Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

ISSN 2278-4136
ISSN 2349-8234
JPP 2014; 2 (5): 126-130
Received: 17-11-2013
Accepted: 18-12-2013

Biswajyoti Patgiri
Department of Rasashastra & Bhaishajya Kalpana, Institute for Post Graduate Teaching and Research in Ayurveda (IPGT&RA), Gujarat Ayurved University, Jamnagar – 361 008, Gujarat, India.

Hardik Soni
Vasu Research Centre (A Division of Vasu Healthcare Pvt. Ltd.), 896/A, GIDC, Makarpura, Vadodara – 390 010, Gujarat, India.

Surendra Bhatt
Vasu Research Centre (A Division of Vasu Healthcare Pvt. Ltd.), 896/A, GIDC, Makarpura, Vadodara – 390 010, Gujarat, India.

Correspondence
Hardik Soni
Assistant Manager, R&D
Vasu Research Centre (A Division of Vasu Healthcare Pvt. Ltd.)
896/A, GIDC, Makarpura, Vadodara – 390 010, Gujarat
Tel: 0265-2657701, 2657702
E-mail: hsoni@vasuresearch.com;
E-mail:hardik_ayurved@yahoo.com

Evaluation of stability study of Ayurvedic formulation – *Rasayana Churna*

Biswajyoti Patgiri, Hardik Soni*, Surendra Bhatt

ABSTRACT

The objective of the present study was to evaluate stability study of *Rasayana Churna*. Accelerated stability study (Temperature: 40 °C ± 2, Relative Humidity (RH): 75% ± 5) and real time stability study (Temperature: 25 °C ± 2, Relative Humidity (RH): 60% ± 5) was conducted as per ICH guideline Q1A (R2). The change in organoleptic parameters, physic-chemical parameters and microbial load was observed 6 month for accelerated stability and 1 year for real time stability study at an interval of 0,1,3,6 and 12 months. Real time stability was comparatively carried out to evaluate actual degradation rate of *Rasayana Churna* with respect to accelerated condition. No change was observed in color, odour and taste of *Rasayana Churna* up to storage of 6 months at accelerated condition. Results of different physico-chemical parameters were taken in consideration to evaluate intercept and slope. Extrapolated shelf life of *Rasayana Churna* was calculated with 10% degradation rate from physico-chemical parameters at accelerated condition 40 °C ± 2 and 75% ± 5 RH. The present investigation supports that the *Rasayana Churna* was suitable at accelerated condition up to 6 month storage. it can be extrapolated that shelf life of *Rasayana Churna* is 25.12 months (2.09 years) for countries which comes under climatic zone I & II and 16.60 months (1.38 years) for countries which comes under climatic zone III & IV. Real time stability data of *Rasayana Churna* showed very good stability up to 1 year.

Keywords: *Rasayana Churna*, accelerated stability study, Real time Stability, Physic-chemical Parameters.

1. Introduction

A reference to various authentic Ayurvedic texts reveals that aspect of shelf-life has been recognized already and some of the *granthas* have actually given guidance regarding factors that make formulations degrade or become unfit for use, and in specific cases in certain dosage forms have actually prescribed the period from the date it was compounded within which such dosage form should be used. Ayurvedic Formulary of India also has given the time period from the date of manufacture within which the formulations should be consumed for best results [1]. In Ayurvedic literatures, "*Saviryata avadhi*" term is mentioned in context of the time period during which the *Virya* (potency) of any drug remains unaffected [2] due to environmental / microbial deterioration.

India has well documented and traditionally well practiced knowledge of Ayurvedic medicines. The most important challenge faced by Ayurvedic formulations arises from lack of complete evaluation of its constituents, due to its complex nature. Evaluation of constituents is necessary to ensure quality, purity and stability of the finished product. 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. So we can say stability study is necessary as an assessment of product quality [3].

There is a two type of stability study one is accelerated stability and second is real time stability. Pharmaceutical products are generally studied for stability profile at accelerated temperature and humidity, the experimental findings of which can be very helpful to predict reliable self-life or expiry date at room temperature by adopting certain assumptions and criterions [4]. Every product has definite shelf-life which depends on various physical, chemical, environmental and biological factors. Real time study is a long procedure. The manufacturer finds it difficult to wait till the drug degrades naturally at room temperature.

Churna (powder) preparations are widely and largely used in pharmacy as well as by practitioners of Ayurveda for different ailments. According to Ayurvedic Pharmaceutical science, *Churna* preparations remain potent up to two months [2] , after which they start degrading gradually thus losing their efficacy. Nevertheless, they don't become de-efficacious to that much level that can't be employed for therapeutic uses. The two months of potency span or shelf-life of *Churna* preparations is explained in the context of the storage techniques and packaging methods of those days of *Sharangadhara* i.e. 13th century A.D. So, storage condition becomes a most important aspect which affects on shelf-life of the product.

Till date, no specific guidelines are available regarding the stability / shelf life estimation of the pure Ayurvedic formulations from any Government organization except a Gazette notification issued by Government of India on 20th October, 2009 with slight modification in the earlier draft notification issued on 26th November, 2005.[5,6] In present study, stability study was carried out to determine shelf-life of *Rasayana Churna*.

2. Materials and Methods

2.1 Test drug – *Rasayana Churna*

Rasayana Churna is a traditional Ayurvedic formulation, which comprises dried powders of three well known rejuvenating drugs viz. dried stem of Guduchi (*Tinospora cordifolia* Miers.), dried fruit of Gokshur (*Tribulus terrestris* Linn.) and dried pericarp of Amalaki (*Emblica officinalis* Gaertn.) in equal proportion **[7]**.

A freshly prepared *Rasayana Churna* was considered for stability study. *Rasayana Churna* was packed in air tight food grade plastic container having aluminum foil covering.

2.2 Storage condition and evaluation parameters

Accelerated stability study and real time stability study was conducted as per ICH guideline Q1. A (R2). **[8]** Storage condition are mentioned as below,

- Accelerated stability : Temperature: 40 °C ± 2, Relative Humidity (RH): 75 % ± 5
- Real time stability : Temperature: 25 °C ± 2 , Relative Humidity (RH): 60 % ± 5

The change was observed during 6 month for accelerated stability and 1 year for real time stability study at an interval of 0,1,3,6 and 12 months. Real time stability was comparatively carried out to evaluate actual degradation rate of *Rasayana Churna* with respect to accelerated condition.

10% degradation was set to extrapolate of the accelerated stability data as the acceptable point. 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. Ambient temperature and humidity for Zone I & II countries are 21 °C/ 45 %RH and 25°C/60%RH respectively. For Zone III & IV countries 30 °C/ 35 %RH and 30 °C/70 %RH respectively. India comes under climatic Zone III & IV. [8]

The following parameters were considered for evaluation of stability study.

- Organoleptic characters like colour, odour and taste
- Physico-chemical parameters like Loss on drying, pH, Total ash, Water soluble extractive value, bitter residue, total saponin and total tannin.
- Microbial load

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

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

2.3 Examination of color, odour and taste

Color: Five gram *Rasayana Churna* was taken into watch glasses and placed against white background in white tube light. It was observed for their color by naked eye.

Odour: Two gram *Rasayana Churna* was smelled.

Taste: A pinch of *Rasayana Churna* was taken and examined for it's taste on taste buds of the tongue.

2.4 Determination of loss on drying

Loss on drying was determined by weighing about 2gm of the powdered material in previously weighed dried petridish (tarred evaporating dish) and dried in an oven at 105-110 ºC, till two consecutive weights, which do not differ by more than 5mg. The weight after drying was noted and loss on drying was calculated. The percentage was expressed as % w/w with reference to air-dried sample **[9]**.

2.5 Determination of pH

Placed accurately weighed 1 g of *Rasayana Churna* in a 100 mL volumetric flask and made up the volume up to 100 mL with distilled water. The solution was sonicated for about 10 minutes. pH was measured with the help of digital pH meter.

2.6 Determination of total ash

The ash value was determined by incinerating about 1 g of the powdered air-dried material, in a previously weighed crucible at gradually increasing heat up to 500-600 ºC until it is carbon free. Then cooled in a desiccator and weighed. The percentage of total ash was calculated and expressed as % w/w of air dried material **[9]**.

2.7 Determination of water soluble extractive value

About 5 g accurately weighed *Rasayana Churna* was macerated in a glass-stopper conical flask. 100 mL chloroform water was added and macerated for 6 h, shaking frequently and then allowed to stand for 18 h then after 24 h it was filtered rapidly and 20 mL of the filtrate was transferred in a tarred flat bottom evaporating dish with a pipette and evaporated to dryness on a boiling water bath. Then evaporating dish was dried at 105ºC for 6 h and then cooled and weighed. From the weight of the residue the percentage of water soluble extractive was calculated and expressed as % w/w with reference to air dried sample **[9]**.

2.8 Estimation of bitter residue

1 g of the test material was taken in a 150 mL conical flask. To it 50 mL of methanol was added. It was refluxed for half an hour on a water bath. Then filtered and collected the methanol extract in a 250 mL beaker. The residue was extracted for another two cycles of extraction. Three (or all the extracts if greater than 3) methanol extracts were pooled and evaporated it to obtain a thick paste (not free flowing) approximate 5 mL volume. Now shake the concentrated extract with three successive cycles of 25 mL hot water or till all the water soluble matter is extracted or dissolved. Above three (or greater than 3) water washed extracts were pooled and transferred it to a separating funnel. This aqueous extract was extracted with minimum 4 cycles of 25 mL of petroleum ether (60-80 ^{0}C).Then extract the petroleum ether washed aqueous extract with 25 mL of ethyl acetate. Ethyl acetate extraction was repeated for another three more cycles. The ethyl acetate extracts were pooled and transferred to a pre-weighed evaporating dish and evaporated to dryness. From the weight of the residue the percentage of bitter residue was calculated and expressed as % w/w with reference to air dried sample [10].

2.9 Estimation of Total Saponin

5 g of test material was weighed in conical flask. To this 50 mL 90%v/v methanol was added. Content was mixed well and refluxed for half an hour. After cooling it was filtered. Wash the residue with 90%v/v methanol till washing were almost colorless. Methanol extract was combined and evaporated on water bath to obtain a thick paste like residue. Residue was treated with 25 mL petroleum ether (60-80 ^{0}C). Petroleum ether layer was separated and discarded. Residue was treated with 25 mL chloroform. Chloroform layer was separated and discarded. Residue was treated with 25 mL ethyl acetate. Ethyl acetate layer was separated and discarded. Then 5 mL 90%v/v methanol was added in residue. Content was shake well to dissolve the residue completely. Now pour this solution drop wise with constant stirring into a beaker containing 25 mL acetone to obtain precipitate. Rinse the flask containing the residue with minimum volume (about 2 mL) of 90%v/v methanol. Decant the organic layer and dry the residue to constant weight. The percentage of total saponin was calculated and expressed as % w/w with reference to air dried sample [11].

2.10 Estimation of Total Tannin

2.10.1 For blank: 300 mL of distilled water was taken in a 500 mL conical flask. 25 mL of indigo sulphonic acid solution was added and mix well. Titrated against 0.02M $KMnO_4$ solution till stable golden yellow color was developed. The burette reading was noted.

2.10.2 For sample: Accurately weighed about 0.05 g of the sample. Material was transferred to a 500 mL conical flask. To it 50 mL of distilled water was added and mixed well to dissolve completely. To this 250 mL of distilled water was added and mixed well, than sonicate it for 10 min. 25 mL of indigo sulphonic acid solution was added and mixed well. Titrated against 0.02M $KMnO_4$ solution till stable golden yellow color was developed. The burette reading was noted. The percentage of total tannin was calculated using following factor.

1 mL of 0.02M $KMnO_4$ is equivalent to 0.00415g of tannin substance [12].

2.11 Microbial load

Microbial load was carried out as per standard procedure mentioned in Indian Pharmacopoeia [13]. It included Total bacterial count, Total Fungal Count, Presence of *Escherichia coli*, *Salmonella spices*, *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.

3. Results

In the accelerated stability study, Temperature: 40°C ± 2, Relative Humidity (RH): 75% ± 5 was maintained up to 6 months. The product was analyzed on 0, 1, 3 and 6 month. No change was noticed in color, odour and taste of *Rasayana Churna* up to storage of 6 months at accelerated condition (Table 1). Results of microbial load of *Rasayana Churna* was complies with Ayurvedic Pharmacopeial limits at initial month and up to 6 month (Table 1).

Table 1: Results of different parameters of *Rasayana Churna* at 40 °C ± 2 and 75% ± 5 RH in different intervals

Sr. No.	Parameters	Initial Month	1st Month	3rd Month	6th Month
1	Colour	Greenish brown	Complies	Complies	Complies
2	Odour	Characteristic	Complies	Complies	Complies
3	Taste	Bitter & Astringent	Complies	Complies	Complies
4	Loss on drying (% w/w)	3.62	4.20	5.21	6.42
5	pH value (1% w/v solution)	5.2	5.7	6.0	6.2
6	Total ash (%w/w)	6.55	6.42	6.34	6.14
7	Water soluble extractive value (%w/w)	57.86	56.17	51.47	54.48
8	Bitter residue (%w/w)	3.86	3.74	3.41	2.81
9	Total saponin (%w/w)	16.27	15.42	15.11	13.41
10	Total tannin (%w/w)	30.12	29.75	28.42	27.42
11	Total bacterial count (CFU/g)	$32x10^3$	$56x10^3$	$24x10^4$	$35x10^5$
12	Total yeast and mould (CFU/g)	$18x10^1$	$20x10^1$	$48x10^2$	$74x10^3$
13	*E. coli*	Absent	Absent	Absent	Absent
14	*S. spp*	Absent	Absent	Absent	Absent
15	*S.aureus*	Absent	Absent	Absent	Absent
16	*P.areuginosa*	Absent	Absent	Absent	Absent

Results of different physico-chemical parameters were taken in consideration to evaluate intercept and slope (Table 2). Extrapolated shelf life of *Rasayana Churna* was calculated with 10% degradation rate from physico-chemical parameters at accelerated condition 40°C ± 2 and 75% ± 5 RH (Table 3).

Table 2: Intercept and slope of different physico-chemical parameters of *Rasayana Churna*

Condition: 40 °C ± 2 and 75% ± 5 RH						
Parameters	**Initial Month**	**1st Month**	**3rd Month**	**6th Month**	**Intercept**	**Slope**
LOD	3.62	4.20	5.21	6.42	3.70	0.46
pH	5.2	5.7	6.0	6.2	0.72	4.80
Total ash	6.55	6.42	6.34	6.14	6.52	0.06
Water soluble extractive	57.86	56.17	51.47	54.48	56.48	0.59
Bitter residue	3.86	3.74	3.41	2.81	3.89	0.18
Total saponin	16.27	15.42	15.11	13.41	16.16	0.44
Total tannin	30.12	29.75	28.42	27.42	30.09	0.46

Table 3: Extrapolated shelf life of *Rasayana Churna* from different physico-chemical parameters

Condition: 40 °C ± 2 and 75% ± 5 RH			
Parameters	**Result at Initial Month**	**Result at 10% Degradation**	**Months when 10% degradation occurs**
LOD	3.62	3.26	0.96
pH	5.2	4.68	4.80
Total ash	6.55	5.89	9.80
Water soluble extractive	57.86	52.07	7.41
Bitter residue	3.86	3.47	2.39
Total saponin	16.27	14.64	3.42
Total tannin	30.12	27.11	6.42
Mean Months at accelerated condition			**5.03**
Climatic zone I & II			**25.15 Months (2.09 years)**
Climatic zone III & IV			**16.60 Months (1.38 years)**

In the real time stability study, Temperature: 25°C ± 2, Relative Humidity (RH): 60% ± 5 was maintained up to 1 year. The product was analyzed on 0, 1, 3, 6 month and 1 year. It was carried out to check results of *Rasayana Churna* on Organoleptic characters, different physico-chemical parameters and microbial load at optimal condition. Results showed stability of *Rasayana Churna* at real time condition in Table 4.

Table 4: Results of different parameters of *Rasayana Churna* at 25 °C ± 2 and 60% ± 5 RH in different intervals

Condition : 25°C ± 2 , RH: 60% ± 5						
Sr. No.	**Parameters**	**Initial Month**	**1st Month**	**3rd Month**	**6th Month**	**1 year**
1	Colour	Greenish brown	Complies	Complies	Complies	Complies
2	Odour	Characteristic	Complies	Complies	Complies	Complies
3	Taste	Bitter & Astringent	Complies	Complies	Complies	Complies
4	Loss on drying (% w/w)	3.62	3.65	3.64	4.41	5.02
5	pH value (1% w/v solution)	5.2	5.2	5.4	5.82	5.91
6	Total ash (%w/w)	6.55	6.57	6.54	6.32	6.27
7	Water soluble extractive value (%w/w)	57.86	57.91	57.58	57.68	56.83
8	Bitter residue (%w/w)	3.86	3.82	3.85	3.75	3.64
9	Total saponin (%w/w)	16.27	16.30	16.21	16.13	15.83
10	Total tannin (%w/w)	30.12	29.89	29.91	29.85	29.27
11	Total bacterial count (CFU/g)	$32x10^3$	$43x10^3$	$45x10^3$	$52x10^3$	$3x10^4$

12	Total yeast and mould (CFU/g)	$18x10^1$	$20x10^1$	$28x10^1$	$34x10^1$	$58x10^1$
13	*E. coli*	Absent	Absent	Absent	Absent	Absent
14	*S. spp*	Absent	Absent	Absent	Absent	Absent
15	*S.aureus*	Absent	Absent	Absent	Absent	Absent
16	*P.areuginosa*	Absent	Absent	Absent	Absent	Absent

4. Discussion

Stability is aimed at assuring that the product remains within specifications established to ensure its identity, strength, quality and purity. It can be interpreted as length of time under specific conditions and storage that a product will remain within the pre-defined limits for all its important characteristics. The main purpose of conducting stability testing of pharmaceutical products is to ensure the efficacy and quality of active compounds in product, to establish shelf life or expiration period and to support the label claim. The stability data on any dosage form includes selected parameters that together form the stability profile. This stability profile is the basis for assigning the storage conditions and shelf life to pharmaceutical products. The design of the stability program for the finished product should be based on the knowledge of the behavior and properties of the drug substance and the dosage form [14-16].

As per ICH guideline, countries comes under climatic zones I and II having climatic condition 21 °C/ 45% RH and 25 °C/60% RH respectively. Countries comes under climatic zones III and IV having climatic condition 30 °C/35 % RH and 30 °C/70% RH. India comes under climatic zone III & IV [8].

On the basis of available data form accelerated stability study, it can be extrapolated that shelf life of *Rasayana Churna* is 25.12 months (2.09 years) for countries which comes under climatic zone I & II and 16.60 months (1.38 years) for countries which comes under climatic zone III & IV. It was calculated with consideration of 10% degradation rate in different physico-chemical parameters. No considerable change was observed in organoleptic characters and microbial load even after 6 months accelerate study.

As per Drug and Cosmetic act, the optimal climatic condition for the storage of medicine is 25 °C/60% RH. If we can maintain same climatic condition for the storage of *Rasayana Churna* then shelf life of *Rasayana churna* is near to 2.09 years. It is matched with the implemented rule namely 161 B to display the date of expiry of the ASU drugs and propose shelf life of *Ayurvedic* formulations i.e. shelf life of *Churna* (fine / course powder drugs) as 2 years.

5. Conclusion

The present investigation supports that the *Rasayana Churna* was suitable at accelerated condition up to 6 month storage. it can be extrapolated that shelf life of *Rasayana Churna* is 25.12 months (2.09 years) for countries which comes under climatic zone I & II and 16.60 months (1.38 years) for countries which comes under climatic zone III & IV. Real time stability data of *Rasayana Churna* showed very good stability up to 1 year.

6. Acknowledgement

Authors are sincerely thankful to the management of Vasu Healthcare Pvt. Ltd. for state of the art testing facility to carry out this research work.

7. References

1. Anonymous. Ayurvedic Pharmacopoeia of India (API). Part I, Vol.1, 1st Ed. Govt. of India, Ministry of Health and Family Welfare, Dept. of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy, New Delhi, 2001.
2. Sastri P. Sharangadhara Samhita written by Acharya sharangadhara with commentary. Published by Choukhambha Orientalia, Varanasi, 4th edition, 2002: 13.
3. K Bankoti, MS Rana, MK Bharadwaj. Accelerated stability study of herbal capsules. *IOSR Journal of Pharmacy* 2012;2(5):1-6.
4. Cannors KA, Amidon GL and Kennon L. Chemical Stability of Pharmaceuticals - A handbook of Pharmacists. John Wiley & Sons, New York, 1979.
5. Anonymous. The Gazette of India, Extraordinary Part-II, Section-3; Sub-section (i) No. 605, New Delhi, Tuesday, 20th October, 2009.
6. Anonymous. The Gazette of India, Extraordinary Part-II, Section-3; Sub-section (i) No. 482, New Delhi, Saturday, 26th November, 2005.
7. Vagbhata. Ashtanga Hridayam, with the commentaries, 'sarvangasundara' of Arunadatta and 'Ayurvedarasayana' of Hemadri collated by Dr. Anna Moreshvara kunte, and Krishna Ramachandra Shastri Navre, edited by pt. Harishastri Paradakar Vaidya, Gopal Mandir Lane, Uttar sthan, chapter 39/160. Varanasi: Krishanadas Academy; 2000. pp. 937.
8. Anonymous. ICH Harmonised Tripartite Guideline. Stability testing of new drug substances and products – Q1A (R2). 2003 Feb.
9. Anonymous. Ayurvedic Pharmacopoeia of India (API). Part I, Vol.1, 1st Ed. Govt. of India, Ministry of Health and Family Welfare, Dept. of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy, New Delhi, 2001; pp. 143.
10. Rajpal V. Standardization of Botanicals. Vol.1, Eastern Publishers, New Delhi, India, 2002, pp. 88.
11. Rajpal V. Standardization of Botanicals. Vol.1, Eastern Publishers, New Delhi, India, 2002, pp. 226.
12. Rajpal V. Standardization of Botanicals. Vol.1, Eastern Publishers, New Delhi, India, 2002, pp. 247.
13. Anonymous. Indian Pharmacopeia. Published by the Indian Pharmacopoeia Commission Ghaziabad, Government of India, Ministry of Health & Family Welfare, New Delhi 2010; Vol. I: pp.37-48.
14. Jain NK. Pharmaceutical Product Development. CBS Publishers and Distributors, New Delhi, 2006, p 272-9.
15. Shah P, Mashru R, Rane Y. Stability testing of Pharmaceuticals-A global perspective. J Pharm Research 2007;6(1):1-9.
16. Shirish SP, Raghunath DP and Mugdha SP. Stability study of a herbal drug. Pharmacology online 2008;1: 20-3.