

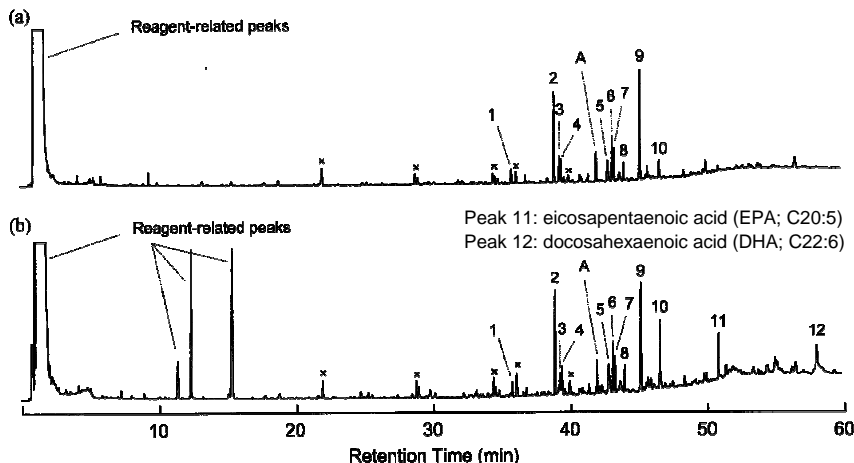
## Highly Sensitive Determination of Lipids in Zooplankton by Reactive Py-GC in the presence of Trimethylsulfonium Hydroxide

**[Background]** Reactive Py-GC in the presence of an organic alkali has been successfully applied to direct analysis of lipids in various biological samples. Furthermore, the use of a sulfonium salt, trimethylsulfonium hydroxide (TMSH), as an alkali reagent enabled to detect thermally-labile polyunsaturated fatty acid (PUFA) components without causing their undesirable isomerization and/or degradation. This note presents the highly sensitive determination of lipids including the PUFA residues in an individual zooplankton sample at levels of a few tenths of µg.

**[Experimental]** *Daphnia galeata* individuals cultured under the standard conditions in the laboratory were used as plankton samples. Dry weights of these individuals ranged from 10 to 60 µg. As derivatizing reagents, a methanol solution of TMSH (0.25 M) and a methanol solution of tetramethylammonium hydroxide (TMAH) (3.8 M) were used. Each dried zooplankton sample was subjected to reactive Py-GC at 400°C in the presence of 2 µl of the organic alkali solution.

**[Results]** Figure 1 shows typical pyrograms of two *D. galeata* individuals, weighing about 60 µg each, obtained by reactive Py-GC in the presence of (a) TMAH and (b) TMSH at 400 °C, respectively. On both of the pyrograms, methyl esters of saturated and unsaturated C14 - 18 fatty acids (peaks 1 - 10) were commonly observed. Here it should be noted that the peaks of EPA (peak 11) and DHA (peak 12) containing 5 and 6

double bonds, respectively, were clearly observed on the pyrogram (b) obtained with TMSH, while they were virtually missing in the pyrogram (a) using TMAH due to their thermal isomerization and/or degradation. This result demonstrates that THM-GC in the presence of TMSH allowed the highly sensitive detection of a series of fatty acid residues including PUFAs even with 5 or 6 double bonds. The peak intensities of the fatty acid components observed using TMSH, permit the rapid determination of the lipid contents and fatty acid compositions in plankton samples.



**Figure 1.** Chromatogram of zooplankton samples obtained by reactive Py-GC at 400°C in the presence of (a) TMAH and (b) TMSH.

Pyrolysis temp. : 400°C, GC oven temp. : 50°C(-5 °C/min)-240°C  
 Separation column : Ultra ALLOY-CW [poly(ethylene glycol)] Length 30 m,  
 0.25 mm i.d., Film thickness 0.25 µm  
 Carrier gas flow : 50 ml/min, Column flow : 1.0 ml/min

\*Contents excerpted from O. Nakanishi, S. Hirao, Y. Ishida, H. Ohtani, S. Tsuge, J. Urabe, T. Sekino, M. Nakanishi, T. Kimoto, *J. Anal. Appl. Pyrolysis* **2003**, 68-69, 187-195.

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Applications : Lipid analysis, Analysis of natural organic compounds

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1-8-14 Saikon, Koriyama

Fukushima-ken 963-8862 JAPAN

Phone: (81)24-935-5100 Fax: (81)24-935-5102

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