Application note

Raman spectroscopy

Thermo Scientific Ramina Process Analyzer – Chemometric model transferability across instruments

Authors

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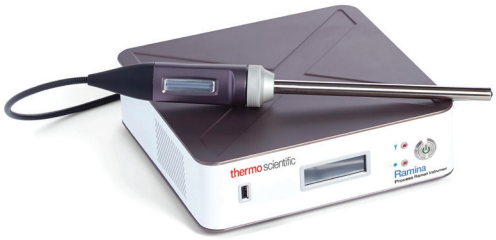

Summary

- The Thermo Scientific™ Ramina™ Process Analyzer represents a breakthrough in process analytical technology (PAT) by providing in-line, real-time and actionable monitoring of multiple analytes in complex systems.
- This paper demonstrates the successful transferability of one chemometric model across 10 different Thermo Scientific Ramina Process Analyzer systems while maintaining an average prediction error of 0.21 g/L, 0.16 g/L and 0.3 g/L for glucose, glutamine, and lactate, respectively in a mixture.
- Measurement accuracy and precision is maintained when applying a chemometric model across multiple instruments, ensuring users do not have to spend time or resources to re-build a model for a new Thermo Scientific Ramina Process Analyzer or new autoclavable probe.

Introduction

The Thermo Scientific Ramina Process Analyzer is a Raman spectroscopy instrument designed to offer rapid, robust, scalable, and reliable identification, quantification, and characterization of molecules during any phase of R&D process development. It is an “all-in-one” instrument utilizing easily exchangeable, autoclavable probes to meet the various analytical needs for, but not limited to, upstream bioprocess monitoring, or characterization of fill and finish products.

Chemometric analysis allows users to develop a data analysis model to monitor the concentration of multiple analytes from their analyzer. There is a significant investment made from a time and resource perspective to build an accurate and robust chemometric model. As a result, it is imperative for a chemometric model to be used between instruments to fully leverage the value of this investment. Once a chemometric model is developed, it can be used with any Thermo Scientific Ramina Process Analyzer to monitor

multiple reactors in parallel with high fidelity of model accuracy. The measurement accuracy and precision maintained when transferring chemometric models across different system hardware ensures that customers do not have to re-build a model when using a new instrument or new autoclavable probe.

Experimental set up

To demonstrate the transferability of a complex chemometric model, we evaluated three relevant analytes: glucose, glutamine and lactate in Gibco™ DMEM growth media. All three analytes occur in bioreactors in the g/L concentration ranges with glucose occurring in the range of 0-12 g/L, glutamine 0-2.5 g/L and lactate 0-20 g/L. A chemometric model was developed by collecting spectra from a set of calibration standards using one Thermo Scientific Ramina Process Analyzer. Within the relevant concentration ranges for each analyte, 24 samples with randomized concentrations were selected using the uniform design method[2]. The model was then applied to spectra from a different set of 8 validation samples, measured using 10 unique Thermo Scientific Ramina Process Analyzers. The acquisition parameters were optimized to maximize the signal-to-noise ratio of the spectral features corresponding to the analyte concentrations. Optimized parameters were 15 second integration time, 450 mW laser power and 10 replicate on-board signal averaging with automatic dark correction. These acquisition parameters resulted in a sample collection period of about 5 minutes. The model was able to predict the concentration of all three analytes in the 8-sample validation set with a high degree of accuracy and precision.

Each Thermo Scientific Ramina Process Analyzer evaluated in this study included a unique hardware set comprised of spectrometer box, fiber optic cables and bioreactor probe tip. Average prediction error for each analyte was 0.21 g/L for glucose, 0.16 g/L for glutamine and 0.3 g/L for lactate. These results demonstrate the excellent transferability of this complex chemometric model across numerous Thermo Scientific Ramina Process Analyzers.

Model analysis

A single PLS model was built using Eigenvector Solo software to predict all three analytes in each mixture. The PLS calibration model was built using 24 calibration samples with 3 replicates per sample. All calibration spectra were collected using one Thermo Scientific Ramina Process Analyzer. The Raman fingerprint region between 870-3096 cm^{-1} was used to build the model. A Savitsky-Golay smoothing filter was applied to remove the random noise and improve the signal to noise ratio. Next, baselines were corrected followed by scattering correction and normalization. In addition, all data were mean-centered before model building. The calibration model was built using the cross-validation strategy of leave-one-sample-out. The 3 replicates for the same sample were carried together in this process. After these crucial pre-processing and cross-validation steps were performed, the resulting optimized model with 4 latent variables was selected. Results of the calibration and cross-validation of the model are shown in Table 1.

	Analyte		
Model Parameter	**Glucose**	**Glutamine**	**Lactate**
RMSEC (g/L)	0.079	0.075	0.146
RMSECV (g/L)	0.095	0.088	0.176
Bias (g/L)	4.79E-05	-1.94E-05	-2.50E-05
CV Bias (g/L)	-2.25E-04	-6.45E-04	-4.42E-03
R^2 Calibration	0.9995	0.9902	0.9994
R^2 Cross-Validation	0.9993	0.9864	0.9991

Table 1. Calibration and cross-validation results for Chemometric model.

Spectra for the 8-sample validation set were subsequently collected on 10 different Thermo Scientific Ramina Process Analyzers. The chemometric model was applied to the validation-set spectra for each Thermo Scientific Ramina Process Analyzer to predict the analyte concentrations. The results discussed below show that high degree of accuracy and precision were maintained for the prediction of all three analytes across all 10 Thermo Scientific Ramina Process Analyzers.

Results

The development of robust chemometric models requires a significant investment of time and resources. To ensure that this investment provides long term value for our customers, we have demonstrated excellent transferability of chemometric models across numerous Thermo Scientific Ramina Process Analyzers. The correlation plot in Figure 1 shows the predicted vs. reference values for glucose (Fig.1a), lactate (Fig.1b) and glutamine (Fig.1c). Each plot contains an overlay of the predicted values for all 10 Thermo Scientific Ramina Process Analyzers. The precision of the chemometric model across 10 Thermo Scientific Ramina Process Analyzers provides customers with consistent results. Furthermore, the accuracy of the model can be seen from the results of Table 2.

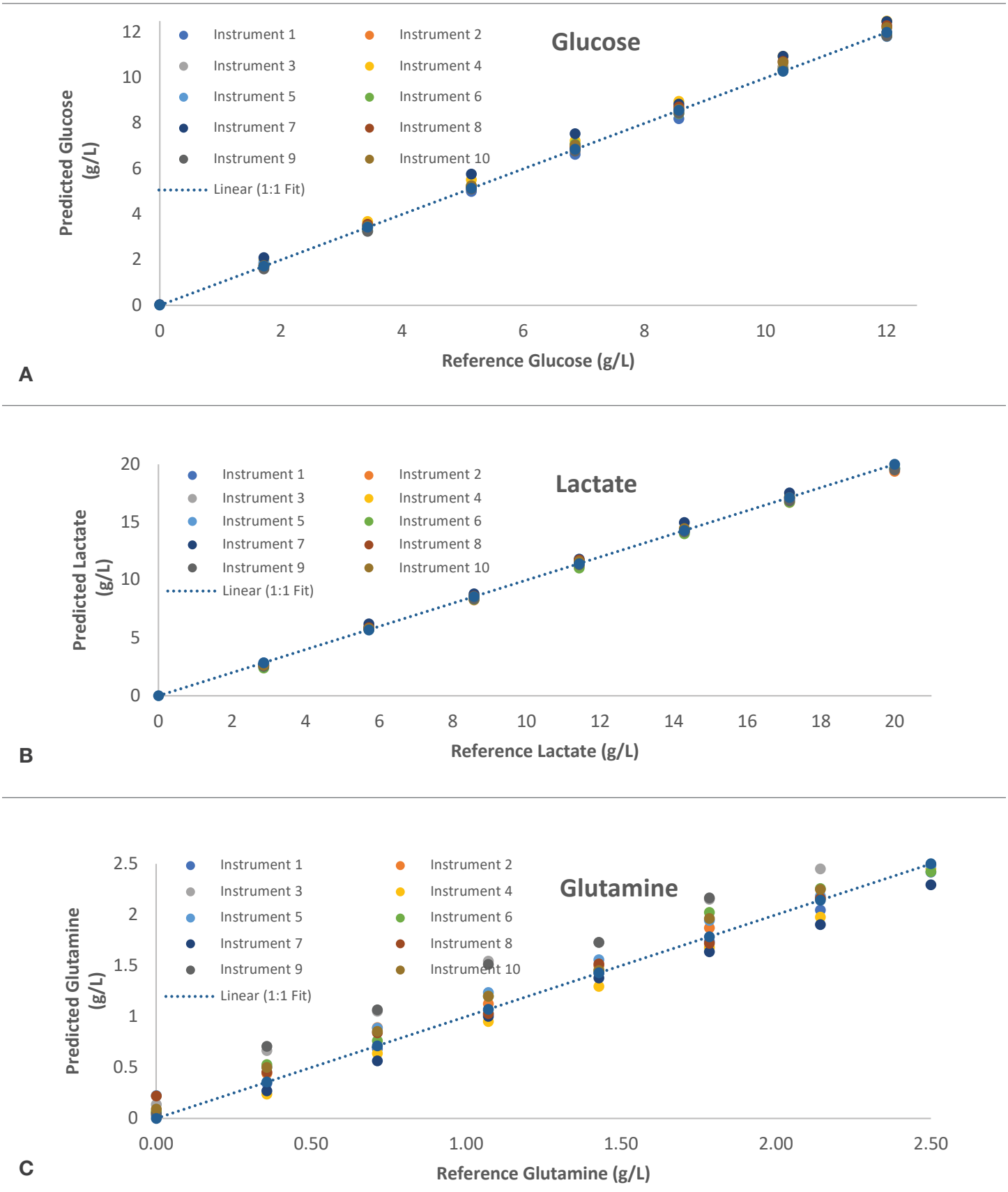

Figure 1. Prediction correlation plot across 10 Thermo Scientific Ramina Process Analyzers.

Hardware	Average prediction error (g/L)		
	Glucose	Glutamine	Lactate
Instrument 1	0.18	0.05	0.20
Instrument 2	0.11	0.13	0.40
Instrument 3	0.12	0.15	0.21
Instrument 4	0.18	0.14	0.26
Instrument 5	0.18	0.08	0.40
Instrument 6	0.40	0.11	0.25
Instrument 7	0.13	0.38	0.32
Instrument 8	0.21	0.11	0.26
Instrument 9	0.47	0.15	0.44
Instrument 10	0.09	0.34	0.23
Average error (across 10 systems)	0.21	0.16	0.30

Table 2. Prediction error (RMSEP) calculated from Chemometric modeling.

Table 2 shows the average prediction error for each analyte on each Thermo Scientific Ramina Process Analyzer and the average error for each analyte across all 10 Thermo Scientific Ramina Process Analyzers. All parameters demonstrate a high degree of measurement accuracy with <0.5 g/L prediction error. Furthermore, in some cases, the prediction error is <0.1 g/L. This level of accuracy is in line with other published results for chemometric modeling[3] and is on par with other relevant measurement techniques for bioreactor monitoring.

With this accuracy and precision, controlling glucose concentrations within a bioreactor in the typical 2-4 g/L range can be readily achieved. Furthermore, through continuous monitoring and feedback utilization, even tighter control is possible leading to improved process and product consistency. Chemometric models developed using Thermo Scientific Ramina Process Analyzer have demonstrated exceptional performance for multi-analyte monitoring in full-scale bioreactor studies. (Real time metabolite monitoring using the Thermo Scientific™ Ramina™ Process Analyzer System and the Thermo Scientific™ 500L HyPerforma™ Dynadrive™ SingleUse Bioreactor (S.U.B.) http://assets.thermofisher.com/TFS-Assets/CAD/Application-Notes/real-time-ramina-app-note.pdf

Conclusion

Customers will benefit from generating chemometric models which are transferrable between multiple Thermo Scientific Ramina Process Analyzers. The ability of the Thermo Scientific Ramina Process Analyzer to utilize the same chemometric model across multiple units provides users with the confidence of measurement accuracy and precision. Advanced signal processing and model optimization may be employed to further increase the level of prediction performance. This example simply provides a benchmarking reference for the development of chemometric models using Thermo Scientific Ramina Process Analyzers.

References

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