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Validation Report: D-Sorbitol/Xylitol Assay Kit (cat. no. K-SORB)

1. Scope

Megazyme's D-Sorbitol/Xylitol Assay Kit (K-SORB) is an enzymatic method used for the rapid measurement and analysis of D-sorbitol/xylitol in foodstuffs and wine. This method was developed in-house and measures D-sorbitol/xylitol in g/L. Methods based on this principle have been accepted by IFU and AIJN.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by the D-Sorbitol/Xylitol Assay Kit (K-SORB).

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

As well as D-sorbitol and xylitol, sorbitol dehydrogenase also oxidises other polyols such as tibitol, iditol and allitol, but with a lower rate. Polyalcohols such as D-mannitol, L-arabitol and dulcitol do not react. Under the assay conditions described in the kit's data booklet, glycerol is oxidised very slowly (approx. 0.4% conversion with 100 µg of glycerol/assay), as is galactitol (approx. 3% conversion with 10 µg of galactitol/assay).

However, the contribution of these substances, if present in the sample, can be taken into account by extrapolation back to the time of addition of the sorbitol dehydrogenase, using the linear "creep rate" of the reaction.

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 D-sorbitol to the sample in the initial extraction steps.

3.2. Working Range

Assay follows the D-Sorbitol/Xylitol Assay Kit (K-SORB) standard procedure. 0.1 mL of D-Sorbitol standard was used as sample, with a range of concentrations (0.01-0.2 g/L D-Sorbitol) which corresponds to 1-20 µg of D-sorbitol per assay. Absorbance A₂ was read after 15 min at 492nm and at 25°C as recommended in the procedure.

The working range is linear between 1-20 µg of D-sorbitol per assay.



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3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, is 0.197 mg/L D-sorbitol or 0.165 mg/L xylitol, which is derived from an absorbance difference of 0.015 with a sample volume of 2.0 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 D-Sorbitol/Xylitol Assay Kit (K-SORB) 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 $k_Q \times s'_0$; where s'_0 is the standard deviation of a number of samples A1 reading. The IUPAC default value for k_Q is 10.
- For D-Sorbitol/Xylitol Assay Kit (K-SORB)

LOD – For 2.0 mL of sample (maximum volume)

D-Sorbitol = 0.250 mg/L

LOQ – For =2.0 L of sample (maximum volume)

D-Sorbitol = 0.800 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 D-Sorbitol/Xylitol Assay Kit (K-SORB) 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 D-sorbitol supplied with the D-Sorbitol/Xylitol Assay Kit (K-SORB) at 0.1 g/L.

Relative Bias $b(\%)$

	n	Ref Material (g/L)	Mean (g/L)	$b(\%)$
D-Sorbitol	21	0.1	0.1002	0.17

3.5. Precision

This report details the reproducibility of the D-Sorbitol/Xylitol Assay Kit (K-SORB), 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
D-Sorbitol	21	0.1	0.1002	0.0004	0.41



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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of D-sorbitol/xylitol 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	D-Sorbitol
Working range (μg in cuvette)	1-20
LOD (mg/L)	0.250
LOQ (mg/L)	0.800
Relative Bias <i>b</i> (%)	0.17
Reproducibility (%CV using D-Sorbitol)	0.41