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DEVLEOPMENT, EVALUATION AND VALIDATION PARAMETER OF MEMORY ENHANCER SYRUP

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ABSTRACT

In psychology, memory is the process in which information is encoded, stored and retrieved. Encoding allows information that is from the outside world to reach our senses in the forms of chemical and physical stimuli. Memory it's an unconscious faculty in which mental impression are retained and reproduced in mind. Memory consist of four process such as learning, retention, recall and recognition. From an information processing perspective there are three main stages such as Encoding, Storage and Retrieval of memory. Loss of memory may develop dementia. The number of phytoconstituents remains present in polyherbal syrup, it is much tedious to establish the quality control and standardization parameters for polyherbal preparations. The present study shows validation parameters and techniques of quality assurance for its quality, efficacy, stability and purity. A different modem analytical technique like HPLC and GC can also be used for the method development of active constituents present in herbal memory enhancer syrup. The method developed with HPTLC for bacoside A.

KEYWORDS: Syrup, HPLC, HPTLC, GC, bacoside A, dementia.

INTRODUCTION

In psychology, memory is the process in which information is encoded, stored, and retrieved. Encoding allows information that is from the outside world to reach our senses in the forms of chemical and physical stimuli. Memory its a unconscious faculty in which mental impressions are retained and reproduced in mind. In this first stage we must change the information so that we may put the memory into the encoding process. Storage is the second

memory stage or process. This entails that we maintain information over periods of time. Finally the third process is the retrieval of information that we have stored. We must locate it and return it to our consciousness. Some retrieval attempts may be effortless due to the type of information.[1]

Memory consists of 4 process

(a) Learning **(b)** Retention **(C)** Recall **(d)** Recognition

From an information processing perspective there are three main stages in the formation and retrieval of memory

- Encoding or registration: receiving, processing and combining of received information
- Storage: creation of a permanent record of the encoded information
- Retrieval, recall or recollection: calling back the stored information in response to some cue for use in a process or activity

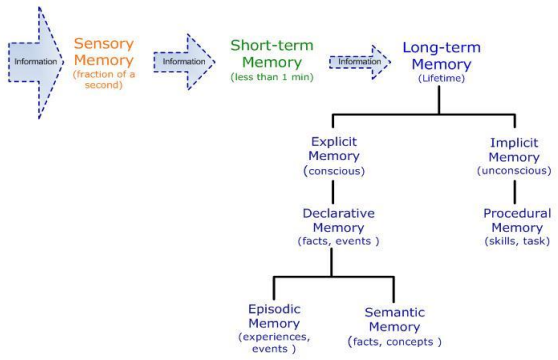

Fig 1: Types of memory

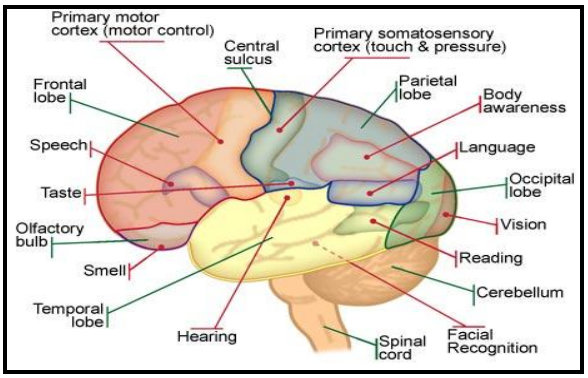

Fig 2: Functions of Brain

Ayurveda is one of the oldest still extant, health traditions in the world. Ayurveda is based on *Sankhya* philosophy which means rational enquiry into the nature of the truth. Sanskrit meaning of *Ayu* is life and *Veda* is knowledge or science. *Charak Samhita* (1000 BC) and *Sushrut Samhita* (100 AD) are the main classics. Ayurveda materia medica gives detailed descriptions of over 1500 herbs and 10,000 formulations. *Madhav Nidan* (800 AD) a diagnostic classic provides over 5000 signs and symptoms. Life in Ayurveda is conceived as the union of body, senses, mind and soul. The concept of *Prakriti* or human constitution plays a central role in understanding health and disease in Ayurveda which is similar to modern pharmacogenomics. With over 400,000 registered Ayurvedic practitioners, Government of India, Department of AYUSH (Ayurveda, Yoga, Unani, Siddha and Homeopathy) has responsibility to regulate quality, education and practice.[2]

Herbal medicines have been enjoying worldwide use. However, one of the impediments in the acceptance of the ayurvedic formulation is lack of standard quality control profiles.[3]

The plants used in Ayurveda and other Indian system of medicines may be of interest to find new leads for treating different diseases. Approaches like high-throughput screening, phytochemical profiling, quality controls and standardization of raw materials and finished products, clinical trials, herbal therapeutics, pharmacokinetics and herbal pharmacovigilance will not only help to prove the rationale of using these systems but also to get maximum benefits of the natural resources.[4]

The chemical constituents of plants vary depending on the species, variety and part of the plant, with conditions of growth (soil, water and temperature) and with the age of the plant. These complexities and variations of chemical content make standardization essential, which can be overcome by using modern analytical techniques.[5] The increasing demand of population and chronic shortage of authentic raw material have made it incumbent, so there should be some sort of uniformity in the manufacturing of ayurvedic medicines so as to ensure quality control and quality assurance. The World Health Organization has appreciated the importance of medicinal plants for public health care in developing nation and evolved guidelines to support the states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety and efficacy.[6]

Herbal medicine is a major component in traditional medicine and a common element in Ayurvedic, Naturopathic, Homeopathic and Allopathic medicine. WHO notes that of 119 plant-drived pharmaceutical medicines, about 74% are used in modern medicine in ways that co-related directly their traditional uses as plant medicines by native cultures.[7] According to recent survey conducted by WHO, approximately 80% of the world population relies mainly on traditional medicines for the primary health care. Herbal medicines according to WHO can be classified into three categories as follows;

- Phytomedicines or phytopharmaceuticals sold as over-the-counter (OTC) products in modern dosage forms such as tablets, capsules and liquids for oral use.
- Dietary supplements containing herbal products also called neutraceuticals, available in modern dosage forms
- Herbal medicines consisting of crude, semi processed or processed medicinal plants. These have a vital place in primary health care in developing countries.[8]

Polyherbal formulations as mentioned in classical texts of Ayurveda are used by number of pharmaceutical companies. It is very difficult to understand the theme of polyherbal preparation as a number of ingredients may vary from 2 to 25 or more. The situation is very further worsened by the multiple uses of these formulations as mentioned in Ayurveda.[9]

Polyherbal preparations are generally the mixtures of extracts, juices, pulps, secretions and exudations of powders of medicinal herbs in solid, liquid or semisolid forms with or without a suitable base. Occasionally, substances from mineral and / or animal sources may be included in them. However, mixture product to which chemically defined active substance have been added, including synthetic compounds /or isolated constituents from herbal materials, are not considered to be herbal. Since the polyherbal medicine is a complex mixture having complicated interactions of compounds, synergy may be expected to play a part. Recent findings regarding synergy in medicinal plants are eye-opener from herbal drug point of view.

Quality assurance (QA) can be defined as the activity of providing the evidence needed to establish confidence that the quality functions are being performed adequately. It is one of the most important departments of pharmaceutical companies that develop and follow internal standard operating procedures (SOPs), directed towards assuring the quality, safety, purity and effectiveness of the drug products.[10,11]

Since number of phytoconstituents remains present in the polyherbal formulation, it is much tedious to establish the quality control and standardization parameters for polyherbal preparation. So, concrete methods of quality control in terms of modern methodologies are needed to be developed for traditional system of medicines. These techniques, when applied on traditional system of medicine then it ensure that the herbal product delivers the required quantity of the quality medicament.[12] Thus today quality assurance is the thrusty area for traditional formulations.

Functions of quality assurance[13]

- Establishes system for ensuring the quality of the raw material and final product.
- It helps to identify and prepare the necessary standard operating procedure relative to control of quality.
- Determine whether the product meets all the applicable specifications.
- Determine whether it was manufactured according to internal standards and the GMPs.

- Quality monitoring audit function, so that it can be able to determine if operation have adequate system, facilities and written procedures to control the quality of product produced.

There are a series of quality assurance activities. Those can be undertaken to provide the documented evidence that the quality function exists for the manufacturing process and further, that the process is under control. A list of some of these activities is given below[14, 15]

- Product stability
- Validated Raw material specifications and their acceptable limits
- Product specifications and their acceptable limits
- Analytical methods
- Training and documentation
- Process validation

Validation provides documentary evidence, which provides a high degree assurance, that any procedure, process, equipment, material, activity or system consistently meets its predetermined specifications and quality attributes in accordance with the principle of GMPs.

The US-FDA defines validation as establishing documented evidence which provides a high degree of assurance that a specific process (such as the manufacturing of pharmaceutical dosage forms) will consistently produce a product meeting it predetermined specifications and quality characteristics. Validation requirements should be applied to the manufacturing facility, its critical services and systems, the manufacturing processes and all analytical test methods used to demonstrate the conformation of the product with its present specification.[15,16]

Process validation is the documented evidence that the process operated within established parameters, can perform effectively and reproducibly to produce a product meeting it's predetermine specification and quality attributes in accordance with the principle of GMP.

Validation is an element of the system of quality assurance, which guarantees for a given medicine

- The reliability and reproducibility of the principle process provided in the dossier.
- The attainment of quality as specified during routine production and packaging.
- The consistency of the active moiety throughout the shelf life of the drug product.[16]

Various types of process validation are described below. The selection of which depends upon a number of consideration like[17]

- Type of data present prior to the validation program,
- The type of data to be generated through the validation,
- Time when it is applied, Status or stage of the product and process development and
- Period or duration of study.

Analytical method validation[18]

Validation of analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications. According to the ICH, typical analytical performance characteristics that should be considered in the validation methods are as follows.

1.Accuracy
2.Precision
3.Linearity
4.Range
5.Detection limit
6.Quantification limit
7.Robustness
8.Specificity

The aim of the work was an attempt to develop memory enhancing syrup of shankhpushpi and Bramhi. Herbal medicines have become a topic of increasing global importance, with both medical and economic implications. Thus motivation for the development and standardization from the point of manufacturing and quality control becomes an essential obligation of today's health care system. This project has been selected as an academia industry symbiosis to thrust the upcoming research programs into industry oriented work to put emphasis on the linkage between knowledge generating institutions and product manufacturing industry. Quality assurances of herbal formulations are done by evaluating parameters such as physical, chemical, microbiological characteristics. Due to the increasing demand for herbal medicine, it is necessary to maintain their quality. During the interaction with Sidhayu research foundation Pvt. Ltd., Nagpur, we came across different formulations that have been developed by them. One of them is herbal memory enhancer syrup. The objective of present study is to carry out different quality assurance parameters of the

formulation herbal memory enhancer syrup prepared by **Sidhayu research foundation Pvt. Ltd., Nagpur.**

MATERIALS AND METHOD

1.1 Material and chemical: Whole plant of *Bacopa monnerrie* was collected from Nagpur local market. The plant specimens were dried and their herbarium sheets were prepared and were authenticated at Department of Botany, R. T. M. Nagpur University, Nagpur. (Herbarium Sheet no. 9884). All chemicals were of analytical grade or HPLC grade. Distilled water was used throughout the studies. Media used for microbial evaluation were obtained from Loba Chemiel Laboratories Pvt. Ltd., Mumbai. Markers were used for analytical method development and quantitative analysis. They were purchased from supplier by Sidhayu Research Pvt. Ltd; Nagpur.

1.2 Preparation of sample[19] 10 mg Bramhi herb was finely powered add 10 ml methanol (1000ug), sonicate for 10 min filtered and made dilutions. Take 1ml from above solution add 10 ml methanol (100ug/ml). Take 1ml from above solution add 10ml methanol (10ug/ml) from the above stock solution the samples were transferred to 10 ml volumetric flask and volume was made up to 10 ml to prepare and absorbance was taken at 540 nm.

1.3 Monograph studies[20] **Total ash value:** The air dried crude drug (2g) was weighed accurately in a silica dish and incinerated at a temperature not exceeding 450º until free from carbon. It was cooled and re-weighed. The percentage of ash with reference to the air-dried drug was calculated.

Total Ash (%) = weight of ash/weight of sample×100

Acid-insoluble ash: The ash (obtained from above) was boiled for 5 min with hydrochloric acid (25mL 2M) the insoluble matter was collected on an ash less filter paper, washed with hot purified water and ignited. The percentage of acid-insoluble ash was calculated with reference to the air-dried drug.

Acid Insoluble Ash (%) = weight of acid insoluble ash/weight of sample×100

Alcohol soluble extractive: The air-dried drug (5g) coarsely powdered was macerated with ethanol (100mL) in a closed flask for 24 h. It was shaken frequently during the first 6 h and was allowed to stand for 18 h. Then filtered rapidly (taking precautions against loss of (ethanol). The filtrate (25mL) was evaporated to dryness in a tared flat- bottomed shallow

dish; dried at 105º and weighed. The percentage of ethanol soluble extractive was calculated with reference to the air-dried drug.

Alcohol Soluble Extractive (%) = Weight of residue\Weight of substance×100

Water soluble extract: The air-dried drug (5g), coarsely powdered, was macerated with chloroform water (100mL) in a closed flask for 24h. It was shaken frequently during the first 6 hand allowed to stand for 18h. Filtered rapidly (take precaution against loss of chloroform water (25mL) of filtrate was evaporated to dried in a tared flat-bottomed shallow dried at 105ºc and weighed. The percentage of water soluble extractive was calculated with reference to air dried drug.

Water Soluble Extractive (%) = Weight of residue\Weight of substance×100

Loss on drying: Crucible were dried in oven and taken weight of dried crucible; then sample (5g) was taken in that dried crucible. Sample was spared in thin uniform layer, then crucible was taken in oven at 105ºc for 4h. Sample was cooled at room temperature and was weight the sample plus crucible. The percentage of loss on dying was calculated with reference to air dried drug.

Loss on Drying (%) = Loss in weight/weight of sample×100

1.4 Formulation of memory enhancer syrup[21]

- **Formula**

Table 1. Formulation of syrup

Sr. No	Ingredients	Quantity
1	Bramhi (Herb)	4.9gm
2	Shankhpushpi (Herb)	19.6gm
3	Citric acid	1.613gm
4	Sodium Methyl paraben	2.118gm
5	Sodium propyl paraben	1.059gm
6	Sugar	784.3gm
7	Colour	0.319gm

- **Method of preparation**

1) Decoction

2) Preparation of sugar syrup followed by addition of preservatives, colournats and flavours.

3) Incorporate sugar solution in concentrated solution of herb which was obtained by decoctions method.

4) Stirred continuously.

1.5 Evaluation of finished product[22]

Physical parameter

The herbal memory enhancer syrup was analysed for the quality consistency considering physical and analytical parameters. The herbal memory enhancer were analysed for physical, chemical, microbiological and stability studies.

pH: The pH value is determined by suitable pH meter using glass rod. Take the pH directly of the syrup sample.

Specific gravity: Unless otherwise specified in the individual monograph, the specific gravity is measured at 25^{O}C. First thoroughly clean the pycnometer with chromic acid and distilled water. Rinse with acetone and dry in oven. Allow to cool in desiccator and weigh. Fill the pycnometer with distilled water. Place the capillary tube on pycnometer. The liquid filled in pycnometer rises in the capillary, so that no air bubble is present in the pycnometer. Wipe the droplets of liquid adhering to the pycnometer by using piece of paper and weigh the pycnometer. Remove the liquid filled in pycnometer and dry it. Fill the pycnometer with the substance under examination. Remove any excess of the substance and weigh.

Total solid content: Take 10g of syrup sample in a tired petridish. Evaporate it on a water bath. Dry in oven at 105˚C. Cool in a desiccator and weight.

Total sugar: First clean the prism of Refractrometer by using water and acetone. Calibrate the apparatus against distilled water. Take the reading on left hand side of the scale for water. Dry the prism and apply the syrup sample directly. Take the reading on left hand side of the scale. Make the necessary correction for determining actual sugar %.

Microbiological Tests[23] Microbiological test were performed for finished product by using E. coli, S. aureus, Salmonella, Pseudomonas, Total microbial plate count and Total yeast and mould as per standard procedure.

Test for Heavy Metals: The herbal memory enhancer syrup was packed in the final containers were evaluated for heavy metal test as per standard procedure.

Stability studies: In stability studies memory enhancer syrup was filled in container and kept in the stability chamber and maintained at 40 ± 2^{0}c and 75 ± 5% RH for one month. At the end of the studies sample was analysed for chemical parameters of finished product. Data was recorded.

Method development and validation of bacoside A in bacopa monneri in memory enhancer syrup formulation.[17, 19, 25]

1. Preparation of reference standard solution: 50ml syrup and 50ml water neutralized with 100% $NaHCO_3$ solution carried extraction with chloroform combined all chloroform layers and evaporated on water bath. Reconstitute with 10ml methanol. Take aqueous layer obtained above evaporate on boiling water bath to obtained soft mass. Add petroleum ether 40-60 ml, sonicate & filter, discard ether & repeat twice. Evaporate residue to dry. Add ethanol & sonicate, boiled, filter repeat 2-3 times, combine all ethanol layer fractions evaporate on water bath add methanol to above residue filter from charcoal to remove green colour combined all fractions methanol evaporate on water bath, add 10ml methanol.

2. Selection of mobile phase

The various mobile phases were tried. Most suitable mobile phase having composition as ethyl acetate: methanol: glacial acetic acid: toluene (3:4:3:1) was selected.

3. Instrumentation and chromatographic condition

The chromatography was performed on (10 cm x 10 cm) aluminum plate coated with (0.2 mm) silica gel 60 F254 (E. Merck, Germany). The samples were applied to the plate as bands width 6 mm by using Camag (Muttenz, Switzerland) Linomat 5 applicator fitted with 100 µl syringe (Hamilton, Switzerland). The rate of application was constant at 150ml/s and space between two bands was 15 mm. The mobile phase was used as ethyl acetate: methanol: glacial acetic acid: toluene: (3:4:3:1). The Linear ascending development of the plate was carried out in twin–trough glass chamber previously saturated with mobile phase for 20 min at room temperature (25º ± 2) and relative humidity (60% ± 5). The length of chromatogram run was 80 mm. After development, the plate was removed and the plate was air dried. The densitometric scanning was performed at 430 nm using Camag TLC scanner 3.

4. Linearity study

Different volumes of syrup (range from 2.4µl–14.4µl), using Linomat 5 sample applicator. The plate was developed and scan the above established chromatographic conditions. The peak area was recorded for each conc. of drug. The conc. and area were subjected to least square regression analysis to calculate calibration curve equation and correlation coefficient (r^2). The linearity was found over the conc. range of 240–1440ng/spot.

The correlation coefficient (r^2) and calibration curve equation were 0.999 and

y=4.844x+48.06 respectively. The linearity range, calibration curve equation and correlation coefficient are given in **Table 8.**

5. Estimation of Bacoside A in Bacopa monnerri

The content of bacoside a in herb was determined by applying an appropriate volume of 2µL and 4µL of test solution and standard solution, respectively. The plate was developed and scanned as perproposed chromatographic conditions. The conc. was determined by linear regression equation. The results are shown in **Table 10.**

5. Accuracy

Accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition method (spiking). Accurately measured amount of standard bacoside A was added on the sample track TLC plate in subsequently increasing conc. of (50ng/spot). The chromatogram was developed and scanned as per proposed chromatographic conditions. The percentage recovery of standard bacoside from the proposed method was calculated. The results and statistical data of recovery studies are show in **Table 11.**

6. Precision (intra - day and inter - day): The precision of the method was determined as intra-day and inter-day variations. Intra-day variations was determined by analyzing 720, 960, 1200 ng/spot of standard solution of sample in triplicate on the same day forth times. Inter-day precision was determined by analyzing 720, 960, 1200 ng/spot of standard solution of bacoside a in triplicate for three consecutive days. The average, standard deviation (S.D.) and percentage relative standard deviation (%RSD) of peak are was calculated. The results are shown in **Table12 and Table13.**

7. Repeatability: The repeatability of sample application was assessed by spotting 9.6µL contain 960ng/spot of standard bacoside on TLC plate (n=6).The plate was developed and scanned as per proposed chromatographic conditions. The average, standard deviation (S.D.) and percentage relative standard deviation (%RSD) of peak are was calculated. The results are shown in **Table 14.**

8. Ruggedness: The ruggedness of method was done at conc. Levels 960ng/spot of working standard solution of bacoside a (n=6). The values of % RSD was lower than 2 indicate the ruggedness of the method. The results are shown in **Table15.**

9. Specificity: The specificity of the method was studied by analyzing standards and

formulation by simultaneously applying on the same plate. The spots of sample formulation were confirmed by comparing R_f values with that of reference standard. The peak purity of individual standard in sample track was assessed by comparing spectra at peak start, peak apex and peak end positions of the spot. The chromatograms of formulation, reference standard and their overlain spectrum are shown, respectively in **Figure 6,7,8.**

RESULT AND DISCUSSION

1.1 λmax determination of bacopa monnirri

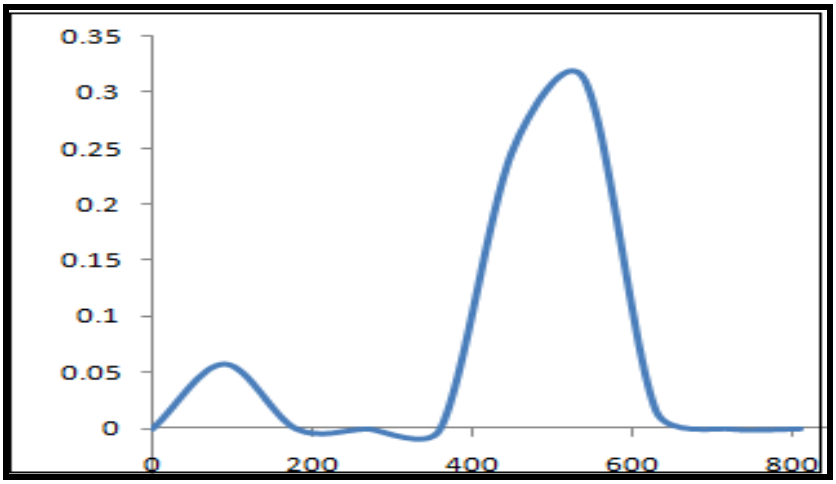

Fig 3. λmax of bacopa monnirri

Determination of UV absorption spectra: After scanning spectra of several dilution for good detection and linear qualification of bramhi in methanol. The lambda max of the drug was found to be at 540 nm.

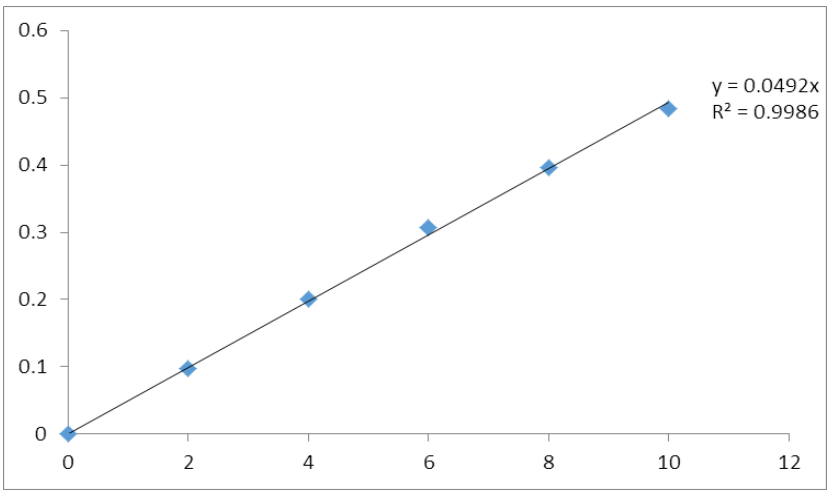

Fig 4. Absorbance spectra of bramhi

From the calibration curve of methanol it was concluded that it obey beer's Lamberd law in conc. range of 1-10 ug/ml the linear equation in methanol was obtained as Y=0.049 and correlation value R^2=0.0996. Correlation coefficient values indicated the liner a correlation between concentration and observation.

1.2 Raw material specification

1. Bramhi[24]

Table 2. Raw material specification for Bramhi

Sr. No.	Specifications	Limits	Observations
1.	Organoleptic Properties		
	State	Dry Herb	Dry Herb
	Colour	Brown	Brown
	Odour and Taste	Characteristic and bitter	Characteristic and bitter
2.	Phytochemical Characterization		
	pH(10% aqueous medium)	8	7.3
	Total ash%	NMT 20%	6.15%
	Acid insoluble ash (AIA) %	NMT 6%	1.66%
	Water Soluble Extractive (WSE)%	NLT 13%	10.2%
	Alcohol Soluble Extractive(ASE)%	NLT 60%	72.45%
	Loss On Drying (LOD) %	NMT7%	5.0%

Raw materials were evaluated for physical and chemical parameters. The results of physical parameters like ash value, pH, loss on drying values, alcohol soluble extractive, water soluble extractive were performed on herb and they were found with in limits for bramhi.

2. Shanhakpushpi

Table 3. Raw material specification for Shanhakpushpi

Sr. No.	Specifications	Limits	Observations
1.	Organoleptic Properties		
	State	Dry Herb	Dry Herb
	Colour	Brown	Brown
	Odour and Taste	Characteristic and bitter	Characteristic and bitter
2.	Phytochemical Characterization		
	pH(10% aqueous medium)	8	7.3
	Total ash%	NMT 20%	6.15%
	Acid insoluble ash (AIA) %	NMT 6%	1.66%
	Water Soluble Extractive (WSE)%	NLT 13%	10.2%
	Alcohol Soluble Extractive(ASE)%	NLT 60%	72.45%
	Loss On Drying (LOD) %	NMT7%	5.0%

Raw materials were evaluated for physical and chemical parameters. The results of physical parameters like ash value, pH, loss on drying values, alcohol soluble extractive, water soluble extractive were performed on herb and they were found within limits for shakhpushpi.

1.3 Finished product evaluation

Table 4. Evaluation of finished product

Sr.no.	Parameter	Limits	Observation
1	Decription	Dark greenish coloured viscous liquid	Dark greenish coloured liquid
2	P_H	5.40	4-6
3	Solid content	71.03%	NLT 50%
4	Total Sugar	60%	NLT 50%
5	Specific gravity	1.3143	1.28-1.33

Memory enhancer syrup was formulated and evaluated for chemical parameter and all the results were found with in the standard limits**.**

1.4 Microbiological Test

Table 5. Microbial specification

1.	Microbiological Test	Observation	Limits
	a) *Staphylococcus aureus*/g	Complies	Absent
	b) *Salmonella* sp./g	Complies	Absent
	c)*Pseudomonas eruginosa*/g	Complies	Absent
	d) *Escherichia coli*	Complies	Absent
	e) Total microbial plate count (TPC)	400 cfu/g	NMT10^5 cfu/g
	f) Total yeast &mould	Less than 1	NMT 10^5 cfu/mg

Microbiological test were performed for finished product and no microbial growth was found for all the microbial species.

1.5 Heavy metal specification

Table 6: Heavy metals specification

1	Heavy Metals	Observation	Limits
	a) Lead	**3.16 ppm**	**NMT 10.0 ppm**
	b) Arsenic	**0.84ppm**	**NMT 3.0 ppm**
	c) Cadmium	**0.006 ppm**	**NMT 0.3 ppm**
	d) Mercury	**0.04 ppm**	**NMT 1.0 ppm**

The herbal memory enhancer syrup was packed in the final containers were evaluated for heavy metal test and all the results were found with in the standard limits.

1.6 Stability studies

Table 7. Stability studies of syrup

Sr.no	Formulation code	Parameter	0 day	15th day	30th day
1	MS-01	Description	Dark greenish	Dark greenish	Dark greenish
2		P_H	5.40	5.39	5.39
3		Specific gravity	1.3143	1.3032	1.3022
4		Total solid	71.03%	71.00%	71.01%

Memory enhancer syrup was didn't show any imperfection on storage condition at 40±20 and 75±5 % for one month. Results for after 30 days indicated that the drug loss was in acceptable limits during the stability test period. Stability was found to be grater.

1.7 Analytical method Validation parameters studies[25]

1. Linearity Study

Table 8. Linearity range of Bacopa Monnerri

Conc. (ng/spot)	240	480	720	960	1200	1440
Area Peak Area	1223.9	2405.4	3562.6	4708.1	5855.3	6995.8

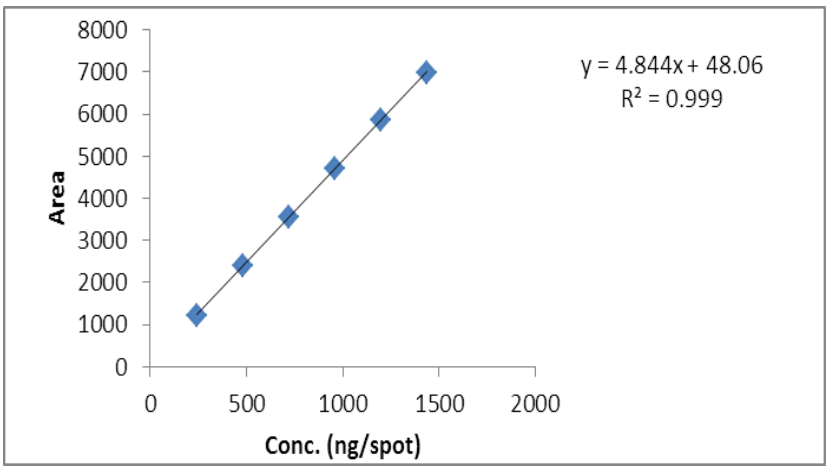

Fig.5 Linearity study of Bacopa Monnerri

Linearity study for bacopa monnerri was done and linearity equation was found to be y=4.844x=48.06 and correlation value was found to be R^2=0.999.

Table 9. Estimation of Bacoside A in Herb

Sr.	Herb	Peak area	Amount found	Amount

No.	(µg/band)		(ng/spot)	found
1	3852.75	5122.5	1047.57	0.0271
2	3852.75	5103.8	1049.53	0.0271
3	3852.75	5132.5	1049.63	0.0272
4	3852.75	5128.2	1048.74	0.0272
5	3852.75	5115.4	1046.12	0.0271
6	3852.75	5123.2	1047.71	0.0271
			Mean	0.0271
			S.D.	0.00007745
			%R.S.D.	0.285

Concentration of bacoside A was determined by linear regression equation. Bacoside A in herb was done and it was found to be 0.0271.

Table 10. Estimation of Bacoside A in Syrup

Sr. No.	Extract (µg/band)	Peak area	Amount found (ng/spot)	Amount found
1	7495.20	5560.0	1137.89	0.0151
2	7495.20	5562.5	1138.40	0.0152
3	7495.20	5530.2	1131.73	0.0151
4	7495.20	5531.4	1131.98	0.0150
5	7495.20	5570.4	1140.03	0.0151
6	7495.20	5564.3	1138.77	0.0152
			Mean	0.0151
			S.D.	0.000125
			%R.S.D.	0.1622

2. Recovery Study

Table 11: Recovery study of Bacoside A

Sr. No.	Sample (ug) [A]	Area [B]	Amount found [C]	Standard added(ng/spot) [D]	Total applied [E]	Area [F]	Total recovered [G]	% recovered [G/E*100]
1	7495.20	5560.0	1137.89	200	1337.89	6414.01	1314.21	98.23
2	7495.20	5560.0	1137.89	250	1387.89	6799.19	1393.72	100.42
3	7495.20	5560.0	1137.89	300	1437.89	7100.24	1455.87	101.25
4	7495.20	5560.0	1137.89	350	1487.89	7480.25	1534.32	101.12
5	7495.20	5560.0	1137.89	400	1537.89	7431.28	1524.21	99.11
6	7495.20	5560.0	1137.89	450	1587.89	7667.04	1572.88	99.11
							Mean	99.25
							SD	1.7851
							%RSD	1.7815

The recovery studies by quantitative estimation of bacoside A was around 100%. This

show of accuracy of the method. The percent amount of bacoside A was found in syrup was 98.23.

3. Precision Study

Table 12. Precision studies (intra - day) for Bacoside A

Conc.[ng/spot]	Intraday[n= 3]			
	Peak area Mean ±S.D.	%R.S.D.	Amount Found	%Amount Found
720	3539.9±23.35	0.65	712.08	98.90
960	4681.54±22.02	0.61	956.54	99.64
1200	5874.23±24.25	0.41	1182.25	98.50

The precision method was determined by intra-day and inter-day variations. The. Intra-day precision was determined by analyzing 720, 960, 1200 ng/spot of sample in triplicate on the same day forth times. The average, standard and relative deviations of peak was calculated which show accuracy of method.

Table 13. Precision studies (inter - day) for Bacoside A

Conc.[ng/spot]	Interday [n= 3]			
	Peak area Mean±S.D.	% R.S.D.	Amount Found	%Amount Found
720	3546.21±22.05	0.62	722.16	100.3
960	4661.10±28.09	0.60	952.32	99.2
1200	5849.24±30.20	0.51	1197.60	99.8

The precision method was determined by intra-day and inter-day variations. The. Inter-day precision was determined by analyzing 720, 960, 1200 ng/spot of bacoside a in triplicate for three consecutive days. The average, standard and relative deviations of peak was calculated. Which show accuracy of method.

4. Repeatability Study

Table 14. Repeatability for Bacoside A

Sr. No	conc.(ng/spot)	Area
1	960	4768.2
2	960	4792.3
3	960	4769.5
4	960	4752.5
5	960	4799.3
6	960	4775.1
	Mean	4776.66
	SD	13.5541
	%RSD	0.28

Repeatability study was done mean, standard deviation, relative standard deviation was calculated. Which show accuracy of method.

5. Ruggedness study

Table 15. Ruggedness for Bacoside A

Drug	Analyst	Ruggedness *(n= 6)*			
		Peak area (960ng/spot)Mean ±S.D.	% R.S.D.	Amount found	%Amount Found
Bacoside A	I	4684.35±24.50	0.52	957.12	99.70
	II	4662.46±28.30	0.60	952.60	99.23

Ruggedness study for bacoside a was done and it was found near to 100%.Which shows the accuracy of method**.**

6. Specificity Study

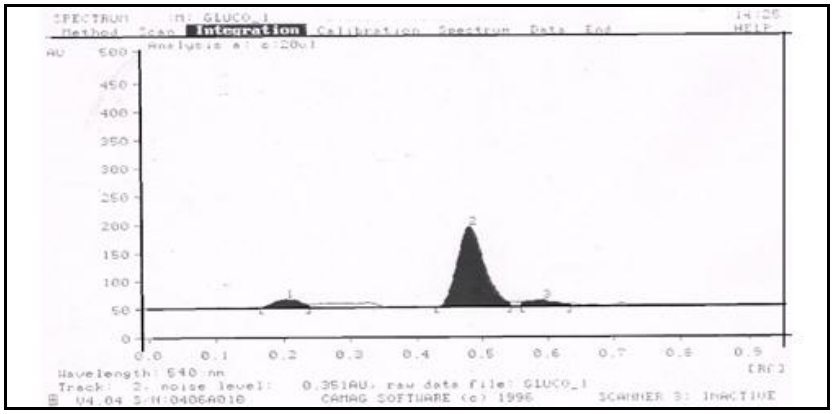

Fig.6 Chromatogram of standard Bacoside A

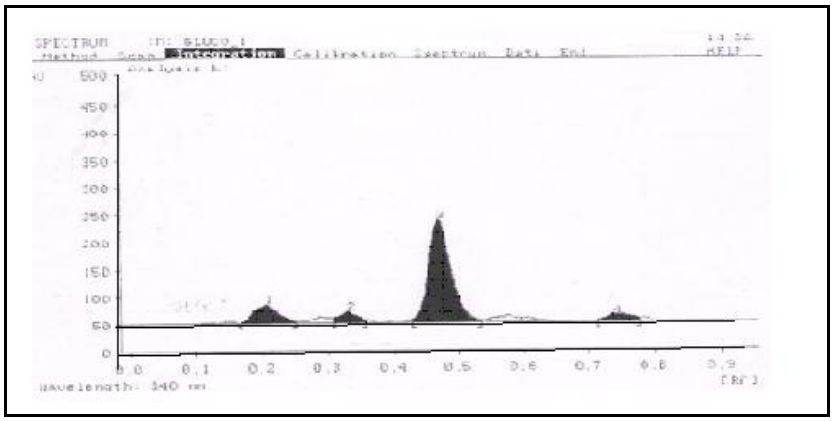

Fig.7 Chromatogram of *formulation (syrup)*

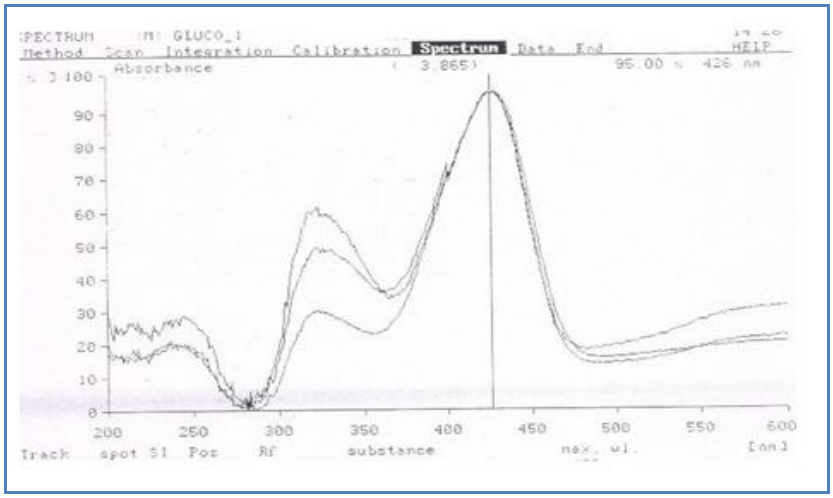

Fig 8. Overlain spectrum of standard, and formulation (syrup)

Specificity study for standard and formulation was done. Rf value of formulation confirmed by comparing it with standard. And it was found with in the acceptable limits.

CONCLUSION

The herbal memory enhancer syrup has been validated with modern tools and techniques of quality assurance for its quality, efficacy, stability and purity. The study of the physicochemical parameters of raw material shows that ingredients used in the manufacturing of herbal syrup were of good quality as per specification. The quantitative estimation of phytoconstituents in the raw materials and finished product shows that the process exhibited no significant loss of active ingredients. The validation parameters were studied for herbal syrup. The result of validation parameters shows that the formulation was validated successfully.

The methods developed with HPTLC for bacoside A are reproducible, sensitive, precise, specific and accurate and can be further applied for the routine quality control tests. The different modern analytical techniques like HPLC and GC can also be used for the method development of active constituents present in herbal memory enhancer syrup.

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REFERENCES

1. Humen information processing Professor Alan Hedge, Cornell University, August 2013.

2. Demenetia etiology and epidemiology a systemic review and The Swedish Council on Technology Assessment in Health Care volume 1 june 2008; 223.
3. Alzheimer's Disease Facts and Figures Includes a Special Report on humens and Alzheimer's Disease. 2014.
4. Patwardhan B, Mashelkar RA. Traditional medicine-inspired approaches to drug discovery: can Ayurveda show the way forward. Drug Discovery Today; 2009; 1-8.
5. Dhalwal K, Birada YS, Shinde VM, Mahadik KR, Rajani M. Phytochemical evaluation and validation of a polyherbal formulation using HPTLC. Phcog Mag: 2008; 4(14): 89-95.
6. Mukherjee PK, Wahile A. Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. J Ethnopharm 2005; 103: 25–35.
7. Therapeutics Letter 25. Herbal medicines An Evidence Based Book Available. 1998:http://www.interchange.ubc.ca/jauca as accessed on 9/9/ 2013.
8. Mohapathra P, Shirwaikar A. Aswatha Ram HN. Standardization of a Polyherbal Formulation. Phcog Mag. 1998; 4(13): 65-69.
9. Bolton S, Ed., In; pharmaceutical statistics, 3rd Edn. Vol 80, Marcel Dekker, inc., New York, 2000; 90.
10. Lachman, Hanna SA. In: Lachman L, Lieberman HA, Kanig JL. editors. The Theory and practice of industrial pharmacy, 3rd ed. Bombay: Vargese publication house; 1987; 1804.
11. Mukherjee PK. Quality Control of Herbal Drugs. 1sted. New Delhi: Business Horizons Pharmaceutical Publishers; 2002; 496-504.
12. Biyani MK.Eastern pharmacist. 1994; 8: 33.
13. Nash RA. In: Nash RA, Wachter AH, editors. Pharmaceutical Process Validation. 3rded. New York: Marcel Dekker; 2003; 13.
14. Bolton S. Eds. In: Pharmaceutical statistics. 3rded. Vol 80. New York: Marcel Dekker inc., 2000; 44.
15. Traisnel MK, Gayot AT. Drug Dev and Ind Pharm 1995; 21: 79.
16. John YL. In: Ira Berry and Robert Nash, editors. Pharmaceutical Process validation 2nd Ed. New York: Marcel Dekker; 2000. p. 444.Jayram T. Pharma Times 2005; 37: 9-16.
17. United States Pharmacopoeia 32. Validation of Compendial Procedures. <1225>, United States Pharmacopoeial convention INC: Rockville: Maryland: 2008; 734-736.
18. Q2 (R1). ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology: 2005; 15-18.

19. The Ayurvedic Pharmacopoeia of India. The Controller of Publication, Civil lines, Delhi; part I Vol IV. 1st ed 2007; 138-139.
20. Standerdisation of formulations containing bacopa monnerri by standerd analytical techniques, vol II. 2008; 76-79.
21. The Ayurvedic Pharmacopoeia of India. The Controller of Publication Civil lines. Delhi. Part I, Vol II. 1st ed. 2004; 167-169.
22. Indian Herbal Pharmacopoeia. Revised New ed. IDMA, Mumbai; 2002.
23. Khandagale A., Shanbhag D., Screening and standerdisation of bacopa monnieri used as medicine in homeopathy using HPTLC method. IOSR Journal of pharmacy, 2012; 52-56.
24. Joyti S. G. , Harisha C.R, Chandola H. M, A comparative study of roots of Bacopa monneria L pennel and Bacopa floribunda International Journal of Pharmacognosy and Phytochemical Research, 2012; 4: 8-11.
25. Dowell A., Davidsons, G., Ghosh D., Validation of Quantitative HPLC method for Bacosides keenmind, Hindwi Research article, 2015; 1155.