ÚOCHB AV ČR
IOCB PRAGUE

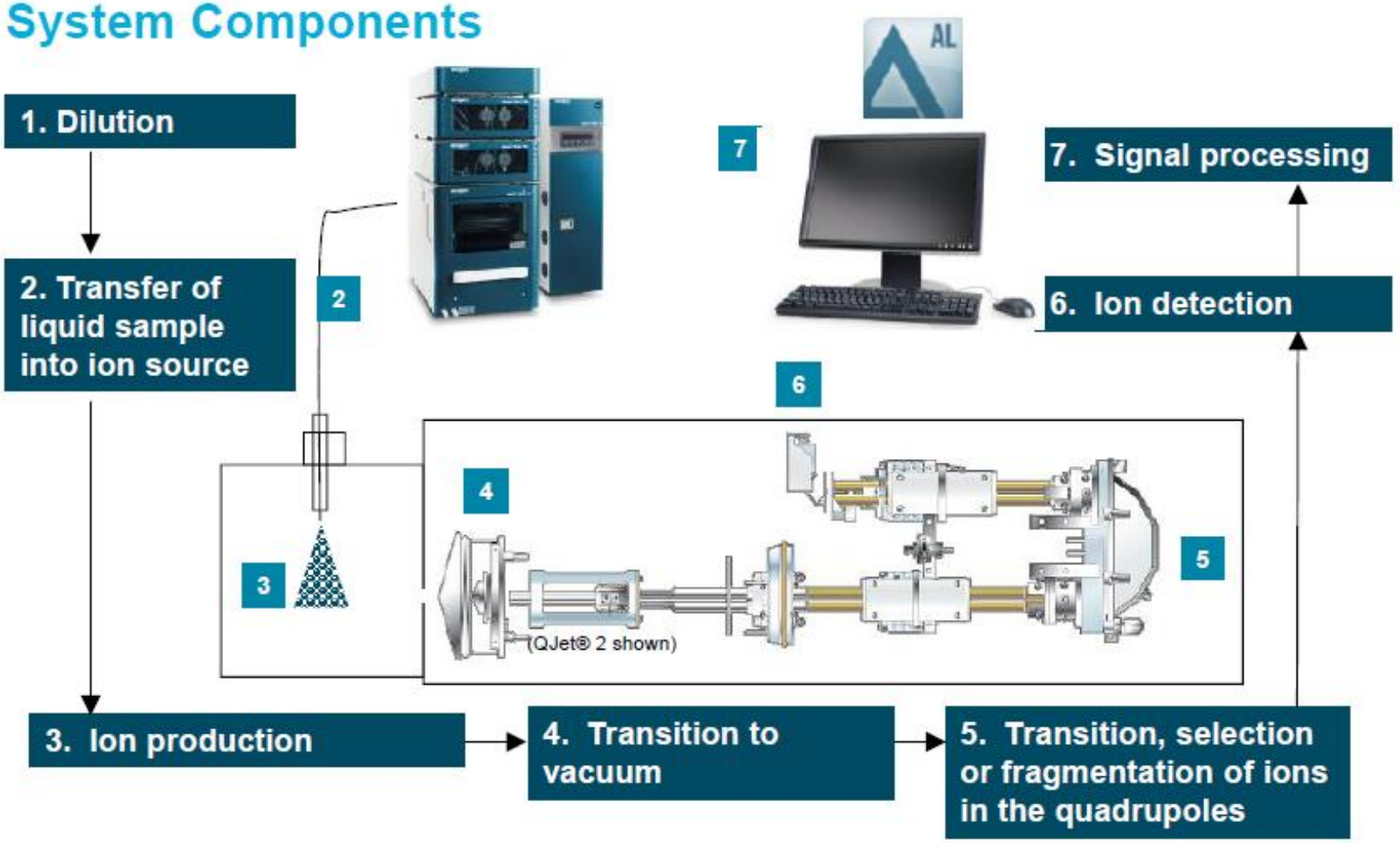
Quantitative LC/MS on a triple quad

Karel Čížek | June 13, 2022

Photo of the instrument

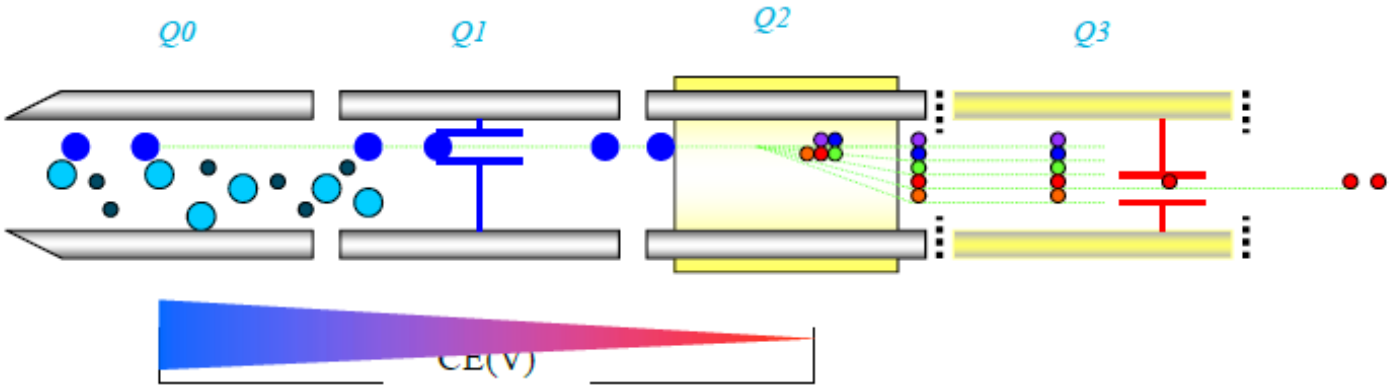
QTRAP: Scheme of the instrument

System Components



Basic mode of operation for quantification

MRM (Multiple Reaction Monitoring)



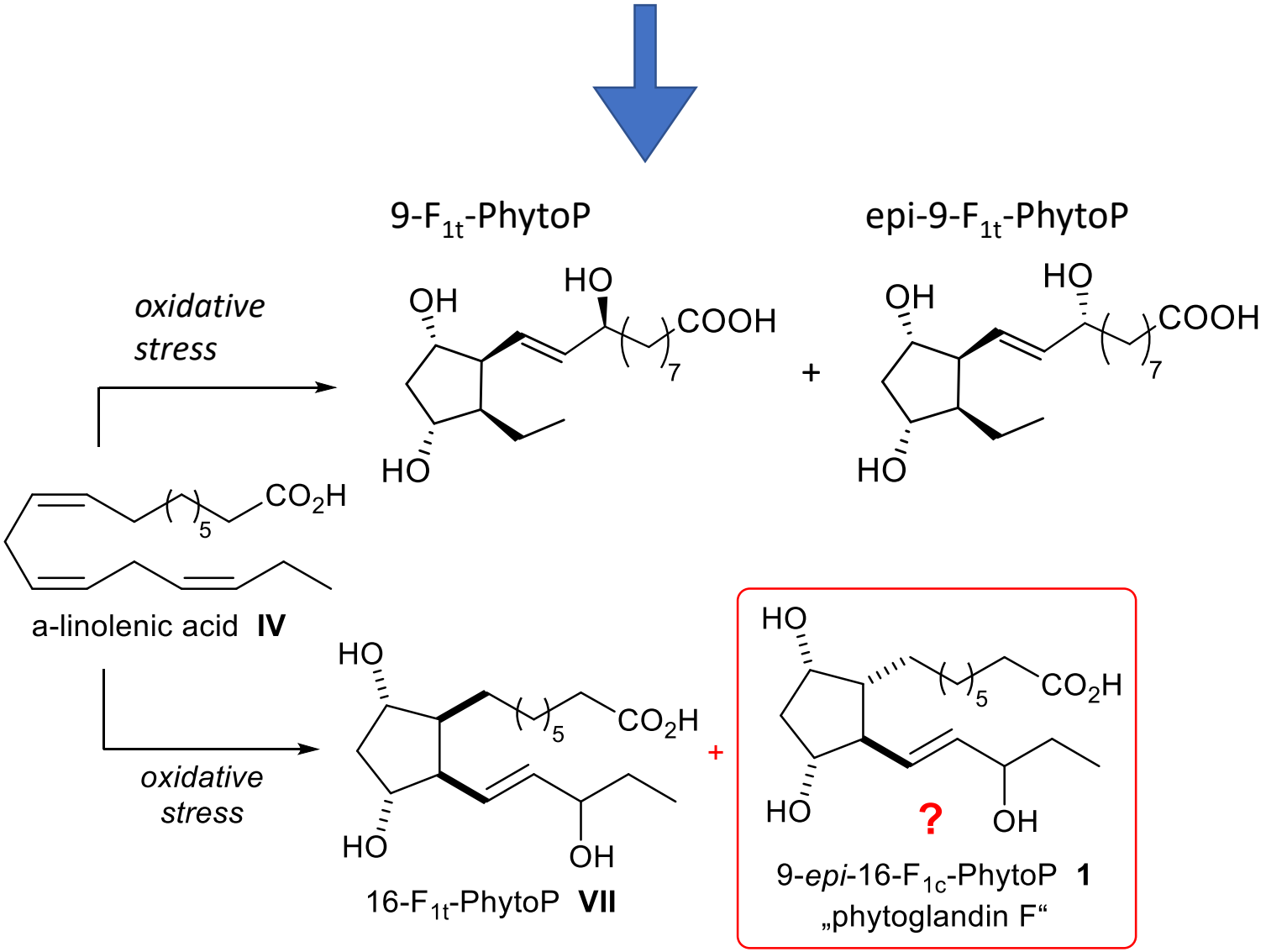
	Q1	Q3	Dwell time
1	453	254	20ms
2	685	885	20ms
3	453	254	20ms
4	396	274	20ms
5	1098	870	20ms
6	464	222	20ms
7	987	274	20ms
8	887	870	20ms

1

Identification of phytoprostanes in aged vegetable oil

Formation of phytoprostanes in aged vegetable oil

Non-enzymatic peroxidation of α-linolenic acid in plants

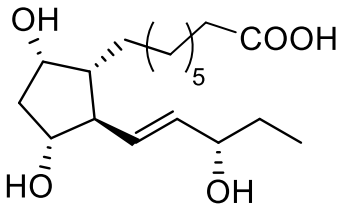


List of standards

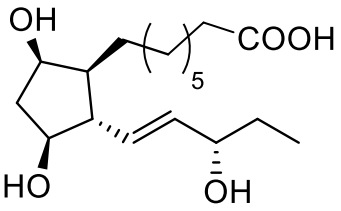
16-F series

16-F_1-PhytoPs

16-F_{1c}-PhytoPs (phytoglandins)

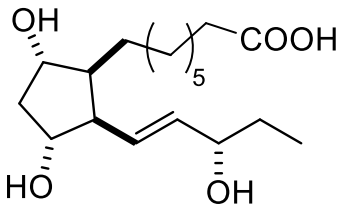


1

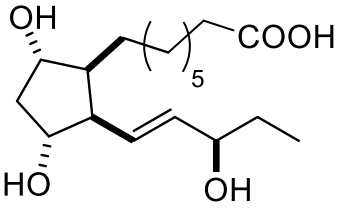


ent-epi-**1**

16-F_{1t}-PhytoPs



VII

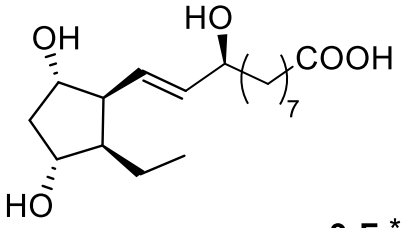


epi-**VII**

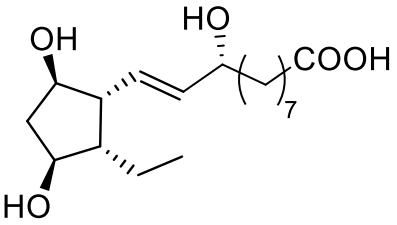
9-F series

9-F_{1t}-PhytoPs

9-F_{1t}-PhytoP

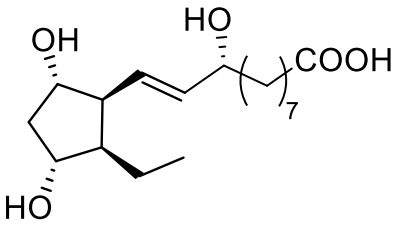


ent-9-F_{1t}-PhytoP

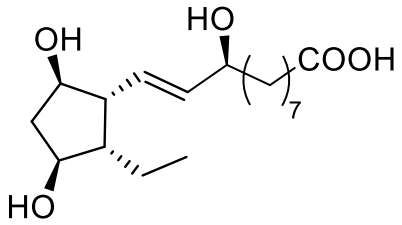


9-F *

epi-9-F_{1t}-PhytoP



ent-epi-9-F_{1t}-PhytoP



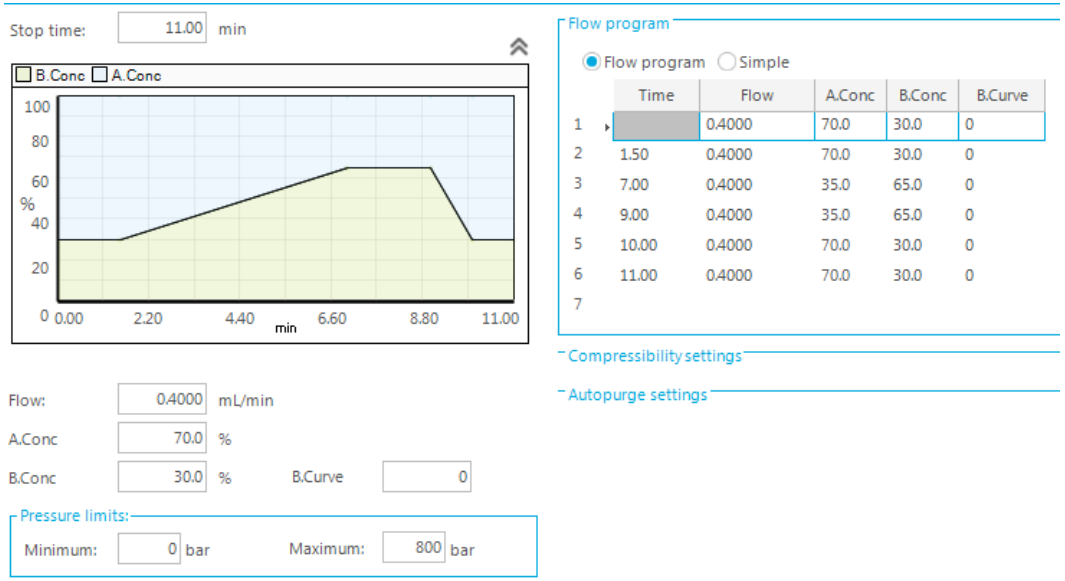
epi-**9-F** *

* Individually prepared enantiomers were used, opposite enantiomers coeluted together.

Experimental conditions

LC-MS method

- Injection volume 10 uL
- Column: Kinetex C18 100 x 2.1 mm, 1.7µm
- MPA: 0.1% FA/H_2O
- MPB: 0.1% FA/MeOH
- Diluent: 50% MeOH +0.1% FA



Sample dissolution

1. Addition of 200 µL of 50% MeOH + 0.1% FA
2. Vortexing for 5 min
3. Sonication for 10 min
4. Filtration through Micro-Spin Filtration Tube at 14 000 g for 5 min

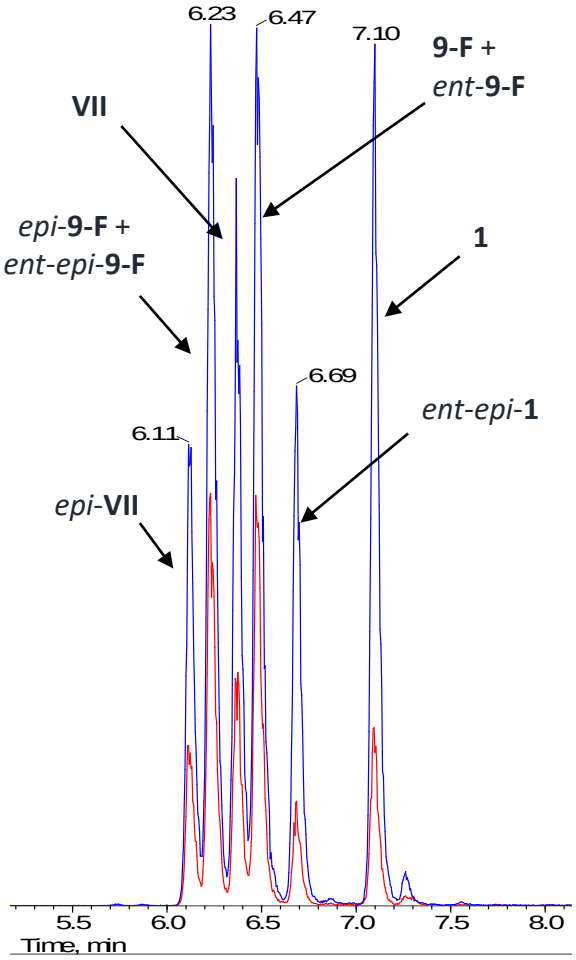
Detection of phytoprostanes in standard mixture

16-F and 9-F standard MIX (100nM of each compound)

Both 9-F and 16-F

327.1 > 283.1

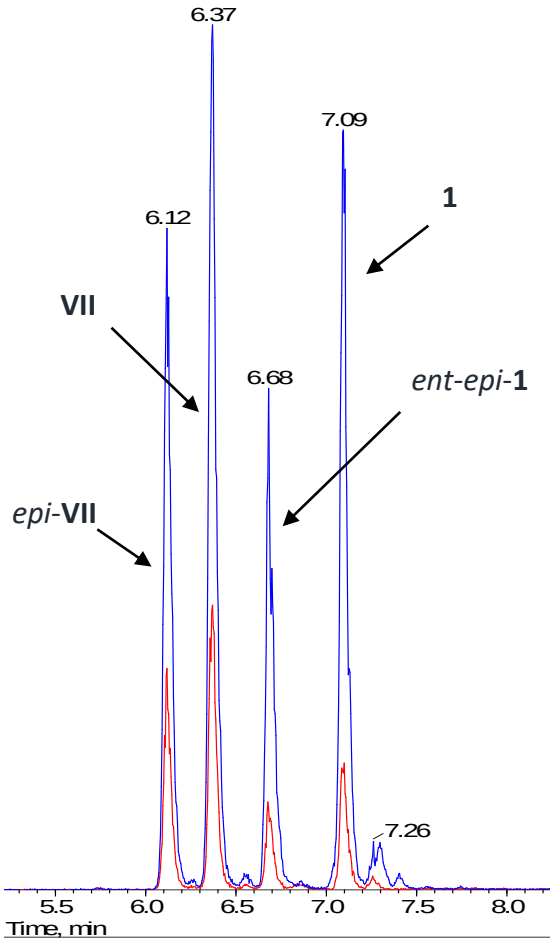
327.1 > 265.0



16-F

327.1 > 225.0

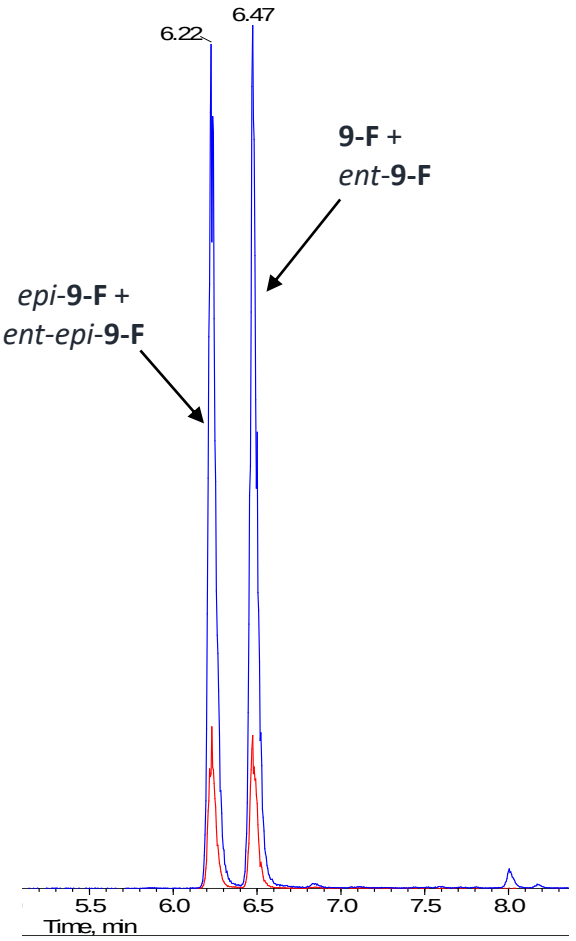
327.1 > 251.1



9-F

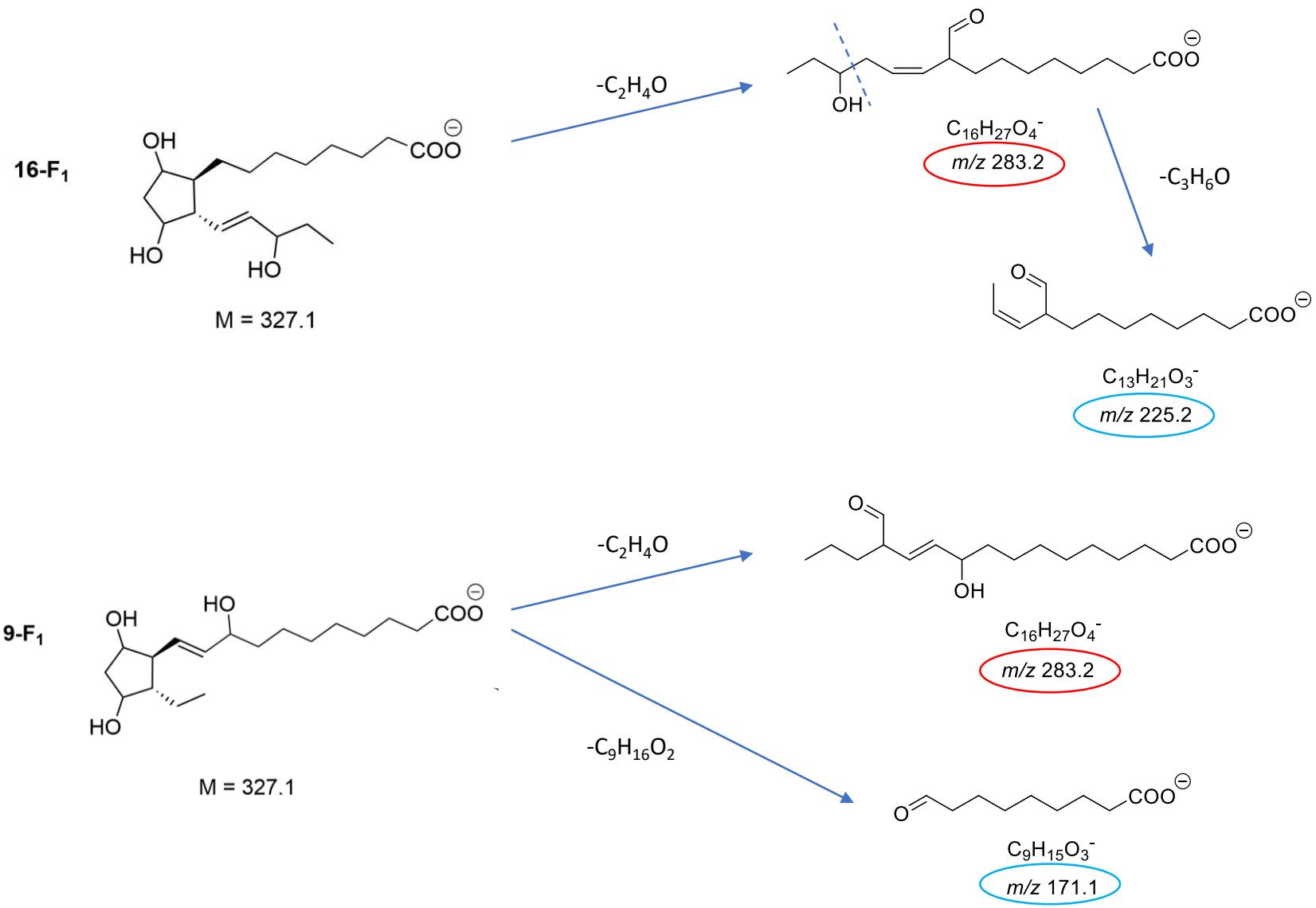
327.1 > 171.2

327.1 > 119.0



Formation of specific and non-specific fragments

Characteristic fragmentation



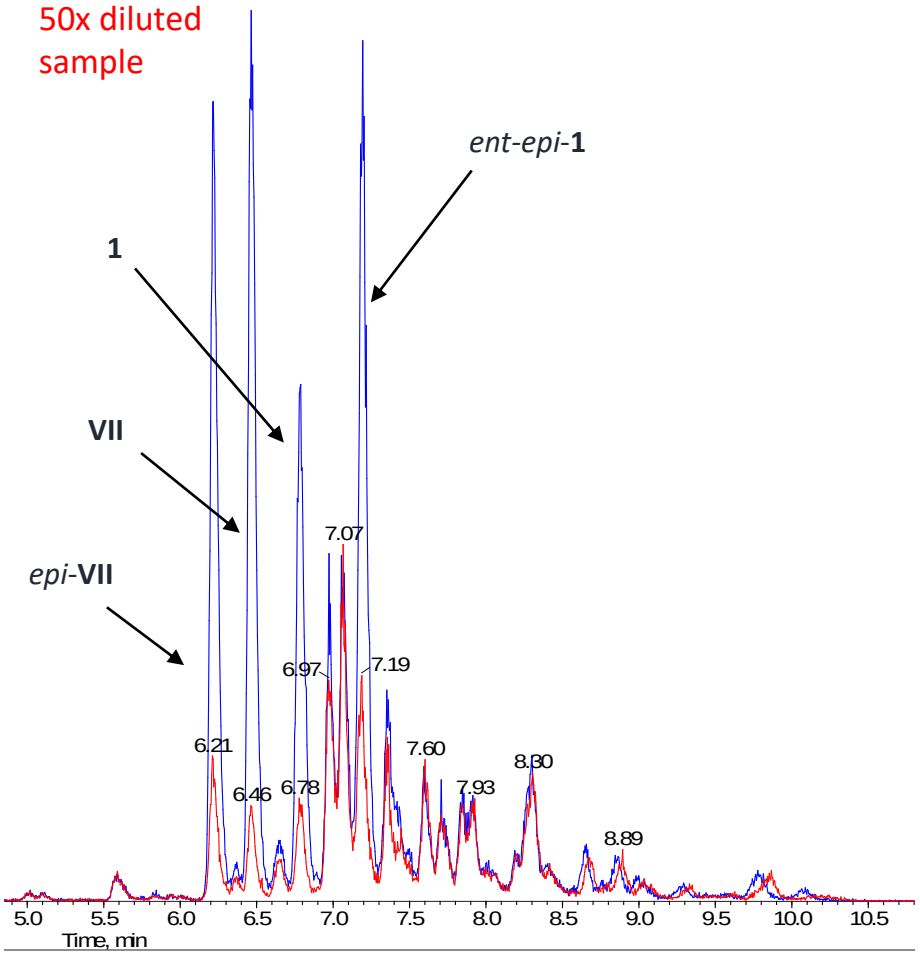
Identification of phytoprostanes in walnut oil

50× diluted + 100 nM of each 16-F and 9-F standard

16-F

327.1 > 225.0

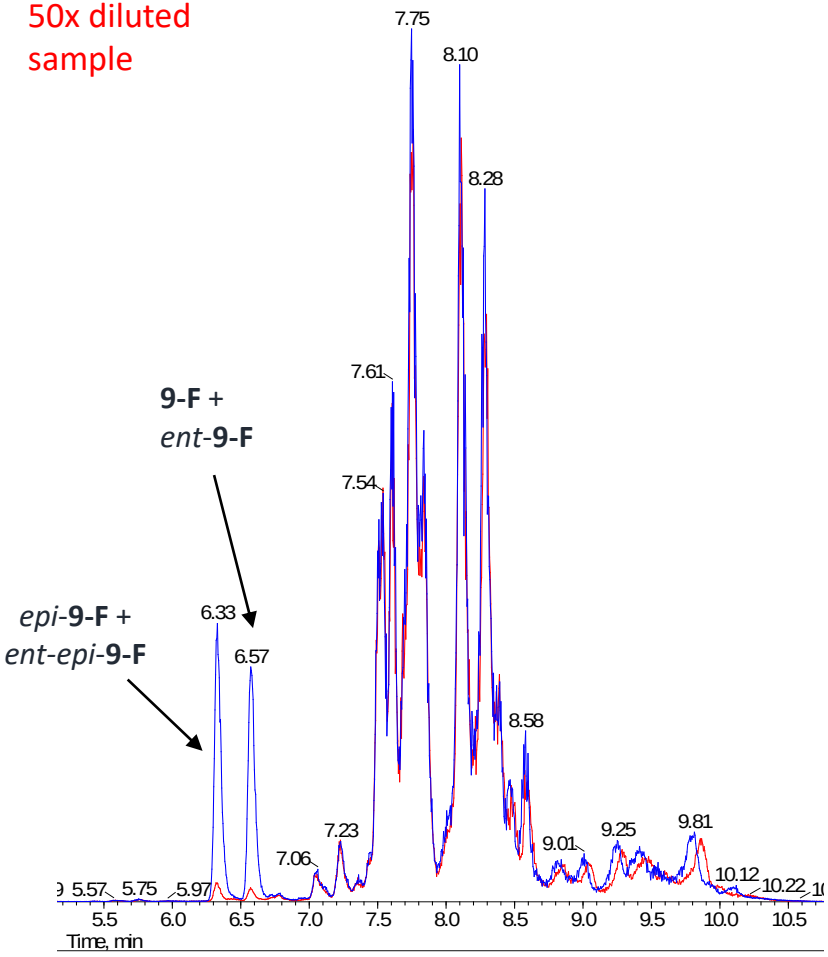
50x diluted sample + standard addition



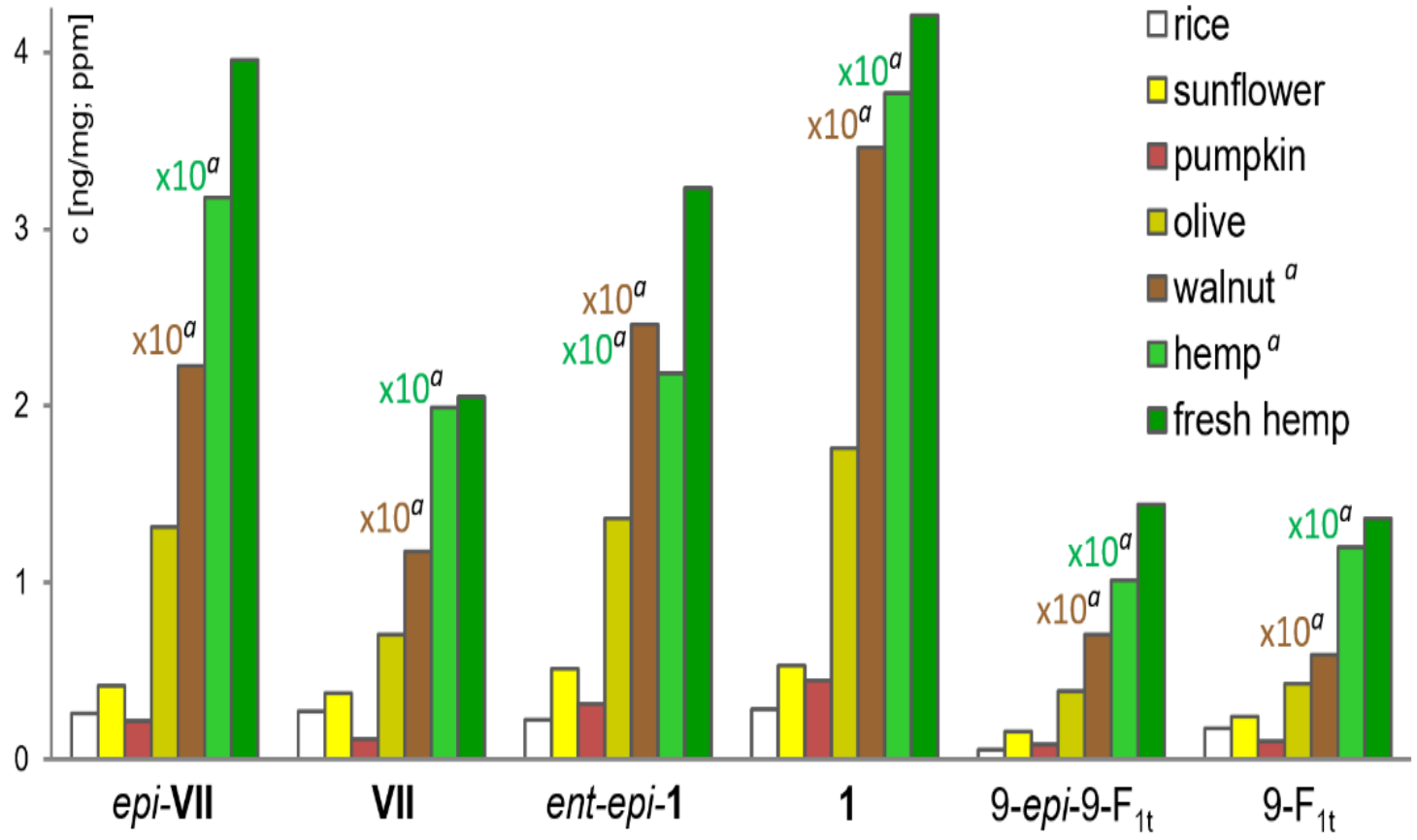
9-F

327.1 > 171.2

50x diluted sample + standard addition



Content of phytoprostanes in aged vegetable oils



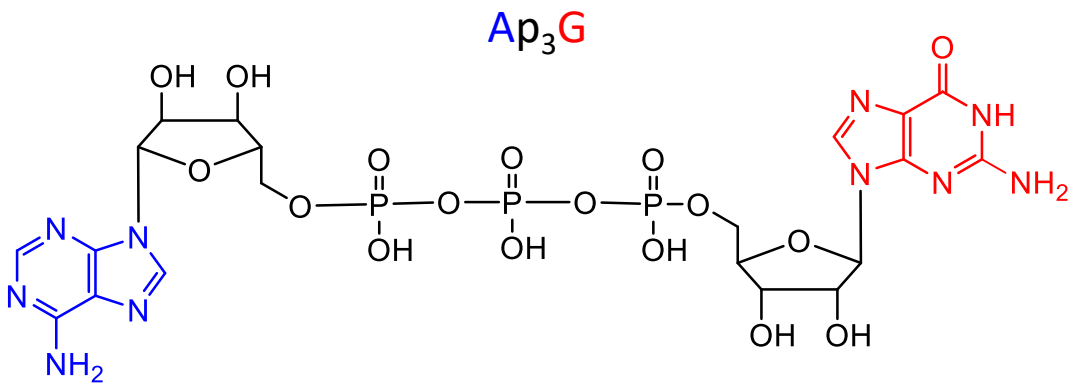
[a]Levels shown 10× less to keep the scale.

Smrček J. et al., Chem. Eur. J. 2021, 27, 1–8

2

Monitoring of nucleotides and dinucleotides in bacteria

Mixture of 20 compounds



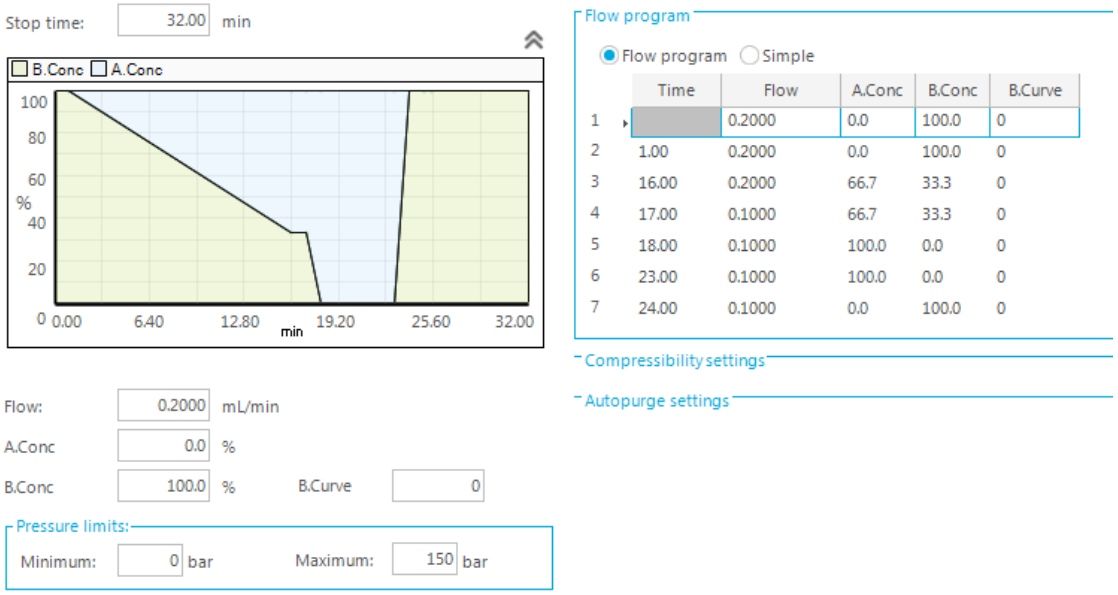
RNA Caps: $Ap_{2\text{-}5}A$, $Ap_{3\text{-}5}G$, $Gp_{3\text{-}4}G$, m^7Gp_3G

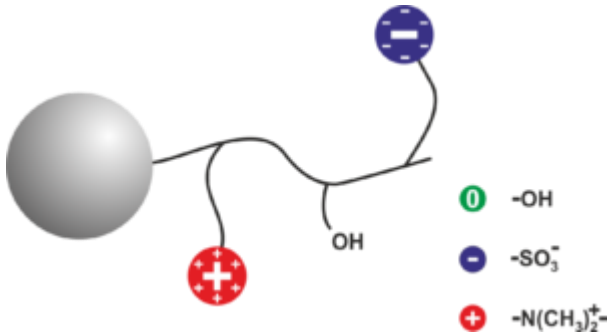
FAD, NAD, NADH, CoA,

AMP, ADP, ATP , GDP, GTP and IMP

Experimental conditions

- Column: iHILIC – (P) Classic 100 x 2.1 mm, 5µm + pre-column
- MPA: 10 mM CH_3COONH_4 / H_2O, pH = **9.0** + **5µM medronic acid**
  MPB: 90% ACN + **5µM medronic acid**
- Diluent: 10 mM CH_3COONH_4 or H_2O

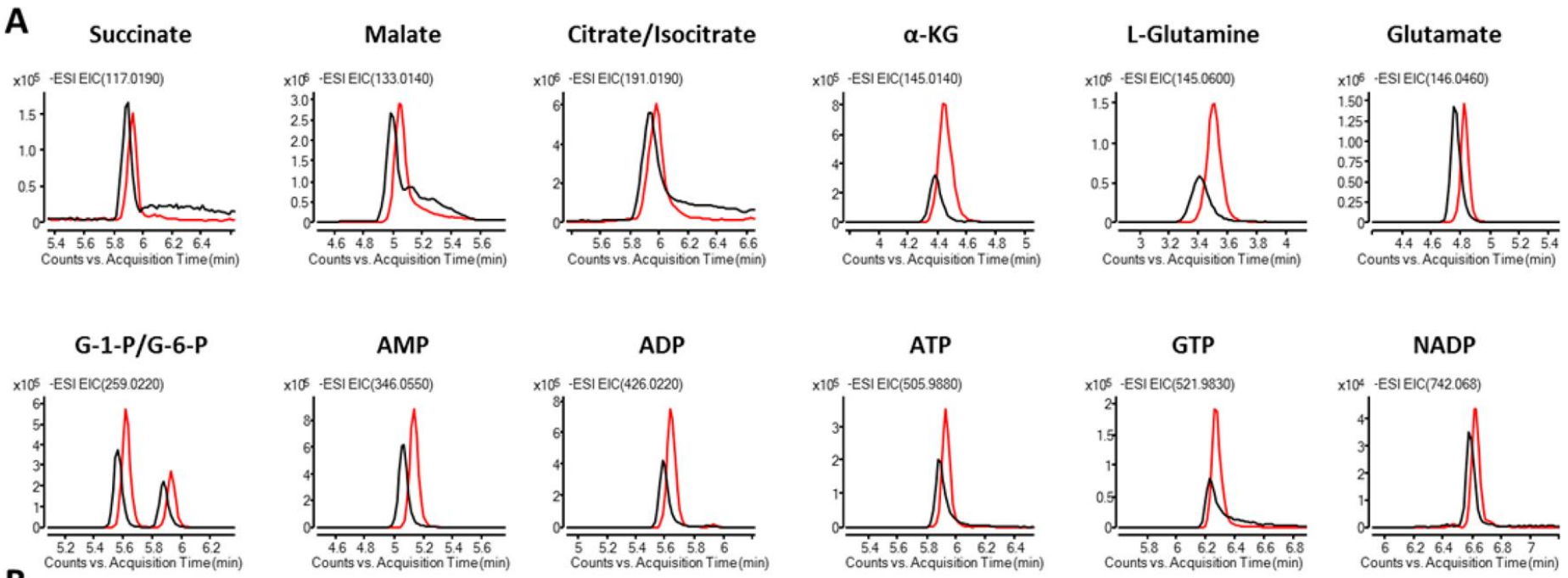


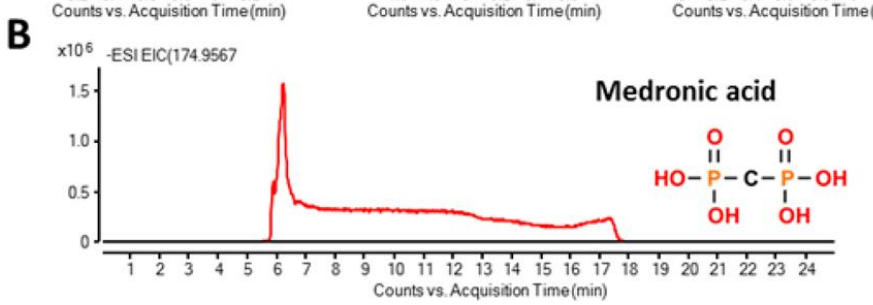


iHILIC®-(P) Classic

Charge Modulated Diol HILIC columns polymer based, 5 µm available in PEEK, stainless steel (SS), PEEK-lined SS (PEEK-SS) stable at basic pH with range pH 1-10 ultra-low bleeding (www.hilicon.com)

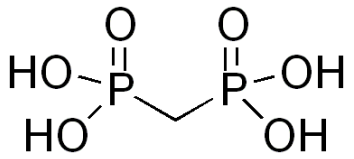
Medronic acid as a mobile phase additive





Control

5 µM Medronic acid

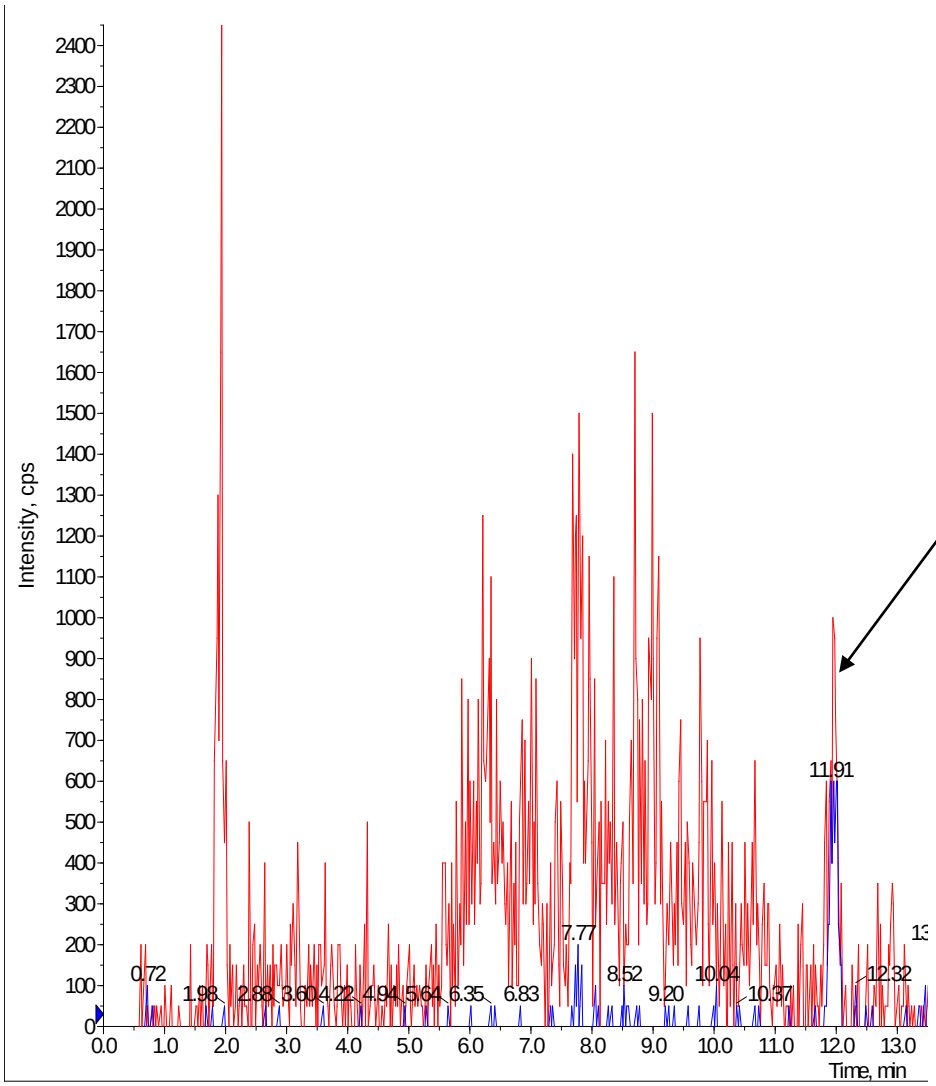


Hsiao J.J. et al. Anal. Chem. 2018, 90, 9457–9464

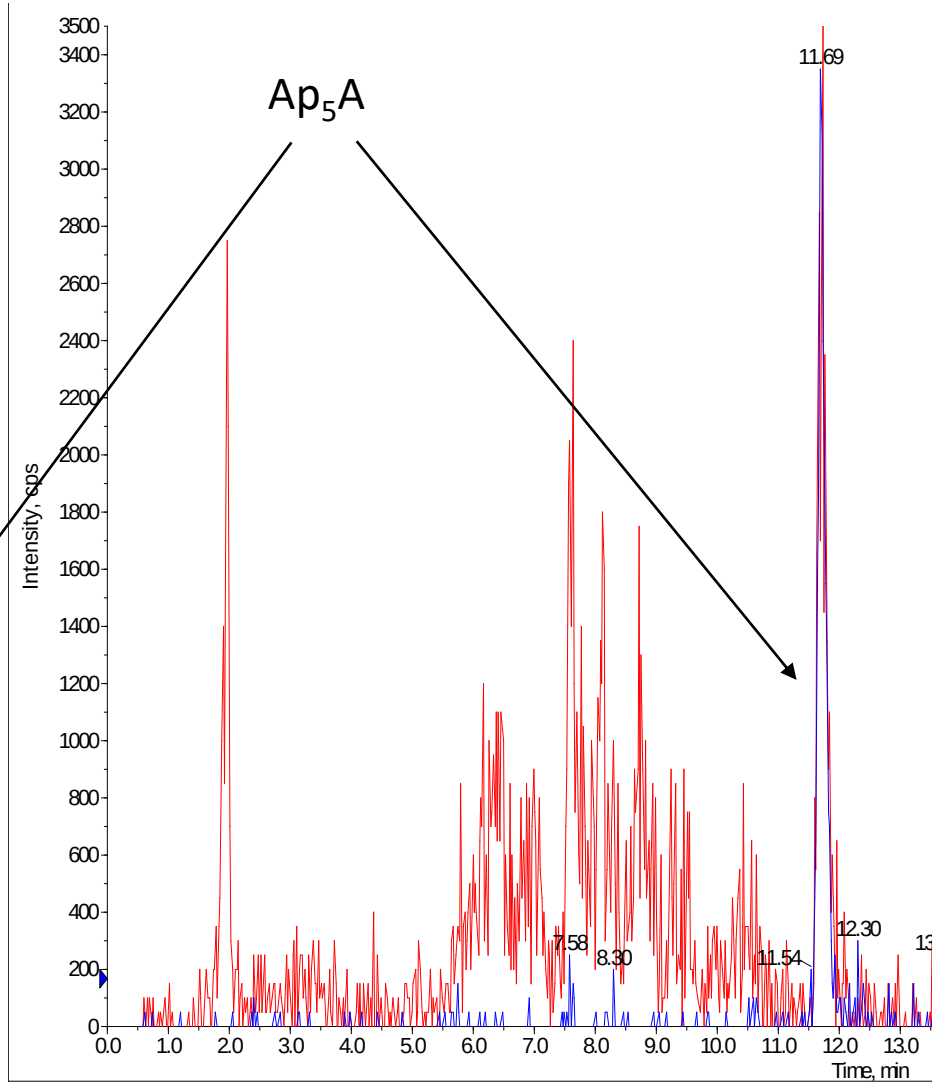
Ap_5A: 100nM

Diluent: Matrix

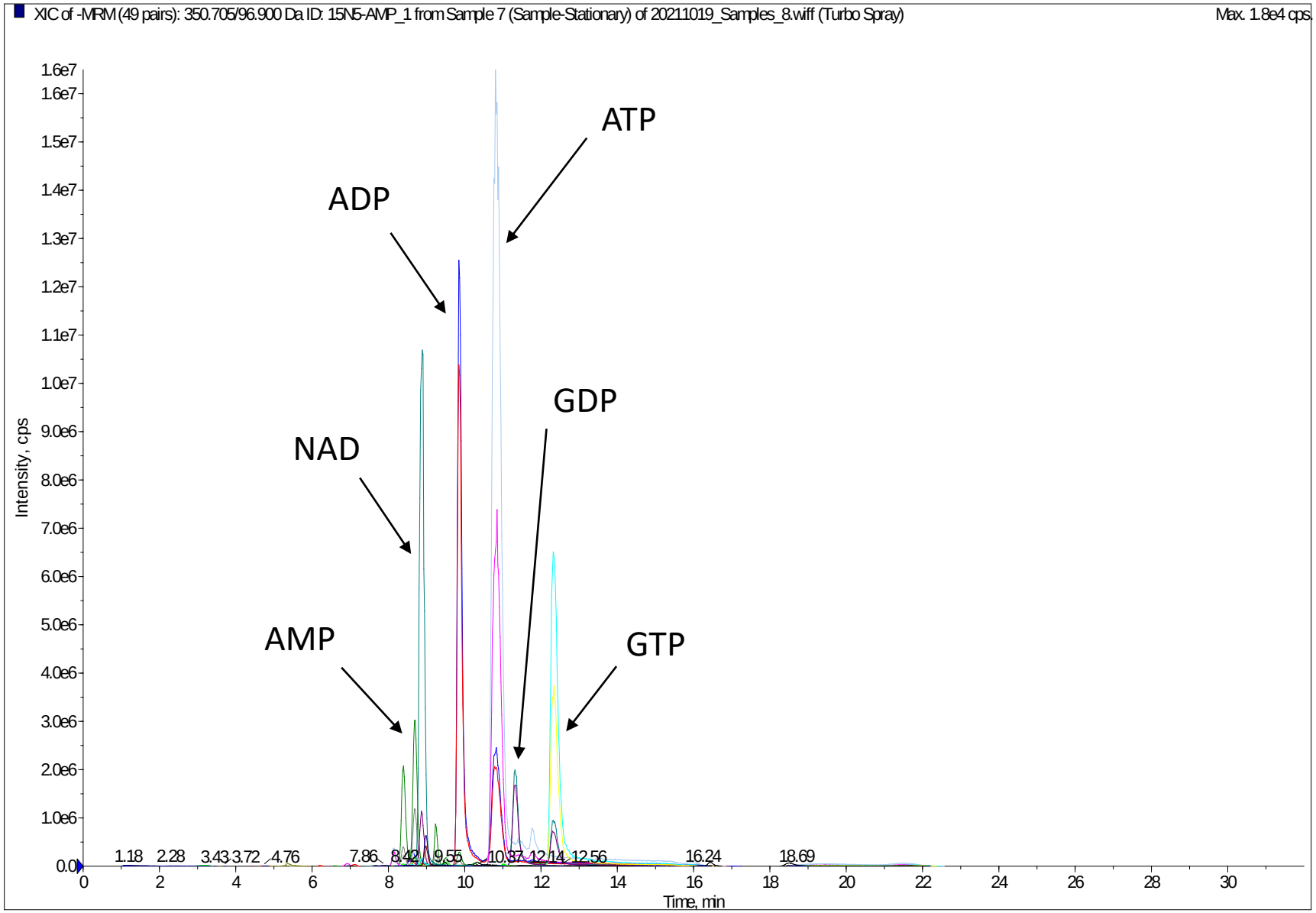
MP



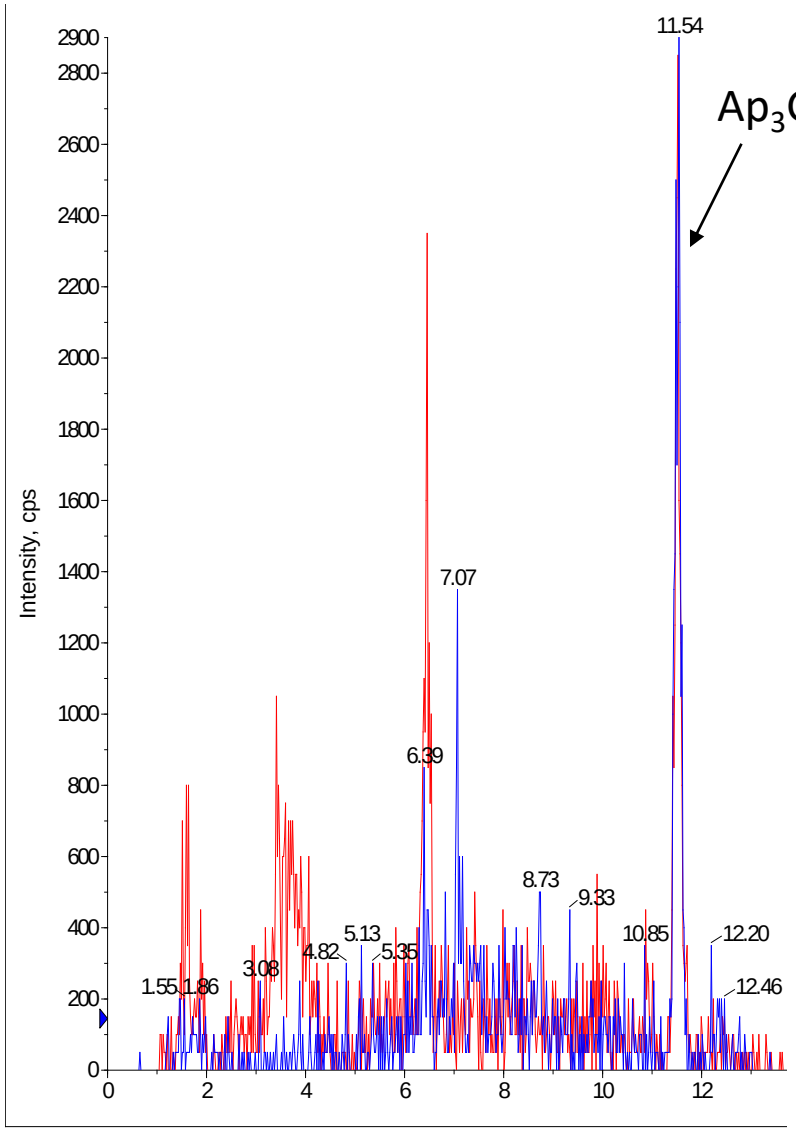
MP + **5µM medronic acid**

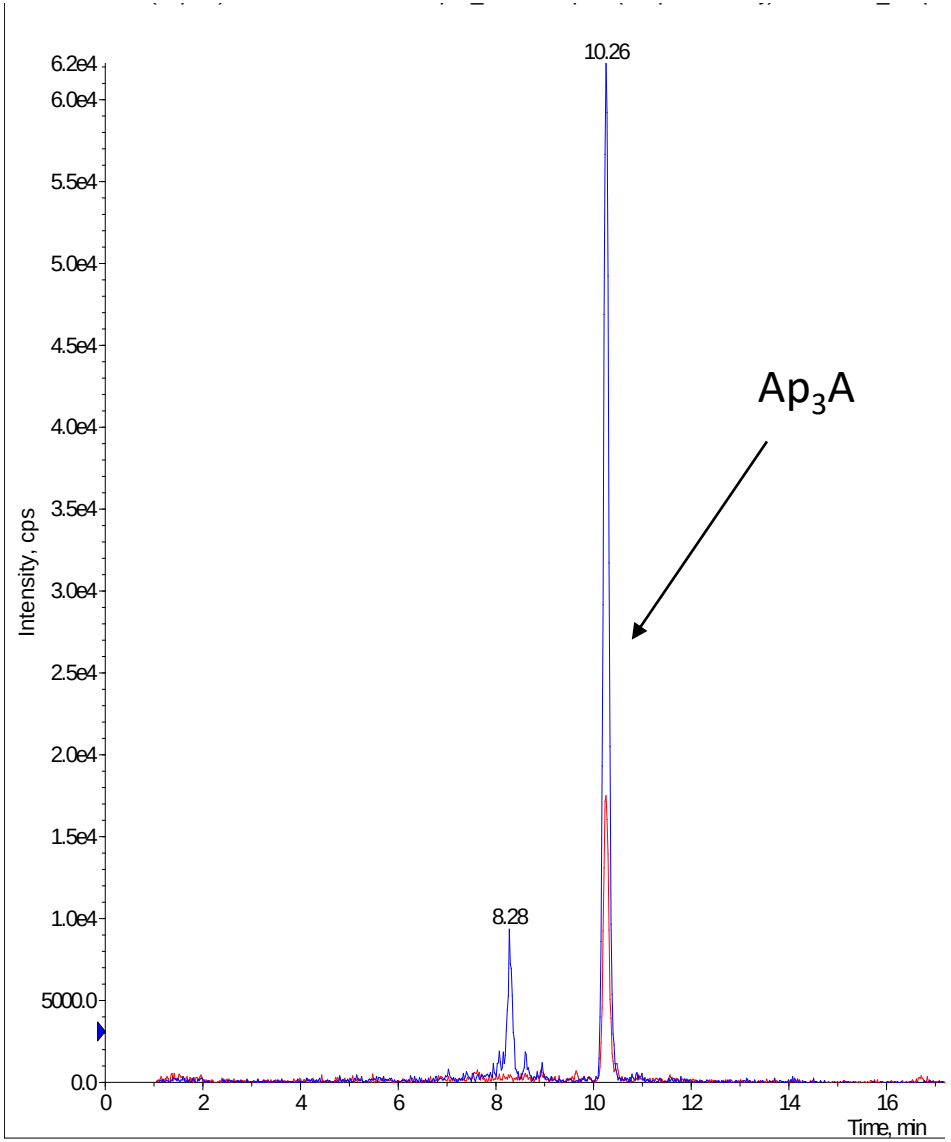


Stationary phase of bacteria (E. coli) growth



Stationary phase of bacteria (E. coli) growth



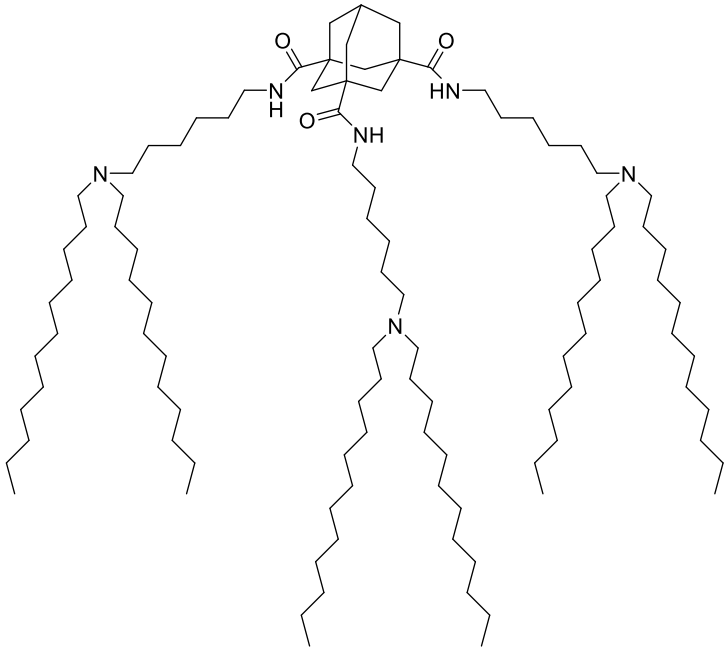


3

Determination of targeted lipidoids

Structures of XMaNs – Adamante-based lipidoids

XMaN6 (C6)



Chemical Formula: $C_{103}H_{202}N_6O_3$
Exact Mass: 1571,58384
Molecular Weight: 1572,78800

MW

C5: 1530.7

C6-lin: 2053.6

C6-lin-est: 2160.8

NH-C6-5-6: 1668.5

Et7: 2005.2

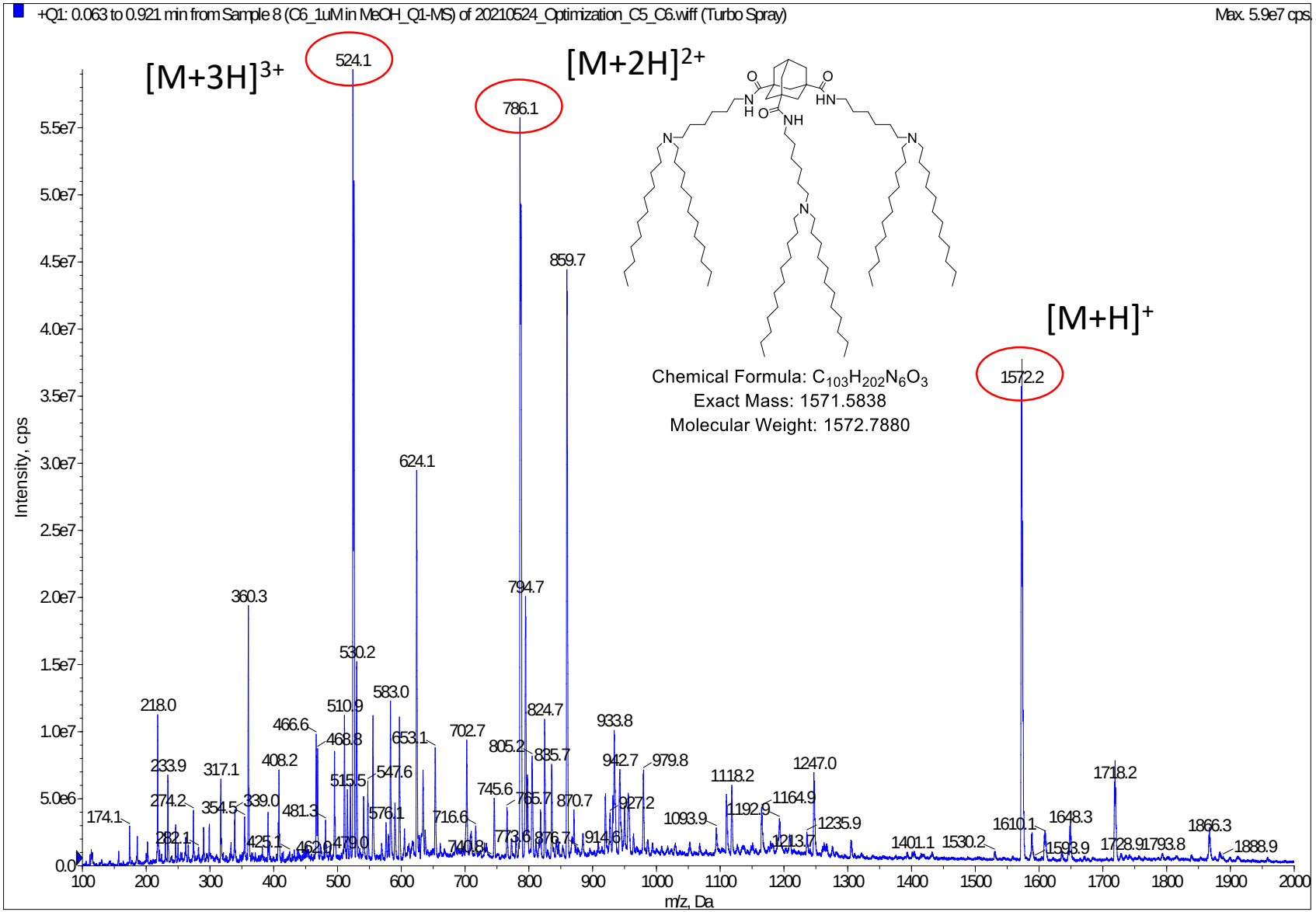
Met7: 1921.0

MC3:641.6

SM-102: 710.7

Hejdankova Z. et al., Adv. Funct. Mater. **2021**, 2101391

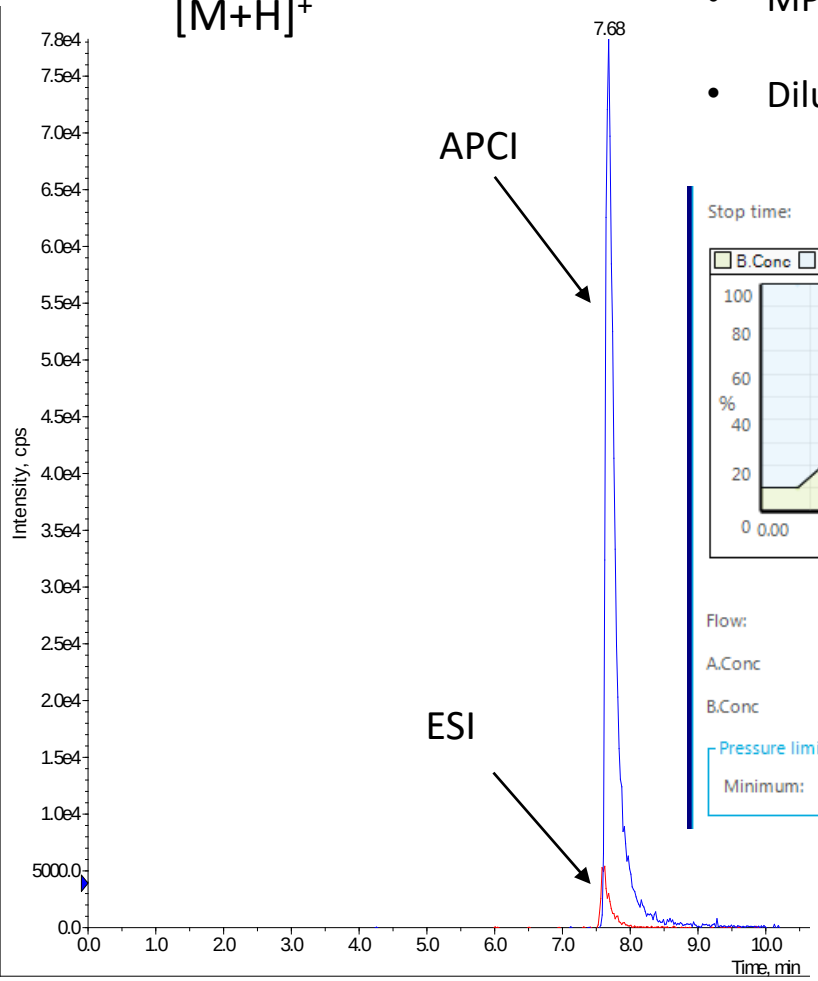
XMaN6 (C6) at ESI



Experimental conditions

XMaN6 (C6): 1µM

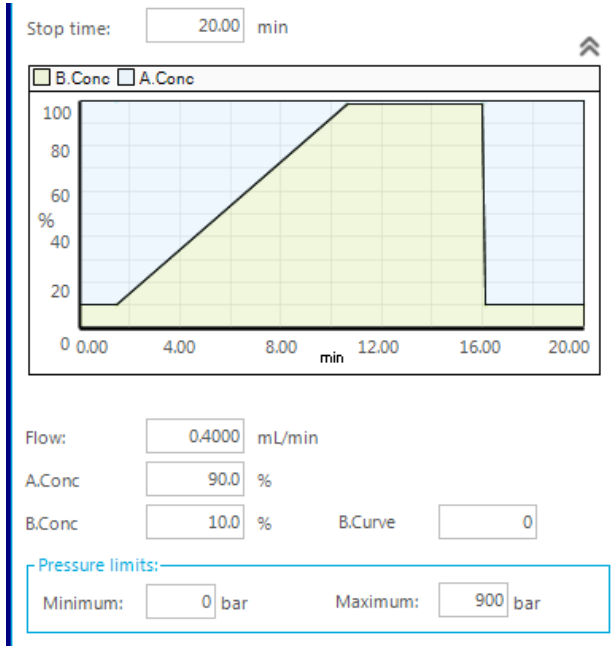
$[M+H]^+$

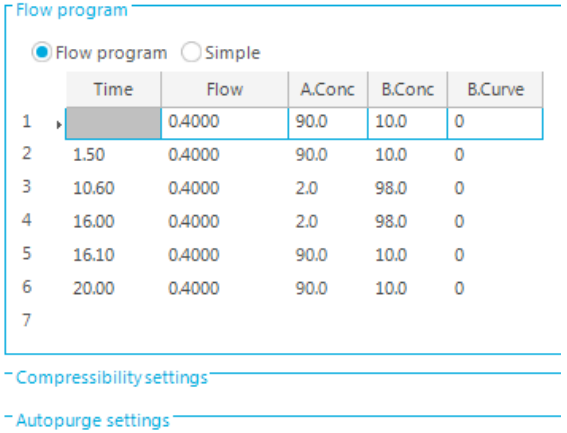


- Column: ACQUITY BEH C18 1.7µm 50 x 2.1 mm + pre-column

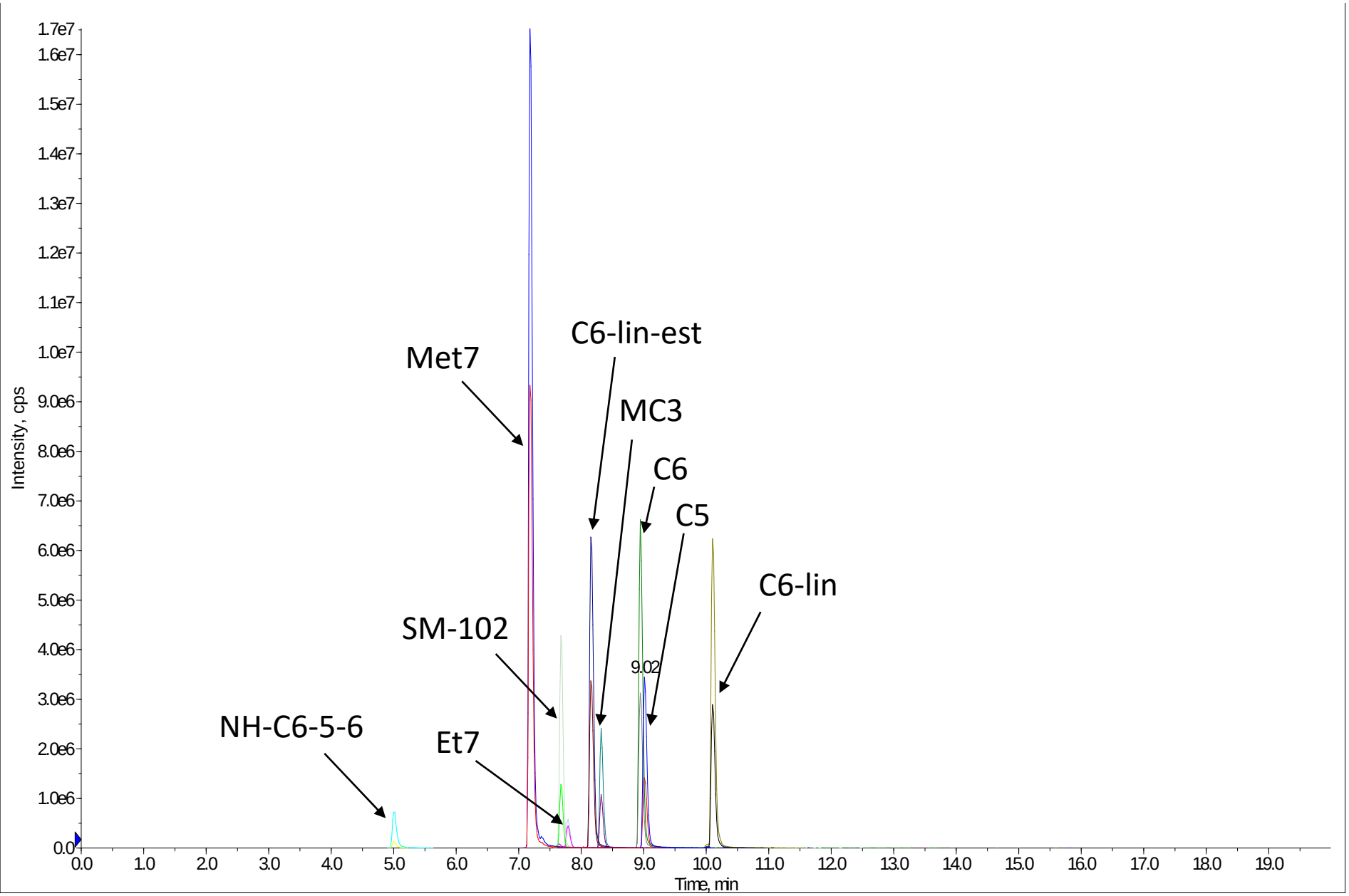
- MPA: ACN/H_2O (6:4) + 20 mM $HCOONH_4$ + 0.1% HCOOH
- MPB: IPA/MeOH/ACN (220:5:25) + 20 mM $HCOONH_4$ + 0.1% HCOOH

- Diluent: IPA/ACN (6:4) + 0.2% FA

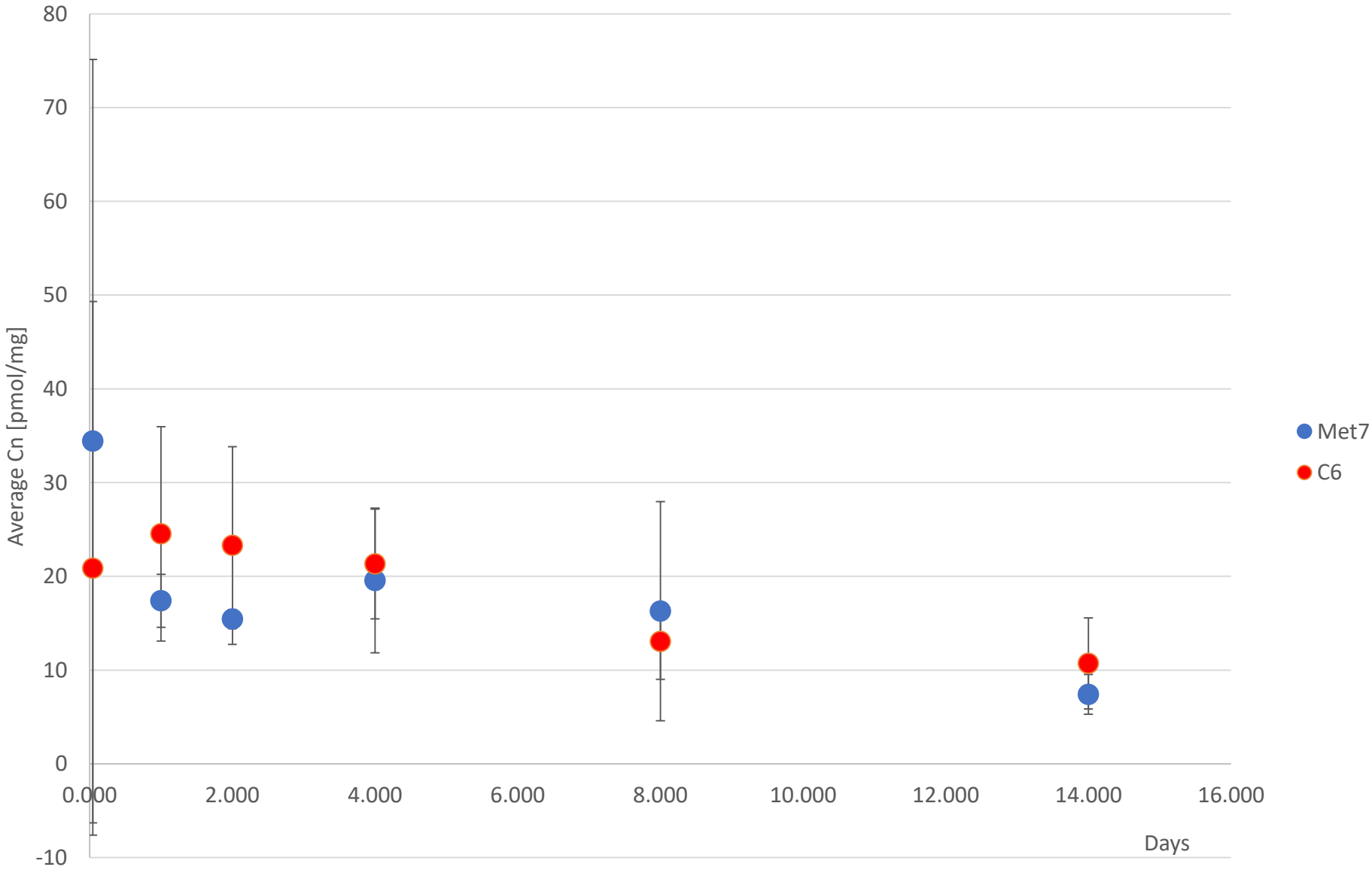




MIX 9: 100 nM


Intensity, cps
1.7e7
1.6e7
1.5e7
1.4e7
1.3e7
1.2e7
1.1e7
1.0e7
9.0e6
8.0e6
7.0e6
6.0e6
5.0e6
4.0e6
3.0e6
2.0e6
1.0e6
0.0
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
16.0
17.0
18.0
19.0
Time, min
Met7
C6-lin-est
MC3
C6
C5
C6-lin
SM-102
9.02
NH-C6-5-6
Et7

Determination of Met7 and C6 in mouse liver



Thank you for your attention !