SeaHARRE-5: lab E

«The Bodø method»

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Storage & extraction

- **Samples stored for 10 months at -30°C in original packing**
- **Each freeze-dried filter torn to pieces and soaked in 1 ml cold (-30°C) 30 % methanol in acetone, back in freezer**
- **Extraction over night, filtered, analysed by HPLC at the same day (or weekend)**

HPLC instrument

- Agilent 1100 HPLC with quaternary pump, cooled autosampler with enlarged injection loop, thermostatted column, diode array detector

HPLC method

- **Injection: 50 μl of 1 ml extract, without mixing**
- **Two columns: 2 x ACE C18 5 μm, 250 x 4.6 mm with separate guard column**
- **Detection at 390, 420, 450 and 480 nm**
- **Calibration by injecting various volumes of the conc. pure standards**

HPLC method – cont.

- **Solvents: 1 M ammonium acetate, methanol, acetone, hexane**
- **Gradient over 130 minutes from 20 % ammonium acetate in methanol to 60 % hexane in acetone (to rinse the column after each sample)**

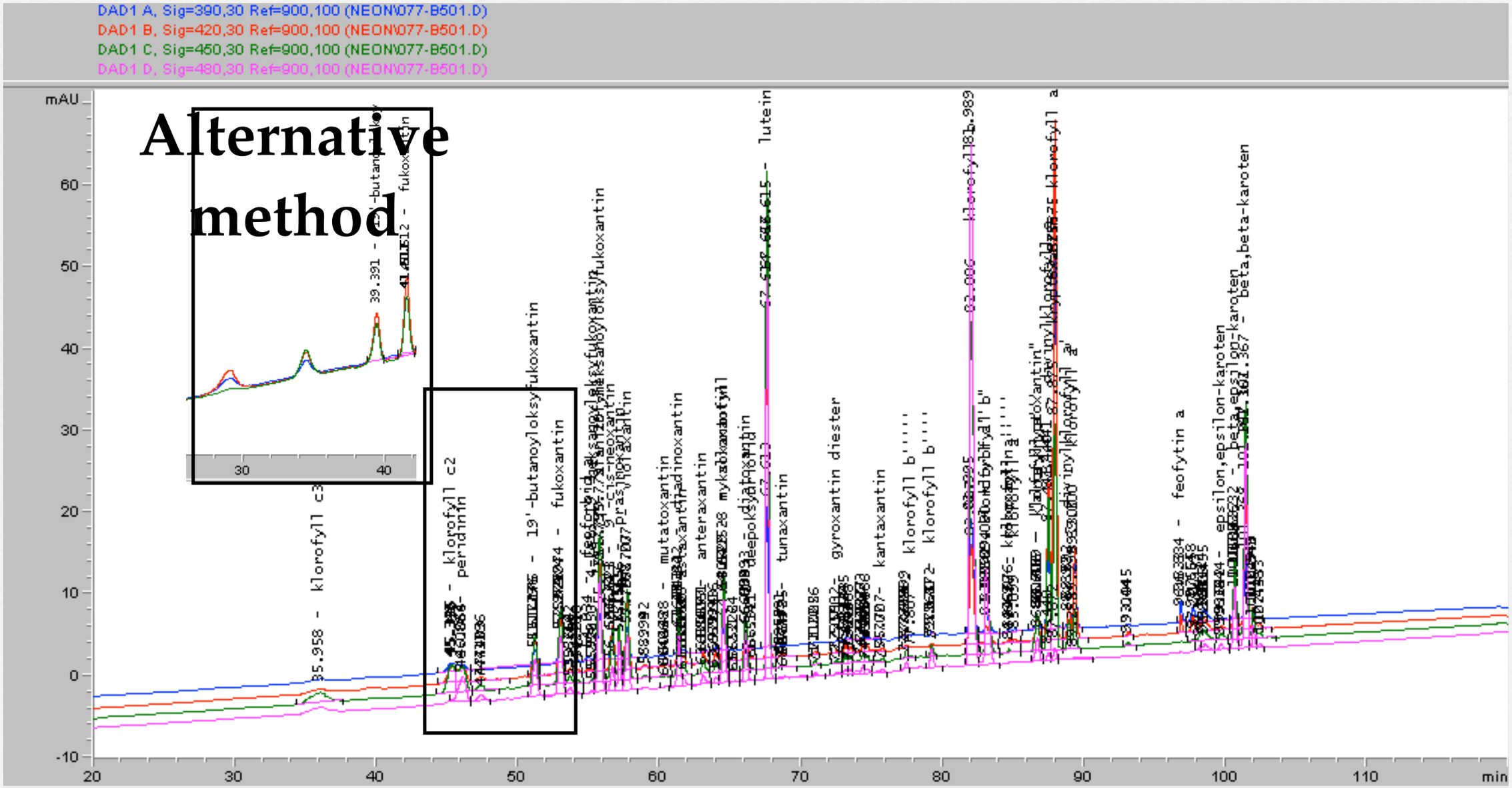
Alternative method

- **Single column: Brownlee (Perkin Elmer) C18 5 μm, 220 x 4,6 mm with guard column**
- **Separation not as good as double ACE column**
- **Analysed, but not summarised due to lack of technician & lack of time**

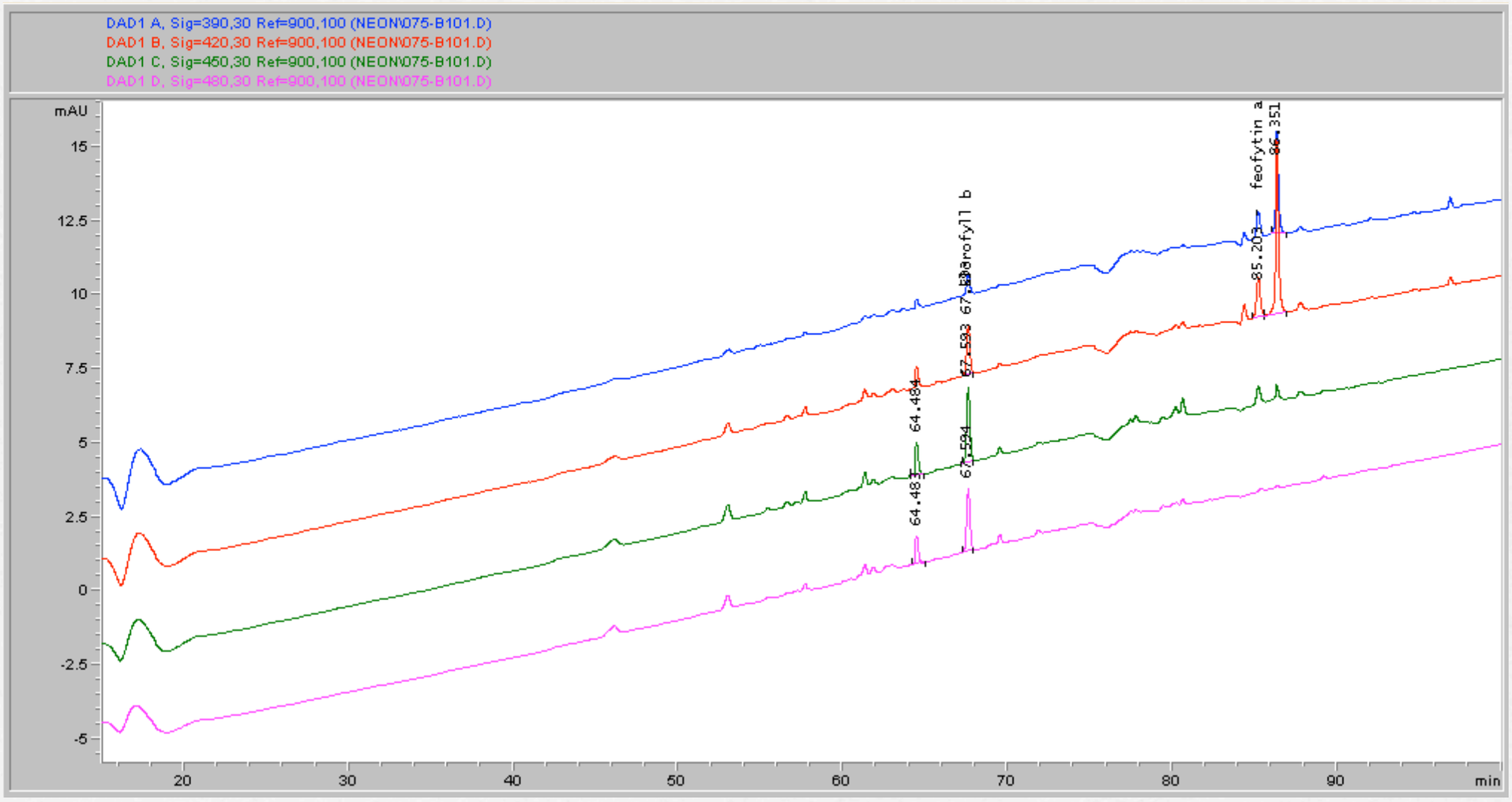
Pigment separation

- Methods not checked for DV/MV-chl cs
- Not separating chl c2/MgDVP; DV/MV-chl bs; lutein/zeaxanthin
- Only alternative method separate chl c2/ peridinin satisfactory
- Calibration at the best wavelenght for each pigment (390/420/450/480 nm)

DHI-mix # 105

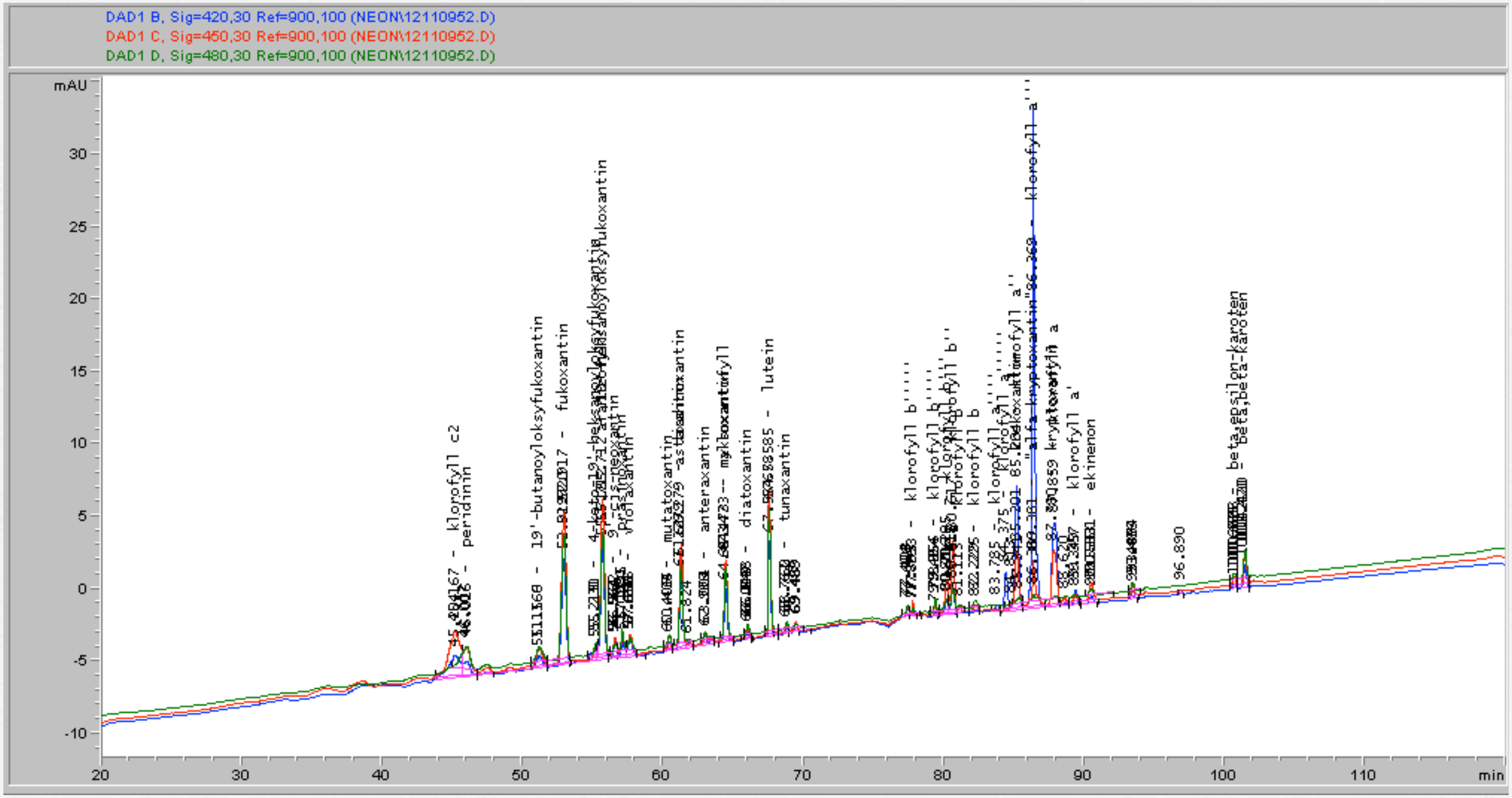

DAD1 A, Sig=390,30 Ref=900,100 (NEON\077-B501.D)
DAD1 B, Sig=420,30 Ref=900,100 (NEON\077-B501.D)
DAD1 C, Sig=450,30 Ref=900,100 (NEON\077-B501.D)
DAD1 D, Sig=480,30 Ref=900,100 (NEON\077-B501.D)
mAU
min
Alternative method
85.958 - klorofyll c3
19'-butanoyloksyfukoxantin
fukoxantin
klorofyll c2
peridinin
mutatoxantin
anteraxantin
tunaxantin
gyroxantin diester
kantaxantin
klorofyll b'''''
klorofyll b''''
klorofyll a
feofytin a
epsilon-karoten
beta,epsilon-karoten
beta,beta-karoten

Natural sample: AG09



- (Sorry for wrong identities here)

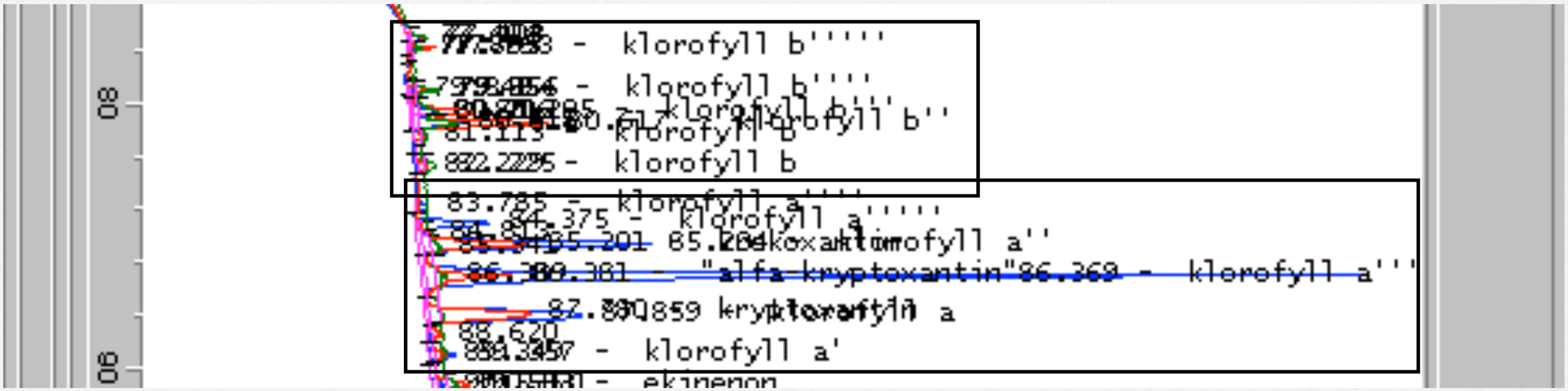
Natural sample: K34



Chlorophylls a/b

- No DV-chl a observed in natural samples
- Both chl a and chl b separated into several peaks (epimer, allomer(s), …)

Example: K34


klorofyll b'''''
klorofyll b''''
klorofyll b
klorofyll a'''''
"alfa-kryptoxantin"
klorofyll a'''
klorofyll a'
ekinenon

Chlorophyll problems

- **<u>Not</u> observed in DHI standards after evaporation and re-dissolving**
- **<u>Not</u> observed in DHI mixes 104, 105, 106**
- **Observed in natural samples & Sigma standards**

Chl. problems – cont.

- <u>Sigma standard</u> found 98 % pure at HPL, 44 % in Bodø (if main peak chl a)
- <u>Natural samples:</u> Probably artefacts during storage/freeze-drying/extraction
- Not present in extracts at other labs?
- <u>Or:</u> Not shown in their HPLC methods?

tirsdag 13. april 2010