Lactose and D-Galactose (Enzymatic method)
GEA Niro analytical method A 18 c

1. Principle

Lactose is hydrolyzed to D-glucose and D-galactose at pH 6.6 in the presence of the enzyme β-galactosidase and water (1).

(1) Lactose + H_2O D-glucose + D-galactose

D-galactose is oxidized at pH 8.6 by nicotinamide-adenine dinucleotide (NAD) to D-galactonic acid in the presence of the enzyme β-galactose dehydrogenase (Gal -DH) (2).

(2) D-galactose + NAD^+ D-galactonic acid + NADH + H^+

The amount of NADH formed in reaction (2) is stoichiometric to the amount of lactose and D-galactose respectively. The increase in NADH is measured by means of its light absorbance at 340 nm.

2. Scope

The method is to be used for milk powders, liquid milk and milk concentrates.

3. Apparatus

1. Spectrophotometer Perkin Elmer, Lambda 2, UV/VIS.
2. Analytical balance - capable of weighing to 0.0001 g.
3. Water bath - thermostatically controlled to 70°C ± 1°C.
4. 100 ml volumetric flask.
5. 50 ml Erlenmeyer flask with stopper.
6. Glass funnel - short stem 65 mm, diameter 50 mm.
7. Pleated filter paper - 11 cm, S&S No. 589.
8. Micropipette (Socorex) & microtip.
9. Volumetric pipettes 5 - 7 - 10 ml.
10. Weighing dish, disposable.
11. Disposable cuvettes, Brand Cat. No. 7590 05.
12. Parafilm "M"

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4. Chemicals

1. Lactose/D-galactose UV-Test No. 176 303, Boehringer Mannheim.

   1.1 Bottle 1 containing 600 mg of lyophilisate, citrate buffer pH 6.6, NAD approx. 35 mg, magnesium sulphate and stabilizers.
   This solution is stable for 3 months at 4°C.

   1.2 Bottle 2 containing 1.7 ml of a β-galactosidase suspension.

   1.3 Bottle 3 containing 34 ml of a solution of potassium diphosphate buffer, pH 8.6 and stabilizers.

   1.4 Bottle 4 containing 1.7 ml of a galactose dehydrogenase suspension.

   1.5 Bottle 5 containing lactose standard.

   Store all bottles at 4°C, see packing label for recommended storage time.
   Bring all bottles to room temperature before use.

2. Zinc sulphate heptahydrate, p.a. Merck.
   $ZnSO_4$, 7 H_2O: R 36 and S 24.

3. Potassium hexacyanoferrate(II) trihydrate, p.a.
   Merck. K_4 $(Fe(CN)_6)$, $3H_2O$.

4. Sodium hydroxide Titrisol 0.1 N, p.a. No. 9956 Merck.
   NaOH: R 36/37 and S 26.

R ≈ DK risk sentences.

S ≈ DK safety sentences.

5. Reagents

1. Solution 1:
   Dissolve contents of bottle 1 with 7.0 ml of deionized water.
   Solution 1 is stable for 3 months at 4°C.

2. Suspension 2:
   Use bottle 2.

3. Solution 3:
   Use bottle 3.

4. Suspension 4:
   Use bottle 4.

Bring all solutions and suspensions to room temperature before use.

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5. Carrez-I solution:
   Dissolve 3.60 g of potassium hexacyanoferrate-II in 100 ml of deionized water.
6. Carrez-II solution:
   Dissolve 7.20 g of zinc sulphate in 100 ml of deionized water.
7. 0.1 N NaOH:
   Dilute 0.1 N Titrisol to 1000 ml with deionized water.

6. Procedure

1. Weigh out an amount of sample corresponding to a final expected concentration of lactose and D-galactose between 0.2 and 1.0 g/l.
   If the amount is unknown, weigh out 0.2 to 0.4 g with an accuracy of 0.1 mg in a 100 ml volumetric flask.
2. Add approx. 60 ml water and incubate for 15 min. at 70°C in a water bath; shake from time to time.
3. Add 5 ml of Carrez-I, 5 ml of Carrez-II and 10 ml of 0.1 mol/l NaOH. Shake vigorously after each addition.
4. Adjust to room temperature and fill up to the mark with water.
5. Mix the solution and filter it through a paper filter into an Erlenmeyer flask. Re-filter the first few ml of the filtrate, as these are usually unclear.
6. Carry out a blank test, proceeding as described above, using all the reagents but omitting the sample.
7. Add the amounts specified in the scheme to disposable cuvettes. Treat the blank test of the reagent as a sample. Read absorption against water using the flow-through cuvette.

Pipette into cuvettes	Blank lactose samples ml	Lactose sample ml	Blank D-galactose sample ml	D-galactose sample ml
Solution 1	0.200	0.200	0.200	0.200
Suspension 2	0.050	0.050	-	-
Sample solution	-	0.100	-	0.100
Mix and incubate for 20 minutes and add:				

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Solution 3	1.000	1.000	1.000	1.000
Deionized water	2.000	1.900	2.050	1.950
Mix and read the absorbances (A_1) for the solutions after approx. 2 minutes. Start the reaction by addition of:				
Suspension 4	0.050	0.050	0.050	0.050
Mix and wait until the reaction has stopped (approx. 15 min.) and read the absorbances (A_2) of the solutions. If the reaction has not stopped after 15 minutes, continue to read the absorbances at 5 minute intervals until the absorbance increases constantly over 5 min.				

8. Determine the absorbance differences (A2 - A1) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the corresponding sample:

   $?A = (A_2 - A_1)_{sample} - (A_2 - A_1)_{blank}$

   It follows for: $?A_{D\text{-}galactose}$ (from 'D-galactose sample')
   and $?A_{lactose + D\text{-}galactose}$ (from 'lactose sample')

   The difference of these values stands for $?_{Alactose}$

9. The measured absorbance differences should, as a rule, be at least 0.100 absorbance units to achieve sufficiently accurate results. If that is not the case, adjust the sample volume or weigh in a larger amount of the sample. If the absorbance difference is too high, dilute the sample further.

10. If the reagent blank shows significant amounts of lactose or D-galactose, correct the sample results.

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7. Result

$$c\ in \frac{g}{l} = \frac{V \times MW \times F}{\varepsilon \times d \times v \times 1000} \times \Delta A$$

c = concentration, g/l sample solution
V = final volume, ml
MW = molecular weight g/mol
F = dilution factor
v = sample volume, ml
d = light path, cm
ε = extinction coefficient of NADH at 340 nm = 6.3 $(l \times mmol^{-1} \times cm^{-1})$
ΔA = absorbance difference of sample minus absorbance difference of blank

Lactose:

$$c\ in \frac{g}{l} = \frac{3.300 \times 342.3 \times F}{6.3 \times 1.00 \times 0.100 \times 1000} \times \Delta A_{lactose}$$

$$c\ in \frac{g}{l} = 1.793 \times F \times \Delta A_{lactose}$$

$$c\ in\ \% = \frac{17.93 \times F \times ml}{w} \times \Delta A_{lactose}$$

D-galactose:

$$c\ in \frac{g}{l} = \frac{3.300 \times 180.16 \times F}{6.3 \times 1.00 \times 0.100 \times 1000} \times \Delta A_{D-galactose}$$

$$c\ in \frac{g}{l} = 0.9437 \times F \times \Delta A_{D-galactose}$$

$$c\ in\ \% = \frac{9.437 \times F \times ml}{w} \times \Delta A_{D-galactose}$$

where

F = dilution factor
ml = original dilution, ml
w = weight of milk powder
?A = absorbance difference of sample minus absorbance difference of blank

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If the sample has been diluted during preparation, the result must be multiplied by the dilution factor F.

Detection limits of 0.2 mg/l for lactose and 1 mg/l for D-galactose are derived from the absorbance difference of 0.010 measured at 340 nm and a maximum sample volume v = 0.5 ml.

In a double determination using one sample solution (1 g diluted to 100 ml) a difference of 0.05 - 0.1% for D-galactose and 0.15 - 0.25% for lactose can be expected.
If the sample is diluted further during sample preparation, the expected difference has to be multiplied by the dilution factor.

8. Remarks

1. Control the method by analysing the standard samples frequently.
2. If the conversions of lactose and of D-galactose have been completed according to the time given in the scheme, it can be concluded (in general) that no interference has occurred. If interference is suspected, use the standard addition for control or consult the reference.

9. Reference

- Methods of Biochemical Analysis and Food Analysis, Boehringer Mannheim
- GEA Niro Research Laboratory

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