ISSN: 2322 - 0902 (P)
ISSN: 2322 - 0910 (O)

International Journal of Ayurveda and Pharma Research

Review Article

A REVIEW ON ANALYTICAL PARAMETERS OF *SNEHA KALPANA*

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Article info

Article History:
Received: 17-04-2026
Accepted: 19-05-2026
Published: 07-06-2026

KEYWORDS:
Taila kalpana, Dravadravya, Standardisation, *Sneha kalpana*.

ABSTRACT

The method of preparation of *Taila kalpana* ensures the incorporation of active constituents of drugs taken in different forms viz, liquid form (*Dravadravya*), in the form of a paste (*Kalka*), into *Taila* in a unique ratio which make the preparation unique as the process ensures both lipid soluble and water-soluble constituents in the final product. The lipophilic nature of the final product makes it therapeutically more effective when compared to other dosage forms in a wide range of diseases. Those measures which are being taken throughout the manufacturing process and quality control which leads to the reproducible quality of a particular product are known as standardisation. It is the confirmation of the identity, quality, purity, safety and reproducibility of medicines. Standardisation of herbal medicines is the process of setting up of standards, parameters, definitive qualitative and quantitative values which can measure the efficacy, safety and reproducibility of the medicine prepared. Various analytical parameters are applied in the standardisation procedures of *Sneha kalpana*. This review is about the analytical parameters of *Sneha kalpana*, its significance and utility in the health care system.

INTRODUCTION

Sneha kalpana is a medicine par excellence, offering high shelf life, unmatched therapeutic utility due to the oil/ ghee base and different modes of drug administration. *Sneha kalpana* is derived from two words, *Sneha*, and *Kalpana*. *Sneha* means oleagenous substance and *Kalpana* means formulations. Formulations prepared with oleaginous substances are included under *Sneha kalpana*. It includes *Taila kalpana* and *Ghritha kalpana*. *Ghritha kalpana* is prepared by incorporating herbal drugs with ghee and *Taila kalpana* is prepared with *Taila* mostly *Tila taila* (sesame oil). As *Sneha kalpana* including *Ghritha* and *Taila kalpana*, are indicated for different therapeutic purposes with different routes of administration, including internal administration, topical administration, nasal administration and parenteral administration like *Vasthi,* the need of analysis of these preparations for its quality is very important.

Access this article online

Quick Response Code

https://doi.org/10.47070/ijapr.v14i6.3573

And almost all the analytical parameters are designed to check the molecular composition like fatty acid structure, double bonds and the weight of fatty acids present on the oil. Because the metabolic fate of *Taila* depends on the amount of unsaturation, weight of fatty acid, configuration and position of double bond present in the oil. This is the era when greater advancements are happening in the development of newer drug delivery systems. As part of this liposomal drug delivery system is being acknowledged recently. As *Sneha kalpana* is an oleaginous formulation, researches have to be taken place in association with standardisation parameters of *Sneha kalpana.*

Review of analytical methods

Refractive index

It is the ratio of the sine of the angle of incidence to the sine of angle of refraction of a beam of light passing from air to the substance[1]. It is the ratio of velocity of light in vacuum to the velocity of light in the substance to be measured[2]. Refractive index varies with temperature and wavelength[3]. For oils it increases with the increase in unsaturation and also chain length of fatty acids[4]. In a study published in American Journal of Oil Chemists' Society, it was proved that refractive index of edible oils increases as the rancidity of the oil progresses. Refractive index is

proven to use as a quality control measure to find adulteration of oils[5]. Butyro-Refractometer or Abbe Refractometer are commonly is used to check refractive index

Moisture content

Moisture content can refer to any volatile substances including water in materials. Moisture content of any substance can be determined by gravimetric and chemical methods. Chemical method for moisture analysis includes chemical reaction takes place between water and reagent. The most well-known method based on this principle is the Karl Fischer titration (KFT) and the advantage of this test is it is specific for water[6].

Specific gravity

Principle

Specific gravity of a liquid is the weight of a given volume of the liquid at 25°C compared to the weight of an equal volume of water at the same temperature. Equivalently, it is the ratio of the density of a substance to the density of a reference substance (water) for the same given volume. Specific gravity of a liquid depends on density of oil [7].

Viscosity

Viscosity of a liquid is related with its resistance to flow. It is the resistance of a fluid (liquid or gas) to a change in shape, or movement of neighbour portions relative to one another under strain[8]. It is perceived as the resistance to flow. Viscosity is of two types which are, dynamic viscosity and kinematic viscosity.

Dynamic viscosity

Dynamic viscosity is the force needed to make the fluid flow at a certain rate. The unit of dynamic viscosity is centi-poise.

Kinematic viscosity

Kinematic viscosity tells how fast the fluid is moving when a certain force is applied. The dose of Kinematic viscosity is centi stokes[9]. It is the quotient of dynamic viscosity and the density of the liquid at the same time and temperature.

Viscosity increases with the molecular weight and decreased with increasing unsaturation level of the oil and temperature[10]. Oils with low viscosity values indicate that they are light weighted and so probably highly unsaturated[2]. The high value might be as a result of suspended particles.

Test for presence of sesamolin (baudouin test)

Sesamolin is a lignan, present in sesame oil. The development of pink colour with furfural solution in the presence of HCl indicates the presence of sesame oil [11]. The colour is produced on account of reaction of furfural with sesamolin present in sesame oil. It helps to identify adulteration of sesame oil.

Saponification value

The saponification value of an oil is stated in terms of mg. of KOH required to neutralize the fatty acids resulting from the complete hydrolysis of 1gm of oil [12].

Significance

Lower the saponification value, larger will be the molecular weight of fatty acids. When the molecular weight of fatty acids increases, a greater number of milligrams of KOH is required to neutralize the total fatty acids present in the oil. Also, it varies with the composition of fatty acids [13]. As volatile oils are having lesser molecular weight an increase in saponification value in oil can be attributed to the increase in the volatility of the oils. It helps to identify the adulteration in oils[14]. The value itself indicates the quality of the oil because it shows the presence of lower molecular weight components [15].

Iodine value/ Iodine absorption value/ Iodine number/ Iodine index

The iodine value of an oil/fat is the number of grams of iodine absorbed by 100gm of the oil/fat, when determined by using Wij's solution[16]. Chemically Wij's solution is Iodine monochloride in which chlorine is a constituent of halogen group which is highly reactive. And when it is added into a fat it will easily get attached to the double bond present in the fatty acids. The amount of halogen that can be combined is dependent upon the amount of unsaturation which is manifested as double bonds in the fatty acids of the oil [17,18].

Significance

The value determines the amount of unsaturation. The higher the iodine value, more will be the number of double bonds or more will be the unsaturation present in the oil. As unsaturation make the oil highly reactive towards water, oxygen or other metals or external factors, for the oil with high iodine value, susceptibility towards rancidity will be more [19]. The value itself indicates the quality or oxidative stability of oil in terms of unsaturation [20].

Acid value /acid number/ neutralization number/ acidity

Acid value is the measure of number of carboxylic groups in a chemical compound such as fatty acids [21]. It is defined as the number of milligrams of KOH required to neutralize the free fatty acids present in one gram of fat [22]. It is a crucial sign of the quality of vegetable oil. Acid value is determined by directly titrating the oil/fat in an alcoholic medium (methanol, diethyl ether) against standard potassium hydroxide/sodium hydroxide solution.

It is a measure of the number of fatty acids, which is liberated by hydrolysis of triglycerides due to the action of moisture, temperature or lipolytic

enzyme lipase. It is the measure of carboxylic group in a compound, here in this case it is fatty acid [23,24].

Significance

The amount of rancidity of the provided fat is indicated by the acid value. Rancified oils provide high acid levels. It is a relative measure of rancidity as free fatty acids are normally formed during decomposition of triglycerides. Acid value of an oil also rises with age[25]. The value is also expressed as percent of free fatty acids as percentage of oleic acid, lauric, linoleic and palmitic acids.

Free fatty acid

Free Fatty Acids (FFA) are formed by the hydrolysis of oils. They are not bound or esterified to a glycerol molecule [26]. Crude oils and fats in natural form, not refined, contain small amounts of FFA, which are usually removed during the refining process [27,28].

Significance

FFA are not desirable in edible oils because, they lower the oxidative stability of the product, increase acidity and lead to off-flavour formation [29].

Rancidity

Rancidity is defined as the natural process occurring in fats and oils on reaction with oxygen or moisture resulting in chemical and physical changes in the oil. It is the characteristic, unpalatable odour and flavour of edible fats and oils following oxidative or hydrolytic degradation. Rancidification is the breakdown of fats and other lipids by oxidation and/or hydrolysis. It can be caused due to microorganisms, moulds, moisture sometimes oxygen or light may cause it [30,31,32].

Rancidity happens in two ways which are oxidative rancidity and hydrolytic rancidity.

Oxidative rancidity

In oxidative type of rancidity, the hydrogen atom nearer to double bond generates reactive oxygen species like hydro peroxide and free radical such as hydro peroxyl, super oxides and hydroxyls[33,34]. Oxidative rancidity is occurred by the oxidation of fatty acids by addition of oxygen at carbon double bonds present in the fatty acids. It is easy to break the carbon double bonds present in a fatty acid. As the double bonds are found more in oils with unsaturated fatty acids, oxidative rancidity rapidly happens in oils with more unsaturation in it. This type of rancidity destroys fat soluble vitamins in the oil[35]. The process of oxidative rancidity is catalysed by light and metal ions.

Oxidative rancidity can cause severe health hazards. Mono aldehydes are present in rancid oils which is a decomposition product of poly unsaturated fatty acids. And it is found to be carcinogenic [36].

This type of rancidity happens in three stages; initiation, propagation and termination. In initiation, where double bonds break and forms free radicals or reactive oxygen species or hydrogen peroxyls and hydroxyls. This stage depends on presence of nickel, oxygen etc. During propagation, the reactive oxygen produces hydroperoxides. During termination there forms volatile and non-volatile chemicals like aldehydes, ketodiens and dimers which causes the specific odour to the oils. Consumption of rancid oils may cause accelerated ageing, obesity and raised cholesterol levels [37,38].

Significance

The higher concentrations of these molecules in the body may generate oxidative stress in the cells. These accumulated free radicals generate hydro peroxides which is a primary oxidative product which can hamper digestive enzymatic activity and can be even mutagenic mitochondrial dysfunction, and also may cause degenerative diseases like Alzheimer's disease on a later period [39,40].

Hydrolytic rancidity

Hydrolytic rancidity is the breaking down of lipid into fatty acid and glycerol in presence of water and it is the commonest way of rancidity of oils. The process of hydrolytic rancidity increases rapidly in presence of enzyme lipase, water and heat [41].

Kreies test

Kreies test is the test adopted for check the rancidity of an oil as per Ayurveda pharmacopoeia on India. Detection of ephedrine aldehyde on shaking of 10 ml oil along with 10ml HCl and 10ml 0.1N Phloroglucinol. Ephedrine is a primary oxidation product of oils or fats. The development of pink colour in the mixture while shaking indicate that the oil is rancid [42].

Composition of fatty acids is particularly important in relation to oxidative stability of fat. The more unsaturated and less saturated a fat is, the faster the oxidation reaction proceeds [43]. Linolenic acid is oxidized the fastest, followed by linoleic and oleic acids in case of sesame oil [41].

Steps to be taken for prevention of rancidification of oils:

- Addition of anti-oxidants while packing of oils
- Keep the oil un exposed to light or temperature
- Air tight packing of bottles
- Filling inert gas inside the bottle to remove oxygen
- Keeping oil in amber coloured bottle.
- Vacuum packing [43]

Peroxide value

Peroxide value of an oil is the index used to quantify the amount of hydroperoxides present in fats and oils. Hydroperoxides, which are shown to be toxic to humans, are the primary oil oxidation products formed during the initial stages of oxidation of fats.

Their content is commonly expressed as the "peroxide value" in milli equivalents peroxidic oxygen per kg of sample [44]. These are formed by auto oxidation and free radical chain reaction[45]. High values of PV are indicative of high levels of oxidative rancidity of the oils and also suggest absence or low levels of antioxidants. Because presence of anti-oxidants may prevent the formation of peroxides in the oil or can delay the process of rancidification [46].

Sometimes evaluating oil quality or rancidity based on only peroxide value can be misleading. Because low peroxide value does not necessarily indicate low level of oxidation. Instead, sometimes it could be due to the advanced level of oil oxidation during which primary oxidation products are converted to secondary oxidation product which cannot be identified by the test for peroxide value [47]. During those instances, oil may present a lowered peroxide value but an increased acid value [48].

Thin layer chromatography

It is a chromatographic technique based on separation of active constituents of a sample in different colours taken place between two phases; stationary phase and mobile phase. The adsorbent will be the stationary phase usually precoated aluminium plates. Works on the principle of adsorption chromatography [49].

It is used for qualitative and quantitative analysis of plant products. It is useful in analysis of Alkaloids, Glycosides, Isoprenoids, Lipid components. Identification of components is achieved by identification of spots on comparison with similar spot in a standard sample. The identification of component is carried out by calculating Rf value/retention factor. Rf value of a component in TLC is the ratio of distance travelled by the component to the distance travelled by the mobile phase over the stationary phase. Rf value is unique for a specific component in a specific mobile phase [49].

The major components of TLC include

- Mobile phase
- Stationary phase
- Solvent system.

An advanced version of thin layer chromatography is the High-performance Thin Layer Chromatography which too works on the same principle but highly sensitive and computerized and the results are being generated with the assistance of a software.

HPTLC (High performance thin layer chromatography)

High performance thin layer chromatography is an aid for the qualitative and quantitative evaluation of the components present in a sample. The evaluation method is done with the aid of a coordinated software programming, which ensures the efficiency, reliability and reproducibility of the method.

It is used for the quantitative and qualitative analysis of pharmaceuticals. Helps in detecting adulteration

- Identification of active ingredients.
- Efficient method of separation, isolation, purification and identification of the constituents of a mixture and also to determine the purity and standardization of herbal drugs.
- In general, chromatography involves moving a preparation of the materials to be separated, the "test preparation", over a stationary phase.
- The molecules in the test preparation will have different interactions with the stationary phase leading to separation of similar molecules.
- Solute undergoes distribution between the mobile phase and stationary phase.
- Test molecules which display tighter interactions with the stationary phase will move slowly than those with weaker interactions.

Three components

- Stationary phase
- Mobile phase
- Solvent system

Stationary phase

It is the surface over which the sample to be tested is placed and the active constituents in the sample is being separated. Usually, silica coated plates of different sizes are used as stationary phase. HPTLC works on the basis of adsorption of active constituents over the stationary phase. It is on the basis of affinity of the active constituent in the sample towards the stationary phase the components got separated and being lodged at different places. The components having more affinity will lodge at nearer place of sample application and travel a lesser distance. The components with lesser affinity will travel longer distance and lodge at a distal point of sample application.

Mobile phase

Mobile phase helps in efficient separation of constituents in the given sample. Usually, a mobile phase will be any chemical solvent or a mixture of solvents. It helps the sample to get separated with the active constituents and hence in the adsorption over the stationary phase. A good mobile phase helps in determining the efficiency of separation and chromatographic techniques.

Solvent system

A solvent has to be needed to prepare a sample of application to get proper chromatographic separation. It is a chemical which has the ability to mix the sample to be tested properly and give proper

separation when it is applied over the stationary phase.

HPTLC finger print of any drug or formulation is a finger print of identification which is unique for that particular medicine. After identification of the component by the calculation of Rf value the quantification of components has to be carried out further.

CONCLUSION

The major analytical parameters to standardise the *Taila* and *Ghrita* preparation are acid value, iodine value, saponification value, test for rancidity and chromatographic techniques. All the parameters help to assess the number and nature of fatty acid chains present in the lipid mixture of *Taila* and *Ghritha*. As these oleaginous medicines are being administered both internally and externally, the analysis of these values is very important as the components affect the bodily functions. As Ayurveda is getting wide acceptance around the globe and greater advancements are being happen in the field of Ayurvedic medicines, the standardisation of Ayurvedic medicines is of paramount importance.

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Cite this article as:
Saranya N., Sreeni TV. A Review on Analytical Parameters of Sneha Kalpana. International Journal of Ayurveda and Pharma Research. 2026;14(6):47-53.
https://doi.org/10.47070/ijapr.v14i6.3573
Source of support: Nil, Conflict of interest: None Declared

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