Method 10.2 – Sugar traces: the resorcinol method

1.

This applicable to water, boiler water and effluent and is used to determine the amount of the sugars in the sample. Although the method is more complicated to perform than the phenol-sulphuric acid method (Method 10.1) it is more specific to sugars and will experience less interference from other hydrocarbons. The method is applicable to sucrose contents of up to 200 mg/kg.

2. Principle

Due to variations in the resorcinol reagent a standard graph is prepared once a day using solutions with a sucrose content of 0 to 200 mg/kg. Each water sample is reacted with resorcinol in a boiling water bath. The absorbance of the resulting solution is read on a spectrophotometer at 480 nm against distilled water.

3. Apparatus

3.1   **Pipettes**: 1, 5×2 cm^3 and 10 cm^3 graduated

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

3.3   **Test tubes** with plastic stoppers: 5×30 cm^3

3.4   **Test tube rack**

3.5   **Spectrophotometer** capable of reading at 480 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**: 4×100 and 2000 cm^3

3.11   **Analytical balance** readable to 0.001 g

3.12   **Boiling water bath**

4. Reagents

4.1   **Ferric chloride hexahydrate** ($FeCl_3 \cdot 6H_2O$)

4.2   Hydrochloric acid (concentrated, 33-40%)

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

4.3   Resorcinol- hydrochloric acid reagent

Resorcinol [$C_6H_4(OH)_2$] is toxic and corrosive to the skin, eyes and respiratory tract. Work in a fume cupboard while wearing gloves and safety glasses during use.

Weigh 0.2 g of resorcinol and 0.3 g of ferric chloride hexahydrate and mix with 1 000 cm^3 concentrated hydrochloric acid. Stir until all the solids have dissolved. Store in a dark bottle to protect against sunlight.

4.4   Benzoic acid solution (0.01%)

Benzoic acid (C_6H_5COOH) is severely irritating to the skin, eyes and respiratory tract and should only be handled in a fume cupboard with gloves while wearing safety glasses.

Weigh 0.20 g benzoic acid and transfer to a 2 000 cm^3 volumetric flask using 1 000 cm^3 distilled water. Stopper and shake vigorously until all the benzoic acid has dissolved. Make to the mark and mix thoroughly.

4.5   Refined sugar: first boiling

5. Procedure

5.1   Standard graph

Weigh 0.5 g refined sugar accurately to 0.001 g. Dissolve in the benzoic acid solution and transfer quantitatively to a 1 000 cm^3 volumetric flask. Make to the mark with benzoic acid and mix thoroughly.

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
10	50
20	100
40	200

Make each flask to the mark with the benzoic acid solution and mix. These standard solutions will last for one month if kept in a refrigerator.

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.

Fill the burette with resorcinol reagent. Add 8 cm^3 of resorcinol reagent from the burette to each test tube. Stopper each test tube and invert three times to mix the contents.

Place the test tubes in a boiling water bath for 5 minutes ± 5 seconds. Remove the test tubes from the water bath and cool in running water for 5 minutes.

Read the absorbance of the solutions in a 10 mm cell in the spectrophotometer at 480 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.

Fill the burette with resorcinol reagent. Add 8 cm^3 of resorcinol reagent from the burette to the test tubes. Stopper the test tubes and invert three times to mix the contents.

Place the test tubes in a boiling water bath for 5 minutes ± 5 seconds. Remove the test tubes from the water bath and cool under running water for 5 minutes.

Read the absorbance of the solution and the blank in a 10 mm cell in the spectrophotometer at 480 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 four 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.

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

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	0.051	0.039
10	50.90	0.207	0.195
20	101.80	0.412	0.400
40	203.60	0.783	0.771

Slope   =   0.0038 AU kg/mg
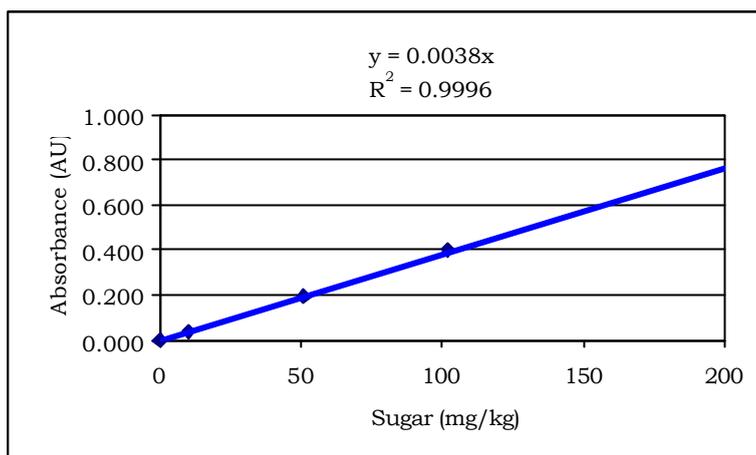
Figure 1: Standard resorcinol graph

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

Sugar in the sample   = $\dfrac{0.466 \text{ AU}}{0.0038 \text{ AU kg/mg}}$

                                 =   122.63 mg/kg

Report as 123 mg/kg

8. References

SASTA (1985). *Laboratory Manual for South African Sugar Factories.* 3rd Edition: 205 - 206, 366 - 368.