Incurred sample accuracy (ISA) assessment: design of experiments based on standard addition

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Incurred Sample Accuracy

- Initial thoughts
- Experimental section (method validation, bioanalysis)
- Design of experiments
- Results and discussion
  - ISR
  - ISA
- Implementation into GLP bioanalysis?

De Boer T and Wieling J. Incurred sample accuracy assessment: design of experiments based on standard addition. *Bioanalysis (2011) 3(9), 983-992*

Initial thoughts – introduction (1)

In bioanalysis, poor data will lead to miscalculated PK parameters → wrong medical treatment

- **Errors in bioanalysis will lead to a measured value X that deviates from true (reference) value μ_T**

Initial thoughts – introduction (2)

Total error affecting the value measured is caused by the sum of random errors and systematic errors [1]

- **Random errors are a measure for precision → expressed as standard deviation (σ)**
  Caused by: e.g. instrumental & sample instability, sample storage, environmental fluctuations
- **Systematic errors are a measure for trueness → expressed as bias (δ)**
  Caused by e.g. insufficient selectivity, matrix effects, non optimal calibration procedures, operator bias, instrumental shifts….
- **Accuracy (total error) = precision + trueness →** $X-\mu = \delta + \sigma$
  where X = measured value and μ = true or reference value

[1] Rozet *et al*. *J. Chromatogr. A* 1158, 111-125 (2007)

Initial thoughts – introduction (3)

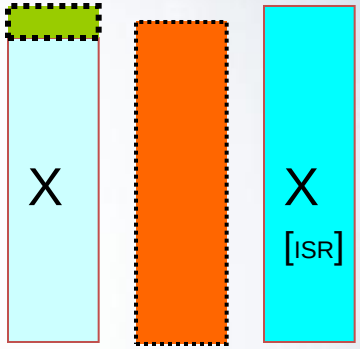
- **To monitor the occurence of random errors, i.e. to examine the reproducibility of the assay: Incurred Sample Reanalysis**

  As the performance of standards and QC's may not adequately mimic that of study samples from dosed subjects

- **If ISR does not detect any instability of the study samples, can it be assumed that there were no systematic errors affecting the initial concentration?**

- **Can the standard addition approach be an acceptable methodology to assess the accuracy of incurred sample concentrations?**

Initial thoughts – introduction (4)



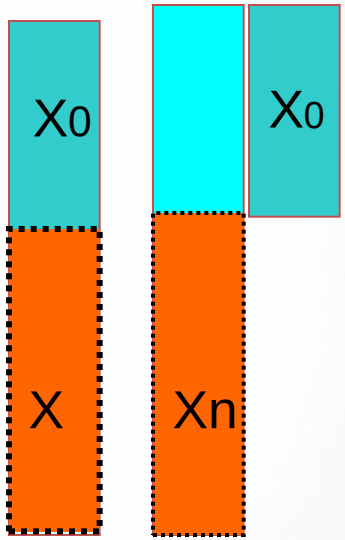
ISR

- **Initial concentration: X**
- **Incurred sample reanalysis: X[ISR]**
- **Reproducibility (random variation): 100 * (X[ISR]-X)/mean**

Initial thoughts – introduction (5)

ISA

- **Standard addition of: X0**
- **Reanalyzed concentration: Xr**
- **Normalized concentration:**
  Xn ≃ Xr-X0 ≃ X



- **If there are no systematic errors affecting the assay: Xn = X (within random variation, σ)**
- **If there are systematic errors: Xn ≠ X**

Accuracy: 100 * (Xn –X)/X

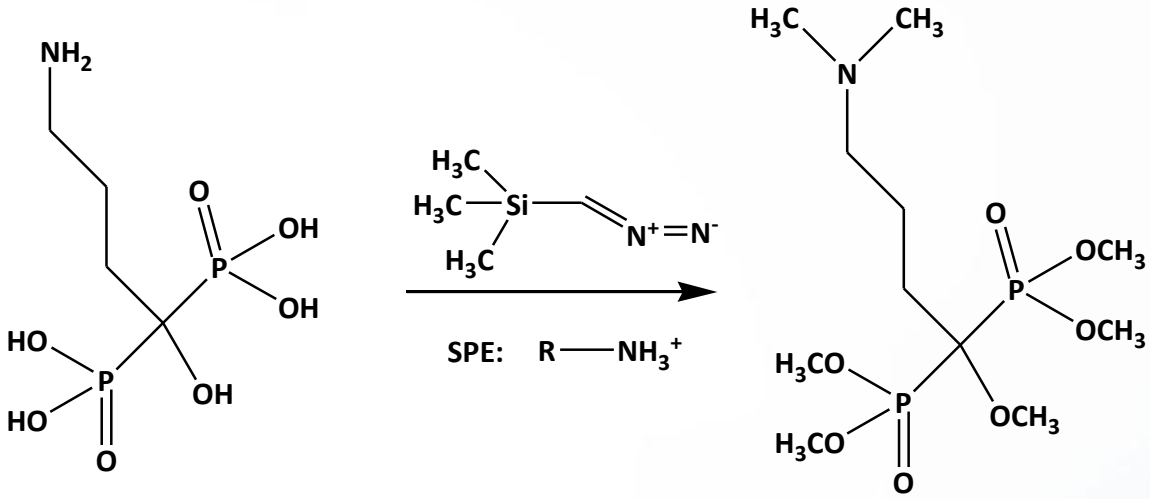
Initial thoughts – experiments

Demonstrate ISR and ISA using study samples from a BE study of alendronate (bisphosphonate) in human urine.

Single blind, randomized, two arms, crossover, pilot bioequivalence study of two formulations of sodium alendronate in healthy female volunteers after single oral dose administration under fasting conditions.

Experimental section (1)

Sample clean-up using a combined off-line SPE - derivatization step



Extremely polar

More lipophilic

Experimental section (2)

Mobile phase	**ammonium formate buffer (150 mM, pH 3.5):ACN; 200 µL/min**
Analytical column	**Xbridge 2.1 x 100 mm 3.5 µm (Hydrophilic Interaction LC)**
Quantitation	**API4000, Turbo Ionspray, positive ionization**
	MRM (N_2 collision gas)

	Q1 (amu)	Q3 (amu)
alendronic acid	348.2	162.9
alendronic acid-d6	354.2	168.1

Experimental section (3)

Results validation

Sample	Spiked concentration (ng/mL)	CV%
LLOQ	1.00	12.5
QClow	3.00	8.1
QCmed	125	5.6
QChigh	800	6.6

Sample	Mean concentration (ng/mL)	%Bias
LLOQ	1.01	0.6
QClow	3.36	12.1
QCmed	139	11.0
QChigh	840	5.0

Selectivity	**No interfering peaks from 6 independent urine lots**
Calibration curves	**Optimal with 1/xx weighing, $r^2 > 0.9960$**
Stability in autosampler	**Extracts stable for 24 hours at 10° C**
Stability after F/T	**No instability after 3 cycles**
Stability at RT	**No instability after 24h at RT**
Carry over	**No carry-over (< 20% of LLOQ)**
Matrix effect (ionisation)	**Variation between 6 independent urine lots <15%**

Experimental section (4)

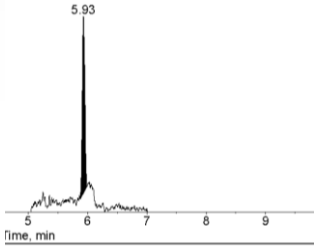
Results bioanalysis

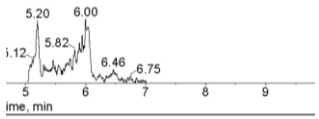
Design: crossover study of 80 subjects; 2x11 PK samples (n=1738)

	Spiked	Mean	CV%	%Bias	Number
QC low	3.00	3.05	6.0	1.8	56
QC med	125	123	6.1	-1.2	56
QC high	800	771	6.1	-3.6	56

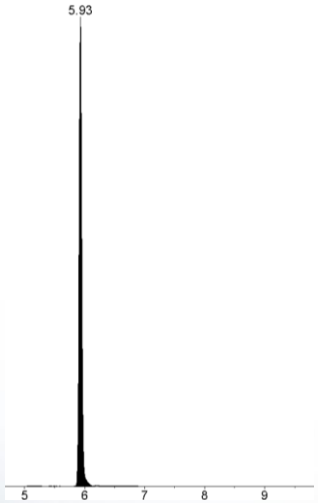
Bioanalysis in 31 consecutive bioanalytical runs: 90.3% of the batches were accepted with 4/6/15 rule LLOQ: 1.00 ng/mL ; R^2 > 0.9843

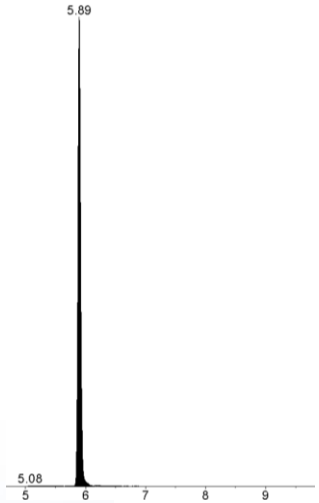
Experimental section (5)

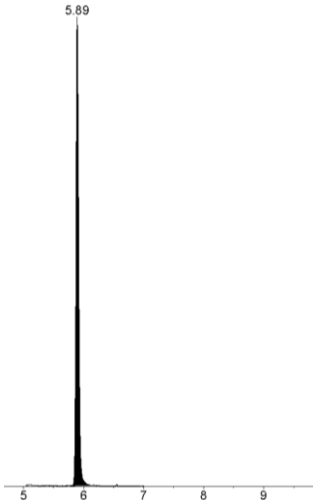




Alendronic acid



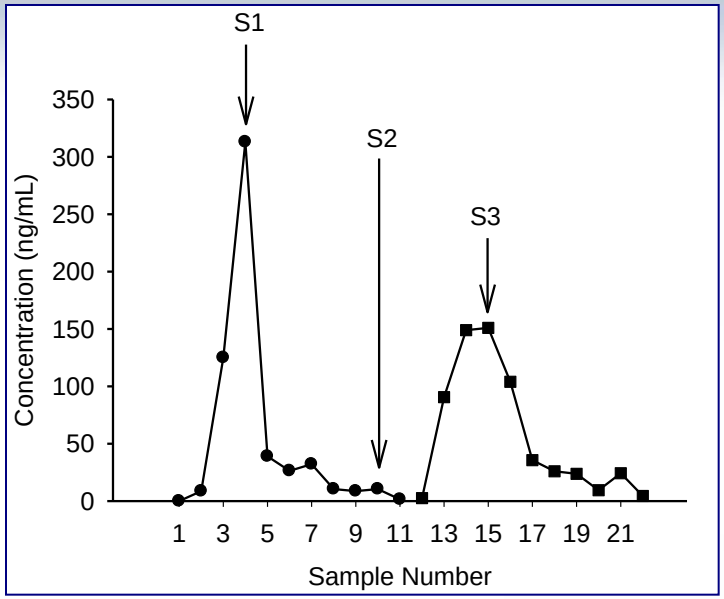




Alendronic acid-d6

LLOQ, 1.00 ng/mL **predose** **“Cmax” (urine), 100 ng/mL**

Design of experiments



Typical urine PK profile (n=22 samples) for both phases of the bioequivalence study (● = arm 1; ■ = arm 2)

- **Reanalysis of samples (n=30) in duplicate (1 run):** ISR
- **Reanalysis of samples (n=30) in duplicate after standard addition with 30 ng/mL:** ISA[30]
- **Reanalysis of samples (n=30) in duplicate after standard addition with 60 ng/mL:** ISA[60]

Results repeat analysis: without (ISR) & with (ISA) standard addition

N	X	X_{R1}	X_{R2}	$\overline{X}_{R1,R2}$	$X_{R[30],1}$	$X_{R[30],2}$	$\overline{X}_{R[30]1,2}$	$X_{R[60],1}$	$X_{R[60],2}$	$\overline{X}_{R[60]1,2}$
1	4.94	4.25	4.27	4.26	33.1	32.8	33.0	59.8	61.1	60.5
2	5.83	5.15	5.14	5.14	33.6	34.4	34.0	62.3	64.7	63.5
3	5.91	6.27	6.05	6.16	34.4	34.9	34.7	62.7	63.1	62.9
4	10.5	10.0	9.91	9.96	39.0	38.1	38.6	66.9	68.0	67.5
5	15.2	14.2	14.2	14.2	42.9	43.5	43.2	72.1	72.9	72.5
6	22.4	22.8	22.3	22.6	52.3	51.2	51.8	81.4	80.8	81.1
7	25.8	23.6	23.1	23.4	52.6	52.4	52.5	82.1	81.8	82.0
8	26.1	28.7	29.1	28.9	58.8	57.0	57.9	85.9	86.3	86.1
9	30.1	34.5	33.4	34.0	63.4	61.7	62.6	92.2	92.1	92.2
10	42.2	37.7	37.7	37.7	65.6	65.7	65.7	94.4	92.7	93.6
11	68.9	63.4	61.7	62.6	88.6	88.1	88.4	117	119	118
12	77.3	68.6	69.5	69.1	95.8	97.4	96.6	125	125	125
13	80.3	83.9	84.5	84.2	111	111	111	143	139	141
14	101	88.4	92.3	90.4	118	119	119	148	147	148
15	106	119	120	120	148	147	148	178	178	178
16	108	96.9	93.6	95.3	119	116	118	143	139	141
17	120	121	119	120	147	149	148	179	178	179
18	149	154	153	154	184	182	183	215	213	214
19	150	145	148	146	171	176	174	203	205	204
20	151	142	141	142	169	171	170	197	198	198
21	156	139	138	138	161	159	160	169	179	174
22	157	141	140	140	167	164	166	197	199	198
23	159	138	144	141	175	170	173	204	200	202
24	242	267	264	266	286	290	288	322	323	323
25	270	280	278	279	299	303	301	335	336	336
26	313	312	307	310	336	340	338	368	360	364
27	330	293	295	294	305	306	306	316	311	314
28	356	371	375	373	400	395	398	437	432	435
29	373	336	341	338	354	357	356	390	387	389
30	575	528	521	524	529	534	532	580	565	573

Repeatability <|6%|

Results repeat analysis: ISR

N	X	X_{R1}	X_{R2}	$\overline{X}_{R1,R2}$	$X_{R[30],1}$	$X_{R[30],2}$	$\overline{X}_{R[30]1,2}$	$X_{R[60],1}$	$X_{R[60],2}$	$\overline{X}_{R[60]12}$
1	4.94	4.25	4.27	4.26	33.1	32.8	33.0	59.8	61.1	60.5
2	5.83	5.15	5.14	5.14	33.6	34.4	34.0	62.3	64.7	63.5
3	5.91	6.27	6.05	6.16	34.4	34.9	34.7	62.7	63.1	62.9
4	10.5	10.0	9.91	9.96	39.0	38.1	38.6	66.9	68.0	67.5
5	15.2	14.2	14.2	14.2	42.9	43.5	43.2	72.1	72.9	72.5
6	22.4	22.8	22.3	22.6	52.3	51.2	51.8	81.4	80.8	81.1
7	25.8	23.6	23.1	23.4	52.6	52.4	52.5	82.1	81.8	82.0
8	26.1	28.7	29.1	28.9	58.8	57.0	57.9	85.9	86.3	86.1
9	30.1	34.5	33.4	34.0	63.4	61.7	62.6	92.2	92.1	92.2
10	42.2	37.7	37.7	37.7	65.6	65.7	65.7	94.4	92.7	93.6
11	68.9	63.4	61.7	62.6	88.6	88.1	88.4	117	119	118
12	77.3	68.6	69.5	69.1	95.8	97.4	96.6	125	125	125
13	80.3	83.9	84.5	84.2	111	111	111	143	139	141
14	101	88.4	92.3	90.4	118	119	119	148	147	148
15	106	119	120	120	148	147	148	178	178	178
16	108	96.9	93.6	95.3	119	116	118	143	139	141
17	120	121	119	120	147	149	148	179	178	179
18	149	154	153	154	184	182	183	215	213	214
19	150	145	148	146	171	176	174	203	205	204
20	151	142	141	142	169	171	170	197	198	198
21	156	139	138	138	161	159	160	169	179	174
22	157	141	140	140	167	164	166	197	199	198
23	159	138	144	141	175	170	173	204	200	202
24	242	267	264	266	286	290	288	322	323	323
25	270	280	278	279	299	303	301	335	336	336
26	313	312	307	310	336	340	338	368	360	364
27	330	293	295	294	305	306	306	316	311	314
28	356	371	375	373	400	395	398	437	432	435
29	373	336	341	338	354	357	356	390	387	389
30	575	528	521	524	529	534	532	580	565	573

Reproducibility: ISR

Results repeat analysis: ISR

N	X	$\bar{X}_{R1R2}$	ISR(%)	$\bar{X}_R[XO] - X_O$ where X_O = 30 ng/mL	ISA(%)	$\bar{X}_R[XO] - X_O$ where X_O = 60 ng/mL	ISA(%)
1	4.94	4.26	-14.8%	2.95	**-39.3**	0.450	**-89.9**
2	5.83	5.14	-12.6%	4.00	**-31.4**	3.50	**-40.0**
3	5.91	6.16	4.1%	4.65	**-20.5**	2.90	**-50.9**
4	10.5	9.96	-5.3%	8.55	-18.1	7.45	**-28.6**
5	15.2	14.2	-6.8%	13.2	-13.2	12.5	-17.8
6	22.4	22.6	0.7%	21.8	-2.7	21.1	-5.8
7	25.8	23.4	-10.0%	22.5	-12.8	22.0	-14.7
8	26.1	28.9	10.2%	27.9	6.9	26.1	0.0
9	30.1	34.0	12.0%	32.6	8.3	32.2	7.0
10	42.2	37.7	-11.3%	35.7	-15.4	33.6	**-20.4**
11	68.9	62.6	-9.7%	58.4	-15.2	58.0	-15.8
12	77.3	69.1	-11.3%	66.6	-13.8	65.0	-15.9
13	80.3	84.2	4.7%	81.0	0.9	81.0	0.9
14	101	90.4	-11.1%	88.5	-11.9	87.5	-12.9
15	106	120	12.4%	118	11.3	118	11.3
16	108	95.3	-12.5%	87.5	-18.5	81.0	**-25.0**
17	120	120	0.0%	118	-1.7	119	-0.8
18	149	154	3.0%	153	2.7	154	3.4
19	150	146	-2.7%	144	-4.0	144	-4.0
20	151	142	-6.5%	140	-7.3	138	-8.6
21	156	138	-12.2%	130	-16.7	114	**-26.9**
22	157	140	-11.4%	136	-13.4	138	-12.1
23	159	141	-12.0%	143	-10.1	142	-10.7
24	242	266	9.3%	258	6.6	263	8.7
25	270	279	3.3%	271	0.4	276	2.2
26	313	310	-1.1%	308	-1.6	304	-2.9
27	330	294	-11.5%	276	-16.4	254	**-23.0**
28	356	373	4.7%	368	3.4	375	5.3
29	373	338	-9.8%	326	-12.6	329	-11.8
30	575	524	-9.3%	502	-12.7	513	-10.8

Reproducibility: ISR: 100% within limits (between -14.8% and 12.4%)

Results repeat analysis: ISA [30]

N	X	$\bar{X}_{R1R2}$	ISR(%)	$\bar{X}_R[XO] - X_O$ where X_O = 30 ng/mL	ISA(%)	$\bar{X}_R[XO] - X_O$ where X_O = 60 ng/mL	ISA(%)
1	4.94	4.26	-14.8%	2.95	**-39.3**	0.450	**-89.9**
2	5.83	5.14	-12.6%	4.00	**-31.4**	3.50	**-40.0**
3	5.91	6.16	4.1%	4.65	**-20.5**	2.90	**-50.9**
4	10.5	9.96	-5.3%	8.55	-18.1	7.45	**-28.6**
5	15.2	14.2	-6.8%	13.2	-13.2	12.5	-17.8
6	22.4	22.6	0.7%	21.8	-2.7	21.1	-5.8
7	25.8	23.4	-10.0%	22.5	-12.8	22.0	-14.7
8	26.1	28.9	10.2%	27.9	6.9	26.1	0.0
9	30.1	34.0	12.0%	32.6	8.3	32.2	7.0
10	42.2	37.7	-11.3%	35.7	-15.4	33.6	**-20.4**
11	68.9	62.6	-9.7%	58.4	-15.2	58.0	-15.8
12	77.3	69.1	-11.3%	66.6	-13.8	65.0	-15.9
13	80.3	84.2	4.7%	81.0	0.9	81.0	0.9
14	101	90.4	-11.1%	88.5	-11.9	87.5	-12.9
15	106	120	12.4%	118	11.3	118	11.3
16	108	95.3	-12.5%	87.5	-18.5	81.0	**-25.0**
17	120	120	0.0%	118	-1.7	119	-0.8
18	149	154	3.0%	153	2.7	154	3.4
19	150	146	-2.7%	144	-4.0	144	-4.0
20	151	142	-6.5%	140	-7.3	138	-8.6
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27	330	294	-11.5%	276	-16.4	254	**-23.0**
28	356	373	4.7%	368	3.4	375	5.3
29	373	338	-9.8%	326	-12.6	329	-11.8
30	575	524	-9.3%	502	-12.7	513	-10.8

Accuracy: 86.7% within limits (i.e. within ±20% similar to ISR criteria)

Results repeat analysis: ISA [60]

N	X	$\bar{X}_{R1R2}$	ISR(%)	$\bar{X}_R[X0] - X_0$ where X_0 = 30 ng/mL	ISA(%)	$\bar{X}_R[X0] - X_0$ where X_0 = 60 ng/mL	ISA(%)
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3	5.91	6.16	4.1%	4.65	**-20.5**	2.90	**-50.9**
4	10.5	9.96	-5.3%	8.55	-18.1	7.45	**-28.6**
5	15.2	14.2	-6.8%	13.2	-13.2	12.5	-17.8
6	22.4	22.6	0.7%	21.8	-2.7	21.1	-5.8
7	25.8	23.4	-10.0%	22.5	-12.8	22.0	-14.7
8	26.1	28.9	10.2%	27.9	6.9	26.1	0.0
9	30.1	34.0	12.0%	32.6	8.3	32.2	7.0
10	42.2	37.7	-11.3%	35.7	-15.4	33.6	**-20.4**
11	68.9	62.6	-9.7%	58.4	-15.2	58.0	-15.8
12	77.3	69.1	-11.3%	66.6	-13.8	65.0	-15.9
13	80.3	84.2	4.7%	81.0	0.9	81.0	0.9
14	101	90.4	-11.1%	88.5	-11.9	87.5	-12.9
15	106	120	12.4%	118	11.3	118	11.3
16	108	95.3	-12.5%	87.5	-18.5	81.0	**-25.0**
17	120	120	0.0%	118	-1.7	119	-0.8
18	149	154	3.0%	153	2.7	154	3.4
19	150	146	-2.7%	144	-4.0	144	-4.0
20	151	142	-6.5%	140	-7.3	138	-8.6
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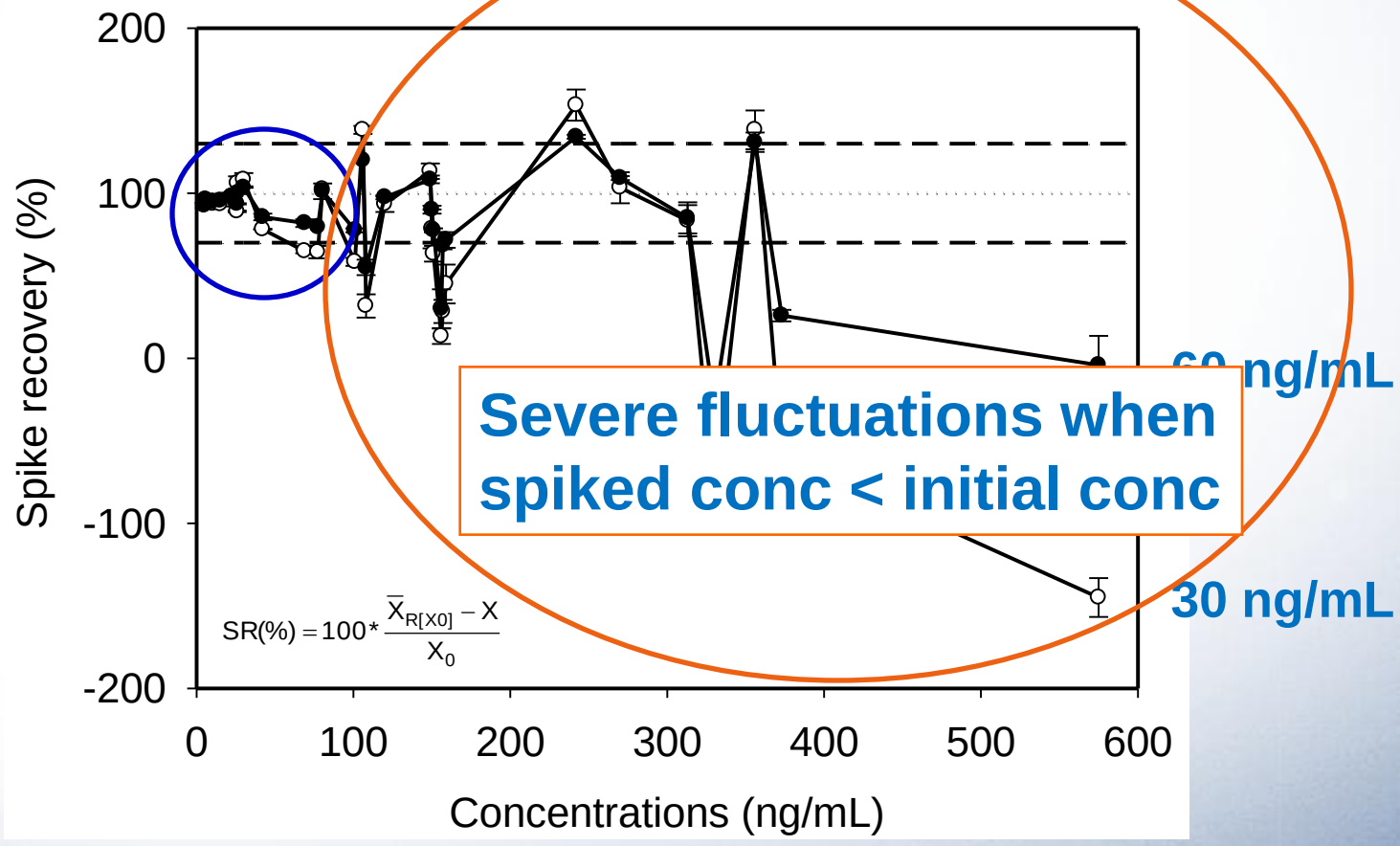
27	330	294	-11.5%	276	-16.4	254	**-23.0**
28	356	373	4.7%	368	3.4	375	5.3
29	373	338	-9.8%	326	-12.6	329	-11.8
30	575	524	-9.3%	502	-12.7	513	-10.8

Accuracy: 73.3% within limits (i.e. within ±20% similar to ISR criteria)

Taking a closer look: calculation of spike recoveries

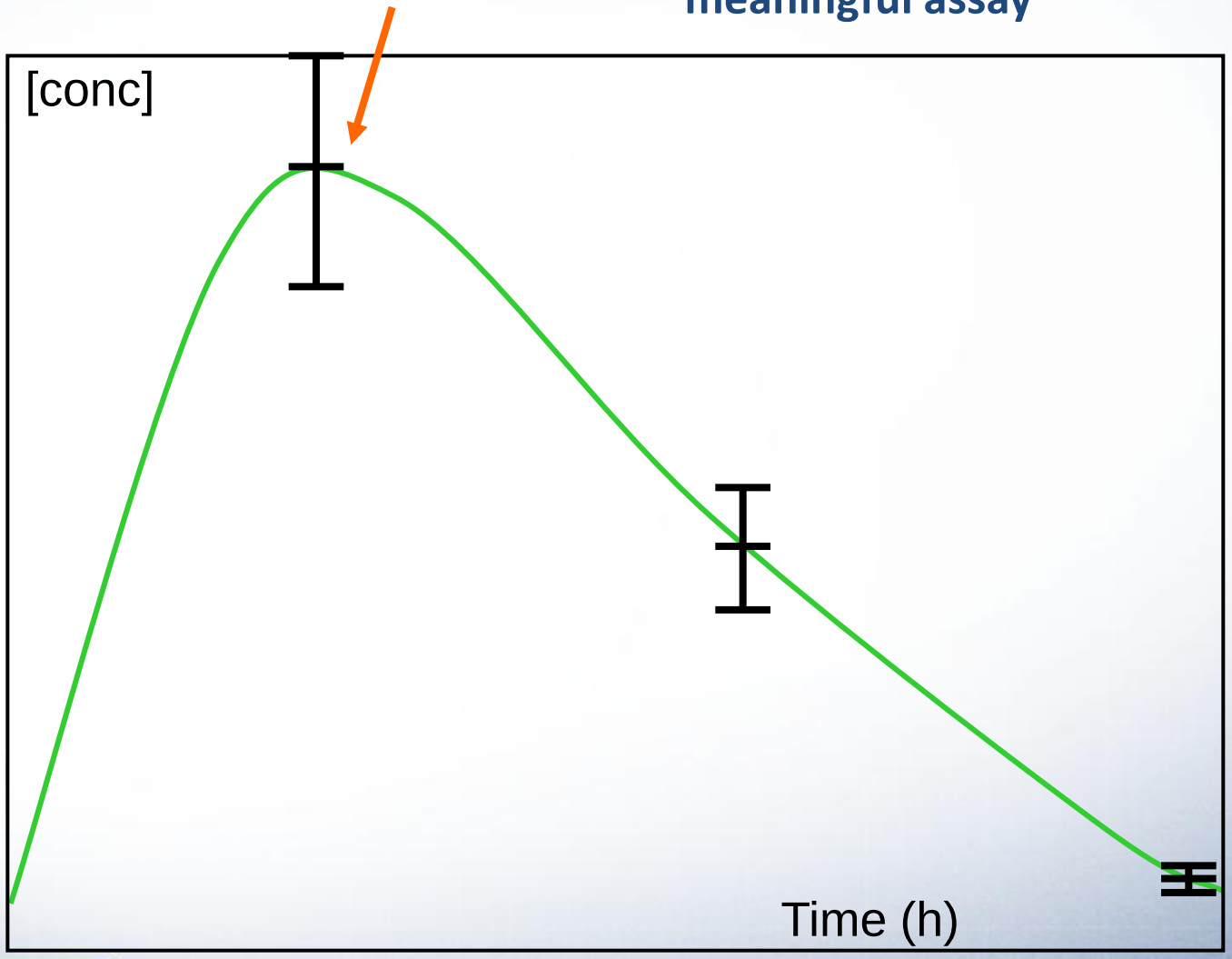
- Spike recovery = 100 * (Xr[30] – X) / 30
- Spike recovery = 100 * (Xr[60] – X) / 60



Taking a closer look:

Too low spike at high concentrations →

Random analytical error may obstruct meaningful assay



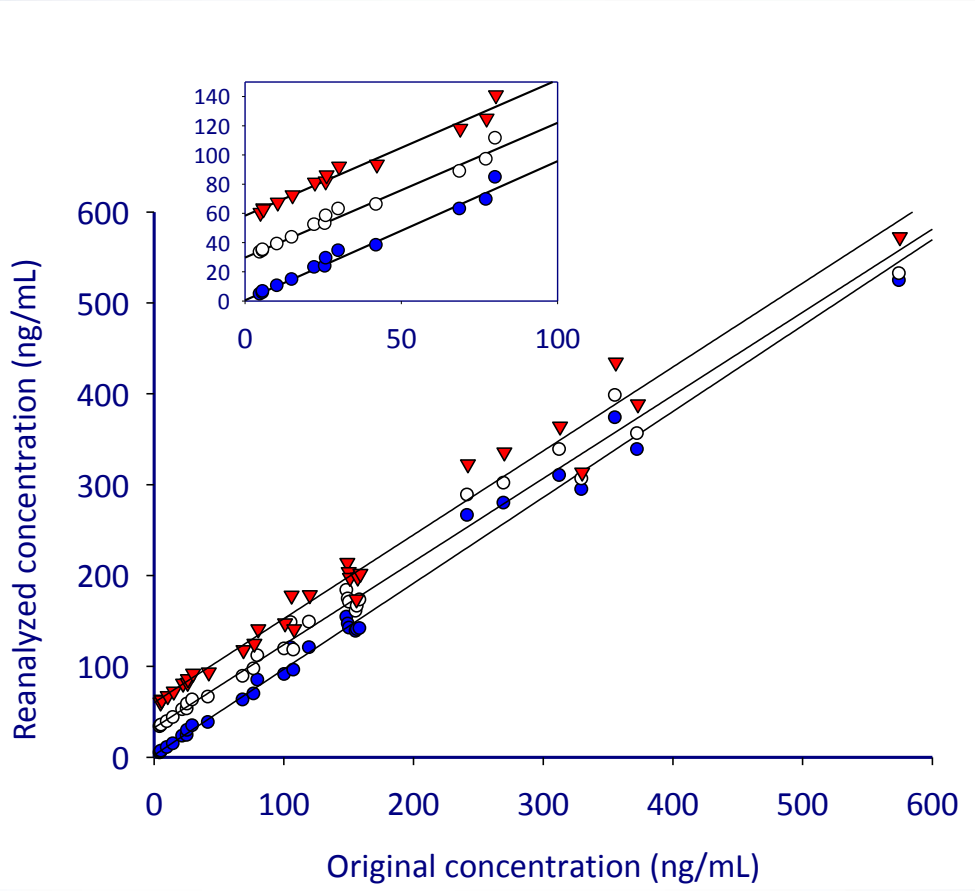
Taking a closer look: correlation between spiked and non-spiked incurred samples

- Hypothesis: initial data are not affected by systematic errors, i.e. normalized data sets (X *vs* ISR *vs* ISA) are equal.

- Calculation of correlation as a first indication of similarity:

  - Pearson's correlation coefficient: information about random errors
  - Slope: information about relative systematic errors
  - Intercept: information about constant systematic errors

Taking a closer look: correlation between spiked and non-spiked incurred samples

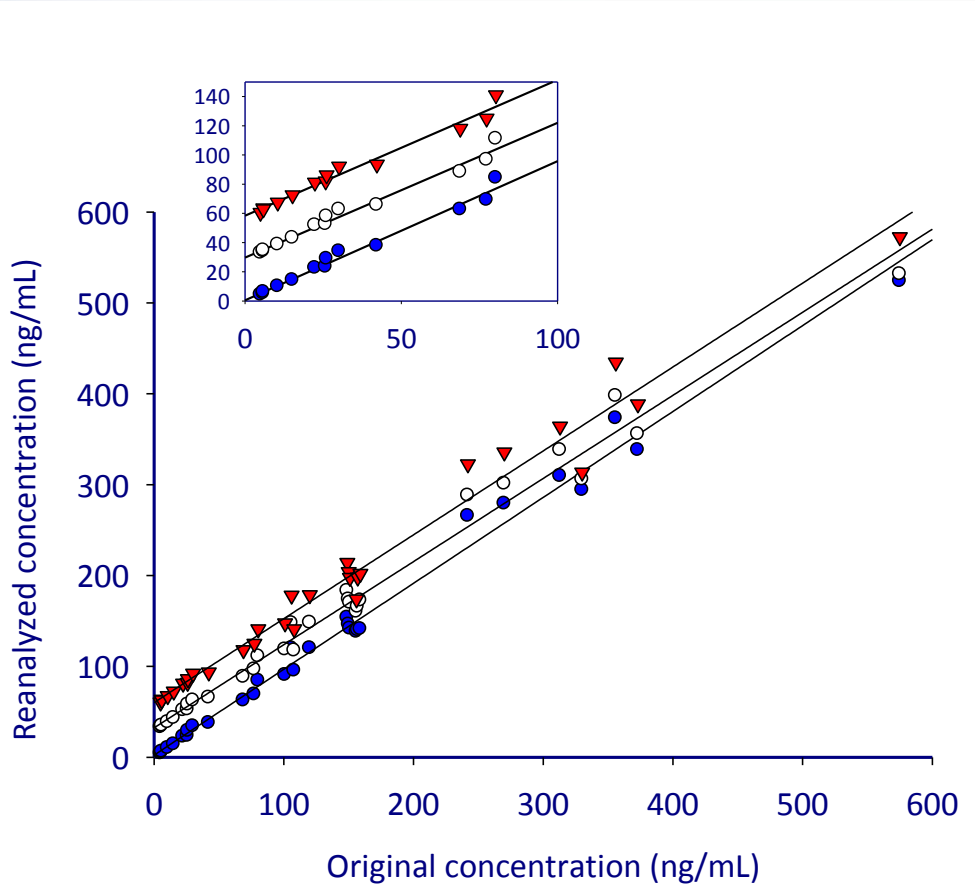


Correlation coefficients:

R=0.9947 (ISR)
R=0.9926 (ISA[30])
R=0.9894 (ISA[60])

No random errors affecting the method → good precision

Taking a closer look: correlation between spiked and non-spiked incurred samples

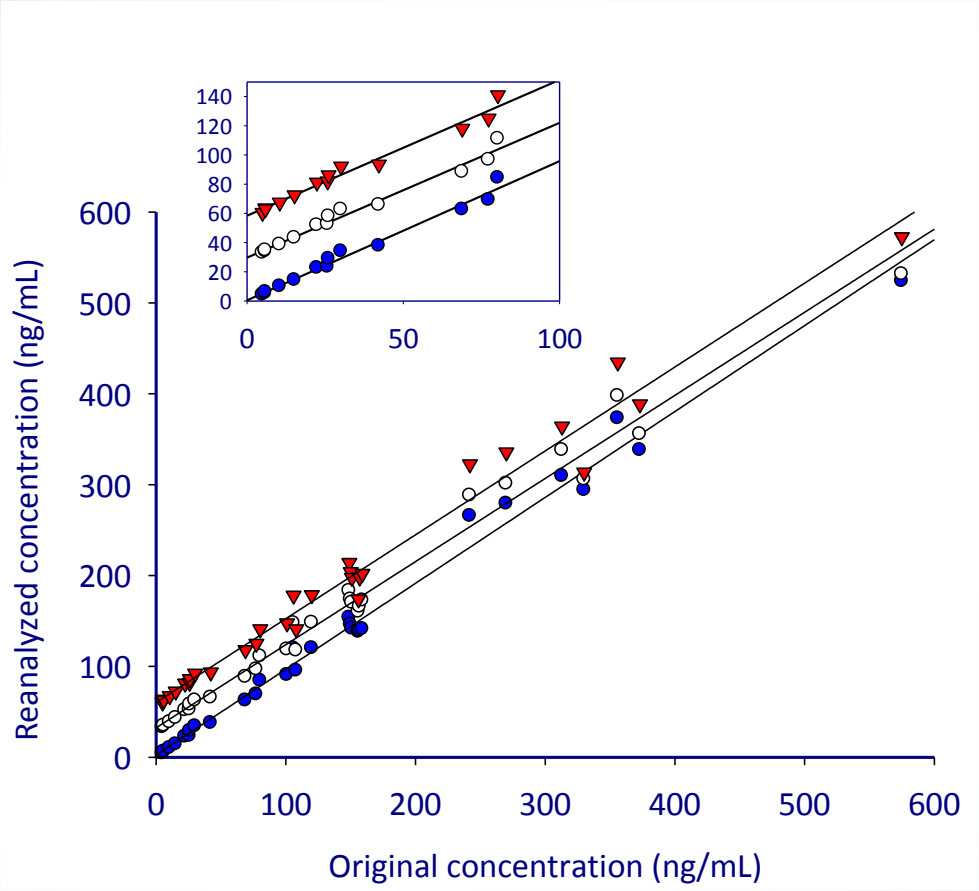


Slopes:

ISR = 0.95 ± 0.02
ISA[30] = 0.92 ± 0.02
ISA[60] = 0.92 ± 0.03

Slopes are approximating 1.0 and are almost parallel
Only minor relative systematic errors affect the method

Taking a closer look: correlation between spiked and non-spiked incurred samples



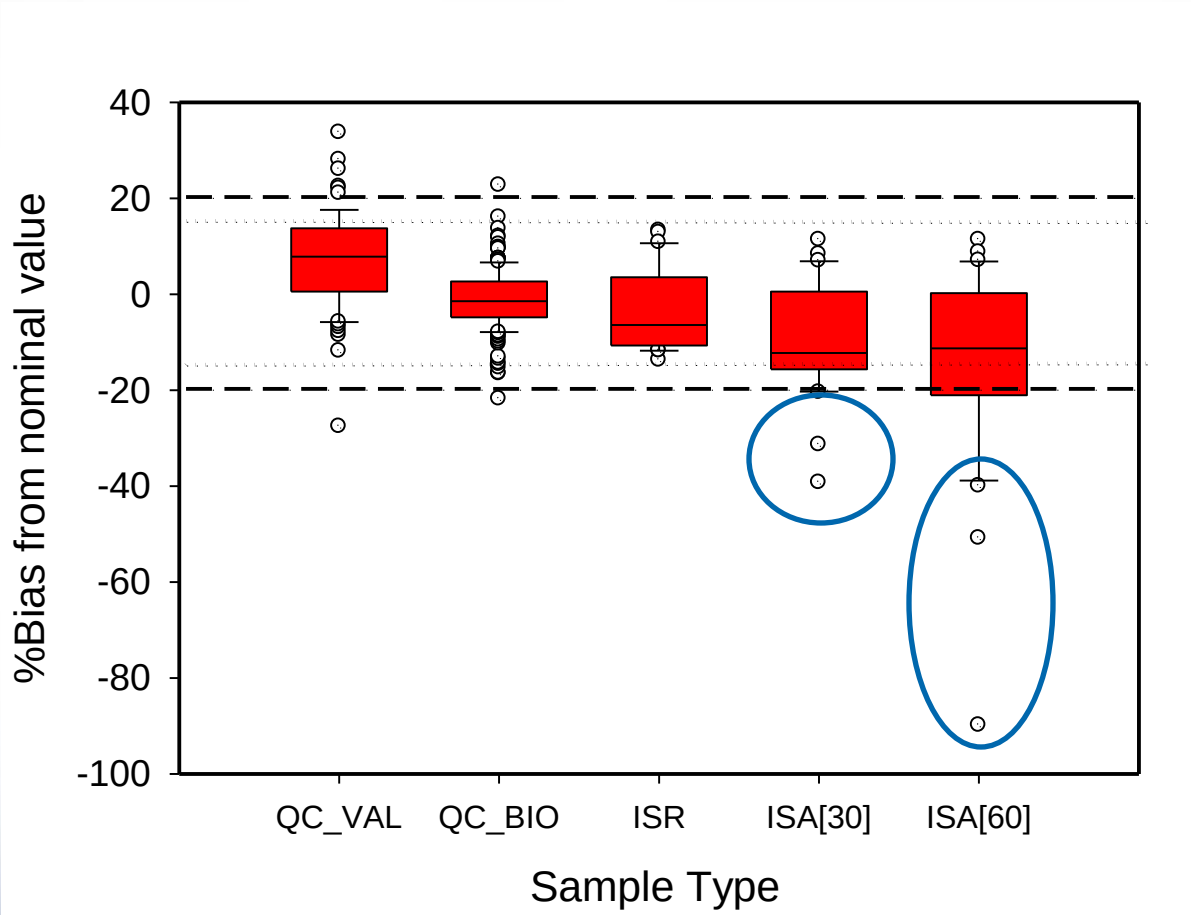
Intercepts:

ISR = 2.34 ng/mL
ISA[30] = 32.4 ng/mL
ISA[60] = 60.2 ng/mL

No constant systematic errors: intercepts correspond with the spiked concentrations.

Implementation into GLP bioanalysis?

- **Do standard addition experiments provide additional information about the performance of the assay?**



Extreme outliers are caused by the low spike recovery results at high concentrations

Conclusion

- Standard addition approach provides additional information about the performance (i.c. accuracy) of the assay

How to proceed….

- **Spiked concentration should however be carefully selected:**
  - Two concentration levels ISA[low] and ISA[high]
  - ISA[low] ~ 3 x Cmin
  - ISA[high] 0.5 x Cmax (undiluted sample)

- **Should ISA be included as an additional validation item (costs?) or should the standard addition experiments be combined with the ISR experiments (in total 10% of number of samples: no additional costs?)**

Thank you for your attention: Questions?