



Frequently Asked Questions

Pectin Assay using *m*-Hydroxydiphenyl

Galacturonic acid residues form the fundamental units of pectin molecules. A quantitative measurement of this acid is used to determine the concentration of pectic substances present in a sample.

The colourimetric assay using *m*-hydroxydiphenyl for analysis of galacturonic acids is quite specific for uronic acids. It can tolerate, for instance, the presence of up to 1000 ppm of sucrose.

REAGENTS:

- Galacturonic acid stock solution:**
Dissolve 100 mg dry galacturonic acid powder in 100 ml distilled water to give a solution concentration of 1 mg/ml. Keep refrigerated. A new solution should be prepared every 4 weeks.
- M/80 Sodium tetraborate in sulphuric acid (0.0125M solution):**
Weigh 1.192 g of sodium tetraborate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) into a 250 ml volumetric flask. Make up to the mark with concentrated sulphuric acid.
- 0.5% sodium hydroxide:**
Weigh 5 g sodium hydroxide into a 1 litre volumetric flask. Make up to the mark with deionised water.
- m*-Hydroxydiphenyl solution (0.15%):**
Weigh 0.15 g *m*-hydroxydiphenyl into a 100 ml volumetric flask. Make up to the mark with 0.5% sodium hydroxide solution. Cover the container with aluminium foil to protect it from the light. Keep refrigerated.

PREPARATION AND MEASUREMENT OF SAMPLES:

Make solutions for calibration curve as follows:

Pipette 2 ml stock galacturonic acid solution into a 100 ml volumetric flask. Make up to the mark with deionised water. The concentration of galacturonic acid in this sample is equivalent to 20 micrograms/ml.

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| 4 ml stock in 100 ml volumetric flask for | 40 micrograms/ml |
| 6 ml " " " " " | 60 micrograms/ml |
| 8 ml " " " " " | 80 micrograms/ml |
| 10 ml " " " " " | 100 micrograms/ml |

Place 16 test tubes in an ice bath to cool. Use three tubes for each sample: two tubes for sample + one tube for blank determination. Keep the sulphuric acid/sodium tetraborate solution in an ice bath throughout the experiment.

Place 1.0 ml of standard or sample into each of three labelled cold test tubes. Allow a few minutes to cool. Add 6.0 ml of the tetraborate solution to each test tube. Mix thoroughly by means of a test-tube stirrer. It is important that the sample plus reagents are mixed properly. Keep test tubes in ice until all samples are

prepared. Heat tubes in a boiling water bath for precisely 6.0 mins. Return tubes to the ice bath. Allow to cool.

Add 0.1 ml (100 microlitres) of *m*-hydroxydiphenyl to the first two tubes to develop colour. Mix thoroughly using the test-tube stirrer. Add (for blank) 0.1 ml (100 microlitres) of 0.5% sodium hydroxide to the third tube. Mix thoroughly. Allow the tubes to stand for 15-20 minutes at room temperature to allow any bubbles formed to dissipate.

Measure absorbance at 520 nm on a spectrophotometer by reading sample against corresponding blank tube with 0.5% sodium hydroxide.

To obtain the absorbance due to *m*-hydroxydiphenyl, subtract the absorbance for sample blank from the total absorbance for sample.

Zero the spectrophotometer with a reagent blank prepared by mixing 10 ml deionised water plus 6.0 ml tetraborate solution and 0.1 ml of 0.5% sodium hydroxide solution.

Plot a calibration curve of Absorbance (y-axis) against concentration of galacturonic acid (x-axis). Use this curve for standardisation.