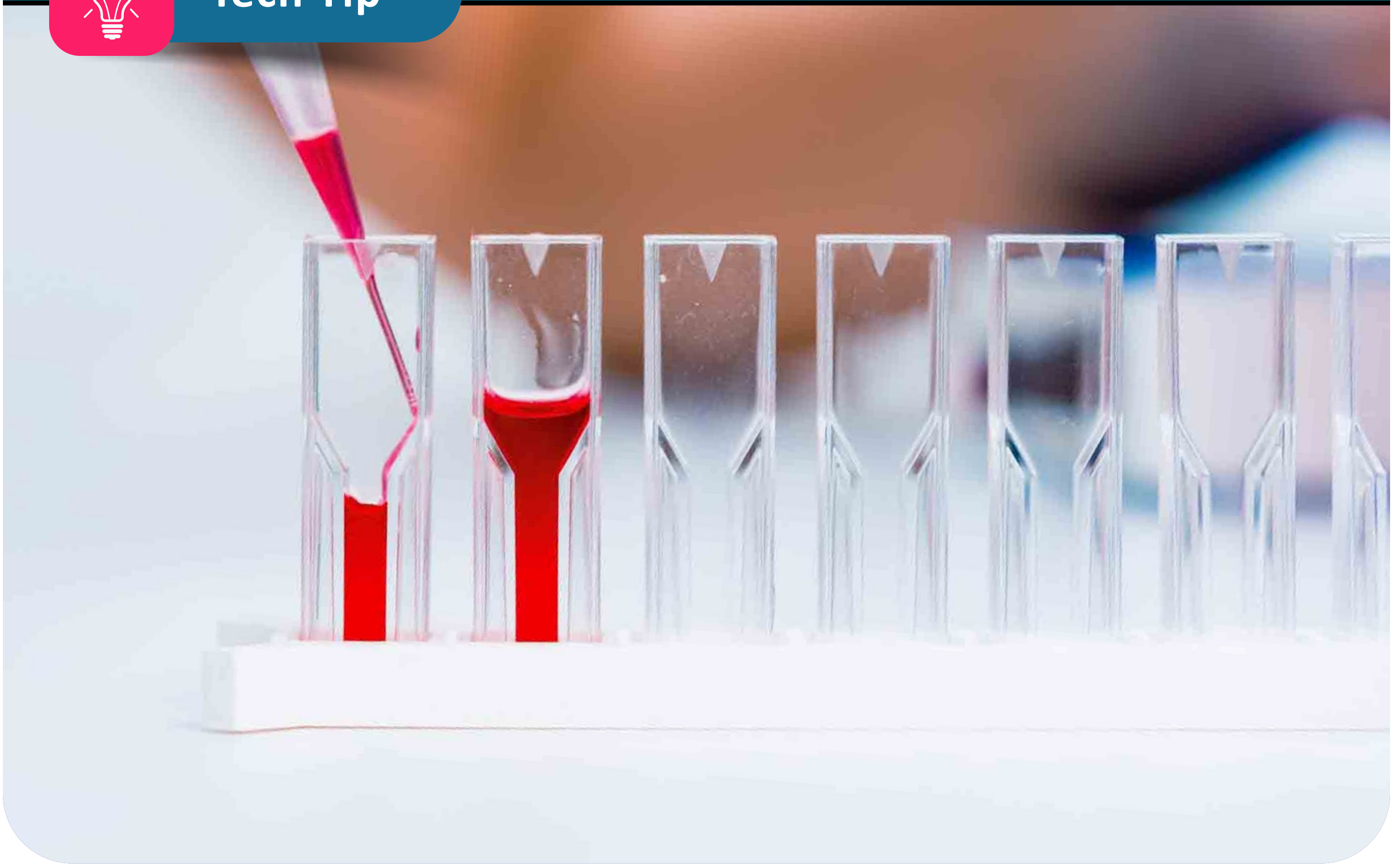
Analyte Validation and Concentration via Raman Peak Ratios

Select Observations and Thought-Provoking Methods

Tech Tip



Introduction

If you have been active in the world of spectroscopy over the last decade, you are already aware that Raman has become a much more widely accepted and streamlined technology for analyte identification.

Once considered too advanced in both theory and instrumentation for most end-users, today the hardware is presented as a handheld device from multiple suppliers, and advances in chemometrics and machine learning have hidden the difficult analyses behind the curtain. Ocean Optics has offered modular and embedded Raman systems for years, and to further enhance our Raman capabilities and portfolio we are also a world leading supplier of Surface Enhanced Raman (SERS) products. These chemistries greatly amplify the analytical Raman signal and therefore allow much lower limits of detection, which is something key to applications such as security and food safety. Ocean Optics has provided many thousands of SERS substrates to users testing for pesticides, explosives, illicit or pharmaceutical drugs, pathogens, fuel markers, and a wide range beyond those.

Raman to Qualify… but to Quantify?

Raman is often treated as a qualification method rather than a quantification method. The peak signatures offer highly repeatable fingerprints for each analyte that can be deconvoluted via any number of numerical methods. This allows detection of complex mixtures and real-world samples that are quite “messy” compared to an idealized laboratory sample. But what about concentration? Can Raman tell us how much of our analyte is present? The answer is a cautious “yes”, and we will take a look at some approaches here.

The most basic approach to quantification via Raman is simple peak intensity. Below we look at a sample of the fuel marker 1,2-di(2-pyridyl)ethylene, nicknamed BPE, in various concentrations. We see the dilutions line up in perfect order and with a very strong linear fit on the 1605cm^{-1} peak intensities.

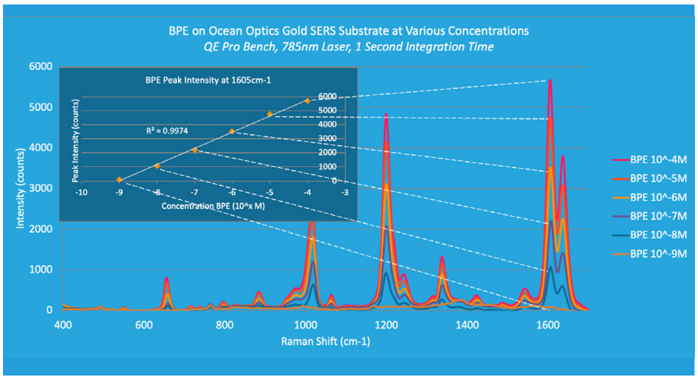


Figure 1

This looks great, but it assumes a very steady optical setup and essentially all experimental parameters as replicable as possible. What if we don’t have a stable setup along with 6 convenient known dilutions to reference?

Baseline Correction

When working in more typical spectroscopy modes such as absorbance or reflectance it is important to account for any optical shifts by performing some type of baseline correction. Peak values, or any values in general, may be skewed by some environmental influence but can be corrected based on some region(s) known to be independent of changing parameters. The same can be done for Raman spectra, and in fact is perhaps the most critical spectral genre to correct if one wishes to extract quantification information.

The example in Figure 2 looks at our BPE analyte with no baseline correction. We can see the spectra hover above the x-axis and have about a 250-count range between them.

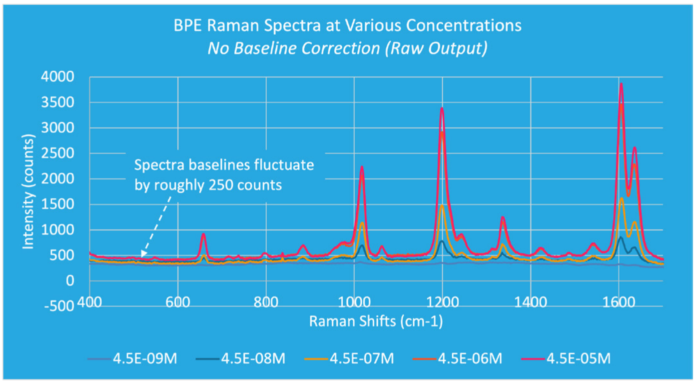


Figure 2

Around 580cm^{-1} there is a nice, Raman-inactive region that is also a relative low-point for all spectra. If we average around those pixels and subtract out across the spectrum, we will get a much cleaner arrangement of our concentration trends.

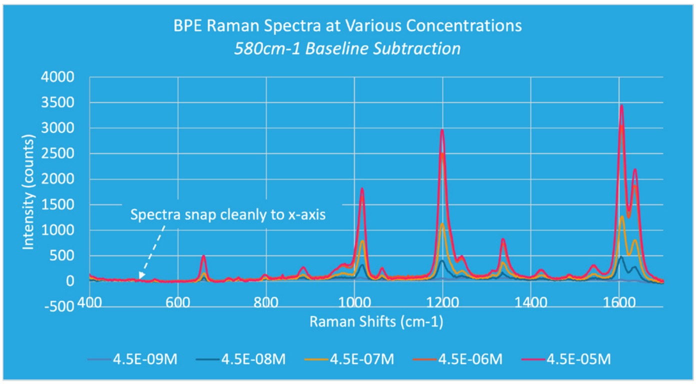


Figure 3

Peak Ratios

Now that our peak values are more meaningful relative to the overall shape of the spectra, let’s take a look at some ratio trends of the major peaks for our various concentration samples. We’ve gone ahead and processed all major peak ratios up front, and will look more closely at two select scenarios within these.

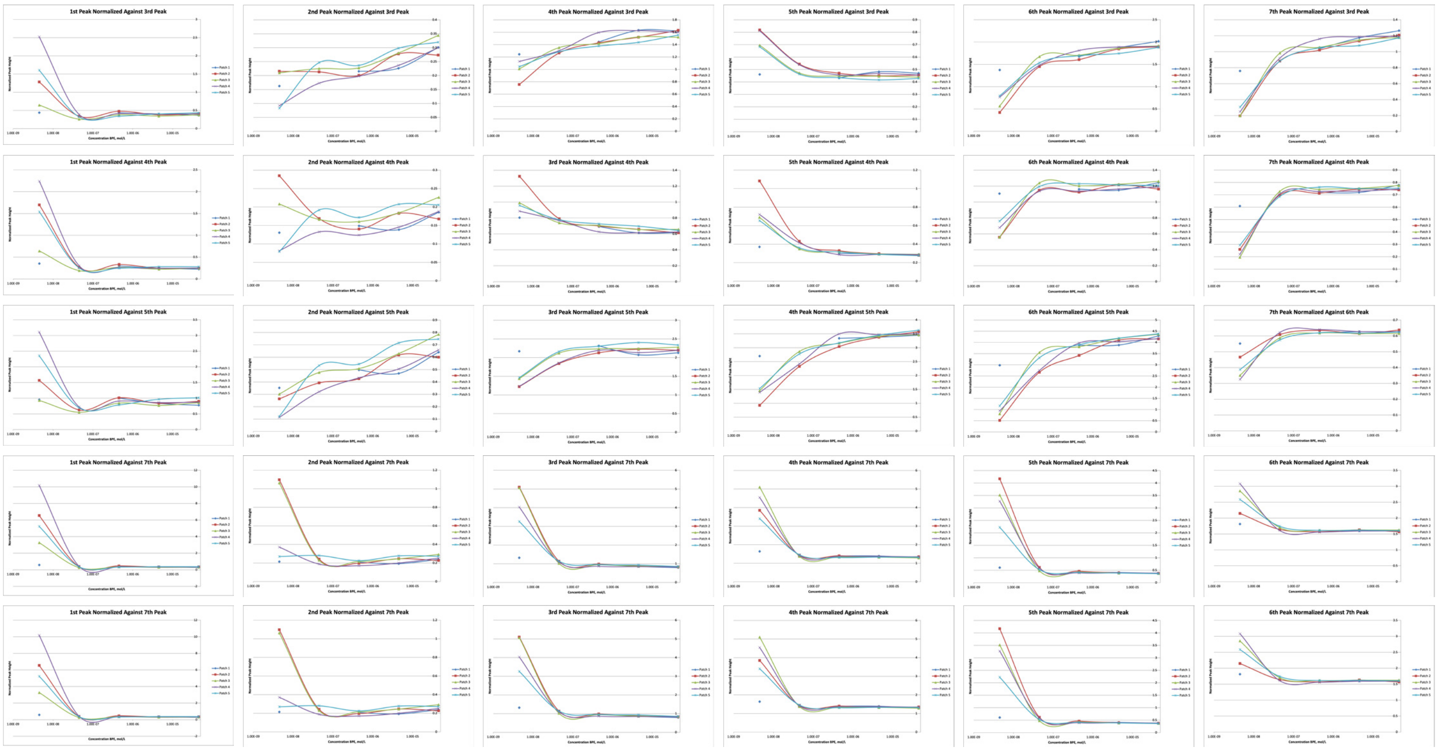


Figure 4: Potential Combinations of Active Peak Ratios for BPE

Validation

If we look at the 1197cm^{-1} peak against the 1634cm^{-1} peak across all 5 BPE dilutions and for 5 replicate runs, there is a highly repeatable ratio for all instances other than the lowest concentration.

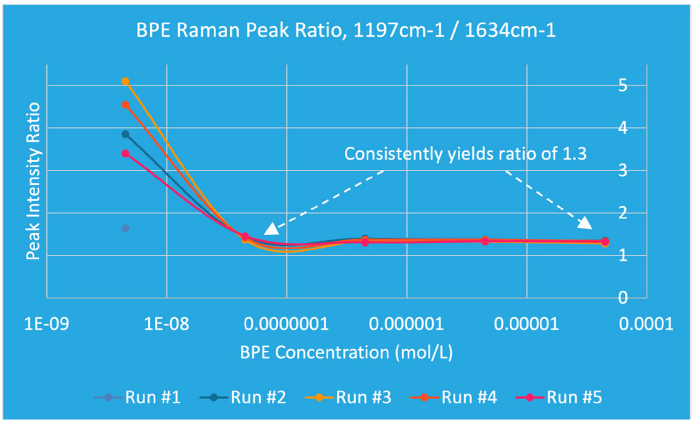


Figure 5

Why is this valuable? This provides a potential sanity check or additional reference on the measurement. If we were working with a government-subsidized fuel using BPE as the active Raman marker at let's say 10^{-6}M, we could use this 1197cm^{-1}/1634cm^{-1} ratio as another layer of confirmation that we have what we're looking for. If that ratio suddenly came out to a wildly different value it would be a flag that something was not right, which may be missed if everything else was looking normal (i.e. someone using a near-identical BPE surrogate to pass the counterfeit check)

Concentration

Now let's take a look at another set of peaks that may give us a slightly different trend behavior. If we still look at the 1634cm^{-1} peak but now ratio that against the 1337cm^{-1} peak, there seems to be a rough yet consistent trend with BPE concentration.

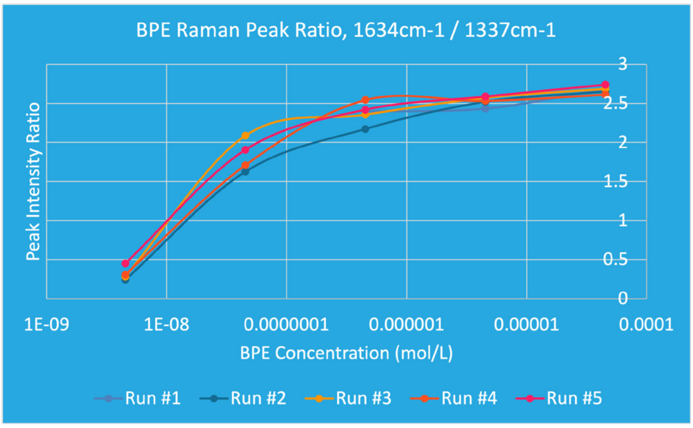


Figure 6

This fit isn't as tight as the first ratio we looked at, but there is enough here to give us some idea of where an unknown sample may fall. If we had taken these known samples as our references and then tested an unknown which yielded a 1634cm^{-1}/1337cm^{-1} ratio less than 2, we may not know the exact concentration but we could make a well-supported guess that it is below 10^{-6}M. This may be enough information for an alarm or basic threshold measurement.

Conclusion

While many will take Raman spectra at face value, we show here how very basic spectral and peak processing can clean up your combined plots and give you some potentially useful information. This gives us a deeper understanding of the spectral outputs and allows us to blend in additional layers of sample validation and rough concentration determination that were previously unavailable. For larger data sets of known conditions, these can be used as training data for PCA correlations and more intricate statistical analysis. Ocean Optics has strong experience processing and understanding spectral responses at the most basic and complex levels. Talk to us about how our applied spectral knowledge can help answer questions about your application.