Combined impactor and x-ray diffraction (XRPD) methodology for characterizing polymorph impurities in inhalation powders

This article presents a combined impactor and x-ray diffraction (XRPD) methodology that can be applied to typical DPI formulations, enabling detection of polymorph impurities at levels comparable to, or better than, corresponding determinations using conventional synchrotron radiation.

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The need to detect polymorph inpurities

The vast majority of marketed inhalation products with solid or semi-solid formulations involve crystalline active pharmaceutical ingredients (APIs). It is well known that a crystalline compound often can exist in more than one crystal modification. If the different crystal modifications are made up of the same chemically identical compound, the various modifications are defined as polymorphs and the general phenomenon is referred to as polymorphism. If solvate formation is involved, the chemical identity is altered and such solvates are generally referred to as pseudopolymorphs. When water molecules are stoichiometrically incorporated in a crystal structure, the solvate form is called a hydrate.

Product formulators and regulatory agencies are well aware that different polymorphs and hydrates of a given mother compound can have different properties that, in turn, can influence product performance and safety. Consequently, regulatory agencies urge the pharmaceutical industry to select the most suitable API crystal modification for the intended dosage form and manufacturing as well as to show that the formulated APIs are stable.

Another driving force for developing sensitive crystal modification detection methodologies is intellectual property. Innovative companies strive to protect their novel products by using state-of-the-art characterization methodologies to aid their claims while generic companies strive for the same methodological performance in order to show that they are not infringing on related patents.

Many inhalation compounds are highly potent, meaning that related products will involve APIs in very low doses, which puts demands on the methodology used to detect and identify the API crystal modification. Examples of such compounds are tiotropium bromide and formoterol fumarate. Both can exist in several different crystal modifications, involving both polymorphs and hydrates.

For tablet formulations, a full range of techniques for polymorph detection and identification are described in literature, of which the most common are spectroscopic techniques (Raman, infrared, near-infrared and solid-state nuclear magnetic resonance) or X-ray powder diffraction (XRPD). However, none of those techniques are directly applicable to detect, for example, the presence of 10% undesired polymorph in a low dose inhalation product. Examples would be dry powder inhalers (DPIs) containing formoterol fumarate (Symbicort, AstraZeneca) or tiotropium bromide (Spiriva, Boehringer Ingelheim).

This article will describe a combined impactor and XRPD methodology that can be applied to adhesive-mixture-based DPIs. This entails detection of polymorph impurities at levels comparable to, or better than, corresponding determinations using synchrotron radiation. It is well known that the high flux, brilliance and stability of synchrotron radiation compared to standard laboratory XRPD leads to improved detection possibilities. However, synchrotron applications have limitations in flexibility during product development as well as in timing, cost and limited environmental control possibilities during support of stability studies.

Figure 1

A typical lactose carrier particle with adhered fine API particles.

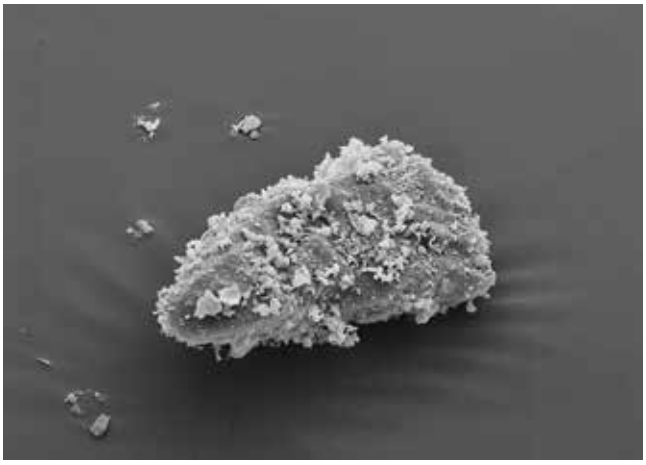
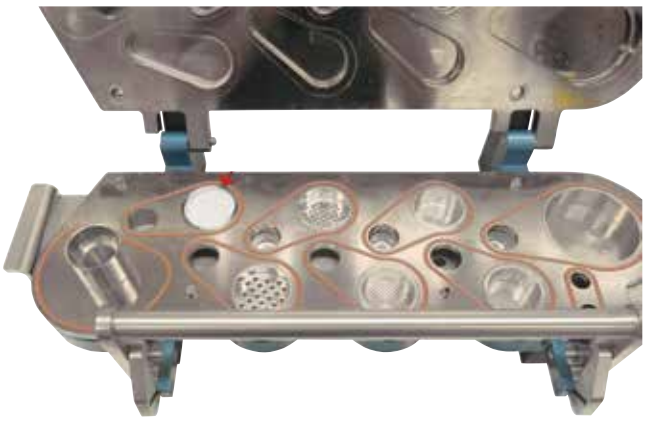


Figure 2

An NGI with its lid open; the red arrow indicates the membrane filter attached up-stream of the Stage 2 nozzles.



Adhesive mixtures and size-based separation of particles via impaction

Powder particles of inhalable size can suffer from poor flow properties. The most common way to improve the situation is to formulate the micronized API into so-called adhesive mixtures, also called ordered mixtures. In an adhesive mixture, the micronized API is mixed with coarse carrier particles (commonly, lactose monohydrate), rendering a mixture for which the fine API particles are adhered onto the surfaces of the carrier particles (Figure 1).

During inhalation, the small API particles will detach from the carrier particles, which will create the respirable fraction of the dose. The respirable fraction of a dose (also called the fine particle fraction) is often specified as particles with an aerodynamic diameter of less than 5 µm. Since these particles are crucial for the clinical effect of the formulation, it is critical that assessment of the aerodynamic size distribution is performed correctly and in a standardized way.

Within the pharmaceutical industry, cascade impactors are widely used for measuring the aerodynamic size distribution from dry powder inhalers and metered dose inhalers. The advantage of using an impactor for fine particle assessment is that different size fractions of the API can be assessed after dose fractionation (e.g., by high pressure liquid chromatography). Other techniques (e.g., laser diffraction) give a size distribution that includes both the API and excipients.

Three impactor types are specified both by the European Pharmacopeia and the United States Pharmacopeia; specifically the Andersen Cascade Impactor (ACI), the Multi-Stage Liquid Impinger (MSLI) and the Next Generation Impactor (NGI). Since the NGI was launched in 2000, it has been the first choice for laboratories within the inhalation area.[1] Its horizontal layout can make it relatively easy to use compared to other impactor types.

In addition to assessing aerodynamic size distribution, the NGI can be used to enrich fine particles from a pharmaceutical formulation for solid state analysis. In doing so, the NGI is set up in the same position as that used during standard fine particle assessments. However, to collect fine particles for x-ray diffraction (XRPD) measurements, the NGI lid is opened and a membrane filter is placed over the jets, over collection cup No. 2, as shown in Figure 2. At 50 L/min, the filter will collect particles less than 8.9 µm and larger particles (e.g., carrier lactose particles) are collected in the inlet (throat), preseparator and on Stage 1. About 30 mg of formulation is required to be collected on the filter for XRPD analysis. After the dose collection is completed, the powder on the filter surface is scraped off onto a zero-background XRPD sample plate. Using this methodology for sample preparation, a formulation with large amounts of carrier lactose can easily be concentrated and thereby be well-suited for XRPD analysis.

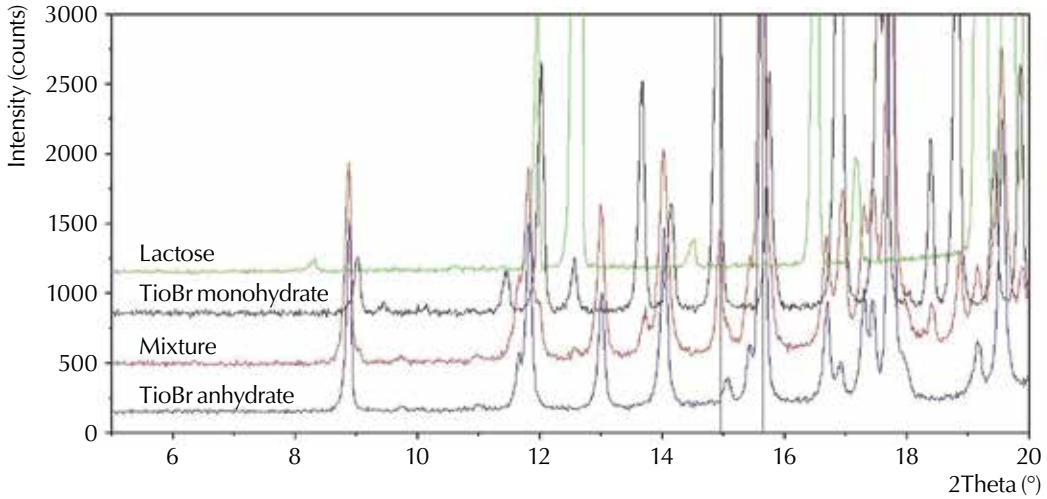
The value of X-ray powder diffraction

Every crystalline form of a given substance gives rise to a unique X-ray diffraction pattern. This means that different crystalline forms of the same substance can be differentiated, i.e., polymorphs, solvates and other forms can easily be identified. The X-ray diffraction pattern is generated by firing X-rays onto a flat powdered sample and registering the diffracted radiation using a detector. When the diffraction angle (Θ) is varied, different lattice planes in the crystal structure will produce diffraction peaks according to Braggs law: $n\lambda = 2d\sin\Theta$, where λ is the wave length of the X-rays, n is an integer number and d is the distance between a given set of lattice planes in the structure.[2,3]

Since every crystalline form produces a unique diffraction pattern, mixtures of crystalline forms will generate diffraction patterns that are a sum of the different components patterns. This can be used for identification and even quantification, e.g., identifying an unwanted polymorph in a polymorphic mixture.[4,5]

Figure 3

Comparison of diffractograms for tiotropium bromide anhydrate (blue), tiotropium bromide monohydrate (black) and α-lactose monohydrate (green). A diffractogram for a 15% mixture of tiotropium bromide monohydrate in anhydrate is also displayed (red). The inserted vertical lines show the positions of the signals used in the quantification method, i.e., the tiotropium bromide monohydrate signal at 14.9°(2Θ) and the tiotropium anhydrate signal at 15.6°(2Θ).



Whereas the peak position is entirely governed by the crystal lattice and Braggs law, peak intensities can be influenced by a number of factors such as sample amount, sample thickness, particle size and particle orientation. Thus, utmost care has to be taken when preparing samples, especially for quantitation purposes.

Using a very thin layer of sample on a zero-background plate is recommended because a thick layer of organic substances would allow penetration of X-rays throughout the sample, resulting in diffraction from various depths in the sample, which would broaden and distort the diffraction peaks. Particle size and particle orientation effects are best handled by reducing the particle size via gentle grinding prior to sample preparation. Care must be taken to avoid excessive grinding which would result in destruction of the crystalline structure of the sample. Spinning of the sample holder during analysis will further improve the reproducibility of diffraction pattern intensities.

For polymorph quantitation purposes, it is most convenient to use an internal standard methodology where a peak from the "undesired" polymorph is compared to a peak from the "desired" polymorph. Since both peaks are taken from the same measurement, the effect of varying sample size from analysis to analysis can be eliminated. It is, of course, desirable to find peaks from the two polymorphs that are intense and non-overlapping with other peaks in the diffractogram.

The signal-to-noise ratio, and thus detectability, can generally be improved by increasing the measurement time, allowing for better statistics of the detected signal. Taking more frequent data points will improve the peak shape, allowing a better determination of peak intensities. The angular range is usually restricted to the peaks of interest for quantitation to keep the total analysis time reasonable, i.e., a couple of hours per sample.

Combined impactor and XRPD methodology: A tiotropium bromide case study

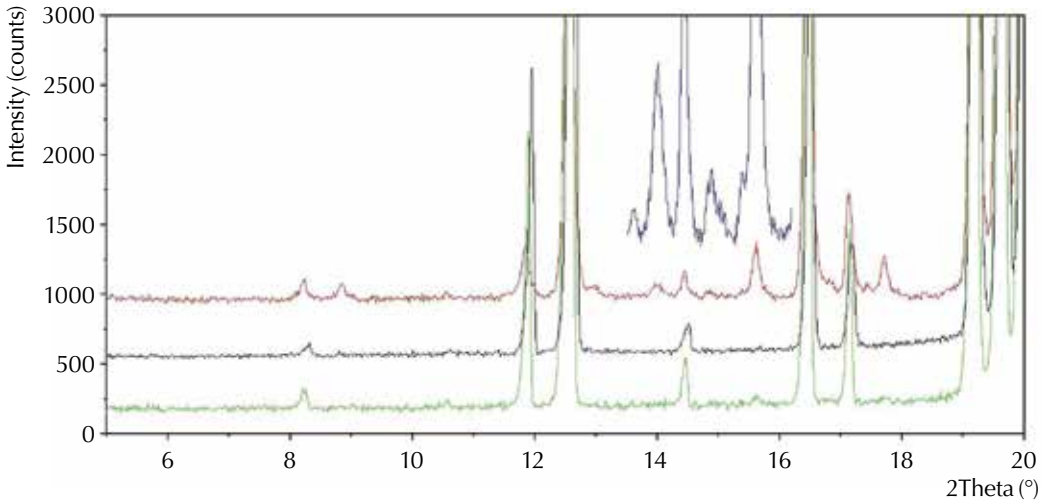
A method was developed to detect and possibly quantify, in this case, the undesired tiotropium bromide monohydrate form in the desired tiotropium bromide anhydrate form in a test formulation blend for inhalation. The total tiotropium bromide concentration in the test formulation was 0.27%. The other 99.73% was an α-lactose monohydrate made up of coarse carrier particles mixed with about 10% of finer particles. The formulation type was an adhesive mixture.

The first step in the development of a quantitative method is to find suitable intense and preferably non-overlapping peaks for the two components, tiotropium bromide anhydrate and tiotropium bromide monohydrate. Interference from α-lactose monohydrate peaks should also be avoided. Diffractograms for the two tiotropium bromide forms, together with a diffractogram for α-lactose monohydrate, are shown in Figure 3. A diffractogram for an approximately 15% mixture of tiotropium bromide monohydrate in tiotropium bromide anhydrate is also shown to better illustrate which peaks could be used for quantitation purposes. A very intense peak at 14.9°(2Θ) from the monohydrate seems suitable, although there is a slight ovelap with a small anhydrate peak. The peak at 18.3°(2Θ) could also be used for the monohydrate detection. This peak is free from overlap but was, in this case, discarded due to the lower intensity. The peak at 15.6°(2Θ) was selected for the anhydrate.

A diffractogram for the 0.27% tiotropium bromide inhalation formulation using standard X-ray diffraction settings is shown in Figure 4. When compared to a diffractogram of pure α-lactose monohydrate, it is clear that no tiotropium bromide anhydrate or monohydrate can be detected.

Figure 4

Diffractograms for 0.27% tiotropium bromide inhalation formulation (green) and α-lactose monohydrate (black), using standard X-ray diffraction settings. Diffractogram for 0.27% tiotropium bromide inhalation formulation after concentration by impaction (red) using standard X-ray diffraction settings. Diffractogram for 0.27% tiotropium bromide inhalation formulation after concentration by impaction (blue) using optimized X-ray diffraction methodology. The peak at 14.9°(2Θ) indicates the monohydrate form.



In order to concentrate the tiotropium bromide content, the coarse carrier lactose particles were removed by impaction in the preseparator and Stage 1 of an NGI. The particles, smaller than 8.9 µm in aerodynamic size, passing through the preseparator and Stage 1, were collected on a filter and transferred to an X-ray diffraction sample plate. This procedure gave a 10-fold increase in tiotropium bromide content. In Figure 4, a diffractogram of the sample after impaction shows characteristic peaks for the tiotropium bromide anhydrate.

To improve the detectability of the monohydrate form, the X-ray diffraction method was optimized by spending more time on each angular position and halving the angular steps between measuring points. The improved methodology increased the signal-to-noise considerably and improved the peak shapes as well. The result is clearly seen in Figure 4, where now traces of the undesired monohydrate form are visible.

Therefore, the combination of concentration by impaction and optimization of the X-ray diffraction methodology allows detection of fairly small amounts of an undesired polymorph in an inhalation formulation of low API content.

A set of calibration mixtures was prepared and analyzed to determine if it was possible to quantify the undesired monohydrate form in concentrations comparable to those obtained after impaction of the 0.27% inhalation formulation.

The calibration blends with lactose were prepared in three steps. First, an anhydrate:monohydrate 85:15 mixture was prepared by gentle grinding in an agate mortar. Then two stock mixtures were prepared by sieving tiotropium bromide and lactose monohydrate fines through a 500 µm net three times. Both stock mixtures contained about 3% tiotropium bromide in total. One of the stock mixtures contained only tiotropium bro-

Figure 5

Calibration mixtures with 2.4% (red), 4.9% (blue), 6.6% (green) and 14.4% (black) monohydrate.

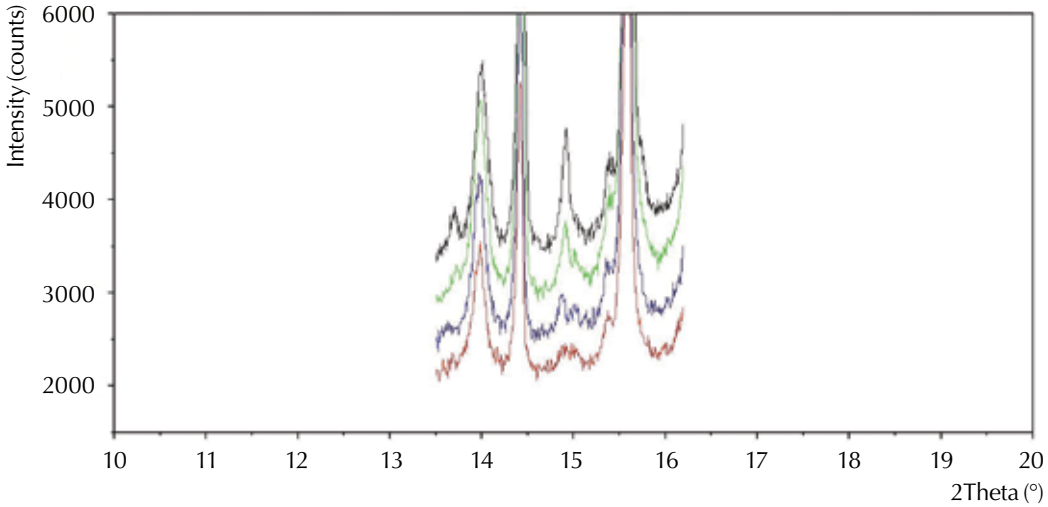
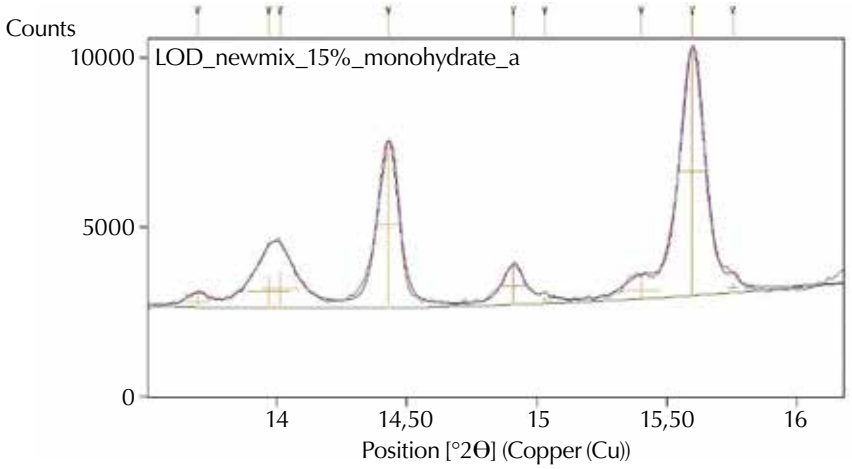


Figure 6

Profile-fitting of 15% monohydrate calibration sample, API and lactose blend; monohydrate signal at 14.9° (2Θ) and anhydrate signal at 15.6° (2Θ).



mide anhydrate and the other contained the 85:15 mix described above. Finally, the two stock mixtures were weighed together in the desired amounts and gently ground in an agate mortar to produce calibration blends with monohydrate contents in anhydrate of 3-15%.

All calibration samples were analyzed with XRPD according to the optimized method; examples are shown in Figure 5. The API/lactose blends were evaluated by applying profile-fitting to the resulting diffractograms (PanAlytical HighScore Plus Software, Version 4.7), to better evaluate the anhydrate and monohydrate peaks that are used for the calculation of monohydrate content. Both these peaks clearly overlap with other minor peaks that are not used in the calculation. Thus, it would be advantageous to diminish the effect of these minor peaks.

Profile-fitting is used to decompose a complicated powder pattern. A mathematical profile function is fitted around observed peak positions. The goal is to obtain better peak parameters completely describing the peaks, even when they partially overlap each other.

Examples of a diffractogram where profile-fitting has been applied are displayed in Figure 6. The peaks used for the determination of monohydrate content can be found at 14.9° (2Θ) (monohydrate) and 15.6° (2Θ) (anhydrate).

Quantification by XRPD in a two-component system relies on determination of peak ratios. In this case, the monohydrate peak at 14.9° (2Θ) and the anhydrate peak at 15.6 ° (2Θ) were chosen for the determination. The peak heights from profile-fitting showed to be the best option for construction of the calibration curves since the precision was clearly superior to the peak area determinations.

The peak intensities are proportional to the content of the components

$$I(M) = k1 * X(M) \text{ and } I(A) = k2 * X(A)$$

where *M* is monohydrate, *A* is anhydrate, *X* is the fraction of the component in the sample and *k1* and *k2* are constants.

The ratio of intensities is used to determine a single constant *K:*

$$\frac{I(M)}{I(A)} = K * \frac{X(M)}{X(A)}$$

When rearranged, this gives:

$$\frac{I(M) * X(A)}{I(A)} = K * X(M)$$

Since both *X(A)* and *X(M)* are known for the calibration samples and the ratio *I(M)/I(A)* can be measured, a plot should result in a straight line with *K* as the slope.

Linear regression, forcing the line through zero was used, giving an equation of the line with Y = 0.9336X (R^2 = 0.9745).

Using the determined *K* and rearranging, it is possible to get an equation for monohydrate content (%) in an unknown sample.

For formulation samples after impaction:

$$\%(M) = 100 * \left(\frac{I(M)}{0.9336 * I(A) + I(M)} \right)$$

Six runs at the low end of the calibration curve for the tiotropium bromide/lactose mixtures were used for determination of noise level. The mean height for the anhydrate peak in the six runs, together with the mean noise level, was used to estimate limit of detection (LOD) and limit of quantitation (LOQ). LOQ was calculated as 10 times the noise level and resulted in a value of 11% tiotropium bromide monohydrate. LOD was determined as LOQ/3-4% monohydrate. The LOD corresponds well with visual observations of diffraction data (Figure 5) for samples with low monohydrate content.

The presented LOD of 4% monohydrate of the total tiotropium bromide content corresponds to 0.01%

of the test product blend, while the LOQ corresponds to 0.03%.

All work was performed under controlled relative humidity (RH) of <30% and in a timely manner to avoid possible transformation of the tiotropium bromide anhydrate form to the monohydrate form. In-depth studies on phase transformation kinetics from tiotropium bromide anhydrate to monohydrate at various RHs were performed prior to the impactor/XRPD method development work to assure safe handling however, those results are not presented in this article.

Some views on alternative methods

As mentioned in the introduction, a range of spectroscopic techniques can be applied for detection and quantification of polymorphs and solvates but none can directly provide the same capacity as the presented combined impactor/XRPD methodology. However, if combined with the impactor concentration step, Raman spectroscopy should theoretically be able to generate the same type of performance as XRPD. Raman has specific advantages in relation to lactose formulations since lactose does not containing any carbonyl or aromatic functional groups as most pharmaceutical small organic compounds do. In other words, Raman spectroscopy has good discriminatory power when studying APIs in lactose formulations as well as crystal component detection capacity similar to XRPD.

Solid-state nuclear magnetic resonance may be of less interest in combination with impaction concentration since it requires large sample volumes. It also may be limited by comparably long measurement times and lack of autosamplers, which can make it a demanding technique for supporting development activities or stability studies involving multiple sample analyses. Near-infrared and infrared may have both worse detection and poorer polymorph and hydrate resolution capacity compared to, for example, XRPD and Raman.

The most promising alternative approach may be analysis using synchrotron radiation. However, just as in the case of solid-state NMR, the technique is not really applicable in situations when frequent and rapid feedback is needed, for example, during formulation and process optimization. Synchrotron analyses normally requires pre-booking of a beam-line station as well as handling of samples in a non-RH-controlled environment. It also requires travelling to the facility in question or shipping samples to personnel and depending on them to ensure that critical sample-handling techniques are completed. If only crystal component detection capacity is considered, the synchrotron option is, of course, a highly recommended alternative. Interestingly, a synchrotron study of polymorphic forms of tiotropium bromide in lactose powder blends has recently been reported and presented a detection limit of 0.4%,[6] i.e., clearly not matching the performance of the impactor/XRPD approach described in this paper.

The combination of impaction concentration and synchrotron analysis would, of course, be the most powerful methodology for detection of API polymorphs in adhesive mixtures. But as explained, such methodology would be more useful for single case investigations, such as patent infringement cases, rather than for routine support during product development.

Conclusions

A generally applicable methodology for detection and quantification of API polymorph impurities in typical DPI adhesive mixtures has been developed, using a test formulation similar to Spiriva as model system.

The method is based on two steps; firstly, concentration of the API content via impaction and, secondly, applying optimized slow scan XRPD for evaluation of the polymorph impurity content. The presented method gave an LOD of 0.01% and an LOQ of 0.03% relative to the total blend.

Once developed, the method has the capacity to run multiple samples per day and is therefore highly suitable for routine support during formulation and process optimization and in stability studies. A critical factor for success, however, is that the presented methodology must be performed in a laboratory that has the ability to control the relative humidity.

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