Linearity

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Outline

I) Introduction

Different types of data;

Different types of assays with regard to outputs;

II) Relationship between quantitative assays and linearity;

How linearity is related to calibration;

III) Basic scheme of demonstration of linearity;

IV) Summary

Definition

CLSI document EP06-A (2003)
LINEARITY: The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the strength (amount) of signal in the region of interest.

ISO document 18113 (2009)
A.3.21
LINEARITY
ability to provide measured quantity values that are directly proportional to the value of the measurand in the sample

Different Types of Data

Scientific classification of data (1946):
Nominal, Ordinal, Interval and Ratio.

Nominal

- Nominal refers to data such as names/categories. For example, five different genotypes. May have numbers assigned, not for arithmetic purpose.
- Easy to remember because nominal sounds like name.

Ordinal

- Ordinal refers to quantities that have an ordering – order matters but not the difference between values. For example, CHF stage I, II, III and IV.
- Easy to remember because ordinal sounds like order.

Different Types of Data

Interval

- Interval data is like ordinal except the difference between two values is meaningful. For example, temperature in Fahrenheit degrees. The difference between 29 and 30 degrees is the same magnitude as the difference 99 and 100 degrees. It is <u>not</u> meaningful to multiply or to divide values.

Ratio

- Ratio has all properties of interval and also has a clear definition of zero. When the result of quantity equals 0, there is none of that quantity. Numbers can be compared as differences; can be multiplied and divided.
- For example, amount of analyte, concentration are ratio variables.

Different Types of Assays

Based on outputs:
Qualitative, Quantitative, Semi-Quantitative

There is no consistency in the definitions (quantitative, semi-quantitative)

Qualitative Assay

- Examinations that have nominal outputs
- For example,
- Test with two outcome (negative, positive);
- Test with few nominal outcomes as genotype.

Different Types of Assays

Based on outputs:
Qualitative, Quantitative, Semi-Quantitative

Quantitative Assay
The amount or concentration of an analyte is measured and expressed as a numerical quantity value in measurements units.

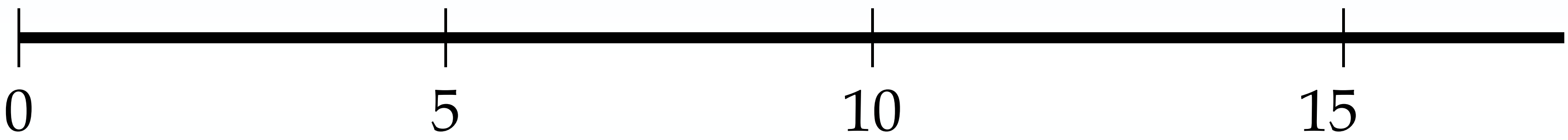
Different definitions
Narrow definition:
quantitative assay=traceable quantitative assay
Broad definition:
quantitative assay=any assay which is not qualitative

Different Types of Assays

Quantitative assay

For example, we have three samples (no random error):

- Sample1 has output of 5 units,
- Sample2 has output of 10 units, and
- Sample3 has output of 15 units.



Interpretation:

- Sample 2 has amount larger than Sample1 by 5 units and Sample 3 has amount larger than Sample 2 by 5 units (these 5 units mean the same amount of analyte);
- Sample 2 has two times more amount than Sample 1 (=10/2); Sample 3 has three times more amount than Sample 1 (=15/5),…..

Different Types of Assays

Based on outputs:
Qualitative, Quantitative, Semi-Quantitative

Semi-Quantitative Assay
No official definitions

- Tests with ordinal categories.
  For example, neg, trace, +, ++, +++
- Tests with ordinal categories and numerical values which are not ratio.
  For example, score for risk with numerical values 2-70.

Different Types of Assays

Scientific Type	Type of assay (ISO, CLSI)
Nominal	Qualitative
Ordinal	Semi-Quantitative
Interval	
Ratio	Quantitative

Two Examples

Linearity

ability to provide measured quantity values that are directly proportional to the value of the measurand in the sample.

Example 1

Consider a device that

$$\mathbf{Conc_{Device} = 0.8 * Conc_{True} + 20}$$

This device is not linear; this device is not quantitative

Indeed,

Sample 1 has $Conc_{Device}$=100, then $Conc_{True}$=100 (100=0.8*100+20)
Sample 2 has $Conc_{Device}$=50, then $Conc_{True}$=37.5 (50=0.8*37.5+20)
Sample 3 has $Conc_{Device}$=25, then $Conc_{True}$=6.25 (25=0.8*6.25+20)
Device is not linear: Sample 1: 100/100=1.0; Sample 2: 50/37.5=1.3
Sample 3: 25/6.25=4.0

Device is not quantitative: using device, Sample 1 has **2 times** more amount of analyte than Sample 2 (100/50=2.0) but true ratio=**2.7** (100/37.5); Sample 2 has **2 times** more amount of analyte than Sample 3 (50/25=2) but true ratio= **6.0** (37.5/6.25)=> No interpretation of numbers

Two Examples

Linearity

ability to provide measured quantity values that are directly proportional to the value of the measurand in the sample

Example 2

Consider a device that

$\mathbf{Conc_{Device} = 0.8 * Conc_{True}}$

This device is linear; this device is quantitative

Indeed,

Sample1 has $Conc_{Device}$=100, then $Conc_{True}$=125 (100=0.8*125)

Sample2 has $Conc_{Device}$=50, then $Conc_{True}$=62.5 (50=0.8*62.5)

Sample3 has $Conc_{Device}$=25, then $Conc_{True}$=31.25 (25=0.8*31.25)

Device is **linear**: Sample1: 100/125=**0.8**; Sample2: 50/62.5=**0.8**

Sample3: 25/31.25=**0.8**

Device is **quantitative**: using device, Sample1 has **2 times** more amount of analyte than Sample2 (=100/50) and true ratio=**2** (125/62.5);

Sample2 has **2 times** more amount of analyte than Sample3 (50/25=2) and true ratio=**2** (62.5/31.25) . Note at $Conc_{True}$=0, $Conc_{Device}$=0.

Linearity and Quantitative Results

If measured quantity is a ratio (as amount, concentration, volume and so on) and the measuring system is **linear**,
then this measuring system produces **quantitative** results.

Linearity is an important characteristic of a quantitative assay.

Less restrictive definitions of linearity

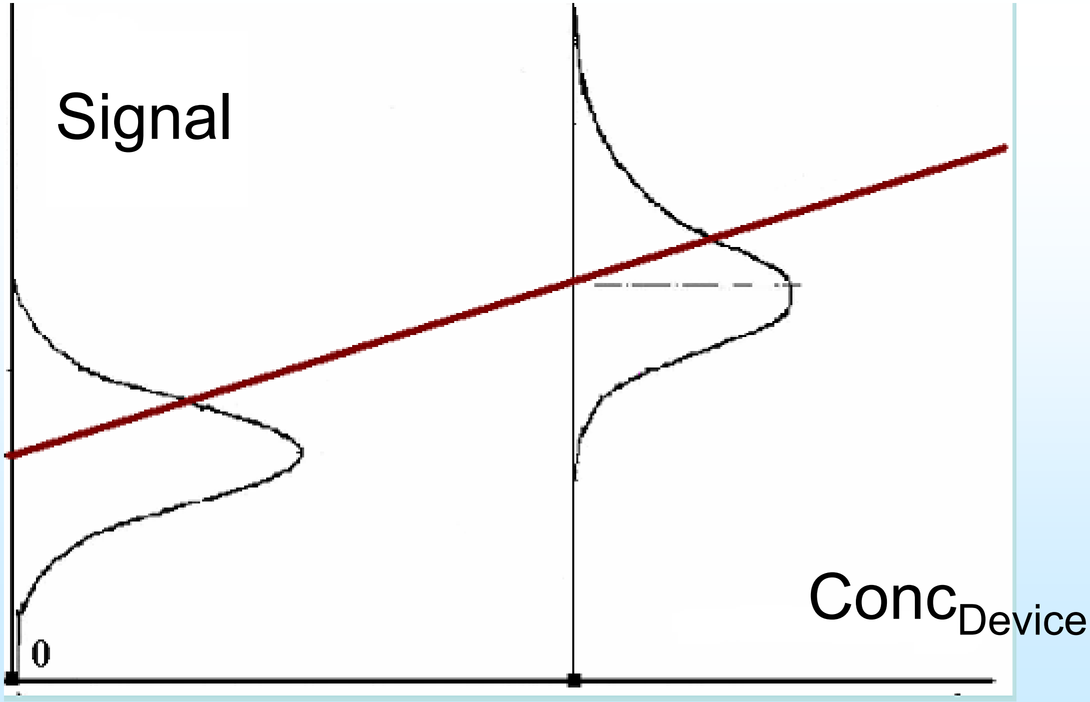
For some applications, users may choose less restrictive definition of linearity:
Ability of a system to provide results that conform to a straight line of the form $Y=a+bX$ within a given range ($a \neq 0$). The system produces results which are not proportional (interval results, not ratio results). The system can be linear after a simple additional transformation (note that FDA is evaluating a final version of the device).

Another less restrictive definition: the system is linear (proportional to the true concentrations) after some monotonic transformation.

How linearity is related to calibration

Calibration is a process that establishes the relationship between the assigned values ($Conc_{Device}$) and the raw instrument signal.

The calibration curve relates the mean of the measured signal to the assigned concentration values ($Conc_{Device}$).



- Linearity is a property related to the final results (not to the raw signals).

- Linearity means a straight-line going through zero between the true value and concentration provided by the device.

- Typical confusion: linearity of the assay = calibration curve is a straight line (it is wrong).

- Consider that there is no random error and the True calibration curve is known. If this true calibration curve will be used, then device is linear.

Linearity is related to how well the device is calibrated.

Working Calibration Curve

Sources of Uncertainties:

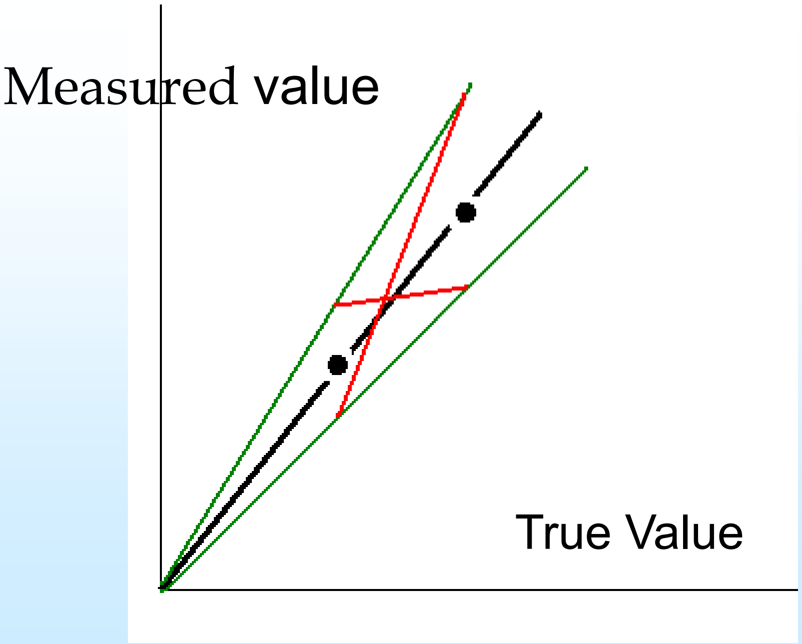
1) Reference materials (RM), assigned values to these materials, number of RMs
2) Number of dilutions
3) Number of replicates (we need to have mean of a signal)
4) Mathematical model
5) Estimation of parameters in the model

This is not a complete list.

How We Do Demonstration of Linearity

Basic points:

1) It is impossible to demonstrate that the system is perfectly linear. Therefore, it should be specified an acceptable deviation from linearity Δ, (for example, ±10%).



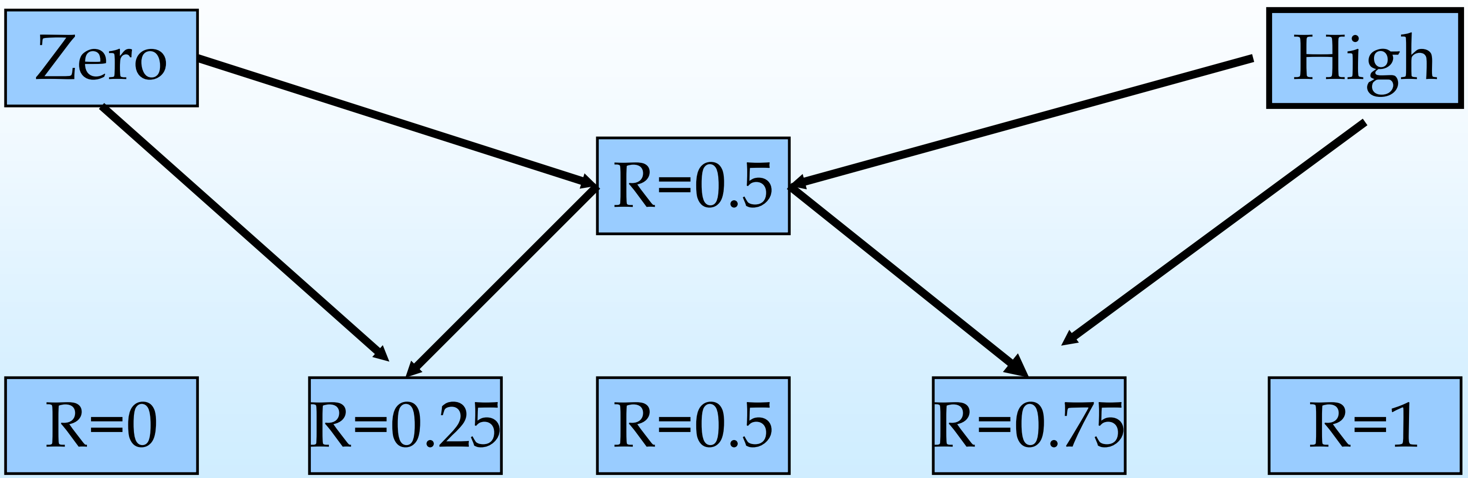
Example:
Δ=20%
The ratio of measured values=
(1+Δ)/(1-Δ)=1.2/0.8=1.5

How We Do Demonstration of Linearity

Basic points:

2) The linearity of a system is demonstrated by testing levels of analyte which are known relatively to each other (not necessary known absolutely).

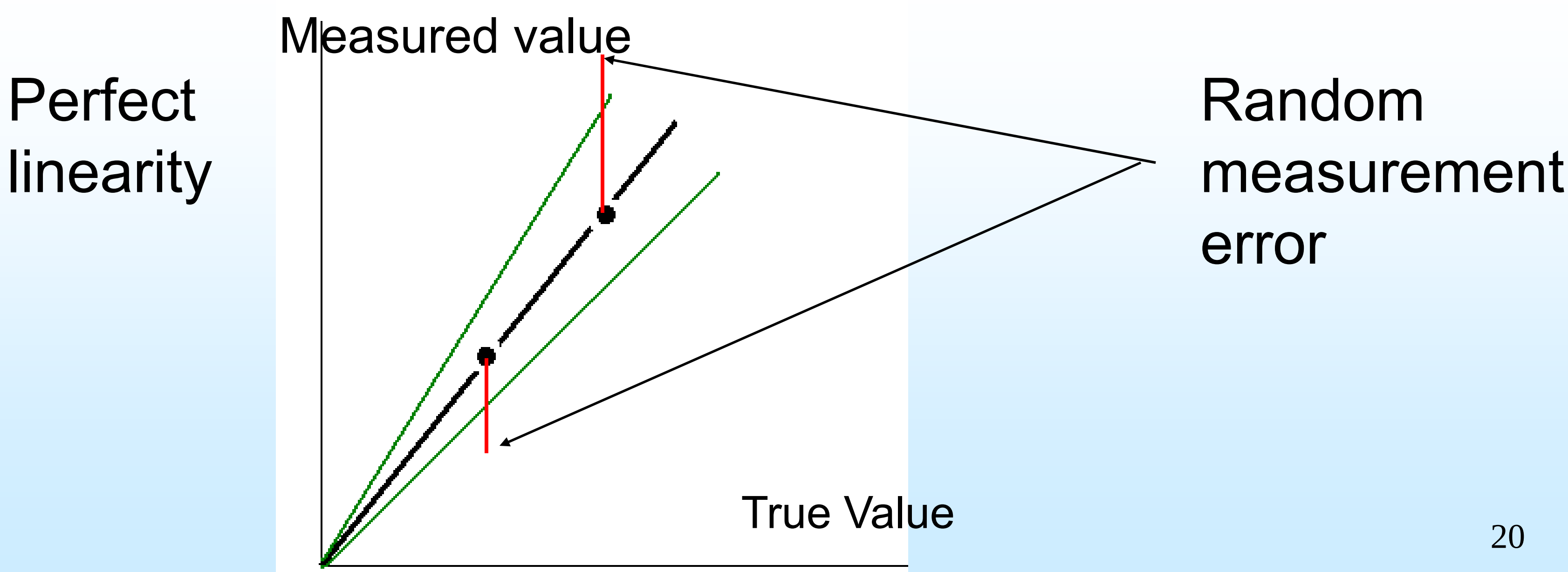
Relative concentrations R (dilutions of High Sample)



How We Do Demonstration of Linearity

Basic points:

3) Random error is present. We need to assess linearity isolated as much as possible from precision.
For this one need to select an appropriate number of replicates (K) for each sample.



How We Do Demonstration of Linearity

Basic points:

3) Random error is present.

Example, %CV of repeatability ≤ 4%, consider that there is no deviation from linearity; in order that all 9 samples were close to the straight line (not more than 5%), one needs to have at least 5 replicates for each sample).

The larger %CV, the larger number of replicates is needed.

How We Do Demonstration of Linearity

Basic Scheme of Linearity Study:

1) At least 9 samples with varying concentrations which are known relative to one another by dilution scheme.
2) These samples are measured by the device K times. An average of K results is close to "$Conc_{Device}$" (Y=Observed value).
3) Relationship of Y vs R (relative concentration) should be close to the straight line with no intercept (deviation not more than Δ)

Hypothetical Example

Claim:
Linearity interval is from 10 to 300 units with Δ=10%.

High Sample was diluted with sample of zero concentration (or close to zero concentration)

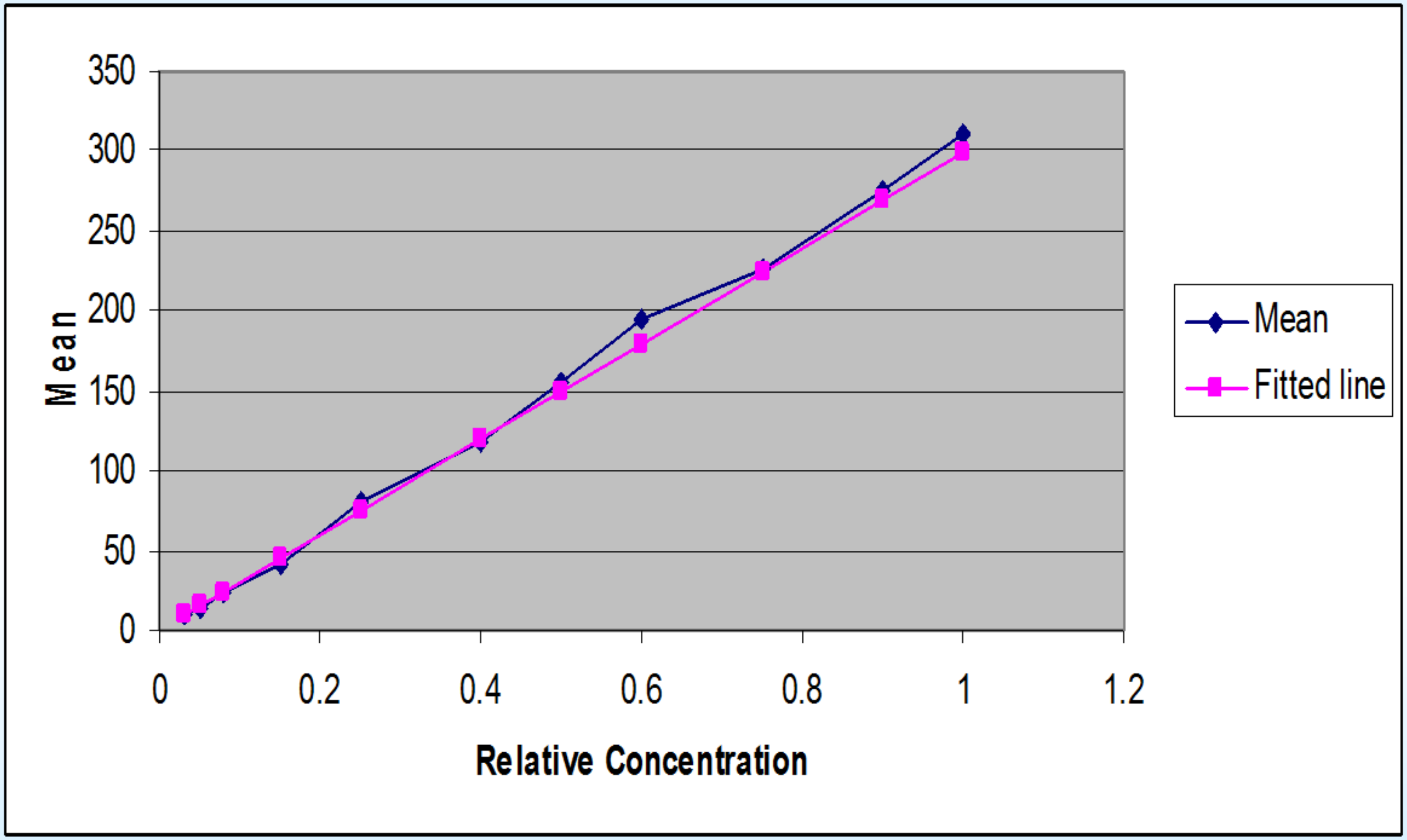
11 different levels

5 replicates at each level

Sample	**R** Relative concentration	**Mean**
1	1	311.6
2	0.9	275.8
3	0.75	226.9
4	0.6	193.7
5	0.5	154.6
6	0.4	117.0
7	0.25	80.7
8	0.15	41.6
9	0.08	23.3
10	0.05	13.6
11	0.03	8.9

Hypothetical Example

Graph: R, Relative Concentration (X) vs Observed Mean (Y)



Best fitted straight line which corresponds to linearity (weighted regression analysis) is **Y=298.4*R**

Hypothetical Example

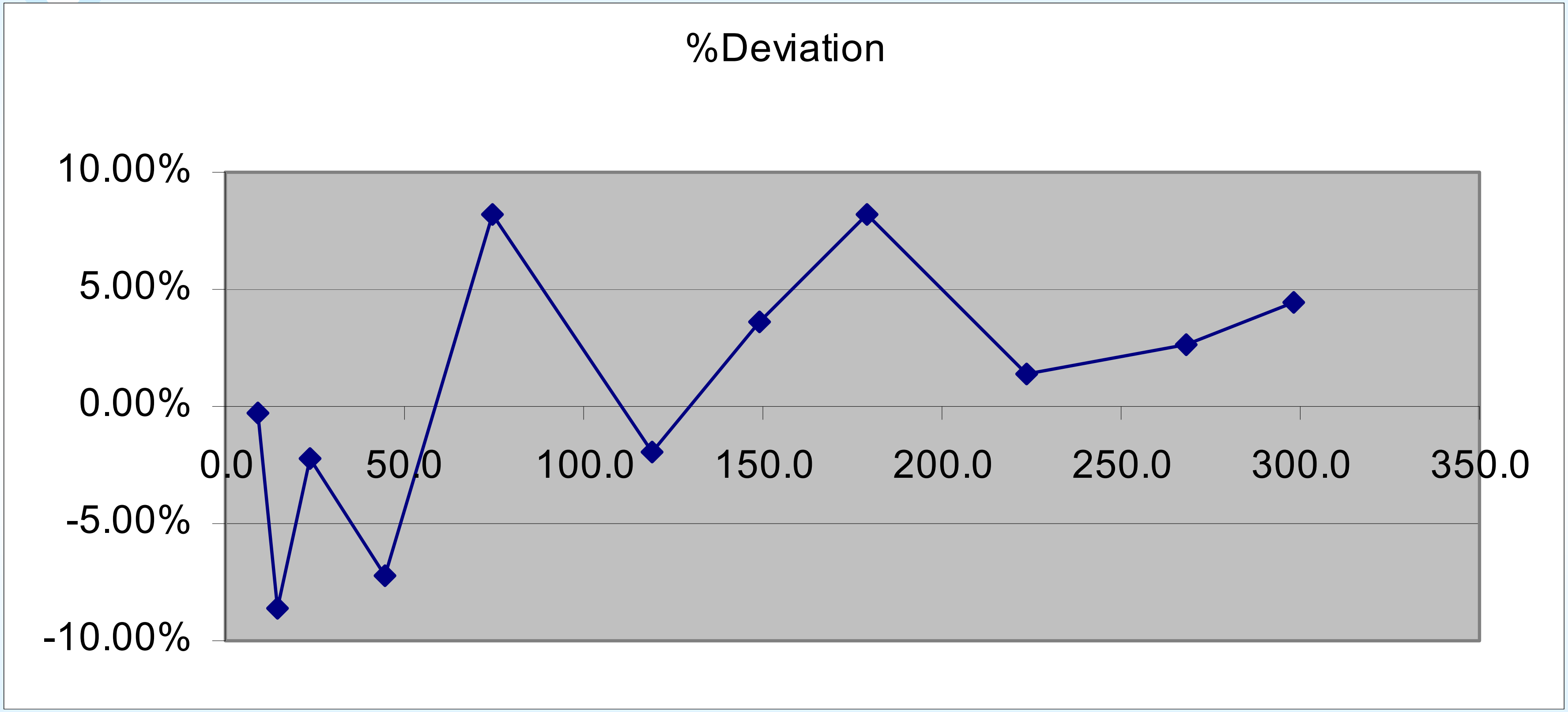
Sample	**R** Relative conc.	**Mean** Mean of 5 replicates	**Predicted** (best fitted line corresp. to linearity)	%Deviation
1	1	311.6	298.4	4.4%
2	0.9	275.8	268.6	2.7%
3	0.75	226.9	223.8	1.4%
4	0.6	193.7	179.1	8.2%
5	0.5	154.6	149.2	3.6%
6	0.4	117.0	119.4	-2.0%
7	0.25	80.7	74.6	8.2%
8	0.15	41.6	44.8	-7.2%
9	0.08	23.4	23.9	-2.2%
10	0.05	13.6	14.9	-8.7%
11	0.03	8.9	9.0	-0.3%

Find a maximum deviation among all values: 8.7% ≤10%

=> **Linearity for 10-300 units is demonstrated with dev. less than 10%**

Hypothetical Example

Graph: Predicted Concentration (X) vs %Dev. from linearity (Y)



Find a maximum deviation among all values: 8.7% ≤10%

=> Linearity for 10-300 units is demonstrated with dev. less than 10%

Additional Notes

1) **Linearity is NOT checking accuracy** (trueness). Calibration verification is related to linearity but it is a different procedure.

- Calibration verification requires testing materials with assigned concentrations (as calibrators, control samples with assigned values, PT samples, patient samples with known values and so on).
- Linearity requires testing samples which are known relative to each other (relative concentration to High sample and Zero sample)

Additional Notes

2) Use of Correlation Coefficient

A common mistake is to assume that a high correlation implies linearity. One can see high deviation from linearity at the lowest and/or highest concentrations and still see a high correlation. Value of correlation depends on the range of levels.

In the hypothetical example, correlation =0.99925.
If Δ=5%, then linearity is not demonstrated.
If Δ=10%, then linearity is demonstrated.

3) IVDMIA Assays

These assays can provide numerical Score results but these results are NOT quantitative => linearity study for the Score is not needed.

Summary

- Linearity is ability to provide results which are proportional to the values of the measurand in a sample
- For quantitative assays, linearity should be demonstrated.
- Linearity can be demonstrated with deviation from linearity Δ more than zero.
- Pay attention to the number of replicates in a linearity study.

Thank you!

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