

The DHI Method

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The DHI Method-



-is basically the HPL method (Van Heukelem & Thomas, 2001)

Extraction solvent: 3 ml 95% acetone with vitamin E as internal standard

Disruption: 10 min in ice cold sonication bath

Soak time: 24 h

Clarification: 0.2 μ m Teflon syringe filter

T (min)	A (%)	B (%)	
0	95	5	
27	5	95	
34	5	95	
35	0	100	
38	0	100	
39.5	95	5	
46	95	5	stop



Detection: Chlide a, Phba, Pht a, and MV & DV Chl a at 665 nm,
The rest of the pigments at 450 nm

Injection procedure: 500 μ l in total; 143 μ l sample (mixed with buffer in the loop)

Calibration procedures: Multipoint calibration with LOQ analysis of all pigments

Pigments quantified:



Alloxanthin
 α -carotene
Aphanizophyll
Antheraxanthin
 β -carotene
 β -Cryptoxanthin
19'-but-fucoxanthin
Chlorophyll *a*
Chlorophyllide *a*
Chlorophyll *b*
Chlorophyll c2
Chlorophyll c3
Canthaxanthin
Crocoxanthin
Diadinoxanthin
Diatoxanthin

Divinyl chlorophyll *a*
Echinenone
Fucoxanthin
Gyroxanthin-diester
19'-hexfucoxanthin
Lutein
Lycopene
Myxoxanthophyll
Neoxanthin
Peridinin
Pheophorbide *a*
Pheophytin *a*
Prasinoxanthin
Violaxanthin
Zeaxanthin





DANAK: The Danish Accreditation and Metrology Fund

“Accreditation is an independent evaluation of a company’s competence and ability to perform certain tasks in compliance with certain specifications. The requirements to accredited companies are laid down in international standards”

DHI Quality System (DHI Quality Manual)

SOP's (Standard Operating Procedures):

- Pipette and balance calibration
- QA of Excel work sheets
- Training of new persons
- How to register samples
- Maintaining documents
- Etc.

Internal Audits: control that we are doing what we have decided and described to do

External Audits: DANAK inspections

Validation:

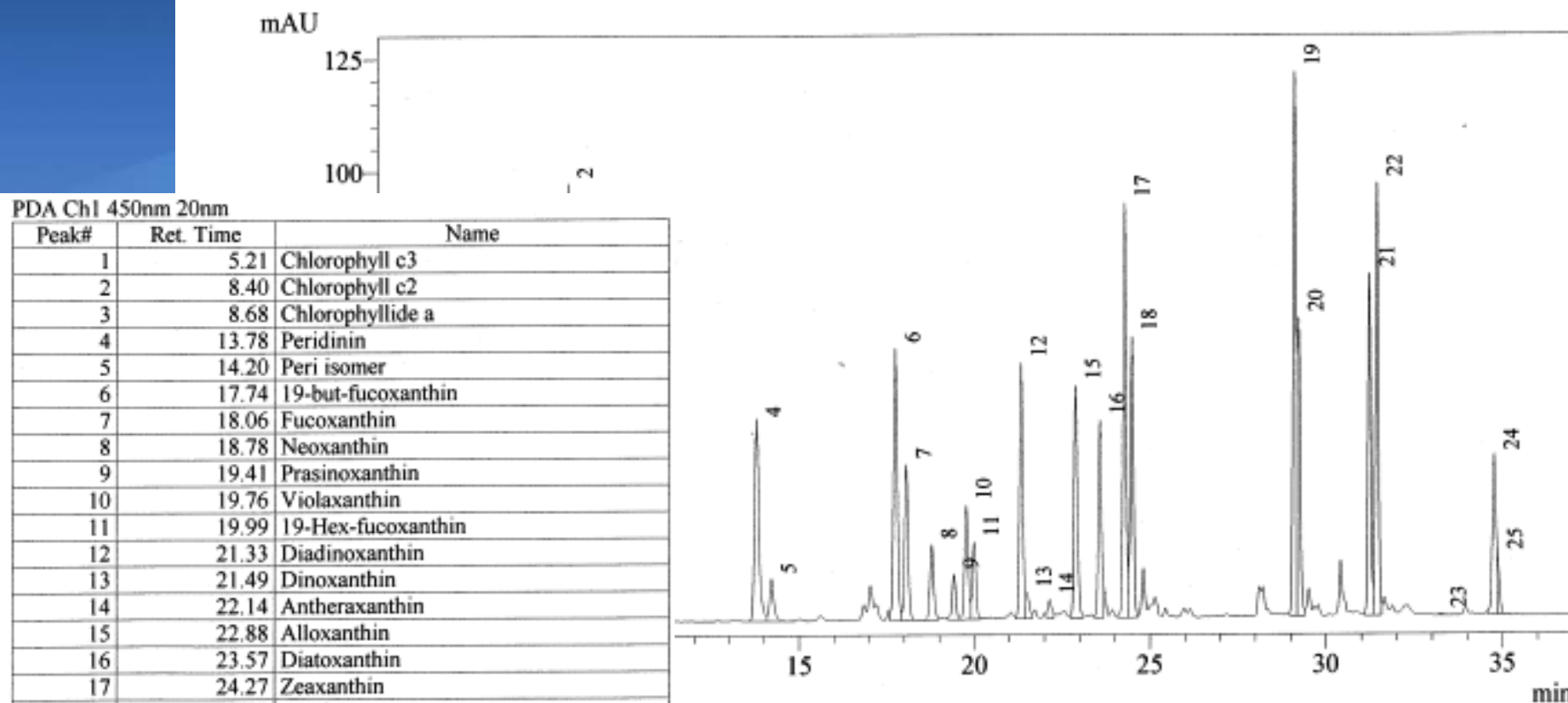
Identity based on mixed pigments:

Elution

Separation

PDA

DHI Mixed pigment; mix-106



Validation:

Control of the precision

(4 injections mixed pigments & Vit e)

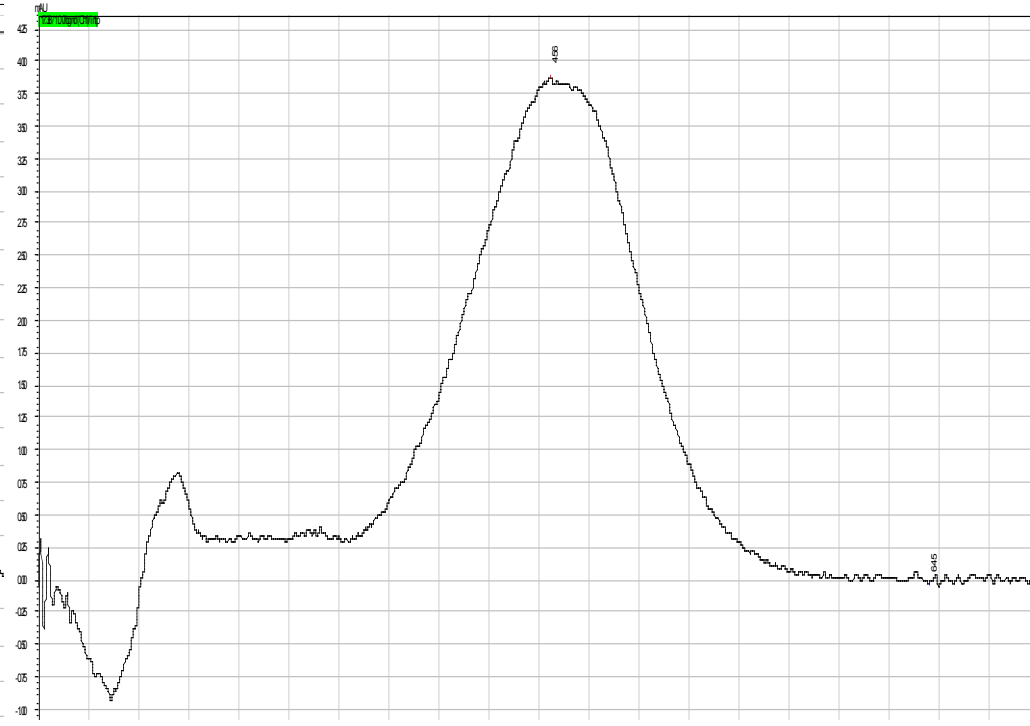
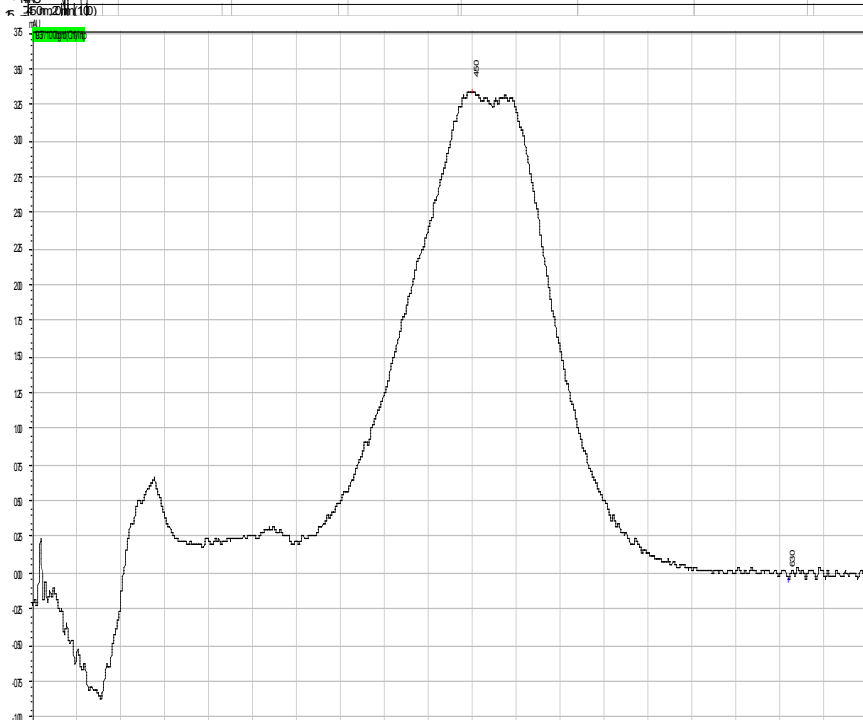
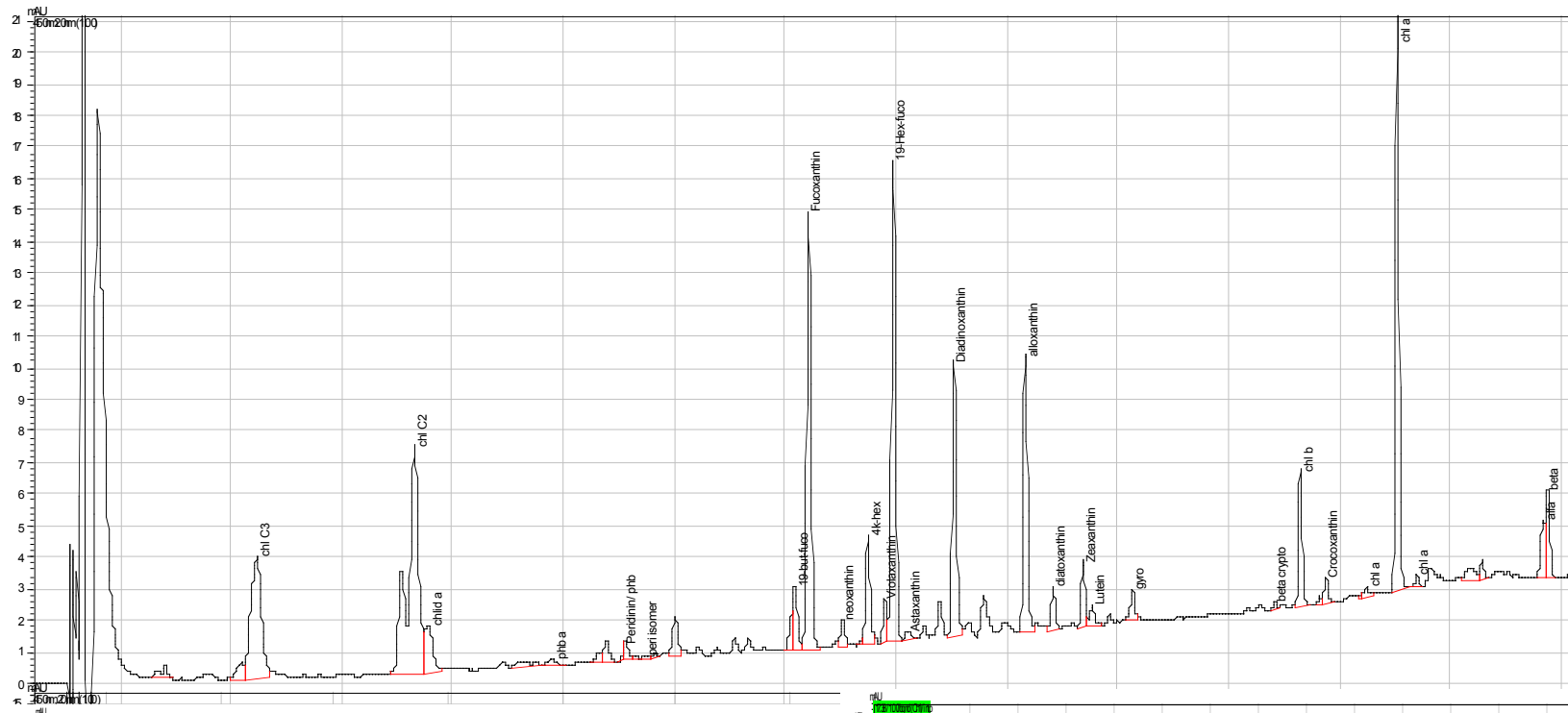
SH2: CV approx. 5 %

Currently CV ~ 1%

Control of the calibration (Chl a)

<5%

Small peaks: Two sentence rule followed; reported if retention time is OK and scan appears correct.



Sample AM

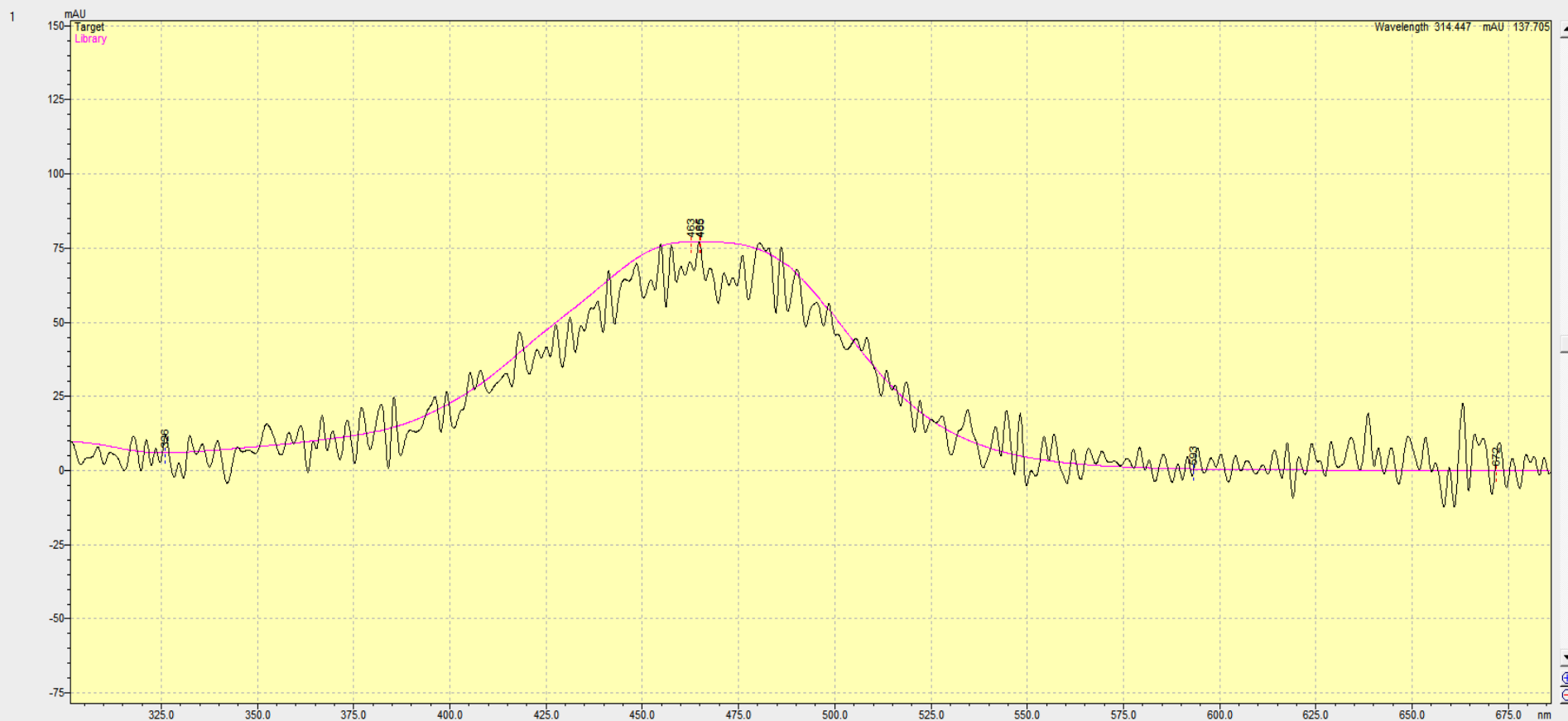


UV Library Search Result

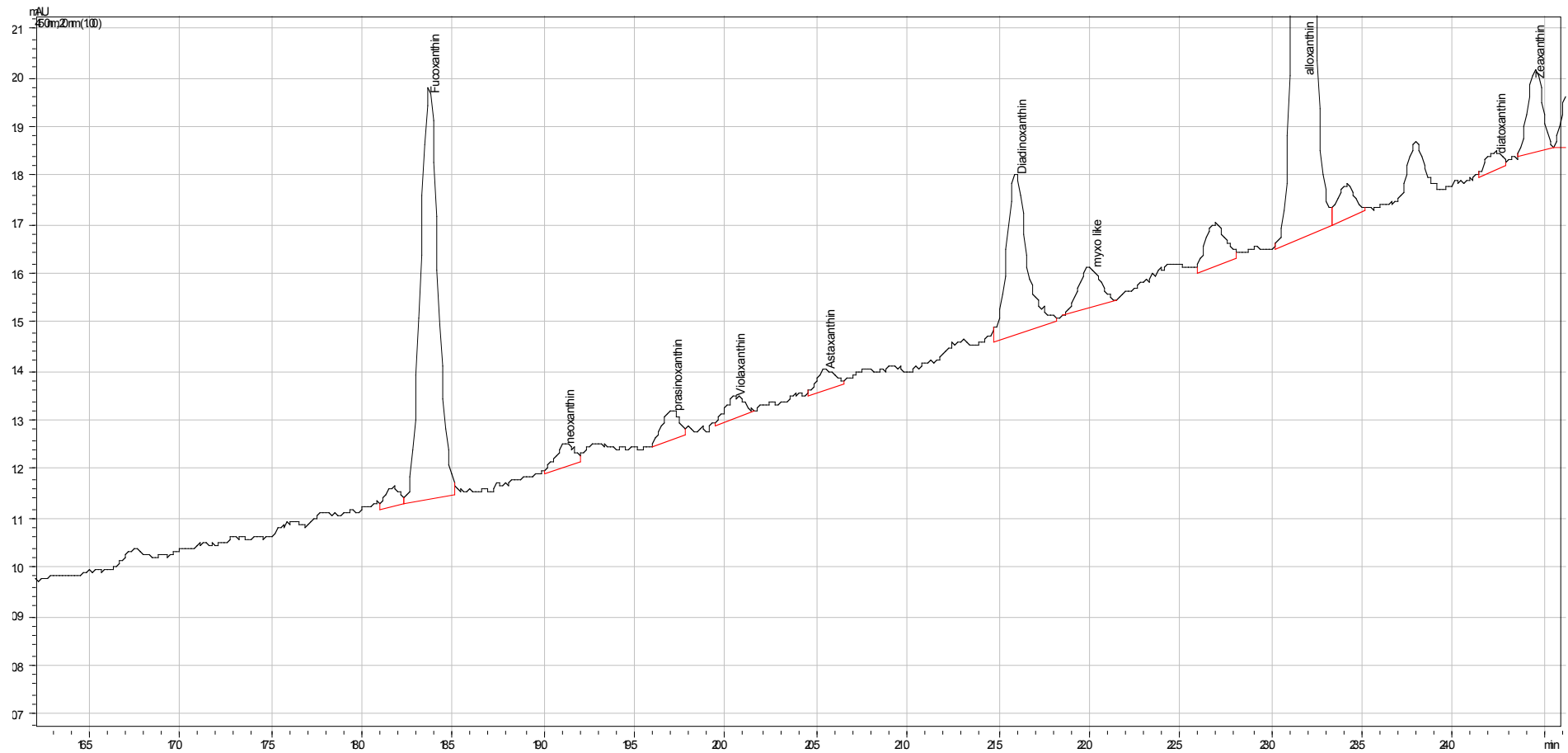
Library Search View

Hit Cnt : 16
Unknown Spectrum Info. Retention Time : 30.08
Lambda max : 464.83 band(Ch1)/intp

#	Compound Name	Ret. Time	Lambda Range	Lambda max	Lambda min	Operation	Similarity	Filename
1	echi	32.895	300-700	465.20/462.66/671.9	325.89/593.10	bgnd/intp	0.989871	bibliotek.lib
2	pras	23.772	300-700	459.90/338.65	323.52/354.09/600.5	bgnd/intp	0.985466	bibliotek.lib
3	peri	16.559	300-700	477.39/328.85/660.9	318.12/357.44/596.1	bgnd/intp	0.979539	bibliotek.lib
4	Fuco	20.840	300-700	452.10/645.16	356.01/548.37	bgnd/intp	0.977293	bibliotek.lib
5	cant	29.319	300-700	477.63/655.16	351.06/348.26/596.5	bgnd/intp	0.972036	bibliotek.lib
6	asta	19.313	300-700	478.80	347.52/621.94	bgnd/intp	0.967681	bibliotek.lib
7	4k-hex-fuco	19.813	300-700			bgnd/intp	0.964763	bibliotek.lib
8	but	24.493	300-700	448.34/469.31/336.1	463.64/351.77/557.2	bgnd/intp	0.957158	bibliotek.lib
9	hex	22.564	300-700	448.51/469.92/335.4	463.04/350.15/560.8	bgnd/intp	0.955706	bibliotek.lib
10	beta	36.982	300-700	451.70/477.57	468.28/354.34/528.7	bgnd/intp	0.954746	bibliotek.lib
11	aphani	17.931	300-700	474.42/505.74/449.2	491.79/454.33/375.2	bgnd/intp	0.954737	bibliotek.lib
12	Zea	26.293	300-700	451.39/478.46	467.92/525.11	bgnd/intp	0.954361	bibliotek.lib
13	dbx	25.702	300-700	452.04/480.05/658.5	469.98/356.97/522.5	bgnd/intp	0.953861	bibliotek.lib
14	myxo	27.552	300-700	475.65/506.48/451.1	492.98/455.79/379.1	bgnd/intp	0.950654	bibliotek.lib
15	allo	26.433	300-700	452.49/481.50/352.5	469.94/355.34/316.0	bgnd/intp	0.949073	bibliotek.lib



Sample A



Sample A; scan of 19'-but-like pigment



UV Library Search Result

Library Search View

Hit Cnt : 16
Unknown Spectrum Info. Retention Time : 18.17
Lambda max : bond(Ch1)/into

#	Compound Name	Ret. Time	Lambda Range	Lambda max	Lambda min	Operation	Similarity	Filename
1	hex	22.564	300-700	448.51/469.92/335.4	463.04/350.15/560.8	bgnd/ntp	0.685257	bibliotek.lib
2	but	24.493	300-700	448.34/469.31/336.1	463.64/351.77/557.2	bgnd/ntp	0.685087	bibliotek.lib
3	4k-hex-fuco	19.813	300-700			bgnd/ntp	0.683210	bibliotek.lib
4	Fuco	20.840	300-700	452.10/645.16	356.01/548.37	bgnd/ntp	0.680418	bibliotek.lib
5	ant	27.524	300-700	446.68/474.74	462.57/351.61/521.3	bgnd/ntp	0.680299	bibliotek.lib
6	Lut	26.489	300-700	446.05/474.04/425.0	461.89/426.37/353.1	bgnd/ntp	0.680192	bibliotek.lib
7	beta crypto	30.524	300-700	450.67/477.16	467.29/313.16/592.0	bgnd/ntp	0.679804	bibliotek.lib
8	beta	36.982	300-700	451.70/477.57	468.28/354.34/528.7	bgnd/ntp	0.679009	bibliotek.lib
9	alfa	34.550	300-700	444.36/472.01/655.0	460.50/347.17/531.0	bgnd/ntp	0.678354	bibliotek.lib
10	Zea	26.293	300-700	451.39/478.46	467.92/525.11	bgnd/ntp	0.678100	bibliotek.lib
11	ddx	23.732	300-700	447.36/476.59/424.1	463.36/424.95/353.1	bgnd/ntp	0.677420	bibliotek.lib
12	crox	29.324	300-700	445.64/474.91/338.3	462.26/350.60/593.5	bgnd/ntp	0.677150	bibliotek.lib
13	dbx	25.702	300-700	452.04/480.05/658.5	469.98/356.97/522.5	bgnd/ntp	0.676757	bibliotek.lib
14	allo	26.433	300-700	452.49/481.50/352.5	469.94/355.34/316.0	bgnd/ntp	0.674581	bibliotek.lib
15	gyro	25.746	300-700	468.46		bgnd/ntp	0.674189	bibliotek.lib

