Method 10.1 – Sugar traces: the phenol-sulphuric acid method

1. Rationale

The method is applicable to water, boiler water and effluent and is used to determine the amount of trace sugars in the sample. Although the method is simpler to perform than the resorcinol method (Method 10.2) it is not specific to sugars and other carbohydrates may interfere. The method is also only applicable to sucrose contents of up to 70 - 80 mg/kg.

2. Principle

A standard graph is plotted using solutions with a sucrose content of 0 to 50 mg/kg. Each water sample is reacted with phenol and sulphuric acid and the absorbance of the resulting solution is read on a spectrophotometer at 490 nm against distilled water.

3. Apparatus

3.1 **Pipettes**: 1, 6 × 2 cm^3 and 10 cm^3 graduated

3.2 **Burette**: 50 cm^3, fast flowing stopcock

3.3 **Test tubes**: 6 × 30 cm^3

3.4 **Test tube rack**

3.5 **Spectrophotometer** capable of reading at 490 nm

3.6 **Optical glass cell**: 10 mm

3.7 **Filter paper**: Whatman No.91, S&S 3000 or equivalent, 185 mm ϕ

3.8 **Funnel**: 100 mm ϕ, stemless

3.9 **Beaker**: 250 cm^3

3.10 **Volumetric flasks**: 5 × 100 and 1 000 cm^3

3.11 **Analytical balance** readable to 0.001 g

4. Reagents

4.1 **Phenol solution** (5%)

Phenol (C_6H_5OH) is a corrosive solid and should be handled with gloves while wearing safety glasses.

Dissolve 5 g of phenol in distilled water and dilute to 100 cm^3 in a volumetric flask.

4.2 Sulphuric acid (concentrated)

Sulphuric acid (H_2SO_4) is a corrosive acid and should be handled with gloves while wearing safety glasses.

4.3 Refined sugar: first boiling

5. Procedure

5.1 Standard graph

Weigh 0.5 g refined sugar accurately to 0.001 g. Dissolve in distilled water and transfer quantitatively to a 1 000 cm^3 volumetric flask and make to the mark.

Pipette the aliquots indicated in Table 1 into 100 cm^3 volumetric flasks to prepare the standard solutions.

Table 1: Standard solutions

Aliquot of stock solution (cm^3)	Sugar in standard solution (mg/kg)
2	10
4	20
5	25
8	40
10	50

Make each flask to the mark with distilled water and mix. Pipette 2 cm^3 of each standard into a series of test tubes, adding one additional test tube with 2 cm^3 of distilled water to serve as the blank. Add 1 cm^3 of the phenol solution to each test tube and shake to mix.

Fill the burette with concentrated sulphuric acid. Add 5 cm^3 of sulphuric acid from the burette to each test tube so that the acid strikes the surface of the liquid in the test tube from straight above. Shake to mix and stand at room temperature for 20 minutes.

Cool the test tubes in running water and shake again to mix. Read the absorbance of the solutions in a 10 mm cell in the spectrophotometer at 490 nm against distilled water as a reference.

5.2 Samples

If the sample is not clear filter through fluted filter paper supported in a funnel which rests directly on a beaker. Pipette 2 cm^3 of clear sample into a test tube. Pipette 2 cm^3 distilled water into another test tube and use as the blank. Add 1 cm^3 of the phenol solution and shake to mix.

Fill the burette with concentrated sulphuric acid. Add 5 cm^3 of sulphuric acid from the burette to the test tubes so that the acid strikes the surface of the liquid in the test tube from straight above. Shake to mix and stand at room temperature for 20 minutes.

Cool the test tubes in running water and shake again to mix. Read the absorbance of the solutions in a 10 mm cell in the spectrophotometer at 490 nm against distilled water as a reference.

6. Expression of Results

6.1 Standard graph

Subtract the absorbance of the blank from the absorbences of the other five solutions. Plot these absorbance (AU) values against the sucrose concentrations (mg/kg). This graph should be a straight line passing through the origin. Calculate the slope (absorbance over concentration) using linear regression and use the slope when determining the amount of sugar in the samples.

6.2 Samples

Subtract the absorbance of the blank from the absorbance of the sample solution. Calculate the sugar content of the sample using the slope of the graph as indicated below.

$$\text{Sugar (mg/kg)} = \frac{\text{Absorbance of the sample (AU)}}{\text{slope (AU kg/mg)}}$$

Report in mg/kg to the nearest unit.

7. Example

7.1 Standard graph

Mass of sugar used = 0.509 g
Concentration of standard solution = 0.509 mM

Table 2: Standard solutions

Aliquot of stock solution (cm^3)	Sugar in standard solution (mg/kg)	Absorbance of solution (AU)	Absorbance of sample (AU)
0	0.00	0.012	0.000
2	10.18	0.150	0.138
4	20.36	0.300	0.288
5	25.45	0.380	0.368
8	40.72	0.608	0.596
10	50.90	0.751	0.739

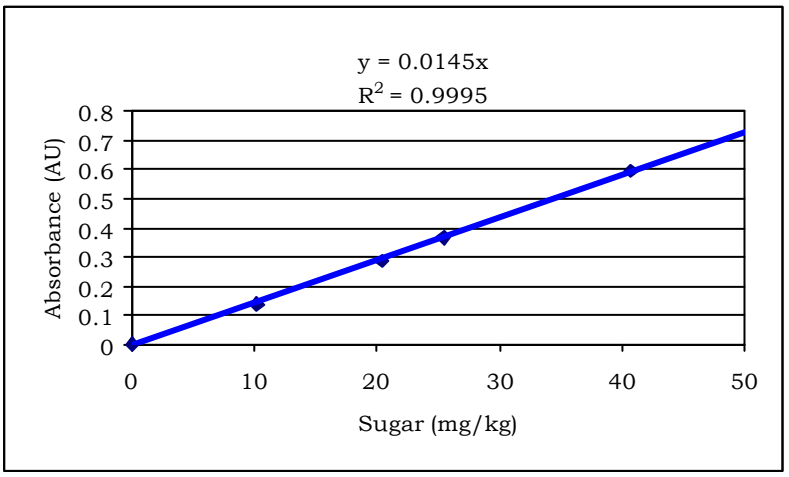

Figure 1: Standard phenol-sulphuric acid graph

Slope = 0.0145 AU kg /mg

7.2 Samples

Absorbance of water	=	0.001 AU
Absorbance of the blank	=	0.012 AU
Absorbance of the solution	=	0.478 AU
Absorbance of the sample	=	(0.478 - 0.001) AU - (0.012 - 0.001) AU
	=	0.466 AU

$$\text{Sugar in the sample} = \frac{0.466\ \text{AU}}{0.0145\ \text{AU kg/mg}}$$

$$= 32.14\ \text{mg/kg}$$

Report as 32 mg/kg

8. References

SASTA (1985). *Laboratory Manual for South African Sugar Factories*. 3rd Edition: 204 - 205, 366 - 367.