

1H qNMR of EPA and DHA Omega-3 Fatty Acids - PLS Regression Models Obtained at 60 and 300 MHz : At-Line and On-Line Monitoring of a Fish Oil Nutritional Supplement Manufacturing Process

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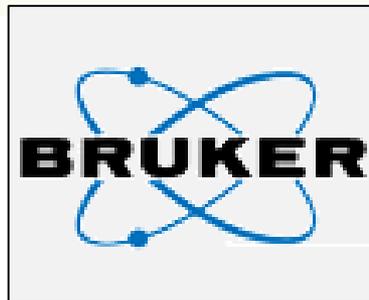
May 23, 2014

Cesena, Italia



Pulsar

60 MHz NMR
3-5 mm Samples
Lab Only



Fourier 60

60 MHz NMR
3-5 mm Samples
Lab Only



MobiLab™ SPECTRA 2T

85+ MHz NMR
3-5 mm Samples
Lab Only



Spinsolve™

42 MHz NMR
3-5 mm Samples
Lab Only



NMReady 60

60 MHz NMR
3-5 mm Samples
Lab Only

**Cryogen Free
Permanent Magnet
High Resolution
Benchtop NMR**



Picospin 45 and 80

45 MHz and 82 MHz NMR
300 micron capillary injection
Lab Only



60 MHz NMR
3-10 mm Samples
Lab or On-Line



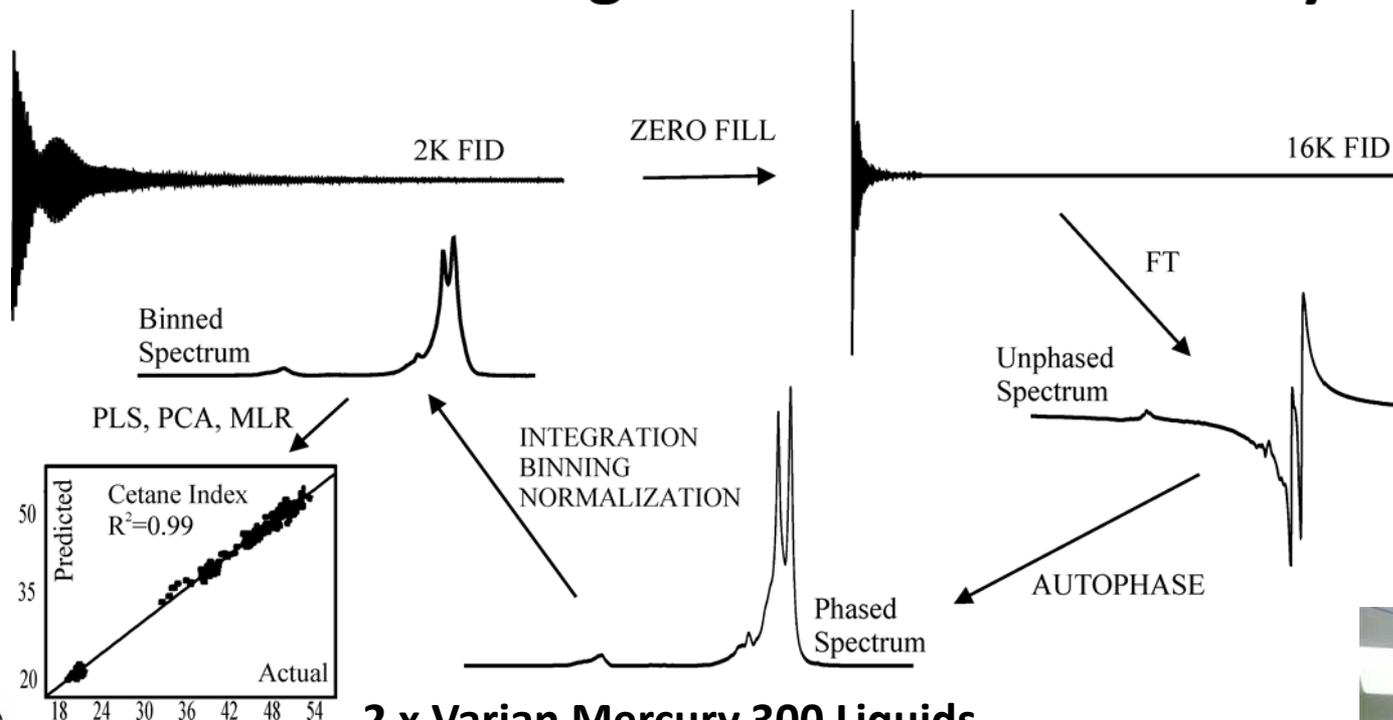
aspectimaging

60 MHz NMR
3-10 mm Samples
Lab or On-Line



60 and 90 MHz
5 mm Samples
Lab Only

NMR Processing for Multivariate Analysis



2 x Varian Mercury 300 Liquids
1 x Varian UnityPlus-200 Solids
Multiple 10/20/40 mm TD-NMR 20-25 MHz
NMR MOUSE

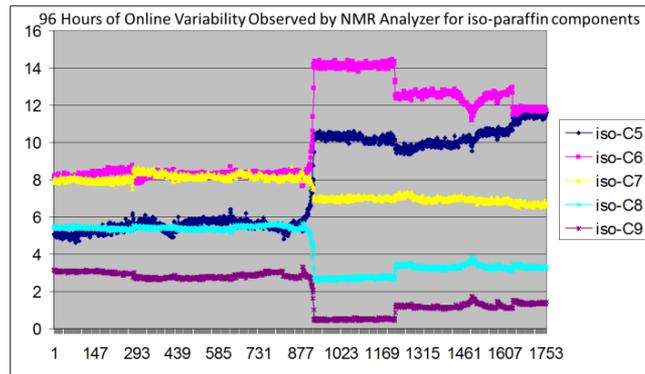
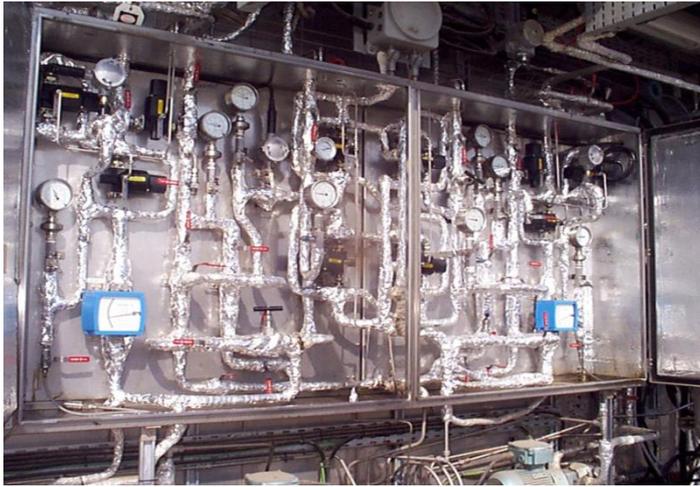
2 x Aspect-AI 60 MHz HiRes-NMR
3 x Qualion 60 MHz HiRes NMR
MicroESR

Shimazu GC

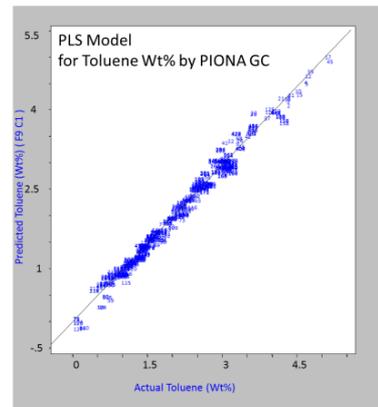
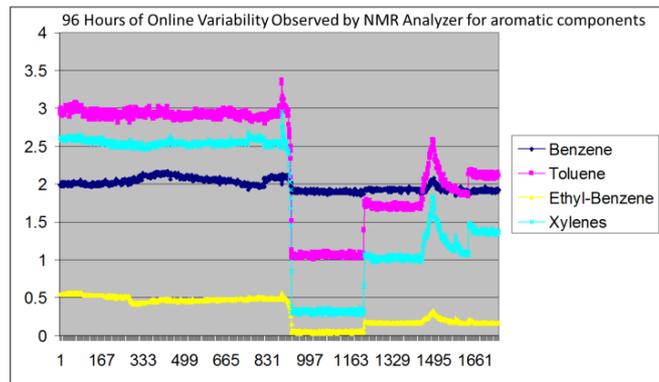
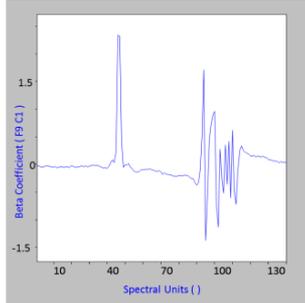
MNova(Verify, qNMR, DB), ACDLabs

Chemometrics: GramsIQ, Eigenvector Solo, Pirouette





Predictive Vector for Toluene PLS Model

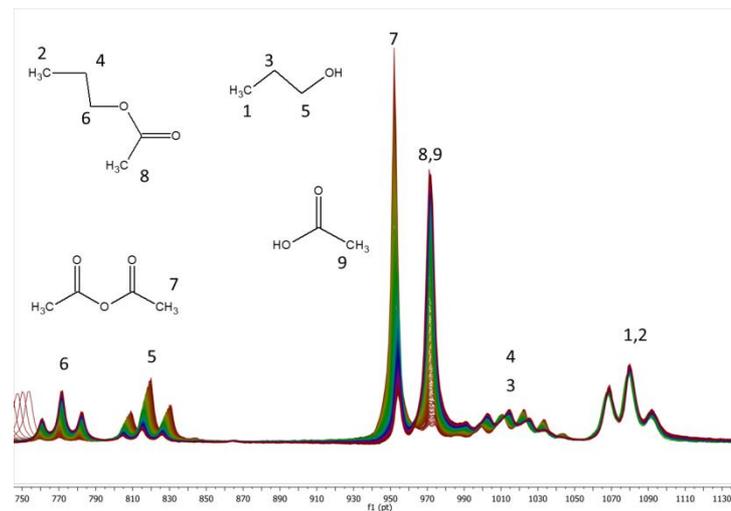
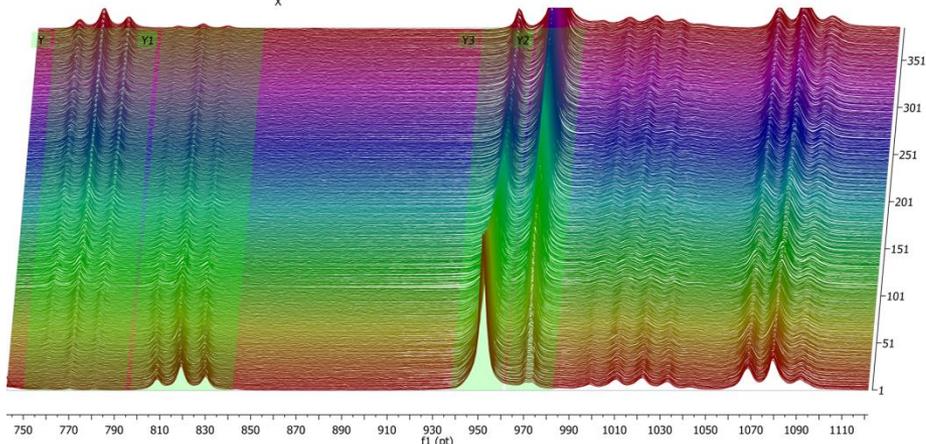
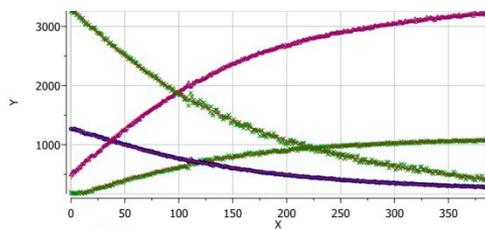
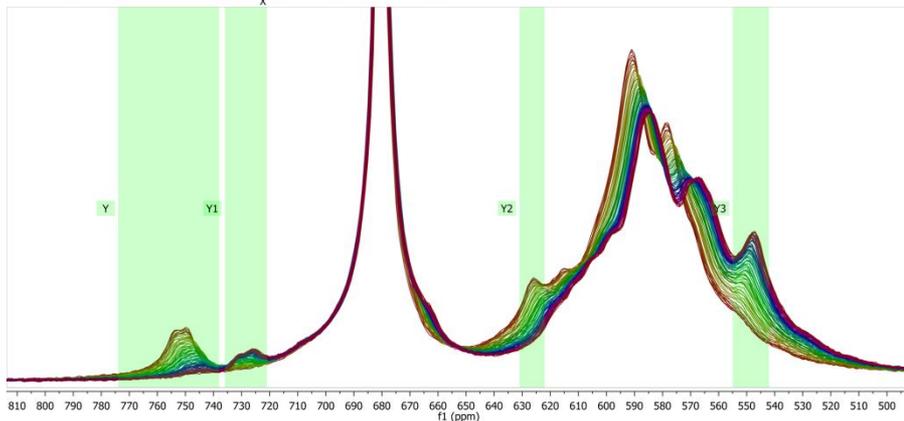
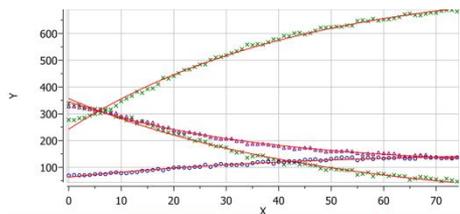


On-Line Process NMR

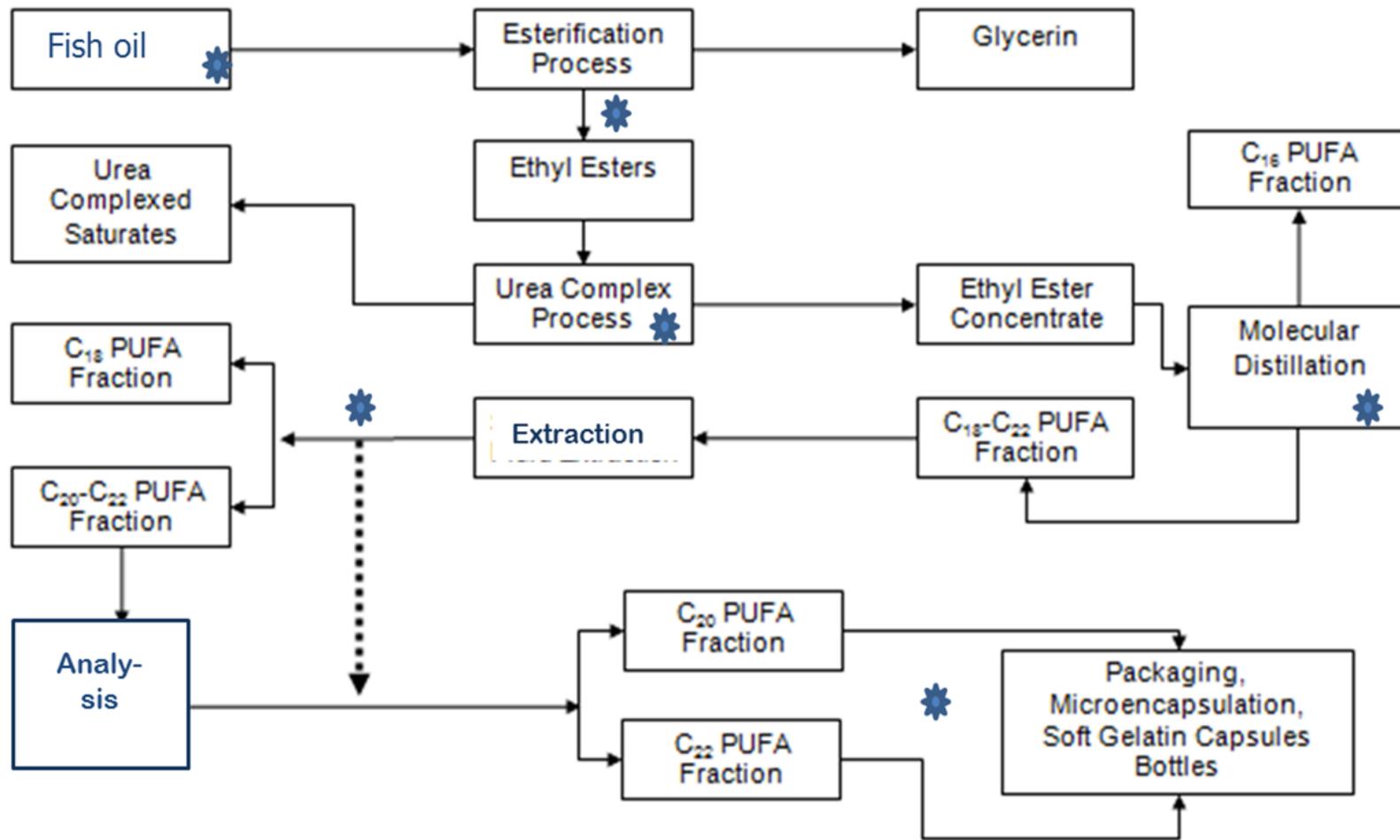
45 Systems Installed 1996-2004

Predominantly in Petroleum
and Petrochemical

60 MHz Reaction Monitoring



Omega 3 Manufacturing Process Sampling Points



★ Samples for NMR Modeling

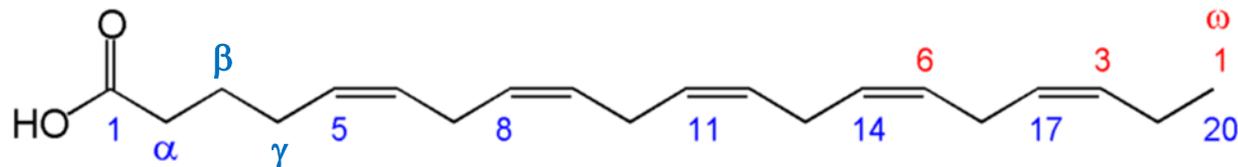
NMR ID	EPA (Area %)	DHA (Area %)	Sample Description
FO3h001	0.64	0.01	First Esterification
FO3h002	21.55	13.34	First Esterification
FO3h003	62.97	15.66	Clathration
FO3h004	29.43	18.16	Mol Dist
FO3h005	14.21	9.54	Pollock Oil
FO3h006	52.74	28.90	Separator
FO3h007	15.21	10.51	PolyUns at Ester
FO3h008	7.18	0.23	First Esterification
FO3h009	16.95	10.04	First Esterification
FO3h010	36.35	16.47	Clathration
FO3h011	61.09	21.26	Mol Dist
FO3h012	13.32	5.95	MSC Pollock Oil
FO3h013	71.78	7.43	Separator
FO3h014	41.40	25.91	PolyUns at Ester
FO3h015	1.19	0.06	First Esterification
FO3h016	11.73	12.23	First Esterification
FO3h017	43.38	19.30	Clathration
FO3h018	6.07	2.78	Clath Raffinate
FO3h019	9.77	0.72	First Esterification
FO3h020	58.93	23.41	Mol Dist
FO3h021	10.62	5.18	MSC Pollock Oil
FO3h022	43.91	21.52	Separator
FO3h023	54.05	28.18	PolyUns at Ester
FO3h024	0.00	0.00	First Esterification
FO3h025	26.97	12.82	First Esterification

NMR ID	EPA (Area %)	DHA (Area %)	Sample Description
FO3h026	44.55	19.30	Clathration
FO3h027	16.69	9.89	First Esterification
FO3h028	6.87	4.53	Crude Pollock Oil
FO3h029	62.79	19.43	Separator
FO3h030	37.29	22.03	PolyUns at Ester
FO3h031	9.71	0.38	First Esterification
FO3h032	32.00	15.10	First Esterification
FO3h033	38.79	26.75	Clathration
FO3h034	41.87	23.25	Mol Dist
FO3h035	35.49	43.99	Separator
FO3h036	0.30	0.00	First Esterification
FO3h037	34.09	8.09	Clathration
FO3h038	15.44	9.82	MonoUns at Ester
FO3h039	60.08	24.52	PolyUns at Ester
FO3h040	8.77	5.75	Crude Salmon Oil
FO3h041	12.41	57.59	Separator
FO3h042	36.36	21.49	PolyUns at Ester
FO3h043	3.79	0.15	First Esterification
FO3h044	29.23	13.78	Clath Raffinate
FO3h045	45.99	33.51	PolyUns at Ester
FO3h046	12.10	5.69	MSC Pollock Oil
FO3h047	24.57	14.32	Separator
FO3h048	45.86	33.61	PolyUns at Ester
FO3h049	6.39	3.68	First Esterification
FO3h050	58.13	24.66	Clathration

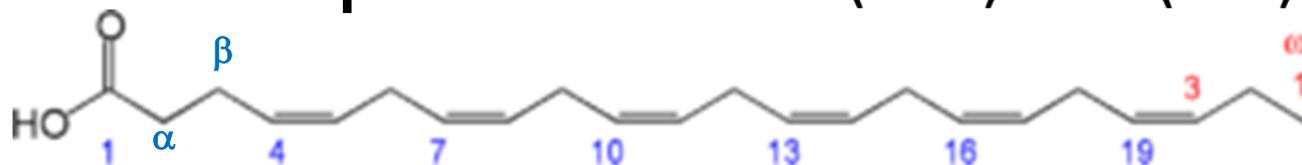
50 sample initial data set from all points in manufacturing process.

First round of PLS regression analysis revealed concentration outliers that were linked to limitations in the GC method. This study was performed on improved GC data values.

Final 50 Sample models were utilized to validate the calibrations on a 24 sample validation set



Eicosapentaenoic Acid (EPA) 20:5(n-3)



Docosahexaenoic Acid (DHA) 22:6(n-3)

Experimental

Varian Mercury - 300 MHz NMR Supercon Spectrometer
4 pulse on pure sample in 5 mm tube, Run Unlocked
In MNova 8.1.2 SPC Files Imported, Stacked, Binned at 3 Hz interval,
Area Normalized to 100, Saved as Transposed Ascii Matrix
PLS Regression Performed Thermo Grams IQ and Eigenvector Solo

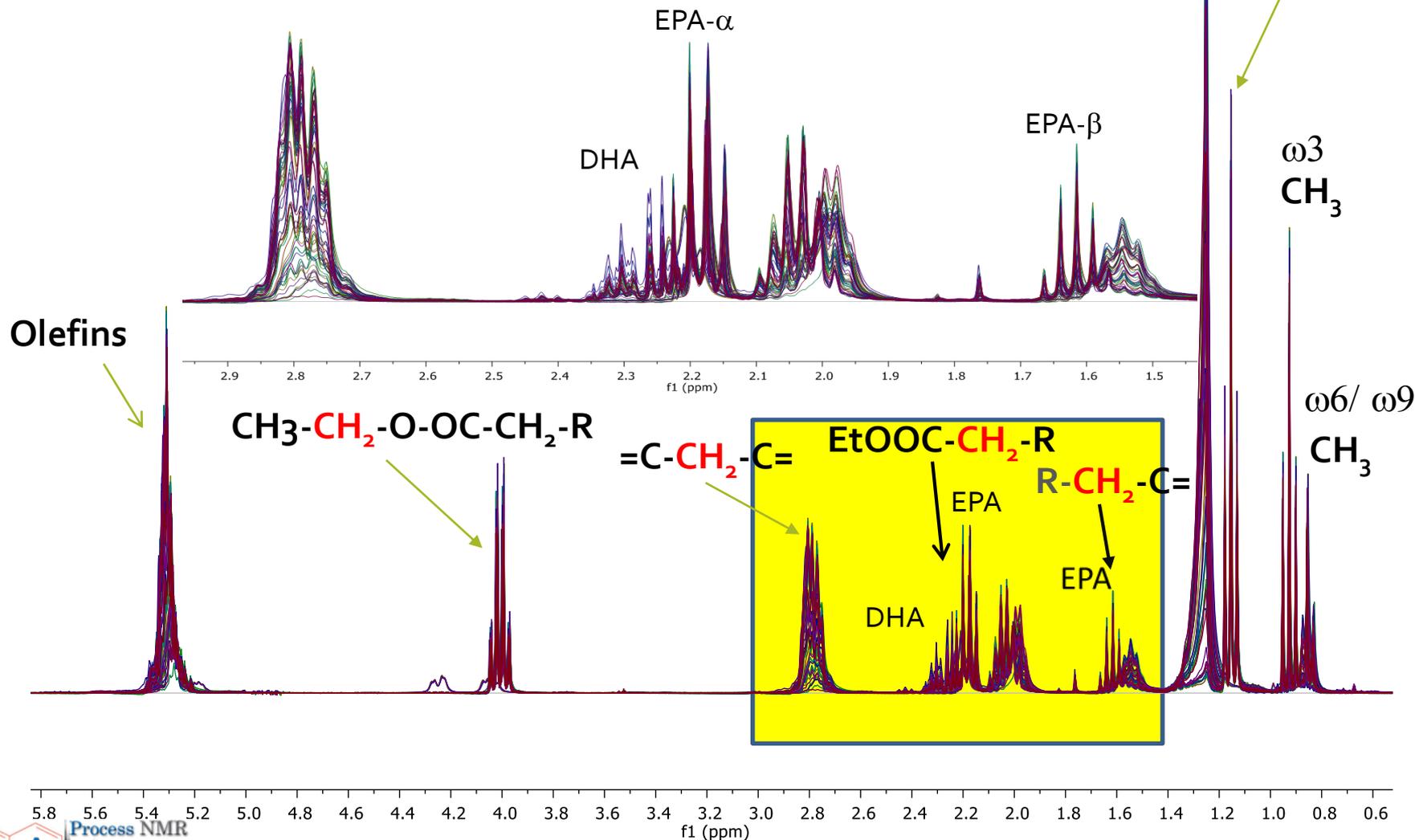
300 MHz ¹H NMR

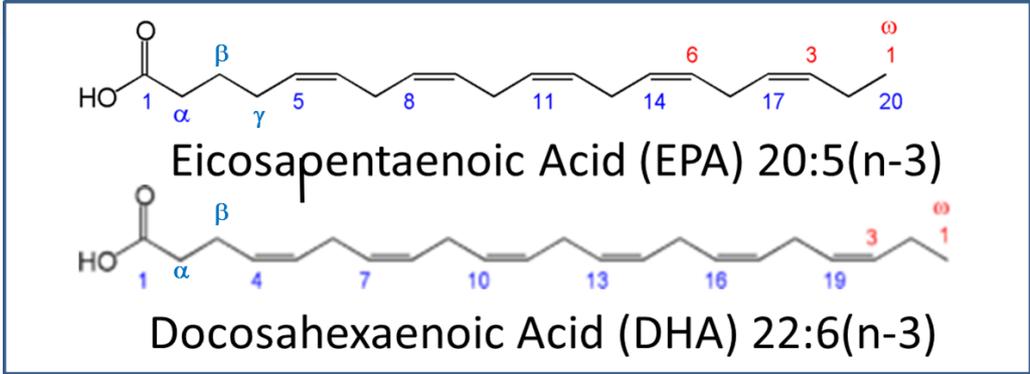
In chain
CH₂

Ethyl
CH₃

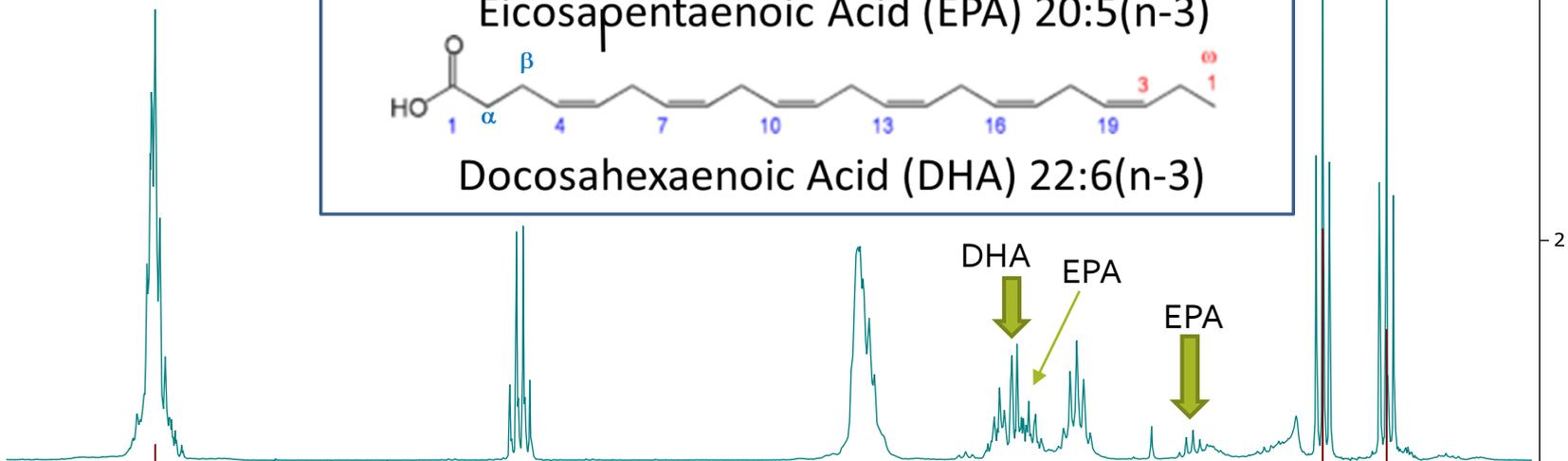
ω3
CH₃

ω6/ ω9
CH₃

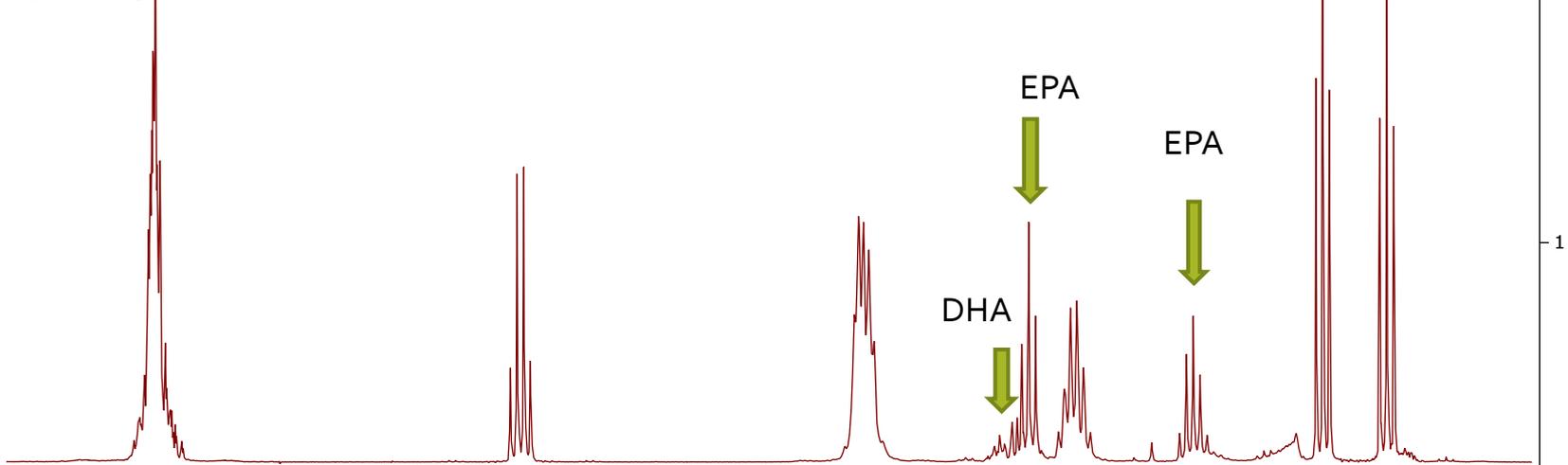




Separator - High DHA



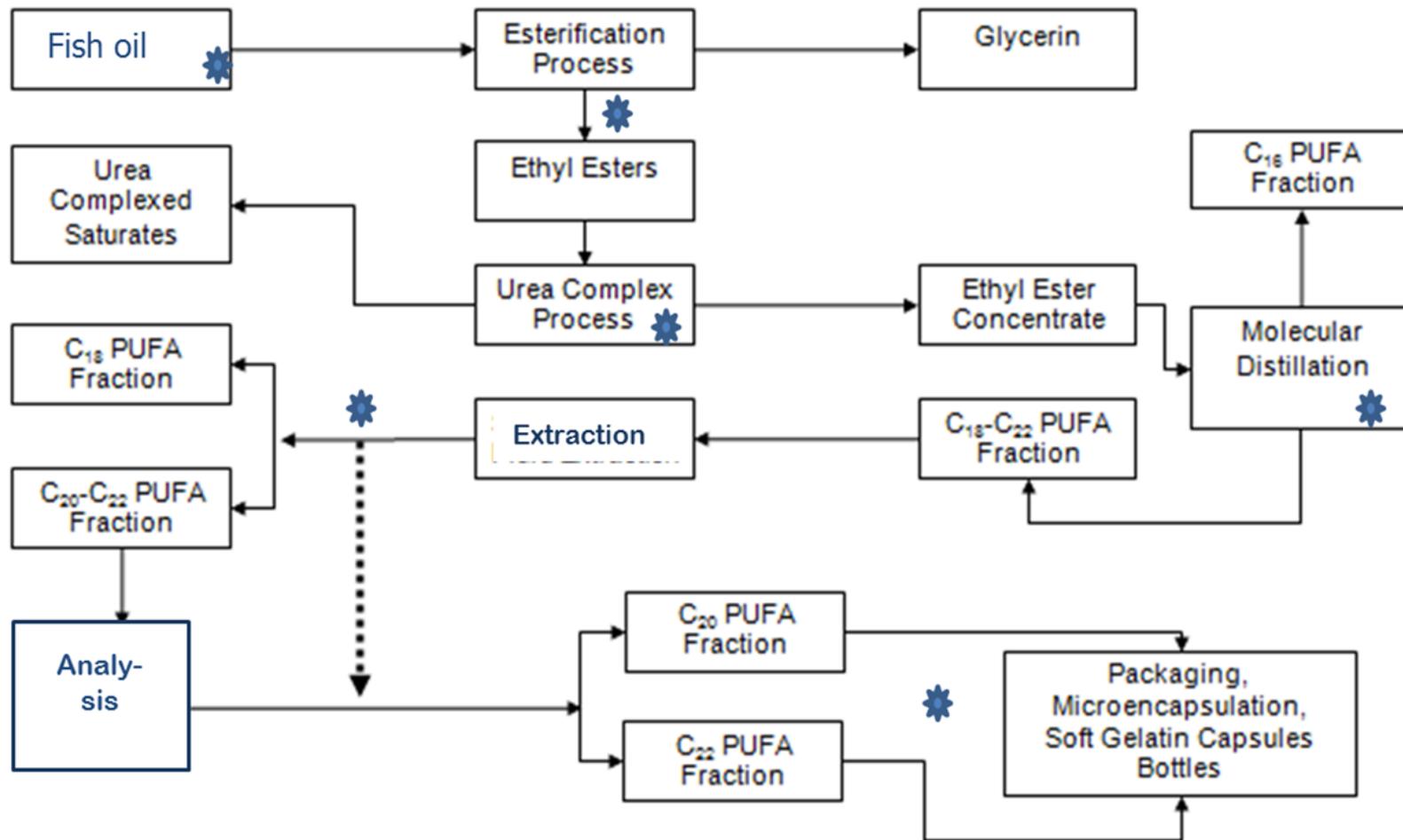
Separator- High EPA



Comparison of High EPA and High DHA PUFA-Ester after Separation

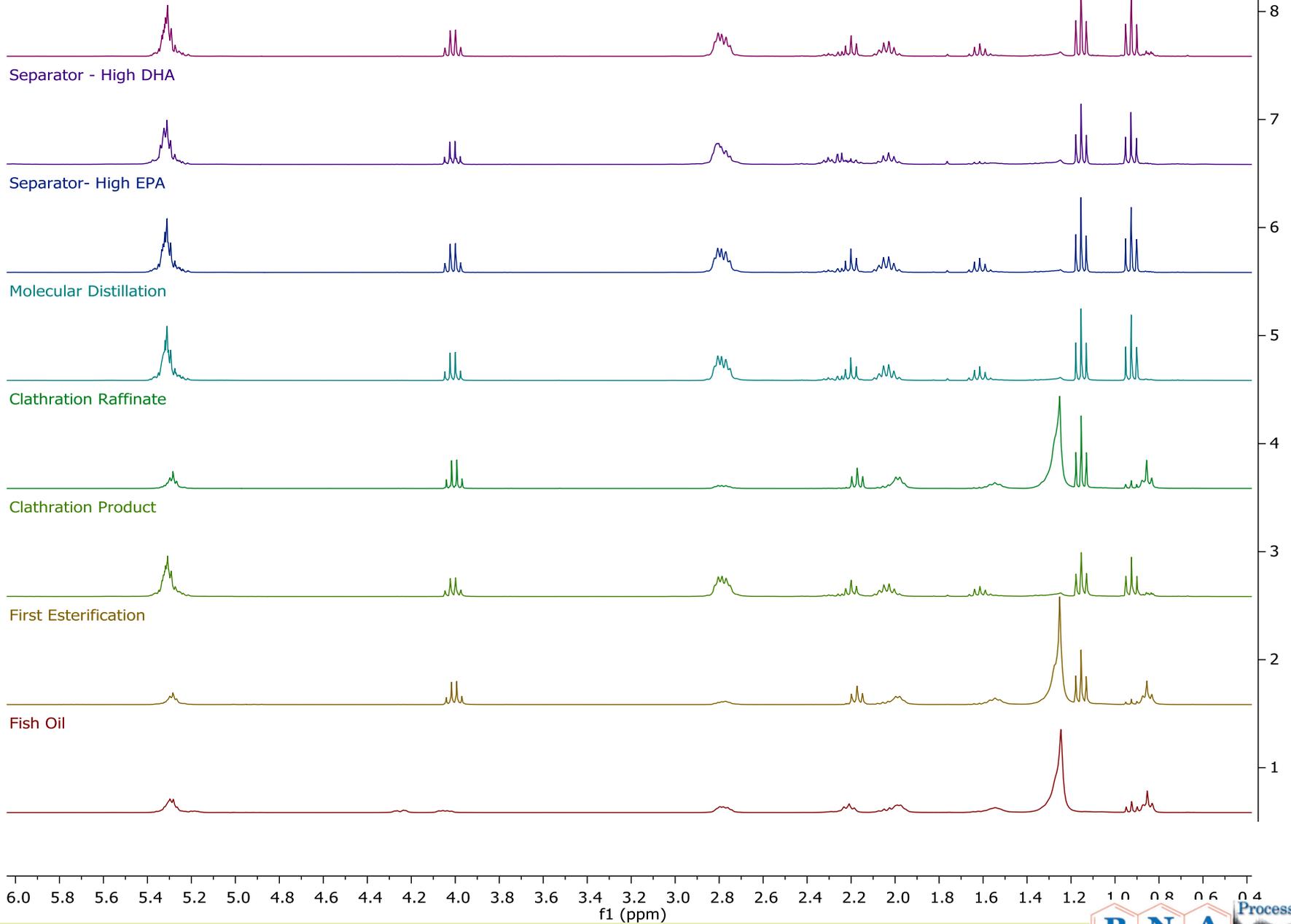
5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6
f1 (ppm)

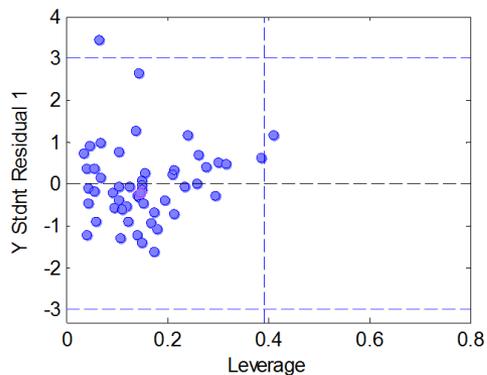
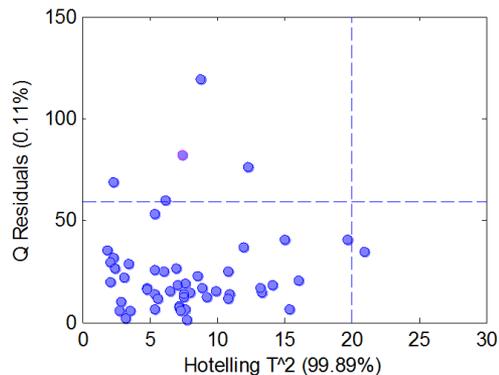
Omega 3 Manufacturing Process Sampling Points



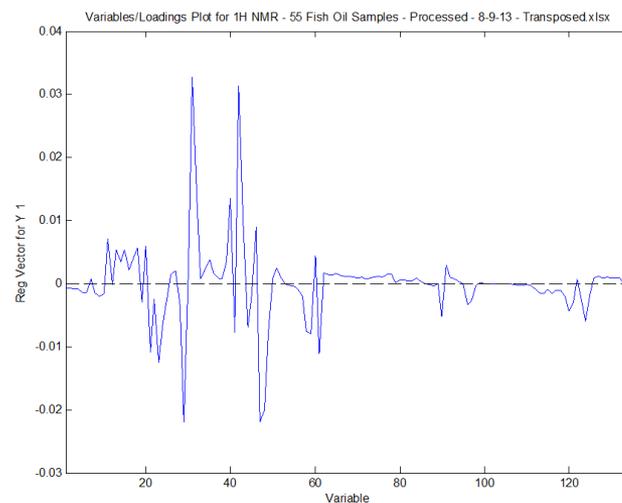
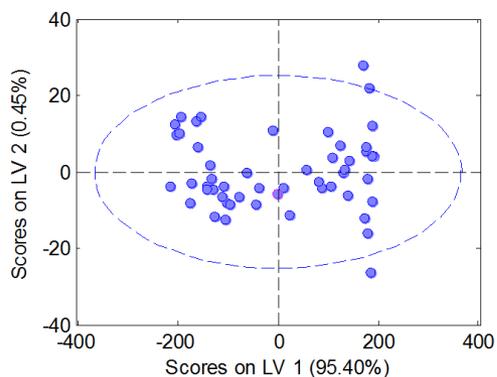
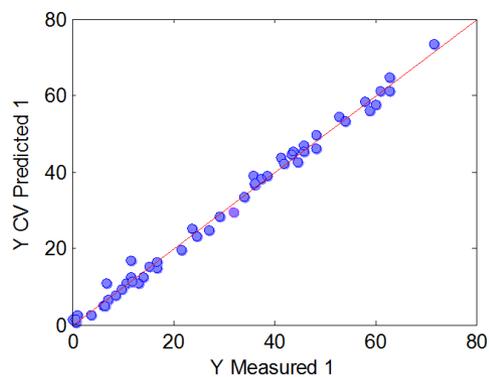
★ Samples for NMR Modeling

PUFA Ester **300 MHz 1H NMR Spectra of Samples at Diferent Points in the Process**

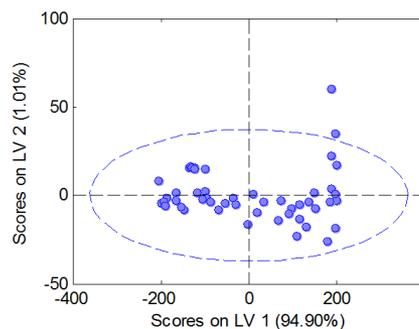
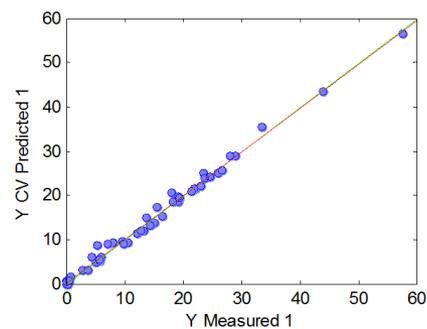
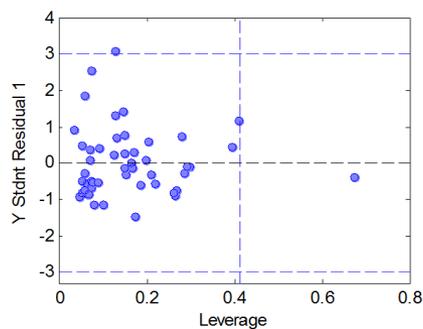
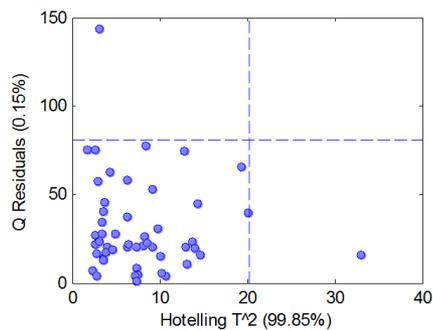




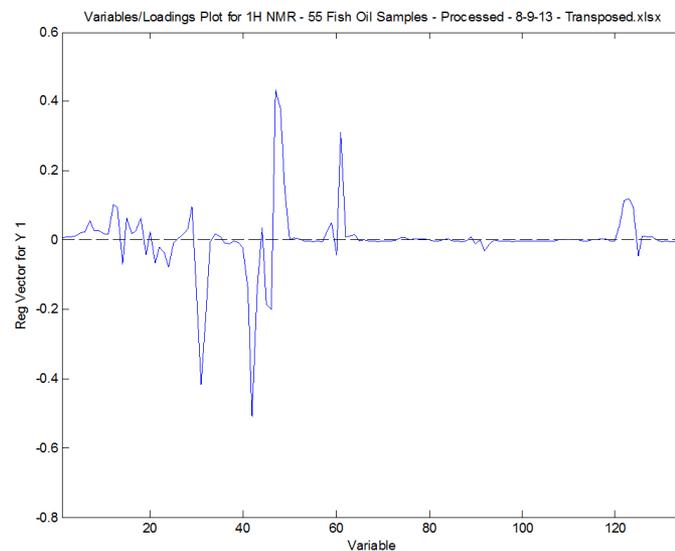
Partial Least Squares calculated with the SIMPLS algorithm
 X-block: 1H NMR - 50 Fish Oil Samples - Transposed.xlsx 50 by 135
 Preprocessing: Mean Center
 Y-block: EPA GC Content.xlsx 50 by 1
 Preprocessing: Autoscale Num. LVs: 8
 Cross validation: venetian blinds w/ 7 splits
 RMSEC: 1.20344 RMSECV: 1.67825
 Bias: -3.55271e-015 CV Bias: -0.0822205
 R² Cal: 0.996441 R² CV: 0.993139



EPA PLS Regression Calibration – 300 MHz



Partial Least Squares calculated with the SIMPLS algorithm
 X-block: 1H NMR - 55 Fish Oil Samples - Transposed.xlsx 50 by 135
 Preprocessing: Mean Center
 Y-block: DHA GC Content.xlsx 50 by 1
 Preprocessing: Mean Center Num. LVs: 8
 Cross validation: venetian blinds w/ 7 splits
 RMSEC: 0.837073 RMSECV: 1.08906
 Bias: 0 CV Bias: 0.0445129
 R² Cal: 0.994978 R² CV: 0.991523



DHA PLS Regression Calibration – 300 MHz

Experimental

Aspect AI – 60 MHz Cryogen-Free NMR Spectrometer

24 pulse on pure sample in 5 mm tube

Locked on ^1H NMR signal

In MNova 8.1.2

SPC Files Imported, Stacked, Binned at 3 Hz interval, Area Normalized to 100

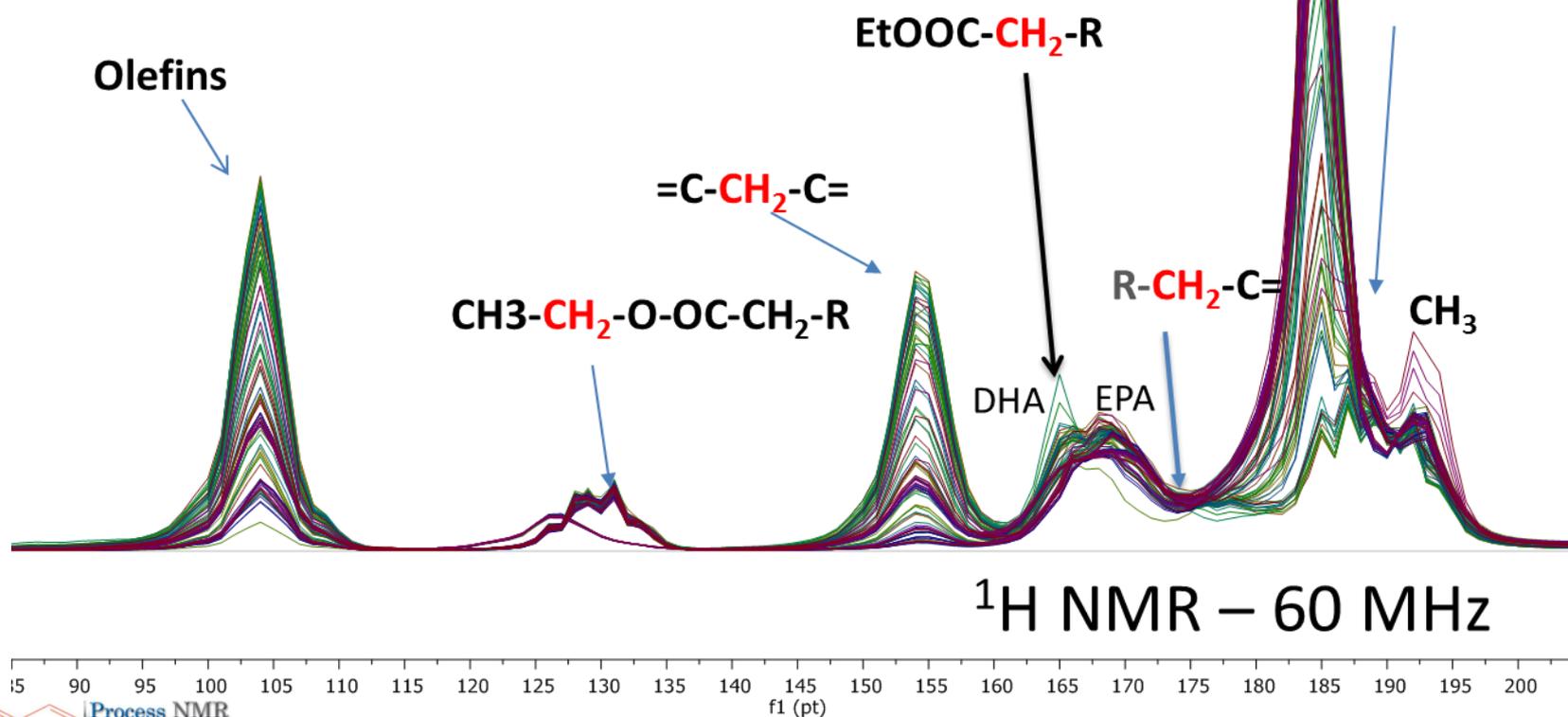
Saved as Transposed Ascii Matrix

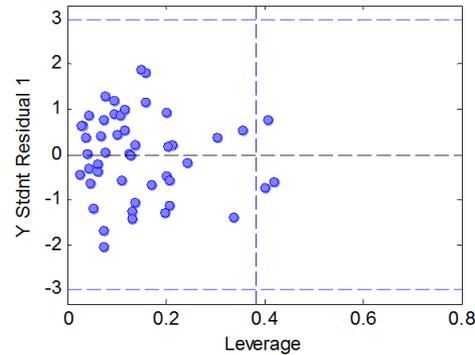
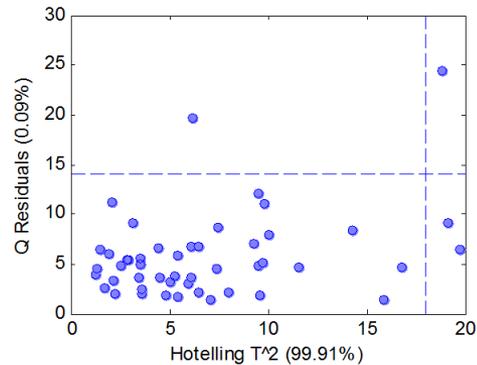
For Peak Integrals Used Advanced Feature – Create Integral Graph from Stacked Plot

PLS Regression Performed Thermo Grams IQ and Eigenvector Solo

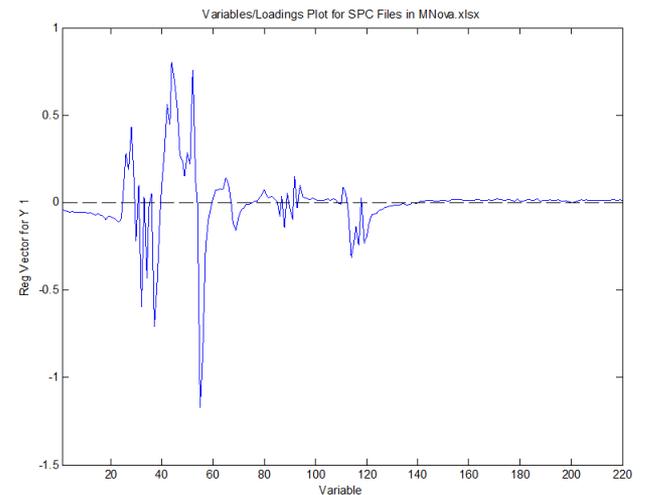
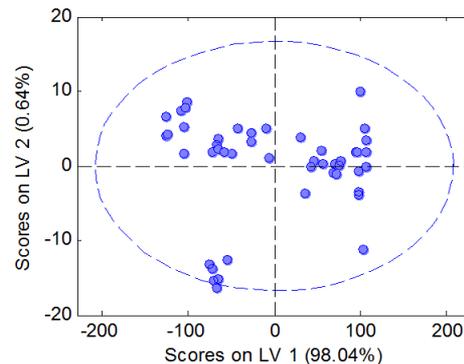
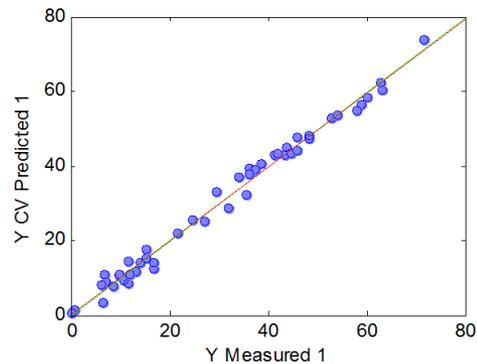
In chain
 CH_2

Ethyl
 CH_3



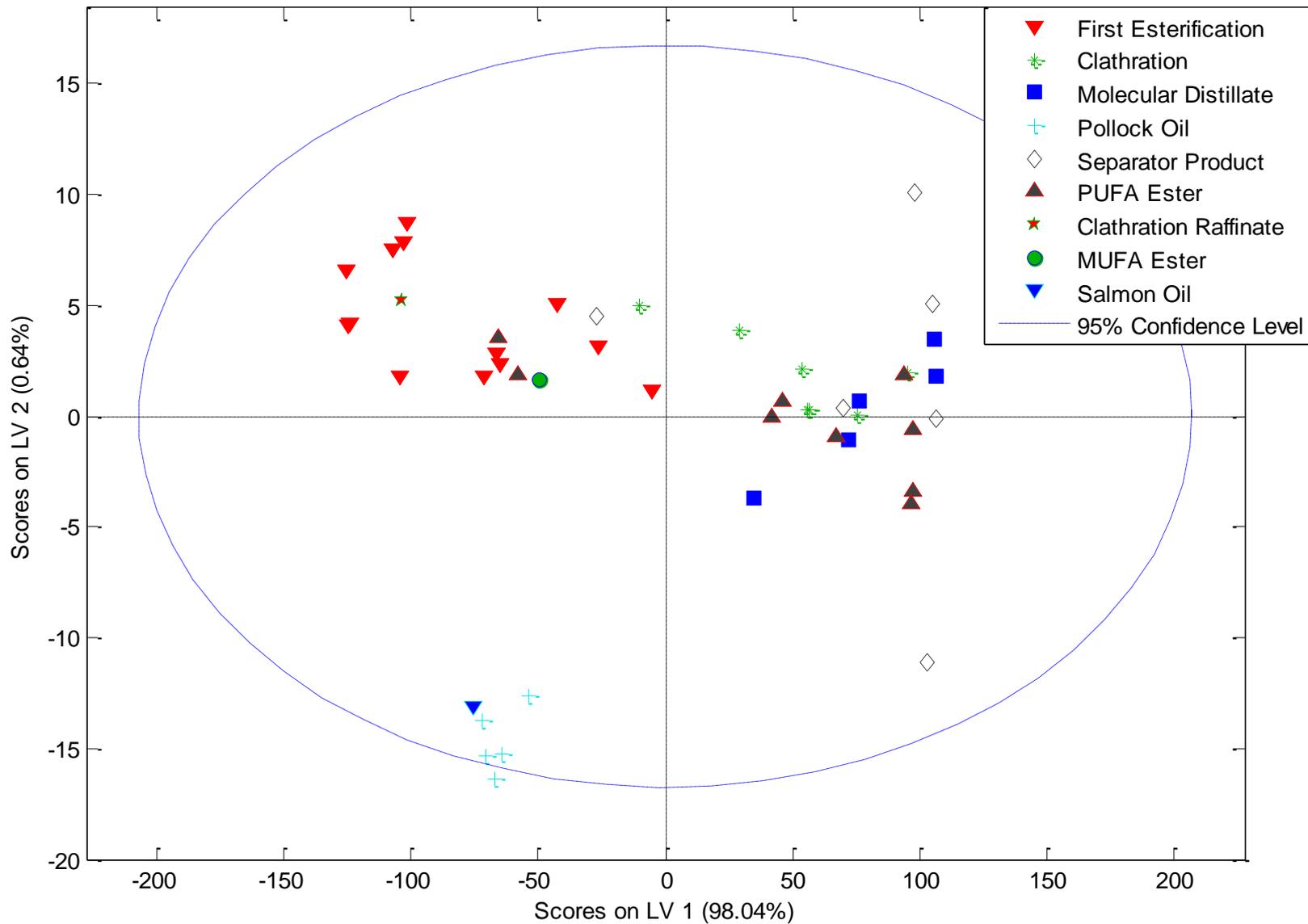


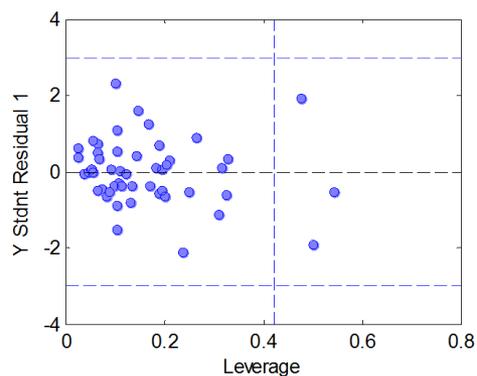
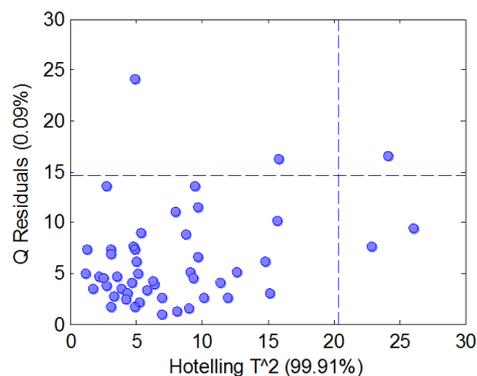
Partial Least Squares calculated with the SIMPLS algorithm
 X-block: SPC Files in MNovo.xlsx 50 by 220
 Preprocessing: Mean Center
 Y-block: EPA GC Content.xlsx 50 by 1
 Preprocessing: Mean Center Num. LVs: 7
 Cross validation: venetian blinds w/ 7 splits
 RMSEC: 1.54867 RMSECV: 2.1229
 Bias: 0 CV Bias: -0.112162
 R² Cal: 0.993826 R² CV: 0.988456



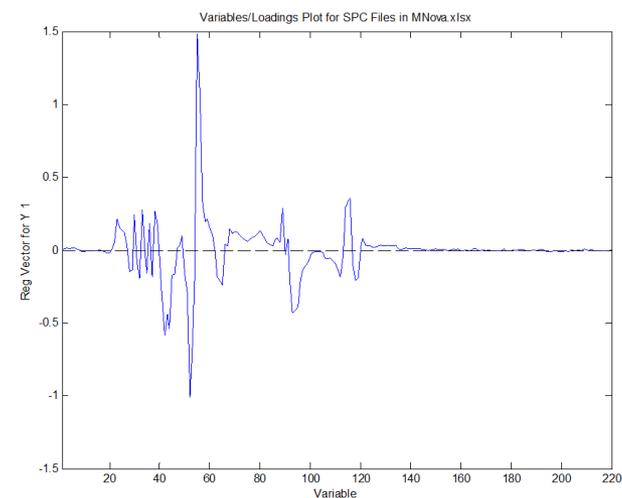
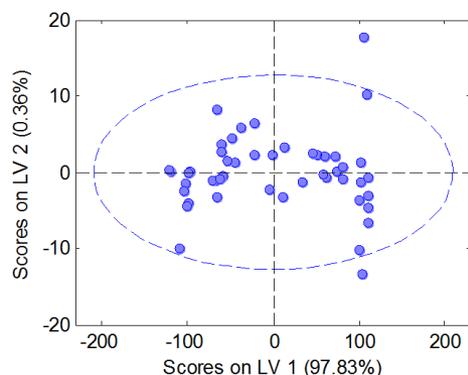
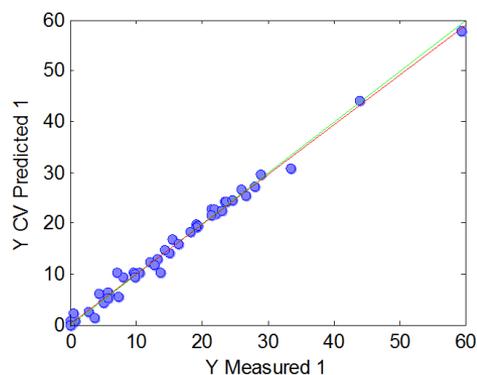
EPA PLS Regression Calibration – 60 MHz

Samples/Scores Plot of SPC Files in MNova.xlsx

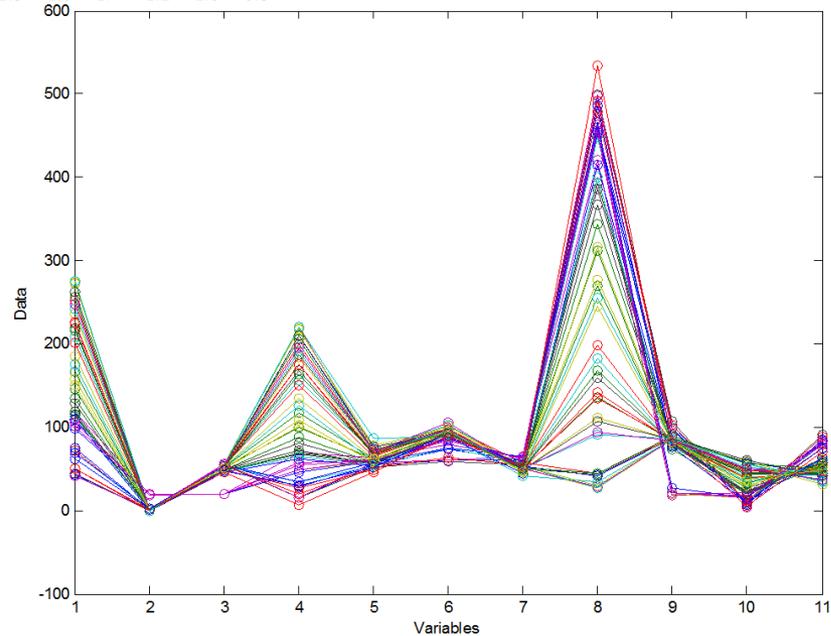
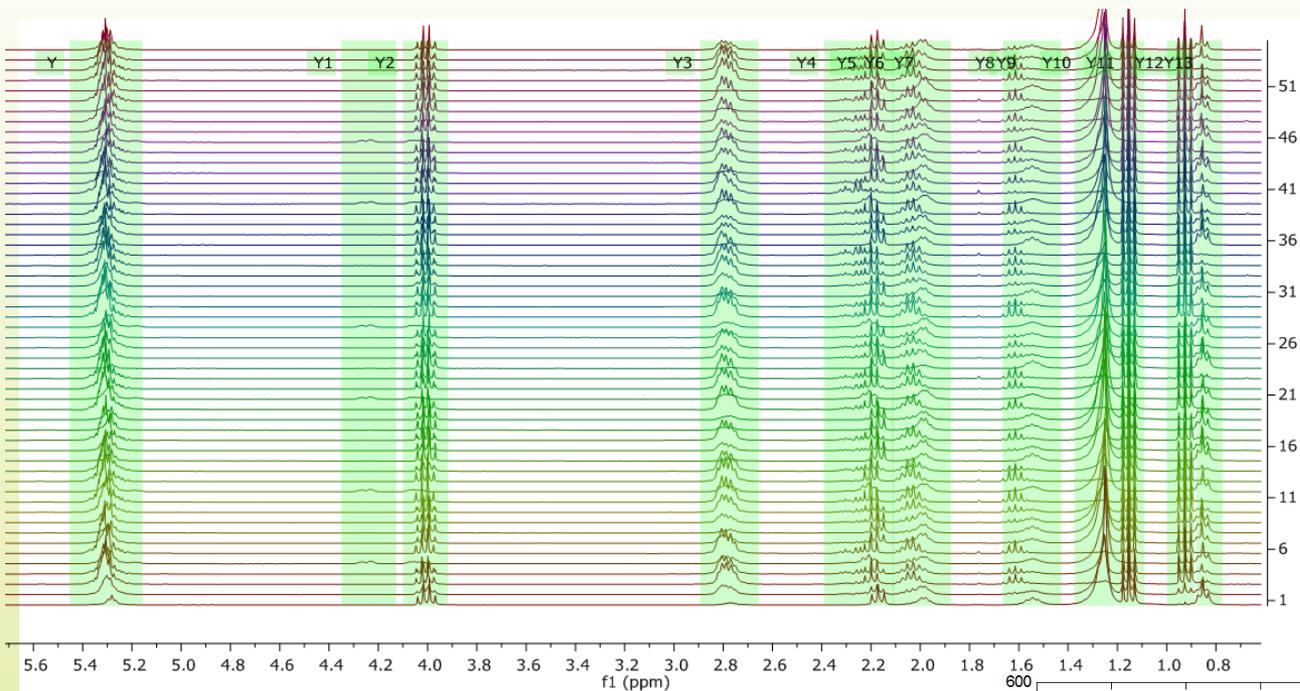




Partial Least Squares calculated with the SIMPLS algorithm
 X-block: SPC Files in MNova.xlsx 49 by 220
 Preprocessing: Mean Center
 Y-block: DHA GC Content.xlsx 49 by 1
 Preprocessing: Mean Center Num. LVs: 8
 Cross validation: venetian blinds w/ 7 splits
 RMSEC: 0.613656 RMSECV: 1.13119
 Bias: 0 CV Bias: -0.118407
 R² Cal: 0.99739 R² CV: 0.99137



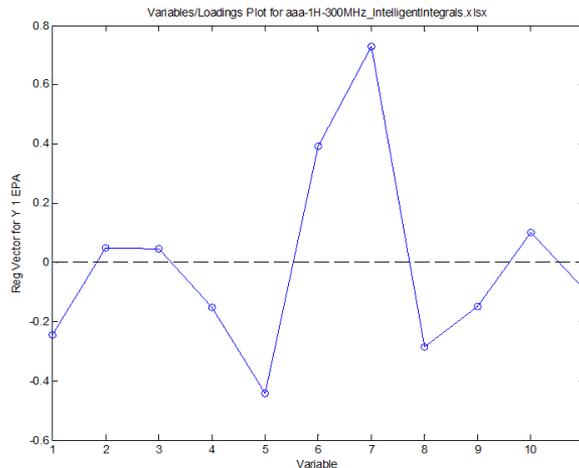
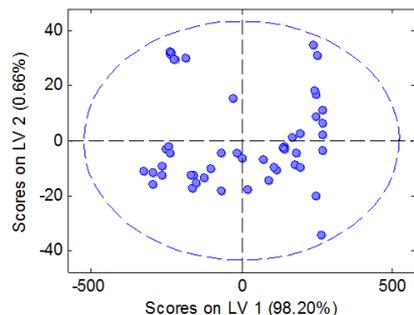
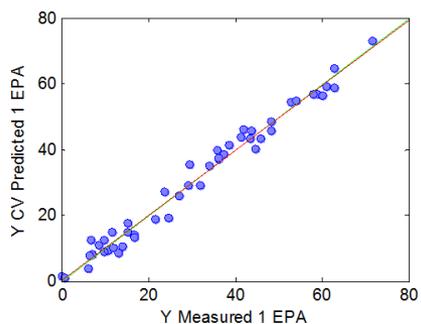
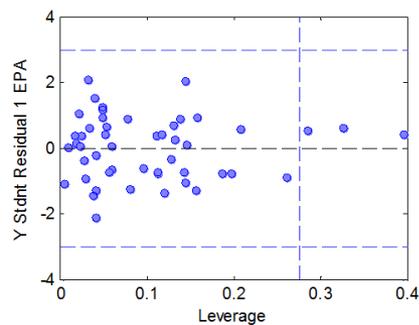
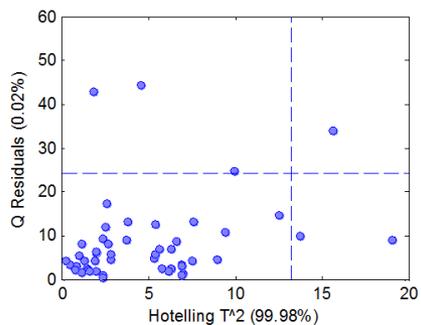
DHA PLS Regression Calibration – 60 MHz



300 MHz ^1H NMR Data

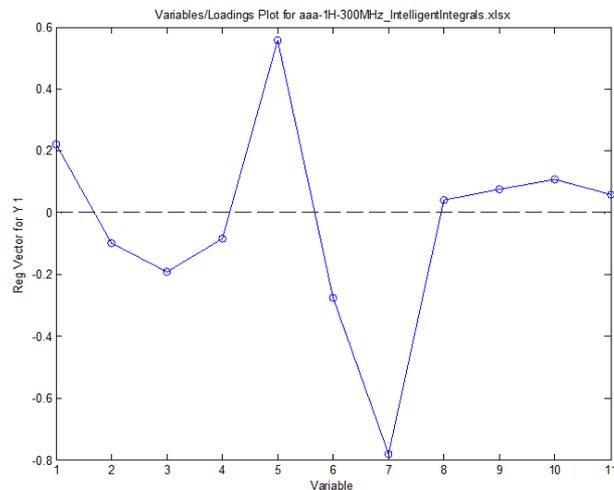
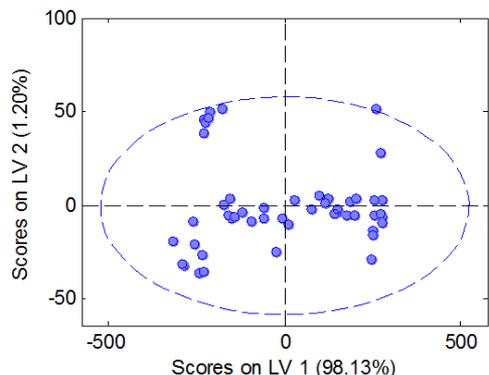
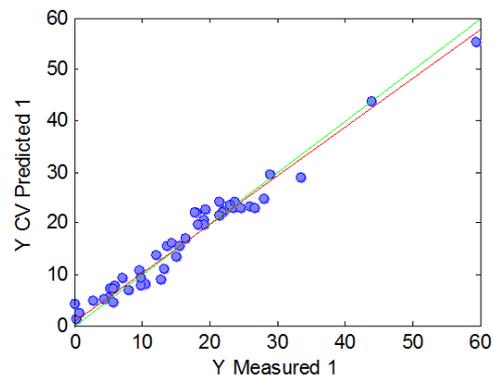
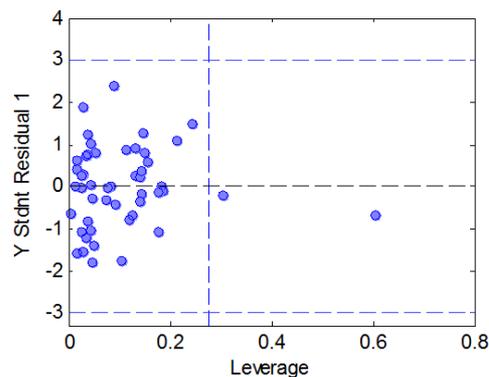
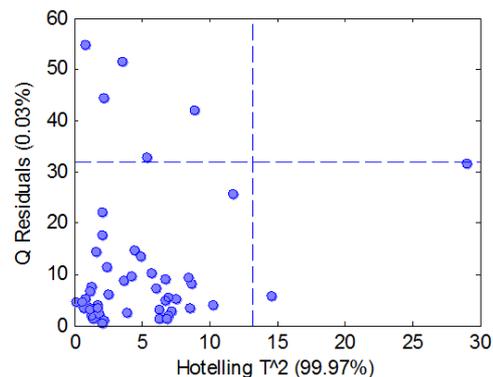
Stacked and integrated.

Integrals Utilized in PLS Regression
Against EPA/DHA content by GC



Partial Least Squares calculated with the SIMPLS algorithm
 X-block: aaa-1H-300MHz_IntelligentIntegrals.xlsx 49 by 11
 Preprocessing: Mean Center
 Y-block: EPA GC Content.xlsx 49 by 1
 Preprocessing: Mean Center Num. LVs: 5
 Cross validation: venetian blinds w/ 7 splits
 RMSEC: 2.20056 RMSECV: 2.70883
 Bias: 1.77636e-014 CV Bias: -0.116845
 R² Cal: 0.987561 R² CV: 0.98129

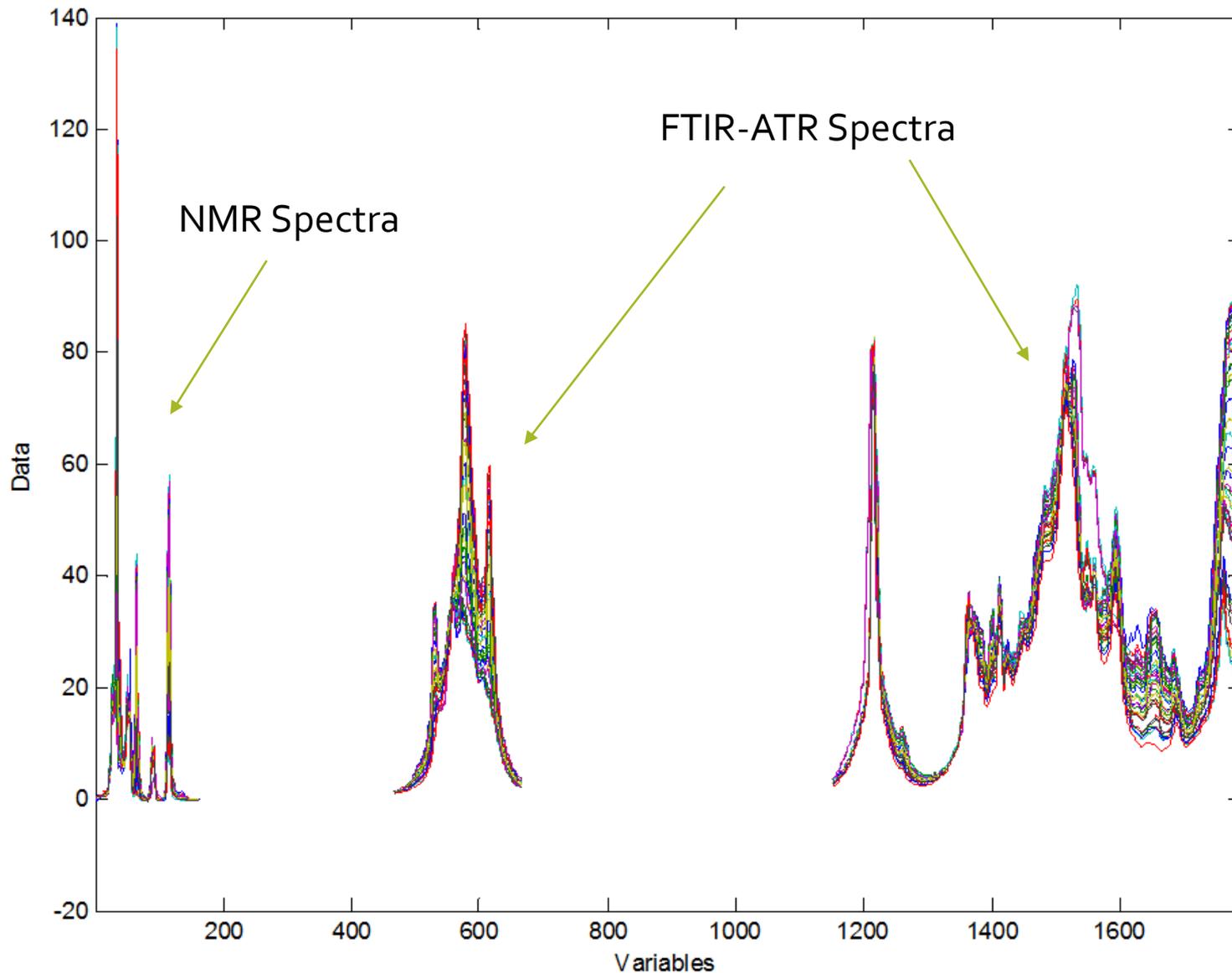
EPA PLS Regression Calibration – Peak Integrals



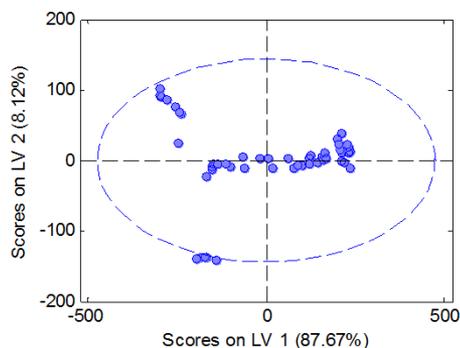
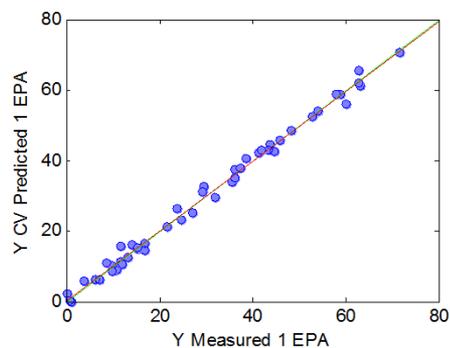
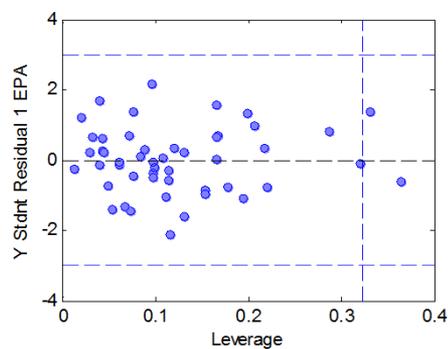
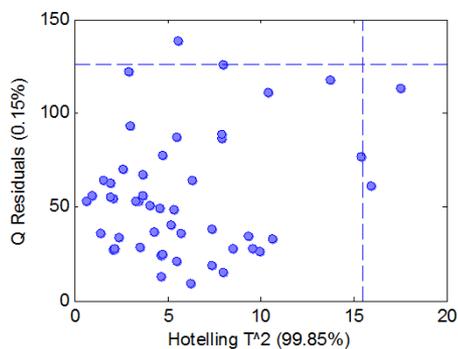
Partial Least Squares calculated with the SIMPLS algorithm
 X-block: aaa-1H-300MHz_IntelligentIntegrals.xlsx 49 by 11
 Preprocessing: Mean Center
 Y-block: DHA GC Content.xlsx 49 by 1
 Preprocessing: Mean Center Num. LVs: 5
 Cross validation: venetian blinds w/ 7 splits
 RMSEC: 1.8631 RMSECV: 2.24092
 Bias: -1.24345e-014 CV Bias: 0.00464222
 R² Cal: 0.974603 R² CV: 0.963534

DHA PLS Regression Calibration – Peak Integrals

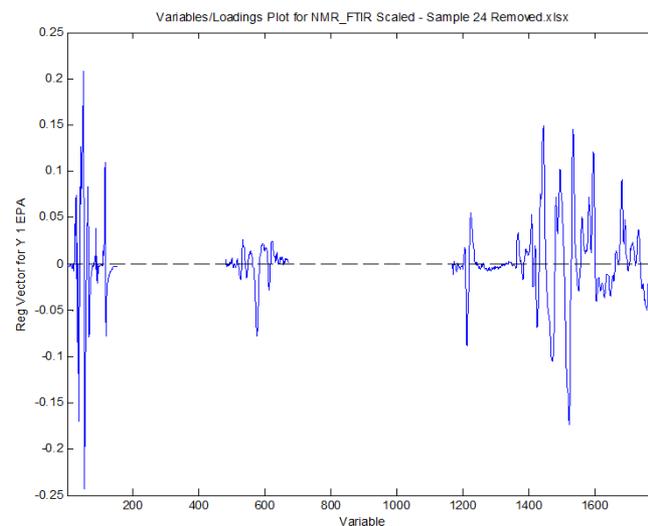
Fusion of ^1H NMR and FTIR-ATR Spectra – absolute intensity scaling



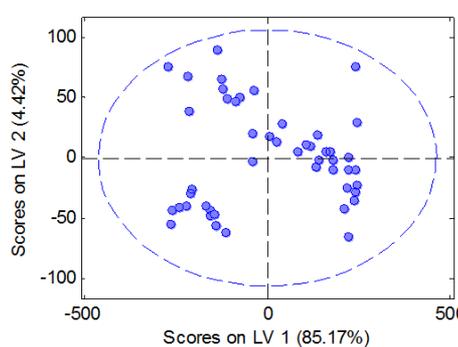
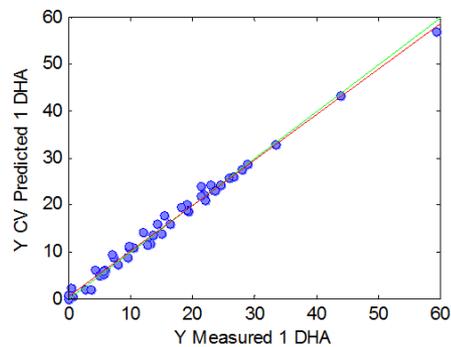
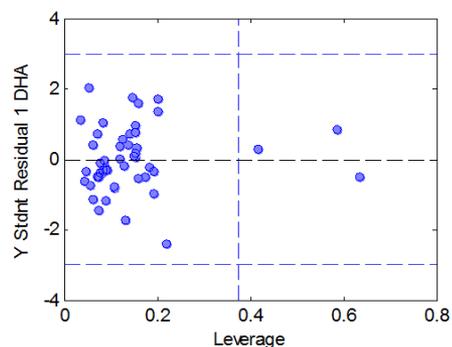
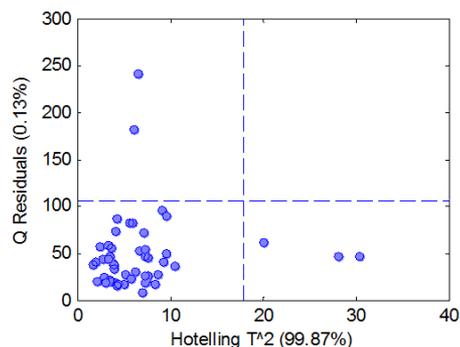
EPA Correlation with Combined and Scaled 1H NMR and FTIR-ATR



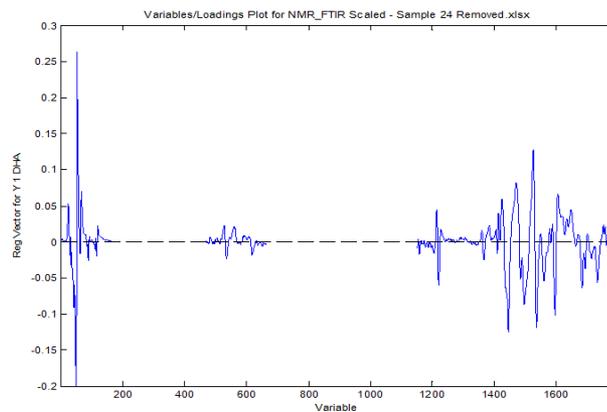
Partial Least Squares calculated with the SIMPLS algorithm
X-block: NMR_FTIR Scaled - Sample 24 Removed.xlsx 49 by 977
Preprocessing: Mean Center
Y-block: EPA GC Content - Sample 24 Removed.xlsx 49 by 1
Preprocessing: Mean Center Num. LVs: 6
Cross validation: venetian blinds w/ 7 splits
RMSEC: 1.16039 RMSECV: 1.62525
Bias: 3.55271e-015 CV Bias: -0.0432842
R² Cal: 0.996587 R² CV: 0.993315



DHA Correlation with Combined and Scaled 1H NMR and FTIR-ATR



Partial Least Squares calculated with the SIMPLS algorithm
X-block: NMR_FTIR Scaled - Sample 24 Removed.xlsx 49 by 998
Preprocessing: Mean Center
Y-block: DHA GC Content - Sample 24 Removed.xlsx 49 by 1
Preprocessing: Mean Center Num. LVs: 7
Cross validation: venetian blinds w/ 7 splits
RMSEC: 0.677763 RMSECV: 1.08301
Bias: -5.32907e-015 CV Bias: 0.010442
R² Cal: 0.996822 R² CV: 0.992286



Regression Results for Various NMR Approaches

PLS Regression Data	EPA Content (Wt%)	DHA Content (Wt%)
	R ² , SECV (Wt%)	R ² , SECV (Wt%)
60 MHz NMR	0.988, 2.13	0.991, 1.13
300 MHz NMR	0.993, 1.68	0.992, 1.09
Peak Integral Data	0.981, 2.71	0.964, 2.24
Fused - NMR-FTIR	0.993, 1.62	0.992, 1.08

24 Sample Validation Set		GC	NMR		GC	NMR	
Sample	Sample ID	DHA	DHA	Difference	EPA	EPA	Difference
1	Finished Product	20.64	20.71	0.07	47.77	45.53	-2.24
2	Finished Product	20.93	19.84	-1.09	33.22	32.74	-0.48
3	Mol Dist	23.94	25.7	1.76	35.49	36.01	0.52
4	First Esterification	4.5	3.81	-0.69	6.75	4.22	-2.53
5	Mol Dist	33.56	33.21	-0.35	46.39	44.56	-1.83
6	Mol Dist	12.63	12.11	-0.52	28.85	26.48	-2.37
7	First Esterification	23.09	22.76	-0.33	43.55	42.18	-1.37
8	Clathration	23.79	23.9	0.11	37.59	35.23	-2.36
9	Clathration	12.53	12.66	0.13	19.12	20.33	1.21
10	First Esterification	0.27	0.38	0.11	8.22	7.79	-0.43
11	First Esterification	0.15	0.67	0.52	4.18	3.62	-0.56
12	First Esterification	8.57	8.55	-0.02	19.12	17.25	-1.87
13	Crude Fish Oil	5.6	10.23	4.63	11.29	15.95	4.66
14	Crude Fish Oil	4.92	6.34	1.42	10.76	8.88	-1.88
15	Finished Product	23.36	24.27	0.91	56.9	57.17	0.27
16	Clathration	0.67	1.74	1.07	1.82	1.85	0.03
17	Crude Fish Oil	5.87	4.99	-0.88	13.34	13.23	-0.11
18	Clathration	14.59	15.15	0.56	30.63	7.09	-23.54
19	Clathration	20.8	20.71	-0.09	45.13	41.61	-3.52
20	Clathration	26.39	27.61	1.22	53.72	46.93	-6.79
21	Separator	20.59	20.86	0.27	62.27	61.34	-0.93
22	Separator	36.98	35.92	-1.06	42.69	44.45	1.76
23	First Esterification	0.09	5.27	5.18	0.3	-0.52	-0.82
24	Separator	13.59	12.83	-0.76	25.28	26.77	1.49
			SEV=	0.81		SEV =	1.64

*** - Viscosity Related Spectrum Issue and/or M-Distance Outlier

- Possible GC Error

Further Steps:

Models have been expanded from 74 samples to 250 samples better covering extreme EPA and DHA content samples

Validated against 50 samples provided by customer.

Validation was passed and 50 Validation Samples were included into the models.

NMR project found problems with the EPA GC analysis that were not known to the customers' laboratory and engineering personnel.

Near-Future Steps:

Laboratory system being purchased to provide rapid (2 minute analysis). Reduces GC to validation method and increases throughput of laboratory 20 fold.

On-line system will be purchased and placed into the process with a 6 stream sample system for real-time monitoring.

Models will be expanded to include full fatty acid distribution of the sample by ^1H NMR correlation to the full GC data provided for 300 samples.

New project on Manufacturing process of Polyalphaolefins is in final stages of feasibility and validation – expect on-line 60 MHz system in combination with TD-NMR for viscosity measurement.

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**Daniel Edwards
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Cosa-Xentaur**

**Practical Applications of NMR in Industry Conference
February 9-12, 2015
San Diego, CA**

<http://www.panicnmr.com/>