Journal of Chromatography, 233 (1982) 297—300
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 1420

Note

Analysis of oxime-trimethylsilyl derivatives of organic acids on OV-1701 fused silica capillary column

A.P.J.M. DE JONG

Department of Clinical Chemistry, Academic Hospital, Free University, De Boelelaan 1117, 1081 HV Amsterdam (The Netherlands)

(Received April 23rd, 1982)

Recently Tanaka et al. [1] argued the statement that the diagnosis of organic aciduria by gas chromatography (GC) alone is much easier and not inferior to a diagnosis by gas chromatography—mass spectrometry (GC—MS). Essential for this GC diagnosis is the analysis of the organic acid profile on at least two packed columns with different stationary phases, e.g. SE-30 and OV-17.

However, the urinary organic acid fraction is so complex that high-resolution capillary GC should be used. Even then the analysis should be performed on two columns with stationary phases differing in polarity, to enhance the identification reliability. Up to now moderately polar capillary columns suitable for the analysis of trimethylsilyl (TMS) derivatives of organic acids were not available.

In this paper I report the excellent performance of an OV-1701 fused-silica capillary column for analyzing complex mixtures of oxime-TMS derivatives of organic acids. OV-1701 is a new stationary phase very comparable with OV-17. This is demonstrated on an organic acid profile from pooled normal urine. A tabulation of methylene units (MU) of more than 30 normally occurring compounds, separated on an OV-1701 fused-silica capillary column is given. A comparison is made between these MU values and those of the same compounds obtained by Tanaka et al. [1] on a packed OV-17 column.

MATERIALS AND METHODS

Urine samples

Urine of ten normal children was pooled (1 ml each) and creatinine was determined (4.8 mmol/l). An aliquot of 1.9 ml of urine was taken and 79.2 μg

0378-4347/82/0000—0000/$02.75

of 3-phenylpropionic acid (internal standard) were added. The extraction and derivatization (oxime-TMS) were carried out according to standard methods as described elsewhere [2].

Gas chromatography—mass spectrometry

The experiments were performed on a Jeol JMS D-100 GC—MS system with a JMA-0231 data system.

The GC column was a 25 m × 0.22 mm I.D. OV-1701 wall-coated fused-silica capillary column (Chrompack, Middelburg, The Netherlands). The GC column was directly introduced into the ion source. The carrier gas (helium) velocity was approximately 40 cm sec^{-1}. Samples were introduced by a modified all-glass sampling device [3] and separated using temperature programming: initial temperature 80°C, programming rate 8°C/min, final temperature 280°C. Injection port temperature was 275°C. The MS conditions were: electron energy 23 eV, emission current 0.6 mA, and source temperature 250°C. For qualitative analysis (identification) 0.5 μl was injected.

To study the column performance, 0.5 μl of the sample was injected with a split ratio of 20 : 1 and the intensity of the ion *m/z* 73, characteristic for all TMS derivatives of organic acids, was recorded. The multiplier voltage was set to −1500 V. The sensitivity was set to 1 V full scale, ten times the lowest sensitivity.

RESULTS AND DISCUSSION

Fig. 1 shows the mass chromatogram of oxime-TMS derivatives of organic acids of pooled urine of ten normal children.

The performance of the OV-1701 fused-silica capillary column is demonstrated by the inertness for TMS derivatives of organic acids for very small amounts injected. In Fig. 1 peak 11 corresponds to approximately 47 pmol of phenylpropionic acid (internal standard) injected. Compounds with a concentration of less than 1% of the internal standard, e.g. benzoic acid (peak 8) 0.6 pmol injected, show a perfect peak shape.

Table I lists the compounds and their MU values on OV-1701 fused-silica capillary column. In Table I a comparison has been made with the corresponding MU values on an OV-17 packed column as given by Tanaka et al. [1]. The MU values on OV-1701 and OV-17 are very comparable. The aliphatic acids tend to shift to higher MU values on OV-1701, whereas the aromatic organic acids shift to lower MU values on OV-1701 compared to OV-17. For urea-di-TMS there is a very noticeable difference of 1.25 MU between the two stationary phases.

The lower MU values for aromatic acid TMS derivatives on OV-1701 can possibly be ascribed to the lower content of phenyl groups in the stationary phase for OV-1701 as compared to OV-17: 7% and 50%, respectively [4].

The remarkable improvement of the performance of the OV-1701 column with respect to the OV-17 column could be ascribed to the different deactivating methods used. OV-17 columns are normally prepared by deactivating the wall with Carbowax. OV-1701 columns are prepared by deactivating the wall by a high-temperature reaction of polysiloxane with silanol groups on the

TABLE I

METHYLENE UNITS OF SOME ORGANIC ACID OXIME-TMS DERIVATIVES ON OV-1701 CAPILLARY COLUMN

GC peak in Fig. 1	Compound	MU values		
		OV-1701	OV-17*	Shift
1	α-Hydroxyisobutyric acid	11.03	10.74	0.29
2	Lactic acid	11.21	10.95	0.26
3	Glycollic acid	11.50	11.27	0.23
4	Glyoxylic acid-oxime	11.97	11.96	0.01
5	Pyruvic acid-oxime	12.08	12.11	−0.03
6	2-Methyl-3-hydroxybutyric acid	12.52	12.25	0.26
7	3-Hydroxyisovaleric acid	12.63	12.35	0.28
8	Benzoic acid	13.38	13.73	−0.45
9	Succinic acid	14.18	14.02	0.16
10	Urea	14.76	13.50	1.25
11	Phenylpropionic acid (internal standard)	15.25	—	—
12	Adipic acid	16.17	15.97	0.20
13	Methyladipic acid	16.42	16.20	0.22
14	2-Hydroxymethyl-5-carboxylfurane	16.72	—	—
15	2-Hydroxyglutaric acid	16.78	—	—
16	3-Hydroxy-3-methylglutaric acid	16.89	16.48	0.41
17	Pyroglutamic acid	17.00	16.80	0.20
18	3-Hydroxyphenylacetic acid	17.08	17.33	−0.25
19	2-Ketoglutaric acid	17.33	17.06	0.27
20	4-Hydroxyphenylacetic acid	17.53	17.66	−0.13
21	2,5-Dicarboxylfurane	17.89	—	—
22	4-Hydroxymandelic acid	18.81	18.42	0.39
23	Vanillic acid	18.93	19.08	−0.15
24	Homovanillic acid	19.09	19.32	−0.13
25	Azelaic acid	19.15	—	—
26	Citric acid	19.31	18.69	0.62
27	Isocitric acid +	19.53	18.86	0.67
	3-hydroxyphenyl-3-hydroxypropionic acid	19.53	—	—
28	Vanilmandelic acid +	20.14	—	—
	4-hydroxyphenyllactic acid	20.14	19.93	0.21
29	Palmitic acid	21.11	20.90	0.21
30	Hippuric acid (mono-TMS)	21.28		0.18
31	3-Hydroxysebacic acid	22.87	—	—
32	Oleic acid	22.87	22.87	0
33	Stearic acid	23.10	22.90	0.20
34	4-Hydroxyhippuric acid (di-TMS)	24.43	24.56	−0.13

*Ref. 1.

glass surface [5]. Probably TMS esters are hydrolyzed by the hydroxy groups of the Carbowax deactivation layer. This assumption is enforced by the fact that highly retained TMS esters on Carbowax-deactivated OV-17 capillary columns do not elute, whereas TMS esters with a short retention time elute normally.

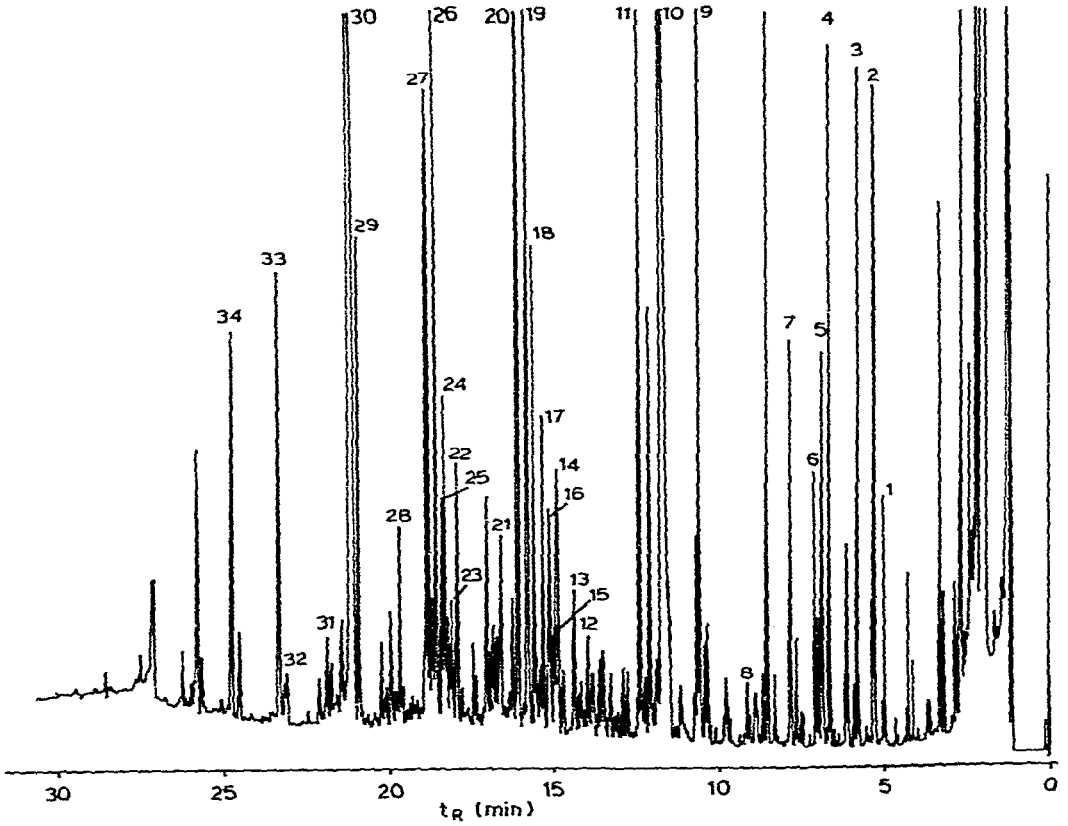

Fig. 1. Organic acid oxime-TMS derivative profile of pooled urine of ten normal children analyzed on a OV-1701 fused-silica capillary column. Peak numbers refer to Table I.

REFERENCES

1 K. Tanaka, D.G. Hine, A. West-Dull and Th.B. Lynn, Clin. Chem., 26 (1980) 1839.
2 C. Jacobs, M. Bojash, E. Mönch, D. Rating, H. Siemes and F. Hanefeld, Clin. Chim. Acta, 111 (1981) 169.
3 A.P.J.M. de Jong, J. High Resol. Chromatogr. Chromatogr. Commun., 3 (1981) 125.
4 K. Grob and G. Grob, J. High Resol. Chromatogr. Chromatogr. Commun., 5 (1982) 13.
5 R. de Nijs, Research and Development, Chrompack, The Netherlands, personal communication.