Supplementary Information (SI) for RSC Advances.

Supporting Information

Intact Quantitation and Evaluation of a PEG-Glycosulfopeptide as a Therapeutic P-Selectin Antagonist

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Supplementary Figures

Figure S1. Schematic of the flow path of the ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) system designed for the analysis of high molecular mass analytes in aqueous solutions using hydrophilic interaction liquid chromatography (HILIC). (A) Configuration of HILIC-MS during sample loading. (B) Configuration of HILIC-MS during elution and intact MS analysis.

Figure S2. Mass spectrometric analysis of PEG10-GSnP-6 under various analytical conditions. Deconvoluted mass spectra of PEG10-GSnP-6 analyzed using (A) butylene carbonate as an additive, (B) 5 mM ammonium acetate/acetic acid (pH 3.8) solvent system with the ion transfer temperature at 275°C, (C) 5 mM ammonium formate/ammonia (pH 8.3) solvent system with MS resolution at 240,000.

Figure S3. Analysis of intact, reduced alpha synuclein in solution. (A) Extracted chromatogram using HILIC. (B) Deconvoluted full mass spectrum. (C) Detected charged states using ESI-Orbitrap-MS.

Figure S4. Analysis of intact, reduced thioredoxin in solution. (A) Extracted chromatogram using HILIC. (B) Deconvoluted full mass spectrum. (C) Detected charged states using ESI-Orbitrap-MS.

Figure S5. Analysis of intact ubiquitin in solution. (A) Extracted chromatogram using HILIC. (B) Deconvoluted full mass spectrum. (C) Detected charged states using ESI-Orbitrap-MS.

Figure S6. Analysis of intact aprotinin in solution. (A) Extracted chromatogram using HILIC. (B) Deconvoluted full mass spectrum. (C) Detected charged states using ESI-Orbitrap-MS.

Figure S7. Analysis of intact enfuvirtide in solution. (A) Extracted chromatogram using HILIC. (B) Deconvoluted full mass spectrum. (C) Detected charged states using ESI-Orbitrap-MS.

Figure S8. Analysis of intact, sulfated big gastrin in solution. (A) Extracted chromatogram using HILIC. (B) Deconvoluted full mass spectrum. (C) Detected charged states using ESI-Orbitrap-MS.

Figure S9. Reproducibility of retention times following multiple injections of PEG10-GSnP-6 extracted from spiked plasma.

Figure S10. MS/MS spectrum of a native murine plasma protein employing higher-energy collisional dissociation (HCD) with stepped collision energies (20,30,40) in negative mode.

Figure S11. MS/MS spectrum of a native murine urine protein employing higher-energy collisional dissociation (HCD) with stepped collision energies (10,20,30) in negative mode.

Figure S12. Binding of U-937 cells to human P-selectin-Fc chimera assessed by flow cytometry. Binding was detected by PE-conjugated anti-Fc. (A) Determination of optimal P-selectin-Fc chimera to 300,000 U-937 cells. U-937 cells incubated without P-selectin displayed no binding. (B-C) Inhibition of binding by anti-PSGL-1 (1:5), anti-P-selectin (2 μg), or EDTA (20 mM). Representative histograms are shown from 3 experiments.

Figure S1.

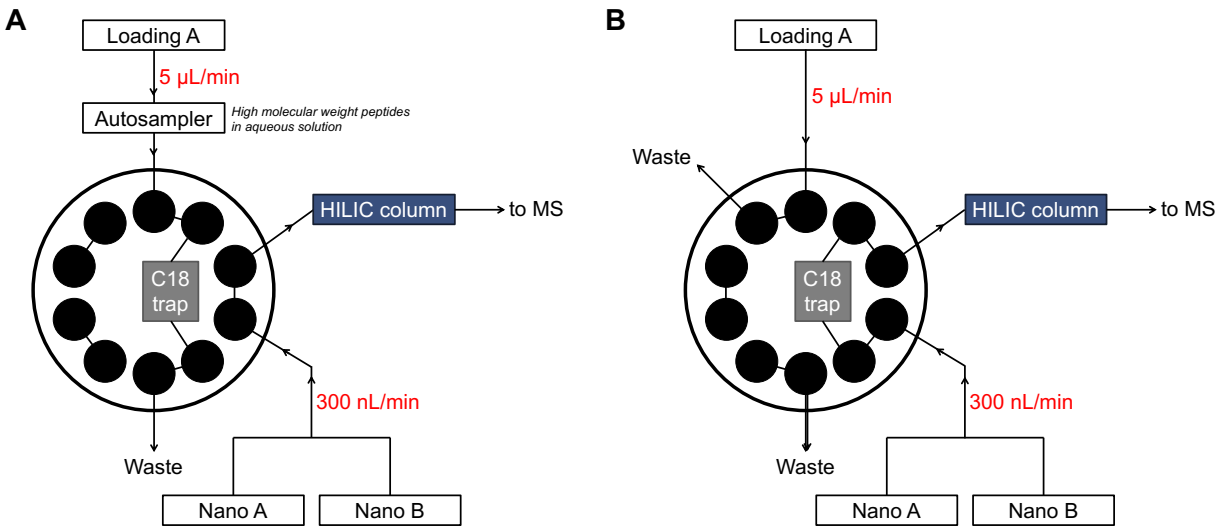

A
Loading A
5 µL/min
Autosampler
High molecular weight peptides in aqueous solution
HILIC column
to MS
C18 trap
300 nL/min
Waste
Nano A
Nano B
B
Loading A
5 µL/min
Waste
HILIC column
to MS
C18 trap
300 nL/min
Waste
Nano A
Nano B

Figure S2.

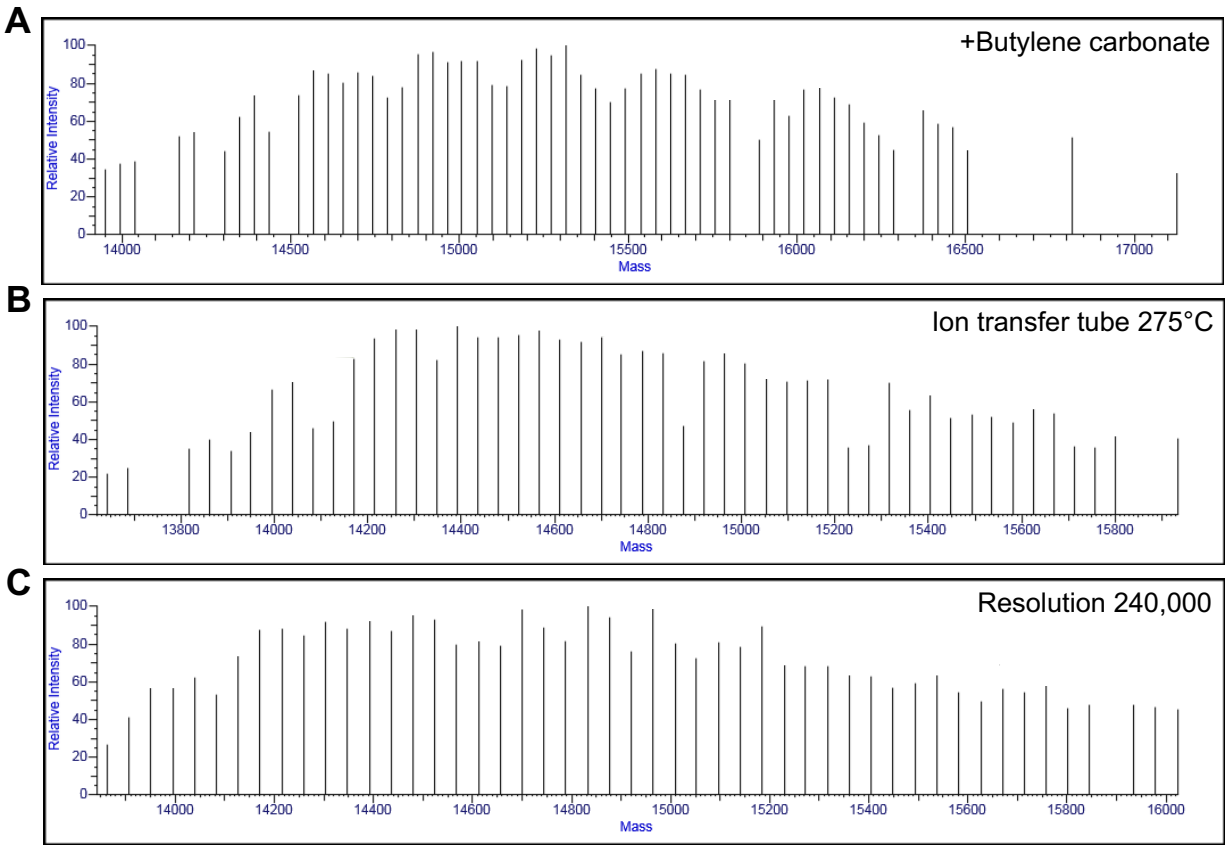

A
+Butylene carbonate
Relative Intensity
Mass
B
Ion transfer tube 275°C
Relative Intensity
Mass
C
Resolution 240,000
Relative Intensity
Mass

Figure S3.

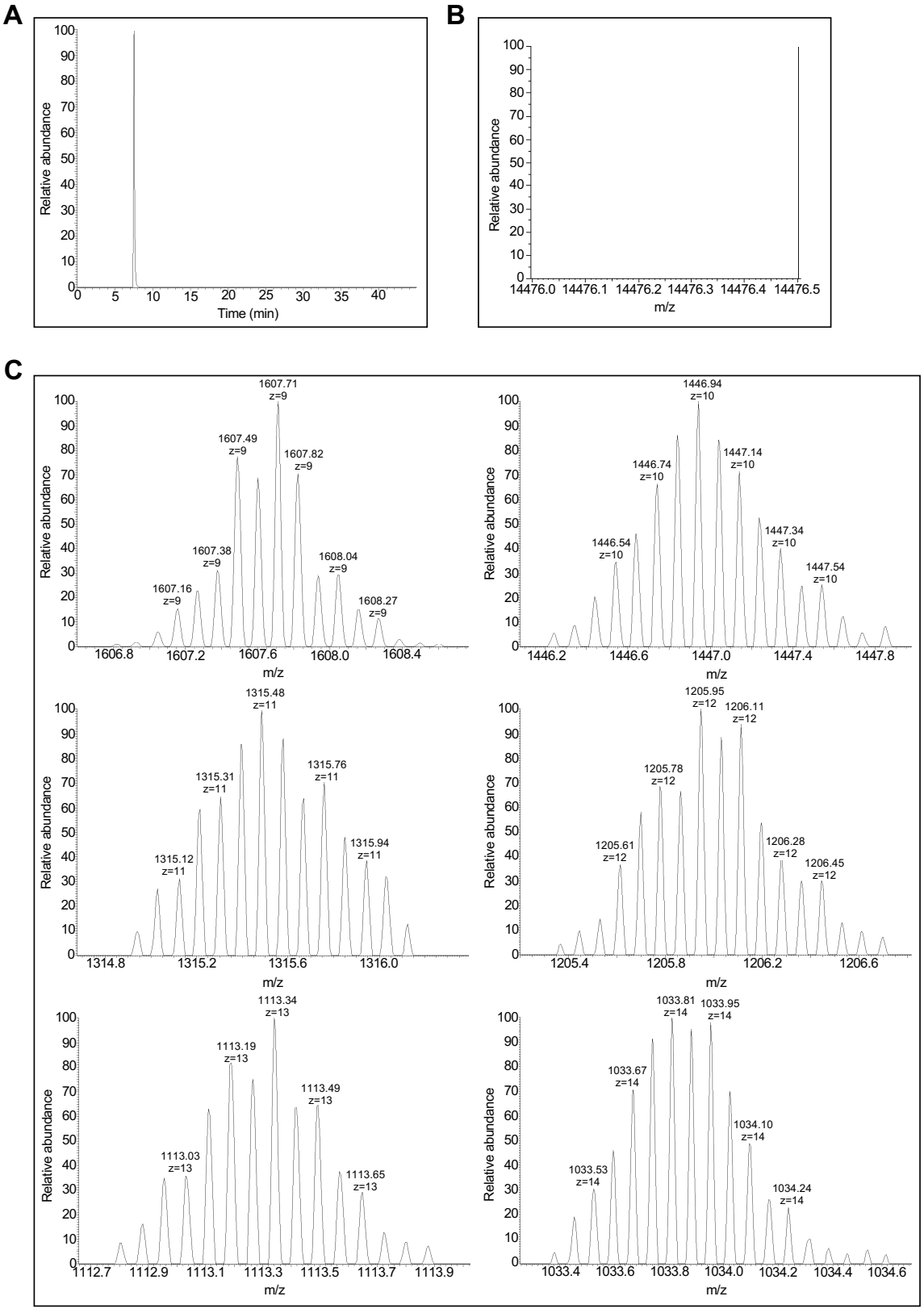

A
Relative abundance
Time (min)
B
Relative abundance
m/z
14476.0 14476.1 14476.2 14476.3 14476.4 14476.5
C
1607.71 z=9
1607.49 z=9
1607.82 z=9
1607.38 z=9
1608.04 z=9
1607.16 z=9
1608.27 z=9
1446.94 z=10
1446.74 z=10
1447.14 z=10
1446.54 z=10
1447.34 z=10
1447.54 z=10
1315.48 z=11
1315.31 z=11
1315.76 z=11
1315.12 z=11
1315.94 z=11
1205.95 z=12
1206.11 z=12
1205.78 z=12
1205.61 z=12
1206.28 z=12
1206.45 z=12
1113.34 z=13
1113.19 z=13
1113.49 z=13
1113.03 z=13
1113.65 z=13
1033.81 z=14
1033.95 z=14
1033.67 z=14
1034.10 z=14
1033.53 z=14
1034.24 z=14
Relative abundance
m/z

Figure S4.

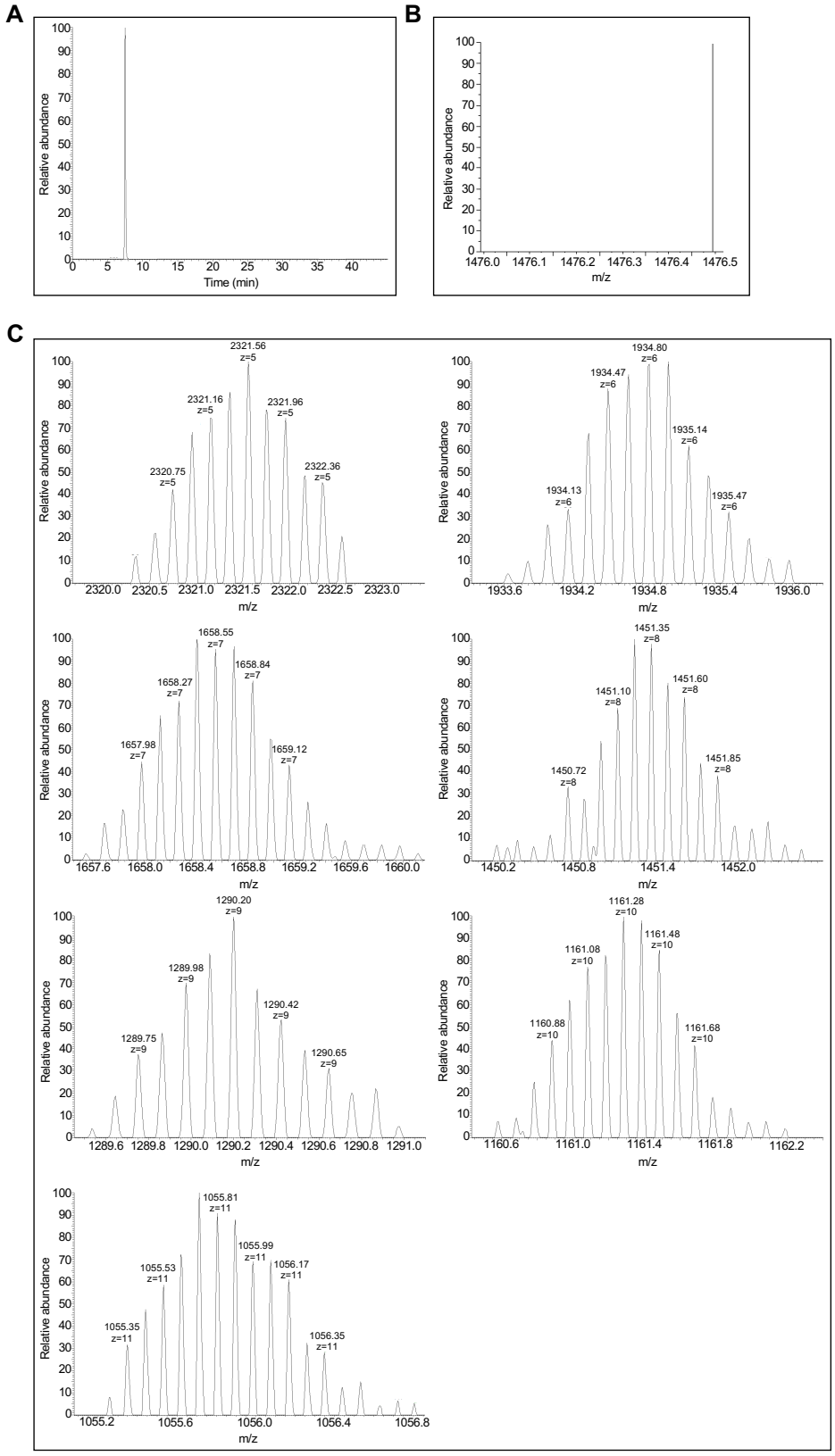

A
Relative abundance
Time (min)
B
Relative abundance
m/z
C
2321.56 z=5
2321.16 z=5
2321.96 z=5
2320.75 z=5
2322.36 z=5
1934.80 z=6
1934.47 z=6
1935.14 z=6
1934.13 z=6
1935.47 z=6
1658.55 z=7
1658.27 z=7
1658.84 z=7
1657.98 z=7
1659.12 z=7
1451.35 z=8
1451.10 z=8
1451.60 z=8
1450.72 z=8
1451.85 z=8
1290.20 z=9
1289.98 z=9
1290.42 z=9
1289.75 z=9
1290.65 z=9
1161.28 z=10
1161.08 z=10
1161.48 z=10
1160.88 z=10
1161.68 z=10
1055.81 z=11
1055.53 z=11
1055.99 z=11
1056.17 z=11
1055.35 z=11
1056.35 z=11
Relative abundance
m/z

Figure S5.

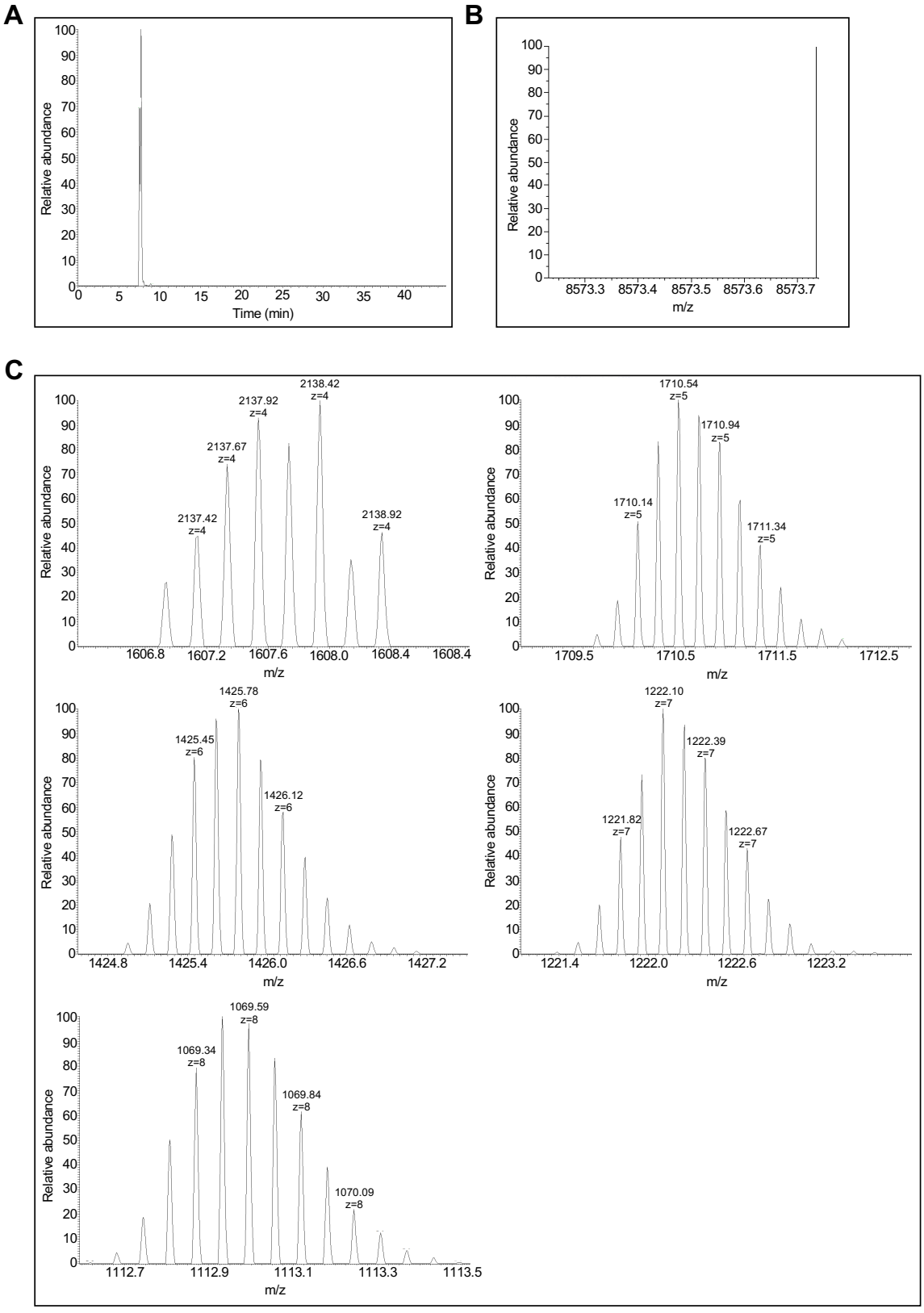

A
Relative abundance
Time (min)
B
Relative abundance
m/z
8573.3
8573.4
8573.5
8573.6
8573.7
C
2138.42 z=4
2137.92 z=4
2137.67 z=4
2137.42 z=4
2138.92 z=4
1710.54 z=5
1710.94 z=5
1710.14 z=5
1711.34 z=5
1425.78 z=6
1425.45 z=6
1426.12 z=6
1222.10 z=7
1222.39 z=7
1221.82 z=7
1222.67 z=7
1069.59 z=8
1069.34 z=8
1069.84 z=8
1070.09 z=8
m/z

Figure S6.

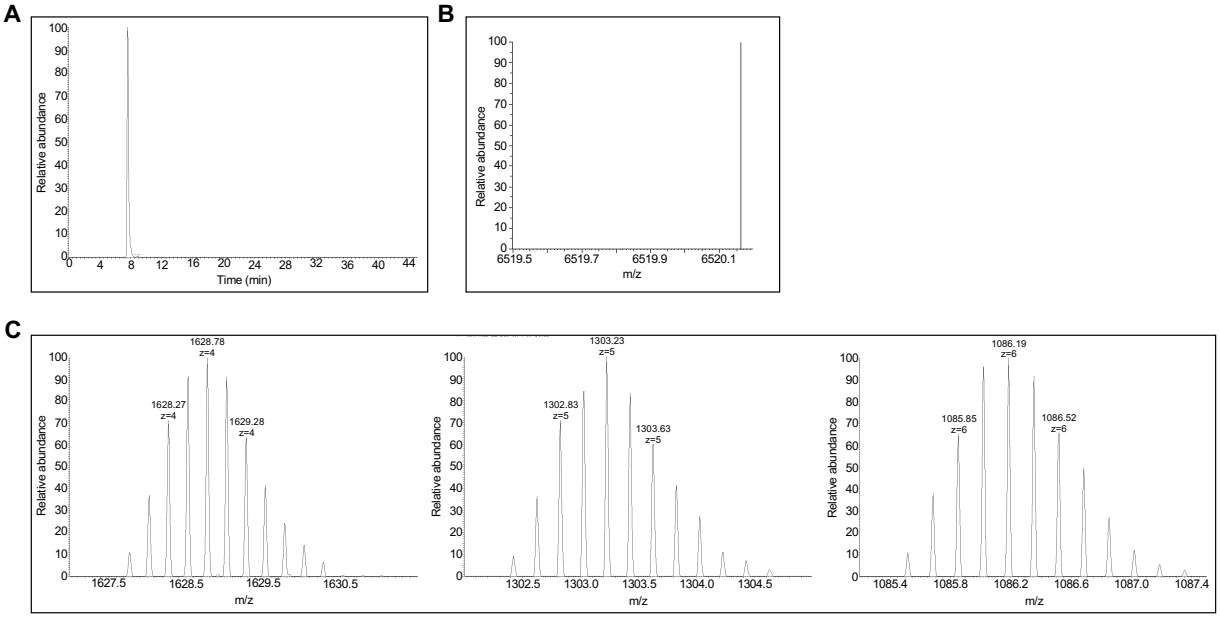

A
Relative abundance
Time (min)
B
Relative abundance
m/z
6519.5
6519.7
6519.9
6520.1
C
1628.78
z=4
1628.27
z=4
1629.28
z=4
1303.23
z=5
1302.83
z=5
1303.63
z=5
1086.19
z=6
1085.85
z=6
1086.52
z=6
Relative abundance
m/z

Figure S7.

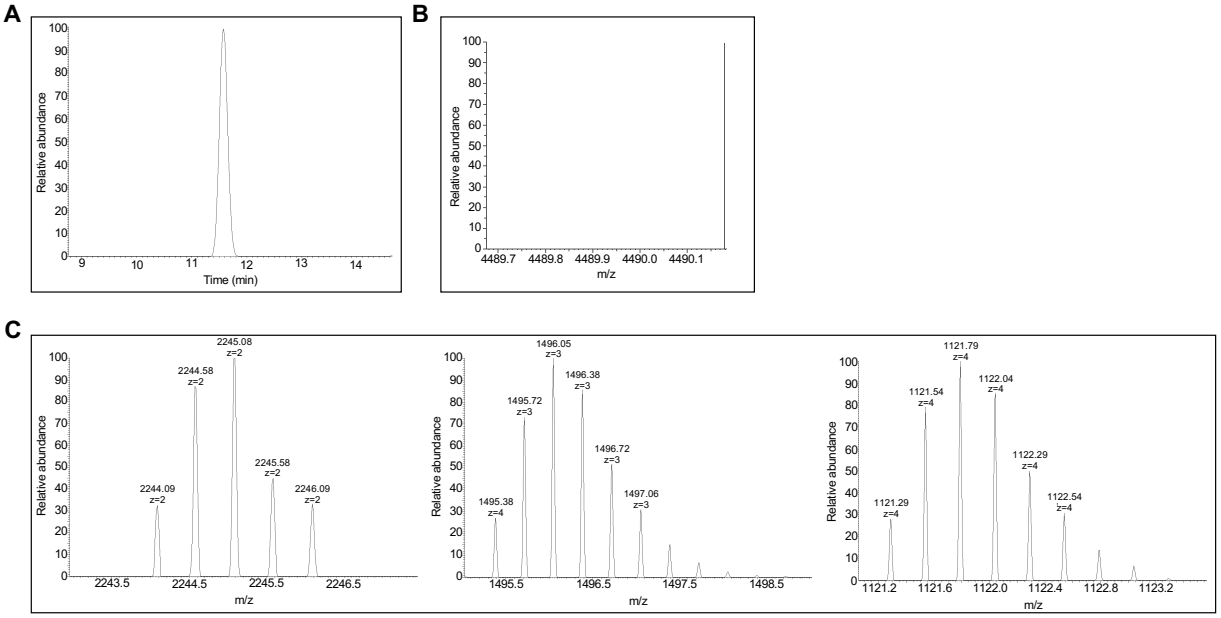

A
Relative abundance
Time (min)
B
Relative abundance
m/z
4489.7
4489.8
4489.9
4490.0
4490.1
C
2245.08
z=2
2244.58
z=2
2244.09
z=2
2245.58
z=2
2246.09
z=2
1496.05
z=3
1496.38
z=3
1495.72
z=3
1496.72
z=3
1495.38
z=4
1497.06
z=3
1121.79
z=4
1121.54
z=4
1122.04
z=4
1122.29
z=4
1121.29
z=4
1122.54
z=4

Figure S8.

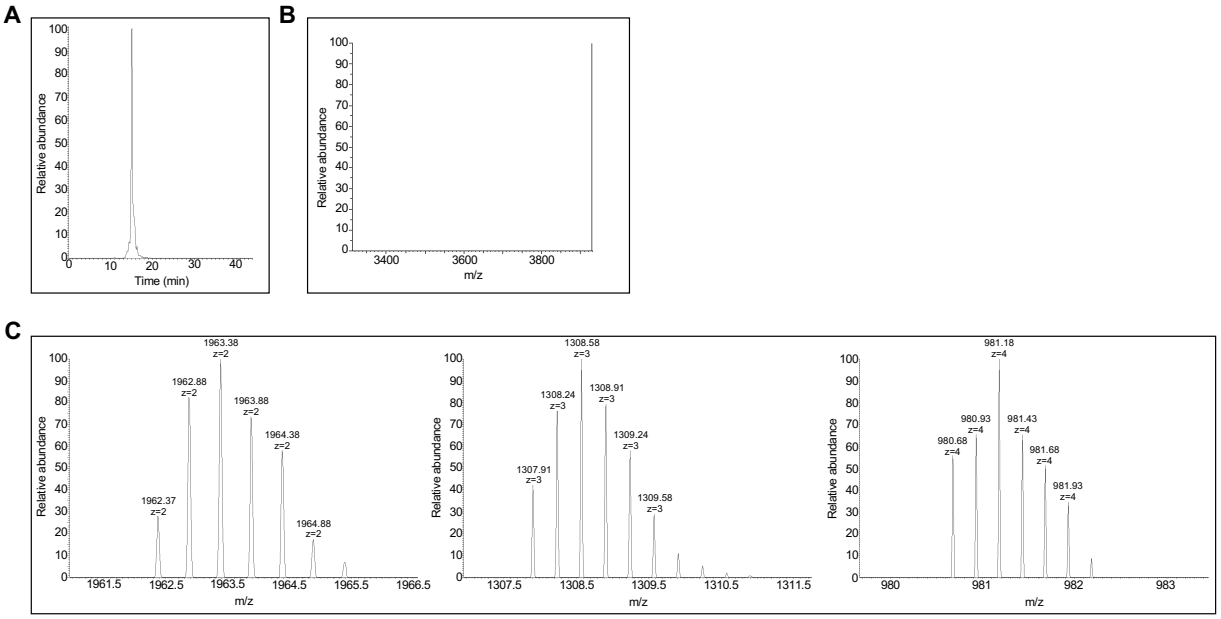

A
Relative abundance
Time (min)
B
Relative abundance
m/z
C
Relative abundance
1963.38 z=2
1962.88 z=2
1963.88 z=2
1964.38 z=2
1962.37 z=2
1964.88 z=2
m/z
1308.58 z=3
1308.24 z=3
1308.91 z=3
1309.24 z=3
1307.91 z=3
1309.58 z=3
m/z
981.18 z=4
980.93 z=4
981.43 z=4
980.68 z=4
981.68 z=4
981.93 z=4
m/z

Figure S9.

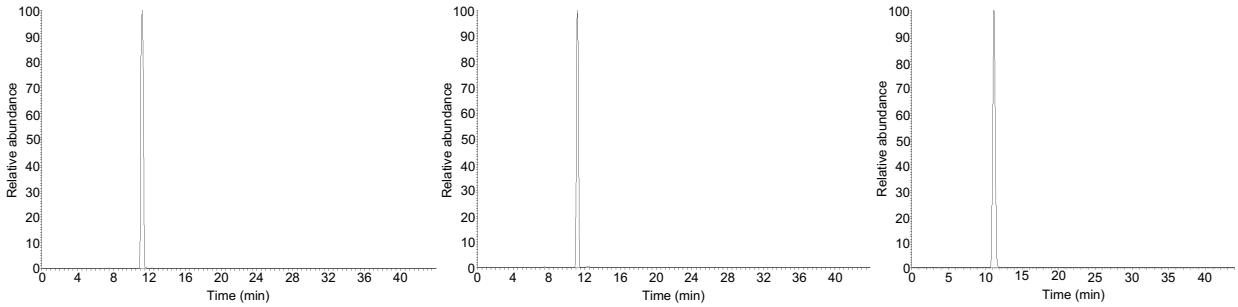

Relative abundance
Time (min)
Relative abundance
Time (min)
Relative abundance
Time (min)

Figure S10.

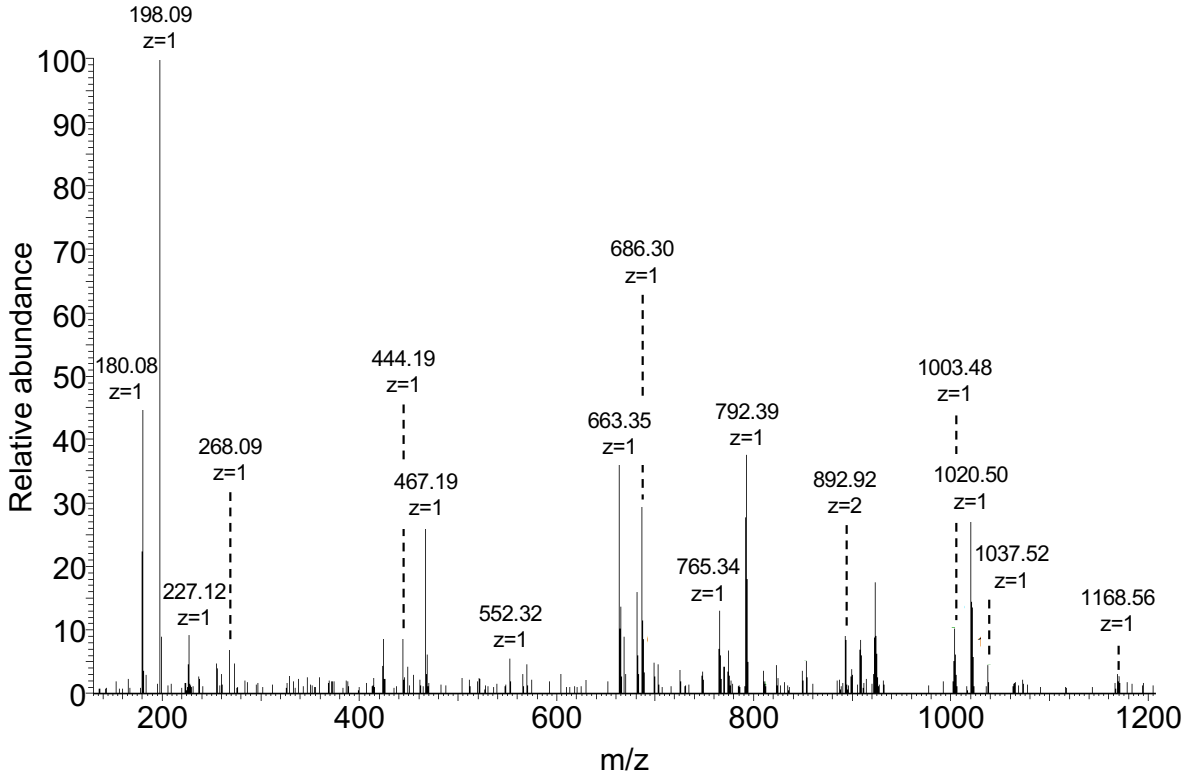

198.09
z=1
180.08
z=1
268.09
z=1
227.12
z=1
444.19
z=1
467.19
z=1
552.32
z=1
686.30
z=1
663.35
z=1
765.34
z=1
792.39
z=1
892.92
z=2
1003.48
z=1
1020.50
z=1
1037.52
z=1
1168.56
z=1
Relative abundance
m/z
0
10
20
30
40
50
60
70
80
90
100
200
400
600
800
1000
1200

Figure S11.

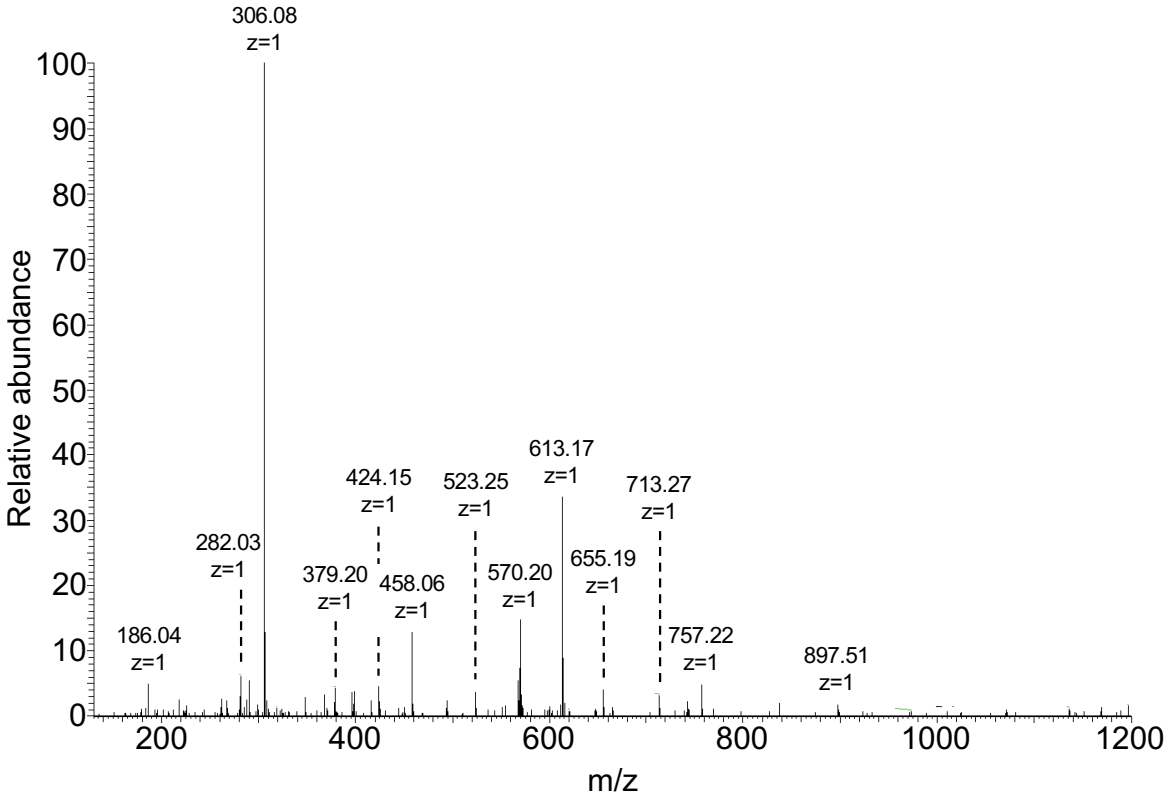
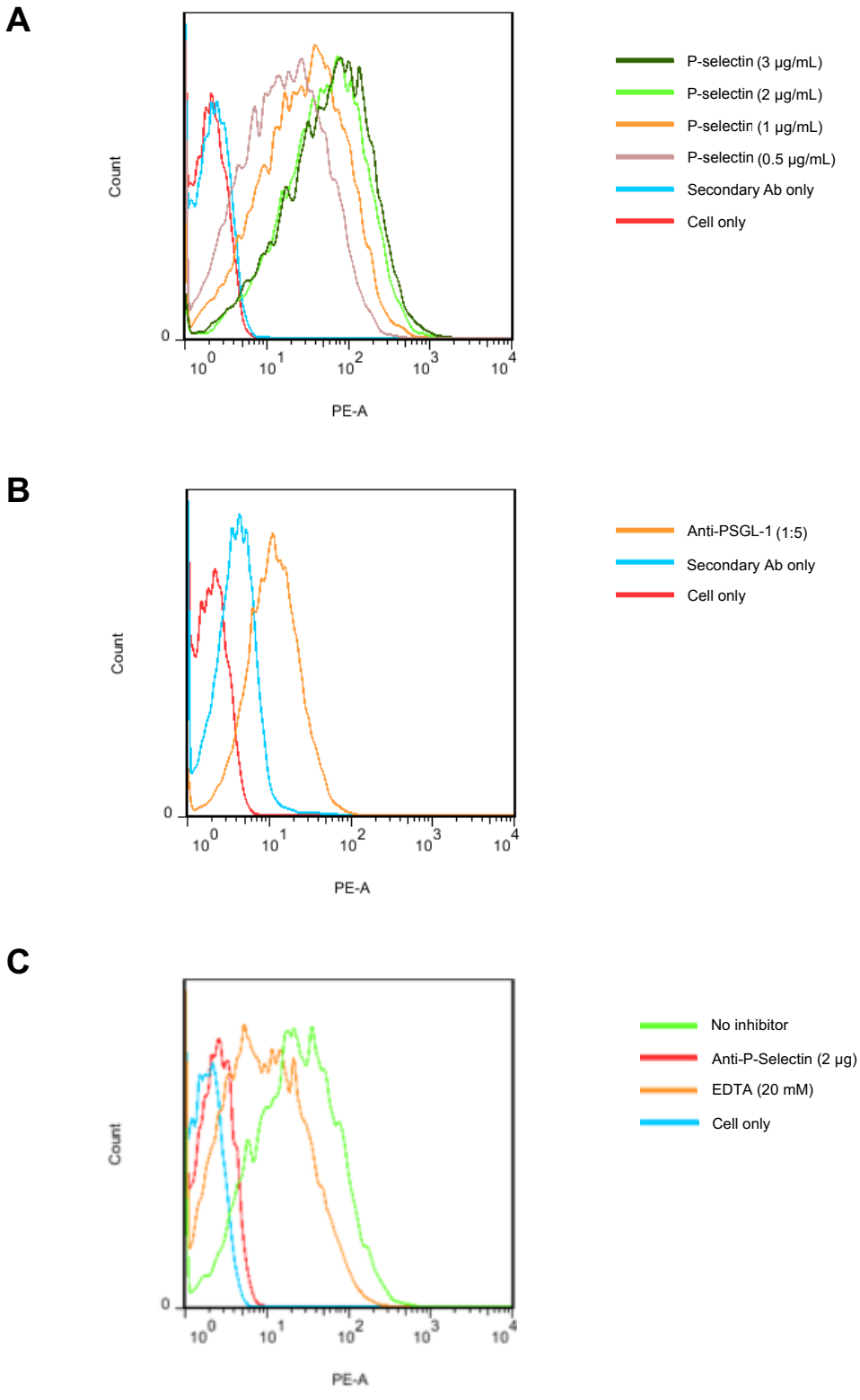

306.08
z=1
613.17
z=1
424.15
z=1
523.25
z=1
713.27
z=1
282.03
z=1
379.20
z=1
458.06
z=1
570.20
z=1
655.19
z=1
186.04
z=1
757.22
z=1
897.51
z=1
Relative abundance
m/z
0
10
20
30
40
50
60
70
80
90
100
200
400
600
800
1000
1200

Figure S12.


A
Count
0
10^0 10^1 10^2 10^3 10^4
PE-A
P-selectin (3 µg/mL)
P-selectin (2 µg/mL)
P-selectin (1 µg/mL)
P-selectin (0.5 µg/mL)
Secondary Ab only
Cell only
B
Count
0
10^0 10^1 10^2 10^3 10^4
PE-A
Anti-PSGL-1 (1:5)
Secondary Ab only
Cell only
C
Count
0
10^0 10^1 10^2 10^3 10^4
PE-A
No inhibitor
Anti-P-Selectin (2 µg)
EDTA (20 mM)
Cell only