METHOD VALIDATION IN ANALYTICAL CHEMISTRY

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Agenda

1. **Basic statistics:**
- Overview of statistics
- Terms and Concepts
2. **Method validation:**
- Introduction
- Definitions
- Performance characteristics
- Application of method validation
- Documentation
- Conclusion.

Over View Of Basic statistics:

- **Statistics Definition**

- The scientific study of numerical data based on variation in nature.

  (Sokal and Rohlf)

- A set of procedures and rules for reducing large masses of data into managable proportions allowing us to draw conclusions from those data.

  (Mc Carthy)

Over View Of Basic statistics:

- ***Why Use Statistics?***

1. identify patterns
2. distinguish true differences from random variation
3. allows *hypothesis testing*

Over View Of Basic statistics:

- ***Types of Statistics:***

A. **Descriptive :**

- used to organize and describe a Sample.
- Using for ex. (tables – Graphs - numerical summaries)

B. **Inferential :**

- used to extrapolate from a sample to a larger population.
- For drawing conclusions

Descriptive Statistics

A. **Measures of Central tendency:**

- MEAN – average.
- MEDIAN -- middle value.
- MODE -- most frequently observed value(s).

B. **Measures of Dispersion:**

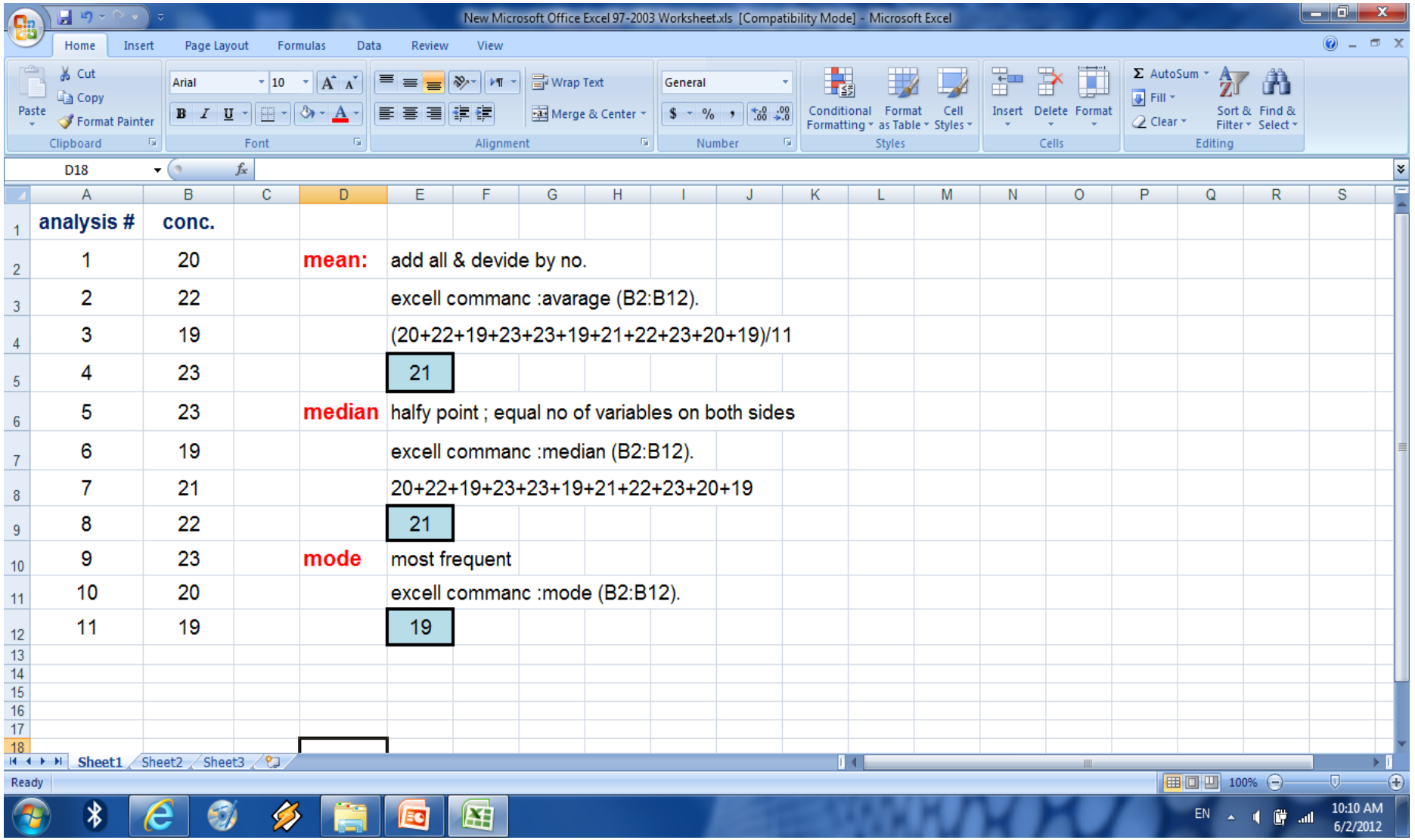
- RANGE .
- STANDARD DEVIATION.
- SKEWNESS.

C. **Measures of Association:**

- correlation

Descriptive Statistics

a . *Measures of Central tendency*



	A	B	C	D	E
1	analysis #	conc.			
2	1	20		mean:	add all & devide by no.
3	2	22			excell commanc :avarage (B2:B12).
4	3	19			(20+22+19+23+23+19+21+22+23+20+19)/11
5	4	23			21
6	5	23		median	halfy point ; equal no of variables on both sides
7	6	19			excell commanc :median (B2:B12).
8	7	21			20+22+19+23+23+19+21+22+23+20+19
9	8	22			21
10	9	23		mode	most frequent
11	10	20			excell commanc :mode (B2:B12).
12	11	19			19

Descriptive Statistics

B. ***Measures of Dispersion***

- ***RANGE :***

  highest to lowest values

- ***STANDARD DEVIATION:***

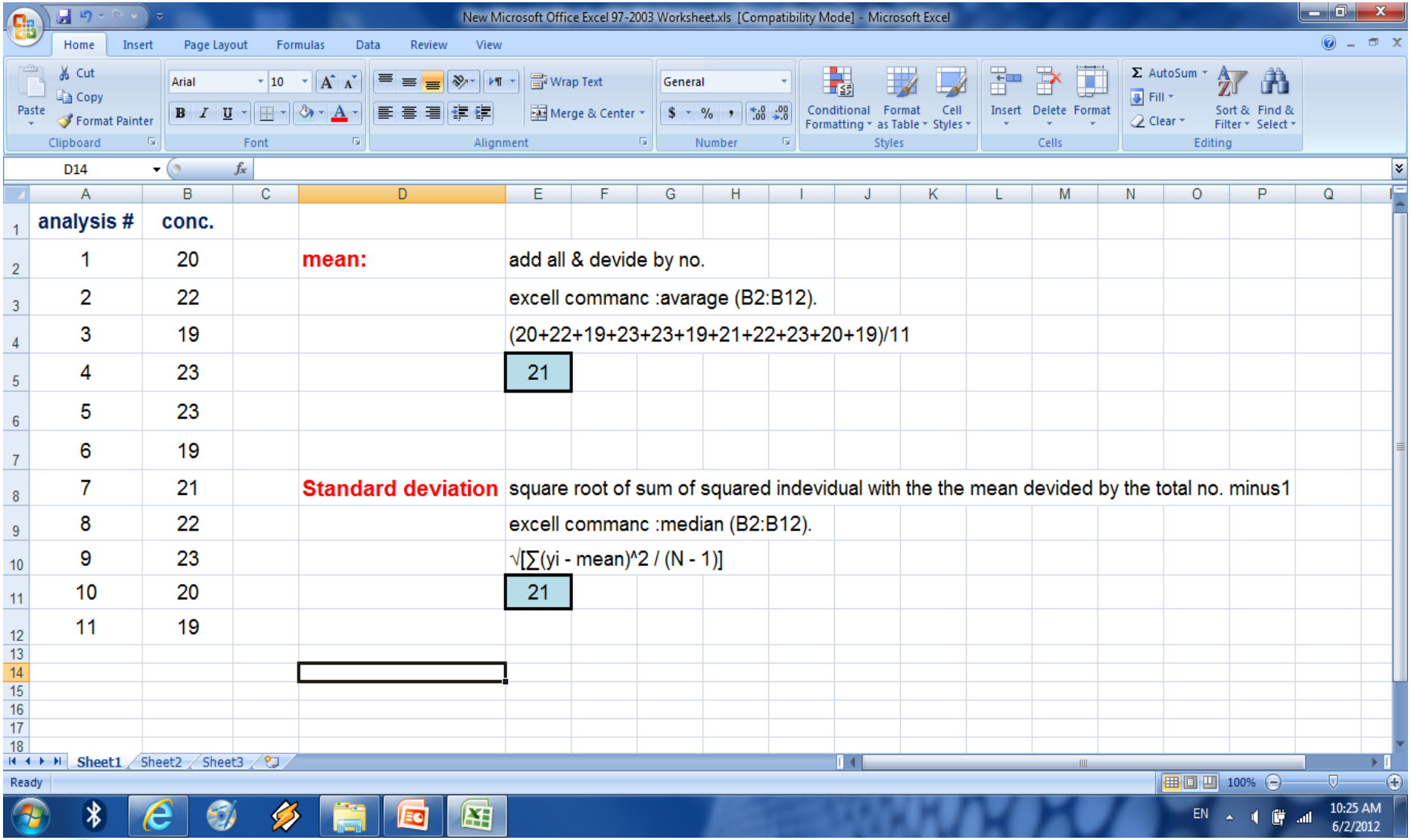
  how closely do values cluster around the mean value

- **SKEWNESS:**

  refers to symmetry of curve

Descriptive Statistics

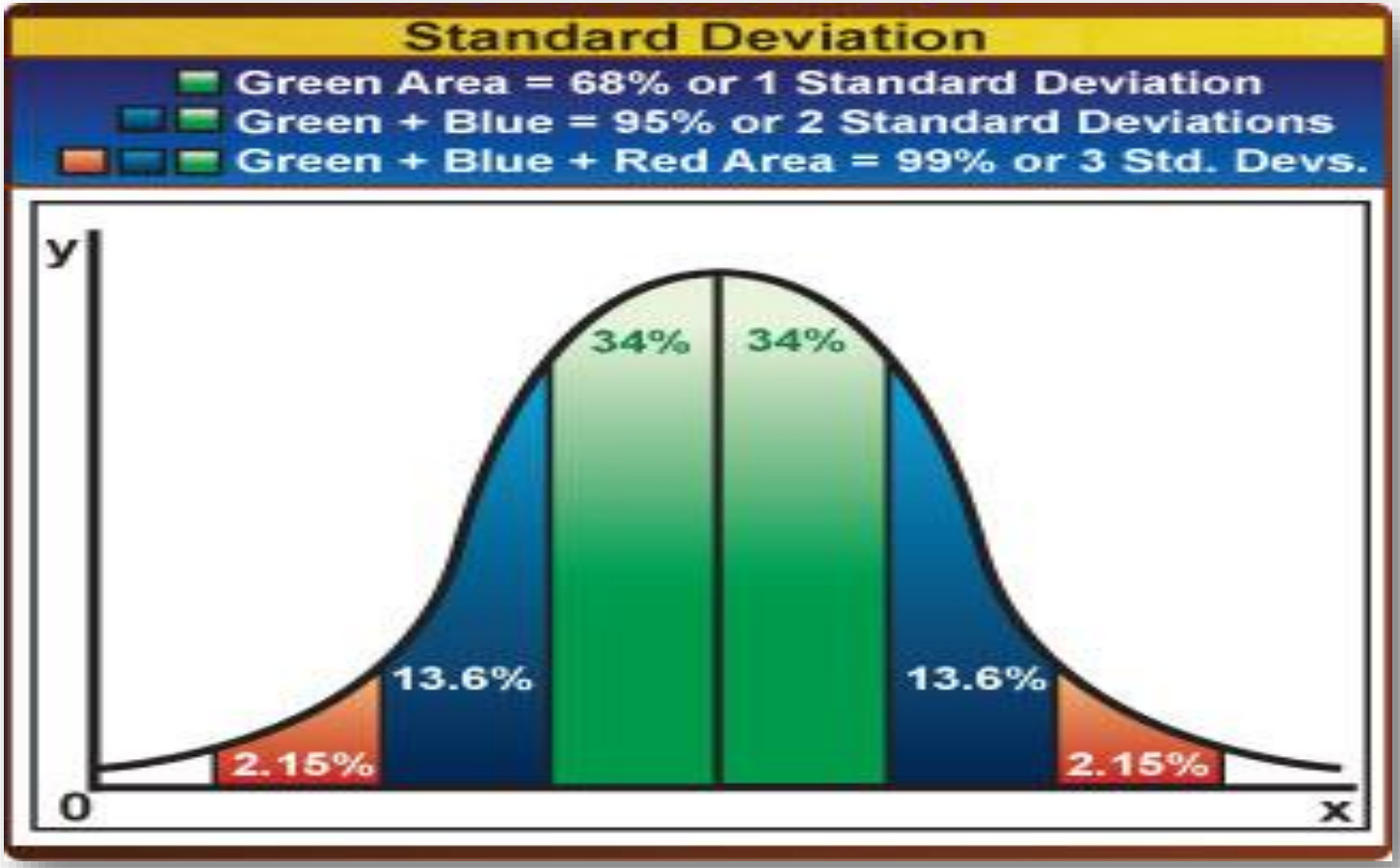
c. *Measures of Dispersion*



	A	B	C	D	E
1	analysis #	conc.			
2	1	20		mean:	add all & devide by no.
3	2	22			excell commanc :avarage (B2:B12).
4	3	19			(20+22+19+23+23+19+21+22+23+20+19)/11
5	4	23			21
6	5	23			
7	6	19			
8	7	21		Standard deviation	square root of sum of squared indevidual with the the mean devided by the total no. minus1
9	8	22			excell commanc :median (B2:B12).
10	9	23			√[∑(yi - mean)^2 / (N - 1)]
11	10	20			21
12	11	19			

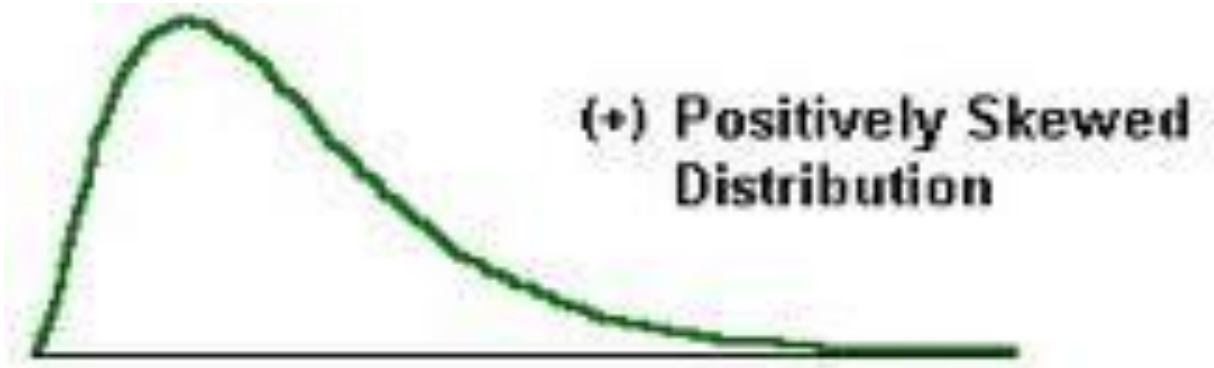
Descriptive Statistics

Standard Deviation



Descriptive Statistics

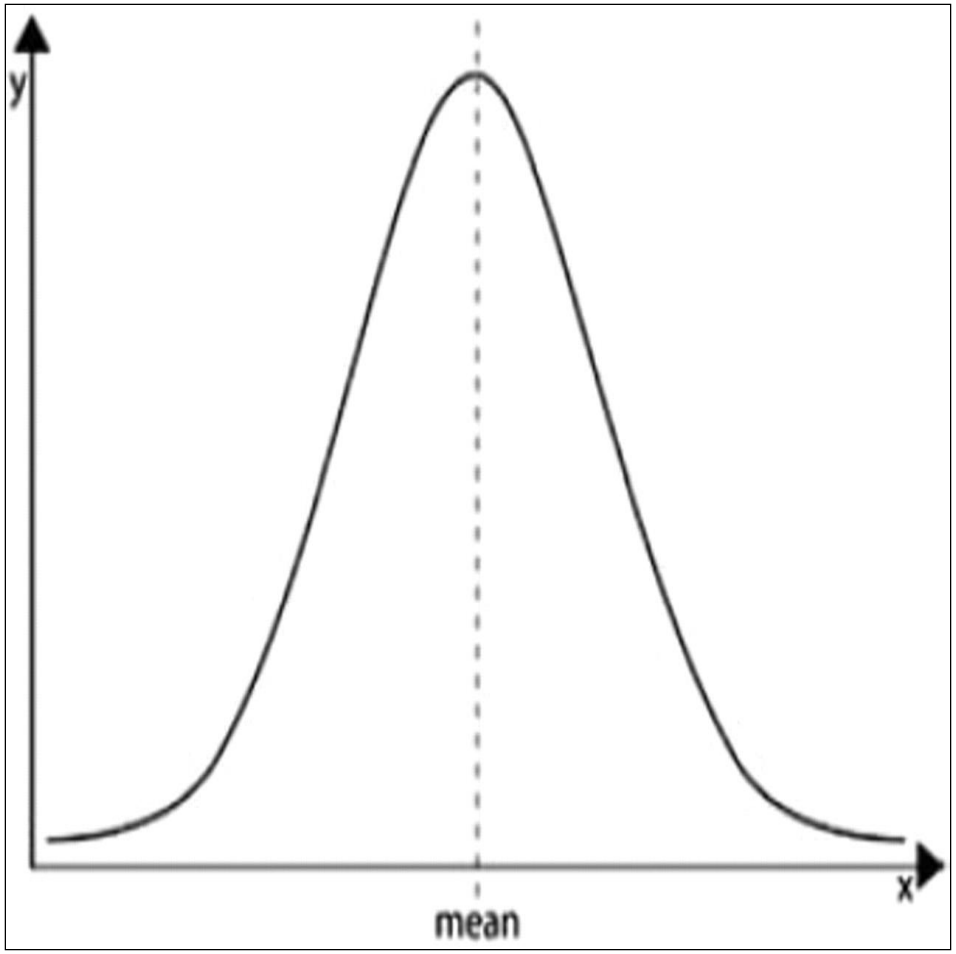
SKEWNESS



Descriptive Statistics

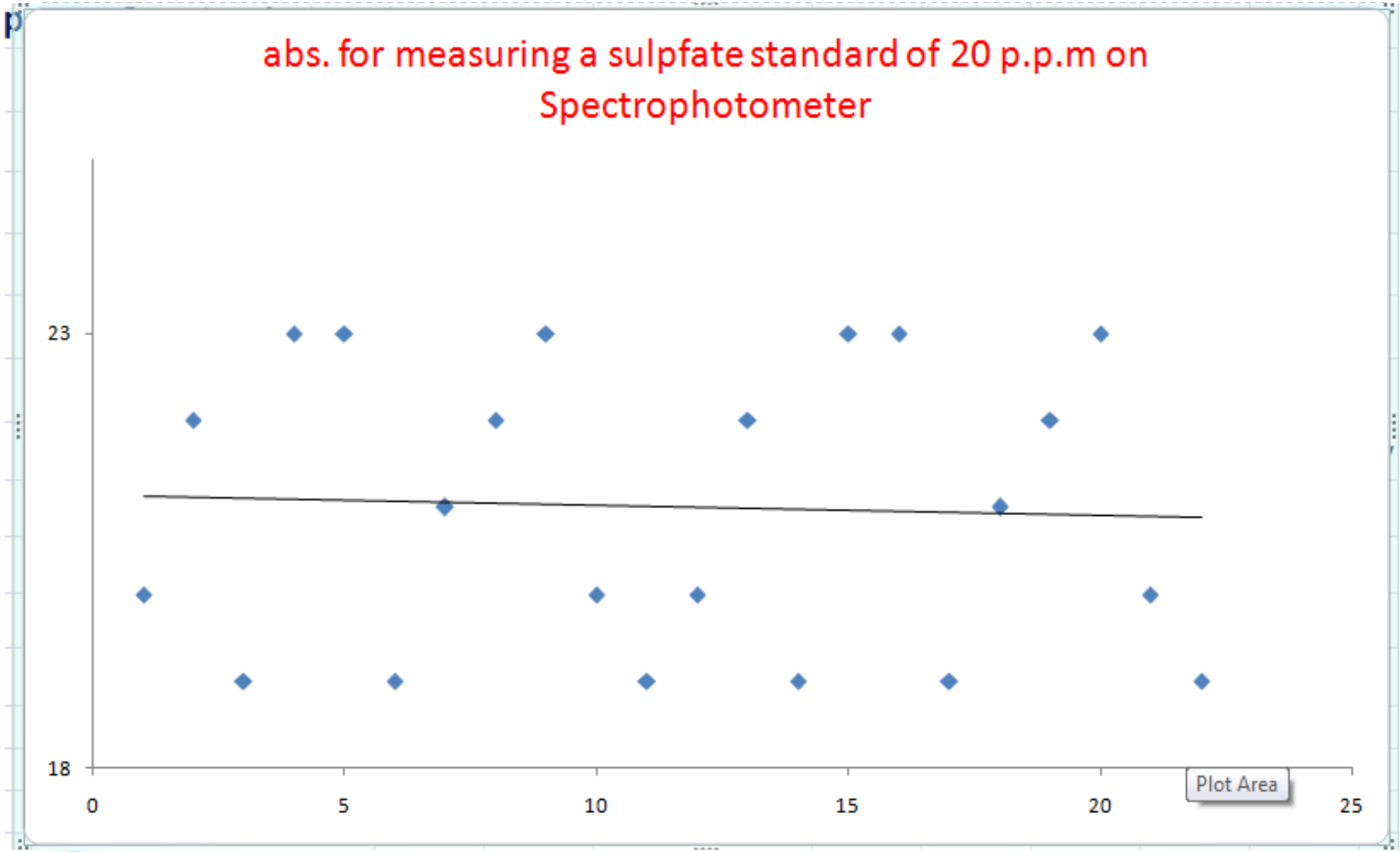
The Normal distribution

- Mean = median = mode
- Skew is zero
- 68% of values fall between 1 SD
- 95% of values fall between 2 SDs



Measures of Association

Correlation



Inferential Statistics

Types of data

- ***Numerical (quantitative)***
- Continuous

(*possible values are in a continuum E.g. measurements in height)*

- Discrete

(number of possible values can be counted) (has a fixed no)

- ***Categorical (qualitative)***
- Ordinal

(rank order, (1st, 2nd, 3rd, etc.))

- Nominal :
- **categorized or labeled data (red, green, blue, male, female)** ***(no order)***

Inferential Statistics

- ***Null Hypothesis (H0):***

- **Is a method of making a decision using data.**

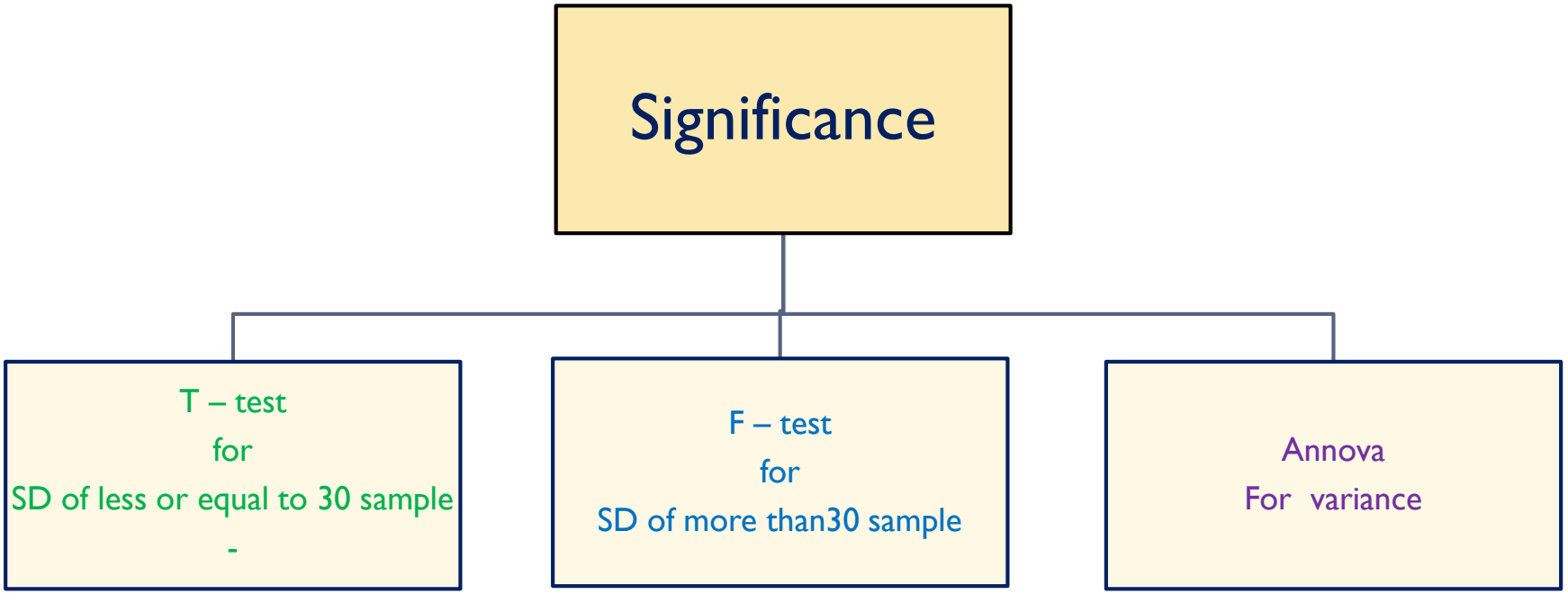
- Statistical hypotheses usually assume no relationship between variables.

  (There is no association between eye color and height)

- If the result of your statistical test is significant, then the original hypothesis ***(H0)***is false and you can say that the variables in your experiment are somehow related ***(H1)*** is retained.

Significant Test

In statistics, a result is called **statistically significant** if it is unlikely to have occurred by chance



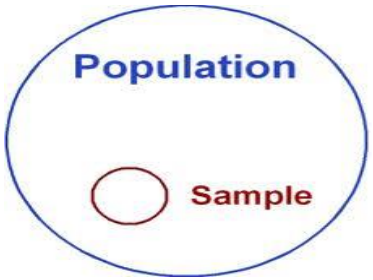
Critical Values for the Student *t*-Test

v 1T	60%	75%	80%	85%	90%	95%	97.5%	99%	99.5%	99.9%	99.95%
v 2T	20%	50%	60%	70%	80%	90%	95%	98%	99%	99.8%	99.9%
1	0.3249	1.0000	1.3764	1.963	3.078	6.314	12.706	31.821	63.657	318.3	636.62
2	0.2887	0.8165	1.0607	1.386	1.886	2.920	4.303	6.965	9.925	22.33	31.596
3	0.2767	0.7649	0.9785	1.250	1.638	2.353	3.182	4.541	5.841	10.21	12.941
4	0.2707	0.7407	0.9410	1.190	1.533	2.132	2.776	3.747	4.604	7.173	8.610
5	0.2672	0.7267	0.9195	1.156	1.476	2.015	2.571	3.365	4.032	5.893	6.869
6	0.2648	0.7176	0.9057	1.134	1.440	1.943	2.447	3.143	3.707	5.208	5.959
7	0.2632	0.7111	0.8960	1.119	1.415	1.895	2.365	2.998	3.499	4.785	5.408
8	0.2619	0.7064	0.8889	1.108	1.397	1.860	2.306	2.896	3.355	4.501	5.041
9	0.2610	0.7027	0.8834	1.100	1.383	1.833	2.262	2.821	3.250	4.297	4.781
10	0.2602	0.6998	0.8791	1.093	1.372	1.812	2.228	2.764	3.169	4.144	4.587
11	0.2596	0.6974	0.8755	1.088	1.363	1.796	2.201	2.718	3.106	4.025	4.437
12	0.2590	0.6955	0.8726	1.083	1.356	1.782	2.179	2.681	3.055	3.930	4.318
13	0.2586	0.6938	0.8702	1.079	1.350	1.771	2.160	2.650	3.012	3.852	4.221
14	0.2582	0.6924	0.8681	1.076	1.345	1.761	2.145	2.624	2.977	3.787	4.140
15	0.2579	0.6912	0.8662	1.074	1.341	1.753	2.131	2.602	2.947	3.733	4.073
16	0.2576	0.6901	0.8647	1.071	1.337	1.746	2.120	2.583	2.921	3.686	4.015
17	0.2573	0.6892	0.8633	1.069	1.333	1.740	2.110	2.567	2.898	3.646	3.965
18	0.2571	0.6884	0.8620	1.067	1.330	1.734	2.101	2.552	2.878	3.610	3.922
19	0.2569	0.6876	0.8610	1.066	1.328	1.729	2.093	2.539	2.861	3.579	3.883
20	0.2567	0.6870	0.8600	1.064	1.325	1.725	2.086	2.528	2.845	3.552	3.850
21	0.2566	0.6864	0.8591	1.063	1.323	1.721	2.080	2.518	2.831	3.527	3.819
22	0.2564	0.6858	0.8583	1.061	1.321	1.717	2.074	2.508	2.819	3.505	3.792
23	0.2563	0.6853	0.8575	1.060	1.319	1.714	2.069	2.500	2.807	3.485	3.768
24	0.2562	0.6848	0.8569	1.059	1.318	1.711	2.064	2.492	2.797	3.467	3.745
25	0.2561	0.6844	0.8562	1.058	1.316	1.708	2.060	2.485	2.787	3.450	3.725
26	0.2560	0.6840	0.8557	1.058	1.315	1.706	2.056	2.479	2.779	3.435	3.707
27	0.2559	0.6837	0.8551	1.057	1.314	1.703	2.052	2.473	2.771	3.421	3.690
28	0.2558	0.6834	0.8546	1.056	1.313	1.701	2.048	2.467	2.763	3.408	3.674
29	0.2557	0.6830	0.8542	1.055	1.311	1.699	2.045	2.462	2.756	3.396	3.659

Terms & Concept

- ***Sample :*** collected data

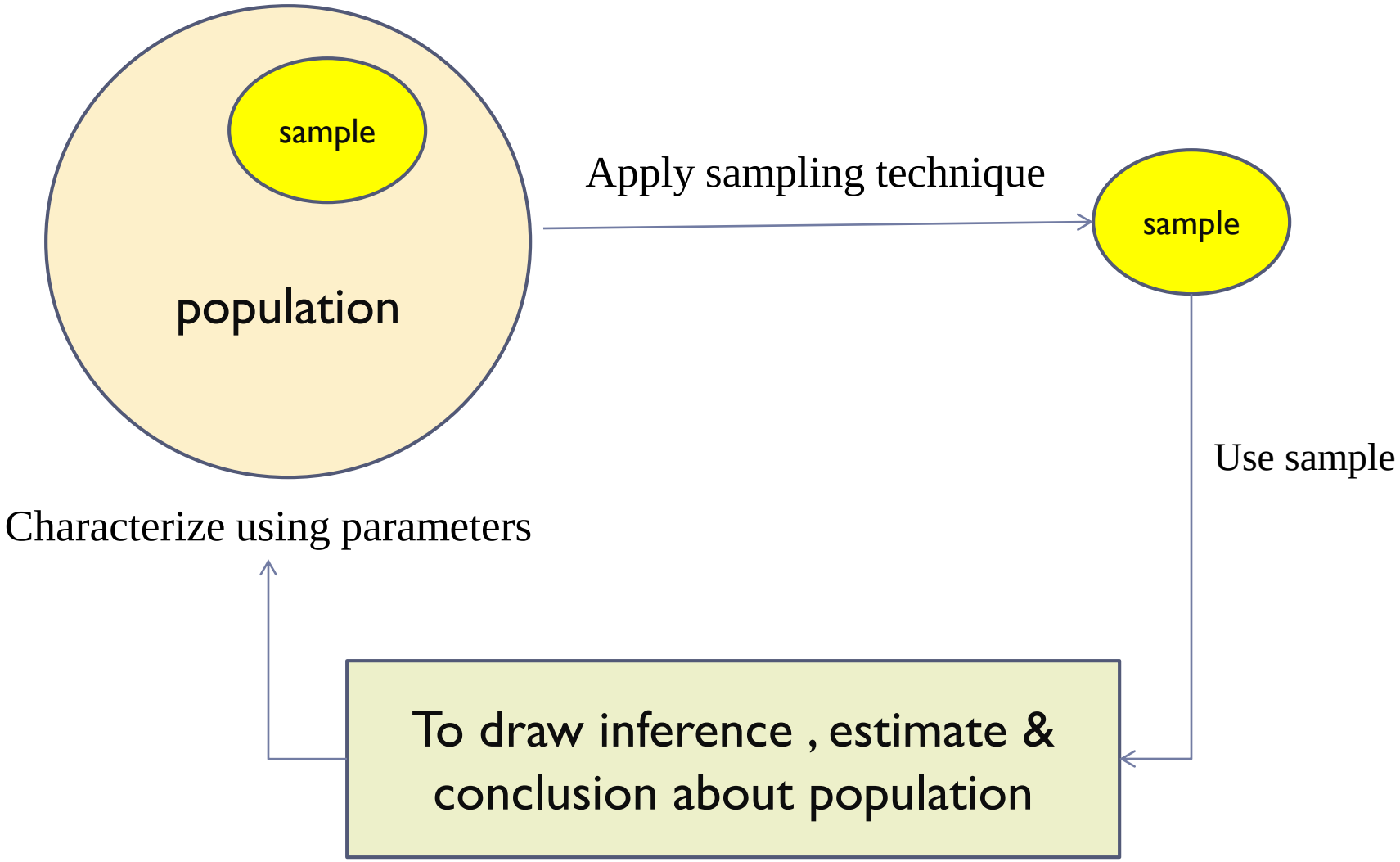
- ***Population :*** all possible data



- **Variable :** a property with respect to which data from a sample differ in some measurable way.

- **variance**: average squared deviation of all possible observations from a sample mean (calculated from sum of squares)

Population and sample



Terms & Concept

- **p value:**

the probability that an observed difference could have occurred by chance

- **confidence interval**:

The range of values we can be reasonably certain includes the true value.

Measure the random error.

- ***Measurement :***

assignment of a number to something

- ***Data :***

collection of measurements

Terms & Concept

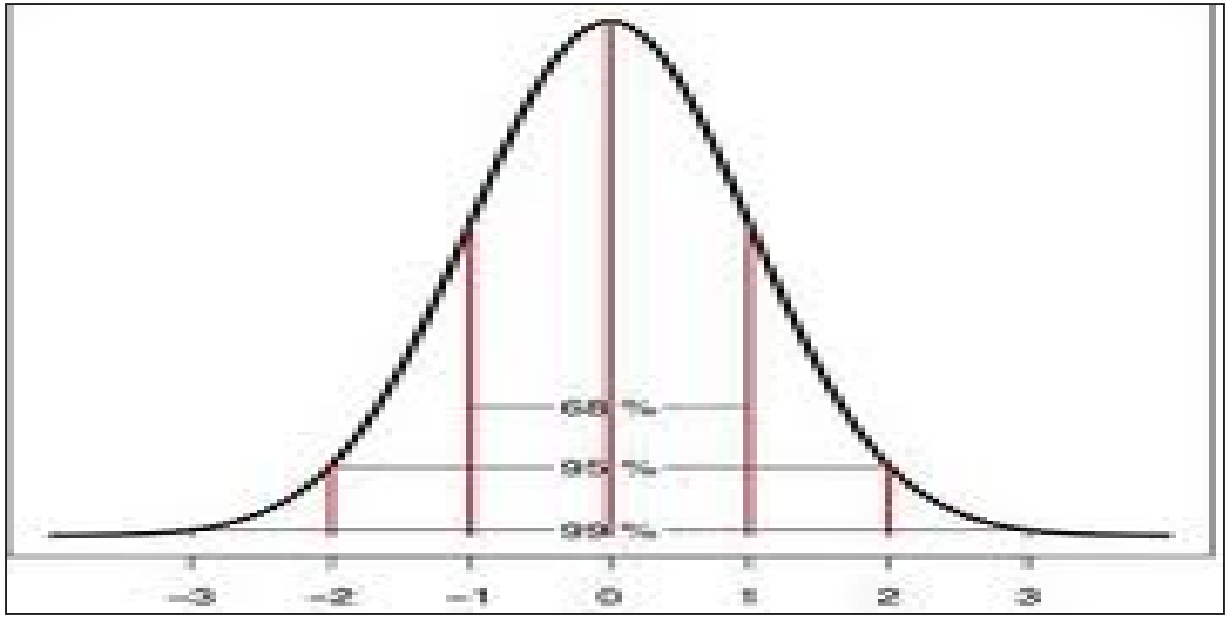
Confidence Interval:

The probability that the unknown population parameter

In confidence interval of 100 trial

Terms & Concept

Confidence Interval:



$$CI = (1-\alpha) \times 100$$

α is The probability that the parameter is not within interval of 100 trial

احتمالية عدم الثقة

Terms & Concept

Types of Error in Experimental Data

Random (indeterminate) Error

Data scattered approx. symmetrically about a mean value.
(dealt with statistically)

Systematic (determinate) Error

Several possible sources. Readings all too high or too low.
Affects accuracy.

Gross Errors

Usually obvious - give “outlier” readings.
Detectable by carrying out sufficient replicate measurements.

Why do Chemical lab. Need Statistics?

- Lab Chemists are concerned with the chemical analysis processes that quantify analytes in different matrices.
- All these processes are subject to
  - systematic variation (e.g. Instrument effects, matrix effect)
  - random variation (e.g. measurement errors)

❖ *Statistics is a tool to help us understand the effects of random variation*

Example problem 1

Ten measurements of phosphate standard on spectrophotometer gave the following values

0.291	0.2898	0.2923	0.302	0.300
0.296	0.2947	0.2986	0.290	0.288

Calculate the average , standard deviation , median

Average=

(0.291 + 0.2898 + 0.2923 + 0.302 + 0.300 + 0.296 + 0.2947 0.2986 +

0.290 + 0.288)/10 = *0.29424*

SD=

∑[(0.291 - *0.29424)^2*) +(0.2898 - *0.29424)^2*) + (0.2923 - *0.29424)^2*) +

(0.302 - *0.29424)^2*) + (0.300 - *0.29424)^2*) + (0.296 - *0.29424)^2*) +

(0.2947 - *0.29424)^2*) +(0.2986 - *0.29424)^2*) + (0.290 - *0.29424)^2*) +

(0.288 - *0.29424)^2*) /9] = *0.004791*

Median = 0.2935

2-Method validation:

Introduction

What is method validation?

1. The process of defining analytical requirements & confirming that the used method has performance characteristics consistent with the application requirements

2. The process of establishing performance characteristics of a method.

3. *The process of verifying that a method is fit for purpose.*

2-Method validation:

- Why is Method validation an important requirements in the practice of chemical analysis ?

1. To reduce the coast *because* Decision = Coast
2. Important for quality control
3. M.V enable the chemist to be sure that the method used is fit for purpose .
4. Uncertainty has to be evaluated and interpreted

(most of the information needed come from validation)

5. M.V is required in ISO 17025 (*clause 5-4-5-2*)

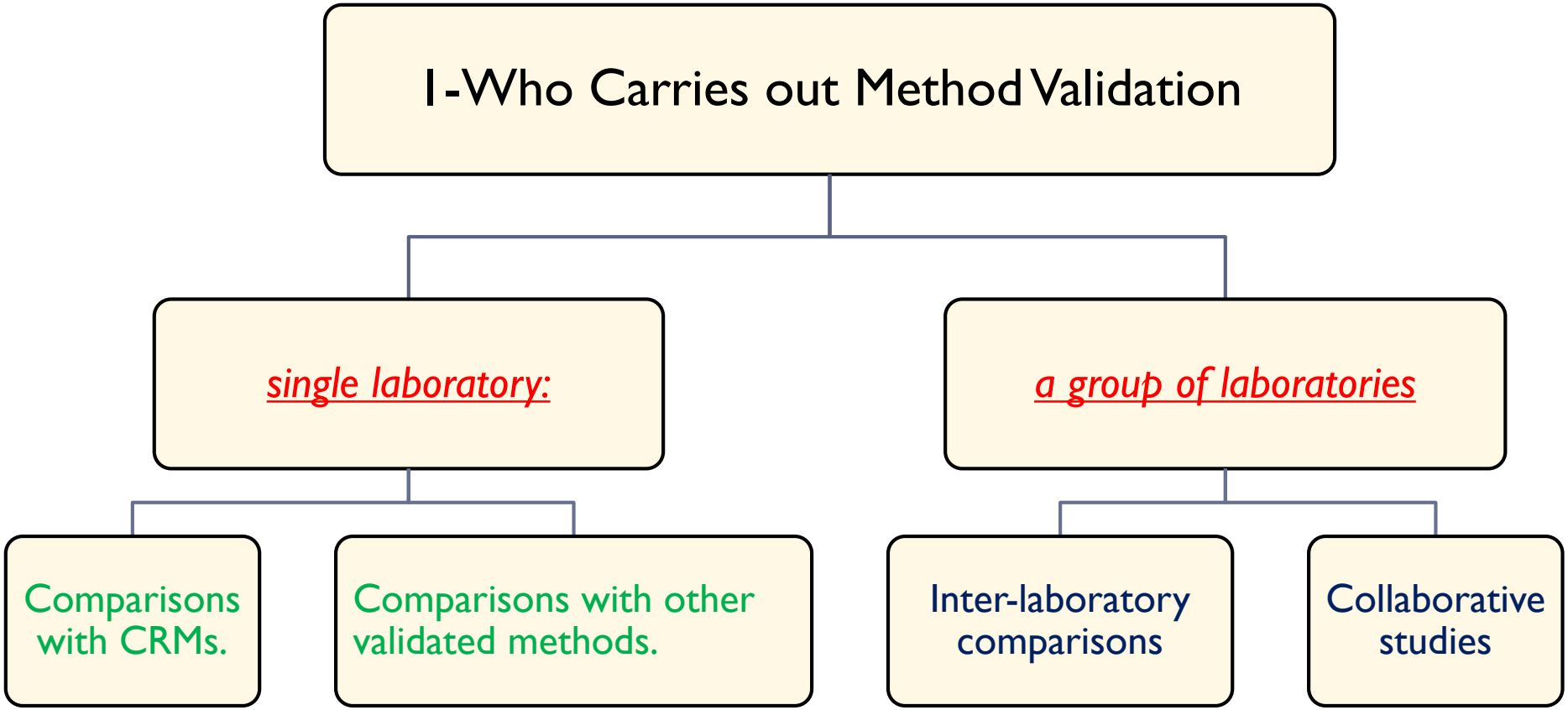
Introduction

When should methods be validated

1. New method development or before introducing a new routine in to routine use.
2. Revision of established methods
3. When established methods are used in different conditions
4. QC indicates method changes and changes outside the scope.
5. Comparison of methods

Introduction

- How should Methods be Validated.



Introduction

❖How should Methods be Validated?

2-The Analytical Requirement

single laboratory:

- Which method is the most suitable?
- What degree of validation is required?
- How the method will be used?

a group of laboratories

- What are the analytes of interest?
- What are the expected concentration levels?
- Are there any interferences?
- How was the sampling done?

Introduction

Advantages and disadvantages of validation

in a single lab. & in a group of lab.

Validation *in a single lab. .*		*Validation in a group of lab..*	
Intra-lab reproducibility.	×	Inter-lab. Reproducibility	✓
Less expensive & fast.	✓	Expensive & slow	×
No compatibility.	√	Difficult to get all to conform	×
Sometimes not acceptable by Regulatory.	×	Some procedures can't be validated	×
No official recognition.	×	Esoteric application	×
		Interested lab. Could be competitors	×

Introduction

❖To What Degree Validation is Required?

1. Balance between coast & time and need to validate.

It may be prefered to subcontract the analysis to another lab.

2. Comply with Customers' requirements

3. Follow Existing experience.

4. Compatibility with other similar standard methods as *(AOAC)*

Definitions

- *Validation*

❑is the process of demonstrating or confirming the performance characteristics of a method of analysis.

- *The performance characteristics of a method of analysis :*

❑are the functional qualities and the statistical measures of the degree of reliability exhibited by the method under specified operating conditions.

- *method of analysis" :*

❑is sometimes assigned to the technique, e.g., liquid chromatography or atomic absorption spectrometry

- *Verification"* :

is confirmation by examination and provision of objective evidence that specified requirements have fulfilled

❖ The tools of validation:

1. Blanks:
   - •Reagent blanks
   - •Sample blank.
2. Samples/test materials
3. Fortified materials/solutions
4. Spiked materials
5. Incurred materials
6. Measurement standards
7. Reference materials
8. Certified Reference materials
10. Statistics
11. Replication

❖ The tools of validation:

1. Blanks:

•*Reagent blanks*

•a blank contains everything except for the analyte,

•(a blank solution that contains the reagents used to dissolve the samples such as acids used for digestion; the reading for this solution is typically substracted from sample readings) .

•*Sample blank.*

•They are matrices without analyte:

•Difficult to obtained.

•Important to estimate interferences.

2. *Samples/test materials:*

- Materials taken from real samples
- Useful because of interference.

3. *Fortified materials/solutions:*

- Materials which are fortified (spiked) with the analyte of the interest.
- Useful for calculation of recovery

4. *Spiked materials:*

- Similar to fortified
  - Fortified ⟷ Spiked

5. *Incurred materials*

- The analyte has essentially alien but added in points prior to the material be sampled.
- Ex: adding of active ingredient in pharmaceutical formulation at the formulation stages

6. *Measurement standards:*

- Any thing in which a particular parameter has been specified to be used for reference or calibration *ex: a calibrated thermometer*

7. *Reference materials :*

- The property or the analyte needs to be stable but not essential to Have the high degree of characterization , tractability and certification

8. *Certified Reference materials:*

- Characterization of parameters in which more strictly controlled than R.M.
- The value of it is certified with uncertainty.
- With very low bias

TraceCERT™
Traceable Certified Reference Materials

BAM

Certificate

Fluka
Riedel-de Haën

This certificate is designed in accordance with ISO Guide 31 [7] and comprises three pages.

Object of certification: **Sulfate, certified anion standard solution**
Fluka Product No.: 80218 (Lot 1259501)
Composition: Na_2SO_4 (high purity quality) diluted in water *Trace***SELECT**™ Ultra (18.2 MΩ cm, 0.22 µm filtered)
Density at 20°C: ρ = 999.3 kg m^{-3} $u_c(\rho)$ = 0.5 kg m^{-3} (using an oscillating u-tube)
Intended use: Calibration of ion chromatography or any other analytical technique
Storing and handling: This reference material shall be stored in the original closed bag between 5°C and 30°C. After opening the bottle should be stored at reduced temperature. The bottle's temperature must be 20°C and the bottle has to be shaken well before every use. We highly recommend using this reference material no longer than 15 months after the aluminium bag was opened.
Expiry date: 10. July 2010 (unopened bottle in aluminized bag, latest use = exp. date + shelf life)
Bottle opening date: (recommended shelf life after opening: 6 months)

Declaration of certified value and uncertainty according to ISO Guide 35 [8] and Eurachem/CITAC Guide [9]

Constituent	Certified value at 20°C	Combined expanded uncertainty [$U = k u_c$; $k = 2$]	Methods of certification
Sulfate	**1005 mg L^{-1}** **1006 mg kg^{-1}**	**8 mg L^{-1}** **8 mg kg^{-1}**	gravimetric preparation and precipitations analysis

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1. Concept of Certification and Traceability Statement

To guarantee highest reliability this certified reference material is certified by two independent certification bodies[4]:

1. Gravimetric preparation using pure materials is a practical realization of concentration units, through conversion of masses and mole fraction to mass fraction[10]. If the purity of the materials is demonstrated and if contamination and loss of material is strictly prevented this approach allows highest accuracy and small uncertainties.
   The certified value of this reference material is based on this approach and directly traceable to the SI unit kilogram. The starting material is measured against a certified reference material (i.e. NIST, BAM or EMPA) followed by gravimetric preparation using balances calibrated with SI-traceable weights. Consequently the value calculated by this unbroken chain of comparisons is traceable to the reference to which the starting material is compared.
2. The bottled solution is certified by BAM (Federal Institute for Materials Research and Testing) using precipitation analysis. The measurements are traced directly to base SI unit: mass and mole.
3. Both values were combined for the certified value of this anion standard solution.

Certificate page 1 of 3

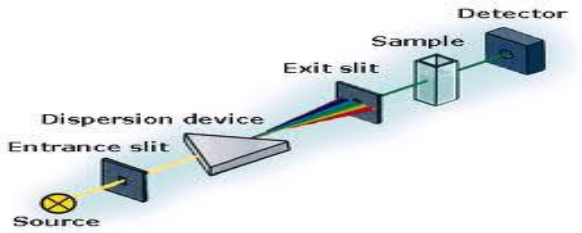
Performance Characteristics

1) Selectivity/Specificity
2) Limit of Detection
3) Limit of Quantitation
4) Linearity
5) Accuracy
6) Trueness
7) Precision
8) Ruggedness (or Robustness)
9) Recovery

Selectivity/Specificity

Selectivity:

The ability of the method to determine accurately the analyte of interest in the presence of other components in a sample matrix under the stated conditions of the test.



Specificity :

a) is a state of perfect selectivity.
b) The ability of the method to measure only what it is intended to measure

note:

- Selectivity & specificity are often used interchangeably
- It is recommended that the term *Selectivity be promoted & that of Specificity be dicouraged*

Selectivity / Specificity

How to establish selectivity?

Compare the response of the analyte in a test mixture with the response of a solution containing only the analyte

Selectivity / Specificity

How to establish selectivity?

The procedure to establish selectivity:

1. Analyze samples and reference materials.
2. Assess the ability of the methods to confirm identity and measure the analyte.
3. Choose the more appropriate method.
4. Analyze samples.
5. Examine the effect of interferences.

Selectivity / Specificity

How to indicate selectivity?

Using selectivity Index.

selectivity Index (b_{an} / b_{int})

b_{an} (slope of calibration curve).

b_{int} (slope of interference)

b_{int} can be determined by application of the procedure on matrix blank and the same blank is spiked with an interferance

Limit of Detection (LoD)

a. The lowest content can be measured with statistics

b. The lowest content if actually present that will be detected and identified

Limit of Detection (LoD)

How to measure LoD?

- LoD = B+3S_0 or 0+3S_0

  B=Blank

  - S_0=standard deviation of 10 measurements

Limit of Detection (LoD)

Expression of the LoD

- Analyze
  - 10 independent sample blanks and get the mean sample blank value (B) **or**
  - 10 independent sample blanks fortified at lowest acceptable concentration.
- Express LoD as the analyte concentration corresponding to
  - B+3s

(s being the sample standard deviation).

Limit of Detection (LoD)

Statistics

❑-Determine Standard Deviation:

$$s = \left\{ \frac{\sum_i (x_i - \bar{x})^2}{\nu} \right\}^{\frac{1}{2}}$$

S: sta ndard deviation

x_i : single measurement

$\bar{x}$: average = $\sum x_i / n$

ν : is the degree of freedom=n-1

❑-Determine the hypothesis:

*determine $T_{calculated} = \dfrac{\bar{x} - \mu}{S/\sqrt{n}}$

*determine $T_{critical}$ (from tables of t distribution)

excel commence TINV(0.05,B15-1)

* If $T_{calculated} < T_{critical}$ ∴ H_o retained & H_1 rejected

a) LOD = $\bar{x}$ + 3S **b) LOD = 0 + 3S**

Limit of Quantitation (LoQ)

- *The Limit of Quantitation is:*

1. the content which is equal or grader than the lowest concentration point on the calibration curve (i.e. what level can be measured)
2. The lowest conc. Of the analyte can be determined with acceptable level of U.C

Limit of Quantitation (LoQ)

Statistics

- Determine Standard Deviation
- Determine the hypothesis

$$\mathbf{LoQ = \bar{x} + 5S\ ;\ 6S\ ;\ 10S}$$

Linearity and Working Range

- *Calibration curve:*

Graphical representation of measuring signal as a function of quantity of analyte.

- *linearity:*

The ability of the method to obtain test results proportional to the concentration of the analyte

Linearity and Working Range

Calibration curve

$y = bx + a$

b = slope

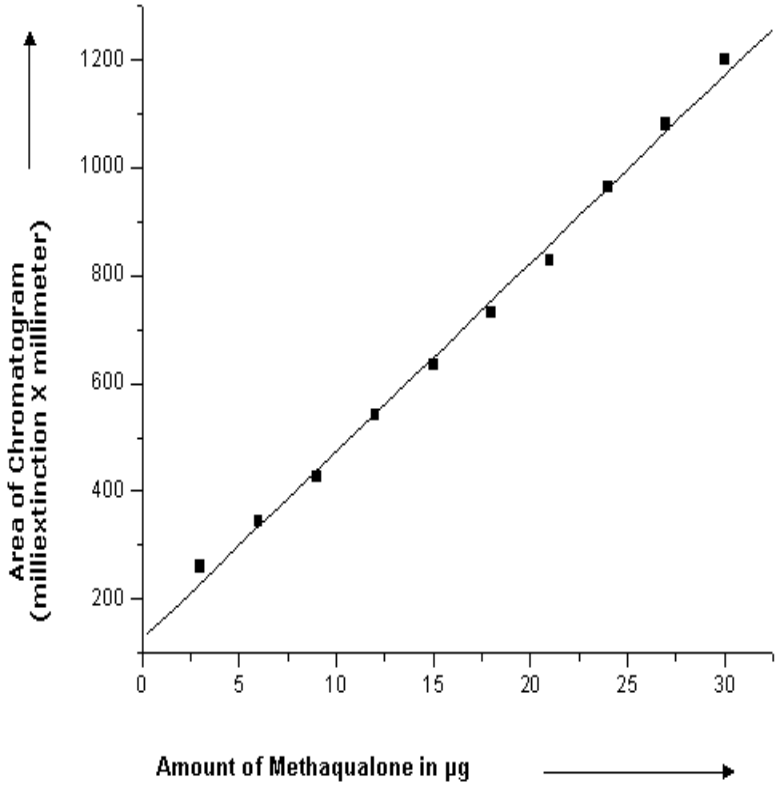
a = intercept

r^2 = correlation coefficient

$r^2 > 0.99$ or 0.98

for very low

concentrations



Linearity and Working Range

procedure

•Analyze Blank plus reference materials or fortified sample blanks at various concentrations

•- Plot measurement response (y axis) against measurand concentration (x axis)

•-Visually examine to identify approximate linear range and upper and lower boundaries of the working range.

•- Calculate appropriate regression coefficient (r).

•- Calculate and plot residual values $S_{residual}$ (difference between actual y value and the y value predicted by the straight line, for each x value).

•-Random distribution about the straight line indicate non-linearity confirms linearity

Accuracy / trueness

- *Accuracy:*

the closeness of a result to a true value

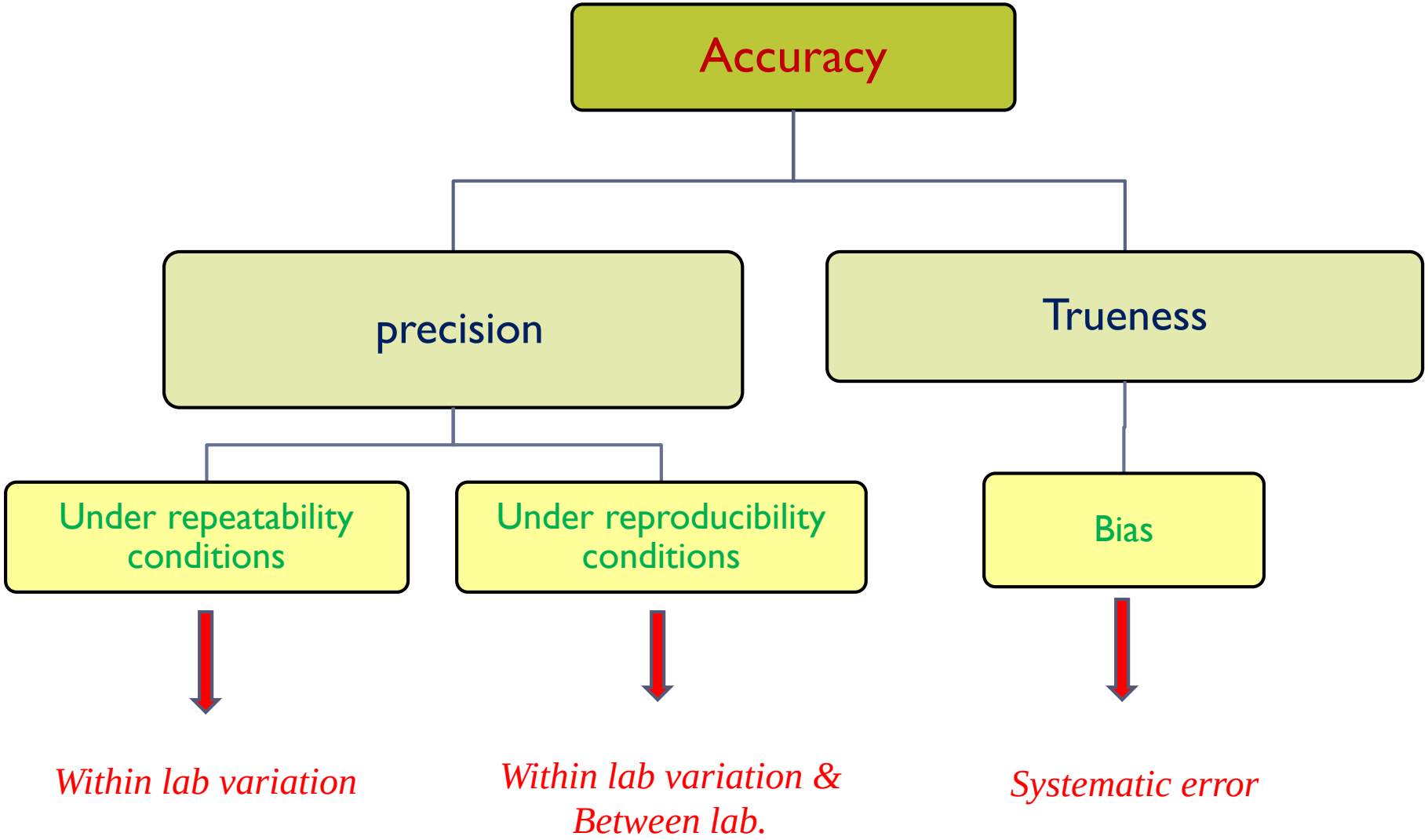
- *Trueness*:

The closeness of agreement between the average value obtained from a large set of test results and an accepted reference value.

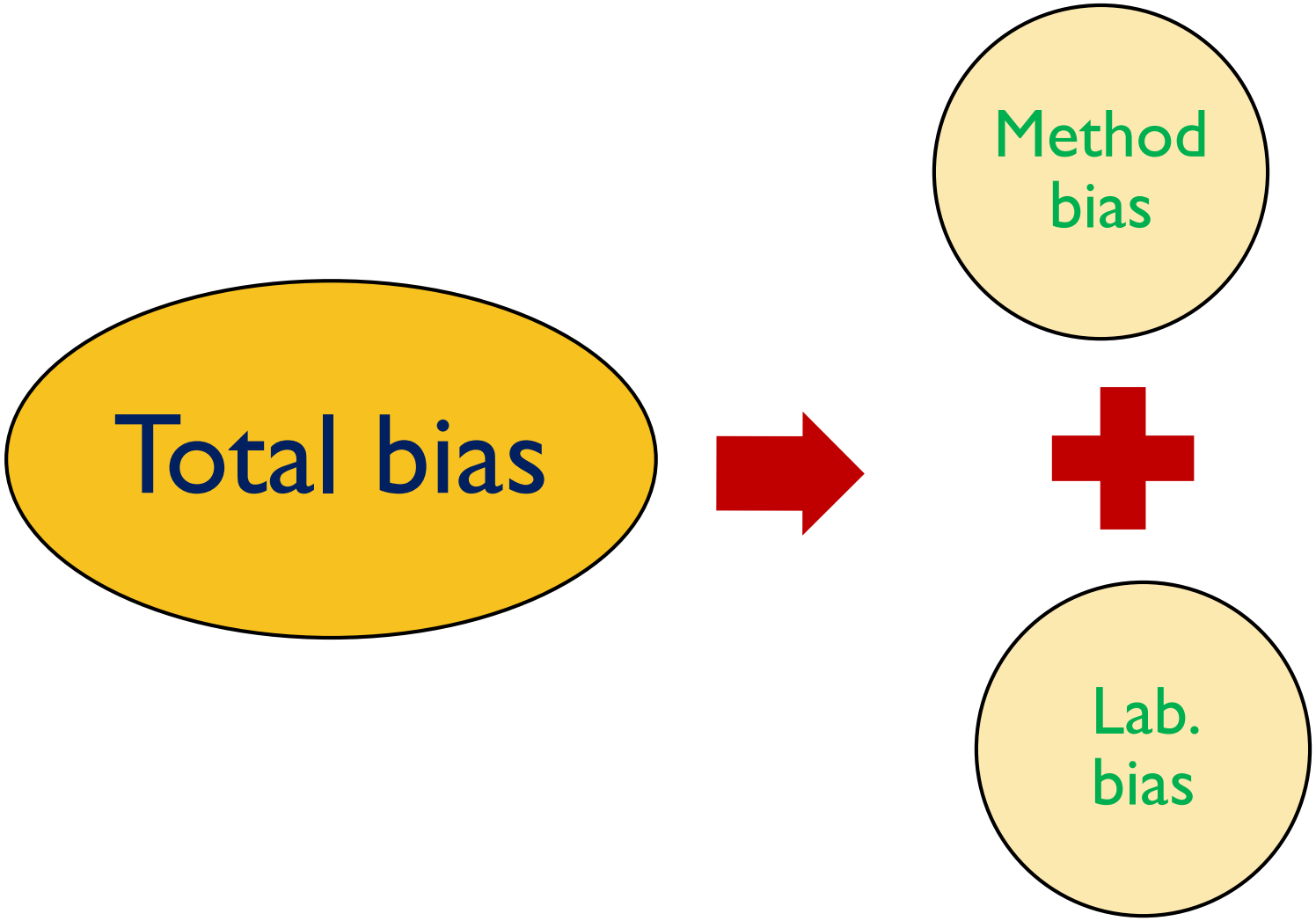
- *Precision*:

how close results are to one another

Accuracy / trueness

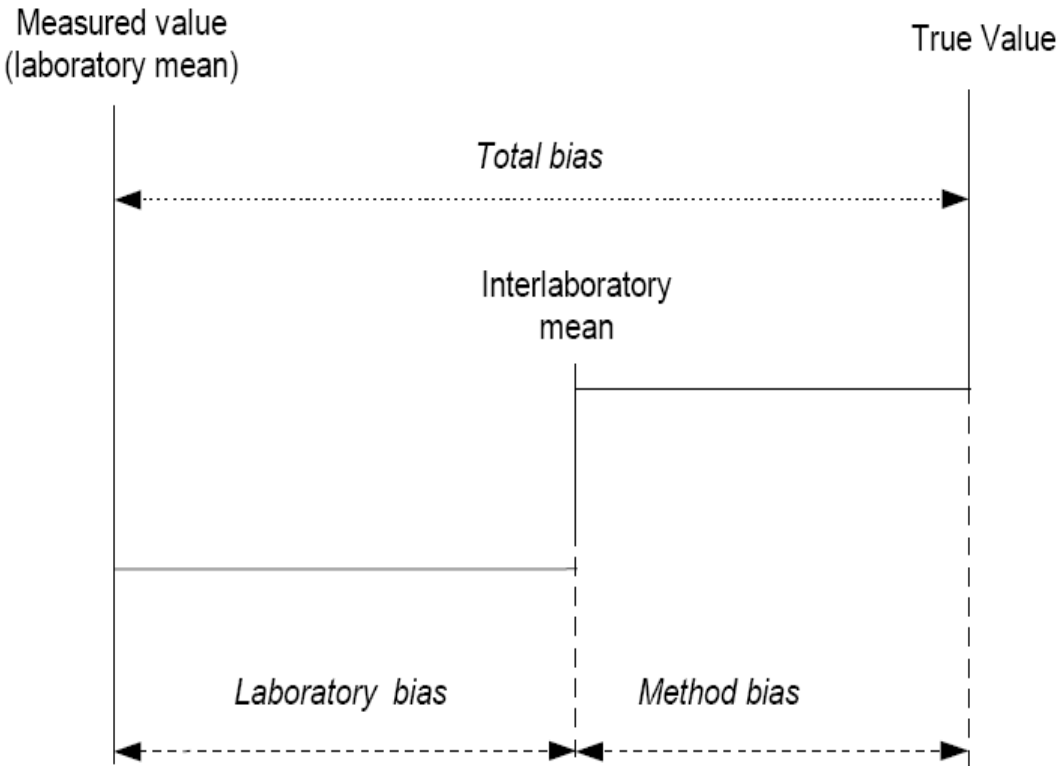


Accuracy/truness

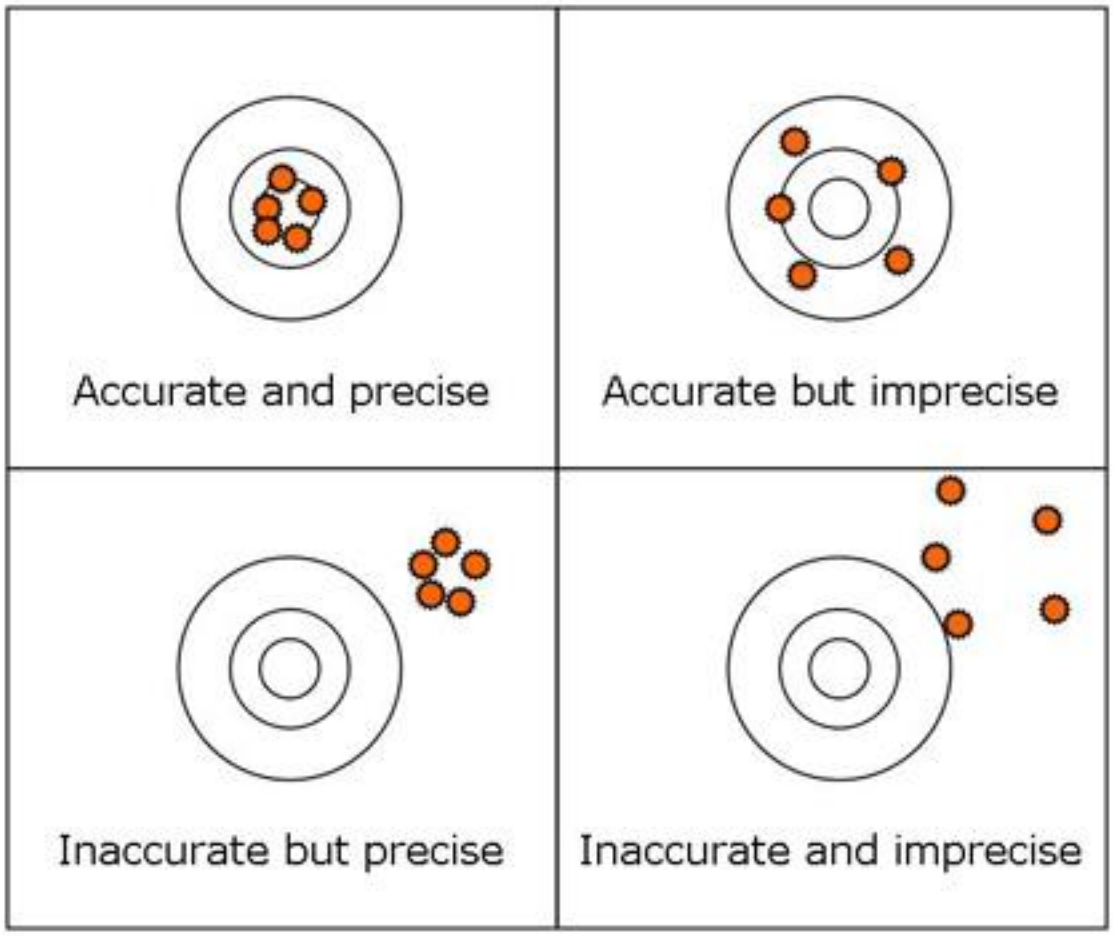


Accuracy/trueness

Interpretation of bias



Accuracy/trueness



Accuracy/truness

Trueness

Expression of the *Trueness*

Trueness → *Recovery*

- Analyze Reagent blank 10 times.
- Analyze reference material 10 times across:

  working range (LoQ – MpL – a 3rd point)
- -Calculate the Mean blank (c_1)
- Calculate the mean value of reference material (c2
- Subtract Mean blank from mean of reference material.
- -Compare with true or accepted true values for the reference material (c3)

Accuracy/truness

Trueness

Statistics

- Determine the hypothesis
- determine the reagent and the analyte mean).
- the bias = $C_2 - C_1$
- Recovery (%) = (C1-C2)/C3 X 100

Precision

The closeness of agreement between independent test results obtained under stipulated conditions.

Precision

under repeatability

1. Same method
2. the same laboratory,
3. the same operator
4. the same equipment
5. within short time intervals

under reproducibility

1. Same method
2. different laboratories
3. different operators
4. using different equipment.

Evaluation of Precision

➢**Use** Standards, reference materials or fortified sample blanks
(across working range LoQ – MpL – a 3rd point).

A. 10times under repeatability conditions

1. Determine repeatability standard deviation & relative standard deviation (s_r , RSD_r) at each concentration

2. Determine the predicted standard deviation (PRSD) & the HORRAT (r)

3. then determine the repeatability limit.

Evaluation of Precision

B. <u>10times under reproducibility conditions</u>

1. Determine reproducability standard deviation & relative standard deviation *(s_r , RSD_r)* at each concentration

2. Determine the predicted standard deviation (PRSD) & the HORRAT (R)

(HORRAT is the ratio between relative standard deviation and the predicted Relative standard deviation)

3. then determine the reproducibility limit. .

Statistics for precision

A. *under repeatability conditions*

- $PRSD_{r = C}^{(-0.15)}$ (C is the mass fraction)

$r = RSD_r / PRSD_r$ $(0.5 < r < 2)$

$r\ limit = t_{\infty} \times \sqrt{2} \times \sigma_r = 2.8 \times \sigma_r$

Statistics for precision

B. reproducibility conditions

$PRSD_R = 2^* C^{(-0.15)}$ (C is the mass fraction)

$r = RSD_R / PRSD_R$ $(0.5 < R < 2)$

$R\ limit = t_\infty \times \sqrt{2} \times \sigma_R = 2.8 \times \sigma_R$

How to convert ppm (mg/l) to decimal fraction

The part P in decimal is equal to the part P in ppm divided by 1000000:

$$P_{(decimal)} = P_{(ppm)} / 1000000$$

Example

Find the decimal fraction of 300ppm:

$P_{(decimal)}$ = 300ppm / 1000000 = 0.0003

How to convert decimal fraction to ppm

The part P in ppm is equal to the part P in decimal times 1000000:

$$P_{(ppm)} = P_{(decimal)} \times 1000000$$

Example

Find how many ppm are in 0.0034:

$P_{(ppm)}$ = 0.0034 × 1000000 = 3400ppm

How to convert percent to ppm

The part P in percent (%) is equal to the part P in ppm divided by 10000:

$P_{(\%)} = P_{(ppm)} / 10000$

Example

Find how many percent are in 6ppm:

$P_{(\%)} = 6ppm / 10000 = 0.0006\%$

How to convert ppm to percent

The part P in ppm is equal to the part P in percent (%) times 10000:0

$P_{(ppm)} = P_{(\%)} \times 10000$

Example

Find how many ppm are in 6%:

$P_{(ppm)} = 6\% \times 10000 = 60000ppm$

Recovery

1. Analyze Matrix blanks or samples unfortified and fortified with the analyte of interest at a range of concentrations 6 times..
2. -Determine recovery of analyte at the various concentration
3. -Fortified samples should be compared with the same sample unfortified.
4. -Or Analyze Certified reference materials (CRM)
5. -Determine recovery of analyte relative to the certified value

2-Statistics for recovery

- Determine each recovery - Recovery (%) = (C1-C2)/C3 X 100
- Determine hypothesis for each concentration recovery.
- Determine the highst and the lowest recovery

(the recovery must be included in the method recovery).

Concentration Recovery limits

Analyte (%)	Unit	Mean Recovery (%)
100	100%	98-102
10	10%	98-102
1	1%	97-103
0.1	0.1%	95-105
0.01	100 ppm	90-107
0.001	10 ppm	80-110
0.0001	1 ppm	80-110
0.00001	100 ppb	80-110
0.000001	10 ppb	60-115
0.0000001	1 ppb	40-120

Source: AOAC (2002). AOAC Requirements for Single Laboratory Validation of Chemical Methods. DRAFT 2002-11-07, \AOACI\eCam\Single-Lab_Validation_47.

Ruggedness and Robustness

- Intra-laboratory study to check changes due to environmental and/or operating conditions
  - Usually it is part of method development
  - Deliberate changes in
    - Temperature
    - Reagents (e.g. different batches)
    - Extraction time
    - Composition in the sample etc……

Ruggedness and Robustness

Ruggedness Test

In any method there will be certain stages , if not carried out sufficiently carefully,

Will have a ***severe effect on method performance*** and may result in the method

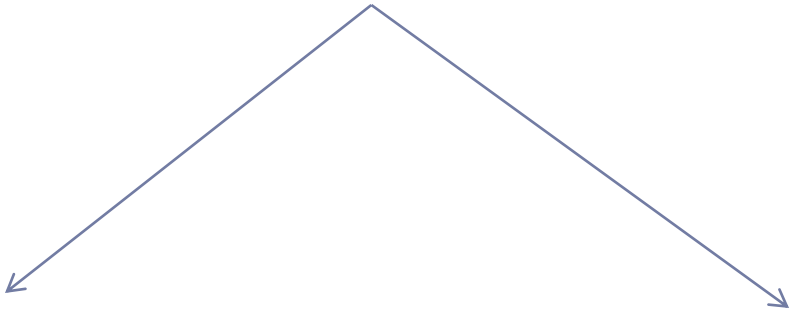
not working at all .

These stages should be identified ,and if possible , their influence on method

performance evaluated using ***Ruggedness Test*** *(some times called* ***robustness test****)*

Ruggedness and Robustness

robustness test



Improvement purpose

QC purpose

Ruggedness and Robustness

evaluation

1. Identify variables which could have a significant effect on the method performance.
2. Analyze R.M or C.R.M
3. Analyze each set of experimental conditions once.
4. Determine the effect of each change of conditions on the mean
5. Rank the variables in order of the greatest effect on the method performance.
6. Design Q.C in order to control the critical variables.
7. Concentrate on these variables for method improvement

Ruggedness and Robustness

Ruggedness Trials

(Plackett-Burman design for 11 factors (N =12))

Exp.	*Factors*											*Response*
	A	B	C	D	E	F	G	H	I	J	K	
1	+	+	-	+	+	+	-	-	-	+	-	y_1
2	-	+	+	-	+	+	+	-	-	-	+	y_2
3	+	-	+	+	-	+	+	+	-	-	-	y_3
4	-	+	-	+	+	-	+	+	+	-	-	y_4
5	-	-	+	-	+	+	-	+	+	+	-	y_5
6	-	-	-	+	-	+	+	-	+	+	+	y_6
7	+	-	-	-	+	-	+	+	-	+	+	y_7
8	+	+	-	-	-	+	-	+	+	-	+	y_8
9	+	+	+	-	-	-	+	-	+	+	-	y_9
10	-	+	+	+	-	-	-	+	-	+	+	y_{10}
11	+	-	+	+	+	-	-	-	+	-	+	y_{11}
12	-	-	-	-	-	-	-	-	-	-	-	y_{12}

Ruggedness and Robustness

Ruggedness Trials
(Youden ,W.J. 1975)

Experiment No.	Factor Combinations	Measurement obtained
1	A B C D E F G	x_1
2	A B c D e f g	x_2
3	A b C d E f g	x_3
4	A b c d e F G	x_4
5	a B C d e F g	x_5
6	a B c d E f G	x_6
7	a b C D e f G	x_7
8	a b c D E F g	x_8

Example (1):

The response of method for analysis Of a parameter (x) for 10 times of sample blank , were:

0.0049 - 0.0051 - 0.0001 - 0.0056 0.0052 - 0.0003 - 0.0002 - 0.0026 - 0.0235 - 0.0004 .

Evaluate the LOQ for your method.

Example (2):

The LOQ of a method to for analysis of parameter (x) was 10 mg/l.

The results of 10 times of analysis of it were :

(10.26 10.32 9.18 10.20 9.65 9.23 10.12 8.25 10.62 10.11)mg/l

And the results of 10 times of analysis of a reagent blank were:

(0.021- - 0.192 - 0.097 - 0.040 - 0.020 - 0.006 - 0.005 - 0.020 0.019 - 0.003) mg/l.

Detect the recovery of the method & detect whether it is accepted or not.

Detect the HORRAT of repeatability & the repeatability limit.

Documentation of Validated Methods

- Documentation of the validation procedure
  - Clear implementation
  - Consistency during application
  - Information has to be easily understood by everyone using the method

The Method Documentation Protocol

- Title
- Scope
- Warning & Safety precautions
- Definitions
- Principle
- Reagents & Materials
- Apparatus and equipment
- Sampling and samples

The Method Documentation Protocol

- Calibration
- Quality Control
- Procedure
- Calculation
- Reporting procedures including expression of results
- Normative references
- Appendix on method validation
- Appendix on measurement uncertainty

Strategy for Method Validation1.

1. Develop a validation protocol, an operating procedure or a validation master plan

2. For a specific validation project define owners and responsibilities.

3. Develop a validation project plan.

4. Define the application, purpose and scope of the method.

5. Define the performance parameters and acceptance criteria.

6. Define validation experiments.

7. Document validation experiments and results in the validation report.

conclusion

- Validation data to give answers and solutions to customer's problems
- Prove "fitness for purpose"
- Is ongoing process
  - New compounds
  - New methods
- prove your ability to detect , identify and quantify analytes reliably


Thank you ...