# Appendix

## 7.1 Chapter 3 – supporting information

### *7.1.1 Peptides and peptide-peptoid hybrids characterisation*

#### *7.1.1.1 P006 – VPTDVGPFAF-$NH_2$*

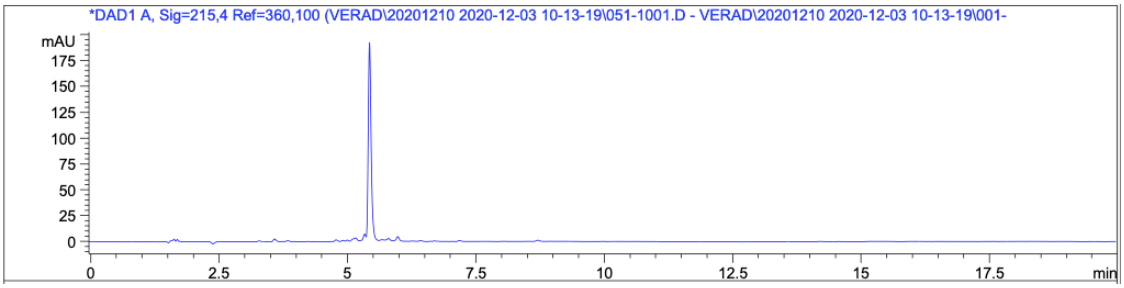



*Figure S 1. HPLC chromatogram (215 nm) of P006. Compound purity based on HPLC peak area analysis: 96.6%.*

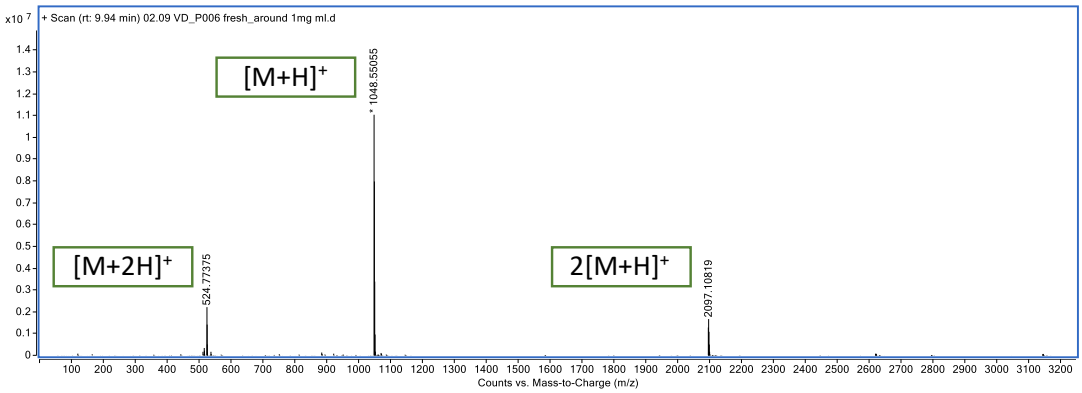



*Figure S 2. Accurate mass spectrum of P006.*

#### *7.1.1.2 LJMU011 – VPTDVGPFA-**4-F-Phe**-$NH_2$*

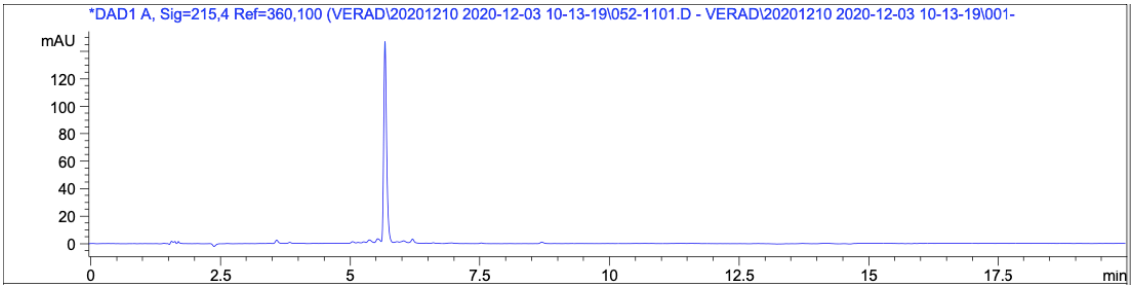



*Figure S 3. HPLC chromatogram (215 nm) of LJMU011.*

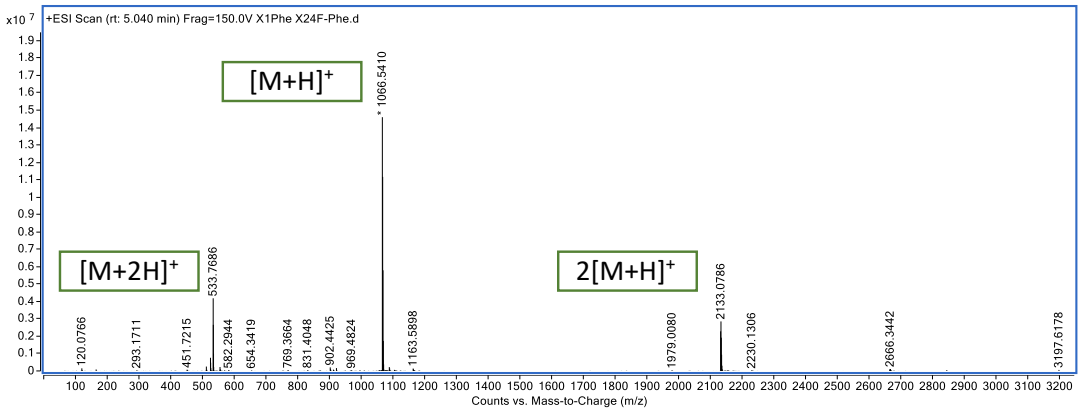



*Figure S 4. Accurate mass spectrum of LJMU011. Compound purity based on HPLC peak area analysis: 95%.*

### *7.1.1.3 LJMU012 – VPTDVGP-**4-F-Phe**-AF-$NH_2$*

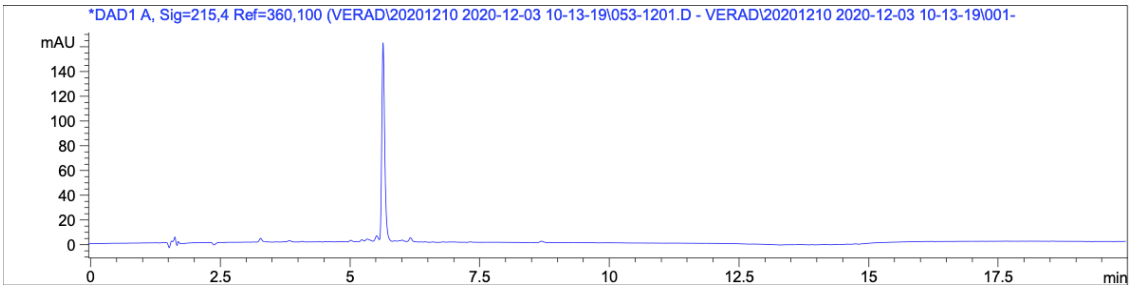



*Figure S 5. HPLC chromatogram (215 nm) of LJMU012. Compound purity based on HPLC peak area analysis: 94.3%.*

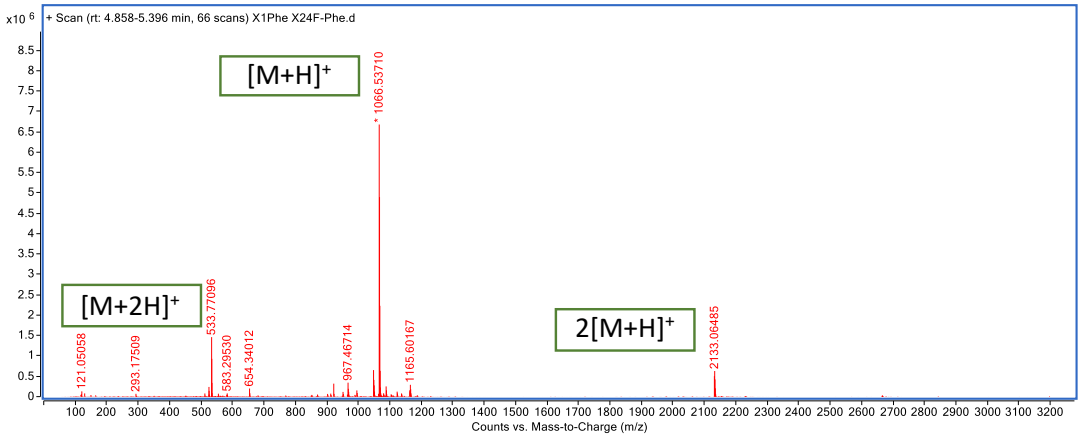



*Figure S 6. Accurate mass spectrum of LJMU012.*

### 7.1.1.4 LJMU013 – VPTDVGP-**4-F-Phe**-A-**4-F-Phe**-$NH_2$

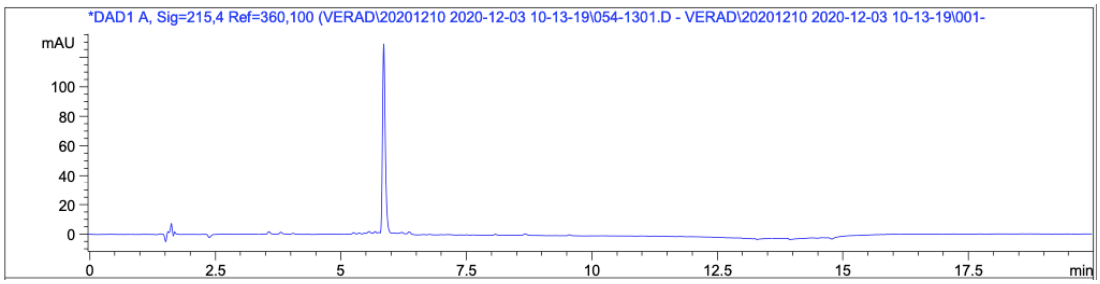



*Figure S 7. HPLC chromatogram (215 nm) of LJMU013. Compound purity based on HPLC peak area analysis: 98%*

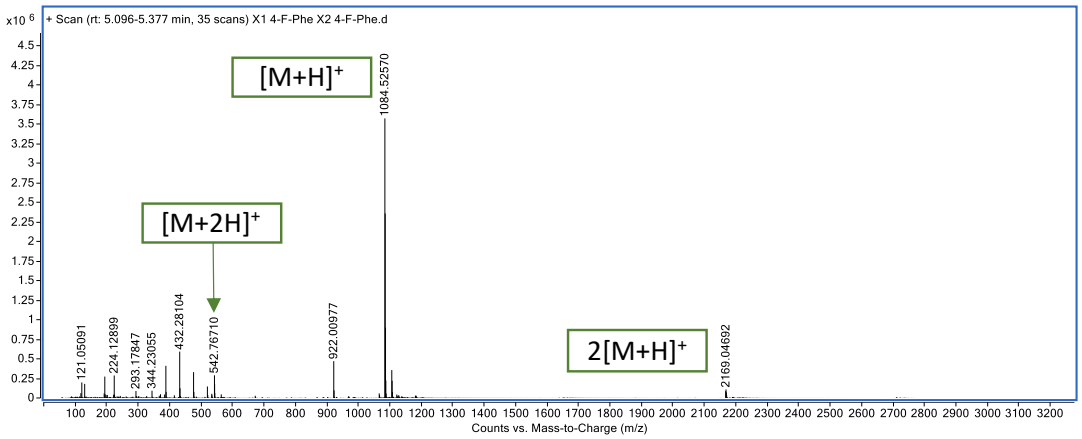



*Figure S 8. Accurate mass spectrum of LJMU013.*

### 7.1.1.5 LJMU014 – VPTDVGPFA-**$F_5$-Phe**-$NH_2$

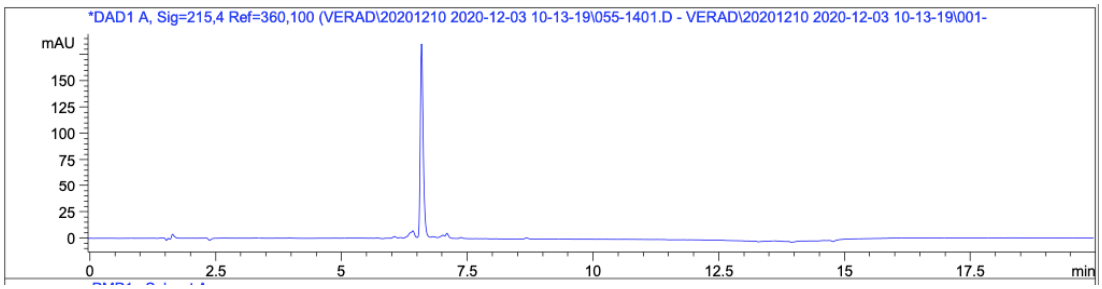



*Figure S 9. HPLC chromatogram (215 nm) of LJMU014. Compound purity based on HPLC peak area analysis: 90.3%*

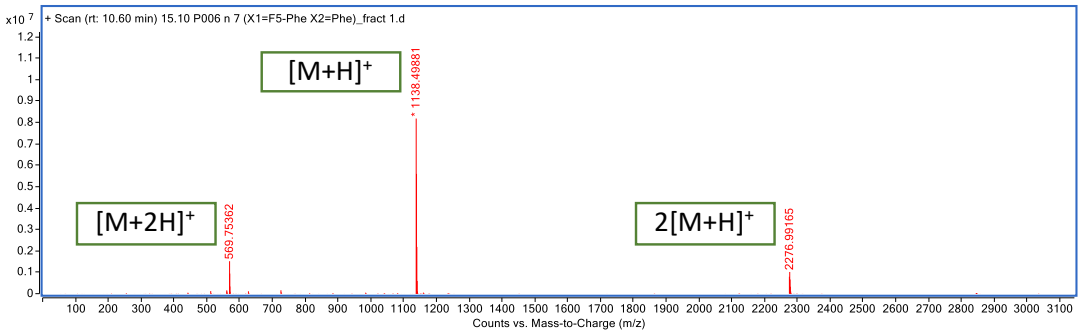



*Figure S 10. Accurate mass spectrum of LJMU014.*

### *7.1.1.6 LJMU015 – VPTDVGP-**F5-Phe**-AF-NH2*

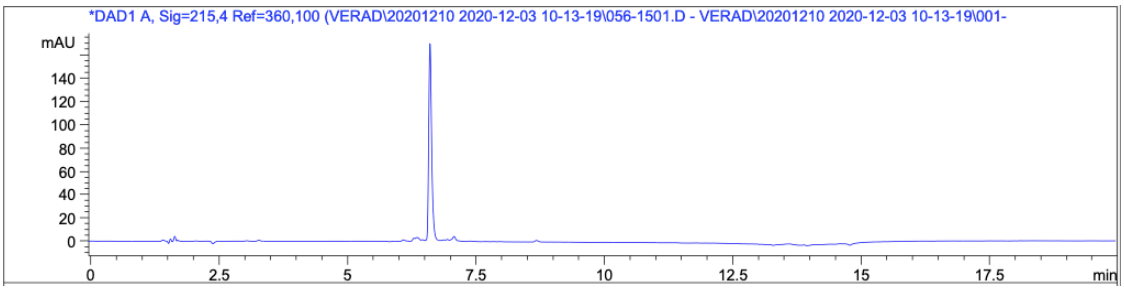



*Figure S 11. HPLC chromatogram (215 nm) of LJMU015. Compound purity based on HPLC peak area analysis: 92.3%*

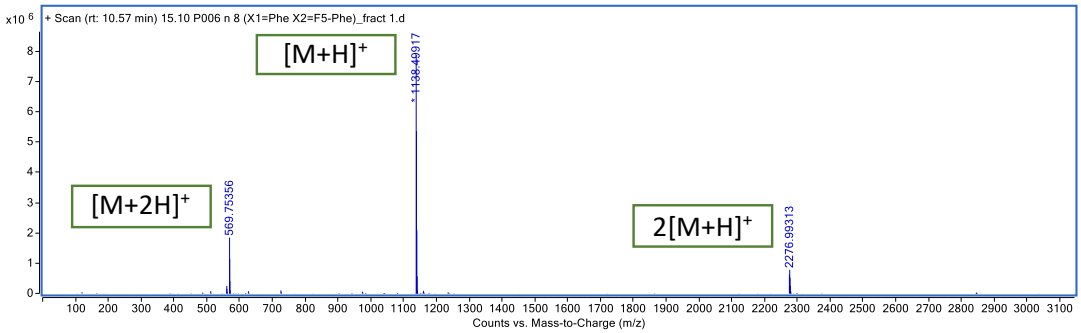



*Figure S 12. Accurate mass spectrum of LJMU015.*

### 7.1.1.7 LJMU016 – VPTDVGP-**$F_5$-Phe**-A-**$F_5$-Phe**-$NH_2$

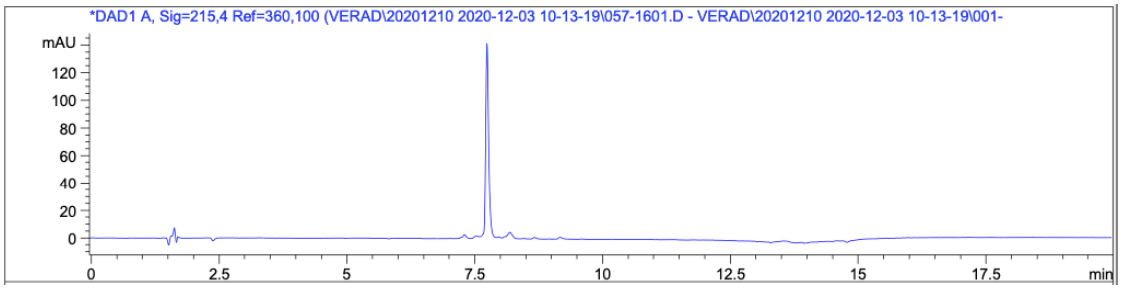



*Figure S 13. HPLC chromatogram (215 nm) of LJMU016. Compound purity based on HPLC peak area analysis: 90%*

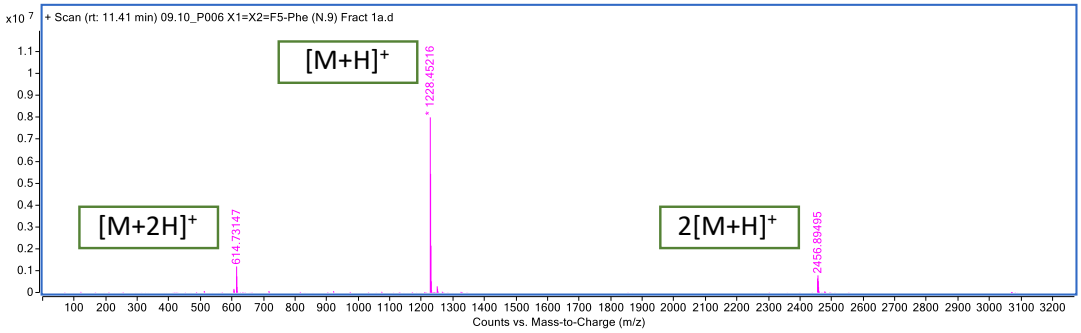



*Figure S 14. Accurate mass spectrum of LJMU016.*

### 7.1.1.8 LJMU017 – **4-F-benzoyl**-VPTDVGPFAF-$NH_2$

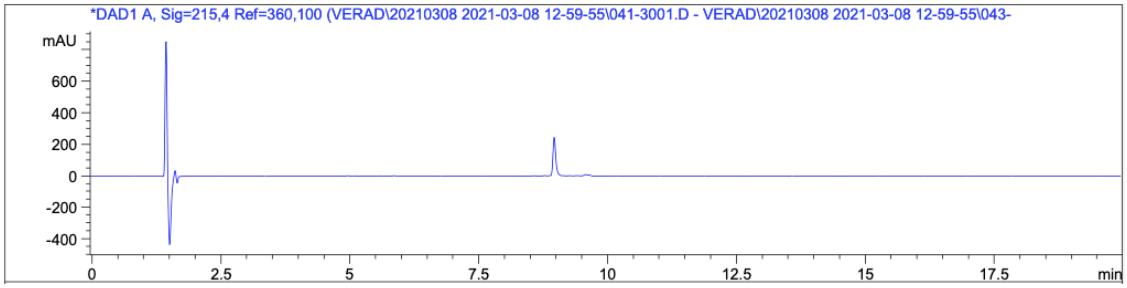



*Figure S 15. HPLC chromatogram (215 nm) of LJMU017. Compound purity based on HPLC peak area analysis: 92.6%*

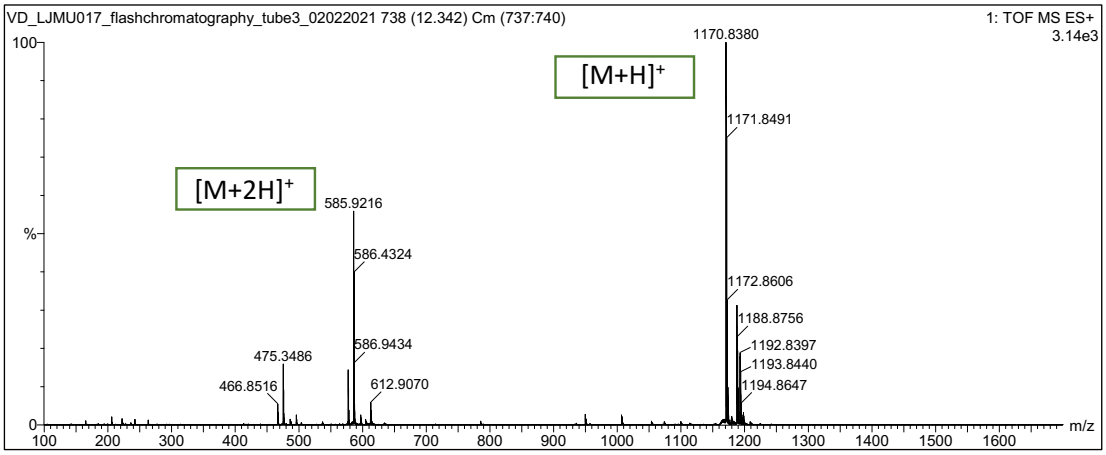



*Figure S 16. MS spectrum of LJMU017.*

### *7.1.1.9 LJMU018 – $F_5$-benzoyl-VPTDVGPFAF-$NH_2$*

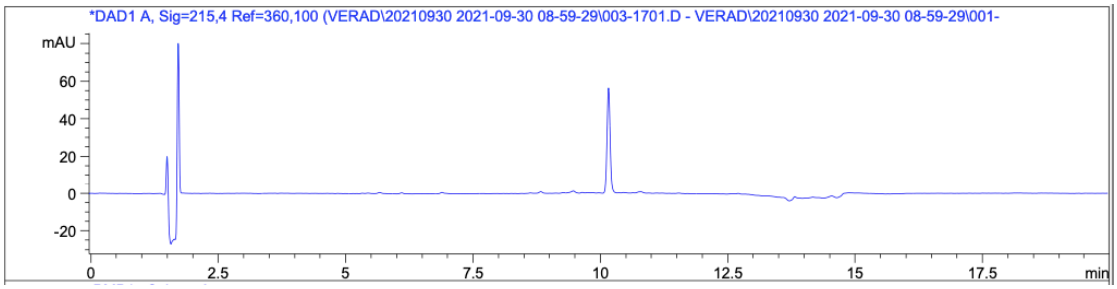



*Figure S 17. HPLC chromatogram (215 nm) of LJMU018. Compound purity based on HPLC peak area analysis: 99.5%.*

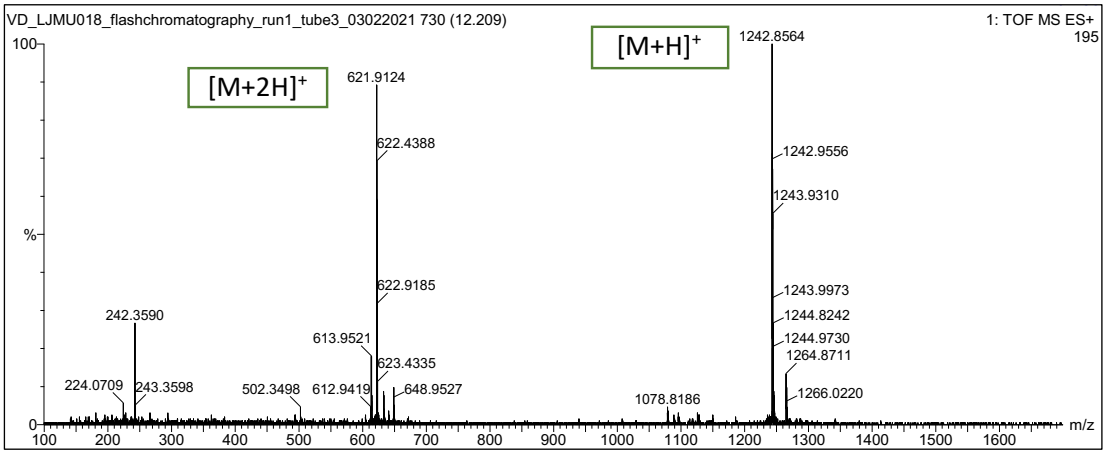



*Figure S 18. MS spectrum LJMU018.*

### 7.1.1.10 LJMU019 – VPTDVGPFA-**NPhe**-$NH_2$

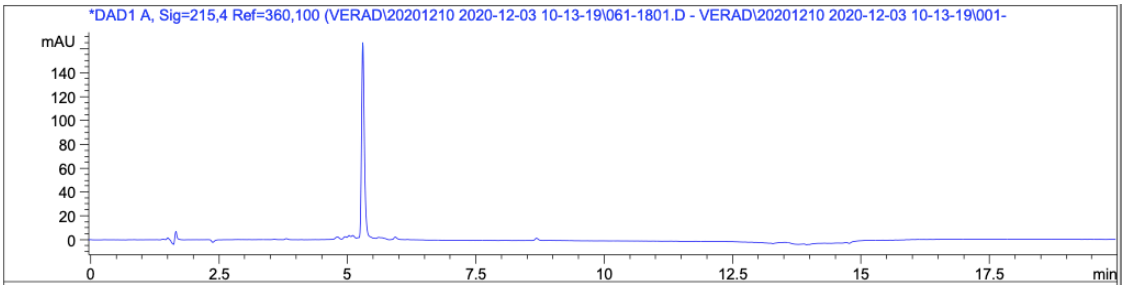



*Figure S 19. HPLC chromatogram (215 nm) of LJMU019. Compound purity based on HPLC peak area analysis: 91.5%.*

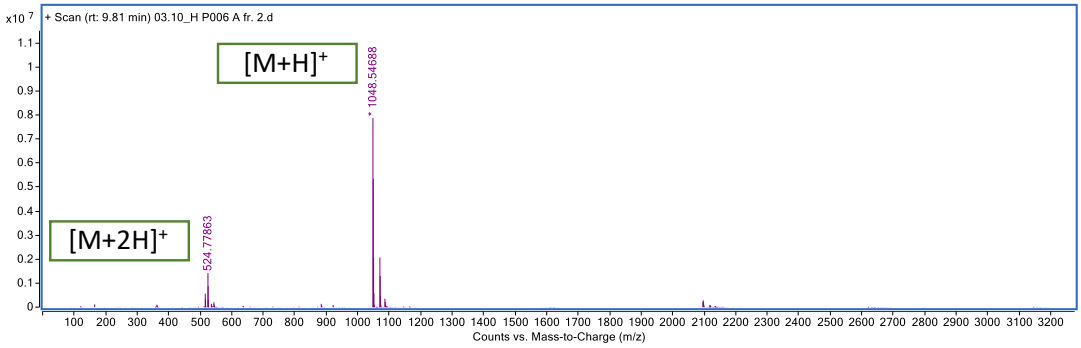



*Figure S 20. Accurate mass spectrum of LJMU019.*

### 7.1.1.11 LJMU020 – VPTDVGPF-**NAla**-F-$NH_2$

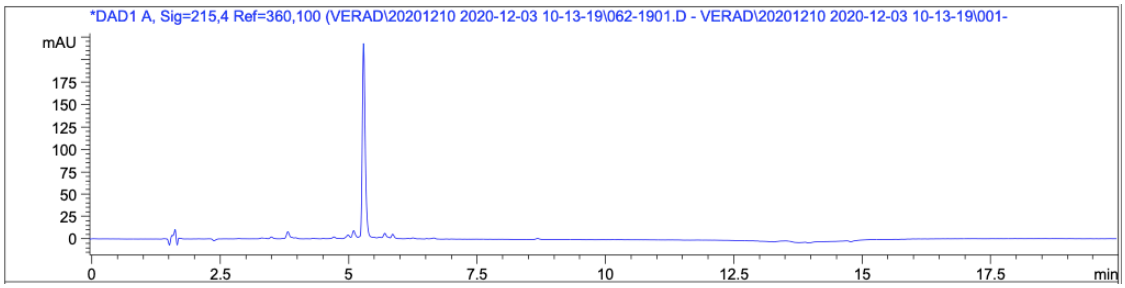



*Figure S 21. HPLC chromatogram (215 nm) of LJMU020. Compound purity based on HPLC peak area analysis: 87.5%.*

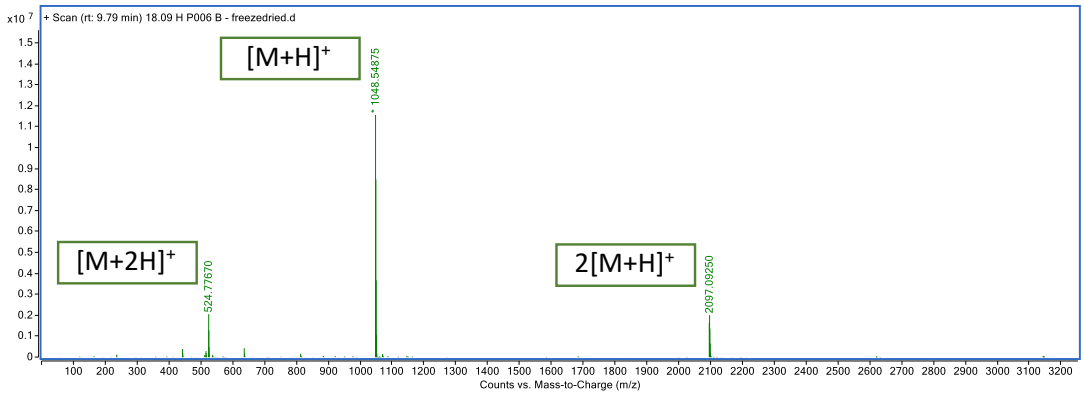



*Figure S 22. Accurate mass spectrum of LJMU020.*

### *7.1.1.12 LJMU021 – VPTDVGP-**NPhe**-AF-$NH_2$*

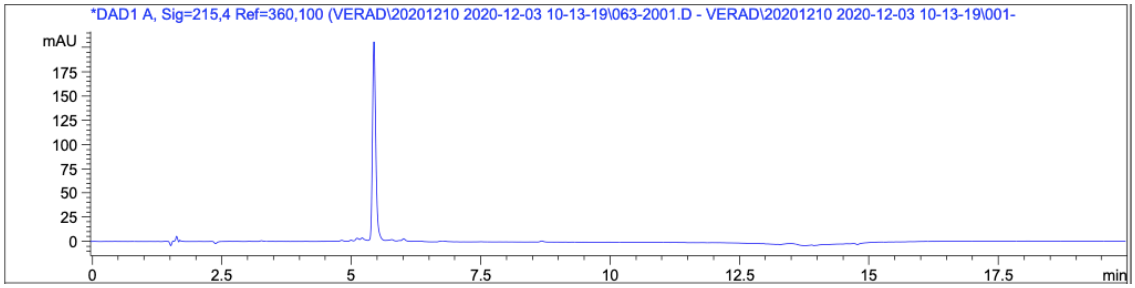



*Figure S 23. HPLC chromatogram (215 nm) of LJMU021. Compound purity based on HPLC peak area analysis: 97.5%.*

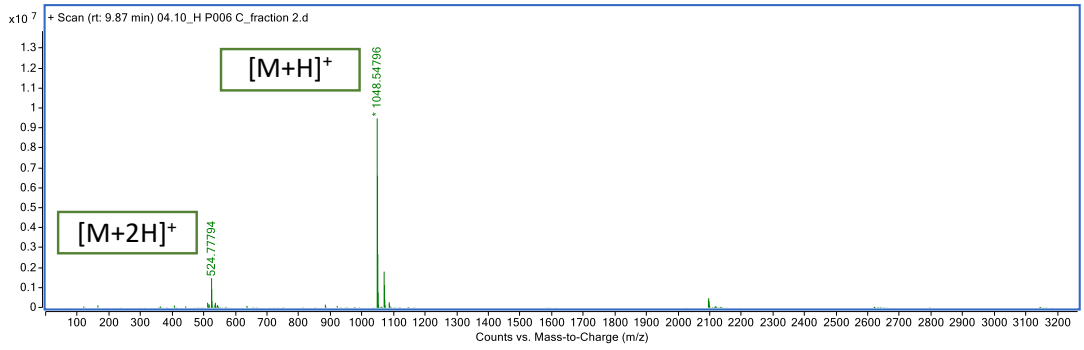



*Figure S 24. Accurate mass spectrum of LJMU021.*

### 7.1.1.13 LJMU022 – VPTD-**NVal**-GPFAF-$NH_2$

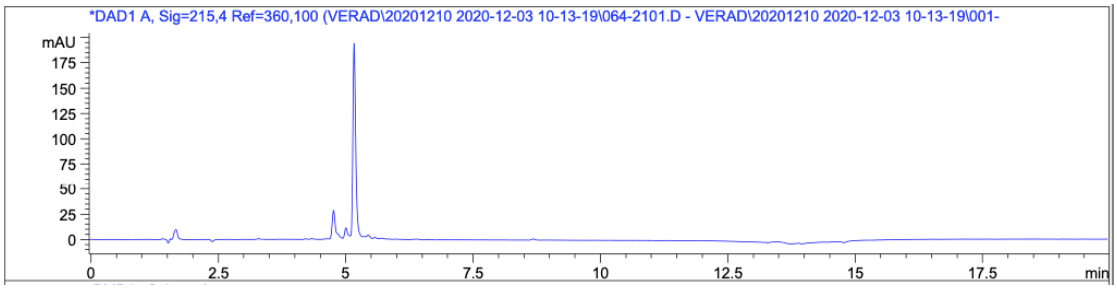



*Figure S 25. HPLC chromatogram (215 nm) of LJMU022. Compound purity based on HPLC peak area analysis: 99.7%. Minor peak attributed to VPTDGPFAF-$NH_2$ due to deletion of NVal.*

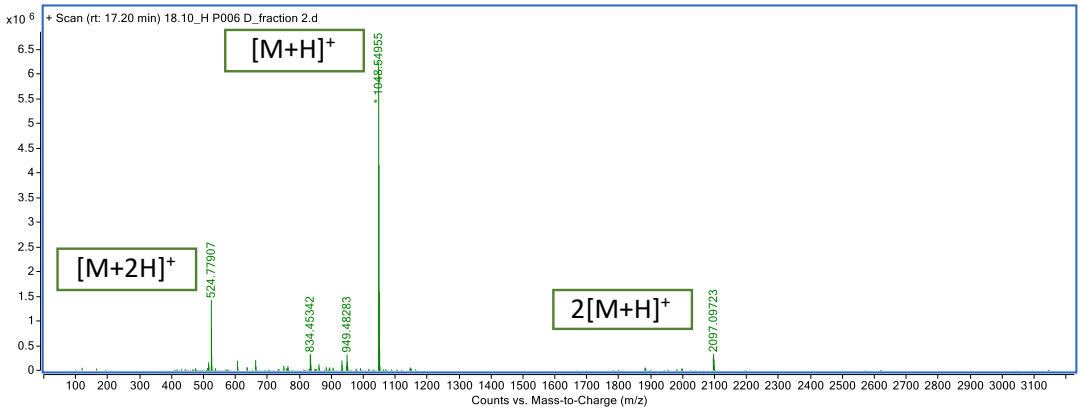



*Figure S 26. Accurate mass spectrum of LJMU022.*

### 7.1.1.14 LJMU023 – VPT-**NAsp**-VGPFAF-$NH_2$

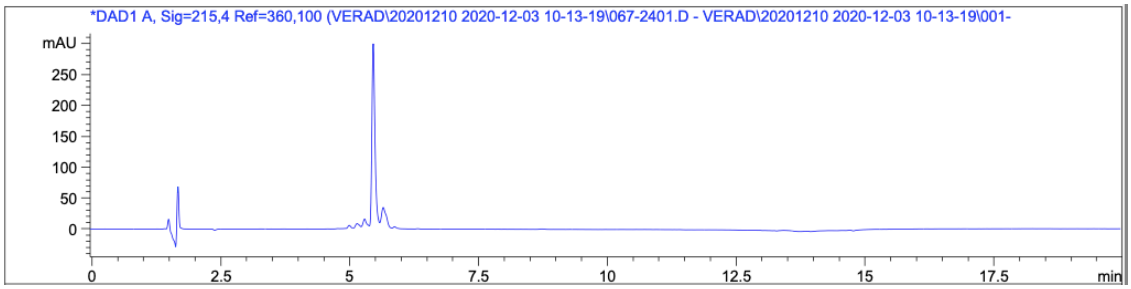



*Figure S 27. HPLC chromatogram (215 nm) of LJMU023. Compound purity based on HPLC peak area analysis: 77%*

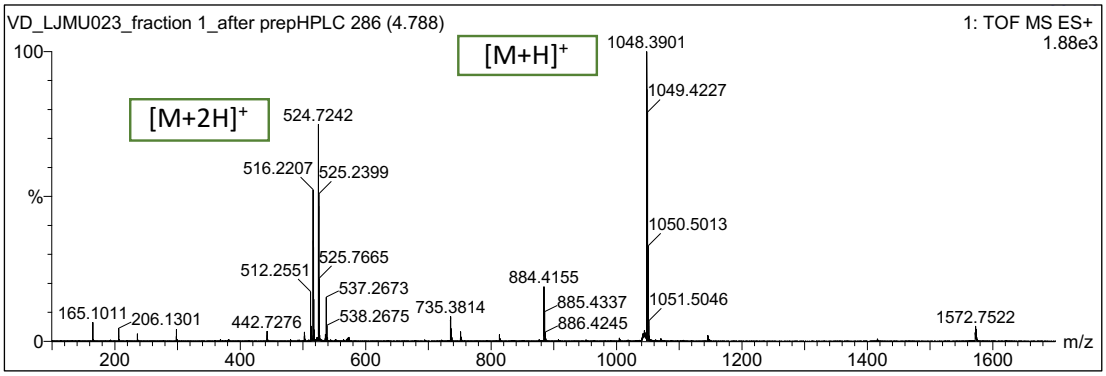



*Figure S 28. MS spectrum of LJMU023.*

### *7.1.1.15 LJMU024 – **NVal**-PTDVGPFAF-$NH_2$*

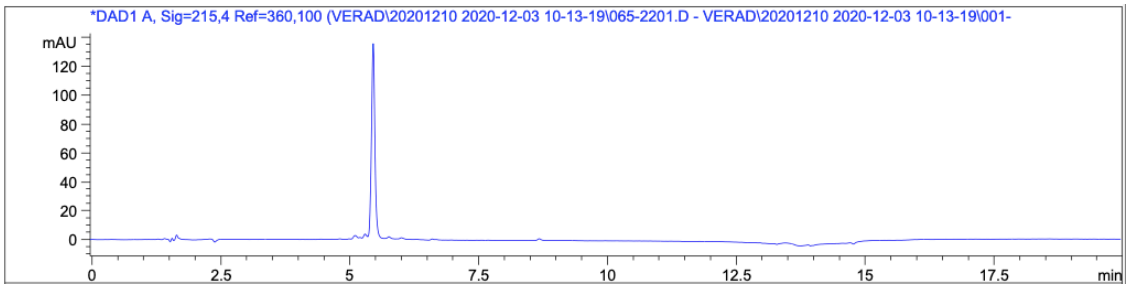



*Figure S 29. HPLC chromatogram (215 nm) of LJMU024. Compound purity based on HPLC peak area analysis: 95.4%.*

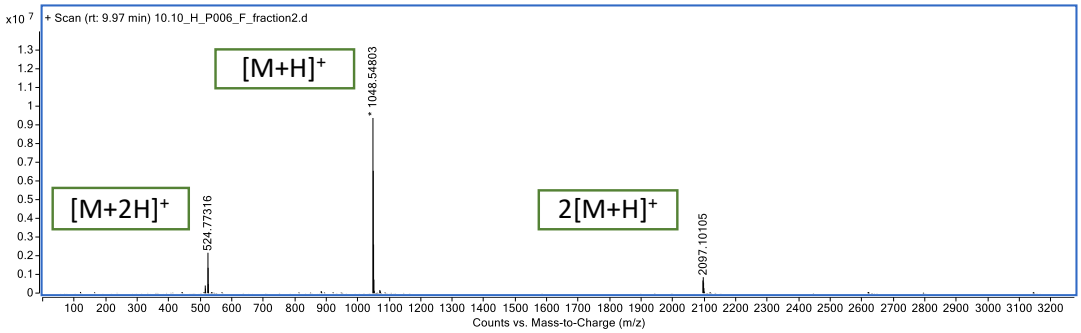



*Figure S 30. Accurate mass spectrum of LJMU024.*

### 7.1.1.16 LJMU025 – TDVGPFAF-$NH_2$

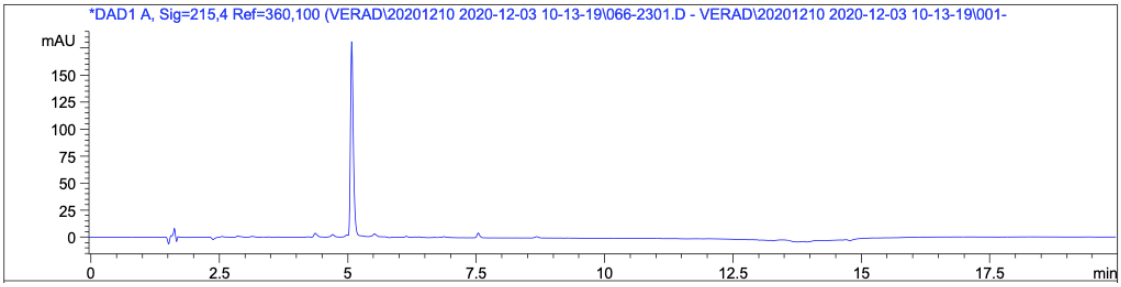



*Figure S 31. HPLC chromatogram (215 nm) of LJMU025. Compound purity based on HPLC peak area analysis: 96%.*

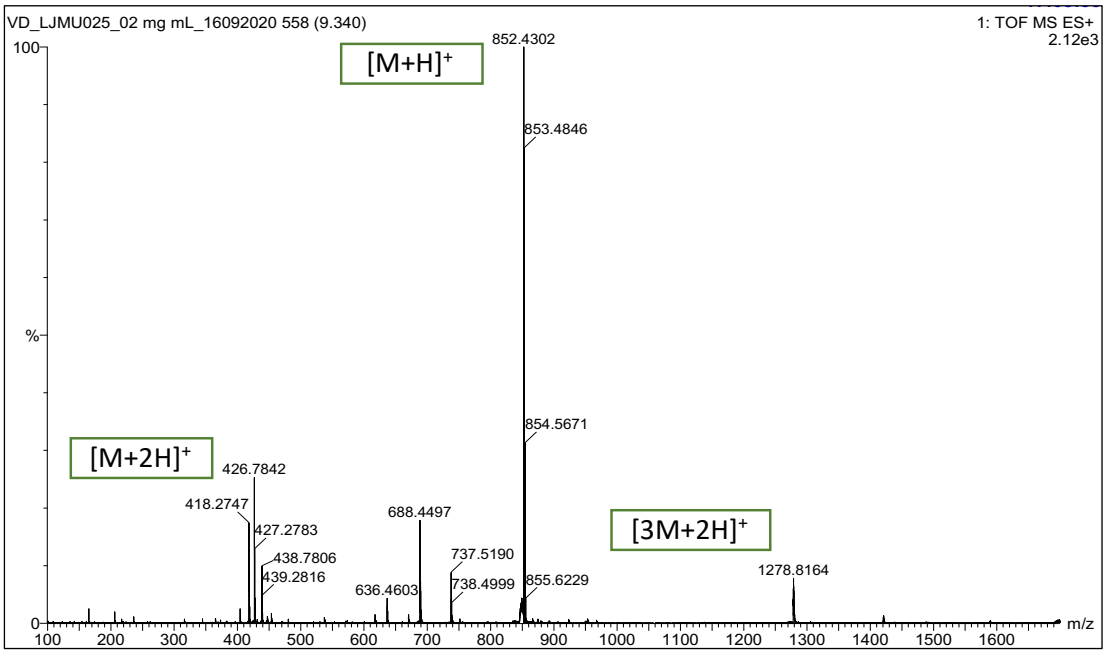



*Figure S 32. MS spectrum of LJMU025.*

### 7.1.1.17 LJMU026 – VP**S**DVGPFAF-$NH_2$

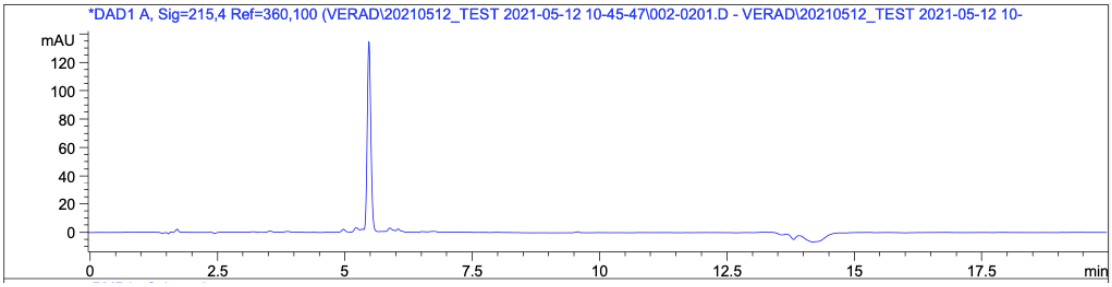



*Figure S 33. HPLC chromatogram (215 nm) of LJMU026. Compound purity based on HPLC peak area analysis: 91.7%.*

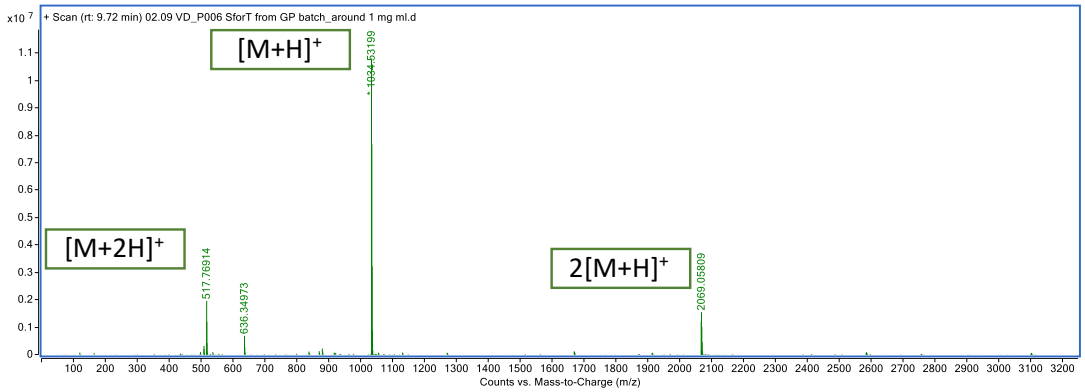



*Figure S 34. Accurate mass spectrum of LJMU026.*

### *7.1.1.18 LJMU027 – **Benzoyl**-VPTDVGPFAF-$NH_2$*

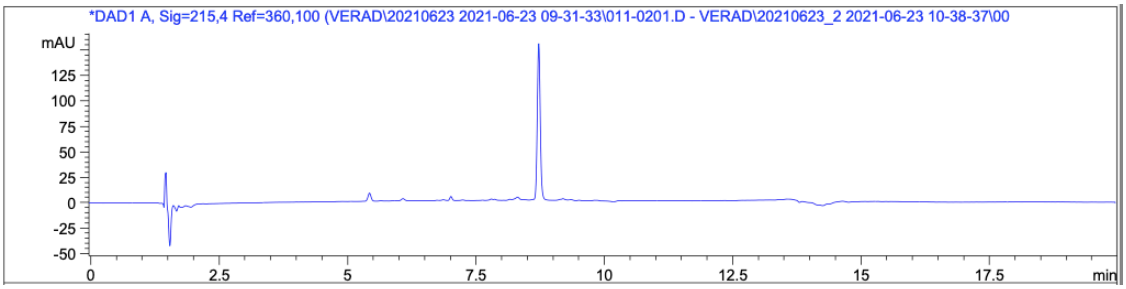



*Figure S 35. HPLC chromatogram (215 nm) of LJMU027. Compound purity based on HPLC peak area analysis: 88.9%.*

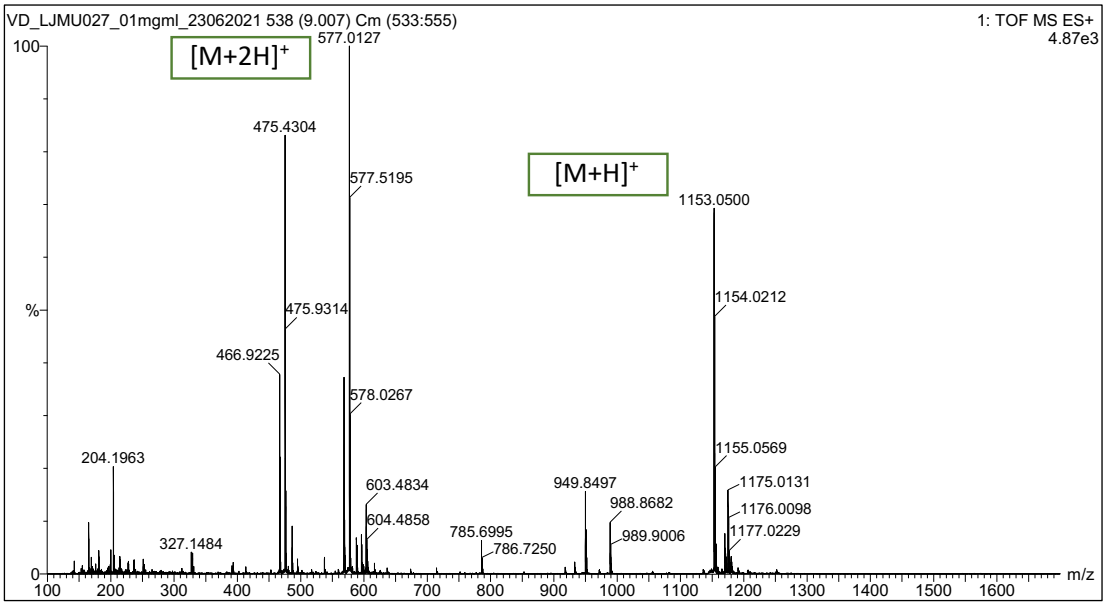



*Figure S 36. MS spectrum of LJMU027.*

### *7.1.1.19 CGRP 8-37 – VTHRLAGLLSRSGGVVKNNFVPTNVGSKAF-$NH_2$*

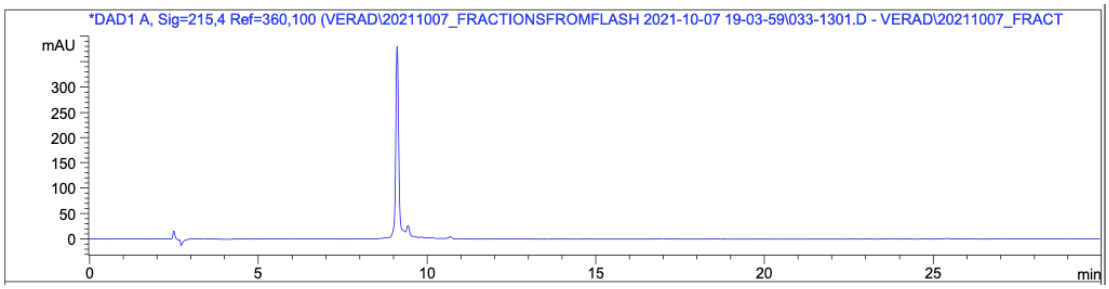



*Figure S 37. HPLC chromatogram (215 nm) of CGRP 8-37. Compound purity based on HPLC peak area analysis: 88.5%.*

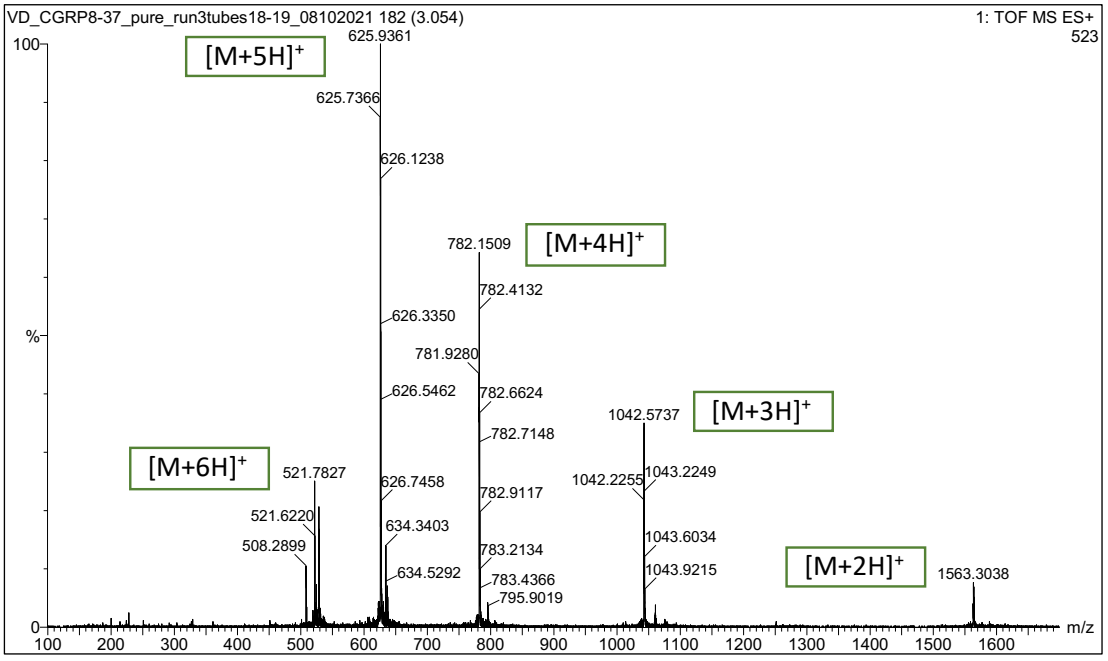



*Figure S 38. MS spectrum of CGRP 8-37.*

### 7.1.2 Experimental LogD (shake-flask method)

#### 7.1.2.1 Calibration curves in PBS aq. solution saturated with 1-octanol

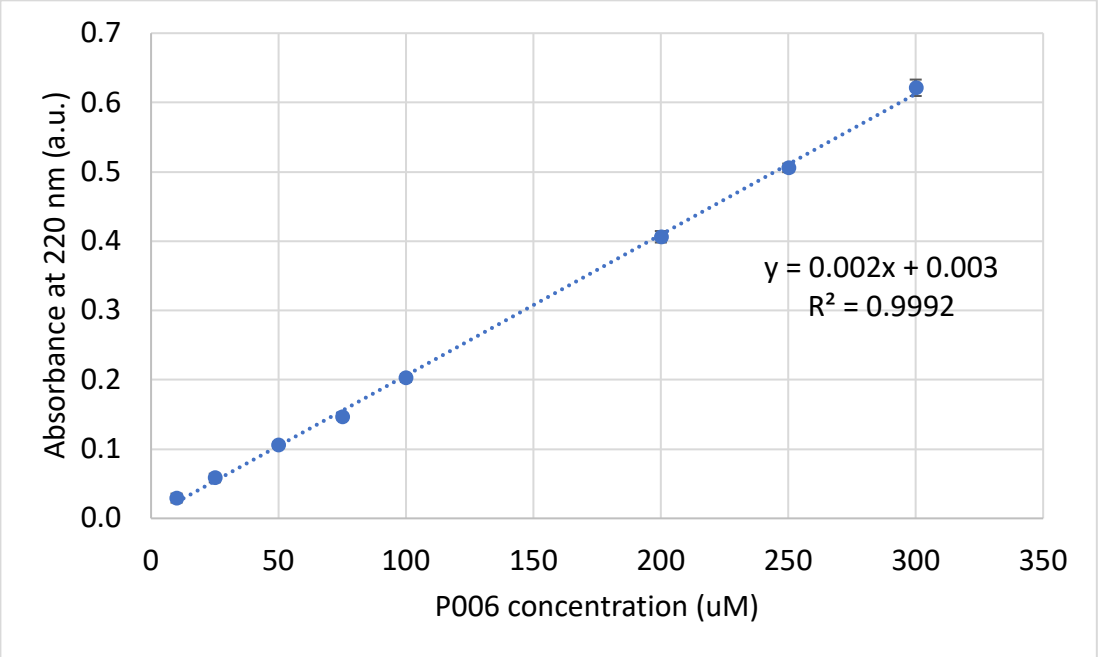



*Figure S 39. P006 calibration curve in aq. PBS solution saturated with 1-octanol. Absorbance detected at 220 nm. Experiment performed in duplicate.*

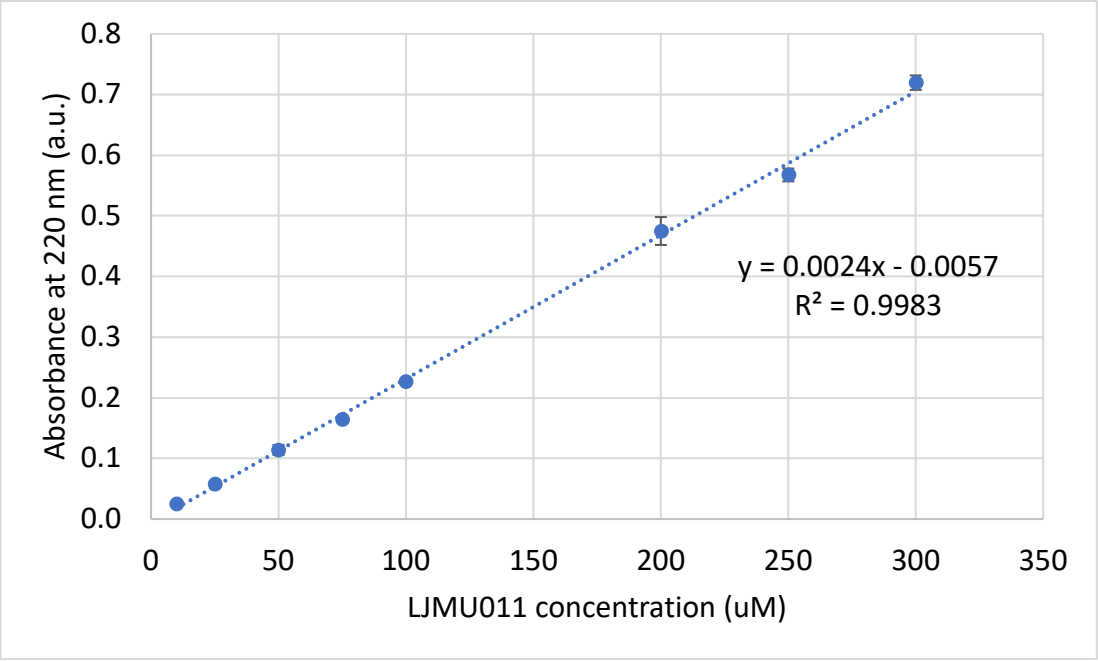



*Figure S 40. LJMU011 calibration curve in aq. PBS solution saturated with 1-octanol. Absorbance detected at 220 nm. Experiment performed in duplicate.*

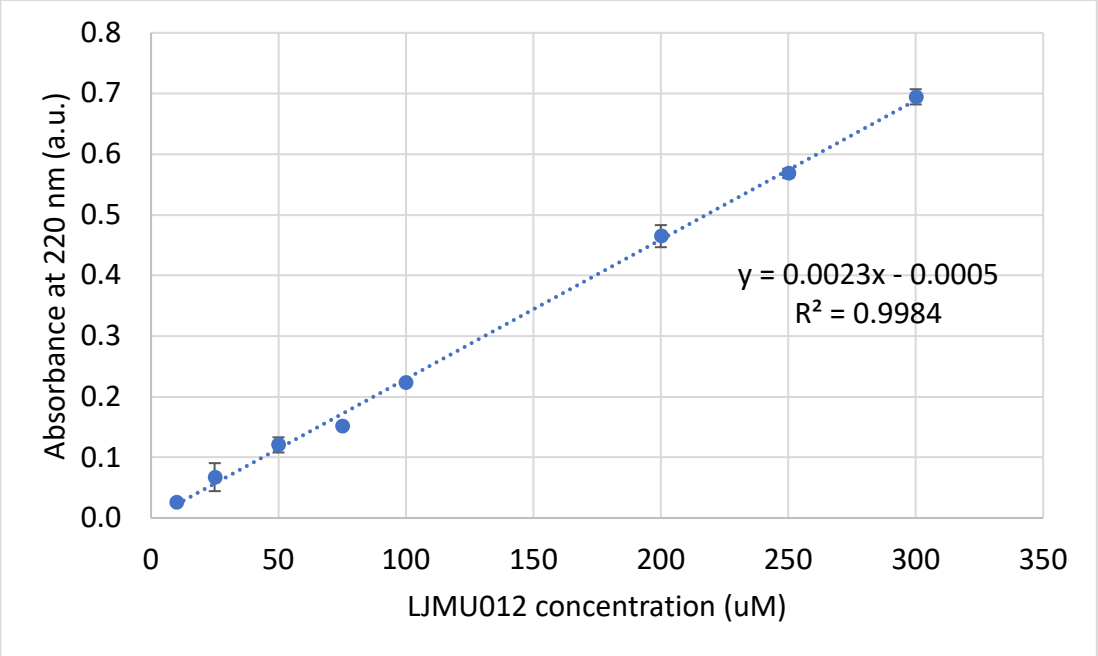



*Figure S 41. LJMU012 calibration curve in aq. PBS solution saturated with 1-octanol. Absorbance detected at 220 nm. Experiment performed in duplicate.*

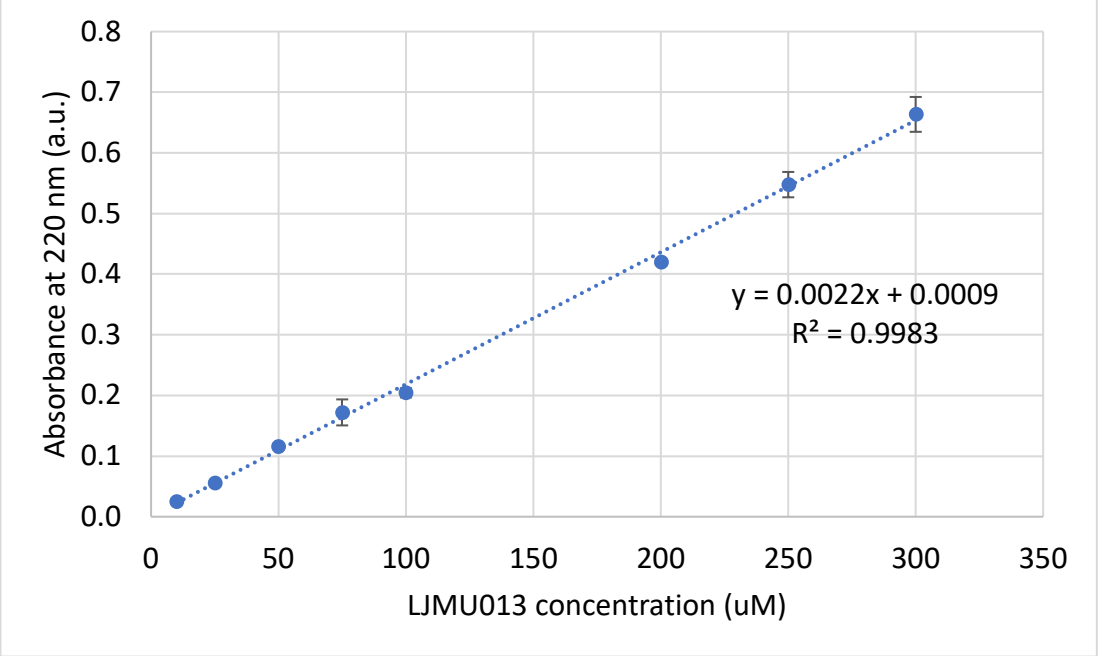



*Figure S 42. LJMU013 calibration curve in aq. PBS solution saturated with 1-octanol. Absorbance detected at 220 nm. Experiment performed in duplicate.*

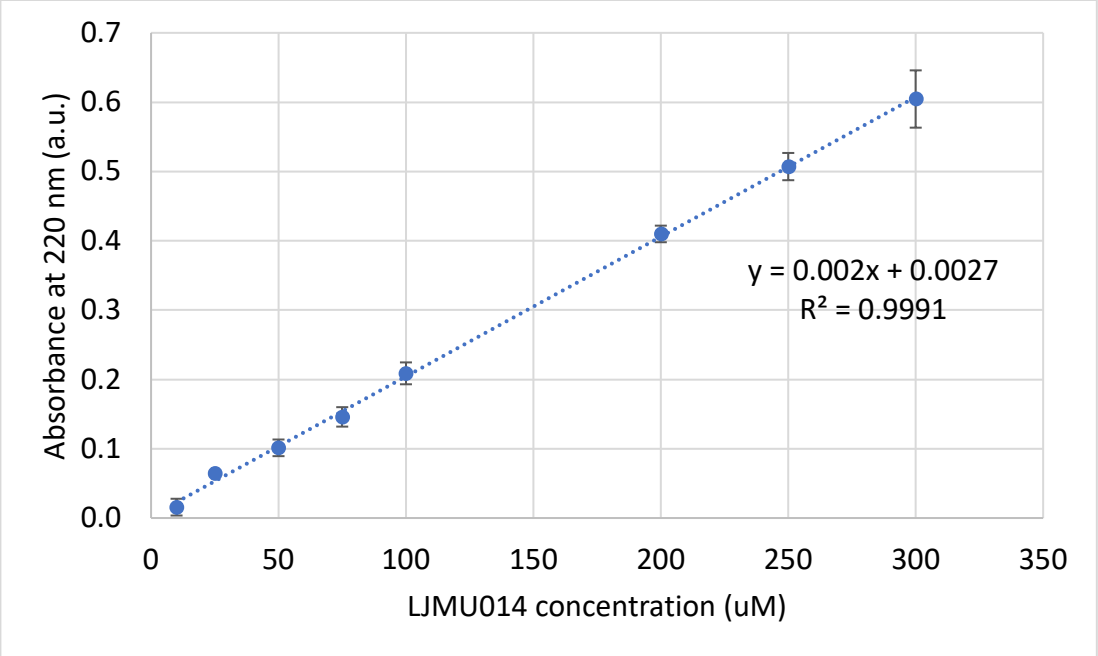



*Figure S 43. LJMU014 calibration curve in aq. PBS solution saturated with 1-octanol. Absorbance detected at 220 nm. Experiment performed in duplicate.*

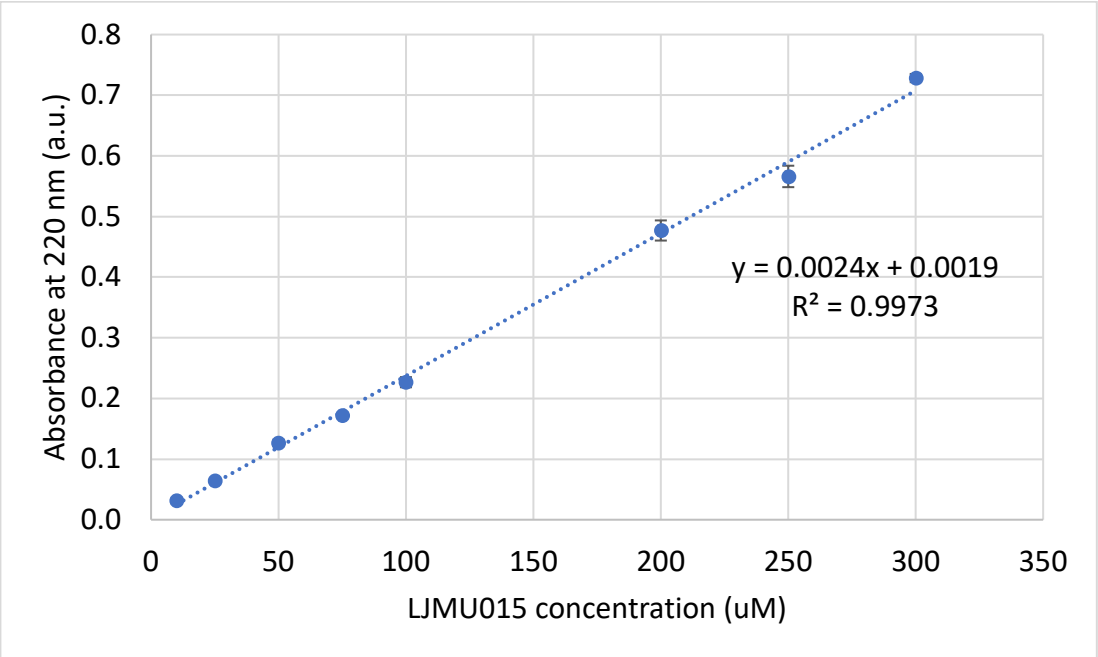



*Figure S 44. LJMU015 calibration curve in aq. PBS solution saturated with 1-octanol. Absorbance detected at 220 nm. Experiment performed in duplicate.*

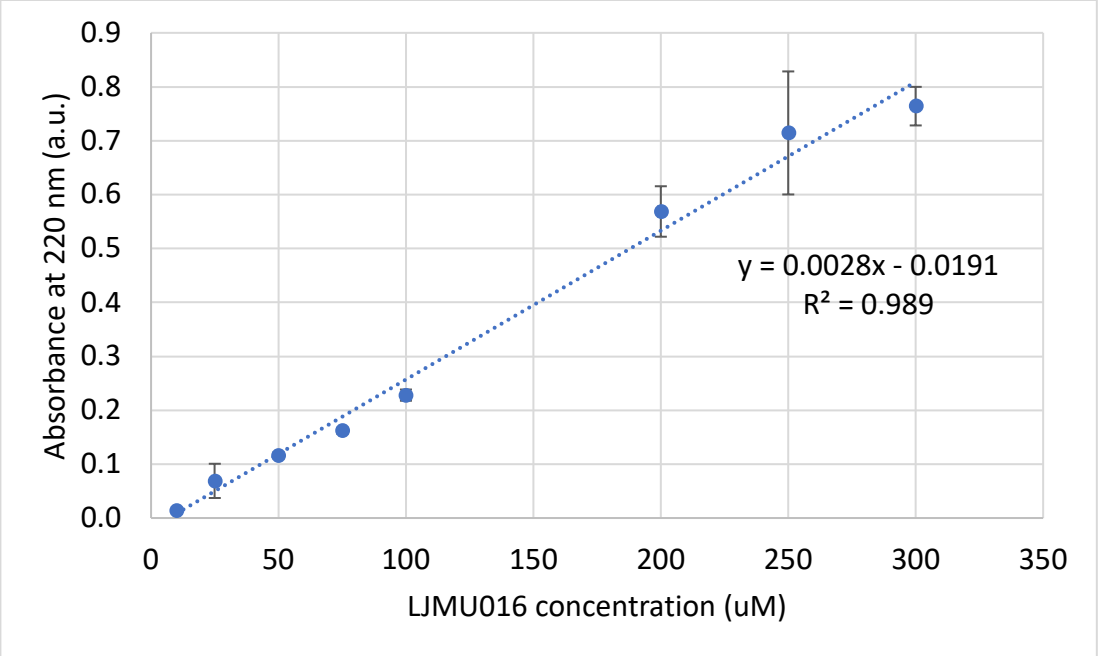



*Figure S 45. LJMU016 calibration curve in aq. PBS solution saturated with 1-octanol. Absorbance detected at 220 nm. Experiment performed in duplicate*

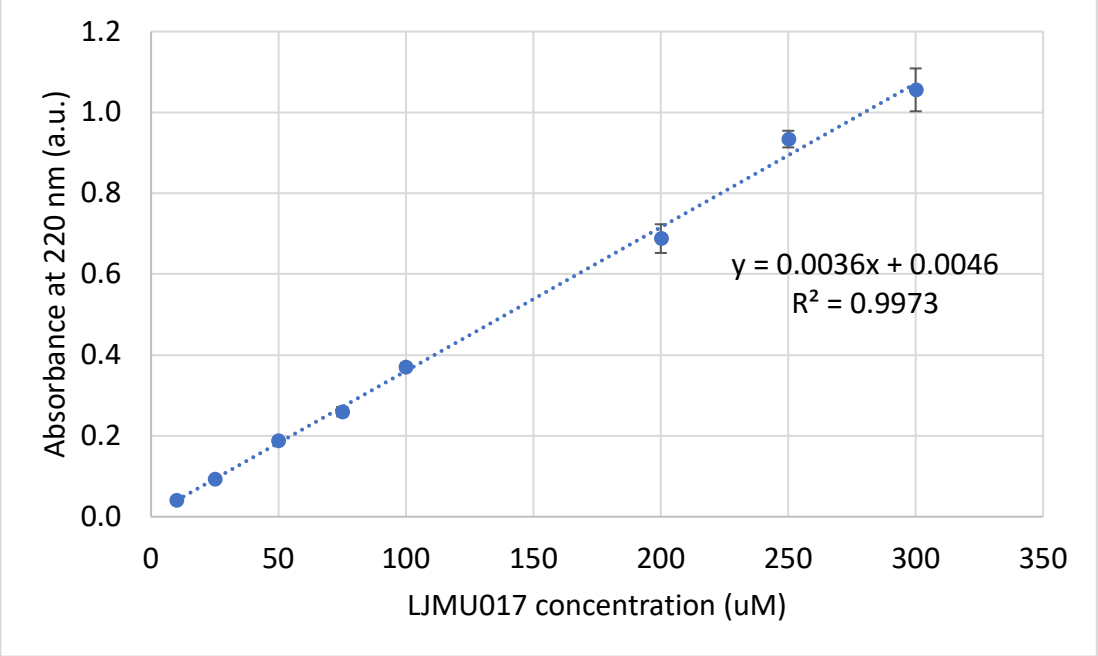



*Figure S 46. LJMU017 calibration curve in aq. PBS solution saturated with 1-octanol. Absorbance detected at 220 nm. Experiment performed in duplicate.*

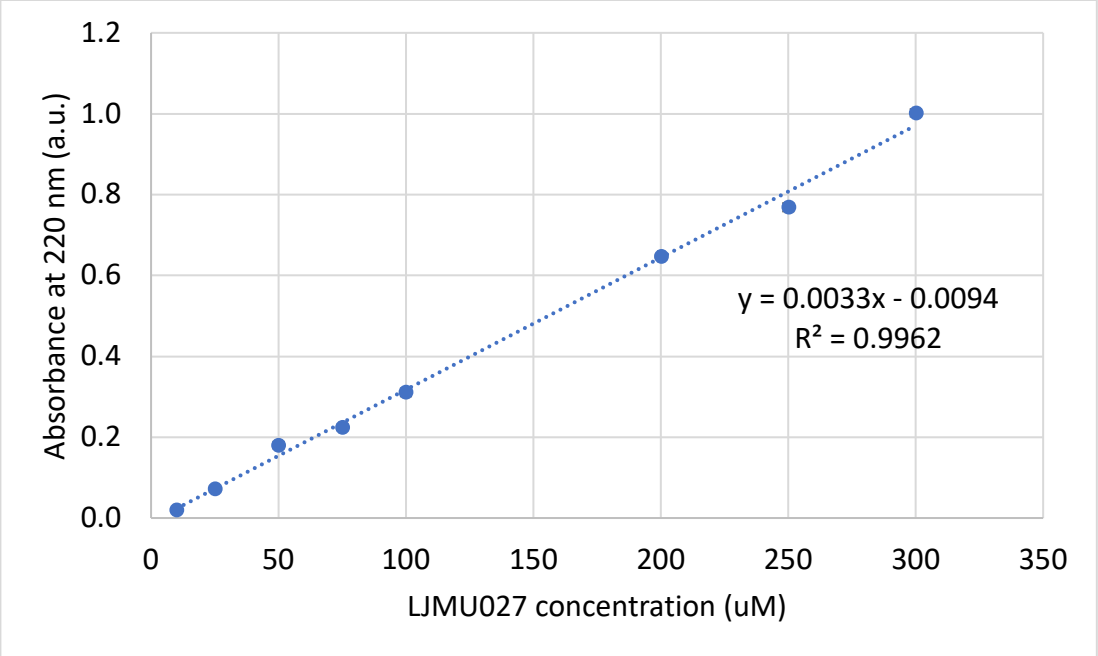



*Figure S 47. LJMU027 calibration curve in aq. PBS solution saturated with 1-octanol. Absorbance detected at 220 nm. Experiment performed in duplicate.*

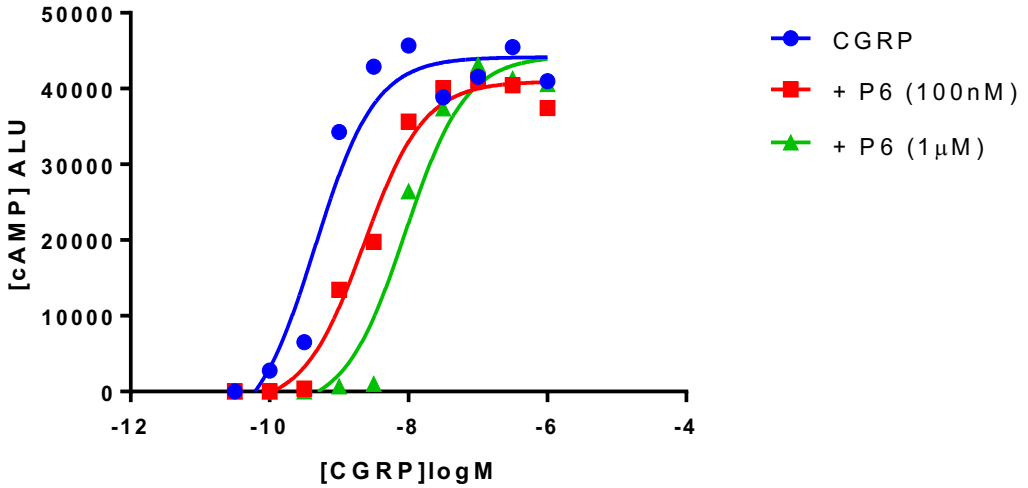
### *7.1.2.2 LogD and cLogP data*

*Table S.1. Lipophilicity assessment – full data set. N.P. = experiment non performed.*

| Entry | RP-HPLC RT (min) | % ACN | Δ RT (min) | Δ % ACN | cLogP (ChemDraw) | miLogP (Molinspiration) | cLogP (Chemicalize) | cLogD, pH 7.4 (Chemicalize) | Experimental LogD, pH 7.4 |
|---|---|---|---|---|---|---|---|---|---|
| **P006** | 5.424 | 34.3 | N.A. | N.A. | -1.4278 | -2.94 | -4.687 | -4.740 | -3.800 |
| **LJMU011** | 5.668 | 35.3 | 0.244 | 1.0 | -0.6222 | -1.70 | -3.058 | -3.160 | -1.120 |
| **LJMU012** | 5.633 | 35.2 | 0.209 | 0.9 | -0.6222 | -1.70 | -3.058 | -3.160 | -1.864 |
| **LJMU013** | 5.850 | 36.0 | 0.426 | 1.7 | -0.4703 | -1.54 | -2.915 | -3.020 | -1.405 |
| **LJMU014** | 6.589 | 39.0 | 1.165 | 4.7 | -0.9228 | -2.41 | -3.973 | -4.030 | -1.972 |
| **LJMU015** | 6.601 | 39.0 | 1.177 | 4.7 | -0.9228 | -2.41 | -3.973 | -4.030 | -1.676 |
| **LJMU016** | 7.735 | 43.6 | 2.311 | 9.3 | -0.4178 | -1.88 | -3.275 | -3.710 | -0.232 |
| **LJMU017** | 8.961 | 48.5 | 3.537 | 14.2 | 2.5966 | -0.83 | -0.607 | -3.700 | -1.200 |
| **LJMU018** | 10.015 | 52.7 | 4.591 | 18.4 | 2.2786 | -0.47 | -0.036 | -3.140 | N.P. |
| **LJMU019** | 5.295 | 33.8 | -0.129 | -0.5 | -0.9316 | -3.65 | -4.963 | -5.020 | N.P. |
| **LJMU020** | 5.288 | 33.8 | -0.136 | -0.5 | -1.1536 | -2.52 | -5.032 | -5.090 | N.P. |
| **LJMU021** | 5.435 | 34.4 | 0.011 | 0.1 | -0.9316 | -2.59 | -4.963 | -5.020 | N.P. |

| Entry | RP-HPLC RT (min) | % ACN | Δ RT (min) | Δ % ACN | cLogP (ChemDraw) | miLogP (Molinspiration) | cLogP (Chemicalize) | cLogD, pH 7.4 (Chemicalize) | Experimental LogD, pH 7.4 |
|---|---|---|---|---|---|---|---|---|---|
| **LJMU022** | 5.160 | 33.3 | -0.264 | -1.0 | -1.1812 | -2.63 | -5.146 | -5.200 | N.P. |
| **LJMU023** | 5.453 | 34.4 | 0.029 | 0.1 | -0.2848 | -3.37 | -4.912 | -4.970 | N.P. |
| **LJMU024** | 5.451 | 34.4 | 0.027 | 0.1 | -1.4856 | -2.47 | -5.145 | -5.160 | N.P. |
| **LJMU025** | 5.073 | 32.9 | -0.351 | -1.4 | -2.6533 | -4.34 | -4.241 | -7.230 | N.P. |
| **LJMU026** | 5.468 | 34.5 | 0.044 | 0.2 | -1.5698 | -3.30 | -5.103 | -5.160 | N.P. |
| **LJMU027** | 8.718 | 47.5 | 3.294 | 13.2 | 2.4536 | -1.00 | -0.749 | -3.850 | -0.300 |

### 7.1.3 Concentration-response curves (cAMP accumulation assay)




$EC_{50}$ CGRP = 0.45nM
$EC_{50}$ CGRP+P6(100nM) = 2.3nM
$EC_{50}$ CGRP+P6 (1μM) = 9.0nM

*Figure S 48. Concentration-response curves previously obtained for P006 (experiment carried out by Professor David A. Kendall in 2017 using DiscoverX Eurofins kit and CHO-K1 CALCRL RAMP1 cells). $K_b$ estimates for P006 = 24 nM and 5.3 nM.*

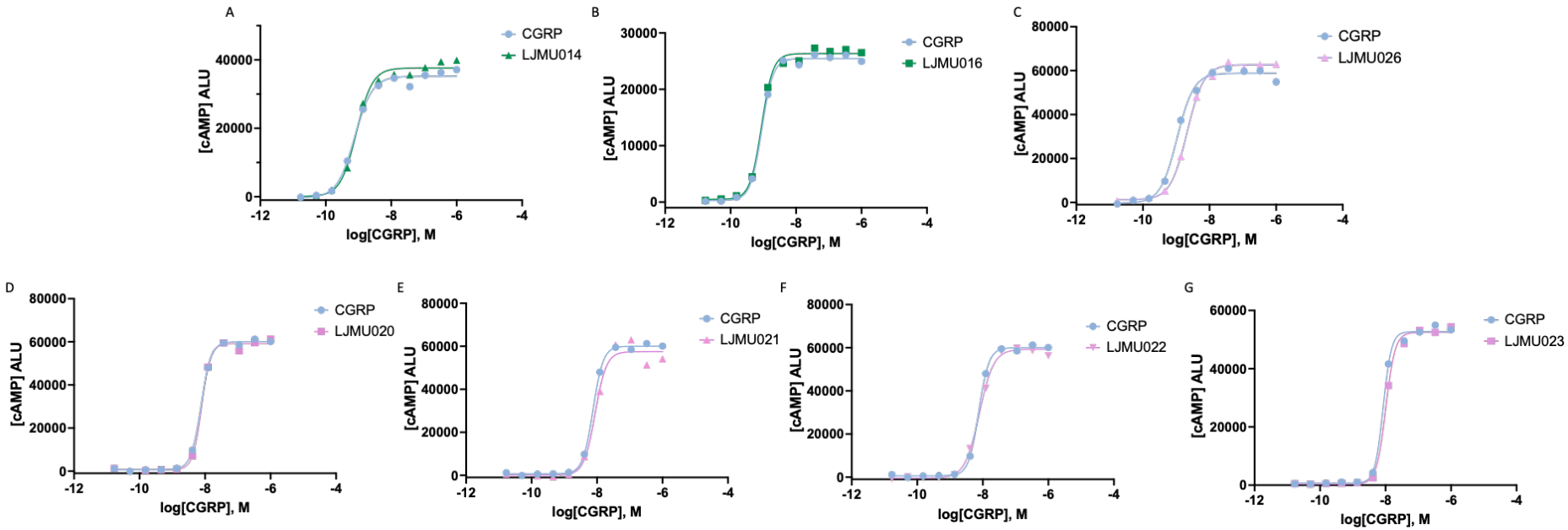



*Figure S 49. Representative cAMP concentration-response curves for inactive peptides. From top left to bottom right: LJMU014 (**A**), LJMU016 (**B**), LJMU020 (**C**), LJMU021 (**D**), LJMU022 (**E**), LJMU023 (**F**).*

## 7.2 Chapter 4 – supporting information

### *7.2.1 Calibration curves for serum stability study*

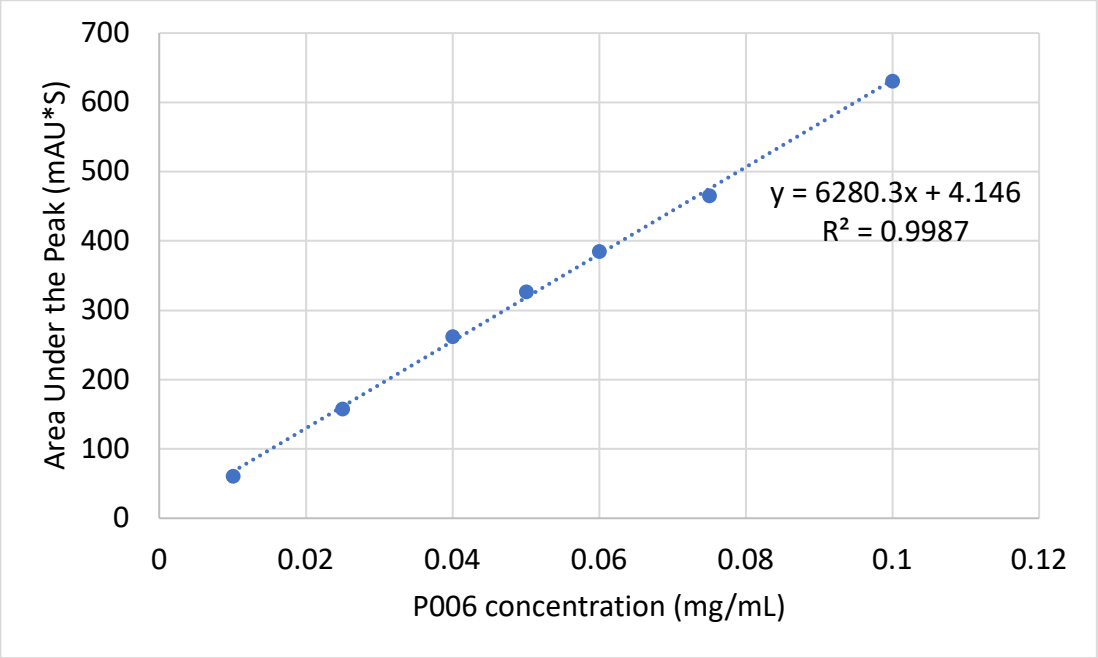



*Figure S 50. P006 calibration curve in serum after protein precipitation with TCA.*

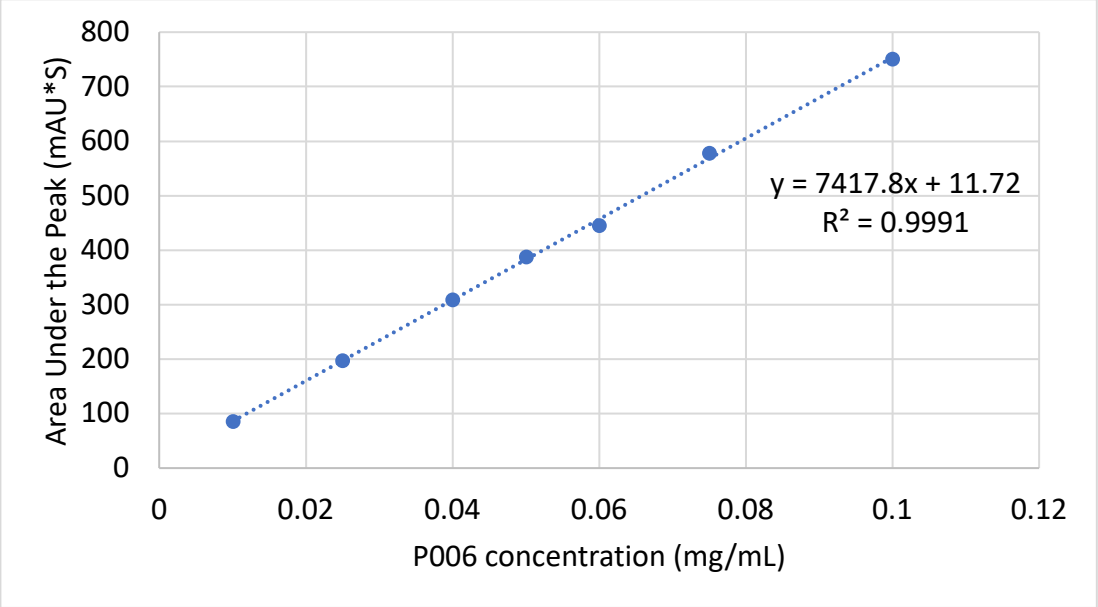



*Figure S 51. P006 calibration curve in serum after protein precipitation with MeOH.*

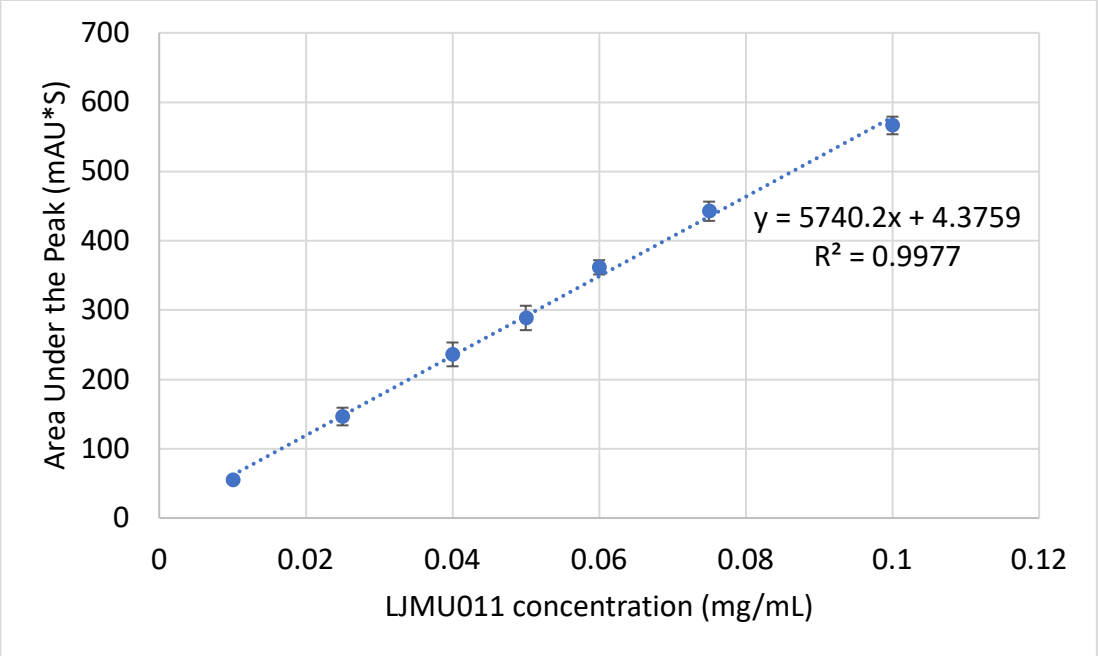



*Figure S 52. LJMU011 calibration curve in serum after protein precipitation with TCA. Experiment performed in duplicate.*

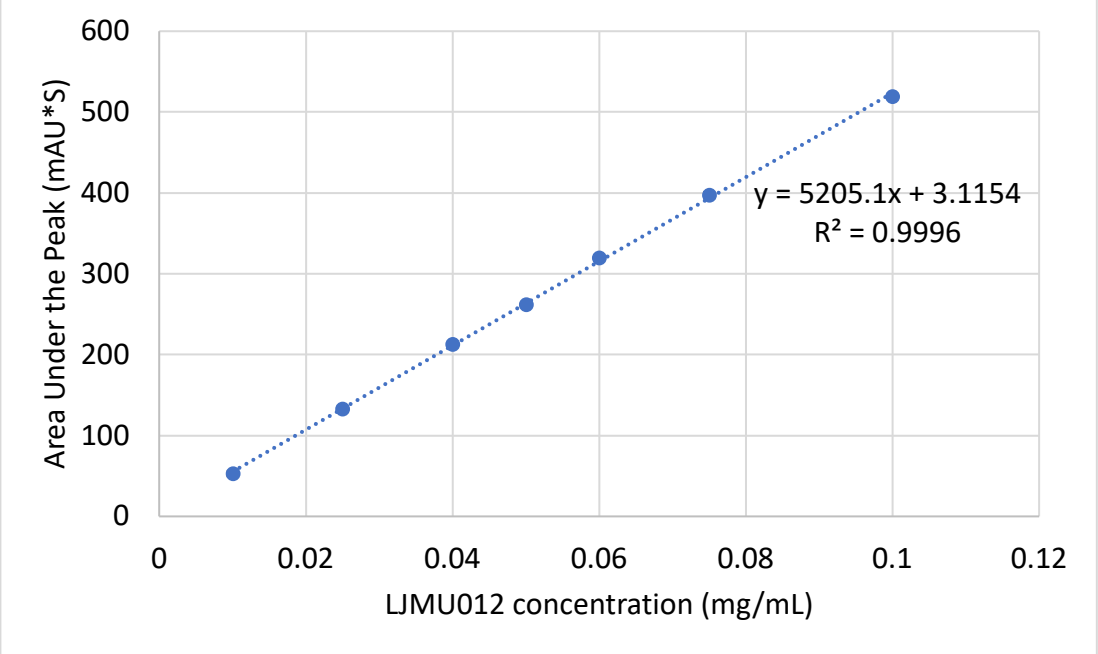



*Figure S 53. LJMU012 calibration curve in serum after protein precipitation with TCA. Experiment performed in duplicate.*

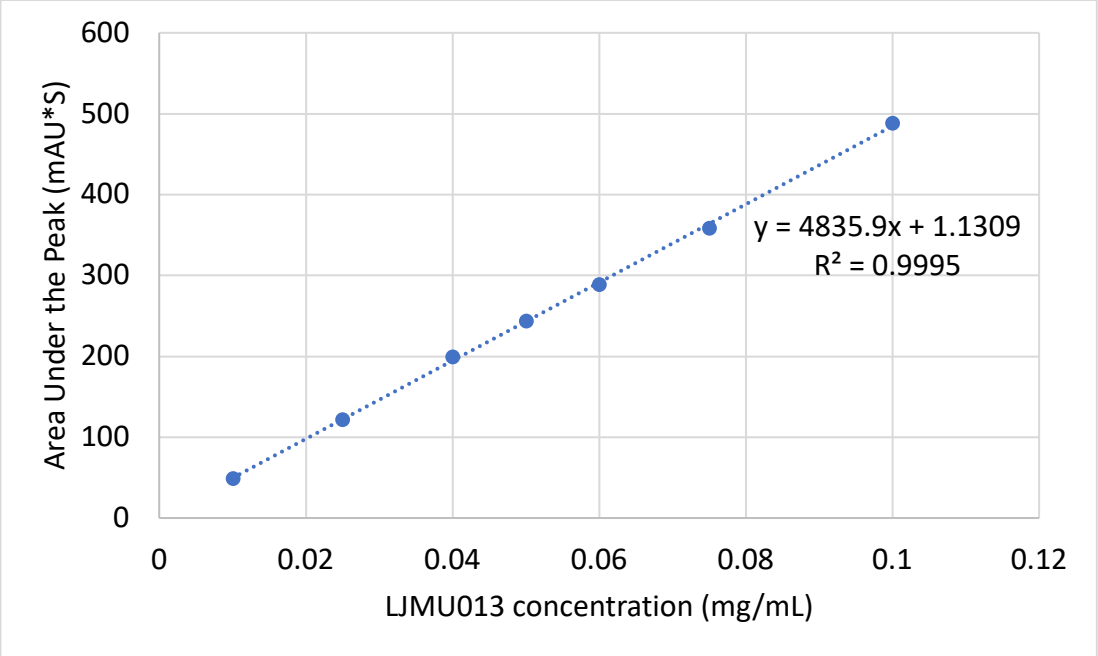



*Figure S 54. LJMU013 calibration curve in serum after protein precipitation with TCA.*

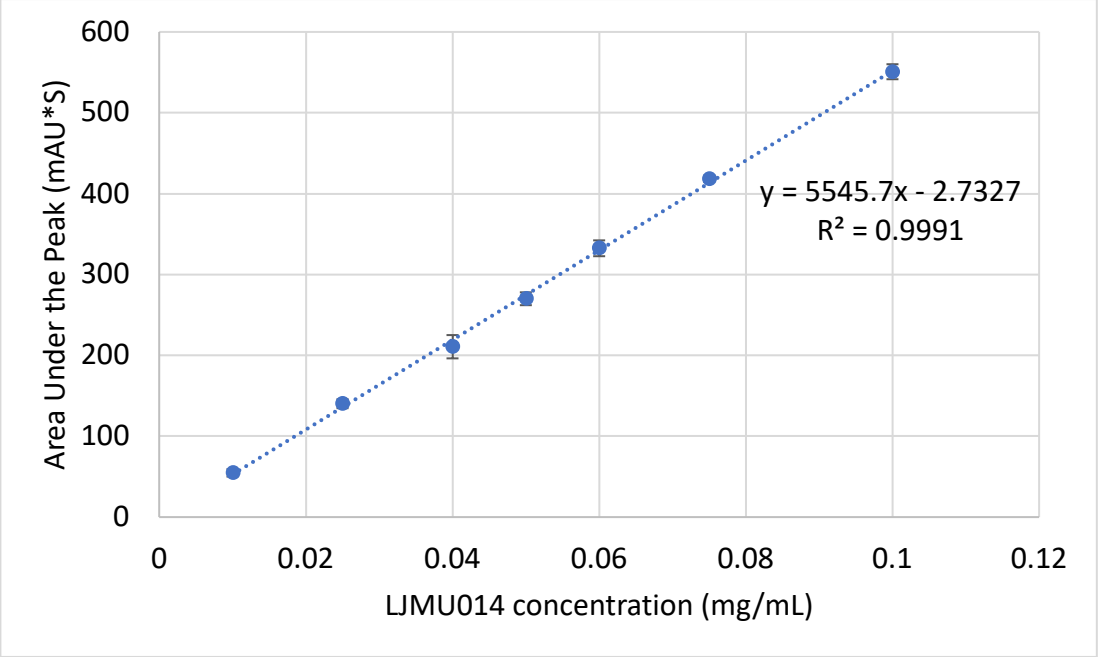



*Figure S 55. LJMU014 calibration curve in serum after protein precipitation with TCA. Experiment performed in duplicate.*

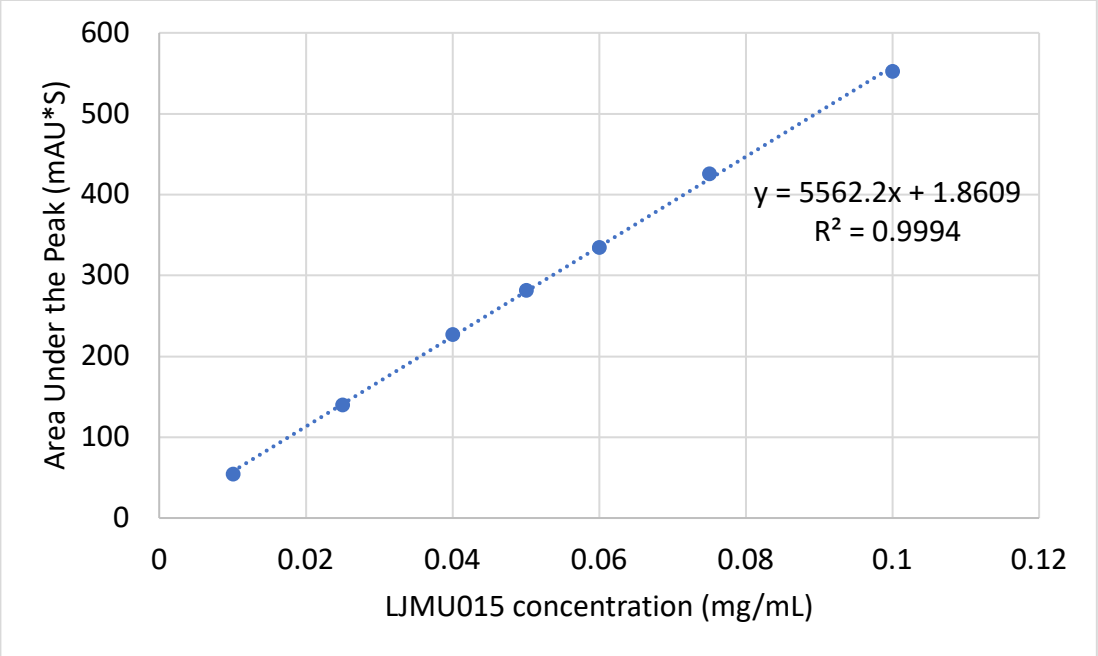



*Figure S 56. LJMU015 calibration curve in serum after protein precipitation with TCA. Experiment performed in duplicate.*

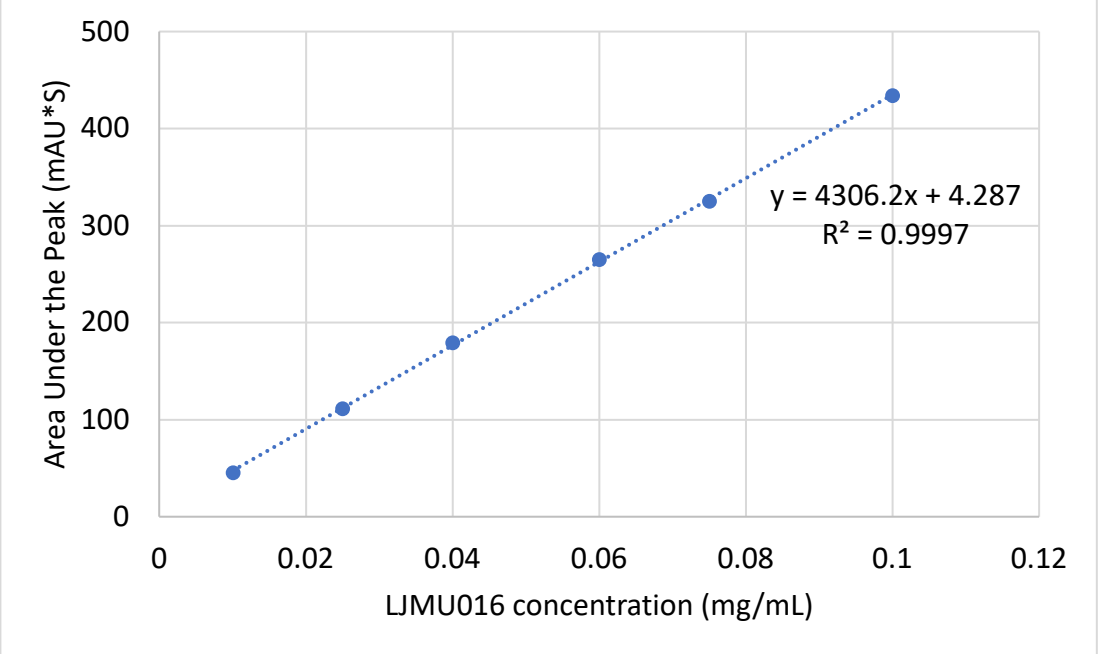



*Figure S 57. LJMU016 calibration curve in serum after protein precipitation with TCA.*

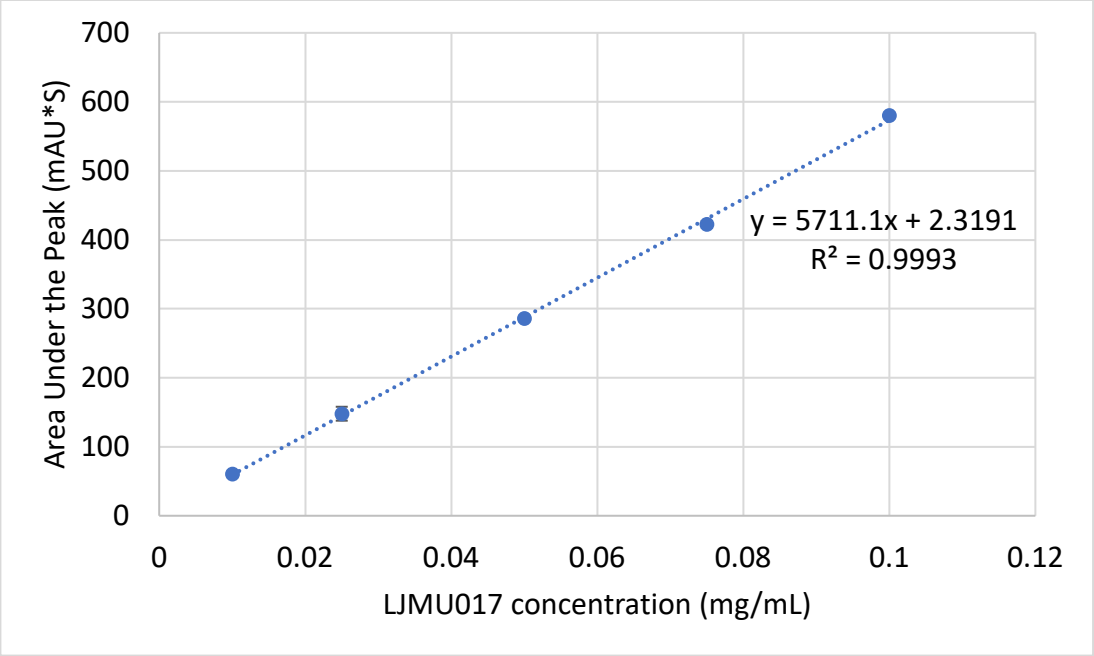



*Figure S 58. LJMU017 calibration curve in $H_2O$.*

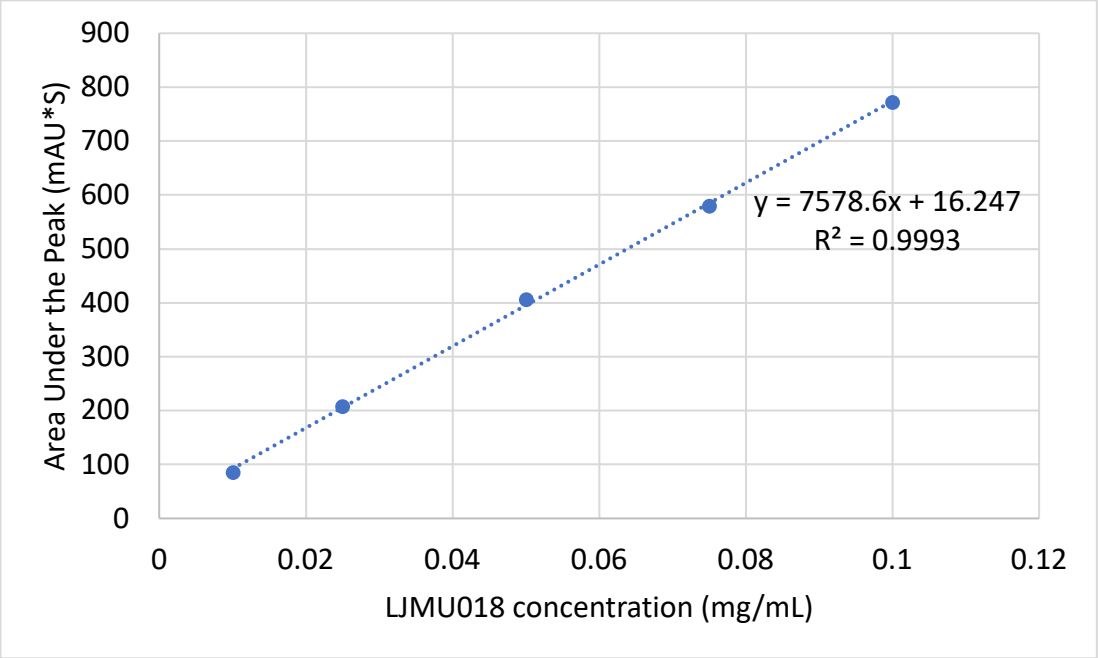



*Figure S 59. LJMU018 calibration curve in $H_2O$.*

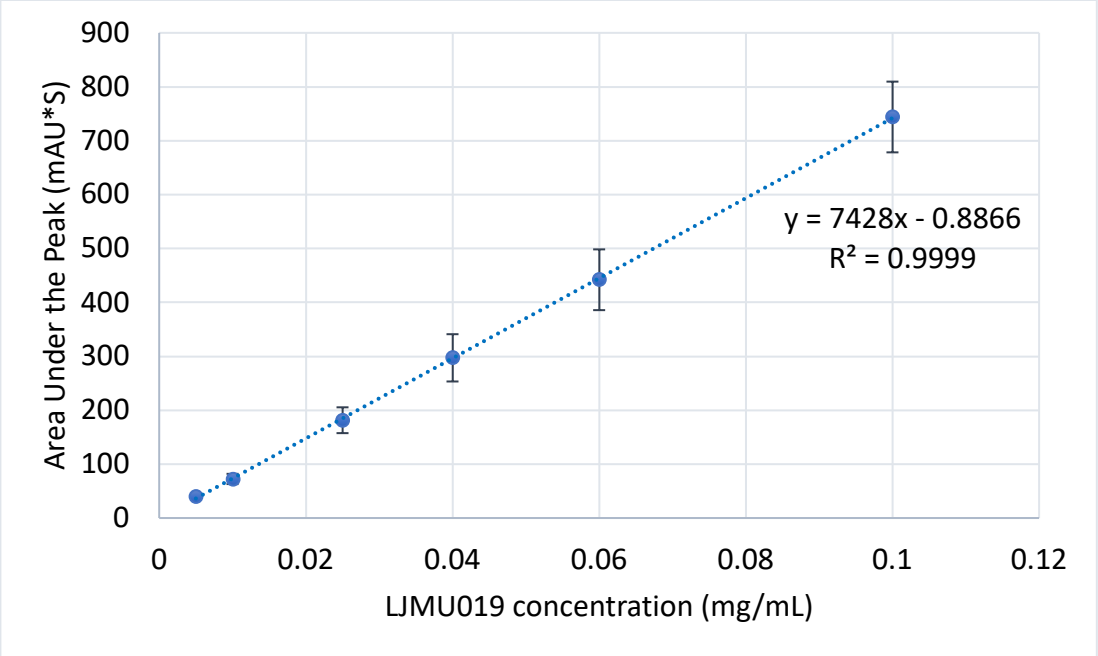



*Figure S 60. LJMU019 calibration curve in serum after protein precipitation with TCA. Experiment performed in triplicate.*

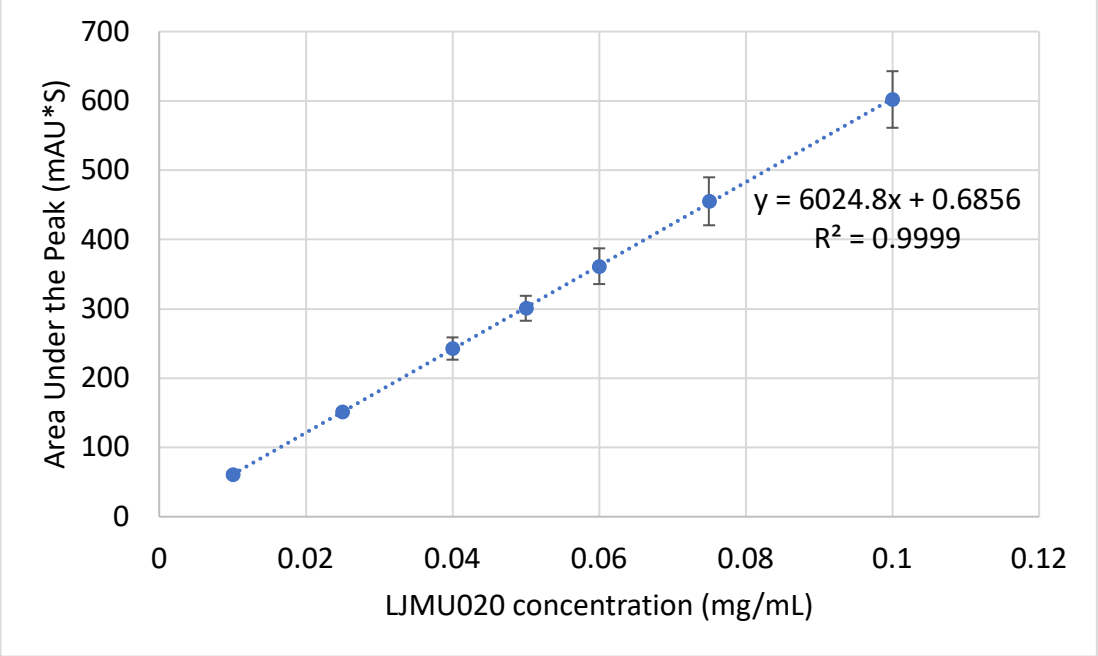



*Figure S 61. LJMU020 calibration curve in serum after protein precipitation with TCA. Experiment performed in triplicate.*

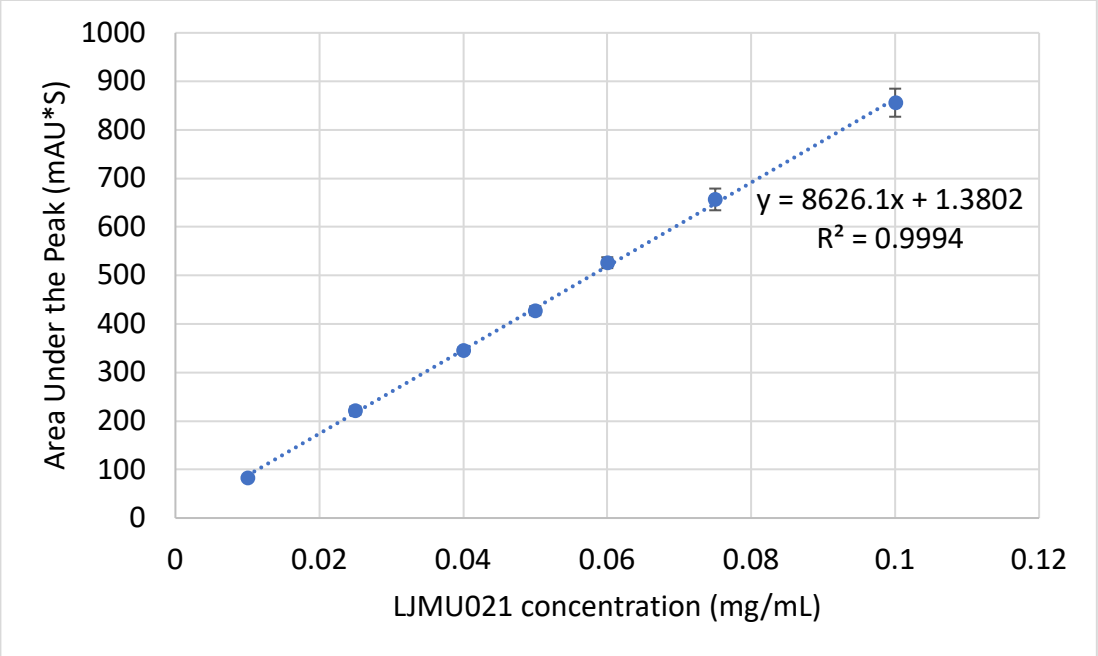



*Figure S 62. LJMU021 calibration curve in serum after protein precipitation with TCA. Experiment performed in duplicate.*

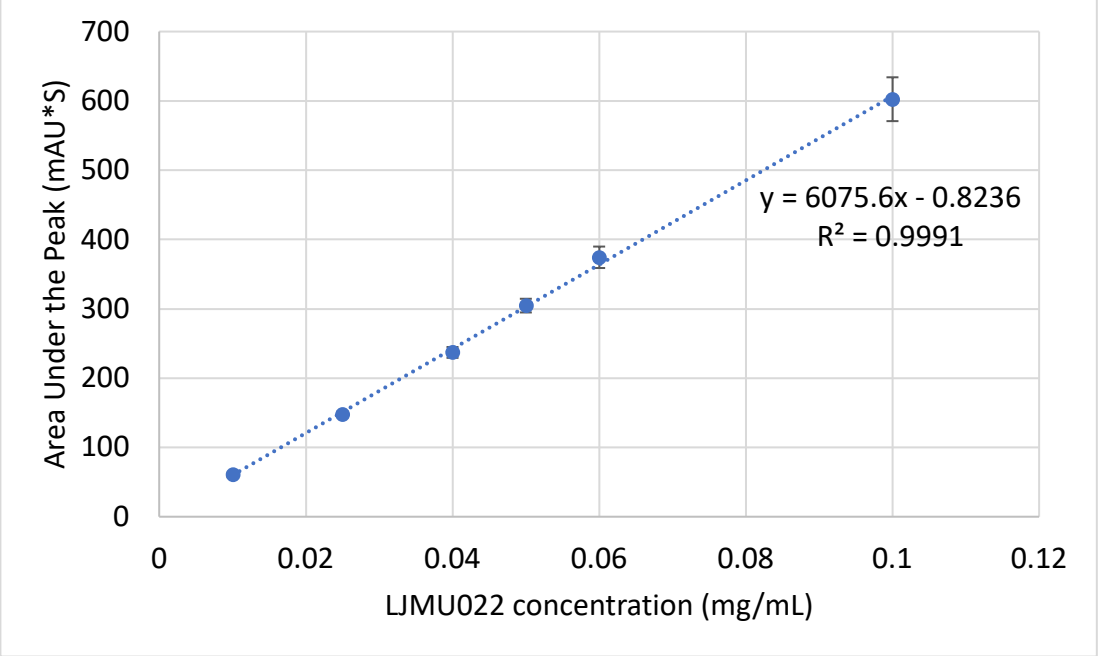



*Figure S 63. LJMU022 calibration curve in serum after protein precipitation with TCA. Experiment performed in duplicate.*

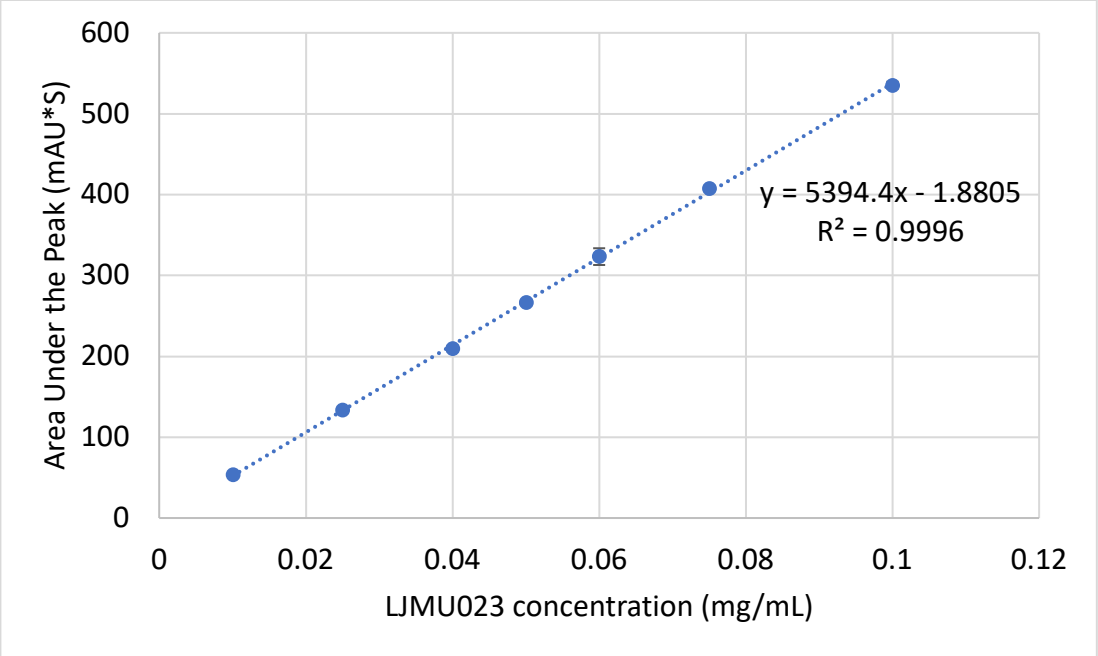



*Figure S 64. LJMU023 calibration curve in serum after protein precipitation with TCA. Experiment performed in duplicate.*

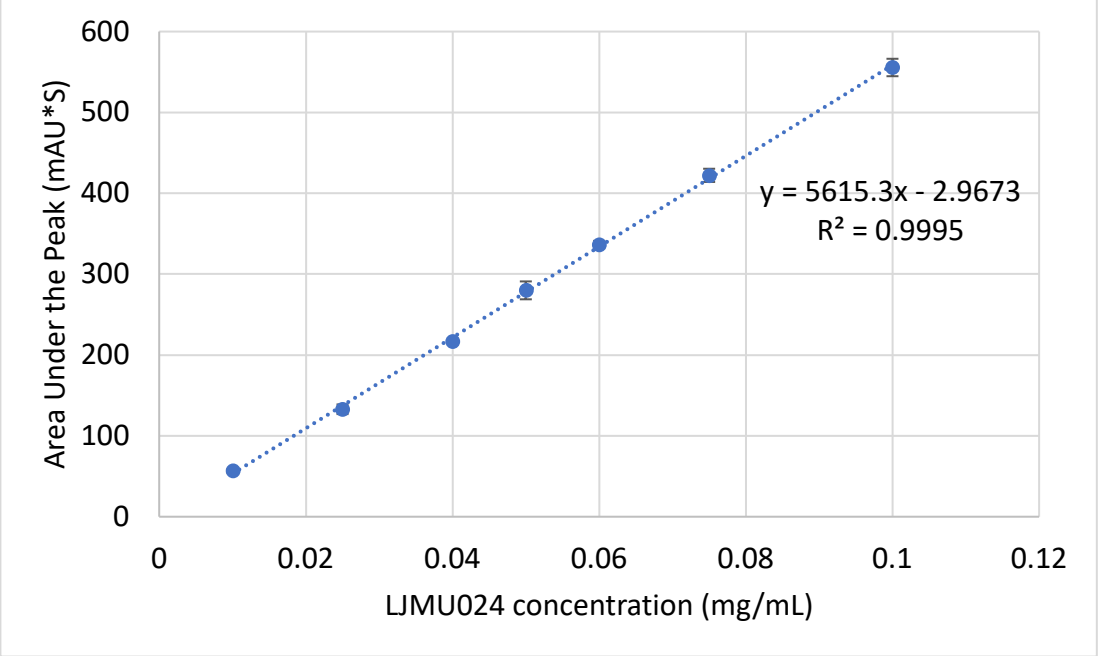



*Figure S 65. LJMU024 calibration curve in serum after protein precipitation with TCA. Experiment performed in duplicate.*

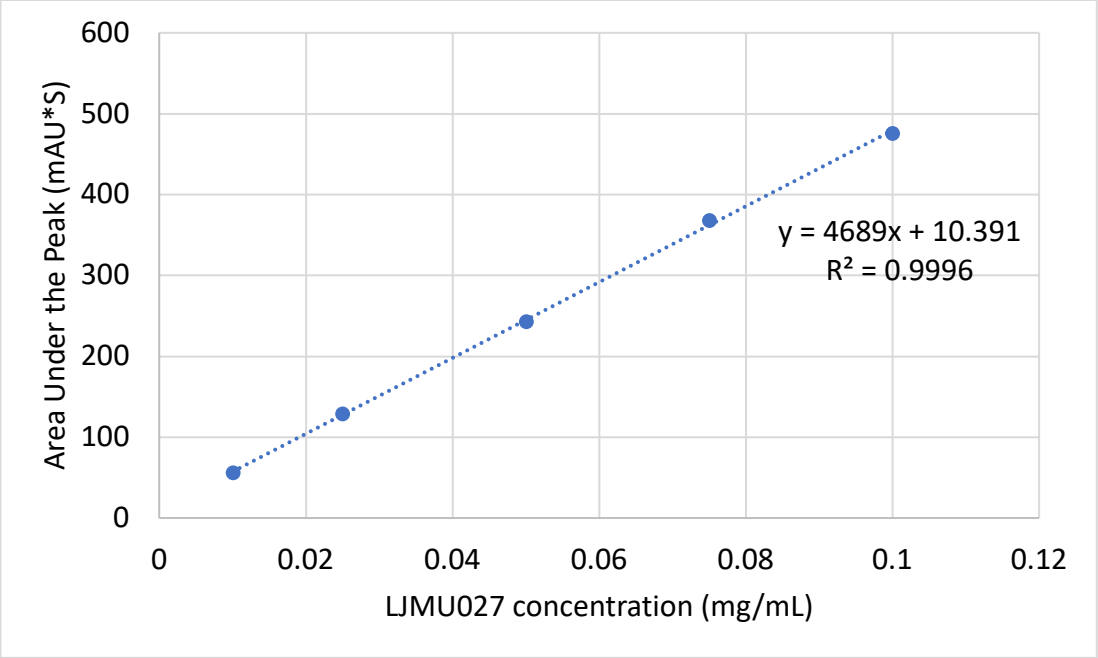



*Figure S 66. LJMU027 calibration curve in $H_2O$.*

### *7.2.2 Stability in human serum – full dataset*

*Table S.2. Stability in human serum. Full dataset expressed as percentage of intact peptide compared to 0 min sample (taken as 100%).*

| | Detected intact peptide (%) | | | |
|---|---|---|---|---|
| | **15 min** | **30 min** | **45 min** | **60 min** |
| **P006** | 61.02 ± 1.43 | 32.55 ± 2.39 | 14.95 ± 1.72 | 4.11 ± 0.55 |
| **LJMU011** | 69.89 ± 1.39 | 33.93 ± 2.72 | 14.56 ± 2.27 | 5.16 ± 1.02 |
| **LJMU012** | 62.92 ± 3.61 | 30.94 ± 2.63 | 11.98 ± 1.90 | 2.82 ± 1.24 |
| **LJMU013** | 63.57 ± 5.16 | 33.95 ± 4.20 | 19.36 ± 3.87 | 3.57 ± 3.34 |
| **LJMU014** | 60.79 ± 3.85 | 29.99 ± 3.04 | 15.25 ± 1.44 | 9.45 ± 1.79 |
| **LJMU015** | 55.68 ± 3.50 | 34.60 ± 2.60 | 18.18 ± 3.94 | 7.05 ± 0.62 |
| **LJMU016** | 69.81 ± 4.13 | 48.34 ± 3.14 | 36.48 ± 2.00 | 32.78 ± 0.72 |
| **LJMU017** | 96.60 ± 3.70 | 90.40 ± 5.10 | 90.10 ± 6.80 | 85.50 ± 4.40 |
| **LJMU018** | 89.95 ± 5.47 | 87.22 ± 2.79 | 79.51 ± 8.57 | 74.95 ± 2.04 |
| **LJMU019** | 64.24 ± 1.04 | 35.85 ± 1.16 | 17.06 ± 5.88 | 4.44 ± 1.68 |
| **LJMU020** | 61.82 ± 5.29 | 30.52 ± 7.27 | 16.18 ± 3.52 | 4.05 ± 1.68 |
| **LJMU021** | 64.20 ± 1.50 | 35.56 ± 0.31 | 16.52 ± 1.50 | 6.69 ± 1.24 |
| **LJMU022** | 68.45 ± 6.22 | 45.63 ± 3.33 | 24.50 ± 0.88 | 13.01 ± 0.32 |
| **LJMU023** | 64.18 ± 2.79 | 38.37 ± 2.53 | 21.20 ± 4.61 | 10.38 ± 3.47 |
| **LJMU024** | 70.73 ± 2.79 | 52.84 ± 1.70 | 42.77 ± 1.73 | 38.04 ± 2.21 |
| **LJMU027** | 101.09 ± 0.68 | 100.33 ± 0.78 | 100.50 ± 0.76 | 94.33 ± 3.27 |

### 7.2.3 First-order reaction plot ln[peptide] versus time

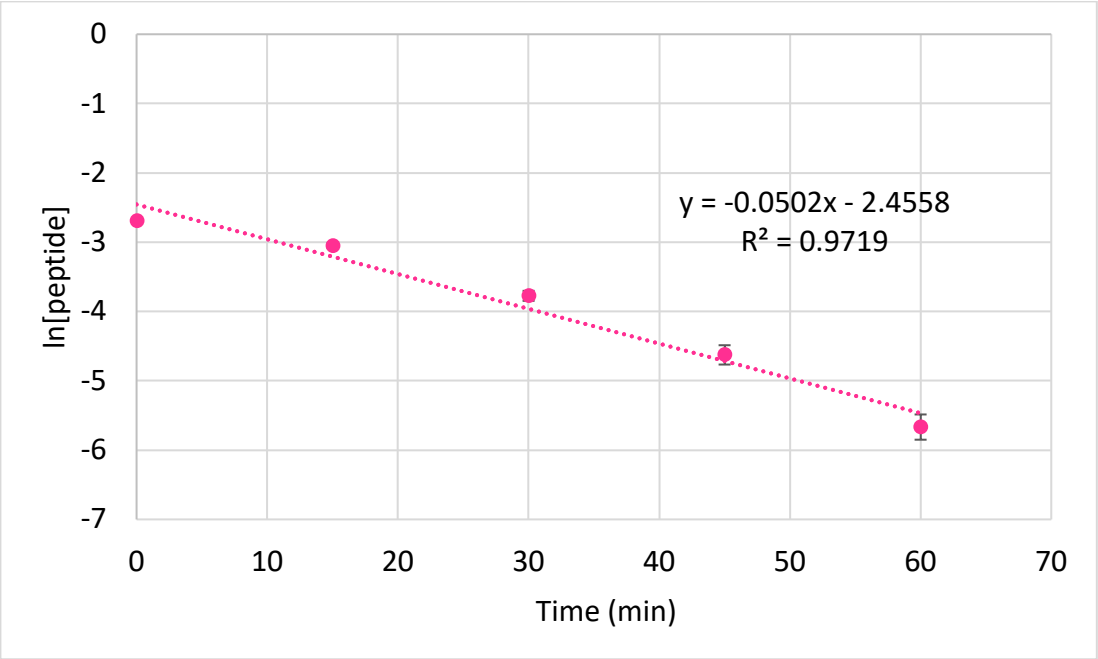



*Figure S 67. First-order reaction plot ln[LJMU011] versus time (min) to determine rate constant.*

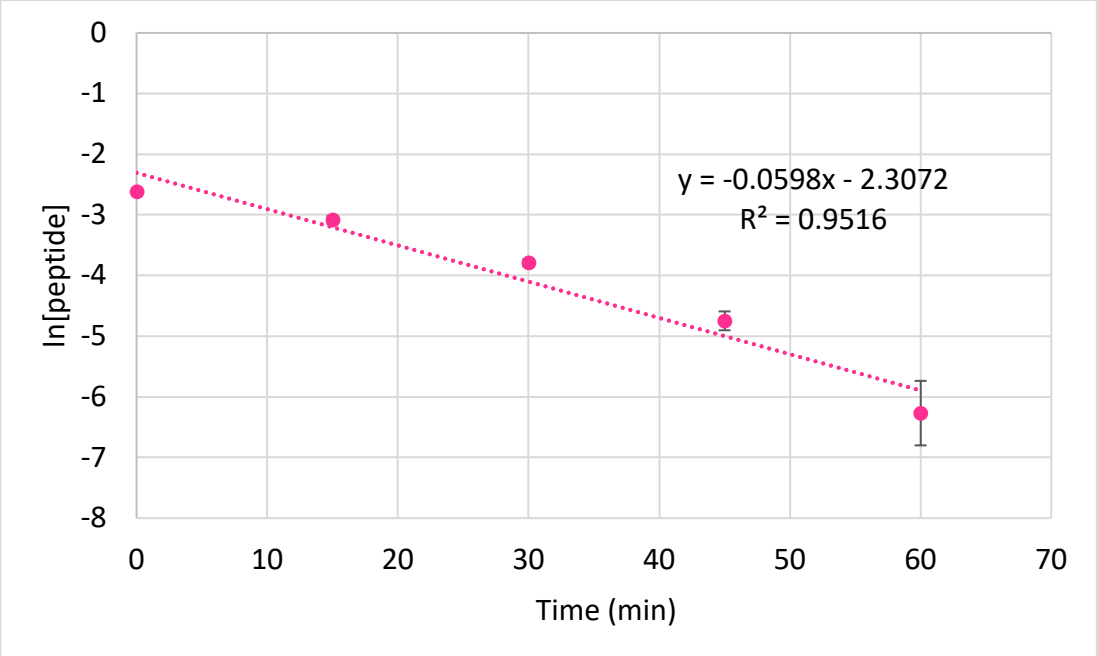



*Figure S 68. First-order reaction plot ln[LJMU012] versus time (min) to determine rate constant.*

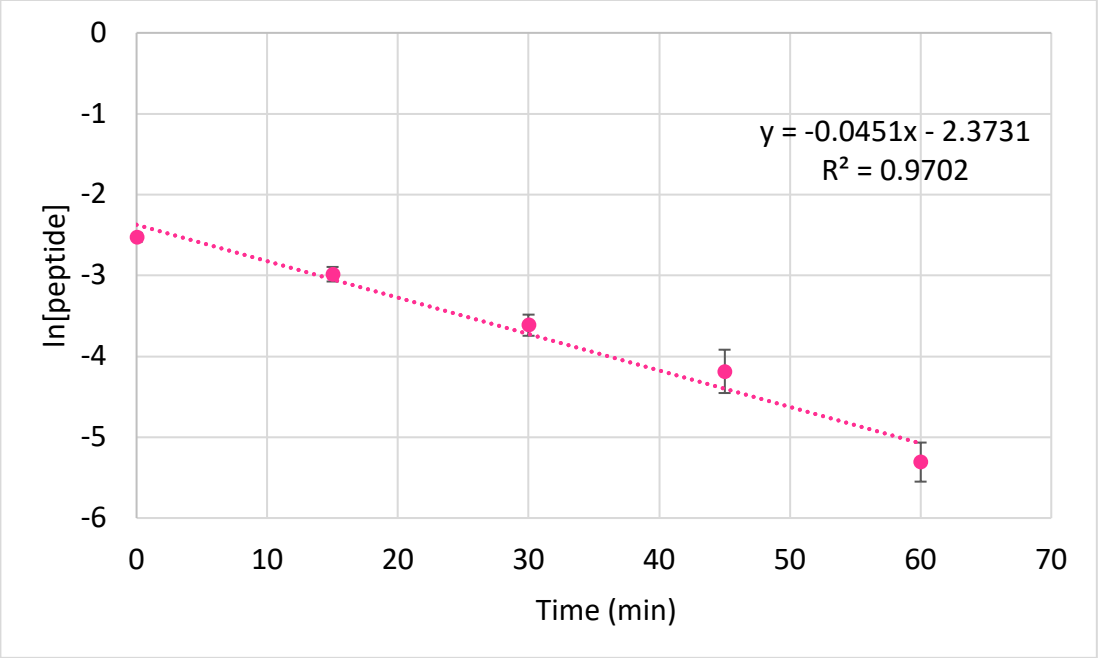



*Figure S 69. First-order reaction plot ln[LJMU013] versus time (min) to determine rate constant.*

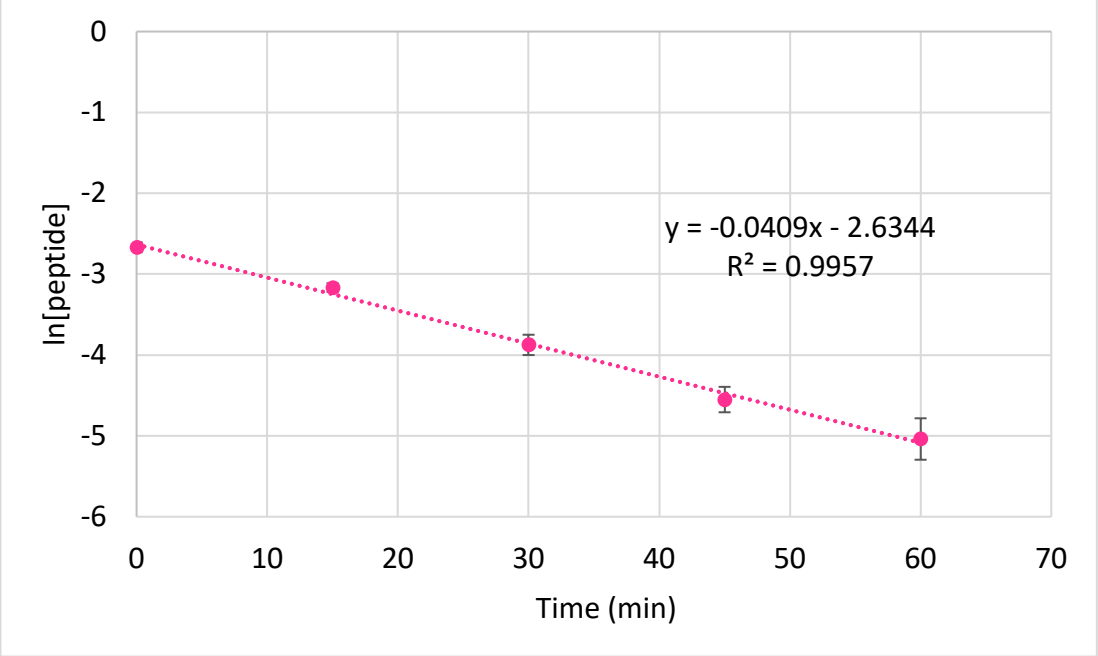



*Figure S 70. First-order reaction plot ln[LJMU014] versus time (min) to determine rate constant.*

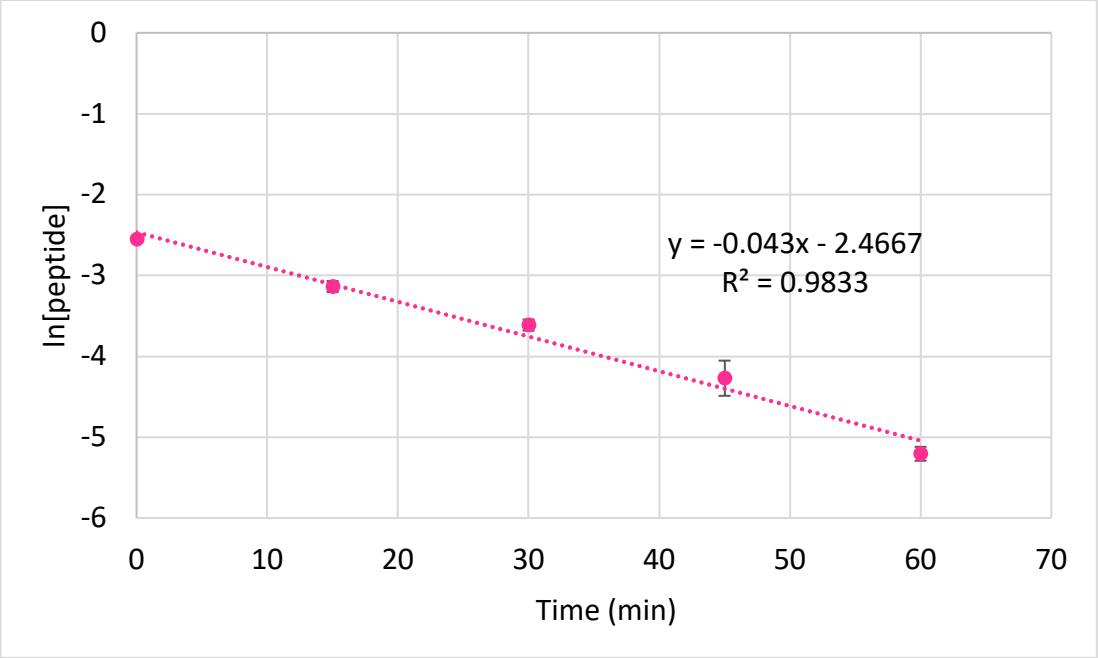



*Figure S 71. First-order reaction plot ln[LJMU015] versus time (min) to determine rate constant.*

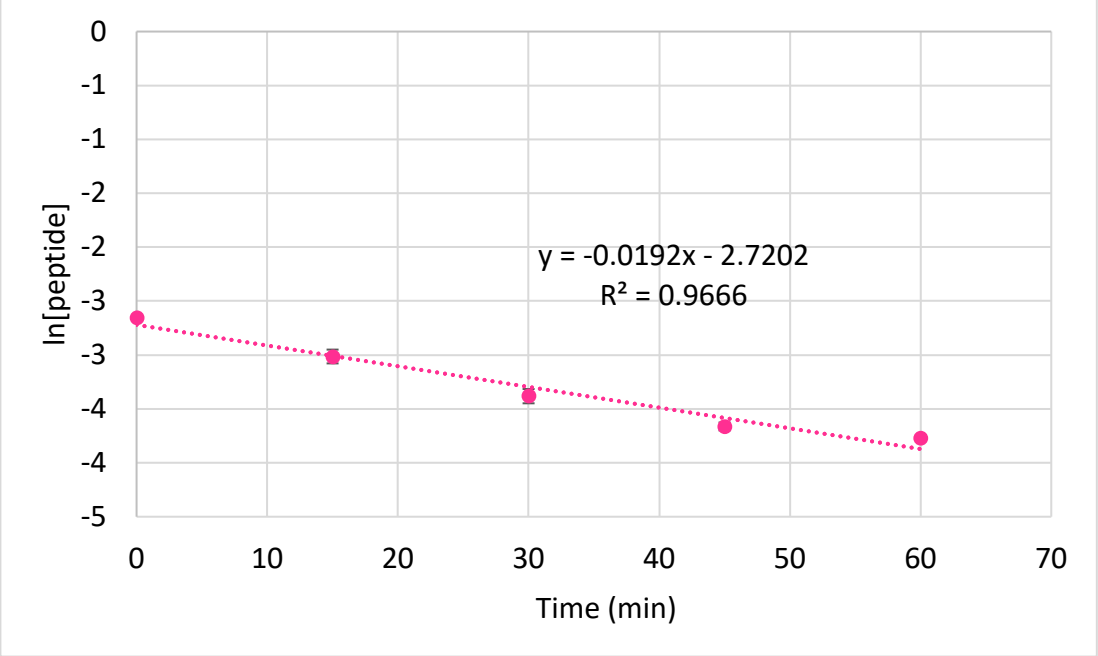



*Figure S 72. First-order reaction plot ln[LJMU016] versus time (min) to determine rate constant.*

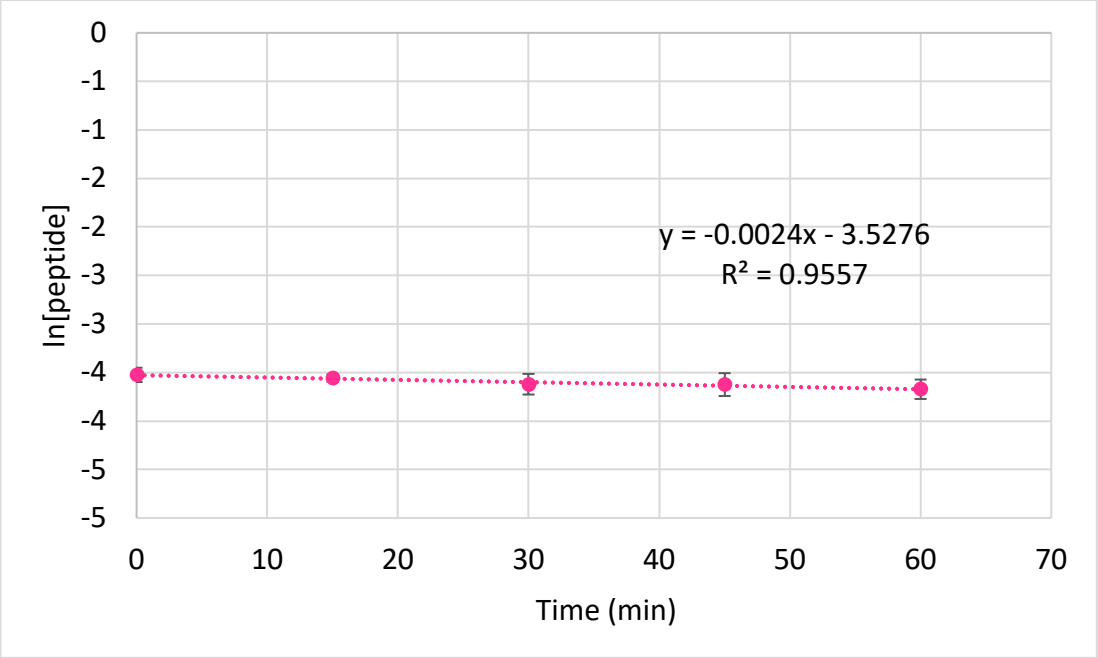



*Figure S 73. First-order reaction plot ln[LJMU017] versus time (min) to determine rate constant.*

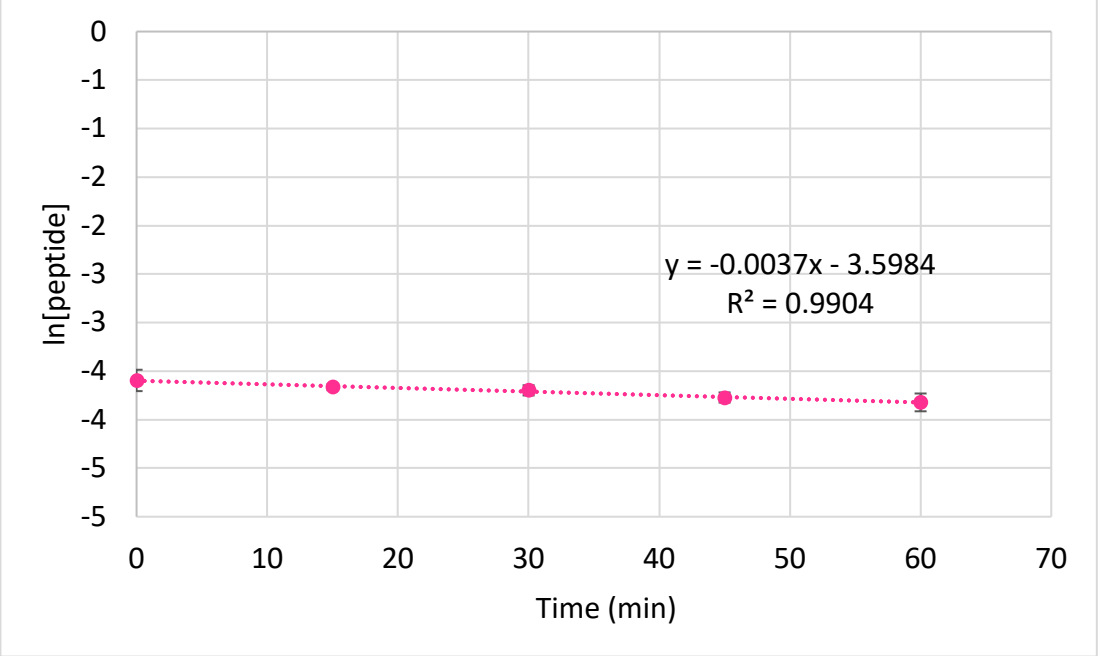



*Figure S 74. First-order reaction plot ln[LJMU018] versus time (min) to determine rate constant.*

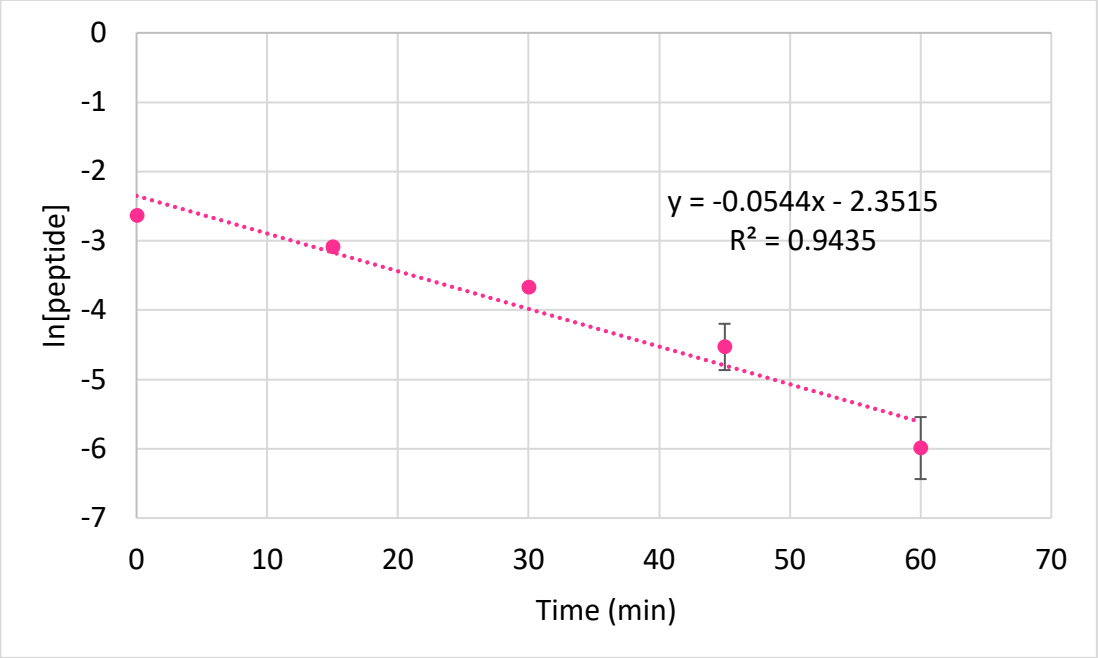



*Figure S 75. First-order reaction plot ln[LJMU019] versus time (min) to determine rate constant.*

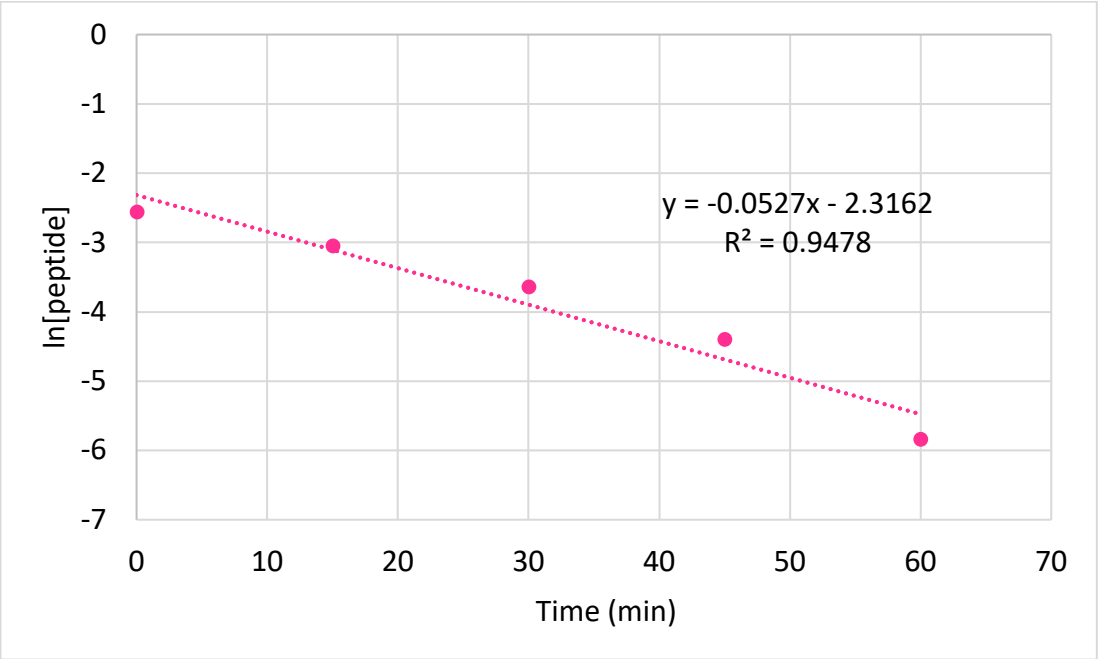



*Figure S 76. First-order reaction plot ln[LJMU020] versus time (min) to determine rate constant.*

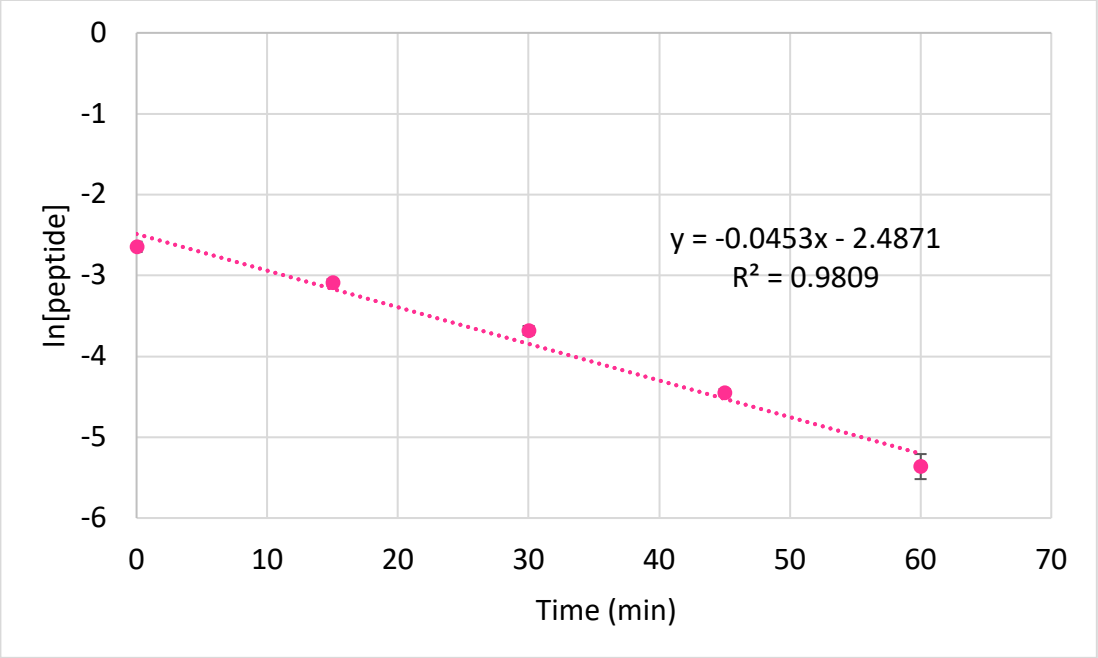



*Figure S 77. First-order reaction plot ln[LJMU021] versus time (min) to determine rate constant.*

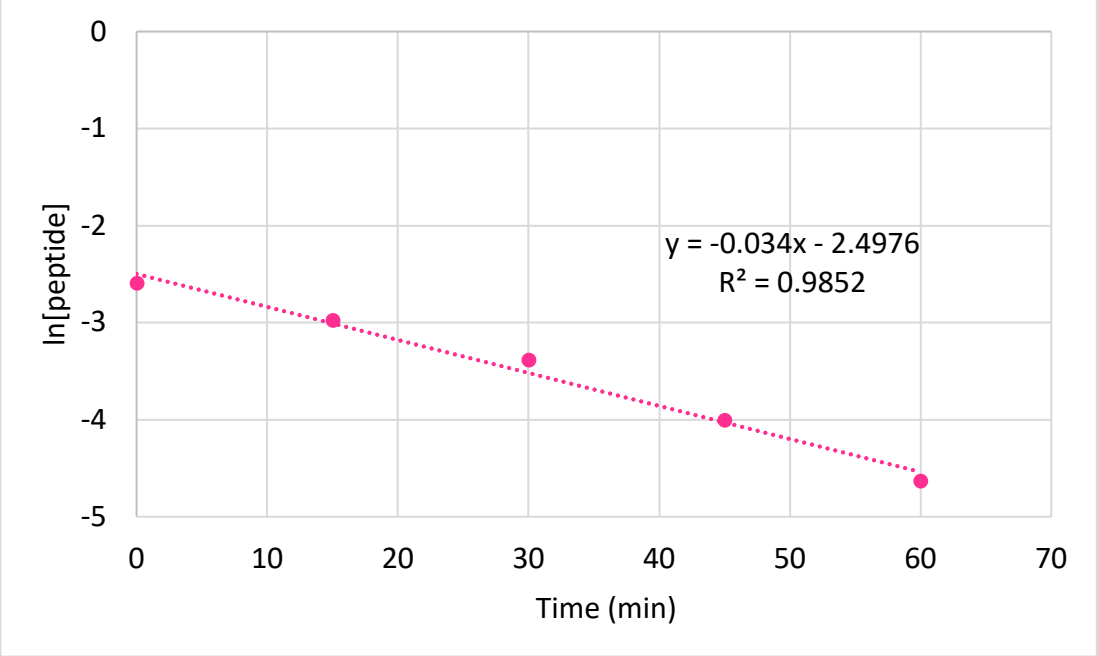



*Figure S 78. First-order reaction plot ln[LJMU022] versus time (min) to determine rate constant.*

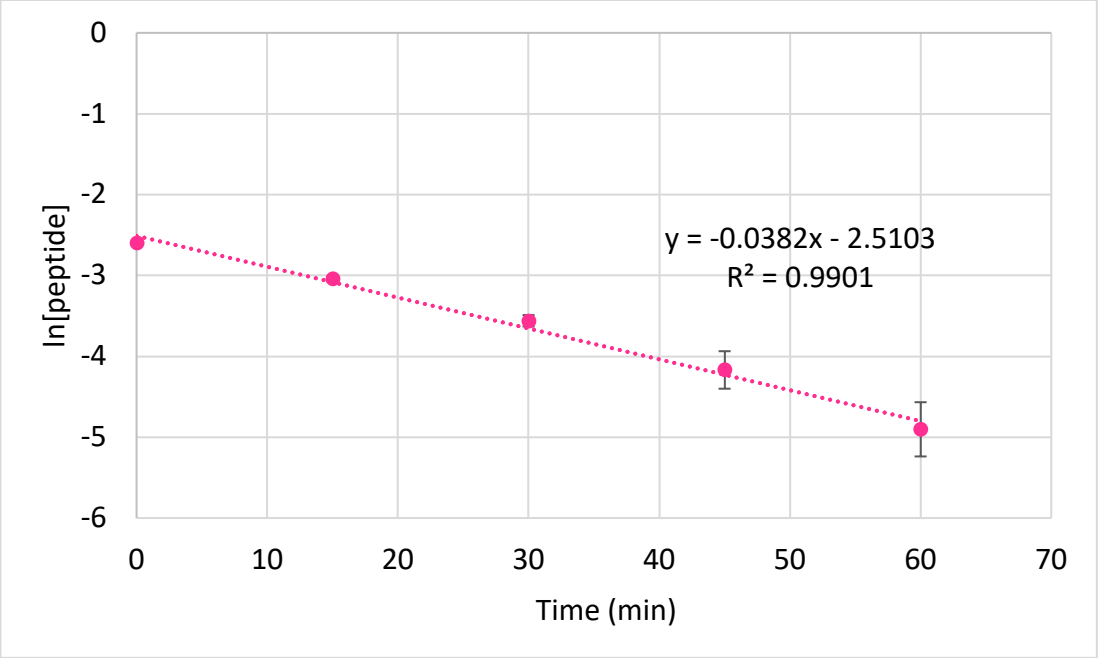



*Figure S 79. First-order reaction plot ln[LJMU023] versus time (min) to determine rate constant.*

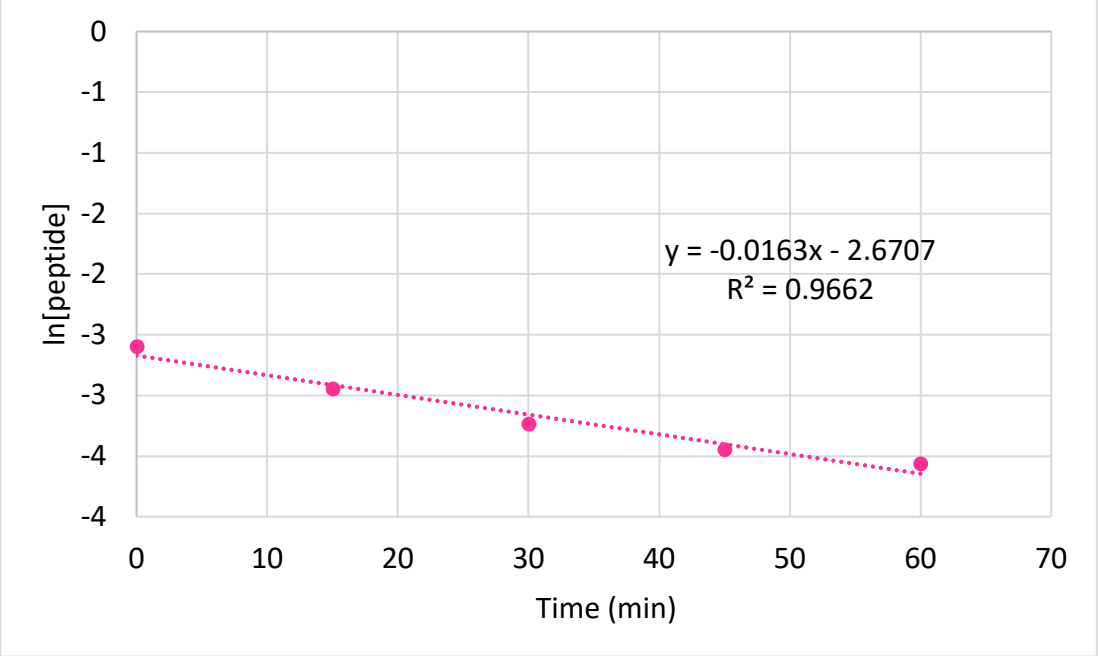



*Figure S 80. First-order reaction plot ln[LJMU024] versus time (min) to determine rate constant*

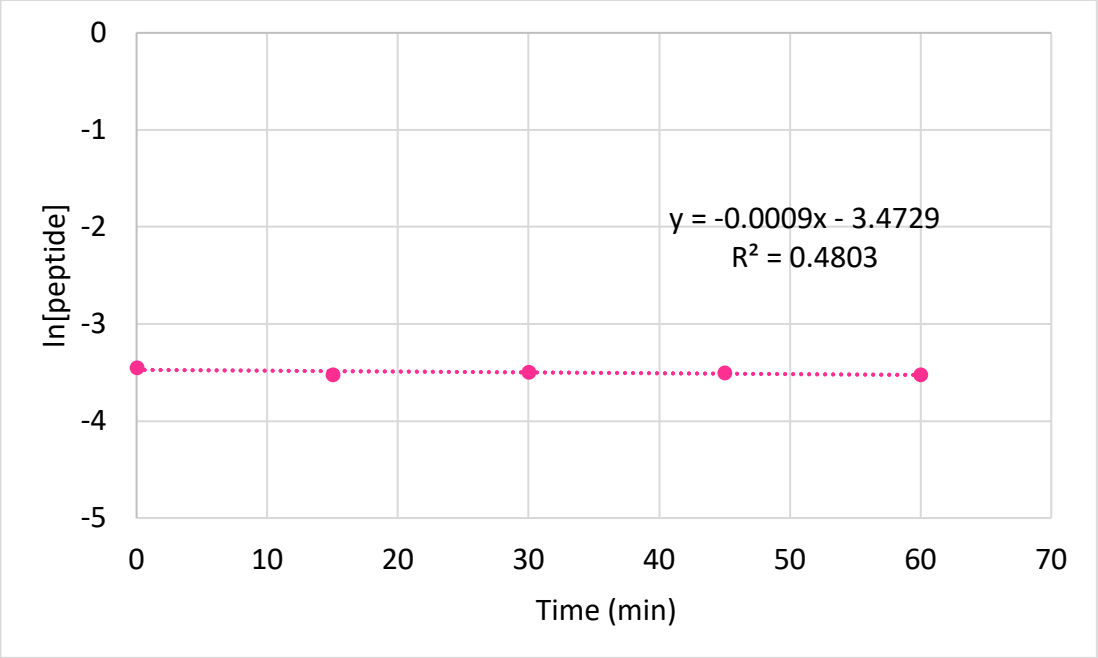



*Figure S 81. First-order reaction plot ln[LJMU027] versus time (min) to determine rate constant.*

## 7.2.4 Metabolite ID – stability study in human serum

### 7.2.4.1 P006 – parent and metabolite identification after 60 min incubation

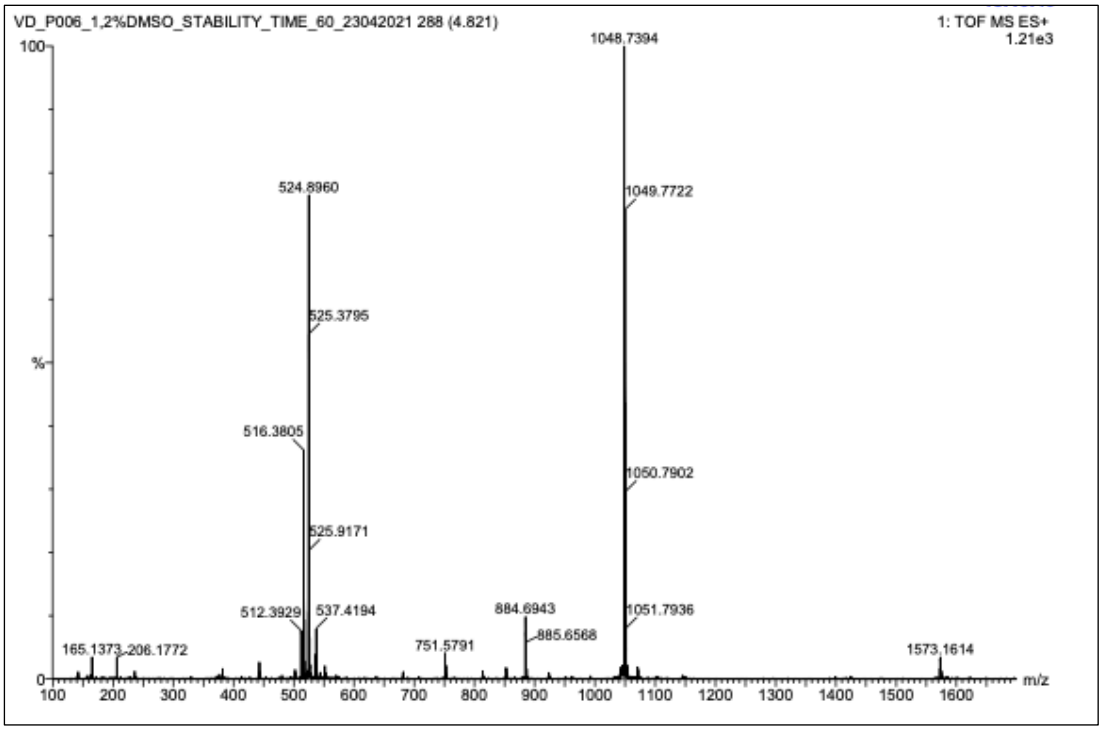



*Figure S 82. LC-MS spectrum of parent peptide detected after 60 min incubation in diluted human serum.*

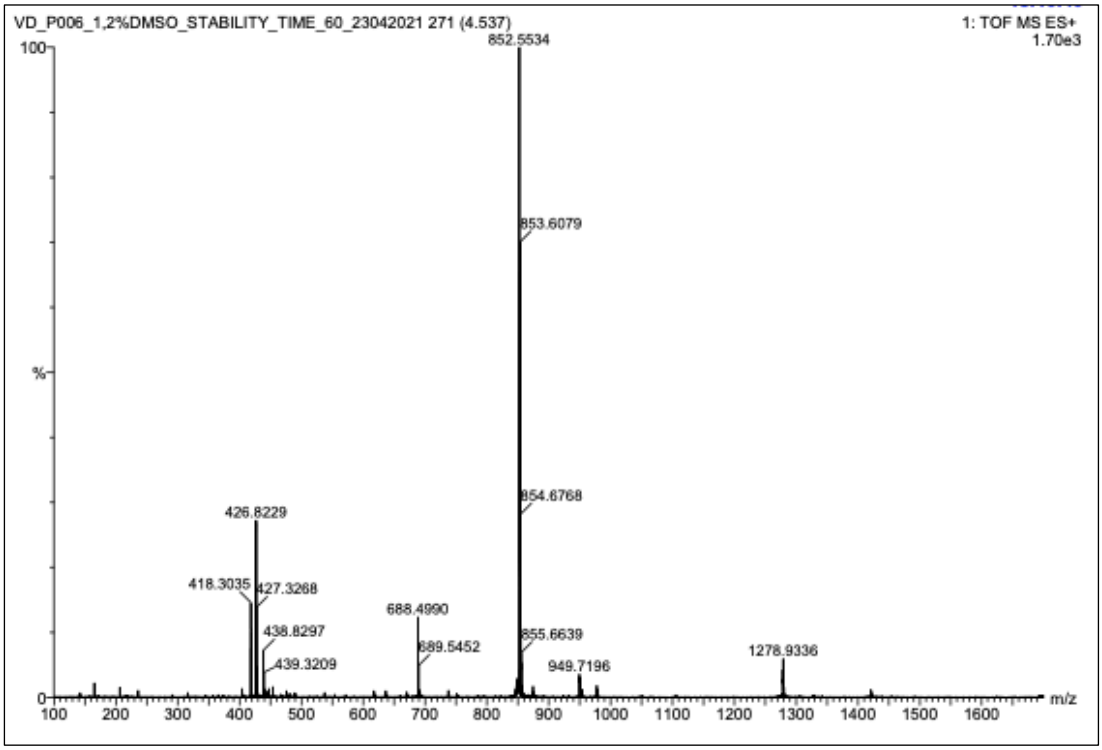



*Figure S 83. LC-MS spectrum of main metabolite detected after 60 min incubation in human serum.*

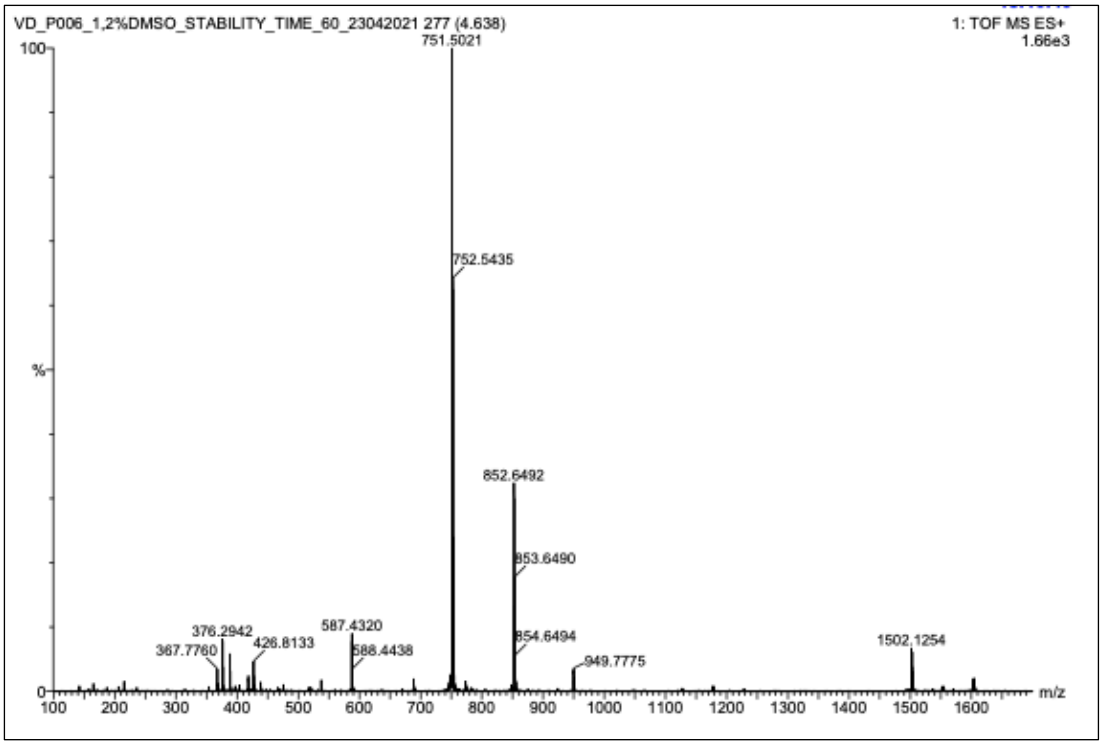



*Figure S 84. LC-MS spectrum of minor metabolite detected after 60 min incubation in human serum.*

### 7.2.4.2 LJMU011 – parent and metabolite identification after 60 min incubation

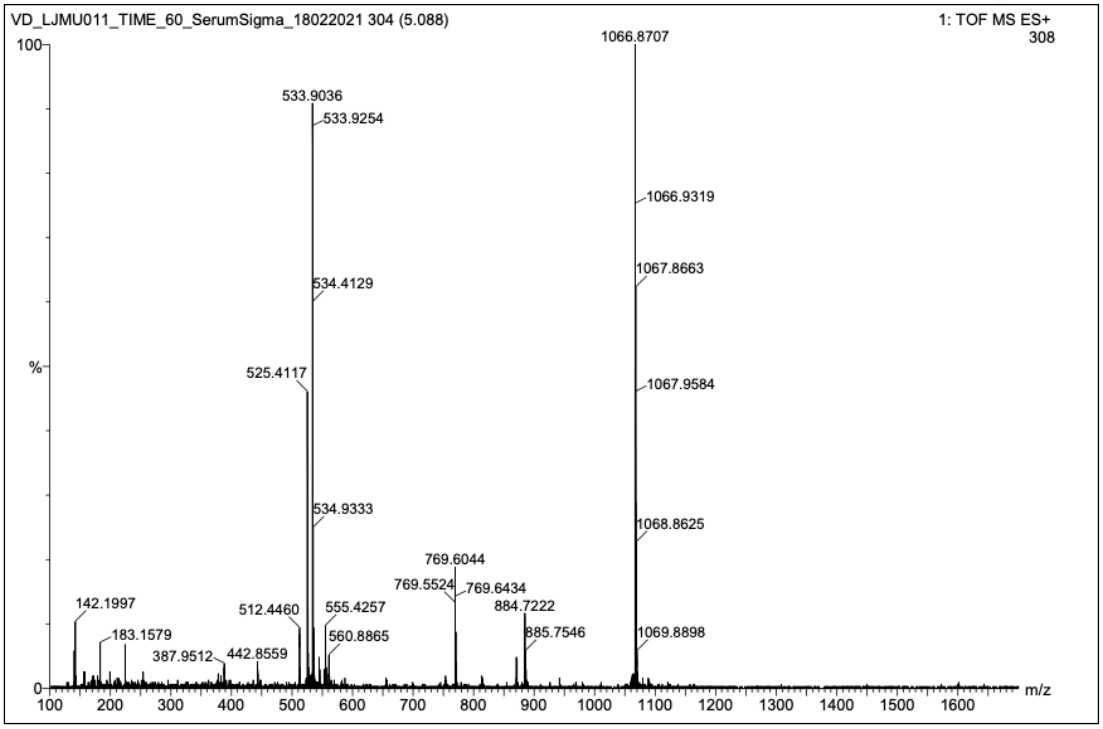



*Figure S 85. LC-MS spectrum of parent peptide detected after 60 min incubation in diluted human serum.*

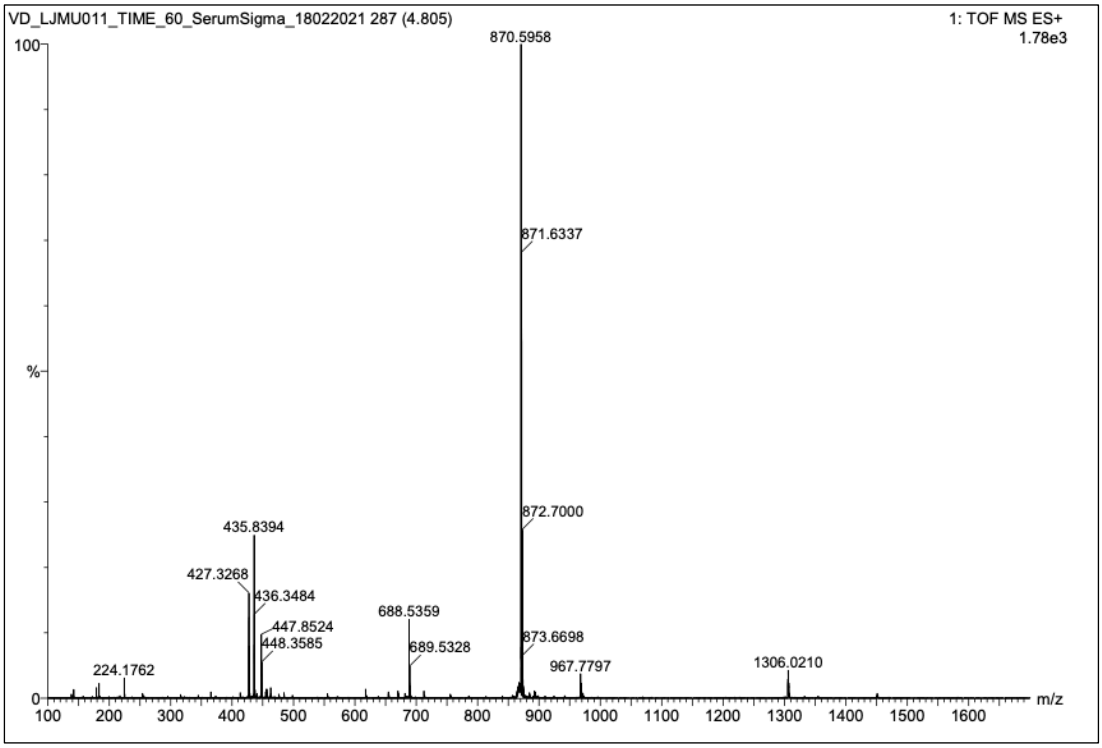



*Figure S 86. LC-MS spectrum of main metabolite detected after 60 min incubation in human serum.*

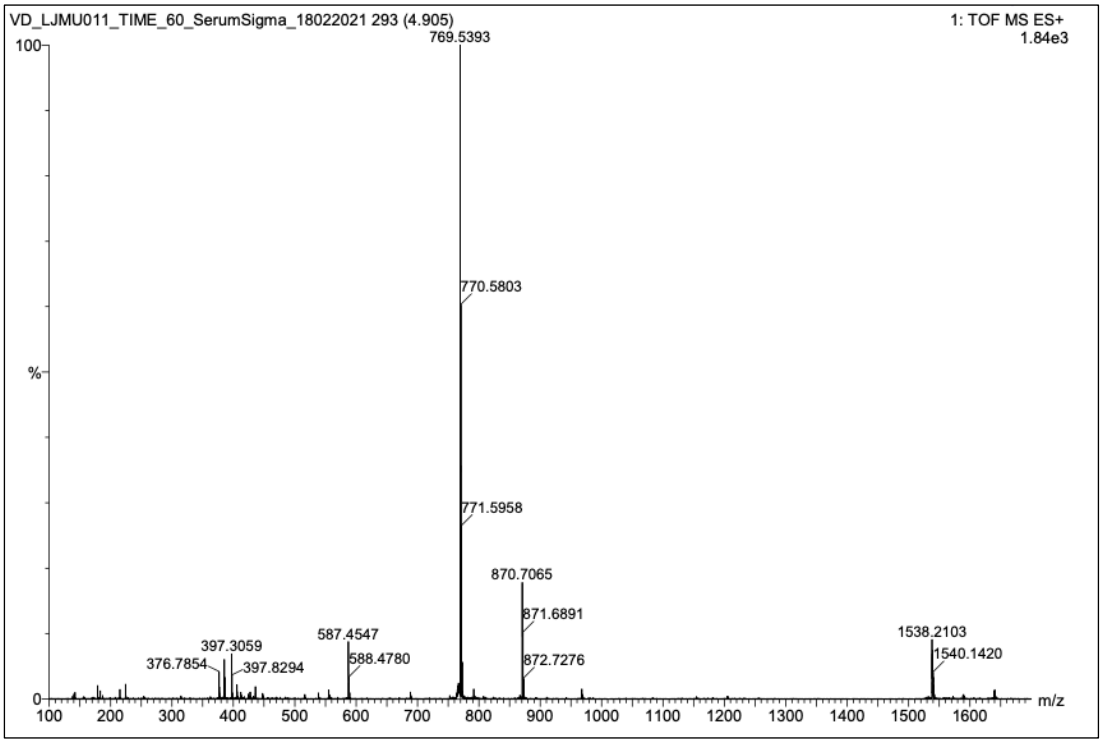



*Figure S 87. LC-MS spectrum of minor metabolite detected after 60 min incubation in human serum.*

### 7.2.4.3 LJMU012 – parent and metabolite identification after 60 min incubation

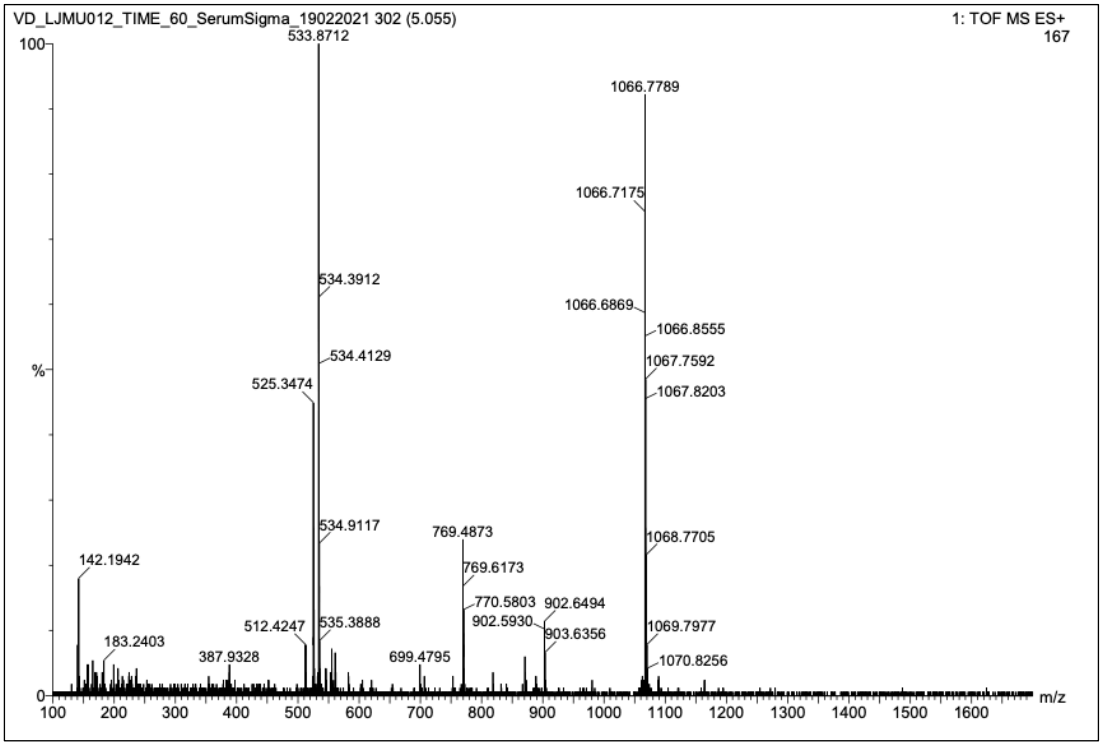



*Figure S 88. LC-MS spectrum of parent peptide detected after 60 min incubation in diluted human serum.*

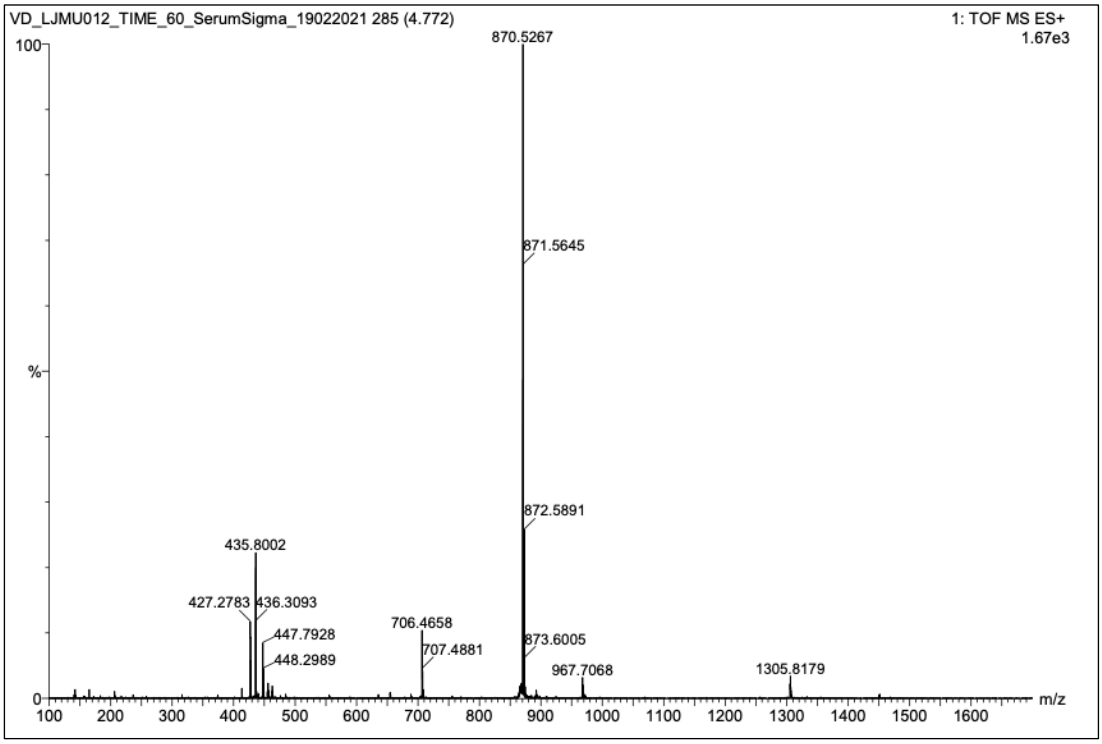



*Figure S 89. LC-MS spectrum of main metabolite detected after 60 min incubation in human serum.*

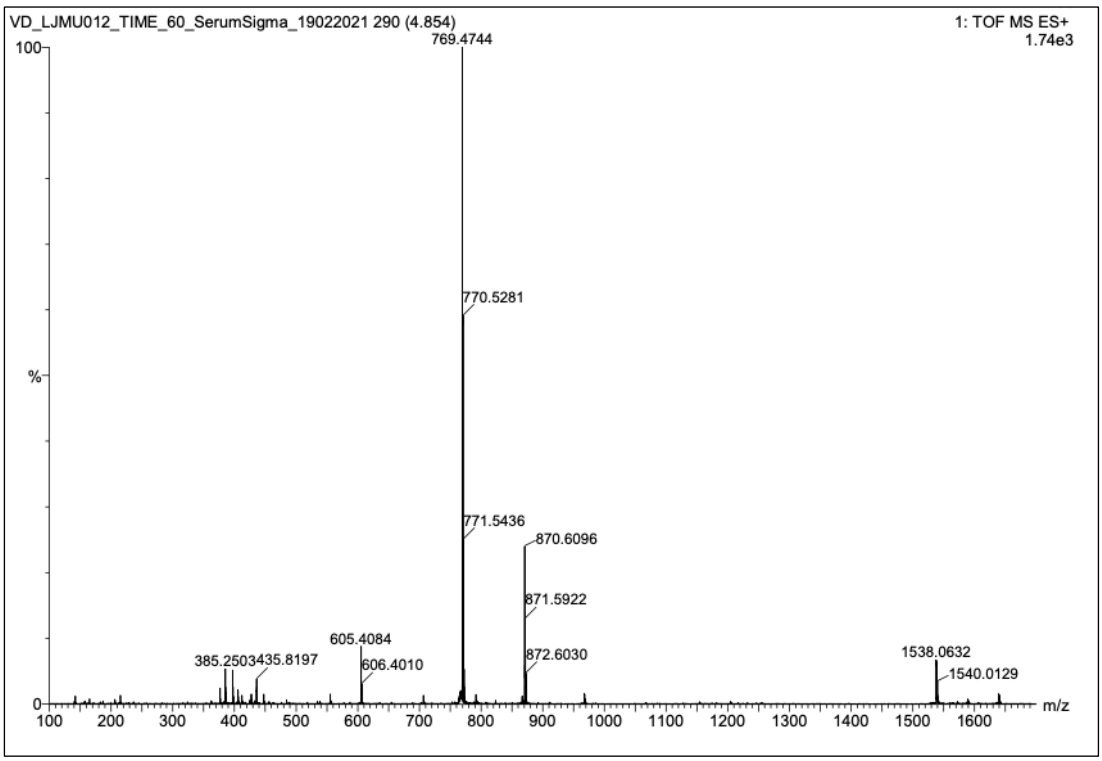



*Figure S 90. LC-MS spectrum of minor metabolite detected after 60 min incubation in human serum.*

### 7.2.4.4 LJMU013 – parent and metabolite identification after 60 min incubation

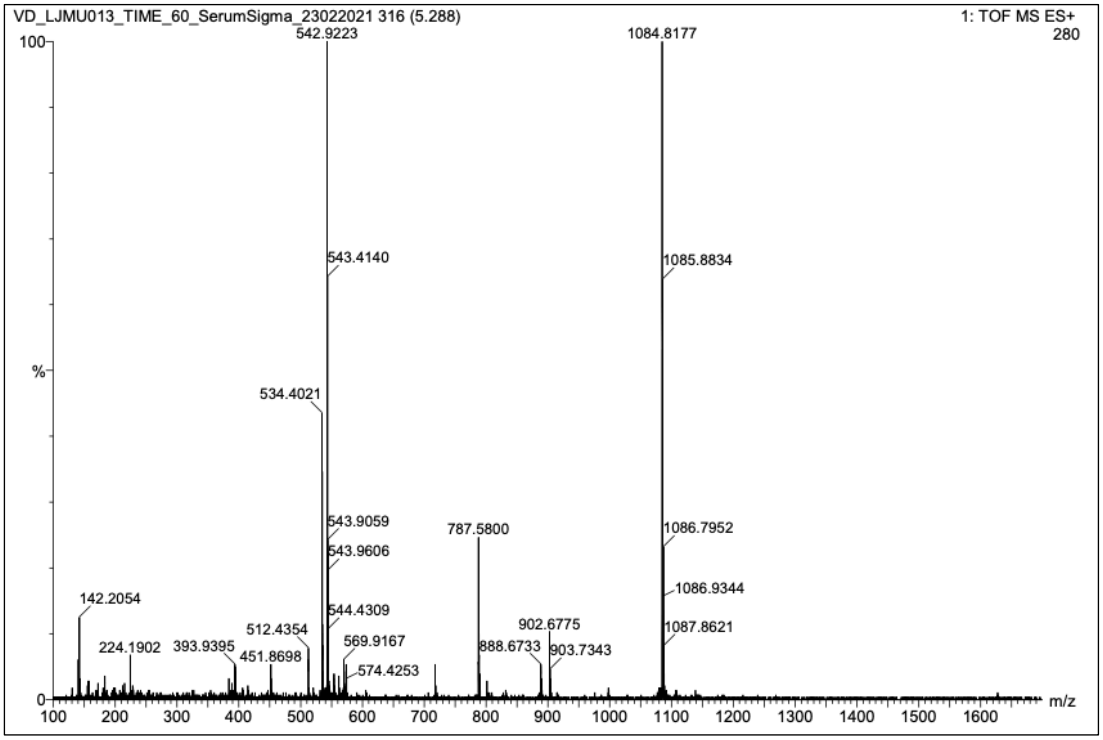



*Figure S 91. LC-MS spectrum of parent peptide detected after 60 min incubation in diluted human serum.*

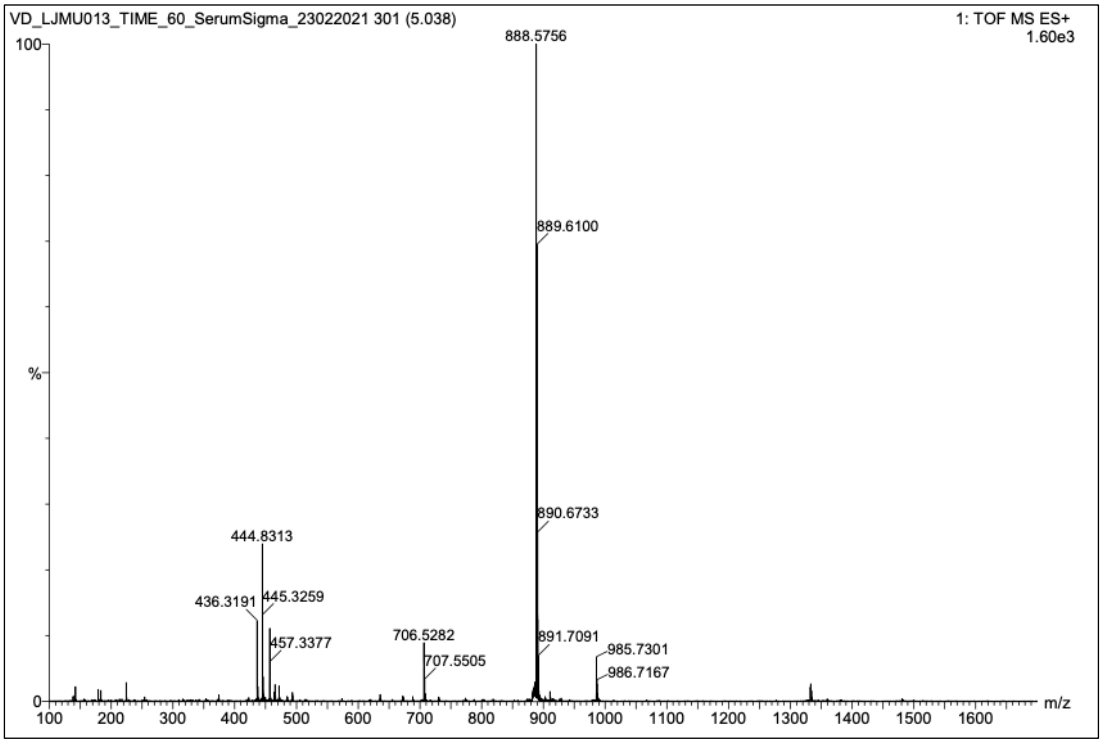



*Figure S 92. LC-MS spectrum of main metabolite detected after 60 min incubation in human serum.*

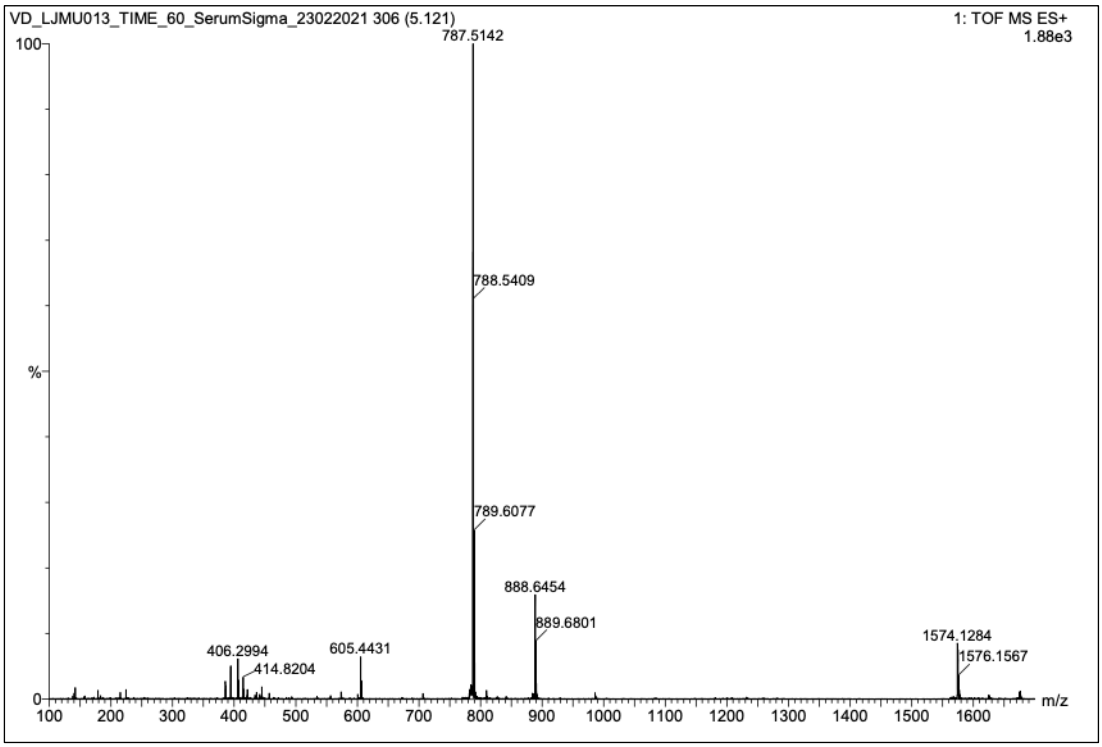



*Figure S 93. LC-MS spectrum of minor metabolite detected after 60 min incubation in human serum.*

### 7.2.4.5 LJMU014 – parent and metabolite identification after 60 min incubation

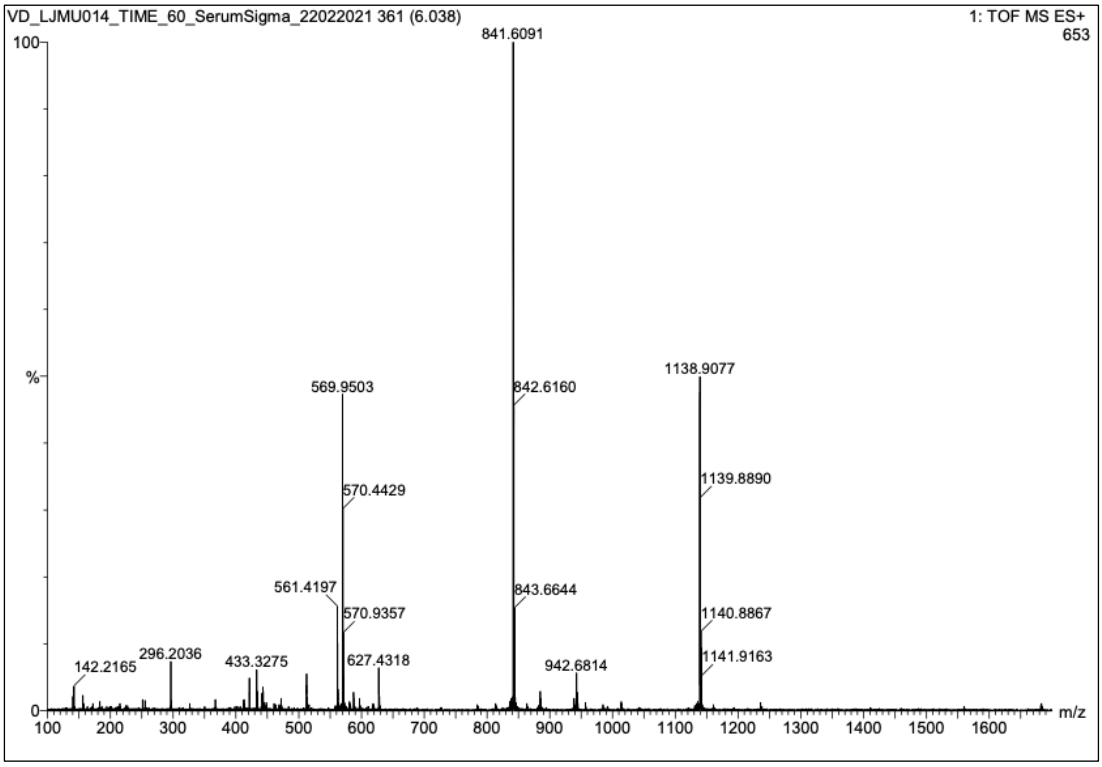



*Figure S 94. LC-MS spectrum of parent peptide detected after 60 min incubation in diluted human serum.*

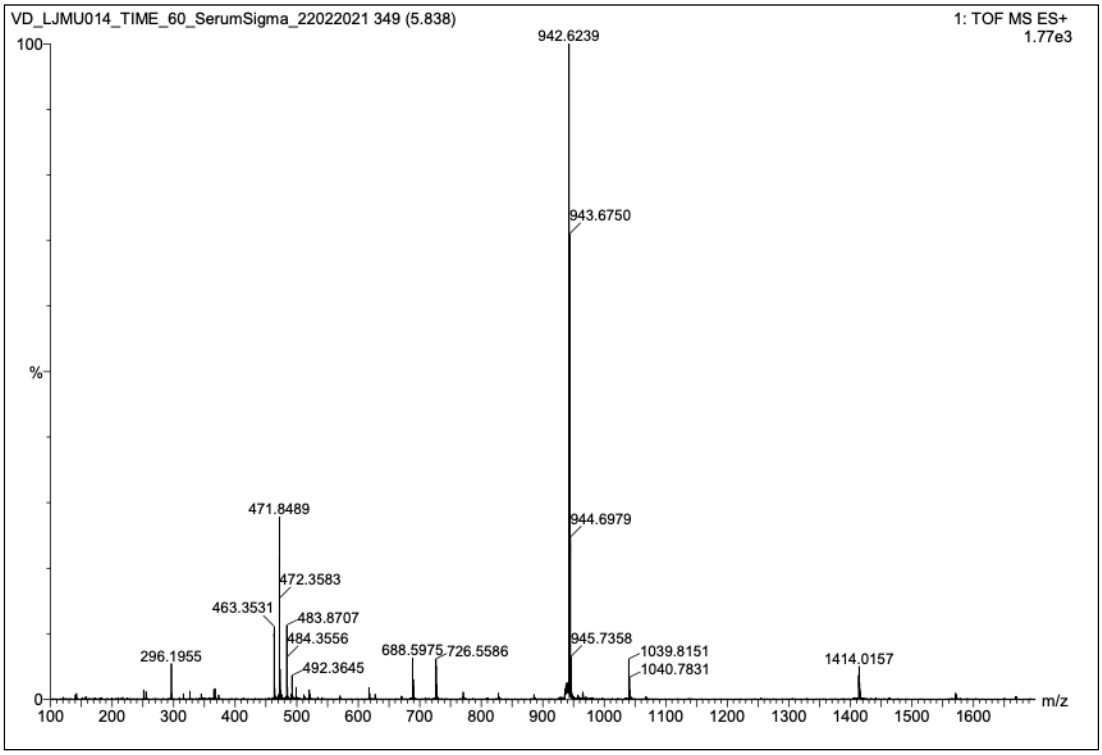



*Figure S 95. LC-MS spectrum of main metabolite detected after 60 min incubation in human serum.*

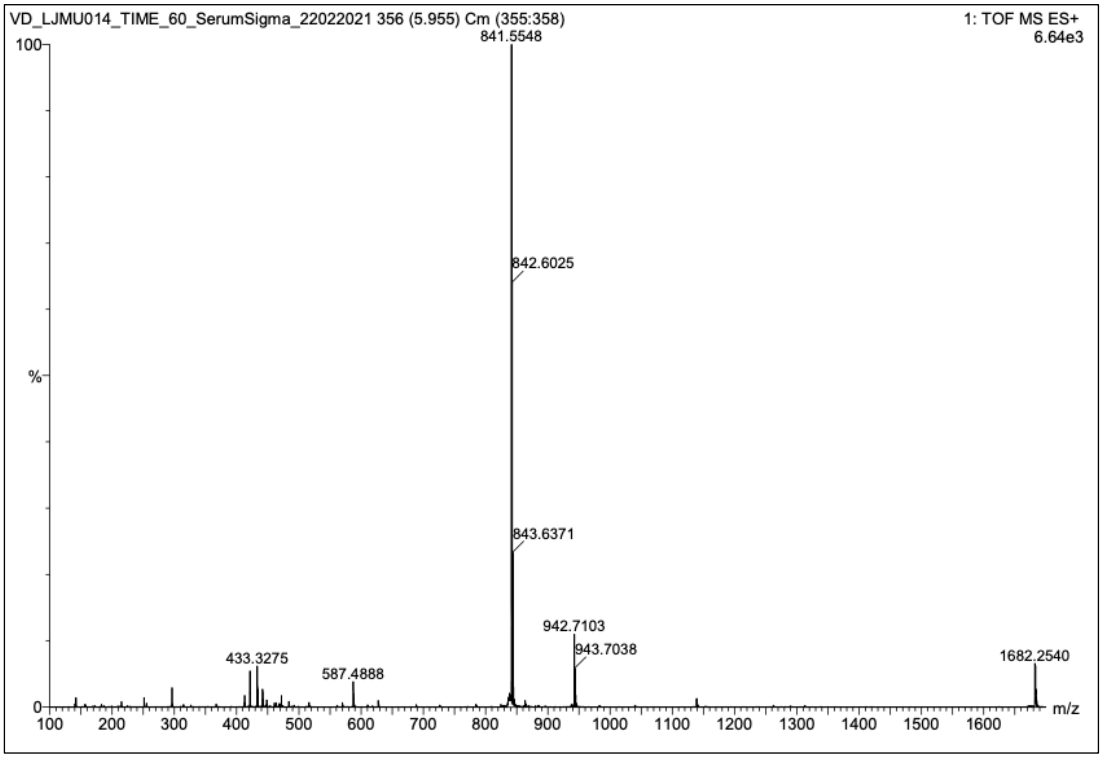



*Figure S 96. LC-MS spectrum of minor metabolite detected after 60 min incubation in human serum.*

### *7.2.4.6 LJMU015 – parent and metabolite identification after 60 min incubation*

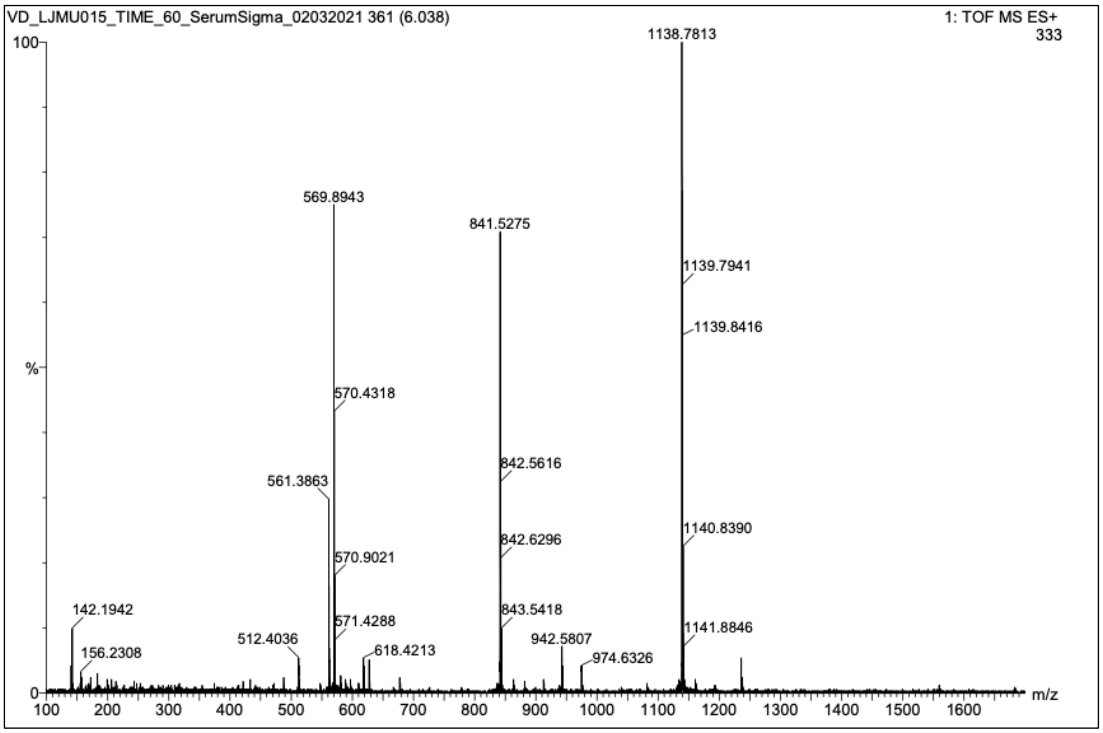



*Figure S 97. LC-MS spectrum of parent peptide detected after 60 min incubation in diluted human serum.*

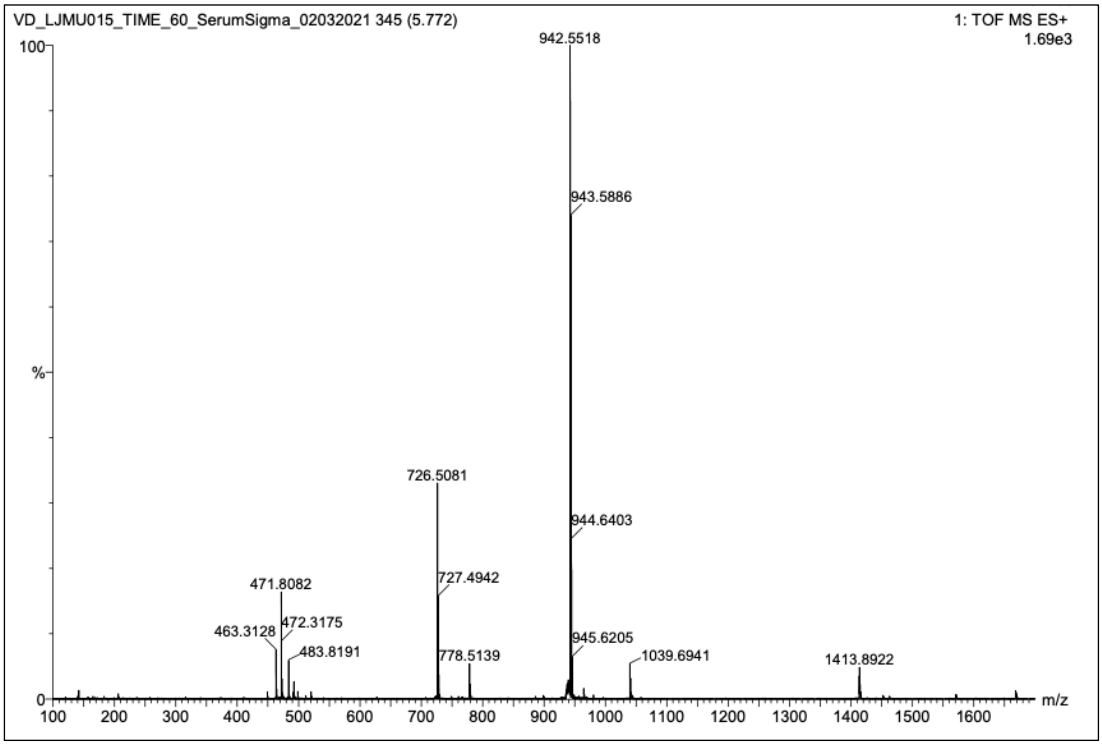



*Figure S 98. LC-MS spectrum of main metabolite detected after 60 min incubation in human serum.*

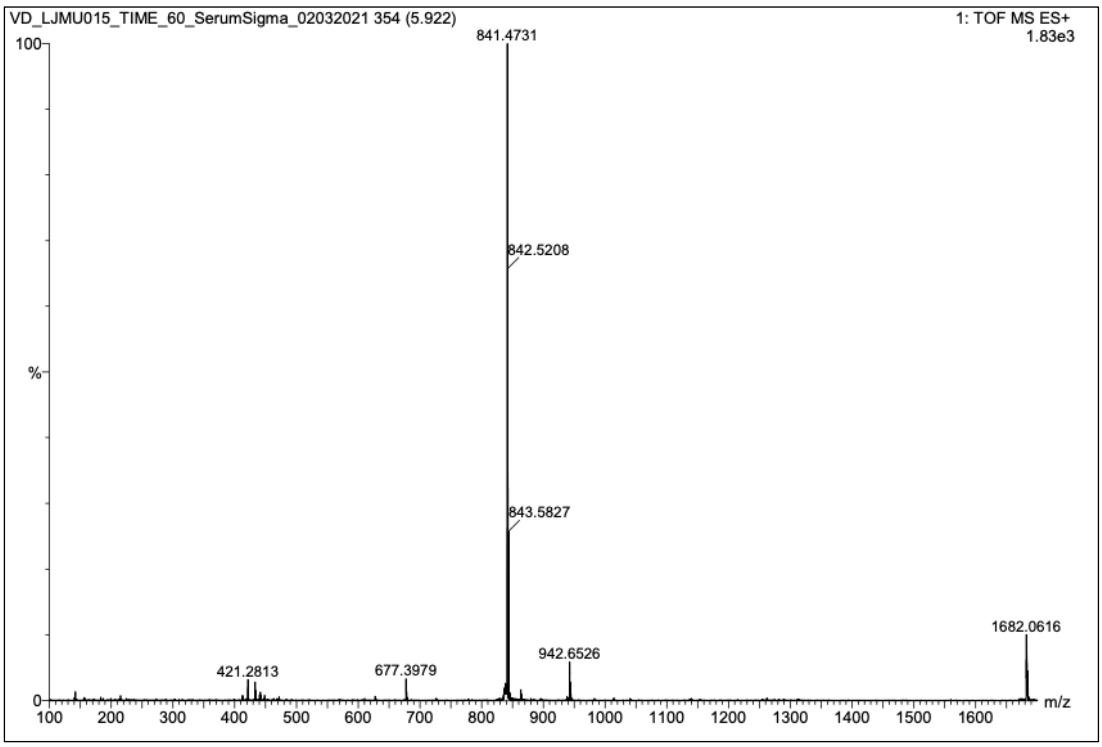



*Figure S 99. LC-MS spectrum of minor metabolite detected after 60 min incubation in human serum.*

### *7.2.4.7 LJMU016 – parent and metabolite identification after 60 min incubation*

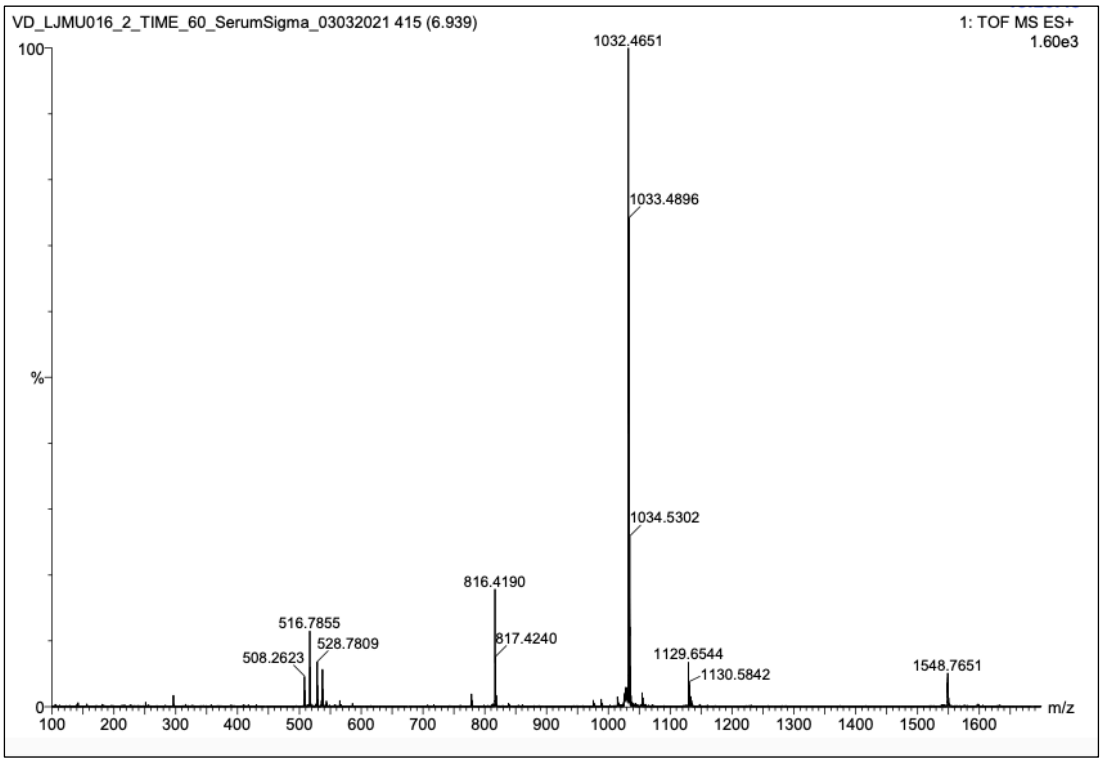



*Figure S 100. LC-MS spectrum of main metabolite detected after 60 min incubation in human serum.*

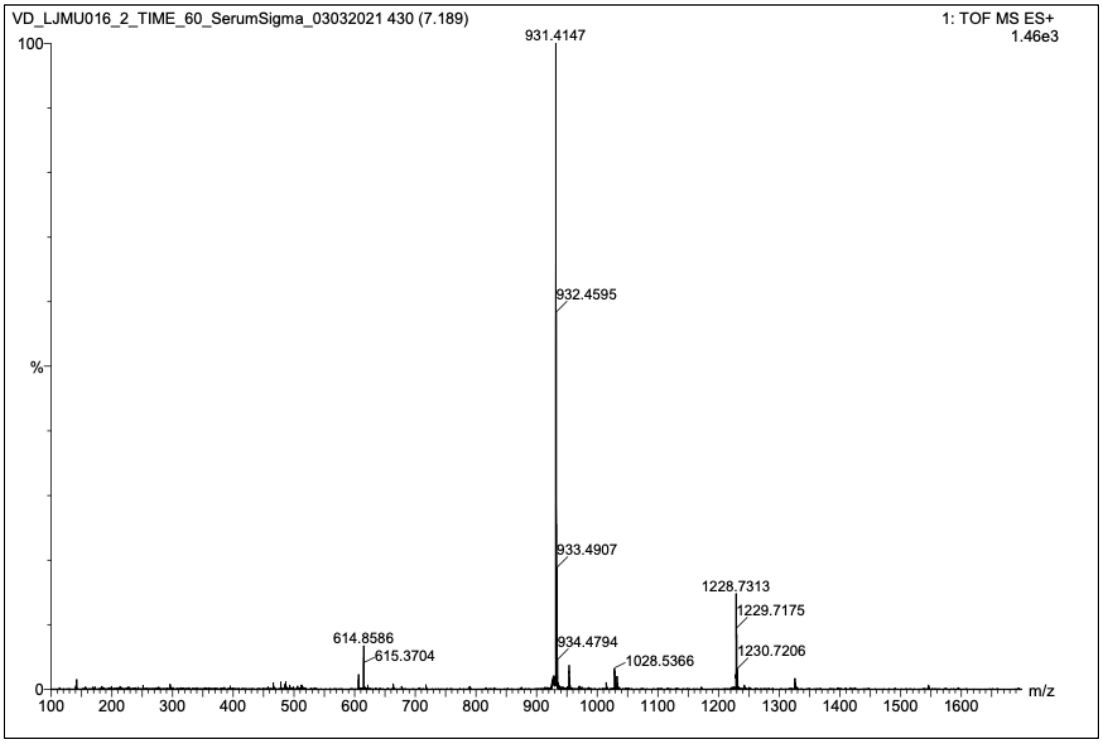



*Figure S 101. LC-MS spectrum of minor metabolite detected after 60 min incubation in human serum.*

### 7.2.4.8 LJMU017 – parent and metabolite identification after 60 min incubation

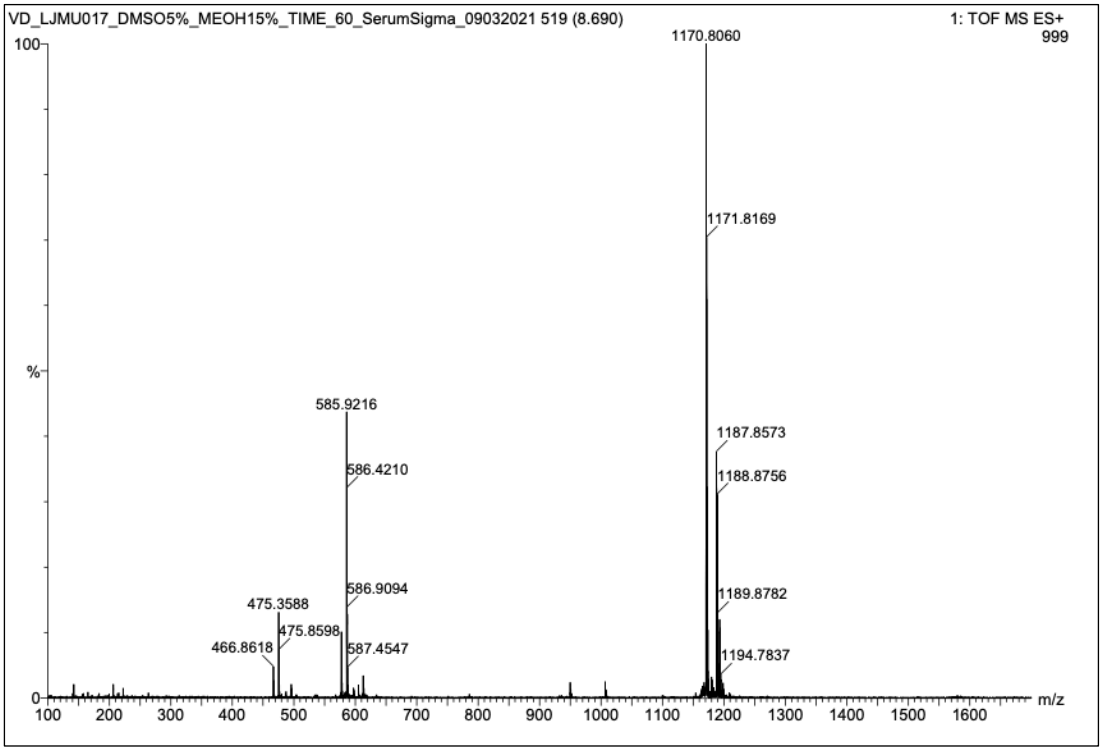



*Figure S 102. LC-MS spectrum of parent peptide detected after 60 min incubation in diluted human serum.*

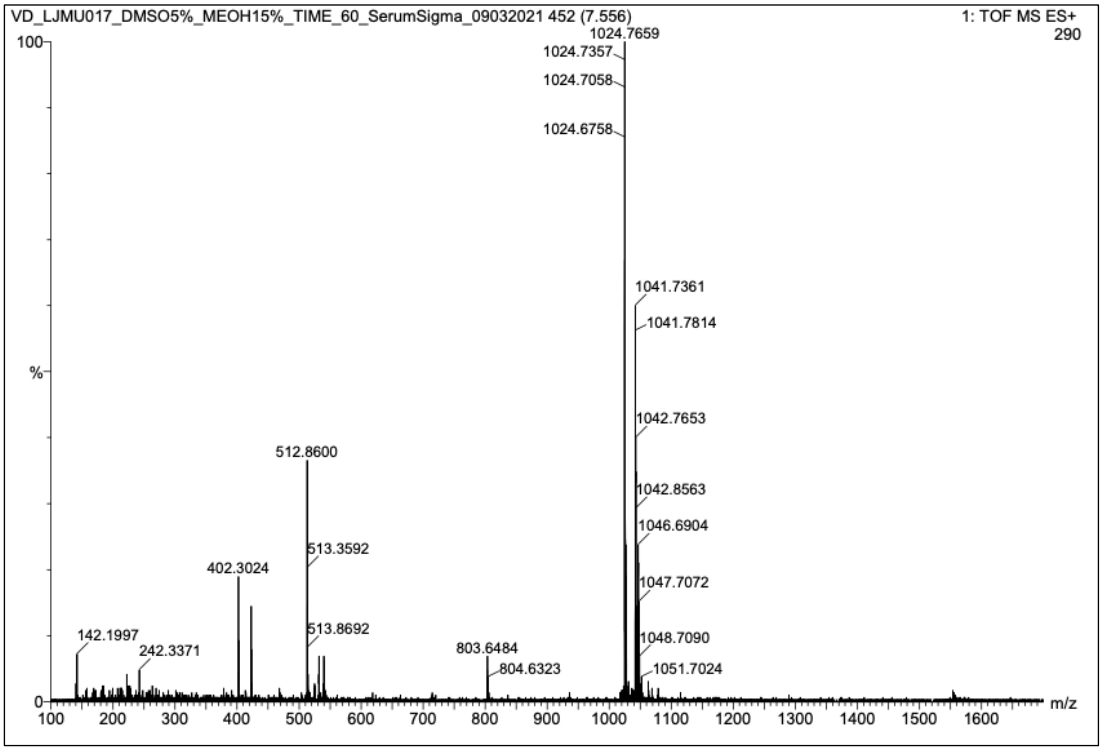



*Figure S 103. LC-MS spectrum of main metabolite detected after 60 min incubation in human serum.*

### 7.2.4.9 LJMU018 – parent and metabolite identification after 60 min incubation

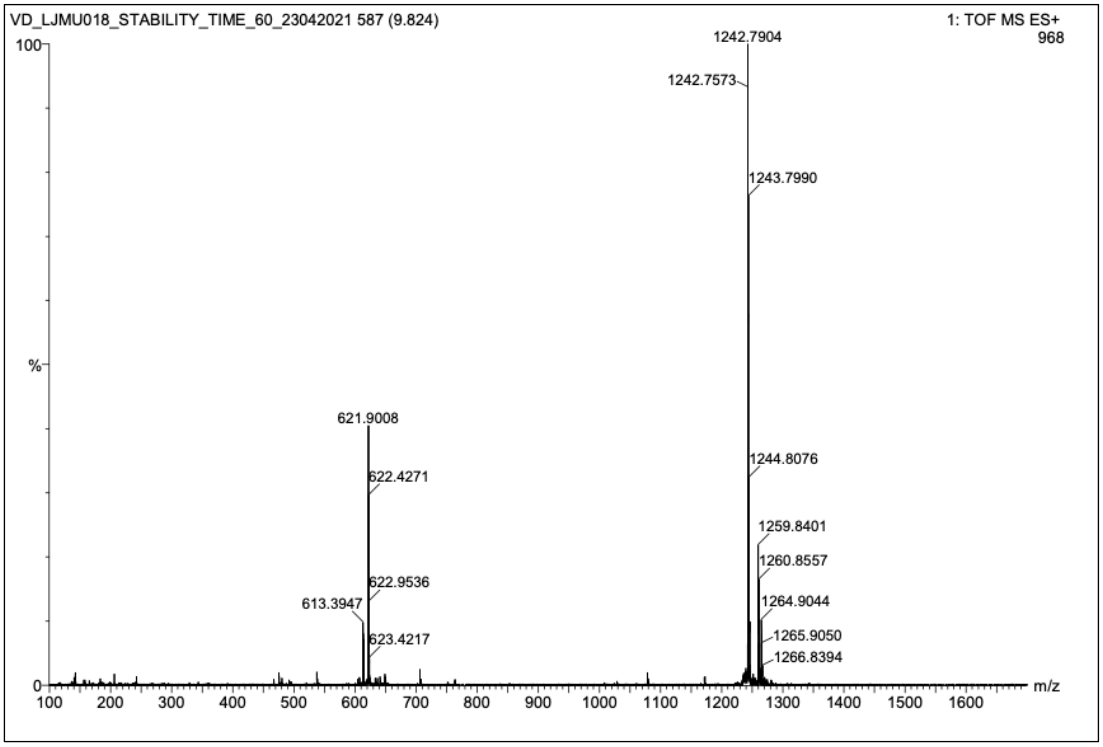



*Figure S 104. LC-MS spectrum of parent peptide detected after 60 min incubation in diluted human serum.*

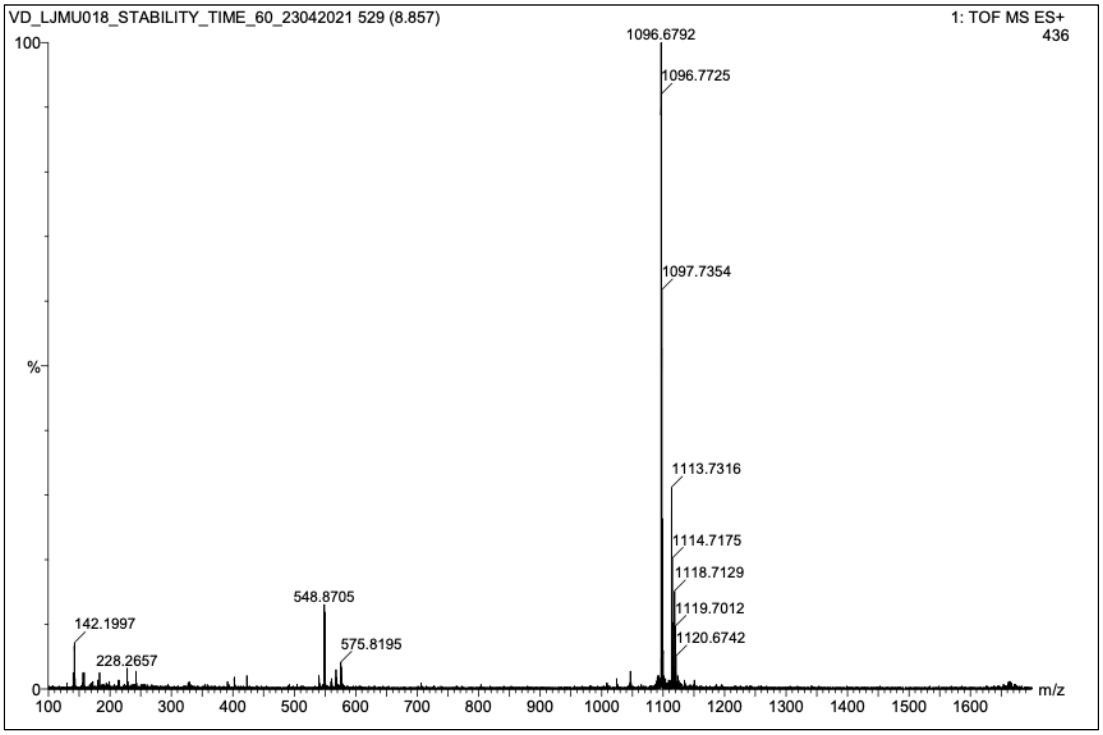



*Figure S 105. LC-MS spectrum of main metabolite detected after 60 min incubation in human serum.*

### *7.2.4.10 LJMU019 – parent and metabolite identification after 60 min incubation*

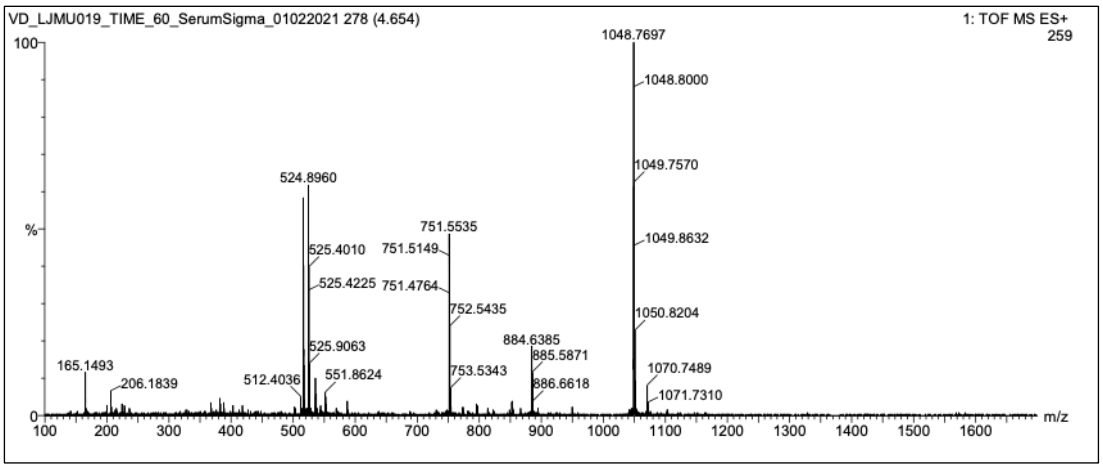



*Figure S 106. LC-MS spectrum of parent peptide detected after 60 min incubation in diluted human serum.*

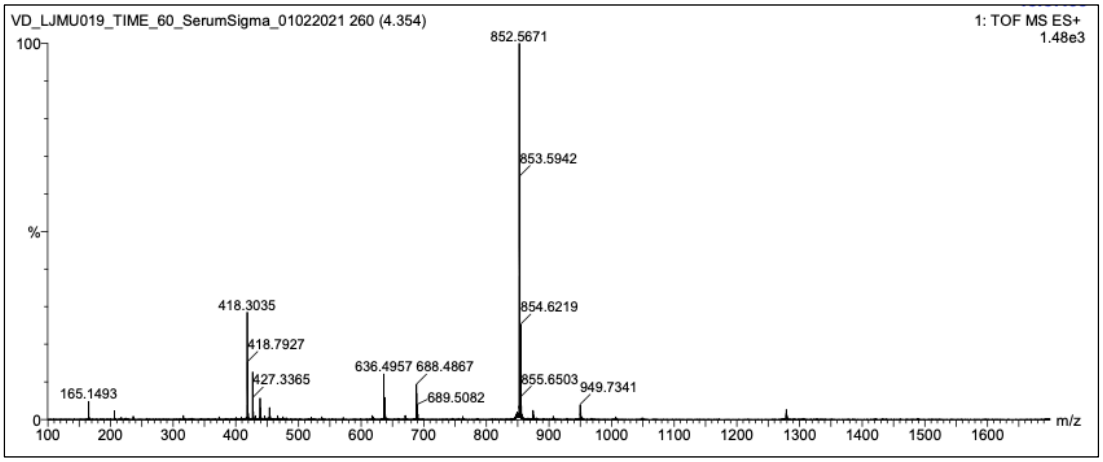



*Figure S 107. LC-MS spectrum of main metabolite detected after 60 min incubation in human serum.*

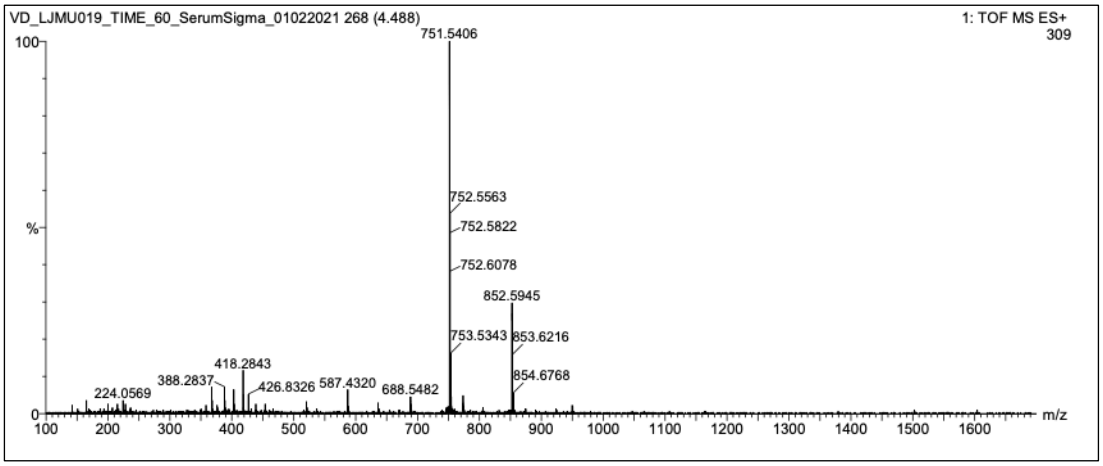



*Figure S 108. LC-MS spectrum of minor metabolite detected after 60 min incubation in human serum.*

### *7.2.4.11 LJMU020 – parent and metabolite identification after 60 min incubation*

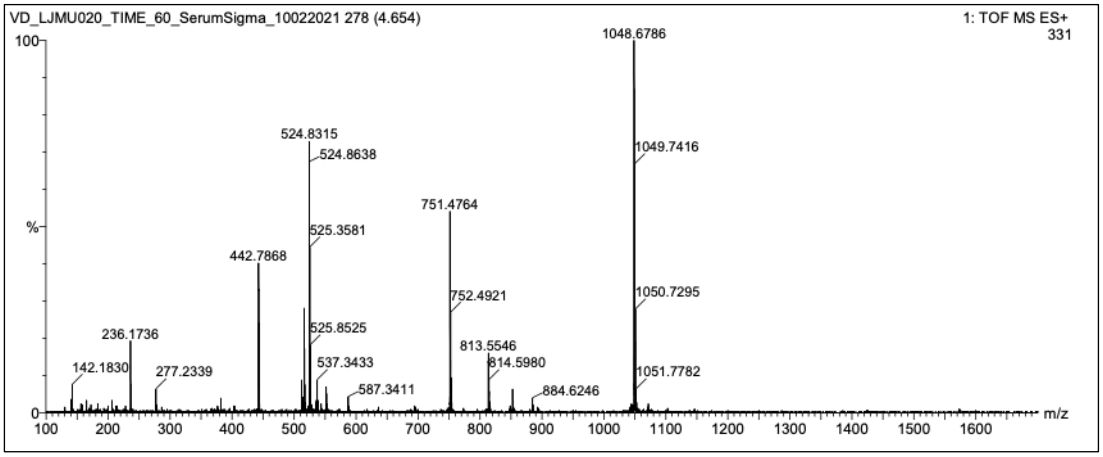



*Figure S 109. LC-MS spectrum of parent peptide detected after 60 min incubation in diluted human serum.*

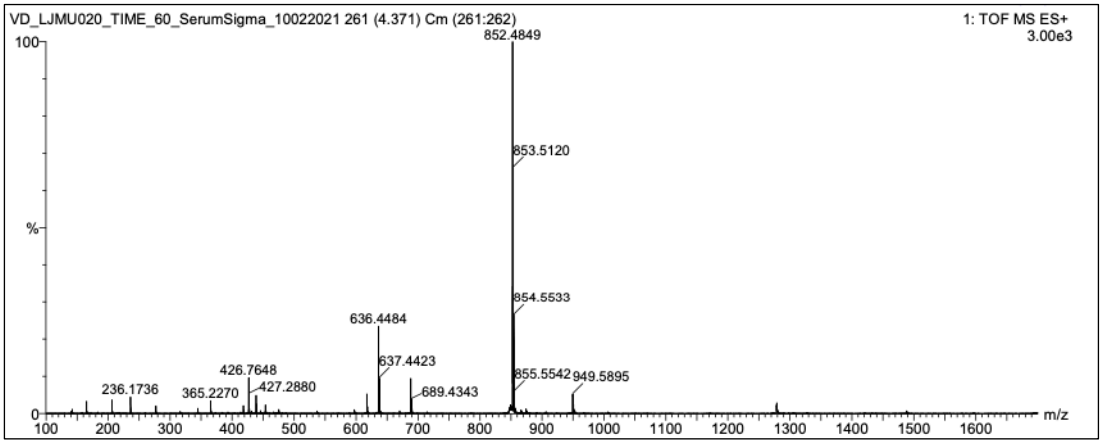



*Figure S 110. LC-MS spectrum of main metabolite detected after 60 min incubation in human serum.*

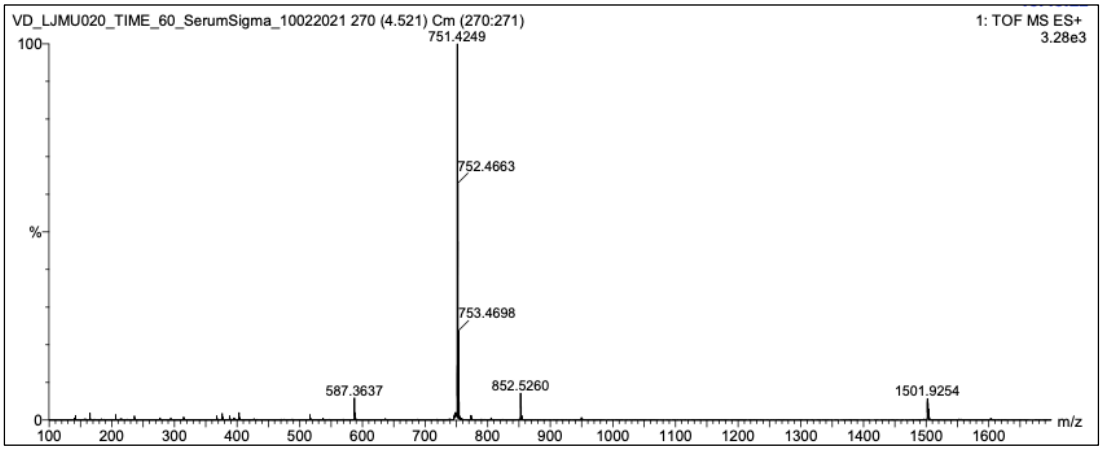



*Figure S 111. LC-MS spectrum of minor metabolite detected after 60 min incubation in human serum.*

### *7.2.4.12 LJMU021 – parent and metabolite identification after 60 min incubation*

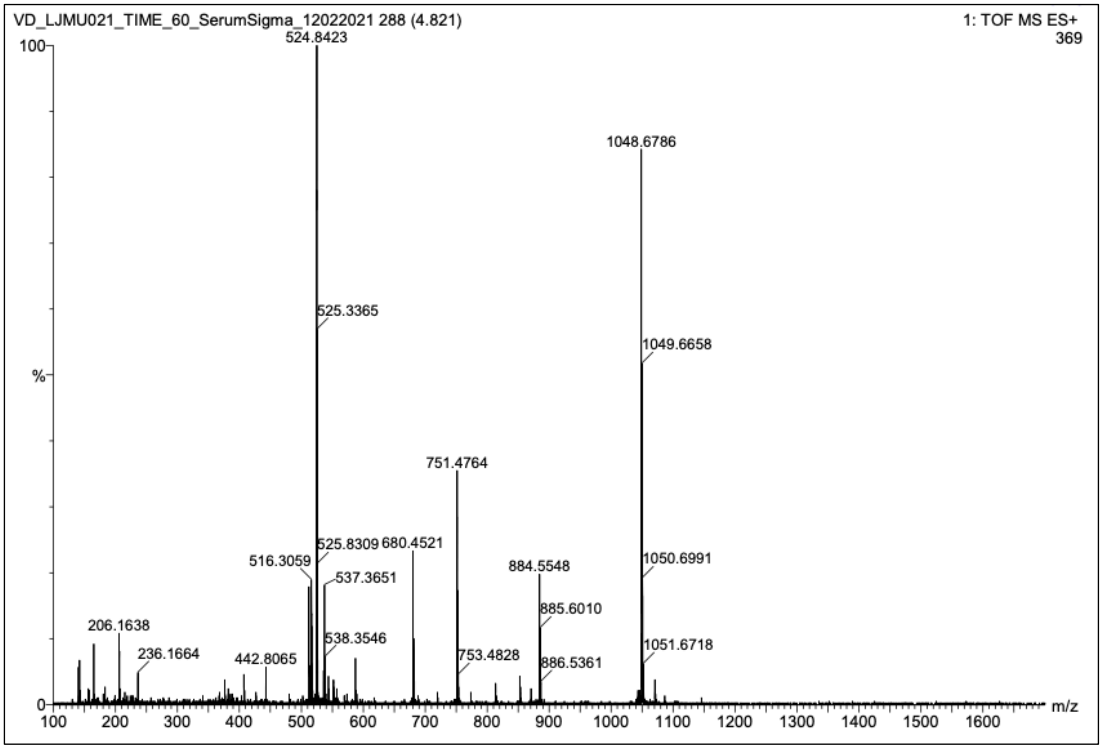



*Figure S 112. LC-MS spectrum of parent peptide detected after 60 min incubation in diluted human serum.*

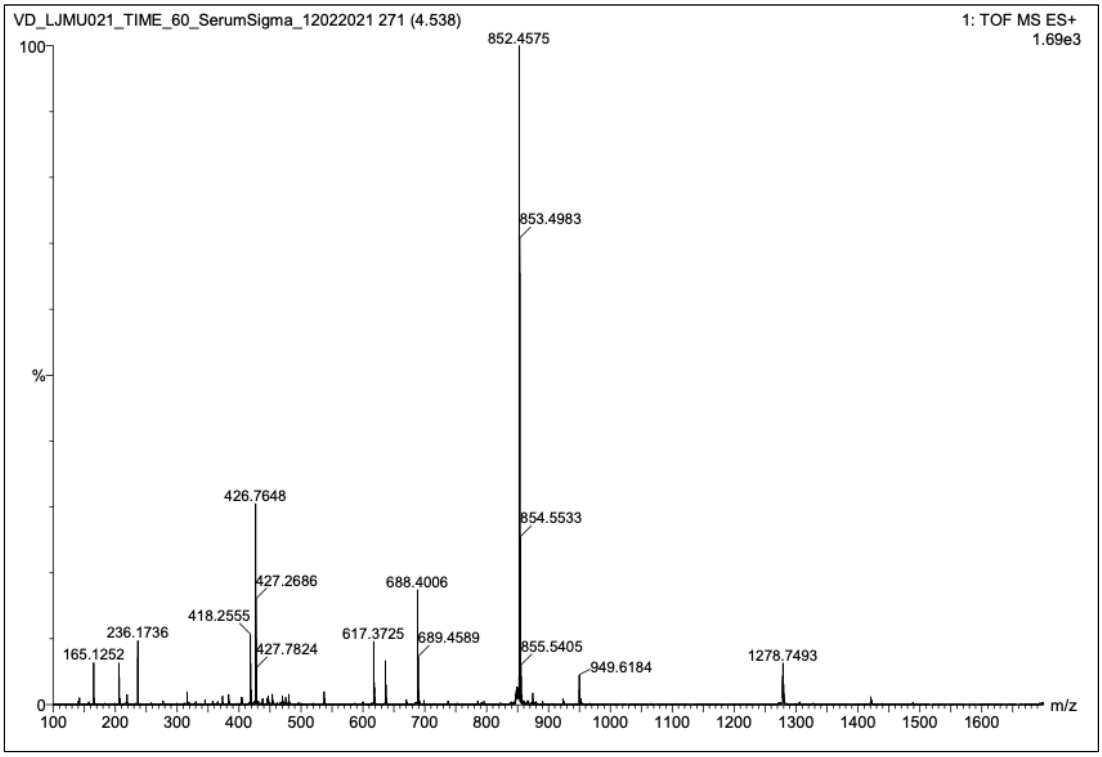



*Figure S 113. LC-MS spectrum of main metabolite detected after 60 min incubation in human serum.*

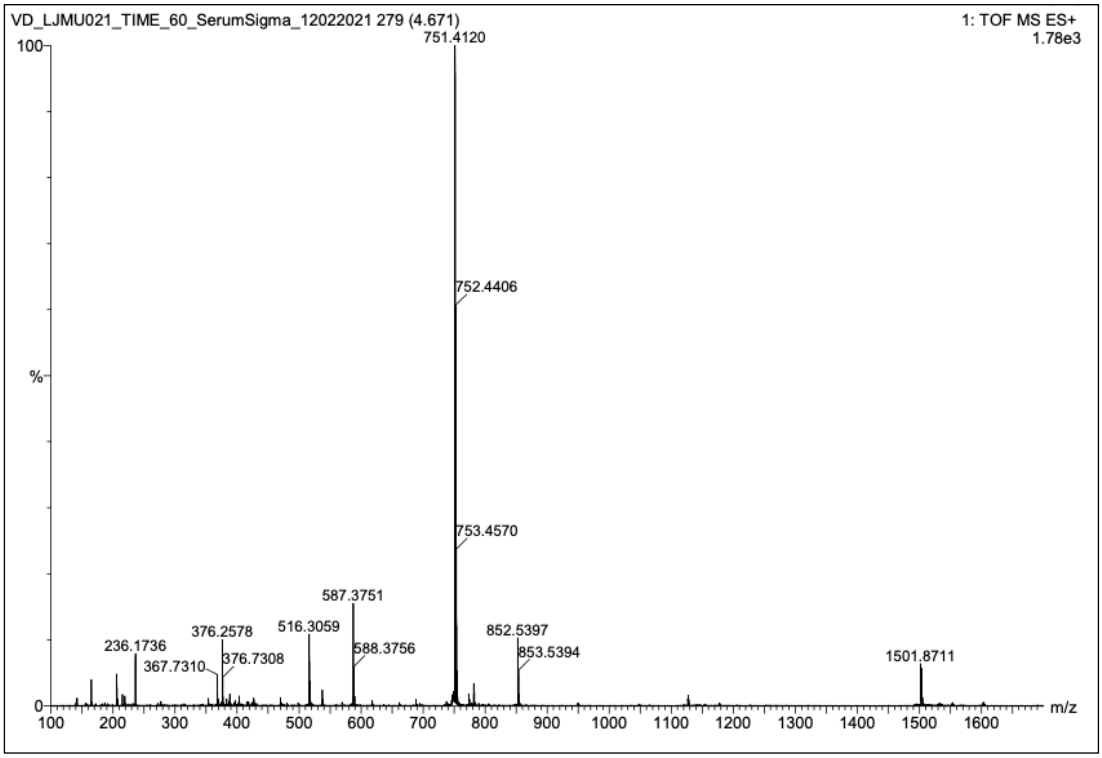



*Figure S 114. LC-MS spectrum of minor metabolite detected after 60 min incubation in human serum.*

### *7.2.4.13 LJMU022 – parent and metabolite identification after 60 min incubation*

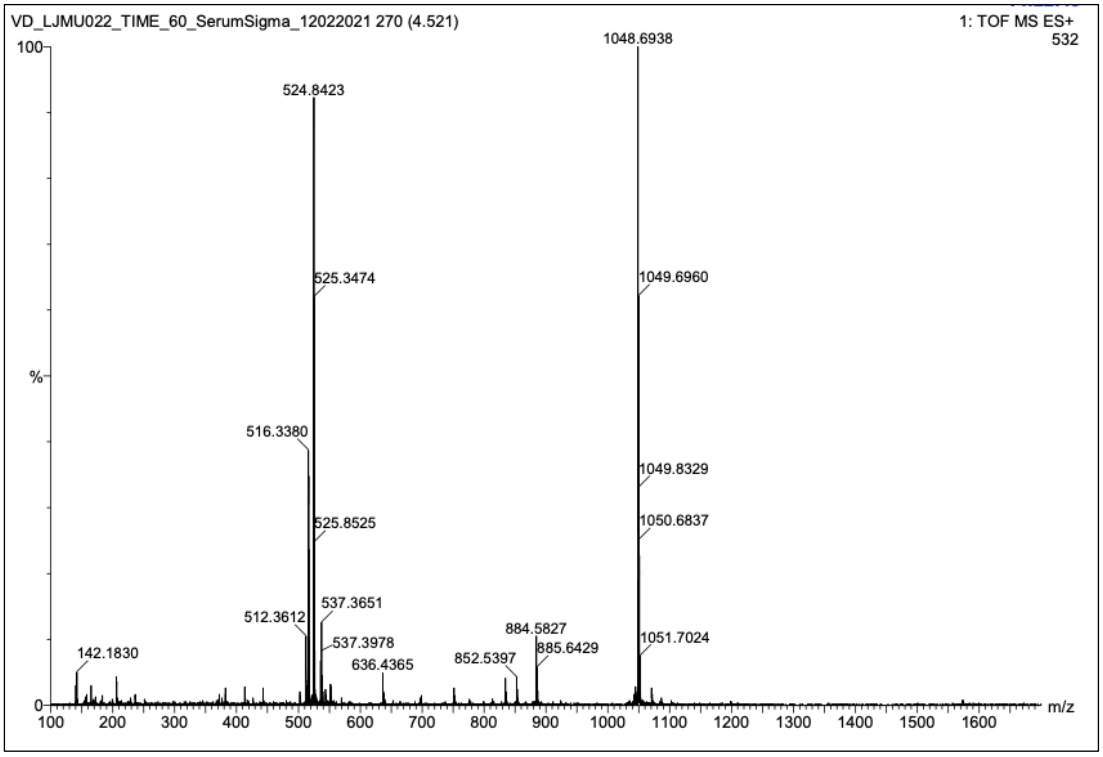



*Figure S 115. LC-MS spectrum of parent peptide detected after 60 min incubation in diluted human serum.*

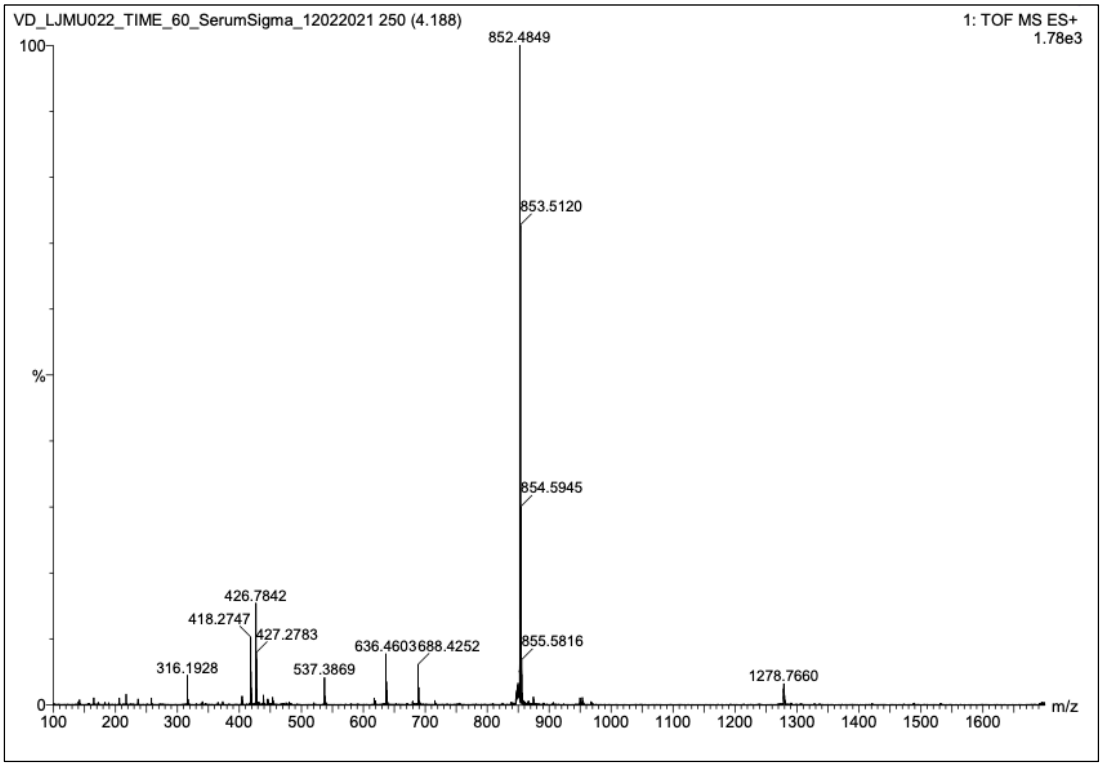



*Figure S 116. LC-MS spectrum of main metabolite detected after 60 min incubation in human serum.*

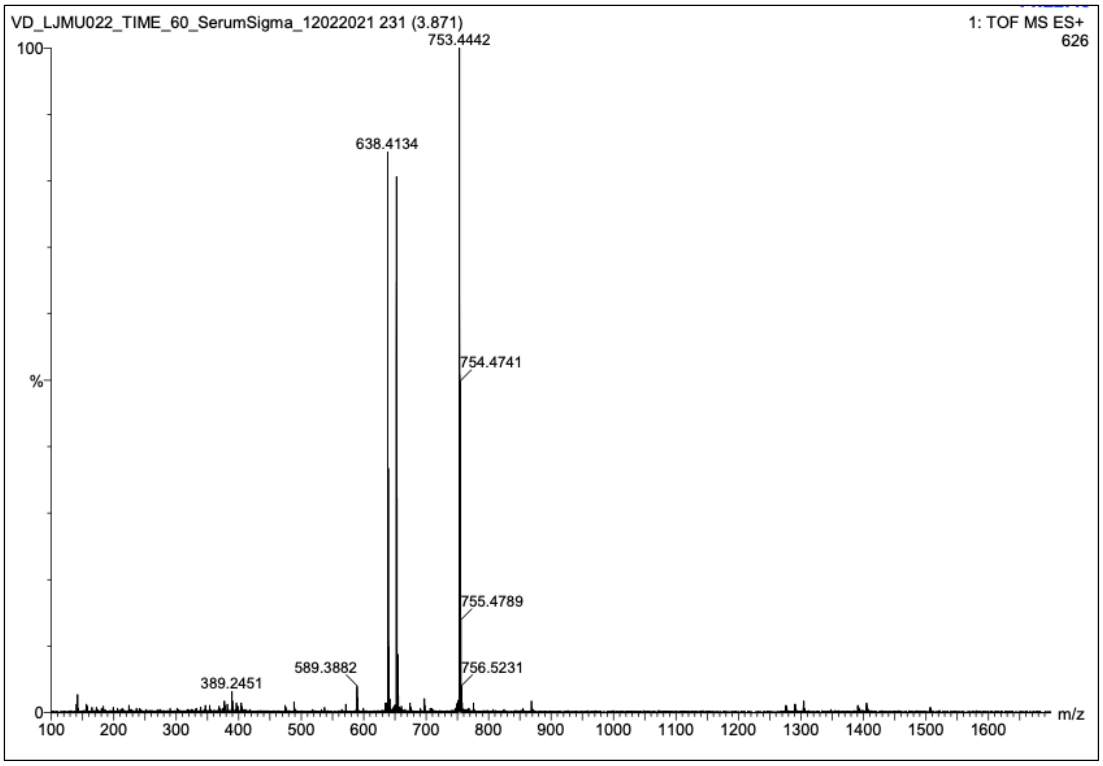



*Figure S 117. LC-MS spectrum of minor metabolite detected after 60 min incubation in human serum.*

### 7.2.4.14 LJMU023 – parent and metabolite identification after 60 min incubation

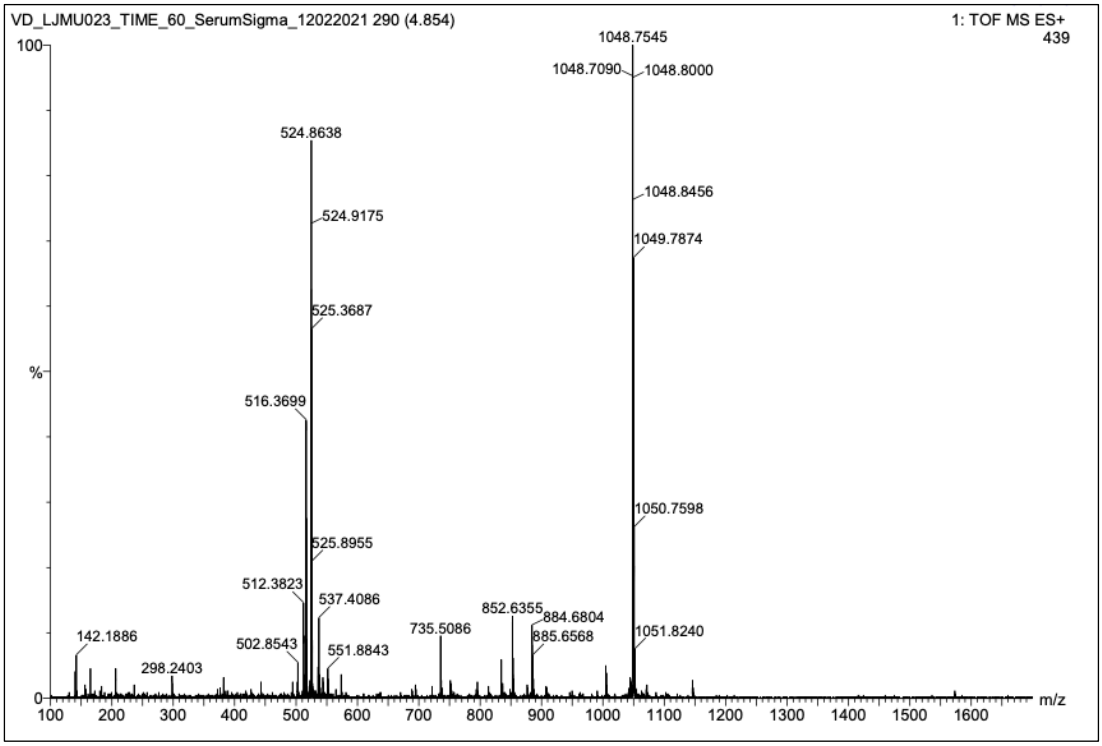



*Figure S 118. LC-MS spectrum of parent peptide detected after 60 min incubation in diluted human serum.*

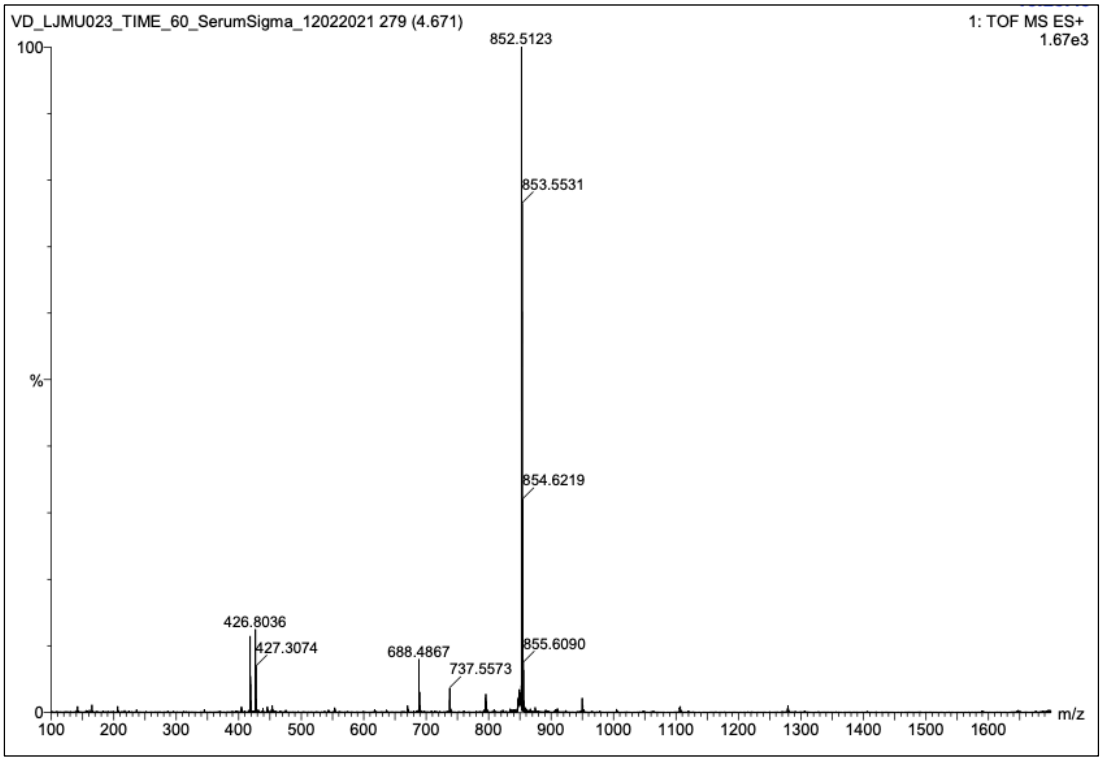



*Figure S 119. LC-MS spectrum of main metabolite detected after 60 min incubation in human serum.*

### *7.2.4.15 LJMU024 – parent and metabolite identification after 60 min incubation*

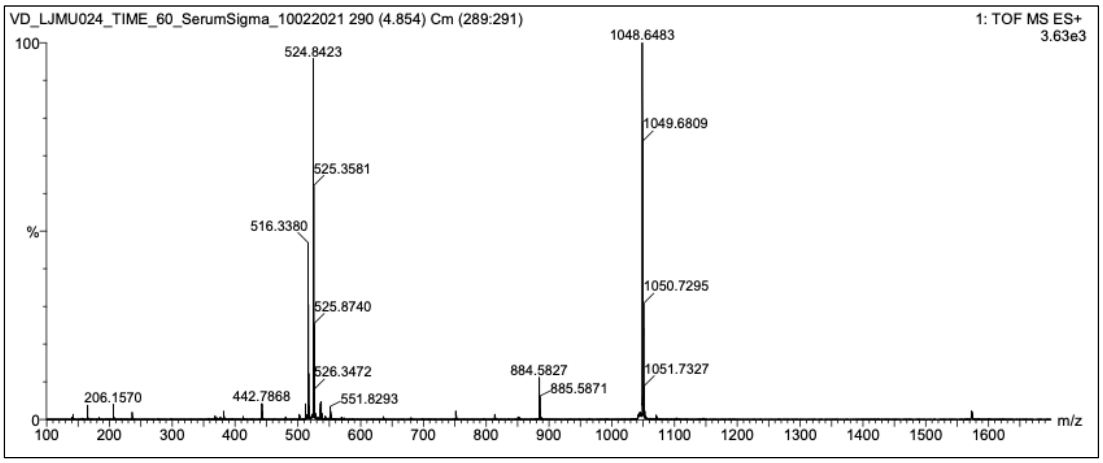



*Figure S 120. LC-MS spectrum of parent peptide detected after 60 min incubation in diluted human serum.*

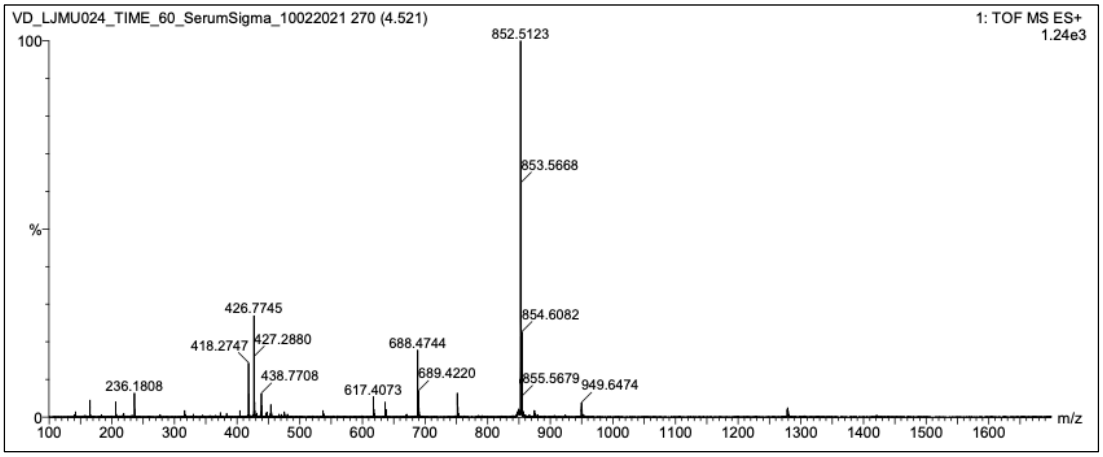



*Figure S 121. LC-MS spectrum of main metabolite detected after 60 min incubation in human serum.*

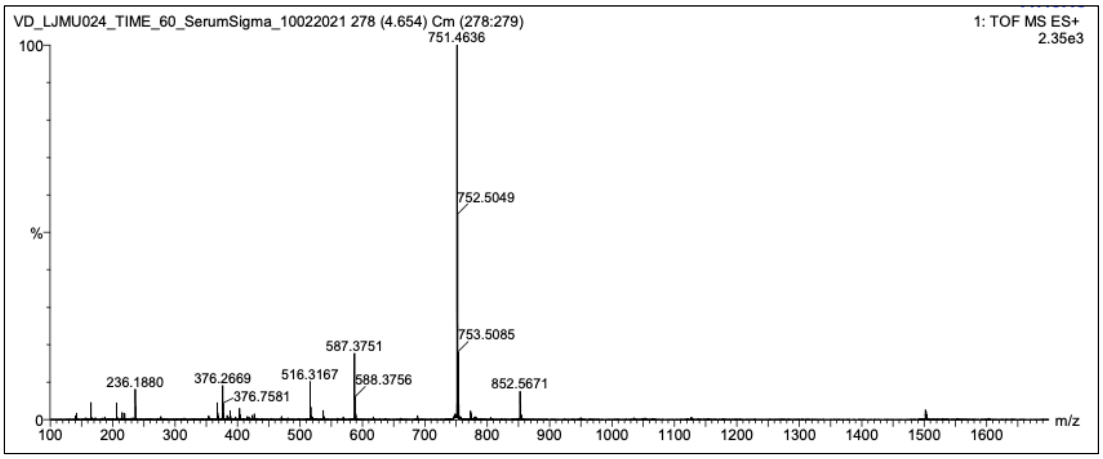



*Figure S 122. LC-MS spectrum of minor metabolite detected after 60 min incubation in human serum.*

### *7.2.4.16 LJMU027 – parent and metabolite identification after 60 min incubation*

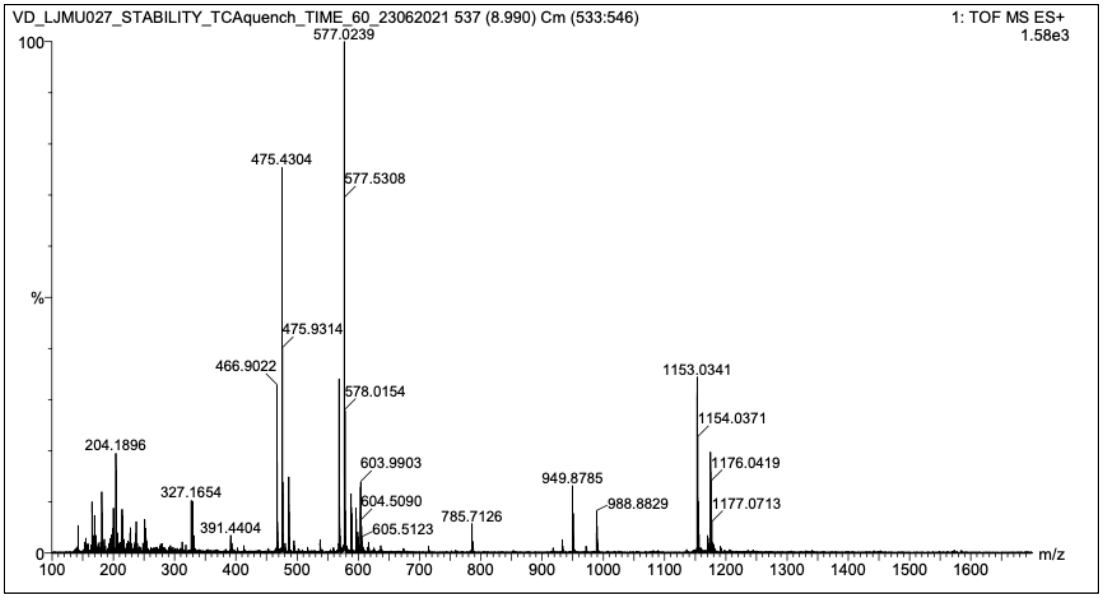



*Figure S 123. LC-MS spectrum of parent peptide detected after 60 min incubation in diluted human serum.*

### 7.2.5 *Calibration curves for plasma protein binding and physicochemical stability studies*

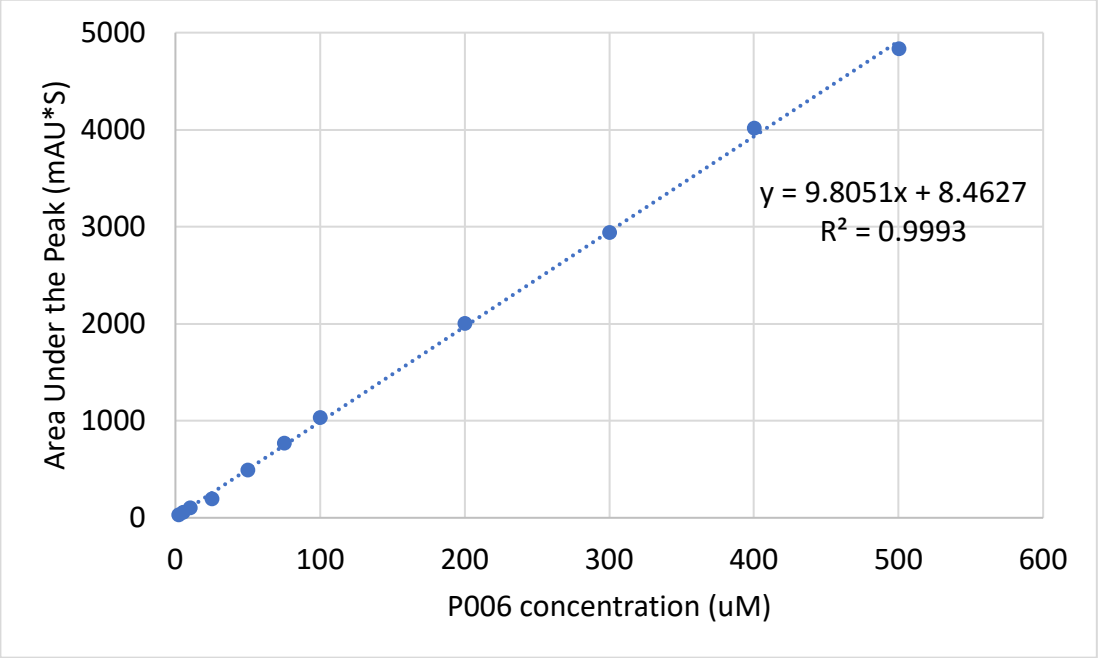



*Figure S 124. P006 calibration curve in PBS aq. solution (5 % DMSO).*

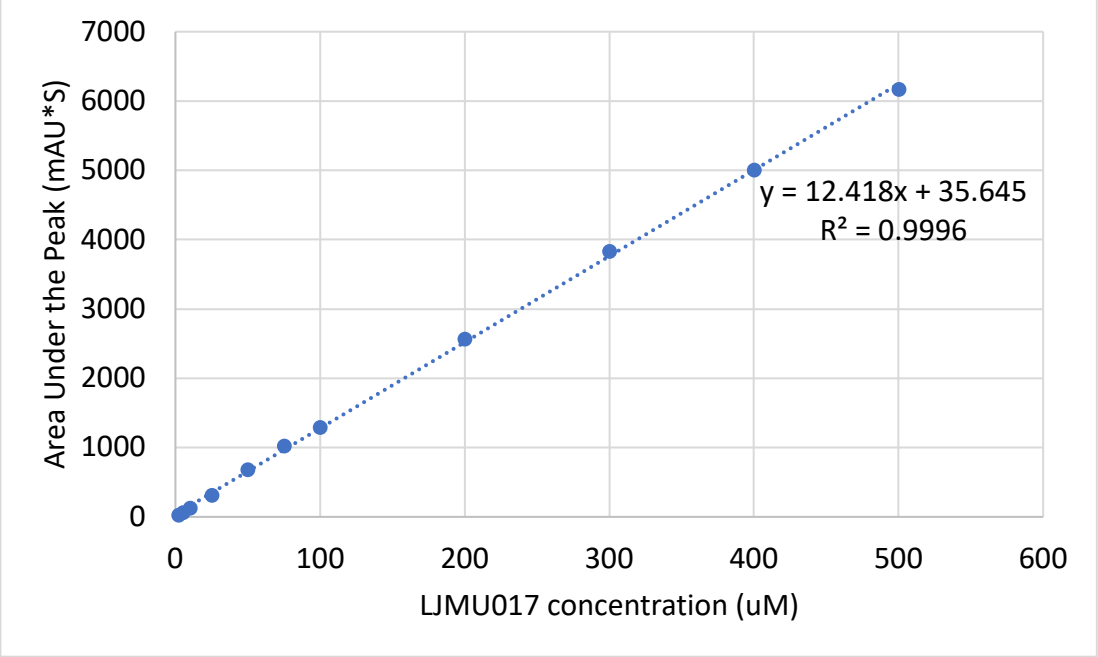



*Figure S 125. LJMU017 calibration curve in PBS aq. solution (5 % DMSO).*

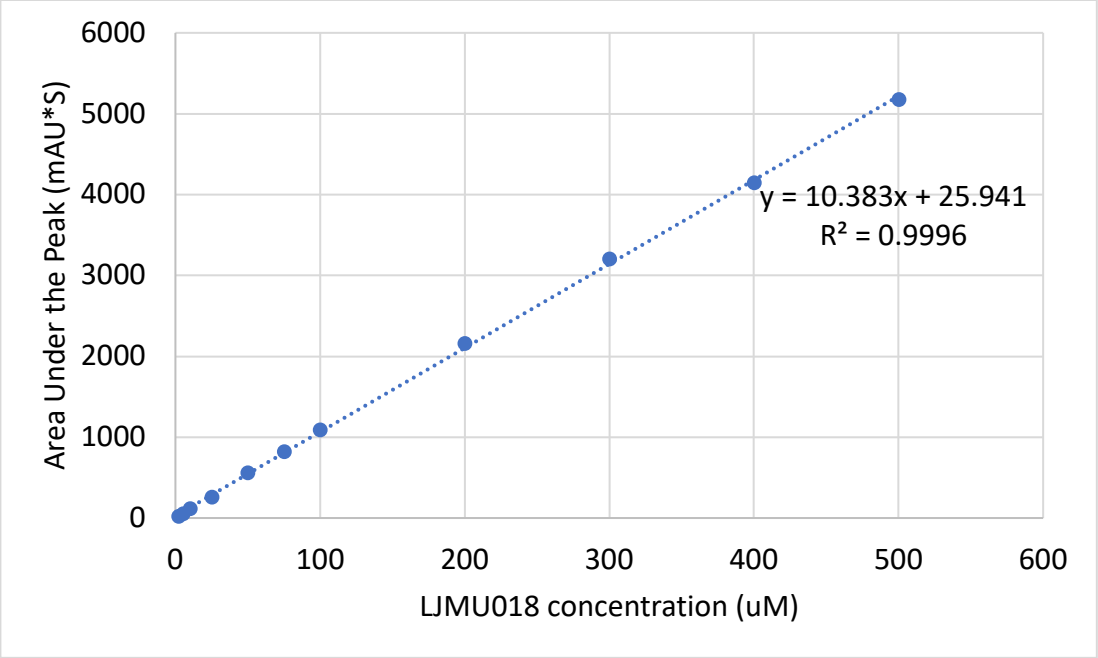



*Figure S 126. LJMU018 calibration curve in PBS aq. solution (5 % DMSO).*

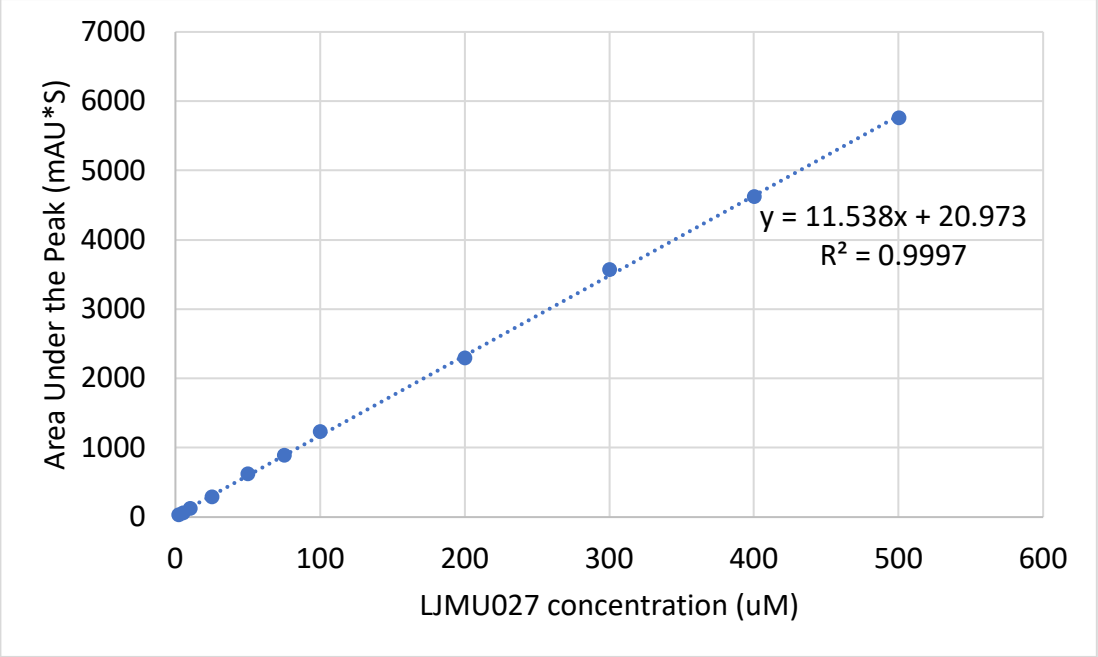



*Figure S 127. LJMU027 calibration curve in PBS aq. solution (5 % DMSO).*

### 7.2.6 Plasma protein binding (PPB) experiments

#### 7.2.6.1 PPB assessment via HSA precipitation method

*Table S.3. HSA precipitation method full dataset (n=3).*

| HSA (μM) | P006 bound (%) | LJMU027 bound (%) | LJMU017 bound (%) | LJMU018 bound (%) |
|---|---|---|---|---|
| **0** | 0.00 | 0.00 | 0.00 | 0.00 |
| **10** | 9.05 ± 1.26 | 9.29 ± 0.32 | 5.53 ± 0.06 | 16.93 ± 1.55 |
| **25** | 6.34 ± 4.08 | 18.24 ± 0.89 | 18.57 ± 0.49 | 25.71 ± 3.86 |
| **50** | 3.26 ± 2.86 | 24.55 ± 0.61 | 30.59 ± 0.04 | 40.24 ± 0.55 |
| **80** | 2.27 ± 3.39 | 40.08 ± 0.94 | 43.15 ± 0.32 | 51.05 ± 0.28 |
| **100** | 3.15 ± 3.03 | 45.17 ± 0.44 | 49.52 ± 0.25 | 58.13 ± 1.56 |
| **150** | 4.75 ± 1.61 | 55.11 ± 0.37 | 60.98 ± 0.11 | 63.64 ± 0.85 |
| **200** | -1.04 ± 3.40 | 62.20 ± 0.52 | 65.98 ± 0.28 | 72.04 ± 0.93 |
| **250** | -1.16 ± 1.74 | 65.28 ± 0.99 | 70.97 ± 0.35 | 75.30 ± 0.96 |
| **400** | 9.90 ± 1.07 | 74.49 ± 0.57 | 78.85 ± 0.40 | 81.94 ± 0.86 |

#### 7.2.6.2 PPB assessment via equilibrium dialysis

*Table S.4. RED method full dataset (n=2).*

| | [Peptide] in white chamber (μM) | [Peptide] in red chamber (μM) | unbound peptide (%) | bound peptide (%) |
|---|---|---|---|---|
| **P006** | 104.73 ± 5.31 | 143.11 ± 6.96 | 73.18 | 26.82 |
| **LJMU027** | 113.29 ± 1.23 | 138.80 ± 4.39 | 81.62 | 18.38 |
| **LJMU017** | 98.02 ± 5.75 | 119.68 ± 6.66 | 81.90 | 18.10 |
| **LJMU018** | 141.55 ± 5.29 | 184.42 ± 5.90 | 76.76 | 23.24 |

#### 7.2.6.3 PPB assessment via ultrafiltration

*Table S.5. Ultrafiltration method full dataset (n=2).*

| | [peptide] in filtrate sol. | Peptide filtrated compared to PBS sample (%) |
|---|---|---|
| **P006** | 55.68 ± 3.08 | 103.52 ± 5.64 |
| **LJMU027** | 9.17 ± 2.28 | 69.66 ± 14.47 |

*Table S.6. Filtration test to assess the extent of nonspecific binding to UF device (n=2).*

| | Filtration | [HSA] (μM) | [peptide] in filtrate sol. | Peptide filtrated compared to PBS sample (%) |
|---|---|---|---|---|
| **LJMU027** | No | 0 | 51.33 ± 0.63 | - |
| **LJMU027** | Yes | 0 | 24.14 ± 2.38 | 48.83 ± 3.91 |
| **LJMU027** | Yes | 100 | 14.35 ± 0.07 | 30.41 ± 0.50 |
| **LJMU027** | Yes | 200 | 16.34 ± 4.43 | 34.17 ± 7.93 |
| **LJMU027** | Yes | 300 | 14.58 ± 6.09 | 30.85 ± 11.84 |
| **LJMU027** | Yes | 400 | 11.93 ± 2.14 | 25.88 ± 3.71 |

## 7.3 Chapter 5 – supporting information

### *7.3.1 Calibration curves for formulation studies*

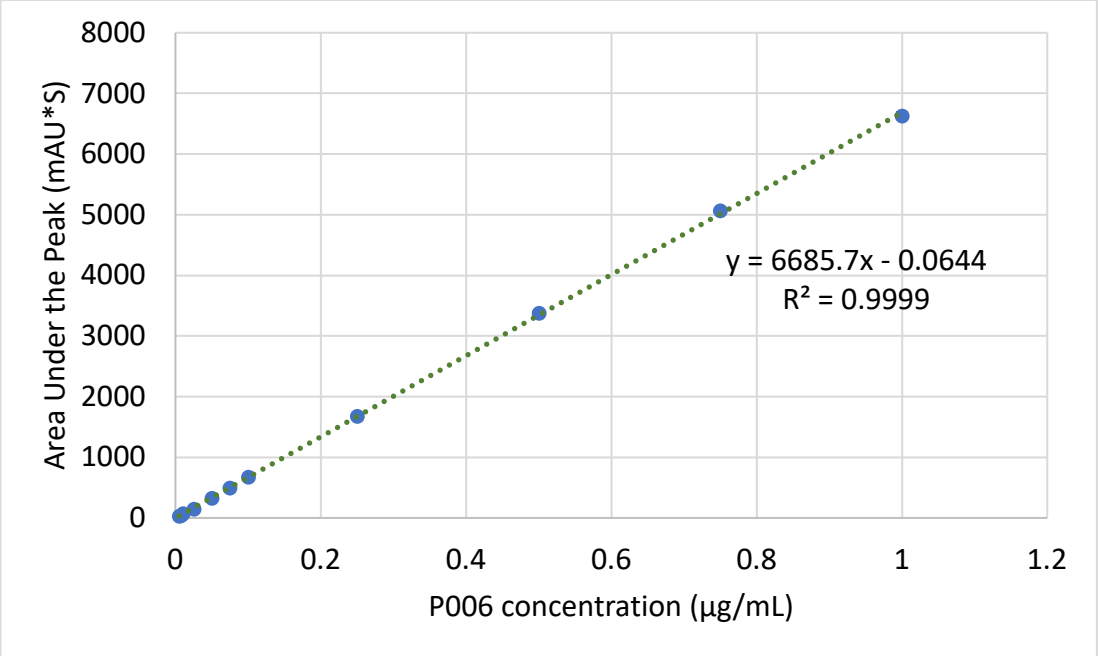



*Figure S 128. P006 calibration curve in $H_2O$.*

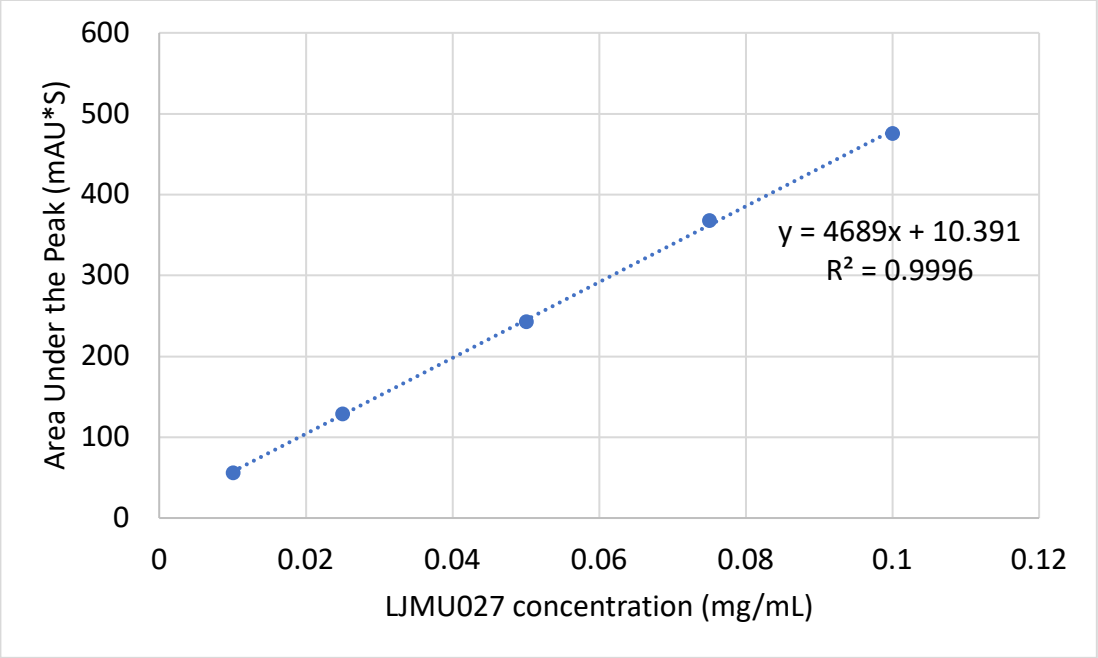



*Figure S 129. LJMU027 calibration curve in $H_2O$.*

### 7.3.2 Table results NPs optimisation

*Table S.7. Taguchi Design of Experiment for blank PLGA NPs optimisation.*

| Exp. No. (n=3) | Amount PLGA (mg) | % PVA | OP:AP ratio | Average size (nm) | PDI | Z-potential (mV) |
|---|---|---|---|---|---|---|
| **1** | 10 | 1 | 01:04 | 121.27 ± 10.21 | 0.20 ± 0.02 | 0.65 ± 1.43 |
| **2** | 10 | 2.5 | 01:07 | 189.43 ± 61.68 | 0.34 ± 0.05 | 0.47 ± 0.43 |
| **3** | 10 | 5 | 01:10 | 251.67 ± 76.45 | 0.62 ± 0.09 | 0.02 ± 0.40 |
| **4** | 20 | 1 | 01:07 | 142.20 ± 5.35 | 0.14 ± 0.04 | 1.64 ± 0.47 |
| **5** | 20 | 2.5 | 01:10 | 204.07 ± 10.92 | 0.20 ± 0.01 | -0.10 ± 0.52 |
| **6** | 20 | 5 | 01:04 | 482.90 ± 48.51 | 0.14 ± 0.00 | 0.35 ± 0.53 |
| **7** | 30 | 1 | 01:10 | 159.30 ± 6.31 | 0.10 ± 0.01 | 0.20 ± 1.84 |
| **8** | 30 | 2.5 | 01:04 | 261.03 ± 13.97 | 0.08 ± 0.02 | 1.29 ± 2.15 |
| **9** | 30 | 5 | 01:07 | 578.00 ± 30.62 | 0.07 ± 0.02 | 0.07 ± 1.03 |