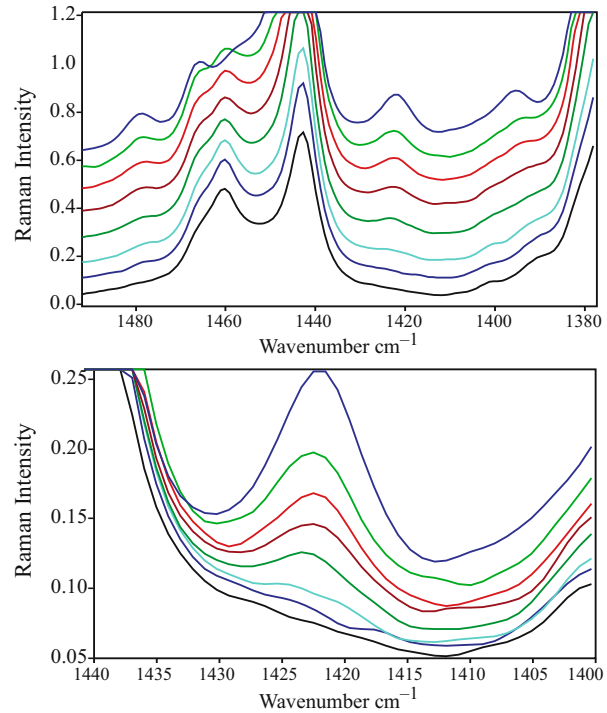

Fig. 10 Example 2. Raman of forms A and B. From top to bottom: form B; 50% form B; 40% form B; 30% form B; 20% form B; 10% form B; 5% form B; form A

The points in the higher range allows for obtaining a better line of calibration in terms of correlation coefficient. In a second experimentation the method of mixing includes a longer grinding time, the correlation coefficient (9 mixtures, single measurement) is quite high 0.9996. The second preparation of the mixtures is quite better as demonstrated visually by Fig. 8 in both ranges 0–50% and 0–15% of form B.

Figure 9 shows the FTIR peak at 3446 cm^{-1} which differentiates form A for mixtures 0–50%. The mixtures are prepared as described above. Samples are dispersed in Nujol between 2 KBr plates.

A peak at 2330 cm^{-1} is present in both forms. The ratio 3446/2330 cm^{-1} was used for the calibration line in the range 0–50% B. Measurements were done in triplicate, the relative standard deviation was <3% for each mixture. The correlation coefficient was found r=0.997. The limit of detection LOD of B is 5%.

Figure 10 shows a part of the Raman spectra of different mixtures B and A, from 5 to 50% and the spectra of pure forms. The laser power is 150 mW. The limit of detection LOD of B is 15%.

Example 3

The determination of a trihydrate in a monohydrate was described in [9]. The thermogravimetric curve permitted for an evaluation of the presence of the trihydrate since trihydrate and monohydrate have separated steps for the loss of the water. Nevertheless, XRPD was chosen for a routine method of quantification. Figure 11 shows the representation of accuracy and the intermediate precision. The accuracy was sufficient. The intermediate precision was designed by the comparison of 8 measurements performed by each analyst using two Scintag instruments with a batch containing 6% of trihydrate. Figure 11 bottom shows that one measurement is quite outside the other 15 measurements. The only possible explanation was the inhomogeneity of the batch.

Example 4

The results presented in Figs 12 and 13 summarize the work published by Giron *et al.* [76, 28]. A new entity

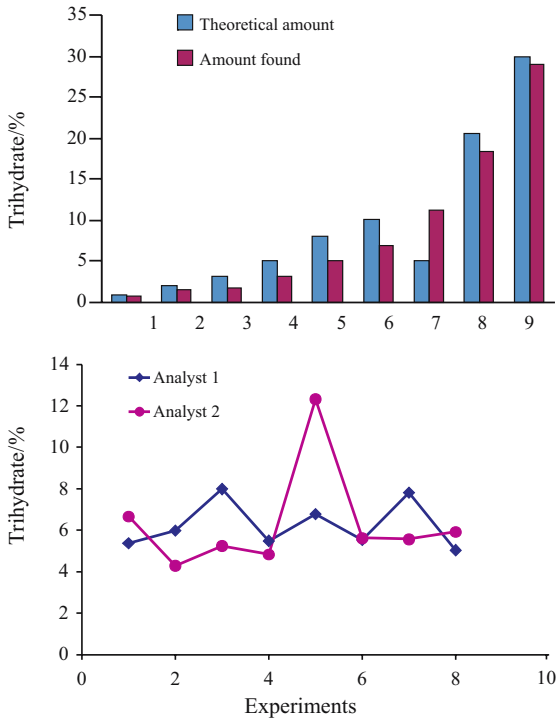

Fig. 11 Example 3. Quantitative determination of a trihydrate in the monohydrate. Top: accuracy. Bottom: Intermediate precision. Homogeneity of the batch

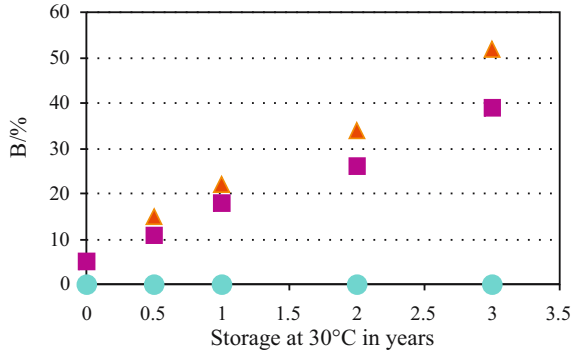

Fig. 12 Example 4 . Monitoring of stability by IR and XRPD [76, 28]. ■ – Batch 2 XRPD 8.6°, ▲ – Batch 2 IR 815/2220 ● – Batch 1 XRPD and IR

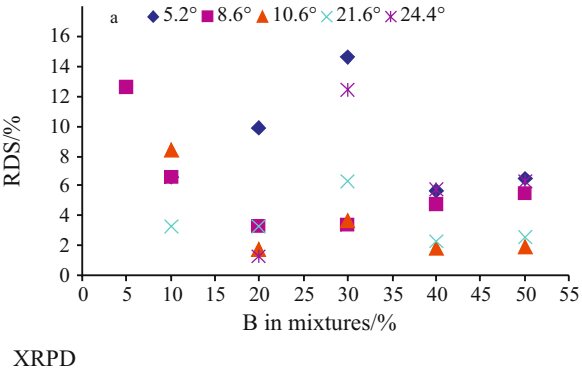

Fig. 13 Example 4. Repeatability, linearity, summary of results. a – XRPD, RSD% of results at different peaks 2 θ at different concentrations in form B. b – % form II found in all stability samples by XRPD for 3 peaks and by IR for 2 wavelengths

was first manufactured as metastable form I and the stable form II was discovered during the polymorphism screening. Form I was monotrop to form II. Two batches of form I were put in formal stability study. Surprisingly a change in XRPD was found after storage of batch 2 while batch 1 remained unchanged. Two techniques were used in parallel for quantitation of both forms: XRPD and IR in Nujol. For XRPD different peaks were checked for repeatability r=0.9923, n=6 and the peak at 8.6° 2θ selected. Figure 13a shows the relative standard deviation (RSD% of 6 measurements) for 5 peaks specific to form II for 6 mixtures. For IR the ratio of the peak at 815 cm^{-1} to the reference peak at 2220 cm^{-1} and the ratio of the peak at 930 cm^{-1} to the reference peak at 2220 cm^{-1} were calculated. Best results for the linearity were found with the peak at 8.6° 2θ for XRPD , r=0.9923, n=6 and 815 cm^{-1} for IR, r=0.9885, n=6. LOQ of 5% were found in XRPD and 15% in IR. The analysis of stability-samples shows a good correlation as demonstrated by Fig. 13b. Figure 12 exemplifies the need of very sensitive method. In batch 1 no trace of form II was detected (LOD 2%). Form II was present in traces in batch 2 as demonstrated by the analysis of sample stored in freezer. These seeds initiate the crystallization and the temperature accelerates the growth of form II.

Example 5

Helmy [35] published a comparison of quantitative determination of polymorphs of Aprepitant (5-[[(2S,3R)-2-[(1R)-1-[3,5-bis(trifluoromethyl)phe-nyl]ethoxy]-3-(4-fluorophenyl)morpholin-4-yl]meth-yl]-1, 2-dihydro-1,2,4-triazol-3-one)) by using XRPD and ATR. In ATR the method selected was the second derivative peak ratio 1140/1272 cm^{-1}. Influence of the pressure was found critical. LOD for both techniques were comparable. Linearity and accuracy were performed. Figure 14 shows a comparison of values obtained for the same mixtures 5 to 15% by both techniques. The results are in good agreement. The results of XRPD are closer to the theoretical values.

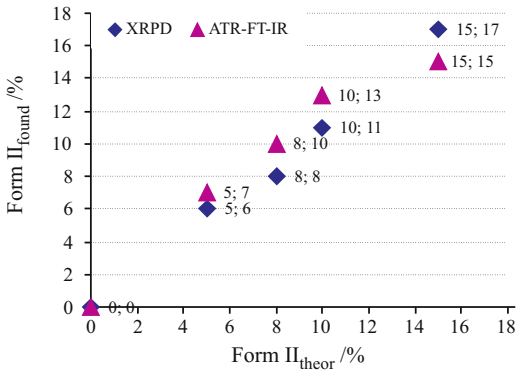

Fig. 14 Example 5. Aprepitant (5-[[(2S,3R)-2-[(1R)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophe nyl)morpholin-4-yl]methyl]-1,2-dihydro-1,2,4-triazol-3-one). Accuracy. Analysis of 4 mixtures with 5, 8, 10, 15% Form II. Mean of 3 determinatons. Comparison of ATR-FTIR and XRPD from data of Helmy *et al.* [35]. ◆ – XRPD, ▲ – ATR-FTIR

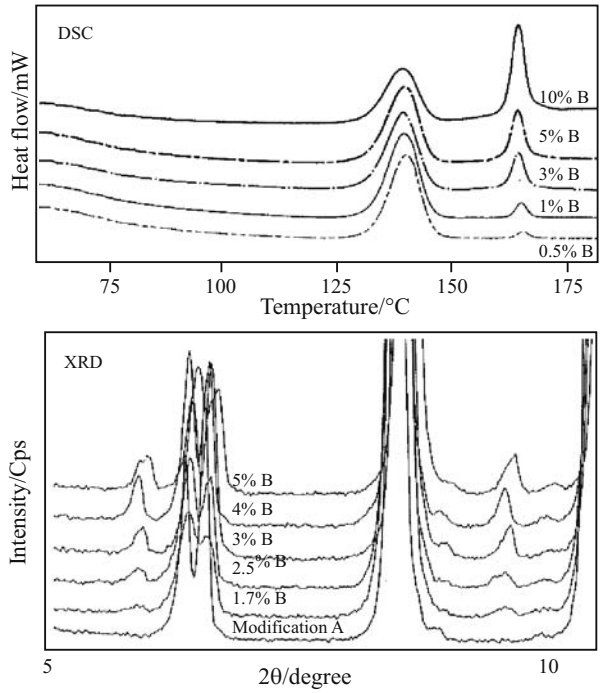

Fig. 15 Example 6. Comparison of sensitivity between DSC and X-ray diffraction

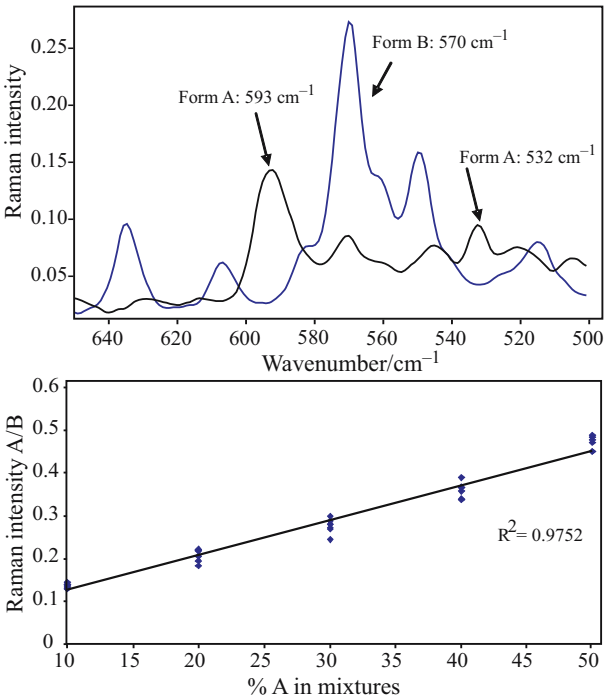

Fig. 16 Example 6. Top: FT-Raman spectra of forms A and B. Bottom: determination of form A in form B. Ratio of peaks intensity at 593 and 570 cm^{-1} *vs.* % A in mixtures A and B

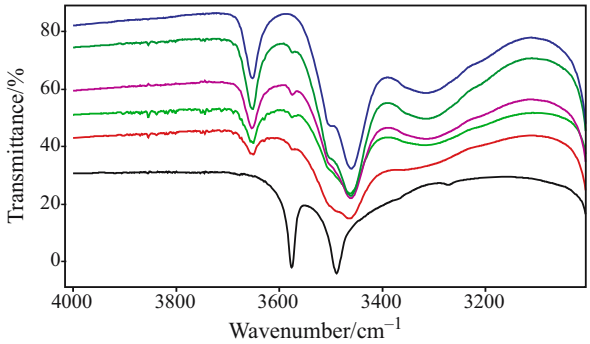

Fig. 17 Example 6. Detection of form B in form A by FTIR in nujol. From top to bottom: 100% form A; 5% form B; 10% form B; 10% form B; 5% form B, 100% form B

Example 6

Figures 15 to 17 correspond to example 6. This substance was first manufactured as metastable form A. Like for example 5, traces of stable form B catalyse the transformation into form A. But in this case the kinetic is extremely fast. Therefore it was necessary to have a very sensitive technique. The DSC melting peak of form B could serve to this objective since a study demonstrated that after melting of form A, since recrystallization into B did not occur in mixtures with small amounts of B. Figure 15 shows a comparison of XRPD and DSC. The LOD of DSC can attain 0.1%. In XRPD the limit with the Scintag was about 2%. IR and Raman of both forms were different. Approx. 10% of B or 10% of A could be detected in mixtures by IR (Fig. 17). The FT-Raman spectra of forms A and B are given in Fig. 16. The detection of form B in form A is difficult and amounts lower than 20% are not detectable. In contrary low amounts of form A can be quantified in form B. A linearity could be found and the ratio between peaks at 593 cm^{-1} *vs.* peak at 570 cm^{-1} reliable for quantitation purposes with a limit of approx. 10%. DSC was the only technique suitable to detect traces of B in A.

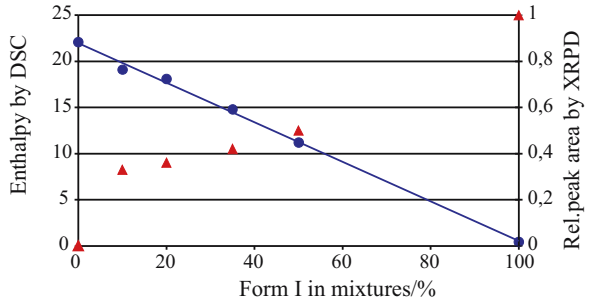

Fig. 18 Example 7. Comparison linearity XRPD and DSC.
● – DSC, △ – XRPD

Example 7

For this substance there was no difference in IR and the XRPD peaks of the two forms overlap except two peaks not very well separated. In DSC an endotherm is present in form I. Figure 18 shows the results of XRPD expressed in ratio of the peaks form I/form II and the DSC results of the endothermic peak of form I *vs.* the amount of form I mixtures of both forms. This figure shows that the DSC is more reliable than XRPD since a linear relationship is found, what is not the case for XRPD.

Example 8

Determination of polymorphs in drug product is limited for low amount of active ingredient in the dosage form. The linearity shown in Fig. 19 corresponds to the determination of the crystalline form in a solid dispersion in which the drug substance is amorphous. A very low strength and the matrix effect of excipients were the limiting factors for a precise LOQ. Figure 20 shows the variation of the repeated measurements. For the determination of the limit of quantitation several mixtures were measured. The relative standard deviation of the peak areas as

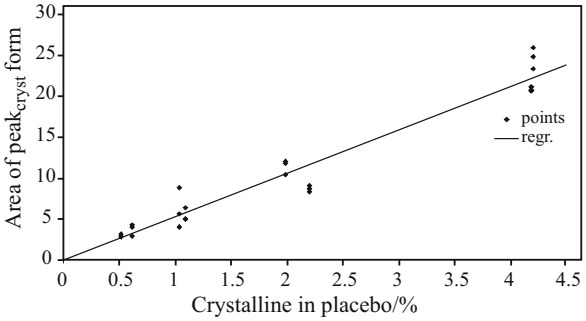

Fig. 19 Example 8. Measurement of crystalline form in placebo mixture of drug product; $y=a+bx$, a=0.0149, b=5.3090, R=0.9734, R^2=0.9475

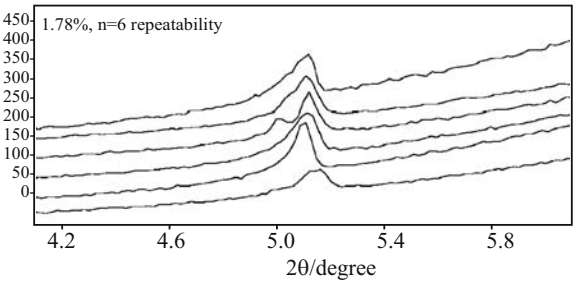

Fig. 20 Example 8. XRPD peak at 5.08 2θ: 6 measurements of the mixture at 1.78% level

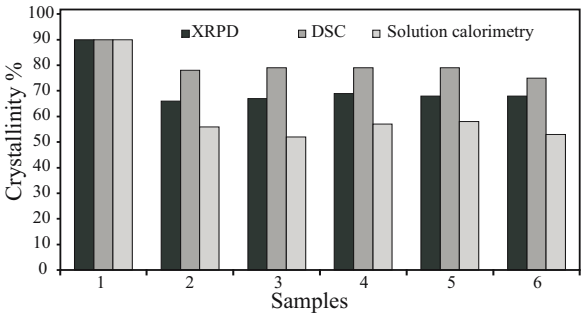

Fig. 21 Example 9. Comparison of XRPD, DSC and solution calorimetry for crystallinity

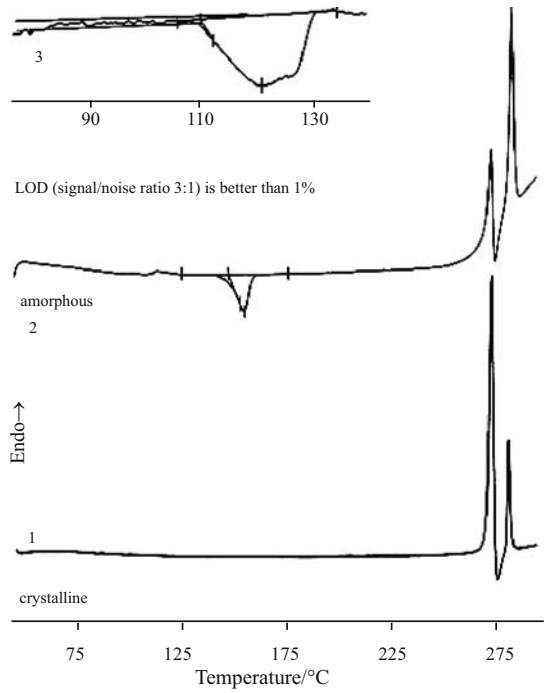

Fig. 22 Example 10. Determination of the amorphous content by DSC down to 1%

Table 3 Example 8. Statistic results obtained with different mixtures at 2% level of the crystalline drug in the placebo mixture of excipients

Mixture%	Peak position (2θ)	Area	Relative STD%	Area/2%	*S*/*N*
1.78	5.08 (*n*=6)	9.0 (*n*=6)	16.5	10.1	14.0 (*n*=6)
1.98	5.06 (*n*=3)	11.0 (*n*=3)	7.1	11.5	7.3 (*n*=3)
2.20	5.07 (*n*=3)	8.7 (*n*=3)	4.0	7.9	10.8 (*n*=3)
2.65	5.15 (*n*=3)	19.5 (*n*=3)	14.5	14.7	18.0 (*n*=3)
Mean				11.1	13
Relative standard deviation of *n*=4 mixtures				26%	36%

well as the determination of the peak height/noise ratio were measured. Table 3 sumarises the results. The LOQ was estimated at 2%.

Example 9

Figure 21 is a comparison of 3 techniques for the measurement of crystallinity of a drug substance: DSC melting enthalpy of the crystalline form, XRPD calculation of crystallinity and solution calorimetry. All methods were validated for linearity and compared for accuracy. Some discrepancy is found. Solution calorimetry gives lower values of crystallinity [9].

Example 10

Figure 22 shows the DSC curves of a drug substance. After milling or after spray drying amorphization is observed. The crystallization exotherm after the T_g of

Table 4 Example 10. Comparison of results of crystallinity obtained by XRPD and DSC

Theoretical amount (spiked samples)	Found X-ray	Found DSC
13.6%	18%	21%
19.7%	18%	21%
29.2%	21%	26%
49.6%	46%	47%
58.5%	54%	50%
79.4%	80%	74%
Production samples		
1	97%	79%
2	91%	71%
3	92%	90%
4	84%	64%
5	83%	85%
6	73%	82%
7	60%	27%
8	24%	27%

the amorphous form allow for determining the amorphous content. XRPD can also be used for crystallinity determination. Both methods were validated. The correlation coefficient for XRPD is 0.99 and LOD is 5%. For DSC the linearity has a correlation coefficient of 0.99 and a LOD of 1%. Table 4 shows the results of analysis of samples by both methods. Good agreement is found for mixtures manufactured in the laboratory (spiked samples). For

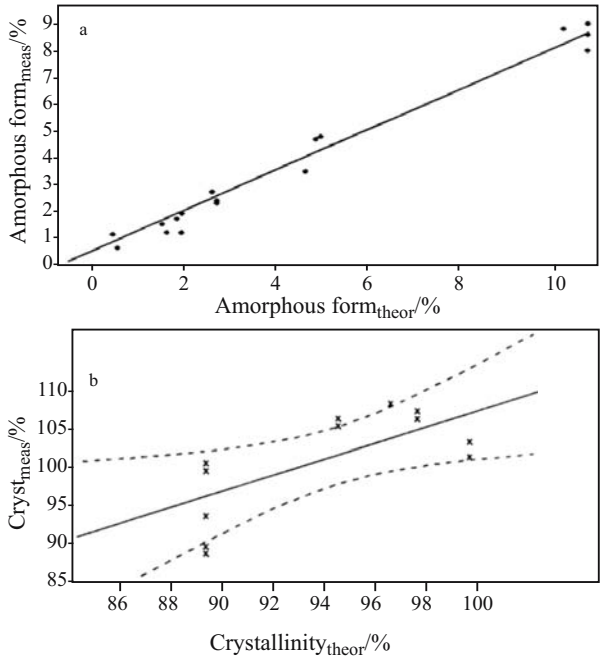

Fig. 23 Example 11. Comparison of accuracy for isothermal calorimetry and XRPD [77]

production samples, there are strong discrepancy in two samples which might be not homogeneous.

Example 11

For a purine derivative [77], XRPD was successfully used for the detection of 4 different polymorphs down to 1–2%. For the quantification of amorphous content isothermal microcalorimetry and XRPD were compared. Figure 23 shows the comparison of the

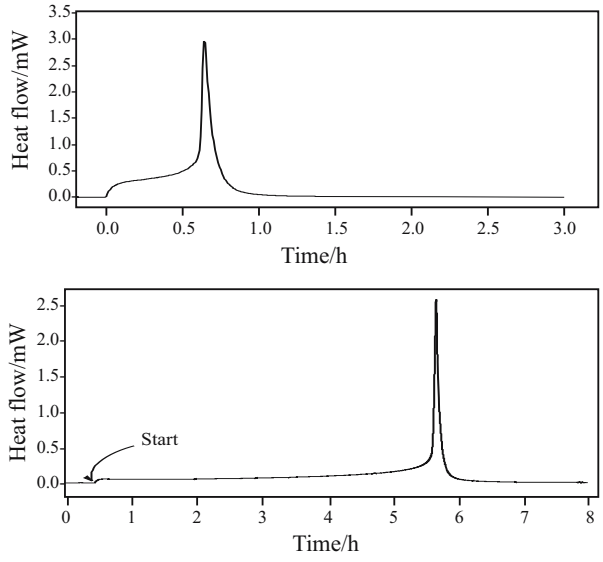

Fig. 24 Example 12. Determination of amorphous content by microcalorimetry. Top: sovent vapor ethanol/water, bottom: solvent vapor dimethylformamide

linearity in the range 0–10% amorphous or 90–100% crystalline. Isothermal calorimetry gave quite better results and the limit of detection was 1%.

Example 12

This example has been selected for the determination of amorphous content by isothermal calorimetry. The duration of recrystallization of amorphous material is dependent on the temperature of decreased glass transition. Different solvents vapors were screened in order to optimize the parameters start of recrystallisation and duration of the analysis. Figure 24 shows the heat flow curves for a sample treated by ethanol/water solvent vapor and by dimethylformamide solvent vapor. With the ethanol/water the correlation coefficient of the linearity was found 0.999 and the relative standard deviation of repeatability for a sample of 16% amorphous content was 2.2%. However the limit of detection was not as low as suitable since the crystallization was too fast. In order to obtain a lower limit of detection dimethylformamide was selected but the time of analysis was longer. The kinetic of crystallization depends also on the particle size. The penetration of solvent vapor in crystalline material is slower and calibration curves of mixtures crystalline/amorphous simulate well the heat flow but not the time of the experimental curve. For samples micronised the crystallization occurs quicker and the time of equilibration may overlap with crystallization. For example approx. 16 h was needed for 400 mg of a 15% mixture amorphous/crystalline compared to approx. 6 h for a real micronised 15% amorphous sample by using dimethylformamide as solvent vapor. Figure 25 shows the calibration curve with a correlation coefficient 0.9994. The repeatability was obtained by calculating the relative standard deviation of 6 measurements. The relative standard deviation was found 2.7% for a sample with a high amorphous content of 16%. The relative standard deviation was found 19% for a sample with a low amorphous content of 1.6%. Accuracy was measured by comparing results obtained from spiking experiments compared to theoretical values calculated as recovery. Recovery values were 105% at level 7% and 119% at level 5%. At level 2% recovery values of 82 and 102% were obtained. Comparison of measurements with ethanol/water as solvent and dimethylformamide as solvent are in very good agreement for a sample measured with both methods: 12.9% compared to 12.7%. Figure 26 shows the heat flow curves for 2, 1.5 and 0.7% amorphous content by using dimethylformamide.

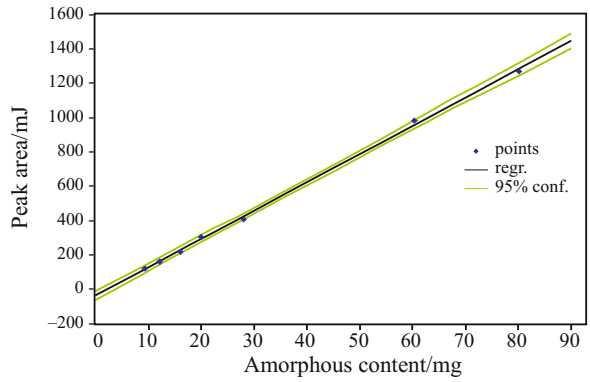

Fig. 25 Example 12 Determination of amorphous content by microcalorimetry by using dimethylformamide as solvent

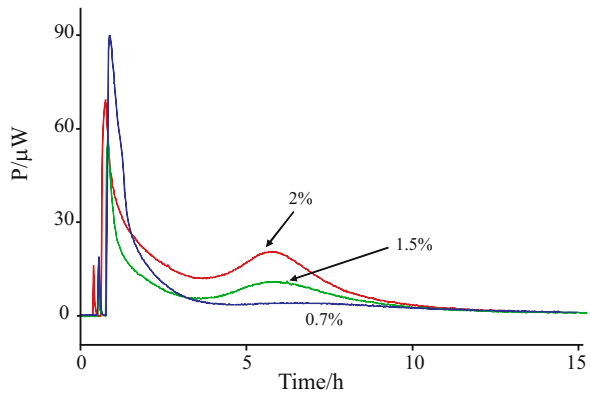

Fig. 26 Example 12. Determination of amorphous content by microcalorimetry. 1.5% is detectable

Conclusions

The examples show that generally XRPD is the method of choice for crystalline forms. Very low limit of detection can be easily obtained. FTIR is easy to be applied but we never observed limit of detection better than 5%. Raman and NIR are growing techniques, Terahertz is newly applied. Thermal analysis techniques extremely sensitive are routinely useful if no kinetic effect appears, preferably for limit methods. For solvates and hydrates classical analytical methods such as GC, TG, Karl Fischer may be more efficient.

In some cases the most sensitive method is not required for routine analysis, e.g. FTIR methodology present in almost analytical laboratories can be sufficient for limit methods in range <10–20%.

For the determination of amorphous content isothermal calorimetry is the reference method. Very low limit of detection can be obtained. However if no recrystallization is induced by solvent vapors, the method cannot be applied and XRPD or alternative methods have to be tried.

Quantitative method development according to ICH is possible if sufficient amount of standard samples polymorphs is available, if the purity and the crystallinity of standards is known.

Storage conditions of standards and mixtures during validation should be guaranteed.

The homogeneity of mixtures, the sample preparation and the particle size are critical parameters. The number of experiments necessary can be extremely high and statistical evaluation of the results has to be done. The repeatability of measurements of analytical mixtures should be a criteria of preparation, e.g. a relative standard deviation less than 5% in a quantification range, 15% for the LOQ.

The stability of polymorphs during measurement should be demonstrated through several measurements e.g. several scans of the same sample.

Every mixture used for calibration should be repeated (e.g. 3 preparations, measured each in triplicate). The number of mixtures should be ≥6 in the considered range.

The influence of the presence of other forms or of the matrix from excipients should be studied for using the calibration model for analysis.

The instrument calibration, parameters should be thoroughly studied and selected.

Recovery studies with spiked samples are easy to be done, but the method has also to be validated by using real samples when possible. Accuracy by using an independent technique for some samples in the range where both techniques can be applied is highly suitable.

References

1 J. K. Haleblian and W. J. McCrone, Pharm. Sci., 58 (1969) 911.
2 J. K. Haleblian, Pharm. Sci., 64 (1975) 1269.
3 International Conference on Harmonization (ICH), Guideline Specification Q6A, Decision Tree: Investigating the need to set acceptance criteria for polymorphism in drug substances and drug products, 1999.
4 Abbreviated New Drug Application (ANDA) Process for Generic Drugs, FDA. gov.
5 W. H. DeCamp, Amer. Pharm. Rev., 4 (2001) 70.
6 L. X. Yu, M. S. Furnes, A. Raw, K. P. Woodland Outlaw, N. E. Nashed, E. Ramos, S. P. F. Miler, R. C. Adams, F. Fang, R. M. Patel, F. O. Holcombe, Y. Chiu and A. S. Hussain, Pharm. Res., 20 (2003) 531.
7 S. A. Raw, M. S. Furness, D. S. Gill, R. C. Adams, F. O. Holcombe and L. Yu, Adv. Drug Del. Rev., 56 (2004) 397.
8 D. Giron, Eng. Life Sci., 3 (2003) 103.
9 D. Giron, S. Garnier and M. Mutz, J. Therm. Anal. Cal., 77 (2004) 709.
10 A. Burger and R. Ramberger, Mikrochim. Acta, II (1979) 259.
11 D. Giron, Labo-Pharma-Probl. Techn., 307 (1981) 151.
12 Polymorphism in Pharmaceutical Solids, H. G. Brittain Ed., Marcel Dekker, New York, 1999.
13 Polymorphism in Molecular Crystals, J. Bernstein, Oxford University Press, Oxford, 2002.
14 M. R. Caira, Design of Organic Solids in Topics in current Chemistry, 98 (1998) 163.
15 R. Hilfiker, Polymorphism: in the Pharmaceutical Industry, 2006, Wiley-VCH, Weinheim.
16 L. Borka and J. K. Haleblian, Acta Pharm. Jugosl., 40 (1990) 71.
17 D. Giron, Thermochim. Acta, 248 (1995) 1.
18 M. Kühnert-Branstätter, Thermomicroscopy in the Analysis of Pharmaceuticals, Pergamon, NY., 1971.
19 M. Pudipeddi and A. T. M. Serajuddin, J. Pharm. Sci., 94 (2005) 929.
20 S. R. Byrn, R. R. Pfeiffer and J. G. Stowell, Solid-State Chemistry of drugs, 2nd Ed., SSCI Inc., West Lafayette, Indiana, 1999.
21 International harmonisation guideline Q2 on validation of analytical procedures, 1996. US29, first supplement, 3614–3617.
22 G. A. Stephenson, The Rigaku Journal, 92 (2005) 2.
23 S. S. Iyengar, N. V. Phadnis and R. Suryanarayanan, Powder Diffraction, 16 (2001) 20.
24 R. Surana and R. Suryanarayanan, Powder Diffraction, 15 (2000) 2.
25 M. Davidovich, J. Z. Gougoutas, R. P. Scaring, I. Vittez and S. Yin, Am. Pharm. Rev., 7 (2004).
26 S. Yin, R. P. Scaring, J. DiMarco, M. Gaella and J. Z. Gougoutas, Am. Pharm. Rev., 6 (2003) 80.
27 S. D. Clas, R. Faizer, R. E. O'Connor and E. B. Vadas, Int. J. Pharm., 121 (1995) 73.
28 D. Giron, B. Edel and P. Piechon, Mol. Cryst. Liq. Cryst., 187 (1990) 297.
29 V. B. Cooper, G. E. S. Pearce and E. R. Petts, J. Pharm. Pharmacol., 55 (2003) 1323.
30 X-ray diffraction, USP 29.
31 D. Giron, J. Therm. Anal. Cal., 64 (2001) 37.
32 D. Giron, J. Therm. Anal. Cal., 73 (2003) 441.
33 ASTME1655-00. 'Standard Practice for IR quantitative Analysis', ASTM International, West Conshohocken, PA.
34 D. E. Bugay, Adv. Drug Deliv. Rev., 48 (2001) 43.
35 R. Helmy, G. X. Zhou, Y. W. Chen, L. Crocker, T. Wang, R. M. Wenslow and A.Vailaya, Anal. Chem., 75 (2003) 605.
36 S. Agatonovic-Kustrin, T. Rades, V. Wu, D. Saville and I. G. Tucker, J. Pharm. Biomed. Anal., 25 (2001) 741.
37 M. Sheikhzadeh, S. Rohan, A. Jutan, T. Manifar, K. Murthy and S. Horne, Pharm. Res., 23 (2006) 1043.
38 V. Koradia, G. Chawwla and A. K. Bansai, Acta Pharmaceutica, 54 (2004) 193.
39 Eur. Ph., 2005, 5, section 2.2.24.
40 EMEA, Guideline for the development and Validation of NIR Methods in the Pharmaceutical Industry, N. Broad, P. Graham, P. Hailey, A. Hardy, S. Holland, S. Hughes, D. Lee, K. Prebble and N. Salton.
41 NIR <1119>, USP 2006, 29, 2979–2983.
42 A. D. Patel, P. E. Luner and M. S. Kemper, J. Pharm. Sci., 90 (2001) 360.
43 M. Otsuka, F. Kato and Y. Matsuda, Analyst, 126 (2001) 1578.
44 Raman <1120>, USP 29 (2006) 2983. Eur. Ph., 2005, 5, section 2.02.

45 R. S. Campbell, N. Sarra, A. C. Williams, I. M. Grimsey and S. W. Booth, J. Pharm. Biomed. Anal., 28 (2002) 1135.
46 L. S. Taylor, Am. Pharm. Rev., 4 (2001) 60.
47 C. J. Strachan, P. F. Taday, D. A. Newnham, K. C. Gordon, J. A. Zeitler, M. Pepper and T. Rades, J. Pharm. Sci., 94 (2005) 837.
48 Y. Hu, H. Wikstrom, S. Byrn and L. S. Taylor, Appl. Spectroscopy, 60 (2006) 977.
49 P. A. Tishmack, D. E. Bugay and S. R. Byrn, J. Pharm. Sci., 92 (2003) 441.
50 M. T. Zell, B. E. Padden, D. J. W. Grant, S. A. Schroeder, K. L. Wachholder, I. Prakash and E. J. Munson, Tetrahedron, 56 (2000) 6603.
51 D. C. Apperley, R. K. Harris, T. Larsson and T. Malmstrom, J. Pharm. Sci., 92 (2003) 2487.
52 R. K. Harris, Analyst, 131 (2006) 351.
53 USP29, 2006, 2666–2667.
54 R. Saklatvala, P. G. Royal and D. Q. M. Craig, Int. J. Pharm., 192 (1999) 55.
55 M. Lappalainen, I. Pitkaenen and P. Harjunen, Int. J. Pharm., 307 (2006) 150.
56 G. M. Venkatesh, M. E. Barnett, C. Owusu-Fordjour and M. Galop, Pharm. Res., 18 (2001) 98.
57 USP Solution calorimetry.
58 P. Royall and S. Gaisford, Curr. Pharm. Biotechnol., 6 (2005) 215
59 M. Anberg, C. Nyström and S. Castensson, Int. J. Pharm., 81 (1992) 153.
60 D. Giron, P. Remy, S. Thomas and E. Vilette, J. Thermal Anal., 48 (1997) 465.
61 M. Anberg, Thermochim. Acta, 248 (1995) 161.
62 K. Fiebich and M. Mutz, J. Therm. Anal. Cal., 57 (1999) 75.
63 H. Ahmed, G. Buckton and D. A. Rawlins, Int. J. Pharm., 130 (1996) 195.
64 K. Kawakami, T. Numa and Y. Ida, J. Pharm. Sci., 91 (2002) 417.
65 S. Garnier, D. Giron and M. Mutz, 'Study of crystallization of drug substances under solvent vapour atmosphere by microcalorimetry', PhandTA7, Innsbruck, Sept. 2003.
66 G. Buckton, Thermochim. Acta, 247 (1994) 117. G. Buckton and P. L. Sheridan, Pharm. Res., 12 (1995) 1025.
67 S. E. Hogan and G. Buckton, Int. J. Pharm., 227 (2001) 57.
68 S. E. Dilworth, G. Buckton, S. Gaisford and R. Ramos, Int. J. Pharm., 284 (2004) 83.
69 V. Lehto, M. Tenho, K. Vaehae-Heikkilae, P. Harjunen, M. Paeaellysaho, J. Vaelisaari, P. Niemelae and K. Jaervinen, Powder Technology, 167 (2006) 85.
70 C. Guftasson, H. Lennholm, T. Iversen and C. Nystrom, Int. J. Pharm., 174 (1998) 243.
71 I. Fix and K.-J.Steffens, Drug Dev. Ind. Pharm., 30 (2004) 513.
72 D. Pratiwi, J. P. Fawcett, K. C. Gordon and T. Rades, Eur. J. Pharm., 54 (2002) 337.
73 P. Royall, C. Huang, S. Jai Tang, J. Duncan, G. Vandevelde and M. Brown, Int. J. Pharm., 301 (2005) 181.
74 T. M. Niemczyk, 2003 'Quantitative analysis of polymorphic mixtures using infrared spectroscopy'. University of New Mexico.
75 T. Okumura and M. Otsuka, J. Pharm.Sci., 94 (2005) 1013.
76 D. Giron, Mol. Cryst. Liq. Cryst., 161 (1987) 77.
77 D. Giron, P. Piechon, C. Goldbronn and S. Pfeffer, J. Therm. Anal. Cal., 57 (1999) 61.

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