
1. Scope
Megazyme’s D-Mannitol/D-Arabitol Assay Kit (K-MANOL) is an enzymatic method used for the measurement and analysis of D-mannitol/D-arabitol in food, beverages and other materials. This novel method was developed in-house and measures both D-mannitol/D-arabitol in g/L.

2. Planning
The purpose of this report is to verify and validate the current method as detailed by D-Mannitol/D-Arabitol Assay Kit (K-MANOL).

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 measures D-mannitol or D-arabitol. It does not distinguish between these polyols. As well as D-mannitol and D-arabitol, ManDH also slowly oxidises sorbitol.

If the conversion of D-mannitol or D-arabitol has been completed within the time specified in the assay (approx. 4 min), it can be generally concluded that no interference has occurred. If the sample contains high levels of D-sorbitol, after the rapid oxidation of the D-mannitol or D-arabitol is complete (3-4 min), there is a second, less rapid and linear increase in absorbance at 340 nm.

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-mannitol or D-arabitol to the sample in the initial extraction steps.

3.2. Working Range
Assay follows the D-Mannitol/D-Arabitol Assay Kit (K-MANOL) standard procedure. 0.1 mL of D-Mannitol standard solution was used as sample, with a range of concentrations (0.02-0.75 g/L) which corresponds to 2-75 μg of D-Mannitol per cuvette.

Absorbance A2 was read after ~ 4 min at 340 nm and at 25°C as recommended in the standard assay procedure.

The working range is linear between 2-75 μg of D-mannitol or D-arabitol per assay.
3.3. LOD and LOQ

The **instrument limit of detection** for the standard manual assay procedure is 0.50 mg/L of D-mannitol or 0.42 mg/L of D-arabitol, which is derived from an absorbance difference of 0.015 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 D-Mannitol/D-Arabinol Assay Kit (K-MANOL) measurement procedure.

- The LOD is the lowest concentration of the analyte that can be detected by the method. LOD is calculated as $3 \times s'_{O}$; where $s'_{O}$ 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 $k_{Q} \times s'_{O}$; where $s'_{O}$ is the standard deviation of a number of samples A1 reading. The IUPAC default value for $k_{Q}$ is 10.

- For D-Mannitol/D-Arabinol Assay Kit (K-MANOL)

  **LOD – For 2.0 mL of sample (maximum volume)**
  - D-Mannitol = 0.170 mg/L
  - D-Arabinol = 0.142 mg/L

  **LOQ – For 2.0 mL of sample (maximum volume)**
  - D-Mannitol = 0.566 mg/L
  - D-Arabinol = 0.473 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.
3.4. Trueness (Bias)
Comparison of the mean of the results (x) achieved with D-Mannitol/D-Arabitol Assay Kit (K-MANOL) method with a suitable reference value (x ref). For this report, Relative Bias is calculated in per cent as: $b(\%) = \frac{x - x_{ref}}{x_{ref}} \times 100$, where the reference material is D-Mannitol standard supplied with the D-Mannitol/D-Arabitol Assay Kit (K-MANOL) at 0.3 g/L.

Relative Bias $b(\%)$

<table>
<thead>
<tr>
<th>Ref Material (g/L)</th>
<th>Mean (g/L)</th>
<th>b(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Mannitol</td>
<td>0.3</td>
<td>0.2995</td>
</tr>
</tbody>
</table>

3.5. Precision
This report details the reproducibility of the D-Mannitol/D-Arabitol Assay Kit (K-MANOL), 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

<table>
<thead>
<tr>
<th>Ref Material (g/L)</th>
<th>Mean (g/L)</th>
<th>Standard Deviation</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-Mannitol</td>
<td>0.3</td>
<td>0.0045</td>
<td>1.51</td>
</tr>
</tbody>
</table>
4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of D-mannitol/D-arabitol 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.

<table>
<thead>
<tr>
<th>Validation Summary</th>
<th>D-Mannitol</th>
<th>D-Arabitol</th>
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<tbody>
<tr>
<td>Working range (µg in cuvette)</td>
<td>2-75</td>
<td>2-75</td>
</tr>
<tr>
<td>LOD (mg/L)</td>
<td>0.170</td>
<td>0.142</td>
</tr>
<tr>
<td>LOQ (mg/L)</td>
<td>0.566</td>
<td>0.473</td>
</tr>
<tr>
<td>Relative Bias b (%)</td>
<td>-0.16</td>
<td>-</td>
</tr>
<tr>
<td>Reproducibility (%CV using kit standard)</td>
<td>1.51</td>
<td>-</td>
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