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Validation Report: D-Fructose/D-Glucose Assay Kit (cat. no. K-FRUGL)

1. Scope

Megazyme's D-Fructose/D-Glucose Assay Kit (K-FRUGL) is an enzymatic method used for the measurement and analysis of D-fructose and D-glucose in plant, food and beverage products. This method was developed in-house and measures both D-fructose and D-glucose in g/L. Methods based on this principle have been accepted by AOAC, EN, NEN, NF, DIN, GOST, OIV, IFU, AIJN, MEBAK and IOCCC.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by D-Fructose/D-Glucose Assay Kit (K-FRUGL).

3. Performance characteristics

The selectivity, working range, limit of detection, limit of quantification, trueness (*bias*) and precision of this kit is detailed in this report.

3.1. Selectivity

This assay is specific for D-glucose and D-fructose.

Interfering substances in the sample being analysed can be identified by including an internal standard. Quantitative recovery of this standard would be expected. Losses in sample handling and extraction are identified by performing recovery experiments, i.e. by adding D-glucose or D-fructose to the sample in the initial extraction steps.

3.2. Working Range

Assay follows the D-Fructose/D-Glucose Assay Kit (K-FRUGL) standard procedure.

0.1 mL of D-fructose and 0.1 mL D-glucose standard was used as sample, with a range of concentrations (0.04-0.8 g/L either sugars) which corresponds to 4-80 µg of either sugar per cuvette.

Absorbance A2 was taken 5 min after the addition of the 1st trigger enzyme (HK/G6P-DH), giving the measurement of D-glucose.

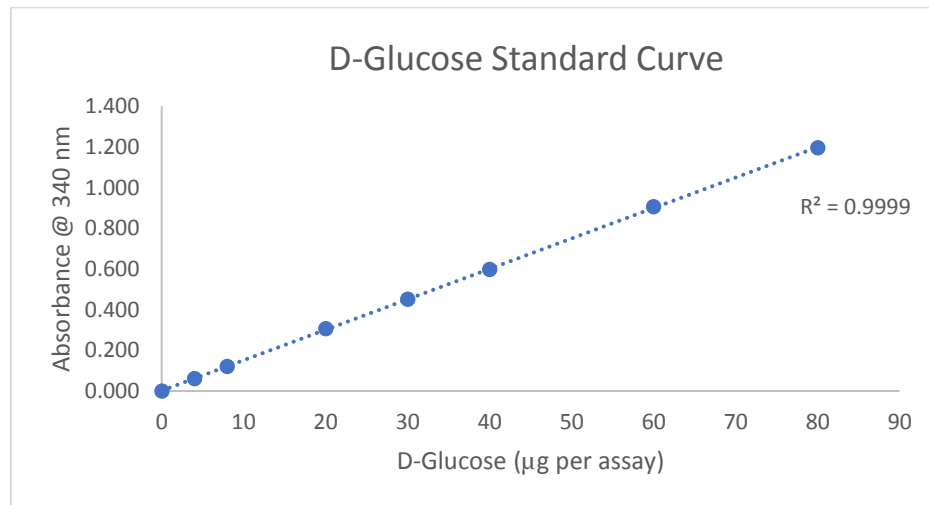
Absorbance A3 was taken 10 min after the addition of the final trigger enzyme (PGI), giving the measurement of D-fructose. Absorbances were read at 340 nm and 25°C as recommended in the procedure.



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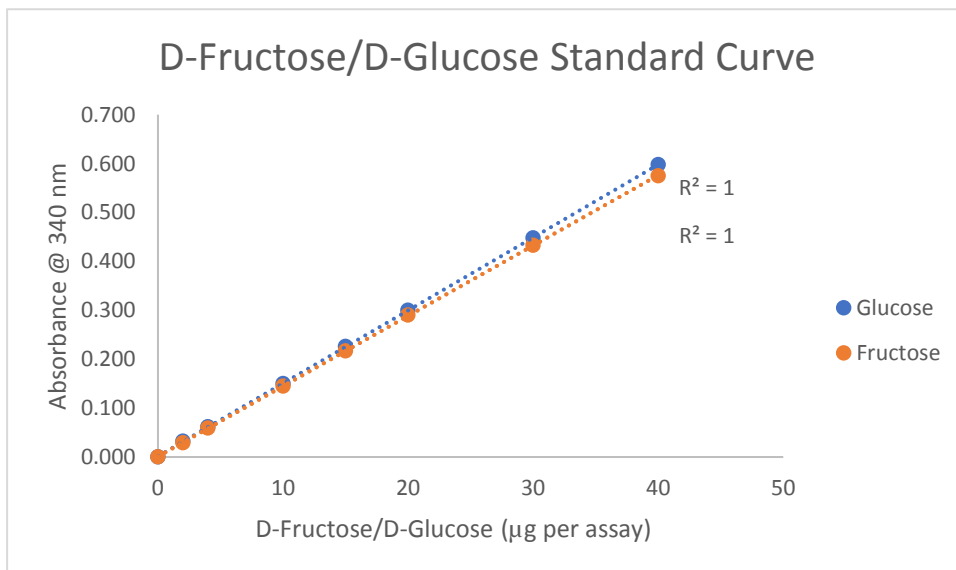
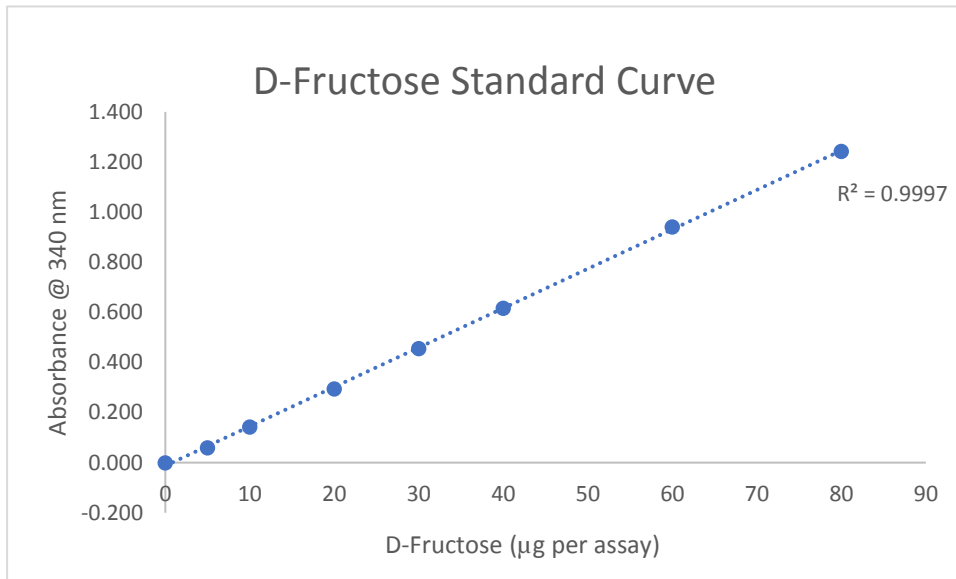
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D-Glucose concentration [µg/assay]	ΔA_{340nm}	D-Fructose concentration [µg/assay]	ΔA_{340nm}
0	0.000	0	0.000
4	0.061	5	0.060
8	0.121	10	0.143
20	0.306	20	0.295
30	0.451	40	0.456
40	0.597	50	0.617
60	0.906	60	0.941
80	1.195	80	1.242





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D-Fructose/D-Glucose mixed standard was also used as sample, with a range of concentrations (0.04-0.8 g/L total sugars) which corresponds to 4-80 µg of total sugars combined per cuvette.



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3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, is 0.663 mg/L, which is derived from an absorbance difference of 0.020 with the maximum sample volume of 2.00 mL.

The **calculated limit of detection (LOD)** and the **calculated limit of quantification (LOQ)** for this report purpose is based on the analysis of samples that have been taken through the whole D-Fructose/D-Glucose Assay Kit (K-FRUGL) measurement procedure.

- The LOD is the lowest concentration of the analyte that can be detected by the method. LOD is calculated as $3 \times s'0$; where $s'0$ is the standard deviation of a number of samples A1 reading.
- The LOQ is the lowest level at which the kit's performance is acceptably repeatable. LOQ is calculated as $kQ \times s'0$; where $s'0$ is the standard deviation of a number of samples A1 reading. The IUPAC default value for kQ is 10
- For D-Fructose/D-Glucose Assay Kit (K-FRUGL)

LOD – For 2.0 mL of sample (maximum volume)

D-Fructose = 0.066 mg/L

D-Glucose = 0.067 mg/L

LOQ – For 2.0 mL of sample (maximum volume)

D-Fructose = 0.232 mg/L

D-Glucose = 0.201 mg/L

* **Note:** The above detection limits are for samples as used in the assay, after sample preparations if required (e.g. deproteinisation). The dilution used in pre-treatment must be accounted for while establishing the detection limits for specific samples.



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3.4. Trueness (*Bias*)

Comparison of the mean of the results (x) achieved with D-Fructose/D-Glucose Assay Kit (K-FRUGL) method with a suitable reference value (x ref). For this report, Relative Bias is calculated in per cent as: $b(\%) = \frac{x - x_{ref}}{x_{ref}} \times 100$, where the reference material is D-fructose and D-glucose supplied with the D-Fructose/D-Glucose Assay Kit (K-FRUGL) at 0.2 g/L of each.

Relative Bias *b*(%)

	n	Ref Material (g/L)	Mean (g/L)	<i>b</i> (%)
D-Fructose	28	0.2	0.1998	-0.11
D-Glucose	28	0.2	0.2023	1.13

3.5. Precision

This report details the reproducibility of the D-Fructose/D-Glucose Assay Kit (K-FRUGL), it is a measure of the variability in results, on different days and by different analysts, over an extended period of time.

For the purpose of this report different lot numbers of the kit standard is used as the reference material.

Reproducibility

	n	Ref Material (g/L)	Mean (g/L)	Standard Deviation	% CV
D-Fructose	28	0.2	0.1998	0.0027	1.35
D-Glucose	28	0.2	0.2023	0.0025	1.23



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Repeatability of this kit can be assessed using wine samples. This is a measure of the variability in results by a single analyst, using real samples, using the same equipment and over a short period of time. The use of wine samples shows one of the many applications of this kit.

Repeatability

Glucose	n	Mean (g/L)	Standard Deviation	%CV
White Wine	6	1.396	0.025	1.79
Red Wine	6	0.4	0.007	1.75

Fructose	n	Mean (g/L)	Standard Deviation	%CV
White Wine	6	2.329	0.047	2.01
Red Wine	6	0.705	0.009	1.28

4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of D-fructose and D-glucose in various matrices. It has been used for many years and is fully automatable for high throughput analysis of samples. Data presented in this report verifies and validates that this method is fit for the purpose intended, which is summarised below.

Validation Summary	D-Fructose	D-Glucose
Working range (µg in cuvette)	4-80	4-80
LOD (mg/L)	0.066	0.067
LOQ (mg/L)	0.232	0.201
Relative Bias <i>b</i> (%)	-0.11	1.13
Reproducibility (%CV using kit standard)	1.35	1.23
Repeatability (%CV using white wine)	2.01	1.79
Repeatability (%CV using red wine)	1.28	1.75