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A REVIEW ON MODERN TECHNIQUES OF PHARMACEUTICAL ANALYSIS

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ABSTRACT

Traditionally, pharmaceutical analysis referred to the chemical analysis of drug molecules. However, over the years, modern pharmaceutical analysis has evolved beyond this to encompass combination techniques, high-throughput technologies, chemo metrics, micro dosing studies, miniaturization and nanotechnology. These analytical advances are now being employed in all stages of drug discovery and the focus of this review will be on how these technologies are being employed within this process. With new, improved and evolving technologies, as well as new applications for existing technology, the search for new drugs for the prevention and treatment of human diseases continues

Keywords: Chromatography, Pharmaceutical Analysis, Constructive Technologies.

INTRODUCTION

Traditionally, pharmaceutical analysis referred to the chemical analysis of drug molecules. However, over the years, modern pharmaceutical analysis has evolved beyond this to encompass combination techniques, high-throughput technologies, chemo metrics, micro dosing studies, miniaturization and nanotechnology. These analytical advances are now being employed in all stages of drug discovery and the focus of this review will be on how these technologies are being employed within this process. With new, improved and evolving technologies, as well as new applications for existing technology, the search for new drugs for the prevention and treatment of human diseases continues [1].

Modes of Chromatography

Modes of chromatography are defined essentially according to the nature of the interactions between the solute and the stationary phase, which may arise from hydrogen

bonding, Vander walls forces, electrostatic forces or hydrophobic forces or basing on the size of the particles (e.g. Size exclusion chromatography).

Different modes of chromatography are as follows:

- Normal Phase Chromatography
- Reversed Phase Chromatography
- Reversed Phase – ion pair Chromatography
- Ion Chromatography
- Ion-Exchange Chromatography
- Affinity Chromatography
- Size Exclusion Chromatography

In this new millennium, the pharmaceutical industry faces new opportunities created by the completion of the Human Genome Project and the increased emphasis on genomics and proteomics in drug discovery. Pathology might now be elucidated by understanding processes at the protein level. Technological advances have focused drug discovery effort towards the search for drugs directed at molecular targets or pathways believed to have a causal role in the disease [1]. Pharmacotherapy is undergoing a paradigm shift as genomics evolves into an important component of diagnosis and treatment. The ability to analyze the entire genome has also led to the identification of more potential drug targets. Small protein drugs are fast becoming an important and rapidly growing segment of the prescription drug market. In addition, recombinant protein drugs are becoming an even more important resource for the physician. Currently, drug discovery efforts are being revolutionized by high-throughput technologies, combinatorial chemistry, genomics, proteomics, informatics and miniaturization [2]. Successful drug discovery and development depends upon close interactions between various disciplines with inputs from biotechnology, biomedical engineering, proteomics and genomics inter alia. Today, pharmaceutical analysis is employed throughout the whole drug discovery and development process. It is used to provide accurate and precise data, not only supporting drug discovery and development but also post-market surveillance. This review illustrates some important developments in the field of modern pharmaceutical analysis [2].

As the pharmacy deals with the health of human beings the drug testing is so far important bellow some modern technology of analysis.

Some modern Technology of analysis

- HPLC
- GC
- Titration
- Acid–base
- Aqueous mixtures
- Indicator
- Potentiometric
- Non-aqueous
- Indicator
- Potentiometric
- Redox (Iodometry, Nitritometry, etc.)
- Other (complexometry, argentometry, etc.)
- UV–vis spectrophotometry
- Microbiological assay (antibiotics)
- IR
- NMR
- Polarimetry
- Fuorimetry
- Atomic absorption spectroscopy
- Polarography
- Gravimetry

Recently, so called Constructive Technology Assessment initiatives belong to the field. Although objects and aims of the activities vary, they have in common that they concern current and future developments of technology, and that they aim at improving the alignment between technological and societal developments, whereby societal developments include the activities of corporations. The methods applied in the field of

Technology Assessment are as diverse as the field itself. They range from forecasting studies to interventions in stakeholders networks. This paper attempts to bring some order in this diversity. This may be useful for experienced practitioners of TA, but even more for new entrants and for educational purposes [2]. The ultimate aim is to develop a methodology of Technology Assessment, specifying in which cases which methods are most appropriate. However, a necessary first step, taking the larger part of this paper, is to classify different methods according to the character and scope [3].

A Future Technology Analysis Framework [2]

Analytical techniques

Titrimetry techniques:

Origin of the titrimetry method of analysis goes back to some- where in the middle of the 18th century. It was the year 1835 when Gay–Lussac invented the volumetric method which subsequently leads to the origin of term titration [3]. Although the assay method is very old yet there are signs of some modernization, i.e., spreading of non-aqueous titration method, expanding the field of application of titrimetric methods to (very) weak acids and bases as well as Potentiometry end point detection improving the precision of the methods. With the development of functional group analysis procedures titrimetric methods have been shown to be beneficial in kinetic mea- surements which are in turn applied to establish reaction rates. There are many advantages associated with these methods which include saving time and labor, high precision and the fact that there is no need of using reference standards [3].

Chromatographic techniques

Thin layer chromatography

Although an old technique yet it finds a lot of application in the field of pharmaceutical analysis. In thin layer chromatography, a solid phase, the adsorbent, is coated onto a solid support as a thin layer usually on a glass, plastic, or aluminum support. Several factors determine the efficiency of this type of chromatographic separation. First the adsorbent should show extreme selectivity toward the substances being separated so as to the dissimilarities in the rate of elution be large. For the separation of any given mixture, some adsorbents may be too strongly adsorbing or too weakly adsorbing. Thin layer chromatography is a popular technique for the analysis of a wide variety of organic and

inorganic materials, because of its distinctive advantages such as minimal sample clean up, wide choice of mobile phases, flexibility in sample distinction, high sample loading capacity and low cost. TLC is a powerful tool for screening unknown materials in bulk drugs. It provides a relatively high degree of assertion that all probable components of the drug are separated [4]. The high specificity of TLC has been exploited to quantitative analytical purpose using spot elution followed by spectrophotometric measurement. TLC has been utilized for the determination of some steroids, pioglitazone, celecoxib and noscapine. TLC plays a crucial role in the early stage of drug development when information about the impurities and degradation products in drug substance and drug product is inadequate. Various impurities of pharmaceuticals have been identified and determined using TLC [4]. Principle of Separation of Different Components Differential affinities (quality of attachment) of the different parts of the analytic towards the stationary and portable stage bring about the differential division of the segments. Fondness, thus, is managed by two properties of the atom: 'Adsorption' and 'Dissolvability'.

Figure No. 01: TLC

Calculating Rf Values In this experiment that all you need to know what is the number of various colors made up the blend, you could simply stop there. In any case, estimations are regularly taken from the plate with a specific end goal to help distinguish the mixes introduce. These estimations are the separation went by the dissolvable, and the separation went by individual spots [6]. At the point when the dissolvable front draws near to the highest point of the plate, the plate is expelled from the measuring glass and the position of the dissolvable is set apart with a different line before it has an opportunity to vanish. The Rf value for each dye is then worked out using the formula:

Rf = distance travelled by component/distance travelled by solvent

The Rf value can be utilized to recognize mixes because of their uniqueness to each compound. When contrasting two distinct mixes under similar conditions, the compound

with the bigger Rf value is less polar in light of the fact that it doesn't adhere to the stationary stage the length of the polar compound, which would have a lower Rf value. Rf values and reproducibility can be influenced by various distinctive variables, for example, layer thickness, dampness on the TLC plate, vessel immersion, temperature, profundity of versatile stage, nature of the TLC plate, test size, and dissolvable parameters. These impacts ordinarily cause an expansion in Rf values. In any case, on account of layer thickness, the Rf value would diminish on the grounds that the portable stage moves slower up the plate [7].

Advantages of TLC

TLC is extremely easy to utilize and reasonable. Students can be shown this method and apply its comparative standards to other chromatographic systems. There are little materials required for TLC (chamber, watch glass, slender, plate, dissolvable, pencil, and UV-light). Therefore, once the best dissolvable is discovered, it can be connected to different procedures, for example, High execution fluid chromatography. TLC can be utilized to guarantee immaculateness of a compound. It is anything but difficult to check the virtue utilizing an UV-light. Recognizable proof of most mixes should be possible basically by checking Rf writing values. You can adjust the chromatography conditions effortlessly to build the improvement for determination of a particular segment.

Figure No. 02: Silica gel -G TLC plate

Disadvantages of TLC

TLC plates don't have long stationary stages. Consequently, the length of partition is constrained contrasted with other chromatographic methods. Additionally, as far as possible is a ton higher. On the off chance that you would require a lower identification

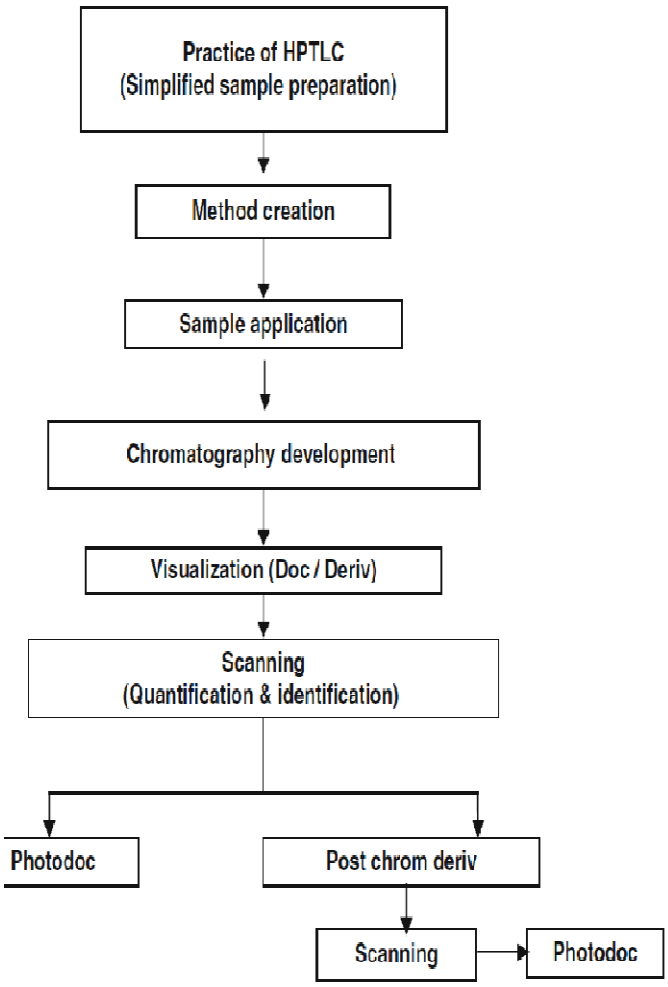
restrict, one would need to utilize other chromatographic procedures. TLC works as an open framework, so elements, for example, moistness and temperature can be outcomes to the consequences of your chromatogram [4].

Table 1: Classification of Chromatographic methods

Stationary phase	Mobile phase	Method
Solid	Liquid	Column, thin-layer, ion exchange, High performance liquid chromatography
Liquid	Liquid	Column, thin-layer, HPLC, paper chromatography
	Gas	Gas – Liquid Chromatography

High performance thin layer chromatography

With the advancement of the technique, high performance thin layer chromatography (HPTLC) emerged as an important instrument in drug analysis. HPTLC is a fast separation tech- nique and flexible enough to analyze a wide variety of samples. This technique is advantageous in many means as it is simple to handle and requires a short analysis time to analyze the complex or the crude sample cleanup. HPTLC evaluates the entire chromatogram with a variety of parameters without time limits. Moreover, there is simultaneous but independent development of multiple samples and standards on each plate, leading to an increased reliability of results. HPTLC has been used to quantitate drugs as ethinyl estradiol and cyproterone, alfuzosin and tramadol and pentazocine.



The word chromatography means 'to show in colour' in greek & was first introduced by Russian biotanist 'micheltswett' to describe that solvent according to adsorbance sequence are resolved into various colour zone. It's a method of analysis in which the flow of solvent or gas promotes the separation of substance by differential migration in a porous absorptive medium. The HPTLC is very useful qualitive analysis method. It is combine arts of chromatography with quickness at moderate cost. It is major advance to TLC principle shorten time duration & better resolution. HPTLC is playing an important role in today analytical world, not in competition to HPLC but as complementary method. One of the most obvious orthogonal features of the two techniques is the primary use of reversed phases in HPLC versus unmodified silica gel in HPTLC, resulting in partition chromatography and adsorption chromatography respectively. Unlike other methods, HPTLC produces visible chromatograms complex information about the entire sample is available at a glance.

Multiple samples are seen simultaneously, So that reference and test samples can be compared for identification [8]. Similarities and differences are immediately apparent and with the help of the image comparison. Several chromatograms can be compared directly, even from different plates. In addition to the visible chromatograms, analog peak data are also available from the chromatogram. They can be evaluated either by the image based software Video scan or by scanning densitometry with TLC Scanner, measuring the absorption and/or fluorescence of the substances on the plate. TLC is an offline technique: the subsequent steps are relatively independent, allowing parallel treatment of multiple samples during chromatography.

Key feature of HPTLC

1. Simultaneous processing of sample and standard - better analytical precision and accuracy less need for Internal Standard.
2. Several analysts work simultaneously.
3. Lower analysis time and less cost per analysis.
4. Low maintenance cost.
5. Simple sample preparation - handle samples of divergent nature.
6. No prior treatment for solvents like filtration and degassing.
7. Low mobile phase consumption per sample
8. No interference from previous analysis – fresh stationary and mobile phases for each analysis -no contamination.
9. Visual detection possible - open system

The procedure of HPTLC involve

1. Sample preparation.

2. Selection of chromatography.

3. Plate, prewashing, conditioning.

4. Sample application

5. Pre-conditioning.

6. Mobile phase.

7. Chromatography development.

8. Detection spot.

9. Scanning & documentation

1. **HPTLC: Separation and Resolution**
2. To which extent various components of a formulation are separated by a given HPTLC system is the important factor in quantitative analysis. It depends on the following factors:
3. Type of stationary phase
4. Type of precoated plates
5. Layer thickness
6. Binder in the layer
7. Mobile phase
8. Solvent purity
9. Size of the developing chamber
10. Saturation of chamber
11. Sample's volume to be spotted
12. Size of the initial spot
13. Solvent level in the chamber
14. Gradient
15. Relative humidity
16. Temperature
17. Flow rate of solvent
18. Separation distance
19. Mode of development[5]

Applications of HPTLC

HPTLC is one of the most widely applied methods for the analysis in pharmaceutical industries, clinical chemistry, forensic chemistry, biochemistry, cosmetology, food and drug analysis, environmental analysis, and other areas. It is due to its numerous advantages, for example, it is the only chromatographic method offering the option of presenting the results

as an image. Other advantages include simplicity, low costs, and parallel analysis of samples, high sample capacity, rapidly obtained results, and possibility ofmultiple detection. Many reports on studies related to clinical medicine have already been published in many journals. HPTLC is now strongly recommended in the analysis of drugs in serum and other tissues [9].

HPTLC in Pharmaceutical Products

HPTLC is also used in analyzing the purity and efficacy of many pharmaceutical preparations and dosage forms. Puranik developed and validated a simple, rapid, and accurate chromatographic methods (HPLC and HPTLC) for simultaneous determination of ofloxacin and ornidazole in solid dosage form. The amount of ofloxacin and ornidazole estimated as percentage of label claimed was found to be 100.23 and 99.61% with mean percent recoveries 100.47 and 99.32%, respectively. Both these methods were found to be simple, precise, accurate, selective, and rapid and could be successfully applied for the determination of pure laboratory prepared mixtures and tablets [10].

HPTLC in Natural Products

The HPTLC technique is rapid, comparatively simple, robust, and extremely versatile. HPTLC not only confirm but also establish its identity. It is also an ideal screening tool for adulterations and is highly suitable for evaluation and monitoring of cultivation, harvesting, and extraction processes and testing of stability. A simple and reproducible method using HPTLC was successfully performed for the quantitative analysis of above diterpenoids in the root bark of Photiniaintegrifolia. In which Diterpenoids 1β, 3α, 8β-trihydroxy-pimara-15-ene 6α, 11, 12, 16-tetrahydroxy-7-oxo-abieta-8, 11, 13-triene and 2α, 19-dihydroxy-pimara-7,15-diene were used as chemical markers for the standardization of Photiniaintegrifolia plant extracts.

A simple HPTLC method has been developed for the simultaneous determination of isoorientin, isovitexin, orientin, and vitexin, both pure and in commercial samples of bamboo leaf flavonoids. It was found that HPTLC is a simple, precise, specific, and accurate and can be used for manufacturing QC of bamboo-leaf flavonoids or for governmental regulatory purposes. Many such reports present the evidence of utilization of HPLTC in

fingerprinting analysis of drugs of natural origin, and hence, the increasing acceptance of natural products is well suited to provide the core scaffolds for future drugs; there will be further developments in the use of novel analytical techniques in natural products drug discovery campaigns [9].

HPTLC in Other Fields

In recent years, HPTLC is a globally accepted practical solution to characterize small molecules in quality assessment throughout the developing world. HPTLC is used for purity control of chemicals, pesticides, steroids, and water analysis. HPTLC is also widely used for analysis of vitamins, water-soluble food dyes, pesticides in fruits, vegetables, and other food stuffs [6].

High-performance liquid chromatography

(HPLC) HPLC is an advanced form of liquid chromatography used in separating the complex mixture of molecules encountered in chemical and biological systems, in order to recognize better the role of individual molecules. It was in the year 1980, HPLC methods appeared for the first time for the assay of bulk drug materials (United States Pharmacopoeia, 1980). As seen in, this has become the principal method in USP XXVII (United States Pharmacopoeia, 2004) and to a lesser extent but one of the most widely used methods also in Ph. Eur. 4 (The European Pharmacopoeia and Council of Europe, 2002). The specificity of the HPLC method is excellent and simultaneously sufficient precision is also attainable. However, it has to be stated that the astonishing specificity, precision and accuracy are attainable only if wide-ranging system suitability tests are carried out before the HPLC analysis [11]. For the reason the expense to be paid for high specificity, precision and accuracy is also high. During the survey of the literature it was observed that among the chromatographic techniques HPLC has been the most widely used system. In liquid chromatography the choice of detection approach is critical to guarantee that all the components are detected. One of the widely used detectors in HPLC is UV detector which is capable of monitoring several wavelengths concurrently; this is possible only by applying a multiple wavelength scanning program. If present in adequate quantity, UV detector assures all the UV-absorbing components are detected.

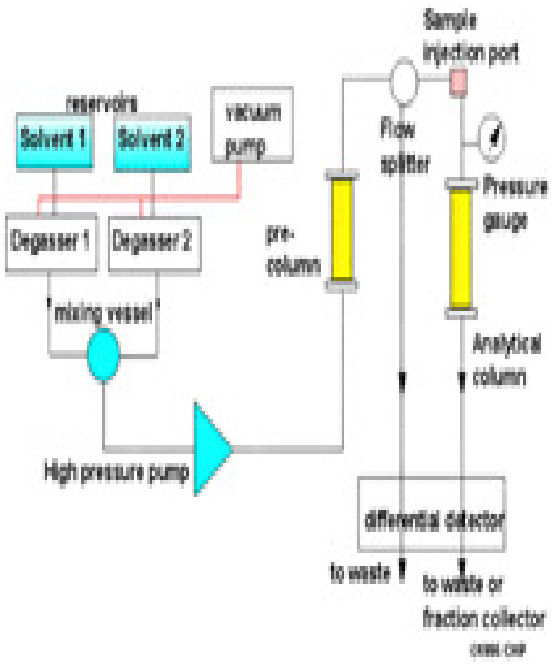


Figure 1: Schematic Representation of HPLC

Figure No. 03: Schematic Representation of HPLC

A photodiode array (PDA) is a lined array of discrete photodiodes on an integrated circuit (IC) chip for spectroscopy. It is placed at the image plane of a spectrometer to allow a range of wavelengths to be sensed concurrently [12]. When a variable wavelength detector (VWD) is used a sample must be injected numerous times, with changing wavelength, to be sure that all the peaks are detected. In the case of PDA, when it is used a wavelength range can be programmed and all the compounds that absorb within this range can be identified in a single analysis. PDA detector can also analyze peak purity by matching spectra within a peak. PDA detector finds its application in the method development of Iloperidone in pharmaceuticals [11]. Detectors A detector in HPLC is placed at the end of the system. Its work is to analyse the solution which is eluting from the column. The concentration of individual component of the analyte is proportional to the electronic signal coming out of the component of the mixture.

Types of Detectors

Detectors are broadly classified into two groups:

Bulk Property Detectors: Bulk property detectors are those that measure the changes in solute and mobile phase in combination. Such detectors show fluctuation in readings even with slight change in mobile phase combination. Examples are: refractive index and conductivity detectors. Due to poor sensitivity and limited range, they are used less despite being universally applicable. In general they are called as non selective detectors because they react to the bulk property of the analyte. Solute Property Detectors: Solute property detectors are also called as selective detectors because they give response for a particular physical or chemical property of the analyte, being ideally independent of the mobile phase. Practically, it is not possible to achieve complete independence from mobile phase but the signal discrimination usually makes it sufficient to work with solvent changes as in gradient elution [15].

Bulk Property Detectors

Electrical Conductivity HPLC Detectors: These detectors senses all the ions, whether they are from a solute, or from the mobile phase. It measures the conductivity of mobile phase along with the solute which needs to be backed-off by suitable electronic adjustments. Thus it is a type of Electrical Conductivity Detector.

Refractive index detectors:

Christiansen effect detector, interferometer detector, thermal lens detector and the dielectric constant detector. They are mostly used for detection of non-ionic compounds that neither fluoresce nor absorb in the UV region. They face the drawback of being less sensitive, need of temperature control and less suitability to gradient elution.

Electrochemical HPLC Detectors:

They are termed as “Electrochemical detectors” for the reason being that they usually measure the current associated with the oxidation or reduction of solutes. They act as amperometer or coulometer in HPLC. They are classified as equilibrium and dynamic detectors. The suitability of these detectors depends on the volumetric characteristics of the solute molecules in the aqueous or organic mobile phase. They are sensitive to changes in the flow rate or composition of the eluent and require working electrode, reference electrode and auxiliary electrode [16]. Light Scattering HPLC Detectors: Light scattering HPLC

detectors are useful for large molecular weight molecules like surfactants, lipids and sugar. It measures the scattered light coming out of the eluent. Low angle laser light scattering detector and the multiple angle laser light scattering detectors are the two types of Light scattering detectors available. They are also called as Evaporative light scattering detector because in this the beam of light by particles of compound remaining after evaporation of the mobile phase. The importance of such type of detector is growing with time because it acts as universal detector and does not require a compound to have a chromophore for the detection.

Solute Property Detectors Ultraviolet/Visible Detectors:

The most common HPLC detectors used are UV detectors because of the fact that most of the compounds absorb in UV or visible region. They give specific response to the class of compounds or particular compounds depending upon the functional group of eluting molecules. The basis of working for optical detectors is the change in intensity when a beam of electromagnetic radiation passes through the detector flow cell. These detectors are of three different types: Fixed wave length detectors, variable wavelength detectors and diode array detectors. Fixed Wave Length [22].

Detectors: Such type of detectors does not allow change in wavelength of the radiation. They are difficult to find nowadays due to limitation of working wavelength. Low pressure mercury lamp is used for very intensive light 254nm.

Variable Wavelength Detectors: Variable wavelength detectors can be adjusted to work on any wavelength over full UV- visible region. The wavelength can be selected at 3nm or less.

Diode Array Detectors: In diode array detector, the sample is subjected to light of all wavelengths generated by the lamp at once. The lights from emission source when collimated by an achromatic lens system, the total light passes through the detector cell onto a holographic grating and then falls onto a diode array which contains 100s of diodes. The chromatogram can be produced using the UV wavelength that was falling on that particular diode at the end of the run. DAD helps to see the response of the analyte at different wavelengths in only single run and thus saves time and energy [23].

Fluorescence HPLC Detectors

Fluorescent detectors are the most selective, sensitive and specific than all other HPLC detectors. Specific wavelength is used to excite and then emit light signal in analyte atoms. They intensity of light is monitored continuously to quantify the analyte concentration. Fluorescence is 10-1000 times sensitive than UV detector for strong UV absorbing compounds. Even a single analyte in the cell can be detected by the fluorescence detector. For some compounds which do not have fluorescence absorbance or low absorbance, they can be treated with fluorescence derivatives such as dansylchloride. The system is easy to operate and relatively stable.

There are three types of Fluorescence detector:

Single Wavelength Excitation Fluorescence Detector: The wavelength of excitation is fixed in such type of detectors and they are made from low pressure mercury lamp.

Multi Wavelength Fluorescence Detector: It contains two monochromators to select wavelength of excitation.

Laser Induced Fluorescence Detector (LIFDs): It shows optical emission from molecules that have excited to higher energy levels by absorption of electromagnetic radiations. It is used as separating tool for polymerase chain reactions, determination of solutes like proteins, nucleic acids, polycyclic aromatic hydrocarbons and toxic elements like cyanide [32].

Mass Spectrometric HPLC Detectors:

The detection by mass spectrometers is based on molecular fragmentation by electric fields and separation is based on the mass to charge ration of the fragmented molecule. They show high selectivity and sensitivity and are used in LC-MS technique for advantage in terms of resolution and sensitivity. The analyte for MSD must be in ionic form.

Properties of Mass spectrometric detectors

1. Co-eluting peaks which are not separated chromatographically can be isolated and separated on the basis of their mass.

2. The molecular mass of unknown molecules can be known.
3. It is fast detection method without the need of retention time validation.
4. It is quantitative as well as qualitative analysis.

Infrared Detector

Infrared detectors are chosen on the basis of property of compound to absorb infrared light i.e. it falls in the region of 4000-690 cm-1. It is a sophisticated instrument and requires Cells or windows made of NaCl or CaF2 and the wavelength scanning is provided by semicircular filter wedges. The detector is suitable for polymer analysis as it differentiate the secondary structure [25].

Other Popular Detectors Used In HPLC:

1. Transport Detectors: The transport detectors have a carrier like metal chain, wire or disc which passes continually through column eluting the analyte of interest from the mobile phase. The solute is adhered on the surface as thin film whereas the mobile phase evaporates. Moving wire and Chain detectors are the two types of transport detectors available for HPLC system.
2. Chiral Detectors: There are compounds and drugs that exist in their enantiomeric form but may have different pharmacological properties. The separation of these enantiomers is possible by the detectors capable of responding to the different chiral forms. They are used for the optically active compounds like amino acids, terpenes and sugars and other compounds. Polarimetry or Optical rotatory dispersion (ORD) and Circular dichroism (CD) are two techniques of chiral detection. ORD detectors are based on differences in refractive index whereas CD detectors differentiate enantiomers by measuring differences between the absorption of right and left handed circularly polarised lights [40].
3. Corona Discharge Detectors: Corona charged aerosol detection (CAD), sometimes referred to as corona discharge detection (CDD) is a newer and unique technique. In this technique, the HPLC column eluent is first nebulized with a nitrogen (or air) carrier gas to form droplets which are then dried to remove mobile phase, producing analyte particles. The principal of working of Corona discharge detectors is the charge transfer diffusionally to the opposing stream of analyte particles when

primary stream of analyte particles is met by a secondary stream of nitrogen (or air). The stream of nitrogen is positively charged and produces required high –voltage in the platinum corona wire for diffusion to take place. The analytes then are further transferred to a collector where it is measured by a highly sensitive electrometer, generating a signal in direct proportion to the quantity of analyte present [7].

Features of Detectors Used In HPLC

1. It must show response for all the components in the mixture.
2. It must show a linear response to the concentration of the analyte.
3. Temperature variation must not affect the response.
4. It must be independent of eluent composition (gradient).
5. It must be capable of tracing even lower concentrations.
6. The peaks must not be widened.
7. It must produce stable.

Spectroscopic techniques

Spectrophotometry

Another important group of methods which find an important place in pharmacopoeias are spectrophotometric methods based on natural UV absorption and chemical reactions. Spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. The advantages of these methods are low time and labor consumption. The precision of these methods is also excellent. The use of UV–Vis spectrophotometry especially applied in the analysis of pharmaceutical dosage form has increased rapidly over the last few years. The colorimetric methods are usually based on the following aspects:

1. Complex-formation reaction.
2. Oxidation-reduction process.
3. A catalytic effect.

It is important to mention that colorimetric methods are regularly used for the assay of bulk materials. For example, the blue tetrazolium assay is used for the determination of

corticosteroid drug formulations. The colorimetric method is also exploited for the determination of cardiac glycosides and is presented in European Pharmacopoeia. Several approaches using spectrophotometry for determination of active pharmaceutical ingredients in bulk drug and formulations have been reported and details of these methods are recorded in table. Derivative spectroscopy uses first or upper derivatives of absorbance with respect to wavelength for qualitative investigation and estimation. The concept of derivatizing spectral data was first offered in the 1950s, when it was shown to have many advantages. However, the technique received little consideration primarily due to the complexity of generating derivative spectra using early UV–Visible spectrophotometers. The introduction of microcomputers in the late 1970s made it generally convincing to use mathematical methods to generate derivative spectra quickly, easily and reproducibly. This significantly increased the use of the derivative technique. The derivative method has found its applications not only in UV-spectrophotometry but also in infrared, atomic absorption, fluorescence spectrometry, and fluorimetry. The use of derivative spectrometry is not restricted to special cases, but may be of advantage whenever quantitative study of normal spectra is problematic. Disadvantage is also associated with derivative methods; the differential degrades the signal-to-noise ratio, so that some form of smoothing is required in conjunction with differentiation.

Near infrared spectroscopy (NIRS)

Near infrared spectroscopy (NIRS) is a rapid and non-destructive procedure that provides multi component analysis of al- most any matrix. In recent years, NIR spectroscopy has gained a wide appreciation within the pharmaceutical industry for raw material testing, product quality control and process monitoring. The growing pharmaceutical interest in NIR spectroscopy is probably a direct consequence of its major advantages over other analytical techniques, namely, an easy sample preparation without any pretreatments, the probability of separating the sample measurement position by use of fiber optic probes, and the expectation of chemical and physical sample parameters from one single spectrum. The major pharmacopoeias have generally adopted NIR techniques. The European Pharmacopoeia in chapter 2.2.40and United States pharmacopoeias (\chapter 1119 United States Pharmacopoeia USP 26 NF 21, 2003 address the suitability of NIR instrumentation for application in pharmaceutical testing. NIR spectroscopy in combination with multivariate

data analysis opens many interesting perceptions in pharmaceutical analysis, both qualitatively and quantitatively [21]. A number of publications describing quantitative NIR measurements of active ingredient in intact tablets have been reported. In addition to the research articles many review articles have been published citing the application of the NIRS in pharmaceutical analysis.

Reversed Phase Chromatography

Reversed phase chromatography has found both analytical and preparative applications in the area of biochemical separation and purification. Molecules that possess some degree of hydrophobic character can be separated by reversed phase chromatography with excellent recovery and resolution. The separation mechanism in reversed phase chromatography depends on the hydrophobic binding interaction between the solute molecule in the mobile phase and the immobilised hydrophobic ligand, i.e. the stationary phase.[8] The actual nature of the hydrophobic binding interaction itself is a matter of heated debate but the conventional wisdom assumes the binding interaction to be the result of a favourable entropy effect. The initial mobile phase binding conditions used in reversed phase chromatography are primarily aqueous which indicates a high degree of organised water structure surrounding both the solute molecule and the immobilised ligand. As solute binds to the immobilised hydrophobic ligand, the hydrophobic area exposed to the solvent is minimised. Therefore, the degree of organised water structure is diminished with a corresponding favourable increase in system entropy. In this way, it is advantageous from an energy point of view for the hydrophobic moieties, i.e. solute and ligand, to associate. [9, 10]. Water adjacent to hydrophobic regions is postulated to be more highly ordered than the bulk water. Part of this ‘structured’ water is displaced when the hydrophobic regions interact leading to an increase in the overall entropy of the system. Separations in reversed phase chromatography depend on the reversible adsorption/desorption of solute molecules with varying degrees of hydrophobicity to a hydrophobic stationary phase.

Choice of Separation Medium

The proper choice of reversed phase medium is critical for the success of a particular application. This choice should be based on the following criteria:

1. The unique requirements of the application, including scale and mobile phase conditions.
2. The molecular weight or size of the sample components.
3. The hydrophobicities of the sample components.
4. The class of sample components.

Sample collection and preparation

The sample should ideally be dissolved in the initial mobile phase. If this is not possible due to stability or solubility problems, formic acid, acetic acid or salt can be added to the sample to increase solubility. These additives do not usually effect the separation so long as the volume of the sample loaded is small compared to the column volume. The only effect when large sample volumes are applied may be an extra peak or two eluting in the void volume after sample injection [24]. Sample preparation is an essential part of HPLC analysis, intended to provide a reproducible and homogenous solution that is suitable for injection onto the column. The aim of sample preparation is a sample aliquot that, Is relatively free of interferences, Will not damage the column, and Is compatible with the intended HPLC method that is, the sample solvent will dissolve in the mobile phase without affecting sample retention or resolution [11].

Detectors

A large numbers of detectors are used for RP-HPLC analysis. However, among these the five dominant detectors used in LC analysis are the electrical conductivity detector, the fluorescence detector, the refractive index detector, mass spectrometry detector and the UV detector (fixed and variable wavelength). These detectors are employed in over 95% of all LC analytical applications [12,13]. The detector selected should be chosen depending upon some characteristic property of the analyte like UV absorbance, fluorescence, conductance, oxidation, reduction, etc. Characteristics that are to be fulfilled by a detector to be used in HPLC determination are:

1. High sensitivity, facilitating trace analysis
2. Negligible baseline noise to facilitate lower detection
3. Low drift and noise level

4. Wide linear dynamic range (this simplifies quantitation)
5. Low dead volume (minimal peak broadening)
6. Cell design that eliminates remixing of the separated bands
7. Insensitivity to changes in type of solvent, flow rate, and temperature
8. Operational simplicity and reliability
9. Tunability, so that detection can be optimized for different compounds
10. Large linear dynamic range
11. Non destructive to sample

Applications

1. Designing a biochemical purification
2. Purification of platelet-derived growth factor (PDGF)
3. Purification of cholecystokinin-58 (CCK-58) from pig intestine
4. Purification of recombinant human epidermal growth factor
5. Process purification of inclusion bodies

Normal Phase Chromatography

In normal phase chromatography, the stationary phase is a polar adsorbent and the mobile phase is generally a mixture of non-aqueous solvents. The silica structure is saturated with silanol groups at the end. These OH groups are statistically disturbed over the whole of the surface. The silanol groups represent the active sites (very polar) in the stationary phase. This forms a weak type of bond with any molecule in the vicinity when any of the following interactions are present [27]

1. Dipole-induced dipole
2. Dipole-dipole
3. Hydrogen bonding
4. π-Complex bonding

These situations arise when the molecule has one or several atoms with lone pair electron or a double bond. The adsorption strengths and hence k' values (elution series) increase in the following order. Saturated hydrocarbon < olefins < aromatics < organic halogen compounds <sulphides< ethers < esters < aldehydes and ketones < amines <sulphones<

amides < carboxylic acids. The strength of interactions depends not only on the functional groups in the sample molecule but also on steric factors. If a molecule has several functional groups, then the most polar one determines the reaction properties. Chemically modified silica, such as the aminopropyl, cyanopropyl and diol phases is useful alternatives to silica gel as stationary phase in normal phase chromatography.The aminopropyl and cyanopropyl phases provide opportunities for specific interactions between the analyte and the stationary phases and thus offer additional options for the optimizations of separations. Other advantages of bonded phases lie in their increased homogeneity of the phase surface [28, 29]. Resolution with water in weak mobile phase may be most conveniently achieved by drying the solvents and then adding a constant concentration of water or some very polar modifier such as acetic acid or triethylamine (TEA) to the mobile phase. The addition of such polar modifiers serves to deactivate the more polar shape as well as the reproducibility of the retention times.

Gas Chromatography

Moving ahead with another chromatographic technique, gas chromatography is a powerful separation technique for detection of volatile organic compounds. Combining separation and on-line detection allows accurate quantitative determination of complex mixtures, including traces of compounds down to parts per trillion in some specific cases. Gas liquid chromatography commands a substantial role in the analysis of pharmaceutical product. The creation of high-molecular mass products such as polypeptides, or thermally unstable antibiotics confines the scope of this technique. Its main constraint rests in the comparative non-volatility of the drug substances therefore, derivatization is virtually compulsory. Recently, gas chromatography has been used for as- say of drugs such as isotretinion , cocaine and employed in the determination of resid- ual solvents in betamethasone valerate [33]. Gas chromatography is also an important tool for analysis of impurities of pharmaceuticals. In recent years GC has been applied to estimate the process related impuri- ties of the pharmaceuticals, residual solvents listed as impurity by the International Conference of Harmonization are analyzed by the GC using a variety of detectors. A gas chromatograph (GC) is an analytical instrument that is utilized to gauge the substance of various segments in a sample. The investigation performed by a gas chromatograph is gas chromatography. Gas chromatography (GC) is a common kind of chromatography used as a

piece of analytical science for segregating and investigating exacerbates that can be vaporized without disintegration. Regular employments of GC are trying the immaculateness of a particular substance, or separating of the distinctive segments of a blend. In a couple of circumstances, GC may help in recognizing a compound. In preparative chromatography, GC can be used to get ready pure compound from a blend. Gas chromatography principle: The specimen arrangement is mixed into the instrument enters a gas stream which transports the sample into a division tube known as the "column." (Helium or nitrogen is used as carrier gas.) The distinctive parts are secluded inside the section. The identifier measures the measure of the part that leaves the section. To quantify an example with an obscure focus, a standard specimen with known concentration is mixed into the instrument. The standard sample top maintenance time (Retention time) and region are contrasted with the test to ascertain the grouping of the obscure sample. A gas chromatograph is a Chemical analysis instrument for isolating chemicals in an complex sample mixture [34]. A gas chromatograph utilizes a course through slender tube known as the column, through which distinctive Chemical constituents of a sample go in a gas stream (transporter gas, portable stage) at various rates relying upon their different chemicals and physical properties and their interaction with a particular column filling, called as stationary phase. As the chemicals leave the end of the column, they are detected and analyzed electronically. The capacity of the stationary stage in the column is to isolate various different components, causing on every component to leave the segment at an different time (retention time). Different parameters that can be utilized to change the order or time of retention are the flow rate of carrier gas, length of column and the temperature [15-19]. In a Gas chromatography analysis, a specific known volume of vaporous or fluid analyte is infused into the "entrance" (head) of the column, usually utilizing a microsyringe. As the carrier gas clears the analyte particles through the column, this development is hindered by the adsorption of the analyte atoms either onto the segment depends or onto pressing materials in the segment. The rate at which the particles progress along the segment depends on upon the quality of adsorption, which in this manner depends on upon the kind of atom and on the stationary stage materials. Since each sort of particle has an alternate rate of development, the distinctive segments of the analyte mix are separated as they advance along the section and achieve the end of the segment at different times (retention time). An indicator is used to moniter the outlet stream from the segment; in this way, the

time at which each part achieves the outlet and the measure of that segment can be resolved. As a rule, substances are recognized by the request in which they rise (elute) from the area and by the retention time of the analyte in the section.

Physical Components of Gas Chromatography

- Autosamplers
- Inlets
- Detectors Autosamplers

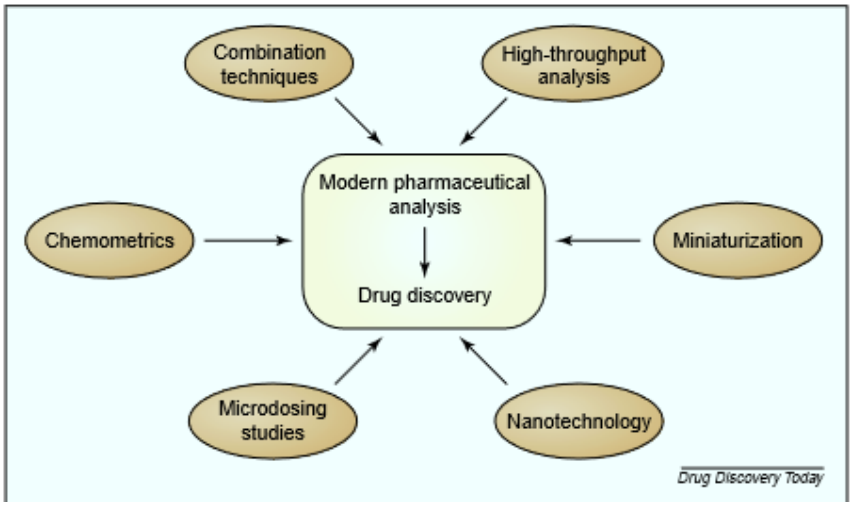
The auto sampler gives the way to bring a sample automatically into the channels. Manual insertion of the sample is possible but no more common. Programmed insertion gives good reproducibility and time-improvement. Inlets the column inlet (or injector) gives the way to bring a sample into a continuous stream of carrier gas. The inlet is a piece of equipment appended to the column head. The common inlet sorts are: S/SL (split/splitless) injector, on-column inlet, PTV injector, and Gas source inlet or gas switching valve, P/T (Purge-and-Trap) system. The decision of carrier gas (portable stage) is very important. The carrier gas must be chemically inert. Generally utilized gasses include nitrogen, helium, argon, and carbon dioxide. The decision of carrier gas is regularly depend upon the sort of indicator which is utilized. The carrier gas framework likewise contains an molecular sieve to expel water and different other impurities. So, helium might be more efficient and give the best separation if flow rates are optimized. Helium is non-combustible and works with a more prominent number of detectors [20]. Thus, helium is the most well-known carrier gas utilized. In any case, the cost of helium has gone up significantly over recent years, causing an expanding number of chromatographers to change to hydrogen gas detectors. There are numerous detectors which can be utilized as a part of gas chromatography. Distinctive detectors will give different sorts of selectivity. A non- selective detector reacts to all mixes aside from the carrier gas, a particular indicator reacts to a range of compounds with a typical physical or chemical property and a particular detector reacts to a one chemical compound. Detectors can likewise be gathered into concentration dependant detectors and mass flow dependant detectors. The sign from a concentration dependant detector is identified with the grouping of solute [21, 22] in the detector, and does not generally crush the sample Dilution of with make-up gas will bring down the detectors reaction. Mass flow dependant detectors

ordinarily decimate the sample, and the sign is identified with the rate at which solute particles enter the detector. The reaction of a mass flow dependant detector is unaffected by make-up gas. Various types of detectors used in GC are:

1. Mass Spectrometer (GC/MS)
2. Flame Ionization Detector (FID)
3. Thermal Conductivity Detector (TCD)
4. Electron Capture Detector (ECD)
5. Nitrogen-phosphorus
6. Flame photometric (FPD)
7. Photo-ionization (PID)

Mass spectrometer (GC/MS) Numerous GC instruments are combined with a mass spectrometer, which is a very good blend. The GC isolates the compounds from each other, while the mass spectrometer distinguishes them in view of their fragmentation pattern. Flame ionization detector (FID) This detector is extremely sensitive towards organic atoms (10-12 g/s = 1 pg/s, linear range: 106-107), yet relative insensitive for a couple of small molecules i.e., N2, NOx, H2S, CO, CO2, H2O. In the event that appropriate measures of hydrogen/air are blended, the burning does not bear the cost of any or not very many particles bringing about a low background signal. In the event that other carbon containing compounds are introduced with this stream, cations will be created in the profluent stream. The more carbon atoms are in the molecule, the more fragments are framed and the more delicate the detector is for this compound. Unfortunately, there is no relationship between the number of carbon molecules and the size of the signal. Subsequently, the individual reaction components for every compound must be experimentally decided for every instrument. Because of the fact that the sample is burnt (pyrolysis), this procedure is not appropriate for preparative GC. Furthermore, a few gasses are typically required to work a FID: hydrogen, oxygen (or compressed air), and a carrier gas [35]. Thermal conductivity detector Thermal Conductivity Detector is less sensitive than the FID (10-5-10-6 g/s, straight range: 103-104), yet is fitting for preparative applications, in light of the way that the example is not annihilated. The acknowledgment relies on upon the relationship between the two gas streams, one containing only the carrier gas, the other one containing the transporter gas and the compound. Really, a carrier gas with a high warm conductivity i.e.,

helium or hydrogen is used to amplify the temperature distinction (and along these lines the distinction in resistance) between two fibers (=thin tungsten wires). The broad surface-to-mass extent permits a fast equilibration to a relentless state. The temperature distinction between the reference and the specimen cell fibers is seen by a Wheatstone bridge circuit [74-85], Electron capture detector (ECD). This detector comprises of a depression that contains two terminals and a radiation source that transmits - radiation (i.e., 63Ni, 3H). The impact amongst electrons and the carrier gas (methane in addition to an inert gas) creates a plasma-containing electrons and positive ions. On the off chance that a compound is available that contains electronegative molecules, those electrons will be "caught" to frame negative particles and the rate of electron accumulation will diminish. The identifier is to a great degree particular for mixes with particles of high electron liking (10-14 g/s), yet has a generally little straight range (102-103). This indicator is every now and again utilized as a part of the investigation of chlorinated mixes i.e., pesticides (herbicides, insecticides), polychlorinated biphenyls, and so forth for which it shows a high sensitivity.[23, 24, 25, 26] Flame photometric (FPD) Flame photometric (FPD) which utilizes a photomultiplier tube to identify spectral lines of the mixes as they are burned in a fire. Compounds eluting off the column are conveyed into a hydrogen energized fire which excites particular components in the molecule, and the excited components (P, S, Halogens, and Some Metals) radiate light of particular characteristic wavelengths. The emitted light is separated and detected by a photomultiplier tube. Specifically, phosphorus emission is around 510-536 nm and sulfur discharge os at 394 nm. [27, 28, 29, 30]. Photo-ionization detector (PID) The Polyarc reactor is an additional to new or existing GC-FID instruments that progressions over each natural compound to methane atoms going before their recognition by the FID. This framework can be used to upgrade the reaction of the FID and think about the recognition of various more carbon-containing mixes [31, 32, 33, 34]. The complete change of mixes to methane and the now indistinguishable reaction in the indicator moreover it additionally disposes of the prerequisite for alignments and gauges since response variables are all equivalent to those of methane.[35, 36, 37]. This checks the fast examination of complex blends that contain atoms where standards are not open [38]. The successive reactor is sold economically as the Polyarc reactor, available online from the Activated Research Company [39, 40, 41, and 42].



Applied field of pharmaceutical Analysis

Examples of combination analytical techniques

GC–MS

HPLC–NMR

LC–MS

CE–NMR

LC/LC–TSP/MS/MS

LC–NMR–MS

HPLC–ESI–MS

GC–ECD/ICP–MS

HPLC–ICP–MS

HPIC–ICP–SFMS

CE–MS

CE–ICP–SFMS

CE–ESI–MS

CE–ICP–MS

CONCLUSION

The main objective of the pharmaceutical drugs is to cure and serve the humans and to make them free from potential illness or prevention of the diseases. For the medicine to serve its intended purpose they should be free from impurity or other inference which might harm humans. The purpose behind this review is to focusing the role of various analytical instruments in the assay of pharmaceuticals and giving a through literature survey of the instrumentation involved in pharmaceutical analysis. It also highlights the advancements of the techniques beginning from the older titrimetric method and reaching the advanced hyphenated technique stages.

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