

Article

# Comparison of Online Comprehensive HILIC $\times$ RP and RP $\times$ RP with Trapping Modulation Coupled to Mass Spectrometry for Microalgae Peptidomics

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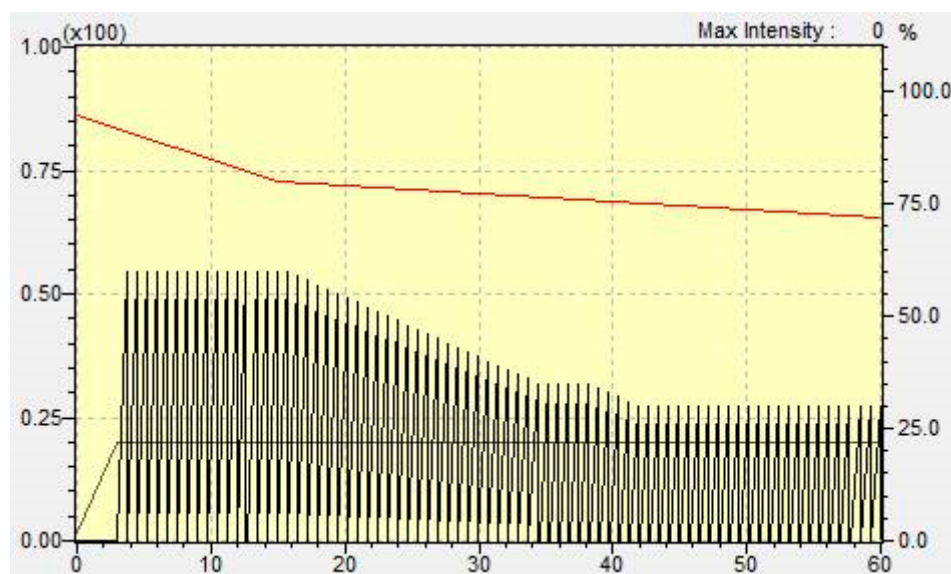
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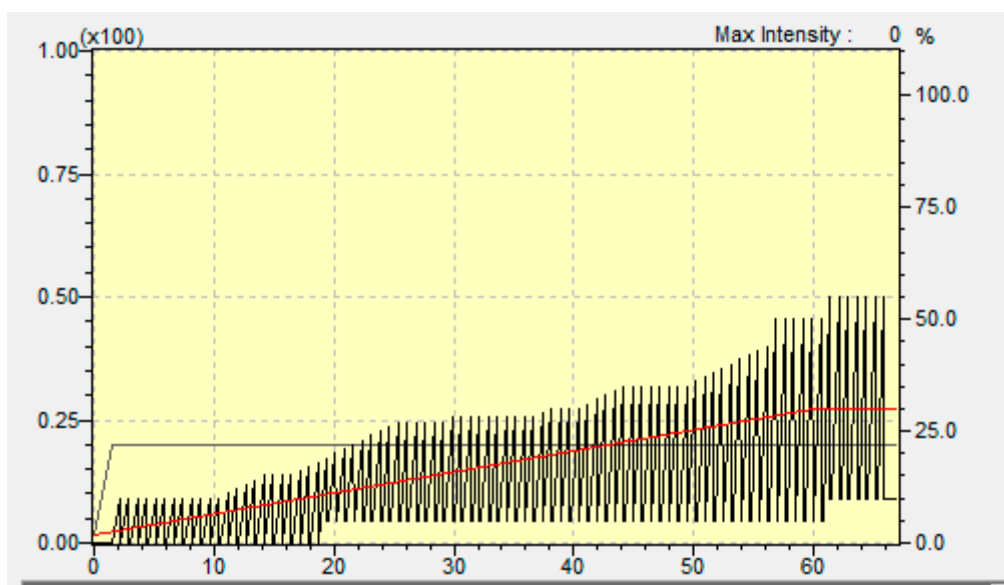
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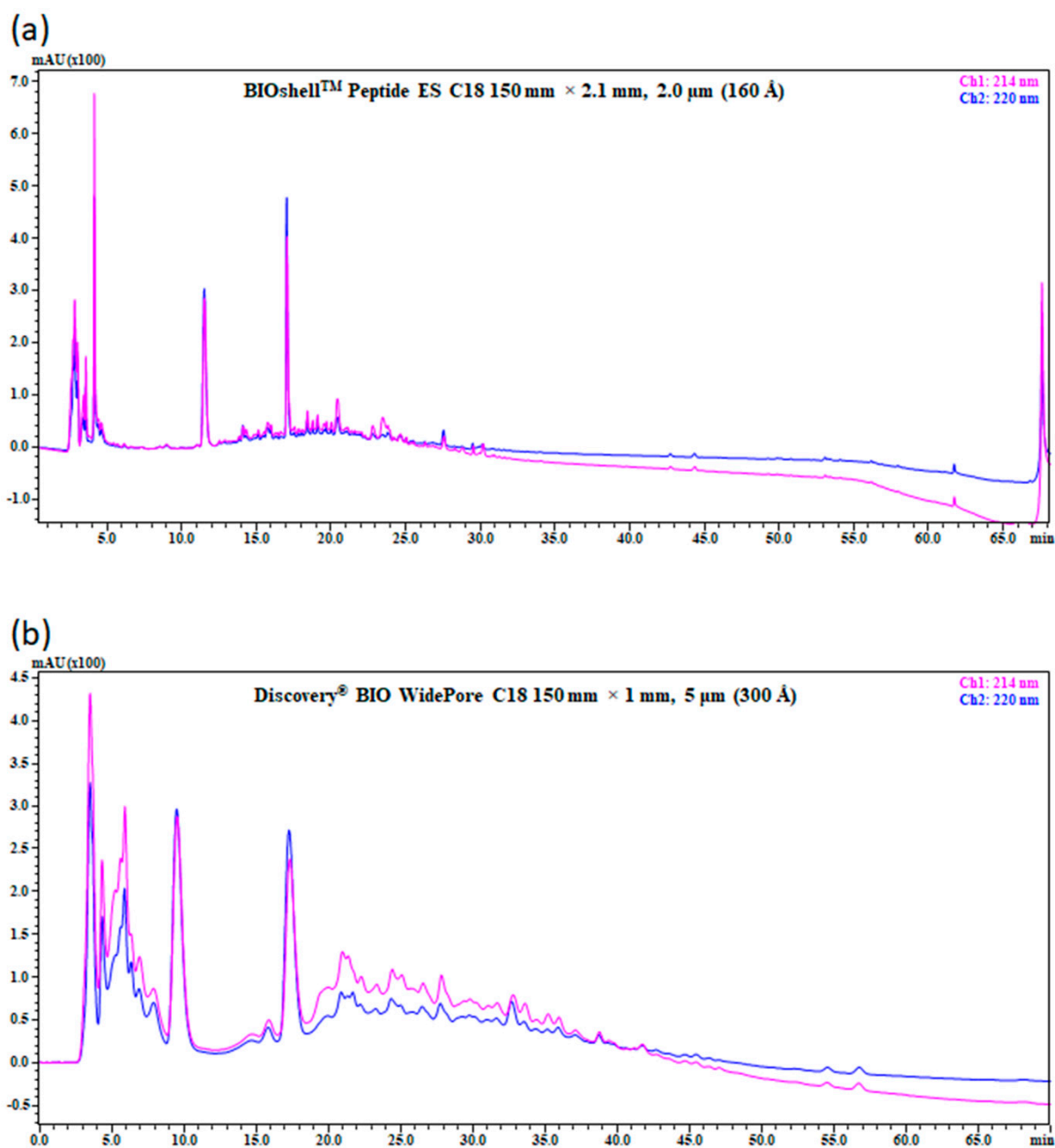
Supplementary Data for the manuscript:



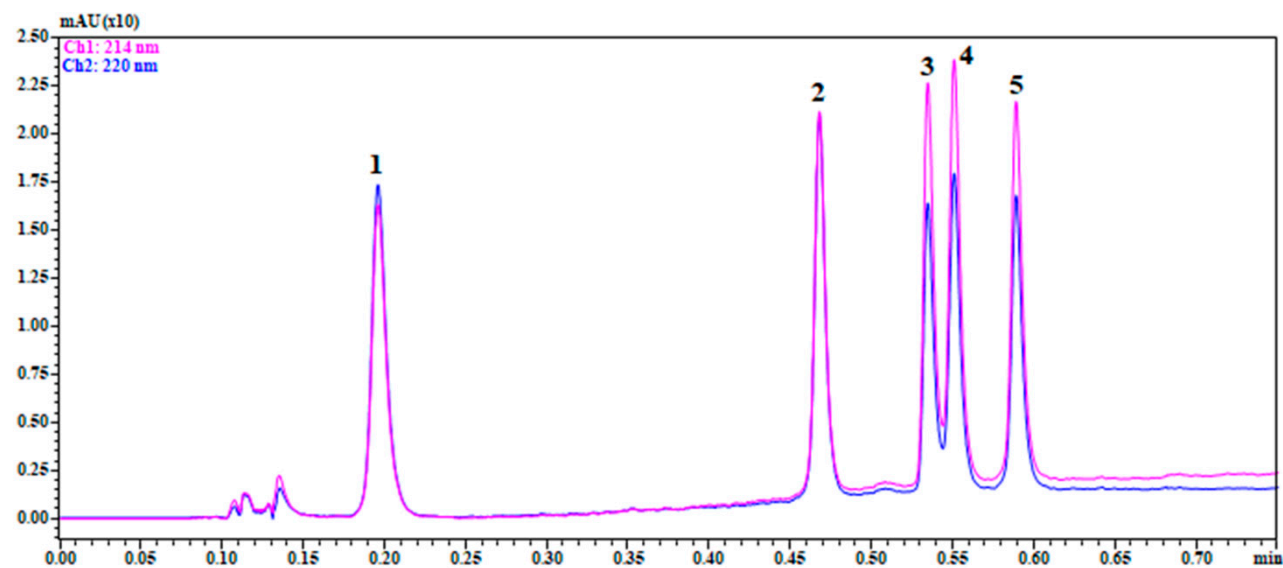
The graph shows the Multi-segment shifting gradient employed in 2D of the HILIC $\times$ RP system. Red line represents the first dimension HILIC gradient, while black line represents the RP gradient in the second dimension. Gradient, and flow rate, is in idle for the first 3 min and starts at 3.00 min.



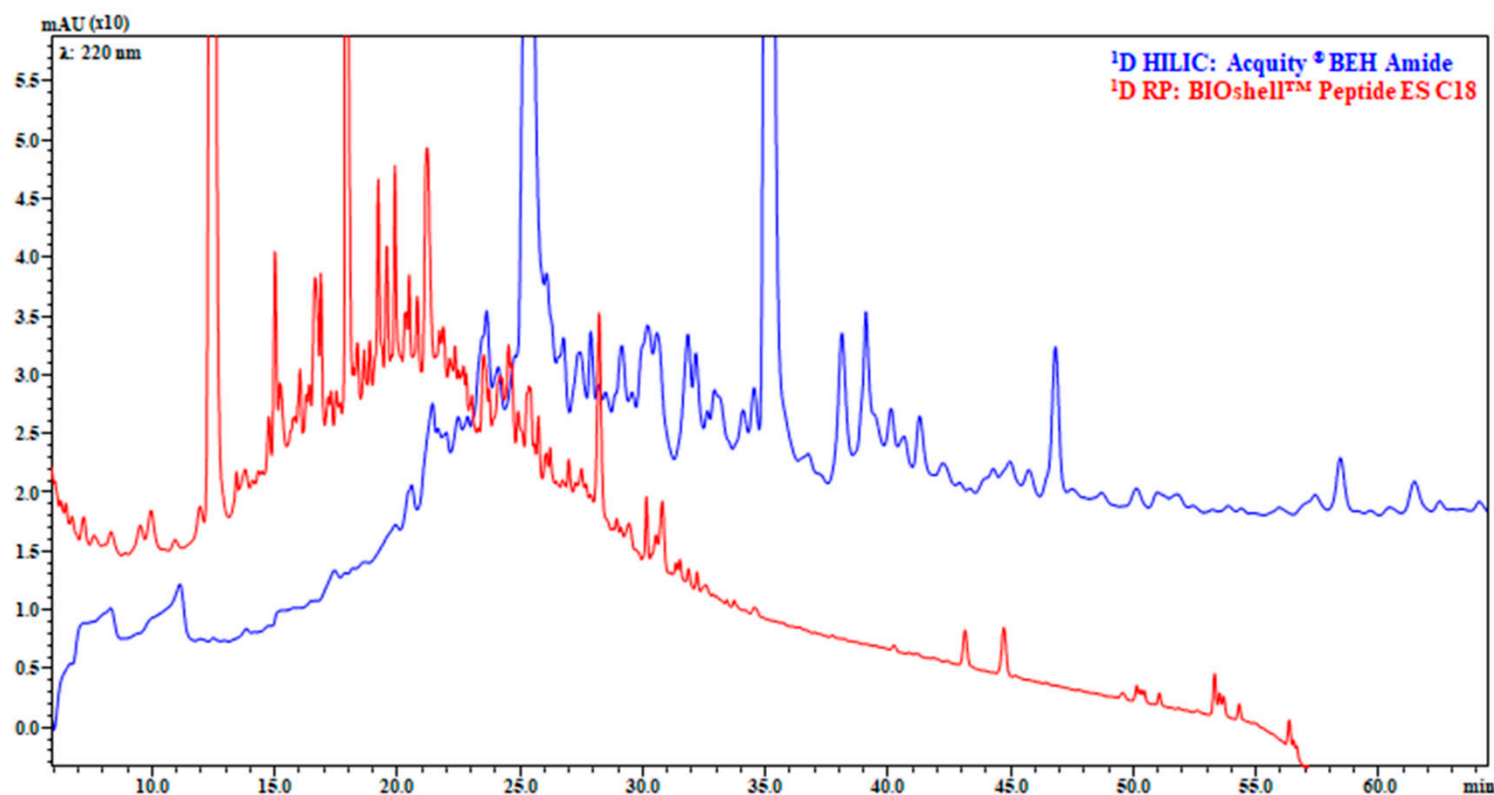
The graph shows the Multi-segment shifting gradient employed in  $^2D$  of the  $RP \times RP$  system. Red line represents the first dimension RP gradient, while black line represents the RP gradient in the second dimension.



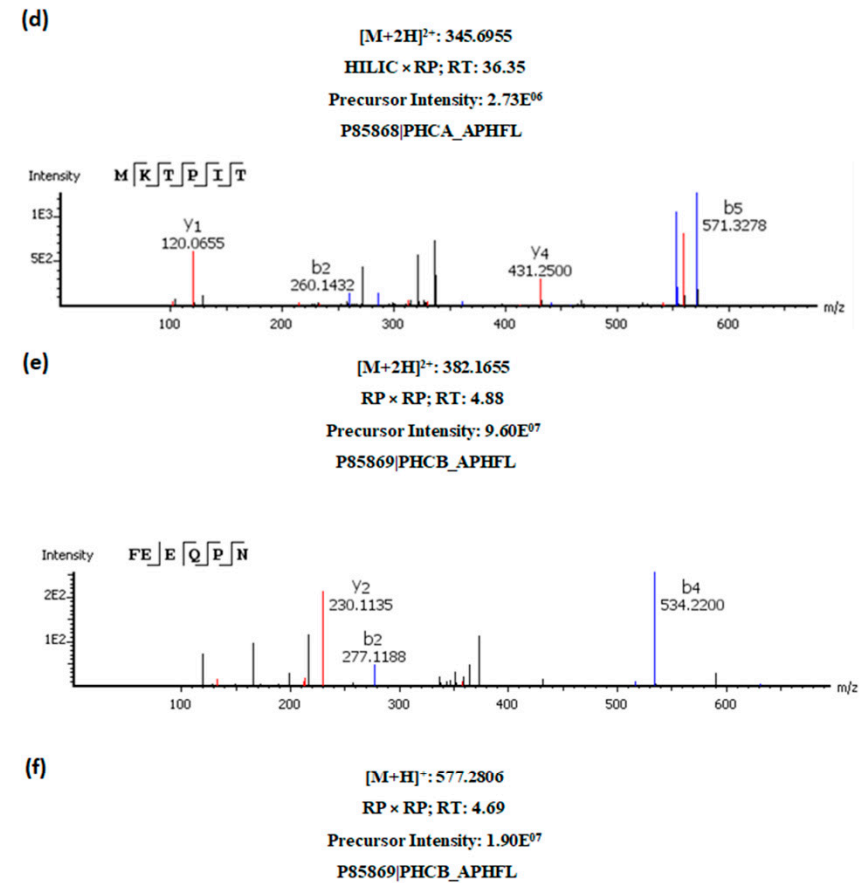
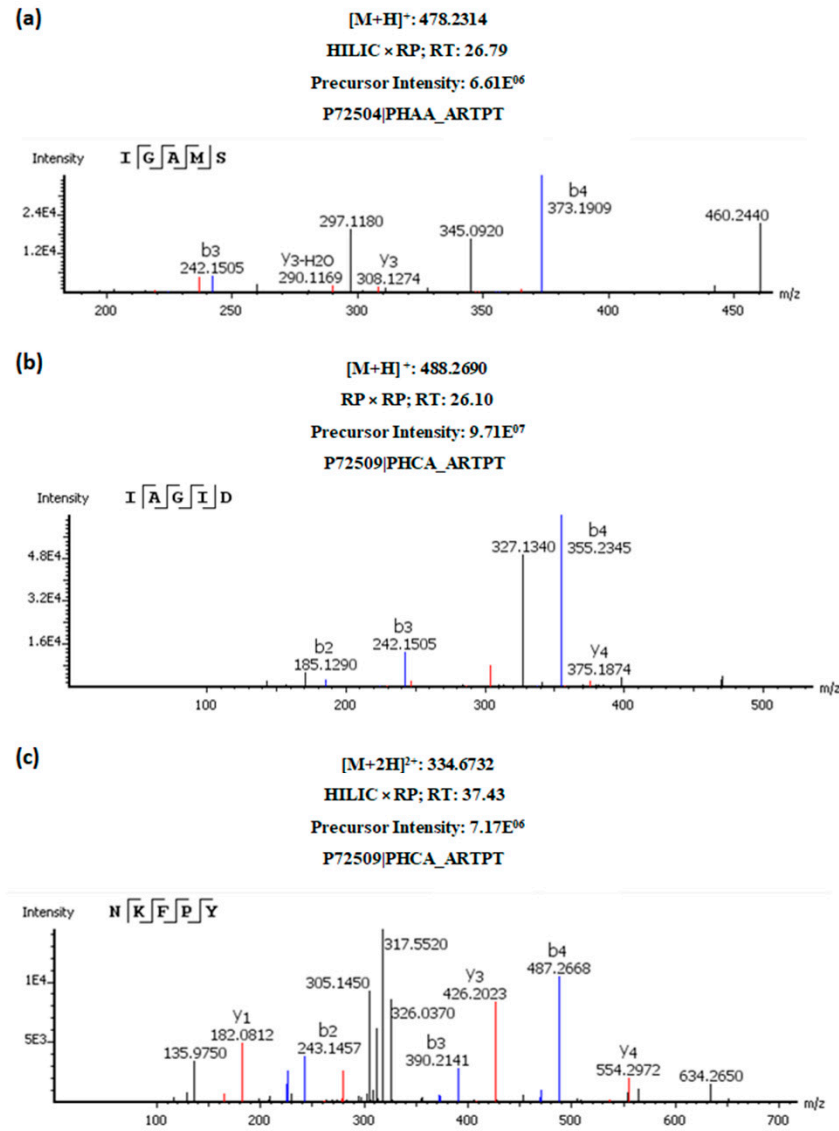
**Figure S1 a,b.** Comparison of separation on two different RP columns: columns: (a): BIOshell™ Peptide ES C18 150 mm × 2.1 mm, 2.0 μm. Flow rate: 0.1 mL/min; gradient: 0–50 min, 5–50% B, 50–60 min, 50–99% B, 99% B held for 1 min, returning to initial conditions in 0.1 min. (b) Discovery® BIO WidePore C18 150 mm × 1 mm, 5 μm (300 Å). Flow rate: 0.06 mL/min; gradient: 0–60 min, 2–30% B, 60–65 min, 30–99% B, 99% B held for 1 min, returning to initial conditions in 0.1 min. Mobile phase (A): 10 mM HCOONH<sub>4</sub> in H<sub>2</sub>O (pH 8.07 adjusted with NH<sub>4</sub>OH), (B): ACN, column temperature: 30°C, injection volume 2 μL. Detection: UV λ 220–214 nm. Sample: Klamath G.I digest.



**Figure S2.** 2D RP of a mixture of five standard peptides. Column: BIOshell™ Peptide ES C18 50 mm × 3.0 mm, 2.7 μm (160 Å). Flow rate: 2.2 mL/min. Mobile phase: 0.1% H<sub>3</sub>PO<sub>4</sub> (B): ACN, column temperature: 50°C, injection volume: 2 μL. Detection: UV λ 220–214 nm. Sample: 1) Gly-Tyr 2) Val-Tyr-Val 3) Met-Enkephaline 4) Leu-Enkephaline 5) Angiotensin II.



**Figure S3.** Enlargement of <sup>1</sup>D HILIC (blue) and <sup>1</sup>D RP (red) chromatograms in which complexity of Klamath G.I digest can be better appreciated. <sup>1</sup>D HILIC analyses conditions: column, Acquity® BEH Amide 150 mm × 2.1 mm, 1.7 μm (130 Å); mobile phase, (A): 5 mM HCOONH<sub>4</sub> in H<sub>2</sub>O (pH 6.02), (B): MeCN; gradient, 0–15 min, 95–80% B, 15–60 min, 80–72% B, 60–62 min, 72–60% B, returning to initial conditions in 0.1 min; Flow rate, 0.1 mL/min; column, 40°C; injection volume, 4 μL. <sup>1</sup>D RP analyses conditions: column, BIOshell™ Peptide ES C18 150 mm × 2.1 mm, 2.0 μm (160 Å); mobile phase, (A): 10 mM HCOONH<sub>4</sub> in H<sub>2</sub>O (pH 9 adjusted with NH<sub>4</sub>OH), (B): MeCN; gradient, 0–60 min, 2–30% B, returning to initial conditions in 0.1 min. Flow rate, 0.1 mL/min, column oven, 40°C; injection volume, 4 μL.



**Figure S4. a–f.** MS/MS spectra revealing identification of peptides from Spirulina (a,b,c) and Klamath (d,e,f) G.I digest

| HILIC × RP-MS/MS_ SAMPLE_KLAMATH G.I. DIGEST |                |        |             |                    |       |                   |               |
|----------------------------------------------|----------------|--------|-------------|--------------------|-------|-------------------|---------------|
| Peptide                                      | Molecular Mass | Length | Error (ppm) | [M+H] <sup>+</sup> | RT    | Accession         | Variable PTM  |
| AAIAGD                                       | 516.2543       | 6      | −3.7        | 517.2597           | 36.97 | P85869 PHCB_APHFL |               |
| AAIAGDA                                      | 587.2914       | 7      | −2.3        | 588.2974           | 37    | P85869 PHCB_APHFL |               |
| AAIAGDAS                                     | 674.3235       | 8      | −3.1        | 675.3287           | 36.96 | P85869 PHCB_APHFL |               |
| AAIAGDASV                                    | 773.3919       | 9      | −0.6        | 774.3987           | 34.12 | P85869 PHCB_APHFL |               |
| FEEQPN                                       | 762.3184       | 6      | −3.4        | 382.1652*          | 53.52 | P85869 PHCB_APHFL |               |
| KTPITE                                       | 687.3803       | 6      | −4.3        | 344.696*           | 52.01 | P85868 PHCA_APHFL |               |
| KTPITEA                                      | 758.4174       | 7      | −4.4        | 380.2143*          | 51.31 | P85868 PHCA_APHFL |               |
| LIDGA                                        | 487.2642       | 5      | −2.7        | 488.2701           | 30.3  | P85868 PHCA_APHFL |               |
| LSNTEL                                       | 675.3439       | 6      | −2.3        | 676.3497           | 34.86 | P85868 PHCA_APHFL |               |
| M(+15.99)KTPI                                | 604.3254       | 5      | −3.4        | 303.169*           | 46.06 | P85868 PHCA_APHFL | Oxidation (M) |
| M(+15.99)KTPIT                               | 705.3731       | 6      | −3.7        | 353.6925*          | 46.79 | P85868 PHCA_APHFL | Oxidation (M) |
| M(+15.99)KTPITE                              | 834.4157       | 7      | −2.1        | 835.4212           | 52.8  | P85868 PHCA_APHFL | Oxidation (M) |
| M(+15.99)KTPITE<br>A                         | 905.4528       | 8      | −4.1        | 453.7318*          | 52.09 | P85868 PHCA_APHFL | Oxidation (M) |
| MKTPI                                        | 588.3305       | 5      | −1.9        | 295.172*           | 36.36 | P85868 PHCA_APHFL |               |
| MKTPIT                                       | 689.3782       | 6      | −2.4        | 345.6955*          | 36.35 | P85868 PHCA_APHFL |               |
| MKTPITE                                      | 818.4208       | 7      | −2.8        | 410.2165*          | 42.39 | P85868 PHCA_APHFL |               |
| QALGTPGAS                                    | 800.4028       | 9      | −1.9        | 401.2079*          | 37.05 | P85869 PHCB_APHFL |               |
| SKFPY                                        | 640.322        | 5      | −1.6        | 641.3282           | 38.64 | P85868 PHCA_APHFL |               |
| SNTEL                                        | 562.2598       | 5      | −3          | 563.2654           | 42.29 | P85868 PHCA_APHFL |               |

|          |          |   |      |           |       |                   |
|----------|----------|---|------|-----------|-------|-------------------|
| TTSTPGNQ | 804.3614 | 8 | -4.9 | 805.3647  | 54.02 | P85868 PHCA_APHFL |
| VAVGVGK  | 628.3908 | 7 | -3.2 | 315.2017* | 46.05 | P85869 PHCB_APHFL |
| VTGTIDQ  | 732.3654 | 7 | -1.3 | 733.3717  | 37.03 | P85869 PHCB_APHFL |
| YSKFPY   | 803.3854 | 6 | -2.7 | 402.6989  | 34.93 | P85868 PHCA_APHFL |

## HILIC × RP-MS/MS\_SAMPLE\_SPIRULINA G.I. DIGEST

| Peptide       | Molecular Mass | Length | Error (ppm) | [M+H] <sup>+</sup> | RT    | Accession         | Variable PTM  |
|---------------|----------------|--------|-------------|--------------------|-------|-------------------|---------------|
| ADSLISGA      | 732.3654       | 8      | -1.2        | 733.3718           | 35.97 | P72509 PHCA_ARTPT |               |
| ADSLISGAA     | 803.4025       | 9      | -2.6        | 804.4077           | 36.72 | P72509 PHCA_ARTPT |               |
| AGDPSIL       | 671.349        | 7      | -4.2        | 672.3535           | 33.01 | P72505 APCB_ARTPT |               |
| AGLEAA        | 530.27         | 6      | -4.6        | 531.2748           | 33.58 | P72509 PHCA_ARTPT |               |
| AIVNDPA       | 698.3599       | 7      | -0.9        | 699.3665           | 41.17 | P72508 PHCB_ARTPT |               |
| ALGTPGSS      | 688.3392       | 8      | -4.7        | 689.3432           | 36.57 | P72508 PHCB_ARTPT |               |
| ANHGL         | 510.255        | 5      | -3.3        | 256.1339           | 43.94 | P72509 PHCA_ARTPT |               |
| ANHGLSGDA     | 840.3726       | 9      | -4.4        | 421.1917           | 52.99 | P72509 PHCA_ARTPT |               |
| ANHGLSGDAA    | 911.4097       | 10     | -5.5        | 456.7096           | 53.77 | P72509 PHCA_ARTPT |               |
| ASEIA         | 489.2435       | 5      | -5.8        | 490.2479           | 38.76 | P72508 PHCB_ARTPT |               |
| ASEIAS        | 576.2755       | 6      | -4          | 577.2805           | 44.75 | P72508 PHCB_ARTPT |               |
| DM(+15.99)EIL | 748.3677       | 6      | -2.8        | 749.3729           | 33.84 | P72508 PHCB_ARTPT | Oxidation (M) |
| DMEII         | 619.2887       | 5      | -1.6        | 620.2950           | 27.01 | P72508 PHCB_ARTPT |               |
| DMEIIL        | 732.3727       | 6      | -0.8        | 733.3795           | 25.55 | P72508 PHCB_ARTPT |               |
| DYAIN         | 594.2649       | 5      | -2.3        | 595.2708           | 35.88 | P72509 PHCA_ARTPT |               |
| FAEQPQ        | 718.3286       | 6      | -2.3        | 719.3342           | 39.56 | P72508 PHCB_ARTPT |               |
| FATGEL        | 636.3119       | 6      | -3.5        | 637.3170           | 28.44 | P72505 APCB_ARTPT |               |
| GIVAGDVT      | 730.3861       | 8      | -1.4        | 731.3924           | 27.62 | P72504 PHAA_ARTPT |               |
| GIVAGDVTP     | 940.5229       | 10     | -3.8        | 941.5266           | 23.96 | P72504 PHAA_ARTPT |               |
| GLVGADAGK     | 786.4235       | 9      | -3.1        | 394.2178*          | 50.11 | P72505 APCB_ARTPT |               |
| IAGGTGPM      | 702.337        | 8      | -0.4        | 703.3441           | 29.1  | P72509 PHCA_ARTPT |               |



|                    |          |    |       |           |       |                   |                  |
|--------------------|----------|----|-------|-----------|-------|-------------------|------------------|
| IAGGTGPM(+15.99)DE | 962.4015 | 10 | 1.8   | 963.4105  | 50.82 | P72509 PHCA_ARTPT | Oxidation<br>(M) |
| IAGGTGPMDE         | 817.364  | 9  | -1.7  | 818.3699  | 30.59 | P72509 PHCA_ARTPT |                  |
| IAGGTGPMDE         | 946.4066 | 10 | 0.6   | 947.4144  | 37.38 | P72509 PHCA_ARTPT |                  |
| IAGID              | 487.2642 | 5  | -2.5  | 488.2702  | 26.1  | P72509 PHCA_ARTPT |                  |
| IAGIDE             | 616.3068 | 6  | -5.6  | 617.3106  | 28.37 | P72509 PHCA_ARTPT |                  |
| IGAMS              | 477.2257 | 5  | -3.4  | 478.2314  | 26.79 | P72504 PHAA_ARTPT |                  |
| ITSNA              | 504.2544 | 5  | -5    | 505.2591  | 37.07 | P72508 PHCB_ARTPT |                  |
| IVKVEL             | 699.4531 | 6  | -2.2  | 350.7330  | 36.02 | P72508 PHCB_ARTPT |                  |
| KSLGTPI            | 714.4276 | 7  | -2.7  | 358.2201  | 39.69 | P72504 PHAA_ARTPT |                  |
| KSLGTPIE           | 843.4702 | 8  | -3.8  | 422.7408  | 44.96 | P72504 PHAA_ARTPT |                  |
| KSLGTPIEA          | 914.5073 | 9  | -2    | 458.2600  | 44.22 | P72504 PHAA_ARTPT |                  |
| LALGTPGSS          | 801.4232 | 9  | -1    | 802.4297  | 31.43 | P72508 PHCB_ARTPT |                  |
| LALGTPGSSV         | 900.4916 | 10 | -0.1  | 901.4988  | 29.21 | P72508 PHCB_ARTPT |                  |
| LALGTPGSSVA        | 971.5287 | 11 | -4    | 972.5322  | 29.97 | P72508 PHCB_ARTPT |                  |
| LDAVN              | 530.27   | 5  | -3.9  | 531.2752  | 35.8  | P72508 PHCB_ARTPT |                  |
| LIAGI              | 485.3213 | 5  | -3.9  | 486.3267  | 20.93 | P72509 PHCA_ARTPT |                  |
| LIAGID             | 600.3483 | 6  | -2.5  | 601.3541  | 23.16 | P72509 PHCA_ARTPT |                  |
| LIAGIDEI           | 842.4749 | 8  | -2.6  | 843.4800  | 23.99 | P72509 PHCA_ARTPT |                  |
| LIAGIDEIN          | 956.5178 | 9  | -2.5  | 479.2650* | 27.73 | P72509 PHCA_ARTPT |                  |
| LIAGIDEINRT        | 1213.667 | 11 | -2.9  | 607.8388  | 39.04 | P72509 PHCA_ARTPT |                  |
| LSGEDAAEA          | 861.3716 | 9  | -5    | 862.3746  | 54.57 | P72504 PHAA_ARTPT |                  |
| LSSTE              | 535.249  | 5  | -3.4  | 536.2544  | 42.32 | P72509 PHCA_ARTPT |                  |
| LSSTEIQ            | 776.3916 | 7  | -1.6  | 777.3976  | 38.1  | P72509 PHCA_ARTPT |                  |
| M(+15.99)GGVAI     | 562.2785 | 6  | -10.1 | 563.2801  | 30.67 | P72505 APCB_ARTPT | Oxidation<br>(M) |
| M(+15.99)QDAIT     | 693.3004 | 6  | -2.5  | 694.3059  | 41.81 | P72505 APCB_ARTPT | Oxidation<br>(M) |
| MKTPL              | 588.3305 | 5  | -2.6  | 589.3362  | 36.65 | P72509 PHCA_ARTPT |                  |
| NKFPY              | 667.3329 | 5  | -1.8  | 334.6732* | 37.43 | P72509 PHCA_ARTPT |                  |
| NKFPYT             | 768.3806 | 6  | -1.6  | 385.1970  | 38.93 | P72509 PHCA_ARTPT |                  |

|                  |          |    |      |          |       |                   |
|------------------|----------|----|------|----------|-------|-------------------|
| NSLGVPI          | 698.3962 | 7  | -5.3 | 699.3998 | 24.72 | P72505 APCB_ARTPT |
| NSLGVPIG         | 755.4177 | 8  | -2.7 | 756.4230 | 26.97 | P72505 APCB_ARTPT |
| RPDVVSPGGN       | 996.4988 | 10 | -4.6 | 499.2544 | 59.09 | P72504 PHAA_ARTPT |
| RPDVVSPGGNA      | 1067.536 | 11 | -4.9 | 534.7726 | 59.86 | P72504 PHAA_ARTPT |
| RPDVVSPGGNAYGEEM | 1676.746 | 16 | -3.2 | 839.3777 | 54.02 | P72504 PHAA_ARTPT |
| SFVTSGE          | 725.3232 | 7  | -0.8 | 726.3299 | 35.89 | P72504 PHAA_ARTPT |
| SLGTPI           | 586.3326 | 6  | -2.7 | 587.3383 | 24.66 | P72504 PHAA_ARTPT |
| SLGTPIE          | 715.3752 | 7  | -1   | 716.3817 | 29.17 | P72504 PHAA_ARTPT |
| SLGTPIEA         | 786.4123 | 8  | 0.7  | 787.4201 | 30.7  | P72504 PHAA_ARTPT |
| SPGEL            | 501.2435 | 5  | -2.8 | 502.2493 | 35.12 | P72504 PHAA_ARTPT |
| TPLTEA           | 630.3224 | 6  | -2   | 631.3285 | 34.38 | P72509 PHCA_ARTPT |
| TTQMQGPN         | 875.3807 | 8  | -2.7 | 438.6964 | 47.03 | P72509 PHCA_ARTPT |
| VAVGVGK          | 628.3908 | 7  | -3.4 | 315.2016 | 47.83 | P72508 PHCB_ARTPT |
| VVSAA            | 445.2536 | 5  | -4.7 | 446.2588 | 30.45 | P72508 PHCB_ARTPT |
| YFATGEL          | 799.3752 | 7  | -1.1 | 800.3816 | 26.21 | P72505 APCB_ARTPT |
| YSDITRPG         | 907.4399 | 8  | -3.9 | 454.7255 | 44.88 | P72505 APCB_ARTPT |

RP × RP-MS/MS\_ SAMPLE\_KLAMATH G.I. DIGEST

| Peptide  | Molecular Mass | Length | Error (ppm) | [M+H] <sup>+</sup> | RT    | Accession         | Variable PTM |
|----------|----------------|--------|-------------|--------------------|-------|-------------------|--------------|
| AAIAGD   | 516.2543       | 6      | -4.4        | 517.2593           | 7.77  | P85869 PHCB_APHFL |              |
| AAIAGDA  | 587.2914       | 7      | -5.6        | 588.2954           | 8.61  | P85869 PHCB_APHFL |              |
| AAIAGDAS | 674.3235       | 8      | -2.1        | 675.3293           | 9.33  | P85869 PHCB_APHFL |              |
| ADARGEF  | 764.3453       | 7      | -3.9        | 383.1784           | 12.33 | P85869 PHCB_APHFL |              |
| DARGEF   | 693.3082       | 6      | -5.3        | 694.3118           | 8.67  | P85869 PHCB_APHFL |              |
| FEEQPN   | 762.3184       | 6      | -2.5        | 382.1655           | 4.88  | P85869 PHCB_APHFL |              |
| IDGATSA  | 633.2969       | 7      | -3.8        | 634.3018           | 5.48  | P85868 PHCA_APHFL |              |
| KTPITEA  | 758.4174       | 7      | -1.3        | 759.4237           | 21.05 | P85868 PHCA_APHFL |              |
| LIDGA    | 487.2642       | 5      | -5.2        | 488.2690           | 19.03 | P85868 PHCA_APHFL |              |
| LIDGATS  | 675.3439       | 7      | -4.5        | 676.3481           | 23.29 | P85868 PHCA_APHFL |              |
| LSNTEL   | 675.3439       | 6      | -2          | 676.3499           | 17.64 | P85868 PHCA_APHFL |              |

|                  |          |   |      |          |       |                   |               |
|------------------|----------|---|------|----------|-------|-------------------|---------------|
| LSTEQ            | 576.2755 | 5 | -3.8 | 577.2806 | 4.69  | P85869 PHCB_APHFL |               |
| M(+15.99)KTPI    | 604.3254 | 5 | -2.7 | 605.3311 | 25.53 | P85868 PHCA_APHFL | Oxidation (M) |
| M(+15.99)KTPIT   | 705.3731 | 6 | -1.4 | 706.3794 | 27.01 | P85868 PHCA_APHFL | Oxidation (M) |
| M(+15.99)KTPITE  | 834.4157 | 7 | -4.9 | 418.2131 | 22.55 | P85868 PHCA_APHFL | Oxidation (M) |
| M(+15.99)KTPITEA | 905.4528 | 8 | -4.7 | 453.7316 | 22.58 | P85868 PHCA_APHFL | Oxidation (M) |
| MKTPI            | 588.3305 | 5 | -2.1 | 295.1719 | 34.64 | P85868 PHCA_APHFL |               |
| MKTPITE          | 818.4208 | 7 | -2.6 | 410.2166 | 31.63 | P85868 PHCA_APHFL |               |
| MKTPITEA         | 889.4579 | 8 | -3.9 | 445.7345 | 32.38 | P85868 PHCA_APHFL |               |
| QALGTPGAS        | 800.4028 | 9 | -2.4 | 801.4082 | 24.79 | P85869 PHCB_APHFL |               |
| SNTEL            | 562.2598 | 5 | -3.4 | 563.2652 | 4.89  | P85868 PHCA_APHFL |               |
| TAVVA            | 459.2693 | 5 | -4.5 | 460.2745 | 16.02 | P85869 PHCB_APHFL |               |
| TPITEA           | 630.3224 | 6 | -3.9 | 631.3273 | 15.35 | P85868 PHCA_APHFL |               |
| TTSTPGNQ         | 804.3614 | 8 | -2.4 | 805.3660 | 6.08  | P85868 PHCA_APHFL |               |
| VAVGVGK          | 628.3908 | 7 | -4.7 | 629.3951 | 32.22 | P85869 PHCB_APHFL |               |
| VSEVAS           | 590.2911 | 6 | -2.2 | 591.2971 | 5.47  | P85869 PHCB_APHFL |               |
| VVSQA            | 502.2751 | 5 | -6.2 | 503.2792 | 12.78 | P85869 PHCB_APHFL |               |
| YSKFPY           | 803.3854 | 6 | -2.5 | 402.6989 | 41.41 | P85868 PHCA_APHFL |               |

RP × RP-MS/MS\_ SAMPLE\_SPIRULINA G.I. DIGEST

| Peptide  | Molecular Mass | Length | Error (ppm) | [M+H] <sup>+</sup> | RT    | Accession         | Variable PTM |
|----------|----------------|--------|-------------|--------------------|-------|-------------------|--------------|
| AGDASV   | 518.2336       | 6      | -2.9        | 519.2394           | 3.65  | P72508 PHCB_ARTPT |              |
| AGDASVLE | 760.3603       | 8      | -0.5        | 761.3672           | 14.46 | P72508 PHCB_ARTPT |              |
| AGDPSI   | 558.2649       | 6      | -4.3        | 559.2698           | 12.19 | P72505 APCB_ARTPT |              |
| AGDPSIL  | 671.349        | 7      | -4.3        | 672.3534           | 23.34 | P72505 APCB_ARTPT |              |
| AGDPSILD | 786.3759       | 8      | -3          | 787.3808           | 17.55 | P72505 APCB_ARTPT |              |
| AIVNDPA  | 698.3599       | 7      | -1.1        | 699.3664           | 17.38 | P72508 PHCB_ARTPT |              |

|                  |           |    |      |           |       |                   |                  |
|------------------|-----------|----|------|-----------|-------|-------------------|------------------|
| AIVNDPAGITPG     | 1123.5873 | 12 | 1    | 1124.5957 | 33.8  | P72508 PHCB_ARTPT |                  |
| ALGTPGSS         | 688.3392  | 8  | -6.8 | 689.3418  | 24.5  | P72508 PHCB_ARTPT |                  |
| ALGTPGSSVAVGVGK  | 1298.7194 | 15 | -2.3 | 650.3655  | 43.41 | P72508 PHCB_ARTPT |                  |
| ANHGL            | 510.255   | 5  | -3.7 | 256.1339  | 16.35 | P72509 PHCA_ARTPT |                  |
| ANHGLSGD         | 769.3354  | 8  | 1.6  | 385.6737  | 15.55 | P72509 PHCA_ARTPT |                  |
| ANHGLSGDA        | 840.3726  | 11 | 0.9  | 421.1918  | 16.41 | P72509 PHCA_ARTPT |                  |
| ANHGLSGDAA       | 911.4097  | 10 | 0.5  | 456.7101  | 17.14 | P72509 PHCA_ARTPT |                  |
| ASEIAS           | 576.2755  | 6  | -4.4 | 577.2803  | 6.75  | P72508 PHCB_ARTPT |                  |
| DM(+15.99)EIL    | 748.3677  | 6  | -2   | 749.3735  | 20.5  | P72508 PHCB_ARTPT | Oxidation<br>(M) |
| DMEIL            | 732.3727  | 6  | -2.4 | 733.3783  | 29.43 | P72508 PHCB_ARTPT |                  |
| DYAIN            | 594.2649  | 5  | -3.1 | 595.2703  | 18.09 | P72509 PHCA_ARTPT |                  |
| EVTAGL           | 588.3119  | 6  | -3.8 | 589.317   | 18.93 | P72505 APCB_ARTPT |                  |
| FAEQPQ           | 718.3286  | 6  | -2.9 | 360.1705  | 17.24 | P72508 PHCB_ARTPT |                  |
| FATGEL           | 636.3119  | 6  | -7.4 | 637.3145  | 24.78 | P72505 APCB_ARTPT |                  |
| FVTSGER          | 794.3922  | 7  | -0.1 | 398.2014  | 28.94 | P72504 PHAA_ARTPT |                  |
| GIVAGDV          | 629.3384  | 7  | -5.1 | 630.3425  | 23.27 | P72504 PHAA_ARTPT |                  |
| GIVAGDVT         | 730.3861  | 8  | -0.8 | 731.3928  | 23.96 | P72504 PHAA_ARTPT |                  |
| GIVAGDVTPIE      | 1069.5656 | 11 | -0.8 | 1070.572  | 29.36 | P72504 PHAA_ARTPT |                  |
| IAGGTGPM         | 702.337   | 8  | 0.4  | 703.3413  | 29.11 | P72509 PHCA_ARTPT |                  |
| IAGGTGPM(+15.99) | 718.332   | 8  | -4.3 | 719.3362  | 21.44 | P72509 PHCA_ARTPT | Oxidation<br>(M) |
| IAGGTGPMMD       | 817.364   | 9  | 2.5  | 818.3695  | 22.38 | P72509 PHCA_ARTPT |                  |
| IAGGTGPMDE       | 946.4066  | 10 | -3.8 | 474.2087  | 18.11 | P72509 PHCA_ARTPT |                  |
| IAGID            | 487.2642  | 5  | -5.2 | 488.2690  | 22.44 | P72509 PHCA_ARTPT |                  |
| IAGIDE           | 616.3068  | 6  | -3.6 | 617.3118  | 20.22 | P72509 PHCA_ARTPT |                  |
| IAGIDEIN         | 843.4338  | 7  | 2.2  | 844.4390  | 27.05 | P72509 PHCA_ARTPT |                  |
| IGAM(+15.99)S    | 493.2206  | 5  | -4.7 | 494.2256  | 13.38 | P72504 PHAA_ARTPT | Oxidation<br>(M) |
| IGAMS            | 477.2257  | 5  | -3.8 | 478.2312  | 23.81 | P72504 PHAA_ARTPT |                  |
| IKANHGL          | 751.4341  | 7  | -3.6 | 376.7230* | 31.91 | P72509 PHCA_ARTPT |                  |

|                  |           |    |      |          |       |                   |                  |
|------------------|-----------|----|------|----------|-------|-------------------|------------------|
| IKANHGLS         | 838.4661  | 8  | -3.8 | 420.2387 | 32.69 | P72509 PHCA_ARTPT | Oxidation<br>(M) |
| IKANHGLSGDA      | 1081.5516 | 11 | -5.6 | 541.7801 | 28.92 | P72509 PHCA_ARTPT |                  |
| ISGAA            | 417.2223  | 5  | -3.4 | 418.2282 | 11.09 | P72509 PHCA_ARTPT |                  |
| ISGAAQA          | 616.318   | 7  | -4.8 | 617.3223 | 16.43 | P72509 PHCA_ARTPT |                  |
| KSLGTPI          | 714.4276  | 7  | -1.9 | 715.4335 | 35.93 | P72504 PHAA_ARTPT |                  |
| KSLGTPIE         | 843.4702  | 8  | -3.3 | 422.7410 | 29.9  | P72504 PHAA_ARTPT |                  |
| KSLGTPIEA        | 914.5073  | 9  | -5   | 458.2586 | 31.43 | P72504 PHAA_ARTPT |                  |
| LALGT            | 473.2849  | 5  | -4.9 | 474.2899 | 32.19 | P72508 PHCB_ARTPT |                  |
| LALGTPG          | 627.3591  | 7  | -6.5 | 628.3623 | 36    | P72508 PHCB_ARTPT |                  |
| LALGTPGSS        | 801.4232  | 9  | -3.4 | 802.4277 | 36.69 | P72508 PHCB_ARTPT |                  |
| LALGTPGSSV       | 900.4916  | 10 | -3.3 | 901.4960 | 39.04 | P72508 PHCB_ARTPT |                  |
| LALGTPGSSVA      | 971.5287  | 11 | -0.2 | 972.5358 | 39.77 | P72508 PHCB_ARTPT |                  |
| LALGTPGSSVAVGVGK | 1411.8035 | 16 | -4.7 | 706.9057 | 51.69 | P72508 PHCB_ARTPT |                  |
| LIAGID           | 600.3483  | 6  | -4.1 | 601.3531 | 33.81 | P72509 PHCA_ARTPT |                  |
| LIAGIDE          | 729.3909  | 7  | -1.2 | 730.3973 | 32.28 | P72509 PHCA_ARTPT |                  |
| LIAGIDEI         | 842.4749  | 8  | -1.2 | 843.4811 | 35.45 | P72509 PHCA_ARTPT |                  |
| LIAGIDEIN        | 956.5178  | 9  | -5.5 | 479.2635 | 34.64 | P72509 PHCA_ARTPT |                  |
| LIAGIDEINRT      | 1213.6666 | 11 | -2.3 | 607.8392 | 40.53 | P72509 PHCA_ARTPT |                  |
| LSGEDAAEA        | 861.3716  | 9  | -2.1 | 862.3771 | 6.91  | P72504 PHAA_ARTPT |                  |
| LSPGEL           | 614.3275  | 6  | -4.8 | 615.3318 | 24.76 | P72504 PHAA_ARTPT |                  |
| LSSTE            | 535.249   | 5  | -4.6 | 536.2538 | 5.86  | P72509 PHCA_ARTPT |                  |
| LSSTEI           | 648.333   | 6  | -4.2 | 649.3376 | 18.16 | P72509 PHCA_ARTPT |                  |
| M(+15.99)KTPL    | 604.3254  | 5  | -3.8 | 303.1689 | 25.31 | P72509 PHCA_ARTPT |                  |
| MKTPL            | 588.3305  | 5  | -5.5 | 589.3345 | 35.14 | P72509 PHCA_ARTPT |                  |
| NKFPY            | 667.3329  | 5  | -5.8 | 668.3364 | 34.42 | P72509 PHCA_ARTPT |                  |
| NKFPYT           | 768.3806  | 6  | -3.4 | 769.3853 | 37.4  | P72509 PHCA_ARTPT |                  |
| QKRPDVVSPGGNA    | 1323.6895 | 13 | -5.2 | 442.2348 | 29    | P72504 PHAA_ARTPT |                  |
| RPDVVSPGGNA      | 1067.5359 | 11 | -0.5 | 534.7723 | 26.82 | P72504 PHAA_ARTPT |                  |
| SFVTSGE          | 725.3232  | 7  | 0.4  | 726.3271 | 19.46 | P72504 PHAA_ARTPT |                  |
| SLGTPIE          | 715.3752  | 7  | 3.9  | 716.3817 | 25.51 | P72504 PHAA_ARTPT |                  |

|             |           |    |      |          |       |                   |
|-------------|-----------|----|------|----------|-------|-------------------|
| SLGTPIEA    | 786.4123  | 8  | 1    | 787.4164 | 27.79 | P72504 PHAA_ARTPT |
| SPGEL       | 501.2435  | 5  | -4.7 | 502.2484 | 12.86 | P72504 PHAA_ARTPT |
| SSVAVGVGK   | 802.4548  | 9  | 5.3  | 402.2348 | 50.25 | P72508 PHCB_ARTPT |
| STAQIDALS   | 904.4501  | 9  | 8.6  | 453.2362 | 23.75 | P72508 PHCB_ARTPT |
| SVINSSDVQGK | 1132.5724 | 11 | 1.3  | 567.2914 | 26.04 | P72505 APCB_ARTPT |
| TTQMQGPN    | 875.3807  | 8  | -3.8 | 438.6960 | 18.64 | P72509 PHCA_ARTPT |
| VAVGVGK     | 628.3908  | 7  | 0.5  | 629.3953 | 31.99 | P72508 PHCB_ARTPT |
| YFATGEL     | 799.3752  | 7  | 2    | 800.3803 | 33.1  | P72505 APCB_ARTPT |
| YLSPGEL     | 777.3909  | 7  | -1.3 | 778.3972 | 30.85 | P72504 PHAA_ARTPT |
| YSDITRPG    | 907.4399  | 8  | -0.3 | 454.7250 | 29.02 | P72505 APCB_ARTPT |

\*Observed as  $[M+2H]^{2+}$

**Table S1.** Complete list of peptide identified in the different samples with HILIC × RP and RP × RP approaches with Database search.



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