**Pharm Methods, 2019; 10(1): 21-26.**
A multifaceted peer reviewed journal in the field of Pharm Analysis and Pharmaceutics
www.phmethods.net | www.journalonweb.com/phm

*Original Article*

# High-performance Thin Layer Chromatography Method Development and Validation for Simultaneous Determination of Phenolic Acids in Selected Indian Bamboo Species

**Jayanta Kumar Maji, Mansi Patel, Snehal Patel, Shital Butani, Priti Mehta***

Department of Pharmaceutical Analysis, Institute of Pharmacy, Nirma University, Ahmedabad, Gujarat, INDIA.

## ABSTRACT

**Background:** Indian bamboo species like *Bambusa arundinaceae* Retz, *Bambusa vulgaris, Schard* and *Dendrocalamus strictus* Roxb is sucessively proved in various pharmacological activities like antihyperglycemic, anti-diabetic, anti-cancer, anti-inflamatory, anti-obesity, anti-fatigue, anti-lipidemic and cardiovascular diseases. Hence, there is a need to optimize selective and sensitive methodologies using sophisticated analytical equipment to accurately quantify the levels of bioactive compounds such as phenolic acids in Indian bamboo species. **Material and Method:** In the present study to decide the robustness of HPTLC analytical method by the factorial design; analyze the four factors (developed distance, saturation time, mobile phase ratio, band length) in terms of in order which associated to the main factors and interaction effects; determine method development various parmeters (precision, accuracy, lineraty, limit of detetion, limit of quantification, etc). **Results:** Phenolic acids chromatographed on top of silica gel 60 $F_{254}$ TLC plates using toluene: ethyl acetate: formic acid: methanol (3:3:0.6:0.8 v/v/v/v) as optimized mobile phase. A prominent spot for chlorogenic acid (ChA), gallic acid (GA), caffeic acid (CA) and ferulic acid (FA) was simultaneously observed with retardation factor ($R_f$) 0.15 ± 0.02, 0.57 ± 0.03, 0.78 ± 0.01, 0.87 ± 0.01 when the densitometric scanning was implemented at 280nm.The linearity for the calibration plots showed $r^2$ = 0.999 with concentration from (100-700 ng/band) for ChA and (50-350 ng/band) for GA, CA, FA simultaneously. **Conclusion:** Developed method successfully employed for the simultaneously quantification of selected Indian Bamboo- species.

**Key words:** Phenolic acids, Factorial design, Bamboo species, HPTLC, Validation.

**Correspondence**
**Dr. Priti J Mehta**
Department of Pharmaceutical Analysis, Institute of Pharmacy, Nirma University, Ahmedabad- 382470, Gujarat, INDIA.
Phone no: +91-9898335567
E-mail: drpritimehta@nirmauni.ac.in
DOI: 10.5530/phm.2019.1.4

## INTRODUCTION

Currently, a number of application have been accomplished on bioactive compounds like phenolics and flavonoids because of several health profit to human beings. The researcher has recommended that a primary root of the never-ending ailments is noticeable to free radical attacks on biomolecules.[1] Thus, plants have been used as an alternative resources because of appreciable amount of natural antioxidant. A natural antioxidant like phenolic acids, such as Chlorogenic acid (ChA) [3-(3,4- dihydroxycinnamoyl) quinic acid], Gallic acid (GA) [3,4,5 trihydroxy benzoic acid], Caffeic acid (CA) [3,4-dihydroxy cinnamic acid] and Ferulic acid (FA) [4-hydroxy-3-methoxy cinnamic acid] (Figure 1) are various biological activity like anti-inflamatory, anti-bacteria, anti-fatigue, anti-mutagenic, anti-fatigue, anti-carcinogenic and radioprotective due to its strong antioxidant and free radical scavenging power.[2-4] Alternatively, various bamboo species reservoir like India (2nd largest production in world after China) now had taken as National Bamboo Mission under the Ministry of Agriculture for selected species like *Bambusa arundinaceae* Retz, *Bambusha vulgaris* Schard and *Dendrocalamus strictus* Roxb due to its pharmacological activities like antihyperglycemic, anti-diabetic, anti-cancer, anti-inflamatory, anti-obesity, anti-fatigue, anti-lipidemic and cardiovascular disease and among others.[5] Bamboo species belongs to the family Poaceae which is the largest genus (70 genera) with close to 1000 species but 130 species growing in India.[6] On the other hand, a literature assessment reflects the phenolic acids fingerprinting pattern in the context of plannar chromatography numerous way like, optimization, validation, simultaneous determination successfully.[7-11] But robustness test is not done simultaneous analytical method development for four antioxidants in favor of various design methodologies "Design of Experiment", like fractional factorial design, central composite design, full factorial design supporting graphical interpretation such as normal probablity plot, pareto chart and response surface method.

As a part of long term research effort aimed at establishing a suitable solvent system for optimizing the separation of four phenolic acids, method development and towards validation for ICH guidelines. This paper presents a detailed study on the simultaneous quantification of phenolic acids from Indian bamboo species. The purpose of this study were to decide the robustness of HPTLC analytical method by the factorial design; analyze the four factors (Developed distance, saturation time, mobile phase ratio, band length) in terms of in order which associated to the main factors and interaction effects; determine method development various parmeters (Precision, accuracy, lineraty, limit of detetion, limit of quantification, etc); establish a reliable HPTLC method for routine determination phenolic acids from Indian bamboo species.

## MATERIALS AND METHODS

### HPTLC Instrumentation

Automatic sample injector (Linomat 5, 100 µl Hamilton syringe, Camag, Switzerland), scanner 3, win CATS planar chromatography manager software version 1.4.2.8.21 (All form CAMAG, Muttenz, Switzerland, UV chamber (Camag, Switzerland), Stationary phase: Aluminium backed precoated silica gel 60 $F_{254}$ (20 × 10 cm, thickness, Merck, Darmstadt, Germany) were used in the study.

### Chemical

Working standard of GA, FA, ChA, CA were purchased from SRL, Gujarat, India. All reagents and chemicals used procured from MERCK specialties Pvt. Ltd., India. Indian bamboo species used in this study was procured from the Dang Forest in the month of September 2018. The plant herbarium authentified (Voucher number- SR- x1, x2, x3/2019) by

Dr. Hitesh A Solanki, Department of Botany, University School of Sciences, Gujarat University, Ahemadabad-38009. Specimen of each drug has been submitted to Pharmacognosy laboratory in Institute of Pharmacy, Nirma University, Gujarat for further reference.

## Preparation of Standard Solution and Investigation of Linearity Curve

Primary standard solution prepared by weighing 10mg of the respective phenolic acid class (ChA, GA, CA, FA) transferred into volumetric flask of 10 ml and dissolved and marked with methanol to obtain concentration 1mg/ml. The final mix working solutions planned by diluting appropriate aliquots of the main standard solutions with methanol into four different sets of 10 ml volumetric flask to reach the concentration ranges 50-350 ng/ml for GA, FA, CA and ChA 100-700 ng/ ml respectively. The standard volume applied to the HPTLC plates in pre assigned experimental condition (10 mm bottom and 15 mm side edges, band length 6 mm). The mobile phase be contained in toluene: ethyl acetate: methanol: formic acid (6:6:1.6:1.2, v/v/v/v) and developed distance was 8.5cm, saturation time for 20 mins before each run. Developed HPTLC plates dried up in a air dryer. The scanning was observed with instrumental densitometric condition like, slit dimension (6.0 × 0.30 mm, micro), scanning speed 20 mm/s, data resolution 100 µm/step, optical filter (Second order), filter factor (Savitsky goloy 7). Evaluation was via peak areas with linset function.

## Analytical Validation

The method acessed in accordance with ICH guidelines Q2 (R1) for evaluation of various statistical parameters.[12] Method repeatability established from RSD values by repeating the process according to guidelines in both inter and intraday in three different concentration level. Accuracy study (% Recovery) performed by reanalyzing samples of three spiked concentration level with (80%, 100%, 120%) and expressed as Relative standard deviation (RSD, %).The sensitivity test done in terms of Detection and Quantification Limit (DL and QL). The DL and QL appraised using the formula DL = 3.3* N/B and QL = 10*N/B; where, 'N' is standard deviation of peak areas of the drug ($n$=3) taken as a measure of noise and 'B' is the slope of corresponding calibration curve. Specificity test confirmed by analyzing and comparing the $R_f$ values and spectra of the spots and it was ascertained by analyzing peak purity of standard drug and bamboo species.

## Experimental design methodology for robustness

To study of the robustness and ruggedness of this proposed method, two level factorial design (FD) applied; four factors full factorial design (24). In the ongoing experiment, four factors were preferred based on the chromatographic intitution and experienced gained during trial error, development Distance (A), saturation time (B), volume in methanol in mobile phase ratio (C), band length (D). All experiments investigated is randomized order to reduce the bias effect of uncontrolled factors according to the experimental sphere of the selected variables. The experiments were performed based on the experimental domain and the responses were recorded in the form of retardation factor of ChA, GA, CA and FA to check the robustness of the method.

## Preparation of Bamboo Species Sample[13]

The extraction procedure for the free and bound phenolic acids for the bamboo species was slightly modified in two different way. Every bamboo species containing {First way- (A)} sample (1g) was agitated with 10 ml (Ethanol -80%) solvent for fifteen minute at 25°C. The extract was centrifuged (10 min, 10,000 rpm and 40°C) and then supernatant was evaporated to dryness using a temperature controlled water birth. The sample was redisollved in 5ml 50% eathanol.

In the 2nd way (B) the residue pellet was dissolved 10ml 2M NaOH and vortexing (3000 rpm, 10 min). The mixture was incubated (1.5 hr, 90°C) with constant agitation at 500 rpm. Afterwords the resultant mixture acidified with 14 ml of 2M HCl under control pH (2-3). The acidified extract was defatted by adding 12 ml n-hexane followed by vortexing (5 min, 2500 rpm) and incubating (25°C, 10 min, 500 rpm). The upper layer hexane was removed after centrifugation (10min, 10,000 rpm). The remaining mixture was dissolved in ethyl acetate (10 ml, at 3000 rpm) and incubated at 25°C for 10 min with constant agitation 500 rpm. The upper layer of ethyl acetate was recovered after centrifugation (10 min, 10,000 rpm) for bound phenolic acids. Ethyl acetate layer was evaporated for dryness in an temperature control water birth. The sample was resuspended in 5 ml 50% eathanol.

## Statistical Tools

Experimental design for robustness study was performed using Design Expert (Versions 11.0.5.0.64-bit), Stat- Ease Inc., Minneapolis, MN, USA statistical software. The remaining calculation for the analysis (Method validation) excuted use of Microsoft Excel 2010 software (Microsoft-USA).

# RESULTS AND DISUSSION

## Development of Optimum Mobile Phase

To obtain high resolution and reproducible peaks, various mobile phase ratio were experimented. The important parameters were found optimum with employ of toluene: ethyl acetate: formic acid: methanol (3:3:0.6:0.8 v/v/v/v) as a mobile phase. The wavelength of 280 nm was selected to be optimal for the simultaneous marker sensitivity. A sharp and well resolved peak was obtained for chlorogenic acid, gallic acid, caffeic acid and ferulic acid at 0.15 ± 0.02, 0.57 ± 0.03, 0.78 ± 0.01, 0.87 ± 0.01 applying 30 min chamber saturation of the respective mobile phase.

## Linearity and Calibration Curve

The linear curve constructed was assessed by its correlation coefficient. In the elevated range of volume (2-14 µl ) solution are applied on HPTLC plate to get concentration in the linear concentration ranges 50-350 ng/ band for GA, FA, CA and ChA 100-700 ng/ band respectively. The linearity of four respective marker was shown in (Figure 2). The calibration curved was determined by linear regression analysis in the favor of sketching peak area against drug quantity per separated brand. The

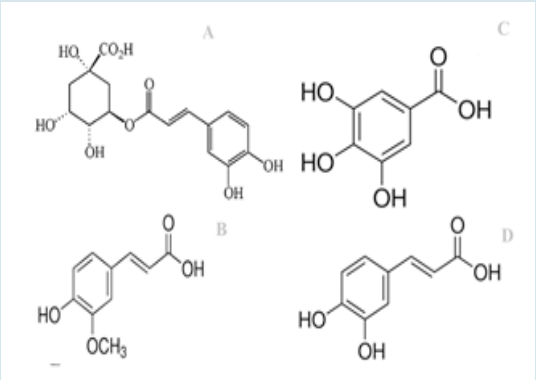



**Figure 1:** The respective phenolic acid image, A = Chlorogenic acid, B = Gallic acid, C = Caffeic acid and D = Ferulic acid.

high correlation coefficients ($r^2$) value indicated of the respective markers (Table 2), no significant differences was observed in the slope of standard curve. The HPTLC plate and chromatogram of respective sample was shown in (Figure 3).

**Table 1: Full Factorial Design Consisting of Sixteen Experiment Runs.**

| Runs | Development Distance (cm) | Saturation time (min) | Mobile phase ratio (ml) | Band length (mm) | Retention factor of Chlorogenic acid | Retention factor of Gallic acid | Retention factor of Caffeic acid | Retention factor of Ferulic acid |
|---|---|---|---|---|---|---|---|---|
| 1 | 7 | 20 | 3.2 | 6 | 0.25 | 0.75 | 0.86 | 0.93 |
| 2 | 7 | 20 | 1.6 | 6 | 0.08 | 0.62 | 0.74 | 0.8 |
| 3 | 7 | 20 | 1.6 | 4 | 0.06 | 0.58 | 0.7 | 0.75 |
| 4 | 9 | 30 | 1.6 | 4 | 0.12 | 0.62 | 0.73 | 0.78 |
| 5 | 7 | 30 | 3.2 | 6 | 0.14 | 0.71 | 0.80 | 0.84 |
| 6 | 9 | 30 | 1.6 | 6 | 0.10 | 0.63 | 0.76 | 0.83 |
| 7 | 9 | 20 | 3.2 | 6 | 0.14 | 0.64 | 0.8 | 0.94 |
| 8 | 9 | 20 | 1.6 | 4 | 0.09 | 0.62 | 0.74 | 0.8 |
| 9 | 9 | 30 | 3.2 | 4 | 0.29 | 0.78 | 0.89 | 0.98 |
| 10 | 9 | 30 | 3.2 | 6 | 0.03 | 0.68 | 0.85 | 0.97 |
| 11 | 7 | 30 | 3.2 | 6 | 0.2 | 0.76 | 0.85 | 0.89 |
| 12 | 9 | 20 | 1.6 | 6 | 0.28 | 0.82 | 0.89 | 0.92 |
| 13 | 7 | 30 | 1.6 | 6 | 0.19 | 0.52 | 0.79 | 0.9 |
| 14 | 7 | 20 | 3.2 | 4 | 0.34 | 0.73 | 0.88 | 0.95 |
| 15 | 9 | 20 | 3.2 | 4 | 0.27 | 0.72 | 0.84 | 0.90 |
| 16 | 7 | 30 | 1.6 | 4 | 0.2 | 0.54 | 0.83 | 0.93 |

**Table 2: Analytical Validation Parameters of Proposed HPTLC Method for Simultaneous Estimation of ChA, GA, CA and FA.**

| Analytical parameters | ChA | GA | CA | FA |
|---|---|---|---|---|
| Calibration range [a] (ng/band) | 100-700 | 50-350 | 50-350 | 50-350 |
| Regression equation | 6.492x + 658.4 | 19.84x + 321.4 | 17.68x + 427.1 | 11.58x -297.1 |
| Coefficient of determination ($r^2$) | 0.996 | 0.990 | 0.998 | 0.990 |
| Standard deviation of slope | 0.5429 | 0.7982 | 0.8195 | 0.4948 |
| Confidence limit of slope [b] | 5.164 -6.493 | 18.892 -19.843 | 15.67 - 17.69 | 10.32-11.53 |
| Standard deviation of intercept | 242.77 | 178.491 | 183.2 | 110.64 |
| Confidence limit of intercept [b] | 594.03-658.41 | 321.41-436.75 | 427.48-448.39 | 110-270 |
| Limit of detection (ng/band) | 5.1847 | 14.78 | 13.26 | 56.64 |
| Limit of quantification (ng/band) | 15.711 | 44.88 | 40.19 | 171.63 |
| Precision study | | | | |
| Repeatability | | | | |
| Interday precision (% RSD) | 1.42 | 0.019 | 1.25 | 0.573 |
| Intraday precision (% RSD) | 1.52 | 0.035 | 1.30 | 0.95 |
| Accuracy (% of recovery) | 95-108 | 92-109 | 95-105 | 94-109 |

HPTLC = high performance thin layer chromatography; ChA = Chlorogenic acid, GA = Gallic acid, CA = Caffeic acid, FA = Ferulic acid

[a] Mean of three determination

[b] Confidence interval at 95% confidence level and four degree of freedom

[c] $n$ = 3 replicates

## Method validation

### *Precision and Accuracy*

Repeatability, precisions (Inter and intraday) were observed performing three time measurements in respetive target concentration level. The precision of establish method was calculated in terms of % RSD of peak area. The precision result was showed here (150ng, 200 ng, 250 ng) for GA, CA, FA and (300 ng, 400 ng, 500 ng) % RSD < 2% respectively. (Table 2), expressing acceptable precision in terms of repeatability both Interday and intraday. Accuracy study was executed by standard addition method using three different levels. Recovery experiment was evaluated by over spotting the drug standard at 80 %, 100 % and 120 % to the pre- analyzed sample and the results were re-analyzed by proposed HPTLC method percentage recovery at all three levels in the range of (95-103.97%), signifying the suitability and applicability of method for routine use of drug analysis (Table 2).

### *Detection Limit (DL) and Quantification Limit (QL)*

The determination of DL and QL was based on the standard deviations of the responses and slopes of constructed calibration curves ($n$ = 3) as described by ICH guidelines Q2 (R1). Limit of detection for ChA, GA, CA and FA were found to be 5.1847 ng/band, 14.78 ng/band, 13.26 ng/band and 56.64 ng/band respectively. Limit of quantification for ChA, GA, CA and FA were found to be 15.711ng/band, 44.88 ng/band, 40.19 ng/band and 171.63 ng/band respectively is indicating good sensitivity of the method.

## Robustness and Design Analysis for Robustness

Two level Factorial design (FD) comprising a total sixteen experiment runs acheived from the design matrix were subjected to experiment in order to generate the response variables (y1,y2,y3,y4) shows in Table 1. All experimental runs were carry outed in randomized order to minimize the effects of uncontrolled factors that may introduce biased responses. Rather than analysis of single coefficient whole model equation was used and for response surface analysis; crucial focus was given to factors whose responses are with or without significance and are considered too. Graphical projection in form of response surfaces showed the correlation of the effect of the factors on the $R_f$ vlues (Table 3). The pareto chart is useful for the investigaing the significance of factors, where effects above the Bonferroni limit are almost certainly significant and the effect above the $t$-value limit are possibly significant and effect below the $t$-value limit are not likely to be signifiant.The Pareto chart for all four drugs reveals that volume of methanol in mobile phase had important effects on retention factor of drugs, in decreasing order C > AC > ACD > DC < CD < A for GA, as shown in Figure 4 E. In three dimensional response surface plots clearly shown increase in methanol content and saturation time had important effects to increase the $R_f$ of all four drugs as shown in (Figure 4, 5). The equation in terms of coaded factors can be used to make predictions regarding the response for given levels of each factor. The coaded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. The statistical finding (Table 3) of the model, $p$ value > 0.05, signal to noise ratio > 4 (Adequate precision), simultaneously the low standard deviation indicates a good relationship between the experimental data and those of the fitted models.

## Assay Study of Indian Bamboo Species

The Indian bamboo species (*Bambusa arundinaceae*, *Bambusa vulgaris*, *Dendrocalamus strictus*) extracts (Free -A, Bound -B) was observed the $R_f$ in the hromatogram in applied developed HPTLC methods. The quantification of the respective phenolic acids is explained in Table 4.

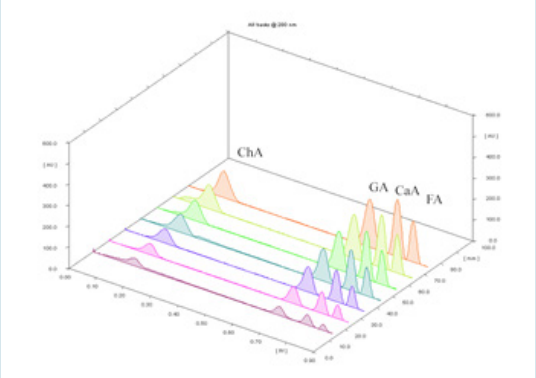



**Figure 2:** 3D chromatogram of 100, 200, 300, 400, 500, 600 and 700 ng/spot ChA and 50, 100, 150, 200, 250, 300 and 350 ng/spot GA, CaA, FA for each Concentration Detected at 280nm.

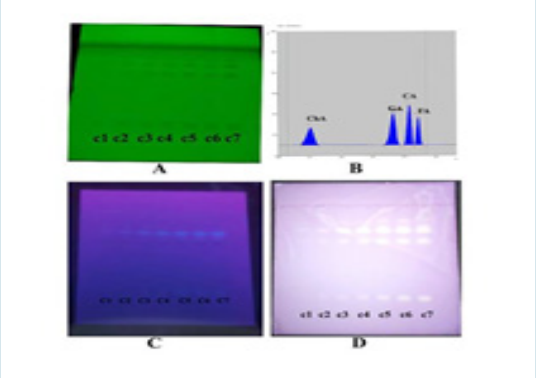



**Figure 3:** Separation Results of Phenolic Acids (A) Short UV (C) Long UV (D) DPPH Visualizing Agent and Corresponding Densitogram by 280nm.

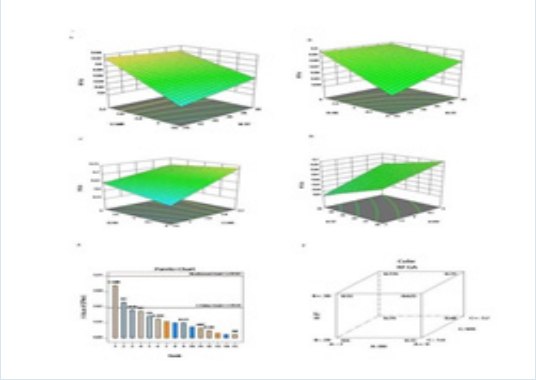


**Figure 4:** Three-Dimensional Response Surface Plot Showing Effect of Factor on $R_f$ Values of FA (A) and (B), GA (C) and (D), (E) and (F) Paretochart and Cube of GA Showing the Interaction of Various Design Factor. FA= Ferulic Acid, GA = Galic Acid.

## CONCLUSION

The method was consecutively established and robustness determination through DOE. Application of full factorial design was applied for simul-

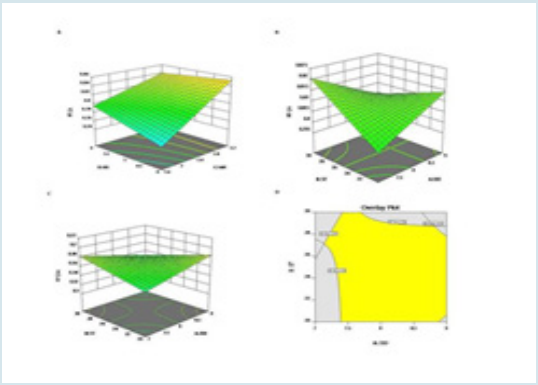


**Figure 5:** Three- Dimensional Response Surface Plot Showing Effect of Factor on Rf Values of CA (A) and (B), ChA (C) and (D) Overlay Plot Showing the Interaction of Various Design Factor. CA= Caffeic Acid, ChA = Chlorogenic Acid.

taneously determination of robusness method displayedslight changes in different factor such as saturation time, mobile phase ratio, develoed distance, band length used a prominent effector on a retardation factor. The developed method can be applied for regular analysis of Indian bamboo species in favor of phenolic acid determination.

## ACKNOWLEDGEMENT

The authors gratefully acknowledge Ministry of Ayush, Govt. of India for funding these research work (Z.28015/21/2016-HP(EMR)-AYUSH-A). The authors are thankful to Institute of Pharmacy, Nirma University, Ahmedabad, India for providing necessary facilities to carry out the research work.

## CONFLICT OF INTEREST

The author(s) declared no potential conflict of interest with respect to the research, authorship, publication of the article.

**Table 3: Predicted Response Models and Statistical Parameters by ANOVA Analysis.**

| Response ($R_f$ value) | Polynomial equation model for retardation factor | Model *p* value | Standard deviation | Adequate precision |
|---|---|---|---|---|
| ChA | 0.1669 + 0.0006A - 0.006B + 0.0156C -0.0006D-0.0169AB +0.0019AC -0.0019BC +0.0069BD-0.0044CD + 0.0244ABC +0.0181BCD | **0.0213** | 0.0152 | 9.49 |
| GA | 0.67 + 0.0187A -0.015B + 0.05125C + 0.0007D + 0.00375 AB -0.035AC - 0.00375AD + 0.02625BC - 0.0215CD + 0.01ABC - 0.0275ACD | **0.0455** | 0.0485 | 6.83 |
| CA | 0.8094 + 0.0031B +0.0369C + 0.0081D - 0.0081AB -0.0044AC + 0.0044AC - 0.0081BD-0.0144CD + 0.0319ABC - 0.0181ACD + 0.0169BCD -0.0019ABCD | **0.0253** | 0.0180 | 11.34 |
| FA | 0.8819 + 0.0081A + 0.0081B + 0.0431C + 0.0156D + 0.0144AC + 0.0094AD -0.0131BC - 0.0081BD + 0.0406ABC - 0.0069ABD - 0.0094ACD + 0.0106BCD | 0.0852 | 0.0325 | 3.69 |

**Table 4: The Respective Phenolic Acids Present in Different Species Bamboo Samples.**

| Samples (For 80% ethanolic extract) (A); (Purified ext.) (B) | % yield of extract | Concentration prepared | Marker determined (ng/spot) | % marker in extract | % of marker in raw powder (gm) |
|---|---|---|---|---|---|
| *Bambusa arundinaceae* (A) | 2.28 | 15.44mg/ml | ChA- 942.514<br>GA- 3.6134<br>FA- 660.937 | ChA- 4.13<br>GA-0.01<br>FA-2.89 | ChA-0.0941<br>GA-0.0002<br>FA-<br>0.0658 |
| *Bambusha arundinaceae* (B) | 6.56 | 4.65mg/ml | ChA- 636.30<br>FA-<br>3111.35 | ChA-<br>0.96<br>FA-<br>4.74 | ChA- 0.0629<br>FA-<br>0.3109 |
| *Bambusha vulgaris* (A) | 2.91 | 2.91mg/ml | ChA-791.69 | ChA-2.73 | ChA- 0.079 |
| *Bambusha vulgaris* (B) | 5.68 | 5.68mg/ml | ChA- 960.50<br>GA – 37.54<br>FA- 1.16 | ChA – 1.69<br>GA – 0.066<br>FA- 0.0020 | ChA – 0.0096<br>GA – 0.0003<br>FA- NA |
| *Dendrocalamus strictus* (A) | 3.80 | 3.80 mg/ml | ChA- 1557.1<br>GA – 19.82 | ChA – 1.69<br>GA – 0.066 | ChA – 0.006<br>GA – 0.0002 |
| *Dendrocalcmus strictus* (B) | 5.51 | 5.51 mg/ml | ChA- 1803.37<br>FA – 845.2 | ChA- 3.27<br>FA – 1.53 | ChA- 0.0180<br>FA – 0.008 |

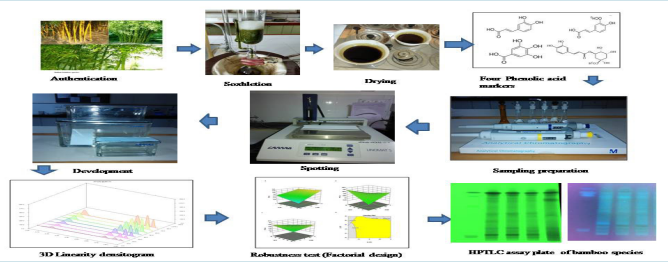
## ABBREVIATIONS

**ChA:** Chlorogenic Acid; **GA**: Gallic Acid; **CA:** Caffeic Acid; **FA:** Ferulic acid; **DOE:** Design of Experiment; **FA:** Factorial Analysis; **HPTLC:** High Performance Thin Layer Chromatography.

## REFERENCES


1. Alara OR, Abdurahman NH, Olalere OA. Ethanolic extraction of flavonoids, phenolics and antioxidants from *Vernonia amygdalina* leaf using two-level factorial design. Journal of King Saud University-Science. 2017;6:220-30.
2. Srinivasan M, Sudheer AR, Menon VP. Ferulic acid: therapeutic potential through its antioxidant property. Journal of Clinical Biochemistry and Nutrition. 2007;40(2):92-100.
3. Cinkilic N, Cetintas SK, Zorlu T, Vatan O, Yilmaz D, Cavas T, *et al.* Radioprotection by two phenolic compounds: chlorogenic and quinic acid, on X-ray induced DNA damage in human blood lymphocytes *in vitro*. Food and Chemical Toxicology. 2013;53:359-63.
4. Shanthakumar J, Karthikeyan A, Bandugula VR, Prasad NR. Ferulic acid, a dietary phenolic acid, modulates radiation effects in Swiss albino mice. European Journal of Pharmacology. 2012;691(1-3):268-74.
5. Goyal AK, Brahma BK. Antioxidant and nutraceutical potential of bamboo: an overview. International Journal of Fundamental and Applied Sciences. 2014;3(1):2-10.
6. Sangeetha R, Diea YKT, Chaitra C, Malavi P, Shinomol G. The Amazing Bamboo : A Review on its Medicinal and Pharmacological Potential. Indian J Nutr. 2015;2(1):1-7.
7. Medić-Šarić M, Jasprica I, Smolčić-Bubalo A, Mornar A. Optimization of chromatographic conditions in thin layer chromatography of flavonoids and phenolic acids. Croatica Chemica Acta. 2004;77(1-2):361-6.
8. Maleš Ž, Plazibat M, Vundać V, Žuntar I, Pilepić K. Thin-layer chromatographic analysis of flavonoids, phenolic acids and amino acids in some Croatian Hypericum taxa. Journal of Planar Chromatography-modern TLC. 2004;17(4):280-5.
9. Wójciak-Kosior M, Matysik G, Soczewiński E. High-performance thin-layer chromatography combined with densitometry for quantitative analysis of phenolic acids in complex mixtures. Journal of Planar Chromatography-modern TLC. 2006;19(107):21-6.
10. Pradhan SK, Gupta RC, Goel R, Preet R. Simultaneous determination of chlorogenic and caffeic acid in *Siegesbeckia orientalis* L.(Xi Xian) by a validated high-performance thin-layer chromatographic method. Journal of Planar Chromatography-modern TLC. 2017;30(6):516-20.
11. Jug U, Glavnik V, Kranjc E, Vovk I. High-performance thin-layer chromatography and high-performance thin-layer chromatography–mass spectrometry methods for the analysis of phenolic acids. Journal of Planar Chromatography-modern TLC. 2018;31(1):13-22.
12. Guideline IH. Validation of Analytical Procedures: Text and Methodology Q2 (R1). International Conference on Harmonization. Geneva, Switzerland. 2005.
13. Krygier K, Sosulski F, Hogge L. Free, esterified and insoluble-bound phenolic acids. 1. Extraction and purification procedure. Journal of Agricultural and Food Chemistry. 1982;30(2):330-4.


## PICTORIAL ABSTRACT




## SUMMARY

- Indian bamboo species is used for management of diabetic and radio-protective agent in cancer.
- Simple, robust, specific and rapid HPTLC method developed and established for the determination of phenolic acids in selected Indian bamboo species.
- Factorial design successfully applied for determination of robustness studies.
- The methods validated as per International Conference on Harmonization (ICH) guidelines.

## ABOUT AUTHORS


**Jayanta Kumar Maji** is working as Research Fellowship, Department of pharmaceutical analysis at Institute of Pharmacy, Nirma University, Gujarat, India.

**Mansi Patel** is working as DST-INSPIRE project Fellow, Department of pharmaceutical Analysis, Gujarat, India.

**Shital Butani** is working as an Associate Professor, Department of Pharmaceutics, IP-NU, Gujarat, India

**Dr. Snehal Patel** is working as an Associate Professor ; Department of Pharmacology, IP-NU, Gujarat, India.

**Dr. Priti J Mehta** is working as HOD in Pharmaceutical Analysis, IP-NU, Gujarat, India.