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Validation Report: Ascorbic Acid Assay Kit (L-Ascorbate) (cat. no. K-ASCO)

1. Scope

Megazyme's Ascorbic Acid Assay Kit (L-ascorbate) (K-ASCO) is for the specific measurement and analysis of L-ascorbic acid in beverages, meat, flour, dairy and vegetable products. This method measures ascorbic acid in g/L and methods based on this kit's principle have been accepted by MEBAK.

2. Planning

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

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

Under the conditions stated in this procedure, the assay is specific for L-ascorbic acid. However, iso-ascorbic acid (D-arabo-ascorbic acid), if present, will be determined simultaneously (but the rate of reaction is slower). In the measurement of iso-ascorbic acid, the incubation time with AAO needs to be increased to approx. 20 min.

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

3.2. Working Range

Assay follows the Ascorbic Acid Assay Kit (L-ascorbate) (K-ASCO) standard procedure. 0.1 mL of L-ascorbic acid was used as a sample, with a range of concentrations (0.005-0.3 g/L ascorbic acid) which corresponds to 0.5-30 µg of ascorbic acid per assay. Absorbance A₂, was read at 578 nm after incubation at 37°C for 8 min as detailed in the procedure.

The working range is linear between 0.5-30 µg of ascorbic acid per assay.



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3.3. Working Range LOD and LOQ

The **instrument limit of detection**, as per kit booklet, is 0.175 mg/L of ascorbic acid which is derived from an absorbance difference of 0.010 with a maximum sample volume of 1.5 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 Ascorbic Acid Assay (L-ascorbate) (K-ASCO).

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

For Ascorbic Acid Assay Kit (L-ascorbate) (K-ASCO)

- **LOD – For 1.5 mL of sample (maximum volume)**
Ascorbic Acid = 0.280 mg/L
- **LOQ – For 2.0 mL of sample (maximum volume)**
Ascorbic Acid = 0.963 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 Ascorbic Acid Assay Kit (L-ascorbate) (K-ASCO) 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 L-ascorbic acid supplied with the Ascorbic Acid Assay Kit (L-ascorbate) (K-ASCO) and prepared to 0.15 g/L of ascorbic acid as detailed in the kit data booklet.

Relative Bias $b(\%)$

	n	Ref Material (g/L)	Mean (g/L)	$b(\%)$
Ascorbic Acid	18	0.15	0.147	-2.00

3.5. Precision

This report details the reproducibility of the Ascorbic Acid Assay Kit (L-ascorbate) (K-ASCO), 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
Ascorbic Acid	18	0.15	0.147	0.0033	2.23



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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of Ascorbic Acid 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	Ascorbic Acid
Working range (μg in cuvette)	0.5-30
LOD (mg/L)	0.280
LOQ (mg/L)	0.963
Relative Bias <i>b</i> (%)	-2.00
Reproducibility (%CV using kit standard)	2.23

