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MODERN PHYSICAL AND CHEMICAL METHODS OF ANALYSIS

Topic 1. Introduction to food analysis.
Topic 2. Chromatography and chromatographic analysis methods
Topic 3. Column and thin layer chromatography
Topic 4. High performance liquid chromatography
Topic 5. Gas chromatography
Topic 6. Mass spectrometry
Topic 7. Understanding spectroscopy. Atomic absorption spectroscopy.
Topic 8. Atomic emission and atomic fluorescence spectroscopy
Topic 9. Infrared spectroscopy
Topic 10. Ultraviolet and nuclear magnetic resonance spectroscopy
Topic 11. Refractometry and calorimetry
Topic 12. Multivariate analysis

Reasons for analyzing foods

1. Food safety
2. Government regulations
   (a) Nutrition labeling
   (b) Standards – mandatory and voluntary
   (c) Food inspection and grading
   (d) Authenticity
3. Quality control
4. Research and development

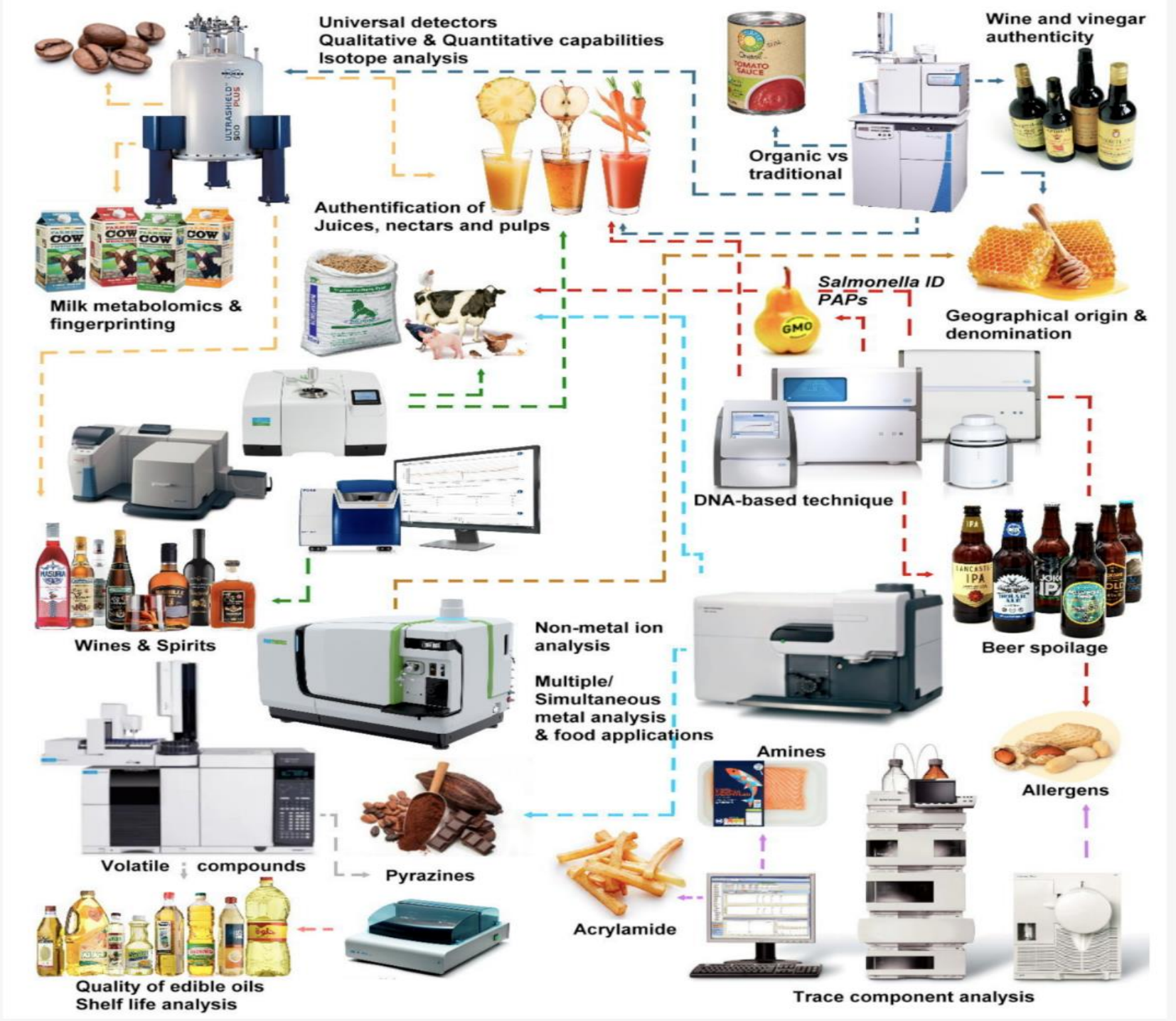
Food analysis

Multiple applications

Different methods

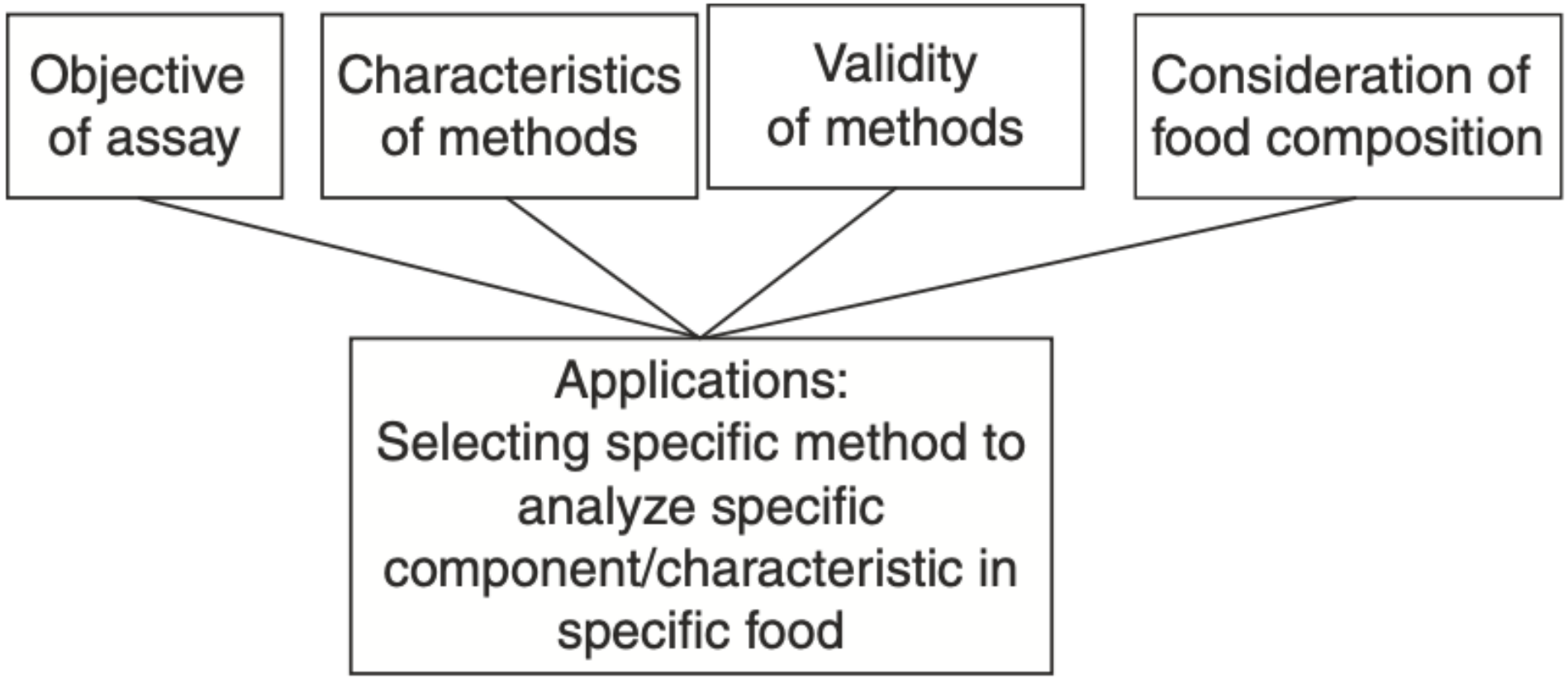
Different stages

Different purposes



MODERN PHYSICAL AND CHEMICAL METHODS OF ANALYSIS

Method selection in food products



Types of samples analyzed in a quality assurance program for food products
Questions to be asked for method selection

Sample type	*Critical questions*
Raw materials	Do they meet your specifications? Do they meet required legal specifications? Are they safe and authentic? Will a processing parameter have to be modified because of any change in the composition of raw materials? Are the quality and composition the same as for previous deliveries? How does the material from a potential new supplier compare to that from the current supplier?
Process samples control	Did a specific processing step result in a product of acceptable composition or characteristics? Does a further processing step need to be modified to obtain a final product of acceptable quality?
Finished product	Does it meet the legal requirements? What is the nutritive value, so that label information can be developed? Or is the nutritive value as specified on an existing label? Does it meet product claim requirements (e.g., "low fat," "gluten free")? Will it be acceptable to the consumer? Will it have the appropriate shelf life? If unacceptable and cannot be salvaged, how do you handle it? (trash? rework? seconds?)
Competitor's sample	What are its composition and characteristics? How can we use this information to develop new products?
Complaint sample	How do the composition and characteristics of a complaint sample submitted by a customer differ from a sample with no problems?

Criteria for choice of food analysis methods: characteristics of a method

Characteristic	*Critical questions*
Inherent properties	
Specificity/selectivity	Is the property being measured the same as that claimed to be measured, and is it the only property being measured? Are there interferences? What steps are being taken to ensure a high degree of specificity?
Precision	What is the precision of the method? Is there within-batch, batch-to-batch, or day-to-day variation?* What step in the procedure contributes the greatest variability?
Accuracy	How does the new method compare in accuracy to the old or a standard method? What is the percent recovery? Is it reproducible between labs?
Applicability of method to laboratory	
Reagents	Can you properly prepare them? What equipment is needed? Are they stable? For how long and under what conditions?
Equipment	Is the method very sensitive to slight or moderate changes in the reagents? Do you have the appropriate equipment? Are personnel competent to operate equipment?
Cost	What is the cost in terms of equipment, reagents, and personnel?
Applicability to food/sample	Destructive or nondestructive? Online or off-line? Official method/approval? Nature of food matrix?
Usefulness	
Time required	How fast is it? How fast does it need to be?
Reliability	How reliable is it from the standpoints of precision and stability?
Need	Does it meet a need or better meet a need? Simplicity of operation?
Personnel	
Safety	Is any change in method worth the trouble of the change?
Procedures	Who will do any required calculations?

Example of a quality management test (for dried pasta)

Component/property being measured	*Name of test*
Quality test done in-house on semolina	
Moisture content	Rapid moisture analyzer
Color (L*, a*, b* determined to calculate Linear E value)	Colorimeter
Quality tests done in-process	
Moisture	Rapid moisture analyzer
Dimensions (after extrusion)	Micrometer and tape measurements by trained personnel
Metal detection	In-line metal detection (ferrous, nonferrous, stainless steel)
Package weight	In-line check with weight scale
Quality tests done on final product	
Moisture	Rapid moisture analyzer
Color L*, a*, b* determined to calculate Linear E value)	Colorimeter
Dimensions (diameter and shape)	Micrometer and tape measurements by trained personnel
Cooking quality	Sensory test by trained personnel (descriptive test; biting of samples)
Label	Visual inspection (with probability sampling methods)

Official Methods in Food Analysis. Who is behind them?

AOAC INTERNATIONAL
Association of Official Agricultural Chemists.

AOAC INTERNATIONAL's Official Methods of Analysis℠ program is the organization's premier program for consensus method development. Methods approved in this program have undergone rigorous scientific and systematic scrutiny and are deemed to be highly credible and defensible.

AACCI
American Association of Cereal Chemist

The AACCI offers check samples such as flours, semolina, and other cereal-based samples, for analyses such as moisture, ash, protein, vitamins, minerals, sugars, total dietary fiber, and soluble and insoluble dietary fiber. Samples also are available for testing physical properties and for microbiological and sanitation analyses.

SMEDP
Standard Methods for the Examination of Dairy Products

NSSP
National Shellfish Sanitation Program

Government regulations regarding the composition of foods often state the official or standard method by which the food is to be analyzed (Example for semolina) (to be included in its **COA** (certificate of analysis)

Properties	*Technical/product data sheet*	*Specification*	*Certificate of analysis*
Chemical			
Moisture	Max value; AACCI	Max value; AOAC/AACCI	Actual value; AACCI
Protein	Min value; AACCI	Min value; AOAC/AACCI	Actual value; AACCI
Ash	Max value; AACCI	Max value; AOAC/AACCI	Actual value; AACCI
Falling number value	Target value, +/–; AACCI		
Enrichment			
Niacin		Max, min, target value; AOAC/AACCI	
Thiamine mononitrate		Max, min, target value; AOAC/AACCI	
Riboflavin		Max, min, target value; AOAC/AACCI	
Ferrous sulfate		Max, min, target value; AOAC/AACCI	
Folic acid		Max, min, target value; AOAC/AACCI	
Physical			
Bran specks	Max value; Internal	Max value; AOAC/AACCI	Actual value; Internal
Black specks	Max value; Internal	Max value; Internal	Actual value; Internal
Color L, a, b		Max L value, Min b value; AOAC/AACCI	Actual values (Hunter)
Color (linear E)			Actual value (calculated from Hunter LAB)
Extraneous matter	Complies with FDA regulations; AACCI		
Insect fragments		Max value; AOAC/AACCI	
Rodent hair		Max value; AOAC/AACCI	
Granulation	Value, +/–. % over #40, 60, 80, 100 sieve. % thru #100 sieve; Rotap	Min, max, target value. % over #40, 60, 80, 100 sieve. % thru #100 sieve; AOAC/AACCI	Actual value. % over #40, 60, 80, 100 sieve. % thru #100 sieve; Rotap
Microbiological			
Standard plate count; Total plate count	Product is considered not ready to eat and requires further processing, so no microbiological guarantees provided	Target value; FDA BAM	
Yeast		Target value; FDA BAM	
Mold		Target value; FDA BAM	
Vomitoxin	Complies with FDA advisory max level	Max value; FDA BAM	
Shelf life	Number of days at recommended storage conditions		

Reasons for analyzing foods

1. Food safety
2. Government regulations
   (a) Nutrition labeling
   (b) Standards – mandatory and voluntary
   (c) Food inspection and grading
   (d) Authenticity
3. Quality control
4. Research and development

… and none of this would be worth it without asessing the quality of data, thus the need of a **Quality Management System** (also called **QA** –Quality Assurance- / **QC** -Quality Control-)

What is a quality management system?

A **quality management system (QMS)** is a set of procedures and processes aimed at ensuring that a product or service meets the desired level of quality and customer expectations. It involves **continuous monitoring and evaluation** of various aspects of a product or service, such as design, production, delivery, and customer satisfaction, to identify and eliminate defects and improve overall quality. The goal of a quality control system is to **deliver consistent and reliable** products or services that meet customer needs and expectations.

Quality assurance (QA) is a systematic and proactive process that aims to ensure that products and services meet desired levels of quality. It is a preventive approach to quality, focused on designing, implementing and maintaining processes and standards to produce products and services that consistently meet customer requirements and expectations.

Quality control (QC), on the other hand, is a reactive and detective approach to quality, focused on finding and fixing defects in products and services through the use of testing, inspection and verification processes. QC is focused on ensuring that the final product meets established quality standards and customer requirements.

In summary, QA is concerned with the overall quality management process, while QC focuses on verifying and ensuring the quality of the final product. Both QA and QC are necessary to maintain and improve the quality of products and services over time.

Need for a quality management system

A quality management system (QMS) is necessary for several reasons:

Customer satisfaction: A QMS helps to ensure that customer requirements and expectations are met, resulting in increased customer satisfaction and loyalty.

Improved processes: By implementing a QMS, organizations can identify inefficiencies in their processes and make improvements, leading to increased efficiency and cost savings.

Increased competitiveness: A QMS helps organizations to meet or exceed industry standards, which can give them a competitive advantage in their market.

Compliance: A QMS can help organizations comply with regulations and standards, such as ISO 9001, reducing the risk of legal or financial penalties.

Consistent quality: A QMS helps organizations to consistently produce high-quality products and services, reducing the risk of defects and improving customer trust in the organization.

Continual improvement: A QMS provides a framework for continuous improvement, allowing organizations to continuously monitor, evaluate, and improve their processes and products.

Requisites for accreditation under ISO 17025

ISO 17025 is an international standard that specifies the general requirements for the competence of testing and calibration laboratories. To be accredited under **ISO 17025, a laboratory must meet the following requirements:**

Technical competence: The laboratory must have the necessary personnel, facilities, equipment, materials, and reference standards to perform tests and calibrations in a competent and accurate manner.

Quality management system: The laboratory must have a quality management system (QMS) in place that covers all aspects of the laboratory's operations, including sample receipt, handling, testing and reporting. The QMS must be based on the ISO 17025 standard and include regular internal audits and continuous improvement.

Test and calibration methods: The laboratory must use recognized and validated methods for tests and calibrations, and ensure that these methods are performed correctly.

Sampling and handling: The laboratory must have procedures in place to ensure that samples are collected, stored, and transported in a manner that does not compromise the quality of the results.

Calibration and maintenance of equipment: The laboratory must calibrate and maintain its equipment to ensure the accuracy of results. Records of calibration and maintenance must be kept.

Requisites for accreditation under ISO 17025 (Cont.)

Results and reporting: The laboratory must report results in a clear, accurate, and traceable manner, and have procedures in place to ensure that results are properly validated and reviewed.

Traceability: The laboratory must have a system in place to ensure the traceability of its measurements to appropriate reference standards.

Confidentiality: The laboratory must have procedures in place to ensure the confidentiality of customer information and test results.

Personnel: The laboratory must have personnel who are technically competent, trained, and knowledgeable in their areas of responsibility.

Other specifics of accreditation

Compliance with regulatory agencies (which specify requisites for method validation):

Foods and drugs administration **(FDA):** For regulating food products and pharmaceuticals.
U. S. Department of Agriculture **(USDA):** For regulating agricultural products.
Environmental Protection Agency **(EPA):** For regulating the allowed amounts of contaminants in specific matrices.

In Mexico: STPS, EMA, PROFEPA, SAGARPA
In Uzbekistan: ??

Method validation under ICH/ FDA/EPA

Selectivity: The method should be able to distinguish the analyte of interest from other substances in the sample. To demonstrate selectivity, an analytical method should have sufficient resolution and sensitivity to distinguish the analyte of interest from other substances that may be present in the sample. This can be achieved through appropriate sample preparation, chromatographic separation, and detection techniques.

Linearity: The method should have a linear response over the specified range of analyte concentrations.
To demonstrate linearity, an analytical method should produce a response that is proportional to the analyte concentration over the specified range. The linearity of a method is usually assessed by analyzing samples at different concentrations and plotting the response against the analyte concentration. A method is considered linear if the resulting plot is a straight line with a slope that is consistent with the response factor of the method.

Limit of detection and limit of quantitation: The method should be able to accurately detect and quantify the analyte of interest at the lowest levels specified.

Limit of detection (LOD) refers to the lowest concentration of an analyte that can be detected by an analytical method with a defined level of confidence. **The LOD is the lowest concentration at which the analyte can be differentiated** from background noise.

Limit of quantitation (LOQ) refers to the lowest concentration of an analyte that can be accurately quantified by an analytical method. **The LOQ is the lowest concentration at which the analyte can be measured** with a defined level of precision and accuracy.

Method validation under ICH/ FDA/EPA (Cont.)

Precision: The method should have consistent results when performed by different analysts or on different days, demonstrating the precision of the method. To demonstrate precision (both in-run and between-run), a method should produce consistent results when repeated under similar conditions. The precision of a method is usually expressed as a statistical parameter such as the standard deviation or coefficient of variation.

Accuracy: The method should produce results that are consistent with the true value of the analyte, demonstrating the accuracy of the method. To demonstrate accuracy, an analytical method should produce results that are close to the true value of the analyte. The accuracy of a method can be determined by analyzing reference samples of known concentration and comparing the results to the expected values. The accuracy of a method is usually expressed as a statistical parameter such as the bias or mean absolute deviation.

Robustness: The method should be able to produce consistent results despite variations in the conditions or parameters used in the analysis. To demonstrate robustness, an analytical method should be tested under deliberate variations in method parameters, such as changes in temperature, pH, or flow rate. The results should be compared to results obtained under normal conditions to determine the effect of the variations on the accuracy and precision of the method.

Blanks: Samples that are processed and analyzed in the same manner as samples, but contain no analyte. They are used to detect and quantify any contamination introduced during sample collection, preservation, and analysis.
They may be **method blanks** where the blanks are taken through the entire process of sampling, extraction and analysis. They may be **extraction blanks**, where the blanks go through extraction and analysis. They may be **instrument blanks**, where the blanks are analyzed as the other samples. If high technology instruments (such as a GC-MS is used, there may be also reagent blanks).

Method validation under ICH/ FDA/EPA (Cont.)

Matrix Spike and Matrix Spike Duplicates: Samples that are spiked with a known amount of an equal or similar substance to the analytes of interest have been added, and are processed and analyzed along with the samples. They are used to evaluate the performance of the analytical method and to assess matrix effects, which are changes in analyte response due to the presence of other compounds in the sample matrix.

Standards: Samples containing **known** concentrations of the analyte of interest. They are used to verify the accuracy of the analytical method and to monitor the precision and bias of the results. In the context of analytical testing, standards serve as a point of comparison for test results. By analyzing a sample containing a known concentration of an analyte and comparing the results to the expected value, analysts can determine the accuracy and precision of their analytical method.

Quality Control Samples: Samples of the drug substance or food product, representing different batches or lots, used to monitor the precision and accuracy of the analytical results over time. QC samples typically represent different lots or batches of a product, and they are analyzed alongside test samples to verify that the analytical method is performing consistently and accurately. The results from QC samples are compared to established acceptance criteria to determine if the method is working as expected. If the results from the QC samples fall outside of the established criteria, the analytical method may need to be modified or revalidated.

Validation of analytical data

Validation of analytical data. Central Tendency

Mean/Average can be defined as the sum of all the numbers divided by the total number of values.

where:

$\bar{x}$ = mean

$$\bar{x} = \frac{x_1 + x_2 + x_3 + \ldots + x_n}{n} = \frac{\Sigma x}{n}$$

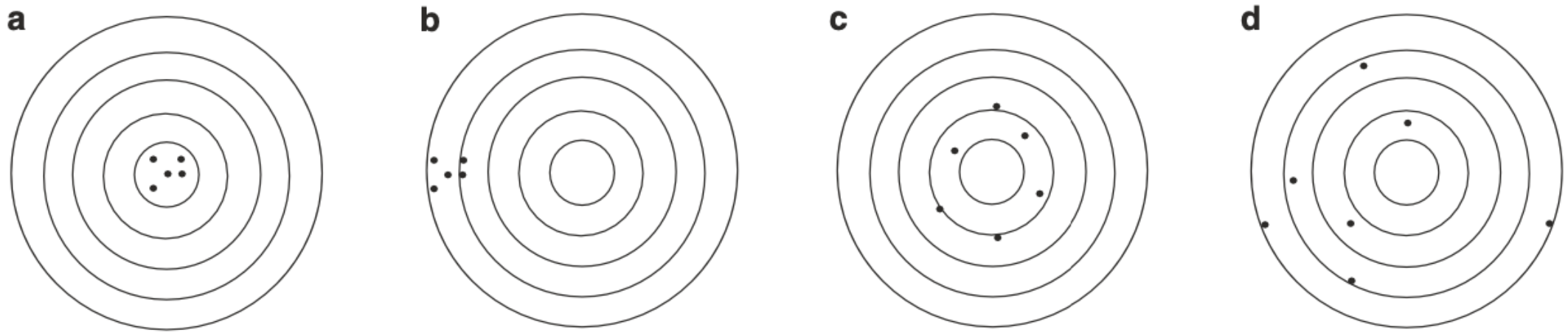
x_1, x_2, etc. = individually measured values (x_i)

n = number of measurements

$$\bar{x} = \frac{64.53 + 64.45 + 65.10 + 64.78}{4} = 64.72\%$$

Median: the midpoint or middle number within a group of numbers

Validation of analytical data. Precission and accuracy



Precision: This parameter is a measure of how reproducible or how close replicate measurements become.

Accuracy: How close a particular measure is to the true or correct value

a. Precise and accurate
b. Precise and non accurate
c. Accurate and non precise
d. Non precise and non accurate

Validation of analytical data. Standard deviation; indicators of dispersion

Standard deviation: a measure of the amount of variation or dispersion of a set of values

$$\sigma = \sqrt{\frac{\Sigma(x_i - \mu)^2}{n}}$$

where:

σ = standard deviation
xi = individual sample values
μ = true mean
n = total population of samples

However, true mean is generrally not known, thus:

SD (Standard deviation) is calculated as:

$$SD = \sqrt{\frac{\Sigma(x_i - \bar{x})^2}{n}}$$

If n>30

$$SD = \sqrt{\frac{\Sigma(x_i - \bar{x})^2}{n-1}}$$

If n<30

Validation of analytical data. Indicators of dispersion

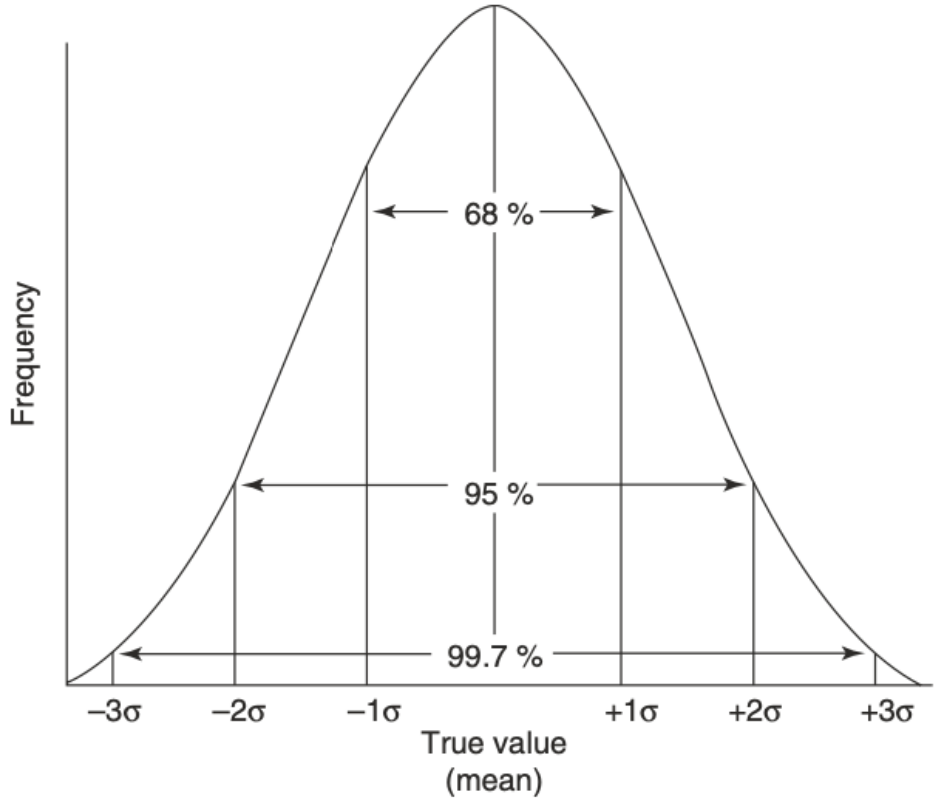
The co-efficient of variation (CV) is a statistical measure of the relative dispersion of data points in a data series around the mean. It represents the ratio of the standard deviation to the mean.

$$\%\,\text{Coefficient of variation}\,(\%\text{CV}) = \frac{\text{SD}}{\bar{x}} \times 100$$

Example

$$\%\,\text{CV} = \frac{0.293}{64.72} \times 100 = 0.453\%$$

Example of normal distribution



Validation of analytical data. Indicators of dispersion

A confidence interval is the mean of your estimate plus and minus the variation in that estimate. This is the range of values you expect your estimate to fall between if you redo your test, within a certain level of confidence. Confidence, in statistics, is another way to describe probability

$$\text{Confidence interval (CI)} = \bar{x} \pm Z \text{ value} \times \frac{\text{standard deviation (SD)}}{\sqrt{n}}$$

$$\text{CI (at 95\%)} = 64.72 \pm 1.96 \times \frac{0.2927}{\sqrt{25}}$$

$$= 64.72 \pm 0.115\%$$

If n>25

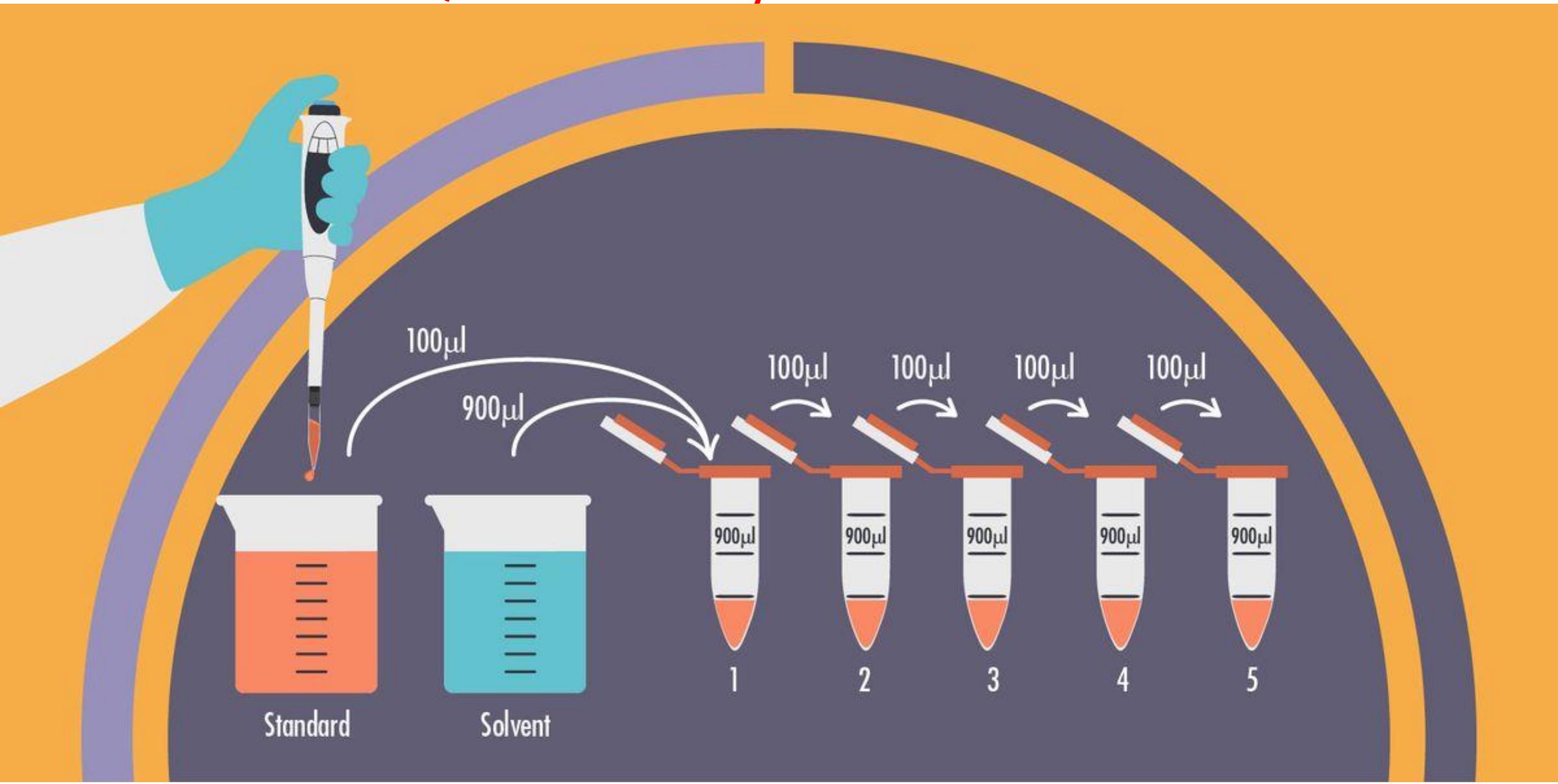
$$\text{CI} = \bar{x} \pm t \text{ value} \times \frac{\text{standard deviation (SD)}}{\sqrt{n}}$$

$$\text{CI (at 95\%)} = 64.72 \pm 3.18 \times \frac{0.2927}{\sqrt{4}}$$

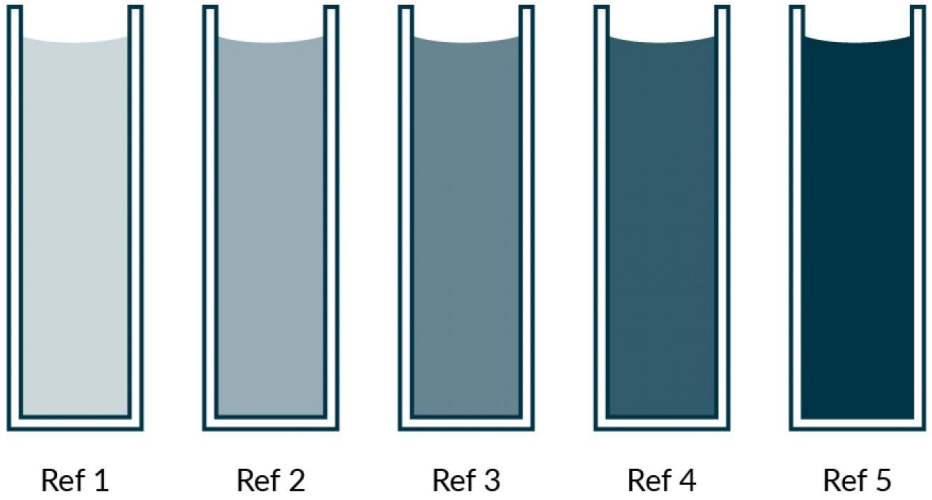
$$= 64.72 \pm 0.465\%$$

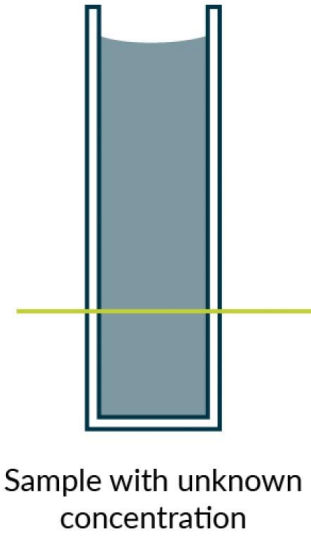
If n<25

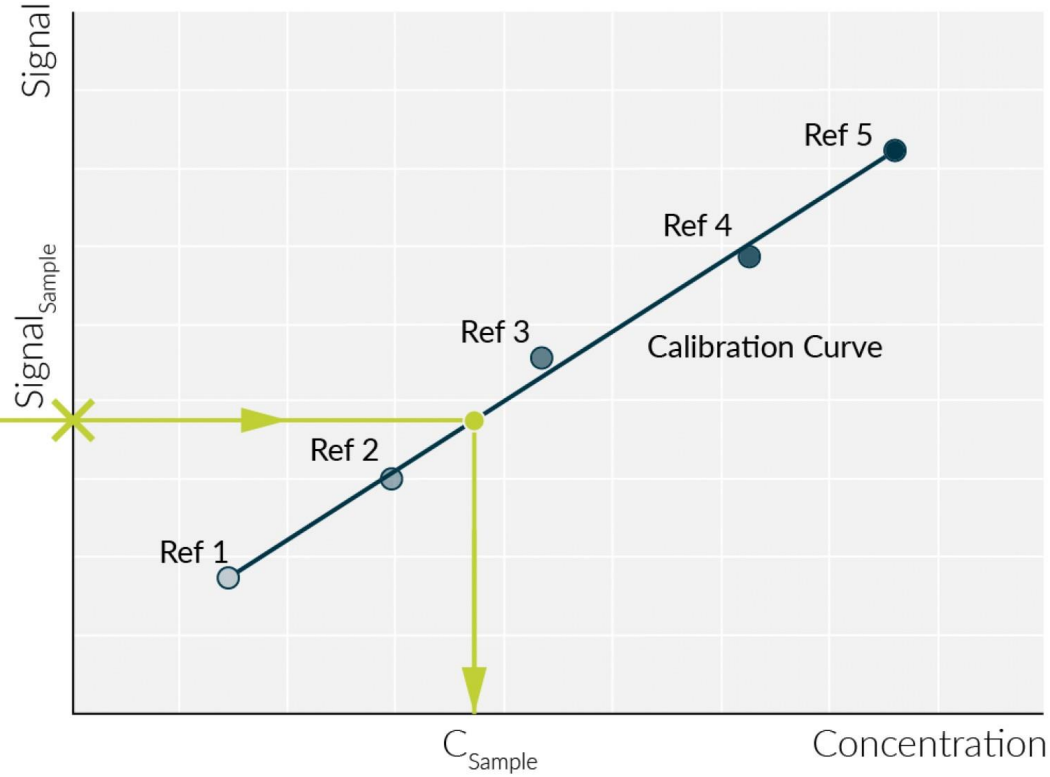
Quantitation of analytical data. Calibration curve



Quantitation of analytical data. Calibration curve

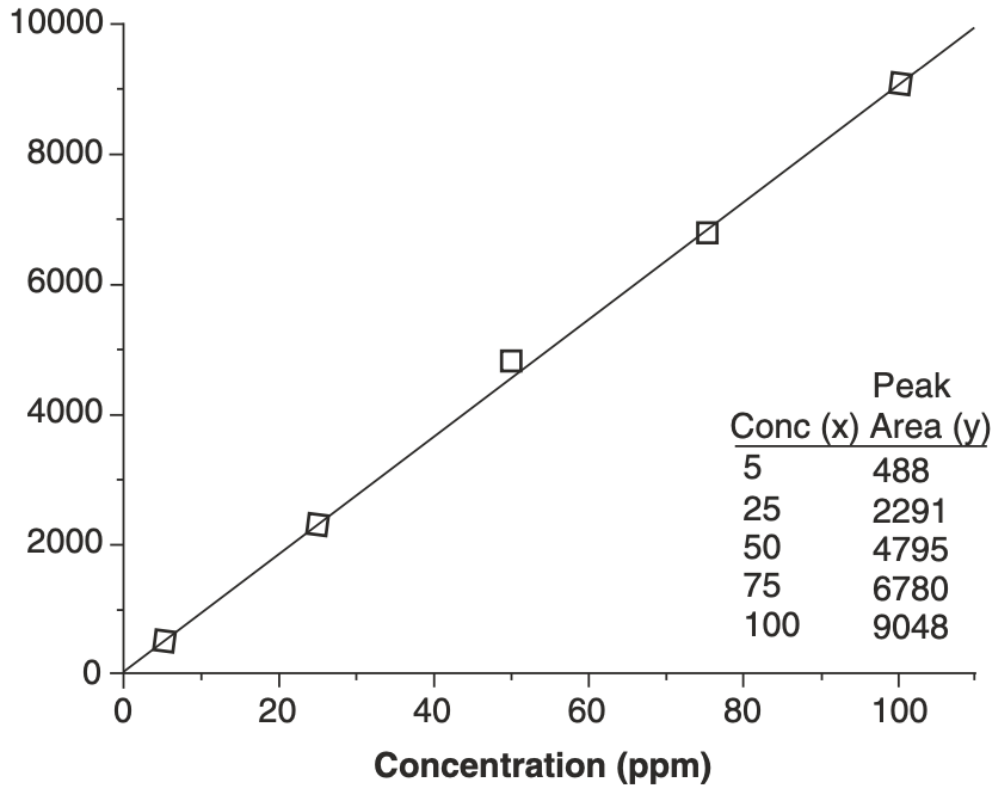






Quantitation of analytical data. Calibration curve

A calibration curve is a way to identify the concentration of an unknown substance. These curves use data points of known substances at varying concentrations, and researchers or developers can use these curves to find where an unknown substance plots.



Conc (x)	Peak Area (y)
5	488
25	2291
50	4795
75	6780
100	9048

$$\text{slope } a = \frac{\Sigma(x_i - \bar{x})(y_i - \bar{y})}{\Sigma(x_i - \bar{x})^2}$$

$$y - \text{intercept } b = \bar{y} - a\bar{x}$$

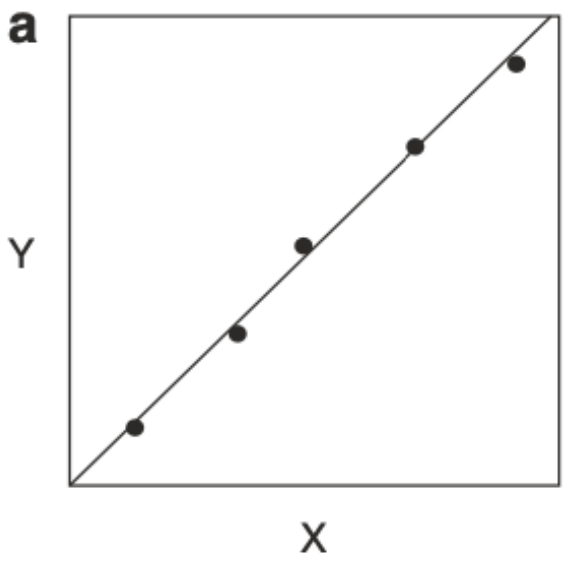
where:

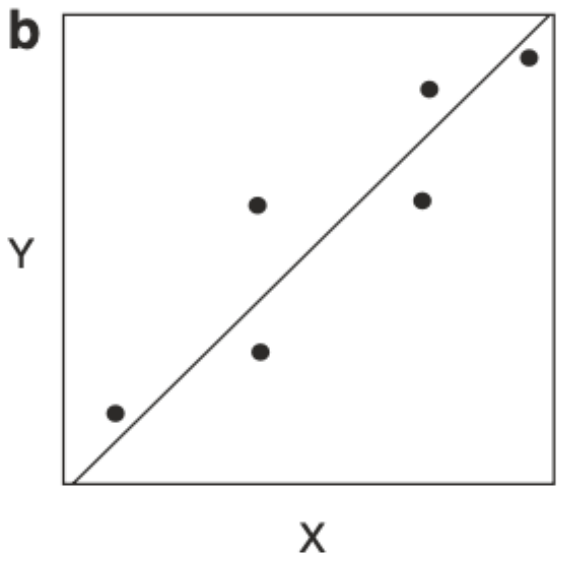
x_i and y_i = individual values

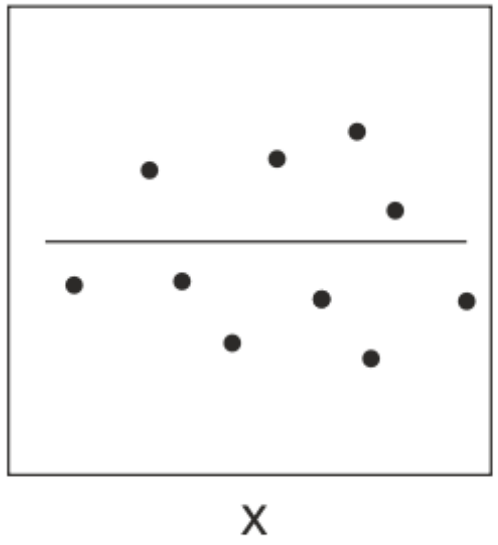
$\bar{x}$ and $\bar{y}$ = means of the individual values

$$y = ax + b \qquad x = \frac{y - b}{a}$$

Quantitation of analytical data. Calibration curve







High linearity (r^2) = 0.999

Not so high linearity (r^2) = 0.93

No linearity (r^2) = 0.2

correlation coefficient =

$$r = \frac{\Sigma(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\left[\Sigma(x_i - \bar{x})^2\right]\left[\Sigma(y_i - \bar{y})^2\right]}}$$

Coefficient of determination (r^2/ R^2): It provides a measure of how well observed outcomes are replicated by the mode

Quality of analytical data. LOQ and LOD

Limit of detection (LOD) –X_{LD}-: the lowest possible amount that we can detect with some degree of confidence (or statistical significance).

$$X_{LD} = X_{Blk} + (3 \times SD_{Blk})$$

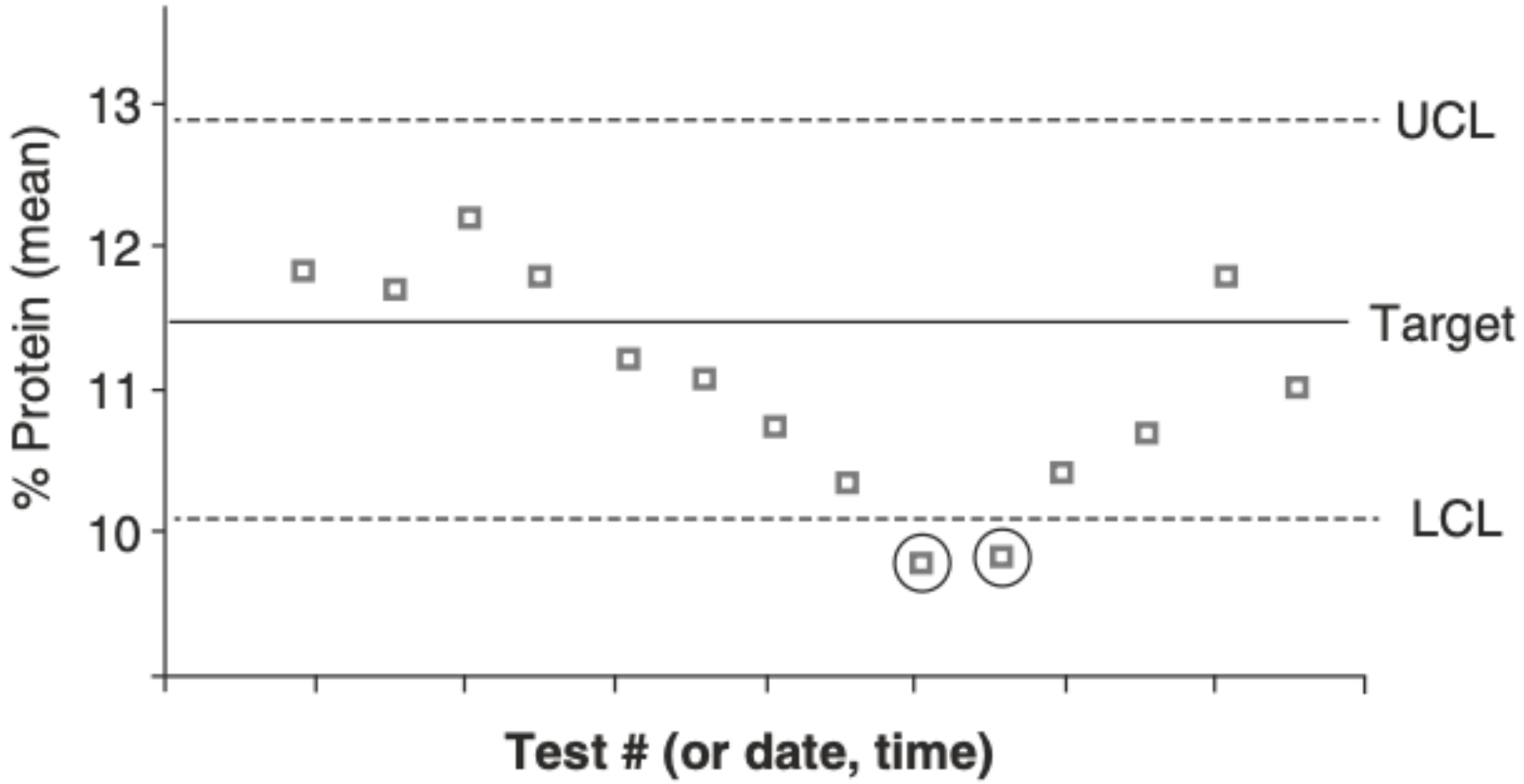
where:

X_{LD} = minimum detectable concentration
X_{Blk} = signal of a blank
SD_{Blk} = standard deviation of the blank readings

$$X_{LQ} = X_{Blk} + (10 \times SD_{Blk})$$

Limit of quantification (LOQ) –X_{LQ}-: the lowest possible amount that we can detect with some degree of confidence (or statistical significance).

Quality of analytical data. Control charts



Quality of analytical data. Sources of error

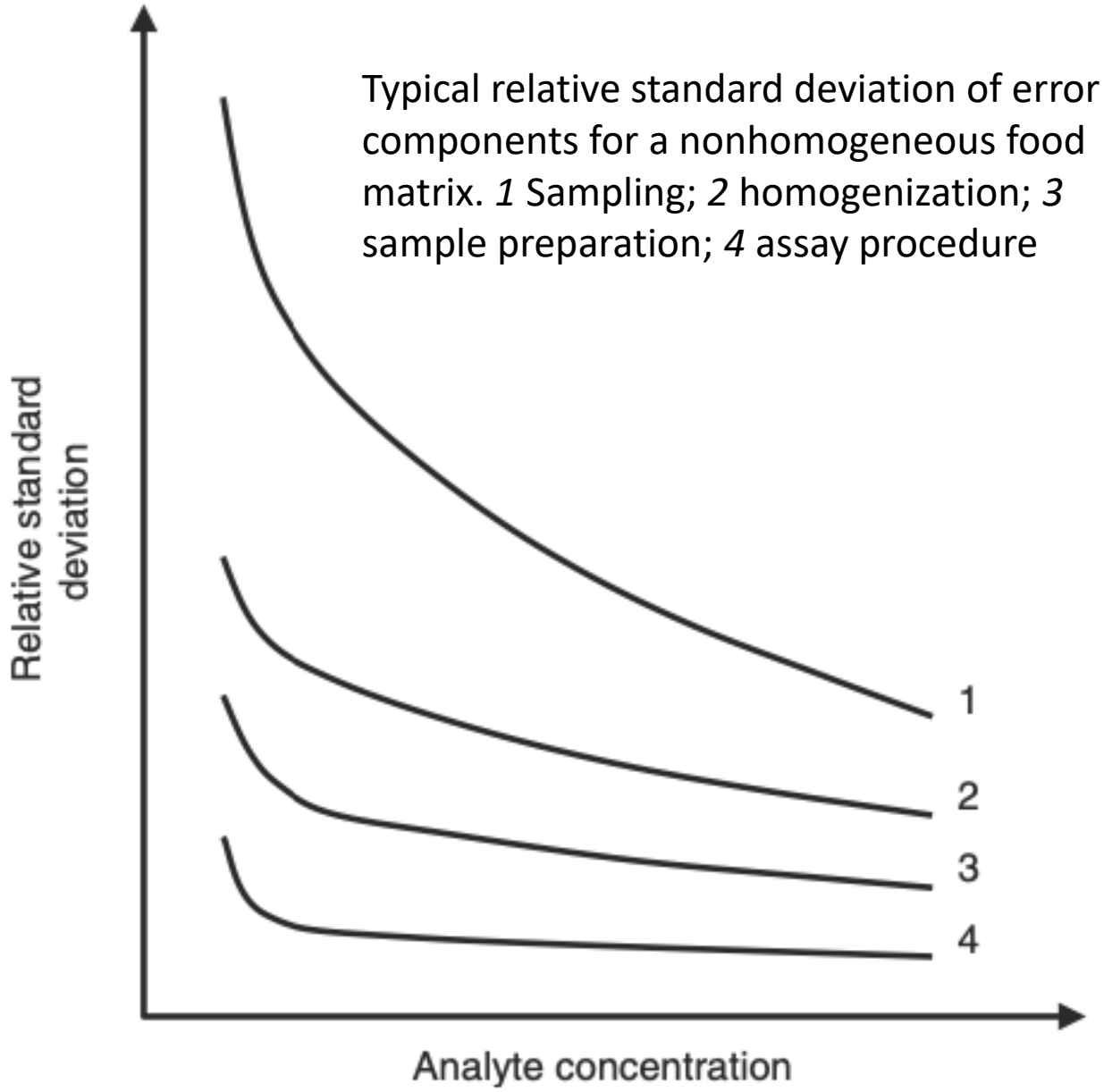
Systematic or determinate error produces results that consistently deviate from the expected value in one direction or the other. For example, one pipete that does not deliver the intended amount of volume.

Random or indeterminate errors are always present in any analytical measurement. This type of error is due to our natural limitations in measuring a particular system. These errors fluctuate in a random fashion and are essentially unavoidable. For example, reading an analytical balance, judging the endpoint change in a titration, and using a pipette all contribute to random error. Background instrument noise, which is always present to some extent, is a factor in random error.

Blunders are easy to eliminate, since they are so obvious. The experimental data are usually scattered, and the results are not close to an expected value. This type of error is a result of using the wrong reagent or instrument or of sloppy technique. Some people have called this type of error the “Monday morning syndrome” error.

Sampling: Essentials.

The small portions taken for analysis are referred to as **samples**, and the entire lot or the entire production for a certain period of time, in the case of continuous processes, is called a **population**. The process of taking samples from a population is called **sampling**.



Typical relative standard deviation of error components for a nonhomogeneous food matrix. *1* Sampling; *2* homogenization; *3* sample preparation; *4* assay procedure

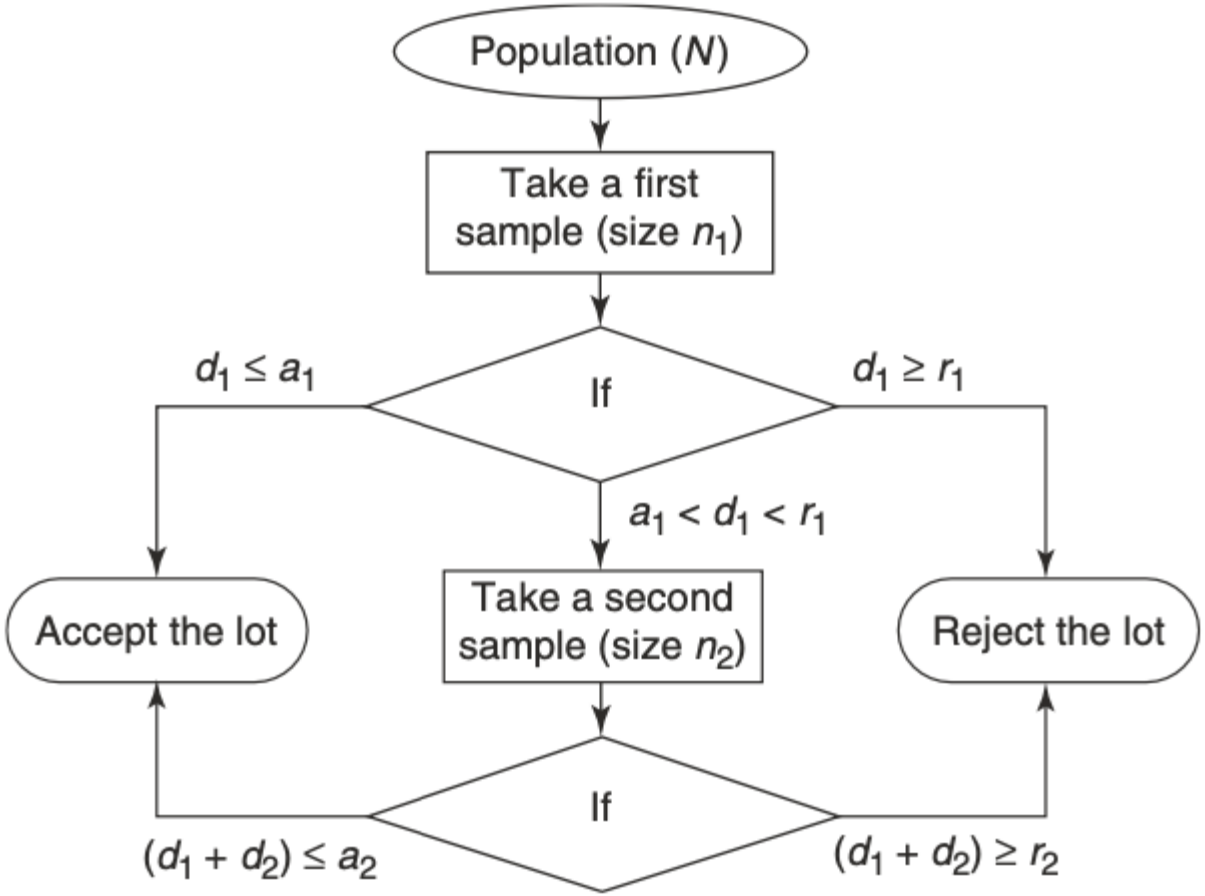
Sampling: Examples of sampling plans.

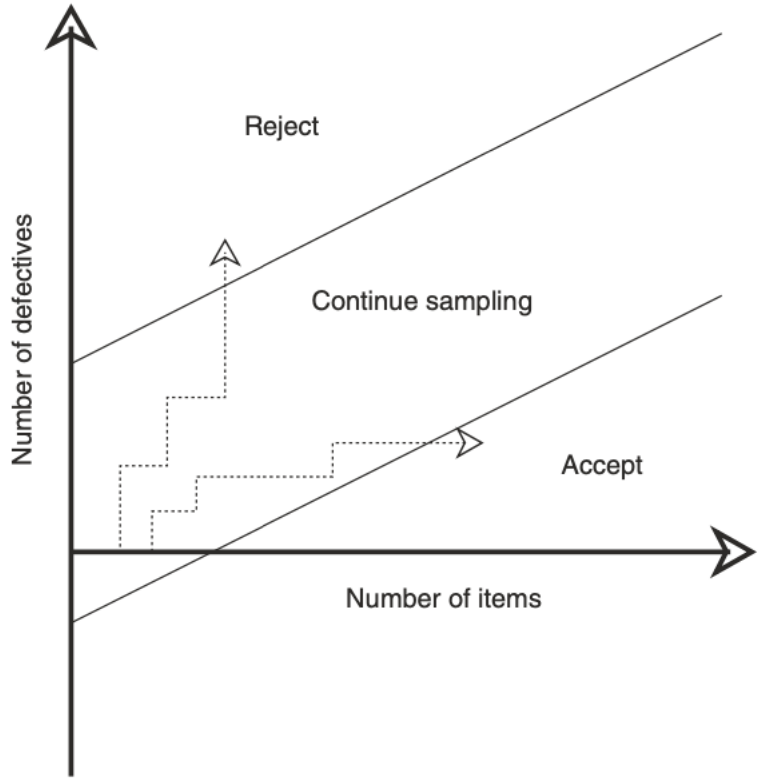
Attribute sampling: Sampling is performed to decide on the acceptability of a population based on whether the sample possesses a certain characteristic or not. The result has a binary outcome of either conforming or nonconforming.

Variable sampling: Sampling is performed to estimate quantitatively the amount of a substance (e.g., protein content, moisture content, etc.) or a characteristic (e.g., color) on a continuous scale. The estimate obtained from the sample is compared with an accept- able value (normally specified by the label, regulatory agencies, or the customer) and the deviation measured.

Acceptance sampling is a procedure that serves a very specific role: to determine if a shipment of products or ingredients has enough quality to be accepted.

Sampling: Examples of sampling plans (acceptance sampling).





N, population size; n_1 and n_2, sample size; a_1 and a_2, acceptance numbers; r_1 and r_2, rejection numbers; d_1 and d_2, number of non-conformities, *Subindices* 1 and 2 represent samples 1 and 2, respectively