Quantitative measurement of active pharmaceutical ingredients using the diffuse reflectance Cary 630 FTIR

Application note

Pharmaceuticals

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Introduction

Fourier transform infrared (FTIR) spectroscopy is commonly used in the pharmaceutical industry for the identification of drug products, as specified in the US, European, Japanese, Chinese, Indian and International Pharmacopoeias. Like all optical spectroscopic techniques, FTIR can also provide quantitative information and can be used to predict the concentration of pharmaceuticals in both solid and liquid states. FTIR follows the Beer-Lambert law, thus the absorbance of a sample is directly proportional to the concentration, the pathlength and the molar absorptivity. Since the molar absorptivity of a molecule is constant and the measurement pathlength can be controlled, the absorbance of a sample is directly proportional to the concentration.

In FTIR spectroscopy, sample preparation and sampling methodology often defines the success of the measurement. For solid samples, the classic transmission measurement technique involves dispersing the sample in potassium bromide powder (KBr) and then placing the salt mixture under pressure to form a transparent pellet. In addition to the difficulties associated with making acceptable KBr pellets, variance in mixing and the

dimensions of the resulting pellet make this technique less useful for quantitative measurement, though it is still used for qualitatively ascertaining the identity of a compound. For ease of use, analytical accuracy and speed of measurement, analysts have turned to other FTIR sampling methodologies for analysis of pharmaceutical compounds that are solids — notably attenuated total reflectance (ATR) and diffuse reflectance infrared Fourier transform (DRIFT).

This application note will demonstrate the effectiveness of the DRIFT method for the quantitative measurement of a powdered sample using the Agilent Cary 630 FTIR system. Mixtures of acetaminophen in corn starch are used to demonstrate the analysis of the active pharmaceutical ingredient (API) in the presence of an excipient.

Analysis of pharmaceutical compounds using the Cary 630 FTIR

The Cary 630 FTIR spectrometer is ultracompact, robust, easy to use, and possesses class-leading performance. With its optimized sampling technology, the Cary 630 is an exceedingly useful spectrometer for QA/QC, analytical services and method development in the pharmaceutical industry. The Cary 630 is well matched to these applications due to its interchangeable sample interfaces that allow easy switching between ATR for qualitative sample identification, and diffuse reflectance for quantitative analysis. Additionally, the Cary 630 is available with Agilent's exclusive DialPath technology, which makes quantitative and qualitative analysis of liquid samples equally quick and easy. With CFR 21 part 11 compliant software and automated IQ/OQ capability, the Cary 630 is well suited to pharmaceutical analysis.

For qualitative analysis, the Cary 630 ATR sampling technology is particularly useful as a replacement for the KBr pellet transmission method, since it allows quick measurement of neat solids without any sample preparation. The system employs a diamond ATR element that eliminates scratching of the window and is highly resistant to damage by chemicals. The innovative sample press ensures proper contact of the sample with the diamond ATR for best spectral results, while at the same time eliminates the possibility of overpressure on the ATR crystal. Since ATR measures infrared absorbance via an evanescent wave that penetrates just a few microns, the sample pathlength is quite short. This makes ATR quite useful for qualitative analysis of solids, but less useful for quantitative analysis of solids.

For quantitative analysis of powdered or coarse grained solids, DRIFT is ideal, since it provides both easy sample preparation and quantitative precision. With the DRIFT technique, diffusely reflected light interacts with sample particles several times before being collected by the instrument collection optics. These multiple interactions give the DRIFT technique a larger sampling volume, decreasing issues associated with heterogeneous mixtures. In some cases, strongly absorbing samples are diluted in KBr before analysis, but the powdered mixture is simply placed into a sample cup eliminating the preparation difficulties associated with the classic transmission pellet-making technique.

Experimental conditions

Five calibration samples ranging from 0 to 10% acetaminophen in corn starch were prepared. These samples were split into two lots. One lot was diluted in dry KBr in a ratio of 1:10 (sample:KBr) before analysis, whereas the second set was measured neat, without any sample preparation. All measurements were carried out on an Agilent Cary 630 FTIR incorporating a diffuse reflectance sample interface. Data was recorded with a 30 second collection time (74 scans) at 4 cm^{-1} resolution. The Cary 630 MicroLab PC software was used for data collection, and calibrations were developed using Agilent Resolutions Pro software.

Results and discussion

Comparison of DRIFT measurements in diluted and undiluted samples

Diffuse reflectance measurement of powdered samples typically results in a relatively long pathlength. Figure 1 shows a pictorial representation of the light path at the sample. It shows that several paths are possible, increasing the interaction of the infrared light with the sample. The long pathlength resulting from these multiple interactions often causes concentrated samples to have absorbance values beyond the dynamic

range of an instrument. Additionally, strong absorbances reduce the amount of light returning to the instrument detector, which results in higher noise.

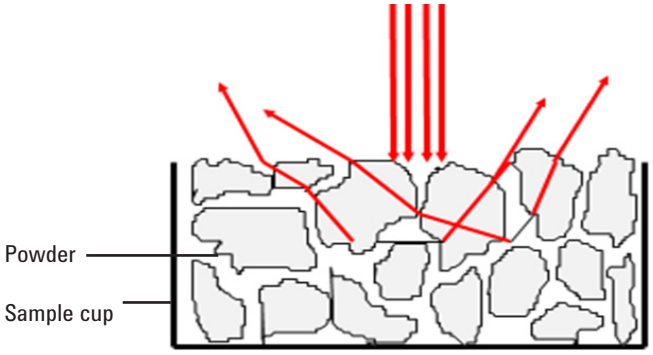

Figure 1. Pictorial diagram of diffuse reflectance sample measurement of a powdered sample

In order to reduce the overall absorbance, samples are often mixed with a non-absorbing, diffusely reflecting salt such as KBr. The salt reduces the concentration of the sample allowing the absorbance values to fall within the linear range. To demonstrate this effect, a sample of acetaminophen in corn starch was measured with and without dilution in dry KBr. Figure 2 shows the measured spectra of the sample with (red) and without (blue) dilution. In the undiluted sample, the absorbance of both the OH stretch near 3400 cm^{-1} and the CO stretch near 1100 cm^{-1} are above 2.0 absorbance units, resulting in a large amount of noise on the strongly absorbing bands.

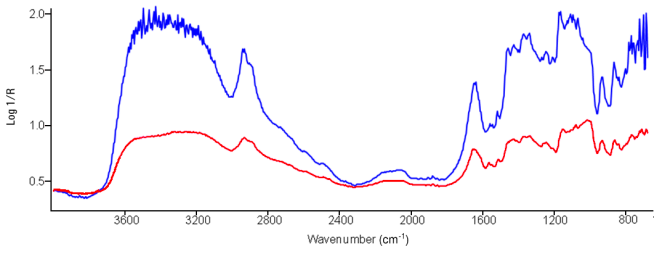

Figure 2. DRIFT measurement of neat (blue) and diluted (red) acetaminophen in corn starch

It should be noted in the above example, that the noise present is on bands associated with the corn starch excipient. The lower intensity bands arising from the acetaminophen are not affected. The most prominent absorbance bands of the acetaminophen at 1559 cm^{-1} and 1513 cm^{-1} are still within their linear range.

Quantitative measurement using diffuse reflectance — diluted samples

Dilution of the 5 calibration standards produced optimized absorbance values between 0.6–0.8 absorbance units for the strongest band as is shown in Figure 3; the calibration curve is shown in Figure 4. The 1559 cm^{-1} acetaminophen band is used for this calibration, and is ratioed to a reference frequency at 1379 cm^{-1} (resulting from corn starch). Acetaminophen has minimal absorbance at 1379 cm^{-1}; this frequency can therefore be used as an internal standard to correct for any pathlength difference caused by particle size variation. The calibration shows excellent linearity and a correlation coefficient of 0.999.

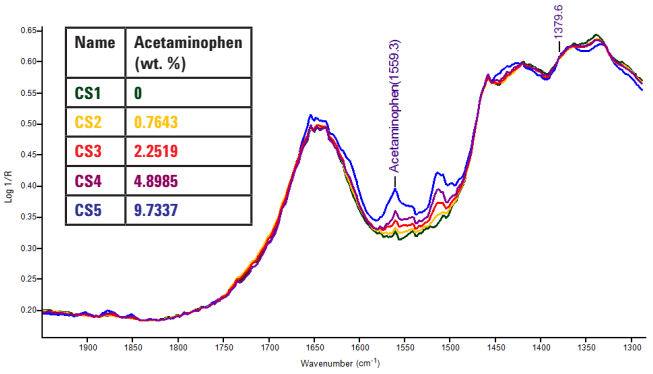

Figure 3. Carbonyl region of the FTIR diffuse reflectance calibration spectra of acetaminophen in cornstarch measured after dilution in KBr powder

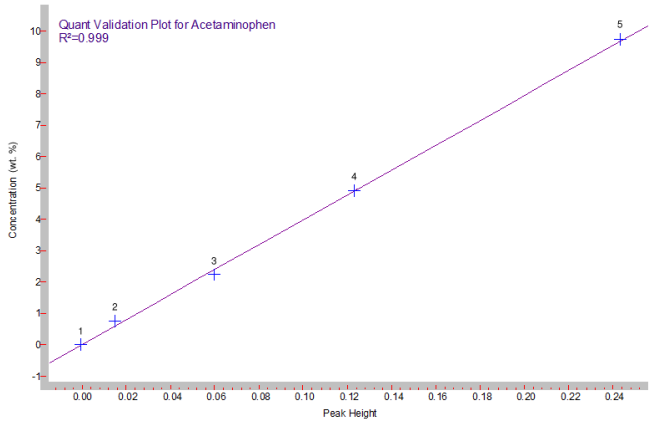

Figure 4. Calibration plot of the acetaminophen peak height at 1559 cm^{-1} ratioed to the 1379 cm^{-1} peak height absorbance. The samples are diluted 10:1 in KBr prior to measurement.

Quantitative measurement using diffuse reflectance — neat samples

The 5 calibration standards were measured without dilution in KBr powder, and the spectra of the calibration set are shown in Figure 5. A quantitative calibration was made by correlating the ratio of the same acetaminophen band area centered at 1559 cm^{-1}, to the corn starch CO overtone near 2100 cm^{-1}. This overtone band was chosen since the 1379 cm^{-1} band that was used for the diluted samples was clearly too absorbing. The calibration plot is shown in Figure 6; a perfect correlation coefficient of 1.000 was found for this calibration.

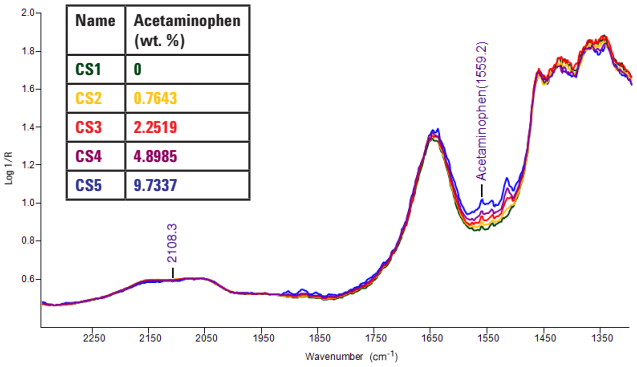

Figure 5. Carbonyl region of the FTIR diffuse reflectance calibration spectra of acetaminophen in cornstarch measured neat without dilution

Conclusions

Diffuse reflectance is shown to be an excellent technique for the quantitative analysis of pharmaceutical solids and powders, eliminating the need to prepare pressed KBr pellets, which

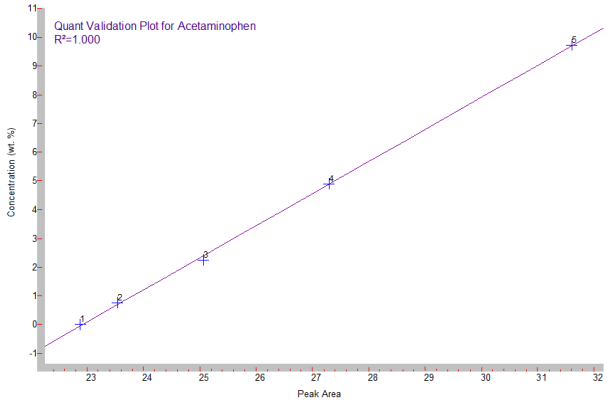

Figure 6. Calibration plot of the acetaminophen peak area absorbance at 1600–1500 cm^{-1} ratioed to the area near 2100 cm^{-1}. The samples were measured neat by diffuse reflectance FTIR.

are commonly used in classic transmission FTIR spectroscopy. In this case, the neat samples produced a calibration which was as accurate as that of the diluted samples. This is due to the fact that the bands of the neat active ingredient remained in the linear absorbance range, while those of the excipient were too absorbing for accurate analysis. In some cases, where both active and excipient bands are too absorbing and thus non-linear, simple dilution in powdered KBr is all that is necessary to get accurate quantitative measurements.

The interchangeable, easy-to-use sampling technologies available for the Agilent Cary 630 FTIR are ideal for quantitative and qualitative analysis in the pharmaceutical industry — DRIFT for quantitative analysis of solids; ATR for qualitative identification of solids; DialPath for quantitative and qualitative analysis of liquid samples.

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Published November 29, 2011
Publication number: 5990-9414EN

Agilent Technologies