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Development of a Stability-Indicating Reverse-phase HPLC Method for M1 Peptide Extracts

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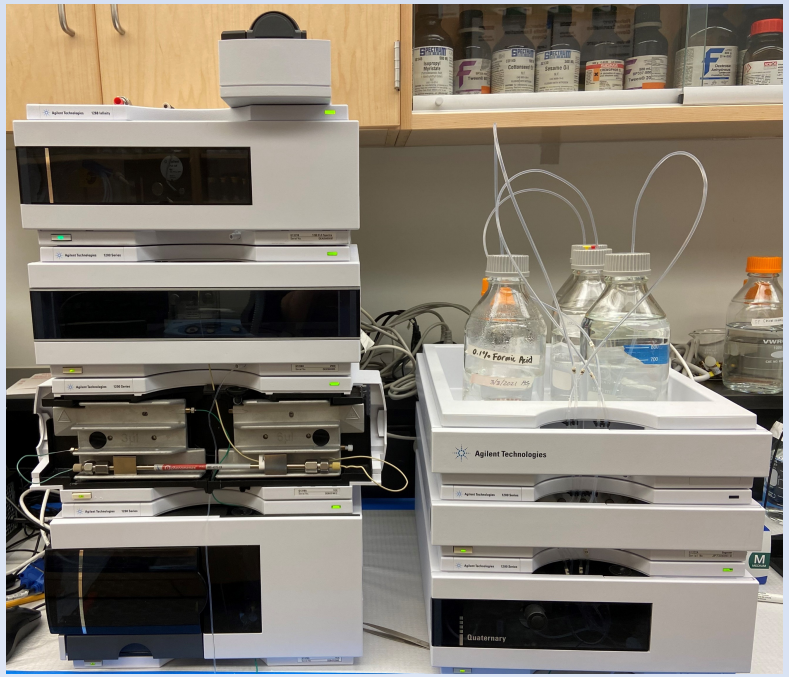
Introduction

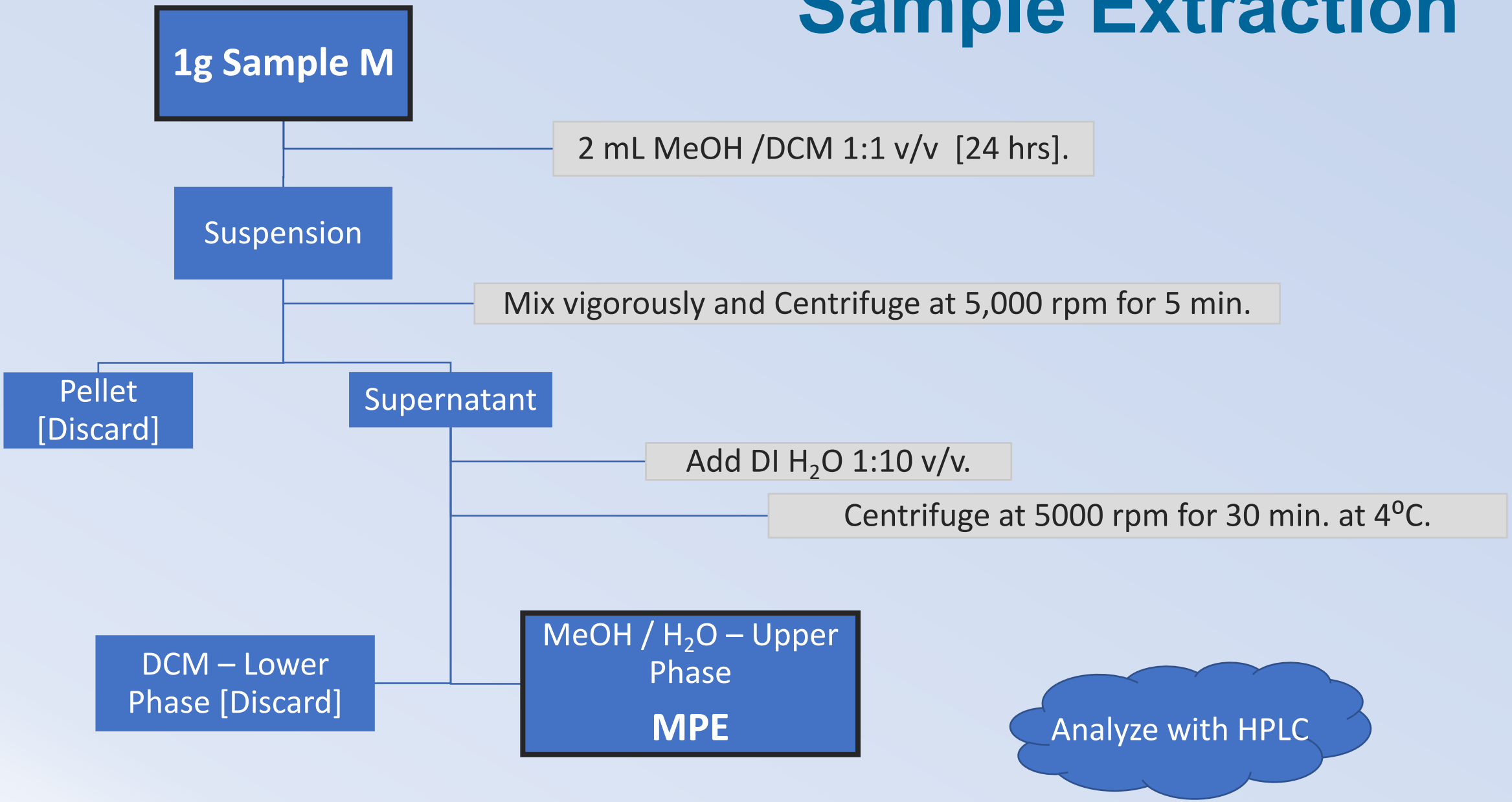
- Cosmonauts are exposed to, and experience radiation induced DNA damage during space travel.
- Tiwari (2019) found this type of DNA damage to be one of the most significant health hazards radiation can create.
    - Necessary to monitor DNA stability during and after travel.
- Recent novel nuclear matrix metalloprotease (nMMP) identification
    - In need of a reliable analysis method.
    - Helena (2018) discusses nMMP presence can be an indicator of both DNA damage and subsequent repair processes.

Introduction

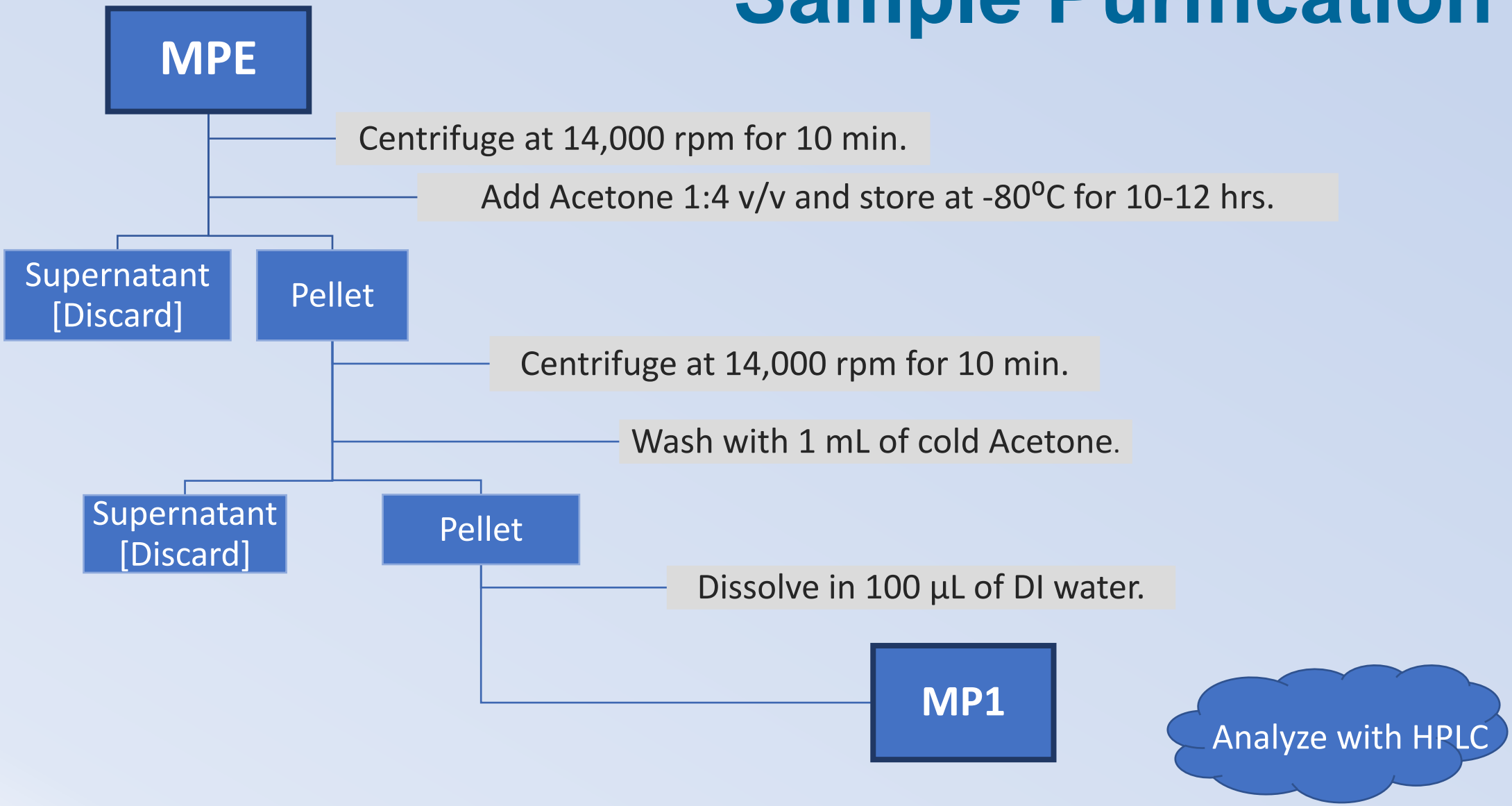


- We have undertaken development of a dependable High-Pressure Lipid Chromatography (HPLC) analyzation method.
  - Our preliminary results are outlined here.
- Only after an acceptable HPLC method is obtained and verified, then sample can be analyzed.
  - Then further research on physicochemical characterization and functional identification of sample M can be done.

Sample Extraction



Sample Purification



HPLC Method Development

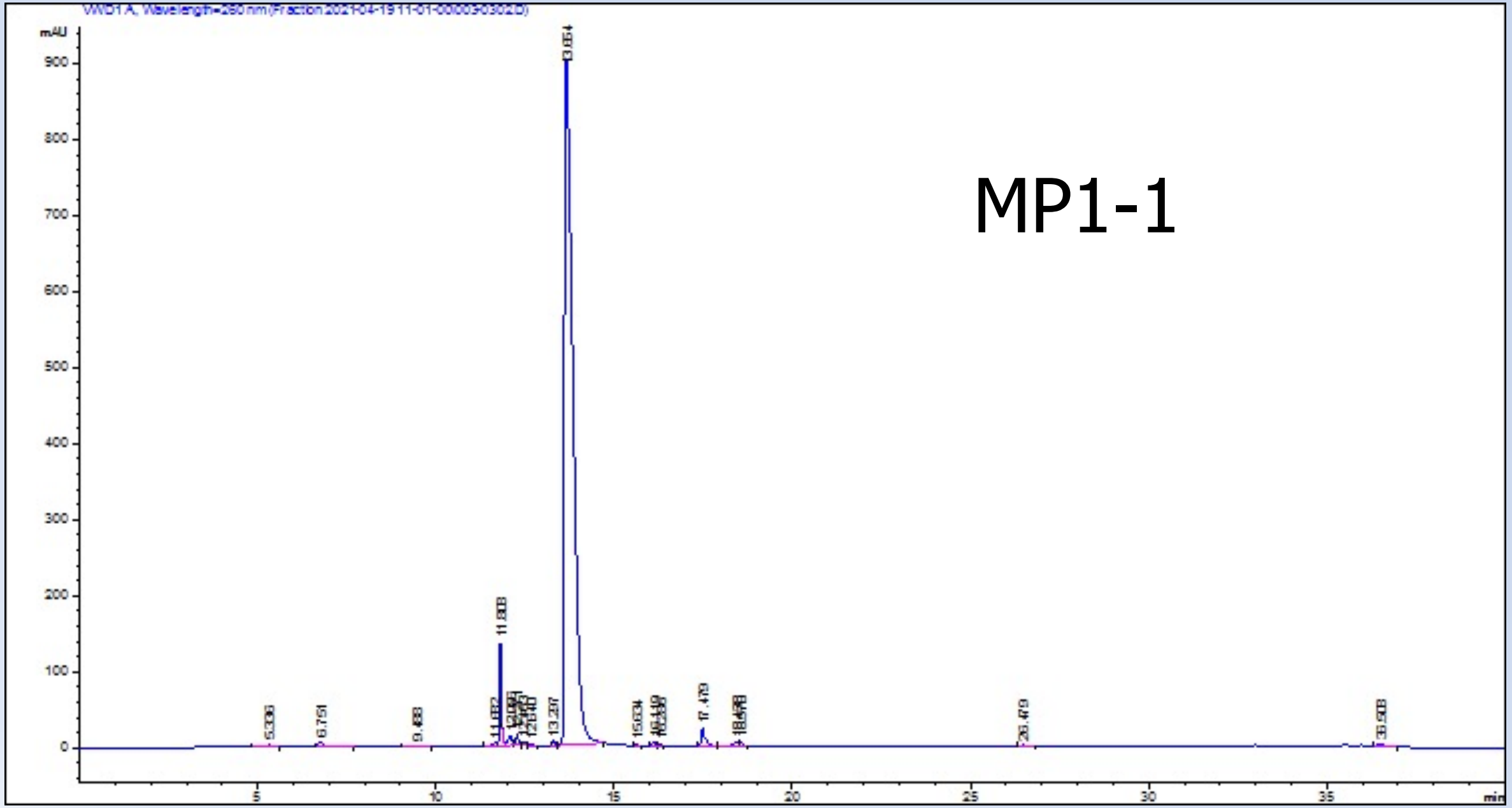
- Began with literature to get an idea of the HPLC method steps that would work for our sample.
- Gradient elution method as shown in Table 1 below.
- First analyze sample M at the crude state then at the purified state.
- Before any HPLC is ran the lines are first washed, then the column itself is washed.
- A blank sample (DI Water) is then injected at the beginning to equilibrate the system.

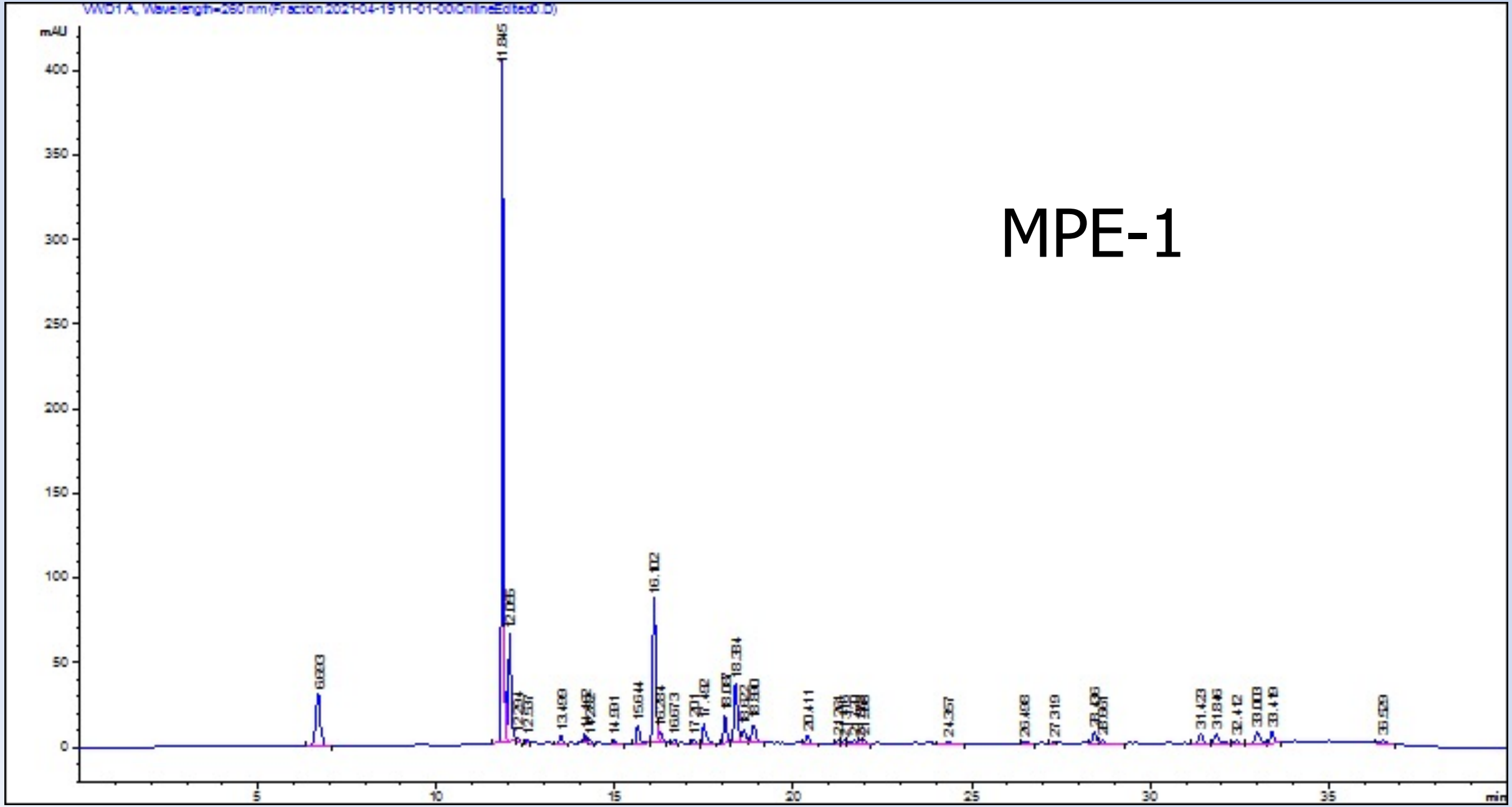
HPLC method details:

Flow rate: 0.5 mL/min
Injection volume: 10 µL
Detection: 260 nm
C18 Column
Each sample is run for 30 min. with a 10 min wash of 100% 0.1% formic acid to follow
Total run time: 40 min.

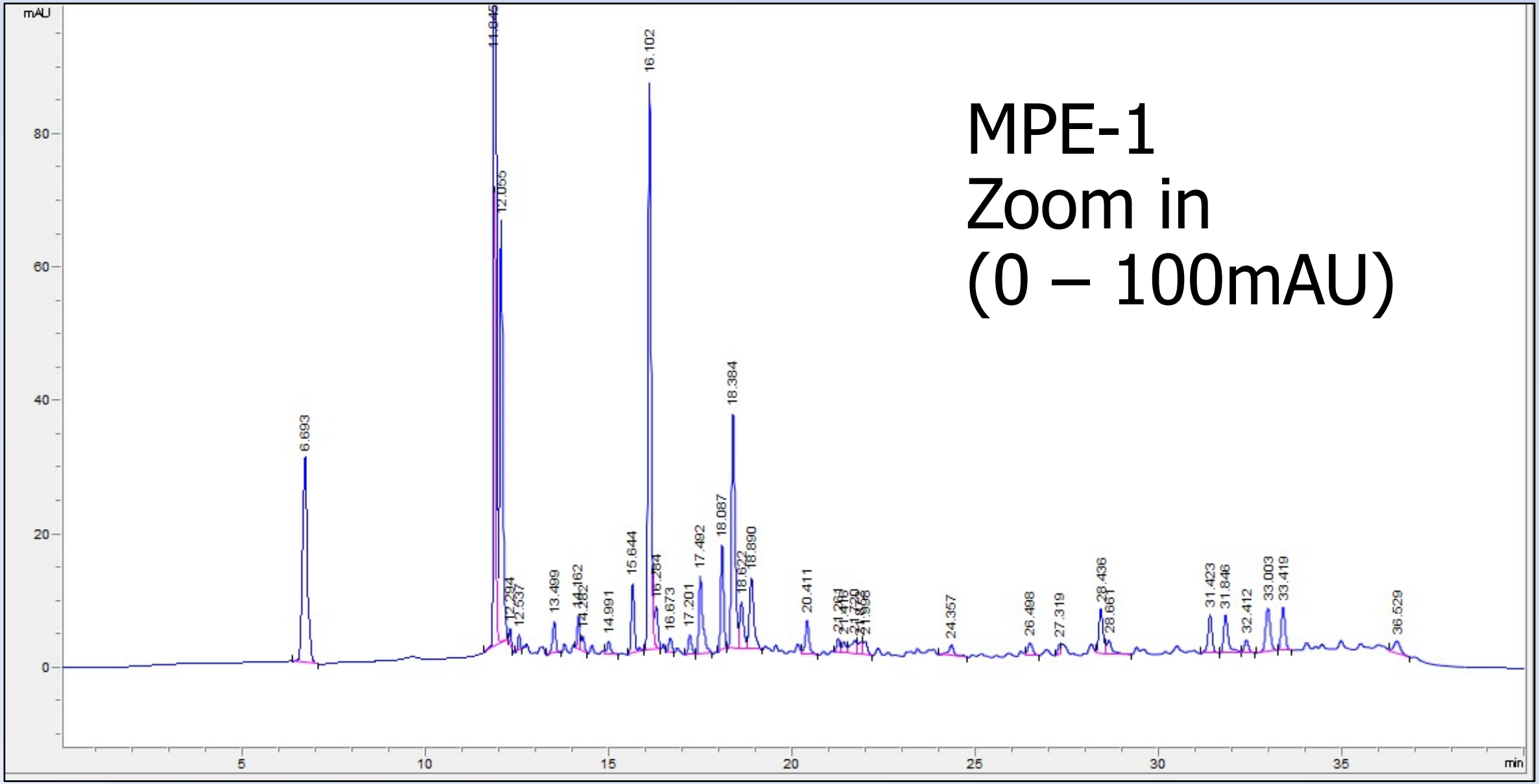
Table 1: Representative HPLC Method

Time (minutes)	0.1% Formic Acid (%)	Acetonitrile (%)
0	100	0
30	100	0
35	20	80
40	100	0


MP1-1
mAU
min


VWD1 A, Wavelength=280 nm (Fraction 2021-04-19 11-01-00\OnlineEdited0.D)
mAU
400
350
300
250
200
150
100
50
0
11.845
6.833
16.102
18.384
5
10
15
20
25
30
35
min

MPE-1


MPE-1
Zoom in
(0 – 100mAU)
mAU
min
6.693
11.945
12.055
12.294
12.537
13.499
14.162
14.991
15.644
16.102
16.284
16.673
17.201
17.492
18.087
18.384
18.522
18.890
20.411
24.357
26.498
27.319
28.436
28.661
31.423
31.846
32.412
33.003
33.419
36.529

Discussion

- Repeated HPLC analysis has shown similar elution profiles.
  - Indicate that sample M is relatively pure as there is only 1 major peak.
  - Indicates a lack of stability of the sample throughout the purification process.
- Analysis of sample after 1 week were also performed.
  - Samples saved at room temperature and $-20^{o}C$ and analyzed with HPLC, all showing similar elution profiles.

Future Studies

- After preliminary results of the HPLC method there are new questions to answer.
  - Mass spectrometry could provide further indication of degradation as well as knowing the mw of each peak would help in identification.
  - Introductory use of the HPLC to analyze the sample after a week of degradation are promising and the stability of the sample would also be a possible pathway of further study.

Acknowledgements

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References

1. Craik, D.J., Troeira Henriques, S., Mylne, J.S., Wang, C.K. (2012). Cyclotide Isolation and Characterization. *Methods in Enzymology, 156,* 37-62. https://doi.org/10.1016/B978-0-12-394291-3.00024-1
2. Helena, J., Margaretha, A., Grobbelaar, S., Magalena Nolte, E., Nel, M., Sean Pepper, M., Coetzee, M., and Mercier A.E. (2018). Deoxyribonucleic Acid Damage and Repair: Capitalizing on Our Understanding of the Mechanisms of Maintaining Genomic Integrity for Therapeutic Purposes. *International Journal of Molecular Sciences, 19,* 1148. 10.3390/ijms19041148.
3. Tiwari, V., Wilson III, D.M. (2019). DNA Damage and Associated DNA Repair Defects in Disease and Premature Aging. *The American Journal of Human Genetics, 105,* 237-257. https://doi.org/10.1016/j.ajhg.2019.06.005.