

Additional file 1. HPLC data for *Bacillus megaterium* samples (the second strategy for the optimization)

Sample No.	Run	DCW ¹ g/l	CN ² Area	CN ³ µg/0.5 ml	CN ⁴ µg/l	Ad ⁵ Area	AD ⁶ µg/0.5 ml	Ad ⁷ µg/l	Total B12 ⁸ µg/l	B12 ⁹ µg/1g DCW
1	4	7.22	0.8113	1.736	8.681	3.0433	18.575	92.8731	101.554	14.065
2	17	7.78	0.1634	0.341	1.748	2.1577	13.169	65.8477	67.5961	8.688
3	7	13.33	5.2111	11.152	55.760	4.8725	29.739	148.695	204.456	15.338
4	13	6.94	0.9422	2.016	10.081	1.1239	6.859	34.2983	44.3801	6.394
5	8	12.5	2.6056	5.576	27.880	2.5268	15.422	77.111	104.992	8.399
6	5	14.44	0.4171	0.893	4.463	1.5532	9.479	47.3994	51.8625	3.591
7	15	11.53	1.6247	3.477	17.384	2.1359	13.036	65.1818	82.5665	7.1610
8	1	5	0.0252	0.0539	0.269	0.3537	2.159	10.7939	11.0636	2.2126
9	14	13.89	0.5146	1.101	5.506	0.8303	5.0677	25.3385	30.8448	2.220
10	12	15.83	1.6864	3.609	18.045	1.2644	7.717	38.5873	56.6323	3.577
11	6	7.22	0.4095	0.876	4.382	0.0419	0.256	1.27867	5.66044	0.783
12	11	11.11	0.78	1.669	8.346	0.9711	5.927	29.6354	37.9817	3.418
13	10	5.56	0.9593	2.052	10.265	0.3744	2.285	11.4258	21.6906	3.901
14	2	8.33	1.9975	4.275	21.373	0.1186	0.723	3.61934	24.9932	3.000
15	3	8.33	0.7795	1.668	8.341	0.8919	5.444	27.2183	35.5592	4.268
16	16	8.89	0.6372	1.36	6.818	0.3946	2.408	12.0416	18.8598	2.121
17	9	5.55	0.8024	1.717	8.586	0.6321	3.858	19.2898	27.8757	5.023

¹Dry cell weight (DCW).

²The peak area of the sample during the retention time of the cyanocobalamin.

³The concentration of the cyanocobalamin (µg) in the concentrated sample from 100 ml culture to 0.5 ml = peak area × 100 (standard concentration) / 46.7277 (the peak area for the standard).

⁴The concentration of the cyanocobalamin in the sample (µg) per liter = the concentration of cyanocobalamin per 0.5 ml (CN³) × 0.5 × 10.

⁵The peak area of the sample during the retention time of the adenosylcobalamin.

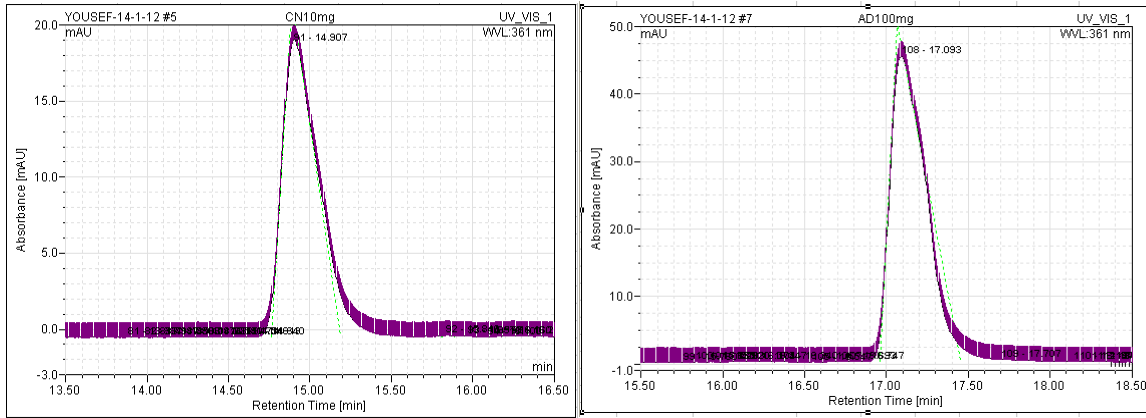
⁶The concentration of the adenosylcobalamin (µg) in the concentrated sample from 100 ml culture to 0.5 ml = peak area × 70.8 (standard concentration) / 11.6 (the peak area for the standard).

⁷The concentration of the adenosylcobalamin in the sample (µg) per liter = the concentration of adenosylcobalamin per 0.5 ml (Ad⁶) × 0.5 × 10.

⁸Total concentration of vitamin B12 (cyanocobalamin & adenosylcobalamin).

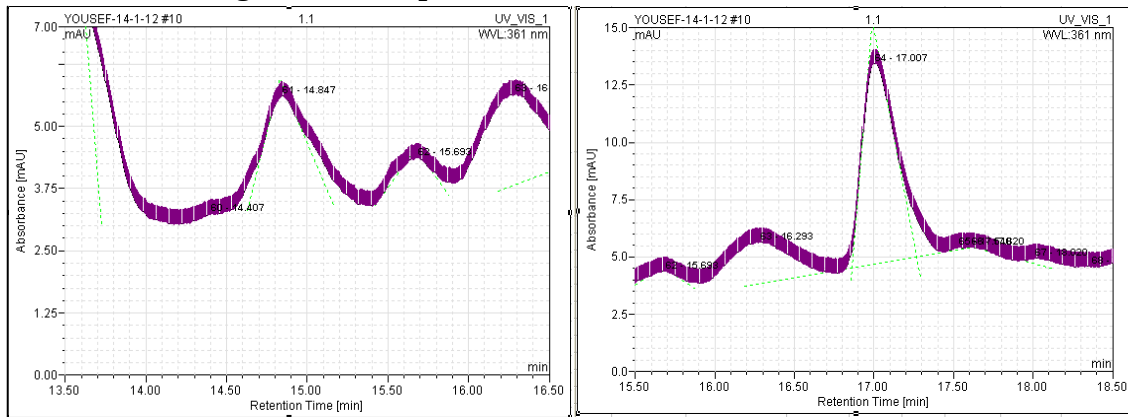
⁹Concentration of vitamin B12 per gram dry cell weight (µg/1g DCW) = Total vitamin B12 (B12⁷) / Dry cell weight (DCW¹).

1. HPLC chromatograms of standards (cyanocobalamin (CN) and adenosylcobalamin)(Ad)



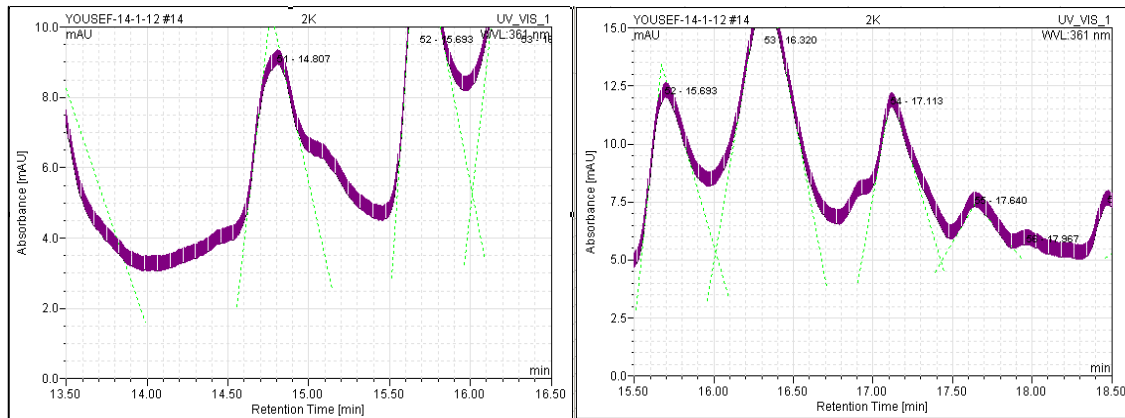
Sample	CN Retention time	CN Area	CN $\mu\text{g/L}$	Ad Retention time	Ad Area	Ad $\mu\text{g/L}$	Total B12 $\mu\text{g/L}$
CN 10mg; 100mg	14.907	5.118; 46.73	--	--	--	--	--
Ad 70.8mg	--	--	--	17.093	11.646	--	--

2. HPLC chromatograms of sample No. 1



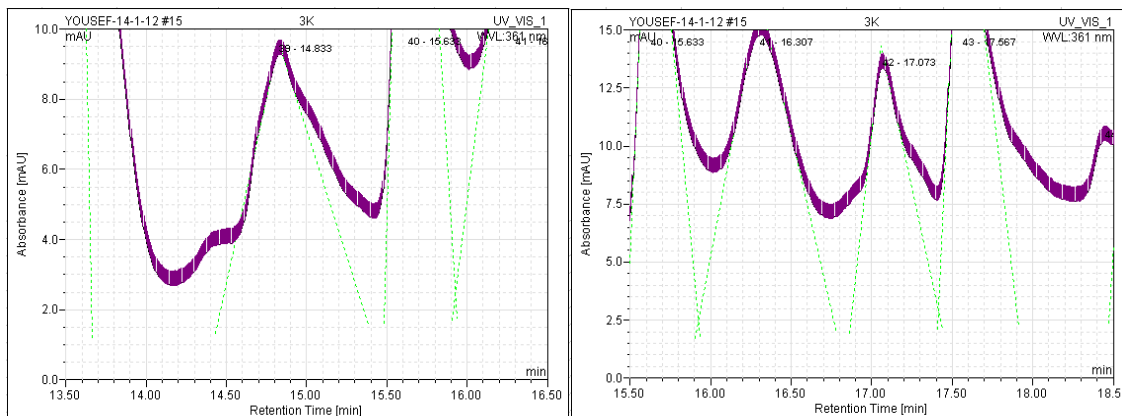
CN Retention time	CN Area	CN $\mu\text{g/L}$	Ad Retention time	Ad Area	Ad $\mu\text{g/L}$	Total B12 $\mu\text{g/L}$
14.847	0.8113	8.68	17.007	3.0433	92.87312	101.5543

3. HPLC chromatograms of sample No. 2



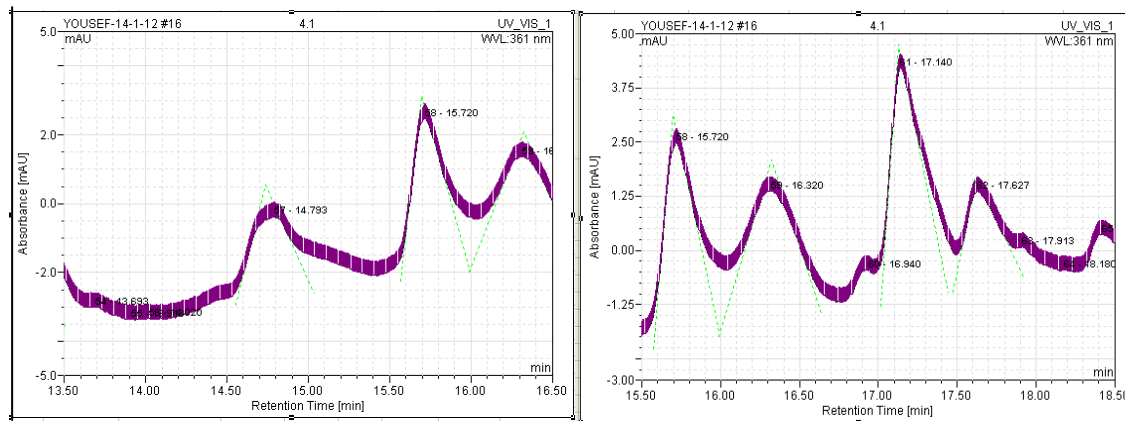
CN Retention time	CN Area	CN $\mu\text{g/L}$	Ad Retention time	Ad Area	Ad $\mu\text{g/L}$	Total B12 $\mu\text{g/L}$
14.807	0.1634	1.7484	17.113	2.1577	65.85	67.596

4. HPLC chromatograms of sample No. 3



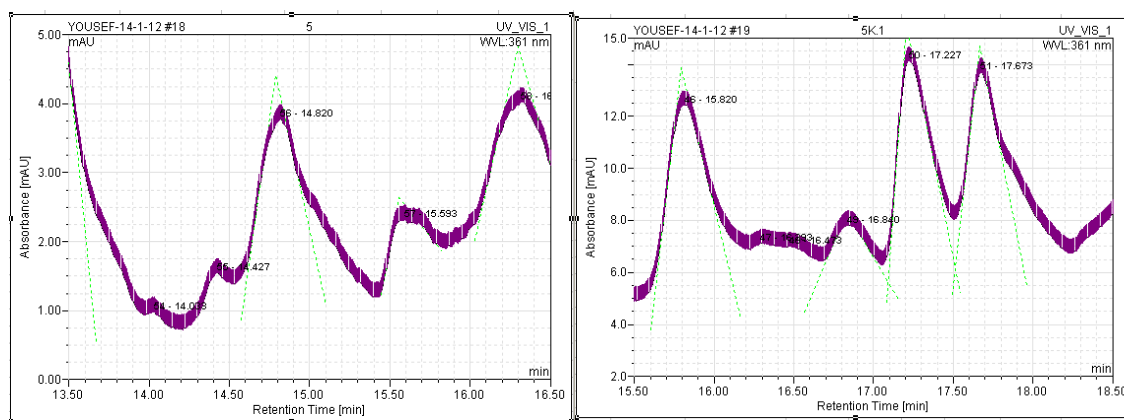
CN Retention time	CN Area	CN $\mu\text{g/L}$	Ad Retention time	Ad Area	Ad $\mu\text{g/L}$	Total B12 $\mu\text{g/L}$
14.833	5.2111	55.76	17.073	4.872	148.69	204.4555

5. HPLC chromatograms of sample No. 4



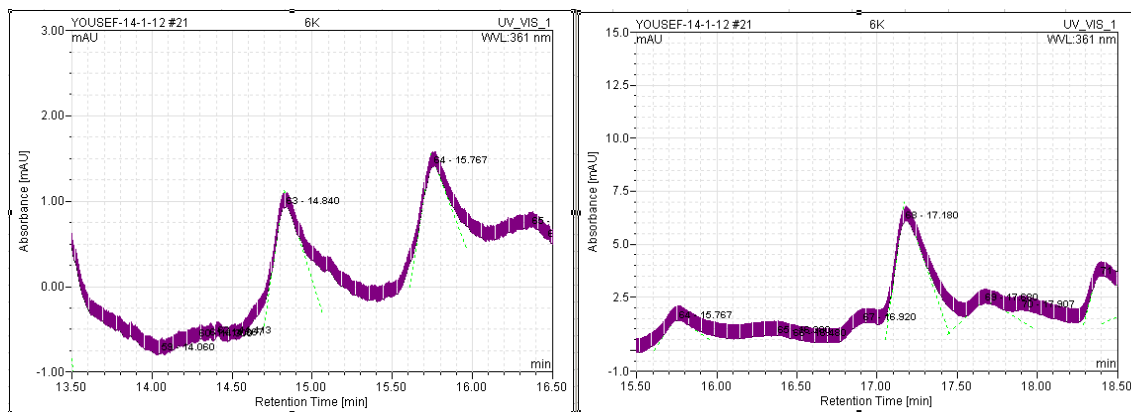
CN Retention time	CN Area	CN $\mu\text{g/L}$	Ad Retention time	Ad Area	Ad $\mu\text{g/L}$	Total B12 $\mu\text{g/L}$
14.793	0.9422	10.08181	17.140	1.1239	34.298	44.38

6. HPLC chromatograms of sample No. 5



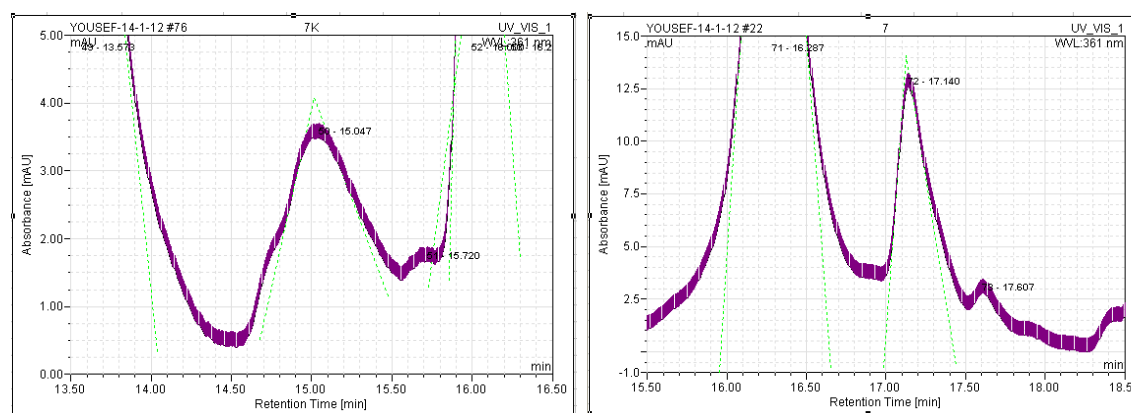
CN Retention time	CN Area	CN $\mu\text{g/L}$	Ad Retention time	Ad Area	Ad $\mu\text{g/L}$	Total B12 $\mu\text{g/L}$
14.820	2.6056	27.88	17.227	2.5268	77.11	104.99

7. HPLC chromatograms of sample No. 6



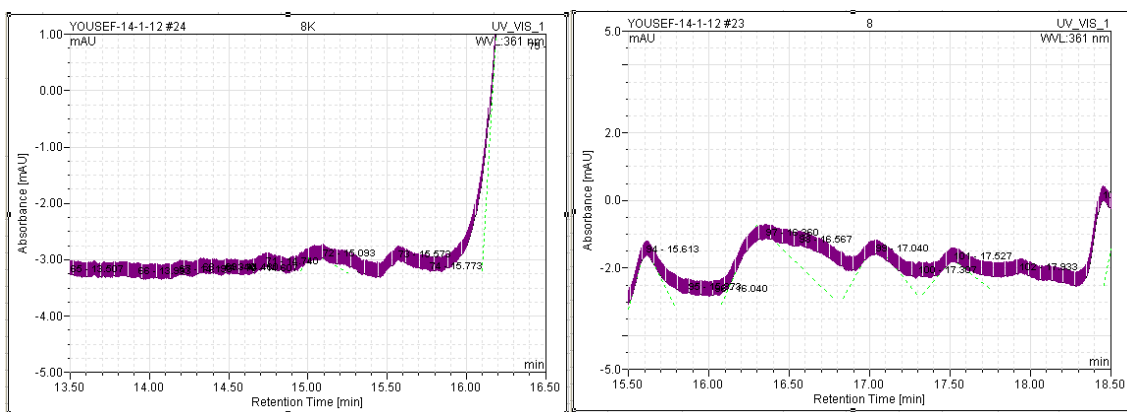
CN Retention time	CN Area	CN μL	Ad Retention time	Ad Area	Ad $\mu\text{g/L}$	Total B12 $\mu\text{g/L}$
14.840	0.4171	4.463	17.180	1.553	47.399	51.862

8. HPLC chromatograms of sample No. 7



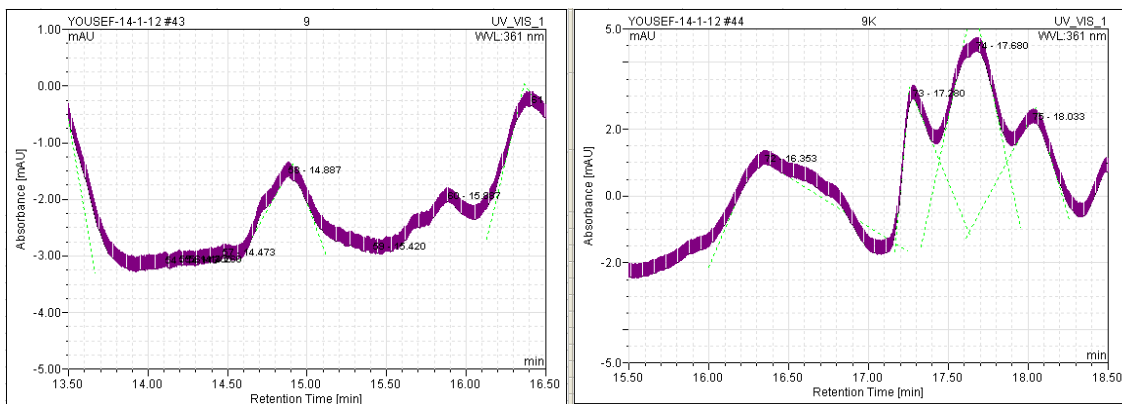
CN Retention time	CN Area	CN μL	Ad Retention time	Ad Area	Ad $\mu\text{g/L}$	Total B12 $\mu\text{g/L}$
15,047	1.6247	17.384	17.140	2.1359	65.181	82.566

9. HPLC chromatograms of sample No. 8



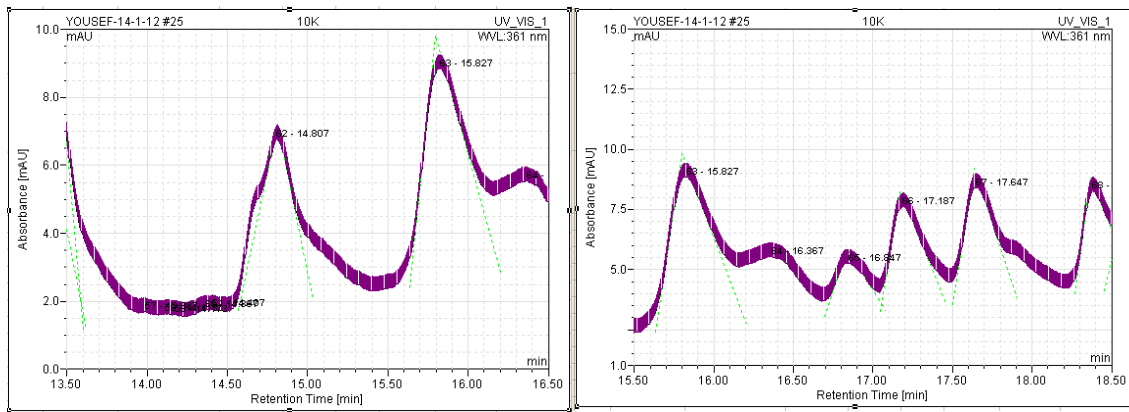
CN Retention time	CN Area	CN μL	Ad Retention time	Ad Area	Ad $\mu\text{g/L}$	Total B12 $\mu\text{g/L}$
15,040	0.0252	0.269	17.040	0.3537	10.79395	11.0636

10. HPLC chromatograms of sample No. 9



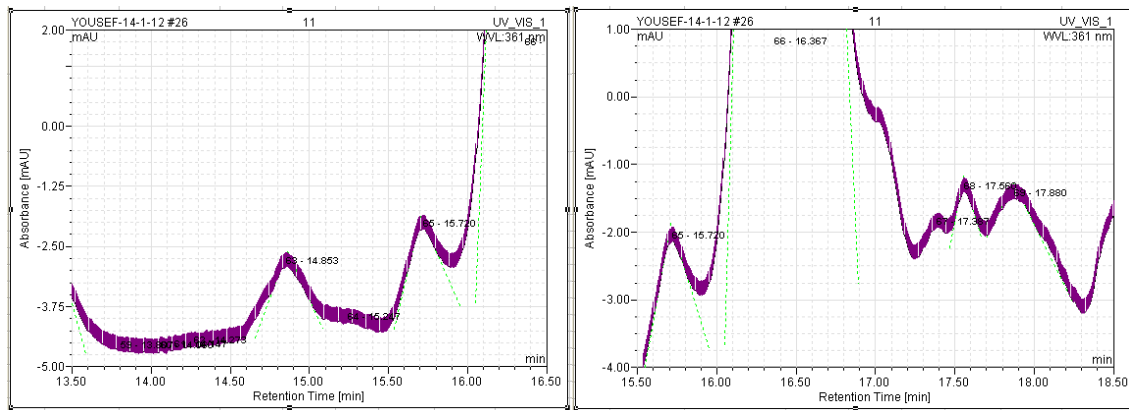
CN Retention time	CN Area	CN μL	Ad Retention time	Ad Area	Ad $\mu\text{g/L}$	Total B12 $\mu\text{g/L}$
14,887	0.5146	5.506	17.280	0.8303	25.338	30.844

11. HPLC chromatograms of sample No. 10



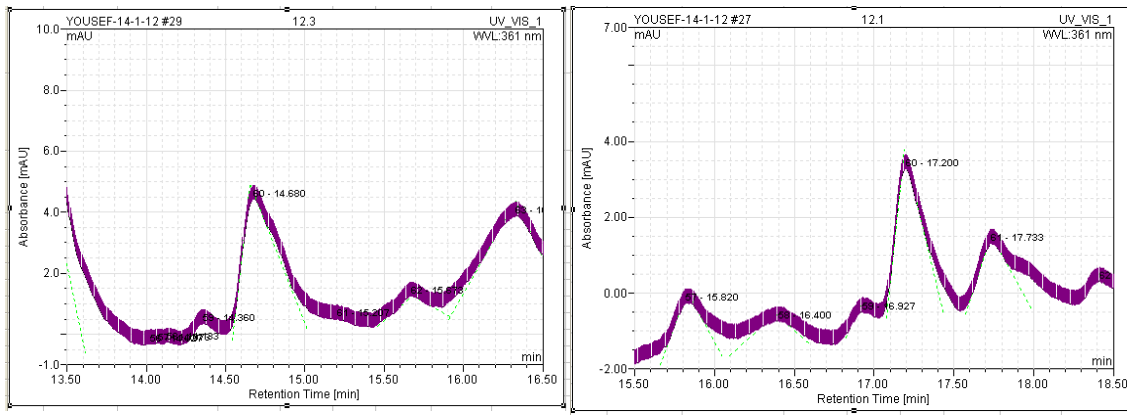
CN Retention time	CN Area	CN μL	Ad Retention time	Ad Area	Ad $\mu\text{g/L}$	Total B12 $\mu\text{g/L}$
14,807	1.6864	18.0449	17.187	1.2644	38.5872	56.63225

12. HPLC chromatograms of sample No. 11



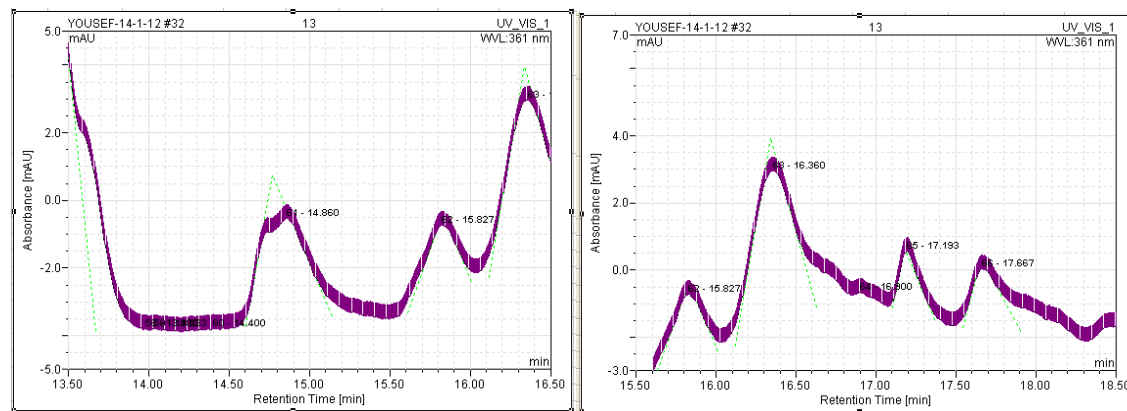
CN Retention time	CN Area	CN μL	Ad Retention time	Ad Area	Ad $\mu\text{g/L}$	Total B12 $\mu\text{g/L}$
14,853	0.4095	4.38176	17.387	0.0419	1.27867	5.660442

13. HPLC chromatograms of sample No. 12



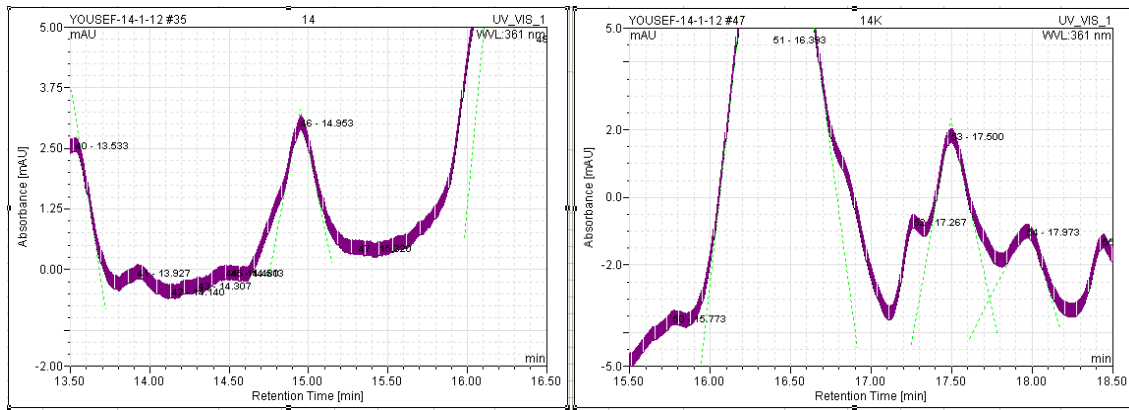
CN Retention time	CN Area	CN μL	Ad Retention time	Ad Area	Ad $\mu\text{g/L}$	Total B12 $\mu\text{g/L}$
14,680	0.78	8.3462	17.200	0.9711	29.6354	37.981

14. HPLC chromatograms of sample No. 13



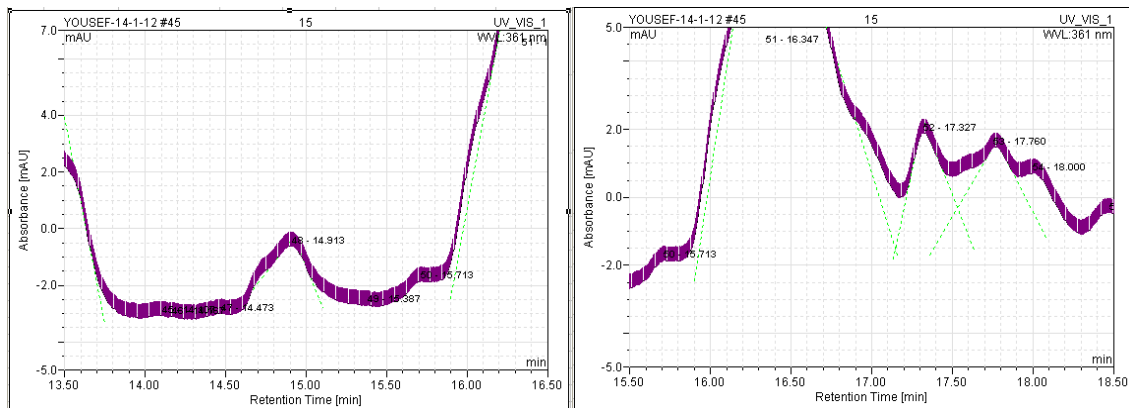
CN Retention time	CN Area	CN μL	Ad Retention time	Ad Area	Ad $\mu\text{g/L}$	Total B12 $\mu\text{g/L}$
14,860	0.9593	10.264	17.193	0.3744	11.4258	21.690

15. HPLC chromatograms of sample No. 14



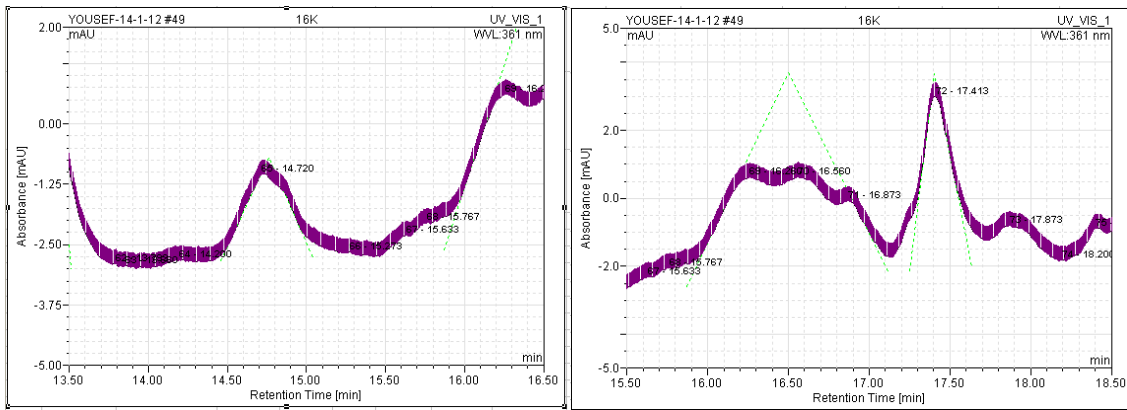
CN Retention time	CN Area	CN μL	Ad Retention time	Ad Area	Ad $\mu\text{g/L}$	Total B12 $\mu\text{g/L}$
14,953	1.9975	21.373	17.267	0.1186	3.61934	24.9931

16. HPLC chromatograms of sample No. 15



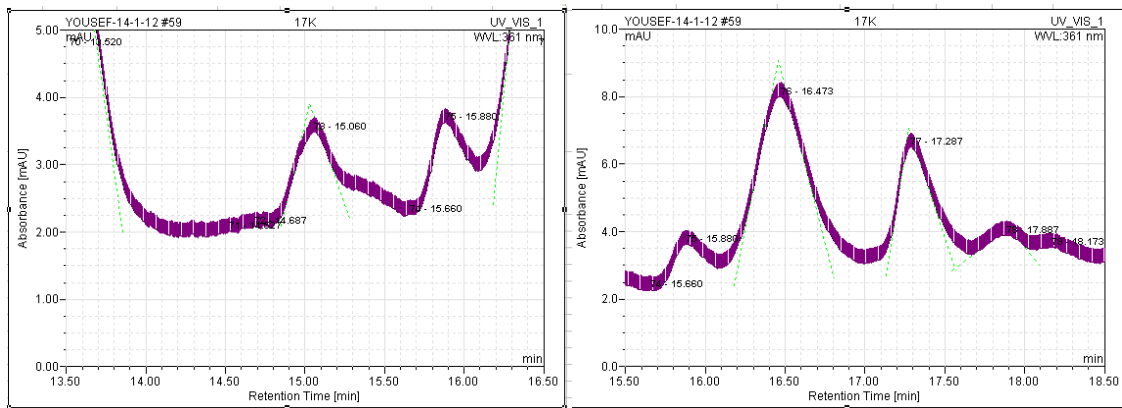
CN Retention time	CN Area	CN μL	Ad Retention time	Ad Area	Ad $\mu\text{g/L}$	Total B12 $\mu\text{g/L}$
14,913	0.7795	8.3408	17.327	0.8919	27.2183	35.5592

17. HPLC chromatograms of sample No. 16

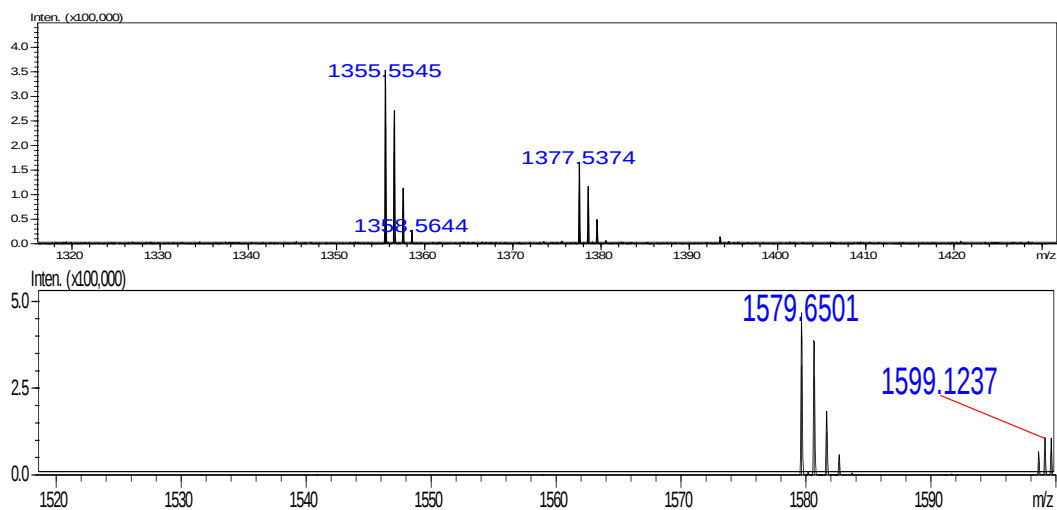


CN Retention time	CN Area	CN μL	Ad Retention time	Ad Area	Ad $\mu\text{g/L}$	Total B12 $\mu\text{g/L}$
14,720	0.6372	6.8182	17.413	0.3945	12.0416	18.69

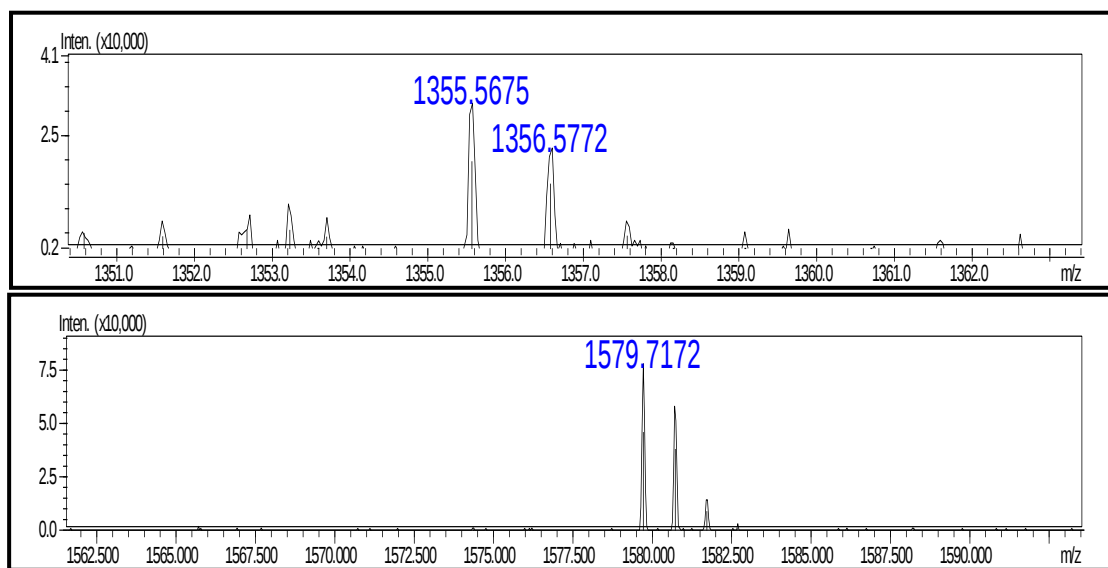
18. HPLC chromatograms of sample No. 17



CN Retention time	CN Area	CN μL	Ad Retention time	Ad Area	Ad $\mu\text{g/L}$	Total B12 $\mu\text{g/L}$
15.060	0.8024	8.585	17.287	0.63209	19.289	27.8756



Mass spectrometry spectrum of the cyanocobalamin standard (1355.5545) and adenosylcobalamin standard (1579.6501)



Mass spectrometry spectrum of the corrinoids purified from *B. megaterium*.