Gulf Carbon Pigment Analysis Method

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INTRODUCTION

Pigments were analyzed using the high pressure liquid chromatographic (HPLC) separation methods of Van Heukelem and Thomas (2001), (VHT) with minor modifications. The method uses a binary gradient separation on a C8 column. The method is capable of separating mono- and divinyl chlorophyll a, zeaxanthin and lutein and partial separation between chlorophyll c_1 and c_2 and mono and divinyl chlorophyll *b*. However, the method does not separate β,β-carotene and β,ε-carotene.

EXTRACTION

Prior to HPLC analysis, filters were lyophilized (freeze-dried) at -47 to -52 °C and 0.100 mbar for 12-15 h (Labconco FreeZone 6) to remove all the water from the filters. After lyophilization, the filters were extracted in 90% acetone (3 ml), vortexed and weighed. The filters were then soaked overnight at -19°C and subsequently vortexed for 1 minute and reweighed. The weighing was done to quantify any weight loss due to evaporation. Our long term comparisons have shown that this loss was negligible. The sample slurry was then transferred to a 5 cc glass syringe and filtered through a 13 mm diameter 0.2 µm PTFE HPLC syringe filter (Alltech, Catalog: 2164) The clarified extract was collected in disposable microcentrifuge tubes (2 ml) and stored at -19°C for a up to 8 hrs. In cases where longer storage was necessary the samples were immediately stored in a freezer at - 80°C until analysis.

ANALYTICAL METHOD

Instrumentation: Pigments are separated using two different methods using two identical HPLC systems. The HPLC systems were comprised of a Waters 600 pump and a photodiode array UV detector Model 2996, with wavelength resolution of 1.2 nm. The photodiode arrays (PDAs) were set to record spectra from 350 to 650 nm. . The Waters proprietary software package MaxPlot was used to acquire a chromatogram, the amplitude of which represented the largest absorbance of each one second spectrum between 408 and 480. The integration software was set to only report peaks greater than 0.0005AU.

Injection Procedure: Immediately prior to injection, a 50:50 mixture was prepared using 350 µL of sample extract and 350 µL of 0.05 M tetrabutylammonium acetate (TBAA) adjusted to pH 6.5. The mixture was then injected to flush and fill a 500 µL injection loop, the contents of which were then injected onto the column.

GRADIENT:

This was essentially identical to the VHT method. The runtime was 31 mins, the column used was a Zorbax Eclipse XDB C8 with a particle size of 3.5 µm and 150 x 4.6 mm

internal dimensions.

CALIBRATION:
The systems were calibrated using pigments from algal cultures or from standards purchased from DHI (Denmark). All regression coefficients (r^2) for major pigment standards were 0.99 or higher. The coefficients of variation (CV) for replicate injections were ~ 2%. Pigment concentrations and purity of each pigment was examined using a dual beam UVvisible spectrophotometer (CARY 300 Bio). The absorption coefficients used were those reported by DHI in the SeaHARRE-2 report (Hooker et al. 2005). All solvents and chemicals used were of HPLC-grade (Fisher Scientific) and analytical grade. For more details please refer to Chakraborty et al chapter in SeaHARRE report 5 (Hooker et al. 2012).

VALIDATION: Efforts were taken at to assure quality control of the data and system performance within expectations. Since the HPLC pigment analysis methods involve manual injection, the injection loop is routinely cleaned with acetone to avoid contamination between samples. In addition, acetone blanks are run following idle periods of more than two weeks to rule out the presence of residual contamination. Multi-point calibrations of all pigments are performed every 2-3 years. Multi-point calibration of chlorophyll-a and fucoxanthin are done every six months (approximately). Mixed pigments from DHI (Denmark) were used to determine fluctuations in retention time for all reported pigments. A minimum variation in retention time was observed. Periodic checks of the stability of the systems are also made every six months at USM. Assessments of the long term stability of the systems, expressed as the average noise calculated according to methods given in the SeaHARRE 2 report (Hooker et al. 2005), yielded a coefficient of variation (CV) in % for replicate injections of 13.7

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