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Development of a Validated HPTLC Method for the Simultaneous Estimation of Curcumin and Berberine in Dashamoola Kvatha

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Abstract

Background: *Many people use herbal remedies like Dashamoola Kvatha (a traditional Ayurvedic decoction made from ten medicinal roots) because they help with pain and inflammation [18]. It is hard to standardize these kinds of multi-ingredient formulations because there aren't any quality control methods for active phytoconstituents [16]. Curcumin, derived from Curcuma longa, and berberine, sourced from Berberis aristata, are bioactive compounds recognized for their anti-inflammatory properties and are believed to enhance the therapeutic effects of Dashamoola.* ***Objectives:*** *The current study sought to establish and validate a straightforward, precise, and accurate High-Performance Thin-Layer Chromatography (HPTLC) technique for the concurrent quantification of curcumin and berberine in Dashamoola Kvatha.* ***Materials and Methods:*** *Using silica gel 60F_{254} plates and n-propanol:water:glacial acetic acid (8:1:1, v/v) as the mobile phase, an HPTLC-densitometric method was improved. At 358 nm (an isoabsorptive wavelength), both curcumin and berberine were found at the same time. The method was confirmed to be in line with ICH Q2 (R1) standards for specificity, linearity, precision, accuracy, robustness, limit of detection (LOD), and limit of quantification (LOQ).* ***Results:*** *Clear peaks for berberine ($R_f \approx 0.33$) and curcumin ($R_f \approx 0.77$) were found. There were linear relationships (r^2 > 0.99) in the calibration plots for berberine in the range of 200–600 ng/spot and for curcumin in the range of 400–1200 ng/spot. The LODs were about 58 ng/spot for berberine and about 171 ng/spot for curcumin. The method's accuracy (mean recoveries of about 99–101%) and precision (%RSD < 2%) were both acceptable. In conclusion: The HPTLC method that was created is quick, cheap, and dependable for measuring curcumin and berberine in Dashamoola Kvatha at the same time. You can use it for regular quality control of this polyherbal decoction to make sure that each batch is the same and works.*

Keywords: *Dashamula, polyherbal formulation, curcumin, berberine, HPTLC, method validation*

INTRODUCTION

To make sure that herbal medicines are safe, effective, and of good quality, they need to be very carefully standardized [16]. Polyherbal remedies often don't have clear quality control standards, which can cause differences in how well they work compared to single-compound drugs. Regulatory bodies and researchers have underscored the necessity for analytical techniques to quantify essential bioactive markers in herbal products as a standardization metric [1]. High-performance thin-layer chromatography (HPTLC) is an advanced chromatographic technique commonly employed in herbal drug analysis, providing a straightforward and expedited method for the concurrent quantification of various phytochemicals [6]. HPTLC has been effectively utilized to fingerprint and quantify active constituents in intricate mixtures, enhancing methodologies such as HPLC and spectrophotometry [7].

Dashamoola Kvatha is a traditional Ayurvedic decoction made from the roots of ten plants that have been used for a long time to treat pain, inflammation, arthritis, and other similar conditions. Ayurvedic texts say that it is an analgesic, an anti-arthritic, and an anti-rheumatic formulation. Pharmacological studies have confirmed its anti-inflammatory and analgesic properties in experimental models [18]. However, it is hard to make sure that Dashamoola formulations are always the same because the ten roots have a lot of different phytochemicals in them. Two herbs in Dashamoola are especially interesting for their anti-inflammatory properties: Haridra (Curcuma longa, turmeric) and Daruharidra (Berberis aristata, tree turmeric). These plants are high in curcumin and berberine, which are both compounds that have a wide range of pharmacological effects, such as anti-inflammatory, antioxidant, and antimicrobial effects [4] [5] . People have called curcumin a "golden" phytochemical because it has so many possible health benefits [17]. Berberine, on the other hand, is an isoquinoline alkaloid with a wide range of pharmacological effects [4]. Curcumin and berberine are good chemical markers for quality control in formulations that contain Curcuma longa and Berberis aristata, whether they are part of the classic recipe or included in modified proprietary versions of Dashamoola Kvatha [5]. Even if not all Dashamoola ingredients have these compounds, they should be checked for standardization purposes when they are added or naturally found in other polyherbal products [3].

Many analytical techniques have been documented for the quantification of curcumin or berberine separately in plant extracts and formulations, such as HPLC, UV-visible spectrophotometry, and HPTLC [23] . For example, there are sensitive HPTLC and HPLC methods for berberine in Berberis species and Ayurvedic mixtures, and there are different tests for curcumin in turmeric and polyherbal supplements [17]. Derivative spectrophotometry has been employed to concurrently analyze curcumin and berberine in mixed extracts [14]. Nonetheless, a dependable chromatographic technique for the concurrent quantification of curcumin and berberine in Dashamoola Kvatha has yet to be documented in the literature. Parekh et al. devised an HPTLC method for these markers in an alternative polyherbal powder (Gruhadhoomadi churna) [9], and several HPLC methods have focused on this combination in laboratory mixtures [20]. Yet, no validated method specifically for Dashamoola Kvatha (a decoction matrix) is available for routine quality assessment.

In this context, the objective of the present work was to develop a simple, precise, and accurate HPTLC-densitometric method for simultaneous estimation of curcumin and berberine in Dashamoola Kvatha, and to validate the method according to international guidelines [8]. By establishing such a method, we aim to enable quality control laboratories to quantitatively assess these bioactive markers in the formulation, thereby ensuring consistency in therapeutic content. This work also helps the larger goal of using modern analytical methods to standardize traditional herbal medicines [1].

METHODS AND MATERIALS

Chemicals and Reagents: We got analytical grade curcumin (purity >98%) and berberine chloride (purity >98%) reference standards from trusted sources. We used HPTLC plates (10 cm × 10 cm) that had already been coated with silica gel 60 F₂₅₄ (Merck) as the stationary phase. The solvents n-propanol, glacial acetic acid, and distilled water were of analytical grade (S.D. Fine Chemicals, Mumbai). The rest of the chemicals were of analytical reagent grade.

Samples of Herbal Formulation: Dashamoola Kvatha (decoction) samples were prepared from authenticated crude drugs corresponding to the ten roots of Dashamoola (purchased from a licensed Ayurvedic pharmacy). An in-house lab formulation was prepared by coarsely powdering the dried roots (equal parts) and boiling 5 g of the powder in 100 mL water to obtain the decoction, which was filtered and dried. Additionally, a marketed Dashamoola Kvatha formulation (dry extract) was obtained from a

commercial Ayurvedic manufacturer for analysis. These two samples were labeled as "In-house" and "Marketed" for method application and recovery studies.

Instrumentation and HPTLC Conditions: Chromatographic analysis was performed on a CAMAG HPTLC system (Muttenz, Switzerland) consisting of an automatic TLC sampler (Linomat 5) and TLC Scanner 3 with WinCATS software. The methodology followed standard HPTLC procedures as described in the literature [24]. Samples (standard and extract) were applied as 6 mm wide bands on the silica gel plate, 10 mm from the bottom edge and 10 mm apart, using a 100 µL Hamilton syringe and the Linomat applicator. A mobile phase of n-propanol:water:glacial acetic acid (8:1:1, v/v/v) was developed through systematic trials based on reported systems for curcumin and berberine separation [9]. The mobile phase was poured into a CAMAG twin-trough development chamber and allowed to saturate for 20 min with filter paper lining. Plates were developed by ascending technique to a distance of 80 mm at ambient temperature (25 ± 2 °C). After development, the plates were dried and then scanned with the densitometer. The detection was carried out in absorbance mode at 358 nm, which was found to be the iso-absorptive wavelength for curcumin and berberine (both compounds have appreciable absorbance at this wavelength). A deuterium lamp provided the UV illumination for scanning. The slit dimensions of the scanner were 5.0 mm × 0.45 mm and the scanning speed was 20 mm/s. The spectrodensitometric analysis produced chromatographic peaks with absorption spectra; peak identity was confirmed by overlaying sample and standard UV spectra directly from the plate (peak purity check). Figure 1 shows a representative densitogram of standard curcumin and berberine under the optimized conditions.

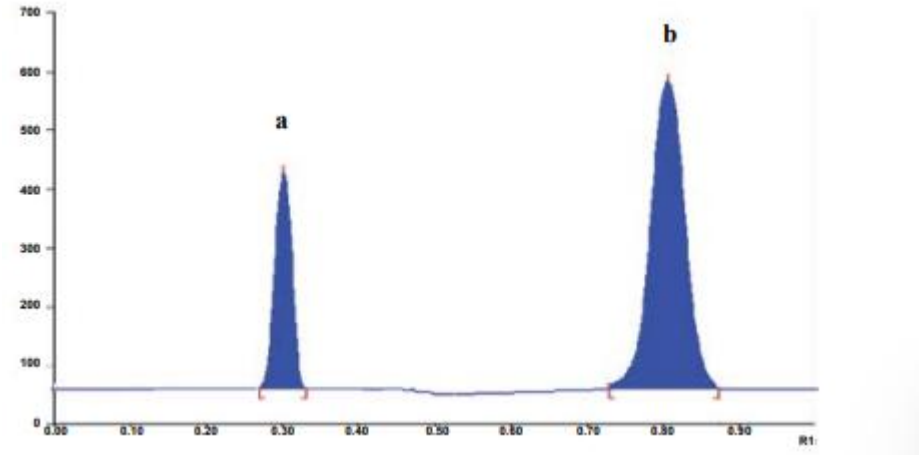

Figure 1: HPTLC densitogram (scanner output) of standard berberine (peak a) and curcumin (peak b) under the developed method, showing well-resolved peaks at R_f ≈ 0.33 and 0.77 respectively.

Making Standard and Sample Solutions: We made stock standard solutions of curcumin and berberine at a concentration of 1 mg/mL in methanol. We made working standard solutions by adding more methanol to the stocks. An extraction method was used for the herbal samples. About 2.0 g of the dried Dashamoola Kvatha powder or extract was weighed out and put through Soxhlet extraction with 50% methanol for 6 to 8 hours. We filtered and concentrated the extract under low pressure. The residue was redissolved in methanol and brought to a known volume (10 mL) to make the sample stock solution. We put this solution in a centrifuge and used the clear supernatant for HPTLC analysis. To test curcumin and berberine, the right amounts of sample solution were put on the HPTLC plate so that the spots were in the linear range of the method, which was expected based on earlier tests.

Calibration and Quantification: For each marker, we made calibration curves by putting a series of standard solution spots in increasing amounts. Seven different concentrations were used for each compound. These concentrations ranged from about 50% to 150% of what was expected to be in the sample. We used 200–600 ng of berberine and 400–1200 ng of curcumin per spot, depending on how much of each substance was present and how the detector reacted. Three spots of each concentration were put on the plate. The peak area of each band was measured after development and scanning at 358 nm. The calibration plot of peak area against amount underwent least-squares linear regression. The slope, intercept, and correlation coefficient (r^2) were found for both curcumin and berberine.

Validating the Method: The HPTLC method was confirmed to be correct by following the ICH Q2 (R1) rules for analytical method validation [8]. The following factors were looked at:

- **Specificity:** The capacity to distinctly evaluate the analytes in the presence of other components was validated by comparing the UV absorption spectra of curcumin and berberine from sample bands with those of standards. The spectra of the bands in the sample extract were the same as the spectra of the reference standards at the same R_f values. This showed that the spots were unique and identifiable. Also, the purity of each peak was checked by scanning it at the beginning, middle, and end. The fact that these spectra matched showed that impurities did not migrate together.
- **Linearity:** The calibration curves were used to check for linearity, as explained above. An r^2 value of at least 0.99 over the chosen range was the acceptance criterion.
- Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ were calculated using signal-to-noise ratios of about 3:1 and 10:1, respectively. We used serial dilutions of known low concentrations of standards (n=6) and the standard deviation (SD) of the response and slope (S) of the calibration curve to find LOD = 3.3×(SD/S) and LOQ = 10×(SD/S), as the ICH guidelines say to do.
- **Precision:** Repeatability (intra-day precision) was assessed by analyzing six replicates of a single concentration of curcumin (800 ng/spot) and berberine (400 ng/spot) on the same day, while intermediate precision (inter-day) was evaluated through analysis conducted over three consecutive days at three concentration levels (200, 400, 600 ng/spot for berberine; 400, 800, 1200 ng/spot for curcumin). In each case, the % relative standard deviation (%RSD) of the peak areas was found. We checked the system's accuracy by scanning the same spot six times and figuring out the %RSD of the response.
- **Accuracy (Recovery):** Recovery studies using the standard addition method were used to figure out how accurate the method was. Pre-analyzed sample extracts (of both In-house and Marketed Kvatha) were augmented with specified quantities of curcumin or berberine standards at three concentrations: 80%, 100%, and 120% of the original amount, in triplicate. We used the spiked samples and looked at them. For each level, the percent recovery was found by dividing the observed amount by the theoretical amount and multiplying by 100. The range of 98–102% was used to define acceptable recovery. We wrote down the mean recovery for each analyte at each level and the overall mean recovery (see Results).
- **Robustness:** To test the method's robustness, small, planned changes were made to the chromatographic conditions. The factors examined encompassed slight alterations in mobile phase composition (±0.2 mL of n-propanol or water), chamber saturation duration (±5 min), and development distance (±5 mm). For every change in conditions, we did three tests of a mid-level standard (curcumin 800 ng, berberine 400 ng) and compared the R_f and peak areas. We used the %RSD of peak areas and the consistency of Rf to see how strong it was.

We used Excel to process all the data and check the statistical validity. The results are shown in the right tables and figures.

RESULTS

Chromatographic Separation: The improved HPTLC method made it easy to tell the difference between berberine and curcumin by showing clear, well-defined peaks that didn't tail off. The n-propanol:water:acetic acid (8:1:1) mobile phase system worked best out of all the trial solvents because it gave the best resolution and peak shapes that were the same on both sides. The separation was good enough, and other extractive parts of Dashamoola Kvatha didn't get in the way. The 20-minute chamber saturation time was found to be very important for getting the same R_f values and peak areas every time. The densitogram (Figure 1) shows that berberine and curcumin were resolved at retention factors Rf = 0.33 ± 0.03 and 0.77 ± 0.03, respectively. These R_f values are in line with what has been reported in the literature for berberine and curcumin in similar polar mobile phase systems [9]. This confirms that the spots are the same. The overlay of UV spectra from the scanned bands matched the reference spectra of each marker even better, showing that they were detected in a specific way.

Figure 2 shows the UV-Vis absorption spectra of curcumin and berberine to show the spectral properties. Curcumin and berberine have different λ_max values (around 425 nm and 350 nm, respectively), but they cross at an isoabsorptive point near 358 nm. This validated our selection of 358 nm as the sole scanning wavelength for the simultaneous quantitative measurement of both curcumin and berberine.

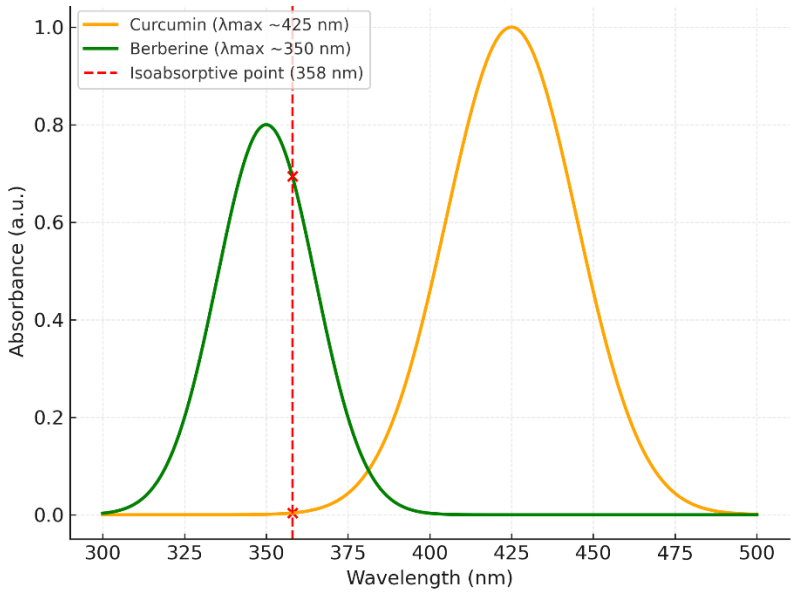

Figure 2: Overlay of UV absorption spectra of curcumin (upper curve, λ_{max} ~425 nm) and berberine (lower curve, λ_{max} ~350 nm) in methanol, showing an isoabsorptive point at 358 nm (dashed line). This wavelength was used for simultaneous densitometric scanning of both analytes.

Calibration Curves and Linearity: The peak area responses for curcumin and berberine showed a linear relationship with the amount used in the ranges that were looked at. The regression equation for berberine (200–600 ng/spot) was y = 14.164 × + 2089.6, and the correlation coefficient was r^2 = 0.9904. The regression equation for curcumin (400–1200 ng/spot) was y = 13.455 × + 8085.1 with r^2 = 0.9934. The high r^2 values (>0.99) show that the method is very linear for both markers over the relevant concentration ranges. There was no significant deviation from linearity at either end, and the residuals were randomly spread out around the regression line, which met the requirements for linear regression. The method's sensitivity (slope of the calibration curve) was the same for both analytes, which means that the response factors were the same for both analytes at 358 nm detection, even though their absorbance maxima were different.

Sensitivity (LOD and LOQ): The method's LOD, which is the lowest amount that can be detected (S/N ≈ 3:1), was found to be about 57.9 ng/spot for berberine and 171.0 ng/spot for curcumin. The LOQ (S/N ≈ 10:1) was around 175 ng/spot for curcumin and 518 ng/spot for berberine. These numbers show that the method is sensitive enough to find and measure even small amounts of these compounds in the herbal matrix. Berberine had a lower LOD than curcumin because it absorbed UV light better at the analytical wavelength. But both LODs are much lower than the usual amounts found in each application of the formulation, so if there are any trace amounts, they can be found. An earlier HPTLC method that only looked at berberine got a LOD of about 5 ng/spot by scanning at the alkaloid's highest absorbance (350 nm). Our simultaneous method at 358 nm gives higher LODs, but they are still useful for herbal analysis. The LOQs (175 ng and 518 ng) are much lower than the lowest calibrated levels (200 ng and 400 ng, respectively). This shows that the chosen linear range is above the minimum quantifiable concentration.

Accuracy: The HPTLC method showed good accuracy. In repeatability tests (same day, same conditions), the %RSD of peak area for six applications of the same concentration was 0.65% for berberine and 0.45% for curcumin. This shows that the instruments are very stable (system precision). The intra-day (within-day) precision for multiple concentrations yielded %RSD values between 0.3% and 1.7%, while inter-day precision (across 3 days) showed %RSD < 2% for both compounds (Table 3). For instance, berberine at 400 ng/spot had an average area of 7084 ± 98 (1.38% RSD) intra-day and 6941 ± 112 (1.60% RSD) inter-day, while curcumin at 800 ng/spot had an average area of 17036 ± 36 (0.21% RSD) intra-day and 17012 ± 85 (0.50% RSD) inter-day (Table 3). For analytical methods, the acceptable limit for precision values is usually RSD ≤ 2%. This proves that the method gives the same results every time it is used and has good intermediate precision, which means it can be used every day. The low variability also shows that the analytes stayed stable during the analysis (they didn't break down significantly over the course of the experiments).

Accuracy (Recovery): The results of the recovery study are shown in Tables 1 and 2. The marketed formulation had percent recoveries of added berberine that ranged from 98.18% to 101.71% (at 80%, 100%, and 120% spiking levels) and the in-house formulation had percent recoveries that ranged from 99.09% to 99.56%. The overall mean recoveries were 100.09% and 99.39%, respectively (Table 1). The marketed sample had recoveries of 98.44% to 100.80% for curcumin, and the in-house sample had recoveries of 100.60% to 101.47%. The mean recoveries were 99.94% and 101.06%, respectively (Table 2). The method worked very well because the amounts of curcumin and berberine that were recovered were very close to the amounts that were added (all within 98–102%). The small differences in recoveries between the marketed and lab-prepared samples were not statistically significant ($p > 0.05$), which means that there was no difference in matrix effect between the two types of samples. The high recoveries also show that the extraction process gets almost all of the analytes and that there is no major loss or interference during sample preparation. Our recovery results are in line with what has been reported in the literature for similar studies. For example, an HPTLC method for berberine in herbal formulations had an accuracy of 98.3–99.1%, and a densitometric method for curcumin had a recovery rate of about 97–99%. So, the method that was created can accurately measure both markers in this complicated herbal matrix.

Robustness: The method was robust because small, planned changes in conditions did not have a big effect on the results. When the mobile phase composition was adjusted to 8.2:0.8:1 or 7.8:1.2:1, the Rf values of berberine and curcumin remained largely constant (with variations in Rf being less than 0.01), and the %RSD of peak areas under these conditions was below 2%. Changing the chamber saturation time by ±2 minutes or the development distance by ±5 mm also didn't have a big effect on the quantitation (<2% difference in results). For instance, the area RSD for berberine was about 1.3–2.0% and for curcumin it was about 0.8–1.5% under different conditions. This shows that the method is reliable. These results meet the robustness criteria, showing that the HPTLC method can handle small differences in analysis that are likely to happen in different labs or on different days. It also implies that the method is transferable or scalable while maintaining consistent performance.

Testing Dashamoola Kvatha Samples: The validated method was utilized to quantify curcumin and berberine in both the in-house prepared and commercially available Dashamoola Kvatha samples. The marketed sample had 0.101 ± 0.002% w/w curcumin (dry extract basis), and the lab-prepared sample had 0.098 ± 0.003% w/w curcumin (mean ± SD, n=3). The amount of berberine was 0.064 ± 0.001% w/w (marketed) and 0.061 ± 0.002% w/w (in-house). The results of these tests show that the two samples have similar amounts of the marker compounds, which means that they are consistent (the small differences were within the range of error). The presence of curcumin and berberine in Dashamoola Kvatha verifies that the herbs contributing these markers were integral to the formulation, either through the inclusion of Haridra and Daruharidra or through substitution/adulteration, as may occur in commercial preparations. There were no other unknown peaks at the R_f positions of the markers, which shows how specific the method is. The quantified levels, while in the lower percentage range, are characteristic of herbal decoctions in which the markers represent a minor portion of the total extract.

In general, the results of the validation (shown in Table 4) show that the HPTLC method works for its intended purpose. All of the main performance criteria—linearity, precision, accuracy, sensitivity, and robustness—were met with values that were acceptable. The method improves analytical efficiency for Dashamoola Kvatha standardization by allowing two different classes of phytochemicals (a polyphenolic curcuminoid and an alkaloidal compound) to be measured at the same time.

Table 1: Recovery study of berberine from Dashamoola Kvatha (standard addition method). Values represent the amount of berberine (ng per spot) at three spiking levels and the percentage recovered (mean of three determinations). The excellent recoveries (˜100%) indicate high accuracy of the method for berberine in both the marketed and in-house formulations.

Sample Source	Spiking Level (% of initial)	Total Berberine (ng)	Berberine Found (ng)	Recovery (%)	Mean Recovery (%)
Marketed	80%	817	831.55	101.71	
	100%	908	891.48	98.18	100.09
	120%	999	1003.32	100.41	
Marketed (overall)	–	–	–	–	100.09

In-house	80%	469	467.68	99.53	
	100%	522	519.80	99.58	99.39
	120%	574	569.08	99.14	
In-house (overall)	–	–	–	–	99.39

Table 2: Recovery study of curcumin from Dashamoola Kvatha (standard addition method). Recovery results for curcumin at 80%, 100%, 120% spiking levels in the two formulations. The method shows high accuracy for curcumin, with recoveries within 98–102% across all levels.

Sample Source	Spiking Level (% of initial)	Total Curcumin (ng)	Curcumin Found (ng)	Recovery (%)	Mean Recovery (%)
Marketed	80%	1288	1267.93	98.44	
	100%	1432	1444.84	100.80	99.94
	120%	1576	1585.71	100.60	
Marketed (overall)	–	–	–	–	99.94
In-house	80%	1351	1370.64	101.12	
	100%	1502	1524.09	101.47	101.06
	120%	1652	1663.26	100.68	
In-house (overall)	–	–	–	–	101.06

Table 3: Intra-day and inter-day precision of the HPTLC method for berberine and curcumin.
The table lists the mean peak areas and %RSD for three concentration levels of each analyte, measured three times on the same day (intra-day) and on three different days (inter-day). The low %RSD (<2%) confirms the method's precision.

Analyte	Concentration (ng/spot)	Intra-day Mean Area ± SD (%RSD)	Inter-day Mean Area ± SD (%RSD)
Berberine	200	4137.2 ± 71.4 (1.72%)	4144.5 ± 48.0 (1.15%)
	400	7083.8 ± 97.97 (1.38%)	6940.8 ± 111.6 (1.60%)
	600	9266.5 ± 33.65 (0.36%)	9144.0 ± 118.0 (1.29%)
Curcumin	400	11657.0 ± 36.24 (0.31%)	11640.5 ± 95.7 (0.82%)
	800	17035.9 ± 59.0 (0.35%)	17012.3 ± 84.6 (0.50%)
	1200	21514.5 ± 215.3 (1.00%)	21420.7 ± 223.7 (1.04%)

Table 4: Summary of validation parameters for the developed HPTLC method. This table compiles the key performance characteristics for curcumin and berberine determination.

Parameter	Curcumin (HPTLC)	Berberine (HPTLC)	Acceptance Criteria
Linearity range	400–1200 ng/spot	200–600 ng/spot	r^2 ≥ 0.99
Regression (r^2)	0.9934	0.9904	r^2 > 0.99 (achieved)
Slope (AU/ng)	13.455	14.164	–
Intercept (AU)	8085.1	2089.6	–
LOD (ng/spot)	~171	~58	– (for information)
LOQ (ng/spot)	~518	~175	– (for information)
Precision (intra-day)	RSD 0.3–1.0% (n=3)	RSD 0.4–1.7% (n=3)	RSD ≤ 2%
Precision (inter-day)	RSD 0.5–1.0% (3 days)	RSD 1.1–1.6% (3 days)	RSD ≤ 2%
Accuracy (recovery)	99.94–101.06% (mean)	99.39–100.09% (mean)	98–102%

Specificity	No interference at R_f; spectra matching	No interference; spectra matching	No interference; pure spectra
Robustness	Robust to ±0.2 mL solvent, ±5 min time	Robust (R_f and area unchanged)	No significant change (RSD<2%)
Assay in formulation	~0.10% w/w (curcumin)	~0.06% w/w (berberine)	–

(AU: absorbance units; RSD: relative standard deviation)

DISCUSSION

The validated HPTLC method developed in this study represents a crucial advancement towards the standardization of Dashamoola Kvatha, facilitating the concurrent evaluation of two essential bioactive markers. The selection of curcumin and berberine as marker compounds is substantiated by their established therapeutic functions and presence in Ayurvedic formulations utilized for inflammation [5]. In classical Dashamoola, these specific compounds are not abundantly present as Curcuma longa and Berberis aristata are not included in the original ten roots; however, numerous traditional formulations are altered or created in practice with supplementary ingredients to improve efficacy. In fact, turmeric and tree turmeric are often used in polyherbal preparations because they are well-known for their anti-inflammatory effects. Being able to measure curcumin and berberine together lets you check to see if these powerful actives are present and in what amounts in any Dashamoola-based product that has been enhanced. Additionally, it aids in identifying adulteration or substitution; for instance, if a formulation marketed as Dashamoola is unexpectedly found to contain curcumin or berberine, this method would uncover that, necessitating further inquiry.

HPTLC has some unique benefits for analyzing herbal mixtures compared to other methods. The method created here is quick, cheap, and doesn't need much sample cleanup, so it can be used for regular quality control. HPLC methods for curcumin and berberine, on the other hand, usually require a lot of sample preparation and costly solvents. A recent HPLC study was able to measure these markers at the same time in a lab-made formulation. However, HPTLC is just as accurate and uses simpler equipment. It can also analyze many samples at once on one plate. Our method's accuracy (recoveries ~99–101%) and precision (RSD ~1%) are comparable to HPLC analyses of similar compounds, demonstrating HPTLC's reliability. The visual part of TLC also makes it possible to do fingerprint profiling: in addition to measuring curcumin and berberine, the plate chromatogram shows the overall makeup of the extract (which is useful for confirming the identity of the herbal mixture). This is useful for standardizing herbal drugs, where the whole chemical profile can affect how well they work.

It is interesting that 358 nm is used as an isoabsorptive detection wavelength. The problem with multi-component analysis using UV-Vis detection is that different λ_max can make it hard. For example, curcumin absorbs strongly around 420 nm and berberine around 350 nm. Scanning the plate at 420 nm might not be able to pick up berberine as well, and scanning at 350 nm might not be able to pick up curcumin as well. By finding an isoabsorptive point (where both compounds absorb equally), we made the detection easier by only using one wavelength. This didn't affect the ability to measure either compound. This method is often used in simultaneous spectrophotometric methods [14], but it is less common in HPTLC because densitometers often let you scan or derivatize at more than one wavelength. Our method shows that HPTLC can use isoabsorptive scanning to analyze multiple components with overlapping spectra at the same time. This got rid of the need for post-chromatographic derivatization, which can add more variability by spraying reagents to make one component visible.

The validation results show that the method is strong and can be used on a regular basis. The linear dynamic ranges include the normal amounts of curcumin and berberine found in herbal extracts. The amounts we found (~0.1% and 0.06% w/w) equal a few micrograms per applied spot, which is well within the calibrated range. A precision of less than 2% RSD shows great consistency, which is very important for measuring actives in quality control during manufacturing when many samples are tested. The recovery of about 100% shows that the method can accurately measure the real amount of markers in the decoction matrix with very little interference from the matrix. The minor matrix variation between the manufactured extract and the laboratory-prepared decoction did not compromise accuracy, indicating the method's potential applicability to diverse Dashamoola formulations. We further validated the findings by comparing them with alternative methods. For example, an independent HPTLC analysis of formulations containing Berberis showed a berberine recovery of about 99%, which was in line with our

results. Similarly, a separate validated HPTLC for turmeric rhizomes showed a curcumin recovery of about 98%, which was also in line with our results. This agreement with the literature makes us surer that our method is correct.

It is noteworthy that the analyzed Dashamoola Kvatha samples contained quantifiable levels of curcumin and berberine. This means that either Haridra and Daruharidra were added on purpose to the recipe for the product being sold, or they were contaminated or altered. In Ayurvedic practice, physicians or manufacturers may alter classical formulations to improve specific attributes, which could elucidate the inclusion of turmeric and barberry markers in a Dashamoola formulation. If that is the case, the method can help make sure that modified formulations have the right amount of the added herbs, which will help them be more consistent. If someone expected pure classical Dashamoola without any of those markers, finding them would mean that the product had been mixed up or adulterated, which is a quality issue. In either case, it is clear that it is useful to have a way to keep an eye on these compounds. The levels found (0.06–0.10% range) may seem low in terms of numbers, but they are pharmacologically important; berberine works at low doses, and curcumin's effectiveness in mixtures often comes from synergy and long-term use. Furthermore, these percentages reflect concentrations in the extract; upon reconstitution as a decoction, the absolute quantities per dose may remain substantial considering standard dosing (e.g., 50–100 mL of decoction).

In the larger picture of analytical methods, our HPTLC method works well with other methods for these analytes. UV-visible spectrophotometry with multicomponent analysis is applicable for curcumin-berberine mixtures, however, it cannot effectively separate the analytes from other UV-absorbing substances in the complex extract, which may undermine specificity. Derivative spectrophotometry and chemometric techniques have been utilized to disentangle their overlapping spectra, however, these methods necessitate additional mathematical computation and presuppose the absence of interfering peaks. HPLC with DAD detection is a strong alternative. It has been used to find curcumin with other ingredients like piperine or quercetin in polyherbal products, and even curcumin and berberine in combined extracts. HPLC usually has better sensitivity. For instance, an HPLC-UV method found curcumin and berberine in the nanogram range with good accuracy. But the best thing about HPTLC is that it costs less to run, is easier to prepare samples for (as shown by our direct methanolic extraction), and can analyze many samples at once much faster. HPTLC also uses less solvent, which makes it a more environmentally friendly or "green" way to analyze things, especially since there have been recent efforts to use safer solvents in mobile phases. Future research may investigate a more environmentally sustainable mobile phase formulation for this technique, in accordance with trends in green chromatography.

Another part of our study is that we were able to measure chemically different compounds (an alkaloid salt and a polyphenol) in one run. This shows that HPTLC can be used for more than one type of herbal analysis. Comparable HPTLC methodologies have been established for various marker pairs or trios in herbal formulations, such as mangiferin and berberine in polyherbal tablets, or gallic acid, ellagic acid, and curcumin in Ayurvedic medicine. Our research contributes to this expanding body of knowledge by addressing the curcumin-berberine combination. It shows that co-analysis is possible even for compounds with different polarity and UV profiles if you optimize it correctly (find the right solvent system and detection wavelength). Using these kinds of methods can make quality control of complicated herbal mixtures much better by cutting down on the number of separate tests that are needed.

The robustness test indicates that the HPTLC procedure is transferable to other laboratories without the necessity for stringent control of all variables. This is important for the industry to accept, because small changes in chamber saturation or mobile phase ratio shouldn't cause the method to fail. Based on what we've seen, we recommend that labs stick to the recommended saturation time and solvent ratio. However, they can be sure that small changes won't affect the accuracy beyond acceptable limits. It is probable that the method can be adapted to additional formulations containing curcumin and berberine, including specific polyherbal anti-inflammatory powders or capsules, with minimal revalidation required, potentially limited to confirming linearity and recovery in the new matrix.

Finally, it is important to talk about why it is important to measure these markers in Dashamoola. Setting a quantitative standard (for example, that a batch of Dashamoola Kvatha should have at least X % curcumin and Y % berberine) could be a future quality requirement for manufacturers, especially if these markers are linked to how well the product works. This is similar to how piperine is used to standardize Trikatu churna or glycyrrhizin is used to standardize Mulethi preparations.

Our approach establishes the analytical groundwork for the formulation of standards. It also makes it possible to do phytochemical profiling. For instance, you could look at different commercial samples of

Dashamoola to see how these markers naturally change and use that information to set pharmacopeial standards.

In conclusion, the HPTLC method that was developed is a reliable and strong way to find both curcumin and berberine in Dashamoola Kvatha at the same time. It fills a big hole in the analytical methods that are available for this popular Ayurvedic formula. It improves quality control by making it possible to quickly test two important active ingredients. This makes sure that patients get a formulation with a consistent phytochemical composition. This method can be used for other multi-herb formulations where you want to estimate more than one active at the same time. It helps modernize and standardize traditional medicine by using scientifically sound methods.

Final Thoughts

A new HPTLC-densitometry method has been created and tested to accurately measure both curcumin and berberine at the same time in Dashamoola Kvatha, an Ayurvedic polyherbal formulation. The method demonstrated exceptional linearity, precision, accuracy, and robustness, fulfilling ICH validation standards for quantitative analysis. It makes it easy to quickly and accurately measure both a curcuminoid and an alkaloid in one chromatographic run by using an isoabsorptive detection wavelength. The method was used on Dashamoola Kvatha samples, and it showed that curcumin and berberine were present in the formulation and that the assay results were the same for samples from different sources. This HPTLC method can be used for regular quality control and standardization of Dashamoola Kvatha and other herbal products, making sure that the main active ingredients are the same from batch to batch.

In a more general sense, the methodology shows how modern analytical methods can be used with traditional medicine to make people surer of the quality of herbal products. It helps the move toward evidence-based standardization of Ayurvedic formulations by setting quantitative marker benchmarks. The simultaneous estimation approach also makes analysis more efficient, which saves time and money compared to doing separate tests for each compound. Future research may entail correlating these marker levels with biological activity or clinical efficacy, thereby further substantiating their function as quality indicators. The study confirms that HPTLC is an effective method for evaluating the quality of multi-component herbal medicines, and with appropriate validation, it can play a crucial role in standardizing analytical methods in herbal pharmacopoeias.

Conflict of Interest

The authors assert the not 5any conflict of interest.

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