International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 1174-1184 www.ijprajournal.com ISSN: 2456-4494

Review on Bio- Analytical Chemistry: A Key to Understanding Biological Molecules

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Date of Submission: 20-08-2025 Date of Acceptance: 30-08-2025

ABSTRACT: Bio-analytical chemistry plays a crucial role in the quantitative measurement of analytes in biological matrices, supporting drug development, therapeutic monitoring, and disease diagnosis. This field employs a range of methodologies, including chromatography (HPLC, GC), mass spectrometry (LC-MS/MS), and electrophoresis (CE). Ensuring the accuracy and reliability of these methods requires rigorous validation, encompassing assessments of specificity, sensitivity, precision, and stability. As bio-analytical chemistry continues to evolve, advancements in miniaturization, high-throughput analysis, and omics technologies (study large sets of biological molecules) are enhancing its capabilities. The development of precise and dependable analytical methods is essential for driving biomedical research and improving healthcare outcomes. By integrating cutting-edge techniques with robust validation protocols, bio-analytical chemistry remains a vital discipline in the pursuit of scientific and medical advancements.

Key words: Bio-analytical chemistry, chromatography (HPLC, GC), mass spectrometry (LC-MS/MS), electrophoresis (CE), method validation, accuracy, precision, sensitivity, specificity, biomedical research, therapeutic monitoring, disease diagnosis, high-throughput analysis, miniaturization.

I. INTRODUCTION:

- Bio-analytical chemistry is the quantitative analysis of drugs in biological fluids (mainly plasma and urine) or tissues. Bio-analytical methods are widely used to quantitative drugs and their metabolites in physiological matrices.
- The determination of drugs concentration in biological fluids is indispensable in pharmaceutical research.
- A bio-analytical methods should allow the quantification of drugs and their metabolites in biological matrices, e.g. plasma, urine, and cerebrospinal fluid, and must be validated with respect to their reliability for the intended use.
- Bio-analytical method validation comprises all criteria determining data quality such as selectivity, accuracy, precision, recovery, sensitivity, and stability. Bio-analytical method validation employed for the quantitative determination of drugs and their metabolites in biological fluids plays a significant role in the evaluation and interpretation of bioavailability, bioequivalence, pharmacokinetic, and toxico-kinetic study data.
- These studies generally support regulatory filings. The quality of these studies is directly related to the quality of the underlying bio-analytical data. Bio-analytical method validation all criteria determining data quality such.
- Chromatographic methods (high-performance liquid chromatography)[HPLC] have been widely used for the bio-analysis of small molecules, with liquid chromatography coupled to triple quadrupole mass spectrometry (LC/MS/MS) being the single most commonly used technology. After developing a method with desired attributes, the method requires validation to establish that it will continue to provide accurate, precise, and reproducible data during study-sample analysis.

FATHER OF BIOANALYTICAL CHEMISTRY:

- IZAAKMAURITS KOLTHOFF

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Figure 1
(February 11 ,1894 – March 4 ,1993)

- He was an analytical chemistry and chemistry educator.
- Known for : Introducing model methods to analytical chemistry .
- Development of "cold process" rubber synthesis .
- His research transformed the ways by which scientists separate, identify, and quantify chemical substances and built the field upon solid theoretical principles and experimental techniques.

AIM AND SCOPE :

Analytical and Bio-analytical Chemistry's mission is the rapid publication of excellent and high-impact research articles on fundamental and applied topics of analytical and bio- analytical measurement science.

It's scope is broad, and ranges from novel measurement platforms and their characterization to multidisciplinary approaches that effectively address important scientific problems.

METHODS OF BIOANALYTICAL CHEMISTRY :

The quantitative determination of substances and their metabolites in biological fluids is known as bio-analysis.

This techniques is employed early in the drug development process to aid drug discovery programmes by proving information on the metabolic fate and pharmacokinetics of compounds in living cells and animals.

TECHNIQUES USED IN BIOANALYSIS : CHROMATOGRAPHIC TECHNIQUES :

HPLC (High Performance Liquid Chromatography)
FPLC (Fast Protein Liquid Chromatography)
CE (Capillary Electrophoresis)

HYPHENATED TECHNIQUES :

LC –MS (Liquid Chromatography -Mass Spectroscopy)
GC – MS (Gas Chromatography - Mass Spectroscopy)
CE – MS (Capillary electrophoresis – Mass Spectroscopy)

CHROMATOGRAPHIC TECHNIQUE :

Chromatography is separation technique that utilizes the principle of differential distribution of components between a stationary phase and a mobile phase to isolate and identify individual substances within a mixture.

It's commonly used for qualitative and quantitative analysis of substances in various fields like chemistry, biology, and environmental science.

- **Mobile phase :** This is the moving fluid(liquid or gas) that carries the mixture through the stationary phase.
- **Stationary phase :** This is the stationary, non-moving material (solid or liquid) that the mixture interacts with.

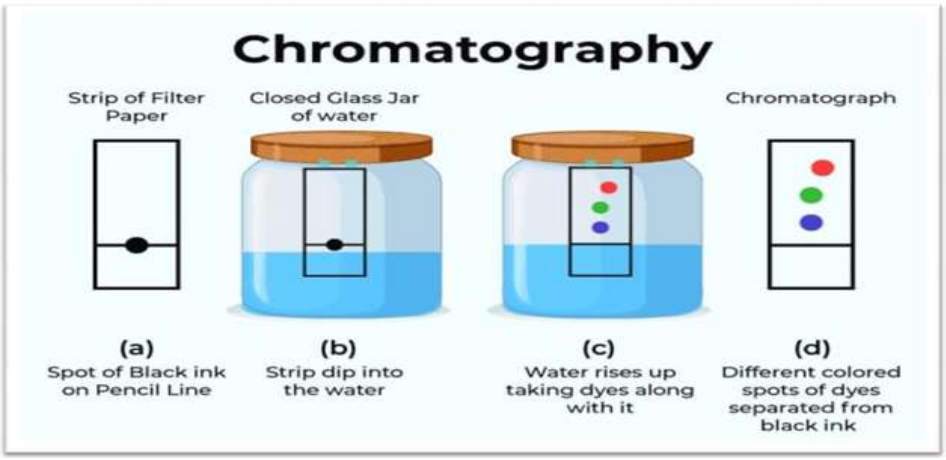

Figure 2 (CHROMATOGRAPHY)

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HIGH -PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC):

HPLC(High-performance liquid chromatography) is an analytical technique for separating, identifying, and quantifying each component in a mixture.

There are three basic types of liquid chromatographic columns:

- Liquid – liquid extraction.
- Solid phase extraction.
- Ion - exchange chromatography.

LIQUID – LIQUID EXTRACTION:

Liquid – liquid extraction, also known as solvent extraction and partitioning, is a technique for separating chemicals or metal complexes based on their relative solubilities in two immiscible liquids, commonly water (polar) and an organic solvent (non- polar).Ex. Benzene & water.

Figure 3 (LIQUID-LIQUID EXTRACTION

SOLID PHASE EXTRACTION:

Solid phase extraction(SPE) is a technique for preparing and purifying samples quickly and selectively.

SPE involves extracting, portioning, and/or absorbing one or more analytes from a liquid sample onto a solid stationary phase.

Figure 4(SOLID PHASE EXTRACTION)

ION – EXCHANGE CHROMATOGRAPHY:

Ion exchange chromatography is a chromatographic separation technique that is based primarily on a compound's net charge and is commonly used to track deamination of proteins and succinimide production.

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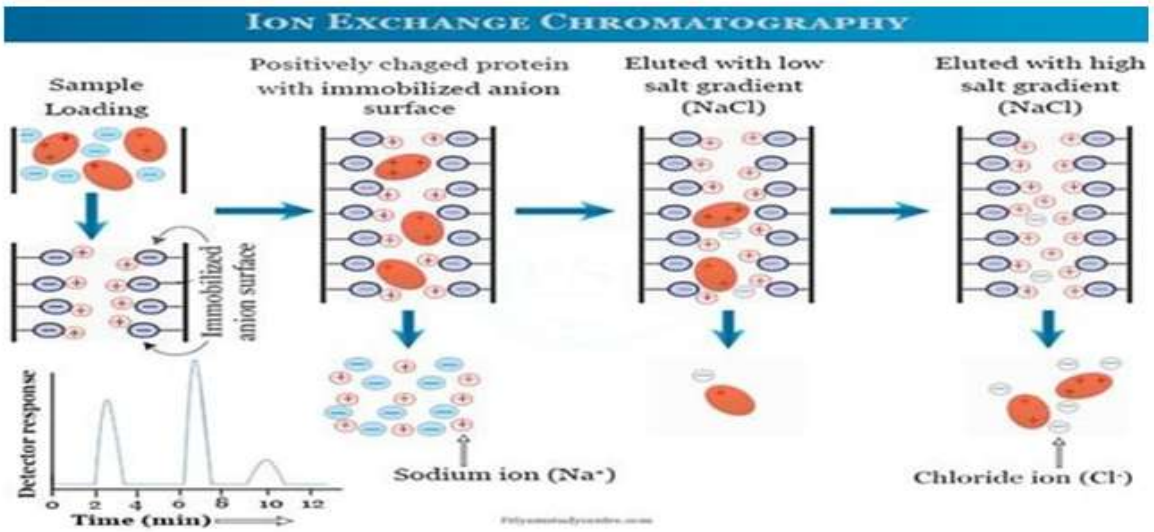

Figure 5 (ION EXCHANGE CHROMATOGRAPHY)

The chromatographic matrix is directly related to positive (cationic) or negative (anionic) charge moieties.

FAST PROTEIN LIQUID CHROMAOGRAPHY (FPLC) :

Fast protein liquid chromatography (FPLC) is a type of medium pressure chromatography in which the speed of the mobile phase passing through the stationary phase is controlled by a pump.

Large molecules such as proteins, nucleotides, and peptides are purified using FPLC.

It can also be used to profile proteins involved in the purification of animalvenoms, and it can detect minor changes in single proteins, which is important in clinical settings.

Polynucleotides can also be detected using FPLC, which is used to purify RNA and plasmid DNA quickly.

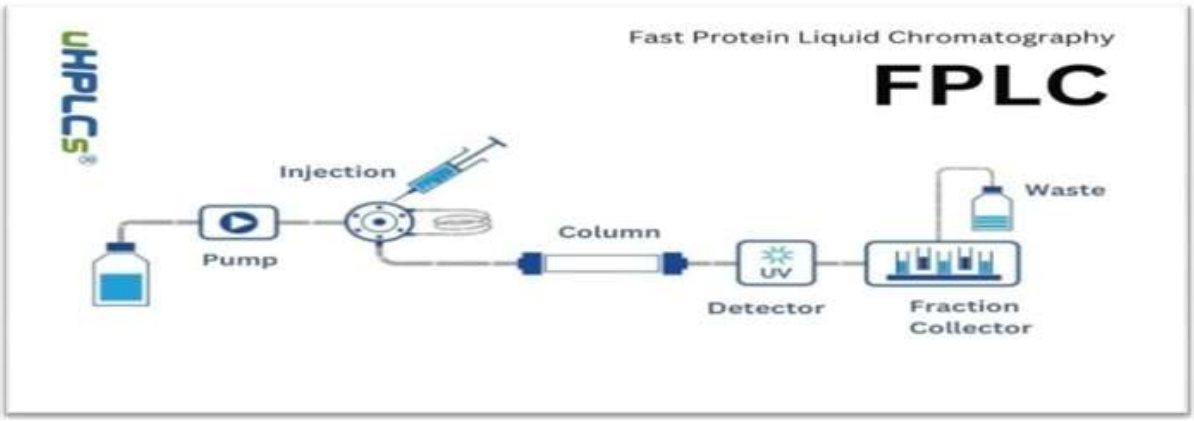

Figure 6 (FAST PROTEIN LIQUID CHROMATOGRAPHY)

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HYPHENATED TECHNIQUES:

Hyphenated techniques, also known as 'squad analytical techniques', involve combing two or more analytical methods, typically a separation technique(like chromatography or electrophoresis) and a detection technique (like mass spectrometry or NMR).

Examples:

Common hyphenated techniques include,

- GC – MS (Gas Chromatography coupled with Mass Spectrometry)
- LC – MS (Liquid Chromatography coupled with Mass Spectrometry)
- LC – NMR (Liquid Chromatography coupled with Nuclear Magnetic Resonance)
- CE – MS (Capillary Electrophoresis coupled with Mass Spectrometry)

LIQUID CHROMATOGRAPHY - MASS SPECTROMETRY (LC – MS):

The technology of bioanalytical Liquid Chromatography–Mass Spectrometry combines liquid chromatography and mass spectrometry.

In laboratories, LC – MS is commonly used to analyse pharmacological compounds , drug products, and biological materials quantitatively and qualitatively.

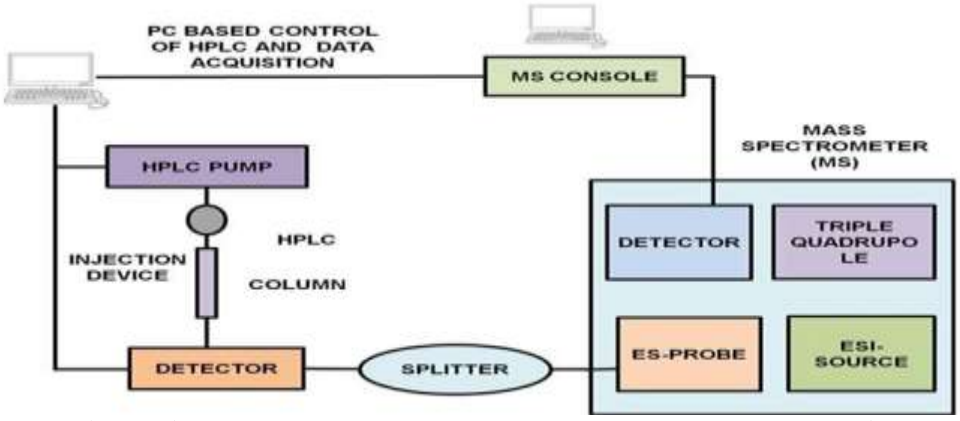

Figure 7(LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY)

GAS CHROMATOGRAPHY - MASS SPECTROMETRY (GC – MS):

Gas chromatography is a technique for separating substances in a mixture by injecting a gaseous or liquid sample into a mobile phase, also known as 'the carrier gas', and 'passing the gas' through a stationary phase.

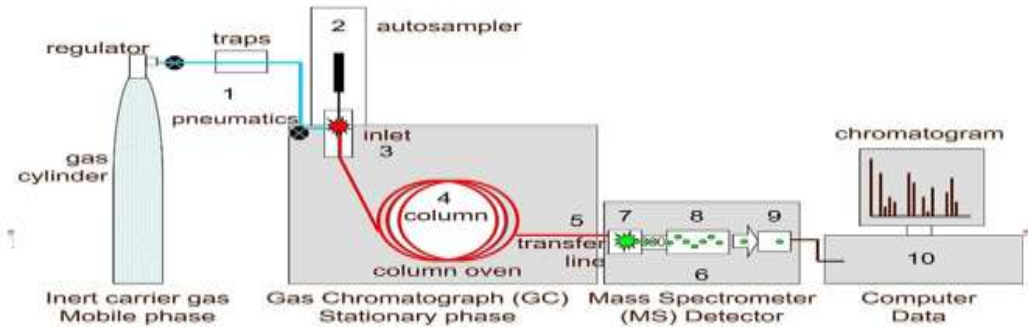

Figure 8 (GC-MS DESIGN)

The stationary phase might be solid or liquid fixed to a solid carrier.

The sample might be either gas or liquid, but it is always injected as gas or vapour onto the column

CAPILLARY ELECTROPHOROSIS – MASS SPECTROMETRY (CE-MS):

CE , or capillary electrophoresis, is a chemical analysis technique that separates

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molecules in an electric field according to size and charge.

Capillary electrophoresis is carried out in a capillary tube with a sub-millimeter diameter that holds a flowing electrolyte solution.

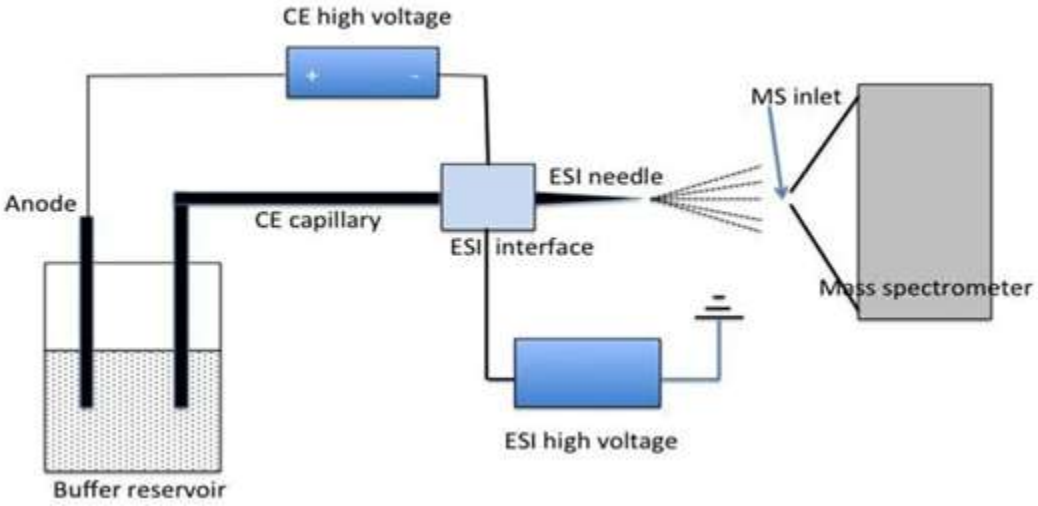

Figure 9 (SCHEMATIC DIAGRAM OF CE-MS)

HIGH PERFORMANCE CAPILLARY ELECTROPHOROSIS (HPCE) :

HPCE has applications in Life Sciences research, drug testing, diagnostics, and biopharmaceutical production.

Proteins, nucleic acids, carbohydrates, viruses and bacteria, chemicals, and a variety of other analytes are detected, separated, and analysed using it.

We employ the HPCE-512, a new instrument from delta DOT that can separate and analyse a wide spectrum of materials, from tiny compounds and amino acids to proteins and Virus – Like Particles.

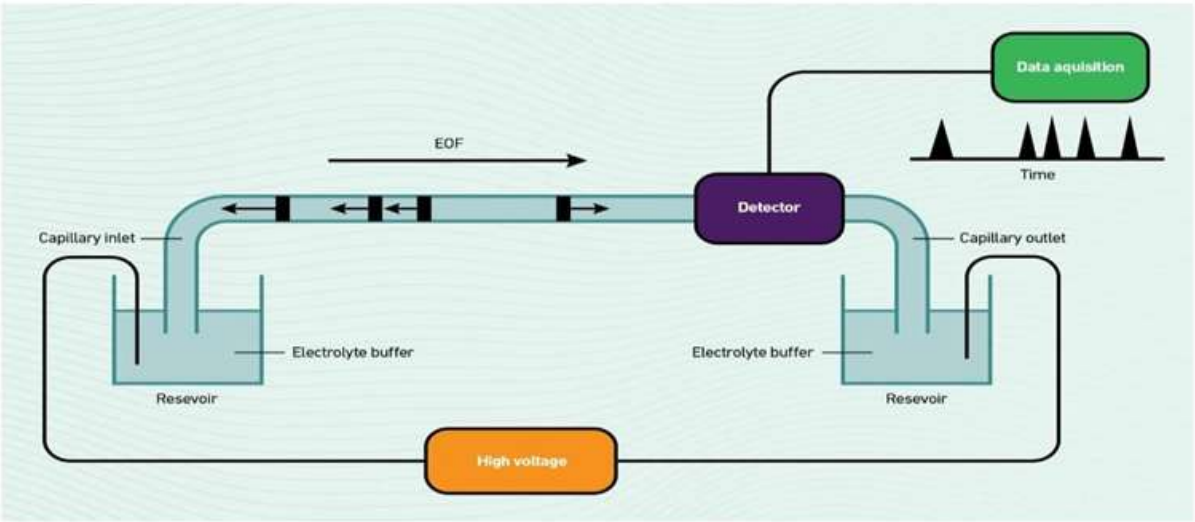

Figure 10(HPCE DESIGN)

BIOANALYTICAL TECHNIQUE:

Bio-analytical techniques are analytical methods used to determine the concentration of drugs or drug metabolites within biological samples like blood, plasma, urine etc.

These techniques are crucial for understanding drug bioavailability, pharmacokinetic studies, and toxicokinetic evaluations.

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Common techniques include chromatography (Liquid chromatography, Gas chromatography), Mass spectrometry (MS), and various ligand-binding assays.

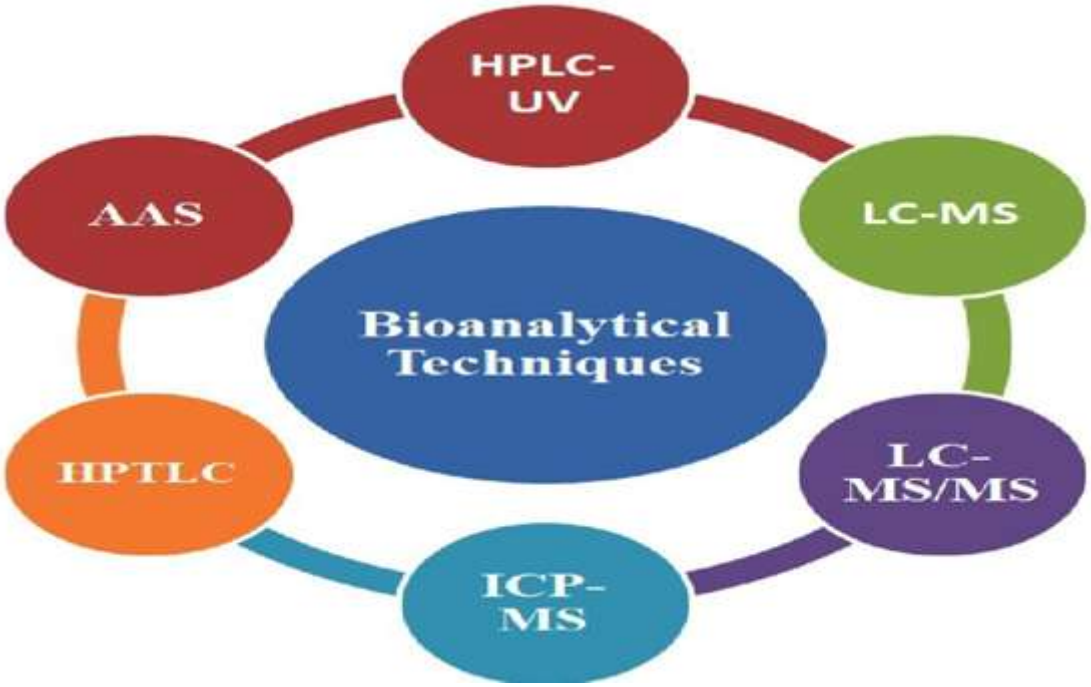

Figure 11(BIOANALYTICAL TECHNIQUES)

BIOANALYTICAL METHOD VALIDATION :
Bio-analytical method validation is a critical process in the development and approval of pharmaceutical products.
Importance of bio-analytical method validation is to ensures accuracy, precision.

TYPES OF BIOANALYTICAL METHOD VALIDATION:
Bio-analytical method validation most widely classified into three types.
A. Full validation
B. Partial validation
C. Cross validation

VALIDATION PARAMETERS:
Accuracy , precision, linearity, selectivity and specificity, limit of detection, limit of qualification, standard curve (calibration curve) , recovery, stability, robustness, ruggedness.

ANALYSIS OF BIOPOLYMER:
BIOPOLYMERS:
Biopolymers are polymeric molecules containing covalently bonded 'monomers' having biological origin.
A significant portion of the components that make up the world around us cone from living organisms, including plants, trees, microbes, and animals.
Biopolymers are materials that have been manufacture from biological sources such as fats, vegetable oil, sugars, resins and proteins and often synthesized from starch, sugar and natural fibres.

IDENTIFICATION AND QUANTIFICATION:
Properties of biopolymers that can be used for their analysis:

- Size
- Mass
- Shape
- Charge
- Isoelectric point
- Electron structure
- Affinity interactions
- Chemical reactivity

PROTEIN:
Proteins are large, complex molecules that play many critical roles in the body.
They do most of the work in cells and are required for the structure, function, and regulation of the body's tissues and organs.

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Proteins are made up of hundreds or thousands of smaller units called amino acids, which are attached to one another in long chains.
There are 20 different types of amino acids that can be combined to make a protein.

STRUCTURE OF PROTEINS:
Structure units – 20 amino acids

- ✓ Primary structure – amino acid sequence (-C - N – bonds).
- ✓ Secondary structure – alpha – helix , beeta – sheet (H – bonds).
- ✓ Tertiary structure – 3D structure (S –S bonds , H – bonds).
- ✓ Quaternary structure – 3D structure of >1 subunits (weak interactions).

URINE ANALYSIS:
NORMAL CHEMICAL COMPOSITION OF URINE:
Urine is an aqueous solution of greater than 95% water ,with a minimum of these remaining constituents in order of decreasing concentration:

- Urea 9.3 g/L
- Chloride 1.87 g/L
- Sodium 1.17g/L
- Potassium 0.750 g/L
- Creatinine 0.670 g/L
- Other dissolved ions, inorganic and organic compounds (proteins , hormones , metabolites)

Degrees of Proteinuria

Normal	Nephritic	Nephrotic
<150 mg/24 hour **OR** <15 mg/mmol	<150-3000 mg/24 hour **OR** 15-300 mg/mmol	>3000 mg/24 hour **OR** >300 mg/mmol

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Table 1(DEGREES OF PROTEINURIA)

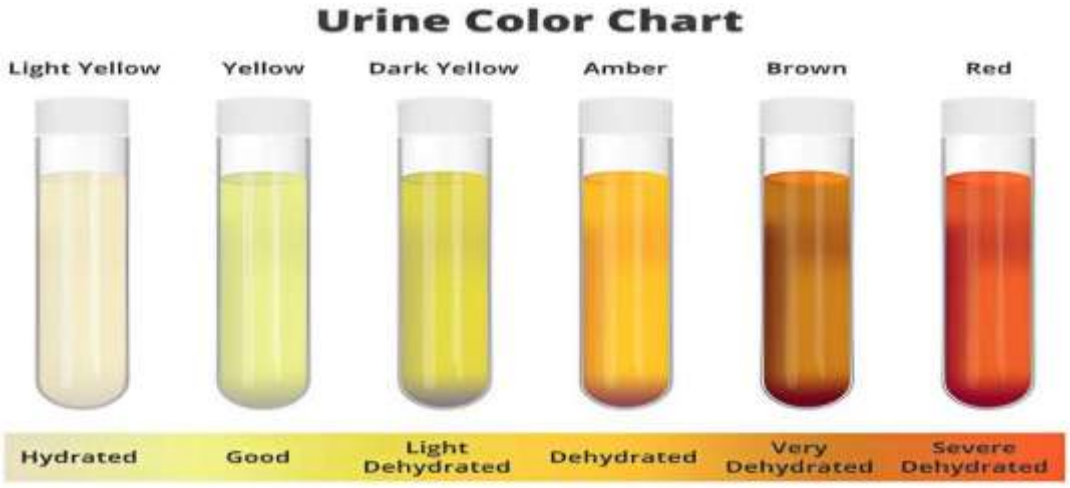

Figure 12(URINE COLOUR CHART)

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NORMAL AND ABNORMAL CONSTITUENTS OF URINE :

CONSTITUENT	NORMAL	ABNORMAL / SIGNIFICANCE
1. Colour	Yellow to amber	Red or reddish- presence of haemoglobin. Greenish- brown or black- caused by bile pigments. The colour of urine darkens upon standing.
2. Appearance	Clear	Milky- fat globules, pus, bacteria.. Smoky- blood cells. Hazy- refrigeration.
3. Reaction	Between 4.6 & 8.0 pH, with an average of 6.0	High acidity- diabetic acidosis, fever, dehydration. Alkaline- urinary tract infection, renal failure.
4. Specific gravity	Between 1.003 and 1.030.	Low (1.001-1.002)-diabetes mellitus. High (over 1.030) –diabetes mellitus ,hepatic disease , congestive heart failure.
5. Odour	Faintly aromatic	Fruity sweet-acetone , associated with diabetes mellitus . Unpleasant – decomposition of drugs , foods, alcohol.
6. Quantity	Around 1000-1500 ml per day	High – diabetes mellitus, diabetes insipidus, nervousness, diuretics, excessive intake. Low- acute nephritis, heart disease, diarrhoea , vomiting. None – uremia, end-stage renal disease(ESRD).
7. Protein	Negative	Positive (proteinuria)-renal disease ,pyelonephritis.
8. Glucose	Negative	Positive(glycosuria)- diabetes mellitus
9. Ketones	Negative	Positive (ketonuria)- uncontrolled diabetes mellitus ,high protein ,low carbohydrate diet ,starvation.
10. Bilirubin	Negative	Positive (biliuria)-liver disease, biliary obstruction,congestive heart failure.
11. Blood	Negative	Positive (hematuria)-renal disease, trauma.
12. Nitrites	Negative	Positive(nitrituria)- bacteriuria.

Table 2(NORMAL AND ABNORMAL CONSTITUENTS OF URINE)

METHOD TO DETECT PROTEINURIA: ELECTROPHOROSIS:

The electrophoretic technique to detect Bence -Jones protein (BJP) in human urine with the limit of detection LOD(Limit of detection) of 1mg/L to BJP, and LOD of 3mg/L to other types of protein .

These results were obtained by immunofixation using Dako antisera on cellulose nitrate followed by further staining with gold.

The technique does not require particular skill and is cheap. Such low levels of LOD allowed them to use un-concentrated urine samples, thereby eliminating the problem of the loss of low molecular weight proteins.

Usually, though it is often necessary to concentrate the urine before electrophoresis because of the low urinary protein level . This is a time-consuming process.

Other methods:

Chromatography, Capillary electrophoresis– Mass

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spectroscopy

EST FOR PROTEIN IN URINE SAMPLE:
HELLER'S TEST:
PROCEDURE:

- In a clean test tube , 2ml of concentrated nitric acid is taken.
- To this 2ml of urine or other sample is added .The sample should be from the sidewall of the test tube in an inclined position in order to form a layer of the sample above the nitric acid.

OBSERVATION: The formation of a white ring at the junction of the two layers.

RESULT:

Figure 13(HELLER' TEST)

BIURET TEST:

PROCEDURE:

- Label 3 test tubes as test , positive and negative.
- In the test tube labelled as 'test' add 1-2ml of sample. The test tube labelled as 'positive' add 1-2 ml of albumin solution. In the test tube labelled as 'negative' add 1-2 ml of distilled water.
- In each test tube add equal volume of 1-2 ml biuret reagent.
- Shake well and let it stand for 5 minutes at room temperature.

OBSERVATION: The development of violet colour in the suspension .

RESULT:

Figure 14(BIURET TEST)

ACCURACY:
DIPSTICK :

- ❖ The multiple urine test stripes is used for the detection of glucose, protein, ketones and acidity/alkalinity in education.
- ❖ It is wide used as an initial screening tool for the evaluation of proteinuria. Its diagnostic accuracy has not yet been sufficiently evaluated.
- ❖ We evaluated its diagnostic accuracy using spot urine albumin/creatinine ratio (ACR) and total protein /creatinine ratio(PCR) in proteinuria.

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Figure 15(URINE TEST STRIP)

- ❖ In a general population– based study, the accuracy of urine dipstick for diagnosis of proteinuria was relatively low.
- ❖ When ACR ≥ 30 mg/g was set as the reference standard for proteinuria,the sensitivity and specificity were 37.1% and 97.3% and 23.3% and 98.9%.

II. CONCLUSION:

- Bio analytical chemistry plays a vital role in the development of modern medicine, pharmacology, and life sciences.
- It provides the tools and methodologies necessary for the qualitative and quantitative analysis of biological samples, enabling the detection of trace – level bio molecules such as drugs, proteins, and nucleic acids.
- Techniques such as chromatography, mass spectrometry and immunoassays have revolutionized the accuracy and sensitivity of bio analytical measurements.
- As research advances, the integration of bio-analytical chemistry with emerging technologies like nanotechnology and biosensors is expected to further enhance diagnostic and therapeutic applications.
- Overall, bio-analytical chemistry serves as a cornerstone in understanding complex biological systems and advancing health-related research.

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