Identification of a pharmaceutical tablet's origin using FT Near-IR and Principal Component Analysis

Application Note

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Introduction

After 10–15 years and $800 million[1], a pharmaceutical company developed a new drug candidate that successfully passed all the Food & Drug Administration (FDA) mandated clinical trials and is now ready for sale to patients in the United States[2]. There is a clear reason for the eagerness of drug companies to manufacture a generic version of its pioneer equivalent once it is no longer under patent protection; in 2006 Pfizer reported over $1.5 billion in sales of its Zyrtec product, which was losing patent protection in September of that year. To protect the consumer, in 1984 the FDA passed the Waxman-Hatch Act[3] that requires generic drugs to maintain the same bioequivalence and pharmaceutical equivalence as their pioneer counterpart, while the excipients can vary.

Both mid-infrared (Mid-IR) spectroscopy, the spectral region from 4,000 cm^{-1} to 400 cm^{-1}, and near infrared (Near-IR) spectroscopy, the spectral region from ~12,500 cm^{-1} to 4,000 cm^{-1}, have the capability of identifying active pharmaceutical ingredients (API) and excipients of pharmaceutical products. Each region has advantages that will be discussed and the use of Near-IR spectroscopy for the differentiation of Zyrtec and its cetirizine generic counter parts will be highlighted.

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Instrumentation

Infrared spectra for this investigation were collected on an Agilent Cary 660 FTIR spectrometer configured for both the Mid-IR and Near-IR (retroreflector glowbar and tungsten halogen sources; KBr and quartz beamsplitters; and both DLaTGS and InGaAs detectors). Chemical imaging data were collected on a 660 FTIR/ 620 FTIR Imaging system with a 64 × 64 focal plane array (FPA)[4] detector utilizing Agilent's ATR-Imaging technique. All data were collected and processed using Resolutions Pro 5.0 software and post-processing chemometric analyses of the Near-IR data were performed using Bio-Rad's KnowItAll software version 8.0.2 with AnalyzeIt MVP[5]. Table 1 shows the FTIR collection parameters used for the experiments.

Table 1. FTIR collection parameters

Parameter	Value
Number of sample scans	32
Number of background scans	64
Spectral resolution (cm^{-1})	4
Apodization	Norton-Beer Medium
Collection symmetry	Symmetrical
Speed (kHz)	5 (DLaTGS) 10 (InGaAs)
Smoothing algorithm	None
Post processing	Automated CO_2 & Water Subtraction
Sampling accessories	Mid-IR: Pike MIRacle Diamond/ZnSe ATR Near-IR: Pike Near-IR Diffuse Reflection Integrating Sphere Micro slide on ATR

Materials and reagents

The active component of Zyrtec tablets is commonly referred to as cetirizine hydrochloride (empirical formula is $C_{21}H_{25}ClN_2O_3$•2HCl). It is also known by the chemical name (±) - [2- [4- [(4-chlorophenyl) phenylmethyl] -1- piperazinyl] ethoxy]acetic acid, dihydrochloride and is a racemic mixture. The compound's molecular weight is 461.82 g/mol, and its chemical structure is shown in Figure 1.[6]

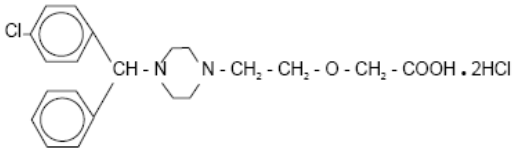

Figure 1. Chemical structure of the active pharmaceutical ingredient in Zyrtec

Cetirizine HCl samples from 6 separate distributors were used in this experiment, with 5 tablets being randomly selected from each container. Each tablet was run in triplicate using both Mid-IR and Near-IR spectroscopy. The tablets were rotated and/or flipped over between repeat spectral collections to change the sampling location. Table 2 lists the sample source information.

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Table 2. Zyrtec/cetirizine samples used

Drug API	Drug Label	Distributor	Lot Number or UPC Number	Manufacturer
Cetirizine 10 mg	Zyrtec	Merck-Medco	23214517220003	Pfizer
Cetirizine 10 mg	Zyrtec OTC	McNeil Consumer Health	123296	McNeil Consumer Health
Cetirizine 10 mg	N/A	Walmart	TME0914	Perrigo (Allagan, MI)
Cetirizine 10 mg	N/A	CVS	21727188	Dr. Reddy's Lab LTD (India)
Cetirizine 10 mg	N/A	Rite Aid	21727138A	Dr. Reddy's Lab LTD (India)
Cetirizine 10 mg	Tradaxine	N/A	N/A	SBL Pharma-ceuticals (Mexico)
Cetirizine 10 mg	Zyrtec	Merck-Medco	23214517220003	Pfizer

Sampling techniques

Mid-IR spectra provide distinct patterns for many compounds and can be used to correlate a functional group to a spectral peak. This information is then used for both qualitative (providing a distinct fingerprint to answer the question, 'What is it?') and quantitative information (using a peak or set of peaks to find out how much of a substance or analyte is present). Depending on the sensitivity of the source, detector, and sampling accessory combination, FTIR spectroscopy can be used to quantitate chemical species from the percent-level down to the single parts-per-billion concentration level. Absorptivity in the Mid-IR region is typically higher than that of the Near-IR region where first, second and third harmonic along with combination frequencies from the fingerprint region are studied.

ATR Sampling Accessory

Attenuated total reflection (ATR) is a popular sampling technique as it virtually eliminates sample preparation for most solids, liquids, and gels. The infrared energy from the spectrometer enters the ATR accessory where it is internally reflected through a crystal upon which a sample is placed. An evanescent wave of the infrared radiation interacts with the sample and causes molecular vibrations, which in turn can be detected by the spectrometer. The ATR's crystal (also referred to as an internal reflective element, abbreviated IRE) is chosen based on the physical properties of the sample in question, such as pH and hardness, the spectral region of interest, as well as the refractive index of both the sample and crystal. A single-bounce Diamond/Zinc Selenide crystal Pike MIRacle ATR accessory (shown in Figure 2) was used to collect the Mid-IR data.

Figure 2. ATR Sampling Accessory from Pike Technologies

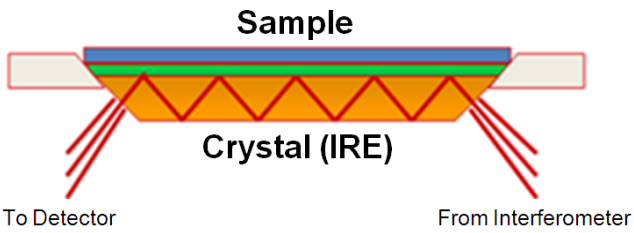


Figure 3. Overview of ATR sampling. A sample is placed on the surface of an internal reflective element crystal. The depth of penetration is typically in the order of a few microns and primarily depends on the infrared wavelength as well as the refractive indices of the sample and the crystal material. Advantageously for ATR, many samples may be analyzed without the need for sample preparation, whether they are solids, liquids, or gels.

Near-IR Integrating Sphere (IntegrateIR)

The second sampling technique used in this study was a Near-IR Integrating Sphere (IntegrateIR) from Pike Technologies, shown in Figure 4.[7] Integrating spheres can be implemented to function from the UV-Visible (when used with Agilent's dispersive spectrometers, such as the Cary UV-Visible-NIR) to the Near & Mid-IR region of the spectrum (as with interferometer-based instruments). Their use is particularly beneficial for the analysis of turbid, translucent, or opaque refractory materials, which exhibit significant scattering effects that limit the application of standard techniques. They are equally beneficial for the study of samples that distort the IR beam, such as lenses. This accessory provides greater than 95% collection efficiency; unlike standard diffuse reflectance accessories, which collect less than 50% of the scattered NIR energy. The accessory contains an internal gold reference that eliminates the need for the collection of a background or reference spectrum.

Typical applications of an integrating sphere accessory include the analysis of:

- surfaces in general
- pharmaceuticals
- thin films and coatings
- optics – lenses, filters or beamsplitters
- color/tint
- clothing, fabrics or materials
- paper
- powders and pastes
- sunscreens.

Agilent Cary 660 FTIR spectrometer is expandable to a spectral range of 50,000–20 cm^{-1} (from the UV to the Far-IR) depending on the combination of the source, beamsplitter, sampling accessory and detector. However, the best signal-to-noise ratio in the Near-IR region for the instrument and IntegrateIR accessory combination is between 11,500–3,850 cm^{-1}. It is noteworthy that accessories (such as an ATR, microscope, integrating sphere, etc.) will only perform as well as the spectrometer powering them. The unmatched optical energy throughput of the 600-IR series is the ultimate complement to these accessories.

Figure 4. IntegrateIR NIR sampling accessory. Samples or sample containers are placed on the integrating sphere for analysis. There is no sample preparation required or cleaning of accessories surface between analyses.

Micro-ATR Imaging

Figure 5. Micro slide on ATR accessory

The last sampling technique used in this study was micro-ATR Imaging to demonstrate product inhomogeneity on a microscopic scale. Spectrochemical Imaging is the most effective way to acquire a chemical specific image of a sample by taking a picture using an infrared sensitive camera Focal Plane Array (FPA)[4] and an FTIR. With this technique, the user will obtain a 2-dimensional chemical image at all wavelengths in a single measurement. This is accomplished by combining the technologies of an FPA[4] and the FTIR. The focal plane array is a 2-dimensional array of infrared sensitive detectors similar to a CCD, and measures a signal at every point from the sample area examined. The FTIR produces simultaneous spectral information at every frequency. The result is a spectrum at every point on the sample, collected simultaneously. Effectively, a full array of spectra is collected in a single scan.[8]

The results include:

- high spatial resolution images (1.1 µm pixel size with Agilent's micro-ATR Imaging), which is not possible to achieve by normal mapping techniques
- fast data collection (analysis times in the order of seconds to minutes as opposed to hours with mapping or linear array experiments)
- high signal-to-noise ratio (each detector element or pixel is completely filled)
- full spectral information from every pixel.

Results and discussion

Mid-IR infrared spectra of the tablet's surface were collected, as shown in Figure 6, on the 660 FTIR with a PIKE MIRacle Diamond/ZnSe ATR sampling accessory. These spectra provide a spectral fingerprint of the cetirizine-containing tablet. Some of the functional groups that result in spectral peaks of interest are listed in Table 3.[9]

Table 3. Functional group spectral analysis

Peak (cm^{-1})	Band Strength	Functional Group Association
1100	Weak	Benzene ring
1200–1400	Weak	Cl-CH; Benzene ring; -C-O-C-
1700	Strong	-C-O-C-; -COOH
2375	Narrow, Medium	$-CO_2$
2800–3000	Narrow, Strong	dinitrophenol, -COOH
3000–3600	Broad, Strong	-OH

Visual examination of Figure 6 (Appendix) reveals that subtle spectral differences exist between the six tablets. These differences may also be highlighted by transforming the spectra to their 2nd derivative spectra. The differences may be attributed to the fact that while the active pharmaceutical ingredient, cetirizine hydrochloride, should remain the same in each tablet, there may be a deviation due to the different excipients. In addition, the spectral response of a solid material collected with an ATR accessory is dependent on intimate contact between the sample and the internal reflective element crystal. Spectral variations may result from the tablet's surface heterogeneity as well as variations in the amount of pressure that is applied to the sample.

While ATR accessories are robust and easy to use, the manual nature of the technique limits its use in sample high-throughput applications as it can still present a bottle neck because for each data collection, the user must clean the crystal and pressure clamp, load a sample, and apply the correct amount of pressure. While usually considered to be a non-destructive technique, due to the application of pressure, ATR can be deemed a destructive technique depending on the sample type under investigation.

Near infrared spectroscopy is an alternative non-destructive spectroscopic analysis technique that provides a data set that can be used to discriminate samples. These samples are typically analyzed 'as-is' and do not require any sample preparation. Multiple phase species can be tested from within a plastic bag, glass vial, or reagent bottle if needed with a rapid analysis time. To improve productivity, an automated transmission tablet analysis stage can be added to the integrating sphere accessory.

The NIR spectra of the cetirizine tablets are shown in Figure 7 (Appendix). While the NIR spectra do not appear to be as information-rich as the Mid-IR spectra upon visible investigation, the information content is very high and the method can be more advantageous for many applications.

The 90 Near-IR sample spectra (6 distributors, 5 tablets from each, 3 replicate spectra from each tablet) were exported directly from Resolutions Pro into Bio-Rad's KnowItAll Analytical System software AnalyzeIt MVP. The multivariate analysis package for principal component analysis was used to perform the computations and generate graphics once the parameters in Table 4 were defined.

Principal Component Analysis (PCA) is a chemometric analysis of a data matrix using an unsupervised statistical method to represent variability in a multivariate data set using factors or principal components[10]. With this data output, human pattern recognition and pattern recognition software can be used to identify the natural clusters in the data and identify outliers. This can provide the researcher with clues as to the reasons for the grouping of data or outliers. This data can further assist with a root cause analysis of a manufacturing process, identify a drug's origin (for example, cetirizine manufacturers), assist in the separation of plastics for recycling, or provide the alcohol content of a beverage[11]. PCA is built on the assumption that relevant variations, the principle

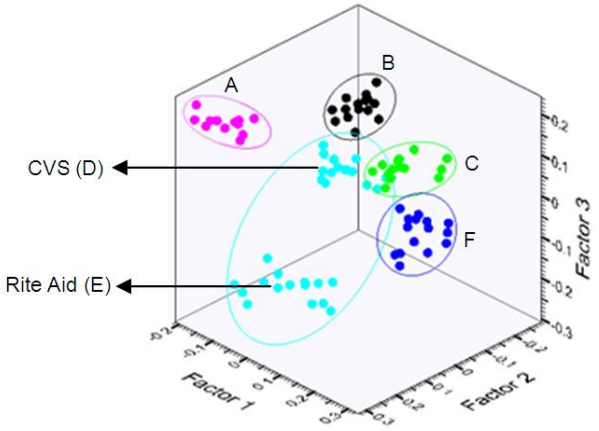
components, imply more important than non-relevant changes, like detector noise[12]. Once the principal component analysis is complete, a training set can then be constructed and used for classification of upcoming samples.

The PCA technique can be applied to Mid-IR data sets; however, the Near-IR data was used for the cluster analysis because of the simplicity and speed of the integrating sphere.

Table 4. PCA parameters

Parameter	Value
Analysis type	Pareto
Number of factors	3
Probability factor	95%
1st Derivative points	7
2nd Derivative points	7
Number of samples	90

The PCA output can be a powerful visual tool for the investigation of the variance of spectral data sets. For example, Figure 8 shows the cluster analysis based on the PCA of the Near-IR spectra of the 30 cetirizine tablet samples with different colors assigned to the different distributors. While some of the clusters overlap, there is still a clear separation of the samples based on its origin. Although the CVS and Rite Aid samples share the same manufacturer, there exists a class separation, which suggests that different excipient formulations are used. The PCA calibration can equally be used to predict the origin of an unknown cetirizine tablet even if there are no distinct physical attributes on the tablet. If desired, a larger and more robust model could be developed from this limited data set by including the results from multiple lots or batches from each supplier as well as spectral data from additional tablet manufacturers.



A - Tradaxine (SBL Pharmaceutical)
B - Zyrtec OTC (McNeal Consumer Health)
C - Zyrtec (Merck Medco—Pfizer)
D - Cetirizine (CVS—Dr. Reddy's Lab)
E - Cetirizine (Rite Aid—Dr. Reddy's Lab)
F - Cetirizine (Walmart—Perrigo)

Figure 8. Three component PCA of Near-IR data

In a third part of the experiment, one of the prescription Zyrtec samples was analyzed on the micro-ATR imaging system to study the component distribution within the tablet's surface. Using this experimental approach, 4,096 Mid-IR spectra were collected simultaneously in seconds to provide a comprehensive understanding of the tablet's surface chemistry. The 3-dimensional absorbance plots in Figure 9 were generated from a single chemical image and represent the same sample location (70 µm × 70 µm) with a 1.1 µm spatial resolution. Each of the 3-D chemical images is generated based on the absorbance at different wavelengths and is particularly useful when studying the distribution of specific functional groups across the area of analysis. This spectroscopic approach reveals that the tablet does not have a homogeneous distribution of ingredients across its surface when viewed on a micron scale. This finding is noteworthy and may help to explain some of the variance seen in the Mid-IR ATR spectra from the cetirizine-containing tablets collected in the first part of this investigation.

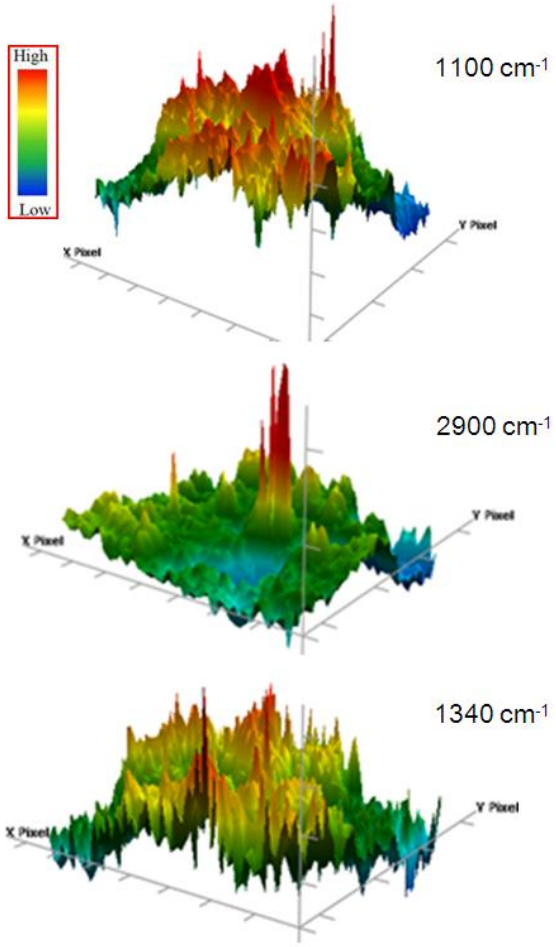


Figure 9. ATR-Imaging (1.1 µm spatial resolution) of Zyrtec tablet

Conclusion

Both Mid-IR and Near-IR spectroscopy can be used for the routine analysis of pharmaceutical tablets. Spectral acquisition using an ATR accessory can be a simple and cost-effective means of obtaining characteristic spectral information. Near-IR sampling with an Integrating Sphere accessory is also an invaluable tool for such analyses as it can be a high throughput and hassle free analytical approach that can often be a time saving replacement to existing chromatography or wet chemistry techniques. Its ability to acquire spectra of a sample through a plastic bag, glass vial, or a reagent bottle can be a significant asset in pharmaceutical applications. In addition, when combined with an easy-to-use chemometric software package such as AnalyzeIt MVP, infrared spectroscopy can be used to perform the rapid screening of chemical species, identify unknown samples or mixtures, and allow users to quickly solve challenging pharmaceutical problems. Agilent's ATR-Imaging can be used to provide the ultimate surface analysis with high spatial resolution with distinct applications with a variety of pharmaceutical applications.

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[4] This product is regulated by the U.S. Department of State under the International Traffic in Arms Regulations, 22 CFR 120-130 ('ITAR'). An export license from the U.S. government is therefore required to export this product from the United States, and other ITAR restrictions apply to the shipment, use, service and other aspects of this product and the FTIR instrument in which it is used.

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Appendix

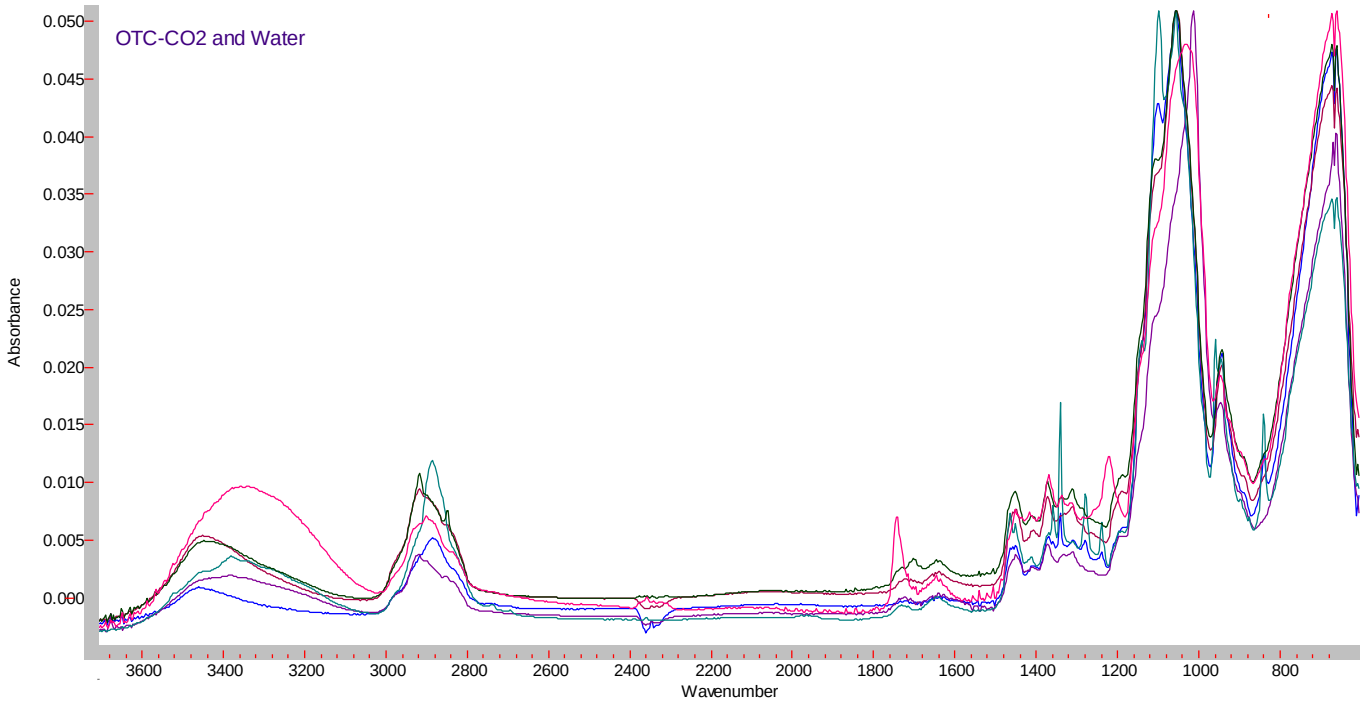

Figure 6. Overlaid Mid-IR spectra of six cetirizine-containing tablet samples (one tablet from each distributor) used in this study

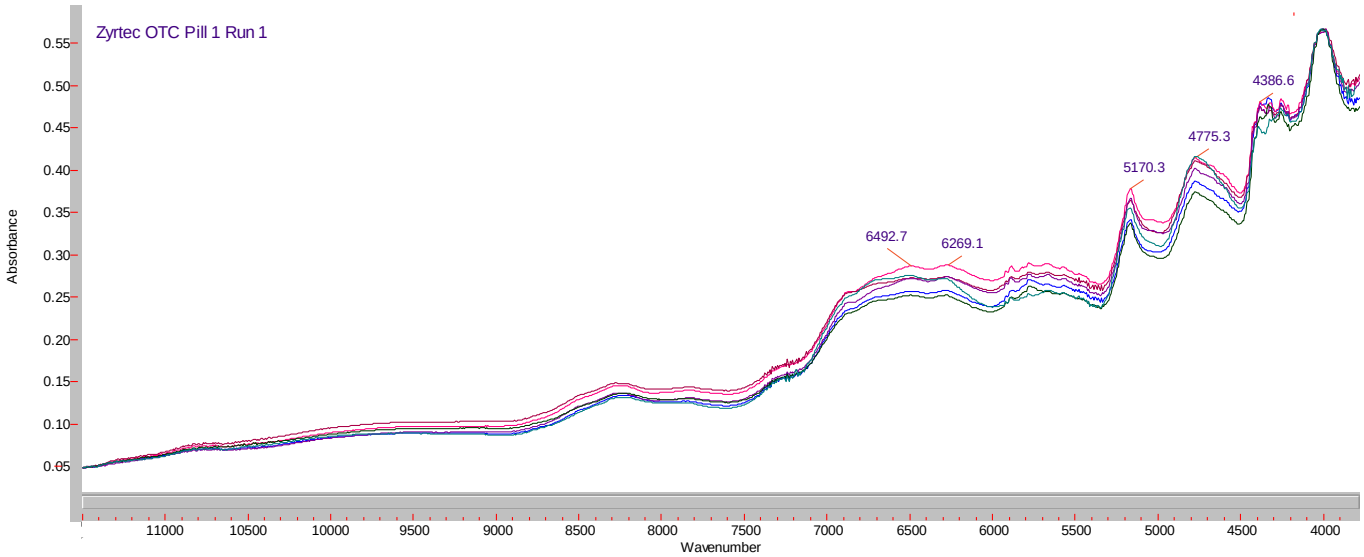

Figure 7. Overlaid Near-IR spectra of six cetirizine-containing tablet samples (one tablet from each distributor) used in this study

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