

Verification Methods for 198 Common Raw Materials Using a Handheld Raman Spectrometer

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Using handheld Raman spectroscopy, methods were developed and evaluated for 198 substances widely used as raw materials. Development of each method required only one sample, for which a single reference spectrum was collected. After methods were built, they were directly transferred to a separate handheld Raman spectrometer. All method and sample pairings were tested, resulting in a total of nearly 40,000 challenges. A 100% correct acceptance rate and a 99.9% correct rejection rate were achieved.

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Pharmaceutical manufacturers are seeking ways to maintain and improve product quality while reducing manufacturing costs, including in the analytical testing of raw materials. Movement toward 100% inspection of raw materials as well as increased production volumes and the desire for lean manufacturing are additional motivations to find more cost-effective testing technologies.

Raman spectroscopy's inherent selectivity makes it an effective option for qualitative verification of pharmaceutical raw materials (1–3). Until recently, Raman spectroscopy was difficult to implement because of the technical challenge of detecting the relatively weak but highly selective Raman scattering effect. During the past 10 years, improvements in the sensitivity of charge-coupled device (CCD) detectors, the increased availability of longer-wavelength diode lasers, and developments in Rayleigh rejection filters have made Raman spectroscopy suitable for routine applications. Moreover, miniaturization of optical components has enabled the development of portable, handheld Raman spectrometers capable of robust material verification through transparent packaging materials such as glass and plastic (4).

US Pharmacopoeia General Chapter <1120> recognizes Raman spectroscopy as one of the accepted technologies for material identity verification, asserting that “Because the Raman spectrum is specific for a given compound, qualitative Raman measurements may be used as a compendial ID test, as well as for structural elucidation” (5). As this statement suggests, the inherent characteristics of Raman spectra, namely discrete peaks corresponding to specific molecular-



Figure 1: TruScan handheld material identity verification system based on Raman spectroscopic technology.

bond vibrations, enable verification of a wide range of raw materials.

In addition to its high degree of molecular selectivity, Raman spectroscopy is relatively insensitive to physical and environmental factors such as particle size, humidity, and contributions from packaging material. This insensitivity combined with the high molecular selectivity allows for very streamlined method development when using Raman. In fact, a method can be typically based on a single reference spectrum (4).

The high molecular selectivity and external factor insensitivity of Raman spectroscopy help eliminate problems associated with transferring methods between multiple instruments. Careful instrument engineering to provide a consistent spectroscopic platform to acquire and share data, with thorough system response characterization, allow a method developed on one device to be shared seamlessly with any other device, thereby saving time and cost (4).

Portable, field-based alternatives offer many operational advantages over laboratory testing, but it is important to consider challenges arising when such a technology is in use such as the inability to control the testing environment (temperature, humidity, ambient lighting, etc.). Instruments used in the field should be expressly designed for field use and be able to automatically compensate for external variables such as ambient lighting and sampling geometry, and spectroscopic differences in analyzed materials.

An important final consideration when evaluating any analytical methodology is the method performance that can be achieved. In the context of identity testing, specificity (i.e., selectivity) is the only figure of merit that must be demonstrated through comprehensive validation (6). When making an identity assessment on the basis of spectral data, the unknown measurement is examined in relation to one or more reference spectra. The final assessment can be performed

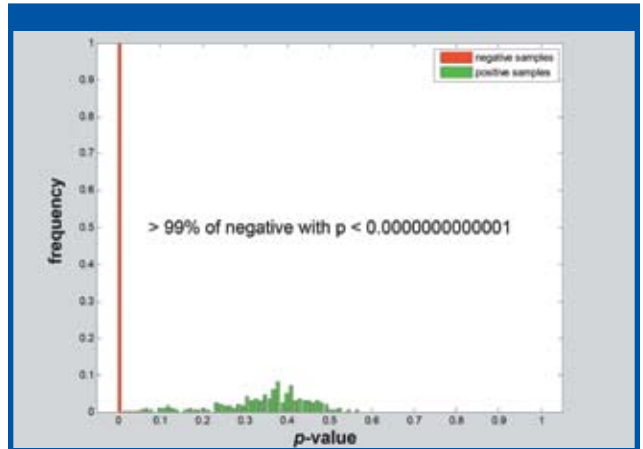


Figure 2: Histogram of p -values for true positive testing and false positive testing of 198 materials.



Figure 3: Confusion matrix summary of p -values for device challenges on 198 materials.

manually by comparing peak locations, but usually a full-spectrum automated analysis is performed. Clearly, the algorithmic approach used to render a final decision plays a role in determining the level of specificity that can be achieved when performing an analysis. Common approaches for spectral comparison include hit quality index (HQI) and spectral correlation (7). These methods are useful for quick similarity assessments; unfortunately, they generally do not provide a direct interpretation in the context of spectral identity testing. Furthermore, even when these techniques produce a favorable correlation coefficient, misidentification may occur because these metrics are not particularly sensitive to discrepancies between spectra of interest (4).

An alternative to HQI and spectral correlation is to use a fully multivariate probabilistic approach that looks for spectroscopic discrepancies between the test and reference spectrum in question. Under a null hypothesis that the test

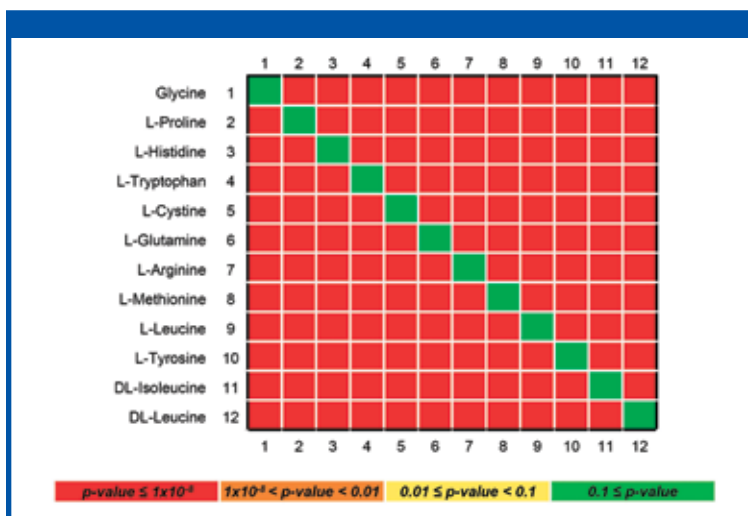


Figure 4: Confusion matrix for amino acid subset.

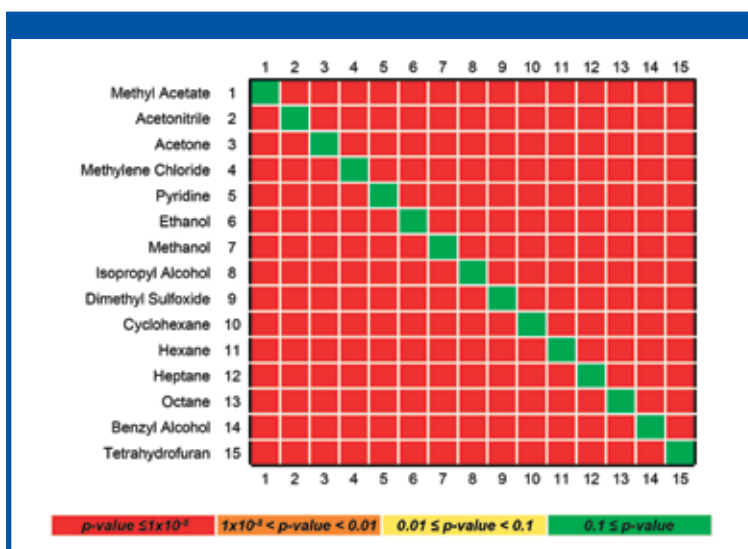


Figure 5: Confusion matrix for organic solvent subset.

and reference spectra are the same within the measurement uncertainty, the comparison is distilled into a traditional p -value (small p -values indicate greater discrepancy between test and reference spectra). If no significant discrepancies are found (i.e., p -value $>$ 0.05), then the device deems the test spectrum consistent with the reference spectrum, and a pass result is declared. If discrepancies are found—other than those expected to arise from the uncertainty in the measurement alone—then the test material cannot be authenticated against the reference and a fail result is declared.

In the present study, the broad applicability of portable Raman spectroscopy for raw material identity verification is evaluated by examining a range of 198 Raman-active substances widely used as raw materials in the manufacture of pharmaceutical, consumer health, and personal care products. Each verification method was developed using one reference spectrum in a fraction of the time typically required for near-infrared (NIR) method development. Many diverse categories of materials were tested (see sidebar, “Categories of

materials”). A thorough assessment of selectivity was achieved by evaluating each material against all methods to fully assess the performance of each method.

Experiment

This study used five handheld material-identity verification systems (TruScan, Ahura Scientific, Wilmington, MA, see Figure 1). These instruments use Raman spectroscopy as the analytical technique and contain a 785-nm NIR external cavity-stabilized, thermoelectrically cooled laser as the light source. The laser typically operates at 300 mW output and automatically adjusts if necessary to avoid detector saturation. These instruments also include a single-pass dispersive spectrometer, a cooled CCD detector array, and dielectric edge filters for Rayleigh rejection. Each instrument has an integrated barcode reader for selecting methods and capturing sample lot/batch/other identifiers. Table I shows specifications for the TruScan Raman material identity verification system.

Acquiring a spectrum is facilitated by using an auto-acquisition mode that monitors incoming data in real time and automatically adjusts the control settings (e.g., laser power, exposure time, number of exposures) such that spectra of comparable statistical resolving power are achieved from one measurement to the next. This adaptive approach to spectroscopy ensures consistent statistical performance of the instrument and embedded decision algorithms regardless of operating conditions.

Four of the instruments (hereafter called reference devices) were used to acquire reference spectra for the 198 materials included in this study (see sidebar, “Materials tested”). To obtain the reference spectra, a reference sample of each material contained in a borosilicate glass vial (Kimble Glass, Vineland, NJ) was placed at the correct distance from the device’s laser aperture using a vial-holder accessory. All data acquisition was performed in auto-mode. For test spectra, most analyses are complete in less than one minute. Reference-grade spectra used for method development are also generated using an auto-mode; however, these spectra have higher standards for signal-to-noise ratio than a standard test spectrum and therefore require a longer data acquisition time. Most auto-mode reference spectra are acquired in less than 30 min. The scans that did not auto-terminate within 45 min were manually stopped at 45 min to prevent photobleaching.

Reference spectra can be transferred between TruScan devices using a compact flash card or a standard web browser that addresses the device via a TCP/IP connection. Using these approaches, the reference spectra were transferred

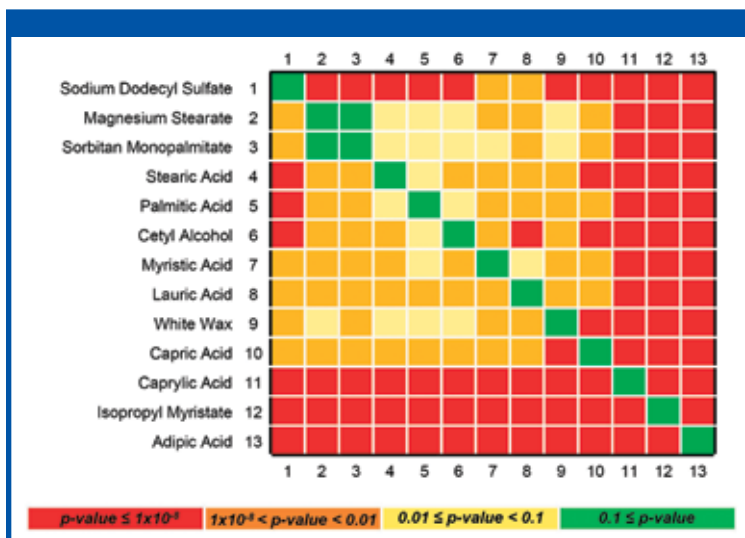


Figure 6: Confusion matrix for long-chain hydrocarbon subset.

Categories of materials.

Alcohol/acid/base	Flavor/fragrance
Amino acid	General inorganic
Antibiotic	General organic
Antifoam	Monomer/polymer
Antioxidant	Polyol
Active pharmaceutical ingredient	Preservative/antiseptic/biocide
Binder	Solvent
Buffer	Sweetener
Diluent	Thickener/stabilizer/emulsifier
Emollient/lubricant/coating	Vitamin/mineral
Fatty acid	

from the four reference devices to the test device. Once all of the reference spectra had been transferred to the test device, the TCP/IP utility WebAdmin was used to generate a new method for each of the 198 materials using only one reference spectrum for each method. In the context of the TruScan device, methods are analytical tasks based on stored reference spectra that the instrument's software executes to determine whether a material's identity can be verified.

To challenge the methods, a test sample of each material was placed in a borosilicate glass vial, and the vial was inserted into the test device's vial holder. For each test sample, a spectrum was acquired using auto-mode. The onboard processor then evaluated the spectrum acquired for each test material against the method developed for that material, and also against all the methods created for the rest of the 198 materials, for a total of 39,204 evaluations of test spectra against methods. In addition to the pass and fail results normally presented by the test device, the p -value was recorded for each test sample–method evaluation pair.

Results and discussion

The handheld Raman material-identity verification system

shows a pass result whenever test of a method against a material produces a p -value ≥ 0.05 . In general, testing a method against the “wrong” material produces a p -value far less than 0.05, resulting in a fail.

Figure 2 provides a summary in histogram form of the p -values generated in this study for all pairings of the 198 materials tested. The green bars represent results obtained when testing materials against methods corresponding to the same materials (true positive testing), and the red bars represent analyses achieved when testing materials against other methods (false-positive testing). To view both sets of results in the same graph, the y -axis of the histogram represents the fraction of samples in each bin rather than the total frequency of samples in each bin. The results from true positive testing, in green, are all above the pass threshold with p -values ranging from 0.052 to > 0.5 .

Though the false-positive testing did reveal a few negative samples with values as high as 0.15, well above the pass threshold and therefore false positives, the frequency with which this occurred is so low that negative samples with p -values > 0.05 are not visible in the histogram. Conversely, negative samples presented in the histogram reveal that more than 99% of pairings where methods were tested against a different material, the p -value obtained was less than 10^{-13} , considerably less than the pass threshold, and a definitive fail result. This demonstrates the extreme degree of selectivity achievable with handheld Raman spectroscopy.

The data in the histogram in Figure 2 were also encoded as a confusion matrix (see Figure 3), thus providing a different view of the information. Figure 3 contains 198 rows and 198 columns (39,204 method–test material pairings), color coded red for p -values < 0.05 (corresponding to “fail” results) and green for p -values ≥ 0.05 (corresponding to “pass” results). The green along the diagonal in Figure 3 shows where the test device declared pass results when a method was tested against its corresponding material. The red shown on the vast majority of off-diagonal positions indicates where the device declared a fail result when testing a method against the wrong material. As can be seen in Figure 3, for all but six pairings, the test device indicated pass when the material's method was tested against the same material, and fail when tested against all others.

The false-positive results for the six pairings shown in Figure 3 are results of the extremely high similarity in composition and/or molecular structure of the materials in each pair. One example is safflower oil, which has a very high concentration of linoleic acid. Other examples are povidone and crospovidone which have the same structure except for a cross linkage in crospovidone. The slight differences between the materials in each pair do not result in significant differences in their Raman spectra.

To enhance the granularity of p -value results, the range was

Materials tested *

1,3-Propanediol	D-(+)-Maltose monohydrate	Magnesium stearate	Propionic acid
2-(N-morpholino)ethanesulfonic acid (MES)	D-(+)-Mannose	Magnesium sulfate	Propyl paraben, sodium salt
2,2'-Asobis(2-methylpropionitrile)	D-(+)-Trehalose	Malic acid	Propylene glycol
2-amino-2-hydroxymethyl-1,3-propanediol (TRIS)	D-(+)-Xylose	Maltitol	Pyridine
3-(N-morpholino)propanesulfonic acid (MOPS)	Dextrin from corn	Mannitol	Retinoic acid (vitamin A acid)
4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)	Dextromethorphan hydrobromide	Meglumine	Safflower oil
6-Caprolactone monomer	Dextrose anhydrous	Menthol	Simethicone
Acesulfame K	Dextrose monohydrate	Methanol	Sodium acetate
Acetaminophen	Diethylene glycol	Methyl acetate	Sodium acetate trihydrate
Acetic acid	Dimethicone (PDMS)	Methyl ethyl ketone (MEK)	Sodium benzoate
Acetone	Dimethyl sulfoxide	Methyl methacrylate	Sodium bicarbonate
Acetonitrile	Disodium edetate dihydrate	Methyl paraben, sodium salt	Sodium carbonate
Acrylic acid	DL-Ethionine sulfone	Methylene chloride	Sodium citrate
Adenine hydrochloride	DL-Isoleucine	Microcrystalline cellulose (Avicel)	Sodium cyclamate
Adipic acid	DL-Leucine	Myristic acid (tetradecanoic acid)	Sodium dodecyl sulfate (SDS)
Ammonium acetate	DL-Norleucine	Myristyltrimethylammonium bromide	Sodium L-ascorbate
Ammonium chloride	Epinephrine HCl	Naproxen	Sodium metabisulfite
Ammonium phosphate disbasic	Ethanol	n-Butylamine	Sodium molybdate
Ammonium sulfate	Ethyl acrylate	N-Cyclohexyl-2-aminoethanesulfonic acid (CHES)	Sodium molybdate dihydrate
Ampicillin	Ethyl cellulose (Ethocel)	N-Cyclohexyl-2-hydroxyl-3-aminopropanesulfonic acid (CAPSO)	Sodium phosphate dibasic
Aspartame	Ethylene diamine tetracetic acid (EDTA)	Niacin (B3)	Sodium phosphate monobasic monohydrate
Benzaldehyde	Ethylene glycol	Niacinamide (B3)	Sodium pyrophosphate dibasic
Benzalkonium chloride	Fish oil	Nitric acid	Sodium saccharin
Benzoic acid	Folic acid (B9)	Nylon 6	Sodium succinate dibasic
Benzyl alcohol	Fructose	Nylon 6/6	Sorbic acid
Beta-carotene	Fumaric acid	Octane	Sorbitan monopalmitate
Beta-cyclodextrin	Glucosamine hydrochloride	Octylphenol ethoxylate (Triton X-100)	Sorbitol
Biotin (B7)	Glycerol	Oleic acid	Stearic acid (octadecanoic acid)
Caffeine	Glycine	Orange oil	Succinic acid
Calcium carbonate	Heptane	Palmitic acid (hexadecanoic acid)	Sucrose
Calcium hydrogenphosphate dihydrate	Hexane	Peanut oil	Sulfanilamide
Calcium phosphate dibasic	Hydrogen peroxide	Penicillin G potassium salt	Sulfuric acid
Calcium sulfate	Hydroxypropyl methyl cellulose phthalate	Penicillin G sodium	Tartaric acid
Calcium sulfate dihydrate	Ibuprofen	Penicillin V potassium	Taurine
Capric acid (decanoic acid)	Imidazole	Phenol	Tetrahydrofuran (THF)
Caprylic acid (octanoic acid)	Isopropyl alcohol (IPA)	Phosphoric acid	Thiamine hydrochloride(B1)
Carnauba wax	Isopropyl myristate	Poly(vinyl alcohol)	Thiamine pyrophosphate
Cetyl alcohol (1-Hexadecanol)	Lactic acid	Polyethylene glycol (polyethylene oxide) (380-420)	Thymine
Cimetidine	Lactose monohydrate	Polyethylene terephthalate	Thymol
Citric acid monohydrate	L-Arginine	Polysorbate 20 (Tween 20)	Titanium (IV) dioxide
Citric acid anhydrous	L-Arginine monohydrochloride	Polyvinylpyrrolidone (PVP or Povidone)	Tocopherol (E)
Corn starch	Lauric acid (dodecanoic acid)	Potassium carbonate	Toluene
Cremophor EL	L-Cystine	Potassium citrate tribasic monohydrate	Tributyl citrate
Crospovidone	L-Glutamine	Potassium citrate tribasic monohydrate	Triethyl citrate
Cyclohexane	L-Histidine	Potassium nitrate	Triethylene glycol
D-(+)-Arabinose	Limonene	Potassium phosphate dibasic	TRIS hydrochloride
D-(+)-Fucose	Linoleic Acid	Potassium phosphate monobasic	Urea
	L-Leucine	Potassium sodium tartrate tetrahydrate	Vanillin
	L-Methionine		White wax (bees wax)
	L-Proline		Xylitol
	L-Tryptophan		Zinc sulfate heptahydrate
	L-Tyrosine		

* Materials sourced from Fisher Scientific (Pittsburgh, PA), Fluka (Milwaukee, WI), ISP Technologies (Wayne, NJ); Sigma Aldrich (St. Louis, MO) Spectrum Chemical (Gardena, CA), and Supelco (Bellefonte, PA).

Table 1: Instrument specifications.

Spectral range	250–2900 cm^{-1}
Resolution	7–10 cm^{-1} max
Excitation wavelength λ	785 nm, cooled, external cavity stabilized
Output power	~300 mW max
Laser line width	<2 cm^{-1} FWHM (<0.12 nm)
Exposure options	AUTO
Detector	2048 element silicon CCD, thermoelectrically cooled
Spectrometer	Single-pass dispersive
Probe working distance	18 mm
Spot size	2 mm diameter

more finely divided in the following compound class studies, allowing a more detailed evaluation of the differentiating capability of the device's embedded decision algorithms.

Amino acids. Of the 198 test materials, 12 were amino acids. Figure 4 shows p -value ranges for the pairings of amino acids. The green indicates p -values ≥ 0.1 , a more stringent qualification than the test device's normal threshold of 0.05 for a pass result. For all amino acids, the test device correctly verified each material. The test device also correctly rejected nonmatching materials, with p -values $\leq 1 \times 10^{-8}$.

Solvents. Test materials also included 15 organic solvents. Figure 5 shows p -value ranges for the test pairings of these solvents. The green area indicates p -values ≥ 0.1 , again, a more stringent qualification than the test device's normal "pass" threshold of 0.05. For this category, the test device correctly verified each material. It also correctly rejected nonmatching materials, with p -values $\leq 1 \times 10^{-8}$, as indicated by red squares in Figure 5.

Long-chain hydrocarbons. The p -values for 13 long-chain hydrocarbons are shown in Figure 6. Unlike the other categories of test substances discussed previously, some of these pairings resulted in higher p -values when a method was tested against a similar material in this class of compounds. For larger molecules such as these, the Raman signal corresponding to the long hydrocarbon chain portion of the molecule can dominate the signal making spectral features arising from other functional groups difficult to detect. This is in contrast to small molecules where single atomic or functional group substitutions are easily detected (e.g. acetone/methyl ethyl ketone and calcium sulfate/magnesium sulfate).

In almost all cases, the long-chain hydrocarbon pairings produced exclusive (completely selective), pass verdicts with p -values > 0.1 , indicated by green in Figure 6. The one exception was the magnesium stearate–sorbitan monopalmitate pairing. In this case, the Raman signal from the long-chain

hydrocarbon (differing between the two materials by only one carbon) overwhelms differences present in the rest of the molecule. About half of the negative pairings produced very low p -values $\leq 1 \times 10^{-8}$, indicated by red in Figure 6. Nineteen pairings of long-chain hydrocarbons resulted in p -values in the range of 0.01 to 0.1 (yellow in Figure 6). Off-diagonal p -values in this range represent cases in which clear gaps in selectivity between methods exist (p -value > 0.05) or where the pairings are not so far removed, lying in the 0.01–0.05 range, that there is risk for inadequate selectivity. It is important to identify materials like these during method validation to identify potential false positives and assess their degree of risk. Another group, the 54 pairings indicated by orange in Figure 6, produced p -values in the range 1×10^{-8} to 0.01 which are of significantly less concern during method validation and robustness testing.

Conclusion

This study demonstrates the general applicability of handheld Raman spectroscopy for pharmaceutical raw material identification through extensive characterization of method performance for 198 raw materials commonly used in the pharmaceutical and related industries. Method development required only one sample of each material. In nearly 40,000 method challenges, a 100% correct positive identification rate and 99.9% correct rejection rate was achieved.

Handheld Raman spectroscopy offers an attractive alternative to other analytical techniques for identity verification of raw materials. The technology's specificity for a wide range of materials, its ease of method development and validation, and the ability to directly transfer methods between instruments, enable handheld Raman spectroscopy to both improve the quality of raw-materials testing while reducing cost.

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