

Supplementary Information

Total Wash Elimination for Solid Phase Peptide Synthesis

Jonathan M. Collins*, Sandeep K. Singh, Travis A. White, Drew J. Cesta, Colin L. Simpson, Levi J. Tubb & Christopher L. Houser

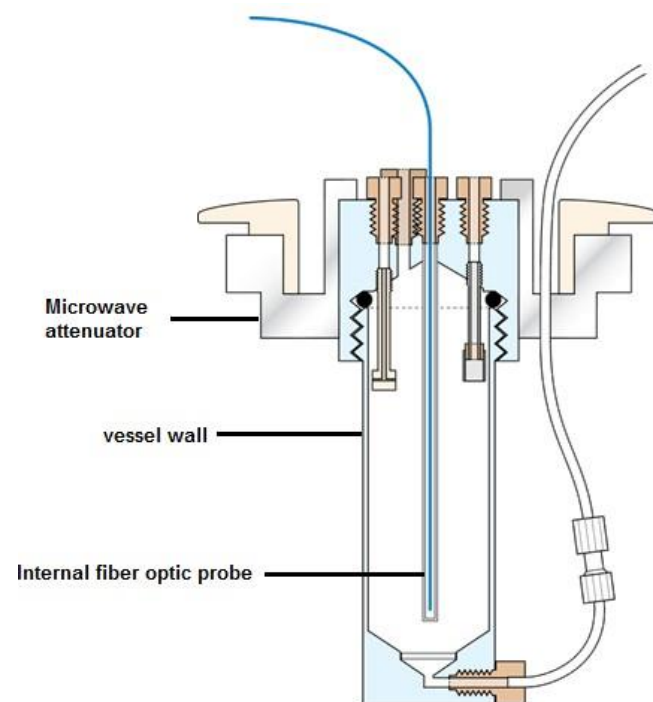
Peptide Synthesis Research, CEM Corporation, 3100 Smith Farm Rd, Matthews, NC 28104, USA

Table of Contents

1. Materials	Page 2
2. Supplementary Fig. 1 Microwave SPPS reaction vessel schematic	Page 3
3. Supplementary Table 1 Results from wash-free and wash based Fmoc SPPS	Page 4
4. Supplementary Table 2 Total synthesis time and total waste volume from automated microwave peptide/protein synthesis using wash-free methodology	Page 4
5. Purity Reports and UPLC-MS Chromatograms for Table 1	Page 5-26
6. Purity Reports and UPLC-MS Chromatograms for Supplementary Table 1	Page 27-37
7. C.A.T. GmbH Epimerization Data for 25 mmol Liraglutide Production Run	Page 38-54
8. Supplementary Table 3 Stepwise method details for wash-free 0.1 mmol research scale synthesis	Page 55
9. Supplementary Table 4 Stepwise method details for wash-free 25 mmol production scale synthesis	Page 56
10. Supplementary Table 5 Stepwise method details for residual pyrrolidine quantification by GC-FID	Page 57
11. GC-FID calibration curve	Page 59
12. GC-FID chromatograms	Page 60-61

Materials

The following Fmoc amino acids were obtained from CEM Corporation and contain the indicated side chain protecting groups: Ala, Asn(Trt), Arg(Pbf), Asp(OMpe), Cys(Trt), Gln(Trt), Glu(OtBu), Gly, His(Boc), Ile, Leu, Lys(Boc), Phe, Pro, Met, Ser(tBu), Thr(tBu), Trp(Boc), Tyr(tBu), and Val. Fmoc-Lys(palmitoyl-Glu-OtBu)-OH, Oxyma, Rink Amide ProTide and preloaded Wang ProTide resins were also obtained from CEM Corporation. Fmoc-Rink Amide MBHA PS resin was obtained from MilliporeSigma. *N,N'*-Diisopropylcarbodiimide (DIC), pyrrolidine, piperidine, trifluoroacetic acid (TFA), 3,6-dioxa-1,8-octanedithiol (DODT), and triisopropylsilane (TIS) were obtained from Sigma-Aldrich. Dichloromethane (DCM), *N,N*-Dimethylformamide (DMF), *N*-methylpyrrolidone (NMP), anhydrous diethyl ether (Et₂O), and acetic acid were obtained from VWR. LC-MS grade water (H₂O) and LC-MS grade acetonitrile (MeCN) were obtained from Fisher Scientific. *N*-butylpyrrolidine (NBP) was obtained from CEM Corporation.



Supplementary Fig. 1 Microwave SPPS reaction vessel schematic.

Supplementary Table 1 Results from wash-free and wash based Fmoc SPPS.

Entry	Peptide	Deprotection Temp.	Deprotection Time	Crude Purity (Wash-free)	Crude Purity (Wash based)
1	⁶⁵⁻⁷⁴ ACP	110 °C	80 sec	91%	91%
2	Liraglutide	110 °C	80 sec	73%	74%
3	Semaglutide	110 °C	80 sec	71%	74%
4	¹⁻⁴² β-amyloid	110 °C	80 sec	69%	69%
5	Liraglutide	80 °C	8 min	72%	--
6*	Liraglutide	90 °C	10 min	77%	78% [#]

*Synthesized at 25 mmol scale; [#]80 °C/4 min Deprotection method was used

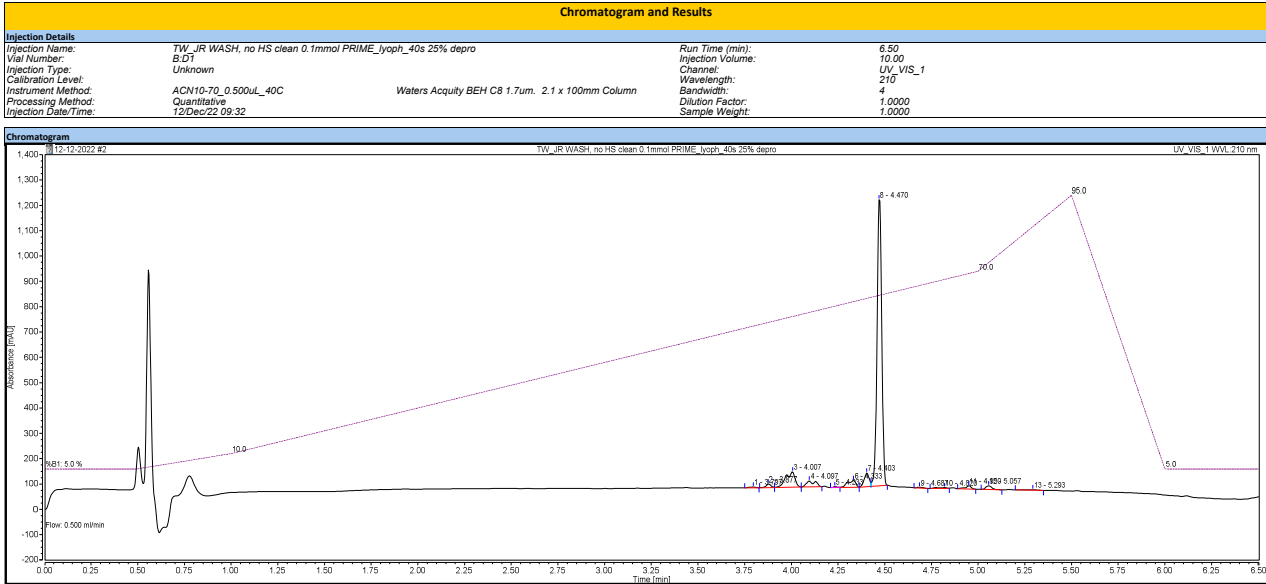
Supplementary Table 2 Total synthesis time and total waste volume from automated microwave peptide/protein synthesis using wash-free methodology.

Entry	Peptide/Protein	Total Synthesis Time	Total Waste Volume
1	JR	38 min	43 mL
2	⁶⁵⁻⁷⁴ ACP	35 min	39 mL
3	Liraglutide	1 h 52 min	135 mL
4	¹⁻⁴² β-Amyloid	2 h 29 min	179 mL
5	Proinsulin 86-mer	10 h 40 min	687 mL
6	Barstar 89-mer	10 h 56 min	700 mL
7	Semaglutide	1 h 51 min	135 mL

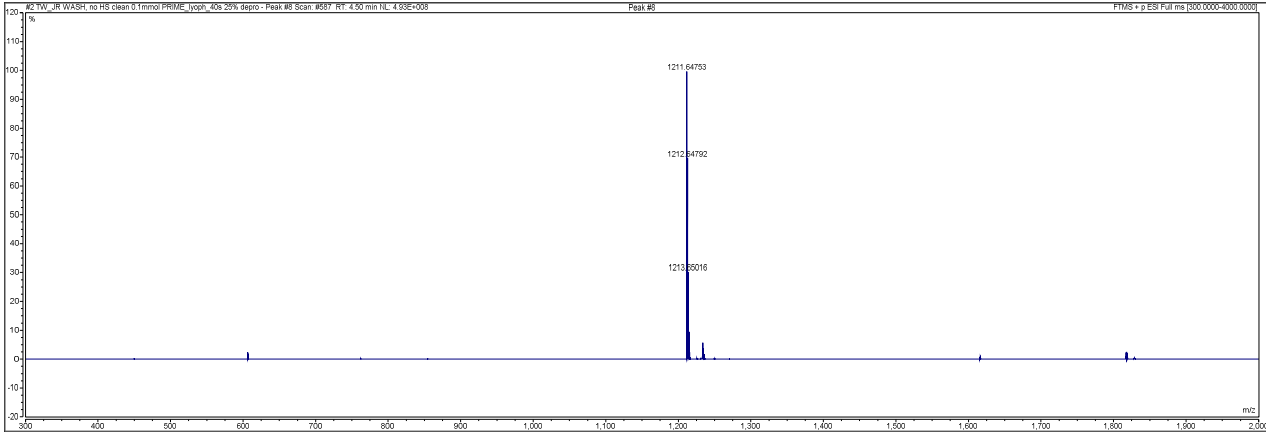
JR-Table 1, entry 1

Instrument: FRNTNR2_1 Sequence: 12-12-2022

Page 1 of 1



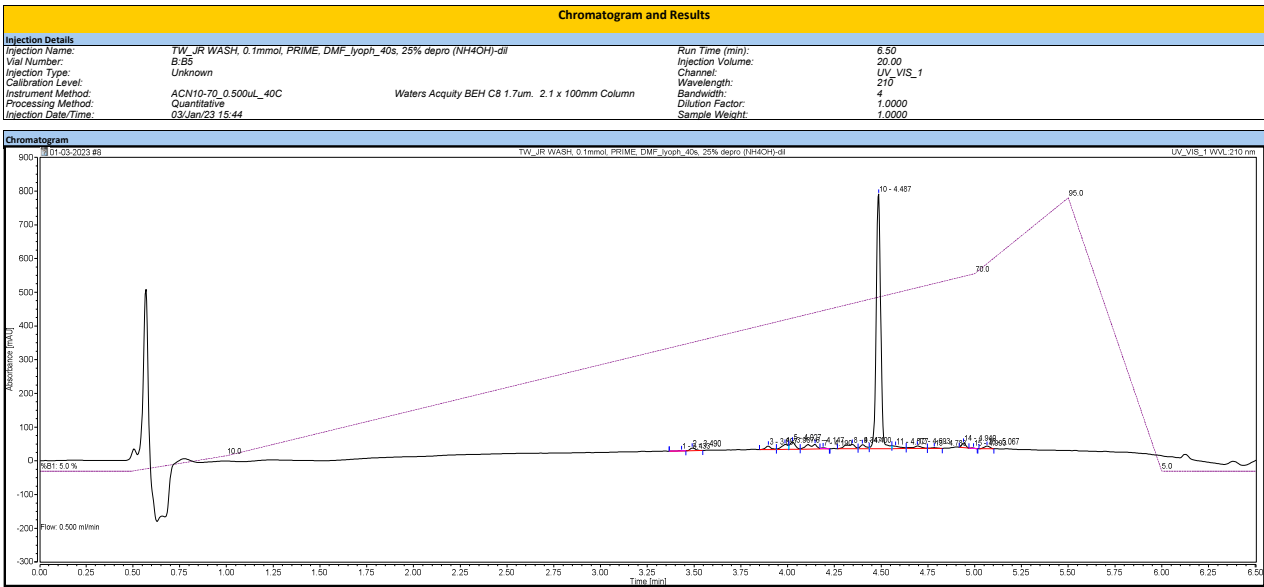
No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	3.797	0.118	2.924	0.28	0.22
2	3.877	0.395	14.209	0.92	1.05
3	4.007	3.423	80.637	8.87	4.39
4	4.097	1.286	22.981	3.01	1.70
5	4.253	0.061	7.721	0.14	0.20
6	4.333	0.452	27.105	3.33	2.01
7	4.403	1.647	53.861	3.86	5.99
8	4.470	32.639	1243.249	77.03	83.70
9	4.557	0.106	3.182	0.25	0.24
10	4.820	0.130	2.933	0.42	0.23
11	4.959	0.434	12.958	1.08	1.93
12	5.057	0.548	14.919	1.29	1.11
13	5.293	0.150	1.959	0.35	0.15
Total:		42.657	1349.170	100.00	100.00



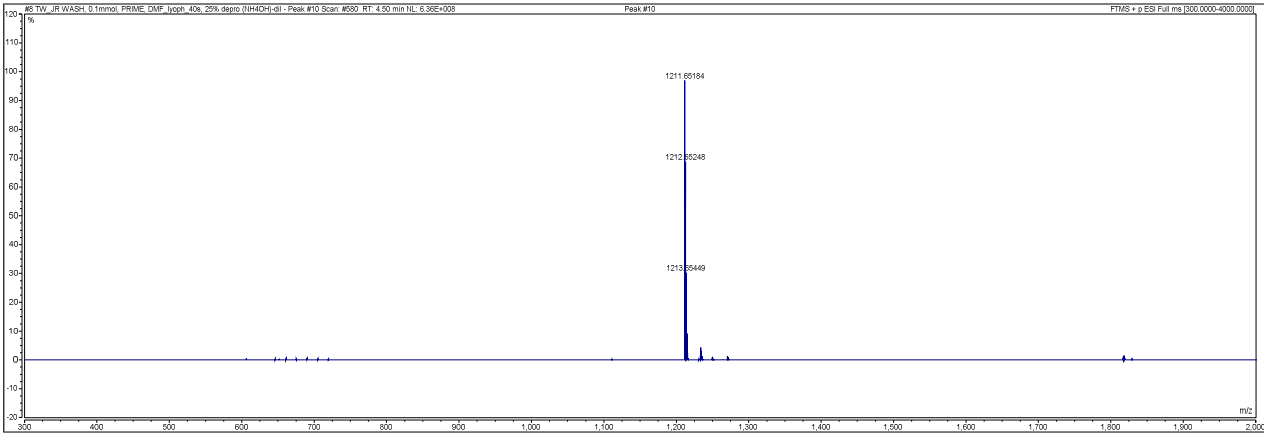
JR-Table 1, entry 2

Instrument: F9000R2_1 Sequence: 01-03-2023

Page 1 of 1



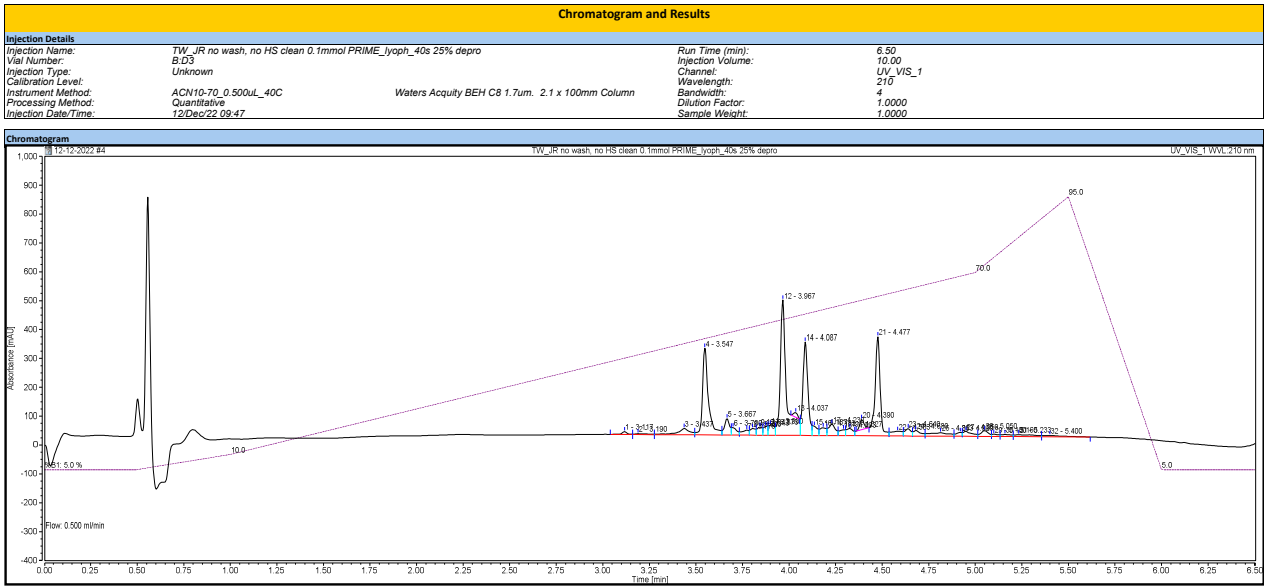
No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	3.433	0.024	0.669	0.09	0.08
2	3.490	0.257	7.349	1.91	0.38
3	3.689	0.357	10.464	1.50	1.18
4	3.987	0.580	14.690	2.19	1.65
5	4.027	0.716	21.431	2.98	2.41
6	4.147	0.850	13.361	3.98	2.50
7	4.190	0.028	1.980	0.18	0.22
8	4.347	0.753	12.638	2.84	1.41
9	4.400	0.405	11.638	2.84	1.41
10	4.487	20.914	755.001	78.98	84.82
11	4.579	0.394	7.146	1.49	0.80
12	4.603	0.234	7.156	1.40	0.80
13	4.783	0.101	2.284	0.38	0.26
14	4.940	0.331	13.367	1.26	1.58
15	4.993	0.033	1.886	0.12	0.18
16	5.067	0.290	7.658	1.09	0.86
Total:		26.486	890.167	100.00	100.00



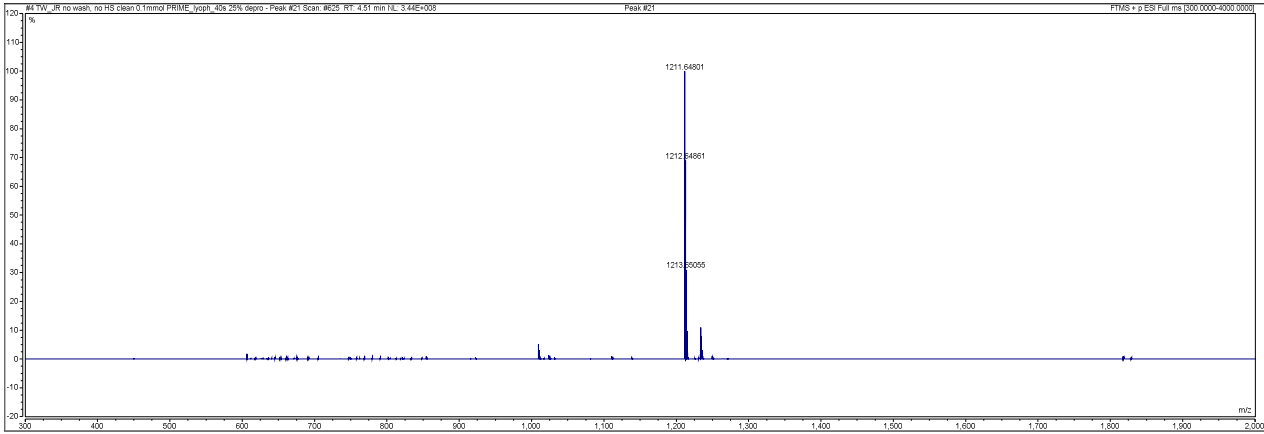
JR-Table 1, entry 3

Instrument:FRNTR2_1 Sequence:12-13-2022

Page 1 of 1



No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	3.117	0.337	10.044	0.44	0.49
2	3.190	0.248	2.977	0.32	0.15
3	3.547	1.854	22.465	2.17	1.00
4	3.547	17.102	300.340	14.33	14.79
5	3.667	1.738	56.857	2.31	2.79
6	3.700	0.638	23.957	1.08	1.23
7	3.773	0.781	17.442	0.97	0.86
8	3.803	0.926	23.219	0.99	1.13
9	3.873	0.781	30.635	1.01	1.39
10	3.957	1.312	35.091	1.09	1.73
11	4.037	18.189	488.501	23.47	23.07
12	4.037	0.405	15.786	0.52	0.78
13	4.087	10.052	323.366	12.97	15.92
14	4.183	1.026	32.102	1.36	1.35
15	4.233	1.076	26.822	1.39	1.32
16	4.233	1.683	29.530	2.17	1.95
17	4.233	0.682	20.237	0.96	1.00
18	4.367	1.972	33.210	1.23	1.73
19	4.367	1.052	22.238	1.36	1.24
20	4.390	0.471	15.723	0.51	0.73
21	4.477	12.797	343.269	16.51	16.90
22	4.553	1.176	16.066	1.45	0.79
23	4.650	0.866	25.893	1.43	0.95
24	4.650	0.886	18.893	1.43	0.94
25	4.813	1.468	12.023	1.89	0.59
26	4.813	0.471	15.723	0.51	0.73
27	4.960	0.959	17.613	0.91	0.83
28	5.050	0.931	19.980	1.20	0.98
29	5.180	0.295	7.319	0.41	0.33
30	5.180	0.295	7.319	0.55	0.33
31	5.233	0.830	6.757	1.07	0.33
32	5.400	0.560	4.471	0.72	0.22
Total:		77.497	2031.120	100.00	100.00

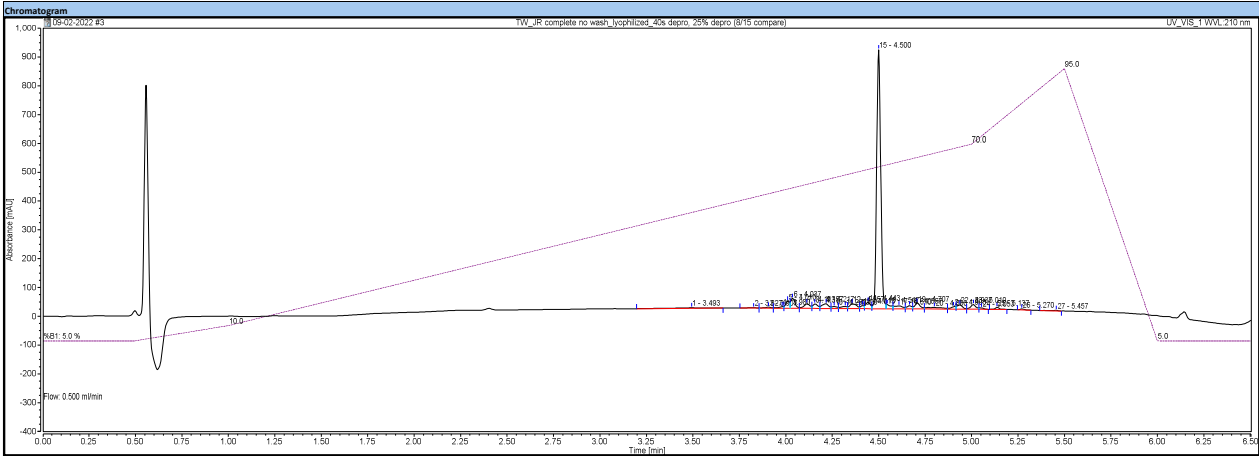


JR-Table 1, entry 4

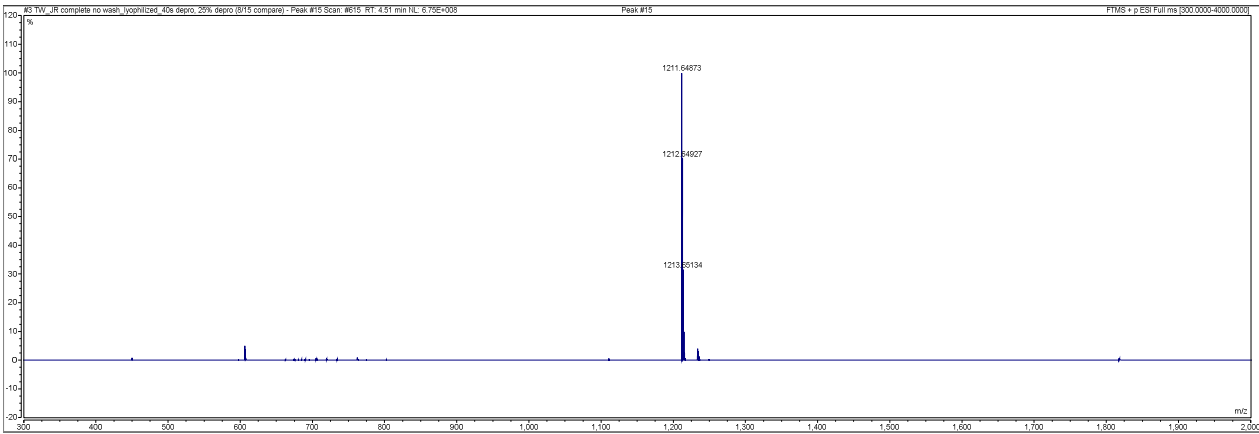
Instrument:FRONTNR2_1 Sequence:09-03-2022

Page 1 of 1

Chromatogram and Results					
Injection Details					
Injection Name:	TW_IR complete no wash_lyophilized_40s depro, 25% depro (8/15 compare)			Run Time (min):	6.50
Vial Number:	Y:AB			Injection Volume:	2.50
Injection Type:	Unknown			Channel:	UV_VIS_1
Calibration Level:				Wavelength:	210
Instrument Method:	ACN/H ₂ O 70:30.500uL_40C			Bandwidth:	4
Processing Method:	Quantitative			Dilution Factor:	1.0000
Injection Date/Time:	02/Sep/22 10:11			Sample Weight:	1.0000



No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	3.493	0.408	2.292	1.23	0.19
2	3.827	0.071	1.408	0.21	0.12
3	3.907	0.143	3.153	0.33	0.31
4	3.980	0.180	3.617	0.34	0.47
5	4.010	0.595	24.853	1.79	2.07
6	4.037	0.971	34.923	2.77	3.80
7	4.113	0.589	15.817	1.77	1.90
8	4.137	0.476	15.807	1.43	1.32
9	4.157	0.665	16.154	2.43	1.36
10	4.260	0.218	6.504	0.65	0.54
11	4.320	0.299	7.285	0.90	0.61
12	4.340	0.119	1.237	0.16	0.13
13	4.413	0.242	10.382	0.70	0.88
14	4.443	0.637	22.768	1.91	1.90
15	4.500	22.752	859.216	68.58	74.31
16	4.547	0.427	14.247	1.28	1.20
17	4.610	0.382	10.374	1.66	0.86
18	4.657	0.532	16.379	1.88	1.19
19	4.707	0.724	20.483	2.18	1.71
20	4.800	0.559	5.903	1.88	0.49
21	4.815	0.219	8.136	0.83	0.70
22	4.937	0.506	14.969	1.82	1.28
23	5.010	0.542	16.771	1.94	1.39
24	5.043	0.138	4.634	0.41	0.39
25	5.137	0.155	6.172	0.47	0.51
26	5.270	0.083	3.072	0.25	0.26
27	5.457	0.088	2.109	0.26	0.18
Total:		33.285	1200.396	100.00	100.00



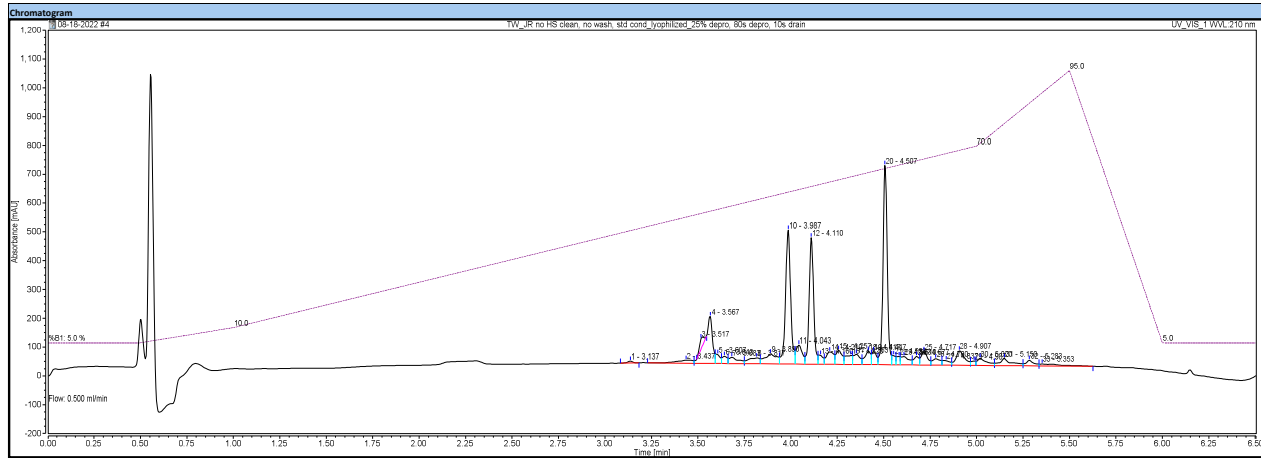
JR-Table 1, entry 5

Instrument: FRNTNR2_1 Sequence: 08-18-2022

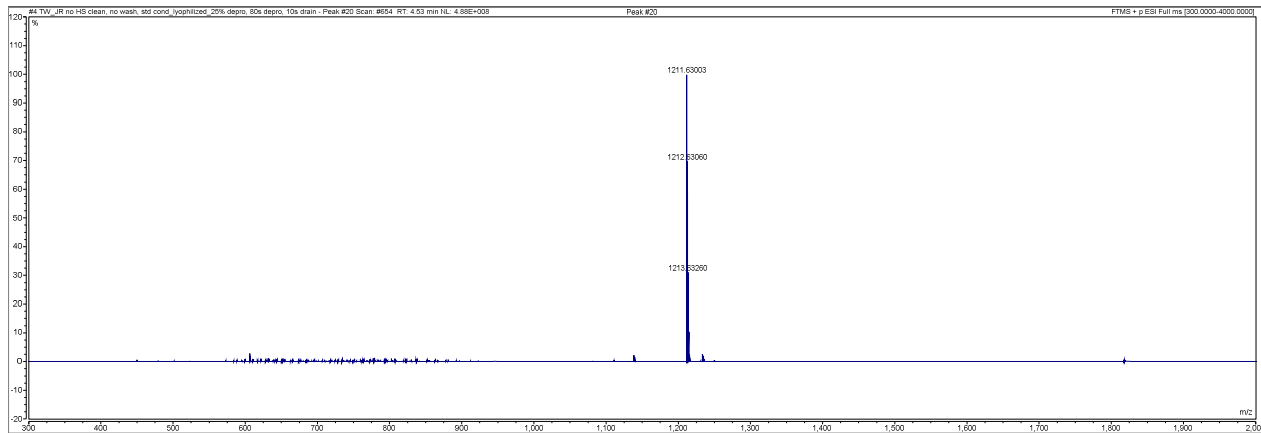
Page 1 of 1

Chromatogram and Results

Injection Details			
Injection Name:	TW_IR no HS clean, no wash, std cond_lyophilized_25% depro, 80s depro, 10s drain	Run Time (min):	6.50
Vial Number:	YIC9	Injection Volume:	20.00
Injection Type:	Unknown	Channel:	UV_VIS_1
Calibration Level:	ACN10-70, 0.500uL_40C	Wavelength:	210
Instrument Method:	Quantitative	Bandwidth:	4
Processing Method:		Dilution Factor:	1.0000
Injection Date/Time:	18/Aug/22 09:05	Sample Weight:	1.0000



No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	3.137	0.213	6.345	0.23	0.24
2	3.437	1.115	10.422	1.22	0.40
3	3.517	0.905	26.427	0.99	1.38
4	3.567	7.963	162.319	8.69	6.16
5	3.607	0.916	31.984	1.00	1.19
6	3.643	0.807	24.795	0.88	0.94
7	3.683	2.80	21.685	1.40	0.82
8	3.817	1.438	20.604	1.57	0.78
9	3.890	2.582	34.562	2.92	1.31
10	3.987	14.817	454.996	16.18	17.64
11	4.043	2.581	66.011	2.92	2.50
12	4.110	12.206	438.890	13.33	16.65
13	4.160	0.931	30.968	1.02	1.18
14	4.210	2.003	43.164	2.19	1.64
15	4.267	1.923	48.956	2.10	1.86
16	4.323	1.394	31.065	1.52	1.18
17	4.353	1.446	34.800	1.58	1.32
18	4.417	1.695	46.118	1.85	1.75
19	4.447	1.358	46.028	1.48	1.75
20	4.507	18.439	691.641	20.13	26.25
21	4.553	0.765	32.158	0.79	1.22
22	4.583	0.662	28.825	0.72	1.09
23	4.610	1.455	28.518	1.59	1.08
24	4.647	0.863	29.963	0.97	1.14
25	4.717	1.837	46.895	2.01	1.78
26	4.780	1.085	21.859	1.18	0.83
27	4.837	0.745	15.594	0.82	0.59
28	4.907	2.671	49.980	2.92	1.90
29	4.987	0.417	14.721	0.46	0.56
30	5.020	1.441	25.030	1.57	0.94
31	5.150	1.772	25.152	1.93	0.95
32	5.283	0.929	18.931	1.01	0.72
33	5.353	0.878	6.449	0.96	0.24
Total:		91.601	2635.299	100.00	100.00

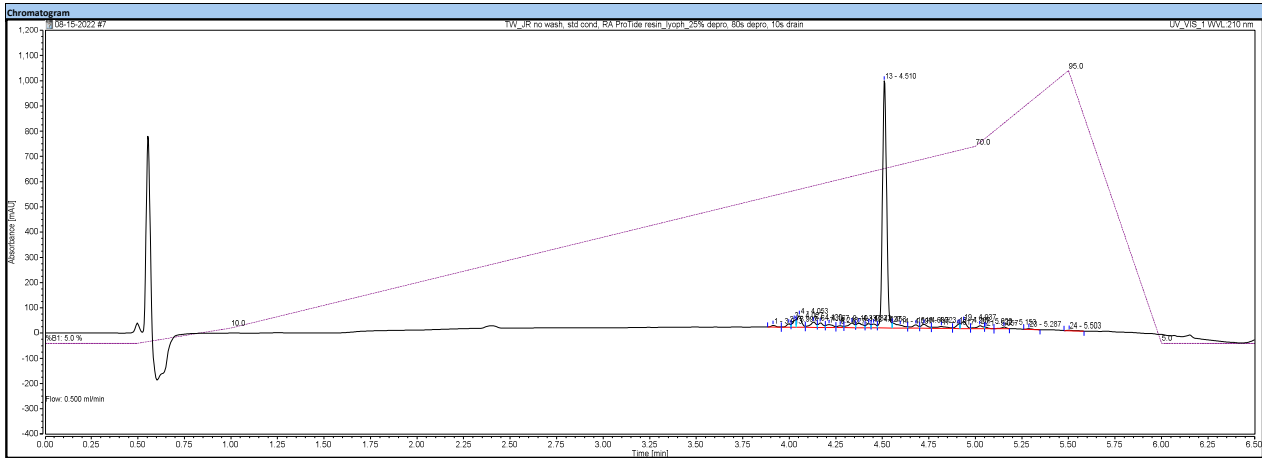


JR-Table 1, entry 6

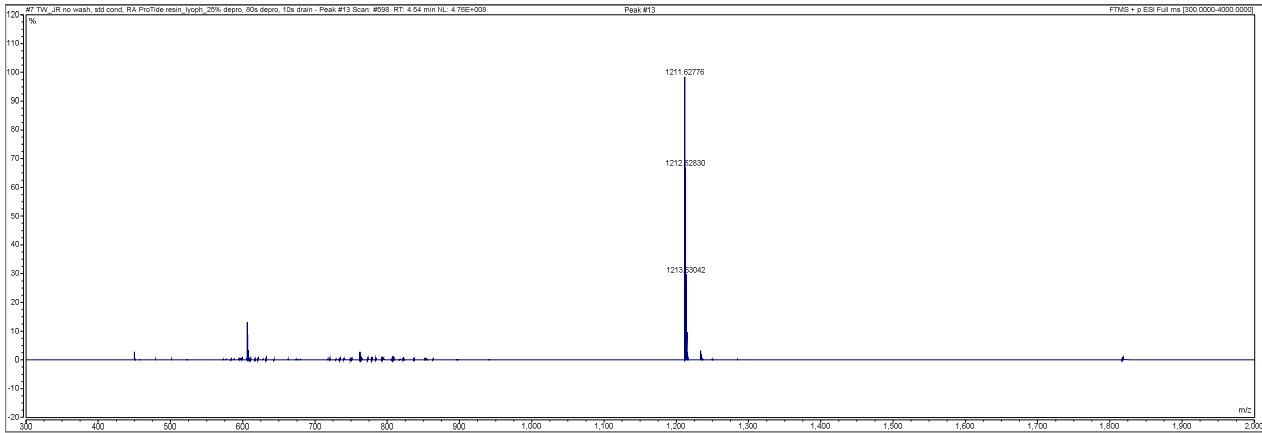
Instrument: FRNTNR2_1 Sequence: 08-15-2022

Page 1 of 1

Chromatogram and Results					
Injection Details					
Injection Name:	TW_JR no wash, std cond. RA ProTide resin_lyoph_25% depro, 80s depro, 10s drain			Run Time (min):	6.50
Vial Number:	YB6			Injection Volume:	5.00
Injection Type:	Unknown			Channel:	UV_VIS_1
Calibration Level:	ACN10-70_0.500uL_40C			Wavelength:	210
Instrument Method:	Waters Acquity BEH C8 1.7um. 2.1 x 100mm Column			Bandwidth:	4
Processing Method:	Quantitative			Dilution Factor:	1.0000
Injection Date/Time:	15/Aug/22 14:41			Sample Weight:	1.0000



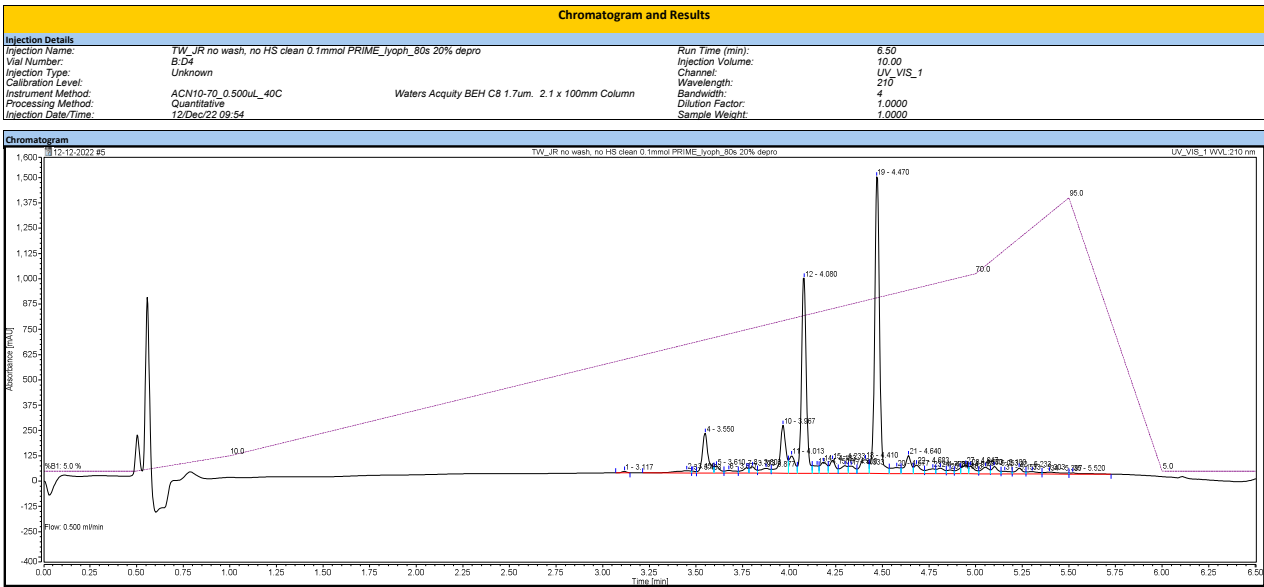
No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	3.913	0.177	6.607	0.48	0.50
2	3.997	0.326	12.984	0.89	0.98
3	4.027	0.587	27.980	1.59	2.12
4	4.063	0.287	46.630	3.50	3.53
5	4.130	0.735	21.080	2.03	1.58
6	4.167	0.597	19.473	1.49	1.38
7	4.213	0.474	11.702	1.29	0.88
8	4.243	0.240	6.994	0.65	0.53
9	4.337	0.717	18.967	1.55	1.43
10	4.373	0.582	18.737	1.55	1.42
11	4.423	0.430	16.539	1.20	1.20
12	4.463	0.430	16.539	1.20	1.20
13	4.510	25.180	978.844	68.45	73.99
14	4.580	0.941	11.069	2.56	0.84
15	4.680	0.913	13.444	1.41	1.04
16	4.723	0.596	15.712	1.62	1.19
17	4.817	0.388	7.625	1.01	0.57
18	4.837	0.923	28.440	2.56	2.13
19	5.024	0.497	11.585	1.36	0.90
20	5.063	0.183	5.883	0.36	0.45
21	5.153	0.200	6.884	0.54	0.50
22	5.267	0.085	2.808	0.23	0.21
23	5.503	0.085	1.806	0.23	0.14
Total:		36.785	1322.894	100.00	100.00



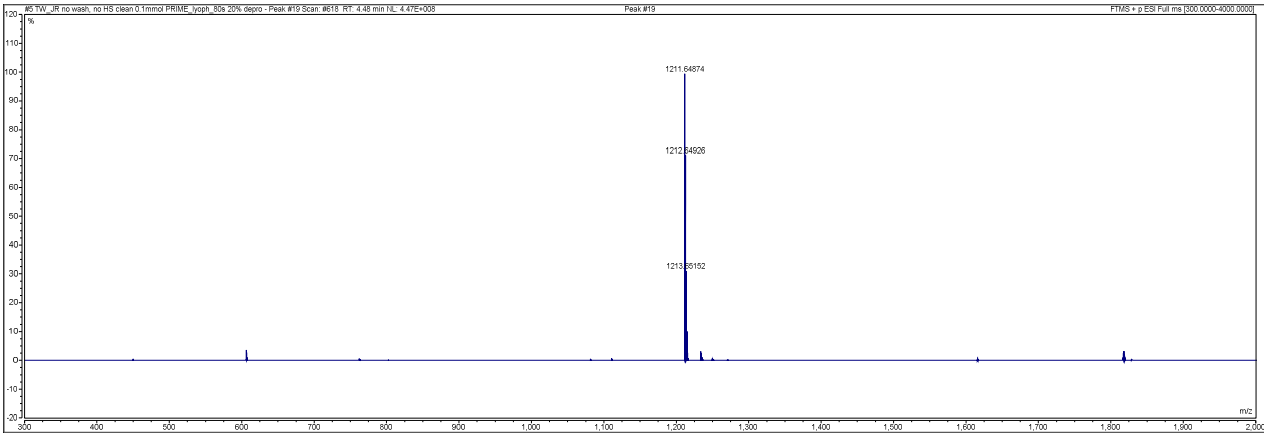
JR-Table 1, entry 7

Instrument: F9000R2_1 Sequence: 12-12-2022

Page 1 of 1



No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	3.117	0.201	7.631	0.15	0.20
2	3.450	1.090	11.292	0.83	0.28
3	3.483	0.212	8.645	0.12	0.22
4	3.550	7.095	196.035	5.40	5.05
5	3.610	1.170	34.487	0.89	0.34
6	3.677	0.814	34.188	0.62	0.34
7	3.773	0.967	26.790	0.74	0.69
8	3.803	1.113	31.859	0.85	0.82
9	3.897	1.405	41.829	1.07	0.81
10	3.967	8.384	236.790	6.38	5.11
11	4.013	3.020	85.659	2.30	2.21
12	4.080	28.467	964.517	21.88	24.87
13	4.150	1.353	36.826	1.03	0.95
14	4.233	2.276	85.701	1.73	1.68
15	4.293	2.348	85.150	1.78	1.68
16	4.300	1.652	38.044	1.26	0.98
17	4.353	1.253	36.959	0.95	0.91
18	4.410	3.424	87.517	2.62	1.74
19	4.470	44.199	1466.113	33.64	37.80
20	4.577	1.749	29.927	1.34	1.28
21	4.640	3.328	88.427	2.53	2.28
22	4.683	1.748	42.565	1.33	1.10
23	4.770	1.245	23.551	0.95	0.61
24	4.810	1.265	27.535	0.96	0.61
25	4.863	0.721	18.143	0.55	0.47
26	4.917	0.601	17.108	0.45	0.41
27	4.917	0.601	45.057	0.45	0.41
28	4.973	1.354	37.562	1.03	0.97
29	5.053	1.318	36.184	1.01	0.92
30	5.100	1.330	36.184	1.01	0.93
31	5.154	0.693	28.510	0.53	0.32
32	5.203	0.822	11.289	0.63	0.28
33	5.303	0.822	11.289	0.63	0.28
34	5.397	0.955	9.038	0.73	0.23
35	5.520	0.525	5.906	0.40	0.15
Total:		131.398	3878.288	100.00	100.00



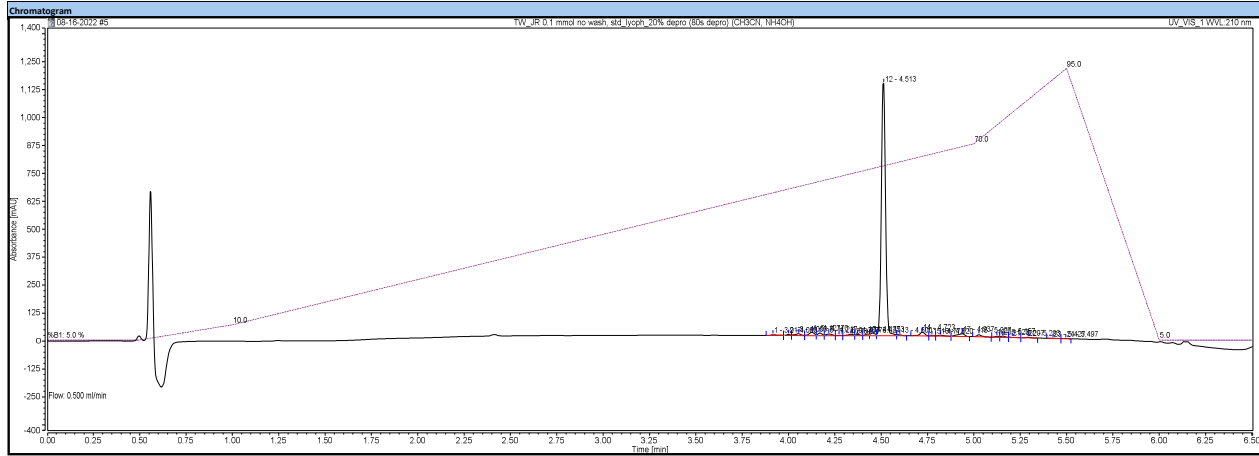
JR-Table 1, entry 8

Instrument FRTNTR2_1 Sequence 09-16-2022

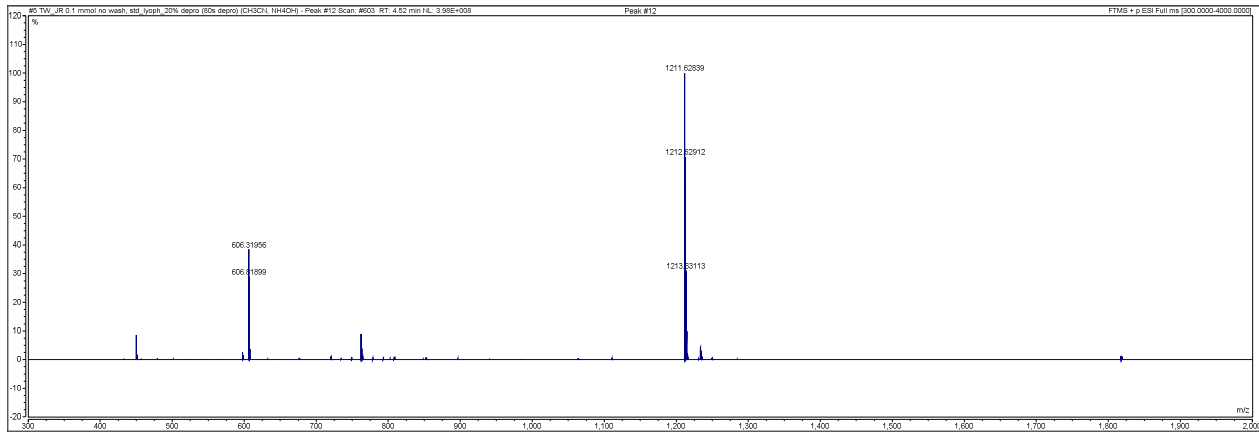
Page 1 of 1

Chromatogram and Results

Injection Details			Run Time (min):	6.50
Injection Name:	YW_JR 0.1 mmol no wash, std_lyoph_20% depro (80s depro) (CH3CN, NH4OH)		Injection Volume:	3.00
Vial Number:	Y.C3		Channel:	UV_VIS_1
Injection Type:	Unknown		Wavelength:	210
Calibration Level:			Bandwidth:	4
Instrument Method:	ACH10-70_0.500uL_40C		Dilution Factor:	1.0000
Processing Method:	Quantitative		Sample Weight:	1.0000
Injection Date/Time:	16/Aug/22 15:34		Waters Acquity BEH C8 1.7um, 2.1 x 100mm Column	



No.	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	3.917	0.147	3.597	0.43	0.28
2	4.000	0.119	4.483	0.35	0.35
3	4.053	0.312	8.298	0.91	0.65
4	4.127	0.417	14.812	1.88	1.16
5	4.170	0.309	11.835	0.90	0.93
6	4.217	0.280	7.293	0.76	0.57
7	4.215	0.038	1.248	0.11	0.10
8	4.337	0.221	6.392	0.64	0.50
9	4.373	0.138	5.510	0.46	0.43
10	4.447	0.141	7.494	0.50	0.58
11	4.453	0.323	12.017	0.94	0.94
12	4.513	28.673	1128.485	86.42	86.52
13	4.600	0.131	4.151	0.38	0.33
14	4.743	0.553	17.345	1.61	1.36
15	4.775	0.050	1.620	0.14	0.13
16	4.820	0.172	4.704	0.50	0.37
17	4.937	0.517	11.696	1.50	0.92
18	5.027	0.222	9.104	0.79	0.71
19	5.123	0.110	4.476	0.32	0.35
20	5.157	0.157	5.568	0.46	0.44
21	5.207	0.050	1.117	0.15	0.09
22	5.253	0.065	1.892	0.19	0.15
23	5.427	0.028	0.872	0.08	0.07
24	5.497	0.023	0.830	0.07	0.07
Total:		34.335	1274.837	100.00	100.00

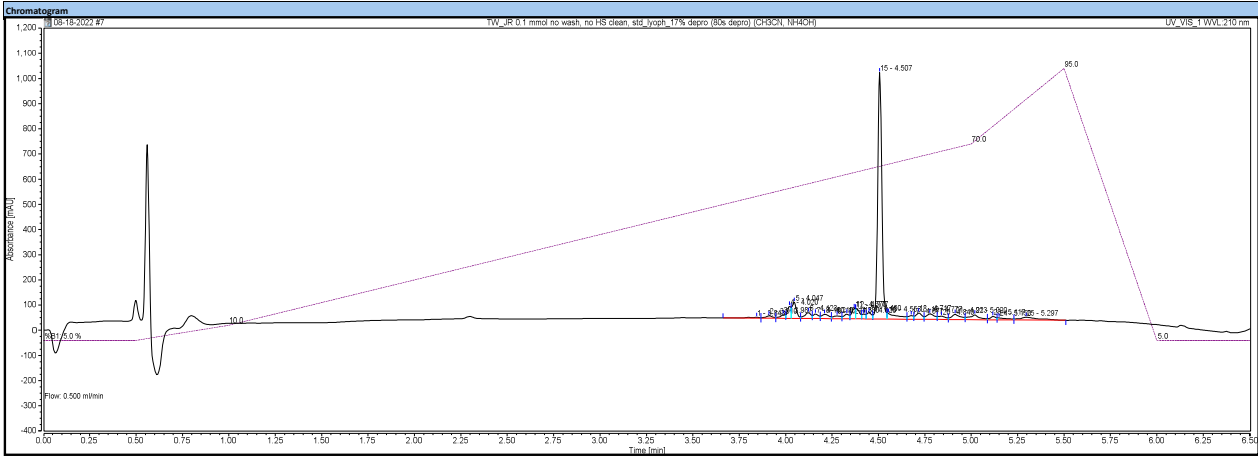


JR-Table 1, entry 9

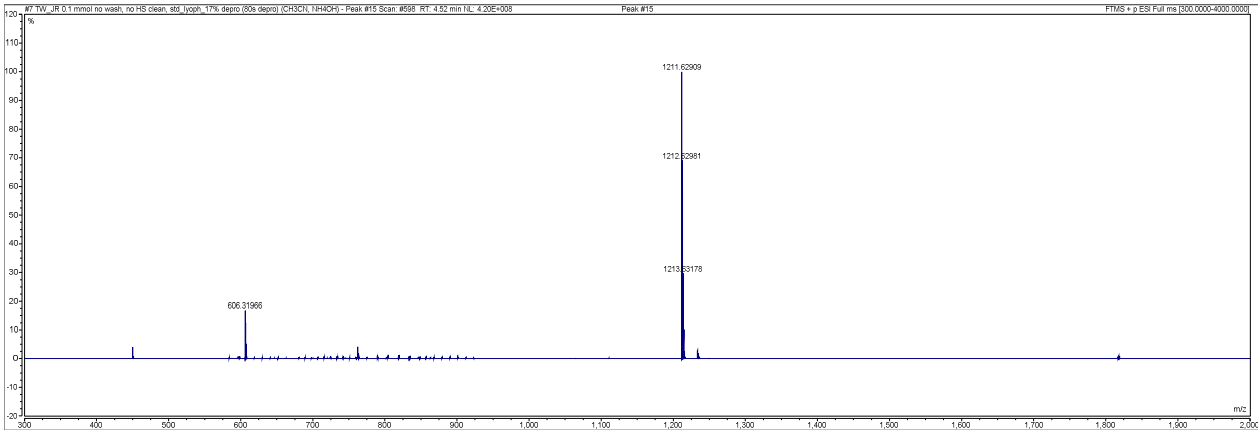
Instrument: FRNTN2_1 Sequence: 08-18-2022

Page 1 of 1

Chromatogram and Results				
Injection Details				
Injection Name:	YW_JR 0.1 mmol no wash, no HS clean, std_lyoph_17% depro (80s depro) (CH3CN, NH4OH)			Run Time (min): 6.50
Vial Number:	Y:44			Injection Volume: 5.00
Injection Type:	Unknown			Channel: UV_VIS_1
Calibration Level:				Wavelength: 210
Instrument Method:	ACN10-70_0.500uL_40C			Bandwidth: 4
Processing Method:	Quantitative			Dilution Factor: 1.0000
Injection Date/Time:	18/Aug/22 11:38			Sample Weight: 1.0000



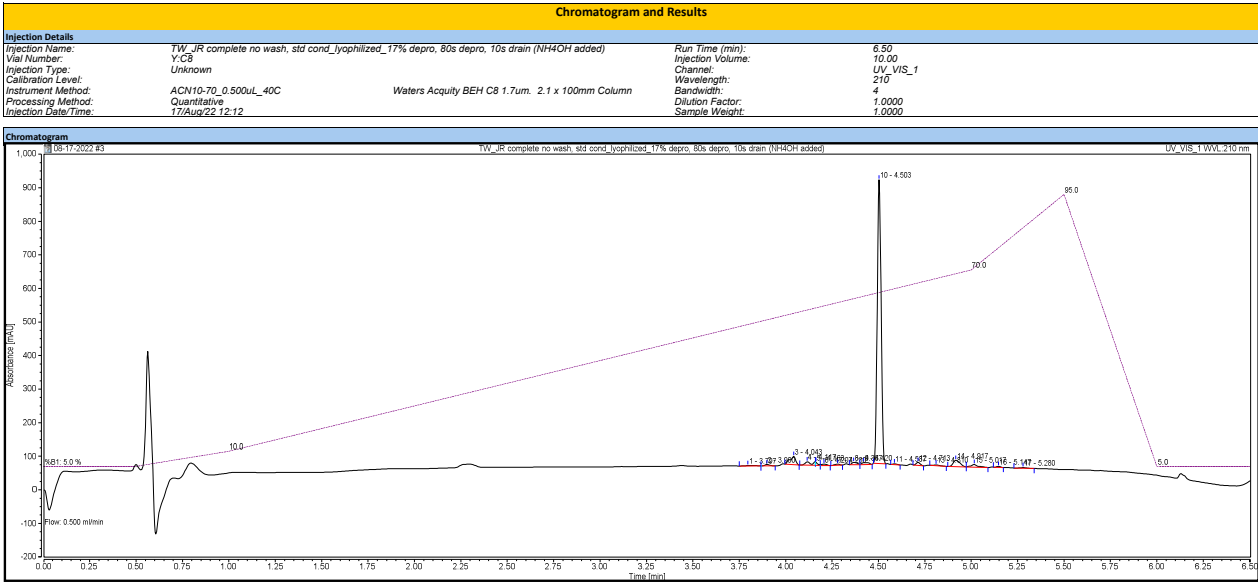
No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	3.843	0.270	3.030	0.60	0.20
2	3.910	0.421	9.526	0.94	0.64
3	3.987	0.455	13.799	1.01	0.92
4	4.020	1.056	48.692	2.35	3.26
5	4.047	1.794	64.296	3.99	4.30
6	4.125	0.909	23.262	2.02	1.56
7	4.160	0.594	12.814	1.32	1.19
8	4.210	0.750	16.877	1.67	1.13
9	4.277	0.528	10.803	1.17	0.72
10	4.330	0.607	18.490	1.35	1.24
11	4.370	0.907	40.959	2.02	2.74
12	4.377	0.991	38.958	2.20	2.00
13	4.420	0.425	19.143	0.94	1.28
14	4.450	0.744	24.798	1.65	1.66
15	4.507	239.358	978.391	56.39	65.43
16	4.553	1.478	22.551	3.29	1.51
17	4.677	0.441	13.802	0.98	0.92
18	4.717	0.978	25.191	2.17	1.88
19	4.777	1.096	21.265	2.44	1.42
20	4.840	0.612	12.512	1.36	0.84
21	4.913	1.134	20.232	2.42	1.35
22	5.040	1.181	19.055	2.63	1.29
23	5.117	0.463	13.541	1.03	0.91
24	5.150	0.474	7.741	1.05	0.54
25	5.297	1.307	10.634	2.91	0.71
Total:		44.972	1495.242	100.00	100.00



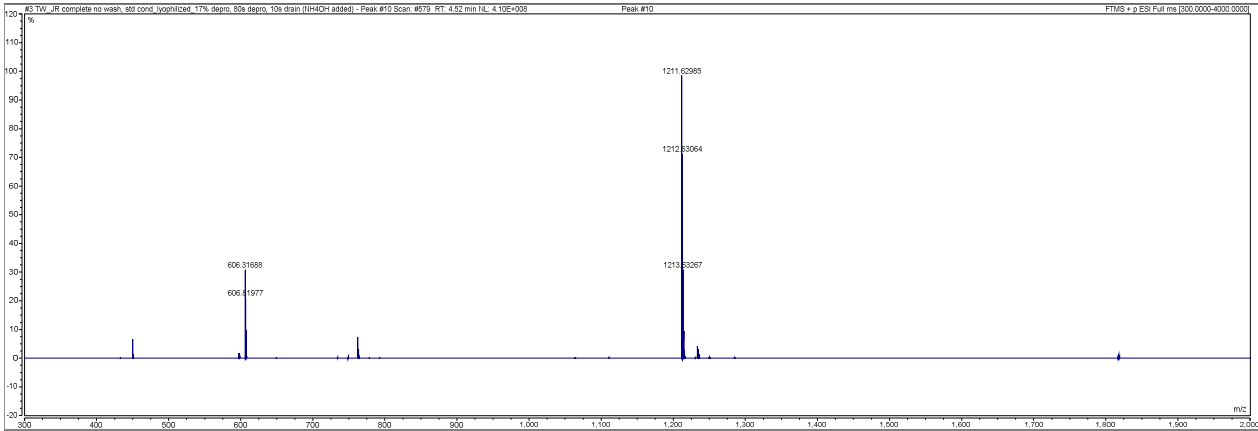
JR-Table 1, entry 10

Instrument: FRNTNR2_1 Sequence: 06-17-2022

Page 1 of 1



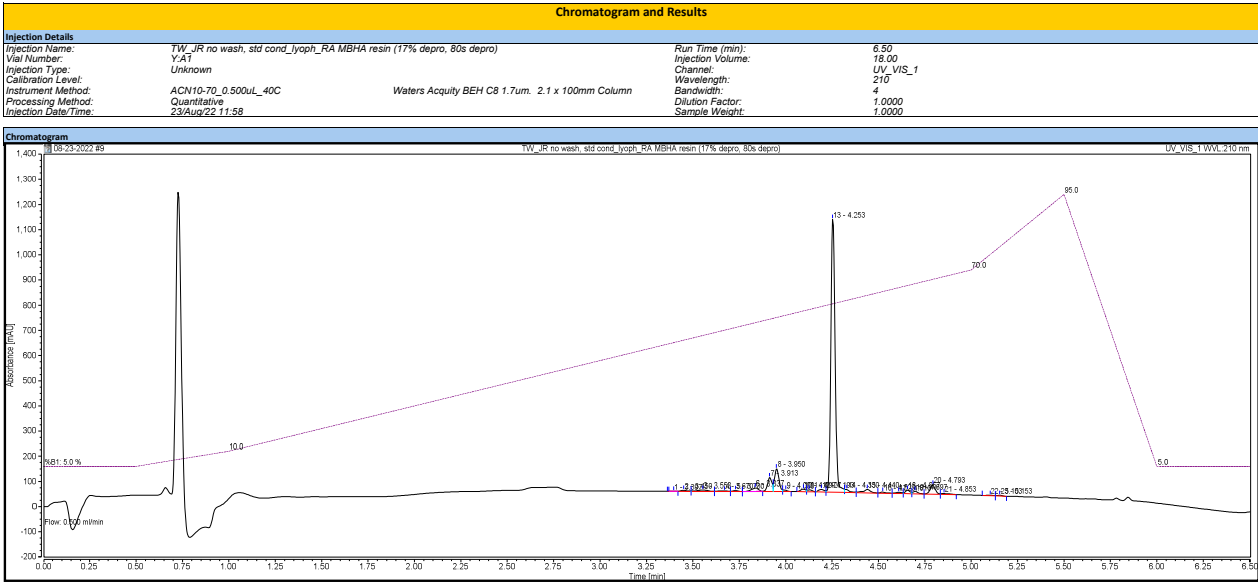
No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	3.797	0.073	1.287	0.30	0.13
2	3.900	0.119	4.151	0.49	0.43
3	4.043	0.692	25.154	3.66	2.62
4	4.117	0.423	10.081	1.24	1.05
5	4.163	0.132	9.312	0.54	0.97
6	4.207	0.146	4.372	0.50	0.45
7	4.280	0.104	3.176	0.43	0.33
8	4.367	0.226	7.029	0.93	0.73
9	4.420	0.250	5.855	1.03	0.61
10	4.503	20.381	845.103	83.71	87.90
11	4.587	0.069	2.766	0.29	0.29
12	4.713	0.212	8.985	0.87	0.93
13	4.810	0.666	2.246	0.27	0.23
14	4.917	0.794	19.032	3.26	1.98
15	5.017	0.330	8.060	1.35	0.84
16	5.147	0.655	2.771	0.23	0.29
17	5.280	0.076	2.074	0.31	0.22
Total:		24.349	961.454	100.00	100.00



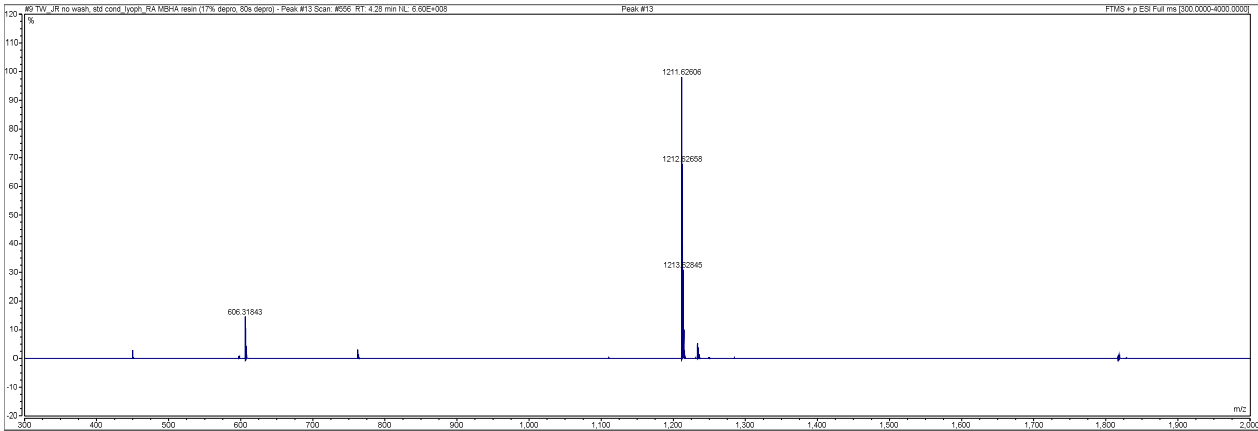
JR-Table 1, entry 11

Instrument: FRNTN2_1 Sequence: 08-23-2022

Page 1 of 1



No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	3.397	0.012	0.510	0.03	0.04
2	3.450	0.226	4.618	0.58	0.33
3	3.583	0.414	5.369	1.00	0.40
4	3.670	0.150	2.316	0.38	0.17
5	3.740	0.125	4.157	0.32	0.30
6	3.837	0.364	13.490	1.45	0.97
7	3.913	0.618	55.758	4.16	3.99
8	3.950	2.458	90.169	6.31	6.45
9	4.000	0.172	5.345	0.44	0.43
10	4.097	0.309	9.872	0.79	0.71
11	4.127	0.237	7.509	0.61	0.54
12	4.193	0.314	10.598	0.86	0.76
13	4.253	28.598	1084.314	73.46	77.57
14	4.330	0.386	11.374	0.99	0.81
15	4.440	0.646	13.663	1.66	0.98
16	4.523	0.175	4.495	0.45	0.32
17	4.610	0.198	4.714	0.51	0.34
18	4.657	0.404	14.042	1.04	1.00
19	4.697	0.329	10.395	0.85	0.74
20	4.793	1.184	36.172	3.04	2.59
21	4.853	0.179	3.264	0.46	0.23
22	5.103	0.077	1.895	0.20	0.14
23	5.153	0.092	2.959	0.24	0.21
Total:		38.929	1397.796	100.00	100.00

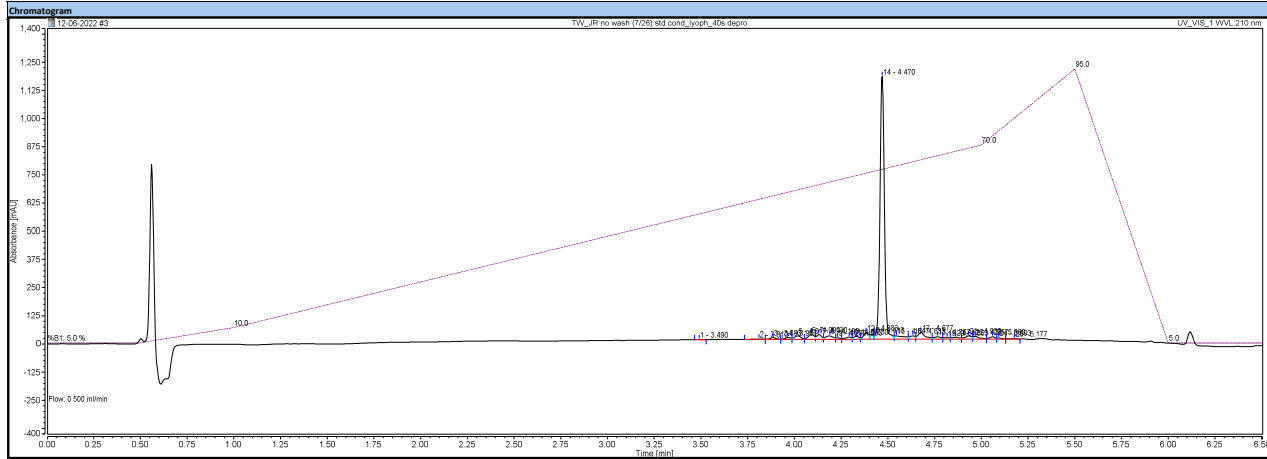


JR-Table 1, entry 12

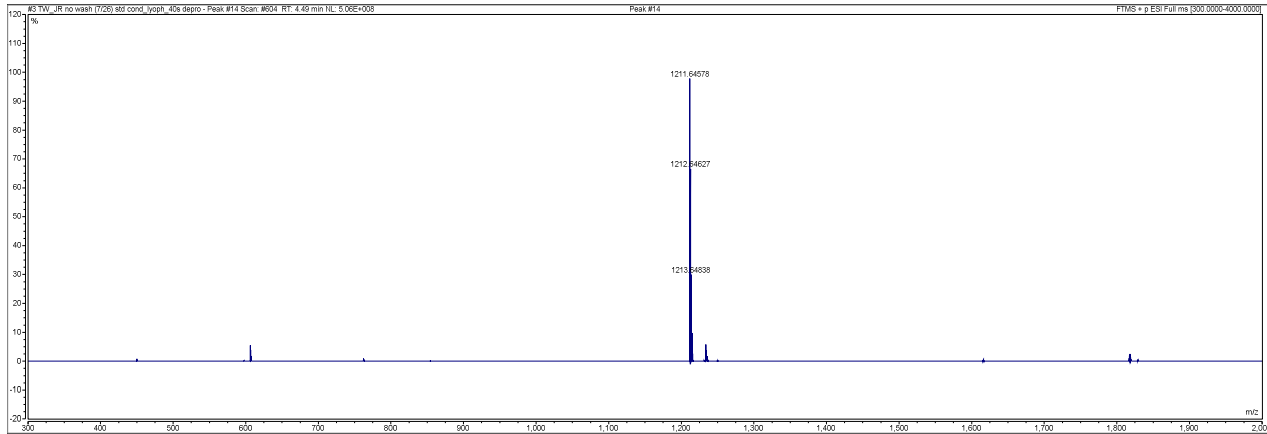
Instrument: FPRNTNR2_1 Sequence: 12-06-2022

Page 1 of 1

Chromatogram and Results					
Injection Details					
Injection Name:	TW_JR no wash (7/26) std cond_lyoph_40s depro			Run Time (min):	6.50
Vial Number:	B:A6			Injection Volume:	5.00
Injection Type:	Unknown			Channel:	UV_VIS_1
Calibration Level:				Wavelength:	210
Instrument Method:	ACN10-70_0.500uL_40C			Bandwidth:	4
Processing Method:	Quantitative			Dilution Factor:	1.0000
Injection Date/Time:	06/Dec/22 10:38			Sample Weight:	1.0000
Waters Acquity BEH C8 1.7um, 2.1 x 100mm Column					



No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	3.490	0.034	1.235	0.08	0.08
2	3.810	0.113	1.831	0.26	0.12
3	3.883	0.306	8.209	0.70	0.57
4	3.957	0.297	8.543	0.68	0.58
5	4.017	0.616	15.639	1.41	1.08
6	4.090	0.826	22.910	1.50	1.58
7	4.130	0.644	21.631	1.48	1.47
8	4.200	0.170	5.184	0.39	0.42
9	4.230	0.385	9.659	0.89	0.88
10	4.293	0.421	12.919	0.97	0.98
11	4.383	0.958	30.942	2.20	2.10
12	4.410	0.465	21.775	1.07	1.48
13	4.410	32.077	1156.785	73.89	72.25
14	4.547	0.924	14.631	2.12	0.99
15	4.623	0.489	20.838	1.15	0.98
16	4.717	1.384	38.822	0.94	2.19
17	4.767	0.467	10.709	1.07	0.73
18	4.817	0.260	6.822	0.50	0.46
19	4.863	0.407	8.822	0.34	0.97
20	4.930	0.577	14.254	1.32	0.97
21	4.967	0.489	12.198	0.81	0.83
22	5.060	0.344	10.779	0.70	0.70
23	5.093	0.127	4.837	0.29	0.33
24	5.177	0.057	1.117	0.13	0.08
Total:		43.529	1471.666	100.00	100.00

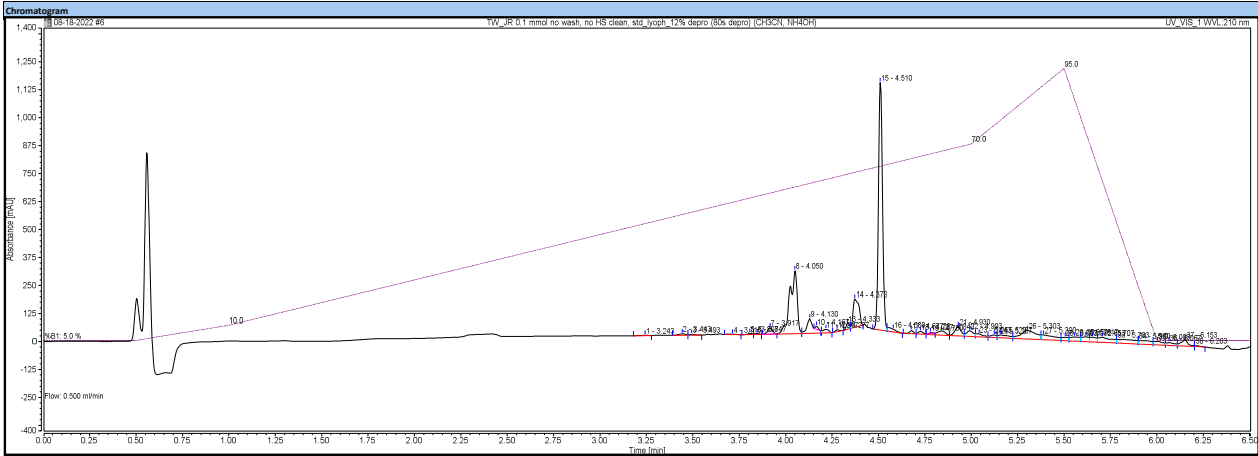


JR-Table 1, entry 13

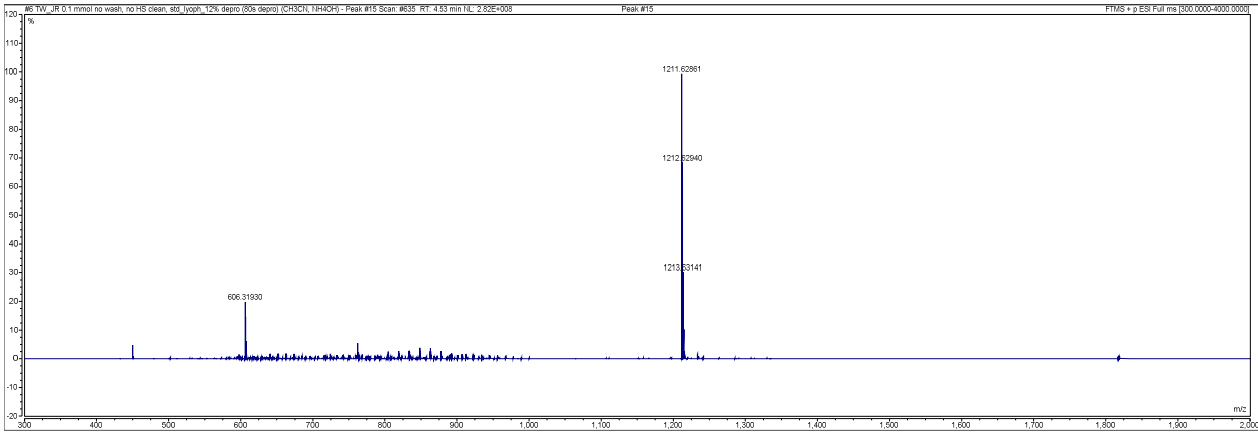
Instrument: FRNTNFR2_1 Sequence: 08-18-2022

Page 1 of 1

Chromatogram and Results			
Injection Details			
Injection Name:	YW_IR 0.1 mmol no wash, no HS clean, std_lyoph_12% depro (80s depro) (CH3CN, NH4OH)		Run Time (min): 6.50
Vial Number:	Y:A3		Injection Volume: 25.00
Injection Type:	Unknown		Channel: UV_VIS_1
Calibration Level:			Wavelength: 210
Instrument Method:	ACN10-70_0.500uL_40C		Bandwidth: 4
Processing Method:	Quantitative		Dilution Factor: 1.0000
Injection Date/Time:	18/Aug/22 11:31		Sample Weight: 1.0000



No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	3.243	0.065	1.514	0.09	0.07
2	3.443	0.315	6.501	0.42	0.31
3	3.493	0.155	2.083	0.20	0.18
4	3.807	0.105	2.083	0.14	0.10
5	4.050	0.150	5.026	0.20	0.23
6	4.130	0.119	3.351	0.16	0.17
7	4.217	0.867	28.957	1.73	1.34
8	4.333	13.098	281.235	17.30	13.34
9	4.373	2.657	64.962	3.32	2.08
10	4.510	0.564	15.437	0.33	0.65
11	4.580	0.233	8.776	0.25	0.73
12	4.677	0.529	26.504	0.70	1.26
13	4.750	4.592	125.311	6.06	5.94
14	4.840	27.852	1106.878	36.73	32.40
15	4.930	0.376	11.919	0.50	0.69
16	5.033	0.380	14.468	0.50	0.69
17	5.152	0.376	11.919	0.50	0.69
18	5.303	0.073	2.830	0.10	0.14
19	5.373	1.184	18.729	1.56	0.89
20	5.507	0.081	2.830	0.10	0.14
21	5.553	1.058	21.333	1.40	1.20
22	5.707	0.599	12.632	0.79	0.60
23	5.753	0.599	12.632	0.79	0.60
24	5.900	0.865	12.253	1.14	0.58
25	6.003	3.671	38.109	2.85	1.81
26	6.053	0.614	14.765	0.81	0.70
27	6.153	0.069	1.823	0.43	1.03
28	6.203	0.125	3.383	0.17	0.16
Total:		75.722	2108.590	100.00	100.00

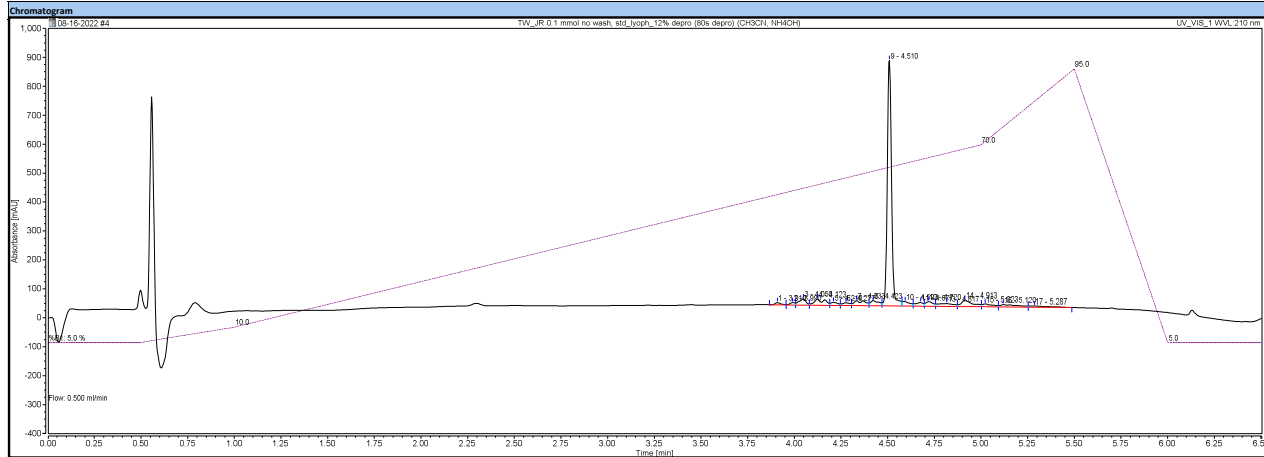


JR-Table 1, entry 14

Instrument:FRNTR2_1 Sequence:08-16-2022

Page 1 of 1

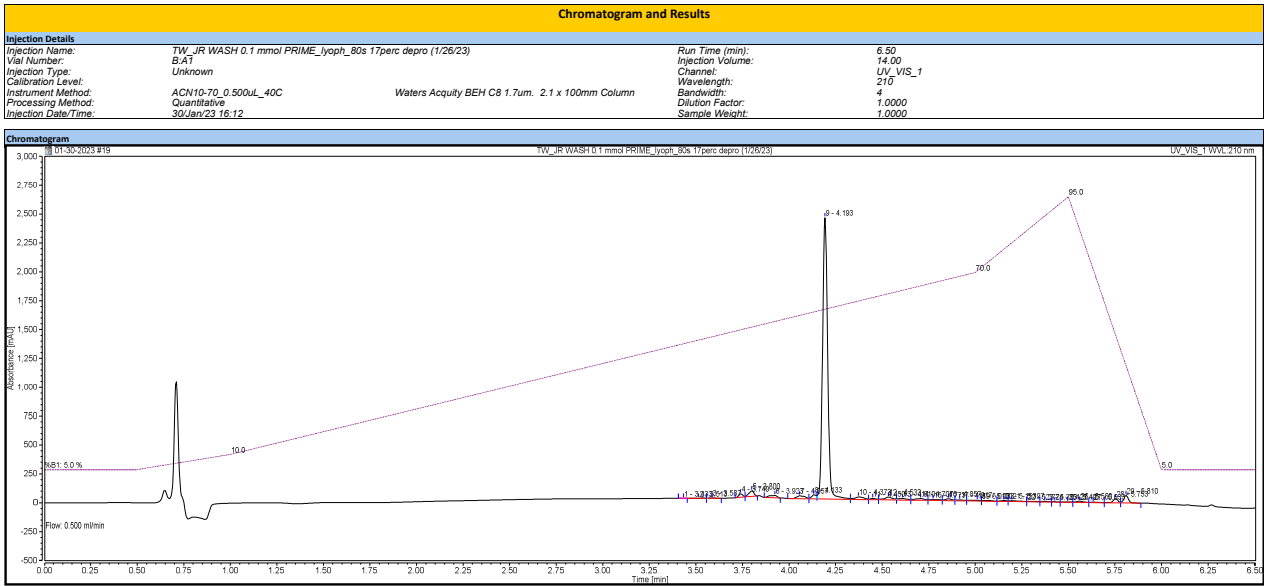
Chromatogram and Results					
Injection Details					
Injection Name:	TW_JR 0.1 mmol no wash, std_lyoph_12% depro (80s depro) (CH3CN, NH4OH)			Run Time (min):	6.50
Vial Number:	Y:CT			Injection Volume:	5.00
Injection Type:	Unknown			Channel:	UV_VIS_1
Calibration Level:				Wavelength:	210
Instrument Method:	ACN10-70_0.500uL_40C			Bandwidth:	4
Processing Method:	Quantitative			Dilution Factor:	1.0000
Injection Date/Time:	16/Aug/22 14:38			Sample Weight:	1.0000
Waters Acquity BEH C8 1.7um. 2.1 x 100mm Column					



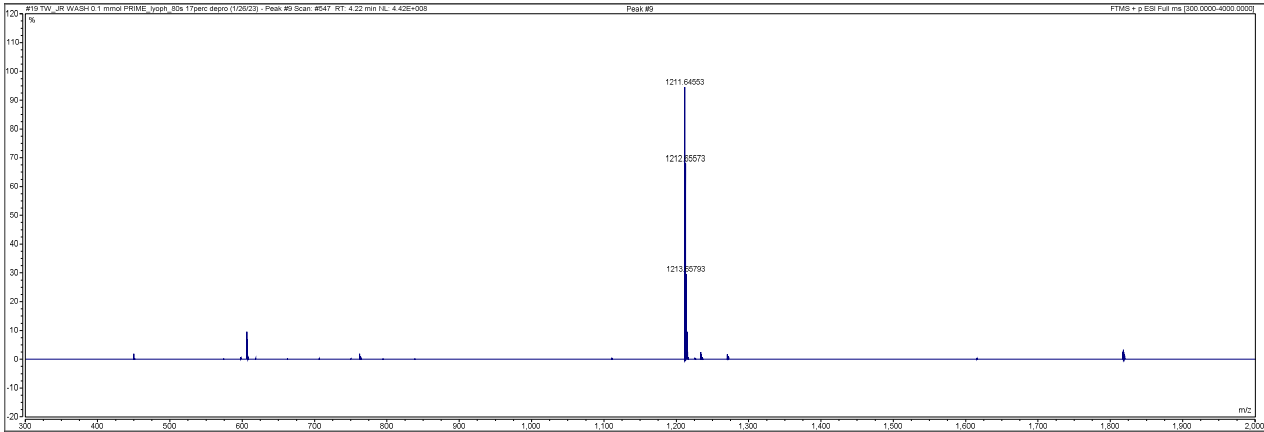
JR-Table 1, entry 15

Instrument:FRNTR2_1 Sequence:01-35-2023

Page 1 of 1



No.	Retention Time		Area		Height		Relative Area		Relative Height	
	min		mAU*min		mAU		%		%	
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	3.433		0.009		0.388		0.01		0.01	
2	3.513		0.155		1.806		0.18		0.08	
3	3.597		0.144		3.681		0.18		0.13	
4	3.740		0.235		29.846		0.77		1.04	
5	3.800		0.952		46.533		1.60		1.61	
6	3.923		1.222		20.933		1.04		0.72	
7	4.057		1.317		25.488		1.38		0.89	
8	4.133		1.033		36.483		1.08		1.77	
9	4.193		77.116		2438.487		80.54		84.86	
10	4.373		1.281		22.213		1.34		0.77	
11	4.450		0.384		10.250		0.40		0.30	
12	4.540		0.354		10.250		0.40		0.30	
13	4.610		0.792		13.037		0.83		0.45	
14	4.707		0.916		14.604		0.96		0.51	
15	4.773		0.916		7.580		0.96		0.20	
16	4.857		0.696		16.630		0.73		0.58	
17	4.917		0.416		8.869		0.44		0.23	
18	5.010		0.433		8.307		0.41		0.23	
19	5.033		0.387		5.485		0.41		0.19	
20	5.117		0.307		7.381		0.32		0.28	
21	5.197		0.474		7.280		0.50		0.25	
22	5.277		0.195		2.410		0.20		0.08	
23	5.373		0.203		3.890		0.21		0.14	
24	5.479		0.097		2.497		0.10		0.08	
25	5.497		0.141		7.167		0.15		0.09	
26	5.603		0.258		10.582		0.32		0.14	
27	5.693		0.258		4.187		0.32		0.14	
28	5.753		1.142		37.152		1.20		1.29	
29	5.810		1.871		65.089		1.96		2.27	
Total:			95.280		2871.096		100.00		100.00	

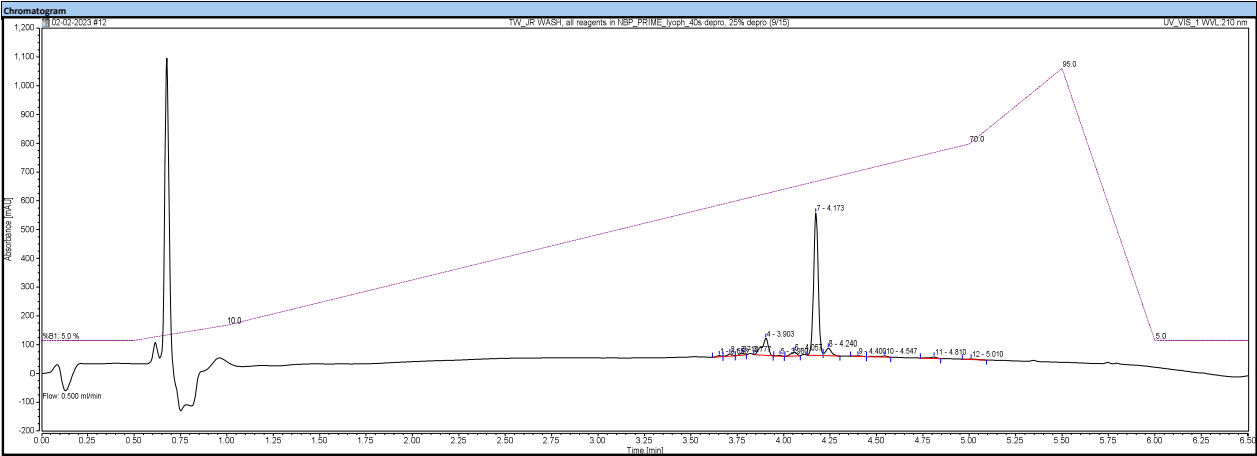


JR-Table 1, entry 16

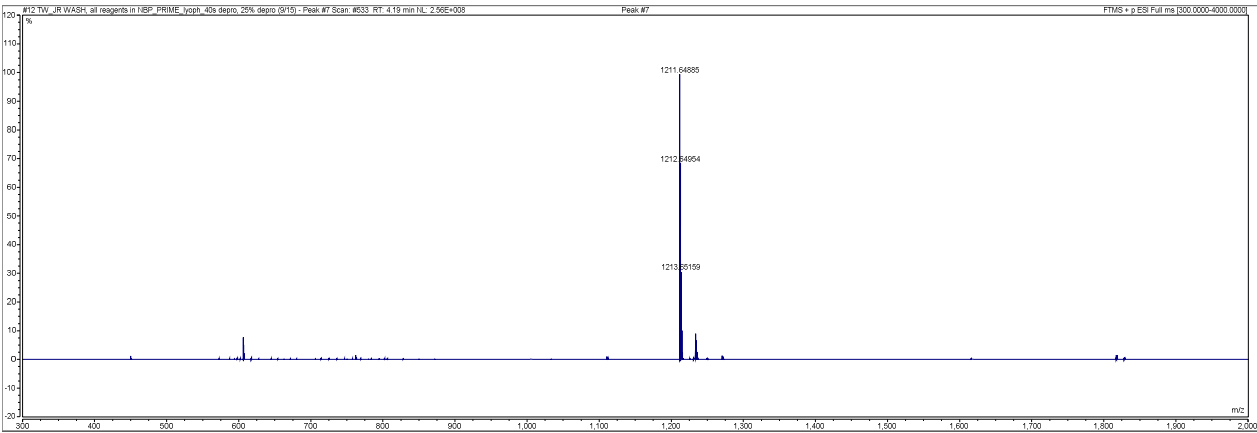
Instrument: FRNTNFR2_1 Sequence: 02-02-3523

Page 1 of 1

Chromatogram and Results			
Injection Details			
Injection Name:	YW_JR WASH: all reagents in NBP_PRIME_lyoph_40s depro, 25% depro (9/15)		Run Time (min): 6.50
Vial Number:	8.44		Injection Volume: 10.00
Injection Type:	Unknown		Channel: UV_VIS_1
Calibration Level:			Wavelength: 210
Instrument Method:	ACN10-70_0.500uL_40C		Bandwidth: 4
Processing Method:	Quantitative		Dilution Factor: 1.0000
Injection Date/Time:	02/Feb/23 16:00		Sample Weight: 1.0000



No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	3.653	0.120	4.341	0.62	0.69
2	3.710	0.229	7.848	1.19	1.25
3	3.777	0.194	6.380	0.95	1.05
4	3.903	0.397	59.729	12.41	9.49
5	3.980	0.070	2.437	0.36	0.45
6	4.057	0.493	13.708	2.55	2.19
7	4.173	4.438	494.998	73.98	78.63
8	4.240	0.946	26.874	4.90	4.27
9	4.400	0.068	1.269	0.43	0.28
10	4.549	0.018	0.189	0.43	0.67
11	4.810	0.192	5.112	1.00	0.81
12	5.010	0.094	1.526	0.49	0.24
Total:		19.320	629.507	100.00	100.00



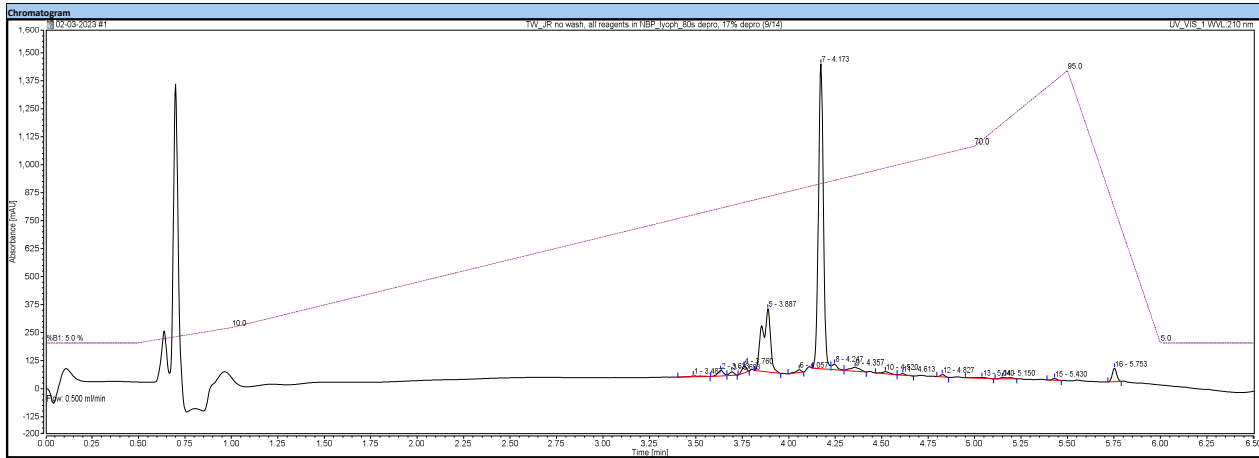
JR-Table 1, entry 17

Instrument:FRONTNR2_1 Sequence:02-03-2023

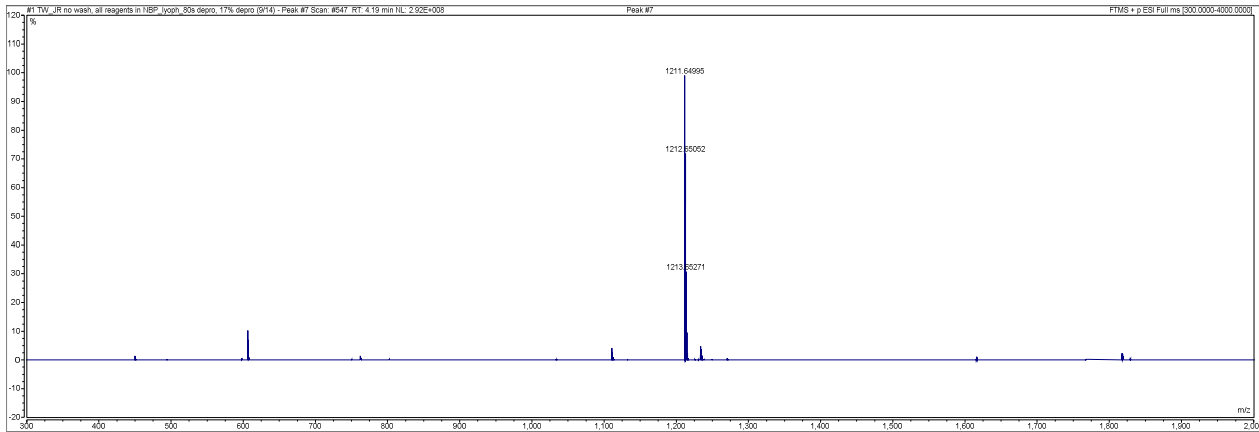
Page 1 of 1

Chromatogram and Results

Injection Details			
Injection Name:	TW_JR no wash, all reagents in NBP_lyoph_80s depro, 17% depro (9/14)	Run Time (min):	6.50
Vial Number:	843	Injection Volume:	15.00
Injection Type:	Unknown	Channel:	UV_VIS_1
Calibration Level:		Wavelength:	210
Instrument Method:	ACN10-70_0.500uL_40C	Bandwidth:	4
Processing Method:	Quantitative	Dilution Factor:	1.0000
Injection Date/Time:	03Feb/23 08:40	Sample Weight:	1.0000



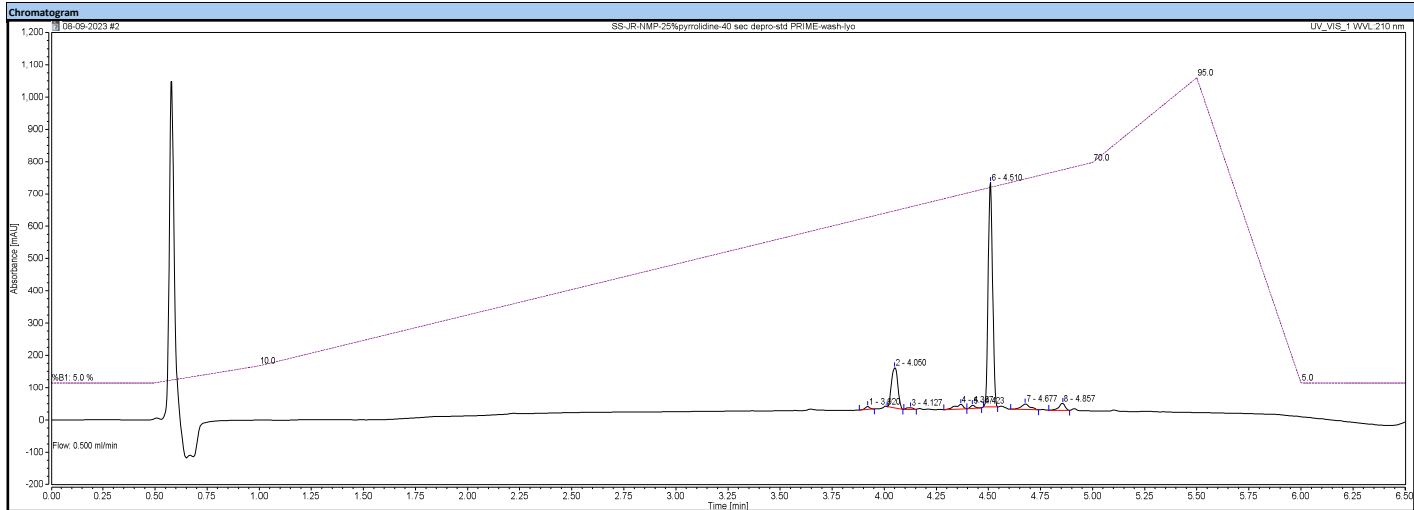
No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	3.487	0.326	4.500	0.50	0.24
2	3.633	0.903	25.699	1.39	1.37
3	3.693	0.461	16.279	0.71	0.87
4	3.780	0.476	30.411	1.70	1.62
5	3.887	14.569	281.506	22.49	14.97
6	4.057	0.422	12.753	0.65	0.69
7	4.173	41.940	1361.088	64.74	72.89
8	4.247	0.855	25.490	1.82	1.38
9	4.357	1.097	18.055	1.82	1.38
10	4.520	0.418	9.893	0.65	0.51
11	4.613	0.205	6.592	0.32	0.28
12	4.813	0.205	10.562	0.42	0.38
13	5.040	0.289	3.486	0.42	0.19
14	5.130	0.394	6.096	0.52	0.42
15	5.430	0.219	7.913	0.34	0.32
16	5.753	1.677	59.962	2.59	3.19
Total:		64.784	1880.307	100.00	100.00



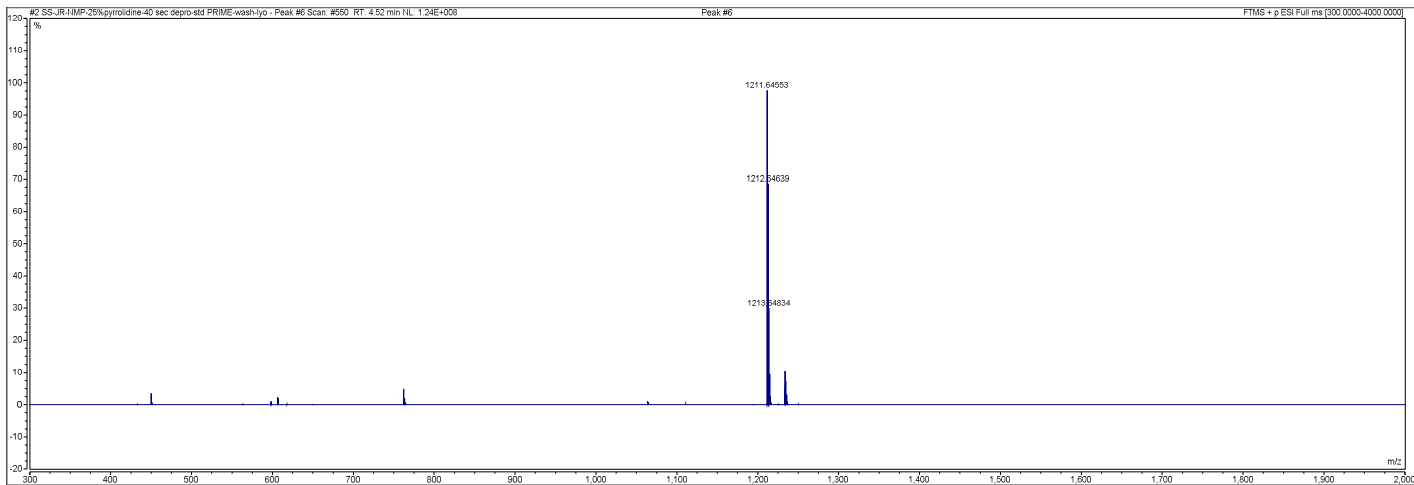
JR-Table 1, entry 18

Chromatogram and Results

Injection Details			Run Time (min):		
Injection Name:	SS-JR-NMP-25%pyrrolidine-40 sec depro-std PRIME-wash-lyo		6.50		
Vial Number:	G.F5		Injection Volume:		
Injection Type:	Unknown		10.00		
Calibration Level:			Channel:		
Instrument Method:	ACN10-70_0.500uL_40C		Wavelength:		
Processing Method:	Quantitative		210		
Injection Date/Time:	08/Aug/23 11:24		Bandwidth:		
			4		
			Dilution Factor:		
			1.0000		
			Sample Weight:		
			1.0000		

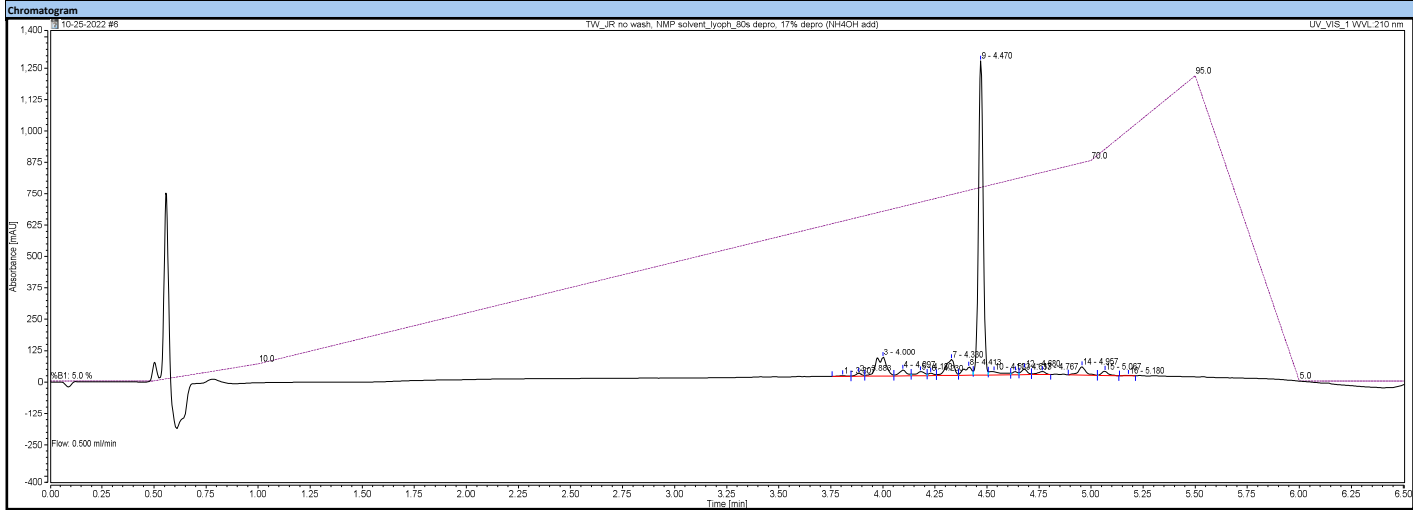


No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	3.920	0.245	9.301	1.00	1.04
2	4.050	4.345	124.242	17.81	13.85
3	4.127	0.189	4.994	0.78	0.86
4	4.367	0.653	14.334	2.68	1.60
5	4.423	0.246	9.510	1.01	1.06
6	4.510	17.105	695.162	70.12	77.52
7	4.577	0.869	17.473	3.55	1.95
8	4.857	0.741	21.770	3.04	2.43
Total:		24.392	896.786	100.00	100.00

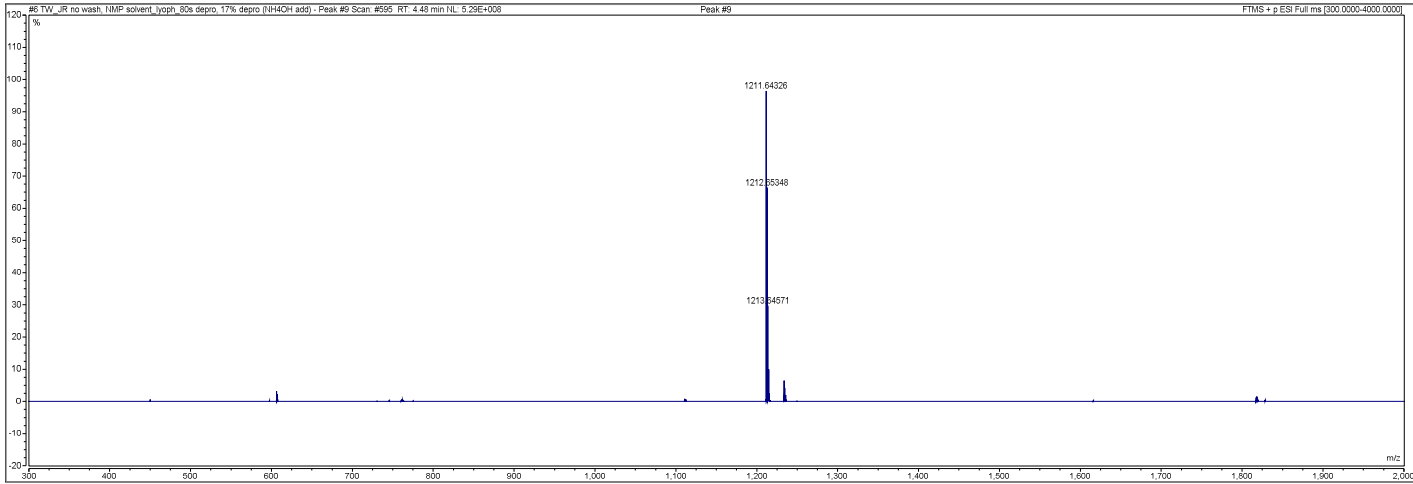


Chromatogram and Results

Injection Details			Run Time (min):	6.50
Injection Name:	TW_JR no wash, NMP solvent, lyoph_80s depro, 17% depro (NH4OH add)		Injection Volume:	4.00
Vial Number:	Y:B5		Channel:	UV_VIS_1
Injection Type:	Unknown		Wavelength:	210
Calibration Level:			Bandwidth:	4
Instrument Method:	ACN10-70_0.500uL_40C	Waters Acquity BEH C8 1.7um, 2.1 x 100mm Column	Dilution Factor:	1.0000
Processing Method:	Quantitative		Sample Weight:	1.0000
Injection Date/Time:	25-Oct-22 12:12			

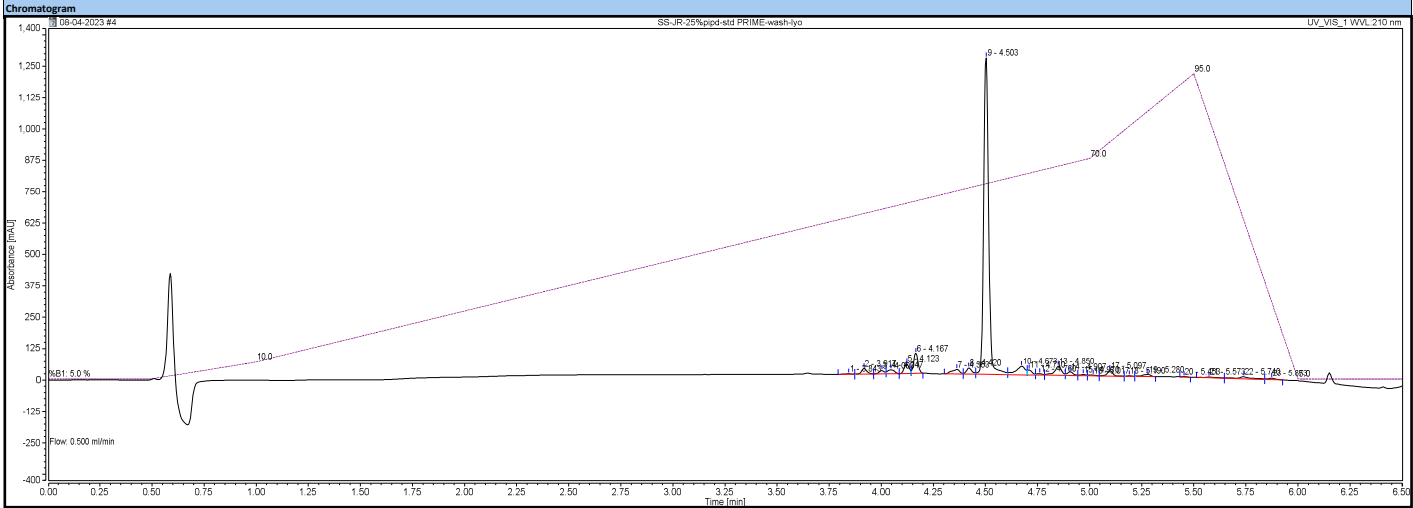


No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	3.807	0.150	2.841	0.31	0.18
2	3.883	0.410	12.353	0.84	0.77
3	4.000	4.459	75.131	9.17	4.69
4	4.097	0.960	23.089	1.97	1.44
5	4.180	0.770	15.997	1.58	1.00
6	4.230	0.298	9.802	0.61	0.61
7	4.330	2.919	63.925	6.00	3.97
8	4.413	1.476	32.593	3.03	2.04
9	4.470	32.406	1251.204	66.61	78.18
10	4.533	1.003	13.055	2.09	0.82
11	4.637	0.376	12.471	0.77	0.78
12	4.680	0.752	23.307	1.55	1.46
13	4.767	0.541	12.106	1.11	0.76
14	4.957	1.435	33.868	2.95	2.12
15	5.067	0.620	17.085	1.27	1.07
16	5.180	0.076	1.938	0.16	0.12
Total:		48.649	1600.366	100.00	100.00

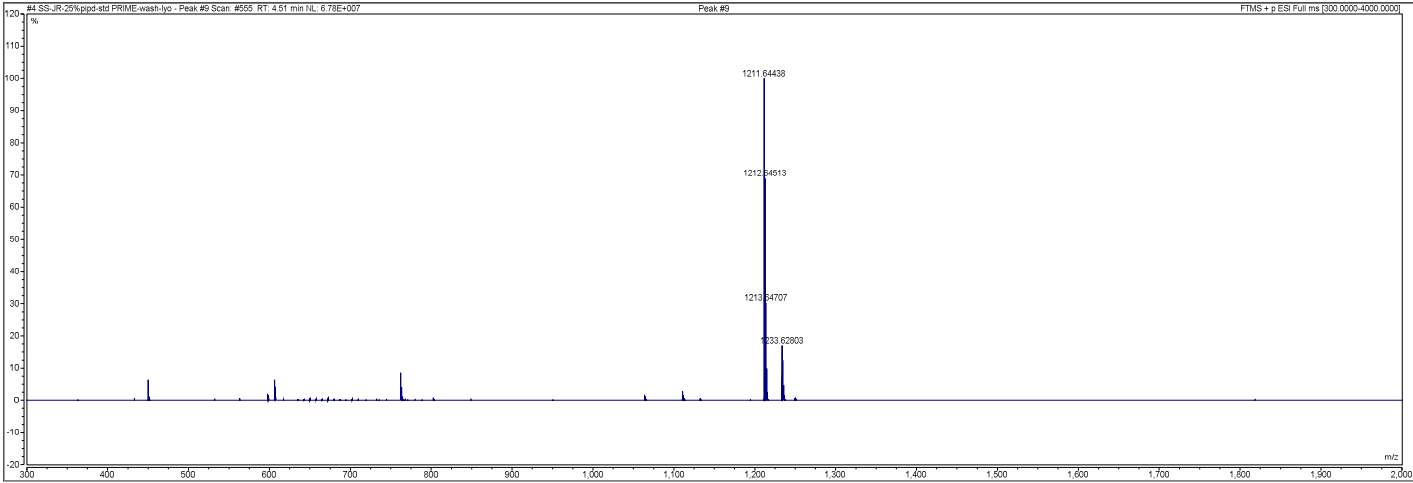


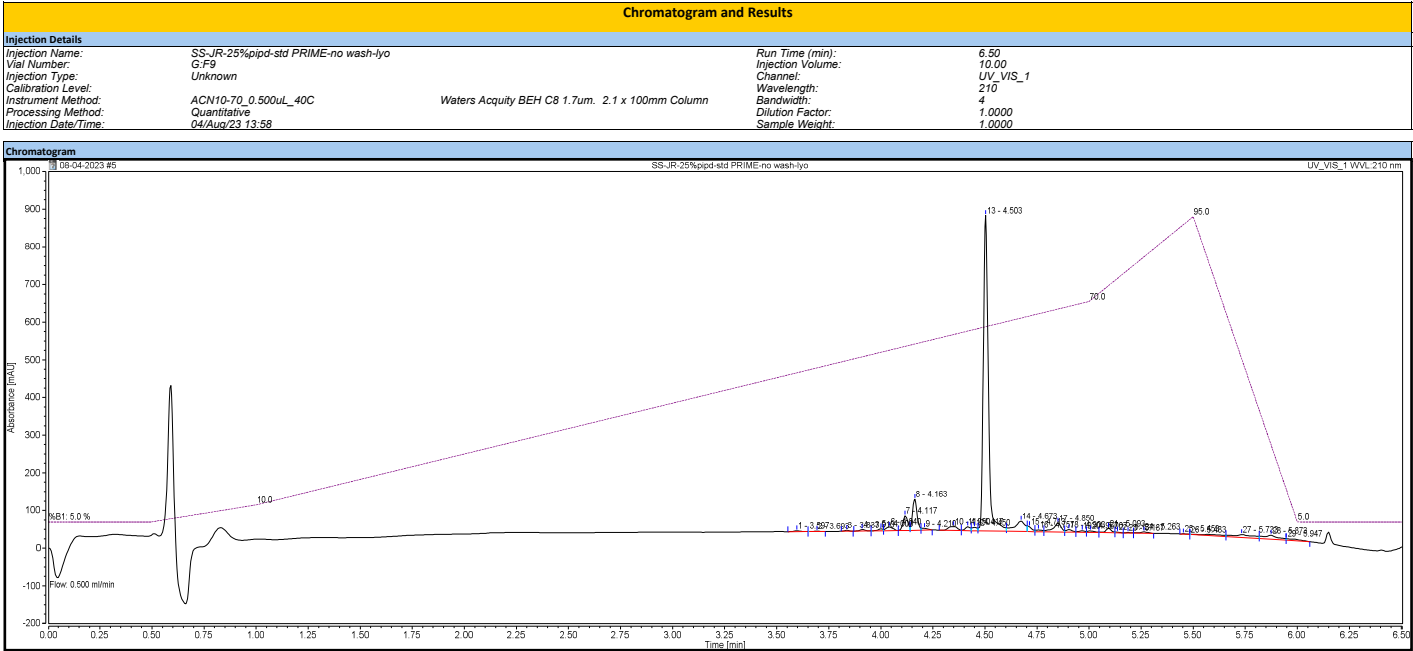
Chromatogram and Results

Injection Details			Run Time (min):	6.50
Injection Name:	SS-JR-25%ppd-std PRIME-wash-lyo		Injection Volume:	10.00
Vial Number:	G.F8		Channel:	UV_VIS_1
Injection Type:	Unknown		Wavelength:	210
Calibration Level:			Bandwidth:	4
Instrument Method:	ACN10-70_0.500uL_40C	Waters Acquity BEH C8 1.7um. 2.1 x 100mm Column	Dilution Factor:	1.0000
Processing Method:	Quantitative		Sample Weight:	1.0000
Injection Date/Time:	04/Aug/23 13:51			



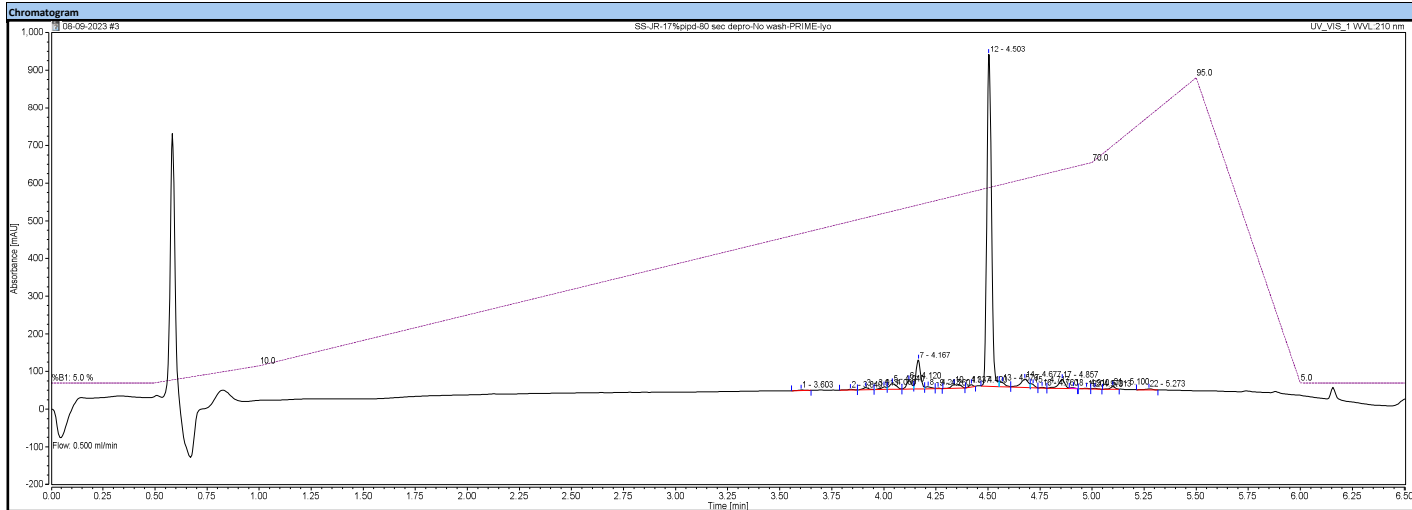
No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	3.843	0.071	1.834	0.15	0.11
2	3.917	0.671	22.617	1.40	1.37
3	4.003	0.403	13.517	0.84	0.82
4	4.047	0.598	16.173	1.24	0.98
5	4.123	1.076	39.256	2.24	2.38
6	4.167	1.963	79.603	4.09	4.63
7	4.363	0.792	18.217	1.95	1.11
8	4.420	0.748	25.076	1.58	1.52
9	4.503	34.879	1260.708	72.59	76.50
10	4.573	1.793	34.595	3.73	2.10
11	4.710	0.521	20.282	1.08	1.23
12	4.760	0.189	5.495	0.39	0.33
13	4.850	1.456	37.227	3.03	2.26
14	4.907	0.474	14.853	0.99	0.90
15	4.970	0.148	4.536	0.31	0.28
16	5.017	0.115	2.398	0.24	0.15
17	5.097	0.661	22.190	1.38	1.35
18	5.190	0.055	1.974	0.11	0.12
19	5.280	0.393	8.980	0.82	0.54
20	5.453	0.040	1.722	0.08	0.10
21	5.573	0.182	3.025	0.38	0.18
22	5.740	0.588	8.192	1.22	0.50
23	5.873	0.230	5.586	0.48	0.34
Total:		48.048	1648.034	100.00	100.00



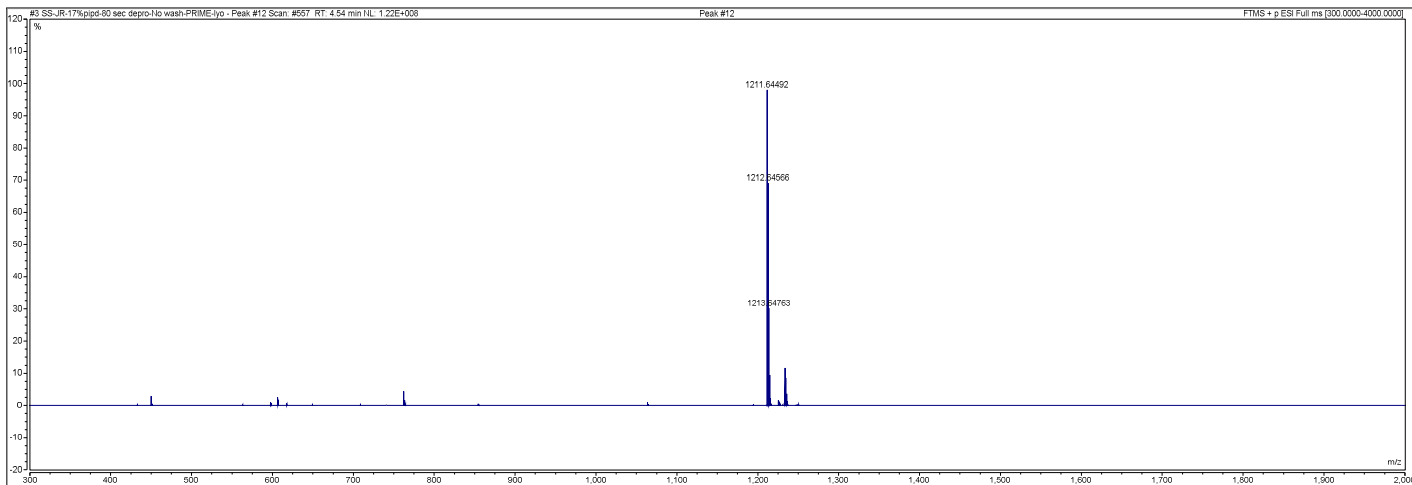


Chromatogram and Results

Injection Details			
Injection Name:	SS-JR-17%ppd-80 sec depro-No wash-PRIME-Iyo		Run Time (min): 6.50
Vial Number:	G.F6		Injection Volume: 10.00
Injection Type:	Unknown		Channel: UV_VIS_1
Calibration Level:			Wavelength: 210
Instrument Method:	ACN10-70_0.500uL_40C	Waters Acquity BEH C8 1.7um. 2.1 x 100mm Column	Bandwidth: 4
Processing Method:	Quantitative		Dilution Factor: 1.0000
Injection Date/Time:	08/Aug/23 11:31		Sample Weight: 1.0000

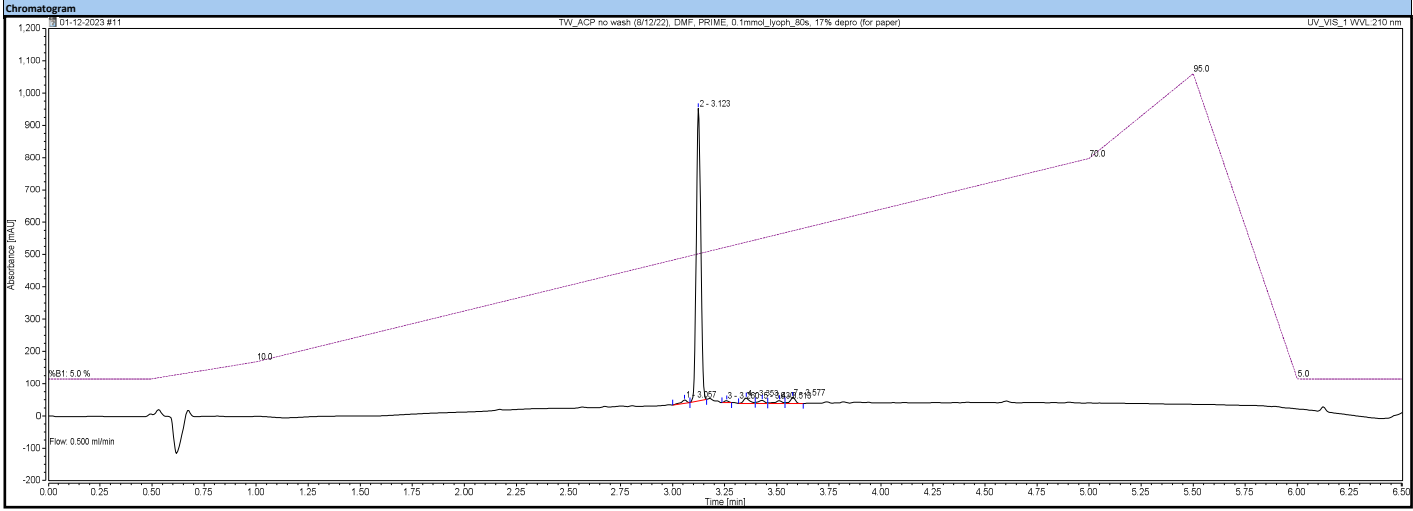


No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	3.603	0.057	1.569	0.19	0.14
2	3.840	0.057	1.692	0.19	0.15
3	3.813	0.168	5.447	0.56	0.49
4	4.000	0.215	6.738	0.72	0.60
5	4.040	0.579	14.596	1.93	1.31
6	4.120	0.628	22.344	2.09	2.00
7	4.167	1.924	76.692	6.41	6.58
8	4.213	0.169	4.845	0.56	0.43
9	4.260	0.027	1.279	0.09	0.11
10	4.337	0.480	9.333	1.60	0.84
11	4.420	0.125	6.048	0.41	0.54
12	4.603	22.670	881.125	75.48	79.01
13	4.570	0.389	11.078	1.30	0.99
14	4.677	0.941	22.214	3.13	1.99
15	4.717	0.204	8.453	0.68	0.76
16	4.760	0.049	2.322	0.16	0.21
17	4.857	0.918	24.111	3.06	2.16
18	4.910	0.044	2.109	0.15	0.19
19	4.973	0.057	2.001	0.19	0.18
20	5.013	0.049	1.842	0.16	0.17
21	5.100	0.178	6.936	0.59	0.62
22	5.273	0.105	2.418	0.35	0.22
Total:		30.034	1115.192	100.00	100.00

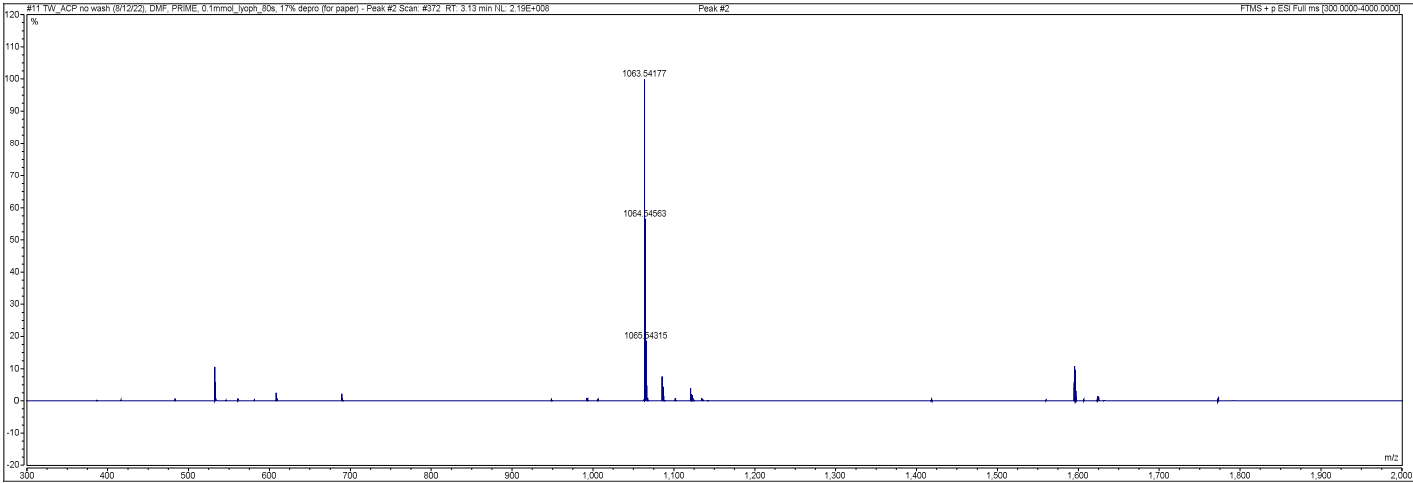


Chromatogram and Results

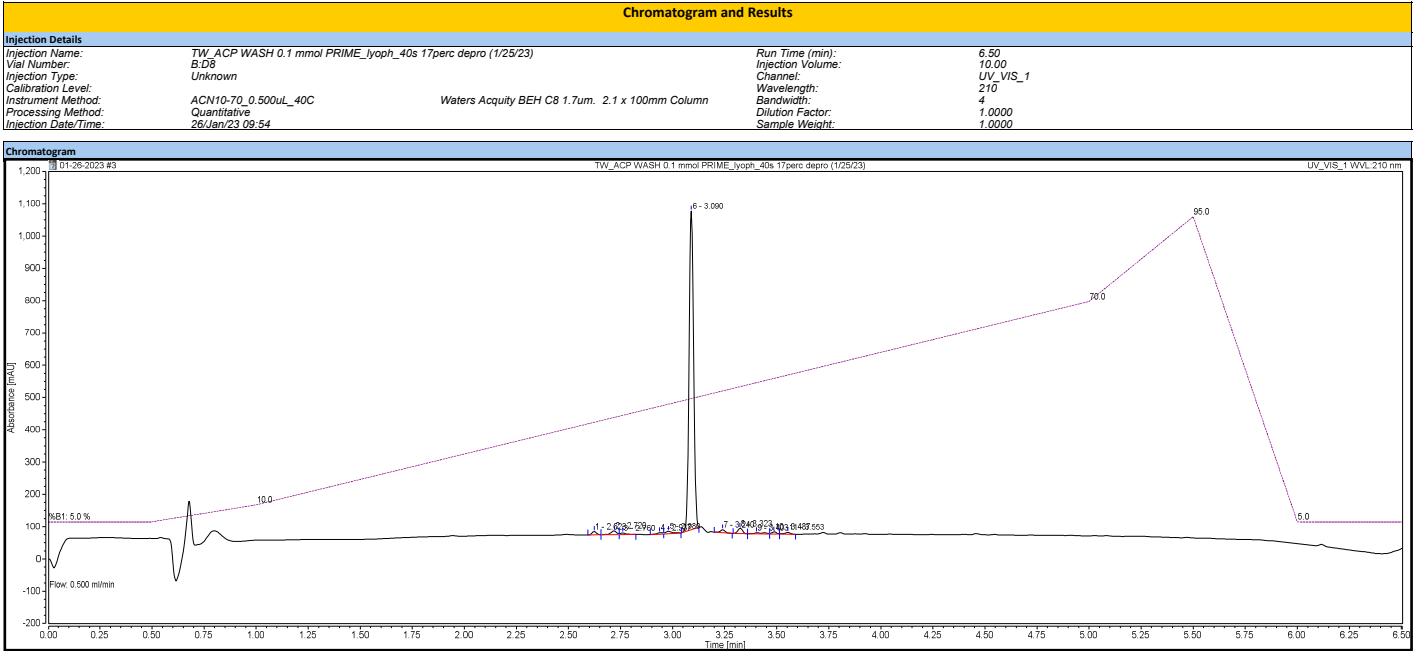
Injection Details		
Injection Name:	TW_ACP no wash (8/12/22), DMF, PRIME, 0.1mmol_lyoph_80s, 17% depro (for paper)	
Vial Number:	B.44	
Injection Type:	Unknown	
Calibration Level:		
Instrument Method:	ACN10-70_0.500uL_40C	Waters Acquity BEH C8 1.7um. 2.1 x 100mm Column
Processing Method:	Quantitative	
Injection Date/Time:	12/Jan/23 15:22	
Run Time (min):	6.50	
Injection Volume:	10.00	
Channel:	UV_VIS_1	
Wavelength:	210	
Bandwidth:	4	
Dilution Factor:	1.0000	
Sample Weight:	1.0000	



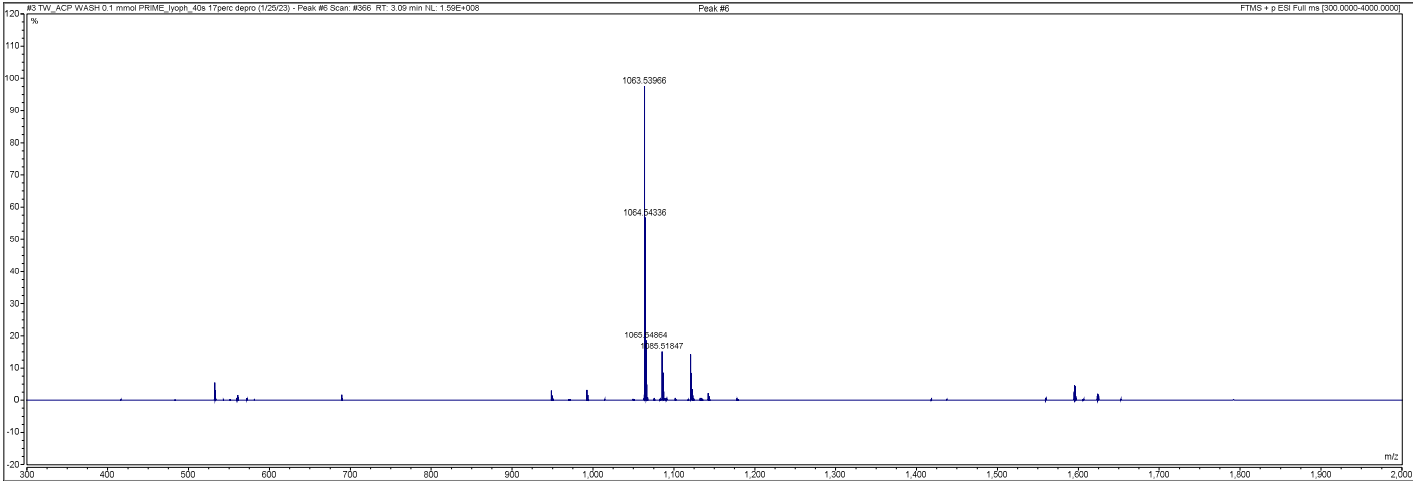
No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	3.057	0.340	10.548	1.36	1.08
2	3.123	22.689	906.700	90.72	92.63
3	3.260	0.141	5.888	0.59	0.60
4	3.353	0.593	17.188	2.39	1.76
5	3.430	0.347	10.120	1.39	1.03
6	3.513	0.359	9.151	1.43	0.94
7	3.577	0.542	19.199	2.17	1.96
Total:		25.011	978.294	100.00	100.00



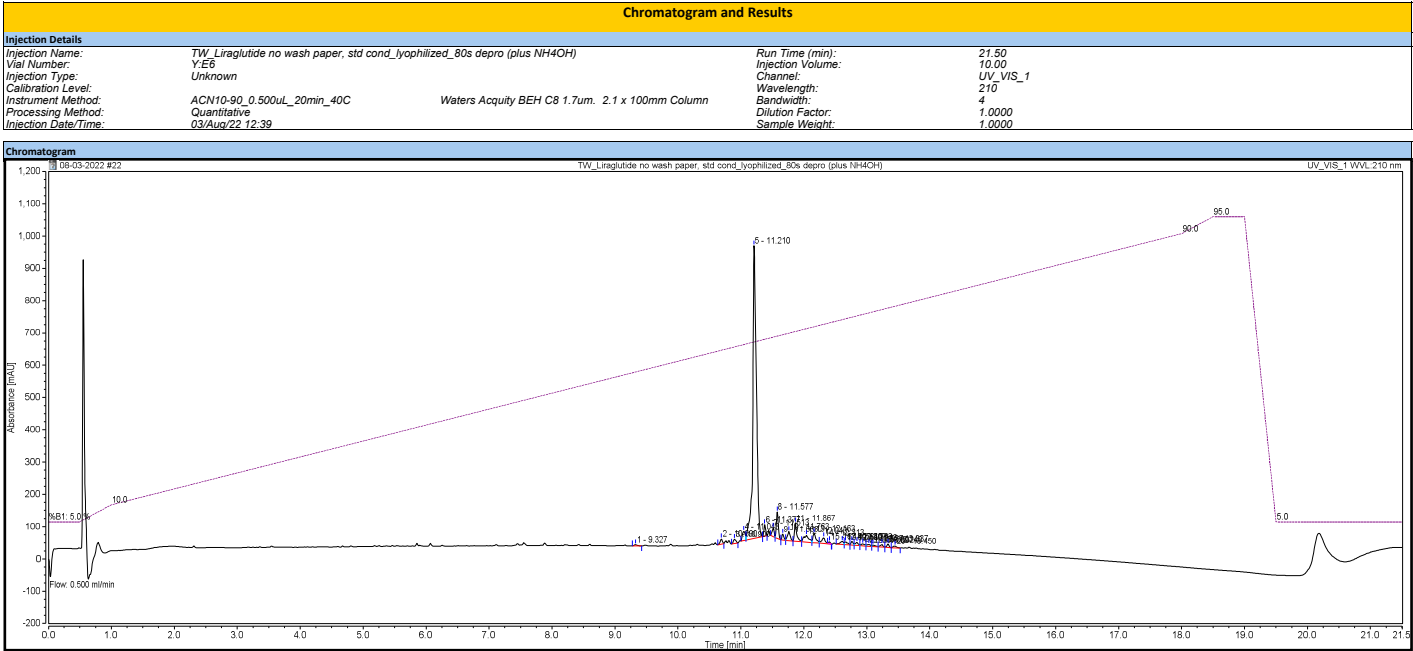
65-74 ACP-Supplementary Table 1, entry 1, wash-based



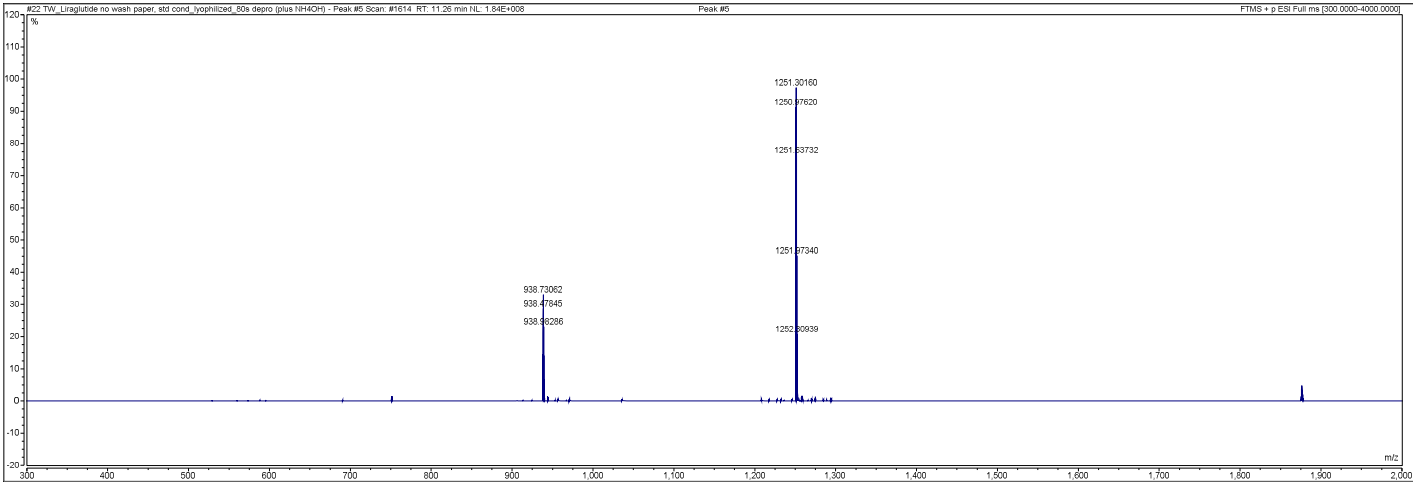
No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	2.623	0.260	10.565	0.95	0.99
2	2.720	0.376	11.464	1.38	1.07
3	2.760	0.159	5.096	0.58	0.48
4	2.937	0.139	4.334	0.51	0.40
5	2.980	0.270	7.559	0.99	0.71
6	3.090	24.892	987.624	90.84	92.27
7	3.230	0.232	9.686	0.92	0.90
8	3.423	0.446	16.864	1.63	1.58
9	3.403	0.230	3.789	0.84	0.35
10	3.487	0.190	7.130	0.69	0.67
11	3.553	0.186	6.198	0.68	0.58
Total:		27.403	1070.309	100.00	100.00



Liraglutide-Supplementary Table 1, entry 2, wash-free



No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	9.327	0.195	3.629	0.24	0.26
2	10.690	0.659	16.736	0.80	1.22
3	10.900	0.635	13.241	0.77	0.97
4	11.043	1.448	28.682	1.75	2.09
5	11.210	60.946	906.570	73.75	66.20
6	11.377	1.115	35.586	1.32	2.60
7	11.513	2.906	26.690	1.24	1.95
8	11.577	1.415	61.873	3.52	5.88
9	11.663	0.638	18.528	0.77	1.35
10	11.763	1.415	23.670	1.71	2.06
11	11.863	2.365	26.670	2.89	4.14
12	12.043	2.980	20.975	2.40	1.53
13	12.163	1.610	28.690	1.95	2.09
14	12.313	0.196	17.783	1.43	1.30
15	12.407	0.196	1.863	0.21	0.43
16	12.610	0.593	9.864	0.72	0.72
17	12.663	0.518	9.096	0.63	0.66
18	12.763	0.418	11.468	0.51	0.64
19	12.843	0.532	9.468	0.64	0.67
20	12.937	0.427	6.056	0.52	0.44
21	13.030	0.369	5.491	0.45	0.40
22	13.097	0.236	4.375	0.35	0.32
23	13.240	0.405	8.107	0.49	0.58
24	13.337	0.435	10.617	0.53	0.78
25	13.450	0.350	5.577	0.42	0.41
Total:		82.644	1369.505	100.00	100.00



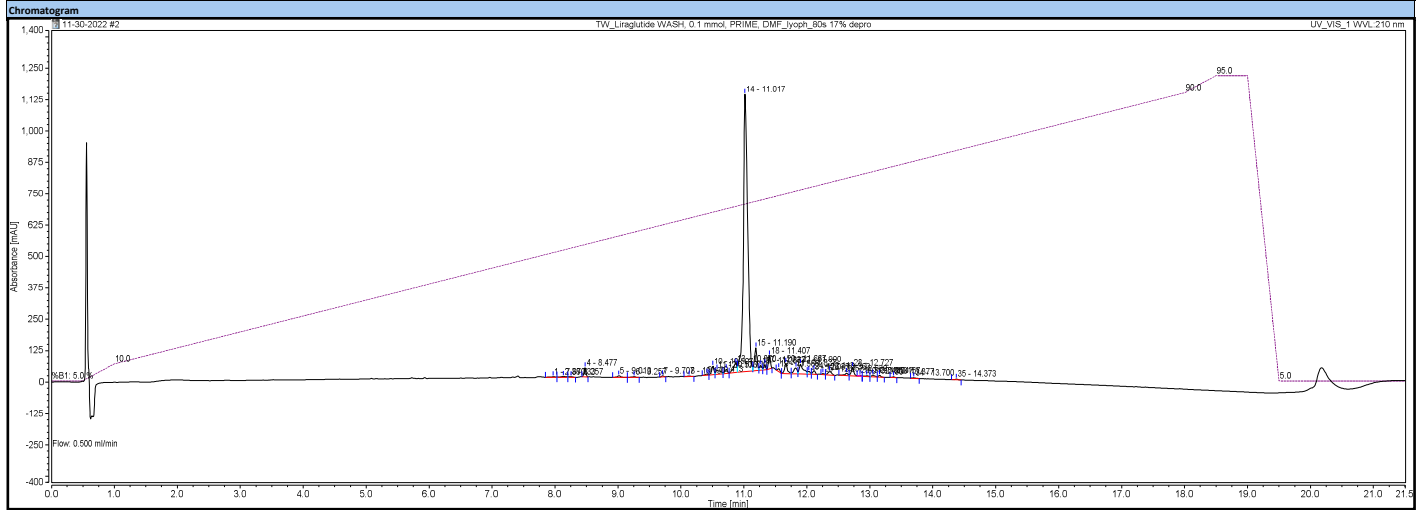
Liraglutide-Supplementary Table 1, entry 2, wash-based

Instrument: PRNTH2, 1 Sequence: 11-30-2022

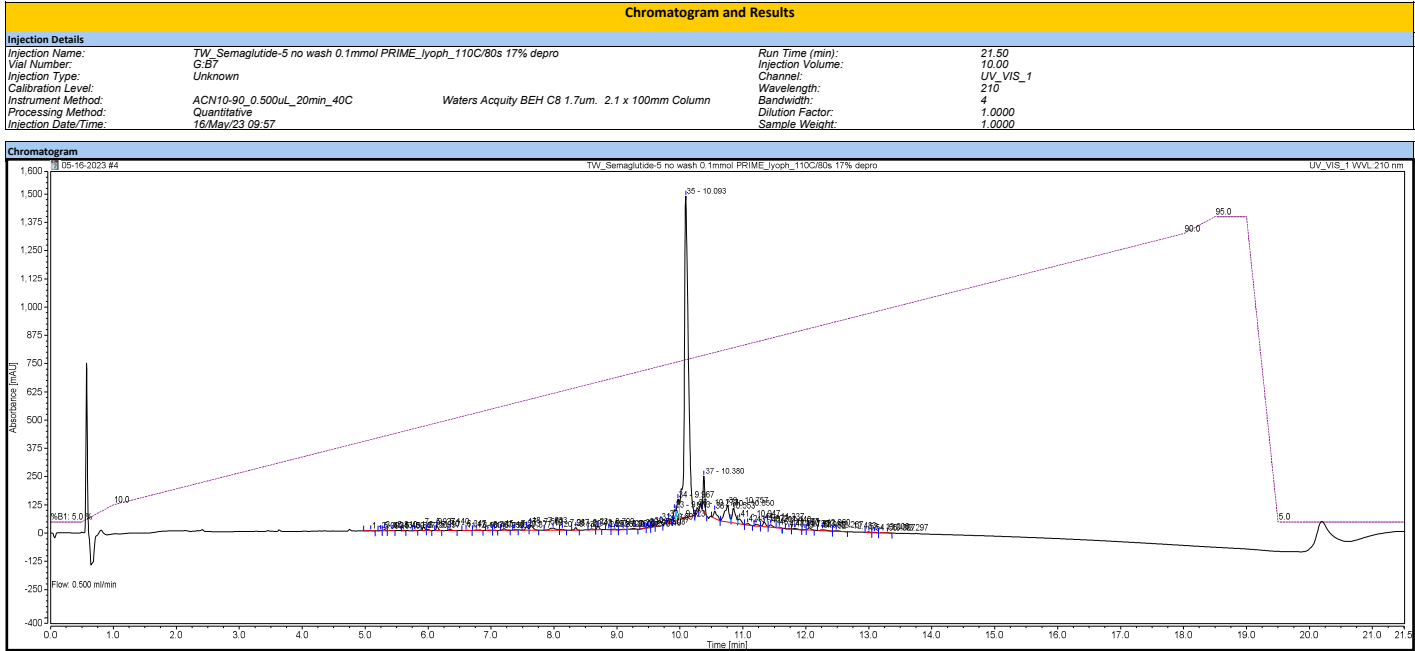
Page 1 of 1

Chromatogram and Results

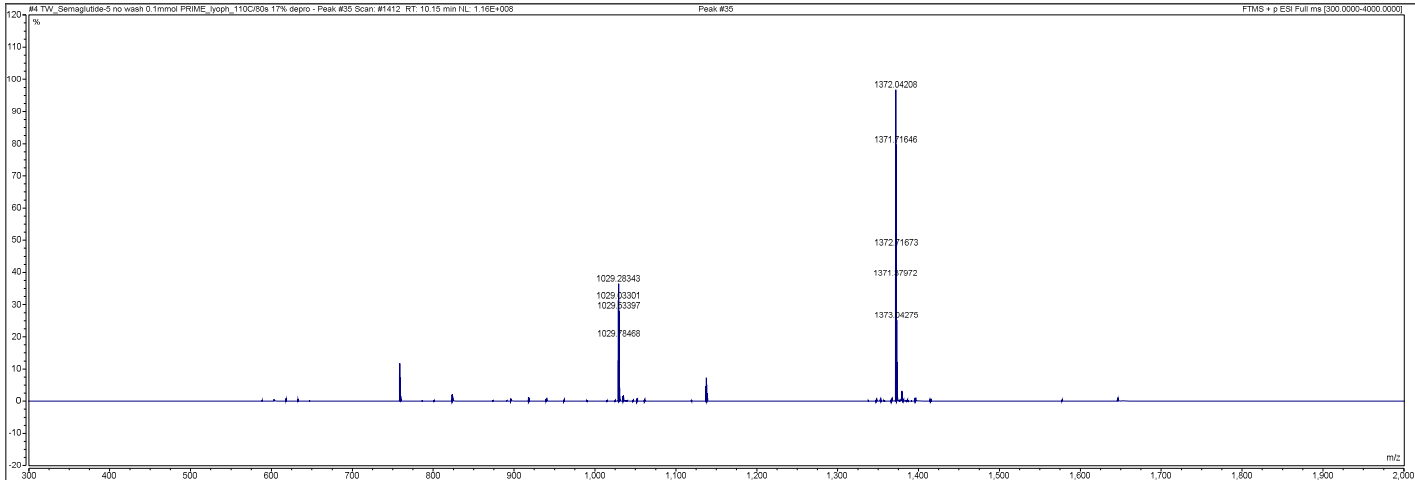
Injection Details			
Injection Name:	TW_Liraglutide WASH, 0.1 mmol, PRIME, DMF, lyoph_80s 17% depro		Run Time (min): 21.50
Vial Number:	Y:A3		Injection Volume: 10.00
Injection Type:	Unknown		Channel: UV_VIS_1
Calibration Level:			Wavelength: 210
Instrument Method:	ACN10-90_0.500uL_20min_40C		Bandwidth: 4
Processing Method:	Quantitative		Dilution Factor: 1.0000
Injection Date/Time:	30/Nov/22 11:12		Sample Weight: 1.0000



Semaglutide-Supplementary Table 1, entry 3, wash-free



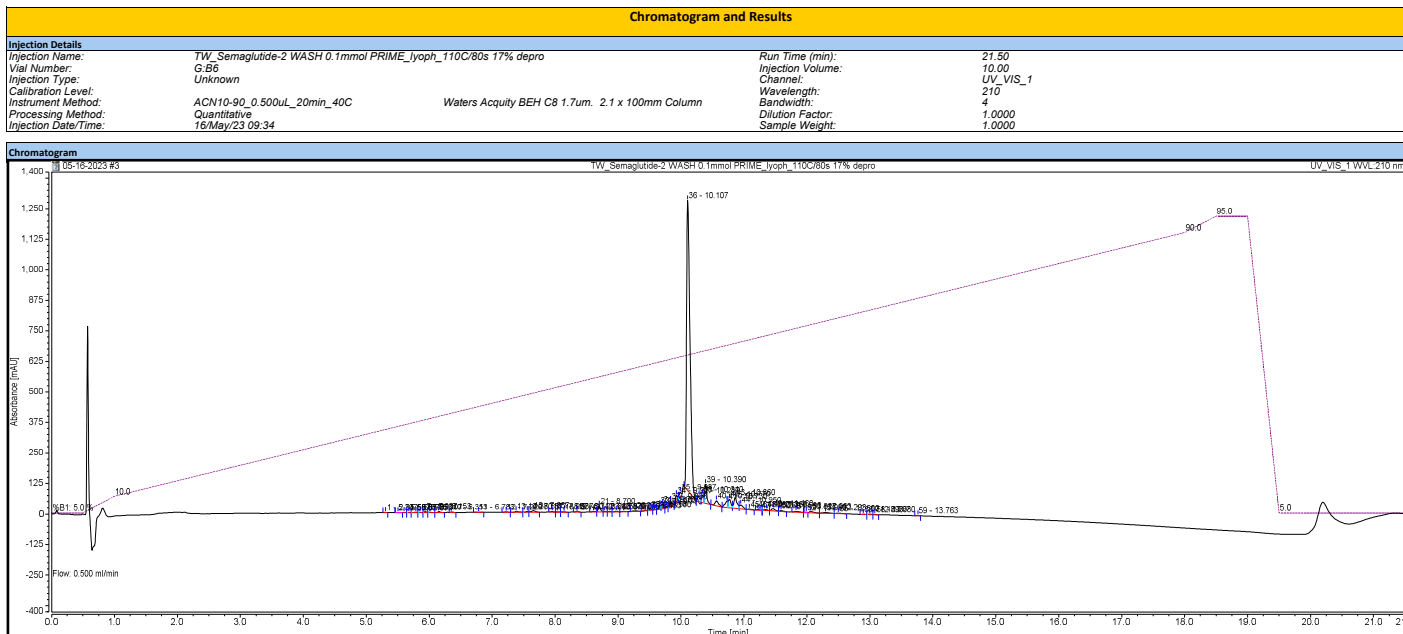
No.	Retention Time		Area		Height		Relative Area		Relative Height	
	min		mAU*min		mAU		%		%	
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	5.090		0.141		2.000		0.10		0.09	
2	5.243		0.097		3.228		0.07		0.14	
3	5.310		0.211		5.579		0.14		0.25	
4	5.430		0.089		1.372		0.06		0.06	
5	5.607		0.068		2.379		0.05		0.11	
6	5.790		0.065		1.496		0.04		0.07	
7	5.927		0.099		17.135		0.41		0.76	
8	5.997		0.239		4.943		0.16		0.22	
9	6.140		0.737		19.810		0.50		0.88	
10	6.347		0.279		6.949		0.19		0.31	
11	6.603		0.101		1.962		0.07		0.09	
12	6.767		0.238		2.735		0.16		0.12	
13	6.950		0.069		0.904		0.05		0.04	
14	7.053		0.063		1.812		0.04		0.08	
15	7.203		0.333		3.376		0.23		0.33	
16	7.377		0.084		2.267		0.06		0.10	
17	7.563		0.743		15.568		0.51		0.69	
18	7.633		1.269		23.272		0.86		1.03	
19	7.987		1.087		9.666		0.74		0.43	
20	8.130		0.225		3.808		0.15		0.17	
21	8.343		0.415		11.316		0.28		0.50	
22	8.597		0.305		4.768		0.21		0.21	
23	8.700		0.687		18.443		0.45		0.73	
24	8.870		0.092		2.080		0.06		0.09	
25	8.960		0.212		3.675		0.14		0.16	
26	9.097		0.164		2.063		0.11		0.09	
27	9.270		0.367		4.949		0.25		0.22	
28	9.440		0.112		2.558		0.08		0.11	
29	9.503		0.163		4.159		0.11		0.16	
30	9.577		0.293		8.959		0.20		0.40	
31	9.697		1.296		19.336		0.88		0.86	
32	9.823		0.971		18.430		0.66		0.82	
33	9.913		1.999		50.749		1.36		2.24	
34	9.967		4.113		91.301		2.80		4.04	
35	10.093		104.729		1423.867		71.25		62.98	
36	10.277		0.733		22.867		0.50		1.01	
37	10.380		5.157		158.542		3.51		7.01	
38	10.553		2.079		33.104		1.41		1.46	
39	10.757		5.859		73.924		3.99		3.27	
40	10.850		3.949		65.791		2.69		2.91	
41	10.947		1.363		22.253		0.93		0.98	
42	11.077		0.392		6.619		0.27		0.29	
43	11.207		0.525		9.464		0.36		0.42	
44	11.337		1.141		23.204		0.78		1.03	
45	11.440		0.699		11.068		0.48		0.49	
46	11.577		0.158		3.576		0.11		0.16	
47	11.730		0.082		1.389		0.06		0.06	
48	11.823		0.359		3.999		0.24		0.18	
49	11.983		0.084		2.323		0.06		0.10	
50	12.080		0.562		10.166		0.38		0.45	
51	12.267		0.573		4.935		0.39		0.22	
52	12.483		0.312		2.643		0.21		0.12	
53	13.000		0.144		3.272		0.10		0.14	
54	13.087		0.055		1.282		0.04		0.06	
55	13.297		0.124		1.337		0.08		0.06	
Total:			146.994		2260.664		100.00		100.00	



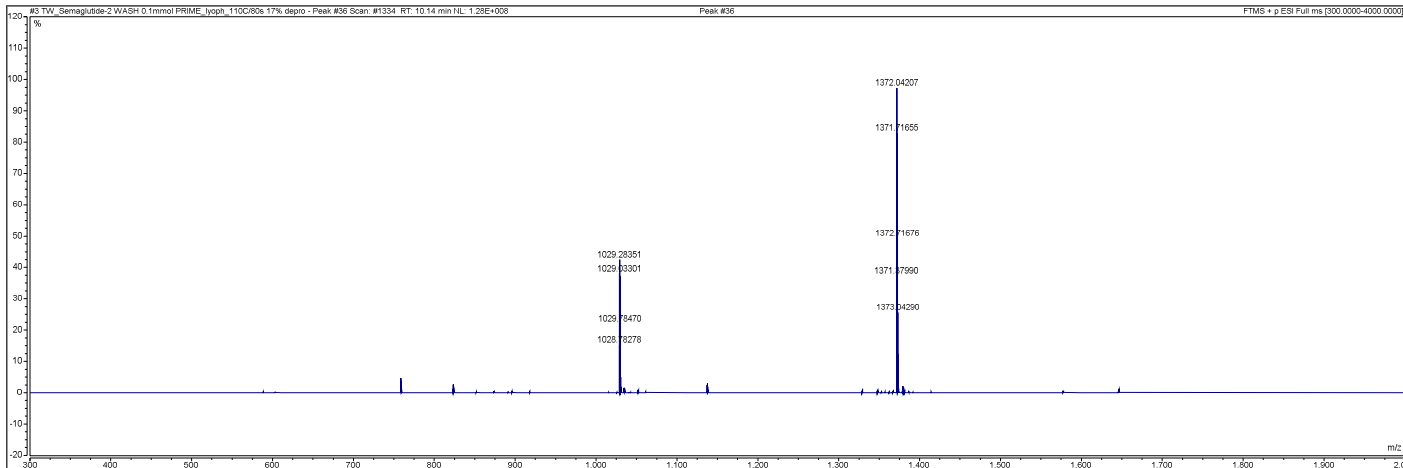
Semaglutide-Supplementary Table 1, entry 3, wash-based

Instrument FRRNTR2_1 Sequence 05-16-2023

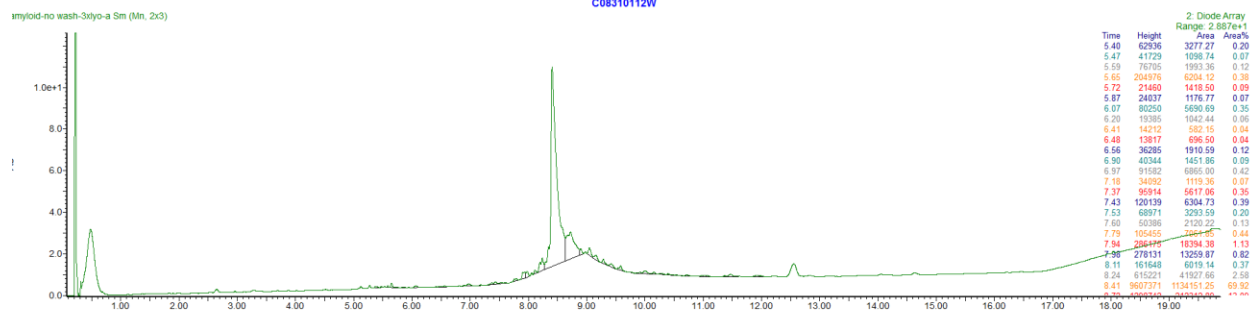
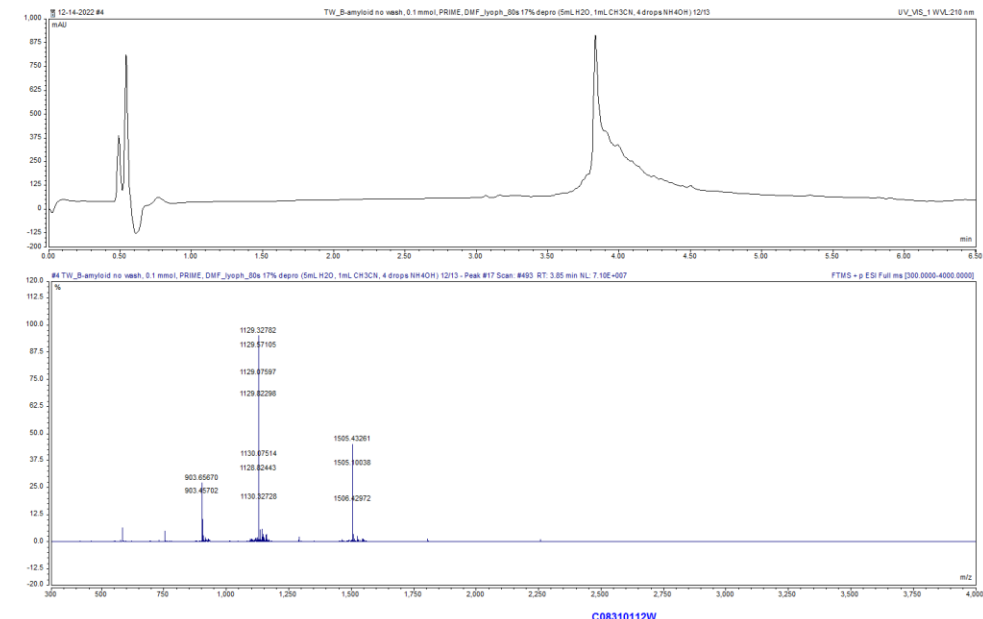
Page 1 of 1



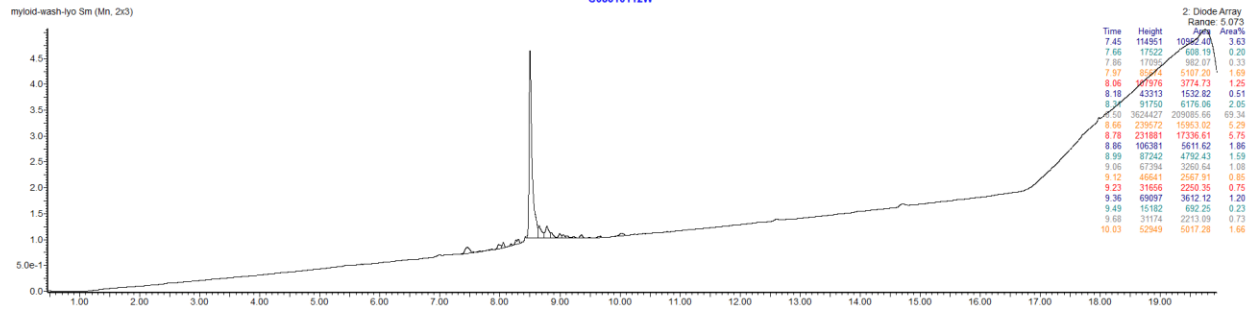
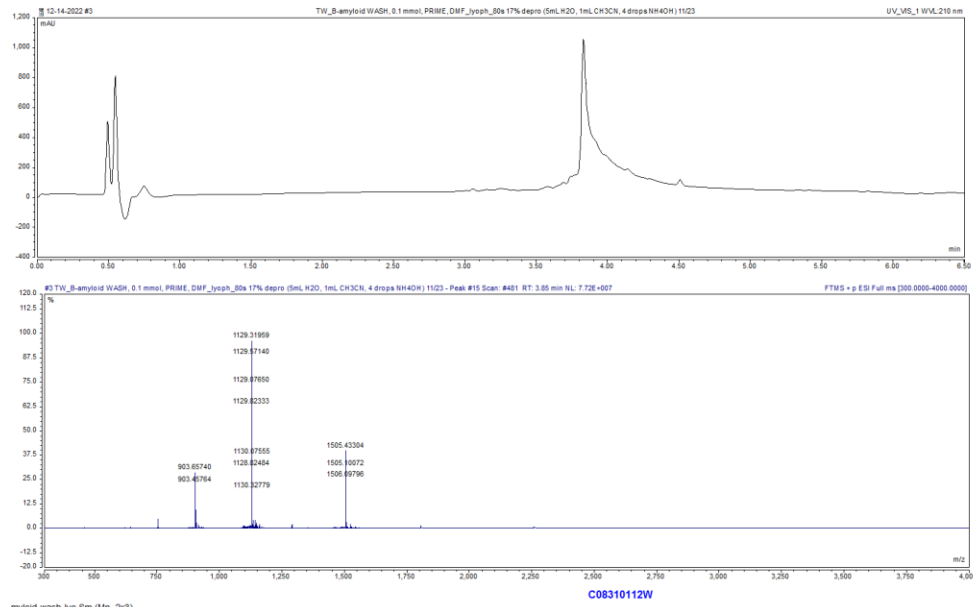
No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	5.307	0.051	1.460	0.05	0.08
2	5.507	0.036	0.641	0.04	0.04
3	5.603	0.065	1.579	0.07	0.09
4	5.677	0.051	1.062	0.05	0.06
5	5.787	0.104	1.581	0.10	0.09
6	5.853	0.064	1.120	0.06	0.07
7	5.937	0.163	4.656	0.16	0.27
8	6.007	0.077	1.939	0.08	0.11
9	6.153	0.163	4.454	0.16	0.26
10	6.353	0.089	2.547	0.09	0.15
11	6.783	0.077	0.999	0.08	0.08
12	7.220	0.057	1.353	0.05	0.08
13	7.380	0.229	4.420	0.23	0.26
14	7.557	0.137	3.422	0.14	0.20
15	7.657	0.148	4.135	0.15	0.41
16	7.957	0.123	2.453	0.12	0.14
17	8.037	0.074	1.684	0.07	0.10
18	8.143	0.076	1.082	0.08	0.06
19	8.347	0.191	4.840	0.19	0.28
20	8.810	0.140	1.742	0.14	0.10
21	8.700	1.031	24.572	1.04	1.43
22	8.787	0.090	2.016	0.09	0.12
23	8.870	0.087	1.717	0.09	0.10
24	8.970	0.161	2.795	0.16	0.16
25	9.090	0.127	1.821	0.13	0.11
26	9.283	0.201	2.526	0.20	0.15
27	9.433	0.137	3.817	0.14	0.22
28	9.510	0.246	6.174	0.25	0.36
29	9.580	0.095	2.323	0.09	0.13
30	9.683	0.823	16.178	0.83	0.94
31	9.707	0.687	18.487	0.69	1.07
32	9.770	0.184	6.784	0.18	0.39
33	9.833	0.857	21.519	0.86	1.25
34	9.927	1.028	27.580	1.03	1.60
35	9.987	0.751	20.175	0.76	1.17
36	10.107	74.065	1187.759	74.57	68.98
37	10.287	1.159	25.026	1.17	1.45
38	10.340	1.147	31.966	1.15	1.86
39	10.390	3.117	77.856	3.14	4.52
40	10.563	1.352	20.694	1.36	1.20
41	10.720	1.401	27.169	1.41	1.58
42	10.770	1.684	36.224	1.70	2.10
43	10.880	2.578	45.238	2.60	2.63
44	10.950	0.991	15.722	1.00	0.91
45	11.087	0.139	2.592	0.14	0.15
46	11.213	0.116	3.076	0.12	0.18
47	11.260	0.020	0.822	0.02	0.05
48	11.360	0.306	4.409	0.31	0.26
49	11.460	0.716	10.011	0.72	0.58
50	11.590	0.217	3.236	0.22	0.19
51	11.827	0.241	2.908	0.24	0.17
52	11.990	0.020	0.721	0.02	0.04
53	12.063	0.460	6.432	0.46	0.37
54	12.293	0.412	3.646	0.41	0.21
55	12.500	0.199	2.000	0.20	0.12
56	12.893	0.057	1.174	0.06	0.07
57	12.997	0.106	2.319	0.11	0.13
58	13.080	0.046	1.128	0.05	0.07
59	13.763	0.048	1.215	0.05	0.07
Total:		99.323	1721.838	100.00	100.00



¹-⁴²β- amyloid-Supplementary Table 1, entry 4, wash-free

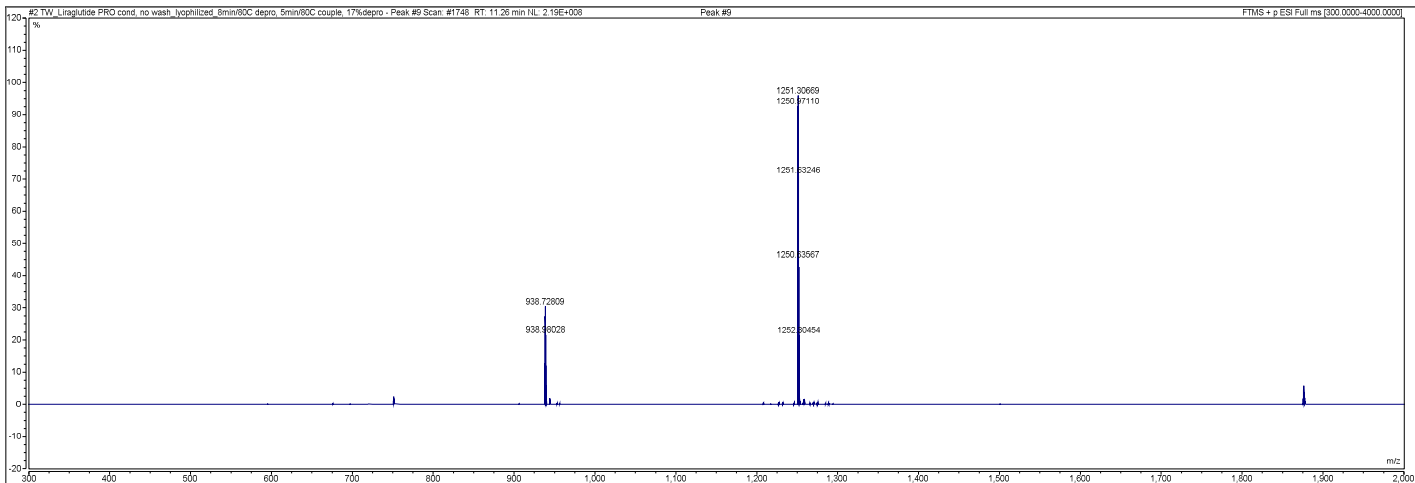
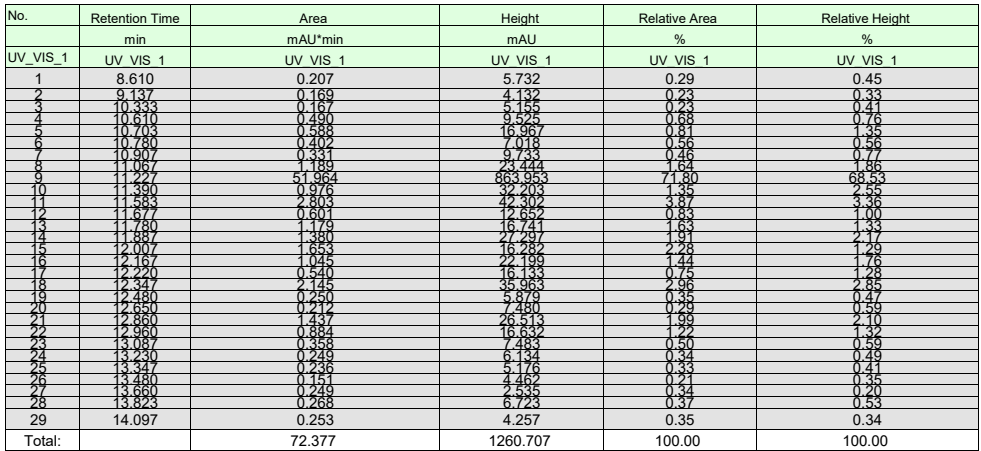


$1-42\beta$ - amyloid-Supplementary Table 1, entry 4, wash-based



Instrument:FRNTNR2_1 Sequence:08-19-2022

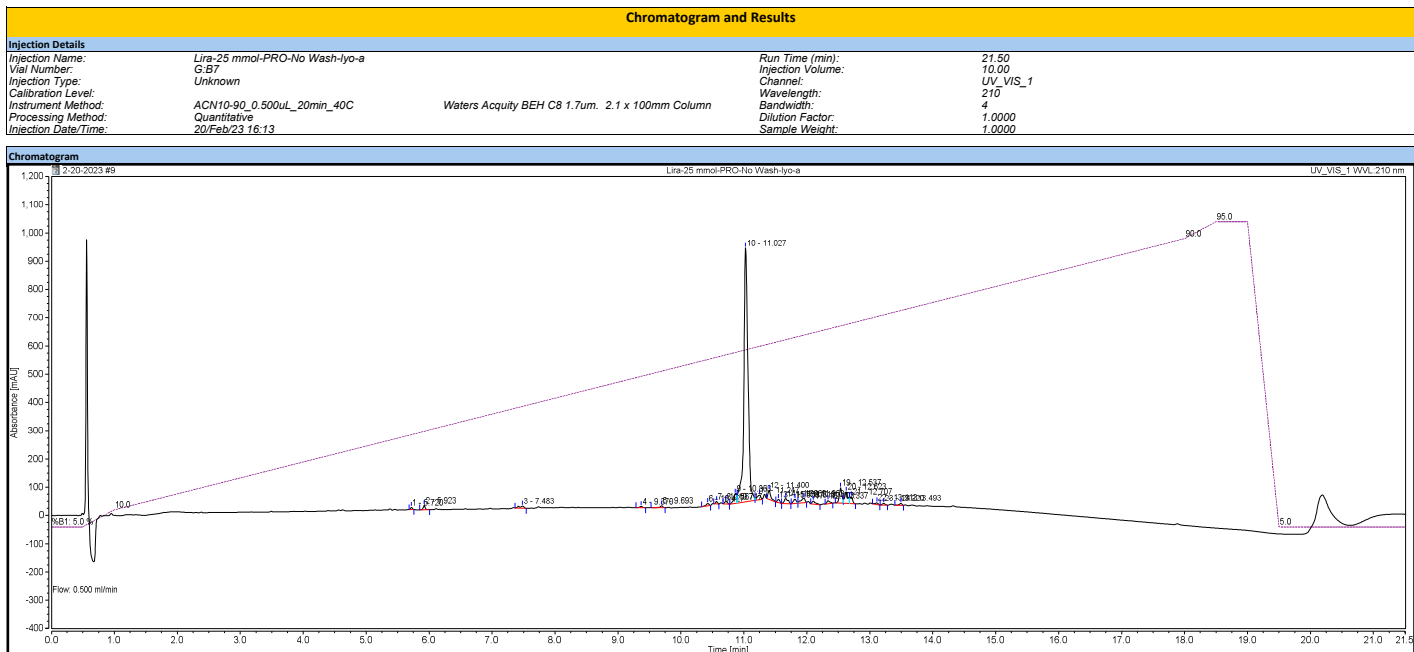
Injection Details				
Injection Name:	TW_Liraglutide PRO cond, no wash_lyophilized_8min/80C depro, 5min/80C couple, 17%depro		Run Time (min):	21.50
Vial Number:	Y:A6		Injection Volume:	10.00
Injection Type:	Unknown		Channel:	UV_VIS_1
Calibration Level:			Wavelength:	210
Instrument Method:	ACN10-90_0.500uL_20min_40C	Waters Acquity BEH C8 1.7um. 2.1 x 100mm Column	Bandwidth:	4
Processing Method:	Quantitative		Dilution Factor:	1.0000
Injection Date/Time:	19/Aug/22 08:36		Sample Weight:	1.0000



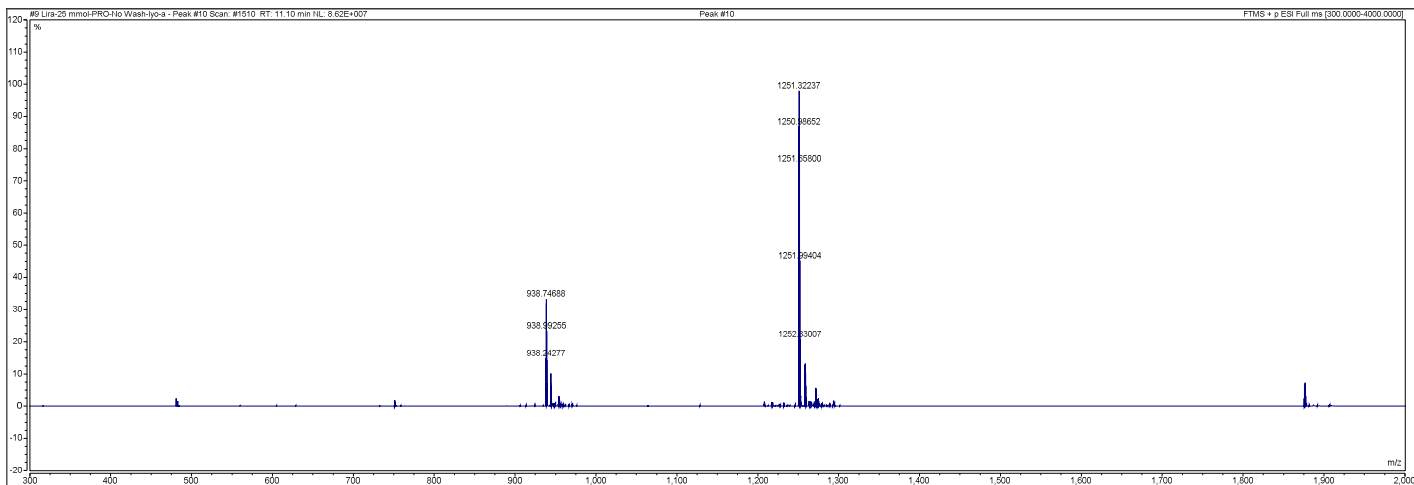
Liraglutide-Supplementary Table 1, entry 6, wash-free

Instrument: FRRNTR2_1 Sequence: 2-20-2023

Page 1 of 1



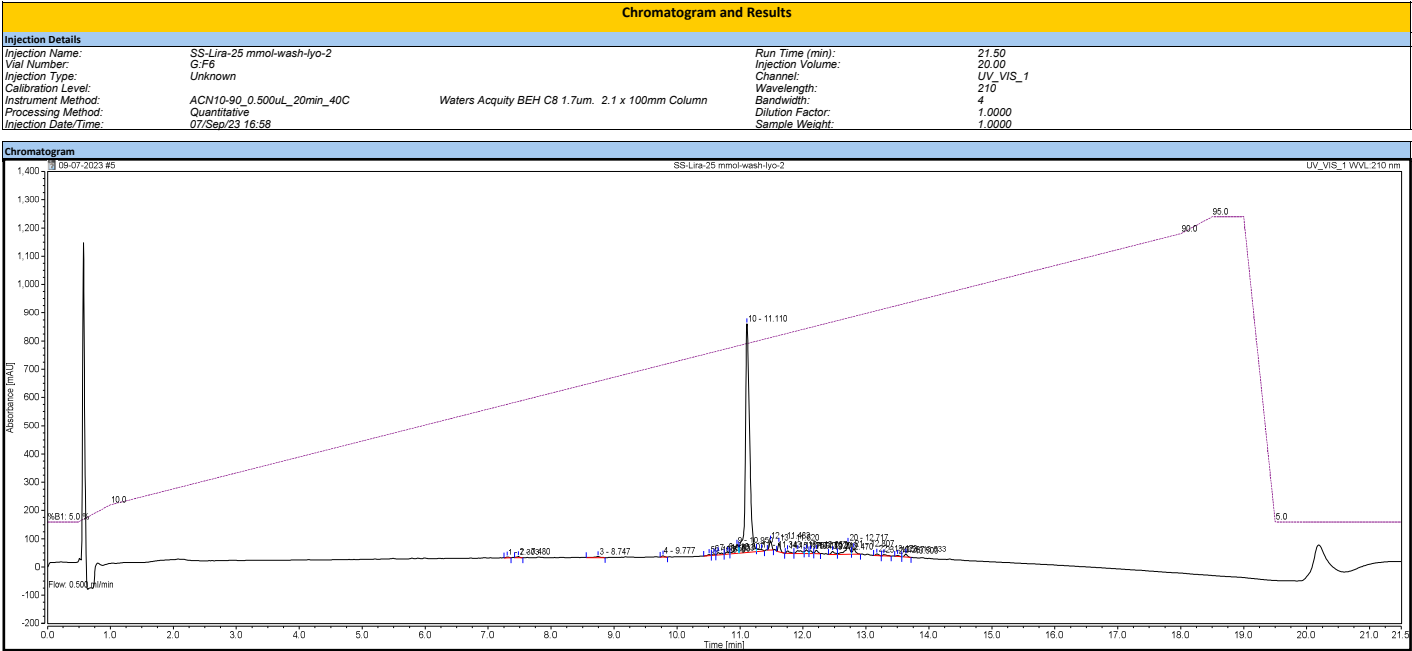
No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	5.720	0.247	8.253	0.28	0.65
2	5.923	0.547	15.411	0.62	1.21
3	7.493	0.669	8.091	0.76	0.64
4	9.370	0.260	4.626	0.29	0.36
5	9.693	0.373	6.818	0.42	0.54
6	10.427	0.405	8.416	0.46	0.66
7	10.567	0.264	8.814	0.30	0.69
8	10.717	0.457	9.322	0.52	0.73
9	10.863	1.781	33.278	2.02	2.62
10	11.027	68.273	698.468	77.31	70.66
11	11.247	1.255	16.512	1.39	1.30
12	11.400	1.609	32.916	1.82	2.59
13	11.550	0.499	11.960	0.57	0.94
14	11.663	1.262	18.845	1.43	1.48
15	11.813	0.832	14.524	0.94	1.14
16	11.953	1.198	15.183	1.36	1.19
17	12.103	0.627	10.468	0.71	0.82
18	12.337	0.562	10.964	0.64	0.66
19	12.537	2.575	53.856	2.92	4.24
20	12.623	2.473	40.285	2.80	3.17
21	12.707	1.234	24.468	1.40	1.92
22	13.113	0.322	6.104	0.36	0.48
23	13.220	0.286	6.226	0.32	0.49
24	13.493	0.331	7.698	0.37	0.61
Total:		88.309	1271.506	100.00	100.00



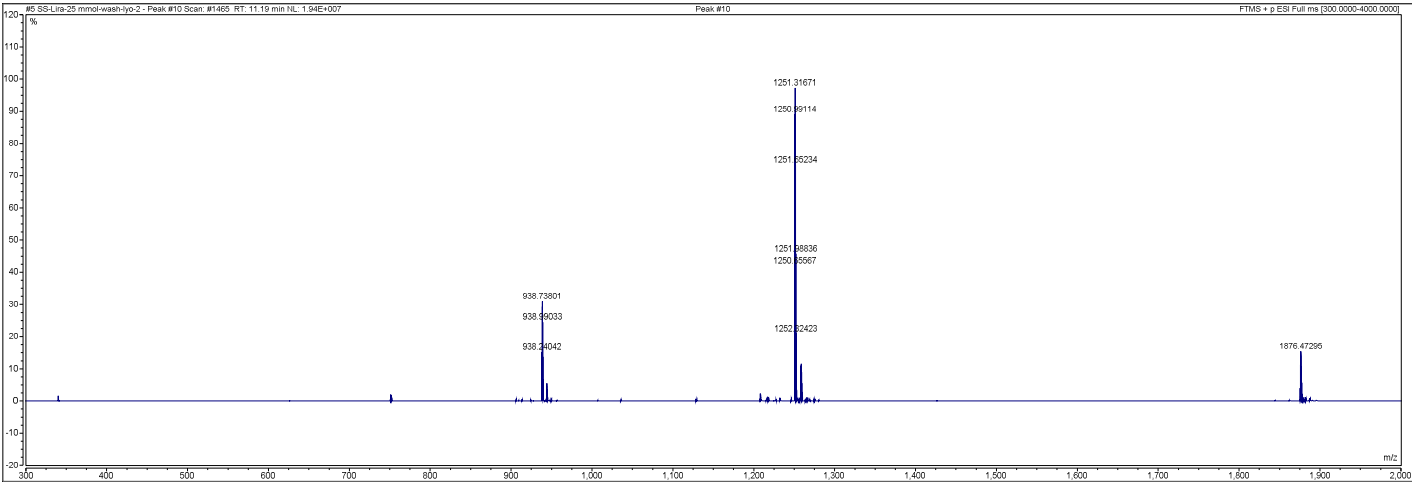
Liraglutide-Supplementary Table 1, entry 6, wash-based

Instrument:FRNTHRP2_1 Sequence:09-07-2023

Page 1 of 1



No.	Retention Time	Area	Height	Relative Area	Relative Height
	min	mAU*min	mAU	%	%
UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1	UV_VIS_1
1	7.303	0.163	3.280	0.20	0.30
2	7.480	0.204	4.142	0.26	0.38
3	8.747	0.355	3.943	0.44	0.38
4	9.777	0.180	3.956	0.20	0.36
5	10.510	0.202	4.213	0.25	0.38
6	10.593	0.184	5.076	0.23	0.46
7	10.653	0.559	8.593	0.70	0.78
8	10.807	0.487	7.563	0.61	0.69
9	10.950	1.879	29.070	2.35	2.64
10	11.110	62.628	810.016	78.38	73.44
11	11.343	0.754	6.177	0.94	0.56
12	11.483	1.382	32.582	1.73	2.95
13	11.620	1.233	31.290	1.54	2.84
14	11.757	0.546	9.736	0.68	0.88
15	11.917	1.059	10.764	1.33	0.98
16	12.057	0.720	13.527	0.90	1.23
17	12.120	0.513	10.108	0.64	0.92
18	12.213	0.627	12.162	0.75	1.10
19	12.470	0.668	10.681	0.84	0.97
20	12.717	2.997	40.009	3.75	3.63
21	12.807	1.127	20.684	1.41	1.88
22	13.173	0.345	6.110	0.43	0.55
23	13.287	0.343	4.941	0.43	0.45
24	13.503	0.207	4.046	0.26	0.37
25	13.633	0.559	10.274	0.70	0.93
Total:		79.898	1102.915	100.00	100.00





Certificate of Analysis

Analysis number: 9998020-2/23

Analysis: Determination of optical purity of amino acid derivatives and peptides via GC-MS

Sample name: Lira-25 mmol-no wash-purified

Date of shipment: 13.04.2023
Sample receipt: 17.04.2023

Company: CEM
Contact person: Dr. Sandeep Singh

Date of completion: April 20, 2023

Completed:

(Operator)

Approved:

(Quality Assurance)

Out of Specification:

OOS is confirmed or defined by the customer. Investigation will be pursued in an stepwise approach: when all parameters of the analysis are checked and the analysis is verified, in a second approach further analyses are performed. If the results of the first analysis are not verified, root cause is defined, corrective action is done and if possible preventive action is taken.



Analysis number 9998020-2/23	Lira-25 mmol-no wash-purified
---------------------------------	-------------------------------

Method description in accordance to SOP: A.0.3. /rv 200114

If Fmoc and DNP are present protective groups, they need to be cleaved.
The peptide / amino acid derivative is hydrolyzed in 6N DCl in D₂O. (In case of presence of Asn, it will be hydrolyzed to Asp, respectively Gln/Pyr to Glu, and so detected and determined).
If necessary an antioxidant and/or scavenger is added. After completion of hydrolysis excess of reagent is removed and the sample is esterified with deuteriochloride in methyl alcohol. In accordance to the column specification homologue alcohols are possible. After evaporation of excess of reagent the residue is acylated using trifluoroacetic anhydride or pentafluoropropionic anhydride. If histidine is to be determined, the -NH of the imidazol group is derivatized with propyl or butyl chloroformate in a separate step. The residue is dissolved and injected.

Calculation of enantiomeric purity:

$$\% D = \frac{Area_D}{Area_D + Area_L} * 100$$

Change Control:

Minor changes that are not in accordance to the method description are mentioned in the report. Major changes need to be approved.

System Suitability Test:

SST is running before each sequence. Results must meet the acceptance criteria.
For sensitive parameters performance qualification is determined weekly and must meet the acceptance criteria.

Data based on generic validation Revision 010213

Analyte:	:	Free proteinogenic amino acids
Standard deviation	:	≤± 0.1% (at ≤ 1.5% Enantiomer) (For Cys and amino acids linked on to Cys possibly higher)
Limit of detection	:	<<0.1%
Limit of quantitation	:	0.10%
Range	:	0.10 - 5 % Enantiomer

The standard deviation of m-1 contribution that must be considered for some amino acids, is substance and matrix specific. Thus, also the standard deviation of the result for those amino acids could be higher. For amino acids which show complex mass pattern, such as Trp and Nal, standard deviation could be even higher than 0.3%.

If the LOQ of generic validation cannot be met due to substance-specific influences, the result will be reported as < estimated LOQ >.

We assure that the analysis is performed in accordance to the GMP guidelines and in accordance to ICH guideline Q2(R1)



230103

A_0_3_e

Analysis number
9998020-2/23

Lira-25 mmol-no wash-purified

Results:

A.0.3. /rv 200114

The listed amino acid(s) were identified via retention time and mass spectra.

The identity of the main component(s) comply.

The following impurity of the optical antipode was found:

Alanine	0.10 % D-Enantiomer
Valine	<0.10 % D-Enantiomer
Threonine	<0.10 % D-Threonine <0.10 % D-allo Threonine <0.10 % L-allo Threonine
Isoleucine	<0.10 % D-Isoleucine <0.10 % D-allo-Isoleucine 0.14 % L-allo-Isoleucine
Leucine	0.13 % D-Enantiomer
Serine	<0.10 % D-Enantiomer
Aspartic acid	0.18 % D-Enantiomer
Phenylalanine	0.14 % D-Enantiomer
Glutamic acid	0.25 % D-Enantiomer
Tyrosine	<0.10 % D-Enantiomer
Lysine	0.13 % D-Enantiomer
Arginine	0.10 % D-Enantiomer
Tryptophan	n.d. *)
Histidine	0.47 % D-Enantiomer

Notes:

*) Tryptophan could not be determined.

Most likely, it was completely decomposed during sample preparation procedure. Only small differences in the sample matrix can lead to decomposition.

The method is generically validated. However it may not meet all requirements for the release of drug substances and drug products. It is to prove if substance specific validation is required.

230103

A_0_3_e

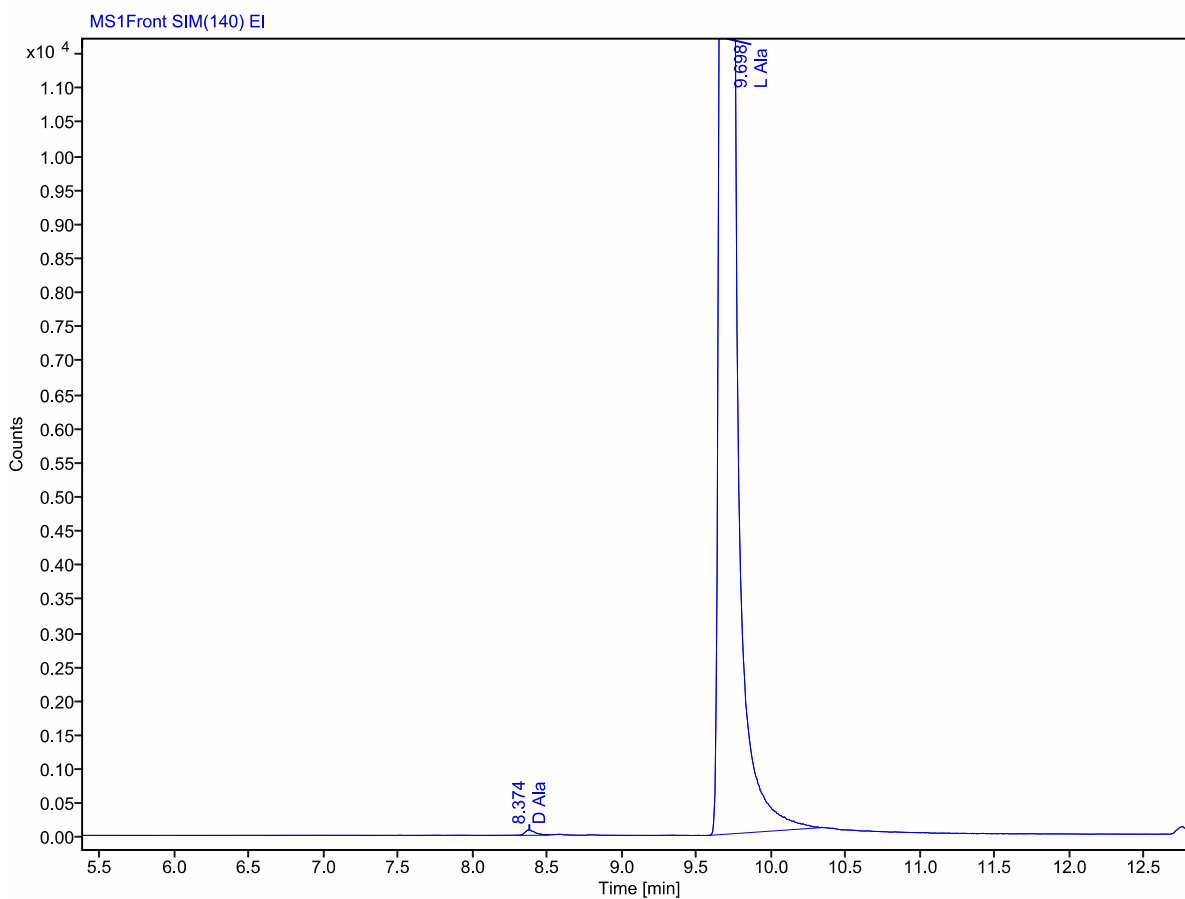
Chromatogram Report



Data file /HP9/Results/2023_04/XY020_2.rslt\XY020-02-01_1A1.dx
Sample name Lira-25 mmol-no wash-purified
Instrument HP#9 **Location** 94
Injection date 04/19/2023 13:57:49 **Injection volume [µl]** 0.200
Acq. method S1419_ET_AAA_SIM_B.amx **Acq. operator** Verena Pohl (verena)
Analysis method ET_AAA.pmx

Signal: MS1Front SIM(140) EI

RT [min]	Type	Width [min]	Area	Height	Area% Name
8.374	MM m	0.22	383.5	78.4	0.10 D Ala
9.698	BM m	0.77	383402.5	96088.1	99.90 L Ala



E-Signature

Level	Level Name	Signed By	Date	Comment	Meaning
1	Approver	Verena Pohl (verena)	2023-04-20 11:02:57+02:00		Approved

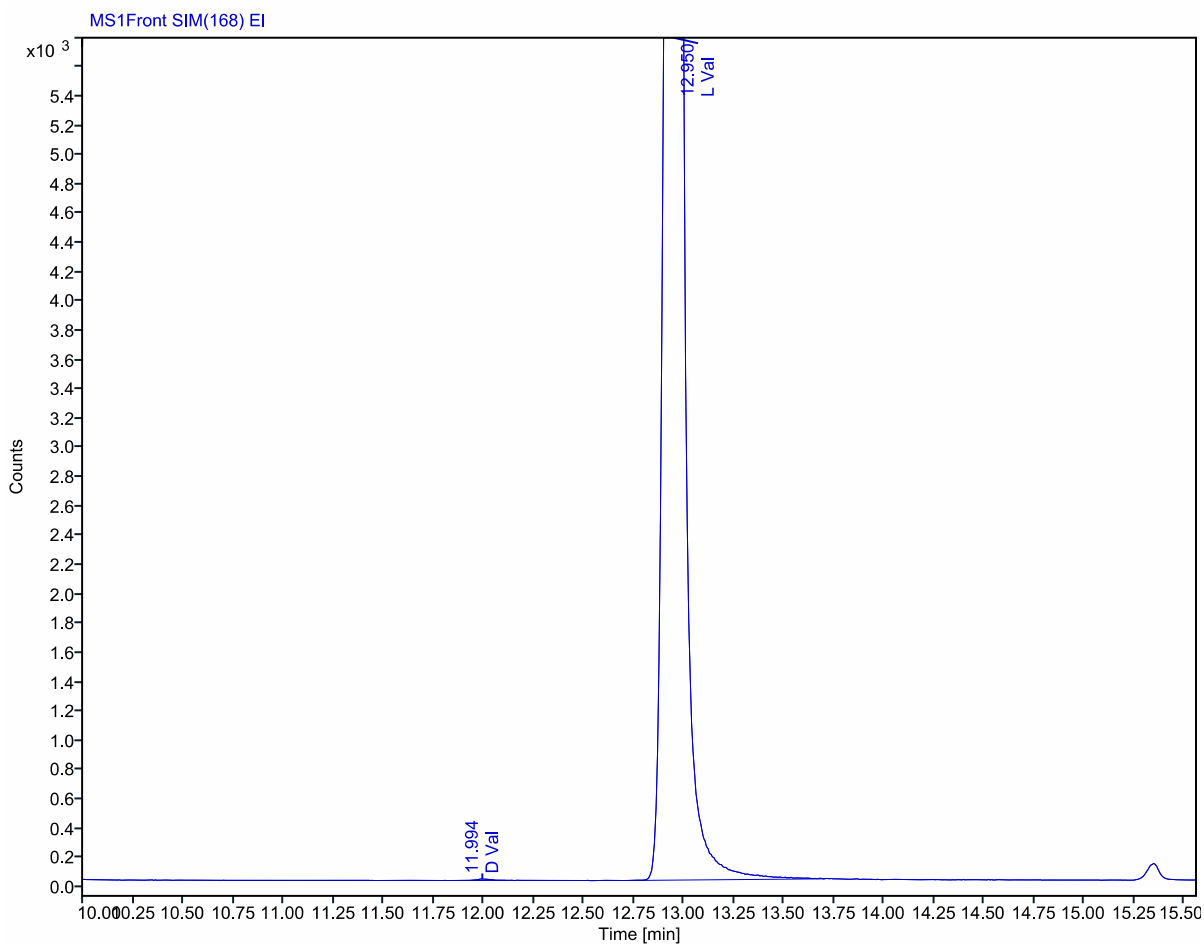
Chromatogram Report



Data file /HP9/Results/2023_04/XY020_2.rsl\XY020-02-01_1A1.dx
Sample name Lira-25 mmol-no wash-purified
Instrument HP#9 **Location** 94
Injection date 04/19/2023 13:57:49 **Injection volume [µl]** 0.200
Acq. method S1419_ET_AAA_SIM_B.amx **Acq. operator** Verena Pohl (verena)
Analysis method ET_AAA.pmx

Signal: MS1Front SIM(168) EI

RT [min]	Type	Width [min]	Area	Height	Area%	Name
11.994	MM m	0.18	45.4	9.6	0.04	D Val
12.950	BBA	0.98	117125.7	26393.9	99.96	L Val



E-Signature

Level	Level Name	Signed By	Date	Comment	Meaning
1	Approver	Verena Pohl (verena)	2023-04-20 11:02:57+02:00		Approved

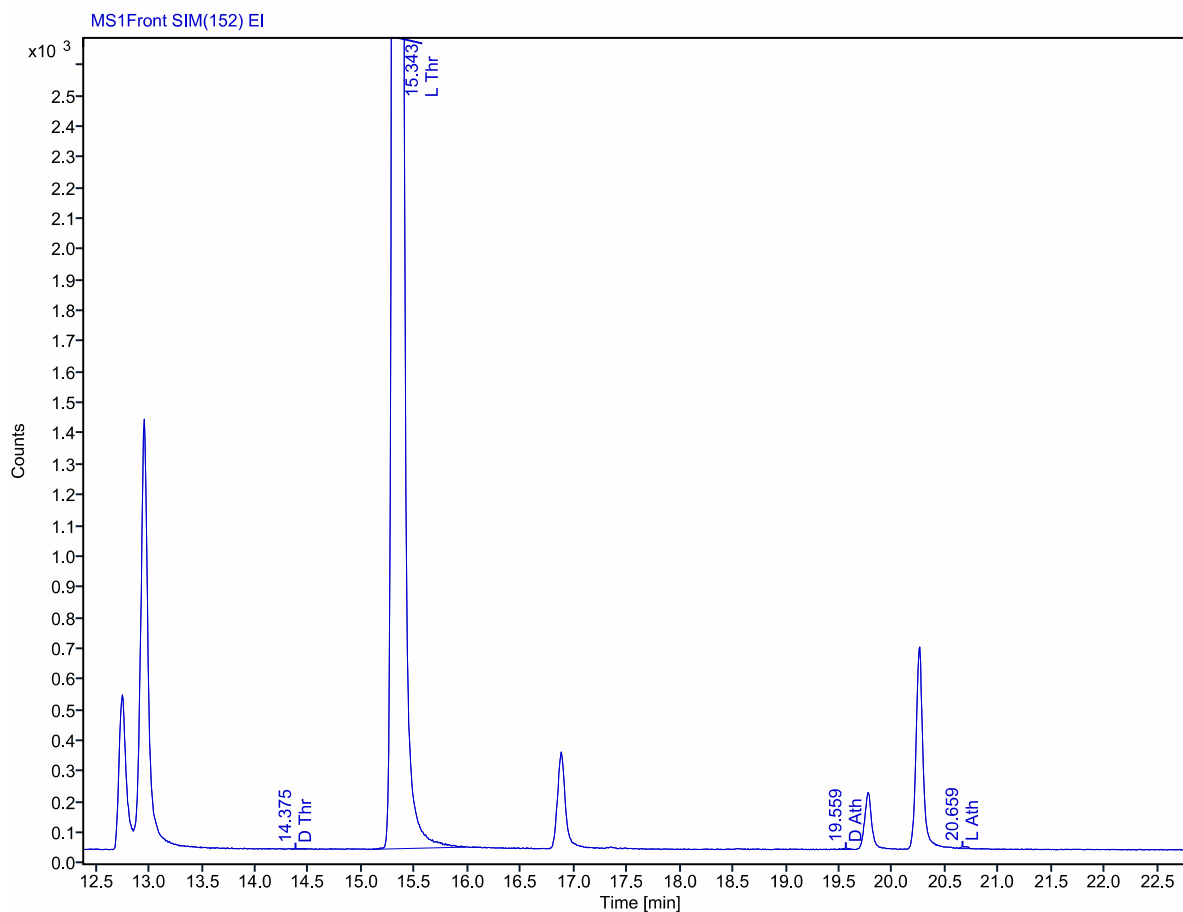
Chromatogram Report



Data file /HP9/Results/2023_04/XY020_2.rslt\XY020-02-01_1A1.dx
Sample name Lira-25 mmol-no wash-purified
Instrument HP#9 **Location** 94
Injection date 04/19/2023 13:57:49 **Injection volume [µl]** 0.200
Acq. method S1419_ET_AAA_SIM_B.amx **Acq. operator** Verena Pohl (verena)
Analysis method ET_AAA.pmx

Signal: MS1Front SIM(152) EI

RT [min]	Type	Width [min]	Area	Height	Area% Name
14.375	MM m	0.1	8.7	2.4	0.01 D Thr
15.343	BBA	0.9	102719.5	24501.5	99.94 L Thr
19.559	MM m	0.1	19.2	5.6	0.02 D Ath
20.659	MM m	0.1	30.3	7.0	0.03 L Ath



E-Signature

Level	Level Name	Signed By	Date	Comment	Meaning
1	Approver	Verena Pohl (verena)	2023-04-20 11:02:57+02:00		Approved

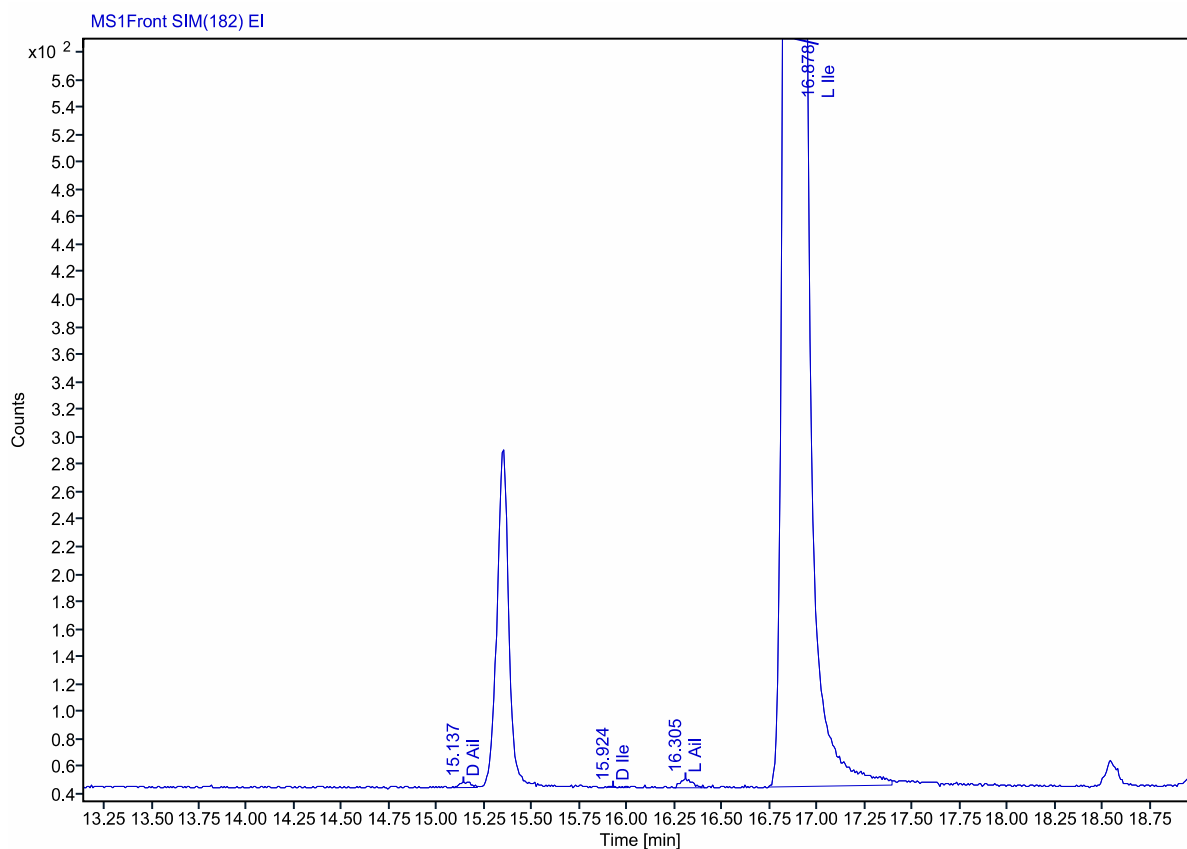
Chromatogram Report



Data file /HP9/Results/2023_04/XY020_2.rslt\XY020-02-01_1A1.dx
Sample name Lira-25 mmol-no wash-purified
Instrument HP#9 **Location** 94
Injection date 04/19/2023 13:57:49 **Injection volume [µl]** 0.200
Acq. method S1419_ET_AAA_SIM_B.amx **Acq. operator** Verena Pohl (verena)
Analysis method ET_AAA.pmx

Signal: MS1Front SIM(182) EI

RT [min]	Type	Width [min]	Area	Height	Area%	Name
15.137	MM m	0.13	17.3	3.9	0.08	D Ail
15.924	MM m	0.11	3.4	0.8	0.02	D Ile
16.305	MM m	0.16	29.0	7.0	0.14	L Ail
16.878	MM m	0.65	21029.7	4326.8	99.76	L Ile



E-Signature

Level	Level Name	Signed By	Date	Comment	Meaning
1	Approver	Verena Pohl (verena)	2023-04-20 11:02:57+02:00		Approved

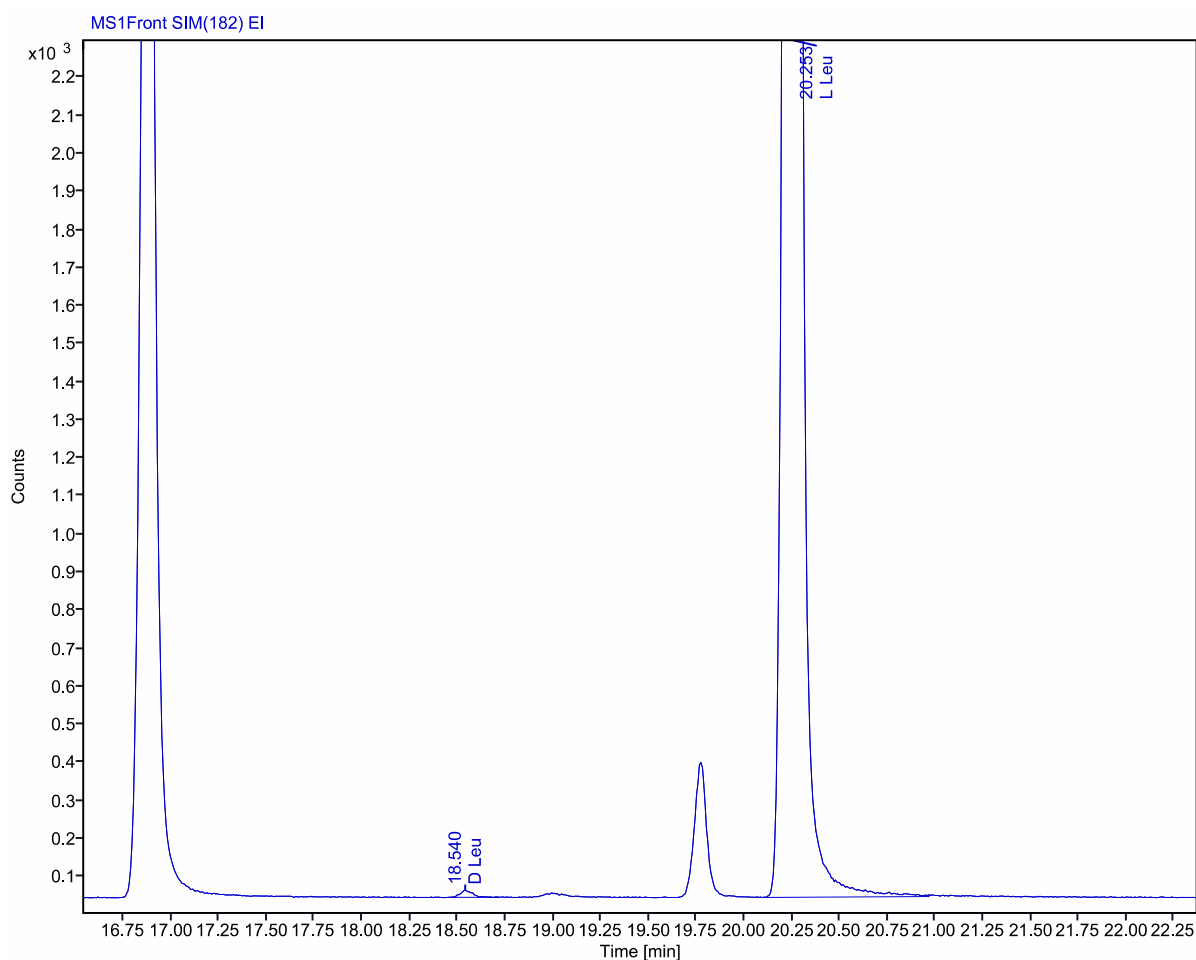
Chromatogram Report



Data file /HP9/Results/2023_04/XY020_2.rslt\XY020-02-01_1A1.dx
Sample name Lira-25 mmol-no wash-purified
Instrument HP#9 **Location** 94
Injection date 04/19/2023 13:57:49 **Injection volume [µl]** 0.200
Acq. method S1419_ET_AAA_SIM_B.amx **Acq. operator** Verena Pohl (verena)
Analysis method ET_AAA.pmx

Signal: MS1Front SIM(182) EI

RT [min]	Type	Width [min]	Area	Height	Area%	Name
18.540	MM m	0.3	82.4	18.7	0.13	D Leu
20.253	MM m	0.9	63765.5	14655.5	99.87	L Leu



E-Signature

Level	Level Name	Signed By	Date	Comment	Meaning
1	Approver	Verena Pohl (verena)	2023-04-20 11:02:57+02:00		Approved

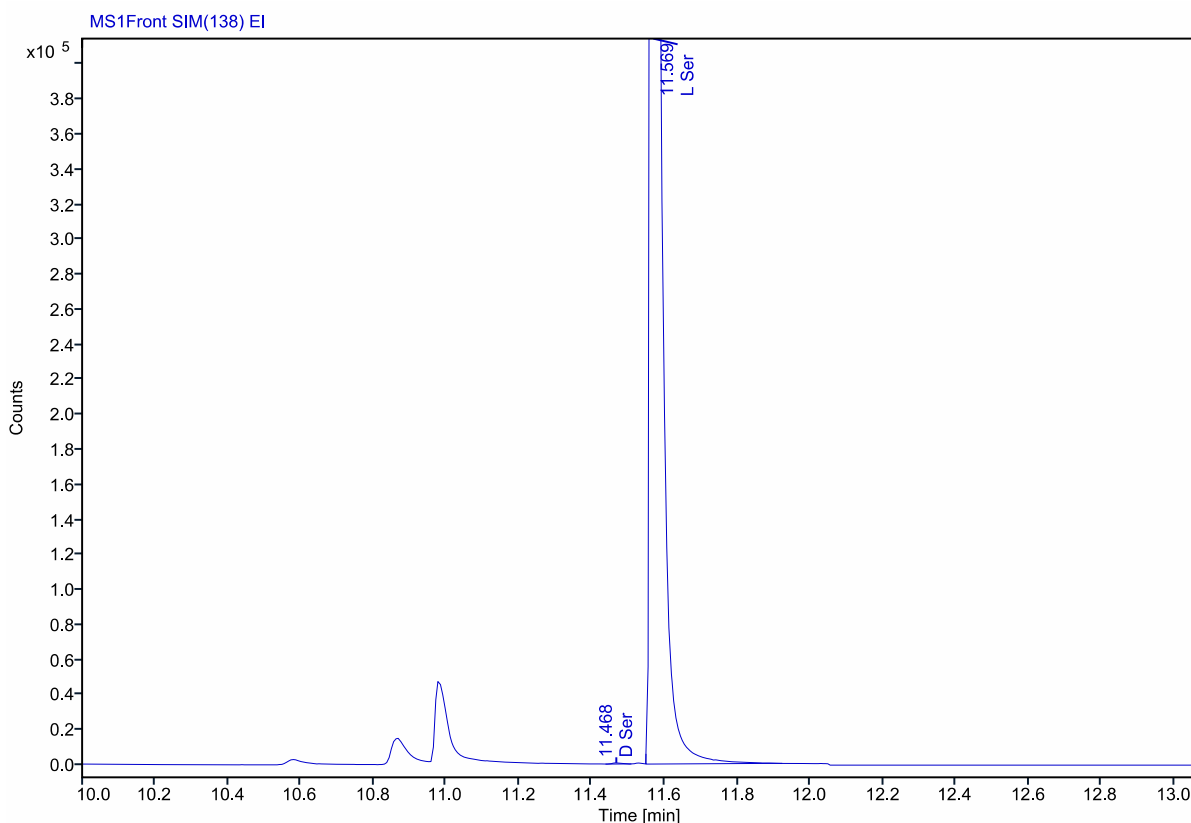
Chromatogram Report



Data file /HP7/Results/2023_04/XY020_4.rsl\XY020-02-01_1A2.dx
Sample name Lira-25 mmol-no wash-purified
Instrument HP#7 **Location** 92
Injection date 04/19/2023 17:55:12 **Injection volume [µl]** 0.100
Acq. method S1382_ET_AAA_SIM_A.amx **Acq. operator** Verena Pohl (verena)
Analysis method ET_AAA.pmx

Signal: MS1Front SIM(138) EI

RT [min]	Type	Width [min]	Area	Height	Area% Name
11.468	MM m	0.07	1106.6	639.8	0.05 D Ser
11.569	MM m	0.37	2014579.7	1131560.8	99.95 L Ser



E-Signature

Level	Level Name	Signed By	Date	Comment	Meaning
1	Approver	Verena Pohl (verena)	2023-04-20 09:16:16+02:00		Approved

Chromatogram Report

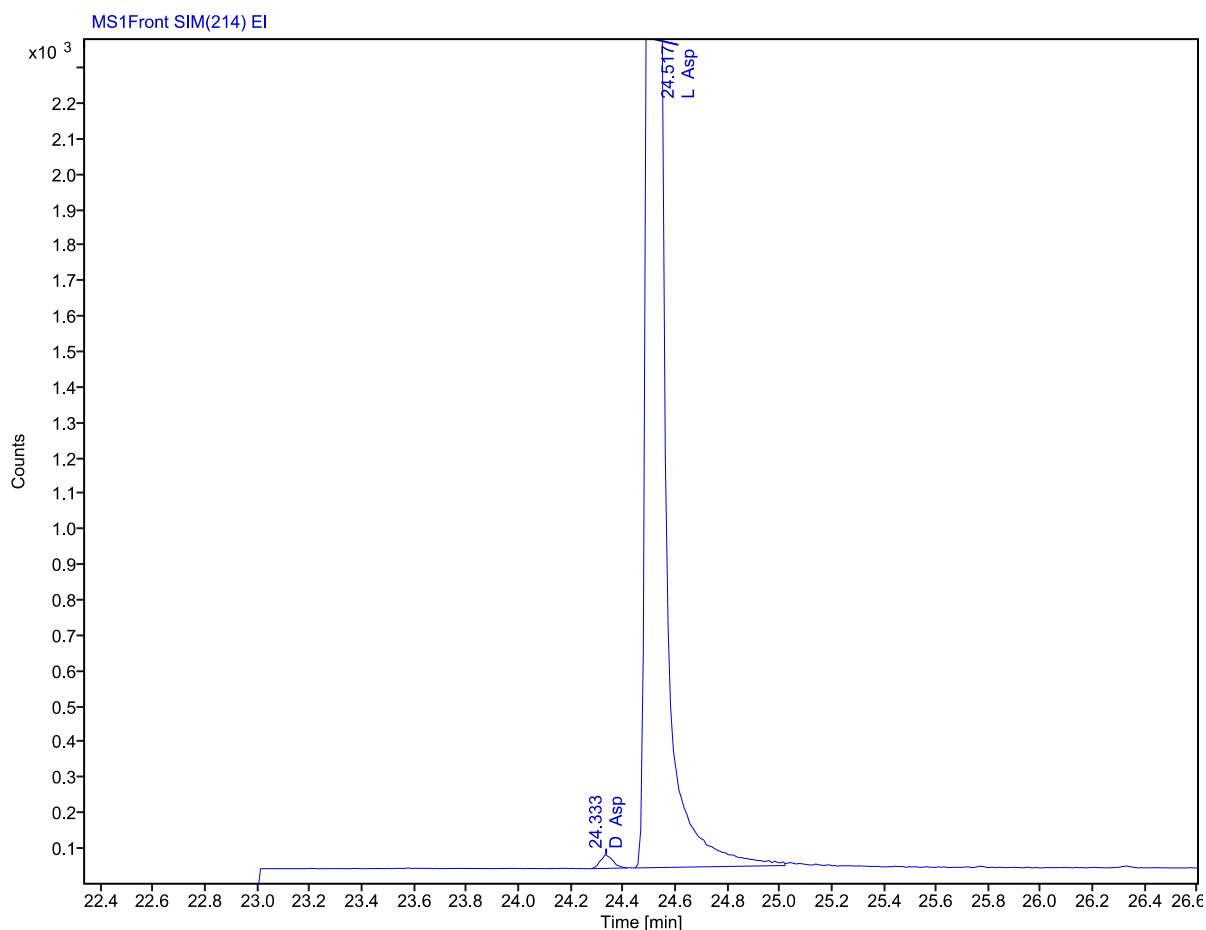


Data file /HP9/Results/2023_04/XY020_2.rsl\XY020-02-01_1A1.dx
Sample name Lira-25 mmol-no wash-purified
Instrument HP#9 **Location** 94
Injection date 04/19/2023 13:57:49 **Injection volume [µl]** 0.200
Acq. method S1419_ET_AAA_SIM_B.amx **Acq. operator** Verena Pohl (verena)
Analysis method ET_AAA.pmx

Signal: MS1Front SIM(214) EI

RT [min]	Type	Width [min]	Area	Height	Area%	Name
24.333	BB	0.14	126.3	39.8	0.41	D Asp
24.517	BM m	0.57	30997.5	10850.6	99.59	L Asp

M-1 contribution of 0.23% must be subtracted



E-Signature

Level	Level Name	Signed By	Date	Comment	Meaning
1	Approver	Verena Pohl (verena)	2023-04-20 11:02:57+02:00		Approved

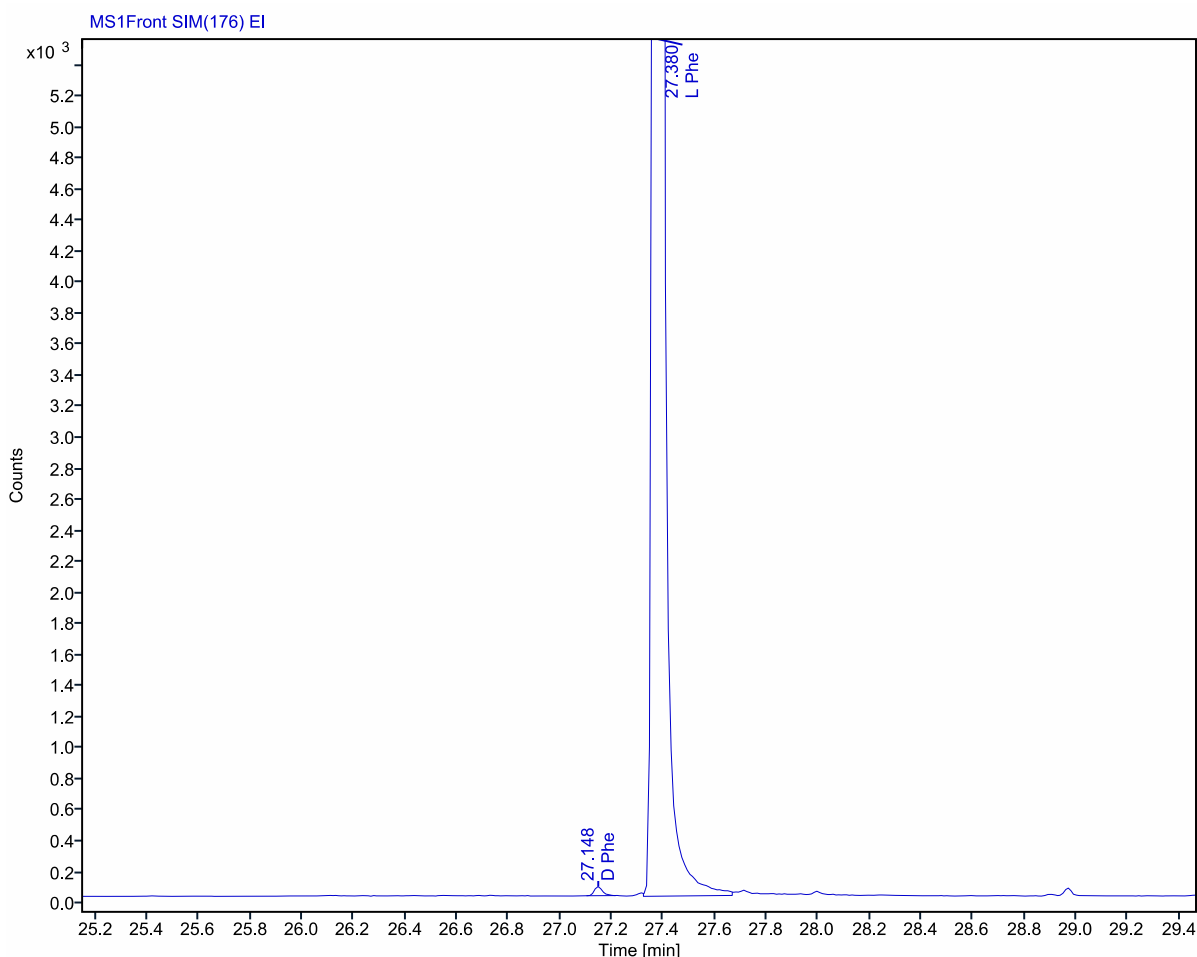
Chromatogram Report



Data file /HP9/Results/2023_04/XY020_1.rsl\XY020-02-01_1EVY1.dx
Sample name Lira-25 mmol-no wash-purified
Instrument HP#9 **Location** 92
Injection date 04/19/2023 12:22:24 **Injection volume [µl]** 0.200
Acq. method S1419_ET_AAA_SIM_B.amx **Acq. operator** Verena Pohl (verena)
Analysis method ET_AAA.pmx

Signal: MS1Front SIM(176) EI

RT [min]	Type	Width [min]	Area	Height	Area% Name
27.148	MM m	0.11	127.9	58.4	0.14 D Phe
27.380	MM m	0.34	91904.9	50673.0	99.86 L Phe



E-Signature

Level	Level Name	Signed By	Date	Comment	Meaning
1	Approver	Verena Pohl (verena)	2023-04-20 11:12:24+02:00		Approved

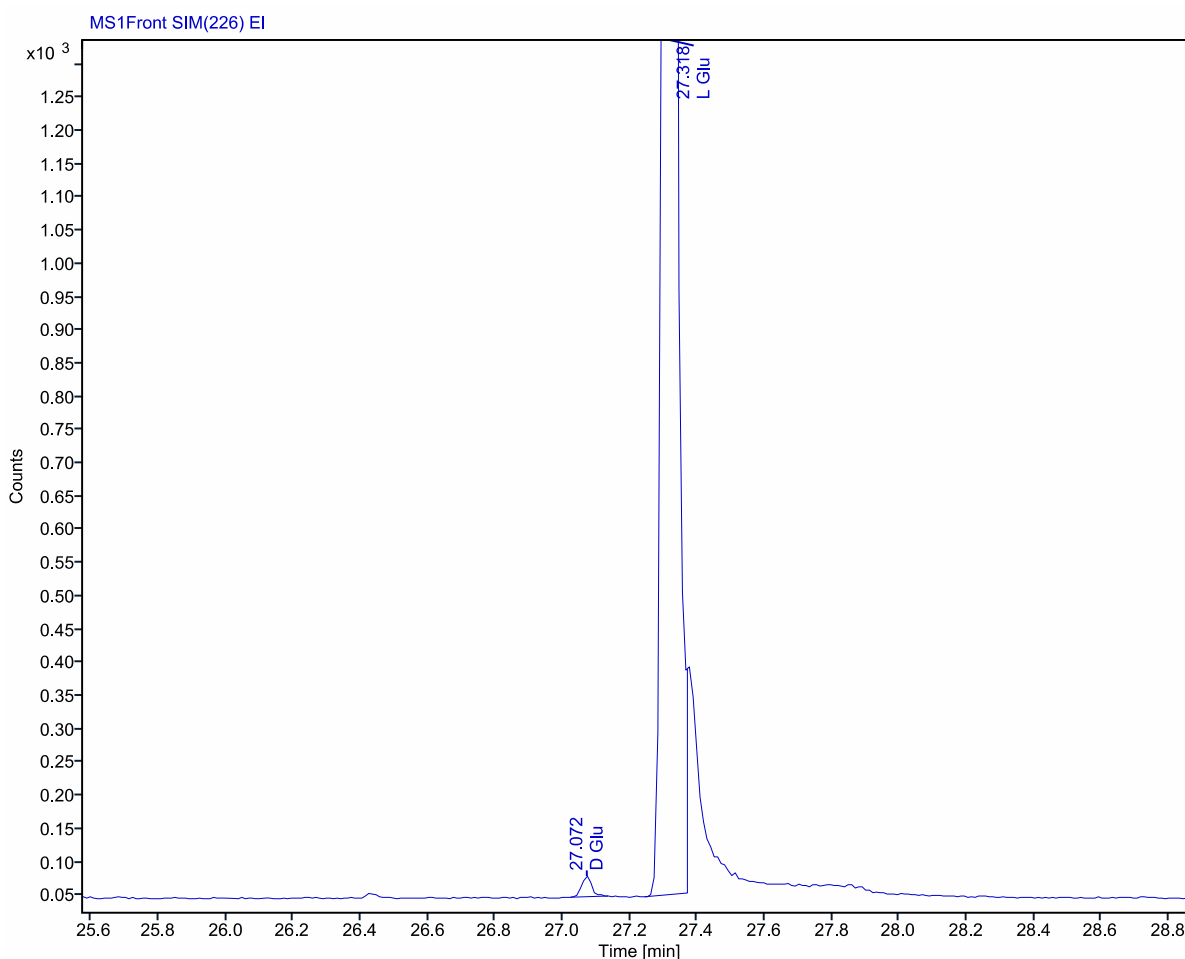
Chromatogram Report



Data file /HP9/Results/2023_04/XY020_1.rslt\XY020-02-01_1EVY1.dx
Sample name Lira-25 mmol-no wash-purified
Instrument HP#9 **Location** 92
Injection date 04/19/2023 12:22:24 **Injection volume [µl]** 0.200
Acq. method S1419_ET_AAA_SIM_B.amx **Acq. operator** Verena Pohl (verena)
Analysis method ET_AAA.pmx

Signal: MS1Front SIM(226) EI

RT [min]	Type	Width [min]	Area	Height	Area% Name
27.072	MM m	0.11	63.9	31.1	0.25 D Glu
27.318	MM m	0.13	25337.8	15858.9	99.75 L Glu



E-Signature

Level	Level Name	Signed By	Date	Comment	Meaning
1	Approver	Verena Pohl (verena)	2023-04-20 11:12:24+02:00		Approved

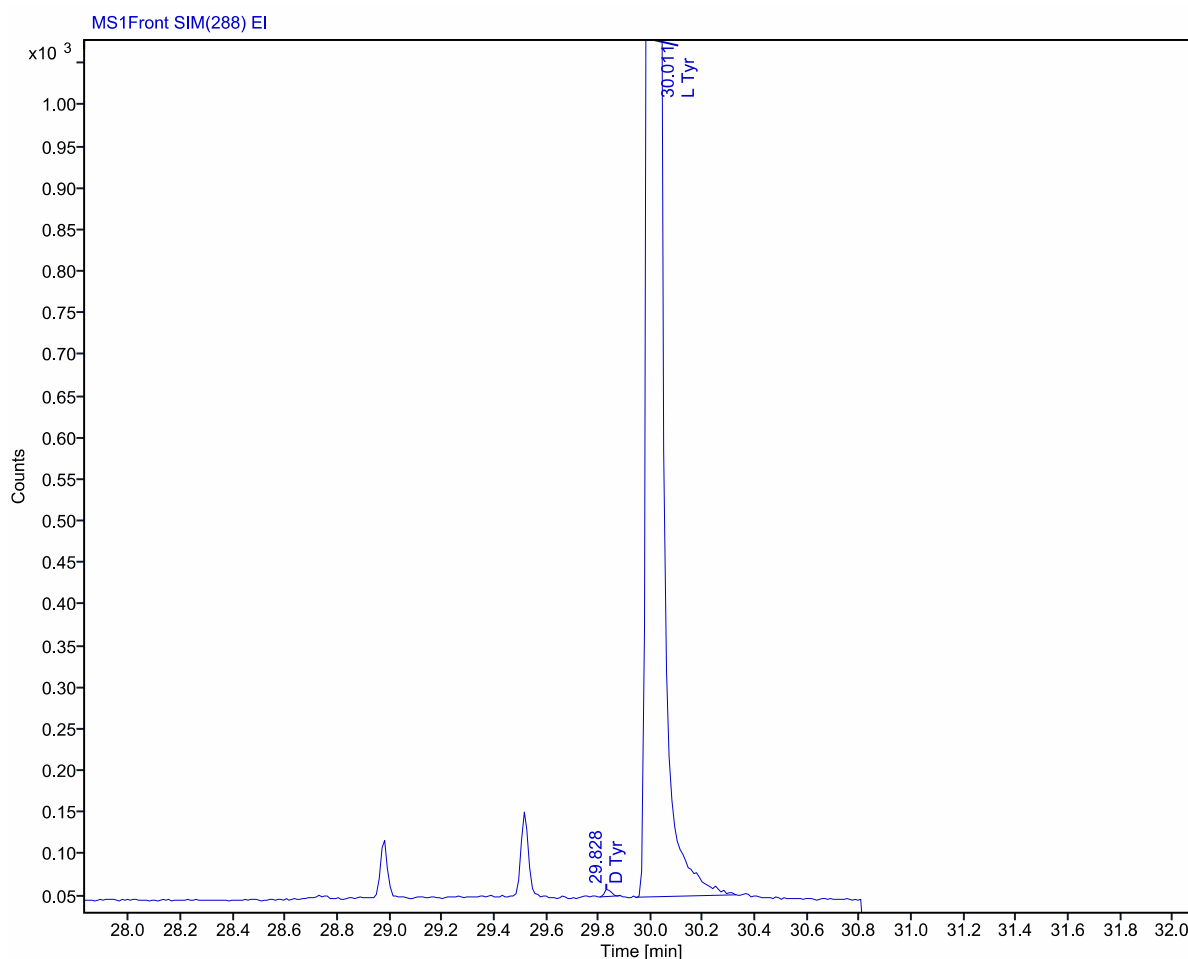
Chromatogram Report



Data file /HP9/Results/2023_04/XY020_1.rsl\XY020-02-01_1EVY1.dx
Sample name Lira-25 mmol-no wash-purified
Instrument HP#9 **Location** 92
Injection date 04/19/2023 12:22:24 **Injection volume [µl]** 0.200
Acq. method S1419_ET_AAA_SIM_B.amx **Acq. operator** Verena Pohl (verena)
Analysis method ET_AAA.pmx

Signal: MS1Front SIM(288) EI

RT [min]	Type	Width [min]	Area	Height	Area% Name
29.828	MM m	0.08	14.9	8.5	0.07 D Tyr
30.011	MM m	0.39	21385.3	9850.0	99.93 L Tyr



E-Signature

Level	Level Name	Signed By	Date	Comment	Meaning
1	Approver	Verena Pohl (verena)	2023-04-20 11:12:24+02:00		Approved

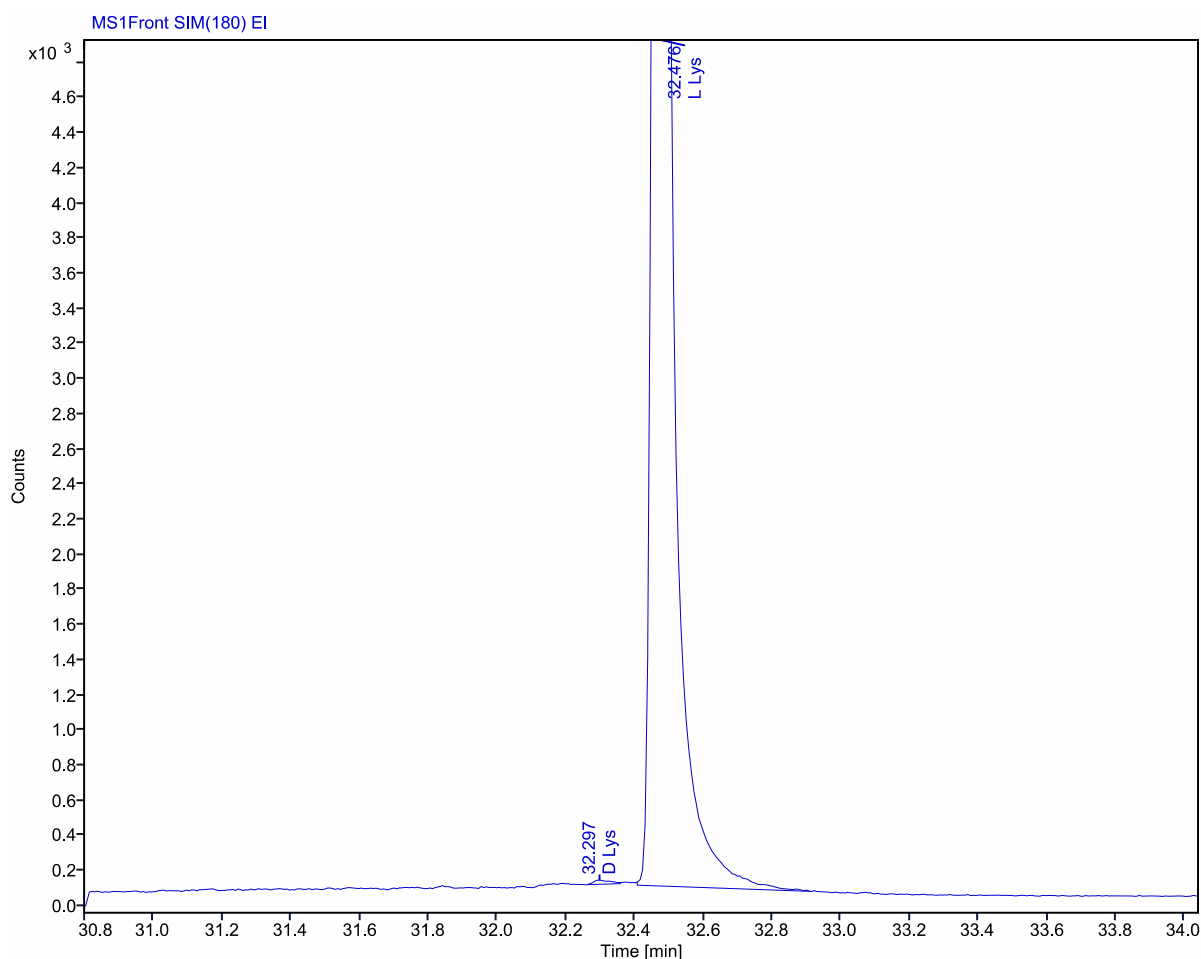
Chromatogram Report



Data file /HP9/Results/2023_04/XY020_1.rsl\XY020-02-01_1EVY1.dx
Sample name Lira-25 mmol-no wash-purified
Instrument HP#9 **Location** 92
Injection date 04/19/2023 12:22:24 **Injection volume [µl]** 0.200
Acq. method S1419_ET_AAA_SIM_B.amx **Acq. operator** Verena Pohl (verena)
Analysis method ET_AAA.pmx

Signal: MS1Front SIM(180) EI

RT [min]	Type	Width [min]	Area	Height	Area% Name
32.297	MM m	0.10	77.4	22.9	0.13 D Lys
32.476	MM m	0.50	60475.5	22431.5	99.87 L Lys



E-Signature

Level	Level Name	Signed By	Date	Comment	Meaning
1	Approver	Verena Pohl (verena)	2023-04-20 11:12:24+02:00		Approved

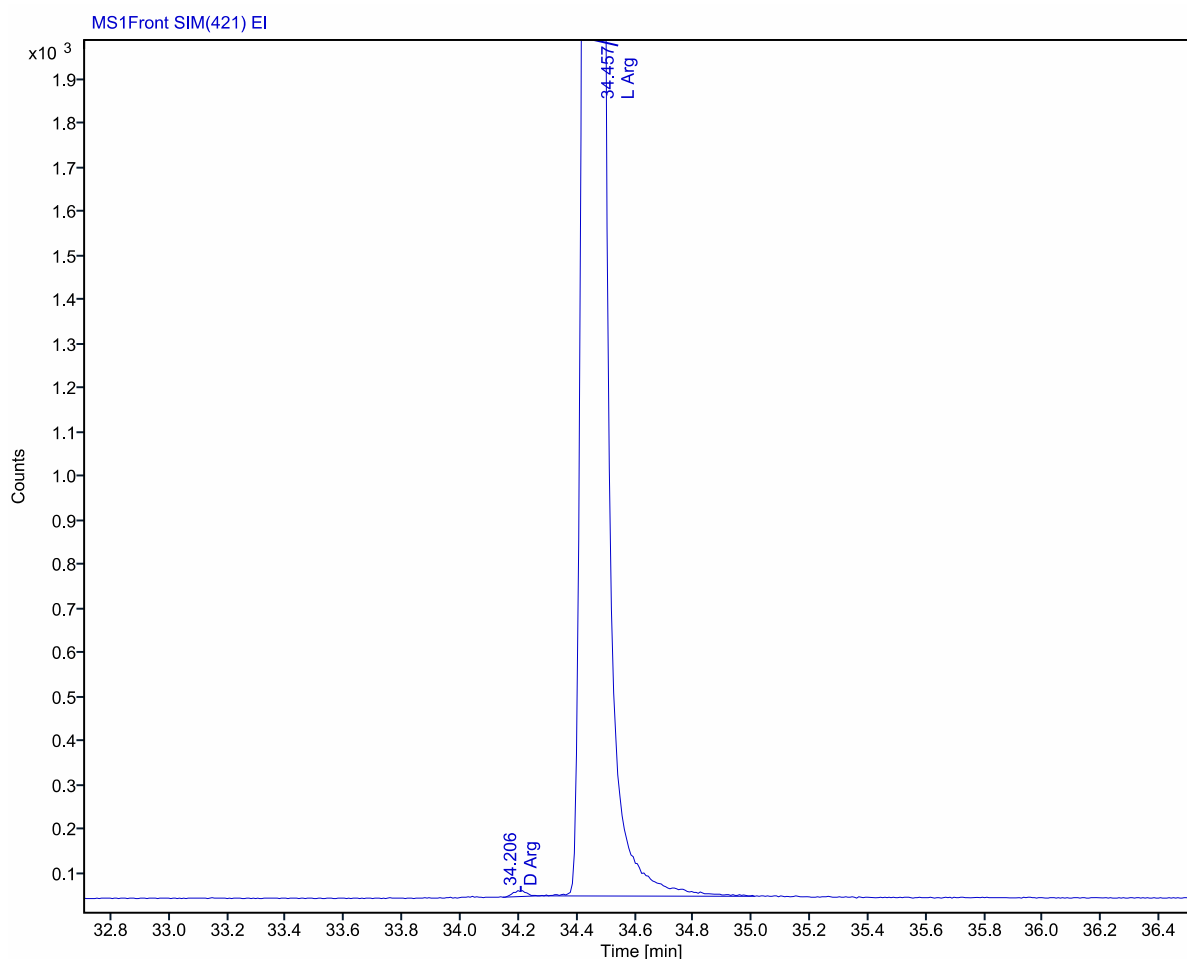
Chromatogram Report



Data file /HP9/Results/2023_04/XY020_2.rsl\XY020-02-01_1A1.dx
Sample name Lira-25 mmol-no wash-purified
Instrument HP#9 **Location** 94
Injection date 04/19/2023 13:57:49 **Injection volume [µl]** 0.200
Acq. method S1419_ET_AAA_SIM_B.amx **Acq. operator** Verena Pohl (verena)
Analysis method ET_AAA.pmx

Signal: MS1Front SIM(421) EI

RT [min]	Type	Width [min]	Area	Height	Area% Name
34.206	MM m	0.12	42.5	11.4	0.10 D Arg
34.457	BBA	0.75	40578.5	10879.9	99.90 L Arg



E-Signature

Level	Level Name	Signed By	Date	Comment	Meaning
1	Approver	Verena Pohl (verena)	2023-04-20 11:02:57+02:00		Approved

Chromatogram Report

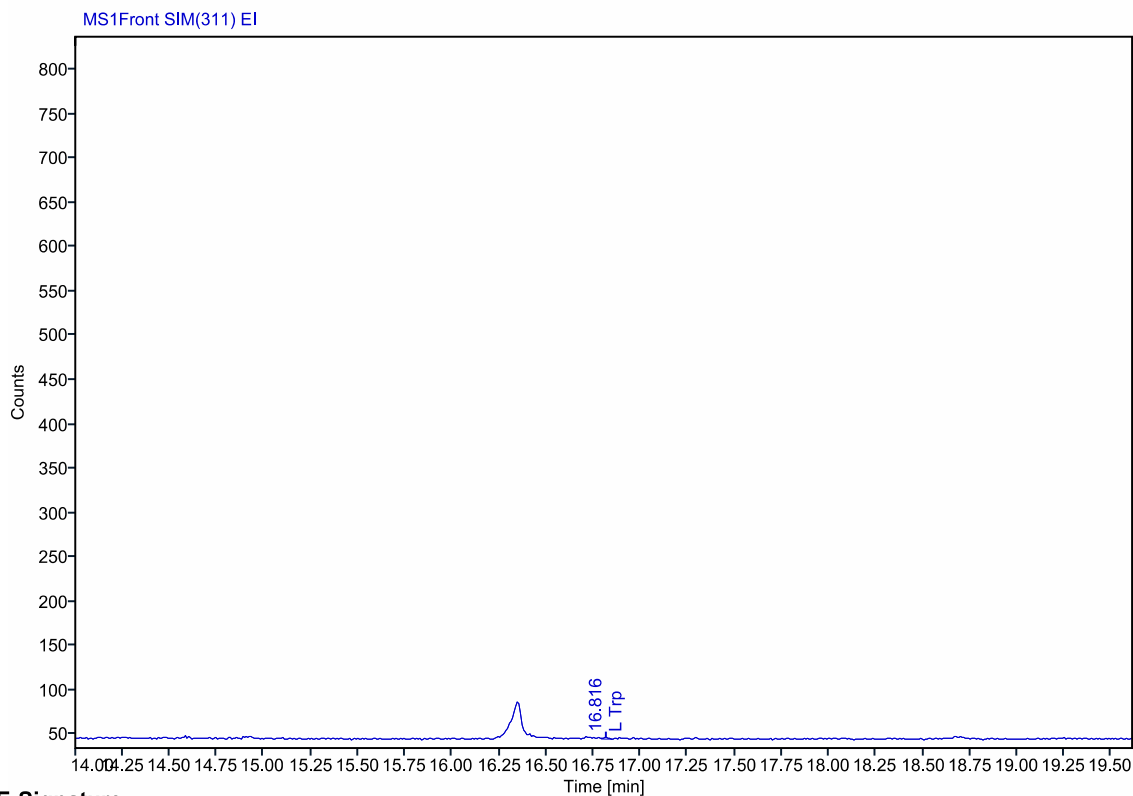


Data file /HP9/Results/2023_04/XY020_3.rsl\XY020-02-01_1EVY2.dx
Sample name Lira-25 mmol-no wash-purified
Instrument HP#9 **Location** 92
Injection date 04/19/2023 23:29:10 **Injection volume [µl]** 1.000
Acq. method S1419_ET_AAA_WRH_A.amx **Acq. operator** Verena Pohl (verena)
Analysis method ET_WRH.pmx

Signal: MS1Front SIM(311) EI

RT [min]	Type	Width [min]	Area	Height	Area%	Name
16.816	MM m	0.06	3.9	2.0	100.00	L Trp

M-1 contribution of 0.17% must be subtracted



E-Signature

Level	Level Name	Signed By	Date	Comment	Meaning
1	Approver	Verena Pohl (verena)	2023-04-20 09:04:51+02:00		Approved

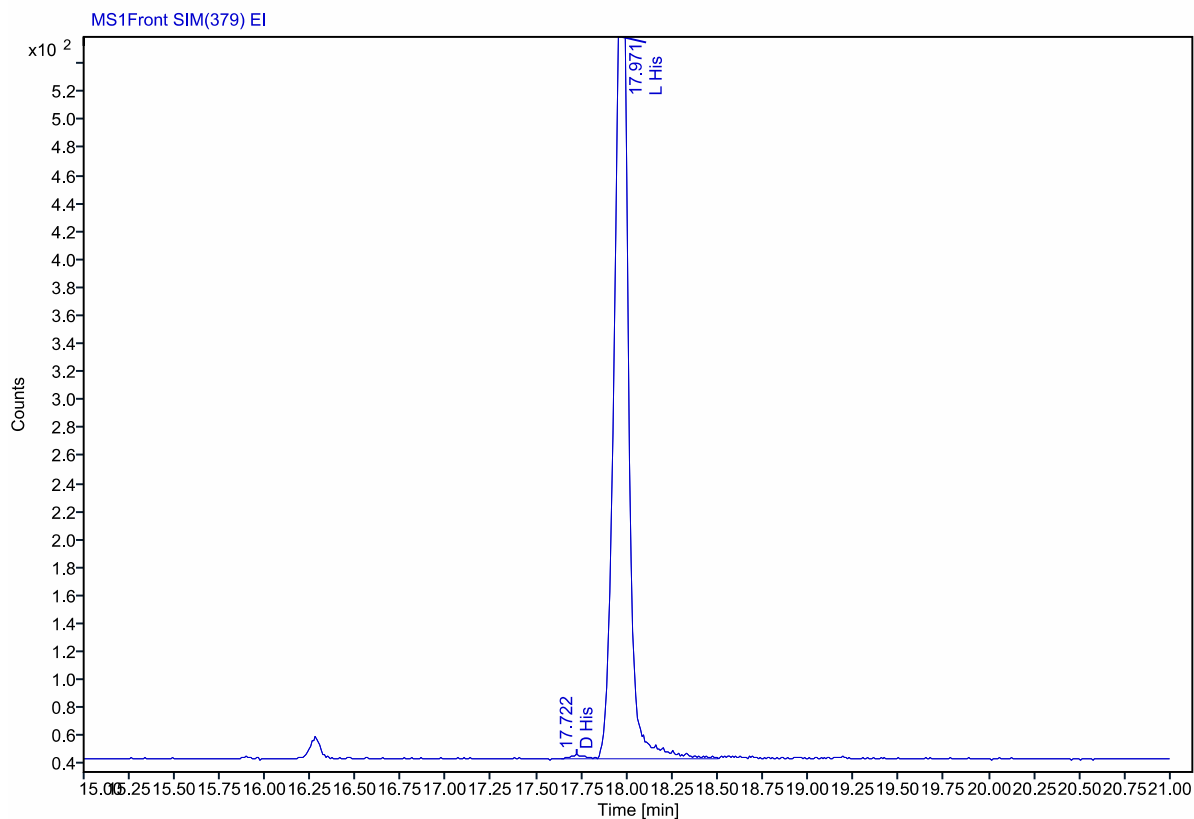
Chromatogram Report



Data file /HP9/Results/2023_04/XY020_5.rslt\XY020-02-01_1A3.dx
Sample name Lira-25 mmol-no wash-purified
Instrument HP#9 **Location** 94
Injection date 04/20/2023 11:32:42 **Injection volume [µl]** 0.200
Acq. method S1419_ET_AAA_WRH_A.amx **Acq. operator** Verena Pohl (verena)
Analysis method *ET_WRH.pmx

Signal: MS1Front SIM(379) EI

RT [min]	Type	Width [min]	Area	Height	Area%	Name
17.722	MM m	0.17	15.2	3.1	0.47	D His
17.971	MM m	0.69	3207.2	615.0	99.53	L His



E-Signature

Level	Level Name	Signed By	Date	Comment	Meaning
1	Approver	Verena Pohl (verena)	2023-04-20 13:48:53+02:00		Approved

Supplementary Table 3 Stepwise method details for wash-free 0.1 mmol research scale synthesis.

Wash-free method for 0.1 mmol synthesis (using one-pot methodology)	
Cycle steps	Operations
1. Resin swelling	1.1. Load 0.1 mmol resin in 30 mL reaction vessel 1.2. Add DMF for resin swelling: 4 mL 1.3. Start bubbling (from bottom of reaction vessel) with N ₂ 1.4. Start resin swelling method: 5 min/RT followed by drain to remove DMF
2. First Deprotection	2.1. Add DMF: 3.5 mL 2.2. Add deprotection reagent: 0.75 mL (17% pyrrolidine in DMF) Total deprotection volume in reaction vessel = 4.25 mL 2.3. Start deprotection microwave method: 80 sec/110 °C 2.4. Start bubbling (from bottom of reaction vessel) with N ₂ 2.5. Start headspace flushing (from top of reaction vessel) with N ₂ flow rate at 3.5 L/min
3. Drain	3.1. Drain all leftover reagents and byproducts. <i>Deprotected peptide resin is left behind in the reaction vessel.</i>
4. Coupling	4.1. Add reagents for coupling in the following order: Amino acid: 1 mL (0.5 M in DMF) DIC: 1 mL (0.75 M in DMF) Oxyma: 1.5 mL (0.26 M in DMF) Total coupling volume in reaction vessel = 3.5 mL 4.2. Start coupling microwave method: 30 sec/RT + 60 sec/105 °C 4.3. Start bubbling (from bottom of reaction vessel) with N ₂
5. Deprotection	5.1. Add deprotection reagent: 0.75 mL (17% pyrrolidine in DMF) to the undrained post-coupling mixture in step 4 Total deprotection volume in reaction vessel = 4.25 mL 5.2. Start deprotection microwave method: 80 sec/110 °C 5.3. Start bubbling (from bottom of reaction vessel) with N ₂ 5.4. Start headspace flushing (from top of reaction vessel) with N ₂ flow rate at 3.5 L/min
6. Drain	6.1. Drain all leftover reagents and byproducts. <i>Deprotected peptide resin is left behind in the reaction vessel.</i>
Repeat steps 4-6 for all remaining Fmoc-amino acid residues in the sequence	
7. Final deprotection	7.1. Repeat step 5 To remove Fmoc protection from the final amino acid residue in the sequence

At the end of final Fmoc deprotection, the peptide resin is collected from the reaction vessel for cleavage and analysis.

Supplementary Table 4 Stepwise method details for wash-free 25 mmol production scale synthesis.

Wash-free method for 25 mmol synthesis	
Cycle steps	Operations
1. Resin swelling	1.1. Load 25 mmol resin in 3 L reaction vessel 1.2. Add DMF for resin swelling: 500 mL 1.3. Start stirring and bubbling (from bottom of reaction vessel) with N ₂ flow rate at 10 L/min 1.4. Start resin swelling method: 10 min/RT followed by drain to remove DMF
2. Deprotection	2.1. Add deprotection reagent: 50 mL (15% pyrrolidine in DMF) 2.2. Add DMF to clean reagent line with purge: 250 mL Total deprotection volume in reaction vessel = 475 mL 2.3. Start deprotection microwave method: 10 min/90 °C 2.4. Start stirring and bubbling (from bottom of reaction vessel) with N ₂ flow rate at 10 L/min 2.5. Start headspace flushing (from top of reaction vessel) with N ₂ flow rate at 85 L/min
3. Drain	3.1. Drain all leftover reagents and byproducts. <i>Deprotected peptide resin is left behind in the reaction vessel.</i>
4. Coupling	4.1. Add reagents for coupling in the following order: Amino acid: 200 mL (0.5 M in DMF) DIC: 50 mL (4 M in DMF) Oxyma: 225 mL (0.33 M in DMF) Total coupling volume in reaction vessel = 475 mL 4.2. Start coupling microwave method: 5 min/80 °C 4.3. Start stirring and bubbling (from bottom of reaction vessel) with N ₂ flow rate at 10 L/min
5. Drain	5.1. Drain all leftover reagents and byproducts. <i>Fmoc-protected peptide resin is left behind in the reaction vessel.</i>
Repeat steps 2-5 for all remaining Fmoc-amino acid residues in the sequence	
6. Final deprotection	6.1. Repeat step 2 To remove Fmoc protection from the final amino acid residue in the sequence

At the end of final Fmoc deprotection, the peptide resin is collected from the reaction vessel for cleavage and analysis.

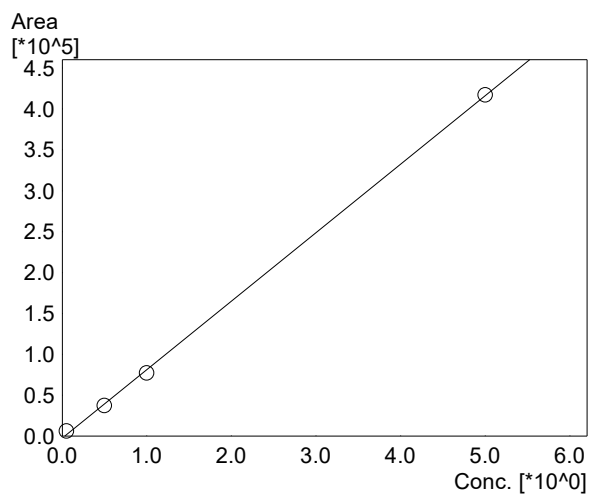
Supplementary Table 5 Stepwise method details for residual pyrrolidine quantification by GC-FID.

Residual pyrrolidine experiment	
Cycle Steps	Operation
1. Resin Swelling and loading	1.1 Resin (0.1 mmol) was preswelled in DMF (8 mL) for 10 min and then added to a clean and dry reaction vessel. 1.2 The reaction vessel was drained, and washed with 10 mL DMF
2. First Deprotection	2.1 Add DMF: 3.5 mL 2.2 Add deprotection reagent: 0.75 mL (17% pyrrolidine in DMF). Total deprotection volume in the reaction vessel = 4.25 mL 2.3 Start deprotection microwave method: 80 sec/110 °C 2.4 Start bubbling (from bottom of reaction vessel) with N ₂ 2.5 Start headspace flushing (from top of reaction vessel) with N ₂ flow rate at 3.5 L/min
3. Drain	Drain all reagents through frit using N ₂ pressure from top of vessel. <i>Deprotected peptide resin is left behind in the reaction vessel.</i>
4. Coupling	4.1 Add reagents for coupling in the following order: Amino acid: 1 mL (0.5 M in DMF), DIC: 1 mL (0.75 M in DMF), and Oxyma: 1.5 mL (0.26 M in DMF). Total coupling volume = 3.5 mL 4.2 Start coupling microwave method: 30 sec/RT + 60 sec/105 °C 4.3 Start bubbling (from bottom of reaction vessel) with N ₂
5. Deprotection	5.1 Add deprotection reagent: 0.75 mL (17% pyrrolidine in DMF) to the undrained post-coupling mixture in step 4. Total deprotection volume in the reaction vessel = 4.25 mL 5.2 Start deprotection microwave method: 80 sec/110 °C 5.3 Start bubbling (from bottom of reaction vessel) with N ₂ 5.4 Start headspace flushing (from top of reaction vessel) with N ₂ flow rate at 3.5 L/min
6. Drain	6.1 Drain all reagents through frit using N ₂ pressure from top of vessel. <i>Deprotected peptide resin is left behind in the reaction vessel.</i>
7. Repeat steps 4–6 twice more	7.1 By the end of step 7, the method will have completed 1 first deprotection, 3 regular deprotections, and 3 couplings; leaving the reaction vessel in a drained post-deprotection state.
8. Add DMF and collect sample for GC- analysis	8.1 After drain is complete, add 3.5 mL of DMF (equivalent volume to coupling mixture) to the resin in the reaction vessel

	8.2 Remove reaction vessel from microwave cavity and allow temperature to cool from 56°C to 25°C 8.3 Open reaction vessel and pipette 100 μL of the solution to a vial while avoiding the uptake of resin beads. 8.4 The aliquot was diluted 100-fold by serial dilution with HPLC-grade IPA (1:10, repeated twice) and then inject into the GC-FID for analysis
--	--

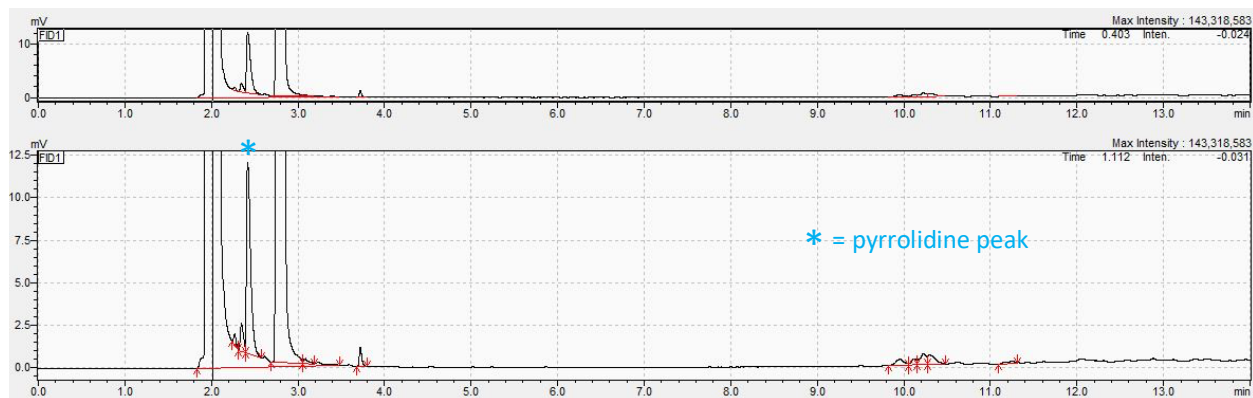
==== Shimadzu LabSolutions Calibration Curve ====

ID# : 1
 Name : Pyrrolidine
 Quantitative Method : External Standard
 Function : $f(x)=83769.2 \cdot x - 2634.55$
 Rr1=0.9997946 Rr2=0.9995893 RSS=4.476877e+007
 MeanRF: 9.129169e+004 RFSD: 2.597406e+004 RFRSD: 28.451719
 FitType : Linear
 ZeroThrough : Not Through
 Weighted Regression : None
 Detector Name : FID1



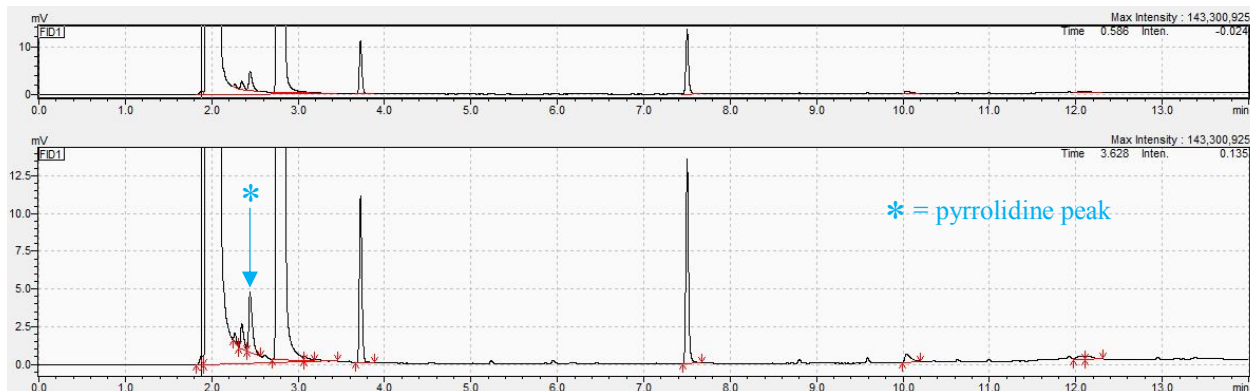
#	Conc.(Ratio)	MeanArea	Area
1	0.05	6493	6493
3	0.5	37366	37366
4	1	77141	77141
6	5	417149	417149

LabSolutions – Representative pyrrolidine standard, 0.5% pyrrolidine in DMF



Peak#	Ret. Time	Area	Height	Conc. (ppm)	Name
1	2.018	647922548	143053652		IPA
2	2.268	1147	608		
3	2.347	4203	1625		
4	2.42	37366	11221	4775	pyrrolidine
5	2.811	4561418	1476155		DMF
6	3.079	1114	280		
7	3.72	2053	1089		
8	9.954	2227	351		
9	10.116	1392	360		
10	10.228	3326	645		
11	10.301	3397	559		
12	11.245	1019	126		

LabSolutions – Real Sample #1, optimized conditions, 80 s deprotection, 10 s drain, headspace flushing ON



Peak#	Ret. Time	Area	Height	Conc. (ppm)	Name
1	1.885	1530	569		
2	2.019	651578198	142819863		IPA
3	2.269	1039	578		
4	2.348	4211	1647		
5	2.443	11436	3957	1680	pyrrolidine
6	2.812	4523719	1459906		DMF
7	3.08	1098	276		
8	3.722	22481	10964		
9	7.503	28507	13477		
10	10.046	2642	545		
11	12.046	1137	186		
12	12.153	1286	212		