IJMPR 2019, 3(5), 15-22

International Journal of Modern Pharmaceutical Research

www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Review Article

SJIF Impact Factor: 5.273

REVIEW ON SIGNIFICANCE OF ANALYTICAL TESTS IN DRUGS AND FORMULATIONS

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Received on: 30/07/2019
Revised on: 20/08/2019
Accepted on: 10//09/2019

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ABSTRACT

Ayurvedic pharmaceutical industries that are flourishing now a day's, they are demanding the standardization at every step of pharmaceutical processing. Also for the researcher, standardisation of drugs play a very important role, as many of times we are confused that which test we have to apply and once the tests are applied then the problem of how to evaluate and interpret the result started. Many organisations and pharmacopeia are there which have set up the suitable specific standardization parameters to evaluate the crude drugs and their finished products. These include various evaluation technique such as- pharmacognostical, physicochemical, phytochemical, analytical, biological and bio technological. Analytical methods are defined as the set of techniques that allow us to know qualitatively and /or quantitatively the composition of any material and chemical state in which it's located. Nowadays, application of several modern analytical techniques has been done for evaluating the raw drugs and *ayurvedic* formulations to ensure their quality, safety and efficacy. If the raw drug is not of the standard quality the medicine would be of no use, as nowadays there are many adulterations which are done with the drugs that can detoriate the quality of our medicines, so it is very important to standardise the raw drugs also. As *ayurvedic* formulations many a times have different methods of their preparation and these medicines are again many a times prepared at the clinics by the *ayurvedic acharya* themselves. So it is a very important step to standardise these formulation so that every time we will come with a same quality of medicine. So it is very important for the *ayurvedic acharyas*, to know the importance of these tests and for which point of evaluation of drug and for which dosage form which type of test is to be done. This paper is henceforth stated to explore the significance of some of these analytical tests- why they are done, which test is to be done for which dosage form, what their result depicts, what's the importance of doing that analytical test for the herbal and mineral formulations and drugs.

KEYWORDS: *Ayurvedic* Formulations, Significance, Standardization, Analytical Techniques.

INTRODUCTION

Ayurvedic pharmaceutical industries that are flourishing now a day's, are demanding standardization at every step of pharmaceutical processing. Many organizations had set up suitable specific standardization parameters to evaluate the crude drugs and their finished products. These include various evaluation technique such as- pharmacognostical, physicochemical, phytochemical, analytical, biological and bio technological. Analytical methods are defined as the set of techniques that allow us to know qualitatively and/or quantitatively the composition of any material and chemical state in which it's located. Nowadays, application of several modern analytical techniques had been inevitable for evaluating the *ayurvedic* formulations to ensure quality, safety and efficacy. This paper is henceforth stated to tell the significance of some of those analytical test- why they are done, what their results depicts, what's the importance of doing that analytical tests for the herbal and *rasa aushadi's.*

AIM AND OBJECTIVES

Aim

To assess the importance of the analytical tests.

Objectives

1. To review about the name of the analytical methods.
2. To review about the use of the analytical methods.
3. To review about the importance of the analytical method.

Analytical Tests

The analytical test can be broadly divided into four categories such as:

A. Physical test
B. Chemical test
C. Chromatographic test
D. Spectrophotometric

Physical Test

A physical test is a qualitative or quantitative procedure that consists of determination of one or more characteristics of a given formulation or raw drug, their process or service according to a specified procedure. Often this is part of an experiment.[1] Some of the physical tests used for the standardization of drugs are:

Viscosity

Used For

Liquid Dosage form.

Definition

Viscosity is the resistance offered to the liquid to flow from one place to another i.e. capillary like syrups had viscosity more than that of the water.[2] Viscosity is the physical quantitative measure, which can be used as a measure in the standardization of sample liquid. Viscosity of one solution or preparation must remain same at a given temperature and pressure.

Significance

- Viscosity plays a very important role in the stability and;
- Pour-ability.
- As viscosity of the depression medium increases, the terminal settling velocity decreases thus the dispersed phase settle at a slower rate and they remain dispersed for longer time yielding stability to the suspensions[3] but they did not be so much viscous that its pour-ability decreases and inconvenience to the patients for dosing increases.
- To find out if any type of adulteration is done. For example- if any other oil is adulterated in the sesame oil it will change its viscosity. So from that we can find out the quality of the drugs we are using.
- And uniformity of formulations can be maintained.

Example

Avaleha, *Arishta*, Oil, *Kwatha*, etc.

Refractive index

Used for

Liquid dosage form.

Definition

The working principle of refractive index is based on the critical angle. The refractive index of a substance with reference to air is the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a beam of light passing from air into the substance.[4]

Significance

- As every medium have the different tendency of refracting back the light rays entering to it. So every different substance shows different refractive index.
- So it is very important for the adulteration and identification of the drug.
- For e.g.- as it is already known that sesame oil have the refractive index of 1.74. So when we buy sesame oil from market we will check its refractive index if it is 1.74[5]. That means it is sesame oil but if it values varies then that means some kind of adulteration is done.

Example

Arishta, *Asava*, Oil, etc.

PH

Used For

Solid and Liquid Dosage form (in solid samples we make suspension of them).

Definition

Ph value is measured by a scientific instrument called as ph meter that measures the hydrogen ion concentration in a solution indicating its acidity or alkalinity[4]. Knowledge of ph to greater or lesser accuracy is useful in many situations including accurse of chemical laboratory work.

Significance

- For stability of the substance and shelf life of the drug.[6] Suppose the ph value of the formulation changes after some time that means some detoration of the product has done.
- Physiological suitability of the substance like.[7]

✓ In absorption of drugs the drugs which have acidic ph are absorbed through the stomach and which having alkaline ph get absorbed through intestines. So we can say about the pharmacokinetic of the drug by knowing their ph value.

✓ Also one more suitable example of the ph value for the physiological suitability is for the formulations which are used externally on the skin must possess acidic ph ranging from 4-6 as that of the skin otherwise if it is alkaline it can disturbed the acidic mantle of the skin.[8]

Example

Avaleha, *Arishta*, Oil, Tablet, Ointments, etc.

Disintegration

Used for

Solid dosage form (tablets- coated, uncoated, sustained release). Chewable tablets are exempted from this test.

Definition

The disintegration test is performed to find out the time takes for a solid oral dosage form like a tablet or capsule to completely disintegrate i.e. penetration of tablet by an aqueous liquid, disintegration of internal bonds and break down of tablets. For the purposes of this test the

disintegration does not imply complete dissolution of the unit or even of its active constituent. Complete disintegration is defined as that state in which a soft mass having no palpably firm core leave no residue of the unit, except fragments of insoluble coating or capsule shell, remaining on the screen of the test apparatus or adhering to the lower surface of the discs.[9]

Significance

- It helps in optimisation of manufacturing variables such as compressional force and dwell time.
- It helps in pre-formulation stage to the formulator.
- It is simple in process control tool to ensure uniformity from batch to batch and among different tablets.

Example

Tablets, Capsules.

Friability

Used for

Solid Dosage form (Tablets).

Definition

Friability is the tendency for a tablet to chip, crumble or break following compression. This tendency is normally confined to uncoated tablets and surfaces during handling or subsequent storage. Friability testing is used to test the durability of the uncoated tablets during packing processes and transit. This involves repeatedly dropping a sample of tablets over a fixed time, using a rotating drum with a baffle. The result is inspected for the broken tablets, and the percentage of tablet mass lost through this process[10] and if it is above the limits that means they are more friable.

Significance

- Tablets need to be hard enough such that they do not break up in the bottle, but friable enough that they disintegrate in the GIT.
- For the Tablet Designing. If tablet designing is poor they get break before reaching the patient
- The binder used is sufficient or not
- Moisture content

Example

Uncoated tablets.

Tablet Hardness

Used for

Uncoated tablet.

Definition

The tablet hardness is defined as the structural integrity and the breaking point of a tablet based on their shape, chemical properties, binding agent and pressure applied during the compression force.[11] The term friable and hardness appear to be same but they are actually different- Hardness is the resistance to scratch, deformation, abrasion or cutting when pressure is applied to it and Friability is the tendency to crumble or pulvarized.[12]

Significance

- It plays a vital role in both product development and subsequent quality control.
- To determine the need for pressure adjustments on the tableting machine.
- Hardness can affect the disintegration. So, if the tablet is too hard, it may not disintegrate in the required period of time.
- And if the tablet is too soft, it will not withstand the handling during subsequent processing such as coating and packaging.
- In general if the tablet hardness is too high, we first check its disintegration before rejecting the batch. And if the disintegration is within limit, we accept the batch.

Tapped Density

Used For

Solid Dosage form.

Definition

Tapped density is achieved by mechanically tapping a measuring cylinder containing a powder sample. Tapped density is a increased bulk density attained after mechanically tapping a container containing the powder sample.[4,13]

Significance

- One important characteristics is maximum packaging density of a powder is achieved under the influence of well-defined external applied forces.
- This parameter is very important in packaging the material.
- During tapping, the sample, the smaller particles try to fit themselves in gaps of larger particles and hence the total volume of powder reduces.

Example

Powder, etc.

Specific Gravity

Used For

Liquid dosage form.

Definition

Specific gravity is the ratio of the density of a substance to the density of a reference substance; equivalently it is the ratio of the mass of a substance to the mass of a reference substance for the given volume.[4,14] Every liquid medium has different specific gravity at specific temperature and pressure.

Significance

- To know about the Adulteration.
- For the Identification of the sample because specific gravity of a substance remain same for that sample under same conditions.

- To decide the dosage of different liquid formulations that how much mass of the substance is present in that specific volume.

Example
Kwatha, Arishta, Oil, *Swarasa*, etc.

Moisture Content (Loss on Drying)
Used For
Solid dosage form.

Definition
Moisture content is defined as percentage of water content present in that drug. It is defined as the percentage of the sample's original wet weight. It is measured by using moisture analyser or drying oven by evaporating the water and then calculate the amount of moisture by weighing the amount of water evaporated from it.[14]

Significance
- Shelf Life of that drug
- For testing the Stability of the drug
- An excess of water in medicinal plant materials will encourage microbial growth, the presence of fungi or insects, and deterioration following hydrolysis. Limits for water content should therefore be set for every given plant material.
- This is especially important for materials that absorb moisture easily or deteriorate quickly in the presence of water.

Example
Crude drug, *Choorna*, Tablet.

Chemical Test
A chemical test is a qualitative or quantitative procedure designed to identify, quantify, or characterise a chemical compound or chemical group. As these tests are used for the qualitative and quantitative identification of chemical constituents they are stated under this categorie.[15]

Saponification Value
Used For
Fatty dosage form.

Definition
The saponification value is the number of mg of potassium hydroxide required to neutralize the fatty acids, resulting from the complete hydrolysis of 1g of the oil or fat.[4]

Significance
- It is a measure of the average molecular weight (or chain length) of all the fatty acids present. The smaller the molar mass of the fat higher the saponification value. So with the help of saponification value we can find out the nature of the fats present in them.
- There is one method for the soap formation in which saponification process is done for the Soap formation. In that we needed the saponification value of the fats as according to it base is added to them. If we have to use any siddha oil for the soap formation by finding its saponification value we can prepare the soap of it.
- To find out the adulteration. As mineral oils are not saponified and the vegetable and animal oil have the high saponification value. So if mineral oil is adulterated into the vegetable oil we can find out it by saponification value.[16]

Example
Sneha kalpana like- Oil, Ghee.

Rancidity
Used For
Fatty Dosage form.

Definition
Rancidity generally is the complete or incomplete oxidation or hydrolysis of fats and oils when exposed to air, light, or moisture or by bacterial action, resulting in unpleasant taste and odor.[17] The deterioration or degradation of fats is termed as 'Rancidity'. The test depends upon the formation of a red colour when oxidized fat is treated with conc. Hydrochloric acid and a solution of phloroglucinol in ether. The compound in rancid fats responsible for the colour reaction is epihydrin aldehyde. All oxidized fats respond to the kreis test and the intensity of the colour produced is roughly proportional to the degree of oxidative rancidity.[4] Among the chemical methods, Kreis test is a promising one for early detection of rancidity, particularly aldehydes with a characteristic odour impact. The colour development in the test is critical and requires optimization.

Significance
- To determine the oxidative fats in the oils and fats which are considered harmful on ingestion.
- Rancidification can also detract from the nutritional value of food, as some vitamins are sensitive to oxidation.[18]
- Shelf life.

Example
Sneha kalpana- like ghee, oil, etc.

Acid Value
Used For
Fatty dosage form.

Definition
It is defined as number of mg of Potassium hydroxide required to neutralize the free fatty acid present in oil or fat. Generally rancidity cause more liberation of free fatty acids.[4]

Significance
- To know about the rancidity. More the free fatty acid present in the sample is indicator of the rancidity of that substance.
- For the shelf life assessment.

Examples
Sneha kalpana like- Oil, Ghee.

Ash Value
Used For
Solid dosage form, Crude drugs.

Definition
The total ash method is designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself, and "non-physiological" ash which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.[4]

Then they are evaluated for Acid Insoluble Ash and Water Soluble Ash. Acid insoluble ash is the residue obtained after boiling the total ash with the dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth. Water soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water. Acid insoluble ash is the amount of the residue after treatment of the total ash with dilute hydrochloric acid.[19]

Significance
- Quality and Purity of crude drug
- Adulteration
- For the *bhasma kalpana* acid insoluble ash is less and water soluble ash is more and for crude drugs vice versa.

Example
Crude drug, Tablets, *Choorna*, *Bhasma*, etc.

Alcohol content
Used for
Alcoholic formulations.

Definition
Alcohol by volume (abbreviated as ABV, abv, or alc/vol) is a standard measure of how much alcohol (ethanol) is contained in a given volume of an alcoholic beverage (expressed as a volume percent).[20-22] It is defined as the number of millilitres (mL) of pure ethanol present in 100 mL (3.4 fl. oz) of solution at 20 °C (68 °F).

Significance
- To determine the alcohol % in some preparation.
- So that these medicines cannot be exploit by the population.
- In ayurvedic formulations this limit is set up to 5-10%.

Examples
Asava and *Arishta.*

Chromatographic Test
Chromatography is a laboratory technique which is used for the separation of a mixture. The mixture is allowed to dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture have different speeds of traveling, causing them to separate. The separation is based on this differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient thus result in differential retention on the stationary phase and thus affect the separation.[23]

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for the later use, and is thus a form of purification. Analytical chromatography is done normally with the smaller amounts of material and is for establishing the presence or measuring the relative proportions of the analytes in a mixture. The two are not mutually exclusive.[24]

TLC
Used For
Non-volatile Solid and Liquid samples.

Definition
Thin-layer chromatography (TLC) is a chromatography technique used to separate non-volatile mixtures.[25] Chromatography works on the principle that different compounds will have different solubility and adsorption to the two phases between which they are to be partitioned. Thin Layer Chromatography (TLC) is a solid-liquid technique in which the two phases are a solid (stationery phase) and liquid (moving phase).

Significance
- TLC is utilised in identification of drugs, their adulteration and their chemical constituents.
- TLC is a method for analysing mixtures by separating the compounds in the mixture.
- TLC can be used to help determine the number of components in a mixture.
- The identity of compounds and;
- The purity of a compound.

Example
Crude drug, any powder, etc

HPTLC
Used For
Non-volatile Solid and liquid samples.

Definition
HPTLC (Higher Performance Thin Layer Chromatography) is an enhanced form of thin layer chromatography. A number of enhancements can be

made to the basic method of thin layer chromatography to automate the different steps to increase the resolution achieved and to allow more accurate quantitative measurements.[26]

Significance

HPTLC rapidly gaining importance in several fields of science like-

- Pharmaceutical analysis
- Biochemistry
- Pharmacokinetic studies
- Quantitative measurements of the compounds
- It is a both qualitative and quantitative test.

Example

Raw drug, Powder, etc.

GC

Used For

Volatile, Nonpolar substance.

Definition

GC is a common type of chromatography used in analytical chemistry for separating and analysing compounds that can be vaporized without decomposition. Separation of the components are according to their boiling point. By comparing the retention time of the peak we can conclude for the identification of unknown compounds.[27] In combination with MS we can identify the peak compound.

Qualatative Analysis

Generally chromatographic data is presented as a graph of detector response (y-axis) against retention time (x-axis), which is called a chromatogram. This provides a spectrum of peaks for a sample representing the analysts present in a sample eluting from the column at different times. Retention time can be used to identify analysts if the method conditions are constant. Also, the pattern of peaks will be constant for a sample under constant conditions and can identify complex mixtures of analysts. However, in most modern applications, the GC is connected to a mass spectrometer or similar detector that is capable of identifying the analysts represented by the peaks.

Quantative Analysis

The area under a peak is proportional to the amount of analysts present in the chromatogram. By calculating the area of the peak using the mathematical function of integration, the concentration of an analysts in the original sample can be determined. Concentration can be calculated using a calibration curve created by finding the response for a series of concentrations of analysts, or by determining the relative response factor of an analysts. The relative response factor is the expected ratio of an analysts to an internal standard (or external standard) and is calculated by finding the response of a known amount of analysts and a constant amount of internal standard (a chemical added to the sample at a constant concentration, with a distinct retention time to the analysts). In most modern GC-MS systems, computer software is used to draw and integrate peaks, and match MS spectra to library spectra.

Significance

- GC helps in identifying a compound.
- Testing of the purity of a particular substance, or;
- Separating the different components of a mixture (the relative amounts of such components can also be determined).
- GC is a physical separation method in where volatile mixtures are separated.

Example

For non-polar molecules like- oil, fats.

Spectrometric Test

A spectrometer is a scientific instrument used to separate and measure spectral components of a physical phenomenon. Spectrometer is a broad term often used to describe instruments that measure a continuous variable of a phenomenon where the spectral components are somehow mixed.[28]

MS

Used For

Solid and Liquid sample.

Definition

Mass spectrometry (MS) is an analytical method of characterizing matter, based on the determination of molecular masses of individual species present in a sample The results of MS is displayed as a graph called fragmentation spectrum and presented in the form of the abundances of the ions formed and classed in increasing order of their nominal mass as close to their exact masses. Operating under identical conditions, the fragmentation is reproducible and therefore is a means of characterizing the studied compound.[29]

Significance

- MS has both qualitative and quantitative uses.
- These include identifying unknown compounds, determining the isotopic composition of elements in a molecule, and determining the structure of a compound by observing its fragmentation.
- Also we can quantify the amount of a compound in a sample.
- Pharmacokinetics is often studied using mass spectrometry because of the complex nature of the matrix.
- Several software packages are used to conduct searches in spectral libraries in which the main peaks of known compounds are encoded.

Example

Any unknown powder, etc.

XRD

Used For

Fluid, Powder and Crystals.

Definition

XRD is analytical technique used for the identification of crystalline phases of various materials and the quantitative phase analysis subsequent to the identification. XRD is one of the most important non-destructive tools to analyse all kinds of matter-ranging from fluids, to powder and crystals.[30]

Significance

- XRD techniques are used for the identification of crystalline phases of various materials and the quantitative phase analysis subsequent to the identification.
- XRD techniques are superior in elucidating the three dimensional atomic structure of crystalline solids.
- XRD is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimension.
- It is a powerful and rapid (<20 min) technique for identification of an unknown mineral. They have certain limitations like-

✓ Homogeneous and single phase material is best for identification of unknown.
✓ Must have access to a standard reference file of inorganic compounds.
✓ Requires tenths of a gram of a material which must be ground into a powder.
✓ For mixed materials, detection limit is~2% of sample.
✓ For unit cell determination, indexing of patterns for non-isometric crystals systems is complicated.
✓ Peak overlay may occur and worsen for high.

Examples

Kajjali and *Rasa parpati* comparison, etc. as in this the compound may e same ut the crystalline structure may be different so we need to do XRD.

XRF

***Used* For**

Fluids, Powder, and Crystals.

Definition

XRF is the emission of characteristic "secondary" (or fluorescent) X-rays from a material that has been excited by bombarding with high energy X-rays or gamma rays. XRF analysers determine the chemistry of a sample by measuring the fluorescent (or secondary) X ray emitted from a sample when it is excited by a primary X-ray source. Each of the elements present in a sample procedures a set of characteristics fluorescent X rays that is unique for that specific element.[31]

Significance

- The phenomenon is widely used for elemental analysis and chemical analysis.
- XRF Spectroscopy is an excellent technology for qualitative and quantitative analysis of material composition.
- XRF is particularly well suited for investigations that involve- bulk chemical analyses of major and race elements.
- XRF also have some limitations like-

✓ They cannot be able to distinguish variations among isotopes of an elements, so these are done with other instrument.
✓ In practise, most commercially available instruments are very limited in their ability to precisely and accurately measure the abundances of elements with Z<11.

Examples

Powders and Pellets of the same ingredients, etc., in this XRF is done as there would be no change in the crystalline structure.

CONCLUSION

1. Each test has its own significance in the standardization of drugs.
2. They have both pre formulations and post formulations importance.
3. According to the need and requirement what we want to identify from the study the type of analytical test should be decided.
4. Each formulation should be tried to standardize for the uniformity of them.

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