BRIEF REVIEW OF STABILITY STUDY- A RECENT TREND OF AYURVEDIC PHARMA INDUSTRY W.S.R. TO ACCECLERATED STABILITY STUDY OF KVGAP'S HAEMOCARE SYRUP

Abstract

Stability studies are new quality control paradigms which ensure better safety, quality and efficacy of proprietary medicines throughout their shelf life under controlled storage conditions of temperature and humidity. Accelerated stability testing of an ayurvedic proprietary medicine: KVGAP'S Haemocare syrup was done as per guidelines of Ayurvedic Pharmacopeia of India. KVGAP'S Haemocare Syrup were manufactured in 3 pilot batches, packed in the container and closure system proposed for marketing and stored at elevated stress conditions (Temperature 40 ± 2 °C, Relative humidity 75 ± 5 %.) The reference samples were stored at a temperature of less than 10 ^{0}c. Samples were evaluated at the intervals of 0, 3 and 6 months for organoleptic parameters, Physico-chemical Parameters**,** TLC, Microbial analysis and test for specific pathogens. On analysis, samples did not showed differences from the initial values of physico chemical parameters beyond the specified limits (25 percent), Neither new spots were seen in TLC plates, nor did the existing spots disappear. The product's appearance, taste, odor and color remained unchanged throughout the study period. No growth of microbes were detected and specific micro-organisms were absent in the samples throughout the study period. Hence the product remains stable in accelerated storage conditions. Shelf life of the Haemocare syrup was estimated to be 4.18 years if stored in accelerated stress conditions. This study can provide valuable information to design stability studies of ayurvedic formulations.

Keywords: Ayurvedic Pharma Industry, Haemocare Syrup, Organoleptic Parameters,

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I. INTRODUCTION

Ayurveda (Literal meaning: Science of life) with at least 2000 years of history is an Indian system of medicine. It is gaining popularity across the globe by virtue of its unique scientific principles, prevention and healing potentials. Being a holistic system of medicine, it can offer newer possibilities and dimensions for the future of medicine. One of the prime components of Ayurvedic system of medicine is medicines, which are of different origin: herbal, mineral and animal. Ayurvedic manufacturing industry is upraising, since past decades with an exponential growth in the number of manufacturing companies ans subsequent increase in number of formulations in market (both classical: prepared according to the formulae described in the books of *Ayurveda* and proprietary: patented products). Proprietary medicines are the popularly marketed formulations, numbers of such formulations are in thousands.

Like any industry, Ayurvedic drug manufacturing industry is also under the strict radar of regulatory authorities to ensure the quality of prepared medicines. Stability studies are new quality control paradigm which ensures better safety, quality and efficacy of proprietary medicines throughout their shelf life under controlled storage conditions of temperature and humidity. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, light and to establish a re-test period for the drug substance or a shelf life for the drug product and recommended storage conditions[1]. ICH guidelines[2] for stability testing provided framework for the stability studies in international realm. The stability study guidelines for Ayurvedic products were framed in Ayurvedic Pharmacopeia of India [3] which is official book prescribing standards for Ayurvedic products. Amongst types of stability studies two main types are Real time stability study and accelerated stability study. Real time stability studies are conducted in recommended storage conditions (30 ± 2 °C while the relative humidity was 60 ± 5 %) and accelerated stability studies a product is stored at elevated stress conditions (Temperature 40 ± 2 °C, while the relative humidity was 75 ± 5 %.)

Legal responsibilities and urge to provide quality products has driven the present study which was designed to evaluate the accelerated stability testing of an Ayurvedic proprietary medicine: KVGAP'S Haemocare Syrup, manufactured by GMP certified KVG Ayurveda Pharma and Research centre, Sullia, Dakshina Kannada district, Karnataka. KVGAP'S Haemocare syrup is a natural haematinic containing ingredients like *Kharjura* (Dates), *Draksha* (Grapes), *Gairika* (Red ochre) and other 15 herbs. It is usually prescribed at a dose of 10 ml Bid. It is indicated mostly as haematinic and tonic. Stability study guidelines were derived from Ayurvedic Pharmacopoeia of India and also from CCRAS (Central Council for Research in Ayurvedic Sciences) published book: Laboratory guide for the analysis of Ayurveda and Siddha formulations.[4]

II. MATERIALS AND METHODS

1. **Test Drug:** KVGAP'S Haemocare Syrup was manufactured in KVG Ayurveda Pharma and Research Centre, Ambateadka, Sullia adhering to the strict GMP guidelines.

2. **Contents of KVGAP'S Haemocare Syrup:**

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Figure 1: Showing Ingredients of KVGAP'S Haemocare Syrup

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Table 1: Showing Ingredients of KVGAP'S Haemocare Syrup
Each 5 ml of syrup contains:

Drug	Botanical Name	Amount
Draksha	*Vitis vinifera*	187mg
Kharjura	*Phoenix sylvestris*	112 mg
Ashwagandha	*Withania somnifera*	75mg
Bhoomiyamalaki	*Phyllanthus niruri*	75mg
Gairika	*Red ochre*	75 mg
Guduchi	*Tinospora cordifolia*	75 mg
Manjishta	*Rubia cordifolia*	75 mg
Mandukaparni	*Centella asiatica*	75 mg
Punarnava	*Boerrhavia diffusa*	75 mg
Sariva	*Hemidesmus indicus*	75 mg
Haritaki	*Terminalia chebula*	60 mg
Vibhitaki	*Terminalia bellirica*	60 mg
Amalaki	*Emblica officinalis*	60 mg
Yashhimadhu	*Glycyrrhiza glabra*	56 mg
Haridra	*Curcuma longa*	37.5 mg
Shunti	*Zingiber officinale*	37.5 mg
Vacha	*Acorus calamus*	30 mg
Pippali	*Piper longum*	7.5 mg
Sita	*Sugar*	2600 mg

- **Selection of batches:** KVGAP'S Haemocare Syrup was manufactured in 3 pilot batches after following methods and procedures used for commercial scale production batches. Batch size of manufactured pilot batches were $1/10^{th}$ of the commercial batch. The overall quality of the pilot batches were representative of the commercial batches.

- **Container and Closure system:** The stability studies were conducted on the dosage form packaged in the container and closure system proposed for marketing (Including appropriate packaging and container).

- **Storage Conditions:**The accelerated stability study was conducted as per the ICH guidelines. The samples are stored in stability chambers and temperature maintained during the study period was 40 ± 2 °C, while the relative humidity was 75 ± 5%. The reference samples for the above study were stored at a temperature less than $10\ ^0$c.

- **Frequency of withdrawal of the Sample:** The samples were withdrawn from the stability chamber at the intervals of 0, 3, 6 months and evaluated for relevant parameters.

- **Parameters for Evaluation:** The samples were evaluated for the following parameters at the interval of 0, 3 and 6 months**.**

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- **Organoleptic characters**
  - Colour
  - Odour
  - Taste
  - Appearance
- **Physico-chemical Parameters**
  - pH[5]
  - Viscosity[6]
  - Total solid[7]
  - Sp.gravity[8]
  - Total sugars[9]
  - Reducing sugar[10]
  - Non reducing sugar[11]
  - Refractive index[12]
  - Brix value
- **TLC**
- **IV. Microbial analysis [13]**
  - Total viable aerobic count
  - Total fungal count
  - Total Enterobacteriaceae count
- **Test for specific pathogen [14]**
  - Staphylococcus Aureus
  - Escherichia coli
  - Pseudomonas aeruginosa
  - Salmonella Species

III. RESULTS

Table 2: Showing Physicochemical parameters of KVGAP'S Haemocare Syrup - Reference Samples

Parameters	0 Month			%	S.D (+/ -)	S.E(+/ -)	t value	p value	Remarks
PH	5.28	3 months	4.68	11.30	0.488	0.345	2.12	**<0.05**	**S**
		6 months	5.30	-0.38	0.666	0.471	0.05	**>0.05**	**NS**
Viscosity	7.91	3 months	9.15	13.55	0.434	0.307	4.95	**<0.05**	**S**
		6 months	10.17	22.25	1.469	1.039	2.67	**<0.05**	**S**
Total solid	49.10	3 months	46.93	4.43	3.117	2.204	0.58	**>0.05**	**NS**

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		6 months	47.13	4.01	3.117	2.204	1.09	**>0.05**	**NS**
Sp.gravity	1.19	3 months	1.17	1.55	0.007	0.005	1.48	**>0.05**	**NS**
		6 months	1.18	0.70	0.007	0.005	2.12	**>0.05**	**NS**
Total sugars	49.67	3 months	51.76	4.05	1.085	0.767	3.35	**<0.05**	**S**
		6 months	51.71	3.95	0.906	0.641	3.91	**<0.05**	**S**
Reducing sugar	2.80	3 months	4.31	34.98	2.489	1.760	1.05	**>0.05**	**NS**
		6 months	4.28	34.58	2.366	1.673	1.08	**>0.05**	**NS**
Non reducing sugar	46.87	3 months	47.18	0.66	1.541	1.089	0.20	**>0.05**	**NS**
		6 months	47.43	1.19	1.035	0.732	0.44	**>0.05**	**NS**
Refractive index	1.40	3 months	1.40	0.43	0.005	0.004	3.93	**<0.05**	**S**
		6 months	1.40	0.36	0.005	0.004	1.73	**>0.05**	**NS**
Brix value	48.50	3 months	47.00	3.09	3.066	2.168	0.83	**>0.05**	**NS**
		6 months	47.37	2.34	3.066	2.168	0.64	**>0.05**	**NS**

Chart 1: Showing physicochemical parameters of KVGAP'S Haemocare Syrup- Reference Samples

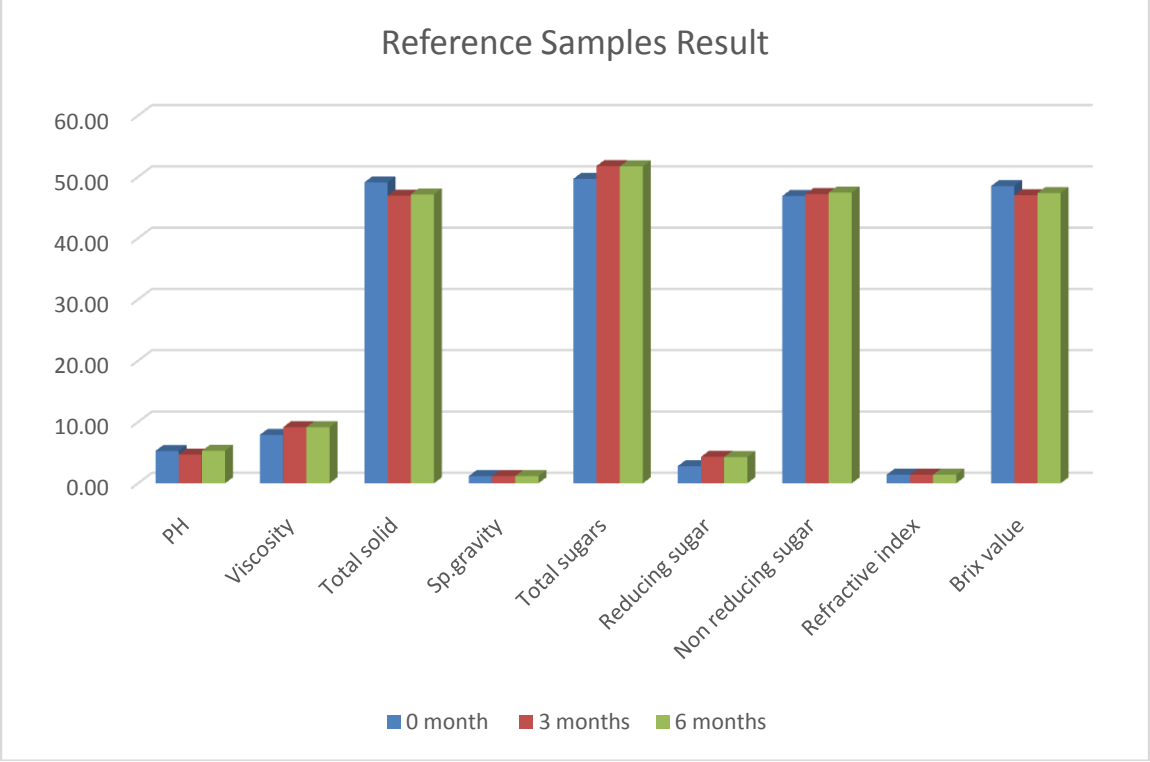

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Table 3: Showing Physicochemical parameters of KVGAP'S Haemocare Syrup – Accelerated Stability study Samples

Parameters	**0 Month**			**%**	**S.D (+/-)**	**S.E (+/-)**	**t value**	**p value**	**Remarks**
PH	5.28	3 months	4.79	9.28	0.395	0.279	1.39	**>0.05**	**NS**
		6 months	5.17	2.08	0.719	0.508	0.32	**>0.05**	**NS**
Viscosity	7.91	3 months	8.49	7.29	0.588	0.416	1.29	**>0.05**	**NS**
		6 months	9.16	15.84	0.335	0.237	3.53	**<0.05**	**S**
Total solid	49.10	3 months	48.57	1.09	6.784	4.797	0.19	**>0.05**	**NS**
		6 months	49.77	1.36	6.332	4.478	0.18	**>0.05**	**NS**
Sp.gravity	1.19	3 months	1.37	15.94	0.332	0.235	0.94	**>0.05**	**NS**
		6 months	1.18	0.14	0.015	0.011	0.15	**>0.05**	**NS**
Total sugars	49.67	3 months	52.47	5.64	0.675	0.478	3.52	**<0.05**	**S**
		6 months	51.54	3.78	0.822	0.581	2.18	**<0.05**	**S**
Reducing sugar	2.80	3 months	4.28	52.86	2.511	1.776	1.06	**>0.05**	**NS**
		6 months	4.39	56.90	2.171	1.535	1.13	**>0.05**	**NS**
Non reducing sugar	46.87	3 months	48.19	2.82	2.251	1.592	0.76	**>0.05**	**NS**
		6 months	46.48	0.82	1.066	0.754	0.20	**>0.05**	**NS**
Refractive index	1.40	3 months	1.40	0.48	0.003	0.002	4.00	**<0.05**	**S**
		6 months	1.39	0.64	0.002	0.001	2.86	**<0.05**	**S**
Brix value	48.50	3 months	46.67	3.78	2.309	1.633	5.42	**<0.05**	**S**
		6 months	47.33	2.41	3.055	2.160	4.92	**<0.05**	**S**

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Chart 2: Showing physicochemical parameters of KVGAP'S Haemocare Syrup - Accelerated stability study Samples

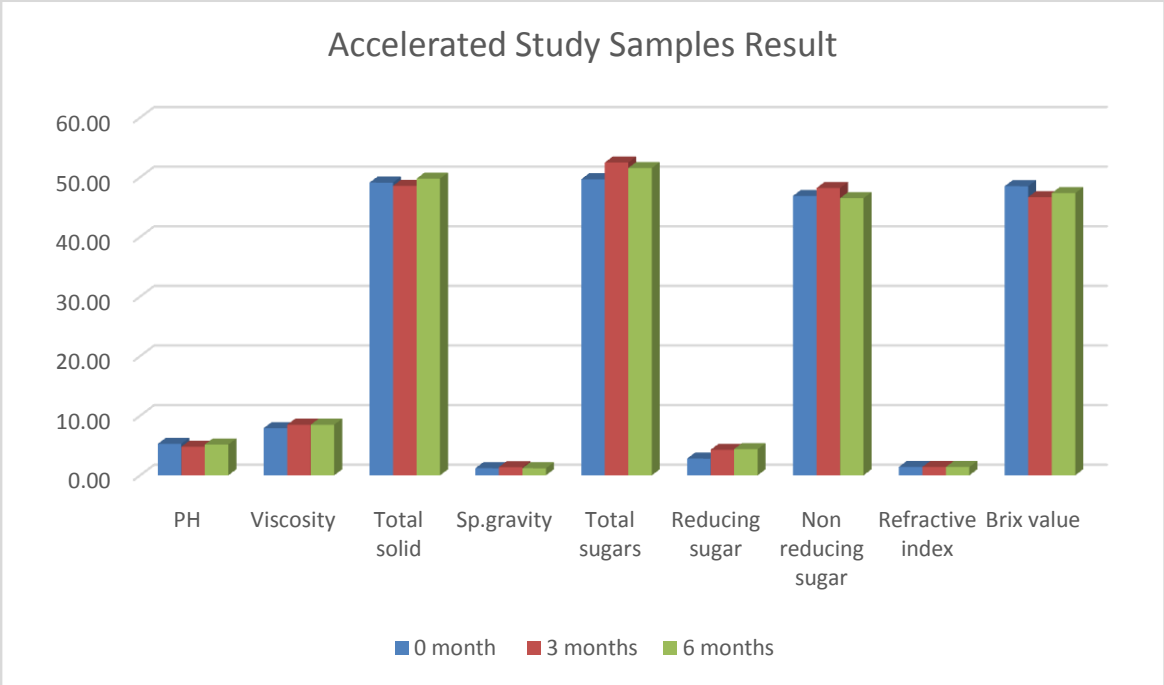

Table 4: Showing comparative result of physicochemical parameters of KVGAP'S Haemocare Syrup

Physicochemical parameters	Reference Samples: Group A	Accelerated Stability study samples: Group B	SE	T Value
PH	5.09	5.08	0.035	0.047
Viscosity	9.08	8.52	0.359	2.498
Total solid	47.72	49.15	0.791	0.996
Sp.gravity	1.18	1.25	0.082	0.999
Total sugars	51.05	51.23	0.240	0.193
Reducing sugar	3.80	3.82	0.072	0.042
Non reducing sugar	47.16	47.18	0.642	0.013
Refractive index	1.40	1.40	0.001	0.759
Brix value	47.62	47.50	0.242	0.122

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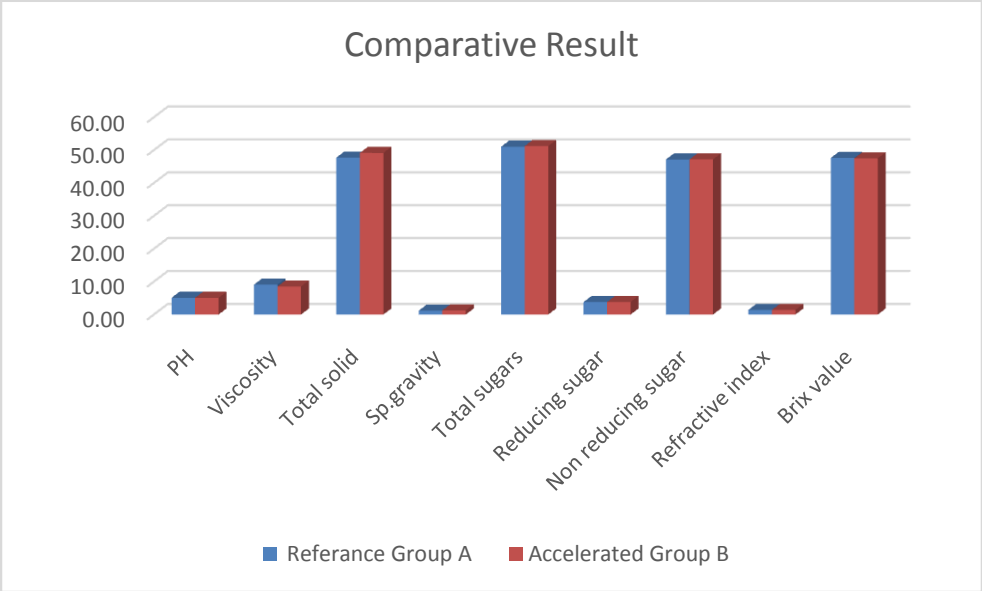
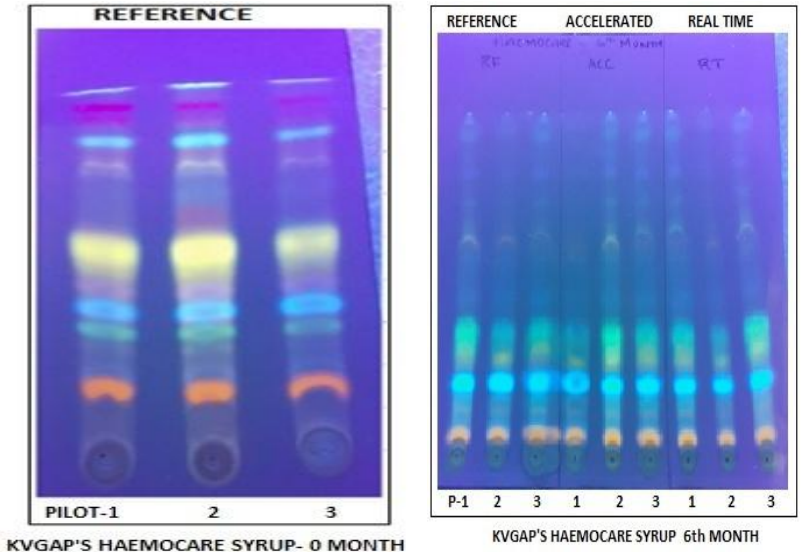
Chart 3: Showing comparative result of physicochemical parameters of KVGAP'S Haemocare Syrup



TLC

Solvent of extraction: Ethyl acetate
Mobile Phase : Toluene: Ethyl acetate: Formic acid (7: 3: 0.1)
Detection : Under UV at 366nm
Applied volume : 20µl



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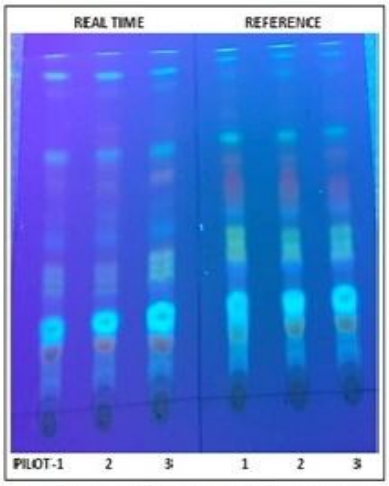

KVGAP'S HAEMOCARE SYRUP- 3rd MONTH

1. Microbiology Report Of Kvgap's Haemocare Syrup

Table 5: Showing the Microbial Analysis report of KVGAP'S Haemocare Syrup (Reference samples)

Sl. No	Parameters	0 Month	3 months	6 months	Specifications	Test Method
1	Total viable aerobic count	NIL	NIL	NIL	$<10^5$ cfug-1	API Part 1 vol 8, first edition 2011
2	Total fungal count	NIL	NIL	NIL	103 g-1	API Part 1 vol 8, first edition 2011
3	Total Enterobacteriaceae count	NIL	NIL	NIL	103 g-1	API Part 1 vol 8, first edition 2011

Table 6: Showing the specific pathogens report of KVGAP'S Haemocare Syrup (Reference Samples)

Sl. No	Parameters	0 Month	3 months	6 months	Specifications	Test Method
1	Staphylococcus Aureus	NIL	NIL	NIL	absent	API Part 1 vol 8, first edition 2011
2	Escherichia Coli	NIL	NIL	NIL	10 g-1	API Part 1 vol 8 first edition2011
3	Pseudomonas aeruginosa	NIL	NIL	NIL	Absent	API Part 1 vol 8 , first edition2011
4	Salmonella Species	NIL	NIL	NIL	Absent	API Part 1 vol 8 , firstedition 2011

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2. Microbiology Report of Accelerated Stability study samples

Table 7: Showing Microbial analysis report of KVGAP'S Haemocare Syrup (Accelerated Stability study samples)

Sl. No	Parameters	0 Month	3 months	6 months	Specifications	Test Method
1	Total viable aerobic count	NIL	NIL	NIL	$<10^5$ cfug^{-1}	API Part 1 vol 8, first edition 2011
2	Total fungal count	NIL	NIL	NIL	103 g-1	API Part 1 vol 8, first edition 2011
3	Total Enterobacteriaceae count	NIL	NIL	NIL	103 g-1	API Part 1 vol 8, first edition 2011

Table 8: Showing Specific pathogens report of KVGAP'S Haemocare Syrup (Accelerated stability study Samples)

Sl. No	Parameters	0 Month	3 months	6 months	Specifications	Test Method
1	Staphylococcus Aureus	NIL	NIL	NIL	absent	API Part 1 vol 8, first edition 2011
2	Escherichia Coli	NIL	NIL	NIL	10 g^{-1}	API Part 1 vol 8, first edition 2011
3	Pseudomonas aeruginosa	NIL	NIL	NIL	Absent	API Part 1 vol 8, first edition 2011
4	Salmonella Species	NIL	NIL	NIL	Absent	API Part 1 vol, first edition 2011

Table No 09: Showing approximate Period for 10% Degradation in Reference Sample

PhysicoChemical Parameters	Initial	10% Degradation	Approximate months for 10% Degradation
PH	5.28	4.80	144.00
Viscosity	7.91	7.19	1.91
Total solid	49.10	44.64	6.00
Sp.gravity	1.19	1.08	6.00
Total sugars	49.67	45.15	13.26
Reducing sugar	2.80	2.55	1.03
Non reducing sugar	46.87	42.61	45.38

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Refractive index	1.40	1.27	6.00
Brix value	48.50	44.09	6.00
Mean Months			25.51

Table 10: Shelf Life (Reference samples)

Months	Multiplication Factor	Shelf Life (Months)	Years
25.51	3.33	84.94	7.07

Table 11: Approximate Period for 10% Degradation in Accelerated stability study Samples

PhysicoChemical Parameters	Initial	10% Degradation	Approximate months for 10% Degradation
pH	5.28	4.80	26.18
Viscosity	7.91	7.19	3.44
Total solid	49.10	44.64	6.00
Sp.gravity	1.19	1.08	6.00
Total sugars	49.67	45.15	14.44
Reducing sugar	2.80	2.55	0.96
Non reducing sugar	46.87	42.61	66.69
Refractive index	1.40	1.27	6.00
Brix value	48.50	44.09	6.00
Mean Months			15.08

Table 12: Showing Shelf Life (Accelerated stability study Samples)

Months	Multiplication Factor	Shelf Life (Months)	Years
15.08	3.33	50.21	4.18

IV. DISCUSSION

Stability studies are mandatory requirements for marketing proprietary formulations. Hence stability study of KVGAP'S Haemocare syrup was conducted in accordance with API guidelines. Results showed no significant change in odor, taste and appearance of KVGAP'S Haemocare syrup after storing for 6 months under accelerated conditions. Physicochemical profiles (pH, Viscosity,Total solid,,Sp.gravity, Total sugars, Reducing sugar, Non reducing sugar, Refractive index,and Brix value) of the samples during the study period of 6 months didn't show much differences. The variations in the physicochemical parameters did not vary beyond 25 percent of the initial value. TLC showed no appearance of new spots and existing spots did not disappeared in subsequent months in comparison with the initial TLC plates. Microbiological analysis of the samples showed no signs of microbial growth and specific pathogens were absent in all the samples. Based on the degradation rate of the physico chemical parameters, shelf life of KVGAP'S Haemocare syrup was calculated. The reference samples shelf life was estimated to be 7.07 years. Estimated shelf life of Accelerated study samples was 4.18 years. The prescribed shelf life of Syrups as per Drug and Cosmetic act-

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1945 is 2 years [5]. Increased shelf life of the accelerated temperature samples can be attributed to the standard operative procedure followed in the preparation and proper packing of the formulation.

Though this study can profoundly pronounce the stability and shelf life an Ayurvedic proprietary formulation KVGAP'S Haemocare syrup, the way ahead of ayurvedic pharma industry is still challenging. The complexity of the ayurvedic formulation with multiple number of the ingredients makes it difficult to identify and quantify the marker compounds. For the Mammoth ayurvedic pharma industry with thousands of proprietary medicines, compiling and establishing stability studies of their products seems to be an herculean task .

V. CONCLUSION

Stability study marks an important milestone in the recent advancement of pharma industry. Ayurvedic pharma industry, major stakeholder with approximately 8000 GMP certified manufacturing units which markets thousands of proprietary medicines every year. Stability studies, mandated by the regulatory authorities aims at testing and ensuring that product remains stable throughout its shelf life in regulated storage conditions. That implies product should not be degraded with respect to its physicochemical properties beyond the specified limits. Though guidelines are available for stability studies, a viable model is the need of the hour. An accelerated stability study of KVGAP'S Haemocare syrup tries to present a model for the stability study of Ayurvedic proprietary products. 3 pilot batches of KVGAP'S Haemocare syrup were manufactured, stored in accelerated storage conditions (temperature 40 ± 2 °C, relative humidity 75 ± 5%.)and tested periodically at the interval of 0, 3 and 6 months. The samples were analyzed for its physico chemical parameters, TLC profile and microbiological analysis. Results showed samples did not showed differences from the intial values of physico chemical parameters beyoed the specified limits (25 percent). Neither new spots were seen in TLC plates, nor did the existing spots disappear.

The product's appearance, taste, odor and color remained unchanged throughout the study period. No growth of microbes were detected and specific micro-organisms were absent in the samples throughout the study period. Hence the product remains stable in accelerated storage conditions. Shelf life of the Haemocare syrup was estimated to be 4.18 years if stored in accelerated stress conditions. For the ayurvedic medicine manufacturers who are in the pursuit of viable stability study models, this study can provide valuable information to design stability studies of their formulation. Stability studies can instill new breath of air to the futuristic trends in pharmaceutical sciences by establishing and ensuring quality, safety and efficacy of medicines thus ensuring patient compliance and safety.

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