



Operating procedure

24 NOV 2006 Revision: 02

Method : CEofix CDT linearity

1. Purpose

The purpose of this test is to check the linearity and LOQ for analyzing 4-sialo Tf in an Apo-Transferrin sample with CEofix™ CDT kit.

2. Method

2.1. Material and

- CEofix™ CDT kit (Analisis 10-004760)
- Apo-Transferrin (Sigma T4382)
- TBS: Tris Saline Buffer (150 mM NaCl, 50 mM Tris adapted with HCl to pH 8)
- BSA: Bovine Serum Albumine (Sigma A4503)
- NaHCO₃ (Merck 6329)
- P/ACE MDQ with UV detector at 200 nm.
- General lab equipment.

Prepare:

- **TBS/BSA solution:** TBS with 20 mg/mL BSA.
- **Carb/Fe solution:** add 16 mg NaHCO₃ to 1 mL Fe solution (10-004765). This solution has to be prepared daily.

2.2. Sample preparation and analysis

Make a solution of 3 mg/mL of Apo-Transferrin in the TBS/BSA solution. Make dilutions to obtain 1.5, 0.75, 0.3, 0.15 and 0.075 mg/mL of Apo-Transferrin in TBS/BSA.

Dilute 50 µL of sample with 150 µL of Carb/Fe solution.

Follow kit instructions and analyze on P/ACE MDQ.

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3. Result

See addendum 1 and figure 1.

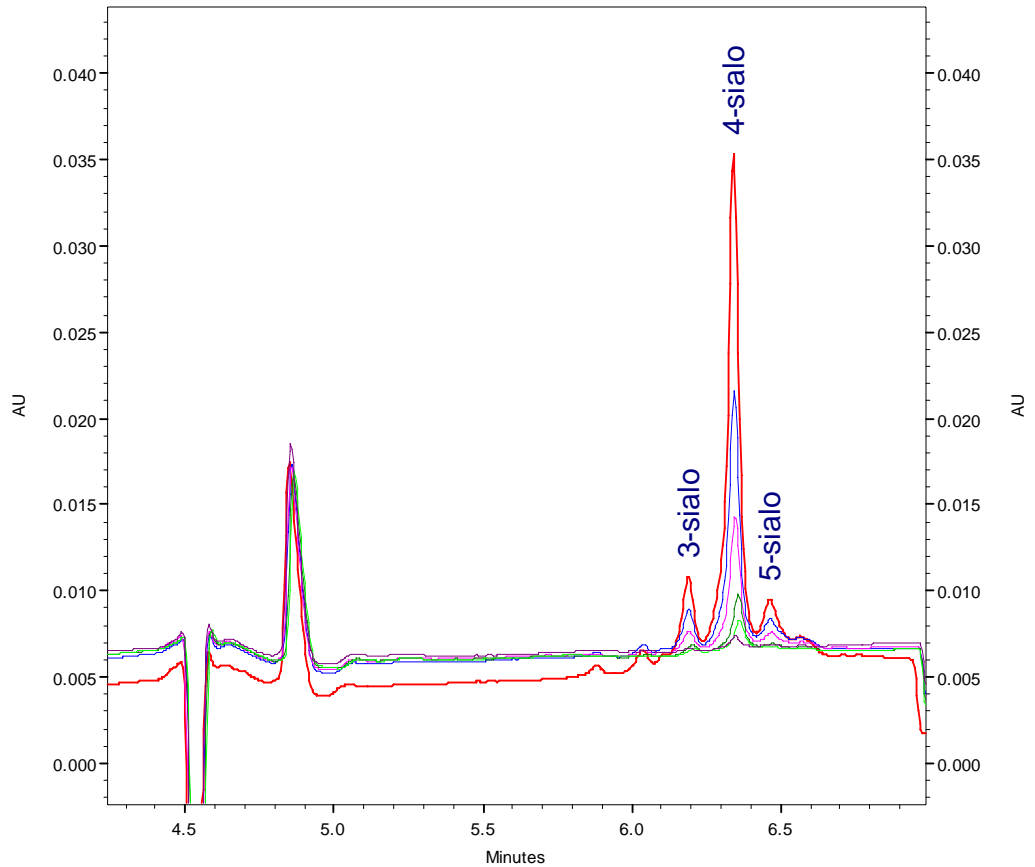


Fig. 1 Apo-Tf analyzed at 3, 1.5, 0.75, 0.3 and 0.075 mg/mL

Linearity see addendum 2.

4. Conclusion

Excellent linearity ($R^2=0.9999$) for 4-sialo Tf determination between 3 and 0.075 mg/mL of Apo Tf.

LOQ: %RSD 8.23 (N=5) at 0.075 mg/mL. LOD may be estimated at 1/3 or 0.025 mg/mL.

Remarks:

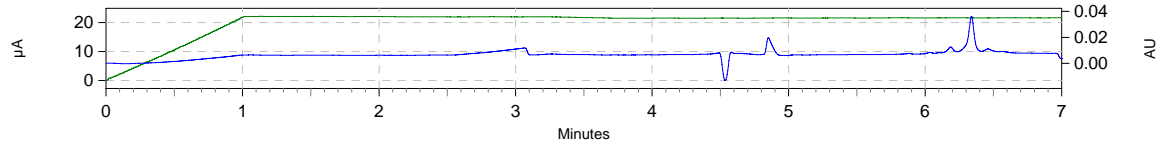
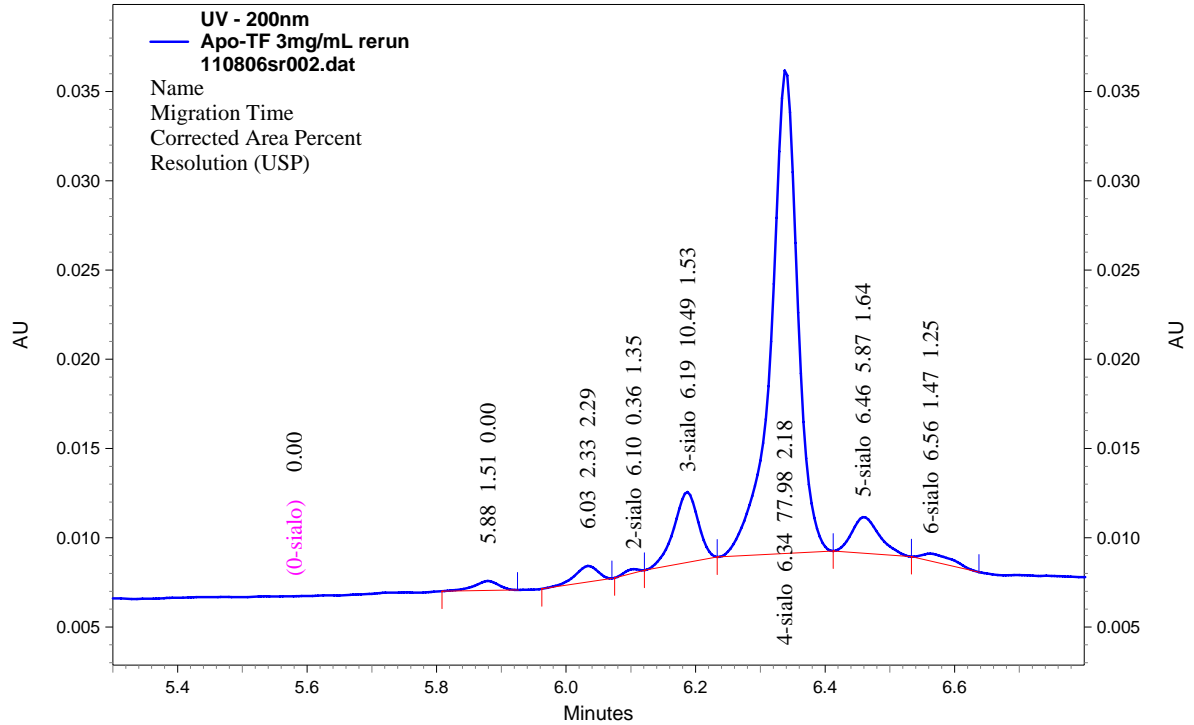
- The LOQ may be adapted by adapting the dilution of the sample in the Carb/Fe solution.
- This LOQ is not valid for an individual isoform in a sample. Here we can go down to a LOQ of 0.4% (RSD 12%) which represents an area of 325 and an estimated concentration <0.012 mg/mL.

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Addendum 1

Data file: D:\32Karat\Projects\CDT\linearity\110806sr002.dat
 Method: D:\32Karat\Projects\CDT\Method\CDT_meth_96well_plate.met
 Sample: Apo-TF 3mg/mL rerun
 Acquired: 08/11/2006 09:34:34
 Printed: 08/11/2006 14:10:15
 Sequence: D:\32Karat\Projects\carchon\sept2006\2.seq
 MDQ setup: cdt (Offline)

CDT - ANALIS R&D



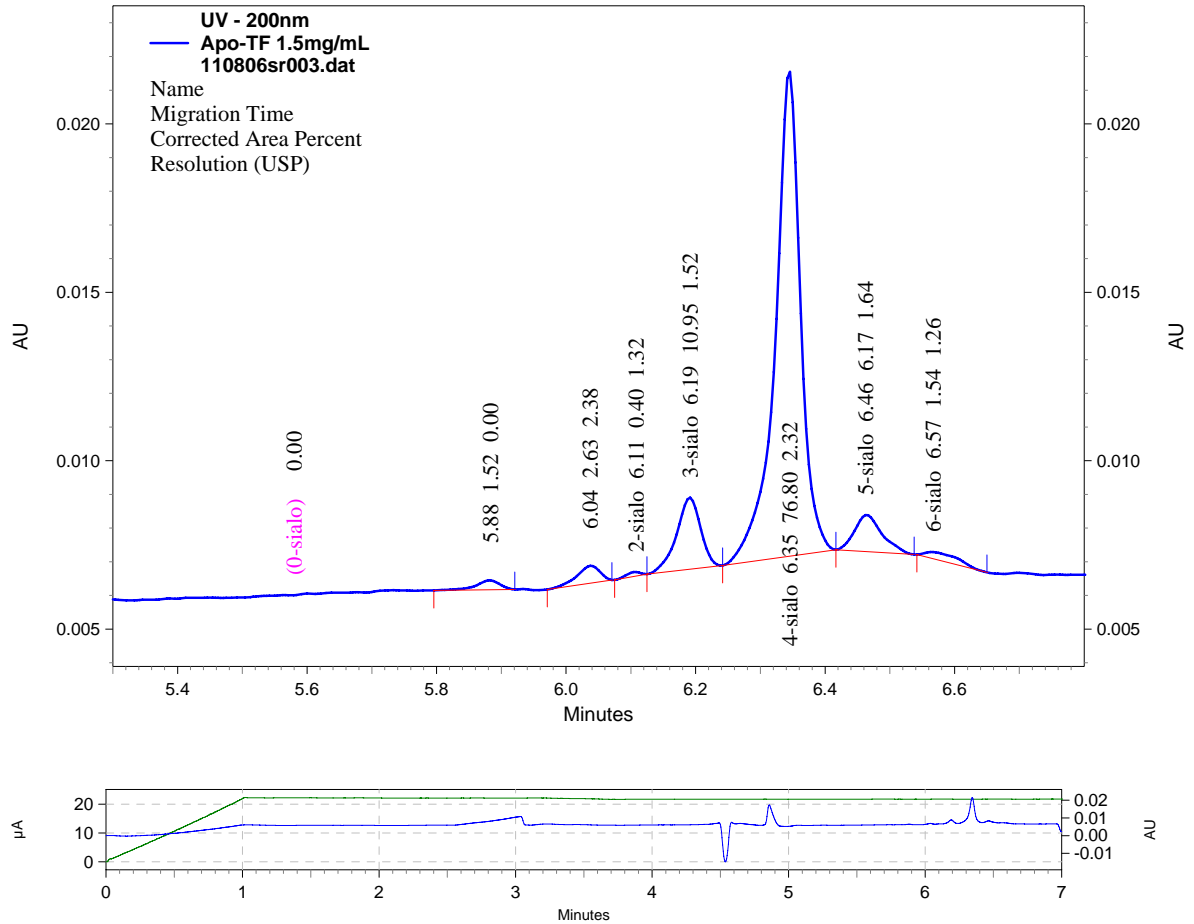
UV - 200nm
 Results

Pk #	Name	Area	Corr Area %	Corr M. T.	Expected MT
	0-sialo		0.00	5.35	5.58
1		1398	1.51	5.64	0.00
2		2210	2.33	5.79	0.00
3	2-sialo	344	0.36	5.86	5.85
4	3-sialo	10222	10.49	5.94	5.93
5	4-sialo	77858	77.98	6.08	6.08
6	5-sialo	5975	5.87	6.20	6.20
7	6-sialo	1516	1.47	6.30	6.30

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Data file: D:\32Karat\Projects\CDT\linearity\110806sr003.dat
 Method: D:\32Karat\Projects\CDT\Method\CDT_meth_96well_plate.met
 Sample: Apo-TF 1.5mg/mL
 Acquired: 08/11/2006 09:52:52
 Printed: 08/11/2006 14:21:03
 Sequence: D:\32Karat\Projects\carchon\sept2006\2.seq
 MDQ setup: cdt (Offline)

CDT - ANALIS R&D



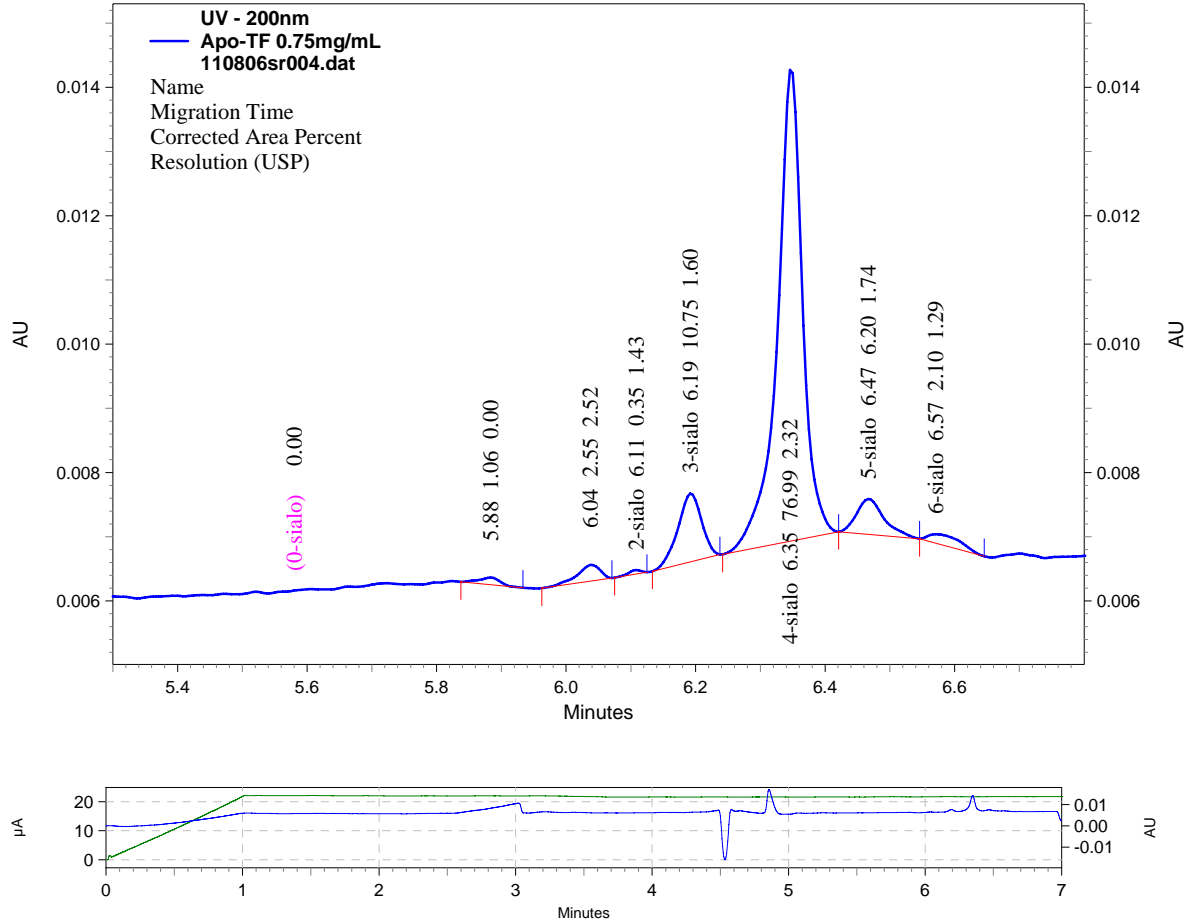
UV - 200nm
 Results

Pk #	Name	Area	Corr Area %	Corr M. T.	Expected MT
	0-sialo		0.00	5.35	5.58
1		721	1.52	5.64	0.00
2		1281	2.63	5.78	0.00
3	2-sialo	198	0.40	5.85	5.85
4	3-sialo	5472	10.95	5.93	5.93
5	4-sialo	39341	76.80	6.08	6.08
6	5-sialo	3217	6.17	6.19	6.20
7	6-sialo	814	1.54	6.29	6.30

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Data file: D:\32Karat\Projects\CDT\linearity\110806sr004.dat
 Method: D:\32Karat\Projects\CDT\linearity\cdt apo.met
 Sample: Apo-TF 0.75mg/mL
 Acquired: 08/11/2006 10:13:11
 Printed: 08/11/2006 14:23:34
 Sequence: D:\32Karat\Projects\carchon\sept2006\2.seq
 MDQ setup: cdt (Offline)

CDT - ANALIS R&D



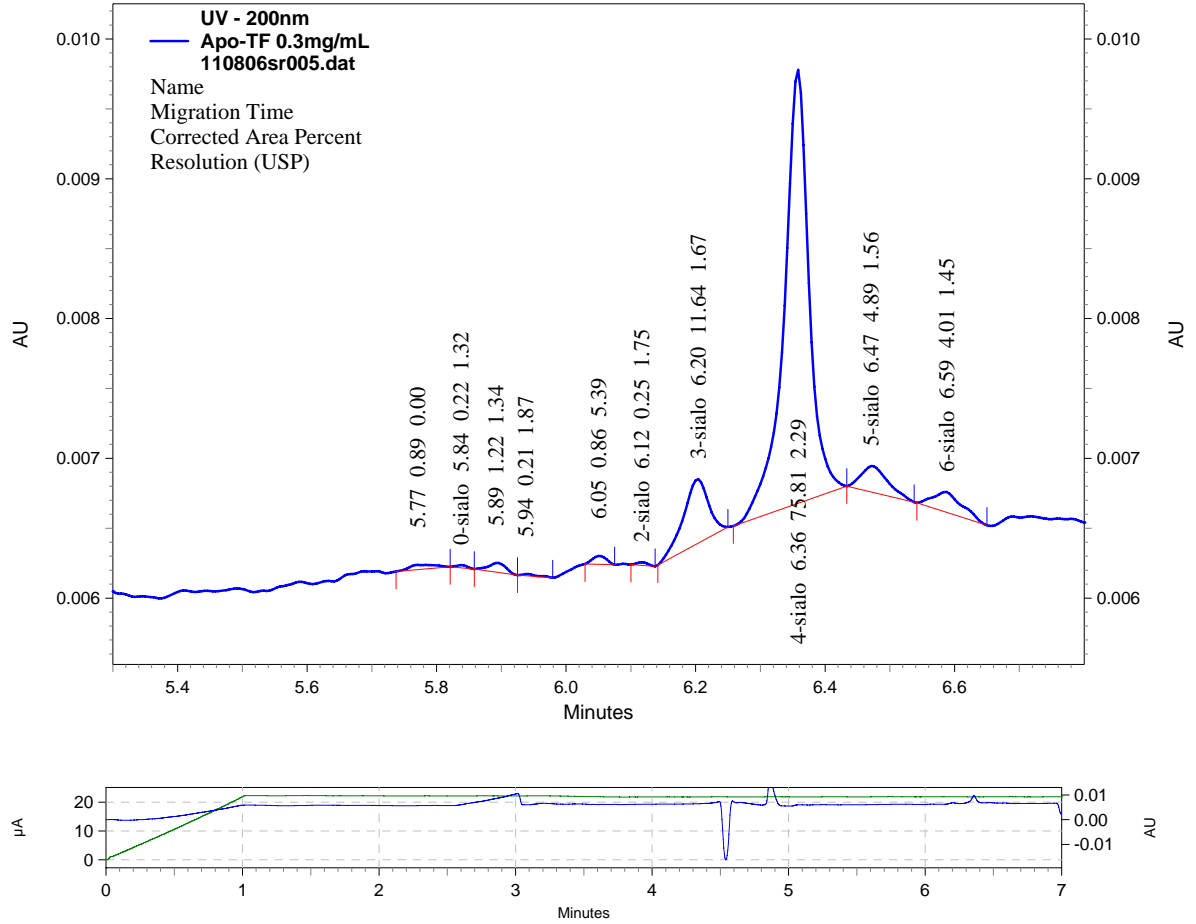
UV - 200nm
 Results

Pk #	Name	Area	Corr Area %	Corr M. T.	Expected MT
	0-sialo		0.00	5.35	5.58
1		253	1.06	5.64	0.00
2		627	2.55	5.78	0.00
3	2-sialo	86	0.35	5.85	5.85
4	3-sialo	2706	10.75	5.93	5.93
5	4-sialo	19865	76.99	6.08	6.08
6	5-sialo	1629	6.20	6.20	6.20
7	6-sialo	562	2.10	6.30	6.30

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Data file: D:\32Karat\Projects\CDT\linearity\110806sr005.dat
 Method: D:\32Karat\Projects\CDT\linearity\cdt apo.met
 Sample: Apo-TF 0.3mg/mL
 Acquired: 08/11/2006 10:26:25
 Printed: 08/11/2006 14:24:35
 Sequence: D:\32Karat\Projects\carchon\sept2006\2.seq
 MDQ setup: cdt (Offline)

CDT - ANALIS R&D



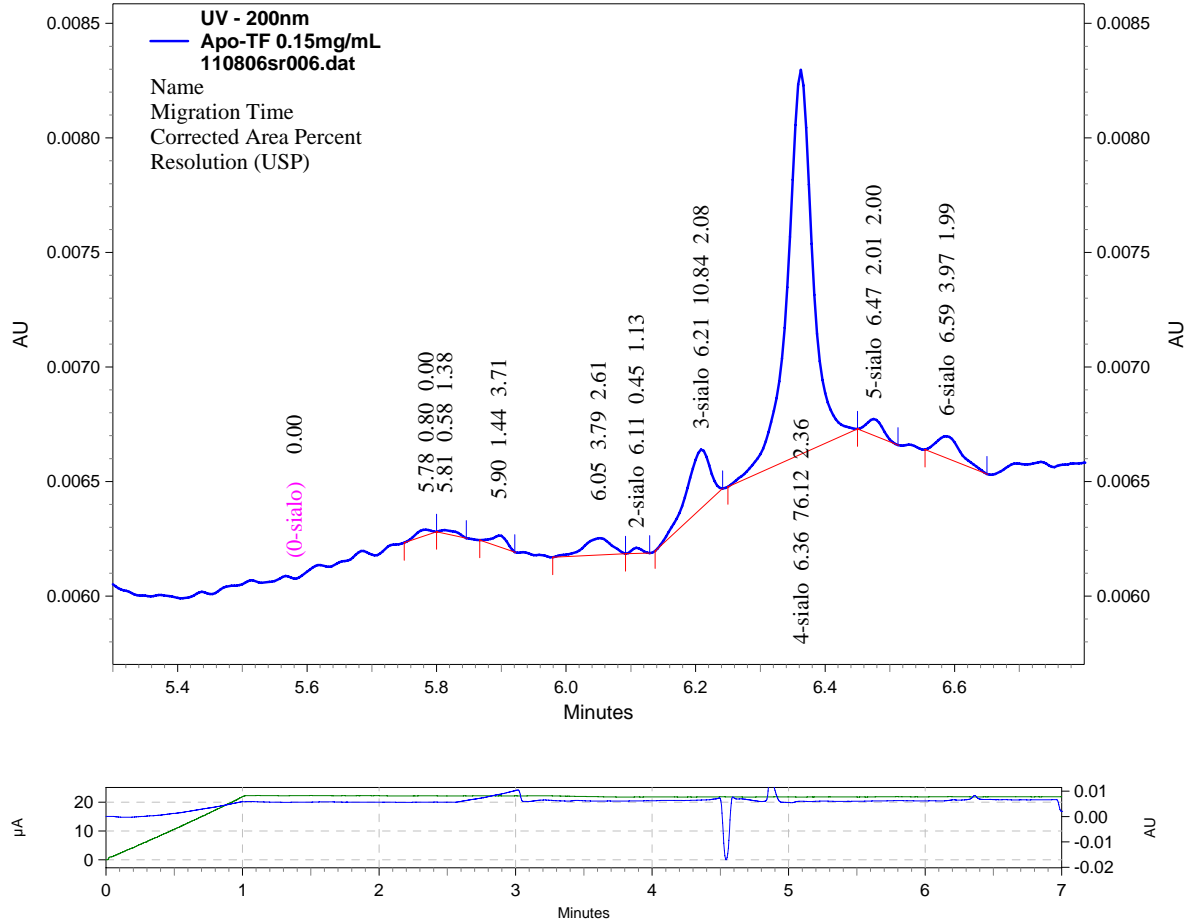
UV - 200nm
 Results

Pk #	Name	Area	Corr Area %	Corr M. T.	Expected MT
1		89	0.89	5.52	0.00
2	0-sialo	22	0.22	5.58	5.58
3		124	1.22	5.63	0.00
4		21	0.21	5.68	0.00
5		90	0.86	5.79	0.00
6	2-sialo	26	0.25	5.85	5.85
7	3-sialo	1245	11.64	5.93	5.93
8	4-sialo	8309	75.81	6.08	6.08
9	5-sialo	545	4.89	6.19	6.20
10	6-sialo	455	4.01	6.30	6.30

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Data file: D:\32Karat\Projects\CDT\linearity\110806sr006.dat
 Method: D:\32Karat\Projects\CDT\linearity\cdt apo.met
 Sample: Apo-TF 0.15mg/mL
 Acquired: 08/11/2006 10:40:50
 Printed: 08/11/2006 14:26:06
 Sequence: D:\32Karat\Projects\carchon\sept2006\2.seq
 MDQ setup: cdt (Offline)

CDT - ANALIS R&D



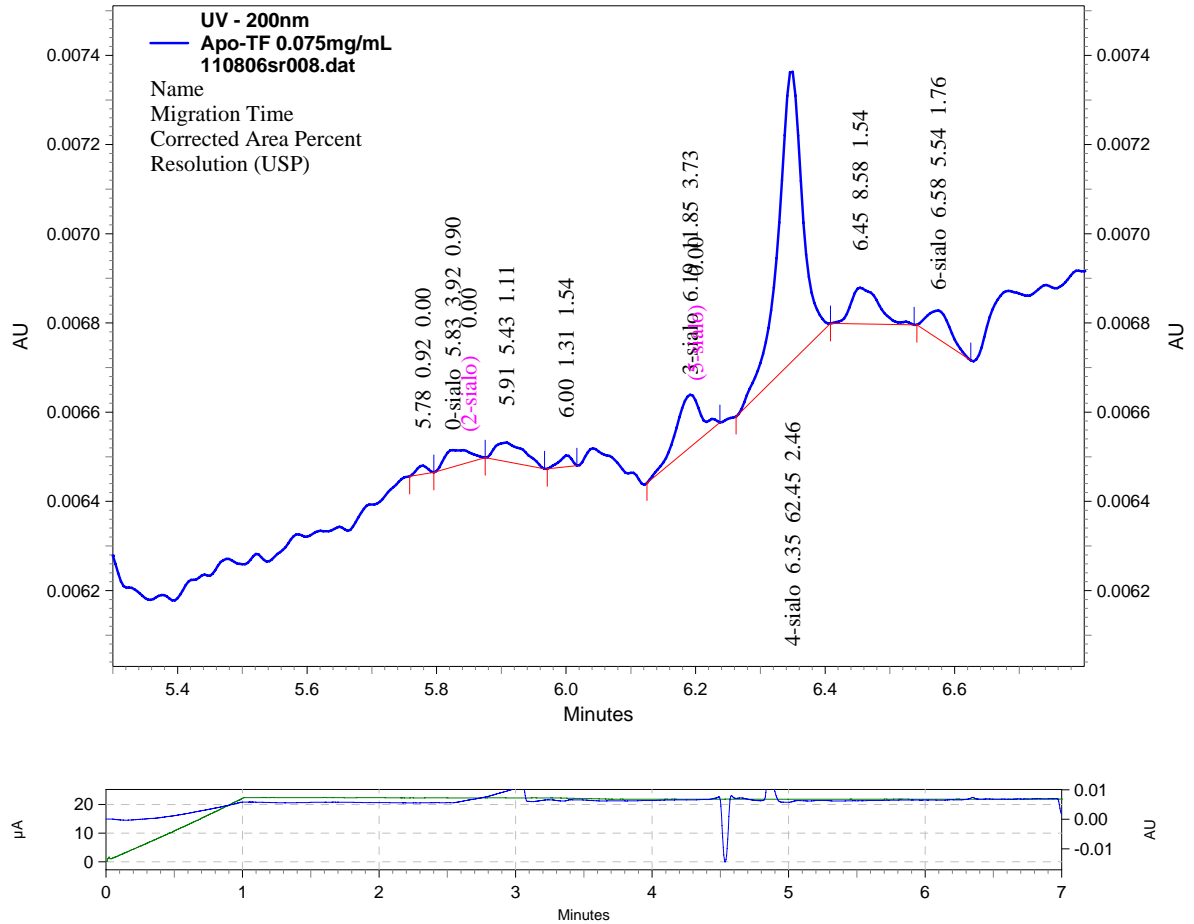
UV - 200nm
 Results

Pk #	Name	Area	Corr Area %	Corr M. T.	Expected MT
	0-sialo		0.00	5.33	5.58
1		44	0.80	5.53	0.00
2		32	0.58	5.55	0.00
3		81	1.44	5.63	0.00
4		218	3.79	5.78	0.00
5	2-sialo	26	0.45	5.84	5.85
6	3-sialo	640	10.84	5.93	5.93
7	4-sialo	4607	76.12	6.08	6.08
8	5-sialo	124	2.01	6.19	6.20
9	6-sialo	249	3.97	6.30	6.30

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Data file: D:\32Karat\Projects\CDT\linearity\110806sr008.dat
 Method: D:\32Karat\Projects\CDT\linearity\cdt apo.met
 Sample: Apo-TF 0.075mg/mL
 Acquired: 08/11/2006 11:58:09
 Printed: 08/11/2006 14:26:36
 Sequence: D:\32Karat\Projects\carchon\sept2006\2.seq
 MDQ setup: cdt (Offline)

CDT - ANALIS R&D



UV - 200nm
 Results

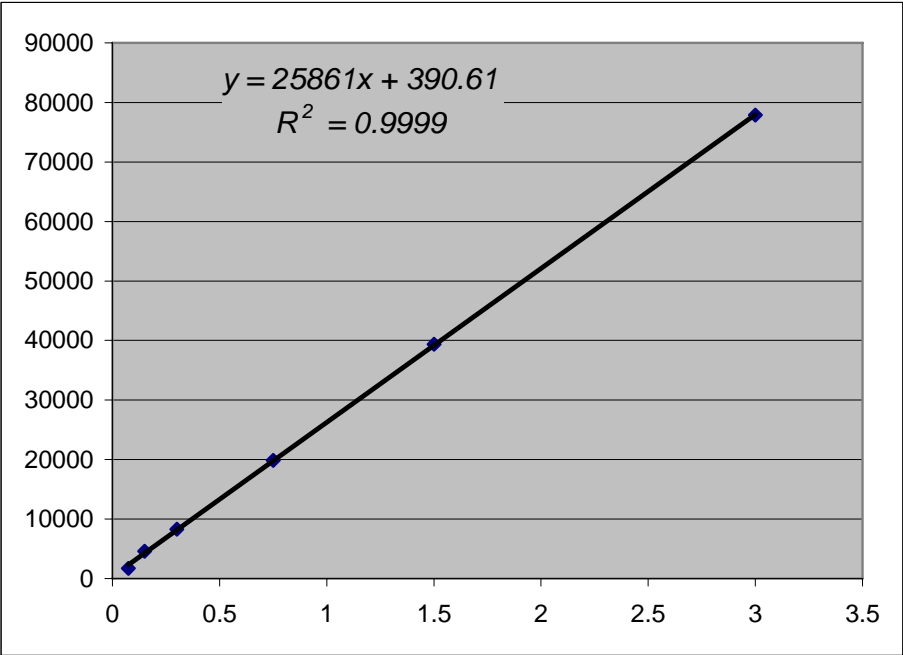
Pk #	Name	Area	Corr Area %	Corr M. T.	Expected MT
1		23	0.92	5.53	0.00
2	0-sialo	99	3.92	5.58	5.58
	2-sialo		0.00	5.60	5.85
3		139	5.43	5.66	0.00
4		34	1.31	5.74	0.00
5	3-sialo	318	11.85	5.93	5.93
	5-sialo		0.00	5.94	6.20
6	4-sialo	1719	62.45	6.08	6.08
7		240	8.58	6.18	0.00
8	6-sialo	158	5.54	6.30	6.30

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Addendum 2

Linearity:

Tf mg/mL	Area 4-sialo Tf
3	77858
1.5	39341
0.75	19865
0.3	8309
0.15	4600
0.075	1719



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