PHARMACOGNOSY AND PHYTOCHEMISTRY

Dr. Galvina Pereira

Department of Pharmaceutical Sciences and Technology

Institute of Chemical Technology, Mumbai

Week 10

Lecture48

Week 10: Lecture 48: Physical Methods of Quality Control of Herbal Drugs (Part 1)

Hello everyone, and welcome to session 3 of the NPTEL course in Pharmacognosy and Phytochemistry. This week, we are delving into the quality control of herbal drugs. Previously, in the first two sessions, we have seen what quality control is, as well as the different types of quality control. The quality control methods, depending on their output, can be divided into qualitative methods and quantitative methods.

The qualitative methods are more of a yes-no approach. Is the compound present? Or not? Is a compound present or not? Is the drug adulterated or not?

For example, is the sample given to you genuine or not? Is it authentic or not? Whereas quantitative methods deal more with ranges or numerical values. If a compound is present in a plant, how much of it is present? If a leaf is given, what is the range of stomatal values?

These are more numeric values. Qualitative will tell you what it is. Quantitative will tell you how much it is. Now, depending upon the nature, your quality control methods are classified into different types. And in the previous session, we have already covered organoleptic methods, macroscopic methods, as well as microscopic methods of drug analysis.

In this particular session, what we are going to see are more physical methods of drug evaluation. Now, physical methods of drug evaluation are actually the methods which use the physical attributes of the drug. This could be their moisture content. This could be their state. This could be their melting point.

So let us see what values or what quality control tests we see when I say we are doing the physical methods of evaluation. The physical methods of evaluation, as per the official books, include loss on drying, extractive values, ash values, foreign organic matter, foaming index, solubility, refractive index, viscosity, optical activity, and melting, boiling, as well as congealing point. So let's start understanding each of these methods a little bit in depth and how these methods of evaluation help us in assessing the quality of drugs. So we first move to a loss on drying. Now, this is a very simple method.

This method simply means that whenever you take a drug and dry it, how much weight of the drug or what percentage of the drug's weight is evaporated or lost. Now, this method is generally used for the quantification of moisture because moisture is an inevitable part of herbal drugs. Whenever you buy a fresh drug from the market, it is bound to have some moisture. It is only after drying this A drug becomes a little stiff and more storable or increases its shelf life.

When you get this drug from the market, it is fresher. It is richer in moisture, and the chances of deterioration are high because of this moisture content. Whereas, when dried, the moisture content is low. Take, for example, most of the spices we use in the kitchen; they are dry. So once the moisture content is reduced, what you observe is that their shelf life increases because, in that case, the enzymes, microbial activity, and all other activities that lead to deterioration significantly decrease. So moisture content is an important value that needs to be determined, especially for drugs such as digitalis and rauwolfia.

and drugs that deteriorate due to enzymatic activity. Do you recall your glycosides? When your emulsin, amylase, or other enzymes are active, water provides a medium for them to facilitate these reactions, and as a result, the deterioration process continues. But once you dry the drug, since moisture is absent, your enzymes in the dry state do not function well. At the same time, reactions in the dry state are very slow.

And as a result, because of that, your rate of deterioration also decreases. So most of the drugs are dried and then kept in the stores. So even in the pharmaceutical industry, when you are trying to procure a raw herbal material, you will get it in the dried form. Now, once you get this herbal material in the dried form, we do a quality control test to ensure how

much moisture is left or how much moisture it is still bound to it. So in order to ensure that or in order to check that we do what is called as loss on drying.

In addition to moisture loss on drying also accounts for solvents which are there certain times take for example extraction of oil. The last batch of castor oil or sometimes even your oils are extracted using solvent and later on the solvents are evaporated. What if they are not evaporated completely? The loss on drying will tell you how much this volatile solvents like your ether, alcohol are there when you heat it at a higher temperature like 100 degrees Celsius above. Similarly, also you can determine the volatile substances.

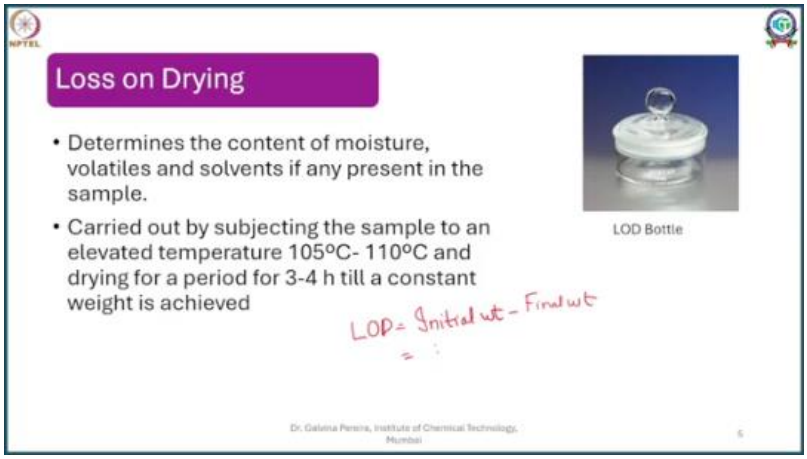
This includes your essential oils and many essential substances which have a low boiling point. So loss on drying accounts for moisture. It accounts for solvents and it accounts for the volatile present in your drug. Now for this, what is done is your drug, especially if you go by a pharmacopoeia method, is kept in what is called as a LOD bottle. So you can see here this is a LOD bottle.

It has a wide mouth to facilitate evaporation by increasing the surface area. So you can take about 5 to 10 grams of sample or 1 gram of sample. The pharmacopoeias generally specify how much quantity of the drug is to be taken and how long it should be kept for drying. So generally, a temperature of about 105 to 110 degrees Celsius is recommended whenever you are performing loss on drying. Why?

Because this is well above the boiling point of water, and your moisture will also evaporate. So you are going to put your sample in this LOD bottle, keep the stopper open to facilitate the evaporation of solvent, water, and volatiles at a temperature of 105 to 110 degrees Celsius for a period of about 3 to 4 hours. Now this process continues, or you have to keep drying until you achieve a constant weight. For example, initially, you take 1 gram of the drug. After one hour, that becomes about 900 milligrams.

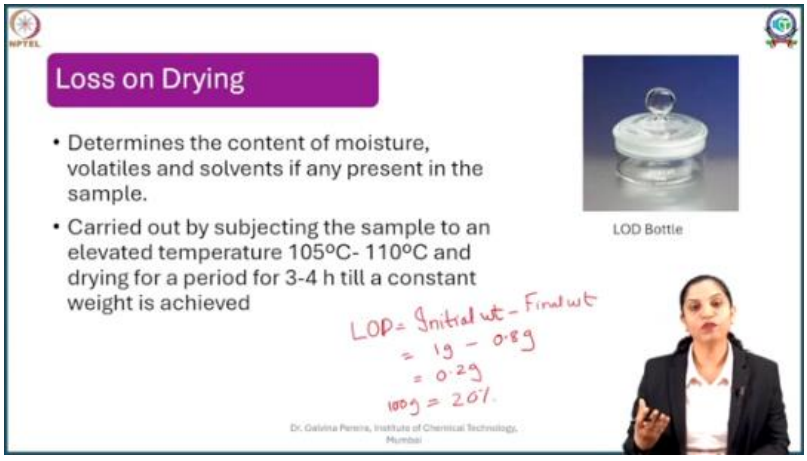
So you have already lost 100 milligrams of the content as moisture or as volatiles. So if you check it after three hours and it has lost 100 milligrams, just keep it for half an hour more and check it again. If it is losing more, you have to keep repeating the process until it achieves a constant weight. So once your constant weight is achieved, what you are going

to do is take the initial weight and then subtract it from the final weight. For example, initially, you have taken 1 gram, and now the final weight is 0.8 grams.



So what has happened is 0.2 grams is something which is lost, that is about 1 gram. 200 milligrams of the drug substance has kind of volatilized or evaporated. So if 1 gram gives me about 0.2 grams of residue, 100 grams will give me about 20%. So, that is 20% of my drug was actually moisture or volatiles. So, this will give me an estimate.

So, say for example, if you see the Indian Pharmacopoeia, if you see acacia, for acacia they say the loss on drying, if you do it for 1 gram of drug, should not be more than 15%. So, if it is more than 15%, in this case, it is 20%, say for example. In that case, the moisture is more than required. That means some deterioration is bound to happen. And in that case, the drug is not fit for use.



We'll go to the next value. The next value is extractive values. Now, extractive values are generally done when no other methods are there for quantification of phyto constituents. Now, different solvents are used. So what is extractive?

Extractive is the preparation of extracts with certain solvents. Say, for example, when you want to prepare your tea, what are you going to do? You're going to take the tea and boil it in water. So what is the solvent for tea? It's water, which dissolves all your tannins.

So if I have to check how much tannin is coming into the water when I boil tea, that is called your water-soluble extractive. So water-soluble extractive value or extractive value is based on a phenomenon called 'like dissolves like.' That means if I have polar compounds, they will dissolve in a polar solvent like water. If I have non-polar compounds, they will dissolve in a non-polar solvent like petroleum ether or ether. If I have more of these mid-polar solvents, I can use what is called your—

alcohol to determine the extractive values. So, for example, if I decide to measure the extractive value of tea, how do I do it? There are different methods, but if you follow the pharmacopoeia method, what you have to do is take about 5 grams of the drug. And these 5 grams of the drug you have to take in 100 ml of solvent.

Now, this solvent is specified by pharmacopoeia. For example, amla has its ethanol-soluble extractive value as well as water-soluble extractive value, which has been specified by the Indian pharmacopoeia. So, how are you going to determine this ethanol-soluble extractive value? You are going to take about 5 grams of amla and then extract it with approximately 100 ml of ethanol. This process is generally done at room temperature with intermittent stirring for a period of 24 hours.

For the first 3 hours, it is stirred or shaken intermittently, and then for the remaining hours, it is just kept as it is. You allow the solvent to seep in, and by diffusion, most of the soluble compounds will come out. Now, what is done is the extract is filtered, similar to how you filter your tea. The extract is filtered and evaporated. Generally, a representative amount.

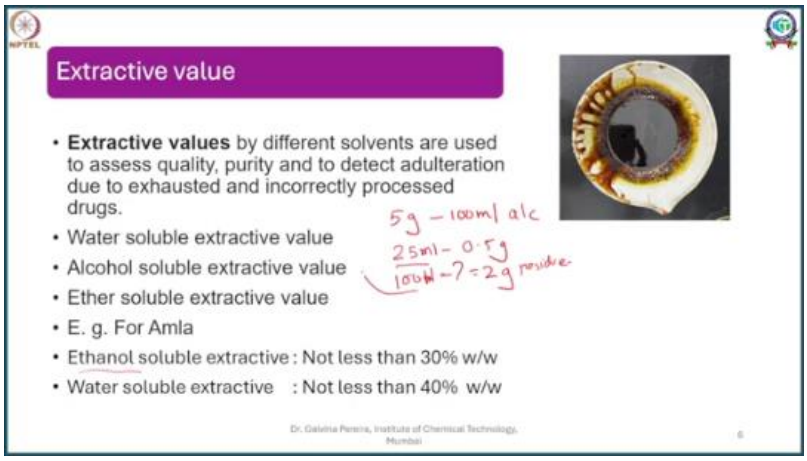
So, from 100 ml, we can evaporate, for example, 25 ml. A representative amount of solvent is evaporated from that, and then the weight of the residue left on the dish is measured. For

example, if I am evaporating this, you can see on the porcelain dish that I am getting a certain residue. So, how much residue is this 25 ml of solvent giving me? I will calculate that. If this residue is given by this much extract, I can calculate it in terms of percentage.

So let's just do a simple example. Say, for example, 5 grams of amla—I extracted it in 100 ml of alcohol. Then I filtered it, and from that, 25 ml of amla, I took it in this evaporating dish and evaporated it completely. After evaporating, say, for example, I get about 0.5 grams of residue.

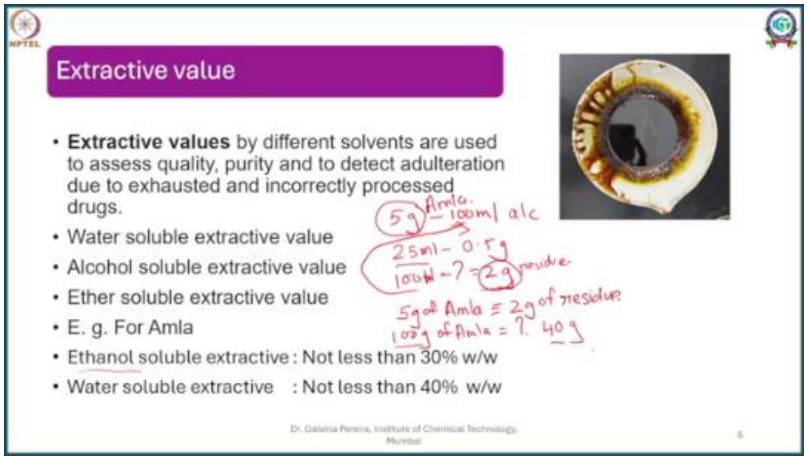
So if I am getting 0.5 grams of residue from 25 ml, then 100 ml will give me how much? So, 100 ml. Gives me how much? So you can do a cross multiplication. So you get 25 versus 100.

So 0.5 grams is equal to 2 grams. So if 25 ml gives me 0.5 grams, then 100 ml is going to give me 2 grams of residue. Now, carefully, this 100 ml has actually been taken from here. So this 2 grams is.



came in from this 5 gram of amla. So 5 gram of amla if it is going to give me 2 gram of residue 100 gram of amla that is I am converting it into percentage. Gives me how much? So again I can do a cross multiplication and what you find it here is. So 5 20s are 100.

So 2 20s are say about 40 gram. So what you get here is 40%. When I am taking 100, it's all in the percentage. So when I am telling about 40%, what does that mean? That means my extractive value.



So when I take 100 gram of amla, 40 gram of it is going to come out in my ethanol. The Indian pharmacopoeia says that my ethanol soluble extractive value should not be less than. is it less than 30 no it is 40 that means my amla is of good quality now why is this test done in certain cases people give exhausted drugs that means imagine if a vendor has already extracted your tea from your tea powder and sold you exhausted tea powder what is going to happen next time you boil it No longer in your water you are going to get the tea extract or you will get a very lesser value of tea extract. That means it is going to fall within or it is going to fall below the range.

If it falls below the range, that means it is a substandard drug. So, this kind of test is generally used to check most often the exhausted drugs or drugs which are adulterated by soil or other substances that do not yield any phytoconstituents. Now, moving on to the next value, the next value is called the ash value or residue on ignition. Ash value is a term more often used by Indian or European pharmacopoeias, whereas residue on ignition is the terminology commonly used by the US pharmacopoeia. So,

Why do you need to ash the drug, or why is this particular value carried out? Ash value, or when you ash a particular substance, you will see most of the organic matter is lost. So, we have seen plants, we have seen animals. What happens is the majority of them are made up of proteins, water, and nucleic acids. Most of them are what are called organic substances.

Now, the basis of organic substances are carbon, hydrogen, nitrogen, sulfur, and phosphorus. So, these react or combust in the presence of oxygen to form what are called corresponding oxides: carbon dioxide, nitrogen dioxide, and sulfur dioxide. And they are

all converted into what is called a gaseous state. Now, what is left behind is more inorganic matter, and this inorganic matter chiefly consists of minerals and metals. Now, from where do these minerals and metals arise?

Now, minerals are thought to originate from physiology. See, we are not only made up of organic matter in our body; we also have inorganic matter. Similarly, if you see in plants, take for example a molecule such as chlorophyll. They will have a molecule or an atom of magnesium. Or even for that matter, when you talk about ergastic cell contents, calcium oxalates.

There are many drugs, such as Colocasia or Arjuna bark, as we see. They are rich in calcium oxalates. So, they have a lot of calcium. Physiologically, even your water contains sodium, and all these micronutrients are part of the plant. So, definitely, these minerals are going to be left behind when you ash them.

And this is how you see them. So, this is how you get ash, and this ash or ashing of your plant is done in a specialized apparatus or assembly or a furnace or a batti called a muffle furnace. So, for that, you require very elevated temperatures. Some pharmacopoeias specify temperatures between 400 to 600 degrees Celsius. Some of them even specify up to 800 degrees Celsius.

So depending upon the method, you're going to take your drug into a silica, quartz, or platinum crucible and put it in the muffle furnace and set the temperature. Now, initially, as the carbonaceous matter burns, you will see a smoky flame of carbon monoxide, carbon dioxide, and so on. Later on, what you will see is something like this. Can you see a little red portion? So all your carbonized matter will also burn as CO2, and then it forms a grayish-white ash.

So this grayish-white ash is precisely what we want. So we have to continue ashing or heating it until you obtain a constant weight, and the weight that you get on ashing is referred to as your total ash value. So, say, for example, you incinerate 1 gram of drug and get 10 milligrams of ash. Convert that into a percentage, and that is your ash value. Now, in different pharmacopoeias, different ash values are mentioned.

The main ash values mentioned are total ash. So when you incinerate the drug, what you get is called your total ash value. Now, in addition to that, you also have acid-insoluble ash. You have your water-soluble ash and your sulfated ash. Now, what are these different types of ash?

Now, acid-insoluble ash. I'll just give you an example. In some cases, your drugs are mixed with silica, mud, soil, or stones. In that case, even if you incinerate, your stone won't carbonize. It's made up of minerals.

So, In order to determine whether it is physiological ash—that is, the ash coming from the minerals and metals present in plants—or non-physiological ash—that is, the ash attributed to iron, metal, stones, or any extraneous material added. So, you have to distinguish it. For this purpose, we do what is called acid-insoluble ash. So, acid-insoluble ash—take, for example, physiological (I am just putting it as 'P'),

such as calcium, sodium, and then you have non-physiological. Take, for example, silicates. Now, what is going to happen, or what is acid-insoluble ash? You are going to boil this ash with hydrochloric acid—about 25 ml of dilute hydrochloric acid. When you do that, your calcium will get converted into calcium chloride, and sodium will get converted into sodium chloride.

Now this is dilute acid and these are salts. So your salts are definitely going to dissolve. So once they dissolve, We will filter it. So on the filter paper, what will remain is your silicates.

So silicates, your pieces of soil, rocks are not going to react with HCL and as a result, the non-physiological ash remains. So as acid insoluble ash is more of a measure of non-physiological ash, whereas your water soluble ash is more of a measure of your physiological ash. Now, why you do the next one is sulfated ash. Sulfated ash is done in a manner when you obtain the ash, certain times the minerals or metals are closely associated and the drug is unorganized. Take for example, acacia, trigacanth, which are forming lump.

Whereas your organic matter that is your plant powder, root powder, fruit powder is more carbonaceous. Your acacia or unorganized drug because of their nature or certain resins because they form clumpy mass they don't decompose. ash easily so there's lot of

carbonized matter which remains over a period of time now in order to facilitate ashing in order for us to make it easier to ash we do what is called as sulfated ash so once the ash is done we add little bit say just 0.5 ml of sulfuric acid some pharmacopoeia specify 1 ml and then you heat it again Now you heat it slowly because it's going to generate sulfuric acid fumes. So at a very slow heating rate, you go on elevating the temperature till all the fumes are gone.

And then what you get is most of the minerals; you will get them as sulfates. And that is what is referred to as your sulfated ash. So sulfated ash is not done for all the drugs. It is done for drugs which are more resistant to ashing due to their nature. Now, a few examples of ash value.

So if you see, the total ash value for amla should not exceed more than 5%. But if you see, the acid-insoluble ash is 2%. Why is this? Because this is physiological plus non-physiological, whereas this is only taking into account more of a non-physiological ash.

Now, moving on to the next value: foreign organic matter. Now, this is very important.

Certain times, certain drug substances are said to be adulterated or substituted, or they may contain certain extraneous matter or even non-plant matter for that fact. Now, in this case, what is done is we try to find out this value called foreign organic matter. Now, foreign organic matter contains a number of clauses. Now, foreign organic matter may include a plant part which is other than that specified. Now, say for example, your drug is acacia gum.

What if the bark of acacia is seen in that or your drug for example is arjuna bark. In that case, what if people add stem or root in that? So any plant part other than specified is a foreign organic matter. Other plant, instead of Arjuna, I add something like your Behda. So I mix Arjuna and Behda.

What is going to happen is the plant other than specified is also there in your drug. So you see that is also a kind of foreign organic matter. Certain times the drugs are infested with worms, ants, insects or bacteria or fungus. So any other living organism or fungus or bacteria inside it is also there. Certain times due to improper storage you will find even

animal excreta as a part of your drug. Even that is treated as your foreign organic matter and in the last case certain time the pieces of packaging material those plastic bags cupboards or even certain pins or you know something that is used to fasten your the thread pieces might fall into your drug all of them are treated as foreign organic matter so foreign organic matter is generally done for a whole drug rather than powder because in powder it is difficult to find. So once your powder is in ultra-fine state it becomes difficult because even your foreign organic matter has been comminuted to a fine size. Still, there is a possibility that you can detect. So

Whenever it is an entire drug, you can easily pick out, you know, you can understand, no, this is animal excreta, this is a fly; you can pick it up. But when it is microscopy, you can see here, say, for example, clove powder. Now, clove powder is from a plant. It is a case where you use a flower bud. Now, this flower bud doesn't have stone cells.

So, if I see the microscopy or if I scan the microscopy of clove, I should see more of floral parts like pollen. I should see petals. But at certain times, imagine this. These are a group of stone cells. Now, these stone cells are not present in flowers, but they are present in stems.

So what has happened is, if I am seeing this, then this is precisely what it is. So if I am seeing this in the powder, that indicates apart from the clove flowers, the clove stems have also been powdered along with it just to adulterate the drug. So that is how you detect the foreign organic matter in the drug as well as in the powder. Now, here are a few references if you wish to delve deeper into the methods. Apart from that, you can see your pharmacopoeias as well as your WHO monographs.

So, thank you everyone for your patient listening. Thank you.