The Vernet Lab HPLC Method

Scripps Institution of Oceanography
La Jolla, CA

Wendy Kozlowski
Maria Vernet, PI

Method Application

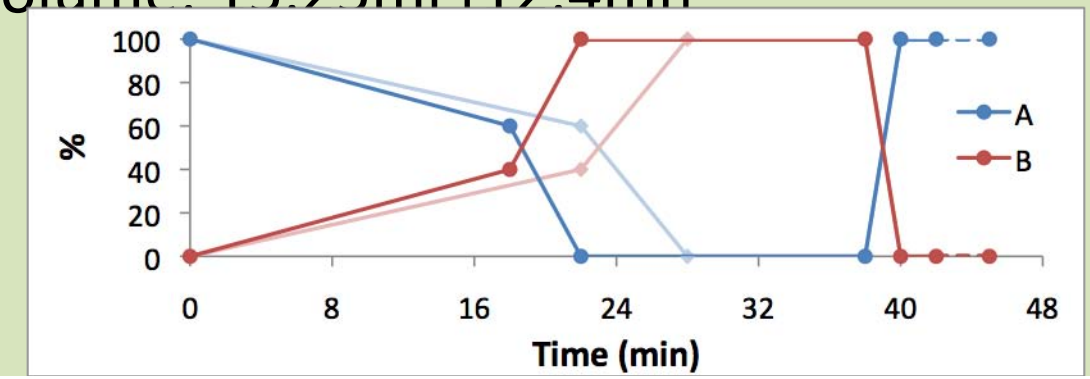
- Historical (1994-2008)
  - ~1000 field samples per year
  - Analyzed IN field
    - 1 – 6 month period per year
    - at Palmer Station / onboard research vessels
  - Primarily Vernet projects
  - Primarily Mesotrophic Antarctic Waters (0.5 – 220 km offshore)
  - Purpose
    - Biomass & Composition
    - PP modeling
    - Optical modeling

Method Application

- Looking Forward
  - Fewer samples (several hundred)
  - Mix of Field and Cultures
  - Run at SIO lab
  - Primarily Vernet projects +
    SIO grad student projects
  - Still primarily Antarctic species +
    some coastal CA samples

Hardware & Method Overview

- Agilent 1100 Series HPLC
  - DAD (440nm) [VWD (440nm) & FLD (440nm Ex, 660nm Em)]
  - Waters Symmetry C8 150mm x 4.6µ, 3.5µ, 100Å, 25°C
  - Vacuum degasser
  - Refrigerated sample tray (4°C)
- Zapata *et al.* (2000)
  - Solvent A: 50:25:25 MeOH:MeCN:0.25M Pyridine
  - Solvent B: 20:60:20 MeOH:MeCN:Acetone
  - Re-equilibration volume: 15.25ml [12.4ml]
  - 1.0ml / min



SH5 Sample Handling and Extraction

- Samples received by HPL 2/2/09 (Liq N_2 → -80°C)
- SH5 samples extracted 2/6 – 2/9
- EE samples extracted 2/10 – 2/13
- Analysis (except re-injections) complete 24-48h after extraction
- Possible Issue: missing material
  - 17*/72 (SH5) and 18/77 (EE) not folded in half
  - 1/72 had hole torn through foil and filter

Extraction Details

Sample Set	Extraction Solvent [ml]		Solvent Added	Net Percent	Mode‡ and Time of Disruption	Soak Time [h]	Clarification Procedure
	Added	HPLC					
SH5	2.96§	2.96	90% Acetone	83.7% Acetone*	Sonic Probe 12s	24	0.45µ Whatman nylon "Puradisk" syringe filter
EE	1 to 1.95§ (25mm)	1 to 1.95	90% Acetone	73.8% - 76.0% Acetone*	Sonic Probe 12s	24	0.45µ Whatman nylon "Puradisk" syringe filter
	4.90§ (47mm)	4.9	90% Acetone	83.0% Acetone**	Sonic Probe 12+s	24	0.45µ Whatman nylon "Puradisk" syringe filter

§ ± 0.01 (water calibration of pipets and dispensettes used)

* Based on 0.22ml water in 25mm glass fiber filter

**Based on 0.41ml water in 47mm glass fiber filter

‡ SH5: Branson Digital (400W) - 25% amplitude - 2s/1s pulse

‡ SIO: Misonix XL2000 (100W) - 10s - power “8” - no pulse

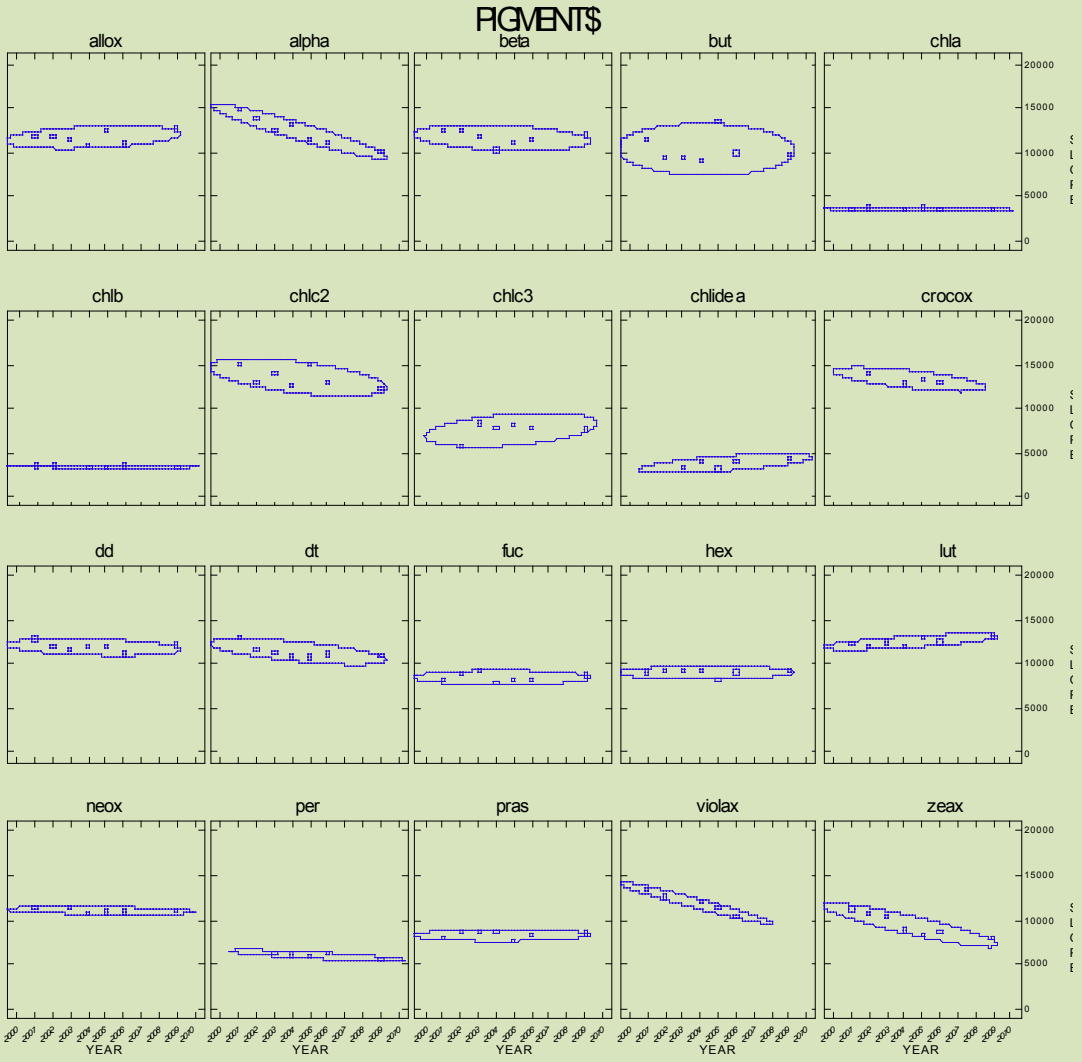
Analysis Details

- Injection Procedures
  - 500µl sample extract, 400µl H_2O
  - amber 2.0ml vial with pre-slit septa
  - not >6 h prior to sample injection
  - 400µ (100-600µl if needed) injected onto 900µl loop
  - needle wash in 90% acetone before and after injection
- Quantification Procedures (DAD/VWD)
  - In Chemstation (Rev. A.10.02) visually inspect each peak's integration
  - Save modifications to batch file
    - Integration problem areas: dt/zeax/lut/lycop/unk caro
  - Output Peak Name, RT, Area using EXCELDDE macro (Don Grothen)
  - Manually* match peaks in consecutive runs using RT
  - Apply calibration information and quantify in Excel

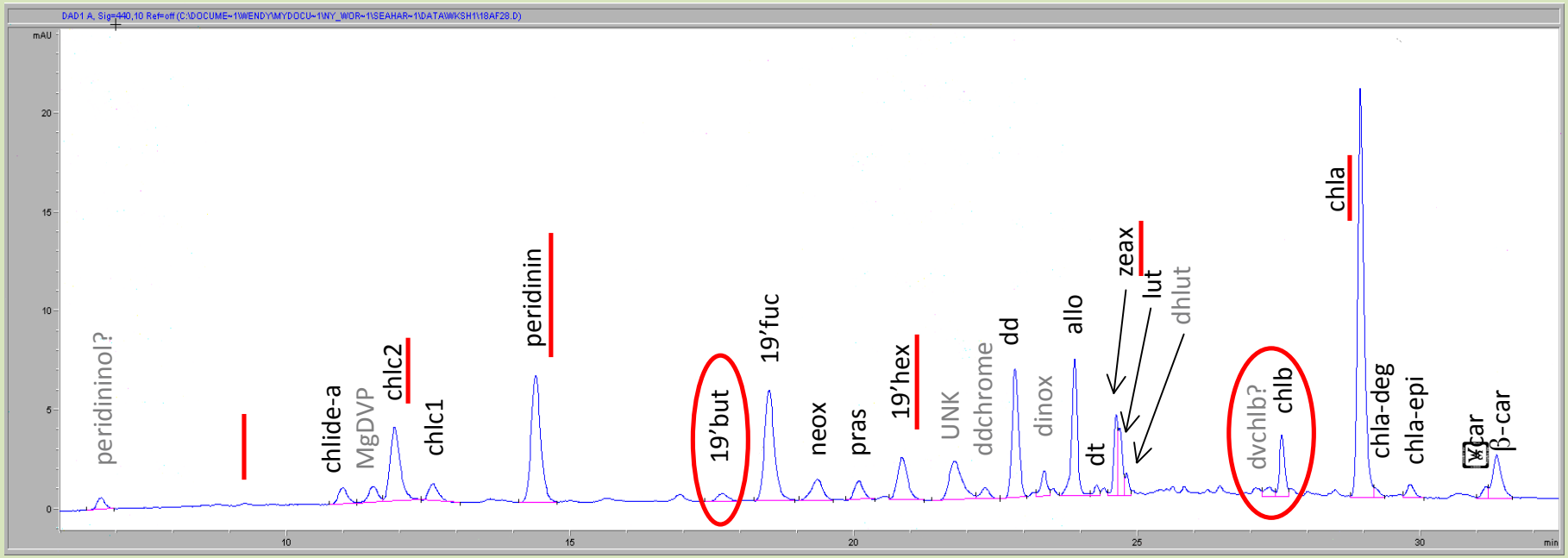
Calibration

- Standards from DHI:
  - chlc3, chlide-a, chlc2, per / but, fuc, neox, pras, hex / dd, allo, dt / zeax, lut, [illegible]car, β-car
  - Use DHI concentrations
- Standards from Sigma Chemical:
  - chla ([illegible]87.67 @ 664nm) chlb ([illegible]51.36 @ 646nm)
  - Powder → 90% acetone
  - Check concentrations spectrophotometrically
- 5pt injection series
  - (0.4 – 160ng, pigment dependent)

Calibration Response

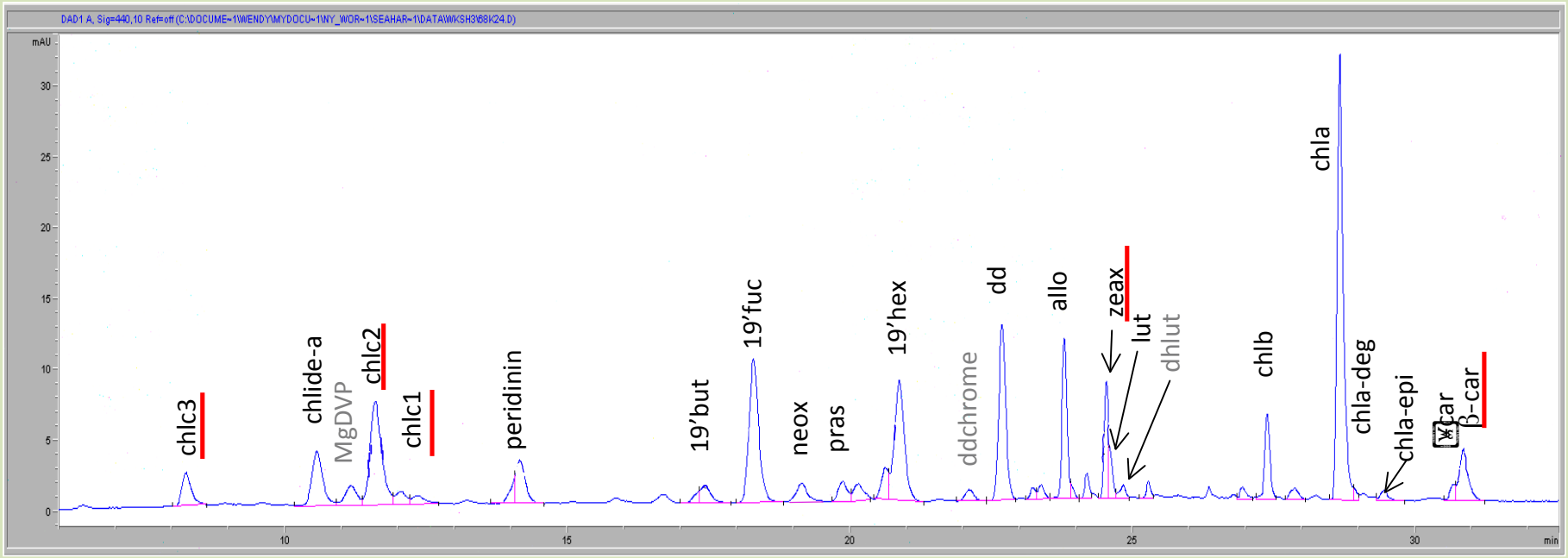


SH5 Sample "Challenge" Chromatogram



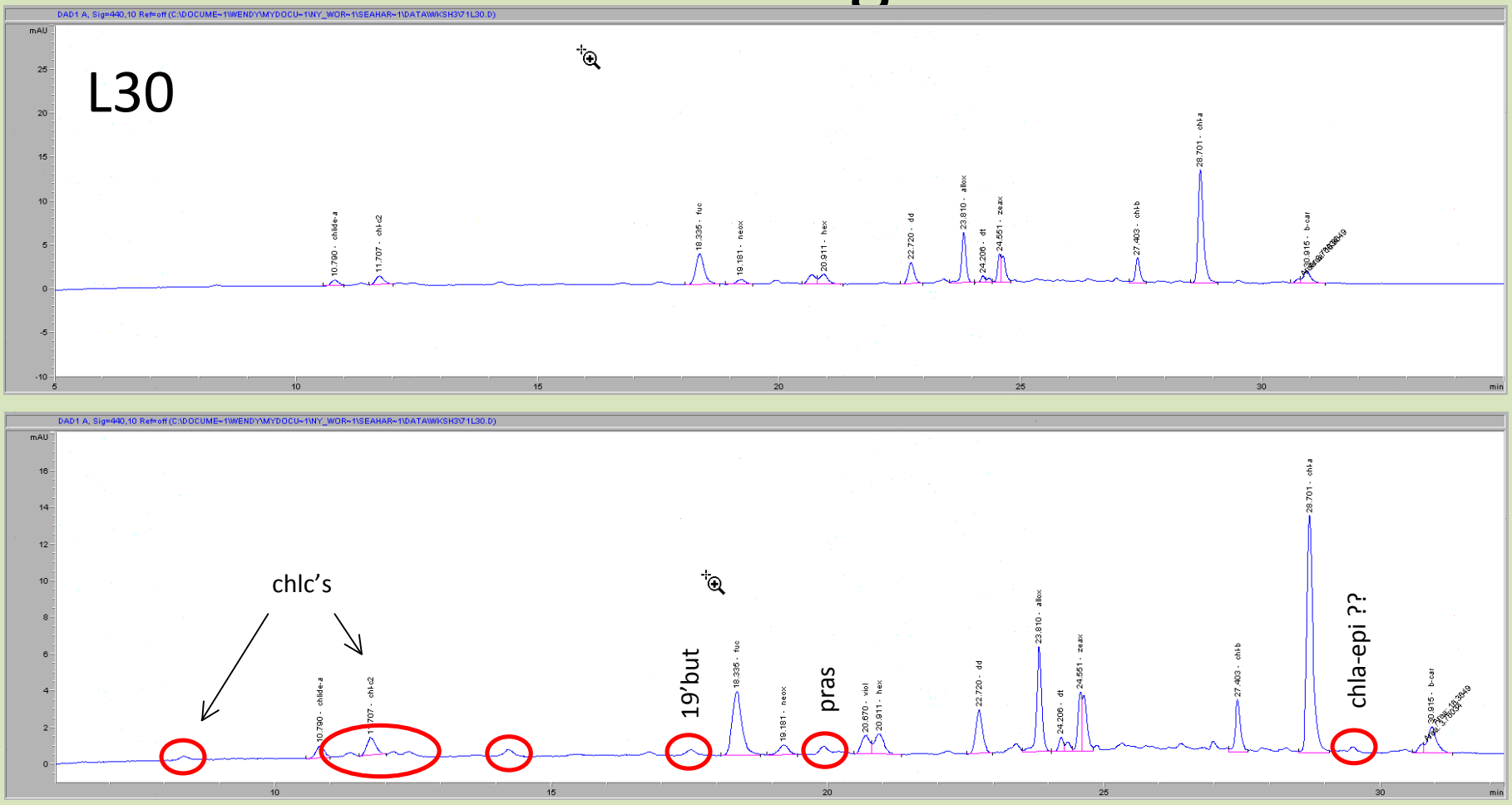
- AF28
- APD's >20: chlc3 (55), chlc2 (53), per (32), 19'but (NR) , hex (69), zeax (41), Tchlb (34), Tchla (29)

SH5 Sample “Good” Chromatogram

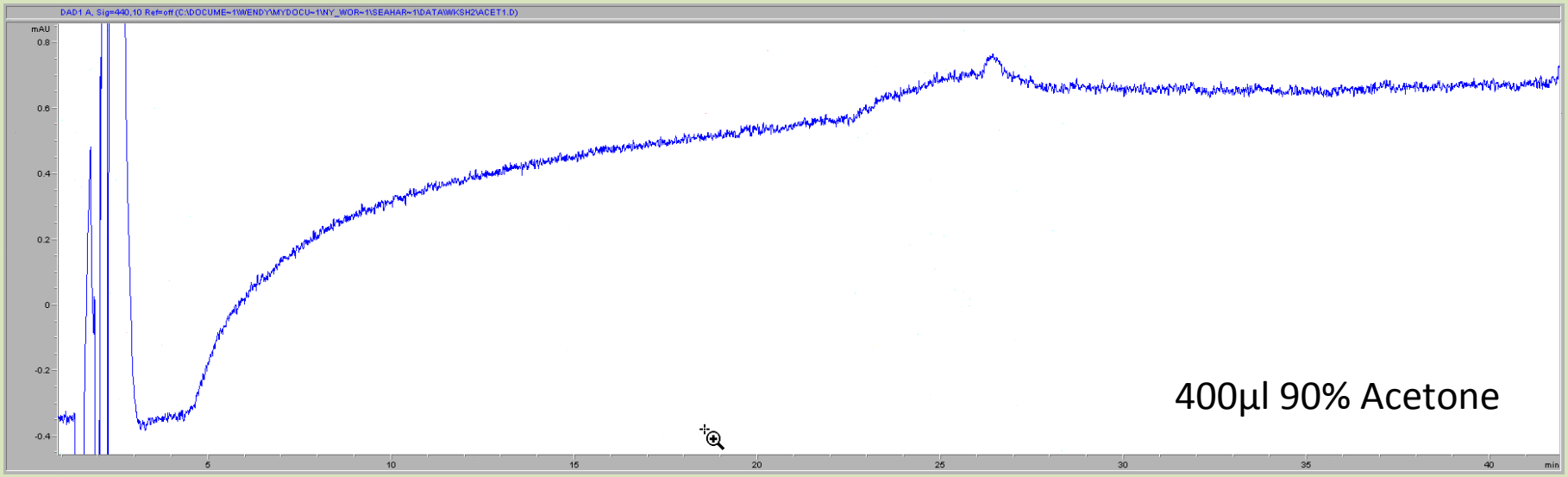


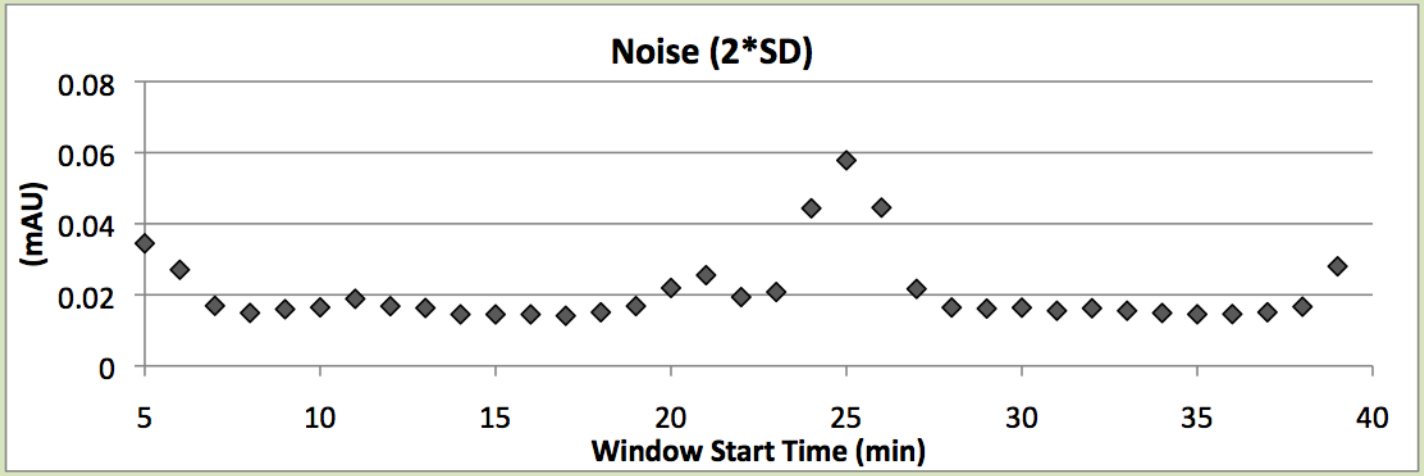
- K25
- APD’s >20: Tchlc (22), zeax (49), β-car (24)

SH5 Low Concentration Chromatogram



QC Consideration: Signal Noise





Method Summary

- Strengths
  - Average accuracy 22.4% across primary pigments
  - APD's >25% include Chla, Chlb, DD, Allo & Fuc – important, prevalent peaks for water types we commonly sample. But and Hex APD's ~ 30%.
- Weaknesses
  - Chlc's and mid-chromatogram area – lut/zeax

Improvements

- Methods:
  - Add internal standard
  - Incorporate injection sequence to add water immediately prior to injection
- Integration / Quantitation / QC
  - Incorporate SNR calculations into protocol for LOD / LOQ
  - Small peak area integration and calibration
  - Peak infor export improvement

THANK YOU

QC Consideration: Signal Noise

- HPL DAD
- USAP DAD
- USAP VWD