Original Research Article (Experimental)

A rapid HPTLC method to estimate piperine in Ayurvedic formulations

Alok K. Hazra a, Banti Chakraborty a, Achintya Mitra b, Tapas Kumar Sur c, * a
Division of Pharmaceutical Chemistry, R.K.M.A Quality Testing Laboratory, Kolkata, 700103, India
b National Research Institute of Ayurvedic Drug Development, CCRAS, Kolkata, 700091, India
c Department of Pharmacology, I.P.G.M.E & R, Kolkata, 700020, India

A R T I C L E   I N F O
Article history:
Received 11 April 2017
Received in revised form 28 June 2017
Accepted 20 July 2017
Available online 11 October 2018

Keywords:
Piper
Piperine
Alkaloid
HPTLC
Ayurveda

A B S T R A C T

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 (pipil), Piper nigrum (marich) and Piper chaba (chat). 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 342nm.

Results: The Rf 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.

© 2017 Transdisciplinary University, Bangalore and World Ayurveda Foundation. Publishing Services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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 (pipil), Piper nigrum (marich), Piper chaba (chat), etc. as mentioned in Ayurvedic Formulary of India. Piperine, an alkaloid (1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-petadienyl]piperidine; C13H17NO2) (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...
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 60F254 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*, *Sringlyadi 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
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, 60F254, 10 × 10 cm) using Camag Linomate V applicator. The plates developed up to 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 Rf 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.

![Fig. 3. Standard curve of piperine.](image)

**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 (%) | pH  |
|----------------|--------------------|--------------------|---------------|------------------------|-------------------------------|-------------------------------|-----|
| *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 ± 0.99 |

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.](image)
2.6. Calibration curve

Aliquots of standard solution of piperine were applied in dupli-cates 20 ng, 30 ng, 40 ng and 50 ng over the silica gel 60 F254 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*...

### Table 2

| Samples | Number of ingredients in formulation [12] | Piperine containing herbs (%) in test formulation | Piperine content in IP* | 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 | 3b | 33% & 33%** | – | 2.27 ± 0.83 |
| Sitopadali churna | 5c | 13%** | – | 2.81 ± 0.52 |
| Hingavastaka churna | 8d | 12.5%** & 12.5%*** | – | 7.09 ± 0.73 |
| Avipattikara churna | 14e | 0.75%*** & 0.75%*** | – | 0.16 ± 0.43 |
| Sringayadi churna | 3f | 33%*** | – | 3.62 ± 0.58 |
| Talisadya churna | 8g | 43% & 83%*** | – | 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, Paribhasaprakaran; 16); c – AFI, Part I, Section 7:34, 116 (Sarngadharamshita, Madhyamakhanda, Adhyaya 6; 134–135 ½); d – AFI India, Part I, Section 7:37, 117 (Bhaisajyaratnavali, Agramandayadirodhikara; 37); e – AFI, Part I, Section 7:02, 106 (Bhaisajyaratnavali, Agramandayadirodhikara; 37); f – AFI, Part I, Section 7:31, 115 (Sarngadharamshita, Madhyamakhanda, Adhyaya 6; 42 ½); g – AFI, Part I, Section 7:13, 109 (Sarngadharamshita, 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.

Fig. 5. HPTLC chromatographic scanning of all samples.
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 mg/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.
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.

Source of funding

We hereby declared that the work done in the article was self funded. Hence, the source of funding is self-funded.

Conflict of interest

None.

Acknowledgements

The authors are grateful to the Secretary, Ramakrishna Mission Ashram, Narendrapur for providing all the facilities for research.

References

[1] Subrat N, Iyer M, Prasad R. The Ayurvedic medicine industry: current status and sustainability. New Delhi: Ecotech Services Pvt. Ltd.; 2002.
[2] Wal P, Wal A, Gupta S, Sharma G, Rai AK. Pharmacovigilance of herbal products in India. J Young Pharm 2011;3:256–8.
[3] Farah MH, Olsson S, Bate J, Lindquist M, Edwards R, Simmonds MS, et al. Botanical nomenclature in pharmacovigilance and a recommendation for standardization. Drug Saf 2006;29:1023.
[4] WHO. Guidelines for assessing quality of herbal medicines with reference to contaminants and residues. Geneva: World Health Organization; 2007.
[5] The Ayurvedic Pharmacopoeia of India. New Delhi: Ministry of Health and Family welfare, Dept. of AYUSH; 2007.
[6] Pathak N, Khandelwal S. Cytoprotective and immunomodulating properties of piperine on murine splenocytes: an in vitro study. Eur J Pharmacol 2007;576:160–70.
[7] Singh A, Duggal S. Piperine: review of advances in pharmacology. Int J Pharm Sci Nanotech 2009;2:615–20.
[8] Srinivasan K. Black pepper and its pungent principle-piperine. A review of diverse physiological effect. Curr Rev Food Sci Nutr 2007;47:735–48.
[9] Ahmad N, Fazal H, Abbasi BH, Farooq S, Ali M, Khan MA. Biological role of Piper nigrum L. (black pepper): a review. Asian Pac J Trop Biomed 2012;2:1945–53.
[10] Majeed M, Badmeev V, Rajendran R. Use of piperine as a bioavailability enhancer. United States Patent No.5. 972. 1999. p. 382.
[11] Patil U, Singh A, Chakraborty A. Role of piperine as a bioavailability enhancer. Int J Recent Adv Pharma Res 2011;4:16–23.
[12] Ayurvedic formulary of India. Part-1. 2nd ed. New Delhi: Ministry of Health and Family welfare, Dept. of AYUSH; 2003.
[13] Ayurvedic Pharmacopoeia of India. Part-1. 1st ed. New Delhi: Ministry of Health and Family welfare, Dept. of AYUSH; 2006.
[14] Tapadiya G, Merku M, Deolake U, Khadabadi S, Saboo S, Sahu K. Quantitative estimation of Piperine from pharmaceutical dosage form by HPTLC. Asian J Pharm Clin Res 2009;2:47–50.
[15] Chakraborty B, Hazra AK, Mondal S, Mitra A, Sur TK. Identification and quantification of piperine in different Ayurvedic formulations by HPTLC techniques. Kolkata: Society for Ethnopharmacology of India; March 19, 2016. p. 14.
[16] ICH guidelines, validation of analytical procedure. www.nihs.go.jp.
[17] Adhikari A, Hazra AK, Sur TK. Detection of arecoline by simple HPTLC method in Indian non-tobacco pan masala. J Adv Pharm Tech Res 2015;6:195–9.
[18] Zaveri M, Khandhar A, Patel S, Patel A. Chemistry and pharmacology of Piper longum L. Int J Pharma Sci Rev Res 2010;5:67–76.