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The Review Study On Analytical and Bioanalytical Techniques for Drug Design

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ABSTRACT:
The clinical course of the bio analytical method changes, it is necessary to establish a balance or relationship between different methods in order to compare the results of different methods. Cross validation is used to accomplish this. This is an important first step to ensure the quality and reliability of the development process. Bio analytical development is important in the development process for drug discovery and commercial approval. Bio analysis of metabolites is important to study the efficacy, toxicity and bioavailability of the drug. Therefore, characterization requires the use of various analytical methods such as chromatography, mass spectrometry, electrophoresis and spectroscopy to supplement the required information. This article attempts to improve the knowledge of art.

KEYWORD: Bio analytical , Analytical, Drug discovery, Drug design, Mass spectroscopy, LC-MS, GC, NMR, HPLC

I. INTRODUCTION :

Drug discovery is the process of identifying potential new treatments using a combination of computational, experimental, interpretive and clinical models(1).The development and discovery of drug includes preliminary research based on cell and animal models and human trials of drug finally entering the approval phase of drugs that have been released. Today, drug discovery includes drug identification, drug chemistry and improvement of these compounds in terms of compatibility, selectivity, potency, metabolic stability and oral bioavailability(2).Analytical methods are a method used to determine the chemical or physical properties of drugs, chemicals or compounds. Many types of technologies are used for analysis, from simple weighing to the use of special equipment .Analytical methods are efficient and quantitative determination of compounds using various techniques such as titration, spectroscopy, chromatography and gravimetric analysis(3). Analytical methods are a method used to determine the chemical or physical properties of chemicals, drugs or compounds. Many types of technologies are used for analysis, from simple weighing to the use of special equipment(4). Bio analytical methods are quantitative measurements of drugs and/or metabolites or biomarker concentrations in biological fluids (such as blood, plasma, blood, urine, and saliva)or tissue extracts(5).Bio analytical methods usually involve the extraction of analytes from chemical samples, liquid chromatography separates the needs of endogenous products and metabolites that may cause matrix effects or selective problems, and is often in the form of tandem mass spectrometry MS, which aims to improve analytical selectivity and sensitivity. When establishing a quantitative bio analytical LC-MS method, all three sometimes need to be carefully considered as a common process. Triple Quadrupole Analyzer Liquid Chromatography-Mass Spectrometer/Mass Spectrometer (LC-MS/MS) is still the method of choice for Target Quantitative Analysis(6). Some techniques commonly used in bio analytical studies include Hyphenated techniques: 1)LC–MS (liquid chromatography–mass spectrometry), 2)GC–MS

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(gas chromatography–mass spectrometry), 3)LC–DAD (liquid chromatography–diode array detection), 4)CE–MS (capillary electrophoresis–mass spectrometry)Chromatographic methods :1)HPLC (high performance liquid chromatography), 2)GC (gas chromatography), 3)UPLC (ultra performance liquid chromatography), 4) Supercritical fluid chromatography(7).

PRINCIPLE:

The necessary parameters to ensure acceptance of bio analytical method validation performance are accuracy, precision, selectivity, sensitivity, reproducibility, and stability. Select a specific description of the bio analytical procedure to be written. This can take the form of maternal awareness, questionnaires, reports and/or general birthing procedures. Each step in the bio analytical method, from the writing of the fabric to the measurement and time of the examination, must be examined to determine which media, matrix, fabric, or process changes may affect the estimation of the analytes in the matrix. Substrate changes due to the body's structure should also be taken into account. In the case of LC-MS-MS-based techniques, it is often important to take steps to ensure that the software is free of the effects matrix throughout the process if the elements of the matrix are set to those used in the first. Verification a point.

IMPORTANCE OF DRUG DISCOVERY:

A preclinical drug discovery program's objective is to produce one or more clinical candidate molecules with sufficient evidence of biologic activity at a disease-relevant target, sufficient safety, and sufficient drug-like qualities to be tested in humans. Because many molecules do not progress through the full process due to issues with safety, kinetics, potency, intellectual property protection, or other variables, the majority of discovery programmes aim to develop more than one candidate molecule(9).New diseases, the emergence of drug resistance, and our growing understanding of medical issues, which enables the treatment of previously incurable ailments, are just a few of the numerous factors that make new medications significant. Sometimes a previously utilised drug might be used for a new purpose instead of a new drug being needed(10).The introduction of new diseases and chronic ailments has led to an increase in drug use in daily life in recent years. Additionally, there is a critical need for new and safer pharmacological treatments because of the way we live today (i.e., exposure to toxic toxins and microorganisms). As a result, drug discovery and development will inevitably advance at an exponential rate, and it is realistic to anticipate that huge pharmaceutical corporations will quickly emerge. Pharma experts have established themselves as pillars of numerous R&D facilities, including foundations and public and commercial organisation(11).Drug discovery is the process by which novel candidate pharmaceuticals are found in the domains of medicine, biotechnology, and pharmacology(12).

DRUG DISCOVERY PROCESS:

From discovery to approval, the expensive process of drug discovery typically takes more than ten years. Notably, the cost and approval time vary based on the ailment being treated and the medicine being developed. A treatment that is urgently needed and would typically undergo a priority review process will have its review process take significantly longer than a drug that just offers marginal enhancements over currently marketed therapies. (13).The development of science and technology helped to shift the focus of drug discovery away from the conventional drug discovery process, which involved using medicinal plants, and towards small molecule synthesis, which was thought to be easily synthesised, with relatively good chemical stability and straightforward characterization of reactivity(14).A small molecule screen typically entails the systematic screening of hundreds or thousands of compounds with a molecular weight of less than 500 against a biological target(15).The pharmaceutical sector, which generates and analyses numerous biological libraries using HTS, uses the process extensively. The procedure's objective is to find compounds that can be turned into a therapeutic lead because they affect a certain biological process. This would make it possible for the pharmaceutical sector to manufacture expensively huge molecules(16).A carefully constructed culture-based HTS model should be cost-effective, easy to execute, have a high sensitivity and low signal-to-noise ratio, and be at least micro-scale capable of scaling to production scale. HTS are often carried out in 96-well microplate format with liquid dispensing and plate handling robotics for automation, but many are switching to 384-well plates and larger in an effort to hasten the discovery process(17).The type of screening, the target, and the assay employed will determine the adaption or format of the screening

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(96, 384, or 768) though. Since it is impossible to accurately simulate biological processes in small-scale models, cell proliferation experiments where the goal of the screen is to measure tumour growth or growth inhibition will not be suitable in 768-microplate(18).pharmaceuticals is a very alluring and more affordable alternative due to the sluggish pace of new drug development, the high associated cost, and the high rate of late-stage failures. Finding new therapeutic uses for already-marketed, low-risk medications is a process known as drug repurposing, which has the potential to shorten the time required for drug development and to lower associated costs(19).Commercially speaking, drug repurposing may not be as appealing as alternative methods due to the restrictive intellectual property (IP) rules that regulate it(20).It is challenging to obtain IP protection for such relationships under the law because the majority of innovative medication target illness associations are probably confirmed by publications or internet databases Accessibility to advanced information and genomic databases has accelerated the creation of novel computational techniques(21).

II. METHOD :

ANALYTICAL METHOD:

LIQUID CHROMATOGRAPHY:

LC is a method of separation where liquid serves as the mobile phase. HPLC is the name given to modern LC, which typically uses very fine packing particles and a high pressure. In HPLC, the sample is driven through a column that is filled with a stationary phase made up of irregularly shaped or spherically shaped particles, an aporous monolithic layer, or a porous membrane by a liquid under high pressure (the mobile phase). The polarity of the mobile and stationary phases has historically led to the divishich the stationary phase is more polar than the mobile phase (for example, using toluene as the mobile phase and silica as the stationary phase), and the contrary (for example, using a mixture of water and methanol as the mobile phase and C18 = octadecylsilyl as the stationary phase). Ironically, there are fewer in the "normal phase on of HPLC into two distinct sub-classes. RPLC is therefore employed significantly more in applications. Affinity chromatography is used, which is based on the selective non-covalent interaction of an analyte and particular molecules. Though not extremely sturdy, it is very specific. It is frequently used in biology to purify proteins with tags attached. These fusion proteins are marked with substances that selectively bind to the stationary phase, such as His-tags, biotin, or antigens. Following purification, some of these tags are often taken off to get the pure protein. The affinity of a bio molecule for a metal (Zn, Cu, Fe, etc.) is frequently used in affinity chromatography. Columns are frequently created manually. As an initial stage, conventional affinity columns are utilised to flush out undesirable bio molecules. However, there are HPLC methods that do make use of affinity chromatography characteristics. Affinity for Immobilised Metal Based on the relative affinity for the metal, chromatography can be used to separate the aforementioned compounds (e.g., Dionex IMAC). These columns can frequently be loaded with various metals to produce a column with a specific affinity. A separation method known as supercritical fluid chromatography uses a fluid as the mobile phase that is both above and reasonably close to its critical temperature and pressure(22).

MASS SPECTROSCOPY:

The 96-well plates contained 25 L of the eluates from the gel filtration size exclusion chromatography mixed with 25 litres of water. With a Micromass LCT time-of-flight or a Quattro I triple quadrupole mass spectrometer, each outfitted with a Gilson 215 and liquid handler a Gilson 841 micro injector, a 5-L aliquot from each sample was analysed by mass spectrometry using automated ESI/MS methods in both positive and negative ionisation modes. The samples were introduced into the mass spectrometer using a Hewlett-Packard 1100HPLC system at a flow rate of 50 L/min with a carrier solvent of 1:1 water-acetonitrile with 0.025% formic acid. The de solvation gas (N2), nebulizing gas (N2), and electrospray source temperatures were all kept at 80, 120, and ambient temperatures, respectively. The voltage of the nozzle-skimmer was held constant at 20 V. Data from mass spectrometers were collected in the 100–1200 m/z range. Mass spectra were typically collected for 0.75 to 1 minute. Using in-house MS processing software, the various scans were merged, background subtracted, smoothed, baseline subtracted, and centroided.45 The entire analysis time for the 33 plates utilised in the RGS4 screen was approximately 6 days. The total analysis time for each well was approximately 2.5 minutes, or 4 hours per 96-well plate. The processed spectra were automatically and effectively evaluated for the presence of [M + H]+, [M + 2H]2+, and [M + NH4]+ ions consistent with a chemical in the

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combination using in-house MS interpretation software that features a clever background subtraction algorithm46. The programme reported the results in an Excel spreadsheet after ranking the observed hits according to a weighted signal-to-noise ratio scale(23).

BIOANALYTICAL TECHNIQUES:

Liquid Chromotography-Mass Spectroscopy (LS-MS):

A method that combines mass spectrometry and liquid chromatography is known as bio analytical liquid chromatography-mass spectrometry. For the quantitative and qualitative examination of pharmacological compounds, drug products, and biological materials, laboratories frequently utilise LC-MS. Bio availability, bioequivalence, and pharmacokinetic data evaluation and interpretation have benefited greatly from the use of LC-MS. Biological samples are identified using LC-MS during all stages of a drug's technique development for research and quality control(24). In the pharmaceutical business, LC-MS is a widely used method for the simultaneous measurement of numerous analytes, including medicines, metabolites, and endogenous biomarkers(25).LC-MS is an analytical method that combines sensitive and accurate mass spectral detection with high resolution chromatographic separation. An important advancement in the history of chromatography is the combination of LC-MS.In LC-MS, mass spectroscopy aids in the analysis of a sample's structural clarity and elemental composition. It is a potent technology with great sensitivity and selectivity that is utilised for numerous applications.It is most frequently employed in the field of bioanalysis and is extensively used in pharmacokinetic investigations of drugs. When it comes to the components in the many elements of phenotypic cloning, particularly in the field of molecular pharmacognosy, LC-MS also play a role in pharmacognosy(26).The analytical chemistry technique known as liquid chromatography-mass spectrometry (LC-MS) combines the mass analysis capabilities of mass spectrometry (MS) with the physical separation capabilities of liquid chromatography (or HPLC). Due to the synergistic enhancement of each technique's particular capabilities, coupled chromatography-MS systems are widely used in chemical analysis. While mass spectrometry offers spectrum information that may aid to identify (or confirm the suspected identification of) each separated component, liquid chromatography separates mixtures containing many components(27).

PRINCIPLE OF LC-MS:

Utilising an HPLC, the LC-MS technique separates the mixture's distinct components first, followed by ionisation and separation of the ions based on their mass/charge ratio. The separated ions are then sent to an identification and quantization device, such as a photo or electron multiplier tube detector. The ion source is a crucial part of any MS analysis since it essentially facilitates the effective creation of ions for analysis. Ion sources for ionising intact molecules include ESI (Electronspray Ionisation), APCI (Atmospheric Pressure Chemical Ionisation), and others. The choice of ion source also depends on whether the target analyte is polar or non-polar chemically(28).

APPLICATION OF LC-MS:

Because liquid chromatography can separate delicate and complicated natural mixtures, whose chemical composition needs to be clearly established (e.g., biological fluids, environmental materials, and pharmaceuticals), the combination of MS with LC systems is appealing. Analysis of volatile explosive residue is another application for LC-MS(29). Because it provides for fast molecular weight confirmation and structural identification, LC-MS is frequently employed in drug development. The generation, testing, and validation of a discovery from a wide range of items with possible applications is accelerated by these features. Peptide mapping, glycoprotein mapping, lipodomics, natural product dereplication, bioaffinity screening, in vivo drug screening, metabolic stability screening, metabolite identification, impurity identification, quantitative bioanalysis, and quality control are just a few of the highly automated methods used in LC-MS applications for drug development(30).

More than 85% of naturally occurring chemical compounds are polar and thermally labile, making LC-MS one of the most used chemical analysis methods today. GC-MS cannot analyse these samples. For instance, HPLC-MS is thought to be the best analytical method for proteomics and pharmaceutical labs(31).

NMR:

Molecules are studied using nuclear magnetic resonance spectroscopy (NMR), which records how radiofrequency electromagnetic radiation interacts with molecule nuclei when

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positioned in a high magnetic field.Chemical shifts, multiplicity (the interaction of nearby nuclei), integrals, intramolecular connections, and a wide range of dynamic processes, such as molecular movements in solution, chemical exchange, and ligand binding, are just a few examples of the structural information that NMR offers that is particularly rich and unique(32) . It was challenging to interface LC with NMR and MS until recent years. However, many of the issues with physically placing the LC and MS close to the NMR magnet have since been resolved overcome(33). Practically, a post-columnsplitter is used to achieve a straightforward hyphenation of LC to NMR and MS. By using a 1-2 m capillary, this sends 90–95% of the flow to the NMR and the remaining portion to the MS. The valve-switching interface known as the BNMI (Bruker NMR-MassSpectrometry Interface) is a potent substitute(34). These integrated LC-NMR-MS systems are very adaptable and work as needed. In fact, longer NMR experiments might frequently use the LC-MS.By applying a set of pre-established groundrules, skilled optimisation of the chromatographic separation conditions considerably enhances the generation of LC-NMR-MS data on a regular basis(35).The logical answer is frequently to optimise the chromatographic resolution because doing so might be time-consuming and counterproductive. The NMR assignments and the MS data combined frequently result in clear structural elucidation(36).

GAS CHROMATOGRAPHY:

An analytical tool called a gas chromatograph (GC) is used to determine the composition of different parts of a sample (37).In analytical science, gas chromatography (GC) is a popular type of chromatography that is used to separate and study compounds that may be vaporised without disintegrating. Regular uses of GC include determining a substance's cleanliness or identifying the separate components of a blend (38). For isolating compounds in a complicated sample mixture, a gas chromatograph is a chemical analytical tool. In a gas chromatograph, different chemical components of a sample flow through a thin tube known as the column at different rates depending on their different chemical and physical properties and their interaction with a specific column filling, known as the stationary phase (39).Gas chromatography (GC) is unquestionably the best technology for analysing taste matrices since they are extremely complex and need to be separated before being measured and characterisedfor their analytes.(40).

HPLC METHOD:

The analytical chemistry method of high-performance liquid chromatography (HPLC), formerly known as high-pressure liquid chromatography, is used to separate, recognise, and quantify each component in a mixture. It uses pumps to move a column of solid adsorbent material through a pressurised liquid solvent solvent carrying the sample mixture. The adsorbent material and each component in the sample interact slightly differently, resulting in various flow rates for the various components and their separation as they exit the column (41). Both in the laboratory and in the field of clinical science, HPLC has several uses. Because it is a dependable method for obtaining and ensuring product purity, it is a widely utilised technology in the pharmaceutical industry (42). HPLC has been used for manufacturing (for example, in the production of pharmaceutical and biological products), legal (for example, in the detection of performance-enhancing drugs in urine), research (for example, in the separation of the components of a complex biological sample or of similar synthetic chemicals from each other), and medical (for example, in the detection of vitamin D levels in blood serum) purposes(43). Despite the fact that HPLC can create products of incredibly high quality (purity), it isn't always the main technique employed in the manufacturing of bulk medicinal ingredients.(44). Only 15.5% of syntheses, according to the European Pharmacopoeia, use HPLC.(45). The American Pharmacopoeia, however, uses it in 44% of syntheses.(46). In modern analytical chemistry, high-performance liquid chromatography is one of the most potent technologies available. Any material that can dissolve in a liquid can have its constituents separated, identified, and quantified using this technique. The most precise analytical technique, HPLC, is frequently used to assess the stability of pharmacological items as well as their quantitative and qualitative composition(47).

GC-MS METHOD:

The analytical technique known as gas chromatography-mass spectrometry (GC-MS) combines the separation capabilities of gas-liquid chromatography with the detection capabilities of mass spectrometry to identify various compounds within a test sample.(48).In order to increase the efficiency of sample studies, gas chromatography-

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mass spectrometry (GC-MS), a hybrid analytical technique, combines the separation powers of GC with the detection features of MS. While GC can distinguish between volatile and nonvolatile components in a sample, MS aids in fragmenting the components and identifies them based on their mass.(49). The analytical method of gas chromatography mass spectrometry (GC-MS), which combines the two potent methods of gas chromatography and mass spectrometry, is used to separate, recognise, and quantify volatile chemicals. It is hence ideal for examining the numerous molecules with low molecular weight. GC-MS analysis is most frequently employed with volatile and semi-volatile substances, while it can be utilised with solid, gaseous, and liquid samples as well (50).An analytical technique called gas chromatography-mass spectrometry (GC-MS) combines the advantages of gas chromatography and mass spectrometry to identify various compounds in a test sample(51).

APPLICATION OF GC-MS:

Application areas for GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, food and flavor analysis, and identification of unidentified samples, including those of material samples collected from Mars during probe trips as early as the 1970s(52).To identify drugs and/or poisons in biological samples from suspects, victims, or the deceased, forensic toxicologists frequently utilize GC-MS. As part of sample preparation for drug screening, GC-MS techniques typically use liquid-liquid extraction, in which target chemicals are removed from blood plasma(53). Forensics, petrochemical, chemical, agricultural, tobacco, pharmaceutical, healthcare, energy, mining, and environmental research, to name a few, all use GC-MS to identify unidentified volatile chemicals. For instance, drug testing can be done for a variety of reasons, including pathology, healthcare, and anti-doping for both humans and animals.(54).

III. CONCLUSION :

This review is aimed in focusing the role of various bio analytical techniques in pharmaceuticals and gives a thorough literature survey of the bio analytical methods and instruments in drug analysis and has been reported by LC, MS, NMR, GC, HPLC Methods. Bio analysis is now widely accepted and is allowed to work as an essential part of pharmacokinetic/pharmaco dynamic characterization of new medicinal products , from the moment of discovery to all stages of medicine development. Chapter importance and application of bio analytical parameters discusses drug discovery and development to help develop safer and more effective drugs while reducing development time and cost.

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