



"Advances in Pharmaceutical Analysis: A Review of Modern Analytical Techniques"

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Abstract : Analytical techniques serve a cornerstone role in the pharmaceutical industry, ensuring the identity, purity, strength, and safety of pharmaceutical products. The review presented herein is a comprehensive summary of the main analytical techniques utilized in pharmaceutical analysis, ranging from traditional and instrumental techniques. Key techniques addressed are titrimetric techniques, spectroscopy (UV-Vis, IR, NMR), chromatography (TLC, HPLC, GC), and electrophoresis, and advanced hyphenated techniques such as LC-MS and GC-MS. Each technique is critically assessed according to its basic principles, applications, strengths, and limitations in drug quality control and formulation development. Additionally, the report discusses the significance of validation parameters, regulatory requirements, and the role of analytical techniques in ensuring conformity to pharmacopeial standards. By bringing together both conventional and advanced techniques, the book brings to the limelight the pivotal role of analytical chemistry in driving innovation in pharmaceutical sciences.

IndexTerms - Pharmaceutical analysis, Analytical techniques, Titrimetric analysis, Spectroscopy (UV-Visible, Infrared, NMR, Raman, Atomic, X-ray), Chromatography (Thin Layer Chromatography, High Performance Liquid Chromatography, Gas Chromatography, High Performance Thin Layer Chromatography, Paper Chromatography, Ion Chromatography), Mass spectrometry (MS), Hyphenated techniques (LC-MS, GC-MS), Quality control, Drug formulation, Regulatory standards, Pharmacopeial compliance, Method validation, Drug development, Active pharmaceutical ingredients (APIs), Pharmaceutical quality assurance

I. INTRODUCTION

Pharmacology and clinical sciences have long shaped pharmaceutical research, chemistry is the engine behind advancement in both fields. Recent years have seen a redefining of drug discovery by the combined efforts of chemistry, pharmacology, microbiology, and biochemistry. Thanks to the multidisciplinary cooperation between biologists and chemists, which promotes innovation by shared knowledge, the development of new therapeutic agents is no more the outcome of chemists' inventiveness.[1]

The path of drug development starts with the identification of a new molecule showing possible therapeutic action against a particular condition. Active pharmaceutical ingredients (APIs) such as these go through synthetic and characterization procedures. Later analytical investigations are carried out to produce preliminary data on their safety and therapeutic efficacy—necessary steps in determining interesting drug candidates for additional in-depth review.[1]

Pre-discovery investigation seeks to identify disease's underlying causes. This covers investigating changes in genes, interactions between proteins, and the behavior of compromised cells. Scientists investigate how these alterations interfere with regular biological processes and help to advance diseases. Equipped with this understanding, researchers create compounds specifically targeting these aberrant systems. Effective such compounds may progress to become possible drug candidates or APIs, so marking the first turning point in the complicated process of pharmaceutical development.[1]

From an analytical point of view, methods for pharmaceutical analyses are far less more complex than methods used in drug analysis and their metabolites in bio samples such as blood, plasma, hair, or urine. However, the ultimate Measurement of a drug substance's concentration. formulations is as important as determination in complex matrices, as the pharmaceutical product quality and patient health correlate directly. In the drug development and pharmaceutical regulation, chemical testing plays a central role to guarantee the optimal efficacy and safety to patients. For this purpose, sound strategies for quality control (qualitative and quantitative analyses, purity test, chiral resolution, related substance and stoichiometric determination) are of first relevance to the pharmaceutical industry. Pharmaceutical research is advancing increasingly complex molecules and drug formulations, and every new and highly selective. The analytical method thus has broad prospects. interest. Therefore, pharmaceutical quality control must provide application of the correct analytical methods, where a trend is noted to use faster and more efficient methods with cost savings and reduction of solvent consumption.[2]

In chemical analysis drug analysis is beneficial to the Isolation, estimation, and determination of chemicals. Natural and synthetic source chemicals. Those compounds are typically constitute up-to one or more chemical substances. The analytical chemistry process begins with two general divisions that cover qualitative and quantitative analysis. In qualitative analysis only the available samples are measured, and in quantitative analysis the overall number of parts in a compound ought be identified. For example; the analysis of wide variety of Compound or product analysis is of value in drug analysis because it includes the life. Nowadays, large number of drugs has been introduced into the market, and demand for drugs is increasing day by day. The newly developed drugs are a type of new variety or either they are modified version of available drugs. The drugs are characterized in relation with the

drugs for sale, and on offer Pharmacopoeia. Pharmacopoeia is therefore employed in the drug development was necessary to report about the better therapeutic agents for withdrawal in the market. Some times During the course of development of drug agents, analytical characterization of Certain drugs fail to get listed in the pharmacopoeias. Therefore, in such instances Preparation is the basis of novel drug development. most important analytical methods. There are several compounds throughout the drug development process artist-designed, and they can easily set up their structure and behavior, and also helps in determining the impurities in a compound. Therefore, if all the safety measures have been observed to reach the target, then the drug, then the bioassays of drugs will performed to find that how it will work, and functions analytically. The scientist from recent years were aimed at the small molecules which are organic in character, as well as the natural or synthetic sources.[3]

As the different channels through which pharmaceutical products can be bought continue to grow and diversify, so too is there a corresponding increase in the need for ensuring the maintenance of the quality of such products, a need that is increasingly complex and urgent. The advent and rapid growth of use of the internet have revolutionized the shopping behavior of consumers to their products. Increasingly, consumers are opting for online pharmacies, perceiving them as one of the major sources for the procurement of their medicines. Online pharmacies offer a degree of convenience to many consumers that traditional retail outlets are not in a position to offer, coupled with the promise of significant cost savings. While the United States Food and Drug Administration (FDA) acknowledges and values the benefits that accrue to consumers through legitimate online pharmacies, the agency is concerned with the dangers of patient harm that can be caused by the purchase of drugs over the internet.[4]

The internet provides a fertile platform for businesses and individuals who would like to bypass the regulatory protections that have been put in place and enforced by the FDA. As a result of its size and comparatively unregulated status, the internet presents a challenge to the FDA, and it becomes hard for them to ensure that any product purchased over the internet has passed the rigorous current good manufacturing practices (cGMP) necessary for safety and quality. Additionally, there is no guarantee that such products carry the correct active ingredients, are entirely free from harmful impurities that may cause health issues, or have been processed and transported to ensure their effectiveness and safety. In the majority of cases, products purchased over the internet are not FDA-approved, and this presents a serious issue as to their overall safety and performance for consumers.[4]

FDA's Center for Drug Evaluation and Research sponsored this research in the Office of Compliance to evaluate the quality of a small number of drugs purchased online from international sources. Traditional test methods, such as potency, content uniformity, chromatographic purity and drug release rate, were used to evaluate compliance, and non-traditional methods, such as NIR, NIR imaging and TGA, were investigated for discrimination ability and providing complementary quality information.[4]

Analytical Techniques

Titrimetric Analysis: Titrimetric analysis continues to be of utmost significance and utility in the analytical chemistry laboratory as a satisfactory analytical reagent. Its usefulness even persists despite growing dependence upon purely physical analysis techniques, often involving the use of instrumentation that is not just highly sophisticated but also quite costly.[5]

Titrimetric methods remain most favored in the estimation of bulk drug substances and contribute to around 70% of their coverage in the European Pharmacopoeia (EP). Furthermore, in the United States Pharmacopoeia (USP) terminology, it is pertinent to note that over 40% of low molecular weight organic compounds are being estimated by techniques encompassing aqueous or non-aqueous titration. In practice, titrimetric methods have remained the most common and routine method of choice in pharmaceutical analysis. Such dominance has been of particular relevance since the advances brought about in physicochemical measurement assays, the popularity of non-aqueous titration methods, and the determination of endpoints by means of potentiometric methods. Such advances have not only increased but also diversified to a very large extent the scope and versatility of titrimetric methods, of which the domain of pharmaceutical analysis has been a major gainer. The European Pharmacopoeia and United States Pharmacopoeia are aggressively embracing a wide range of analytical methods to maintain the quality of drugs, which range from a wide range of methods such as titration, spectrometry, and chromatography, and many more. The details of these methods, along with the corresponding percentage utilization, are detailed in Table 1, which refers to the editions of the European Pharmacopoeia (The European Pharmacopoeia and Council of Europe, 2002) and the United States Pharmacopoeia (United States Pharmacopoeia, 2004).[5]

Chromatography: Thin Layer Chromatography (TLC): In the present has been made to explain the Basic principles and applicability of Thin Layer Chromatography. (TLC) compared to other analytical methods. Because TLC takes less time marked by the consumption process, with economy, and with the potential to be enacted with lowered or lessened resource usage. This method, which is renowned for being complicated, has a wide area of application in many areas. pharmaceutical analysis. Carried out with accuracy 32 amino acids can be differentiated by TLC. It also has a broad application in identification of the impurities within a compound provided. This may be used as an preliminary analytical method preceding HPLC. The theory of TLC is very simple, and samples are only slightly Pretreatment. Thin Layer Chromatography, or TLC for short, may be used efficiently to keep track of and observe the progress of a reaction, identify compounds present in a substance with TLC It is also used in an attempt to properly distinguish between isomeric compounds that are in a mixture. Many traditional methods that are used in the practice of industrial chemistry, specifically with regards to environmental applications, food chemistry, toxicology, water, inorganic, pesticide analysis, purity of dyes, cosmetics, plant material, and herbal Analysis depend on TLC as the preferred method.[6]

A. PAPER CHROMATOGRAPHY: THE HISTORY OF PC AS AN ANALYTICAL TECHNIQUE AND THE MOST IMPORTANT DEVELOPMENTS THAT FOLLOWED AFTER APPLYING PC TO A LARGE NUMBER OF CHEMICAL INVESTIGATIONS ARE DISCUSSED. THE STATE OF THE ART OF PC IS TAKEN INTO ACCOUNT WITH SPECIAL REFERENCE TO SOLVENT SYSTEMS; PAPER AND TYPE OF PAPER AND THE MOST IMPORTANT APPLICATION ARE TAKEN INTO ACCOUNT AS WELL. A BRIEF OVERVIEW OF THE LITERATURE HAS BEEN PLOTTED ON THE COMPARISON OF THE TWO CLOSELY RELATED TECHNIQUES THAT ARE PAPER- AND THIN-LAYER CHROMATOGRAPHY AND THE ADVANTAGES OF EACH TECHNIQUE HAVE BEEN EMPHASIZED. FROM THIS LITERATURE REVIEW, IT WOULD APPEAR THAT PC IS NOW USED VERY WIDELY ALL OVER THE WORLD, ESPECIALLY IN NON-UNITED STATES NATIONS, FOR THE SEPARATION OF A LARGE NUMBER OF ORGANIC AND INORGANIC COMPOUNDS THAT ARE MOSTLY METAL IONS AND AMINO ACIDS. THE FUTURE APPLICATION OF PC WILL DEPEND ON THE AVAILABILITY OF SHORT-FIBER PAPERS AND THOSE WITH GEL FILTRATION AND PERMEATION CHARACTERISTICS.[7]

B. HIGH PERFORMANCE LAYER CHROMATOGRAPHY: HPLC IS NOW USED EXTENSIVELY FOR SEPARATIONS AND PURIFICATIONS IN A BROAD INDUSTRY BASE LIKE PHARMACEUTICALS, FOOD AND POLYMER AND ENVIRONMENTAL INDUSTRIES AND BIOTECHNOLOGY INDUSTRIES. IT IS ACCOMPLISHED BY INJECTION OF SMALL VOLUME OF LIQUID SAMPLE IN STREAM OF FLOWING LIQUID (REFERRED TO AS THE MOBILE PHASE) WHICH FLOW DOWN A COLUMN PACKED WITH PARTICLES OF STATIONARY PHASE. SEPARATION OF MIXTURE INTO ITS COMPONENTS DEPENDS ON THE VARYING DEGREES OF RETENTION OF EACH COMPONENT IN THE COLUMN. HPLC IS ONE OF THE TYPES OF LIQUID CHROMATOGRAPHY, I.E., THE MOBILE PHASE IS LIQUID. REVERSED-PHASE HPLC IS MOST FREQUENT FORM OF HPLC. THE TERM REVERSED-PHASE HAS BEEN USED AS THE MOBILE PHASE IS HIGHLY POLAR, AND STATIONARY PHASE IS HIGHLY NON-POLAR. HPLC EQUIPMENT CONSISTS OF SOLVENT RESERVOIR, PUMP, INJECTOR, COLUMN, DETECTOR, AND INTEGRATOR OR ACQUISITION AND DISPLAY SYSTEM. CENTRAL UNIT OF THE SYSTEM IS THE COLUMN IN WHICH THE SEPARATION TAKES PLACE. INFORMATION THAT CAN BE OBTAINED WITH THE ASSISTANCE OF HPLC ARE IDENTIFICATION, QUANTIFICATION, AND RESOLUTION OF THE COMPOUND. APPLICATIONS OF NOTE ARE IN THE FIELD OF PHARMACEUTICALS, FOOD, RESEARCH, MANUFACTURE, FORENSIC, AND BIO-MONITORING OF POLLUTANTS.[8]

C. HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY: HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY, ALSO REFERRED TO AS HPTLC, IS THE RESULT OF EVOLUTION RELATIVE TO CONVENTIONAL THIN-LAYER CHROMATOGRAPHY, OFTEN REFERRED TO AS TLC. HPTLC EMPLOYS VERY EFFICIENT CHROMATOGRAPHIC LAYERS THAT SIGNIFICANTLY ENHANCE THE SEPARATION PROCESS AND RELIES ON THE MOST ADVANCED EQUIPMENT AND TECHNOLOGY AT EVERY STAGE OF THE ANALYTICAL PROCESS. THE SOPHISTICATED METHOD COMPRISES A SERIES OF VERY CRUCIAL PROCEDURES LIKE THE ACCURATE AND PRECISE SPOTTING OF THE SAMPLES ON THE CHROMATOGRAPHIC PLATES, THE REPRODUCIBLE AND UNIFORM DEVELOPMENT OF THE CHROMATOGRAMS WITH ENSURED RESULTS, AND THE APPLICATION OF COMPUTERIZED SYSTEMS FOR DATA ACQUISITION AND ASSESSMENT, ENHANCING ANALYSIS TO BE MORE ROBUST.[9]

HPTLC, OR HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY, IS A VERY ADVANCED AND HIGHLY DEVELOPED ANALYTICAL METHOD THAT ORIGINATED FROM SCIENTIFICALLY BASED METHODS THAT PROVIDE QUALITATIVE AS WELL AS QUANTITATIVE ANALYSES. THE UNIFORM PROCEDURES USED IN THE METHOD PROVIDE HIGH RELIABILITY, REPRODUCIBILITY, AND OUTSTANDING ACCURACY IN APPLICATIONS. HPTLC, THEREFORE, CAN ACHIEVE THE STRICT AND HIGH QUALITY STANDARDS THAT ARE DEMANDED IN MODERN ANALYTICAL LABORATORIES, BUT STILL OFFER ENHANCED RESOLUTION AND GREATER PRECISION IN QUANTITATIVE MEASUREMENTS THAT ARE NECESSARY IN ACCURATE SCIENTIFIC RESEARCH AND ANALYSIS.[9]

D. GAS CHROMATOGRAPHY: GAS CHROMATOGRAPHY, COMMONLY ABBREVIATED AS GC, IS A HIGHLY POWERFUL AND SOPHISTICATED TECHNIQUE EMPLOYED FOR THE SEPARATION OF VOLATILE COMPONENTS IN A MIXTURE. THIS IS DONE ON THE BASIS OF DIFFERENTIAL DISTRIBUTION OF THESE COMPONENTS BETWEEN TWO PHASES, A STATIONARY PHASE, AND A MOBILE PHASE. DUE TO ITS INHERENT SIMPLICITY, VERY GOOD SENSITIVITY, AND ADDITIONAL ANALYTICAL POWER, GAS CHROMATOGRAPHY HAS PROVEN TO BE A VERY USEFUL INSTRUMENT IN MODERN CHEMICAL ANALYSIS.[10] THE COLUMN AND THE STATIONARY PHASE ARE THE CORE PART OF THE GC SYSTEM, AND THEY ARE REFERRED TO AS THE "HEART" OR "BRAIN" OF THE CHROMATOGRAPH SINCE THEY CARRY OUT THE SEPARATION PROCESS. IN THE MAJORITY OF GC ARRANGEMENTS, THE SAMPLE IS VAPORIZED AND INTRODUCED INTO THE INLET OF A COLUMN OF SEPARATION, WHICH COULD BE PACKED WITH HIGHLY DIVIDED SOLID OR COATED WITH A THIN LAYER OF LIQUID STATIONARY PHASE.[10] SINCE THE DIFFERENT COMPONENTS OF THE SAMPLE MOVE THROUGH THE COLUMN VIA AN INERT GAS, EITHER HELIUM, NITROGEN, OR HYDROGEN, THEIR RESPECTIVE INTERACTION WITH THE STATIONARY PHASE IS QUITE DIFFERENT BASED ON THE INDIVIDUAL CHEMICAL CHARACTERISTICS CONTAINED WITHIN EACH COMPONENT. DUE TO THIS DIFFERENCE IN INTERACTIONS, DIFFERENT TRAVEL SPEEDS EXIST FOR THE COMPONENTS AS THEY MOVE THROUGH THE COLUMN, THUS CREATING AN EFFICIENT SEPARATION OF THE INDIVIDUAL COMPONENTS. ONCE THE INDIVIDUAL COMPOUNDS ARE SEPARATED FROM THE COLUMN, THEY ARE THEN SENT THROUGH A DETECTOR, WHICH PRODUCES SIGNALS PROPORTIONAL TO EACH COMPOUND PRESENT. THE RETENTION TIME, OR HOW LONG IT TAKES FOR EACH INDIVIDUAL COMPONENT TO PASS THROUGH THE COLUMN, IS USED AS A QUALITATIVE BASIS FOR THEIR IDENTIFICATION.[10] THE ONGOING INNOVATIONS AND DEVELOPMENTS IN COLUMN TECHNOLOGY HAVE LED TO INCREDIBLE BREAKTHROUGHS IN THE EFFICIENCY AND RESOLUTION OF THE WELL-ESTABLISHED GAS CHROMATOGRAPHY, OR GC. AMONGST SUCH DEVELOPMENTS, PERHAPS THE MOST IMPORTANT BREAKTHROUGH HAS BEEN THE DEVELOPMENT OF CAPILLARY COLUMNS. THESE SORTS OF SPECIALIZED COLUMNS POSSESS HIGHLY STRUCTURED STATIONARY PHASES, WHICH POSSESS MUCH MORE SEPARATION POWER COMPARED TO THE CONVENTIONAL PACKED COLUMNS USED EARLIER. SUCH REVOLUTIONARY DEVELOPMENTS, DUE TO SUCH INNOVATIVE IMPROVEMENTS, HAVE GREATLY INCREASED THE VERSATILITY OF GAS CHROMATOGRAPHY, OPENING THE WAY TO MORE APPLICATION IN VARIOUS RESEARCH AND INDUSTRIAL SETTINGS AS WELL.[10]

E. ION CHROMATOGRAPHY: ION CHROMATOGRAPHY (IC) IS A STATE-OF-THE-ART ANALYTICAL METHOD OF THE PREVIOUS CENTURY, AND DURING THE PAST 20 YEARS OR SO, THE PREFERRED METHOD METHOD FOR THE DETERMINATION OF INORGANIC ANIONS AND SMALL ORGANIC ANIONS. IC IS ALSO WIDELY APPLIED FOR THE DETERMINATION THERE ARE INORGANIC CATIONS; HOWEVER, THIS IS BECAUSE THERE ARE A RANGE OF ADVANCED AND HIGHLY EVOLVED SPECTROSCOPIC METHODS THAT INCORPORATE A RANGE OF ELEMENTS OF ANALYSIS (PARTICULARLY INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY ICP/MS, IN WHICH THE FUNCTION OF IC BECOMES ANCILLARY WITHIN THE REALM OF CATION ANALYSIS.[11]

IC CAN BE TERMED A SOUND ESTABLISHED AND MATURE ANALYSIS TECHNIQUE, AND IT CAN BE PRESUMED THAT IT HAS COME TO A POINT WHERE NEW DEVELOPMENTS AND ADVANCEMENTS HAVE BEEN MADE. CAN BE VIEWED AS TAKING MORE GRADUAL AND INCREMENTAL STEPS THAN UNDERGOING ANY FUNDAMENTAL OR DRAMATIC CHANGES. BUT TAKE A CLOSER LOOK AT THE RECENT TRENDS AND THE GENERAL SITUATION, A RESEARCH ON CURRENT TRENDS INDICATES THAT THE COMPANY CALLED IC IS CONTINUALLY MAKING RAPID AND OUTSTANDING PROGRESS IN BOTH ITS BUSINESS PROCESSES AND STRATEGIC APPROACHES. THOSE THAT ENTAIL PURSUING COMPLETELY NEW PATHS. THE PRIMARY FUNCTION OF THIS OVERVIEW WILL ATTEMPT TO CONCENTRATE ON SOME OF THESE IMPORTANT DEVELOPMENTS AND EMERGING TRENDS THAT HAVE ONLY RECENTLY ARISEN AND BECOME EVIDENT.[11]

IN A CAREFUL AND CONSCIOUS ATTEMPT TO GIVE THE MUCH CLEARER AND TRUER MENTAL PICTURE OF THE CONCEPT OF IC, AND TO ATTEMPT TO GIVE A DEEP SENSE OF EXPECTATION ABOUT THE THRILLING NEW PATHS WHICH THIS CONCEPT IS BOUND TO FOLLOW IN THE NOT-TOO-DISTANT FUTURE, IT IS IMPORTANT TO RECORD THE FOLLOWING FACT. FOR CLEARLY, THE MOST CRITICAL AREAS TO BE ADDRESSED AMONG AREAS OF OVERWHELMINGLY CRUCIAL IMPORTANCE IN WHICH THE MOST INTENSE AND ASSURED IC DEVELOPMENT AND IC FUTURE EXISTS ARE THE EVOLUTION OF NEW STATIONARY PHASES, THE TREND OF MINIATURIZATION, AND THE EXTENSION OF PEAK CAPACITY, WHICH TO A LARGE EXTENT IS ACHIEVED THROUGH THE USE OF COMPLEX ELUTION PROFILES AND THE CHEMOMETRICS INVOLVED SEPARATION OPTIMIZATION, HYPHENATED IC SYSTEMS, AND THE APPLICATION OF IC IN BIOANALYSIS. ALL THE ABOVE TOPICS ARE DISCUSSED IN DETAIL BELOW.[11]

I. SPECTROSCOPY

A. UV VISIBLE SPECTROSCOPY: ULTRAVIOLET (UV) SPECTROSCOPY IS ONE OF THE OLDER AND NEWER ANALYTICAL METHODS IN THE PHARMACEUTICAL SCIENCES WITH A HISTORY OF USE DATING BACK OVER 35 YEARS. THE METHOD IS USED PRIMARILY TO ANALYZE COLORLESS COMPOUNDS BY TRACKING THEIR MONOCHROMATIC LIGHT ABSORPTION IN THE NEAR-ULTRAVIOLET REGION OF THE ELECTROMAGNETIC RADIATION SPECTRUM FROM 200 TO 400 NM IN WAVELENGTH.[12]

PHARMACEUTICAL ANALYSIS INVOLVES A COMPLETE SET OF COMPLEX TESTS TO ASCERTAIN FUNDAMENTAL PROPERTIES LIKE THE IDENTITY, POTENCY, QUALITY, AND PURITY OF DIFFERENT DRUG SUBSTANCES. AMONG ALL THIS WIDE RANGE OF TESTING, THERE IS A MAJOR FOCUS ON THE USE OF UV SPECTROSCOPY, AN IMPORTANT ANALYTICAL METHOD THAT DEALS WITH THE TESTING OF RAW MATERIALS, INTERMEDIATES, AND THE FINAL DRUG PRODUCTS AT DIFFERENT VARIED STAGES OF THE DRUG MANUFACTURING PROCESS.[12]

THE UNDERLYING PRINCIPLE THAT THE PROCESS OF UV SPECTROPHOTOMETRY IS BASED UPON REVOLVES AROUND THE TRANSMISSION OF A BEAM OF LIGHT, AND IN THIS CASE, A SPECIFIC WAVELENGTH OF LIGHT, THROUGH A SAMPLE SOLUTION. WHEN THIS SPECIFIC FORM OF LIGHT IS CONCENTRATED AND SENT THROUGH A CUVETTE WITH THE SAMPLE DISSOLVED IN THE CORRECT SOLVENT, A PORTION OF THIS LIGHT IS ABSORBED BY THE CHEMICAL COMPOUND FOUND IN THE SAMPLE. THE UNABSORBED LIGHT PROCEEDS ONWARD AND IS SENT TO A PIECE OF EQUIPMENT REFERRED TO AS A PHOTOELECTRIC CELL, WHERE THE RADIANT ENERGY OF THIS LIGHT IS SUBSEQUENTLY CONVERTED TO AN ELECTRICAL SIGNAL IN ORDER TO BE FURTHER ANALYZED. THIS ELECTRICAL SIGNAL IS THEN READ—MOST COMMONLY WITH THE HELP OF EITHER A GALVANOMETER OR DIGITAL METER—SO THAT THE SUBSTANCE'S ABSORBANCE CAN BE DETERMINED WITH ACCURACY.[12][19]

ULTRAVIOLET-VISIBLE SPECTROSCOPY IS A WELL-DEVELOPED TECHNIQUE APPLIED TO THE RECORDING OF THE ABSORBANCE SPECTRA OF VARIOUS SOLUTIONS AND, IN SOME CASES, SOLID SAMPLES AS WELL. THE HIGH SENSITIVITY, EASE OF USE, AND HIGH RELIABILITY OF THE TECHNIQUE MAKE IT AN INVALUABLE AND ESSENTIAL ANALYTICAL TOOL, PARTICULARLY IN PHARMACEUTICAL QUALITY CONTROL AND SCIENTIFIC RESEARCH.[12]

B. INFRA-RED SPECTROSCOPY: INFRARED (IR) SPECTROSCOPY IS A VERY EFFECTIVE ANALYTICAL METHOD THAT PROVIDES RICH AND COMPREHENSIVE INFORMATION ABOUT THE MOLECULAR FORMULATION OF COMPOUNDS. IN A WIDER PERSPECTIVE, NEAR-INFRARED (NIR) SPECTROSCOPY HAS GAINED MUCH POPULARITY AND EXPOSURE OVER THE LAST FEW YEARS, ESPECIALLY IN PROCESS ANALYSIS. NIR SPECTROSCOPY IS PARTICULARLY USEFUL IN THE PHARMACEUTICAL SECTOR FOR A WIDE RANGE OF ESSENTIAL ACTIVITIES LIKE IDENTIFICATION OF RAW MATERIALS, CONTROL OF PRODUCT QUALITY, AND IN SITU CONTINUOUS PROCESS MONITORING.[13]

OUTSIDE OF THE DRUG INDUSTRY, NIR SPECTROSCOPY HAS FOUND WIDESPREAD USE IN BIOTECHNOLOGY, GENOMICS, PROTEOMICS, INTERACTOMICS, TEXTILE MONITORING, FOOD QUALITY ANALYSIS, PLASTICS, AND POLYMERS, INSECT DETECTION, FORENSIC SCIENCE, CRIME SCENE ANALYSIS, MILITARY USE, AND EVEN FOR ASTRONOMICAL SPECTROSCOPY.[13]

NEAR-INFRARED (NIR) SPECTROSCOPY, A SPECIFIC SUBDISCIPLINE OF THE BROAD FIELD OF VIBRATIONAL SPECTROSCOPY, IS EFFECTIVE IN THE NEAR-INFRARED REGION OF THE ELECTROMAGNETIC SPECTRUM. THIS PARTICULAR RANGE IS APPROXIMATELY FROM 750 TO 2500 NANOMETERS (NM). IT IS IMPORTANT TO UNDERSTAND THAT THIS RANGE FALLS RIGHT ON THE THRESHOLD WITH THE VISIBLE SPECTRUM. MANY ORGANIC COMPOUNDS, AND CERTAIN INORGANIC COMPOUNDS, POSSESS FAVORABLE CHARACTERISTICS WITH REGARD TO ABSORPTION, REFLECTANCE, OR TRANSMISSION WHEN LIGHT IN THIS SPECIFIC RANGE OF WAVELENGTHS IS USED TO ILLUMINATE THEM. BUT NIR ABSORPTION BANDS ARE BROAD, TYPICALLY OVERLAPPING, AND TYPICALLY 10 TO 100 TIMES WEAKER THAN MID-INFRARED FUNDAMENTAL ABSORPTION BANDS. BUT THE TECHNIQUE IS VERY EFFECTIVE DUE TO ITS SPEED, NONDESTRUCTIVE CHARACTER, AND ABILITY TO ANALYZE SAMPLES WITH LITTLE OR NO PREPARATION.[13]

C. NMR SPECTROSCOPY: OVER THE PAST DECADES, NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY HAS ARISEN AND BECOME ONE OF THE MOST IMPORTANT AND INDISPENSABLE ANALYTICAL TOOLS FOR QUESTIONING AND PROBING A VAST ARRAY OF METABOLIC PROCESSES. WHILE IT MUST BE CONCEDED THAT NMR IS LESS SENSITIVE THAN MASS SPECTROMETRY, IT MAKES UP FOR THIS WITH A NUMBER OF UNIQUE STRENGTHS AND ADVANTAGES THAT RENDER IT A MOST VALUABLE ANALYTICAL TOOL IN THE DISCIPLINE OF METABOLOMICS. SOME OF ITS REMARKABLE STRENGTHS ARE ITS REMARKABLE ABILITY TO DEFINE AND CHARACTERIZE COMPLEX MOLECULAR STRUCTURES WITH PRECISION, THE USE OF NEW ISOTOPE-FILTERED TECHNIQUES THAT ENABLE SELECTIVE INTERROGATION AND ANALYSIS OF INDIVIDUAL SINGLE MOLECULES, AND ITS REMARKABLE ABILITY TO MEASURE AND DETERMINE POSITIONAL ISOTOPOMER DISTRIBUTIONS IN COMPLEX BIOLOGICAL MIXTURES BY USING ADVANCED MULTINUCLEAR AND MULTIDIMENSIONAL EXPERIMENTAL TECHNIQUES.[14]

PERHAPS ONE OF THE MOST REMARKABLE AND ASTOUNDING CAPABILITIES OF NUCLEAR MAGNETIC RESONANCE, OR NMR, IS CERTAINLY ITS AMAZING ABILITY TO CONDUCT IMAGING IN VIVO, I.E., WITHIN A LIVING ORGANISM. THIS IMAGING IS SPATIALLY SELECTIVE, AND AS A RESULT SCIENTISTS AND RESEARCHERS ARE ABLE TO SEE AND DYNAMICALLY MONITOR THE DIFFERING METABOLIC PROCESSES THAT ARE BEING CARRIED OUT WITHIN THE TISSUES OF THE LIVING ORGANISM. THIS REAL-TIME VIEWING GIVES FANTASTICALLY USEFUL INFORMATION REGARDING BIOLOGICAL FUNCTIONS. ADDITIONALLY, WHEN NMR IS UTILIZED IN CONJUNCTION WITH STABLE ISOTOPE TRACERS, IT IS AN AMAZINGLY POTENT AND VERSATILE TOOL THAT ALLOWS US TO INVESTIGATE FURTHER THE DYNAMICS AND COMPARTMENTALIZATION OF INTRICATE METABOLIC PATHWAYS AND NETWORKS. THESE AREAS ARE OF VITAL IMPORTANCE TO OUR UNDERSTANDING OF BIOLOGICAL SYSTEMS, BUT OUR EXISTING UNDERSTANDING IS VERY FAR FROM COMPREHENSIVE AND MUCH MORE NEEDS TO BE EXPLORED AND ANALYZED.[14][20]

IN THIS COMPREHENSIVE REVIEW, WE ARE ADDRESSING THE VARYING APPLICATIONS AND TECHNIQUES PERTAINING TO THE VARIOUS ONE-DIMENSIONAL (1D) AND TWO-DIMENSIONAL (2D) NUCLEAR MAGNETIC RESONANCE (NMR) TECHNIQUES. SPECIFICALLY, WE ARE ADDRESSING TECHNIQUES SUCH AS DIRECT DETECTION AND ISOTOPE-EDITED STRATEGIES FOR METABOLITE PROFILING, AND FOR THE DESCRIPTION OF THEIR ISOTOPOMER DISTRIBUTIONS. THESE TECHNIQUES ARE APPLIED VIA THE PLATFORM OF STABLE ISOTOPE-RESOLVED METABOLOMICS (SIRM), IN WHICH THE ANALYTICAL FUNCTIONALITY IS SIGNIFICANTLY INCREASED. WE ALSO STRESS THE INDISPENSABLE ASPECT OF THE VARIED SAMPLE PREPARATION PROTOCOLS, SUCH AS PREPARATIVE CRYOQUENCHING, METABOLITE RECOVERY METHODOLOGIES, AND CHEMOSELECTIVE DERIVATIZATION PROTOCOLS. THESE PROTOCOLS ARE SIGNIFICANTLY RESPONSIBLE FOR THE REPRODUCIBLE PROCUREMENT OF CORRECT, DEPENDABLE, AND MEANINGFUL DATA CRITICAL FOR NMR-BASED METABOLOMIC STUDIES.[14]

D. MASS SPECTROMETRY: MASS SPECTROMETRY, OR SIMPLY MS, IS A SCIENTIFIC TECHNIQUE THAT IS APPLIED WIDELY IN SCIENTIFIC RESEARCH AND A BROAD RANGE OF INDUSTRIES FOR IDENTIFICATION AND CHEMICAL CHARACTERIZATION OF CHEMICAL COMPOUNDS. THIS IS DONE THROUGH THE SEPARATION PROCESS OF ELECTRICALLY CHARGED PARTICLES CALLED IONS IN THE GAS PHASE. THE WHOLE PROCESS OF MASS SPECTROMETRY STARTS AT A VERY SIGNIFICANT PART CALLED THE ION SOURCE WHERE THE MOLECULES OF THE ANALYTE, THE SAMPLE TO BE ANALYZED, GO THROUGH A PROCESS OF CONVERSION INTO IONS. IN OTHER CASES, THE ION SOURCE HAS A DUAL FUNCTION BY PROVIDING THE IONIZATION OF SOLID OR LIQUID SAMPLES TO GET THEM READY FOR ION FORMATION IN THE GAS PHASE. THE CONVERSION IS IMPORTANT BECAUSE IT MAKES THESE OTHERWISE DIFFICULT-TO-ANALYZE SAMPLES AVAILABLE TO ANALYTICAL ANALYSIS.[15][21]

ONCE THE GAS IS IONIZED, THE GAS-PHASE IONS PRODUCED ARE THEN FED INTO THE MASS ANALYZER, A HIGHLY ADVANCED DEVICE THAT IS USED SPECIFICALLY TO DISTINGUISH THE IONS ACCORDING TO THEIR MASS-TO-CHARGE RATIO, ALSO KNOWN AS m/z . THE DISTINCTION IS EITHER DONE IN SPACE OR ALONG THE TIME AXIS, AND DEPENDING ON THE SPECIFIC TYPE OF MASS ANALYZER USED BY THE ANALYSIS, THIS DISTINCTION IS DONE. AFTER THIS DISTINCTION, THE DISTINGUISHED IONS ARE THEN DETECTED USING AN ION DETECTOR, WHICH GENERATES ELECTRICAL SIGNALS PROPORTIONAL TO THE NUMBER OF IONS IN CONTACT WITH ITS SURFACE AREA.[15]

THE SIGNALS ARE THEN TREATED TO PRODUCE A MASS SPECTRUM, A GRAPHICAL PLOT THAT PLOTS THE RELATIVE ABUNDANCE OF IONS AGAINST m/z VALUES. MASS SPECTRA TYPICALLY APPEAR AS HISTOGRAMS, WITH A PEAK FOR EACH DISTINCT ION SPECIES. THE IONS CAN BE INTACT MOLECULES, MOLECULAR FRAGMENTS, OR OTHER SPECIES THAT ARE PRODUCED WHEN THE MOLECULES ARE IONIZED.[15]

MASS SPECTROMETRY PROVIDES AN EXTREMELY POWERFUL TOOL FOR THE DIRECT IDENTIFICATION OF NUMEROUS COMPOUNDS BASED ON THE MEASUREMENT OF THEIR MOLECULAR WEIGHTS TOGETHER WITH THEIR INTRINSIC FRAGMENTATION PATTERNS. THIS CAPABILITY RENDERS IT AN EXTREMELY SELECTIVE AND RELIABLE MEANS OF QUALITATIVE ANALYSIS, FURTHER ESTABLISHING IT AS A GOOD ANALYTICAL TECHNIQUE.[15]

E. X-RAY SPECTROSCOPY: X-RAY SPECTROSCOPY IS A SET OF SOPHISTICATED ANALYTICAL METHODS THAT EMPLOY THE STIMULATION OF SUBSTANCES BY X-RAYS TO EXAMINE AND INVESTIGATE THE ATOMIC AS WELL AS MOLECULAR STRUCTURE OF A RANGE OF COMPOUNDS. THE PROCEDURE OF THIS PROCESS IS AN INTERESTING ONE; IT IS THE PROCESS OF INTERACTION IN WHICH, WHEN ATOMS ARE STIMULATED BY X-RAYS, THE INNER-SHELL ELECTRONS CAPTURE ENERGY FROM THIS STIMULATION AND THEN MOVE TO HIGHER ENERGY LEVELS. AFTER SUCH EXCITING, WHEN THESE EXCITED ELECTRONS FINALLY MOVE TO THEIR ORIGINAL, MORE STABLE, AND LOWER-ENERGY POSITIONS, IN THE PROCESS, THEY EMIT CHARACTERISTIC X-RAYS. THROUGH THE ANALYSIS OF EMITTED X-RAYS, SCIENTISTS ARE ABLE TO COLLECT VALUABLE INFORMATION REGARDING BOTH SPATIAL ARRANGEMENT AND ATOMIC COMPOSITION OF THE SAMPLE UNDER ANALYSIS.[16][25]

THERE ARE TWO BROAD CLASSES OF METHODS EMPLOYED IN THE CASE OF X-RAY SPECTROSCOPY:-WAVELENGTH-DISPERSIVE X-RAY SPECTROSCOPY, ALSO REFERRED TO AS WDXS, IS A SOPHISTICATED ANALYTICAL TECHNIQUE THAT EXAMINES X-RAYS OF SPECIFIC WAVELENGTHS. ANALYSIS IS CONDUCTED AFTER DIFFRACTING THE X-RAYS WHEN THEY PASS THROUGH A CRYSTALLINE SUBSTANCE. THE UNIQUE FEATURES OF THE TECHNIQUE ALLOW IT TO PROVIDE HIGH SPECTRAL RESOLUTION. WDXS IS THEREFORE BEST SUITED FOR PRECISE ELEMENTAL ANALYSIS IN A WIDE RANGE OF APPLICATIONS.[16][25]

ENERGY-DISPERSIVE X-RAY SPECTROSCOPY, OR EDXS OR EDS, IS AN EXTREMELY SOPHISTICATED TECHNIQUE OF DIRECT DETERMINATION OF ENERGIES OF EMITTED X-RAYS. IT IS AN ANALYSIS TECHNIQUE USED TO PROVIDE QUICK AND COMPLETE ANALYTICAL ANALYSIS, WITH EXACT DETAILS ABOUT MATERIAL COMPOSITION. IT IS USED PREDOMINANTLY TOGETHER WITH ELECTRON MICROSCOPY, INCREASING BOTH TECHNIQUES' CAPABILITIES AND PROVIDING FURTHER EXTENSIVE ANALYSIS.[16]

BOTH OF THESE TECHNIQUES ARE OF UTMOST SIGNIFICANCE AS REGARDS DEFINING AND DISCOVERING THE ATOMIC STRUCTURE OF DIFFERENT COMPOUNDS, ESPECIALLY MACROMOLECULES, SINCE MACROMOLECULES ARE BIG AND COMPLICATED STRUCTURES.[16]

ONE OF THE BEST-KNOWN AND MOST WIDELY ACCEPTED APPLICATIONS OF THE METHOD KNOWN AS X-RAY SPECTROSCOPY IS, IN FACT, X-RAY CRYSTALLOGRAPHY. THIS PARTICULAR TECHNIQUE UTILIZES X-RAY DIFFRACTION EXTENSIVELY IN A VERY INTRIGUING PHENOMENON. WHEN A CRYSTAL IS BOMBARDED WITH A CONCENTRATED BEAM OF X-RAYS, THE X-RAYS ARE DIFFRACTED AS THEY BOUNCE OFF THE REGULARLY SPACED ATOMS OF THE CRYSTAL LATTICE STRUCTURE. BY MEASURING THE ANGLES AT WHICH THE X-RAYS ARE DIFFRACTED WITH HIGH ACCURACY, AND BY MEASURING THE INTENSITIES OF THE DIFFRACTED BEAMS, SCIENTISTS ARE ABLE TO DEDUCE AND CONCLUDE THE COMPLEX THREE-DIMENSIONAL STRUCTURE OF LARGE AND COMPLEX MOLECULES, SUCH AS PROTEINS AND NUCLEIC ACIDS, RESPONSIBLE FOR PLAYING KEY ROLES IN BIOLOGICAL PROCESSES.[16]

THE FUNDAMENTAL EQUATION USED IN THE X-RAY DIFFRACTION TECHNIQUE IS KNOWN AS BRAGG'S LAW AND CAN BE EXPRESSED MATHEMATICALLY AS:

$$n\lambda = 2d \sin \theta$$

Where

n is a positive integer that denotes the order of reflection.

λ stands for the unique wavelength of X-rays.

d is the distance between atoms in a crystal lattice, and

θ is the angle of incidence (or reflection).

Bragg's Law is a mathematical model that allows scientists to compute the structure of atoms in a crystal lattice to a very high degree of precision. It is this ability that makes X-ray crystallography such a useful and critical tool of both structural biology and materials science.[16]

Raman Spectroscopy: In 1928, the great Indian physicist Chandrashekhara Venkata Raman discovered something revolutionary about the phenomenon known as inelastic scattering of light. This incredible discovery ultimately became well known as the Raman Effect. The Raman Effect graphically describes the change in the wavelength of a small amount of light that is scattered by molecules, resulting in frequencies that are different from those of the incident light that caused the process to occur. It should be noted that the degree or amount of this change in wavelength is directly related to the chemical nature and properties of the particular molecules that are causing the scattering effect.[17]

Raman spectroscopy takes fullest advantage of this special effect by closely scrutinizing the scattered light in a concerted attempt to provide valuable information regarding the molecular vibrations taking place in the sample. These vibrations also provide very valuable information that is critical in understanding different aspects of the molecule, including its structure, symmetry, bonding character, and the electronic environment surrounding it. Thus, by virtue of its sophisticated analytical methodologies, Raman spectroscopy provides qualitative and quantitative analysis of a single chemical compound, and it is therefore possible to make a detailed assessment of its properties.[17][22]

Atomic Spectroscopy: Spectroscopy is the study of how matter interacts with electromagnetic radiation in a variety of forms and properties. When the interactions are studied specifically for the purpose of making an analysis, the method is called spectrometry.[18]

ATOMIC SPECTROSCOPY IS AN OVERARCHING TERM THAT ENCOMPASSES A VARIETY OF TECHNIQUES THAT ARE PARTICULARLY DESIGNED TO ANALYZE AND QUANTIFY THE ELEMENTAL COMPOSITION OF A SAMPLE. THE VARIETY OF TECHNIQUES EMPLOYED IN THE FIELD INCLUDES A VARIETY OF IMPORTANT AND WELL-KNOWN TECHNIQUES, SUCH AS ATOMIC ABSORPTION SPECTROSCOPY (BETTER REFERRED TO AS AAS), ATOMIC EMISSION SPECTROSCOPY (BETTER REFERRED TO AS AES), ATOMIC FLUORESCENCE SPECTROSCOPY (REFERRED TO AS AFS), X-RAY FLUORESCENCE (BETTER REFERRED TO AS XRF), AND INORGANIC MASS SPECTROMETRY (BETTER REFERRED TO AS MS).[18][23]

AAS, AES, AND AFS DEAL WITH THE PROCESS OF INTERACTION BETWEEN VALENCE ELECTRONS OF AN ATOM IN THE GASEOUS STATE AND UV-VISIBLE LIGHT, LEADING TO ABSORPTION, EMISSION, OR FLUORESCENCE AND GIVING INFORMATION ABOUT THE CONCENTRATION AND ELEMENT NATURE.[18]

IN X-RAY FLUORESCENCE (XRF), ENERGETIC PARTICLES INTERACT WITH THE INNER-SHELL ELECTRONS OF AN ATOM AND CAUSE ELECTRONIC TRANSITIONS THAT RESULT IN CHARACTERISTIC X-RAY PHOTONS. THE TECHNIQUE IS PARTICULARLY EFFICIENT FOR ANALYZING AND QUANTIFYING ELEMENTS IN SOLID AND POWDERED SAMPLES.[18][24]

INORGANIC MASS SPECTROMETRY (MS) IS A HIGH-TECH ANALYTICAL METHOD THAT INVOLVES IONIZATION OF THE SAMPLE'S ATOMS. THIS LEADS TO THE FORMATION OF IONS, WHICH ARE SUBSEQUENTLY METICULOUSLY SEPARATED ACCORDING TO THEIR MASS-TO-CHARGE RATIO, OTHERWISE KNOWN AS M/Z. THIS SEPARATION IS CARRIED OUT IN AN ELECTRIC OR MAGNETIC FIELD, ENABLING ACCURATE MEASUREMENT AND EXAMINATION. DUE TO THIS ADVANCED PROCESS, TRACE ELEMENT ANALYSIS CAN BE CONDUCTED WITH INCREDIBLE SENSITIVITY, IN ADDITION TO ISOTOPIC ANALYSIS, WHICH HELPS US FURTHER KNOW THE ELEMENTAL COMPOSITION OF MATERIALS.[18]

COMBINED, THESE ATOMIC SPECTROSCOPIC METHODS HAVE A VITAL ROLE IN A VARIETY OF APPLICATIONS INCLUDING ENVIRONMENTAL ANALYSIS, CLINICAL DIAGNOSIS, MATERIALS ANALYSIS, AND DRUG ANALYSIS.[18]

II. CONCLUSION

Analytical techniques form the backbone of the assessment of pharmaceuticals in order to evaluate the quality, safety, effectiveness, and homogeneity of drug products. Since the drugs become increasingly complex, there has been a significant boost in the demand for precise, reproducible, and advanced analytical techniques. The fundamental techniques such as UV-Visible spectroscopy, infrared (IR) spectroscopy, nuclear magnetic resonance (NMR), mass spectrometry (MS), high-performance liquid chromatography (HPLC), and gas chromatography (GC) are the prominent tools for the qualitative and quantitative analysis of drugs and metabolites.

All analytical techniques exhibit distinctive strengths and weaknesses, and hence the choice of a suitable technique is of paramount significance, based on the nature of the sample and the information sought. Techniques such as LC-MS and GC-MS significantly improve the sensitivity and selectivity of analysis, enabling one to fully understand complex pharmaceutical formulations.

Ongoing developments in analytical instrumentation, automation, and computation have drastically improved the accuracy and efficiency of pharmaceutical testing. These advances, in addition to simplifying the compliance with rules, also help in the manufacture of safer and more effective drug products. Hence, the implementation of sophisticated analytical methods is crucial in the pharmaceutical sector, as it serves as a foundation to all phases of drug discovery, development, through quality control, to post-marketing surveillance..

REFERENCES

- [1] Siddiqui MR, AlOthman ZA, Rahman N. Analytical techniques in pharmaceutical analysis: A review. *Arabian Journal of chemistry*. 2017 Feb 1;10:S1409-21.
- [2] Bonfilio RB, De Araujo MB, Salgado HR. Recent applications of analytical techniques for quantitative pharmaceutical analysis: A review. *WSEAS Trans. Biol. Biomed*. 2010 Oct 1;7(4):316.
- [3] Sharma S, Singh N, Ankalgi AD, Rana A, Ashawat MS. Modern trends in analytical techniques for method development and validation of pharmaceuticals: A review. *J. Drug Deliv. Ther*. 2021 Jan 2;11:121-30.
- [4] Westenberger BJ, Ellison CD, Fussner AS, Jenney S, Kolinski RE, Lipe TG, Lyon RC, Moore TW, Revelle LK, Smith AP, Spencer JA. Quality assessment of internet pharmaceutical products using traditional and non-traditional analytical techniques. *International journal of pharmaceutics*. 2005 Dec 8;306(1-2):56-70.
- [5] Qarah N, El-Maaiden E. Spectrophotometric/titrimetric drug analysis. *In Drug Formulation Design* 2023 Jan 5. IntechOpen.
- [6] Bele AA, Khale A. An overview on thin layer chromatography. *International Journal of Pharmaceutical Sciences and Research*. 2011 Feb 1;2(2):256.
- [7] Zweig G, Sherma J. Paper chromatography—past, present and future. *Journal of Chromatographic Science*. 1973 Jun 1;11(6):279-83.
- [8] Ali AH. High-performance liquid chromatography (HPLC): A review. *Annals of advances in chemistry*. 2022 Jun 20;6(1):010-20.
- [9] Sonia K, Lakshmi KS. HPTLC method development and validation: An overview. *Journal of Pharmaceutical Sciences and Research*. 2017 May 1;9(5):652.
- [10] Rahman MM, Abd El - Aty AM, Choi JH, Shin HC, Shin SC, Shim JH. Basic overview on gas chromatography columns. *Analytical separation science*. 2015 Dec 7:823-34.
- [11] Michalski R. Ion chromatography applications in wastewater analysis. *Separations*. 2018 Feb 26;5(1):16.
- [12] Shinde G, Godage RK, Jadhav RS, Manoj B, Aniket B. A review on advances in UV spectroscopy. *Research Journal of Science and Technology*. 2020;12(1):47-51.
- [13] Aenugu HP, Kumar DS, Srisudharson NP, Ghosh S, Banji D. Near infra red spectroscopy—An overview. *International Journal of ChemTech Research*. 2011 Jun;3(2):825-36.

- [14] Fan TW, Lane AN. Applications of NMR spectroscopy to systems biochemistry. *Progress in nuclear magnetic resonance spectroscopy*. 2016 Feb 1;92:18-53.
- [15] Urban PL. Quantitative mass spectrometry: an overview. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*. 2016 Oct 28;374(2079):20150382.
- [16] Nag N, Sasidharan S, Saudagar P, Tripathi T. Fundamentals of spectroscopy for biomolecular structure and dynamics. In *Advanced Spectroscopic Methods to Study Biomolecular Structure and Dynamics* 2023 Jan 1 (pp. 1-35). Academic Press.
- [17] Das RS, Agrawal YK. Raman spectroscopy: Recent advancements, techniques and applications. *Vibrational spectroscopy*. 2011 Nov 1;57(2):163-76.
- [18] Sahin D. Atomic spectroscopy. In *Modern Spectroscopic Techniques and Applications* 2019 Oct 8. IntechOpen.
- [19] Vogt C, Wondergem CS, Weckhuysen BM. Ultraviolet-visible (UV-vis) spectroscopy. In *Springer handbook of advanced catalyst characterization* 2023 May 18 (pp. 237-264). Cham: Springer International Publishing.
- [20] Tampieri A, Szabó M, Medina F, Gulyás H. A brief introduction to the basics of NMR spectroscopy and selected examples of its applications to materials characterization. *Physical Sciences Reviews*. 2021 Feb 3;6(1):20190086.
- [21] Dubey RD, Ahmad AR, Paroha S, Gupta RK, Sahu PK, Verma S, Daharwal SJ, Prasad Reddy SL. Mass Spectroscopy: A Versatile Analytical Technique. *Research Journal of Science and Technology*. 2011 Apr 28;3(2):55-64.
- [22] Orlando A, Franceschini F, Muscas C, Pidkova S, Bartoli M, Rovere M, Tagliaferro A. A comprehensive review on Raman spectroscopy applications. *Chemosensors*. 2021 Sep 13;9(9):262.
- [23] Lewen N. The use of atomic spectroscopy in the pharmaceutical industry for the determination of trace elements in pharmaceuticals. *Journal of pharmaceutical and biomedical analysis*. 2011 Jun 25;55(4):653-61.
- [24] Bendicho C, Lavilla I, Pena-Pereira F, Romero V. Green chemistry in analytical atomic spectrometry: a review. *Journal of Analytical Atomic Spectrometry*. 2012;27(11):1831-57.
- [25] Besley NA. Density functional theory based methods for the calculation of X-ray spectroscopy. *Accounts of Chemical Research*. 2020 Jul 2;53(7):1306-15.

