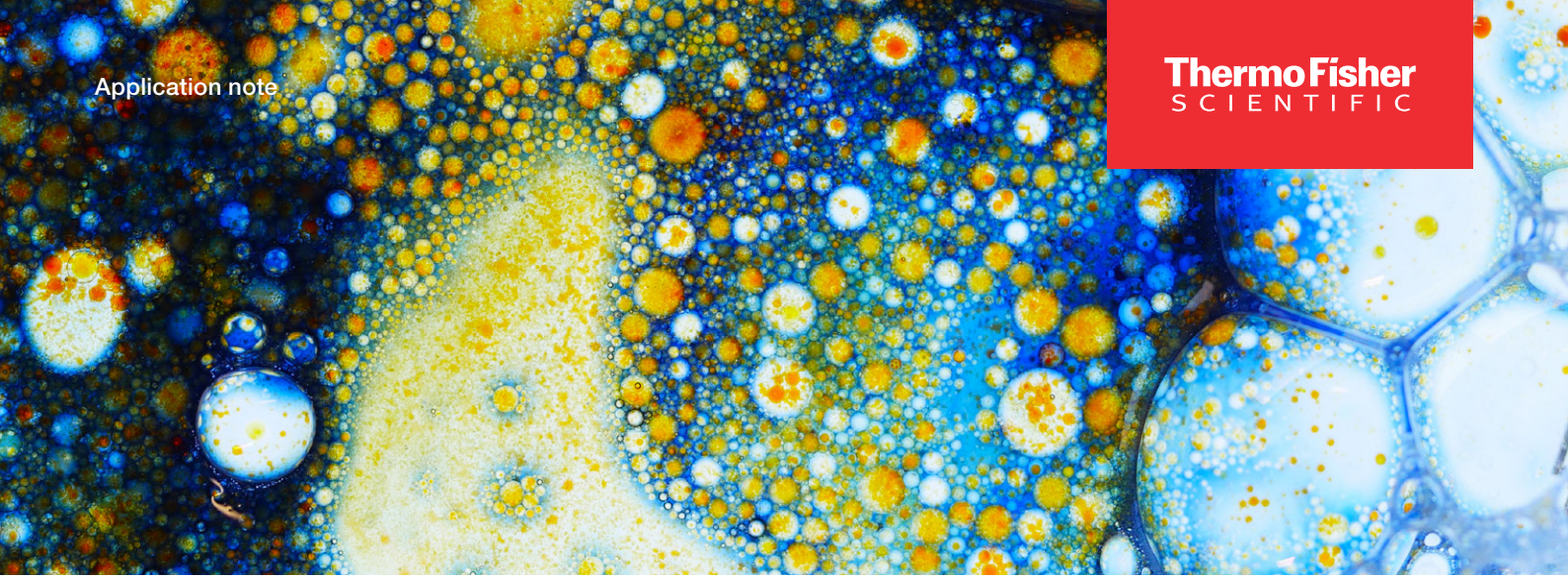

Application note

Enabling real-time release of final products in manufacturing of biologics

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Keywords

DXR3 SmartRaman, spectrometer, biopharmaceutical, GMP, real-time release testing, QbD, RTRT, manufacturing, multi-attribute testing

Introduction

Biopharmaceuticals (or biologics) are manufactured using biological-expression systems (such as mammalian, bacterial, and insect cells) and have spawned a large and growing biopharmaceutical industry (BioPharmaceuticals). The structural and chemical complexity of biologics, combined with the intricacy of cell-based manufacturing, imposes a huge analytical burden to correctly characterize and quantify both processes (upstream) and products (downstream). In small-molecule manufacturing, advances in analytical and computational methods have been extensively exploited to generate process analytical technologies (PAT) that are now used for routine process control, leading to more efficient processes and safer medicines.

Raman spectroscopy is a vibrational spectroscopy technique with several useful properties (non-destructive, non-contact, high molecular-specificity, and robustness) that make it particularly suited for PAT applications in which molecular information (composition and variance) is required.

Typical good manufacturing practice (GMP) operations involve performing an extensive set of tests according to approved specifications before the material is released to the market or for further processing. Recent ICH guidelines (ICH Q8, Q9, Q10, and Q11), however, suggest an alternative real-time release strategy to provide assurance of product quality prior to release. Real-time release testing uses the principles of the pharmaceutical Quality by Design (QbD) to optimize release and stability testing. A combination of manufacturing process understanding, process control, and product knowledge can be used to demonstrate that the material was made according to GMP.

The exact approach to real-time release testing (RTRT) will vary depending on the process requirements. The RTRT strategy may be based on control of process parameters, monitoring of product attributes, or on a combination of both at appropriate steps throughout the process. Critically, the RTRT strategy should be based on a firm understanding of the process and the relationship between process parameters, in-process material attributes, and product attributes.

Quality, cost, and speed are the major drivers for implementing in-line monitoring, at-line monitoring, and real-time release.

Here, we review some of the most important applications of Raman spectroscopy to the manufacturing and analysis of biopharmaceuticals. This article covers two aspects of the biopharmaceutical-manufacturing process: identity/variance testing of raw materials and cell culture media; and multi-attribute product testing of a biologic drug product or final product testing of a biologic drug product.

Raw material characterization

Acceptance of raw materials today is often predicated on small-scale functional testing and/or limited analytical methods, which may not be representative of at-scale performance. This leads, in some cases, to fluctuating process outputs and, in extreme cases, not meeting predefined release criteria. Furthermore, many clinical products are developed using a small number of batches resulting in a narrow range of raw material variation and thus a limited process understanding. Especially in upstream cell culture, the unforeseen variability of various components of the cell culture media can impact a product's micro-heterogeneity and its critical quality attributes (CQA).

Multi-attribute tests for high-risk raw materials may include identity test, quantitative test for the concentration of key ingredients in a raw material, batch-to-batch variability test, and degradation tests.

One high-risk raw material encountered in biologics manufacturing is cell culture media. Identification of cell culture media samples by traditional liquid chromatography (LC) methods, such as amino acid or vitamin analysis, has high costs and requires significant analytical expertise and laboratory space. Raman spectroscopy offers many potential benefits, such as low cost, portability, and potentially limited skill required to operate the instruments.

Buffers are another set of critical raw materials used in downstream manufacturing. Osmolality is a measure of concentration and is considered a critical quality attribute and critical process parameter in bioprocessing. The yield and quality of a biologic are highly dependent on the optimization of the downstream process. Identity testing along with osmolality of buffers can be carried out using a multi-attribute method based on principal component analysis and partial list squares. Rapid testing of buffers through single-use flexi bags can be carried out using the fiber optics probe of the Thermo Scientific™ DXR3 SmartRaman Spectrometer at the point of use with no need for sample preparation.

Final product identity testing

Final product identification of biologics pre- and post-shipment is another regulatory requirement. Product testing for identity through different kinds of primary packaging (glass vials, syringes, glass bottles) poses a significant analytical challenge in the manufacturing of biologics. Fill finish sites may not have the necessary analytical expertise to carry out the tests and may have to send the samples to the parent site or external lab for testing, incurring time and money.

Moreover, biologics or small molecule drug products would also have to undergo retesting upon importation either from a third country in the EU member state or the USA when drug products have been sent to the USA from other countries. A full list of tests is typically carried out, including final product identity testing. For biopharma manufacturers, this involves either sending the samples back to the parent site for analysis or employing third-party labs in the country of import. This increases significant costs and delays in the delivery of highly needed drug products.

End product identity testing/final product identity testing of biologics after fill-finish or pre-shipping to the fill-finish line is carried out by a variety of analytical techniques depending on the molecule/registration dossier.

For example, the verification test for biologic proteins is peptide mapping—a long-established workflow for protein identification using LC/mass spectrography (MS). This complex separation technique requires protein extraction and clean-up, enzyme digestion, one or more stages of liquid chromatography, and two phases of mass spectrometry before the final spectrum is matched against protein databases. Although it is a standard methodology, peptide mapping necessitates an analytical lab with qualified technical resources, entails extensive time for preparation, and introduces significant costs in solvents, columns, and analytical equipment.

The DXR3 SmartRaman Spectrometer, with its high sensitivity and resolution, allows characterization of the drug product by evaluating the fingerprint region of the molecule. Therefore, the DXR3 SmartRaman Spectrometer's unique capability with sampling flexibility ensures repeatable measurements, and subsequent analysis allows rapid method development and deployment.

We ran a feasibility study for multinational drug manufacture whereby the primary goal was to set up a rapid multi-attribute end product test to differentiate 15 different types of drug products and determine the concentration of the two preservatives in the drug products.

For this feasibility test we were given 15 different types of biologic drug products that varied in concentration from 0.5 mg/mL to 6 mg/mL. Concentration of two preservatives A and B ranged from 0.85 mg/mL to 5.0 mg/mL and 0.42 mg/mL to 3.91 mg/mL respectively.

These commercial drug products were supplied in their native glass vials varying in size and volume. A picture of such glass vials is shown below (Figure 1).

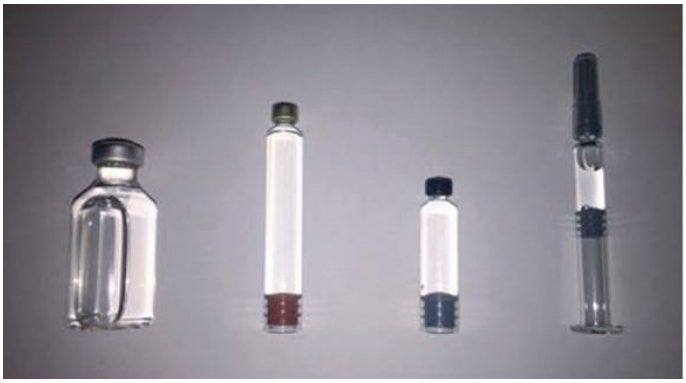

Figure 1. Typical native glass vials.

Reversed-phase high-performance liquid chromatography (HPLC) is currently used for the final product identity test and quantitative measurement of two preservatives in the final drug product.

DXR3 SmartRaman Spectrometer with universal sampling plate and 180-degree sampling module was used to acquire spectra of 15 drug products. To acquire each spectrum, a 532 nm laser with 40 mW power and 1 minute of scanning time was used. Ten spectra were acquired for each sample to accommodate the variability of glass vials and scattering effects.

DXR3 SmartRaman Spectrotometer offers excellent selectivity, repeatability, and full wavelength range to characterize biologics based on the characteristic band assignment (Table 1 and Figure 2).

Band frequency (cm^{-1})	Region	Vibrational mode	Protein structure assignments
870–1,150	Backbone, skeletal stretch	C_α-C, C_α-C_β, C_α-N	Secondary structure elements: α-helix, β-sheets, less-ordered structure
1,200–1,340	Amide III	N-H in-plane, Cα-N stretch	Hydrogen bonding, secondary structure
1,400–1,480	Side chain deformations	CH_2 and CH_3 deformations	Local environments, intermolecular interactions of side chains
1,510–1,580	Amide II	N-H deformations and C-N stretch (observed in UVRR and not conventional Raman spectra)	Local environments, intermolecular interactions of side chains
1,630–1,700	Amide I	C=0 stretch N-H in-plane bending	Secondary structure elements: α-helix, β-sheet, less-ordered structure

Table 1. Characteristic Raman band assignment.

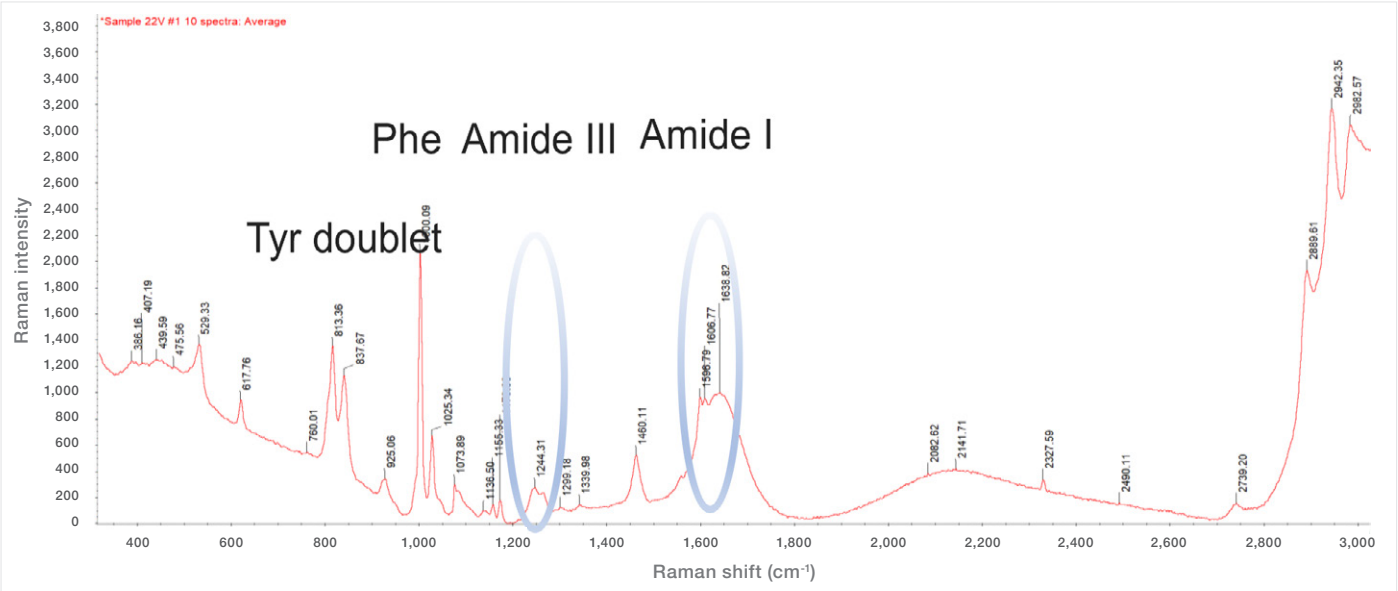

Figure 2. DXR3 SmartRaman spectrum showing characteristic bands of a biologic drug product.

Figure 3 shows the spectra of a sample containing a drug product against its placebo. It is imperative to establish that technique chosen for a feasibility study. In this case, Raman spectroscopy is sensitive enough to detect the differences between the drug product and its placebo. DXR3 SmartRaman Spectrometer offers high sensitivity to determine the significant differences between placebo and actual drug products.

Figure 4 is showing spectra of different classes of drug products. These spectra were utilized to build the discriminant analysis method on the Thermo Scientific™ TQ Analyst™ Software. TQ Analyst Software is a validated qualitative and quantitative method building software offering full compliance for pharmaceutical applications.

The discriminant analysis classification technique can be used to determine the class or classes of known materials that are most similar to an unknown material by computing the unknown's distance from each class center in Mahalanobis distance units. The discriminant analysis technique is typically used to screen incoming materials or final products to determine if they are compound/molecule a, b, or c.

Discriminant analysis methods typically specify at least two classes of known materials, but the method also works with only one class. Multiple standards may be used to describe each class (at least one class must contain two or more standards). Multiple regions of the spectrum may be used for the analysis.

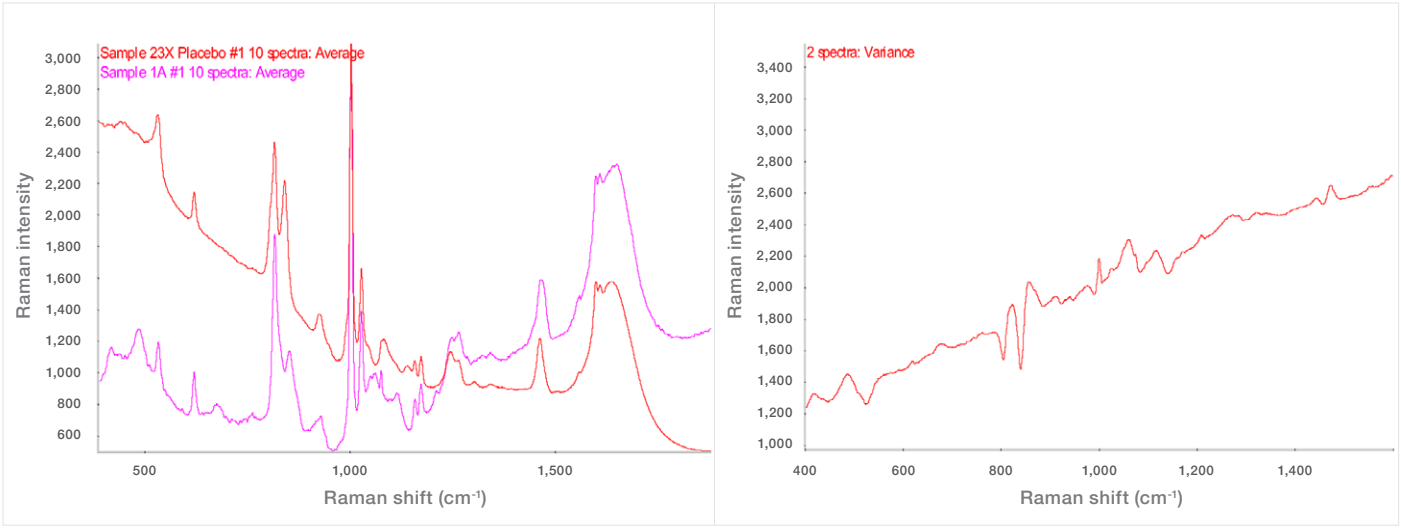

Figure 3. Raman spectra of drug product and its placebo and variance spectrum.

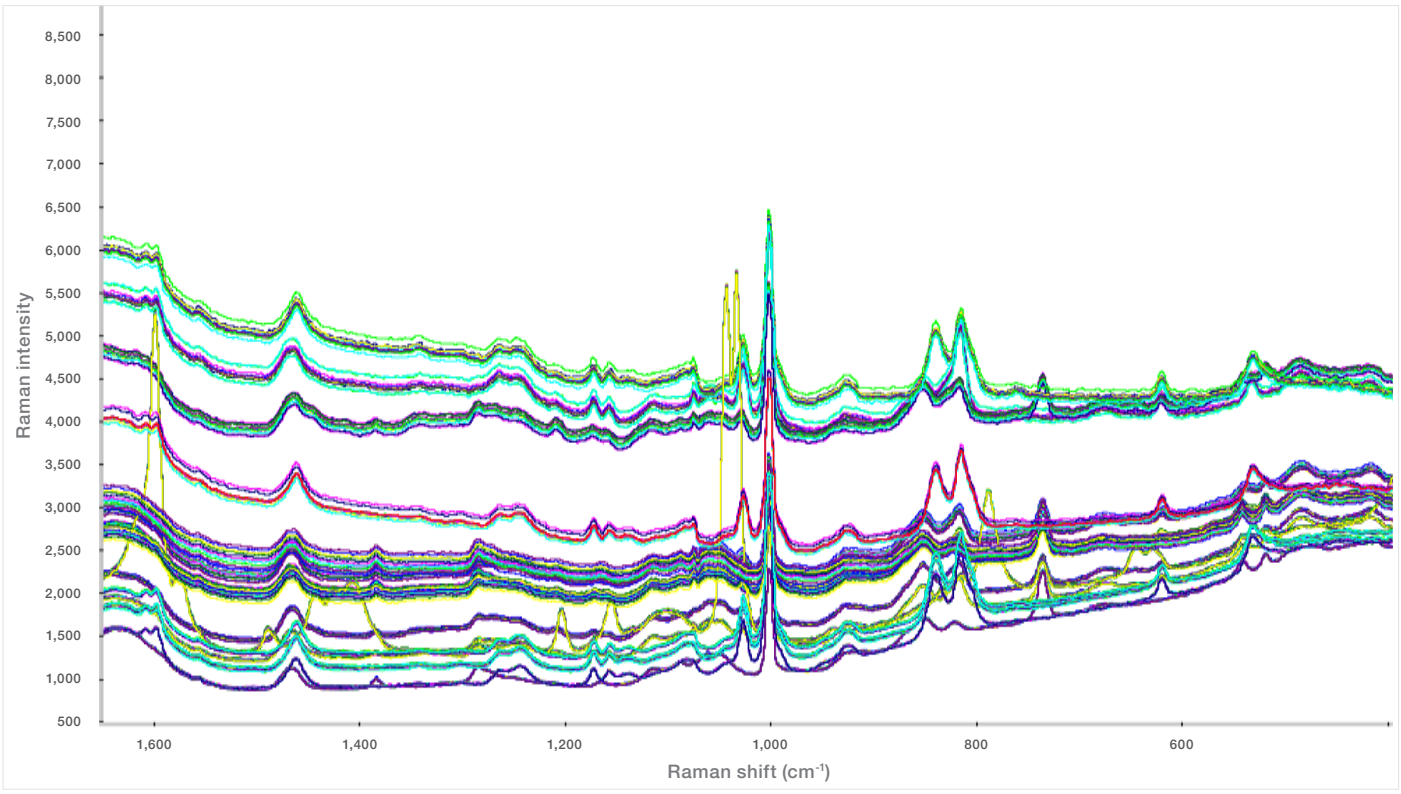

Figure 4. Raman spectra of different classes of drug products.

What does discriminant analysis do?

A discriminant analysis method applies the spectral information in the specified region or regions of an unknown sample spectrum to a stored calibration model to determine which class of standards is most similar to the unknown.

When the method is used to analyze an unknown sample or a class, the software performs a principal component analysis on the spectra of the standards and uses those results to determine score values for the unknown sample spectrum. The score plots are used to produce Mahalanobis distance values, which in turn are used to rank the classes.

The result of a discriminant analysis is the name of the class or classes that are most similar to the spectrum of the unknown sample. The Mahalanobis distance between the unknown sample and each reported class can also be reported. The closer each distance value is to zero, the better is the match.

After cross-validation, principal component scores plot revealed the class differentiation and the report indicated that all the classes of the different products were correctly identified with no mismatches to indicate false positives.

Quantitative analysis of biologics for preservative A and preservative B

As part of this feasibility study, our client also wanted to determine if the DXR3 SmartRaman Spectrometer test could be utilized to replace the HPLC test for measuring the concentration of two preservatives in their drug products. The level of preservative A was 0.85 mg/mL to 3.07 mg/mL and that of preservative B was 0.32 mg/mL to 2.57 mg/mL.

Pure samples of preservatives A and B were acquired as references, and to ascertain their presence in the final drug formulation.

Actual class	Mismatch	Calculated class	Calculated distance	Next class	Next distance
Product D		Product D	0.5809	C	4.5556
Product A		Product A	1.9869	I	12.9617
Product B		Product B	1.3796	E	25.1324
Product C		Product C	0.5417	D	3.8568
Product D		Product D	0.8466	M	9.0495
Product I		Product I	1.7709	A	13.9064
Product M		Product M	0.5284	S	3.3881
Product O		Product O	0.2244	X	17.3044
Product R		Product R	0.5419	C	4.4691
Product T		Product T	0.5944	X	2.3213
Product X		Product X	0.79	T	3.1646
Product S		Product S	1.1837	M	3.0829
Product N		Product N	1.0954	U	15.1798
Product U		Product U	0.1603	T	9.1738
Product S		Product S	1.8544	N	22.1624

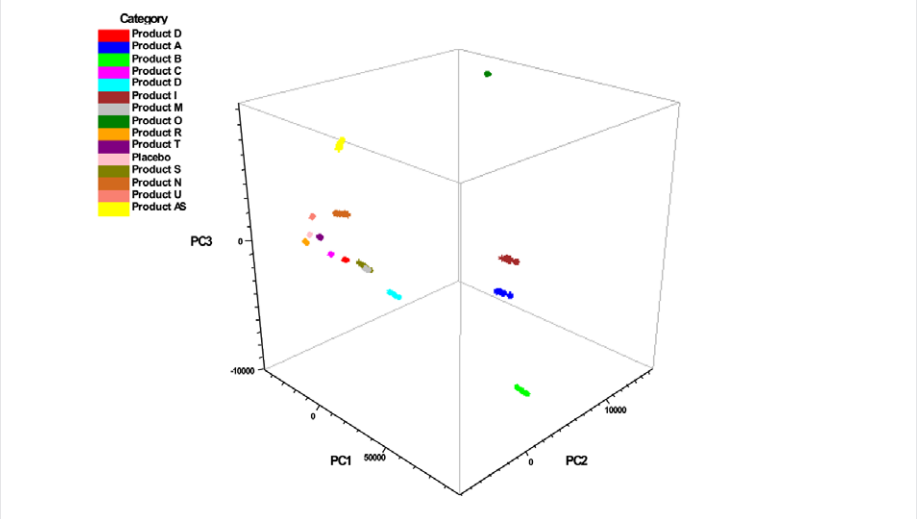

Figure 5. Analysis of preservative A and preservative B.

Samples of varying concentrations as per table 1 were acquired using the same parameters as of spectra acquired for identity test through 3 mL vial. Figure 6 is showing the spectra of the drug product with the two preservatives.

Four standards with the reference values were supplied in 3 mL and 10 mL vials and a validation sample to test the model for 3 mL and 10 mL vials.

Four spectra per standard were acquired and used to build the chemometric method. The final drug product samples were scanned with a DXR3 SmartRaman Spectrometer to acquire spectra in the range of 3500 to 50 cm-1 and captured with a single exposure of the CCD, avoiding stitching artifacts. The sample time took approximately 1 minute. Three spectra were collected per sample. The sample spectra were loaded into TQ Analyst Software for chemometric analysis using a partial least squares (PLS) method.

	Preservative A (mg/mL)	Preservative B (mg/mL)
Standard 1 3 mL and 10 mL	0.85	0.42
Standard 2 3 mL and 10 mL	1.27	1.12
Standard 3 3 mL and 10 mL	1.57	1.75
Standard 4 3 mL and 10 mL	3.07	2.57
Validation – 3 mL	1.57	1.75

Table 2. Calibration and validation sample.

	PLS results for 3 mL Cartridge	
	Preservative A (mg/mL)	Preservative B (mg/mL)
Validation sample: 3 mL	1.58 actual 1.57	1.71 actual 1.75
Real Sample in solution: 3 mL	1.56 actual 1.55	1.69 actual 1.77
Real sample in suspension: 3 mL	0.72 actual 0.69	1.23 actual 1.58

Table 3. Validation result for 3 mL sample.

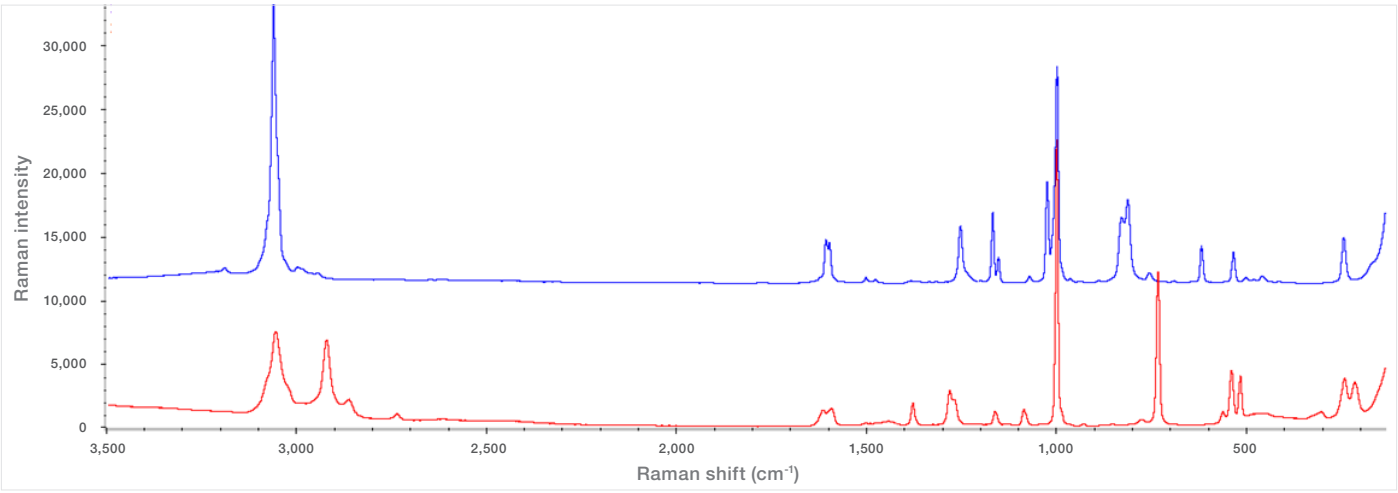

Figure 6. Spectrum in blue is from pure preservative A and spectrum in red is from pure preservative B.

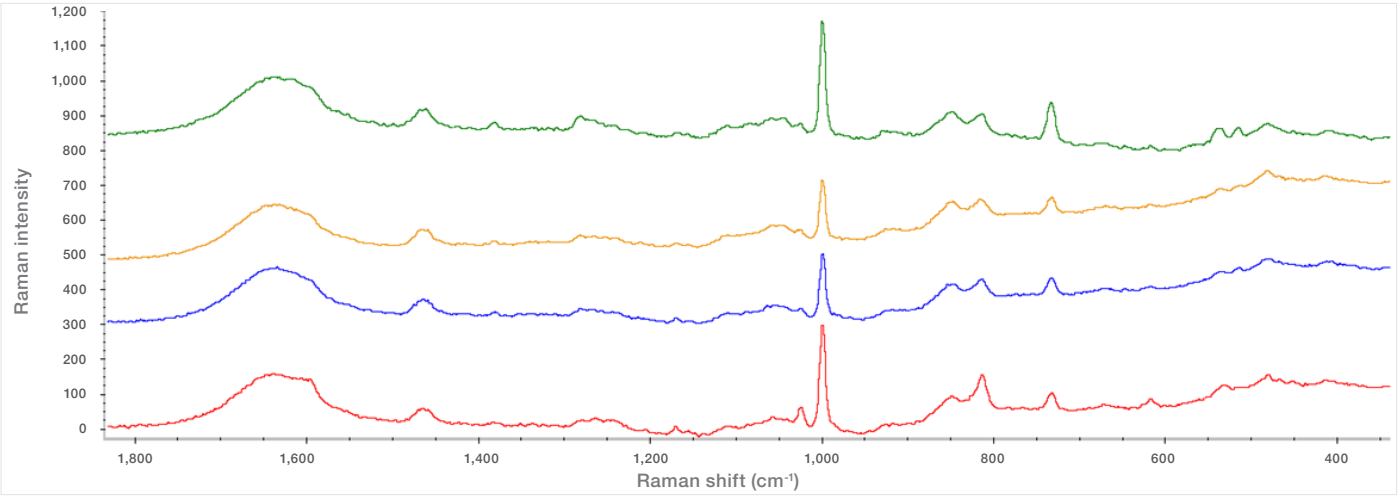

Figure 7. Spectra showing varying concentration of preservatives in final drug product.

Results

PLS analysis of the final drug product samples revealed excellent predictive capabilities within the range of materials tested. The spectra used to develop the PLS method for 3 mL cartridge are shown on calibration plots (Figure 8 and Figure 9) that compare the calculated preservative concentrations versus the actual concentrations. The calibration plot can be used to determine how well the method predicts the actual preservative concentrations in the samples. The plot developed by the chemometric method resulted in a correlation coefficient of 0.998 for preservative A. Root mean square error of calibration (RMSEC) was 0.0425 mg/mL, and the Root mean square error of prediction (RMSEP) calculated was 0.0372 for preservative A. The additional method for preservative B resulted in in a correlation coefficient of 0.999. The RMSEC was 0.0316 mg/mL, and the calculated RMSEP was 0.0496. The method was able to accurately predict the 3 mL validation sample and a real sample in solution (Table 3). The prediction can be improved when suspensions are allowed to settle and liquid phase is analyzed.

When 10 mL vial calibration samples were added to the above PLS method, method performance remained the same and was able to accurately predict the validation samples (Table 4).

Conclusions

A multi-attribute test to establish Final product identification and predicting concentrations of preservatives was done with the DXR3 SmartRaman Spectrometer by developing a discriminant analysis method and partial least square method. The final drug product identification test is part of release testing and current methods used are time-consuming and laborious. This Raman technique successfully demonstrates the ability to measure and monitor preservative concentrations either in the lab environment or at the line. The method developed shows excellent correlation with actual preservative concentrations with errors comparable to the reference analysis method. This application demonstrates the continued capability of the DXR3 Raman Spectrometer to be successfully used in bioprocess environments for implementing multi-attribute final product testing of biologics. Apart from the examples shown here, DXR3 SmartRaman Spectrometer can be used to implement at-line control strategies to monitor protein concentration, excipients concentration, and critical quality attributes like osmolality and pH. Many such examples are cited in the literature for Raman applications in biopharma manufacturing.

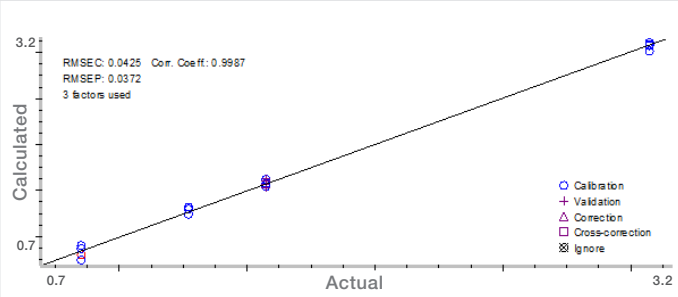

Figure 8. PLS model for preservative A —3 mL cartridge.

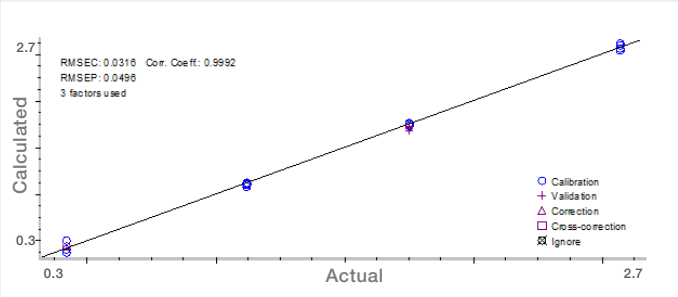

Figure 9. PLS model for preservative B —3 mL cartridge.

	PLS 3 mL cart and 10 mL vials	
	Preservative A (mg/mL)	Preservative B (mg/mL)
Validation sample: 3 mL	1.58 actual 1,57	1.71 actual 1,75
Real sample in solution: 3 mL	1.56 actual 1.55	1.65 actual 1.77
Real sample in suspension: 3 mL	0.80 actual 0.69	1.21 actual 1.58
Real sample in suspension: 10 mL	0.73 actual 0.68	1.32 actual 1.57

Table 4. Validation results for 3 mL 10 mL vials.

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