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RESEARCH ARTICLE

# Quality control of marketed herbal products of *Asparagus racemosus* Willd. through high performance thin layer chromatography (HPTLC) analysis

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## Abstract

*Asparagus racemosus* Willd. is a valuable medicinal plant which is used all over the world. There are several marketed products of *A. racemosus.* The high demand for this herb has increased the risk of adulteration in its commercial products. The adulterated herbal products might pose serious ill effects on health. Therefore, it is necessary to check the quality of marketed products in terms of the presence of their major bioactive compounds. The present study aimed to carry out the qualitative and quantitative analysis of Shatavarin IV in marketed products of *A. racemosus* through a validated high performance thin layer chromatography (HPTLC) method. Ten marketed products were analysed and all of them had shown the presence of Shatavarin IV which was quantified. The identification and quantification were done by taking a standard Shatavarin IV as reference. The Shatavarin IV was detected at $R_f$ 0.4±0.05 and showed maximum absorption at 425 nm. The Shatavarin IV was quantified using a 6-point calibration curve having a standard deviation of 3.89 % with an $R^2$ value of 0.9968. The amount of Shatavarin IV varied between 1.47±0.25 to 2.69±0.51 mg/g on a dry weight basis which is a normal range in the raw plant materials. Thus, the present findings would be a simple, reliable and cost-effective method for the quality determination of herbal products of *A. racemosus.* The developed HPTLC chromatograms would serve as a reference for the quality assessment of commercial products of *A. racemosus* in future.

## Keywords

*Asparagus racemosus*, Shatavarin IV, HPTLC, Quality control, Adulteration, Herbal products.

## Introduction

*Asparagus racemosus* has various therapeutic and medicinal properties like antioxidant activity, anti-inflammatory activity, cardioprotective activity, anticancer activity, antiulcer activity etc. due to the presence of a major glycoside, Shatavarin IV (1, 2). The plant has been placed in the 'endangered' category in its natural habitat due to its varied uses and high demand (3). The ever-increasing market value of *A. racemosus* is due to rise in its national and international demand (4). The high market value may lead to the production of adulterated products.

In general, there are 27% of adulterated herbal products available in the market all over the globe and in India, there are 31 % adulterated herbal products reported (5). The adulterated herbal products may result in unpredictable adverse effects on the health of users (6). Nowadays, the use of chromatographic techniques is prevalent for the quality assessment of herbal products (7). There are several chromatographic techniques like high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), gas chromatography (GC) etc. used largely but HPTLC is the most potential technique due to its reliability and simplicity (8).

Therefore, in the present study, a validated HPTLC method has been employed to assess the qualitative and quantitative determination of Shatavarin IV in selected marketed herbal products of *A. racemosus*.

## Materials and Methods

### *Sample, standard and chemicals*

The herbal products or processed powder of *A. racemosus* were procured from the market. To protect the vendors, the samples were renamed from ARMP-1 to ARMP-10. The standard Shatavarin IV was purchased from Natural Remedies Private Limited, Bangalore, India. Chemicals like ethyl acetate, methanol, sulfuric acid and water (HPLC grade) were purchased from Merk Life Science Private Limited, Mumbai, India. Anisaldehyde was purchased from HiMedia Laboratories Private Limited, Mumbai, India. Acetone and acetic acid glacial were procured from Merck Specialities Private Limited, Mumbai, India. The HPTLC silica gel 60 $F_{254}$ (10×20 cm) plates were procured from Merck KGaA, Darmstadt, Germany. The samples and standards were prepared by dissolving a known amount in methanol. The samples were prepared by dissolving in methanol (100 mg/ml) and allowed to stand in a water bath for 1 hr at 60˚C. The aliquot was then filtered through a 0.22 µm syringe filter and was stored for further analysis.

### *HPTLC analysis conditions*

The chromatographic method is adopted from standard procedure (9) with slight modification and validated in the current study for analysis. The standard and the extracted solution were spotted on pre-coated silica gel 60 $F_{254}$ (10×20 cm) HPTLC plates as the stationary phase for the chromatographic analysis. A 100 µL syringe and a CAMAG Linomat V sample applicator were used to apply the samples and standards on the plates. Utilizing a nitrogen aspirator at a rate of 25 kPa, the samples and standards of band length 8 mm were applied. There was a gap of 12 mm between the bands. The linear ascending plate development was carried out using a 20×10 cm twin-trough glass chamber (CAMAG) that had been saturated for 20 min at room temperature in the mobile phase of the ratio of ethyl acetate-methanol-water (7.5:1.5:1, v/v/v). The bands were allowed to run up to a distance of 90 mm. The developed plates were viewed in a CAMAG UV cabinet at 254 and 366 nm wavelength of UV light and derivatized using anisaldehyde–sulfuric acid reagent at 110 °C in a hot-air oven for 5 min. The CAMAG TLC scanner 4 with winCATS Software was used to scan the plate (Slit dimension: 6.00 x 0.45) at a wavelength of 425 nm. The spectra of Shatavarin IV were taken in the range of 300-700 nm for both standard and herbal samples. The Shatavarin IV in marketed samples was quantified using a calibration curve after identifying through retardation factor ($R_f$) and spectral comparison between the standard and marketed samples.

### *Method Validation*

The method of quantitative analysis was validated in terms of linearity, the limit of detection (LOD) and quantification (LOQ), precision, stability and recovery tests according to the guidelines set by International Conference on Harmonization, ICH (10). The linear curve was made by six different concentrations of Shatavarin IV and the regression equation was obtained for quantitative analysis. The precision test was done by injecting the replicate solution of standard for 3 times (0, 3, 6hrs) within a day and injecting the same solution of standard for three consecutive days (0, 24, 48hrs) in the stability test. The recovery test was done by the method of standard addition.

## Results

### *Method validation*

The calibration curve showed a good linearity with a coefficient of determination ($R^2$) of 0.9968 and a regression equation of y=14.25x+779.45 for quantitative estimation (Fig. 1A). The LOD and LOQ were found to be 24 and 72 ng/band respectively (Table 1). The relative standard deviation (RSD) of precision and stability were determined to be 1.63% and 1.69% respectively (Table 2). The recovery rate was found to be 97 % for Shatavarin IV (Table 3).

**Table 1.** Sensitive analysis and calibration curve of Shatavarin IV

| Component | linear range | Regression equations | $R^2$ | LOD (ng) | LOQ (ng) |
|---|---|---|---|---|---|
| Shatavarin IV | 72-432 | y=14.25x+779.45 | 0.9968 | 24 | 72 |

LOD: Limit of detection; LOQ: Limit of quantification

**Table 2.** Intra-day and inter-day precision of Shatavarin IV by HPTLC method

| Component | Precision | Amount applied (ng) | Amount found (ng) | Mean | RSD (%) |
|---|---|---|---|---|---|
| Shatavarin IV | Intra-day | 705 | 703 | 701 | 1.63 |
| | | | 699 | | |
| | | | 701 | | |
| | Inter-day | 705 | 703 | 702.33 | 1.69 |
| | | | 700 | | |
| | | | 704 | | |

RSD: Relative standard deviation

### *Qualitative and quantitative estimation of Shatavarin IV*

The Shatavarin IV was detected at $R_f$ of 0.4±0.05 by comparing the bands with the chromatogram of standard (Fig. 2). The 2D densitogram of the Shatavarin IV has been

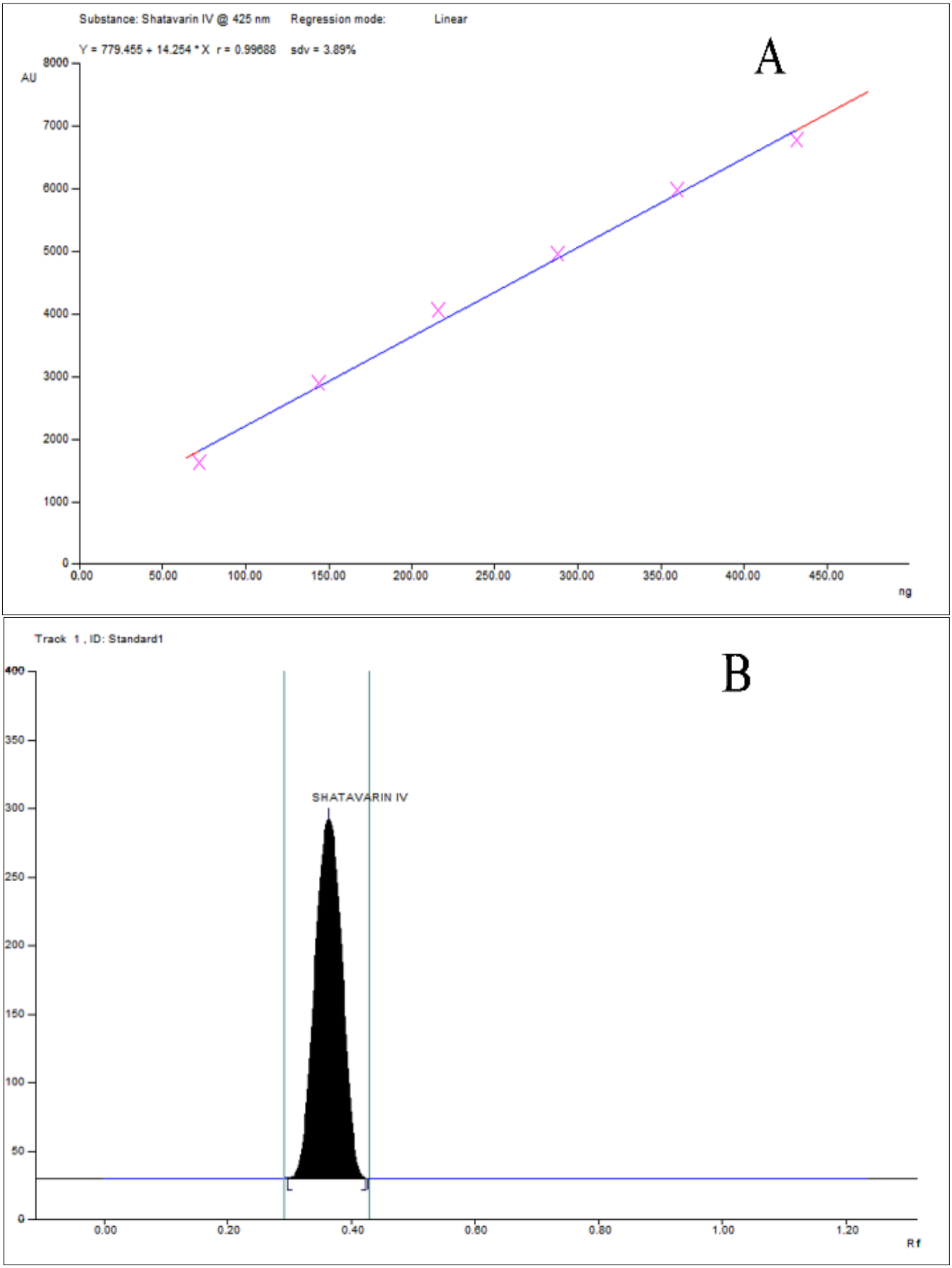



**Fig. 1.** Calibration Curve (**A**) and 2D densitogram (**B**) of Shatavarin IV through HPTLC.

given in Fig. 1B. The 3D densitogram and developed HPTLC chromatograms has been given in the Fig. 3A. The spectral confirmation was also done to confirm the Shatavarin IV bands (Fig. 3B). The 2D densitogram of the marketed products are given in the Fig. 4. The quantity of Shatavarin IV was in the range of 1.47±0.25 to 2.69±0.51 mg/g on a dry weight basis (Table 4).

**Table 3.** Recovery study of Shatavarin IV by HPTLC method

| Component | Original amount (ng) | Added Amount (ng) | Found (ng) | Recovery rate (%) | Average recovery (%) | RSD (%) |
|---|---|---|---|---|---|---|
| | | | 888.56 | 96.06 | | |
| | | 200 | 897.78 | 97.05 | 97.17 | 0.96 |
| | | | 910.32 | 98.41 | | |
| | | | 1005.45 | 98.09 | | |
| Shatavarin IV | 725 | 300 | 995.67 | 97.13 | 97.57 | 0.39 |
| | | | 999.43 | 97.50 | | |
| | | | 1102.22 | 97.97 | | |
| | | 400 | 1099.37 | 97.72 | 97.55 | 0.43 |
| | | | 1090.78 | 96.95 | | |

RSD: Relative standard deviation

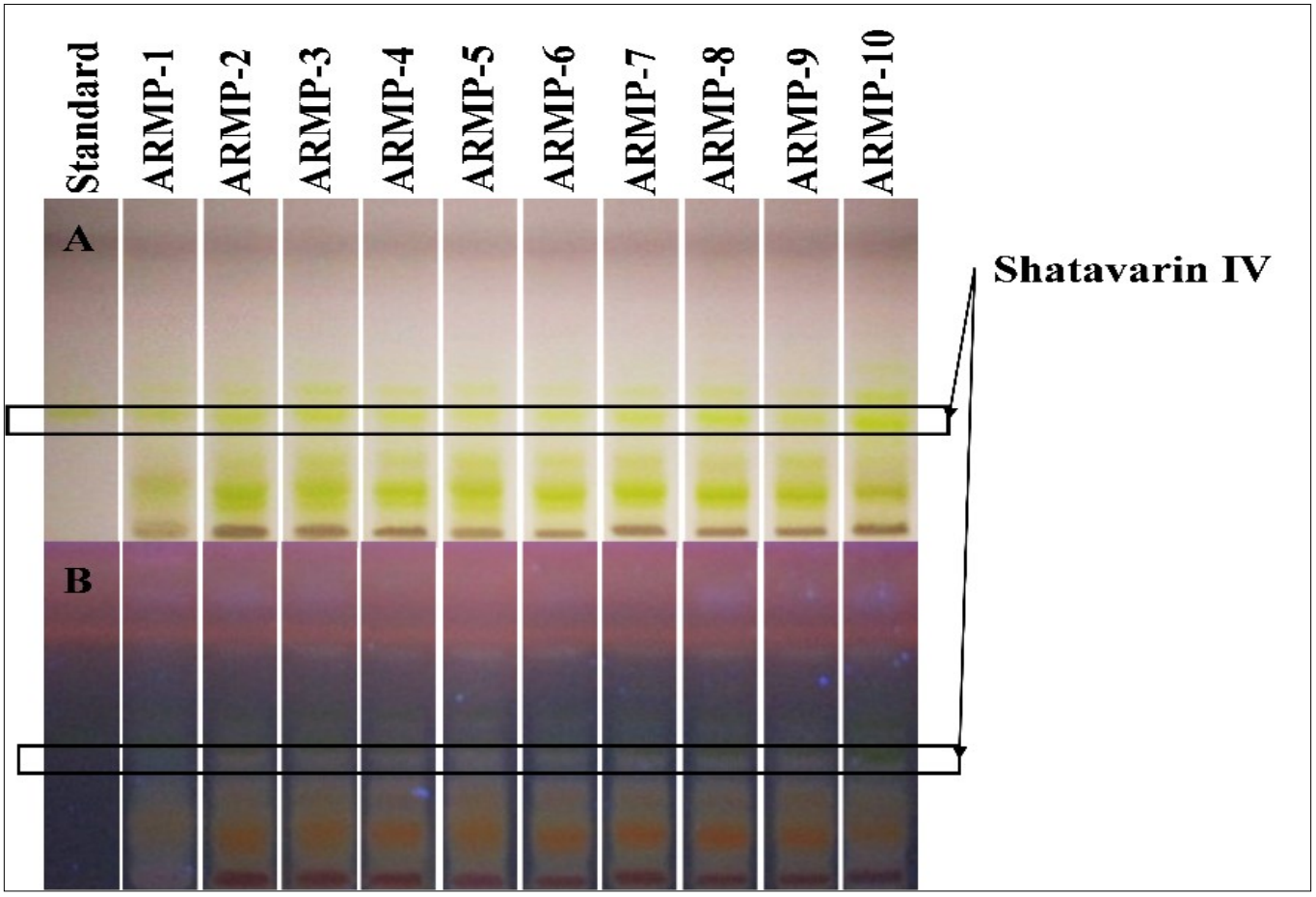



**Fig. 2.** HPTLC Chromatogram after derivatization at UV light 366 nm wavelength (**B**) and in normal florescent light (**A**).

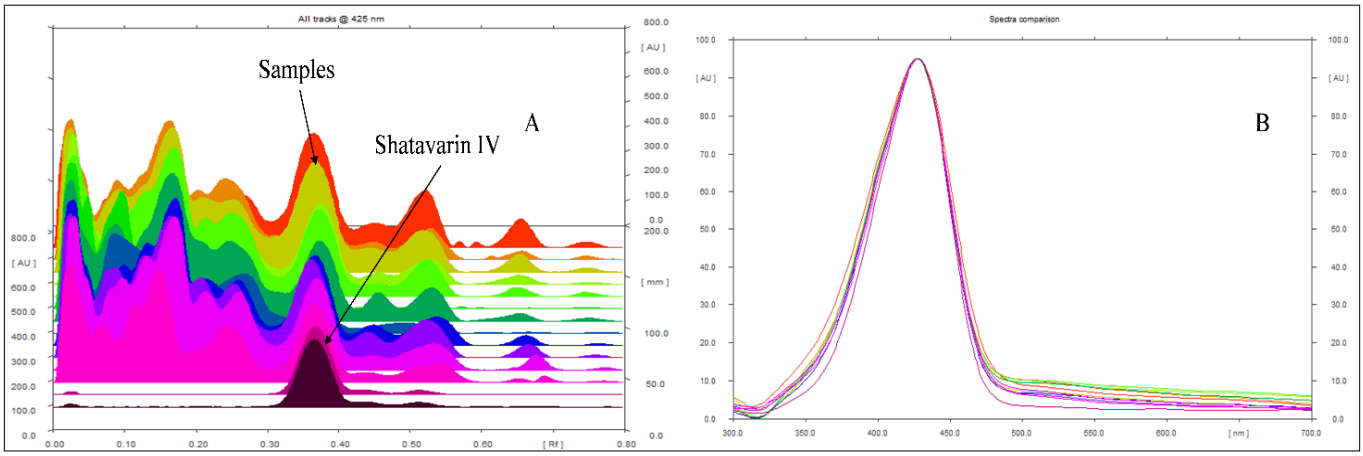
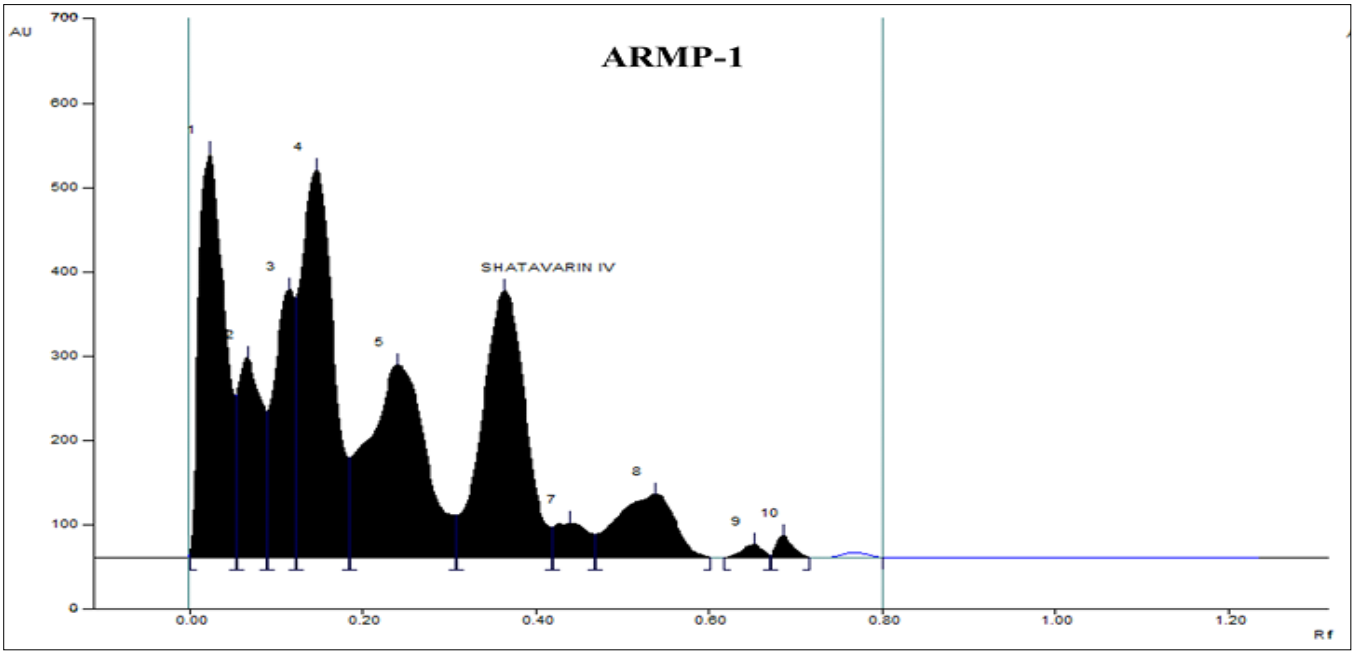
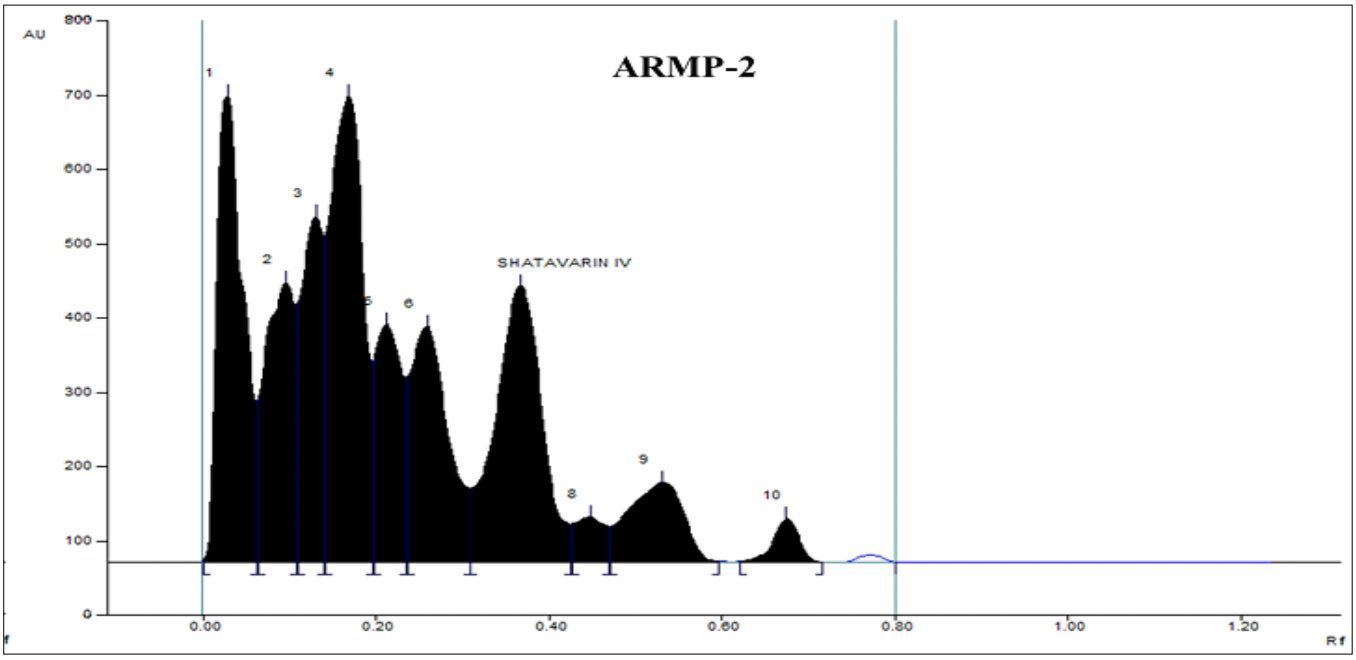
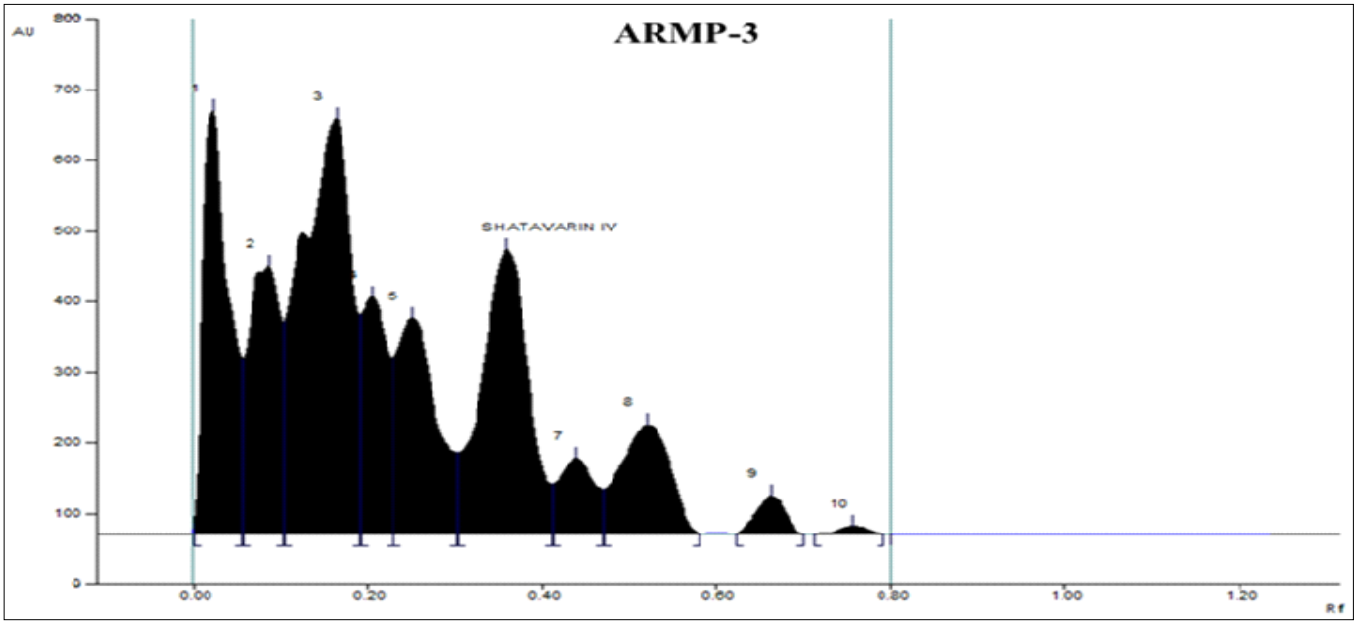
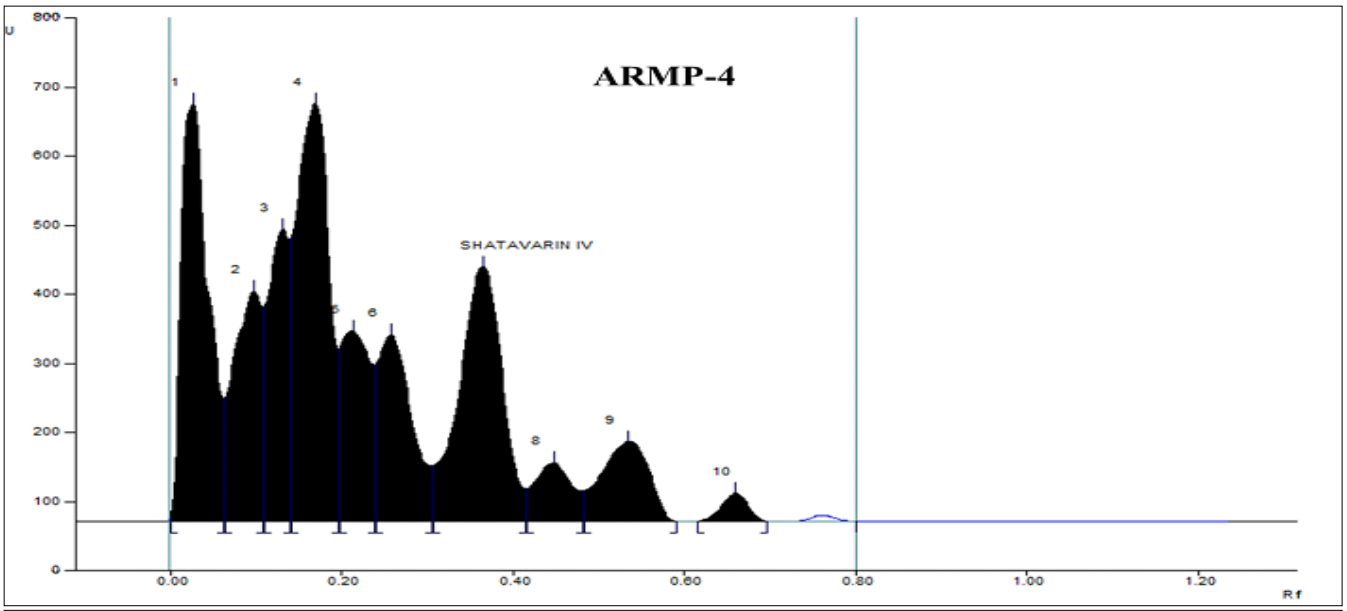


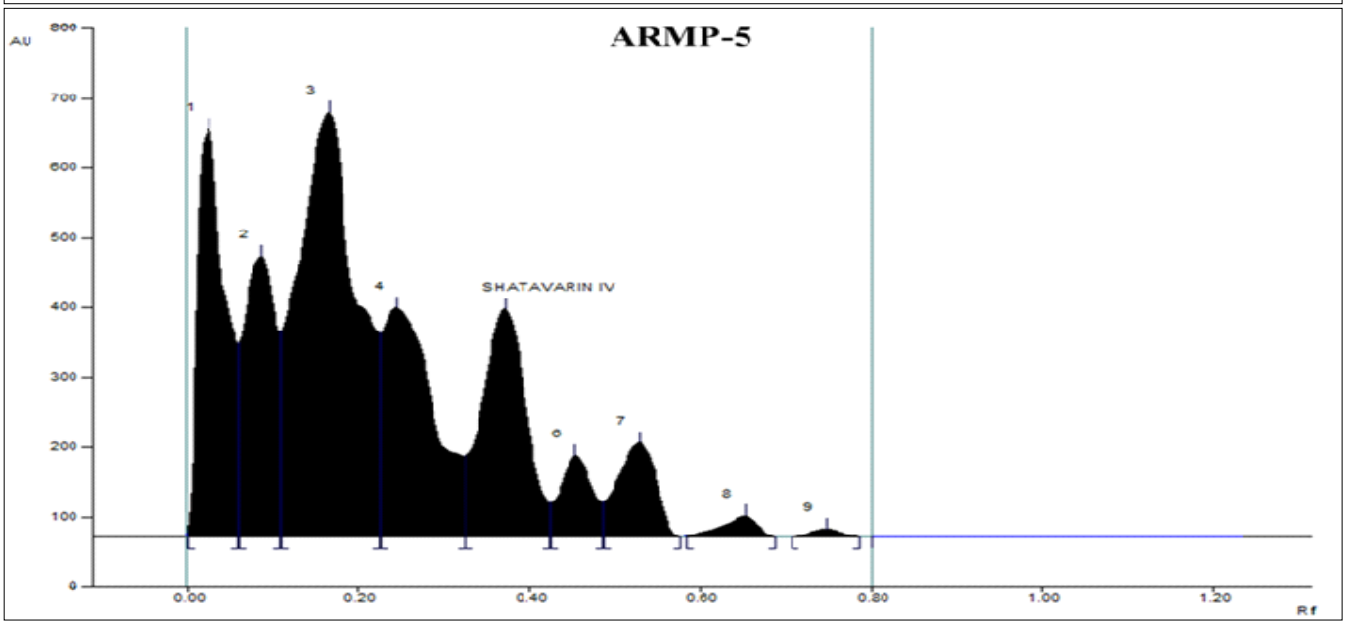

**Fig. 3.** 3D Densitogram (**A**) and overlay Spectra (**B**) of marketed products and Shatavarin IV.

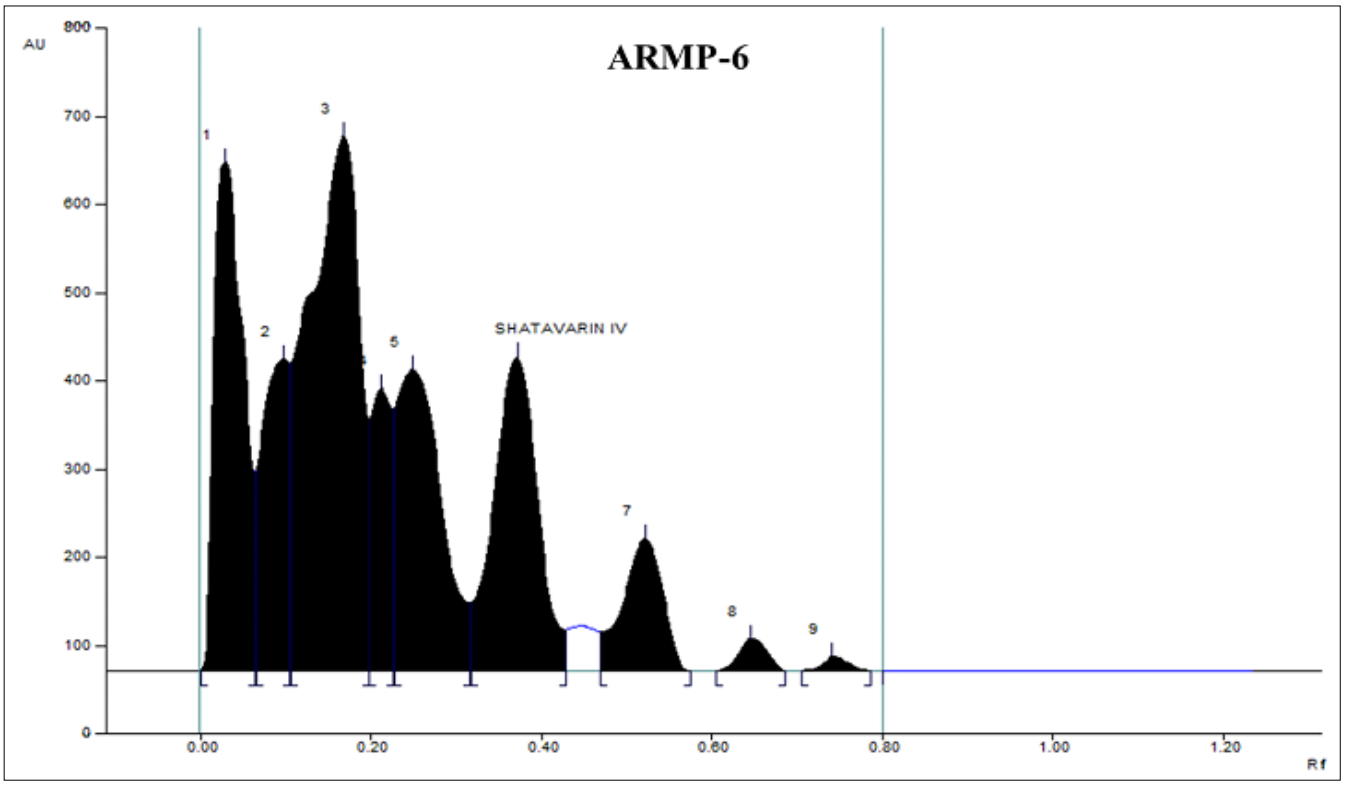

ARMP-1
AU
Rf
SHATAVARIN IV
1
2
3
4
5
7
8
9
10
0
100
200
300
400
500
600
700
0.00
0.20
0.40
0.60
0.80
1.00
1.20

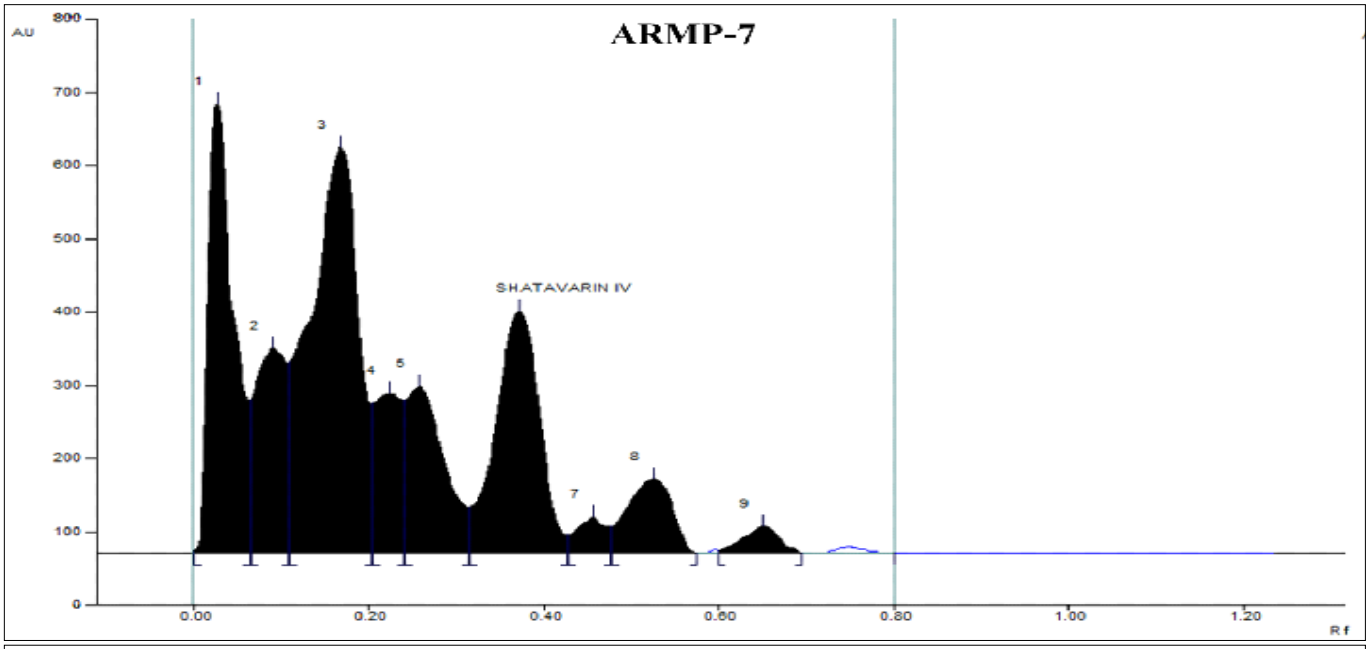

ARMP-2
AU
Rf
SHATAVARIN IV
1
2
3
4
5
6
8
9
10
0
100
200
300
400
500
600
700
800
0.00
0.20
0.40
0.60
0.80
1.00
1.20

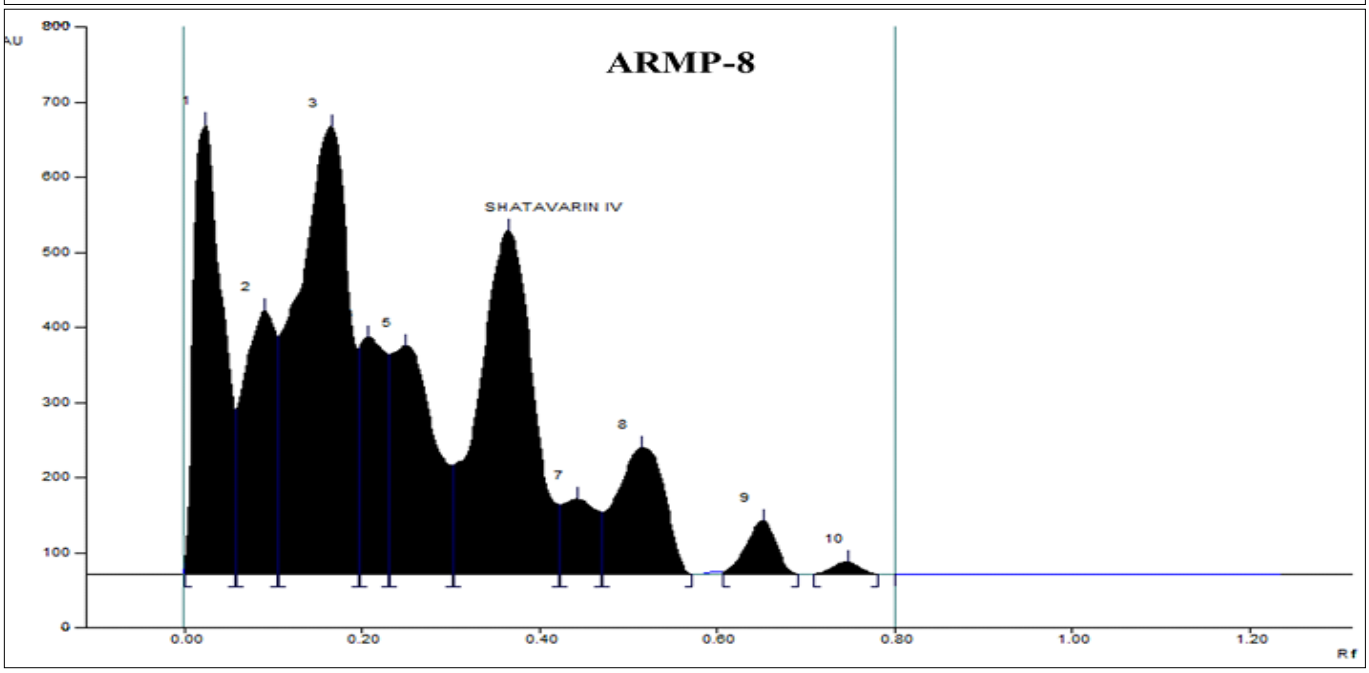

ARMP-3
AU
Rf
SHATAVARIN IV
1
2
3
5
7
8
9
10
0
100
200
300
400
500
600
700
800
0.00
0.20
0.40
0.60
0.80
1.00
1.20

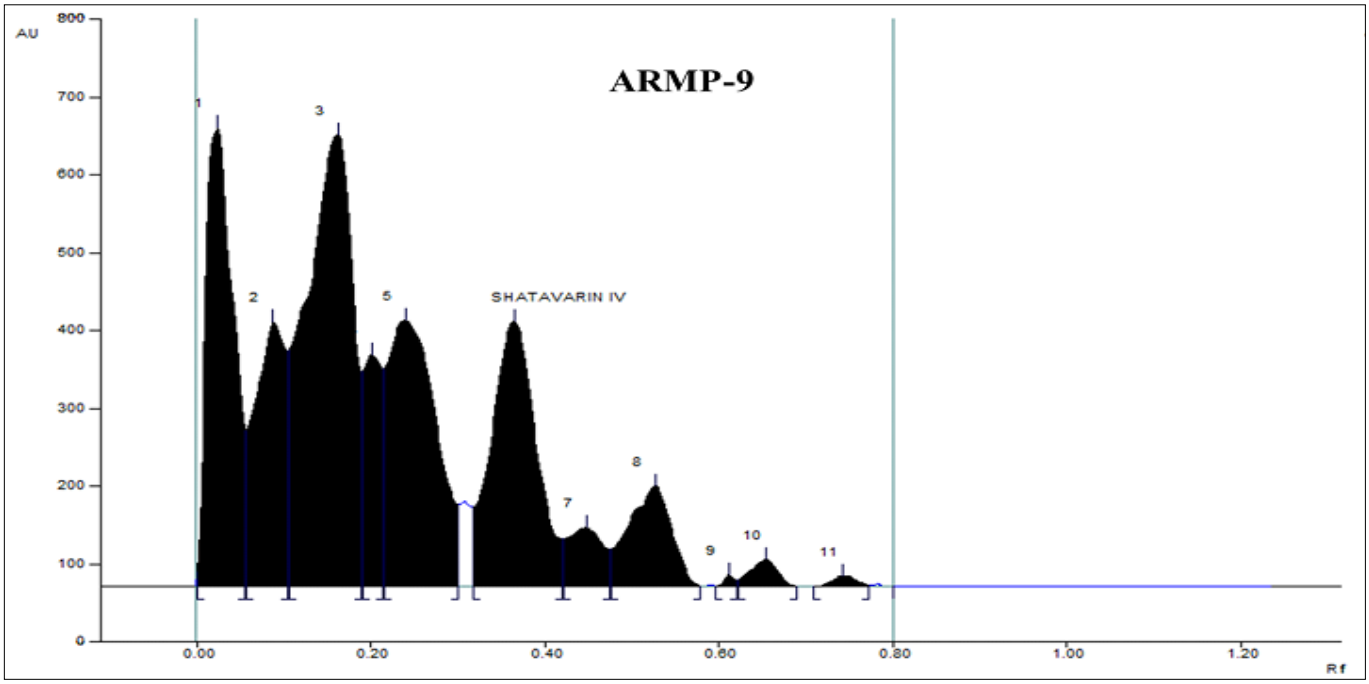

ARMP-4
SHATAVARIN IV
1
2
3
4
5
6
8
9
10
800
700
600
500
400
300
200
100
0
0.00
0.20
0.40
0.60
0.80
1.00
1.20
Rf


ARMP-5
AU
SHATAVARIN IV
1
2
3
4
6
7
8
9
800
700
600
500
400
300
200
100
0
0.00
0.20
0.40
0.60
0.80
1.00
1.20
Rf


ARMP-6
AU
SHATAVARIN IV
1
2
3
5
7
8
9
800
700
600
500
400
300
200
100
0
0.00
0.20
0.40
0.60
0.80
1.00
1.20
Rf


ARMP-7
AU
800
700
600
500
400
300
200
100
0
1
2
3
4
5
7
8
9
SHATAVARIN IV
0.00
0.20
0.40
0.60
0.80
1.00
1.20
Rf


ARMP-8
AU
800
700
600
500
400
300
200
100
0
1
2
3
5
7
8
9
10
SHATAVARIN IV
0.00
0.20
0.40
0.60
0.80
1.00
1.20
Rf


ARMP-9
AU
800
700
600
500
400
300
200
100
0
1
2
3
5
7
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9
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11
SHATAVARIN IV
0.00
0.20
0.40
0.60
0.80
1.00
1.20
Rf

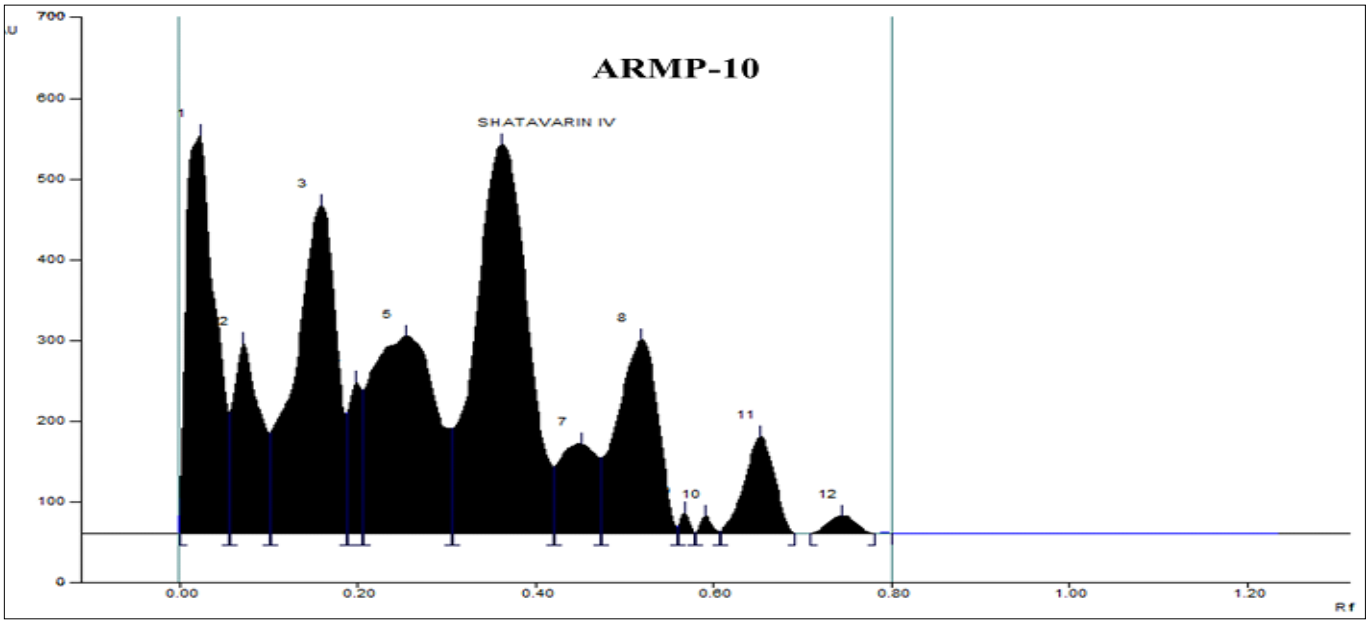


**Fig. 4.** 2D HPTLC densitogram of marketed products of *A. racemosus.*

**Table 4.** The amount of Shatavarin IV determined in Marketed products of *A. racemosus* in terms of mg/g on dry weight basis (n=3)

| Code | Product type | Shatavarin IV (mg/g, Mean±SD) |
|---|---|---|
| ARMP-1 | Shatavari (Tablet) | 1.47±0.25 |
| ARMP-2 | Shatavari Powder | 1.95±0.19 |
| ARMP-3 | Shatavari Churna | 2.07±0.33 |
| ARMP-4 | Shatavari Powder | 1.78±0.20 |
| ARMP-5 | Shatavari (Capsule) | 1.60±0.89 |
| ARMP-6 | Shatavari Powder | 1.79±0.73 |
| ARMP-7 | Shatavari Powder | 1.59±0.29 |
| ARMP-8 | Shatavari Roots Powder | 2.69±0.51 |
| ARMP-9 | Shatavari Powder | 1.64±0.75 |
| ARMP-10 | Shatavari Churna | 2.62±0.35 |

SD-Standard deviation

## Discussion

The method validation criteria of the present HPTLC method shown its robustness for use in the quantification process of Shatavarin IV. LOD is the minimum concentration of the analyte that can be detected and LOQ is the minimum concentration of the analyte which can be quantified. The Shatavarin IV in the marketed samples was above the minimum ranges of LOD and LOQ, hence quantified in the samples. The precision and stability were also within the acceptable range of RSD. The recovery of the Shatavarin IV was good in the present HPTLC method.

The marketed product, ARMP-8 showed the highest concentration of Shatavarin IV i.e., 2.69±0.51 mg/g on dry weight basis and the lowest concentration of 1.47±0.25 mg/g on dry weight basis was observed in ARMP-1. However, all the marketed products found to have Shatavarin IV i.e., none of the products has been adulterated in terms of Shatavarin IV content. In the previous studies, it has been seen that the roots of *A. racemosus* procured from Natural Remedies, Bangalore, India have shown a Shatavarin IV content of 1.30 mg/g (11). According to an HPLC/ESI-MS/MS assessment of commercial *A. racemosus* products, the variation of Shatavarin IV ranged from 0.08 mg/g to 2.94 mg/g on dry weight basis (12). They have studied both multi and single ingredient marketed products including crude extract, capsule, syrup, tablet etc. Similarly, the content of Shatavarin IV was quantified to be $4.8\times10^4$ mg/g in polyherbal formulation using HPTLC (13). The previous reports suggested that HPTLC method could be applied successfully for quantification of Shatavarin IV in aqueous extract and polyherbal formulation of *A. racemosus* (14). Further, several researchers have used HPTLC method for analysing the bioactive constituents in marketed herbal products of important medicinal plants (15-18). Besides HPTLC, some other chromatographic method like HPLC and HPLC-Q-TOF-MS/MS have also been used for determination of Shatavarin IV in *A. racemosus* samples and marketed products (19, 20). However, HPTLC is still one of the most flexible, reliable and economical separation techniques that is best suited for the evaluation of botanicals and herbal medicines (21, 22). Considering the fact that, Shatavarin IV lack chromophores and thus, required a pre-column derivatisation as well as costly detectors like evaporative light scattering detection (ELSD) which is a laborious and costly affair (23). Therefore, HPTLC is a simple and cost-effective method for analysis of Shatavarin IV with post chromatographic derivatization as describe in the present study.

## Conclusion

The present study dealing with the determination of Shatavarin IV in marketed products of *A. racemosus* Willd. is a simple and reliable approach for quality control assessment which would be used as reference in future research in the said field. The method used in the present study could be applied for the identification and quantification of Shatavarin IV in a variety of formulations including capsule, tablet and powder of *A. racemosus*.

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## Authors contributions

BBC and BMP did the experimental work, studied the literature, prepare the original draft of manuscript. AR, SJ, AS, TS helped in literature study and manuscript writing. PCP reviewed the manuscript and SN conceptualised, designed and arranged the financial requirement for the research. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest**: **:** Authors do not have any conflict of interests to declare.

**Ethical issues**: None

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