ELSEVIER

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Journal of Pharmaceutical and Biomedical Analysis 31 (2003) 597–600

JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS

www.elsevier.com/locate/jpba

Short communication

Determination of dexamethasone acetate in cream by HPLC

C.V. Garcia *, A.R. Breier, M. Steppe, E.E.S. Schapoval, T.P. Oppe

Departamento de Produção e Controle de Medicamentos, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Av. Ipiranga, 2752, Porto Alegre 90610-000, RS, Brazil

Received 31 January 2002; received in revised form 5 September 2002; accepted 10 October 2002

Abstract

The aim of this research was to validate a high performance liquid chromatographic method for the quantitative determination of dexamethasone acetate contained in cream preparation. A MetaSil octadecyl silane (250 × 4.6 mm, 5 μm) column, a methanol: water (65:35; v/v) mobile phase (1.0 ml min^{-1}) and an UV detector (set at 254 nm) were used to evaluate the parameters: linearity, precision, accuracy, specificity, as well as, quantitation and detection limits. The calibration curve showed a correlation coefficient of 0.9999. The precision was demonstrated by the relative standard deviation (RSD) of 0.53. The recovery test resulted in an average of 97.85%, what confirmed the accuracy of the method. The quantitation and detection limits determined were 1.41 and 0.47 μg ml^{-1}, respectively. The specificity test showed there was no interference in the drug peak.
© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Dexamethasone; Validation of method; HPLC; Quality control

1. Introduction

Dexamethasone is a synthetic glucocorticoid frequently employed in the diagnosis of adrenal diseases in addition to many inflammatory and immunological processes [1]. It is inserted in European Pharmacopoeia (2002) [2], where spectrophotometric method is used for its determination. However, this method is subject to many interferences. The United States Pharmacopeia (2002) [3] and The Japanese Pharmacopoeia (1996) [4] show high performance liquid chromatographic methods for dexamethasone acetate (DA) bulk substance and injectable suspension [3] determination. The former uses phenyl column and water:acetonitrile as mobile phase, the latter, octadecyl silane column and the same mobile phase, but no one of these official codes shows any method for cream preparation.

High performance liquid chromatography (HPLC) is a useful tool to analyse samples of complex nature, like ointments and creams, because it provides not only the separation and determination, but also eliminates most interference problems [5].

In the literature, few HPLC methods are proposed for the analysis of DA in creams [8], ointments [9] and tablets [10]. In contrast to two

* Corresponding author. Tel.: +51-331-65214; fax: +51-331-65378.

E-mail address: cassiavi@starinfo.net (C.V. Garcia).

0731-7085/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
PII: S0731-7085(02)00695-7

of these methods [8,9], the method reported here has a simpler sample preparation, without chloroform extraction [9] or evaporation steps [8], and even so, it demonstrated to be sensitive and efficient. Furthermore, the previous methods [8,9] utilize water:acetonitrile as mobile phase and octadecyl silane columns, while the tablets method [10] uses water:methanol mobile phase and the same column reported above. This mobile phase was tested in this work.

The aim of this study is to validate a simple and fast HPLC method for the quantitative determination of DA in cream, since there is still no official one. The validation procedure will follow the ICH guidelines [6]. The validation parameters evaluated were linearity, specificity, precision, accuracy and quantitation and detection limits.

2. Materials

2.1. Samples

Commercially available cream containing DA 0.1% (Globo, Belo Horizonte, Brazil).

2.2. Chemicals

Methanol used for preparation of mobile phase, and as diluent, was liquid chromatography grade (Merck, Darmstadt, Germany). Water was purified using a Millipore system (São Paulo, Brazil). DA, 100.28% (Valquímica, São Paulo, Brazil) was used as the external standard. The excipients of the placebo cream, Emulgade® (mixture of cetearyl alcohol, ceteareth-20, sorbitan stearet, POE 20 sorbitan oleate) (Henkel, São Paulo, Brazil), cetostearyl alcohol, octyldodecanol, octyl stearate, diazolidinyl urea, propylene glycol, methylparaben, propylparaben and butylated hydroxytoluene, were all obtained from Galena (São Paulo, Brazil).

2.3. Instrumentation and chromatographic conditions

The liquid chromatograph consisted of a Shimadzu LC-10 A with a SPD-10A variable-wavelength UV detector (set at 254 nm), a C-R6A integrator, a LC-10AS solvent delivery pump and a Rheodyne injection valve with a 20 μl loop (Shimadzu, Kyoto, Japan). The column was a MetaSil octadecyl silane 250×4.6 mm, 5 μm (MetaChem Technologies, Torrance, USA). Mobile phase used was methanol:water (65:35; v/v), at flow rate of 1.0 ml min^{-1}. The instrument was operated at ambient temperature and the detector sensitivity used was 0.05 AUFS.

3. Methods

3.1. Preparation of calibration curve

Aliquots of 1.0; 2.0; 4.0; 6.0; 8.0; 10.0 and 12.0 ml of a 0.5 mg ml^{-1} solution of standard DA were transferred to 100 ml volumetric flasks and diluted to volume with methanol. The final concentrations obtained were 5.0; 10.0; 20.0; 30.0; 40.0; 50.0 and 60.0 μg ml^{-1}, respectively. Each solution was prepared three times. The volume injected was 20 μl.

3.2. Preparation of samples

An amount of commercial cream containing the equivalent to 3 mg of DA was weighted and transferred to a 100 ml volumetric flask, with 20 ml of methanol. It was diluted to volume with the same solvent and shaken for 10 min. So, this solution was filtered and 20 μl were injected into the HPLC column. This procedure was done six times in order to evaluate the precision of the method.

3.3. Recovery test

In order to determine the accuracy of the method, aliquots of 1.0, 2.0 and 3.0 ml of a 1.5 mg ml^{-1} DA standard solution (1.5 mg, 3.0 mg and 4.5 mg, respectively, corresponding to 50.0, 100.0 and 150.0% of the sample concentration) were added to three commercial samples solutions, respectively, prepared as cited in Section 3.2. Each solution was prepared in duplicate and injected three times.

3.4. Specificity test

The specificity was evaluated by assaying an amount of placebo cream containing the same excipients as the commercial product, cited in Section 2.2. The solutions were prepared in the same way did for commercial sample (Section 3.2).

4. Results and discussion

The main objective of the validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose [6]. There are some validation characteristics that need to be evaluated, which are: linearity, specificity, accuracy, precision, detection and quantitation limits, and robustness. There is no need to evaluate all these parameters. It is the responsibility of the analyst to select those considered relevant for each test procedure [7].

In this work, a methanol:water mobile phase was chosen because of the high solubility of DA in this solvent and the lower toxicological risks of it, when compared with acetonitrile. So, two proportions were studied: 70:30 (v/v) like a previous reported study [10] and 65:35 (v/v). The second alternative showed to be better, since it improved the separation of the peaks in the sample chromatogram and kept a great retention time for the drug peak.

Under the experimental conditions described, a standard calibration curve of DA was constructed and it demonstrated to be linear over a concentration range of 5–60 μg ml^{-1}. The correlation coefficient obtained for the line was 0.9999, indicating good linearity. The representative linear equation was: $y = 14\,785x + 25\,834$ where x is the concentration in μg ml^{-1} and y is the peak area in mV s. The data were validated by means of the analysis of variance, which demonstrated significative linear regression and no-significative linearity deviation ($P < 0.05$). The quantitation and detection limits determined were 1.41 and 0.47 μg ml^{-1}, respectively. These low values indicated the sensitivity of this HPLC method.

The results obtained in the determination of DA in commercially available samples are in the Table 1. It was found an excess of 25.0% in the amount labeled, however, since there is no official reference values for cream, it is difficult to say that these samples are reproved. The low relative standard deviation (RSD) of 0.53 showed the precision of the developed method. Fig. 1 show a representative chromatogram of commercial sample solution. The DA retention time was about 8.5

Table 1
Results of quantitative determination of DA in cream by HPLC

Sample	Quantity of dexamethasone acetate (1 mg g^{-1} cream)		
	%	Average	RSD
1	124.7	125.4	0.53
2	125.3		
3	125.6		
4	126.9		
5	124.8		
6	125.4		

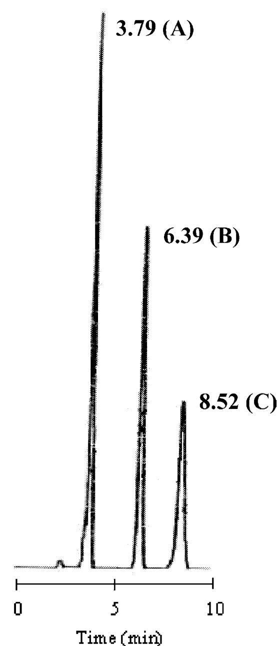

Fig. 1. Chromatogram of cream containig dexamethasone acetate. Chromatographic conditions: MetaSil octadecyl silane (250 × 4.6 mm; 5 μm); mobile phase: methanol: water (65:35 v/v); flow rate: 1.0 ml min^{-1}; injection volume: 20.0 μl; detection UV 254 nm and ambient temperature. (A) Methylparaben; (B) propylparaben; (C) DA.

Table 2
Recovery of standard solution added to commercially available samples

Amount added (mg)	Amount found (mg)	Percentage of recovery[a]	RSD
1.50	1.48	98.70	1.72
3.00	2.95	98.24	0.38
4.51	4.35	96.60	0.07

[a] Each value is an average of two determinations.

min. This is a good value to routine procedure in quality control and it allows to analyse a large number of samples in the same day. The other two peaks in the chromatogram were the preservative methylparaben and propylparaben, in this order.

The recovery test resulted in 97.85% of mean recovery with low RSDs (less than 2.0%). The data of this test can be observed in Table 2. These values show the great accuracy of the method, which is an important parameter in determination methods.

The specificity test demonstrated there was no interference in the drug peak. The chromatogram obtained did not show any other peak besides those from the preservatives, which were present in the sample chromatogram. So, it proved that the peak at 8.5 min was not suffering interference from any other excipient. The specificity is very important, since the cream is a complex matrix and contains a lot of excipients that could cause problems with the resolution of the peaks. By the way, there is the alert about oily excipients that could harm the analysis by clogging up the column [5]. During the development of this study, it was not observed.

5. Conclusion

The proposed HPLC method demonstrated to be simple, linear, accurate, precise, specific and sensitive. It can be used to determine DA in creams, since there is no official method for this drug in that pharmaceutical form.

Acknowledgements

The authors thank to FATEC by the financial support and LEPCQ.

References

[1] A. Goldfien, Adrenocorticoesteróides e antagonistas córtico-supra-renais, in: B.G. Katzung (Ed.), Farmacologia Básica e Clínica, sixth ed, Guanabara Koogan, Rio de Janeiro, 1995, pp. 450–461.
[2] European Pharmacopoeia, fourth ed., Council of Europe, Strasbourg, 2002.
[3] United States Pharmacopeia, 25th ed., United States Pharmacopeial Convention, Rockville, 2002, pp. 511–515.
[4] The Japanese Pharmacopoeia, 13th ed., Society of Japanese Pharmacopoeia, Tokyo, 1996.
[5] P. Williams, E. Biehl, J. Pharm. Sci. 70 (1981) 530–534.
[6] International Conference on Harmonisation of Technical Requeriments for Registration of Pharmaceuticals for Human Use (ICH) Q2B: Guideline on Validation of Analytical Procedure—Methodology, 1996.
[7] J. Ermer, J. Pharm. Biomed. Anal. 24 (2001) 755–767.
[8] H. Tokunaga, T. Kimura, J. Kawamura, Chem. Pharm. Bull. 32 (1984) 4012–4016.
[9] F. Belliardo, A. Bertolino, J. Liq. Chromatogr. 4 (1981) 293–298.
[10] M. Santoro, E. Govato, E. Hackmann, Anal. Lett. 26 (1993) 925–935.