Determination of lactose, saccharose and fructose/glucose A-25.2

Key words

Instrumental HPTLC - quantitative analysis - densitometry (absorbance) - post chromatographic derivatization - process control - food analysis - carbohydrates - biosynthesis-monitoring - antibiotics

Scope

The method was developed to monitor the biosynthesis of antibiotics. Fermentation broth (culture medium) is diluted, filtered and chromatographed without further processing. The plate is then derivatized with aniline-diphenylamine-phosphoric acid reagent, and evaluated densitometrically.

The accuracy attainable is 2-3 % (CV at n = 10 on different plates).

Literature

F. Kreuzig, J. Liquid Chromatog. **6,** 1227-1236 (1983)

Advantages of using planar chromatography for this analytical task

- Extremely easy sample preparation
- Low running costs
- Speed and reliability of method
- Elimination of interferences that can be caused by enzyme inhibitors in enzymatic methods

Chemicals

Methanol
Water
1-Butanol
Acetic acid
Aniline
Phosphoric acid 85%

Standards:

Lactose
Saccharose
Fructose
Glucose

Sample preparation

The culture medium (fermentation broth) is diluted with methanol - water 1:1 until the concentration (total sugar) is about 0.5%. The solution is filtered and chromatographed immediately.

Standard solutions

Dissolve 10 mg each of lactose, saccharose, fructose or glucose* to 10 mL in methanol - water 1:1 and dilute this solution 1:2 with methanol - water 1:1 (500 ng/µL).

Application scheme:

U1	S1	U2	U3	S2	U4	U5	S3	U6	U1	S1	U2	...	
	200			600			1000			200			µL/band
	100			300			500			100			ng carbohydrate each

Layer

HPTLC plates Merck silica gel 60 F_{254}, 20 x 10 cm

Sample application

Bandwise with CAMAG Automatic TLC Sampler III, band length 4 mm, distance between tracks 6 mm, distance from side 15 mm, distance from lower edge 10 mm = 17 applications.

Chromatography

In CAMAG Twin-Trough chamber with 1-butanol - acetic acid -water 80:100:15 with chamber saturation, migration distance 50 mm, running time about 30 min.

After chromatography, the plate is dried for 20 min at 115°C. Then the chromatogram can be stored. The following derivatization is carried out when densitometric evaluation is feasible within one hour.

* In this method fructose and glucose are not separated. If they are to be determined separately, application method A-07.3 (NH_2-bonded silica) must be used.

Derivatization

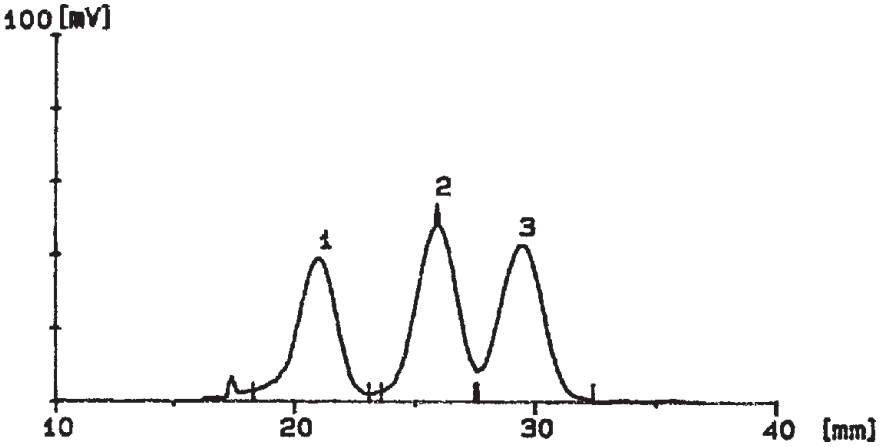
By dipping for 3 s with CAMAG Chromatogram Immersion Device in diphenylamine reagent (3 mL aniline + 3 g diphenyl amine + 15 mL conc. phosphoric acid made up to 150 mL with methanol) followed by heating at about 115°C for about 15 min. The colors are as follows:

Lactose: greyish blue
Saccharose: brownish green
Fructose: rust brown

(Glucose lies between saccharose and fructose.)

Densitometric evaluation

With CAMAG TLC Scanner and CATS evaluation software; scanning by absorbance at 620 nm.



Densitogram of 1 lactose, 2 saccharose, 3 fructose