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Contents

B.Pharm VIII sem UNIT IV Basic Statistics & Validation of analytical methods & equipments:

1 Validation: Analytical Methods: ICH Guidelines

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

VALIDATION OF ANALYTICAL PROCEDURES: TEXT AND METHODOLOGY
Q2(R1)

Current *Step 4* version
Parent Guideline dated 27 October 1994
(Complementary Guideline on Methodology dated 6 November 1996
incorporated in November 2005)

This Guideline has been developed by the appropriate ICH Expert Working Group and has been subject to consultation by the regulatory parties, in accordance with the ICH Process. At Step 4 of the Process the final draft is recommended for adoption to the regulatory bodies of the European Union, Japan and USA.

Q2(R1)
Document History

First Codification	History	Date	New Codification **November 2005**

Parent Guideline: Text on Validation of Analytical Procedures

Q2	Approval by the Steering Committee under *Step 2* and release for public consultation.	26 October 1993	Q2
Q2A	Approval by the Steering Committee under *Step 4* and recommendation for adoption to the three ICH regulatory bodies.	27 October 1994	Q2

Guideline on Validation of Analytical Procedures: Methodology developed to complement the Parent Guideline

Q2B	Approval by the Steering Committee under *Step 2* and release for public consultation.	29 November 1995	in Q2(R1)
Q2B	Approval by the Steering Committee under *Step 4* and recommendation for adoption to the three ICH regulatory bodies.	6 November 1996	in Q2(R1)

Current *Step 4* version

Q2A and Q2B	The parent guideline is now renamed Q2(R1) as the guideline Q2B on methology has been incorporated to the parent guideline. The new title is "Validation of Analytical Procedures: Text and Methodology".	November 2005	Q2(R1)

VALIDATION OF ANALYTICAL PROCEDURES:
TEXT AND METHODOLOGY

ICH Harmonised Tripartite Guideline

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PART I:

TEXT ON VALIDATION OF ANALYTICAL PROCEDURES

ICH Harmonised Tripartite Guideline

Having reached Step 4 of the ICH Process at the ICH Steering Committee meeting on 27 October 1994, this guideline is recommended for adoption to the three regulatory parties to ICH

1. Introduction

This document presents a discussion of the characteristics for consideration during the validation of the analytical procedures included as part of registration applications submitted within the EC, Japan and USA. This document does not necessarily seek to cover the testing that may be required for registration in, or export to, other areas of the world. Furthermore, this text presentation serves as a collection of terms, and their definitions, and is not intended to provide direction on how to accomplish validation. These terms and definitions are meant to bridge the differences that often exist between various compendia and regulators of the EC, Japan and USA.

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. A tabular summation of the characteristics applicable to identification, control of impurities and assay procedures is included. Other analytical procedures may be considered in future additions to this document.

2. Types of Analytical Procedures to be Validated

The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

- Identification tests;
- Quantitative tests for impurities' content;
- Limit tests for the control of impurities;
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

Although there are many other analytical procedures, such as dissolution testing for drug products or particle size determination for drug substance, these have not been addressed in the initial text on validation of analytical procedures. Validation of these additional analytical procedures is equally important to those listed herein and may be addressed in subsequent documents.

A brief description of the types of tests considered in this document is provided below.

- Identification tests are intended to ensure the identity of an analyte in a sample. This is normally achieved by comparison of a property of the sample (e.g., spectrum, chromatographic behavior, chemical reactivity, etc) to that of a reference standard;

- Testing for impurities can be either a quantitative test or a limit test for the impurity in a sample. Either test is intended to accurately reflect the purity characteristics of the sample. Different validation characteristics are required for a quantitative test than for a limit test;

- Assay procedures are intended to measure the analyte present in a given sample. In the context of this document, the assay represents a quantitative measurement of the major component(s) in the drug substance. For the drug product, similar validation characteristics also apply when assaying for the active or other selected component(s). The same validation characteristics may also apply to assays associated with other analytical procedures (e.g., dissolution).

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics which should be considered are listed below:

Accuracy

Precision

Repeatability

Intermediate Precision

Specificity

Detection Limit

Quantitation Limit

Linearity

Range

Each of these validation characteristics is defined in the attached Glossary. The table lists those validation characteristics regarded as the most important for the validation of different types of analytical procedures. This list should be considered typical for the analytical procedures cited but occasional exceptions should be dealt with on a case-by-case basis. It should be noted that robustness is not listed in the table but should be considered at an appropriate stage in the development of the analytical procedure.

Furthermore revalidation may be necessary in the following circumstances:

- changes in the synthesis of the drug substance;
- changes in the composition of the finished product;
- changes in the analytical procedure.

The degree of revalidation required depends on the nature of the changes. Certain other changes may require validation as well.

TABLE

Type of analytical procedure characteristics	IDENTIFICATION	TESTING FOR IMPURITIES quantitat.	 limit	ASSAY - dissolution (measurement only) - content/potency
Accuracy	-	+	-	+
Precision				
Repeatability	-	+	-	+
Interm.Precision	-	+ (1)	-	+ (1)
Specificity (2)	+	+	+	+
Detection Limit	-	- (3)	+	-
Quantitation Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

- signifies that this characteristic is not normally evaluated

+ signifies that this characteristic is normally evaluated

(1) in cases where reproducibility (see glossary) has been performed, intermediate precision is not needed

(2) lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s)

(3) may be needed in some cases

GLOSSARY

1. ANALYTICAL PROCEDURE

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc.

2. SPECIFICITY

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

This definition has the following implications:

Identification: to ensure the identity of an analyte.

Purity Tests: to ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.

Assay (content or potency):
to provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.

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.

This is sometimes termed trueness.

4. PRECISION

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.

Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

4.1. Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision .

4.2. Intermediate precision

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

4.3. Reproducibility

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

5. DETECTION LIMIT

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

6. QUANTITATION LIMIT

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

7. LINEARITY

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.

8. RANGE

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

9. ROBUSTNESS

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

PART II:

VALIDATION OF ANALYTICAL PROCEDURES: METHODOLOGY

ICH Harmonised Tripartite Guideline

Having reached Step 4 of the ICH Process at the ICH Steering Committee meeting on 6 November 1996, and incorporated into the core guideline in November 2005, this guideline is recommended for adoption to the three regulatory parties to ICH

INTRODUCTION

This document is complementary to the parent document which presents a discussion of the characteristics that should be considered during the validation of analytical procedures. Its purpose is to provide some guidance and recommendations on how to consider the various validation characteristics for each analytical procedure**.** In some cases (for example, demonstration of specificity), the overall capabilities of a number of analytical procedures in combination may be investigated in order to ensure the quality of the drug substance or drug product. In addition, the document provides an indication of the data which should be presented in a registration application .

All relevant data collected during validation and formulae used for calculating validation characteristics should be submitted and discussed as appropriate.

Approaches other than those set forth in this guideline may be applicable and acceptable. It is the responsibility of the applicant to choose the validation procedure and protocol most suitable for their product. However it is important to remember that the main objective of validation of an analytical procedure is to demonstrate that the procedure is suitable for its intended purpose. Due to their complex nature, analytical procedures for biological and biotechnological products in some cases may be approached differently than in this document.

Well-characterized reference materials, with documented purity, should be used throughout the validation study. The degree of purity necessary depends on the intended use.

In accordance with the parent document, and for the sake of clarity, this document considers the various validation characteristics in distinct sections. The arrangement of these sections reflects the process by which an analytical procedure may be developed and evaluated.

In practice, it is usually possible to design the experimental work such that the appropriate validation characteristics can be considered simultaneously to provide a sound, overall knowledge of the capabilities of the analytical procedure, for instance: specificity, linearity, range, accuracy and precision.

1. SPECIFICITY

An investigation of specificity should be conducted during the validation of identification tests, the determination of impurities and the assay. The procedures used to demonstrate specificity will depend on the intended objective of the analytical procedure.

It is not always possible to demonstrate that an analytical procedure is specific for a particular analyte (complete discrimination). In this case a combination of two or

more analytical procedures is recommended to achieve the necessary level of discrimination.

1.1. Identification

Suitable identification tests should be able to discriminate between compounds of closely related structures which are likely to be present. The discrimination of a procedure may be confirmed by obtaining positive results (perhaps by comparison with a known reference material) from samples containing the analyte, coupled with negative results from samples which do not contain the analyte. In addition, the identification test may be applied to materials structurally similar to or closely related to the analyte to confirm that a positive response is not obtained. The choice of such potentially interfering materials should be based on sound scientific judgement with a consideration of the interferences that could occur.

1.2. Assay and Impurity Test(s)

For chromatographic procedures, representative chromatograms should be used to demonstrate specificity and individual components should be appropriately labelled. Similar considerations should be given to other separation techniques.

Critical separations in chromatography should be investigated at an appropriate level. For critical separations, specificity can be demonstrated by the resolution of the two components which elute closest to each other.

In cases where a non-specific assay is used, other supporting analytical procedures should be used to demonstrate overall specificity. For example, where a titration is adopted to assay the drug substance for release, the combination of the assay and a suitable test for impurities can be used.

The approach is similar for both assay and impurity tests:

1.2.1 Impurities are available

For the assay , this should involve demonstration of the discrimination of the analyte in the presence of impurities and/or excipients; practically, this can be done by spiking pure substances (drug substance or drug product) with appropriate levels of impurities and/or excipients and demonstrating that the assay result is unaffected by the presence of these materials (by comparison with the assay result obtained on unspiked samples).

For the impurity test, the discrimination may be established by spiking drug substance or drug product with appropriate levels of impurities and demonstrating the separation of these impurities individually and/or from other components in the sample matrix.

1.2.2 Impurities are not available

If impurity or degradation product standards are unavailable, specificity may be demonstrated by comparing the test results of samples containing impurities or degradation products to a second well-characterized procedure e.g.: pharmacopoeial method or other validated analytical procedure (independent procedure). As appropriate, this should include samples stored under relevant stress conditions: light, heat, humidity, acid/base hydrolysis and oxidation.

- for the assay, the two results should be compared;

- for the impurity tests, the impurity profiles should be compared.

Peak purity tests may be useful to show that the analyte chromatographic peak is not attributable to more than one component (e.g., diode array, mass spectrometry).

2. LINEARITY

A linear relationship should be evaluated across the range (see section 3) of the analytical procedure. It may be demonstrated directly on the drug substance (by dilution of a standard stock solution) and/or separate weighings of synthetic mixtures of the drug product components, using the proposed procedure. The latter aspect can be studied during investigation of the range.

Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares. In some cases, to obtain linearity between assays and sample concentrations, the test data may need to be subjected to a mathematical transformation prior to the regression analysis. Data from the regression line itself may be helpful to provide mathematical estimates of the degree of linearity.

The correlation coefficient, y-intercept, slope of the regression line and residual sum of squares should be submitted. A plot of the data should be included. In addition, an analysis of the deviation of the actual data points from the regression line may also be helpful for evaluating linearity.

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.

For the establishment of linearity, a minimum of 5 concentrations is recommended. Other approaches should be justified.

3. RANGE

The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by 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.

The following minimum specified ranges should be considered:

- for the assay of a drug substance or a finished (drug) product: normally from 80 to 120 percent of the test concentration;
- for content uniformity, covering a minimum of 70 to 130 percent of the test concentration, unless a wider more appropriate range, based on the nature of the dosage form (e.g., metered dose inhalers), is justified;
- for dissolution testing: +/-20 % over the specified range;

e.g., if the specifications for a controlled released product cover a region from 20%, after 1 hour, up to 90%, after 24 hours, the validated range would be 0-110% of the label claim.

- for the determination of an impurity: from the reporting level of an impurity[1] to 120% of the specification;
- for impurities known to be unusually potent or to produce toxic or unexpected pharmacological effects, the detection/quantitation limit should be commensurate with the level at which the impurities must be controlled;

  Note: for validation of impurity test procedures carried out during development, it may be necessary to consider the range around a suggested (probable) limit.

- if assay and purity are performed together as one test and only a 100% standard is used, linearity should cover the range from the reporting level of the impurities[1] to 120% of the assay specification.

4. ACCURACY

Accuracy should be established across the specified range of the analytical procedure.

4.1. Assay

4.1.1 Drug Substance

Several methods of determining accuracy are available:

a) application of an analytical procedure to an analyte of known purity (e.g. reference material);

b) comparison of the results of the proposed analytical procedure with those of a second well-characterized procedure, the accuracy of which is stated and/or defined (independent procedure, see 1.2.);

c) accuracy may be inferred once precision, linearity and specificity have been established.

4.1.2 Drug Product

Several methods for determining accuracy are available:

a) application of the analytical procedure to synthetic mixtures of the drug product components to which known quantities of the drug substance to be analysed have been added;

b) in cases where it is impossible to obtain samples of all drug product components , it may be acceptable either to add known quantities of the analyte to the drug product or to compare the results obtained from a second, well characterized procedure, the accuracy of which is stated and/or defined (independent procedure, see 1.2.);

c) accuracy may be inferred once precision, linearity and specificity have been established.

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[1] see chapters “Reporting Impurity Content of Batches” of the corresponding ICH-Guidelines: “Impurities in New Drug Substances” and “Impurities in New Drug Products”

4.2. Impurities (Quantitation)

Accuracy should be assessed on samples (drug substance/drug product) spiked with known amounts of impurities.

In cases where it is impossible to obtain samples of certain impurities and/or degradation products, it is considered acceptable to compare results obtained by an independent procedure (see 1.2.). The response factor of the drug substance can be used.

It should be clear how the individual or total impurities are to be determined e.g., weight/weight or area percent, in all cases with respect to the major analyte.

4.3. Recommended Data

Accuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g., 3 concentrations/3 replicates each of the total analytical procedure).

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.

5. PRECISION

Validation of tests for assay and for quantitative determination of impurities includes an investigation of precision.

5.1. Repeatability

Repeatability should be assessed using:

a) a minimum of 9 determinations covering the specified range for the procedure (e.g., 3 concentrations/3 replicates each);

or

b) a minimum of 6 determinations at 100% of the test concentration.

5.2. Intermediate Precision

The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. The applicant should establish the effects of random events on the precision of the analytical procedure. Typical variations to be studied include days, analysts, equipment, etc. It is not considered necessary to study these effects individually. The use of an experimental design (matrix) is encouraged.

5.3. Reproducibility

Reproducibility is assessed by means of an inter-laboratory trial. Reproducibility should be considered in case of the standardization of an analytical procedure, for instance, for inclusion of procedures in pharmacopoeias. These data are not part of the marketing authorization dossier.

5.4. Recommended Data

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

6. DETECTION LIMIT

Several approaches for determining the detection limit are possible, depending on whether the procedure is a non-instrumental or instrumental. Approaches other than those listed below may be acceptable.

6.1. Based on Visual Evaluation

Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods.

The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

6.2. Based on Signal-to-Noise

This approach can only be applied to analytical procedures which exhibit baseline noise.

Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit.

6.3 Based on the Standard Deviation of the Response and the Slope

The detection limit (DL) may be expressed as:

$$DL = \frac{3.3\,\sigma}{S}$$

where σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways, for example:

6.3.1 Based on the Standard Deviation of the Blank

Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

6.3.2 Based on the Calibration Curve

A specific calibration curve should be studied using samples containing an analyte in the range of DL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

6.4 Recommended Data

The detection limit and the method used for determining the detection limit should be presented. If DL is determined based on visual evaluation or based on signal to noise ratio, the presentation of the relevant chromatograms is considered acceptable for justification.

In cases where an estimated value for the detection limit is obtained by calculation or extrapolation, this estimate may subsequently be validated by the independent analysis of a suitable number of samples known to be near or prepared at the detection limit.

7. QUANTITATION LIMIT

Several approaches for determining the quantitation limit are possible, depending on whether the procedure is a non-instrumental or instrumental. Approaches other than those listed below may be acceptable.

7.1. Based on Visual Evaluation

Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods.

The quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

7.2. Based on Signal-to-Noise Approach

This approach can only be applied to analytical procedures that exhibit baseline noise.

Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably quantified. A typical signal-to-noise ratio is 10:1.

7.3. Based on the Standard Deviation of the Response and the Slope

The quantitation limit (QL) may be expressed as:

$$QL = \frac{10\,\sigma}{S}$$

where σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways for example:

7.3.1 Based on Standard Deviation of the Blank

Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

7.3.2 Based on the Calibration Curve

A specific calibration curve should be studied using samples, containing an analyte in the range of QL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

7.4 Recommended Data

The quantitation limit and the method used for determining the quantitation limit should be presented.

The limit should be subsequently validated by the analysis of a suitable number of samples known to be near or prepared at the quantitation limit.

8. ROBUSTNESS

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g., resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used.

Examples of typical variations are:

- stability of analytical solutions;
- extraction time.

In the case of liquid chromatography, examples of typical variations are:

- influence of variations of pH in a mobile phase;
- influence of variations in mobile phase composition;
- different columns (different lots and/or suppliers);
- temperature;
- flow rate.

In the case of gas-chromatography, examples of typical variations are:

- different columns (different lots and/or suppliers);
- temperature;
- flow rate.

9. SYSTEM SUITABILITY TESTING

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated. See Pharmacopoeias for additional information.

2 Basic Statistics

2.1 Measures of Central Tendency

Measures of central tendency, also known as measures of location, are typically among the first statistics computed for the continuous variables in a new data set.The main purpose of computing measures of central tendency is to give one an idea of what is a typical or common value for a given variable.The common measures of central tendency are 1) Arithmetic Mean 2) Median 3) Mode 4) Weighed Arithmetic Mean 5) Harmonic Mean 6) Geometric Mean .

1. **Arithmetic Mean** The arithmetic mean is calculated by adding up all the values and dividing by the number of values. The mean of a population is denoted by the Greek letter mu ($\boldsymbol{\mu}$) while the mean of a sample is typically denoted by a bar over the variable symbol: The mean of x would be designated $\bar{x}$ and pronounced "x-bar."
   For example, if we have the following values of the variable x: 93, 88, 97, 100, 103. We calculate the mean by adding them up and dividing by 5 (the number of values):
   $\boldsymbol{x = (93 + 88 + 97 + 100 + 103)/5 = 481/5 = 96.2}$
   Summation notation, $\sum$, which defines a statistic by expressing how it is calculated. The difference is the symbol for the mean itself Population mean $\boldsymbol{\mu}$ and sample mean $\bar{x}$. The mean of a data set, as expressed in summation notation, is:

$$\boxed{\bar{x} = 1/n \sum_{i=1}^{n} x_i} \tag{2.1}$$

2. **Median** The median of a data set is the middle value when the values are ranked in ascending or descending order. If there are n values, the median is formally defined as the ((n+1)/2)th value. If n = 7, the middle value is the ((7+1)/2)th or fourth value. If there is an even number of values, the median is the average of the two middle values. This is formally defined as the average of the (n/2)th and ((n/2)+1)th value. If there are six values, the median is the average of the (6/2)th and ((6/2)+1)th value, or the third and fourth values.:
   Odd number of values:
   1, 2, 3, 4, 5, 6, 7 median = 4
   Even number of values:
   1, 2, 3, 4, 5, 6 median = (3+4)/2 = 3.5
   so, for the above data, median is 97.
   The median is a better measure of central tendency than the mean for data that is asymmetrical or contains outliers. This is because the median is based on the ranks of data points rather than their actual values: 50 percent of the data values in a distribution lie below the median, and 50 percent above the median, without regard to the actual values in question. Therefore it does not matter if the data set contains some extremely large or small values, because they will not affect the median more than less extreme values.

3. **Mode** the mode, which refers to the most frequently occurring value. The mode is most useful in describing categorical data. For example, if the the numbers below reflect the favored news sources of a group of students, where 1 = english newspapers, 2 = local newspapers, 3 = television and 4 = Internet:
   1, 1, 2, 2, 2, 2, 3, 3, 3, 3, 3, 3, 3, 4,4,4,4,4,4,4,4,4,4,4,4.

We can see that the Internet is the most popular source because 4 is the most common value in this data set. In a symmetrical distribution such as the normal distribution, the mean, median, and mode are identical. In an asymmetrical or skewed distribution they differ, and the amount by which they differ is one way to evaluate the skewness of a distribution.

4. **Weighted Arithmetic Mean** A weighted mean is a kind of average. Instead of each data point contributing equally to the final mean, some data points contribute more "weight" than others. If all the weights are equal, then the weighted mean equals the arithmetic mean. Weighted means are very common in statistics, especially when studying populations.
   To find the weighted mean:

$$\text{Weighted Mean} = \frac{\sum_{i=1}^{n}(x_i \times w_i)}{\sum_{i=1}^{n} w_i} \tag{2.2}$$

   The natural abundance of Carbon occurs as isotope-12, 99% and isotope-13, 1%. If the atomic weight of Carbon element is calculated as simple arithmetic mean, that is equal weightage for both isotopes, then it would be $\mathbf{12 + 13 = 12.5}$.
   When atomic weight is computed giving weightage based on actual occurance, then using the weighted arithmetic mean formula: $\mathbf{[(99)(12) + (1)(13)]/100 = 12.01}$ The actual atomic weight of carbon is 12.01.

5. **Harmonic Mean** Harmonic Mean is the reciprocal of the arithmetic mean of the reciprocals.It is the number of observations, divided by the sum of reciprocals of the observations. It is appropriate for situations when the average of rates is desired. The harmonic mean is involved in many situations where rates, ratios, geometry, trigonometry etc considered, the harmonic mean provides the truest average.The Harmonic mean is always the lowest mean.

$$\boldsymbol{HM = \frac{N}{1/x_1 + 1/x_2 + 1/x_3 + \cdots + 1/x_n}} \tag{2.3}$$

   Where, x_i= Individual score
   N = Sample size (Number of scores)
   To find the Harmonic Mean of 1,2,3,4,5,6,7,8.
   So, Harmonic Mean = 2.94.

6. **Geometric Mean** The geometric mean is a type of mean or average, which indicates the central tendency or typical value of a set of numbers by using the product of their values. The geometric mean is defined as the nth root of the product of n numbers.

$$\boldsymbol{GM = \left\{\prod_{n=1}^{k} x_n\right\}^{1/k}} \tag{2.4}$$

   # What is the geometric mean of 3, 5 and 7?
   $\mathbf{\sqrt[3]{3 \times 5 \times 7} = 4.72}$
   The PI symbol in statistics means to multiply a series of numbers. The definition

says to multiply k numbers and then take the kth root. The geometric mean only works with positive numbers. Negative numbers could result in imaginary results depending on how many negative numbers are in a set. Most uses of the geometric mean involve real data, such as the length of physical objects or the number of people responding to a survey.
If a measurement of population growth shows 50 at time 0, 100 after one day, and 200 after two days, the geometric mean (100) is more meaningful than the arithmetic mean (116.7).The geometric mean is always less than or equal to the arithmetic mean, and is meaningful for data with logarithmic relationships.

$$\sqrt[3]{50 \times 100 \times 200} = [\log 50 + \log 100 + \log 200]/3 = \text{antilog}[2] = 100 \qquad (2.5)$$

2.2 Measures of Dispersion

Dispersion refers to how variable or "spread out" data values are: for this reason measures of dispersions are sometimes called "measures of variability" or "measures of spread."

1. **The Range** The range, which is the difference between the highest and lowest values. Often the minimum (smallest) and maximum (largest) values are reported as well as the range. For the data set (93, 88, 97, 100, 103), the minimum is 88, the maximum is 103, and the range is (103 - 88) = 15.

2. **The Variance and Standard Deviation** For continuous data, the most common measures of dispersion are the variance and standard deviation. Both describe how much the individual values in a data set vary from the mean or average value.The variance is the average of the squared deviations from the mean, and the standard deviation is the square root of the variance. The variance of a population is signified by σ^2, and the standard deviation as σ, while the sample variance and standard deviation are signified by s^2 and s, respectively.
Here for σ^2 calculation $\bar{x}$ is assumed to be equal to μ as it is calculated from large number of samples. Written in summation notation, the formula to calculate the sum of all deviations from the mean and squared for a data set with n observations and divide their sum by n, the number of cases, to get the average deviation or variance for a population:

$$\boxed{\sigma^2 = \frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{x})^2} \qquad (2.6)$$

Another way to calculate σ^2

$$\boxed{\sigma^2 = \frac{n(\sum x^2) - (\sum x)^2}{n^2}} \qquad (2.7)$$

The sample formula for the variance requires dividing by n - 1 rather than n because we lose one degree of freedom when we calculate the mean. The formula for the variance of a sample, notated as s^2 , is therefore:

$$\boxed{s^2 = \frac{1}{(n-1)}\sum_{i=1}^{n}(x_i - \bar{x})^2} \qquad (2.8)$$

Another way to calculate sample variance s^2:

$$\boxed{s^2 = \frac{n(\sum x^2) - (\sum x)^2}{n(n-1)}} \tag{2.9}$$

The square root of the variance is called the standard deviation and is signified by σ for a population and s for a sample. The formula for a population standard deviation is:

$$\boxed{\sigma = \sqrt{\sigma^2} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{x})^2}} \tag{2.10}$$

The formula for the sample standard deviation is:

$$\boxed{s = \sqrt{s^2} = \sqrt{\frac{1}{(n-1)}\sum_{i=1}^{n}(x_i - \bar{x})^2}} \tag{2.11}$$

3. **Degrees of Freedom:** In statistics, the number of degrees of freedom is the number of values in the final calculation of a statistic that are free to vary.

4. **Coefficient of Variation**
   The coefficient of variation (CV), a measure of relative variability, and makes it possible to compare variability across variables measured in different units. . The CV is calculated by dividing the standard deviation by the mean, then multiplying by 100.

$$\boxed{CV = \frac{s}{\bar{x}} \times 100} \tag{2.12}$$

5. **Standard Error of Mean(SEM)**
   SEM is usually estimated by the sample standard deviation divided by the square root of the sample size.

$$\boxed{SE_{\bar{x}} = \frac{s}{\sqrt{n}}} \tag{2.13}$$

   This estimate may be compared with the formula for the true standard deviation of the sample mean:

$$\boxed{SE_{\bar{x}} = \frac{\sigma}{\sqrt{n}}} \tag{2.14}$$

   .

2.3 Checking it Out!

1. Calculate the measures of dispersion with following data:55, 48, 63, 39, 44.

Table 2.1: Measures of Dispersion Calculation

x data	$(x - \bar{x})$	$(x - \bar{x})^2$
55	5.2	27.04
48	-1.8	3.24
63	13.2	174.24
39	-10.8	116.64
44	-5.8	33.64
$\sum = \mathbf{249}$	$\sum = \mathbf{0}$	$\sum = \mathbf{354.8}$
$\bar{x} = \mathbf{49.8}$	0	$\sigma^2 = \mathbf{70.96}$
$\bar{x} = \mathbf{49.8}$	0	$\sigma = \mathbf{8.4237}$
$\bar{x} = \mathbf{49.8}$	0	$s^2 = \mathbf{88.7}$
$\bar{x} = \mathbf{49.8}$	0	$s = \mathbf{9.4180}$

2. For the following data: 119, 98, 101, 88, 104, 102, 108, 108, 93, 112. Calculate a) the mean, b) the standard deviation, c) the variance, d) the coefficient of variation, e) the range, and f) the median.

3. Compute the arithmetic mean, geometric mean, and harmonic mean of the following set of data. 3, 5, 7, 13, 17, 29 , 57.

4. If the weights are 2, 1, 1, 3, 3, and 2 for the numbers 5, 7, 8, 11, 14, and 44 compute the weighted average and variance.

2.4 Confidence Interval

A confidence interval, in statistics, refers to the probability that a population parameter will fall between two set values for a certain proportion of time. Confidence intervals measure the degree of uncertainty or certainty in a sampling method. A confidence interval can take any number of probabilities, with the most common being a 95% or 99% confidence level.
The extreme values of the interval are called the confidence limits. The term 'confidence' implies that we can assert with a given degree of confidence, i.e. a certain probability, that the confidence interval does include the true value. The size of the confidence interval will depend on how certain we want to be that it includes the true value: the greater the certainty, the greater the interval required.

Factors affecting the width of the confidence interval include the size of the sample, the confidence level, and the variability in the sample. A larger sample size normally will lead to a better estimate of the population parameter.
Figure 2.1 shows the sampling distribution of the mean for samples of size n. If we assume that this

distribution is normal, then 95% of the sample means will lie in the range given by:

$$\mu - 1.96(\sigma/\sqrt{n}) < \bar{x} < \mu + 1.96(\sigma/\sqrt{n}) \tag{2.15}$$

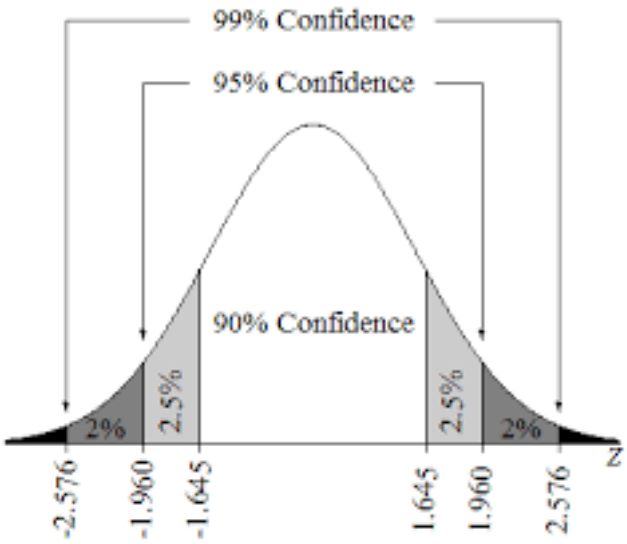

Figure 2.1: The sampling distribution of the mean, showing the range within which 95% of sample means lie.

(When we use z- table to check that the proportion of values between z = -1.96 and z = 1.96 is 0.95 - looking it up in reverse). In reality we are unlikely to know σ exactly. When the sample is large, σ can be replaced by its estimate, 's'. Other confidence limits are sometimes used, in particular the 99% and 99.7% confidence limits.
For large samples, the confidence limits of the mean are given by

$$\bar{x} \pm zs/\sqrt{n} \tag{2.16}$$

where the value of z depends on the degree of confidence required. For 95% confidence limits, z = 1.96 For 99% confidence limits, z = 2.58 For 99.7% confidence limits, z = 2.97

2.5 Tests of Significance or Hypothesis Testing

Hypothesis testing is an act in statistics whereby an analyst/statistician/researcher tests an assumption regarding a population parameter.
Every test of significance begins with a null hypothesis H_0. For a new drug testing, the null hypothesis might be that the new drug is no better, on average, than the current drug. We would write H_0: there is **no difference** between the two drugs on average.
The alternative hypothesis might be that the new drug has a different effect, on average, compared to that of the current drug. We could write H_a: the two drugs have different effects, on average - **it could be better or worse.** - But, it should be remembered that the test decision (better or worse) should be 'priori' before setting experiment.
The final conclusion once the test has been carried out is always given in terms of the null hypothesis. We either "reject H_0 in favor of H_a" or "do not reject H_0"; we never conclude "reject H_a", or even

"accept H_a". The significance level for a given hypothesis test is a value for which a P-value less than or equal to is considered statistically significant. Typical values for are 0.1, 0.05, and 0.01.
Type I error, is rejecting a null hypothesis when it is actually correct and accepting alternate hypothesis.
Type II error is accepting a null hypothesis when the alternate hypothesis should have been accepted.
To minimize the probability of Type I error, the significance level is generally chosen to be small.

2.6 t-tests [paired, unpaired]

Comparison of an experimental mean with a known value

$$t = \frac{(\bar{x} - \mu)\sqrt{n}}{s} \tag{2.17}$$

If |t| (i.e. the calculated value of 't' without regard to sign) exceeds a certain critical value then the null hypothesis is rejected. The critical value of t for a given significance level can be found from t - table.
where $\bar{x}$ = sample mean, s = sample standard deviation and n = sample size.
Problem:
In a new method for determining Tramadol (pain reliever & habit forming drug) in tablets with label claim the following values were obtained:
50.9, 50.7, 48.1, 49.6, 49.1 mg/tablet
Is there any evidence of systematic error?
The mean of these values is 49.68 and the standard deviation is 1.16. Adopting the null hypothesis that there is no systematic error, i.e. that $\mu = 50$, Eq. (2.17) gives:

$$t = \frac{(49.68 - 50)\sqrt{5}}{1.16} = -0.6168$$

From Table , the critical value is $t_{4,0.05} = 2.78$. Since the observed/calculated value of | t | is lesser than the critical value the null hypothesis is retained: there is no evidence of systematic error.
Comparison of two experimental means
Problem:
In a comparison of two methods for the determination of Lorazepam (treatment of anxiety)in tablet dosage forms, the following results mg/tablet were obtained. A new analytical method is tested by comparing it with those obtained by using a standard/regular method.:
Method 1: 1.1, 1.3, 0.9, 0.8, 1.1 mean = 1.04; standard deviation 0.1949
Method 2: 0.8, 0.9, 09, 0.7, 0.8 mean = 0.82 ; standard deviation 0.0836
For each method five determinations were made. Do these two methods give results which differ significantly at P = 0.05?
The two methods give two sample means, $\bar{x}_1$ and $\bar{x}_2$. The null hypothesis is that the two methods give the same result, i.e. H_0 : $\mu_1 = \mu_2$, or $\mu_1 - \mu_2 = 0$, so we need to test whether $(\bar{x}_1 - \bar{x}_2)$ differs significantly from zero. If these standard deviations are not significantly different, a pooled estimate, s, of the standard deviation can first be calculated using the equation:

$$s^2 = \frac{(n_1 - 1){s^2}_1 + (n_2 - 1){s^2}_2}{(n_1 + n_2) - 2} \tag{2.18}$$

To decide whether the difference between the two means, $\bar{x}_1$ and $\bar{x}_2$, is significant, i.e. to test the null hypothesis, $H_0 : \mu_1 = \mu_2$, the statistic 't' is calculated from:

$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{s\sqrt{1/n_1 + 1/n_2}} \tag{2.19}$$

where t has ($n_1 + n_2 - 2$) degrees of freedom.

From Eq. (2.18), the pooled value of the standard deviation is given by:

$$s^2 = \frac{(|4 \times 0.1949^2)|+|4 \times 0.0836^2)|}{(5+5)-2} = 0.02248$$

so s = 0.1499.
From Eq. (2.19):

$$t = \frac{(1.04 - 0.82)}{0.1499\sqrt{1/5 + 1/5}} = 2.38$$

There are eight degrees of freedom, the critical value is $t_{8,0.05} = 2.31$. Since the experimental value of |t| is greater (very slightly) than table value, the difference between the two results is significant at the 5% level and the null hypothesis is rejected.

Paired t-test:

The paired sample t-test, is a statistical procedure used to determine whether the mean difference between two sets of observations is zero. In a paired sample t-test, each subject or entity is measured twice, resulting in pairs of observations.

Problem:

Two analytical methods are compared by analysing with both the methods, the table gives the results of determining the drug XXX concentration (% w/w) in tablets. Tablets from five different batches were analysed to see whether the results obtained by the two methods differed. Each batch is giving **a pair** of measurements, that is one value for each method. By taking the difference, d, between each pair of results given by the two methods, and testing whether n paired results are drawn from the same population, that is H_0 : μ_d = 0.

$$t = \frac{\bar{d}\sqrt{n}}{s_d} \tag{2.20}$$

where $\bar{d}$ and s_d are the mean and standard deviation respectively of d values, the differences between the paired values. (Eq.(2.20) is similar to Eq. (2.17).) The number of degrees of freedom of t is n - 1.

Test whether there is a significant difference between the results obtained by the two methods in table 2.2.

These differences have mean, $\bar{d} = -3.024$, and standard deviation, $s_d = 1.3688$. Substituting in Eq. (2.20), with n = 5, gives |t| = 4.9399. The table value is $t_4 = 2.7763$ (P = 0.05). Since the calculated value of | t | is greater than table value the null hypothesis is regected: the methods do give significantly different results for the XXX concentration.

Table 2.2: Analysis Data

Batch	Method 1	Method 2	Difference(I-II)
1	185.17	189.29	- 4.12
2	186.19	190.66	-4.47
3	187.12	189.73	-2.61
4	188.33	191.23	-2.9
5	188.41	189.43	-1.02

3 Statistics: Online Video Links

Links from youtube on statistics learning in hyperlink format.
Click the colored box & Watch.

Documents

1. Lisa Sulivan - Nonparametric Tests

Sites to look for complete statistics learning resources

1. Khan Academy - Statistics - 67 videos

On Line Videos

1. Types of Data: Nominal, Ordinal, Interval/Ratio - Statistics Help
2. Introduction to Statistics (1.1)
3. Types of Sampling Methods (4.1)
4. Bar Charts, Pie Charts, Histograms, Stemplots, Timeplots (1.2)
5. Causation vs Association, and an Introduction to Experiments (3.1)
6. Statistics 101: ANOVA, A Visual Introduction
7. Statistics 101: One-way ANOVA, A Visual Tutorial
8. Statistics 101: One-way ANOVA, Understanding the Calculation
9. Linear Regression - Fun and Easy Machine Learning
10. Correlation & Regression: Concepts with Illustrative examples
11. Statistics made easy ! ! ! Learn about the t-test, the chi square test, the p value and more
12. Mode, Median, Mean, Range, and Standard Deviation (1.3)
13. The Normal Distribution and the 68-95-99.7 Rule (5.2)
14. A Gentle Introduction to Non-Parametric Statistics (15-1)

Video Links from zstastics.com

1. Categorical Data I: Proportions testing | Z test | Chi Squared test
2. Teach me STATISTICS in half an hour!
3. Descriptive Statistics: The Mean
4. Arithmetic Mean | Geometric Mean | Harmonic Mean

5. Descriptive Statistics: The median
6. Descriptive Statistics: The Mode
7. Variance and Std Deviation | Why divide by n-1?
8. Standard Error (of the sample mean) | Sampling | Confidence Intervals | Proportions
9. What is the Coefficient Of Variation?? (+ examples!)
10. What is skewness? A detailed explanation (with moments!)
11. What is Kurtosis? (+ the "peakedness" controversy!)
12. What are Quartiles? Percentiles? Deciles?
13. What are "moments" in statistics? An intuitive video!
14. Range | Interquartile Range (IQR) | Box and whisker plot
15. What are degrees of freedom?!? Seriously.
16. What is Regression? | SSE, SSR, SST | R-squared | Errors
17. Regression II - Degrees of Freedom EXPLAINED | Adjusted R-Squared
18. Regression Output Explained
19. Likelihood | Log likelihood | Sufficiency | Multiple parameters
20. Maximum Likelihood Estimation (MLE) | Score equation | Information | Invariance
21. Hypothesis testing (ALL YOU NEED TO KNOW!)
22. ANOVA: One-way analysis of variance
23. Non-parametric tests - Sign test, Wilcoxon signed rank, Mann-Whitney

4 References

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