

Short Contribution

HPLC Determination of Phytoplankton Pigments Using N,N-Dimethylformamide

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The suitability of N,N-dimethylformamide (DMF) as an extractant for the standard reverse-phase HPLC method was examined using algal cultures. Good pigment separations and recovery were achieved with 20% (volume %) addition of an ion-pairing solution in an injection. While slight amounts of degradation products of chlorophyll *a*, i.e., chlorophyllide *a*, allomeric and epimeric forms, were produced, adequate attention to filtration and extraction prevents the formation of degradation products, confining them to an acceptable level. Because of its strong extractability, which expedites the extraction process, DMF is an efficient solvent for HPLC analysis of phytoplankton pigments.

Keywords:

- Pigments,
- HPLC,
- N,N-dimethylformamide,
- phytoplankton,
- chlorophyll *a*.

1. Introduction

High-performance liquid chromatography is proving to be an extremely useful tool in studying a range of aspects of phytoplankton ecology. Quantification of chlorophyllous and carotenoid pigments provides information on biomass, taxonomic composition, physiological state, photoadaptation, grazing processes and detritus of algal origin (e.g., Gieskes, 1991). Their accurate measurement involves a number of procedures. Pigment extraction is one of the most important steps. Among various solvents for chlorophyll *a* (Chl *a*), N,N-dimethylformamide (DMF) is known for its power of extraction and efficiency in various taxonomic groups (e.g., Moran and Porath, 1980; Speziale *et al.*, 1984; Suzuki and Ishimaru, 1990). Furthermore, DMF shows the best extractive ability for accessory pigments among the commonly used solvents, such as 90% acetone or methanol (Suzuki *et al.*, 1993; Wright *et al.*, 1997). Although 90% acetone is most widely used as an extractant in both conventional fluorometry and HPLC, it can lead to an underestimation of Chl *a*. The underestimation is serious in extraction by soaking, and even mechanical treatments of samples, such as homogenization and sonication, result in some losses of materials (Wright *et al.*, 1997).

In spite of its high performance, DMF is at present seldom used as an extractant for HPLC, probably due to safety considerations in view of its toxicity. Although Suzuki

et al. (1993) described a simplified HPLC method using DMF, separations of some polar pigments were insufficient. This was mainly because the method did not use ion-pairing reagents (Mantoura and Llewellyn, 1983). In this communication we examine the suitability of DMF for the standard reverse-phase HPLC method using ion-pairing reagents, paying special attention to the degradation of chlorophyll *a*. The test was conducted using phytoplankton cultures including a diatom *Phaeodactylum tricorutum* with a high chlorophyllase activity.

2. Materials and Methods

The examinations were done using clonal cultures of *P. tricorutum*, a cryptophyte *Chroomonas salina*, a haptophyte *Pleurochrysis carterae* and a chlorophyte *Dunaliella tertiolecta*. Cells were grown in the f/2 medium (Guillard and Ryther, 1962) at 20°C under illumination of ca. 60 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$ supplied by daylight type fluorescence tubes with a photoperiod of 12:12 h LD. The cultures were harvested in the mid to late exponential growth phase. Care was taken to take the cell suspension into a flask before filtration, avoiding residual materials around culture bottles. Cell suspensions were filtered onto 25 mm Whatman GF/F filters using gentle suction of 150 mmHg (vacuum). Immediately after the filtration the filter papers were folded once and blotted between pieces of filter paper to reduce the excess water content. From the filtration through the whole assay process the samples were handled under dim light or in darkness to prevent photodegradation of the pigments.

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