ORIGINAL ARTICLE

Development and Validation of Reversed-phase High-performance Liquid Chromatography Method for Simultaneous Estimation of Desonide and *Curcumin* in Topical Dosage Form

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Abstract

A reversed-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated to simultaneously determine curcumin and desonide in both bulk and pharmaceutical dosage forms. This method is characterized by its simplicity, sensitivity, speed, specificity, precision, and accuracy. The mobile phase consisted of a mixture with a ratio of 65:35 v/v, and the flow rate was maintained at 0.8 mL/min. Chromatographic separation was achieved using a C18 column (250 × 4.6 mm – 5 µm p.s), with ultraviolet detection performed at 240 nm. An injection volume of 20 µL was used. The retention times for desonide and curcumin were 3.45 and 7.51 min, respectively, resulting in distinct and well-defined chromatographic peaks. The respective percentage recoveries were 99.56% and 99.87%. The suggested approach demonstrated strong linearity, accuracy, and precision, and it was effectively used to determine the medicines in pharmaceutical dosage forms. The current approach was created and successfully used to ascertain the quantities of curcumin and desonide in a combined formulation after it was statistically validated in compliance with the ICH recommendations.

Key words: Curcumin, desonide, ICH guidelines, reversed-phase high-performance liquid chromatography, ultraviolet-spectroscopy, validation

INTRODUCTION

Curcumin, the active compound found in the perennial herb *Curcuma longa*, commonly known as turmeric, belongs to the polyphenol class.[1] It is also referred to as diferuloylmethane. Turmeric's yellow-pigmented portion consists mainly of curcuminoids, which share a chemical structure similarity with curcumin.[2,3] The primary curcuminoids in turmeric include curcumin I, II, III, demethoxycurcumin and the recently discovered cyclocurcumin.[4] Commercially, Curcumin I comprises around 77%, Curcumin II approximately 17%, and Curcumin III about 3%. The curcuminoid complex goes by various names such as Indian saffron, yellow ginger, yellow root, kacha haldi, ukon, or natural yellow.[5] Curcuminoids constitute about 3–5% of turmeric. Curcumin was initially identified as 1,6-heptadiene-3,5-dione-1,7-bis(4-hydroxy-3-methoxyphenyl)-(1E,6E) in 1815 and obtained in crystalline form in 1870.[6] Lampe verified and synthesized the feruloylmethane backbone of curcumin in 1910. Both acute and chronic inflammation can be suppressed by curcumin. By decreasing histamine levels and maybe boosting the adrenal glands' endogenous cortisone production, it lessens inflammation. Curcumin also demonstrated anti-inflammatory properties on human vascular cells *in vitro*.[7]

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Due to its glucocorticoid efficacy, desonide has been utilized extensively to treat dermatose-infected inflammatory and pruritic illnesses. Desonide is an excellent treatment for a variety of skin diseases that include redness, swelling, itching, and pain. Desonide is an essential component of topical corticosteroids.[8] Many topical formulations available in the market, such as lotions, creams, gels, ointments, foams, and hydrogels, include desonide.[9] Desonide exhibits anti-inflammatory, antipruritic, and vasoconstrictive effects similar to other topical corticosteroids. The medication attaches itself to glucocorticoid receptors in the cytosol. When it reaches the nucleus, this complex attaches itself to DNA's genetic components. Numerous genes are both repressed and activated by this.[10]

A literature review indicated that numerous spectroscopic techniques exist for determining curcumin and desonide separately.[11-15] There is a lack of readily available methods for simultaneously quantifying curcumin and desonide in both bulk and pharmaceutical dosage forms. Therefore, the aim of this study was to develop and validate a user-friendly, rapid, accurate, reproducible, and cost-effective reversed-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous determination of curcumin and desonide in topical formulations.

MATERIALS AND METHODS

Materials

All chemicals and reagents utilized in the research were of analytical grade. Curcumin and desonide were generously provided by Aurobindo Pharmaceutical Ltd., Hyderabad. The study utilized the Shimadzu Prominence System (SPD-20AT, Shimadzu) for analysis. Chromatograms and data were recorded using Spinchrom CFR Software.

Methods

Preparation of mobile phase

Following multiple trials, a mobile phase consisting of methanol and acetonitrile in a ratio of 65:35 v/v was selected, as it provided satisfactory resolution and peak characteristics. The mobile phase was prepared by combining methanol and acetonitrile in the specified ratio in a volumetric flask. Subsequently, it was filtered through a 0.45 µ nylon membrane (Millipore) and degassed using an ultrasonic bath.

Preparation of standard solution

To prepare the standard stock solution, 10 mg of desonide and 20 mg of curcumin were separately transferred into 100 mL volumetric flasks containing a mixture of Methanol and acetonitrile in a ratio of 65:35 v/v. The volume was adjusted to 100 mL to achieve concentrations of 100 µg/mL for desonide and 200 µg/mL for curcumin, respectively.

Selection of analytical wavelength

The efficacy of the HPLC method, relying on ultraviolet (UV) spectrophotometric determination, hinges on the careful selection of the detection wavelength. Standard solutions underwent scanning between 200 and 400 nm using a UV spectrophotometer. After overlaying spectra of desonide and curcumin, 240 nm was chosen as the detection wavelength.

Optimized chromatographic condition

In the current study, the separation of desonide and curcumin was accomplished using a Grace C18 column (250 × 4.6 mm, 5 µ) with a mobile phase composed of methanol and acetonitrile in a 65:35 ratio. The flow rate was set at 0.8 mL/min, and UV detection was performed at a wavelength of 240 nm at ambient temperature. Desonide eluted at 3.45 min, while curcumin eluted at 7.51 min.

Sample preparation

1 mL of nano gel formulation was mixed with methanol and volume was adjusted to 10 mL (1:10), then it was centrifuged at 10,000 RPM for 15 min. The un-dissolved gel was settled at the bottom and supernatant was used for the estimation of free desonide and curcumin in the formulation. The absorbance was recorded if it was >1.000 then another 1:10 dilution was made up and absorbance was re-recorded.

Method validation[16-19]

The method underwent validation in accordance with the ICH Q2 (R1) guidelines to assess specificity, recovery, precision, linearity, robustness, limit of detection (LOD), and limit of quantification (LOQ).

System suitability

Desonide and curcumin working standard solutions were tested for system suitability test by injecting 10 µL into the chromatographic column as per the proposed chromatographic conditions. Parameters, such as theoretical plates, resolution, tailing factor, and capacity factor were evaluated to confirm system suitability.

Linearity and range

From the stock solutions of desonide and curcumin, aliquots were pipetted into volumetric flasks, mixed, and then diluted to 10 mL using the mobile phase, accordingly, to obtain solutions within the desired concentration range- 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, and 25 µg/mL of desonide - 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 µg/mL of curcumin. These 10 solutions were evaluated for linearity.

Precision

Solutions of concentrations 2.5, 12.5, and 25 µg/mL for desonide and 5, 25, and 50 µg/mL for curcumin were evaluated

for intraday variability for 2 times while interday variability was evaluated on 3 days to confirm the precision of the method.

Accuracy

Accuracy is commonly expressed as percent recovery through the assay of known added amounts of the analyte. In this study, a recovery study was conducted using the standard addition method, where known amounts of standard drugs were added to a known concentration of commercial pharmaceutical products. The standard drug was added at three different concentrations: 80%, 100%, and 120% of the pre-analyzed sample concentration. The mixtures were then analyzed using the proposed method.

Sensitivity

The LOQ and LOD were calculated using the following equations,

$LOD = 3.3\sigma/S$

$LOQ = 10\sigma/S$

Where σ = Standard deviation of response

S = Slope of calibration curve.

Robustness

Intentional alterations in the composition of the mobile phase and flow rate were implemented to evaluate the robustness of the proposed method. The study was conducted at concentrations of 25 µg/mL for desonide and 50 µg/mL for curcumin.

Assay of drugs in topical dosage form

The prepared test sample solutions were chromatographed using the mobile phase at a flow rate of 0.8 mL/min. The amounts of both drugs were calculated from the peak areas obtained in the chromatogram.

RESULTS AND DISCUSSION

The formulation required the use of an RP-HPLC method for drug estimation because desonide and *curcumin* were dissolved in polar solvents, such as methanol and acetonitrile. Testing different mixtures was part of the early trials. Refer table 1 for optimized chromatographic conditions. However, good separation of Desonide and *Curcumin* with sharper peaks and satisfactory system suitability parameters were observed with mobile phase - methanol and acetonitrile at a composition of 65:35%. Preliminary batches also helped to optimize chromatographic conditions. It was validated that the system suitability parameters were within the permissible range, as indicated in Table 2 and Figures 1,2,5.

Table 1: Optimized chromatographic condition

S. No.	Parameter	Conditions used for analysis
1	Column	Cosmosil C18 (250 mm×4.6 ID, particle size 5 micron)
2	Mobile phase	Methanol and Acetonitrile (65:35)
3	Flow rate	0.8 mL/min
4	Detection wavelength	240 nm
5	Sample volume	20 µL
6	Column temperature	Ambient

Method validation

The analytical method that was developed underwent validation in accordance with the guidelines of the ICH, taking into account several criteria, such as linearity, precision, accuracy, recovery studies, LOQ, LOD, robustness, ruggedness, and specificity.

Linearity and range

Linearity was evaluated by screening nine solutions of concentration range as mentioned in Tables 3 and 4. Responses at various respective concentrations were found to be linear, refer figures 3 and 4. Desonide and *Curcumin* had linear regression equations with correlation coefficients of 0.999 and 0.999, respectively, of y = 37.73x–9.592 and y = 30.41x–0.050.

Precision

Tables 5 and 6 display the intraday and interday precision study data for curcumin and desonide. The RSD values were <2.0% for both the intra- and inter-day precision studies, indicating the accuracy of the suggested estimation approach.

Table 2: Evaluation of system suitability parameters

Name of drug	RT (min)	Resolution	Th. Plate	Asymmetry factor
Desonide	3.45	4.38	3728	1.09
Curcumin	7.51	0.00	5436	1.17

Table 3: Linearity data for Desonide

S. No.	Concentration (µg/mL)	Area (mean±SD)	%RSD
1	2.5	809.25±6.58	0.77
2	5	1640.56±5.97	0.54
3	7.5	2473.12±15.23	0.44
4	10	3287.20±19.44	0.53
5	12.5	4125.50±26.31	0.75
6	15	4917.24±22.62	0.66
7	17.5	5732.35±32.02	0.64
8	20	6523.41±29.45	0.62
9	22.5	7361.86±21.59	0.74
10	25	8123.76±24.39	0.82

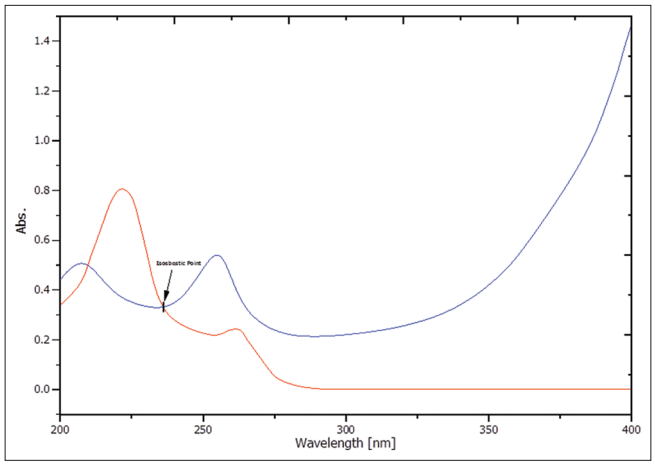

Figure 1: Overlay ultraviolet spectra of Desonide and *Curcumin*

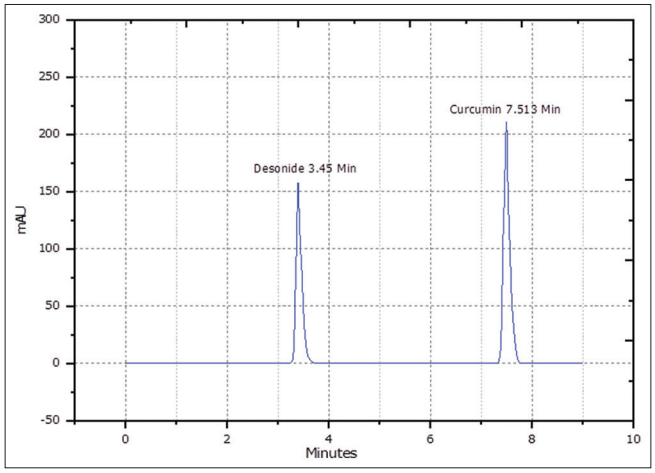

Figure 2: Chromatogram for standard solution of Desonide and *Curcumin*

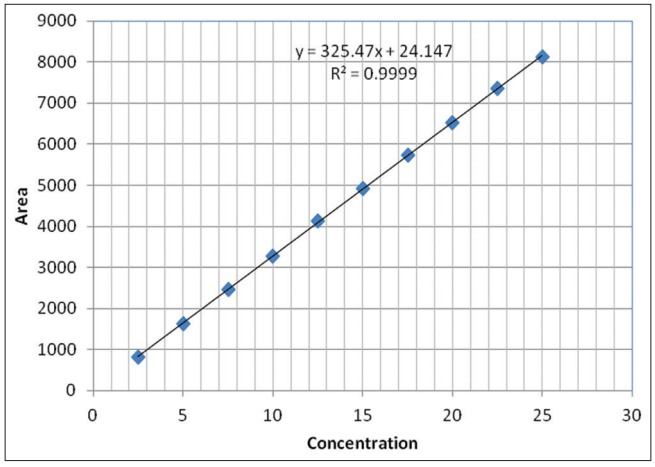

Figure 3: Calibration curve for Desonide

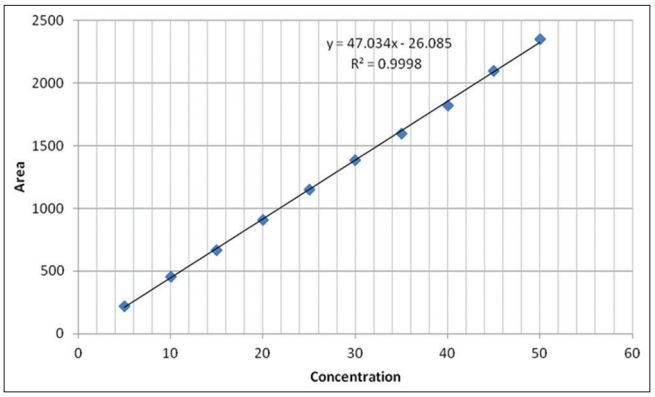

Figure 4: Calibration curve for *Curcumin*

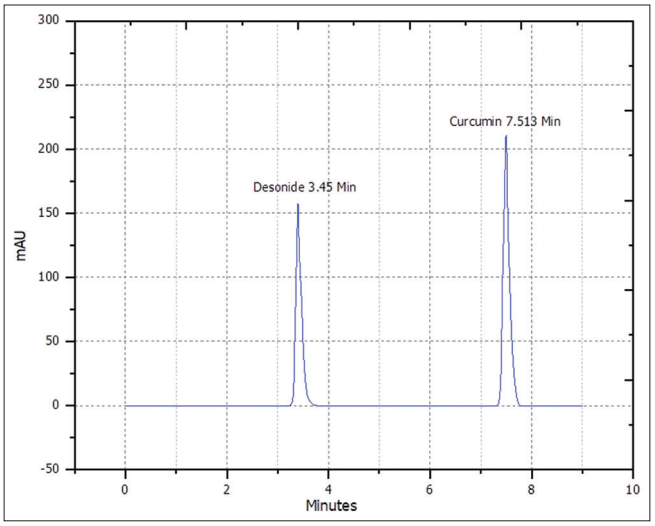

Figure 5: Chromatograph for test sample of Desonide and *Curcumin*

Table 4: Linearity data for Curcumin

S. No.	Concentration (µg/mL)	Area (mean±SD)	%RSD
1	5	221.12±1.58	0.67
2	10	454.21±4.71	0.64
3	15	668.84±5.11	0.79
4	20	911.11±6.44	0.71
5	25	1154.24±12.32	0.78
6	30	1384.61±14.12	0.64
7	35	1599.52±20.11	0.45
8	40	1823.64±19.25	0.80
9	45	2101.80±18.89	0.84
10	50	2354.53±16.81	0.93

Accuracy and recovery study

The accuracy is evaluated by determination of the % assay of Desonide and *Curcumin*. The mean percent recovery was found to be satisfactory with mean values of 99.87 and 99.56, respectively, for Desonide and *Curcumin*. Percent mean recovery and percent RSD mentioned in table 7. The mean percent recovery was found to be satisfactory with mean values of 99.87 and 99.56, respectively, for Desonide and *Curcumin.*

Table 5: Intra-day precision study

Time	Desonide			Curcumin		
	Concentration (µg/ml)	Area±SD	% RSD	Concentration (µg/ml)	Area±SD	% RSD
Morning	2.5	810.21±3.68	0.84	5	226.54±3.51	0.62
	12.5	3304.52±11.31	0.72	25	912.25±10.8	0.68
	25	7404.83±24.55	0.56	50	2036.17±18.22	0.57
Evening	2.5	811.07±4.21	0.81	5	232.12±2.41	0.81
	12.5	3308.62±14.58	0.57	25	914.25±8.11	0.41
	25	7414.23±21.67	0.54	50	2034.23±16.25	0.93

Table 6: Inter-day precision study

Day	Desonide			Curcumin		
	Concentration (µg/mL)	Area±SD	% RSD	Concentration (µg/mL)	Area±SD	% RSD
Day 1	2.5	818.74±4.68	0.87	5	232.75±6.11	0.91
	12.5	3310.34±12.47	0.88	25	909.85±12.83	0.78
	25	7426.10±26.76	0.96	50	2041.18±15.32	0.83
Day 2	2.5	819.21±5.51	0.79	5	230.25±4.48	1.04
	12.5	3320.33±19.08	0.81	25	922.53±10.02	0.86
	25	7429.44±20.67	0.94	50	2042.63±20.38	0.84

Table 7: Accuracy and recovery study

Set	Desonide				Curcumin			
	Sample Conc. (µg/mL)	Standard added (µg/mL)	% Mean recovery	% RSD	Sample Conc. (µg/mL)	Standard added (µg/mL)	% Mean recovery	% RSD
80	100	80	99.6570	0.54	30	24	99.7195	0.71
100	100	100	99.8684	0.35	30	30	99.4685	0.38
120	100	120	99.7253	0.61	30	36	99.3908	0.43

Table 8: Robustness study

Robust condition	Desonide		Curcumin	
	Area±SD	% RSD	Area±SD	% RSD
Flow rate (mL/min)				
0.6	3342.32±15.87	0.45	904.36±9.14	0.56
1.0	3299.25±23.65	0.52	924.33±15.23	0.68
Wavelength change				
221	3301.57±25.06	0.61	914.65±12.58	0.72
425	3412.87±24.88	0.53	949.56±5.65	0.49

Sensitivity

desonide and curcumin, the LOD, and LOQ were established. For desonide and curcumin, the corresponding LOD values were determined to be 0.4134 µg/mL and 0.1454 µg/mL. Conversely, the LOQ for desonide and *curcumin* was determined to be 0.9554 µg/mL and 0.3364 µg/mL, respectively.

Robustness

The impact of minute but purposeful changes to the chromatographic settings was assessed to examine the robustness. The results of robustness study shown in table 8. UV detection wavelength (±2 nm) and flow rate (varying by ±0.2/min) were the circumstances under investigation.

Table 9: Assay study

Drug	Drug content (%) (*n*=3)	% RSD
Desonide	100.18±0.52	0.72
Curcumin	101.26±0.46	0.86

Assay of drugs in topical dosage form

To find the drug content, an assay of medications in topical dosage form was done. The results in Table 9 demonstrate how well the suggested method works for analyzing desonide and *curcumin* in medicinal dose forms.

CONCLUSION

A pharmaceutical dosage form's desonide and *curcumin* may be estimated simultaneously using the RP-HPLC method, which was designed and proven to be linear, accurate, robust, and precise. These medications' pharmacological dosage forms can be routinely quantitatively analyzed using this technology. No degradation products interfered with the drug peak observations.

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