International Journal of Phytomedicine 6 (2014) 417-424
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Validated HPTLC method for standardization of an ayurvedic powdered formulation-Panchsakar churna

Kumar Puspendra[1,2*], Jha Shivesh[2], Patel Durgawati[3], Alam Sanjar[1]

***Corresponding author:**

Kumar Puspendra

[1]KIET School of Pharmacy, KIET Group of Institutions, Ghaziabad, 201206. Uttar Pradesh, India.
[2]Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi-835215. Jharkhand, India.
[3]Department of Chemistry, Government Nagarjuna Post Graduate College of Science, Raipur, 492010. Chhattisgarh, India.

Abstract

Panchsakar Churna is a marketed ayurvedic powdered formulation containing *Cassia angustifolia* and *Terminalia chebula* as the main ingredients. Present study aims to standardize Panchsakar Churna based upon chromatographic and spectral studies. Methods: This study developed an efficient and reliable high performance thin layer chromatographic method for quantification of Sennoside B and Gallic acid in Panchsakar Churna. The developed method was validated for linearity, accuracy, precision, sensitivity and purity. Results: Linearity of peak area was tested in the range 0.310-3.100µg/spot (r=0.9835) and 0.200-2.000 µg/spot (r=0.9929), Accuracy was assessed with recovery and the recoveries were 99.909-100.330 % and 99.690-100.562 % (n=3 for each sample), Precision were 0.257-2.580 and 0.425-2.201, sensitivity was determined with respect to limit of detection and limit of quantification, limit of detection were 73.7634 ng/spot and 50.1556 ng/spot, while limit of quantification were 245.8781ng/spot and 167.1853ng/spot, purity of the spot were 0.9984-0.9999 (r) and 0.9977-0.9998 (r) respectively for Sennoside B and Gallic acid. Conclusion: Sennoside B and Gallic acid can be used as possible marker compound for standardization of Panchsakar Churna.

Keywords : Panchsakar Churna; HPTLC; Standardization; Sennoside; Gallic acid.

Introduction

Ayurveda is one of the indigenous systems of medicine. It has been practiced in India since ancient times. This plant based system of medicine has already gained worldwide attention due to its safety and efficacy. With the growing need for safer drugs, attention has been drawn to their quality, efficacy and standards of the Ayurvedic formulations. [1, 2, 3] *Panchsakar Churna* is a popular poly herbal Ayurvedic preparation. It consists of a fine powder of *Swarnapatri* (*Cassia angustifolia* Vahl.)-1part, *Haritaki* (*Terminalia chebula* Retz.)-1part, *Madhurika* (*Foeniculum vulgare* Mill.)-1part, *Shunthi* (*Zingiber officinale* Rosc.)-1part and *Saindhav lavana*-1part. *Panchsakar Churna* is used in the constipation, piles and other abdominal diseases [4, 5]. Sennosides found in *Panchsakar Churna* are stimulant purgatives which exert their action by increasing the smooth-muscle tone in the wall of the large intestine and have only minor effects on the small intestine [6, 7]. Gallic acid is a major component of ingredients of *Panchsakar Churna*, which has been widely used as herbal drug in constipation and anti ulcerogenic agent [8]. The need of new chemical entities for health care is explored and served through the plant sources [9]. Recently, High performance thin layer chromatography (HPTLC) has been widely employed for the quantification of secondary metabolites. The technique has been applied for the first time for the estimation of Sennoside B and Gallic acid in *Panchsakar Churna.* HPTLC has the advantage which provides identification of the analytes by in-situ spectrum scanning along with R_f comparison with the standard.

Materials and Methods

Sennoside B

Chemicals and Materials: Calcium Sennoside B (99% pure) marker was purchased from Yucca enterprises, Mumbai to carry out the quantification by HPTLC. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India. The HPTLC plates Silica gel 60 F 254 (20 cm 20 cm) were purchased from E. Merck KGaA (Darmstadt, Germany)

HPTLC instrumentation: The chromatographic estimation was performed by spotting standards and extracted samples on precoated silica gel aluminium plate 60F-254 (10 cm 10 cm with 250µm thickness, E. Merck, Darmstadt, Germany, supplied by Anchrom Technologies, Mumbai, India) using a Camag Linomat IV sample applicator (Camag, Muttenz, Switzerland) and a 100µl Hamilton syringe. The samples, in the form of bands of length 5mm, were spotted 12mm from the bottom, 12mm from left margin of the plate and 10mm apart, at a constant application rate of 15 sec/µl using nitrogen aspirator. Plates were developed using a mobile phase consisting of Ethyl acetate: Isopropanol: Formic acid (3:1.8:0.2v/v). Linear ascending development was carried out in 10

cm 10cm twin trough glass chamber (Camag Muttenz, Switzerland) equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 30 min at room temperature. The length of chromatogram run was 7.4cm approximately; 15ml of the mobile phase (7.5 ml in trough containing the plate and 7.5 ml in the other trough) was used for each development, which required 20 min. It results in better apparent resolution with more convenient capability of the detecting device to perform integration of peak area and peak height, Subsequent to the development, HPTLC plates were dried in a current of air with the help of an air-dryer. The slit dimension settings of length 4.00 mm and width 0.45 mm, and a scanning speed of 20 mm/s was employed. Densitometric scanning was performed on Camag TLC scanner III in the absorbance/reflectance mode at 254 nm and operated by winCATS Planar Chromatography Manager. The source of radiation utilized was Deuterium (D2) lamp. Concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light. Calculation was done via comparison of peak area with standard track. For spectrum scanning the slit dimension settings of length 4.00 mm and width 0.45 mm, and a scanning speed of 100 nm/s, absorbance/ reflectance mode at 200 to 700 nm was employed.

Development of the optimum mobile phase: The composition of the mobile phase for development of chromatographic method was optimized by testing different solvent mixtures of varying polarity. The mobile phase Ethyl acetate: Isopropanol: Formic acid (3:1.8:0.2v/v) gave good resolution, sharp and well defined peak at R_f value 0.50 for Sennoside B. Well defined spots were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature. Shown in Figure 1 and Figure 2.

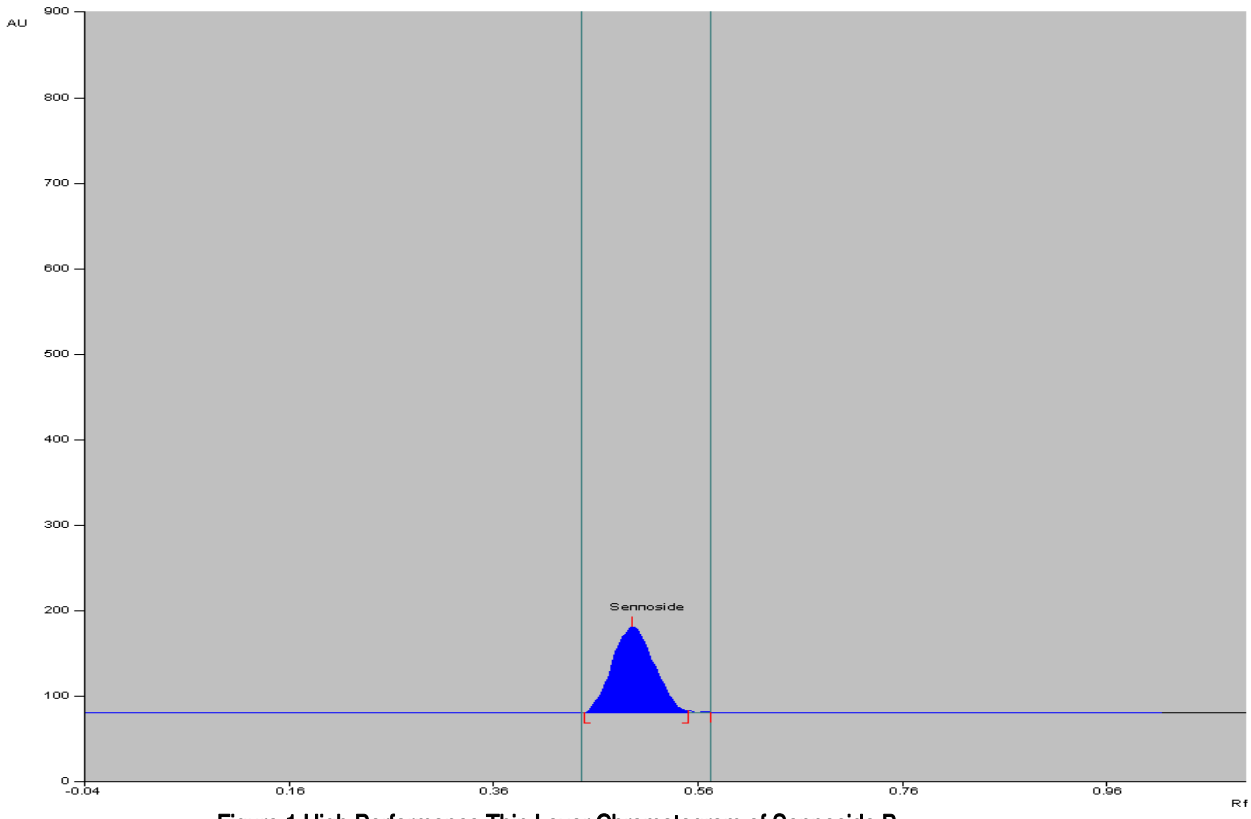


Figure 1 High Performance Thin Layer Chromatogram of Sennoside B

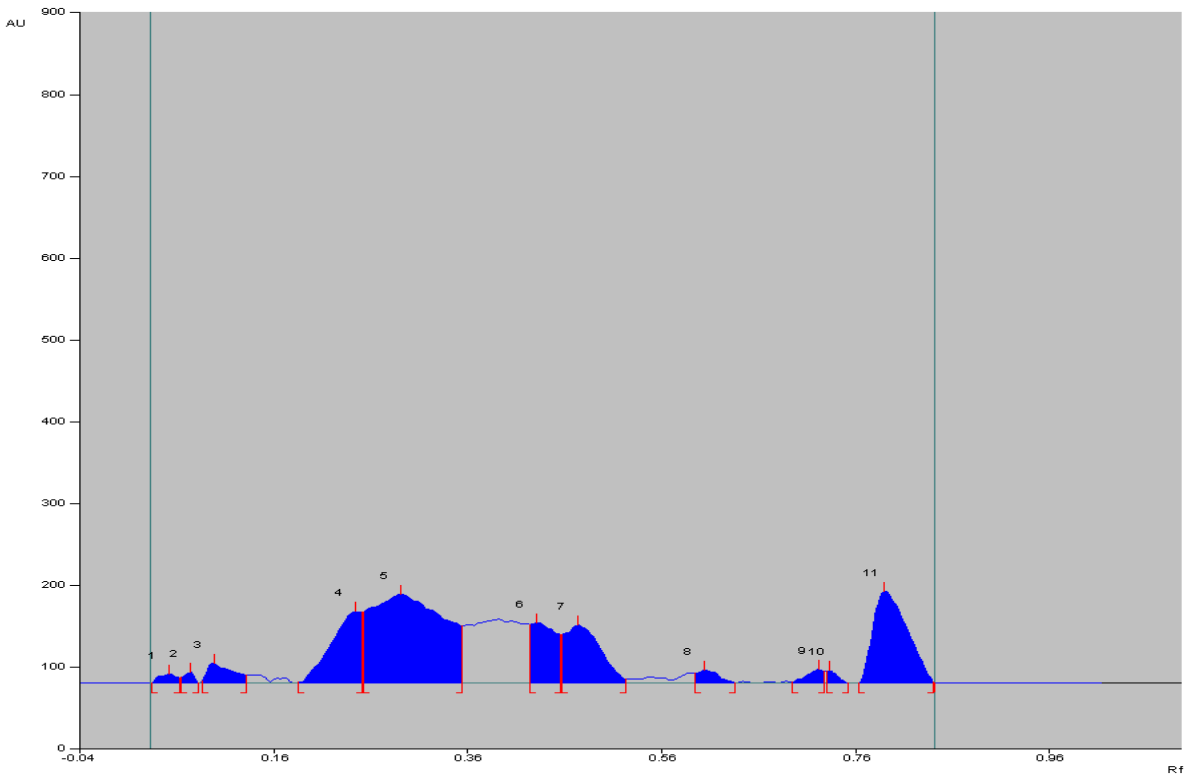
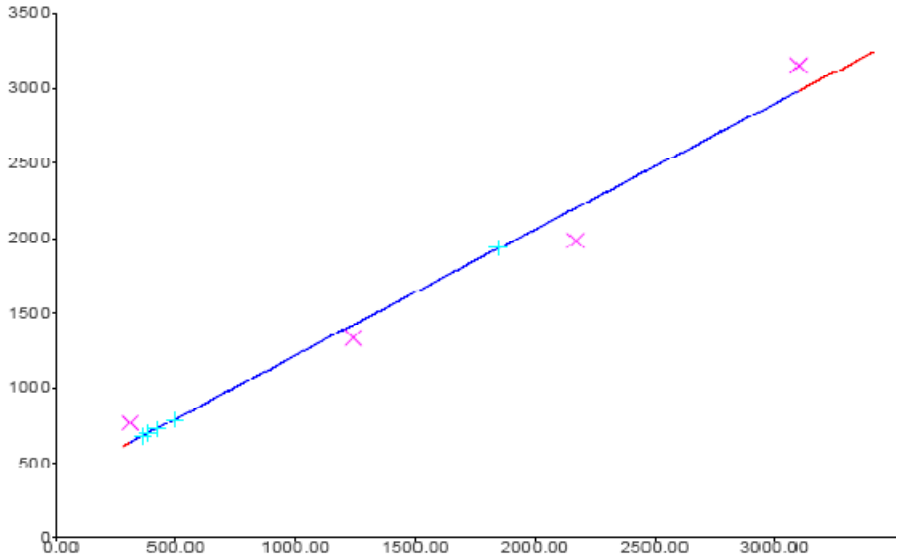


Figure 2 High Performance Thin Layer Chromatogram for Aqueous methanolic extract of Panchsakar Churna

Calibration plot of Sennoside B: A stock solution of 1.550mg/10ml of Sennoside B was prepared in methanol. Different volumes of stock solution, 1µl, 4µl, 7µl and 10 µl were spotted on HPTLC plate to obtain concentrations of 0.310, 1.240, 2.170 and 3.100 µg per spot of Sennoside B respectively. The data of peak area versus drug concentration were treated by linear least-square regression (Graph 1)



Graph 1: Calibration plot of Sennoside (peak Area versus drug concentration)

Analysis of Sennoside B in Samples: To determine the content of Sennoside B in the formulation the powder (150mg) was macerated with Aqueous methanol {(50%v/v) (10ml)} for 24 hours and filtered through whatman filter paper no. #1 and volume was

made up to 10 ml. The resulting solution was analyzed for Sennoside B content. The filtered solution was applied on HPTLC plate followed by development and scanning. Analysis was repeated in triplicate.

Purity of Spot in chromatogram: The spot obtained on the chromatogram were analysed at wavelength 254 nm at three points in the standard as well as in sample i.e. in the point start (S) to middle (M), middle and finally in the middle to end (E). Figure 3 shows the purity of the spot which is scanned at wave length 254nm with value of r (S, M) and r (M, E)

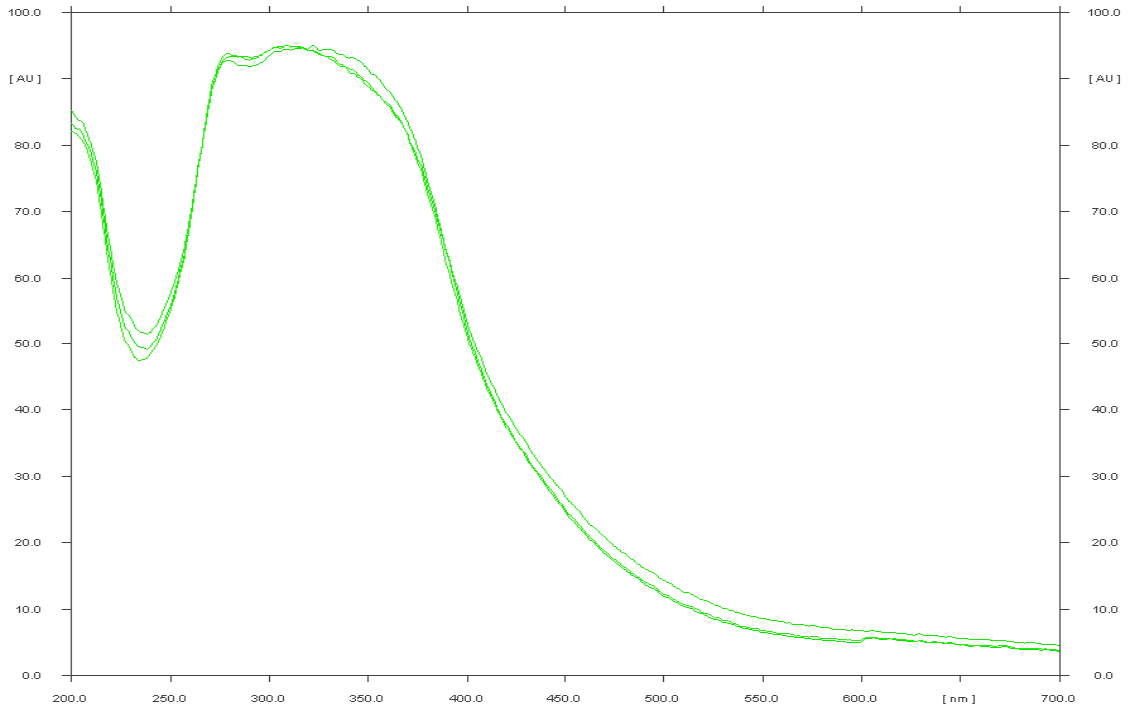


Figure 3 Purity spectra of Sennoside B.

Recovery Studies: The accuracy of the method was evaluated with recovery test. The sample solutions were prepared to contain Sennoside B and 30µg/ml standard Sennoside B were added. Development and scanning of the HPTLC plate was repeated in triplicate with same instrumental condition and solvent system.

Precision: Different amount of Sennoside B covering low, medium and higher ranges of the calibration curve were spotted on the HPTLC plate. These spots were analyzed by using above described HPTLC method. In order to control the scanner parameters, one spot was analyzed several times. By analyzing the same spot several times (n = 3) the precision of the scanning device and the HPTLC method was evaluated in terms of percentage Coefficient of Variation (%CV) (Table 1)

Table 1: Precision of the analysis for Sennoside B

Amount of Sennoside B spotted (µg)	Amount detected (µg) (Mean±S.D., *n*=3)	Precision (%CV)
0.310	0.4508±0.00915	2.029
1.240	1.1703±0.03020	2.580
2.170	1.8868±.023310	1.235
3.100	3.3120±0.00850	0.257

Sensitivity: The sensitivity of the method was determined with respect to LOD, LOQ, linearity range and correlation coefficient. Solutions containing 0.310-3.100µg of Sennoside B were spotted on HPTLC plate. The LOD was calculated as 3 times the noise level and LOQ was calculated as 10 times the noise level.

Gallic acid

Chemicals and Materials: Gallic acid (99% pure) marker was obtained from Natural Remedies Pvt. Ltd., Bangalore, India. All chemicals and reagents used were of analytical grade and were

purchased from Merck Chemicals, India. The HPTLC plates Silica gel 60 F 254 (20 cm 20 cm) were purchased from E. Merck KGaA (Darmstadt, Germany)

HPTLC instrumentation: The chromatographic estimation was performed by spotting standards and extracted samples on precoated silica gel aluminum plate 60F-254 (10 cm 10 cm with 250µm thickness, E. Merck, Darmstadt, Germany, supplied by Anchrom Technologies, Mumbai, India) using a Camag Linomat IV sample applicator (Camag, Muttenz, Switzerland) and a 100µl Hamilton syringe. The samples, in the form of bands of length 4 mm, were spotted 12 mm from the bottom, 10 mm from left margin of the plate and 10mm apart, at a constant application rate of 10 sec/µl using nitrogen aspirator. Plates were developed using a mobile phase consisting of Ethyl Acetate: Toluene: Formic acid (3.5:1:0.5 v/v). Linear ascending development was carried out in 10 cm 10 cm twin trough glass chamber (Camag Muttenz, Switzerland) equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 30 min at room temperature. The length of chromatogram run was 7.1 cm. approximately; 15ml of the mobile phase (7.5 ml in trough containing the plate and 7.5 ml in the other trough) was used for each development, which required 15 min. It results in better apparent resolution with more convenient capability of the detecting device to perform integration of peak area and peak height, Subsequent to the development, HPTLC plates were dried in a current of air with the help of an air-dryer. The slit dimension settings of length 4.00 mm and width 0.45 mm, and a scanning speed of 20 mm/s was employed. Densitometric scanning was performed on Camag TLC scanner III in the absorbance/ reflectance mode at 254 nm and operated by winCATS Planar Chromatography Manager. The source of radiation utilized was D2 and W lamp. Concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light. Evaluation was via peak heights and peak area with linear regression. For spectrum scanning the slit dimension settings of length 4.00 mm and width 0.45 mm, and a scanning speed of 100 nm/s, absorbance/ reflectance mode at 200 to 700 nm was employed.

Development of the optimum mobile phase: The composition of the mobile phase for development of chromatographic method was optimized by testing different solvent mixtures of varying polarity. The mobile phase Ethyl Acetate: Toluene: Formic acid (3.5:1:0.5 v/v) gave good resolution, sharp and well defined peak at R_f value 0.65 for Gallic acid. Well defined spots were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature. Shown in Figure 4.and Figure 5.

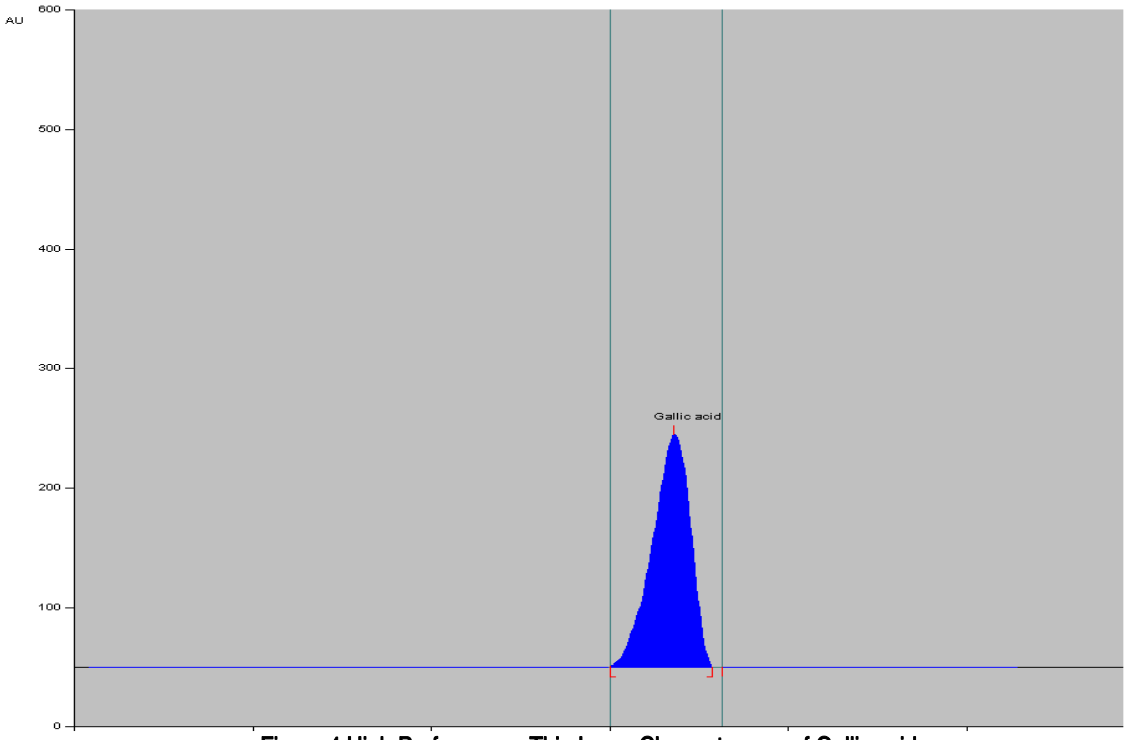


Figure 4 High Performance Thin Layer Chromatogram of Gallic acid

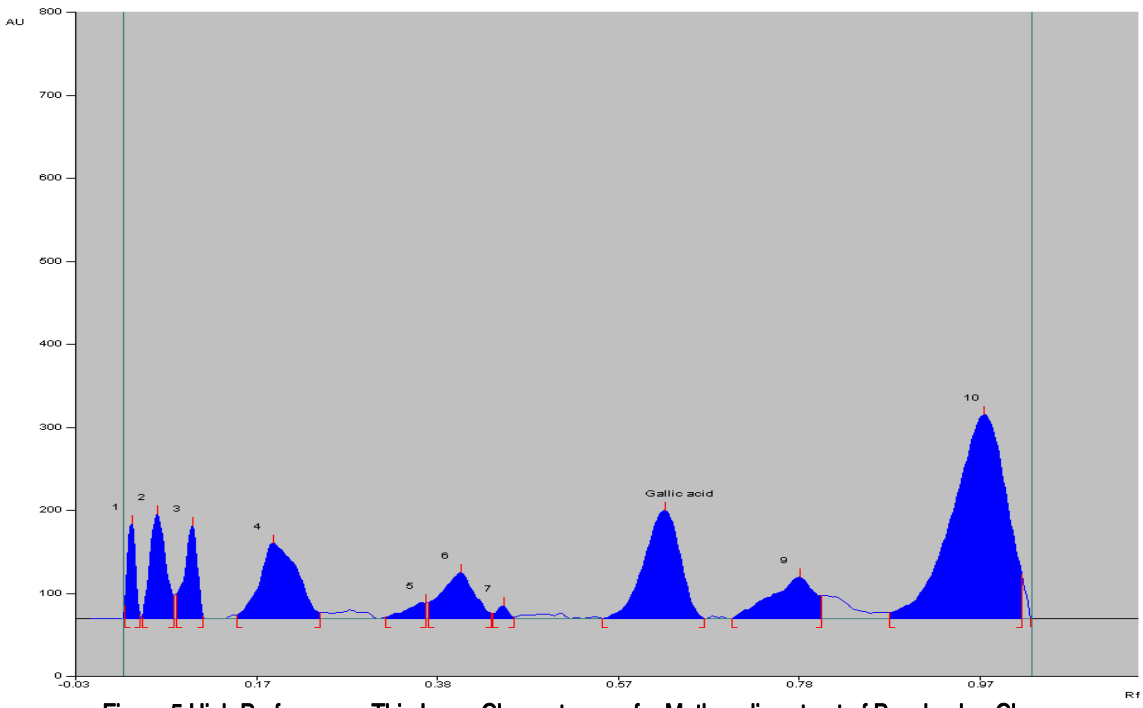
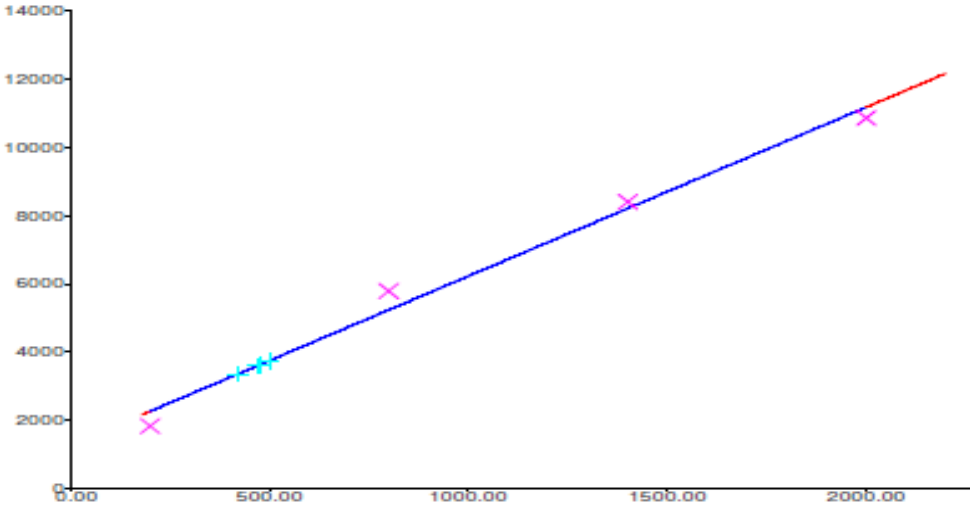


Figure 5 High Performance Thin Layer Chromatogram for Methanolic extract of Panchsakar Churna

A stock solution of 2.001mg/10ml of Gallic acid was prepared in methanol. Different volumes of stock solution, 1µl, 4µl, 7µl and 10 µl were spotted on HPTLC plate to obtain concentrations of 0.200, 0.800, 1.400 and 2.000 µg per spot of Gallic acid respectively. The data of peak area versus drug concentration were treated by linear least-square regression (Graph 2)



Graph2: Calibration plot of Gallic acid (peak Area versus drug concentration)

Analysis of Gallic acid in Samples: To determine the content of Gallic acid in the formulation, the powder (300mg) was macerated with methanol (10ml) for 24 hours and filtered through whatman filter paper no. #1 and volume was made up to 10 ml. The resulting solution was analyzed for Gallic acid content. The filtered solution was applied on HPTLC plate followed by development and scanning. Analysis was repeated in triplicate.

Purity of Spot in chromatogram: The spot obtained on the chromatogram were analyzed at wavelength 254 nm at three points in the standard as well as in sample i.e. in the point start to middle , middle and finally in the middle to end . Figure 6 shows the purity of the spot which is scanned at wave length 254nm with value of r (S, M) and r (M, E)

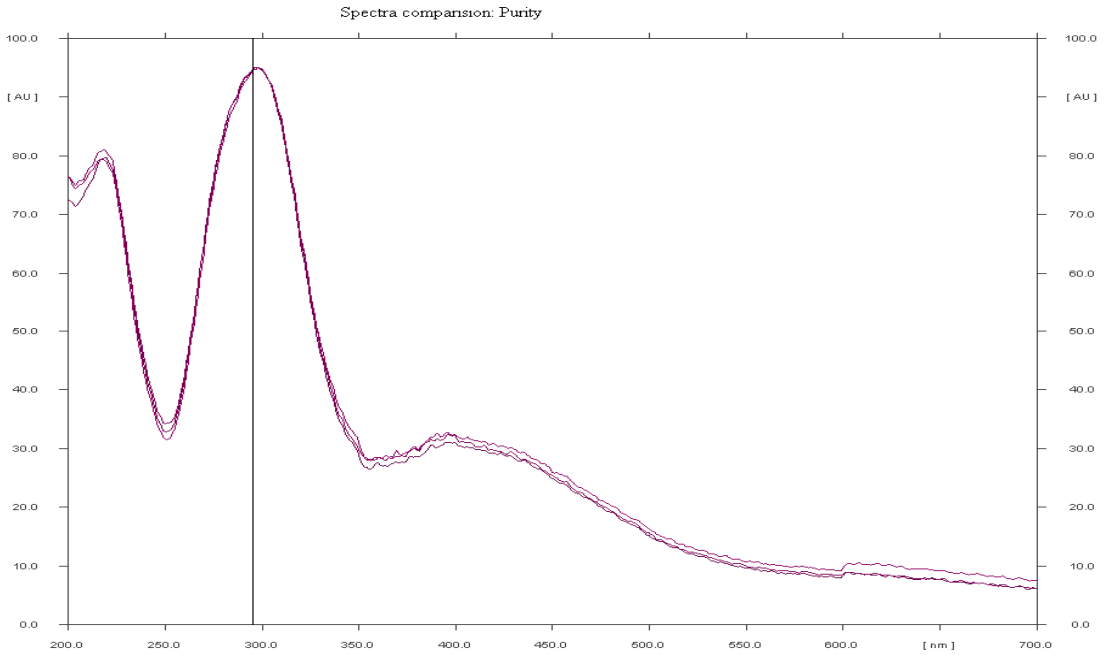


Figure 6 Purity spectra of Gallic acid

Recovery Studies: The accuracy of the method was evaluated with recovery test. The sample solutions were prepared to contain Gallic acid and 20µg/ml standard Sennoside B were added. Development and scanning of the HPTLC plate was repeated in triplicate with same instrumental condition and solvent system.

Precision: Different amount of Gallic acid covering low, medium and higher ranges of the calibration curve were spotted on the HPTLC plate. These spots were analyzed by using above described HPTLC method. In order to control the scanner parameters, one spot was analyzed several times. By analyzing the same spot several times (n = 3) the precision of the scanning device and the HPTLC method was evaluated in terms of percentage Coefficient of Variation (%CV) (Table 2)

Table 2: Precision of the analysis for Gallic acid

Amount of Gallic acid spotted (µg)	Amount detected (µg) (mean±S.D., *n*=3)	Precision (%CV)
0.200	0.2090±0.00415	1.990
0.800	0.8674±0.01910	2.201
1.400	1.4337±0.00610	0.425
2.000	1.9481±.01100	0.564

Sensitivity: The sensitivity of the method was determined with respect to LOD, LOQ, linearity range and correlation coefficient. Solutions containing 0.200-2.00 µg of Gallic acid were spotted on HPTLC plate. The LOD was calculated as 3 times the noise level and LOQ was calculated as 10 times the noise level.

Results

Calibration curves shows a good linear relationship over the concentration range 0.310-3.100 µg/ spot and 0.200-2.000 µg/spot with respect to peak area (n=3) for respectively. No significant difference was observed in the slopes of standard curves.

Calibration curves show a good linear relationship over the selected concentration range. No significant difference was observed in the slopes of standard curves. Regression value for Sennoside B and Gallic acid were found to be 0.9835 and 0.99290 respectively at 254nm.

Analysis of Sennoside B and Gallic acid in Samples

A single spot at R_f 0.50 was observed in the chromatogram of the aqueous methanolic extract and 0.65 was observed in the chromatogram of the methanolic extract of the formulation respectively for Sennoside B and Gallic acid. There was no interference from other constituents in the formulation. The Sennoside B content was found to be 4.4088, 6.5143, 5.6075 and 5.3656µg/gm; Gallic acid content was found to be 3.6061, 3.0787, 3.4647 and 3.3869µg/gm in formulation IH, M1, M2 and M3 respectively on the basis of peak area and overall average Sennoside B content for all formulation was found to be 5.4740±0.5546µg/gm and Gallic acid content was found to be 3.3841±0.1548µg µg/gm of Panchsakar Churna.

Purity of the Spot in chromatogram

Purity of the spot which is scanned at wave length 254nm with value of r (S,M) within the range 0.9995-0.9999 and 0.9996-0.9998; r (M,E) within the range 0.9984-0.9997 and 0.9977-0.9994 respectively for Sennoside B and Gallic acid.

Linearity

The calibration curve was found to be linear in the range of 0.310-3.100µg and 0.200-2.00 µg respectively for Sennoside B and Gallic acid. Peak area and concentration was subjected to least-square linear regression analysis to calculate the calibration equation and correlation coefficients. The regression data shows a good linear relationship over the concentration range, which were 0.9835 and 0.9969 respectively for Sennoside B and Gallic acid.

Accuracy

After recovery studies for Sennoside B, accuracy of the method were found to be 100.330%, 99.909%, 100.171% and 100.180% in formulation IH, M1, M2 and M3 respectively; while 100.383%, 99.930%, 99.690% and 100.562% in formulation IH, M1, M2 and M3 respectively for Gallic acid.

Precision

Precision values were found to be in the range of 0.257-2.580 and 0.425-2.201 for respectively for Sennoside B and Gallic acid in terms of %CV.

Sensitivity

Under the experimental conditions employed, the lowest amount of drug which could be detected (LOD) was found to be 73.7634 ng/spot and 50.1556 ng/spot, the lowest amount of drug which could be quantified (LOQ) was found to be 245.8781ng/spot and 167.1853 ng/spot on the basis of peak area under the curve for Sennoside B and Gallic acid respectively.

Conclusion

Sennoside B and Gallic acid are the major constituents and the main pharmacologically active principles in Panchsakar Churna. The analytical method developed in this study is specific for sennosides B and Gallic acid, which allowed the quantification of these two compounds. Because of the strict requirement of extensively validated method by regulatory authorities, linearity, accuracy, precision, sensitivity and purity testing is now widely applied in the pharmaceutical world. Results of these validation parameters showed that the method was susceptible enough to standardize the Panchsakar Churna; the developed method can be used for routine standardization of Panchsakar Churna.

Acknowledgement

Authors are thankful to Birla Institute of Technology, Mesra, Ranchi for providing the research facility to carry out the research work.

References

[1]. Elamthuruthy AT, Shah CR, Khan TA, Tatke PA, Gabhe SY. Standardization of marketed *Kumariasava* an Ayurvedic *Aloe vera* product, J of Pharm and Biomed Anal. 2005; 37: 937–941. (url: http://www.sciencedirect.com/science/article/pii/S0731708504006326)

[2]. Mukherjee PK. Quality control of Herbal Product, 1st ed. New Delhi, India, Buisness Horizons Ltd., 2002. p. 6-7.

[3]. Kumar P, Jha S, Standardization of an Ayurvedic powdered formulation by modified Lycopodium spore and Spectrophotometric method, Ind J Trad Knowledge. 2011; 10(4): 604-607. (url: http://nopr.niscair.res.in/bitstream/123456789/12817/4/IJTK%2010%284%29%20604-607.pdf)

[4]. Pathak RR. Ayurved Sarsangrah. 12th ed. Allahbad, India: Shree Baidyanath Ayurved Bhawan Ltd.; 2003. p. 589.

[5]. Kumar P, Jha S, Naved T. Pharmacognostical characterization of an Ayurvedic powdered formulation: Panchsakar Churna, Int J Res in Pharm and Chem. 2011; 1(4): 1034-1041. (url: http://www.ijrpc.com/files/000035.pdf)

[6]. Shao WS, Hsiu TS. Validated HPLC method for determination of sennosides A and B in senna tablets, J of Pharm and Biomed Anal. 2002; 29: 881–894. (url: http://www.sciencedirect.com/science/article/pii/S073170850200208X)

[7]. Bhattacharjee SK. A handbook of medicinal plant, Jaipur, India, Pointer Publisher; 2004. p. 74-79.

[8]. Jeganathan NS, Kannan K. HPTLC method for estimation of Ellagic acid and Gallic acid in Triphala churnam formulations, Res J of Phytochem. 2008; 2(1): 1-9. (url: http://scialert.net/qredirect.php?doi=rjphyto.2008.1.9&linkid=pdf)

[9]. Mukherjee PK, Wahile A. Integrated approaches towards drug development from Ayurveda and other Indian system of medicine, J of Ethnopharmacol. 2006; 103(1): 25-35. (url: http://www.sciencedirect.com/science/article/pii/S0378874105006525)