

# Smart Notes



## D-Glucose concentration in beverages

Changes in concentrations of carbohydrates such as glucose, lactose, and fructose can have a negative effect on the manufacturing process as well as product quality, nutrition, safety, and shelf life.<sup>1</sup> Of special concern is the concentration of D-glucose, which can impact those suffering from diabetes. These concerns highlight why D-glucose concentration testing is highly recommended by Title 21 of the US FDA Code of Federal Regulations.<sup>2</sup> Enzymatic methods utilizing UV-Vis measurements are well-suited to test D-glucose concentration because it is highly specific, provides reliable results, is easily run, and is cost-efficient.

### How do I determine D-Glucose concentration in beverages using UV-Vis spectrophotometry?

D-Glucose concentration in beverages can be measured by quantifying the absorbance of an enzymatic reaction at 560 nm. In the Glucose Colorimetric Detection Kit, glucose oxidase (GOx) reacts with D-glucose to produce hydrogen peroxide. In the presence of horseradish peroxidase (HRP), hydrogen peroxide reacts with a substrate to produce a colored product, which absorbs light at 560 nm. The Thermo Scientific™ GENESYS™ 150 includes Thermo Scientific™ VISION*lite*™ Software that provides users an easy method programming experience and guides you through data collection, data processing, and report printing.

#### Materials needed

GENESYS 150 UV-Vis Spectrophotometer with VISION*lite*, Glucose Colorimetric Detection Kit, and disposable plastic 10 mm cuvettes.



## Procedure

1. Follow the procedure in the Glucose Colorimetric Detection Kit to complete the enzyme assay. In short, fill the microcuvette with 80  $\mu\text{l}$  of standards/controls/samples, then add 100  $\mu\text{l}$  each of HRP, substrate and glucose oxidase solution and incubate for 30 minutes at room temperature.
2. Figure 1 shows how to make a new method for the glucose assay in the VISION<sup>lite</sup> Software. Select Quant and define the following parameters: measurement wavelength -560 nm; units - g/L; curve type - linear with intercept; and define your standard concentration range.
3. Run Autozero and select "Measure standards" in the upper portion of page. The program will prompt you to run the blank and then measure all the listed standard solutions. Save the new method for future use.
4. After generating the calibration curve, select the icon "Go to samples mode" and measure samples. The program will prompt you to run the blank and then measure all of the listed samples. If samples are diluted with assay buffer, then enter the dilution factor (DF) in a designated column in the sample list.

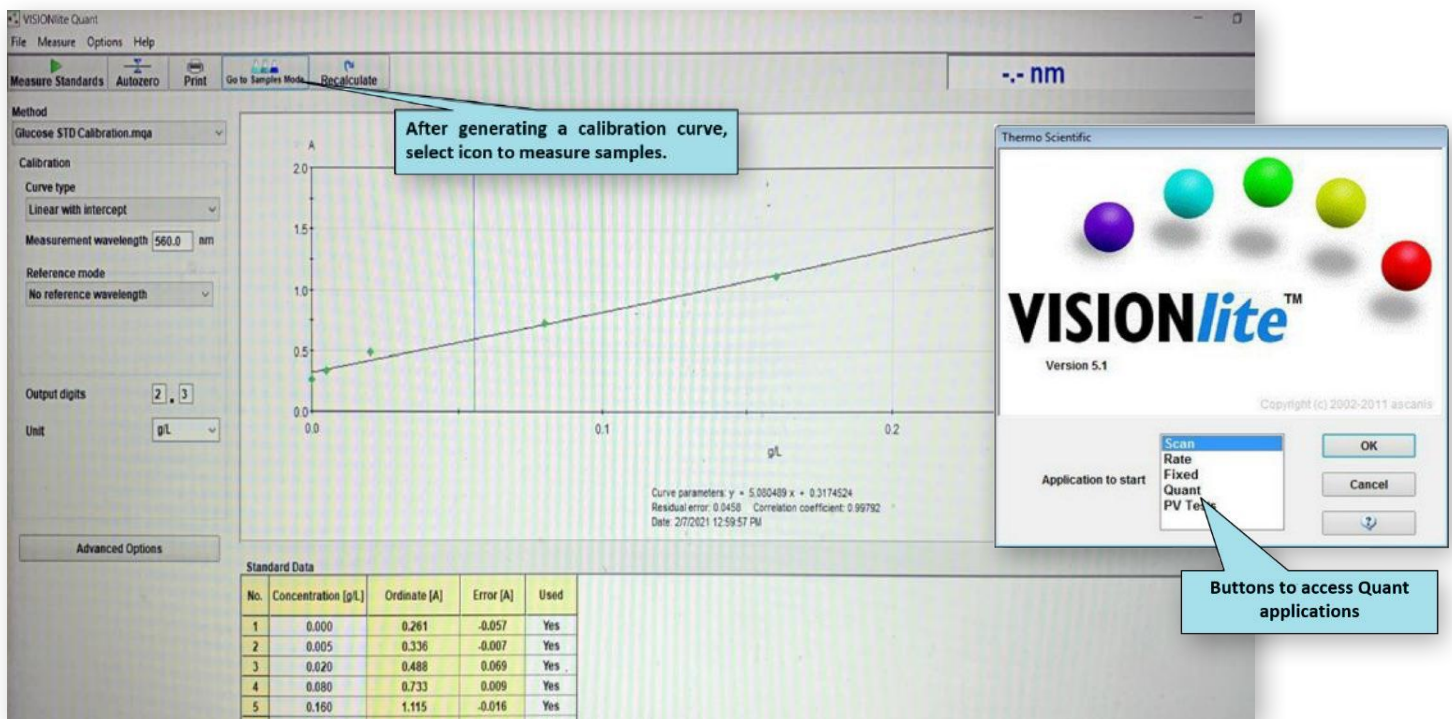


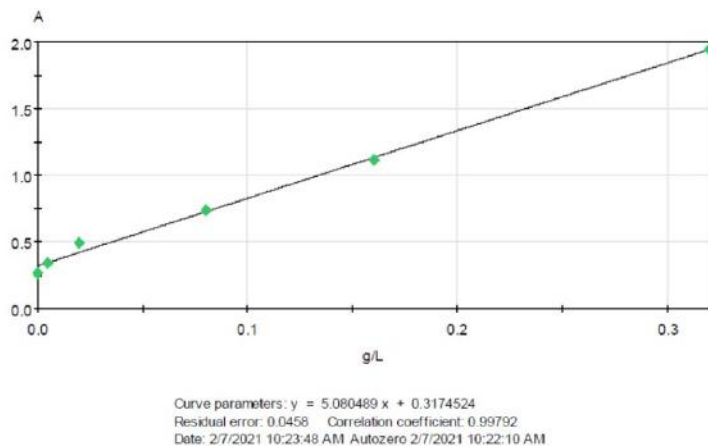
Figure 1. VISION<sup>lite</sup> guides to set up a new standard calibration in Quant method.

## Results

Figure 2 shows a linear calibration plot with a dynamic range 0.005 to 0.32 g L<sup>-1</sup> of D-glucose. Three Gatorade solutions with known concentrations of sugar were tested 63 g/L, 49 g/L, and 24 g/L. The sugar content in Gatorade is equal parts D-glucose and fructose, so we expect the enzyme assay to report the D-glucose concentration as half of the known sugar concentration. As expected, the VISION/ite method reported concentrations of 29.7 g/L, 25.2 g/L, and 13.2 g/L respectively. GENESYS 150 allows the user to identify and quantify D-glucose concentration sugar content present in beverages.

## References

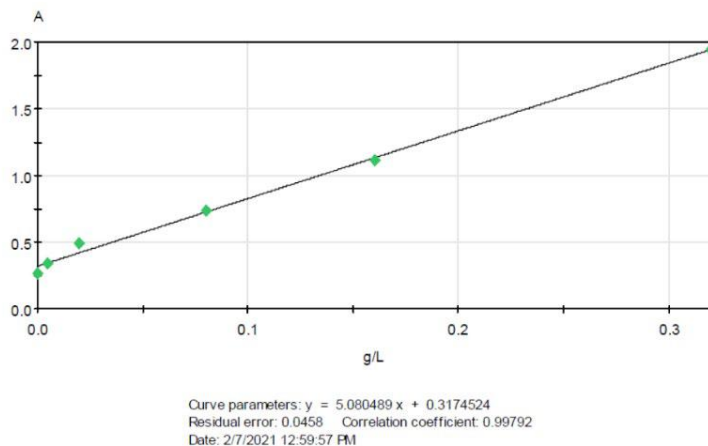
1. Goldfein, K.R. and Slavin, J.L. (2015), Why Sugar Is Added to Food: Food Science 101. COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY, 14: 644-656.
2. [https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=168.120&SearchTerm=glucose#:~:text=\(b\)%20The%20food%20shall%20meet,calculated%20on%20a%20dry%20basis](https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=168.120&SearchTerm=glucose#:~:text=(b)%20The%20food%20shall%20meet,calculated%20on%20a%20dry%20basis)



### Standards

No.	Concentration [g/L]	Ordinate [A]	Error [A]	Used
1	0.000	0.261	-0.057	Yes
2	0.005	0.336	-0.007	Yes
3	0.020	0.488	0.069	Yes
4	0.080	0.733	0.009	Yes
5	0.160	1.115	-0.016	Yes
6	0.320	1.945	0.001	Yes

Figure 2. Linear calibration plot with a dynamic range of 0.005 to 0.32 g L<sup>-1</sup> D-glucose.



Sample	Dilution Factor	Ordinate [A]	Concentration [g/L]
Gatorade 63g/L Sugar	450	0.653	29.721
Gatorade 24g/L Sugar	300	0.541	13.194
Gatorade 50g/L Sugar	450	0.602	25.204

Figure 3. Gatorade glucose level determination.



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