Development and validation of HPLC method for analysis of dexamethasone acetate in microemulsions

Maria Cristina Cocenza Urban¹, Rubiana Mara Mainardes², Maria Palmira Daflon Gremião¹*

¹Departamento de Fármacos e Medicamentos, Faculdade de Ciências Farmacêuticas de Araraquara, Universidade Estadual Paulista “Julio de Mesquita Filho”, ²Departamento de Farmácia, Universidade Estadual do Centro-Oeste

INTRODUCTION

Since 1948, with the introduction of cortisone and later hydrocortisone (1951), anti-inflammatory steroids have become a prominent pharmacological class. They are currently the drugs of choice for the treatment of several diseases, despite their adverse side effects (Avery, Woolfrey, 1997).

The use of topical steroid preparations represented a major advance in dermatology. Dexamethasone ace-
tate (9-fluoro-17β,21-trioxy-16α-methylpregna-1,4-diene-3,20-dione 21-acetate monohydrate) is a steroid indicated for the treatment of several pathologies due to its anti-inflammatory and immunosuppressor effects (Figure 1). This steroid is frequently incorporated in ointments, creams, lotions, aerosols and microemulsions (Gasco et al., 1988; Hashigushi et al., 1997; Vianna et al., 1998; Lehmann et al., 2001).

Microemulsions are isotropic and thermodynamically stable solutions, generally composed of a combination of three to five components: oil, water, surfactant, cosurfactant and active substance (Constantinides, Scalart, 1997). The microemulsions show great potential as drug delivery systems because they can improve the solubility, absorption and therapeutic efficacy of the drug (Formariz et al., 2005).

Surfactants are extensively used to stabilize drug delivery systems. Commonly they are molecules self-assembled in water or in oil, leading to the formation of a well defined microstructure. Even a single surfactant can display a rich variety of structures that depends on several parameters, such as water content and temperature. Nevertheless, these microheterogeneous systems can interfere with drug separation and detection, and an adequate analytical method is needed to analyze the drug carried by these systems.

Capillary electrophoresis has been used to determine of dexamethasone (Guo et al., 2004) in pharmaceutical dosage forms. Thin-layer chromatographic-densitometric method is also described for the analysis of dexamethasone in an ointment (Krzek et al., 2005). Another method for analysis of dexamethasone acetate in ointments reported is flow-injection chemiluminescence (Wu, Lv, 2007). Although these methods are versatile tools in pharmaceutical analysis, they are time-consuming. HPLC remains the analytical method of choice, especially for analysis for topical formulations, owing to their complex composition. Few HPLC methods are described on the literature for the analysis of dexamethasone acetate in creams and ointments (Capella-Peiró, 2002, Garcia et al., 2003) and in other pharmaceutical forms (Vianna et al., 1998; Milojevic et al., 2002). HPLC is very useful for analysis of complex samples, such as ointments and creams, as it provides drug separation, determination and the elimination of most interference problems (Williams et al., 1981). However, no HPLC method for the analysis of the dexamethasone in microemulsion has been described.

The validation of an analytical method must demonstrate that it fulfills all the requirements of the analytical applications, ensuring the reliability of the results. For this reason, the tests must show that its specificity, linearity, precision, sensitivity, accuracy and limit of quantification are adequate for the analysis (ICH, 2003; British Pharmacopoeia, 2001; ANVISA, 2003; USP, 2004).

The aim of this study was to develop a simple, rapid, specific, precise and accurate reversed-phase HPLC method for the determination of dexamethasone acetate in microemulsions. The parameters used to validate the method were linearity, specificity, precision, accuracy and limit of quantification.

**MATERIAL AND METHODS**

**Materials**

Dexamethasone acetate (Purifarma, São Paulo, Brazil) (99%) was used without further purification. The microemulsion was composed of isopropyl myristate (Henrifarma, São Paulo, Brazil) as the oily phase, PPG-5 Ceteth-20 (Croda, São Paulo, Brazil) as surfactant and distilled water.

Methanol (HPLC grade - Mallinckrodt, USA) was used to prepare the mobile phase and to dilute the samples. Water was obtained by distillation.

**Methods**

**Instrumentation and chromatographic conditions**

The method was performed on a Shimadzu System consisting of: Solvent Delivery Module LC-9A, Ultraviolet-Visible Spectrophotometric Detector Module SPD-6AV, Column Oven Module CTO-6A and System Controller Module SCL-6B with a Rheodyne injection valve with a 20 µL loop attached.

Isocratic Chromatographic separations were carried out in a stainless steel Merck Lichrospher 100 RP-18 colu-

![FIGURE 1 - Chemical structure of dexamethasone acetate.](image-url)
Development and validation of HPLC method for analysis of dexamethasone acetate in microemulsions

Method Validation
The method was validated in accordance with International Conference on Harmonization guidelines (ICH-2003) for validation of analytical procedures.

Specificity and Selectivity
These parameters were determined by comparing the chromatograms of the dexamethasone acetate standard, drug-loaded microemulsion and microemulsion without drug.

Linearity
The linearity was determined from the triplicate analytical curves obtained by HPLC analysis of dexamethasone acetate standard solutions.

Accuracy
The accuracy was determined by the standard addition method. Amounts of 5.0; 10.0; 15.0 µg.mL⁻¹ of the dexamethasone acetate standard were added to the microemulsions samples in which 10.0 µg.mL⁻¹ of the drug had been incorporated previously. The final concentrations of the fortified solutions were 15.0, 20.0 and 25.0 µg.mL⁻¹ of dexamethasone. The recovery experiments were performed in triplicate for each concentration.

Precision
The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). The intra-day precision was calculated as the relative standard deviation (RSD) of results from ten standard samples, during the same day, and the inter-day precision was studied by comparing the assays on two different days. Ten sample solutions (15.0 µg.mL⁻¹) were prepared and assayed, and the standard deviation (SD) and RSD were calculated.

Limit of quantification
The lower limit of quantification was the smallest analytical concentration which could be measured with precision and accuracy.

RESULTS AND DISCUSSION
In this study, a HPLC method for quantitative analysis of dexamethasone acetate in microemulsion was developed and validated.

Specificity and Selectivity
The specificity and selectivity describe the capacity
of the analytical method to measure the drug in the presence of impurities, excipients, degradation products or matrix components (ICH, 2003; Brasil, 2003; USP, 2004). These parameters were determined by comparing the chromatograms of the dexamethasone acetate standard, drug-loaded microemulsion and microemulsion without drug.

The chromatogram of the dexamethasone acetate standard presented a peak in the time retention of 6.7 (Figure 2B). The chromatogram of dexamethasone-loaded microemulsion sample (Figure 2C) showed a peak and retention time similar to dexamethasone acetate standard (Figure 2B). The components of the microemulsion do not interfere with the analysis, therefore no peak is observed in the region of the main peak of dexamethasone (Figure 2A). The chromatogram peaks are well resolved, indicating the high specificity of the method. The retention time of 6.7 min is a good value for routine procedures in quality control. In fact, compared to values obtained elsewhere for analysis of dexamethasone acetate in creams (8.5 min - Garcia et al., 2003) and ointments (11.7 min - Zivanovic et al., 2005), the present method proved advantageous, with a shorter retention time.

**Linearly**

The analytical curve for dexamethasone acetate standard was constructed by plotting the area under the curve (AUC) of the main peak versus drug concentration. It was found to be linear over a wide concentration range (2.0-30.0 µg.mL⁻¹) with a correlation coefficient of 0.9995. The straight line equation obtained from the experimental results was found to be (Equation 3):

\[ y = 40456.425x + 6537.444 \]  

The data were validated by analysis of variance, which demonstrated significant linear regression and non-significative deviation from linearity \((P < 0.05)\). The RSD of the slope and of the intercept of the three lines were 1.95 % and 2.9 %, respectively.

Thus, this HPLC method can be considered to show adequate linearity in the concentration range (2.0-30.0 µg.mL⁻¹) for quantitative analysis of dexamethasone acetate under the experimental conditions described.

**Accuracy**

Accuracy is one of the most important parameters of an analytical methodology and it can be expressed as the percent recovery of known amounts of drug added to a sample. The recoveries were determined by adding known amounts of the dexamethasone acetate reference substance (5.0 µg.mL⁻¹, 10.0 µg.mL⁻¹ and 15.0 µg.mL⁻¹) to the microemulsion sample (10.0 µg.mL⁻¹). The results presented in Table I refer to the average of three assays.

**TABLE I - Analytical recovery of dexamethasone standard solution added to sample**

| Amount added (µg.mL⁻¹) | Recovery µg.mL⁻¹ ± SD | % ± SD |
|------------------------|------------------------|-------|
| 5.0                    | 5.6 ± 0.2              | 112 ± 1.3 |
| 10.0                   | 11.2 ± 1.2             | 112 ± 6.1 |
| 15.0                   | 17.1 ± 1.6             | 114 ± 6.5 |
for each concentration. The results are in good agreement with acceptable values for the validation of an analytical procedure (recovery = 80-120 %) (Brittain, 1998; ANVISA, 2003).

**Precision**

The precision refers to the variability of the results in repeated analyses of the sample under identical experimental conditions. The method was validated by evaluating the intra- and inter-day precision. The precision was calculated from an average of ten determinations of a homogeneous sample (USP, 2004). The intra- and inter-day precision assays were expressed as relative standard deviation (RSD) 0.89 and 0.43, respectively, indicating that the method presents a good precision (Brittain, 1998). The detailed precision data are shown at Table II.

**Limit of quantification**

The lower limit of quantification was determined to be 2 µg.mL\(^{-1}\), with a relative standard deviation lower than 10%.

**CONCLUSION**

The results show that the HPLC method presented here can be considered suitable for the analytical determination of dexamethasone acetate in microemulsions, owing to its high selectivity and specificity, linearity in the concentration range used and high precision and adequate accuracy at the concentrations studied.

**ACKNOWLEDGEMENT**

The authors thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and Rede Nanobiotec for their financial support.

**REFERENCES**

AVERY, M.A.; WOOLFREY, J.R. Antiinflammatory steroids. In: WOLFF, M.E. *Burger’s Medicinal Chemistry and Drug Discovery*. 5.ed. New York: John Wiley & Sons, 1997. cap.65, p.281-376.

BRASIL. Resolução RE n.899, de 29 de maio de 2003. A Agência Nacional de Vigilância Sanitária aprova guia para validação de métodos analíticos. *Diário Oficial da União*, Brasília, 2003b. Available at: <http://www.anvisa.gov.br>. Access on: April 15th. 2006.

BRITISH PHARMACOPOEIA. London: The Stationery Office, 2001. v.2, p.A437-A8.

BRITTAIN, H.G. Validação de métodos analíticos não cromatográficos. *Pharm. Tech.*, v.6, p.4-9, 1998.

| Theoretical concentration (µg.mL\(^{-1}\)) | Concentration | Intra-day | Inter-day |
|---------------------------------------------|---------------|-----------|-----------|
|                | µg.mL\(^{-1}\) | %         | µg.mL\(^{-1}\) | %         |
| 15.00          | 15.5          | 103.7     | 15.2       | 101.5     |
| 15.00          | 15.6          | 104.1     | 15.3       | 102.1     |
| 15.00          | 15.3          | 102.1     | 15.3       | 102.3     |
| 15.00          | 15.5          | 103.7     | 15.3       | 102.0     |
| 15.00          | 15.3          | 102.3     | 15.3       | 101.9     |
| 15.00          | 15.2          | 101.6     | 15.4       | 102.4     |
| 15.00          | 15.4          | 102.5     | 15.4       | 102.4     |
| 15.00          | 15.2          | 101.5     | 15.4       | 103.0     |
| Average        | 15.4          | 102.4     | 15.4       | 102.5     |
| Standard Deviation (µg.mL\(^{-1}\))       | 0.14          | 0.06      |
| Relative Standard Deviation (%)            | 0.88          | 0.41      |

**TABLE II - Analysis of intra- and inter-day precision assays**
CAPELLA-PEIRÓ, M.E.; GIL-AGUSTI, M.; MONFERRER-PONS, L.; ESTEVE-ROMERO, J. Direct injection micellar liquid chromatographic method for the analysis of corticosteroids in creams, ointments and other pharmaceuticals. *Anal. Chim. Acta.*, v.454, p.125-135, 2002.

CONSTANTINIDES, P.P.; SCALART, J.P. Formulation and physical characterization of water-in-oil microemulsions containing long- versus medium-chain glycerides. *Int. J. Pharm.*, v.158, p.57-68, 1997.

FORMARIZ, T. P., URBAN, M. C. C., SILVA-JR, A. A., GREMIÃO, M. P. D., OLIVEIRA, A. G. Microemulsões e fase líquidas cristalinas como sistemas de liberação de fármacos. Revista Brasileira de Ciências Farmacêuticas, v.41, p.301-312, 2005.

GARCIA, C.V.; BREIER, A.R.; STEPPE, M.; SCHAPOVAL, E.E.; OPPE, T.P. Determination of dexamethasone acetate in cream by HPLC. *J. Pharm. Biomed. Anal.*, v.31, p.597-600, 2003.

GASCO, M.R.; GALLARATE, M.; PATTARINO, F. On the release of prednisone from oil in water microemulsions. *Farmaco*, v.43, p. 325-350, 1988.

GUO, D.; CHEN, N.N.; YANG, X.X.; HOU, L.B. Determination of dexamethasone sodium phosphate content in fuyankang cream by high-performance capillary electrophoresis. *Di Yi Jun Yi Da Xue Xue Bao*, v.24, p.839-840, 2004.

HASHIGUSHI, T.; YASUTAKE, T.; MANAKO, T.; OTAGIRI, M. *In vitro* percutaneous absorption of prednisolone derivates based on solubility parameter. *Int. J. Pharm.*, v.158, p.11-18, 1997.

INTERNATIONAL CONFERENCE ON HARMONIZATION (ICH); validation of analytical procedures: Methodology, Q2B (CPMP/ICH/281/95), 1995. Available at: <http://www.ich.org>. Access on: 30th. Aug. 2006.

KRZEK, J.; MASLANKA, A.; LIPNER, P. Identification and quantification of polymyxin B, framycetin, and dexamethasone in an ointment by using thin-layer chromatographic with densitometry. *J. AOAC Int.*, v.88, p.1549-1554, 2005.

LEHMANN, L.; KEIPERT, S.; GLOOR, M. Effects of microemulsion on the stratum corneum and hydrocortisone penetration. *Eur. J. Pharm. Biopharm.*, v.52, p.129-136, 2001.

MILOJEVIC, Z.; AGBABA, D.; ERIC, S.; BOBERIC-BOROJEVIC, D.; RISTIC, P.; SOLUJIC, M. High-performance liquid chromatographic method for the assay of dexamethasone and xylometazoline in nasal drops containing methyl p-hydroxybenzoate. *J. Chromatogr. A.*, v. 949, p.79-82, 2002.

WILLIAMS, P.; BIEHL, E.R. High-pressure liquid chromatographic determination of corticosteroids in topical pharmaceuticals. *J. Pharma. Sci.*, v.70, p.530-534, 1981.

WU, F.; LV, J. Flow injection chemiluminescence detection and solvent extraction for human skin ointment dexamethasone acetate absorption analysis and the reaction mechanism study. *Talanta*, v.72, p.1811-1817, 2007.

ZIVANOVIC, L.; ZECEVIC, M.; MARKOVIC, S.; PETROVIC, S.; IVANOVIĆ, I. Validation of liquid chromatographic method for analysis of lidocaine hydrochloride, dexamethasone acetate, calcium dobesilate, butylhydroxyanisol and degradation product hydroquinone in suppositories and ointment. *J. Chromatogr. A.*, v.1088, p.182-186, 2005.

Received for publication in 23 de novembro de 2007. Accepted for publication in 06 de outubro de 2008.