

PHAZIR™ and SpectralProbe™

Determination of Ethanol in Wine



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Abstract

Polychromix offers self-contained spectrometers that can be rapidly deployed for at-line analysis of process parameters. While the procedures described below have been developed for the SpectralProbe POD™, the principles apply to any NIR spectrometer offered by Polychromix. The goals of this paper are to demonstrate the capability of quantitatively measuring ethanol in wine using within an acceptable accuracy and repeatability.

Introduction

Ethanol content is one of the most important process parameters in ongoing monitoring of fermentation processes of alcoholic beverages. Current techniques to measure ethanol content in fermented beverages, such as wine include HPLC, GC, refractometry and ebullimetry. Spectroscopic techniques such as NIR offer a rapid and accurate alternative to these more time-consuming or expensive methods. Samples can be determined directly at-line, with minimal sample processing.

Partial Least Squares (PLS) is a method commonly used in the NIR community to correlate values with spectral changes in the spectrum. Because it is a correlation method, the resulting accuracy depends upon low-noise spectra, representative samples and on the accuracy of the reference method.

In the following discussion, a method is presented to determine the added ethanol content in a white wine. PLS models were developed based on spectra obtained using NIR instruments capable of being part of an at-line process. Two instruments were evaluated for their predictive ability by reporting the accuracy and repeatability of resulting PLS models.

Experimental

PHAZIR M1624

Increasing aliquots of ethanol were added to a white wine matrix over a range of 2-20% (w/w), in 40 mL glass vials. Samples for NIR testing were then transferred to 1 mm path length cuvettes, and spectra were collected using the PHAZIR M1624. Spectra were collected in triplicate for two positions of the cuvettes. The spectral range for the PHAZIR M1624 is from 1595-1624 nm, but the effective wavelength range used for model development was limited to 1619-1821 nm. Spectral evaluation and PLS model development was done using PHAZIR Method Generator (PHAZIR MG).

Sample Preparation

Samples were made up of varying ratios of ethanol to the white wine matrix. A total of 10 samples were made for the training data set, by adding from 0 to 20% w/w ethanol. The wine was added directly to the 40 mL borosilicate glass vials and weighed on an analytical balance. Then increasing amounts of ethanol were added to the wine samples, and weight added of ethanol was recorded. Weights were recorded to the nearest 0.1 mg. The calibration values are shown in Table 1. A sample was pipetted from each vial into the 1 mm path length cuvette for NIR measurement.

Sample	%EtOH (w/w)
1	0
2	2.48
3	5.11
4	6.53
5	9.88
6	11.79
7	14.82
8	15.92
9	17.75
10	20.33

Table 1 Experimental design.

Spectra were taken using both the **SpectralProbe™**, and the **miniSpectralProbe™**. The spectrometers were stabilized for 60 minutes and wavelength calibrated before spectra were collected. The reference spectrum is a 15-scan average of an empty cell (no sample or cuvette present). Each sample spectrum is the result of 8 scans through the cuvette. The spectra were taken in triplicate with both orientations of the cuvette. This resulted in a minimum of 6 spectra for each sample. Spectral data were collected using the POD Windows Utility program (POD Mon), the software integrated with the SpectralProbe POD.

Materials

- 95% Ethanol solution (ENG Scientific, Lot# 707060)
- PCX white wine (made from kit)
- 1 mm path length rectangular quartz cells with screw caps (New Era Enterprises, Inc.)
- 40 mL pre-cleaned EPA vials with Teflon septa.

Equipment

- SpectralProbe POD M1624
- SpectralProbe POD mini1624
- POD Windows Utility program (POD Mon)
- PHAZIR Method Generator™ v. 1.5.7

Results and Discussion

Spectra were collected simultaneously on the SpectralProbe POD and the SpectralProbe miniPOD. Discussion of the results presented will show results from the miniPOD, but the results obtained from the larger unit are similar.

In either case, the spectra were collected from samples (Table 1) in triplicate using the POD Mon software. The resulting spectra were imported into PHAZIR MG for analysis and model development.

A subset of the spectra generated is shown in Figure 1. The absorption bands between 1619-1821 nm were found to be relevant for PLS model development. Note that the strength of the 1693 nm absorption band increases with ethanol content.

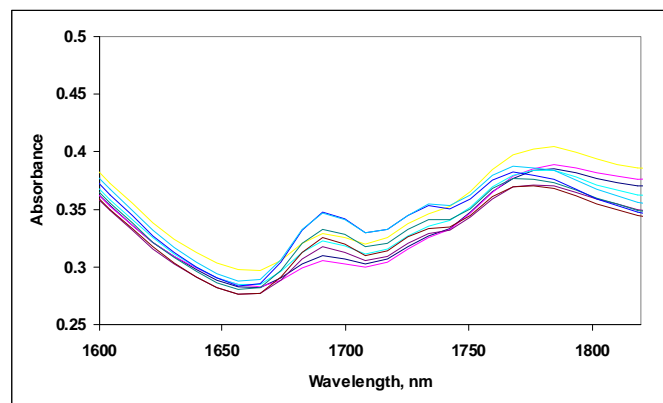


Figure 1 Spectra of a subset of samples showing the relevant wavelength range for PLS model development.

Frequently, spectra are manipulated to remove variations that are not related to the property of interest, such as wide and overlapping bands in the NIR, or particle size and packing differences. These additional preprocessing conditions were optimized to improve separation between the different quantitation levels, and to minimize PLS prediction errors. In this study, no preprocessing was required, except to truncate the applicable wavelength range from 1619-1821 nm. This eliminated the overwhelming water peak at 1940 nm, where absorbance values were too high to obtain good signal. Peaks present between 2174- 2371 nm were also observed, but did not improve the calibration model when included.

A 3 factor PLS model was then developed from the spectral data. Leave-five-out cross-validation using PHAZIR MG resulted in root-mean square error (RMSE) for the training data set of 0.09 %ethanol, and a correlation (R^2) of 0.999 for the miniPOD, and root-mean square error (RMSE) for the training data set of 0.11% w/w ethanol, and a correlation (R^2) of 0.999 for the SpectralProbe POD. These errors are comparable or better with

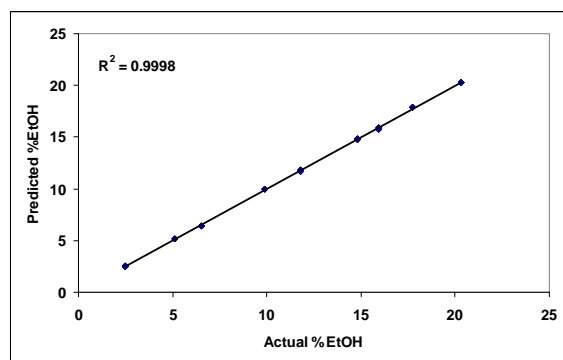


Figure 2 Predicted versus added %ethanol for mini POD data. RMSE of cross validation= 0.09% w/w

other published methods.

Unit	Factors in PLS model	RMSECV, %w/w	Repeatability SD, %w/w
SpectralProbe POD	3	0.11	0.01
Mini POD	3	0.09	0.01

Table 2 Results obtained for both units. Effective wavelength range for PLS models was 1619-1821 nm.

Note that the results shown in Table 2 are similar for both POD units. Repeatability on the replicate measurements was similar at 0.01% ethanol (w/w). These results compare favorably with published results on traditional laboratory benchtop systems.

Competitive Comparison

A similar study was performed by Thermo-Nicolet on ethanol in wine using the Antaris FT-NIR. Ethanol was determined over concentrations of 4-27%. A 4-factor PLS model was generated from 2nd derivative preprocessed data, and gave a RMSECV of 0.26% with an R^2 of 0.99.

Anton Paar performed a similar study of their Alcolyzer Wine. They report that concentration of 0-20 %v/v ethanol can be determined with an accuracy of 0.1% v/v. Precision for this instrument of 0.01 %v/v at 1 standard deviation.

Conclusions

Accurate and precise calibrations for ethanol in wine can be reliably made using either of the POD units. The RMSE of calibrations are similar and produce robust PLS calibrations for added ethanol between 2 and 20% w/w. Accuracy is comparable between the units, as well as comparable to, if not better, than other published methods. As well the POD units have the added advantage of rapid assessment in prediction.

These calibrations are most likely to be used for initial assessment of ethanol from multiple containers or batch processes where multiple samples need to be taken to monitor a fermentation process. The combination of the POD system and NIR has the advantage of precision and short analysis time for system control.