# APPENDICES

## APPENDIX A

## TOTAL SUGAR ANALYSIS

The phenol-sulfuric acid method is a simple and rapid colorimetric method to determine total carbohydrates in a sample. The method detects virtually all classes of carbohydrates, including monosaccharide, disaccharide and polysaccharide. In hot acidic medium, glucose is dehydrated to hydroxymethyl furfural. This forms a yellow colored product with phenol and absorption maximum at 490 nm (Dubois *et al*., 1956).

**Procedure**

1. Prepare standard glucose stock 100 mg in 100 ml of water. Working standard, 10 ml of stock diluted to 100 ml with distilled water.
2. Pipette out 500 µl of the sample solution in test tube .
3. Add 500 µl of 5% phenol to test tube.
4. Add 2.5 ml of 96% sulfuric acid to test tube and mixed with vortex mixer.
5. Incubate 10 min and measure at 490 nm. Calculate the amount of total sugar present in the sample solution using the standard curve.

Absorbance corresponds to 0.5 ml of the test tube = x mg of glucose

1 ml of the sample solution contain = (x/0.5) x 100 mg of glucose

= % of total sugar

**Table 1A** Absorbance at 490 nm of glucose standard

| Glucose concentration | Absorbance (490 nm) | | | |
|---|---|---|---|---|
| | Duplicate1 | Duplicate2 | Duplicate3 | Mean |
| 0 | 0 | 0 | 0 | 0 |
| 50 | 0.391 | 0.339 | 0.343 | 0.358 |
| 100 | 0.834 | 0.785 | 0.658 | 0.759 |
| 150 | 1.256 | 1.263 | 1.215 | 1.245 |
| 200 | 1.92 | 1.658 | 1.612 | 1.730 |
| 250 | 2.449 | 1.963 | 2.001 | 2.138 |

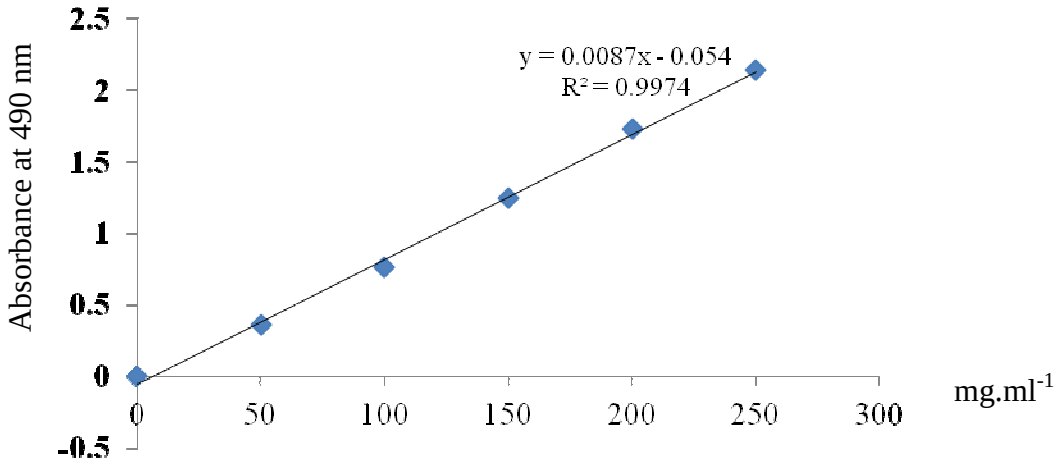


**Figure 1A** Standard curve of glucose