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Original Research Article (Experimental)

A rapid HPTLC method to estimate piperine in Ayurvedic formulations

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ABSTRACT

Background: *Trikatu*, *Sitopaladi*, *Hingavastaka*, *Avipattikara*, *Sringyadi* and *Talisadya* are very popular Ayurvedic (*churna*) medicines practiced in India; however, unfortunately, they possess several quality control issues.
Objective: The aim of this study was to find out a simple, accurate and sensitive HPTLC method for the detection and quantification of marker molecule, piperine (alkaloid) on these Ayurvedic formulations for standardization.
Materials and methods: Methanolic extraction (reflux) was performed from the above six *churnas* as well as three single ingredients *Piper longum* (*pipul*), *Piper nigrum* (*marich*) and *Piper chaba* (*chai*). HPTLC was done using piperine as a standard. The mobile phase was a mixture of toluene-ethyl acetate (7:3, v/v) and detection at 342λ.
Results: The R_f was detected at 0.39. Piperine was quantified in all samples. *P. nigrum* showed higher piperine than *P. longum* and *P. chaba*. The maximum piperine was noted in *Hingavastaka churna* and followed by *Sringyadi churna*, *Sitopaladi churna*, *Talisadya churna*, *Trikatu churna* and *Avipattikara churna*.
Conclusion: This method can be successfully employed for standardization and quantitative analysis of piperine in Ayurvedic formulations (*churnas*) and also be helpful to clinicians and pharmacists to draw significant role of piperine present in all these samples.

1. Introduction

In recent years, herbal formulations have achieved widespread acceptability as therapeutic agents for several chronic diseases such as diabetes, arthritis, liver diseases, gastrointestinal disorders, cough and cold, memory loss and immunodeficiency [1]. These medicines are readily available in the market from health food stores without prescriptions and have been widely used in India, China, USA, and have fairly good market all over the world [2]. The validation of herbal products is a major public health concern both in developed and resource-poor countries, where fakers sell adulterated herbal medicines. It is feasible that the introduction of scientific validation would control the production of impure or poor quality herbal products and would eventually ensure their rational use [3,4]. In Ayurveda, different powder (*churna*) formulations such as *Trikatu churna*, *Sitopaladi churna*, *Hingavastaka churna*, *Avipattikara churna*, *Sringyadi churna* and *Talisadya churna* are most commonly used from ancient times to treat asthma, cough and cold, tuberculosis, fever, indigestion, chronic rhinitis/sinusitis and other inflammatory and respiratory disorders [5]. Interestingly, it has been noted that in all these formulations one or more herbal ingredients have their origin from the family Piperaceae, like *Piper longum* (*pipul*), *Piper nigrum* (*marich*), *Piper chaba* (*chai*), etc. as mentioned in Ayuvedic Formulary of India. Piperine, an alkaloid (1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-petadienyl]piperidine; $C_{17}H_{19}NO_3$) (Fig. 1) is the active principle in this group of herbs [6].

Modern research confirmed that piperine is helpful in reducing inflammation, improving digestion, and relieving pain and asthma [7–9]. It improves the bioavailability of other nutritive substances including β-carotene, curcumin, selenium, pyridoxine, glucose and amino acids [10,11]. Although these Ayurvedic herbal powder formulations are very popular as Ayurvedic medicines, unfortunately

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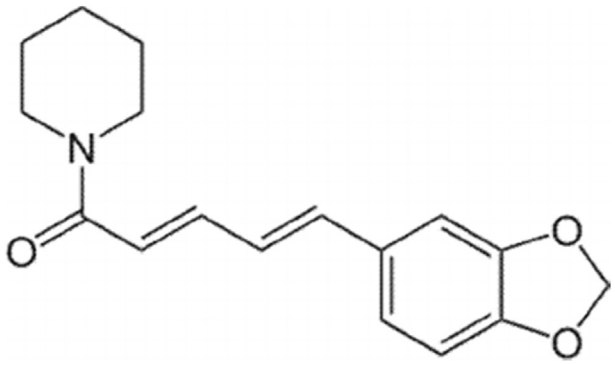

Fig. 1. Chemical structure of piperine.

these lack establishment of piperine content which is mainly contributed by ingredient plants from Piperaceae family. Therefore, the aim of this study was to establish a quality control tool for efficacy and consequently develop a simple HPTLC method for the estimation of piperine in Ayurvedic formulations (*churna*) that contain herbs from Piperaceae family.

2. Materials and methods

2.1. Chemicals and reagents

TLC plates coated with silica gel $60F_{254}$ for HPTLC were purchased from Merck, Germany and piperine was purchased from Sigma, USA. All other chemicals, reagents and solvents were used are of AR grade.

2.2. Test samples

Root and fruit from *P. longum* (*pipul*), fruit from *P. nigrum* (*marich*), stem from *P. chaba* (*chai*) were obtained from market and Ayurvedic powdered formulations such as *T. churna*, *Sitopaladi churna*, *H. churna*, *A. churna*, *Sringyadi churna* and *T. churna* were obtained from Ramakrishna Mission Ayurvedic Hospital, Narendrapur, Kolkata. All these powdered formulations were prepared following the instructions of *Granthokta* as mentioned in the Ayurvedic Formulary of India [12]. All the raw botanicals as well as *churna* formulations were authenticated by microscopic and macroscopic examinations. The physicochemical parameters for quality standards were evaluated for each raw material and Ayurvedic formulations as mentioned in Ayurvedic Pharmacopoeia of India [13].

2.3. Preparation of test samples

1 g of each raw material and *churna* sample was taken separately, refluxed with 20 ml of methanol for 30 min, filtered through Whatmann filter paper no. 41 and this procedure was repeated thrice. The pooled filtrate was concentrated and volume was adjusted to 10 ml with methanol in a volumetric flask. Aliquot of each extract was further diluted 50% for quantification by HPTLC.

2.4. Standard piperine solution

Standard stock solution was prepared by dissolving 10 mg of piperine in 10 ml methanol and sonicated, which yields a solution of concentration 1 mg/ml. Working standard was prepared from this stock solution at concentration 10 μg/ml.

2.5. Instrumentation and chromatographic condition

A Camag HPTLC system comprising of Linomate V automatic sample applicator with Camag TLC Scanner 3 and Camag WinCAT software were used for detection and quantification of piperine in the single herbs and Ayurvedic formulations. The

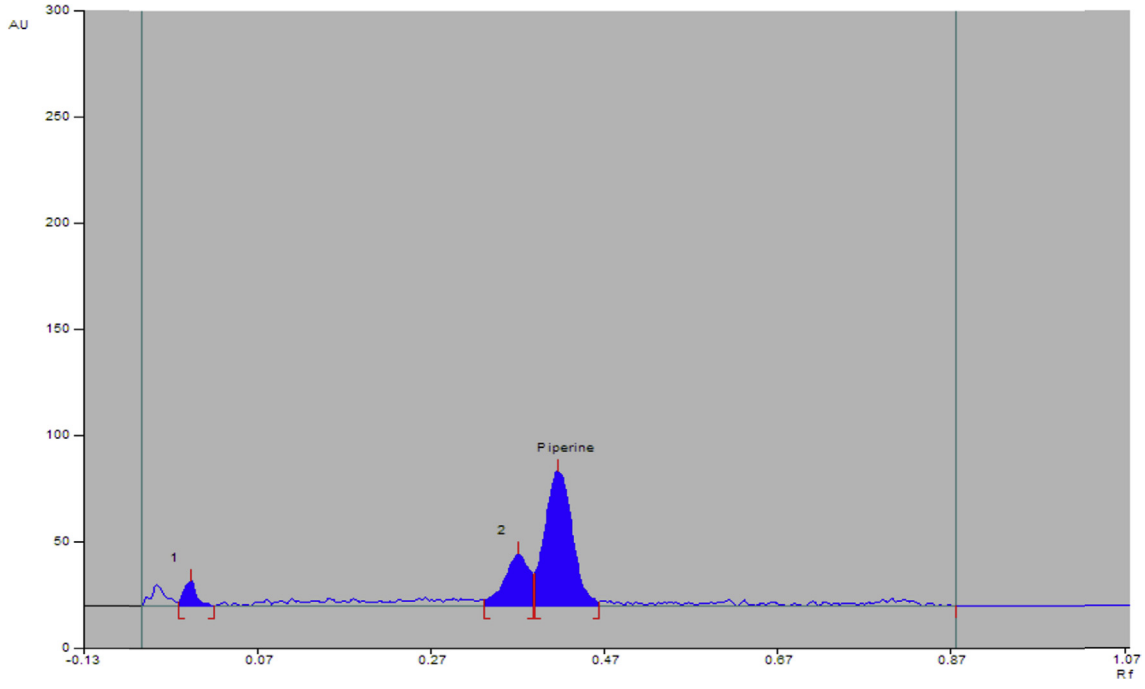

Fig. 2. Chromatogram of standard piperine.

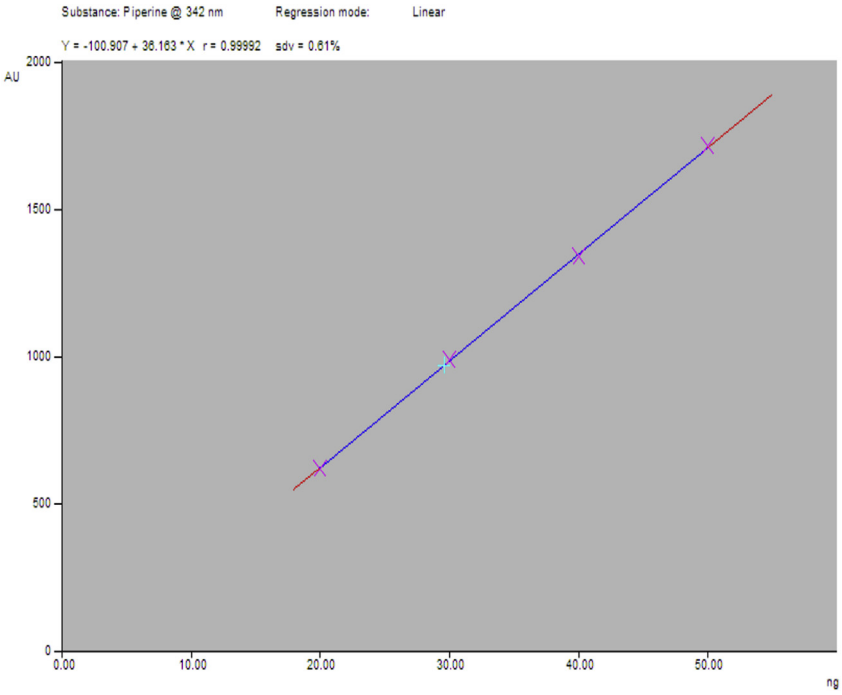

Fig. 3. Standard curve of piperine.

Table 1
Quality parameters of Ayurvedic drugs and raw plant ingredients.

	Foreign matter (%)	Loss on drying (%)	Total ash (%)	Acid insoluble ash (%)	Alcohol soluble extractive (%)	Water soluble extractive (%)	*p*H
P. longum root	0.48 ± 0.016	–	2.85 ± 0.08	1.26 ± 0.03	6.88 ± 0.18	12.81 ± 0.28	–
P. longum fruit	1.63 ± 0.034	–	4.39 ± 0.06	0.32 ± 0.01	9.41 ± 0.22	12.4 ± 0.37	–
P. nigrum fruit	0.21 ± 0.001	–	3.90 ± 0.05	0.45 ± 0.02	7.83 ± 0.16	11.23 ± 0.54	–
P. chaba stem	0.12 ± 0.002	–	7.17 ± 0.16	1.54 ± 0.07	3.20 ± 0.18	5.79 ± 0.69	–
Trikatu churna	–	10.05 ± 0.84	4.98 ± 0.27	0.64 ± 0.02	5.49 ± 0.27	8.08 ± 0.55	4.81 ± 0.61
Sitopaladi churna	–	4.18 ± 0.68	26.21 ± 0.36	25.31 ± 0.82	2.89 ± 0.14	42.32 ± 0.98	9.65 ± 0.78
Hingavastaka churna	–	4.61 ± 0.59	18.23 ± 0.55	1.74 ± 0.09	12.35 ± 0.26	25.94 ± 0.74	6.52 ± 0.37
Avipattikara churna	–	5.83 ± 0.74	3.61 ± 0.28	0.52 ± 0.03	22.18 ± 0.38	54.16 ± 0.61	5.72 ± 0.85
Sringyadi churna	–	8.29 ± 0.88	18.83 ± 0.74	2.04 ± 0.03	8.08 ± 0.57	29.54 ± 0.92	5.82 ± 0.63
Talisadya churna	–	2.22 ± 0.15	8.64 ± 0.69	8.53 ± 0.16	12.71 ± 0.41	68.52 ± 0.77	7.62 ± 0.59

N = 6 in each test; Results are mean ± standard deviation.

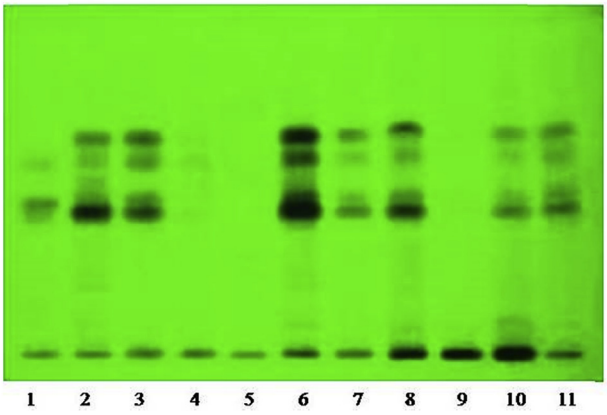

Fig. 4. HPTLC plate of samples. Track 1 = Piperine standard, Track 2 = *P. longum* fruit, Track 3 = *P. nigrum* fruit, Track 4 = *P. longum* root, Track 5 = *P. chaba* stem, Track 6 = *Hingavastaka churna*, Track 7 = *Trikatu churna*, Track 8 = *Sringyadi churna*, Track 9 = *Avipattikara churna*, Track 10 = *Sitopaladi churna*, Track 11 = *Talisadya churna*.

standard solutions and test samples were spotted in the form of bands (8 mm bandwidth) with 100 µl Hamilton syringe on pre-coated silica gel plates (Merck, $60F_{254}$, 10 × 10 cm) using Camag Linomate V applicator. The plates developed upto 80 mm with a solvent system (toluene: ethyl acetate = 7:3, v/v) in Camag glass twin-trough chamber previously saturated with mobile phase vapor for 30 min at 25 °C. The densitometric scanning was performed on Camag TLC Scanner 3 at absorbance 342 nm (deuterium lamp, slit dimension 5.0 × 0.45 µm) and operated by multilevel WinCATS planar chromatography manager software [14,15]. Spots were well resolved in the chromatogram of extracts of samples from single herbs or Ayurvedic powder formulations, and the spot of standard piperine was at R_f value 0.39 (Fig. 2). The amount of piperine present in the samples was calculated using calibration curve of standard piperine and expressed as mg/g of dry samples. The experiments were repeated thrice to confirm results.

Table 2
Piperine content in ingredients and Ayurvedic samples.

Samples	Number of ingredients in formulation [12]	Piperine containing herbs (%) in test formulation	Piperine content in IP[a]	Piperine (mg/g)
P. longum root	1	100%	–	0.29 ± 0.42
P. longum fruit	1	100%	0.4–1%	1.48 ± 0.95
P. nigrum fruit	1	100%	2.5%	1.94 ± 0.77
P. chaba stem	1	100%	–	0.22 ± 0.61
Trikatu churna	3[b]	33%* & 33%**	–	2.27 ± 0.83
Sitopaladi churna	5[c]	13%**	–	2.81 ± 0.52
Hingavastaka churna	8[d]	12.5%* & 12.5%**	–	7.09 ± 0.73
Avipattikara churna	14[e]	0.75%* & 0.75%**	–	0.16 ± 0.43
Sringyadi churna	3[f]	33%**	–	3.62 ± 0.58
Talisadya churna	8[g]	4%* & 8%**	–	2.62 ± 0.64

N = 6 in each test; Results are mean ± standard deviation; * means *Piper nigrum* fruit & ** means *Piper longum* fruit; a = Indian Pharmacopeia; b = AFI, Part I, Section 7:14, 110 (*Bhaisajyaratnavali, Paribhasaprakarana*; 16); c = AFI, Part I, Section 7:34, 116 (*Sarngadharasamhita, Madhyamakhanda, Adhyaya* 6; 134–135 ½); d = AFI India, Part I, Section 7:37, 117 (*Bhaisajyaratnavali, Agnimandyadirogadhikara*; 37); e = AFI, Part I, Section 7:02, 106 (*Bhaisajyaratnavali, Amlapittadhikara*; 24–25); f = AFI, Part I, Section 7:31, 115 (*Sarngadharasamhita, Madhyamakhanda, Adhyaya* 6; 42 ½); g = AFI, Part I, Section 7:13, 109 (*Sarngadharasamhita, Madhyamakhanda, Adhyaya* 6; 130–131 ½); AFI = The Ayurvedic Formulary of India, Part I, 2nd revised Edition, Govt of India, Ministry of Health & Family Welfare, Department of Indian Systems of Medicine & Homeopathy, New Delhi. 2003.

2.6. Calibration curve

Aliquots of standard solution of piperine were applied in duplicates 20 ng, 30 ng, 40 ng and 50 ng over the silica gel 60 F_{254} plate as described earlier. The plate was developed and analyzed to generate calibration equation for quantification of piperine in samples (Fig. 3).

2.7. Method validation

ICH guidelines were followed for the method validation of the analytical procedures [16,17]. The method was validated for precision, repeatability and accuracy. The repeatability of the method was checked by repeated scanning of the same spot of piperine (40 ng), six times and was expressed as co-efficient of variance (% CV). The variability of the method was studied by analyzing aliquots of piperine (20–50 ng) on the same day and on different days and the outcome data were expressed as %CV. The recovery studies were done at three levels (50%, 100% and 150% addition). The percent recovery and average percent recovery was calculated for studying accuracy of method.

2.8. Data analysis

The data were represented as mean ± standard deviation. Descriptive statistics were conducted wherever it was applicable.

3. Results and discussion

Piperine is a common marker compound present in several species of Piperaceae family such as *P. longum*, *P. nigrum*, *P. chaba*

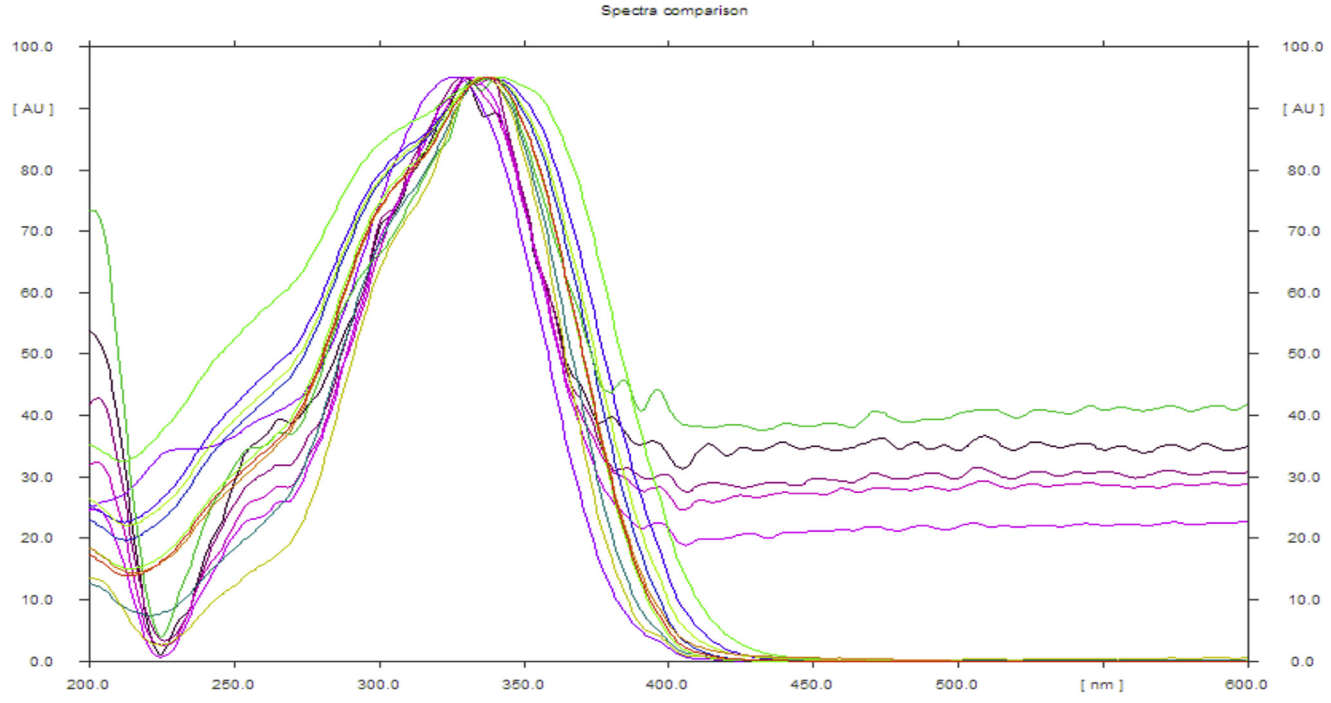

Fig. 5. HPTLC chromatographic scanning of all samples.

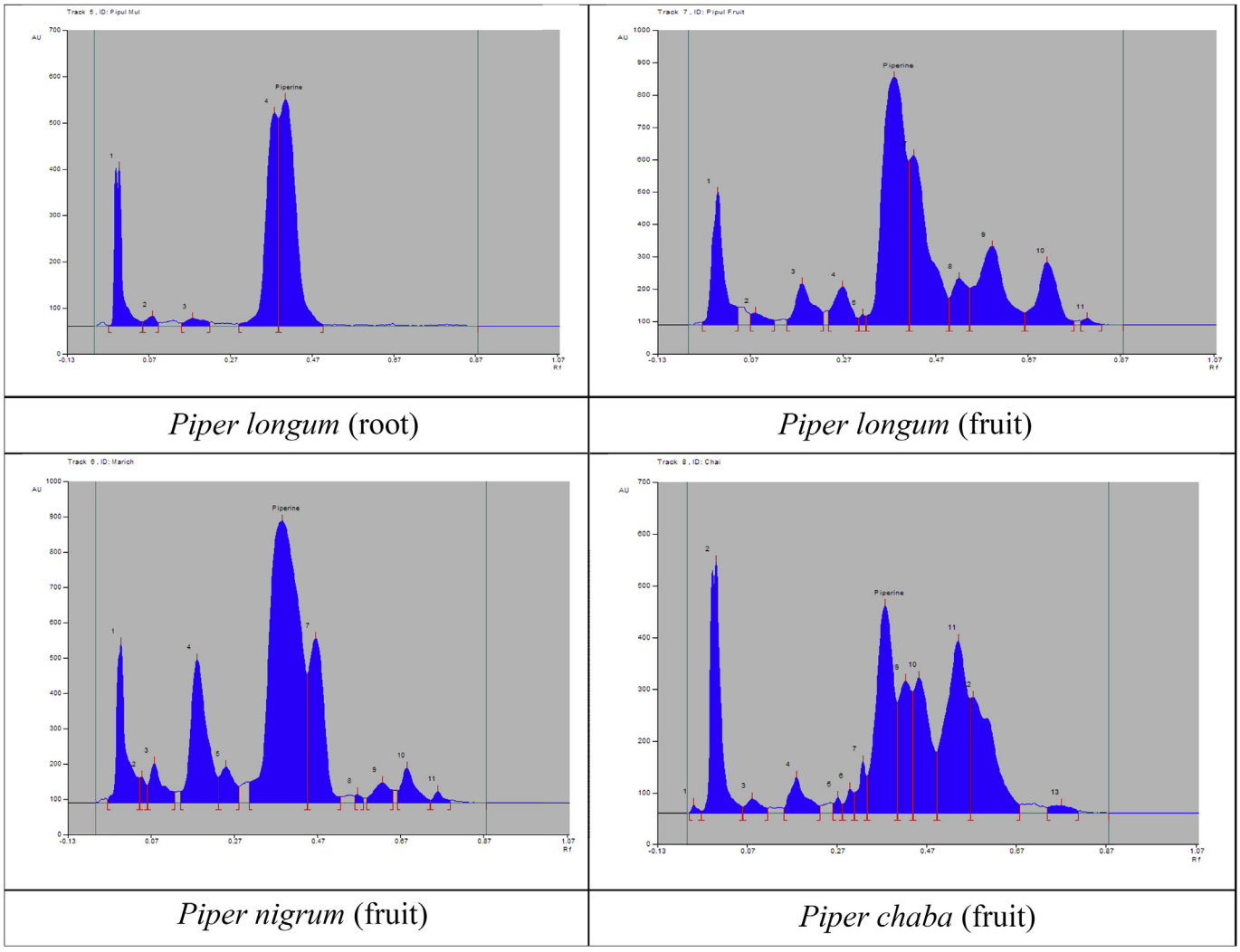

Fig. 6. HPTLC chromatograph of single herbs (*Piper sp.*).

etc. This alkaloid is tasteless, but its stereoisomer, chavicine, is the active ingredient in black pepper that provides its characteristic taste. Loss of pungency during storage of black pepper is attributed to the slow isomerization of chavicine into piperine [18]. Piperine is considered as a known marker compound that is usually assayed to authenticate *pipul*, *marich* etc. abundantly used in compound formulation of Ayurvedic drugs from ancient times. The physicochemical properties of the test samples were permitted within the limit values (Table 1) and assure for their qualities [13].

The HPTLC procedure was optimized with a view to develop a stability indicating assay method. The solvent system of the mobile phase having toluene: ethyl acetate (7: 3, v/v) gave dense, compact and well separated spots of the single herbal ingredients as also Ayurvedic formulation at 342 nm (Fig. 4). The limit detection for piperine and the limit of quantification was found to be 20 ng and 0.228 μg/ml respectively. These values are considered to be good enough for a reasonable accuracy in most of the laboratories worldwide. *P. longum* fruit exhibits 1.48 ± 0.95 mg/g of piperine, whereas root contains only 0.29 ± 0.42 mg/g. Moreover, piperine concentration was found 1.94 ± 0.77 mg/g in *P. nigrum* fruit and only 0.22 ± 0.61 mg/g in *P. chaba* stem. The assay values were found to be within the standard acceptable limits and so the method can be adopted for estimation of piperine in Ayurvedic formulations (Fig. 3).The peak area and concentration was subjected to least square linear regression analysis to calculate the calibration equation $Y = 100.9 + 36.16X$ and regression coefficient (r^2) was 0.9999 (Fig. 2).

Table 2 denoted maximum piperine quantified in the Ayurvedic formulation of *H. churna* (7.09 ± 0.73 mg/g) followed by *Sringyadi churna* (3.62 ± 0.58 mg/g), *Sitopaladi churna* (2.81 ± 0.52 mg/g), *T. churna* (2.62 ± 0.64 mg/g), *T. churna* (2.27 ± 0.83 mg/g) and *A. churna* (0.16 ± 0.43 mg/g). This is the first attempt to standardize these Ayurvedic formulations in the view point of piperine as a marker compound. All the samples denoted R_f as 0.39 and matched with piperine standard (Fig. 5). The quantities and compositions of *Piper* sp. present in Ayurvedic *churnas* are different from each other. This is the primary attempt to quantify piperine in single herbs as well as six well known powder Ayurvedic formulations. The HPTLC chromatograms of single herbal ingredients (Fig. 6) as well as Ayurvedic formulations (Fig. 7) were represented for better understanding that there is a co-relation between piperine and the herbs belongs to Piperaceae family.

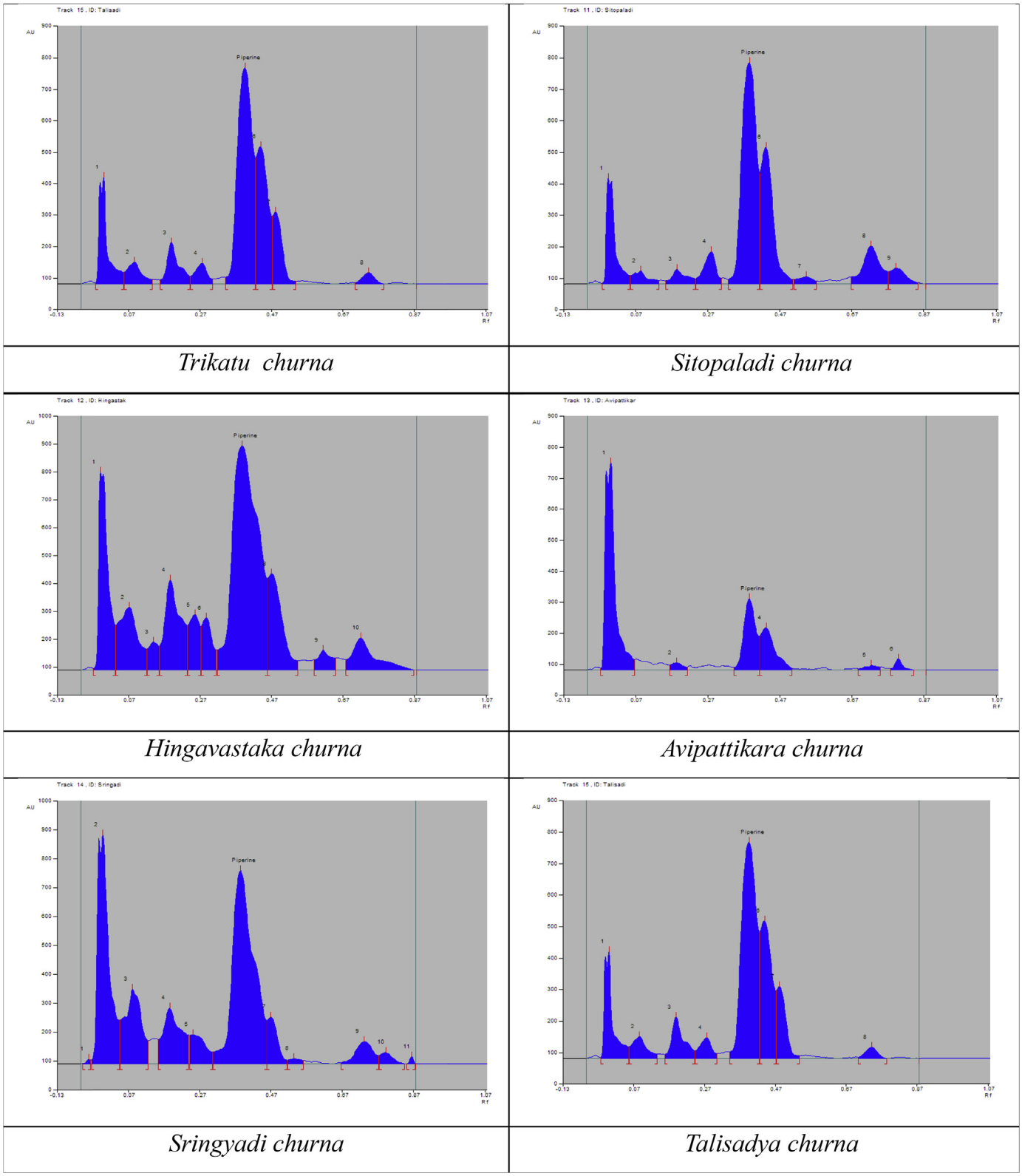

Fig. 7. HPTLC chromatograph of Ayurvedic *churna* formulation.

Linearity studies were carried out and there exists linearity in the concentration range of 10–50 μg/ml for piperine. The good average recovery values obtained in recovery studies indicate that the proposed method is accurate for estimation of drug in Ayurvedic powder (*churna*) formulation. Thus, the developed method was found to be accurate, precise, suitable and cost effective for the estimation of piperine in Ayurvedic formulation containing the *Piper* sp.

4. Conclusion

This HPTLC method can be successfully employed for standardization and quantitative analysis of piperine in Ayurvedic formulations (*churnas*) as well as raw materials and also be helpful to clinicians and pharmacists to draw significant role of piperine present in all these samples.

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Conflict of interest

None.

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