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# Development of fingerprint for single component analysis of an ayurvedic formulation (*Sitopaladi Churna)* by High Performance Liquid Chromatography

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## Abstract

*Sitopaladi Churna. Is a most trusted and sold Ayurvedic formulations among the Ayurvedic medicines, Piperine is one of the major constituent of Sitopaladi Churna. The process of development of HPLC fingerprints for Sitopaladi Churna extract is introduced in detail.  The three laboratory batches and three marketed batches were taken in this study to estimate the % of piperine in this indigenous formulation. The selection of a suitable chromatographic system, the screening for important parameters, and gradient optimization to method validation, and an integrated and universal HPLC fingerprint approach was performed. This improves the separation quality of the fingerprint. The detection wavelength of piperine was 342 nm.  The results of the method validation, based on the relative standard deviation of relative retention times and relative peak areas, were acceptable. Calibration curves showed good linear regression ($R^2 > 0.9935$) within test range. The LODs and the LOQs for the piperine were 0.027055 mg/ml and 0.081985 mg/ml. This strategy is used for the estimation of piperine in Sitopaladi Churna formulation and identifies and assessed its quality.*

**Key Words:** Sitopaladi Churna, Herbals, Piperine, HPLC, Fingerprint, Validation.
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## INTRODUCTION

Herbal formulations show the number of problems when quality aspect is considered. This is because of nature of the herbal ingredients and different secondary metabolites present therein. Mainly, variation in the chemical profile of the herbal due to intrinsic and extrinsic factors (growing, harvesting, storage and drying processes) [1-3].

Chromatographic fingerprint have been suggested to check for authenticity or provide quality control of herbal medicine [4]. Chromatography has the advantage of separating a complicated

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System into relatively simple sub-systems and then presenting the chemical patterns of herbal medicine in the form of a chromatogram. The World Health Organization (WHO) accepts fingerprint chromatography as an identification and quality evaluation technique for medicinal herbs since 1991 [5]. Fingerprints can be a unique identification utility for herbs and their different species) [6-7], and can be used for modeling pharmaceutical activities [8]. Now, chromatographic fingerprint technique plays an important role in controlling the quality of TCM for the systemic characterization of compositions of samples and focusing on the identification and assessment of the stability of the components [9]. The Patent proprietary Ayurvedic medicines are sold over the counter in pharmacies; these products appear to represent a major share of branded traditional medicine in India. Nevertheless systems like Ayurveda still need to gain an empirical support of modern medical sciences to make them credible and acceptable for all. An innovative research effort to define the advantage of traditional system of medicine with respect to their safety and efficacy could result in a better utilization of these complementary systems of medicine. Sitopaladi Churna. Is a most trusted and sold Ayurvedic formulations among the Ayurvedic medicines. Sitopaladi churna contains sugar, *Bombusa bombos* (Banslochan), Piper longum (Pipali), Elattaria cardamomum (Elaichi), Cinnomomum zeylanicumm (Tvak). It is used for different disorders like Intercostal neuralgia and pleurodynia, Asthma due to disorder of bile, Numbness of tongue, Digestive impairment, Burning sensation in palm and soles [10].

Sitopaladi Churna is mentioned in sarangdhara samhita madhymakhanda adhyay 6,134-135 1/2(an old Ayurvedic classical text). Piperine is one of the major constituent of Sitopaladi churna. Molecular formula $C_{17}H_{19}NO_3$ density 1.193 g/cm3, melting point 130°C, in animal studies, piperine also inhibited other enzymes important in drug metabolisms [11].

## MATERIAL AND METHODS

**Instrumentation**
Experiments were performed on a HPLC system Simadzu-10AT$_{VP}$, binary gradient equipped with detector Simadzu UV -VIS SPD-10 A$_{VP}$, software Spinchrom, Chennai. The separations were performed on column Merck's [Lichrospher 100, C-18 (250 x 4.6 mm) and ODS RP-18 (250 x 4.6 mm, 5μ particle size)] using methanol mobile phase with flow rate 1.2 mL min−1. Detector was set at 342 nm and attenuation adjusted to 0.000 AUFS. UV detector, Spectra of extracts was recorded with a UV-2101PC UV scanning spectrophotometer (Shimadzu) and Microsoft Excel 2002 were used for calculations.

**Herbs, Chemicals and reagents**
Crude drugs were procured from local market and identification was conformed by macroscopic and microscopic characters. All the herbs procured from the local market All the chemicals and solvents were used of AR grade; Standard Piperine (98%) was procured from Lancaster (England). methanol (MeOH) was procured from Merck and used as mobile phase.

**Preparation of the formulations:**
Sitopaladi Churna, three laboratory batches (named SCL-I, SCL-II and SCL III) were prepared in the institutional laboratory according to reported method of Ayurvedic formulary of India. The available commercially brand SCM-A, SCMB and SCM-C of Sitopaladi Churna was procured from local Pharmacy.

**Preparation of reference solution of Piperine:**
Accurately weighed Piperine (10 mg) was transferred in 100 ml volumetric flask and dissolved in and diluted to 100 ml with ethanol. The final solution contained 100 µg of the Piperine per ml of the solution.

**Standard plot of Piperine**
Serial dilutions containing 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg/ml piperine in ethanol were prepared from a stock solution of piperine (10 mg/100ml). Each dilution was chromatographed on HPLC and area under the peak of piperine recorded (Table I). Retention time of piperine was observed to be 2.543 min. A standard curve of piperine was prepared by plotting the actual amount of piperine present in 10 ml of the dilutions against the area under the peaks of piperine observed by injecting above serial dilutions. The intercept and the slope of the standard plot was observed to be 53.145 and 121.18, respectively, with coefficient of correlation as 0.9935 ($R^2$).

**Sample preparation**
Accurately weighed 5 gm of powdered Sitopaladi Churna was taken and refluxed with 60 ml of ethanol for 1 hour. The extract was filtered and re-refluxes the marc left with 40 ml of ethanol for another 1 hours. The previous filtrate was filter and combined. The ethanolic extract of Sitopaladi Churna Concentrated under vacuum till the semisolid mass was obtained. It was finally dissolved and made up the volume up to 100 ml with ethanol and filtered through sintered glass funnel (G-2) by vacuum filtration assembly. The filtrate was centrifuged at 2000 rpm for 30 minutes, the supernatant was collected and volume was made with ethanol.

The same procedure was performed for, the separately powdered drug of Piper longums (Pippali) and piperine extract. Since the other ingredient of the formulations does not contain piperine so they were not included in the present study. Each of the solutions was subjected to HPLC and the area under the peak of piperine was recorded. The amount of piperine was calculated in the test material using the regression equation. Table 2 shows the mean results from 3 separate extractions and estimations done for each test material.

**Chromatographic conditions** [12]
The chromatographic runs were performed at a flow rate of 1.2 ml/min, a column temperature of $30^0$C, a detection wavelength of 342nm, and an injection volume of 20µg.

## RESULTS AND DISCUSSION

**Optimization of HPLC Condition**
For giving the most chemical information and better separation in the chromatograms, the column, mobile phase, detection wavelength and conditions for gradient elution were investigated in this study.

Two kinds of reversed-phase columns, C18 column (250mm×4.6mm, 5µm) and ODS RP-18 (250 x 4.6mm were investigated, the ODS RP-18 (250 x 4.6mm, 5µm) column was found to be more suitable and gave good peak separation and sharp peaks. The effect of mobile phase composition on chromatographic separation was investigated and found there was a sharp peak obtained by methanol as compared to ethanol so the mobile phase selected as methanol Because

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of the long retention time of some of the late-eluting peaks in isocratic runs, gradient elution was employed in HPLC analysis The wavelength for the detection of the constituents in the formulation was selected by the UV. There are some different peaks obtained and different wavelength but peaks were not well in shape & not well separated. Therefore, 342 nm was selected as detection wavelength (Figure 1).
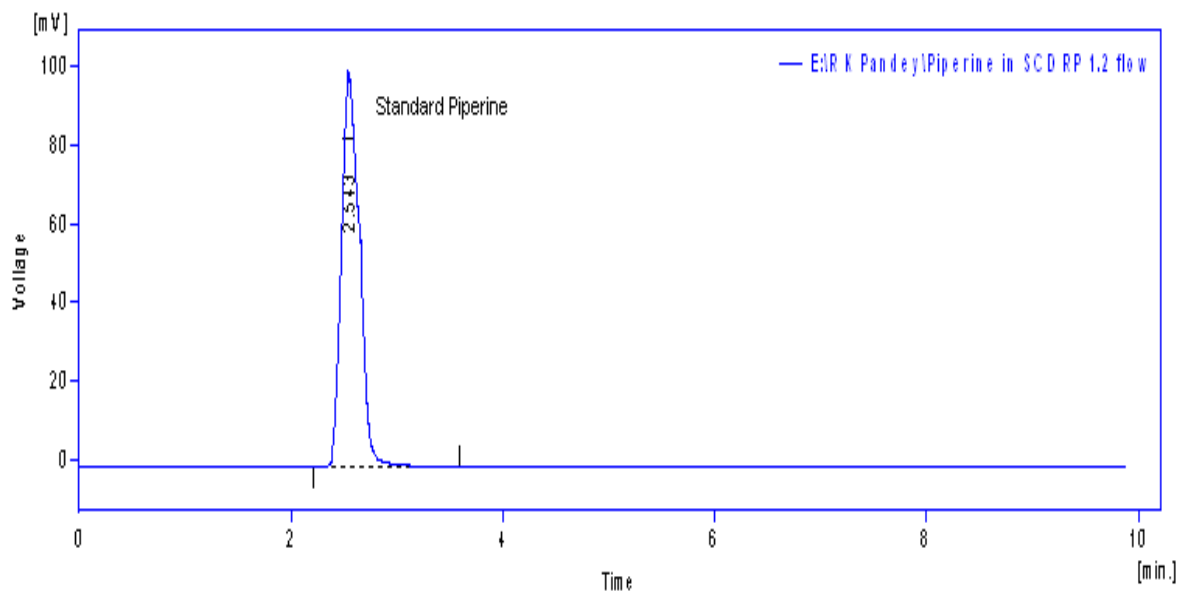


**Figure 1: RP-HPLC Chromatogram of standard Piperine at 342 nm**

**Method validation of quantitative analysis**
The method was validated in terms of linearity, limits of detection and quantification (LODs and LOQs), precision, repeatability and recovery test. [13]
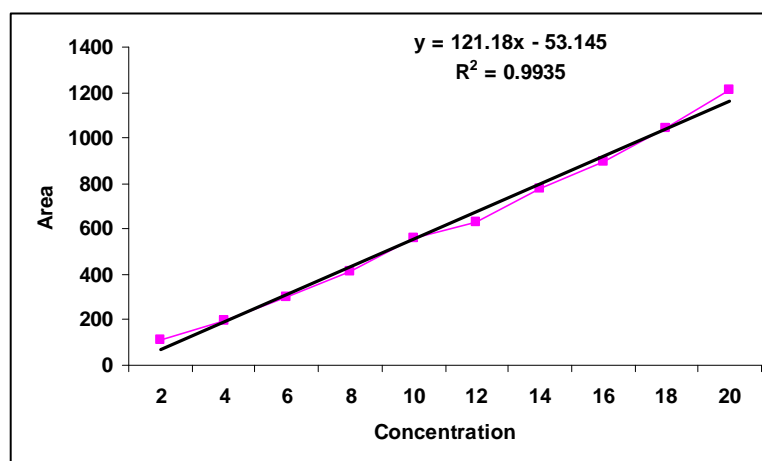


y = 121.18x - 53.145
$R^2$ = 0.9935

**Figure 2: Calibration curve of standard Piperine**

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## Linearity

Linearity was examined with standard solutions. A mixed stock solution consisted of piperine was prepared. 2,4,6,8,10,12,14,16,18,20 µg/ml of the stock solution each was put into a 10 ml volumetric flask and adjusted with Ethanol for the standard curves. Each calibration curve contained six different concentrations and was performed in triplicate. An aliquot (20µ) of each standard working solution was subjected to HPLC-UV analysis the linearity was established by plotting the peak area (y) versus concentration (x) of piperine. Calibration curves showed good linear regression ($R^2 > 0.9935$) within test range (Figure 2).

The LODs and LOQs under the present HPLC-UV method were determined at signal-to-noise ratios (S/N) of 3 and 10, respectively. Standard solution containing piperine as a reference compounds was diluted to a series of appropriate concentrations with ethanol and an aliquot (20µl) of the diluted solution was injected into HPLC for analysis.

## Precision

The LODs and the LOQs for the piperine was 0.027055 mg/ml and 0.081985 mg/ml. Intra- and inter-day variations were utilized to determine the precision. The intra-day variation was determined by analyzing the six samples of (SCL) & SCM within 1 day and inter day variation was determined on three consecutive days. To confirm the repeatability, six different working solutions prepared from the same sample of each batch of SCL & SCM were analyzed. Variations were expressed as relative standard deviations (R.S.D.). Table 1 showed the results of the tests of precision and repeatability. It indicated that the R.S.D. values of the overall intra- and inter-day variations were less than 0.462 in piperine, 0.865643% in SCL, and1.051% in SCM and the repeatability was less than 0.2311 in piperine 0.075113 in SCL and 1.031483 % in SCM for the Piperine in the formulation.

**Table 1- Precision of intra-day, inter-day and repeatability HPLC measurements for marker compound in Sitopaladi churna**

| Formulations | Intra-day | | Inter-day | | Repeatability | |
|---|---|---|---|---|---|---|
| | Content (mg/g) | RSD (%) | Content (mg/g) | RSD (%) | Content (mg/g) | RSD (%) |
| SCL-I | 16.84 | 0.865643 | 16.81 | 0.865643 | 16.84 | 0.0751 |
| SCL -II | 16.86 | 0.517998 | 16.79 | 0.52063 | 16.85 | 0.07506 |
| SCL III | 16.73 | 0.365073 | 16.76 | 0.36456 | 16.83 | 0.3114 |
| SCM-A | 15.72 | 0.810159 | 15.35 | 0.83173 | 15.79 | 0.4578 |
| SCM -B | 15.91 | 0.237313 | 15.31 | 0.24728 | 15.83 | 0.5006 |
| SCM -C | 15.37 | 1.033 | 15.29 | 1.051 | 15.43 | 1.0314 |
| *P. longum* (fruit) | 17.76 | 0.462 | 17.42 | 0.346 | 17.61 | 0.2311 |

## Recovery

The recovery test was determined by standard addition method. Piperine were spiked into the Each Sample, and then, processed and quantified in accordance with the established procedures the results of recovery test were summarized in Table 2. The average recoveries of the piperine in SCL were 98.16-99.68% and 94.99-98.01% and 99.68 in *Piper longum* extract. The R.S.D values were less than0.037 in all batches of SCL and 0.030 in SCM.

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**Table 2- Recovery for piperine compound in Sitopaladi churna**

| Formulations | Recovery (mg/g) | | | | |
|---|---|---|---|---|---|
| | Original | Spiked | found | % Recovery | RSD (%) |
| SCL-I | 16.85 | 16.86 | 33.45 | 98.44 | 0.022 |
| SCL –II | 16.84 | 16.84 | 33.64 | 99.68 | 0.037 |
| SCL III | 16.73 | 16.69 | 33.12 | 98.16 | 0.023 |
| SCM-A | 15.71 | 15.71 | 31.11 | 98.01 | 0.030 |
| SCM –B | 15.9 | 15.9 | 31.01 | 94.99 | 0.016 |
| SCM –C | 15.37 | 15.36 | 30.42 | 97.96 | 0.011 |
| *P. longum* (fruit) | 17.62 | 17.42 | 34.98 | 99.83 | 0.012 |

## CONCLUSION

The reported RPHPLC-UV method was precise, accurate and sensitive enough for quantitative evaluation of piperine in Sitopaladi Churna by HPLC fingerprint and quantitative analysis. The content of the piperine was not less than 16.73 & 16.76 in intraday and intraday respectively as compare to marketed formulation. This strategy can be utilized for the estimation of piperine & chemical standardization of Sitopaladi Churna formulation & other herbal formulations containing piperine.

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