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A Systematic Procedure for Screening Counterfeit Pharmaceutical Tablet Coatings and Cores Utilizing Infrared Spectroscopy

Adam Lanzarotta[a,*], John Crowe[a], Sara Andria[a]

[a] *Forensic Chemistry Center, United States Food and Drug Administration, Cincinnati, OH 45237*

Abstract

Suspect counterfeit tablet coatings and cores have been examined and compared to authentic coatings and cores using attenuated total internal reflection Fourier transform infrared (ATR-FT-IR) spectroscopy. The analytical approach described in this study is currently employed on a routine basis by the Forensic Chemistry Center (FCC) as an initial screen prior to confirmatory analyses.

Keywords: counterfeit, FT-IR, forensic

1. Introduction

A thorough analysis of a suspect counterfeit product requires the entire sample (packaging components and dosage form) to be examined using a multidisciplinary approach, which includes several orthogonal techniques [10, 6, 1, 9, 2, 4, 3]. Many of the techniques used by the FCC for counterfeit analysis have been listed in Table 1 of Reference 7. The type and number of required techniques are typically determined on a case-by-case basis. However, ATR-FT-IR spectroscopy is one technique that is employed in the analysis of nearly all of the suspect counterfeit tablets received by the FCC. The technique is often employed early in an investigation as an initial screen because it is fast and accurate. Furthermore, it can accommodate the as-received state of the sample and allows both the coating and core to be examined. It is the intent of this report to provide an example of the FCC's counterfeit tablet screening procedure using ATR-FT-IR spectroscopy.

2. Materials and Methods

2.1. Materials

All pharmaceutical tablets discussed in this study were received by the FCC through criminal investigations. Examination of the tablets involved the use of tweezers, razor blades, a fine-pointed stainless steel probe, microscope slides, and chloroform. All analytical data were collected shortly after the sample was received in the laboratory. Physical characteristics of the suspect tablet (i.e. color, shape, debossing) were compared to tablets currently approved for sale in the United States using websites that have "Pill Identifying" searching capabilities, such as http://pillbox.nlm.nih.gov/, www.drugs.com, and www.rxlist.com.

2.2. Instrumentation

Macro ATR spectra were collected on a Thermo Nicolet 8700 main bench FT-IR spectrometer, which contained a potassium bromide beam splitter and a deuterium triglycine sulfate (DTGS) detector. The spectrometer was outfitted with a Durascope ATR accessory (Smith's Detection), which was equipped with a diamond-coated ZnSe internal reflection element (IRE). Spectra were the result of 64 co-additions at 4 cm^{-1} resolution.

Suspect spectra were searched against commercial and user-created libraries. Commercial libraries contained spectra that were collected on several different instruments and with different collection parameters. FCC user-created libraries contained spectra that were collected on FCC instrumentation with identical collection parameters, which allowed unknowns to be compared to standards, and suspect counterfeits to be compared to authentics with a much higher degree of confidence. FCC user-created libraries contain spectra of authentic coatings and cores, counterfeit coatings and cores, active pharmaceutical ingredients (APIs), excipients, and many other materials.

2.3. Sample Preparation

Tablet coatings were examined *in situ* by placing one side (preferably the side with little or no debossing) of the intact

*Corresponding author: Adam Lanzarotta, Phone: 513-679-2700 ext. 2236. Email: adam.lanzarotta@fda.hhs.gov

Figure 1: Images of the Case 1 suspect tablet.

tablet on top of the IRE. Force was applied to the top of the tablet using the pressure arm on the ATR accessory, which allowed the bottom of the tablet to achieve intimate contact with the IRE. Care was taken while lowering the pressure arm because excessive force caused the tablet to break and insufficient force resulted in an unacceptable signal-to-noise ratio. If the tablet broke before a satisfactory spectrum was collected, a fraction of the coating, which was larger than the diameter of the IRE, was removed from the core and placed onto the IRE using micro tools such as tweezers and fine-pointed probes. The pressure arm was then lowered to provide the force recommended by the manufacturer.

After the tablet coating was examined, the tablet was cut in half using a razor blade. The coating was removed from one of the halves using micro tweezers, a fine-pointed probe, and/or a razor blade. The remaining half tablet core was then ground with a mortar and pestle. Approximately 5 mg of the ground core was placed directly onto the IRE. Force was applied using the pressure arm and a spectrum was collected.

The remainder of the ground tablet core was added to 1-3 mL chloroform. After gentle shaking, the solution was either left to sit overnight or centrifuged so that the insoluble compounds would settle at the bottom of the vial. Several drops of the supernatant were then placed onto a clean glass microscope slide, which was left to air dry. The resulting residue was scraped off of the microscope slide with a razor blade and placed directly onto the IRE. Force was applied using the pressure arm and a spectrum was collected.

A small amount of the appropriate reference standard was recrystallized in chloroform. Several drops of the solution were then placed onto a clean glass microscope slide, which was left to air dry. The resulting residue was scraped off of the microscope slide with a razor blade and placed directly onto the IRE. Force was applied using the pressure arm and a spectrum was collected.

3. Discussion

3.1. Case 1

Visible images of the Case 1 suspect tablet have been provided in Figure 1. The blue, diamond-shaped tablet debossed "Pfizer" on one side and "VGR 100" on the other side was found to be a suspect Viagra 100 mg tablet using the "Pill Identifying" feature of the websites described above. The infrared spectrum of the Case 1 suspect tablet coating has been provided in Figure 2(a). The suspect spectrum was first searched using all libraries over the full spectral range, which yielded the top fifteen spectra with which it shared the highest unbiased, statistical correlation. Thirteen of the fifteen library spectra were counterfeit tablet coatings, one of which has been provided in Figure 2(b). An authentic Viagra 100 mg coating spectrum has been provided in Figure 2(c). The suspect coating spectrum and stored counterfeit coating spectrum shared several similarities between 3700 and 2700 cm^{-1} and between 1800 and 600 cm^{-1}. However, there were differences that indicated the suspect coating formulation was slightly different from the archived counterfeit coating formulation. For example, the 1743 and 1657 cm^{-1} bands were shifted and exhibited a different band ratio in the suspect coating spectrum. There were more significant differences between the suspect and authentic Viagra 100 mg coating spectra. For example, the suspect coating spectrum contained unique bands at 3676, 1657 and 1014 cm^{-1} (among others) and the authentic coating spectrum contained unique bands at 3393 and 1051 cm^{-1}. Overall, it was concluded that the suspect coating spectrum exhibited characteristics that were similar to a counterfeit coating spectrum archived by the FCC, and was not consistent with an authentic Viagra 100 mg coating spectrum.

Once it was concluded that the suspect coating spectrum was not consistent with the authentic coating spectrum, the suspect coating formulation was determined. All FCC authentic and counterfeit libraries were therefore removed from the search queue in order to limit the results to individual components. Out of the 15 library search matches, the suspect coating spectrum (Figure 3(a)) was most consistent with talc (Figure 3(b)). The free OH stretching and Si-O-Si stretching vibrations

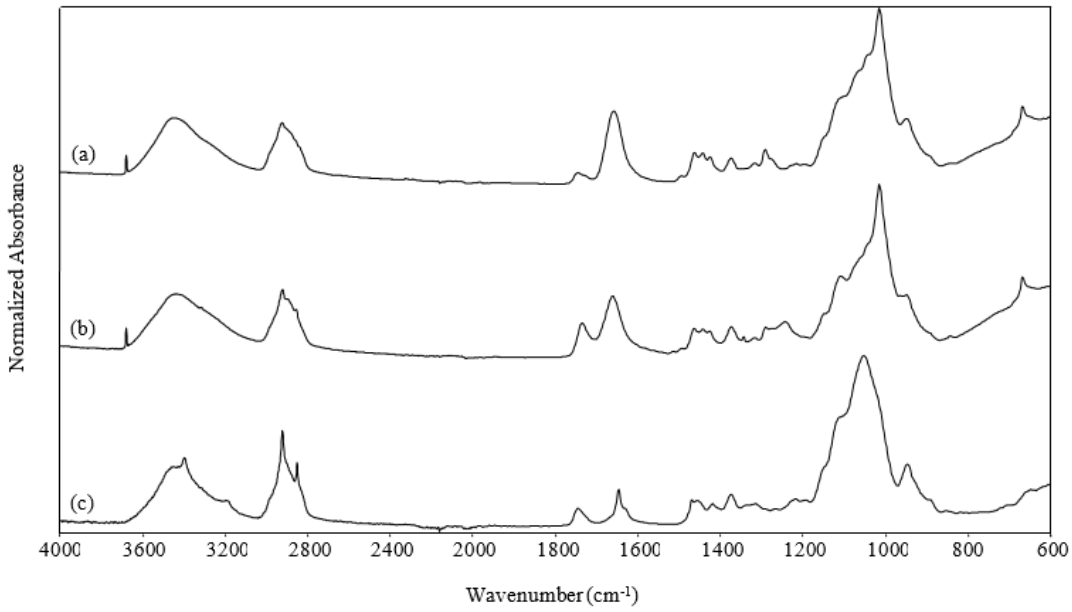

Figure 2: Infrared spectra of the Case 1 suspect tablet coating (a), an archived counterfeit coating (b), and an authentic Viagra 100 mg coating (c).

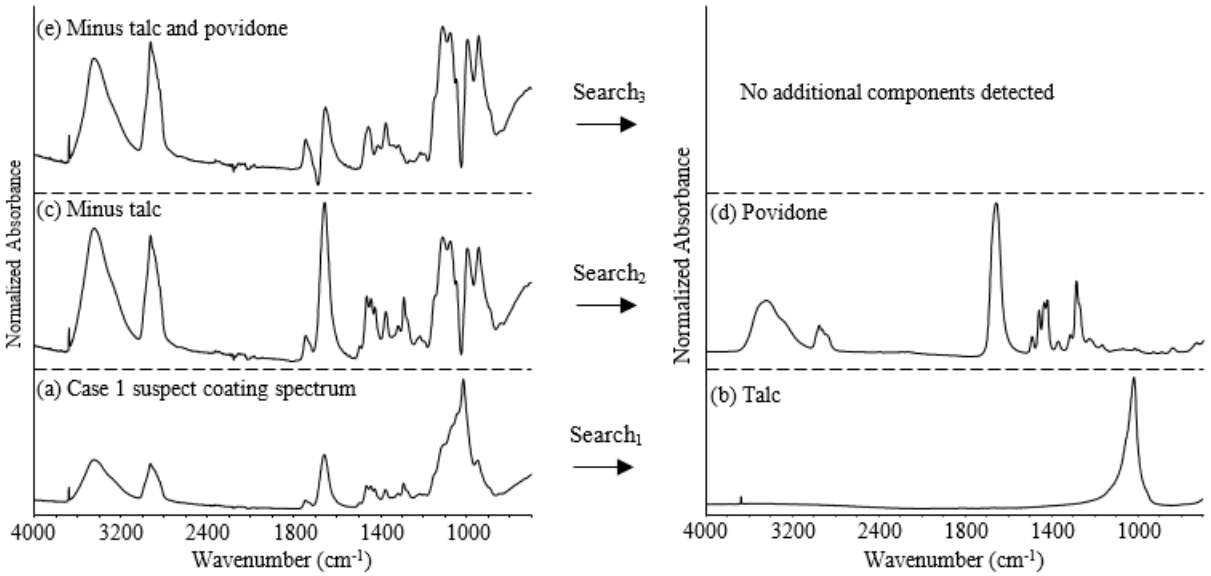

Figure 3: Spectral subtraction performed on the Case 1 suspect tablet coating spectrum.

characteristic of talc are located at 3676 and 1015 cm^{-1}, respectively [7]. The standard spectrum of talc was then spectrally subtracted from that of the suspect coating spectrum using a process described by Lanzarotta et al. [3] The resultant spectrum (Figure 3(c)) was searched using the same libraries over the full spectral range. Of the 15 library search matches, the suspect spectrum was most consistent with povidone (polyvinyl pyrrolidone), a spectrum of which is shown in Figure 3(d). The cyclic amide C=O stretching, a CH_2 symmetric scissoring deformation (CH_2 next to the N), a CH_2 symmetric scissoring deformation, a CH_2 symmetric scissoring deformation (CH_2 next to C=O), and the C-N stretching vibrations characteristic of povidone are located at 1657, 1493, 1462, 1424 and 1290 cm^{-1}, respectively [5]. After an additional subtraction (Figure 3(e)) no other components were detected, which may have been due to the overwhelming presence of spectral artifacts (derivative-shaped bands around 1700 and 1000 cm^{-1}) that are characteristic by-products of the subtraction process (the peak characteristic of talc at 3676 cm^{-1} and the peak characteristic of povidone at 1657 cm^{-1} were retained in the subtraction spectrum because

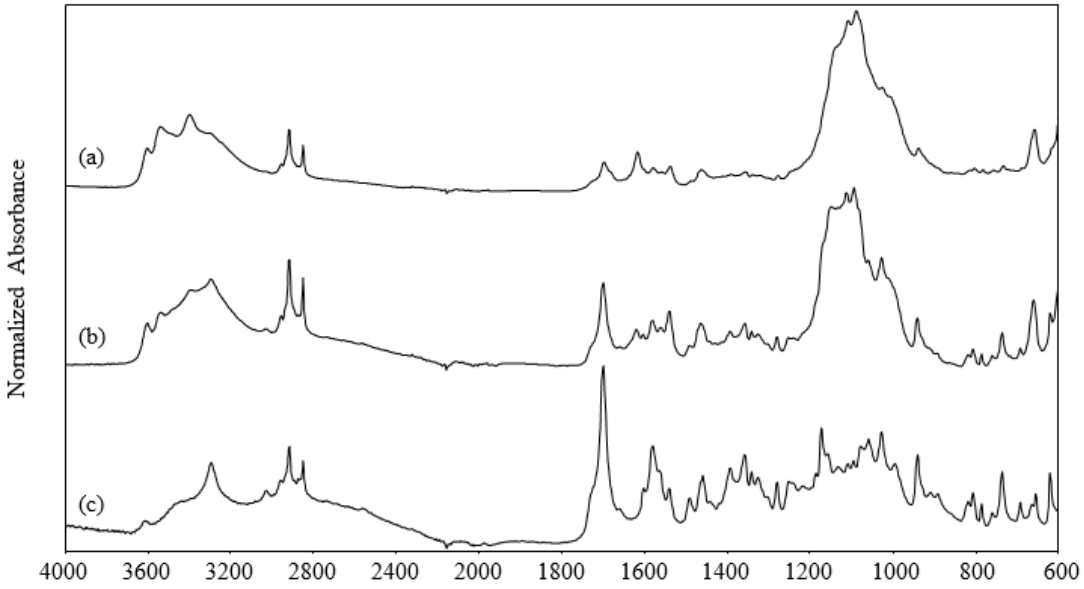

Figure 4: Infrared spectra of the Case 1 suspect tablet core (a), an archived counterfeit core (b), and an authentic Viagra 100 mg core (c).

of slight peak position and relative peak intensity differences between the suspect and standard spectra). It is also possible that the suspect coating did not contain additional components, or contained additional components at a concentration below the detection limit of the instrument employed.

The infrared spectrum of the Case 1 suspect tablet core has been provided in Figure 4(a). The spectrum was searched using all libraries over the full spectral range. Thirteen of the fifteen matches were archived counterfeit tablet cores. One of the top-matched counterfeit core spectra and an authentic Viagra 100 mg core spectrum have been illustrated in Figures 4(b) and 4(c), respectively. Although the suspect core has nearly a peak-for-peak match with the counterfeit core, there are several band shape and intensity differences between the two spectra. For example, most bands in the suspect core spectrum are poorly resolved compared to the bands in the counterfeit core spectrum. The resolution differences, which are due to the two tablets having similar components at different relative concentrations, are most significant between 1500 - 1200 cm^{-1} and between 900 - 600 cm^{-1}. The band shape differences between the suspect and counterfeit core spectra are most noticeable in the region between 1200 - 1000 cm^{-1}. Specifically, differences in the strong, broad band envelope centered around 1100 cm^{-1} may be attributed to the suspect and counterfeit cores having different ingredients, or the same ingredients with a different crystalline structure. More significant differences were observed between the suspect and authentic Viagra 100 mg core spectra. The suspect core spectrum contained several unique peaks between 1200 - 1000 cm^{-1} and at 3604, 3544, 3400, 1619 and 659 cm^{-1}. The authentic Viagra 100 mg core spectrum contained several unique peaks between 1200 - 1000 cm^{-1}. Overall, it was concluded that the suspect core spectrum exhibited characteristics that were similar to a counterfeit core spectrum archived by the FCC and was not consistent with an authentic Viagra 100 mg core spectrum.

The suspect core formulation was determined using the same library searching process described above, which involved removing all FCC authentic and counterfeit libraries from the search queue. The suspect core spectrum (Figure 5(a)) was most consistent with calcium sulfate hemihydrate (Figure 5(b)). The following bands characteristic of calcium sulfate hemihydrate were observed in the suspect core spectrum: OH stretching vibrations at 3605 and 3551 cm^{-1}, an OH bending vibration at 1618 cm^{-1}, SO_4 antisymmetric stretching vibrations at 1141, 1112 and 1087 cm^{-1} and an SO_4 symmetric stretching vibration at 1008 cm^{-1} [8]. In order to determine if additional components were present, calcium sulfate hemihydrate was spectrally subtracted from the suspect core spectrum. The resultant spectrum, illustrated in Figure 5(c), was searched using the same libraries. Magnesium stearate (Figure 5(d)) was the top match. Figures 5(c) and 5(d) both had peaks at precisely 2956, 2918 and 2850 cm^{-1}, which were attributed to CH_3 antisymmetric stretching, CH_2 antisymmetric stretching, and CH_2 symmetric stretching vibrations, respectively. Since these absorptions are commonly observed in many different long-chain CH_2-containing molecules, the exact identity of the compound giving rise to these absorptions could not be determined.

Since the long CH_2-chain containing molecule could not be further identified, additional subtractions were not performed. Instead, a region of the subtracted spectrum was searched that did not include the intense CH_2 stretching vibrations that strongly influenced the results of the previous search. Seven of the fifteen hits for this region included sildenafil citrate, which is the API in authentic Viagra 100 mg tablets. Sev-

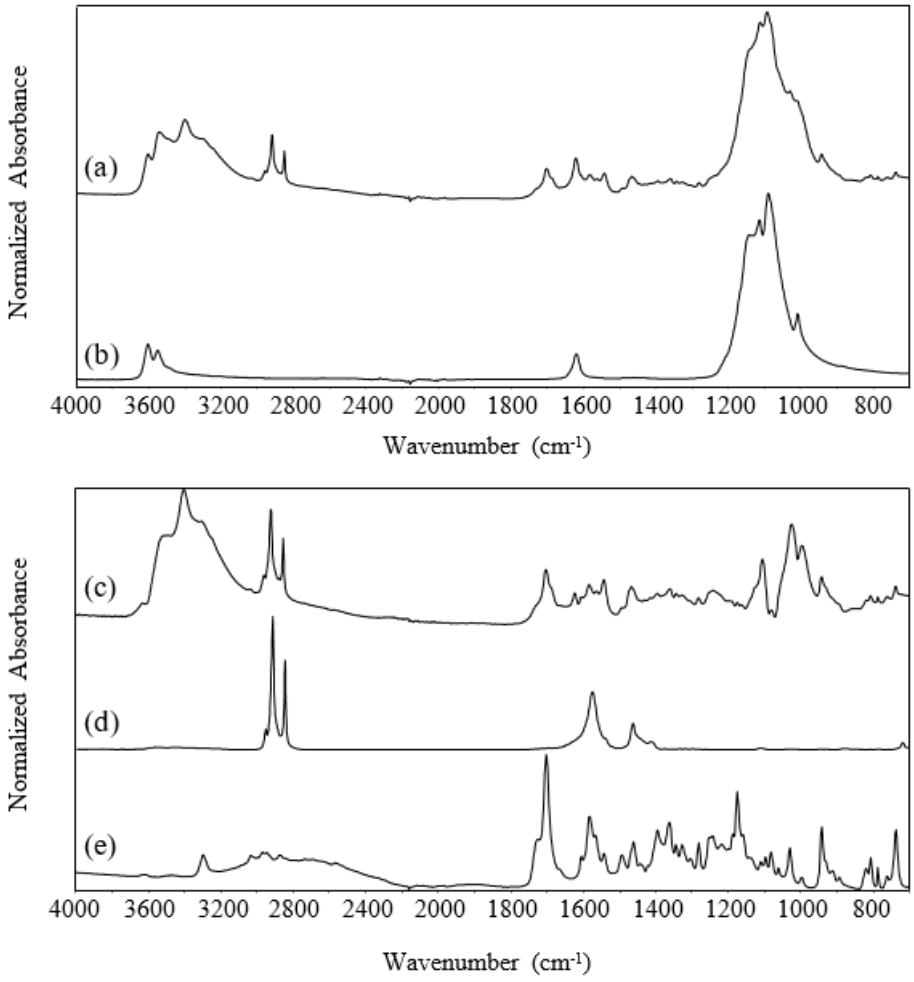

Figure 5: The infrared spectrum of the Case 1 suspect tablet coating (a) compared to the infrared spectrum of calcium sulfate hemihydrate (b). The spectral subtraction of the Case 1 suspect tablet coating and calcium sulfate hemihydrate (c) compared to the infrared spectra of magnesium stearate (d) and sildenafil citrate (e).

eral strong bands characteristic of sildenafil citrate (Figure 5(e)) were observed in the residual spectrum, which included secondary amide N-H stretching, secondary amide C=O stretching, SO_2 antisymmetric stretching and SO_2 symmetric stretching vibrations at 3299, 1701, 1357 and 1172 cm^{-1}, respectively. However, many of the weaker bands characteristic of sildenafil citrate were poorly resolved or not observed due to the spectral artifacts caused by the subtraction process. As a result, there was a lower degree of confidence that sildenafil citrate was present in the suspect tablet core compared to a scenario where the suspect spectrum shared a peak-for-peak match with the standard.

In order to increase the confidence that sildenafil citrate was present, a chemical extraction of the API was performed. An ideal solvent would dissolve only the API and would leave the excipients un-dissolved. However, such a solvent may not exist when a formulation contains an API and one or more excipients with similar solubility properties. Since the majority of the interference was caused by calcium sulfate hemihydrate, it was important that the extraction solvent favored solubility properties of sildenafil citrate while not favoring those of calcium sulfate hemihydrate (e.g. chloroform). An infrared spectrum of the chloroform extract dried residue is provided in Figure 6(a). The spectrum was searched below 2000 cm^{-1}, which avoided the strong peaks at 2918 and 2850 cm^{-1}, which were likely attributed to the long CH_2-chain containing molecule previously discussed. The search resulted in no suitable matches.

Since sildenafil citrate was detected using the spectral subtraction process described above, the suspect residue spectrum was compared to a standard spectrum of sildenafil citrate (Figure 6(b)). The suspect residue spectrum appeared to exhibit several bands characteristic of sildenafil citrate. However, significant peak shape differences and shifting were observed. For instance, the secondary amide N-H stretching and C=O stretching vibrations were shifted in the suspect spectrum to 3298 and 1687 cm^{-1}, respectively. Among others, these differences indicated that the suspect residue may be present as a freebase form of sildenafil citrate and/or it may have a different crystalline

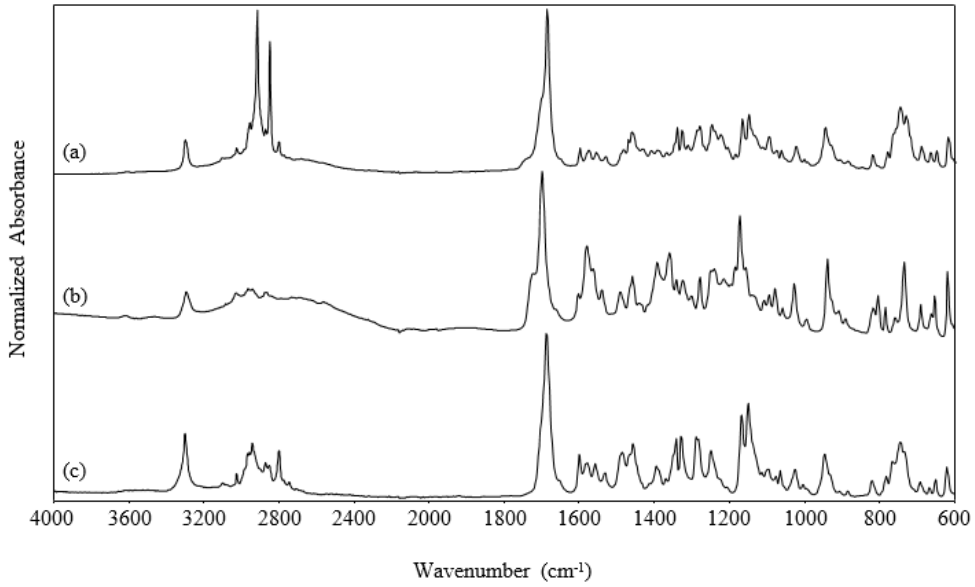

Figure 6: Chloroform extraction of the Case 1 suspect tablet core (a) compared a standard spectrum of sildenafil citrate (b) and a spectrum of sildenafil citrate recrystallized with chloroform (c).

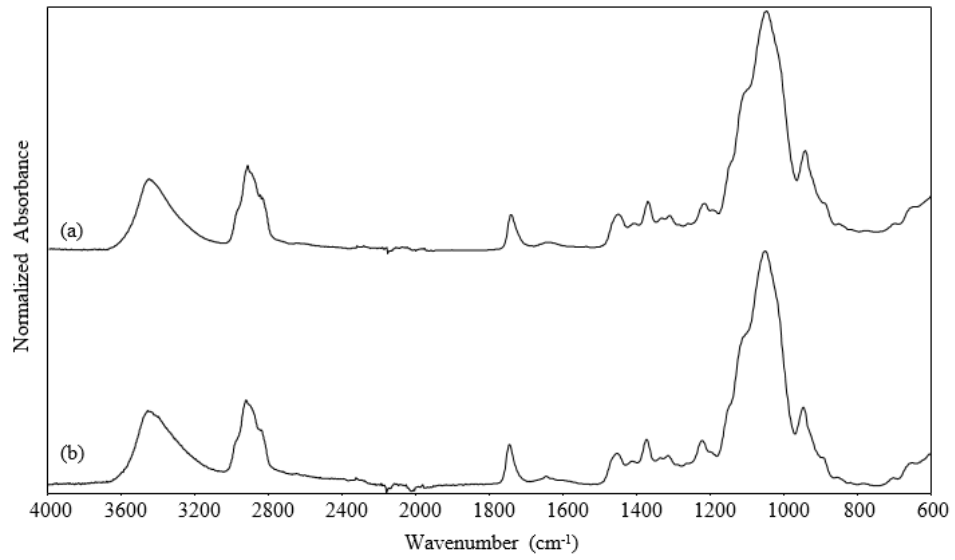

Figure 7: Infrared spectrum of the Case 2 suspect tablet coating (a) compared to the infrared spectrum of an authentic Viagra 100 mg tablet coating (b).

structure compared to the sildenafil citrate standard, which was likely caused by dissolving the suspect sample in chloroform. In order to confirm this hypothesis, the sildenafil citrate standard was recrystallized using chloroform. A spectrum of the recrystallized standard, which has been illustrated in Figure 6(c), was added to the FCC user-created libraries. The suspect residue spectrum was then searched again below 2000 cm^{-1} using all libraries. The recrystallized sildenafil citrate spectrum was the top match. Although much of the functional group region (between 4000 - 2000 cm^{-1}) was obscured by the CH_2 stretching vibrations of the long CH_2 chain-containing compound, the N-H stretching vibration at 3298 cm^{-1} was consistent with that of the recrystallized standard. Further, the suspect spectrum was nearly a peak-for-peak match with the standard spectrum below 2000 cm^{-1}, including the secondary amide C=O stretching vibration at 1687 cm^{-1}. These results made it possible to conclude that sildenafil citrate was present with a high degree of confidence. Overall, it was concluded that 1) the infrared spectrum of the Case 1 suspect tablet coating was not consistent with the infrared spectrum of the authentic Viagra

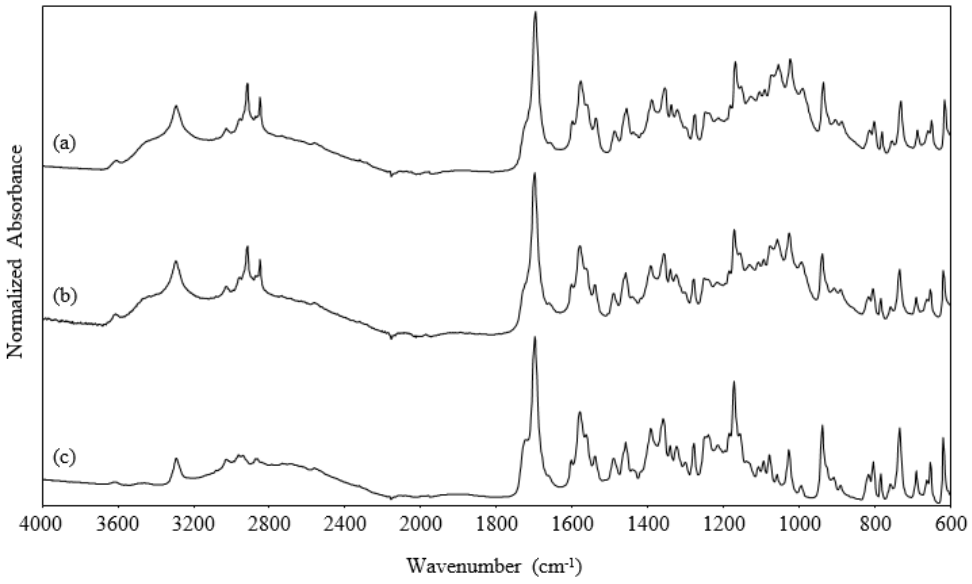

Figure 8: Infrared spectrum of the Case 2 suspect tablet core (a) compared to the infrared spectra of an authentic Viagra 100 mg tablet core (b) and sildenafil citrate (c).

100 mg tablet coating, 2) the infrared spectrum of the Case 1 suspect tablet core was not consistent with the infrared spectrum of the authentic Viagra 100 mg tablet core, and 3) the Case 1 suspect tablet core was consistent with the presence of sildenafil. However, the salt/base form of sildenafil in the tablet could not be determined because sildenafil citrate recrystallizes as the freebase form of sildenafil using chloroform.

3.2. Case 2

The Case 2 suspect tablet had the same shape, color and debossing characteristics as the Case 1 suspect tablet. It was therefore also considered to be a suspect Viagra 100 mg tablet. The infrared spectrum of the Case 2 suspect tablet coating (Figure 7(a)) was searched against all libraries. All fifteen hits consisted of authentic Viagra 25 mg, 50 mg and 100 mg coating spectra. The search resulted in coating spectra from multiple dosage forms because manufacturers often use the same or similar coating formulations for more than one product. Among others, the suspect coating spectrum exhibited the following bands characteristic of the authentic Viagra 100 mg coating spectrum (Figure 7(b)): OH stretching, ester C=O stretching and C-O stretching vibrations at 3454, 1745 and 1051 cm^{-1}, respectively.[1]

The infrared spectrum of the Case 2 suspect tablet core (Figure 8(a)) was searched against all libraries. All fifteen hits consisted of authentic Viagra 25 mg, 50 mg and 100 mg core spectra. Multiple dosage forms resulted for this search because different dosages of the same product often have the same ingredients but at different concentrations or, as in the case with Viagra, the same formulation with more material. As a result, two or more dosages may have spectra with the same band positions and only a slight difference in band intensities, which may result in a high search correlation between the two spectra. The suspect core spectrum exhibited a peak-for-peak match with the authentic Viagra 100 mg core spectrum (Figure 8(b)). Among others, the suspect core spectrum shared the following bands characteristic of the authentic formulation: amide N-H stretching, CH_2 antisymmetric stretching, CH_2 symmetric stretching, amide C=O stretching and C-O stretching vibrations at 3294, 2917, 2850, 1700 and 1026 cm^{-1}, respectively.

Concluding that a suspect tablet core is consistent with the authentic tablet core does not necessarily indicate that it contains the correct API. Therefore, it was still necessary (if possible) to spectroscopically determine if the API is present. The infrared spectrum of the Case 2 suspect tablet core was searched once the FCC authentic and counterfeit libraries were removed from the search queue. Seven of the fifteen hits were sildenafil citrate, a spectrum of which has been provided in Figure 8(c). Spectral subtraction was not required to confirm the presence of the API since a peak-for-peak match was observed from 2000 - 1250 cm^{-1} and 900 - 600 cm^{-1} between the suspect tablet spectrum and the sildenafil citrate standard spectrum. Overall, it was concluded that 1) the infrared spectrum of the Case 2 suspect tablet coating was consistent with the infrared spectrum of the authentic Viagra 100 mg coating, 2) the infrared spectrum of the Case 2 suspect tablet core was consistent with the infrared spectrum of the authentic Viagra 100 mg core, and 3) the Case 2 suspect tablet core was consistent with the presence of sildenafil citrate, the API in authentic Viagra 100 mg tablets.

[1]The composition of the Case 2 suspect tablet coating and core was determined using spectral subtractions. However, the results have not been provided here because both the suspect coating and core were consistent with an authentic coating and core, which have proprietary formulations.

4. Conclusion

The infrared analysis procedure employed by the FCC for the examination of counterfeit pharmaceutical tablet coatings and cores has been described. The purpose of this study was to illustrate the process on a small sample set that accurately represents many samples received by the FCC. Two examples have been provided - one example where the suspect counterfeit tablet was not consistent with the authentic tablet, and one example where the suspect counterfeit tablet was consistent with the authentic tablet. Other common samples examined by the FCC include but are not limited to 1) a suspect tablet with an incorrect API that gives a similar desired effect (e.g. a suspect Cialis 20 mg tablet that contains sildenafil citrate instead of tadalafil, the correct API in Cialis 20 mg tablets), 2) a suspect tablet with no API, and 3) a suspect tablet with two or more APIs.

5. Declaration of Conflicting Interest

The authors declare that there is no conflict of interest.

6. Disclaimer

An earlier version of this content was previously published as a Laboratory Information Bulletin in July of 2013 and has since been peer reviewed prior to publication with the Journal of Regulatory Science.

7. Acknowledgments

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8. Article Information

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