

HPLC Pigment Analysis SeaHarre-5

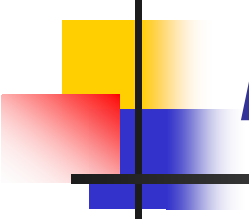
***Dalhousie University
Method***

by

Claire Normandeau

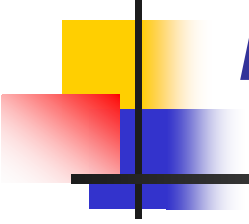
John Cullen

Markus Kienast



Extraction

- 2 ml 100% MeOH (calculated with the relative density)
- I.S. vit E acetate diluted in solvent (0.125 mg.ml^{-1})
- Storage 2h at -20°C
- Sonication 20 sec
- Centrifugation at 13 000 rpm



Injection

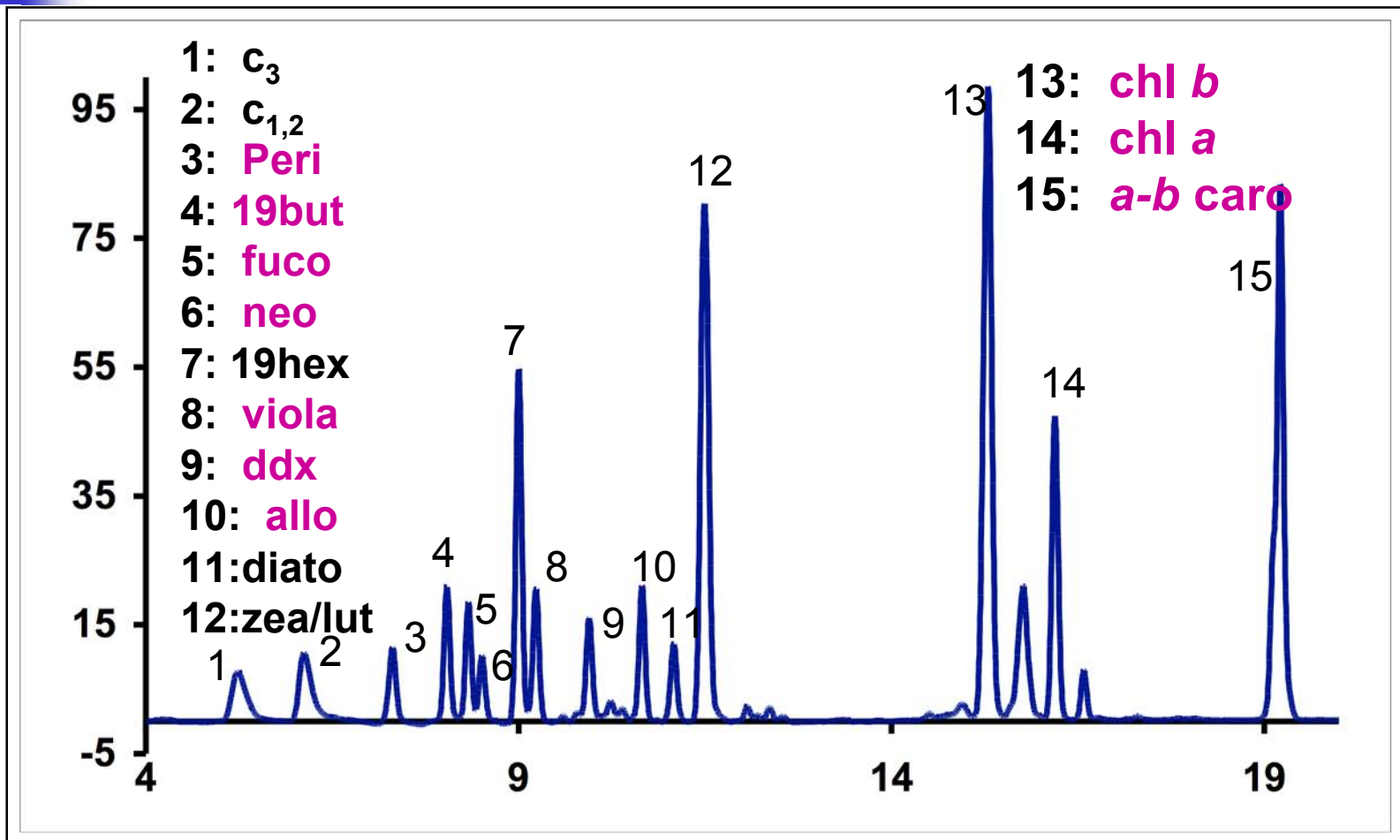
- 231 μ l sample + 69 μ l buffer (DHI)
- Mix 3 times in 500 μ l loop
- Inject 300 μ l
- Sequence:
 - Methanol
 - chl a
 - Mixed standard
 - vit E
 - no more than 14 samples

HPLC system-Wright et al.

- Agilent 1100
- PDA and fluorescence detectors
 - Carotenoids: 450 nm
 - Chl *a* and pheopigments: 436 nm
- C18 column, 4.6 x 150 mm, 5 μ m particle size
- Cooled autosampler (4 °C)
- No thermostat on column

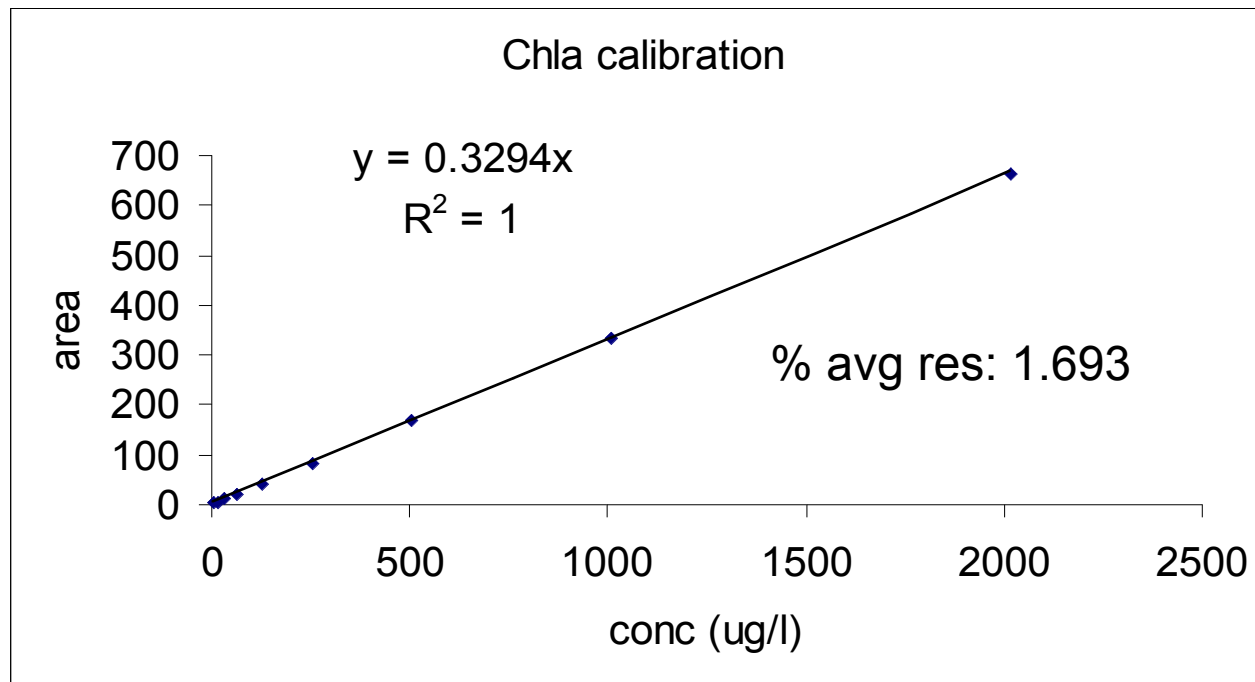


Peak identification



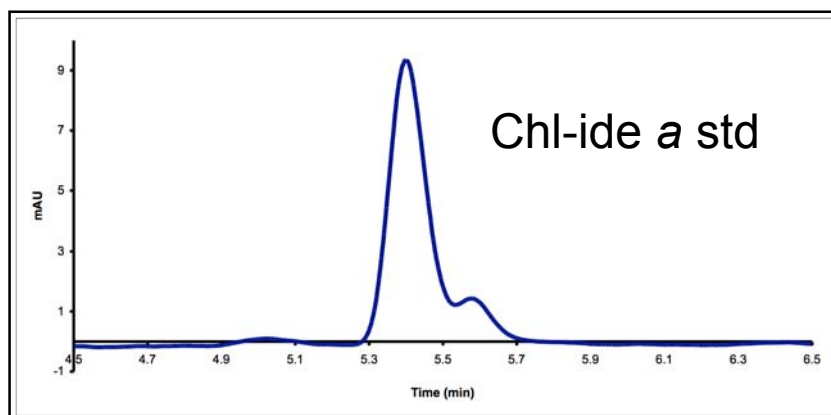
Calibration

- DHI standards using the conc. provided
- 5 dilutions

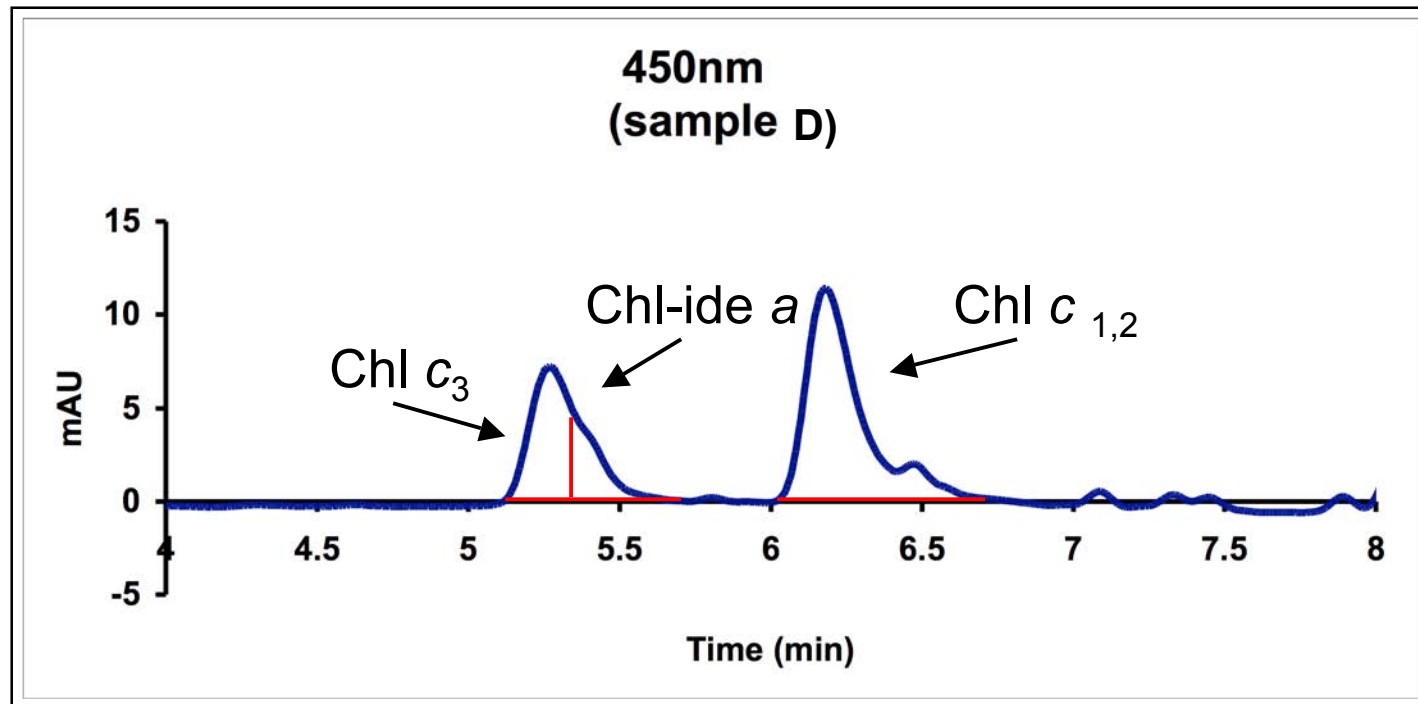


% absolute residuals on calibrations

Chla	1.693	19but	2.684
chlde a-fluo	6.238	19hex	1.341
chlde a- 436nm	3.717	ddx	0.31
chlc3	1.649	diato	1.89
chlc2	0.884	allo	0.885
peridinin	1.409	zea	1.466
fuco	0.648	chlb	1.045
a-carot	5.626	b-carot	1.774

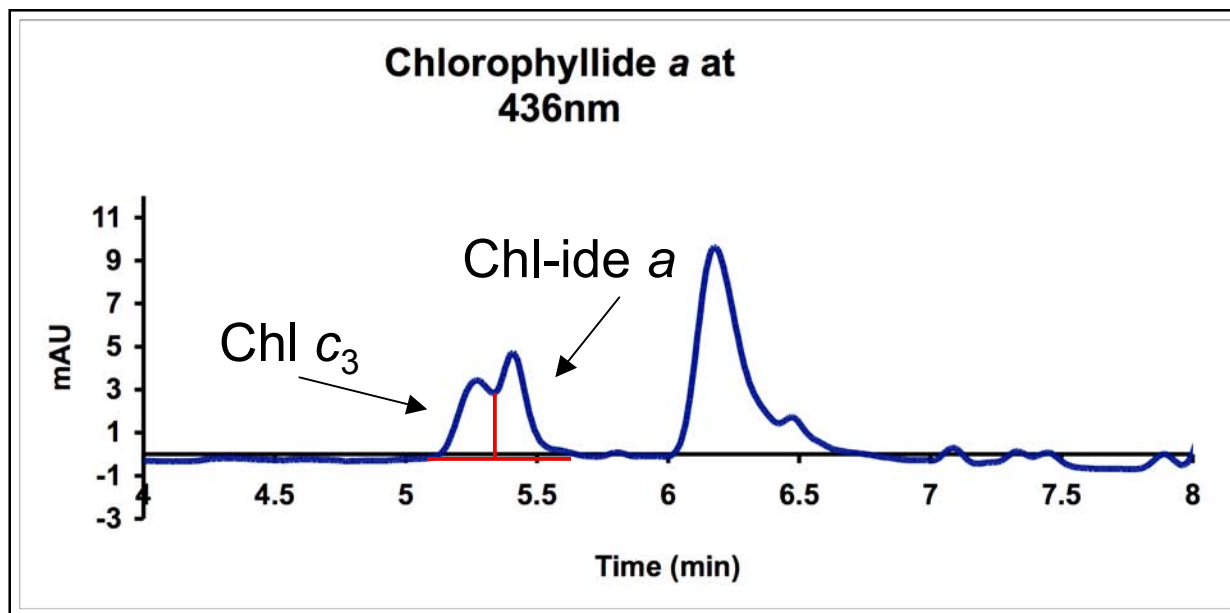


Difficulty integrating chl c_3 , chl $c_{1,2}$ and chlorophyllide a



Chl $c_{1,2}$
 ϵ : 6.29%
apd: 8.23%

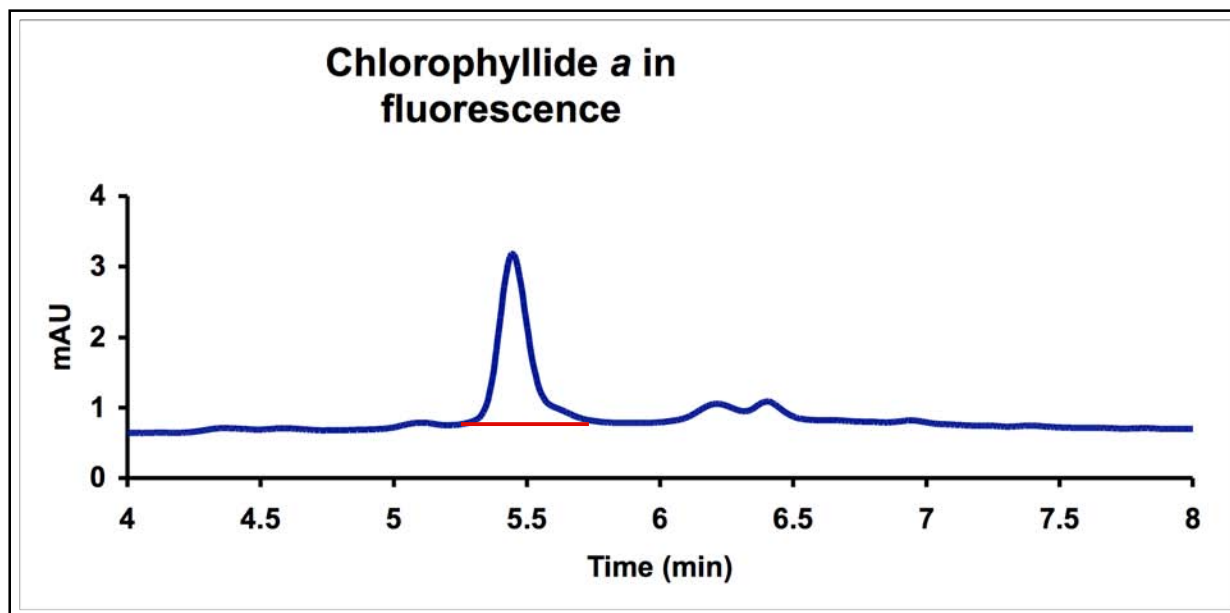
Chl c_3
 ϵ : 6.37%
apd: 8.29%



Chl-ide a 436nm:

ξ : 5.73 %

apd: 7.60 %

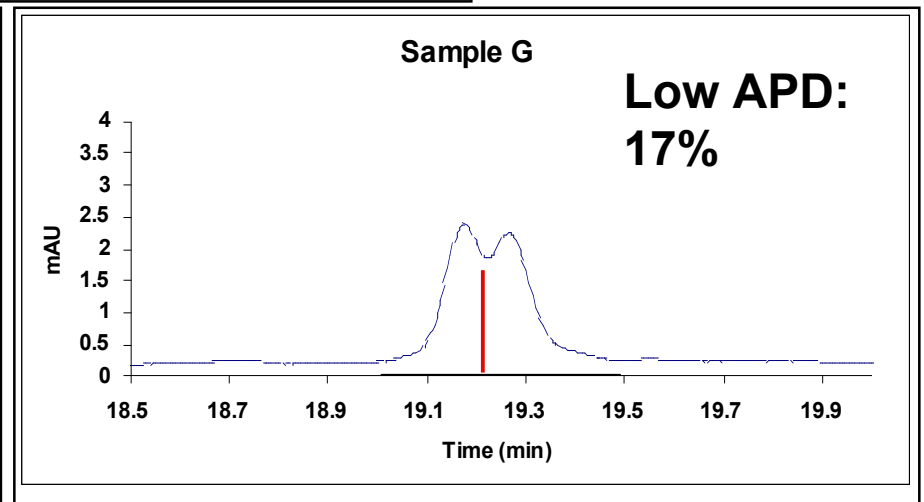
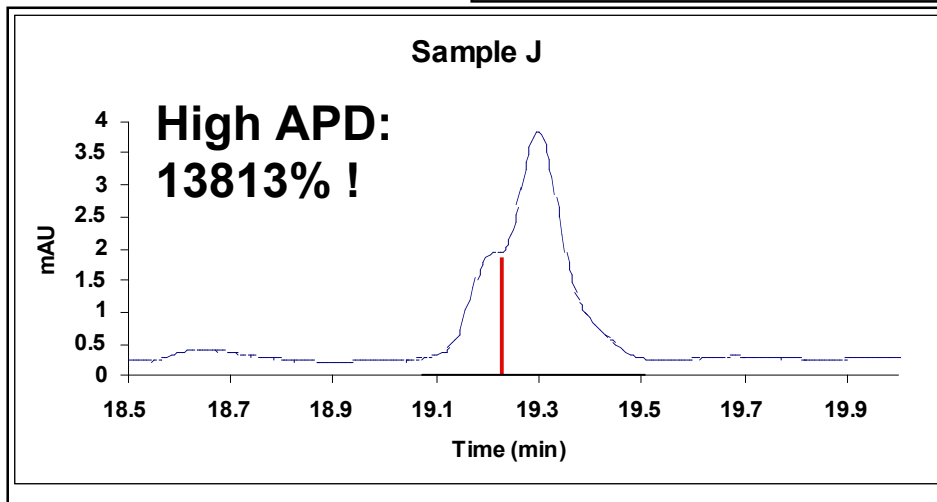
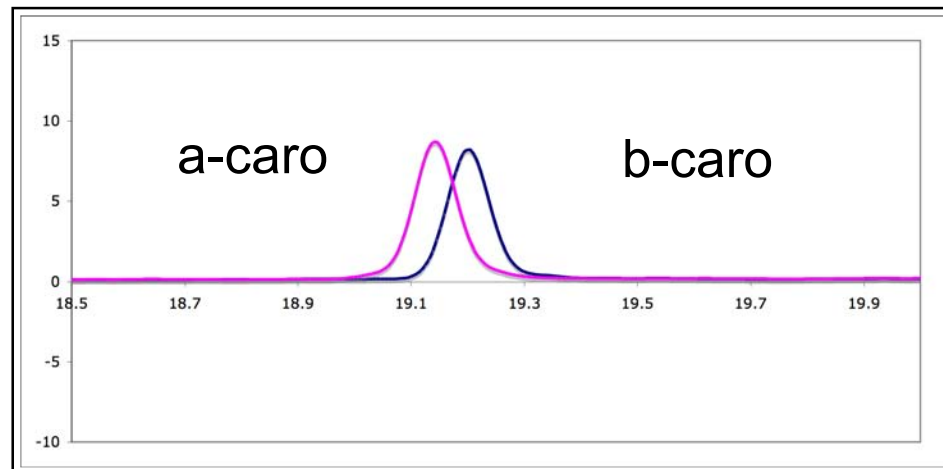


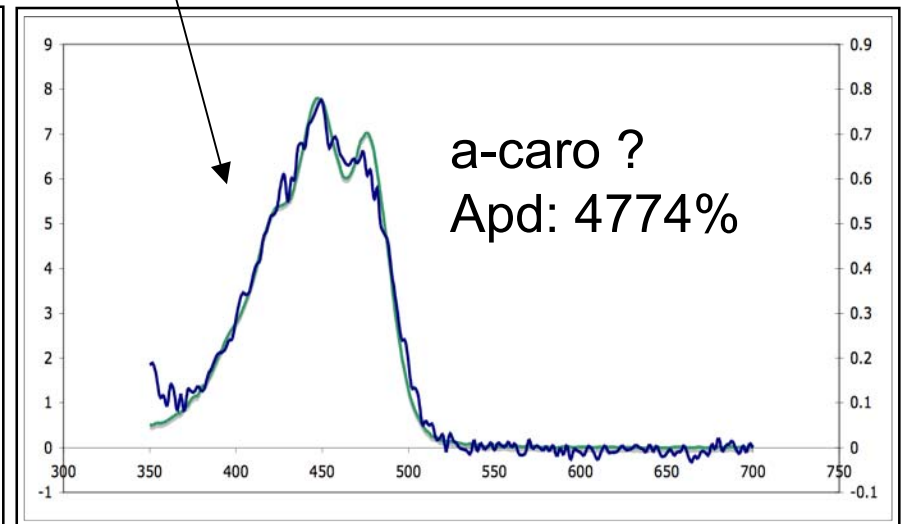
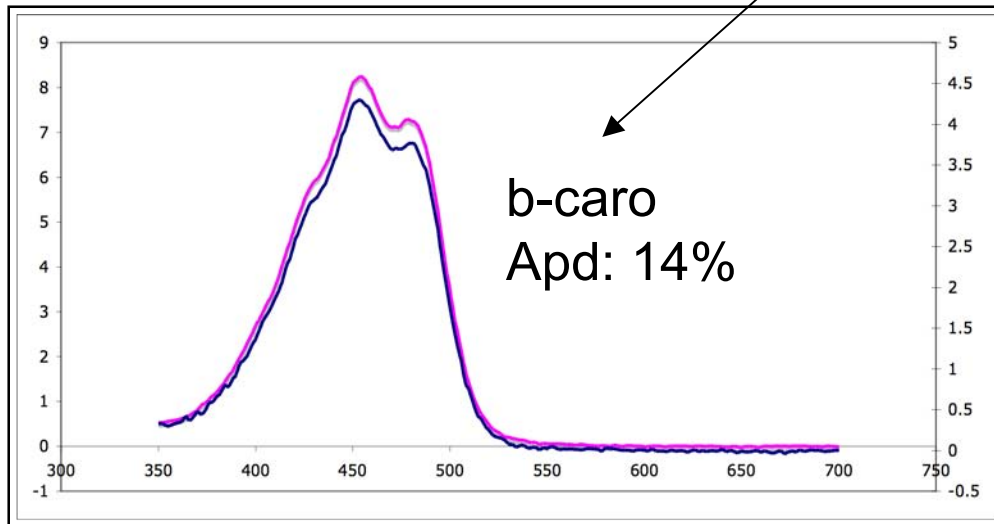
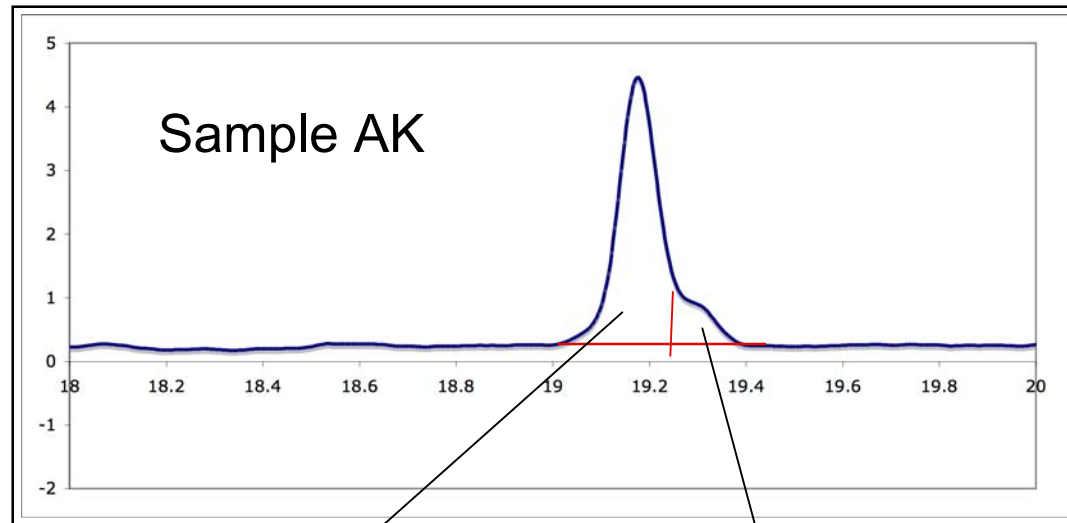
Chl-ide a FL:

ξ : 6.74 %

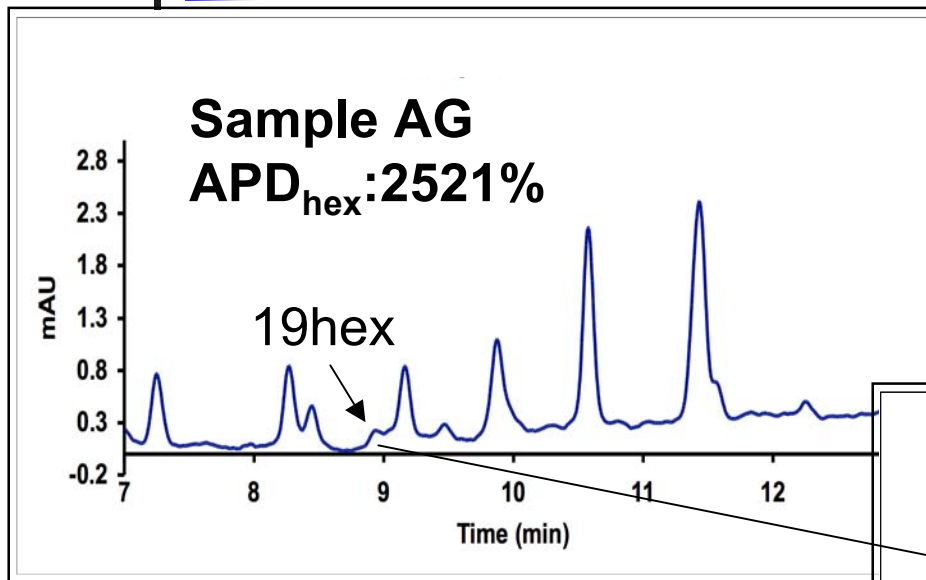
apd: 8.91 %

Coelution between *a*-carotene and *b*-carotene



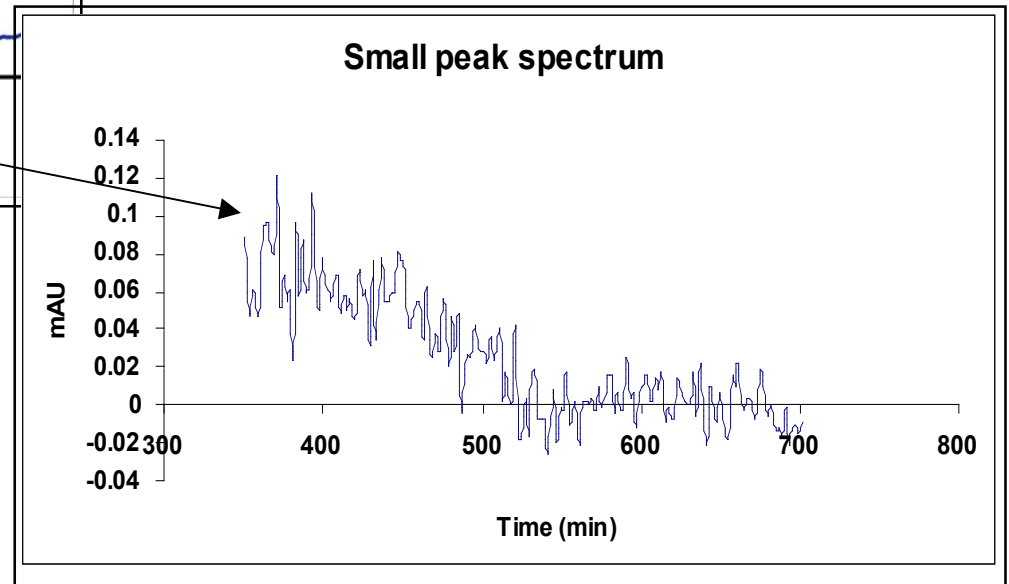


Small peaks at LOD

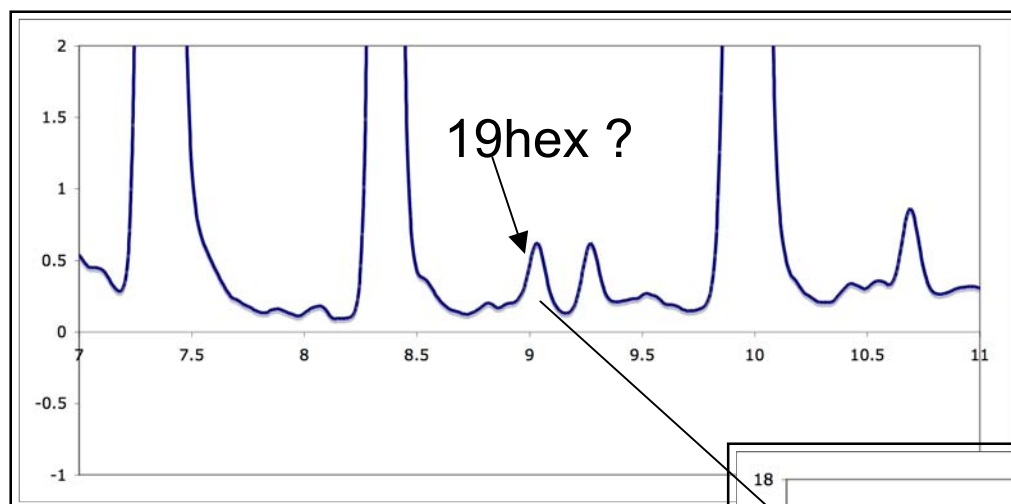


Larger APD on:

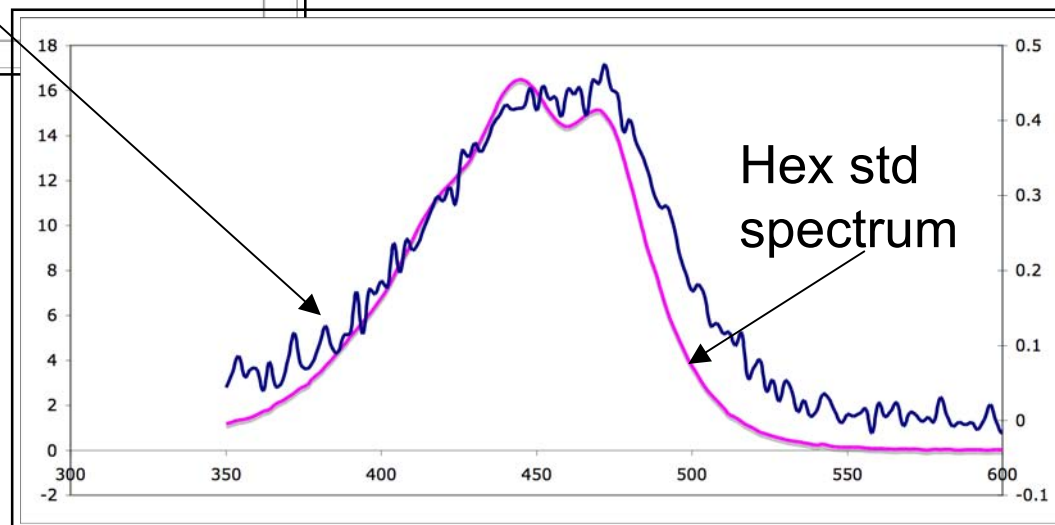
- But -fuco
- Hex -fuco
- Diato

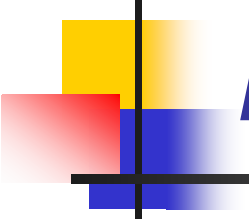


Small peaks at LOQ



Sample AA
APD_{hex}: 214%





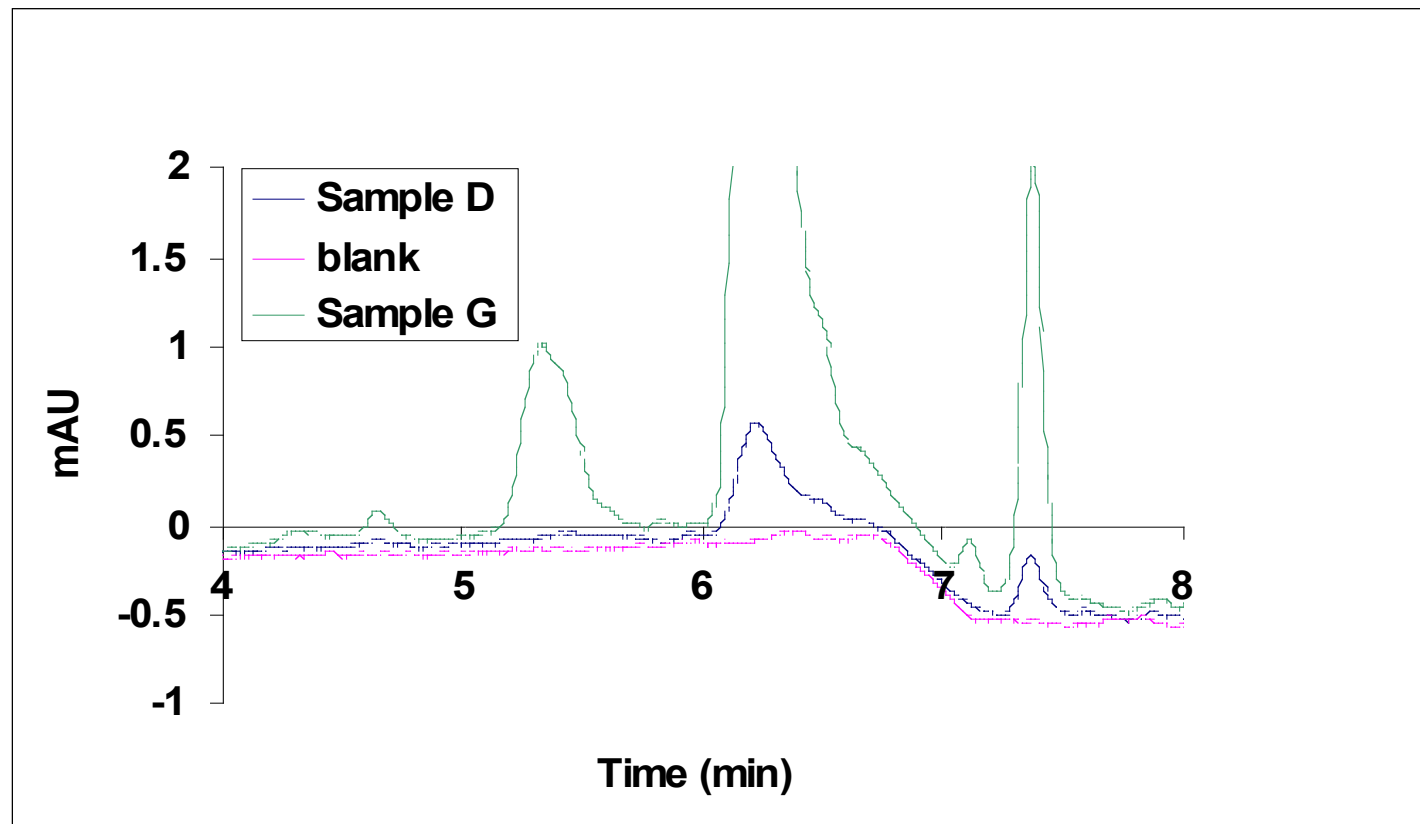
Ideas for improvement

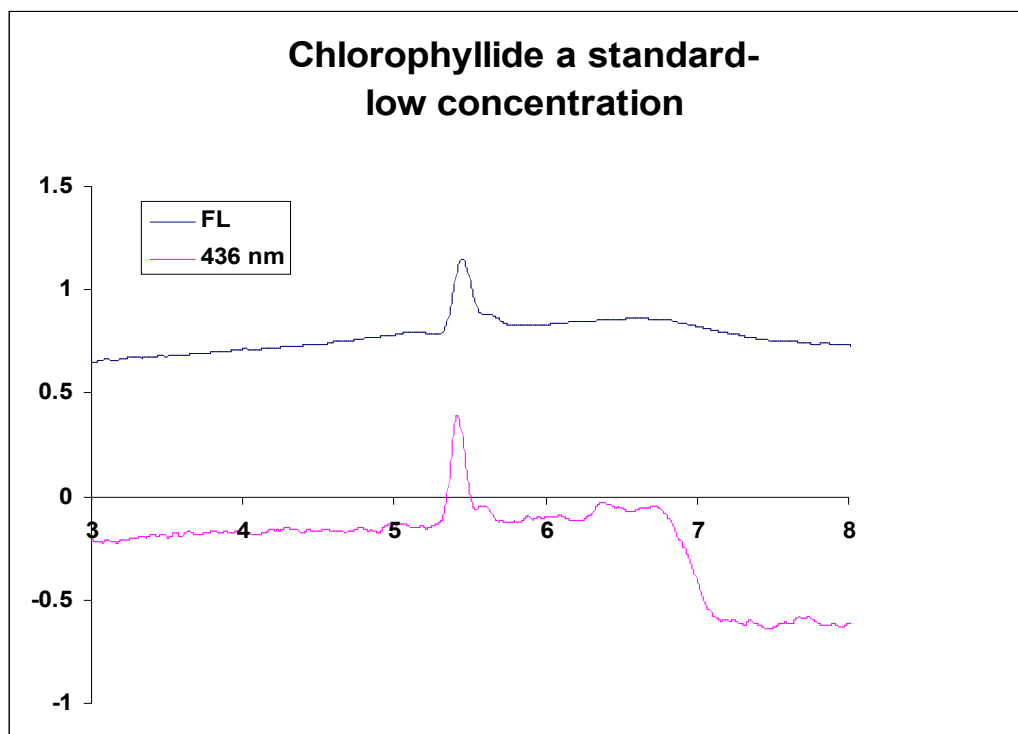
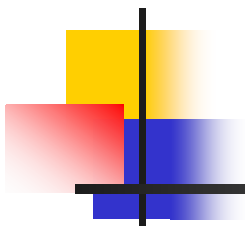
- Quantify no lower than LOQ and use two sentences rule
- Agree on how to integrate difficult pigments (chlorophyllide *a*, chl $c_{1,2}$, c_3 and *a-b* carotene)



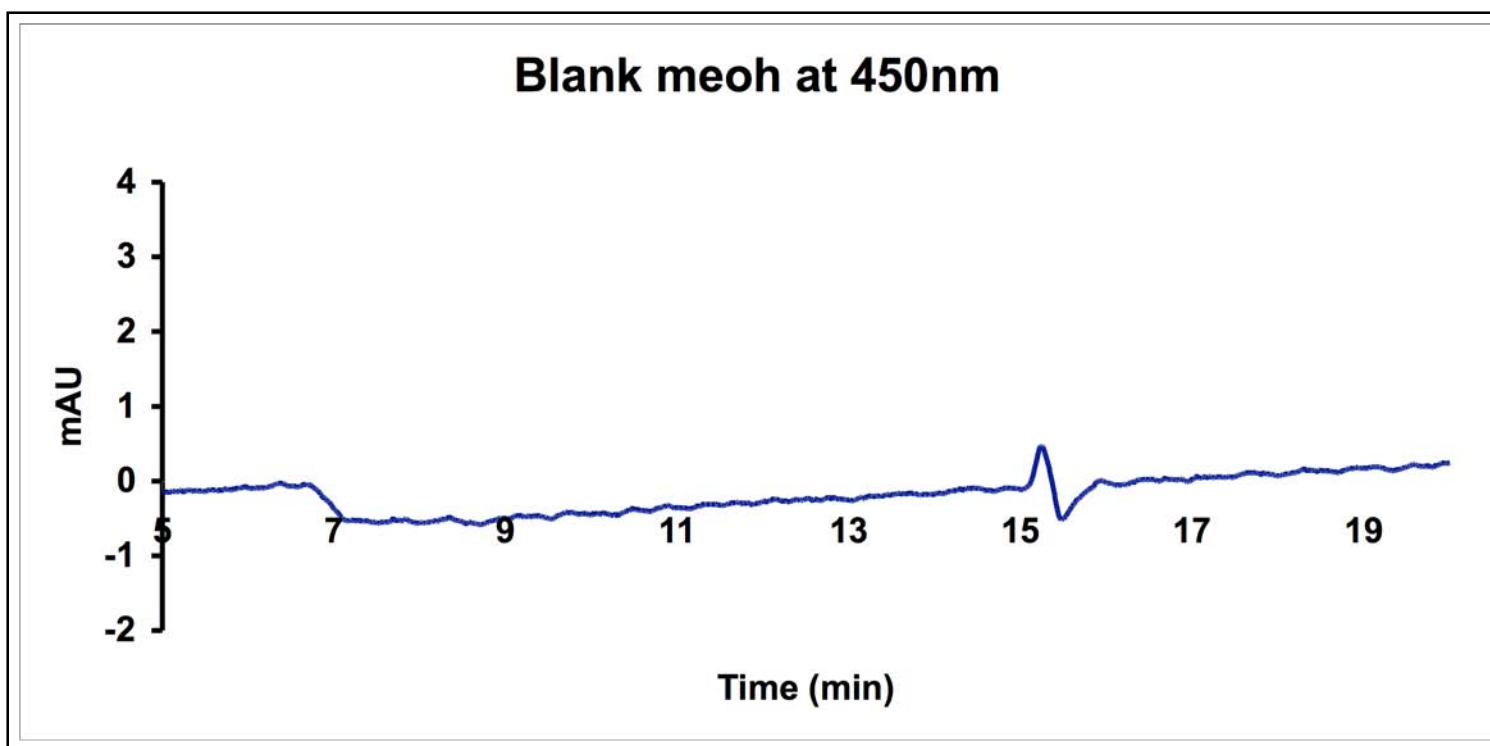
Thank you!

Chlorophyll c2 integration

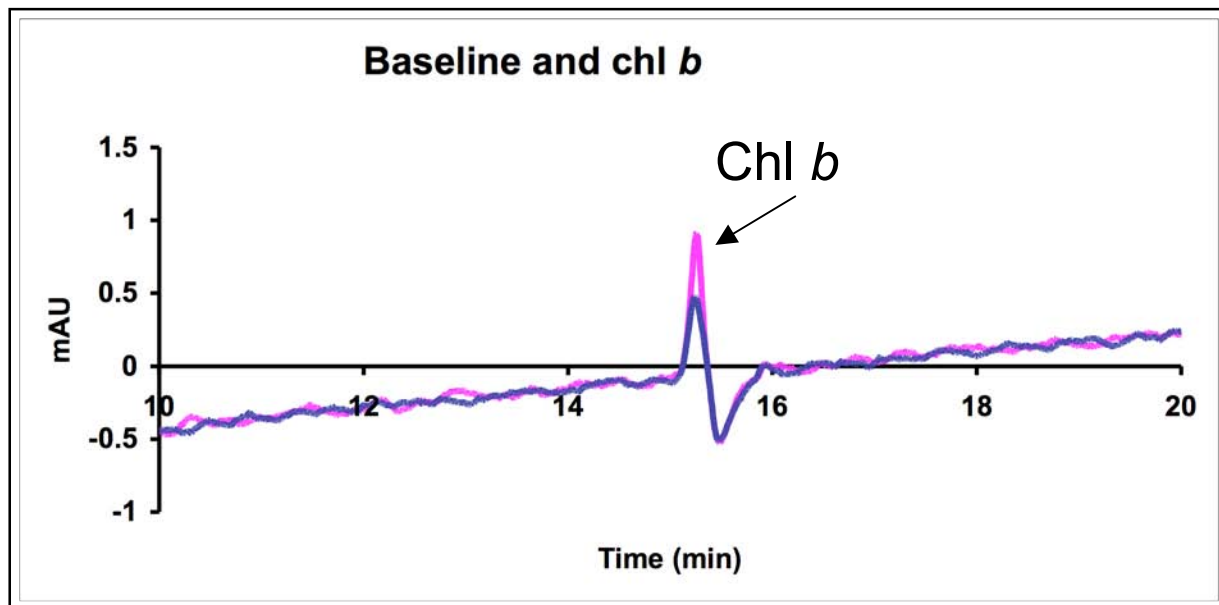
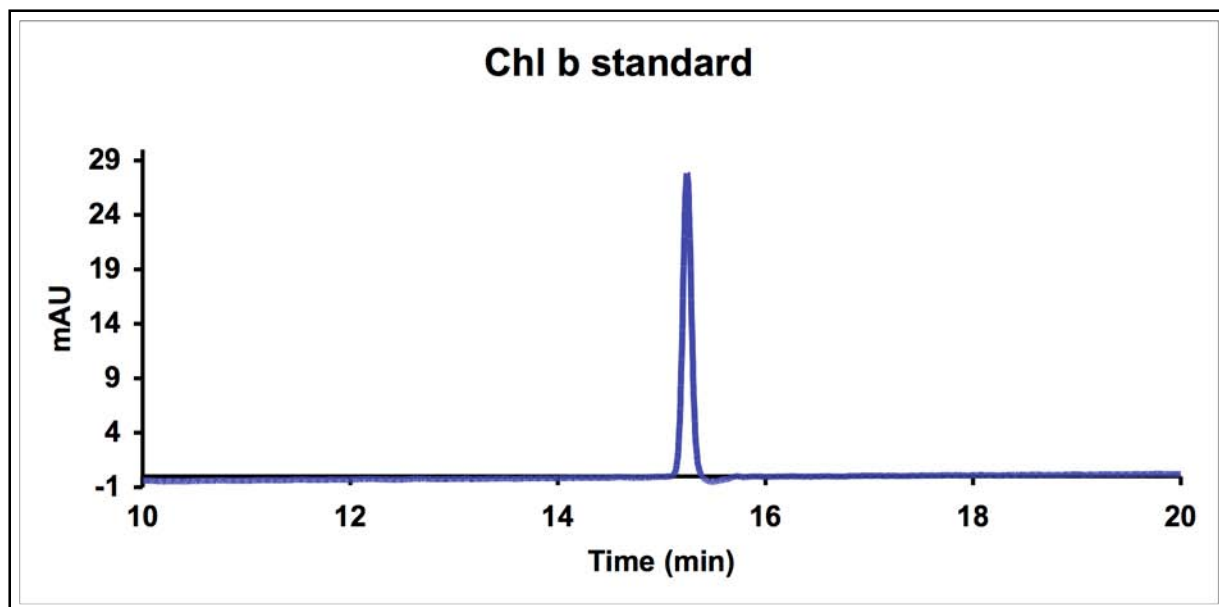




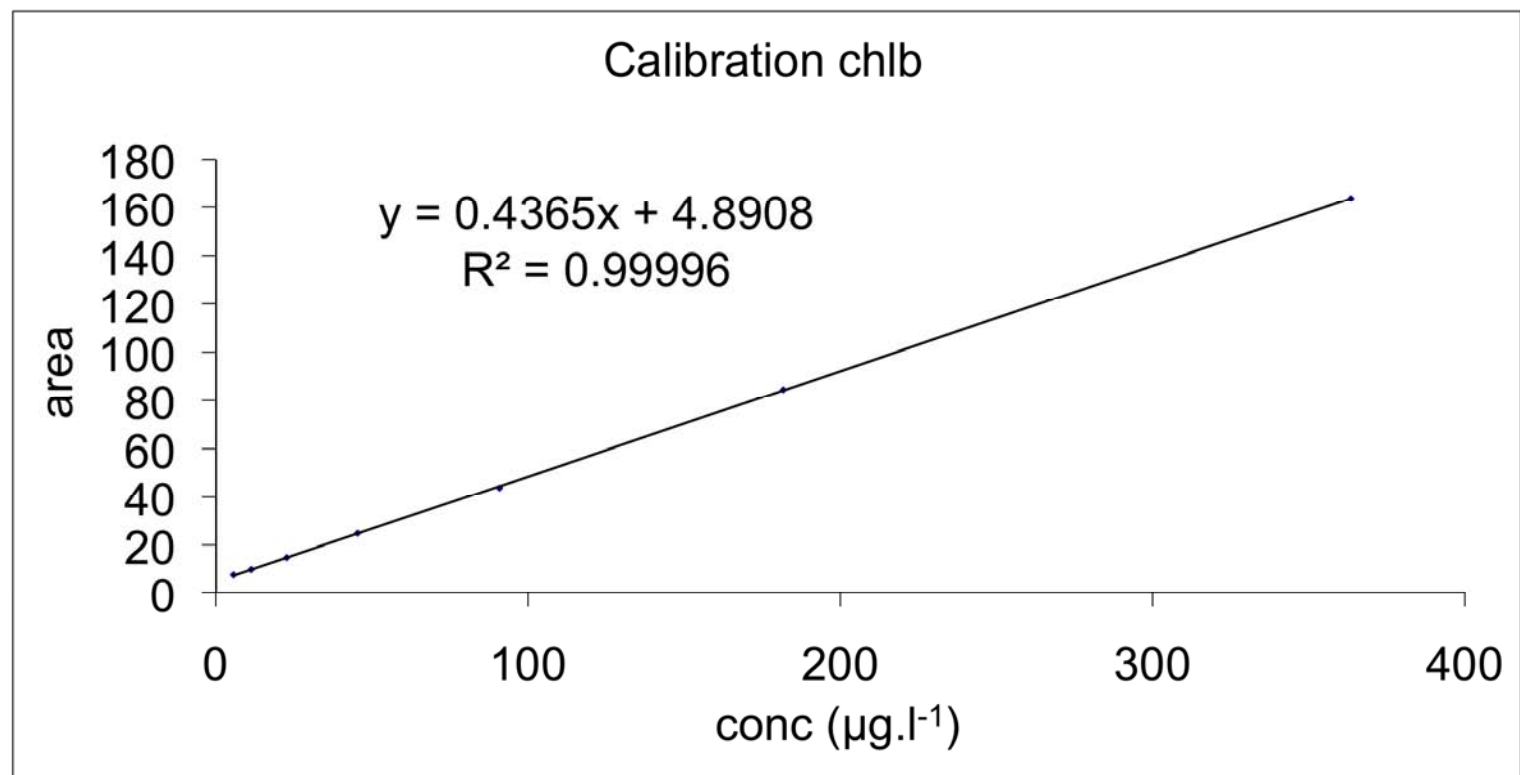
Baseline close-up

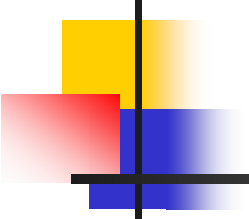


Chlorophyll b



Chlorophyll b calibration





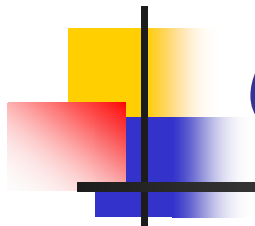
HPLC solvent system program (Wright et al, 1991)

Time (min)	Flow Rate (ml.min ⁻¹)	% A	% B	% C
0	1	100	0	0
4	1	0	100	0
18	1	0	20	80
21	1	0	100	0
24	1	100	0	0
29	1	100	0	0

A: 80:20 MeOH: 0.5 M ammonium acetate (pH 7.2)

B: 90:10 acetonitrile:H₂O

C: ethyl acetate



Quantification

Pigment concentration =

$$\frac{\text{Peak area}}{\text{Cal. factor}} * \frac{\text{Volume extraction}}{\text{Volume filtered}} * \frac{\text{Area vit E std}}{\text{Area vit E sample}}$$