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Validation Report: D-Gluconic Acid/D-Glucono-δ-lactone Assay Kit (cat. no. K-GATE)

1. Scope

Megazyme's D-Gluconic Acid/D-Glucono-δ-lactone Assay Kit (K-GATE) is an enzymatic method used for the rapid measurement and analysis of D-Gluconic Acid/D-Glucono-δ-lactone in foods and beverages.

This D-Gluconic Acid/D-Glucono-δ-lactone method was developed in-house and measures D-gluconic acid/D-glucono-δ-lactone in g/L. Methods based on this assay principle have been accepted by ISO, DIN and GOST.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by D-Gluconic Acid/D-Glucono-δ-lactone Assay Kit (K-GATE).

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-gluconic acid.

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-gluconic acid to the sample in the initial extraction steps.

3.2. Working Range

Assay follows the D-Gluconic Acid/D-Glucono-δ-lactone Assay Kit (K-GATE) standard procedure. 0.1 mL of sodium D-gluconate standard was used as a sample, with a range of concentrations (0.008-0.5 g/L D-Gluconic Acid) which corresponds to 0.8-50 µg of D-gluconic acid per assay. Absorbance A2 was read after 5 min, at 340 nm and at 25°C as recommended in the procedure.

The working range is linear between 0.8-50 µg of D-gluconic acid/D-glucono-δ-lactone per assay.

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3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, is 0.792 mg/L of D-Gluconic Acid 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 D-Gluconic Acid/D-Glucono-δ-lactone Assay (K-GATE).

- 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 10 replicates.

- For D-Gluconic Acid/D-Glucono-δ-lactone Assay Kit (K-GATE)
- **LOD – For 2.0 mL of sample (maximum volume**)
  D-Gluconic Acid = 0.119 mg/L

  LOQ – For 2.0 mL of sample (maximum volume)
  D-Gluconic Acid = 0.395 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.

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3.4. Trueness (*Bias*)

Comparison of the mean of the results (x) achieved with the D-Gluconic Acid/D-Glucono-δ-lactone Assay Kit (K-GATE) 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 sodium D-gluconate supplied with the D-Gluconic Acid/D-Glucono-δ-lactone Assay Kit (K-GATE) and prepared to 0.25 g/L of D-gluconic acid.

Relative Bias *b*(%)

	n	Ref Material (g/L)	Mean (g/L)	*b*(%)
D-Gluconic Acid	24	0.25	0.2490	-0.40

3.5. Precision

This report details the reproducibility of the D-Gluconic Acid/D-Glucono-δ-lactone Assay Kit (K-GATE), 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
D-Gluconic Acid	24	0.25	0.2490	0.0038	1.53

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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of D-gluconic Acid/D-glucono-δ-lactone in various matrices. It is a novel method 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-Gluconic Acid
Working range (µg in cuvette)	0.8 - 50
LOD (mg/L)	0.119
LOQ (mg/L)	0.395
Relative Bias *b*(%)	-0.40
Reproducibility (%CV using kit standard)	1.53