

Analysis of Soft fruit for health benefits: Progress so far

Sean Conner



The James
Hutton
Institute

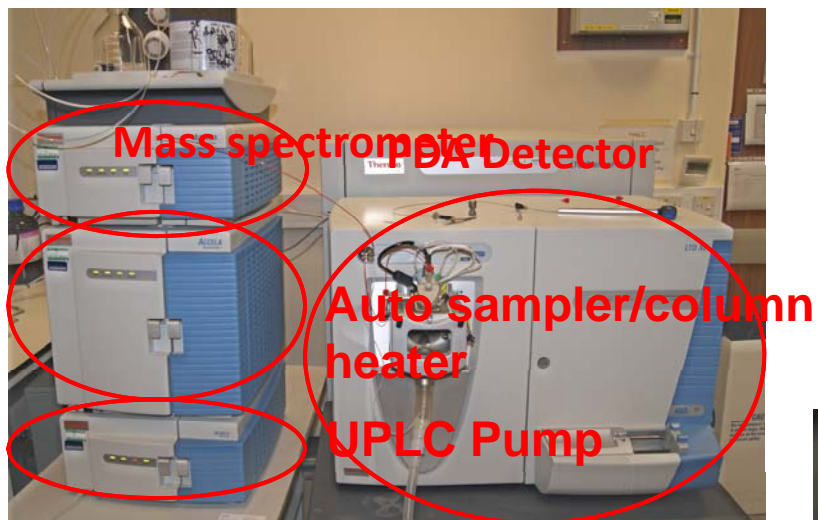


The Interreg IVB
North Sea Region
Programme



C  Future-proofing berryfruit
CLIMAFRUIT

LC-MS Analysis



Composition of LC-MS System



Electrospray ionization (ESI) Positive & negative mode

Method Development

- Extraction protocol
- LC-MS method
- Processing Methods
- Metabolite Identification
- Calibration Curves

Extraction Protocol

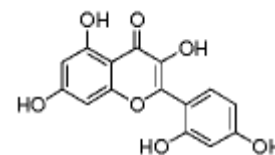


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3 ml 60:40 water/methanol 1%
acetic acid 0.1 mg/ml morin
(internal standard)
100 mg freeze dried (FD)
powder

Shaken for 60 minutes at 30 °C



morin

LC-MS Methods Developed

- Raspberry
- Blackcurrant
- Blackberry

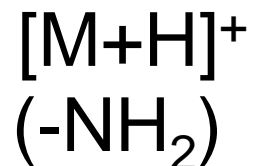


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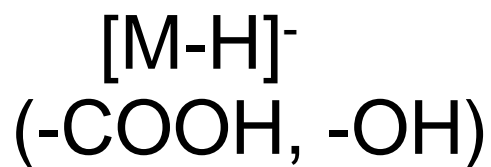


Positive or Negative mode?

Basic Molecules



Acidic Molecules

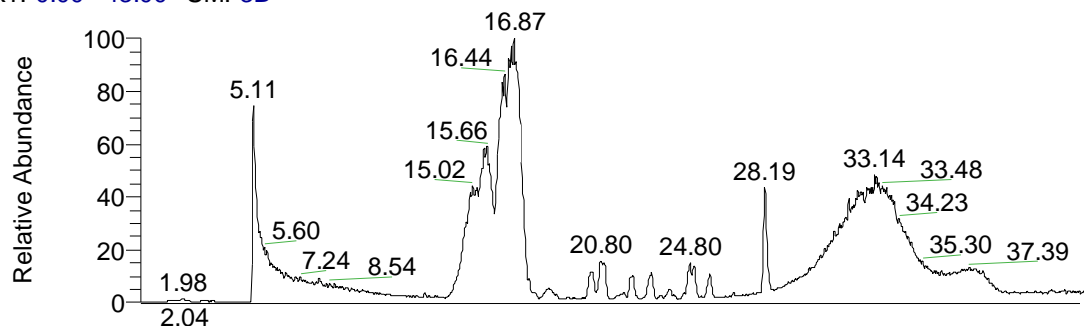


- Anthocyanins - positive
- Flavanols - negative
- Ellagitannins - negative
- Organic acids - negative

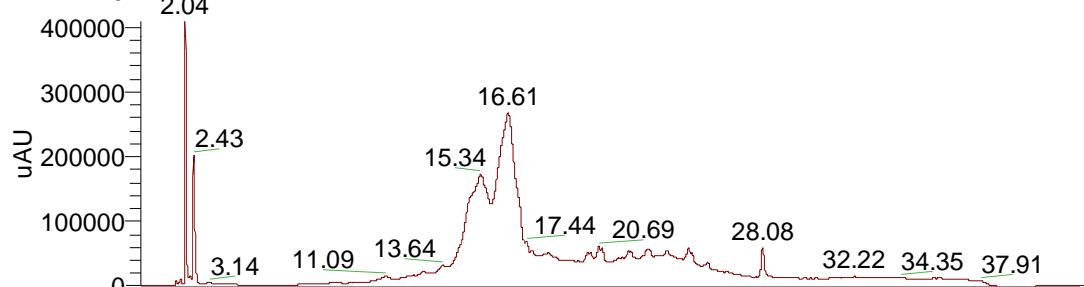
LC-MS Chromatogram FD Blackcurrant

Positive mode

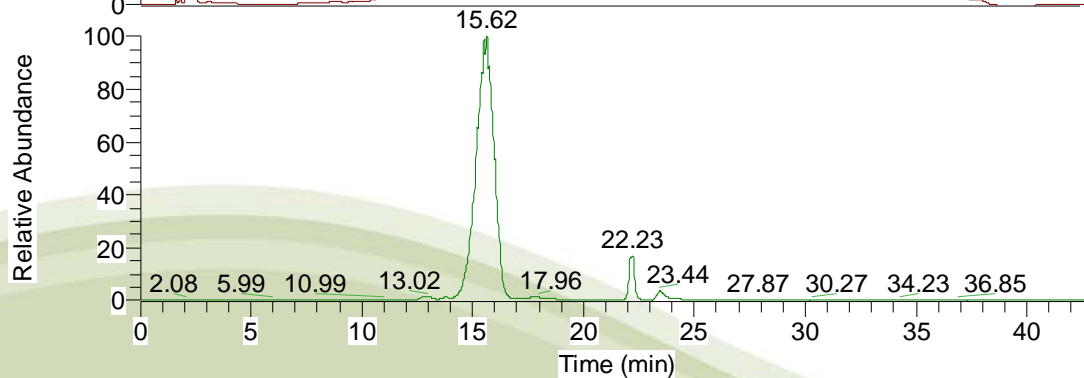
RT: 0.00 - 43.00 SM: 3B



MS
chromatogram



PDA
chromatogram



Selected ion
Chromatogram
m/z 611

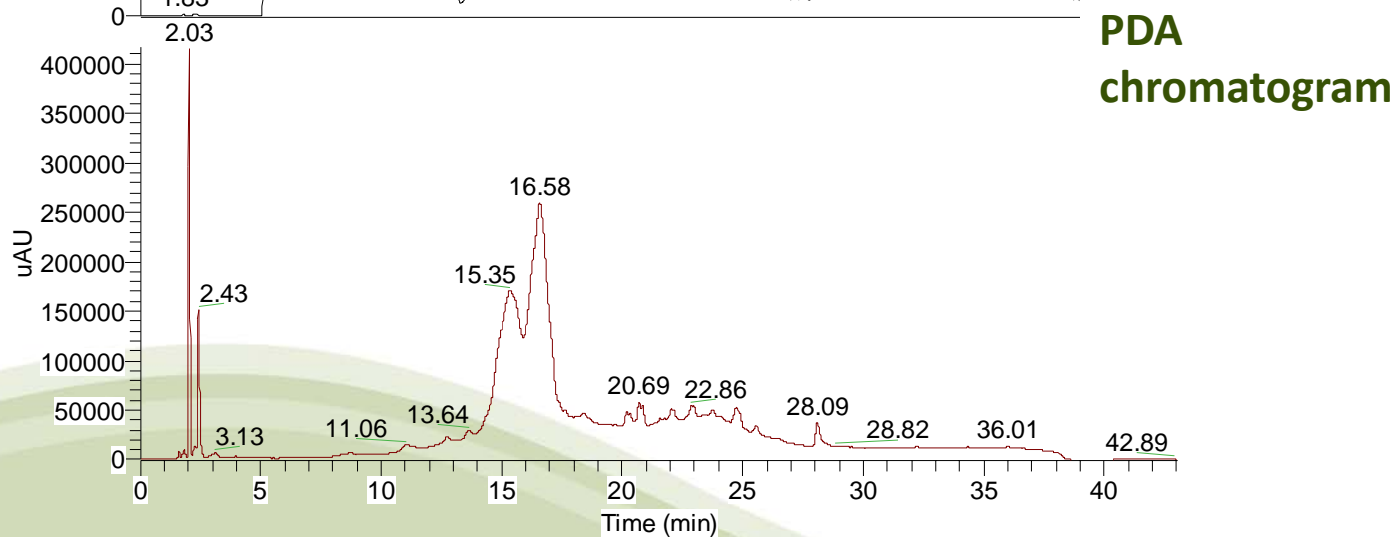
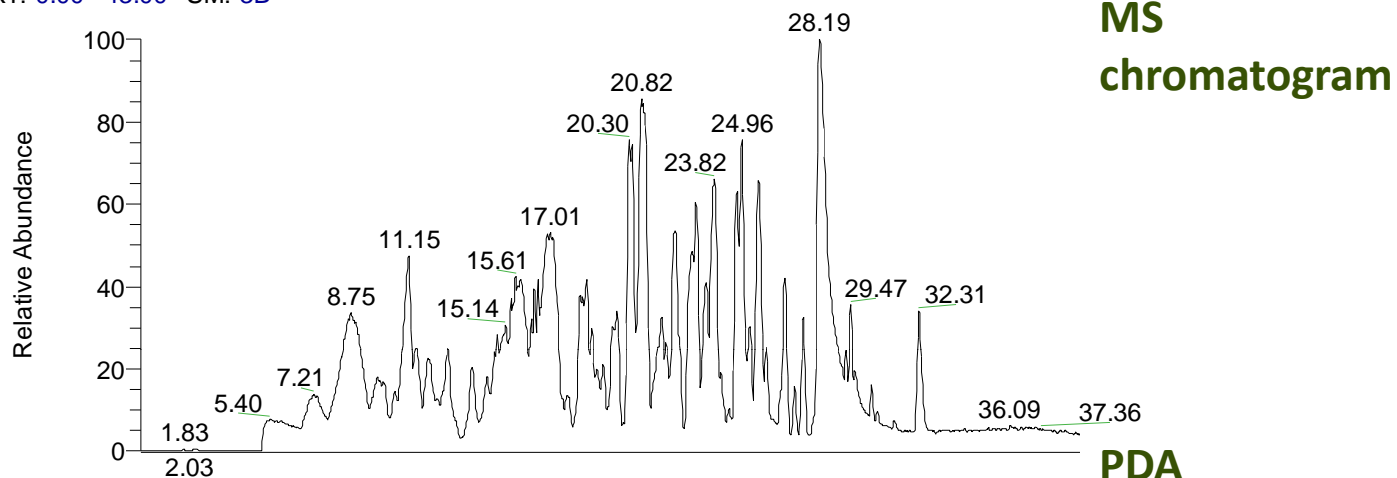
LC-MS Chromatogram FD Blackcurrant

Negative mode

RT: 0.00 - 43.00 SM: 3B

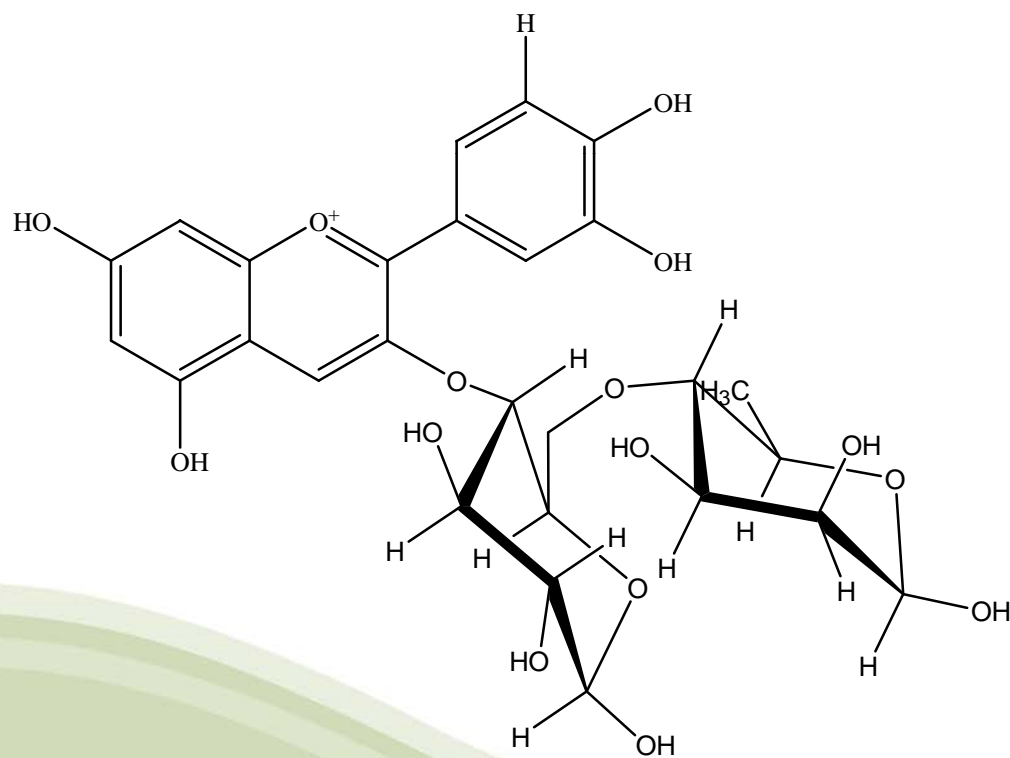


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Peak Identification

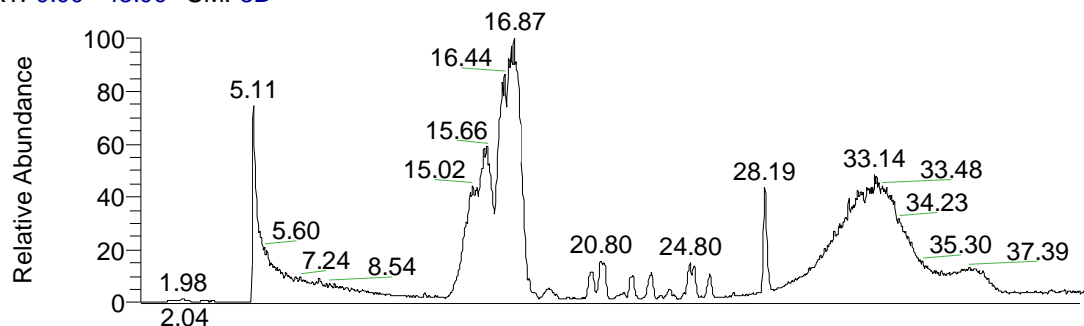
delphinidin-3-rutinoside ($m/z \sim 611$)



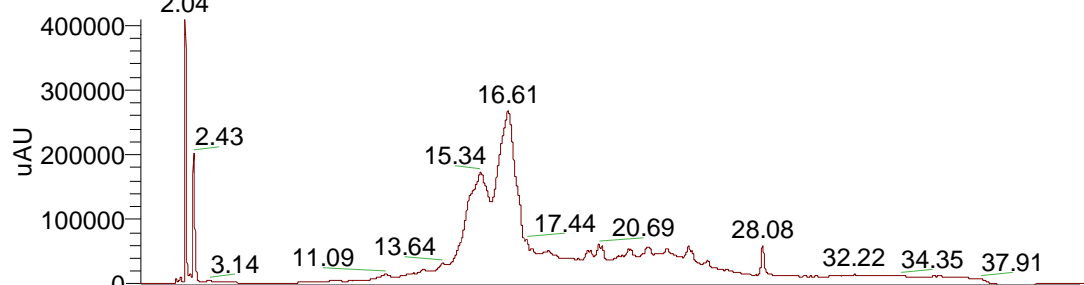
LC-MS Chromatogram FD Blackcurrant

Positive mode

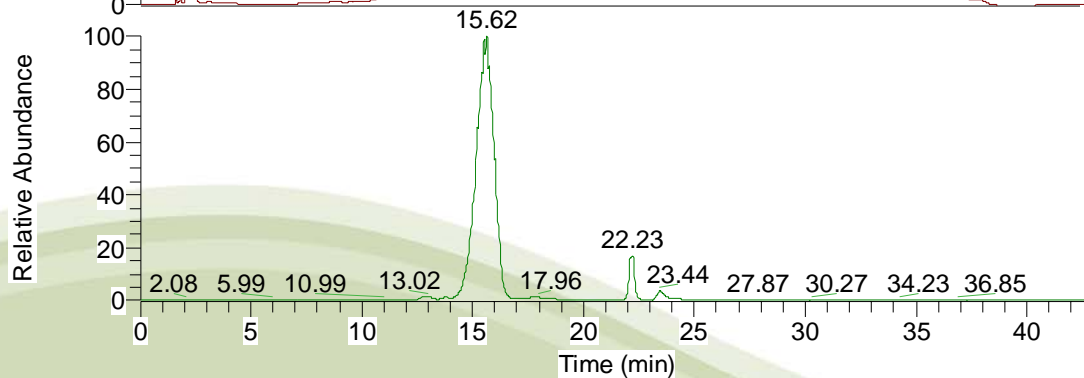
RT: 0.00 - 43.00 SM: 3B



MS
chromatogram



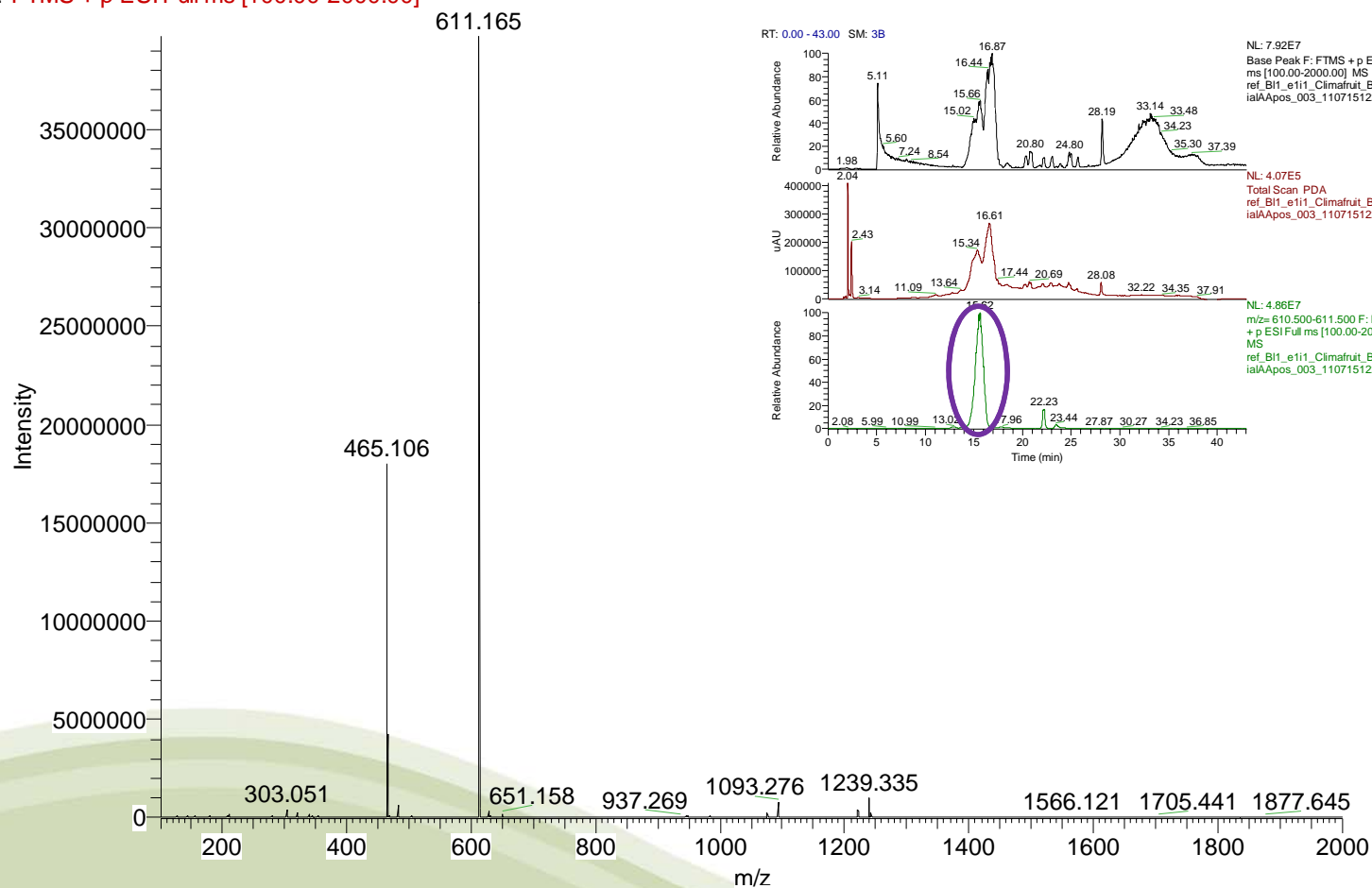
PDA
chromatogram



Selected ion
Chromatogram
m/z 611

Mass spectrum of peak at 15.62 mins

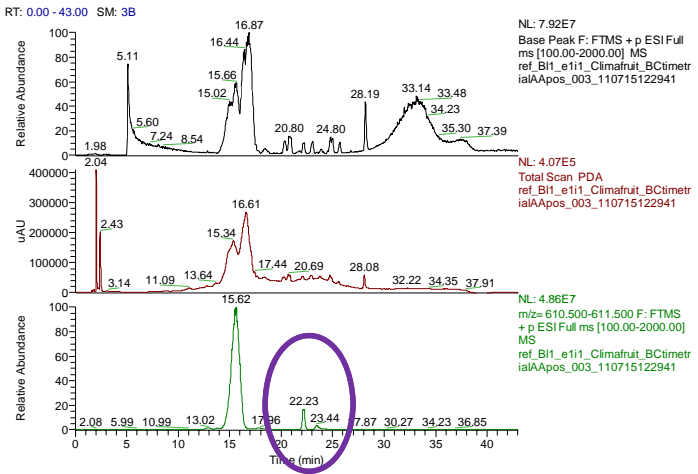
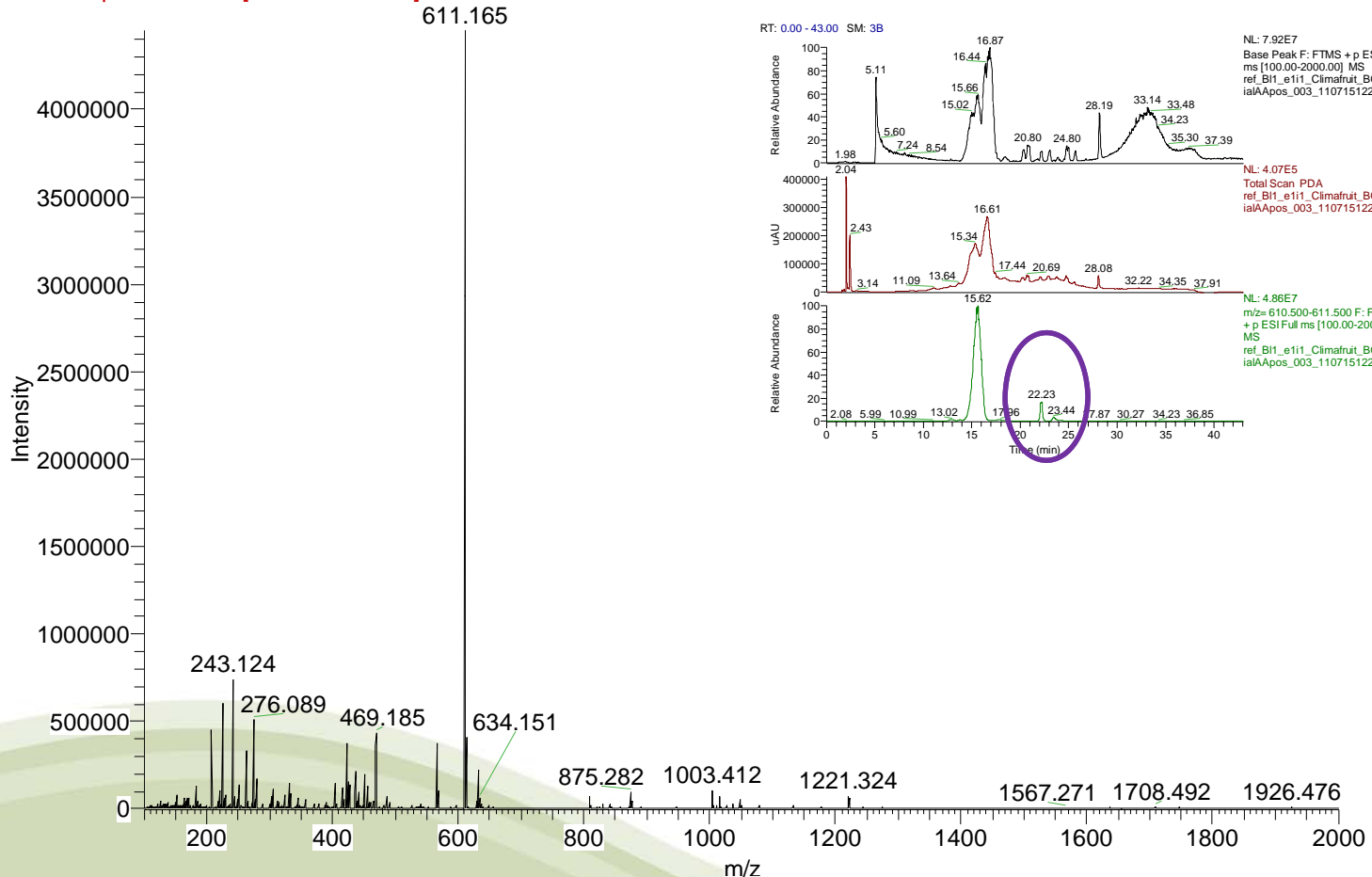
ref_BI1_e1i1_Climafuit_BCtmetrialAApos_003_110715122941 #3476 RT: 15.62 AV: 1 SB: 1 13.98 , 16.38 NL: 3.97E7
F: FTMS + p ESI Full ms [100.00-2000.00]



Mass spectrum of peak at 22.23 mins

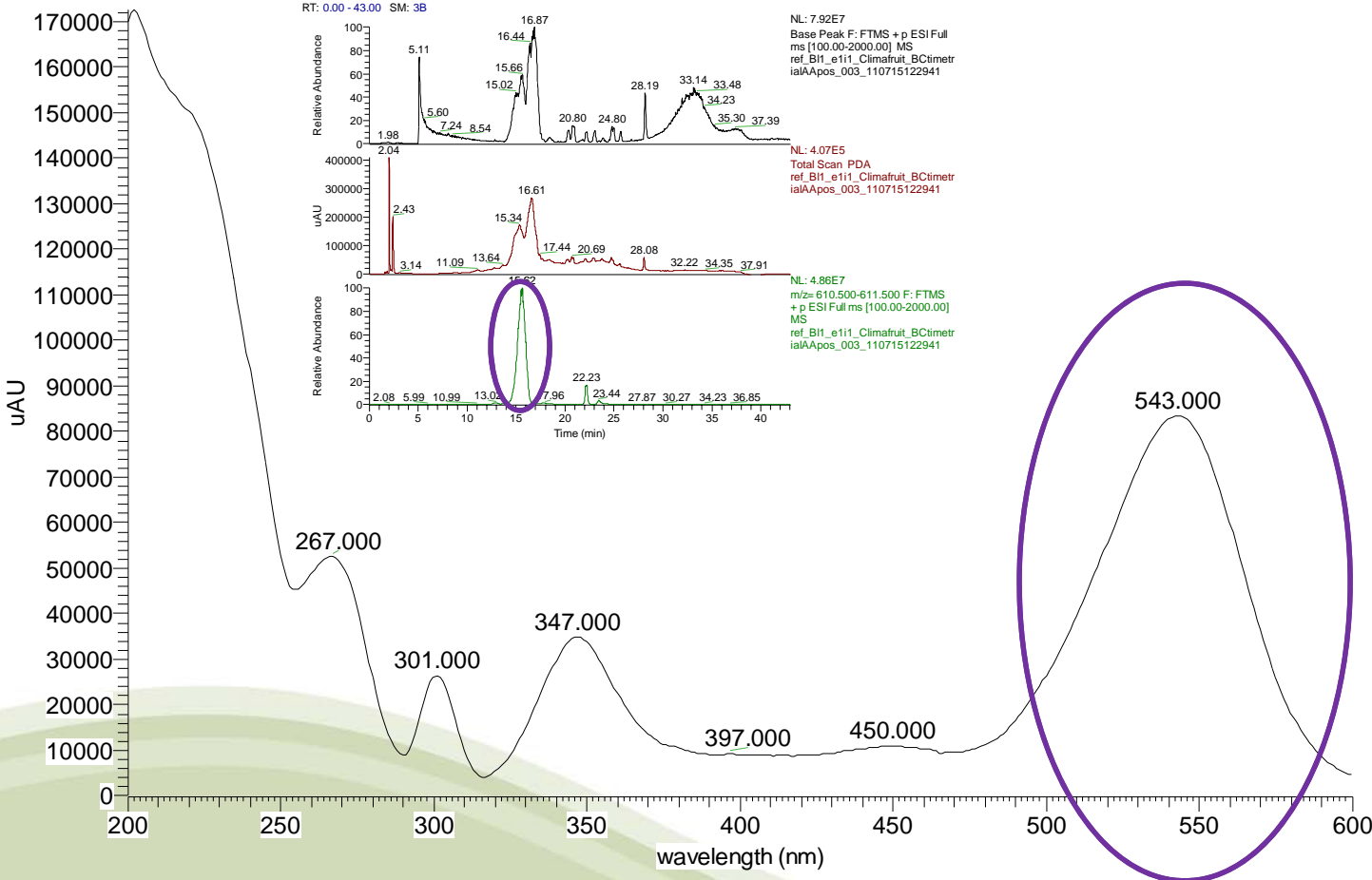


ref_BI1_e1i1_Climafuit_BCtimetrialApos_003_110715122941 #5026 RT: 22.12 AV: 1 SB: 1 13.98 , 16.38 NL: 4.45E6
F: FTMS + p ESI Full ms [100.00-2000.00]



PDA of peak at 15.62 mins

ref_BI1_e1i1_Climafruit_BCtmetrialAApos_003_110715122941 #4627 RT: 15.42 AV: 1 SB: 2 13.98 , 16.38 NL: 1.72E5
F: FTMS + p ESI Full ms [100.00-2000.00]



Processing methods

- Unique identifiers
- Retention time
- Mass to charge ratio
- All three fruits of interest have generated chromatograms containing in excess of 100 metabolites in both positive & negative mode
- Processing and data mining time consuming

Progress to date

- Method development – Complete
- Blackcurrant Samples 2010 harvest -
AARHUS UNIVERSITY Faculty of Agricultural Sciences
- Harvest time trial – complete
- Traditional cultivar trial – extracted
- Organic cultivar trial – extracted
- Blackcurrant Samples
Swedish University of Agricultural Sciences - selected 6
cultivars (sampled 2008,2009,2010) - extracted

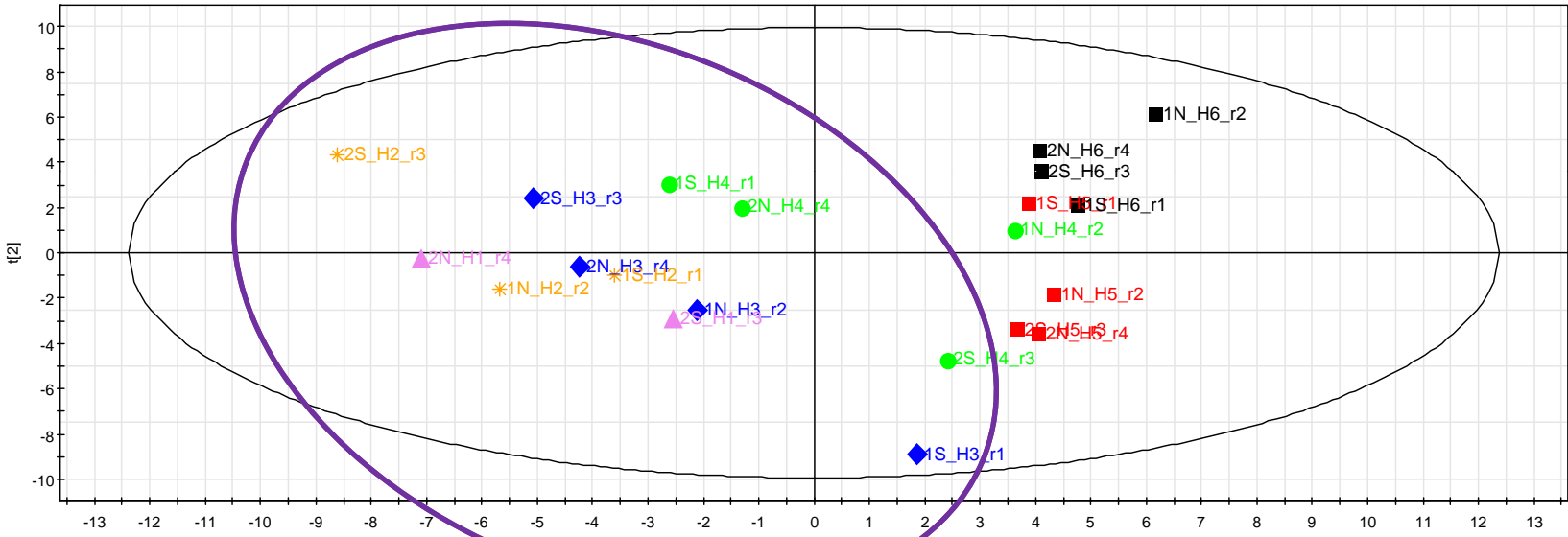
Blackcurrant harvest time trial results



“Untargeted” approach - PCA positive mode



Harvest Time Trial Pos Samples_Long.M2 (PCA-X)
 t[Comp. 1]/t[Comp. 2]
 Colored according to Obs ID (SAMPLEINJECSumb)



- 3_aug
- 5_aug
- ◆ 10_aug
- * 12_aug
- ▲ 17_aug
- 29_jul

Increased polyphenols

Hotelling T2 (0.95)



Targeted analysis - Anthocyanins Harvest time trial (Ben Connan)



Anthocyanin Content mg/100mg FDM

