

The USM Method

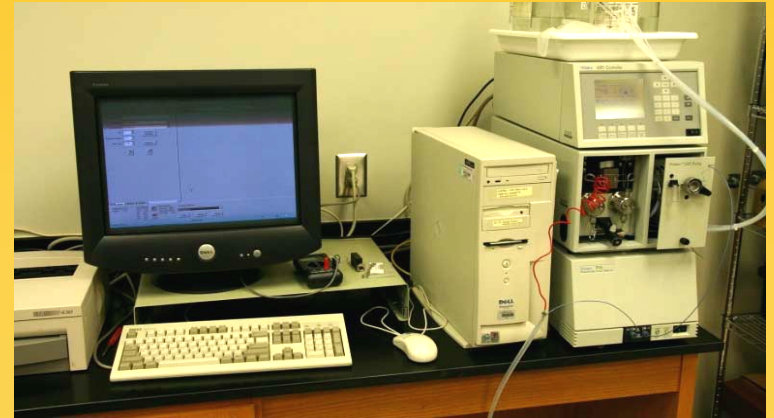


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Instrumentation & Method

2 identical systems: Waters- 2998 PDA; 600E pump system; manual injector



- Wright et al(1991) (Modified)

A= Methanol + 0.125M ammonium acetate + 0.0125% BHT,
B= Acetonitrile + 0.011% BHT, C= Acetone, D= Water. 34 mins ,
Column - Altima HP C18 5 μ - 250mm

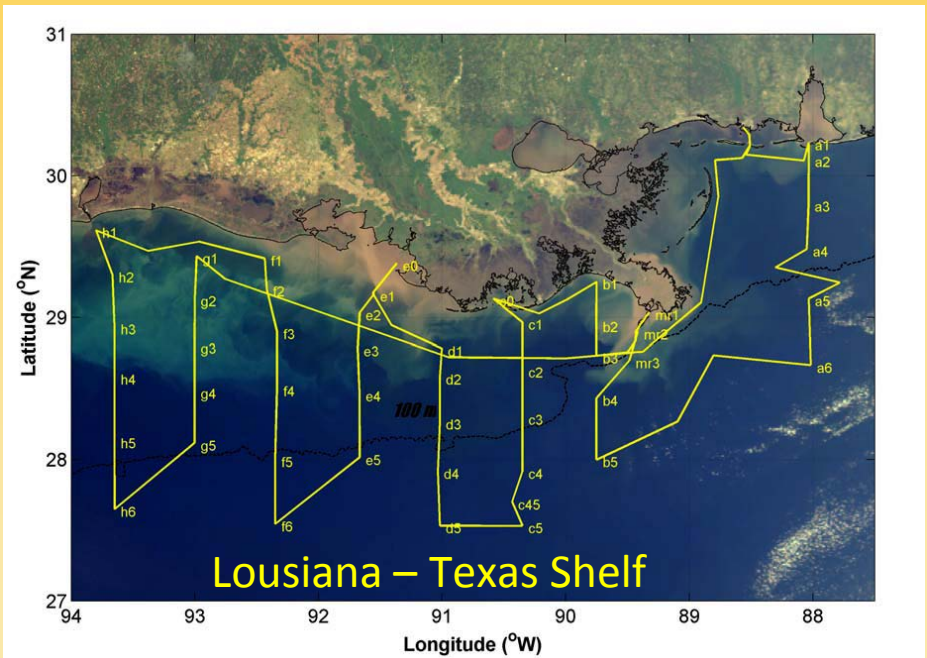
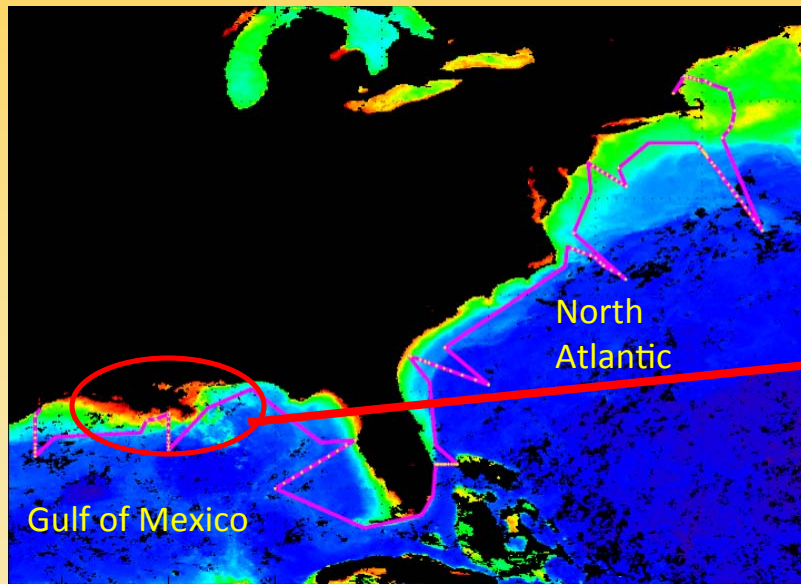
- Van Heukelem and Thomas (2001) (Modified)- 31 mins run

Samples are freeze dried (12-15 h) and extracted in 90 % Acetone -
analyzed within 24 h from the time of extraction

Short term sample storage = -19 °C (max 18 hrs)
= -80 °C (max 2weeks)

Applications

- Scientific research and publication, SeaBASS
- All water types: open ocean, coastal and estuarine, lake samples



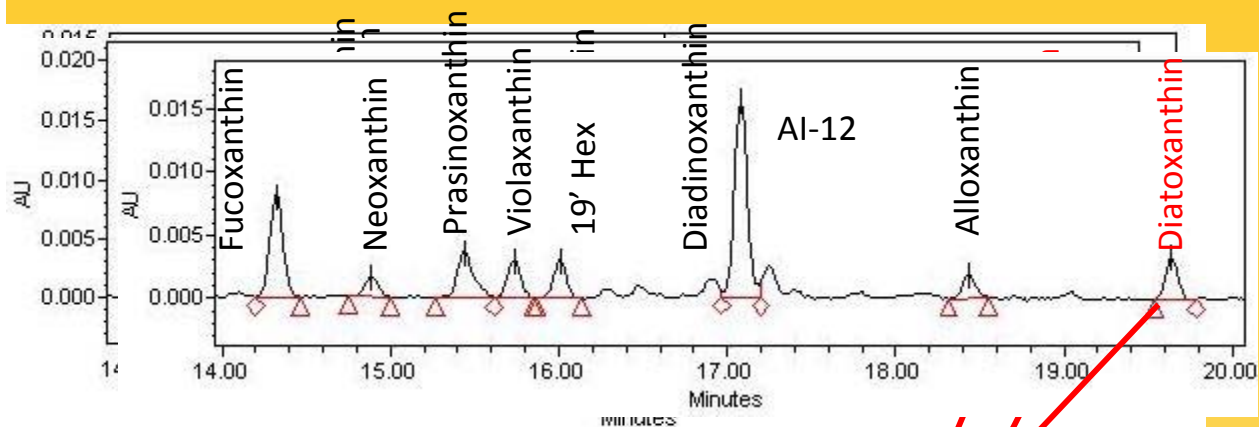
Analysis

- Maxplot (plots largest absorbance in each spectrum between 409 and 480nm)
- Acceptance and rejection based on
 1. Retention time
 2. Library spectral match
- Visually inspect integration of all peaks for each individual chromatogram

Case studies

- Diatoxanthin problem
- Fucoxanthin spectral inconsistencies in DHI mix for C18

Diatoxanthin Problem



Retention Time

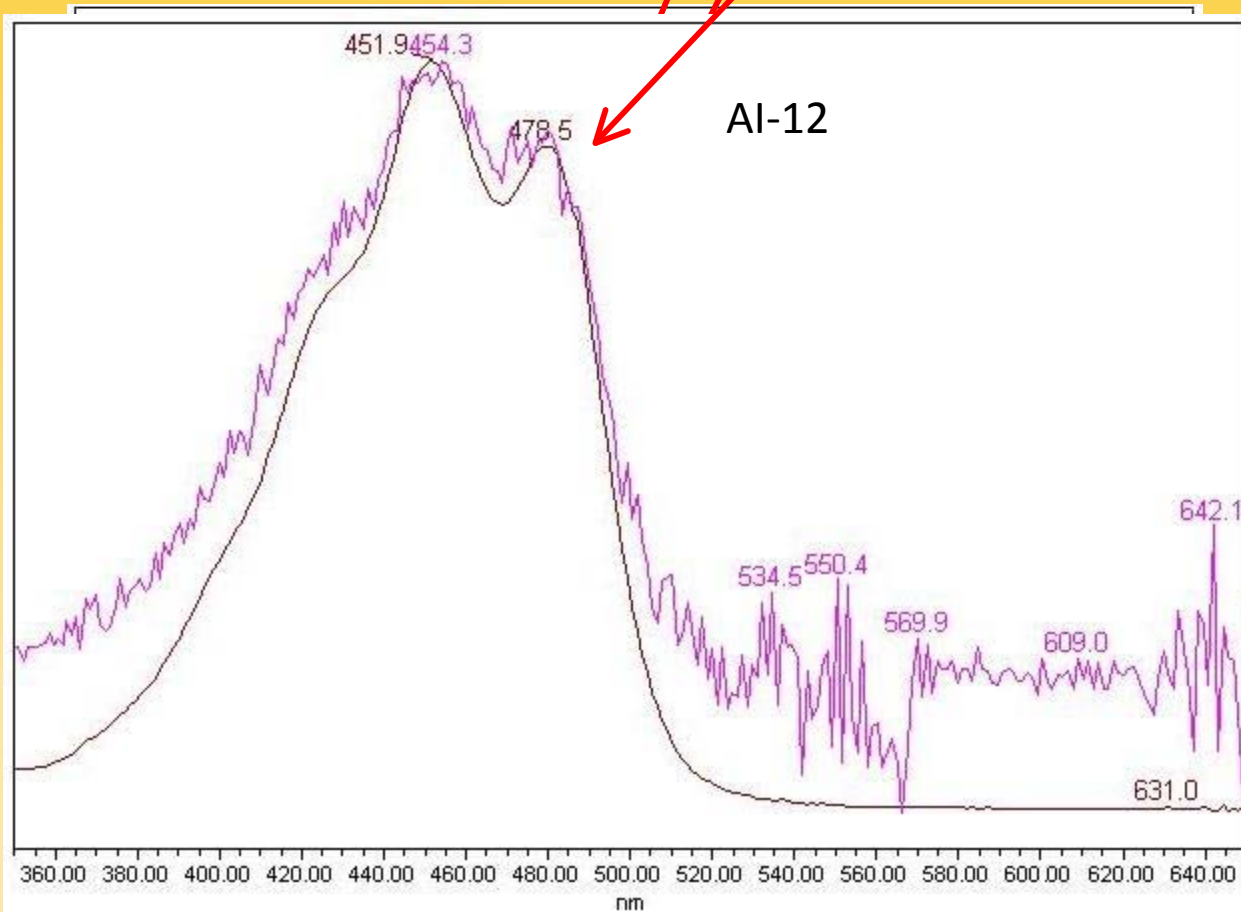
Al01= 19.643 mins

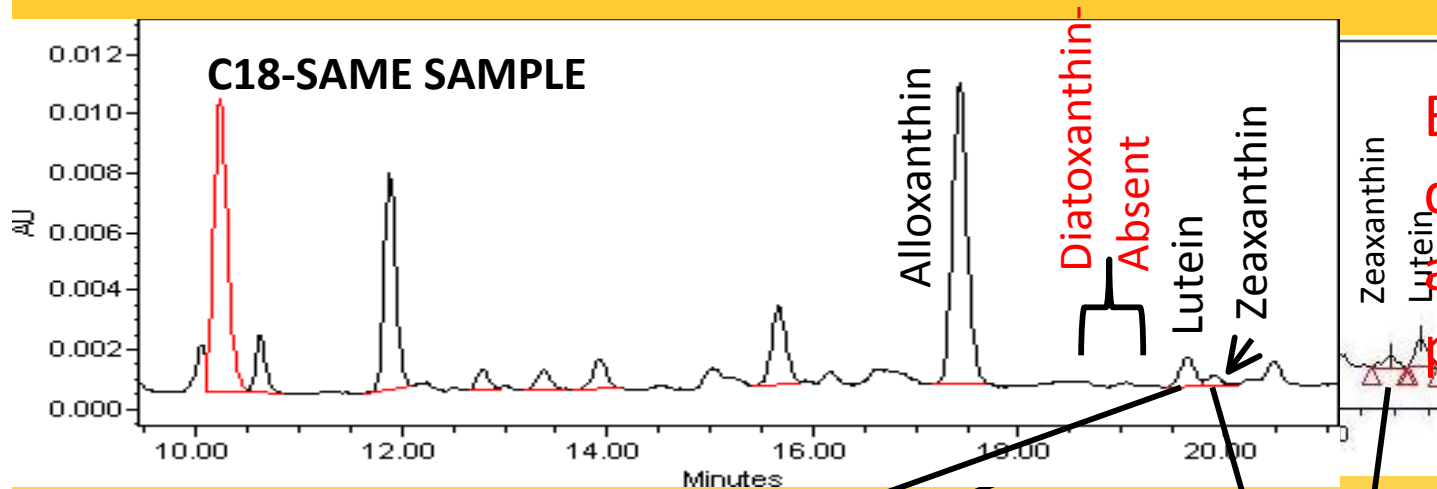
Al10= 19.630 mins

Al12= 19.638 mins

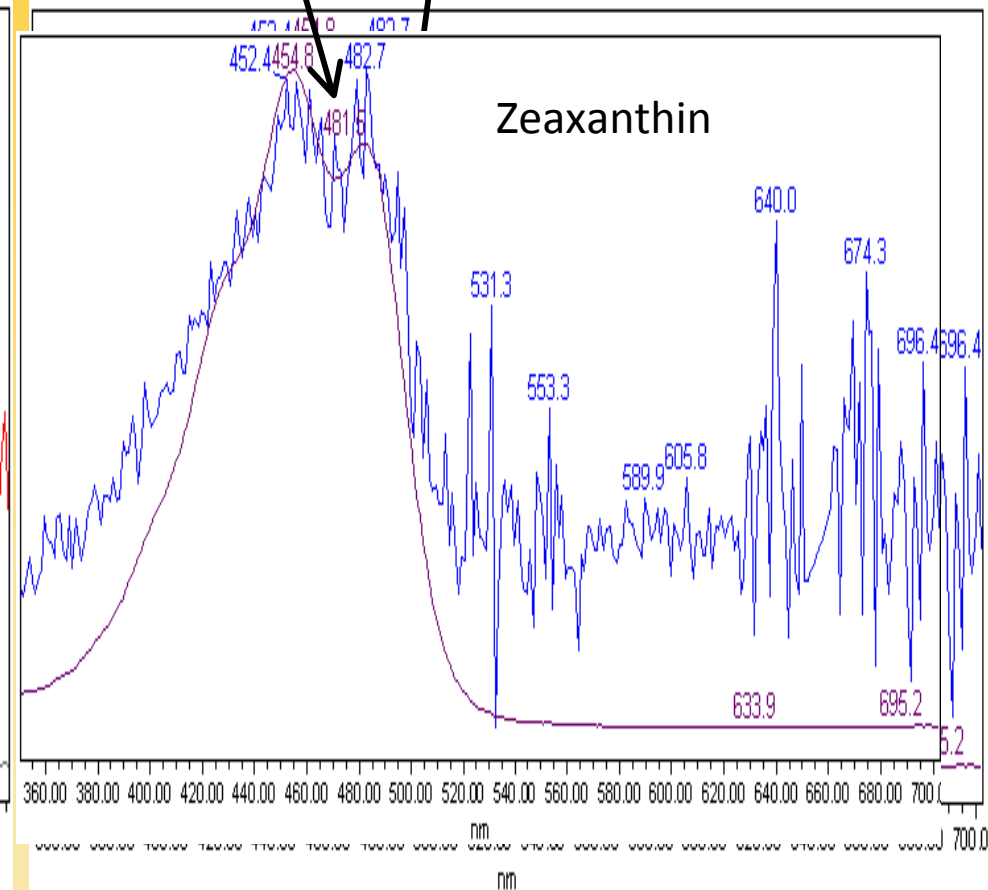
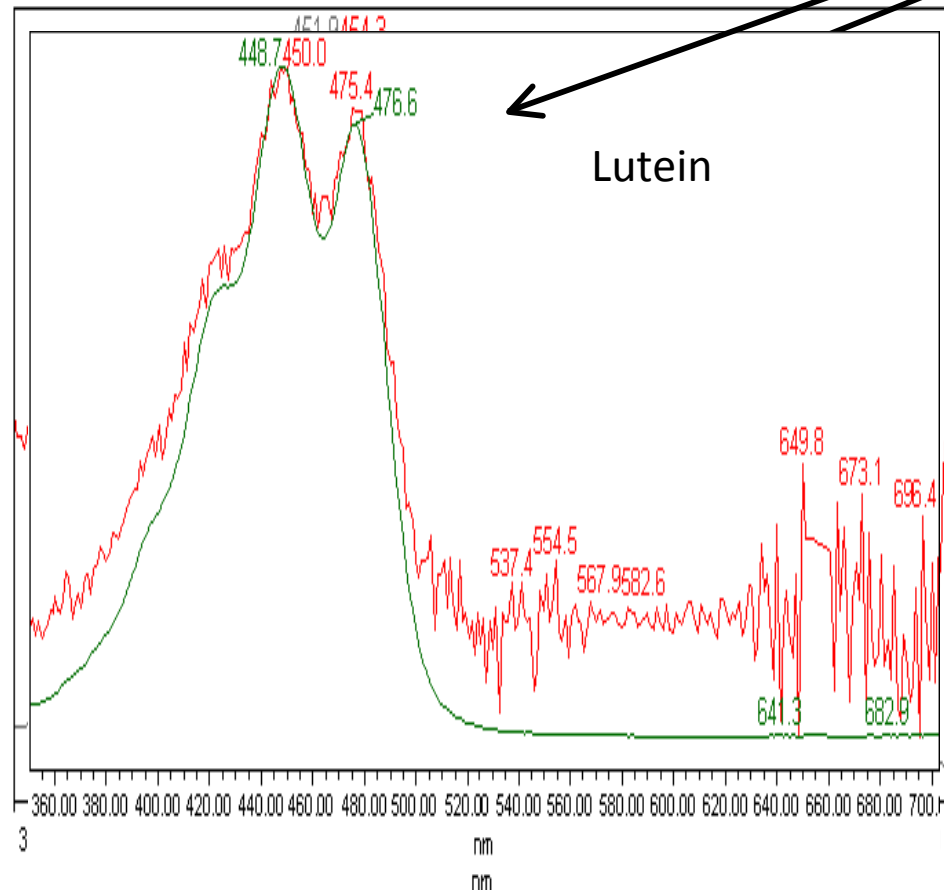
Avg. RT Diato = 19.7 mins

Avg. RT Zea = 20.07 mins



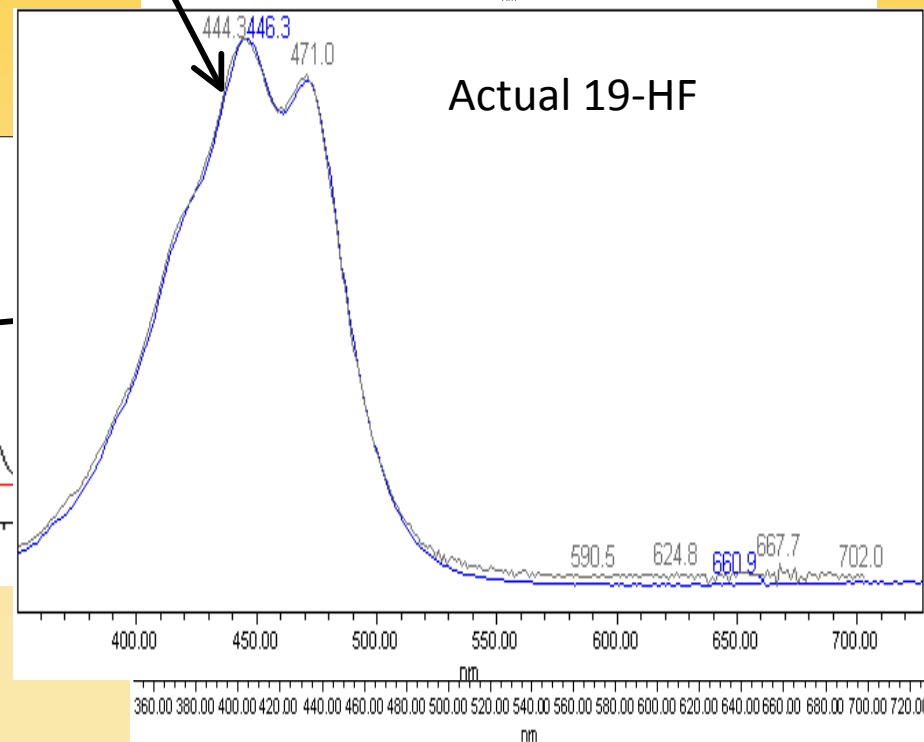
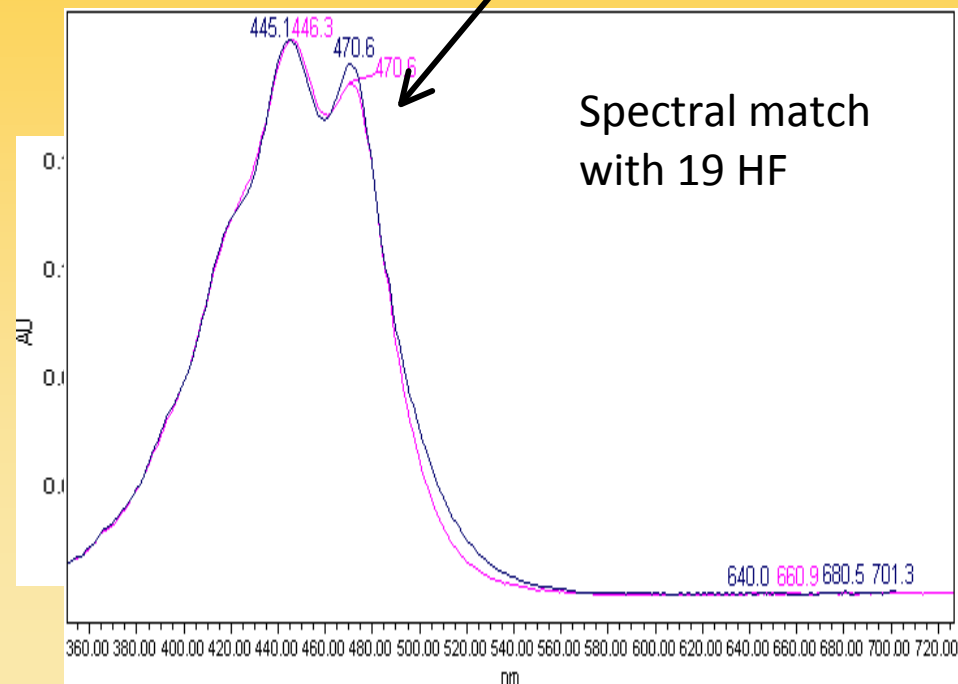
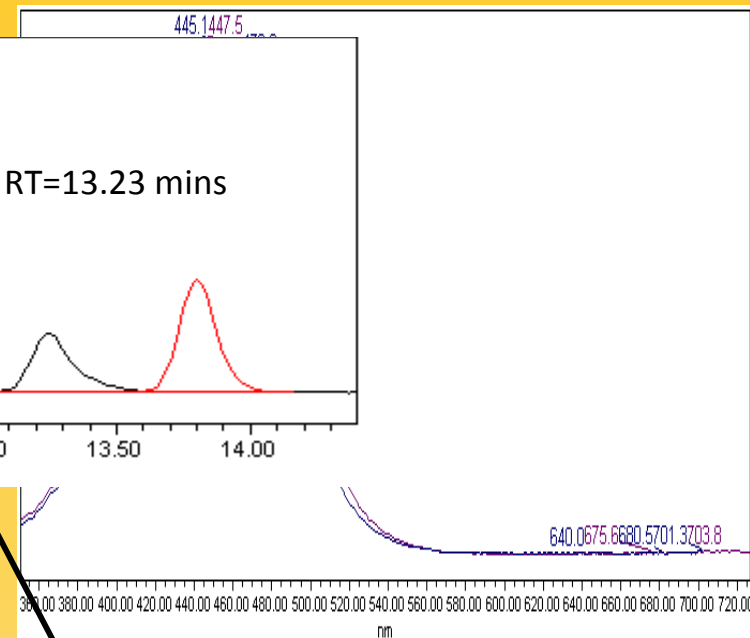
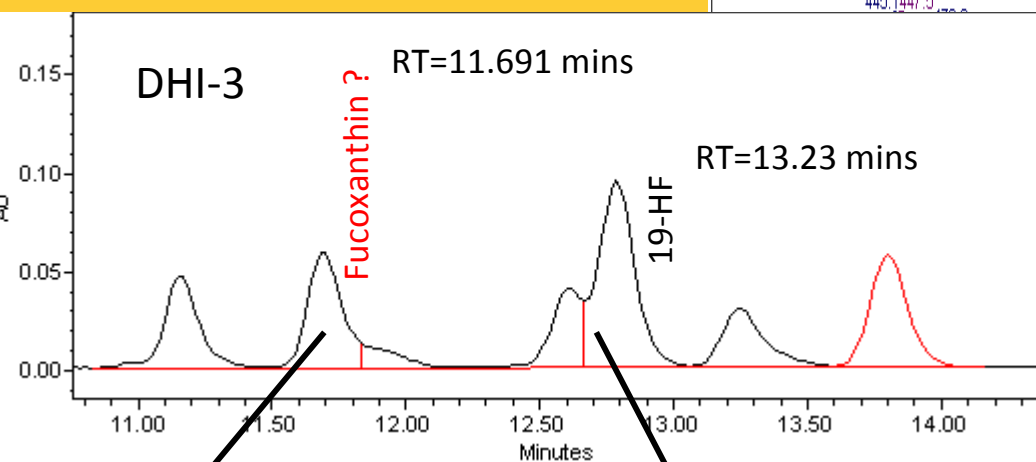
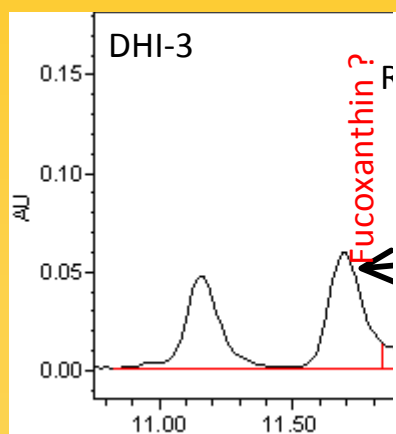


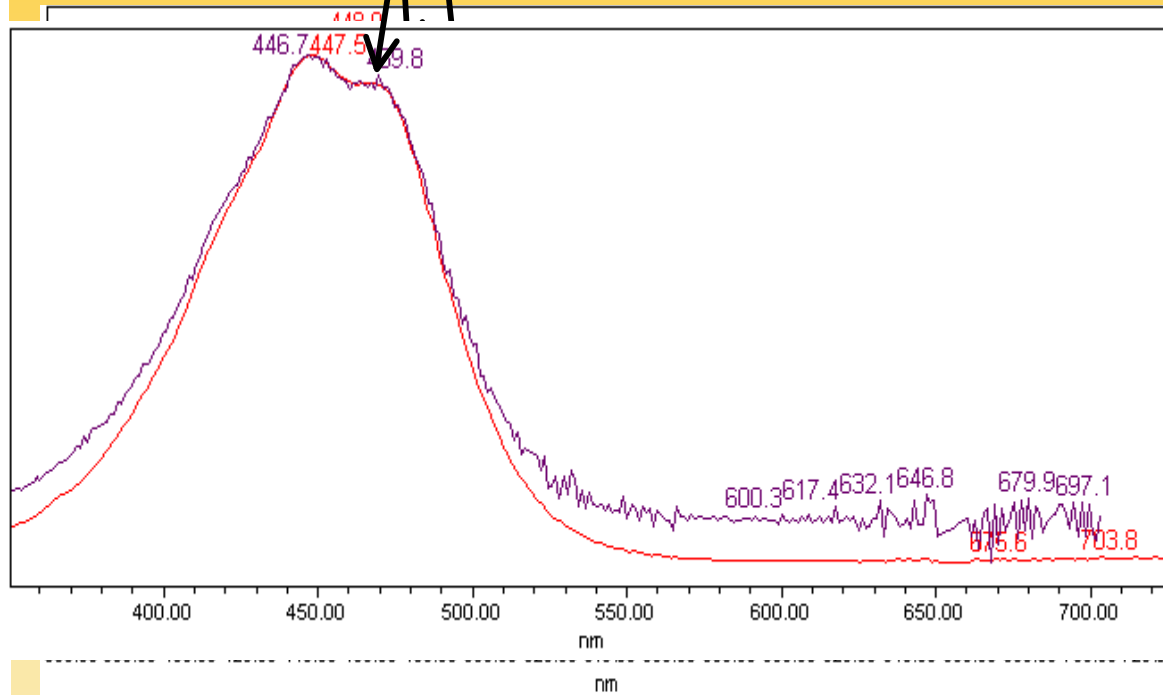
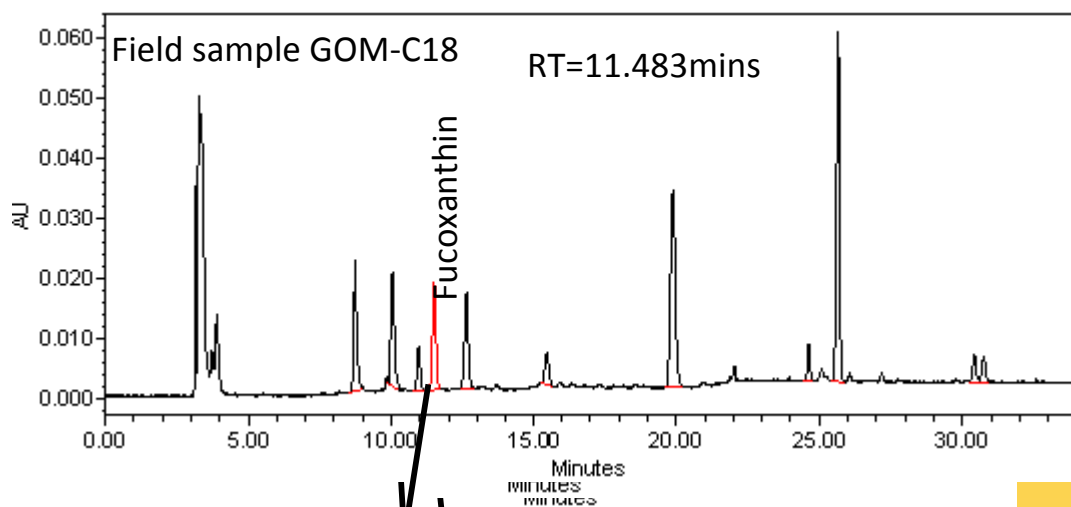
B31B1C18C8
 does show w
 any Diato
 peak and Zea



- Diatoxanthin has been a major concern in our analysis-particularly C8
 - Having two methods helps
 - C18- Diatoxanthin → Lutein → Zeaxanthin
 - C8- Diatoxanthin → Zeaxanthin → Lutein
-
- But for SeaHARRE-5 samples were analyzed from a single method point of view

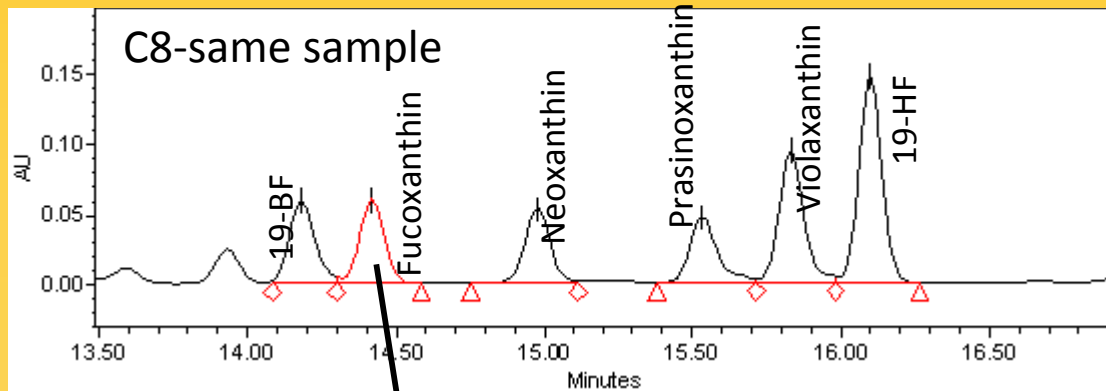
Curious case of Fucoxanthin in DHI mix – C18



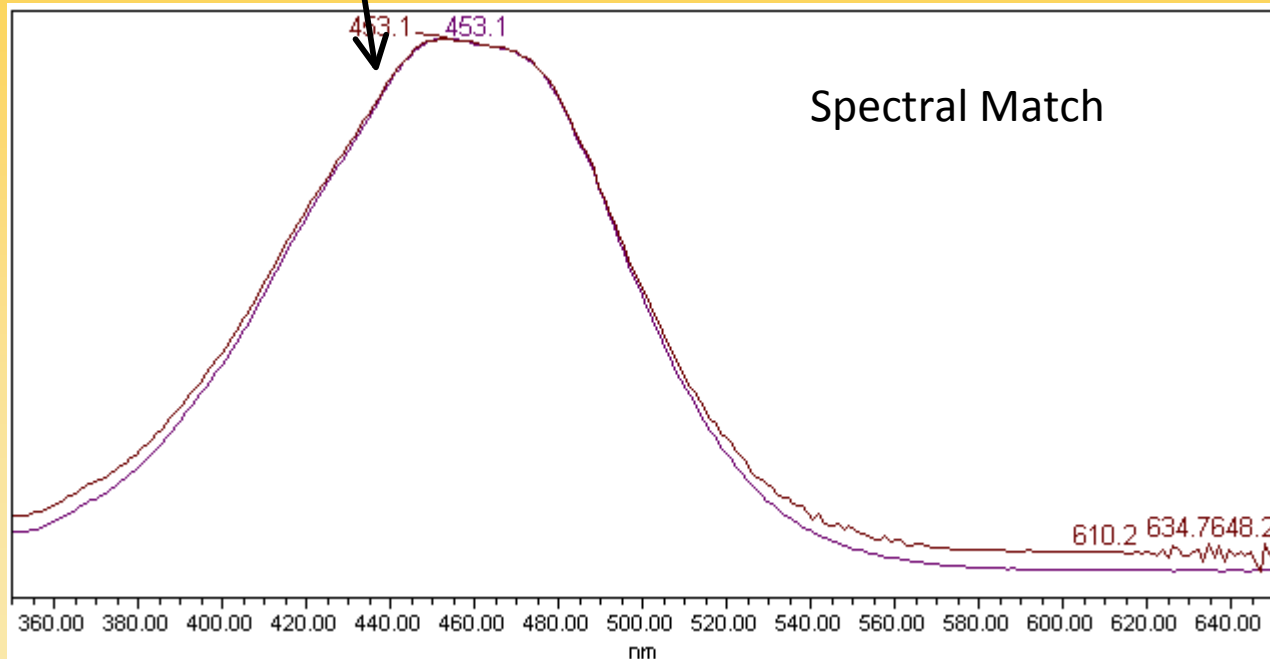


For other samples
SeaHARRE5,
Field samples
both RT and
Spectra almost
identical

Only observed in C18 method.



DHI mix - C8
method
Fucoxanthin has
perfect RT and
spectral match



Issues during SeaHARRE-5

- Pressure problems
- Hardware-pump replaced, wiring replaced
- Freeze Dryer problems
- Long storage –Gaps due to hardware issues and research cruise participation.

Quality Assurance

- **System and Column stability**

1. Long term stability – Noise calculation- every 6 months CV (%)= 13.7 – C8, 34 - C18
2. Pressure – column stability
3. Peak shape

- **Replicate injections-sample reproducibility**

1. Standards- MVChlorophyll-a, Fucoxanthin.
2. DHI mix
3. Field samples

- **Injection precision:** Pipettes $\pm 2\%$ and Syringe = $\pm 1\%$
(based on weight)

Advantages & Disadvantages

- Advantages
 - Freeze drying – thanks to Jay Pinckney
 - Injection precision: we weigh the buffer and the sample for every injection to verify sample and buffer volumes
 - Two simultaneous HPLC gives us better confidence in reported data
- Disadvantages
 - Manual Injection – Labor intensive
 - Ambiguity in Diatoxanthin in C8 at low SNR - similar issues with 19'But and peridinin
 - Chl-c's broad peaks and peak tailing
 - We can make it better by reducing the sample to buffer ratio
 - Still working on it... for an ideal ratio

Acknowledgements

- Donald Redalje
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THANK YOU