Rapid Analysis of Sugars Using Discrete Analyzers

Mari Kiviluoma, Leena Kaski, Sari Hartikainen, Leala Thomas and Annu Suoniemi-Kähärä*
*Author, corresponding address annu.suoniemi-kähärä@thermofisher.com

Poster Note 71752

Introduction

Thermo Scientific™ Gallery™ and Thermo Scientific™ Arena™ analyzers can automatically measure several sugars from a wide range of sample types. Typical sample materials include fruit juices, wine and must, and other fermentation process samples. These measurements are performed as part of process control, during raw material inspection, as a quality control measurement, or as a measurement to support label claims.

The reason laboratories have chosen automated methods are ease-of-use, specificity, and optimized applications in which very low volumes of reagents are used resulting in a low cost per test. Discrete cell technology offers faster, reproducible results since multiple tests can be done on a single sample without the need for extra method changeover time. Most homogenous liquid samples can be measured without pretreatment. All necessary analysis steps are automated, providing true walk-away time for the operator.

Thermo Scientific ready-to-use system reagents are available for D-Glucose, D-Fructose, D-Glucose + D-Fructose, D-Glucose + D-Fructose + Sucrose, Sucrose (Total Glucose) and Lactose (Glucose). In addition, Thermo Scientific discrete analyzers are designed to adapt to the laboratory's own applications.

Materials and Methods

Thermo Scientific system reagents are ready-to-use liquids where reagent volumes are optimized for the application and can complete up to 350 tests/ kit. All reagent vials are bar-coded containing information for the material lot and expiration date. The instrument will sound an alarm when the reagent is almost finished and offers automatic calibration when a new reagent vial is added. In addition to reagent on-board stability, the instrument has the capability to trace reagents linked with long term storage of results, associated calibrations, and reagent lot data.

Calibrator and Control Samples

Thermo Scientific calibration solutions are also ready-to-use. A sugar combination standard can be used for Glucose, Fructose, and Total Glucose calibrations. A separate standard for Lactose is available.

Instruments and Applications

Thermo Scientific Gallery and Gallery Plus instruments are pre-programmed with enzymatic sugar methods in the analyzer's application library.

In sugar analysis, each reagent is always dispensed with extra reagent to ensure contamination free analysis. Samples are also dispensed with extra sample which is discarded to ensure accurate and contamination free dispensing. All methods are performed at 37 °C and reaction end products are measured at 340 nm using an additional 600 nm side wavelength for verification. Applications are designed to use a 1:50 pre-dilution which minimizes the matrix effect. Since these analyzers allow the insertion of up to 4 reagents per test, complicated methods can be automated, such as that used for D-Glucose + D-Fructose + Sucrose analysis. For Sucrose (Total Glucose) and Lactose (Glucose), a separate Glucose measurement is required to calculate the concentrations of sucrose and lactose. Calculations are done automatically by the instrument.

Measuring Range

Measuring ranges for each sugar kit are shown in Table 1. Applications are designed using automated dilutions to achieve these ranges.

TABLE 1. Measurement ranges for the sugar kits.

Kit	Test Limit Low, g/L	Test Limit High, g/L
D-Glucose	0.1	160
D-Frutose	0.7	200
D-Glucose+D-Fructose	0.4	200
D-Glucose+D-Fructose+Sucrose	0.24	200
Sucrose (Total Glucose)	0.1	100
Lactose (Glucose)	0.05	15

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Results and Discussions

Method Comparison Studies

Glucose, fructose, sucrose, and a combination of all sugar methods were compared against the HPLC method. Analyzed sample types ranged from dry white wines to sweet liqueurs. Glucose and fructose were measured from white, red, and rosé wines in parallel to the FTIR (Fourier transform infrared spectroscopy) technique. The number of samples and the associated concentrations used in the various comparison studies are shown in Table 2. Comparison graphs are shown in Figures 1-6. When concentrations are compared, an $r^2 = 0.984$ or better was obtained.

TABLE 2. The number of samples and their concentration range in method comparison studies.

Analyte	Samples in the HPLC Comparison	Concentration Range, g/L in HPLC Comparison	Samples in the FTIR Comparison	Concentration Range, g/L in FTIR Comparison
D-Glucose	450	0.0–105.0	120	0.04–27.5
D-Frutose	390	0.0–264.0	110	0.04–86.6
D-Glucose+D-Fructose+Sucrose	390	1.6–192.5		
Sucrose (Total Glucose)	0.1	100		

FIGURE 1. D-Glucose method comparison between discrete analysis (DA) and HPLC.

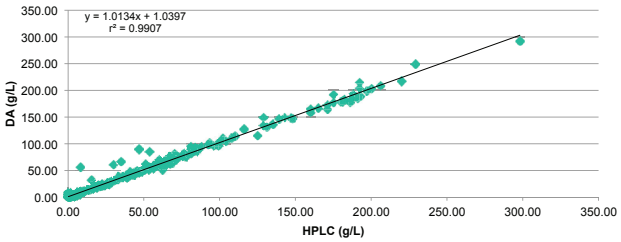


FIGURE 2. D-Glucose method comparison between discrete analysis (DA) and FTIR.

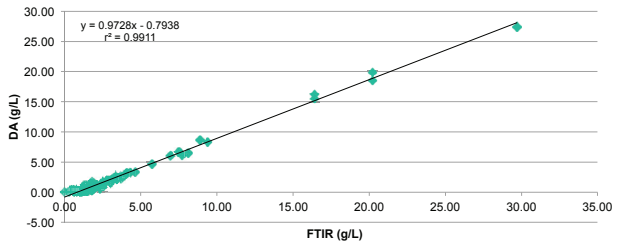


FIGURE 3. D-Fructose method comparison between discrete analysis (DA) and HPLC.

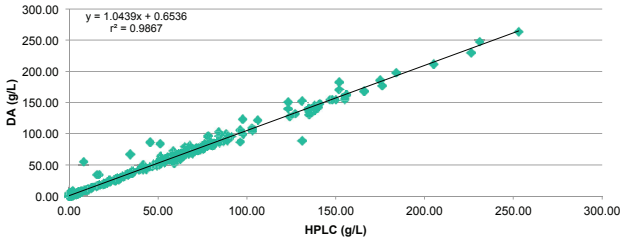


FIGURE 4. D-Fructose method comparison between discrete analysis (DA) and FTIR.

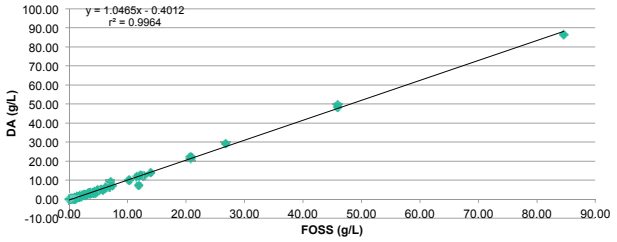


FIGURE 5. Glucose + Fructose + Sucrose method comparison between discrete analysis (DA) and HPLC.

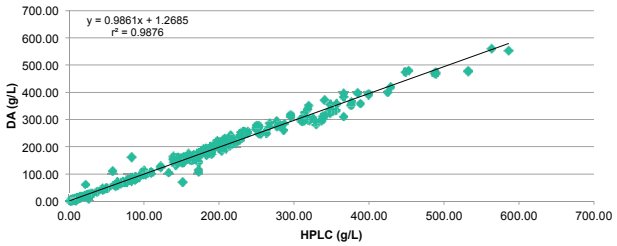
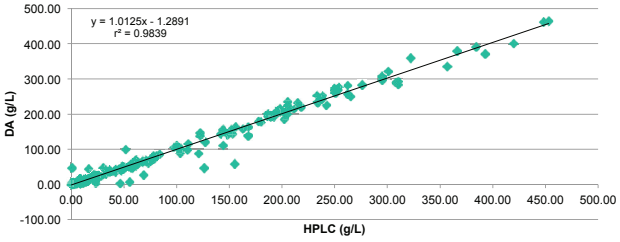


FIGURE 6. Sucrose (Total Glucose) method comparison between discrete analysis (DA) and HPLC.

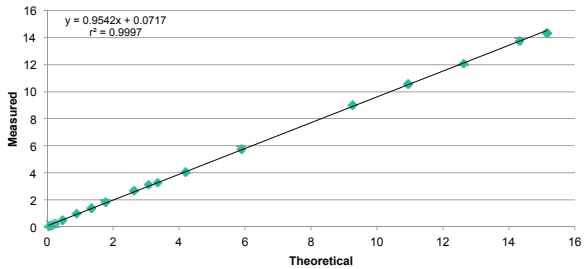


Linearity Performance and Calibration Data

Linearity performance data for lactose is shown in Figure 7. Data was primarily generated using water based solutions. A plot of the theoretical concentration vs. the measured concentration is shown.

For each method, a linear calibration fitting was chosen. The analyzer is designed with an automated calibrator dilution function, allowing the insertion of additional calibration points if required.

FIGURE 7. Lactose (Glucose) linearity performance data.



Precision Studies

Results of the precision studies are shown in Table 3. The method coefficient of variation (CV) is typically around 1% within a run and improves with higher concentrations. Total precision is typically under 2%.

Analysis Speed

The automated operating system allows laboratories to simultaneously measure multiple analytes while reducing total analysis time and increasing efficiency. Maximum capacity is dependent upon the analyzer model, varying from 200 photometric tests per hour with the Gallery analyzer to 350 photometric tests per hour with the Gallery Plus analyzer. For example, the Gallery Plus analyzer, is capable of performing 77 D-Glucose + D-Fructose + Sucrose tests per hour with first results available in less than 20 minutes.

Conclusion

Performance data from the enzymatic tests for D-Glucose, D-Fructose, D-Glucose + D-Fructose, D-Glucose + D-Fructose + Sucrose, Sucrose (Total Glucose) and Lactose (Glucose) is presented. Methods are fully automated using the Thermo Scientific Gallery and Arena discrete analyzers. The data shows that methods are accurate and repeatability is very good, for example, a within run CV as low as 0.5% can be achieved for D-Glucose analysis. The linearity of methods is wide due to the automated pre and post-dilution features of the analyzers. As shown within these studies, the automated discrete analyzer methods correlate well with the commercially available HPLC and FTIR methods. Reagents are ready-to-use, saving a technician's time and reducing errors. Volume optimized kit sizes and on-board stability minimize the amount of reagent used and result in an increase in cost efficiency. Multiple tests can be performed on a single sample, speeding up the analysis completed in laboratories. Once loaded, all steps of the analysis are fully automated. When testing D-Glucose + D-Fructose + Sucrose, first results are available in less than 20 minutes.

TABLE 3. Method precision data for the Gallery analyzers.

Kit	Gallery analyzer						
D-Glucose		Mean 12.5 g/L		Mean 34.6 g/L		Mean 69.4 g/L	
		SD	CV %	SD	CV %	SD	CV %
	Within run	0.101	0.8	0.253	0.7	0.371	0.5
	Between run	0.166	1.3	0.508	1.5	1.104	1.6
	Total	0.194	1.6	0.567	1.6	1.165	1.7
D-Fructose		Mean 8.0 g/L		Mean 37.7 g/L		Mean 79.0 g/L	
		SD	CV %	SD	CV %	SD	CV %
	Within run	0.094	1.2	0.367	1.0	0.452	0.6
	Between run	0.055	0.7	0.101	0.3	0.631	0.8
	Total	0.109	1.4	0.381	1.0	0.776	1.0
D-Glucose+ D-Fructose		Mean 5.46 g/L		Mean 44.17 g/L		Mean 72.57 g/L	
		SD	CV %	SD	CV %	SD	CV %
	Within run	0.042	0.8	0.417	0.9	0.419	0.6
	Between run	0.038	0.7	0.396	0.9	0.924	1.3
	Total	0.057	1.0	0.575	1.3	1.015	1.4
D-Glucose+ D-Fructose+ Sucrose		Mean 5.61 g/L		Mean 44.56 g/L		Mean 88.12 g/L	
		SD	CV %	SD	CV %	SD	CV %
	Within run	0.062	1.1	0.552	1.2	0.715	0.8
	Between run	0.067	1.2	0.473	1.1	2.287	2.6
	Total	0.091	1.6	0.727	1.6	2.396	2.7
Sucrose (Total Glucose)		Mean 1.91 g/L		Mean 25.11 g/L		Mean 45.10 g/L	
		SD	CV %	SD	CV %	SD	CV %
	Within run	0.031	1.6	0.345	1.4	0.301	0.7
	Between run	0.007	0.3	0.216	0.9	0.646	1.4
	Total	0.031	1.6	0.406	1.6	0.712	1.6
Lactose (Glucose)		Mean 0.30 g/L		Mean 0.93 g/L		Mean 2.30 g/L	
		SD	CV %	SD	CV %	SD	CV %
	Within run	0.004	1.5	0.009	1.0	0.015	0.6
	Between run	0.008	2.6	0.012	1.3	0.018	0.8

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