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Development of HPTLC Method for Simultaneous Determination of Quercetin and Curcumin in Polyherbal Formulation

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Abstract:

A new simple, precise, rapid and selective high-performance thin-layer chromatographic (HPTLC) method has been developed for the simultaneous determination of Quercetin and Curcumin in Ayurvedic formulations. The stationary phase silica gel G60F254 was selected for separation and the sample was developed using a mixture of Toluene: Ethyl acetate: Formic acid in the ratio 4.5:3.8:0.1 v/v as mobile phase. The Rf value of Quercetin and Curcumin was found to be 0.49 and 0.58 respectively. Linearity was discovered in the concentration range of 200 to 1000 ng/spot for Quercetin and 200 to 1000 for Curcumin the correlation coefficient value is 0.9979 and 0.9981. The results of analysis were validated in terms of accuracy and precision. The LOD was determined to be 4.1ng and 1.99ng/spot of Quercetin and Curcumin respectively. LOQ was determined to be 12.77ng and 6.03ng/spot of Quercetin and Curcumin respectively. The proposed HPTLC method provides a faster and cost-effective quantitative control for routine analysis of Quercetin and Curcumin.

Keywords: Curcumin, Quercetin, Polyherbal formulation, HPTLC

INTRODUCTION:

Herbal remedies have been utilised as therapies for a variety of ailments since ancient times. Flavonoids, glycosides, triterpenoids, lipids, oils, steroids, and organic acids are among the active chemical components found in natural goods, and they may be responsible for their widespread pharmacological activity. Although the Ayurveda system of medicine is beneficial to humanity, effective standardising tools for assessing its quality, quantity, and efficiency are still lacking. Chromatographic techniques are accurate and successful in standardising and quantifying key biomarker chemicals from single drug and polyherbal formulations, according to WHO recommendations. [1-2]

HPTLC is a concept that encompasses a widely established technique based on scientific facts, as well as the application of proven qualitative and quantitative analysis methods.[1]. Higher separation efficiencies, shorter analysis times, smaller mobile phase quantities, and efficient data processing are all advantages. HPTLC is required to create methodologies for the rapid, precise, and accurate identification and quantification of active ingredients or marker compound/s as a qualitative and quantitative goal to assess the authenticity and intrinsic quality of the product [3-4].

Quercetin (QCN) belongs to the flavanol family of flavonoids. QCN can be found in a variety of fruits and vegetables. QCN has antioxidative, anticarcinogenic, anti-inflammatory, antiaggregatory, and vasodilating properties, among others.[6] Curcumin (diferuloylmethane), a key active component of the culinary spice turmeric, has been utilised to treat a variety of diseases in Indian and Chinese medicine. Curcumin is a polyphenol that has anti-inflammatory, antioxidant, antiproliferative, and wound-healing effects [5]. The current research focuses on development and validation of polyherbal formulations. HPTLC analysis was used to standardise the formulation by employing curcumin and quercetin as biomarkers.

MATERIALS AND METHODS:

Equipment: For this study, a CAMAG TLC system with a Linomat-5 applicator, CAMAG TLC scanner, and Shimadzu single pan balance was used.

Chemicals:

Quercetin (K-Shipra biotech, M.P,) Curcumin (Biomed, Goa), *Divya madhunashini vati*, (Patanjali, Belagavi), Toluene, Ethyl acetate, Formic acid (all reagents of analytical grade) and silica gel 60F254 precoated TLC aluminium plates [E-Merk].

Standard solution of Curcumin:

To make a stock solution of curcumin, 10 mg of correctly weighed curcumin was liquified in methanol and the amount was increased to 10 ml with methanol to achieve a final concentration of 1 mg/ml.

Standard solution of Quercetin:

To make a stock solution of quercetin, 10 mg of correctly weighed quercetin was liquified in methanol and the amount was made up to 10 ml with methanol to achieve a final concentration of 1 mg/ml

Preparation of sample solution:

Divya madhunashini vati tablets were used to make the sample solution. Weighed and finely pulverised twenty tablets Approximately 500mg of powder was transferred to a 50ml volumetric flask, liquified in methanol, capacity filled up with methanol, and filtered under vacuum through a 0.45 m Millipore nylon membrane filter.

Chromatographic conditions:

The analysis was carried out on HPTLC silica gel G60F254 plates with a 20 cm x 10 cm size and a fluorescent

indicator. The plate was cleaned by predeveloping it with methanol to the top and drying it in a 1050C oven for 5 minutes. Using a CAMAG, sample and standard zones were put to the layer as bands. Linomat 5 automated spray-on applicator with a 100µl syringe and settings of 6 mm band length, 4 l/sec application rate, 4 mm gap between bands, 6.5 mm distance from plate side edge, and 2 cm distance from plate bottom.

Calibration curve:

To construct a calibration curve, a 2, 4, 6, 8, and 10µl standard solution of Quercetin and Curcumin was put to a TLC plate. Chromatographic conditions were used to create the chromatograms.

Method validation:

The suggested analytical method was validated according to Q2 of the International Conference on Harmonization (ICH).

Linearity:

The prepared standard stock solution was diluted to produce linearity standard solutions with Quercetin and Curcumin concentrations of 200-1000 ng/spot and 200-1000 ng/spot, respectively. Three different sets of such solutions were available. Each set was examined in order to arrive at a conclusion. To determine the method's linearity, the standard deviation (SD), coefficient of determination (r^2), slope, and intercept of the calibration curves were calculated.[7]

Repeatability:

The method's repeatability was tested by scanning the same spot three times for curcumin (600 ng) and quercetin (800 ng) and calculating the coefficient of variance (%CV).

Specificity

The method's specificity was determined by determining the peak purity of the standard and test samples. The Rf values and spectra of the separated bands were compared to those of the standards at three levels: peak start, peak apex, and peak end of the spot, to validate the spot of each standard in the sample.[4]

Limit of Detection and Limit of Quantification:

Standard s3olutions in various dilutions of curcumin and quercetin, as well as methanol and the blank, were used to determine the limit of detection and limit of quantification, which were computed using the signal to noise ratio. The LOD was set to 3:1 (SD/S) and the LOQ to 10:1 (SD/S); S stands for slope of the calibration curve and SD stands for standard deviation of the regression line's Y-intercept. [2-4]

Precision:

Intraday and Interday precision were measured to assess precision. three bands of both sample and reference solutions per plate on three plates (intra-day precision) and three consecutive days (inter-days precision) at three distinct concentrations of 600, 800, 1000 ng/spot for quercetin and 600,800,1000 ng/spot for curcumin. These three different concentration levels of standard and sample solutions were used to perform intra-day (repeatability) and inter-day (intermediate precision) precisions, and all results were expressed as mean ± RSD (%).[2]

Accuracy:

Recovery experiments were used to determine accuracy. The recovery study was carried out by injecting 20, 40, and 60% of standard Curcumin and Quercetin into the pre-analysed samples externally. The experiment was carried out three times and duplicated on the plate. This was done to see how well medicines recovered at different degrees of formulation [9,10].

Robustness:

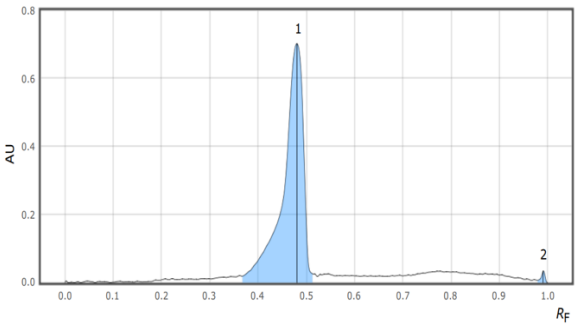
The robustness of the method was determined by making deliberate modest wavelength modifications and estimating the percent divergence from the original method [2].

RESULTS AND DISCUSSION:

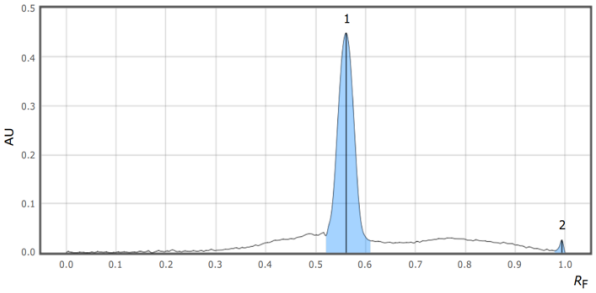
HPTLC method for simultaneous estimation of CMN and QCN:

The mixture several mobile phases were tried to separate spots of Quercetin and Curcumin. Toluene/ethyl acetate/formic acid (4.5:3.8:0.1) was utilised as a mobile phase which gave good resolution. A TLC sampler was used to apply the sample. The TLC plate was then developed in solvent over an 8-cm development distance (migration time of 15 minutes). The plates were then dried before densitometric analysis. Rf obtained for quercetin and curcumin is 0.49 and 0.57 respectively.

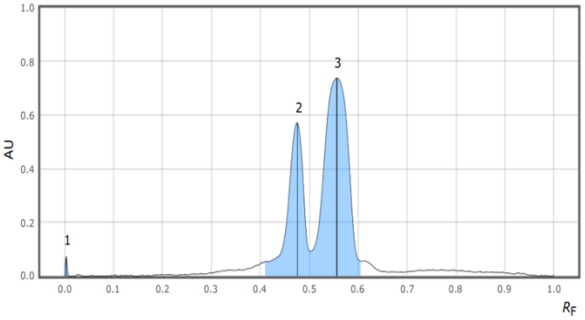
QUERCETIN



CURCUMIN



Sample



Method of Validation parameters for estimation of biomarker compound by HPTLC

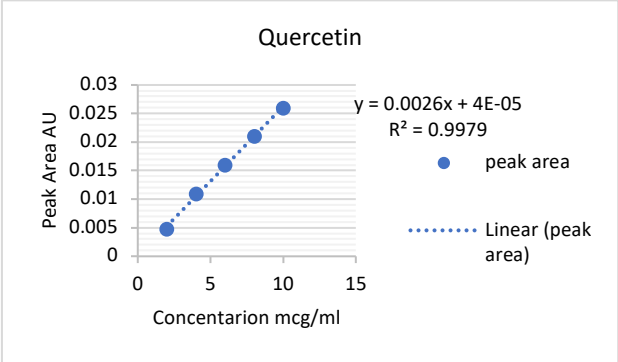
Parameters	Quercetin	Curcumin
Range	200-1000 ng/spot	200-1000ng/spot
Specificity	Specific	Specific
Limit of Detection(ng/spot)	4.1	1.99
Limit of Quantification(ng/spot)	12.77	6.03
Repeatability study (n=7) (%CV)	0.16	0.10

Calibration curve and Linearity:
The calibration curve was created by graphing the peak area ratios of Curcumin and Quercetin against the calibration standard concentrations.
Linearity was established within concentration range 2-10 µg/ml. Linear regression data showed a good linear relationship over concentration range.

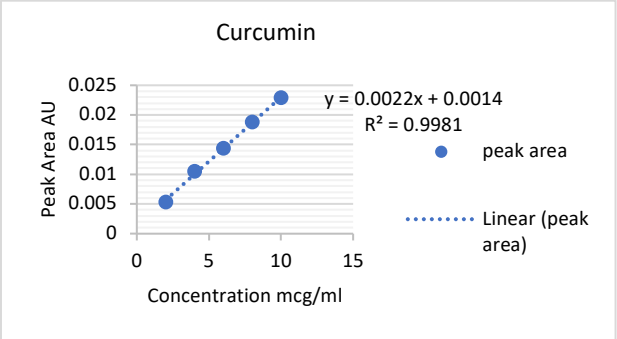
Linearity regression data for calibration curve

Parameters	Value (Quercetin)	Value (Curcumin)
Linearity Range	200-1000ng/spot	200-1000ng/spot
Correlation of coefficient (According to area)	0.9988	0.9981
Slope	0.0026	0.0028

The correlation coefficient r^2 was 0.9979 for Quercetin.



The correlation coefficient r^2 was 0.9981 for Curcumin.



Precision: Intermediate precision were studied separately and shown below

Intermediate Precision

Intraday Precision

Biomarker	Concentration (ng/spot)	Peak area ± SD (n=3)	%CV
Quercetin	600	0.01677 ± 1.5	0.09
	800	0.02179 ± 3.05	0.14
	1000	0.02660 ± 6.45	0.24
Curcumin	600	0.01446 ± 7.5	0.52
	800	0.01880 ± 8.7	0.46
	1000	0.02280 ± 8.5	0.37

Interday Precision

Biomarker	Concentration (ng/spot)	Peak area ± SD (n=3)	%CV
Quercetin	600	0.01674 ± 6.1	0.36
	800	0.02197 ± 7.5	0.34
	1000	0.02669 ± 5.6	0.21
Curcumin	600	0.01438 ± 1.5	0.11
	800	0.01876 ± 2.0	0.11
	1000	0.02282 ± 5.7	0.03

Recovery studies
Curcumin had a percent recovery of 99.3%, 97.71%, and 98.81%, while quercetin had a percent recovery of 98.3%, 100.4%, and100.49%. Curcumin had a 98.6 percent recovery rate, while quercetin had a 99.73 percent recovery rate.

Robustness of method:
The time from sample application to scanning varied from 0, 20, 40, 60 mins. The standard deviation of peak areas was calculated for each parameter and % RSD was determined to be less than 3 %.

BIOMARKER	Nominal amt (ng/band)	Calculated amt (ng/band) mean±SD	%RSD	% Recovery	%Mean
Quercetin	40	39.74±0.20	0.52	99.3	
	60	58.63±0.55	0.94	97.71	98.6
	80	79.05±0.5	0.63	98.81	
Curcumin	40	38.3±0.60	0.62	98.3	
	60	60.8±0.81	0.41	100.4	99.73
	80	81.5±0.49	0.16	100.49	

CONCLUSION:

The Rf values of Quercetin and Curcumin differ significantly, hence this analytical approach can be used to estimate both compounds simultaneously. The approach was validated and found to be repeatable and specific for the examination of pharmaceutical formulations without causing any interference. Low reagent volume, speed and simplicity of sample treatment, and adequate precision and accuracy are all advantages of the developed approach. Furthermore, the approach used has the advantage of allowing many sample spots to be applied to the HPTLC plate. The methods can be used for the routine simultaneous analysis of the Quercetin and Curcumin in pharmaceutical preparations.

List of Abbreviations:

HPTLC: High performance thin layer chromatography; Rf: Resolution factor; QCN: Quercetin CMN: Curcumin; RSD: Relative standard Deviation; Ng: Nano gram; SD: Standard deviation; CV: Coefficient of variation; µl: Microlitre; R^2: Regression coefficient.

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