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Review Article

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A Review - Challenges Faced By an Analyst for Developing Method for Analysis and Standardization of Drugs

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ABSTRACT

The development of the pharmaceuticals brought a revolution in human health. These pharmaceuticals would serve their intent only if they are free from impurities and are administered in an appropriate amount. To make drugs serve their purpose, various chemical and instrumental methods were developed at regular intervals, which are involved in the estimation of drugs. These pharmaceuticals may develop impurities at various stages of their development, transportation and storage, which make the pharmaceutical risky to be administered thus; they must be detected and quantitated. For this analytical instrumentation and methods play an important role. This review highlights the role of the analytical instrumentation and the analytical methods in assessing the quality of the drugs. The review highlights a variety of analytical techniques such as chromatographic, spectroscopic, photometric and thermo analytical methods that have been applied in the analysis of drugs.

Keywords: Challenges, Impurity, Problems faced, Analytical techniques, Standardization of drug

INTRODUCTION

The numbers of drugs introduced into the market are increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Very often, there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. There is a scope, therefore to develop newer analytical methods for such drugs.

Analytical methods development and validation play important roles in the discovery, development, and manufacture of pharmaceuticals. Pharmaceutical products formulated with more than one drug, typically referred to as combination products, are intended to meet previously unmet patients need analytical method development and validation by combining the therapeutic effects of two or more drugs in one product. These combination products can present challenges to the analytical chemist responsible for the development and validation of analytical methods. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency, and performance of drug products. Identification and quantification of impurities is a crucial task in

pharmaceutical process development for quality and safety. Related components are the impurities in pharmaceuticals, which are unwanted chemicals that remain with the active pharmaceutical ingredients (APIs) or develop during stability testing, or develop during formulation or upon aging of both APIs and formulated APIs to medicines. The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products. Various analytical methodologies are employed for the determination of related components in pharmaceuticals. There is a great need for development of new analytical methods for quality evaluation of new emerging the drug.⁽¹⁾

Basic criteria for new method development of drug analysis

- The drug or drug combination may not be official in any pharmacopoeias.
- A proper analytical procedure for the drug may not be available in the literature due to patent regulations.
- Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients.
- Analytical methods for the quantitation of the drug in biological fluids may not be available.
- The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.

Different analytical techniques

Sr.No.	Different analytical techniques
1.	Chromatographic techniques <ul style="list-style-type: none"> High Performance liquid chromatography (HPLC) Paper chromatography Thin Layer Chromatography (TLC) Gas Chromatography (GC)
2.	Spectroscopic method <ul style="list-style-type: none"> Infrared spectroscopy (IR) Nuclear magnetic resonance (NMR) spectroscopy
3.	Photometric techniques <ul style="list-style-type: none"> Flame Photometry.
4.	Thermoanalytical technique <ul style="list-style-type: none"> Thermogravimetric Analysis (TGA) Differential Scanning Calorimetry (DSC)

CHROMATOGRAPHIC TECHNIQUES

High Performance liquid chromatography (HPLC)

HPLC is a type of liquid chromatography (LC) which is a separation technique where analysis is separated by virtue of differing solubility's between a liquid mobile phase and a liquid or solid stationary phase. In HPLC, the mobile phase forced through a reusable column by means of a pumping system and the stationary phase is porous particles that are densely packed inside the column. The reversed phase HPLC (RP-HPLC) is the most widely used in analytical techniques in the European pharmacopoeia. It is applied for identification; test the purity of bulk drugs and for quantitative analysis of the main compounds in the samples and their related impurities. In RP-HPLC the stationary phase is a

hydrophobic legend chemically bonded onto a particulate support. RP-HPLC is generally used to separate small polar to semi-polar molecules.

Problems faced during HPLC drug analysis

- To the use of the conventional columns which have low resolution power and consume long run time (e.g. the run time was 100 min. in HPLC official method for Roxithromycin (ROX). The column is the only device in HPLC system, which actually separates an injected mixture. Column packing materials are the 'media' producing the separation and properties of this media are of primary importance for successful separations.
- The use of the gradient elution is the other reason behind the disadvantages of some official HPLC analytical methods because of its own

disadvantages as the long time needed for column equilibration.

- c) Limited choice of detectors, base-line drift on varying the eluent, lower signal-to-noise and signal-to-background ratios, spur peaks (impurities in weak eluent) and increased instrument complexity.
- d) Furthermore, some of official HPLC methods in are using sample solvent different than that which used as mobile phase, which can affects the analysis results of some drugs. Addition to unsuitable column temperature which is used in some official HPLC method (e.g. 15 C° and 60 C° for analysis of ROX and Doxycyclin (DOX) respectively).⁽²⁾

Paper Chromatography

This is probably the first and the simplest, type of chromatography that people meet. A drop of a solution of a mixture of dyes or inks is placed on a piece of chromatography paper and allowed to dry. The mixture separates as the solvent front advances past the mixture. Filter paper and blotting paper are frequently substituted for chromatography paper, if precision is not required. Separation is most efficient if the atmosphere is saturated in the solvent vapour some simple materials that can be separated by using this method are inks from fountain and fibre-tipped pens, food colourings and dyes. The components can be regenerated by dissolving them out of the cut up paper. The efficiency of the separation can be optimised by trying different solvents and this remains the way that the best solvents for industrial separations are discovered (some experience and knowledge of different solvent systems is advantageous). Paper chromatography works by the partition of solutes between water in the paper fibres (stationary phase) and the solvent (mobile phase). Common solvents that are used include pentane, propanone and ethanol. Mixtures of solvents are also used, including aqueous solutions, and solvent systems with a range of polarities can be made. A mixture useful for separating the dyes on smarties is a 3:1:1 mixture (v/v) of butan-1-ol: ethanol: ammonia solution. As each solute distributes itself (equilibrates) between the stationary and the mobile phase, the distance a solute moves is always the same fraction of the distance moved by the solvent. This fraction is variously called the retardation factor or the retention ratio and is given the symbol R or R_f . It is possible that two solutes have the same R_f values using one

solvent, but different values using another solvent (this occurs with some amino acids). This means that if a multi component system is not efficiently separated by one solvent the chromatogram can be dried and run again using a second solvent.

Problems faced during paper chromatography drug analysis

- a) Large quantity of sample cannot be applied on paper chromatography.
- b) In quantitative analysis paper chromatography is not effective.
- c) Complex mixture cannot be separated by paper chromatography.
- d) Less Accurate compared to HPLC or HPTLC.

Thin layer chromatography (TLC)

TLC is similar to paper chromatography, but the stationary phase is a thin layer of a solid such as alumina or silica supported on an inert base such as glass, aluminium foil or insoluble plastic. The mixture is 'spotted' at the bottom of the TLC plate and allowed to dry. The plate is placed in a closed vessel containing solvent (the mobile phase) so that the liquid level is below the spot. TLC has advantages over paper chromatography in that its results are more reproducible, and that separations are very efficient because of the much smaller particle size of the stationary phase. The solvent ascends the plate by capillary action, the liquid filling the spaces between the solid particles. This technique is usually done in a closed vessel to ensure that the atmosphere is saturated with solvent vapour and that evaporation from the plate is minimised before the run is complete. The plate is removed when the solvent front approaches the top of the plate and the position of the solvent front recorded before it is dried (this allows the R_f value to be calculated). TLC has applications in industry in determining the progress of a reaction by studying the components present and in separating reaction intermediates. In the latter case a line of the reaction mixture is 'painted' across the TLC plate instead of a single spot, and the line of product after separation is cut out of the plate and dissolved in an appropriate solvent. Many spots are not visible without the plates being 'developed'. This usually involves spraying with a solution that is reversibly adsorbed or reacts in some way with the solutes. Two examples of developing solutions are iodine in petroleum ether (useful for identifying aromatic compounds, especially those with electron donating

groups e.g. $C_6H_5NH_2$) and ninhydrin (useful for identifying amino acids). Iodine vapour is also used to develop plates in some cases. Alternatively, specially prepared plates can be used that fluoresce in ultraviolet light. The plates are used in the normal manner, but once dried they are placed under an ultraviolet lamp. Solute spots mask fluorescence on the surface of the plate i.e. a dark spot is observed. Some compounds have their own fluorescence, which can be used for identification or retardation factors can be used to identify known solutes.

Problems faced during TLC drug analysis

- It is dependent on another analytical technique to determine quantities of components in a mixture
- TLCs from low temperature reactions may give misleading results, when used to qualitatively monitor reactions.
- If the drug is impure, a longer TLC plate will show all the compounds in the mixture as it has more time to separate.
- A disadvantage is it will take a bit longer and contamination will cause a wrong spot to appear on the TLC plate.⁽³⁾

Gas chromatography (GC)

This technique uses a gas as the mobile phase, and the stationary phase can be either a solid or a non-volatile liquid (in which case small inert particles such as diatomaceous earth are coated with the liquid so that a large surface area exists for the solute to equilibrate with). If a solid stationary phase is used the technique is described as gas-solid adsorption chromatography, and if the stationary phase is liquid, it is called gas-liquid partition chromatography. The latter is more commonly used, but in both cases the stationary phase is held in a narrow column in an oven and the stationary phase particles are coated onto the inside of the column.

Problems faced in GC drug analysis

- The samples analysed are limited to those that are volatile or can be made volatile (reaction to form a volatile derivative)
- The samples must be thermally stable to prevent degradation when heated.
- Cannot be used to prepare samples for further analysis once separated.
- Problems can be encountered when injecting the sample.

- It is difficult to measure and inject such small samples accurately without
- Evaporation of the sample. For e.g. the rubber seal through which the sample is injected may leak leading to loss of the sample.
- Small pieces of the rubber septum may be adsorbed onto the column giving 'ghost peaks'.
- The sample may be injected directly into the heated part of the injector so vaporisation may not occur (and the sample is lost).⁽⁴⁾

SPECTROSCOPIC METHOD

Infrared (IR) spectroscopy

Infrared Spectroscopy is an analytical method that measures the absorbance of a select band of electromagnetic radiation by a sample. The wavelength of radiation that is absorbed is characteristic of different types of chemical bonds. The graphical representation of the absorption of the electromagnetic radiation by the sample makes an IR spectrum. Organic chemists use infrared spectroscopy as a means of identifying various functional groups within compounds. This information is used to determine the structure of compounds. IR is usually used in combination with other techniques, especially nuclear magnetic resonance (NMR) spectroscopy. IR detects frequencies of infrared light that are absorbed by a molecule. Molecules absorb these frequencies of light because they correspond to frequencies of vibrations of bonds in the molecule.⁽⁵⁾

Problems faced in IR spectroscopy drug analysis

- Enantiomers cannot be distinguished as their spectrums are identical.
- Extensive method development is required before technique can be used as a truly rapid analysis technique.
- Development of method requires a specialist operator with computing knowledge.
- Instruments are expensive.⁽⁶⁾

Nuclear magnetic resonance (NMR) spectroscopy

There are two general types of NMR instrument; continuous wave and Fourier transform. Early experiments were conducted with continuous wave (CW) instruments, and in 1970 the first Fourier transform (FT) instruments became available. This type now dominates the market, and currently we

know of no commercial CW instruments being manufactured now. Continuous Wave (CW) NMR instruments, Continuous wave NMR spectrometers are similar in principle to optical-scan spectrometers. The sample is held in a strong magnetic field, and the frequency of the source is slowly scanned (in some instruments, the source frequency is held constant, and the magnet field is scanned). These systems are currently obsolete except for a few wide line experiments that are performed in specialty solid-state NMR applications. Fourier Transform (FT) NMR instruments, the magnitude of the energy changes involved in NMR spectroscopy are very small. This means that, sensitivity can be a limitation when looking at very low concentrations. One way to increase sensitivity would be to record many spectra, and then add them together. As noise is random, it adds as the square root of the number of spectra recorded. For e.g. if one hundred spectra of a compound were recorded and summed, then the noise would increase by a factor of ten, but the signal would increase in magnitude by a factor of one hundred - giving a large increase in sensitivity. However, if this is done using a continuous wave instrument, the time needed to collect the spectra is very large (one scan takes two to eight minutes). In FT-NMR, all frequencies in a spectral width are irradiated simultaneously with a radio frequency pulse. A single oscillator (transmitter) is used to generate a pulse of electromagnetic radiation of frequency ω_0 , but with the pulse truncated after only a limited number of cycles (corresponding to a pulse duration τ), this pulse has simultaneous rectangular and sinusoidal characteristics. It can be proven that the frequencies contained within this pulse are within the range $\pm 1/\tau$ of the main transmitter frequency ω_0 . Following the pulse, the nuclei magnetic moments find themselves in a non-equilibrium condition having precessed away from their alignment with the applied magnetic field. They begin a process called "relaxation", by which they return to thermal equilibrium. A time domain emission signal (called a free induction decay (FID)) is recorded by the instrument as the nuclei magnetic moments relax back to equilibrium with the applied magnetic field. A frequency domain spectrum that we are familiar with is then obtained by Fourier transformation of the FID.⁽⁷⁾

Problems faced in NMR spectroscopy drug analysis

- NMR is presently relatively unstandardized on parameters such as design, slice selection techniques, R_f parameters and imaging algorithms, making for difficulties in reliability and comparison from machine to machine and centre to centre.
- Because of its newness, its interpretation is also difficult and identifying the absence and presence of pathology and nature of pathology is not always easy or possible.
- The time necessary to acquire data is greater than that in Fourier transform.
- NMR is not currently used under this condition.
- Because of lengthy data acquisition time and a narrow gantry for the head some patients experiencing the feeling of claustrophobia.
- NMR is expensive.⁽⁸⁾

PHOTOMETRIC TECHNIQUE

Flame photometry

Flame photometry relies upon the fact that the compounds of the alkali and alkaline earth metals can be thermally dissociated in a flame and that some of the atoms produced will be further excited to a higher energy level. When these atoms return to the ground state they emit radiation which lies mainly in the visible region of the spectrum. Each element will emit radiation at a wavelength specific for that element the number of atoms returning to the ground state. This is in turn proportional to the absolute quantity of the species volatilized in the flame i.e. light emitted is proportional to sample concentration. It can be seen that if the light emitted by the element at the characteristic wavelength is isolated by an optical filter and the intensity of that light measured by a photo-detector, then an electrical signal can be obtained proportional to sample concentration. Such an electrical signal can be processed and the readout obtained in an analogue or digital form.

A simple flame photometer consists of the following basic components:

- The burner:** a flame that can be maintained in a constant form and at a constant temperature.
- Nebuliser and mixing chamber:** a means of transporting a homogeneous solution into the flame at a steady rate.

- c) **Simple colour filters** (interference type): a means of isolating light of the wavelength to be measured from that of extraneous emissions.
- d) **Photo-detector**: a means of measuring the intensity of radiation emitted by the flame

Problems faced in flame photometry drug analysis

- a) The flame produced is noisy and turbulent.
- b) The readings obtained are non-reproducible since the sizes of droplets vary.
- c) Only small portion of small reaches to flame.
- d) Due to large wastage, fewer atoms are excited and consequently the emission intensity is weak. This lowers the sensitivity of the estimation.⁽⁹⁾

THERMO GRAVIMETRIC TECHNIQUE

Thermo gravimetric (TG) analysis

A technique whereby the weight of a substance, in an environment heated or cooled at a controlled rate, is recorded as a function of time or temperature. Thus, the data obtained from a TG experiment are displayed as a thermal curve with an ordinate display having units of weight (or weight per cent) and the abscissa may be in units of either temperature or time. [The abbreviation TG has been used, but should be avoided, so that it is not confused with T_g (glass transition temperature)]. Many types of materials can be characterized by techniques of thermo gravimetric and there are numerous applications of TG for materials characterization by the quantitative weight losses that occur in specified temperature regions of the TG thermal curve (see Table 1). In most TG studies, mass loss is read directly in units of weight percent of the original sample quantity.

The results from thermo gravimetric analysis may be presented by:

- (1) Mass versus temperature (or time) curves, referred to as Thermogravimetric curve
- (2) Rate of mass loss versus temperature curve, referred to as Derivative Thermogravimetric (DTG).

The results of a TG experiment may be used, in many cases, as "compositional analysis". A common example of this is the assignment of moisture content of polymers and coals. Another example would be the determination of residual solvent in many pharmaceutical compounds. The determination of ash value or ash residues also fall into this category since the remaining weight is read directly as weight or

weight percent. Also, by using the techniques of TG, can determine the purity of a mineral, inorganic compound, or organic material. TGA can be used to evaluate the thermal stability of a material. In a desired temperature range, if species is thermally stable, there will be no observed mass change. TGA also gives the upper use temperature of a material.

Problems faced in TG drug analysis

- a. Drift effect or buoyancy effect (weight gain with temperature)
- b. External vibrations
- c. External heat
- d. Thermocouple (position and decomposition)
- e. Heat of reaction
- f. Thermal conductivity
- g. Mass and packing of sample
- h. Slow recorder/readout equipment.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) is one of the thermo-analytical techniques. A calorimeter measures the heat into or out of a sample. A differential calorimeter measures the heat of sample relative to a reference. A differential scanning calorimeter does all of the above and heats the sample with a linear temperature ramp. DSC is a technique in which the difference in the amount of heat required to increase the temperature of a sample and reference are measured as function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. Generally, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. Only a few mg of material are required to run the analysis. DSC is the most often used thermal analysis method, primarily because of its speed, simplicity, and availability. It is mostly used for quantitative analysis.

Problems faced in DSC drug analysis

- a. Furnace heating rate
- b. Recording or chart speed
- c. Furnace atmosphere
- d. Geometry of sample holder/location of sensors
- e. Sensitivity of the recording system
- f. Composition of sample containers.⁽¹⁰⁾

CONCLUSION

The pharmaceuticals serve their intent only if they are free from impurities and are administered in an

appropriate amount. The pharmaceuticals may develop impurities at various stages of their development, transportation and storage, which make the pharmaceutical risky to be administered. Thus, the impurities must be detected and quantitated. For this, analytical instrumentation and methods play an important role. This review highlights the role of the

analytical instrumentation and the analytical methods in assessing the quality of the drugs. Hence, this review will help analyst to know analytical techniques such as chromatographic, spectroscopic, photometric and thermo analytical methods for method development and standardisation of drugs and the problems he may face during method development.

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