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

1. Scope

Megazyme's D-Mannose/D-Fructose/D-Glucose Assay Kit is an enzymatic method used for the measurement and analysis of D-mannose, D-fructose and D-glucose in plant products and in acid hydrolysates of polysaccharides. This novel method was developed in-house and measures each sugar in g/L.

2. Planning

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

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

The assays are specific for D-glucose, D-fructose and D-mannose.
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, D-fructose and, or, D-mannose to the sample in the initial extraction steps.

3.2. Working Range

Assay follows the D-Mannose/D-Fructose/D-Glucose Assay Kit (K-MANGL) standard procedure. 0.1 mL of D-glucose, D-fructose plus D-mannose standards were used as samples, with a range of concentrations (0.05-0.8 g/L total sugars) which corresponds to 5-80 µg of total sugars 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 8-10 min after the addition of the trigger enzyme (PGI), giving the measurement of D-fructose.
Absorbance A4 was taken 20 min after the addition of the final trigger enzyme (PMI), giving the measurement of D-mannose. Absorbances were read at 340 nm and 25^{o}C as recommended in the procedure.

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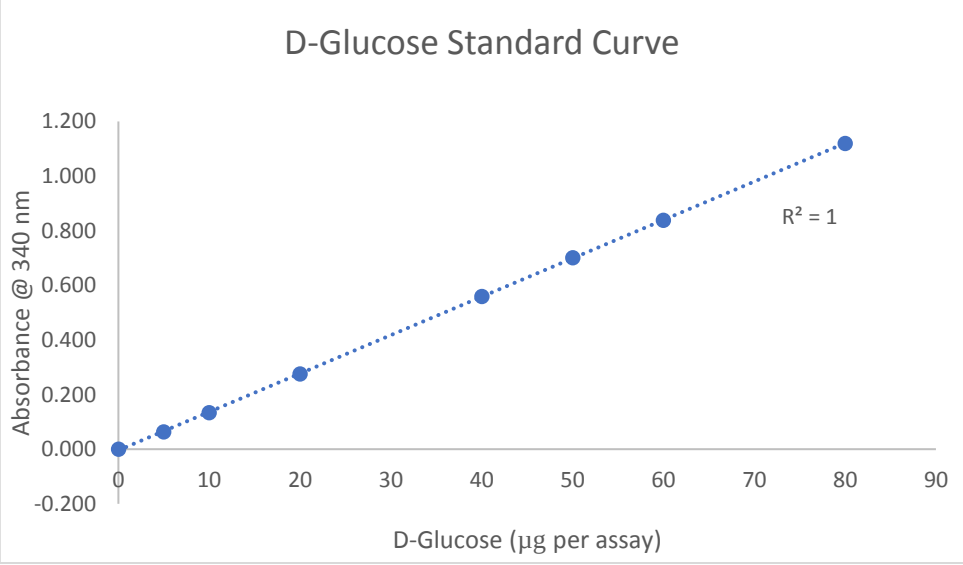
D-Glucose concentration [µg/assay]	ΔA_{340nm}		D-Fructose concentration [µg/assay]	ΔA_{340nm}		D-Mannose concentration [µg/assay]	ΔA_{340nm}
0	0.000		0	0.000		0	0.000
5	0.064		5	0.071		5	0.067
10	0.134		10	0.145		10	0.134
20	0.276		20	0.282		20	0.269
40	0.560		40	0.563		40	0.544
50	0.701		50	0.706		50	0.678
60	0.838		60	0.843		60	0.818
80	1.120		80	1.124		80	1.086



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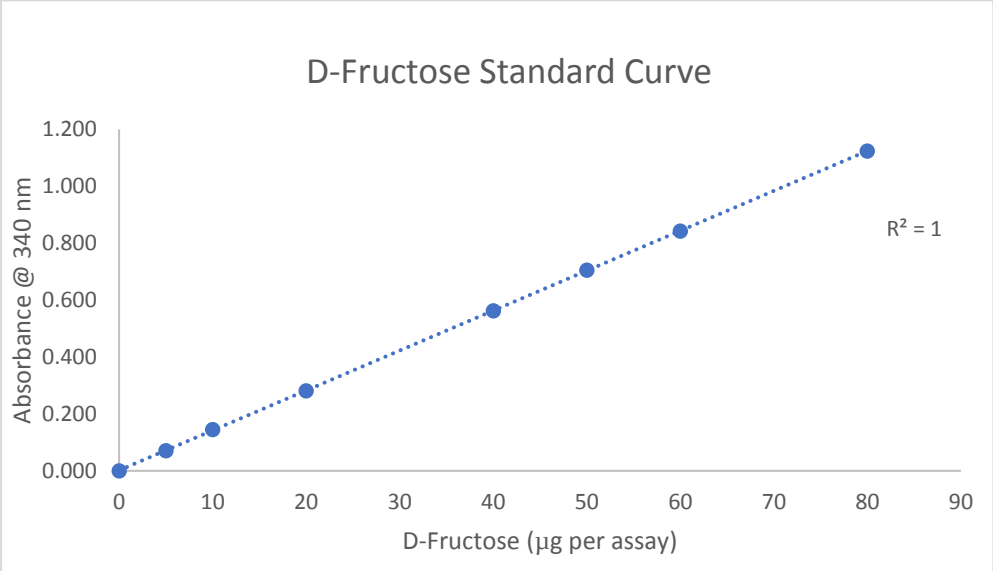

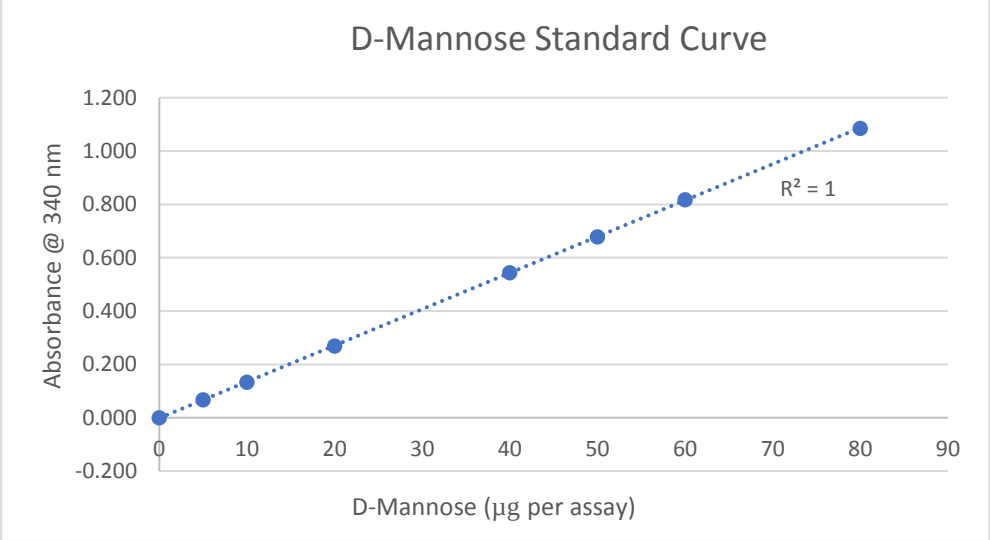

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3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, is 0.721 mg/L D-glucose or 0.733 mg/L D-mannose, 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-Mannose/D-Fructose/D-Glucose Assay Kit (K-MANGL) procedure.

- The LOD is the lowest concentration of the analyte that can be detected by the method. LOD is calculated as 3 x 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 x 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-Mannose/D-Fructose/D-Glucose Assay Kit (K-MANGL)

  LOD – For 2.0 mL of sample (maximum volume)
  D-Glucose = 0.070 mg/L
  D-Fructose = 0.200 mg/L
  D-Mannose = 0.100 mg/L

  LOQ – For 2.0 mL of sample (maximum volume)
  D-Glucose = 0.220 mg/L
  D-Fructose = 0.700 mg/L
  D-Mannose= 0.300 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 the D-Mannose/D-Fructose/D-Glucose Assay Kit (K-MANGL) method with a suitable reference value (x ref). For this report, Relative Bias is calculated in per cent as: ***b*(%) = x – xref / xref x 100,** where the reference material is the D-Mannose/D-Fructose/D-Glucose Assay Kit (K-MANGL) standard at 0.1 g/L of each sugar, D-fructose, D-glucose and D-mannose.

Relative Bias *b*(%)

	n	Ref Material (g/L)	Mean (g/L)	*b*(%)
D-Glucose	14	0.1	0.1013	1.257
D-Fructose	14	0.1	0.0994	-0.65
D-Mannose	14	0.1	0.0987	-1.264

3.5. Precision

This report details the reproducibility of the D-Mannose/D-Fructose/D-Glucose Assay Kit (K-MANGL), 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-Glucose	14	0.1	0.1013	0.0022	2.15
D-Fructose	14	0.1	0.0994	0.0029	2.91
D-Mannose	14	0.1	0.0987	0.0034	3.47

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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of D-glucose, D-fructose and D-mannose 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-Glucose	D-Fructose	D-Mannose
Working range (µg in cuvette)	4-80	4-80	4-80
LOD (mg/L)	0.07	0.2	0.1
LOQ (mg/L)	0.22	0.7	0.3
Relative Bias *b*(%)	1.26	-0.65	-1.26
Reproducibility (%CV using kit standard)	2.15	2.91	3.47