Prasino…
Prasino!
Prasino?

The Search for Prasinoxanthin's True Identity

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Why Prasino?

Table 24	[Pras]
C	1
D	51.3 2
E	46.1 2
G	77.8 9
H	95.6 4
I	83.3 9
K	47.9 0
L	41.9 8
N	
O	84.5 9
T8	57.8 9
T18	41.7 6
U	
A' Average	66.7 1
A+ Average	60.2 7

- **Results for Prasino Field APDs from Table 24**
- From the combined SeaHarre 5 results, it was apparent that HPL differed from most of the community by reporting prasinoxanthin was not present in any of the SH5 samples.
- HPL found a clear peak at prasino's retention time but rejected it due to the mismatch with library spectra.
- Might have HPL's extraction procedure corrupted these peaks?

Prasino Experiment

	Acetone (100%)	Water	Prasino	Filter
Control	2.5ml	250ul	250ul	No
Tr A	2.5ml	250ul	250ul	Blank
Tr B	2.5ml	100ul	250ul	Sample-small peak
Tr C	2.5ml	100ul	250ul	Sample-large peak

- Natural sample filters were duplicates from a prior sample set
- Peaks at prasino's retention time did not match the spectra and determined to be false positive
- 3 replicates per treatment
- Final concentration in all tubes was ~ 90% acetone

Calculating Prasino Spike to Mimick Size Found in Natural Sample

- Initial Dilution of Stock

250ul prasino stock * spec concentration / (5ml EtOH + .25ml{stock} *1000)
= *0.162* ng/ul of prasino

- In Extraction Tube

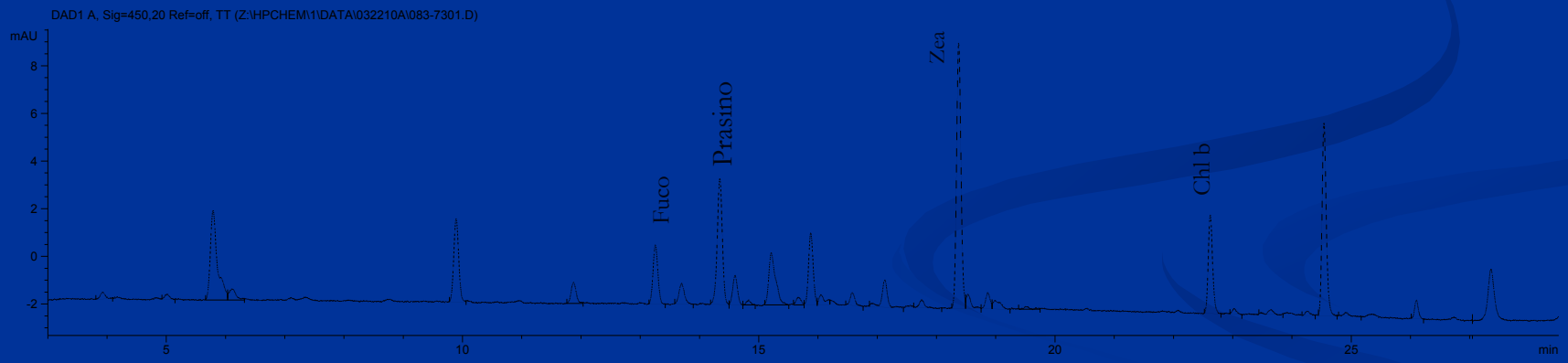
2.5ml acetone + 0.25ml (total volume) water + *0.25*ml EtOH with prasino
= 3ml TV

0.162ng/ul x (0.25 ml * 1000) = 40.5ng in extraction tube
40.5 / 3ml TV = 13.5ng/ml = 0.135ng/ul
0.135ng/ul * 150ul/injection = 2.025 ng/inj

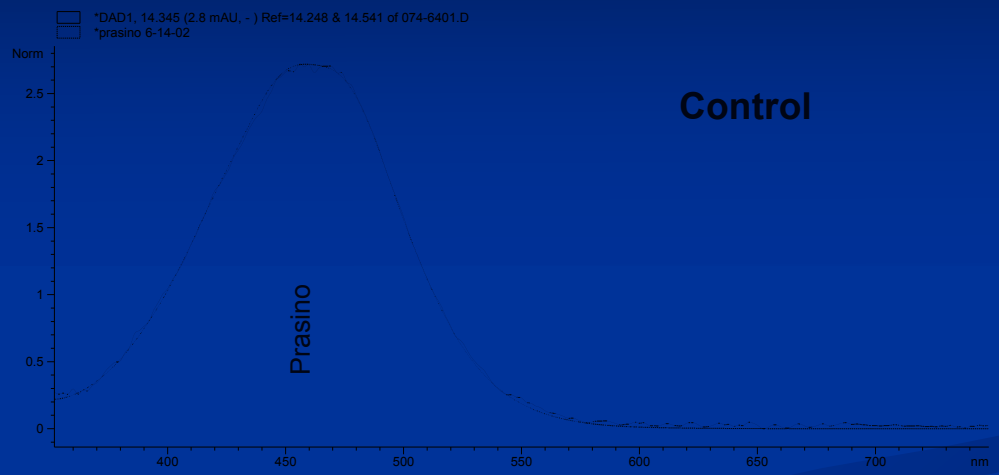
Procedure

- Added acetone and water to tubes. Chilled 30 minutes
- Added filters to appropriate tubes, then prasino. Chilled one hour.
- Sonicated only tubes with filters. Chilled three hours.
- Filtered slurry from tubes through 45um syringe filter. Filled HPLC vials with 500ul of extract and placed vials in autosampler.

Typical Chromatogram at 450 nm

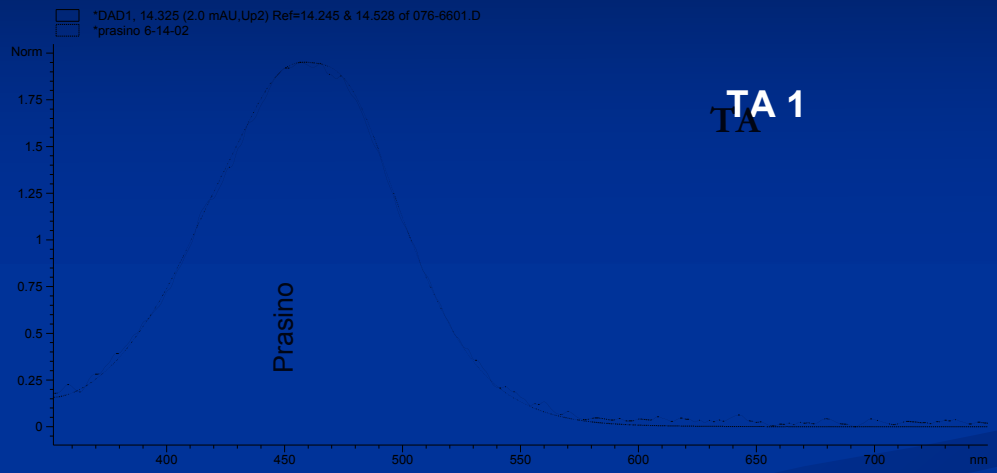


Results from Control



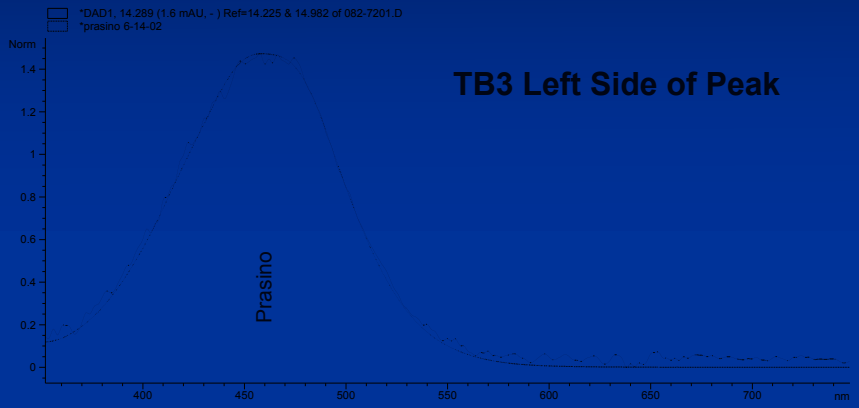
- Average prasino amount = 2.059ng/inj
- Average recovery = 101%
- All Control injections show clear prasino spectral match
- Prasino presence without distortion to spectra.
- No other contaminates found around perimeter or inside of peak.

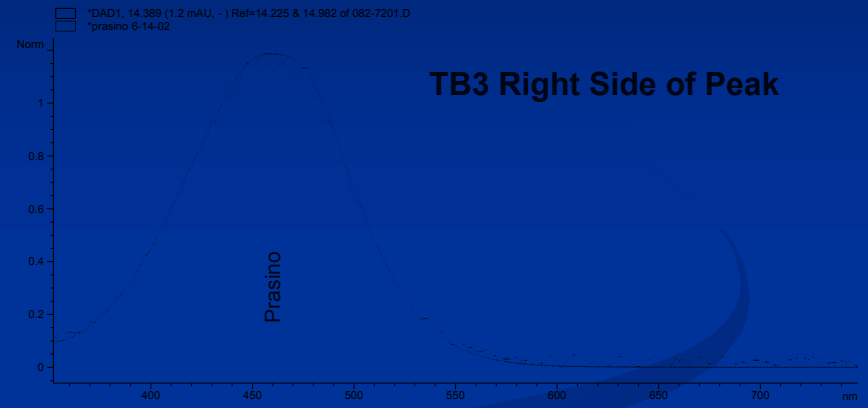
Results from Treatment A with Blank Filter



- Average prasino amount = 2.092ng/inj
- Average recovery = 103%
- All Treatment A injections show clear prasino spectral match
- Prasino presence without distortion to spectra.
- No other contaminates found around perimeter or inside of peak

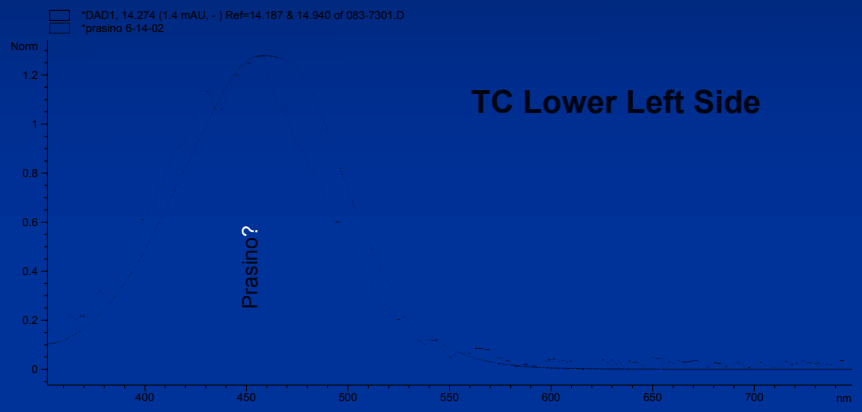
Results from Treatment B with Small False Positive

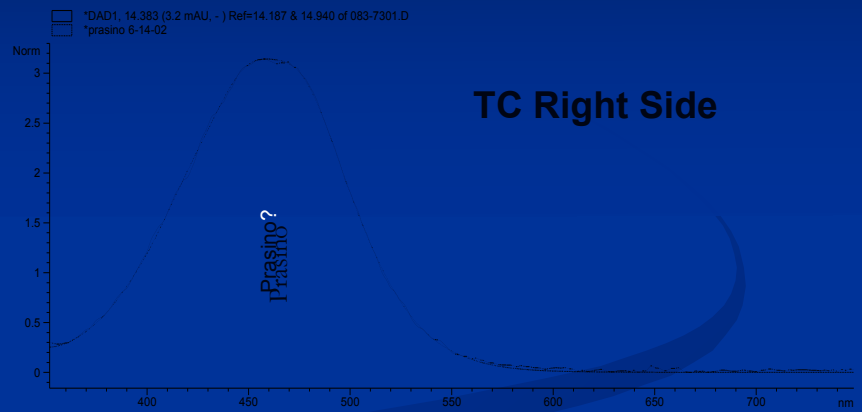




- Average prasino amount = 2.208ng/inj
- All Treatment B injections show prasino spectral match
- Prasino presence with little distortion to spectra.
- Slight to no other contaminates found around perimeter or inside of peak

Results from Treatment C with Large False Positive





- Average prasino amount = 3.467ng/inj
- All Treatment C injections show contamination in reference to prasino spectra in Library
- Prasino presence with much distortion to spectra on lower left side.
- Prasino present but contamination hinders the ability to accurately intergrate

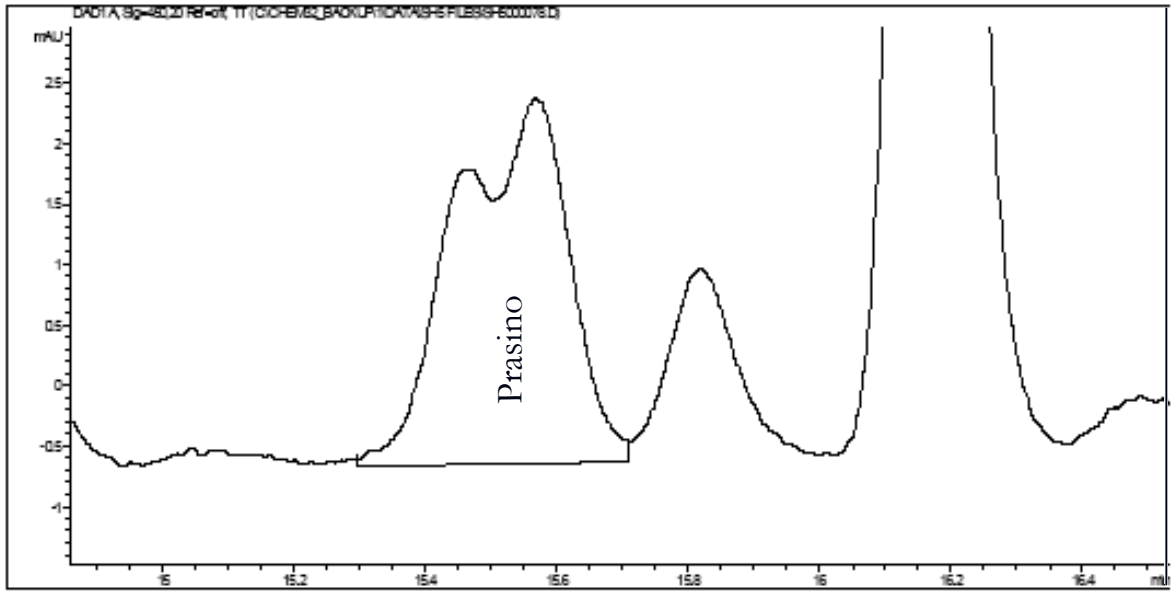
Conclusion

- Extracts in the control group showed clear, strong prasino spectral matches. The extraction process did not alter prasino's spectra in any way.
- Treatment A with blank filters, again, showed clear prasino matches with spectral library. The presence of a GF/F filter did not alter prasino's spectral match.
- Treatment B with known peaks at prasino's RT indicates with small false positive prasino peaks, the spike swamped any mal effect contaminates would have on quantifying and reporting prasino's amount.

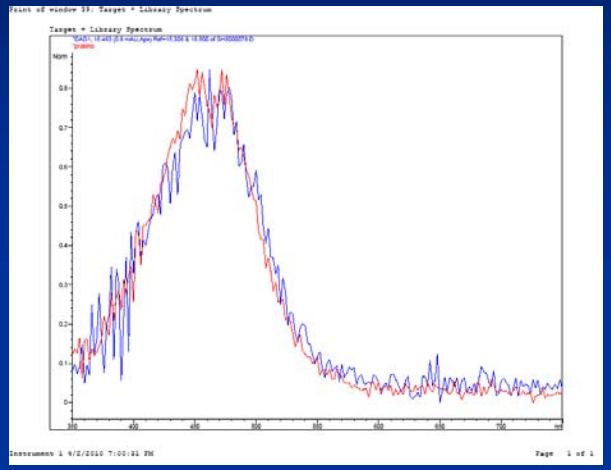
Conclusion from Treatment C

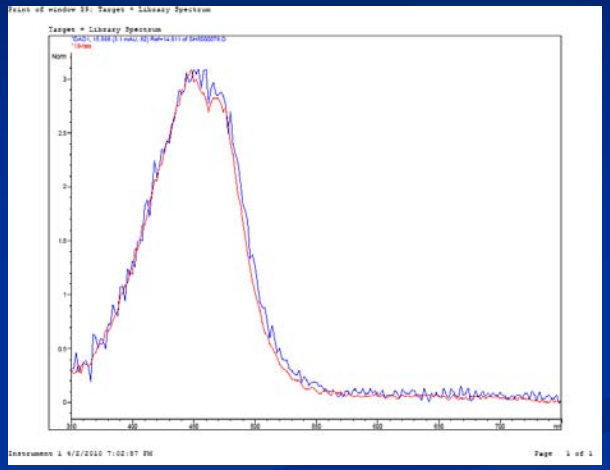
- Treatment C chromatograms clearly showed well shaped prasino peaks, without any indication of contamination.
- Observation of spectral match at sample apex confirmed prasino's presence. However, further spectral checks indicated non-prasino presence.
- With contamination/co-elution occurring within visually well defined peaks, it is recommended several points of spectral match be confirmed prior to final acceptance or rejection of pigment identification.

Neeley's Split Prasino Peak



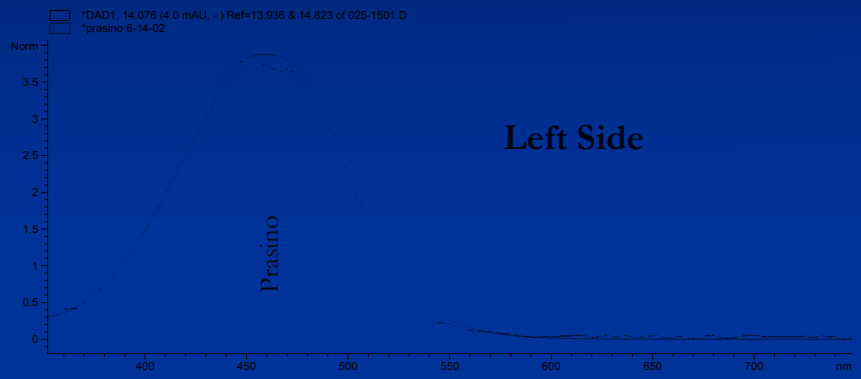
Left and Right Side of Split Peak

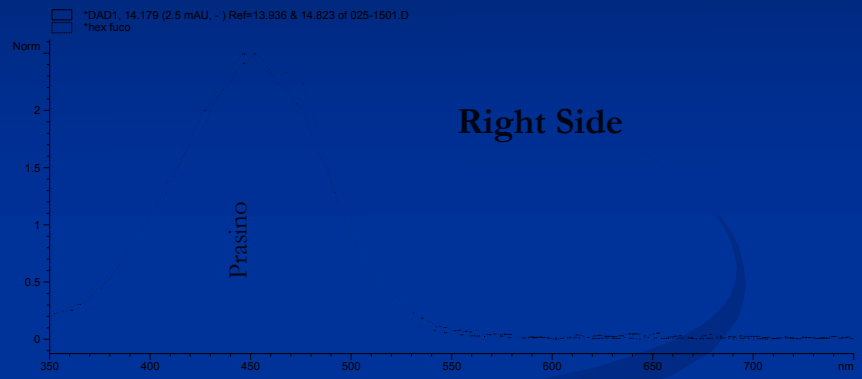




- Left side of peak shown with prasino library spectra and right side with 19-hex

HPL's Left and Right Side of Site D

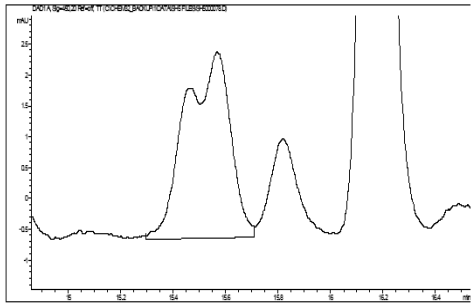


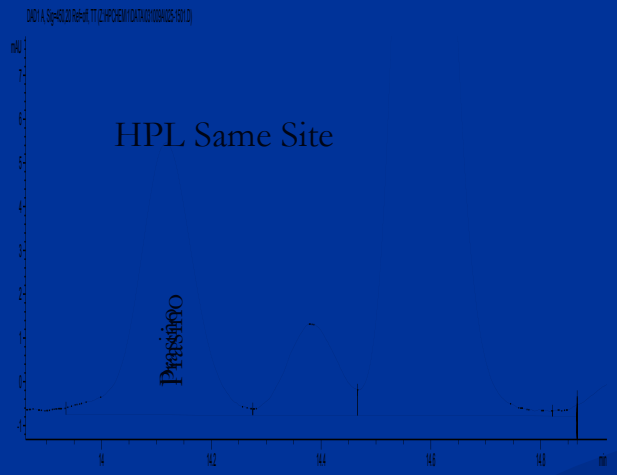


- As with split peak, left side of peak shown with prasino library spectra and right side with 19-hex
- Apex matched closest with 19-hex
- HPL with co-eluted peak

Mechanical Differences Between Labs?

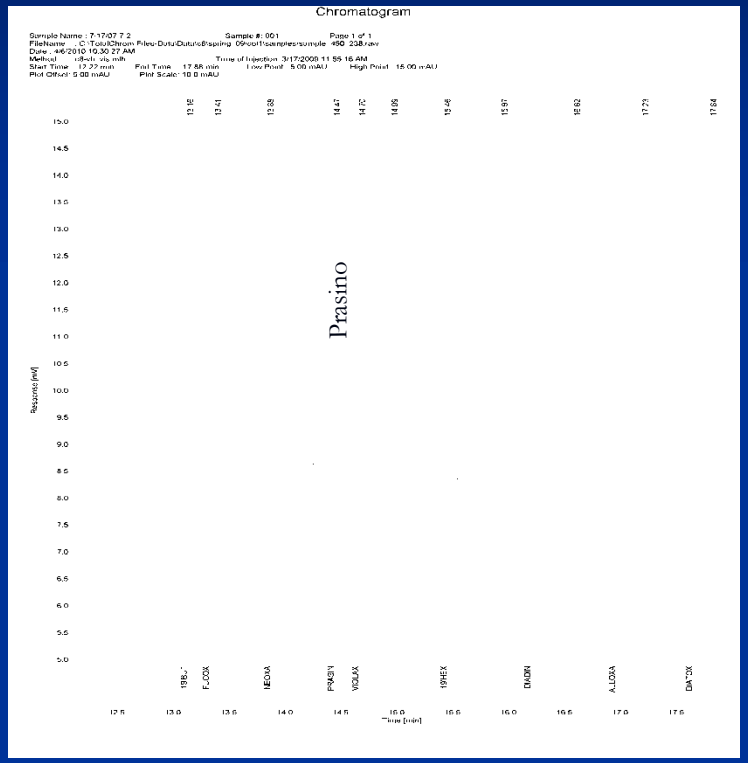
Neeley's Split Prasino

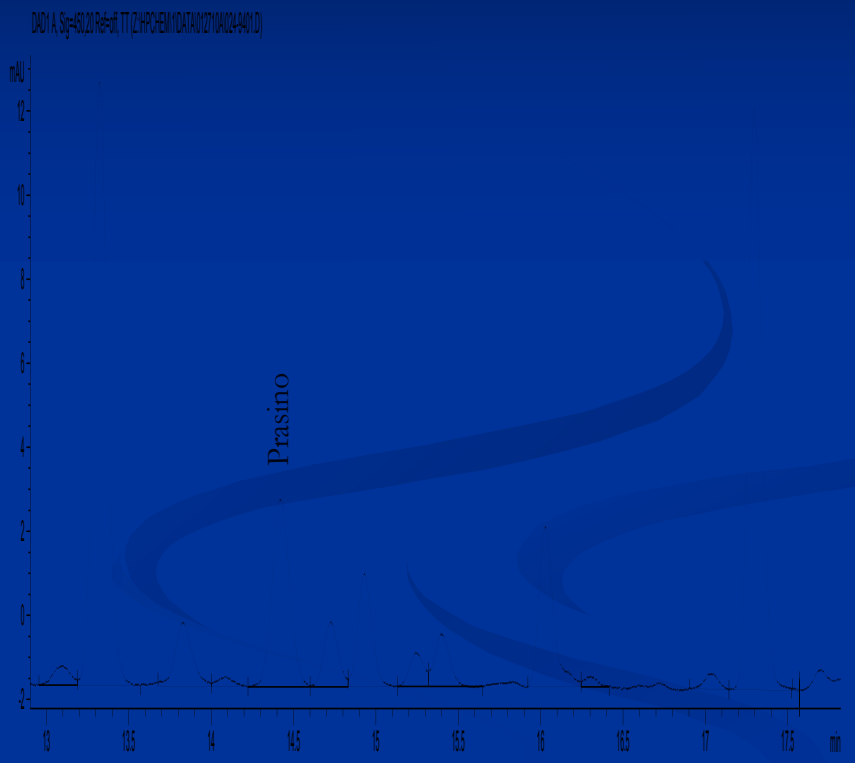




- Is Neeley's split peak appearing as a co-elution for HPL's?
- Are we seeing differences between HPLC method implementation?

Another Look at Prasino





- Client's chromatogram

HPL's chromatogram (duplicate)

Final Thoughts

- For HPL, several spectral matches around perimeter and inside of peak are necessary before rejecting or accepting prasino as present in a sample.
- By far, the majority of prasino peaks have been rejected by HPL.
- Are we (HPL) seeing a co-elution others are seeing as split or separate peaks altogether?
- Is this issue with prasino a factor of method implementation differences? Software or equipment limitations?
- Is HPL the only lab having this issue of possible co-elution?
- Where do we go from here?

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