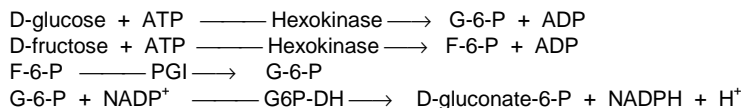


UV method for approx. 32 assays each

 For laboratory use only
 Store between +2 and +8°C

The method is contained in the Austrian, German, Italian, Swiss food laws and in European regulation. Recommended e.g. by IFU, AIJN, MEBAK, OICCC, OIV. Standardized by DIN, EN, GOST, NEN, NF. Approved by AOAC.

Principle



Ref.: Schmidt, F.H. (1961) Die enzymatische Bestimmung von Glucose und Fructose nebeneinander, Klinische Wochenschrift 39, 1244-1247

Assay performance

Wavelength: 340 nm (NADPH), $\epsilon = 6.3 \text{ l} \times \text{mmol}^{-1} \times \text{cm}^{-1}$
 Light path: 1.00 cm (glass or plastic cuvettes)
 Temperature: +20 to +25°C
 Assay volumes: 3.020 ml (D-glucose),
 3.040 ml (D-fructose)
 Measurement: against air or against water
 Sample solution: 1 to 100 µg D-glucose + D-fructose in 0.100 to 2.000 ml sample solution

Reagents

- # 1: Powder mixture with triethanolamine buffer, pH approx. 7.6, approx. 80 mg NADP, approx. 190 mg ATP, magnesium sulfate (for stability see pack label). *Dissolve contents of bottle # 1 with approx. 31 ml redist. water.* The solution is stable for 1 month at +2 to +8°C, resp. for 2 months at -15 to -25°C.
- # 2: Approx. 0.7 ml hexokinase (HK) / glucose-6-phosphat dehydrogenase (G6P-DH) suspension (approx. 200 U / 100 U) in ammonium sulfate (for stability see pack label). *The suspension is ready for use.* Swirl bottle carefully before the suspension is pipetted.
- # F: Approx. 0.7 ml phosphoglucose isomerase (PGI) suspension (approx. 490 U) in ammonium sulfate (for stability see pack label). *The suspension is ready for use.* Swirl bottle carefully before the suspension is pipetted.

In addition (not contained in the kit):

Standard solution D-glucose, anhydrous, ultrapure, 0.5 g/l for test control only.
 Standard solution D-fructose, ultrapure, 0.5 g/l for test control only.

The reagents for the determination of D-glucose and D-fructose are not hazardous. The general safety rules for the work in chemical laboratories should be applied. After use the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

Procedure

Pipette into cuvettes:	Blank	Standard ¹	Sample ²	Rerun assay ³	Assay with internal standard ⁴	High sensitive assay ⁵
Tea buffer, NADP, ATP solution # 1	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000 ml	1.000 ml
Sample solution⁶ (e.g. 0.05 to 0.5 g D-glucose+D-fructose/l)	-	-	0.100 ml	0.200 ml	0.100 ml	2.000 ml
Standard solution ⁶ (e.g. 0.5 g D-gluc. or D-fru./l)	-	0.100 ml	-	-	0.100ml	-
Redist. Water	2.000 ml	1.900 ml	1.900 ml	1.800 ml	1.800 ml	-
Mix⁷, after approx. 3 min read the absorbances (A₁). Add:						
HK/G6P-DH suspension # 2	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml
Mix⁷, after approx. 10 to 15 min read the absorbances (A₂). Repeat absorbance reading after another 2 min⁸. Add:						
PGI suspension # F	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml	0.020 ml
Mix⁷, after approx. 10 to 15 min read the absorbances (A₃).						

Notes

- Run a "standard" to see "accidents" in analysis. The measurement of the standard is not necessary for calculating results.
- This assay together with the blank is a single determination.
- In the case of a double determination, run two assays with different sample volumes. The absorbance differences measured have to be proportional to the sample volumes (calculate with resp. volumes).
- Recovery = $[(\Delta A_{\text{sample+standard}} - \Delta A_{\text{sample}}) / \Delta A_{\text{standard}}] \times 100 [\%]$
- Assay recommended in the case of trace level compound analysis, with sample volume increased up to 2.000 ml (0.0004 to 0.05 g/l D-glucose, resp. D-fructose, resp. D-glucose + D-fructose).
- Before dispensing, rinse the enzyme pipette, resp. the tip of the piston pipette with sample resp. with standard solution.
- e.g. with a plastic spatula, or after closing the cuvette with Parafilm (trademark of American Can Co., Greenwich Ct., USA)
- The reaction has stopped when the absorbance is constant. If the reaction has not stopped, continue to read absorbances until they increase constantly over 2 min. Extrapolate absorbances to the time of the addition of HK/G6P-DH (suspension # 2).

Calculation

$$\Delta A_{D\text{-glucose}} = (A_2 - A_1)_{\text{sample, resp. standard}} - (A_2 - A_1)_{\text{blank}}$$

$$\Delta A_{D\text{-fructose}} = (A_3 - A_2)_{\text{sample, resp. standard}} - (A_3 - A_2)_{\text{blank}}$$

$$c = (V \times MW \times \Delta A) / (\epsilon \times d \times v \times 1000) \quad [\text{g D-glucose or D-fructose/l sample solution}]$$

$$c = (3.020 \times 180.16 \times \Delta A) / (6.3 \times 1.00 \times 0.100 \times 1000) = \mathbf{0.8636 \times \Delta A} \quad [\text{g D-glucose/l sample solution}]$$

$$c = (3.040 \times 180.16 \times \Delta A) / (6.3 \times 1.00 \times 0.100 \times 1000) = \mathbf{0.8693 \times \Delta A} \quad [\text{g D-fructose/l sample solution}]$$

If the sample has been diluted during preparation, multiply the result with the dilution factor F.

When analyzing samples which are weighed out for sample preparation, calculate the content from the amount weighed:

$$\text{Content}_{D\text{-gluc/D-fruc}} = \frac{C_{D\text{-gluc/D-fruc}} [\text{g/l sample solution}]}{\text{weight}_{\text{sample}} [\text{in g/l sample solution}]} \times 100 [\text{g/100 g}]$$

Sample preparation

If the sample has one of the characteristics below, which hamper the test, please follow the corresponding sample preparation procedure:

1. Dilute *clear, colourless and almost neutral liquid samples* to get a sample solution with 0.05 to 0.5 g D-glucose + D-fructose /l.
2. Filter or centrifuge *turbid solutions*, dilute (see pt. 1).
3. Degas *samples containing carbon dioxide*, e.g. by filtration, or add NaHCO₃ until the solution is slightly alkaline, dilute (see pt. 1).
4. Adjust *acid (esp. slightly coloured) solutions* with KOH or NaOH to approx. pH 7, incubate a few minutes, or dilute (see pt. 1) without pH adjustment in the case of colourless samples.
5. Treat "*strongly coloured solutions*" used undiluted with PVPP, e. g. 1 g/100 ml, mix, incubate a few minutes, filter.
6. Crush (corn size < 0.3 mm) or homogenize *solid or semi-solid (pasty) samples*, extract with water, or dissolve in water, filter and dilute (see pt. 1) if necessary.
7. Extract *fat containing samples* with hot water at a temperature above the melting point of fat, e.g. in a 100 ml volumetric flask. Adjust to +20°C, fill volumetric flask to the mark. Store in ice or in refrigerator for approx. 15 resp. 30 min, filter.
8. Clarify *samples containing protein* with Carrez reagents
Weigh sufficient quantity of solid or pasty sample into 100 ml volumetric flask, add approx. 60 ml water. Or pipette liquid sample into 100 ml volumetric flask containing approx. 60 ml water. Add, and mix after each addition, 5 ml Carrez-I-solution (3.60 g K₄[Fe(CN)₆] x 3H₂O = potassium hexacyanoferrate(II)/100 ml), 5 ml Carrez-II-solution (7.20 g ZnSO₄ x 7 H₂O = zinc sulfate hepta-hydrate/100 ml). Adjust to pH 7.5 to 8.5 by the addition of e.g. 10 ml NaOH (0.1 M). Fill the flask to the mark, mix and filter.
9. Deproteinize *samples containing protein* with perchloric acid **only in the absence of sucrose and maltose**.

Assay characteristics

1. **Specific** for D-glucose and D-fructose. In the analysis of commercial D-glucose, D-glucose monohydrate and D-fructose results of < 100 % have to be expected because the materials absorb moisture.
2. **Sensitivity:** 0.2 mg/l D-glucose or D-fructose
($\Delta A = 0.005$; $v = 2.000$ ml; $V = 3.020/3.040$ ml)
3. **Detection limit:** 0.4 mg/l D-glucose or D-fructose
($\Delta A = 0.010$; $v = 2.000$ ml; $V = 3.020/3.040$ ml)
4. **Linearity:** from 1 µg D-glucose + D-fructose/assay ($v = 2.000$ ml; $V = 3.020/3.040$ ml)
to 100 µg D-glucose + D-fructose/assay ($v = 0.100$ ml; $V = 3.020/3.040$ ml)
5. **Precision:** $\Delta A = \pm 0.005$ absorbance units
CV = approx. 1 to 2 %
Fruit juice: $r = 0.42 + 0.027 \times C_{D\text{-glucose in g/l}} [\text{g/l}]$
 $r = 0.15 + 0.033 \times C_{D\text{-fructose in g/l}} [\text{g/l}]$
 $R = 1.0 + 0.042 \times C_{D\text{-glucose in g/l}} [\text{g/l}]$
 $R = 1.05 + 0.045 \times C_{D\text{-fructose in g/l}} [\text{g/l}]$
Wine: $r = 0.056 \times C_{D\text{-glucose, resp. D-fructose in g/l}} [\text{g/l}]$
6. **Interferences:** none known.
7. **Technical Information:** the reagents can also be used for the determination of sucrose (with additional β -fructosidase).