Organic acids

Standard 1 analysis of silylated organic acids in urine

Application Note

Metabolomics

Authors

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Introduction

GC/MS with an Agilent CP-Sil 8 CB Low Bleed/MS column separates 37 silylated organic acids in urine in 70 minutes.
Conditions

Technique: GC-capillary

Column: Agilent CP-Sil 8 CB Low Bleed/MS, 0.25 mm x 30 m fused silica WCOT (df = 0.25 μm) (Part no. CP5860)

Temperature:
- 50 °C (1 min) → 80 °C, 10 °C/min;
- 80 °C → 150 °C, 1.7 °C/min;
- 150 °C → 220 °C, 3.5 °C/min;
- 220 °C → 290 °C, 20 °C/min;
- 290 °C (10 min), hold

Carrier Gas: He, 80 kPa (0.8 bar, 12 psi)

Injector: Splitter, T = 270 °C

Detector: MS, T = 270/220 °C

Sample Size: 1.0 μL

Concentration Range: 2 - 10 ng / component

Solvent Sample: hexane + silylation reagent

Sample preparation: After addition of the internal standards heptanoylglycine and tropic acid and 6N HCl to 2 mL of urine, the sample is extracted twice with 4 mL ethyl acetate, containing n-C_{23} and n-C_{24}. Centrifugation and drying with sodium sulfate is followed by evaporation to almost dryness. The residue is diluted with 0.5 mL toluene and dried. Directly after that 200 mL silylation reagent (BSTFA, pyridine and TMCS) is added. After reaction (30 min at 60 °C), 0.5 mL hexane is added and this mixture is transferred to an autosampler vial.

Peak identification:

1. 2-phenylbutyric acid
2. lactic acid
3. caproic acid (hexanoic acid)
4. glycolic acid
5. pyruvic acid
6. β-hydroxybutyric acid
7. α-hydroxybutyric acid
8. methylmalonic acid
9. 2-hydroxyscaproic acid
10. γ-hydroxybutyric acid
11. benzoic acid
12. octanoic acid (caprylic acid)
13. ethylmalonic acid
14. succinic acid
15. glyceric acid
16. fumaric acid
17. glutaric acid
18. 3-methylglutaric acid
19. decanoic acid (capric acid)
20. malic acid
21. adipic acid
22. 2-hydroxyphenylacetic acid
23. 3-phenylactic acid
24. 2-hydroxyglutaric acid
25. 3-hydroxy-3-methylglutaric acid
26. α-ketoglutaric acid
27. 4-hydroxyphenylacetic acid
28. suberic acid
29. cis-aconitic acid
30. citric acid
31. homogentisic acid
32. sebacic acid
33. ascorbic acid
34. n-C_{23}
35. n-C_{24}
36. heptanoylglycine (I.S.)
37. tropic acid (3-hydroxy-2-phenylpropionic acid, I.S.)

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