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Validation Report: Tartaric Acid Assay Kit (cat. no. K-TART)**1. Scope**

Megazyme's Tartaric Acid Assay Kit (K-TART) is used for the rapid measurement and analysis of tartaric acid in wine, fruit juice and other materials. This method was developed in-house and measures tartaric acid in g/L. Methods based on this principle are used widely in the wine industry.

2. Planning

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

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 tartaric acid in white wines, red wines and fruit juices. L-malic acid, D-malic acid, L-lactic acid and D-lactic acid do not react or interfere with the tartaric acid assay when present at concentrations of 2 g/L or less.

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 tartaric acid to the sample in the initial extraction steps.

3.2. Working Range

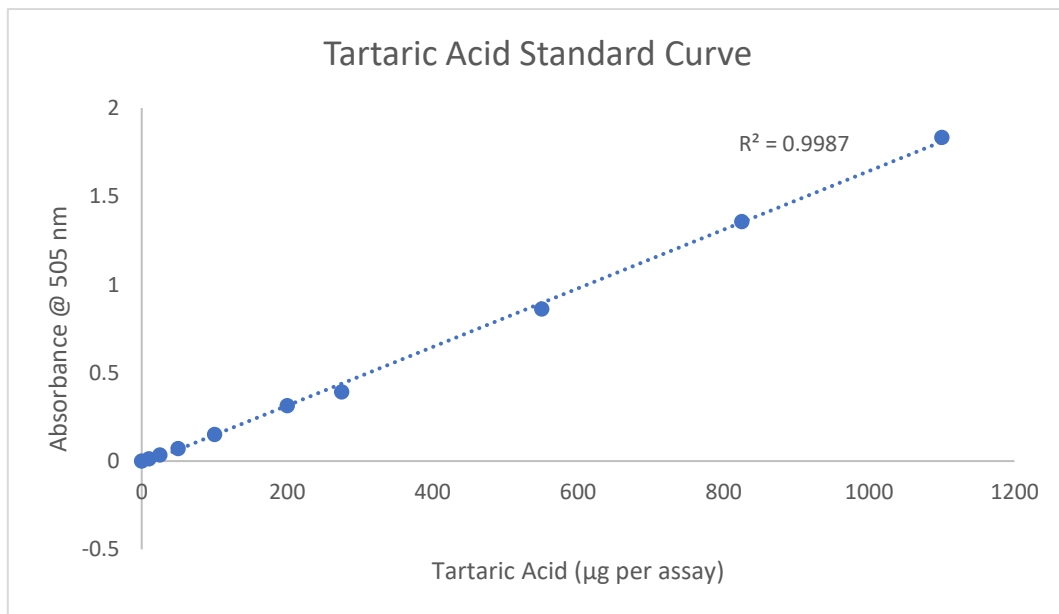
Assay follows the Tartaric Acid Assay Kit (K-TART) standard procedure. 0.1 mL of tartaric acid standard was used as sample, with a range of concentrations (0.15-11 g/L tartaric acid) which corresponds to 15-1100 µg of tartaric acid per cuvette. Absorbance A₂ was read after 4 min, at 505 nm and at 25°C or 37°C as recommended in the procedure.



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Tartaric Acid Concentration [$\mu\text{g}/\text{assay}$]	$\Delta A_{505\text{nm}}$
0	0.000
10	0.013
25	0.034
50	0.071
100	0.150
200	0.314
275	0.391
550	0.862
825	1.357
1100	1.833





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3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, ~ 108 mg/L of tartaric acid which is derived from an absorbance difference of 0.020 with the maximum sample volume of 0.10 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 Tartaric Acid Assay Kit (K-TART) procedure.

- The LOD is the lowest concentration of the analyte that can be detected by the method. LOD is calculated as $3 \times s'0$; 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 $kQ \times s'0$; where $s'0$ is the standard deviation of a number of samples A1 reading. The IUPAC default value for kQ is 10.
- For Tartaric Acid Assay Kit (K-TART)

LOD – For 0.1 mL of sample (maximum volume)

Tartaric Acid = 122 mg/L

LOQ – For 0.1 mL of sample (maximum volume)

Tartaric Acid = 398 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 Tartaric Acid Assay Kit (K-TART) method with a suitable reference value (x ref). For this report, Relative Bias is calculated in per cent as: $b(\%) = \frac{x - x_{ref}}{x_{ref}} \times 100$. The reference material for this purpose is tartaric acid supplied with the Tartaric Acid Assay Kit (K-TART) at 5.0 g/L.

Relative Bias *b*(%)

	n	Ref Material (g/L)	Mean (g/L)	<i>b</i> (%)
Tartaric Acid	13	5.0	5.0214	0.43

3.5. Precision

This report details the reproducibility of the Tartaric Acid Assay Kit (K-TART), 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 are used as the reference material.

Reproducibility

	n	Ref Material (g/L)	Mean (g/L)	Standard Deviation	%CV
Tartaric Acid	13	5.0	5.0214	0.0623	1.24

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	1.763	0.102	5.77
Red Wine	12	1.603	0.065	4.08



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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of tartaric 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	Tartaric Acid
Working range (μg in cuvette)	15-1100
LOD (mg/L)	122
LOQ (mg/L)	398
Relative Bias <i>b</i> (%)	0.43
Reproducibility (%CV using tartaric acid)	1.24
Repeatability (%CV using white wine)	5.77
Repeatability (%CV using red wine)	4.08