

Reversed-Phase- HPLC Method Development and Validation for Simultaneous Determination of Dexamethasone and Curcumin in Bulk Mixtures

Ekta Panchal^{1,2}, Shiv Kumar Yadav^{2*}, Neha Jain¹, Mahima Chauhan³, Deepika Yadav², Archana Sharma^{1*}

¹Amity Institute of Pharmacy, Amity University Uttar Pradesh, Sector 125, Noida 201303, INDIA.

²Anangpuria School of Pharmaceutical Sciences, Pt. B. D. Sharma University of Health Sciences, Alampur, Faridabad 121004, Haryana, INDIA.

³HIMT College of Pharmacy College, Greater Noida, Uttar Pradesh, 201301, INDIA.

Correspondence

Dr. Archana Sharma

Associate Professor, Amity Institute of Pharmacy, Amity University Uttar Pradesh, Sector 125, Noida 201313, INDIA.

E-mail: asharma22@amity.edu

Dr. Shiv Kumar Yadav

Professor, Anangpuria School of Pharmaceutical Sciences, Pt. B. D. Sharma University of Health Sciences, Alampur, Faridabad 121004, Haryana, INDIA.

E-mail: shiv.yadav@anangpuria.com

ABSTRACT

Introduction: The combination of curcumin and dexamethasone can improve anti-inflammatory benefits, lower the dosage of corticosteroids needed, and offer a more comprehensive therapeutic strategy with fewer side effects in treating rheumatoid arthritis. **Objective:** This research work aimed to develop a specific and reproducible RP (Reversed-Phase)–HPLC analytical method with a PDA detector for the qualitative and quantitative determination of dexamethasone and curcumin in a bulk mixture. **Methods:** The RP technique was employed in which the HPLC instrument was equipped with a PDA detector and a C18 analytical column (250 × 4.6 mm, 5µm) at 30°C temperature. The mobile phase used in this work consisting 0.1 % Orthophosphoric acid: Acetonitrile at the specified ratio of 30:70 accompanied by a defined flowrate of 1mL/min with an injection volume of 10µL. The spectrum detected for the diluted simple solution at wavelength of 280nm using UV visible spectroscopy for good resolution. **Results:** This method resulted in two distinct peaks of dexamethasone and curcumin with a retention time of 3.16 and 4.16 minutes respectively and all studied parameters which ensure the suitability of the developed method were analyzed and fall within the acceptable criteria. The sensitivity was detected at 0.232µg/ml & 0.704µg/ml, respectively, for dexamethasone while, for curcumin it was found to be 0.065µg/ml and 0.198µg/ml, respectively. **Conclusion:** The validation methodology was developed by ICH guidelines for its linearity, accuracy, precision, robustness, LOD, & LOQ. From the results, it can be concluded that the developed method was found to be satisfactory in terms of reproducibility and accuracy. The chromatogram shows two distinct peaks for both drugs and further, it could be effectively utilized in the quantitative and qualitative estimation of both drugs for any pharmaceutical research.

Keywords: RP-HPLC, Dexamethasone, Curcumin, Validation, Qualitative, Quantitative.

History

- Submission Date: 03-02-2026;
- Review completed: 16-06-2026;
- Accepted Date: 26-08-2026.

DOI : 10.5530/pj.2026.18.137

Article Available online

<http://www.phcogj.com/v18/i3>

Copyright

© 2026 Phcogj.Com. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

INTRODUCTION

Dexamethasone is commonly used in the treatment of arthritis due to its potent anti-inflammatory properties. Arthritis is characterized by inflammation of the joints, leading to pain, stiffness, and reduced mobility¹. Dexamethasone works by suppressing the inflammatory response, thereby reducing swelling and pain associated with arthritis. It can be administered orally in tablet form, injected directly into the affected joint, or given intravenously depending on the severity and type of arthritis being treated². In some cases, it may also be prescribed in combination with other medications to provide maximum relief. Although dexamethasone is prominently effective in relief from arthritis proper guidance from health professionals is mandatory due to its long-term side effects³. The structures of dexamethasone and curcumin are represented in Figure 1.

Curcumin, an active phytoconstituent derived from the rhizomes of *Curcuma longa* (*Nigella sativa* L.) (Turmeric) belonging to the Zingiberaceae family, exhibits a plethora of therapeutic properties. Its multifaceted nature encompasses antimicrobial, anti-inflammatory, antioxidant, wound healing, pro-apoptotic, anti-proliferative, antiparasitic, antimalarial, antinociceptive, and anticancer effects, in addition to chemo-preventive capabilities⁴. In human participants, curcumin demonstrates modulation of various cellular

signaling pathways, including pro-inflammatory cytokines, prostaglandin E₂, apoptotic proteins, cyclooxygenase-2, 5-lipoxygenase, and C-reactive protein, attributed to its pleiotropic activities. These observations underscore curcumin's significant potential as a therapeutic agent across a spectrum of medical conditions. Despite the manifold pharmacological benefits of curcumin, its efficacy is impeded by challenges such as limited bioavailability, swift metabolism, abbreviated half-life, inadequate absorption, and rapid systemic elimination⁵.

To surmount these hurdles, numerous investigations have explored amalgamation strategies and formulations pairing curcumin with other pharmacologically active agents. One such pairing involves dexamethasone, designed to potentiate curcumin's anti-inflammatory efficacy within biological contexts. By capitalizing on synergistic interactions and mitigating the limitations inherent to curcumin monotherapy, these combination modalities exhibit promise in augmenting the therapeutic efficacy of curcumin-based interventions⁶. The combination of dexamethasone and curcumin in arthritis shows potential for synergistic anti-inflammatory effects, which can be analyzed and quantified using Reverse Phase –High Performance Liquid Chromatography (RP-HPLC), offering insights into their individual concentrations and interaction dynamics for optimized therapeutic outcomes. Further research utilizing RP-HPLC

Cite this article: Ekta P, Shiv K Y, Neha J, Mahima C, Deepika Y, Archana S. Reversed-Phase-HPLC Method Development and Validation for Simultaneous Determination of Dexamethasone and Curcumin in Bulk Mixtures. Pharmacogn J. 2026;18 (3):221-228.

can elucidate the pharmacokinetic profile and bioavailability of this combination therapy in arthritis management⁷.

MATERIALS AND METHODS

Chemicals

Dexamethasone sourced from UniChem Laboratories Ltd., Ghaziabad, India, and curcumin procured from Sun Pharma, India, both facilitated by GLR Innovations, were utilized in the study. High-performance liquid chromatography (HPLC) grade methanol, orthophosphate, and acetonitrile were obtained from Merck India Ltd., Mumbai, ensuring high purity without additional purification. All solvent preparations adhered to stringent HPLC-grade standards. Methanol, the diluent, underwent filtration through 0.22 μm membrane filters before utilization in the analysis process. To ensure optimal performance, all prepared solvents underwent sonication for approximately 15 minutes before analysis, enhancing their homogeneity and stability for accurate analysis.

Instrumentation

All standards and samples were analyzed on an RP-HPLC instrument which was equipped with a PDA detector (Shimadzu Corp., Kyoto, Japan). The analytical column employed for the chromatographic separation was C18, 250 $\times$ 4.6 mm, 5 μm bead size. All the evaluation parameters and interpretation were examined and detected by using software Liquid Chromatography (LC) solution v.1.24 Spinchrom-1.

Methods

Solubility Assessment: Different solvents were used to investigate the solubility of dexamethasone and curcumin. The solubility assessments of dexamethasone and curcumin were done by using different solvents i.e. HPLC grade distilled water, Phosphate Buffer Saline (PBS pH 7.4), ethanol, methanol, and chloroform. For the investigation of the solubility profile with selected solvents, the saturated solution was firstly prepared by dissolving the excess quantity of drugs (i.e.

dexamethasone and curcumin) in 10ml of each solvent. After that, at the room temperature of $25 \pm 1^\circ\text{C}$, the prepared saturated drug solutions were agitated for 24 hrs. at 100rpm using a magnetic stirrer. The obtained specimens were then centrifuged at 10,000 rpm for about 10 minutes. The clear supernatant was recovered via a 0.22 μm syringe membrane filter which was further analyzed through a UV-visible spectrophotometer (Shimadzu, UV-1800, Japan). The received findings were then examined and noted.

Spectral Analysis: The requisite absorption maxima of Dexamethasone (40 $\mu\text{g/mL}$) and Curcumin (10 $\mu\text{g/mL}$) solutions prepared in methanol: water at the ratio of 2:1 was analyzed in between the range from 200 nm to 400 nm using a UV spectrophotometer.

Chromatographic conditions: Chromatographic analysis was carried out using stationary phase as C18 (250 $\times$ 4.6 mm, 5 μm particle size) column at 30 $^\circ\text{C}$ temperature. Separation of dexamethasone and curcumin was isocratically done using a mobile phase consisting of Solvent A (0.1% Orthophosphoric Acid) and Solvent B (Acetonitrile) which was prepared using a 30:70(v/v) proportion of solvent A and B respectively at a specified flow rate of 1 mL/min with injection volume 10 μL ^{8,9}.

Preparation of standard stock solution of dexamethasone and curcumin: To prepare the standard solutions separately of both drugs, accurately weighed 10mg quantity of dexamethasone and curcumin, respectively was transferred to a 100 ml volumetric flask. After that, they were dissolved in 100ml methanol (filtered through 0.22 μm syringe filters) to produce a stock solution of 100 $\mu\text{g/mL}$, which was further sonicated to dissolve¹⁰.

Preparation of sample dilution for dexamethasone and curcumin: Specific dilutions were carried out from stock solutions to make the sample solutions. Firstly, take 1ml of stock solution, and dilute up to 10ml with diluent and the concentration was found 10 $\mu\text{g/mL}$ then filtered through a 0.22 μm filter and injected in HPLC instrument. Similarly, all the other sample solutions were prepared from the range of 10–60 $\mu\text{g/mL}$ using methanol as diluent¹¹.

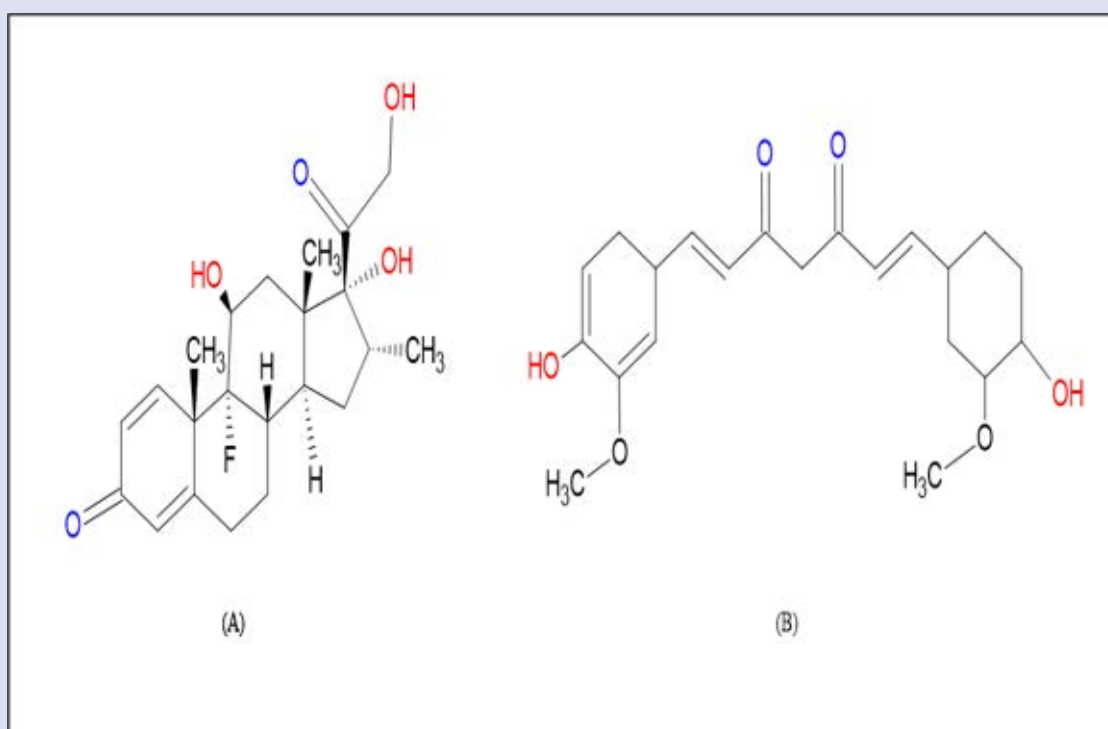


Figure 1. Chemical structure of dexamethasone (A) and curcumin (B)

Method development and optimization: This RP-HPLC method for dexamethasone and curcumin was developed and validated according to Q2-ICH guidelines. Further, it was evaluated for precision, linearity, robustness, accuracy, LOD, and LOQ.

Specificity: One of the important aspects of the HPLC technique is its specificity, defined as the method's capacity to determine the amount of analyte precisely and free from disturbance from other predicted ingredients. By introducing the blank solution, standard, and samples independently via injection, the chromatograms were recorded that demonstrate the specificity of the procedure.

Linearity: During the analytical method development process, the term linearity is associated with the ability to produce test results (within the predefined range) which are directly proportional to the amount of analyte. The range, from the lowest to highest levels of analyte with acceptable precision, accuracy, and linearity, is known as the method's range. For Dexamethasone and Curcumin, a linearity range of 10–60 µg/ml was selected. Subsequently, each dilution was filtered through a 0.22 µm filter before injection¹².

Intra-day and inter-day precision: The evaluation of precision parameters was performed by inter-day and intra-day variation studies. For the intra-day precision studies, the analytical test results received from six repeated injections of standard (within one day) were collected, and the response factor of drug peaks and percentage RSD (Relative Standard Deviation) were then calculated. Similarly, for the inter-day precision studies, the analytical test results received from six replicated injections of the standard were collected for six sequential days which was then followed for the response factor of drug peaks and percentage RSD. The repeatability parameter of the analytical method was further assessed using a 40 µg/ml concentration of analyte and injecting the sample six times, and the percentage RSD was calculated.

Accuracy/Recovery Studies: The method's accuracy was assessed using the standard recovery percentage. The recovery tests were performed using a standard drug solution which was mixed with the pre-analyzed sample at three different concentration levels: 50%, 100%, and 150%. Using this procedure, the test sample was mixed with a known concentration of the standard drug.

50% Accuracy: Given 10mg standard of each compound was dissolved in a 100ml volumetric flask and made up the volume to a 100ml with diluent and concentration found 100 µg/ml. Further, take 2ml of stock solution and dilute up to 10ml with diluent and the concentration was found 20 µg/ml then filtered through a 0.22 µm filter and injected in HPLC instrument. Inject duplicate injections of a standard solution into HPLC.

100% Accuracy: Given 10mg standard of each compound was diluted in a 100ml volumetric flask and made to a 100ml volume using diluent. The concentration was found to be 100 µg/ml. Further, take 4 ml from the stock solution and dilute up to 10ml with diluent, the concentration was found to be 40 µg/ml and filtered through a 0.22 µm filter.

150% Accuracy: Given 10mg standard of each compound was diluted in a 100ml volumetric flask and made to a 100ml volume using diluent. The concentration was found to be 100 µg/ml. Further, take 6ml from the stock solution and dilute up to 10ml with diluent, the concentration was found to be 60 µg/ml and filtered through a 0.22 µm filter.

Robustness: In an analytical method the term robustness is defined as the ability to stay unaffected due to minor changes but determined modifications in method parameters during routine use. By examining the sample and purposefully changing the procedure parameters, the resilience was investigated. The percentage RSD was used to indicate how the responses to the drugs changed. The method's robustness was examined using wavelength ± 5 nm and flow rate ± 0.1 ml variations^{4,8}.

Sensitivity

Detection Limit or Limit of Detection (LOD)

An individual analytical procedure's LOD is referred to as the smallest amount of analyte in a sample that can be determined but not generally quantified as an exact figure. To calculate LOD, results obtained from linearity evaluation were used. The linearity plot's slope was also determined from the above received findings. The standard deviation (SD) of the responses was calculated for each of the ten replicate measurements of the same concentration (20 µg/mL).

The LOD is represented as:

$$\text{LOD} = 3.3\sigma/S$$

Quantitation Limit or Limit of Quantitation (LOQ)

In the analytical process, the term quantitation limit is referred to as the lowest amount of analyte that can be quantitatively analyzed with sufficient precision and accuracy.

The LOQ is represented as:

$$\text{LOQ} = 10\sigma/S$$

Where,

σ = Relative Standard Deviation (of the response)

S = the slope of the calibration curve (of the analyte)^{6,17}

RESULT AND DISCUSSION

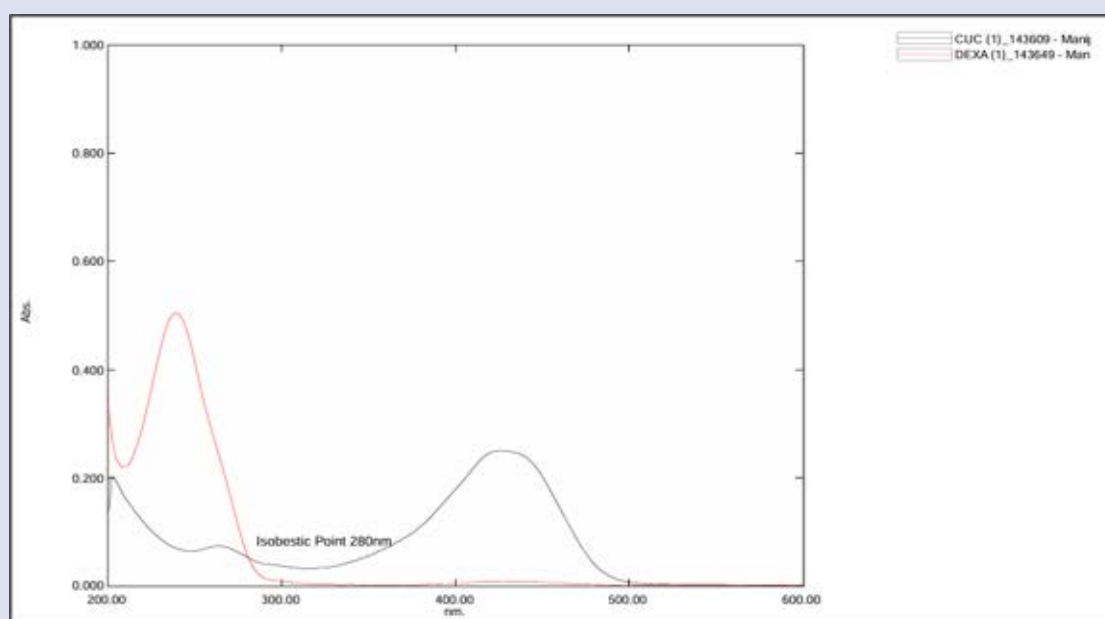
The results of this study revealed the solubility assessment, chromatographic conditions, spectral analysis and Reversed-Phase-HPLC Method development for the simultaneous determination of dexamethasone and curcumin. Both drugs are strongly soluble in organic solvents. A UV absorption maximum of dexamethasone and curcumin was identified at 280 nm. The chromatographic conditions were analyzed for the simultaneous detection of both drugs with retention times (3.155 min and 4.160 min) and showed excellent specificity, linearity (R^2 values of 0.9994 and 0.999), precision (low % RSD), and accuracy (recovery rates of 99-101%). Robustness testing displayed stability under slight variations in operational parameters, and sensitivity analysis revealed the method's ability to detect low concentrations. Overall, the method is reliable, precise, and suitable for routine analysis of dexamethasone and curcumin in pharmaceutical formulations. The accuracy of the method was confirmed by recovery studies, which showed outstanding recovery rates ranging from 99% to 101%, indicating the method's accuracy in quantifying both drugs. The robustness tests demonstrated that the method remained stable even when small changes were made to the operational parameters, such as wavelength and flow rate. Finally, the sensitivity of the method was validated by determining the limits of detection (LOD) and quantification (LOQ), confirming its suitability for routine analysis of dexamethasone and curcumin.

Solubility Assessment: The solubility of dexamethasone and curcumin was evaluated in different solvents viz. ethanol, methanol, chloroform, PBS pH 7.4, and HPLC-grade distilled water. The quantitative results of both drugs are mentioned in Table 1. As per the results, dexamethasone and curcumin demonstrated the highest solubility in ethanol and methanol, and slight solubility in PBS 7.4.

Spectral Analysis: The absorption maxima of Dexamethasone and Curcumin were detected at 239nm and 426nm, respectively. The overlapping of dexamethasone and curcumin spectrum was analyzed during scanning through UV spectroscopy which showed the development of an isobestic point at 280nm as shown in Figure 2. Thus, absorption maxima of 280 nm were selected for the HPLC study due to greater resolution efficacy at ambient temperature conditions^{13,14}.

Table 1. Solubility studies of Dexamethasone and Curcumin in different solvents

Dexamethasone			
S. No.	Name of solvent	Solubility (mg/ml)	Solubility
1	Ethanol	112.82±0.445	Freely soluble
2	Methanol	110.88±0.441	Freely soluble
3	Chloroform	5.820±0.029	Slightly soluble
4	Phosphate Buffer 7.4	1.150±0.004	Slightly insoluble
5	Water	0.080±0.000	Practically insoluble
Curcumin			
S. No.	Name of solvent	Solubility (mg/ml)	Solubility
1	Ethanol	34.690±0.105	Soluble
2	Methanol	33.910±0.049	Soluble
3	Phosphate Buffer 7.4	13.970±0.077	Sparingly soluble
4	Chloroform	4.470±0.003	Slightly soluble
5	Water	0.230±0.296	Practically insoluble

**Figure 2.** The Isobestic point of Dexamethasone and Curcumin via UV Spectroscopy

Chromatographic Conditions: The current investigation aimed to design a simplified isocratic Reverse Phase-HPLC method for the simultaneous detection of dexamethasone and curcumin in the bulk mixture. The retention time for dexamethasone and curcumin were found to be 3.155 min and 4.160 min, respectively, by using 40 µg/ml. The chromatogram on HPLC analysis of the blank and standard solution of curcumin (40 µg/ml) and dexamethasone (40 µg/ml) was optimized and evaluated by the suggested procedure. The HPLC analysis of the standard chromatogram is depicted in Figure 3. The retention time, % purity and peak area detected are shown in Table 2.

Method Validation and Optimization

Specificity: The originated chromatogram of the standard mixture solution of blank and dexamethasone curcumin combination depicted in Figures 3(A) and 3(B), respectively, shows that the peaks developed in the chromatogram at the working concentration are due to the presence of drugs as blank has not represented any peaks at the same retention time of dexamethasone and curcumin. Thus, it can conclude the specificity of the developed method¹⁵.

Linearity: For Dexamethasone and curcumin, a calibration curve was drawn across a concentration range from 10-60 µg/ml was precisely

measured, and each dilution was filtered through a 0.22 µm filter before being injected. The calibration curve for curcumin and dexamethasone was obtained by using a 10- 60 µg/ml solution. The area was measured at 280 nm as an isobestic point. The graph of a calibration curve, indicated the regression equation at ($Y = 21177x - 6817$) and R^2 value was 0.9994 and regression equation ($Y = 130237x - 30629$) and R^2 value was 0.999 for dexamethasone and curcumin, respectively, which shows good linearity as shown in Figure 4(A) and 4(B). The other related statistical parameters i.e. slope (m) and intercept (C) involved in the determination of dexamethasone and curcumin shown in Table 3. All the results and findings related to the determination of both drugs fall within the acceptable criteria indicating the linearity in the data¹⁶.

Precision: The inter-day and intra-day variation studies were demonstrated for the precision of this method. The intra-day and inter-day variation studies were done six repeated times using standard for six days consecutively and % RSD along with response factor of drug peaks were calculated. From the resulting data, it was concluded that the developed method was precise. To determine the repeatability of this developed method the injection containing 40 µg/ml concentration was injected six times and the % RSD was calculated. The low value of % RSD contributed to the precision of this developed method. The repeatability ($n=6$) Mean (µg/ml) Standard Deviation and RSD (%) were found to be within the acceptable range which is shown in Table 4¹⁷.

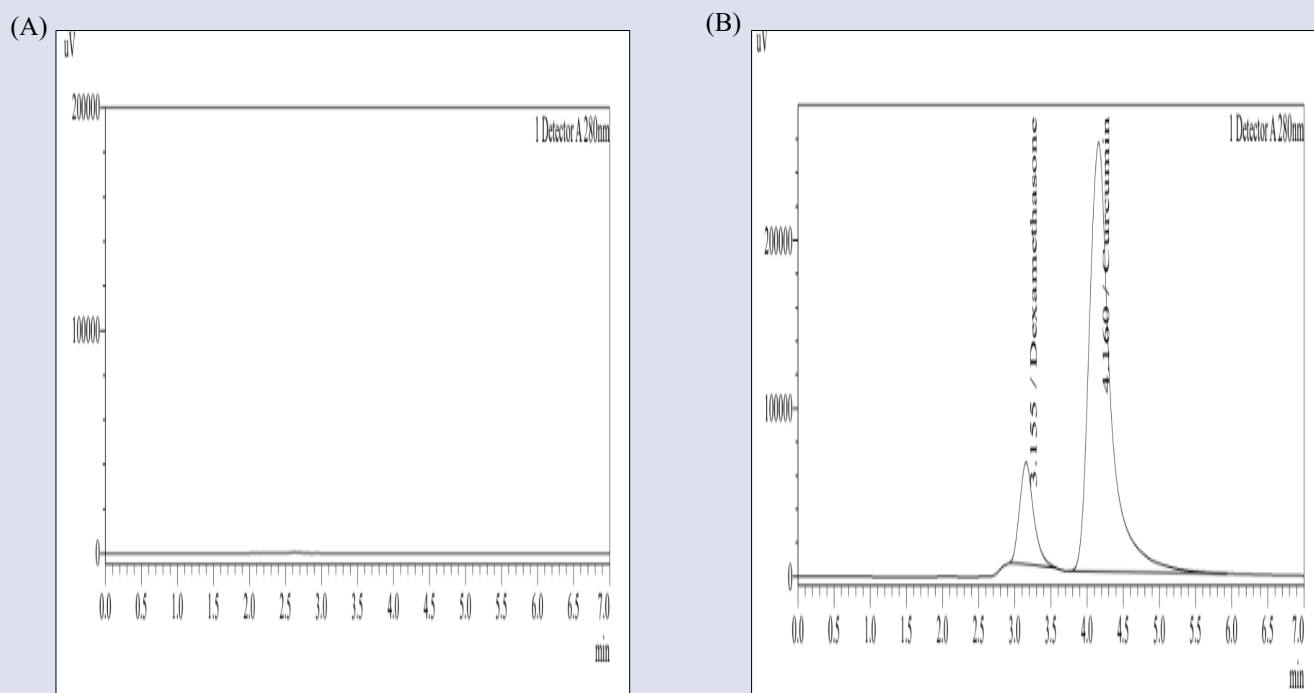


Figure 3. (A) Chromatogram of blank, **(B)** Simultaneous chromatogram of standard Dexamethasone and Curcumin

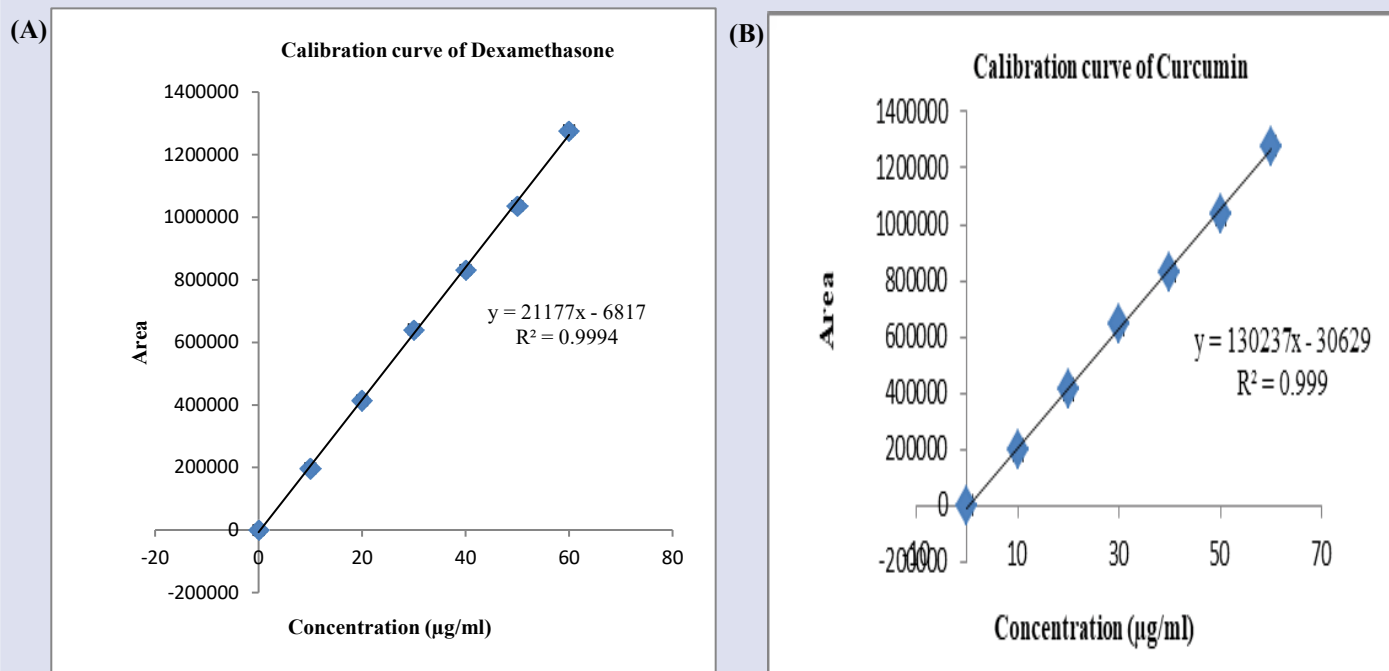


Figure 4. (A) The standard calibration curve for Dexamethasone using RP-HPLC, **(B).** The standard calibration curve for curcumin using RP-HPLC

Table 2. Data of Simultaneous Dexamethasone and Curcumin

S. No.	Name	Conc. (µg/ml)	Ret. Time	% Purity	Area
1	Dexamethasone	40	3.155	98.34	818103
2	Curcumin	40	4.160	99.21	5191167

Table 3. Statistical parameters of standard curve for quantification of Dexamethasone and Curcumin

Statistical parameters	Dexamethasone Results	Curcumin Results
Regression equation ($y = mx + c$)	$Y = 21177x - 6817$	$Y = 130237x - 30629$
Intercept (C)	6817	30629
Slope (m)	21177	130237

Table 4. Precision parameters for dexamethasone and curcumin by optimized method (n=6)

Drug	Repeatability Mean ($\mu\text{g/ml}$) $\pm$ SD	% RSD
Dexamethasone	97.54 $\pm$ 0.85	0.87
Curcumin	99.39 $\pm$ 1.35	1.36
Inter-day precision (n=6)		
Dexamethasone	98.62 $\pm$ 0.76	0.77
Curcumin	98.72 $\pm$ 1.39	1.41
Intra-day precision (n=6)		
Dexamethasone	98.85 $\pm$ 1.14	1.15
Curcumin	100.17 $\pm$ 1.52	1.52

Table 5. The recovery data of the drugs in bulk mixture

Dexamethasone				
	Conc. ($\mu\text{g/ml}$)	Area	Amount Recovered ($\mu\text{g/ml}$)	% Recovery
50%	20	417600	20.04	100.21
	20	420074	20.16	101.79
	20	414003	19.87	99.36
Mean		417226		100.12
SD		3052.76		0.72
% RSD		0.73		0.72
	Conc. ($\mu\text{g/ml}$)	Area	Amount Recovered ($\mu\text{g/ml}$)	% Recovery
100%	40	851570	40.53	101.33
	40	851570	40.53	101.33
	40	843763	40.17	100.41
Mean		848968		101.03
SD		4507.37		0.53
% RSD		0.53		0.53
	Conc. ($\mu\text{g/ml}$)	Area	Amount Recovered ($\mu\text{g/ml}$)	% Recovery
150%	60	1262405	59.93	99.89
	60	1270772	60.33	100.55
	60	1273637	60.46	100.77
Mean		1268938		100.40
SD		5836.28		0.46
% RSD		0.46		0.46
Curcumin				
	Conc. ($\mu\text{g/ml}$)	Area	Amount Recovered ($\mu\text{g/ml}$)	% Recovery
50%	20	2552277	19.83	99.16
	20	2551957	19.83	99.15
	20	2552455	19.83	99.17
Mean		2552230		99.16
SD		252.35		0.010
% RSD		0.01		0.010
	Conc. ($\mu\text{g/ml}$)	Area	Amount Recovered ($\mu\text{g/ml}$)	% Recovery
100%	40	5182396	40.03	100.07
	40	5182396	40.03	100.07
	40	5190810	40.09	100.23
Mean		5185201		100.12
SD		4857.83		0.09
% RSD		0.09		0.09
	Conc. ($\mu\text{g/ml}$)	Area	Amount Recovered ($\mu\text{g/ml}$)	% Recovery
150%	60	7880848	60.75	101.24
	60	7929853	61.12	101.87
	60	7880242	60.74	101.24
Mean		7896981		101.45
SD		28469.60		0.364
% RSD		0.36		0.36

Table 6. The robustness data for dexamethasone and curcumin

Robustness data of Dexamethasone with deliberate change in Flow rate						
Robustness_ 0.9ml				Robustness_ 1.1ml		
Conc.(µg/ml)	Area	Amount Recovered (µg/ml)	% recovery	Area	Amount Recovered (µg/ml)	% recovery
40	846202	40.28	100.70	823915	39.23	98.07
40	844284	40.19	100.47	823771	39.22	98.05
40	845563	40.25	100.63	826474	39.35	98.37
Mean	845350		100.60	824720		98.17
SD	976.63		0.12	1520.71		0.18
% RSD	0.12		0.11	0.18		0.18
Robustness data of Dexamethasone with deliberate change in Wavelength						
Robustness_ 275nm				Robustness_ 285nm		
Conc.(µg/ml)	Area	Amount Recovered (µg/ml)	% Recovery	Area	Amount Recovered (µg/ml)	% recovery
40	838379	39.91	99.78	838110	39.90	99.75
40	834448	39.73	99.31	836002	39.80	99.50
40	840241	40.00	100.00	837056	39.85	99.62
Mean	837689		99.55	837056		99.62
SD	2957.44		0.33	1054.00		0.12
% RSD	0.35		0.33	0.13		0.12
Robustness data of curcumin with deliberate change in Flow rate						
Robustness_ 0.9ml				Robustness_ 1.1ml		
Conc.(µg/ml)	Area	Amount Recovered (µg/ml)	% recovery	Area	Amount Recovered (µg/ml)	% recovery
40	5105357	39.44	98.59	5061742	38.10	97.75
40	5154193	39.81	99.53	5068294	39.15	97.88
40	5129775	39.62	99.06	5075018	38.20	98.01
Mean	5129775		99.06	5068351		97.88
SD	24418.00		0.47	6638.19		0.13
% RSD	0.48		0.47	0.13		0.13
Robustness data of curcumin with deliberate change in Wavelength						
Robustness_ 275nm				Robustness_ 285nm		
Conc.(µg/ml)	Area	Amount Recovered (µg/ml)	% recovery	Area	Amount Recovered (µg/ml)	% recovery
40	5158029	39.84	99.60	5081629	39.25	98.13
40	5122472	39.57	98.92	5066286	39.14	97.84
40	5140251	39.70	99.26	5073958	39.19	97.99
Mean	5140251		99.26	5073958		97.99
SD	17778.50		0.34	7671.50		0.15
% RSD	0.35		0.34	0.15		0.15

Table 7. The standard curve parameters, LOD, LOQ for dexamethasone and curcumin

	Standard curve equation	Correlation coefficient	Linear range (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)
Dexamethasone	Y= 21177x – 6817	0.999	10-60	0.232	0.704
Curcumin	Y= 130237x – 30629	0.9994	10-60	0.065	0.198

Accuracy

The accuracy of the developed method was assessed using the standard recovery percentage. To conduct recovery tests, a standard drug solution was added to the previously analyzed sample at the three different concentration levels (50%, 100%, and 150%). Using this procedure, the test sample was mixed with a known concentration of the standard drug. All the recovery data-related parameters like peak area, amount of drug added, amount received, percent recovery, mean percent SD, and % RSD were stated in Table 5. The results and findings related to this evaluation fell within the limits. The percentage recovery ranges from 99-101 percent which shows a good recovery amount with an appropriate accuracy profile of the proposed method¹⁶.

Robustness: By examining the sample and purposefully changing the procedure parameters, the resilience was investigated. The percentage RSD was used to indicate how the responses to the drugs changed. The method's robustness was examined using wavelength ± 5 nm and flow rate ± 0.1 ml variations. By examining the sample with a lower concentration (i.e. 40 µg/ml) and purposefully changing the procedure parameters, the resilience was investigated. The percentage RSD was

used to indicate how the responses of the drug changed. The method's robustness was examined by varying the wavelength and flow rate which is stated in Table 6¹⁸.

Sensitivity: Following ICH recommendations, the developed method's LOD and LOQ were examined. There are various methods available for figuring out the LOD and LOQ, depending on whether an instrumental or non-instrumental procedure is used. One of the techniques used here was mentioned as,

$$\text{LOD} = 3.3[\text{SD}/S]$$

$$\text{LOQ} = 10[\text{SD}/S]$$

Where,

SD= standard deviation of the intercept

S = mean of slope in the standard curve

The LOD and LOQ of this method were determined based on the SD of the response and slope (S) of the standard curve at levels of the LOD and LOQ⁴. The results were obtained within the range that is stated in Table 7.

CONCLUSIONS

From the results, it can be concluded that the above developed method for simultaneous qualitative and quantitative estimation of dexamethasone and curcumin is a cost-effective, simple, specific, rapid, and reproducible technique. The linearity, precision, accuracy, robustness, specificity, and all the calibration parameters were validated as per the ICH recommendations which represent good resolution peaks and research findings with less retention time. This proposed method could be utilized for the estimation of different pharmacokinetic parameters for this combination of drugs along with the quality control test evaluations.

ACKNOWLEDGMENTS

The authors are highly thankful to Anangpuria School of Pharmaceutical Sciences, Alampur, Faridabad, Haryana for providing the necessary support and equipped platform to perform this research work.

REFERENCES

- Buckman TA, Sakyi SA, Yeboah-Mensah K, Antwi MH, Darban I, Owusu-Brenya L, et al. Demographic, Clinical Profile of Rheumatoid Arthritis Patients and Their Association with Disease Severity in Ghana. *Int J Rheumatol*. 2024;2024(1):9. Available from: <https://doi.org/10.1155/2024/6639079>
- Yi Z, Ran Y, Chen X, Tong Q, Ma L, Tan Y, et al. Tea polyphenol carrier-enhanced dexamethasone nanomedicines for inflammation-targeted treatment of rheumatoid arthritis. *J Mater Chem B*. 2023;11(48):11505-18. Available from: <https://doi.org/10.1039/d3tb02316h>
- Han Y, Huang S. Nanomedicine is more than a supporting role in rheumatoid arthritis therapy. *J Control Release*. 2023;356:142-61. Available from: <https://doi.org/10.1016/j.jconrel.2023.02.035>
- Patil N, Mahajan H. Development and validation of RP-HPLC method for simultaneous qualitative and quantitative estimation of curcumin and quercetin in bulk mixture. *Indian J Pharm Educ Res*. 2022;56(1):247-54. Available from: <https://www.ijper.org/sites/default/files/IndJPhaEdRes-56-1-247.pdf>
- Rahmani AH, Alsahli MA, Aly SM, Khan MA, Aldebasi YH. Role of curcumin in disease prevention and treatment. *Adv Biomed Res*. 2018;7(1):38. Available from: https://doi.org/10.4103/abr.abr_147_16
- Kagathara C, Odedra K, Vadia N. Development of HPTLC method for the simultaneous estimation of quercetin, curcumin, and ascorbic acid in herbal formulations. *J Iran Chem Soc*. 2022;19(10):4129-38. Available from: <https://doi.org/10.1007/s13738-022-02586-9>
- Ponnusamy C, Krishnaswami V, Pandian S, Natesan S. In-vitro Drug Release and Ex-vivo Corneal Permeation of Dexamethasone and Curcumin Nanodispersions Using Facilitated Simultaneous RP-HPLC Technique. *Ana Chem Lett*. 2018;8(3):311-20. Available from: <https://doi.org/10.1080/22297928.2018.1445555>
- Kumar VK, Singh A. A stability indicating RP-HPLC method for related impurities of dexamethasone in tobramycin and dexamethasone otic suspension. *Int J Health Sci*. 2022(6):119-32. Available from: <https://doi.org/10.53730/ijhs.v6nS6.9177>
- ParabGaonkar V, Hullatti K. Quality assessment and RP-HPLC method development for estimation of curcuminoids in Curcuma longa: A Quality by Design approach. *J Liq Chromatogr Relat Technol*. 2021;44(1-2):95-102. Available from: <https://doi.org/10.1080/010826076.2020.1848862>
- Xiao H, Sedlářik V. A rapid and sensitive HPLC method for simultaneous determination of irinotecan hydrochloride and curcumin in co-delivered polymeric nanoparticles. *J Chromatogr Sci*. 2020 Jul 24;58(7):651-60. Available from: <https://doi.org/10.1093/chromsci/bmaa033>
- Nasr ZA, Soliman MM, Mohamed EH, Fouad FA. Assessment of the greenness of micellar HPLC method for rapid separation and simultaneous estimation of chlorpheniramine maleate in presence of some co-administered drugs in three pharmaceutical dosage forms using single run. *Acta Chromatogr*. 2021 Sep 7;34(2):138-49. Available from: <https://doi.org/10.1556/1326.2021.00883>
- Vaz GR, Clementino A, Bidone J, Villetti MA, Falkembach M, Batista M, Barros P, Sonvico F, Dora C. Curcumin and quercetin-loaded nanoemulsions: Physicochemical compatibility study and validation of a simultaneous quantification method. *Nanomaterials*. 2020 Aug 22;10(9):1650. Available from: <https://doi.org/10.3390/nano10091650>
- Tagde P, Nagpal K, Kulkarni GT. A new Validated RP-HPLC Method for simultaneous quantification of Raloxifene and Curcumin. *Res J Pharm Technol*. 2022;15(9):4218-25. Available from: <https://rjptonline.org/AbstractView.aspx?PID=2022-15-9-69>
- Mittha JN, Kalshetti MS. RP-HPLC method for simultaneous estimation of tobramycin and dexamethasone in eye drop. *Res J Pharm and Technol*. 2022;15(3):1282-6. Available from: <https://indianjournals.com/article/rjpt-15-3-057>
- Akhtar M, Gul S, Shamim S, Siddiqui R. Simultaneous determination of moxifloxacin with NSAIDs in API, dosage and serum by reverse phase high-performance liquid chromatography: Application to in vitro drug interactions. *Pak J Pharm Sci*. 2021 Nov 1;34(6). Available from: <https://pubmed.ncbi.nlm.nih.gov/35034869/>
- Smerikarova M, Bozhanov S, Mateeva A, Maslarska V. Development of simple HPLC-UV method for the simultaneous determination of repaglinide, dexamethasone, and remdesivir, and its application to synthetic mixture and human plasma. *Curr Pharm Anal*. 2023 Oct 1;19(8):662-72. Available from: <https://doi.org/10.2174/0115734129263384230928052923>
- Saad MN, Essam HM, Elzanfaly ES, Amer SM. A two-step optimization approach: Validated RP-HPLC method for determination of gatifloxacin and dexamethasone in ophthalmic formulation. *J Chromatogr Sci*. 2020 Jun 5;58(6):504-10. Available from: <https://doi.org/10.1093/chromsci/bmaa013>
- Fonseca-Santos B, Gremião MP, Chorilli M. A simple reversed phase high-performance liquid chromatography (HPLC) method for determination of in situ gelling curcumin-loaded liquid crystals in in vitro performance tests. *Arab J Chem*. 2017 Nov 1;10(7):1029-37. Available from: <https://doi.org/10.1016/j.arabjc.2016.01.014>

Cite this article: Ekta P, Shiv K Y, Neha J, Mahima C, Deepika Y, Archana S. Reversed-Phase- HPLC Method Development and Validation for Simultaneous Determination of Dexamethasone and Curcumin in Bulk Mixtures. *Pharmacogn J*. 2026;18 (3):221-228.