Application note

Introduction to Method Transfer between Raman Spectrometers

Author

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Industry/Application:

Quantitative analysis, transfer of analytical methods (calibrations) between instruments, pharmaceutical manufacturing

Products used:

Thermo Scientific™ DXR3 SmartRaman+ Spectrometer, ASA (Automatic Sampling Array) accessory, TQ Analyst Software

Goal:

This document aims to provide a brief introduction to the transfer of analytical methods between Raman spectrometers.

Keywords:

Raman spectroscopy, chemometrics, quantitative analysis, analytical methods, models, method transfer, calibration transfer

Key Benefits:

Options for transferring methods (calibrations) between Raman instruments expanding their use.

Introduction

The sampling advantages of Raman spectroscopy, along with the extensive chemical and physical information that can be extracted from Raman spectra, makes it an appealing analytical technique serving a variety of fields. The process of extracting answers from spectra can be relatively simple or involved depending on the information sought, the complexity of the Raman spectrum, and the breadth of the analytical goal. Analysis of these Raman spectra can be performed using “chemometrics,” which is a broad term for the use of mathematical processing to extract information from experimental data. Falling within the chemometrics space is multivariate data analysis. Multivariate data analysis can be used with Raman spectral data as well as data from other forms of vibrational spectroscopy to generate analytical methods (models). These analytical methods utilize previously collected spectral information (databases) to determine properties or characteristics of unknown samples based on acquired spectra. These methods can be qualitative in nature with the goal of identification or classification, or they can be quantitative where the result is a numerical value indicating the amount of a component present in the sample. The diversity of methods is mirrored by the variety of applications.

The development of analytical methods represents an investment of time and resources. For this reason, it is particularly important, especially for larger scale implementations, that once a method has been generated using a primary instrument it can then be transferred for use with secondary instruments. This is referred to as method transfer. Since there are many different types of applications that use analytical methods, there is also a range of what will be required for method transfer. This note offers a brief introduction to the general concepts of method transfer. Different tools for method transfer have been developed and studied quite extensively, but the details and the specifics of implementation are well beyond the scope of this document.[1,2]

The focus here will be on the transfer of methods between instruments, but a second part of method transfer involves changes, unintentional or intentional, in analysis conditions. Just as instrumental differences can affect the results, altering the sampling or measurement conditions can also introduce errors. This is often considered part of method maintenance. It may involve the aging of instruments or repairs to instruments or even changes in the way samples are prepared or presented for analysis. The concepts for method maintenance and method transfer are often related but the source of deviations leading to measurement errors are not necessarily the same.

A Raman spectrum typically consists of two axes. The x-axis units are typically wavelength or wavenumber (cm^{-1}) and are generally displayed as a Raman shifted spectrum (referenced to the wavelength of the laser). The y-axis is an expression of Raman intensity in various units. In an ideal world, Raman instruments would all produce exactly the same spectrum from the same sample. However, this is not the case, so it is necessary to recognize the possible variations and do what can be done to account for them.[2,3]

A calibration of both the spectrograph and the laser wavelength provides accurate and reproducible x-axis values. This typically involves using standards with known wavelengths such as neon bulbs and standards like polystyrene. Shifts in peak positions can have a noticeable effect on chemometric methods. An example of this is shown in Figure 1 where the results of shifting peak positions (+/- 4 cm^{-1}) results in significant deviations (up to 26.5%) in the results obtained from a quantitative method. With this method, a small shift (< 1 cm^{-1}) has a relatively small effect on the results but as the shift gets larger the effect rapidly increases. The magnitude of the effect of peak shifts will depend on the method and the type of preprocessing used.

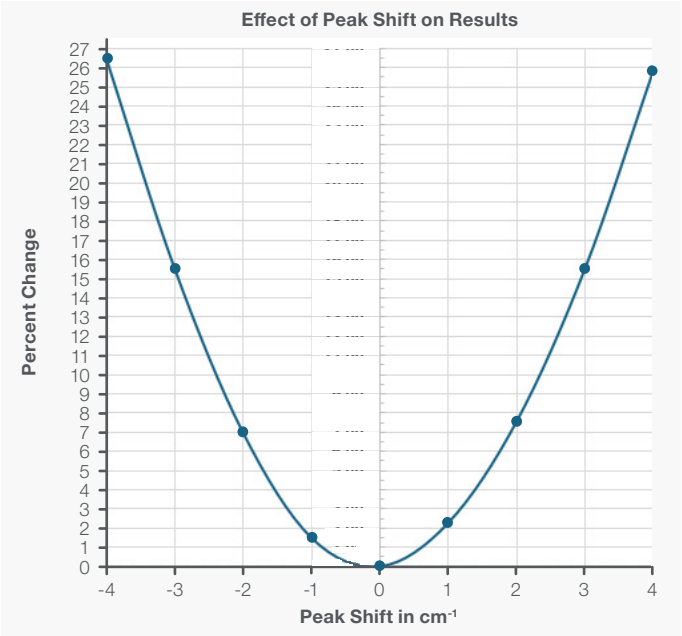

Figure 1. Effect of peak shift on the results obtained from a partial least squares analytical method.

Raman intensity is important particularly for quantitative analysis because it varies directly with the amount of material present. Many factors other than concentration affect intensity: laser power, sample focus, sample preparation, detector response, optical components, etc. While methods for standardizing intensities using NIST standards and calibrated white light sources have been used, that does not solve all the problems of intensity variations between instruments. It is advisable to plan for a way that fits within the specifics of the application, to allow for use of peak ratios or another method to normalize the intensities of the Raman spectra.

Different approaches to method transfer

Direct transfer method: The method is developed solely on the primary instrument and then directly transferred to secondary instruments.

- Direct transfer does not require collection of standards on secondary instruments.
- It involves less time and effort.
- Successful direct method transfer is not always possible.
- Method transfer success depends on the method and analysis requirements.

It is important to optimize methods to minimize the effects of variance through techniques like preprocessing (normalization, baselines, etc.—there are too many preprocessing options to detail here)[2,4] or the selection of other parameters (spectral ranges, etc.).

Global methods (full calibration): This requires collecting reference spectra on both the primary instrument and secondary instruments.

- This is a large all-inclusive method.
- A robust model, it incorporates instrument-to-instrument variations
- Because it is so inclusive, it requires more time to collect the spectral data on each instrument.
- The method must be updated with each new instrument.

Correction or standardization: This method uses a limited number of correction or transfer spectra collected on secondary instruments.

- Many different options exist for this approach—as with direct transfer, there are too many to detail here[3,5,6,7] — but they include univariate and multivariate approaches.
- Correction spectra are used to generate corrections to "adjust" results from the primary method.
- Standardization spectra are used to generate transfer functions for standardizing spectra.
- This method requires fewer secondary spectra than a full Global Method.

To illustrate a couple of these concepts, quantitative methods were developed using a Thermo Scientific™ DXR3 SmartRaman+ Spectrometer. The transfer of methods to other DXR3 SmartRaman+ instruments were investigated.

Experimental

Samples used in this investigation were aqueous solutions of acetaminophen with concentrations between 0 – 10 mg/ml (see Figure 2). The standards and test solution (2.604 mg/ml) were analyzed in glass vials using a custom sample holder for the ASA (Automatic Sampling Array) accessory used with the DXR3 SmartRaman+ spectrometer. The excitation source was a 532 nm laser. This testing involved a primary instrument and 5 secondary instruments. Spectra were collected from the standards as well as the test sample on all 6 of these instruments. A partial least squares method was generated using TQ Analyst software. Preprocessing of the spectra included spectral normalization of peak intensities using the water peak at 3416 $cm^{-1}$and using first derivatives to minimize baseline offsets.

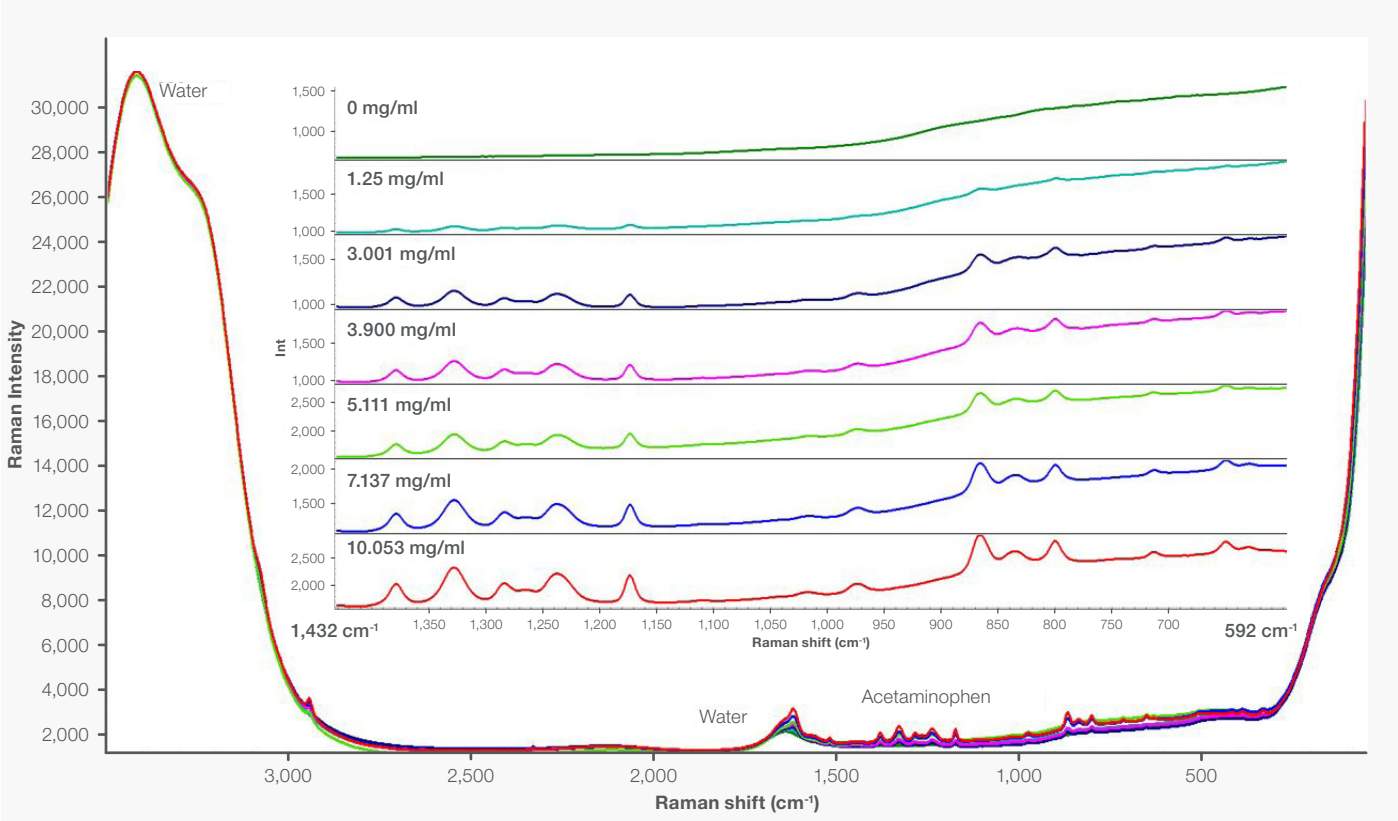

Figure 2. Representative Raman spectra from the acetaminophen solutions.

	Primary	Secondary #1	Secondary #2	Secondary #3	Secondary #4	Secondary #5
Region 1	-1.40%	-3.60%	-17.99%	19.44%	-11.51%	31.22%
Region 2	1.58%	0.00%	-6.52%	-1.33%	-9.17%	-7.44%

Table 1. Percent differences from the expected concentration of the test solution (2.604 mg/ml) for the primary instrument and all 5 secondary instruments. Region 1 is 1432–592 cm^{-1} and Region 2 is 2960–2930 cm^{-1}.

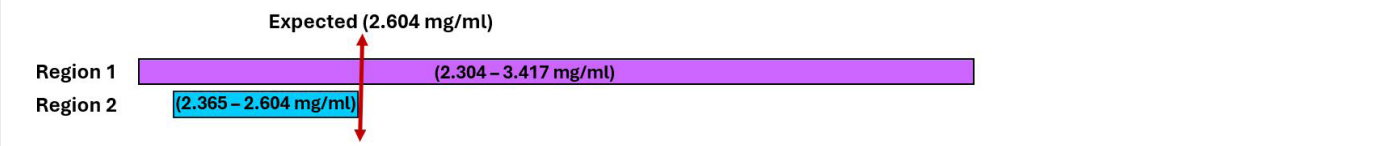

Figure 3. Range of values for the test solution (2.604 mg/ml) determined on the primary instrument and all 5 secondary instruments. Region 1 is 1432–592 cm^{-1} and Region 2 is 2960–2930 cm^{-1}.

	Primary	Secondary #1	Secondary #2	Secondary #3	Secondary #4	Secondary #5
Direct	-1.58%	0.00%	-6.52%	-1.33%	-9.17%	-7.44%
Global	1.58%	6.08%	0.35%	0.09%	-1.92%	-1.96%
Correction	0.83%	2.42%	-4.17%	1.08%	-6.85%	-5.10%

Table 2. Percent differences between the values determined for the test solution and the expected value (2.604 mg/ml) for the primary instrument and all 5 secondary instruments using the direct transfer method, the global method, and the correction method.

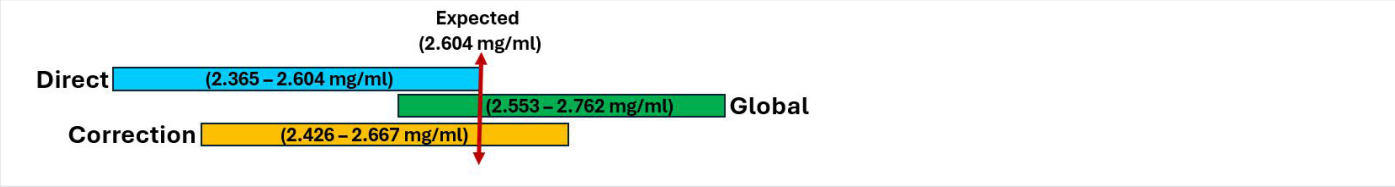

Figure 4. Range of values for the test solution (2.604 mg/ml) determined on the primary instrument and all 5 secondary instruments using the direct transfer method, the global method, and the correction method.

One important parameter that was optimized for method transfer was the selection of the spectral region used for the method. The initial region selected was in the fingerprint region (1432–592 cm^{-1}) and was selected due to the presence of multiple acetaminophen peaks. However, the success of direct transfer of the method from the primary instrument to the secondary instruments was dependent on the preprocessing and specific parameters used. Using the test solution (2.604 mg/ml), the secondary instruments gave values from 2.304 to 3.417 mg/ml (up to a 31% difference). An analysis of the spectral variance in the normalized spectra showed that the C-H stretching region was more consistent, so the method was recalibrated using the region at 2960–2930 cm^{-1}. This time the secondary instruments gave values ranging from 2.365 to 2.604 mg/ml (0–9.2% difference). A comparison of the results is shown in Table 1 and Figure 3. Figure 3 graphically compares the range of values obtained, and Table 1 shows a comparison of how close the values were to the expected value as percent differences. Using the second region shows a considerable improvement in the direct transferability of method.

Multiple vial-holder being loaded into the ASA (Auto Sampling Array) accessory for measurement.

To illustrate how different approaches to method transfer compare, a couple of simple examples were developed. The direct transfer method has already been discussed in the previous section. A global method was also generated by collecting all the standards on the primary as well as all the secondary instruments and using all the reference spectra in the analytical method. The other example is a correction method where the direct transfer method is used but a correction is applied to the results based on a limited number of correction spectra collected on the secondary instruments. The correction is applied post-prediction and is a mathematical fit based on the correction spectra collected on the secondary instruments. In this case the correction was a first order fit. The test solution (2.604 mg/ml) was run on all the instruments, and the calculated intensities based on the different methods were compared. Figure 4 shows a comparison of the range of values obtained from the different method transfer options. Table 2 shows the percent differences between the calculated and expected concentrations of the test solution for each of the instruments. The global method gave the best results in that it has the smallest range and is centered closest to the expected value, but it also required the greatest number of spectra. Using the correction spectra with the direct transfer method improved the results over the direct transfer method itself. Whether the extra work involved in the global method or the correction method is warranted for the enhancement of results depends on the goals of the application, the way methods are implemented, and the analysis requirements.

Conclusions

This was intended as a general introduction to the concepts of method transfer between Raman spectrometers. Method transfer in vibrational spectroscopy has been studied extensively, and a vast number of techniques have been developed and investigated. The examples presented here just illustrate a very small part of a vast subject. An important key to success is to use high quality spectra generated from a Raman spectrometer such as the DXR3 SmartRaman+ spectrometer and then to optimize the analytical method. The next step is to consider what is going to be required for method transfer. While direct method transfer is the most appealing approach because it represents the most straightforward approach with the least effort, it may be the exception rather than the rule. Constructing a global method is a more rigorous approach but is not always practical from both an effort and an implementation standpoint. Correction or standardization method transfer are tempting alternatives because they often require less data compared to a full global method. The example presented here was a correction method transfer approach. The standardization approaches were not addressed here but they use transformations that strive to “standardize” the spectra themselves. There are many options on how to implement method transfer, and in the end, the choice will depend on the specific application.

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