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Optimization of HPTLC densitometric method for curcuminoids and polyphenolics in an ayurvedic *Emblica officinalis* and *Curcuma longa* based Nishamalaki formulation by Box-Behnken design

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The present study focuses on developing a simplified, specific, and accurate high performance thin layer chromatographic (HPTLC) method for the quantitative and qualitative determination of ellagic acid, gallic acid, and curcuminoids (bisdemethoxycurcumin, demethoxycurcumin, and curcumin) in Nishamalaki Ayurvedic formulation. Pre-coated silica gel 60 F_{254} aluminum-backed plates were used as the stationary phase in the chromatographic technique development, and the optimized mobile phase was toluene: dichloromethane: glacial acetic acid: formic acid (6:4:1.6:0.9% v/v/v/v) with double development in linear ascending mode. The detection wavelength for quantification for ellagic and gallic acid was 280 nm, and curcuminoids (bisdemethoxycurcumin, demethoxycurcumin, and curcumin) were 430 nm. The optimized mobile phase showed optimum separation between peaks for ellagic acid, gallic acid, and curcuminoids (bisdemethoxycurcumin, demethoxycurcumin, and curcumin) at R_F of 0.12±0.02, 0.21±0.02, 0.55±0.02, 0.69±0.02 and 0.82±0.02 respectively. Chromatographic conditions were optimized using the Box-Behnken design. Various variables, such as, the volume of formic acid and glacial acetic acid, and chamber saturation time, that are likely to impact R_F were identified for further optimization. The volume of glacial acetic acid may be regarded as a critical method parameter, which caused the greatest change in the R_F value and was the important factor among the three factors. The linear range was 600-1800 ng/band for all markers (r^2 greater than 0.98). The limit of detection (LOD) and quantification (LOQ) measured indicated the method's sensitivity. For all markers, the recovery percentage reveal acceptable accuracy, and the method was repeatable and reproducible from precision measurements with less than a 2% relative standard deviation. The optimized method was precise, specific, accurate, robust and reproducible for quantifying ellagic acid, gallic acid, and curcuminoids (bisdemethoxycurcumin, demethoxycurcumin, and curcumin) in the quality-control testing of botanical extract along with Nishamalaki ayurvedic formulation.

Keywords: Ayurvedic formulation, BBD, *Curcuma longa*, *Emblica officinalis*, HPTLC, Nishamalaki, Validation

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As the popularity of Ayurveda and natural product grows, so does the concept of traditional blend formulation in health care and nutrition. Additionally, clinical research testing with detailed toxicity studies are required for licensing plant based formulations to be available as products globally. Moreover, natural botanical based products are used by more than 80% of the rural population for treatment in India. The rural population uses various preventive and treatment base approaches for diabetes mellitus[1]. Diabetes mellitus in India, is often managed and supported through the use of natural ingredients and phytochemicals derivatives[2-4]. Despite the many pharmaceutical treatments that are currently accessible, dietary intervention continues to be the primary pillar for the prevention and treatment of type 2 diabetes[4]. Plants possessing polyphenolic compounds have antioxidant action and are proven to lessen the impact of diabetes through a variety of mechanisms[5,6].

Oxidative stress is a crucial factor in the pathophysiology of diabetes with diabetic vascular problems[2-3,7-9]. Nishamalaki (NA) an Ayurvedic combination, has demonstrated metabolic and oxidative support associated with diabetes. Traditional medicine has made reference to the *Curcuma longa* L. plant's root as a source of curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin), having antioxidant action and improving glucose metabolism in the body, leading to antidiabetic activity[10,11]. Similarly, NA also comprises of

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fruits of *Emblica officinalis*, EO (Indian Gooseberry, Amla) and has great antidiabetic potential in traditional medicine[11-16].

According to studies, curcuminoids also exhibit antioxidant, anti-inflammatory, and immunomodulatory properties[11-13]. Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], is the major polyphenol in *C. longa* and other curcuminoids. The number of methoxy groups on aromatic rings varies between demethoxycurcumin and bisdemethoxycurcumin. Curcuminoids regulate blood sugar levels via different mechasnims, *viz.*, inhibiting α-amylase and α-glucosidase[10,12], stimulates the insulin release from pancreatic cells[10,11,13,14], blockade of liver gluconeogenesis, glycolysis and glycogenesis process. Consuming curcumin reduces the chance of developing diabetes as it has been proven in studies to have insulin-sensitizing effects[11,14-16]. Furthermore, research on the effects of *Curcuma* has focused on its renoprotective, prophylaxis of cataract, antioxidant, anti-inflammatory, immunomodulatory, antibacterial, and anti-carcinogenic properties.

Commonly referred to as Amla, the fruit of the Euphorbiaceae plant *Emblica officinalis* Gaertn. (Syn: *Phyllanthus emblica* L.) has great medicinal potential in traditional Indian medicine. In STZ-induced T2DM mice, the *Emblica officinalis* fruit is reported to lower blood glucose levels, improve glycemic status, reduce oxidative stress and also linked to the prevention of insulin resistance by a high-fructose diet[7]. According to the literature, *E. officinalis* fruits have ethnobotanical benefits; antibacterial, antioxidant, adaptogenic, hepatoprotective, anticancer, renoprotective, induces insulin release, gastroprotective, immunomodulator, and antiulcerogenic properties[7,17,18]. A potent antioxidant and immunity booster is gallic acid, a naturally occurring phenolic acid[19]. Gallic acid and Ellagic acid slow vitamin C degradation, reduce oxidative stress, major contributor to diabetes mellitus and diabetic complications[19,20]. Additionally, research shows that ellagic acid, reduces inflammation and insulin resistance, thereby managing the macrovascular and microvascular consequences of diabetes[21].

World Health Organization has focused on using appropriate standards and sophisticated analytical techniques to guarantee the quality of medicinal plant products that may result in synergistic effects on diabetes mellitus[22]. Thus, chemical fingerprints acquired by chromatographic methods are used to prove the chemical integrity of herbal formulation and are highly recommended for quality control of herbal products[22]. Herbs with numerous chemical constituents impose a significant difficulty in standardization[23,24]. HPTLC is an efficient technology for standardization, fingerprinting and quantification. Since HPTLC can analyze multiple samples concurrently (*i.e.,* on the same plate), unlike HPLC, it is frequently used as a routine analytical technique for herbals and has several benefits, including better resolution, a minute quantity of mobile phase requirement, speed, and a cost-effective analysis for each sample. Additionally, samples can be applied automatically, and repeated scanning is feasible by altering the scanning parameters[23,24].

A Box-Behnken design (BBD)-based HPTLC approach for detecting five markers in the NA formulation has been provided. The BBD is a popular response surface design for examining how various factors affect responses[25]. According to the literature study, chromatographic methods, including HPLC[26-32], HPTLC[33-39] have been published for estimating each marker, including ellagic acid, gallic acid, bisdemethoxycurcumin, demethoxycurcumin, curcumin but none have been described for estimating all five markers at once. As a result, the current study introduces a HPTLC method for the simultaneous quantification of five markers, including ellagic acid, gallic acid, curcumin, demethoxycurcumin, and bisdemethoxycurcumin in NA formulation and plant extracts. The suggested HPTLC technique may be used for regular examination of NA ayurvedic formulation as well as quality control testing.

Materials and Methods

Materials

Curcuma longa extract (CLE) and *Emblica officinalis* extract (EOE) were used for the study. The standard curcumin (CUR), bisdemethoxycurcumin (BDMC), and desmethoxycurcumin (DMC) compounds were purchased from USP (MD, USA). The extracts were authenticated by proximate analysis. The ellagic acid (EA) and gallic acid (GA) were obtained from Sigma-Aldrich (St. Louis, US). The NA formulation blended in the ratio of CLE to EOE (1:1), similar to traditional use[7].

Instrumentation and software

Samples were applied with a semi-automatic sample applicator (Linomat V, Camag, Switzerland) and a 100 µL syringe (Camag, Bonaduz, Switzerland). The

scanning and recording of the densitogram were done using a Camag TLC Scanner IV densitometer. The plate after application was developed in a twin-trough chamber (10 x 10 cm). For peak integration, CamagWinCATS software version (1.4.6) was utilized. The sample and standard solution were sonicated in an ultrasonic bath (Frontline FS-4, Mumbai, India). The UV cabinet (dual-wavelength UV lamp) and a TLC visualizer (Camag, Muttenz, Switzerland) flat bottom were utilized in the investigation. Calculations were performed using Microsoft Excel 2010 software to quantify extracts by analysis of linear regression and computation of various validation parameters.

Preparation of standard stock and working standard solution

Accurately weighed 5.0 mg of each standard; EA, GA, and curcuminoids (CUR, BDMC, and DMC) were transferred into volumetric flasks (5 mL) and diluted in methanol up to the mark (1000 µg/mL). The standard working solution of each marker were prepared from the stock solution to final concentration (100 µg/mL). All these solutions, both stock and working standard, were further preserved at 4-6°C.

Chromatographic conditions

Standard and samples were applied as bands (bandwidth 8 mm) using a Hamilton syringe on aluminum backed HPTLC plate precoated with silica gel 60 F_{254}, (10 cm × 10 cm, 100 µm thickness; E. Merck, Darmstadt, Germany) with a sample applicator. The mobile phase components were thoroughly mixed before use. The time needed for chamber saturation with vapours of mobile phase was for 20 min. Further cold air dryer was used to dry the plates. Densitometric scanning was performed in absorbance reflectance mode with a halogen tungsten light at 430 nm and a deuterium lamp at 280 nm. The measurements for the slit were 6 mm × 0.45 mm, the scanning rate was 20 mm/s, and the data resolution was 100 µm/step. To ensure that the separation is repeatable, the results were evaluated in order to identify the best separation and spot migration.

Optimization of chromatographic conditions by BBD

Preliminary trials initially were performed to identify the criticality of factors. Solvents of varying polarity, such as toluene, dichloromethane, glacial acetic acid, and formic acid in varying ratios, were utilized to optimize the mobile phase. Further, a multivariate technique utilizing design of experiment is adopted to study the effect of various factors simultaneously on responses. Response surface design, BBD is the ideal experimental design strategy for modeling and optimization by understanding the major effects of the factors and their interactions[25,40]. On the basis of preliminary experiments, the primary factors, volume (mL) of glacial acetic acid (A), volume of formic acid (B), and time for chamber saturation (C) were the variables chosen for the optimization procedure. R_F values for EA, GA, and curcuminoids (CUR, BDMC, and DMC) were chosen as responses. Randomization was employed in the experimental trials to mitigate the potential influence of uncontrolled factors that may introduce partiality into the measurements. A three-factor BBD demands 17 experiments, including five central points. The factor and levels selected for optimization were volume of glacial acetic acid (1.4 and 1.8 mL), volume of formic acid (0.6 and 1.2 mL), and time required for chamber saturation (15 and 25 min). For the current optimization investigation, several computations were carried out using the polynomial model with factors and interaction terms and Design-Expert software, trial version 11 (Stat-Ease Inc., Minneapolis). ANOVA functionality in design expert software was used to establish the statistical validity of polynomials. Additionally, 3-D response surface graphs and 2-D contour plots were generated. The response data from the experiment were then quantitatively compared with the value predicted for model validation.

Method validation

In reference with International Conference on Harmonization (ICH) standards Q2 (R1), the developed HPTLC method was validated for linearity, precision, accuracy, LOD, LOQ, and robustness[41]. The regression coefficient was utilized to evaluate the linear correlation of all markers over the range of concentration between 600-1800 ng/band for EA and GA at 280 nm and BDMC, DMC, and CUR at 430 nm by executing five replicate measurements. Further, Bartlett's testing was done to evaluate the variance along regression line showing homoscedasticity or heteroscedasticity[42]. In accordance to ICH guidelines, Limit of Detection and Limit of Quantification were calculated based on the calibration curve's slope and standard deviation of response (intercept). Recovery studies assessed the precision of verifying the method recovery at three distinct levels, namely 50%, 100%, and 150%. This was carried out by spikinga known amount of standard (EA, GA, or CUR) to the pre-quantified samples, and computing the recovery and

percentage RSD for each marker using the recommended method, in triplicate. The repeatability and intermediate precision investigations were performed to establish the method's reproducibility. The measured peak area observed was expressed as a percentage of the relative standard deviation (%RSD). The same concentration was applied six times to test for repeatability, and then further scanning was done and results were given as a percentage of RSD (%RSD). On the same day, three repetitions of the concentrations (600, 1200, and 1800 ng/band for EA, GA, and curcuminoids (BDMC, DMC, and CUR) were run to achieve intraday precision. Similarly, utilizing the above mentioned concentrations of all markers specified, an investigation of intermediate precision was carried out on different days. By evaluating the markers and extracts, the method's specificity was ascertained. The bands for EA, GA, BDMC, DMC, and CUR were verified by comparison of the R_F and spectra with standard. Purity of peak was then determined by comparison of the spectra at peak starting point (S), peak apex point (M), and peak ending point (E) positions. The reported HPTLC method's robustness was assessed concerning the response, R_F, and peak area, under a variety of conditions, including the following: Volume of toluene (± 0.2 mL); Saturation time (± 2 min); solvent front (80 ± 2 mm); alteration in wave length for EA, GA (280 ± 2 nm) and for BDMC, DMC, CUR (430 ± 2 nm).

Fingerprinting and quantification of markers in Ayurvedic formulation

CLE and EOE, 50 mg of each, after accurately weighing was taken into a volumetric flask (50 mL), further dissolution in methanol and the final volume was equalized upto the mark with methanol. Further sonication was carried out for 10 min followed by centrifugation (5000 rpm) for 5 min. After that the supernatant layer was collected and filtered.

Accurately weighed 50 mg of Nishamalaki formulation similarly was taken into volumetric flask (50 mL), followed by dissolution in methanol and final volume was adjusted up to the mark with methanol. After 10 min of sonication, it was centrifuged for 5 min at 5000 rpm. Then after, the supernatant layer was collected and filtered. After being prepared as previously explained, 5 µL of the sample solution was applied on the plate and then developed using the identical circumstances as those mentioned for standard. Each marker's concentration in the extract was determined by linear regression analysis after scanning the bands at their respective R_F values for EA and GA peak at 280 nm and BDMC, DMC, respectively and CUR at 430 nm.

Results and Discussion

The suggested HPTLC technique showed no interference from the botanical extract and was found to be easy to use, sensitive, specific, economical, and time-saving. The procedure was validated in accordance with ICH recommendations.

Chromatographic development

The spectra of the markers when recorded from 200 to 700 nm wavelength range revealed detection wavelength of 280 nm to achieve adequate peak areas for the EA and GA, and 430 nm for BDMC, DMC and CUR. After conducting literature research, different mobile phases were tried, including toluene, n-hexane, dichloromethane, methanol, formic acid, glacial acetic acid and ethyl acetate[43-44]. As a result, these solvents were utilized in various combinations and ratios during the early research.

Optimization using BBD

Due to its adaptability, BBD was selected for improving separation by acquiring a better knowledge of the primary with interaction impacts of factors; volume of glacial acetic acid (A), volume of formic acid (B), and Chamber saturation time (C), selected for optimization by preliminary studies. A quadratic model with the least PRESS value was selected and an adjusted R^2 value nearer to 1 showed greater match. ANOVA further validated the model, and the outcomes of all the statistical parameters were as per the acceptance criteria resulting in a model that was best fitted as shown in Supplementary Table S1. Reproducibility of the model was measured by % CV value found to be below 10% and signal to noise ratio was expressed as adequate precision showing value above 4 revealing acceptable results. At a p-value of 0.05, the F-ratio was employed to assess the model's significance in terms of the variance of each component that made up the error term. The model's P-value was within acceptable limits[25].

The experimental data matched the polynomial equation satisfactorily in this case, as seen by the corrected R^2 values, which was in acceptable limits of R^2 (0.80) (Supplementary Table S1). The final equation of the actual elements and factors, is as per Table 2. A positive sign in the table denotes a synergistic impact, while an antagonistic relationship

was denoted by negative sign between the inputs and the response[25]. The RSD graphs Fig. 1 (a-o) revealed that with the elevation in volume of glacial acetic acid, R_F values of EA, GA, CUR increased, while the volume of formic acid increased the R_F values of all the markers. However, chamber saturation time for chamber saturation displayed a negligible effect on EA retardation factor.

The Design Expert software used numerical optimization to determine the ideal conditions for chromatographic development. Various restrictions for factors and responses were applied. The software offered various potential options, all of which had the appropriate response value, following the criteria. Three of these prediction options with desirability values close to or equal to 1 were chosen. The agreement between measured and predicted responses was checked to determine the validity of the proposed model. The measured value of optimal condition that was comparatively closer to the anticipated value, with decreased error and high desirability[25]. Thus, the optimized mobile phase, toluene: dichloromethane: glacial acetic acid: formic acid (6:4:1.6:0.9, *v/v/v/v*) ultimately showed good separation in the peaks and the peaks were observed at retardation factors of 0.12, 0.21, 0.55, 0.69 and 0.82 for EA, GA, BDMC, DMC and CUR, respectively (Fig. 2).

Method validation

The proposed HPTLC method was further subjected to validation by ICH guidelines[42-45]. The concentration range demonstrated linearity, with a correlation coefficient of 0.9941 for EA, 0.9854 for GA, 0.9942 for BDMC, 0.9861 for DMC, and 0.9834 for CUR, respectively, according to the measured signal in the calibration plots (peak area vs. concentration) using ordinary least squares regression analysis (Supplementary Fig. S1). Fig. 2 depicts a 3D densitogram demonstrating the linearity of GA and EA at 280 nm and for curcuminoids (BDMC, DMC and CUR) at 430 nm. Further heteroscedasticity of variance for the peak area of each standard marker as response was further statistically evaluated by Bartlett's test resulted in the calculated χ^2 value less than the tabulated (0.05, 4) χ^2 critical value = 9.488 (Table 2). To measure the degree of variance among the peak area of five determinations, the residual values of each marker were computed, and mean residual values for EA, GA, BDMC, DMC, and CUR were in the range of -72.92 to 110.17, 161.84 to

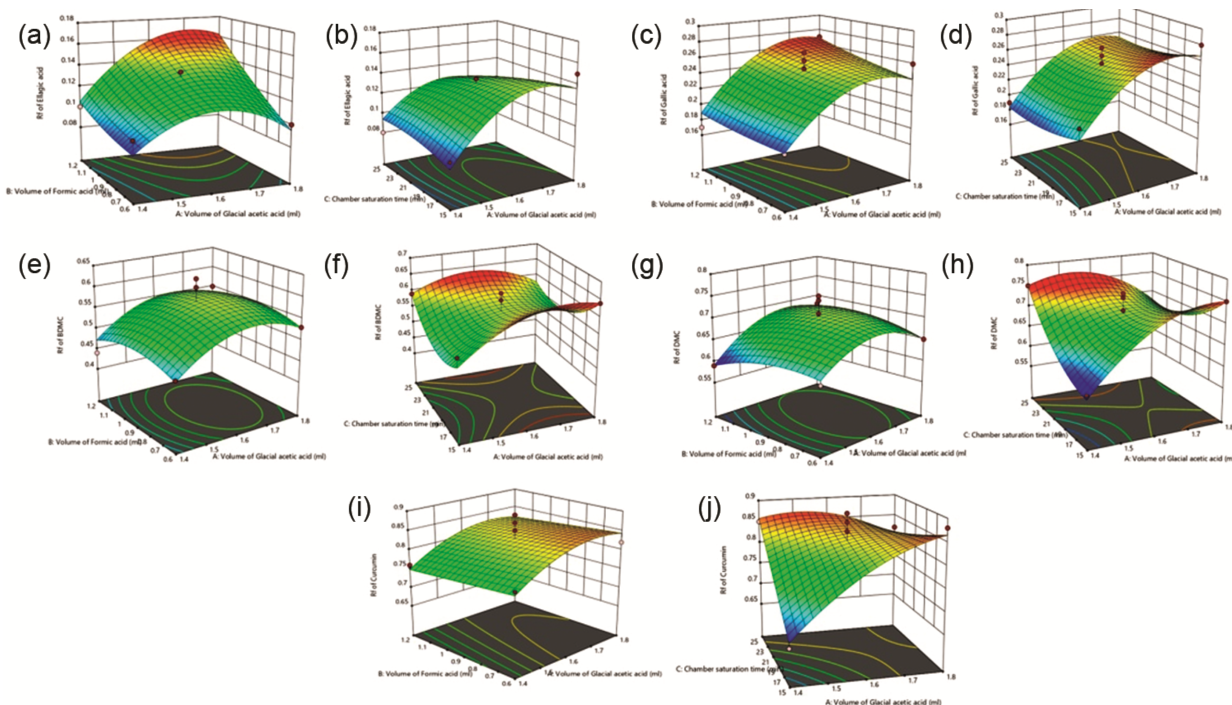

Fig. 1 — Three-dimensional plots for responses: (a) R_F of EA as function of A and C while fixed factor C(b) R_F of EA as function of A and C while fixed factor C (c) R_F of GA as function of A and C while fixed factor C (d) R_F of GA as function of A and C while fixed factor C(e) R_F of BDMC as function of A and C while fixed factor C (f) R_F of BDMC as function of A and C while fixed factor C; (g) R_F of DMC as function of A and C while fixed factor C; (h) R_F of DMC as function of A and C while fixed factor C; (i) R_F of CUR as function of A and C while fixed factor C; (j) R_F of CUR as function of A and C while fixed factor C

444.85, -19.38 to 250.47, -620.58 to 529.36 and 110.31 to 705.35 (Supplementary Fig. S2). The LOD and LOQ for all five markers indicate the sensitivity of the suggested method as shown in Table 1. The reproducibility of the procedure was indicated by the sample applicator's repeatability, precision studies (intraday and interday) which revealed a % RSD below 2% for all the markers, demonstrating that the analytical technique was appreciably precise (Table 1). The mean percent recovery found for EA,

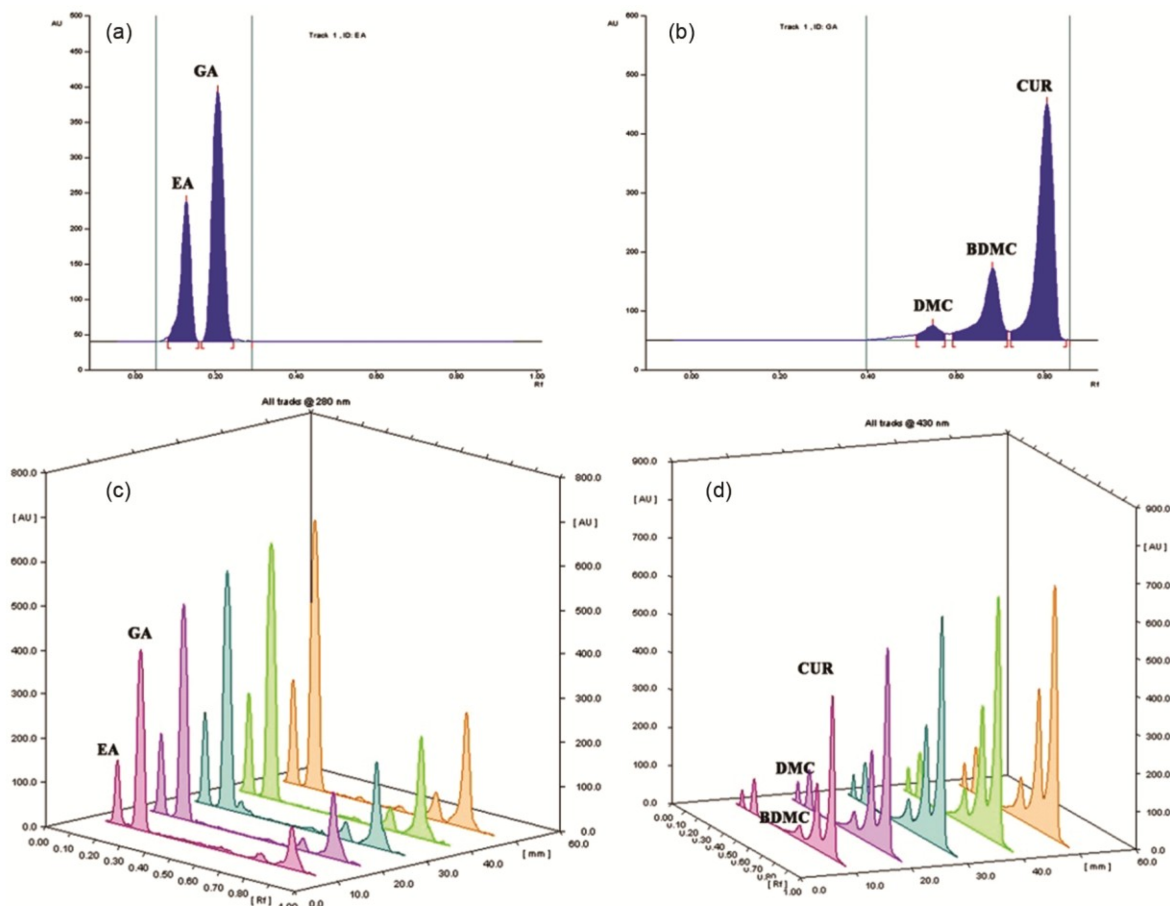

Fig. 2 — HPTLC chromatograms and 3D densitograms for linearity of compounds: EA, GA at 280 nm (a,c) and BDMC, DMC, CUR at 430 nm (b,d).

Table 1 — Validation parameters for EA, GA, BDMC, DMC and CUR by HPTLC method

Parameters	EA	GA	BDMC	DMC	CUR
Linear regression parameters					
Calibration range (ng/band)	600-1800	600-1800	600-1800	600-1800	600-1800
Regression equation	y = 2.4645x+1402.3	y = 7.196x+6228.6	y =4.1837x-0.9942	y =9.0128x-0.9861	y = 9.4951x+5511.2
Correlation coefficient	0.9941	0.9854	0.9942	0.9861	0.9834
Bartlett's test	0.00050	0.0001	0.0001	6.823	1.0960
Limit of detection [ng/band]	40.542	4.612	9.770	3.581	3.657
Limit of quantification [ng/band]	122.854	13.975	29.607	10.852	11.083
Precision (Peak area±%RSD)					
Repeatability	0.928	0.913	1.001	0.912	0.797
Intraday precision	0.11-0.34	0.09-0.53	0.28-1.02	0.10-0.53	0.11-0.16
Interday precision	0.62-0.79	0.21-0.45	0.20-0.88	0.26-0.67	0.14-0.54
Accuracy (Percent Recovery±%RSD)					
Concentration spiking (50%)	99.35±0.11	99.64±0.07	97.87±0.30	100.78±0.16	99.48±0.14
Concentration spiking (100%)	99.79±0.17	99.78±0.06	99.62±0.17	102.17±0.11	99.11±0.03
Concentration spiking (150%)	100.12±0.07	101.04±0.09	99.68±0.15	100.19±0.12	96.98±0.02

GA, BDMC, DMC, and CUR were within the range of 99.35 – 100.12, 99.64 – 101.04, 97.87 – 99.68, 100.19 – 102.17 and 96.98 – 99.48 in ayurvedic botanical extract respectively and the % RSD was also below 2% revealing the proposed methods accuracy. An intentional alteration of numerous parameters, such as the mobile phase composition, the time required for chamber saturation, the solvent front, and the detection wavelength, proved the method's robustness by showing that the changes had no appreciable effect on the RF of any markers or peak area (% RSD 0.16 - 2.16) (Table 1).

The densitograms of the botanical extract obtained were compared with the standard densitogram using the proposed method. It was found that all markers in the extract showed peaks at the same R_F values for EA, GA, BDMC, DMC, and CUR, respectively (Fig. 3 & Fig. 4), like the standard. The overlay spectra showed that the purity of peak for all the markers in the plant-based extract at the peak starting position, peak apex position, and peak ending position of the spot displayed that peak purity was greater than 0.99 for all markers in the botanical extract[42-44] (Supplementary Fig. S3).

Fingerprinting and quantification

According to the fingerprinting results, EA, GA, BDMC, DMC and CUR were all clearly separated. The R_F values for each marker, 0.12 for EA, 0.21 for GA, 0.55 for BDMC, 0.69 for DMC, and 0.82 for CUR, were similar to the standard markers. The visual representation of the developed plate was viewed under UV mode at 254 nm is indicated as black spots for EA and GA (Fig. 3 & Fig. 4) and a green fluorescence band for BDMC, DMC, and CUR at 366 nm (Fig. 3). HPTLC 3D Densitogram for fingerprinting of EA and GA at 280 nm and BDMC, DMC, and CUR at 430 nm is as shown (Fig. 4 a,b).

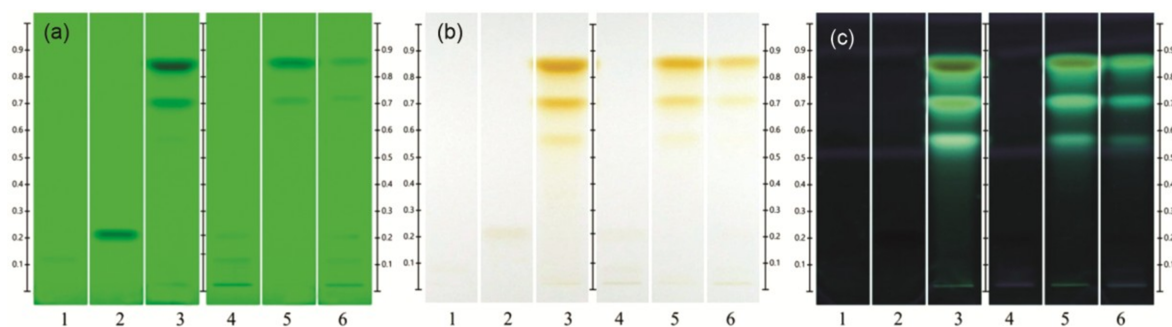

Fig. 3 — Photographic image for fingerprinting of Ellagic acid and Gallic acid, BDMC, DMC and Curcumin with extract and formulation (a) 254 nm, (b) 366 nm and (c) white light; Track details: T1-Ellagic acid, T2-Gallic acid, T3-Curcuminoids, T4-*Emblica officinalis* extract, T5-*Curcuma longa* extract, T6-NA formulation

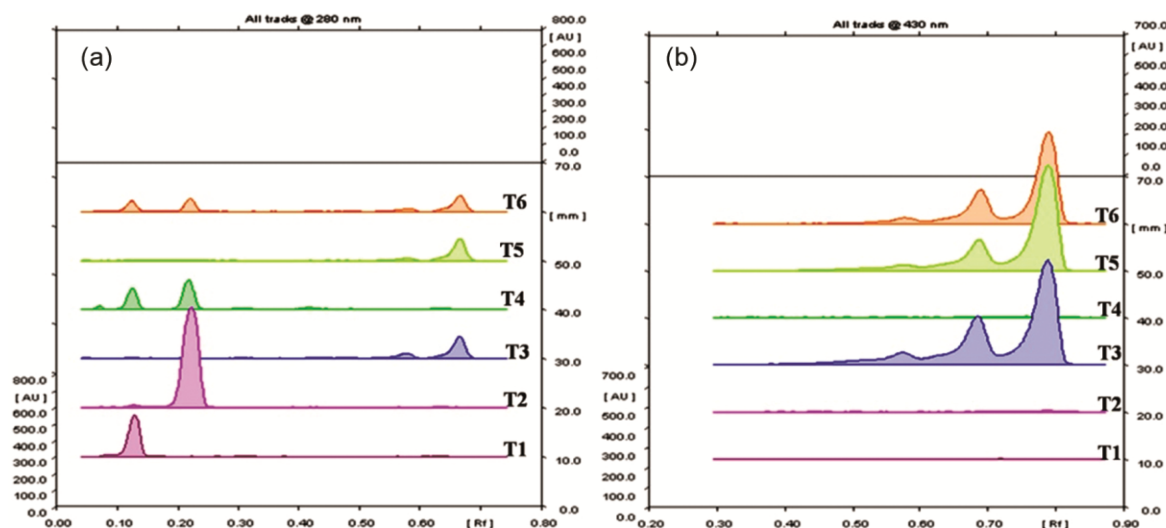

Fig. 4 Densitometric UV spectra of standards, extracts and NA formulation: (a) at 280 nm and (b) at 430 nm Track detailsT1-gallic acid, T2-ellagic acid, T3-Curcuminoids standard, T4-*Emblica officinalis* extract, T5-*Curcuma longa* extract, T6-NA formulation

Table 2 — % content of analytes in extracts and formulation

Samples	EA	GA	Total Curcuminoids (CUR+BDMC+DMC)
Emblica officinalis extract	0.845±0.192	11.561±1.172	ND
Curcuma longa extract	ND	ND	95.734±3.197
Nishamalaki formulation	0.251±0.087	1.539±0.417	4.639±0.921

The mean amount in nishamalaki formulation (%) for EA, GA, and total curcuminoids (BDMC, DMC, and CUR) were found to be 0.251±0.087, 1.539±0.417 & 4.639±0.921 while for *Emblica officinalis* extract, the mean amount (%) found for EA, GA, 0.845±0.192, 11.561±1.172 respectively and the *Curcuma longa* extract the mean amount of total curcuminoids found, 95.734±3.197 (Fig. 3 & Fig. 4, Table 2).

Conclusion

A specific HPTLC method was developed to simultaneously quantify EA, GA, and total curcuminoids (BDMC, DMC, and CUR) in Ayurvedic formulation and was found to be a acurate and precise. Additionally, BBD enabled the simultaneous investigation of several variables, and amongst all, the volume of glacial acetic acid is a crucial variable that must be controlled for the best outcome. Further validation of the method was performed as per ICH guidelines Q2 (R1) where the results were found to be within the acceptance criterion for all validation parameters. Furthermore, this proposed HPTLC method may be used for quality control testing and analysis of botanical formulation and combinations as blended with *Curcuma longa* extract and *Emblica officinalis* extract similar to a traditional ayurvedic formulation like Nishamalaki.

Supplementary Data

Supplementary data associated with this article is available in the electronic form at https://nopr.niscpr.res.in/jinfo/ijtk/IJTK_23(08)(2024)719-728_SupplData.pdf

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Conflict of Interest

According to the writers, they have no conflicts of interest. This attests to the fact that the text has not been submitted for publication elsewhere and is an original work. Furthermore, we attest that all references have been properly cited and that no information, including tables and figures, has been taken verbatim from other publications. All authors have given their consent for this manuscript to be submitted to the Indian Journal of Traditional Knowledge.

Author Contributions

The idea was conceived and developed by KGP and AG who verified that analytical methods. RRP and LH contributed in planning, designing and supervising the experiments. The research work was performed by KCP along with data collection and data processing. All the authors contributed in interpretation of the result and findings. SG and KCP wrote the manuscript with feedback from all the authors supporting shaping the manuscript.

Data Availability

The data that support the findings of this study are available in the supplementary material of this article or at request from the corresponding author.

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