Pharmacogn. J.
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Original Article

Shelf Life Evaluation and Comparative HPTLC Profile of Hridya Yoga Churna

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ABSTRACT

Background: Quality, safety and efficacy of medicines used form the backbone of every health care system. Shelf-life is the length of time, after which all substances start to degrade in their qualities and become unsuitable for consumption or sale. The saviryatavadhi of churna is considered to be 2 months according to ayurvedic classics. The use of modern packaging technology and preservatives have increased the shelf life period of ayurvedic medicines and the shelf life period of churna is 2 years according to Rule 161-B, Drugs and Cosmetics Act. To ascertain the quality, hridya yoga churna was taken up to assess its shelf life period. **Methods:** Accelerated stability study (includes evaluation of organoleptic and physicochemical parameters, assay of active constituents and microbial load) and Comparative HPTLC (High Performance Thin Layer Chromatography) were done to evaluate the shelf life of the Hridya yoga churna. Atomic absorption spectrophotometry was done to analyze the heavy metal content in the sample. **Results and Conclusion:** In the present study the shelf life period of Hridya yoga churna is found to be 3 years and 7 months. Similar R_f values obtained in HPTLC analysis of hridya yoga churna initially and after six months showed the minimum deterioration of the product. The presence of heavy metals namely arsenic, cadmium and lead were not detected and mercury was present within permissible limits.

Key words: Accelerated stability, Shelf life, Hridya Yoga, Churna, HPTLC.

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DOI : 10.5530/pj.2016.3.10

INTRODUCTION

Shelf life or "Saviryata avadhi" means the time period during which the potency (Virya) of a drug remains unaffected due to environmental factors or from microbial contamination. Different dosage forms have different shelf life period. Pancha vidha kashaya kalpanas (five different dosage forms) are described in therapeutic system of Ayurveda, which is further classified into upakalpanas. These include different dosage forms like Swarasa (juice), Kwatha (decoction), Kalka (paste), Churna (powder), Vati (tablet), Taila (oils), Lepa-malahara (ointment), Asava and Arista (self generated alcoholic preparations), Avaleha (linctus), Bhasma (incinerated powder), Pisti (paste) etc. Among them some have very short shelf life period, Eg. Swarasa- which is to be used immediately after preparation, and some of them have long shelf life, Eg. Asava, Arishta, Bhasma etc. The information regarding the concept of Shelf life (Saviryatavadhi) for different dosage forms are found scattered in Brhatrayi. According to Acharya Charaka a drug can be utilized for therapeutic purposes until it retains its fragrance, color, taste etc.[1] During the medieval period authors attempted to compile the information regarding the concept of shelflife and Saviryata avadhi of different dosage forms are available in texts like Vangasena Samhita,[2] Sharangadhara Samhita,[3] Yogaratnakara[4] etc. The uses of modern packaging technology and preservatives have increased the shelf life period of ayurvedic medicines. The saviryata avadhi of churna given in Sarngadharasamhita is 2 months.[3] Shelf-life defined for Churna according to Rule 161-B, Drugs and Cosmetics Act is 2 years. Stability study provides evidence on how quality of a drug substance or product varies with time under influence of variety of environmental factors such as, temperature, humidity and light and also to establish a retest period for the drug substance or product and recommended storage conditions.[5] Considering the developments in pharmaceutical industry an attempt has been made to evaluate the shelf life of Hridya yoga churna of Bhavamisra.

MATERIALS AND METHODS

Preparation of the study drug

The individual drugs of the classical hridya yoga were collected and identified. Individual powder microscopy was done at Pharmacognosy unit, IPGT and RA, Jamnagar to prove the authenticity of the drug. All the drugs were powdered separately and the powder was sieved through mesh size #85. All the seven drugs were taken in equal quantity and mixed together to make the formulation. The formulation was packed in air tight food grade containers. Four containers of 50 g each were packed and stored in accelerated stability study chamber. Composition of the hridya yoga churna is given in Table 1.

Accelerated stability study

Accelerated stability study was conducted as per ICH guideline Q1. A(R2).[6] Samples were stored at 40°C ± 2°C, and 75 ± 5% relative humidity. Samples were withdrawn at the intervals of 1, 3, and 6 months. Basic analytical parameters including loss on drying at 110°C,[7] ash value,[8] pH value,[9] water soluble extractives,[10] methanol soluble extractives,[11] percentage of tannins,[12] alkaloids[13] and saponins[14] were evaluated at regular intervals. 10% degradation was set as the acceptable point to extrapolate the accelerated stability data. Real time aging factor 5 and 3.3 was used for extrapolation of shelf life for climatic Zone I & II countries and climatic Zone III & IV countries respectively. India comes under climatic zone III & IV.

Number of months when 10% degradation was occurred was calculated using following formula:

$$\text{Months when 10\% degradation occurs} = \frac{[0\ \text{month assay value} - \{(0\ \text{month assay value} \times 10/100\}] - \text{Intercept}}{\text{Slope}}$$

Table 1: Composition of Hridya Yoga

Drugs	Botanical source	Part Used	Quantity
Haritaki	*Terminalia chebula* Retz	Fruit rind	All drugs in equal quantity
Vacha	*Acorus calamus* Linn	Rhizome	
Rasna	*Alpinia galanga* (L.) Willd	Root	
Pippali	*Piper longum* Linn	Fruit	
Shunthi	*Zingiber officinale* Roscoe	Rhizome	
Shathi	*Hedychium spicatum* Sm. in A. Rees	Rhizome	
Pushkaramoola	*Inula racemosa* Hook.f	Root	

Table 2: Organoleptic parameters of Hridya Yoga at different intervels

Organoleptic Parameters	Initial	1st month	3rd month	6th month
Taste	Pungent and Bitter	OK	OK	OK
Colour	Creamish	OK	OK	OK
Odour	Charcteristic	Charcteristic	Charcteristic	Charcteristic

Table 3: Physico chemical parameters of Hridya Yoga at different intervels

Physico chemical parameters	Initial	1st month	3rd month	6th month
LOD (%)	9.51	9.24	11.28	11.79
Ash Value (%)	4.91	5.18	4.94	4.46
Acid Insoluble Ash (%)	0.63	0.68	0.65	0.62
Water Soluble extractive (%)	22.33	23.01	24.11	24.82
Alcohol Soluble extractive (%)	29.21	28.15	27.66	28.73

Table 4: Assay of Active constituents of Hridya Yoga at different intervels

Assay of Actives	Initial	1st month	3rd month	6th month
Tannin (%)	8.19	7.20	7.42	7.12
Alkaloids (%)	0.17	0.16	0.14	0.14
Saponin (%)	5.86	5.75	4.87	4.82

Table 5: Microbial load of Hridya Yoga

Microbial Test	Initial	6th month	Permissible limits as per WHO
Total plate count (cfu/g)	1447 cfu/g	1656cfu*/g	Not more than 10^5/g
Total fungal count (cfu/g)	94 cfu/g	96 cfu/g	Not more than 10^3/g
Escherichia coli	Absent	Absent	10/g
Pseudomonas aeruginosa	Absent	Absent	Absent
Staphylococcus aureus	Absent	Absent	Absent
Salmonella Spp.	Absent	Absent	Absent

* cfu-colony forming unit.

Table 6: Heavy Metal Analysis of Hridya Yoga

Heavy Metal by AAS	Results	Permissible limits as per WHO
Arsenic	ND (Not Detected)	Not more than 3 ppm*
Lead	ND	Not more than 10 ppm
Mercury	0.217 ppm	Not more than 1 ppm
Cadmium	ND	Not more than 0.3 ppm

*parts per million.

Table 7: Intercept and Slope

Parameters	Intercept	Slope
LOD	9.35	0.44
Ash Value (%)	5.11	0.094
Acid Insoluble Ash (%)	0.67	0.004
Water Soluble extractive (%)	22.54	0.409
Alcohol Soluble extractive (%)	28.54	0.042
Tannin (%)	7.78	0.126
Alkaloids (%)	0.165	0.005
Saponin (%)	5.80	0.19

Table 8: Approximate period for 10% Degradation

Parameters	Initial	10% Degradation	Months required for 10% degradation
LOD	9.51	8.559	1.78
Ash Value (%)	4.91	4.42	7.35
Acid Insoluble Ash (%)	0.63	0.57	25.75
Water Soluble extractive (%)	22.33	20.10	5.95
Alcohol Soluble extractive (%)	29.21	26.29	53.59
Tannin (%)	8.19	7.37	3.25
Alkaloids (%)	0.17	0.15	2.4
Saponin (%)	5.86	5.27	2.77
Mean Months			12.855 Months

Table 9: Extrapolation of Shelf life

Drug	Mean Months for 10% degradation	Multiplication Factor	Shelf life	
			Months	Years
	12.855	3.33	42.81	3 Years 7 Months

Table 10: R_f at 254 nm

Track 1	Track 2
0.14	0.14
0.32	0.32
0.46	0.46
0.61	0.61
0.75	0.75
0.82	0.82
0.96	0.96

Table 11: R_f at 366 nm

Track 1	Track 2
0.14	0.14
0.32	0.32
0.38	0.38
0.42	0.42
0.46	0.46
0.52	0.52
0.69	0.69
0.75	0.75

Table 12: R_f at 540 nm

Track 1	Track 2
0.07	0.07
0.14	0.14
0.23	0.23
0.32	0.32
0.46	0.46
0.52	0.52
0.61	0.61
0.75	0.75
0.90	0.90
0.96	0.96

Heavy metal Analysis

Heavy metal analysis was performed using Atomic absorption sprectrophotometer as per the methods described in WHO guidelines.[15] Atomic absorption spectroscopy (AAS) is a spectroanalytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state.

Microbial load

Estimation of Microbial load was carried out as per standard procedure mentioned in Indian Pharmacopoeia initially and at 6 months. It included Total bacterial count, Total Fungal Count, Presence of Escherichia coli, Salmonella species, Pseudomonas aeruginosa and Staphylococcus aureus. Pure culture of Escherichia coli (NCIM: 2065; ATCC: 8739), Salmonella Spp. (NCIM: 2257 NCTC: 6017), Pseudomonas aeruginosa (ATCC 9027), Staphylococcus aureus (ATCC 6358) were obtained from NCIM Pune. The media used for the microbial limit test were of HiMedia Pvt. Ltd.

HPTLC Analysis

The HPTLC (High Performance Thin Layer Chromatography) finger print profile of methanolic extract of Hridya yoga was taken on aluminium plate coated with silica gel 60 F254 (E. Merck) as adsorbent and employing CAMAG Linomat 5 applicator. The mobile phase used was Toluene: Ethyl acetate: Formic acid (10:3:1). For derivatization Anisaldehyde Sulphuric acid reagent was used and heated at 100 ± 5°C till the spots appeared. The plate was dried and visualized under UV 254 nm, 366 nm and 540 nm. HPTLC finger prints were taken initially and after 6 months.

RESULTS AND DISCUSSION

The main purpose of conducting stability testing of pharmaceutical products is to ensure the efficacy and quality of active compounds in product and to establish shelf life or expiry period. No considerable changes were observed in organoleptic characters (Table 2) and microbial load (Table 5) of the Hridya yoga churna even after 6 months. Results of different physico-chemical parameters (Table 3) and active constituent assays (Table 4) were taken in consideration to evaluate intercept and slope (Table 7). Extrapolated shelf life of Hridya yoga *Churna* (Table 9) was calculated with 10% degradation rate (Table 8) from physicochemical parameters at accelerated condition 40°C ± 2 and 75% ± 5 RH. On the basis of available data from accelerated stability study, it can be extrapolated that shelf life of *Hridya yoga Churna* is 42.81 months (3.07 years) for countries which come under climatic zone III & IV.

Ayurvedic medicines were alleged to contain heavy metals and for the purpose of establishing the safety and efficacy, heavy metal analysis of the Hridya yoga churna was carried out (Table 6). The presence of arsenic, lead and cadmium were not detected in the Hridya yoga churna. Even though Mercury was detected (.217 ppm), it was within the permissible limits.

HPTLC finger print studies of methanolic extract of Hridya yoga were carried out and chromatogram was developed and detected using the UV visible chamber, initially and after 6 months. Seven spots were identified at UV 254 nm in the chromatogram as depicted in Figure 1 and 2. The corresponding R_f values of the seven components are 0.14, 0.32, 0.46, 0.61, 0.75, 0.82, 0.96 (Table 10). Eight spots were identified at 366 nm

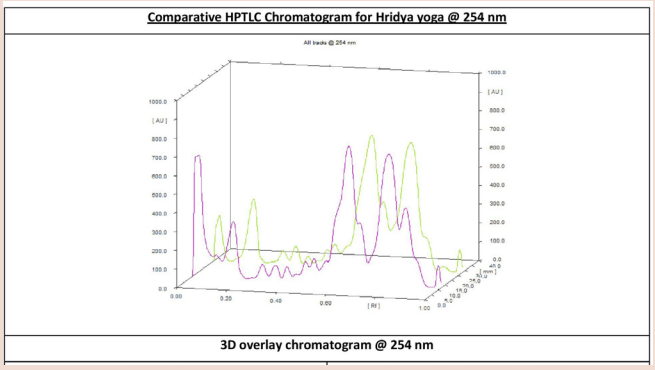

Figure 1: Comparative HPTLC Chromatogram for Hridya yoga at 254 nm.

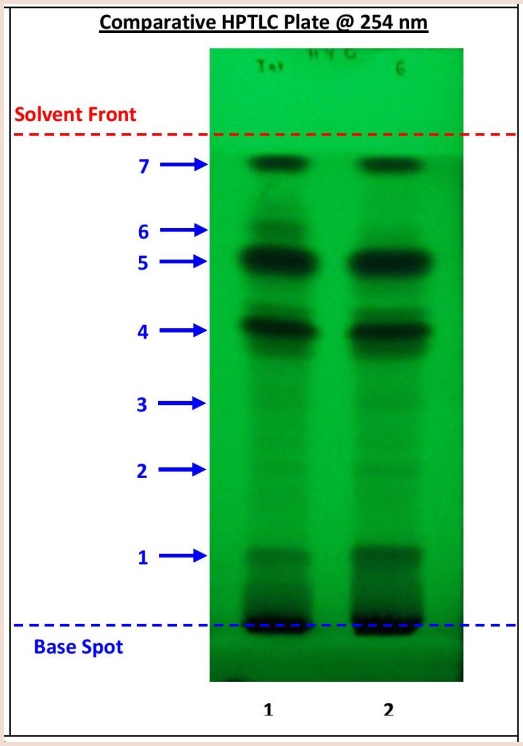

Figure 2: Comparative HPTLC plate at 254 nm.

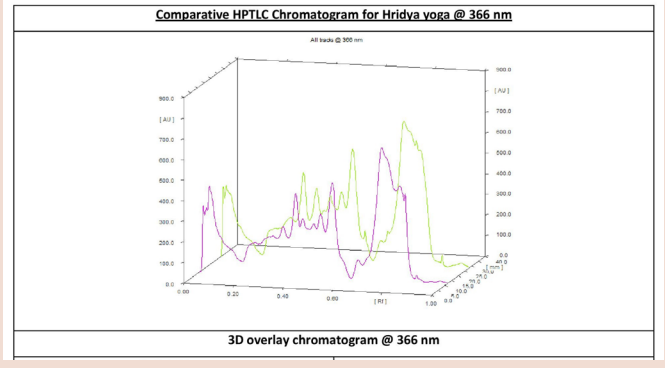

Figure 3: Comparative HPTLC Chromatogram for Hridya yoga at 366 nm.

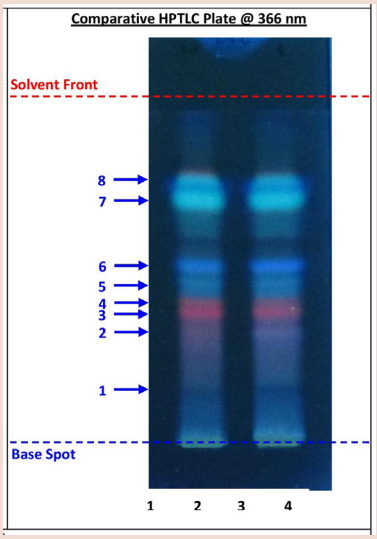

Figure 4: Comparative HPTLC plate at 366 nm.

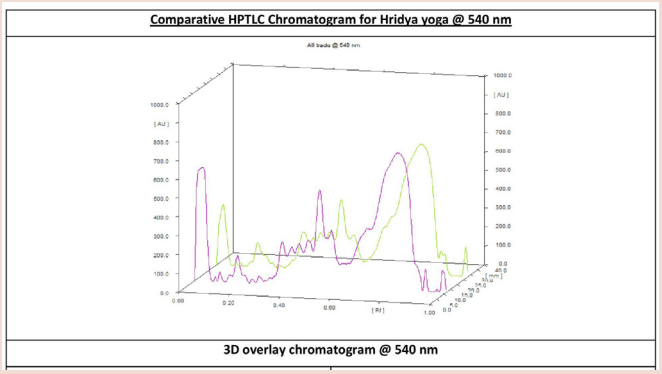

Figure 5: Comparative HPTLC Chromatogram for Hridya yoga at 540 nm.

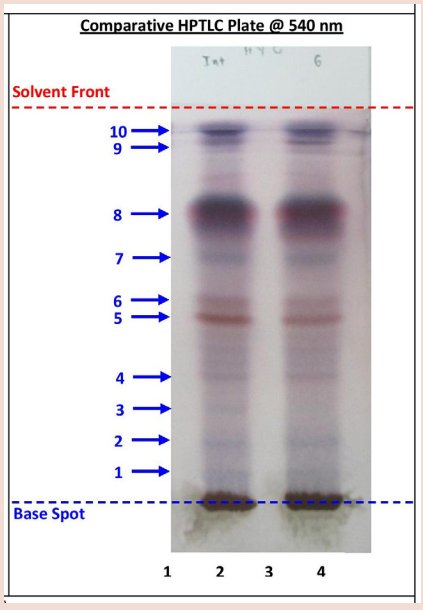

Figure 6: Comparative HPTLC plate at 540 nm.

(Figure 3 and 4). The corresponding R_f values are 0.14, 0.32, 0.38, 0.42, 0.46, 0.52, 0.69, 0.75 (Table 11). Ten spots were identified at 540 nm (Figure 5 and 6). The corresponding R_f values are 0.07, 0.14, 0.23, 0.32, 0.46, 0.52, 0.61, 0.75, 0.90, 0.96 (Table 12). All the spots identified in the hridya yoga extract initially were identified at six months also which shows the minimum deterioration of the product.

CONCLUSION

Shelf−life defined for Churna at Rule 161−B, Drugs and Cosmetics Act is 2 years. In the present study the quality of Hridya yoga churna is assessed by evaluation of shelf life. On the basis of the accelerated stability study conducted the shelf life period of Hridya yoga churna is found to be 3 years and 7 months. Similar R_f values obtained in HPTLC analysis of hridya yoga churna initially and after six months showed the minimum deterioration of the product. The presence of heavy metals namely arsenic, cadmium and lead were not detected and mercury was present within permissible limits.

ACKNOWLEDGEMENT

Vasu Research Centre, Vadodara. Dr Galib. Asst. Professor, Dept.of Rasasastra & Bhaishajyakalpana, IPGT and RA, Jamnagar.

CONFLICT OF INTEREST

The author decalre no conflict of interest.

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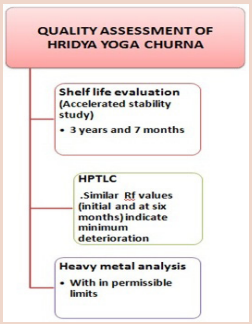
PICTORIAL ABSTRACT



SUMMARY

- The quality of Hridya yoga churna was evaluated by assessment of shelf life.
- On the basis of the accelerated stability study conducted, the shelf life period of Hridya yoga churna is found to be 3 years and 7 months
- Similar R_f values in HPTLC analysis(initial and at six months) also supports the findings.

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