

[3] Analysis:

Instrument:	HPLC Waters 626 pump, Gilson 231 Autoinjector, Alltech Alltima C18 column (250 x 4.6mm, 5um packing), Waters 996 diode array detector, Hitachi F1000 fluorescence detector, Waters Millennium software
Precision:	Coefficient of variation estimated as being between 5 and 15% for major pigments based on analyses of replicate samples.
Comments	-

[4] Results:

Quality of Data	Good.
Known Problems:	Chlorophyll c1& c2 & MgDVP not resolved. 19'-hexanoyloxyfucoxanthin & 4-keto-19'-hexanoyloxyfucoxanthin not resolved. Chl a and divinyl Chl a not resolved, but the latter is not present to our knowledge (tested using another method)

[5] Brief description of analytical methods

Method: Pigments were extracted by sonication for 1 min in 1.5ml methanol using a 4mm dia probe at 50W power in semi-darkness. Extracts were filtered using Milcrofiltration Systems MFS Nylon, 3mm dia, 0.45 um filters and analysed by the method of Wright *et al* (1991) using a Waters 626 pump, Gilson 231 autoinjector (with the sample stage refrigerated at -10°C), Alltech Alltima C18 column (at 30.0°C) and a Waters 996 diode array detector. Pigments were identified by comparison of their retention times and spectra with those of mixed standards obtained from known cultures (Jeffrey and Wright, 1997) that were injected with each batch of samples. Peaks were integrated using Waters Millennium software.

Pigment calibration Pigments were isolated from well-characterised sources (Jeffrey and Wright, 1997) and calibrated using the internal standard procedures of Mantoura and Repeta (1997) by adding approximately 130 ng β -apo-8'-carotenal (Fluka) to each sample before extraction.

References:

- Jeffrey, S.W., Wright, S.W. (1997) Qualitative and quantitative analysis of SCOR reference algal cultures *in* Jeffrey, S.W., Mantoura, R.F.C., Wright, S.W. (eds.) (1997) *Phytoplankton pigments in oceanography: Guidelines to modern methods*. ISBN 92-3-103275-5. UNESCO, Paris, p. 343–360
- Mantoura, R.F.C., Repeta, D.J. (1997) Calibration methods for HPLC *in* Jeffrey, S.W., Mantoura, R.F.C., Wright, S.W. (eds.) (1997) *Phytoplankton pigments in oceanography: Guidelines to modern methods*. ISBN 92-3-103275-5. UNESCO, Paris, p. 407–428
- Wright, S.W., Jeffrey, S.W., Mantoura, R.F.C., Llewellyn, C.A., Bjornland, T., Repeta, D., Welschmeyer, N. (1991) Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton. *Marine Ecology Progress Series* **77**, 183-196.

[6] Comments:

HPLC Chlorophyll-a is the only pigment currently available for cruises AU 9101AU9303, AU 9404, AU9407, and AU 9501. Chlorophyll-a and other Pigments listed above are available from cruises AU 9604 and AU 9706.

There were no significant method changes in the methodology between 1991 and 1998.