Determination of estriol in serum during pregnancy A-22.2

Key words

Instrumental HPTLC - quantitative analysis - prechromatographic derivatization - densitometry (fluorescence) - clinico-chemical diagnostics - steroids - estriol

Scope

Estriol is present in serum mainly in form of its conjugates (16-glucuronide, 3-glucuronide, 3-sulfate and 3-sulfate-16-glucuronide).

Estriol is liberated from its conjugates by ß-glucuronidase, extracted by solid phase, and derivatized with dansyl chloride. Following chromatography on silica gel with toluene - dioxane - methanol 8:2:1, it is determined quantitatively by fluorescence measurement.

The limit of quantification of this procedure is 2 µg/L estriol in serum. The results correlate outstandingly well with those obtained by radioimmunoassay (RIA).

Literature

W. Funk, Fresenius Z. Anal. Chem. **318**, 206-219 (1984).

F. Arndt, Thesis, Giessen University, Faculty of Human Medicine, 1983.

Advantages of using HPTLC for this analytical task

- Substantially greater cost effectiveness than RIA, particularly if the daily (weekly) number of such analyses is only small.

Chemicals

ß-Glucuronidase (Merck) 40 U/mL
Sodium hydroxide 1N
ClinElut (Analytichem Int. USA No. 1001)
Dichloromethane
Isopropanol
Acetone
Dansyl chloride
Proline (3.5 mg/mL water bidist.)
Methanol
n-Hexane
Toluene
Dioxane
Paraffin oil

Standard: Estriol

Sample preparation and derivatization

a) Enzymatic cleavage and extraction:

- Add 20 µL ß-glucuronidase to 500 µL serum, homogenize and incubate the mixture for 2 h at 37°C.
- Adjust to pH 8.5 with 1N sodium hydroxide, pour onto ClinElut cartridges, and allow to adsorb for 3 min.
- Elute with 4 mL dichloromethane - isopropanol 9:1, repeat it after 4 min.
- Evaporate the eluate to dryness in a stream of nitrogen.
- Dissolve residue in 1.5 mL acetone, transfer to an Eppendorf reaction vessel, and evaporate again to dryness.
- Dissolve residue in 100 µL acetone.

b) Derivatization:

- Mix the residue dissolved in 100 µL acetone with 20 µL 0.2% dansyl chloride solution and 10 µL 1N sodium hydroxide, shake the mixture for 15 s.
- Add 50 µL acetone, shake, and incubate for 60 min in the dark at room temperature.
- Add 20 µL proline solution, swirl and incubate again for 15 min in the dark at room temperature. (The excess dansyl chloride dissolves).
- Use supernatant phase for application onto the HPTLC plate. (10 µL = 25 µL human serum).

Standard solution

Dissolve 10 mg estriol in 100 mL acetone, dilute 1:100 with acetone and derivatize in the same way as the samples.

Layer

HPTLC plates silica gel Merck 60 F_{254}, 20x10 cm, prewashed with methanol.

Sample application

With CAMAG Linomat as 7 mm bands, distance between tracks 3 mm, distance from left edge 15 mm, distance from lower edge 7 mm, delivery rate 5 s/µL = 18 applications per plate.

Application pattern:

S1	U1	S2	U2	S3	U3	S4	U4	S1	U1	...
1	10	2	10	4	10	8	10	1	10	... µL/Band

S1- S4 = standard in different concentrations; U1-U4 = unknowns

Alternatively the Automatic TLC Sampler can be used.

Chromatography

In CAMAG Horizontal Developing Chamber 20x10 cm with toluene - dioxane - methanol 8:2:1 with chamber saturation, migration distance 5 cm, running time 13 min, R_F about 0.36.

While still wet, dip the plate with the CAMAG Chromatogram Immersion Device for 1 s in a solution of paraffin oil - n-hexane 5:1. The fluorescence intensity is increased tenfold and stabilized for several hours.

Densitometric evaluation

With CAMAG TLC Scanner and CATS evaluation software; scanning by fluorescence at 313/>400 nm.

The limit of quantification for estriol is 100 pg/zone, corresponding to a serum concentration of about 2 µg/L.

Discussion

Table 1: Estriol level in the serum (µg/L)

	Total estriol (conjugated and unconjugated)	unconjugated estriol
normal cycle	0.25 - 0.37	
week 25 of pregnancy	50	2 - 6
week 40 of pregnancy	200	7 - 25

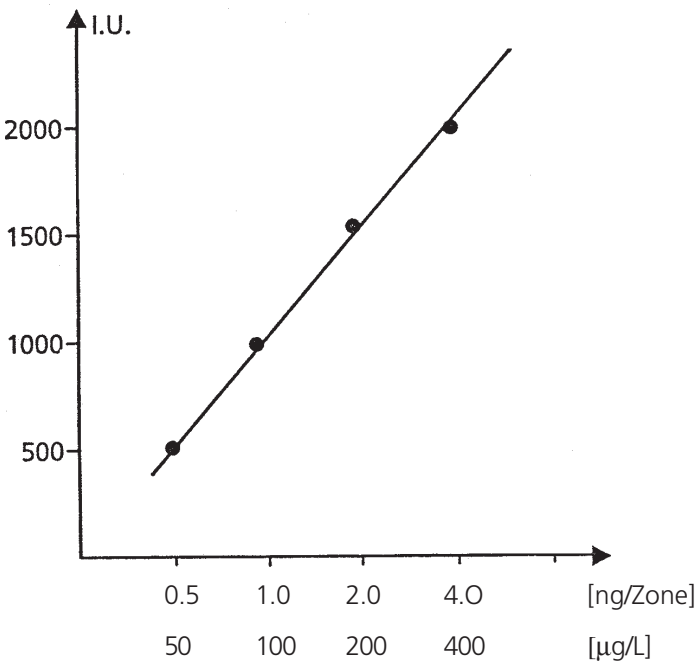

Fig. 1 Calibration curve for estriol

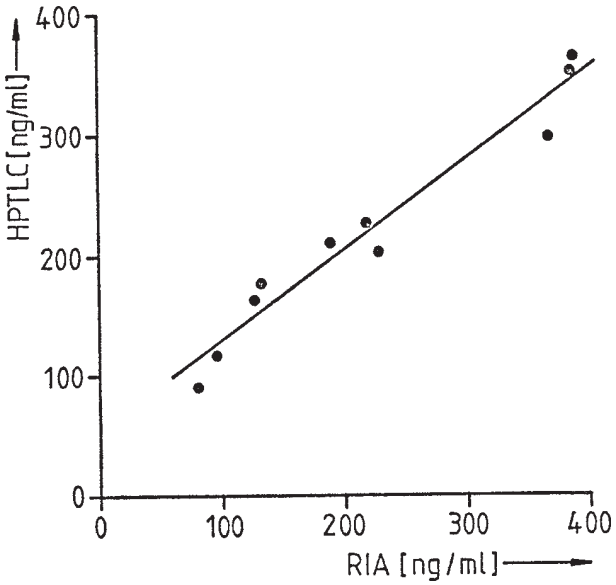

Fig. 2 Comparison with radioimmunoassay

Results obtained by quantitative HPTLC determination of 10 different sera compared with those found by RIA. Orthogonal regression yields a correlation coefficient of r = 0.9778 and confirms the good correlation of the two analytical procedures.