Challenges with Equivalence Testing According to USP <1032>

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Topics

- Introduction
- USP 1032 Equivalence Test Approach (Brief Introduction)

- The Slope Challenge
- The Correlation Challenge
- The Precision Challenge
- The Tolerance Interval Challenge
- The 5PL Challenge

- Conclusions

Introduction

Introduction

- Stegmann Systems is deeply convinced that Equivalence Testing is THE superior method for qualifying assay runs in comparison to any older methods (especially in comparison to Difference Testing)

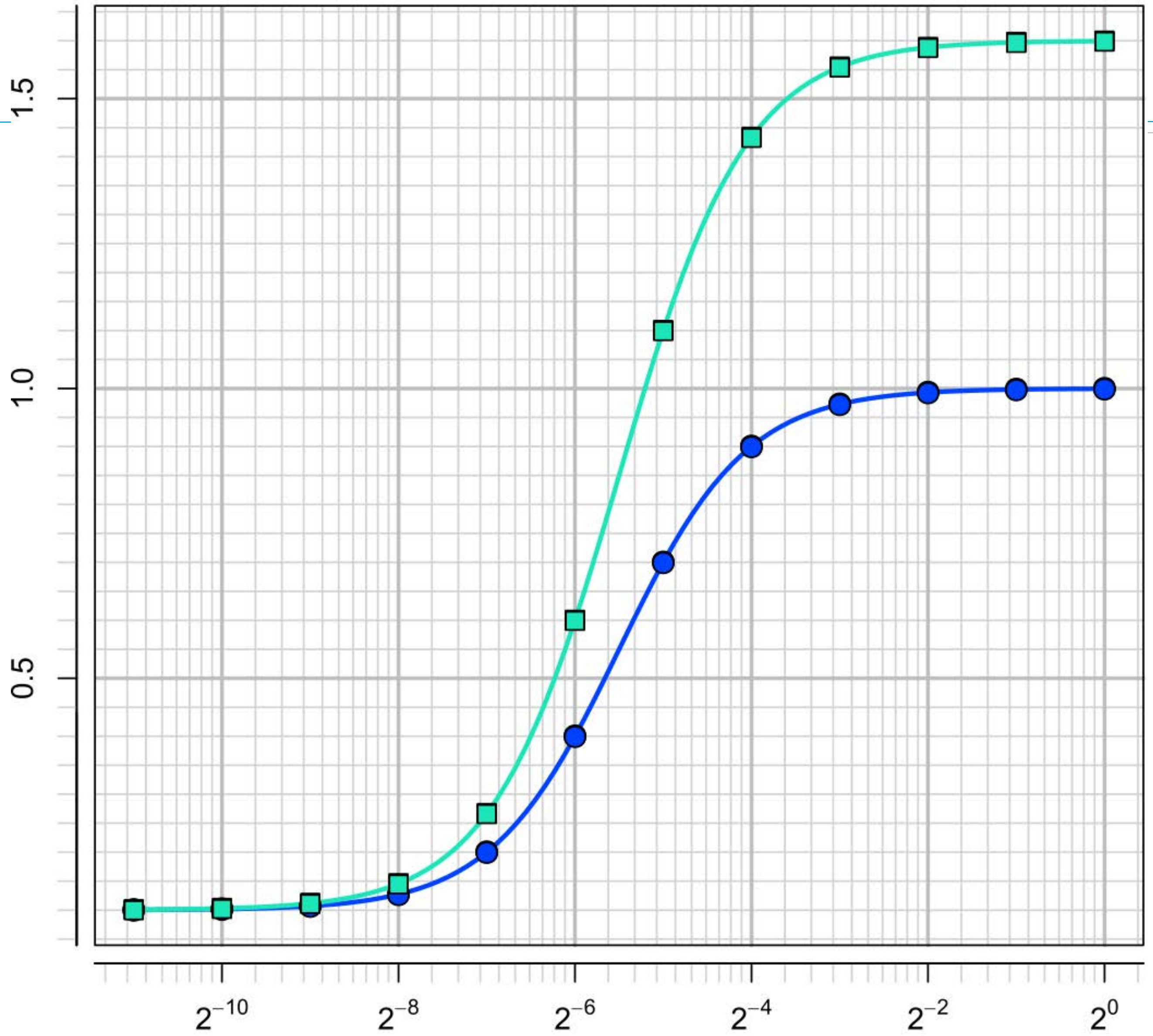
- Stegmann Systems is part of the community of Bioassay Experts and plays its role as a software vendor. It is not the business of Stegmann Systems to develop new methods. Focus is the implementation.

- Stegmann Systems has no interest in harming especially the USP <103x> guidance. We don’t want to be offensive in any way. Nevertheless we feel responsibility for our clients and our impression was, there is discussion required.

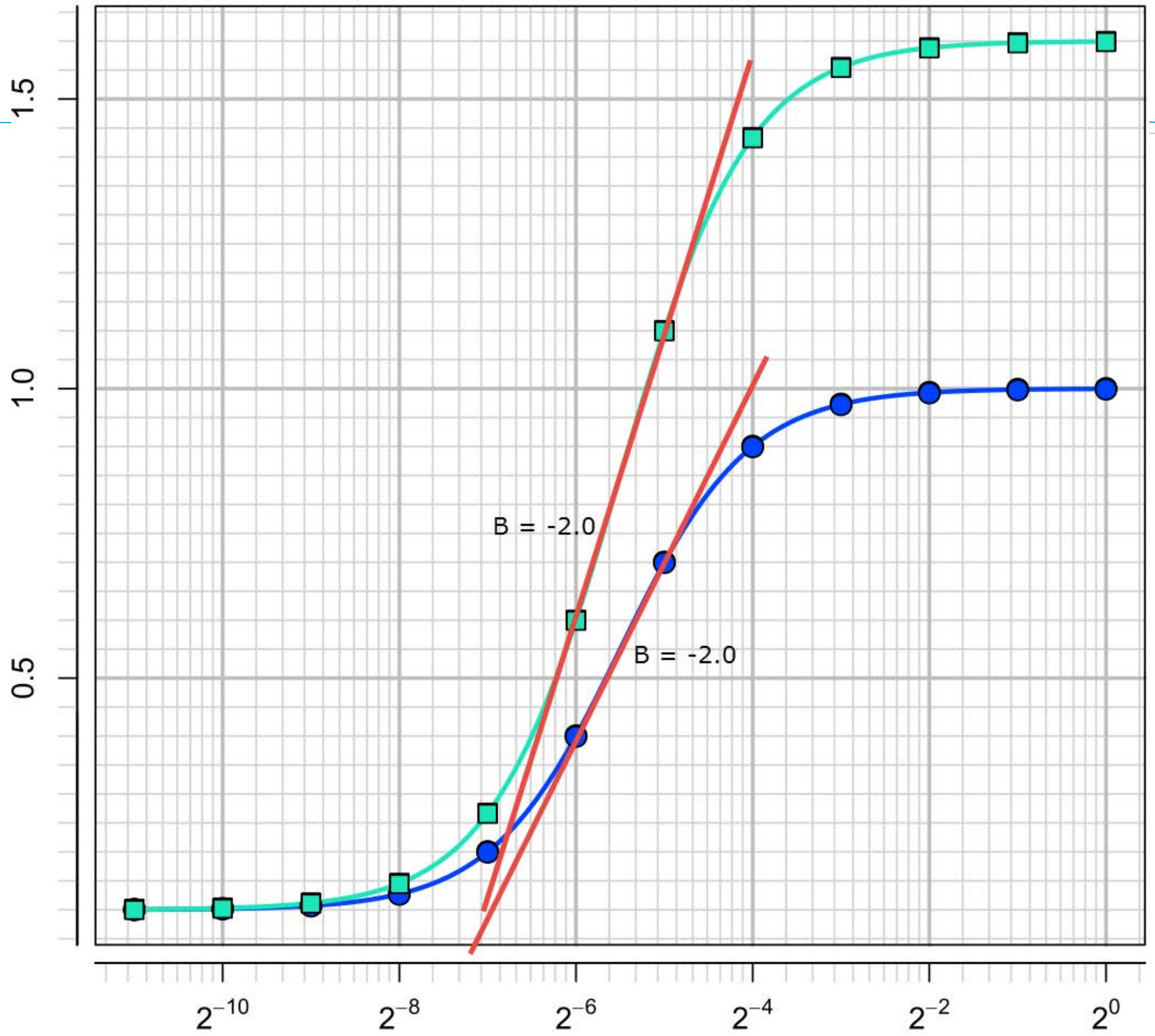
Here is, what happened…

Introduction

- CASSS Bioassays 2016, Vendor Talk by Stegmann Systems.
- The talk is about Equivalence Margin Development with PLA 3.0.
  In the conclusion of the talk a statement has been made, that a developed test system should be able to distinguish the following two curves.
- The data presented was simulated from a 4-parameter model with the following parameters
- Lower Asymptote $D_1 = D_2 = 0.1$
- Slope $B_1 = B_2 = -2.0$
- Inflection Point $C_1 = C_2 = -5.5$
- Upper Asymptote $A_2 = 1.6$ (60% higher than A_1)
  $A_1 = 1.0$



$D_1 = D_2 = 0.1$
$B_1 = B_2 = -2.0$
$C_1 = C_2 = -5.5$
$A_2 = 1.6$
$A_1 = 1.0$



$D_1 = D_2 = 0.1$
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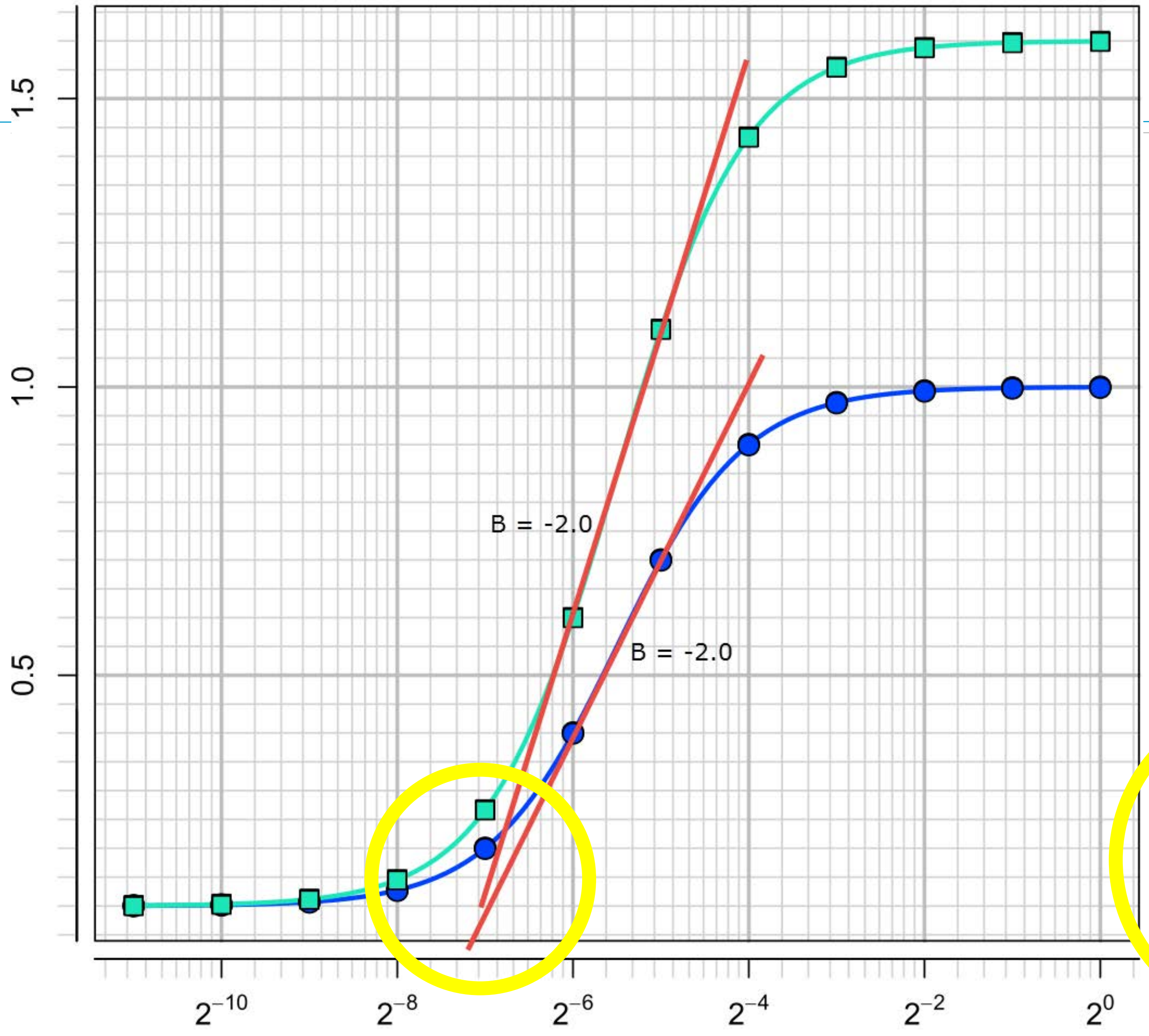


Do you think identical slopes should intersect?

$D_1 = D_2 = 0.1$

$B_1 = B_2 = -2.0$

$C_1 = C_2 = -5.5$

$A_2 = 1.6$

$A_1 = 1.0$

USP <1032> Equivalence Test Approach

USP <1032> Equivalence Testing

- Test System
  - System Suitability Tests
  - Sample Suitability Tests: Similarity of Test and Standard

- Test System Development
  - Tolerance Interval Approach
  - Sound Knowledge

- This talk will only focus 4- and 5-parameter logistic fits and the Tolerance Interval Approach

Test Domains

The USP explicitly states a number of tests, but allows to use further tests beyond the guidance.

- „USP Domain of Tests“ – those tests are stated in the guidance
- „Extended Domain of Tests“ – those tests are beyond the guidance but usually well-known

USP Domain of Tests for 4PL and 5PL		Extended Domain (Examples)
System Suitability	*Sample Suitability (Similarity)*	Various Tests
• Asymptotes • Slope • EC50 • Asymmetry Parameter • Potency Control Sample	• Difference of Parameter Estimates • Ratios of Parameter Estimates	• Asymptote Range (upper – lower) • Asymptote Ratios (upper/lower) • (Universally) Scaled Parameter Differences
• Goodness of Fit • Precision	• Parallelism Sum of Squares	• Sum of Squares for various ANOVA elements

USP Equivalence Testing – Critical Aspects

1. B is identified as the Slope Parameter in 4-Parameter Fits and only subsets of parameter estimates are used to conclude similarity

   → **The Slope Challenge**

2. The parameter by parameter approach neglects correlation effects

   → **The Correlation Challenge**

3. Equivalence Margins developed with the Tolerance Interval Method are allowed to be reused with different sample size

   → **The Precision Challenge**

4. The development of Equivalence Margins is performed with Tolerance Intervals below 100%

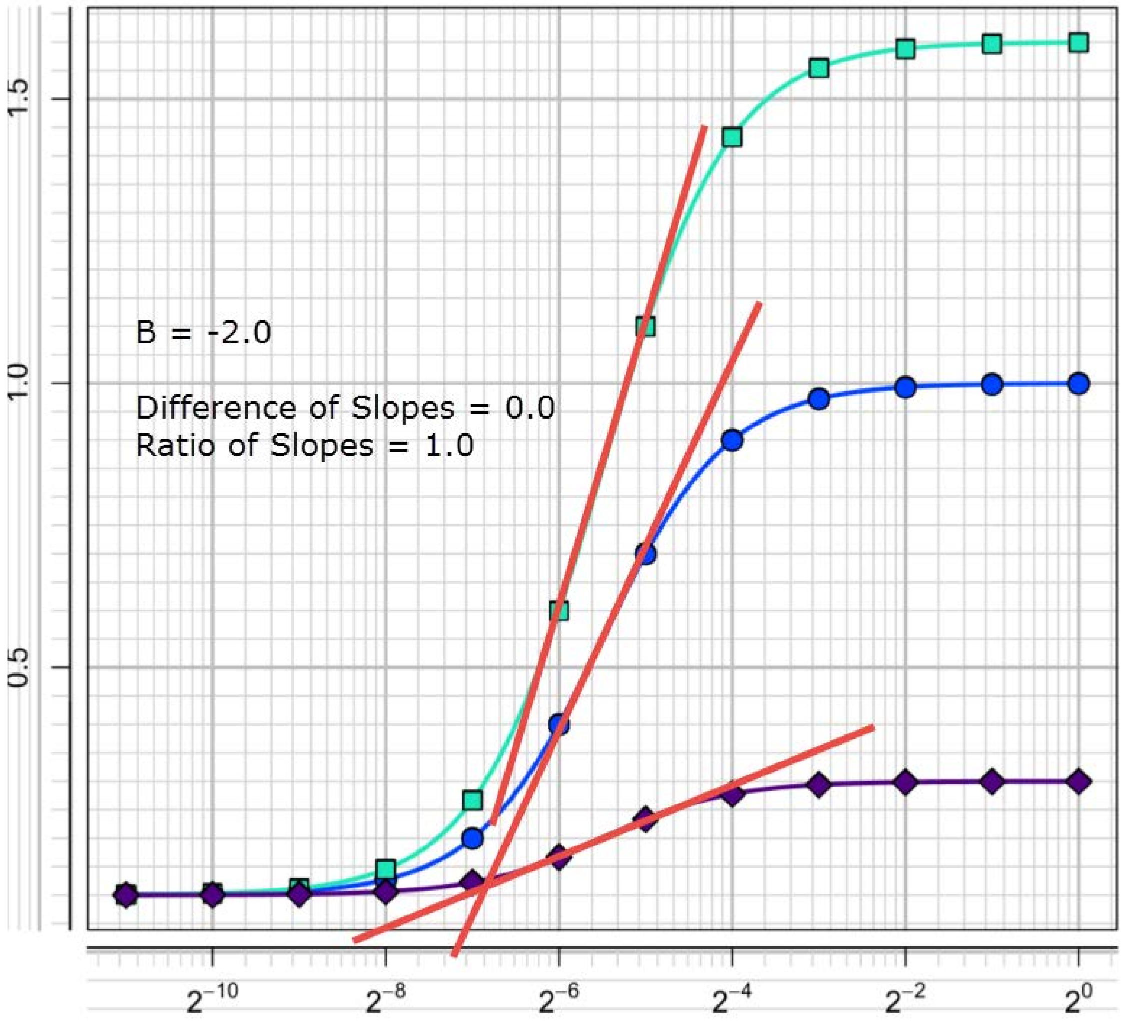
   → **The Tolerance Interval Challenge**

5. The assumptions are transferred to the 5-Parameter Fit Functions

   → **The 5PL Challenge**

The Slope Challenge

The „Slope Parameter“ B



$$f(z) = D + \frac{A - D}{1 + b^{-B(z-C)}}$$

D = Lower Asymptote
A = Upper Asymptote
C = log(EC50)
B = “Slope Parameter”, “Hill-Slope/Factor”?

b = Logarithm Base (e.g. 2, e, 10)

USP <1030> Definition of Parallelism

Parallelism (of concentration- response curves): *A quality in which the concentration-response curves of the Test sample and the Reference Standard are identical in shape and differ only by a horizontal difference that is a constant function of relative potency.*

Notes […]

3. The assessment of parallelism depends on the type of function used to fit the response curve. Parallelism for a nonlinear assay using a four-parameter logistic fit means that (a) the slopes of the rapidly changing parts of the Test and Reference Standard curves (that is, slope at a tangent to the curve where the first derivative is at a maximum) should be similar; and (b) the upper and lower asymptotes of the response curves (plateaus) should be similar. For straight-line analysis, the slopes of the lines should be similar. **[J]**

The true Slope S at the inflection point

S is the true slope in the inflection point. Calculated by solving the first derivative for second derivative = 0 (at Dose = C)

$$\boldsymbol{S = \frac{1}{4} \ln b \cdot (A - D) \cdot B}$$

- Parameter B is NOT proportional to S since A-D is not constant.

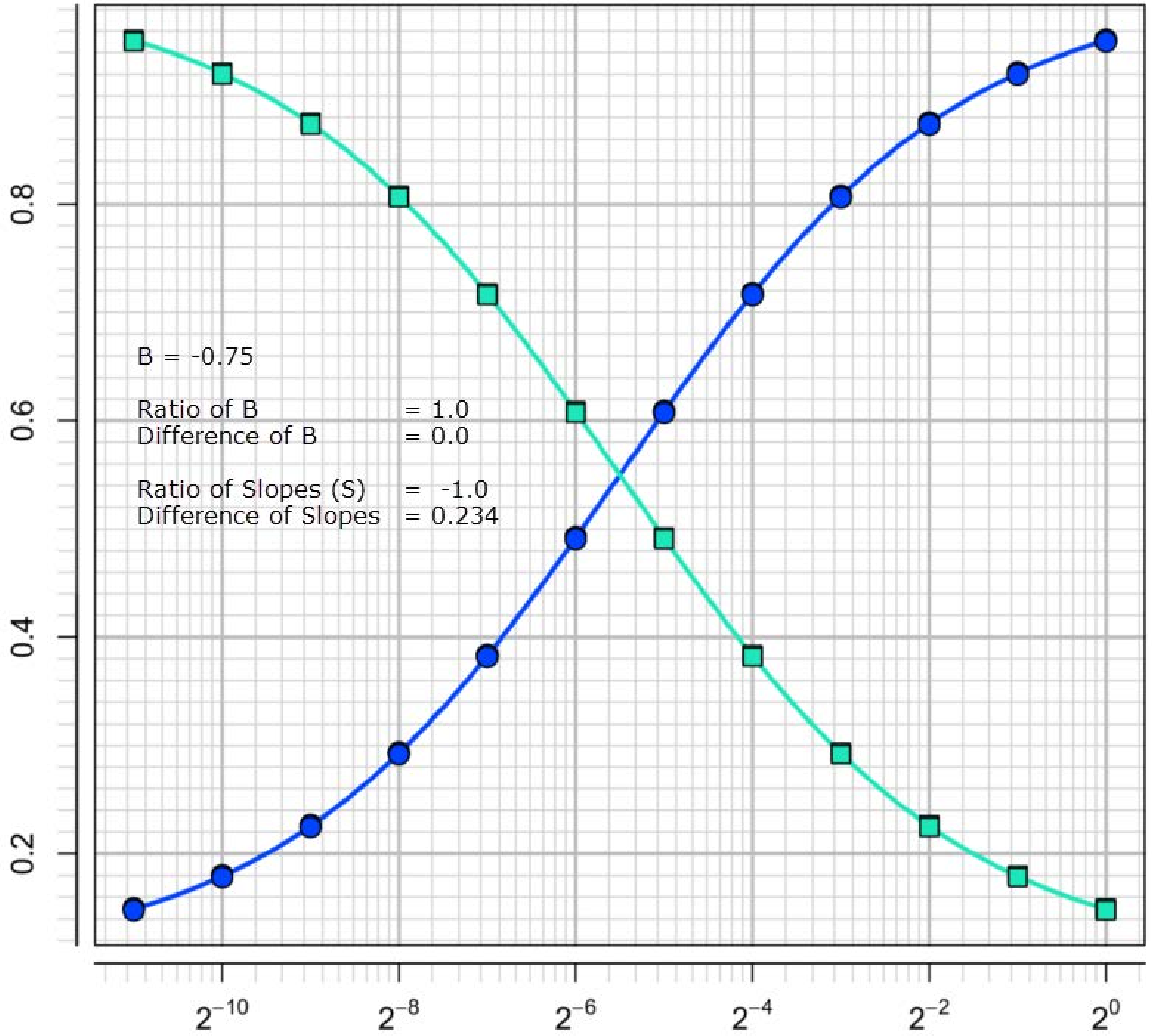
- **The comparison of B values (Ratio, Differences) states nothing about the similarity of slopes in the inflection point !**
- **Unfortunately this is a important assumption of the Similarity Approach in USP <1032>.**

B is not the slope !

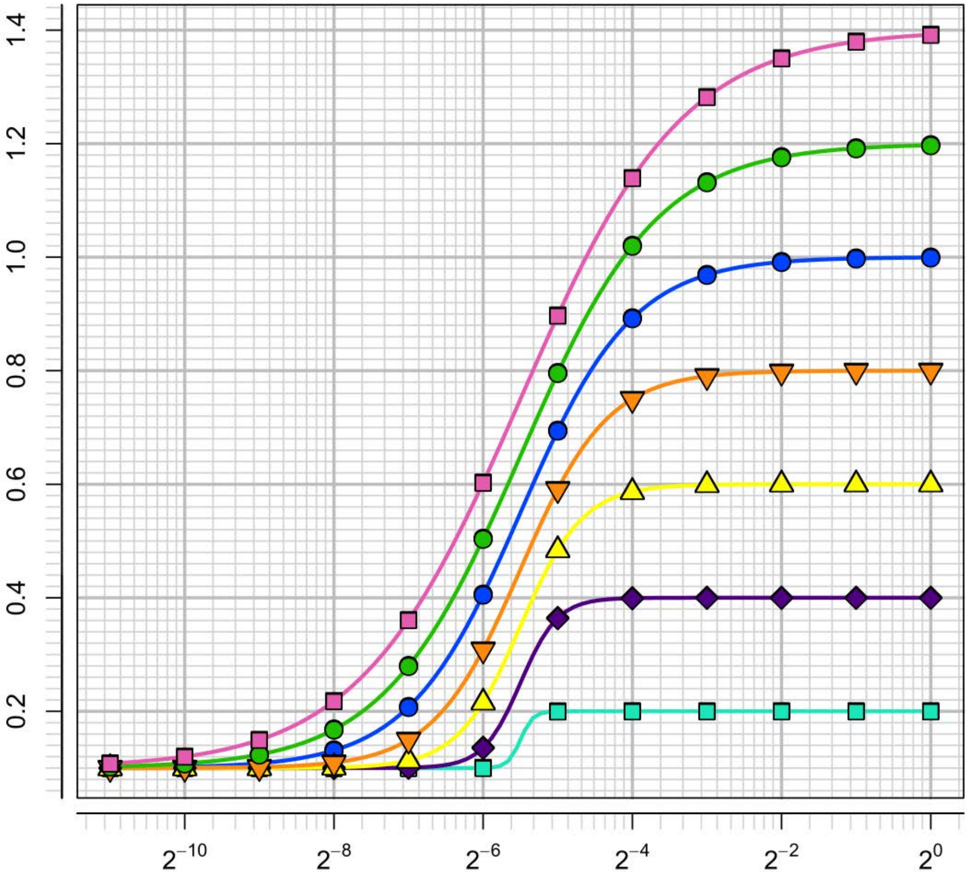
…nor is it sufficient to describe the steepness in the inflection point or the curve shape.

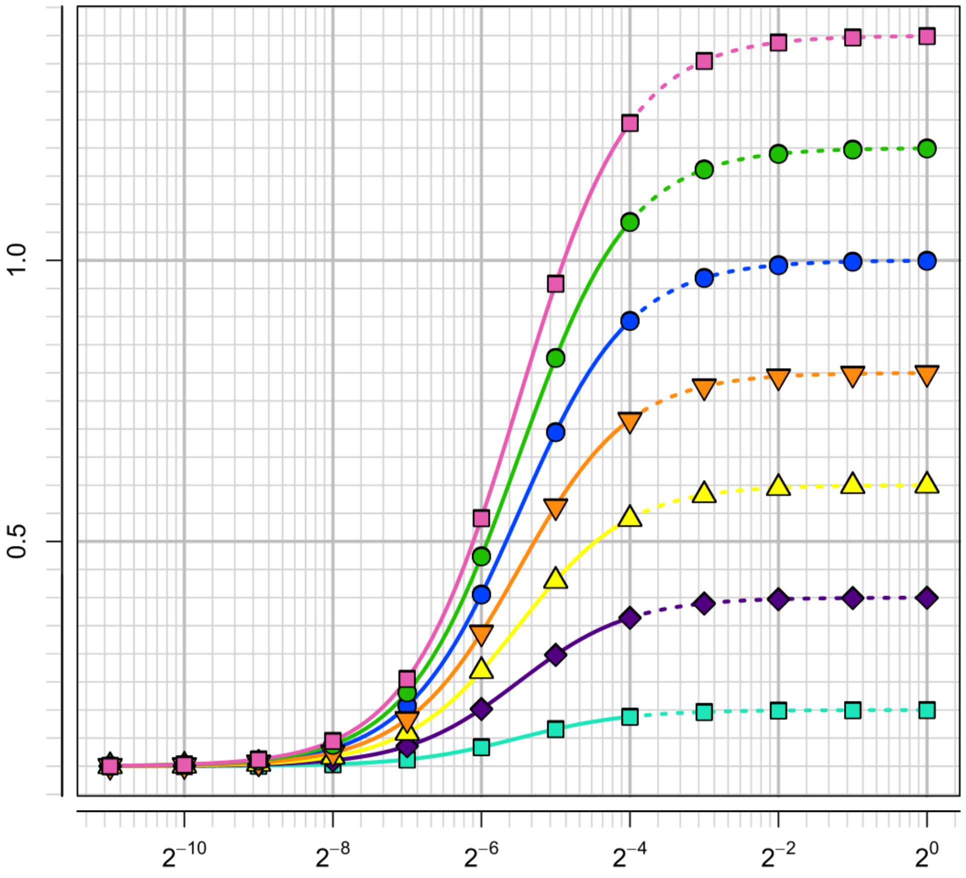
- The description of B as the „slope at the inflection point“ seems to be a historic error flowing through the literature of the last decades.
- Curves with a ratio of B of 1.0 or a difference of B of 0.0 can cross each other with any angle up 90°.
- **USP <1032> is build around the B value as „slope parameter“ while the guidance seems to assume this has the same impact as using S.**

B is not the slope!



Constant S vs. Constant B

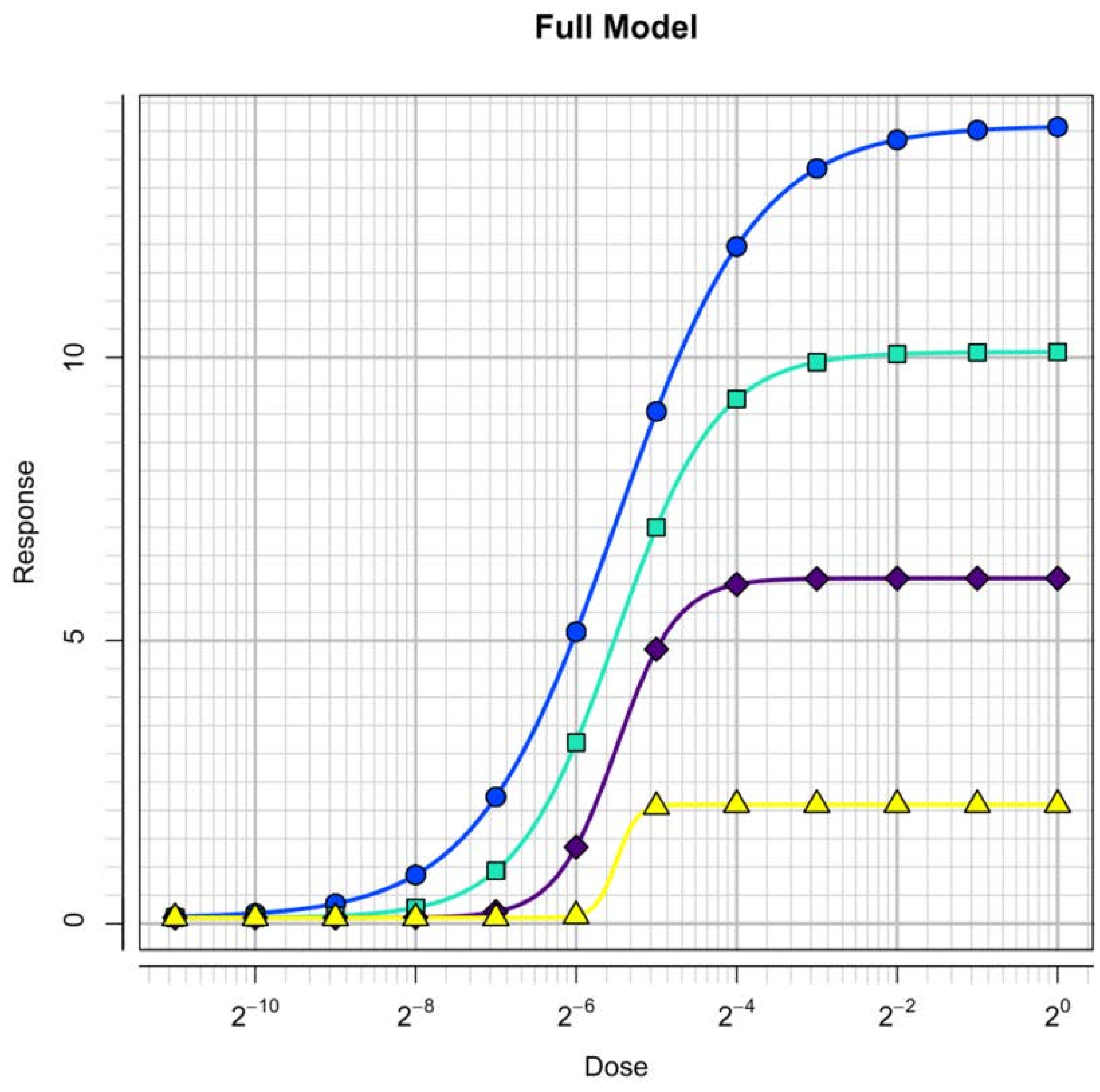




Development of S

S = -4.0

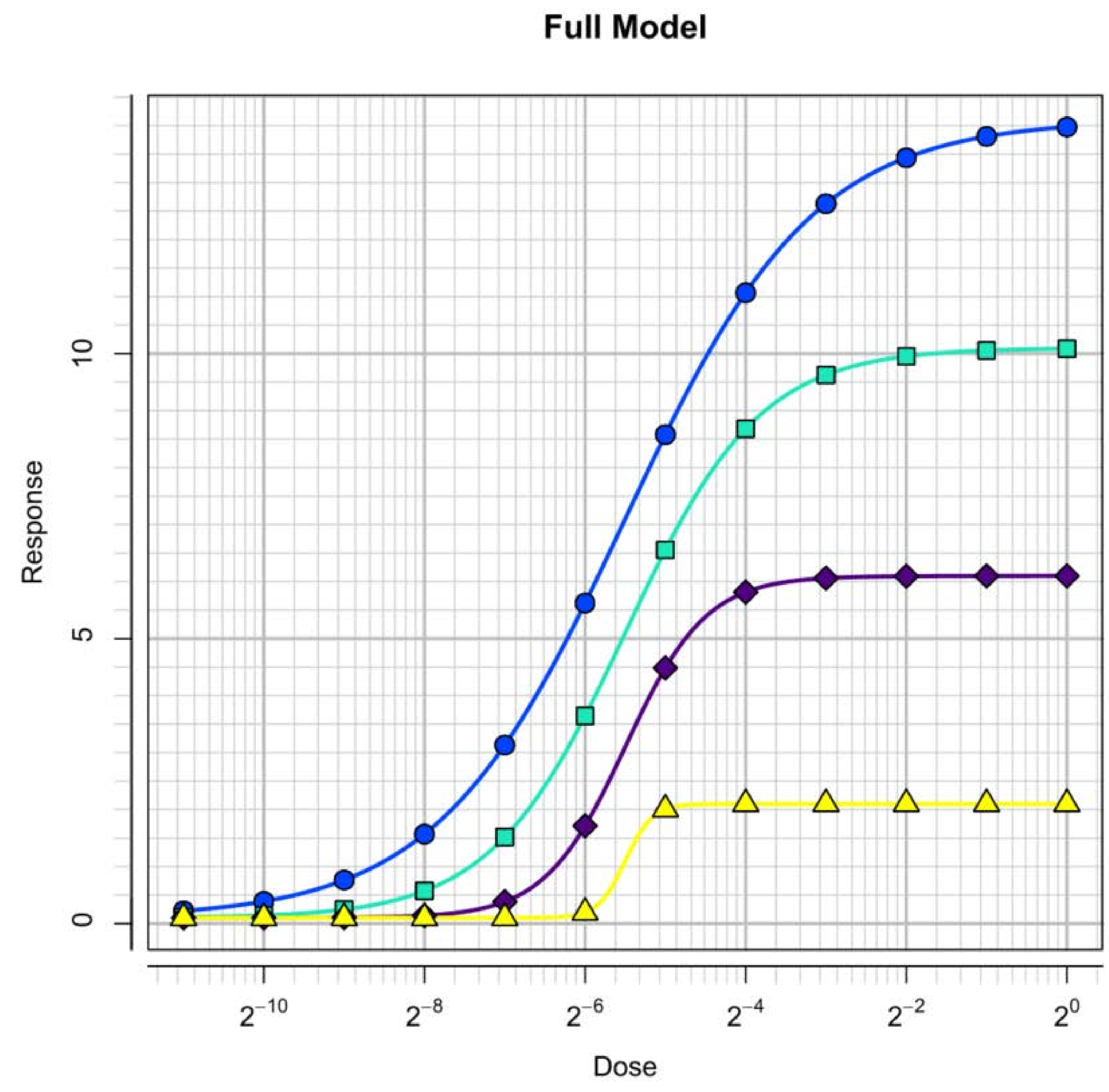
B_1 = -1.65
B_2 = -2.31
B_3 = -3.85
B_4 = -11.54



Development of S

S = -3.0

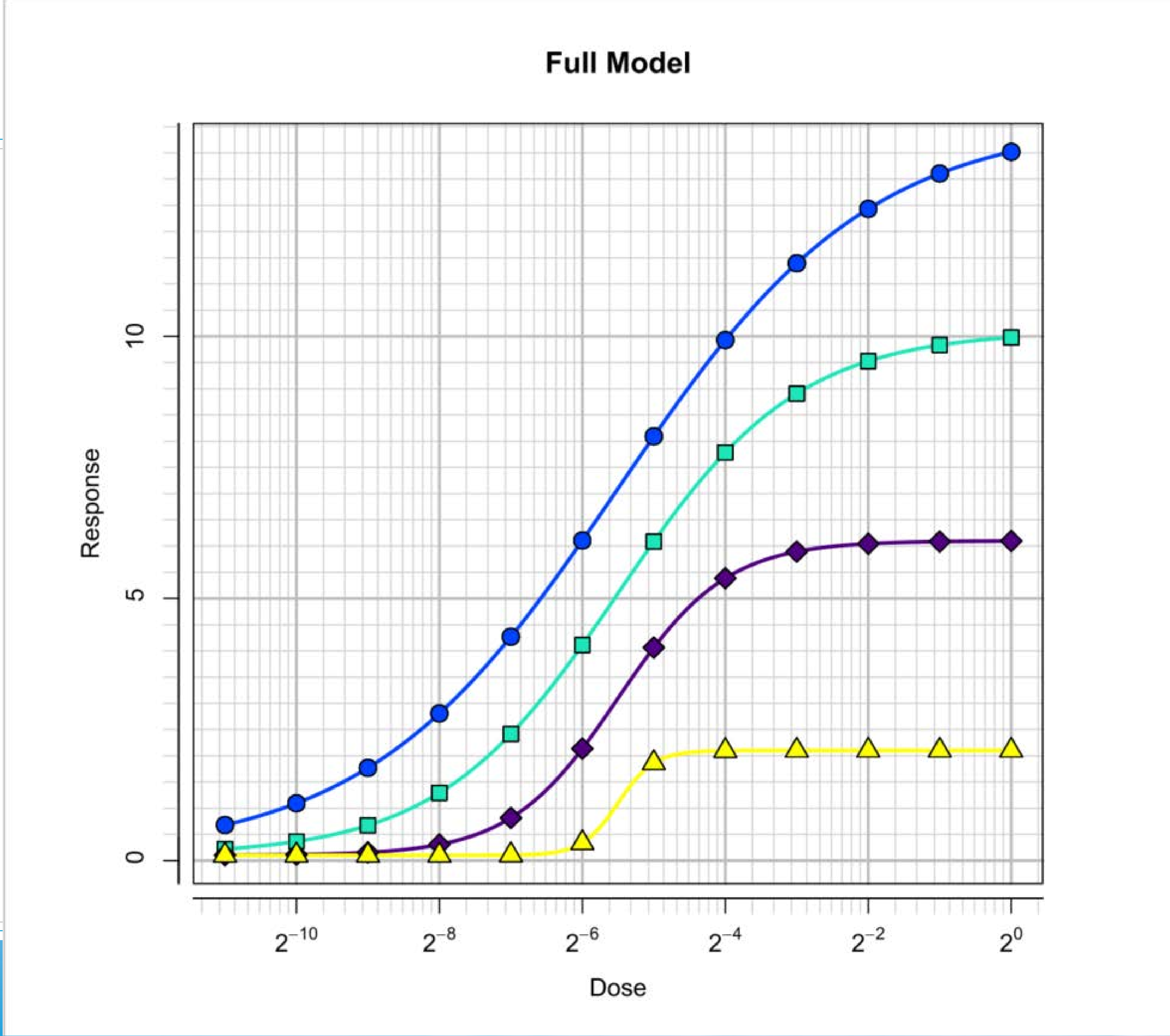
B_1 = -1.24
B_2 = -1.73
B_3 = -2.89
B_4 = -8.66



Development of S

S = -2.0

B_1 = -0.82
B_2 = -1.15
B_3 = -1.92
B_4 = -5.77

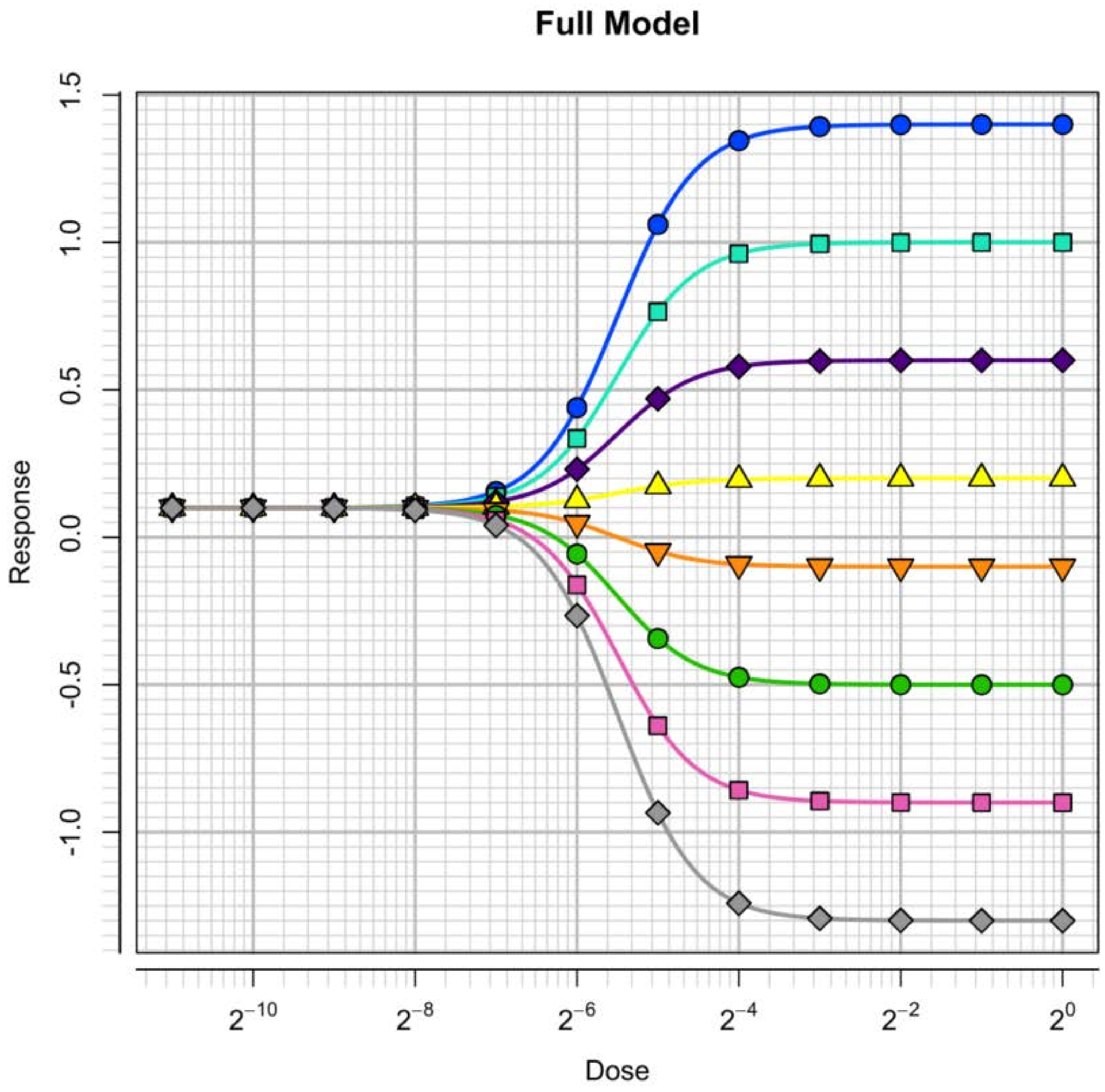


Development of B

B = -3.0

$S_1 = -0.68$
$S_2 = -0.47$
$S_3 = -0.26$
$S_4 = -0.05$
$S_5 = 0.10$
$S_6 = 0.31$
$S_7 = 0.52$
$S_8 = 0.73$

$\log_2(EC90) - \log_2(EC10) = -2.11$

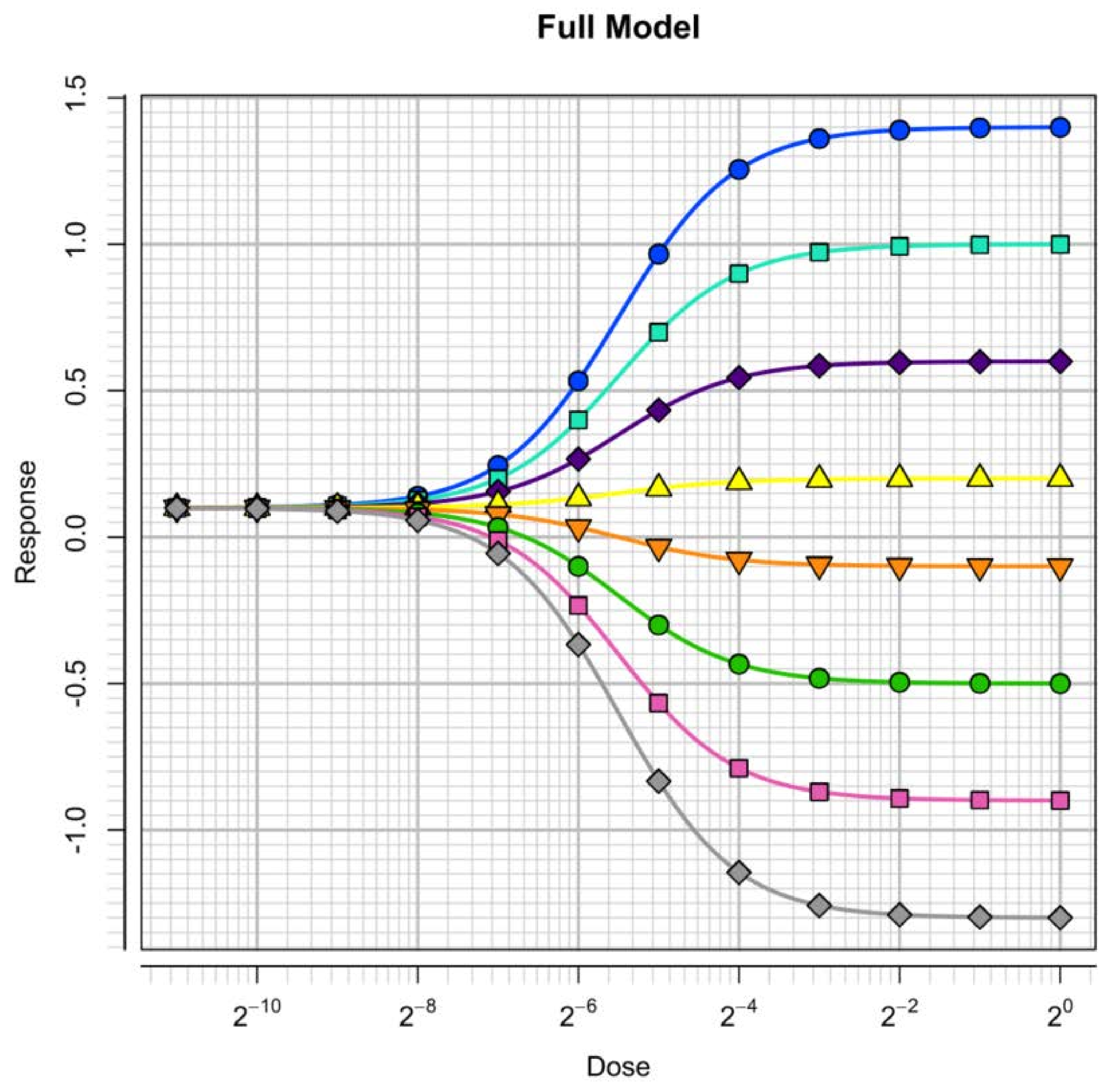


Development of B

B = -2.0

$S_1 = -0.45$
$S_2 = -0.31$
$S_3 = -0.17$
$S_4 = -0.03$
$S_5 = 0.07$
$S_6 = 0.21$
$S_7 = 0.35$
$S_8 = 0.49$

$\log_2(EC90) - \log_2(EC10) = -3.17$

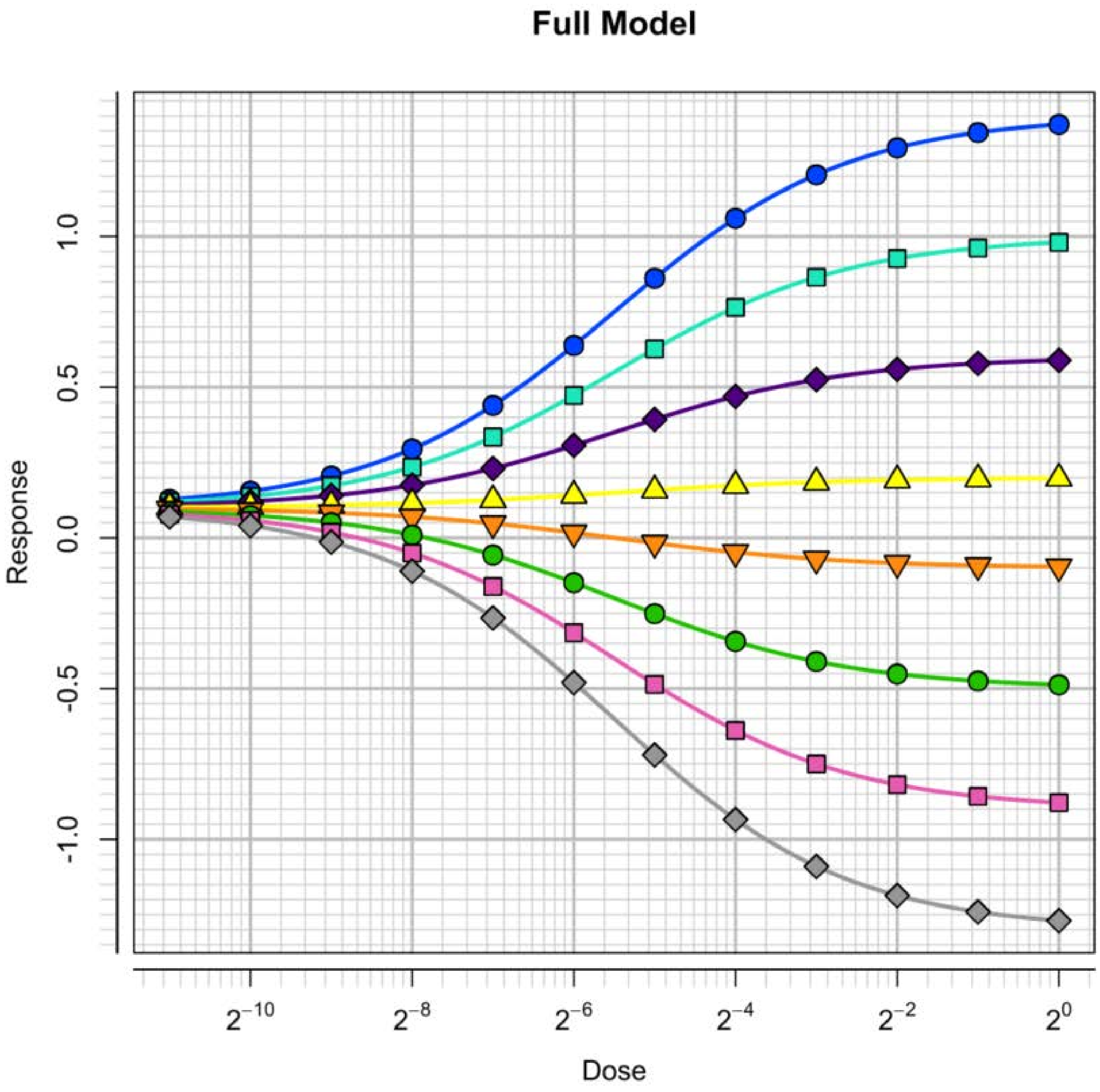


Development of B

B = -1.0

$S_1 = -0.23$
$S_2 = -0.16$
$S_3 = -0.09$
$S_4 = -0.02$
$S_5 = 0.03$
$S_6 = 0.10$
$S_7 = 0.17$
$S_8 = 0.24$

$\log_2(EC90) - \log_2(EC10) = -6.34$



So, what is B?

B is a measure of the width of the dynamic portion of the curve:

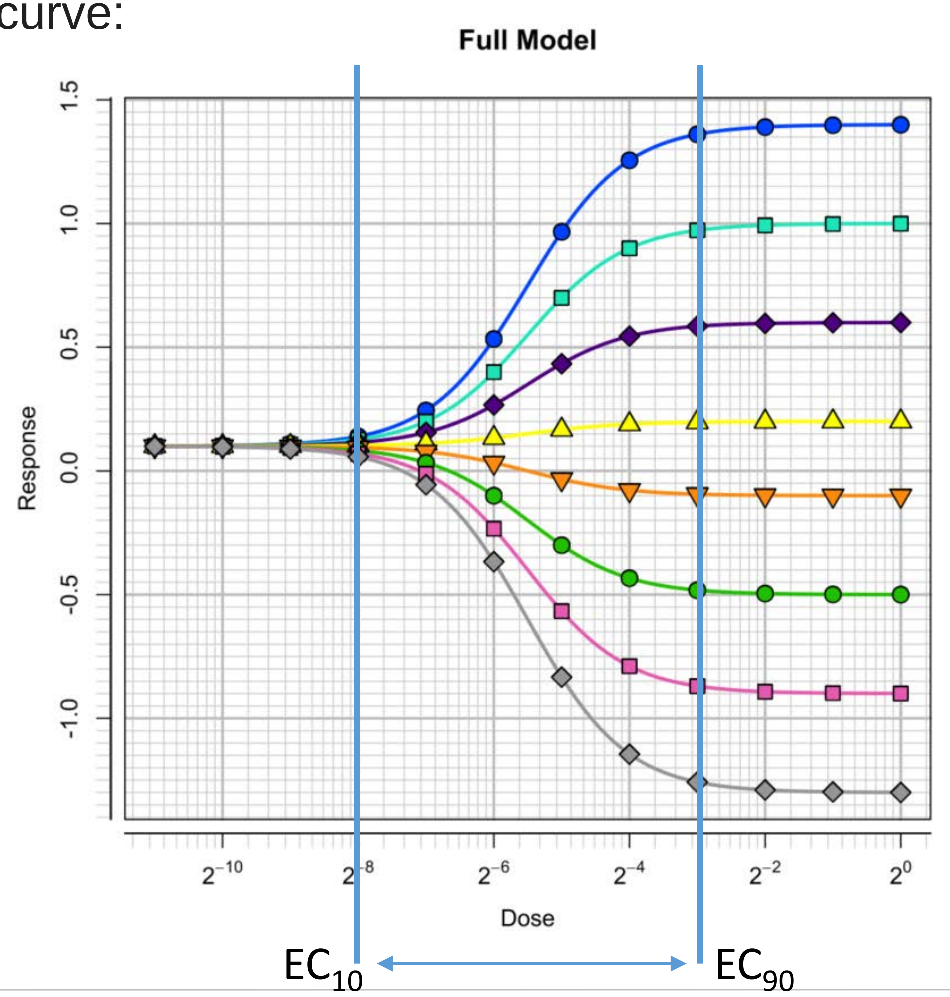
$$\log_b(ECn) - \log_b(ECm) = \frac{\log_b\left(\frac{(1-n)\cdot m}{(1-m)\cdot n}\right)}{-B}$$

Examples: $\log_b(EC90) - \log_b(EC10) = \log_b(81) / -B$

$\log_b(EC95) - \log_b(EC05) = \log_b(361) / -B$

→ Difference is independent of A, C and D.

→ B is a reciprocal measure of the width of the dynamic region of the fit



Risk Associated with the Slope Challenge

- **USP Guidance demands only „*some parameters*“ of the curve as acceptance criteria.**

- Acceptable System Suitability and Sample Suitability qualifies a run!

Challenge:

A test system developed in accordance to the guidance should be able to securely disqualify e.g. a “flat” line

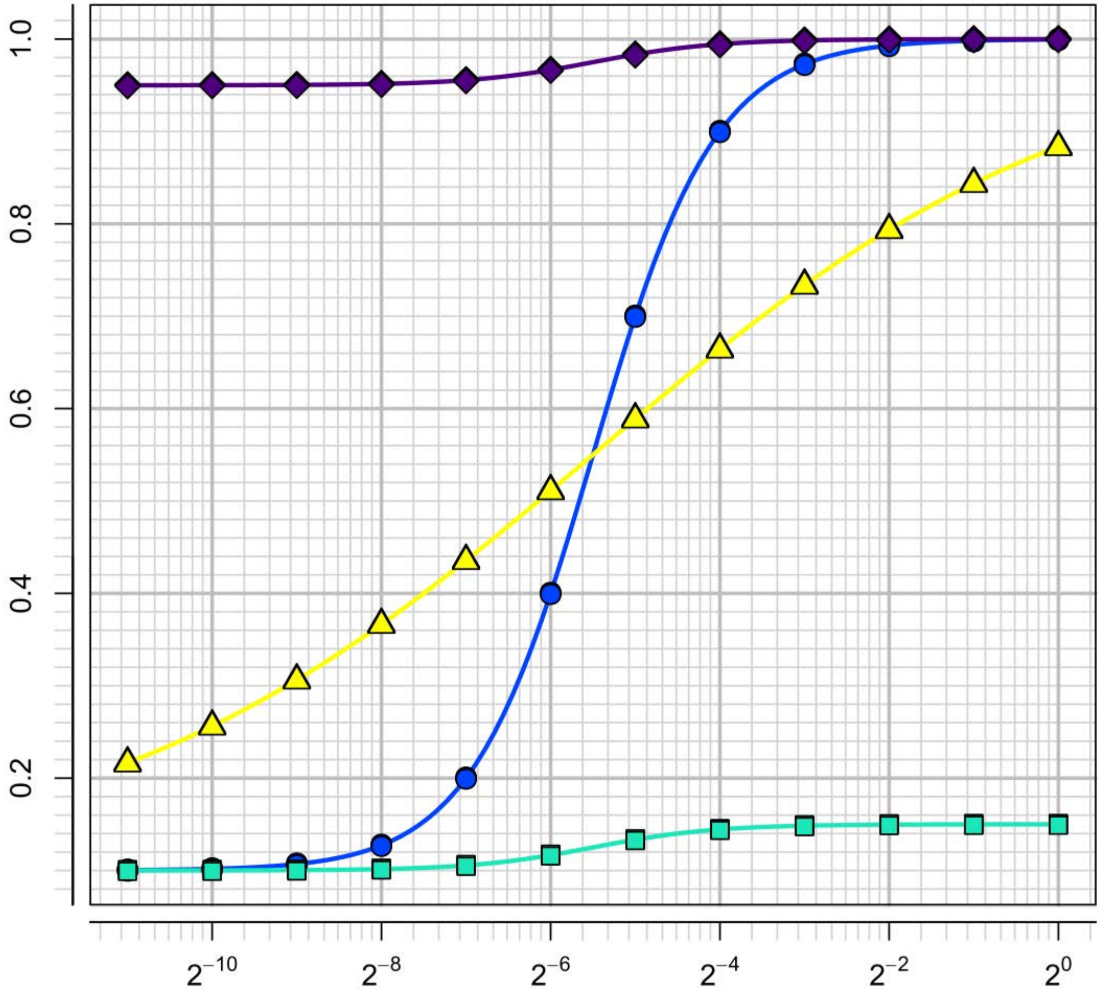
Risk Associated with the Slope Challenge

PLA 3.0

Example for System Suitability Testing

Simulation with 4 curves (blue = historical data)

	Historical (blue)	Purple upper „flat“	Green lower „flat“	Yellow „crossing“
A	1.0	1.0	0.15	1.0
B	**-2.0**	**-2.0**	**-2.0**	**-0.5**
C	-5.5	-5.5	-5.5	-5.5
D	0.1	0.95	0.1	0.1

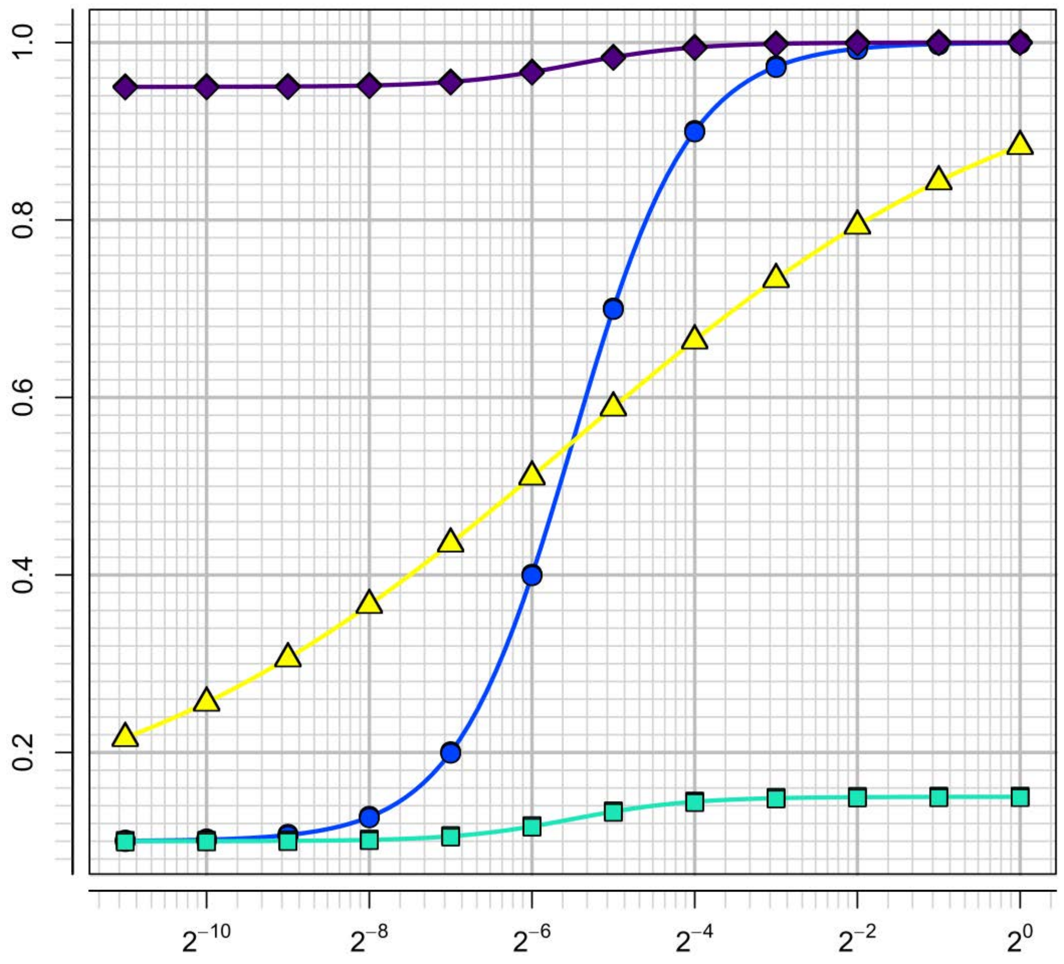


Test system will be developed with historical data (blue line). A test system will be constructed within the „USP domain of tests“.

Which test system is capable of disqualifying all of the three challenge curves?

Risk Associated with the Slope Challenge

Test System	lower „flat“ line	upper „flat“ line	crossing line
A	Not Accepted	**Accepted**	**Accepted**
B	**Accepted**	**Accepted**	Not Accepted
D	**Accepted**	Not Accepted	**Accepted**
A & B	Not Accepted	**Accepted**	Not Accepted
A & D	Not Accepted	Not Accepted	**Accepted**
D & B	**Accepted**	Not accepted	Not accepted
A & B & D	**Not Accepted**	**Not Accepted**	**Not Accepted**



Slope Challenge: Conclusions

→ **It is not sufficient to conclude similarity in the inflection point from the value of the B parameter**

→ **It is required to test ALL of the parameters of the model** otherwise the system may not be able to correctly disqualify an inacceptable curve. This is in contrast to the statements in the USP 1032.

→ Developed test systems may need revision if not all of the parameter estimates are covered!

The Correlation Challenge

The Correlation Challenge

- In non-linear systems parameters are not independent of each other.

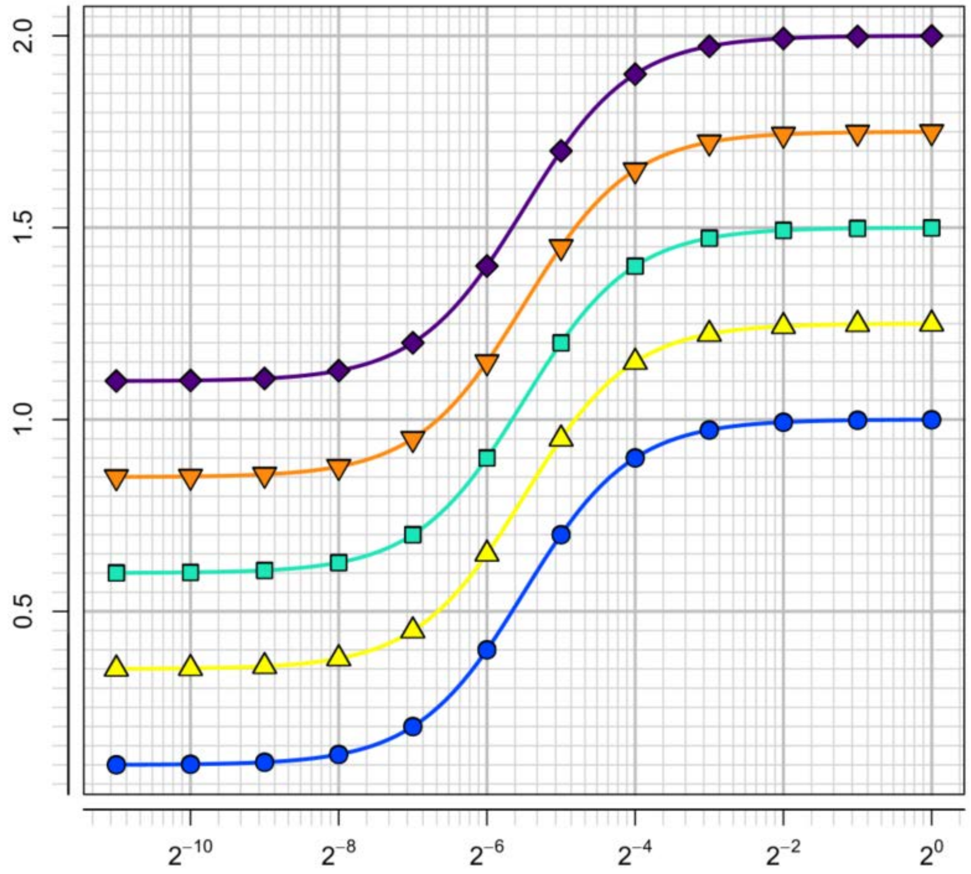
- USP <1032> describes how to develop a test system from historical assay data, by developing equivalence margins for every parameter of the system.

- According to the USP this test system is developed using the parameters of the model fit. For 4PL these parameters are
  - Lower Asymptote D
  - Upper Asymptote A
  - „Slope Parameter“ B
  - EC50

If you develop parameters according to this the following may happen…

The Dataset

The example dataset is a perfect dataset of historical data. It covers 99% of the acceptable ranges. The Dose Response Curve shifts along the response axis but the dynamic range of the curves is constant.



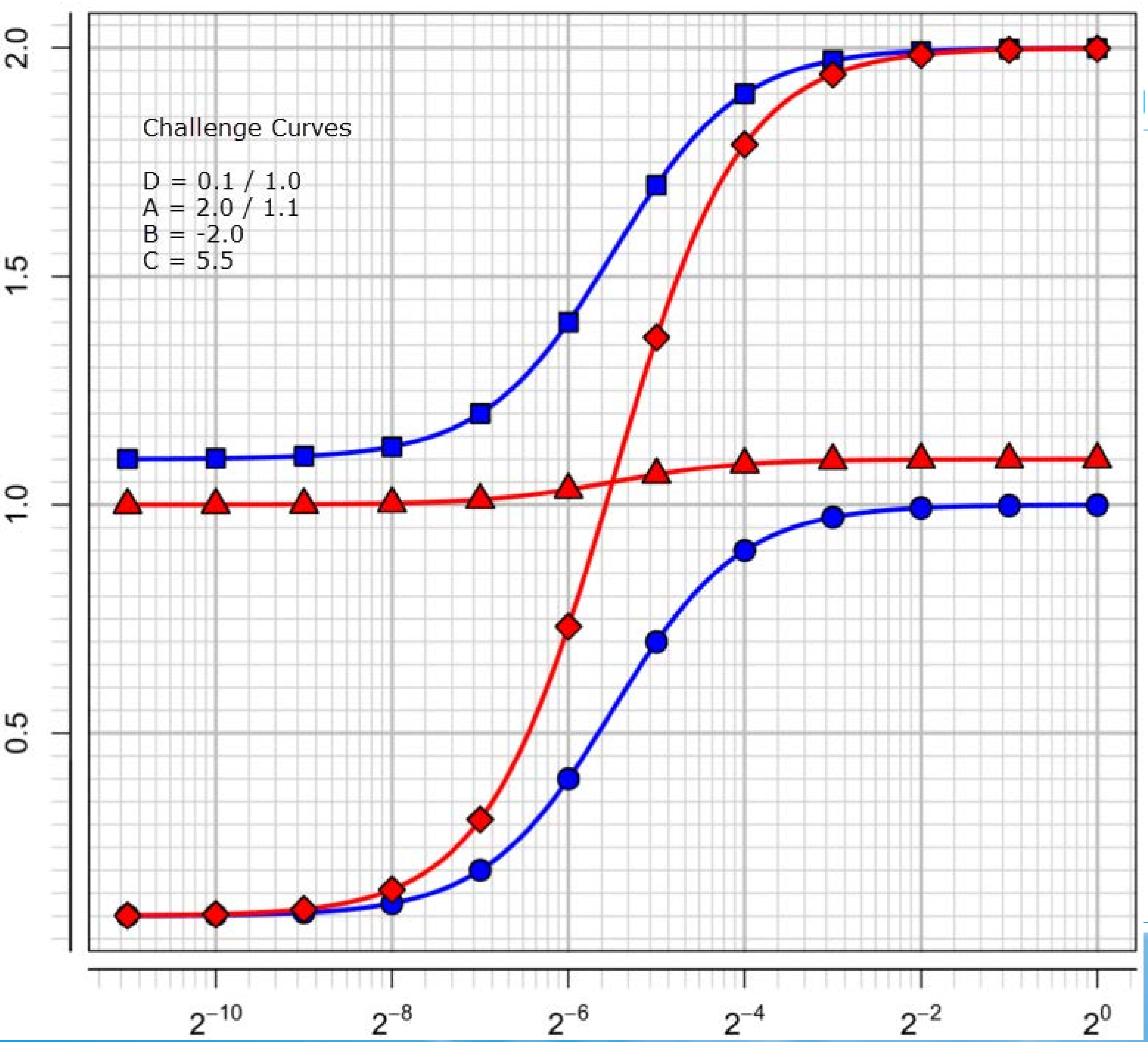
Simulation Data:

- $D_1 - D_5$ 0.1 .. 1.1 (delta: 0.25)
- $A_1 - A_5$ 1.1 .. 2.1 (delta: 0.25)
- B -2.0
- C 5.5

Hypothetical Parameter Confidence Intervals = ± 0.05

Challenge Curves

- Blue Curves = outer curves of historical data
- Red Curves = challenge curves
  - Curve A = steep curve
  - Curve B = „flat“ curve



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Challenge Curves

The presented challenge curves are accepted if the USP guideline is followed and even if **all** instead of „some” parameters are used.

Equivalence Margins		Curve A	Curve B
D	0.095, 1.150	0.1	1.0
A	1.050, 2.150	2.1	1.1
B	-1.950, -2.050	-2.0	-2.0
C	5.450, 5.550	5.5	5.5

The curves are not covered by the historic data but will be accepted.
Nothing is introduced within the USP Domain of Tests that helps disqualifying those curves in System Suitability testing.

Correlation Challenge - Conclusions

→ The neglect of parameter correlation allows dose response curves to be accepted that are not covered by the original (historical) data.

  → There is no way to get around this, when working within the „USP Domain of Tests“

→ This information loss is compensated by using additional tests (Extended Domain of Tests)

- The intrinsic dependency in the example is A – D = 1.0.
  It could be fixed by introduction of A-D as an equivalence test.
- In many cases the intrinsic dependencies are more complex. E.g. A ~ const*D

→ If dependencies are neglected similarity may not be concluded.

This risk gets much more relevant due to the slope misinterpretation:
it can no longer be concluded that curves with identical B are “similar” in the inflection point.

The Precision Challenge

The Precision Challenge

The USP <1032>, Chapter 4.7 states :

> *Equivalence testing has practical advantages compared to difference testing, including that **increased replication** yielding improved assay precision will increase the chances that samples will pass the similarity criteria; that decreased assay replication or precision will decrease the chances that samples will pass the similarity criteria; and that sound approaches to combining data from multiple assays of the same sample to better understand whether a sample is truly similar to Standard or not are obtained.* **[F]**

According to this statement it can be concluded, that the same similarity criteria may be applied to samples with increased replication.

The Precision Challenge

- Modern software estimates parameter confidence intervals from the Asymptotic Standard Error which is calculated by the Non-linear optimizers (e.g. Marquardt-Levenberg)
  - Used by SAS, R, Graphpad, Mathematica etc.
- Confidence Intervals from parameter estimates, differences and ratios of parameter estimates are calculated by

$$\mathrm{CI} = [l, u] = \begin{cases} \left[p - \mathrm{SE}_p \cdot \mathrm{t}_{\alpha, df_{\mathrm{E}}}, p + \mathrm{SE}_p \cdot \mathrm{t}_{\alpha, df_{\mathrm{E}}}\right], & \text{parameter estimates } p \\ \left[-\left|p_1 - p_2\right| - \mathrm{SE}_{p_1 - p_2} \cdot \mathrm{t}_{\alpha, df_{\mathrm{E}}}, \left|p_1 - p_2\right| + \mathrm{SE}_{p_1 - p_2} \cdot \mathrm{t}_{\alpha, df_{\mathrm{E}}}\right], & \text{differences of parameter estimates } p_1 - p_2 \\ \mathrm{Fieller}\left(p_1, p_2, \mathrm{SE}_{p_1}, \mathrm{SE}_{p_2}, \mathrm{t}_{\alpha, df_{\mathrm{E}}}\right), & \text{ratio of parameter estimates } \frac{p1}{p2} \end{cases}$$

- When Tolerance Intervals are constructed from these Confidence Intervals they are functions of the sample size:

$$\mathrm{TI} = \left[\min\{l_1, l_2, \ldots, l_n\}, \max\{u_1, u_2, \ldots, u_n\}\right] = \mathrm{TI}\left(df_{\mathrm{E}}, \ldots\right)$$

The Precision Challenge - Conclusions

- Equivalence Margins calculated from parameter estimates confidence intervals by asymptotic standard error are only valid for a specific sample size!
- Increased sample size with original margins allow curve shapes, which are not supported by the data. **Since all parameters are affected at once**, this change has significant impacts on the shape of accepted curves.
    - With increasing precision increasingly strange curve shapes get acceptable.

The Tolerance Interval Challenge

The Tolerance Interval Challenge

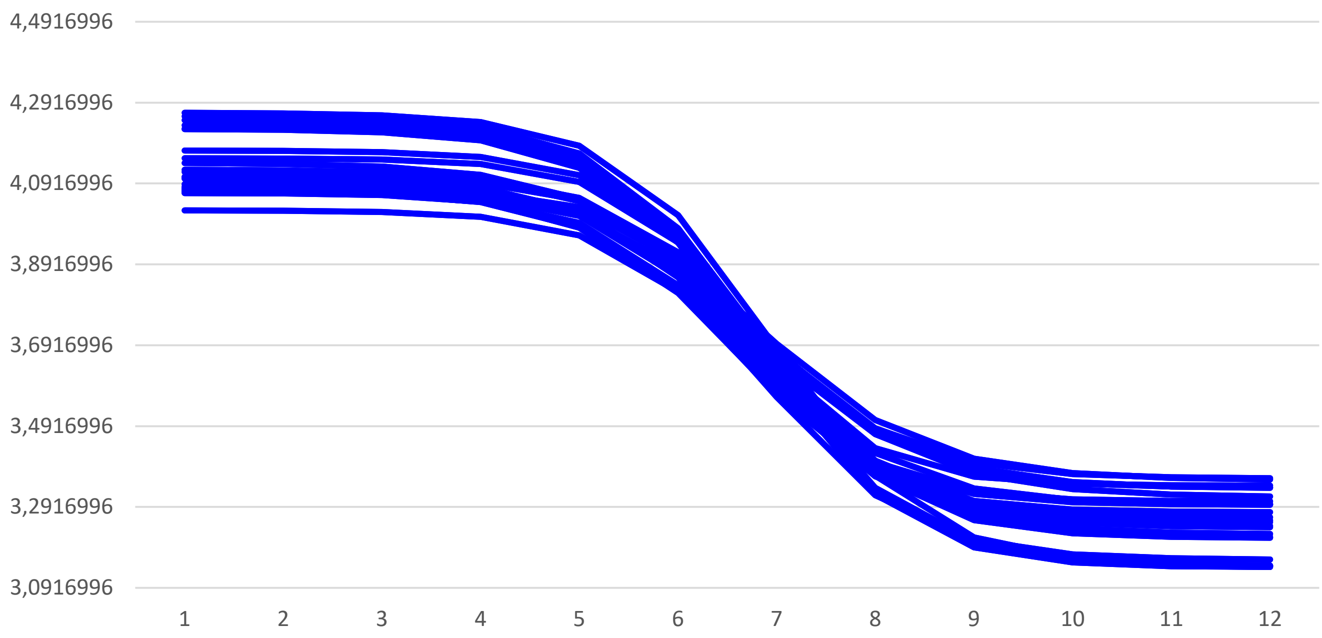
- According to USP Historic Data Covering 99% of the Acceptable Range should be selected to develop Equivalence Margins with the Tolerance Interval Approach

- The Tolerance Interval Approach accepts only a defined percentage of the historic data sets per parameter estimate (e.g. 95%)

If you develop parameters according to this the following will happen…

Historic Dataset

- 24 Historic Assays, Customer Data
- Develop Equivalence Margins with different Tolerance Intervals (100%, 95%, 90%)
- Then verify the acceptance of these historic assays by the developed Equivalence Margins



Acceptance of Historic Data TI = 100%

- Tolerance Interval Approach with Parameter Estimates

Strategy Verification Result: Development Data

Test	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Equivalence Test: Parameter Estimate A	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Parameter Estimate B	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Parameter Estimate C	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Parameter Estimate D	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Difference of Parameter Estimates A	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Difference of Parameter Estimates B	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Difference of Parameter Estimates D	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Ratio of Parameter Estimates A	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Ratio of Parameter Estimates B	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Ratio of Parameter Estimates D	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Scaled Parameter A	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Scaled Parameter B	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Scaled Parameter D	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Additional Test: Sum of Squares of Non-Linearity	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Additional Test: Sum of Squares of Non-Parallelism	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Strategy Test Result	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Expected Test Result	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Strategy correctly qualifies Assay	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Acceptance of Historic Data TI = 95%

- Tolerance Interval Approach with Parameter Estimates
- TI = 95%: 14 out of 24 runs are accepted, 10 historic runs are excluded

Strategy Verification Result: Development Data

Test	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Equivalence Test: Parameter Estimate A	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✗
Equivalence Test: Parameter Estimate B	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓
Equivalence Test: Parameter Estimate C	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓
Equivalence Test: Parameter Estimate D	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Difference of Parameter Estimates A	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Difference of Parameter Estimates B	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Difference of Parameter Estimates D	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓
Equivalence Test: Ratio of Parameter Estimates A	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Ratio of Parameter Estimates B	✓	✓	✗	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Ratio of Parameter Estimates D	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓
Equivalence Test: Scaled Parameter A	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Scaled Parameter B	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Scaled Parameter D	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓
Additional Test: Sum of Squares of Non-Linearity	✓	✓	✓	✓	✓	✓	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Additional Test: Sum of Squares of Non-Parallelism	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓
Strategy Test Result	✓	✓	✗	✗	✗	✓	✗	✗	✓	✓	✓	✓	✗	✓	✓	✗	✓	✓	✓	✓	✗	✗	✓	✗
Expected Test Result	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Strategy correctly qualifies Assay	✓	✓	✗	✗	✗	✓	✗	✗	✓	✓	✓	✓	✗	✓	✓	✗	✓	✓	✓	✓	✗	✗	✓	✗

Acceptance of Historic Data TI = 90%

- Tolerance Interval Approach with Parameter Estimates
- TI = 90%: ONLY 8 out of 24 runs are accepted, 16 historic runs are excluded

Strategy Verification Result: Development Data

Test	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Equivalence Test: Parameter Estimate A	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓	✗	✓	✓	✓	✓	✗	✓	✓	✗
Equivalence Test: Parameter Estimate B	✓	✓	✓	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✗	✓	✓
Equivalence Test: Parameter Estimate C	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✗	✓	✓	✓	✗	✓	✓
Equivalence Test: Parameter Estimate D	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✗	✓	✓	✓	✗	✓	✓	✓
Equivalence Test: Difference of Parameter Estimates A	✗	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Difference of Parameter Estimates B	✓	✓	✓	✗	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Difference of Parameter Estimates D	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓
Equivalence Test: Ratio of Parameter Estimates A	✗	✓	✓	✗	✓	✓	✓	✓	✓	✗	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Ratio of Parameter Estimates B	✓	✓	✗	✓	✗	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓
Equivalence Test: Ratio of Parameter Estimates D	✓	✓	✗	✓	✓	✓	✗	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓
Equivalence Test: Scaled Parameter A	✗	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Scaled Parameter B	✓	✓	✓	✗	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Equivalence Test: Scaled Parameter D	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓
Additional Test: Sum of Squares of Non-Linearity	✓	✓	✓	✓	✓	✓	✗	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✗	✓	✓	✓
Additional Test: Sum of Squares of Non-Parallelism	✓	✓	✓	✗	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✗	✓	✓
Strategy Test Result	✗	✓	✗	✗	✗	✓	✗	✗	✓	✗	✓	✗	✗	✓	✓	✗	✗	✗	✗	✓	✗	✗	✓	✗
Expected Test Result	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Strategy correctly qualifies Assay	✗	✓	✗	✗	✗	✓	✗	✗	✓	✗	✓	✗	✗	✓	✓	✗	✗	✗	✗	✓	✗	✗	✓	✗

The Tolerance Interval Approach Challenge – Conclusions & Fixes

PLA 3.0

- According to USP you should select 99% or more of the acceptable ranges to develop the margins
- The Tolerance Interval Approach disqualifies much more curves than expected, when TI < 100%
- Reasons:
  - Neglect of parameter correlation
  - Historic Datasets are excluded per parameter

→ Consider to set the Tolerance Interval to 100%, to accept all previously accepted curves.

The 5PL Challenge

5PL

- The problems shown get much more complex with 5PL

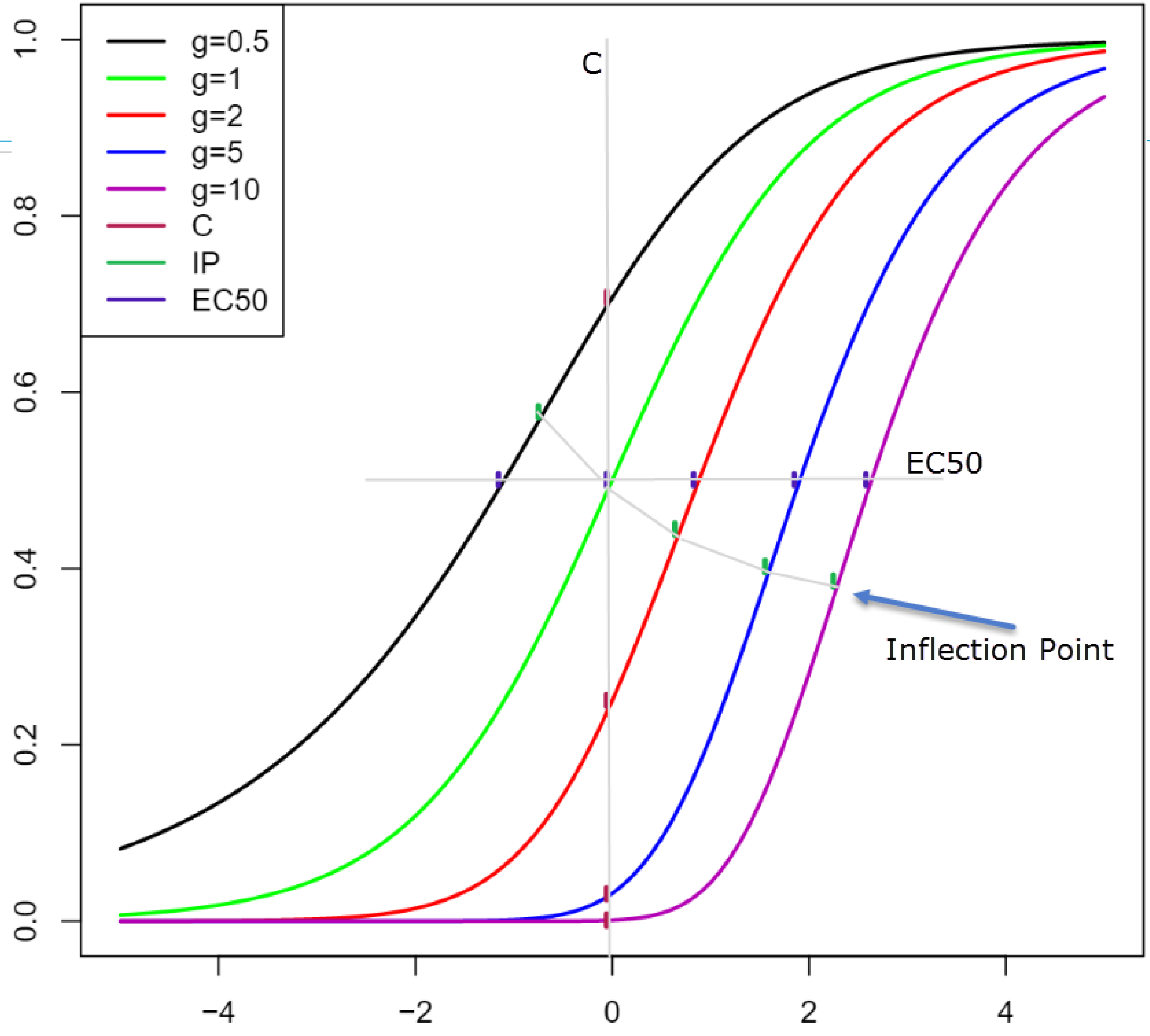
	2PF	4PF	5PF
Slope (Transformed Model) in Inflection Point (S)	$\frac{1}{4} \cdot \ln(b) \cdot B$	$\frac{1}{4} \cdot \ln(b) \cdot (A - D) \cdot B$	$\ln(b) \cdot (A - D) \cdot B \cdot (1 + \frac{1}{g})^{-g-1}$
Slope (Transformed Model) EC50	$\frac{1}{4} \cdot \ln(b) \cdot B$	$\frac{1}{4} \cdot \ln(b) \cdot (A - D) \cdot B$	$\ln(b) \cdot (A - D) \cdot B \cdot g \cdot (2^{\frac{1}{g}} - 1) \cdot (2^{\frac{1}{g}})^{-g-1}$
Slope (Transformed Model) C	$\frac{1}{4} \cdot \ln(b) \cdot B$	$\frac{1}{4} \cdot \ln(b) \cdot (A - D) \cdot B$	$\ln(b) \cdot (A - D) \cdot B \cdot g \cdot 2^{-g-1}$
Inflection Point (Transformed Model)	C	C	$C + \frac{1}{B} \cdot \log_b(g)$
$\log_b$(EC50)	C	C	$C - \frac{1}{B} \cdot \log_b(2^{\frac{1}{g}} - 1)$

5PL

- Graphic shows a variation of g
  - A, B, C, D = constant
- Green Curve (g = 1.0) = 4PL

USP <1032>, Chapter 4.7

- "*Similarity measures may be based on the parameters of the concentration-response curve and may include the slope for a straight parallel-line assay; intercept for a slope-ratio assay; the slope and asymptotes for a four-parameter logistic parallel-line assay; or the slope, asymptotes, and nonsymmetry parameter in a five-parameter sigmoid model*." [D]



Conclusions

Conclusions

- A number of problems have been identified that require attention.

• Slope Challenge	the „slope parameter“ B does not characterize the curve shape in the inflection point which is a base assumption of USP <1032>
• Correlation Challenge	allows unintended curve shapes due to the systematic neglect of dependencies and information loss by the parameter by parameter approach.
• Precision Challenge	may apply inappropriate equivalence margins to qualify the curves
• Tolerance Interval	excludes to many acceptable assays
• 5PF Challenge	parameter correlation

→Companies running Equivalence Test methods may need to reassess their test systems.

Recommendations

- **Every** parameter estimate of the model has to be covered by at least one Equivalence Test!
- Check intrinsic dependencies with variables beyond the USP Domain (e.g. dynamic range)!
- Use the Tolerance Interval Approach with TI = 100%!
- Do not reuse Equivalence Margins which have been calculated from confidence intervals of parameter estimates of assays with different sample sizes!
- Be VERY cautious when using the 5PL function with Equivalence Margins due to correlation effects!

Thank you!

Questions? Visit our booth!

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