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Standardization of Navayasa churna for its Piperine content by UV Spectrophotometric and HPTLC methods

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ABSTRACT

Navayasachurna(NC) is an important Ayurvedic classical preparation used to treat Anaemia (pandu), Jaundice (kamala), Urinary disorder (prameha), Carbuncle (pidaka), Heart diseases (hrdroga), Skin diseases(kustha), and Piles (arsa). Pepper is one of the ingredients of NC which consist of both Piper nigrum and Piper longum. Three different batches of NC were prepared by following the procedure as given in The Ayurvedic Formulary of India. In the current experiment, piperine was estimated by UV spectrophotometric and HPTLC methods. The piperine content by UV methods was found to be 0.78, 0.52 and 0.90% w/w in Piper nigrum, Piper longum and NC respectively. Whereas by HPTLC, piperine content was found to be 3.92, 7.67 and 1.40% w/w respectively in Piper nigrum, Piper longum and NC. HPTLC is the most sensitive technique as compared to UV method in the analysis of piperine content in NC.

Key words: HPTLC, navayasachurna, piperine, UV

INTRODUCTION

WHO estimates that 70% of the population in the developing countries rely on herbal or traditional medicines for their primary health care [1]. Traditional medicines are getting popularised even in the developed countries also. Ayurveda is our traditional system of medicine in India and various kinds of preparations are being used since vedic period [2]. They are believed to be safer, efficacious, and with lesser side effects [3].

Navayasachurna is one of the most important classical preparations in Ayurveda. NC is used to treat pandu, kamala, prameha, pidaka, hrdroga, kushta and arsa. Both the varieties of pepper such as *Piper nigrum* and *Piper longum* are used in NC preparation. Black and long peppers stimulate skin and tongue and therefore can be used in topical application also [4]. In Ayurvedic system of medicine, the unripe fruits of long pepper are used in digestive disorders and to treat skin diseases [5]. Literature survey has not revealed any information regarding the determination of Piperine content in NC and hence the current experiment describes the standardization of both the crude peppers and churna by suitable methods.

MATERIALS AND METHODS

Procurement of raw materials:
The crude drugs were procured from the local market of Udupi and were authenticated by the botanist Dr.Usharani S Suvarna, Head of the Department, Mahatma Gandhi Memorial College, Udupi, Karnataka India.

Procurement of Marker compound:
Standard piperine was obtained from Sigma Aldrich. All other chemicals used in the experiment were of analytical grade.

Instruments:
UV spectrophotometer (Schimadzu, Japan) and HPTLC (Linomat IV applicator, CAMAG Scanner using Wincats software, Switzerland) were used in the experiment.

Preparation of churna:
The raw material was dried in a hot air oven to remove moisture content and then they were ground to a fine powder using a blender. Required quantities of raw materials were weighed and mixed uniformly to obtain churna.The composition of churna consists of ten ingredients as shown in the Table 1 [6].

Table 1. Composition of Navayasachurna

Sanskrit Name	Official Name	No. of Parts
Sunthi	*Zingiberofficinale*	1
Marica	*Piper nigrum*	1
Pippali	*Piper longum*	1
Haritaki	*Terminalia chebula*	1
Vibhitaka	*Terminalia bellirica*	1
Amlaki	*Phyllanthusemblica*	1
Musta	*Cyperusrotundus*	1
Vidanga	*Embeliaribes*	1
Chitraka	*Plumbagozeylanica*	1
LauhaBhasma	*Iron Bhasma*	9

Preparation of standard solution:
An accurately weighed Piperine (100mg) was dissolved in methanol and volume was made up to the mark i.e., 100ml. 1ml of the solution was diluted with methanol to 100ml in a volumetric flask to give 10mcg/ml Piperine solution [7].

Preparation of sample extract:
Reflux the powdered churna (1 gram) with 60ml methanol for one hour. Filter the extract. Reflux the marc left with 40ml of methanol for one hour. Filter and combine the filtrate. Concentrate the methanol extract on a water bath till a semisolid mass is obtained. The residue was dissolved in 75ml of methanol and filtered after dissolving and make up the volume. This was centrifuged at 2000rpm for 20min, the supernatant was collected into a 100ml volumetric flask and the volume was made up with methanol. The same procedure was followed for the powdered samples of *Piper nigrum* and Piper *longum*[8].

Determination of Piperine by UV Spectrophotometric method
Preparation of calibration curve:
A series of calibrated volumetric flasks of 10ml were taken and standard solution of Piperine was pipetted out into them and solutions of concentrations 1mcg/ml, 2mcg/ml, 5mcg/ml, 10mcg/ml and 20mcg/ml were prepared. The absorbance was measured at absorption maxima 342.5nm [7], methanol was used as the blank. Absorption maxima was recorded for each solution.The linear correlation between these concentrations (x- axis) and absorbance (y-axis) was graphically presented.

Estimation of Piperine content in NC and Piper species:
Solutions of concentrations 1mcg/ml and 10mcg/ml from the churna extract and *Piper nigrum* extract; 5mcg/ml and 25mcg/ml from the *Piper longum* extract were prepared. The absorbance of these solutions was recorded at 342.5nm and the concentrations were determined from the standard curve.

Determination of Piperine by High Performance Thin Layer Chromatography (HPTLC) method
HPTLC plate of required dimension was taken and a mixture of toluene: diethyl ether: dioxane (9:3:1) was taken in a chamber as mobile phase [9] and the chamber was allowed to saturate. The standard and sample solutions were applied on the HPTLC plate in a band form using an applicator. The plate was placed in the chromatographic chamber which was previously saturated with the mobile phase. The chromatogram was developed and the HPTLC plate was screened using HPTLC scanner and the data was recorded and analysed.

RESULTS AND DISCUSSION

Determination of Piperine by UV Spectrophotometric method:

The piperine content of both the species piper scuh as *Piper nigrum* and *Piper longum* including the churna were determined by UV spectrophotometric method. Piperine obeys Beer-Lamberts law in concentration range 10-15 mcg/ml at the maxima 342.5nm. The correlation coefficient R^2 was calculated where the value 0.99754 indicates a good linearity between the concentration and absorbance as represented in Figure 1.

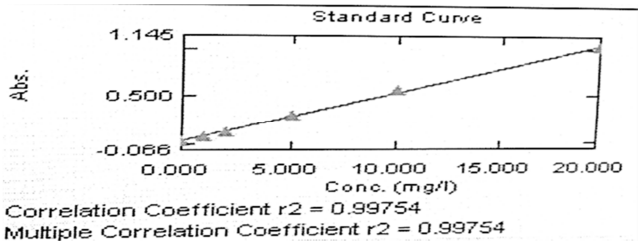

Figure 1. Calibration curve for Piperine

For estimating the standard solutions by UV spectrophotometry, methanol was used as blank and the optimum wavelength used was 342.5nm. The concentrations of standard solutions taken were 1mcg/ml, 2mcg/ml, 5mcg/ml, 10mcg/ml and 20mcg/ml and their absorbance was found to be 0.075, 0.125, 0.298, 0.574 and 1.028 respectively. In accordance with the Beer- Lambert's law, as the concentration of the samples increases, so does their absorbance. Solutions of the churna extract of concentrations 1mcg/ml and 10mcg/ml were prepared and their absorbance was found to be 0.081 and 0.494 respectively.

Solutions of the *P. nigrum* extract of concentrations 1mcg/ml and 10mcg/ml were prepared and their absorbance were found to be 0.073 and 0.432 respectively. Similarly, for *P.longum* extract of concentration 5mcg/ml and 25mcg/ml was made and absorbance was observed to be 0.301 and 1.366 respectively. The amount of Piperine content in raw materials and churnaare shown in the Table 2.

Table 2. Piperine content in crude drugs and churna by UV Spectrophotometric method

Sl. No.	Sample	Piperine content (% w/w)
1	*Piper nigrum*	0.7837
2	*Piper longum*	0.5253
3	Churna	0.9066

Determination of Piperine by HPTLC method:

Chromatograms for Standard Piperine, *P. nigrum*, *P. longum* and Navayasachurna are represented in the Figures 2-5 respectively. The amount of piperine in all the samples are given in Table 3.

Table 3. Piperine content in crude drugs and churna by HPTLC

Sl. No.	Sample	R_f value	Area	Piperine (% w/w)
1	Standard	0.58	17088.3	87.94
2	*P.nigrum*	0.57	15276.1	3.92
3	*P. longum*	0.61	31319.1	7.67
4	Churna	0.57	5445.2	1.40

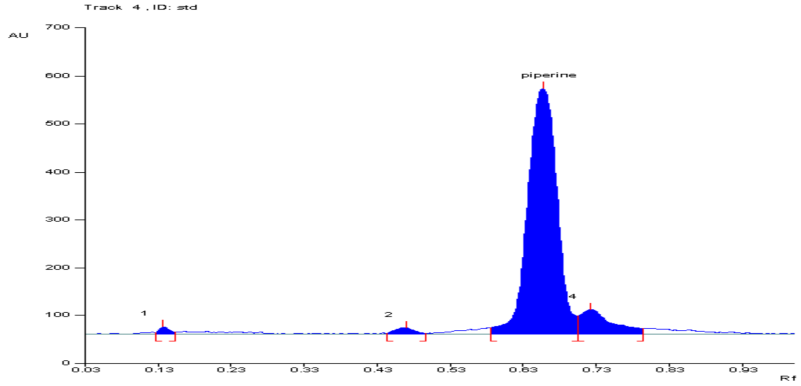

Figure 2. Chromatogram of Standard Piperine

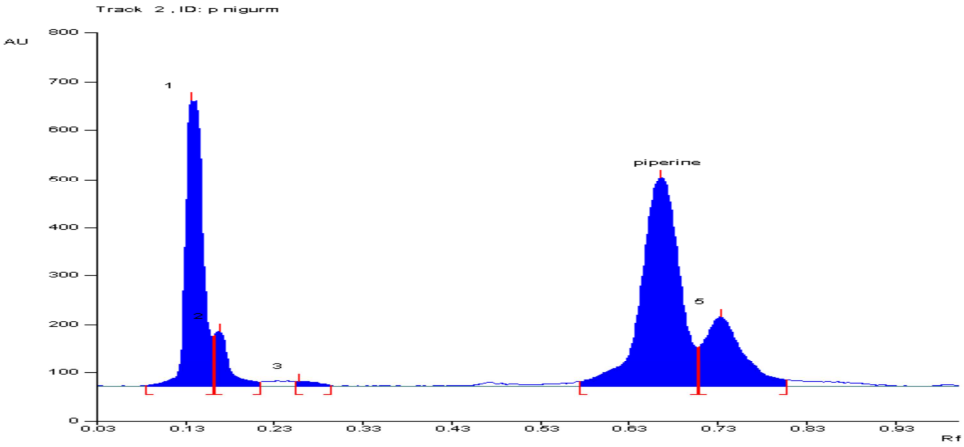

Figure 3. Chromatogram of *P. nigrum*

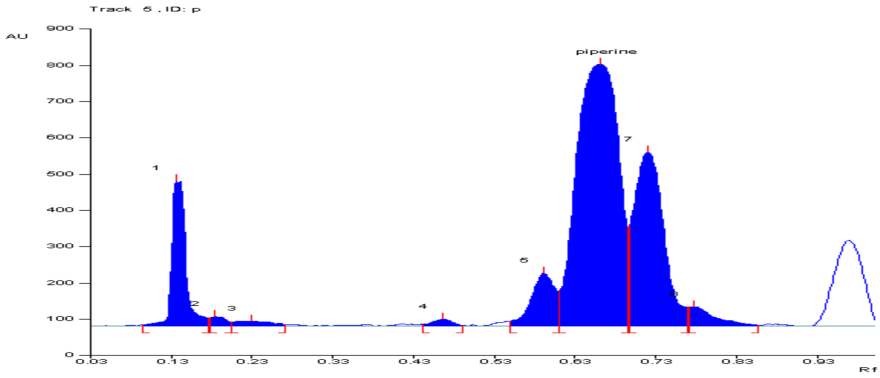

Figure 4. Chromatogram of *P. longum*

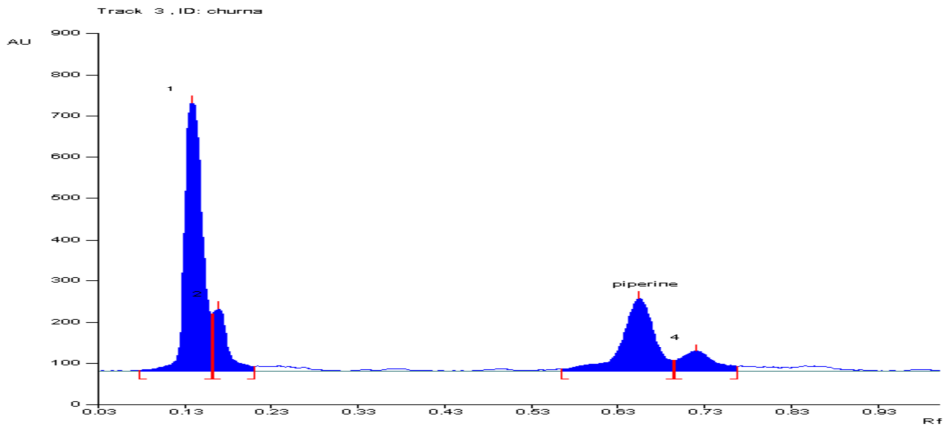

Figure 5. Chromatogram of Navayasachurna

CONCLUSION

We found a lot of difference in piperine content which was observed in results from both the methods. Errors and loss of piperine can occur during extraction procedure while estimating by UV method. Whereas, HPTLC is the most sensitive and accurate method and therefore, can be routinely employed in the determination of piperine content of Navayasachurna samples in Quality Control laboratories of a manufacturing unit.

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