

QUANTIFICATION OF STEROLS IN AMNIOTIC FLUID BY GAS CHROMATOGRAPHY COUPLED TO MASS SPECTROMETRY

Amaral, C.^{*#}, Valongo, C.[#], Quelhas, D.[#], Tomaz, C. T.^{*}; Cardoso, M. L.^{#φ}

^{*} Centro de Investigação em Ciências da Saúde (CICS), Universidade da Beira Interior

[#] Centro de Genética Médica Dr. Jacinto Magalhães (CGMJM), INSA

^φ Departamento de Bioquímica, Faculdade de Farmácia, Universidade do Porto

Introduction: Cholesterol and cholesterol precursors [7-dehydrocholesterol (7-DHC), desmosterol, lathosterol] are important biochemical markers of cholesterol biosynthesis, and their quantification is useful for the diagnosis of disorders of cholesterol biosynthesis pathway. The most frequent disease of this group is Smith-Lemli-Opitz syndrome (SLO) resulting from deficiency of the enzyme 7-dehydrocholesterol reductase (DHCR7), responsible for the last step of the cholesterol biosynthesis. The diagnosis is based on the detection of markedly elevated levels of 7-dehydrocholesterol and 8-dehydrocholesterol in body fluids and tissues and is characterized by a pattern of multiple congenital malformations, which can be detected before birth by prenatal ultrasound.

Several methods were described in literature for sterols quantification. Among them gas chromatography-mass spectrometry (GC-MS) with selected-ion monitoring (SIM) is the most common one.

Material and Methods: Sterols in 100 amniotic fluid samples were quantified by GC-MS-SIM. The samples were grouped by gestational age between 13 and 22 weeks of pregnancy. The method was adapted from literature and it is based on the alkaline hydrolysis of sterol esters, extraction of free sterols, derivatization and quantification of sterols. Epicoprostanol was used as internal standard. The method was linear for cholesterol ($r_2 > 0.999$), 7-DHC ($r_2 > 0.998$), desmosterol ($r_2 > 0.999$) and lathosterol ($r_2 > 0.999$). Within-run and between-run coefficients of variation were 1.8% and 2.8% for cholesterol, 0.5% and 3.5% for 7-DHC, 2.3% and 3.2% for desmosterol and 2.2% and 2.6% for lathosterol, respectively.

Results: The concentration range in $\mu\text{mol/L}$, of each sterol in amniotic fluid samples (mean \pm SD) was: for cholesterol 22.540 ± 2.304 at 13 weeks and 55.443 ± 2.590 at 22 weeks; for 7-DHC was 0.004 ± 0.002 at 13 weeks and 0.152 ± 0.015 at 22 weeks; for desmosterol was 0.151 ± 0.049 at 13 weeks and 0.688 ± 0.046 at 22 weeks; for lathosterol was 0.025 ± 0.008 to 13 weeks and 0.691 ± 0.073 at 22 weeks, respectively.

Conclusion: The method is suitable for identification, separation and quantification of cholesterol, 7-dehydrocholesterol, desmosterol and lathosterol in amniotic fluid. The results showed that as long as pregnancy goes on, the concentration of cholesterol in amniotic fluid increases, as well as its precursors. This fact is related with the increased need of cholesterol of the developing fetus. The range of reference of each sterol in amniotic fluid was calculated for different gestational age (13 to 22 weeks) and it will be useful for validation of results of biochemical prenatal diagnosis of inborn errors of sterol biosynthesis other than SLO.

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Key words: amniotic fluid; cholesterol; 7-dehydrocholesterol, desmosterol and lathosterol.