Mare Purum Project

Novia University of Applied Sciences

Report: Evaluation of using NIR, FTIR spectrometers in measurements of anaerobic digestion components.

Elena Sherman, Ngoc Dinh

Vaasa, 15.8.2013

Contents

1 Introduction

In this task, the interested objects are chemical constituents in a typical laboratory scale anaerobic digester and their concentration. By using near infrared (NIR) and FTIR spectrometers, spectral data of samples in the digestion progress were collected. With the help of the method partial least-squares (PLS), a model was computed by regression of a set of spectral data. Then, another set of the spectral data were applied to the model as the validation. The accuracy of the model is assessed based mainly on the coefficient of determination and RMSECV.

2 About IR

Infrared spectrums are emitted by any objects have temperature and our body can detect as heat. It is a portion of electromagnetic spectrum that include various radiation such as radio, microwave, X-rays, visible lights, ect. One source of radiation can emit many kinds of spectrum. One example is the natural sun at zenith which provides infrared radiation, visible light and ultraviolet rays. Each type of radiation is categorized by their unique range of wavelengths and energy. Infrared lights' wavelength is longer than the visible lights and shorter than microwave. IR radiations cause rotations and vibration movements in molecules.

Moreover, the infrared is also divided into many regions of wavelengths such as near-infrared, mid-infrared and far-infrared. However, the number of regions, their name and their wavelengths are different between the research fields and systems.

IR is applied in many scientific, engineering devices and home appliances as well.

3 Materials, methods before calibration

3.1 Samples

32 sample bottles which were taken out from the laboratory had been frozen to preserve their chemical components and prevent the activity of microorganisms. These samples were collected from four batch fermentations located in Novia's laboratory. The fermentation's inputs are mainly pig manure, waste from raw fish industrial treatment and waste from greenhouse plants and bacteria from Stormossen.

3.2 Pure components for spectroscopic measurements

Three components choosen for spiking are acetate, propionate and ammonium. The reasons to choose these ions because they can be found with relatively high concentrations in samples and we are interested in control of these ions' concentration because ammonium at high concentration can kill bacteria and volatile acid relates directly to the amount of methane gas produced. With each ion CH_3COO^-, $C_3H_5O_2^-$ and NH_4^+, their salts were prepared with ion concentration from 0.5 g/L to 5g/L at a step size of 0.5g/L. Three pairs of salt choosen are: Sodium acetate CH_3COONa and magnesium acetate

$(CH_3COO)_2Mg$; ammonium chloride NH_4Cl and ammonium sulfate $(NH_4)_2SO_4$; sodium propionate $C_3H_5O_2Na$ and calcium propionate $(C_3H_5O_2)_2Ca$. For sodium acetate, ammonium chloride and calcium propionate, the wanted concentrations of ion acetate, ammonium and propionate respectively are 1, 2, 3, 4 and 5 g/L. For magnesium acetate, ammonium sulfate and sodium propionate, the wanted concentrations of ion acetate, ammonium and propionate respectively are 0.5, 1.5, 2.5, 3.5 and 4.5 g/L. For the ease of preparing, a 5g/L solution of each salt was made and then we diluted these salt solutions with distilled water to get the wanted concentration of ion.

First, we calculated the amount (volume) of needed 5g/L solution to obtain 5 solutions of different concentration:

$$n_1 = n_2 = c_1 \times V_1 = c_2 \times V_2$$

$$\rightarrow V_1 = \frac{c_2 \times V_2}{c_1}$$

For example, below is the calculation for 0,5g/L:

$$V_1 = \frac{c_2 \times V_2}{c_1} = \frac{0.5\frac{\text{g}}{\text{L}} \times 50\text{mL}}{5\frac{\text{g}}{\text{L}}} = 5\text{mL}$$

Table 1 below shows the results of calculation for the needed volume of 5g/L solution and the volume of distilled water to obtain the needed ion concentration.

Table 1: Mixing table to get the interested ions concentration from a 5g/L solution

[Ion] [g/L]	**Volume of 5g/L solution** [mL]	**Volume of distilled water added** [mL]	**Total volume** [mL]
0.5	5	45	50
1	10	40	50
1.5	15	35	50
2	20	30	50
2.5	25	25	50
3	30	20	50
3.5	35	15	50
4	40	10	50
4.5	45	5	50
5	50	0	50

For the series of concentration are 0.5, 1.5, 2.5, 3.5 and 4.5 g/L, the total needed volume of 5g/L solution is 5+15+25+35+45=125 mL ; for the series 1, 2, 3, 4 and 5 g/L, the total needed volume of 5g/L solution is 10+20+30+40+50=150 mL. We prepared each salt with size of 200mL of 5g/L ion concentration. The mass salts needed were calculated:

	Ion[g]	Salt[g]
1 mole of salt	M_{ion}	M_{salt}
A mole of salt in 1L	5	x
B mole of salt in 200ml	1	y

$$\frac{M_{\text{ion}}}{M_{\text{salt}}} = \frac{5}{x} = \frac{1}{y}$$

$$\rightarrow \quad y = \frac{M_{\text{salt}}}{M_{\text{ion}}}$$

Table 2 below shows the mass of salt needed for a 200 mL solution of 5g/L ion concentration.

Table 2: Mass of salts needed for 5g/L ion concentration

	M_{ion} [g]	**M_{salt}[g]**	**y [g]**
Sodium acetate	59	82.03	1.390
Magnesium acetate	118	214.305	1.816
Ammonium chloride	18	53.5	2.972
Ammonium sulfate	36	132.06	3.668
Sodium propionate	73	95.99	1.315
Calcium propionate	146	186.078	1.275

We also prepared pure components with lower concentration. With Sodium acetate, Ammonium chloride and Calcium propionate, the set of concentration is: 0.05; 0.15; 0.25; 0.35 and 0.45. With Magnesium acetate, Ammonium sulfate and Sodium propionate, the set of concentration is: 0.1; 0.2; 0.3; 0.4 and 0.5. For the ease of preparation, a solution of 5g/L ion concentration of each salts were prepared to be diluted to get the lower concentration. New pure salts were filled in 2mL Eppendorf tubes. This time we also applied the formula $V_1 = \frac{c_2 \times V_2}{c_1}$ to get the volume of the needed 5g/L solution. The volume of needed 5g/L and the volume of distilled water which were used to prepare these salts are presented in the table 3:

Table 3: Mixing tables for pure components

[Ion][g/L]	0.05	0.15	0.25	0.35	0.45	0.1	0.2	0.3	0.4	0.5
V solution of 5g/L [µL]	20	60	100	140	180	40	80	120	160	200
V distilled water[µL]	1980	1940	1900	1860	1820	1960	1920	1880	1840	1800
Total V µL	2000	2000	2000	2000	2000	2000	2000	2000	2000	2000

3.3 Spiking solutions

Samples would be added with ions acetate, propionate and ammonium. Spiking solution used are sodium propionate, ammonium sulfate and magnesium acetate with ion concentration of 100g/L. Each of the 32 samples was divided into 14 sub-samples with volume of 1mL each which later were spiked. The volume of each pure spiking solution for each sub-sample was calculated based on its coded values in the cube of a central composite design. With this design, the correlation between components can be broken. Table 4 gives the codes and spike size.

Table 4: Spike codes and spike size

	Spiking scheme (coded)			Spike size [g/L]		
	Ammonium	Acetate	Propionate	Ammonium	Acetate	Propionate
0	-1	-1	-1	0.00	0.00	0.00
1	-1	-1	1	0.00	0.00	0.50
2	-1	1	-1	0.00	0.50	0.00
3	-1	1	1	0.00	0.50	0.50
4	1	-1	-1	0.50	0.00	0.00
5	1	-1	1	0.50	0.00	0.50
6	1	1	-1	0.50	0.50	0.00
7	1	1	1	0.50	0.50	0.50
8	0	0	-1	0.25	0.25	0.00
9	0	0	1	0.25	0.25	0.50
10	0	-1	0	0.25	0.00	0.25
11	0	1	0	0.25	0.50	0.25
12	-1	0	0	0.00	0.25	0.25
13	1	0	0	0.50	0.25	0.25

Appendix 1 shows one example of how to calculate the spiking volume from spike size. In table 5, spiking volume of each component is presented. We can see that the volume of each component in one type of spiking solution is proportional with each other with a factor of 1 or 0.5, for the easy of prepare, we prepared 13 spiking solutions with proportions of components within one spiking solution as shown in table 5. As spiking, we added one spiking solution to one sub-samples with total volume represented in table 5.

Table 5: Spiking volume, portions and total volume

	Spiking volume [mL]			Proportion			Total volume [µL]
#	Ammonium	Acetate	Propionate	Ammonium	Acetate	Propionate	
0	0.00000	0.00000	0.00000	0	0	0	0.000
1	0.00000	0.00000	0.00503	0	0	1	5.03
2	0.00000	0.00503	0.00000	0	1	0	5.03
3	0.00000	0.00505	0.00505	0	1	1	10.10
4	0.00503	0.00000	0.00000	1	0	0	5.03
5	0.00505	0.00000	0.00505	1	0	1	10.10
6	0.00505	0.00505	0.00000	1	1	0	10.10
7	0.00508	0.00508	0.00508	1	1	1	15.22
8	0.00251	0.00251	0.00000	1	1	0	5.03
9	0.00253	0.00253	0.00505	0.5	0.5	1	10.10
10	0.00251	0.00000	0.00251	1	0	1	5.03
11	0.00253	0.00505	0.00253	0.5	1	0.5	10.10
12	0.00000	0.00251	0.00251	0	1	1	5.03
13	0.00505	0.00253	0.00253	1	0.5	0.5	10.10

3.4 Preparation of spiking solution

As seen from the table above, the needed amount of master solution is small, we prepared 100mL of each spiking solution. Calculation for mass of salts for spiking solution is similar to that of 5g/L. The mass of each salt calculated is shown in table 6.

	Ion[g]	**Salt[g]**
1 mole of salt	**M_{ion}**	**M_{salt}**
A mole of salt in 1L	100	x
B mole of salt in 100ml	10	y

$$\frac{M_{\text{ion}}}{M_{\text{salt}}} = \frac{5}{x} = \frac{10}{y}$$

$$\rightarrow \quad y = \frac{M_{\text{salt}}}{M_{\text{ion}}} \times 10$$

Table 6: Mass of salts for spiking solutions

Salt	M_{ion} [g]	M_{salt}[g]	y [g]
Magnesium acetate	118	214.305	18.161
Ammonium sulfate	36	132.06	36.683
Sodium propionate	73	95.99	13.149

After spiking, these sub-samples were centrifuged and measured by the NIR and Mid-IR spectroscopy two times each sample.

3.5 Recalculation of components concentration

As said before, the ions ammonium, acetate and propionate are found in the digester input. After spiking, the amount of these ions increased, as well the volume of the samples, which leads to a change in the concentration of each ion. The initial ion concentration in 00- samples were measured in Ketek laboratory and can be found in Excel file attached. Below is the formula for calculating the ion concentration after spiking:

$$c_{1.\text{ion}} = \frac{c_{0.\text{ion}} \times V_{sa} + c_s \times V_{\text{s}}}{V_{\text{sa}} + V_{\text{ts}}}$$ with $c_{0.\text{ion}}$, $c_{1.\text{ion}}$ and c_s is the ion concentration in the pre-spike (00) sample, the post-spike and the spiking solution; V_{sa}, V_{s} and V_{ts} is the volume of the sample before spike, the spiking solution containing that ion and the total spiking solution. For instance, the concentration of ion acetate in the sample F01SA06SP03 after spiking is calculated:

$$c_{\text{acetate}} = \frac{2443\frac{\mu\text{g}}{\text{mL}} \times 1\text{mL} + 100\frac{\text{g}}{\text{L}} \times 0.00505\text{mL} \times \frac{1000\mu\text{g}}{1\text{mg}}}{1\text{mL} + 0.01010\text{mL}} = 2918.526\frac{\mu\text{g}}{\text{mL}}$$

Total volatile fatty acid TVFA includes acid acetic and acid propionic so the new concentration of TVFA derives from the addition of acetate and propionate.

3.6 Filenames

Data from the spectrometers has been saved on the computer in needed format as ASC and dat which are format that Matlab can read well. A filename containing the paths to these files were made.

4 Calibration

4.1 NIR Calibration and Results

Spectra range of NIR data: 908-1682 nm.

For each ion, 6 models were computed:

Model 1: Pure components

Model 2: Spiked samples

Model 3: Process samples

The calibration data and validation data are taken from the NIR measurement of samples and pure components. We wrote a script in Matlab to split spectra data to 2 parts: calibration data as every first

and second samples or pure components and the validation data as every third samples or pure components.

Model 4: Calibration data from spiked samples, validation data from process samples

Model 5: Calibration data from process samples, validation data from pure salts

Model 6: Calibration data from spiked samples, validation data from pure salts.

Table 7: PLS calibration models for acetate

	Pure on pure	Proc. on proc.	Spike on proc.	Proc. on pure	Spike on pure	Spike on spike
# spectra in calibration	76	44	832	64	832	556
# spectra in validation	38	20	64	114	114	276
# LVs.	7	6	9	6	9	4
Cross validation[splits]	8	6	10	8	10	10
RMSEC[mg/L]	372	294	420	410	420	556
RMSECV[mg/L]	686	362	434	474	434	559
RMSEP[mg/L]	1007	709	388	3210	1283	602
R^2 Prediction	0.338	0.547	0.81	0.04	0.01	0.55
Bias[mg/L]	1.3E-11	-1.2E-11	4.1E-11	-1.9E-12	4.1E-11	3.4E-12

Table 8: PLS calibration models for propionate

	Pure on pure	Proc. on proc.	Spike on process	Proc. on pure	Spike on pure	Spike on spike
# spectra in calibration	76	44	832	64	832	556
# spectra in validation	38	20	64	114	114	276
# LVs.	3	6	5	6	5	4
Cross validation[splits]	8	6	10	8	10	10
RMSEC[mg/L]	937	252	376	282	376	381
RMSECV[mg/L]	996	378	378	337	378	386
RMSEP[mg/L]	206	365	433	1701	1293	381
R^2 Prediction	0.22	0.46	0.25	0.011	0.00	0.44
Bias[mg/L]	1.1E-12	-6.1E-12	1.1E-11	-4.5E-13	1.1E-11	-2.0E-12

Table 9: PLS calibration models for TVFA

	Pure on pure	Proc. on proc.	Spike on process	Proc. on pure	Spike on pure	Spike on spike
# spectra in calibration	76	44	832	64	832	556
# spectra in validation	38	20	64	114	114	276
# LVs.	8	6	5	6	5	10*
Cross validation[splits]	8	6	10	8	10	10
RMSEC[mg/L]	304	771	1324	932	1324	833
RMSECV[mg/L]	785	1027	1331	1081	1331	880
RMSEP[mg/L]	942	1675	1242	5881	3638	902
R^2 Prediction	0.656	0.63	0.74	0.01	0.07	0.86
Bias[mg/L]	6.2E-12	-4.0E-11	6.1E-11	-5.4E-12	6.1E-11	-1.0E-10

Table 10: PLS calibration models for ammonium

	Pure on pure	Proc. on proc.	Spike on proc.	Proc. on pure	Spike on pure	Spike on spike
# spectra in calibration	76	44	832	64	832	556
# spectra in validation	38	20	64	114	114	276
# LVs.	5	8	3	6	3	14*
Cross validation[splits]	8	6	10	8	10	10
RMSEC[mg/L]	289	102	267	156	267	130
RMSECV[mg/L]	378	132	269	184	269	160
RMSEP[mg/L]	266	298	399	1072	1425	184
R^2 Prediction	0.955	0.63	0.52	0.48	0.01	0.87
Bias[mg/L]	-1.3E-11	-5.0E-12	6.1E-12	-4.5E-13	6.1E-12	-6.3E-12

(*) choose another LV but not the suggested one

4.2 MIR calibration and Results

For the each component, 6 models were made. All on All means calibration data and validation data are from splited process samples, spiked samples and pure salts. Results of calibration models are shown in tables 11; 12; 13 and 14:

Table 11: Calibration and validation for acetate

	Pure on Pure	Proc. on Proc.	Spike on Proc.	Proc. on pure	Spike on Pure	All on All
# spectra in calibration	88	44	831	64	831	628
# spectra in validation	42	20	64	130	130	312
RMSEC [mg/L]	132	384	359	352	410	404
RMSECV [mg/L]	292	462	367	427	412	421
RMSEP [mg/L]	302	393	381	1185	1012	406
R^2	0.97	0.81	0.83	0.22	0.69	0.82
CV Bias [mg/L]	10.6	-22.3	-0.3	2.1	-0.1	3.2
Latent Variables	9	3	6	4	4	7

Table 12: Calibration and validation of ammonium

	Pure on Pure	Proc. on Proc.	Spike on Proc.	Proc. on pure	Spike on Pure	All on All
# spectra in calibration	88	44	831	64	831	628
# spectra in validation	42	20	64	130	130	312
RMSEC [mg/L]	85	43	117	68	117	110
RMSECV [mg/L]	106	82	120	99	120	116
RMSEP [mg/L]	117	221	84	618	831	109
R^2	0.99	0.92	0.94	0.72	0.85	0.95
CV Bias [mg/L]	2.07	-2.57	-0.01	-6.38	-0.01	-0.45
Latent Variables	4	6	7	6	7	8

Table 13: Calibration and validation of propionate

	Pure on Pure	Proc. on Proc.	Spike on Proc.	Proc. on pure	Spike on Pure	All on All
# spectra in calibration	88	44	831	64	831	628
# spectra in validation	42	20	64	130	130	312
RMSEC [mg/L]	437	359	404	370	404	401
RMSECV [mg/L]	582	442	407	374	407	405
RMSEP [mg/L]	573	344	406	319	614	419
R^2	0.79	0.43	0.36	0.76	0.65	0.36
CV Bias [mg/L]	0.89	-6.82	-0.05	-7.95	-0.05	-0.01
Latent Variables	5	3	3	2	3	3

Table 14: Calibration and validation of TVFA

	Pure on Pure	Proc. on Proc.	Spike on Proc.	Proc. on pure	Spike on Pure	All on All
# spectra in calibration	88	44	831	64	831	628
# spectra in validation	42	20	64	130	130	312
RMSEC [mg/L]	184	415	893	708	893	951
RMSECV [mg/L]	226	828	909	930	909	966
RMSEP [mg/L]	193	1218	976	3131	2378	963
R^2	0.98	0.90	0.86	0.37	0.83	0.84
CV Bias [mg/L]	-4.13	-53.00	-1.18	-6.78	-1.18	0.15
Latent Variables	5	7	6	5	6	5

Scatter plots of concentration from calibration and validation for each model and each constituent are represented in appendix 2.

Appendix

Appendix 1: Examples how to calculate spiking volume from spike size for sub-samples # 9

9

Given

$$\frac{x \cdot 100}{1 + (x + y + z)} = 0.25$$

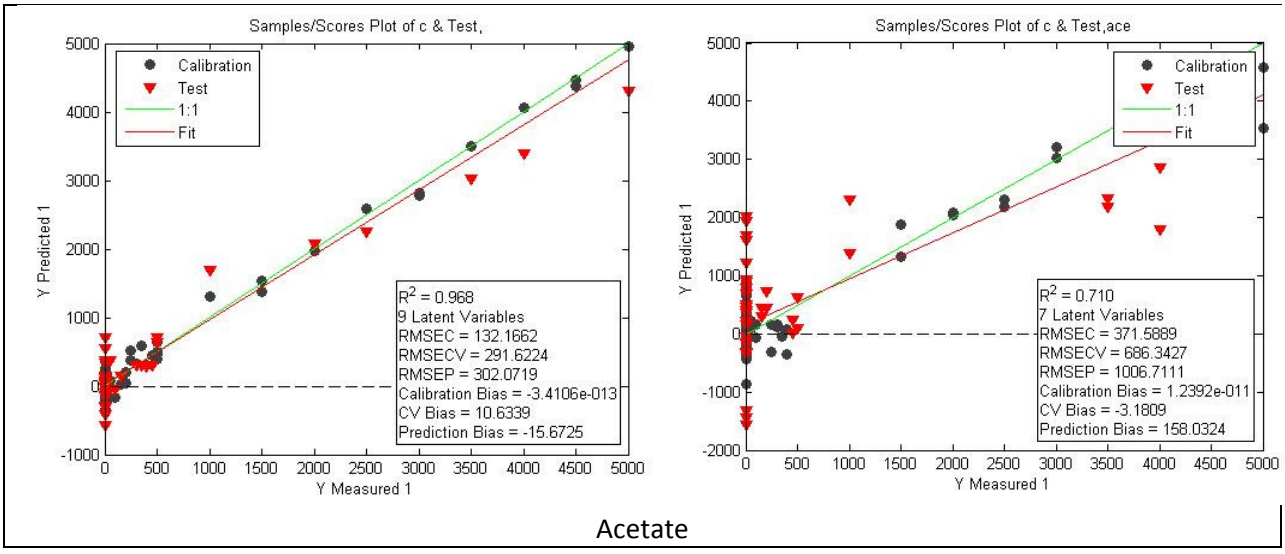
$$\frac{y \cdot 100}{1 + (x + y + z)} = 0.25$$

$$\frac{z \cdot 100}{1 + (x + y + z)} = 0.5$$

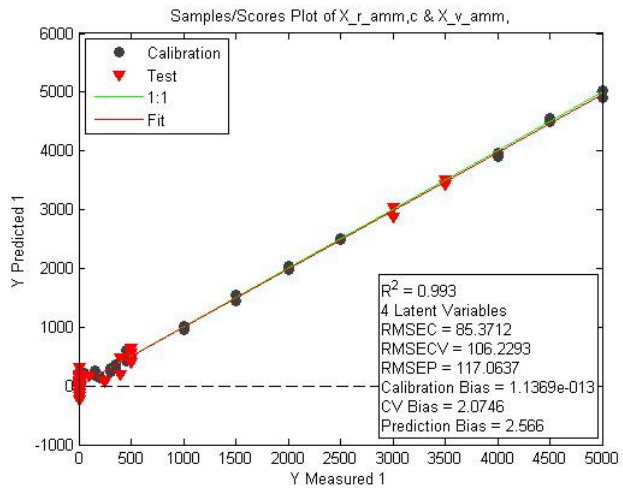
$$\text{Find}(x,y,z) \rightarrow \begin{pmatrix} 0.002525252525252525252525 \\ 0.002525252525252525252525 \\ 0.005050505050505050505051 \end{pmatrix}$$

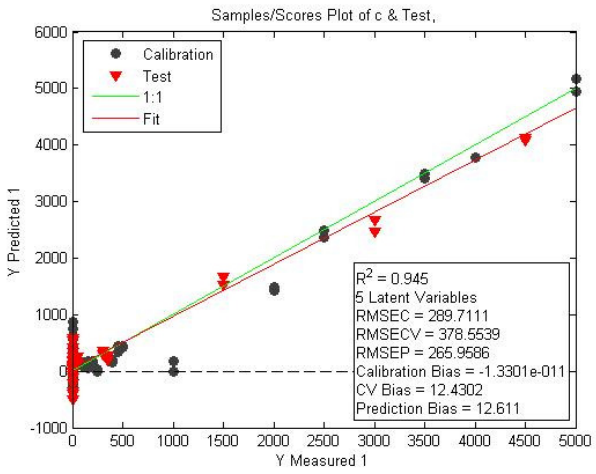
Appendix 2 : MIR and NIR scatter plots

1 Pure on pure, left: MIR, right: NIR

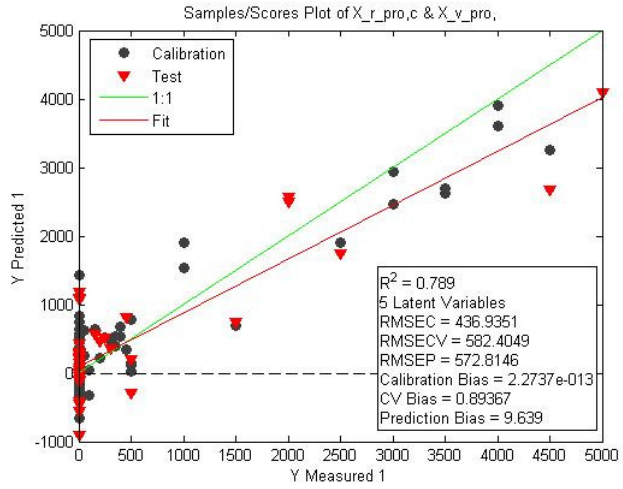


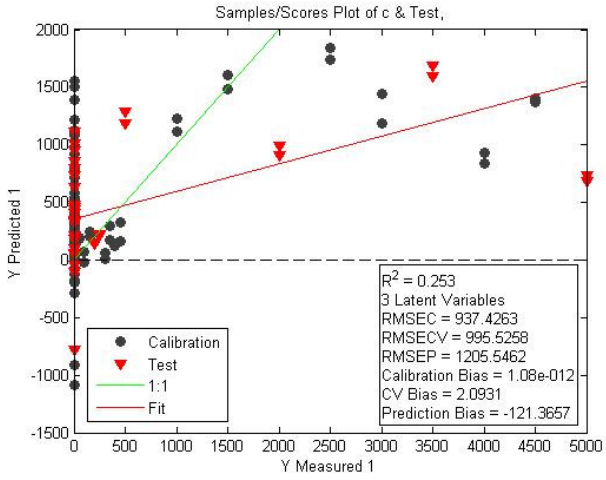
Acetate



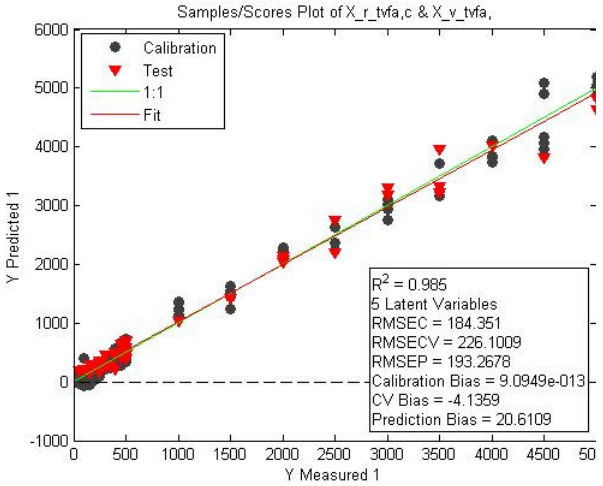


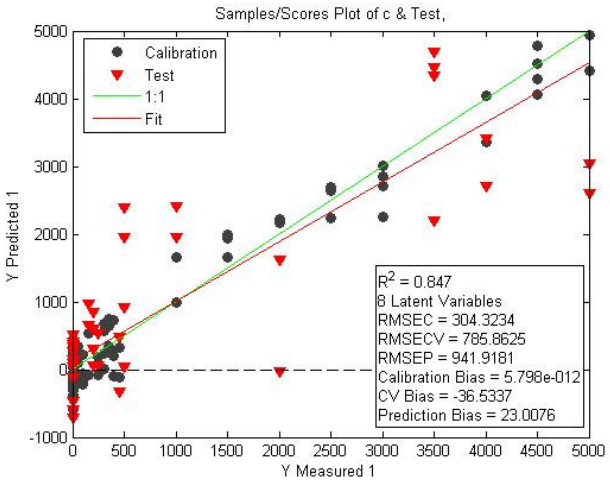
Ammonium





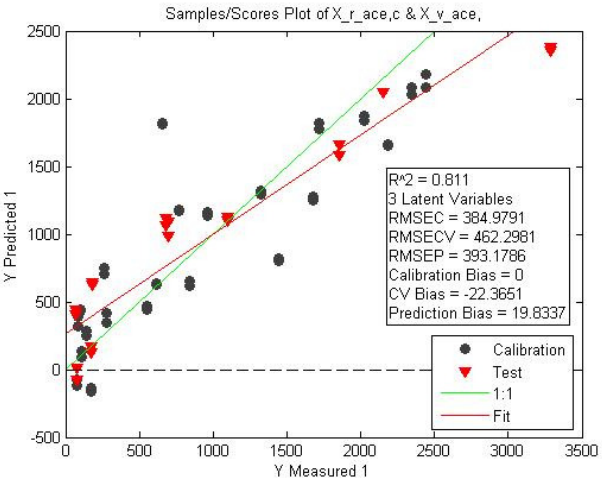
Propionate

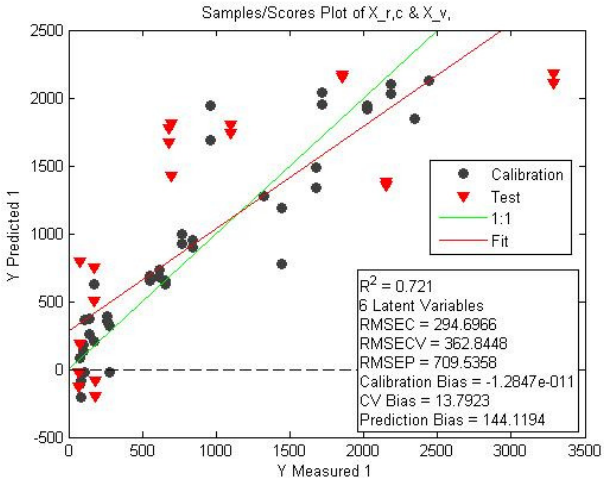




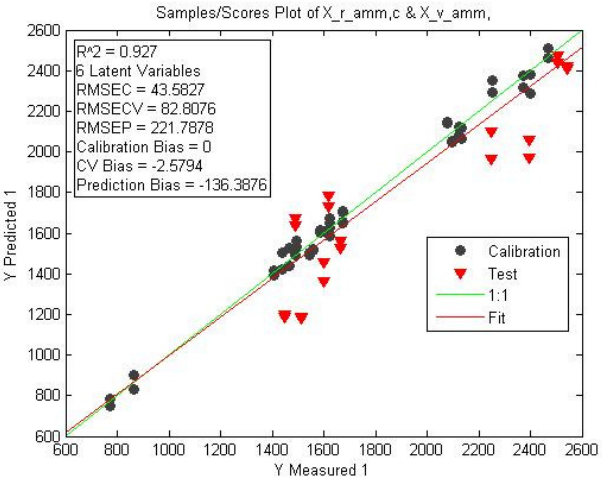
TVFA

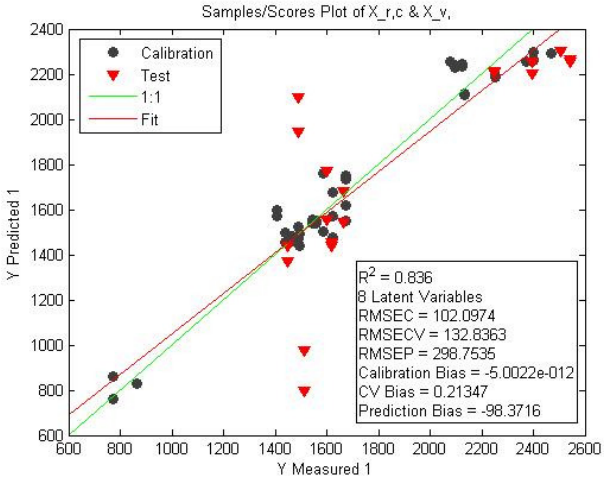
2 Process on process, left: MIR, right: NIR



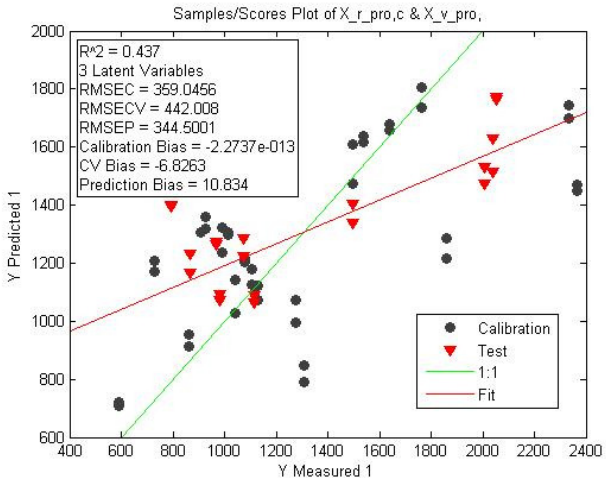


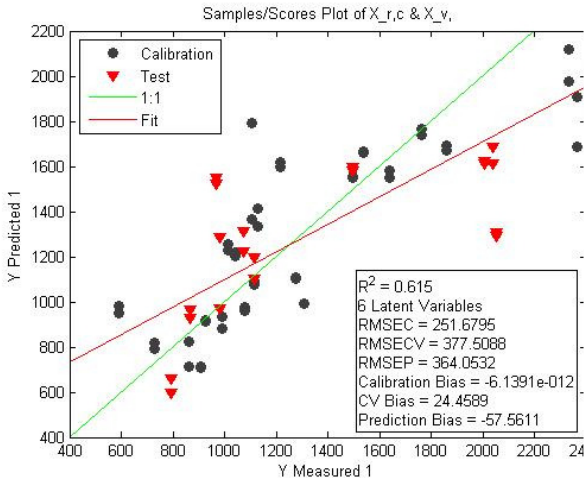
Acetate



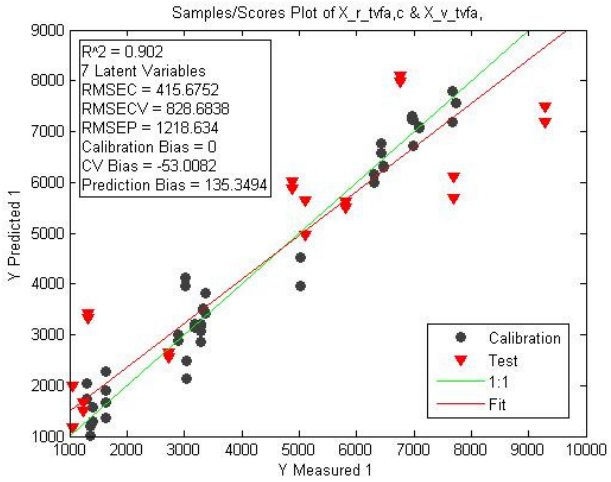


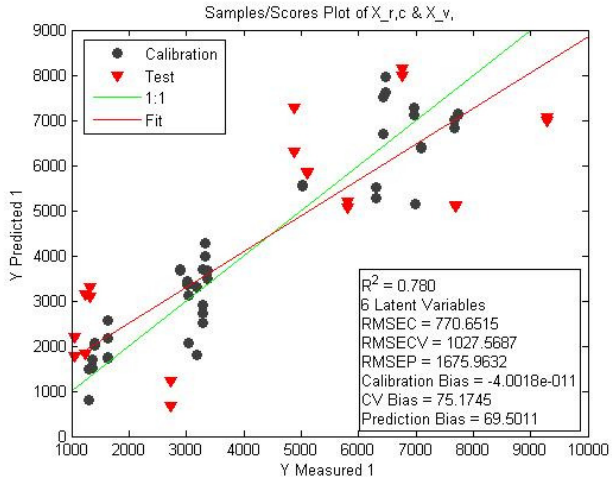
Ammonium





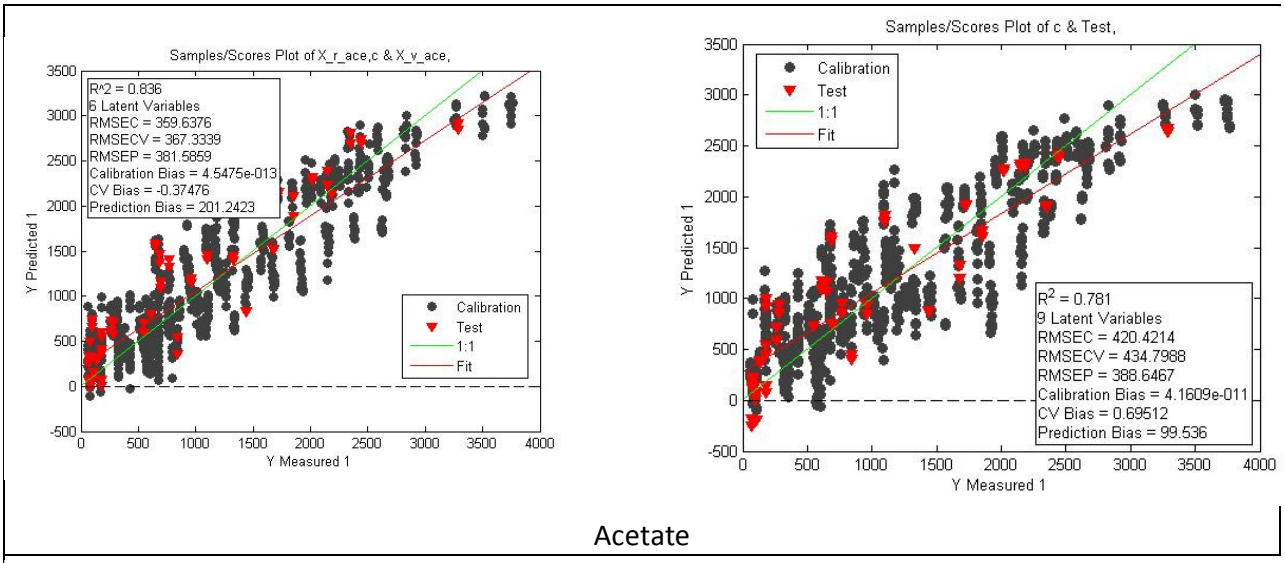
Propionate



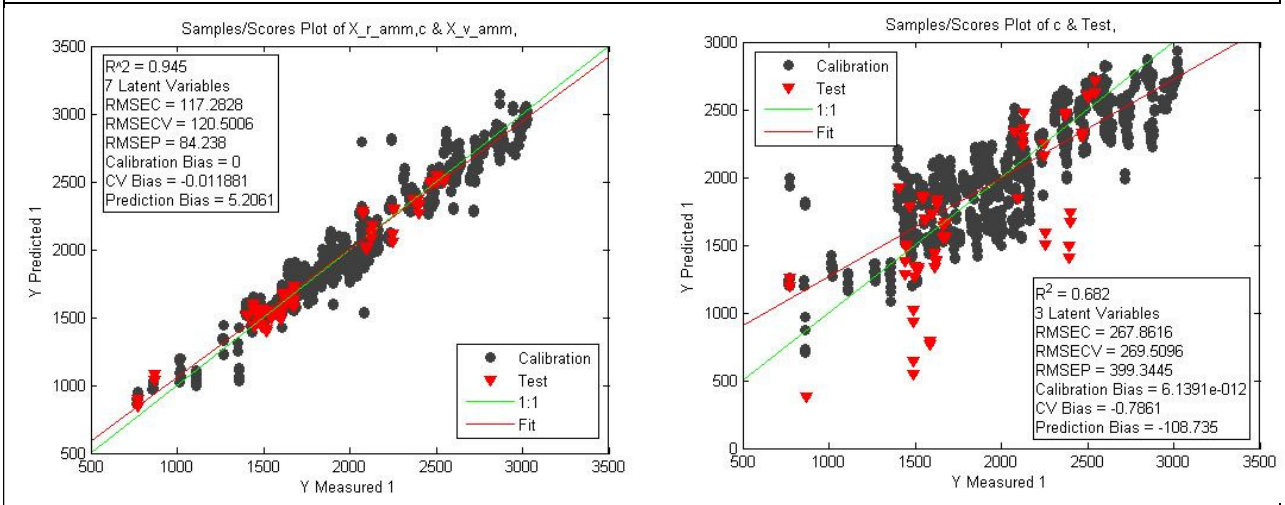


TVFA

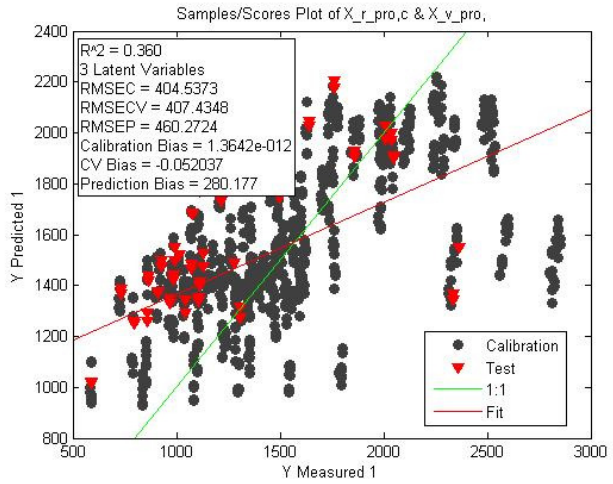
3 Spike on process , left: MIR, right: NIR

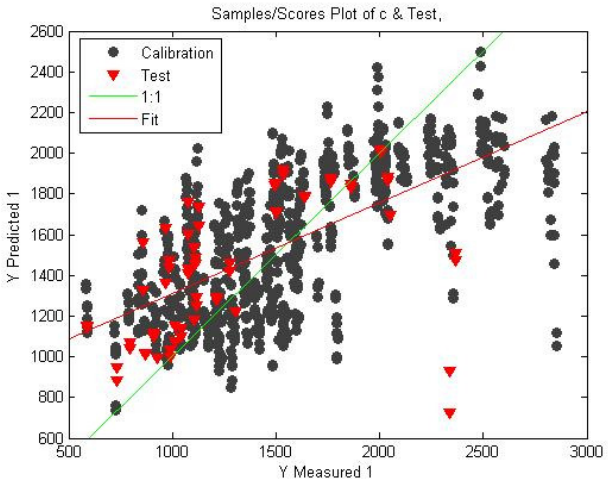


Acetate

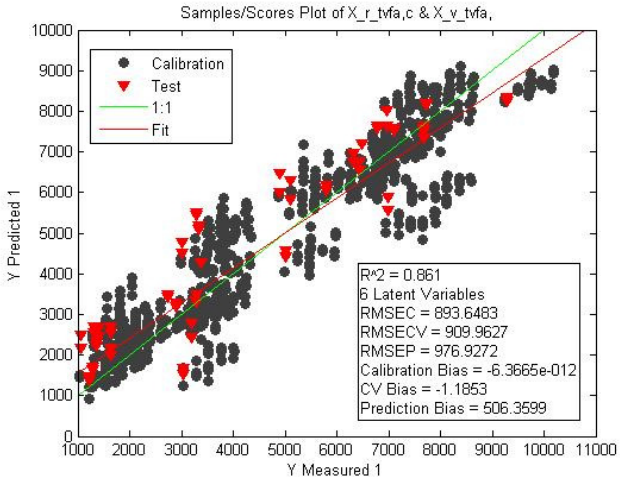


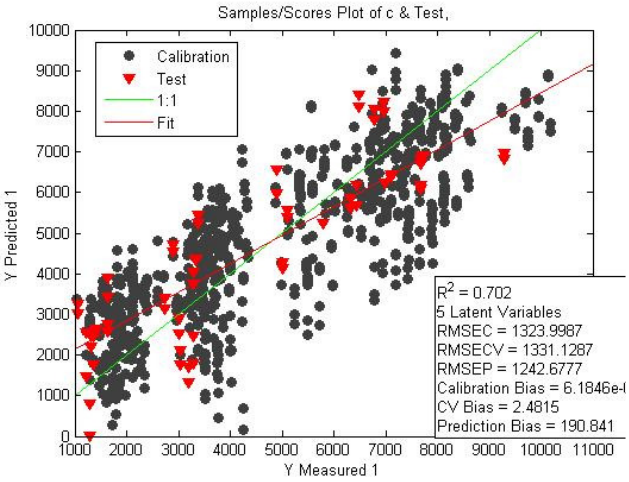
Ammonium





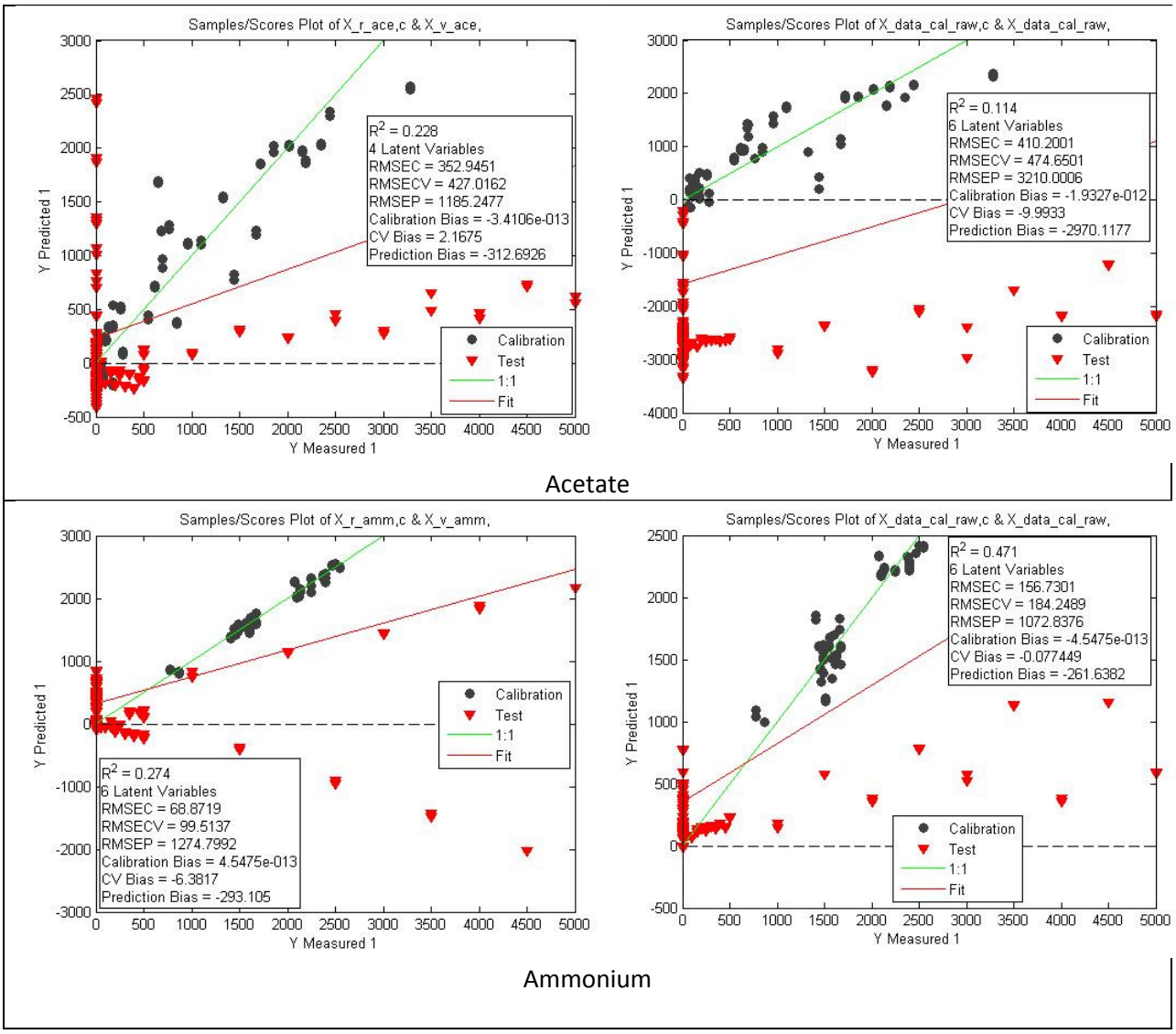
Propionate

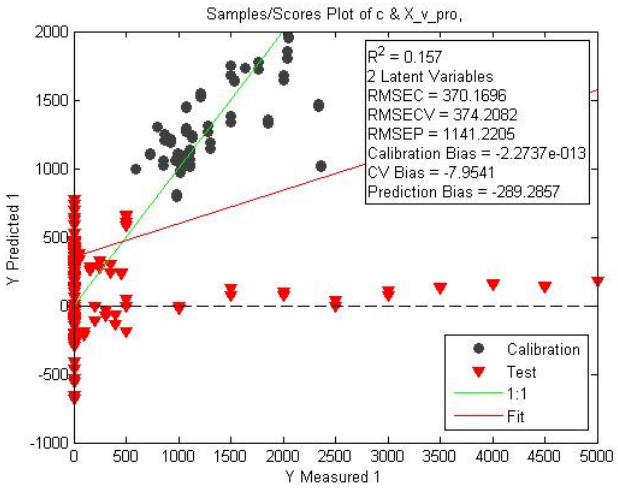


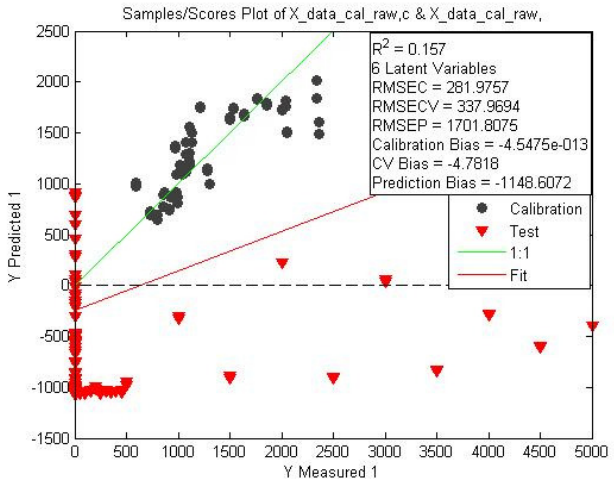


TVFA

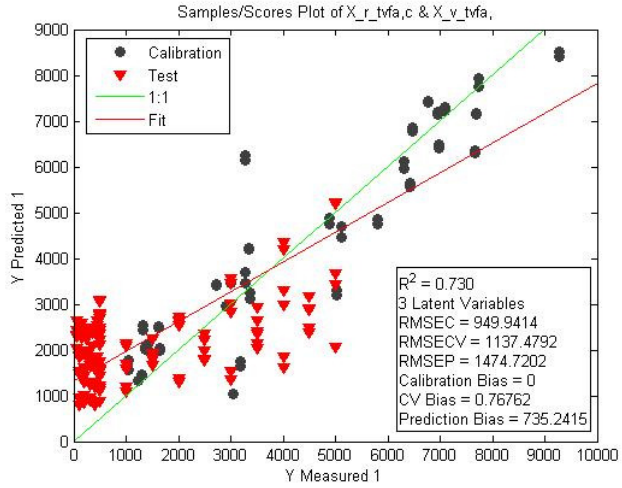
4 Process on pure, left: MIR, right: NIR

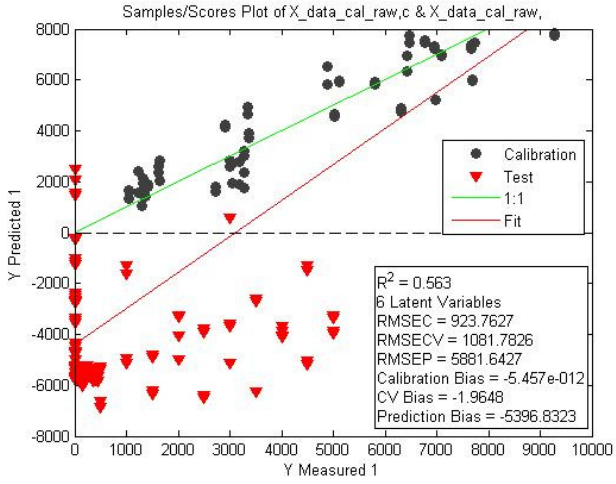






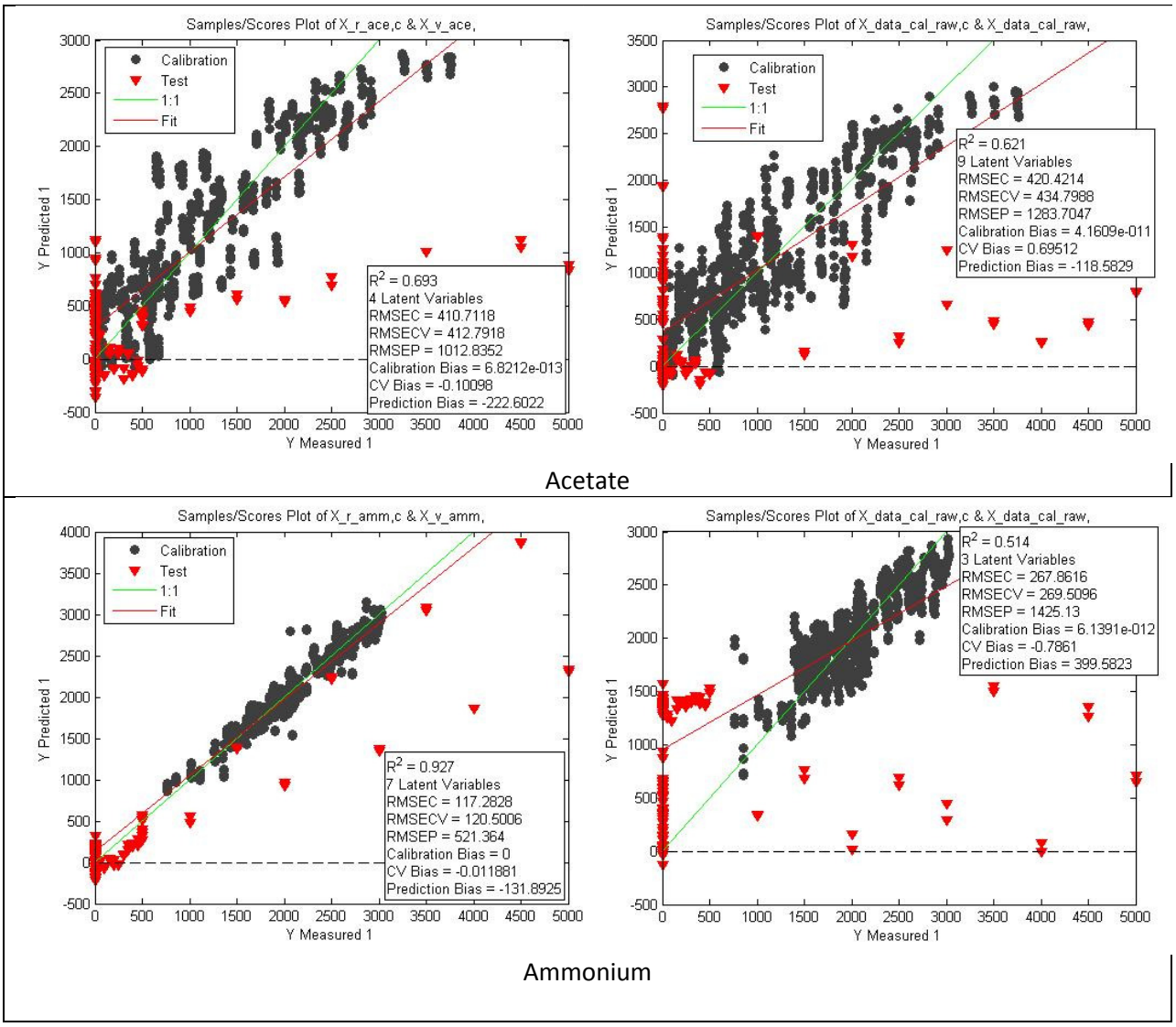
Propionate

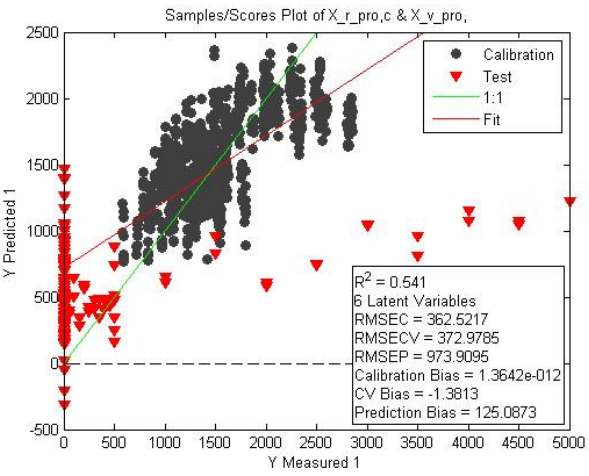


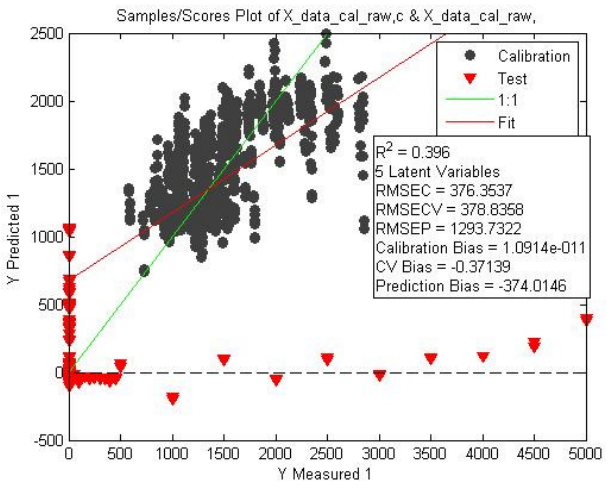


TVFA

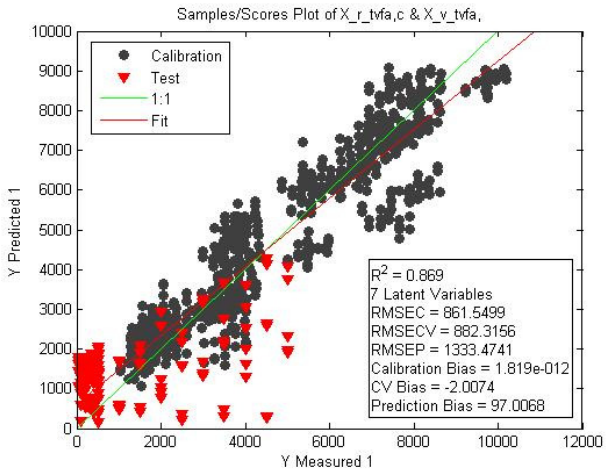
5 Spike on pure, left: MIR, right: NIR

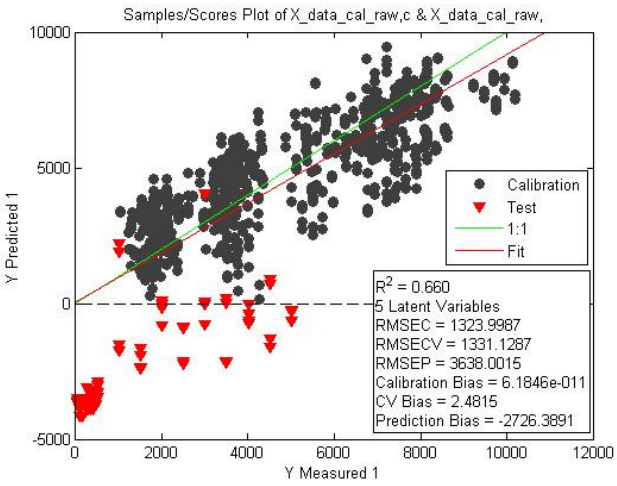






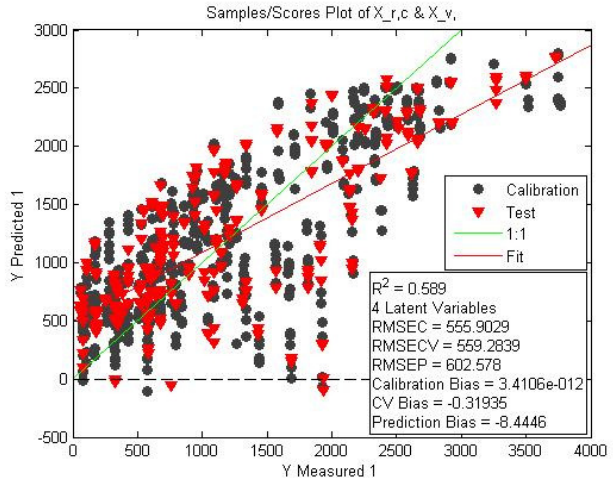
Propionate



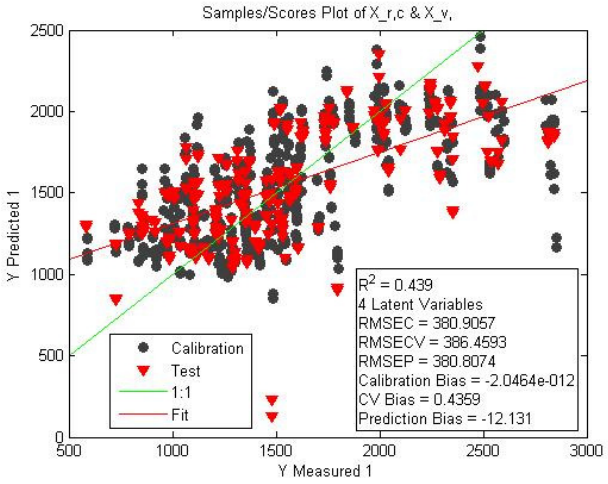


TVFA

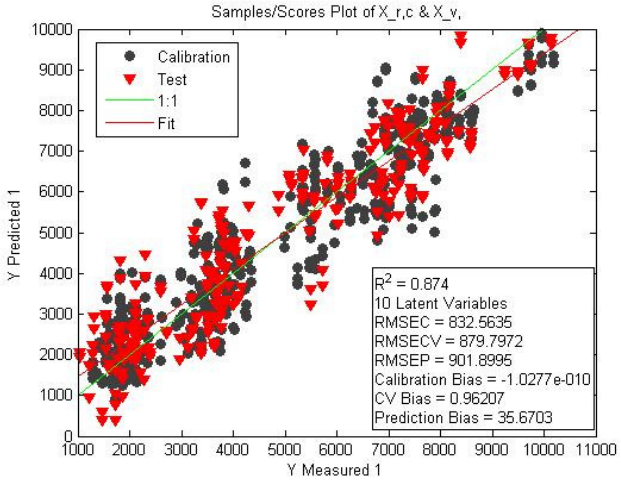
6 Spike on spike NIR



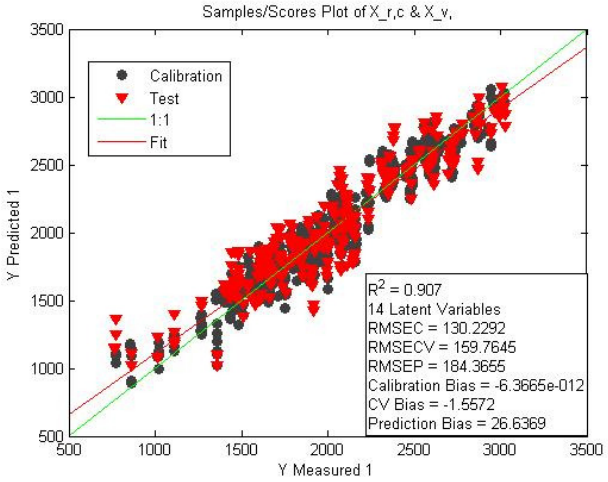
Acetate



Propionate

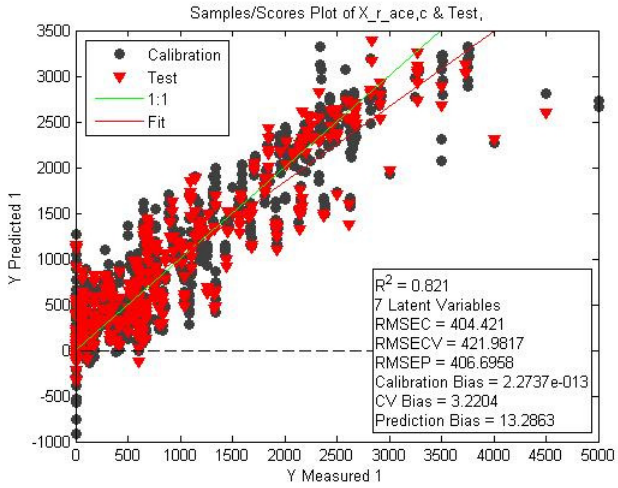


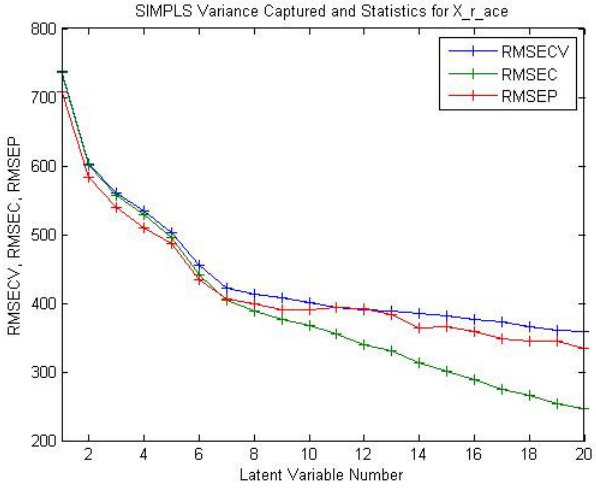
TVFA



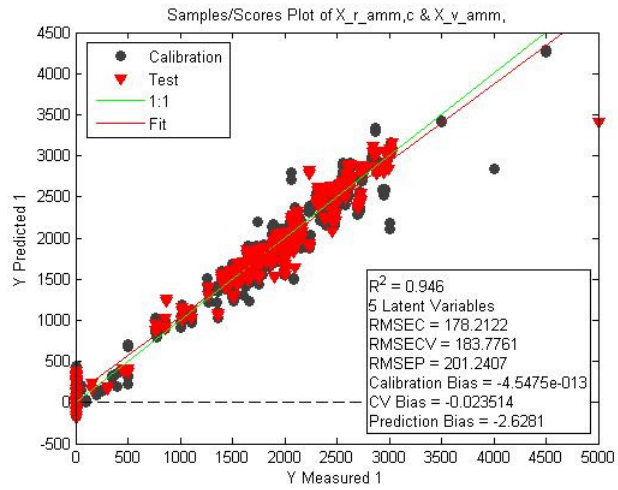
Ammonium

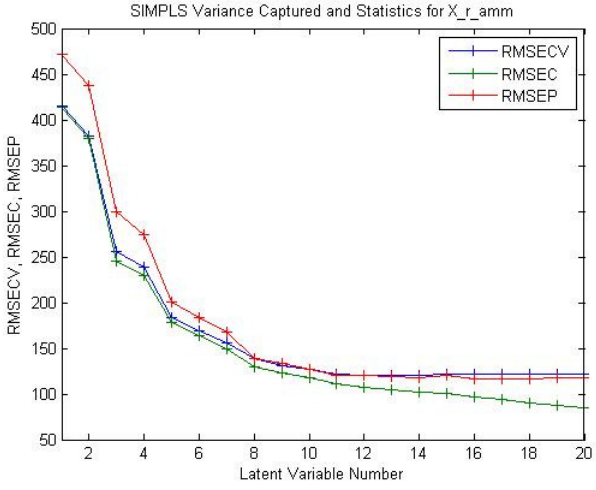
7 All on All MIR



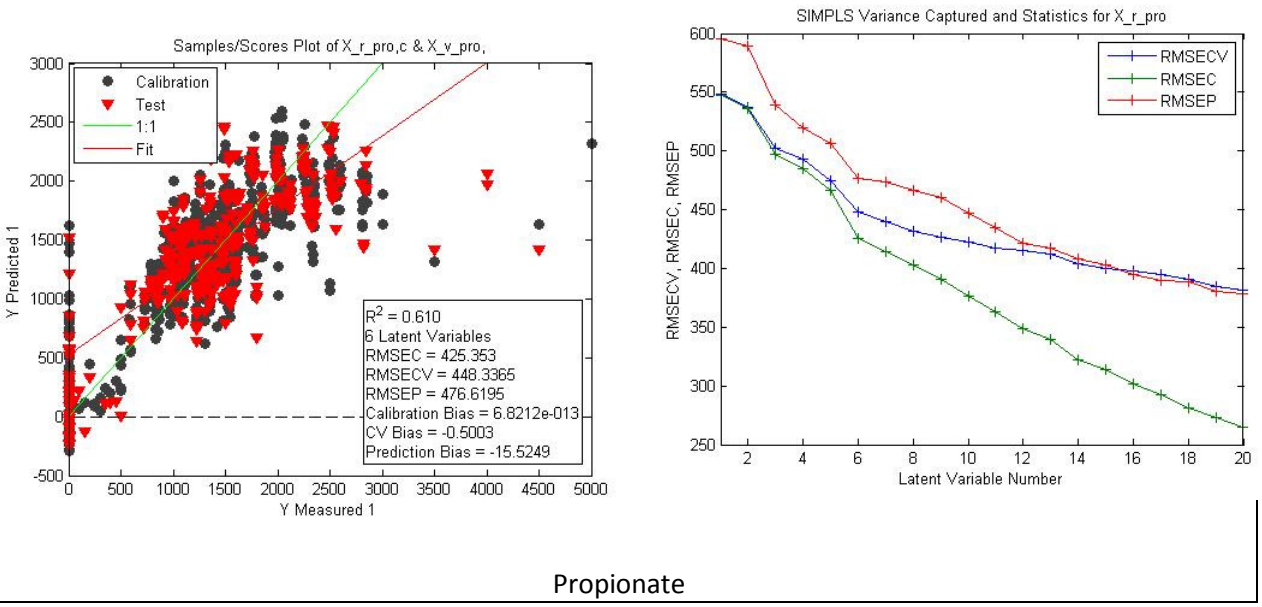


Acetate

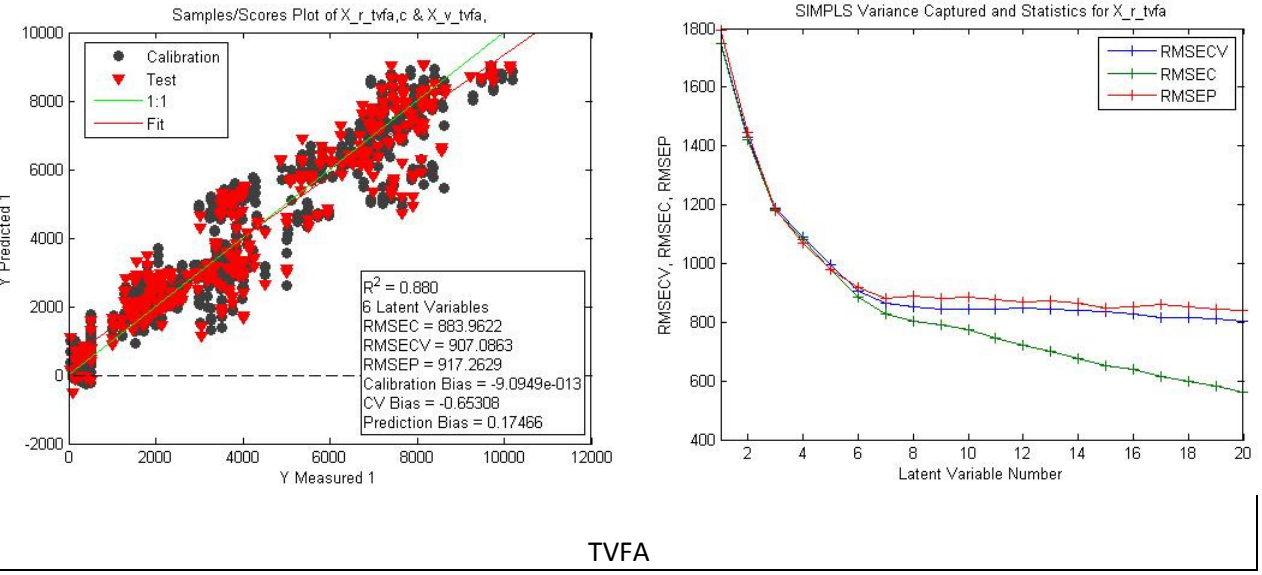




Ammonium



Propionate



TVFA

Bibliography

J.Dahlbacka, T.Lillhonga, & M.Döring. (2013). Designed orthogonal sample spiking based calibrations for quantitative liquid phase measurements with near infrared spectroscopy in an anaerobic digestion process. *Journal of near infrared spectroscopy*(21), 11-22.

M.Döring. (2010). *Theoretical aspects of anaerobic digestion and near-infrared spectroscopy.* Vaasa.