

DETERMINATION OF SHATAVARIN-IV IN *ASPARAGUS RACEMOSUS* BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

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Abstract : Standardization of herbal medicines and plant ingredients with reference to their active marker is the need of the time. We tried to standardize *Asparagus racemosus* using high performance thin layer chromatography method (HPTLC) for its active compound, shatavarin IV. A simple, sensitive HPTLC method was developed for the determination of shatavarin IV in *Asparagus racemosus* extract. The R_f value of shatavarin IV were found to be 0.44. The amount of shatavarin IV present in the test extract is 0.23/100mg. Shatavarin IV showed good linearity in the concentration range of (32.20-1030.50 $\mu\text{g/mL}$), with a correlation coefficient of 0.999. The average percentage recovery for shatavarin IV is 99.60 %. The proposed method is reproducible and statistically validated. The system was successfully used to investigate the presence of the shatavarin IV in *Asparagus racemosus* plant parts as well as to analyze their content in market products.

Keywords: *Asparagus racemosus*, Shatavarin IV, HPTLC analysis, Method validation.

Introduction

Asparagus racemosus Willd. (Liliaceae), commonly known as 'Shatavari', is a much-branched, spinous under shrub found growing wild in tropical and sub-tropical parts of India. In Indian system of medicine *Asparagus racemosus* is an important medicinal plant and its root paste or root juice has been used in various ailments and as health tonic.^{1,2} *Asparagus racemosus* is a well known Ayurvedic rasayana which prevent ageing, increase longevity, impart immunity, improve mental function, vigor and add vitality to the body and it is also used in nervous disorders, dyspepsia, tumors, inflammation, neuropathy, hepatopathy.³ Reports indicate that the pharmacological activities of *Asparagus racemosus* root extract include antiulcer,⁴ antioxidant,⁵ antidiarrhoeal,^{6,7} antidiabetic⁸ and immuno-modulatory activities.⁹

The major active constituents of *Asparagus racemosus* are steroidal saponins (Shatavarins I-IV) that are present in the roots. Shatavarin IV is a glycoside of sarsasapogenin having two molecules of rhamnose and one molecule of glucose. Shatavarin IV has been reported to display significant activity

as an inhibitor of Core 2 GlcNAc transferase in cell free assays and recently to exhibit immuno-modulation activity against specific T-dependent antigens in immuno compromised animals.¹⁰

Materials and Methods

Plant Materials

The root of the *Asparagus racemosus* were picked up in the month of February-March from the campus. Collected crude drugs were authenticated from the Botany Dept., Dr. H. S. Gour Vishwavidyalaya, Sagar. collected crude druge were authenticated from the Botany Dept. Dr. H.S. Gour Vishwavidyalaya, Sagar, M.P. (India).

HPTLC Plates

The precoated and preactivated TLC plates (E. Merk No.OB575863) of silica Gel 60F₂₅₄ with the support of aluminium sheets having thickness of 0.25 mm and size 20 x 20 cm were cut into smaller size according to required dimensions.

Selection of Solvent

Solvent system (10 mL), ethyl acetate: methanol: water (75:15:10) was poured into Camag

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Twin Trough Chamber and allowed for 15 min. for chamber saturation. After chamber saturation the TLC plates were developed in the solvent system for 20 min. up to 9 cm length, by ascending technique. The plates were removed from the chamber after the development and dried.

Standard Shatavarin IV Solution

Standard shatavarin IV was obtained from Natural Remedies Pvt. Ltd. Bangalore, India. Accurately weighed quantity (5 mg) of shatavarin IV was dissolved in methanol and made up to 10 mL (0.5 mg/mL) with methanol in a volumetric flask.

Sample Preparation

Accurately weighed 100 mg of *Asparagus racemosus* extract was transferred into a 250 mL beaker then 50 mL of methanol was added and boiled on a water bath for 10-15 min. After cooling the liquid was filtered through 42 wattman filter paper. The process was repeated for three more times and the fractions were combined and made up to 100 mL.

Application of Standard and Sample

Standard solutions of shatavarin IV and sample solutions were applied to the plates as 6 mm bands by means of CAMAG LINOMAT IV an automatic sample application device. The quantity of sample applied was 20 μ L. After development and drying of plates, evaluation of both standard and samples were performed.

Post Chromatographic Treatment of Plates

The detection of spots in the TLC plates was carried out by viewing at 254, 366 nm and also by spraying vanillin sulphuric acid reagent (Dissolve 1g of Vanillin in 100 mL ethanol and 5 mL of concentrated sulphuric acid keeping ethanol under the cold condition) The TLC plate heated at 100°C for 5-10 min. and observed in visible light.

Method Validation

Calibration Curve

Standard shatavarin IV solutions concentrations range (32.20-1030.50 μ g/mL) were

analyzed for studying the linearity and the area count obtained for these solutions.

Limit of Detection and Limit of Quantitation

In order to estimate the limit of detection (LOD) and lower limit of quantitation (LLOQ), blank methanol was spotted six times following the same method. The signal to noise ratio (S/N) was determined. LOD was considered as 3:1 and LLOQ as 10:1.

Recovery Studies

The accuracy and precision of the method were studied by performing experiments by standard addition technique. Three different levels of standards (0.3 μ g, 0.6 μ g and 0.9 μ g) were added to a previously analyzed sample, each level being repeated thrice. The percentage recovery was calculated from amount of shatavarin IV found.

Results

The R_f value of shatavarin IV in standard and samples were found to be 0.44 (Yellow spots). The chromatographic plate was shown in **Plate 1**. The amount of shatavarin IV present in the methanolic extract in 20 μ L is 0.46 μ g; therefore 100 mg of extract contain 0.23 mg of shatavarin IV. The estimation of shatavarin IV in *Asparagus racemosus* are shown in **Table 1**. The signal to noise ratios 3:1 and 10:1 were considered as LOD and LLOQ respectively. The LOD and LLOQ were found to be 100 and 400 ng/spot. Shatavarin IV showed good linearity in the concentration range of (32.20-1030.50 μ g/mL), with a correlation coefficient of 0.999. **Figure 1** showed linearity graph between peak areas vs. concentration. The mean percentage recovery obtained for shatavarin IV was 99.60% showed in **Table 2**. Low value of standard deviation and coefficient of variation are indicative of high precision of the method.

Discussion

A critical analysis of the literature revealed that this herb finds widespread use in several traditional systems of medicine. The number of analytical reports for the determination of shatavarin IV is comparatively small. Only few methods are described in the literature. All of them show major

disadvantage, As part of our efforts to developed analytical method suitable for the standardization of shatavarin IV in *Asparagus racemosus*, this study had several aims to develop and validate a system for accurate, rapid and direct determination of the shatavarin IV in *Asparagus racemosus*; to investigate the distribution of these compounds in different plant parts and finally to analyze several commercial products to determine their composition and quality.

We tried to standardize *Asparagus racemosus* using HPTLC for its active compound, shatavarin IV. The method provides good resolution and separation of shatavarin IV from other constituents of *Asparagus racemosus*. The proposed HPTLC method is rapid, simple and accurate for quantitative monitoring of shatavarin IV in *Asparagus racemosus*.

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Table 1. Estimation of Shatavarin IV in *Asparagus racemosus*

Replicate	Sample Area	Shatavarin IV (μg)
1	1764179	0.046
2	1763057	0.046
3	1763914	0.046
Average	1763716 \pm 586	0.046 \pm 0.0

Mean \pm SD, n = 3

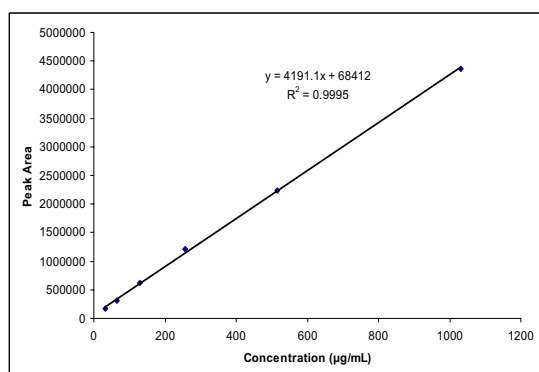


Figure 1. Linearity graph of shatavarin IV

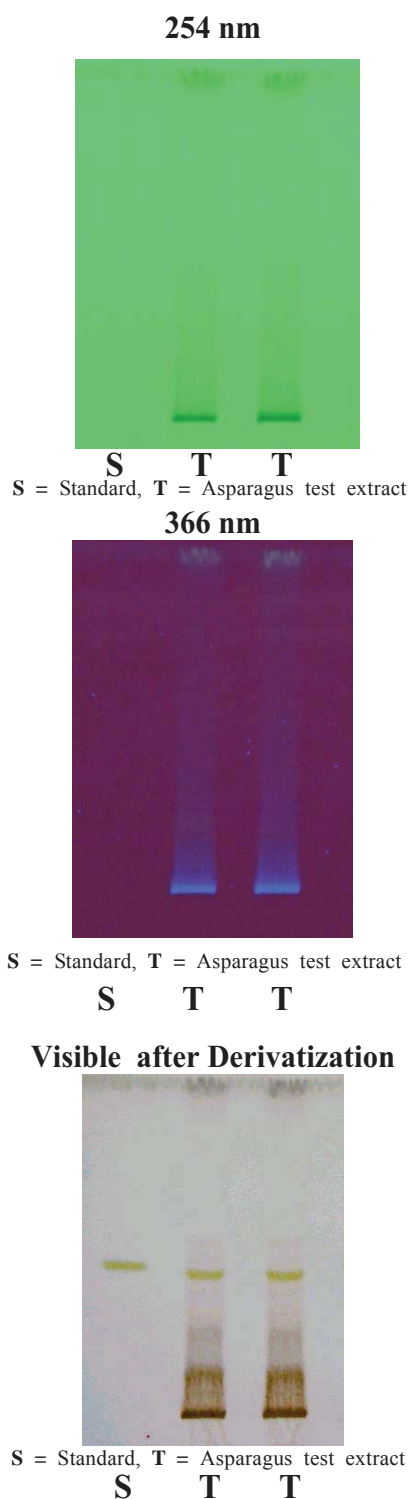


Plate 1. HPTLC Chromatogram of Standard Shatavarin IV and *Asparagus racemosus* test extract

Table 2. Results of recovery analysis

Sample	Amount of shatavarin IV present in (µg)	Amount of shatavarin IV added to A (µg)	Total shatavarin IV taken (A+B) (µg)	Total shatavarin IV found (µg)	% Recovery D/C x100 (Mean)	% Mean recovery
	A	B	C	D		
<i>Asparagus racemosus</i>	0.046	0.3	0.346	0.342 ± 0.04	98.84	
		0.6	0.646	0.648 ± 0.02	100.30	99.60
		0.9	0.946	0.943 ± 0.03	99.68	

Mean ± S.D., n = 3

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