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STABILITY STUDY OF HERBAL IRON TONIC

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ABSTRACT

The stability study of pharmaceutical products plays a major role in determining the shelf life of a new drug as well as new formulation under particular storage condition. Objective of this study was to investigate and ensure quality of herbal iron formulation. Accelerated stability study of temperature 40°C±2, relative humidity 75%±5 of the herbal iron preparation was evaluated through different parameters like physiochemical and microbial study. Stability studies are used to maintain the quality, safety and efficacy of pharmaceutical product throughout its shelf life. Here, stability studies are conducted as per ICH guidelines.

KEYWORDS

New formulation, Stability, Storage, Label, Quality and Shelf life.

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INTRODUCTION

The herbal syrup is a blood- building and iron-boosting tonic meant to nourish and restore over a longer period of time. To increase iron level and improves the health of the organ that transport blood, absorb iron, and remove waste. And while its meant to support healthy iron level not to necessarily treat iron deficiency[1]. Herbal iron is packed with nature's most powerful herbs containing plant based iron so our body will get recognized and absorbed[2]. In this particular recipe, we start with a decoction of Dried Moringa leaves, Stinging nettle leaf, Astragalus root, Sarsaparilla root, Curry leaves, Bhringraj, with little amount of water and then we added a base of honey, blackstrap molasses and alcohol for preservative purpose.

Stability studies are one of the important factors to be determined during the development of both new drugs as new formulation. Stability studies of a pharmaceutical product become stable when physical, chemical, microbiological characteristics remain unchanged. Shelf life of the products can be defined as the substance reduced to 90% of its original concentration shelf life of a pharmaceutical product helps in predicting the expiry date, quality and stability under suitable condition.

The data obtained from stability testing method is an major requirement for regulatory authorities to get approval of any new drug or formulation.

The shelf-life prediction is a major role for the pharmaceutical product development of all the dosage forms and also it is utilized. Objective of this study Accelerated stability study of temperature 40˚C±2, relative humidity 75%±5 of the herbal iron preparation was evaluated through different parameters like physiochemical and microbial study[3]. Stability studies of pharmaceutical products are considered as pre-requisite for the acceptance and approval of any pharmaceutical products. These studies are required to be conducted in a planned way following the guidelines issued by ICH, WHO or other agencies[4].

Importance of stability studies

Product instability of active drug may lead to under medication due to the lowering of the drug in dosage form.

During the decomposition of the drug or product it may lead to toxic products.

During the marketing from one place to another during the transportation the drug has the compatibility to change its physical properties.

In stability may be due to changing in physical appearance through the principles of kinetics are used in predicting the stability of drug there different between kinetics and stability study[4].

METHODOLOGY

Types of stability studies on drug substances

A comprehensive pharmacopoeia protocol (USP) prescribes the criteria for acceptable levels of physical, chemical, microbiological, therapeutic and toxicological stability studies.

Physical stability

The physical parameters like Colour, Dissolution, Appearance, Odour, and Taste should be checked for its stability to determine the efficacy and safety of the product.

Chemical stability

Chemical changes due to air, pressure, temperature and atmosphere was checked for its stability.

Microbiological stability

The microbial stability of a product should remain unchanged because it may cause hazardous effect to the drug product.

Therapeutic stability

The therapeutic effect (Drug Action) remains unchanged.

Toxicological stability

The animal studies should performed to determine the toxicological stability[4].

Types of stability studies

The type of stability studies and its storage conditions with respective time period were shown in Table No.1.

STABILITY TESTING METHODS

Stability testing is a procedure performed for all the pharmaceutical products at various stages of the product development. In the early stages, the stability testing is performed by the accelerated stability studies which mainly are performed at high temperature humidity. The accelerated stability study is easy to predict the degradation of the drug within short period of time. In the accelerated stability studies mainly the drug is performed at long-term storage. During this elevated temperatures are used to determine the products shelf-life. Types like Real-time stability testing, accelerated stability testing, Retained sample stability testing and cyclic temperature stress testing.

Accelerated stability testing

This type of stability testing is done at higher temperatures and that decomposition the product is determined. The information is used to predict the

shelf life or used to compare their relative stability of alternative formulations. The accelerated stability study is easy to predict the shelf life thus reduces the duration to know the stability of the substance. In addition to temperature, stress conditions are applied such as moisture, light, pH and gravity. Due to the measurement of instability time is also reduced in comparison to the real-time testing. For the accelerated stability studies the stability projections are done at four different stress temperatures. However, projections are obtained when denaturing stress temperatures are avoided. The accelerated stability studies are easily predicted by the Arrhenius equation.

$$K = Ae^{-Ea/RT}$$

K=Specific rate constant A= Specific gravity factor or Arrhenius factor

Ea= Energy of activation R= Real gas constant 4.184j/mol. kT= Absolute temperature k.

In this method the drugs are stored at temperature 45°C. These studies are to be done at room temperature and at refrigerator temperatures. During different intervals the samples are collected and examined for the stability. The sampling is done at weakly once per month. The products which degrade very fast for them regular sampling at short duration of time should be done. When the temperature increases the decomposition of the substance is also very rapid. As per ICH and WHO the storage condition for accelerated stability studies is 40°C ± 2°C 75%RH ± 5% RH. If the product is unstable on the prescribed temperature and humidity intermediate conditions are used i.e. 30°C±2°C 65% RH±5% RH. FDA prescribes the sampling testing for 0, 1, 2, 3 and 4 weeks respectively. WHO prescribes for 0, 1, 2, 3, 4, and 6 months. ICH prescribes the test to be performed for every 3 months in a year, 6 months in 2 years and yearly thereafter. These accelerated tests are mainly done for photochemical stability and moisture absorption. This test is performed for all the pharmaceutical preparations but mainly this is a test used for dispersed systems like pharmaceutical emulsions and suspensions[4].

CONCEPT OF STABILITY IN HERBAL MEDICINE

Stability is the capability of a specific formulation in a particular container/closure system to remain within its physical, chemical, microbiological, toxicological, therapeutic specifications, and is always expressed in terms of shelf life. The shelf life of a product can be defined as the time duration up to which it is expected to retain 90% of its active ingredients (label claim) when stored in recommended condition. The purpose of stability testing is to provide evidence of how the quality of a pharmaceutical product changes with time due to impact of a variety of environmental factors, temperature, humidity and light and product-related factors, namely, container closure system and packaging materials.

A type of stability is discussed in below Table No.2.

PROTOCOL FOR STABILITY TESTING

The protocol is a document describing the basic components of a well-controlled stability study which depends on the type of herbal formulation[3].

Selection of batches and samples

In general, this selection should constitute a random sample from pilot or production batches that may involve a single batch or 2-3 batches.

Test attributes

The tests that monitor the quality, potency, purity, and identity that are expected to vary upon storage are chosen as stability tests.

Analytical procedures

Procedures given in the official compendia should be followed and if alternate methods are to be used, they need to be duly validated.

Acceptance criteria

This should be fixed beforehand in the form of statistical limits for the results manifested in computable terms and pass or fail for qualitative tests.

Storage conditions

These are based upon the marketing climatic zone of the drug as depicted.

Storage period

It generally extend from minimum of 3 or 6 months in accelerated and stress testing and up to 12, 18, or 60 months in ongoing or follow-up stability testing.

Testing frequency

It should be sufficient to establish the stability profile of the formulation Test schedules for different types of stability testing.

Sampling plan

It involves devising for the number of samples to be placed in the stability chambers and taking out of the charged batch so as to cover the entire study.

Container closure system

The testing in actual containers as well as closures scheduled for marketing, are to be tested separately with proper orientation of storage of containers.

Evaluation

The data on quantitative attribute is analyzed to determine the time duration at which 95% one-sided confidence limit for the mean curve intersects the acceptance criterion.

Statements, labeling

A storage statement, retest period, and re-test date based on the stability evaluation of the herbal formulation should be established for the labeling[3,5].

Post formulation studies

Physicochemical Parameters

Physical appearance (i.e., colour, odour, taste), pH, Weight/ml and Specific Gravity of formulated syrup were also checked).

Organoleptic Evaluation

Syrup was also evaluated for physical appearance, taste, odour and colour.

Colour

Herbal iron tonic in quantity of 5ml was taken into watch glasses. A perfectly white background was used and the sample was observed for colour with naked eye in white tube light shown as like Figure No.1.

Odour

Sample of 2ml of herbal iron tonic was smelled for odour test.

Taste

A pinchful of herbal iron tonic was tasted on taste buds of tongue[6].

pH

For pH determination 1ml of herbal iron tonic with 100ml distilled water in a 100ml volumetric flask. The solution was sonicated for about 10 minutes. The pH was measured for every week in a month with the help of digital pH meter.

Viscosity

The viscosity of herbal iron tonic formulation was determined at 25˚C by using Brookfield viscometer.

Solubility

Herbal iron syrup was prepared and solubility was checked by absorbing clarity of solution visually.

Analysis of Particle Size and Zeta potential

Particle size is measure random changes in intensity of light scattered from the formulation and zeta potential is determination for colloidal stability. The mean diameter and zeta potential of sample are measured by dynamic light scattering (DLS) the particles were suspended in water to a final concentration of 100µg/ml. The measurement was done in disposable polystyrene cuvettes at 25^{o}C with a detection angle of 90^{o}C. The particle size and zeta potential were determined.

Microbiological testing

Microbial test was carried out as per standard procedure mentioned in Indian pharmacopoeia. It shows presence or absence of E.coli, Klebsiella pneumoniae, Staphylococcus aureus in the sample syrup[7,2]. The medium used for microbial test was Mueller Hinton agar.

RESULTS AND DISCUSSION

The design of stability program should be based on knowledge of behavior, properties of the drug substance and dosage form. The result proves that the drug product remains within specifications established to ensure its identity, strength, quality and purity.

Analysis of particle size and Zeta potential

The mean diameter and zeta potential of the samples were measured by Dynamic light scattering (DLS) using zeta sizer (Nano ZS 90, Malvern Instruments, United Kingdom).The particles were suspended in water to a final concentration of 100µg/ml. The measurement was done in

disposable polystyrene cuvettes at 25ºC with a detection angle of 90ºC shown in Table No.3. The particle size, Polydispersity index (PDI) and zeta potential were determined.

Viscosity

The Viscosity of the sample was determined at 25ºC using the Brookfield viscometer, Model Dve-Viscometer at 10 rpm (spindle number S63).

Report

At 10 rpm: %15.1 torque, 180cp

Sedimentation volume

The sample was stored in 50ml measuring cylinder and observation was made every 10 min for every weak. The sedimentation volume was then calculated using the following equation as per Table No.4.

$$\text{Sedimentation Volume} = \frac{\text{Ultimate volume } (H_u)}{\text{Initial Volume } (H_o)}$$

pH

pH of the sample was found out using a digital pH meter.

Redispersibility

The Redispersibility was determined by studying a number of strokes to redisperse the formed sediment at the end of 7 the day of storage of the formulation.

QUANTITATIVE EVALUATION

FTIR (Fourier Transform Infrared Spectroscopy)

FTIR spectrum was used for identification of compound. The FTIR analysis of reference standard.

UV Spectroscopy (Ultra violet visible spectroscopy)

The spectral evaluation of herbal extracts was done on UV spectrophotometer (shimadzu). Purified extract solution and reference standard were collectively dissolved in distilled water and the spectrum of resulting solution were taken with range of 200 to 800nm to determine the maximum absorbance (λ max).

Limit test

Limit test of iron is based on the reaction of iron in ammonical solution with thioglycolic acid in presence of citric acid to form iron thioglycolate complex. The sample colour intensity is more than that of standard colour intensity was observed.

Discussion

The finished formulation was dark brown in colour with characteristic odour and taste. The stability data on any dosage form includes selected parameters that together form the stability profile which forms the basis for assigning the storage conditions and shelf life to pharmaceutical products[2]. It seems impossible to conduct real time stability study which requires longer time but accelerated stability testing were performed to report shelf life of herbal formulation.

Table No.1: Types of stability studies

S.No	Types of stability studies	Storage conditions	Minimum time period (Months)
1	Long Term	25±2°C and 60±5% RH or 30±2°C and 65±5% RH	12
2	Intermediate	30±2°C and 65±5% RH	6
3	Accelerated	40±2°C and 75±5% RH	6

Table No.2: According to USP types of stability

S.No	Type	Condition to be maintained
1	Chemical	Chemical integrity and labeled potency
2	Physical	Appearance, palatability, Uniformity
3	Microbiological	Sterility
4	Therapeutic	Drug action remains unchanged
5	Toxicological	No increase in toxicity

Table No.3: Report of particle size and zeta potential

S.No	Sample	Physical Nature	Avg. Particle size (d.nm)	P PDI	Avg. Zeta Potential (mV)
1	Sample syrup	Liquid	1769	0.389	-12.2

Table No.4: Report of sedimentation volume

S.No	Sample	Time (min)	Sedimentation volume			
			First week	Second Week	Third week	Fourth week
1	Syrup	0	1	1	1	1
2	Syrup	5	0.98	0.98	0.99	0.99
3	Syrup	10	0.97	0.98	0.98	0.98
4	Syrup	20	0.96	0.97	0.96	0.96
5	Syrup	30	0.96	0.97	0.96	0.96
6	Syrup	40	0.96	0.97	0.96	0.96
7	Syrup	50	0.96	0.97	0.96	0.96
8	Syrup	60	0.96	0.97	0.96	0.96
9	Syrup	75	0.96	0.97	0.96	0.96
10	Syrup	90	0.96	0.97	0.96	0.96

Table No.5: Report of ph value

S.No	Sample	First week	Second week	Third week	Fourth week
1	Syrup	6.57	6.71	6.68	6.47

Table No.6: No of strokes

S.No	Sample	First week	Second week	Third week	Fourth week
1	Syrup	12	8	9	12

Table No.7: Report of colour and odour

S.No	Week	Colour		Odour	
		Atmosphere	Refridgerator	Atmosphere	Refridgerator
1	First week	Light brown	Dark brown	Acidic odour	Acidic odour
2	Second week	Light brown	Dark brown	Acidic odour	Acidic odour
3	Third week	Light brown	Dark brown	Acidic odour	Acidic odour
4	Fourth week	Light brown	Dark brown	Acidic odour	Acidic odour

Microbiological test

Table No.8: Report of microbial test

S.No	After 1day	Observation	After 30 days	Observation
1	E.coli	Absent	E.coli	Absent
2	Klebsiella pneumoniae	Absent	Klebsiella pneumoniae	Absent
3	Staphylococcus aureus	Absent	Staphylococcus aureus	Absent

Figure No.1: Colour

Figure No.2: Viscosity

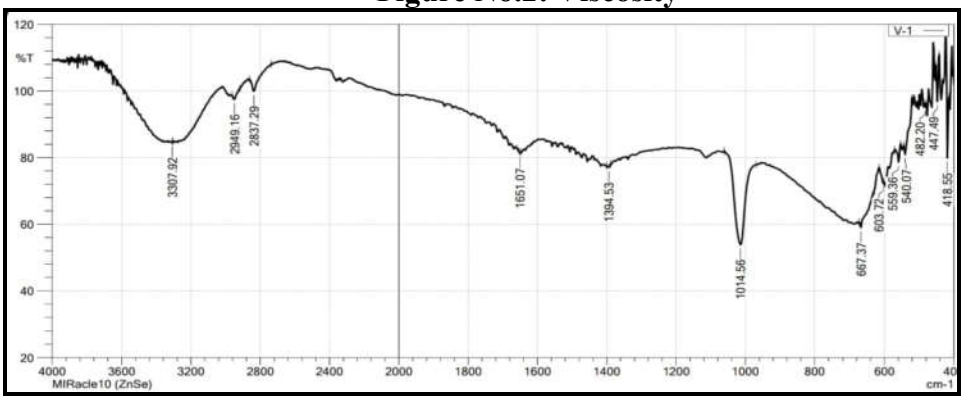

Figure No.3: FTIR

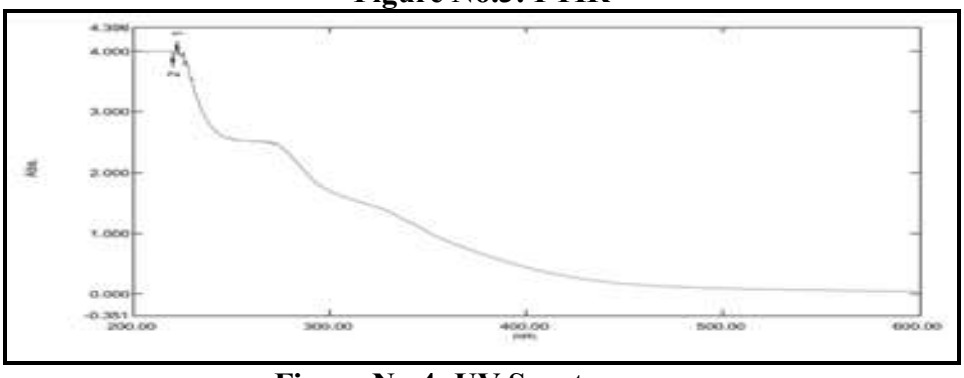

Figure No.4: UV Spectroscopy

Figure No.5: Microbial test

Figure No.6: Limit test

CONCLUSION

Herbal iron formulation was appropriate and stable under accelerated condition of storage upto 6months. The results of stability studies of the various parameters like visual appearance, nature, pH of the formulation showed that there was no significant variation when stored at appropriate temperature and relative humidity. It was concluded that, stability studies are done to determine the shelf life, expiry date, proper storage condition and to suggest some labeling instructions.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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