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Fingerprinting and Stability Studies of Shatavari Churna: An Ayurvedic Formulation

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ABSTRACT

Asparagus racemosus (AR) Wild (family Liliaceae) is commonly known as Shatavari, it is an important medicinal plant of tropical and subtropical India. Roots of *Asparagus racemosus* Wild (Shatavari in vernacular) are widely used in Ayurveda as Rasayana for immunostimulation, galactogogue and also in treatment of conditions like ulcers and cancer. Literature survey indicates that no fingerprinting and stability studies were carried out; hence present study was undertaken for the same. UV and HPTLC studies were carried out for fingerprinting of Shatavarin IV a steroidal saponin. HPTLC study were carried out on aqueous and alcoholic extracts using Camag HPTLC system equipped with Linomat V applicator, Camag TLC scanner 3 and CATS 4 software for interpretation of data. Alcohol extract revealed 9 phytoconstituents with Rf 0.04, 0.17, 0.25, 0.28, 0.34, 0.42, 0.68, 0.79 and 0.93, while aqueous extract revealed 7 phytoconstituents with Rf 0.06, 0.12, 0.16, 0.28, 0.34, 0.42 and 0.93, Band with Rf 0.34 in both extracts correspond to that of standard Shatavarin IV (Rf 0.35). The specificity was confirmed by overlaying the spectra of standard Shatavarin IV (max 426nm), with the absorption spectrum obtained from the corresponding band in the track of alcohol and aqueous extracts. Stability studies reveals that the selected Shatavari churna formulation was found stable during the entire stability studies over a duration of about 6 months at 45^0C and 75% relative humidity, the stability was analyzed over physical characteristics, physicochemical parameters and major active constituents i.e. Shatavarin IV.

INTRODUCTION

In recent years, plant derived products are increasingly being sought out as medicinal products, nutraceuticals and cosmetics and are available in health food shops and pharmacies over the counter as self medication or also as drugs prescribed in the non-allopathic systems[1,2]. Herbal medicines widely used in health care in both developed and developing countries are complex chemical mixtures prepared from plants and are limited in their effectiveness because they are poorly absorbed when taken orally[3]. According to an estimate of the World Health Organization (WHO), about 80% of the world population still uses herbs and other traditional medicines for their primary health care needs[4]. Herbal formulations have reached widespread acceptability as therapeutic agents for diabetics, arthritics, liver diseases, cough remedies, memory enhancers and adoptogens[5]. As per WHO definition, there are three kinds of herbal medicines: raw plant material, processed plant material and medicinal herbal products. Herbal drugs are finished labelled products that contain active ingredients such as aerial or underground parts of plant or other plant material or combination thereof, whether in the crude state or as plant preparations. The use of herbal medicines has increased remarkably in line with the global trend of people returning to natural therapies[6]. Herbal medicine products are dietary supplements that people take to improve their health and are sold as tablets, capsules, powders, teas, extracts and fresh or dried plants[7]. Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles[8]. Quality evaluation of herbal preparation is a fundamental requirement of industry and other organization dealing with Ayurvedic and herbal products. Herbal drugs regulations in India as well as an overview of regulatory status of herbal medicine in USA, China, Australia, Brazil, Canada and Germany have been reported [9]. According to WHO guidelines, an herbal product needs to be standardized with respect to safety before releasing it into the market. Standardization is a system that ensures a predefined amount of quantity, quality & therapeutic effect of ingredients in each dose[10]. Shatavari consists of tuberous roots of *Asparagus racemosus*. It is rich in saponin glycosides. Shatavari is a galactogogue and a powerful nutritive tonic and rejuvenative, which can be given to a person with any type, constitution, sex, age. Charaka has categorized it as balya, promoting strength[11]. Shatavari (tuberous roots of *Asparagus racemosus*, family Liliaceae) is also known as Shatmuli or Shatpadi and is found distributed throughout tropical Asia, Africa and Australia. In India, it is found in Himalaya up to an altitude of 1300 to 1400 m and all tropical parts of India.

The roots are traditionally used as diuretic, tonic, antidiabetic, in gout, female genitourinary tract disorders, as styptic, anti-ulcer, intestinal disinfectant and astringent in diarrhea, nervine tonic, in sexual debility for spermatogenesis, lactic disorders, haematuria, bleeding disorders and hyperacidity[12]. Quantitative estimation of chemical markers of each ingredient in the poly herbal preparation required ideal separation technique. For herbal preparations (including polyherbal), there is an urgent need for scientific proof/validation with chemical standardization protocols/procedures, biological assays, animal models and clinical trials. HPTLC thus offers major advantages over other commonly available conventional chromatographic techniques. The proposed method was validated on the basis of its selectivity, linearity, limit of detection (LOD) and limit of quantification (LOQ) according to ICH requirements [13-16]. HPTLC profile is quite helpful in setting up of standards for evaluating the purity and quality of Ayurvedic preparations. This will be helpful to overcome batch to batch variations in different Ayurvedic preparations[17].

MATERIALS AND METHODS

Plant material

Raw material i.e. Shatavari roots were collected from Herbal Garden of Priyadarshini J. L. Chaturvedi College of Pharmacy, 846, New Nandanwan, Nagpur, Maharashtra and was authenticated by Dr. Mrs. Chaturvedi, Professor and Head of Department, Department of Botany, Rashtra Sant Tukdoji Maharaj, Nagpur University, Nagpur. Herbarium is deposited in the Department on 24-03-2013 with number as 9900.

The collected roots were washed and cut into small pieces and dried for 17 days in shade followed by drying in hot air oven for about 5-6 hrs. Dried roots were powdered and passed through mesh 16.

Formulation profile

Formulation 1(Marketed): It is in powdered form, purchased from local market in Nagpur. Coded as **SCM-I**.

Formulation 2 (Marketed): It is in powdered form, purchased from local market in Nagpur. Coded as **SCM-II**.

Formulation 3 (Laboratory): It is in powdered form, prepared in Laboratory of Natural Product, Sudhakarrao Naik, Institute of Pharmacy, Pusad, and Coded as **SCL**.

The raw material (Shatavari root) used for preparation was collected from the Herbal Garden and was authenticated by Department of Botany, Rashtra Sant Tukdoji Maharaj, Nagpur University, Nagpur. The Formulation of Shatavari Churna was prepared as per The Ayurvedic Formulary of India12. The powders should completely pass through 355 μm I. S. sieve (sieve number 44) and not less than 50 percent pass through 180 μm I. S. sieve (sieve number 85).

Methods

UV–visible spectroscopy

UV-Visible double beam spectrophotometer (UV-1800, SHIMADZU Co, Japan) with 1cm matched quartz cells, Micropipette of Variable volume 10-1000 μL (Gene Pete Co.) and Digital balance (Citizen Co.) were used.

It is the branch of science dealing with the study of interaction between Electromagnetic radiation and matter. It is a most powerful tool available for the study of atomic and molecular structure/s and is used in the analysis of wide range of samples. Optical spectroscopy includes the region on electromagnetic spectrum between 100 A and 400 μm[18-19]. UV-Visible spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis. It involves measuring the amount of ultraviolet or visible radiation absorbed by a substance in solution. Instrument which measure the ratio, or function of ratio, of the intensity of two beams of light in the U.V-Visible region are called Ultraviolet-Visible spectrophotometers. In qualitative analysis, organic compounds can be identified by use of spectrophotometer, if any recorded data is available, and quantitative spectrophotometric analysis is used to ascertain the quantity of molecular species absorbing the radiation. Spectrophotometric technique is simple, rapid, moderately specific and applicable to small quantities of compounds. The fundamental law that governs the quantitative spectrophotometric analysis is the Beer -Lambert law. Beer's law: It states that the intensity of a beam of parallel monochromatic radiation decreases exponentially with the number of absorbing molecules. In other words, absorbance is proportional to the concentration. Lambert's law: It states that the intensity of a beam of parallel monochromatic

radiation decreases exponentially as it passes through a medium of homogeneous thickness. A combination of these two laws yields the Beer-Lambert law.

Beer-Lambert law: When beam of light is passed through a transparent cell containing a solution of an absorbing substance, reduction of the intensity of light may occur. Mathematically, Beer-Lambert law is expressed as

$$\mathbf{A=a\ b\ c}$$

Where, **A**=absorbance or optical density

a=absorptivity or extinction coefficient

b=path length of radiation through sample (cm)

c=concentration of solute in solution.

Both b and a are constant so a is directly proportional to the concentration c.

When c is in gm/100 ml, then the constant is called A (1%, 1 cm)

$$A = A\ (1\%\ /1\ cm)\ bc$$

Quantification of medicinal substance using spectrophotometer may carried out by preparing solution in transparent solvent and measuring it's absorbance at suitable wavelength. The wavelength normally selected is wavelength of maximum absorption (λ_{max}), where small error in setting the wavelength scale has little effect on measured absorbance. Ideally, concentration should be adjusted to give an absorbance of approximately 0.9, around which the accuracy and precision of the measurements are optimal. The assay of single component sample, which contains other absorbing substances, is then calculated from the measured absorbance by using one of three principal procedures. They are, use of standard absorptivity value, calibration graph and single or double point standardization. In standard absorptive value method, the use of standard A (1%, 1 cm) or E values are used in order to determine its absorptivity. It is advantageous in situations where it is difficult or expensive to obtain a sample of the reference substance. In calibration graph method, the absorbances of a number of standard solutions of the reference substance at concentrations encompassing the sample concentrations are measured and a calibration graph is constructed. The concentration of the analyte in the sample solution is read from the graph as the concentration corresponding to the absorbance of the solution. The single point standardization procedure involves the measurement of the absorbance of a sample solution

and of a standard solution of the reference substance. The concentration of the substances in the sample is calculated from the proportional relationship that exists between absorbance and concentration.

$$C_{test} = (A_{test} \times C_{std})/A_{std}$$

Where C_{test} and C_{std} are the concentrations in the sample and standard solutions respectively and A_{test} and A_{std} are the absorbances of the sample and standard solutions respectively.

For assay of substance/s in multi component samples by spectrophotometer; the following methods are being used routinely, which includes

• Simultaneous equation method
• Derivative spectrophotometric method
• Absorbance ratio method (Q-Absorbance method)
• Difference spectrophotometry
• Solvent extraction method[20].

Method validation

Validation is concerned with assuring that a measurement process produces valid measurements. Results from method validation can be used to judge the quality, reliability and consistency of analytical results. It is an integral part of any good analytical practice. A measurement process producing valid measurements for an intended application is fit for purpose. Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. Analytical methods need to be validated or revalidated, before their introduction into routine use. Whenever the conditions change for which the method has been validated (e.g., an instrument with different Characteristics or samples with a different matrix); and whenever the method is changed and the change is outside the original scope of the method.

Nowadays, there are several international renowned organizations offering guidelines on Method validation and related topics.

• American Society for Testing and Material (ASTM)

• Codex Committee on Methods of Analysis and Sampling (CCMAS)
• European Committee for Normalization (CEN)
• Cooperation on International Traceability in Analytical Chemistry (CITAC)
• European Cooperation for Accreditation (EA)
• Food and Agricultural Organization (FAO)
• United States Food and Drug Administration (FDA)
• International Conference on Harmonization (ICH) [21].

Thin Layer Chromatography- High Performance Thin Layer Chromatography

Out of many chromatographic methods, thin layer chromatography (TLC) is widely used for the rapid analysis of drugs and their preparations. With the advancement of high performance TLC plates, introduction of new stationary phase and the instrumentalization of techniques, TLC has been transformed into modern sensitive and high performance analytical methods. TLC came into usage as a result of pioneering work of Stahl has been in practice for decades. Despite the introduction of other chromatographic techniques like HPLC, TLC still holds its relevance. Even though it is highly incredible that TLC will a major chromatographic technique. It also seems increasingly unlikely that the technique will ever fall into disuse in stark contrast to paper chromatography, which has now attained the status of lost art. There are a number of factors that make TLC a very suitable technique is the time required for the demonstration of most of the characteristic constituents of drug by TLC is very short. TLC provides both quantitative as well as qualitative information regarding the active constituent. It is suitable for monitoring the identity and purity of drug thus detecting adulteration and substitution. With the aid of suitable separation techniques, TLC can be used to analyze drug combinations and phytochemical preparations. Identification of unknown components of the sample, manufacturing impurities or decomposition products in drug substance or formulation and drug metabolites in biological specimen is done. TLC is a separation technique in which stationary phase consists of an appropriate material which is spread as uniform thin layer on glass, plastic or metallic plate. Solution of analytes is deposited on the plate prior to development. The separation is based on adsorption; partition, ion exchange or combination of this mechanism is carried out by migration of solutes in a solvent or suitable mixture of solvent through the thin layer. The TLC profile was

developed for the selected Ayurvedic formulation Shatavari Churna of Marketed and laboratory formulation.

Instrumentation

The apparatus for TLC / HPTLC consist of the following components:

TLC plate

Traditionally TLC has been performed using a number of stationary phases (silica gel, alumina, cellulose, kiesulguhr and polyamides) coated onto glass, plastic or aluminium foil. TLC plate is prepared by following methods:

Spraying methods
Spreading methods
Immersion methods.

Preconditioning of plates

It may be necessary to wash plates prior to separation. This can be done by migration of appropriate solvents. The plate may also be impregnated. At the time of use the plate may be activated if necessary by heating at 100-120°C for 1 hour.

Developing chamber

A chromatographic tank with a flat bottom and twin trough of inert transparent material of suitable size and provided with tightly fitting lid. For horizontal development tank is provided with a trough for mobile phase and it additionally contains a device for directing the mobile phase to stationary phase.

Sample applicators

Micropipettes, microsyringes, calibrated disposable or linomat applicator system (HPTLC) are the system that are used for the proper application of sample solution onto the plates and thus ensure better resolution.

Development techniques

In beginning TLC was performed by simply placing the plate in a glass tank containing appropriate amount of solvent, which was allowed to migrate to the required distance needed to obtain the desired separation. There is a large number of development techniques derived in order to improve the type of separation afforded by basic TLC techniques. These methods include continuous, multiple development programmed multiple development and automated multiple development. However circular, anti-circular, radial, triangular and centrifugal development can also be used.

Detection and quantification

In TLC detection is based on the human eye aided by vast array of selective spraying reagents and the use of plates impregnated with fluorescent indicators, which allow compounds to be detected by fluorescence quenching. For quantitative evaluation, especially in HPTLC, a range of TLC UV/visible scanners are available which are capable of operating in one of many several modes. Thus scanners are available to measure absorbance, fluorescence and fluorescence quenching, thus obtain spectra of individual spot *in situ*. Apart from this type of detector many are described including video camera and computer based image processing system as an alternative to scanning densitometry. Thus over the last decade considerable innovation has taken place in the area of instrumental TLC and HPTLC greatly increase the potential of technique. However it must be emphasized that in order to get the best result using these quantitative technique much more control is needed at all stage of sample application and chromatography. The recent search in autospotters has really enhanced the precision of technique applicators like Linomat and can apply up to 2-99 µl in a streak.

Densitometry is *in situ* instrumental measurements of visible, UV absorbance and fluorescence quenching. The scanner converts the spot/ band on the layer into a chromatogram consisting of peaks similar in appearance to that of HPLC chromatogram. The portion of scanned peak on the recorder chart is related to Rf values of spots on the layer and the peak height or area is related to the concentration of substance in the spot. It is still unlikely that TLC, in any of its forms, will ever displace HPLC and GLC from their current positions, especially given the huge investments that have been made in these techniques. However it is now the case that for many applications

TLC represents an alternative to other systems. Certainly it is no longer possible to say about TLC that it is a low resolution, semiquantitative or quantitative technique, with poor sensitivity, useful for chemists, but not a real quantitative tool for analyst and chromatographers. Most of the steps in quantitative TLC can be automated and therefore TLC made a very attractive preposition.

Fluorescence detection devices are used either for direct measurement of fluorescence or the inhibition of fluorescence whereas visualization agents include various agents that are used to detect the separate spots by spraying, exposure to vapour or immersion.

Preparation of samples and TLC plates

All the samples were prepared by dissolving weighed quantity (5 mg per 10 ml solvent) of extracts in respective solvent. The solvent systems were developed using primary solvent system selection method by running the plates on trial basis in different solvent systems. The plates were prepared by dissolving 30 gm Silica gel G in 100 ml of distilled water to get slurry which was spread over glass plates. The thickness of gel layer was 0.25-5 mm.

Development of chromatogram

About 2 mm of adsorbent from each edge of the plate was removed to give a sharply defined edge. Apply 2-5 µl volumes of 10 mg/ml solutions of the sample extract in an organic solvent to the plate with the aid of template. The spot size should be about 0.3 cm. For weak solution several applications may be necessary and each spot should be allowed to dry before applying another volume of solution to the same spot. It is better to use single spot of solution of different strength. Thus spot size is more uniform and results are more consistent. Allow the solvent to evaporate and plates are placed in developing tank. This preparation is done 30 min. before insertion of the plate. The tank should be lined with filter paper dipping into the developing solvent to eliminate edge effect. As the solvent to rise a distance of 10-15 cm, remove the plate and allow to dry using heat or current of air. The plates are sprayed with suitable spraying reagents. The solvent systems used and their relative resolution property is shown below.

Development of stability studies

The purpose of stability study was to provide evidence on the quality of drug substance or drug products varies with time under the influence of a variety of environmental factor such as temperature, humidity and light recommendation for storage conditions. Stability study was designed to increase the rate of chemical degradation or physical change of an active drug substance or drug product by using exaggerated storage conditions[22].

All the selected formulation batches of ShCL, ShCM-I and ShCM-II were subjected to stability studies at accelerated conditions at 45^0C at 75% relative humidity for six month. The sample were withdrawn at 1, 3 and 6 months interval of time and evaluated for previously developed parameter colour, odour, taste, moisture content and content of phytoconstituents i.e. Shatavarin IV.

Extraction and Isolation

Tuberous roots of *Asparagus racemosus* (250 g) were powdered, defatted with Hexane and repeatedly extracted by maceration with 90% methanol at room temperature for 24 h. The total methanolic extract was evaporated on a water bath to obtain a concentrated liquid syrupy mass (100 ml) which was dissolved in 10% methanol. The resulting solution was partitioned with Chloroform, Ethyl acetate and n-Butanol, successively. The n-Butanol extract was dried on a water bath and the dried fraction was dissolved in minimum quantity of 90% methanol to load on the column as the sample. Silica gel G (60-120) was used for packing the column and eluted with Ethyl acetate: Methanol: Water (8:1:1 v/v) as mobile phase. Multiple fractions, each of 27 ml were collected and out of which eluted fraction 10 to 22 showed presence of Shatavarin IV. Shatavarin IV containing fractions was confirmed by TLC using Ethyl acetate: Methanol: Water (7.5:1.5:1 v/v) as developing solvent and the spots were visualized by dipping the plate with Vanillin-Sulfuric acid reagent. All the fractions (10-22) were mixed and dried at room temperature.

RESULTS AND DISCUSSION

A simple, valid and rapid UV fingerprint method has been developed for estimation of Shatavarin-IV for Shatavari formulations and raw ingredient. All the chemicals and solvents

used were of Analytical Grade, Shatavarin-IV was procured from Sigma-Aldrich USA through Sigma- Aldrich Corporation Bangalore, India. Shatavarin-IV in Shatavari churna was estimated against standard marker component by using UV- visible spectrophotometer, Shimadzu UV-1700. The standard solution of Shatavarin-IV was prepared by accurately weighing 10 mg of Shatavarin-IV and transferred in 100 ml capacity volumetric flask, which was dissolved and diluted up to 100 ml with methanol. The concentration was made 100 µg / ml of solution. About 2, 4, 6, 8 and 10 ml of Standard solution of Shatavarin-IV was pipette out and transferred in volumetric flask and were diluted such that the final concentration ranges between 2-10 µg / ml in a series of five volumetric flasks. The absorbances of Shatavarin-IV were measured at 426 nm against methanol. The observations are summarized in Table No 1 and Calibration curve in Figure No 1.

Table No 1. Calibration data of Shatavarin-IV in Shatavari churna.

Concentration in µg / ml	Absorbance
2	0.328
4	0.645
6	0.979
8	1.330
10	1.664

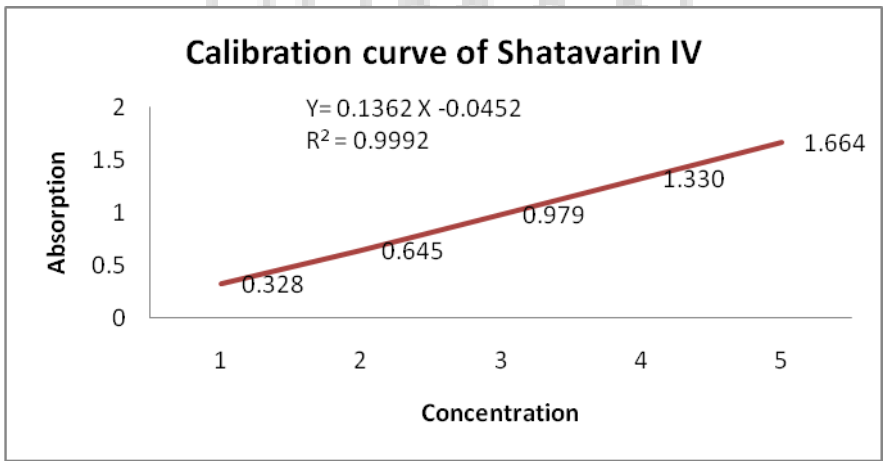

Figure No 1. Calibration curve of Shatavarin-IV

Accurately weighed 1 gm of extract (One laboratory formulation, two marketed preparations and *Asparagus racemosus*) was transferred into a 250 ml beaker then 50 ml of methanol was added and boiled on water bath for 10-15 minutes. After cooling the liquid was filtered through Whatman filter paper. The process was repeated three times and the fractions were combined and made up to 100 ml. Precision of method was determined with the product. An amount of the product powder equivalent to 100% of the label claim of shatavarin-IV was accurately weighed and assayed. The repeatability of sample application and measurement of absorbance for active compound were expressed in terms of Relative Standard Deviation (RSD) and Standard Error. Method repeatability was obtained from RSD value by repeating the assay in same day for intra-day precision, for inter-day precision, the assay were performed on different days.

In order to estimate the Limit of Detection (LOD) and Limit of Quantitation (LOQ), blank method was run many times, the signal to noise ratio was determined. LOD was considered as 3:1 and LOQ as 10:1. LOD and LOQ were experimentally verified by diluting known concentration of shatavarin-IV until the average responses were approximately three or ten times the Standard Deviation of the response for six replicate determinations.

For recovery, the pre-analyzed samples were spiked with extra 50, 100 and 150 % of the standard Shatavarin-IV and the mixtures were analyzed by the proposed method. The experiment was conducted six times. This was done to check the recovery of the drug at different levels in the formulations. Table No 2 and Table No 3.

Table No 2. Recovery of Shatavarin-IV in Shatavari churna

Drug added to analyte (%)	AVG	Recovery (%)	SD	RSD (%)
50	13.85	98.52	±0.1526	0.0451
100	18.89	99.12	±0.3281	0.1821
150	24.94	99.56	±0.1692	0.0152

Table No 3. Optical and Regression characteristic of Shatavarin-IV

Parameters	Observations
Absorption maximum	426 nm
Beer's law limit	2-10µg/ml
Correlation coefficient (r^2)	0.9992
Recovery	99.06
LOD	3.66
LOQ	11.11
Regression of equation	y= 0.1362x-0.0452
Slope (a)	0.1326
Intercept (b)	0.0452
Precision (%RSD)	
Repeatability	0.2152
Intra-day precision	0.2815
Inter-day precision	0.8695

The extract of batch of laboratory and marketed formulations of Shatavari churna was transferred in 10 ml volumetric flask. Absorbance for aliquots of each was noted at 426 nm. The corresponding concentration of Shatavarin-IV against respective absorbance value was determined by using calibration curve summarized in Table No 4.

Table No 4. Estimation of Shatavarin-IV in formulation and crude drug

Name		Shatavarin-IV content [Mean (n=3)±SD]	SEM
Asparagus racemosus		0.641±0.001	0.00015
Shatavari Churna	ShCL	0.582±0.002	0.00026
	ShCM-I	0.462±0.001	0.00051
	ShCM-II	0.493±0.002	0.00037

Development of HPTLC fingerprint method for Shatavarin-IV

The standard Shatavarin-IV was purchased from Sigma-Aldrich USA through Sigma-Aldrich Corporation Bangalore, India. All the chemicals and reagents used were of Analytical grade. The standard Shatavarin IV solution was prepared by accurately weighing 5 gm of shatavarin IV and was dissolved in methanol and made up to 10 ml in a volumetric flask (0.5 mg/ ml) were refluxed with 60 ml of methanol for 1 hour. The extract was filtered and the marc was again refluxed with 40 ml of methanol for another 1 hour. The previous filtrate was filtered and combined. The methanol extract was concentrated under vacuum till a semisolid mass was obtained. It was finally dissolved and the volume made up to 100 ml with methanol and filtered through sintered glass funnel (G-2) by vacuum filtration assembly. The filtrate was centrifuged at 2000 rpm for 30 minutes, the supernatant was collected and volume was made up with methanol. The instrumentation and chromatographic conditions were:

Spotting device:	Linomat V semi sample spotter; Camag
Syringe:	100 µL Hamilton
TLC chamber:	Glass twin through chamber (20x10x4 cm); Camag
Densitometer:	TLC scanner 3 linked to win cats software V.4.06; Camag
HPTLC plates:	10 x10 cm, 0.2 mm thickness Precoated with silica gel 60
Wavelength:	462 nm
Solvent system:	N-Butanol: acetic acid: water (4:1:5)
Temperature:	25^0C

The HPTLC chromatogram is shown in Figure No 2.

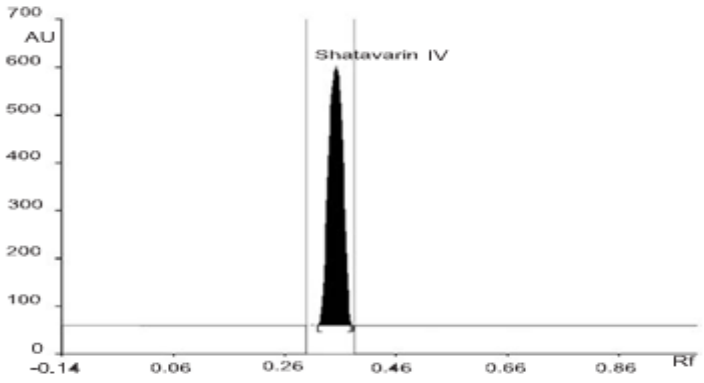

Figure No 2. HPTLC chromatogram of standard Shatavarin

Calibration curve of Shatavarin was prepared by preparing the stock solution of Shatavarin in methanol. Different volumes of stock preparation were spotted on TLC plates. The data of peak areas plotted against corresponding concentration were treated by least-square regression analysis method validation. Repeatability of the sample application and measurement of peak area were carried out using many replicates of the same spot was expressed in terms of percent Relative Standard Deviation (% RSD). The intra and inter day variation for the determination of Shatavarin IV was carried at three different concentration levels of 200, 400, 600 ng/spot and observations are summarized in Table No 5.

Table No 5. Intra and Inter-day precision of HPTLC method for Shatavarin

Principle component	Amount	Intra-day precision		Inter-day precision	
	ng/spot	SD of Area	% RSD	SD of Area	% RSD
Shatavarin IV	200	0.01281	0.00195	0.2795	0.04215
	400	0.02551	0.00120	0.23158	0.01192
	600	0.05124	0.00100	0.20012	0.00651

Robustness of the method was done at a specific concentration for Shatavarin by introducing small change in the mobile phase volume, duration of mobile phase saturation and activation of pre-washed TLC plates with methanol, the effect on the result were examined. In order to estimate the LOD and LOQ blank methanol was spotted LOD was considered 3:1 and LOQ as 10:1. LOD and LOQ were experimentally verified by diluting the known concentrations of shatavarin until the average responses were approximately 3 or 10 times the standard deviation of the response determinations. The specificity of the method was confirmed by analyzing the standard drugs and extract. The spot for Shatavarin in the sample was confirmed by comparing the R_f values and spectra with that of both the standard. The peak purity of the Shatavarin was assessed by comparing the spectra at three different levels. For recovery the pre-analyzed samples were spiked with extra 50, 100 and 150% of the standard Shatavarin and the mixtures were re-analyzed by the proposed method. The experiment was repeated many times, Table No 6 and Table No 7.

Table No 6. Recovery for Shatavarin in Shatavari churna

Drug added to analyte	Concentration found	SD	% Recovery	% RSD
50	432.16	±0.0125	96.16	0.00325
100	530.60	±0.1125	97.30	0.00561
150	729.24	±0.5142	98.31	0.12056

Table No 7. Validation parameters of method of Shatavarin by HPTLC

Parameters	Data of Shatavarin
Linearity range	100-600 ng/spot
Correlation coefficient	0.9995±0.00041
Limit of Detection	2.52
Limit of Quantitation	7.41
Recovery	97.25
Precision (%RSD)	
Repeatability	0.001586
Inter-day precision	0.237066
Intra-day precision	0.029853
Robustness	Robust
Specificity	Specific

For estimation of Shatavarin the appropriate aliquots of piperine extract of each laboratory marketed batch and *Asparagus racemosus* raw ingredient withdrawn in 10 ml volumetric flask separately. The filtered solution was applied on the TLC plate followed by development and scanning. A single spot at R_f =0.44 was observed in chromatogram of Shatavarin along with other component as shown in Figure No 3. The total Shatavarin content observed in summarized in Table No 8.

Table No 8. Shatavarin content in the formulation and crude drug.

Sample	Shatavarin content	RSD
Asparagus racemosus	0.51±0.012	0.1541
ShCL	0.50±0.051	0.1954
ShCM-I	0.45±0.037	0.3512
ShCM-II	0.42±0.015	0.5872

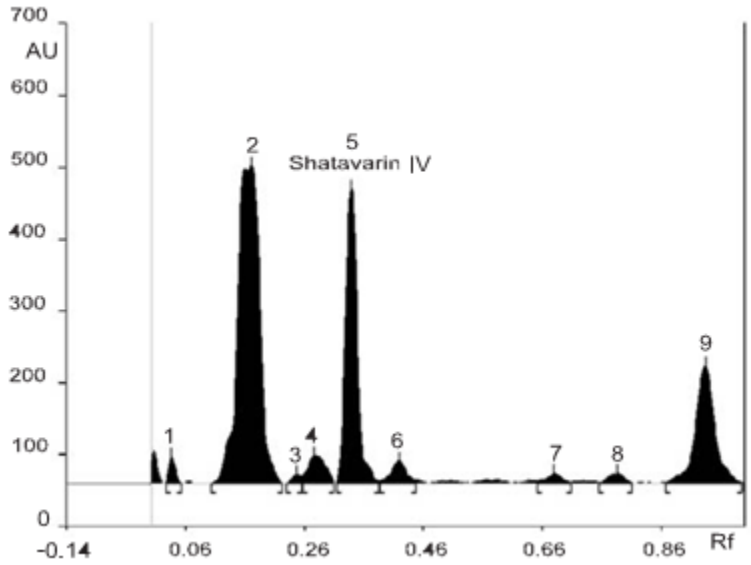

Figure No 3. HPTLC chromatogram of Shatavarin in Shatavari churna

Development of stability studies of Shatavari churna

The laboratory batch and two marketed preparations of Shatavari churna were subjected for development of valid stability testing method at accelerated condition at 45^0C at 75% relative humidity for six months. The Shatavari churna sample were withdrawn at intervals of time (1, 3 and 6 months) and evaluated by previously developed method for various parameters like colour, odour, taste, moisture content and shatavarin content. The changes in value of above parameters with respect to developed parameters were recorded and summarized in Table No 9.

Table No 9. Development of Stability testing of Shatavari churna.

Parameter	Duration	Accelerated stability testing of formulation			Inference
		ShCL	ShCM-I	ShCM-II	
Colour	0 1 3 6	Grayish white NC NC NC	Grayish white NC NC NC	Grayish white NC NC NC	No change
Odor	0 1 3 6	Characteristic NC NC NC	Characteristic NC NC NC	Characteristic NC NC NC	No change
Taste	0 1 3 6	Sweet NC NC NC	Sweet NC NC NC	Sweet NC NC NC	No change
Moisture content	0 1 3 6	8.0±0.18 7.9±0.15 7.5±0.24 7.2±0.58	10.0±0.25 9.8±0.14 9.4±0.19 9.1±0.15	9.5±0.32 9.4±0.12 9.1±0.17 8.9±0.21	Very less change
Shatavarin content	0 1 3 6	0.582±0.002 0.580±0.004 0.578±0.002 0.577±0.010	0.462±0.001 0.461±0.009 0.458±0.015 0.454±0.030	0.493±0.002 0.491±0.005 0.488±0.014 0.483±0.009	Very less change

CONCLUSION

UV fingerprint method was developed for estimation of shatavarin IV in Shatavari churna. The shatavarin IV content of all the formulations shows close proximities amongst each other and recovery studies (99.06%) are indicative of reproducibility of method. Hence, the present developed method is simple, accurate, rapid, precise, reproducible and sensitive and can be used for routine fingerprint for estimation of shatavarin IV in shatavari churna.

Fingerprint method for Shatavari churna with TLC Densitometric methods i.e. HPTLC using shatavarin IV as standard was developed. A good linear relationship was obtained over a concentration range of 100-600 ng/ spot. The correlation coefficient (r^2) value was found to be 0.9995±0.00041, indicates good linearity between the concentration and peak area. The Limit of Detection and Limit of Quantification for shatavarin IV were calculated, which were considered good enough for a reasonable accuracy. The RSD was found to be less than 1% for both intra-day precision and inter-day assay precision. The low value indicates robustness of the method. The amount of shatavarin IV was calculated using the calibration graph. The content of shatavarin IV shows close proximities. The recovery studies were carried out for the accuracy parameter. The mean % recovery (97.25) and % RSD of three levels (0.00325, 0.00561 and 0.12056) were calculated and found to be within the limit, which indicates significant precision of method. The developed HPTLC method is simple, rapid, precise, sensitive and accurate for routine estimation of Shatavarin IV in Shatavari churna. The statistical analysis proved that the method is reproducible and efficient for the analysis of shatavarin IV.

The accelerated stability studies reveal that the sensory characters, physicochemical character and the shatavarin IV content determined as per the developed and described method previously, shows very less change. It was found that the selected marketed formulations and formulation prepared in laboratory are found to be stable even at accelerated conditions.

REFERENCES

1. Gautam V, Raman RMV, Ashish K. Exporting Indian healthcare (Export potential of Ayurveda and Siddha products and services) Road beyond boundaries (The case of selected Indian healthcare systems).Gautam V, Raman RMV, Ashish K. (eds.) Export-Import Bank of India; Mumbai: 2003; p 4–54.
2. Sagar Bhanu PS, Zafar R, Panwar R. Herbal drug standardization. The Indian Pharmacist 2005; 4(35):19-22.
3. Amit J, Sunil C, Vimal K, Anupam P. Phytosomes: A revolution in herbal drugs. The Pharma Review 2007; 11-13.
4. http://www.who.int/research/en.
5. Patel PM, Patel NM, Goyal RK. Quality control of herbal products. The Indian Pharmacist 2006; 5(45):26-30.
6. Vaidya ADB, Devasagayam TPA. Current status of herbal drugs in India: An overview. J Clin Biochem 2007; 41(1):1–11.
7. http://www.morethanvitamins.co.uk/herbal-remedies-c-24.html.
8. Yadav NP, Dixit VK. Recent approaches in herbal drug standardization. Int J Integr Biol 2008; 2:195-203.
9. Sachan V, Kohli Y, Gautam R. Regulatory issues for herbal products – a review. Pharmainfo.net, 12/16/2009; http://www.pharmainfo.net/justvishal/publications/regulatoryissues-herbal-products-review.
10. Zafar R, Panwar R, Sagar Bhanu PS. Herbal drug standardization: The Indian Pharmacist 2005; 4(36): 21-25.

11. Iyengar M.A., Study of Crude Drugs, Published by Prof. Dr. M. A. Iyengar, Dept. of Pharmacognosy, College of Pharmaceutical Sciences, Manipal, Printed at Manipal Press Ltd., Manipal, 10, 108-109 (2001).
12. Khare CP. Indian Medicinal Plants. Berlin: Springer Verlag; 2007
13. Gupta V, Jain UK (2011) Status of piperine content in Ayurvedic formulation: method standardization by HPTLC. Res J Pharm Biol Chem Sci 2: 524-531.
14. Mukherjee P.K. (2005) Quality Control of Herbal Drugs. New Delhi: Business Horizons 741.
15. Ong E.S. (2004) Extraction methods and chemical standardization of botanicals and herbal preparations. J Chromatogr B Analyt Technol Biomed Life Sci 812:23-33.
16. Guideline on Validation of Analytical Procedure- Methodology (1996) Geneva: International Conference on Harmonization.
17. Meena A.K., Rao M.M., Panda P., Kiran, Yadav A., et al. (2010) Standardization of Ayurvedic polyherbal formulation, Pancasama Churna. Int J Pharmacog Phytochem Res 2: 11-14.
18. Willard-Hobart H, Merritt Jr Lynne L, Dean John A (1974) Instrumental Methods of Analysis. (5th Edn), Von Nostrand, University of Michigan.
19. Chatwal GR, Anand S (2002) Instrumental Methods of Chemical Analysis. (5thedn), Himalaya Publishing House, New Delhi.
20. Davidson AG (2002) Ultraviolet-visible absorption spectrophotometry. In Beckett A H, Stenlake J B, (4thedn), Practical Pharmaceutical chemistry. CBS Publishers and distributors, New Delhi, 275-278.
21. Patil KM, Bodhankar SL (2005) High-performance thin-layer chromatographic determination of lamotrigine in serum. J Chromatogr B Analyt Technol Biomed Life Sci 823: 152-157.
22. Singh B. V., Chemistry of Milk and Milk Products, Muzaffarnagar: Asian Publisher; 1965: 143.