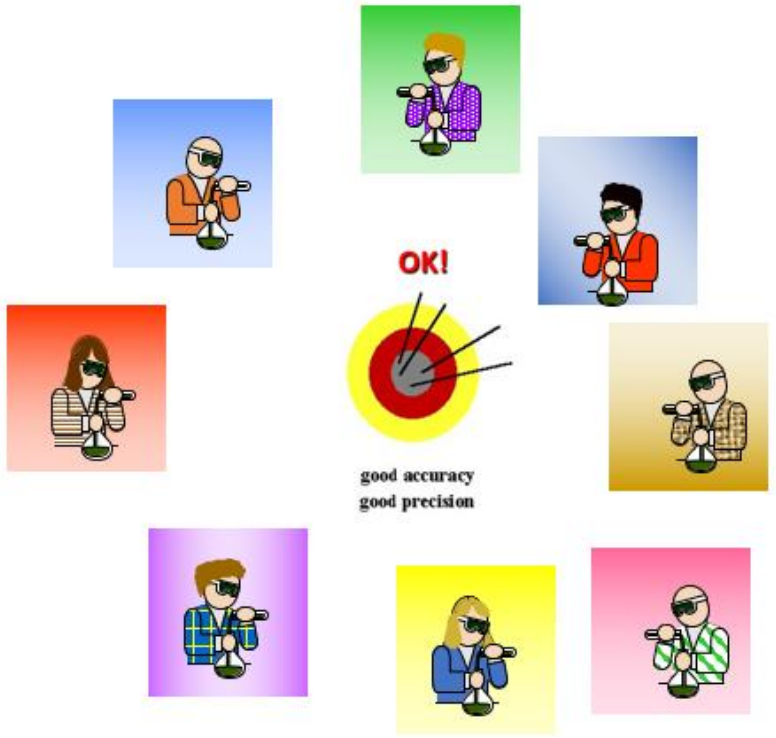
MSA and Optimization of a UHPLC Measurement System



EUROPE DISCOVERY SUMMIT
EXPLORING DATA INSPIRING INNOVATION

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JMP Academic Program

Problem description & Goal

In a chemical company **SPC revealed significant batch to batch raw material variation** resulting in product **quality problems**
Analysis of all supplied batches is necessary however impossible using slow standard GC method.
A fast UHPLC analytic method is under development but not ready yet for validation because of strong measurement variation

Goal: Specification of robust & optimal settings for UHPLC method so that validation is possible

JMP Case Study Library

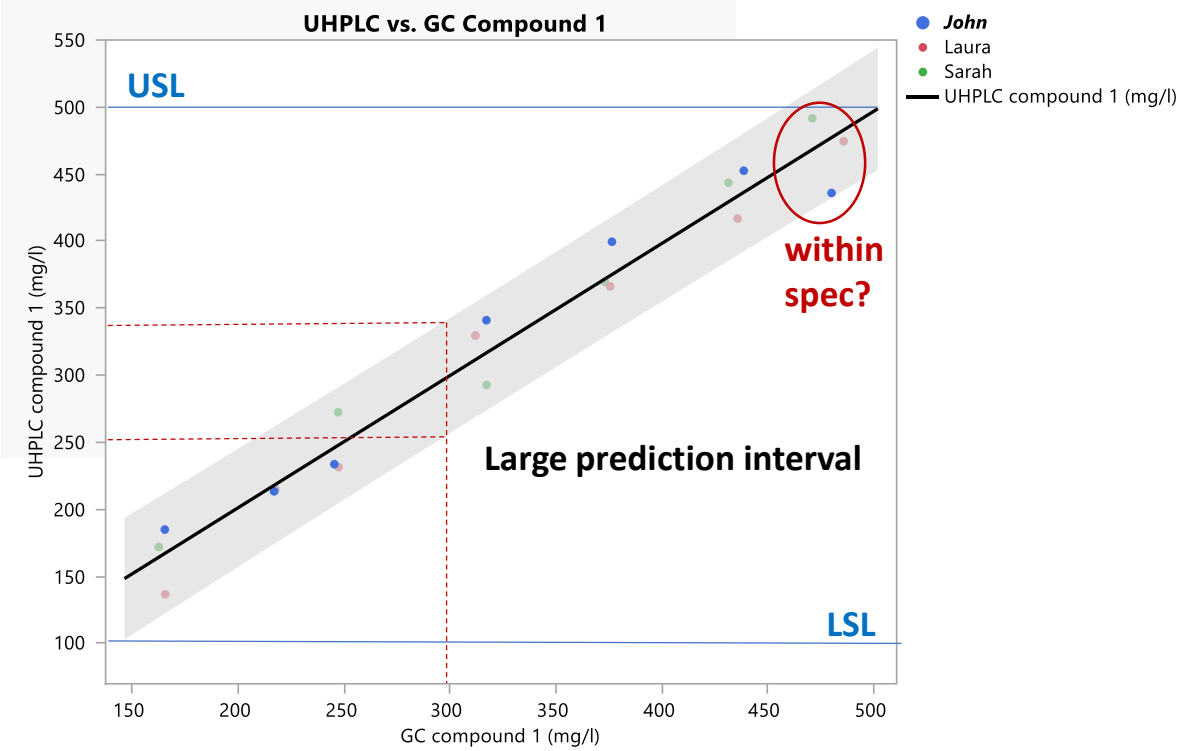
Teaching resources

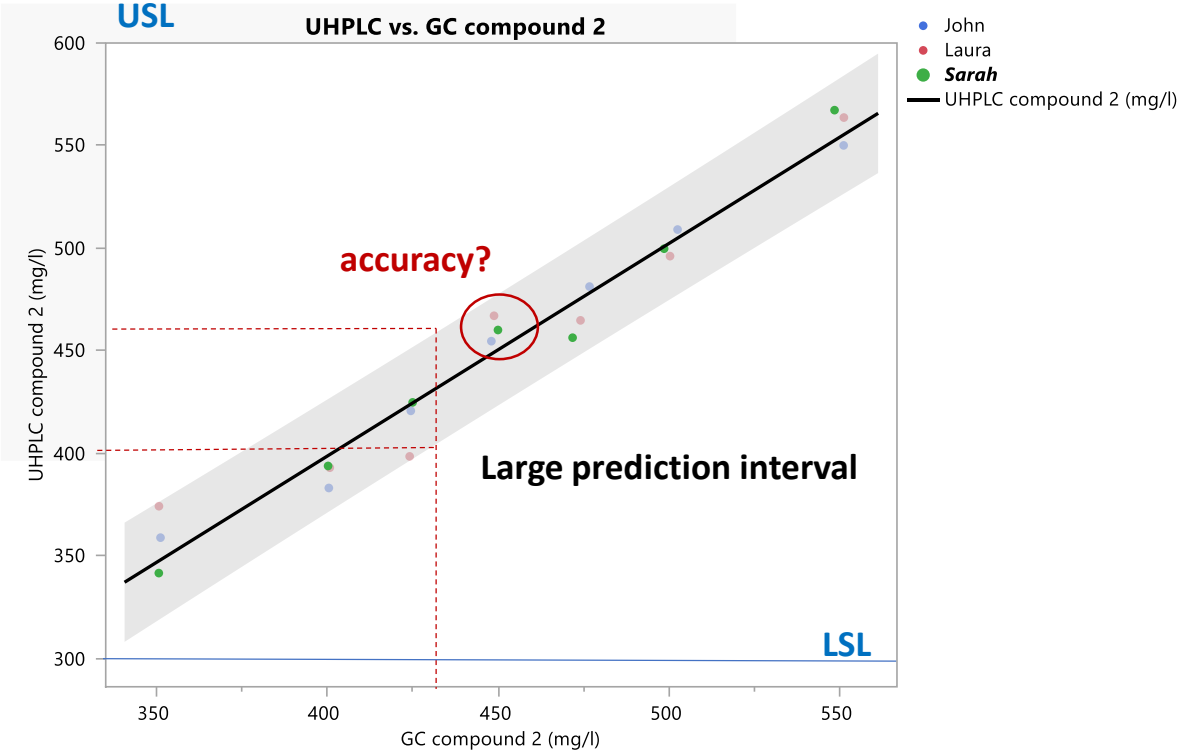
- Free teaching material
- Managed by JMP Academic Team, developed with JMP users from industry and academia
- Add real-world scenarios for practical problem solving to many university courses
- Include data sets, background and task, solution, exercises

"Manufacturing Excellence in Pharma – Part 1, 2, 3"

- Series of three independent case studies (SPC, MSA, DOE)
- Will be added to the Case Study Library at *jmp.com/cases* soon
- Already available on request (volker.kraft@jmp.com)

Screening UHPLC vs. GC for Compound 1 & 2





GC accurate & precise standard method, but too slow

UHPLC method faster, but high operator variation

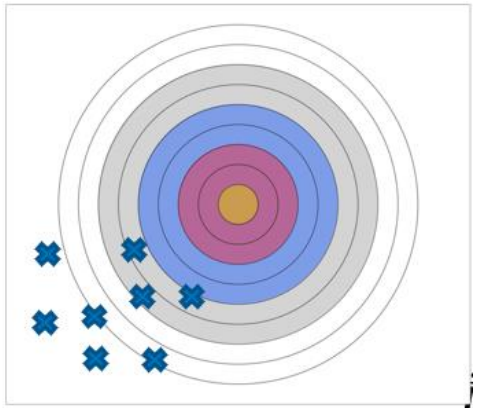
→ **MSA study**

Statistical Problem Solving

Problem → **Cause** → **Solution**

High variation UHPLC measurement system

Biased and Not Precise



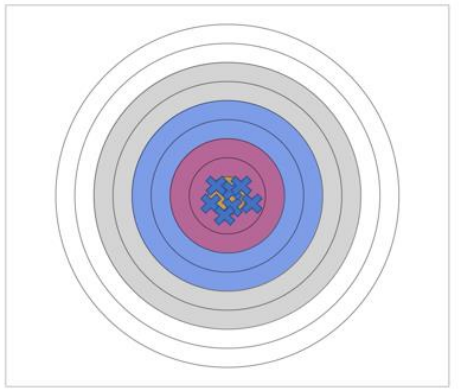
MSA – Gauge R&R

DOE

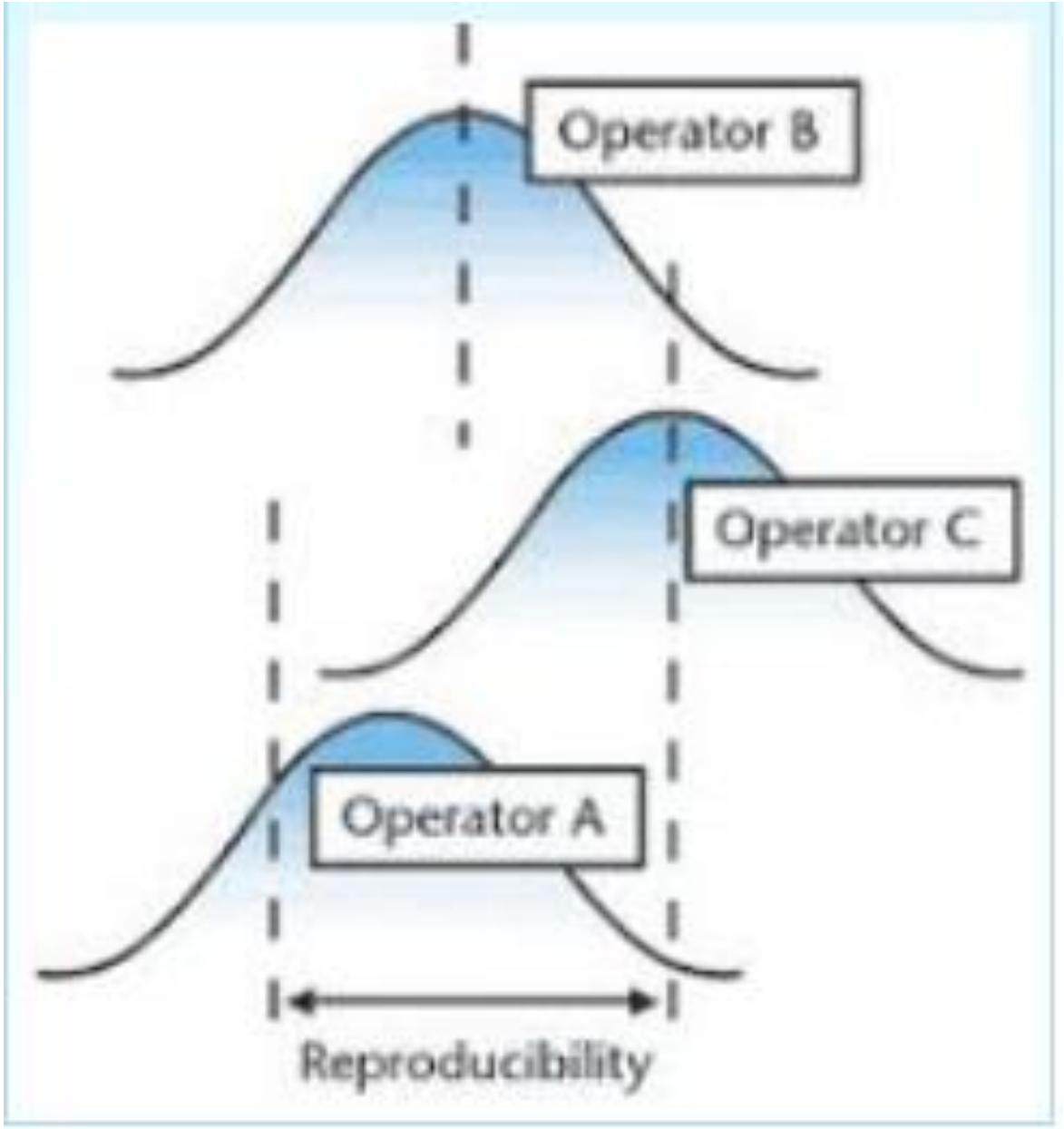
Variation root cause(s)

Optimal & robust settings UHPLC measurement system

Accurate and Precise



Measurement System Analysis (MSA)



MSA - Gauge R&R study

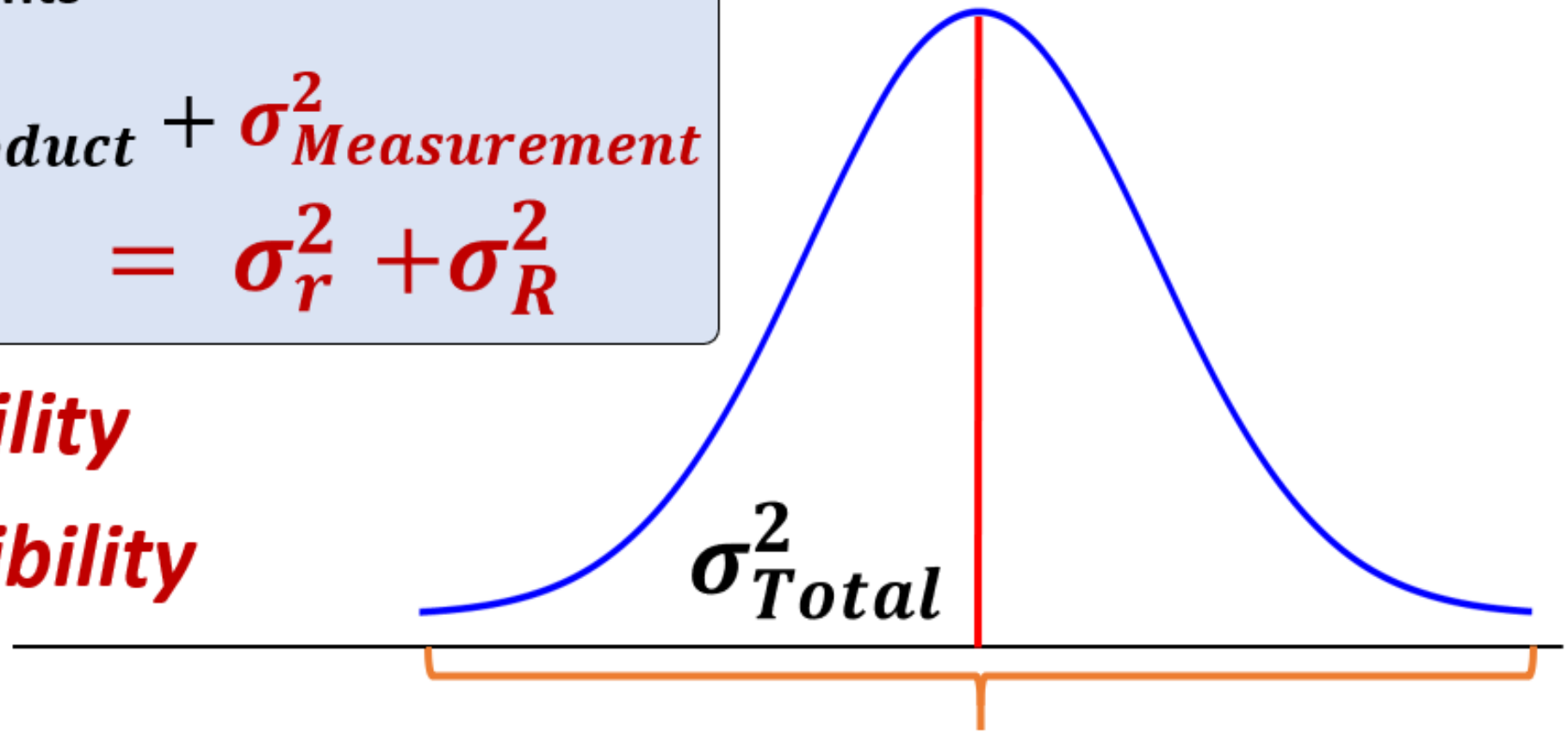
Variance Components

$$\sigma^2_{Total} = \sigma^2_{Product} + \sigma^2_{Measurement}$$

$$= \sigma^2_r + \sigma^2_R$$

σ_r ***Repeatability***

σ_R ***Reproducibility***



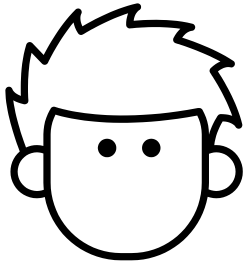
Measurement system can detect process variation

% Gauge R&R = $\sigma_{Measurement} / \sigma_{Total} < 10\%$

Gauge R&R study UHPLC

Between operator variation → reproducibility

John



Batch 1 2x

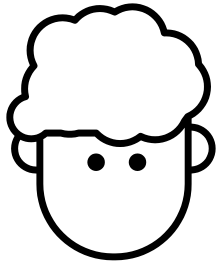
Batch 2 2x

Product variation

Batch 3 2x

Batch 4 2x

Laura



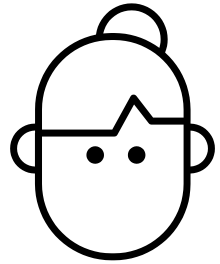
Batch 1 2x

Batch 2 2x

Batch 3 2x

Batch 4 2x

Sarah



Batch 1 2x

Batch 2 2x

Batch 3 2x

Batch 4 2x

Within operator variation → repeatability

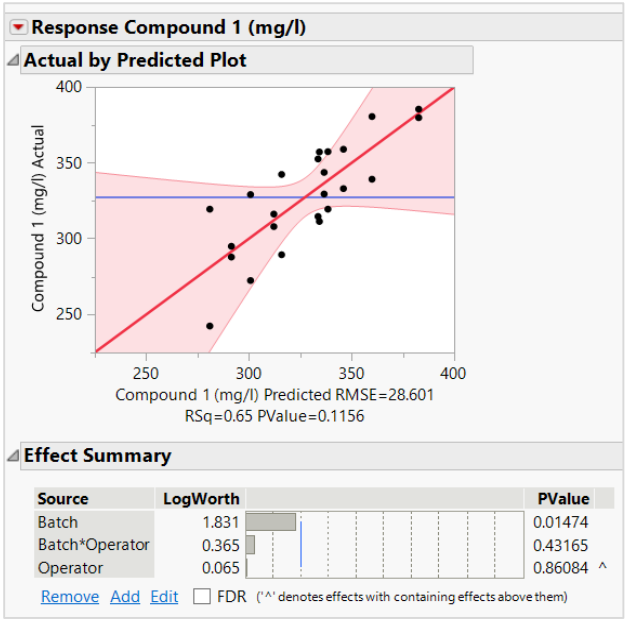
MSA - GAUGE R&R UHPLC ANALYSIS

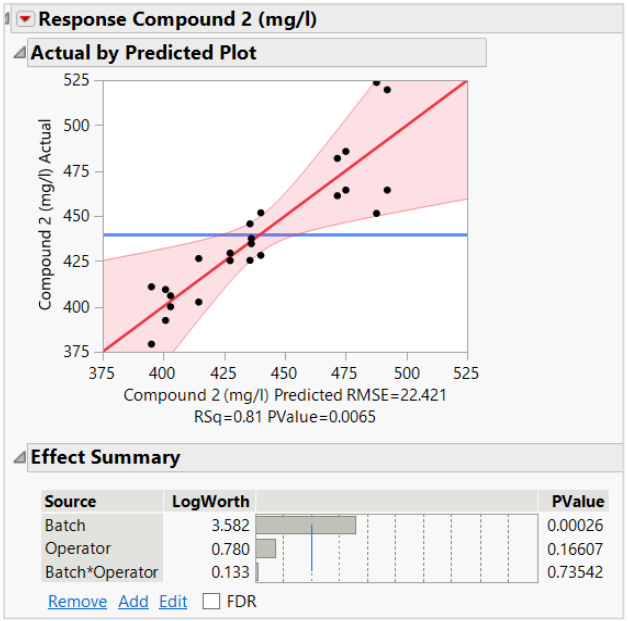
JMP DEMO

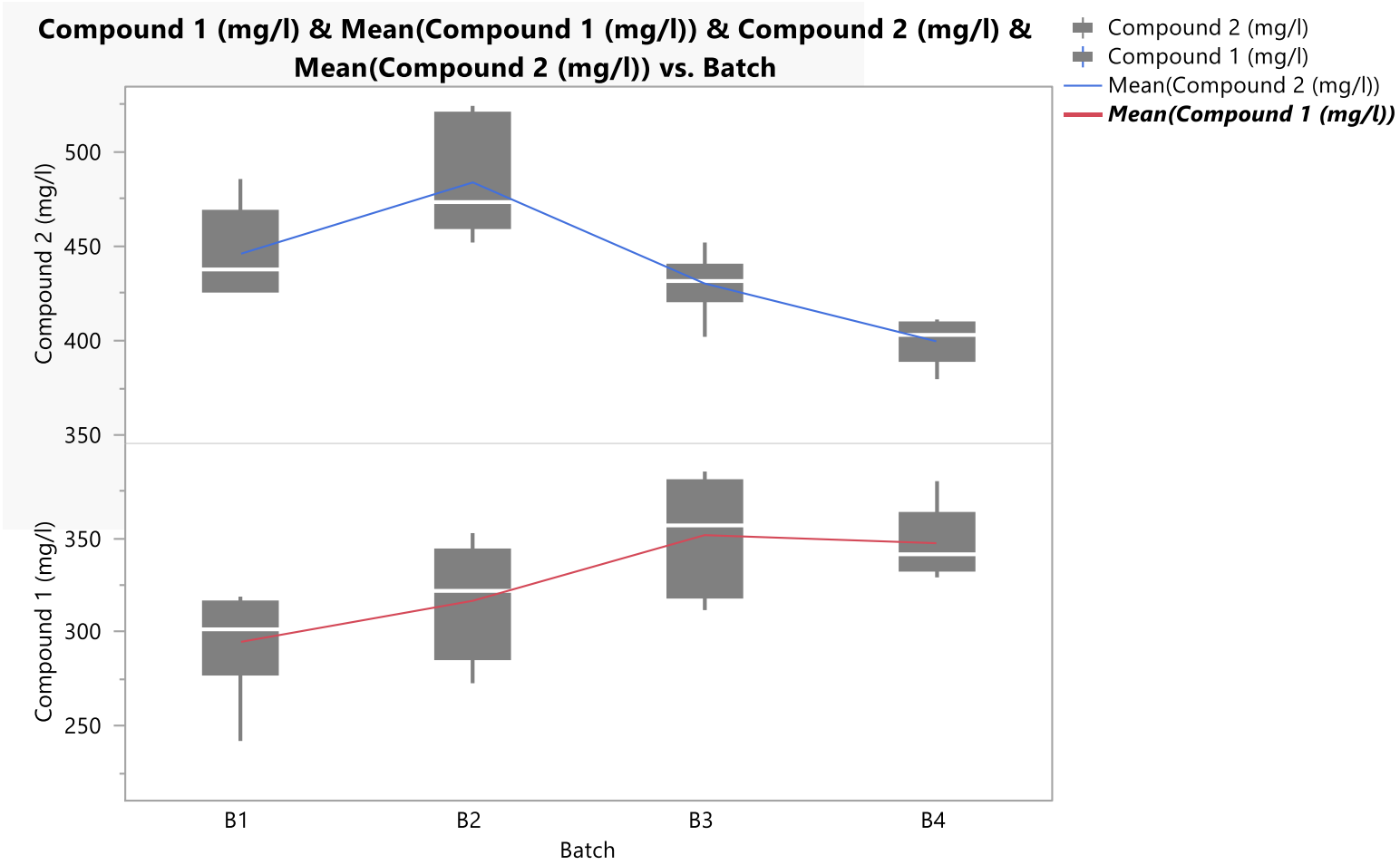
Full Factorial DOE Gauge R&R study UHPLC

	Batch	Operator	Compound 1 (mg/l)	Compound 2 (mg/l)
1	B3	John	380	435
2	B1	Laura	316	446
3	B2	Sarah	353	461
4	B4	Laura	333	411
5	B1	John	319	486
6	B1	John	242	464
7	B2	Sarah	315	482
8	B3	John	385	438
9	B2	Laura	329	524
10	B1	Sarah	295	425
11	B4	Sarah	380	392
12	B1	Sarah	288	429
13	B4	John	344	400
14	B1	Laura	308	425
15	B2	Laura	272	451
16	B3	Laura	311	428
17	B3	Laura	357	452
18	B4	John	329	406
19	B3	Sarah	357	402
20	B3	Sarah	319	426
21	B2	John	289	520
22	B2	John	342	464
23	B4	Laura	359	379
24	B4	Sarah	339	409

ANOVA Results for compound 1 and compound 2



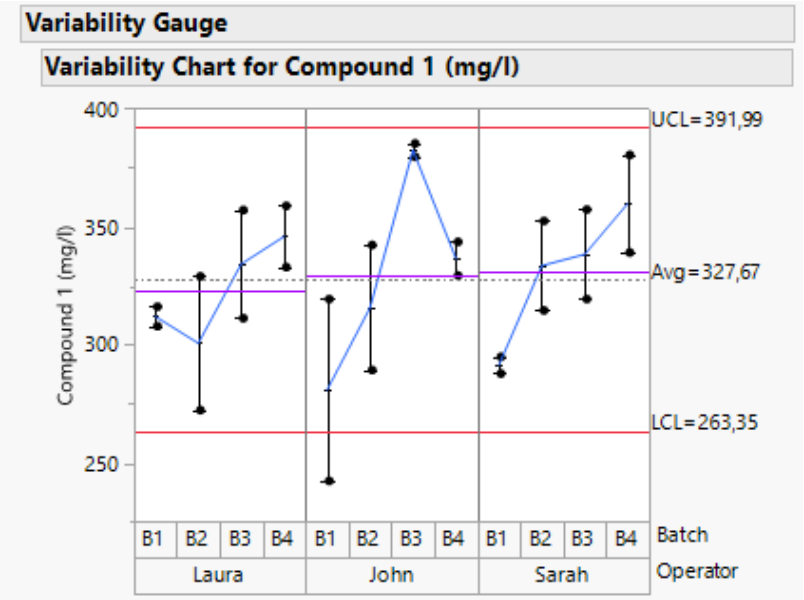




Significant difference between batch averages

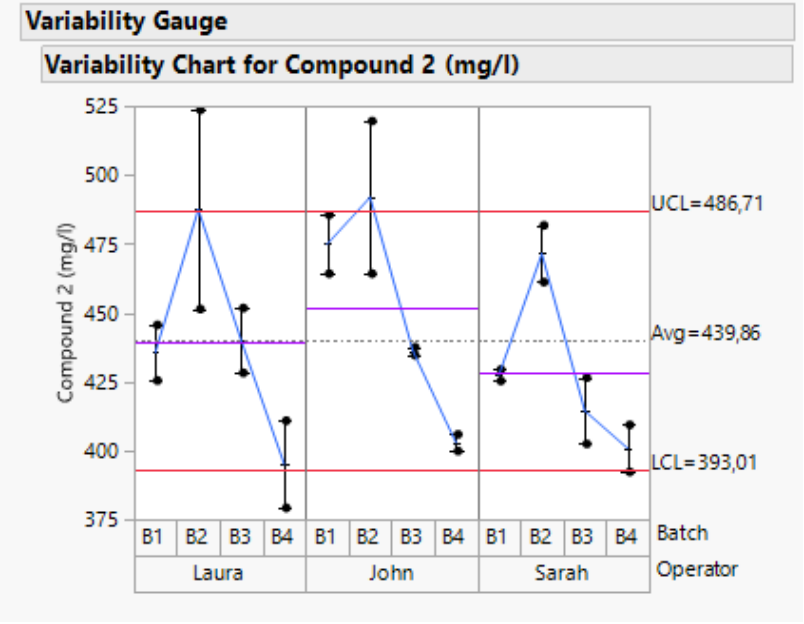
detected by individual UHPLC measurements?

Variability Chart of Compound 1 & Compound 2



John has more variation

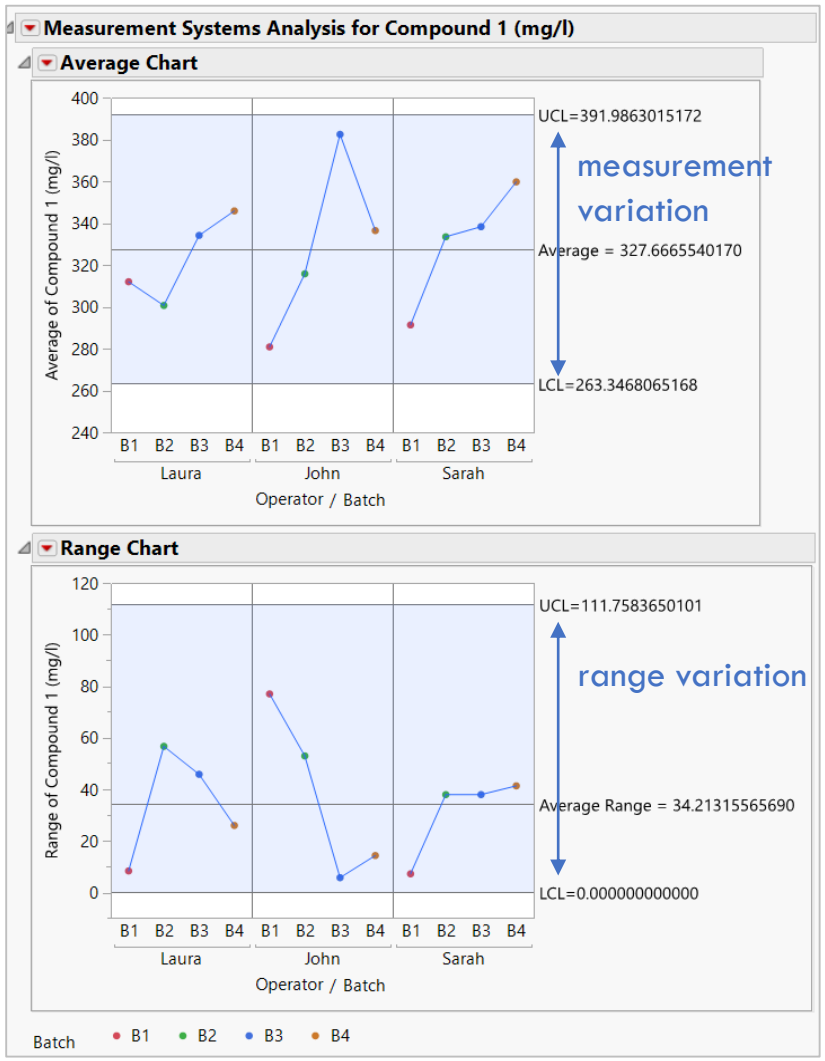
variation not consistent across batches

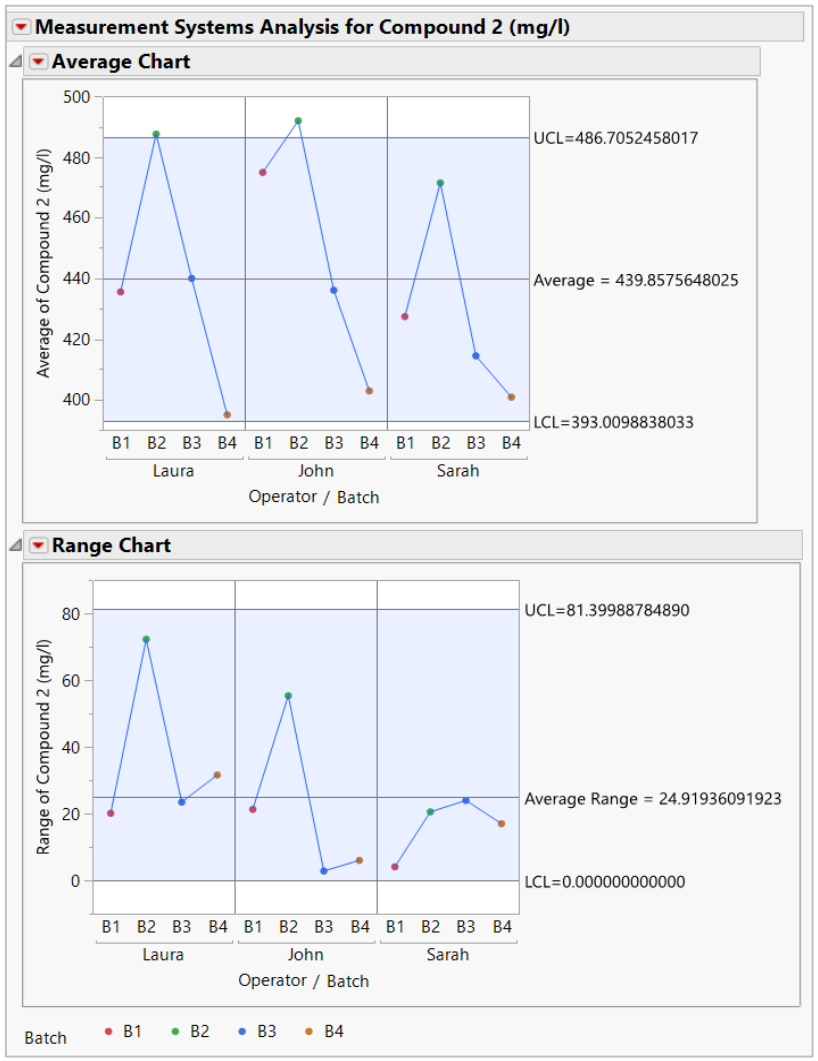


Laura has more variation

variation not consistent across batches

Average & Range Chart of Compound 1 and Compound 2

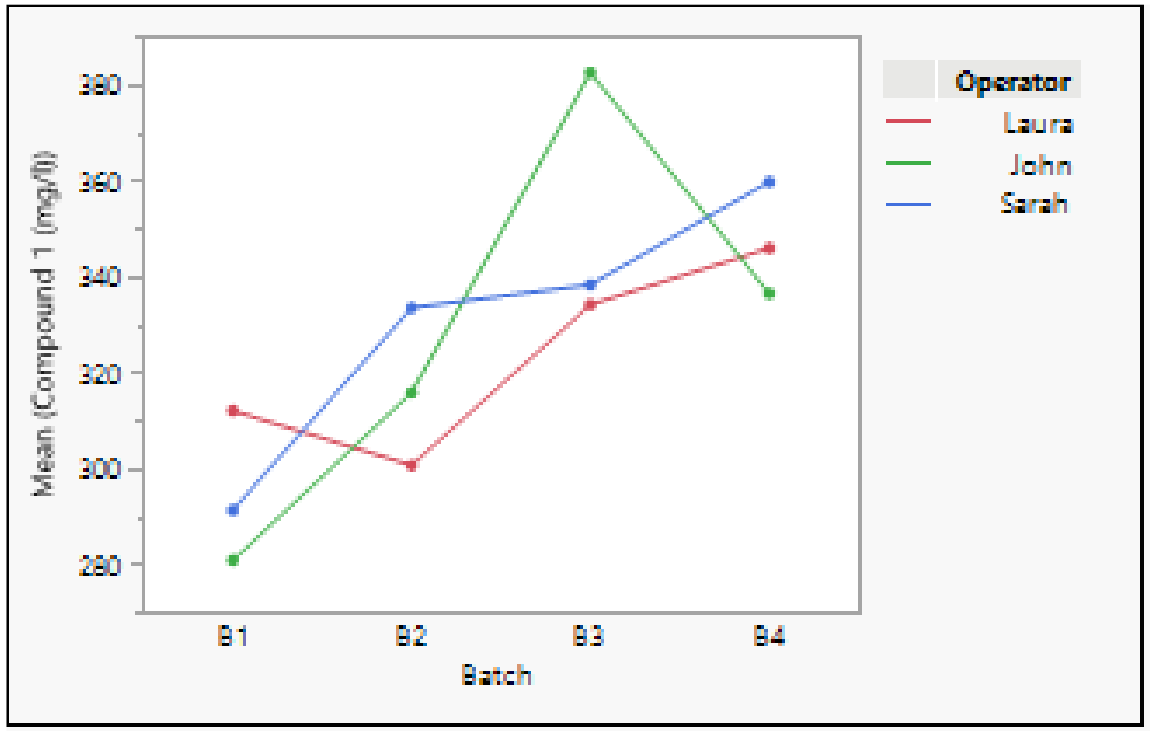


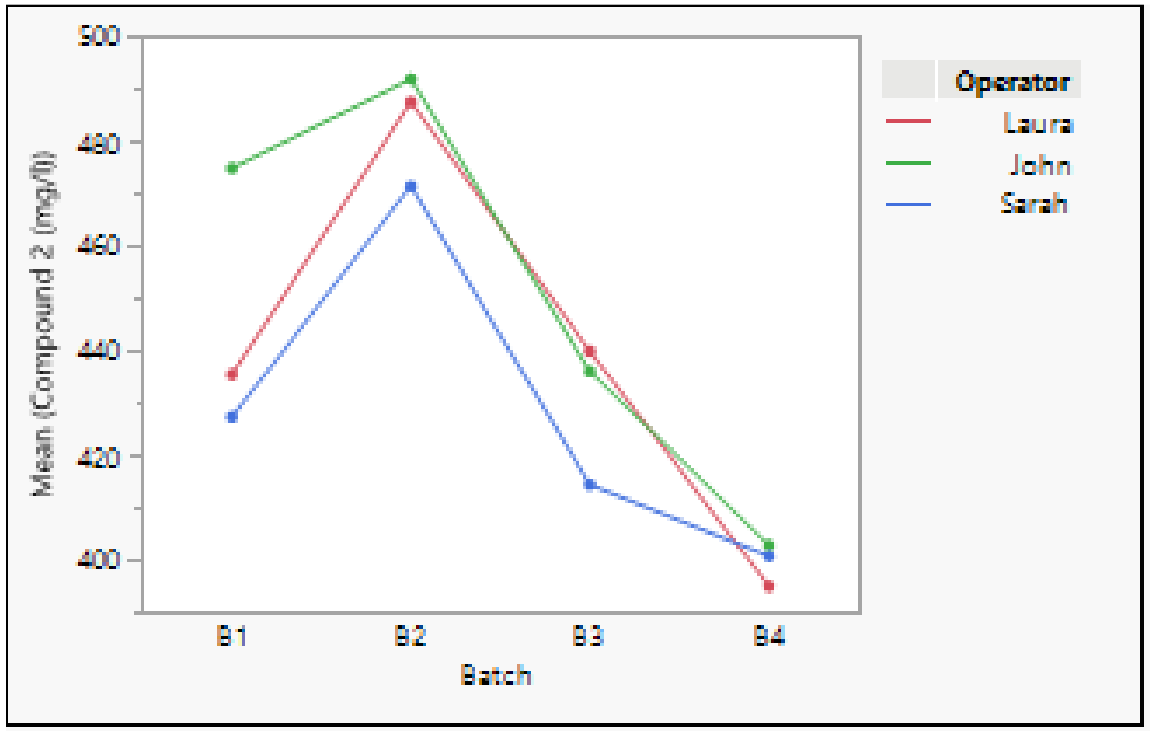


Individual UHPLC measurements cannot detect quality shifts between batches!

No significant difference in measurement range between operators

Parallelism Plots for Operator and Batch for Compound 1 and Compound 2





Lines are not parallel and there is a major crossing

indication interaction between operators and batches

→ serious reproducibility issue that requires further investigation!

EMP Gauge R&R Results Compound 1 and Compound 2

EMP Gauge R&R Results

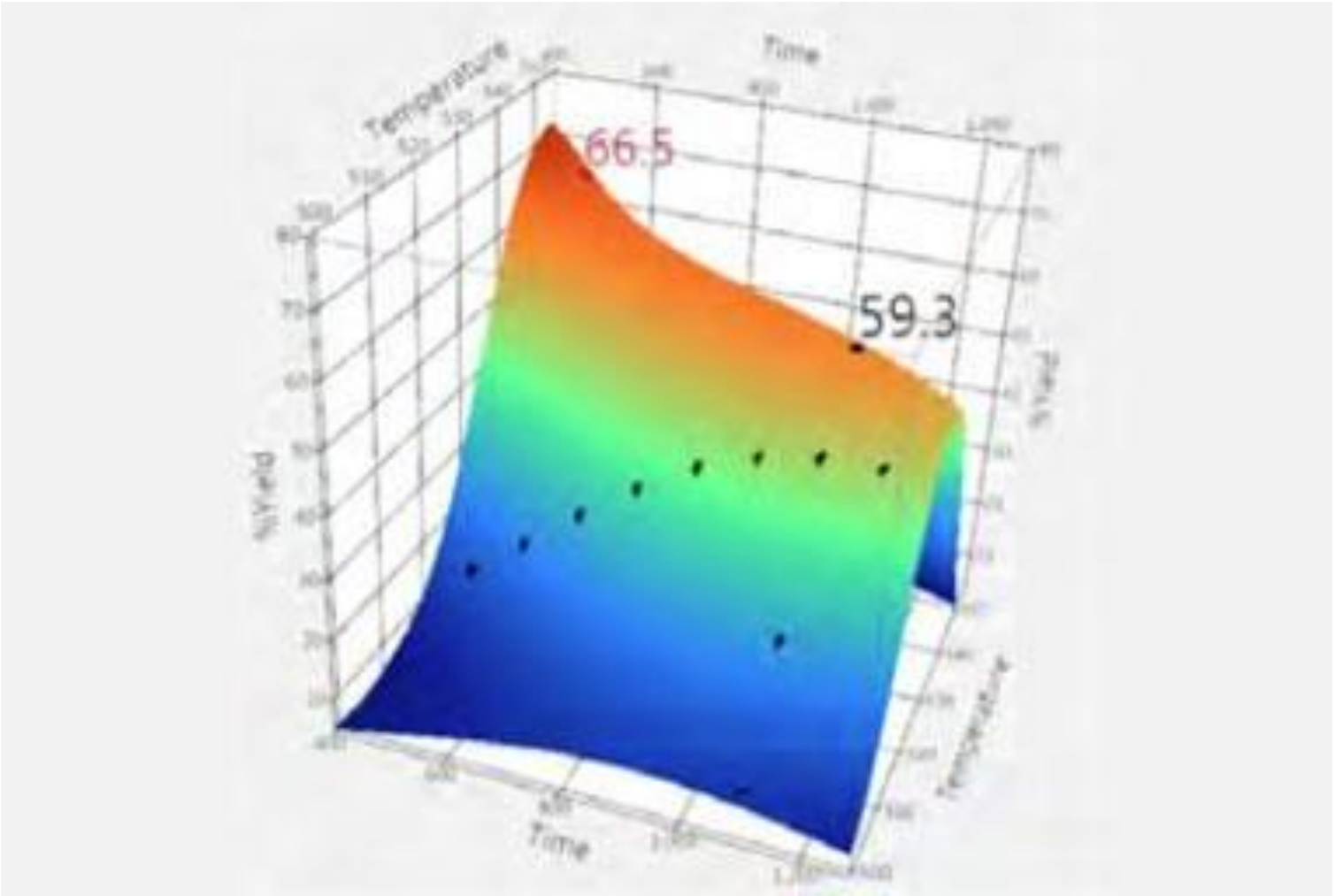
Component	Std Dev	Variance Component		% of Total	20 40 60 80
Gauge R&R	27.670253	765.6429		56.3	
Repeatability	27.670253	765.6429	σ_r^2	56.3	
Reproducibility	0.000000	0.0000	σ_R^2	0.0	
Product Variation	24.391369	594.9389	σ_P^2	43.7	
Interaction Variation	0.000000	0.0000		0.0	
Total Variation	36.886065	1360.5818	σ_{Tot}^2	100.0	

EMP Gauge R&R Results

Component	Std Dev	Variance Component	% of Total	20 40 60 80
Gauge R&R	22.598991	510.7144	30.7	
Repeatability	20.817847	433.3827	26.1	
Reproducibility	8.793841	77.3316	4.7	
Product Variation	33.928918	1151.1715	69.3	
Interaction Variation	0.000000	0.0000	0.0	
Total Variation	40.766234	1661.8858	100.0	

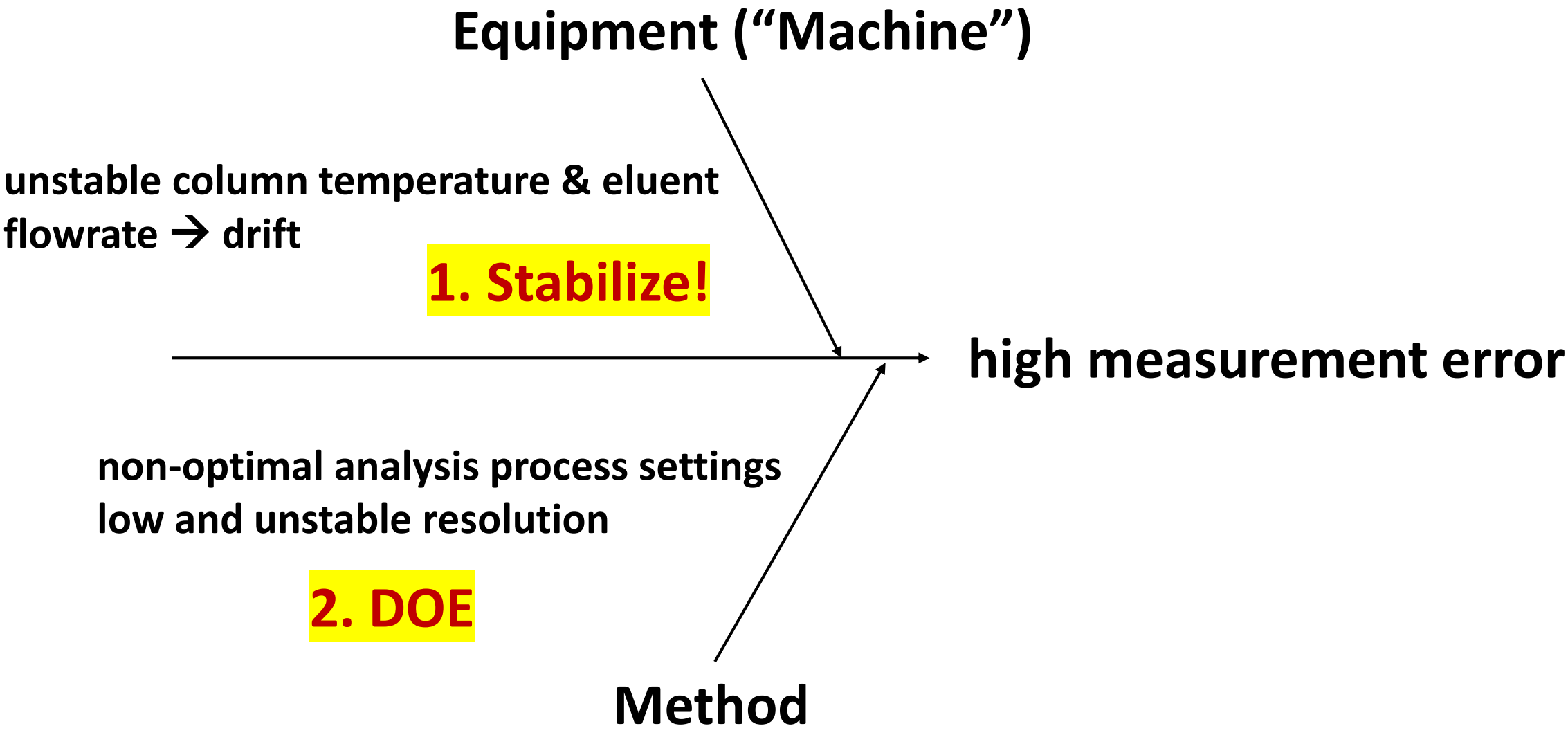
Main task for improving the UHPLC measurement system is optimization of the repeatability

→ Specify robust UHPLC process settings



Optimisation UHPLC by Design of Experiments

Root Cause Analysis



Goal

Y = compound concentration in standard sample (mg/l)

Model Y = F(UHPLC control factors)

Specify optimal, robust UHPLC control settings

Achieve Quality P/T ratio criterion

Y → match target compound concentration <= 10% tolerance

standard sample 1: Y = 300 +/- 20 mg/l

standard sample 2: Y = 450 +/- 15 mg/l

Model

Main effects (X_1, X_2,…X_i) & all quadratic effects (X^2)

Temperature column 25 – 35 °C

Eluent Flowrate 5 – 15 ml/min

Gradient → four continuous factors %Acetonitrile/ml (%ACN/ml)

- %ACN (V = 0ml) 5 – 20%
- %ACN (V = 1ml) 5 – 20%
- %ACN (V = 5ml) 35 – 70%
- %ACN (V = 6ml) 35 – 70 %

Wavelength UV detector 192 – 270 nm

Select interaction effects X_i*X_i

Temperature * Eluent Flowrate

Eluent Flowrate * all gradient factors %ACN

Custom Design

DOE UHPLC OPTIMIZATION

JMP DEMO

Optimization UHPLC - DOE

25 run Custom Design

Y_1 Y_2

	Temperature column (°C)	Eluent Flowrate (ml/min)	%ACN (V = 0ml)	%ACN (V = 1ml)	%ACN (V = 5ml)	%ACN (V = 6 ml)	Wavelength (nm)	300 mg/l compound 1 measured	450 mg/l compound 2 measured
1	30	10	5	12,5	35	35	270	290	418
2	25	5	12,5	12,5	52,5	52,5	192	495	544
3	35	15	20	12,5	35	35	231	244	363
4	25	5	20	5	35	35	231	549	597
5	35	5	12,5	12,5	35	70	231	236	371
6	25	15	5	12,5	70	70	231	208	426
7	35	5	20	12,5	52,5	35	270	171	396
8	30	5	5	5	52,5	70	270	268	370
9	25	5	20	20	70	70	270	386	650
10	25	5	5	20	35	35	270	414	476
11	30	5	20	20	35	52,5	192	248	442
12	30	5	12,5	5	70	35	192	315	393
13	25	10	20	5	70	52,5	231	475	528
14	25	15	12,5	5	35	52,5	270	364	411
15	25	10	5	12,5	52,5	70	192	342	514
16	25	15	20	20	52,5	35	192	279	473
17	35	15	12,5	20	70	70	270	231	415
18	35	5	5	20	70	52,5	231	145	340
19	30	10	12,5	20	52,5	35	231	299	441
20	35	15	5	5	70	35	192	102	339
21	30	15	5	20	35	70	192	117	369
22	35	15	5	20	52,5	52,5	231	113	418
23	30	15	20	5	52,5	70	231	280	292
24	35	10	20	5	35	70	192	302	426
25	30	15	20	12,5	70	52,5	270	181	318

Optimization UHPLC - DOE

Design evaluation

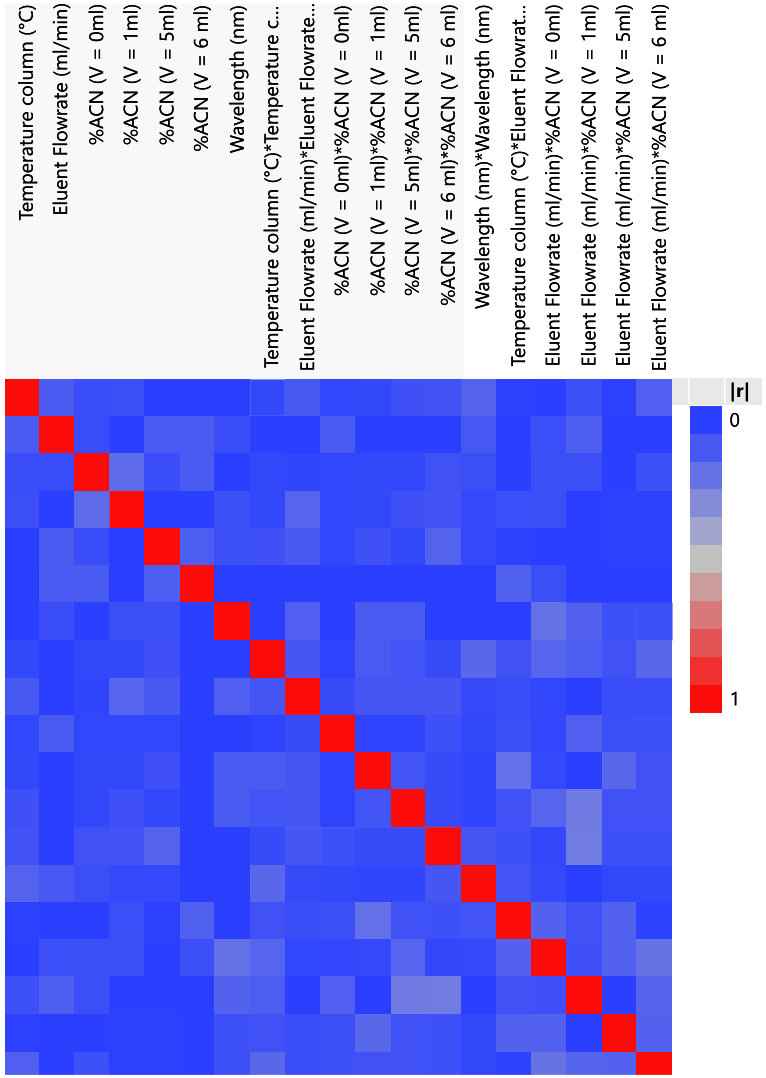
Design Evaluation

Power Analysis

Significance Level 0,05

Anticipated RMSE 1

Term	Anticipated Coefficient	Power
Intercept	1	0,153
Temperature column (°C)	1	0,873
Eluent Flowrate (ml/min)	1	0,924
%ACN (V = 0ml)	1	0,914
%ACN (V = 1ml)	1	0,878
%ACN (V = 5ml)	1	0,883
%ACN (V = 6 ml)	1	0,904
Wavelength (nm)	1	0,844
Temperature column (°C)*Temperature column (°C)	1	0,417
Eluent Flowrate (ml/min)*Eluent Flowrate (ml/min)	1	0,34
%ACN (V = 0ml)*%ACN (V = 0ml)	1	0,389
%ACN (V = 1ml)*%ACN (V = 1ml)	1	0,422
%ACN (V = 5ml)*%ACN (V = 5ml)	1	0,418
%ACN (V = 6 ml)*%ACN (V = 6 ml)	1	0,397
Wavelength (nm)*Wavelength (nm)	1	0,454
Temperature column (°C)*Eluent Flowrate (ml/min)	1	0,793
Eluent Flowrate (ml/min)*%ACN (V = 0ml)	1	0,796
Eluent Flowrate (ml/min)*%ACN (V = 1ml)	1	0,766
Eluent Flowrate (ml/min)*%ACN (V = 5ml)	1	0,804
Eluent Flowrate (ml/min)*%ACN (V = 6 ml)	1	0,771



Power OK

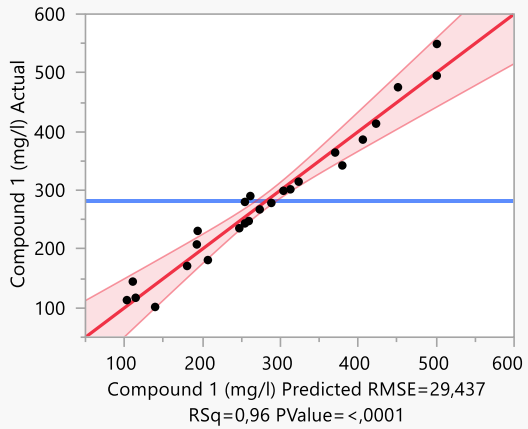
Collinearity negligible

Optimization UHPLC - DOE

Analysis DOE results Compound 1

Response Compound 1 (mg/l)

Actual by Predicted Plot



Effect Summary

Source	LogWorth	PValue
Temperature column (°C)(25,35)	8,128	0,00000
Eluent Flowrate (ml/min)(5,15)	4,564	0,00003
Temperature column (°C)*Eluent Flowrate (ml/min)	4,463	0,00003
%ACN (V = 0ml)(5,20)	3,351	0,00045
%ACN (V = 0ml)*%ACN (V = 0ml)	3,043	0,00091
Eluent Flowrate (ml/min)*Eluent Flowrate (ml/min)	2,728	0,00187
Temperature column (°C)*Temperature column (°C)	2,719	0,00191
%ACN (V = 1ml)(5,20)	2,700	0,00200
%ACN (V = 5ml)(35,70)	1,762	0,01729
Eluent Flowrate (ml/min)*%ACN (V = 0ml)	1,696	0,02011

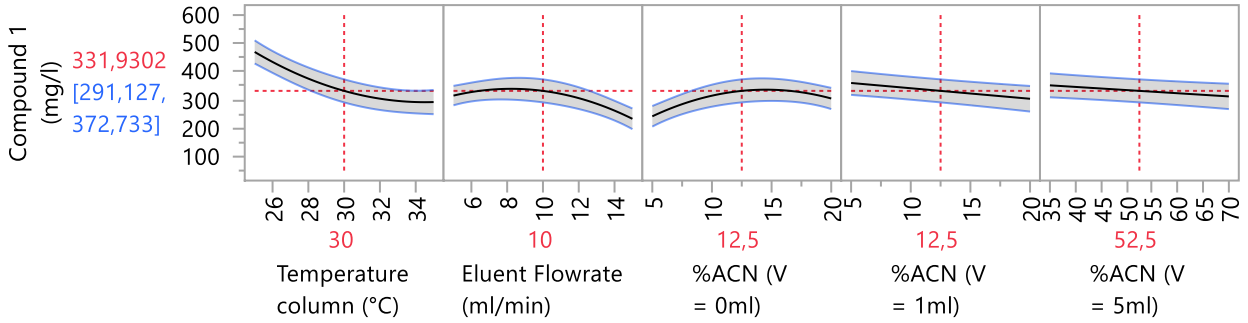
Summary of Fit

RSquare	0,964036
RSquare Adj	0,938348
Root Mean Square Error	29,43681
Mean of Response	282,149
Observations (or Sum Wgts)	25

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	331,93018	19,0243	17,45	<,0001 *
Temperature column (°C)(25,35)	-88,60203	7,253126	-12,22	<,0001 *
Eluent Flowrate (ml/min)(5,15)	-41,04818	6,72579	-6,10	<,0001 *
%ACN (V = 0ml)(5,20)	31,538215	6,91661	4,56	0,0004 *
%ACN (V = 1ml)(5,20)	-27,9162	7,368386	-3,79	0,0020 *
%ACN (V = 5ml)(35,70)	-19,58999	7,257906	-2,70	0,0173 *
Temperature column (°C)*Temperature column (°C)	49,041669	12,8711	3,81	0,0019 *
Eluent Flowrate (ml/min)*Eluent Flowrate (ml/min)	-57,74613	15,11408	-3,82	0,0019 *
%ACN (V = 0ml)*%ACN (V = 0ml)	-58,44641	13,94525	-4,19	0,0009 *
Temperature column (°C)*Eluent Flowrate (ml/min)	47,894533	8,026571	5,97	<,0001 *
Eluent Flowrate (ml/min)*%ACN (V = 0ml)	20,435806	7,795239	2,62	0,0201 *

Prediction Profiler

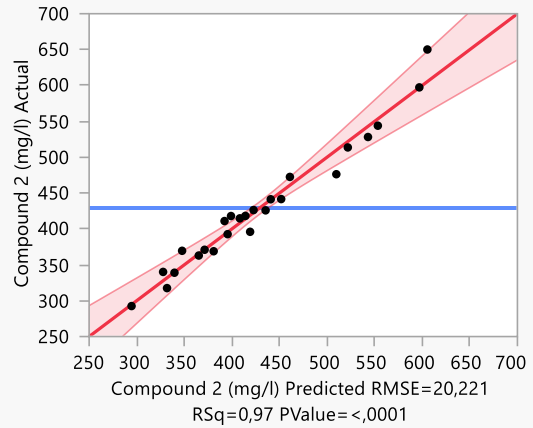


Optimization UHPLC - DOE

Analysis DOE results Compound 2

Response Compound 2 (mg/l)

Actual by Predicted Plot



Effect Summary

Source	LogWorth	PValue
Temperature column (°C)(25,35)	8,397	0,00000
Temperature column (°C)*Temperature column (°C)	5,394	0,00000
Eluent Flowrate (ml/min)(5,15)	5,185	0,00001
Temperature column (°C)*Eluent Flowrate (ml/min)	4,292	0,00005
Eluent Flowrate (ml/min)*%ACN (V = 0ml)	3,799	0,00016
Eluent Flowrate (ml/min)*Eluent Flowrate (ml/min)	3,779	0,00017
%ACN (V = 0ml)(5,20)	3,305	0,00050
%ACN (V = 1ml)(5,20)	3,026	0,00094
Eluent Flowrate (ml/min)*%ACN (V = 1ml)	2,036	0,00921

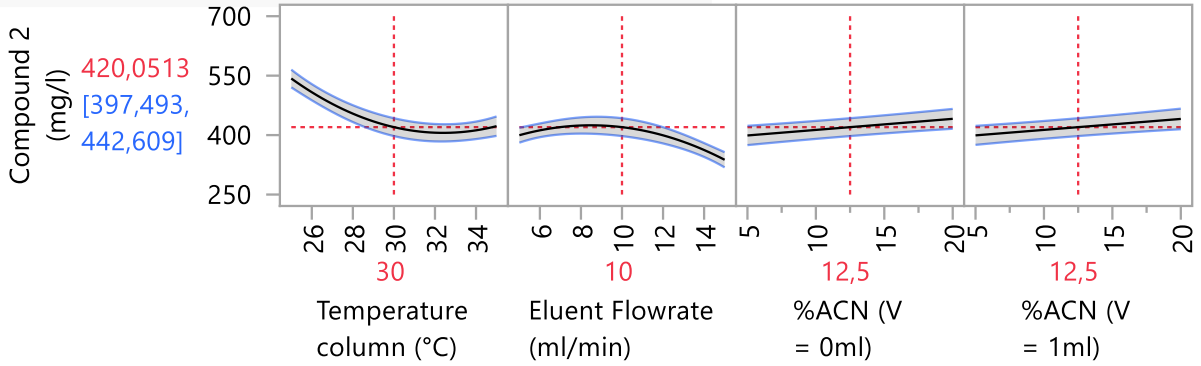
Summary of Fit

RSquare	0,965345
RSquare Adj	0,944551
Root Mean Square Error	20,22141
Mean of Response	429,1664
Observations (or Sum Wgts)	25

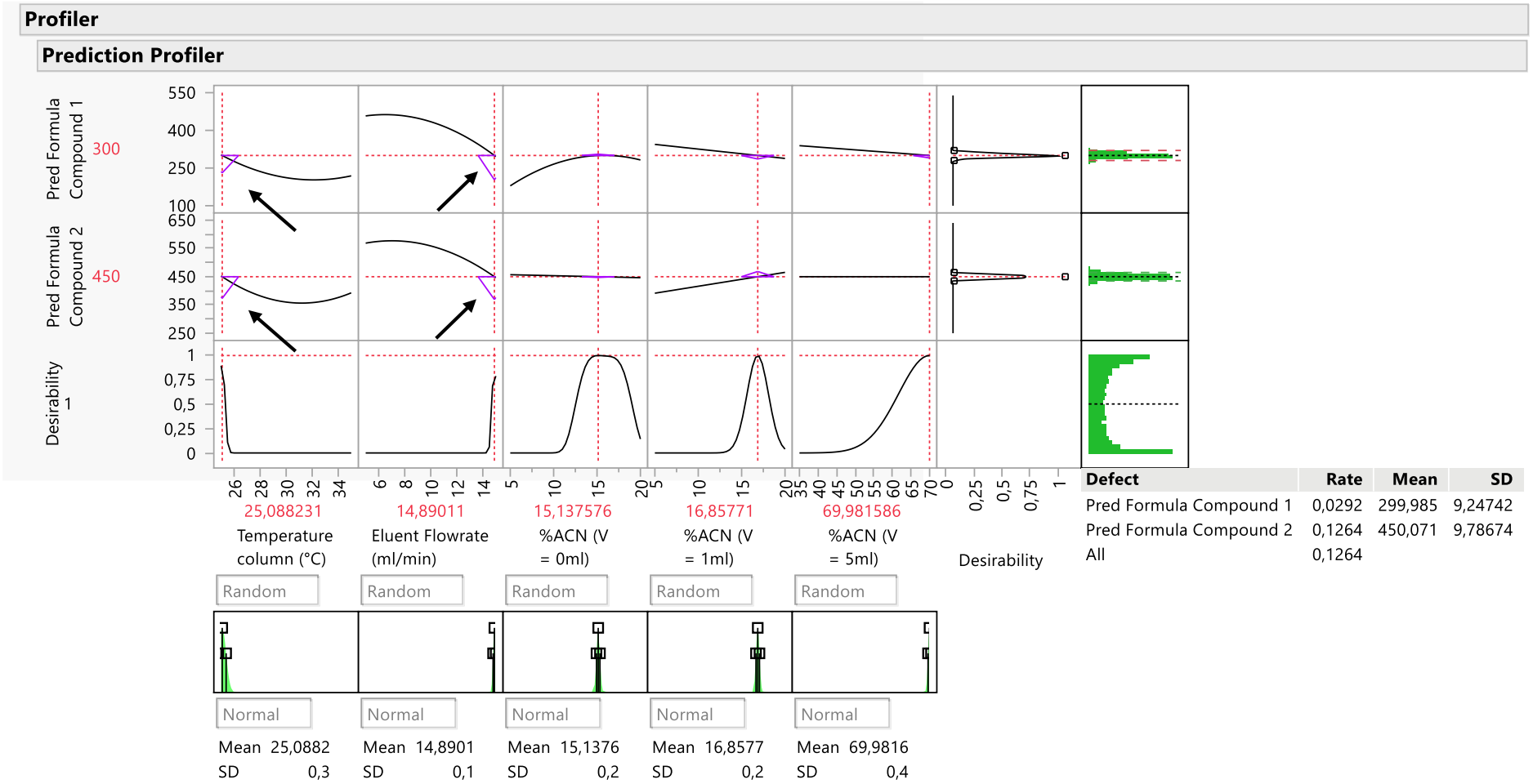
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	420,05129	10,58335	39,69	<,0001 *
Temperature column (°C)(25,35)	-60,12599	4,983292	-12,07	<,0001 *
Eluent Flowrate (ml/min)(5,15)	-31,01553	4,595035	-6,75	<,0001 *
%ACN (V = 0ml)(5,20)	20,982086	4,745405	4,42	0,0005 *
%ACN (V = 1ml)(5,20)	20,746393	5,057633	4,10	0,0009 *
Temperature column (°C)*Temperature column (°C)	62,591934	8,896357	7,04	<,0001 *
Eluent Flowrate (ml/min)*Eluent Flowrate (ml/min)	-51,33267	10,3199	-4,97	0,0002 *
Temperature column (°C)*Eluent Flowrate (ml/min)	30,884169	5,518854	5,60	<,0001 *
Eluent Flowrate (ml/min)*%ACN (V = 0ml)	-26,84522	5,37124	-5,00	0,0002 *
Eluent Flowrate (ml/min)*%ACN (V = 1ml)	16,522124	5,531272	2,99	0,0092 *

Prediction Profiler



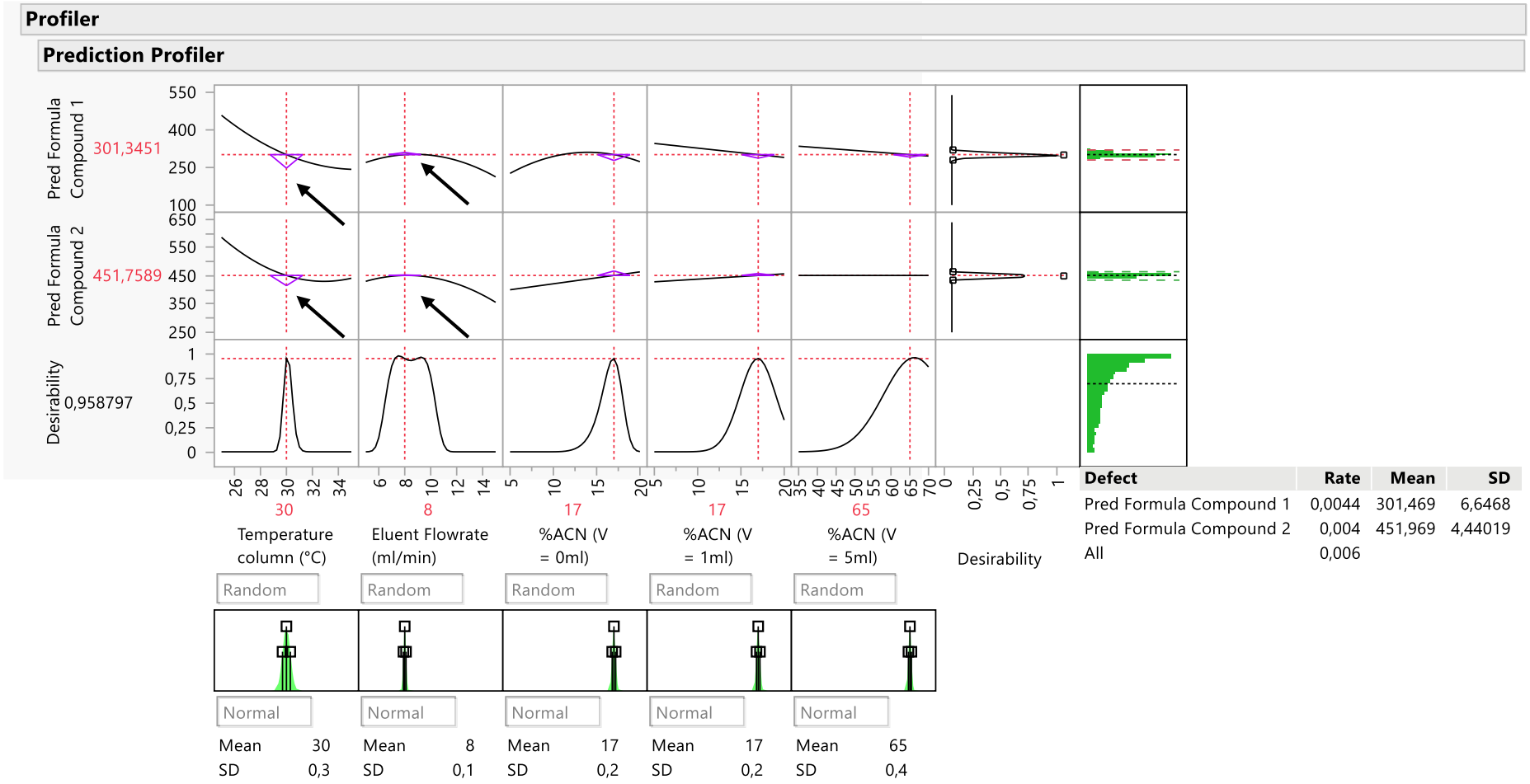
Optimal (& Robust) UHPLC Settings



Defect	Rate	Mean	SD
Pred Formula Compound 1	0,0292	299,985	9,24742
Pred Formula Compound 2	0,1264	450,071	9,78674
All	0,1264		

No robust settings → too high sensitivity

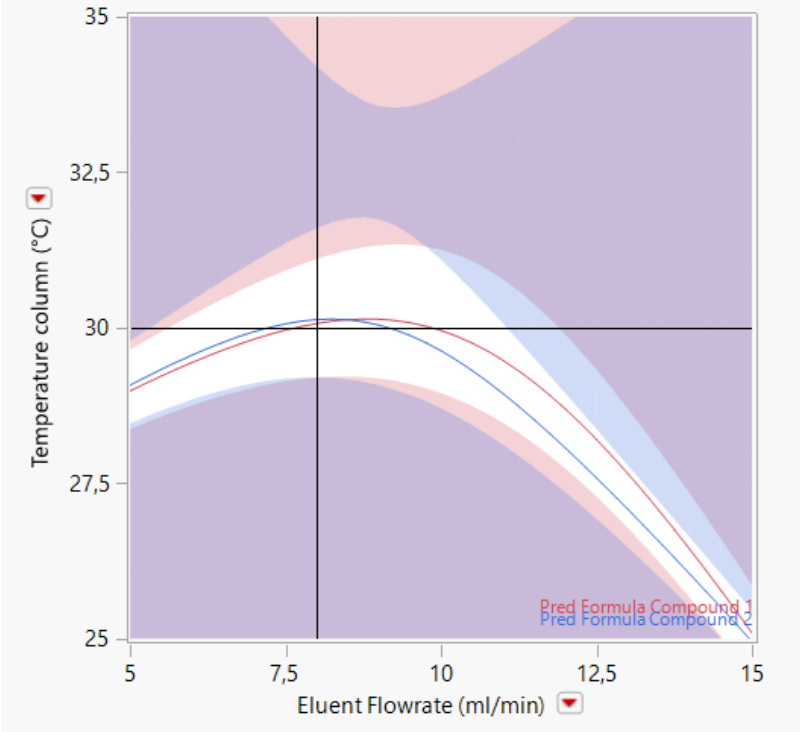
Optimal & Robust UHPLC Settings

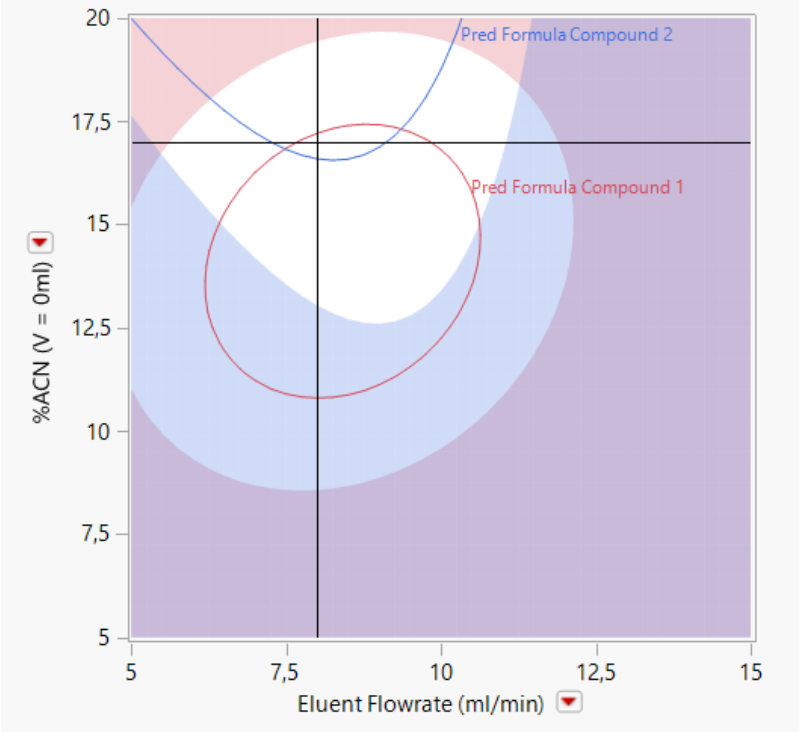


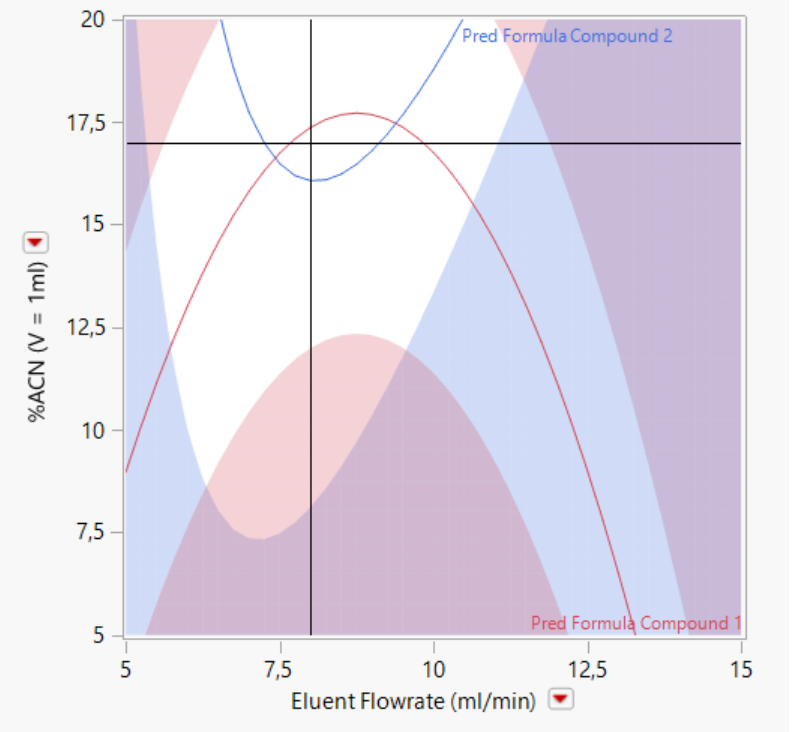
Defect	Rate	Mean	SD
Pred Formula Compound 1	0,0044	301,469	6,6468
Pred Formula Compound 2	0,004	451,969	4,44019
All	0,006		

More robust settings

Optimal & Robust UHPLC Settings







Contour Profiler

VALIDATION EXPERIMENTS

Full Factorial DOE MSA UHPLC - VALIDATION

	Batch	Operator	Instrument	Compound 1 (mg/l)	Compound 2 (mg/l)
1	B1	John	GC	301	451
2	B1	John	GC	304	448
3	B1	John	UHPLC	302	449
4	B1	John	UHPLC	296	450
5	B1	Laura	GC	286	442
6	B1	Laura	GC	292	446
7	B1	Laura	UHPLC	289	443
8	B1	Laura	UHPLC	290	440
9	B1	Sarah	GC	295	451
10	B1	Sarah	GC	298	447
11	B1	Sarah	UHPLC	300	450
12	B1	Sarah	UHPLC	301	449
13	B2	John	GC	318	486
14	B2	John	GC	321	480
15	B2	John	UHPLC	310	481
16	B2	John	UHPLC	324	482
17	B2	Laura	GC	336	479
18	B2	Laura	GC	336	488
19	B2	Laura	UHPLC	333	486
20	B2	Laura	UHPLC	331	483
21	B2	Sarah	GC	335	484
22	B2	Sarah	GC	338	492
23	B2	Sarah	UHPLC	340	488
24	B2	Sarah	UHPLC	336	488
25	B3	John	GC	343	408
26	B3	John	GC	345	406
27	B3	John	UHPLC	346	406
28	B3	John	UHPLC	351	408
29	B3	Laura	GC	353	408
30	B3	Laura	GC	356	411
31	B3	Laura	UHPLC	360	408
32	B3	Laura	UHPLC	347	403
33	B3	Sarah	GC	340	404
34	B3	Sarah	GC	337	397
35	B3	Sarah	UHPLC	335	396
36	B3	Sarah	UHPLC	344	403

GAUGE R&R

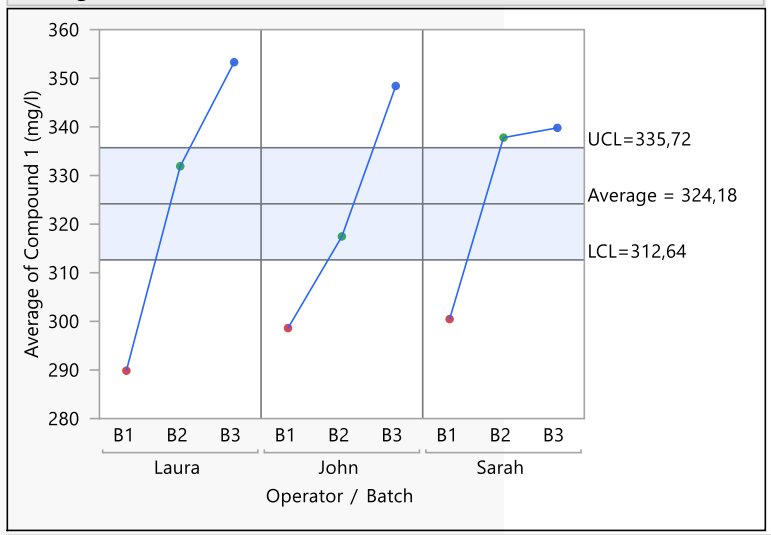
Three batches → batch variation

Three operators → one repeated UHPLC & GC measurement/batch

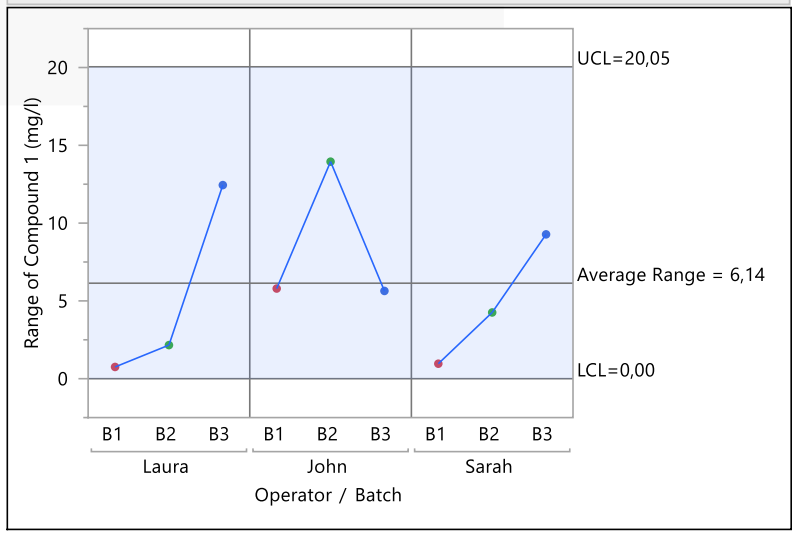
MSA UHPLC VALIDATION – Compound 1

Measurement Systems Analysis for Compound 1 (mg/l) Instrument=UHPLC

Average Chart



Range Chart



Batch ● B1 ● B2 ● B3

EMP Gauge R&R Results

Component	Std Dev	Variance Component	% of Total	20 40 60 80
Gauge R&R	5,401311	29,17416	4,0	
Repeatability	5,401311	29,17416	4,0	
Reproducibility	0,000000	0,00000	0,0	
Product Variation	25,368507	643,56117	89,3	
Interaction Variation	6,941584	48,18559	6,7	
Total Variation	26,849971	720,92092	100,0	

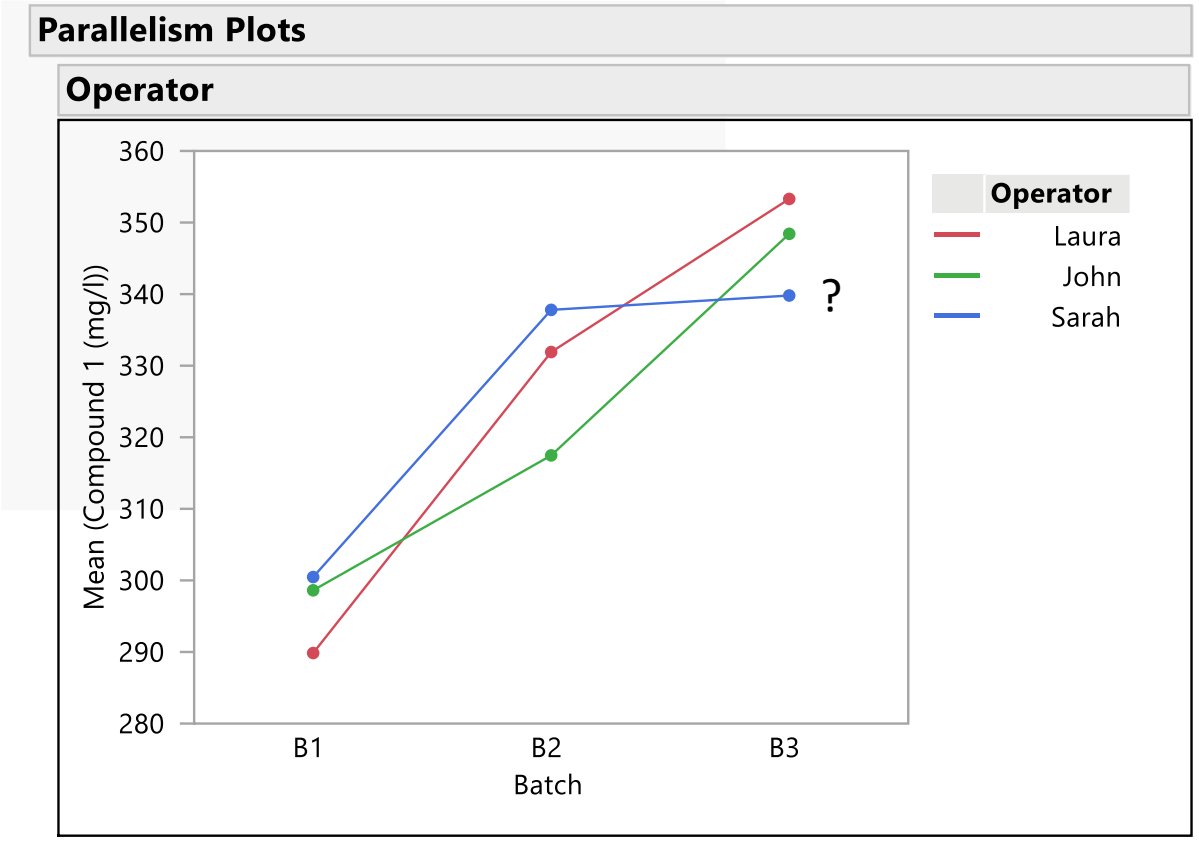
Mainly product variation

Individual UHPLC measurements can detect quality shifts between batches!

Precision/Tolerance ratio = 6*5,40/400 = 8%

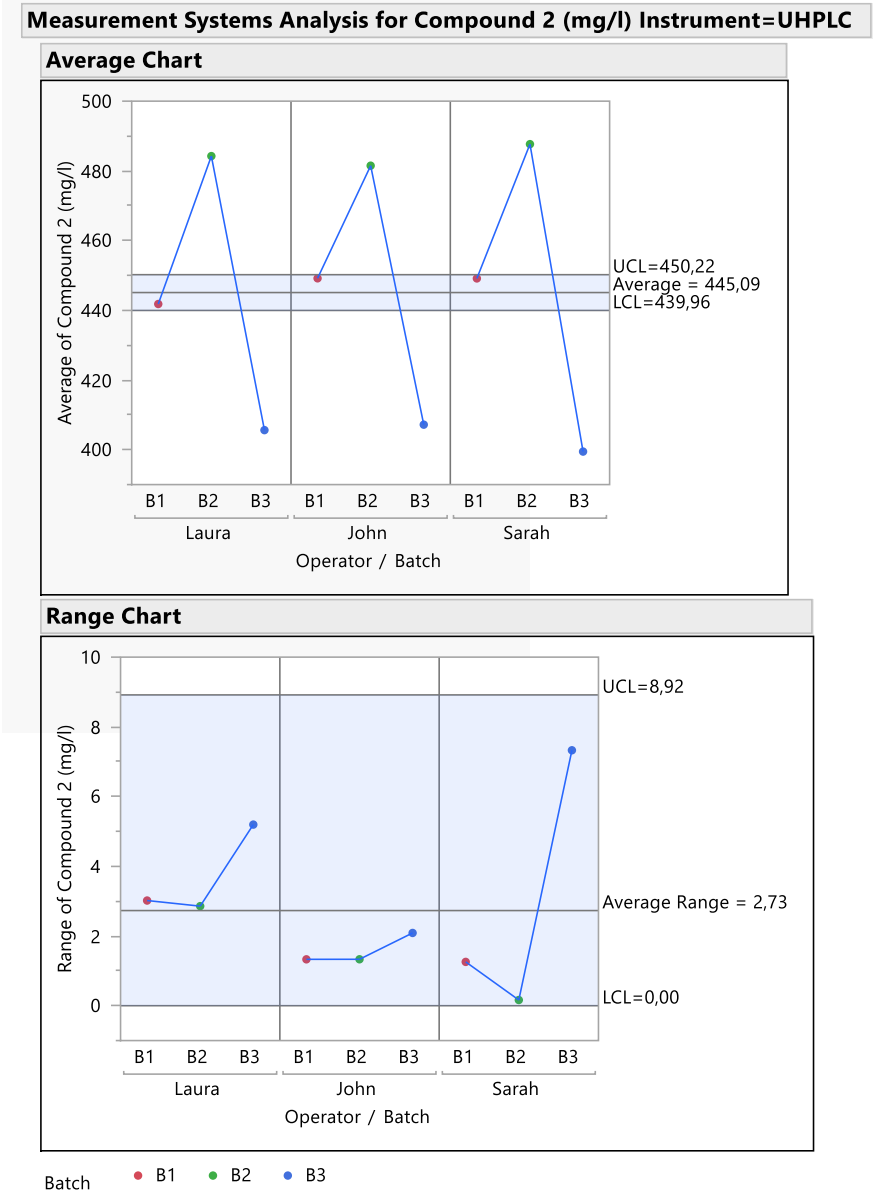
→ Precision OK!

MSA UHPLC VALIDATION – Compound 1



Lines are close & parallel with some crossing indicating small batch – operator interaction

MSA UHPLC VALIDATION – Compound 2



EMP Gauge R&R Results

Component	Std Dev	Variance Component	% of Total	20 40 60 80
Gauge R&R	2,443365	5,9700	0,3664	
Repeatability	2,443365	5,9700	0,3664	
Reproducibility	0,000000	0,0000	0,0	
Product Variation	40,147447	1611,8175	98,9	
Interaction Variation	3,432175	11,7798	0,7229	
Total Variation	40,367900	1629,5673	100,0	

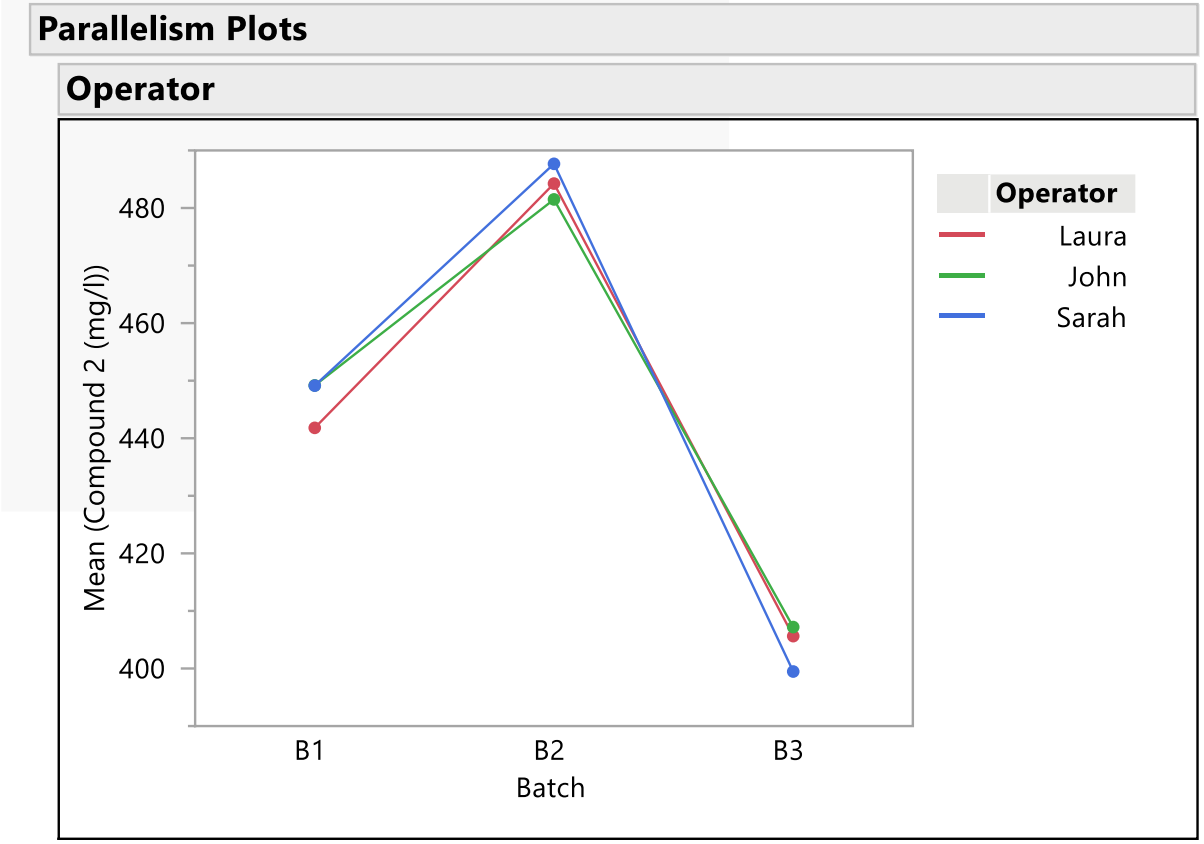
Mainly product variation

Individual UHPLC measurements can detect quality shifts between batches!

Precision/Tolerance ratio = 6*2,44/300 = 5%

→ Precision OK!

MSA UHPLC VALIDATION – Compound 2

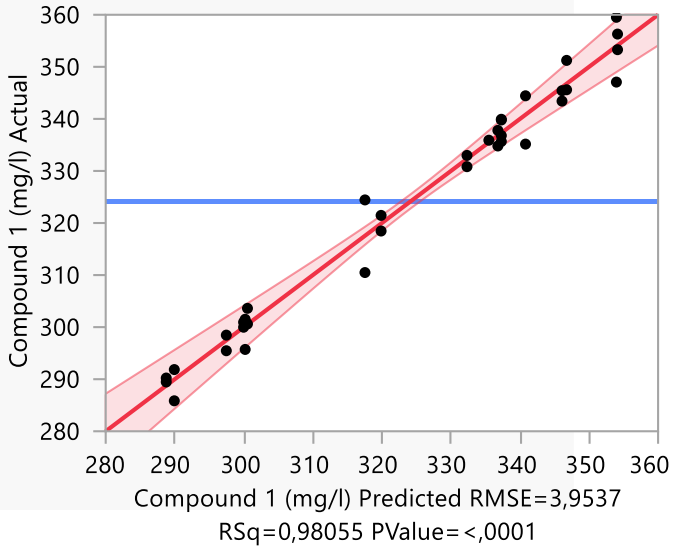


Lines are very close & parallel with no major crossing indicating no operator bias

MSA UHPLC VALIDATION – COMPARISON GC

Response Compound 1 (mg/l)

Actual by Predicted Plot

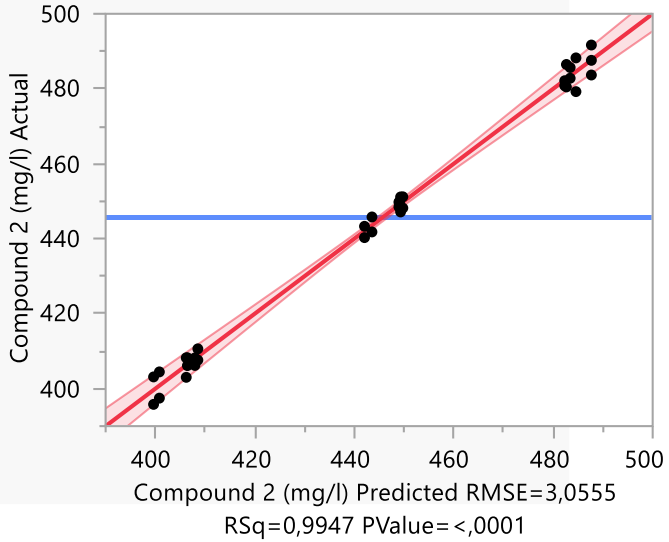


Effect Summary

Source	LogWorth	PValue	
Batch	18,388	0,00000	
Batch*Operator	6,758	0,00000	
Operator	1,254	0,05566	^
Operator*Instrument	0,298	0,50303	
Batch*Instrument	0,191	0,64473	
Instrument	0,000	1,00000	^

Response Compound 2 (mg/l)

Actual by Predicted Plot



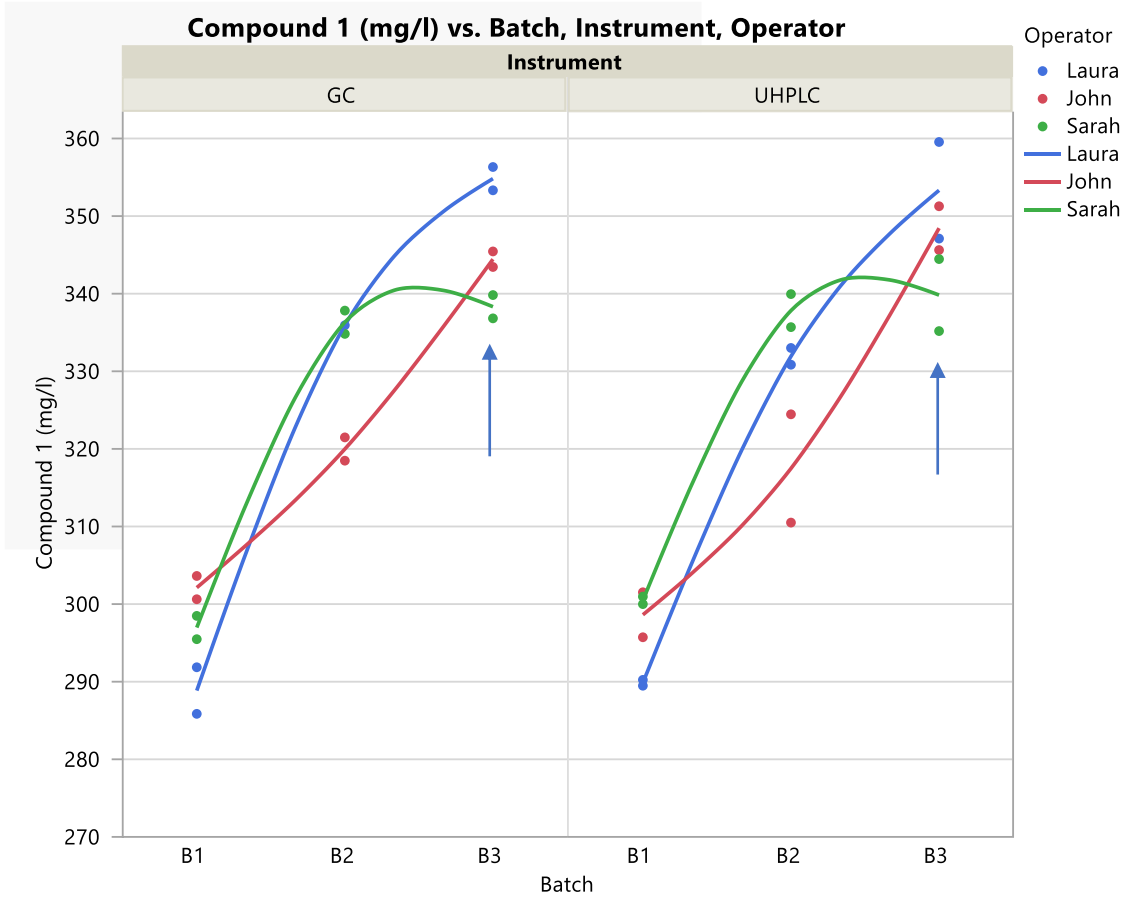
Effect Summary

Source	LogWorth	PValue	
Batch	24,994	0,00000	
Batch*Operator	3,296	0,00051	
Instrument	0,473	0,33686	
Operator	0,367	0,42948	^
Operator*Instrument	0,050	0,89098	
Batch*Instrument	0,050	0,89098	

no significant instrument effect → UHPLC accurate

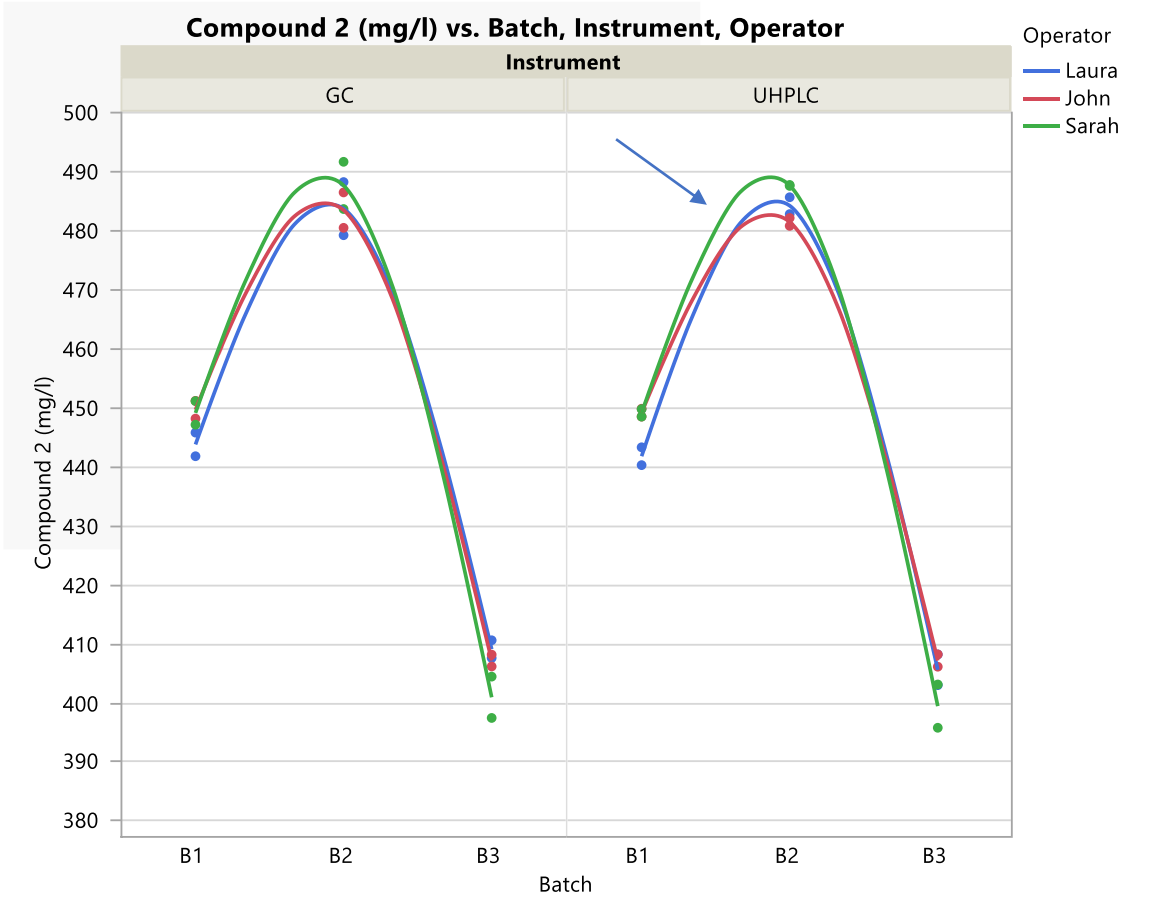
small but significant batch – operator interaction effect

MSA UHPLC VALIDATION – COMPARISON GC



Source	LogWorth	PValue
Batch	10,012	0,00000
Batch*Operator	4,805	0,00002
Operator	1,566	0,02714

Source	LogWorth	PValue
Batch	6,734	0,00000
Batch*Operator	1,881	0,01316
Operator	0,448	0,35629

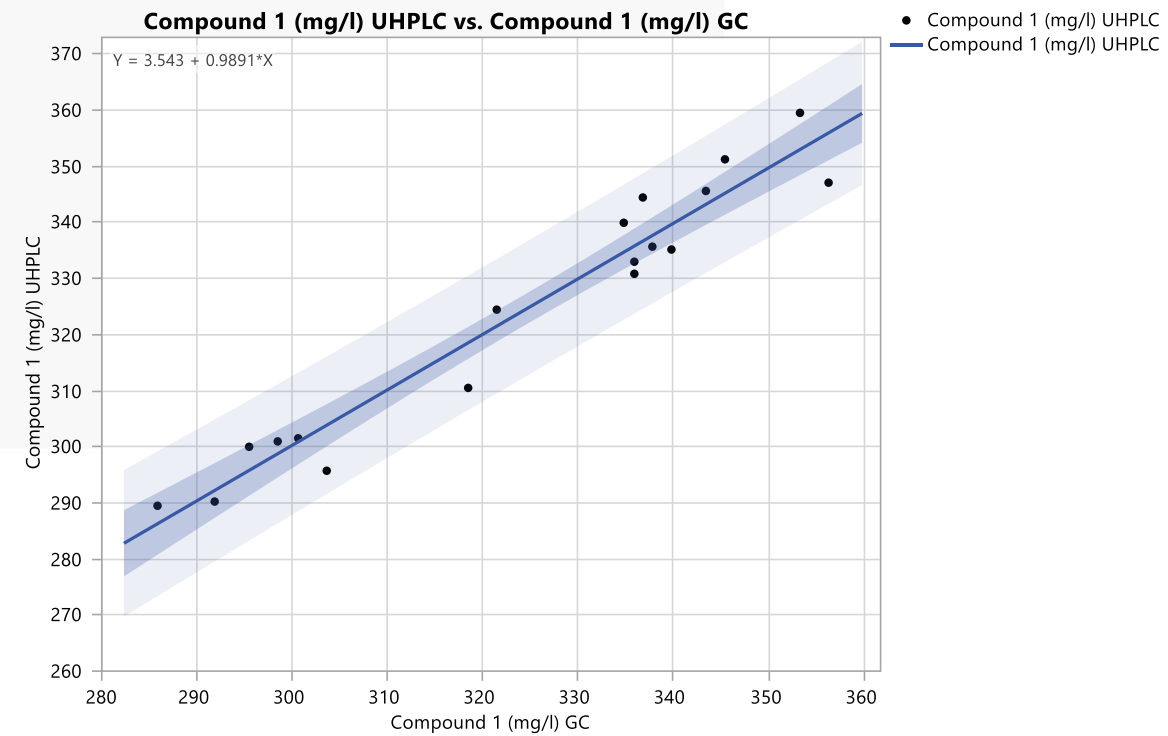


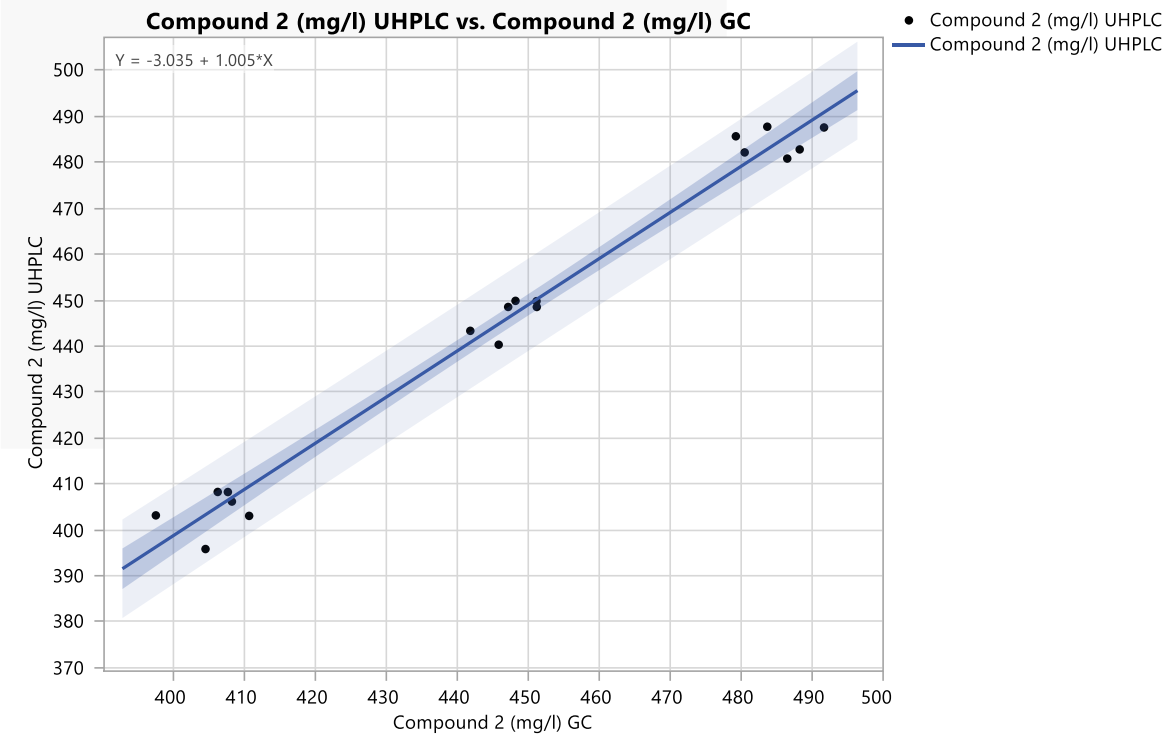
Source	LogWorth	PValue
Batch	9,567	0,00000
Batch*Operator	0,784	0,16442
Operator	0,065	0,86180

Source	LogWorth	PValue
Batch	11,515	0,00000
Batch*Operator	2,087	0,00819
Operator	0,447	0,35750

Batch – operator interaction Compound 1 observed for GC & UHPLC → Sarah?

MSA UHPLC VALIDATION – COMPARISON GC





UHPLC is accurate

No difference with GC standard analysis method