

Simultaneous Quantification of Caffeic Acid and p-Coumaric Acid in *Typha domingensis* Pers. Using a Validated High-Performance Thin-Layer Chromatography–Densitometric Method

Krishna Nayak

Indukaka Ipcowala College of Pharmacy

Veena Patel

`veena.patel@cvmu.edu.in`

Indukaka Ipcowala College of Pharmacy

Mitalben Parmar

The Charutar Vidya Mandal (CVM) University

Dimal Shah

The Charutar Vidya Mandal (CVM) University

Research Article

Keywords: High performance thin-layer chromatography, *Typha domingensis*, Caffeic acid, p-Coumaric acid

Posted Date: September 28th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10874612/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Typha domingensis Pers., a perennial aquatic macrophyte belonging to the family Typhaceae, is widely found in wetlands and marshy habitats across tropical and subtropical regions. Different parts of the plant, including the roots, leaves, and inflorescences, have traditionally been utilized as food and in herbal medicine for treating wounds, inflammation, and bleeding-related conditions. Phytochemical investigations have revealed that the plant contains a diverse range of bioactive compounds, such as phenolic acids, flavonoids, tannins, saponins, glycosides, alkaloids, and steroids. Among these constituents, the hydroxycinnamic acids p-coumaric acid and caffeic acid are recognized as important phenolic markers because of their notable pharmacological properties. Therefore, the present study was undertaken to develop and validate a simple, accurate, precise, and robust high-performance thin-layer chromatography (HPTLC) method for the simultaneous estimation of p-coumaric acid and caffeic acid in *Typha domingensis Pers.* The validation of the proposed analytical method was carried out in accordance with the International Council for Harmonisation (ICH) Q2(R2) guidelines. Chromatographic separation was performed on pre-coated aluminium silica gel 60 F₂₅₄ plates using a mobile phase consisting of toluene: ethyl acetate: formic acid (7:3:0.5, v/v/v). The R_f values obtained for caffeic acid and p-coumaric acid were 0.44 and 0.58, respectively, and densitometric analysis was carried out at a wavelength of 325 nm. The method exhibited excellent response over the concentration range of 200–1200 ng/band, with correlation coefficients (R²) of 0.996 for caffeic acid and 0.997 for p-coumaric acid. The detection limit (DL) was determined to be 67.01 ng/band for caffeic acid and 12.79 ng/band for p-coumaric acid, while the quantitation limit (QL) was 197.02 ng/band and 38.76 ng/band, respectively. Overall, the validated HPTLC method proved to be specific, accurate, precise, and robust for the simultaneous quantification of p-coumaric acid and caffeic acid in both their standard forms and the hydroalcoholic extract of *Typha domingensis Pers.*

1. Introduction

Medicinal plants have been used since ancient times as a valuable source of therapeutic agents for the prevention and management of various diseases [1, 2]. Even today, natural products derived from medicinal plants play a crucial role in drug discovery and development due to the wide variety of bioactive phytoconstituents that exhibit diverse pharmacological properties [3, 4]. According to the World Health Organization (WHO), approximately 80% of the world's population relies on traditional herbal medicines to meet their primary healthcare needs [5]. The growing global acceptance and commercial use of herbal medicines have highlighted the importance of establishing effective quality control measures, standardization protocols, and authentication techniques to ensure the safety, efficacy, purity, and consistency of medicinal plants and herbal formulations [6, 7]. Nevertheless, the phytochemical composition of medicinal plants can vary considerably as a result of factors such as geographical location, climatic conditions, harvesting time, extraction methods, processing procedures, and storage conditions [8, 9]. Consequently, the development of reliable analytical methods for

phytochemical profiling and the quantitative determination of marker compounds has become an indispensable component of herbal drug research and quality assurance [10].

Chromatographic techniques are extensively used for the qualitative and quantitative evaluation of phytoconstituents present in medicinal plants and herbal formulations [11]. Among the various chromatographic approaches, high-performance thin-layer chromatography (HPTLC) has become a widely accepted analytical technique because of its simplicity, rapidity, accuracy, reproducibility, and cost-effectiveness in the standardization of herbal drugs [12, 13]. This method allows the simultaneous analysis of multiple samples while requiring minimal sample preparation and lower solvent consumption. Furthermore, chromatographic fingerprinting combined with densitometric analysis provides reliable identification and quantitative determination of phytochemical markers within complex herbal matrices [14–17]. These advantages have led to the extensive application of HPTLC in routine quality control and phytochemical standardization of medicinal plants and herbal formulations. In addition, several validated HPTLC methods have been reported for the simultaneous estimation of flavonoids, alkaloids, glycosides, phenolic compounds, and other phytoconstituents from medicinal plants, highlighting the versatility and effectiveness of this technique in herbal analysis [18–21].

Typha domingensis Pers., commonly referred to as southern cattail, is a member of the family Typhaceae and is widely distributed in wetlands, marshes, lakeshores, and riverbanks across tropical and subtropical regions [22]. The plant is distinguished by its long, linear leaves, rhizomatous root system, and characteristic cylindrical inflorescences [23]. Various plant parts, including the roots, rhizomes, leaves, pollen, and inflorescences, have long been used for medicinal, nutritional, and ecological applications [24]. Ethnomedicinal studies have documented its traditional use in managing wounds, burns, inflammation, bleeding disorders, skin diseases, diarrhoea, and urinary tract ailments [25, 26]. In addition, traditional systems of medicine have recognized the plant for its haemostatic activity and wound-healing potential [27]. Beyond its therapeutic significance, *T. domingensis* has gained considerable ecological attention because of its phytoremediation capability and its effectiveness in absorbing environmental pollutants from aquatic ecosystems [28].

Phytochemical studies on *T. domingensis* have identified a wide range of biologically active secondary metabolites, including flavonoids, phenolic acids, tannins, alkaloids, glycosides, steroids, triterpenoids, and saponins [29–31]. These phytoconstituents are believed to contribute to the diverse biological and pharmacological properties exhibited by the plant [32]. Extracts obtained from *Typha* species have been reported to exhibit several pharmacological activities, such as antioxidant, antimicrobial, anti-inflammatory, cytotoxic, antidiabetic, hepatoprotective, and wound-healing effects [33–36]. Earlier studies have also demonstrated that *T. domingensis* extracts possess significant antimicrobial and cytotoxic activities, providing scientific support for their traditional medicinal use [37]. In addition, phytochemical profiling has confirmed the presence of numerous bioactive constituents in *T. domingensis* that are associated with potent antioxidant and other biological activities [38]. The antioxidant activity of the plant is primarily attributed to its phenolic compounds and flavonoid

derivatives, which are capable of scavenging reactive oxygen species and reducing oxidative stress-induced cellular damage [39–41].

Among the phenolic compounds reported in medicinal plants, p-coumaric acid and caffeic acid are prominent hydroxycinnamic acid derivatives that occur extensively across a wide range of species [42]. These bioactive compounds exhibit remarkable antioxidant potential along with several pharmacological effects, including anti-inflammatory, antimicrobial, anticancer, hepatoprotective, cardioprotective, and neuroprotective activities [43–45]. Caffeic acid is recognized for its strong free radical scavenging ability and its capacity to inhibit lipid peroxidation and oxidative damage [46]. Likewise, p-coumaric acid has been reported to possess antioxidant, anti-inflammatory, and chemoprotective properties [47]. Owing to their broad distribution in herbal matrices and well-established pharmacological significance, both compounds are widely accepted as important phytochemical markers for the quality control and standardization of medicinal plants [48]. Therefore, the quantitative estimation of these marker compounds is essential for ensuring the identity, purity, consistency, and therapeutic reliability of herbal raw materials and herbal formulations [49].

Analytical methods used for the quality assessment of herbal materials should undergo proper scientific validation to ensure the reliability, accuracy, and reproducibility of the generated results [50]. According to the International Council for Harmonisation (ICH), analytical procedures must be validated for parameters such as specificity, linearity, precision, accuracy, robustness, limit of detection, and limit of quantification before they are adopted for routine analysis [51]. In addition, chromatographic fingerprinting, when combined with validated analytical methods, serves as an important tool for the authentication, quality evaluation, and standardization of medicinal plants and herbal formulations [52].

A review of the available literature indicates that although several studies have described the phytochemical profile and biological activities of *Typha domingensis* Pers., only limited information is available on the development of validated analytical methods for the simultaneous quantification of p-coumaric acid and caffeic acid in its hydroalcoholic extract [37, 38]. To the best of our knowledge, no validated HPTLC-densitometric method has been reported for the simultaneous estimation of these two phenolic biomarkers in *T. domingensis* Pers. Therefore, the present investigation was designed to develop a simple, accurate, precise, sensitive, and robust HPTLC-densitometric method for the simultaneous quantification of p-coumaric acid and caffeic acid in the hydroalcoholic extract of *Typha domingensis* Pers. The proposed method was subsequently validated in accordance with the ICH Q2(R2) guidelines and is expected to serve as a reliable analytical tool for routine quality control, phytochemical standardization, authentication, and the evaluation of batch-to-batch consistency of *T. domingensis*-based herbal raw materials and formulations.

2. Experimental

2.1 Plant material

The female inflorescence of *Typha domingensis* Pers. was collected from marshy wetlands in the Patan district of Gujarat, India. The collected plant material was identified and authenticated based on morphological characteristics with the help of available literature, standard botanical references, and previously published research articles. A voucher specimen of *T. domingensis* Pers. (Voucher Specimen No. IICP/2024/25/TD01) was deposited in the Department of Pharmacognosy and Phytochemistry, Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, Gujarat, India, for future reference. The lower cylindrical portion of the inflorescence, representing the female flower, was selected for the present study. The collected inflorescence, were shade- dried at room temperature until completely dry, which facilitated the easy separation of the female floral material from the supporting stalk. The separated female flowers were carefully collected, stored in airtight containers, and subsequently used for hydroalcoholic extraction for phytochemical and chromatographic studies. *Typha domingensis* Pers. was collected from the wetland regions of Gujarat, India, as shown in Fig. 1.

2.2 Chemicals

The biomarker compounds p-coumaric acid and caffeic acid were procured from Yucca Enterprises (Mumbai, Maharashtra, India). Ethanol procured from Radha Swami Traders, Vadodara (License no.: 37/26–27), Analytical grade -methanol, toluene, ethyl acetate, and formic acid were obtained from SRL Chemicals.

2.3 Preparation of extract of *Typha domingensis* Pers.

Five grams of the dried female inflorescence of *Typha domingensis* Pers. was accurately weighed and transferred to a conical flask. Subsequently, 100 mL of a hydroalcoholic solvent comprising ethanol and water (70:30, v/v) was added, and the mixture was macerated for 24 h with occasional stirring at room temperature. After maceration, the extract was filtered through filter paper, and the filtrate was concentrated by evaporation until a solid residue was obtained. The dried extract was carefully scraped, transferred into an airtight container, and stored under refrigerated conditions until further analysis.

2.4 HPTLC method development

2.4.1 Instrumentation

The study was carried out using CAMAG HPTLC instrumentation (Muttensz, Switzerland), including a CAMAG Linomat 5 sample applicator, CAMAG TLC Scanner 4, an ultraviolet (UV) cabinet equipped with dual-wavelength UV illumination, and CAMAG winCATS software. An electronic balance (Model ME 204, Mettler Toledo, Columbus, OH, USA) was used for the accurate weighing of all materials. Microsoft Excel 2019 (Microsoft Corporation, Redmond, WA, USA) was employed for extract quantification and the statistical evaluation of method validation parameters.

2.4.2 Preparation of standard solutions of caffeic acid and p-coumaric acid

Stock solutions of the biomarker compounds caffeic acid and p-coumaric acid were prepared separately by accurately dissolving 10 mg of each compound in ethanol and making up the volume to 10 mL in separate volumetric flasks to obtain a concentration of 1000 µg/mL. Subsequently, 1 mL from each stock solution was transferred into a 10 mL volumetric flask and the volume was adjusted with ethanol to obtain a mixed standard solution containing p-coumaric acid and caffeic acid at a final concentration of 100 µg/mL each. The prepared mixed standard solution was stored for subsequent analysis.

2.4.3 Chromatographic conditions

Sample application was carried out using a 100-µL Hamilton micro syringe (Bonaduz, Switzerland) fitted to a CAMAG Linomat 5 applicator equipped with a nitrogen flow system. The samples were applied as bands onto pre-coated silica gel 60 F₂₅₄ aluminium HPTLC plates (10 cm × 10 cm, 100 µm thickness; E. Merck, Darmstadt, Germany). Chromatographic separation was performed using a mobile phase consisting of toluene: ethyl acetate: formic acid (7:3:0.5, v/v/v). Plate development was conducted in a twin-trough glass chamber pre-saturated with the mobile phase for 30 min using filter paper, following the linear ascending development technique. The mobile phase was allowed to migrate to a distance of 90 mm from the point of application. Following development, the plates were removed from the chamber and air-dried for 20 min to remove any residual solvent. The developed chromatograms were initially examined under UV light using a CAMAG UV chamber and subsequently subjected to densitometric scanning at 325 nm with a CAMAG TLC Scanner 4 for the identification and quantification of p-coumaric acid and caffeic acid. The corresponding R_f values and peak characteristics were then recorded.

2.5 Method of Validation

The developed HPTLC method was validated in accordance with the International Council for Harmonisation (ICH) Q2(R2) guidelines [4]. The validation included the evaluation of key analytical parameters such as specificity test, response, accuracy test, precision, lower range (DL, QL) and robustness.

2.5.1 Specificity Test

The specificity of the developed method was evaluated to ensure the accurate identification and quantification of p-coumaric acid and caffeic acid in the presence of other constituents of the plant matrix. The marker compounds in the hydroalcoholic extract of *T. domingensis* were identified by comparing their R_f values with those of the corresponding reference standards and by overlaying the absorption spectra of the sample and standard compounds. Peak purity was further assessed by comparing the recorded spectra at the start (S), apex (M), and end (E) positions of the respective chromatographic bands corresponding to p-coumaric acid and caffeic acid.

2.5.2 Response (Calibration curve)

The response of the developed method was evaluated by applying mixed working standard solutions of p-coumaric acid and caffeic acid over a concentration range of 200–1200 ng/band onto pre-coated silica

gel 60 F254 HPTLC plates. The chromatographic plates were developed under the optimized experimental conditions, and the peak areas of the separated bands were recorded. Calibration curves were constructed by plotting the peak area against the corresponding concentration for each analyte (n = 6).

2.5.3 Accuracy Test

The accuracy of the developed method was evaluated using the standard addition technique at three concentration levels, namely 50%, 100%, and 150%. Known amounts of standard p-coumaric acid and caffeic acid were added to a previously analyzed hydroalcoholic extract of *T. domingensis* Pers. The concentrations of both biomarker compounds were determined from the corresponding peak areas using their respective regression equations. The quantity of each biomarker originally present in the unspiked sample was deducted from the total amount determined, and the results were expressed as percentage recovery.

$$\% \text{ Recovery} = \frac{\text{Amount found} - \text{Original amount}}{\text{Amount added}} \times 100$$

2.5.4 Precision

2.5.4.1 Repeatability Test

The repeatability of the developed method was evaluated by applying 800 ng/band of a homogeneous mixed working standard solution containing p-coumaric acid and caffeic acid (100 µg/mL each) onto the stationary phase. The chromatographic analysis was carried out in six replicates, and the corresponding peak areas and R_f values were recorded. The percentage relative standard deviation (%RSD) of the mean peak areas was calculated to assess the repeatability of the method.

2.5.4.2 Intermediate Precision Test

The intermediate precision of the developed method was assessed by evaluating intraday and interday precision. Three concentrations within the established linearity range, namely 600, 800, and 1000 ng/band of p-coumaric acid and caffeic acid, were analysed in triplicate. Intraday precision was determined by performing the analysis at three different time intervals on the same day, whereas interday precision was evaluated over three consecutive days. The percentage relative standard deviation (%RSD) was calculated from the mean peak areas obtained to determine the precision of the method.

2.5.5 Detection Limit and Quantitation Limit

The detection limit (DL) and quantitation limit (QL) of the developed method were determined in accordance with ICH guidelines using the standard deviation of the response and the slope of the respective calibration curves for p-coumaric acid and caffeic acid. The DL and QL values were calculated using the following equations:

$$DL = 3.3 \times \frac{1 \times \sigma}{S}$$

$$QL = 10 \times \frac{1 \times \sigma}{S}$$

Where σ is the standard deviation of the response, and S is the slope obtained from the calibration curve.

2.5.6 Robustness

The robustness of the proposed HPTLC method was assessed by intentionally introducing small variations in selected analytical parameters, including the detection wavelength (325 ± 2 nm), chamber saturation time (30 ± 5 min), and the composition of the mobile phase (± 1). The effects of these deliberate changes on the peak areas and R_f values were evaluated, and the percentage relative standard deviation (%RSD) was calculated for each parameter to determine the robustness of the developed method.

3. Results and discussion

3.1 Optimisation of chromatographic conditions

Various chromatographic parameters, including the composition of the mobile phase and chamber saturation time, were systematically optimised to achieve efficient chromatographic separation and satisfactory analytical performance. Several solvent systems were evaluated, namely (1) Methanol: water: formic acid (4:5.7:0.3, v/v/v), (2) Toluene: ethyl acetate: formic acid: water (3:3:0.8:0.2, v/v/v/v), (3) Benzene: acetic acid: water (3.7:4.5:1.8, v/v/v), and (4) Toluene: ethyl acetate: formic acid (7:3:0.5, v/v/v), (5) Toluene: Ethyl acetate (7:3, v/v) using different solvent ratios to obtain sharp, symmetrical, and well-resolved peaks for the selected biomarker compounds. When formic acid was omitted from the mobile phase, poor peak resolution accompanied by significant peak tailing was observed. However, the inclusion of formic acid (0.5%, v/v) markedly improved peak symmetry and resulted in better chromatographic resolution. Among the solvent systems investigated, toluene: ethyl acetate: formic acid (7:3:0.5, v/v/v) produced the most satisfactory separation, yielding distinct R_f values of 0.44 for caffeic acid and 0.58 for p-coumaric acid. Chromatographic development was performed in a twin-trough TLC chamber pre-saturated with the optimised mobile phase for 30 min at room temperature.

To determine the optimum detection wavelength, the UV absorption spectra of caffeic acid and p-coumaric acid were recorded (Fig. 2). Both biomarker compounds exhibited maximum absorbance at 325 nm; therefore, densitometric scanning of the developed HPTLC plates was performed directly at this wavelength without the need for derivatisation. The chromatograms obtained at 325 nm displayed well-resolved and distinct peaks for both p-coumaric acid and caffeic acid, confirming the suitability of the optimised chromatographic conditions for their effective separation and detection (Fig. 3). Optimized chromatographic conditions used for simultaneous estimation of caffeic acid and p-coumaric acid by HPTLC method are as shown in Table 1.

Table 1
Optimized chromatographic conditions used for simultaneous estimation of caffeic acid and p-coumaric acid by HPTLC method

Parameters	Conditions
Band width	6 mm
Micro-syringe	100 µl
Pre coated silica gel thickness	250 µm
Plate dimension	10 cm x 10 cm
Development	Linear ascending
Chamber	Twin trough glass chamber
Saturation time	30 minutes
Length of chromatographic run	8 cm
Wavelength	325 nm
Slit dimension	6.00 x 0.45 nm
Mobile phase composition	Toluene: Ethyl acetate: Formic acid (7:3:0.5, v/v/v)

3.2 HPTLC Fingerprinting

The HPTLC fingerprint chromatogram of the hydroalcoholic extract of the female inflorescence of *Typha domingensis* exhibited distinct chromatographic bands corresponding to caffeic acid and p-coumaric acid, with R_f values of 0.44 and 0.58, respectively, when scanned at 325 nm. The fingerprint profile was generated to identify these marker compounds in the extract by comparing their chromatographic characteristics with those of the corresponding reference standards under the optimised chromatographic conditions. The HPTLC fingerprinting profile is presented in Fig. 4.

3.3 Method Validation

The developed HPTLC method was validated in accordance with the ICH Q2(R2) guidelines by evaluating analytical parameters such as specificity test, response, accuracy test, precision, lower range (DL, QL) and robustness.

The specificity of the method was established by comparing the chromatographic profiles and R_f values of the hydroalcoholic extract with those of the corresponding reference standards. The R_f values and resolved chromatographic peaks obtained for p-coumaric acid and caffeic acid in the extract were consistent with those of the standard compounds, confirming the specificity and reliability of the

developed HPTLC method. The peak purity characteristics confirmed the identity and spectral homogeneity of caffeic acid and p-coumaric acid in the hydroalcoholic extract as shown in Fig. 5.

Calibration curves for caffeic acid and p-coumaric acid were established by plotting the peak area against the corresponding concentration over the range of 200–1200 ng/band. Both biomarker compounds exhibited excellent response (calibration curve) as shown in Fig. 6 and the corresponding 3D densitograms of the reference standards and hydroalcoholic extract are presented in Fig. 7, within the selected concentration range. Linear Regression parameters for caffeic acid and p-coumaric acid by HPTLC Method are as shown in Table 2.

Table 2
Linear Regression parameters for caffeic acid and p-coumaric acid by HPTLC Method

Parameters	caffeic acid	p-coumaric acid
Calibration range (ng/band)	200–1200	200–1200
R _f value	0.44	0.58
Regression equation	6.9713x + 4356.9	19.35x – 134.87
Correlation coefficient (R ²)	0.996	0.9975
Standard deviation of slope	0.182744	0.374176
Standard deviation of intercept	137.352	74.99982
Detection Limit (ng/band)	65.01872	12.79089
Quantitation Limit (ng/band)	197.0264	38.76027

The accuracy of the method was assessed using the standard recovery approach at spiking levels of 50%, 100%, and 150%. The percentage recovery of caffeic acid ranged between 99.44% to 101.78%, whereas p-coumaric acid exhibited recoveries ranging from 99.15% and 99.24%. These recovery values confirmed the satisfactory accuracy of the developed HPTLC method. (Table 3)

Table 3
Recovery study of caffeic acid and p-coumaric acid

Phytoconstituents	Level	Concentration present from extract (ng/band)	Concentration of standard added (ng/band)	Concentration recovered (ng/band) $\pm$ SD ^a	% Mean recover $\pm$ SD ^a
	Unspiked	400	-	-	-
Caffeic acid	50%	400	200	596.66 $\pm$ 1.16	99.44 $\pm$ 0.19
	100%	400	400	814.26 $\pm$ 3.70	101.78 $\pm$ 0.46
	150%	400	600	996.61 $\pm$ 2.51	99.66 $\pm$ 0.25
p-coumaric acid	50%	400	200	595.44 $\pm$ 2.81	99.24 $\pm$ 0.46
	100%	400	400	793.21 $\pm$ 1.02	99.15 $\pm$ 0.12
	150%	400	600	992.08 $\pm$ 29.03	99.20 $\pm$ 2.90
^a SD, standard deviation (n = 3)					

The precision study of the developed HPTLC method was evaluated by determining both intraday and intraday precision at concentrations of 200, 800, and 1200 ng/band. For intraday precision, the %RSD values ranged from 0.76% to 1.06% for caffeic acid and from 0.68% to 1.58% for p-coumaric acid. Similarly, the interday precision study showed %RSD values ranging from 0.95% to 1.36% for caffeic acid and from 0.57% to 1.05% and for p-coumaric acid. The low %RSD values obtained in both studies demonstrated the good precision and reproducibility of the developed analytical method. (Table 4)

Table 4
Precision Study of caffeic acid and p-coumaric acid

Phytoconstituents	Concentration (ng/band)	Intraday	Interday		
		Mean area $\pm$ SD ^a	%RSD ^b	Mean area $\pm$ SD ^a	%RSD ^b
Caffeic acid	200	5446.33 $\pm$ 41.86	0.76	5451.67 $\pm$ 53.57	0.98
	800	10132 $\pm$ 107.46	1.06	10016 $\pm$ 95.44	0.95
	1200	12446 $\pm$ 102.50	0.82	12653.33 $\pm$ 172.43	1.36
p-coumaric acid	200	4350.17 $\pm$ 46.45	1.06	4278.27 $\pm$ 24.46	0.57
	800	15440.33 $\pm$ 244.15	1.58	15657 $\pm$ 164.52	1.05
	1200	23774.7 $\pm$ 162.23	0.68	23515.2 $\pm$ 206.58	0.87
^a SD, standard deviation (n = 3)					
^b %RSD, percent relative standard deviation (SD/mean $\times$ 100)					

The robustness of the proposed method was examined by introducing small, deliberate variations in chromatographic parameters, including the detection wavelength, mobile phase composition, and chamber saturation time. Under these modified conditions, the %RSD values for the recorded peak areas remained below 2%, indicating that the method was robust and unaffected by minor experimental variations. (Table 5)

Table 5
Robustness study of p-coumaric and caffeic acid

Parameters	Optimized condition	Change in condition	Mean area $\pm$ SD ^a	% RSD ^b
Caffeic acid				
Wavelength (nm)	325	323	10289 $\pm$ 104.36	1.01
		327	10440.66 $\pm$ 70.44	0.67
Saturation time (min.)	30	25	10371 $\pm$ 107.39	1.03
		35	10412.33 $\pm$ 95.03	0.91
Mobile phase (V/V)	Toluene: ethyl acetate: formic acid (7:3:0.5)	6.5:3.5:0.5	10159 $\pm$ 130.03	1.27
		7.5:2.5:0.5	10376 $\pm$ 106.97	1.03
p-coumaric acid				
Wavelength (nm)	325	323	15780.67 $\pm$ 142.42	0.90
		327	15931.66 $\pm$ 78.57	0.49
Saturation time (min.)	30	25	15546.67 $\pm$ 209.03	1.34
		35	15622.33 $\pm$ 211.86	1.35
Mobile phase (V/V)	Toluene: ethyl acetate: formic acid (7:3:0.5)	6.5:3.5:0.5	15823.33 $\pm$ 96.09	0.60
		7.5:2.5:0.5	15829 $\pm$ 133.29	0.84
^a SD, standard deviation (n = 3)				
^b %RSD, percent relative standard deviation (SD/mean $\times$ 100)				

4. Conclusions

A simple, precise, accurate, robust, and specific HPTLC method was successfully developed and validated for the simultaneous quantification of p-coumaric acid and caffeic acid in the hydroalcoholic extract of the female inflorescence of *Typha domingensis*. The validated method demonstrated excellent linearity, precision, and recovery over the selected concentration range and fulfilled the validation requirements outlined in the ICH Q2(R2) guidelines.

The HPTLC fingerprint profile generated for the hydroalcoholic extract can serve as a reliable tool for the identification and authentication of *Typha domingensis*. In addition, the proposed method can be effectively employed for the routine quality control, phytochemical standardization, and batch-to-batch consistency assessment of herbal extracts and formulations containing p-coumaric acid and caffeic acid as marker compounds.

Abbreviations

HPTLC

High Performance thin Layer Chromatography

ICH

International Council for Harmonisation

DL

Detection Limit

QL

Quantitation Limit

RSD

Relative Standard Deviation

UV

Ultraviolet

TLC

Thin-Layer Chromatography

CAMAG

CAMAG Chromatography System*

R_f

Retardation Factor

TD

Typha domengensis

Declarations

Acknowledgements:

The authors express their sincere gratitude to Indukaka Ipcowala College of Pharmacy, New V.V. Nagar, and Charutar Vidyamandal University for providing the facilities and necessary support to successfully conduct this research work.

Funding:

This work was supported by the Student Startup and Innovation Policy (SSIP) 2.0, Government of Gujarat, under the Proof of Concept (PoC) proposal approved at the Charutar Vidya Mandal University

Innovation and Startup Council (CVMUISC) through a financial grant of ₹35,000.

Conflict of Interest:

Authors declare no conflict of interest.

References

1. Sofowora A. *Medicinal Plants and Traditional Medicine in Africa*. Spectrum Books Ltd; 1993.
2. Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy*. Nirali Prakashan; 2010.
3. Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. *J Nat Prod*. 2007;70(3):461–477.
4. Rates SMK. Plants as source of drugs. *Toxicon*. 2001;39(5):603–613.
5. World Health Organization. *WHO Traditional Medicine Strategy 2014–2023*. Geneva: WHO; 2013.
6. Mukherjee PK. *Quality Control of Herbal Drugs*. Elsevier; 2019.
7. Kunle OF, Egharevba HO, Ahmadu PO. Standardization of herbal medicines. *Int J Biodivers Conserv*. 2012;4(3):101–112.
8. Li S, Han Q, Qiao C, et al. Chemical markers for quality control of herbal medicines. *Chin Med*. 2008;3:7.
9. Bent S. Herbal medicine in the United States. *J Gen Intern Med*. 2008;23(6):854–859.
10. Choudhary N, Sekhon BS. An overview of advances in standardization of herbal drugs. *J Pharm Educ Res*. 2011;2(2):55–70.
11. Reich E, Schibli A. *High-Performance Thin-Layer Chromatography for the Analysis of Medicinal Plants*. Thieme; 2007.
12. Sethi PD. *High Performance Thin Layer Chromatography*. CBS Publishers; 1996.
13. Srivastava MM. *High-performance thin-layer chromatography*. Springer; 2011.
14. Wagner H, Bladt S. *Plant Drug Analysis: A Thin Layer Chromatography Atlas*. Springer; 2009.
15. Sherma J, Fried B. *Handbook of Thin-Layer Chromatography*. CRC Press; 2003.
16. Patel RP, Patel MM. HPTLC method in herbal drug standardization. *J AOAC Int*. 2008;91:114–120.
17. Ferenczi-Fodor K, et al. Validation of planar chromatographic methods. *J AOAC Int*. 2001;84:1265–1276.
18. Shah B, Seth AK. Simultaneous estimation of phytoconstituents using HPTLC. *J Planar Chromatogr*. 2010;23:52–58.
19. Chauhan A, Semwal DK, et al. Analytical methods in herbal standardization. *J Pharm Anal*. 2014;4(6):367–378.
20. Reich E. Modern HPTLC applications in herbal drug analysis. *J AOAC Int*. 2007;90:28–35.
21. Simultaneous quantification of quercetin and solasodine biomarkers from *Solanum nigrum* Linn. berry extract using a validated high-performance thin-layer chromatography-densitometric method.

22. Grace JB, Harrison JS. Biology of *Typha* species. *Aquat Bot.* 1986;23:1–11.
23. Cronquist A. *An Integrated System of Classification of Flowering Plants*. Columbia University Press; 1981.
24. Duke JA. *Handbook of Medicinal Herbs*. CRC Press; 2002.
25. Ahmad M, et al. Traditional medicinal uses of wetland plants. *Ethnobot Res Appl.* 2013;11:203–215.
26. Kizil S, et al. Ethnobotanical uses of *Typha* species. *J Ethnopharmacol.* 2008;117:175–183.
27. Moerman DE. *Native American Ethnobotany*. Timber Press; 1998.
28. Vymazal J. Plants used in constructed wetlands. *Hydrobiologia.* 2011;674:133–156.
29. Harborne JB. *Phytochemical Methods*. Springer; 1998.
30. Evans WC. *Trease and Evans Pharmacognosy*. Elsevier; 2009.
31. Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev.* 1999;12(4):564–582.
32. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants. *Oxid Med Cell Longev.* 2009;2(5):270–278.
33. Rice-Evans CA, Miller NJ. Antioxidant activities of flavonoids. *Free Radic Biol Med.* 1996;20:933–956.
34. Prior RL, Cao G. Antioxidant phytochemicals in fruits and vegetables. *HortScience.* 2000;35(4):588–592.
35. Scalbert A, et al. Dietary polyphenols and prevention of diseases. *Crit Rev Food Sci Nutr.* 2005;45:287–306.
36. Halliwell B. Free radicals and antioxidants. *Lancet.* 1994;344:721–724.
37. Phytochemical, Cytotoxic, and Antimicrobial Evaluation of *Tribulus terrestris* L., *Typha domingensis* Pers., and *Ricinus communis* L.: Scientific Evidences for Folkloric Uses.
38. Phytochemical profiling, in vitro biological activities, and in-silico molecular docking studies of *Typha domingensis*.
39. Heim KE, Tagliaferro AR. Flavonoid antioxidants. *J Nutr Biochem.* 2002;13:572–584.
40. Heleno SA, et al. Phenolic acids: natural sources and bioactivities. *Food Chem.* 2015;173:501–513.
41. Kumar N, Goel N. Phenolic acids: health benefits. *Biotechnol Rep.* 2019;24:e00370.
42. Espíndola KM, et al. Caffeic acid and derivatives. *Front Chem.* 2019;7:541.
43. Pei K, et al. p-Coumaric acid and pharmacological activities. *Molecules.* 2016;21:625.
44. Magnani C, Isaac VLB. Caffeic acid: biological properties. *Int J Pharm Pharm Sci.* 2015;7(11):1–7.
45. Liang YZ, Xie P, Chan K. Quality control of herbal medicines. *J Chromatogr B.* 2004;812:53–70.
46. Rice-Evans C. Mechanism of antioxidant action of phenolics. *Free Radic Biol Med.* 1995;20:933–956.
47. Pei K, et al. Biological applications of p-coumaric acid. *Molecules.* 2016;21:625.
48. Mukherjee PK, et al. Phytochemical standardization of herbal drugs. *J Ethnopharmacol.* 2008;115:1–8.

49. Snyder LR, Kirkland JJ. *Practical HPLC Method Development*. Wiley; 2010.
50. Bakshi M, Singh S. Development of validated analytical methods. *J Pharm Biomed Anal*. 2002;28:1011–1040.
51. International Council for Harmonisation (ICH). *ICH Q2(R2): Validation of Analytical Procedures*. 2023.
52. Gupta M, Sharma PK, Ansari SH, Lagarkha R. Pharmacognostical evaluation and chromatographic fingerprinting of medicinal plants. *Int J Pharm Sci Rev Res*. 2011;8(2):45–52.

Figures



Figure 1

Female inflorescence of *Typha domingensis* Pers.

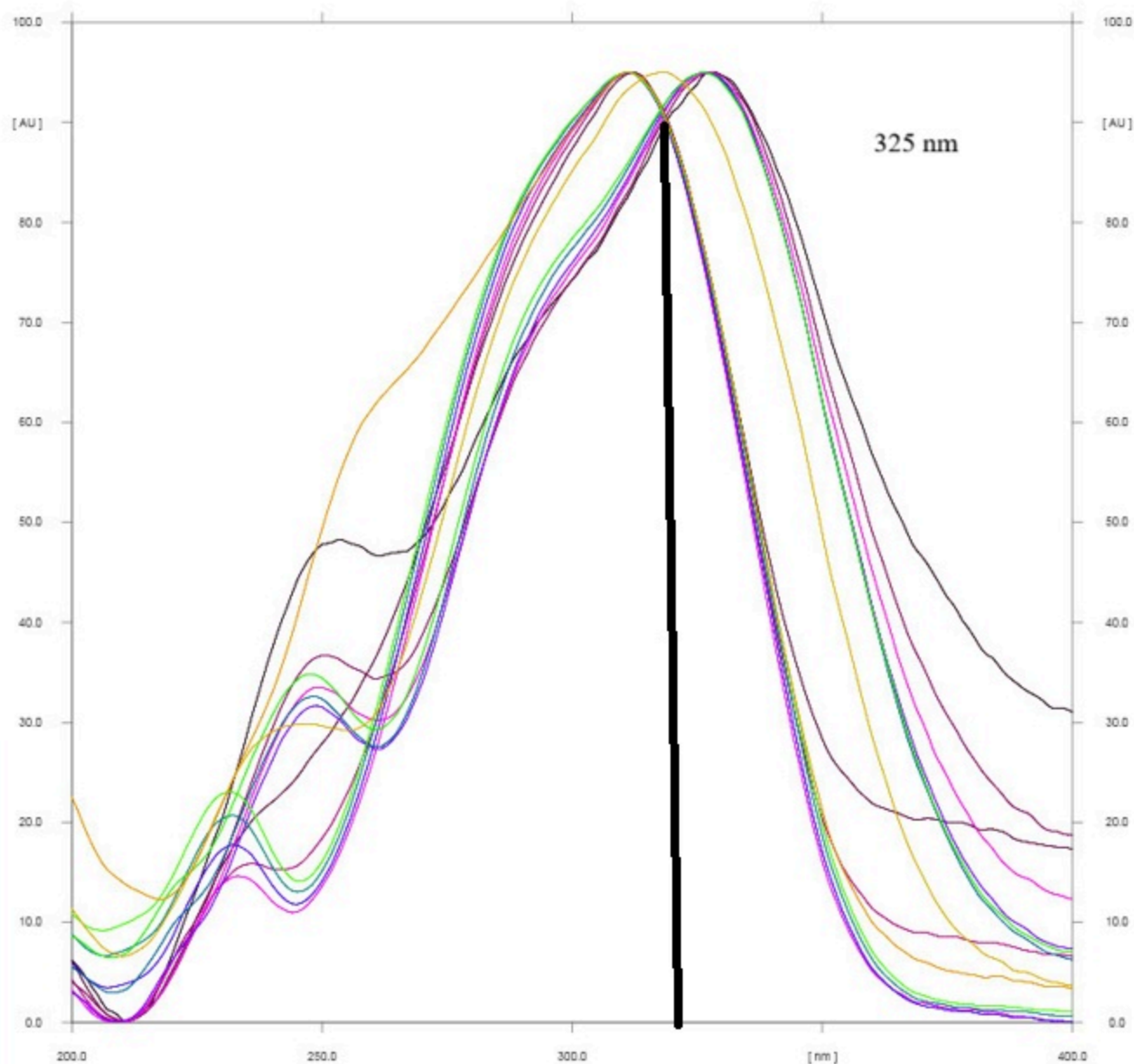


Figure 2

Overlay UV-Visible absorption spectra of p-coumaric acid and caffeic acid.

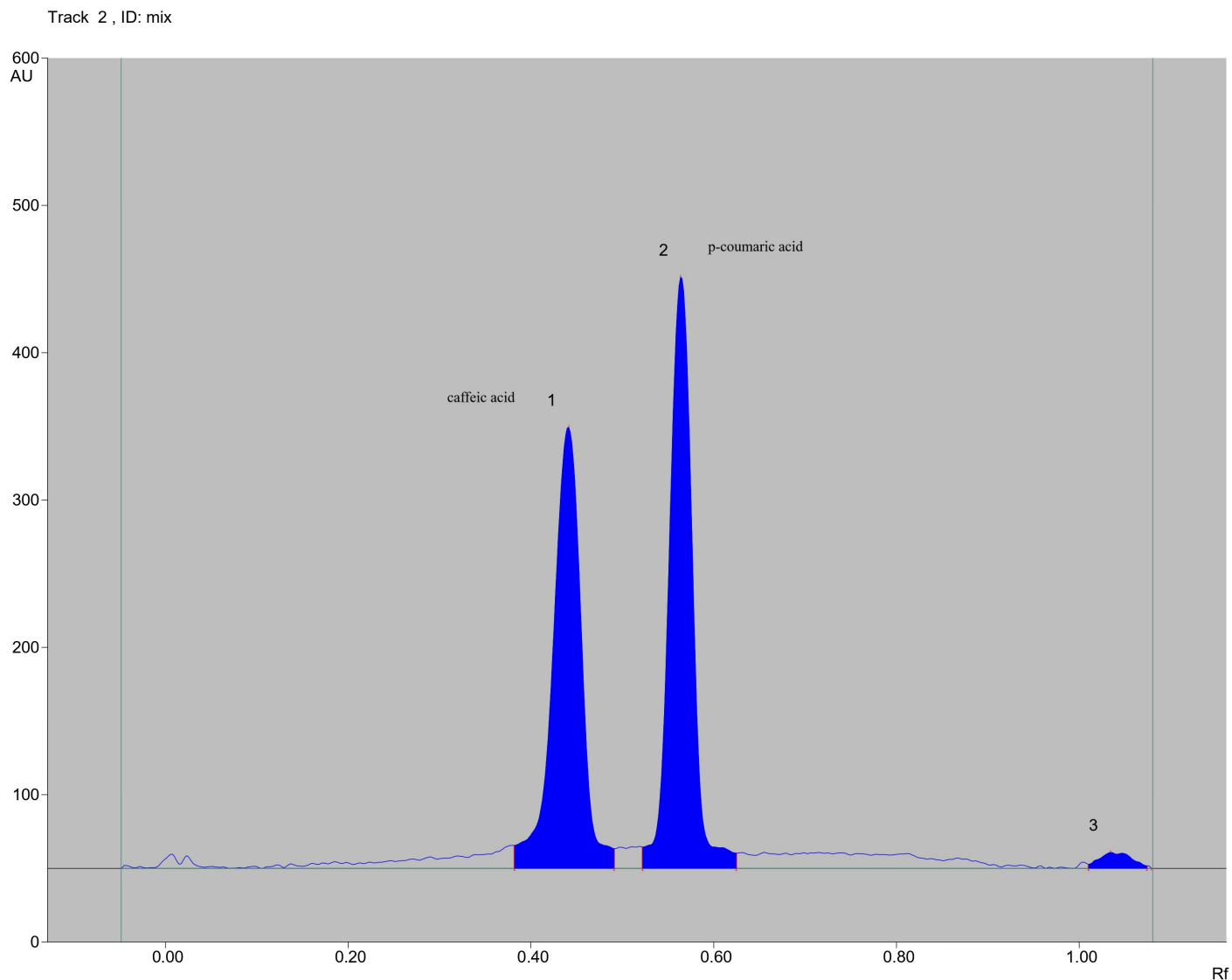


Figure 3

HPTLC densitograms of p-coumaric acid and caffeic acid recorded at 325 nm under optimized chromatographic conditions.

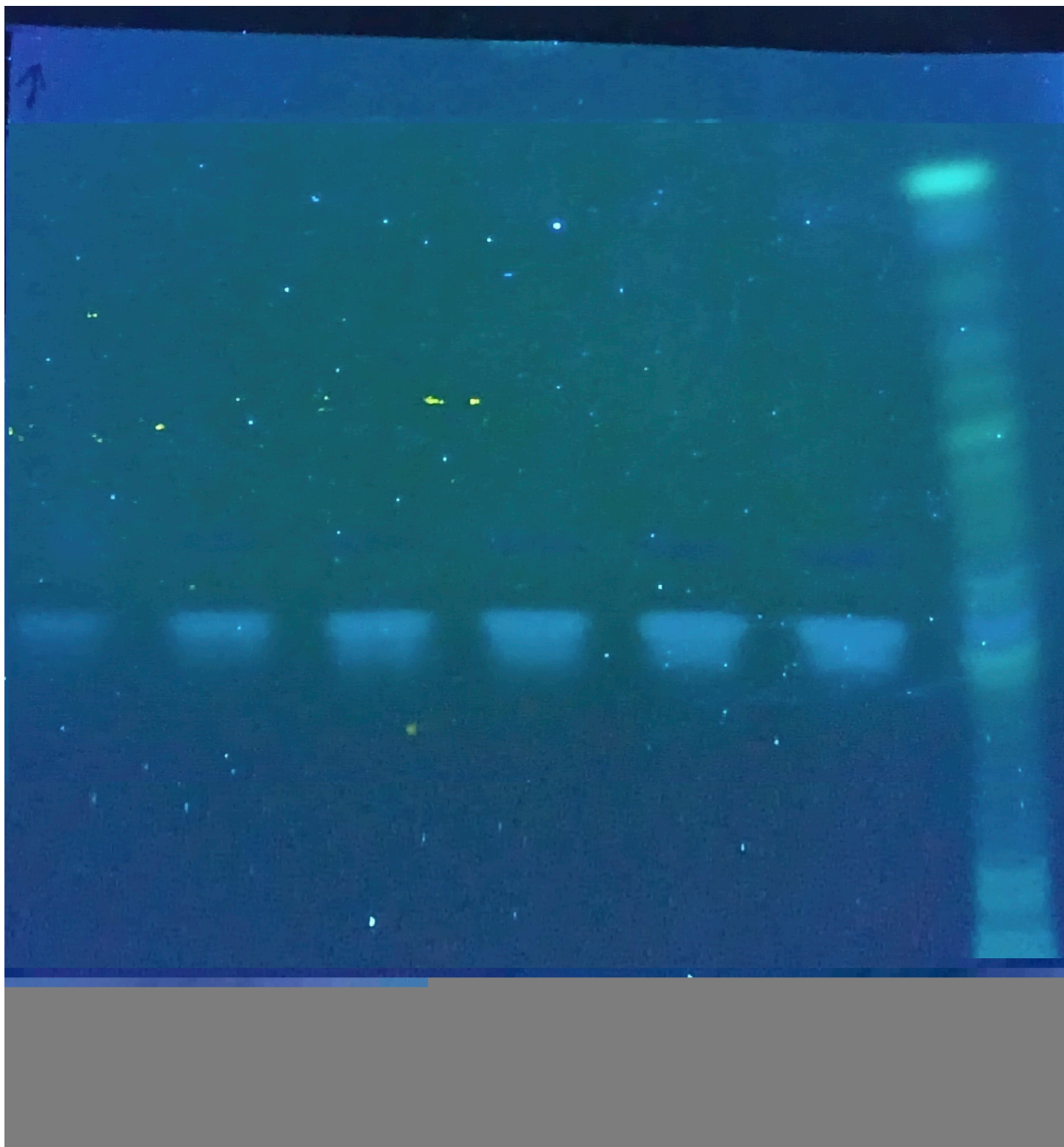


Figure 4

HPTLC fingerprinting profile of the sample showing distinct bands of p-coumaric acid and caffeic acid.

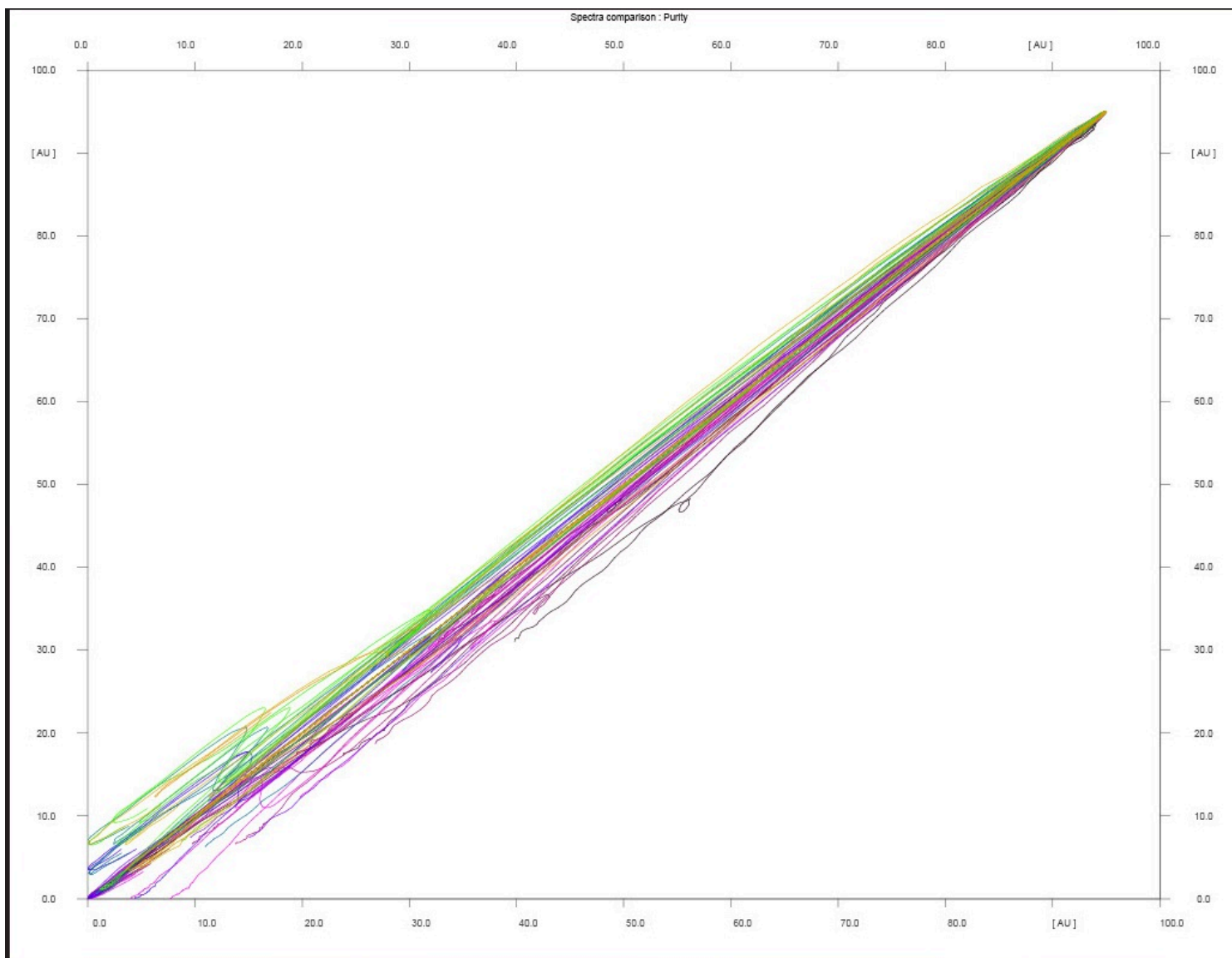
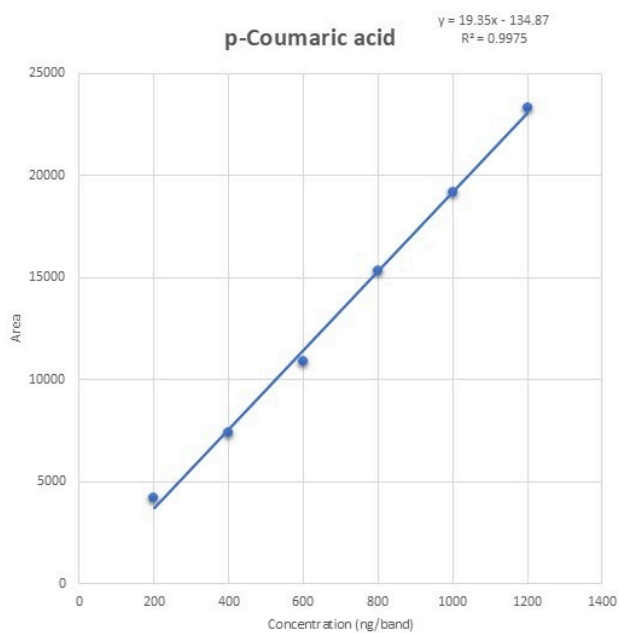
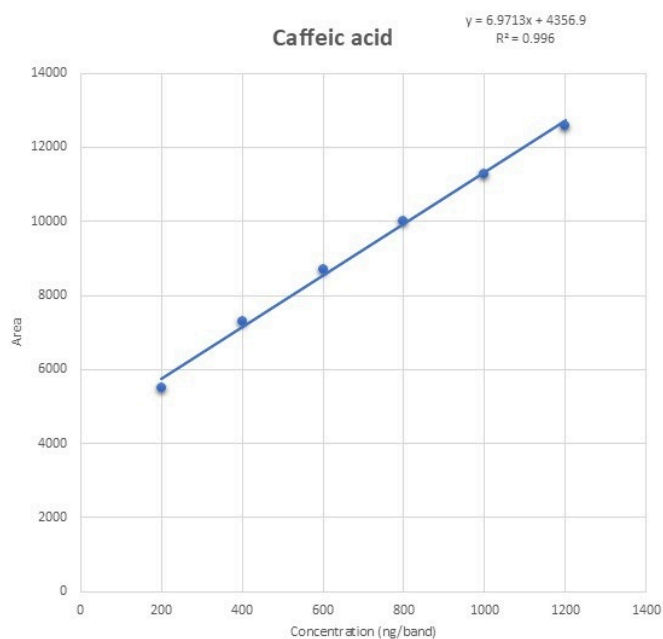


Figure 5

Peak purity assessment of p-coumaric acid and caffeic acid in the hydroalcoholic extract



A



B

Figure 6

Calibration curve of (A)- p-Coumaric acid and (B) Caffeic acid

Image not available with this version

Figure 7

3D densitograms of the reference standards and hydroalcoholic extract