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Research Article

ANALYTICAL STANDARDIZATION OF ABHIJIT TAIL

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ABSTRACT

'Analysis' means the detailed examination, which reveals the minor but important aspects regarding to the drug. Analytical study of a product provides some standards to judge its quality. It is useful to decide future work plan and objective parameters to know the accurate status of drug by conducting the comparative study of various samples during drug preparation.

Excellence of any product is established not merely by the external appearance of the drugs, but also it has to pass with critical analysis in drug testing laboratory. The drug has to be passed through certain characteristics like the potency, uniformity, purity, stability etc. in order to attain its acceptance. The quality of a dosage form should not only be tested at the end but must built into the product right from the moment of receipt of raw materials through processing until the final packaging. Physico-chemical analysis provides the objective parameters to improvise the standards for quality of raw drugs as well as finished products.

INTRODUCTION

As it was observed that no previous work had been carried out concerning the pharmaceutical standardization of Abhijit Tail till date, the formulation was opted for the current research work. Total three samples of Abhijit Tail were prepared under standardized conditions and for assurance of excellence, efficacy and performance, obtained three samples were further subjected for physico-chemical analysis. A very sincere attempt was made to set standardized parameters for the formulation. In the present research work analytical analysis of Abhijit Tail has been studied.

Aim & Objectives

1. Preparation of total three samples of Abhijit Tail as per the text Chakrdatt [1].
2. Physico-chemical analysis of Abhijit Tail.

MATERIAL AND METHODS

Pharmaceutics of Abhijit Tail

To prepare Abhijit Tail, Krishna Til Tail was treated was Yashtimadhu Kalka, Amalaki swaras and Go-Dugdh. In order to maintain the quality and efficacy of the formulation, best quality Krishna Til seeds were procured and Tail was extracted from them. Before Tail Pak, Murcchana was done according to the Bhaishjya Ratnawali [2]. Abhijit Tail was prepared according to the text Chakradatt.

Ingredients of Abhijit Tail were taken in accordance to the basic rule of Sneha Pak [3] i.e. one part of Krishna Til Tail, one forth part of Mulethi Kalka and Four parts of Go-Dugdh and Amalaki Swaras each.

Physico-chemical analysis of Abhijit Tail

Parameters were taken according to "Protocol of testing of Ayurvedic, Siddha, and Unani Medicines", written by Dr. D.R. Lohar, published by Government of India, Department of Ayush, Ministry of Health and Family Welfare, and Pharmacopoeial Laboratory For Indian Medicines, Ghaziabad.

Place of work: Institute of Biomedical and Industrial Research (IBIR), Jaipur

The three samples of oil were subjected to analytical study. Parameters used for the analysis were-Organoleptic tests (Colour, Odour, Touch, Taste)

1. Weight/ml. at 25℃
2. Refractive index at 40℃
3. Viscosity
4. pH value
5. Iodine value
6. Saponification value

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7. Acid value
8. Peroxide value
9. Unsaponifiable matter
10. Test for heavy metals (Lead, Cadmium, Mercury, Arsenic)
11. Test for Aflatoxins (B1,B2,G1,G2)
12. Microbial contamination (Total bacterial count, Total fungal count)
13. Pesticide residue (Organochlorine pesticides, Organophosphorus pesticides, Pyrethroids)
14. HPTLC

pH

The pH value of an aqueous liquid may be defined as the common logarithm of the reciprocal of the hydrogen ion concentration expressed in gram per liter. The pH of a given solution was measured by using digital pH meter.

Refractive Index

The refractive index (η) 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. The Abbe type Refractometer was opted to calculate refractive index.

Specific gravity

Specific gravity of a liquid is the weight of a given volume of the liquid at a specific temperature compared with the weight of an equal volume of water at the same temperature, all weighing being taken in air. Pyknometer was used to calculate S.G. via following equation-

$$\text{Calculation: Specific Gravity} = \frac{\text{weight of liquid sample } \left(\frac{g}{ml}\right)}{\text{Volume of liquid sample } \left(1.0\frac{g}{ml}\right)}$$

Peroxide Value

The peroxide value is the number of mill equivalents of active oxygen that expresses the amount of peroxide contained in 1000 g of the substance.

$$\text{Calculation: Peroxide Value} = \frac{(A-B)\text{ X Normality of sodium thiosulphate X } 1000}{\text{Weight of sample (gm)}}$$

Iodine Value

The Iodine value of a substance is the weight of iodine absorbed by 100 parts by weight of the substance. Method opted was iodine monochloride method.

$$\text{Calculation: Iodine Value} = \frac{(B-A)\text{ X } 0.01269 \text{ X } 100}{\text{Weight of sample (gm)}}$$

Rancidity

It is a natural process of decomposition of fat or oil by either oxidation or hydrolysis. It converts fatty acid of oil into free fatty acids and produce offensive, unpleasant odour and taste.

Saponification value

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

Calculation:

$$\text{Saponification Value} = \frac{(B-A)\text{ X Normality of HCL X } 56.10}{\text{Weight of Sample (gm)}}$$

Acid Value

The acid value is the number of mg potassium hydroxide required to neutralize the free acid in 1 g of the substance.

$$\text{Acid Value} = \frac{\text{Volume of KOH X Normality of KOH X Eq. wt X } 1000}{\text{Weight of sample}}$$

Unsaponifiable matter

The unsaponifiable matter consists of substances present in oils and fats, which are not saponifiable by alkali hydroxides and are determined by extraction with an organic solvent of a solution of the saponified substance being examined.

Boiling point

It was measured by using boiling point apparatus.

Test for Heavy Metal

Atomic Absorption Spectrophotometry (AAS) method was applied to test heavy metal presence.

Microbiological Analysis

Membrane filtration technique was used to determine microbial load limit.

Determination of Pesticide Residues

Equipment and Glassware used- Gas Chromatography- Mass Spectrophotometer, Soxhlet extraction unit, volumetric flask, micro-pipettes.

Test for Aflatoxins

Aflatoxins are closely related group of secondary metabolites shown to be mycotoxin.

HPTLC

HPLC was done to find out the percentage of antioxidant gallic acid in three samples of oil.

Conditions

Stationary phase: Pre-coated silica gel 60 F_{254} aluminium plates
Mobile phase: Toluene: Ethyl acetate: Formic acid (5:5.5:0.5).
Chamber Saturation Time: 20 min.
Reference Standard: 25 mg of Gallic acid was dissolved in 25 ml of methanol and 1 ml of above solution was taken and diluted up to 10 ml of methanol.
Test Solution: 20 gm of drug was extracted by cold maceration in methanol and then the liquid extract was filtered. Volume was made up to 100 ml with methanol.

$$\textbf{Calculation:}\ \text{Percentage of Gallic acid} = \frac{C_{\circ}\text{ X Purity of std}}{C1}$$

Where, C_0= Concentration of sample found from cal. curve in µg and C_1= Concentration of sample applied in µg.

RESULTS

Yield of Abhijit Tail

Table 1 Detail of % yield and % loss of Abhijit Tail

Sample No.	Initial amount	Yield	% yield	% gain
Sample I	7000 ml	7500 ml	107.1	07.1
Sample II	2000 ml	2100 ml	105.00	05.00
Sample III	2000 ml	22500 ml	107.5	07.5

Table 2 Observation and results of Organoleptic parameters

Parameters	Sample 1st	Sample 2nd	Sample 3rd
Appearance	Soft, Viscous	Soft, Viscous	Soft, Viscous
Colour	Yellow	Yellow	Yellow
Odour	Characteristic Odour	Characteristic Odour	Characteristic Odour
Taste	Characteristic	Characteristic	Characteristic
Touch	Unctuous	Unctuous	Unctuous

Table 3 Results of Quality Control test

Parameters	Sample 1st	Sample 2nd	Sample 3rd	Mean
Ph	4.7	4.7	4.7	4.7
Specific gravity(at 27^0C)	0.926	0.924	0.927	0.925
Refractive index (at 27^0C)	1.468	1.469	1.468	1.468
Rancidity	- ve	- ve	- ve	- ve
Peroxide value	2.4	2.6	2.1	2.3
Acid value	4.86	4.36	4.56	4.59
Saponification value	187.46	187.74	187.39	187.53
Iodine value	115	116	115	115.33
Unsaponifiable matter	1.8	1.8	1.9	1.8
Boiling point	229^0C	229^0C	229^0C	229^0C

Table 4 Results of test for heavy metals

Heavy metals	Sample 1st	Sample 2nd	Sample 3rd	Possible limit
Lead	Absent	Absent	Absent	10ppm
Cadmium	Absent	Absent	Absent	3ppm
Mercury	Absent	Absent	Absent	0.3ppm
Arsenic	Absent	Absent	Absent	1ppm

Table 5 Results of test for Aflatoxins

Aflatoxins	Sample 1st	Sample 2nd	Sample 3rd	Possible limit
B1	Absent	Absent	Absent	0.5 PPB
B2	Absent	Absent	Absent	0.1 PPB
G1	Absent	Absent	Absent	0.5 PPB
G2	Absent	Absent	Absent	0.1 PPB

Table 6 Results of test for Microbial contamination

Parameters	Sample 1st	Sample 2nd	Sample 3rd	Possible limit
Total Bacterial Count	5327cfu/gm	5364cfu/gm	5346cfu/gm	100000cfu/gm
Total Fungal Count	<10cfu /gm	<10cfu /gm	<10cfu /gm	1000cfu /gm

Table 7 Results of test for Pesticide residue (Organophosphorus pesticides)

Parameter	Sample 1st	Sample 2nd	Sample 3rd
Organophosphates	Not Detected	Not Detected	Not Detected
Organochlorines	Not Detected	Not Detected	Not Detected
Pyrethroids	Not Detected	Not Detected	Not Detected

Table 8 Results of test for Gallic Acid Estimation by HPTLC

Parameter	Sample 1st	Sample 2nd	Sample 3rd	Mean
% Gallic Acid	0.069	0.066	0.052	0.062

DISCUSSION

- **Organoleptic characters-** The comparably similar organoleptic characters of the samples indicated that the efforts were made to maintain the similar standardized conditions during pharmaceutical procedures.
- **pH-** The mean pH value of samples is 4.7, indicating the acidic nature of the Tail which would have better absorption in acidic media while in case of alkali drug, alkali media is a better one.
- **Specific gravity-** The increased value of S.G. shows that the solid content of drug is higher than the liquid content. Lesser would be the liquid content, lesser would be the chances of microbial contamination of the oil and higher would be the self-life of the oil.
- **Refractive index-** It is also a Characteristic feature of the liquids. It is used to identify the product. If there is any kind of impurity or adulteration, the R.I. value of the product would alter. Thus it acts a parameter of rancidification of the oil.
- **Rancidity-** It converts fatty acid of oil into free fatty acids and produce offensive, unpleasant odour and taste. These free fatty acids make oil unstable and thus rancidity is responsible for reduced self-life of oil or fats.
- Peroxide, Acid, Saponification and iodine values are measured as identification tool of oil and to evaluate the compositional quality and stability of the oil.
- **Peroxide Value-** It gives a measure of the extent to which oil sample has undergone primary oxidation. Oil with higher degree of unsaturation is more susceptible to autoxidation and thus rancidification. Higher the peroxide value, shorter will be the self-life of oil.
- **Acid Value-**.When oil rancidify triglyceroids of oil convert to Fatty acids and glycerols and acid value increases. It indicates hydrolytic rancidity of oil. Higher the acid value higher would be the chances of rancidification and therefore the self-life of oil will reduce.
- **Saponification Value-** Saponification value is inversely proportional to the mean molecular weight or chain length of fatty acid i.e. it is inversely proportional to the no. of free fatty acids and rancidity.
- **Iodine Value-** It is an identification measure of degree of unsaturation in oil. The iodine gets incorporated into fatty acid chain wherever double bonds exist. Hence the measure of iodine absorbed by an oil gives the degree of unsaturation i.e. rancidification. Therefore the self-life of oil will reduce with the increment in iodine value.
- **Heavy metals, Aflatoxins** and **Pesticide residue** were absent in all three samples of oil, showing that the drug is non-toxic and safe for application.
- **Total microbial count** and **Total fungal count** were within permissible limit in all three samples of oil. It depicts that no harmful pathogen was present in the samples and the drug is safe for application.
- **HPTLC-** for authentication of the drug, HPTLC was performed to estimate the % of Gallic acid, a strong anti-oxidant present in Amalaki, in the samples of oil. Samples were simultaneously run with Gallic acid bio-marker and there R_f values were determined relatively. The mean % of Gallic acid of three samples of oil is 0.062

CONCLUSION

- Analytical results demarked that Abhijit Tail is yellow color, soft, viscous, unctuous formulation with characteristic odour and taste.

- pH value denotes its acidic nature.
- There was absence of heavy metals, aflatoxins and pesticide residue while microbial count was found within limit.
- Chromatogrphic estimation by HPTLC method for Gallic Acid depicted that % of Gallic acid was maximum in first sample i.e. 0.069% followed by second sample i.e. 0.066%. The value of third sample was found minimum i.e. 0.052%.

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