Determination of lecithin and sphingomyelin in amniotic fluid (L/S ratio)

A-23.2

Key words

Instrumental HPTLC - quantitative analysis - densitometry (fluorescence) - clinico-chemical diagnostics - derivatization - phospholipids - lecithin - sphingomyelin - L/S ratio - amniotic fluid - high-risk pregnancy

Scope

Determination of the ratio of lecithin to spingomyelin (L/S ratio) in amniotic fluid is a viable method of monitoring and assessing fetal lung maturity. This is particularly important in high-risk pregnancies, for which early confinement is to be expected or aimed at (see table 1). Chloroform extract of amniotic fluid is chromatographed on silica gel, derivatized with manganese (II)-chloride - sulfuric acid reagent, and evaluated densitometrically by fluorescence measurement. The working range of this procedure is 5 - 50 mg/L.

The recovery rate (n=8) is determined to be 93% ± 1.5% for lecithin and 90% ± 2.0% for spingomyelin.

Advantages of using planar chromatography for this analytical task

- Dependable, simple method which is cost-effective for any number of samples.
- Also suitable with slight modifications for other phospholipids.

Chemicals

Chloroform
Methanol
Ethanol
n-Hexane
Sodium chloride
Manganese(II)- chloride
Sulfuric acid conc.
Standards: Lecithin (LEC)
Sphingomyelin (SPH)

Sample preparation

- Centrifuge 3 mL amniotic fluid for 10 min at 3500 rpm.
- Extract 2 mL supernatant phase with 8 mL chloroform. (A small quantity of sodium chloride on the tip of a spatula will prevent emulsification.)
- Evaporate the slightly turbid chloroform phase to dryness under nitrogen.
- Dissolve the residue in 400 µL chloroform - methanol - ethanol 7:5:100.

Standard solutions

0.8 mL (40 mg) from a ampoule containing 250 mgLEC in 5 mL is mixed with 2.6 mL chloroform and 3.6 mL methanol, then made up to 20 mL with n-hexane (2 mg LEC/mL).

20 mg SPH cryst. is dissolved in ethanol - n-hexane 14:6, then made up to 10 mL (2 mg SPH/mL).

0.5 mL of the stock solutions are evaporated to dryness under nitrogen, then the residue is made up to 20 mL with chloroform - methanol - ethanol 7:5:100. The stock solutions are stable for several days at -20°C (50 mg/mL SPH und LEC).

Layer

HPTLC plates Merck silica gel 60 F_{254}, 20 x 10 cm, prewashed with methanol, and dried for 30 min at 110°C.

Sample application

Spotwise with CAMAG Automatic TLC Sampler III, track distance 7 mm, distance from left side 15 mm, distance from lower edge 10 mm = 26 applications per plate.

Application pattern:

U1 S1 U2 S2 U3 S3 U4 S4 U1 S1 ...

Standard mixture (S): 200, 400, 800, 1600 nl; sample (U): 200 nL

Chromatography

In CAMAG Horizontal Developing chamber with chloroform - methanol - water 20:7:1, migration distance 50 mm, running time about 10 min R_f (LEC) about 0.15, R_f (SPH) about 0.08.

No pre-conditioning.

Derivatization

The HPTLC plate is dried in a stream of cold air, dipped for 1 s with the CAMAG Chromatogram Immersion Device in manganese(II)-chloride - sulfuric acid reagent (0.2 g $MnCl_2 \cdot 4\ H_2O$ dissolved in 30 mL water, mixed with 30 mL methanol and 2 mL conc. sulfuric acid), and heated to 120°C for 20 min.

Dipping the plate for 1 s in paraffin oil - n-hexane 1:5 after cooling down to room temperature increases the fluorenscence by a factor of 2 and stabilizes it for several hours.

Densitometric evaluation

With CAMAG TLC Scanner and CATS evaluation software; scanning fluorescence at 366/>400 nm.

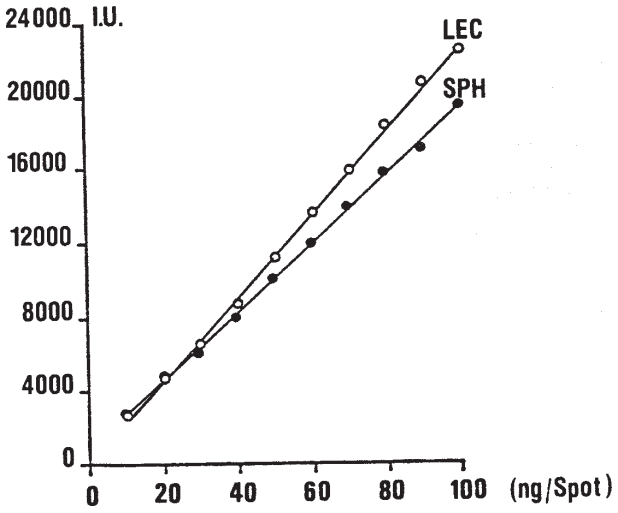

Fig. 1

Evaluation via peak heights with linear regression

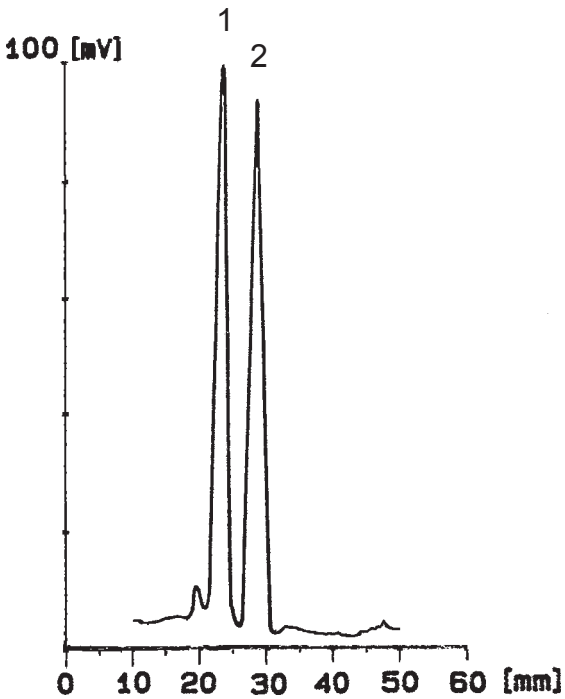

Fig. 2

HPTLC of a standard mixture with SPH (1) and LEC (2), 50 ng each.

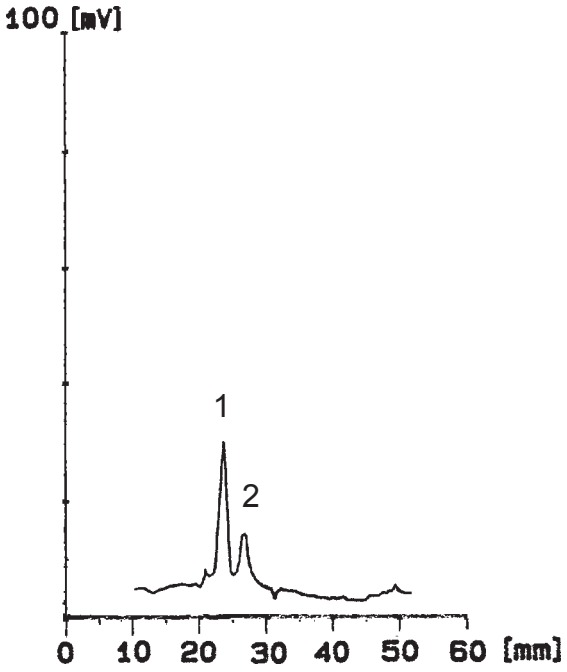

Fig. 3

Example of an immature fetal lung. After amniocentesis of a patient in week 34 of pregnancy the L/S ratio was determined to be 0.5. The LEC content (2) (5.8 mg/L) is appreciably less than the SPH content (1) (11.3 mg/L).

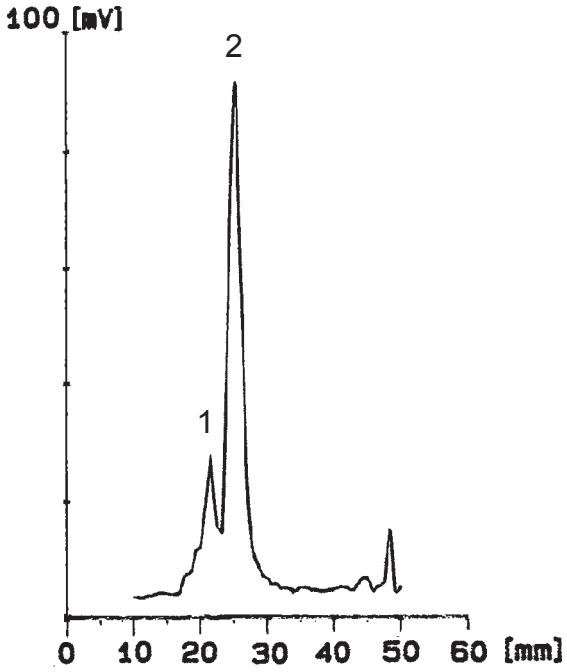

Fig. 4

Analysis of amniotic fluid from the same patient in week 36 of pregnancy. The LEC content (2) (65 mg/L) is now appreciably higher than the SPH content (1) (13 mg/L). The L/S ratio of 4.85 indicates mature fetal lungs.

Table

L/S ratio	Lung status	Status of fetus
1.0	immature	severe RSD*
1.0-1.5	maturing	medium to severe RSD
1.5-2.0	final stage of maturation	slight to medium RSD
≥ 2.0	mature	no RSD

RDS = Respiratory distress syndrom

Literature

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[3] W. Wortmann, B. Wortmann, Anaesthesist **26,** 457f (1977).