Industrial Sucrose Analysis

A simple two-step enzymatic assay for sucrose in aqueous solutions or extracts.

Bulletin Reference	TB – Sucrose – Industrial – GMRD-105 – V.02
Order Code	GMRD-105 (For GM8, GL6)
Reagent Kit Size	80 ml (120 analyser cycles) – GMRD-105
Instruments	GM8 and GL6 series analysers
Samples	Aqueous solutions and extracts.
Sample Volume	10 µl
Analysis Time	20 seconds (from injection)
Working Range	0.5 - 20 %W/V (GM8, GL6)
Reagent Stability	Shelf-life unopened: 9 months stored at 0 - 5°C. Shelf-life reconstituted: Invertase, 3 - 4 days stored at 0 - 5°C. Aliquots may be frozen for extended life.
Note	4 vials of Invertase (β-fructosidase) are provided to maximise kit life. Sample opacity or turbidity presents no problem since the detection method is electrochemical rather than spectrophotometric. Endogenous glucose, if present, should be determined as a sample 'blank', i.e. extract diluted pro-rata in water instead of β-fructosidase. Incomplete hydrolysis may take place for sucrose concentrations greater than 10 %W/V. For greater accuracy at these levels repeat hydrolysis using a 5 µl sample and scale results proportionally.

Principle

i) Sucrose, a disaccharide, is stoichiometrically hydrolysed by Invertase (β-fructosidase) to α-D-glucose and fructose in a simple pre-reaction. Under the special buffer conditions used, mutarotation to β-D-glucose rapidly occurs,

$$\text{Sucrose} \xrightarrow{\textit{Invertase}} (\alpha\text{-D-Glucose}) \xrightarrow{\textit{Phosphate Buffer}} \beta\text{-D-Glucose} + \text{Fructose}$$

ii) In the presence of molecular oxygen, β-D-glucose is oxidised by the enzyme glucose oxidase (GOD) to gluconic acid and hydrogen peroxide,

$$\beta\text{-D-Glucose} + O_2 \xrightarrow{\textit{Glucose Oxidase (GOD)}} \text{D-Gluconic acid} + H_2O_2$$

Under the conditions of the assay, the rate of oxygen consumption is directly proportional to glucose concentration, which relates directly to the original sucrose concentration.