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Review Article

Analytical Techniques for Isoniazid: A Systematic Review of UV Spectrophotometry and HPLC Methods

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ABSTRACT

This study looks at the pharmacological characteristics and analytical techniques of isoniazid (INH), a first-line antibiotic used to treat active Mycobacterium tuberculosis (TB). The bacterial cell wall is disrupted by the prodrug isoniazid, which is activated by the catalytic enzyme peroxidase and inhibits the formation of mycolic acid. With the chemical formula $C_6H_7N_3O$, isoniazid is a hydrazide derivative of isonicotinic acid. A hydrazide group has been added to a pyridine ring to create its structure. Isoniazid is mostly metabolized via acetylation in the liver, is water soluble, and stable under normal circumstances. 2.48 is the pKa for isoniazid. High-Performance Liquid Chromatography (HPLC) and UV-Visible spectroscopy are two analytical methods that are emphasized for their use in assessing isoniazid. UV-Vis spectroscopy works on the basis of electronic transitions, in which electrons are excited by light absorption and the ensuing spectra reveal information about interactions between molecules. Spectrophotometric and simultaneous equation approaches are examples of UV-Vis methods that differ by solvent and detection wavelength (e.g. λ_{max} 263–572 nm). Combinations of isoniazid and rifampicin, for instance, have λ_{max} at 264 and 474 nm in water. Chemicals in biological matrices such as human plasma, serum, and urine can be separated, identified, and quantified using HPLC. It employs a range of mobile phases (such as methanol-acetonitrile-buffer combinations) and columns (such as C8, RP-18) with UV detection at λ_{max} 254–274 nm. The composition of the matrix and the solvent affects retention periods and limits of detection (LOD); LODs as low as 0.023 g/ml have been observed. The significance of UV-Vis and HPLC in TB therapy research and monitoring is highlighted by their adaptability in assessing INH and its combinations. Accurate measurement is made easier by this data, which supports clinical applications and advances pharmaceutical research.

INTRODUCTION

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A first-line antibiotic for both active and latent Mycobacterium tuberculosis (TB) infections is isoniazid (INH). It functions as a prodrug when the enzyme is activated, producing radicals that prevent the formation of mycolic acid, an essential part of the mycobacterial cell wall. Strong bactericidal effects are provided by this mechanism, especially when combined with other TB drugs. With the chemical formula $C_6H_7N_3O$, isoniazid is structurally a hydrazide derivative of isonicotinic acid. Its simple structure allows it to function as a prodrug by substituting a hydrazide group for the pyridine ring. Under typical circumstances, it is stable, soluble in water, and mostly acetylated in the liver. The pKa value of the compound is 2.48. UV-VIS spectroscopy is a trustworthy technique for figuring out solute concentrations in analytical applications by measuring light absorption based on the Beer-Lambert law in the visible, ultraviolet, and near-infrared spectrums. This method is prized for its affordability, speed, precision, and ease of use. A sophisticated chromatography method called High-Performance Liquid Chromatography

(HPLC) is used to separate, identify, and measure substances in mixtures. The analyte's interactions with the stationary phase, mobile phase, and solvent determine the retention periods, which are measured by a detector, a stationary phase column, and a high-pressure pump for the mobile phase. Water and methanol are frequently used solvents in HPLC, and gradient elution improves separation effectiveness. ^[1,2,3]

Isoniazid Drug Profile

Structure of Isoniazid

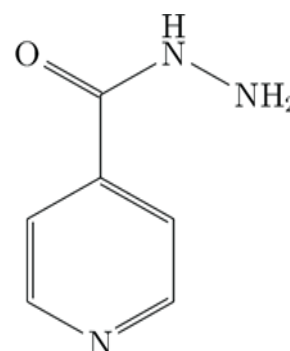


Table 1: Isoniazid Drug Profile

Attribute	Details
Chemical Name	Isonicotinic acid hydrazide
Molecular Formula	$C_6H_7N_3O$
IUPAC Name	4-pyridinecarboxylic acid, hydrazide
Molecular Weight	137.14 g/mol
Melting Point	171.4 °C
λ max (wavelength)	262-270 nm
Solubility	Water, Ethanol, Chloroform, Ether, Benzene.
PKa Value	acidic-2.48
Category	Antitubercular agent
Colour	White, Colourless
Mechanism of action	Inhibit the synthesis of mycolic acid, essential components of the mycobacterial call wall. It is a prodrug activated by the enzyme catalase-Perioxidase (KatG) in Mycobacterium Tuberculosis.
Therapeutic use	First- line treatment and prophylaxis of tuberculosis (TB) . Often used in combination with other anti tuberculosis drugs like Rifampicin, Ethambutol to prevent resistance
Typical Dosage	Adult: 2 mg/kg/day , Children: 10-15 mg/kg/ml.
Adverse Effects	Rash, Fever

Pharmacokinetic	Absorption: Rapidly absorb orally. Distribution: Widely distributed , including cerebrospinal fluid , Metabolism: Acetylated in liver, Excretion: Renal.
Pharmacodynamic	It is an antibiotic used to treat mycobacterial infections.

Principle

The way that chemical compounds absorb UV or visible light and cause electrons to travel between energy levels is measured by UV-visible spectroscopy. Excitation to higher energy states produces absorption spectra, whereas relaxation back to lower states produces emission spectra. This idea is the foundation of molecular spectroscopy. By injecting the sample components

into a column with a porous stationary phase and forcing a liquid mobile phase through at high pressure, High-Performance Liquid Chromatography (HPLC) separates the components of the sample. Solute affinity is the basis for separation; longer retention periods are associated with stronger interactions. When compared to traditional chromatography, HPLC uses smaller particles for faster and more effective analysis. [4,5,6]

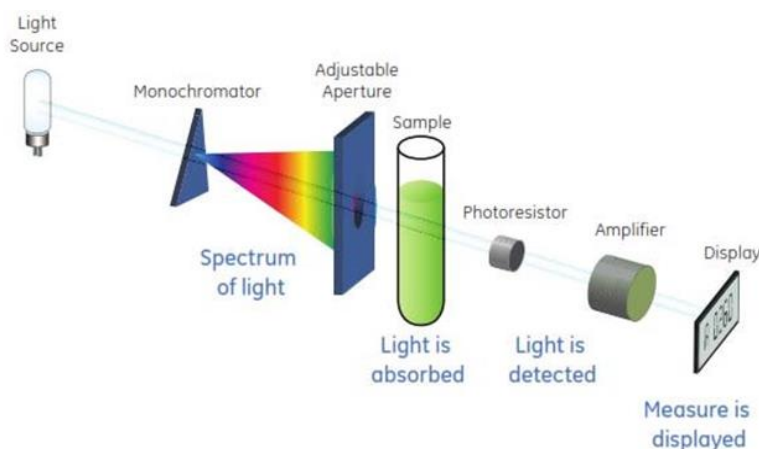


Figure 1: Schematic diagram of UV spectrophotometer

Solvents for UV Spectrometry

Table 2: The wavelength of measurement with the solvent

Solvent	λ (nm)
water	190
Methanol, ethanol	205
glycerol	230
dimethylformamide	270
Acetic acid	270

Absorption intensity transmission and UV spectrum

- 1) Bathochromic shift:** also called a redshift, The migration of absorption towards a longer wavelength.
- 2) Hypochromic shift:** also called a blueshift, happens when the absorption shifts to a shorter wavelength.
- 3) Hyperchromic effect:** The absorption peak's strength increases.
- 4) Hypochromic change:** A decrease in the absorption peak's intensity. [7]

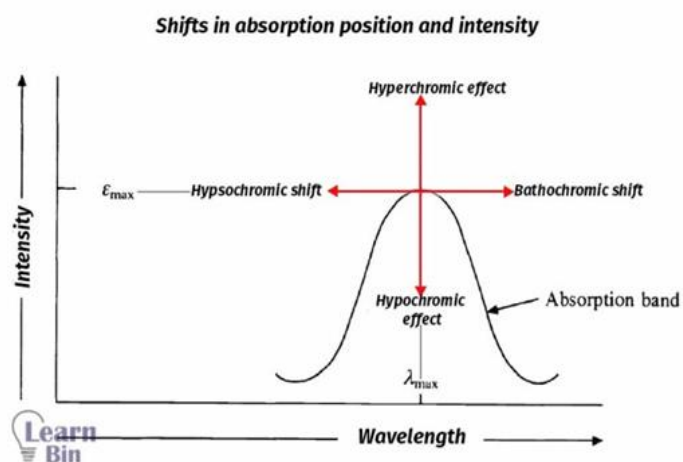


Figure No.2: Shifts in Absorption and

Intensity

- 1) Chromophores
- 2) Auxochrome

Table 3: UV Segments and Their Wavelength Regions

Ultraviolet Segment	Wavelength Range	Region
Extreme ultraviolet	10 nm-121 nm	Extreme Ultraviolet
Far Ultraviolet	122 nm-200 nm	Far Ultraviolet
Middle Ultraviolet	200 nm-300 nm	Middle Ultraviolet
Near Ultraviolet	300 nm-400 nm	Near Ultraviolet

Instrumentation

Light source

- (1) Hydrogen Lamp
- (2) Deuterium Lamp
- (3) Tungsten Lamp
- (4) Xenon Discharge Lamp ^[8]

Monochromator

Sample Cell

Detector ^[9]

Theories of Spectroscopy

Beer's Law

A solution's absorbance (A) is directly proportional to its concentration (c) and route length (b). The equation is $A = a.b.c$. Use $c = A / (ab)$ to get concentration (c), where A stands for absorbance, a for molar absorptivity, and b for path length.

Lambert's Law

The absorbing medium's thickness directly correlates with the light's drop in intensity. ^[10]

Law of Beer-Lambert

According to the Beer-Lambert equation, the sample's absorbance (A) is proportional to its concentration (c) and route length (b). $A = a.b.c$ is the formula, where a is the absorptivity. Absorbance quantifies the amount of light that the sample absorbs. ^[11]

Types of Spectroscopies

1. According to the Study Level:

Molecular Spectroscopy: UV Spectroscopy, Colorimetry, Infrared Spectroscopy, and Fluorimetry

- 1) Atomic Spectroscopy
- 2) Atomic Absorption Spectroscopy
- 3) Flame Photometry

2. Based on the Interaction with Electromagnetic Radiation (EMR)

- 1) Absorption Spectroscopy
- 2) Emission Spectroscopy

3. Based on the Type of Energy Changes Studied

- 1) Electronic Spectroscopy
- 2) Magnetic Spectral Analysis ^[12]

Method development in UV spectroscopy

The evolution of UV spectroscopy techniques

1. Choosing the Wavelength (λ_{max})
2. Creating Standard Solutions
3. Choosing the Solvent
4. Development of the Calibration Curve
5. Verifying the Linearity Range

6. Analysis of the Sample
7. Optimizing Instruments
8. Correction to Baseline
9. Verification of Methods

Validation Parameter

Linearity

Purpose- Identifying if the UV absorbance is proportionate to the analyte concentration is the goal.

Acceptance Criteria-2 (correlation coefficient) must be greater than 0.999. a linear correlation, within a certain range, between concentration and absorbance.

Table 4 : Different Linearity range

Concentration ($\mu\text{g/ml}$)	Absorbance	Solvent
0.75- 3.75 $\mu\text{g/ml}$	721 nm	Methanol and distilled water
1-150 $\mu\text{g/ml}$	262 nm	Water and ethyl acetate
2- 10 $\mu\text{g/ml}$	263 nm	Methanol
2.5- 35 $\mu\text{g/ml}$	344 nm	Methanol
5-15 $\mu\text{g/ml}$	225.66 nm	0.1N HCL
5- 15 $\mu\text{g/ml}$	262.2 nm	Methanol & phosphate buffer
10 $\mu\text{g/ml}$	265 nm	Methanol

Accuracy

Purpose- To measure how close the experimental values are to the true value.

Acceptance Criteria- Accuracy should be within 98%-102% of the expected value for pharmaceutical compounds, although criteria may vary depending on the application and regulatory guidelines.

Precision

Purpose- To evaluate the precision under the same operating conditions over a short period.

Acceptance Criteria- Relative standard deviation (RSD) should be $\leq 2\%$.

Relative standard deviation % = $\frac{\text{standard deviation}}{\text{Mean}} \times 100$

Detection Limit (LOD)

Purpose- To determine the lowest amount of analyte that can be detected but not necessarily quantified.

Acceptance Criteria- LOD can be determined by signal-to-noise ratio, typically $S/N \geq 3$.

The LOD was calculated according to following equation:

$$\text{LOD} = 3.3 \times \text{SD } \sigma$$

Where, SD = standard deviation of response, σ = slope of regression line

Quantitation Limit (LOQ)

Purpose- Finding the lowest concentration of analyte that can be detected but not necessarily quantified is the goal of the detection limit (LOD).

Acceptance Criteria: Signal-to-noise ratio, usually $S/N \geq 3$, can be used to calculate LOD. The following formula was used to determine the LOD:

$\text{LOD} = \frac{3.3 \times \text{SD}}{\sigma}$ where σ is the slope of the regression line and SD is the response standard deviation.

Limit of Quantitation (LOQ)

The goal is to ascertain the minimum quantity of analyte that can be quantitatively quantified with a level of accuracy and precision that is acceptable.

Acceptance Criteria: A signal-to-noise ratio of $S/N > 10$ is normally required for LOQ.

The following formula was used to determine the LOQ:
 $\text{LOQ} = \frac{10 \times \text{SD}}{\sigma}$

LOQ = where σ is the regression line's slope and SD is the response's standard deviation

Robustness

The goal is to determine whether the method can withstand minor, intentional adjustments to its parameters, such as wavelength and solvent composition.

Range

Purpose: To identify the concentration range over which the technique exhibits satisfactory linearity, accuracy, and precision.

Acceptance Criteria: Must meet regulatory requirements or encompass 80%–120% of the intended concentration.

Stability of Analytical Solution

Purpose: To ascertain whether the analytical solution is stable throughout time. **Acceptance Criteria:** Over a predetermined amount of time (e.g., 24 hours), the answer must be within $\pm 2\%$ of the original reading.

ICH Guidelines (ICH Q2R1) for Analytical Procedure and Validation:

The method used to carry out the analysis is referred to as the analytical technique. The procedures required to carry out each analytical test should be thoroughly explained. The preparation of the reagents, the reference standard, the sample, the usage of the equipment, the creation of the calibration curve, use of the formulae for the calculation.

Advantages of UV-Visible spectroscopy

- 1) High accuracy
- 2) Robust operation
- 3) Simplicity
- 4) Cost-effectiveness

Disadvantages of UV-Visible spectroscopy

- 1) Chromophore limitation
- 2) Sample state
- 3) Preparation time

Application Of UV-Vis Spectroscopy:

Detection of Impurities: Identifying contaminants in samples.

Structural Elucidation: Determining the structure of organic compounds through absorption characteristics.

Quantitative Analysis: Measuring concentrations of substances in solutions.

Qualitative Analysis: Identifying compounds based on their UV-Vis spectra.

Chemical Analysis: Analysing various chemical substances and reactions.

Pharmaceutical Analysis: Quantifying active pharmaceutical ingredients and other components.

Dissociation Constants: Measuring the dissociation constants of acids and bases.



Molecular Weight Determination: Estimating the molecular weights of compounds.

HPLC Detection: Serving as a detector in high-performance liquid chromatography (HPLC).^[13]

Table 5: Representative Spectroscopic Method of Analysis Of Isoniazid

Compounds	Method	λ_{max}	Solvent	Linearity $\mu\text{g/ml}$	Accuracy	Precision (% RSD)	LOD $\mu\text{g/ml}$	LOQ $\mu\text{g/ml}$	Ref
Isoniazid, Para amino salicylic acid	UV spectroscopic method	263 nm	Methanol	2-10	-	1.5326	3.3	10	39
Isoniazid, Rifampicin, pyrazinamid	UV spectroscopic method	721 nm	Methanol	0.75-3.75	102.3	0.94	0.006	0.019 8	40
Isoniazid, Pyridoxine Hydrochlorid	UV spectroscopic method	265 nm	Hydrochloric acid	0,9966	98,7909	0,3485	10747	3583 3	41
Isoniazid, Pyridoxine Hydrochlorid	UV spectroscopic method	262.2 nm	Methanol, phosphate buffer	99.69	1.17	0.003	0.003 3	0.011 0	42
Isoniazid, Rifampicin pyrazinamid	UV spectroscopic method	225.6 6 nm	Double distilled water	5-15	100.07	0.63	0.18	0.72	43

High Performance Liquid Chromatography (HPLC)

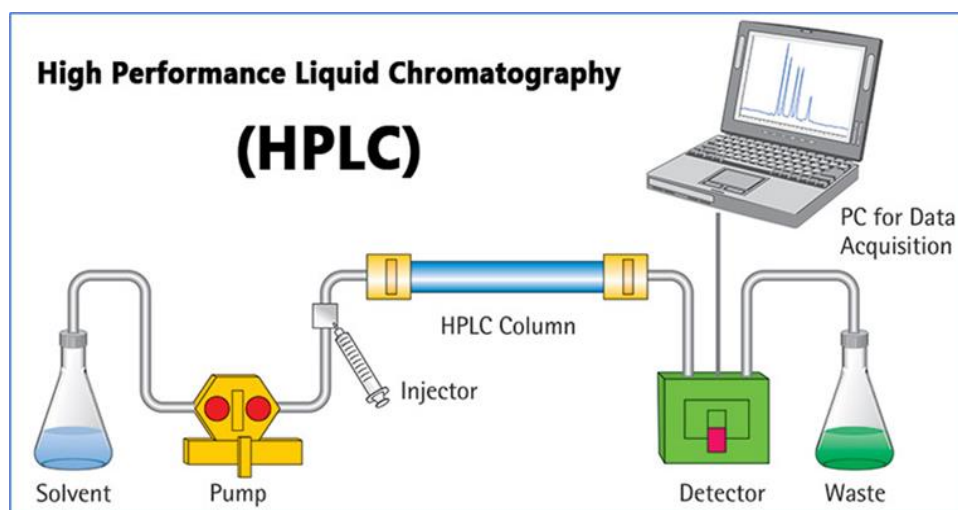


Figure 3: Flow diagram of HPLC

History

Chromatography uses a stationary and mobile phase to separate, identify, and quantify the components of a mixture. A detector, pump, and column are used in HPLC, a kind of column chromatography. Gravity-based flow made early liquid chromatography systems sluggish and ineffective. For some applications, gas chromatography (GC) proved superior, but it was unable to analyze polar, high-mass biopolymers. Building on the work of Martin and Synge (1941), scientists like Cal Giddings and Josef Huber enhanced liquid chromatography in the 1960s by decreasing particle size and raising pressure. Performance had significantly increased by the 1970s thanks to

developments in HPLC instruments and particle technologies, including gas compressor pumps and permeable layer particles. Efficiency gradually increased with smaller particles, but higher pressure became problematic.^[14, 15, 16]

Instrumentation

Solvent Reservoir-

Pump
Sample Injector
Column
Detector

Table 6 : Common HPLC solvents and their important their properties

Solvent	UV cut off	Viscosity	Refractive Index	Boiling Point
Acetone	330 nm	0.30	1.36	56.3
Acetonitrile	190 nm	0.38	1.34	81.6
Chloroform	245 nm	0.57	1.44	61.15
Hexane	195 nm	0.312	1.38	68.7
Propyl alcohol	210 nm	2.3	1.38	97.2
Water	190 nm	1	1.33	100

Type of HPLC

Normal Phase Chromatography

Principle: the non-polar analytes elute first while polar analytes are retained longer by a polar stationary phase.

Application: Best suited for preparative-scale HPLC, ideal for isomeric combination separation, and a backup choice for lipophilic chemicals.

mobile phase: Non-polar solvents, such as hexane and dichloromethane.

stationary phase: Polar materials, such as alumina and silica gel.^[17]

Reverse phase chromatography

principle: is the separation of non-polar analytes from the non-polar stationary phase by hydrophobic interactions.

Application: It works well with a variety of

substances, such as hydrophobic, lipophilic, and nonvolatile molecules.

Stationary phase: Non-polar materials, such as C18 and C8-bonded silica. Polar solvent is the mobile phase.^[18]

Size-Exclusion Chromatography

Principle: Using gel matrices, separation is carried out according to molecular size.

Application: Determining polysaccharide molecular weights and amino acid structures.

mobile phase: Depending on the kind of gel and analyte, the mobile phase may consist of either aqueous or non-aqueous solvents.

Stationary phase: semi-rigid gels such as polystyrene and alkyl-dextran, or gel materials such as agarose, polyacrylamide, and dextran (soft gel).^[19]



Ion-exchange chromatography:

Principle: The interaction between charged solutes and oppositely charged groups on the stationary phase is the foundation of the retention

Application: In biology and industry, common applications include protein purification, water deionisation, carbohydrate analysis, and ionic compound separation.

Mobile phase: Is an aqueous solution that contains salts to regulate ionic strength and buffers to alter pH.

Stationary Phase: A resin or matrix with covalently bonded charged groups, such as cationic (like quaternary ammonium) or anionic (like sulfonic acid) functional groups, is known as the stationary phase. [20]

Affinity Chromatography

Principle: Target molecules and immobilized ligands must form a specific bond in order for separation to occur.

Applications: The separation of biomolecules, enzyme isolation, and protein purification.

Mobil phase: A buffer system that is tailored to the conditions of binding and elution.

stationary phase: A solid matrix having covalently bonded ligands, such as agarose or sepharose, is known as the stationary phase. [21]

Method Development of HPLC

1. Assessing the drug's physicochemical characteristics to inform the choice of solvent and technique.
 2. Choosing the detector, column, and mobile phase, among other basic chromatographic parameters.
 3. Modifying the parameters of the analysis approach in light of initial findings.
 4. Getting the sample ready to make sure it works with the selected technique.
- enhancing the process to increase resolution, accuracy, and precision.

Understanding the physicochemical properties of a drug molecule

Selection of chromatographic conditions

The first step in developing a chromatographic separation method is choosing the beginning conditions, which in reversed-phase separations usually involve a C18 column with UV detection.

Selection of column

The steady retention of the sample is guaranteed by a repeatable stationary phase. Purified silica columns, such as C8 and C18, are frequently employed for basic chemical separation because of their strength and stability in organic solvents. But compounds based on silica can dissolve at pH values higher than 7. For the separation of ionizable substances, such bases and acids, reversed-phase chromatography (RPC) is frequently utilized. Utilizing buffered mobile phases or ion-pairing agents to inhibit ionization and improve separation, it takes use of the polarity and molecular weight of the analyte.

Selection of Chromatographic Mode

The polarity and molecular weight of the analyte determine the chromatographic modes. Using buffered mobile phases or ion-pairing reagents, reversed-phase chromatography (RPC) is a popular method for tiny organic molecules because it efficiently separates ionizable chemicals like bases and acids.

Buffer Selection

The ideal pH range for reversed-phase chromatography on silica-based packing materials is typically between 2 and 8, therefore choosing a buffer is mostly based on that value. When a buffer's pKa is around the target pH—ideally, within two pH units of the intended mobile phase pH—it controls pH the best. The following general rules apply when choosing a buffer:

Table 7: Buffer used in HPLC

Buffer	pKa	Useful pH Range
Ammonium Acetate	4.8	3.8- 5.8

Acetic acid	4.8	3.8-5.8
formic Acid	3.8	2.8-4.8

General Considerations for Buffer Selection

Phosphate buffers dissolve more readily in water and methanol. Chromatographic problems such as tailing and changes in selectivity can be brought on by hygroscopic salt buffers. Ammonium salts dissolve better in organic/water-soluble phases. Microbial development is encouraged by low organic buffered phases, which impacts column performance. Phosphate buffers reduce the life of HPLC columns by dissolving silica at pH >7; organic buffers are recommended

instead. The release of CO₂ causes ammonium bicarbonate buffers to change pH during a 24- to 48-hour period. [22, 23,24]

Concentration of Buffers

Generally, 10–50 mM of buffer concentration is adequate for small molecule analysis. Generally speaking, the mobile phase should not have more than 50% organic content. The most used buffer systems for reversed-phase HPLC are phosphoric acid and its potassium or sodium salts. [25]

Selection of Mobile Phase

Table 8: The overall Study of Technique

Mode	Solvent Type used	Type of compound used
Reverse Phase	Water/Buffer, Methanol	Neutral or non-ionized compounds which can dissolve in water/organic mixtures.
Ion-pair	Water/Buffer, Methanol	Ionic or Ionizable compounds
Normal Phase	Organic solvents	Mixtures of isomers and compounds not soluble in organic/Water mixtures.
Ion exchange	Water/Buffer	Inorganic ions, proteins, nucleic acids, organic acids.
Size exclusion	Water, chloroform	High molecular weight compounds.

Table 9: Mobile Phases Used During the HPLC Method Development Process.

Mobile phase	Ratio	PH
Methanol: potassium dihydrogen phosphate	40: 60	7.0
Methanol: water: acetonitrile: ammonium acetate	44: 30: 25: 1	5.5
Methanol: water: acetonitrile	45: 30: 25	5.5- 6.5

Selection of detectors

Table 10: Detectors Used in HPLC

Detector	Type of compound can be detected
UV-Visible & Photodiode array	Compounds with chromophores, such as aromatic rings or multiple alternating double bonds.
Fluorescence detector	Fluorescent compounds, usually with fused rings or highly conjugated planar system.



Conductivity detector	Charged compounds, such as inorganic ions and organic acid.
Electrochemical detector	For easily oxidized compounds like quinines or amines

Component of validation parameter Internal Diameter (ID):

HPLC columns with a larger internal diameter are utilized in industrial settings, whilst those with a smaller ID have a lower loading capacity but consume less solvent and are more sensitive. [26]

Pore Size:

Larger pores improve kinetics for larger analytes, while smaller pores provide a greater surface area but are better suited for smaller analytes. Because the inner surface area of the particle is significantly bigger than the outer surface, there is a considerable interaction between the analytes and the inner surface.

Pump Pressure:

Pumps come in a variety of pressure capabilities, and the system's effectiveness is correlated with their capacity to provide a steady flow rate. For instance, two micrometers. [27]

System Resolution:

The formula $=2(t_2-t_1)/(W_2-W_1)$ is used to determine the resolution between two components in an HPLC system. where W_1 and W_2 are the peak widths and t_1 and t_2 are the retention times. In general, a resolution factor larger than 2.0 is regarded as appropriate. [28]

System Precision:

The relative standard deviation (RSD) of the peak responses (area or height) following several injections is used to calculate the system precision. If the RSD is 2% or below, five replicates are usually employed; if it is greater than 2%, six repetitions are needed.

Asymmetry factor:

The formula $T=W_{0.05}/2f$ is used to determine the peak asymmetry or tailing factor, where $W_{0.05}$ is the peak width at 5% of the peak height. Reduced resolution and precision are the results of increased asymmetry. [29]

Method of validation parameter

Specificity: The capacity to distinguish the analyte with clarity when anticipated components such as contaminants or degradation products are present is known as specificity. [30]

Range and Linearity: The ability of a method to produce findings proportionate to the analyte concentration within a certain range is measured by its linearity. Typically, to evaluate this, standard solutions at different concentrations are analyzed, and a calibration curve is produced. [31]

Accuracy: Trueness, which is frequently evaluated as accuracy, is the degree to which measured values resemble the true value. By contrasting the measured value with a reference standard, accuracy is ascertained. The usual accuracy limitations for pharmacological compounds and products are 98.0–102.0% and 97.0–103.0%, respectively. An average recovery range of 50–150% can be suitable for determining impurities. [32]

Calculate The Relative Standard Deviation (RSD) By Following The Equation Below:

$$\text{relative standard deviation \%} = \frac{\text{standard deviation}}{\text{Mean}} \times 100$$

Precision: A bio-analytical method's precision is the consistency of results from several measurements of the same homogeneous material under predetermined conditions. It shows the random error. Usually stated as the relative standard deviation (RSD) or coefficient of variation (%CV), it evaluates the variability (scatter) in concentrations from replicate samples.

% CV = standard deviation males X 100 is the formula used to determine the %CV. [33]

Detection Limit (LOD):

The smallest amount of an analyte in a sample that can be detected but not precisely measured is known as the detection limit of an analytical procedure. It is a crucial indicator of the sensitivity of the procedure since it denotes the lowest concentration at which the analyte may be accurately identified.

The LOD was calculated according to following equation: $LOD = 3.3 \times SD \sigma$

Limit of Quantification (LOQ):

The limit of quantification, or LOQ, is a measurement of an analytical technique's ability to accurately and precisely quantify an analyte. It can be computed as follows: $LOQ = 10 \times SD \sigma$

Where, SD = standard deviation of response σ = slope of regression line

Robustness: An analytical procedure is said to be robust if it can withstand minor, deliberate adjustments to its parameters, demonstrating its dependability under typical operating circumstances. [34,35]

System Suitability: Tests for system suitability make sure that the sensitivity, resolution, and repeatability of a chromatographic system are sufficient for the planned analysis. These tests evaluate the system's entire performance, taking into account the electronics, equipment, processes, and samples. Peak resolution, theoretical plate count, peak tailing, and capacity are frequently assessed metrics. [36]

Advantages of HPLC

1. High repeatability.
2. Extremely sensitive
3. Ongoing column effluent monitoring.
4. Useful for sorting and evaluating extremely complicated combinations.
5. Excellent accuracy and efficiency.
6. Rapid resolution.

Disadvantages of HPLC

1. Because HPLC requires expensive equipment and organic solvents, it can be costly.
2. Needs frequent maintenance and a steady power source.
3. It could be difficult to debug or create new techniques.
4. Expensive and complicated operations.

Pharmaceutical Applications

- 1) Dissolution tests of pharmacological dose forms in tablets.
- 2) Regulation of medication stability and assessment of shelf life.
- 3) Determining the Active Component
- 4) Determining the active pharmacological component
- 5) Quality Control for Pharmaceuticals

b) Food Applications

- 1) HPLC is frequently used to ensure quality control and analyze food goods.
- 2) Good for testing chemicals that are labile in complicated matrices.
- 3) Examines pollutants, food additives, and natural substances including carbohydrates, fats, proteins, and amino acids.
- 4) Performs multiresidue testing for pesticides and pollutants.

c) Forensics Applications

- 1) medicines found in biological specimens.
- 2) Performs forensic analysis on textile dyes.
- 3) Identifies cocaine in blood samples and its metabolites.

d) Clinical Applications

- 1) Applied to routine analysis and clinical research.
- 2) Assesses glycated hemoglobin to track diabetes patients' long-term glucose management.
- 3) Aids in determining the origins of poisoning. [37,38]

Table 11: HPLC- based methods described for analysis of Isoniazid in pharmaceuticals and biological samples.

Mobile phase	Column	Detect ion	λ_{max} (nm)	Flow rate (ml/min)	LOD (g/ml)	LOQ (μ g/ml)	Ref.
Methanol:Acetonitrile:buffer(20mM pH2.5 heptanesulfonic acid sodium) (10:8:82 v/v/v)	Synergi Max-RP C12(250 \times 4.6mm,4 μ m)	UV	264	2	0.023	0.1	44
Water :methanol (85:15v/v)	Merck C8,(250 \times 4.6mm,5 μ m)	UV	274	1.2	-	-	45
0.1M phosphate buffer, (pH5 Ortho phosphoric acid):methanol(50:50v/v)	Waters,Symmetry shield RP-18,150(4.6mm cm,5 μ m)	UV	254	0.9	0.150	200	46
0.05M ammonium acetate buffer(pH): acetonitrile (99:1,v/v)	Pinnacle II C18(150 \times 4.6 mm,5 μ m)	UV	275	1.2	-	-	47

CONCLUSION

Isoniazid and its combinations can be analyzed both quantitatively and qualitatively using UV-Vis spectroscopy and HPLC. For routine examination, UV-Vis spectroscopy is quick and easy, while High Performance Liquid Chromatography is more sensitive and precise, especially for complex biological matrices. By facilitating efficient isoniazid monitoring in clinical and research settings, these techniques enhance therapy outcomes and TB treatment approaches.

REFERENCES

1. Govinda Verma, Dr. Manish Mishra. Development and optimization of UV-visible spectroscopy-a review ; Word journal of pharmaceutical research. 2018; Page no. 1170,1171.
2. Miss. Pawar Diksha Lakshman, Trupti Thange. A review of high performance liquid chromatography; International journal of research publication and review. 2022; page no. 3015



3. Priya Sadapha, Kavita Dhamak. Review article on high-performance liquid chromatography (HPLC) method development and validation; *International journal of pharmaceutical sciences review and research*.2022; page no 23.
4. Smit Patel, Aniket Raulji, Diya Pate, Diya Panchal, prof. Mitali Dalwadi, Dr. Umesh Upadhyay. A review on uv visible spectroscopy; *International journal of pharmaceuticle research and application*. 2022; page no 1144.
5. Alekhya Mandru , Jyothi Mane , Ramya Mandapati. A Review on UV-visible spectroscopy; *Journal of pharma insites and research* 2023; page no 91.
6. Abdu Hussen Ali. High performance liquid chromatography (HPLC): a review; *Article in Annals of Advances in Chemistry* · June 2022 ; Page no 11.
7. Kisner, H. , Brown, W. Kavains, G. “ Multiple analytical frequency and standards for least squares analysis of serum lipids. *Anal. Chem*. 1983, 55, 1703.
8. Sargar Komal Bharat, Alfa Jain. A review on advances in uv spectroscopy; *International journal of pharmaceutical research in pharmacy and pharmaceutical sciences*. 2023; page no 19,20.
9. Saiyed Anjumara Alemustufa, Vaghela Kuldeepsinh Hemantsinh, Patel Vruddhi Rashmikant, Mitali Dalwadi, Dr. Chainsesh Shah and Dr. Umesh Upadhyay. A review on uv visible spectroscopy; *Word journal of pharmacy and pharmaceutical science*. 2021; Page no 415.
10. Shraddha khokrale, Jeetu Lulla, Laxmikant Borse. A Review on uv visible spectroscopy’s method devlopment and validation; *International journal of pharmaceutical pharmaceutical sciences*; 2024.
11. Bhagyashri Shivsharan Dhange; A review on uv-visible Spectroscopy; *International journal of novel research and devlopment (IJNRD)*. 2024; Page no 84.
12. Diya Patel, Diya Panchal, kunj Patel, Prof. Mitali Dalwadi, Dr. Umesh Upadhyay. A Review on uv visible spectroscopy; *International journal of creative research thoughts (IJCRT)*. 2022; Page no 409,410.
13. Rachit Shukla, Prashant Kumar Singh, Savita Upadhyay. A comprehensive review of high performance liquid chromatography (HPLC); *International journal of Pharmaceuticle science*. 2023; Page no 313.
14. Ms. Pooja S Murkute, Mr. Paresh H Patil, Dr. Gajanan S Sananp, Mr. Nakul P Kathar, Ms. Aishwarya P Pimple. A review on high performance liquid chromatography (HPLC); *International journal for research trends and innovation*. 2022; Page no 98.
15. Dhara k Patel, Mrs. Mitali Dalwadi. A review on high performance liquid chromatography; *International journal of pharamaceutical research and applications*. 2023; Page no. 2532.
16. Abdu Hussen Ali. High performance liquid chromatography (HPLC): a review; *Article in Annals of Advances in Chemistry* · June 2022 ; Page no 11.
17. Patel Dixita , Dr. Priyanka S. Patil , Dr. Umesh M. Upadhyay. A review on HPLC method development; *International journal of all research education and scientific methods*. 2024; Page no 987.
18. Chetna Malwal , P. B. Jadhav , Vinod Bairagi. A comprehensive review on high-performance liquid chromatography; *International journal of pharmaceuticle*. 2024; Page no 291.
19. Chanchal Bhati, Neha Minocha, Deepika Purohit, Sunil Kumar, Manish Makhija, Sapna Saini, Deepak Kaushik and Parijat Pandey. High performance liquid chromatography:

- Recent patents and advancement; biomedical & pharmacology journal, 2022; Page no 731.
20. S. Karimunnisa Begum , K. Udaya Kumari, S. Sajana. A short review of high-performance liquid chromatography; Mathematical statistician and engineering applications. 2022; Page no 411.
 21. Chetna Malwal, P. B. Jadhav, Vinod Bairagi. A comprehensive review on high-performance liquid chromatography; International journal of pharmaceuticle science .2023; Page no 291.
 22. Azim M D Sabir, Mitra Moloy, Bhasin Parminder S. HPLC method development and validation: A review; International research journal of pharmacy. 2013; Page no 41.
 23. Puneet Narula and Bikash Pal.A comprehensive review on method devlopment by HPLC; World journal of pharmaceutical research. 2021; Page no 1851.
 24. Vijendra H. Rajan. Development and validation of HPLC method - A Review; International journal of current research in pharmacy. 2015; Page no 62.
 25. Ms Pallavi Nemgonda Patil. HPLC Method Development- A review; Journal of pharmaceutical research & education. 2017; Page no 250.
 26. Nazario CE, Silva MR, Franco MS, Lancas FM. Evolution in miniaturized column liquid chromatography instrumentation and applications: An overview. Journal of chromatography; 2015.
 27. Webb PA, Orr C, Camp RW, Olivier JP, Yunes YS. Analytical methods in fine particle technology. Norcross: Micromeritics Instrument Corporation; 1997.
 28. Iverson BD, Garimella SV. Recent advances in microscale pumping technologies: a review and evaluation. Microfluidics and nanofluidics. 2008.
 29. Apawu, Aaron Kwaku. Reversed- phase HPLC determination of allin in diverse varieties of fresh garlic and commercial garlic products; MS thesis. East Tennessee State University, 2009.
 30. Engel E, Ratel J, Blinet P, Chin S T, Rose G, Marriott P J. Benchmarking of candidate detectors for multiresidue analysis of pesticides by comprehensive two-dimensional gas chromatography. Journal of Chromatography. 2013 ; Page no142.
 31. Murugan S, Elayaraja A, Niranjana Babu M, Chandrakala K, Prathap Naik K, Ramaiah P and Chathurya .Vulchi. A Review on method development and validation by using HPLC; International journal of novel trends in pharmaceuticle science. 2013; Page no 80.
 32. Shweta Shinde, Dr. Vikas Rajurkar. Advances in high-performance Liquid chromatography (HPLC) method development and validation: A comprehensive review; Journal of drug delivery and bio-therapeutics. 2024;Page no 83.
 33. Vaishnavi S. Kalamb, Madhuri D. Game, Tejaswini G. Malge, Akash S. Malthankar. HPLC method development and validation: A review. International Journal of Advanced Research in science, communication and technology. 2022; Page no 757.
 34. A. Kirthi, R. Shanmugam1 , M. Shanti Prathyusha1 , D. Jamal Basha . A Review on bioanalytical method development and validation by RP - HPLC; Journal of global trends in pharmaceutical sciences. 2014; Page no 2288.
 35. Dhirendra Kumar Mehta, Mahato Ashok Kumar, Koiri Sonali. HPLC method development and validation: A review ; Wold journal of pharmaceuticle and medical research. 2024; Page no 239.
 36. Patwekar SL, Sakhare R S and, Nilesh N. Nalbalwar. HPLC method development and validation- A general concept; International



- journal of chemical and pharmaceutical sciences. 2015; Page no 13.
37. Adunga Girma Lema, Batiru Megersa Bekele. Review on high performance liquid chromatography Method of development, public health importance and validation; Austin chromatography .2023; Page no.3,4.
 38. S.D. Varhadi, V. A. Gaikwad, R. R. Sali, K. Chambalwar, Vikas Khandekar. A short review on: Definition, principle and applications of high performance liquid chromatography; International journal of pharmacy and pharmaceutical research.2020; Page no 632,633.
 39. S. Vijayraj, V. Sangeetha, B. Om Shanti, And K.P Sampath Kumar; Development and validation of isoniazid and paraamino salicylic acid simultaneously in prepared cocrystal formulation by UV spectrophotometry; International Journal of science and technology; 2013.
 40. Sourav Khawas, sampurna parui, Suddhasattya Dey, Sudip Kr. Mondal Saptarshy sarkar; simultaneous Spectrophotometric Estimation of Rifampicin, isoniazid and Pyrazinamide in their pharmaceuticle dosage form; Asian J. research article ; march -April 2020.
 41. Rida Evalina tarigan Yustifin sarumaha; Determination of isoniazid and Pyridoxine Hydrochlorid levels in tablets with ultraviolet spectrophotometry by successive ratio derivative; international Archives of Medicinal Science and Public Health; 2022.
 42. Lidsa Rimayani Nasution, Muchlisyam Bachri, effendy De Lux Putra; Simultaneous estimation of isoniazid and Pyrodoxine Hydrochlorid in tablet dosage form by spectrophotometry ultraviolet with area under curve method; Asian journal of pharmaceutical and clinical research; 2018.
 43. Luthvia, Muhammad Andry; Simultaneous content analysis of rifampicin,isoniazid,and Pyrazinamide in tablet with dual wavelength method and ratio substation method; Journal Farmasi Sains dan Praktis; November 2022.
 44. Zhifeng ZHOU,Lingyun CHEN, Peng LIC, Mei SHEN, and Fei ZOU; Simultaneous Determination of Isoniazid, Pyrazinamide, Rifampicin and Acetyisoniazid in Human Plasma by High Performance Liquid Chromatography; Analytical Science; November 2010.
 45. A.K.Hemanth Kumar, V. Sudha, Geetha Ramachandran; Simple and Rapid Method for Simultaneous DetAsian Journal of Biomedical and Pharmaceutical Sciencesermination of Isoniazid and Acetyl Isoniazid in Urine by HPLC;2014.
 46. Rohit Bhandari and Indu Pal KAUR; A Sensitive HPLC Method for Determination of Isoniazid in Rat Plasma, Brain, Liver and Kidney; Journal of Chromatography and Separation Techniques; anuary 2012.
 47. Madhavi R., Mohana Krishna A., Shobha Rani G., Mounika D. ; Isoniazid: A Review of Analytical Methods; Asiaon journal of pharmaceuticle analysis; 2015.

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