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Validation Report: Citric Acid Assay Kit (cat. no. K-CITR)

1. Scope

Megazyme's Citric Acid Assay Kit is an enzymatic method used for the rapid measurement and analysis of citric acid in foodstuffs, beverages and other materials. This method was developed in-house and measures citric acid in g/L. Methods based on this principle have been accepted by MEBAK, OIV, EU, ISO2963, AOAC and IFU22.

2. Planning

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

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 citric 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 citric acid to the sample in the initial extraction steps.

3.2. Working Range

Assay follows the Citric Acid Assay Kit (K-CITR) standard procedure. 0.2 mL of citric acid standard was used as sample, with a range of concentrations (0.005-0.5 g/L citric acid) which corresponds to 1-100 µg of citric acid per cuvette. Absorbance A2 was read after 5 min, at 340 nm and at 25°C as recommended in the procedure.

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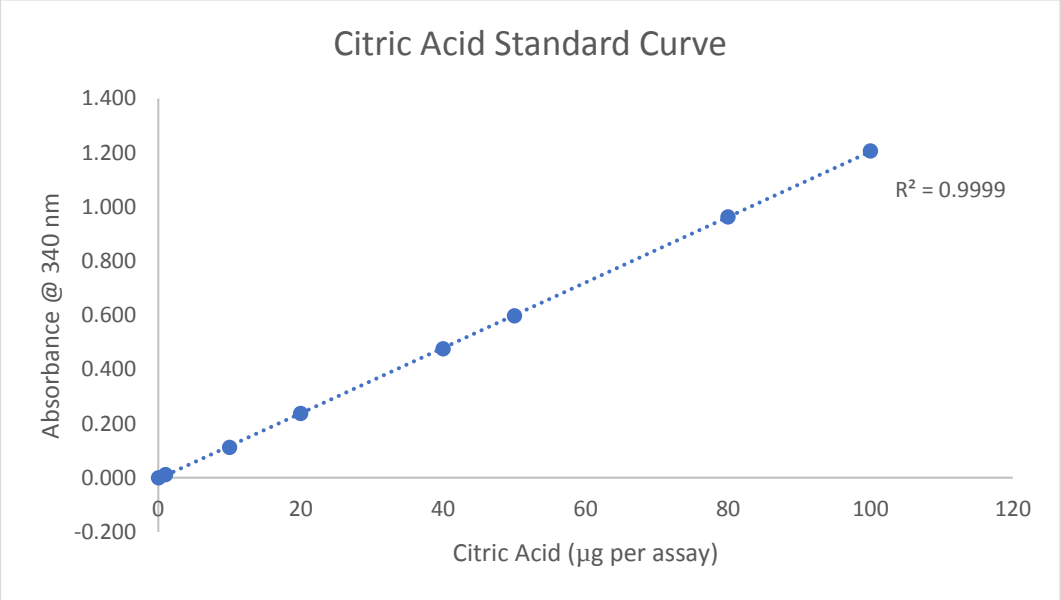
Citric Acid Concentration [µg/assay]	ΔA_{340nm}
0	0.000
1	0.012
10	0.112
20	0.237
40	0.476
50	0.598
80	0.963
100	1.206



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3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, is 0.491 mg/L, which is derived from an absorbance difference of 0.010 with a sample volume of 1.7 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 Citric Acid Assay Kit (K-CITR) 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 Citric Acid Assay Kit (K-CITR)

  LOD – For 1.7 mL of sample (maximum volume)
  Citric Acid = 0.098 mg/L

  LOQ – For 1.7 mL of sample (maximum volume)
  Citric Acid = 0.295 mg/L

* **Note:** The above detection limits are for samples as used in the assay i.e. after any required sample preparation (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 Citric Acid Assay Kit (K-CITR) 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 citric acid supplied with the Citric Acid Assay Kit (K-CITR) at 0.2 g/L

Relative Bias *b*(%)

	n	Ref Material (g/L)	Mean (g/L)	*b*(%)
Citric Acid	24	0.2	0.1983	-0.86

3.5. Precision

This report details the reproducibility of the Citric Acid Assay Kit (K-CITR), 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
Citric Acid	24	0.2	0.198	0.004	1.82

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	0.137	0.004	2.86
Red Wine	12	0.049	0.001	2.13

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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of citric 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	Citric Acid
Working range (µg in cuvette)	1-100
LOD (mg/L)	0.098
LOQ (mg/L)	0.295
Relative Bias *b*(%)	- 0.86
Reproducibility (%CV using citric acid)	1.82
Repeatability (%CV using white wine)	2.855
Repeatability (%CV using red wine)	2.125