CLINICAL INVESTIGATION

Raman Spectroscopy: A Sensitive and Specific Technique for Determining the Accuracy of Compounded Pharmaceutical Formulations

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OBJECTIVES: Raman spectroscopy is a widely used technology to identify chemical unknowns or confirm chemical identity. We have tested Raman spectrometry to identify compounded pharmaceutical formulations. In contrast to the commonly used application mentioned above, compounded pharmaceutical formulations contain a mixture of ingredients, and the Raman spectrometer is being used to correctly identify the composition of the complete pharmaceutical formulation, including the active pharmaceutical ingredient(s). The objective of this pilot study was to document the potential use of Raman spectroscopy as a tool to provide quality control to compounded pharmaceutical formulations.

METHODS: "Testing a test" study design was used to prospectively determine whether Raman spectroscopy could verify the accuracy of compounded pharmaceutical formulations. A total of 9 formulations that are commonly compounded at Cook Children's Health Center were selected for testing. Each of the 9 formulations and 2 blank controls were randomly tested for compounding accuracy in replicate. A total of 110 tests were conducted.

RESULTS: Raman spectroscopy was found to be a reliable test to determine the accuracy of compounded pharmaceutical formulations with a 100% positive predictive value.

CONCLUSIONS: Raman spectroscopy promises to be an excellent tool for compounding pharmacies to provide an objective measure of compounding accuracy to their unique, compounded pharmaceutical formulations.

INDEX TERMS: pharmacy compounding, pharmaceutical formulations, quality control, Raman spectroscopy

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The literature is replete with reports of dosage errors secondary to inappropriately compounded formulations. Such errors may often lead to clinically significant adverse outcomes.^{1–12} A time- and cost-efficient method to objectively identify such errors will minimize adverse outcomes, restore consumer confidence in compounded formulations, and expand the practice of compounding pharmacists.

Many analytical techniques can be used to identify and quantitate chemical compounds or compounded pharmaceutical formulations. One of the preferred techniques is the use of gas or liquid chromatography combined with mass spectrometric detectors. These techniques are

very precise and accurate. However, the cost associated with acquisition and maintenance of these instruments, the time required to develop and validate analytical methods, and the high skill level required of operators limit widespread use in compounding pharmacies. To this end, we have explored analytical techniques that might be used in compounding pharmacies to determine the accuracy of compounded formulations. Such techniques must be precise, accurate, easy to use, and cost-effective for compounding pharmacies to effectively implement as a practice standard. Features of Raman spectroscopy show promise for this technology to be used to determine the accuracy of compounded

Figure 1. The CBEx is a hand-held Raman spectrometer that can identify pharmaceuticals and/or mixtures. The device correlates scattered light patterns of unknown pharmaceutical samples to a library of known pharmaceuticals. As depicted on the display, cocaine (white spectral image) is identified ($r = 0.99$) using a “point and shoot” sampling technique when compared to a scattered light pattern (blue spectral image) from a library of known pharmaceuticals.

pharmaceutical formulations.¹³

Raman spectroscopy is similar to infrared absorption techniques measuring vibrational, rotational, and other low-frequency modes of a molecule. However, Raman spectroscopy uses only a single wavelength of light to characterize the compounded pharmaceutical formulation and collects the resulting scattered light pattern. The resulting light patterns are unique to the compounded pharmaceutical formulation being tested and make it possible to identify product formulation. The primary aim of this prospective study is to document the use of Raman spectrometry as an effective tool to accurately identify select compounded pharmaceutical formulations.

METHODS

Test instrument

A handheld Raman spectrometer (CBEx, Snowy Range Instruments, Laramie, Wyoming) was selected for testing on basis of its relatively

low acquisition cost, user-friendly operation, and sensitivity (Figure 1). Specifically, the spectrometer can test compounded pharmaceutical preparations for identity using a test vial or “point and shoot” sampling. With “point and shoot” sampling, compounded pharmaceutical formulations contained in a variety of packaging, including glass jars, bottles, syringes, and/or polyvinyl tubing and bags, can be easily tested. No removal of test material from packaging is required.

Effective use of the CBEx requires the operator to follow the simple operating instructions and first create or use a library of compounded pharmaceutical formulations that are of interest to detect. Using proprietary software (Peak, Snowy Range Instruments) and the CBEx spectrometer, a unique library can be created that is specific for your compounded pharmaceutical formulations. Simply scan your standard compounded pharmaceutical formulations or mixtures and store them in the CBEx spectrometer for future comparisons. Subsequent unknowns will be scanned and compared to the unique library for identification; scans that meet or exceed the predetermined minimum acceptable correlation will be matched to the stored scattered light pattern that has been linked to the specific compounded pharmaceutical formulation.

Study design

A prospective “testing a test” study design was selected.¹⁴ This study design uses a 2×2 block design (Table 1). One factor includes the standard formulation (i.e., gold standard) with 2 levels, including presence or absence of the pharmaceutical ingredient(s), and the other factor includes test score, including positive or negative identification of the pharmaceutical ingredient(s). The minimum acceptable correlation factor was predetermined to be $r = 0.85$ for accurate identification. Accordingly, the positive predictive value (No. of true positives / No. of true positives + No. of false positives), negative predictive value (No. of true negatives / No. of true negatives + No. of false negatives), sensitivity (No. of true positives / No. of true positives + No. of false negatives), and specificity (No. of true negatives / No. of false positives + No. of true negatives) can be determined.

A total of 9 formulations that are commonly compounded at Cook Children’s Health Care

Table 1. Test Result Definitions Using a 2 × 2 Block Table Design for “Testing a Test”

Test Results	Test Definition
True positive	Raman spectroscopy identified the correct compounded pharmaceutical formulation
False positive	Raman spectroscopy identified the incorrect compounded pharmaceutical formulation
False negative	Raman spectroscopy did not identify the correct compounded pharmaceutical formulation
True negative	Raman spectroscopy did not identify the incorrect compounded pharmaceutical formulation

System, Fort Worth, Texas, were selected for testing based on their frequency of use. These included amiodarone 5 mg/mL, baclofen 5 mg/mL, captopril 1 mg/mL, carvedilol 1.67 mg/mL, hydralazine 4 mg/mL, naproxen 25 mg/mL, nifedipine 4 mg/mL, ursodiol 50 mg/mL, and valine 100 mg/mL. The above-mentioned formulations were compounded and double-checked for accuracy by both the compounding technician and the pharmacist. Each of the respective formulations were tested using the Raman spectrometer, and the resulting scattered light pattern was recorded and stored as the “gold standard” for comparison testing.

Identification

Each of the 9 compounded pharmaceutical formulations and 2 blank controls (i.e., formulations without an active pharmaceutical ingredient) were randomly tested for identification in replicate ($n = 10$). A total of 110 tests were conducted. The results from each Raman test were recorded as either true positive, false positive, false negative, or true negative (Table 2).

RESULTS

Testing results for identification are listed in Table 3. The positive predictive value for use of Raman spectrometry was 100%; the Raman

spectrometer correctly identified all compounded pharmaceutical formulations. The negative predictive value was 91%; Raman spectrometry correctly identified 20 of 22 compounded pharmaceutical formulations that did not contain active pharmaceutical ingredients. Raman testing sensitivity was calculated to be 98% and specificity was 100%.

Overall, Raman testing was accurate and correctly identified 108 of 110 tests. Testing procedures were user friendly, they required instrument operators to have a relatively low bioanalytical skill level, and operational training required limited time. Individual tests can also be completed in less than a minute, in order to not interfere with work flow, and instrument acquisition cost is relatively low. The greatest time required is to establish the library of compounded pharmaceutical formulations for comparison testing; much of that time is ensuring the specific “gold standard” formulation is compounded accurately.

DISCUSSION

A lack of objective end points for determining the accuracy of compounded pharmaceutical formulations has been a significant area of concern to detect and minimize compounding errors. To this end, there remains a significant

Table 2. A 2 × 2 Block Study Design Is Listed for “Testing a Test”

Identification	Pharmaceutical Formulation		PPV or NPV
	Correct Formulation	Incorrect Formulation	
Positive	True positive I	False positive II	PPV*
Negative	False negative III	True negative IV	NPV†
	Sensitivity‡	Specificity§	

NPV, negative predictive value; PPV, positive predictive value

*PPV = No. of true positives (I) ÷ [No. of true positives (I) + No. of false positives (II)]

†NPV = No. of true negatives (IV) ÷ [No. of true negatives (IV) + No. of false negatives (III)]

‡Sensitivity = No. of true positives (I) ÷ [No. of true positives (I) + No. of false negatives (III)]

§Specificity = No. of true negatives (IV) ÷ [No. of false positives (II) + No. of true negatives (IV)]

Table 3. A 2×2 Block Study Design Is Listed for “Testing a Test:”*

Identification	Pharmaceutical Formulation		PPV or NPV
	Correct Formulation	Incorrect Formulation	
Positive	n = 88	n = 0	PPV = 100%
Negative	n = 2	n = 20	NPV = 99%
	Sensitivity = 98%	Specificity = 100%	

NPV, negative predictive value; PPV, positive predictive value

*A total of 110 analyses were performed; the results of testing are listed in the appropriate cell.

need to develop objective testing that can be effectively used in compounding pharmacies. Such measures would reduce the risk of compounding errors and enhance the benefit of compounded prescriptions.

We have introduced the use of Raman spectrometry as a potential tool to meet the need for determining quality control in compounding pharmacies. Our results documented this technique to be 100% predictive. However, the above-mentioned study was limited to only 9 formulations and a total of 110 tests. Nevertheless, this technique shows promise as a useful tool. Further testing is required to validate use and establish limits for usefulness.

We speculate that use of Raman spectrometry may also be used to determine beyond-use dating and document drug diversion. We also looked at this technique in preliminary tests to quantify the content of active pharmaceutical ingredients in compounded pharmaceutical formulations. We tested the 4 mg/mL hydralazine formulation to determine whether Raman spectrometry can determine the concentration of the active ingredient.

We visually inspected the scattered light pattern that resulted from Raman testing and identified 3 significant wavenumbers with the highest peak or intensity level (Figure 2); 764, 1034, and 1373 cm^{-1} . We compounded hydralazine formulations to range in concentration from 1 to 10 mg/mL and tested each formulation using the Raman spectrometer. From each test, we correlated the hydralazine concentration to the measured intensity (mW) for each peak, respectively (Figure 3). A linear correlation was observed throughout the range of hydralazine concentrations. Such results suggest that Raman spectrometry can be used to detect concentrations of active pharmaceuticals in compounded pharmaceutical formulations. However, further testing is required to determine whether such a test will be suitable for stability

testing. We speculate that degradation of active ingredients will lead to the accumulation of degradation products that will alter the spectral pattern. Thus, a decrease in the active peak in combination with an altered spectral pattern will be observed. Such observations may make possible use of this technique for drug stability testing.

We also suggest that Raman spectrometry can be used to detect drug diversion. Since Raman spectrum has been shown to detect active pharmaceuticals, and it has the ability to detect changes in active concentrations, we speculate this technique can be used to detect drug diversion. For example, drugs sent to the floor in polyvinyl bags for infusion therapy can be identified for content and concentration using “point and shoot” sampling upon dispensation and return of partially used bags, etc. Visual inspection of the peaks and respective intensities can detect changes in content and concentration from dispensing.

Figure 2. A unique Raman spectra is depicted for the compounded pharmaceutical formulation hydralazine. Three peaks (A = 764 cm^{-1} , B = 1034 cm^{-1} , and C = 1373 cm^{-1}) were selected to determine the ability to quantitate hydralazine content using Raman spectrometry.

Figure 3. Three representative wavenumbers were selected from the unique scattered light pattern that resulted from Raman analysis of hydralazine (Figure 2). The arbitrary intensities for each wavenumber peak are depicted for each of the respective hydralazine concentrations ranging from 1 to 10 mg/mL. A significant linear correlation between hydralazine concentration and arbitrary intensity was observed for each of the three wavenumbers.

● 754 cm⁻¹; ■ 1034 cm⁻¹; ▲ 1373 cm⁻¹

CONCLUSIONS

Our preliminary study suggests Raman spectrometry can be effectively used to identify pharmaceutical formulations that have been correctly compounded, and can be used to determine the concentration of the active ingredients in a compounded pharmaceutical formulation. We also speculate that Raman spectrometry may be useful to determine drug stability (beyond-use) dating and may be useful to detect drug diversion. However, further studies are needed to further examine the effectiveness of Raman spectrometry applications in compounded pharmaceutical formulations.

Disclosure The authors declare no conflicts or financial interest in any product or service mentioned in the manuscript, including grants, equipment, medications, employment, gifts, and Honoraria. The authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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