MicromATR vision

Confirming Pharmaceutical Active Ingredients by FTIR with the MicromATR Vision™ Diamond ATR Accessory

Pharmaceutical Regulatory Compliance and Quality Assurance/ Quality Control

Introduction

Raw material validation and qualification is performed in the Pharmaceutical industry to confirm the purity and genuine composition of active and excipient raw ingredients. This verification is a critical quality control measure to avoid counterfeiting in globally sourced raw materials and final products. It is also an important quality assurance and quality control measure to avoid contaminated or impure materials from entering a manufacturing or mixing process. Fourier Transform Infrared Spectroscopy (FTIR) is one of the most rapid and convenient techniques to identify and qualify a pharmaceutical ingredient. One particularly useful FTIR sampling technique is attenuated total reflectance (ATR). ATR measurement of a sample requires minimal sample preparation in order to obtain a high quality infrared (IR) spectrum. ATR eliminates sample thickness and other difficulties observed with traditional sample preparation techniques for FTIR measurement. This allows for a greater number of materials to be tested and a much faster turnaround time for lab analysis.

The United States Pharmacopoeia (USP) has recognized FTIR, and its sampling techniques, as highly specific for the identification of a specimen. The USP Section <197> "Spectrophotometric Identification Tests"[1] states: "The IR absorption spectrum of a substance, compared with that obtained concomitantly for the corresponding USP Reference Standard, provides perhaps the most conclusive evidence of the identity of the substance that can be realized from any single test." ATR is recognized as a legitimate technique in the USP section 197, referenced as <197A>, and states that the specimen under examination is "intimately in contact" with the ATR crystal, which is typically a diamond. The MicromATR Vision accessory incorporates an internal viewing scope that allows the analyst to observe the sample making contact with the diamond ATR crystal. This feature is especially useful to confirm the intimate sample to diamond contact of very small amounts of solids, such as single crystals or granules of active pharmaceutical ingredients (API). The video microscopy of MicromATR Vision also allows the observation of crystal habit, an additional discerning property of solid materials. USP <197> also states that ATR can be used as an alternative test method as long as the specimen and UPS Reference Standard for that material are "concomitantly measured". The USP section <851>[2] defines this requirement for FTIR and ATR as measuring the specimen and reference standard in immediate succession. Examples of this USP validation analysis applied to Atorvastatin, a cholesterol-lowering drug, will be described in this application note. Expected variation in the IR bands for pure anhydrous and hydrate forms will be discussed along with the analysis of oxidized Atorvastatin. The ability to differentiate between pure and degraded API products by ATR is analogous to identifying counterfeit drugs with similar composition but not genuine sourced product.

Instrumentation

Figure 1: The MicromATR Vision press tip and diamond ATR puck after measuring a methanol cast film on the ATR. Both parts are easily removed for convenient cleaning, inspection, or for trace sample placement using a stereo microscope.

The MicromATR Vision diamond ATR accessory was used to measure all the specimens. The diamond crystal is impervious to scratching, abrasion, or chipping and allows the collection of spectra to the full available mid-IR frequency range, typically 6000-400cm-1 on most FTIR spectrometers. The spectra were collected using a commercial FTIR spectrometer at 4 cm-1 resolution with a 1 min collection time. ATR accessories produce a high quality spectrum simply by placing a sample directly on the ATR crystal, for powders and polymers the press is used to force the sample into optical contact with the crystal. The diamond ATR plate of the MicromATR is made to be conveniently removed for easy cleaning (Figure 1), or to save the sample material for analysis with other techniques. The press tip magnetically snaps on and off the press assembly for easy cleaning and inspection (Figure 1). These features also allow the placement of trace samples for micro-sampling (i.e. single API crystals, fibers, hairs, or contaminant particles) on the center of the diamond using a stereomicroscope or other viewing aids.

MicromATR Vision™ Benefits

- Simple FTIR analysis with no sample preparation
- Press swings high to accommodate large samples and allow easy cleaning
- The press tip and ATR plate (puck) magnetically snap on/off for convenient cleaning and micro-sampling
- Built in scope camera allows visual confirmation of ATR contact
- Scope images are easily captured using Czitek eSpot video capture software

Materials and Methods

Pure Atorvastatin was sourced directly from the pharmaceutical manufacturer. Sigma-Aldrich anhydrous methanol was used to dissolve the Atorvastatin for sample preparation experiments. The Atorvastatin pure sample (8mg) was dissolved in 0.5mL of methanol and the FTIR analysis was performed before and after exposure to 300uL of a 3% hydrogen peroxide solution heated for 48 hours of 80°C in order to induce oxidation of the Atorvastatin.

Results and Discussion

Traditional FTIR techniques such as KBr discs, nujol mulls, and cast films are recognized sample preparations for all the world's pharmacopeias. They are all transmission IR analysis techniques which have been practiced in IR spectroscopy for the past 65 years or more. The quality of the spectra produced from these techniques is highly dependent on the skill of the analyst. So one can imagine the difficulty matching a poorly prepared reference API material's spectrum to an experimental or in-process API spectrum with unknown purity. Thickness, uniformity, and water vapor interference are the three main factors that can confound these transmission IR measurements. ATR analysis has eliminated each of these skill dependent sample preparation difficulties and has quickly grown in popularity in the pharmaceutical industry. However, there are still some nuances of FTIR that are present in ATR analysis, and need to be understood to avoid improper classification.

Pure API USP Validation

Infrared spectroscopy is very sensitive to crystallinity or polymorphism, and pure API's are often prone to having different polymorphs. Polymorphs have the same chemical structure but with different crystal packing. The crystal packing or crystal structure of a pure API can change over time, due to factors such as heat, hydration, solvation, or physical processes (i.e. grinding, sheer forces). Many of these crystallinity changes are due to the preference of API's to form lower energy hydrogen bonding (H-bonding) orientations. The changes induced by crystallinity in IR spectroscopy are often misinterpreted as impurity or contamination.

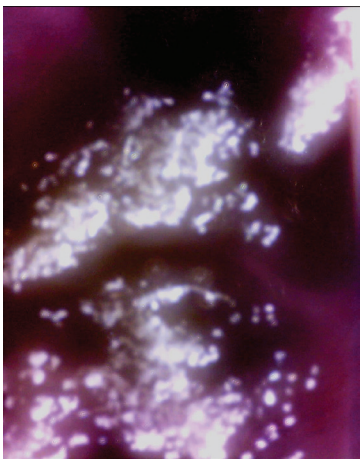

Figure 2: Video micrograph of Atorvastatin observed on MicromATR Vision.

Atorvastatin is a great example of crystallinity changes. Figure 2 is a video micrograph of Atorvastatin obtained directly from the manufacturer observed in direct contact

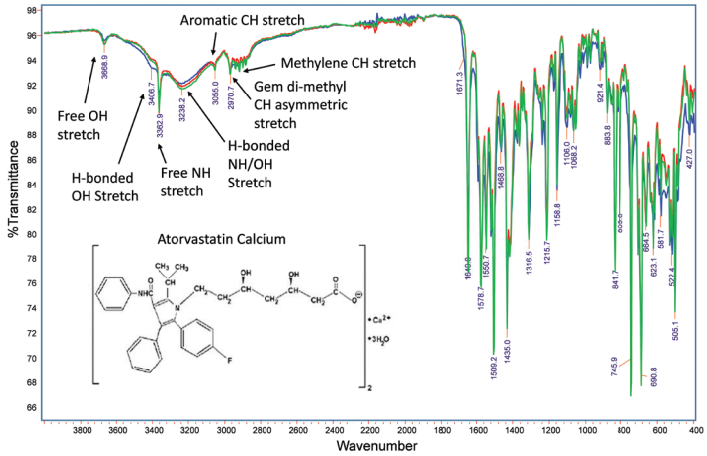

Figure 3: The overlaid FTIR ATR spectra of the Atorvastatin pure reference standard measured "as is" on two separate days (red and green) compared to the ATR spectrum after grinding (blue). The difference observed is representative of sample to sample differences expected from a passing USP qualification.

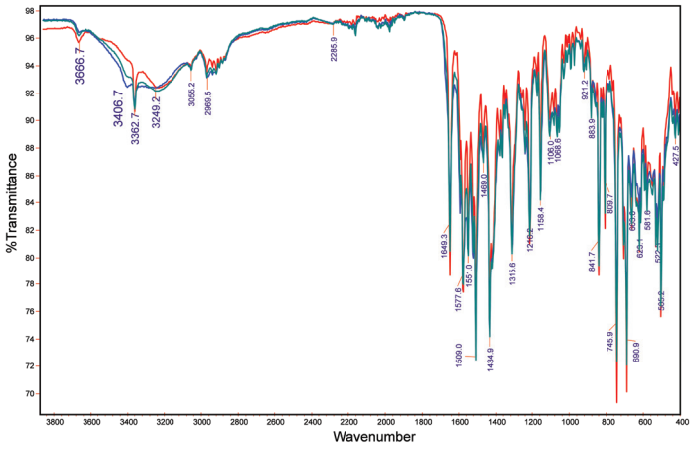

Figure 4: The overlaid FTIR ATR spectra of the Atorvastatin pure reference standard measured "as is" (red), after grinding (green), after second grinding on the ATR plate (blue).

with the diamond ATR crystal. The crystals are finely divided and milled to a small particle size. Figure 3 shows IR spectra of pure Atorvastatin "as is" without any sample preparation in two separate samplings (red and green), and after grinding with a mortar & pestle (blue). The red and green spectra indicate the expected variation from an ideal match of reference USP Atorvastatin and a "concomitantly measured" Atorvastatin sample to be qualified from a process. The blue spectrum in Figure 3 indicates a slight changes in crystallinity due to the grinding process. This same change in crystallinity is observed to progress the longer the sample is measured in repeated sampling on the ATR (Figure 3). The level of change in IR bands in Figure 3 indicates H-bonding differences are occurring as the sample is ground or manipulated on the ATR. The band at 3669cm-1 is consistent with a free OH (not H-bonded) whereas the 3407cm-1 is consistent with a more H-bonded OH group. The more crystalline "free OH" at 3669cm-1 is observed to decrease as the 3407cm-1 band increases as the Atorvastatin changes crystallinity. Likewise the NH stretch group at 3363cm-1 is "free NH" and the H-bonded NH stretch is observed at 3238cm-1. The hydrocarbon CH stretch bands are not affected by changes in crystallinity (Figure 3&4). The differences observed in Figure 4 might be considered a failure match to a USP standard. In such a case USP <197> states that both the reference USP standard and experimental sample be dissolved similarly in methanol or other suitable solvent and re-measured as a fully dried cast film. The methanol cast film on the MicromATR is shown in Figure 5, and overlaid with the Japanese published FTIR spectrum of Atorvastatin measured as a KBr disc. The MeOH cast film produces amorphous Atorvastatin, and the KBr disc spectrum (black) indicates a mixture of the amorphous (red) and crystalline (blue) Atorvastatin. These observations indicate the ATR FTIR measurement of a reference standard induces less crystallinity change to a sample compared to the KBr disc technique.

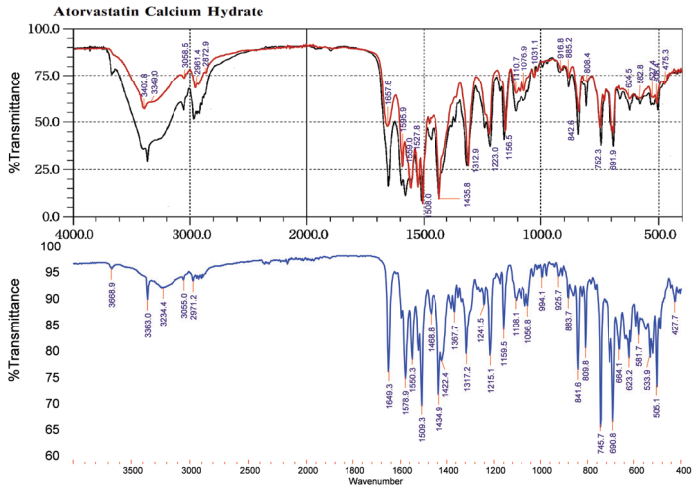

Figure 5: The FTIR ATR spectra of Atorvastatin pure reference standard, measured as a MeOH cast film (red), overlaid with the Japanese pharmacopoeia's publish spectrum of Atorvastatin. The blue spectrum is the FTIR ATR spectrum of Atorvastatin pure reference standard measured "as is" without grinding.

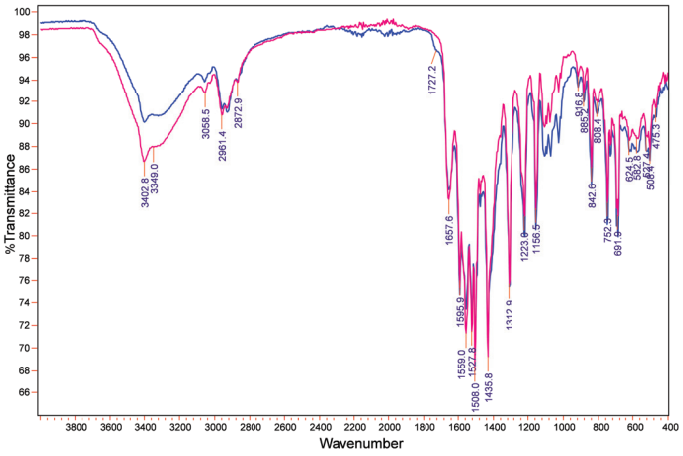

Figure 6: The overlaid FTIR ATR spectra of the Atorvastatin pure reference standard measured as a MeOH cast film (red) and after 48 hours exposure to hydrogen peroxide and heat (blue)

API Oxidation

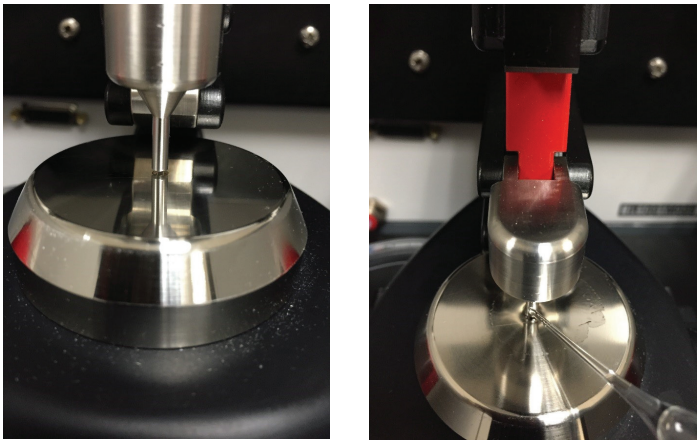

Figure 7: The procedure for casting a film on the MicromATR Vision by placing the press tip low enough to hold the solvent in place over the diamond until dry.

With the differences expected due to crystallinity explained above, what does a true contamination spectrum of an API look like? Oxidation of an API is a common route for failed qualification testing by FTIR or other analytical techniques. Atorvastatin dissolved in methanol was exposed to 3% hydrogen peroxide solution and heated for 48 hours to induce oxidation. The reference pure and oxidized Atorvastatin are measured as MeOH cast films (Figure 6) on ATR using the MicromATR. The MeOH cast films are easily measured with the MicromATR Vision with the press tip placed ~1mm above the diamond as shown in Figure 7. Capillary action holds the MeOH solvent in place so it forms a quality cast film over the diamond. Using a stream of dry air or nitrogen over the ATR puck makes the process faster and guarantees the solvent is completely evaporated. The oxidized Atorvastatin (Figure 6) indicates a new absorbance peak at 1727cm-1 consistent with an oxidation carbonyl stretch from an ester or carbamate functional group. Additional absorbance in the 1150-950cm-1 region of the oxidized Atorvastatin is consistent with C-O-C ether and C-OH alcohol functional groups from oxidation products. Such new peaks observed in the ATR spectra measured "as is" or as a MeOH cast film would indicate the presence of an impurity or degradation products in an API.

Conclusion

We have shown that MicromATR Vision™ ATR sampling accessory produces high quality FTIR spectra with minimal sample preparation. The MicromATR Vision ATR accessory has detachable ATR plates and sample press tips that make cleaning, visual inspection, and micro-sampling more convenient than traditional ATR accessories. The ATR spectra produced can be used to validate API raw materials in pharmaceutical applications and is an accepted USP technique. Differences can be observed in the ATR FTIR spectra of API materials due to crystallinity. If these differences are observed, then the sample can be dissolved in methanol and measured as a dried cast film on the ATR. This produces spectra of the same crystallinity for comparison of a reference API and an experimental sample. Some published pharmacopoeia spectra may contain a mix of crystallinity forms of the API, which could be misinterpreted as a match failure to an experimental API spectrum. Therefore, the experimental sample should be measured immediately after the validated reference pharmacopoeia standard. Oxidized or degraded API material will produce new bands in the carbonyl and C-O stretch regions of the IR spectrum. The oxidation bands are easily distinguishable when compared to the reference pure spectrum of the API. The unique features of the MicromATR Vision™ accessory provide a sensitive and convenient technique to validate the identity of pharmaceutical materials and ensure that out-of-spec materials are caught before entering a manufacturing process or final product formulation.

References:

1. USP 34-NF 29 General Chapter <197>, "Spectrophotometric Identification Test".

2. USP 34-NF 29 General Chapter <851>, "Spectrophotometry and Light Scattering".

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