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Original Article

METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF CURCUMIN AND PIPERINE BY RP-HPLC

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ABSTRACT

Objective: To develop a simple, sensitive, specific, accurate reverse phase high performance liquid chromatographic (RP-HPLC) method for the estimation of curcumin and piperine.

Methods: The separation was done using a column Inertsil–ODS C_{18} (250 mm × 4.6 mm, 5µ particle size) and mobile phase composed of methanol: water (45:55 v/v), flow rate at 1 ml/min and detection was carried out at 282 nm with photodiode array (PDA) detector.

Results: The separation of curcumin and piperine were found to be at the retention time of 2.433 min and 3.095 min, respectively. The method was found to be linear at a concentration range 20-80 µg/ml for curcumin and piperine. The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.05µg/ml and 0.17µg/ml for curcumin and 0.18µg/ml and 0.53µg/ml for piperine respectively. The average percentage recoveries of curcumin and piperine were in the range of 98-100.6%.

Conclusion: A simple and sensitive reverse phase high performance liquid chromatographic method was developed for the estimation of curcumin and piperine.

Keywords: Method development, Method validation, RP-HPLC, Curcumin, Piperine

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INTRODUCTION

Curcumin is principal curcuminoid of indian spice turmeric of ginger *Curcuma longa Linn.* (family: Zingiberaceae). It has antioxidant, anti-inflammatory, anti-fungal, anti-viral action [1] and anti-carcinogenic action [2]. Curcumin is chemically called as 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione, structurally it was shown in fig. 1 [3]. Piperine was first pharmacologically active compound isolated from *Piper longum and Piper nigrum* (family: Piperaceae) [4]. Piperine(1-peperoyl piperidine) exhibits pharmacological activities like anti-hypertensive, anti-platelet [5], and anti-asthamatic, anti-tumor activities [6]. Literature survey revealed that few analytical methods were reported for the estimation of curcumin and piperine. Previously reported methods were U. V. Spectroscopy [7-13], HPLC [14-20], HPTLC [21], UFLC [22], LC-MS/MS [23], GC-MS [24] for curcumin and piperine, individually or in combination with other drugs. Based on literature it was confirmed that few HPLC methods were reported for simultaneous estimation of curcumin and piperine. The present method was simple, sensitive, and selective and developed with low-cost solvent in the mobile phase and simple standard preparation and separation were done at shorter run time and all parameters were validated according to ICH guidelines [25].

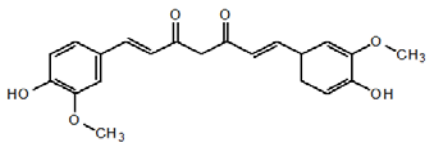


Fig. 1: structure of curcumin

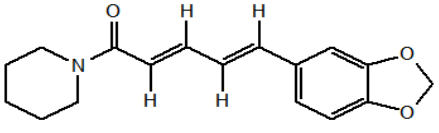


Fig. 2: structure of piperine

MATERIALS AND METHODS

Instrument

The method was developed with the system composed of Waters HPLC, Model No 2690/5 series. The chromatographic separation was done on Inertsil-C_{18} ODS column.

Chemical solutions and reagents

Curcumin and piperine were supplied from spectrum pharmaceuticals Pvt, Ltd, kukatpally, Hyderabad. Orthophosphoric acid, Acetonitrile (HPLC grade), Water (HPLC grade) was purchased from Merck chemicals private limited (Mumbai, India).

Chromatographic conditions

The chromatographic separation was done with different solvents, finally a good resolution was achieved with a mobile phase methanol and water (45:55, v/v) using Inertsil-ODS C_{18} column, at a flow rate 1 ml/min. The chromatographic detection was carried at 282 nm, with PDA detector.

Preparation of standard and working standard solutions

Weigh 10 mg of curcumin and piperine and dissolved in 10 ml of calibrated volumetric flasks separately, add 10 ml of mobile phase and sonicated for 20 min. 0.4 ml was taken from above stock solution and transferred into 10 ml volumetric flask and add 10 ml of mobile phase.

Mobile phase composition

Different trails were done for estimation of curcumin and piperine with the different combination of solvents with different ratios like (90:10, 60:40, 50:50, and 40:60). Finally, methanol and water (45:55, v/v) were selected because of peak symmetry, the good resolution between curcumin and piperine.

Method validation

The method was validated according to ICH guidelines, 2005. Validation parameters were according to guideline of ICH Q2R1. Validation parameters include system suitability, linearity, precision, LOD, LOQ, accuracy and robustness, ruggedness.

Specificity

Specificity was established by comparing analytic chromatographic peak with the blank. No predictable peak was found at the retention time of curcumin and piperine. The data was given in fig. 3, 4, and 5.

Linearity and calibration curve

The linearity range was estimated between 20-80 µg/ml for curcumin and piperine. The calibration curve was plotted between concentration against corresponding peak area using least square method.

Precision

The precision of the method was evaluated with same concentration level, six replicate injections were injected and precision was expressed in terms of intra-day (repeatability) and inter-day precision and was conveyed in terms of percent relative standard deviation (%RSD).

Accuracy (% recovery)

Accuracy of the method was evaluated using standard addition method, where standard was spiked at 50%, 100%, 150% levels and accuracy was estimated with percentage recovery and percentage relative standard deviation (%RSD).

Robustness

Robustness was evaluated by making small deliberate changes in flow rate, temperature, mobile phase composition, provides an indication of the reliability of the method.

Ruggedness

Ruggedness of the method was studied on different HPLC systems, under similar conditions at different times.

LOD and LOQ

LOD and LOQ were estimated using slope (S) and standard deviation of intercept (α) from the calibration curve.

LOD =3.3α/S

LOQ =10α/S

α is the standard deviation of y-intercept and S is a slope from linearity plot.

RESULTS

Optimization of chromatographic conditions

The chromatographic separation was done with various trails using different conditions. Based on theoretical plates, peak shape and back pressure, the column was selected. Finally, the chromatographic separation was carried out using Inertsil-ODS C_{18} column (250 mm×4.6 mm; 5µ particle size) and mobile phase methanol: water (45:55 v/v) at a flow rate 1 ml/min and injection volume 10 µl and detection at 282 nm with PDA detector. The retention time was found to be 2.433 min for curcumin and 3.095 min for piperine. The optimized chromatographic conditions were shown in table 1 and the chromatogram of blank, standard, and sample was shown in fig. 3, 4, 5.

Table 1: Optimized chromatographic conditions for estimation of curcumin and piperine

Parameter	Condition
Stationary phase(Column)	Inertsil-ODS C_{18}(250 mm×4.6 mm, 5µ)
Mobile phase	Methanol: water (45:55 v/v)
Column temperature	Ambient
Detection wave length	282 nm
Injection volume	20 µl
Flow rate	1 ml/min
Run time	10 min
Retention time	2.433 min for curcumin, 3.095 min for piperine

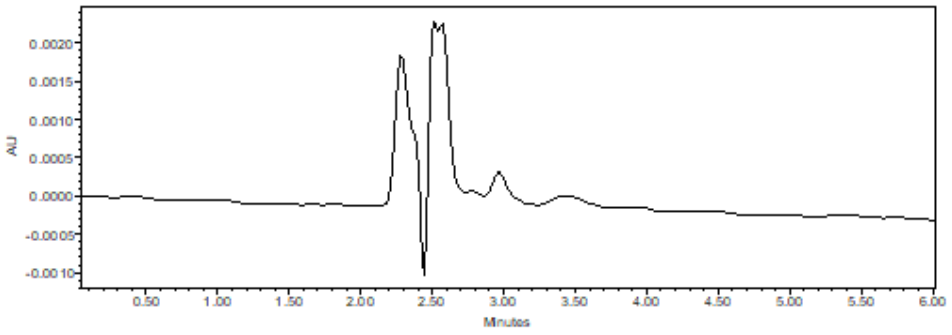


Fig. 3: Typical HPLC chromatogram of blank

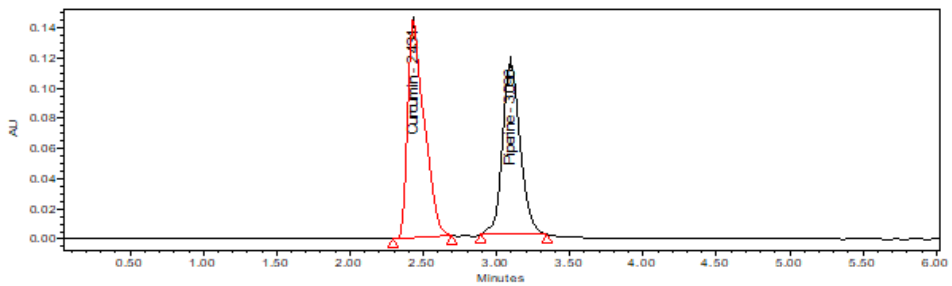


Fig. 4: Typical HPLC chromatogram of standard

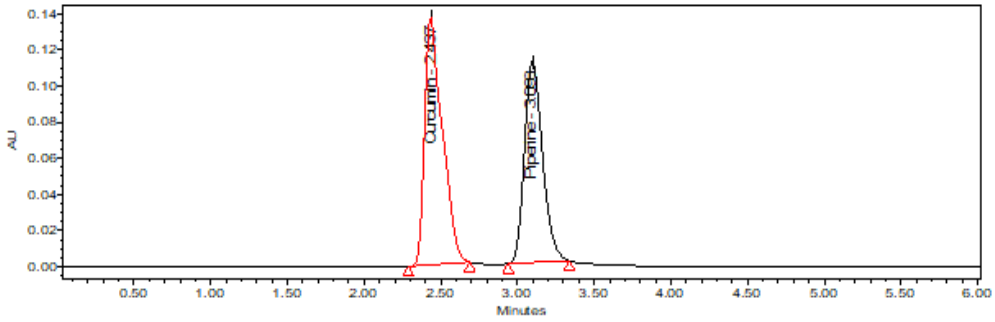


Fig. 5: Typical HPLC chromatogram of sample

Method validation

System suitability

System suitability tests were carried out to make sure that optimized chromatographic conditions are suitable for estimation of curcumin and piperine. Standard solution containing a mixture of curcumin and piperine (40µg/ml) was prepared. Six replicate injections from above standard solutions were injected into column and verify system suitability parameters like theoretical plate count, tailing factor, retention time, resolution, and percentage relative standard deviation of the peak for chromatograms. The developed method had shown more than 2000 theoretical plates, the percentage relative standard deviation for peak area and retention times were less than 2 and tailing factor less than 2, which ensure the suitability of developed method. The results were shown in table 2.

Table 2: System suitability of curcumin and piperine

Parameter	Curcumin	Piperine	Acceptance criteria
Retention time	2.437 min	3.097 min	.....
Theoretical plates	5934.2	8730.4	>2000
Tailing factor	1.24	1.11	<2

Specificity

Specificity was carried out by evaluation of blank, standard and sample injections. The resultant chromatograms of blank, standard and sample were compared, the correlation was good between standard and sample and no interference of excipients in blank with drug was observed. The data was shown in fig. 3, 4, and 5.

Linearity

The linearity of the method was determined at seven concentration levels ranging from 20-80 µg/ml for curcumin and piperine. The regression line equation for curcumin was Y = 32252X – 40399 and for piperine was Y = 25780X + 2765. The correlation coefficient values were found to be 0.999 and 0.999 for curcumin and piperine. The regression data for the calibration curve had shown a good linear relationship over a concentration range 20-80 µg/ml for curcumin and piperine. The results of the regression were summarized in table 3 and 4 and data was shown in fig. 6

Precision

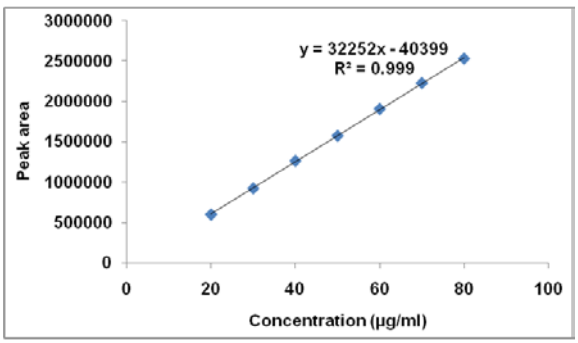
The precision of the developed method was checked in terms of repeatability (intra-day precision) and inter-day precision, which shown percent relative standard deviation (% RSD) less than 2, ensures precision of the developed method. The results were shown in table 5 and 6.

Accuracy

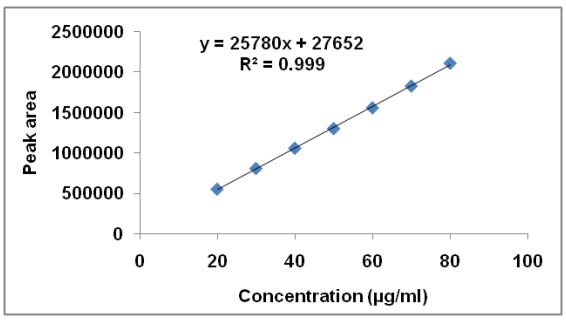
Accuracy of the method was examined by performing the recovery of added standard to the drug product was calculated, and it was found to be 98-100.6% with %RSD less than 2, which indicate the method was accurate. The results were summarized in table 7 and 8.

Robustness

Robustness of the method was checked by the slight variation in flow rate, which shown that no significant changes in the validation parameter. The results of variation in flow rate were given in table 9 and 10.



[A]



[B]

Fig. 6: Standard calibration curve of [A] curcumin [B] piperine

Table 3: Linearity and range of curcumin

S. No.	Concentration(µg/ml)	Peak area
1	20	598203
2	30	921576
3	40	1260823
4	50	1571434
5	60	1904090
6	70	2222634
7	80	2526577
Slope		32252
Y-intercept		40399
Correlation coefficient		0.999

Table 4: Linearity and range of piperine

S. No.	Concentration (µg/ml)	Peak area
1	20	551566
2	30	807869
3	40	1058029
4	50	1301310
5	60	1559184
6	70	1828268
7	80	2110397
Slope		25780
Y-intercept		27652
Correlation coefficient		0.999

Table 5: Intra-day and inter-day precision of the developed method for curcumin (n=6)

Drug	Concentration(µg/ml)	Intra-day precision		Inter-day precision	
		(mean±SD)	%RSD	(mean±SD)	%RSD
Curcumin	40	1266128±3958.6	0.3	1266921±2370.2	0.2

n is number of determinations, SD is standard deviation, RSD is relative standard deviation

Table 6: Intra-day and inter-day precision of the developed method for piperine (n=6)

Drug	Concentration(µg/ml)	Intra-day precision		Inter-day precision	
		(mean±SD)	%RSD	(mean±SD)	%RSD
Piperine	40	1053989±3924.2	0.4	1056681±3304.6	0.3

n is number of determinations, SD is standard deviation, RSD is relative standard deviation

Table 7: Accuracy of the developed method for curcumin (n=3)

Drug name	Level of addition (%)	Amount added (mg)	Drug found (mg/ml) (mean±SD)	% recovery (mean±SD)	% RSD
	50	20	19.9±0.13	99.9±0.7	0.7
Curcumin	100	20	40.02±0.3	100.1±0.6	0.4
	150	20	59.5±0.4	99.1±0.7	0.7

n is number of determinations, SD is standard deviation

Table 8: Accuracy of the developed method for piperine (n=3)

Drug name	Level of addition (%)	Amount added (mg)	Drug found (mg/ml) (mean±SD)	% recovery (mean±SD)	% RSD
	50	20	19.9±0.1	99.4±0.4	0.4
Piperine	100	20	39.7±0.2	99.4±0.5	0.2
	150	20	60±0.1	100±0.13	0.1

n is number of determinations, SD is standard deviation

Table 9: Robustness of curcumin (n=6)

S. No.	Flow rate (ml/min)	Peak area (mean±SD)	Tailing factor (mean±SD)
1	0.8	1294922±3898.9	1.23±0.005
2	1.0	1264795±4420.12	1.24±0.01
3	1.2	1236192±3462.24	1.24±0.01

n is number of determinations, SD is the standard deviation

Table 10: Robustness of piperine (n=6)

S. No.	Flow rate (ml/min)	Peak area (mean±SD)	Tailing factor (mean±SD)
1	0.8	1074930±1902.8	1.11±0.008
2	1.0	1057483±1039.96	1.12±0.005
3	1.2	1026428±2006.41	1.11±0.04

n is number of determinations, SD is standard deviation

Table 11: Ruggedness of curcumin (n=6)

S. No.	System 1		System 2	
	Peak area (mean±SD)	% Assay (mean±SD)	Peak area (mean±SD)	% Assay (mean±SD)
1	1265855±0.22	100.8±0.22	1261805±3793.2	100.4±0.3

n is number of determinations, SD is standard deviation

Table 12: Ruggedness of piperine (n=6)

S. No.	System 1		System 2	
	Peak area (mean±SD)	% Assay (mean±SD)	Peak area (mean±SD)	% Assay (mean±SD)
1	1054355±0.34	99.9±0.34	1056971±2626.7	100.2±0.3

n is number of determinations, SD is standard deviation

Ruggedness

Ruggedness of the method was estimated by comparing the results obtained from two different HPLC systems, which proves that the present assay method was rugged. The results were summarized in table 11 and 12.

LOD and LOQ

LOD and LOQ were calculated from the standard deviation of response and slope of the calibration curve of curcumin and piperine. The LOD and LOQ were found to be 0.05 µg/ml and 0.17 µg/ml for curcumin and 0.17 µg/ml and 0.53 µg/ml for piperine, which shown that the developed method was sensitive and it can detect and quantify at the lower concentration.

DISCUSSION

The present RP-HPLC method is a simple, precise, specific, accurate, linear and robust method for analyzing each component in the sample mixture. The previous study had reported that U. V. Spectroscopy methods [7-13] and HPLC methods [14-20] for curcumin and piperine individually and in combination with other drugs. In the present RP-HPLC method, we used PDA detector which prove selectivity of the method. The method was developed by using different buffer ratios at different flow rates. Finally methanol: water (45:55 v/v) as mobile phase and Inertsil-ODS column as stationary phase was selected and separation was done for curcumin at 2.34 min and piperine at 3.09 min. The method was validated as per ICH guidelines. Literature survey revealed, stability indicating RP-HPLC method for simultaneous estimation of curcumin and piperine with U. V. detector at different mobile phase and different column [16]. The separation was detected at 8.14 min for curcumin and 9.04 min for piperine. In the reported method, separation of curcumin and piperine was 5.71 min and 5.95 min respectively, longer than the present method. In the present method, PDA detector able to identify curcumin and piperine, and enhances the selectivity of the method. The method was validated according to ICH guidelines and results were in compliance of ICH guidelines. The linearity of the method had a good correlation with concentration and peak area. The correlation coefficient of curcumin and piperine was found to be 0.999, which indicates good linear relationship over concentration range 20-80 µg/ml. The % RSD values in intra-day and inter-day precision study were found to be less than 2 for curcumin and piperine, which indicate method was precise. The amount of drug recovery was 99.9% for curcumin and 99.6% for piperine. Hence, the present developed method was said to be suitable for the analysis of drugs in their pharmaceutical dosage form. Compared to the previous method, this method is selective, simple, sensitive, and separation was done at shorter run time.

CONCLUSION

The RP-HPLC method for estimation of curcumin and piperine was found to be simple, sensitive, precise and accurate. The present study of method development and method validation was as per ICH guidelines, and it meets the specific acceptance criteria. It is concluded that the method was precise, specific, linear, accurate, and robust within the limit. The present method development and method validation of curcumin and piperine can be used for intended purpose.

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AUTHORS CONTRIBUTIONS

All authors had contributed equally

CONFLICTS OF INTERESTS

Declaration none

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