

The Use of Raman Spectroscopy in the Detection of Counterfeit and Adulterated Pharmaceutical Products

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Introduction

The counterfeiting of commercial products such as clothing items, hand bags, and movies is an ever-increasing worldwide problem. However, the risk to the public health from purchasing a counterfeit hand bag is minimal compared to the risk associated with a consumer purchasing and consuming a counterfeit pharmaceutical product. The increase in the counterfeiting of pharmaceutical drugs worldwide is of major concern to the Food and Drug Administration (FDA) [1].

Counterfeit pharmaceuticals pose a significant public health and safety risk since they may contain harmful impurities, may be ineffective, and/or have low to no bioavailability. Counterfeit pharmaceuticals may include products that may have the correct ingredients, wrong ingredients, incorrect quantities of the active pharmaceutical ingredients (API), as well as fake packaging. Evidence analyzed by the FDA's Forensic Chemistry Center (FCC) submitted as part of counterfeit investigations has included pharmaceuticals, dietary supplements, cosmetics, and even medical devices. In recent cases, well-organized counterfeiters have become more sophisticated in both the manufacture and distribution of the counterfeit product. Therefore, where many of these counterfeit products were previously found and purchased on the black market and the internet, they are now finding their way into the legal distribution chain [2]. In countries outside the U.S., drug counterfeiting is widespread, and in some cases, may account for more than 50 % of the products sold in those markets [1].

The number of counterfeit investigations conducted by the FDA's Office of Criminal Investigation (OCI) has increased from five per year in the late 1990's to over 20 per year since 2000 [1]. The FDA's Forensic Chemistry Center (FCC) provides forensic analysis of evidence collected as part of OCI's counterfeit investigations. The type of analysis the FCC performs on suspect counterfeit products includes screening or rapid analysis of products to sort legitimate product from suspect counterfeit products. The screening of suspect counterfeit products also generates chemical information which can be used to determine the potential public health risk. Additionally, the

FCC may generate forensic information related to the source or origin of a counterfeit product, for example, how do similar counterfeits relate to each other?

The FCC uses a wide range of analytical methods and instrumental techniques in the screening and sourcing of suspect counterfeit and adulterated pharmaceuticals. An instrumental technique used frequently in the analysis of suspect counterfeit product evidence is Raman spectroscopy. Raman spectroscopy is used to perform a variety of analyses in the pharmaceutical industry, as well as in forensic analysis [3,4,5,6,7,8,9]. The vibrational spectroscopic information obtained from a forensic sample using Raman spectroscopy is complementary to the infrared (IR) spectroscopic information that may be obtained for the same forensic sample. However, the inherent instrumental and sampling advantages of Raman versus IR spectroscopy in some cases, makes Raman a better choice in the analysis of counterfeit and suspect adulterated pharmaceuticals [3,4,5].

Raman spectroscopy provides specific information on the identification of analytes, characterization of sample matrices, and molecular spectroscopic information useful in the structural elucidation of unknowns [3,4]. The technique is rapid and when coupled with sample preparation methodologies (i.e., micro-extractions, fraction collections, small particle analysis), a large amount of information can be obtained from a single piece of forensic evidence.

This paper focuses specifically on the Raman spectroscopic analysis of suspect counterfeit tablets as an example, although the same sample preparations and Raman spectroscopic measurements can be applied to suspect counterfeit capsule formulations and to adulterated pharmaceutical products.

Experimental

All Raman spectra presented and discussed in this paper were collected on a research grade dispersive Raman spectrometer system with both micro- and macro-sampling capabilities. The Raman spec-

trometer system consists of two lasers (633nm and 785 nm), two gratings (high and low resolution) and a CCD detector. The data collection parameters for all the Raman spectral data presented are given in Table 1.

Table 1. Raman spectral data collection parameters

Data Collection Parameter	Setting
Laser	633 nm
Confocal Hole	800 μ m
Slit Width	200 μ m
Grating	950 l/mm
Integration time	10 to 20 sec averaged 2X
Focusing objective	50X
Detector	CCD

The optical microscope attached to the system allows for samples to be visually observed using transmitted, episcopic and/or plane polarized light. The Raman spectrometer system is also coupled with a Fourier Transform Infrared (FT-IR) attenuated total reflectance (ATR) microscope, allowing FT-IR spectroscopic measurements to be made on the same sample.

Analysis of Counterfeit Pharmaceutical Dosage Forms

A pharmaceutical dosage form, such as a tablet, is frequently composed of a coating and a core. The tablet coating can consist of organic components (cellulose, wax, stearates), inorganic components (silicates, TiO_2), and possibly a color component (dye). The tablet core consists of an API, fillers (cellulose, lactose), flow agents (talc, stearates) and disintegrates [10,11]. Depending on the dosage form being analyzed, the API concentration can range from microgram (μ g) to gram (gm) quantities. The variety of dosage formulations, as well as API concentrations that are currently marketed by numerous pharmaceutical companies, provides a unique challenge to the FCC in forensic analysis of suspect counterfeit products. Therefore, a multidisciplinary approach is necessary to gather as much information as possible about a suspect or authentic pharmaceutical dosage form as part of an investigation. Solid-state analysis is an important part of this multidisciplinary approach and Raman spectroscopy is a key instrumental technique used in the analysis of suspect counterfeit products.

In the analysis of suspect counterfeit pharmaceutical dosage forms, Raman spectroscopy has advantages over other instrumental techniques. Raman allows for the qualitative identification of both API's and excipients used to manufacture the product [6,7,8,9]. It should be noted, however, that in some cases pharmaceutical excipients exhibit a weak Raman response and/or may exhibit a large amount of fluorescence. For this reason, both FT-IR and Raman spectroscopies are used at the FCC when determining the excipients used in suspect counterfeit dosage formulations (Figure 1). Figure 1 is a comparison

of the FT-IR attenuated total reflectance (ATR) spectrum and Raman spectrum collected of the same tablet coating. There are significant differences observed between the two spectra in Figure 1. In the FT-IR ATR spectrum (Figure 1a), the main features observed are attributed to the organic components of the coating (e.g., cellulose) and in the Raman spectrum (Figure 1b) the main features observed are attributed to the inorganic components (e.g., titanium dioxide) of the coating material. Raman spectroscopy allows for chemical information to be obtained in the spectral region below 600 cm^{-1} . This region is very useful for the determination of inorganic compounds. The combination of techniques can provide for a complete spectral "snap shot" of the components used in the tablet coating and core formulation.

The Raman spectra collected of an authentic tablet coating and core can be used as a "spectral fingerprint" in the comparison of the authentic to a suspect counterfeit product. Figure 2 is a comparison of the Raman spectrum of an authentic tablet coating (Figure 2b) compared to the Raman spectrum of a suspect counterfeit tablet coating (Figure 2a). Raman spectra of the tablet coatings were obtained directly from the tablets with no sample preparation. The tablets under investigation were placed directly on the Raman microscope stage and the 633 nm laser was focused on the tablet coating. The major spectral features observed in both spectra are due to titanium dioxide (TiO_2). However, upon further visual comparison of the spectral data in Figure 2 (see insert), an additional peak is observed in the

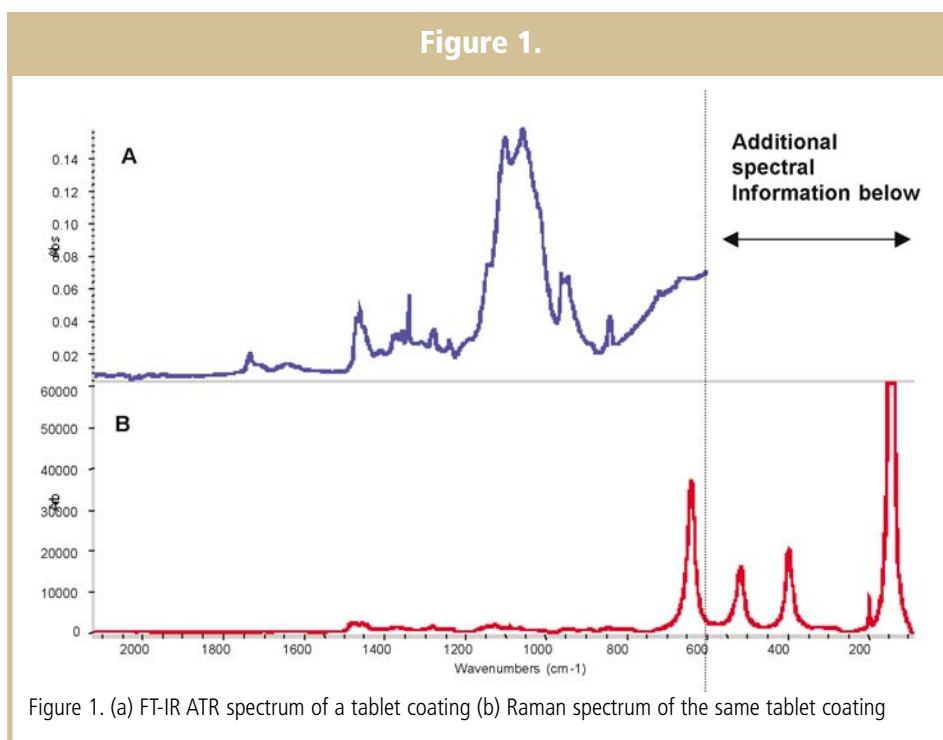


Figure 1. (a) FT-IR ATR spectrum of a tablet coating (b) Raman spectrum of the same tablet coating

Raman spectrum of the suspect tablet coating that is not observed in the authentic product tablet coating.

Figure 3 is a comparison of the Raman spectrum of an authentic tablet core (Figure 3b) and the Raman spectrum of a suspect tablet core (Figure 3a). Raman spectra of the tablet cores were obtained by removing a portion of the tablet coating and then cross-sectioning the tablet core. The cross-section was then placed flat on a glass slide and the 633 nm laser focused on the tablet core. The suspect counterfeit tablet core (Figure 3a) exhibits differences at $\sim 400\text{ cm}^{-1}$, 1100 cm^{-1} and 1400 cm^{-1} , which are not observed in the Raman spectrum of the authentic tablet core (Figure 3b). The Raman spectrum of the suspect counterfeit tablet core can be used to verify the presence of the API

Figure 2.

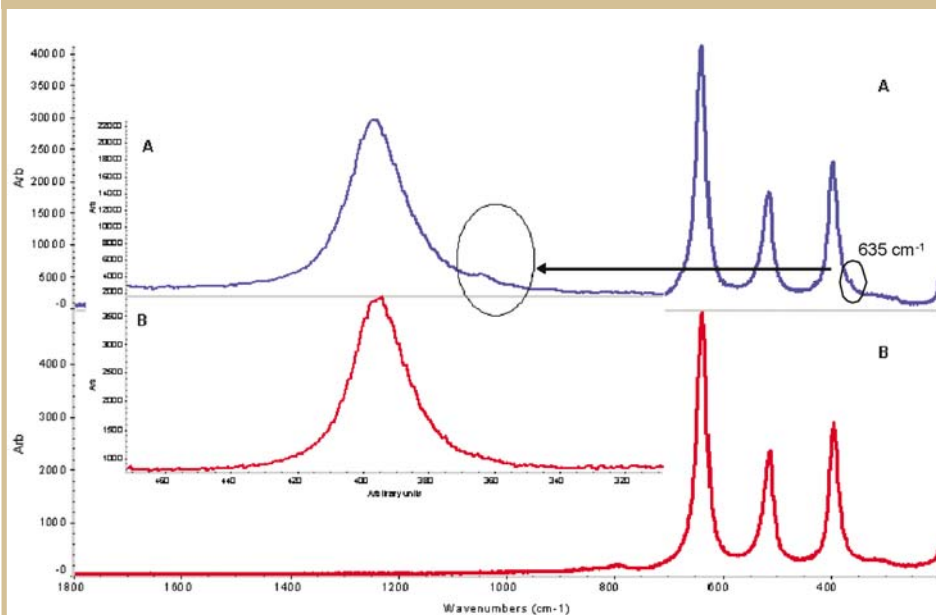


Figure 2. (a) Raman spectrum of a suspect counterfeit tablet coating (b) Raman spectrum of an authentic tablet coating

sampling spot size/spatial resolution of Raman microscopy far exceeds that of FT-IR microscopy. The optical polarizers are set in the optical setup in such a manner that they do not interfere with the collection of the Raman spectral data. This type of analysis can also be automated by using Raman spectral mapping. The PLM - Raman technique is not only useful for determining the excipient components of a pharmaceutical formulation but also for determining the presence of the crystal (polymorphic) forms of the API used in an authentic or suspect counterfeit formulation.

Figure 5 is an example of this kind of analysis. Raman spectroscopy is an ideal technique for determining the presence of different crystalline (polymorphic) forms of an API [6,13,14,15]. Different polymorphic forms of an API may have different solubility profiles, thus leading to different degrees of bioavailability. In general, differences in the crystallinity of a compound will generate differences in the Raman spectral data collected for that specific crystal type. The Raman spectral differences observed can range from very subtle to

used in the manufacture of the authentic and/or suspect counterfeit product. Figure 4 is the Raman spectrum of the suspect counterfeit product compared to the Raman spectrum of the API standard used in the authentic product. Many of the Raman bands that are observed in the API standard spectrum (Figure 4b) can easily be observed in the Raman spectrum of the suspect counterfeit tablet core (Figure 4a).

A disadvantage to using any vibrational spectroscopic technique such as Raman and FT-IR in the analysis of suspect counterfeit dosage forms is performing adequate sampling of the tablet core and coating. Unlike liquid or gas chromatographic techniques, Raman spectroscopy provides for no front-end separation of the individual components in the tablet formulation. This disadvantage can be overcome in several ways. The first is to make use of optical microscopy techniques such as polarized light microscopy (PLM), which allows for the physical separation and classification of different particle types in the tablet core based on their optical properties [12]. The tablet core cross-section is gently broken apart on a glass slide. The slide is then placed on the Raman microscope stage and the sample is then viewed using transmitted and/or plane polarized light. When using plane polarized light, different components in the tablet core formulation can be physically separated by the degree of birefringence that each exhibits using crossed polarizers. A Raman spectrum can then be obtained of each particle type which has been physically separated. Raman microscopy is much better suited to this type of analysis since the

extreme. Two different polymorphic forms of an API are shown in Figure 5. Form 1 (Figure 5a) and form 2 (Figure 5b) can be visually differentiated based on their optical properties. The Raman spectra of each form (Figure 5c) provides additional unique information for spectral differentiation. Therefore, PLM-Raman spectroscopy can be used to verify that the proper crystal (polymorphic) form of the API is present in the sample under investigation.

Figure 3.

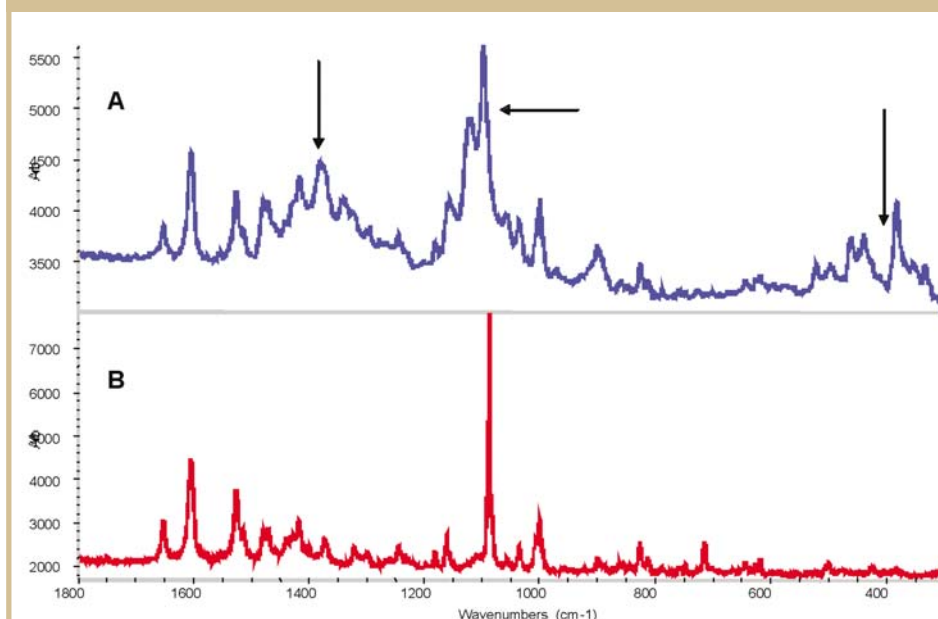


Figure 3. (a) Raman spectrum of a suspect counterfeit tablet core (b) Raman spectrum of an authentic tablet core

Figure 4.

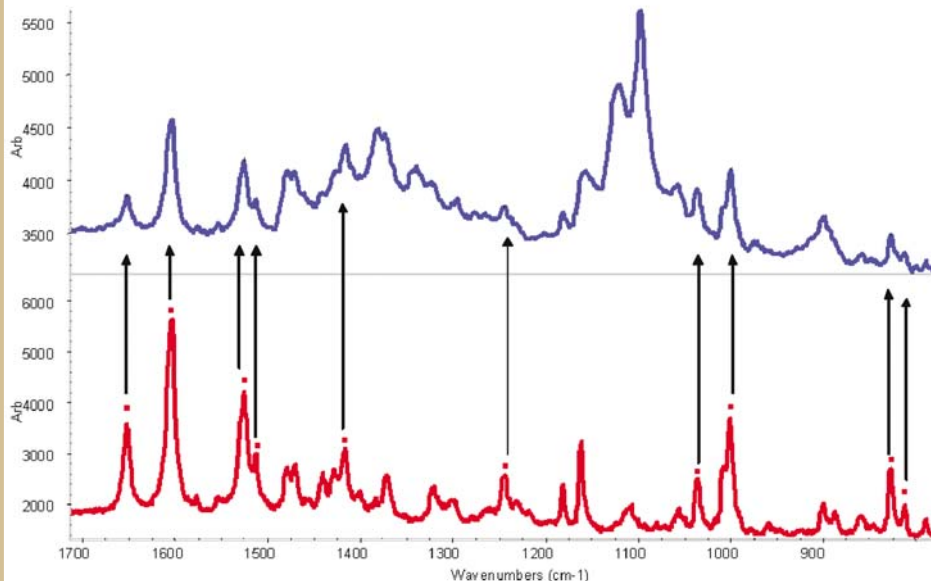


Figure 4. (a) Raman spectrum of a suspect counterfeit tablet core (b) Raman spectrum of the API standard. Peaks used for comparison are marked with arrows

In addition to physical separation of the tablet core components, micro-extractions can be used to isolate the API from the tablet core formulation. When using micro-extractions, care should be taken to compare the Raman spectrum of the extracted API from the tablet core to the Raman spectrum of the extracted/re-crystallized API standard using the same solvent system. This must be done to avoid mis-identifications due to the possible formation of different polymorphic forms of the API due to the solvent used in the extraction.

Micro-extractions allow for small amounts of material to be analyzed when evidence is limited. When dealing with small amounts of material and low levels of API, sensitivity of the Raman measurement may become an issue. In traditional micro-extractions, a small amount of solvent (200 ul - 1ml) may be placed on a glass slide and/or small watch glass as the solvent evaporates the dried material forms residue "rings" (Figure 6A). In this case, the amount of material to be analyzed is dispersed over a relatively large area making it difficult to determine a good sampling area. Recently, coated metal substrates have become available which allow the concentration of small amounts of extracted material [16,17]. The nature of coating allows for the surface tension of the droplet to be maintained while the solvent evaporates (Figure 6b). The dried residue is then a concentrated spot of material which is ideal for measurement using Raman microscopy. Instead of the 200 ul - 1ml deposition, now volumes as low as 20 ul can be deposited and Raman spectra obtained of the dried material. Figure 6c is the Raman spectrum of a 10 ul droplet (~0.4 ug of API) deposited on a slide and dried compared to the Raman spectrum of powdered neat standard of the API (Figure 6d). Good agreement is observed between the Raman spectra of the dried material versus the API standard Raman spectrum. However, minor differences are noted in the Raman spectrum in Figure 6c due to different crystallization conditions between the extracted material and neat powdered API.

Conclusion

Raman spectroscopy is a powerful instrumental technique used in the analysis of suspect counterfeit pharmaceutical dosage forms. The technique provides fundamental spectroscopic information on the API as well as excipients used in specific pharmaceutical formulations, and when combined with FT-IR can provide a complete spectral "snap shot". In most cases, using Raman based analysis, sample preparation is minimal, and when coupled with polarized light microscopy, physical separation of different particles can aid in the analysis of the pharmaceutical formulation. The use of modified metal substrates with micro-extractions allows for small amounts of material to be isolated and analyzed using Raman microscopy.

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Disclaimer

The mentioning of specific products/ instruments in this presentation is for information purposes only and does not constitute an endorsement by either the Food and Drug Administration and/or the Forensic Chemistry Center.

Figure 5.

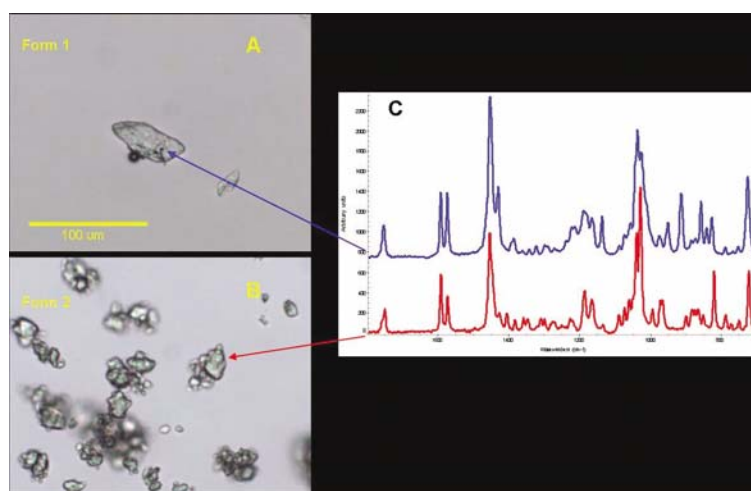


Figure 5. (a) Photomicrograph of API polymorphic form 1 (b) Photomicrograph of API polymorphic form 2 (c) Comparison of the Raman spectra of API polymorphic form 1 to form 2

Figure 6.

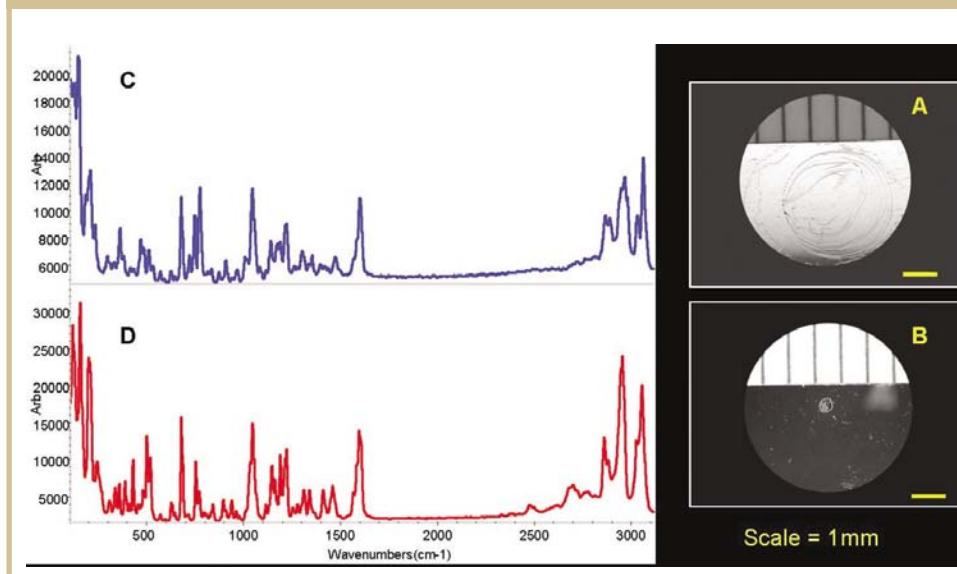


Figure 6. (a) Photomicrograph of dried extract residue on a glass slide (b) Photograph of a dried extract on a modified metal substrate (c) Raman spectrum of ~0.4 ug of drug API deposited on a modified metal substrate (d) Raman spectrum of the API powder standard

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