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Quality control study of a ayurveda herbal preparation: *Samangadi churna*

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Abstract
Samangadi churna is commonly used Ayurveda formulation having 8 herbs. *Samangadi churna* is used to enhance *Medha* (memory), *Aayu*(Longevity), and *Bala* (Muscle strength). Present study is designed to done quality control of *Samangadi churna* using identifing physiochemical parameter and discover a simple, sensitive and accurate HPTLC method for fingerprinting as well as detection and quantification of active marker phytoconstituent gallic acid and plumbagin in *Samangadi churna* for standardization. Physicochemical Parameter of Samangadi Churna reported using method given Ayurvedic Pharmacoepia of India. Methanolic extraction was performed for HPTLC analysis of churna. HPTLC was for gallic acid using mobile phase of Toulene: Chloroform:Methanol(4:4:1 v/v/v) and detection at 366 nm. For identify plumbagin using mobile phase containing Toluene: Ethyl acetate: Formic acid (7:3:0.1 v/v/v). The Rf was detected for gallic acid at 0.42 and plumbagin at 0.7 in *Samangadi churna*. Gallic acid was detected to be 120.8 µg in 150 mg of *Samangadi churna* sample while Plumbagin was detected to be 34.61 µg in 100mg *Samangadi churna* sample. The HPTLC method was developed for determination of gallic acid and plumbagin in *Samangadi churna.* The proposed HPTLC method and physiochemical parameters value can be used for the routine quality control of *Samangadi churna*.

Keywords: *Samangadi churna*, physiochemical, HPTLC, gallic acid, plumbagin, herbal drug Standardization

Introduction
India have a rich heritage of Ayurveda, Yoga, Siddha and Unani. Ayurveda is now much globalised term and people seek management of chronic or cognitive disorders through herbal medicines presumed to be a safe and inexpensive. Stardardisation of Ayurveda medicines in terms of modern pharmaceutics is a developing process and worldwide India can play the leading role in production and standardisation of therapeutically effective Ayurvedic formulations. It needs an amalgamation of traditional concepts with sophisticated modern techniques of standardization. Lack of universal standardisation of a limiting factor for global acceptance of Ayurvedic medicines. Herbal medicines are consider an enhance can be well target to paediatrics age group clinical practice also substantiate the inclination of society towards Ayurveda for common paediatrics aliments. *Lehan* is unique concept of *kaumarbhritya*.*leh* means anything which is lickable having semi-solid in consistency or sticky also known as electauries. It promotes the physical and mental health andmay act as food supplement [1]. *Samangadi churna* is commonly used *lehan* having8 herbal drugs*(Rubiacordifolia, Terminalia chebula, Terminalia bellerica, Emblica officinalis, Bacopa monnieri, Sidacardifolia, Abutionindicumand Plumbago zeylanica) given in* ancient classics. *Samangadichurna*is used to enhance *Medha* (memory), *Aayu* (Longevity), and *Bala* (Muscle strength) [2].

Table 1: Ingredients of *Samangadi Churna* [3].

Plant	Botanical name	Part used	Weight in grams
Manjishtha	Rubia cordifolia	Root	12.5gm
Haritaki	Terminalia chebula	Fruit	12.5gm
Amalaki	Emblica officinalis	Fruit	12.5gm
Vibhitaki	Terminalia bellarica	Fruit	12.5gm
Brahmi	Bacopa monnieri	Whole plant	12.5gm
Bala	Sida cordifolia	Root	12.5gm
Atibala	Abutilon indicum	Root	12.5gm
Chitrak	Plumbago zylenica	Root	12.5gm

Different Ayurveda classics has explained *Samangadi churn* in different disease and with different contents such as *Samangadikwath*, *Samangadi Kashaya*, *Samangadi Churna* etc. [4, 5]. Complex nature and variability of the chemical constituents of herbal drugs is a limiting factor for establishing quality control parameters, therefore the new analytical techniques are expected to help in circumventing this problem.For the routine quality control of herbal medicine various method like HPTLC, Spectrofluromety etc are used [6, 7].

Standardization of HPTLC method could be beneficially used for the purpose of quantity *Samangadichurna* is not reported till date. So, present study was conducted for quality control of the *Samangadi Churna* with respect to its physicochemical properties, and HPTLC quantification and fingerprint study.

Materials and methods

Preparation of samangadi churna

Samangadi churna has eight ingredient *Rubiacordifolia, Terminalia chebula, Emblica officinalis, Terminalia bellarica, Bacopa monnieri, Sidacordifolia, Abutilon indicum*and*Plumbago zylenica* which were purchased from raipur, Chhattisgarh market and authenticated by Prof. P.K Joshi department of Dravyaguna, GAC, Raipur, Chhattisgarh. A fine powder was prepared using sieve no. 120 and taking all herbs in equal proportion as per Ayurvedic Pharmacopia India, 2011. Final product was kept in air tight container till further use.

Phytochemical Study

Physiochemical parametrs

Samangadi churna analysis was done according to the general parameters for *churna* given in the Ayurvedic Pharmacopoeia of India (API, 2011) [6]. such as particle fitness, total ash (%), alcohol-soluble extractive(%), water-soluble extractive(%), loss on drying (%), Acid insoluble ash (%), pH(10 % aqueous solution).

Fingerprinting Analysis of *Samangadi churna* Using HPTLC

2.1. Chemicals and reagents

The reagents, solvents, and chemicals were used are of Analytical Reagent grade. TLC plates HPTLC Si 60F254 10 X10 cm (Merck), Standard Plumbagin (Sigma Aldrich Pvt. Ltd, Mumbai, India) and gallic acid (Molychem, India) was used.

2.5. Instrumentation and chromatographic condition

A Camag HPTLC system comprising of Camag TLC Scanner 4 and Linomate V automatic sample applicator. For detection and quantification of markers in Ayurvedic formulation Camag WinCAT software were used.

Fingerprinting analysis of *Samangadi churna* was done in three parts

1. Fingerprint has developed for *Samangadi churna* and its respective raw materials.
2. Quantification of Gallic acid in *Samangadi churna*
3. Quantification of Plumbagin in *Samangadichurna*

Fingerprint has developed for *Samangadi churna* and its respective raw materials.

Samples: *Samangadi churna* 1.0 g powder and powdered raw materials (eight drugs) is mixed with 10 mL of methanol, sonicated for 10 min, and centrifuged for two minute. The supernatant is used as test solution.

Chromatographic setting:

Developing solvent: Toulene:Chloroform:Methanol(4:4:1 v/v/v).

Sample application: 1 µL each of test solution are applied as 8 mm bands, minimum 2 mm apart and 8 mm from lower edge of plate.

Stationary phase: HPTLC plates Si 60 F254 (Merck), 10x10 cm

Development: 10x10 cm Twin Trough Chamber first saturated by adding add10 mL developing solvent for 20 min with filter paper in the trough,, developing distance 70 mm from lower edge of plate. The HPTLC plate is then dried with a hair dryer (cold air) for 5 min.

Derivatization reagent

Anisaldehyde reagent Preparation: Slowly mixed 85 mL of ice-cooled methanol with 10 mL of acetic acid and 5 mL of sulfuric acid. Allowed the mixture to cool to room temperature, then added 0.5 mL of anisaldehyde (p-methoxy benzaldehyde). Use: Derivatizer (Derivatizer: 3 mL, blue nozzle, spraying level 3), heat plate at 100°C for 3 min

Detection: Examination under UV 366 nm.

Fingerprint has developed for *Samangadi churna*and its respective raw materials done using mobile phase Toulene:Chloroform:Methanol(4:4:1 v/v/v). Number of bands were separated and good resolution was observed at fluorescence mode i.e. at 366 nm and after derivatization with anisaldehyde sulphuric acid with the TLC Visualizer under white light.

Method for Quantification of gallic acid in *Samangadichurna.*

Sample: *Samangadi churna* 1.5 g powdered raw material is mixed with 10 mL of methanol, sonicated for 10 min, and centrifuged for two minute. The supernatant is used as test solution.

Standards: 1 mg of gallic acid are individually dissolved in 10 mL of methanol.

Chromatographic setting:

Developing solvent: Dichloromethane: Ethyl acetate: Formic acid (5:4:1 v/v/v)

Sample application: 1 µL test and standard solution are applied as 8 mm bands, min. 2 mm apart, 8 mm from lower edge of plate.

Stationary phase: HPTLC plates silica gel 60 F254 (Merck), 10x10 cm

Development: 10x10 cm Twin Trough Chamber first saturated by adding add10 mL developing solvent for 20 min with filter paper in the trough, developing distance 70 mm from lower edge of plate. The HPTLC plate is then dried with a hair dryer (cold air) for 5 min.

a. Detection: a) Examination under UV 254 nm.
b. Preparation of 3D chromatogram

Calibration graph was plotted to quantify the amount of gallic acid in the S*amangadichurna* Rf was observed and spectral match was also done to confirm the presence of gallic acid.

In methanolic extract of *Samangadichurna*, HPTLC chromatography profiling was carried out at 254 nm for all samples.

Quantification of plumbagin in *Samangadichurna.*

Sample: *Samangadi churna* 1.0 g powdered raw material is mixed with 10 mL of methanol, sonicated for 10 min, and centrifuged for two minute. The supernatant is used as test solution.

Standards (optional): 1 mg of Plumbagin are individually dissolved in 10 mL of methanol.

Chromatographic setting:

Developing solvent: Toluene: ethyl acetate: formic acid (7:3:0.1 v/v/v)

Sample application: 1 µL test and standard solution are applied as 8 mm bands, min. 2 mm apart, 8 mm from lower edge of plate.

Stationary phase: HPTLC plates silica gel 60 F254 (Merck), 10x10 cm

Development: 10x10 cm Twin Trough Chamber first saturated by adding add10 mL developing solvent for 20 min with filter paper in the trough,, developing distance 70 mm from lower edge of plate. The HPTLC plate is then dried with a hair dryer (cold air) for 5 min.

a. Detection: a) Examination under UV 254 nm.
b. Preparation of 3D chromatogram

Calibration graph was plotted to quantify the amount of Plumbagin in the *Samangadichurna*Rf was observed and spectral match was also done to confirm the presence of Plumbagin in *Samangadichurna*.

In methanolic extract of *Samangadichurna*, HPTLC chromatography profiling was carried out at 254 nm for all samples,

Result

As the part of standardization procedure *Samangadi churna* were tested for relevant physical and chemical parameter. The tested physicochemical parameter such as Particle fitness, alcohol-soluble extractive water-soluble extractive Loss of drying, total ash, acid- insoluble ash, and pH (10% aqueous solution) with their value were reported in Table 1.

Table 1: Physicochemical Parameter of Samangadi Churna

Particle fitness	Fine powder
Loss on drying (%)	4.60
Total ash (%)	7.20
Acid-insoluble ash (%)	2.03
Alcohol-soluble extractive (%)	16.12
Water-soluble extractive (%)	21.40
pH (10% aqueous solution)	4.46

Fingerprint method was developed for S*amangadichurna and* its respective raw materials.Number of bands were separated and good resolution Rf values, color and intensity of prominent peaks observed at fluorescence mode i.e. at 366 and after derivatization with anisaldehyde sulphuric acid at white light. The results obtained are reported in following images (figure 1) and tables (table 2 and 3).

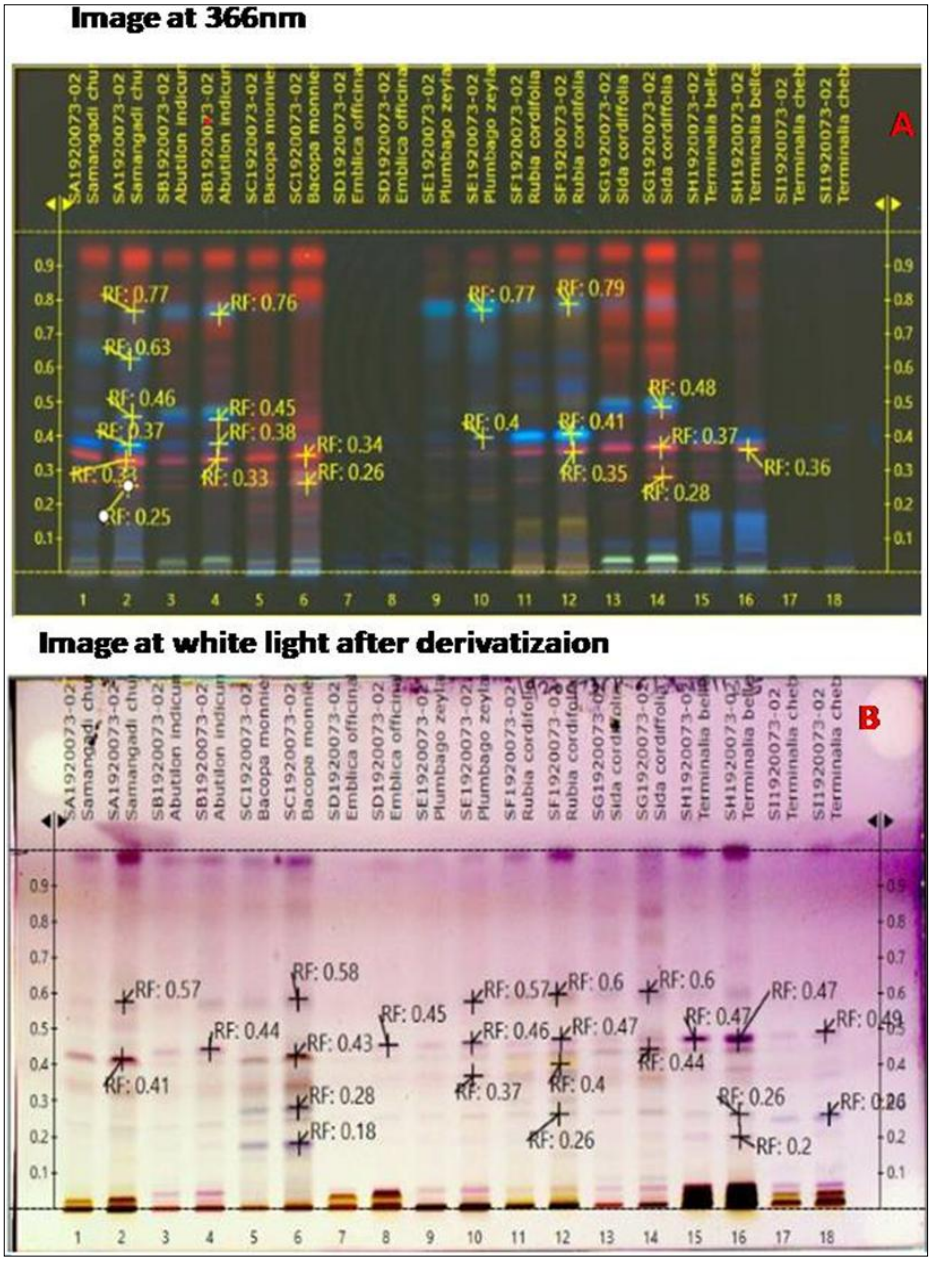

Fig 1: (A) HPTLC Fingerprint at 366 for *Samangadi churna* and its respective raw materials. (B) Image at white light after derivatizaion with Anisaldehyde sulphuric acid (ASR)

Table 2: for respective track number, Rf values, color and intensity of prominent peaks observed

Track No.	No. of Prominent band	Rf (starting form below)	Color	Intensity
2	6	0.25	Red	Medium
		0.33	Reddish pink	Intense
		0.37	Blue	Intense
		0.46	Blue	Low
		0.63	Blue	Low
		0.77	Blue	Low
4	4	0.33	Reddish pink	Intense
		0.38	Blue	Medium
		0.45	Blue	Medium
		0.76	Sky blue	Medium
6	2	0.26	Reddish pink	Medium
		0.34	Red	Intense
10	2	0.4	Blue	Low
		0.7	Sky blue	Intense
12	3	0.35	Pink	Intense
		0.41	Blue	Intense
		0.79	Sky blue	Low
14	3	0.28	Pink	Low
		0.37	Reddish pink	Intense
		0.48	Blue	Medium
16	1	0.36	Pink	Medium

Table 3: for respective track number, Rf value, color and intensity of prominent peak observed at white light after derivatization with anisaldehyde sulphuric acid (ASR)

Track No.	No. of Prominent band	Rf (starting form below)	Color	Intensity
2	2	0.41	Purple	Intense
		0.57	Light purple	Low
4	1	0.44	Pink	Medium
6	4	0.18	Violet	Medium
		0.28	Light violet	Low
		0.43	Purple	Intense
8	1	0.45	Light violet	Low
10	3	0.37	Light violet	Low
		0.46	Pink	Medium
		0.57	Light violet	Low
12	4	0.26	Light violet	Low
		0.40	Yellow	Medium
		0.47	Pink	Medium
		0.6	Pink	Low
14	2	0.44	Pink	Medium
		0.60	Violet	Low
16	3	0.2	Pink	Low
		0.26	Violet	Medium
		0.47	Dark purple	Intense
18	2	0.26	Violet	Medium
		0.49	Pink	Medium

In earlier experiment spiking was done to confirm the presence of gallic acid in *Samangadichurna.* After spiking, increase in area of *Samangadichurna* at gallic acid Rf was observed and spectral match was also done to confirm the presence of gallic acid in *Samangadichurna*. Plate for linearity was

Scanned at 273nm (lambda max of Gallic acid) and calibration graph was plotted to quantify the amount of gallic acid in *Samangadichuuna*. The results obtained are reported in following images (figure 2 and 3). Gallic acid was detected at Rf 0.42 and 120.8 µg gallic acid was detected in 150mg of *Samangadichurna* sample.

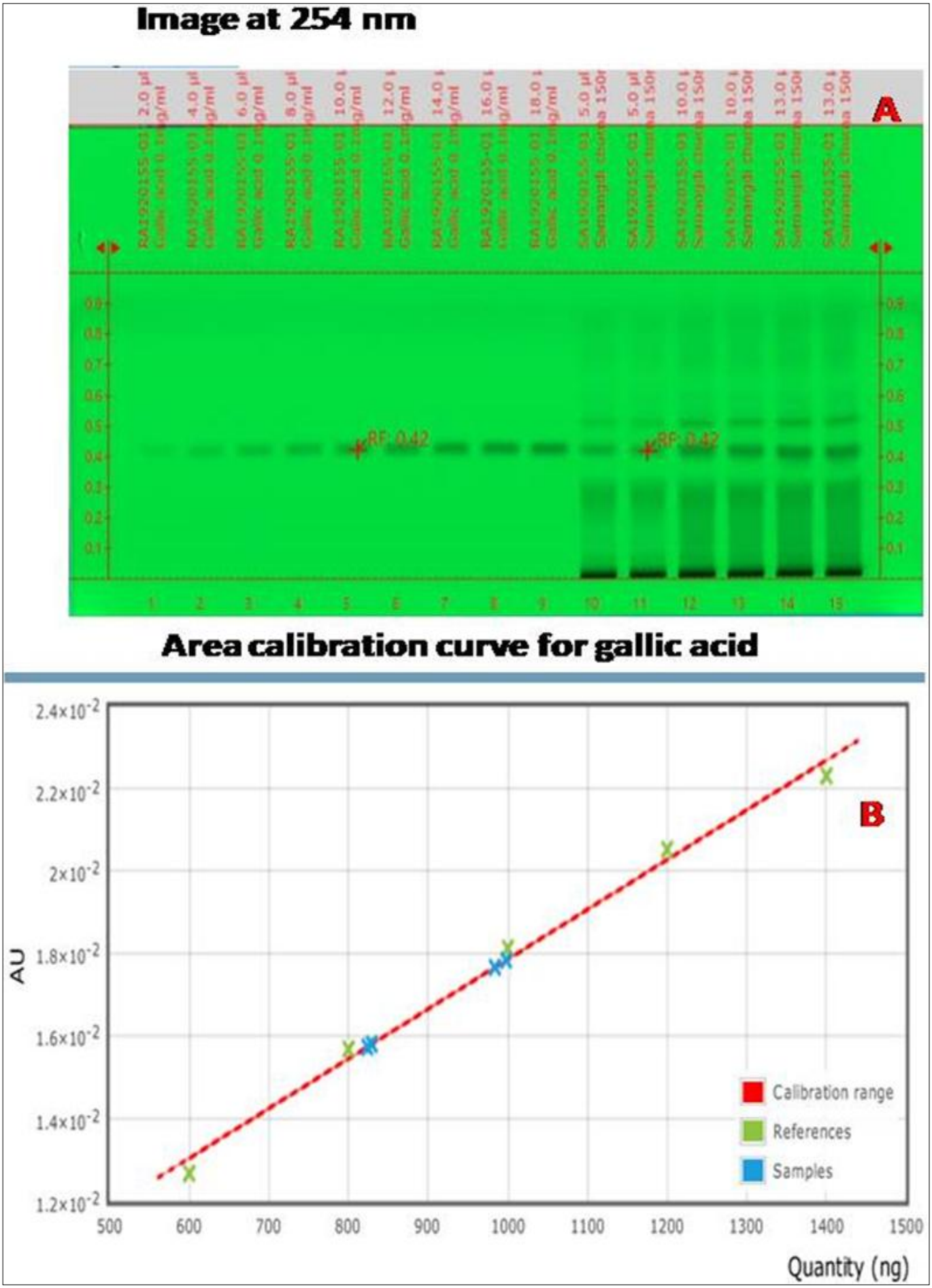

Fig 2: (A) HPTLC Fingerprint Gallic acid and S*amangadichurna* at 254 nm; (B) Area calibration curve for substance gallic acid 273 nm

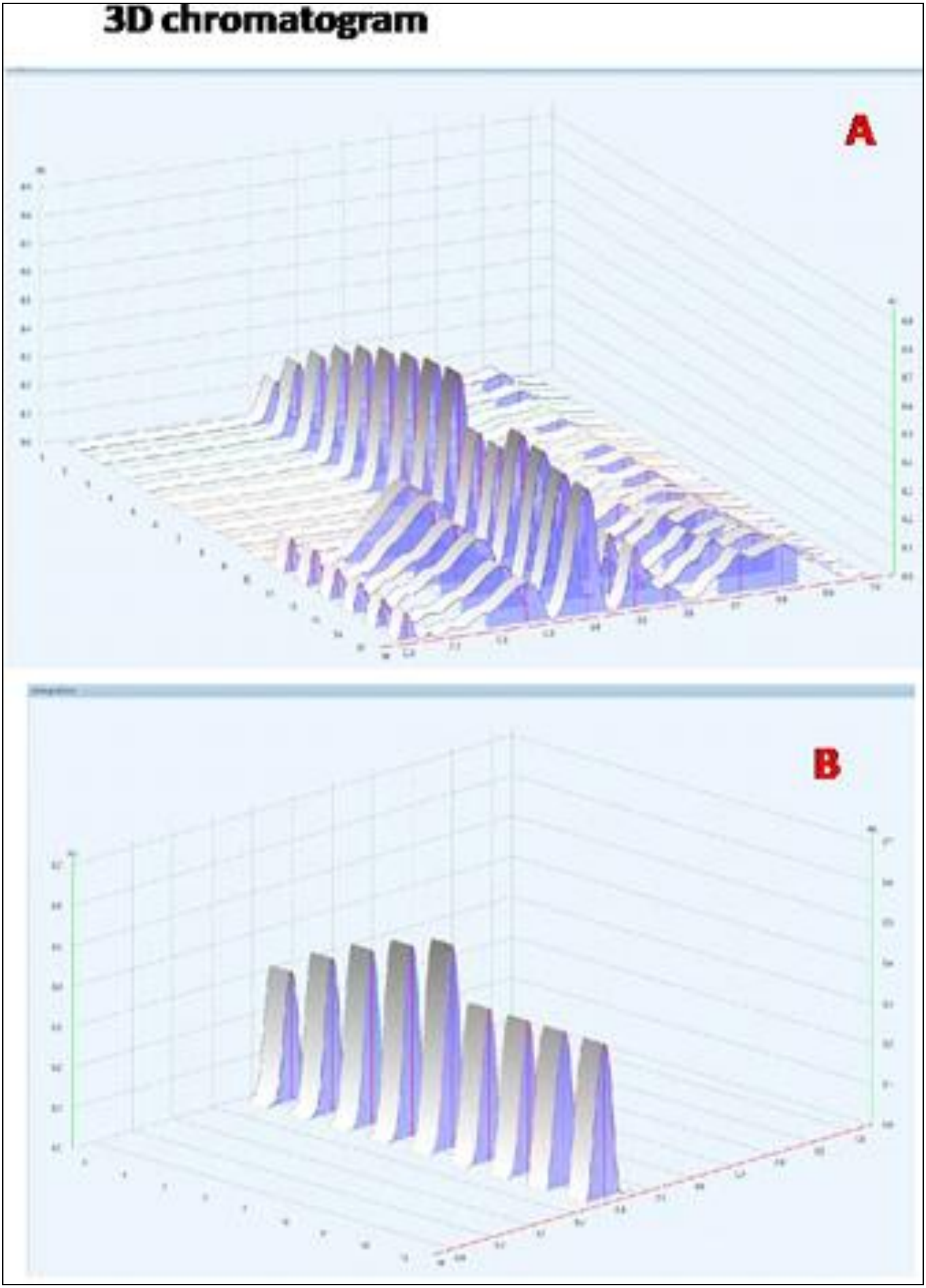

Fig 3: (A) 3D chromatogram for Gallic acid and *Samangadi churna;* (B) 3D chromatogram (Integrated) for separation of gallic acid in *Samangadi churna:*

Spiking experiment was carried out first in earlier experiment, to confirm the presence of plumbagin in *Samangadichurna* as it is a complex polyherbal formulation consisting of eight plants and lot of interference was observed due to presence of chlorophyll bands at plumbagin Rf. After spiking, increase in area of sample was observed and based on area increase response, linearity study was carried out. Scanning was done at 450nm for yellow colored band of plumbagin. In earlier experiment, multi-wavelength scanning was done from 450 to 500nm (as it is complementary color wavelength for yellow color) and maximum area response was observed at 450 nm, therefore this wavelength was chosen for linearity experiment. The results obtained are reported in following images (figure 4 and 5).

Plumbagin was observed at Rf 0.7 in standard and *Samangadi churna* sample. It was detected to be 34.61 µg in 100mg *Samangadichurna* sample.

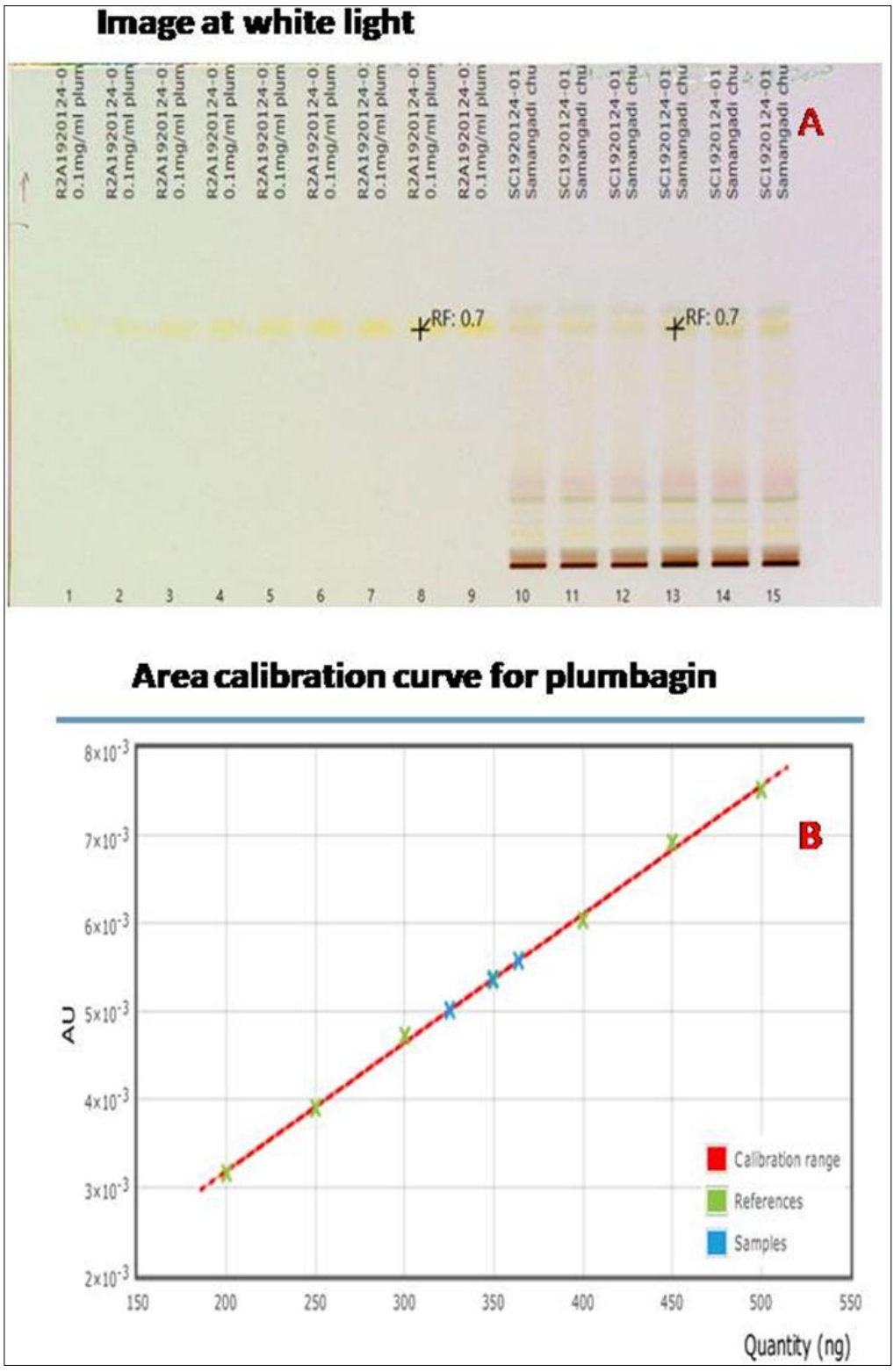

Fig 4: (A) HPTLC Fingerprint at white light for plumbagin and *Samangadichurna*; (B) Area calibration for substance plumbagin result at 450nm.

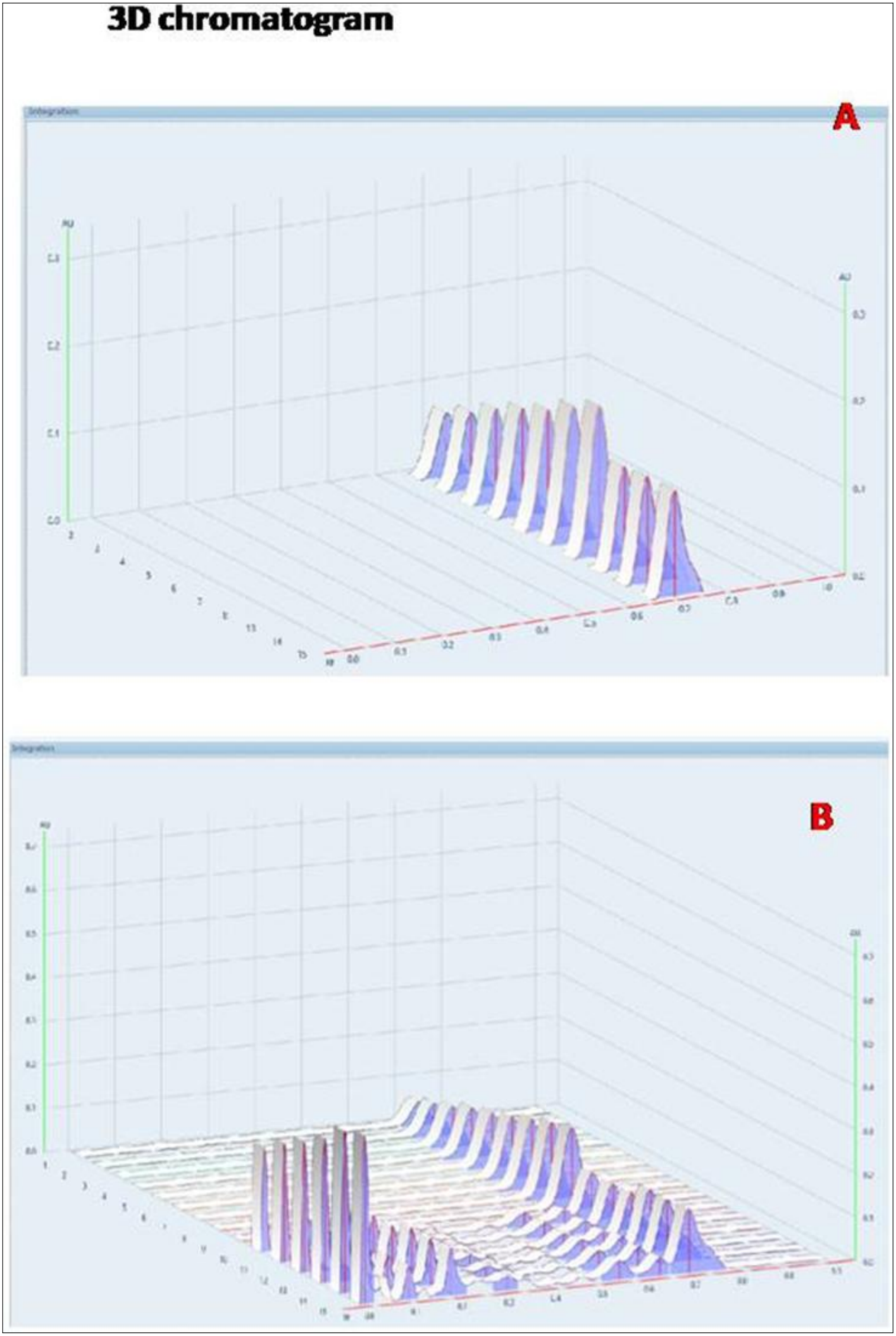

Fig 5: (A) 3D chromatogram for separation of plumbagin in sample along with standard; (B) 3D chromatogram for plumbagin and samangadichurna.

Conclusion

The simple and sensitive HPTLC method was successfully developed for determination of gallic acid and plumbagin in *Samangadichurna*. Development of quality control of *Samangadi Churn* would help in setting a benchmark for further standardisation research. It offered numerous advantages including rapid, less solvent used, less time of analysis, simplicity, multiple sample handling, and less cost per analysis Consequently, this paper first time reported the physiochemical parameters and HPTLC fingerprinting of *Samangadichurna*. HPTLC method could be used as an option method for quantitative analysis of plumbagin content in Ayurvedic formulations containing *Plumbagozylenica*.

Acknowledgement

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