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Validation Report: L-Malic Acid Assay Kit (cat. no. K-LMAL)

1. Scope

Megazyme's L-Malic Acid Assay Kit (K-LMAL) is an enzymatic method used for the rapid measurement and analysis of L-malic acid in foodstuffs, beverages and other materials. This method was developed in-house and measures L-malic acid in g/L. Methods based on this principle have been accepted by AOAC, EEC, EN, NF, NEN, DIN, GOST, OIV, IFU, AIJN and MEBAK.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by the L-Malic Acid Assay Kit (K-LMAL).

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 L-malic 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 L-malic acid to the sample in the initial extraction steps.

3.2. Working Range

Assay follows the L-Malic Acid Assay Kit (K-LMAL) standard procedure. 0.1mL of L-malic acid standard was used as sample, with a range of concentrations (0.005-0.3 g/L L-malic acid) which corresponds to 0.5-30 µg of L-malic acid per cuvette. Absorbance A2 was read after 3 min, at 340 nm and at 25°C as recommended in the procedure.

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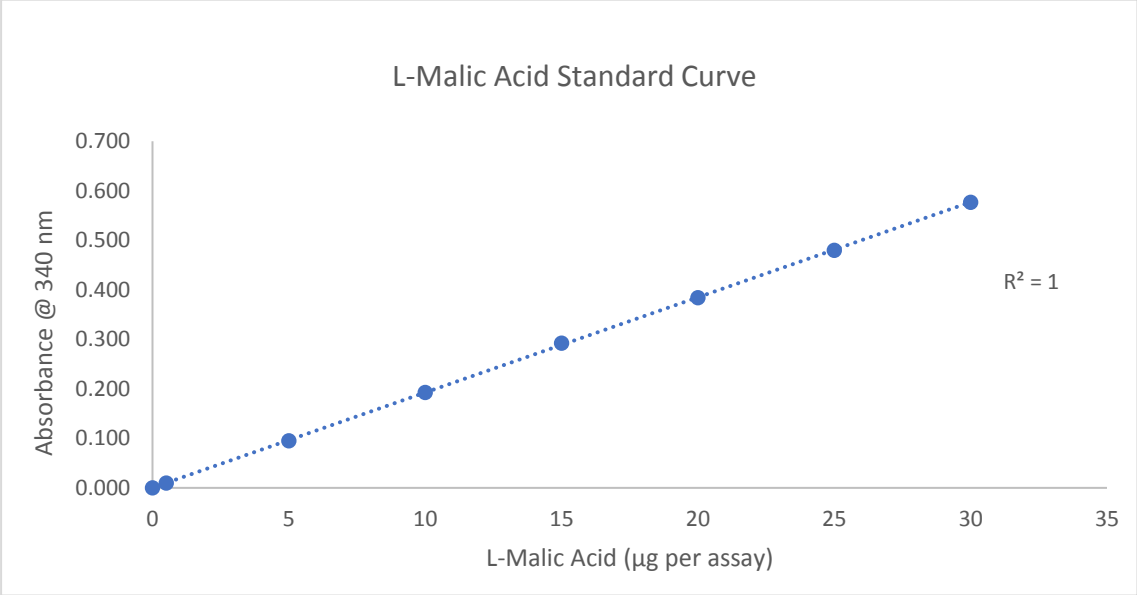
L-Malic Acid Concentration [µg/assay]	ΔA_{340nm}
0	0.000
0.5	0.010
5	0.095
10	0.193
15	0.292
20	0.384
25	0.480
30	0.577



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3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, is 0.25 mg/L, which is derived from an absorbance difference of 0.010 and 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 L-Malic Acid Assay Kit (K-LMAL) 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 L-Malic Acid Assay Kit (K-LMAL)

  LOD – For 2.0 mL of sample (maximum volume)
  L-Malic Acid = 0.025 mg/L

  LOQ – For 2.0 mL of sample (maximum volume)
  L-Malic Acid = 0.100 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 L-Malic Acid Assay Kit (K-LMAL) 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.** The reference material for this purpose is L-malic acid supplied with the L-Malic Acid Assay Kit (K-LMAL) at 0.15 g/L

Relative Bias *b*(%)

	n	Ref Material (g/L)	Mean (g/L)	*b*(%)
L-Malic Acid	16	0.15	0.1507	0.49

3.5. Precision

This report details the reproducibility of the L-Malic Acid Assay Kit (K-LMAL), 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
L-Malic Acid	16	0.15	0.1507	0.0023	1.52

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

	n	Mean (g/L)	Standard Deviation	%CV
White Wine	12	3.093	0.040	1.28
Red Wine	12	0.163	0.004	2.56

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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of L-malic acid 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	L-Malic Acid
Working range (µg in cuvette)	0.5-30
LOD (mg/L)	0.025
LOQ (mg/L)	0.100
Relative Bias *b*(%)	0.49
Reproducibility (%CV using malic acid)	1.52
Repeatability (%CV using white wine)	1.278
Repeatability (%CV using red wine)	2.556