

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 $\mu m)$ (Part no. CP5860)
Temperature	: 50 °C (1 min) \rightarrow 80 °C, 10 °C/min; 80 °C \rightarrow 150 °C, 1.7 °C/mln: 150 °C \rightarrow 220 °C, 3.5 °C/min: 220 °C \rightarrow 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

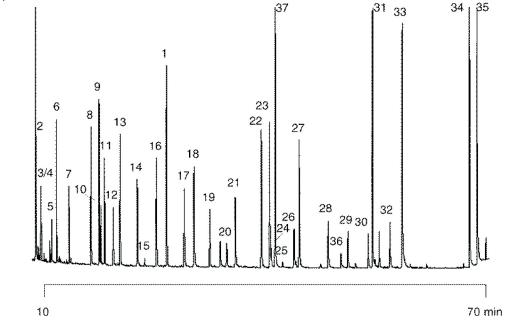
Sample preparation: After addition of the internal standards heptanoylglycine and tropic acid and 6N HCl to 2 ml of urine, the sample is extracted twice with 4 mL ethyl acetate, containing $n-C_{23}$ and $n-C_{24}$. Centrifugation and drying with sodium sulfate is followed by evaporation to almost dryness. 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 transfered to an autosampler vial.

Courtesy : Mr. G. Voortman and Dr. J. Hessels, Twenteborg hospital, Almelo, the Netherlands

Peak identification

- 1. 2-phenylbutyric acid
- 2. lactic acid
- 3. caproic acid (hexanoic acid)
- 4. glycolic acid
- pyruvic acid 5.
- β-hydroxybutyric acid 6. 7. a-hydroxybutyric acid
- 8. methylmalonic acid
- 9. 2-hydroxyisocaproic acid
- 10. y-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-phenyllactic acid
- 24. 2-hydroxyglutaric acid
- 25. 3-hydroxy-3-methylglutaric acid
- 26. a-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-C₂₃
- 35. n-C₂₄
- 36. heptanoylglycine (1.8)

37. tropic acid (3-hydroxy-2-phenylpropionic acid, I.S.)



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