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The advent of robust, rugged, and current Good Manufacturing Practices (cGMP) compliant hand-held Raman spectrometers provides a wealth of opportunities for the analytical pharmaceutical chemist to bring the laboratory to the sample. This paper discusses the use of hand-held Raman spectrometers for the development of qualitative chemical identification methods for a number of well-known pharmaceutical products (tablets and capsules). Methods were developed on two different instruments and transferred to a third instrument for application of the methodology to independently obtained drug products. A novel decision algorithm is presented for the assessment of the correlation between the Raman spectrum of the unknown sample to the spectrum of the authentic reference material. This novel algorithm considers accuracy but more importantly precision (uncertainty/reliability), thus removing human bias that is associated with typical spectral searching approaches. The results presented in this paper show the reliability of developing, validating, and transferring chemical identification assays on hand-held Raman spectrometers.

Index Headings: **Raman spectroscopy; Chemical identification; Remote analysis; Selectivity; Counterfeit drugs; Hand-held instrumentation.**

INTRODUCTION

Current Good Manufacturing Practices (cGMP) require the chemical identity testing of active pharmaceutical ingredients (APIs) and excipients that are ultimately formulated into finished drug products. Additionally, many drug products require chemical identity testing as they are transported from one physical location in the world to another, perhaps from a manufacturing site to a packaging site. As such, chemical identity testing of pharmaceutical materials is one of the most basic, widely performed, and necessary components of pharmaceutical analysis. In light of counterfeit drug products and devices making their way into the supply chain or consumer market,[1–4] chemical identity testing has taken on a new challenge, namely the ability to accurately differentiate between legitimate and counterfeit drug products and/or devices. As such, the conventional approach to chemical identity testing needs to be revisited with modern technology.

Upon a review of the United States Pharmacopeia/National Formulary (USP/NF), the most prevalent technology used for the chemical identity testing of pharmaceutical materials (APIs, excipients, and/or drug products) is infrared (IR) spectroscopy. Through a combined use of USP Chapters <851> and <197> entitled *Spectroscopy and Light Scattering* and *Spectrophotometric Identification Tests*, respectively,[5,6] IR spectroscopy is incorporated into numerous API and drug product monographs for the chemical identification of the material of interest. From a historical perspective, there are a number of reasons that mid-IR is used for the chemical identification of pharmaceutical materials. First and foremost, IR spectroscopy is a technique that probes the vibrational motions of a molecule in which the frequencies of the fundamental molecular vibrations are a direct manifestation of the chemical structure of the material.[7] As such, the IR spectrum acts as a fingerprint of the material. Other reasons that IR spectroscopy is used for chemical identification of pharmaceuticals include the following: (1) IR is a widely used and understood technique, (2) limited amounts of material are required for analysis, (3) modern, relatively inexpensive instrumentation is available with accompanying calibration standards, and (4) the technique is applicable to solids, liquids, gases, and other materials such as pastes, creams/ointments, and medical devices.

Although IR spectroscopy permits the analysis of a multitude of different samples, the technique in many cases requires the preparation of a sample for analysis. Sampling techniques include preparation of an alkali halide pellet (USP <197K>), a neat, liquid sample (USP <197F>), a solution (USP <197S>), or a mineral oil mull (USP <197M>), although attenuated total reflection sampling accessories now allow for the direct analysis of powdered samples (USP <197A>).[6] From a traditional, pharmaceutical analysis perspective, the ability of IR spectroscopy to sample numerous types of samples within a dedicated spectroscopic laboratory equipped with the array of sampling techniques has been viewed as an advantage of the technique. Unfortunately, in today's modern era of pharmaceutical analysis, the need for a laboratory equipped with these sampling techniques can be viewed as a limitation of the technique. Today's pharmaceutical scientists need to have the flexibility to analyze pharmaceutical materials in an array of environments including a traditional laboratory but also on the floor of a production facility, the loading dock in a materials management setting, within processing equipment, at the port of entry for imported products, or within a field setting such as a counterfeit production site or a point of sale such as a pharmacy.

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Over the past three decades, near-infrared (NIR) spectroscopy has been promoted as another type of vibrational spectroscopy applicable for chemical identity testing.[8,9] NIR spectroscopy primarily measures the vibrational frequencies from the combination and overtones of the fundamental modes of vibration.[10] As such, NIR is less selective and less sensitive as compared to the fundamental forms of vibrational spectroscopy such as IR and Raman. Based upon these attributes of NIR, developing methodologies for the chemical identity testing of pharmaceutical materials by NIR has required the building of libraries in which each chemical component within the library is a representation of multiple batches of material. As one can imagine, the generation of a spectral library consisting of multiple components, such as various APIs and excipients, would require a large number (>100) of representative materials for inclusion. Additionally, due to the low selectivity of NIR, significant effort must be made via chemometrics to actually differentiate between structurally similar materials such as lactose monohydrate, lactose anhydrous, and Fast Flo lactose. Finally, validation of the NIR spectral library and transferring of the library from one spectrophotometer to another has been shown to be a daunting task.[11] As such, successful commercial implementation of NIR for chemical identity testing has been implemented in the pharmaceutical industry only with extensive, and continuous, expert oversight of the installation.

Another type of vibrational spectroscopy that is ideal for the chemical identification of APIs, excipients, and drug products/devices is Raman spectroscopy. Like mid-IR spectroscopy, Raman spectroscopy probes the fundamental vibrational motions of a molecule in which the frequencies of the molecular vibrations are a direct manifestation of the chemical structure of the material.[7,12,13] Analogous to an IR spectrum, the acquired Raman spectrum may also act as a fingerprint of the material. One must recall that the total number of fundamental vibrational bands for a nonlinear molecule is equal to $3N - 6$ where N is the total number of atoms within the molecule.[12] From group theory analysis of the molecule, one may determine which fundamental vibrational modes are Raman active and which are IR active.[12] As such, Raman and IR spectroscopy are complementary techniques, allowing one to probe all the vibrational motions associated with a molecule of interest.

One of the distinct advantages of using Raman spectroscopy for the chemical identification of pharmaceutical materials as compared to IR is that in most cases, Raman analysis does not require sample preparation for subsequent analysis. It has been shown that Raman spectra may be acquired on neat material as well as through packaging enclosures including glass vials,[14,15] blister packs,[16,17] and plastic bags.[18] This lack of sample preparation allows Raman spectroscopy to be a very flexible technique in a multitude of testing environments that require routine chemical identity testing of liquids and solids. A perceived hurdle in the use of Raman spectroscopy has been the supposed lack of acceptance of the technique by regulatory bodies. Raman spectroscopy has in fact been incorporated into the USP/NF for many years, being referenced in *Spectroscopy and Light Scattering*, Chapter <851>.[5] More recently, a separate USP chapter has been devoted to Raman spectroscopy: Chapter <1120> *Raman Spectroscopy*.[19] Additionally, as far back as 1998, regulatory bodies have seen the advantage of Raman spectroscopy for the analysis of pharmaceutical materials, including identification within packaged units.[15] More recently, the USP published an in-process revision of Chapter <197> *Spectrophotometric Identification Tests*, proposing a change in the title of the chapter (*Spectroscopic Identification Tests*) as well as the use of alternative methodologies for chemical identification.[20] One such methodology is Raman spectroscopy and similar to existing chemical identification methodologies, Raman can alternatively be used for chemical identification of pharmaceutical materials (API, excipients, drug product) after it is demonstrated that Raman is suitable for the intended application (validation).

Most literature examples demonstrating the use of Raman spectroscopy for pharmaceutical analysis utilize laboratory-based dispersive or Fourier transform based spectrometers. During the past decade, significant improvements have been made in the design, miniaturization, and efficiency of lasers, monochromators, and detectors utilized for Raman spectroscopy.[21] These advancements have led to the development of hand-held Raman spectrometers that approach, if not surpass, the performance characteristics of some laboratory-based systems.[22] Additionally, these same hand-held Raman spectrometers have incorporated 21 CFR Part 11 compliant software, again permitting the use of these instruments in cGMP settings.[†] The advent of hand-held Raman spectrometers now allows the pharmaceutical scientist to bring the laboratory to the sample.

The backdrop has now been established: (1) chemical identity testing of pharmaceutical materials is one of the most widely utilized forms of analysis in the development, manufacturing, and distribution of pharmaceutical products, (2) IR spectroscopy has been the traditional laboratory-based approach, although some field-based spectrophotometers are now available, (3) NIR spectroscopy has not gained wide usage due to less than optimal selectivity, which has required more extensive spectral processing approaches and expert management, (4) Raman is a well-suited, fundamental vibrational spectroscopy approach that has gained regulatory acceptance, and (5) hand-held Raman spectrometers now permit the flexibility of bringing the analyzer to the sample no matter where the sample may reside (loading dock, pristine laboratory, or make-shift counterfeit location). Yet, one hurdle still exists: the development and validation of reliable, cGMP-compliant, Raman-based chemical identification assays that may be utilized across multiple spectrometers in a myriad of field locations.

This paper presents the development of a series of Raman-based, chemical identification methods, which utilized a hand-held spectrometer. These methods were developed for a number of highly prescribed drug products, anti-malarial and antibiotic drug products, as well as tuberculosis (TB) medications. The array of drug products included materials manufactured by innovator and generic manufacturers as well as tablet versus capsule based products of the same API. A key component of the present work is that the chemical

† 21 CFR Part 11 refers to Title 21, Part 11 of the Code of Federal Regulations in which the Food and Drug Administration (FDA) presents guidelines on electronic records and signatures. FDA criteria are presented so that stakeholders (e.g., pharmaceutical manufacturers, analytical instrument companies) can invoke procedures such that electronic records and signatures are considered to be trustworthy, reliable, and equivalent to paper records.

TABLE I. Drug products studied.

	Drug supplier information, Mtd development samples	Validation samples
Anti-malarials		
Mefloquine	Sandoz 250-mg tablet, lot 165880	Sandoz 250-mg tablet, lot 165881
Sulfadoxine + pyrimethamine (Fansidar®)	Roche 500-25 mg tablet, lot B1500-50	Not able to obtain
Anti-tuberculosis		
Rifampicin	Sandoz 300-mg capsule, lot ML061454	Sandoz 300-mg capsule, lot ML080102
Isoniazid	Barr 300-mg tablet, lot 306103	Barr 300-mg tablet, lot 301225
Anti-biotics		
Ciprofloxacin	Ranbaxy 500-mg tablet, multiple lots 1901146, 1917257	Ranbaxy 500-mg tablet, lot 1911392
	Schering 500-mg tablet, lot 5400JP5	Not able to obtain
Erythromycin ethyl succinate	Abbott Labs 400-mg tablet, lot 64993CG21	Abbott Labs 400-mg tablet, lot 57984C621
Erythromycin	Abbott Labs 333-mg tablet, lot 63919AF21	Abbott Labs 333-mg tablet, lot 58820AF21
Amoxicillin	Sandoz 500-mg tablets, lot 147418	Sandoz 500-mg tablets, lot 155610
	Teva 500-mg tablets, lot 418696A	Not able to obtain
	Teva 500-mg capsules, lot 35409529A	Teva 500-mg capsules, lot 35409256A
Highly prescribed drugs		
Celebrex®	G.D. Searle 200-mg capsule, multiple lots C080741, C080744, C080927	Not able to obtain
Lipitor®	Pfizer 10-mg tablets, multiple lots 08618V, 09388V	Pfizer 10-mg tablets, lot 13378V
Nexium®	AstraZeneca 40-mg capsule, lot X1998	AstraZeneca 40-mg capsule, lot X1943
Prozac®	Dista Labs, 20-mg capsule, lot A294452	Dista Labs, 20-mg capsule, lot A453896A
Viagra®	Pfizer 100-mg tablets, lot 8229303	Pfizer 100-mg tablets, lot 8268201
Zetia®	Merck/Schering 10-mg tablet, lot 8EZP506A1	Merck/Schering 10-mg tablet, lot 8EZPS28A1
Zoloft®	Pfizer 100-mg tablet, lot C080884	Pfizer 100-mg tablet, lot C081272

identification methods were developed on two different hand-held Raman spectrometers and these methods were subsequently transferred to a third hand-held spectrometer of the same manufacturer. The methods were then evaluated for the validity of the method development and transfer by assaying different batches of the same drug products used to develop the original methods. The results presented in this paper show the reliability of developing, validating, and transferring chemical identification assays on hand-held Raman spectrometers.

EXPERIMENTAL

Materials. The pharmaceutical drug products used for this study (Table I) were dispensed from licensed pharmacies located within the state of Indiana. The majority of drug products were dispensed as tablets but filled capsules were also obtained. No sample preparation procedures were necessary for Raman analysis. A compressed placebo formulation was generated for the ciprofloxacin formulation in accordance with US Patent 5,286,754, Example 1.[23]

Data Acquisition, Instrumentation, and Software. All spectral data were acquired on three different Ahura Scientific TruScan spectrometers. The units were manufactured at different times using different batches of components. The intact tablets or capsules were placed in a demountable spring-loaded holder, which was attached to the spectrometer. For encapsulated materials, the reference spectrum was acquired directly through the gelatin capsule. Utilization of the demountable holder provided a 2 mm spot size of sample exposure to a 300 mW, 785 nm diode laser. The device has a cooled, 2048 element silicon charge-coupled device (CCD) detector and dielectric edge filters for Rayleigh rejection. A nominal spectral resolution of 6 to 8 cm^{-1} was obtained across the spectral range of 2900 to 250 cm^{-1}. Scan times varied between the various spectral acquisitions for method development (~5 to 45 min) versus routine analysis (~15 to 90 s).

The spectrometer was controlled by version 1.1.1 of TruScan software. Formulation specific methods were developed for each drug product via the embedded web administration software. A standard web browser was connected to the spectrometer over a local area network to associate an authentic reference spectrum with a descriptive method name. All of the calculations were conducted using the TruScan's embedded analysis, with the results being transferred from the remote device to an electronic archive using the Ahura Scientific SyncServer software package. The sync software generated reports in SPC, TXT, JPG, and PDF formats. The sync process facilitates 21 CFR Part 11 electronic records requirements and utilizes standard TCP-IP networking for data communication.

RESULTS AND DISCUSSION

As described by the USP,[6] chemical identification of an unknown material by a spectroscopic measurement such as IR or Raman spectroscopy initially requires acquisition of a reference spectrum for the authentic material. Typically, the reference material is either an authenticated in-house reference standard or a USP reference standard. In the present study, by random fashion, two TruScan hand-held Raman spectrometers were used to develop chemical identification methods for each of the drug products listed in Table I. Prior to analysis of the sample selected for method development, the hand-held Raman spectrometers were turned on and allowed to go through their internal diagnostics tests including a test of system suitability against a polystyrene standard. Subsequently, development of the chemical identification method for each drug product consisted of placing a single tablet or capsule, for a single batch

of material, into the sample holder and acquiring a reference spectrum.

In many cases, the acquisition of the reference spectrum is considered as a simple spectral acquisition. In reality, the acquisition of the reference spectrum is the single most important step in development of a chemical identification assay. Without an authentic reference spectrum, the accuracy of the qualitative chemical identification assay may be questioned. Validation of the assay is obviously the next important step during assay development.

In general, Raman spectra can be affected by ambient lighting, temperature fluctuations, fluorescence, detector noise, and varying laser power. Use of hand-held Raman spectrometers is subject to these same factors but also incurs potential signal variances due to varying operators or extreme environmental conditions from field operation. As such, the reference spectrum not only needs to be an authentic, true representation of the material of interest (accuracy), but the reference spectrum must also account for reliability of the spectral acquisition (intermediate precision). To date, it has been our experience that intermediate precision has not been considered in the commercial development of qualitative chemical identification assays. A fairly recent editorial suggests that a result is not a result without an assessment of reliability (uncertainty).[24] With the advent of real-time monitoring of instrumental fluctuations and consideration of these fluctuations, the intermediate precision of qualitative analysis can be measured and considered in the chemical identification of unknown samples.

As previously mentioned, whether working within a pristine laboratory environment or performing analysis in a field location, acquisition of a Raman spectrum, be it a reference or unknown material, will be subject to variances of spectrometer performance and environmental factors. For example, although most sample holders for Raman spectrometers are designed to reduce external fluorescence (e.g., laboratory light fixtures), the external fluorescent signal may leak through and impact the quality of the resultant measured spectrum. Additionally, most Raman spectrometers are designed to minimize temperature fluctuations although temperature variances do occur and can adversely affect subsequent spectral interpretations. Keeping in mind the varied settings under which spectra may be acquired, robust methodology is required for the acquisition of a reference spectrum that takes into account varying conditions. The spectrometers utilized in this study monitor diagnostic parameters of instrument performance in real time, namely during spectral acquisition. In fact, the length of spectral acquisition for the reference material is dependent upon both the intensity of the spectrum (sample dependent) as well as the measurement uncertainty; together they define the signal-to-noise ratio (SNR) and direct the automated measurement process. Real-time monitoring of measurement variances due to instrument performance, environmental conditions, and/or sample variation during spectral acquisition sets the magnitude of uncertainty tolerated during the chemical identity pass/fail decision. The acquisition and monitoring of real-time measurement uncertainty during spectral acquisition of authentic materials is a novel approach to qualitative chemical identification assays that considers both the precision and accuracy of the developed method.

Utilizing this novel approach to qualitative chemical identification method development, reference spectra were acquired for the eighteen drug products listed in Table I. The spectral acquisition time for each of these drug products varied since each material has significantly different scattering characteristics due to the potency and chemical composition of each drug product. Additionally, some of the drug products produced Raman spectra with underlying fluorescent signals that affected spectral acquisition times. These underlying fluorescent signals did not adversely affect the ability of the developed methods to correctly perform chemical identification (*vide infra*).

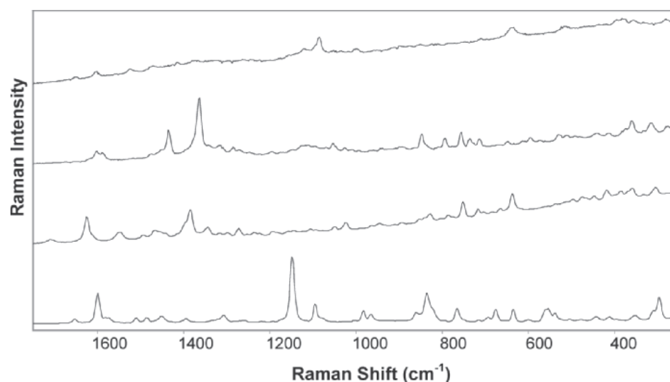

FIG. 1. Acquired reference Raman spectra (top to bottom) for Lipitor, mefloquine, ciprofloxacin (Schering), and Fansidar tablets.

Figure 1 presents a stacked plot of the acquired Raman reference spectra, from one of the two different hand-held Raman spectrometers, for four different drug products: Lipitor, mefloquine, ciprofloxacin (Schering), and Fansidar. Each product exists as a tablet. Different spectral qualities are displayed for each spectrum. The reference Raman spectrum for Fansidar is an ideal spectrum for subsequent spectral comparisons to unknown sample spectra. An excellent SNR is demonstrated as well as the presence of numerous highly resolved peaks and a consistent, flat baseline. In comparison, the reference Raman spectra for mefloquine and ciprofloxacin show intermediate quality. What is immediately evident with the Raman spectra of mefloquine and ciprofloxacin is the offset spectral baseline that is due to a fluorescent signal with superimposed Raman scattering upon this skewed baseline. Additionally, the Raman spectra of mefloquine and ciprofloxacin do not display as many peaks as that for Fansidar nor are the peaks fully resolved. The reference Raman spectrum for Lipitor displays the most challenging spectral qualities for subsequent qualitative chemical identification. Not only does the Raman spectrum for Lipitor display a skewed baseline due to fluorescence, but it also shows very few peaks that are poorly resolved and of low intensity. The concentration of atorvastatin calcium trihydrate in the tablet is very low (10 mg), which immediately makes detection of peaks due to the API challenging. Even so, the spectrum of Lipitor does indeed display peaks due to the API (~1700–1500 and ~1200–1000 cm^{-1}). A comparison of the spectral qualities of all eighteen acquired Raman reference spectra show that they may be classified in one of the aforementioned categories of spectral quality: excellent, intermediate, or challenging.

From the combined guidance of the ICH[25] and USP,[26] validation of qualitative chemical identification assays requires the demonstration of specificity. In the case of methods

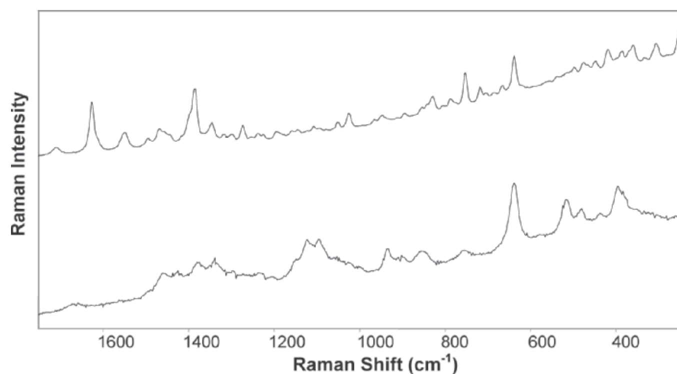

FIG. 2. Comparison of the acquired Raman spectra (top to bottom) for the ciprofloxacin innovator formulation and its placebo.

developed for qualitative chemical identification of drug products, the most common challenges to the developed method (specificity determination) are analysis of a placebo formulation as well as a different formulation of the same API. In this study, we have an innovator formulation for ciprofloxacin, the corresponding placebo, and a generic version of the drug product. All are ideal candidates for validation studies of the ciprofloxacin drug product developed method.

Displayed in Fig. 2 is the reference Raman spectrum of the innovator ciprofloxacin formulation compared to its placebo. The Raman spectrum for the drug product is clearly differentiated from the Raman spectrum of the placebo. From this simple comparison, specificity is demonstrated and one may consider that one aspect of validation of the developed method is completed. The second challenge to the validity of the developed method is to determine whether the method correctly identifies a different batch of the same manufacturer's product. In order to make this assessment, one needs to consider the criteria for "matching" spectra. An additional consideration that is not a validation component, but actually a technology transfer consideration, is the portability of a developed method from one spectrometer to another. In this study, the Raman methods were randomly developed on two different spectrometers and subsequently transferred to a third spectrometer in order to assess the validity of the methods.

The USP outlines a procedure for the comparison of vibrational spectra in order to determine whether the unknown material is chemically identified as a reference/known sample.[6] The procedure specifies that the spectrum of the unknown material must exhibit maxima only at the same wavelengths as that of a similar preparation of the corresponding reference standard. The aforementioned procedure utilizes a potentially biased, non-quantitative human approach. In light of today's computer-based spectrometers, which collect digital spectra, more accurate and precise means of spectral comparisons are certainly possible. One common approach utilizes a spectral library that is populated by reference spectra of authentic materials.

In the traditional spectral library approach, the wavelength correlation between the spectrum of the unknown sample and each component within the spectral library is performed and the best corresponding spectral matches are output for consideration by the operator.[27] This approach is the equivalent to measuring the cosine of the angle between the two spectra, resulting in a correlation coefficient of 1 for an ideal match and 0 for two spectra that are orthogonal to each other. This approach presents a number of challenges to the operator. The first challenge is which mathematical approach should be used to calculate the spectral correlation. Second, in spectroscopy, where noise always exists in measurements, the correlation coefficient can never be unity and therefore, where does one set a correlation threshold for a "pass/fail" correspondence for the spectral comparison?[27] The correlation is merely an angle and is not a probability, so a threshold of 0.95 in no way means 95% likely, 95% confidence, or 95% agreement. An example of the spectral library approach and the challenges associated with the setting of a "pass/fail" threshold is demonstrated in Table II utilizing the drug products presented in Table I.

Utilizing OMNIC™ (Version 7.2, Thermo Electron Corp.) software, a spectral data processing software package, a spectral library was generated using the eighteen Raman reference spectra collected from the two TruScan hand-held spectrometers. Subsequent to spectral library generation, spectra were acquired on a second batch of the materials (Table I) utilizing a third TruScan hand-held spectrometer. In this fashion, not only can the validity of the methods be assessed, but also the transferability of the methods. The OMNIC™ library search software permits the determination of spectral correspondence via a number of different search algorithms.[27] In this study, the correlation[28,29,‡] and absolute difference[27,29,§] search algorithms were used. The correlation search algorithm is the recommended procedure because it removes any effect of offset in the unknown spectrum, eliminating baseline variations, whereas the absolute difference approach puts more weight on small differences between the unknown and library spectra.

Utilizing the correlation search algorithm, each spectrum of "unknown" material (second batch of drug product) that was collected on the third hand-held spectrometer was subjected to the search process. The results of the library searching procedure (Table II) indicated that for seventeen of the eighteen unknown sample spectra, the highest "match value/correspondence" was the reference spectrum correlating to the correct material. From the search algorithms used, the higher the value of the match number, or hit quality index (HQI), the better the correlation between the library and unknown spectra. For the one unique result, namely ciprofloxacin manufactured by Ranbaxy, the spectral search procedure indicated that the best match was to the reference spectrum of ciprofloxacin manufactured by Schering. Although the search procedure correctly ranked seventeen of the drug products, the match values varied significantly: 61.67 to 92.90. The aforementioned challenge of threshold setting remains: what match value constitutes spectral equivalence? Utilizing the absolute difference search algorithm led to significantly poorer results. In this case, only nine of the search procedures identified the highest match value to the correct reference spectrum (Table II). The match values for the corresponding reference spectra were also of significantly lower values: 29.34 to 60.66. These results

‡ $HQI = 100 \times \sqrt{r^2}$, where $r^2 = [(Lib'_m \cdot Unkn'_m)^2]/[(Lib_m \cdot Lib_m) \cdot (Unkn_m \cdot Unkn_m)]$ and Lib' and $Unkn'$ are the three-point smoothed derivative of the library spectrum and unknown spectrum, respectively.

§ $HQI = 100 - 100 * Difference/Spectrum$, where $Difference = \Sigma\, |y_i - mx_i - d_{\min}|$ summed over $i = 1$ to n, $Spectrum = \Sigma\, |y_i - y_{\min}|$ summed over $i = 1$ to n, and $d_{\min} = y_{\min} - mx_{\min}$, m being a scale factor equating the intensity of the unknown spectrum (Y) to the library spectrum (X), and $x_{\min}$ and $y_{\min}$ being the minimum intensity values of the library and unknown spectra, respectively.

TABLE II. **Spectral searching results.**

	Drug supplier information	Search procedure: Correlation algorithm	Search procedure: Absolute difference algorithm	p-value
Anti-malarials				
Mefloquine	Sandoz 250-mg tablet, lot 165880	85.12	Identified Nexium® as best match	0.3067
Sulfadoxine + pyrimethamine (Fansidar®)	Roche 500-25 mg tablet, lot B1500-50	88.56	Identified erythromycin as best match	0.2605
Anti-tuberculosis				
Rifampicin	Sandoz 300-mg capsule, lot ML061454	81.85	29.34	0.2805
Isoniazid	Barr 300-mg tablet, lot 306103	88.47	44.73	0.2944
Anti-biotics				
Ciprofloxacin	Schering 500-mg tablet, lot 5400JP5	83.66	Identified ciprofloxacin by Schering as best match	0.1566
	Ranbaxy 500-mg tablet, multiple lots 1901146, 1917257	84.98, Identified ciprofloxacin by Schering as best match	48.07	0.3342
Erythromycin ethyl succinate	Abbott Labs 400-mg tablet, lot 64993CG21	74.66	47.54	0.2006
Erythromycin	Abbott Labs 333-mg tablet, lot 63919AF21	73.71	60.15	0.1680
Amoxicillin	Sandoz 500-mg tablets, lot 147418	92.90, Sandoz 500-mg tablets	Identified amoxicillin Teva 500-mg tablets as best match	0.2921
	Teva 500-mg tablets, lot 418696A	90.72, Teva 500-mg tablets	Identified amoxicillin Teva 500-mg capsules as best match	0.3038
	Teva 500-mg capsules, lot 35409529A	85.33, Teva 500-mg capsules	60.66	0.2760
Highly prescribed drugs				
Celebrex®	G.D. Searle 200-mg capsule, multiple lots C080741, C080744, C080927	81.60	Identified Lipitor® as best match	0.2820
Lipitor®	Pfizer 10-mg tablets, multiple lots 08618V, 09388V	61.67	35.79	0.2527
Nexium®	AstraZeneca 40-mg capsule, lot X1998	65.28	46.67	0.1648
Prozac®	Dista Labs, 20-mg capsule, lot A294452	77.65	Identified Lipitor® as best match	0.1543
Viagra®	Pfizer 100-mg tablets, lot 8229303	68.90	Identified Lipitor® as best match	0.1592
Zetia®	Merck/Schering 10-mg tablet, lot 8EZP506A1	76.71	Identified Lipitor® as best match	0.2397
Zoloft®	Pfizer 100-mg tablet, lot C080884	88.93	60.24	0.2148

show the obvious challenge to the utilization of traditional spectral searching algorithms that only consider method accuracy and not precision.

An alternative approach to the spectral library wavelength correlation method previously discussed is to evaluate whether the measured spectrum of the unknown sample lies within the multivariate domain of the reference spectrum. The multivariate domain is defined by the uncertainty characteristics of each spectral measurement (reference and sample): the domain in which one would expect spectra of the reference material to lie under the current measurement conditions. These characteristics include exposure settings, instrumental properties such as detector properties, component temperatures, and laser behavior or ambient lighting, as well as the optical properties of the sample itself (low or high scatterer). This approach is different than the traditional assessment as to the simple angle between the unknown sample spectrum and the reference spectrum. Utilizing this novel approach, the critical question in qualitative chemical identity testing, namely whether the unknown spectrum is consistent with the reference spectrum, explicitly takes into account uncertainty of the measurement conditions.[30–32]

This approach to spectral comparison is facilitated by hypothesis testing and subsequent statistical analysis. For example, pharmaceutical identity analysis is a well-defined, closed system that typically involves a known material to be analyzed, e.g., Zoloft, utilizing a known method for analysis of that material. An infinite number of possibilities are not being tested since a known pharmaceutical process is producing a theoretically known material that is being analyzed by a specifically designed method for that known material. As such, the hypothesis: "The drug product from the Zoloft pharmaceutical formulation process is Zoloft" is well defined and may be tested by the null hypothesis ($H_0 =$ Zoloft drug product), and the alternative $H_1 =$ not authentic Zoloft. As with conventional hypothesis tests, evidence against the null is measured using likelihood functions and can be summarized by a p-value. The p-value, also known as the observed level of significance, is known as the smallest level of significance at which the null hypothesis will be rejected assuming the null hypothesis is true.

In the case of chemical identity testing, the *p*-value is the probability of observing the unknown spectrum or one more extreme if the unknown is indeed what it purports to be. Higher *p*-values indicate that any differences between the two spectra are not large relative to the uncertainty of the measurement. In these cases, the measured spectrum is determined to be consistent with the reference spectrum indicating that the null hypothesis is not to be rejected: the unknown sample passes the qualitative chemical identity test. If the *p*-value is too low (<0.05 as the device default), this suggests that the differences between the measured and reference spectra were unlikely to arise from the uncertainty in the measurement alone and as such, one should reject the null hypothesis: the unknown sample fails the qualitative chemical identity test. Utilizing this approach, the qualitative chemical identity test now incorporates not only an accuracy component (use of a specific method for a specific sample), but also precision (*p*-value). The final determination is not unlike chemometric approaches employing Mahalanobis distances (a.k.a. Hotelling statistics), which can also be used to generate *p*-values, but the distinct difference in TruScan's case is that the multivariate models are generated from first principles in real time by the instrument and thus entirely optimized for the measurement at hand. This is very different from standard chemometric methods, which are empirically trained for a specific set of sample properties and measurement conditions. This general framework was previously assessed for general identification performance in Raman, but it has not been evaluated on finished pharmaceutical product.[33] This approach has now been tested for the eighteen drug products outlined in Table I.

Each of the developed methods from the two different handheld Raman spectrometers was transferred to the third spectrometer. After the performance qualification procedure was successfully performed, five tablets from each of the second batches of drug products were tested in order to challenge their corresponding qualitative chemical identity methods. Unfortunately, for four of the drug products, a second batch of material was not able to be sourced, so five different tablets from the method development batch were utilized. Table II presents the mean *p*-values obtained from five tablets from each drug product against their developed methods. It is immediately evident that the *p*-values were greater than 0.05, the cut-off criterion value for pass/fail testing of the unknown spectrum. These results clearly indicate that each set of tablets was correctly assayed against their specific method within the acceptable uncertainty of the overall spectral measurement.

A further review of the results, via a graphical matrix (Fig. 3), reveals that the developed qualitative chemical identity assays are very selective. From Fig. 3, the green boxes along the diagonal demonstrate a *p*-value greater than 0.05, indicating a pass result for the identity of each unknown material as compared to their respective methods. The red elements of the matrix demonstrate that the methodology does not produce false-positive identification for the large majority of assayed drug products. Although Raman spectroscopy is very selective, the matrix does point out that for two different APIs (amoxicillin and ciprofloxacin), off-diagonal elements of the matrix display "pass" *p*-values. Although the innovator version of the ciprofloxacin formulation is correctly identified by its corresponding method, it also passes the generic version of the formulation. One explanation for these results may be that the formulations have very similar excipient blends and as such, the same potency formulations are indistinguishable. From a manufacturing perspective, this false-positive result for each formulation does not present a problem since it is highly unlikely that the same manufacturing site would be generating the two drug formulations. If the methods were being used in an anti-counterfeit operation by law enforcement authorities at a port of entry, these results indicate a limitation of the Raman methodology. On the other hand, it is very interesting to note the selectivity between erythromycin and the ethyl succinate salt of erythromycin. One may initially believe that the Raman spectra of erythromycin and its ethyl succinate salt should be significantly different. Erythromycin is a structurally complex molecule and has similar functional groups to the ethyl succinate counterion. For this reason, as well as potential spectral overlap from excipients present in the two different tablets, selectivity by Raman spectroscopy may be less than ideal. Yet, when each of these materials was assayed by the two methods, each material was correctly identified (green diagonal element, Fig. 3) and, additionally, showed no false-positive results between the two methods. When the two erythromycin materials were assayed by their opposite methods, *p*-values of 1.26×10^{-8} and 2.14×10^{-7} were obtained, clearly indicating the selectivity for these structurally similar APIs and their respective formulations.

The data presented in Fig. 3 result from a single spectrum of the specific drug product assayed against the various reference spectra (methods) of other drug products. Acquisition of reference spectra from different spectrometers, technology transfer of the method to a third spectrometer, and analysis of different batches of the drug product against the methods certainly challenge the multivariate domain/uncertainty approach to qualitative chemical identification. Yet, additional challenges can be devised in an effort to test the selectivity of the Raman methodology and unique data analysis approach. To this end, reference Raman spectra were acquired for ciprofloxacin API and innovator placebo and these methods were added to the array of previously developed methods. Challenges to the methodology considered that the high selectivity (*p*-value $\geq$ 0.05) may be based upon a single sampling of the unknown or the significant chemical differences between the products. To test these challenges, five different Raman spectra were collected for the ciprofloxacin innovator tablet. These spectra were then assayed against the ciprofloxacin innovator tablet method. Table III presents the results and it is very clear that the method displays strong reproducibility for multiple sampling events ($n = 5$). The reported *p*-values for each sampling of the innovator tablet are greater than 0.05 and the relative standard deviation between the results is very low, namely 6.2%. Towards the latter challenge, it was also considered whether the selectivity would be maintained when methods exist for the API and placebo versus the formulated tablet. Additional data in Table III shows that when the ciprofloxacin innovator tablet is tested against API and placebo methods, selectivity is maintained; indeed, very low *p*-values ($<1 \times 10^{-20}$) are obtained. Not only do these results answer the challenge of selectivity with respect to API and placebo, but also against repeated physical sampling and results reproducibility.

The analyses of the three amoxicillin drug products show some of the limitations of Raman spectroscopy for qualitative chemical identification. The same API is present at the same potency (500 mg) for the three drug products, thus forcing

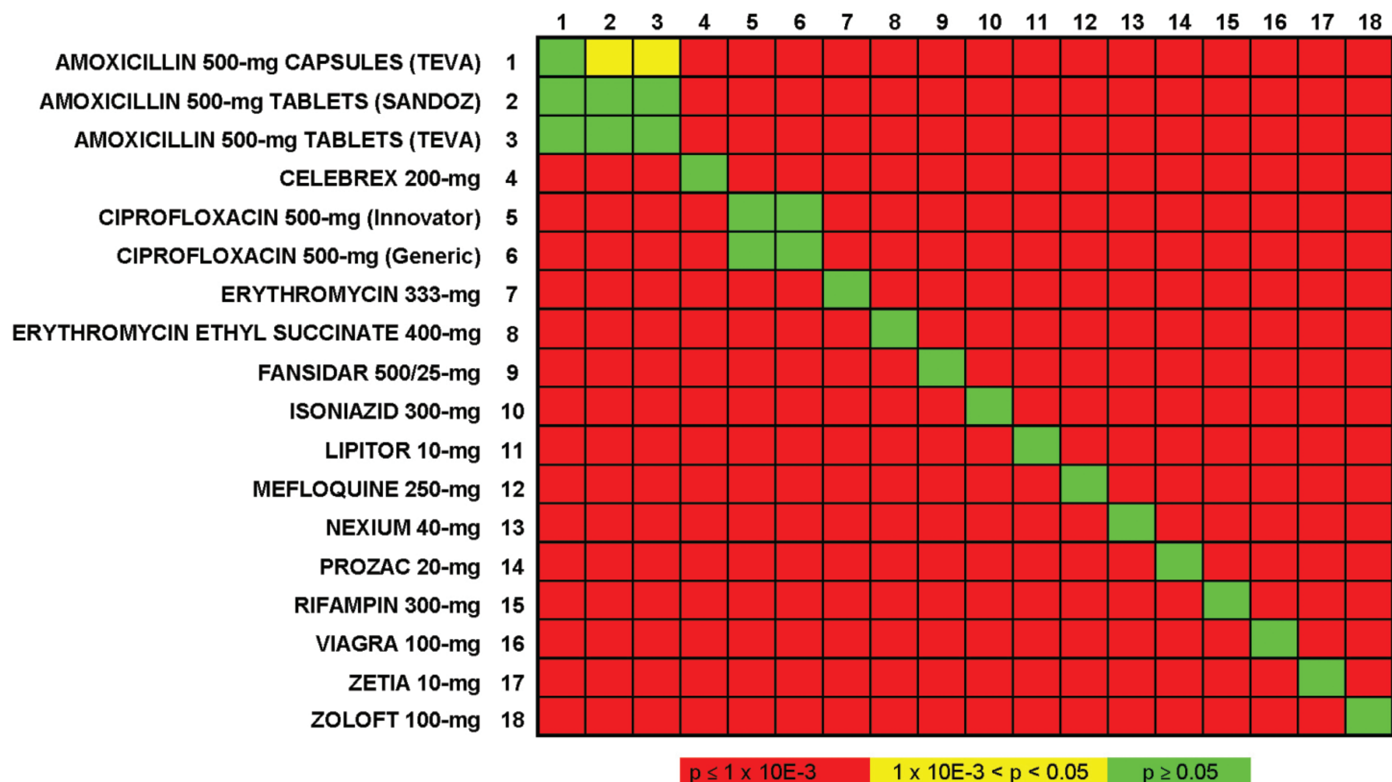

FIG. 3. Comparison of the qualitative chemical identification assays via a selectivity matrix.

formulation differentiation based upon excipient spectral response. Critical examination of Fig. 3 reveals that the method for the Teva capsules only provides correct identification for the corresponding amoxicillin capsules (row one, column one in green vs. columns two and three in yellow). On the other hand, the two tablet methods for amoxicillin (rows two and three) provide correct identification for its corresponding drug product but also false-positive results for the other two formulations. This is unfortunate, especially for the Teva capsule and tablet formulations that may be manufactured at the same site, thus requiring alternative identification assays. It is suspected that the method for the amoxicillin capsules is specific because the Raman spectra are acquired through the capsule, and thus the capsule provides unique spectral response for differentiation from the tablet spectra.

The construction of selectivity matrices like Fig. 3 provides a review of developed methods and their performance. This allows one to determine the method selectivity within the inherent boundaries of the spectroscopic methodology. The information attained via this approach accelerates the development and validation process by illuminating where additional investigational efforts must be applied to ensure method selectivity. The end result is an enhancement of the overall robustness of the method library.

The multivariate domain/uncertainty approach to qualitative chemical identification provides a significant advantage for technology transfer of methods from one instrument to another. The aforementioned spectral acquisition approach incorporates a built-in test of system suitability for wavelength accuracy, which is necessary for the qualitative analysis. From the selectivity matrix (Fig. 3), the results clearly show the direct transferability of methods from the two initial spectrometers to the third unit used for the validation component of the study. Finally, this unique computational approach provides a universal criterion for qualitative chemical identification across multiple Raman spectrometers of the same model/manufacturer.

CONCLUSION

Hand-held Raman spectrometers are an exciting opportunity for analytical chemists in the pharmaceutical industry because the laboratory can now be brought to the samples. The results presented in this paper clearly show that the performance of the hand-held Raman spectrometers provides highly selective,

TABLE III. Selectivity challenges (*p*-values) for ciprofloxacin innovator tablet assayed against various methods.

Method	Sampling number					Mean	RSD
	1	2	3	4	5		
Ciprofloxacin innovator tablet	0.56	0.52	0.52	0.47	0.52	0.52	6.2
Ciprofloxacin generic tablet	0.18	0.11	0.14	0.10	0.16	0.14	24.3
Ciprofloxacin placebo	4.73×10^{-20}	3.93×10^{-23}	3.76×10^{-21}	1.43×10^{-21}	1.04×10^{-21}	1.1×10^{-20}	191.3
Ciprofloxacin API	2.30×10^{-26}	3.04×10^{-30}	1.63×10^{-28}	1.53×10^{-27}	5.70×10^{-30}	4.9×10^{-27}	204.8

excellent signal-to-noise spectra for use in development and utilization of qualitative chemical identification assays necessary for cGMP compliant testing. More importantly, the novel decision algorithm discussed provides a level of accuracy and precision to the qualitative identification process that is not currently available from traditional spectral library searching approaches. The statistically based algorithm provides an objective assessment of the correspondence between the Raman spectrum of the sample of interest (unknown) to the spectrum of the authentic reference material and, for the first time, directly incorporates the uncertainty or reliability in the qualitative identity test.

One of the most exciting outcomes of this work is the fact that the novel decision approach to spectral correlation is not spectrometer dependent. Thus, qualitative chemical identification methods developed on one or more hand-held Raman spectrometers can be transferred to other units located throughout the pharmaceutical company's locations (domestic or world-wide). The fact that methods can now easily be developed in one location and transferred electronically to other world-wide locations presents a breadth of opportunities for the use of the hand-held Raman spectrometers.

One of the most exciting possibilities in the use of the hand-held Raman spectrometers is for counterfeit drug product detection. Upon the development, validation, and transfer of qualitative chemical identification methods for authentic drug products to numerous hand-held Raman spectrometers, these units can then be used to assess the authenticity of seized products. A number of advantages exist by having the ability to perform field analysis: (1) a quick, highly confident initial assessment of the seized materials can be made, (2) the results can dictate further analyses if required for confirmation of suspect material, and (3) most importantly, the newly seized material can be used to develop a new, qualitative chemical identification method and immediately be transferred to other spectrometers in an effort to keep all spectrometers up to date on counterfeit activities. The results presented in this paper show the reliability of developing, validating, and transferring chemical identification assays on hand-held Raman spectrometers.

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