

A simple HPLC method for the determination of halcinonide in lipid nanoparticles: development, validation, encapsulation efficiency, and *in vitro* drug permeation

Clarissa Elize Lopes, Gisele Langoski, Traudi Klein, Priscileila Colerato Ferrari*, Paulo Vitor Farago

Department of Pharmaceutical Sciences, State University of Ponta Grossa, Ponta Grossa, Paraná, Brazil

Halcinonide is a high-potency topical glucocorticoid used for skin inflammation treatments that presents toxic systemic effects. A simple and quick analytical method to quantify the amount of halcinonide encapsulated into lipid nanoparticles, such as polymeric lipid-core nanoparticles and solid lipid nanoparticles, was developed and validated regarding the drug's encapsulation efficiency and *in vitro* permeation. The development and validation of the analytical method were carried out using the high performance liquid chromatography with the UV detection at 239 nm. The validation parameters were specificity, linearity, precision and accuracy, limits of detection and quantitation, and robustness. The method presented an isocratic flow rate of 1.0 mL.min⁻¹, a mobile phase methanol:water (85:15 v/v), and a retention time of 4.21 min. The method was validated according to international and national regulations. The halcinonide encapsulation efficiency in nanoparticles was greater than 99% and the *in vitro* drug permeation study showed that less than 9% of the drug permeated through the membrane, indicating a nanoparticle reservoir effect, which can reduce the halcinonide's toxic systemic effects. These studies demonstrated the applicability of the developed and validated analytical method to quantify halcinonide in lipid nanoparticles.

Uniterms: Halcinonide/encapsulation efficiency. Halcinonide/quantification. Polymeric lipid-core nanoparticles. Solid lipid nanoparticles. Topical administration. Toxicity. High performance liquid chromatography/method validation.

INTRODUCTION

Topical corticosteroids or glucocorticoids are widely employed in dermatosis treatments due to their potent anti-inflammatory and anti-proliferative effects (Hengee *et al.*, 2006; Guichard *et al.*, 2015). However, the use of topical glucocorticoids can result in several local and adverse systemic effects, such as epidermis, dermis, or even subcutaneous tissue atrophy, resulting in irreversible stretch marks and in disorder of the wound-healing processes. Adverse systemic effects include body weight increase, Cushing's syndrome, hypertension, diabetes mellitus, growth retardation, osteoporosis, peptic ulcer, and gastritis (Schacke, Docke, Asadullah, 2002; Senyigit, Ozer, 2012).

Halcinonide (C₂₄H₃₂ClFO₅; MW: 454.96 Da) or (11β,16α)-21-chloro-9-fluoro-11-hydroxy-16,17-[(1-methylethylidene)bis(oxy)]pregn-4-ene-3,20-dione (Figure 1) is a high-potency topical corticosteroid, which is a hydrocortisone derivative and whose modifications alter its absorption, potency, and adverse effects. Halcinonide is classified as a Class II potency corticosteroid (Taheri, Feldman, 2012), whose characteristics include being a white crystalline powder, soluble in acetone and in chloroform, slightly soluble

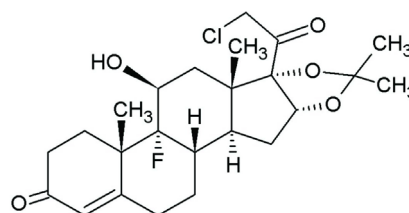


FIGURE 1 - Chemical structure of halcinonide.

*Correspondence: P. C. Ferrari. Departamento de Ciências Farmacêuticas. Universidade Estadual de Ponta Grossa. Av. General Carlos Cavalcanti, 4748 – Uvaranas - 84030-900 - Ponta Grossa, PR, Brazil. Phone: 55-42-3220 3122. E-mail: priscileila@hotmail.com / pcferrari@uepg.br

in alcohol and in ethyl ether, insoluble in water and in hexanes.

Halcinonide is administered by topical via. However, due to its high potency, it displays dose-dependent toxicity and local and systemic adverse effects. In order to reduce the drug toxicity and to improve its action, halcinonide can be encapsulated into nanostructured systems, such as nanoparticles, which promote a controlled release of the drug in specific sites (Schaffazick, Guterres, 2003; Senyigit, Ozer, 2012). Nanostructured systems, such as nanoparticles, are submicron size ranged-usually spherical-drug carriers. Lipid nanoparticles are widely used to carry lipophilic drugs and they are constituted by biocompatible and non-toxic lipids and may to be associated with polymers (Kelidari *et al.*, 2015). They present advantages in the drug delivery control, such as good local tolerability, high encapsulation rate for lipophilic substances, and small particle size, which provide close contact with the stratum corneum. Moreover, they can create a film on the skin surface (Maia *et al.*, 2000).

In order to reduce the halcinonide toxicity in topical use, lipid nanoparticles, such as solid lipid nanoparticles (SLN) and polymeric lipid-core nanoparticles (LCN), were developed. SLNs are a colloidal carrier system for targeting drugs and are appropriate for use in cases of inflamed and damaged skin. They can favor drug penetration in the skin, maintaining a sustained release to avoid systemic absorption. They also reduce skin irritation due to their skin-targeting effect (Chen *et al.*, 2006; Madan *et al.*, 2014; Shah *et al.*, 2015). LCNs are nanostructured devices in which a polymer wall surrounds the lipid core. They can stabilize the encapsulated substances and control the release of the drug to improve its effectiveness, acting as reservoir systems at the epidermis, and retaining themselves at the outermost layers of the skin (Jäger *et al.*, 2007; Venturini *et al.*, 2011; Coradini *et al.*, 2014; Fiel *et al.*, 2014; Brum *et al.*, 2015).

The literature reports just one high performance liquid chromatography (HPLC) method that determines halcinonide from different sample types (Kirschbaum *et al.*, 1980). However, no study involving the quantification of halcinonide entrapped in lipid nanoparticles was submitted for the mandatory validation process. We developed and validated a simple, sensitive, and specific HPLC-UV method to determine the presence of halcinonide in lipid nanoparticles. Subsequently, this validated method was successfully used to quantify the amount of halcinonide encapsulated in these lipid nanostructured systems and to evaluate the *in vitro* drug permeation.

MATERIAL AND METHODS

Chemicals and reagents

Halcinonide 99,2% (Fagron, Anápolis, GO, Brazil), poly(ϵ -caprolactone) (M_w = 10000-14000 g/mol, Sigma-Aldrich, St. Louis, MO, USA), Span 60[®] (sorbitan stearate; Sigma Aldrich, St. Louis, MO, USA), Tween 80[®] (polysorbate 80; Deleware, Porto Alegre, Brazil), capric/caprylic acid triglycerides (Focus Química, São Paulo, Brazil), stearic acid (Fagron, Anápolis, GO, Brazil) and acetone (Vetec, Rio de Janeiro, Brazil) were used to prepare the solid lipid nanoparticles (SLN) and polymeric lipid-core nanoparticles (LCN). HPLC-grade methanol was provided by Sigma-Aldrich (St. Louis, MO, USA). Water was purified in a Milli-Q Plus water purification system (Millipore, Bedford, MA, USA). All other reagents and solvents were of analytical grade.

Apparatus and chromatographic conditions

A Merck-Hitachi Lachrom HPLC (Tokyo, Japan) system, equipped with a D-7000 Interface, an L-7400 UV detector module, a L-7100 quaternary pump, and an integral degasser, was used in the method development. A manual injector (Rheodyne) system, equipped with a 20- μ L injector loop and a 100- μ L syringe (Hamilton, Microliter 710), was used. Data acquisition, analysis, and reporting were performed using ChromQuest 5.0 (Thermo Fisher Scientific, San Jose, CA, USA).

Experiments were performed in the HPLC system described above using a GL Sciences C18 Inertsil[®] ODS3 column (Torrance, CA, USA) of a 5- μ m particle size, 4.6-mm internal diameter, and 150-mm length and a GL Sciences C18 Inertsil[®] ODS3 guard cartridge system (10 mm \times 4 mm, 5 μ m) at 25 \pm 2 °C. The mobile phase, previously filtrated using a 0.45- μ m PTFE membrane and degassed by sonication for 30 min, consisted of methanol and water (85:15 v/v) at an isocratic flow rate of 1.0 mL.min⁻¹. The sample injection volume was of 20 μ L. The halcinonide was monitored at 239 nm. The method run time was of 10 minutes and all experiments were carried out in triplicate.

Sample, standard solution, and system suitability preparation

Halcinonide-loaded LCNs (LCN-HAL; 3.0 mg.mL⁻¹) were prepared with the interfacial deposition method described by Fiel *et al.* (2014). The organic phase, constituted of halcinonide, sorbitan stearate, capric/caprylic acid triglycerides, and poly(ϵ -caprolactone), was

dissolved in acetone at 40 °C and injected into an aqueous phase containing Tween 80 under magnetic stirring. The suspension was submitted to vacuum and to acetone evaporation. All formulations were obtained in triplicate. Unloaded-LCN was also prepared as a negative control.

Halcinonide-loaded SLNs (SLN-HAL; 3.0 mg.mL⁻¹) were obtained by hot homogenization. A lipid phase, constituted by stearic acid and halcinonide, was heated at 70 °C. The aqueous phase, containing Tween 80, was heated at the same temperature and was added to the melted lipid under stirring. The emulsion was kept in the high shear homogenizer Ultra Turrax at 18,000 rpm for 10 min. Then, the nanoemulsion was immediately dispersed into cold distilled water (2–5 °C) stirred for 1 minute at 3,400 rpm to solidify the nanoparticles (Ghadiri *et al.*, 2012). All solid lipid nanoparticles were obtained in triplicate and the unloaded-SLN was also prepared as a negative control.

Method development

The detection wavelength for the HPLC study was set at 239 nm after a spectrometric scanning. The chromatographic conditions were optimized for the halcinonide peak's resolution, varying the mobile phase's composition and proportion. Samples of different formulations were used to optimize the chromatographic conditions to resolve the halcinonide. These samples were filtered with a polytetrafluoroethylene (PTFE) membrane filter (Cromafil® Xtra, 0.45 µm × 25 mm, Macherey-Nagel, Düren, Germany) before their injection into the HPLC system. An appropriate blank was injected before the analysis of every sample. The method was validated and used to determine halcinonide in the LCN and SLN nanoparticles, and to evaluate the *in vitro* drug permeation.

Method validation

To validate the analytical method, the guidelines established by the ICH, International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (2005) and the Brazilian regulation (ANVISA, 2003) were employed (Gomes *et al.*, 2015; Klein, Longhini, Mello, 2012). The method was validated for specificity, linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, precision, and robustness.

Specificity

The specificity was determined by analyzing the chromatograms of unloaded nanoparticles in comparison with those obtained for both halcinonide-containing

formulation nanoparticles (LCN-HAL and SLN-HAL), expecting the confirmation of no interference in the retention time and in the drug quantification due to formulation constituents (Gomes *et al.*, 2015).

The nanoparticles were added in the mobile phase and kept under magnetic stirring during 24 h. Samples were assayed in the supernatant after a suitable dilution and filtration through a 0.45-µm PTFE membrane and injected into the HPLC system.

Linearity

The linearity was determined by the calculation of a regression line from the peak area's plot *versus* the concentration of the working standard solutions prepared at five concentration levels (10.0; 15.0; 20.0; 30.0 and 40.0 µg.mL⁻¹) and it was evaluated by the least-square regression analysis. A stock standard solution (500 µg.mL⁻¹) was prepared daily by dissolving 5.0 mg of halcinonide in a 10-mL volumetric flask using the mobile phase (metanol:water 85:15, v/v). This solution was further diluted in the mobile phase to prepare the five different working standard solutions (ranging from 10 to 40 µg.mL⁻¹). The solutions were injected in the HPLC column in triplicate, keeping the injection volume constant (20 µL) and chromatograms were recorded. The standard deviation value for the slope, Y-intercept, and other statistics of the calibration curve were calculated by linear regression and analysis of variance (ANOVA).

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation (SD) and on the slope (S) of the calibration curve according to the ICH (2005) and Fuster *et al.* (2015) by 3.3 SD/S and 10 DS/S to LOD and to LOQ respectively.

Precision

The precision of the method was determined following the ICH requirements. It was evaluated at three levels: repeatability, intermediate precision, and reproducibility. The repeatability was investigated by testing six sample solutions at 25 µg.mL⁻¹, evaluated in other two different days (reproducibility) and also prepared by two operators (intermediate precision). All analyzes were performed in sextuplicate. The results were reported in terms of relative standard deviation (RSD).

Accuracy

The accuracy was determined by the analysis of three intermediate points of the calibration curve

at different levels: low, intermediate, and high (17.5; 25.0 and 35.0 $\mu\text{g}\cdot\text{mL}^{-1}$, respectively). To determine the recovery known concentration of the stock solution, it was prepared to achieve the final and theoretical drug concentrations. The accuracy value was determined by calculating the percent of halcinonide recovery for these three concentration levels and then determining the RSD (Klein, Longhini, Mello, 2012; Gomes *et al.*, 2015).

Recovery

The recovery was determined in triplicate by the preparation of a halcinonide solution of 200 $\mu\text{g}\cdot\text{mL}^{-1}$, followed by the addition of a new halcinonide solution (500 $\mu\text{g}\cdot\text{mL}^{-1}$), obtaining a final concentration of 700 $\mu\text{g}\cdot\text{mL}^{-1}$. The samples were diluted into 25 $\mu\text{g}\cdot\text{mL}^{-1}$ and the results were evaluated by the RSD.

Robustness

In order to determine the robustness, experimental conditions were deliberately changed to verify the method's reproducibility. It was evaluated by analyzing a halcinonide solution at a 25- $\mu\text{g}\cdot\text{mL}^{-1}$ varying flow rate (0.995 and 1.005 $\text{mL}\cdot\text{min}^{-1}$) and the composition of the mobile phase methanol:water (84:16 and 86:14 v/v). The samples were evaluated in triplicate for each variation. The chromatograms were recorded and compared with the previously reported chromatographic conditions.

Evaluation of encapsulation efficiency

To assess the halcinonide's encapsulation efficiency (EE) in lipid nanoparticles, 500 μl of samples were filtered using an Amicon® Ultra-0.5 mL 10 kDa filter device and centrifuged for 30 min at 40,000 rpm, according to Vijayakumar *et al.* (2016) and Zhang *et al.* (2016). The encapsulation efficiency was determined after the reading of the filtered samples in the HPLC, performed in triplicate and calculated using the equation (1):

$$EE (\%) = \frac{\left(\frac{\text{total drug}}{\text{content}} \right) - \left(\frac{\text{free drug}}{\text{content}} \right)}{\left(\frac{\text{total drug}}{\text{content}} \right)} \times 100$$

In vitro drug permeation

In order to demonstrate the applicability of the validated method and the controlled drug delivery, the *in vitro* drug permeation of free halcinonide and of halcinonide encapsulated into LCN and SLN were evaluated. The skin permeation study was conducted

in vertical diffusion cells, with a receiver compartment containing 12 ml and an available area of 1.77 cm^2 . Strat® M (Merck Millipore) synthetic membranes were used in the study due to their high similarity to human skin. The samples were individually incorporated into a 0.5% Natrosol gel to increase the nanosuspension's viscosity, keeping the drug concentration at 0.1 % in all samples. The membrane was placed between the donor and receiver compartment of the vertical diffusion cells. 1.0 g of sample-containing gel was added into the donor compartment and the system was kept under constant agitation at 37 °C. Considering the lipophilic nature of the sample, the receiver compartment was filled with isopropyl alcohol, ensuring the sink condition. Aliquots of 3.0 mL were withdrawn from the receiver fluid at 1, 2, 4, 6, 8, 12, and 24 hours and the volume was replenished. The cumulative drug that permeated was quantified using the developed and validated HPLC analytical method. The study was performed in triplicate.

RESULTS AND DISCUSSION

Method development and validation

Previous tests were carried out in order to provide a quick and effective method to analyze halcinonide using the HPLC. The investigated chromatographic conditions were mainly related to the mobile phase composition. Initial runs included 60-minute exploratory runs using a mixture of methanol and water as a mobile phase, varying the proportion of methanol from % to 95%.

The mobile phase, composed by methanol:water 85:15 (v/v), provided a symmetric peak for halcinonide with low retention time. The peak was detected at 4.21 min (Figure 2), which is very suitable for routine analyses. In summary, methanol:water (85:15 v/v), a column temperature at 25 ± 2 °C, a sample injection volume of 20 μL , an isocratic flow rate of 1.0 $\text{mL}\cdot\text{min}^{-1}$, a detector set at 239 nm, and a run time of 10 minutes were chosen as suitable chromatographic conditions for further procedures, including the method validation.

The developed method proved itself specific for halcinonide quantification from LCN and SLN (Figure 2), i.e. the peak related to the drug ($t_{\text{R}} = 4.21$ min) did not appear in the inert nanoparticles, indicating no interference due to nanoparticle constituents in the halcinonide quantification.

A linear relationship between the peak area and the halcinonide concentration from 10.0 to 40.0 $\mu\text{g}\cdot\text{mL}^{-1}$ was observed (Figure 3) and the following general linear equation was obtained: $y = -8299.2874 + 43639.9632x$,

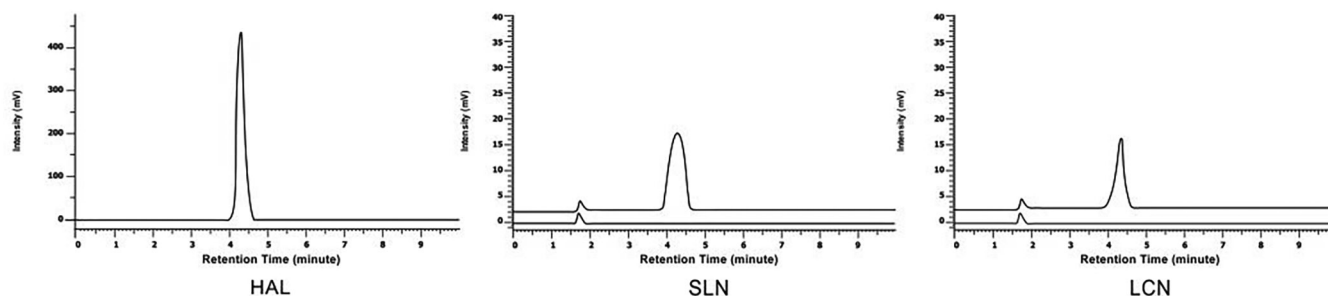


FIGURE 2 - Representative HPLC chromatograms obtained from halcinonide standard solution ($25.0 \mu\text{g.mL}^{-1}$) in methanol:water and halcinonide-loaded and unloaded nanoparticles: LCN and SLN. Mobile phase: methanol:water (85:15 v/v); flow rate: 1.0 mL/min; detection wavelength: 239 nm; column temperature: $25 \pm 2 \text{ }^\circ\text{C}$; and injection volume: 20 μL . HAL: halcinonide. Retention time: 4.21 minutes.

where y is the peak area and x is concentration at $\mu\text{g.mL}^{-1}$. A suitable correlation coefficient ($r=0.9994$) that demonstrates that the method is linear with an r value of nearly 1.0 at the purposed range was recorded. According to the Analytical Methods Committee (AMC), a regression coefficient value near to 1.0 is not necessarily the outcome of a linear relationship and, consequently, the lack-of-fit test should be applied. This test evaluates the variance of the residual values. The ANOVA test to evaluate halcinonide linearity is presented in Table I. The F value for lack of fit was less than the tabulated F value for the 95% confidence level ($\alpha=0.05$), and therefore, according to the ANOVA test, the linear regression showed no lack of fit (Gomes *et al.*, 2015; Klein, Longhini, Mello, 2012).

The lowest halcinonide concentration detected (LOD) and quantified (LOQ) with acceptable precision and accuracy was 0.04 and 0.14 $\mu\text{g.mL}^{-1}$, respectively. These results prove that the chromatographic method is suitable enough to detect and quantify halcinonide at a concentration range of 10.0 to 40.0 $\mu\text{g.mL}^{-1}$.

Precision, expressed as the RSD percentage of replicates, measures the relative errors of the method, as presented in Table II. The method developed for halcinonide quantification showed precision, because the RSD (%) values of all parameters were less than 5.0 %, as established by RE 899/2003 (ANVISA).

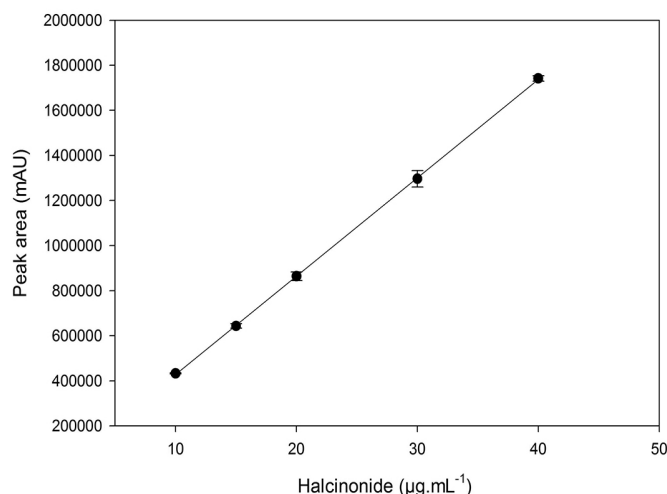


FIGURE 3 - Mean calibration curve obtained to halcinonide using working standard solutions at the concentration range from 10.0 to 40.0 $\mu\text{g.mL}^{-1}$ ($n=3$).

The accuracy of the method refers to the proximity between the mean of the measured values and their real concentrations values (ICH, 2005). The results (Table III) agree with Fuster *et al.* (2015) because the mean recovery values were near to 100 % and the RSD was at less than 2 %, thereby indicating a low variability and a strong agreement between the experimental and theoretical concentration values.

TABLE I – ANOVA linearity results

	<i>SS</i>	<i>DF</i>	<i>MS</i>	<i>F</i>	<i>F_{tab}</i>
Model	1.8581×10^8	1	1.8581×10^8	0.60	3.04
Residual	4.0503×10^9	13	3.1156×10^8	Linear	
Lack of Fit	2.2397×10^8	3	0.7465×10^8	0.195117	2.72
Pure Error	3.8263×10^9	10	3.8263×10^8	No Lack of Fit	

SS: sums of squares; DF: degrees of freedom; MS: mean squares; F: F value of the test; F_{tab}: fixed F value.

TABLE II - Repeatability, reproducibility and intermediate precision data of halcinonide analysis

Sample solution (25.0 µg.mL ⁻¹)	Experimental concentration (µg.mL ⁻¹ ± SD*)	Recovered (%)	RSD** (%)
Repeatability (n=6)	25.90 ± 0.75	103.60 ± 2.99	2.88
Reproducibility (n=6)			
Day 1 (morning)	25.61 ± 0.28	102.45 ± 1.14	1.09
Day 2 (morning)	25.10 ± 0.52	102.09 ± 4.46	4.37
Day 1 (afternoon)	25.23 ± 0.34	100.93 ± 1.36	1.35
Intermediate precision (n=6)			
Analyst 1	25.87 ± 0.83	103.48 ± 3.33	3.21
Analyst 2	25.72 ± 0.33	102.90 ± 1.33	1.29

*SD: standard deviation; **RSD: relative standard deviation.

TABLE III - Accuracy assays for halcinonide analysis

Level of concentration	Theoretical concentration (µg.mL ⁻¹)	Experimental concentration (µg.mL ⁻¹ ± SD*)	Recovered (%)	RSD** (%)
Low	17.5	16.68 ± 0.25	95.31 ± 1.44	1.51
Medium	25.0	24.52 ± 0.18	98.06 ± 0.72	0.73
High	35.0	34.35 ± 0.30	98.14 ± 0.89	0.90

*SD: standard deviation; **RSD: relative standard deviation.

TABLE IV - Recovery assays for halcinonide analysis

Theoretical concentration (µg.mL ⁻¹)	Experimental concentration (µg.mL ⁻¹ ± SD*)	Recovered (%)	RSD** (%)
200	198.48 ± 6.18	99.24 ± 3.09	3.11
700	704.60 ± 22.69	100.65 ± 3.24	3.22

*SD: standard deviation; **RSD: relative standard deviation.

Results of recovery are shown in Table IV. The development method presented a recovery less than 5.0 % of RSD, with a recovery percentage near to 100 %, in accordance to what was established by RE 899/2003 (ANVISA).

The robustness of an analytical procedure measures its capacity to remain unaffected by small, but deliberate, variations in the method parameters, and provides an indication of its reliability during normal usage (ICH, 2005). The method was considered robust because the RSD values were less than 1.54% for the drug content, as summarized in Table V, indicating that the deliberate variation of the method conditions had no significant effect on assay data or on chromatographic performance (Fontana, Bastos, Beck, 2010), not affecting the analysis of halcinonide in lipid nanoparticles.

Encapsulation efficiency

The drug content and encapsulation efficiency (EE) of halcinonide in lipid nanoparticles was carried out by the previously validated HPLC method and the results obtained are presented in Table VI. High percentages of entrapped drug were obtained for lipid nanoparticles in both types. All formulations presented suitable EE values greater than 99%. These values are mainly based on the poor aqueous solubility of halcinonide and on its high partition coefficient (log P=3.31), which leads to high drug load quantities in lipid nanoparticles. The current results are similar to the ones previously reported by Silva *et al.* (2015) on the EE of betamethasone (topical glucocorticoid) in nanoparticles using a similar method for quantification.

TABLE V - Robustness for halcinonide analysis

Conditions	Experimental concentration ($\mu\text{g.mL}^{-1} \pm \text{SD}^*$)	Recovered (%)	RSD** (%)
Flow rate			
0.995 mL.min ⁻¹	25.89 \pm 0.26	103.56 \pm 1.05	1.00
1.005 mL.min ⁻¹	26.00 \pm 0.41	104.03 \pm 1.64	1.54
Mobile phase			
84:16	26.25 \pm 0.24	105.00 \pm 0.95	0.90
86:14	26.48 \pm 0.19	105.93 \pm 0.75	0.70

*SD: standard deviation; **RSD: relative standard deviation.

TABLE VI - Halcinonide-loaded and encapsulation efficiency (EE) for polymeric lipid-core nanoparticles (LCN) and solid lipid nanoparticles (SLN)

Lipid nanoparticle sample	Theoretical concentration ($\mu\text{g.mL}^{-1} \pm \text{SD}^*$)	Experimental concentration ($\mu\text{g.mL}^{-1} \pm \text{SD}$)	EE (% \pm SD)
LCN-HAL	3000	2997.09 \pm 1.14	99.90 \pm 0.04
SLN-HAL	3000	2999.01 \pm 0.10	99.96 \pm 0.01

*SD: standard deviation. HAL: halcinonide.

In vitro permeation study

The permeation profile of pure halcinonide and halcinonide-loaded LCN and SLN are shown in Figure 4. An increase of pure halcinonide percentage in the receptor chambers with time was observed until 12 h. The permeation rates of pure halcinonide from Natrosol gel were about 18% and 50% during 6 and 24 hours,

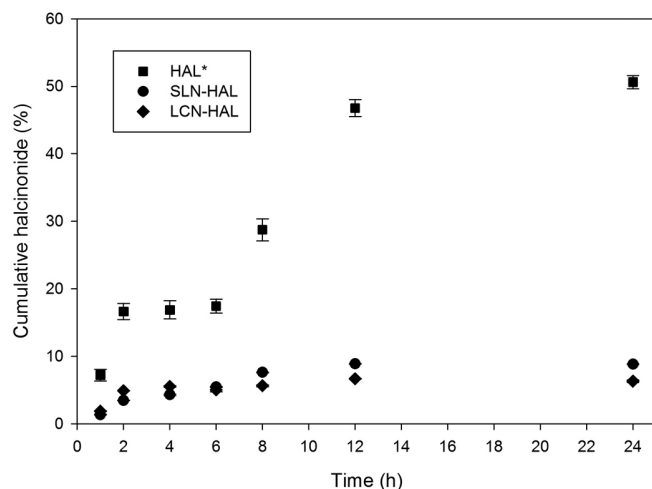


FIGURE 4 - *In vitro* cumulative permeation-time profile of halcinonide and halcinonide-loaded lipid nanoparticles. HAL: halcinonide. *represent statistical different result; Student's *t*-test ($\alpha=0.05$).

respectively. Since the synthetic membrane used has similarities to the human skin, halcinonide can permeate through the stratum corneum barrier due to its lipophilic character, reaching the dermis and being absorbed.

The halcinonide-loaded lipid nanoparticle profile presented no more than 9 % of permeated halcinonide and there was no skin permeation difference between the LCN and the SLN during 24 h ($p>0.05$, *t*-test). This result indicates that despite the nature of the lipid nanoparticles, which could promote the permeation of the drug, the reservoir effect promoted by the LCN and the SLN was essential to control the halcinonide release.

With the purpose of obtaining drug quantification, both for EE and *in vitro* drug permeation, the validated method was successfully applied in the determination of halcinonide in lipid nanoparticles-LCN and SLN-and it can be considered an important tool for the quality control of these promising formulations.

CONCLUSION

A simple and effective HPLC method was developed and validated for the quantitative determination of halcinonide in lipid nanoparticles-LCN and SLN. This method was considered specific, linear, accurate, precise, and robust for a quick determination of halcinonide in lipid nanoparticles according to the ICH guideline and the Brazilian regulation (RE 899/2003). Furthermore, the

drug content and the encapsulation efficiency were quickly and suitably determined, as well as the halcinonide *in vitro* permeation profile, displaying a controlled drug release and indicating a possible reduction of halcinonide toxicity due to its nanoencapsulation.

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