

Influence of primary packaging on stability studies of fludrocortisone acetate tablets

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ABSTRACT

Study objectives: Fludrocortisone acetate is known to be a drug substance that is particularly unstable in various environmental conditions. Therefore, the physical and chemical stability of 55 µg fludrocortisone acetate tablets was investigated in two different packaging materials. The objective of this study was to select appropriate packaging for this product and to determine its shelf life.

Methods: Tablets of fludrocortisone acetate were placed in either blister packs composed of polyvinyl chloride, polyvinylidene chloride and aluminium foil (PVC/PVDC/Al), or of polyamide, Al, PVC and Al (OPA/Al/PVC/Al). Stability was investigated after storage at various temperatures and relative humidity: 25°C/60% RH and 40°C/75% RH for 36 and six months, at predetermined time-intervals and analysed for physical stability (visual appearance, disintegration time, mean mass, hardness) and chemical stability (assay) using a validated high performance liquid chromatography method (HPLC).

Results: The stability-indicating assay method was implemented and completely validated. For three batches of fludrocortisone acetate tablets packaged in PVC/PVDC/Al blisters and stored for 24 months at 25°C, the fludrocortisone acetate content was lower than 95% of the initial content. At 40°C, the fludrocortisone acetate content was lower than 60%. After 36 months at 25°C, no relevant changes were observed for three batches of fludrocortisone acetate tablets packaged in OPA/Al/PVC/Al blisters and the estimated shelf life was more than three years. At 40°C, for all batches tested, the drug remained within specification, i.e. >95%.

Conclusion: The drug stability profile obtained as a function of packaging is consistent with the prediction based on the moisture sensitivity of the drug substance and confirms the capacity of cold-form aluminium blisters to provide an efficient moisture barrier for solid oral forms of fludrocortisone acetate.

KEYWORDS

Drug stability, fludrocortisone acetate, HPLC, packaging, tablets

INTRODUCTION

Fludrocortisone acetate is a synthetic adrenocortical steroid possessing very potent mineralocorticoid properties and high glucocorticoid activity. It is widely and principally used for the treatment of salt-wasting congenital adrenal

hyperplasia [1, 2] and of Addison's disease [3]. More recently, the use of fludrocortisone for severe sepsis and septic shock has been proposed [4].

The assay of fludrocortisone acetate in raw materials and in solid dosage forms has been reported with different analytical methods: direct potential measurement of ion-selective fluoride electrode [5], spectrophotometric procedure [5-8] and several HPLC methods [9-11]. However, potentiometric titration or spectrophotometric methods are not specific enough and do not permit the stability of fludrocortisone acetate in drug products to be followed specifically and accurately.

The chemical structure of fludrocortisone acetate is shown in Figure 1(A). It is highly sensitive to water with hydrolysis of the drug substance leading to the formation of fludrocortisone represented in Figure 1(B). Moreover, other degradation products can occur during prolonged storage of pharmaceutical forms. Fludrocortisone acetate 100 µg (Florinef) tablets are packed commercially

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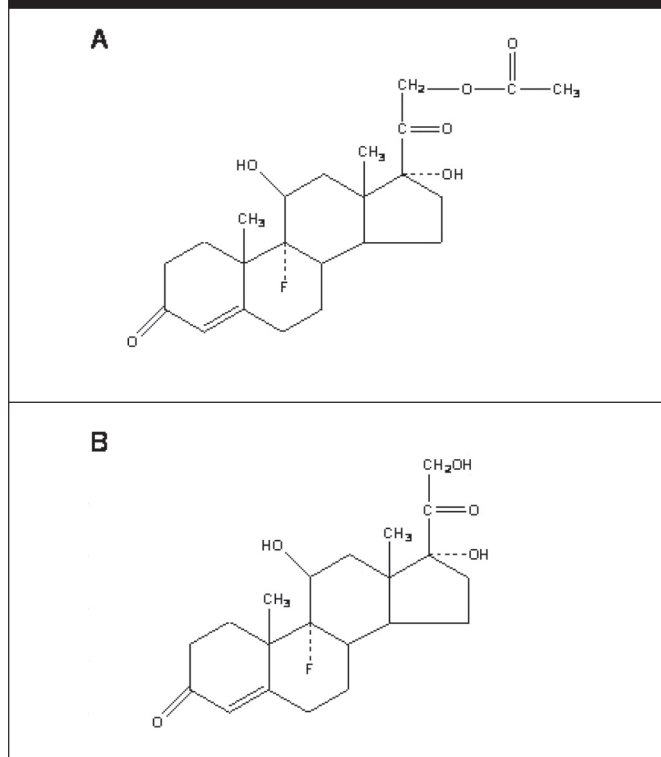
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Figure 1: The chemical structures of fludrocortisone acetate (A) and fludrocortisone (B)



in amber glass bottles with a cotton plug, induction seal and polypropylene caps. However, this packaging is not sufficient for a shelf life of more than 24 months for this product.

The aim of this study was to investigate the stability of fludrocortisone acetate 55 µg tablets (corresponding to 50 µg of fludrocortisone) placed in two different primary packaging materials: PVC/PVDC/Al blisters or OPA/Al/PVC/Al blisters, and subjected to various conditions of temperature and humidity (25°C/60% RH, and 40°C/75% RH) for different periods of time.

MATERIALS AND METHODS

Drugs and chemicals

Fludrocortisone acetate (9-fluoro-11β, 17, 21-trihydroxy-pregn-4-ene-3,20-dione 21-acetate) was purchased from Farmabios (Gropello Cairoli, Italy) and kept in its original container. The tablets, corresponding to fludrocortisone acetate 55 µg tablets, were manufactured by Europhartech (France).

Ethanol, analytical grade, was purchased from Merck (Nogent-sur-Marne, France). Acetonitrile HPLC grade was purchased from Fluka-Riedel-de Haën (Saint-Quentin

Fallavier, France). Water was deionised and filtered through a Milli-Q water purification system from Millipore (Saint-Quentin-Yvelines, France). Microcrystalline cellulose was purchased from Rettenmaier France SARL (Saint-Quentin-Yvelines, France). Anhydrous colloidal silica was purchased from Degussa-Hüls (Courbevoie, France) and magnesium stearate was purchased from Peter Greven (Venlo, The Netherlands).

Packaging

Two container/closure systems were used in the stability studies. The first was a blister pack comprising 200 µm PVC, thermoplastic PVDC of grade 40 g/m² and of 20 µm aluminium foil. These packs were purchased from Cedulose (St Maur des Fossés, France). Packs were formed on the Noack DPN 760 blister packers; the plastic film was unwound from the reel and guided through a pre-heating station on the blister line. The temperature of the pre-heating plates (for the upper and lower plates this is fixed at about 120°C) was such that the plastic became soft and mouldable and cavities were then formed in a forming station using compressed air. Finally, the aluminium foil was heat-sealed at about 170°C.

The second blister material was a Formpack complex comprising 25 µm OPA, 45 µm aluminium and 60 µm 242 g/m² polyvinyl chloride (PVC) with a thickness of 0.13 mm, purchased from Lawson Mardon Charmettes SA (St Maur, France) and heat-sealed with a 20-µm aluminium complex that was also purchased from Lawson Mardon Charmettes SA. The Formpack blisters were formed on a WinPack TR130 blister packer by stretching the laminate mechanically with male dies and moulding the aluminium-plastic compound to form pockets. These were filled with the tablets and then directly sealed at about 180°C with the lidding foil, which is coated on the inside face with a sealing lacquer.

Chromatographic apparatus

The liquid chromatographic system comprised a Thermo Separation Product (TSP) P4000 pump, a TSP AS 3000 auto sampler equipped with a 20-µL loop injector and a TSP UV 2000 absorbance detector (Les Ulis, France). The HPLC system was piloted by PC 1000 software (TSP). The detection wavelength was set at 242 nm. The reversed-phase liquid chromatography was performed on an octadecyl grafted silica Nucleosil C18 4.6 mm-i.d. x 250-mm, 5 µm) stationary phase (Supelco, Saint-Quentin Fallavier, France).

The separation was carried out isocratically with a mobile phase comprising acetonitrile-deionised water (40/60, v/v).

The mobile phase was filtered under vacuum through a 0.45- μm GHP filter. The temperature of the automatic sampler is fixed at 10°C. All separations were performed at room temperature (20 \pm 2°C). Under these conditions and with a flow rate of 1.2 mL/minute, the retention time of fludrocortisone acetate was about 12 minutes.

Sample preparation

A calibration range with five concentration levels (1.76, 1.98, 2.20, 2.42 and 2.64 $\mu\text{g/mL}$) of fludrocortisone acetate was prepared extemporaneously and assayed immediately to prevent all potential degradations of drug substance. For each point of calibration, the appropriate quantity of drug substance was weighed and dissolved with ethanol, in 100-mL volumetric flasks. Fludrocortisone acetate solutions were prepared from the precedent solutions by taking 2 mL, in 50-mL volumetric flasks, and made up to the mark with mobile phase. Finally, fludrocortisone acetate test solutions were obtained by transferring 2.0 mL of the precedent solutions to 20-mL volumetric flasks.

Stability studies

Tablets containing 55 μg fludrocortisone acetate were stored at controlled temperature and relative humidity: 25 \pm 2°C/60% \pm 5% RH, and 40 \pm 2°C/75% \pm 5% RH (mean \pm range) for 36 months and six months, respectively. At each time point of the studies, 20 of these tablets were weighed and introduced into a 125-mL volumetric glass bottle. One hundred millilitres of mobile phase were added precisely to the volumetric glass bottle and the contents submitted to magnetic agitation. At the end of agitation, a homogenised suspension was obtained. From this suspension, 10 mL was transferred to a 20-mL glass centrifuge tube, and centrifuged at 3,500 rpm for 10 minutes. The supernatant was filtered through a 0.45- μm filter, and the first few millilitres were rejected. The final solution consisted of a dilution of 1/5 (v/v) of the supernatant with the mobile phase.

Determination of impurities

Determination of the impurities (synthesis and degradation products of fludrocortisone acetate) was performed against a blank of drug substance at 22 $\mu\text{g/mL}$ freshly prepared in ethanol. Four fludrocortisone acetate tablets and 10.0 mL of ethanol were added to a glass tube with a ground-glass stopper. Using a glass rod with a rounded tip, the tablets were crushed or broken down in order to obtain a homogeneous mixture. The solution was mixed on a vortex mixer for approximately 30 seconds. The solid and liquid phases were separated by centrifuging for 10 minutes at 3,500 rpm at 20°C. The supernatant was

filtered through an Acrodisc GHP Polypro filter with a mesh size of 0.45 μm (Gelman) or equivalent. The first millilitres were discarded (using glass syringes). The fludrocortisone acetate concentration of the solution thus prepared was about 0.0220 mg/mL. A standard solution of fludrocortisone was prepared in ethanol at a concentration of about 0.06 $\mu\text{g/mL}$ and the fludrocortisone content in the drug product then assayed.

Disintegration, hardness, visual appearance

Six dose units were randomly selected from the entire fludrocortisone acetate tablet stock and tested for disintegration in a water-bath at 37°C \pm 2°C, in accordance with the requirements of the European Pharmacopoeia method 2.9.1. (Test A) [12].

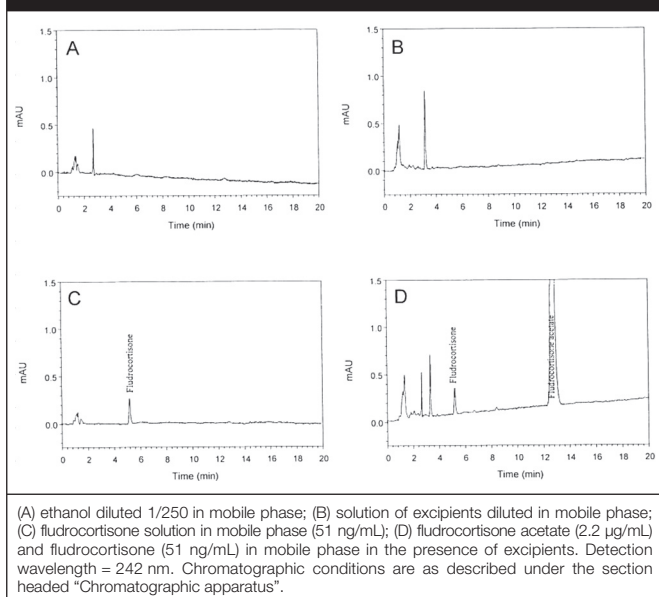
Hardness was determined on 10 tablets using an 8M tablet hardness tester purchased from Dr Schleuniger Pharmatron AG (Switzerland). Analyses were performed in accordance with method 2.9.8 of the European Pharmacopoeia [13]. Appearance was determined organoleptically on the received sample.

RESULTS AND DISCUSSION

Validation of the HPLC method

The analytical method was developed and validated in accordance with recommendations defined by International Conference on Harmonisation (ICH) guidelines [14] before applying it to the stability study. Specificity of the method was tested in the presence of excipients and all the potential impurities comprising related substances or degradation products. The method employed permitted a perfect separation between the main degradation products and fludrocortisone acetate with a resolution factor higher than 2.5. The retention time of fludrocortisone (hydrolysis product) and fludrocortisone acetate was 5.1 \pm 0.02 minutes and 12.7 \pm 0.02 minutes respectively (see Figure 2(C) and Figure 2(D)). The excipients (microcrystalline cellulose, colloidal anhydrous silica and magnesium stearate) did not interfere with the peak of fludrocortisone acetate (see Figure 2(B)). Five standards were prepared at concentrations in the range of 1.76-2.64 $\mu\text{g/mL}$ of fludrocortisone acetate. The linearity was checked on three consecutive days for the same concentration range from different stock solutions prepared in the presence or absence of excipients. The linearity was estimated by least-square regression analysis and accuracy by the determination of confidence interval for the average recovery rate calculated at a significance level of 5%. The intra-day precision (repeatability) was estimated from six determinations at 2.20 $\mu\text{g/mL}$ \pm 5%. The intermediate precision (between-day variability) of the method was

Figure 2: Chromatograms from the various analyses



evaluated by assaying freshly prepared solutions of fludrocortisone acetate at 2.20 µg/mL on three separate days. Once the homogeneity of the variances had been checked, the repeatability and intermediate precision coefficients of variation were calculated. Validation results are summarised in Table 1.

Stability studies of fludrocortisone acetate tablets

It is now well known that the stability of medicinal products is dependent on several chemical and physical factors. The choice and nature of the excipients present in the

tablet formulation can influence the stability of the active ingredient because of chemical interactions [15]. Environmental parameters such as temperature, moisture and light can be responsible for several processes of degradation. Fludrocortisone acetate degradation mechanisms have been partially reported in a previous paper [9]. Generation of the 21-hydroxyl analogue of fludrocortisone, among others, is expected as a result of hydrolysis of the acetate ester; it occurs in hydrolytic solvents and is favoured by high temperature and prolonged storage. Excipients with low residual humidity (<2%) were used because of the sensitivity of fludrocortisone acetate to moisture. The excipients used commercially in tablet formulations of fludrocortisone acetate drug are microcrystalline cellulose as diluent/disintegrant, colloidal anhydrous silica as glidant and magnesium stearate as lubricant. These excipients are widely used in direct compression for manufacturing pharmaceutical formulations. It is important to remember that the excipients used in the formulation are insoluble and chemically inert. However, the absence of degradation products from the excipients as well as fludrocortisone acetate impurities was checked. Requirements for stability studies are defined in several ICH guidelines and include storage conditions of 25°C/60% RH and 40°C/75% RH [16]. A stability study at 40°C/75% RH (accelerated conditions) for six months is required for ensuring the stability of a new drug product. The shelf-life of fludrocortisone acetate 55 µg tablets is defined as the time that the drug product, in specific conditions of storage, remains within specifications established in terms of content of active substance, quality and purity. It is estimated in accordance with ICH guideline Q1E [17].

Table 1: Summary of the validation results from the analytical methods used

	Validation parameters	FA alone	FA in presence of excipients
Linearity	Slope	39200.70	38215.32
	Y-intercept	-2096.60	-151.62
	Linearity R ² value	0.996	0.997
	Y-intercept comparison with 0 (Student's t-test)	1.342 (NS)	0.109 (NS)
	Comparison of ordinates at origin (Student's t-test)	0.931 (NS)	
	Comparison of slope (Student's t-test)	1.050 (NS)	
Accuracy	Confidence interval (0.95)	99.79 ± 0.49%	
Precision	Intra-day variability (n = 6)	% RSD	
	Day 1	1.8	
	Day 2	1.1	
	Day 3	1.2	
	Inter-day variability (n = 18)	1.4	

FA: fludrocortisone acetate drug substance; NS: not significant; RSD: relative standard deviation

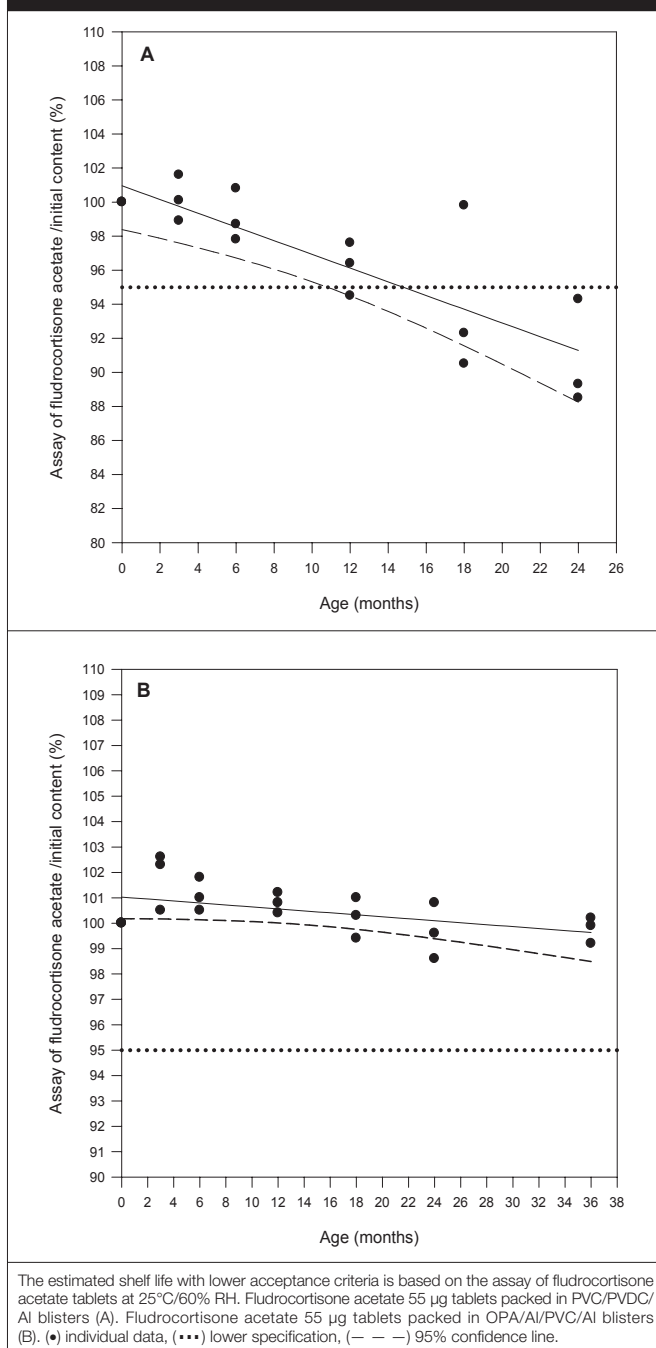
Many types of primary packaging for drug products are available on the market, with different degrees of protection. The choice of the primary packaging must take into account the sensitivity of the drug to light and moisture as well as available packaging facilities. In this study, two types of primary packaging were tested: PVC/PVDC/Al blister offering partial protection against water vapour, gases, and light; and OPA/Al/PVC/Al blister providing a better barrier to environmental stress as reported in the literature [18]. The study described here investigated three batches of fludrocortisone acetate tablets packed in PVC/PVDC/Al blisters and stored for 24 months at 25°C/60% RH and six months at 40°C/75% RH,

and three batches packed in OPA/Al/PVC/Al blisters and stored for 36 months at 25°C/60% RH and six months at 40°C/75% RH.

The expiry date of fludrocortisone acetate 55 µg tablets was estimated by regression analysis of the data by the least-squared method, which includes the calculation of a linear least-squared fit regression line and the 95% confidence limits of that which fitted the regression line in accordance with the methodology described in the literature [17]. If the slope of the fitted regression line is not significantly different from 0, the result indicates no meaningful change in fludrocortisone acetate tablet content. The expiry date information represents the predicted intersection of the lower 95% confidence limit calculated for the regression lines with the lower specification limit.

Figure 3 illustrates the results of regression analysis of the data. On each plot, the specification limits are represented by dotted lines. The data points are represented by black circles corresponding to individual data points. A regression line and a 95% confidence limit curve are also shown. The assay (fludrocortisone acetate content) data from stability testing of fludrocortisone acetate drug product packed in PVC/PVDC/Al blisters at 25°C/60% RH or in OPA/Al/PVC/Al at 25°C/60% RH storage conditions are shown in Figure 3(A) and Figure 3 (B), respectively. The slopes of the regression lines for all three batches packed in OPA/Al/PVC/Al blisters are not statistically different from zero (slope = 0, $p = 0.027$), indicating no meaningful change in the fludrocortisone acetate tablet content. Complementary to these results, no significant change in the quality of fludrocortisone acetate tablets for all batches tested was observed during stability studies regarding their visual appearance, disintegration (<1 minute), and hardness (% change of hardness between beginning (80 ± 10 N) and the ending values: $\approx 3-5\%$). In these conditions, the expiry date of the tablets cannot be predicted and is expected to be much longer than three years. On the contrary, for batches packed in PVC/PVDC/Al blisters, the regression analysis results in a predicted expiry after about 10 months ($p < 0.001$). Moreover, a downward drift of tablet hardness (% change of hardness between beginning: 80 ± 10 N and the ending values: $\approx 30-40\%$) without modification of disintegration properties (<1 minute) was observed in stability studies at 25°C/60% HR after 24 months for the batches tested. In accelerated conditions (40°C/75% RH), after storage for six months, the fludrocortisone acetate content remained higher than 96% for the tablets packed in OPA/Al/PVC/Al blisters and was about 65% for those packed in PVC/PVDC/Al blisters. These results

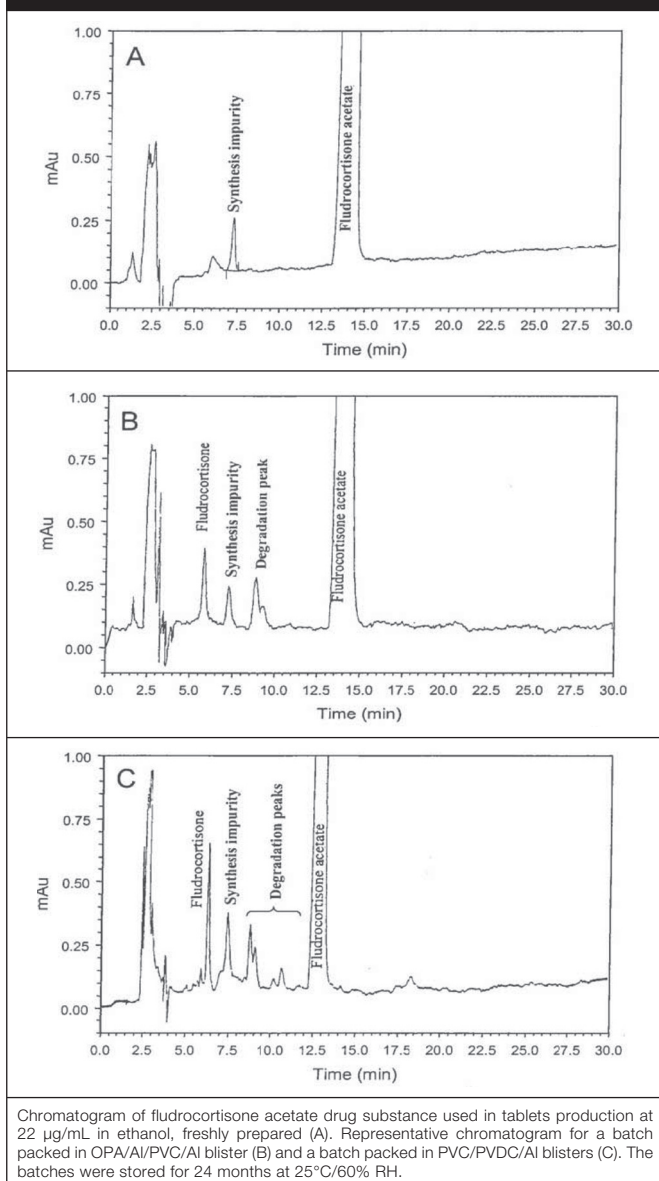
Figure 3: Estimated shelf life of fludrocortisone acetate tablets in the tested packaging



are in accordance with the moisture protection properties of OPA/Al/PVC/Al blisters compared with PVC/PVDC/Al blisters.

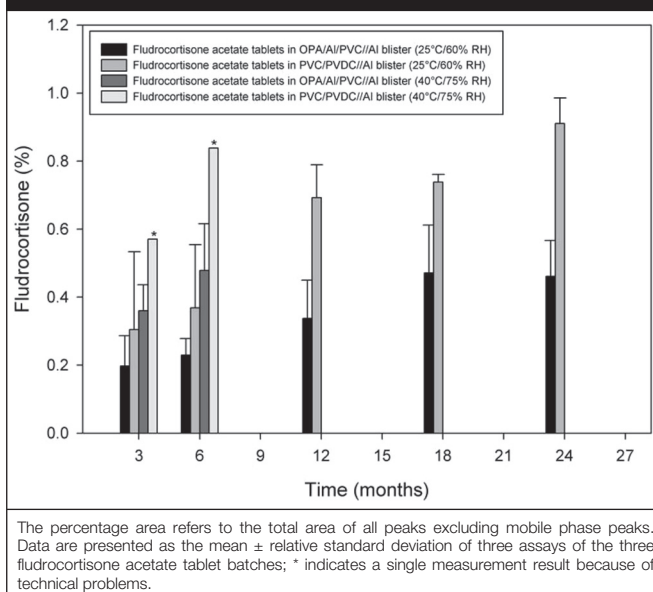
Additionally, the selectivity of the analytical method for impurities was confirmed through long-term and accelerated stability studies. Chromatograms (assay,

Figure 4: Representative chromatograms after storage for 24 months



impurities determination) obtained with fludrocortisone acetate 55 µg tablet batches packed in OPA/Al/PVC/Al blisters and PVC/PVDC/Al after 24 months at 25°C/60% RH are presented in Figure 4(B) and Figure 4(C), respectively. As shown in Figure 5, the results obtained show a significant increase in fludrocortisone after 24 months at 25°C/60% RH in samples in PVC/PVDC/Al blisters compared with samples in OPA/Al/PVC/Al blisters. Samples in PVC/PVDC/Al blisters also showed a dramatic increase in fludrocortisone in comparison with samples in OPA/Al/PVC/Al blisters after six months at 40°C/75% RH. It was concluded that the protection against moisture

Figure 5: Fludrocortisone content in the tested packaging under various conditions



offered by PVC/PVDC/Al blisters is not sufficient and that fludrocortisone acetate tablets need to be packed in OPA/Al/PVC/Al blisters.

CONCLUSION

Firstly, a stability-indicating assay HPLC method was developed to quantify fludrocortisone acetate during stability studies on fludrocortisone acetate 55 µg tablets. This method is simple, selective, sensitive and accurate.

Secondly, the stability of fludrocortisone acetate tablets in two types of primary packaging was studied. Fludrocortisone acetate drug substance is particularly sensitive to environmental conditions (e.g. residual humidity, oxidation and light) as indicated in the literature. Therefore, the packaging that was chosen for the study is used typically to protect pharmaceutical drug products from moisture and other environmental factors. In accelerated conditions (40°C/75% RH), after storage for six months, the content of fludrocortisone acetate remained higher than 96% for tablets packed in OPA/Al/PVC/Al blisters, whereas it was about 65% for those packed in PVC/PVDC/Al blisters. Results of these studies show that only blister packaging made from OPA/Al/PVC/Al gives sufficient protection against fludrocortisone acetate degradation. Fludrocortisone acetate 55 µg tablets are stable for at least three years when packed in OPA/Al/PVC/Al blisters.

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REFERENCES

1. Igarashi Y. Synthetic mineralocorticoid, clinical application of fludrocortisone acetate (Florinef). *Nippon Rinsho*. 1994;52(3):779-86.
2. Van der Kamp HJ, Otten BJ, Buitenweg NS, et al. Longitudinal analysis of growth and puberty in 21-hydroxylase deficiency patients. *Arch Dis Child*. 2002;87(2):139-44.
3. Løvås K, Husebye ES. Replacement therapy in Addison's disease. *Expert Opin Pharmacother*. 2003;4(12):2145-9.
4. Annane D. Glucocorticoids in the treatment of severe sepsis and septic shock. *Curr Opin Crit Care*. 2005;11(5):449-53.
5. Hopkala H, Przyborowski L. Determination of drugs containing fluorine with ion-selective electrode. Part 1: Synthetic glucocorticosteroids. *Pharmazie*. 1988;43(6):422-3.
6. Russo-Alesi, FM. An automated colorimetric method for the determination of steroids in single-tablet assays. *Ann NY Acad Sci*. 1968;153(2):511-24.
7. Ivashkiv E. Spectrophotometric methods for monitoring the microbial transformation of steroids. I. Determination of 9-alpha-fluorohydrocortisone and 9-alpha-fluoro-16-alpha-hydroxyhydrocortisone in fermentation broths. *Appl Microbiol*. 1970;20(2):251-3.
8. European Directorate for the Quality of Medicines & HealthCare. *European Pharmacopoeia*. 6th ed. Strasbourg: EDQM; 2009; (6.0), Monograph 0767. p.2061-2.
9. Ast TM, Abdou HM. Analysis of fludrocortisone acetate and its solid dosage forms by high-performance liquid chromatography. *J Pharm Sci*. 1979;68(4):421-3.
10. Cisternino S, Schlatter J, Saulnier JL. Stability of fludrocortisone acetate solutions prepared from tablets and powder. *Eur J Pharm Biopharm*. 2003;55(2):209-13.
11. Taylor RL, Grebe SK, Singh RJ. Quantitative highly sensitive liquid chromatography-tandem mass spectrometry method for detection of synthetic corticosteroids. *Clin Chem*. 2004;50(12):2345-52.
12. European Directorate for the Quality of Medicines & HealthCare. *European Pharmacopoeia*. 6th ed. Strasbourg: EDQM; 2009 (6.0). p.281-2.
13. European Directorate for the Quality of Medicines & HealthCare. *European Pharmacopoeia*. 6th ed. Strasbourg: EDQM; 2009 (6.0). p.298.
14. International Conference on Harmonisation. Q2 (R1). Validation of analytical procedures: text and methodology. Geneva: ICH; 1997 [cited 2009 June 1]. Available from: www.ich.org/LOB/media/MEDIA417.pdf
15. Serajuddin AT, Thakur AB, Ghoshal RN, et al. Selection of solid dosage form composition through drug-excipient compatibility testing. *J Pharm Sci*. 1999;88(7):696-704.
16. International Conference on Harmonisation. Q1A (R2). Stability testing of new drug substances and products. Geneva: ICH; 2003 [cited 2009 June 1]. Available from: <http://www.ich.org/LOB/media/MEDIA419.pdf>
17. International Conference on Harmonisation. Q1E. Evaluation for stability data. Geneva: ICH; 2004 [cited 2009 June 1]. Available from: www.ich.org/LOB/media/MEDIA415.pdf
18. Lusina M, Cindrić T, Tomać J, et al. Stability study of losartan/hydrochlorothiazide tablets. *Int J Pharm*. 2005; 291(1-2):127-37.