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Validation Report: Acetic Acid Assay Kit (ACS Manual Format) (cat. no. K-ACET)

1. Scope

Megazyme's Acetic Acid Assay Kit (ACS Manual Format) (K-ACET) is an enzymatic method used for the rapid measurement and analysis of acetic acid in foodstuffs, beverages and other materials. This method was developed in-house and measures acetic acid in g/L. Methods based on this principle have been accepted by EN, ISO, ICUMSA, IFU and MEBAK.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by the Acetic Acid Assay Kit (ACS Manual Format) (K-ACET).

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

3.2. Working Range

Assay follows the Acetic Acid Assay Kit (ACS Manual Format) (K-ACET) standard procedure. 0.1 mL of acetic acid standard was used as sample, with a range of concentrations (0.003-0.20 g/L acetic acid) which corresponds to 0.3-20 µg of acetic acid per cuvette. This kit requires the addition of two enzymes. Absorbance A1 was taken 4 min after the addition of the 1st enzyme (L-MDH/CS), and absorbance A2 taken 12 min after the addition of the 2nd enzyme (ACS), all absorbances were read at 340 nm and at 25^{o}C as recommended in the procedure.

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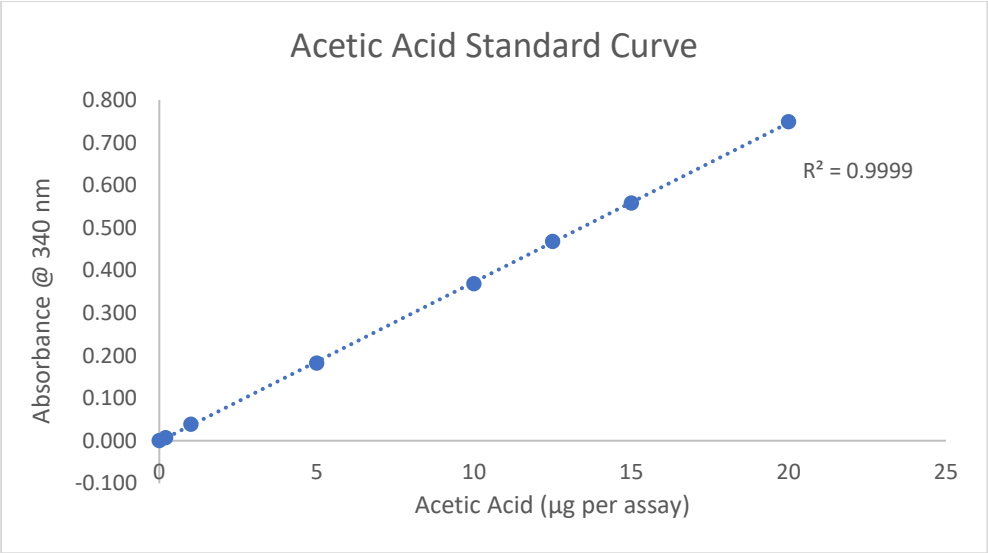
Acetic Acid Concentration [µg/assay]	ΔA_{340nm}
0	0.000
0.2	0.007
1	0.038
5	0.182
10	0.368
12.5	0.467
15	0.558
20	0.749



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3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, is 0.14 mg/L, which is derived from an absorbance difference of 0.010 with 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 Acetic Acid Assay Kit (ACS Manual Format) (K-ACET) 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 Acetic Acid Assay Kit (ACS Manual Format) (K-ACET)

  LOD – For 2.0 mL of sample (maximum volume)
  Acetic Acid = 0.450 mg/L

  LOQ – For 2.0 mL of sample (maximum volume)
  Acetic Acid= 1.5 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 Acetic Acid Assay Kit (ACS Manual Format) (K-ACET) 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 acetic acid supplied with the Acetic Acid Assay Kit (ACS Manual Format) (K-ACET) at 0.1 g/L.

Relative Bias *b*(%)

	n	Ref Material (g/L)	Mean (g/L)	*b*(%)
Acetic Acid	19	0.1	0.1014	1.39

3.5. Precision

This report details the reproducibility of the Acetic Acid Assay Kit (ACS Manual Format) (K-ACET), 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
Acetic Acid	19	0.1	0.1014	0.0008	0.8

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	0.3022	0.009	2.98
Red Wine	12	0.5724	0.011	1.99

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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of Acetic 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	Acetic Acid
Working range (µg in cuvette)	0.3-20
LOD (mg/L)	0.450
LOQ (mg/L)	1.5
Relative Bias *b*(%)	1.393
Reproducibility (%CV using kit standard)	0.803
Repeatability (%CV using White Wine)	2.980
Repeatability (%CV using White Wine)	1.993