

Development and Validation of a Fast RP-HPLC Method for the Determination of Clobetasol Propionate in Topical Nanocapsule Suspensions

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Abstract

A simple and rapid high-performance liquid chromatographic method is validated for the determination of clobetasol propionate in topical nanocapsule suspensions. The method is carried out on an RP-18 column with a mobile phase composed of methanol–water (80:20 v/v) and UV detection at 241 nm. The method validation yields good results with respect to linearity, specificity, precision, accuracy, and robustness. The calibration curve in the range of 5.0–40.0 µg/mL shows a correlation coefficient of 0.9999. Precision (intra-day and inter-day) is demonstrated by a relative standard deviation lower than 1.5%. Accuracy is assessed by the recovery test of clobetasol propionate from sample matrixes (98.33 ± 0.88%). In conclusion, the method is suitable to be applied to assay clobetasol propionate in topical formulations of polymeric nanocapsules, avoiding the use of a buffer solution in the mobile phase.

Introduction

Clobetasol propionate (Figure 1) is a potent topical glucocorticosteroid with a molecular mass of 467.0 Da (1–3). It is a white or almost white crystalline powder that is practically insoluble in water, freely soluble in acetone and in dichloromethane, and sparingly soluble in ethanol (3). The administration of clobetasol propionate is widely used for the treatment of skin disorders such as atopic dermatitis, capillaris dermatitis, and psoriasis (2,4–6). It has been used in clinical practice because of its anti-inflammatory, antipruriginous, and vasoconstrictor activities (6). Prolonged therapy with clobetasol propionate preparations may result in adverse effects like skin atrophy, cutaneous reactivity, and suppression of the hypothalamic-pituitary-adrenal axis (4). Furthermore, the use of greasy and high-residual topical formulations (creams and ointments) could reduce patient compliance in long-term therapies (7).

In the nanotechnology field, some studies have been reported in literature on the preparation of clobetasol propionate-loaded solid lipid nanoparticles and liposomes (8–10). However, no

studies on the development of polymeric nanoparticles containing clobetasol propionate have been reported so far. This way, a novel pharmaceutical dosage form for topical administration of this drug consisting of clobetasol propionate-loaded nanocapsule suspensions is under development by our research group in order to reduce the irritation of the treated area and/or to allow its formulation in hydrophilic vehicles. Nanocapsules are polymeric nanoparticles composed of an oily core surrounded by a thin polymer wall in which the drug could be dissolved in the oil core, dispersed within the particle, or adsorbed at the interface particle/water (11). The small size of these carriers facilitates their formulation in dermatological products and enables comfortable application to the skin (12–13).

Some high-performance liquid chromatographic (HPLC) and spectrophotometric methods have been reported to assay clobetasol propionate in topical products (solutions, shampoos, and creams), liposomes, and solid lipid nanoparticles (4,9,14–17). The United States Pharmacopoeia (17) presents an HPLC method to assay clobetasol propionate in topical solution. However, this method cannot be applied to nanocapsule formulations due to the use of a mobile phase (acetonitrile–0.05 M phosphate buffer–methanol, 95:85:20 v/v) to dilute the samples. This sample preparation does not allow the release of clobetasol propionate encapsulated in the nanocapsules, considering its high aqueous solvent concentration. In fact, literature does not show any validation of an HPLC method for quantitative determination of clobetasol propionate in nanocapsule suspensions. Thus, the aim of the present study was to develop and validate a simple and reliable HPLC method for clobetasol propionate assay in topical nanocapsule suspensions, avoiding the use of a buffer solution in the mobile phase.

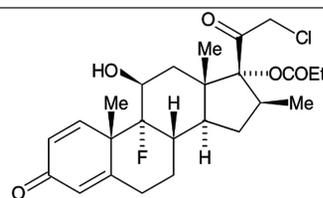


Figure 1. Chemical structure of clobetasol propionate.

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Experimental

Materials and reagents

Clobetasol propionate was obtained from Neo Química (Goiás, Brazil). HPLC-grade acetonitrile and methanol were acquired from Tedia (São Paulo, Brazil). Poly- ϵ -caprolactone (PCL) and sorbitan monostearate (Span 60) were purchased from Sigma Aldrich (São Paulo, Brazil). Caprylic/capric triglyceride mixture was delivered from Brasquim (Porto Alegre, Brazil). Polysorbate 80 (Tween 80) was supplied by Henrifarma (São Paulo, Brazil) and acetone by Vetec (Rio de Janeiro, Brazil). All chemicals and solvents presented were pharmaceutical-grade and were used as received. Clobetasol propionate-loaded nanocapsule suspensions were prepared by interfacial deposition of preformed polymer method as described by Fessi and co-workers (18). The formulations were prepared with (0.5 mg/mL) and without clobetasol propionate.

Apparatus and chromatographic conditions

Two HPLC systems were used in this study, which was performed at room temperature ($25 \pm 1^\circ\text{C}$). HPLC A was employed to carry out all the validation study. HPLC B was used in order to compare the results obtained by two different apparatus as an intermediate precision. HPLC A consisted of a Shimadzu LC-10A system (Kyoto, Japan) equipped with a model LC-10AD pump, an UV-VIS SPD-10A Module, an SLC-10A system controller, and RP-18 Gemini column (250 mm \times 4.60 mm, 5 μm particle size, 110 \AA pore diameter); and HPLC B consisted of a Shimadzu LC-20A system equipped with a model LC-20AT pump, an SPD-M20A PDA detector, a CBM-20A system controller, SIL-20A auto sampler, and RP-18 Gemini (250 mm \times 4.60 mm, 5 μm particle size, 110 \AA pore diameter). The mobile phase consisted of a methanol–water (80:20 v/v) at isocratic flow rate (1 mL/min) until 9.0 min of run. The injection volume was 20 μL . Detection was performed at 241 nm.

Sample preparation

Nanocapsule suspensions used for the evaluation of all parameters were freshly prepared. 1.0 mL of nanocapsule suspensions was diluted with acetonitrile to a concentration of 20.0 $\mu\text{g/mL}$. The use of acetonitrile was necessary to dissolve the nanocapsules and to release the entire drug from the nanocapsules. The resulting solution was filtered through a 0.45- μm membrane and injected in the HPLC system ($n = 3$).

Standard solution

Stock standard solution (0.5 mg/mL) was prepared by dissolving 25.0 mg clobetasol propionate in 50.0 mL of methanol. From this solution, a working standard of 20 $\mu\text{g/mL}$ was prepared by using 5 mL of the stock standard solution in 25.0 mL of mobile phase. In addition, the stock standard solution was diluted, as necessary, with the mobile phase to give five standard solutions with different concentrations of clobetasol propionate (5.0, 10.0, 20.0, 30.0, and 40.0 $\mu\text{g/mL}$), which were used in the linearity study. All solutions were filtered (0.45 μm) before being injected ($n = 3$) into the HPLC system.

Method validation

Validation was carried out assessing the following parameters:

linearity, range, specificity, precision, accuracy, and detection and quantification limits, according to the International Conference on Harmonization (ICH) guidelines (19).

Specificity

Specificity was evaluated by analyzing solutions containing all the components of the clobetasol propionate-loaded NC suspensions, except the drug (blank NC suspensions). The system response was examined for the presence of interference or overlaps with clobetasol propionate responses.

Linearity, limits of detection, and quantification

Linearity was evaluated by the injection and analysis of five concentrations of standard solutions in clobetasol propionate concentrations of 5.0, 10.0, 20.0, 30.0, and 40.0 $\mu\text{g/mL}$, as described in the "Preparation of the standard solution" section. Three independent calibration curves were constructed, and linearity was evaluated by the least-squares regression analysis. Limits of detection (LOD) and quantification (LOQ) were calculated directly from the calibration plot. LOD and LOQ were calculated as $3.3 \sigma/S$ and $10 \sigma/S$, respectively, where σ is the standard deviation of intercept and S is the slope of the calibration plot (19).

Precision

Repeatability (intra-day precision) was evaluated by measuring, in triplicate, six different samples at the same concentration (20.0 $\mu\text{g/mL}$) under the same experimental conditions and on the same day. Intermediate precision was calculated from results obtained by the analysis of samples with the same concentration (20.0 $\mu\text{g/mL}$) on three different days (inter-day precision) or using two different HPLC apparatus (HPLC A and HPLC B, inter-apparatus precision). Precision (repeatability and intermediate precision) was expressed as relative standard deviation [RSD (%)].

Accuracy

Accuracy was evaluated assaying, in triplicate, samples of known concentrations (NC suspensions) spiked with three different concentrations of standard solution (5.0, 10.0, and 20.0 $\mu\text{g/mL}$) at three different levels (lower, medium, and upper concentration), giving sample solutions with concentrations of 15.0, 20.0, and 30.0 $\mu\text{g/mL}$. Recovery (%) was calculated from differences between the peak areas obtained for spiked and unspiked solutions.

Robustness

Robustness was evaluated by the deliberate variation of the mobile phase, flow rate, and wavelength. Sample solutions were evaluated for each variation of the method conditions.

Results and Discussion

HPLC has been widely studied in pharmaceutical analysis, including drug assay in products based on nanotechnology (20–21). Nanoparticle suspensions are complex matrixes com-

posed of at least polymer, oil, and surfactants. This way, the analytical method to assay drugs in these systems must be carefully developed and validated to demonstrate its suitability. In this work, chromatographic conditions were adjusted in order to obtain efficient routine analysis. Methanol was chosen instead of acetonitrile as the organic solvent to compose the mobile phase due to its lower cost. Three proportions of the mobile phase (methanol–water) were evaluated: 70:30 (v/v), 80:20 (v/v), and 90:10 (v/v). These proportions showed retention times for clobetasol propionate of 15.40, 6.75, and 4.13 min, respectively. All proportions of mobile phase showed adequate free-from-tailing peaks of clobetasol propionate. However, considering our goal was to obtain a run time less than 10 min, the proportion 70:30 (v/v) was discarded. Between the proportions 80:20 (v/v) and 90:10 (v/v), the former presented a higher number of theoretical plates ($N = 8710$) compared to the latter ($N = 7387$), which led us to choose the proportion 80:20 (v/v) for the following studies. This choice was also reinforced by the relative higher retention time under these conditions aiming to use the same conditions, with previous validation, to carry out future drug release and in vitro skin permeation studies. In the next step, we evaluated the use of a stationary phase with a different length (RP-18 Gemini column, 150 mm \times 4.60 mm, 5 μ m particle size, 110 Å pore diameter), which showed a retention time lower than 4 min and a lower number of theoretical plates ($N = 3845$). In order to test the influence of the pH of the mobile phase, we used orthophosphoric acid (20 % w/v) to adjust the apparent pH of the mobile phase to pH 3.0 and pH 5.0. The mobile phase without pH adjustment showed apparent pH 6.9. No influence of pH was observed on retention time, number of theoretical plates, and asymmetry. The increase of the flow rate to 1.2 mL/min was also evaluated. Under these conditions, the retention time of clobetasol propionate was 5.65 min, which represented a low decrease compared to the use of a flow rate of 1.0 mL/min. This higher flow rate led to an increase in the HPLC system pressure (more than 150 kgf), which could reduce the column life. We also evaluated the possible precipitation of the sample after its redispersion in the mobile phase. No precipitation of any component of the formu-

lations was observed because of their low concentration in the sample as well as the high amount of organic solvent (methanol) in the mobile phase. In addition, no significant increase on the pressure of the HPLC system during the analyses was observed (even after more than 300 injections of sample solutions in the same column over six months), which could be related to the precipitation of some material at the front part of the column. Regarding the peak shape, it was not observed any significant change in the clobetasol propionate peak by using acetonitrile (sample solvent) instead of mobile phase (standard solution solvent), as can be visualized in Figure 2A–2B. Thus, the mobile phase composed of methanol and water in the proportion 80:20 (v/v) at a flow rate of 1.0 mL/min was considered reliable, suitable, and adequate with a retention time for clobetasol propionate of 6.75 min (Figure 2) and run time of 9 min. Compared to the U.S. Pharmacopeia method (17) of assaying clobetasol propionate in topical solutions, the developed method avoids the use of a buffer solution in the mobile phase, which contributes to the lifetime increase of columns and other components of the chromatographic system.

Regarding the specificity evaluation, the chromatograms shown in Figure 2 demonstrate that the method is specific, and no interference from the excipients was observed. In order to confirm this absence of interference, a peak-purity evaluation using the photodiode array (PDA) was carried out. These analyses showed that no impurities and/or excipients were co-eluting with the clobetasol propionate peak.

Good linearity was observed in the 5.0–40.0 μ g/mL range. The linear equation obtained by the least-square method was $y = 38640.69x - 14382.43$ and showed an adequate determination coefficient ($r^2 = 0.9999$). The validity of the assay was verified by analysis of variance. This revealed that the regression equation was linear ($F_{\text{calculated}} = 13655 > F_{\text{critical}} = 4.96$, $P = 5\%$) with no linearity deviation ($F_{\text{calculated}} = 0.43 < F_{\text{critical}} = 3.71$; $P = 5\%$). In addition, the *t*-test of the *y*-intercept ($t_{\text{calculated}} = -2.69$, $p > 0.05$) showed that it did not differ significantly from zero. LOD and LOQ were 0.45 and 1.38 μ g/mL, respectively.

Repeatability (intra-day precision) and intermediate precision (inter-day and inter-apparatus precision) are given in Table I. All data are lower than the acceptance criterion of 2%. Regarding

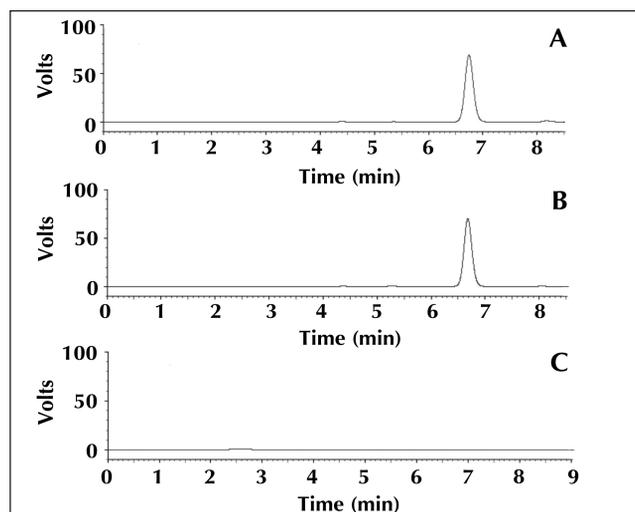


Figure 2. Chromatograms obtained from (A) clobetasol propionate reference substance (20 μ g/mL), (B) clobetasol propionate-loaded nanocapsule suspensions (20 μ g/mL), and (C) unloaded nanocapsule formulations (placebo formulation).

Table I. Results From the Repeatability* and Intermediate Precision† of the Method

	Theoretical amount (μ g/m)	Experimental amount (μ g/mL \pm SD)	% Recovered	RSD (%)
Intra-day (n = 6)	20.0	20.05 \pm 0.33	100.23 \pm 1.64	0.37
Inter-day				
Day 1 (n = 3)	20.0	19.85 \pm 0.22	99.24 \pm 1.09	1.10
Day 2 (n = 3)	20.0	19.87 \pm 0.10	99.35 \pm 0.49	0.49
Day 3 (n = 3)	20.0	19.93 \pm 0.23	99.64 \pm 1.17	1.17
Mean \pm SD (n = 9)	20.0	19.88 \pm 0.04	99.41 \pm 0.21	0.21
Inter-apparatus				
HPLC A (n = 3)	20.0	20.17 \pm 0.25	100.85 \pm 1.20	1.22
HPLC B (n = 3)	20.0	19.93 \pm 0.23	99.65 \pm 1.15	1.15
Mean \pm SD (n = 6)	20.0	20.04 \pm 0.25	100.20 \pm 1.25	1.25

* Intra-day precision.

† Inter-day and inter-apparatus precision.

the accuracy evaluation, good recoveries (97–100%) were obtained (Table II).

Regarding the evaluation of robustness, the deliberate variation of the method conditions had no significant effect on assay data or on chromatographic performance, indicating the robustness of method. The results from robustness testing are presented in Table III. With respect to the composition of HPLC mobile phase, no significant influence in % content of clobetasol propionate was found when changing the mobile phase composition to 75:25 (methanol–water) and 85:15 and also flow rates at 1.00 ± 0.10 mL/min. The effect of wavelength was studied by varying ± 4 nm.

In order to demonstrate the applicability, clobetasol propionate-loaded nanocapsule suspensions were assayed (three batches) using the conditions described in this study. The determination of drug content in sample solutions showed results according to the theoretical value (0.500 ± 0.005 mg/mL; 0.515 ± 0.005 mg/mL; 0.510 ± 0.005 mg/mL). RSD values were lower than 2.0% from triplicate analysis of each suspension, which indicates a precise analytical methodology.

Conclusions

A rapid, specific, and reliable HPLC method has been developed and validated for the assay of clobetasol propionate in topical nanocapsule suspensions, which are complex polymeric mixtures. The analytic methodology proposed is simple, precise, accurate, and linear in the concentration range of 5.0–40.0 $\mu\text{g/mL}$. Furthermore, the method involves the use of a simple mobile phase without buffer solution and minimum sample preparation.

Table II. Results from Accuracy Determination of the Method

Known sample	Amount of clobetasol propionate		Recovery (%)	RSD (%)
	Added $\mu\text{g/mL}$	Found $\mu\text{g/mL}$		
10.0 ± 0.03	5	14.67 ± 0.06	97.81 ± 0.55	0.57
10.0 ± 0.03	10	19.87 ± 0.14	99.35 ± 0.76	0.77
10.0 ± 0.03	20	29.35 ± 0.05	97.83 ± 0.49	0.50

Table III. Results from Study of Method Robustness

Conditions	% Clobetasol propionate	RSD (%)
Recommended conditions*	99.38	0.24
Mobile phase (methanol–)		
75:25	99.16	0.13
85:15	99.21	0.72
λ (nm)		
237	99.45	0.64
245	99.88	0.75
Flow rate (mL/min)		
0.90	99.75	0.63
1.10	99.39	0.41

* The recommended chromatographic conditions were: RP-18 Gemini column (250 mm \times 4.60 mm, 5 μm particle size, 110 \AA pore diameter) with methanol–water 80:20 (v/v) as mobile phase at a flow rate of 1.0 mL/min and UV detection at 241 nm.

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