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Simultaneous determination of methylparaben, propylparaben, hydrocortisone acetate and its degradation products in a topical cream by RP-HPLC

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Abstract

A novel reversed-phase high-performance liquid chromatographic method with UV spectrophotometric detection was developed and validated for the determination of compounds in topical cream. The method describes determination of active component hydrocortisone acetate (HCA), its degradation products hydrocortisone (HC) and cortisone acetate (occurring in formulation after long-term stability tests) and two preservatives presented in the cream-methylparaben and propylparaben, using dexamethasone as an internal standard. The chromatographic separation was performed on a 5 μm SUPELCO Discovery C18 125 × 4-mm ID column. The optimised mobile phase for separation of all the compounds consists of methanol, acetonitrile and water (15:27:58, v/v/v), with the analysis time less than 13 min. The method was applicable for routine analysis (assays and stability tests) of active compound HCA, preservatives and degradation products in pharmaceutical product—topical cream Hydrocortizone cream 1%.
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Keywords: Liquid chromatography; Pharmaceuticals; Degradation products; Methylparaben; Propylparaben; Hydrocortisone acetate

1. Introduction

Hydrocortisone acetate (HCA, Fig. 1) is a corticosteroid designated chemically as pregn-4-ene 3,20-dione, 21-(acetyloxy)-11,17-dihydroxy-(11B). HCA has antiinflammatory, antipruritic and vasoconstrictive properties. The mechanism of the antiinflammatory activity of topical corticosteroids is generally unclear. However, corticosteroids are thought to induce phospholipase A2 inhibitor proteins, preventing arachadonic acid release and the biosynthesis of potent mediators of inflammation. Absorption of HCA is thought to be 1–5% of the administered dose. The absorption of HCA may be higher in areas such as the face, groin, axilla or on injured or inflamed skin,

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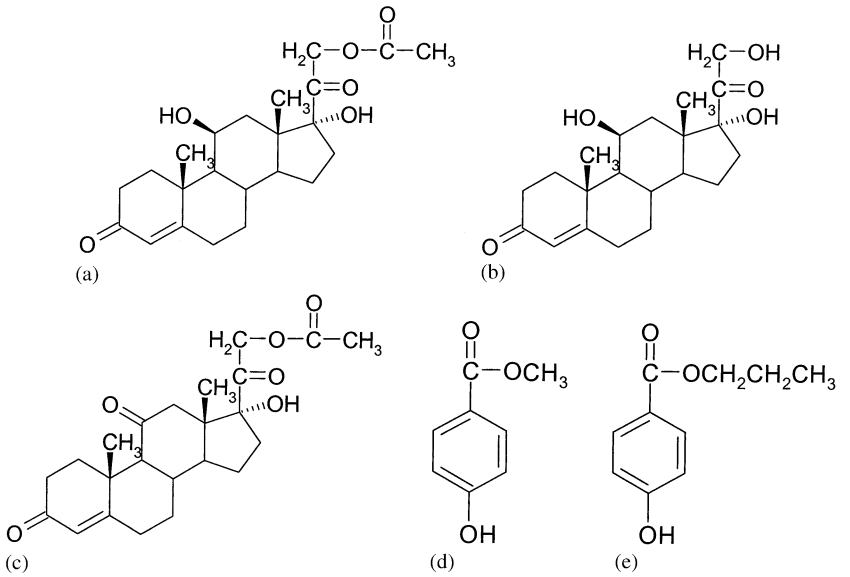

Fig. 1. Major components and degradation product of HC cream 1% (a) HCA; (b) HC; (c) CA; (d) methyparaben; (e) PP.

such as the lesions of atopic dermatitis [1]. Although the chemical stability of the compound is relatively high, several potential impurities are described in different pharmacopoeias. Two main impurities—hydrocortisone (HC, Fig. 1) and cortisone acetate (CA, Fig. 1) may occur in pharmaceutical formulations after a long-term storage.

Methylparaben (MP) and propylparaben (PP) (Fig. 1) are effective antibacterial and antifungal agents, which are commonly used as preservatives in foods, beverages, cosmetics and pharmaceuticals [2]. MP and PP are used together since they have a synergistic effect [3].

Recently there have been a number of reports dealing with various analytical methods for the determination of HCA, such as UV spectrometry [4,5], coulometry [6], mass spectrometry [7], high performance thin-layer chromatography [8], densitometry [9], colorimetry [10] and high performance liquid chromatography (HPLC) [11–17] as well.

Only one HPLC method for simultaneous determination of HCA and its degradation products HC and CA can be found in literature [17], but not in the presence of preservatives MP and PP. HPLC analysis of MP and PP is frequently described in the literature [2,3,18], but we did not find any HPLC method dealing with simultaneous determination of all five components—HCA, HC, CA, MP and PP in pharmaceutical preparations. The current United States Pharmacopoeia—USP 24 [19] or European Pharmacopoeia, third ed. [20] specify a HPLC method for the determination of HCA and its one degradation product CA, but not simultaneously in the presence of second degradation product HC and preservatives MP, PP.

The aim of the presented study was to develop and validate a new HPLC method for the determination of the five different compounds in a topical cream-active component HCA, two preservatives MP and PP and two degradation products of HCA—HC and CA, using internal standard. Thereafter, this method has been successfully applied for the separation, quantification and stability study of all compounds in the pharmaceutical formulation—Hydrocortizone cream 1%.

2. Experimental

2.1. Material and reagents

The in-house secondary standards were used during this study. Standard of HCA was obtained

from Herbacos-Bofarma Ltd (Bochemie Group, Pardubice, Czech Republic); standards of HC, CA, MP, PP and internal standard dexamethasone from Sigma Aldrich Co. (Prague, Czech Republic). All compounds have been assayed against the European Pharmacopoeia CRS reference standards (Council of Europe, Strasbourg).

All standard solutions were prepared in acetonitrile. The final concentrations of the sample or reference standards were about 250 μg ml^{-1} of HCA, 25 μg ml^{-1} for MP, 12.5 μg ml^{-1} for PP, 5 μg ml^{-1} for HC, 5 μg ml^{-1} for CA and 10 μg ml^{-1} for internal standard dexamethasone.

Acetonitrile (Chromasolv, for HPLC) and methanol (Chromasolv, for HPLC) were obtained from Sigma Aldrich Co. All other chemicals were reagent grade from Merck (Darmstadt, Germany).

The deionised water was purified by a Milli-Q system (Millipore, MA) and meets the European Pharmacopoeia specifications.

Hydrocortizone cream 1% was supplied from Herbacos-Bofarma Ltd (Bochemie Group). The cream is composed of the following excipients: Propyleneglycolum, Trolaminum 10%, Brij 72, Alcohol cetylicus, Helianthi oleum, Adeps solidus, Propylis galas and Carbomerum.

2.2. Instrumentation

The HPLC system, consisting of a binary pump LCP 4100 (Ecom, Prague, Czech Republic), autosampler Waters 717 plus, variable wavelength UV detector Waters 486 (Waters, Milford, MA) and PC for data processing, was controlled by chromatographic software CSW, version 1.7 for Windows (Data Apex s.r.o., Prague, Czech Republic).

Analyses were performed on a 5 μm SUPELCO Discovery C18 125×4 mm ID column (Sigma-Aldrich) with pre-column SupelGuard 20×4 mm ID, 5 μm (Sigma-Aldrich).

The optimal mobile phase for separation of HCA, MP, PP, HC and CA was a mixture of methanol, acetonitrile and water (15:27:58, v/v/v). Mobile phase was degassed before application by means of helium.

The finally selected and optimised conditions were as follows: injection volume 10 μl, the mobile phase isocratically pumped at a flow rate 0.8 ml min^{-1} at ambient temperature, the detection wavelength 238 nm.

2.3. Sample preparation

An accurately weighed portion (≈ 0.5 g) of the pharmaceutical cream containing HCA and other compounds was transferred into a 50 ml centrifuge tube and supplemented with 20.00 ml of internal standard (10 μg ml^{-1} solution of dexamethasone in acetonitrile). The mixture was placed into the ultrasonic bath for 10 min and then centrifuged at 3000 rpm for 15 min. A volume of 10 μl of supernatant was analysed by HPLC.

Identification of peaks in the cream samples was based on the comparison of retention times of compounds in standard solutions. Peak identity was confirmed by UV–vis spectra.

3. Results and discussion

3.1. Method development and optimisation

The main criteria for developing a successful HPLC determination of HCA, preservatives and degradation products in topical cream were as follows: the method should be stability indicating, free of interference from excipients, robust and straightforward enough for routine use in quality control laboratory.

The first step was to find the appropriate wavelength, which is usually a compromise for different compounds with different absorption maximum. The main factor is the sensitivity of determination of degradation products, presented in a very low concentration especially in the beginning of the stability tests. Therefore the 238 nm was chosen.

The internal standard—dexamethasone (Fig. 2)—was chosen after testing of several different compounds. The optimisation of mobile phase was firstly done with a binary mixture of water and acetonitrile or methanol. It was found that it is not possible to separate all compounds, therefore a combination of three different solvents had to be used. The optimal composition was tested using different speed of mobile phase.

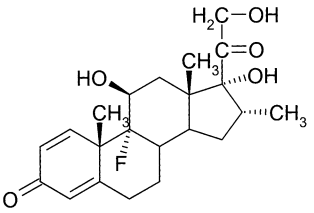

Fig. 2. International standard-dexamethasone = 9-Fluoro-11b,17,21-trihydroxy-16a-methylpregna-1,4-dien-3,20-dione.

Finally, for sufficient separation of HCA, HC, CA, MP and PP, the mobile phase was composed of methanol, acetonitrile and water (15:27:58, v/v/v). Using a 5 μm packing of the column SUPELCO Discovery C18 and increasing the flow rate to 0.8 ml min^{-1}, the analysis time for standards of all compounds was about 13 min (Fig. 3).

3.2. Analytical parameters and validation

The optimised method was validated by a standard procedure to evaluate if adequate accuracy, precision, selectivity and linearity had been achieved. Accuracy was determined using spiked placebo solutions, three preparations each, two injections of each preparation, at the concentration levels described in Section 2.1. The same concentrations were used for evaluation of the precision. Relative standard deviation (RSD) values were calculated for repeated standard injections (system precision) as well as repeated injections of multiple sample preparations (method precision). Short-term stability of standards was evaluated by comparison of response factors of fresh and stored standards. Visual inspection of chromatograms of standards and placebo solutions was conducted to ensure selectivity. Linearity was determined from standard solutions in the 20–150% range at six different concentrations. The method validation results obtained under the final conditions are shown in Table 1. The method meets all common requirements for accuracy, precision and linearity.

3.3. Analysis of degradation products

In the pharmaceutical formulation containing HCA, a small amount of degradation products HC and CA can be found after a long-term storage. For the determination the actual amount

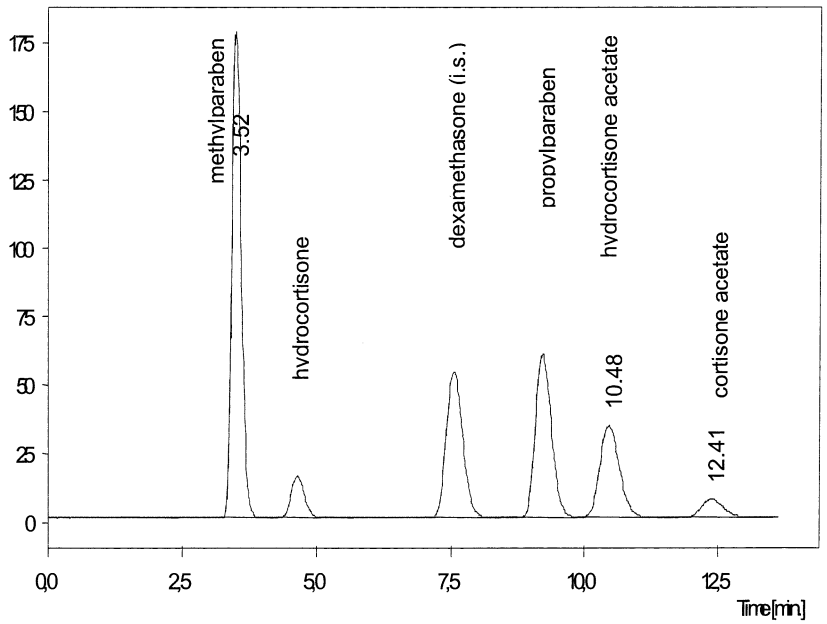

Fig. 3. Chromatogram of separation of standard solutions for all compounds.

Table 1
Method validation results

Validation step	Parameter	HCA	MP	PP	HC	CA	Criteria
System precision	R.S.D.[a]	0.25%	0.22%	0.21%	0.58%	0.62%	$X < 2\%$
Method precision	R.S.D.[b]	1.46%	1.24%	1.32%	1.98%	1.89%	$X < 2\%$
Accuracy	Spiked recovery[b]	99.55%	100.84%	99.33%	101.48%	99.10%	$X = 100 \pm 3$
	Recovery R.S.D.[b]	1.98%	0.74%	1.95%	1.76%	1.43%	$X < 2\%$
Linearity ($n = 6$)[c]	Correlation coefficient	0.999986	0.999984	0.999992	0.999993	0.99918	$X > 0.9990$
Sample stability[d]	% Change in response factors	0.19%	0.33%	0.19%	0.99%	0.21%	$X < 2\%$

[a] Six injections.
[b] Three preparations each, two injections of each preparation.
[c] At 20, 50, 80, 100, 120, 150% levels (for 250 μg ml^{-1} of HCA, 25 μg ml^{-1} of MP, 12.5 μg ml^{-1} of PP, 5 μg ml^{-1} of HC, 5 μg ml^{-1} of CA).
[d] Two-day stability data for all compounds.

of the degradation products, it is necessary to know that they occur in a very low concentration level (~ 100–1000 lower) in comparison with the active compound HCA. This is also the reason why generally methods for determination active compounds and methods for determination of degradation products are often different. In our case, we finally found sufficient separation of all compounds and therefore we could include the whole procedure into one step. Under the optimised conditions, the selectivity of the determination is sufficient and the retention time for HC and CA is far enough from the other compounds presented in the sample. Limit of detection (LOD) for 10 μl injection of HC standard (at signal to noise $S/N = 3$) was 0.009 μg ml^{-1}, limit of quantification (LOQ) (at signal to noise $S/N = 10$) was 0.031 μg ml^{-1}, LOD for CA was 0.012 μg ml^{-1}, LOQ for CA 0.040 μg ml^{-1}.

3.4. Determination in pharmaceutical product

The chromatogram in Fig. 4 was obtained using the described HPLC method with a sample (topical cream Hydrocortizone cream 1%) after a long-term stability test (stored 6 months in original packaging at a temperature 25 ± 2 °C and relative humidity $60 \pm 5\%$). All compounds presented in the sample—HCA, both preservatives, two degradation products and internal standard—are clearly separated. The average determined amounts of the HCA, MP and PP in Hydrocortizone cream 1% ($n = 3$) were 101.8 ± 0.3, 105.0 ± 0.8 and $104.0 \pm 0.9\%$ of the labelled amount, respectively. The amount of the degradation product HC and CA was relatively very low, it was found to be only 0.283 and 0.191% of the amount of the active compound HCA, respectively.

The results of accelerated stability tests (formulation stored 6 months in original packaging at a temperature 40 ± 2 °C and relative humidity $60 \pm 5\%$) were: amounts of the HCA, MP and PP 101.5 ± 0.5, 104.0 ± 0.9 and $104.0 \pm 0.8\%$ of the labelled amount, respectively. The amount of the degradation product HC and CA was found to be 0.365 and 0.235% of the amount of the active compound HCA, respectively.

4. Conclusion

The objective of this study was to develop and validate a simple HPLC method with UV spectrophotometric detection on a SUPELCO Discovery C18 column for the determination of HCA, MP, propylparabene, HC and CA in a topical cream using commercially available dexamethasone as an internal standard.

The total analysis time was less than 13 min. Method has been validated, the results obtained were precise and accurate and the limits of detection of degradation product were sufficiently low.

Method can use for routine analysis (batch analysis and stability tests) of compounds in pharmaceutical products containing active com-

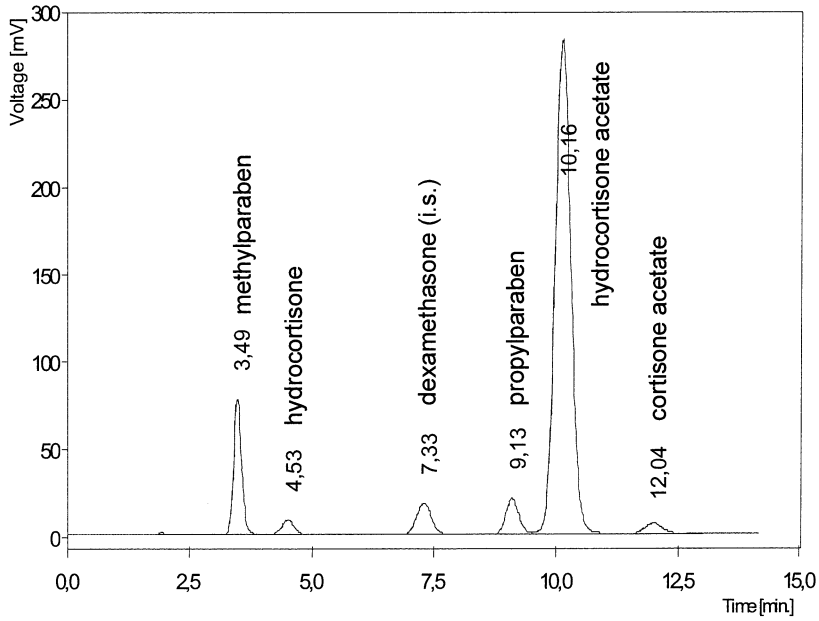

Fig. 4. Chromatogram of all compounds in topical emulgel after 6 months stability test.

pound HCA, preservatives MP+PP and degradation products of the active compound.

This method was successfully applied for the identification, quantitative analysis and stability tests of all major compounds in a topical cream—Hydrocortizone cream 1%.

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References

[1] Available from: http://www.dnd.ca/health.
[2] S.H. Kang, H. Kim, J. Pharm. Biomed. Anal. 15 (1997) 1359–1363.
[3] M.D. Kreuz, A.L. Howard, D. Ip, J. Pharm. Biomed. Anal. 19 (1999) 725–730.
[4] D. Bonazzi, V. Andrisano, R. Gatti, V. Cavrini, J. Pharm. Biomed. Anal. 13 (1995) 1321–1329.
[5] M.I. Walash, N.A. Zaghari, M. Rizk, S. Toubar, Farmaco 42 (1987) 81–90.
[6] K. Nikolic, M. Medenica, M. Bogavac, L. Arsenijevic, Farmaco 46 (1991) 623–626.
[7] F. Bevalot, Y. Gaillard, M.A. Lhermitte, G. Pepin, J. Chromatogr. B 740 (2000) 227–236.
[8] J. Sherma, B.P. Whitcomb, K. Brubaker, J. Planar Chromatogr. Mod. TLC 3 (1990) 189–190.
[9] J. Jarzebinski, E. Lugowska, K. Czaja, Acta Pol. Pharm. 43 (1986) 260–263.
[10] M. Rizk, N. Zakhari, S. Toubar, M.I. Walsh, Acta Pharm. Fenn. 93 (1984) 129–134.
[11] A.R. Lea, J.M. Kennedy, G.K.C. Low, J. Chromatogr. 198 (1980) 41–47.
[12] C. Valenta, H. Janout, J. Liq. Chromatogr. 17 (1994) 1141–1146.
[13] G.J. Bhounsule, V.S. Gorule, G.V. Patil, Indian Drugs 29 (1992) 594–597.
[14] A. Shalaby, M. Shahjahan, J. Liq. Chromatogr. 14 (1991) 1267–1274.
[15] D.M. Hailey, A.R. Lea, J. Assoc. Anal. Chem. 64 (1981) 235–239.
[16] R.D. Marini, A. Pantella, M.A. Bimazubute, P. Chiap, P. Hubert, J. Crommen. Chromatographia 55 (2002) 263–269.

[17] J.C. Reepmeyer, J. Liq. Chromatogr. Relat. Technol. 24 (2001) 693–709.
[18] D. Kollmorgen, B. Kraut, J. Chromatogr. B 707 (1998) 181–185.
[19] United States Pharmacopoeia XXIV, The United States Pharmacopeial Convention, Inc., Rockville, MD, 2000, p. 546.
[20] European Pharmacopoeia 1997, third ed., Council of Europe, Strasbourg, 1996, p. 978–979.