Cholesterol and bile acids

Application Note

Clinical Research

Authors
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**Conditions**

Technique : GC-capillary

Column : Agilent CP-Sil 8 CB, 0.22 mm x 25 m fused silica
  WCOT CP-Sil 8 CB (0.12 µm) (Part no. CP7711)

Temperature : 250 °C (1 min) → 290 °C, 4 °C/min, 290 °C (15 min)

Carrier Gas : He, 140 kPa (1.4 bar, 20 psi), 33 cm/s

Injector : Splitter, 1:10

Detector : FID

Sample Size : 1 µL

Courtesy : Dr. J.A.J.M. Bakkeren, St. Radboud Hospital, Nijmegen, The Netherlands
see also Application Note 51

**Peak identification**

1. cholesterol
2. lithocholic acid (l.S.)
3. chenodeoxycholic acid
4. cholic acid
5. dihydroxycoprostanic acid
6. trihydroxycoprostanic acid
Bile profiles
Chromatogram A shows the bile profile of a healthy human. Chromatogram B shows the bile profile of a human with the cerebro-hepato-renal syndrome of Zellweger. Here the deviating bile acids di- and trihydroxycoprostanic acid are present.

Sample preparation
Add 100 μL internal standard (0.4 mg lithocholic acid/mL) to 1 mL bile or serum. Deproteinize with hot 100% ethanol (for bile: 5 mL; for serum: 10 mL). Wash the precipitate with 100% ethanol (2x). Evaporate the supernatant to dryness under N₂ at 50 °C and hydrolyze with 1 mL 25% KOH in ethylene glycol during 15 min at 200-210 °C.

Add 1 mL methanol and 1 mL 20% NaCl. Extract 4 x (bile) or 6x (serum) with 5 mL petroleum ether. Add 0.8 mL 6 N HCl + 8 mL 20% NaCl to the water/methanol layer. Extract 3x with 5 mL diethylether. Evaporate the diethylether layer to dryness under N₂ at 40-50°C, then methylate with 4 mL 3 N HCl/ methanol overnight at room temperature.

Add 4 mL 20% NaCl and extract 2 x with 7 mL diethylether. Evaporate the diethylether layer to dryness under N₂ at 40-50° C and acetylate with a mixture of acetic acid and acetic anhydride (1:1) and 2 drops of HClO₄ during 15 min at room temperature. Add 10 mL 20% NaCl and extract 2 x with 5 mL diethylether. Evaporate the diethylether layer to dryness under N₂ at 40-50°C and dissolve in 100 μL acetone.