Lipid Biomarkers

Introduction

This document covers the lipid biomarker data included in files LIPBOT and LIPSAP. These files contain identical parameters collected by either conventional water sampling or the use of stand-alone pumps.

Sample Acquisition

Water samples were taken from the water bottles on the CTD rosette sampler or from the ship’s surface pumped non-toxic seawater supply. Sample volumes of 5-15 litres were filtered for analysis. Challenger Oceanics stand-alone pumps (SAPs) were deployed at various depths. These are self contained units which pump large volumes of sea water through a filter package. The SAP filters were subsampled to give volumes pumped through the subsample of the same order as the water samples.

The filters used were Whatman GF/Fs which had been combusted at 380°C for more than 12 hours.

Sample Storage

Sample filters were immediately immersed in 2:1 (v:v) chloroform:methanol and stored at -20°C until analysed. For cruise Charles Darwin 60, the samples were originally intended for pigment analysis and consequently they were stored in acetone not chloroform:methanol.

Analytical Methods

The methods used for sample extraction and analysis have been described in Conte et al (1992) and Conte and Eglington (1993). Briefly, an internal standard mixture was added to the sample prior to extraction in 2:1 (v:v) chloroform:methanol. The non-lipid components and sea salts were removed by washing using the method of Folch et al (1957).

The extract was concentrated using a centrifugal evaporator, taken up in chloroform and any residual water removed by passing the extract through a Pasteur pipette ‘minicolumn’ filled with 1 cm³ combusted (380°C for >12 hours) Na₂SO₄.

Samples were then transesterified using 5% methanolic HCl (55°C, 12 hours, Christie (1982)), and the transesterified products were extracted into hexane. Acyl complex lipids were broken down in the transesterification step into their component fatty acid and alcohol (for wax esters); monomers and any ethyl alkenoates present were converted to methyl alkenoates.

An aliquot of the total transesterified extract was then silylated using BSTFA in pyridine (55°C, 1 hour) for analysis by gas chromatography and GC-mass spectrometry for analysis of the alkenones and alkyl alkenoates.

The transesterified, silylated extracts were gas chromatographed on a 50 m x 0.32 mm Chrompak CPSil5CB (Chrompak) column. The GC used was a Carl Erba 5160 fitted with on-column injection and was programmed from 50-150°C at 10°C/minute and from 150-310°C at 4°C/minute.

Compounds were identified by GC-MS of selected samples and comparison of relative retention times, and quantified using the Minichrom (VG Systems) PC-based chromatography software.
Purity of peaks was checked by GC-MS, although the extremely low abundances of these compounds in most samples precluded confirmation of absolute purity. Undetected minor co-eluants, as well as analytical errors, will contribute to between sample variability.

The data are presented as percentage contribution of individual compounds to the sum total C37+C38 alkenones and C36 alkenoates. Methyl alkenoates include those compounds originally present as ethyl esters. The standard deviation of water column concentration and indices is given for replicate sample analyses.

**Data Warnings**

During the BOFS project, it became apparent that data collected using SAPs did not compare well with data collected using conventional water bottles, with SAPs underestimating the quantity of particulate material. This may be easily explained in terms of loss of trapped material from the filters or overestimation of the volume of water sampled (clogged filters).

Consequently, data from SAPs should be interpreted bearing in mind that the values quoted may quite possibly be underestimates.

**References**


