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A COMPLETE REVIEW ON THE REPORTED METHODS OF UV SPECTROSCOPY OF ISOSORBIDE MONONITRATE TABLETS

Anand Gurjar[1*], Ms. Purvi Ramanauj[2], Dr. Pragnesh Patani[3]

[1*]Student, Khyati College of Pharmacy, Palodia, Ahmedabad.
[2]Associate Professor, Khyati College of Pharmacy, Palodia, Ahmedabad.
[3]Principal, Khyati College of Pharmacy, Palodia, Ahmedabad.

***Corresponding Author:** Anand Gurjar
*Student, Khyati College of Pharmacy, Palodia, Ahmedabad, Email: anandgurjar2273@gmail.com

Abstract: Isosorbide mononitrate (ISMN) is a nitrate ester used in the angina pectoris and chronic heart failure. It has potent vasodilator properties. The HPLC (High Performance Liquid Chromatography) is the most popular method for the analysis of the Isosorbide Mononitrate drug. The other analytical methods are LC (Liquid Chromatography), UV-visible spectroscopy and GC (Gas Chromatography). The various reported analytical methods having their own advantages and limitations. HPLC is widely used for the precise and sensitive quantification of tablet formulations, often enhanced by coupling with detectors like UV, fluorescence, or mass spectrometry. Spectrophotometric methods provide a cost-effective and straightforward approach for routine analysis of APIs in tablets by measuring absorbance or transmittance at specific wavelengths. Gas chromatography is utilized for the precise analysis of volatile components and specific stability studies in tablet formulations. Liquid chromatography enables precise quantification of active ingredients and impurities in tablet formulations. chromatographic techniques are preferred for comprehensive analysis while spectroscopic methods are valued for their simplicity, cost-effectiveness and speed in routine analysis.

Keywords: Isosorbide Mononitrate, High Performance liquid Chromatography, Spectroscopy, Liquid Chromatography, Gas Chromatography, Analytical Methods

1. Introduction

Pharmaceutical analysis is a field within pharmaceutical sciences that focuses on the methods and techniques used to identify, quantify, and assess the quality of drugs and pharmaceuticals. There are two primary types of analysis: Qualitative & Quantitative.[1] The qualitative analysis focuses on identifying the components or substances present in a sample at the other side the qualitative analysis focus on the amount or concentration of a specific substance in a sample. Pharmaceutical analysis is vital for safeguarding patient health by ensuring that drugs are safe, effective, and of high quality. Pharmaceutical analysis relies on a diverse array of techniques to analyse the drug.[2] The choice of technique depends on factors such as the nature of the sample, the desired information, sensitivity, specificity, and cost. The roles of pharmaceutical analysis are identification & characterizing the potential drug, ensuring the quality & consistency of drug products, determine the shelf life & storage conditions and investigating counterfeit drugs. Here are the methods of pharmaceutical analysis shown in the figure.[3]

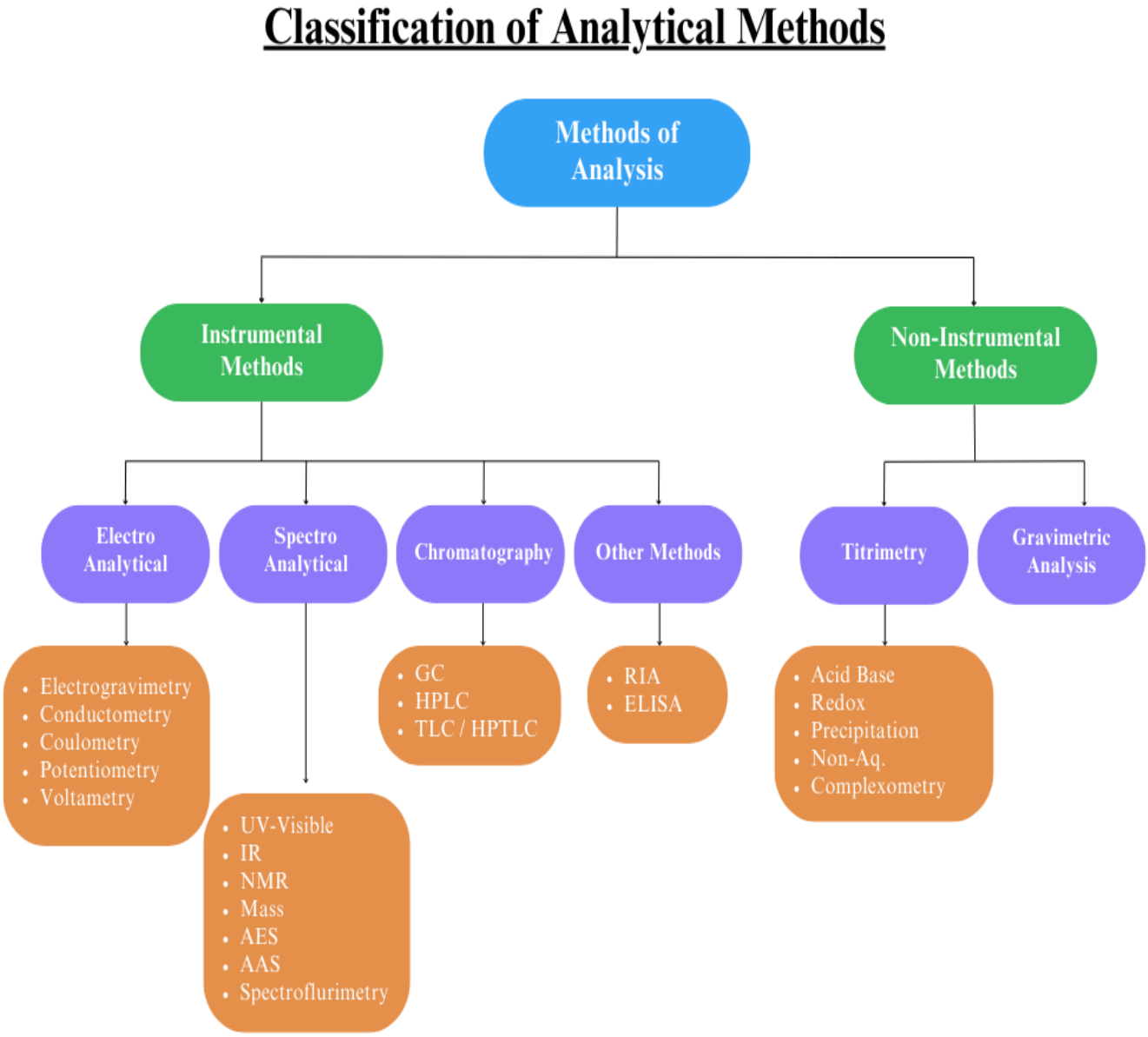

FIGURE 1 CLASSIFICATION OF ANALYTICAL METHODS

1.1 Introduction to Spectroscopy

Spectroscopy is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. The advantage of these methods are low time and labour consumption with excellent precision. When electromagnetic radiation interacts with the matter it can be absorbed, emitted, or scattered.[4] There are three basic principles of the spectroscopy:

1. Energy absorption: The sample can absorb specific wavelengths or frequencies of this radiation. This absorption corresponds to energy transitions within the atoms or molecules of the sample.

2. Energy emission: The sample can emit radiation after absorbing energy. This emitted radiation can also provide information about the sample's composition.

3. Scattering: Radiation can be scattered by the sample in different directions. Analysing the scattered light can reveal details about the sample's structure. Spectroscopy is important in pharmaceuticals for drug discovery, quality control, and manufacturing, ensuring drug safety and efficacy.[5]

There are eight methods of the spectroscopy in pharmaceutical analysis:

1) **Ultraviolet-Visible (UV-Vis) Spectroscopy**: UV spectroscopy involves measuring the absorption of ultraviolet light by a substance. It provides information about its molecular structure

and concentration, by analysing how light is absorbed at different wavelengths. UV spectroscopy can identify specific functional groups and quantify compounds in various samples. UV spectroscopy is efficient, requiring minimal sample preparation and fast results.[6]

2) **Infrared (IR) Spectroscopy**: IR spectroscopy measures the absorption of infrared light by a substance, which causes vibrational transitions in its molecular bonds. This technique provides detailed information about molecular structure, functional groups, and bonding environments. IR spectroscopy is particularly effective for studying molecular vibrations and identifying specific chemical groups within a compound.[7]
3) **Nuclear Magnetic Resonance (NMR) Spectroscopy**: NMR spectroscopy analyses the magnetic properties of atomic nuclei, providing detailed information about molecular structure and dynamics. This technique is highly effective for determining molecular structure, identifying functional groups, and elucidating chemical environments. It requires relatively large sample quantities and can be complex to interpret due to the intricacies of nuclear spin interactions.[8]
4) **Mass Spectroscopy:** Mass spectrometry (MS) identifies and quantifies molecules based on their mass-to-charge ratio. It ionizes compounds and measures the resulting ions' mass, providing detailed information about molecular weight, structure, and composition.[9]
5) **Raman Spectroscopy:** Raman spectroscopy analyses vibrational and rotational transitions in molecules by measuring the inelastic scattering of monochromatic light. This technique provides information about molecular bonding, structure, and dynamics. It is particularly useful for studying materials and complex biological systems.[10]
6) **Florescence Spectroscopy:** Fluorescence spectroscopy measures the emission of light from a sample after it absorbs excitation light. This technique is highly sensitive and used for detecting and quantifying fluorescent molecules in various applications.[11]
7) **X-ray Crystallography:** X-ray crystallography determines the atomic and molecular structure of a crystal by analysing the pattern of X-ray diffraction as the beam passes through the crystal. The resulting diffraction pattern provides precise information about the arrangement of atoms within the crystal lattice. This technique is crucial for elucidating the 3D structures of complex molecules, including proteins and pharmaceuticals. It is essential for understanding molecular interactions and designing new drugs.[12]
8) **Atomic Absorption Spectroscopy:** Atomic Absorption Spectroscopy (AAS) measures the concentration of metal ions in a sample by detecting the amount of light absorbed by free atoms in a gaseous state. This technique is highly sensitive and effective for trace metal analysis in various samples, including pharmaceuticals. It provides accurate quantification of metals based on their characteristic absorption lines.[13]

1.2 Theory of UV-Visible Spectroscopy

The UV-Visible spectroscopy is basically based on the the interaction between the light and matter. The light interacts with solutions and homogeneous media primarily through reflection, absorption, and transmission. The UV-Visible Spectroscopy involves two laws Beer's Law & Lambert's Law which are as follow:

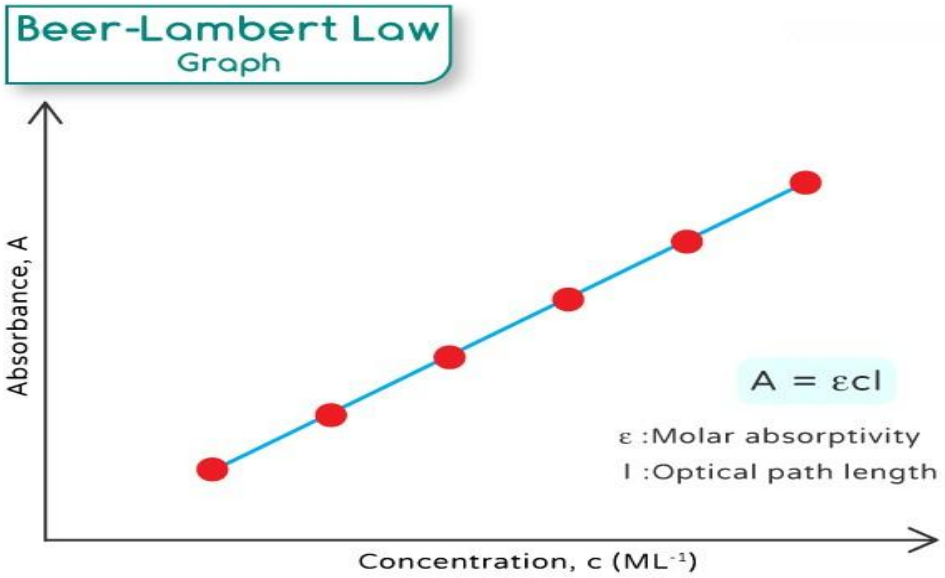

FIGURE 2 BEER-LAMBERT LAW GRAPH

- **Beer's Law :** The Beer's law stats that "The absorption of light by a sample is proportional to its path length and concentration." It describes the relationship between the absorbance of light by asolution and the concentration of the solute, along with the path length of the light through the solution.

- **Lambert's Law :** The Lambert's law stats that "The rate of decrease of intensity of radiation with the thickness of the absorbing medium is directly propotional to the intensity of the incident radiation" it is used for describe the absorption of light by a homogeneous absorbong medium.[14]

The Beer and Lambert's law are similar that stats that the amount of energy absorbed or transmitted by a solution's molar absorptivity and the concentraion of solute.[15] The formula is as follow:

$A = \varepsilon Lc$
Where ;
A is the amount of light absorbed for a particular wavelength by the sample
ε is the molar extinction coefficient
L is the distance covered by the light through the solution
c is the concentration of the absorbing species

2. Isosorbide Mononitrate : Drug Profile [17] [18] [19]
The Isosorbide Mononitrate drug belongs to the nitrates category used for the nitrate therapy in angina pectroris. Angina pectoris is defined as substernal chest pain, pressure, or discomfort that is typically exacerbated by exertion or emotional stress. The major symptoms are ischemia, low cardiac output, myocardial infraction, decresed pulse & diaphoresis.[16] The Isosorbide Mononitrate tablet contain not less than 90 per cent and not more than 110 per cent of $C_6H_9NO_6$. The usual strength of isodorbide mononitrate is 10mg, 20mg, 40mg and 60mg.

IUPAC Name: 8-nitrooxy-2,6-dioxabicyclo[3.3.0]octan-4-ol

Molecular Formula: $C_6H_9NO_6$

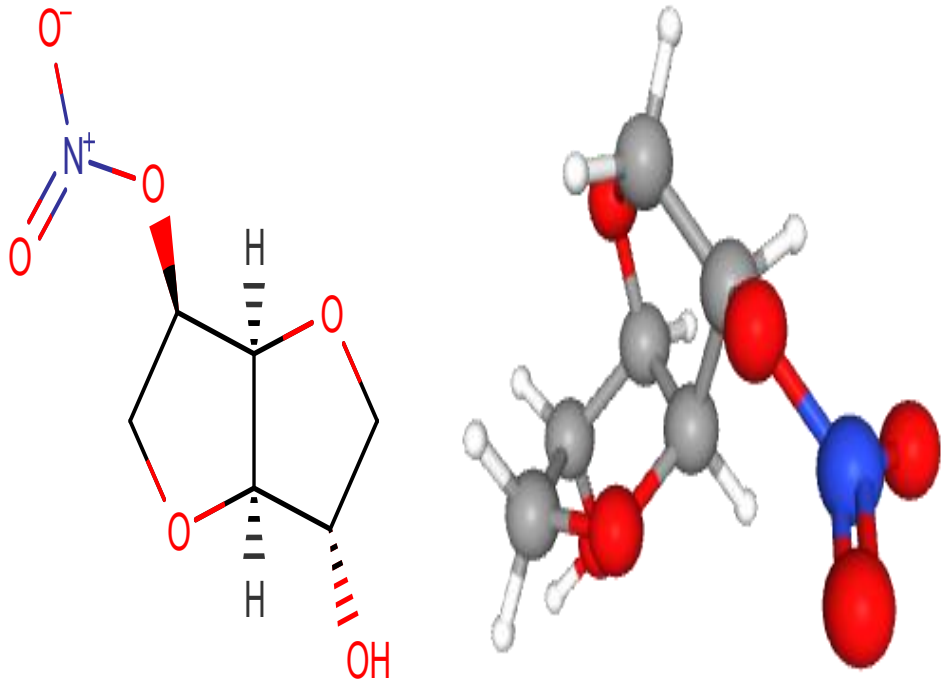

FIGURE 3 : STRUCTURE OF ISMN

Molecular Weight: 191.14 g/mol
Structure: Isosorbide Mononitrate is a nitrate ester of isosorbide, which is a cyclic sugar alcohol. The structure of isosorbide mononitrate includes a nitrate group attached to one of the hydroxyl groups of the isosorbide molecule. The basic structure of isosorbide mononitrate is a six-membered ring with two hydroxyl groups and one nitrate group.

Synonyms: Isosorbide 5-Mononitrate, Isosorbide 5-Nitrate, Monosorbitrate, ISMN.
Appearance: White or off-white crystalline powder.
Melting Point: 88-91°C
Solubility: Soluble in water, ethanol, and other organic solvents.
Stability: Light sensitive, PH sensitive & temperature sensitive.
Pka: First Pka = 4.5, Second Pka = 7.5
Route of Administration: Oral
Pharmacokinetics: ISMN is rapidly absorbed from GIT. It follows dose linear kinetics, the C_{max} is reached within 30 to 60 minutes. The volume of distribution is around 0.4 to 0.6 L/kg. it is having low plasma protein binding. The ISMN is primarily metabolized by the liver and converted into isosorbide which further undergo excretion. About 93% of drug is excreted by urine in 48 hrs.
Bioavailability: 40-50%
Plasma Protein Binding: Low plasma protein binding (approx.,10%).
Half Life: 5 Hrs
Volume of Distribution: 0.4 to 0.8 Liter per kilogram (L/kg).

3. Reported Methods of Isosorbide Mononitrate

Selecting an assay method for a pharmaceutical product involves evaluating the method's sensitivity, specificity, and accuracy to ensure reliable quantification. The method should be compatible with the drug's chemical properties and formulation, and capable of distinguishing the active ingredient from impurities. Additionally, consider the practical aspects, such as the complexity of sample preparation, the required equipment, and the cost. The chosen method should also align with regulatory requirements and industry standards for quality control.

SR NO.	API/DOSAGE FORM	METHOD	SUMMERY	REFERENCE
1.	Tablet	Liquid Chromatography	**Column**: A stainless-steel column (25cm x 4.6mm) packed with end capped octyldecylsilane bounded to porous silica **Mobile phase**: mixture of equal volume of methanol & water **Flow rate**: 1ml/min Spectrophotometer set on 225nm.	20
2.	Bulk Material	High Performance Liquid Chromatography - tandem mass spectrometry	**Column**: ZORBAXXDB-C18(4.6×50mm,5µm) **Mobile phase**: acetonitrile and 2mM ammonium acetate in water with an isocratic elution of 90:10 (v/v). **Injection volume**: 5µL **Flow rate**: 0.35mL/ min	21
3.	Bulk Material	Gas Chromatography-mass spectrometry	**Column**: HP-5 (50 m, 0.32 mm, 0.17 mm) **Mobile phase**: The standard stock solutions of IS, ISDA and IS2A5N were prepared in diethyl ether at a concentration of 1 mg/ml. **Injection volume**: 0.1 ml **Flow rate**: 2ml/min	22
4**.**	Bulk Material	Liquid Chromatography	**Column**: Merck Purosphere RP18e column (150 cm x 4.6 mm, 5µm) **Mobile phase**: Water: acetonitrile (80:20 v/v) **Injection volume**: 0.5 ml **Flow rate**: 1 mL/min	23
5.	Tablet	UV-Visible spectrophotometry	**Apparatus**: Perkin Elmer lambda 25 UV-Visible spectrometer. **Solvent**: Distilled water **Standard solution**: Standard solution of ISMN (1mg/ml) was prepared in distilled water. **Wavelength**: 540 nm	24

6.	Bulk Material	UV-Visible spectroscopy	**Apparatus**: UV visible 1601 Shimadzu double beam spectrophotometer. **Solvent**: Glacial acetic acid **Standard solution**: 0.2 gm of Isosorbide mononitrate standard and add 5 ml water and add sufficient glacial acetic acid to produce 25 ml **Wavelength**: 405 nm	25
7.	Bulk Material	RP-High Performance Liquid Chromatography	**Column**: reverse phase Shiseido C18 column. **Mobile phase:** Phosphate Buffer (pH: 4): Methanol (30:70% V/V) **Injection volume**: 10µl **Flow rate**: 1 ml/min	26
8.	Bulk Material	High Performance Liquid Chromatography	**Column**: Hitachi model 635-T HPLC (15 cm x 4 mm x 0.45 µm) **Mobile phase**: Methanol: water (25:75) **Injection volume**: 10µl **Flow rate**: 0.5 ml/min.	27
9.	Bulk Material	High Performance Liquid Chromatography	**Column**: ODS [Phenomenex Bou clone C_{18}] (250mm x 4.0mm) **Mobile phase**: Acetonitrile and water (6:4) **Injection volume**: 0.5 ml **Flow rate**: 1ml/min	28
10.	Tablets	UV-Visible Spectroscopy	**Apparatus**: UV Spectrophotometer **Solvent**: Phosphate buffer (PH: 6.8) **Standard solution**: Standard solution of ISMN was prepared by dissolving in Phosphate buffer. **Wavelength**: 220nm	29
11.	Bulk Material	Liquid Chromatography	**Column**: Zorbax Eclipse XDB-C18 **Mobile phase**: Methanol and 10 mmol/L Ammonium acetate **Injection volume**: 10µl **Flow rate**: 1ml/min	30

12.	Bulk material	High Performance Liquid Chromatography	**Column**: An C18 column (5µm, 250 ×4.6 mm) **Mobile phase**: methanol and water (25:75, v/v) **Injection volume**: 0.5 ml **Flow rate**: 1 ml/min	31
13.	Bulk Material	High Performance Liquid Chromatography	**Column**: CTO-10 AS VP **Mobile phase**: water and acetonitrile (80:20 v/v) **Injection volume**: 0.5 ml **Flow rate**: 0.8 ml/min	32
14.	Bulk Material	Liquid Chromatography – tandem mass spectrometry	**Column**: C_8 analytical column (100 mm × 2.1 mm i.d.) **Mobile phase**: Water and acetonitrile (80:20 v/v) **Injection volume**: 0.5 ml **Flow rate**: 1.3ml/min	33
15.	Bulk Material	Liquid Chromatography	**Column**: Pinnacle II Cyano column **Mobile phase**: 4.15×10^{-2} mol/L sodium dodecyl sulphate and 0.02 mol/L sodium dihydrogen phosphate with 10% (v/v) of 1-propanol at pH 7.0. **Injection volume**: 10µl **Flow rate**: 25 µg/mL	34
16.	Bulk Material	High Performance Liquid Chromatography	**Column**: ACQUITY UPLC™ BEH C_{18}(100 mm × 2.1 mm, 1.7 µm) **Mobile phase**: acetonitrile–water (20:80, v/v) **Injection volume**: 10µl **Flow rate**: 0.3 ml/min	35

4. Conclusion

The analysis of Isosorbide Mononitrate employs a range of methods, each offering distinct advantages and limitations. The HPLC offers high sensitivity, specificity, and resolution. Capable of quantifying a wide range of active ingredients and impurities in complex matrices. But it requires complex instrumentation and sample preparation. More time-consuming and costly compared to some other methods. The LC provides good separation and quantification of compounds and can be coupled with different detectors for enhanced analysis. Similar to HPLC, it can be complex and expensive. Not as simple as UV-Visible Spectroscopy for some applications. The UV-Visible Spectroscopy is simple, rapid, and cost-effective. It is ideal for routine analysis of APIs based on absorbance at specific wavelengths. Minimal sample preparation needed. But it is limited to compounds that absorb UV or visible light. Less specific and sensitive compared to chromatographic methods. GC excels in analysing volatile compounds but is less versatile for non-volatile components commonly found in

tablet formulations. As a result, the choice of analytical method depends on the specific requirements of the analysis, including the nature of the compounds, required sensitivity, and available resources.

References

1. Mr. Mahesh S. P., Dr. Rvindra R. P., Dr. Shailesh. S. C., Dr. Sanjay J S., Dr. Sandip D F., "Analytical Method Development and Validation: A Review", International Journal of Pharmaceutical and Biological Science Archive, **2019,** Volume 7, Issue 3, Page No. 70-81.
2. Ramanlal N. K., Komal P. J., "Analytical Method Development and Validation: A Review", International Journal of Creative Research Thoughts, **2021**, Vol 9, Issue 8, ISSN: 2320-2882.
3. Proma M., Debarupa D. C., Prithviraj C., Bhupendra S., Nihar R. B., "Different Ultraviolet Spectroscopy Methods Study on Its Application from The Viewpoint of Analytical Chemistry", Asian Journal of Pharmaceutical and Clinical Research, **2021**, Vol 14, Issue 9, 2021, 1-11
4. Smit P., Aniket R., Diya P., Diya P., Prof. Mitali D., Dr. Umesh U., "A Review on "UV Visible Spectroscopy", International Journal of Pharmaceutical Research and Applications, **2022**, Vol 7, Issue 5, ISSN: 2456-4494, Pg 1144-1151.
5. Raj A. M., Patel H., Mitali D., Chainesh S. and Umesh U., "UV Spectroscopy and Its Applications: A Review", World Journal of Pharmacy and Pharmaceutical Sciences, **2021**, Volume 10, Issue 11, 454-469
6. Masoom R. S., Zeid A. Al O., Nafisur R., "Analytical Techniques in Pharmaceutical Analysis: A Review", Arabian Journal of Chemistry, **2013,** 10, S1409-S1421
7. KARTHIKA.B. R., Mr. NISHAD V.M., Dr. PRASOBH G.R, "An Overview on Infrared Spectroscopy", International Journal of Research Publication and Reviews, **2022**, , Vol 3, no 4, pp 526-55.
8. Ioannis P. G., Anastassios T., Vassiliki E., Klimentini B., "Nuclear Magnetic Resonance (NMR) Spectroscopy: Basic Principles and Phenomena, and Their Applications to Chemistry, Biology and Medicine", Chemistry Education: Research and Practice in Europe, **2002**, Vol 3, No 2, pp 229-252.
9. Devin J. S., Sierra J., Benjamin J. B., Abraham K. B.-T., "Applications of Mass Spectrometry for Clinical Diagnostics: The Influence of Turnaround Time", Anal Chem, **2020**, 92(1): 183–202.
10. Paul R., Safa G., Dina G., "Raman Spectroscopy, Review", International Journal of Engineering and Technical Research, **2016**, , Volume-6, Issue-1, ISSN: 2321-0869,pp 2454-4698.
11. Aswathy B., Irene T., Kavitha G., Elessy A., "Fluorescence spectroscopy and its applications: A Review", International Journal of Advances in Pharmaceutical Analysis, **2018**, Vol 08, Issue 01, ISSM: 2277-9353, pp 1-8.
12. Jeffrey R. D., "X-Ray Crystallography of Chemical Compounds", Life Sci., **2010**, 86(15-16): 585–589.
13. Santhosh P., Dr. Satish K., Dr. Arunabha M., "Atomic Absorption Spectroscopy: A Short Review", EPRA International Journal of Research and Development, **2021**, Vol 6, Issue 9, ISSN: 2455-7838.
14. Shraddha K., Jeetu L., Laxmikant B., "A Review of the UV-Visible Spectroscopy's Method Development and Validation", International J Of Pharmaceutical Sci, **2024,** Vol 2, Issue 6, 527-538
15. Diya P., Diya P., Kunj Patel1, Mitali D., Dr. Umesh U., "A Review on UV Visible Spectroscopy", International Journal of Creative Research Thoughts, **2022**, Volume 10, Issue 10 October 2022 | ISSN: 2320-2882
16. Khushboo k., Ashoka B. VL, Mohammad A., "Angina Pectoris: A Review on Current and Future Treatment Strategies", Zeichen Journal, **2022**, Vol 8, Issue 2, ISSN No: 0932-4747
17. Indian pharmacopeia, Vol. 2, Ghaziabad: The Indian Pharmacopeia Commission; **2022**, Isosorbide Mononitrate Tablets; Pg 2644.
18. Durg bank online, "Isosorbide Mononitrate,Drug Bank Accession Number DB01020", https://go.drugbank.com/drugs/DB01020, Accessed on 17 August **2024**.

19. National Centre for Biotechnology Information. "PubChem Compound Summary for CID 27661, Isosorbide Mononitrate" PubChem, https://pubchem.ncbi.nlm.nih.gov/compound/Isosorbide-Mononitrate. Accessed 17 August **2024**.
20. Indian pharmacopeia, Vol. 2, Ghaziabad: The Indian Pharmacopeia Commission; **2022**, Isosorbide Mononitrate Tablets; Pg 2646.
21. YinpingZ., Aijing L., Ranran J., Mengyi W., Ni W., Chunyan L., Zhihui H., Haitang H., Hongyun W., Qing H., "DeterminationofIsosorbide-5-Mononitrate in Human Plasma by High-Performance Liquid Chromatography-Tandem Mass Spectrometry and Its Application to a Bioequivalence Study", Journal of Analytical Methods In Chemistry, **2022**, Volume 2020, Article ID 1753265.
22. Marinkovic V. D., Smiljana S. M., Jovan M. N., Jožef J. Č., Danica A., and Dobrila Ž., "Gas chromatography-mass spectrometry determination of isosorbide 5-mononitrate and related impurities in raw materials and dosage formulations." Journal of pharmaceutical and biomedical analysis **1997**, 16, no. 3: 425-429.
23. Himanshu S. K., Mohan M. M., Milind S. B., Asmita C. N., and Rajen D. S., "LC determination of isosorbide-5-mononitrate in human plasma." Chromatographia 69, **2009**, no. 9: 1035-1039.
24. Anjaly P. and P.N. Sanjay P., "New Spectrophotometric Method for the Estimation of Isosorbide Mononitrate in Bulk and Tablet Formulation", Oriental Journal of Chemistry, **2013** ¸ Vol. 29, No. (2): Pg. 771-775, ISSN: 0970-020 X.
25. Dilshad H., Naveed S., Naqvi S.B., "Assay of New Formulations of Isosorbide Mononitrate By Using UV Spectro Photometer", World Research Journal of Medicine, **2013**, Received: November 07, 2013; Accepted: December 12, 2013.
26. Nidhi S P., Dr. Dulendra P D, Dr. Shailesh V L., Dr. Sachin B N., "Stability indicating RP-HPLC method development and validation for simultaneous estimation of aspirin and isosorbide mononitrate in pharmaceutical dosage form", The Pharma Innovation Journal, **2018,** ISSN (E): 2277- 7695, ISSN (P): 2349-8242, 7(4): 217-234.
27. Nobuyasu M., Chie S., Emiko M., Demji S., Y. Yamanka, "Determination of Isosorbide Mononitrate by High Performance Liquid Chromatography and Their Stability in Aqueous Solution", Journal of Chromatography, **1983**, 264 (1983) 159-163.
28. Fang -Ying T., Chin-Jung, C., Chun-Sheng C., "Determination of Isosorbide Nitrate And its Analogues in Pharmaceuticals by High Performance Liquid Chromatography", Journal of Food and Drug Analysis, **1994**, 1997, 2(4): 271-280.
29. Gundawar R., Penjuri S. C. B., V. Ravi, Damineni S., Sandeep K., "Design, Development and Evaluation of Isosorbide Mononitrate Orally Disintegrating Tablets", International Journal of Pharma Research and Health Sciences, **2020**, 8 (2): 3147-50, DOI:10.21276/ijprhs.2020.02.03
30. LI, Peng-fei; LIU, Li-hong; MA, Ping; SUN, Jian-zi; TONG, Wei-hang; YANG, Jing-yan; WANG, Wen-Jian, "LC-MS/MS Determination of Isosorbide 5-Mononitrate in Human Plasma", Chinese Journal of Pharmaceutical Analysis, **2008**, Vol 28, No.4, pp. 528-531(4).
31. Liandong H., Yanjing S., Na G., Hu, Liandong, "Determination of Isosorbide Mononitrate (ISMN) And Its Two Related Substances in Isosorbide Mononitrate and Sodium Chloride Injection." Semantic Scholar, **2013** Corpus ID: 212592247.
32. Rajan K V., Sanjay G., "A validated High Performance Liquid Chromatography Method for Analysis of Isosorbide Mononitrate in Bulk Material and Extended-Release formulations", Journal of Pharmaceutical Analysis, **2002**, Vol 30, Issue 3, Pg 583-591.
33. Lara C. S., Lina S.O.B. O., Gustavo D. M., Gabriel G., Alberto D. S. P., Gilberto D. N., "Quantification of Isosorbide Mononitrate in Human Plasma by Liquid Chromatography-Tandem Mass Spectrometry Using Atmospheric Pressure Photoionization.", Journal of Chromatography B, **2006**, Vol 832, Issue 2, Pg 302-306.
34. Ning L., Chun-lan L., Ning-wei L., Yu-ming D., "A Novel Micellar Per Aqueous Liquid Chromatographic Method for Simultaneous Determination of Diltiazem Hydrochloride,

Metoprolol Tartrate and Isosorbide Mononitrate in Human serum.", Journal of Chromatography B, **2014**, Vol 967, Pg 90-97.

35. Xiaohong S., Xiaoqin C., Shuang C., Feng Q., Xiumei L., Famei L., "High Performance Liquid Chromatography- Electrospray Ionization Mass Spectrometric Determination of Isosorbide 5-Mononitrate in Human Plasma", Journal of Chromatography B, **2007**, Vol 846, Issue 1-2, Pg 323-328.