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CHEMICAL STANDARDIZATION AND QUANTIFICATION OF *PIPERIN* FROM METHANOLIC EXTRACT OF *PIPER NIGRUM* BY HPLC METHOD ON THE BASIS OF ISOLATED MARKERS

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ABSTRACT

A simple, precise, fast, selective and reproducible HPLC method has been developed for quantification of piperine present in total amount in seeds of *Piper nigrum*. The analytes were resolved by using mobile phase acetonitrole (5.0%) and acetic acid in water on an Waters 515 HPLC on Merk RP – 18 column (5µ, 250 x 4.00 mm ID) by UV detection at 254 nm. Piperine obtained from petroleum ether extract was used as standard (marker). The aim of this work was to develop a RP HPLC method with UV detection for quantification of piperine in methanolic extract. Quantity of piperine obtained from methanolic extract was calculated.

Key words : *Piper nigrum* seeds, Petroleum ether extract, Methanolic extract, Marker compounds, Standardization.

INTRODUCTION

Lack of standardization of polyherbal drug formulation is a serious problem in validating efficacy and maintaining quality control for manufacture of traditional herbal drug.

P. nigrum (Piperaceae) commonly known as *Kali mirch* (black pepper) and *Gol mirch* have chemical contents within the range of 3 to 8 g /100 g, whereas the content of the minor alkaloids piperyline and piperettine have been estimated as 0.2- 0.3 and 0.2-1.6 g /100 g, respectively[1]. The plant grows abundantly throughout in tropical parts of India and in many parts of Sri Lanka, China and Africa. It has extensive culinary use for flavoring,

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condiment and preservating agents. The pungent principles, volatile oils and phenolic compounds are responsible for various biological activities. The fruit extract showed selective anthelmintic activity against cestodes[2]. Piperine displayed CNS depressant activity in mice, antipyretic effect in rabbits given typhoid vaccine and analgesic activity in tail clip pressure in mice[3]. It also showed febrifugal activity in rats[4]. It is prescribed in dyspepsia, flatulence, diarrhoea and as a febrifuge in intermittent fevers[5]. It is also indicated in viral hepatitis[6].

During the past decades, dramatic increase in manufacturing and use of herbal drugs is observed. Standardization of these herbal formulations is an important feature as it is the prerequisite for the quality. The International Organization for Standardization (ISO)[6], the American Spice Trade Association (ASTA)[7] and the Association of Official Analytical Chemists (AOAC) have published spectroscopic methods for piperine determination, involving UV-Vis absorbance measurements at the piperine absorption maximum near 343 nm. In order to get more specific determination of piperine (excluding the other pepper alkaloids) a number of HPLC methods have been described[10, 11]. For this purpose, there is a strong need to adopt a modern technique apart from the conventional parameters of quality control.

No HPLC method has so far been reported for the determination of percentage of pieperine in methanolic extract of *P. nigrum.* So the present study focuses on isolation of chemical markers from pet. ether extract, standardization and quantification of piperine obtained from methanolic extract using marker as standard.

EXPERIMENTAL

Material and methods

The seeds of *P. nigrum* were collected from the local market of Jammu and authenticated by Regional Research Lab., Jammu.

Preparation of petroleum ether extract

500.0 g of coarse powder was extracted by Soxhlet method [12] in pet. ether for 10 h. The resulting extract was clarified by filtration. The clear filtrate was distilled under reduced pressure.

Preparation of methanolic extract

100.0 g of coarse powder was extracted by Soxhlet method in methanol for 8 h.

The extract was clarified by filtration and concentrated.

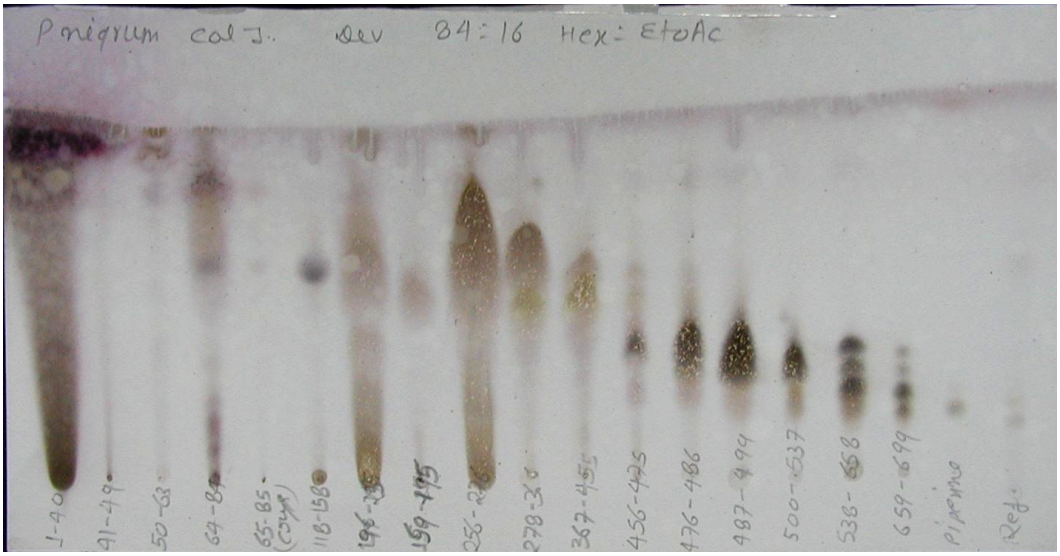

Fig. 1: Typical chromatogram of fractions of pet. ether extract of *P. nigrum* powered seeds in hexane : ethyl acetate (84 : 16) solvent

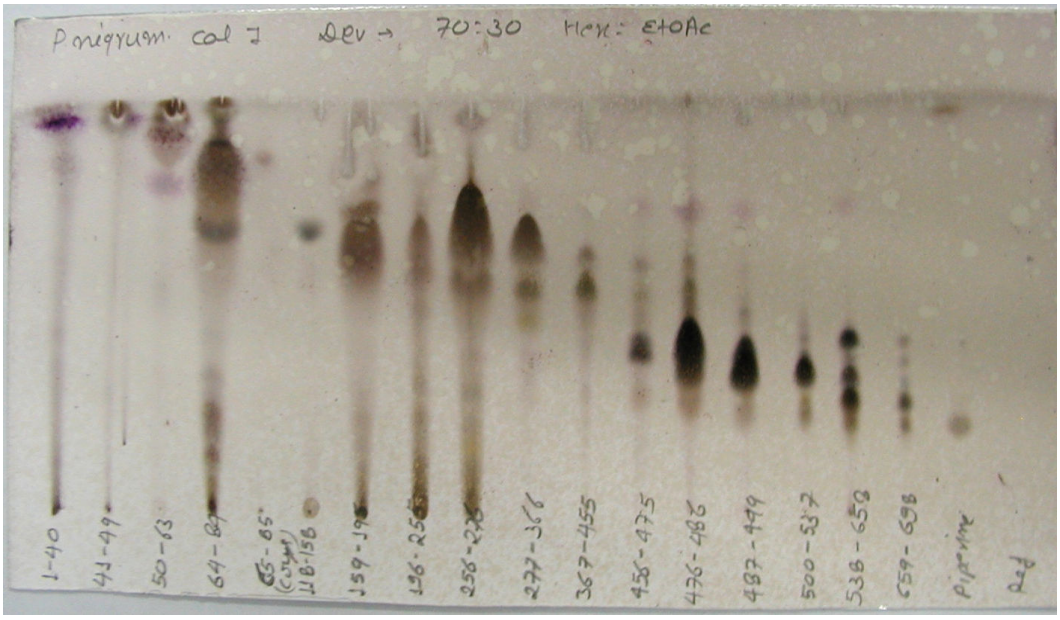

Fig. 2: Typical chromatogram of fractions of pet. ether extract of *P. nigrum* powered seeds in n-hexane : ethyl acetate (70 : 30) solvent

Isolation of marker (piperine) from pet. ether extract by column chromatography

A glass column of two inch in diameter was packed with 300.0 g of silica gel (100-200 mesh) in n – hexane. 25 g of pet. ether extract was adsorbed on 55.0 g of silica gel. The adsorbed material was charged into the column. The column was first eluted with n – hexane and than ethyl acetate and then with n-hexane : ethyl acetate mixture by gradually increasing the polarity with addition of ethyl acetate. Finally, the column was eluted with ethyl acetate.

800.0 fractions of 100 mL each were collected and pooled on the basis of TLC patterns. The TLC plates were developed in n-hexane : ethyl acetate in the ratio of 84 : 16 / 70 : 30. The developed chromatogram was visualized by spraying the plates with 1% cerric ammonium sulphate followed by heating at 110 for 10 min. (Fig. 1 and 2).

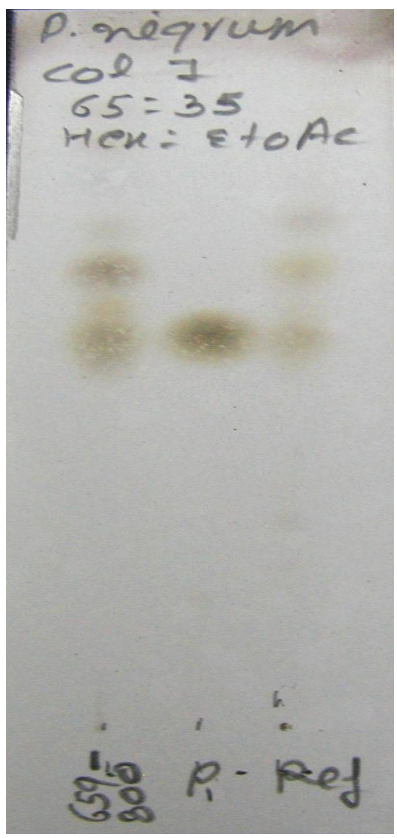

Fig. 3: Typical chromatogram of fractions (659-800) of pet. ether extract of *P. nigrum* powered seeds in n-hexane : ethyl acetate (65 : 35) solvent

Fraction 659-750 were eluted with n–hexane : ethyl acetate in the ratio of 95 : 5. These fractions was developed with 35 : 65 n-hexane : ethyl acetate on TLC. A chromatogram was obtained showing number of spots. One spot was most prominent than others (Fig. 3). The fractions were pooled, dried and residue kept for crystallization in methanol. Crystals thus obtained was characterized as piperine on basis of IR, NMR and Mass spectroscopy.

Preparation of methanolic extract solution

0.0102 g methanolic extract was dissolved in 2 mL methanol (HPLC grade). It was centrifuged and filtered through 0.45µm Millipore filter paper.

Preparation of standard solution

Marker piperine isolated from pet. ether extract was used for preparation of standard solution. For this, 0.0050 g of piperine was dissolved in 10 mL of HPLC grade methanol.

Preparation of calibration curve

Out of total amount of standard solution; 1, 2, 3, 4, 5 and 6 µL were injected in HPLC system and a chromatogram of standard was recorded (Fig. 4). The peak area ratios of standard piperine were calculated and calibration curve was plotted (Fig. 5) against concentration of piperine.

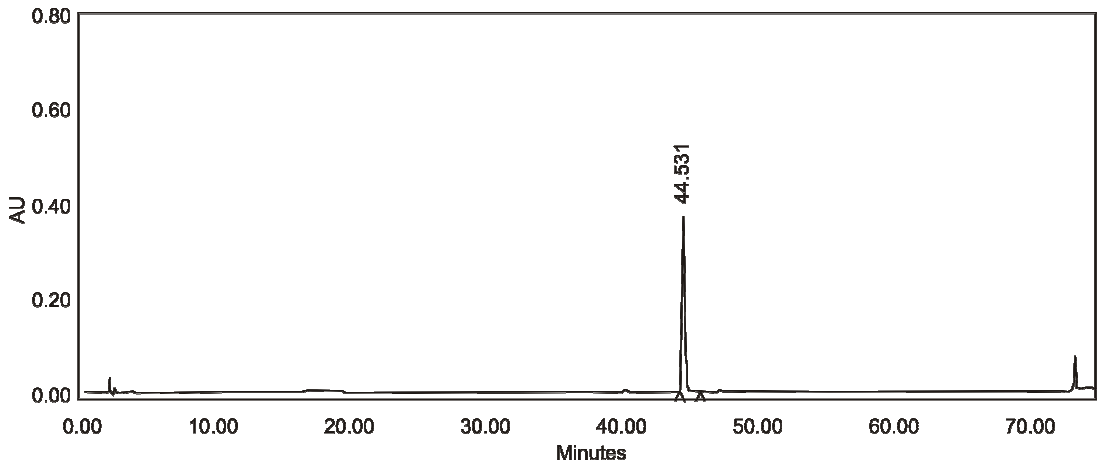

Fig. 4 : HPLC pattern of piperine (marker) isolated from pet. ether extract of *P. nigrum*

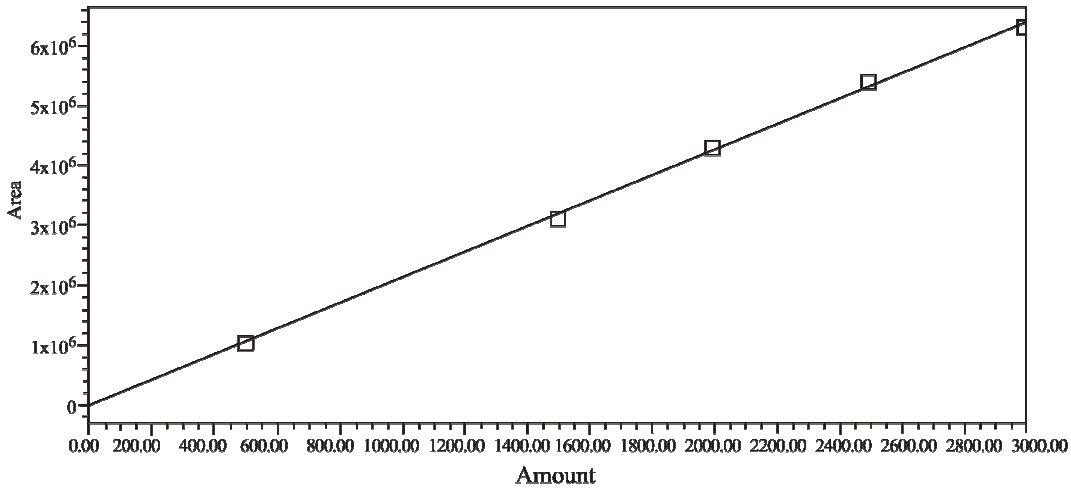

Fig. 5 : Calibration curve [HPLC pattern of piperine (marker) isolated from pet. ether extract of *P. nigrum*]

RESULTS AND DISCUSSION

Analysis

For analysis, a Waters 515 HPLC system having two pump control module was used. It has an automatic sampling unit that is Waters 717 plus auto-sampler, a column oven, a photodiode array, Waters 2996 and temperature control module second. Waters empower software was used for analysis and data processing. The samples were analyzed at 30ºC on Merk RP–18 column (5µ, 250 x 4.00 mm ID) by UV detection at 254 nm. The mobile phase consisting of acetonitrile (5.00%) and acetic acid in water was delivered at a flow rate of 1.0 mL / min using different gradient (Table 1).

Table 1. Ratio of developing solvent (acetic acid in water : acetonitrile 5%) at different time intervals

Time (min.)	Percentage of acetic acid	Percentage of acetonitrile
0.0	92	8.0
1.0	92	8.0
2.0	25	75.0

Cont…

Time (min.)	Percentage of acetic acid	Percentage of acetonitrile
3.0	25	75.0
4.0	92	8.0
5.0	92	8.0

Extractive value

The amount of powder of *P. nigrum* seeds taken for petroleum ether extract was 500 g. and the extract, obtained was 34 g. So the extractive value for pet. ether extract was 6.8%. Again in the case of methanolic extract the amount of powder of *P. nigrum* seeds was 100 g and extract obtained was 13.1 g. So, the extractive value for this was 13.1 g.

Quantification of piperine in methanolic extract

10.0 µL of previously prepared methanolic extract was injected into HPLC system. HPLC analysis showed that 10.0 µL methanolic extract contains 2345808 ng of piperine. So 0.012 g extract, which was dissolved in 2µL methanol for analysis contained 45.90% of piperine

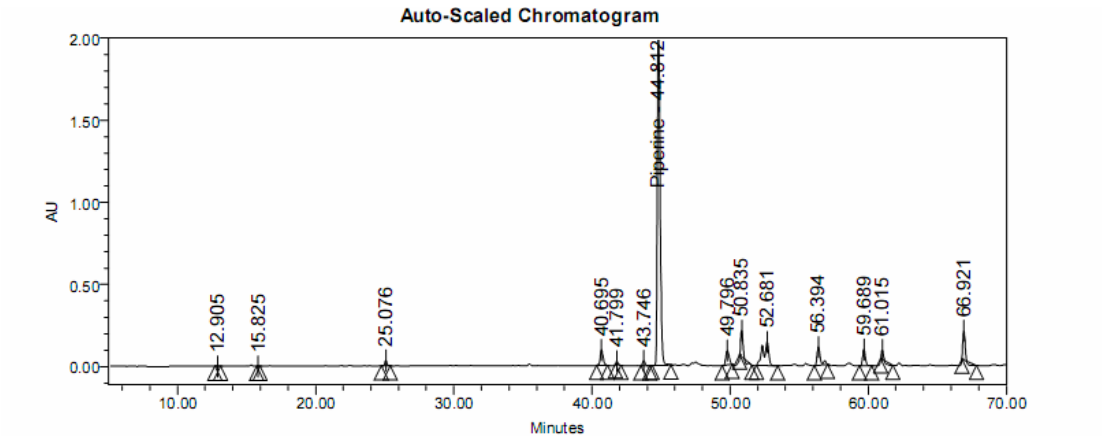

Fig. 6 : HPLC pattern of piperine in methanolic extract of *P. nigrum*

Table 2. Quantification of piperine in methanolic extract of *P. nigrum*

Peak name	RT	Area(µV*sec)	%Area	Height (µV)	Amount	Units
Piperine	44.812	29976215	64.33	2174328	23458.087	ng

As the extractive value of methanolic extract was 13.1 g, so total amount of piperine present in seeds is 6.0 % (Fig. 6 and Table 2).

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REFERENCES

1. D. R. Tainter and A. T. Grenis, Spices and Seasonings, VCH Publishers Inc., NY, (1993) p. 53-68.
2. M. C. Srivastava et al., Ind. J. Pharm**., 30,** 65 (1968).
3. L. E. Bang et al., Arch. Parmacol. Res**., 7**, 127 (1984).
4. J. N. Purseglove et al., Spices, Vol II, Longman London Press (1981) p. 8.
5. P. S. Tathed et al., Selected Medicinal Plants of India, Personal Communication., Chemexcil, (1992) p. 188.
6. ISO 5564, International Organization for Standardization, Geneva, (1982).
7. E. Cliffs, Official Analytical Methods, American Spice Trade Association, NJ, Method 12. 1, 3rd Edn., URL Linkage, (1985).
8. T. Lupina and H. Cripps, J. Assoc. Off. Anal. Chem., **70**, 112 (1987).
9. A. B. Wood, M. L. Barrow and D. J. James, J. Chromatogr Flavour Fragr., **3**, 55 (1988).
10. P. S. Variyar and C. Bandyopadhyay,, Chromatographia**, 39**, 743 (1994).
11. M. Verzele, F. Van Damme, G. Schuddinck and V. Vyncke, J. Chromatography, **471**, 335 (1989).
12. F. Soxhlet, Polytechnisches J (Dingler's), **232**, 461, (1879).

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