

Moving toward 100% raw material inspection with a handheld Raman spectrometer

Robert L Green and Christopher D Brown
Ahura Scientific, Inc., 46 Jonspin Road, Wilmington, MA 01887, USA

Pharmaceutical manufacturers are under pressure to minimize the current costs of incoming raw material inspection as well as increase their testing capacity to accommodate expanded production volume and more stringent regulatory requirements. Current spectroscopic techniques for raw material inspection are described and compared. A handheld Raman spectrometer, suitable for use by non-technical personnel, was shown to be an attractive option for identity testing of most raw materials used by pharmaceutical manufacturing facilities.

Key words: Raman spectroscopy, material verification, raw material identification, incoming inspection, handheld spectrometer, portable Raman

Introduction

Pharmaceutical manufacturers are facing mounting pressure to reduce costs, improve quality control, and increase productivity. Since inspection and verification of incoming raw materials is a significant portion of manufacturing costs, companies are exploring cost-reduction opportunities in this area. In addition to minimizing the current costs of raw material inspection, companies need to perform even more tests to accommodate increased production volume and regulatory requirements as they move towards 100% inspection of incoming raw materials.

An assessment of the workflow associated with incoming raw material inspection reveals numerous opportunities to improve both the efficiency of the testing process and the quality of test results. For the majority of raw materials, containers are opened in order to remove samples for testing. The samples are transferred to the QC laboratory for analysis using appropriate instrumental or wet chemical methods. While awaiting test results, incoming materials are not available for use in the production process. Additionally, this process carries risks of contamination caused by removal of test samples, mislabeling of test samples, and production delays if the QC laboratory has a higher than normal work load.

In recent years, the availability of portable and handheld test instruments has created the potential for moving analytical testing of raw materials from the laboratory to the manufacturing floor.

Current instrumental techniques for raw material verification

The most common analytical techniques used in QC laboratories for identification of raw materials are HPLC (high performance liquid chromatography, NIR (near infrared) and mid-IR (mid-infrared) spectroscopies, as well as wet chemical methods. Raman spectroscopy has also been proven effective and efficient for raw material identification, in-process analysis, and final product authentication¹, and both the USP and EP now recognize Raman spectroscopy as a viable technique for compendial identification².

The Raman light scattering effect, discovered in 1928, has been historically difficult to detect because the scattering phenomenon is very weak. The increased availability of longer wavelength diode lasers, charge coupled devices, and Rayleigh rejection filters has generated renewed enthusiasm for Raman spectroscopy by increasing its sensitivity and decreasing nuisance signal contribution from fluorescence. Today, Raman spectroscopy is a practical laboratory technique, and modern Raman instrumentation is faster, more robust, and less expensive than earlier versions.³ Additional advances in component miniaturization and embedded software algorithms have enabled development of decision-capable handheld Raman solutions for environments beyond the laboratory.

Operational attributes of infrared and Raman spectrometers

A significant advantage of handheld spectrometers (e.g. the Raman instrumentation used in this study) is their ability to quickly verify material identity at the point of need, which minimizes the time between receiving the material and its

Corresponding author: Dr Christopher D. Brown, Ahura Scientific, Inc., 46 Jonspin Road, Wilmington, MA 01887, USA. Tel: (978) 642-1140
Email: cbrown@ahurascientific.com

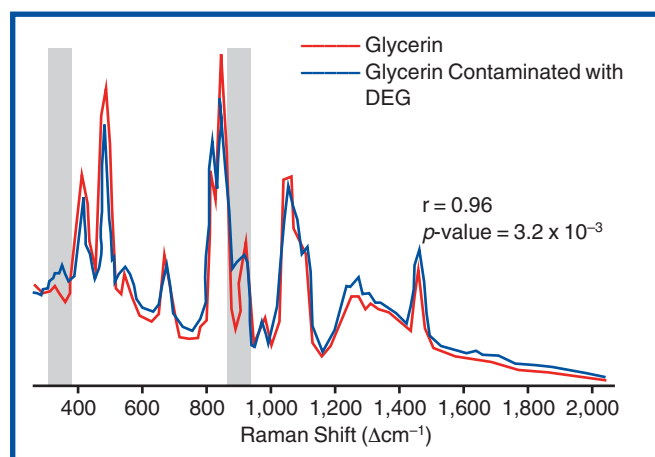


Figure 1. Raman reference spectrum of pure vs contaminated glycerin.

release for manufacturing. The ability to acquire Raman spectra through transparent packaging, such as plastic bags^{1,2}, eliminates the risk of contamination created by opening the packaging to extract a sample. While both Raman and NIR spectra can be acquired through transparent packaging, NIR measurements can produce markedly different spectra as a result of subtle variations from one container to another. In comparison to solids sampling with Raman and NIR, mid-IR techniques require direct contact with the material being tested and are therefore not capable of acquiring spectra through packaging. Raman is unique amongst the three techniques in its ability to measure liquids through a container in a backscattering geometry. This inherent capability negates the need for additional transmission optics or immersed sensors wetted by the sample, as in NIR or mid-IR.

Analytical characteristics of infrared and Raman spectrometers

Because every chemical compound with covalent bonds produces a characteristic pattern of Raman shifts, Raman spectra offer a high degree of selectivity, making them particularly effective for identity testing. For example, the Raman spectra of acetyl salicylic acid and acetaminophen consist of distinct, well-defined peaks that can be used to chemically fingerprint and therefore identify each compound.

Compared to Raman spectra, NIR spectra are less distinct, with broader peaks that result in poorer selectivity and may require computationally intensive methods to detect differences. Additionally, when using NIR, physical attributes of the sample can affect the spectrum and interfere with the straightforward chemical identification desired. Variability in the optics and other components of NIR instruments can result in spectral differences of the same order of magnitude as the compounds being tested. As a result, transferring a NIR method from one instrument to another may require a multitude of reference spectra and/or tuning of method parameters.

Methods of quantifying spectral differences

The most common approach to spectral comparison is to calculate the wavelength correlation, which is equivalent to measuring the cosine of the angle between the two spectra. The resulting correlation coefficient, r , is equal to 1 when the spectra are in perfect correspondence and 0 when they are orthogonal. Although the correlation coefficient provides some indication of the similarity between two spectra, is not particularly sensitive to discrepancies between spectra, and values other than 0 or 1 have no direct interpretation. Despite these deficiencies, a regulatory guidance on selecting a correlation threshold states, “Unless otherwise justified, a [correlation] threshold below 0.95 is not acceptable ...”⁴. An example of how using a correlation threshold to compare spectra can yield erroneous results is shown in **Figure 2**. A Raman reference spectrum of pure glycerin is compared to the spectrum of an “unknown” substance composed of glycerin contaminated with 20% diethylene glycol (DEG). In spite of clear differences in the highlighted areas of the spectra, the correlation coefficient is 0.96, would identify the unknown as glycerin if a threshold of 0.95 were used.

The handheld Raman units used in this study employ an alternative approach to wavelength correlation that evaluates whether the measured spectrum lies within the specified multivariate domain of the reference spectrum (or spectra). The multivariate domain is defined by the uncertainty characteristics of each measurement, including exposure settings, instrument and environmental properties (e.g. temperature, dark current, ambient light) and the optical properties of the sample itself. Rather than comparing the bulk spectra, this approach looks for spectral features that contradict the reference spectrum given the uncertainties of the measurement. Like most statistical tests, the analysis is distilled into a p -value, which in this case is the probability that the observed differences between the sample and reference spectra arose simply by chance due to the uncertainty of the measurement. High p -values signify a high probability that spectral differences arise only from the uncertainty of measurement, meaning that the measured spectrum is consistent with the reference. In such cases the instrument returns a “Pass” result. Low p -values (0.05 is the Pass/Fail threshold for the device) indicate a low probability that discrepancies between spectra arise solely from measurement uncertainty, meaning that the test sample is spectrally and chemically different from the reference. In the example of **Figure 2**, the p -value is 3.2×10^{-3} , which results in the instrument returning a “Fail” result.

Experimental design

Six handheld Raman spectrometers (TruScan, Ahura Scientific, Inc., Wilmington, MA) were used in this work. Three TruScan devices (hereafter referred to as “reference devices”) were used to acquire spectra from 32 common pharmaceutical raw materials (Sigma-Aldrich, St. Louis,

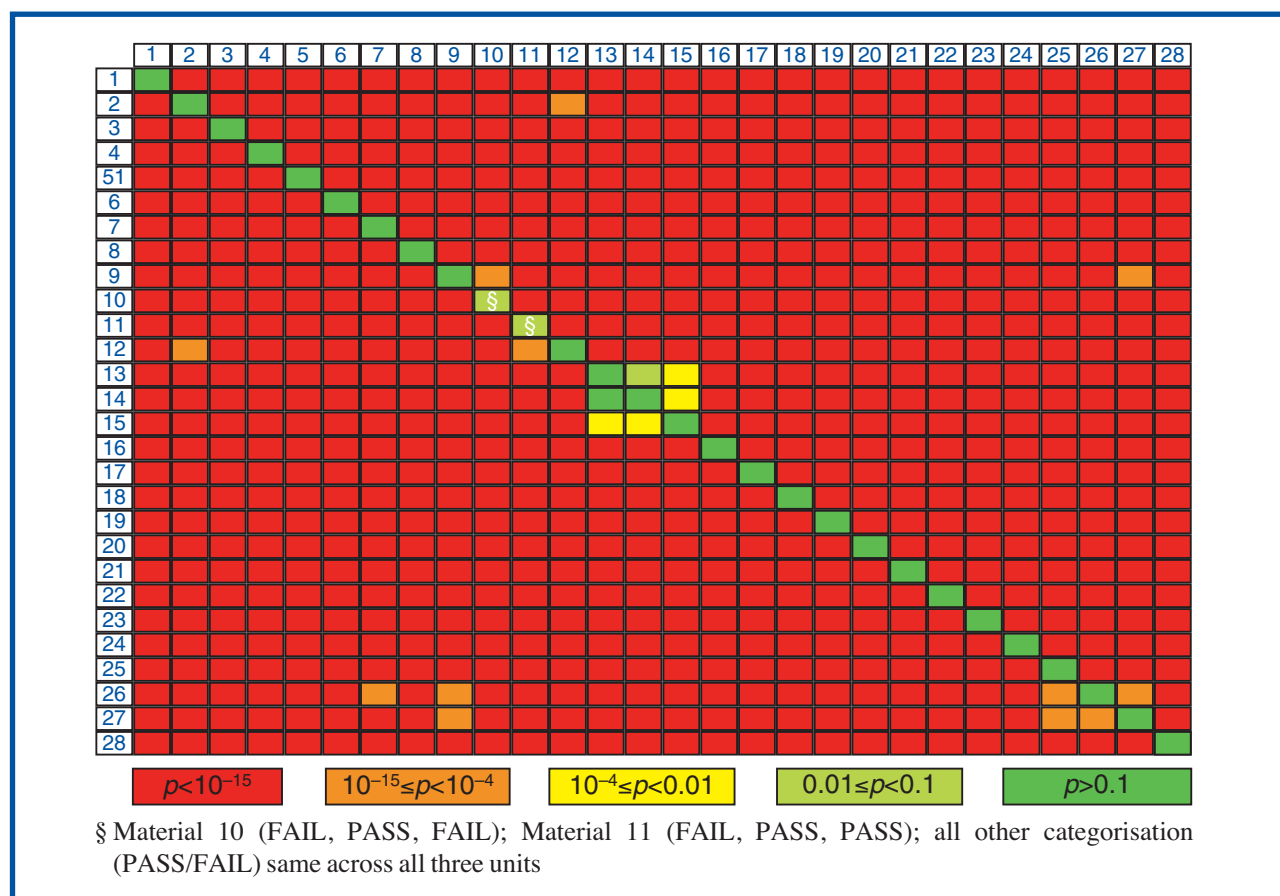


Figure 2. p -values for each sample-method measured across all three test devices.

MO) to evaluate the applicability of the technology for raw material inspection. For each of the 32 raw materials, a single reference spectrum was acquired by one of the three reference devices. Reference spectra were acquired by placing a sample of each material in a borosilicate glass vial (VWR, West Chester, PA) and the data were collected through the wall of the vial. The reference spectra were then consolidated upon a single master unit for method development. Using a web-based software utility, a method for each material was generated. In the context of TruScan, a method is a statistical comparison of a known reference spectrum versus a sample spectrum to verify the identity of the incoming material. The final method library was then cloned to the two remaining TruScan devices (for a total of three “test devices”).

To prepare test samples to challenge the methods, samples of approximately 2g of each test material were sealed in 2mm thick polyethylene bags in an effort to emulate expected-use scenarios (measurement through plastic bags) for incoming material inspection. Three measurements of each sample were made, one with each of the three test devices. To thoroughly evaluate the *specificity* of the Raman spectrometers, each sample spectrum from each of the three test devices was evaluated against the entire method library of 32 raw materials. For example, the cellulose sample was evaluated against the cellulose method and against the methods for all other raw materials. Using the probability approach described previously, each of the three test devices calculated a unique p -value for all of the 32 sample-method pairs.

Results and discussion

During the collection of reference spectra, it was determined that colloidal silica, talc, sodium carboxymethyl cellulose, and hydroxypropylmethyl cellulose did not produce an adequate signal to acquire spectra in a practical period of time for handheld deployment. The remaining 28 materials and their average measurement times are listed in **Table 1**. The variations in measurement time are function of characteristics of the material (Raman cross section, etc.) and range from 1 s to more than 6 min, with the average being less than 1 min.

As indicated in the Experimental Design section, a p -value for each sample-method pair was generated for measurements across all three test devices. The p -values were individually examined and then averaged for presentation purposes. **Figure 2** plots the p -values for each averaged pair. Note that the values range from $p < 10^{-15}$ to $p > 0.1$. The default threshold for “Pass” is $p \geq 0.05$, which indicates the spectrum of the tested sample is consistent with the reference spectrum given the uncertainty of the measurement. The values along the diagonal of **Figure 2** represent cases where the sample was tested against its own corresponding method, which should produce a “Pass” result. All other values arise from the sample being tested against one of the other raw material methods, which should produce a “Fail” result.

With the exception of ethyl cellulose and hydroxypropyl cellulose (items 10 and 11 in **Table 1**), the p -values along the diagonal of **Figure 2** are all greater

Table 1. Common pharmaceutical raw materials used for reference spectra and their average measurement times using handheld Raman spectrometers.

	Material	Average measurement time (seconds)*
1	Titanium (IV) oxide, anatase	1
2	a-Lactose monohydrate	15
3	Polyvinylpyrrolidone	69
4	Dextrose anhydrous	11
5	Dextrose monohydrate	18
6	Sodium bicarbonate	9
7	Potassium bicarbonate	11
8	Calcium carbonate	6
9	Cellulose	36
10	Ethyl cellulose	284
11	Hydroxypropyl cellulose	390
12	Dextrin from corn	49
13	Calcium stearate	79
14	Magnesium stearate	54
15	Stearic acid	42
16	Citric acid	22
17	Potassium citrate tribasic monohydrate	21
18	Sodium citrate	21
19	(+)-Sodium L-ascorbate	8
20	Sulfanilamide	1
21	Acetaminophen	5
22	Sodium phosphate monobasic	15
23	Sodium phosphate dibasic	29
24	Calcium phosphate dibasic	35
25	Trimagnesium phosphate	124
26	Zinc sulfate	4
27	Calcium sulfate	14
28	Calcium sulfate dihydrate	7

* rounded to nearest second

than 0.1, indicating these materials easily pass as being consistent with the method reference spectrum. For ethyl cellulose and hydroxypropyl cellulose, p -values were between 0.01 and 0.1, which are very close to the default threshold of 0.05. As a result, the three test devices returned different pass-fail results for these materials. Further inspection of the spectral data revealed subtle features in the unknown samples that were not found in the reference materials. To determine if the polyethylene bags could be the cause of the discrepancy, a spectrum of a polyethylene bag was examined and found to contain bands corresponding to the extra peaks in the unknown spectra. These data suggest that the very weak Raman signals and long measurement times for these cellulose

materials created conditions that resulted in subtle interference from the polyethylene bags.

Examination of the off-diagonal elements in **Figure 2** confirms the excellent selectivity of the technology as evidenced by $p < 10^{-15}$ for the overwhelming majority unknown-methods pairs. The only materials lacking acceptable selectivity are the alkali metal stearates. Both calcium and magnesium stearate are readily differentiated from stearic acid ($p < 0.01$), but they cannot be differentiated from each other. While smaller molecules differing only in their cation can be easily differentiated with the handheld Raman system (e.g. bicarbonate and sulfate), the large stearate molecule produces the majority of the Raman signal, which minimizes spectral differences caused by the cations. In this case, Raman spectroscopy would verify that the incoming material was stearate, and secondary testing would determine the cation.

Conclusion

Handheld Raman spectroscopy is an excellent alternative to traditional incoming raw material verification by high performance liquid chromatography, wet chemical methods, and NIR and mid-IR spectroscopy. The excellent specificity of Raman spectroscopy, which when coupled with intelligent on-board algorithms, reduces the time and effort to develop and validate methods. Furthermore, methods loaded onto different handheld Raman systems produce consistent data and material verification without loading additional spectra or performing other customization.

In addition to their analytical characteristics, today's handheld Raman solutions are environmentally robust and can be used by expert spectroscopists as well as non-technical personnel. This is in contrast to Raman instruments of the past, which were bulky, slow, expensive and delicate. Based on the study of common pharmaceutical materials presented in this article, the handheld Raman spectrometer offers an attractive option for achieving 100% inspection of most raw materials used by pharmaceutical manufacturing facilities.

References

1. Vankeirsbilck T. *et al.* Applications of Raman spectroscopy in pharmaceutical analysis. *Trends in Analytical Chemistry*, 2002; **21**: 869–877.
2. US Pharmacopeia <1120>: Raman spectroscopy, and EP 2.2.48: Raman spectrometry
3. Smith E, Dent G. *Modern Raman spectroscopy – a practical approach*, John Wiley & Sons, Chichester, UK, 2005.
4. European Medicines Agency (EMA). Note for Guidance on the Use of Near Infrared Spectroscopy by the Pharmaceutical Industry and the Data Requirements for New Submissions and Variations, London, UK, 2003.