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RESEARCH ARTICLE

Chemical Fingerprinting of an Ayurvedic Formulation containing Indigenous Drugs of C.G.

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ABSTRACT:
Chitrakadi Churna is a most trusted oral ayurvedic formulation among the ayurvedic medicines; plumbago zeylanica is one of the constituent of Chitrakadi Churna. The present study summarizes fingerprinting approach based on high performance liquid chromatography for Chitrakadi Churna formulation. The Two laboratories made batches and two marketed batches were taken in this study to estimate the % of *plumbagin* in this indigenous formulation. The selection of a suitable chromatographic system, the screening for important parameters to method validation, and an integrated and universal HPLC fingerprint approach was performed; this improved the separation quality of the fingerprint. The detection wavelength of *plumbagin* was found to be 331 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 > 0.9989) within test range. The LODs and the LOQs for the *plumbagin* were was 0.317mg/ml and 1.0632mg/ml mg/ml .This reported method proves its usefulness for chemical standardization of crude drug and Chitrakadi Churna formulation by means of estimation of *plumbagin*.

KEYWORDS: Chitrakadi Churna, *Plumbagin*, HPLC, Validation

INTRODUCTION:
The quality and chemical content of herbs vary greatly due to many factors such as species variation, geographical source, cultivation, harvest, storage, and processing [1]. Better scientific methodologies are still needed to evaluate and assess medicinal herbs and their products .In recent years, the use of chromatographic chemical fingerprinting for the identification and quality control of medicinal herbs has attracted a lot of interest [2–5]. Analysis of chromatographic profiles, generally with the goal of making a classification, is known as 'fingerprinting'.

Fingerprinting using chromatographic methods is also one of the requirements proposed by US Food and Drug Administration (FDA) for botanicals [6] and The European Agency for the Evaluation of Medicinal Products for herbal preparations. It is of importance because the chromatographic fingerprints are unique and represent powerful tools for the comparison, classification, identification, and evaluation of samples [7]. However, due to the complex fingerprints of herbal samples and chromatographic variations, accurate analysis and interpretation of the chromatograms in chemical fingerprinting still pose a great challenge to analysts.

Churnas are ayurvedic preparations. The plant ingredients of Chitrakadi Churna viz. *Plumbago zylanica, Piper longum , Zingiber officinale, P.nigrum, Piper chhaba ,* Asafetida and *A.gravelens,* powdered and passed through sieve 100. Chitrakadi Churna is used for different disorders of digestive system like anorexia, impairment of metabolism and bile disorders. Chitrakadi

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Churna is mentioned in old Ayurvedic classical text and official books like Sharangadharasamhita madhyama khanda 6/108, Ayurvedic Formulary of India. Plumbagin or 5-hydroxy-2-methyl-1, 4-naphthoquinone is an organic compound with formula $C_{11}H_8O_3$. It has a number of antimicrobial properties (8-10).

MATERIAL AND METHODS:

Instrumentation

Experiments were performed on a HPLC system Shimadzu-10AT_{VP}, binary gradient equipped with detector Shimadzu UV -VIS SPD-10 A_{VP}, software Spinchrom, Chennai. The separations were performed on Merck's column [Lichrospher 100, C-18 (250 x 4.6 mm) and ODS RP-18 (250 x 4.6 mm, 5µ particle size)] using Methanol: acetic acid (0.5%v/v) mobile phase with flow rate 1.2 ml min−1. Detector was set at 331 nm, attenuation adjusted. Microsoft Excel 2002 was used for calculations.

Herbs, Chemicals and reagents

Crude drugs were procured from local market and identification was confirmed by macroscopic and microscopic characters in the Department of Pharmacognosy. All the herbs procured from the local market all the chemicals and solvents were used of AR grade; Standard Plumbagin (98% pure) used for the study. Methanol (MeOH) and acetic acid was procured from Merck and used as a mobile phase.

Preparation of the formulations:

Chitrakadi Churna, three laboratory batches (named CKL-I and CKL-II) were prepared in the institutional laboratory according to reported method of Ayurvedic formulary of India. The available commercially brand CKM-A and CKM-B of Chitrakadi Churna was procured from local Pharmacy.

Preparation of reference solution of Plumbagin:

Accurately weighed Plumbagin (10 mg) was transferred to 100 ml volumetric flask and dissolved in and diluted to 100 ml with methanol. The final solution contained 100 µg of the plumbagin per ml of the solution.

Standard plot of Plumbagin

Serial dilutions containing 4 to 20µg/ml plumbagin in methanol were prepared from a stock solution of plumbagin (10 mg/100ml). Each dilution was chromatographed on HPLC and area under the peak of plumbagin recorded. Retention time of plumbagin was observed to be 3.817 min. A standard curve of plumbagin was prepared by plotting the actual amount of plumbagin present in 10 ml of the dilutions against the area under the peaks of plumbagin observed by injecting above serial dilutions. The intercept and the slope of the standard plot were observed to be 47.39and 106.1, respectively, with coefficient of correlation as 0.9989 (R^2).

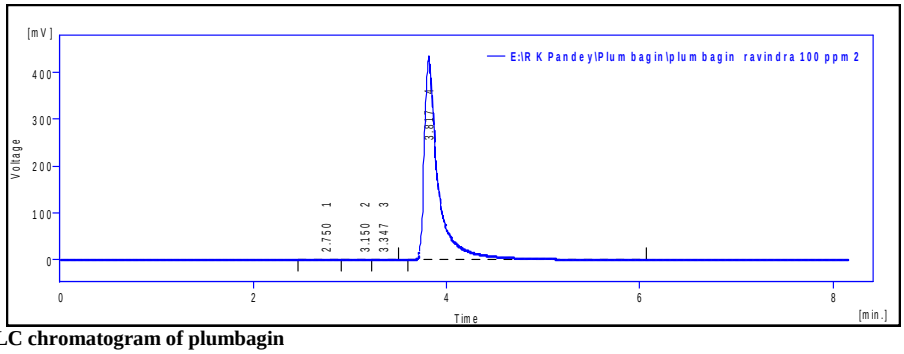

Figure 1- RP HPLC chromatogram of plumbagin

Sample preparation

Accurately weighed 1 gm of powder for the separately powdered crude drug of *Plumbago* zeylanica was refluxed with 60 ml of methanol for 1 hour. The extract was filtered and the marc left was re-refluxed with 40 ml of methanol for another 1 hours. The previous filtrate was filtered and combined. The ethanol extract of Chitrakadi Churna was Concentrated under vacuum till a semisolid mass was obtained. It was finally dissolved and the volume made up to 100 ml with methanol 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 methanol.

Each of the solutions was subjected to HPLC and the area under the peak of plumbagin was recorded. The amount of plumbagin was calculated in the test material using the regression equation.

Chromatographic conditions
The chromatographic runs were performed at a flow rate of 1.2 ml/min, a column temperature of 30^0C, a detection wavelength of 331 nm .

RESULT AND DISCUSSION:

Optimization of HPLC Condition
For better separation in the chromatograms, the column, mobile phase, detection wavelength and conditions for gradient elution were investigated in this study. 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: acetic acid as compared to methanol alone so the mobile phase selected as methanol: acetic acid (0.5%v/v) wavelength for constituents in the formulation was selected by the UV. There are some different peaks obtained and different wavelength but peaks were neither well in shape nor well separated. Therefore, 331 nm was selected as detection wavelength for plumbagin.

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.

Linearity: Linearity was examined with standard solutions. A mixed stock solution consisted of plumbagin was prepared. 4 to 20 µg/ml of the stock solution each was put into a 10 ml volumetric flask and adjusted with Ethanol for the standard curves contained six different concentrations and was performed in triplicate.

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 plumbagin as a reference compounds was diluted to a series of appropriate concentrations with ethanol and an aliquot of the diluted solution was injected into HPLC for analysis.

Precision: The LODs and the LOQs for the Plumbagin was 0.317 mg/ml and 1.0632mg/ml .Intra- and inter-day variations were utilized to determine the precision. The intra-day variation was determined by analyzing the six samples of CKL & CKM 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 CKL & CKM were analyzed. Variations were expressed as relative standard deviations (R.S.D.). It indicated that the R.S.D. values of the overall intra- and inter-day variations were less than 0.30 % Plumbagin and the repeatability was less than 0.013% in Plumbagin in the formulations.

Recovery: The recovery test was determined by standard addition method in accordance to ICH guidelines. Plumbagin was spiked into the each sample, and then, processed and quantified in accordance with the established procedures the results of content and recovery test of plumbagin were summarized in Table 1 and 2. The average recoveries of the plumbagin were not less than 99.81% and The R.S.D value was under limit. **(Figure 1)**

Estimation of Plumbagin in formulation
The content of Plumbagin present in raw material was found to 1.62± 0.074w/w. The content of Plumbagin in laboratory batch and marketed formulations of Chirakadi Churna is discussed in
(Table2).

Table 1: % Recovery for Plumbagin in Chitrakadi Churna (n=6)

Excess drug added to the analyte (%)	Recovery (%)	SD	R.S.D. (%)
50	99.64	0.014	0.061
100	99.89	0.039	0.30
150	99.92	0.01	0.017

Table 2: Estimation of Plumbagin (% (w/w)) in formulations and crude drug

S. No.	Name		Plumbagin Content % (w/w)	SEM
1.	*P.zeylanica*		1.62± 0.074	0.01900
2.	Chitrakadi Churna	CKL-I	0.656 ± 0.0010	0.00066
3.		CKL-II	0.433 ± 0.0026	0.00012
4.		CKM-A	0.188 ± 0.0015	0.00003
5.		CKM-B	0.174 ± 0.0020	0.00058

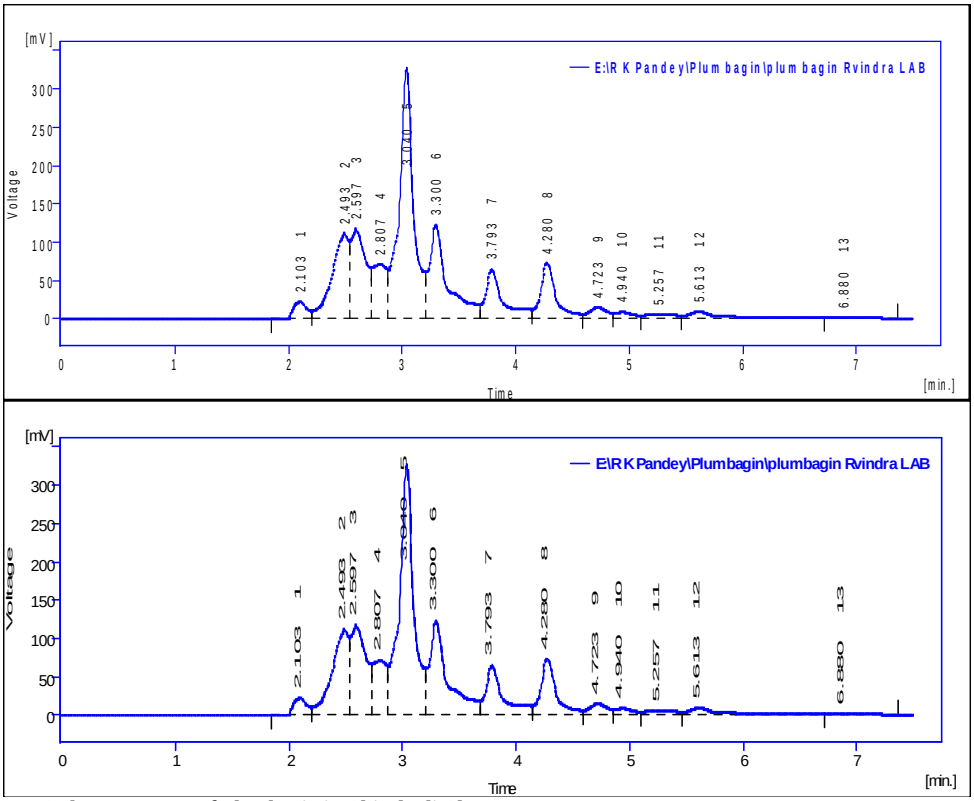

Figure 2- RP HPLC chromatogram of Plumbagin in Chirakadi Churna

CONCLUSION:

The reported RPHPLC-UV method is precise, accurate and sensitive enough for quantitative evaluation of plumbagin in Chitrakadi Churna. The method can be used to determine the purity of the crude drug available from various sources by detecting the related impurities. It is proposed for the analysis of the drug and the degradation products in stability samples .This method can potentially differentiate herbs or complex samples based on their chromatographic profiles. It can also determine the similarity between samples. Therefore, it is a potential useful tool in ensuring the quality and safety of herbal products containing plumbagin.

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