APPLICATION NOTE

The Transferability of Chemometric Models Built on HyperFlux™ PRO Plus to the Process Guardian™ Raman Spectrometer

INTRODUCTION

Tornado Spectral Systems has recently introduced the Process Guardian™ Raman spectrometer (PGR), which is a process-ready version of their HyperFlux™ PRO Plus (HFPP) system. Customers who are considering adding this new system are interested in knowing whether their existing models can be transferred to the Process Guardian™ Raman. Fortunately, the two systems are based on the same high-throughput and high-resolution High Throughput Virtual Slit (HTVS™) technology, making model transfer straightforward. To demonstrate the transferability of models between the two systems, we conducted an experiment using 30 samples containing glucose, lactate, and glutamine in DMEM growth media to simulate bioprocess conditions. We measured these samples using both the HyperFlux™ PRO Plus and Process Guardian™ Raman systems, with calibration concentrations ranging from 0-6 g/L for glucose and lactate, and 0-4 g/L for glutamine. The two spectrometers used in this study were units that happened to be available for this work. They were not pre-selected to enhance success. The HFPP used for this work was produced in 2016 and the PGR was produced in 2022. This simulates quite well the circumstances that would be presented to a typical user tasked with model transfer. No pre-matching of the instruments was done. Our results demonstrate that chemometric models can be easily transferred between the HyperFlux™ PRO Plus and Process Guardian™ Raman spectrometers using common preprocessing techniques. This ease of transfer is directly related to the enhanced capability of the Tornado HTVS™ technology. This finding is important for customers who are considering deployment of the Process Guardian™ Raman, as they can have confidence that their existing models will work seamlessly with the new system.

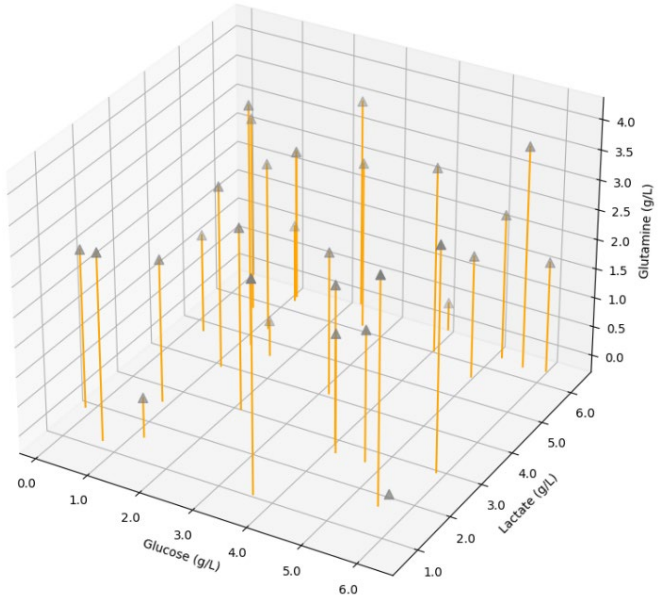

Figure 1 3D scatterplot of design of experiments for the concentrations of glucose, lactate, and glutamine for each sample

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EXPERIMENT AND DATA ANALYSIS

Design of Experiments - 30 samples containing mixtures of glucose, lactate, and glutamine were created as random mixtures of the 3 components diluted into Gibco™ DMEM growth media. The coefficient of correlation between glucose, lactate, and glutamine in the samples generated was ensured to be below a value of 0.1 (see Figure 1).

Experimental Conditions - A Tornado Raman Spectrometer System HyperFlux™ PRO Plus and a Process Guardian™ Raman system both equipped with a 785-nm laser were used for these experiments. Measurements were made using short-focus Tornado Hudson bio immersion probes. The integration times of the spectrometers were set to yield a suitable spectrum every 15 seconds (1000ms exposure time x 15 averages). The laser power was set to 495mW.

Materials - Mixtures of glucose (Sigma-Aldrich), lactate (Sigma-Aldrich), and glutamine (Sigma-Aldrich) were prepared in Gibco™ DMEM growth media (ThermoFisher) in ranges of approximately 0 – 6 g/L.

Data Analysis and Visualization - Data analysis and visualization were performed with PEAXACT multivariate analysis software from S-PACT GmbH. The spectral data range selected was 800-1800 cm^{-1}, which contains the relevant Raman bands for the metabolites of this study. Spectral pre-processing was rubber band baseline correction, Savitzky-Golay smoothing, and Standard Normal Variate (SNV) for scatter correction and normalization. The calibration models were selected based on leave-one-out cross validation, resulting in a 5-latent-variable Partial Least Squares (PLS) regression models for each of the three target metabolites.

RESULTS

The results of the model transfer can be seen in Figures 2, 3, and 4 below. Models were made from the data collected solely on the HFPP and the models were used to predict values from spectra solely collected on the PGR. These results demonstrate seamless transfer that requires no model adjustments. All metabolite calibrations demonstrate a high degree of measurement accuracy with ≤ 0.12 g/L prediction error.

This is an expected level of accuracy for these metabolites in a bioprocess. The increase in RMSEP from the RMSECV is less than 20% in all three models for the three metabolites. This difference is within expected limits for any reliable model when comparing RMSECV (calibration data) and RMSEP (validation data) statistics. It is also worth noting that no significant biases were observed.

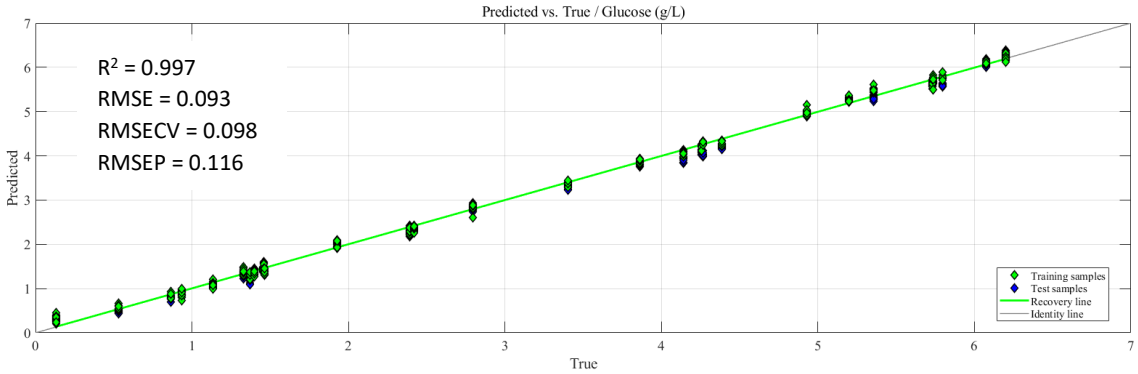

Figure 2 Calibration plot for glucose (g/L). Calibration samples (HFPP) in green and prediction samples (PGR) in blue.

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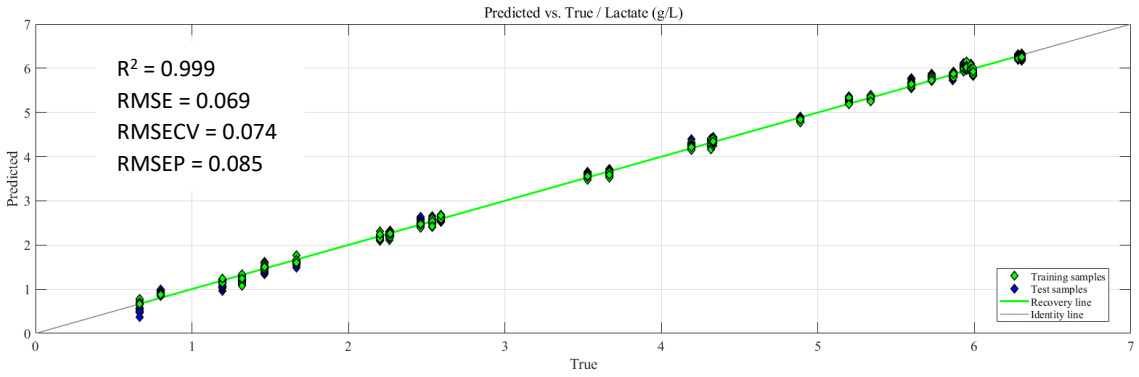

Figure 3 Calibration plot for lactate (g/L). Calibration samples (HFPP) in green and prediction samples (PGR) in blue

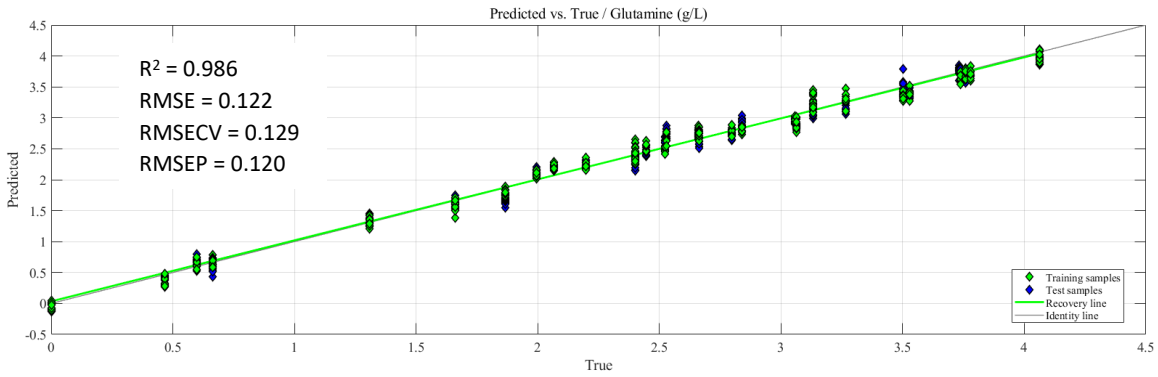

Figure 4 Calibration plot for glutamine (g/L). Calibration samples (HFPP) in green and prediction samples (PGR) in blue

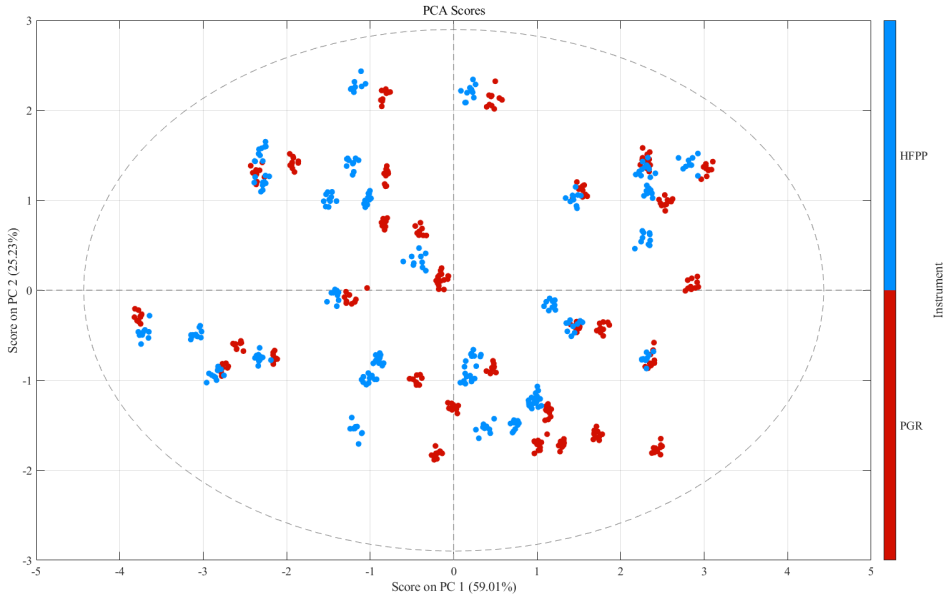

Figure 5 PCA scores for PC1 vs PC2 with HyperFlux™ spectra scores coloured in blue and Process Guardian™ spectra scores coloured in red.

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The principal component analysis (PCA) scores plot for PC2 vs. PC1 for all spectra collected is shown in Figure 5. If there were significantly large differences between the spectra of the HFPP and PGR, the scores plot above would be able to separate the spectra from the two systems. As can be seen in the plot, there is no simple boundary that separates spectra collected by the HFPP from the spectra collected by the PGR. This demonstrates excellent instrument sameness which is at the core of model transferability. The robustness of this seamless model transfer is even more noteworthy when considering the fact that a five-factor model was used. This number of factors would provide opportunity to incorporate idiosyncratic instrument information that would likely diminish performance of the transferred model if it was significant.

CONCLUSION

This experiment demonstrates that chemometric models can be easily transferred between the HyperFlux™ PRO Plus and Process Guardian™ Raman spectrometers (Figure 6) without extraordinary data manipulation. Only common preprocessing techniques that would be used routinely in normal model construction were applied in this study. This finding is significant for customers considering deploying a new PGR system, as it provides assurance that their existing models will work seamlessly. Our experiment used 30 samples containing mixtures of glucose, lactate, and glutamine in DMEM growth media, and the results showed a high degree of measurement accuracy with a prediction error of ≤ 0.12 g/L.

This simulates a typical workflow for a user with an HFPP who wants to deploy a PGR in process for a similar application. The enhanced capability of the Tornado HTVS™ technology makes model transferability straightforward. This experiment demonstrates the transferability of models between two Tornado spectrometers and highlights the benefits of the Tornado HTVS™ technology, not just for bioprocess monitoring, but also for small molecule pharmaceuticals, polymers, gas analysis, and fine chemicals.

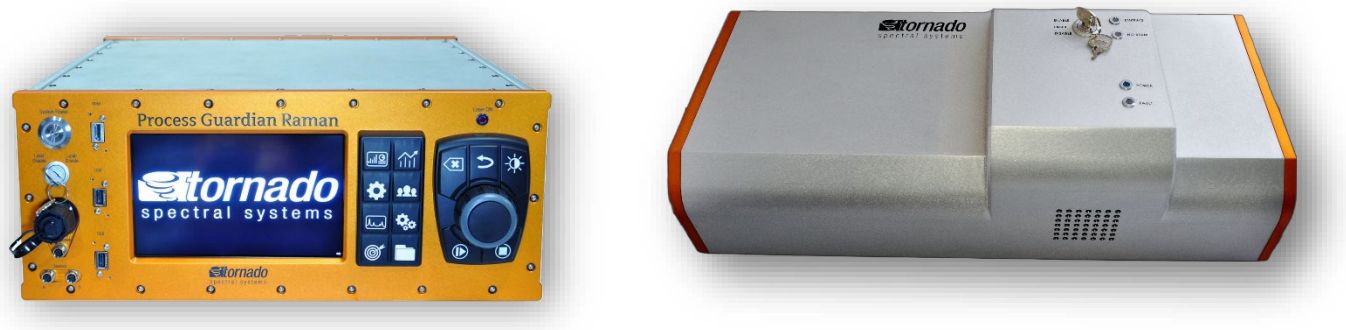

Figure 6 Tornado Raman Spectrometers: (L) The Process Guardian™ and (R) the HyperFlux™ PRO Plus Raman Analyzer.

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