Enhancing the Flexibility of the NGC™ Chromatography System: Addition of a Refractive Index Detector for Wine Sample Analysis

Tech Note

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Sugar and Organic Acid Analysis

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Abstract

In this study, we present a chromatographic workflow for the quantitation of wine constituents using a Bio-Rad NGC Chromatography System equipped with an Aminex® HPX-87H HPLC Column. Chromatographic identification and quantitation of compounds in food substances often requires simultaneous monitoring of multiple optical signals, such as UV/Vis, refractive index, fluorescence, or light scattering. We demonstrate the ability of the NGC System to effectively integrate an external refractive index (RI) detector, in addition to the standard multi-wavelength UV detector that comes with the system. The integration of an RI detector can significantly expand the capabilities of the NGC System and could facilitate a wider adoption of chromatographic workflows in the food and biofuel industries.

Introduction

This study presents an application of external detector analysis relevant to the food and beverage industry, specifically the monitoring of fermentation kinetics and maturation of wines. During the fermentation process, the sugar (fructose) content of wine progressively decreases with its conversion to ethanol. In addition, determination of the levels of lactic acid permits monitoring of its conversion from malic acid, which is an important step in the fermentation process, as it allows the introduction of distinctive flavor characteristics to the product. Hence, analyses of wine constituents are routinely performed to ensure the quality of the final product.

We have developed a chromatographic workflow for the analysis of wine constituents using the Aminex HPX-87H Column (mixed-mode/ion exclusion) and a combination of UV and RI detectors. In order to optimize component separation, we carried out a sample cleanup prior to analysis using activated charcoal to remove background interference from factors such as tannins. The amounts of fructose and lactic acid eluted from the Aminex Column were monitored using the NGC Multi-Wavelength Detector at 190 and 210 nm, respectively, while the amounts of ethanol were monitored using an external RI detector.

Using the NGC System in conjunction with an external RI detector, we generated a sugar, organic acid, and ethanol profile of four different wine samples. Comparison of each of the wine constituents to standard enzymatic assays demonstrated near equivalent performance of our chromatographic workflow.

Materials and Methods

System

Chromatography was performed using an NGC Quest™ Plus System equipped with F10 gradient pumps, an inject valve, and a multi-wavelength detector connected in series with an external RI detector (Figure 1). The RI detector signal output was transmitted to the system using the NGC Signal Import Module (SIM). Data were analyzed using Bio-Rad's ChromLab™ 2.0 Software.

Sample volumes of 25 µl were injected onto an Aminex HPX-87H HPLC Column (300 × 7.8 mm) maintained at 65°C with a ThermaSphere TS-130 Column Heater (Phenomenex, Inc., Torrance, CA). The samples were isocratically eluted with 5.0 mM sulfuric acid at a flow rate of 0.6 ml/min. Eluates were monitored using the integrated multi-wavelength UV (190 and 210 nm) and external RI detectors.

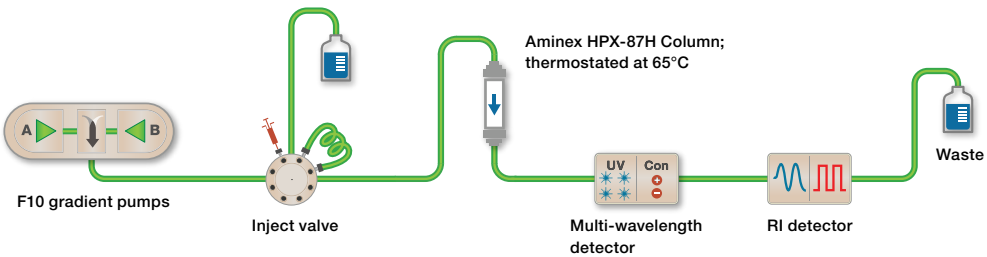

Fig. 1. Schematic of the NGC Quest System, Aminex Column with column heater, and UV multi-wavelength and RI detectors in series.

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Sugar and Organic Acid Reference Solutions

Reference solutions for fructose and lactic acid were prepared in deionized water and analyzed to generate three-point calibration curves. The concentration of lactic acid ranged from 0.5 mg/ml to 2.0 mg/ml, and the concentration of fructose ranged from 1.0 mg/ml to 25.0 mg/ml. The reference solutions were analyzed individually to determine the retention times for each compound across a concentration range relevant for wine samples.

Wine Samples

The fructose and organic acid composition of four varieties of wine, varying in sugar, organic acid, and ethanol content, was analyzed.

Table 1. Wine samples analyzed for fructose and organic acid composition.

Wine Sample	Characteristics
Cabernet Sauvignon	Primary fermentation
Petite Syrah	Secondary fermentation
Chardonnay	Stuck fermentation
Sonoma Sangiovese	Complete fermentation

Sample Treatment

To remove background interference from tannins and phenolic compounds, powdered activated charcoal was added to the wine samples to decolorize them. The samples were incubated at room temperature overnight, with constant stirring. The charcoal was removed by centrifugation.

Peak Identification and Concentration Determination

Data for individual wine samples were evaluated using ChromLab Software. Solutions of fructose and lactic acid were used to generate chromatograms to facilitate peak identification of wine samples by relative retention times. Peak integration for fructose was performed on the 190 nm and RI chromatograms and for lactic acid on the 210 nm chromatogram. The areas under the relevant peaks were quantitated and the three-point reference curves were used to calculate the concentration of each component.

Results and Discussion

Sample Treatment

Wine contains tannins and phenolic compounds, which contribute to high background interference and poor resolution of fructose and organic acids. Consequently, it was necessary to minimize the presence of these compounds prior to analysis. Treatment with activated charcoal prior to sample injection was found to sufficiently lower background and improve resolution to permit quantitation (Figure 2).

Determination of Analysis Wavelengths

Since fructose and lactic acid absorb strongly at 190 nm and 210 nm, respectively, these wavelengths were selected for quantitation. Furthermore, ethanol provided a strong signal in the RI trace (Figure 3), which potentially could be used for further quantitation.

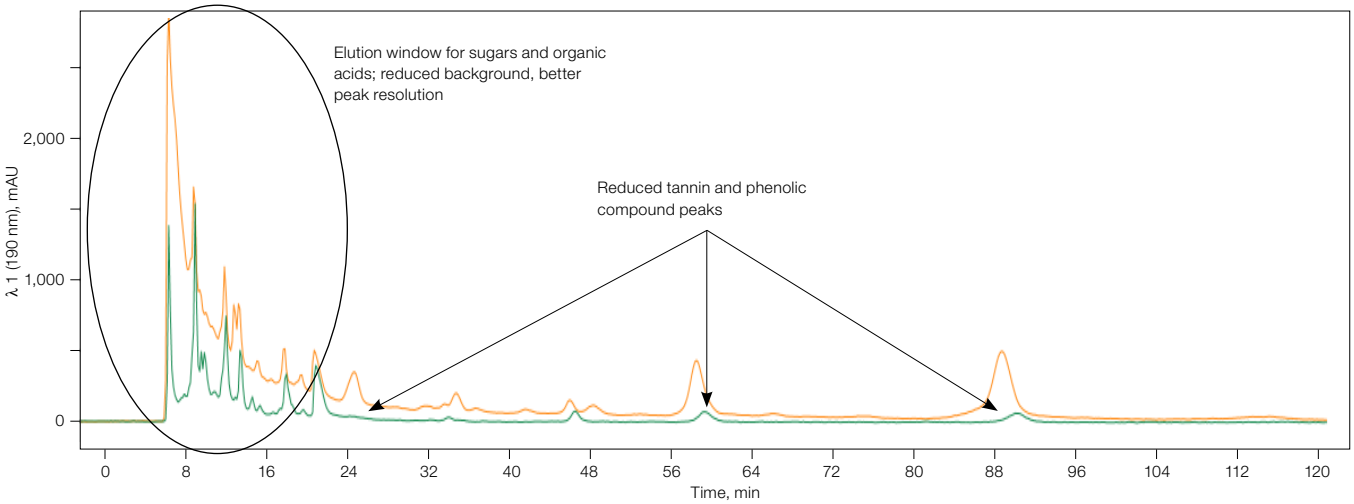


Fig. 2. Treatment of a sample with activated charcoal to remove background interference. Comparison of an untreated wine sample (—) and the sample treated with activated charcoal (—) at 190 nm.

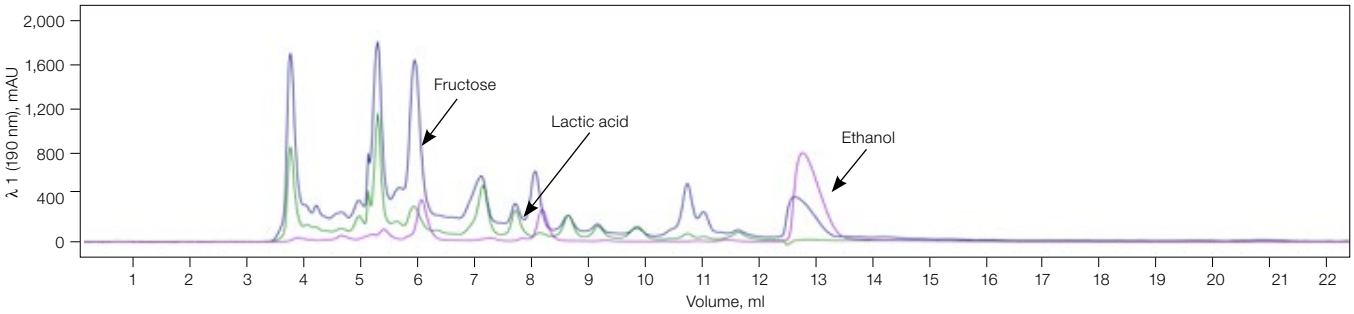


Fig. 3. Chromatogram of a primary fermentation sample. Fructose, 190 nm trace (—); lactic acid, 210 nm trace (—); ethanol, RI detector trace (—).

Linearity of the Reference Solutions

In order to determine the concentrations of fructose and lactic acid, it was necessary to construct corresponding calibration curves for each component of interest using reference solutions. Calibration curve linearity was validated at three different concentrations for each sample type. The concentrations of the reference solutions chosen covered the entire concentration range of each component in the samples investigated (Table 2). A calibration curve for each component was constructed by linear regression of the observed peak area vs. concentration (Figure 4).

Table 2. Concentration range of reference solutions.

Component	Detection	Range, mg/ml	Slope	R^2
Fructose	UV (190 nm)	1–25	20.352	0.9853
	RI	1–25	7.9174	0.9998
Lactic acid	UV (210 nm)	0.5–2	42.669	0.9983
	RI	0.5–2	5.177	0.9995

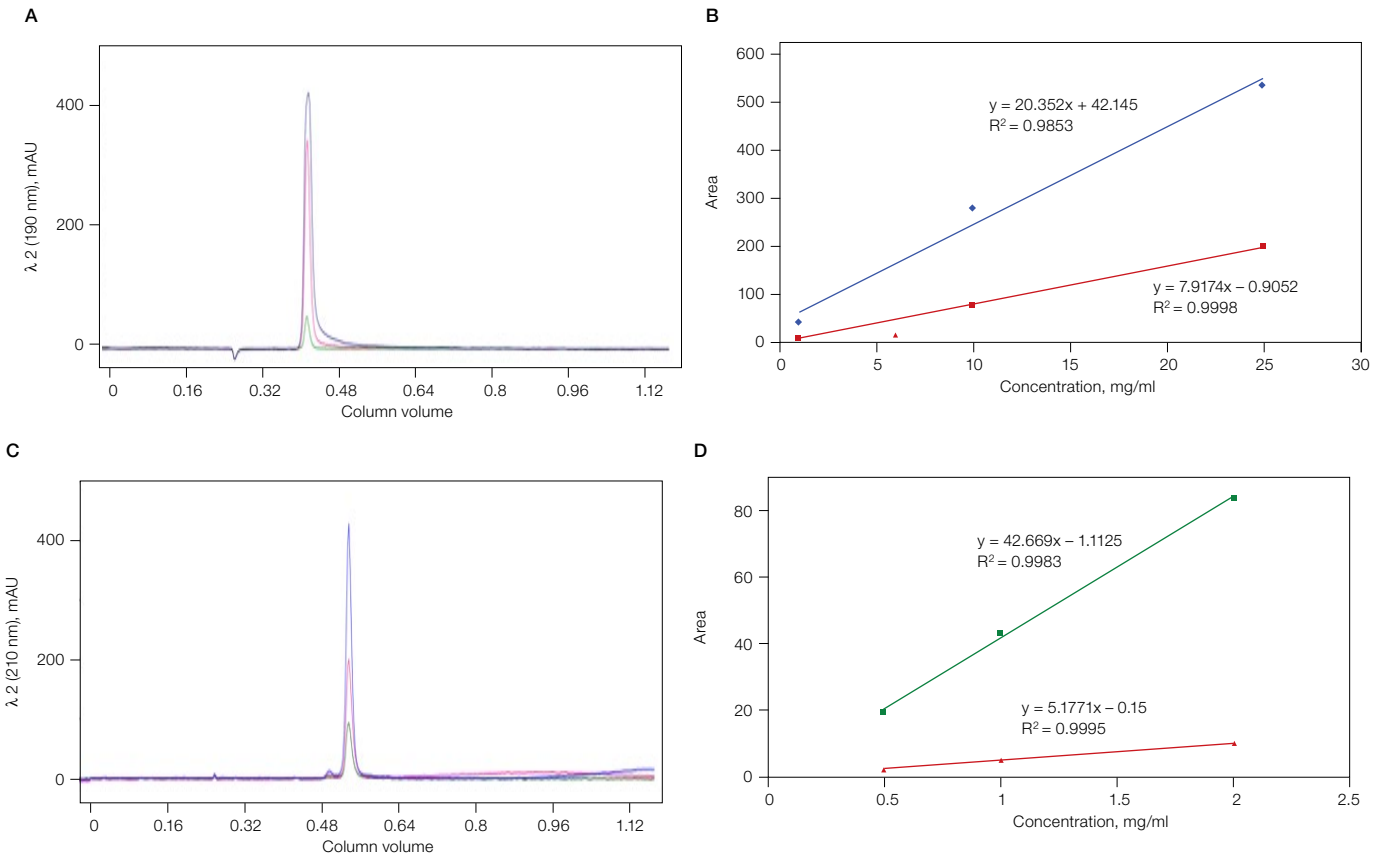


Fig. 4. Linearity of the reference solutions. A, overlay chromatograms of fructose (190 nm) at 25 mg/ml (—), 10 mg/ml (—), and 1 mg/ml (—) concentrations; **B**, fructose calibration curves for the 190 nm (—) and RI (—) traces; **C**, overlay chromatograms of lactic acid (210 nm) at 2 mg/ml (—), 1 mg/ml (—), and 0.5 mg/ml (—) concentrations; **D**, lactic acid calibration curve for the 210 nm (—) and RI (—) traces.

The coefficient of the regression curves (the slope) and the squares of the correlation coefficient (R^2) were calculated from plots of the reference solutions. Calibration curves were linear for all components investigated.

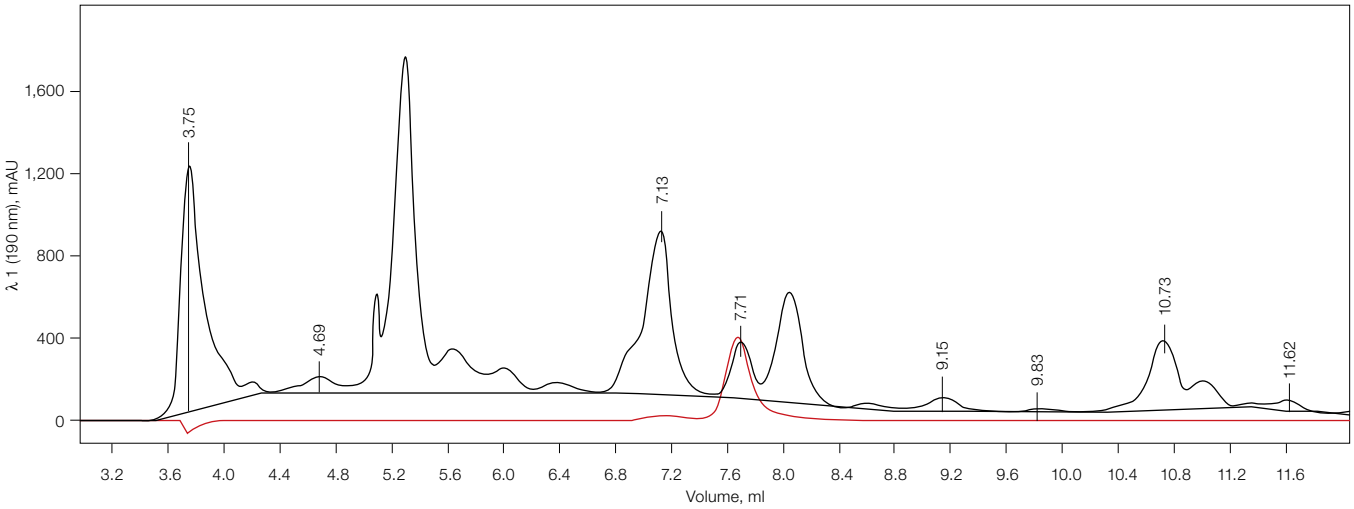
Peak Identification and Concentration Calculations

The chromatograms of the reference solutions were overlaid with those of wine samples to identify the fructose and lactic acid peaks (Figure 5A). Peak areas corresponding to the analytes of interest were calculated using the peak integration evaluation function in ChromLab Software (Figure 5B). The reference solution calibration curves were then used to determine the concentrations of each component (Table 3).

Comparison of NGC Data with Enzymatic Evaluation

The concentration of major sugars and organic acids in wines can be analyzed by standard enzymatic assays. Enzymatic assays of the wine samples in this study were performed by ETS Laboratories (St. Helena, CA). The concentration of sugars and acids measured in this study using the NGC System with external RI detector showed excellent agreement with the values provided by ETS.

A



B

Add Run... | Remove Run... | Save Analysis | Stack | Overlay | Peak Integration | Annotate

Runs/Traces | Peaks

Run: 2013_Cabernet Sauvignon_Primary Fermentation_2, Type: λ 1 (190 nm)

Peak Number	Start (ml)	End (ml)	Retention Time (min)	Retention Volume (ml)	Height (mAU)	Area (ml*mAU)	Relative Area (%)	Width at Half Height (ml)	Peak Asymmetry	Fractions
1	4.40	4.91	7.81	4.69	72.97	19.43	2.58	0.21	0.84	
2	4.96	5.52	8.82	5.29	1665.93	324.24	43.12	0.16	0.77	
3	5.57	5.85	9.39	5.63	210.46	45.40	6.04	0.23	3.46	
4	5.90	6.22	10.01	6.01	117.80	24.84	3.30	0.21	1.90	
5	6.27	6.69	10.63	6.38	35.03	7.47	0.99	0.21	2.61	
6	6.74	7.35	11.87	7.13	800.20	190.78	25.37	0.19	0.60	
7	7.53	7.87	12.85	7.71	254.69	44.05	5.86	0.16	1.18	
8	7.95	8.33	13.42	8.05	525.70	95.69	12.73	0.18	1.79	

Fig. 5. Peak integration evaluation in ChromLab Software. A, overlay chromatograms of a reference solution (—) and primary fermentation wine sample (—); **B**, peak integration data using ChromLab Software. Highlighted box represents the calculated peak areas.

Table 3. Comparison of the NGC System and standard enzymatic assay data.

Wine Sample	Component	NGC System Concentration, mg/ml	Enzymatic Assay Concentration, mg/ml
Cabernet Sauvignon (primary fermentation)	Fructose	0.209	0.2
	Lactic acid	0.99	0.98
Petite Syrah (secondary fermentation)	Fructose	9.49	9.4
	Lactic acid	0.89	0.98
Chardonnay (stuck fermentation)	Fructose	24.44	25.4
	Lactic acid	—	<0.05
Sonoma Sangiovese (complete fermentation)	Fructose	0.4	0.4
	Lactic acid	—	<0.05

—, too low to detect.

External RI Detector

The NGC System was integrated with an external RI detector to evaluate the concentration of fructose in two of the wine samples tested (Table 4). The analog signal output of the RI detector was converted to a digital input and transmitted to the system using the NGC SIM. The data were evaluated using ChromLab Software (Figure 6). The concentration of fructose measured was found to be comparable to that determined by UV (NGC) and enzymatic (ETS) quantitation, providing further confirmation of our analysis.

Table 4. Concentration of fructose evaluated by the RI detector.

Wine Sample	Component	RI Detector (with NGC System), mg/ml	Enzymatic Assay, mg/ml
Petite Syrah (secondary fermentation)	Fructose	9.56	9.4
Chardonnay (stuck fermentation)	Fructose	24.15	25.4

Conclusion

The NGC Systems offer a versatile and expandable platform for chromatography that can be configured for the separation and analysis of complex samples. We have demonstrated the ability of the NGC Quest Plus Chromatography System to utilize an Aminex Column to reliably evaluate the concentrations of fructose and lactic acid in wine samples. The proposed workflow was rapid and provided quantitative results consistent with those determined by standard enzymatic methods. A wide variety of food- and biofuel-related analyses could be performed using chromatographic separation, taking advantage of ChromLab Software for the precise quantitation of analytes. Furthermore, analysis of samples requiring multiple spectroscopic measurements can benefit from the ability of the NGC System to integrate external detectors.

Acknowledgements

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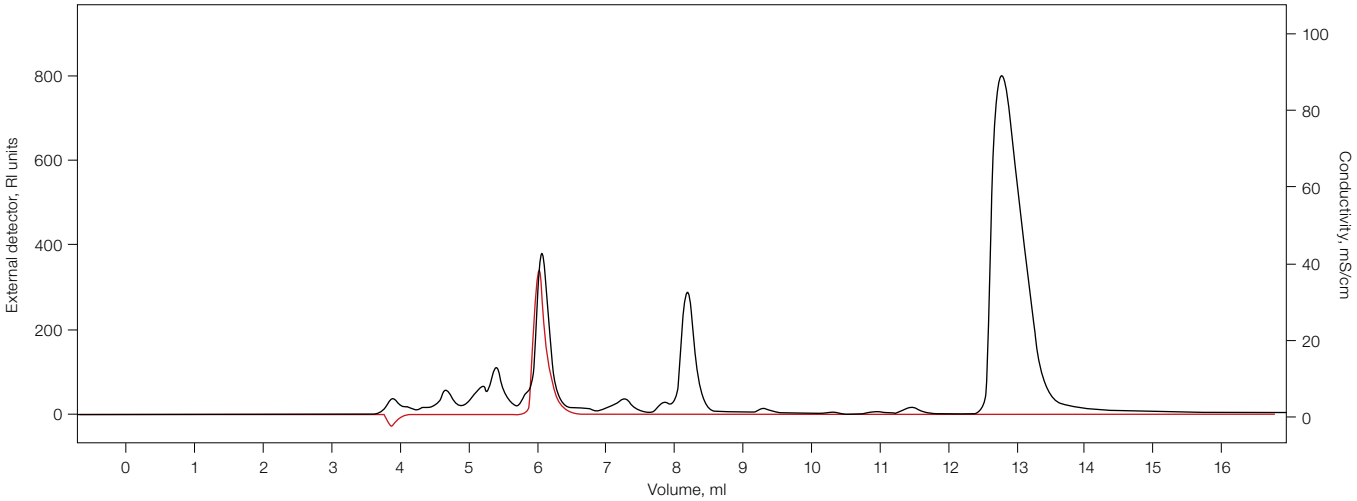

Fig. 6. ChromLab Software evaluation of RI detector data. Overlay chromatograms of a reference fructose solution (—) and secondary fermentation wine sample (—).

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