ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
20 (1999) 405–409

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Short Communication

Validation of a reversed-phase liquid chromatographic method for the determination of hydrocortisone phosphate disodium in a gel formulation

M.J. Galmier [a], E. Beyssac [b], J. Petit [a,*], J.M. Aiache [b], C. Lartigue [a]

[a] *Groupe de Recherche en Biodynamique du Médicament, Laboratoire de Chimie Analytique et de Spectrométrie de Masse, Faculté de Pharmacie, BP38, 63001, Clermont-Ferrand Cédex, France*
[b] *Laboratoire de Biopharmacie, Faculté de Pharmacie, BP 38, 63001, Clermont Ferrand Cédex, France*

Received 26 November 1997; received in revised form 8 September 1998; accepted 20 September 1998

Keywords: Hydrocortisone phosphate disodium; Gel; HPLC; Method validation

1. Introduction

A new pharmaceutical formulation, a gel containing 1% (w/w) hydrocortisone phosphate disodium (HPD) has been developed. Hydrocortisone (11,17,21-trihydroxypregn-4-ene-3,20-dione) is a steroid required for several metabolic processes. A lot of LC methods have been reported for measuring hydrocortisone in biological fluids [1–9] and pharmaceutical formulations [10–14]. Some analytical methods for the analysis hydrocortisone acetate have been published [11,15], but none for hydrocortisone phosphate disodium. As reported, methods used to measure hydrocortisone led to important interferences with the excipients involved in the gel, and a new method was developed.

The method reported in this paper for the determination of hydrocortisone phosphate disodium in a pharmaceutical formulation was validated following the analytical performance parameters required by the USP XXII.

2. Experimental

2.1. Materials

HPD was purchased from Roussel UCLAF. The gel and placebo gel were manufactured in our laboratories. The composition of the gel was a mixture, in water, of polycarbophil excipients with preservatives compounds. HPLC grade methanol was purchased from Merck (Darmstadt, Germany). Water was prepared by reverse osmosis and de-ionization using a MilliQ system (Millipore, Saint-Quentin, Yvelines, France). The

* Corresponding author. Fax: +33-4-73263874.
E-mail address: lab.chimie.anal@u-clermont1.fr. (J. Petit)

PII: S0731-7085(99)00031-X

phosphate buffer 0.02 M (pH 7.5) was prepared using disodium hydrogen phosphate (Merck), sodium dihydrogen phosphate (Prolabo, Paris, France) and 1 M sodium hydroxide (Titrisol, Merck).

2.2. Chromatographic system

The chromatography was carried out using an LCM1 instrument (Waters Assoc., Saint-Quentin, Yvelines, France) equipped with a power supply, an autosampler and a UV spectrophotometric detector connected to a data collection system (Millennium).

The analytical column used was Lichrospher RP18, 5 μm, (4 mm × 25 cm) in conjunction with a Lichrospher RP18, 5 μm, guard column from Merck.

The mobile phase was isocratic with phosphate buffer 0.02 M, methanol (40:60, v/v) and was filtered through a 0.45-μm filter (Millipore). The flow-rate was 0.5 ml min^{-1} which produced a maximum column back pressure of 1550 p.s.i. (1 p.s.i. = 6.9×10^3 Pa). The injection volume was 10 μl and the total run time was 15 min. The detection wavelength was 240 nm.

2.3. Standard preparation

Working standard solution (10 μg ml^{-1}) was prepared by dissolving 10 mg of hydrocortisone phosphate disodium in 100 ml of de-ionized water. A 1.0-ml aliquot of this solution was transferred to a 10-ml volumetric flask and diluted to volume with de-ionized water (equivalent to 100% nominal label claim).

2.4. Sample preparations

The gel sample (100 mg) was transferred to a 100-ml volumetric flask and diluted to volume with de-ionized water. A portion of the resulting solution (10 μg ml^{-1}) was then transferred into a polypropylene autosampler vial for analysis.

2.5. Method validation

The method was validated in compliance with the analytical performance parameters required by the USP XXII for LC method validation. The following parameters were evaluated: specificity; linearity and range; accuracy and precision (intra- and interassay). Solutions of HPD to 60, 80, 100, 120 and 140 μg ml^{-1} were prepared in de-ionized water and were used as spiking solutions to prepare placebo gel samples and standard solutions equivalent to 60, 80, 100, 120 and 140% of nominal label claim (10 μg ml^{-1}). These different samples were prepared on three days and analysed by three analysts with different phosphate buffer solutions.

3. Results and discussion

3.1. Specificity

Chromatograms of placebo gel samples, of placebo gel spiked with HPD and of placebo gel spiked with HPD and a degradation product of HPD (hydrocortisone base:1 ng ml^{-1}) are shown in Fig. 1. No peaks were found in the region where HPD elutes in either chromatogram, indicating specificity of the method against matrix interferences or degradation products of HPD.

3.2. Linearity

The linearity was studied with placebo gel samples (15 samples) and standard solutions (15 samples). The data were fitted to the model $y = ax + b$ using least-squares regression (Table 1).

The existence of the slopes and the verification of the linear model were calculated using the *F*-Snedecor test. Comparison of intercepts and slopes obtained with standard solutions and placebo gel samples were performed using the Student's *t*-test. The intercepts were also compared with the Student's *t*-test to the theoretical value of zero.

As shown in Table 1, the linearity is satisfactory using standard solutions and spiked placebo. No significant difference appears (at the 95% confidence limits) between the results obtained with standard solutions and placebo gel samples and the intercepts are not significantly different with the theoretical value of zero.

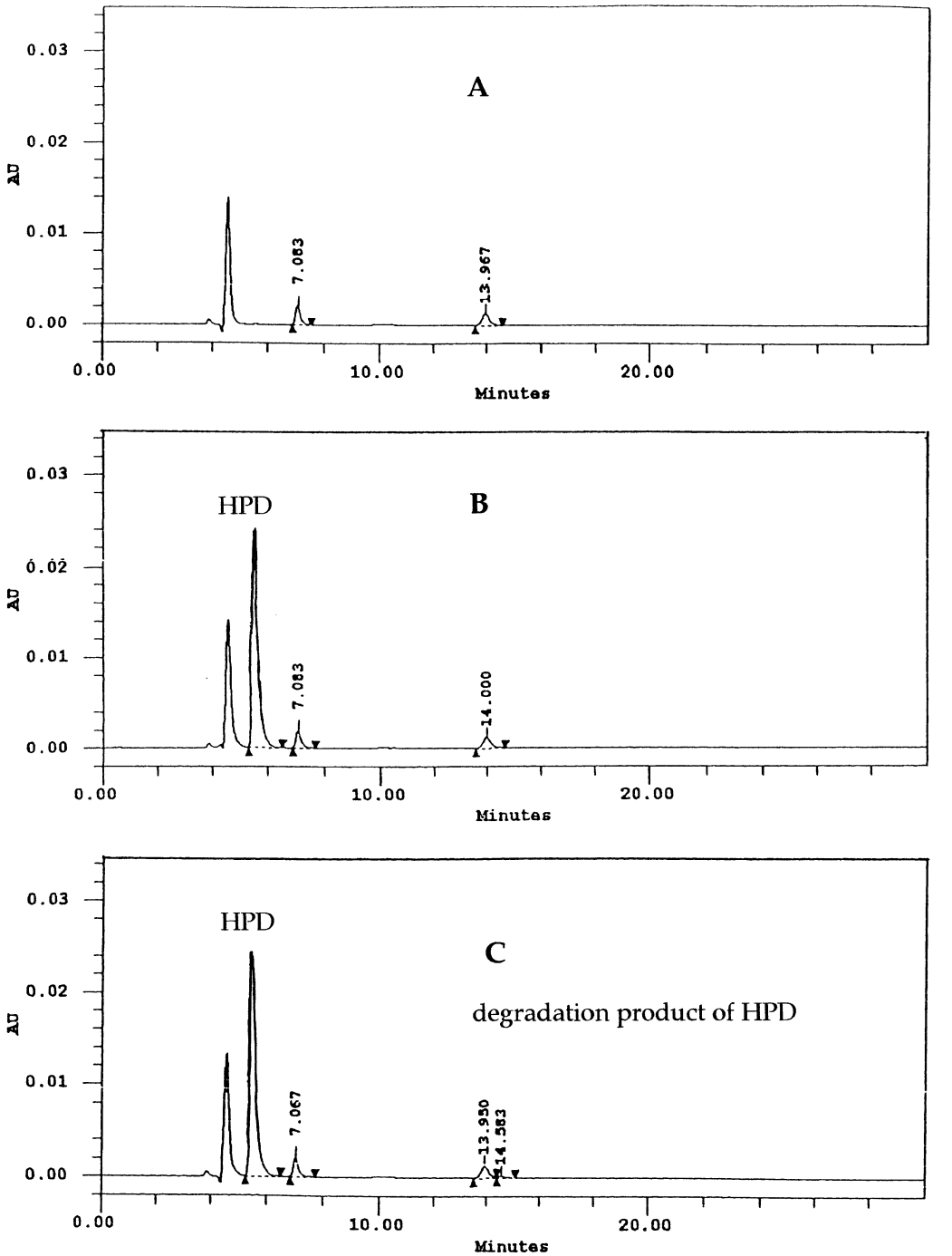

Fig. 1. Chromatograms of placebo gel samples (A), placebo gel spiked with HPD (B) and placebo gel spiked with HPD and a degradation product of HPD (C).

Table 1
Linearity[a]

	Standard solutions	Spiked placebo		
Slope (a)	34 464.94	32 286.48		
Intercept (b)	−411	15839		
Correlation coefficient (r)	0.9957	0.9968		
	Calculated values		Tabulated values	Significance
Existence of slope (F Snedecor test)	1499	2023	4.67	HS
Linear model (F Snedecor test)	0.56	0.34	3.71	NS
Comparison of intercepts (Student test)	1.37		2.056	NS
Comparison of slopes (Student test)	1.91		2.056	NS
Comparison of intercepts with the theoretical value of zero (Student test)	0.04	2.12	2.16	NS

[a] HS: highly significant ($P<0.01$), NS: no significance ($P>0.05$).

As a consequence of the primary assumptions of single point analysis being a linear response through the range of interest and no bias in the intercept, the single point calibration of standard solution can be used for analysis.

3.3. Accuracy and precision

The results of the placebo gel samples ($n=15$) were analysed with the single point calibration of the standard solution (Tables 2 and 3).

Accuracy, defined as $M \pm (\mathrm{SD}/\sqrt{n})\, t$ is $99.62 \pm 1.42\%$ where M is the mean potency value from recovery testing, SD is the standard deviation and the Student's t is $t\ (0.05,\ 14) = 2.14$, and n replicates.

Precision evaluated under the same conditions of work (intra-assay) and under different experimental conditions (interassay) are expressed by the coefficients of variation (CVr for intra-assay and CVR for interassay) according to the Caporal-Gauthier method [16].

Precision is 0.29% for intra-assay and 1.62% for interassay.

4. Conclusion

The method reported here for the determination of HPD was validated in compliance with the USP XXII analytical performance parameters. As the release requirement for the dosage form is $\pm 5\%$ of nominal label claim, the method was validated over a range of 60–140% of nominal label claim (10 μg ml^{-1}). Single point calibration was chosen for analysis because of its simplicity and wide use in the pharmaceutical industry for content uniformity analysis. The assay provides a basis for determination concentration of HPD in other matrices.

Table 2
Accuracy results

Added (mg)	Found (mg)	Recovery (%) individual values
6.00	6.20	103.33
5.90	6.21	105.25
6.00	5.85	97.50
8.10	8.35	103.09
8.10	8.07	99.63
8.00	8.10	101.25
10.00	9.78	97.80
9.80	9.74	99.39
9.90	9.63	97.27
12.00	11.83	98.58
12.10	11.85	97.93
12.20	12.17	99.75
14.00	13.93	99.50
13.90	13.40	96.40
14.10	13.77	97.66

Table 3
Precision results[a]

Added (mg)	Found (mg)	Recovery (%) individual values	Variance	CV[c] (%)
	9.78	97.80		
	9.86	98.60	Intra-assay	CV*r*
10.00	9.99	99.90	(S^2r)[b]	0.29
	9.93	99.30	0.29	
	9.91	99.10		
	9.82	98.20		
	9.81	98.10	Intergroup	
10.00	9.92	99.20	(S^2g)[b]	
	9.80	98.00	1.32	
	9.82	98.20		
	9.86	98.60		
	9.98	100.20		
	10.00	100.40	Interassay	CV*R*
9.96	10.00	100.40	(S^2R)[b]	1.62
	10.02	100.60	1.61	
	10.04	100.80		
	10.07	101.10		

[a] $M°$ (mean of recovery) $= 99.32 \pm 1.11$.
[b] $S^2R = S^2r + S^2g$.
[c] $\mathrm{CV}r = 100 \times Sr/M°$, $\mathrm{CV}R = 100 \times SR/M°$.

References

[1] M. Hariharan, S. Naga, T. van Noord, E.K. Kindt, J. Chromatogr. B 124 (1993) 195–201.
[2] G.J. Samaan, D. Porquet, J.-F. Demelier, D. Biou, Clin. Biochem. 26 (3) (1993) 153–158.
[3] J. Lykkesfeldt, S. Loft, H.E. Poulsen, J. Chromatogr. B 660 (1) (1994) 23–29.
[4] M. Dolezalova, Clin. Chim. Acta 231 (2) (1994) 129–137.
[5] G.L. Lensmeyer, C. Onsager, I.H. Carlson, D.A. Wiebe, J. Chromatogr. A 691 (1–2) (1995) 239–246.
[6] M. Bidart, G. Lesgards, J. Liq. Chromatogr. 18 (4) (1995) 725–738.
[7] Y.P. Qin, Q. Yu, Z.G. Luo, Yaowu Fenxi Zazhi 15 (4) (1995) 9–12.
[8] T. Okumura, Y. Nakajima, T. Takamatsu, M. Matsuoka, J. Chromatogr. B 670 (1) (1995) 11–20.
[9] A. Santos Montes, R. Gonzalo Lumbreras, R. Izquierdo Hornillos, J. Chromatogr. B 673 (1) (1995) 27–33.
[10] S. Zhang, J. Wang, Y. Xu, R. Yu, Zhongguo Yaoke Daxue Xuebao 24 (2) (1993) 86–88.
[11] C. Valenta, H. Janout, J. Liq. Chromatogr. 17 (5) (1994) 1141–1146.
[12] L. Ayllon, M. Silva, D. Perez-Bendito, J. Pharm. Sci. 83 (8) (1994) 1135–1141.
[13] H.M. Dodds, D.J. Maguire, R.H. Mortimer, R.S. Addison, G.R. Cannell, J. Liq. Chromatogr. 18 (9) (1995) 1809–1820.
[14] J.E. Kountourellis, C.K. Markopoulou, K.O. Ebete, J.A. Stratis, J. Liq. Chromatogr. 18 (17) (1995) 3507–3517.
[15] G.J. Bhounsule, V.S. Gorule, G.V. Patil, Ind. Drugs 29 (13) (1992) 594–597.
[16] J. Caporal-Gautier, J.M. Nivet, P. Algranti, M. Guilloteau, M. Histe, M. Lallier, J.J. N'Guyen-Huu, R. Russotto, STP Pharma 2 (4) (1992) 205–226.