

Arlenda European Academy Courses 2006

Validation of analytical methods.

Agenda

1. Validation Criteria in regulatory documents
 1. Pharmaceutical industry
 2. Other areas
2. Objective of analytical Method and objective of validation
3. The only statistics you need: make simple
4. Practically, from the experiments to the report.
5. Demonstration
 1. E-noval
 2. Seelva
6. Conclusion

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Two distinct sets of regulations

→ **Pharmaceutical industry** : ICH (1995-1996)
International Conference on Harmonisation

- Texte ICH (Q2A & Q2B) : “*terminology and methodology*”
- FDA Bioanalytical Method Validation

→ **Other areas** : ISO 5725, 17025, NCCLS, GUM

- 2002/657/EC (SANCO) Guide on performance of analytical methods (09/2002)

> Guide SFSTP 2003: First attempt to harmonise all approaches

Pharmaceutical Industry

Validation of (bio)analytical methods

- Pharmacopeial Forum - current concept for the validation of compendial assays (1986 + Current revision)
- Federal Code of Regulations - Guidelines for submitting samples and analytical data for method evaluation (21CFR 10.90, 1987)
- Pharmacopeial Forum - Validation of compendial assays - Guidelines (1988) USP XXI (1989) and XXII (1990)
- FDA:Center for Drug Evaluation and research - Validation of chromatographic methods - Reviewer guidance (1994)
- ICH-Q2A / ICH-Q2B: Validation of analytical procedures : definitions and terminology / methodology (1995)
- FDA - Analytical procedure and method validation (2000)
- FDA, Bioanalytical Method Validation (2001)

Other areas

- Note CEE (III-844-87, 1989)
- Groupe d'experts de la Pharmacopée Européenne
- 2002/657/EC (SANCO) Guide on performance of analytical methods (09/2002)
- ISO 5725:Accuracy (trueness and precision) of measurement methods and results
- ISO/IEC 17025:2005: General requirements for the competence of testing and calibration laboratories
- GUM = NF ENV 13005. Guide pour l'incertitude de mesure

Documents examined today

- ICH Q2A, Terminology and Definition, 1995
- ICH Q2B, Methodology, 1996
- FDA, Bioanalytical method validation, May 2001
- ISO 5725:Accuracy (trueness and precision) of measurement
- ISO/IEC 17025:2005
- GUM=NF ENV 13005.Guide pour l'incertitude de mesure

Those documents are not harmonized

- SFSTP, Validation of quantitative analytical procedure, Harmonization of approaches, May 2003

Validation Criteria: need a guide?

ICH Q2A	ICH Q2B	FDA May 2001	ISO	SFSTP May 2003
Specificity	Specificity	Specificity	Spec./Select.	Specificity/Selectivity
Linearity -----	----- Linearity	----- Response Function	Response Function	Linearity Response Function
Accuracy/Trueness -----	----- Accuracy	----- Accuracy/Trueness	Accuracy Trueness	Accuracy Trueness
Precision Repeatability Intermediate Precicion Reproductibility	Precision Repeatability Intermediate Precicion Reproductibility	Precision Intra-run Inter-Batch Cross-Validation	Uncertainty Reproducibility	Precision Repeatability Intermediate Precicion Reproductibility
LLOQ	LLOQ	LLOQ & ULOQ	LLOQ&ULOQ	LLOQ & ULOQ
LOD	LOD	LOD	LOD	LOD

Spirit of regulatory documents

Method or results?

- ICH & FDA: “method should be validated for the intended use or application.”
- ICH & FDA: “The acceptability of analytical **data** corresponds directly to the criteria used to validate the method.”

Paradoxically, ICH and FDA documents never mention the quality of the **results** produced by analytical methods.

Criteria listed are about method performance.

Results are the very reason (intent of use) of an analytical method.

Only SFSTP clearly mention the results as objective of analytical method and validation.

Spirit of regulatory documents

Method or results?

- ICH & FDA documents reasoning (**analyst** point de vue):

“If the method is “good” then the results it will produce will be good”

- ISO & SFSTP documents reasoning (**customer** point de vue):

“What matters are the results. If results obtained are “good”, then only a “good” method can produce them.”

The difference is subtle... and important. The decision is made on the same criteria at the end, but in a different way.

Harmonization of the criteria

Validation criteria

Specificity

Response function

Linearity

Trueness

Accuracy

Precision

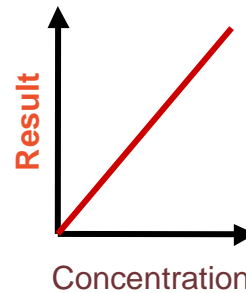
Limit of detection (LOD)

Limit of quantitation
(LOQ)

other ...

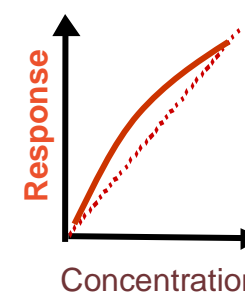
Linearity / Response function

Required linearity



Linearity

Not required linearity



Response function

Linearity

Linearity (ICH Q2A)

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte.

What is a test result ?

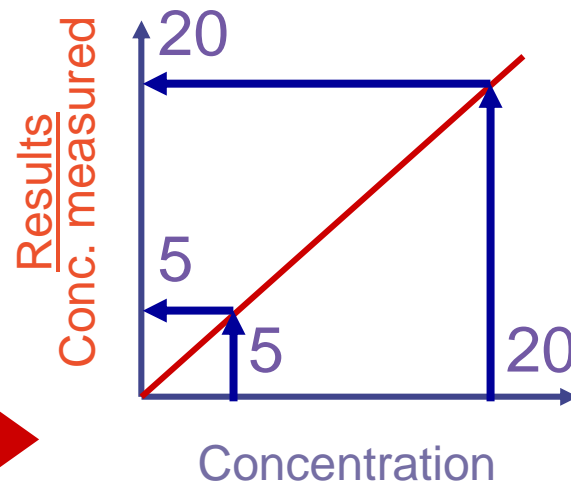
Instrument response ?

or

Concentration ?

Amount ?

Required linearity



Linearity

signal ?

or

a result ?

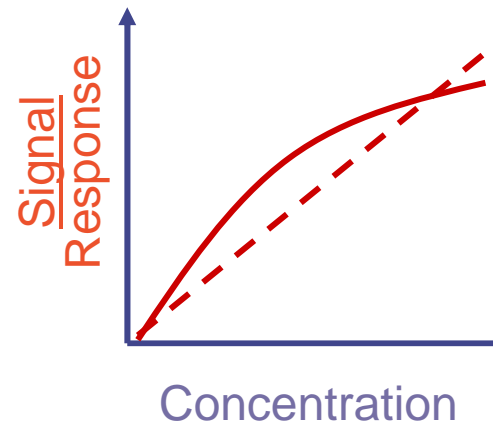
What is a test data ?

Linearity (ICH Q2B)

Linearity should be evaluated by signals as a function of analyte concentration.

In some cases, to obtain linearity between assays and sample concentrations, the test data may have to be subjected to a mathematical transformation prior to the regression.

Non required linearity



Linearity

Linearity (ICH Q2A)

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Confusion between linearity and response function

Linearity (ICH Q2B)

Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content.

In some cases, to obtain linearity between assays and sample concentrations, the test data may have to be subjected to a mathematical transformation prior to the regression.

Linearity SFSTP 2003

- *Linearity (of the results)*

The linearity of an analytical procedure is its ability within a definite range to obtain **results** directly proportional to the concentrations (quantities) of the analyte in the sample.

The linearity criteria must only be applied to the results [**calculated concentration** = f(introduced concentration)], not to the responses [signal = f(introduced concentration)].

Response Function / Calibration Curve

- FDA May 2001 doesn't content the word "Linearity" anymore
"A calibration (standard) curve is the relationship between instrument response and known concentrations of the analyte."
- "The simplest model that **adequately** describes the concentration-response relationship should be used. Selection of weighting and use of a complex regression equation should be justified."

Response Function / Calibration Curve

- FDA May 2001
“Microbiological and immunoassay standard curves are inherently nonlinear....
The concentration-response relationship is most often fitted to a 4- or 5-parameter logistic model, although others may be used with suitable validation.”

Response Function / Calibration Curve

- ICH

“In some cases, to obtain linearity between assays and sample concentrations, the test data may have to be subjected to a mathematical transformation prior to the regression”

Rather contradictory

....

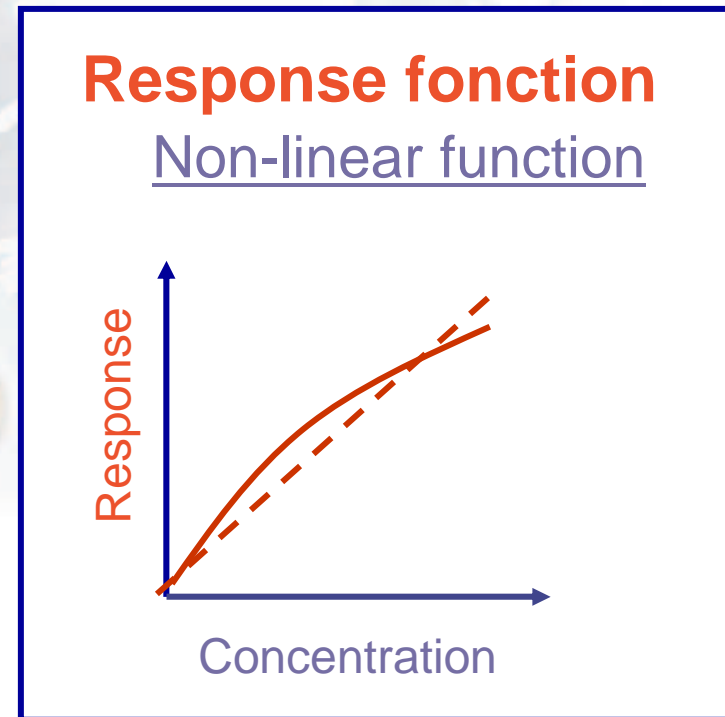
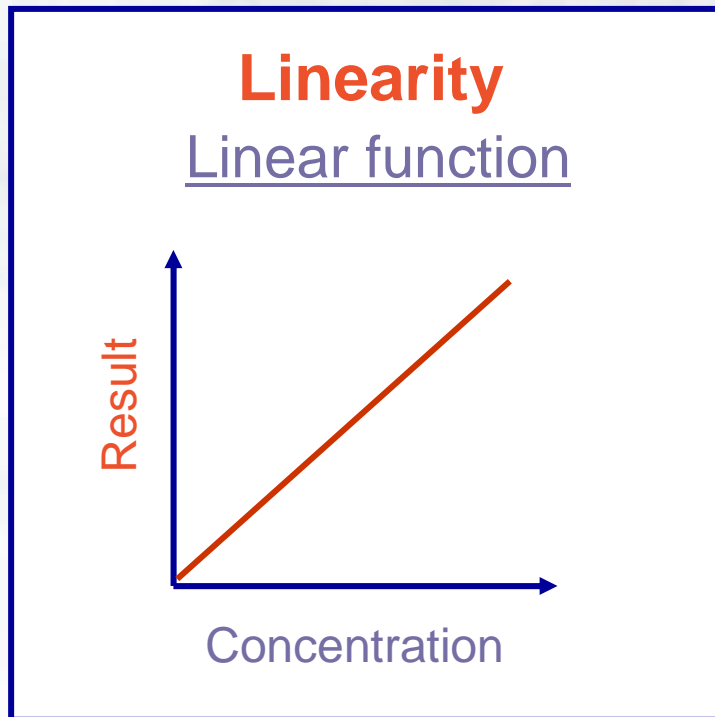
Some analytical procedures, such as immunoassays, do not demonstrate linearity after any transformation. In this case, the analytical response should be described by an **appropriate function** of the concentration (amount) of an analyte in a sample.”

Response Function / Calibration Curve

- SFSTP
“The response function can be linear (straight line), but non-linear models, sometimes induced by the detection method or by the wide concentration range, can also be observed. The response function must however be monotonous, i.e. strictly increasing or decreasing.”

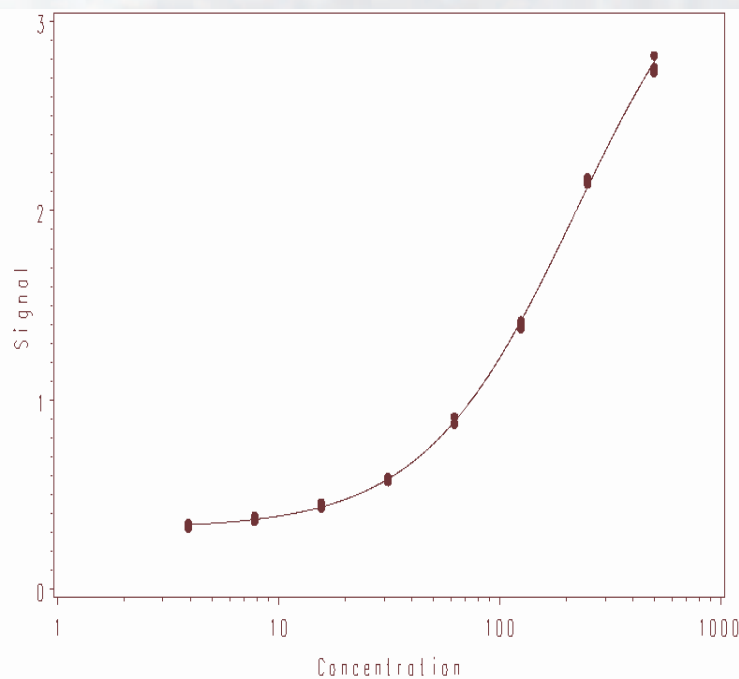
Linearity / Response function

A linear relationship **COULD** exist between the response and the concentration but **MUST** exist between the result (calculated concentration) and the introduced concentration

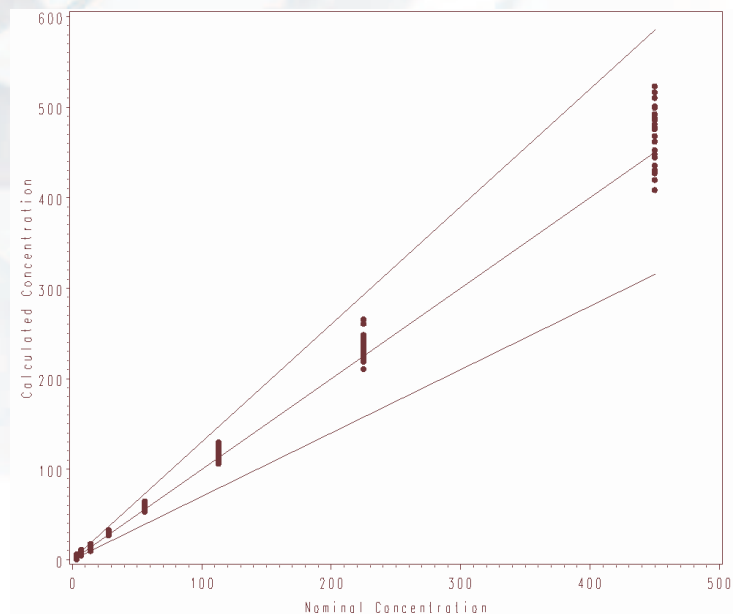


Example with an ELISA assay

A nonLinear Signal Response Function...



...gives Linear result



What experiments for Standard curve?

ICH: “For the establishment of linearity, a minimum of five **concentrations** is recommended. Other approaches should be justified.”

FDA: “The matrix-based standard curve should consist of a minimum of six standard points, excluding blanks, **using single or replicate** samples. The standard curve should cover the entire range of expected concentrations.

> NOTE: **Replicates are MORE important than the number of levels. Without replicates impossible to judge adequacy of a model.**

Accuracy, Trueness and Precision

Validation criteria

Specificity

Response function

Linearity

Trueness

Accuracy

Precision

Limit of detection (LOD)

Limit of quantitation
(LOQ)

other ...

Accuracy - Precision - Trueness

STAT.

Total error = Bias + Standard deviation

Total error = Systematic error + Random error

ISO

Accuracy = Trueness + Precision

ICH

Accuracy ? = Accuracy + Precision

Accuracy according to ICH

Accuracy (ICH Q2A) = ISO definition of accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between **the value** which is accepted either as a conventional true value or an accepted reference value and **the value found**.

Confusion between accuracy and trueness

Accuracy (ICH Q2B) = ISO definition of trueness

Recommended Data (4.3)

Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference between **the mean** and **the accepted true value** together with the confidence intervals.

Accuracy according to FDA

- **Accuracy:** The degree of closeness of **the** determined value to the nominal or known true value under prescribed conditions. This is sometimes termed *trueness*. **Contradictory**
- The **accuracy** of an analytical method describes the closeness of **mean** test results obtained by the method to the true value (concentration) of the analyte.... The deviation of the mean from the true value serves as the measure of accuracy.

>Same confusion as in ICH

Accuracy according to SFSTP

- *Accuracy*

The accuracy of an analytical procedure expresses the closeness of agreement between **the** value found and the value which is accepted either as a conventional true value or an accepted reference value. The closeness of agreement observed is the resultant of the **sum** of the systematic and random errors; in other terms, the total error linked to the **result**. Consequently, **the accuracy is the expression of the sum of the trueness and precision.**

Trueness according to SFSTP

Trueness (bias)

The trueness of an analytical procedure expresses the closeness of agreement between the **mean** value obtained from a series of measurements and the value which is accepted either as a conventional true value or an accepted reference value (international standard, standard from a pharmacopoeia).

The measure of trueness is generally expressed in terms of recovery and of absolute or relative bias (**systematic** error). It must again be noted that the trueness was also called “accuracy” or “accuracy of the mean”. Nevertheless, this use is not recommended

Precision according to SFSTP

- *Precision*

“The precision of an analytical procedure expresses the closeness of agreement (**dispersion** level, relative standard deviation) between a series of **measurements** obtained from multiple sampling of the same homogeneous sample (independent essays) under the prescribed conditions. It gives some information on **random** errors and it can be evaluated at three levels: repeatability, intermediate precision (within laboratory) and reproducibility (between laboratories).”

Precision according to ICH Q2A

- “The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.”

> Same definition in FDA

Precision according to FDA

But...

FDA: “Precision is further subdivided into **within-run, intra-batch precision or repeatability**, which assesses precision during a single analytical run, and **between-run, interbatch precision or repeatability**, which measures precision with time, and may involve different analysts, equipment, reagents, and laboratories.”

- ⇒ Refers to within/between runs/batches instead of repeatability and intermediate precision
- ⇒ Use twice repeatability for within and between runs/batches

Reporting Precision according to ICH Q2B

“The standard deviation, relative standard deviation (coefficient of variation), and **confidence interval** should be reported for each type of precision investigated.”

⇒ Confidence interval OF repeatability and intermediate precision?

⇒ Rather difficult to obtain and useless

⇒ Or Confidence Interval USING repeatability and intermediate precision

⇒ Make greater sense.

⇒ Suggested by FDA: “A confidence interval approach yielding comparable accuracy and precision is an appropriate alternative.”

Uncertainty

- Type Uncertainty $u(x_i)$:
 - Uncertainty of the result of a measurement process expressed by a standard deviation.
- Composed Uncertainty $u_c(y)$:

$$U_c(y(x_1, x_2, \dots)) = \sqrt{\sum_{i=1, n} u(y, x_i)^2}$$

- x_1, x_2, \dots, x_n being independent variables or noise sources
- Expanded Uncertainty U :
 - Uncertainty around a measure with a fixed confidence level.

$$U = k \cdot u_c(y)$$

- k : expansion factor depending on the level

How to quantify the Uncertainty

- 2. Estimer l'incertitude en utilisant une analyse inter-laboratoire (AOAC ou ISO 5725, GUM).
 - ◆ → fidélité (répétabilité, fidélité intermédiaire, reproductibilité)
 - ◆ → Justesse (bias)
 - évaluer les sources d'incertitudes \neq test inter-laboratoire (prélèvement, pré-traitement, homogénéisation, stabilité,...)
 -
- 2bis. Estimer l'incertitude sur une méthode validée en interne (GUM).

Comment?

 - - Estimation de la fidélité totale disponible (par ex : QC analysé plusieurs fois sur une période de temps, avec opérateurs et instruments différents ou écart-type obtenu à partir de répétitions sur différents échantillons)

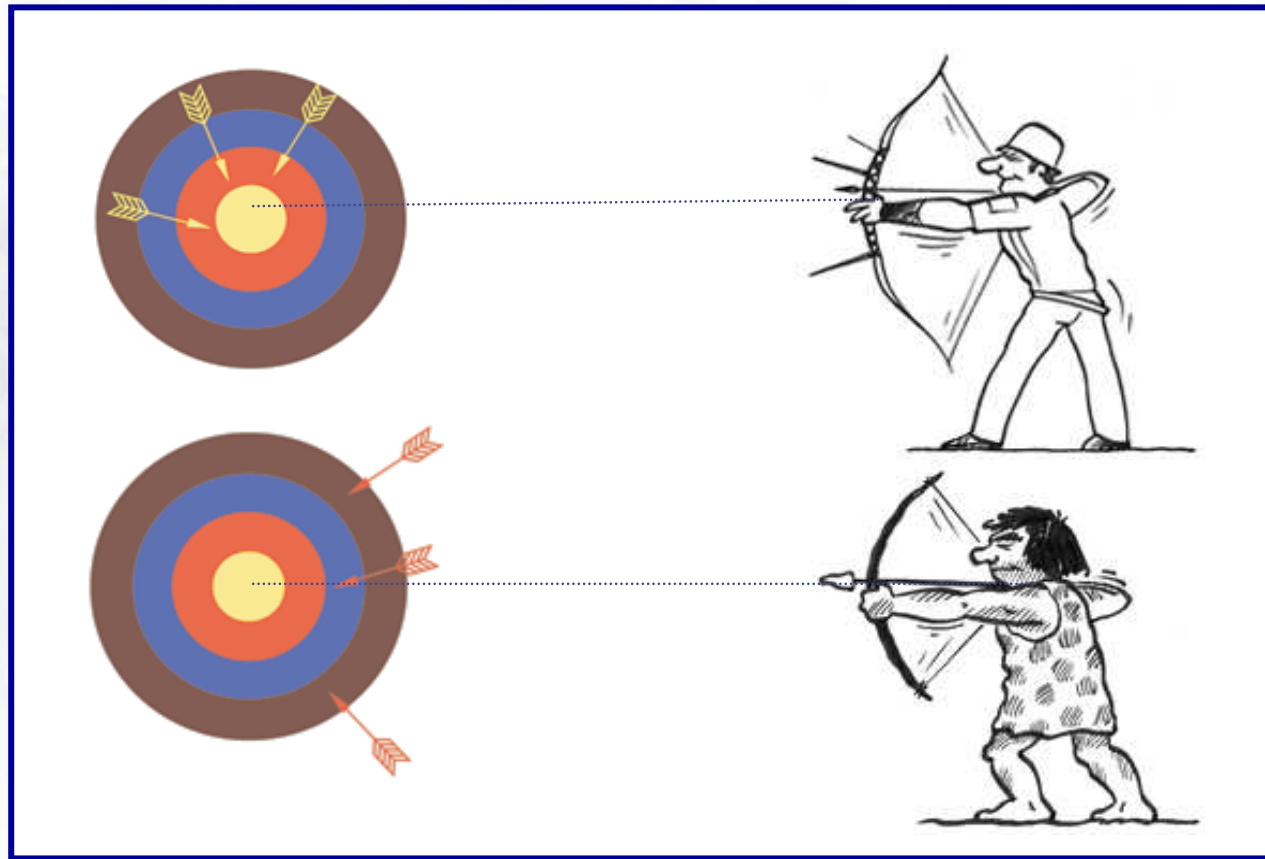
Fundamental differences between ICH, FDA, SFSTP and ISO definitions

	Criterion
Statistics	<p>Total error = bias + std deviation</p> <p>Total error = Systematic error + random error</p>
SFSTP/ISO	<p>Accuracy = Trueness + precision</p> <p>= total error</p>
ICH/FDA	<p>Accuracy = trueness ?</p> <p>Accuracy ? = accuracy + precision</p> <p>Total error = ?</p>

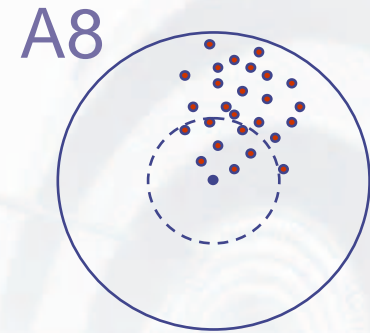
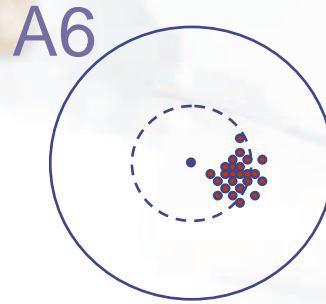
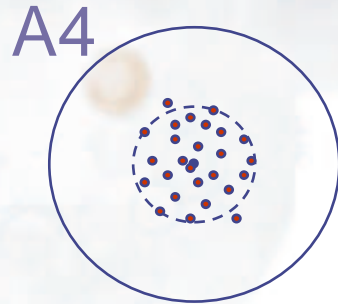
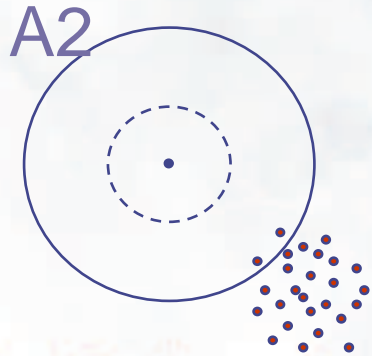
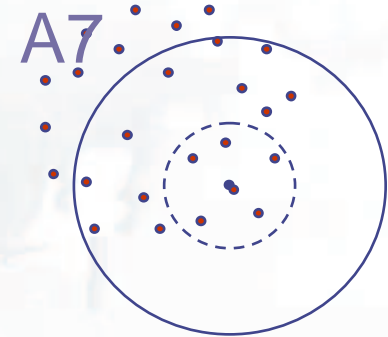
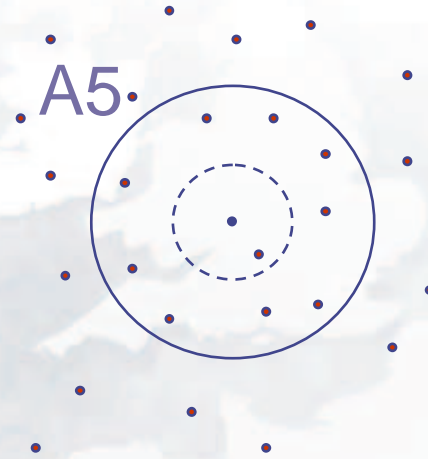
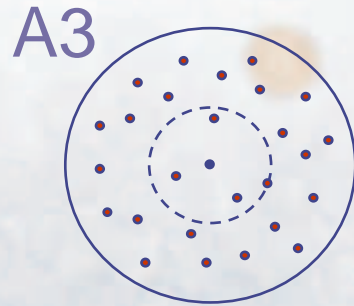
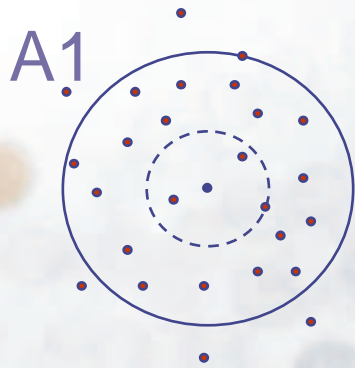
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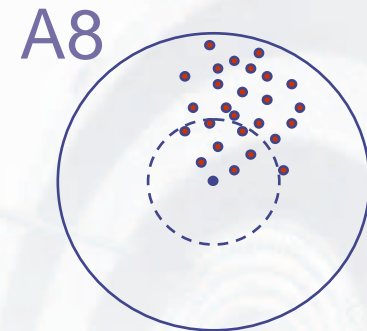
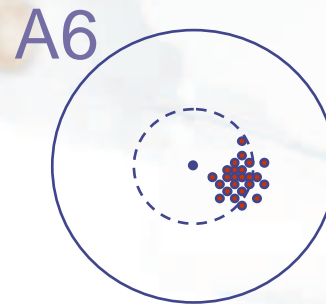
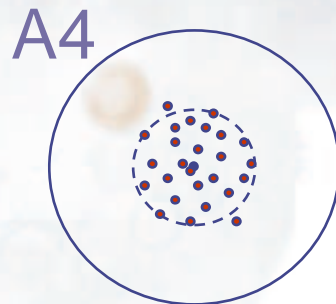
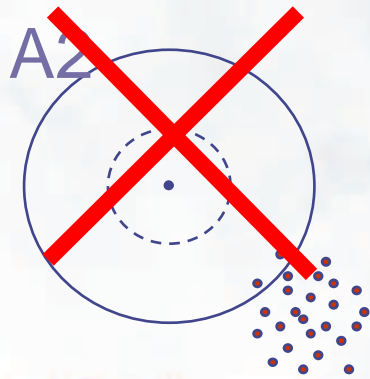
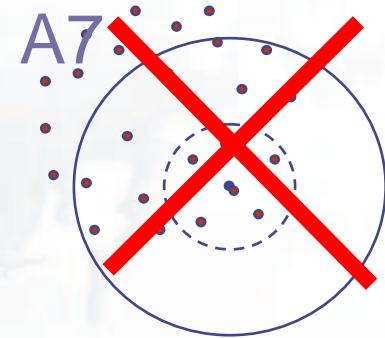
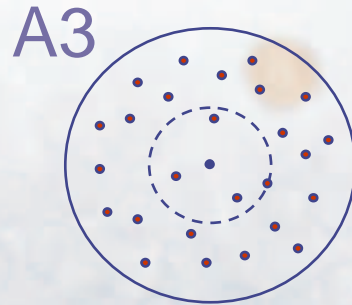
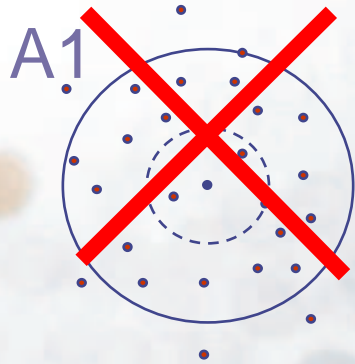
Objective of an analytical method



Results from various archers



Which archer to select?



What decision are we taking ... ?

Objective of analytical method

The objective of an analytical procedure is to be able to determine as accurately as possible each of the unknown quantity that the laboratory will have to quantify.

$$X \leftrightarrow \mu_T$$

X = measured value
or result

μ_T = true unknown value

Objective of validation

The objective of validation is to give to the laboratory as well as to the regulatory bodies **guarantees** that every single measure that will be performed in routine will be **close enough to the unknown « true value »** of the sample.

Objective of validation

Close enough means that the difference between the **result** and the unknown « **true value** » will be within the acceptance limits $[-\lambda, \lambda]$

$$|X - \mu_T| < \lambda$$

λ = acceptance limit, that is fixed *a priori*
(for example 15%).

Objective of validation

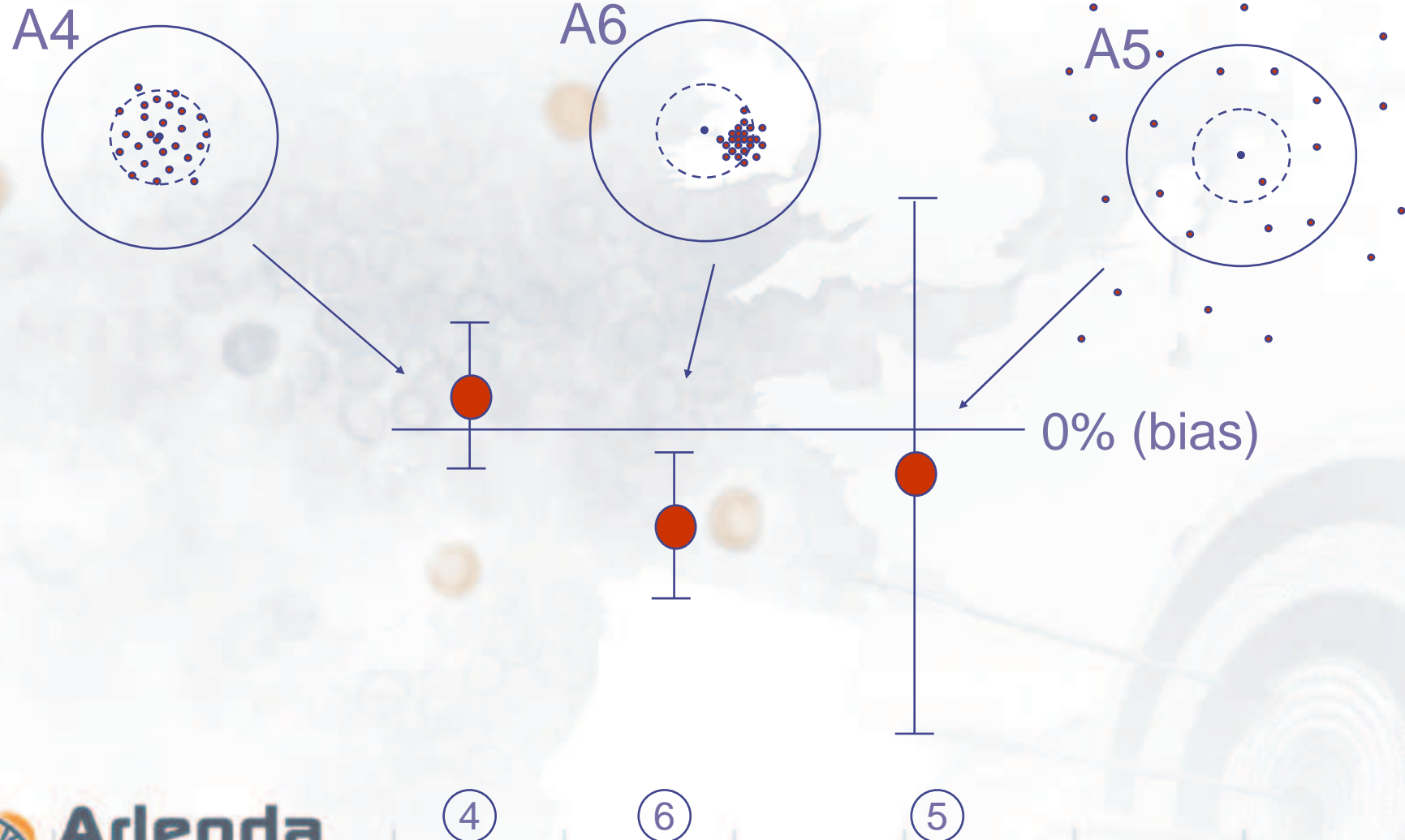
Guarantee means that it is very likely that, the result will be close enough to the unknown «true value».

$$\Pr\left[|X - \mu_T| < \lambda\right] \geq \alpha$$

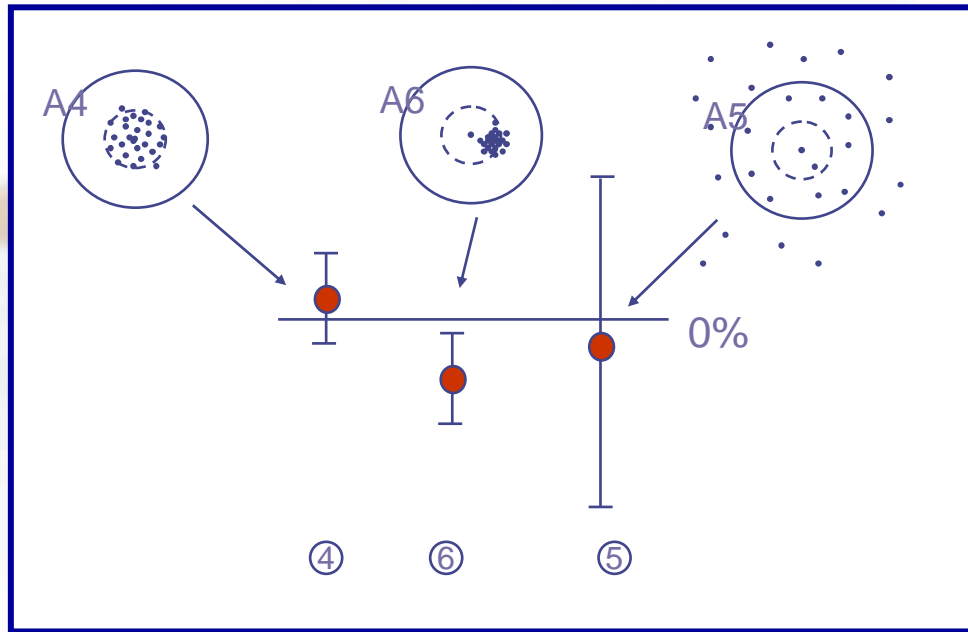
α = minimum probability that a result will fall within acceptance limits

$1 - \alpha$ = a priori maximal risk

How to make the decision ?



Decision based on the Null Hypothesis



$$H_0 : \mu_M = 0$$

Rule :

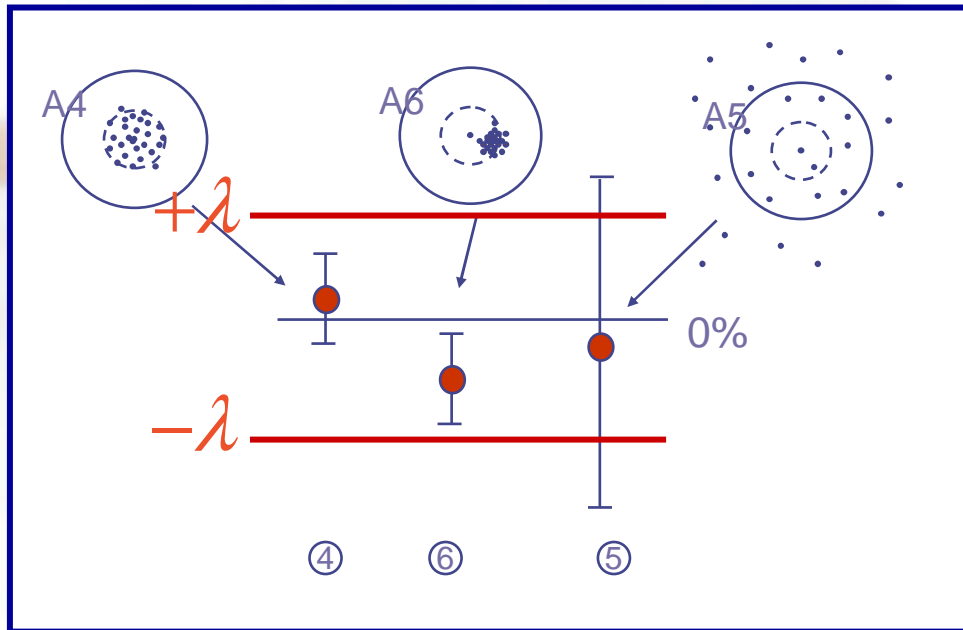
The procedure is declared as accurate when the 95 % confidence limits of the mean bias include the 0 % value.

Conclusions :

The procedures 4 and 5 are thus « accurate »

The greater the variance, the less precise the method, the more likely it is that the confidence interval contains the 0 % value

Decision rule



Conclusions :

The procedures 4 and 6 are valid

Solution :

To fix acceptance limits according to the intended use of the procedure (ex: $\lambda = 5\%$)

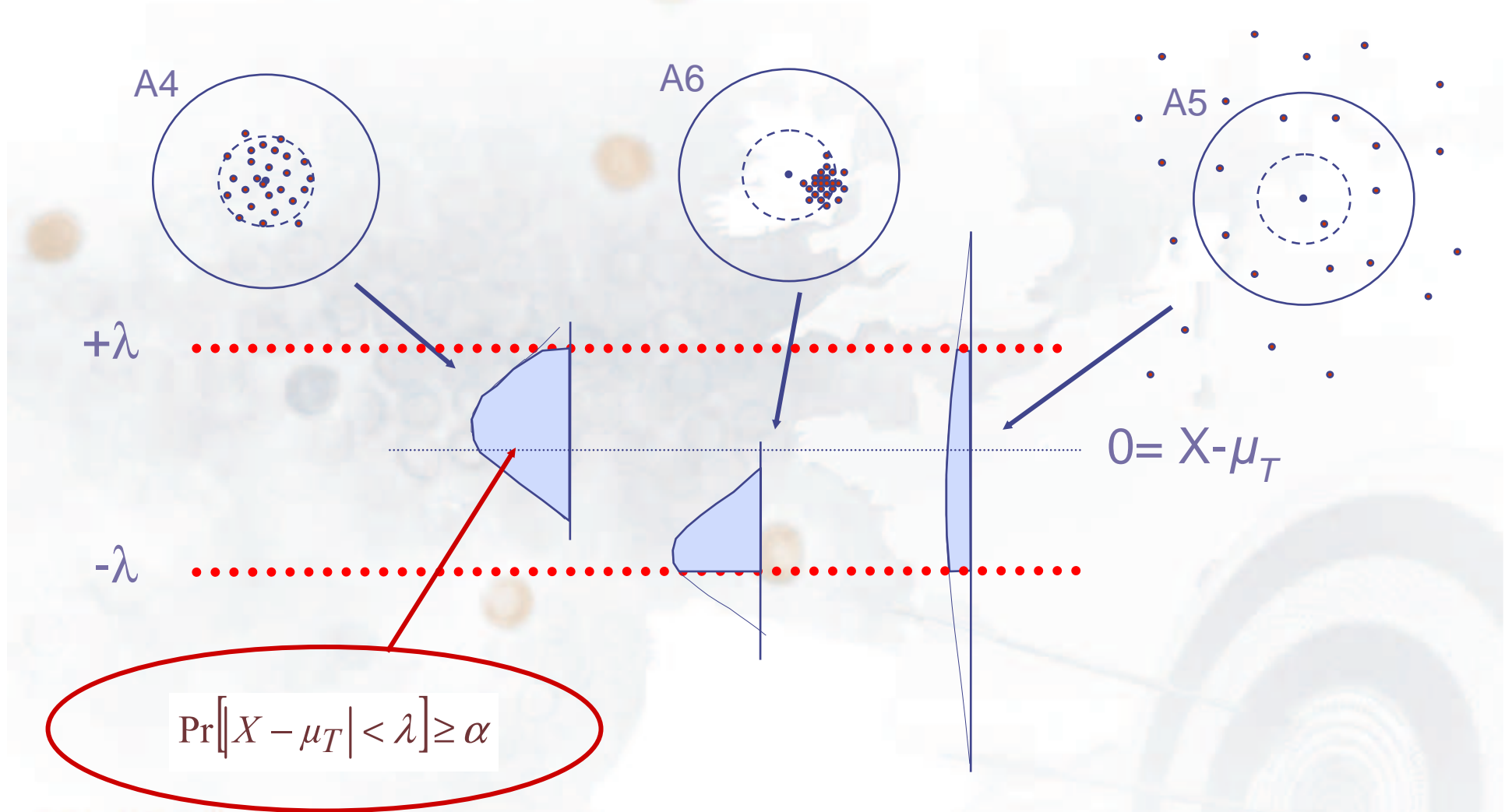
Question :

What decision?

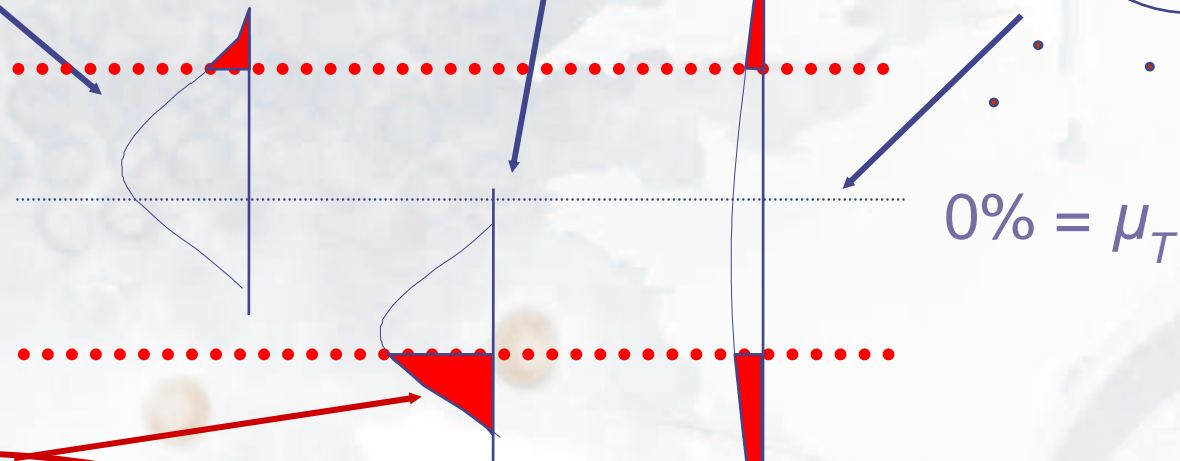
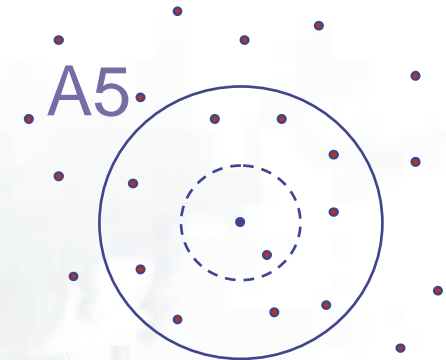
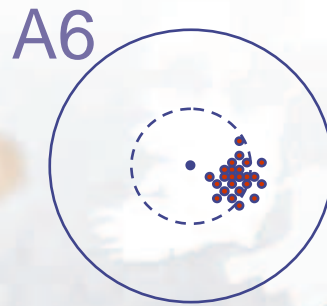
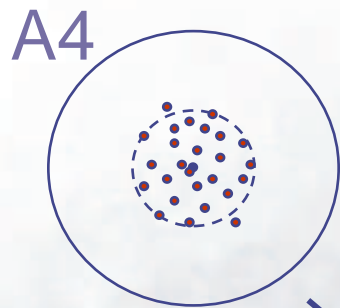
What acceptance limit ?

What risk?

Decision based on the probability...



Decision based on the probability...



$$\Pr\left[|x_i - \mu_T| > \lambda\right] \leq \beta$$

In order to minimize the risk

④

⑥

⑤

Decision in regulatory documents?

- ICH:
“...confirming that the analytical procedure provides an **acceptable** degree of linearity, accuracy, and precision when applied to samples containing amounts of analyte within or at the extremes of the specified range of the analytical procedure.”

No recommendation for Decision in ICH

Decision in regulatory documents?

Method

FDA Bioanalytical Methods:

“The **mean** value should be within **15%** of the actual value except at LLOQ, where it should not deviate by more than 20%

...

The **precision** determined at each concentration level should not exceed **15%** of the coefficient of variation (CV) except for the LLOQ

Mean < 15% & Precision < 15% -THEN- Results in [-15%, 15%]?

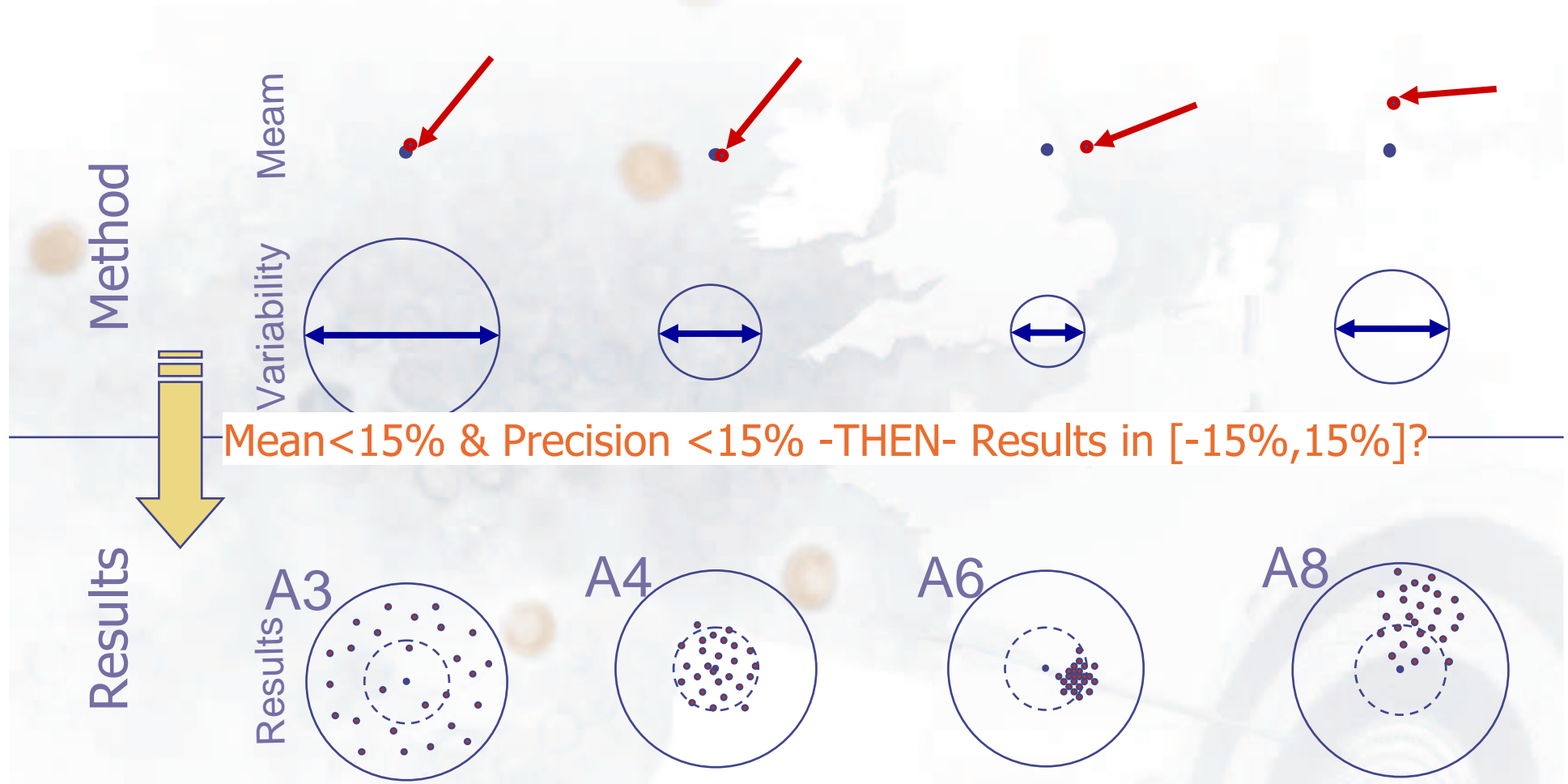
Results

At least **67%** (4 out of 6) of QC samples should be within **15%** of their respective nominal value

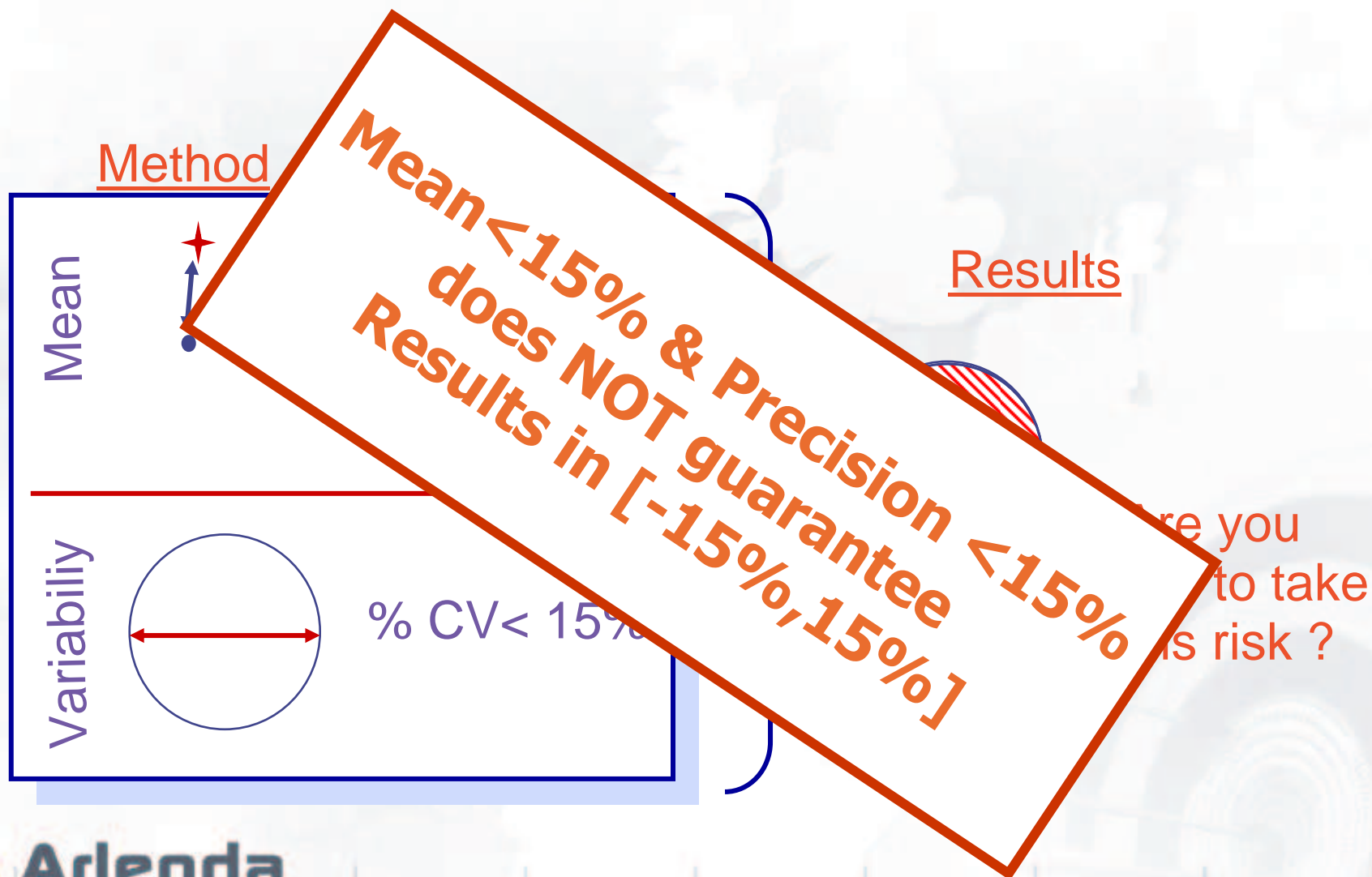
...

Matrix-based standard **calibration samples: 75%**, or a minimum of six standards, **when back-calculated** (including ULOQ) should fall within $\pm 15\%$, except for LLOQ, when it should be $\pm 20\%$ of the nominal value.

Targets / Archers



Total Error or Measurement Error concept



Why it does NOT work?

ICH, Q2A

3. ACCURACY

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

$$x_i = \mu_T + \text{Bias} + \text{Precision}$$



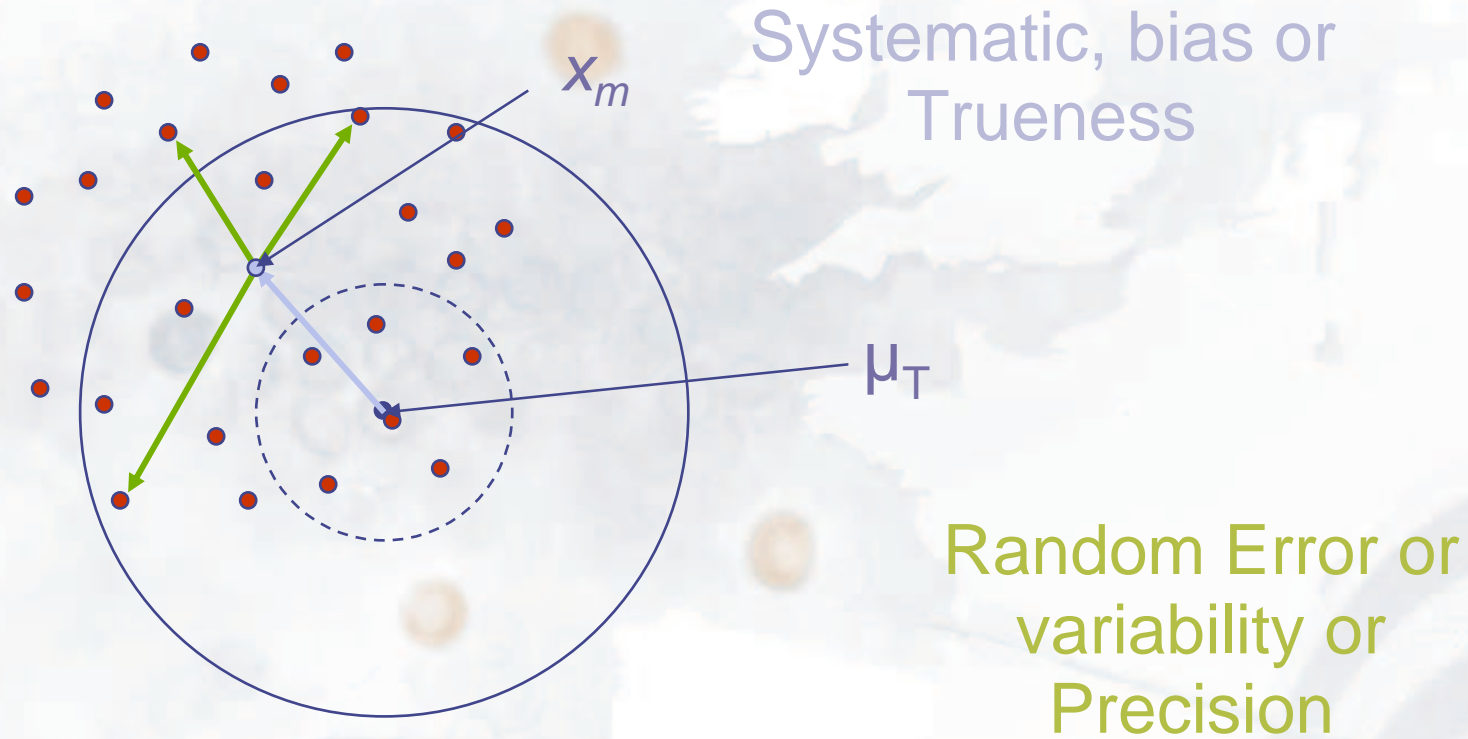
$$x_i - \mu_T = \text{Bias} + \text{Precision}$$

$$= \text{Accuracy}$$

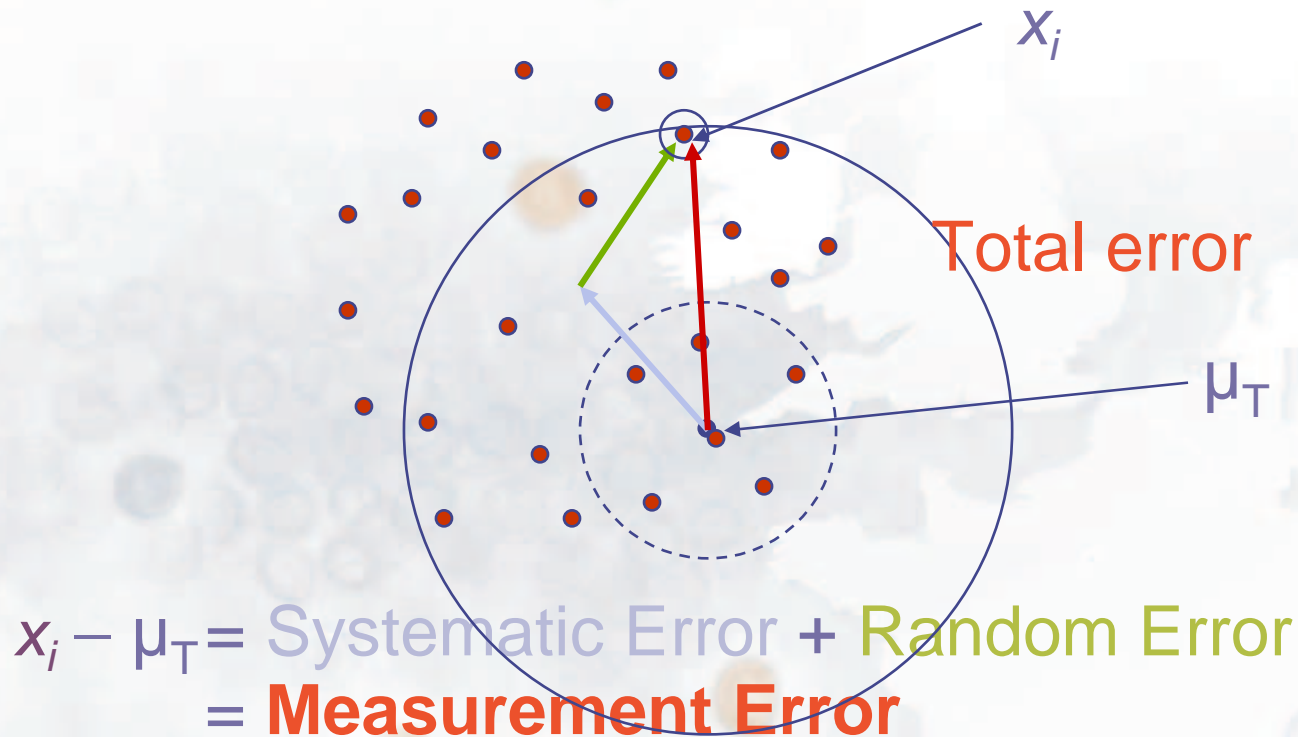
$$= \text{Total Error}$$

$$= \text{Measurement Error}$$

Total or Measurement Error



Total or Measurement Error

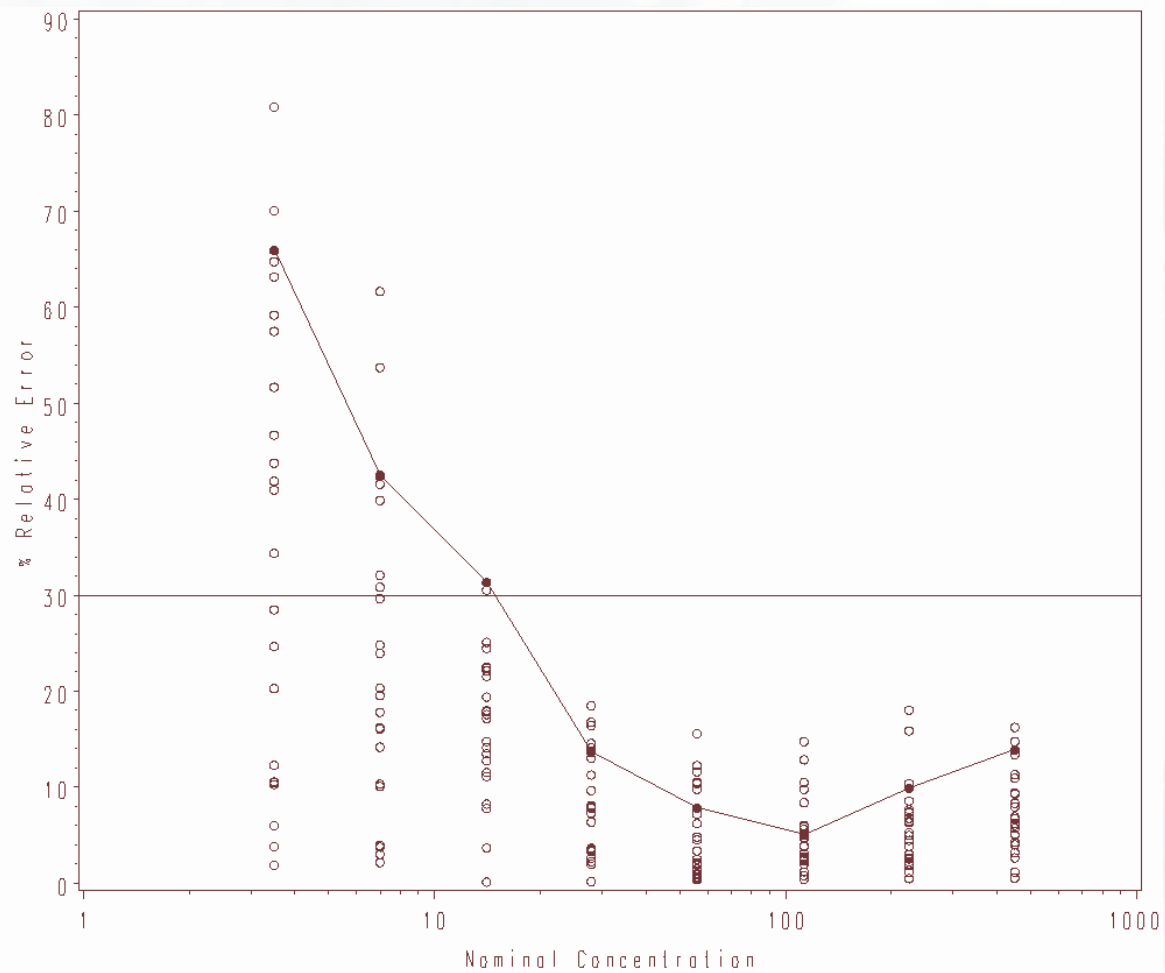


3. ACCURACY

ICH, Q2A

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

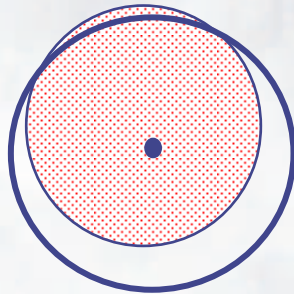
Total or Measurement Error



Measurement Error for Decision

Results

Method



Results in [-15%, 15%]
GUARANTEE
Mean < 15% & Precision < 15%

% Bias < 15%

Var

Preliminary conclusions

1. “Good” Methods do NOT provide necessarily “good” results
2. “Good” results can only be obtained with “good” methods

Make a decision on the results, the very reason of an analytical quantitative method.

This way, it will guarantee your method is valid

HOW?

Agenda

1. Objective of analytical Method and objective of validation
2. Validation Criteria in regulatory documents
 1. Pharmaceutical industry
 2. Other areas
3. The only statistics you need: make simple
4. Practically, from the experiments to the report.
5. Demonstration
 1. E-noval
 2. Seelva
6. Conclusion

The objective of validation becomes...

The objective of the **validation** phase is to evaluate whether, **given the estimates** of bias and precision, the **expected proportion** of measures that will fall within the acceptance limits is greater than a predefined proportion, say β , i.e.:

$$E_{\hat{\mu}, \hat{\sigma}} \left\{ P \left[|X - \mu_T| < \lambda \mid \hat{\mu}_M, \hat{\sigma}_M \right] \right\} \geq \beta$$

Estimated performance

However ...

There exist no exact solution for:

$$E_{\hat{\mu}, \hat{\sigma}} \{ P[|X - \mu_T| < \lambda] | \hat{\mu}_M, \hat{\sigma}_M \} \geq \beta$$

An easy alternative to make a decision, as proposed by other authors, is to compute the

β -expectation tolerance interval (Mee, 1984):

$$E_{\hat{\mu}_M, \hat{\sigma}_M} \{ P_X [\hat{\mu}_M - k \hat{\sigma}_M < X < \hat{\mu}_M + k \hat{\sigma}_M | \hat{\mu}_M, \hat{\sigma}_M] \} = \beta$$

where k is determined so that the expected proportion of the population falling within the interval is equal to β .

Decision in validation

If $\beta = \alpha$ and if the β -expectation tolerance interval is included within the acceptance limits, then the expected proportion of measurements within the acceptance limits is greater or equal to β , i.e. the method is accepted at level β .

if

$$\begin{cases} \hat{\mu}_M - k\hat{\sigma}_M > -\lambda \\ \text{and} \\ \hat{\mu}_M + k\hat{\sigma}_M < +\lambda \end{cases} \Rightarrow E_{\hat{\mu}, \hat{\sigma}} \{P[|x_i - \mu_T| < \lambda] | \hat{\mu}_M, \hat{\sigma}_M\} \geq \beta = \alpha$$



Tolerance or Confidence intervals ?

Confidence Interval

= **Description** of results obtained in the **past** experiments

- Centered on the mean/bias

= Mean \pm kSD

Tolerance Interval

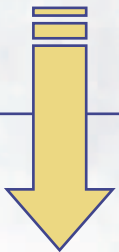
= **Prediction** of results to be obtained in **future** experiments

- take into account the uncertainty of the mean

\sim Mean \pm k(SD + SD_{mean})

What value for Acceptance Limits?

Method



Results

FDA Bioanalytical Methods:

“The **mean** value should be within **15%** of the actual value except at LLOQ, where it should not deviate by more than 20%

...

The **precision** determined at each concentration level should not exceed **15%** of the coefficient of variation (CV) except for the LLOQ

Remember, the intent of the text was:

Mean < 15% & Precision < 15% => Results in [-15%, 15%]

At least **67%** (4 out of 6) of QC samples should be within **15%** of their respective nominal value

...

Matrix-based standard **calibration samples: 75%**, or a minimum of six standards, **when back-calculated** (including ULOQ) should fall within **±15%**, except for LLOQ, when it should be **±20%** of the nominal value.

What value for Acceptance Limits ?

Define the acceptance limits

- on the results
- based on the intended use of the results
- NOT based on the method
The results are used, not the method.....
- On the risk it may constitutes for customer and laboratory

Decision, a conclusion

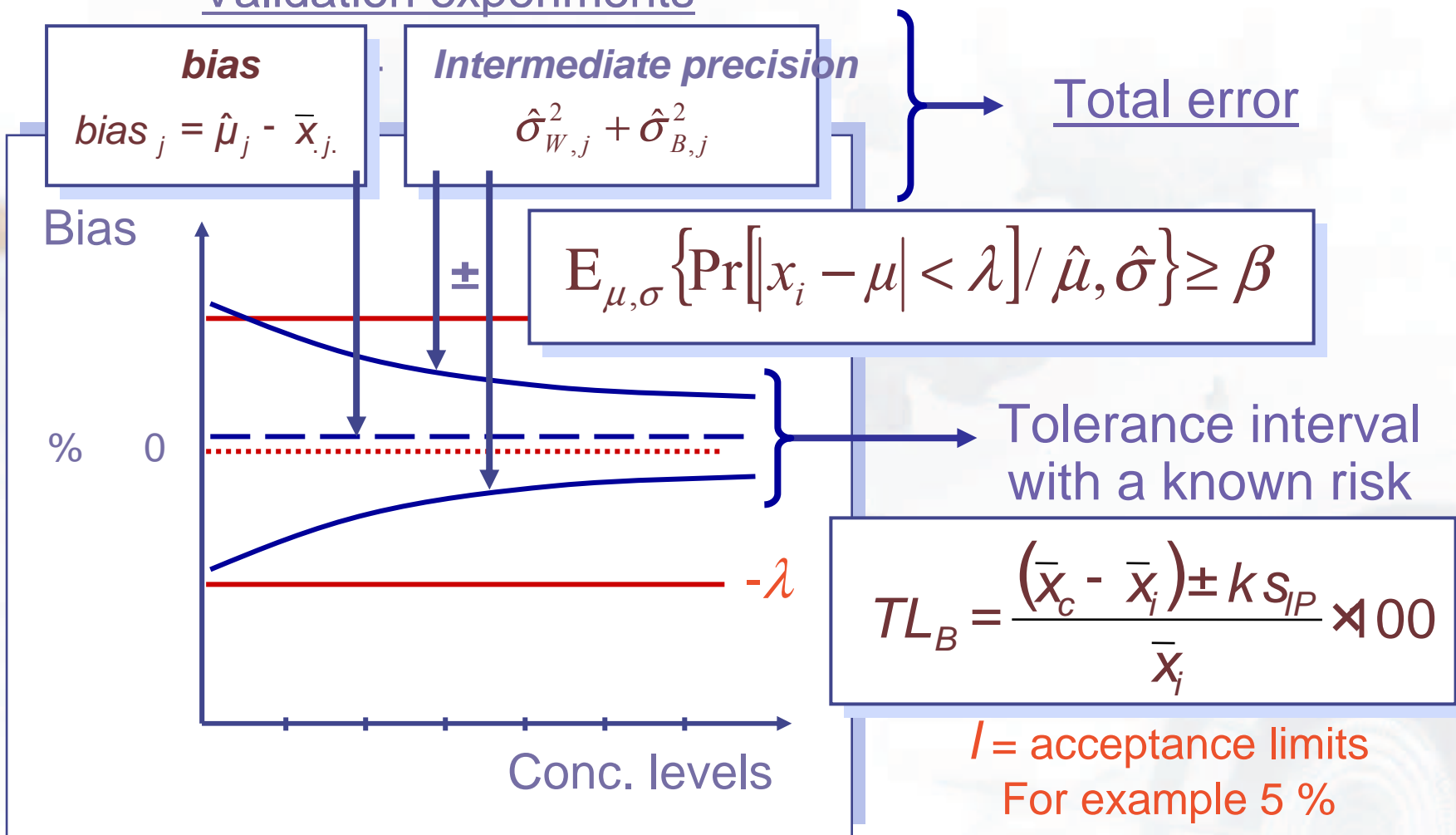
- Expected Proportion of results within **Acceptance** limits (β) should be $\geq 80\%$ to guarantee that at least 90% of the **runs** (γ) will be accepted with the 4-6- λ rule.

$$\beta \geq 80\% \Rightarrow \gamma \geq 90\%$$

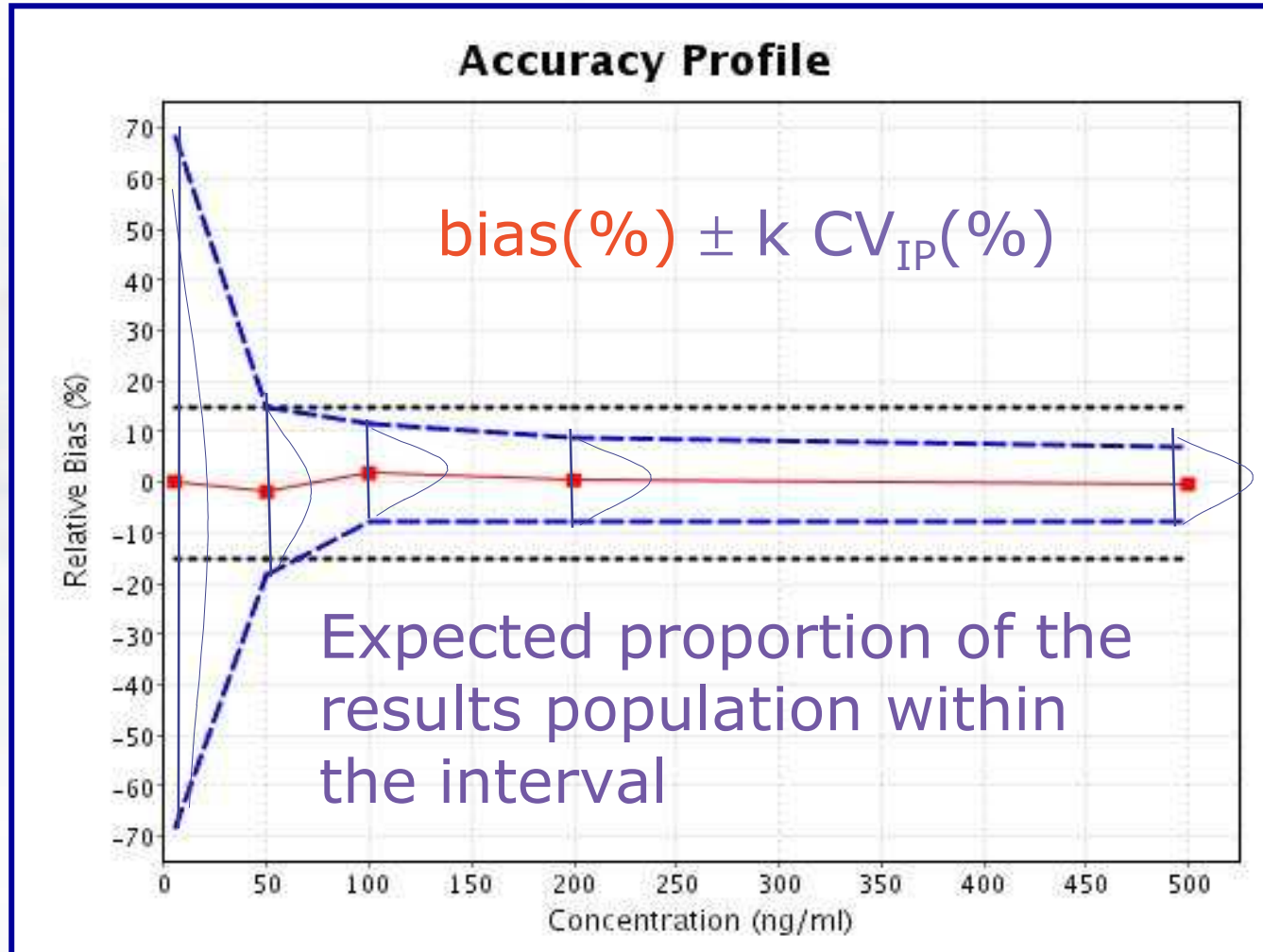
- Taking $\beta = 80\%$ makes **validation** and **routine** decision rules consistent.

Decision tool : accuracy profile

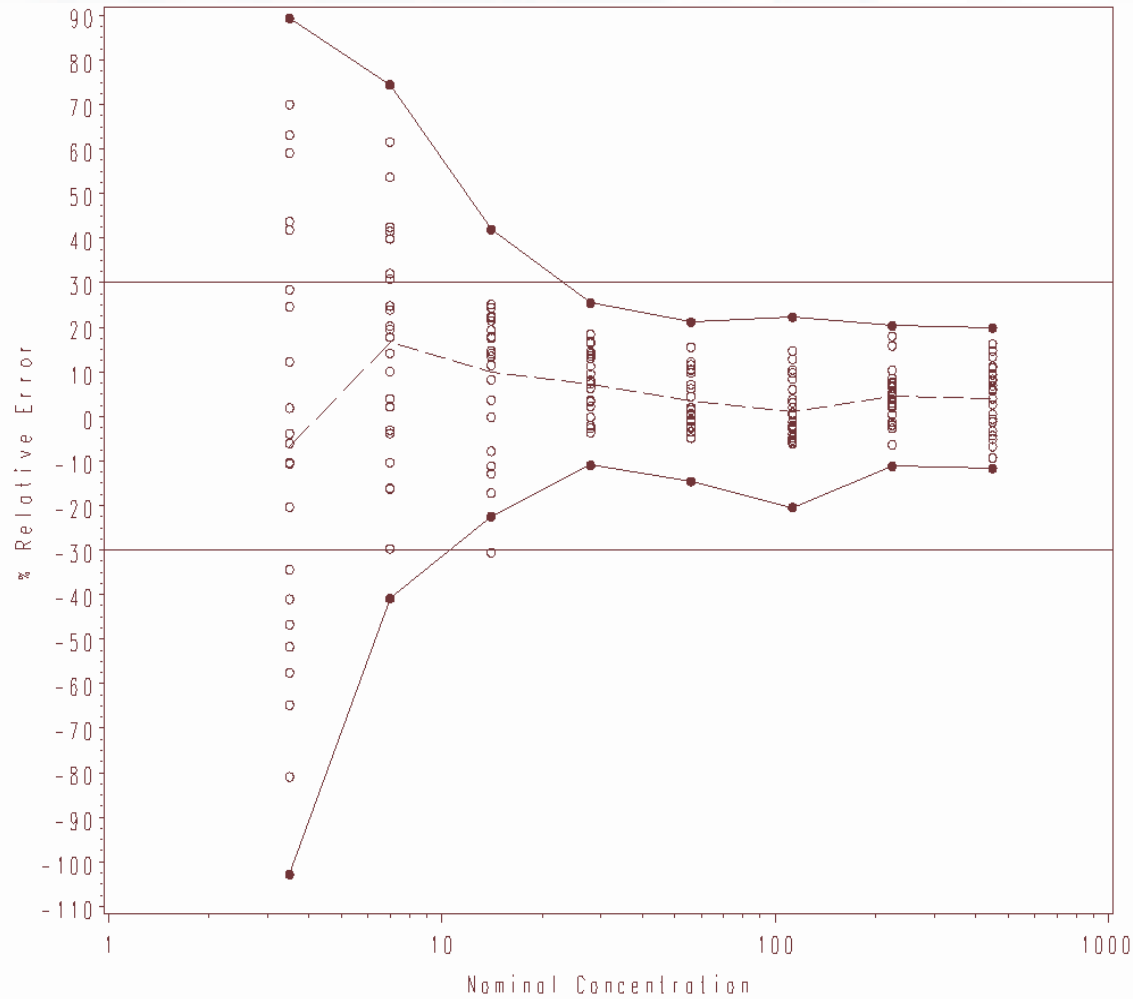
Validation experiments



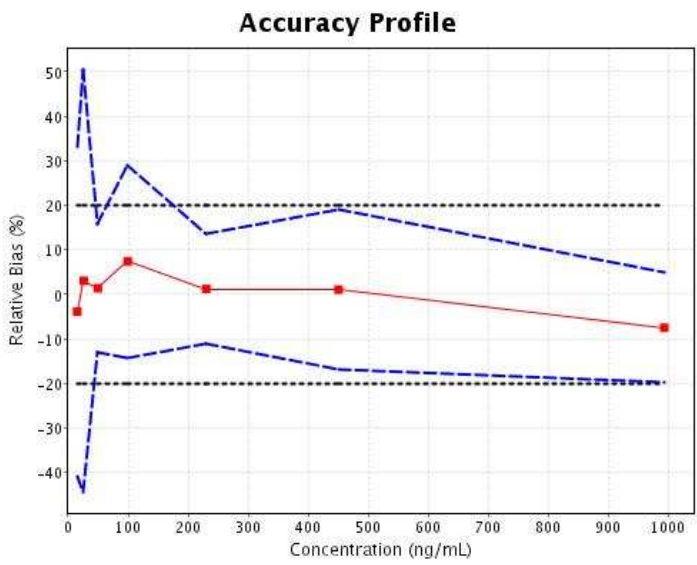
Decision tool : accuracy profile



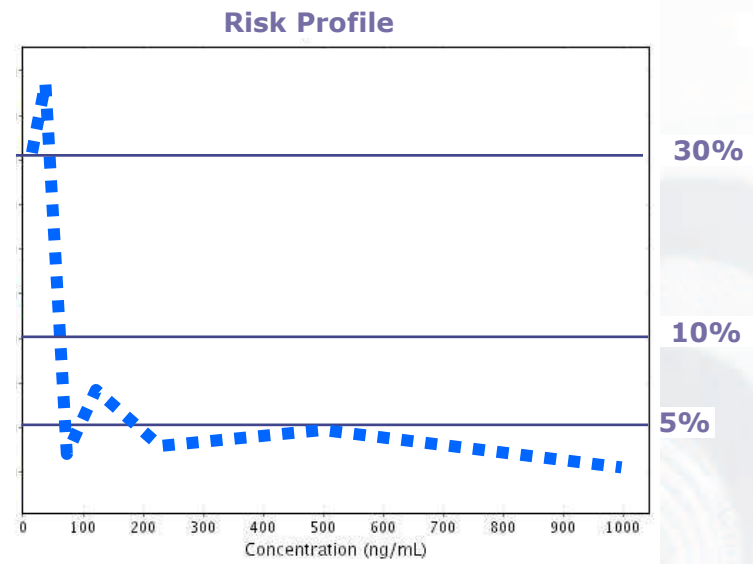
Decision tool : accuracy profile



A flexible alternative: Risk profile



Pr(measurement outside limits)



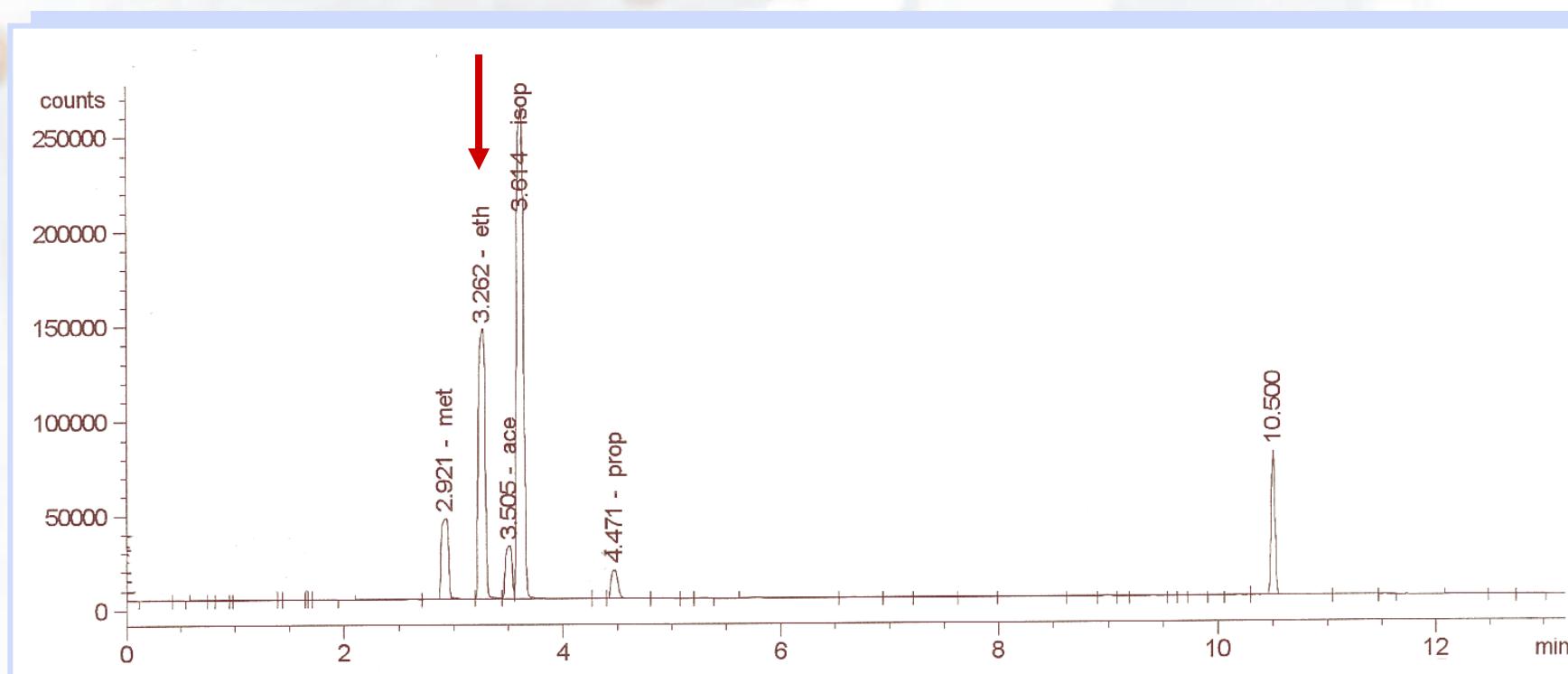
Be more flexible with the risk,
not with the acceptance limits.

Conclusions

1. The focus should be on the accuracy (measurement error) of results provided by the method.
2. If the results are « good » then is the method and criteria are fulfilled.
3. Acceptance limits for accuracy of results must be based on the use of the data.
4. Be conscious of the risks
5. Make validation decision and routine decision consistent

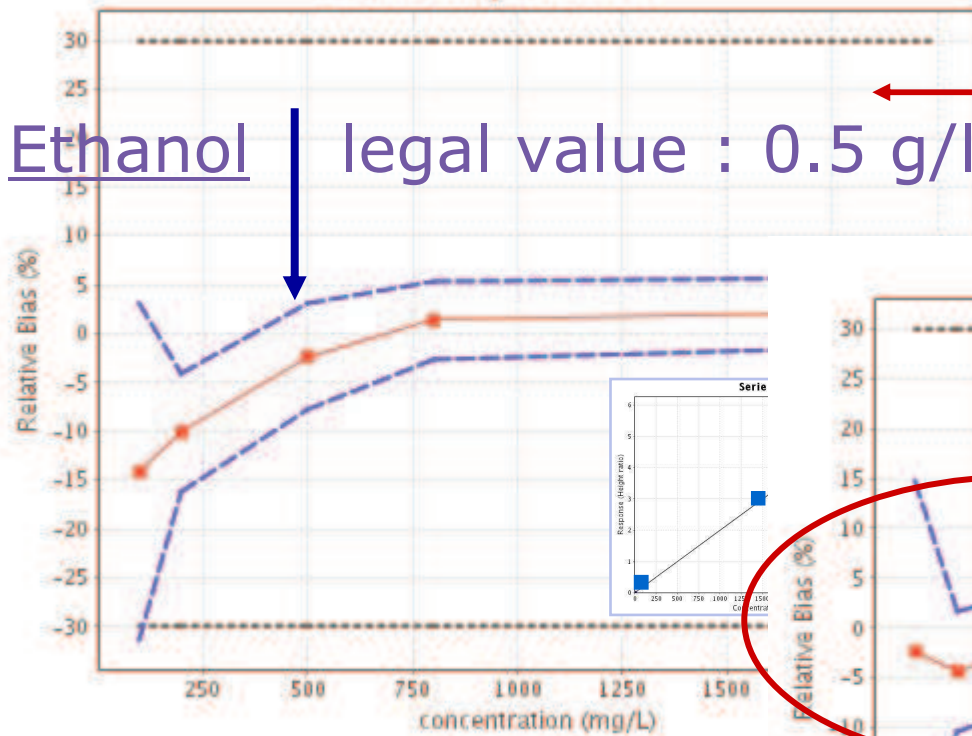
Validation : Example 1

Determination of ethanol, in plasma by Head-space/GC/FID

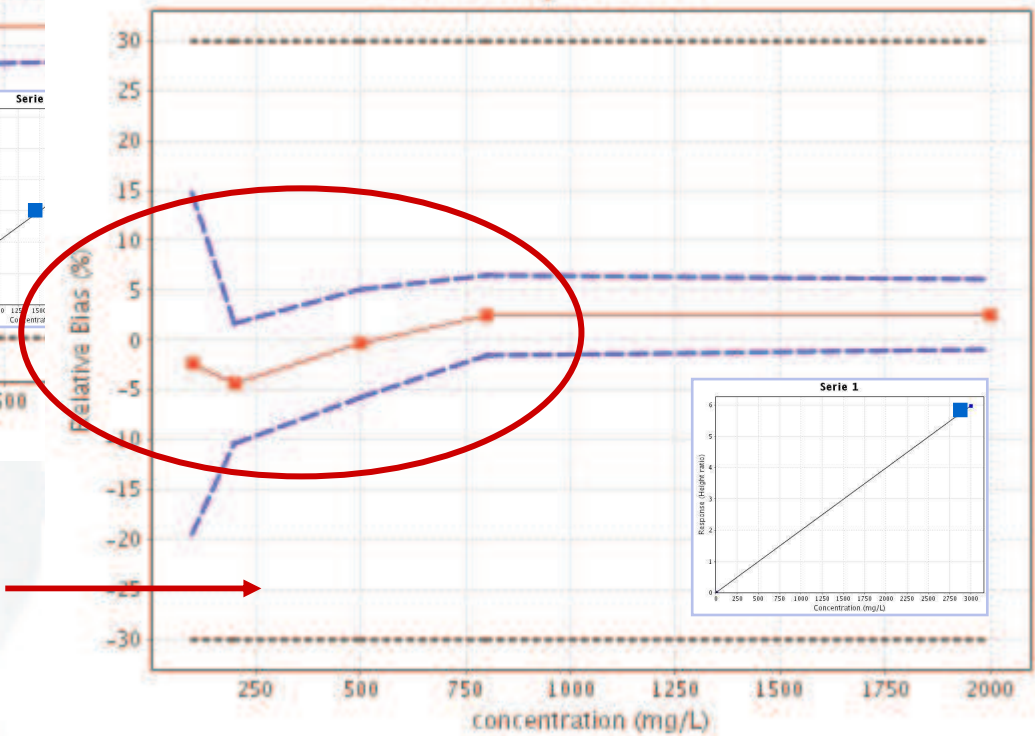


Validation : Example 1

Accuracy Profile



Accuracy Profile



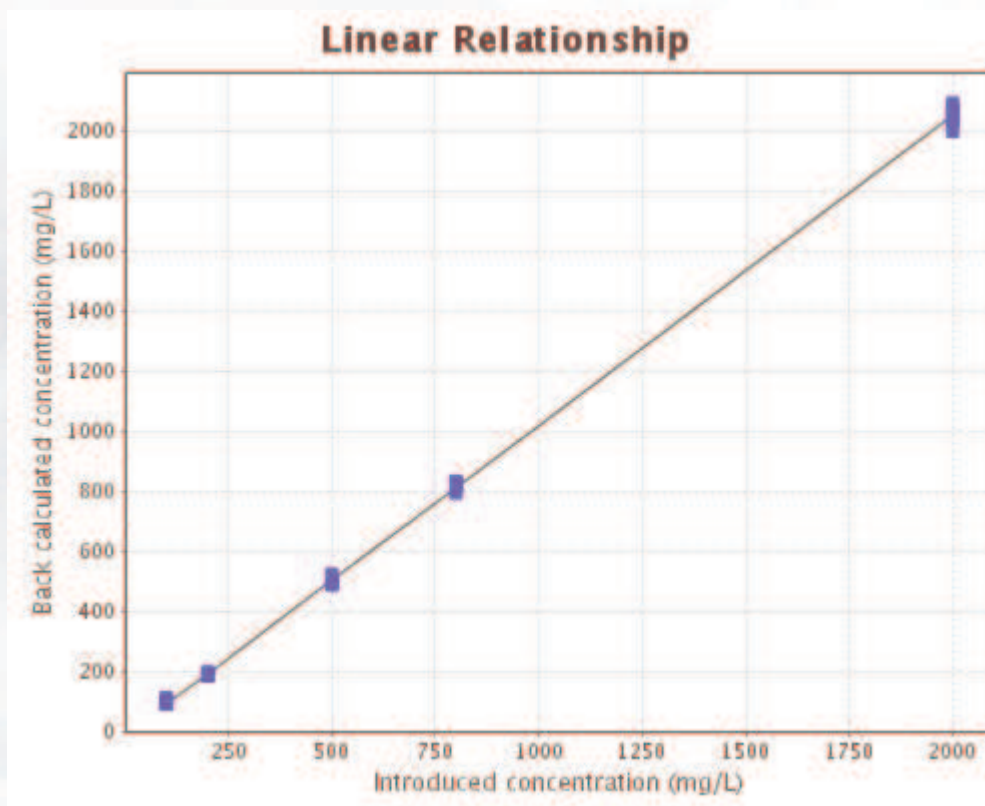
Validation : Example 1

Trueness

Concentration level (mg/L)	Mean introduced concentration (mg/L)	Mean back-calculated concentration (mg/L)	Absolute bias (mg/L)	Relative bias (%)	Recovery (%)
Precision					
Accuracy					
100.0	100.0	97.67	-2.332	-2.332	97.67
200.0	200.0	191.2	-8.8	-4.4	95.6
300.0	300.0	298.5	-1.5	-0.5	99.5
400.0	400.0	380.58	-19.42	-4.855	95.145
500.0	500.0	471.1	-28.9	-5.78	91.4
1000.0	1000.0	980.58	-19.42	-1.942	98.058
2000.0	2000.0	1981	-19	-0.95	99.05
3000.0	3000.0	2851.7	-148.3	-4.943	98.35
4000.0	4000.0	3981	-19	-0.475	99.525
5000.0	5000.0	4981	-19	-0.38	99.62
10000.0	10000.0	9810	-190	-1.9	98.1
20000.0	20000.0	19810	-190	-0.95	99.05
30000.0	30000.0	29810	-190	-0.633	99.367
40000.0	40000.0	39810	-190	-0.475	99.525
50000.0	50000.0	49810	-190	-0.38	99.62
100000.0	100000.0	98100	-1900	-1.9	98.1
200000.0	200000.0	198100	-1900	-0.95	99.05
300000.0	300000.0	298100	-1900	-0.633	99.367
400000.0	400000.0	398100	-1900	-0.475	99.525
500000.0	500000.0	498100	-1900	-0.38	99.62
1000000.0	1000000.0	981000	-19000	-1.9	98.1
2000000.0	2000000.0	1981000	-19000	-0.95	99.05
3000000.0	3000000.0	2981000	-19000	-0.633	99.367
4000000.0	4000000.0	3981000	-19000	-0.475	99.525
5000000.0	5000000.0	4981000	-19000	-0.38	99.62
10000000.0	10000000.0	9810000	-190000	-1.9	98.1
20000000.0	20000000.0	19810000	-190000	-0.95	99.05
30000000.0	30000000.0	29810000	-190000	-0.633	99.367
40000000.0	40000000.0	39810000	-190000	-0.475	99.525
50000000.0	50000000.0	49810000	-190000	-0.38	99.62
100000000.0	100000000.0	98100000	-1900000	-1.9	98.1
200000000.0	200000000.0	198100000	-1900000	-0.95	99.05
300000000.0	300000000.0	298100000	-1900000	-0.633	99.367
400000000.0	400000000.0	398100000	-1900000	-0.475	99.525
500000000.0	500000000.0	498100000	-1900000	-0.38	99.62
1000000000.0	1000000000.0	981000000	-19000000	-1.9	98.1
2000000000.0	2000000000.0	1981000000	-19000000	-0.95	99.05
3000000000.0	3000000000.0	2981000000	-19000000	-0.633	99.367
4000000000.0	4000000000.0	3981000000	-19000000	-0.475	99.525
5000000000.0	5000000000.0	4981000000	-19000000	-0.38	99.62
10000000000.0	10000000000.0	9810000000	-190000000	-1.9	98.1
20000000000.0	20000000000.0	19810000000	-190000000	-0.95	99.05
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5000000000000000.0	5000000000000000.0	4981000000000000	-19000000000000	-0.38	99.62
10000000000000000.0	10000000000000000.0	9810000000000000	-190000000000000	-1.9	98.1
20000000000000000.0	20000000000000000.0	19810000000000000	-190000000000000	-0.95	99.05
30000000000000000.0	30000000000000000.0	29810000000000000	-190000000000000	-0.633	99.367
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300000000000000000000.0	300000000000000000000.0	298100000000000000000	-1900000000000000000	-0.633	99.367
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1000000000000000000000.0	1000000000000000000000.0	981000000000000000000	-19000000000000000000	-1.9	98.1
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400000000000000000000000.0	400000000000000000000000.0	398100000000000000000000	-1900000000000000000000	-0.475	99.525
500000000000000000000000.0	500000000000000000000000.0	498100000000000000000000	-1900000000000000000000	-0.38	99.62
1000000000000000000000000.0	1000000000000000000000000.0	981000000000000000000000	-19000000000000000000000	-1.9	98.1</

Validation : Exemple 1

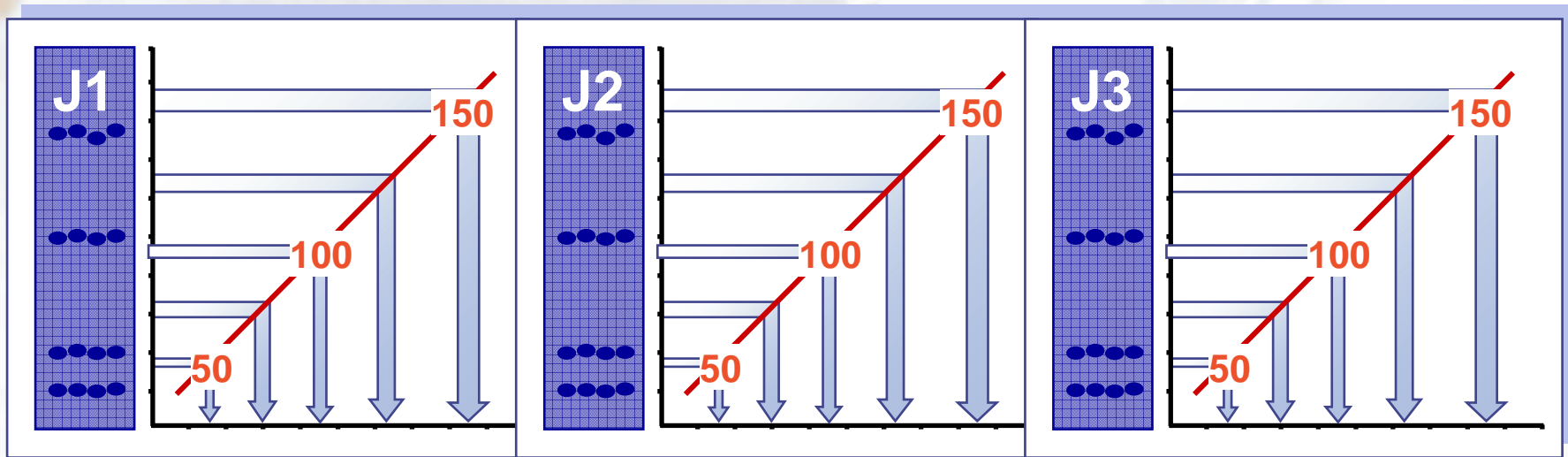
Linearity



$$Y = -10.65 + 1.031 X \quad r^2 = 0.9995$$

Example 2

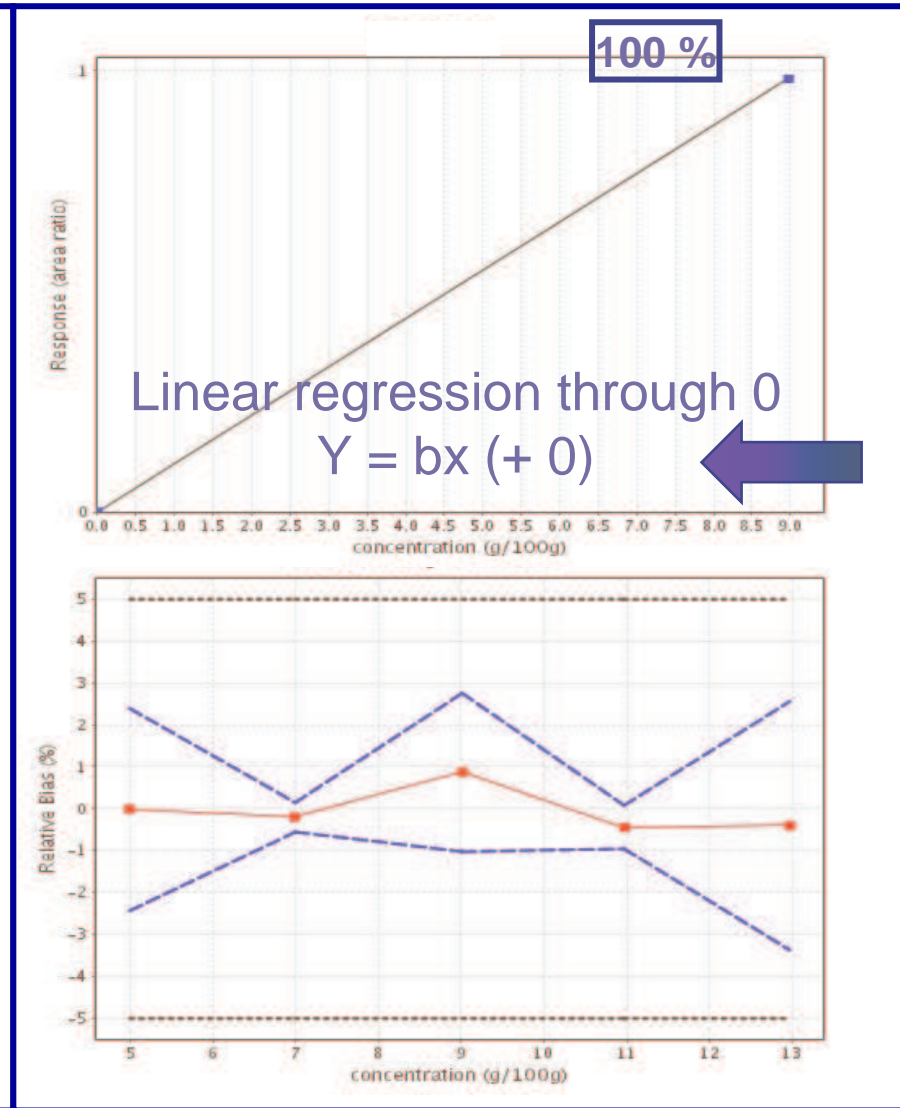
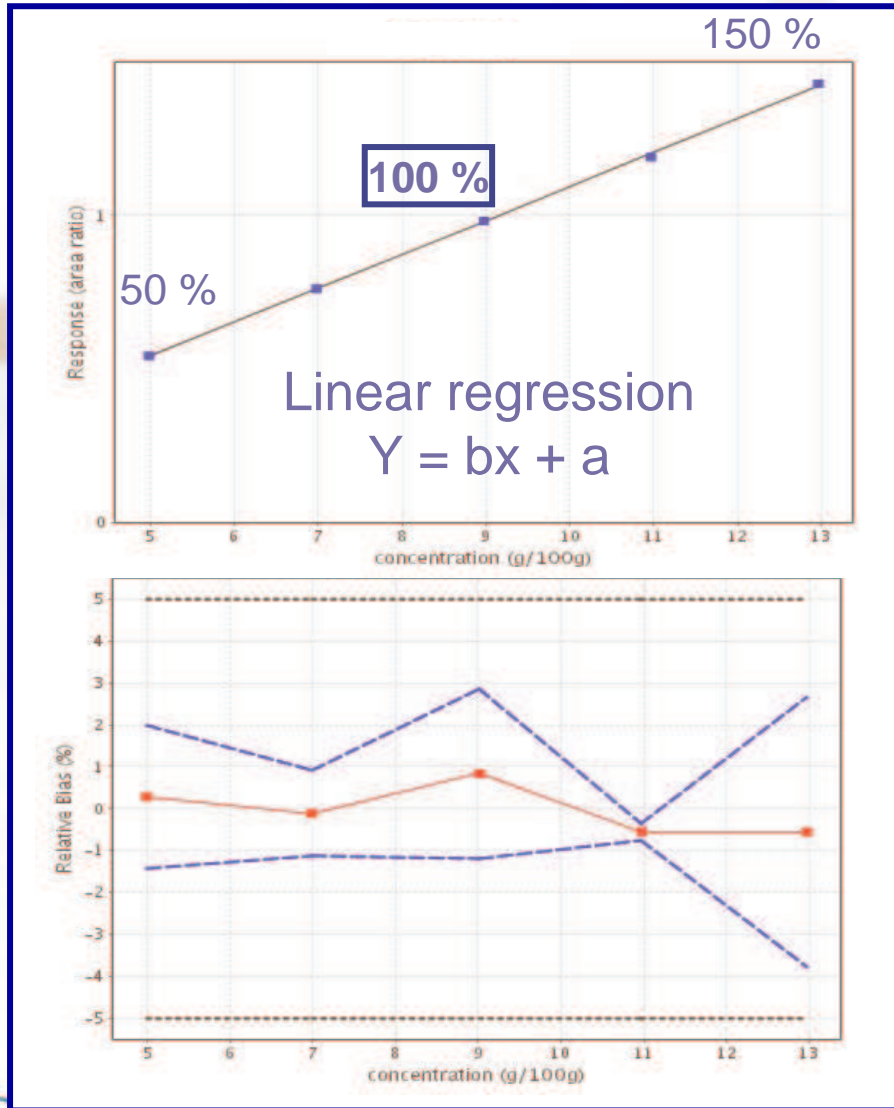
Determination of cineol in an ointment by gaz chromatography after LLE



50 Calibration standards

● Validation standards

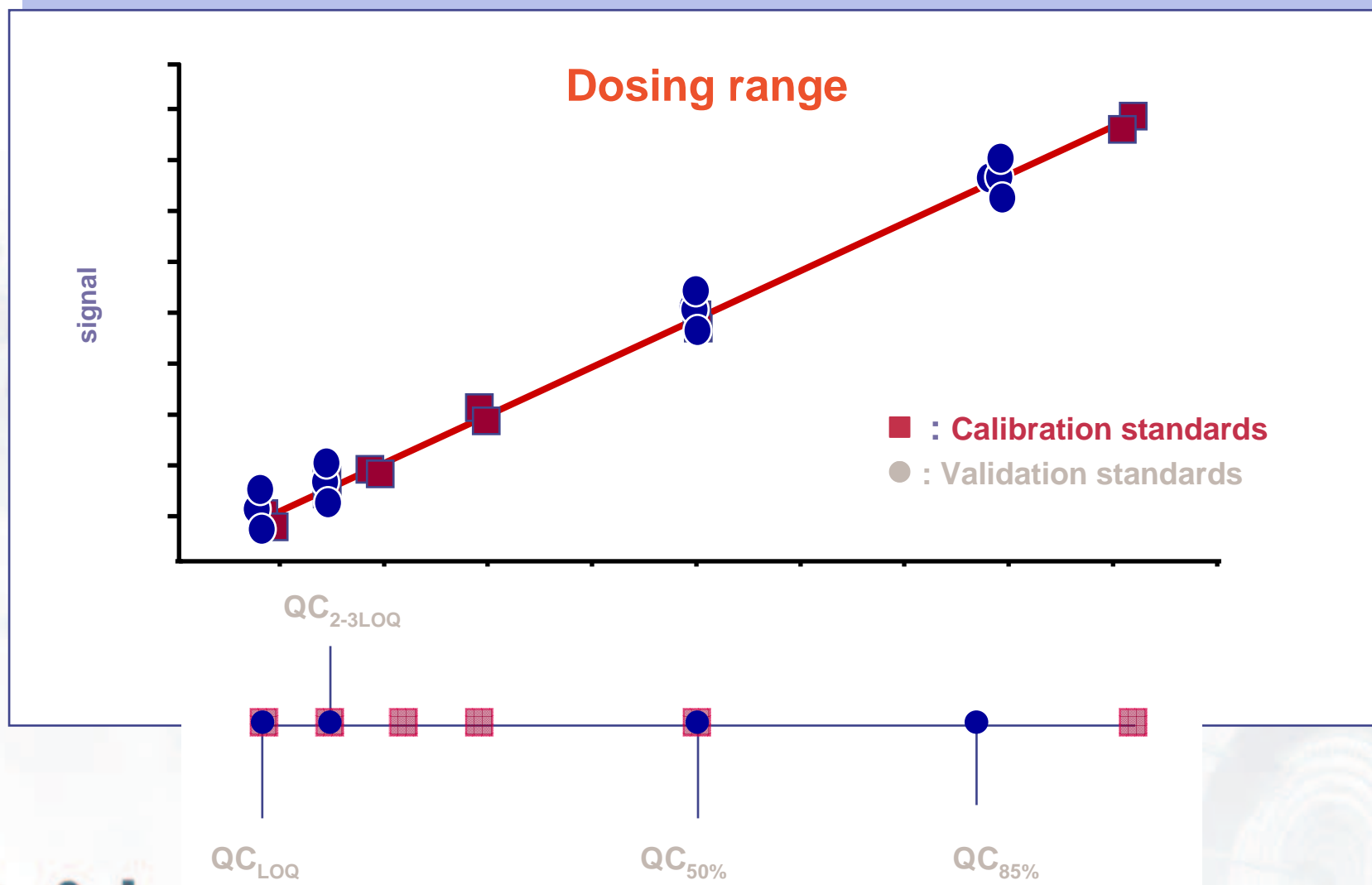
Example 2



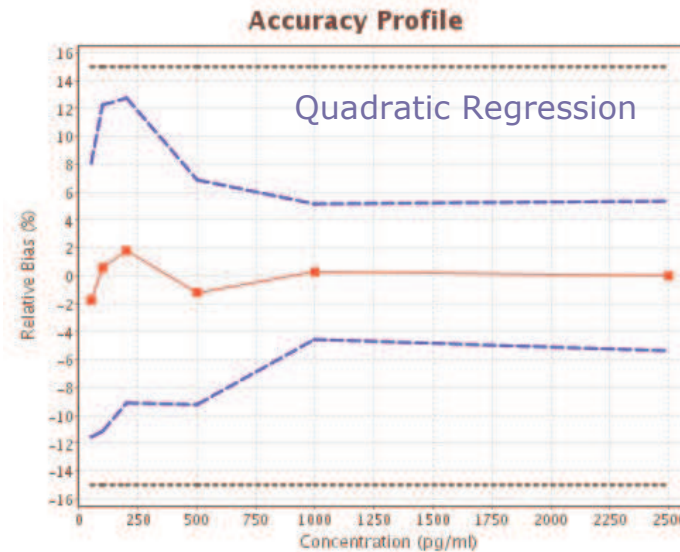
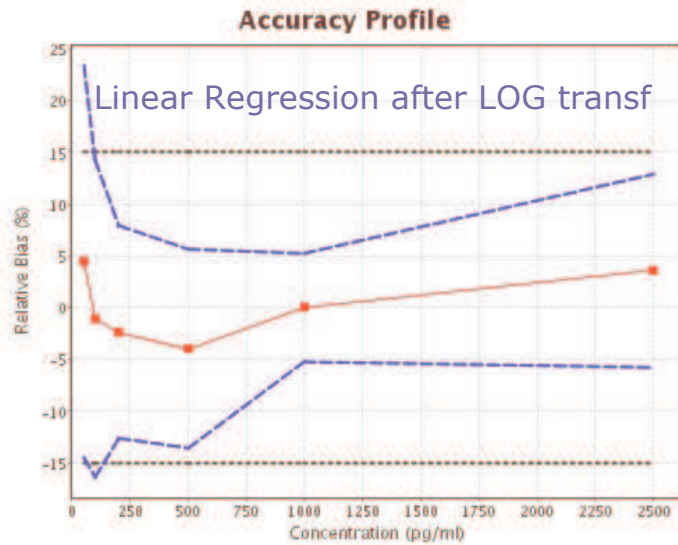
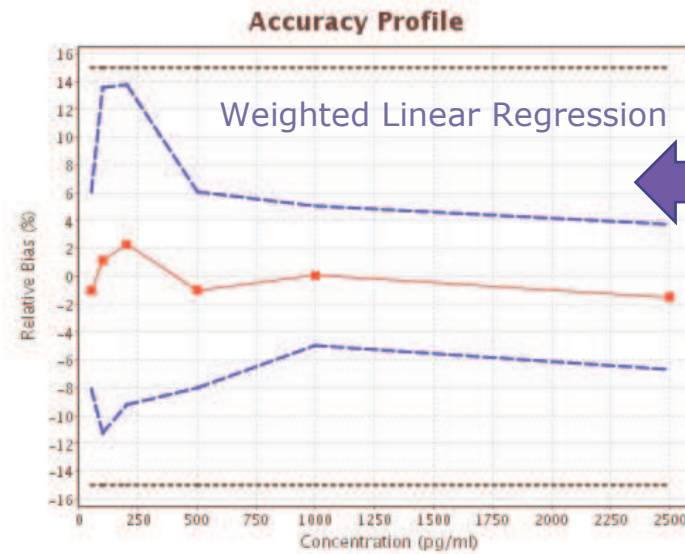
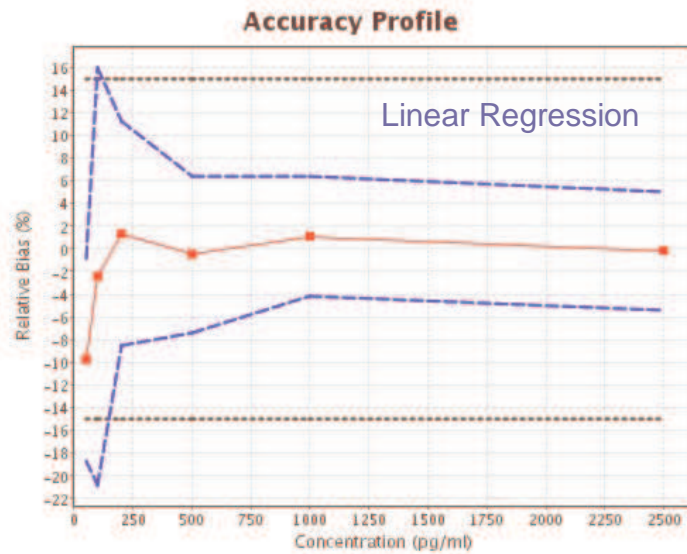
Example 3

Determination of loperamide in human plasma by liquid chromatography coupled to tandem mass spectrometry

Example 3



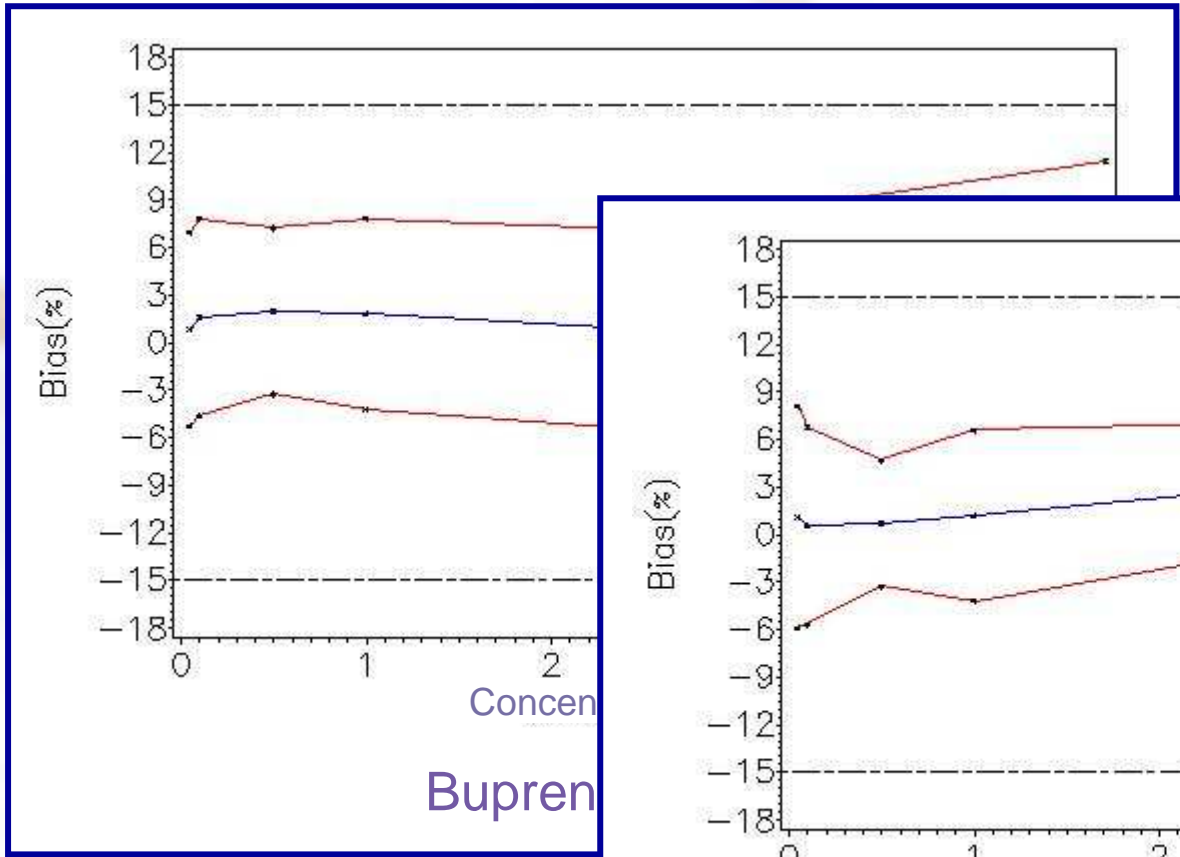
Example 3



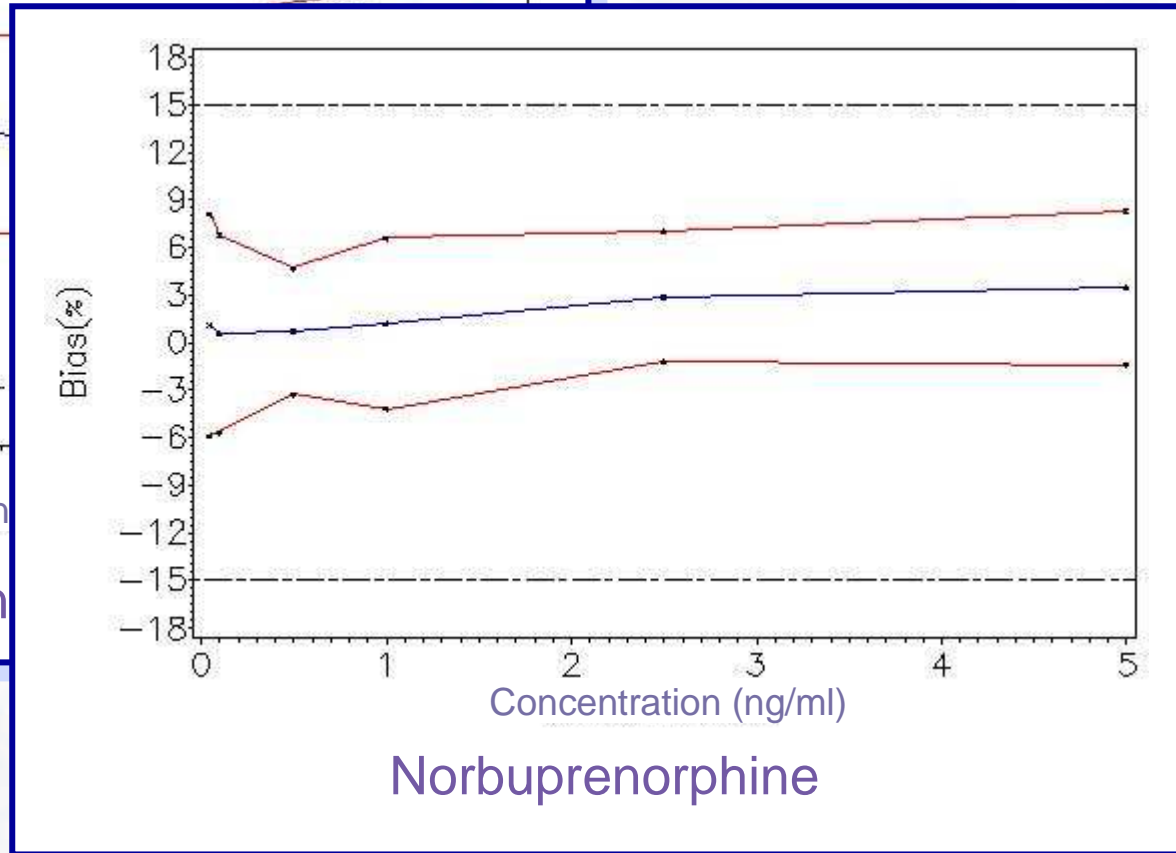
Example 4 : Quality control

Determination of buprenorphine and its N-dealkylated metabolite norbuprenorphine in human plasma by liquid chromatography coupled to tandem mass spectrometry

Example 4 : Quality control



Buprenorphine



Norbuprenorphine

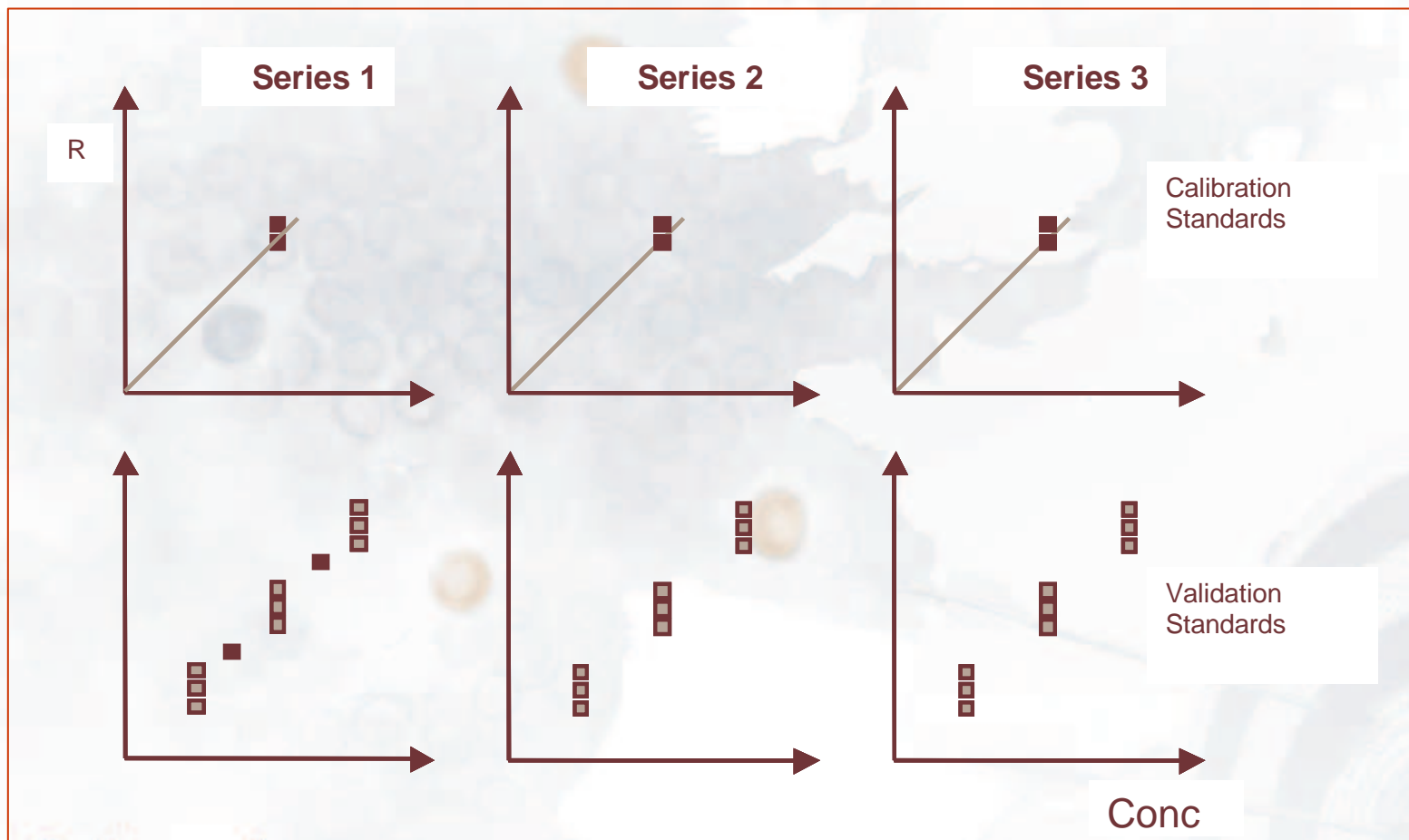
Agenda

1. Objective of analytical Method and objective of validation
2. Validation Criteria in regulatory documents
 1. Pharmaceutical industry
 2. Other areas
3. The only statistics you need: make simple
4. Practically, from the experiments to the report.
5. Demonstration
 1. E-noval
 2. Seelva
6. Conclusion

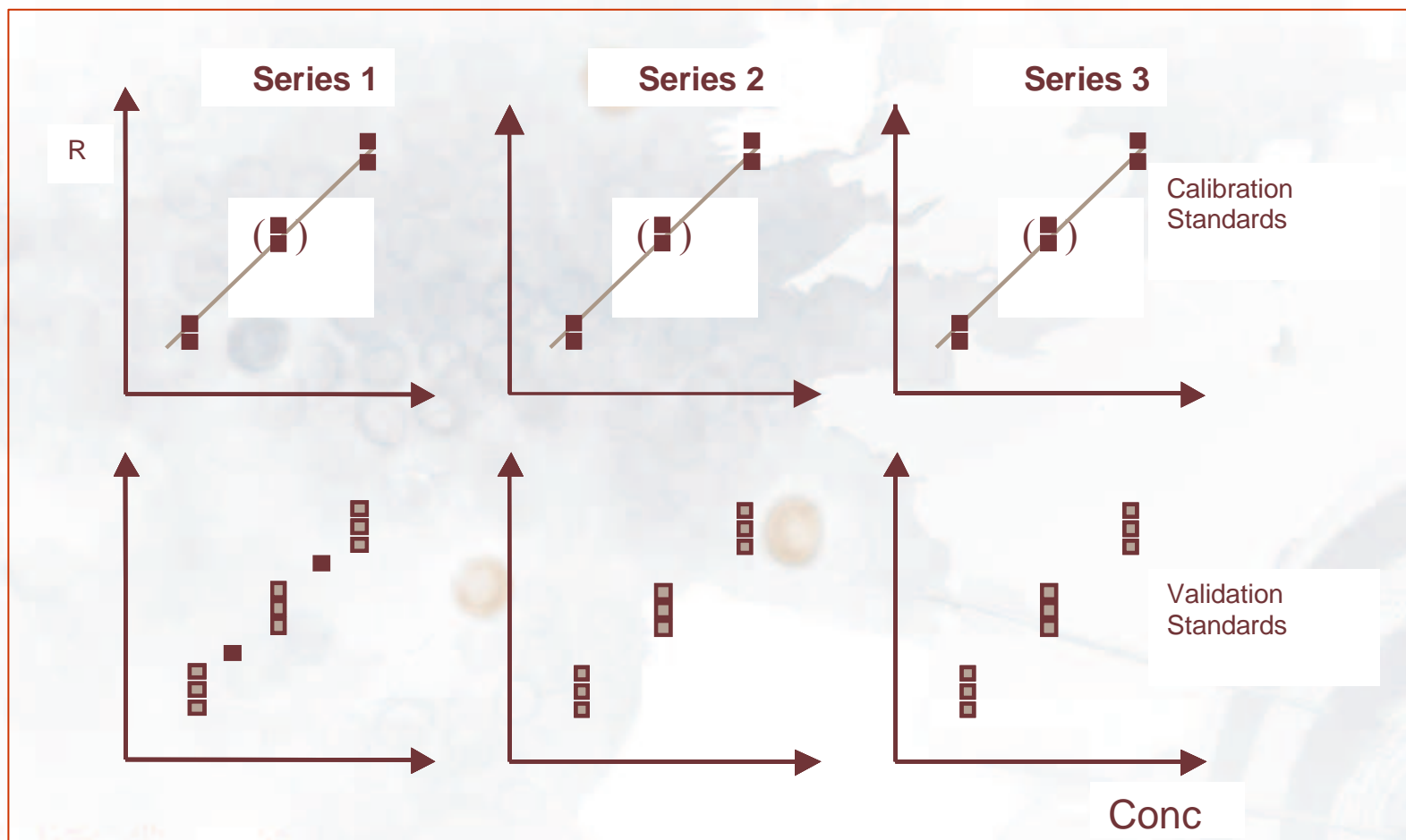
Design for criteria assessment

- Golden rule 1: “All in One” design is recommended.
 - Statistically more powerful
 - Less expensive, shorter in time
 - Use nested designs (runs, reps per runs)
 - All criteria assessed in one experiment
- Golden rule 2: “simulate” routine practice.
 - Factors used should reflect routine practice
 - Calibration & levels as in routine
 - Same sources of variation

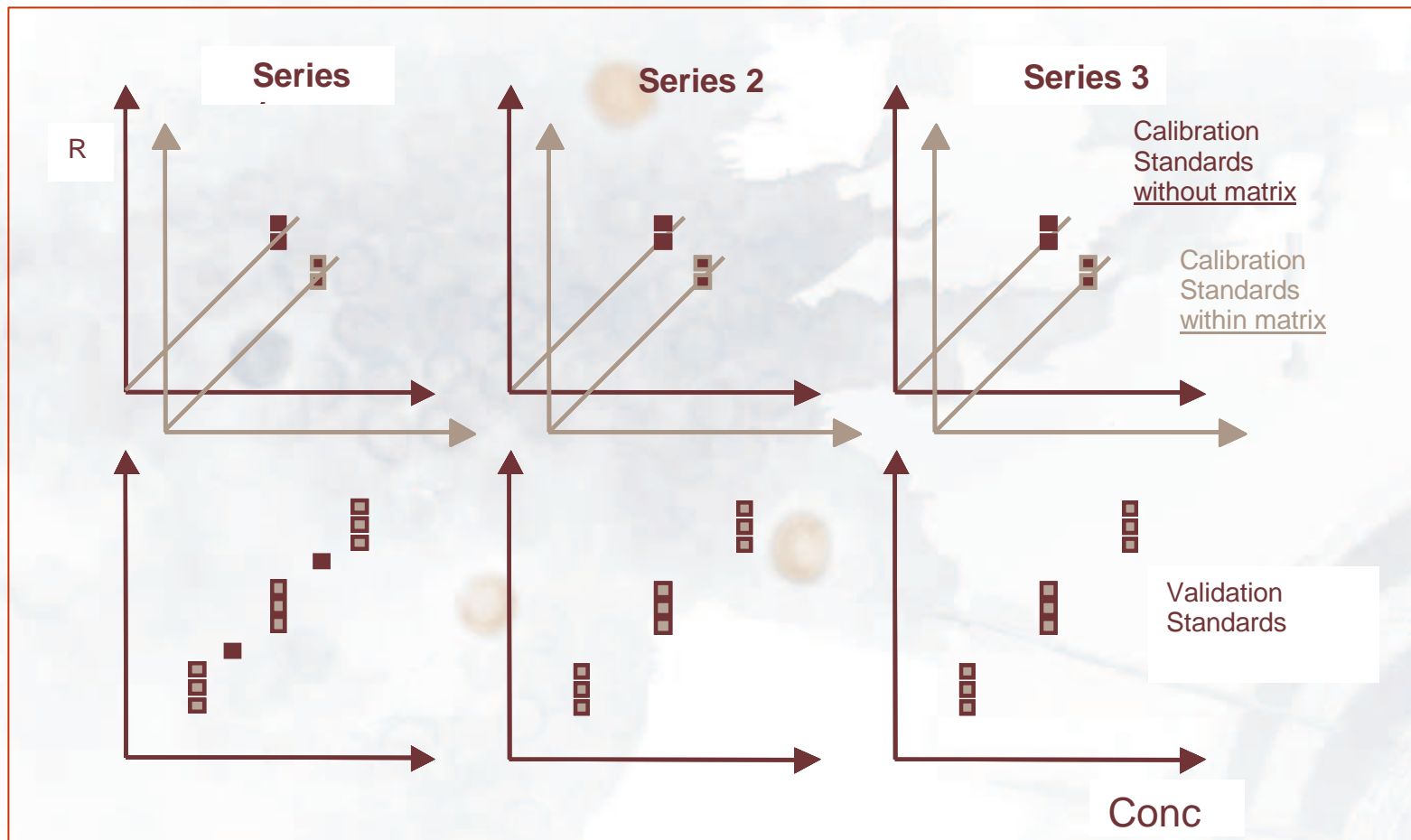
“All in One” design



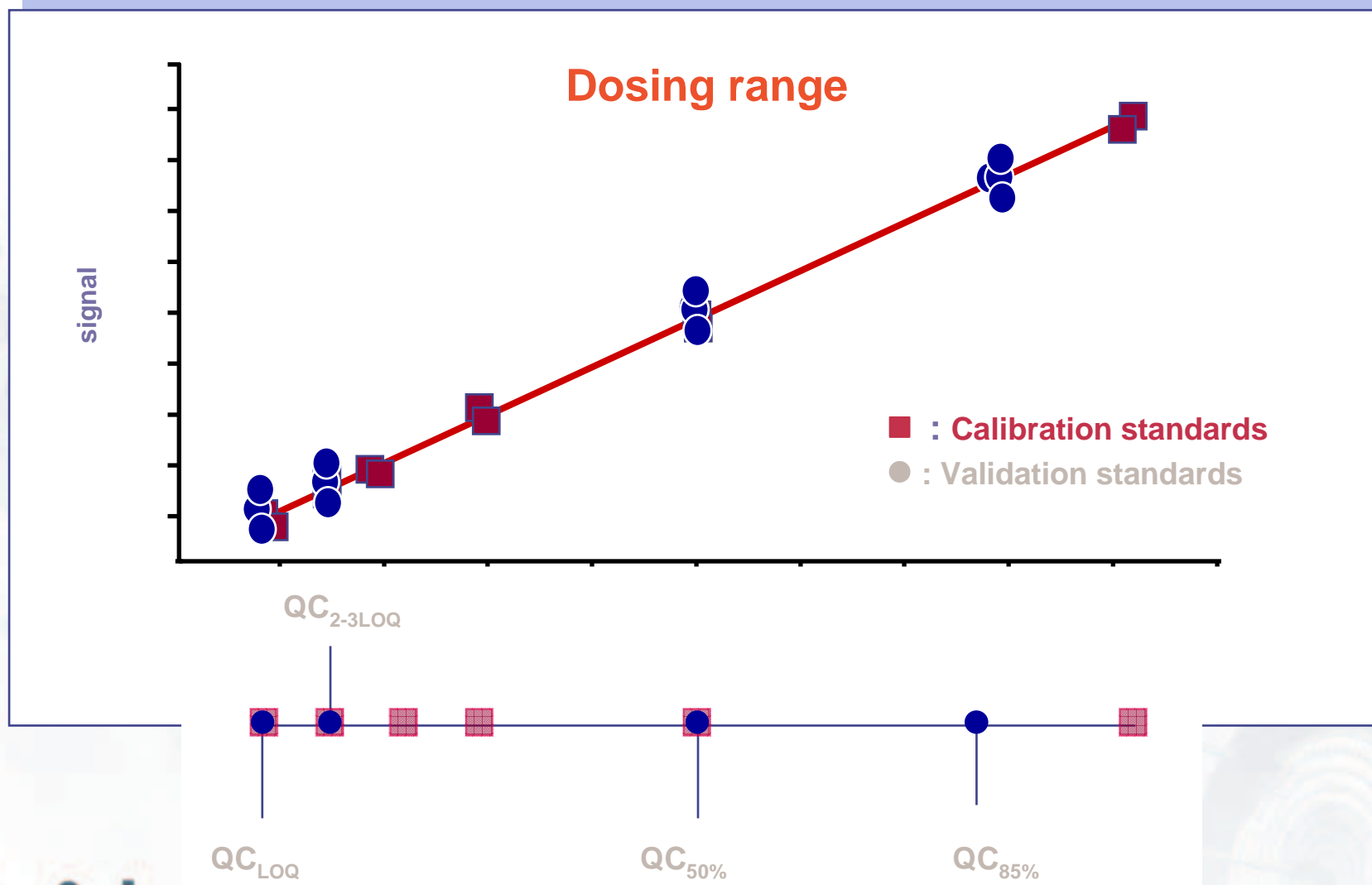
“All in One” design



“All in One” design



“All in One” design



“All in One” design, Minimum sample size

		PROTOCOL				
Standards	Concentration Levels	V1	V2	V3	V4	V5
CS. Calibration out Matrix	Low		2		2	
	Mid	2	(2)	2	(2)	
	High	(2)	2	(2)	2	
CS. Calibration in Matrix	Low				2	2
	Mid			2	(2)	(2)
	High			(2)	2	2
	Additional					(2)
VS. Validation In matrix	Low	3	3	3	3	3
	Mid	3	3	3	3	3
	High	3	3	3	3	3
Minimum Number of series		3	3	3	3	3
Total Number of experiments (minimum)		33	45	39	63	45

“All in One” design, Nested Design

	<u>SERIES</u>					
	1	2	3	4	5	6
Day	1	1	2	2	3	3
Operator	A	B	A	B	A	B

Example of experimental conditions for estimating the intermediate precision.

=> The routine sources of variation should be varied from one series to the other using adequate DOE.

“All in One” design

Nested Design Optimal Sample Size

%RSD W	1%		2%		3%		4%		5%	
	I	J	I	J	I	J	I	J	I	J
1%	4	3	4	3	4	3	4	4	5	9
	5	3	5	3	5	3	5	4	6	7
2%	4	3	4	3	4	3	4	6	8	9
	5	3	5	3	5	3	5	6	9	7
3%	4	4	4	6	5	6	7	10	--	
	5	3	5	3	6	5	8	9		
4%	7	10	9	8	--		--		--	
	8	7	10	6						

Table showing the minimal sample size, i.e. number of runs (I) and replicates per run (J) required when dealing with acceptance limits [-10%, +10%] for various a priori values of within-run (%RSD W) and between-run variances (%RSD B). This table has been computed by simulation, assuming a potential small bias of 2%.

The results

1

Calibration Standards

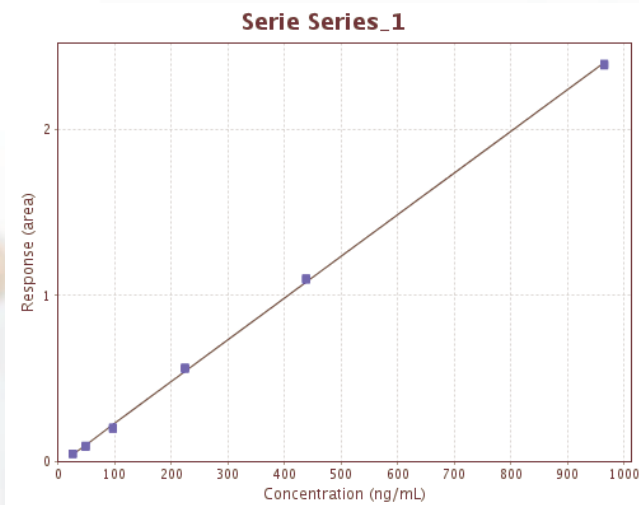
Conc.	Serie1	Serie2	Serie3
25,3533	0,0485	0,0358	0,0449
25,3533	0,0448	0,0402	0,0415
48,2417	0,0959	0,1025	0,0987
48,2417	0,0870	0,0993	0,0892
96,4833	0,1974	0,2046	0,2036
96,4833	0,2057	0,1996	0,2082
223,8496	0,5589	0,5371	0,5095
223,8496	0,5667	0,5066	0,5756
437,8235	1,1041	0,9963	1,1725
437,8235	1,0961	1,0568	1,1772
964,8333	2,3960	2,2877	2,4528
964,8333	2,3861	2,2500	2,3147

Validation Standards

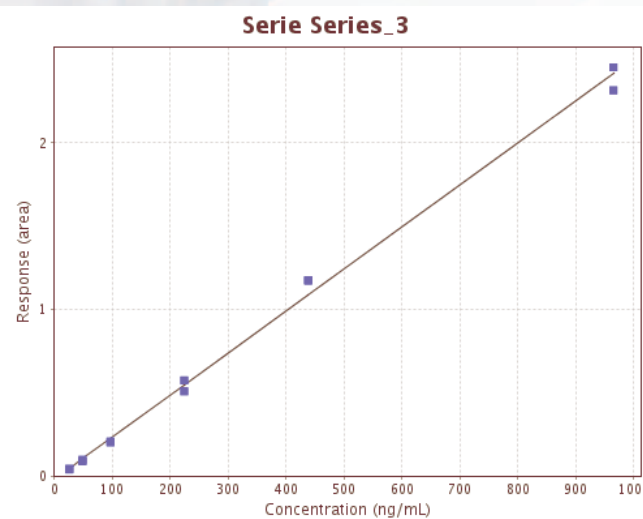
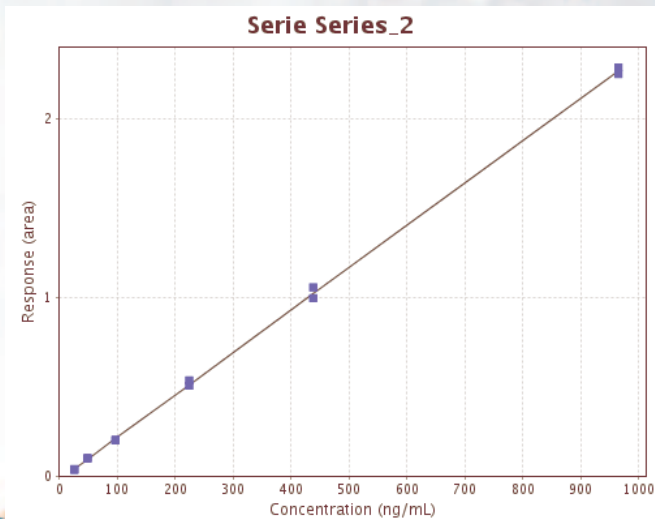
Conc.	Serie1	Serie2	Serie3
25,3533	0,0439	0,0371	0,0444
25,3533	0,0488	0,0422	0,0457
25,3533	0,0480	0,0461	0,0502
25,3533	0,0484	0,0448	0,0474
48,2417	0,0949	0,0922	0,0956
48,2417	0,0926	0,0916	0,1023
48,2417	0,0887	0,0854	0,1007
48,2417	0,1015	0,0918	0,1092
437,8235	0,9873	0,9718	1,0392
437,8235	1,0136	1,0322	1,1132
437,8235	1,0288	1,0342	1,1419
437,8235	1,0173	1,0319	1,0751
838,6479	2,0220	1,9252	2,1271
838,6479	1,9901	2,0284	2,2127
838,6479	2,0937	2,0127	2,2699
838,6479	2,0189	2,0273	2,2546

Response Function Fit by Series

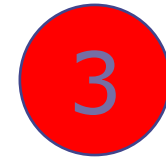
2



- Fit one model per series
- Replicates per series are required
- Graphically represent the standard curves



Response Function Parameters estimates



Model fitted $Y = a + bX + \varepsilon$

	Intercept	Slope	r^2	Residual d.f.
Series_1	-1.932E-02	2.510E-03	0.9996	10
Series_2	-1.758E-02	2.373E-03	0.9995	10
Series_3	-1.386E-02	2.520E-03	0.9963	10

r^2 = coefficient of determination; d.f. = degrees of freedom

Other Models can be envisaged

Model type	Equation	Parameters
Through the origin	$Y = \beta X$	β
Linear	$Y = \alpha + \beta X$	α, β
Quadratic	$Y = \alpha + \beta X + \gamma X^2$	α, β, γ

Response Function Transformations

- Transformations of the data (X & Y) often improve the quality of the results.
- Logarithmic (Base 10) and Square Root are most often used
- Permitted by regulatory documents
- Only with Linear Model!

Other Linear models

$$\text{Log(Ratio)} = \alpha + \beta \text{Log(Concentration)}$$

$$\sqrt{\text{Ratio}} = \alpha + \beta \sqrt{\text{Concentration}}$$

Response Function Weighting

- The weighting of the data often improve the quality of the results.
- Recommended strategy: weighting as a function of the X (conc., Amount)
- Permitted by regulatory documents

Examples of classical weights

$$W_i = \frac{1}{X}, \quad W_i = \frac{1}{X^2}, \quad W_i = \frac{1}{X^{-3}}$$

Response Function Models available

Some classical Linear Models are available

1. Linear regression through 0 using highest Level
2. Linear regression through 0 using a specified level
3. Linear regression
4. Weighted linear regression
5. Linear regression after (base 10) LOGARITHM transformation of both concentration and response
6. Linear regression after SQUARE ROOT transformation of both concentration and response
7. Quadratic regression
8. Weighted quadratic regression

Back Calculate the Results

- After a model has been fitted to the data by series. Keep the same model!
- Calculate the back calculate Concentrations/amounts using the inverse relation.
- Using the estimated parameters.

Compute the Trueness

- By **Level** of concentration/amount compute the average concentration /amount back calculated.

$$\hat{\mu}_j = \frac{1}{\sum_{i=1}^p n_{ij}} \sum_{i=1}^p \sum_{k=1}^{n_{ij}} x_{ijk,calc}$$

- Compute the various Trueness representations

$$biais_j = \hat{\mu}_j - \bar{x}_{.j.}$$

$$biais(\%)_j = 100 \times \frac{\hat{\mu}_j - \bar{x}_{.j.}}{\bar{x}_{.j.}}$$

$$Recovery(\%)_j = 100 \times \frac{\hat{\mu}_j}{\bar{x}_{.j.}}$$

Trueness - Summary

Concentration level (ng/mL)	Mean introduced concentration (ng/mL)	Mean back-calculated concentration (ng/mL)	Absolute bias (ng/mL)	Relative bias (%)	Recovery (%)
25.4	25.35	25.33	-2.016E-02	-7.950E-02	99.92
48.2	48.24	45.57	-2.669	-5.533	94.47
437.8	437.8	428.6	-9.192	-2.099	97.90
838.6	838.6	850.4	11.77	1.404	101.4

Precision - Summary

- Repeatability Variance:
- Intermediate Precision Variance:

$$\hat{\sigma}_{W,j}^2$$

$$\hat{\sigma}_{W,j}^2 + \hat{\sigma}_{B,j}^2$$

Concentration level (ng/mL)	Mean introduced concentration (ng/mL)	Repeatability (RSD%)	Intermediate precision (RSD%)
25.4	25.35	4.897	6.352
48.2	48.24	3.701	3.701
437.8	437.8	3.035	4.414
838.6	838.6	2.563	4.622

Uncertainty and « β -expectation tolerance interval »

Uncertainty

$$u(Y)^2 = s_R^2 + u(\hat{\delta})^2$$

=

β -expectation tolerance variance

$$\hat{\sigma}_{Tol}^2 = s_M^2 + u^2(\hat{\delta})$$

So

Expanded Uncertainty U

=

Semi-Length of β -expectation tolerance Interval

Uncertainty - Summary

Concentration level (ng/mL)	Mean introduced concentration (ng/mL)	Uncertainty of the bias (ng/mL)	Uncertainty (ng/mL)	Expanded Uncertainty (ng/mL)	Relative Expanded Uncertainty (%)
25.4	25.35	0.6921	1.753	3.506	13.83
48.2	48.24	0.5154	1.858	3.716	7.704
437.8	437.8	8.966	21.31	42.61	9.732
838.6	838.6	19.63	43.45	86.90	10.36

Accuracy Summary

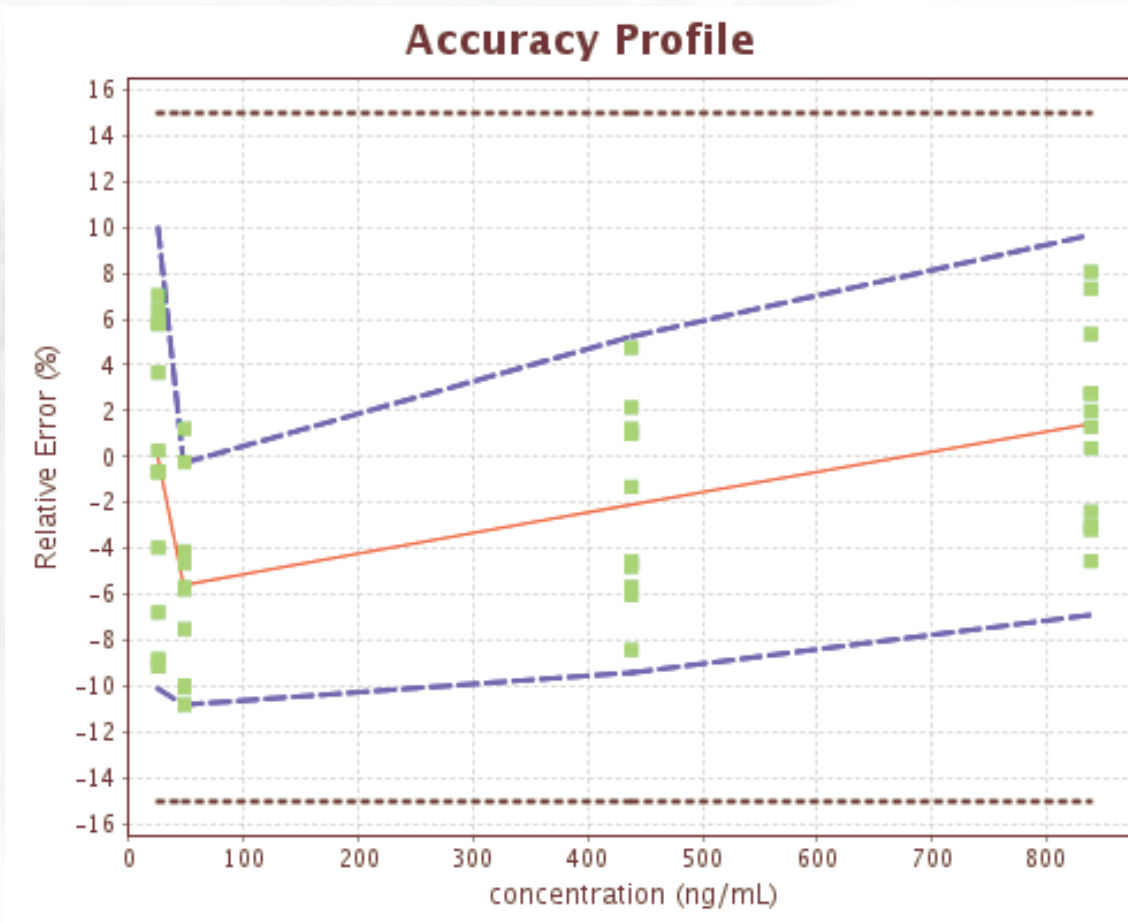
Accuracy Summary Table with Beta-expectation Confidence limits expressed:

1. In absolute Bias
2. In relative Bias.
3. By Concentration Level

Concentration level (ng/mL)	Mean introduced concentration* (ng/mL)	beta-expectation tolerance limit (ng/mL)	Relative Beta-expectation tolerance limit (%)
25.4	25.35	[22.79 , 27.87]	[-10.10 , 9.940]
48.2	48.24	[43.03 , 48.11]	[-10.80 , -0.2704]
437.8	437.8	[396.7 , 460.6]	[-9.404 , 5.205]
838.6	838.6	[781.0 , 919.8]	[-6.870 , 9.677]

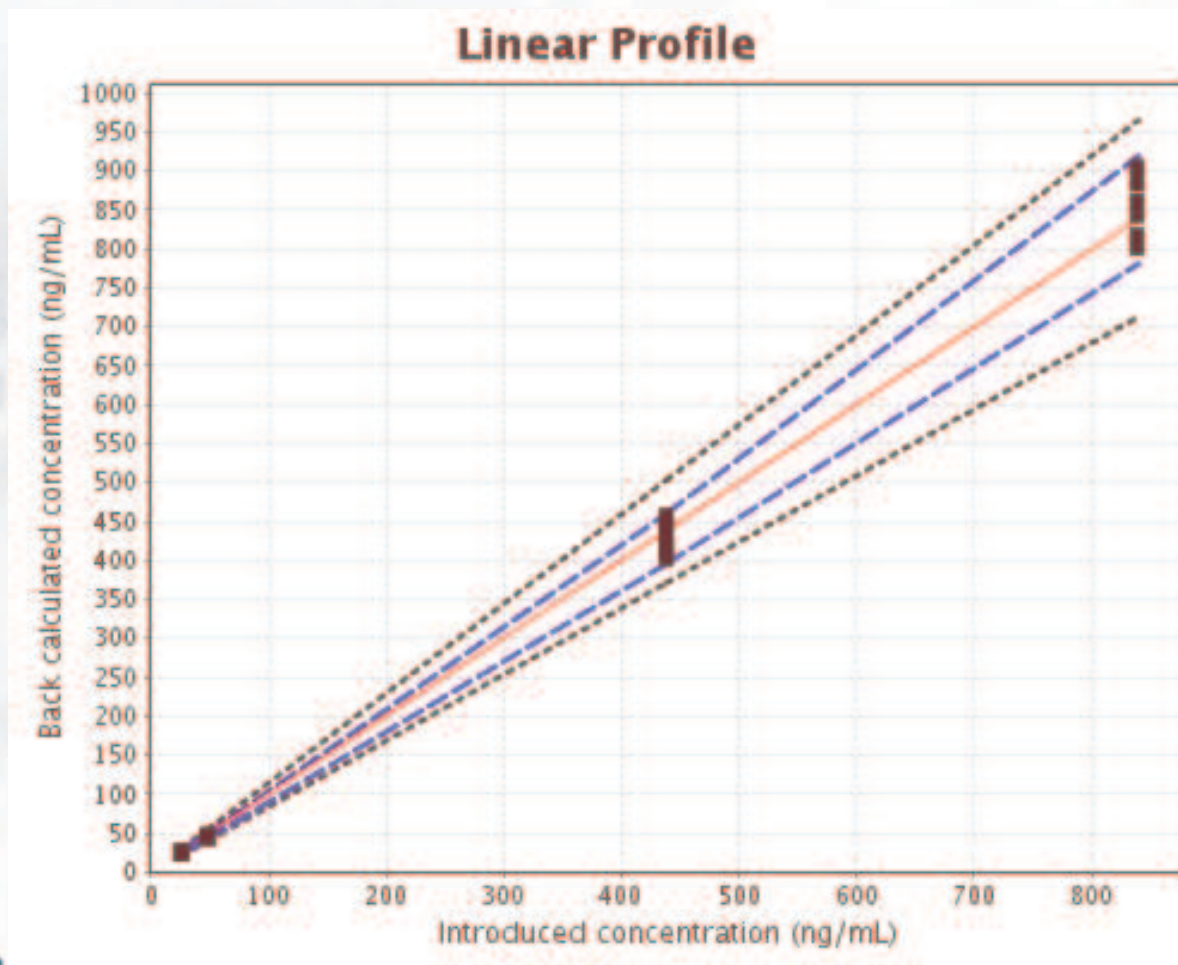
Graph the Accuracy Profile

- In Relative error



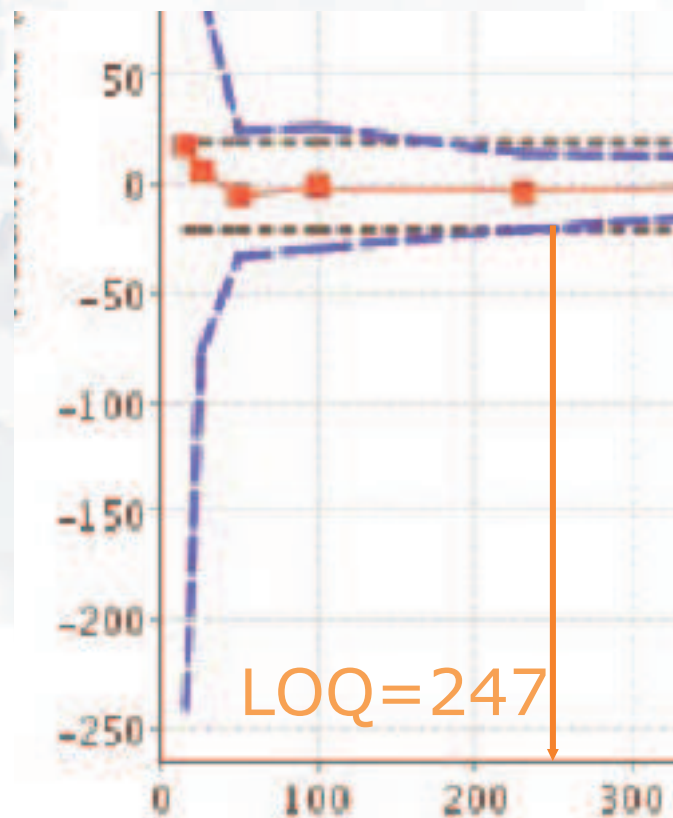
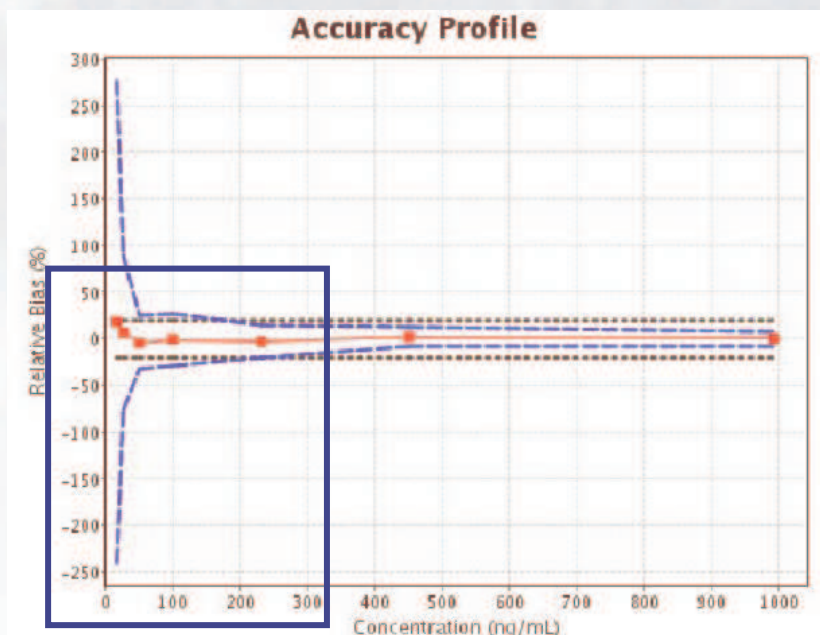
Graph the Accuracy Profile

- In Absolute value



The Limits of Quantitations

- Intersection Between Acceptance Limits and Confidence Limits

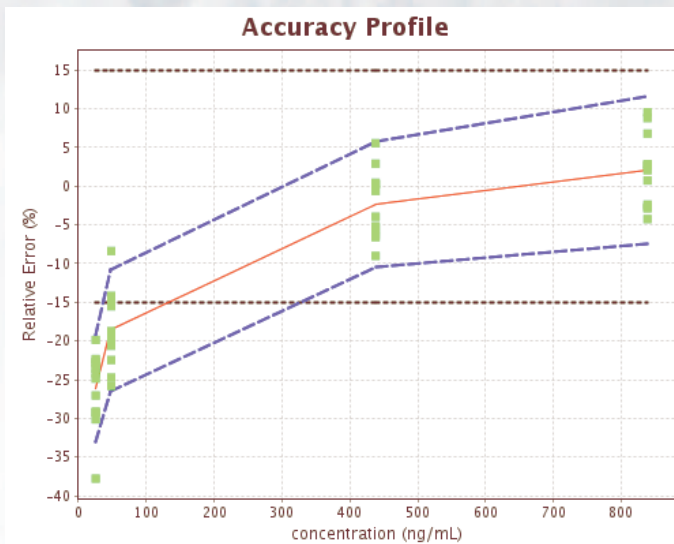


Choice of Best Calibration Model

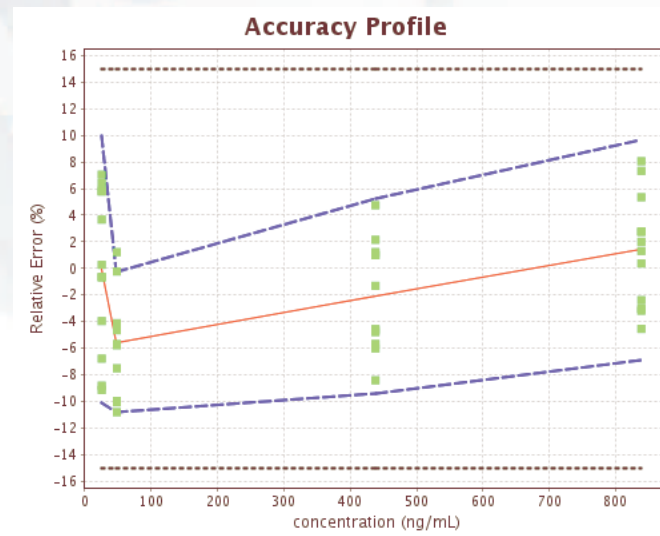
Compare the Accuracy profiles obtained by the various calibration models. The Accuracy profile reflects the behavior of the procedure with respect to the objectives.

Select the Model that gives the best results.

No Intercept Model

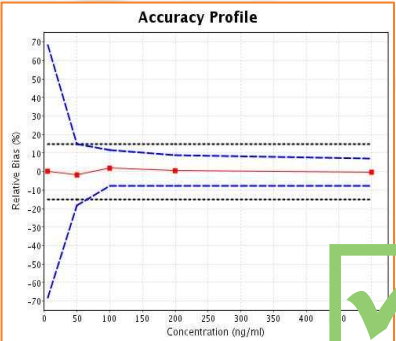
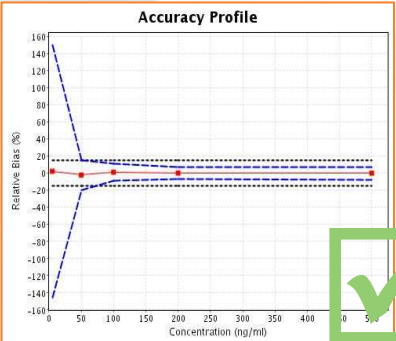
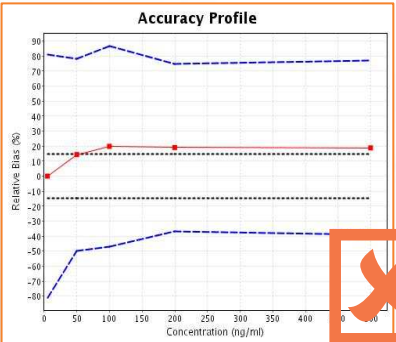


Linear Model



Decision Process

Calibration Curves

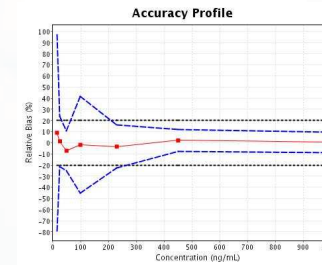
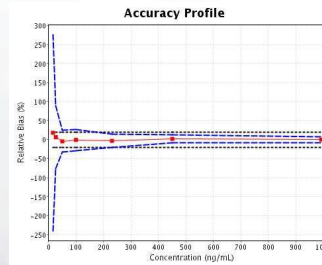


⇒ diagnostics

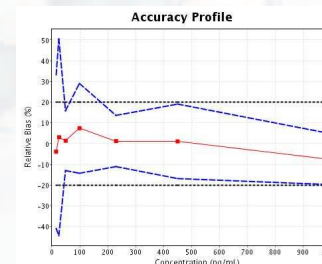
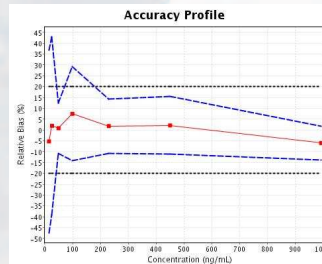
⇒ selection

Decision Process

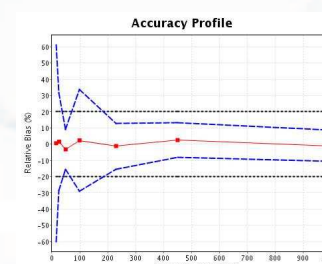
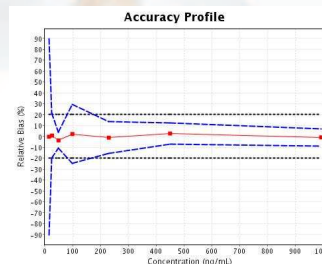
Linear



Log-Log linear



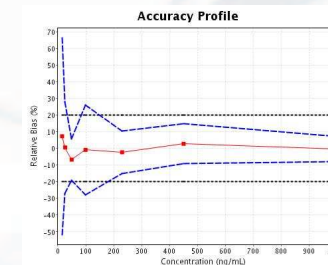
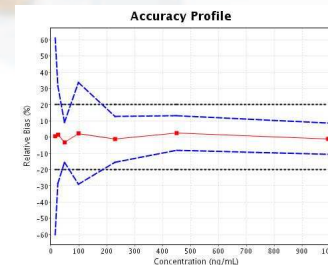
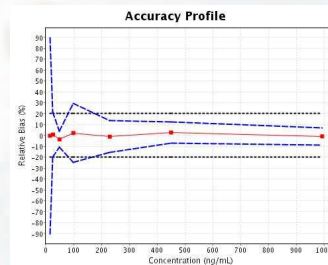
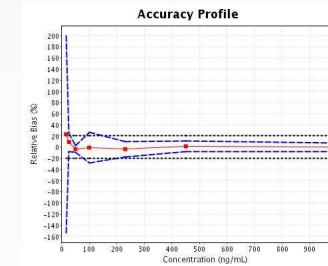
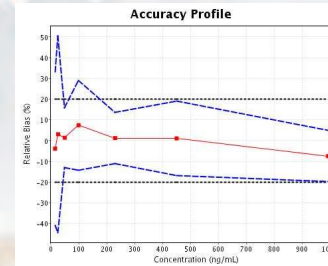
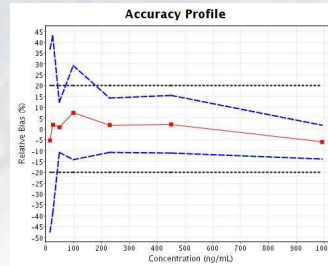
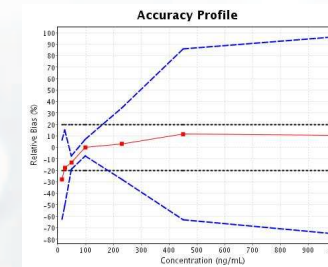
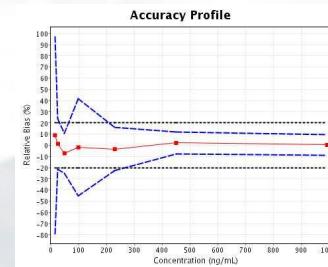
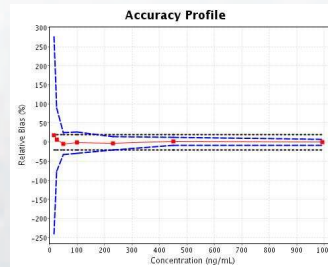
Sqrt-Sqrt Linear



Not Weighted

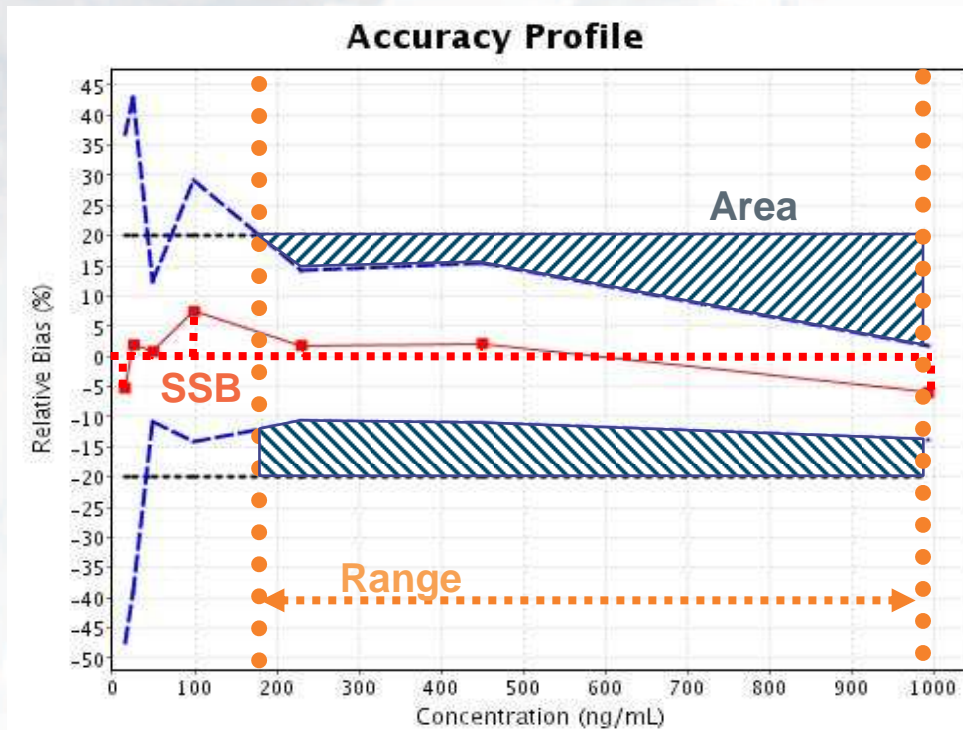
Weighted

Choice of Best Calibration Model

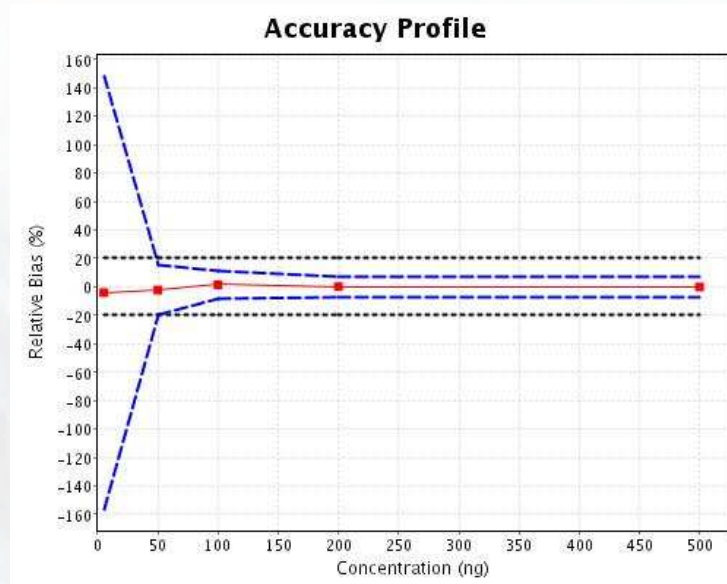


Criteria For selecting the Best Model

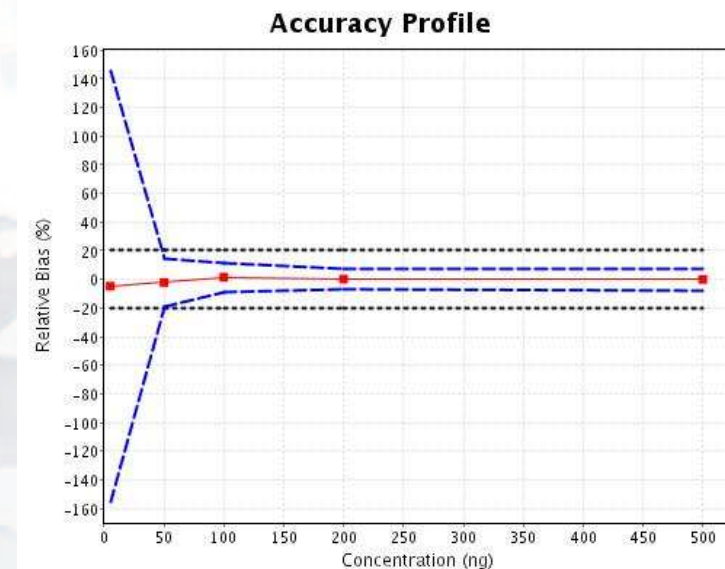
- Three Criteria to envisage:
 - Precision Area
 - Range
 - Bias (SSB)



Accuracy Index for selecting Calibration in or out of Matrix



OUT Matrix



IN Matrix

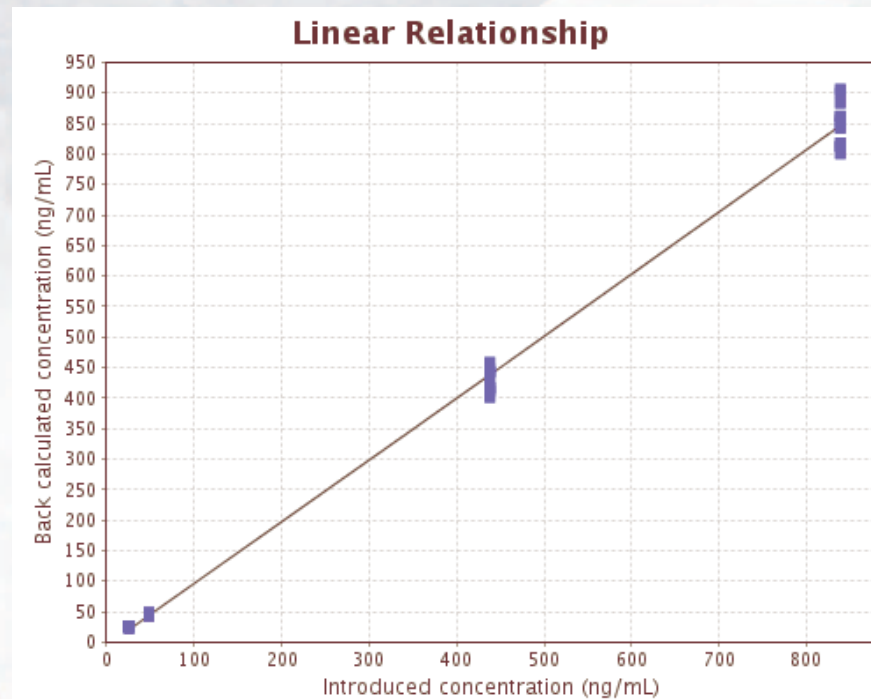
In this example there is no difference in Accuracy
=> Use the easiest method

Advantages

- Decision \Leftrightarrow Objective
- Error rate controlled by the user
- Maximum Likelihood Estimates
- LOQ estimated with accuracy profile

Linearity

- The **linearity** of an analytical method is the ability within a definite range to obtain results directly proportional to the concentration (quantity) of the analyte in the sample.
- A linear regression model is fitted on the back-calculated concentrations as a function of the introduced concentrations.



Many thanks for your attention.

And now a demonstration of our industrial solutions:



for physico-chemical methods



for ligand-binding assays