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Validation Report: L-Arabinose/D-Galactose Assay Kit (cat. no. K-ARGA)

1. Scope

Megazyme's L-Arabinose/D-Galactose Assay Kit, (K-ARGA) is a simple, reliable and accurate UV method for the measurement and analysis of L-arabinose and D-galactose in various materials including foods, feeds, beverages and plant products. This novel L-Arabinose/D-Galactose method was developed in-house and measures L-arabinose/D-galactose in g/L.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by L-Arabinose/D-Galactose Assay Kit (K-ARGA).

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 assay is specific for L-arabinose and D-galactose.

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 L-arabinose or D-galactose to the sample in the initial extraction steps.

3.2. Working Range

Assay follows the L-Arabinose/D-Galactose Assay Kit (K-ARGA) standard procedure. 0.1 mL of L-arabinose or D-galactose standard was used as a sample, with a range of concentrations (0.04-0.8 g/L) which corresponds to 4-80 µg of L-arabinose or D-galactose per assay. Absorbance A2 was read after 6 min for D-galactose, and after 12 min for L-arabinose, both at 340 nm and at 25°C as recommended in the procedure.

The working range is linear between 4-80 µg of L-arabinose or D-galactose per assay.

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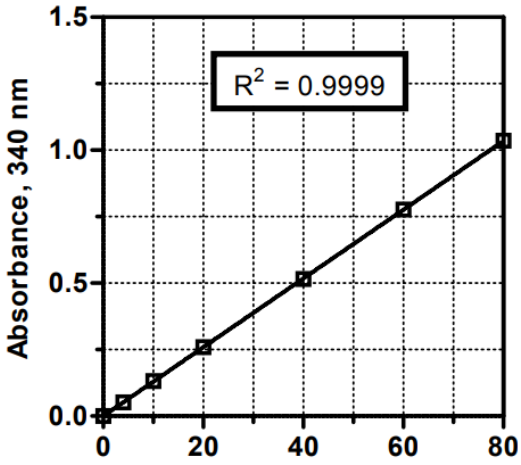
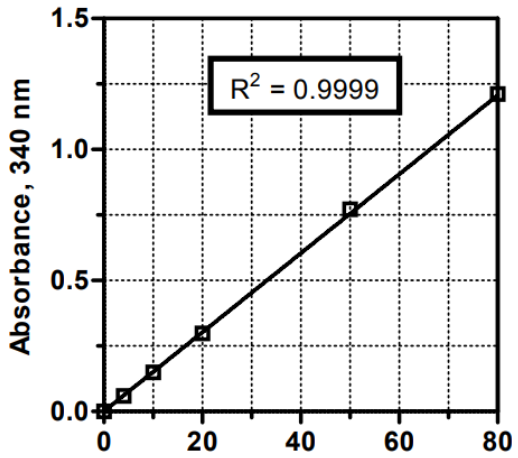


[D-Galactose] µg per final reaction solution



[L-Arabinose] µg per final reaction solution

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3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, is 0.577 mg/L of L-arabinose, or 0.692 mg/L of D-galactose, which is derived from an absorbance difference of 0.020 with a maximum sample volume of 2.0 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 standard procedure of the L-Arabinose/D-Galactose Assay (K-ARGA).

- The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as 3 x σ of the blank sample solution absorbance and 10 x σ of the blank sample solution absorbance, respectively, where σ is the standard deviation of the absorbance values from (at least) 10 replicates.

- For L-Arabinose/D-Galactose Assay Kit (K-ARGA)

  LOD – For 2.0 mL of sample (maximum volume)
  L-Arabinose = 0.058 mg/L
  D-Galactose = 0.069 mg/L

  LOQ – For 2.0 mL of sample (maximum volume)
  L-Arabinose = 0.202 mg/L
  D-Galactose = 0.208 mg/L

* **Note**: The above detection limits are for samples as used in the assay, after any sample preparation, if required. The dilution used in pre-treatment must be accounted for while establishing the detection limits for specific samples.

3.4. Trueness (*Bias*)

Comparison of the mean of the results (x) achieved with the L-Arabinose/D-Galactose Assay Kit (K-ARGA) with a suitable reference value (x ref). For this report, Relative Bias is calculated in per cent as: ***b*(%) = x – xref / xref x 100.** The reference material for this purpose is L-arabinose and D-galactose standards supplied with the L-Arabinose/D-Galactose Assay Kit (K-ARGA) both at 0.40 g/L.

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Relative Bias *b*(%)

	n	Ref Material (g/L)	Mean (g/L)	*b*(%)
L-Arabinose	16	0.40	0.3988	-0.30
D-Galactose	26	0.40	0.3956	-1.10

3.5. Precision

This report details the reproducibility of the L-Arabinose/D-Galactose Assay Kit (K-ARGA), it is a measure of the variability in results on different occasions, by different analysts, over an extended period of time.

Reproducibility

	n	Ref Material (g/L)	Mean (g/L)	Standard Deviation	% CV
L-Arabinose	16	0.40	0.3988	0.0052	1.30
D-Galactose	26	0.40	0.3956	0.0036	0.90

4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of L-Arabinose/D-Galactose in various matrices. Data presented in this report verifies and validates that this method is fit for the purpose intended, which is summarised below.

Validation Summary	L-Arabinose	D-Galactose
Working range (µg in cuvette)	4-80	4-80
LOD (mg/L)	0.058	0.069
LOQ (mg/L)	0.202	0.208
Relative Bias *b* (%)	-0.30	-1.10
Reproducibility (%CV using kit standard)	1.30	0.90