



Organic acids

Application Note

Metabolomics

Authors

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Introduction

GC/MS with an Agilent CP-Sil 8 CB Low Bleed/MS column separates 28 silylated organic acids in urine in 70 minutes.



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Conditions

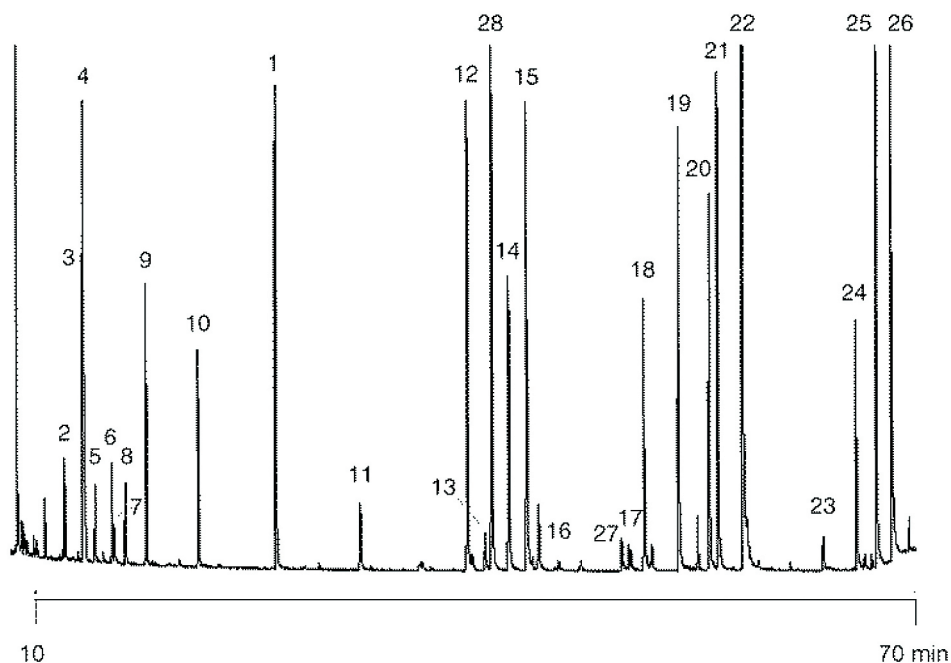
Technique : GC-capillary
Column : Agilent CP- Sil 8 CB Low Bleed/M, 0.25 mm x 30 m fused silica WCOT (df = 0.25 µm) (Part no. CP5860)
Temperature : 50 °C (1 min) → 80 °C, 10 °C/min;
80 °C → 150 °C, 1.7 °C/min;
150 °C → 220 °C, 3.5 °C/min;
220 °C → 290 °C, 20 °C/min;
290 °C (10 min), hold
Carrier Gas : 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

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

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-C₂₃ and n-C₂₄. 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 µL 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. oxalic acid
3. 3-hydroxy-2-methylpropionic acid
4. 2-hydroxyisovaleric acid
- 5,6. B-ketobutyric acid (acetoacetic acid)
- 7,8. acetoacetic acid methylester (methyl acetoacetate)
9. 2-hydroxy-3-methylvaleric acid
10. phenylacetic acid
11. glutaconic acid
12. m-hydroxybenzoic acid
13. 3-hydroxyglutaric acid
14. 3-hydroxyphenylacetic acid
15. p-hydroxybenzoic acid
16. N-acetyl-L-aspartate
17. orotic acid
18. homovanillic acid (HVA)
19. 3,4-dihydroxyphenylacetic acid (DOPAC)
20. VMA
21. p-hydroxyphenyllactic acid
22. ascorbic acid
23. N-acetyl-L-tyrosine
24. 5-hydroxyindoleacetic acid (5-HIAA)
25. n-C₂₃
26. n-C₂₄
27. heptanoylglycine (I.S.)
28. tropic acid (3-hydroxy-2-phenylpropionic acid, I.S.)



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This information is subject to change without notice.

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