Cholesterol and bile acids

Application Note

Clinical Research

Authors
Agilent Technologies, Inc.
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 patient 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.