Supelco®
Analytical Products

Application Note

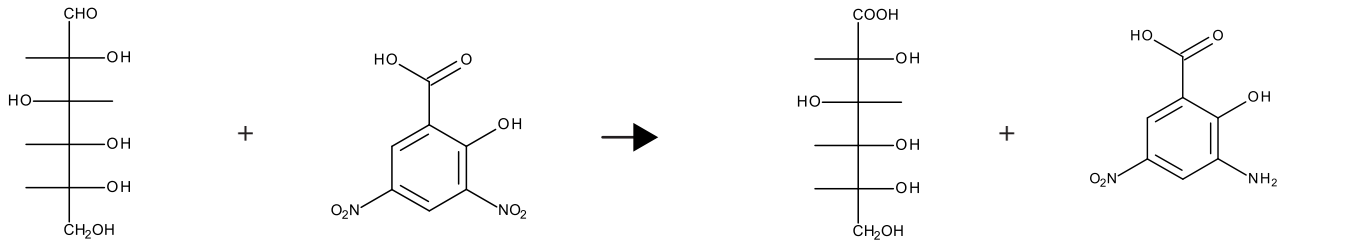
Determination of Sugars in Beverages

Photometric determination using 3,5-dinitrosalicylic acid (DNSA) after acid hydrolysis

Introduction

In the context of a health-conscious diet, the sugar content of foodstuffs plays an important role. In many legal regulations, such as e.g. the Food Information Regulation (FIR) of the European Union, it is also prescribed that foodstuff labels must list the sugar content of the product.[1] The determination of the sugar content of food products is thus obligatory. Due to their high sugar content, this is particularly relevant in connection with fruit juices and soft drinks. Efforts are currently under way to create statutory provisions aimed to reduce the sugar content of such products or to impose special taxes on them, since an excessive uptake of sugar due to the consumption of these products is considered as one of the main causes of metabolic disorders and obesity [2].

One well-known method for the detection of sugars is the chemical reaction with 3,5-dinitrosalicylic acid. Reducing sugars such as glucose reduce the yellow 3,5-dinitrosalicylic acid to red-brown 3-amino-5-nitrosalicylic acid, the color of which is determined photometrically at 580 nm. Fruit juices and soft drinks frequently contain added sugar, mainly sucrose, made from sugar cane or sugar beet. Sucrose is a disaccharide and not a reducing sugar and can hence not be directly detected by the reaction principle described above. It can, however, be relatively easily cleaved by acid hydrolysis into the reducing monosaccharides glucose and fructose. To cleave the disaccharide, hydrochloric acid is added to the sample and the solution is boiled, after which sodium hydroxide solution is added to yield a basic pH value. Then 3,5-dinitrosalicylic acid is added and the color reaction is performed in the boiling solution.



D-Glucose | 3,5-dinitrosalicylic acid (yellow) | D-Gluconic acid | 3- amino-5-nitrosylicylic acid (red-brown)

Figure 1: Reaction of DNSA with sugars [3]

Experimental

This Application Note describes the determination of sugars in beverages. The method is preprogrammed on the corresponding Spectroquant® Prove photometers with firmware version 1.5 or above. Nevertheless, a user-calibration is recommended to obtain the most accurate results.

Method

Non-reducing sugars are converted into reducing sugars by acid hydrolysis. These sugars reduce 3,5 dinitrosalicylic acid (DNSA) to the red-brown 3 amino-5 nitrosalicylic acid, which is determined photometrically. The same concentration of different sugars may result in slightly different signals of absorption. The pre programmed method in the Spectroquant® Prove spectrophotometers is calibrated with the reference substance glucose.

Measuring range

0–200 g/l sugar as glucose (method no. 314)

Sample material

Beverages (e.g. fruit juices and soft drinks)

MERCK

Reagents, Instruments and Materials:

Cat. No.	Description
Reagents	
D0550	3,5-Dinitrosalicylic acid (Sigma)
110164	Hydrochloric acid 6 mol/l EMPROVE®
105590	Sodium hydroxide solution about 32 % for analysis EMSURE®
Only necessary if calibration is performed:	
108337	D(+)-Glucose anhydrous for biochemistry
Instruments	
For sugar measurement one of the following Spectroquant® photometers is necessary:	
1.73026	Spectroquant® VIS Spectrophotometer Prove 100 plus
1.73027	Spectroquant® UV/VIS Spectrophotometer Prove 300 plus
1.73028	Spectroquant® UV/VIS Spectrophotometer Prove 600 plus

Also first generation Prove instruments are compatible and preprogrammed with this method.

Software for data maintenance

The Spectroquant® Prove Connect to LIMS software package provides an easy way to transfer your data into an existing LIMS system. This software can be purchased under:

Cat. No.	Description
Y11086	Prove Connect to LIMS
Materials	
114944	Rectangular cell 50 mm
114901	Flat-bottomed long tubes with screw caps

Pipettes for pipetting volumes of 2.0 and 8.0 ml
Analytical balance

Standard laboratory glass ware
(measuring flasks, beakers, etc.)

Water bath
Ice bath

Only necessary for turbid solutions:

Funnel
Filters

Only necessary for sparkling samples (one of the following is sufficient):

Ultrasonic bath
Stirrer
Nitrogen gas

Analytical approach

Reagent preparation

DNSA-reagent (0.05 mol/l)
In a 100-ml volumetric flask: dissolve 1.1406 g 3,5 dinitrosalicylic acid in approx. 80 ml H_2O, make up to 100 ml with distilled water and mix. If the reagent is not completely dissolved filtrate the solution using a filter.

Sodium hydroxide solution 2.5 mol/l
In a 250-ml volumetric flask: mix 78 g of sodium hydroxide solution 32 % with approx. 150 ml distilled water, cool to room temperature, make up to 250 ml with distilled water and mix

Sample preparation

- Dilute the sample 1:200 with distilled water. Therefore, pipette 0.5 ml sample into a 100-ml volumetric flask and make up to 100 ml with distilled water.
- Filter turbid samples.
- Expel CO2 from samples (either by ultrasonic bath, stirring or introducing nitrogen gas).

Preparing the measurement solutions

Reagent blank (prepare daily)

- Pipette **2.0 ml distilled water** into a 20 ml closable test tube.
- Add **2.0 ml hydrochloric acid 6 mol/l** with a pipette and mix.
- Place test tube for exactly **10 min in a water bath 95 ± 5 °C**.
- Add **8.0 ml sodium hydroxide solution 2.5 mol/l** with a pipette and mix.
- Add **2.0 ml DNSA reagent** with a pipette and mix.
- Place for exactly **5 min in a water bath 95 ± 5 °C**.
- Cool for exactly **10 min in an ice-/water-bath**.
- Leave to stand for **10 min at room temperature** (to avoid fogging up of the cell).

Measurement sample

- Pipette **2.0 ml prepared sample solution** into a 20 ml closable test tube.
- Add **2.0 ml hydrochloric acid 6 mol/l** with a pipette and mix.
- Place test tube for exactly **10 min in a water bath 95 ± 5 °C**.
- Add **8.0 ml sodium hydroxide solution 2.5 mol/l** with a pipette and mix.
- Add **2.0 ml DNSA reagent** with a pipette and mix.
- Place for exactly **5 min in a water bath 95 ± 5 °C**.
- Cool for exactly **10 min in an ice-/water-bath**.
- Leave to stand for **10 min at room temperature** (to avoid fogging up of the cell).

Measurement

- Open the method list (<Methods>) and select method No. 314 “Sugar”.
- It is necessary to zero the method for each measurement series: Open the method, tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. Fill the 50-mm rectangular cell with distilled water. After prompting, insert the filled rectangular cell into the cell compartment. The zero adjustment is performed automatically. Confirm the performance of the zero-adjustment procedure by clicking on <OK>.
- Subsequently perform the reagent blank one time for each measurement series: Tap the <Settings> button and select the <REAGENT BLANK> menu item. Fill the 50-mm rectangular cell with the reagent blank and insert the cell into the cell compartment. The measurement is performed automatically. Accept the reagent blank by activating the <User RB> field and confirm with <OK>.

- After the reagent blank has been measured, fill the measurement sample into the same or a matched 50-mm rectangular cell and insert the cell into the cell compartment. The measurement starts automatically.
- Read off the result in g/l from the display.

Data transfer Prove spectrophotometers (optional)

After measurement transfer the values measured on the Prove spectrophotometer using Prove Connect to LIMS.

Calibration

The reaction times of this method are crucial for the result. Therefore, it is recommended to perform a calibration for each measurement series so that the reaction times of the standards and the samples are the same.

Furthermore, a user-calibration may also be useful if a reference substance other than glucose is used.

In this chapter we explain step-by-step how to prepare user-defined calibration using the prove instruments.

Preparing the calibration standards

Calibration can be carried out with practically any analytical pure sugar, that is either already reducing or can be cleaved to provide a reducing sugar. Glucose is frequently used as a reference substance. To prepare a standard solution, dissolve exactly 1.000 g Glucose in 80 ml distilled water in a volumetric flask and make up to 100 ml with distilled water. This stock solution (10 g/l) can then be diluted as required.

For details on how to prepare the calibration standards see the following table.

	Glucose Concentration					
concentration	**0 g/l (E0)**	**40 g/l**	**80 g/l**	**120 g/l**	**160 g/l**	**200 g/l**
10 g/l glucose stock solution [ml]	0.0	2.0	4.0	6.0	8.0	10.0
	Pipette into a 100-ml volumetric flask and make up to the mark with distilled water.					

Preparing the measurement solutions for the calibration standards

When performing the calibration, it is recommended to prepare all measurement solutions (standards and sample solutions) at the same time so that all reaction times are the same. This procedure ensures getting the most accurate results.

- **Dilute each standard solution 1:200** with distilled water. Therefore, pipette 0.5 ml standard solution into a 100-ml volumetric flask and make up to 100 ml with distilled water.
- Pipette **2.0 ml of each diluted standard solution** into separate 20 ml closable test tubes.
- Add **2.0 ml hydrochloric acid 6 mol/l** with a pipette and mix.
- Place test tube for exactly 10 min in a water bath 95 ± 5 °C
- Add **8.0 ml sodium hydroxide solution 2.5 mol/l** with a pipette and mix.
- Add **2.0 ml DNSA reagent** with a pipette and mix.
- Place for exactly **5 min in a water bath 95 ± 5 °C.**
- Cool for exactly **10 min in an ice-/water-bath.**
- Leave to stand for **10 min at room temperature** (to avoid fogging up of the cell).

User-defined method calibration

- Open the method list (<Methods>) and select method No. 314 “Sugar”.
- It is recommended to zero the method each new working day. To do this, open the method, tap the <Settings> button and select the <ZERO ADJUSTMENT> menu item. Follow the instructions shown on the display to proceed.
- Tap the **<Settings>** button.
- Select the **<RECALIBRATION>** menu item. An input mask pops up.
- Tap twice on **<+>** in the numerical keyboard to create two additional input lines.
- Select the **“Absorbance“** field in the **“E0“** line (selected fields are shown in a blue frame).

  Fill the prepared calibration solution E0 into a 50-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.
- Select the **“Conc.“** field in the **“1“** line and enter the concentration of **40 g/l** for the first calibration solution.

  Select the **“Absorbance”** field in the **“1“** line. Fill calibration solution 1 into a 50-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.
- Select the **“Conc.“** field in the **“2“** line and enter the concentration of 80 g/l for the second calibration solution.

  Select the **“Absorbance“** field in the **“2“** line. Fill calibration solution 2 into a 50-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.
- Select the **“Conc.“** field in the **“3“** line and enter the concentration of 120 g/l for the third calibration solution.

  Select the **“Absorbance“** field in the **“3“** line. Fill calibration solution 3 into a 50-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.
- Select the **“Conc.“** field in the **“4“** line and enter the concentration of 160 g/l for the fourth calibration solution.

  Select the **“Absorbance“** field in the **“4“** line. Fill calibration solution 4 into a 50-mm rectangular cell and insert cell into the cell compartment. The

measurement starts automatically. The measured absorbance is shown in the display.

- Select the **"Conc."** field in the **"5"** line and enter the concentration of 200 g/l for the fifth calibration solution.

  Select the **"Absorbance"** field in the **"5"** line. Fill calibration solution 5 into a 50-mm rectangular cell and insert cell into the cell compartment. The measurement starts automatically. The measured absorbance is shown in the display.

- Activate the **<U-CAL on>**, **<linear>** and **<Fit E0>** fields. Optionally enter a batch number for the calibration, selecting the **<Lot number>** field to do so.

- Once all calibration solutions have been measured, save the calibration by pressing **<OK>**.

Results

The results obtained with the method described above were compared to the sugar concentrations stated on the package of the tested beverages.

Beverage	Sugar Concentration [g/l]		Recovery Rate [%]
	DNSA method	manufacturer specification	
White Grape Juice	161	160	101
Red Grape Juice	155	158	98
Apple Juice	106	102	104
Orange Juice	90	90	100
Multivitamin Juice	119	119	100
Lemonade	93	91	102
Orangeade	94	92	102
Cola	106	106	100

The measured values match to those of the manufacturer specification. The recovery rates for all tested beverages are almost 100 %.

Interferences

There are several substances known to interfere the sugar determination with the DNSA reagent. These include several amino acids (e.g. tryptophan, cysteine, histidine, tyrosine, or hydroxyproline) and oxygen. The interference of dissolved oxygen can be reduced by the addition of sodium sulfite. [3, 4]

Analytical Quality Assurance

The objective of analytical quality assurance (AQA) is to secure correct and precise measurement results.

AQA is recommended before each measurement series. To check the measurement system (test reagents, measurement device, and handling) a self-prepared glucose standard solution can be used. For details on how to prepare the standards see chapter "calibration - preparing the standard solutions". Sample-dependent interferences (matrix effects) can be determined by means of standard addition.

For details on how to perform the AQA check see the instrument-specific manuals.

Conclusion

The DNSA method is an easy and fast way to analyze the sugar concentration in your sample. The measurement can be performed without high instrumental expense.

The results are accurate and match the sugar concentrations specified by the manufacturer.

For more information
SigmaAldrich.com/photometry

References

1 REGULATION (EU) No 1169/2011 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 25 October 2011 on the provision of food information to consumers, L 304/18.

2 German Federal Government, "Gesunde Ernährung - Weniger Zucker, Fette und Salz in Fertigprodukten", 19 Deck 2018

To be found at: https://www.bundesregierung.de/breg-de/aktuelles/weniger-zucker-fette-und-salz-in-fertigprodukten-1562296

3 A.S. Verma, S. Das, A. Singh, Laboratory manual for biotechnology, 1st edition, 2014.

4 R.S.S. Teixeira et al, Carbohydr Res., 2012, 363, 33-37.

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