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Research paper

Stability of fludrocortisone acetate solutions prepared from tablets and powder

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Abstract

To assess the stability of fludrocortisone acetate oral solutions prepared from tablets and powder at three temperatures over a 60-days period. Solutions of fludrocortisone acetate 40 μg/ml were prepared from commercially available 0.05-mg tablets and powder in ethanol 17% v/v. They stored in an amber glass prescription bottles at +4, +23 and +40°C shielded from light. The concentrations of fludrocortisone acetate were determined in duplicate by high-performance liquid chromatography at 0, 1, 7, 14, 30, 50 and 60 days. The initial and final pH of solutions were compared. The recovery of fludrocortisone acetate from tablets was determined. The times (t_{90}) needed for fludrocortisone acetate to fall to 90% of it's initial concentration were calculated by a linear regression analysis to allow the determination of the expired dates. The recovery of fludrocortisone acetate from tablets was 78 ± 3%. The t_{90} expressed with 95% confidence limits were 2 ± 1 and 22 ± 3 days for the solutions prepared from tablets and stored at +23 and +4°C, respectively, whereas t_{90} were 11 ± 2 days and at least 60 days for the solutions prepared with the powder and stored at +23 and +4°C, respectively. No color or odour changes were observed during the study period. The initial pH of the solutions prepared from tablets and powder were 7.7 and 6.9, respectively. No change of pH values was observed at the end of the 60 days. Significant degradation of fludrocortisone acetate occurred in formulations stored at +23°C. Fludrocortisone acetate 40 μg/ml solutions prepared from tablets and powder were stable 19 days and at least 60 days, respectively, when stored at +4°C. The solution prepared from powder is the best in term of stability and final concentration which is independent on the fludrocortisone acetate recovery.

Keywords: Fludrocortisone acetate; Stability; Oral solutions

1. Introduction

The destruction of the adrenal cortex cause adrenal dysfunction which is characterized by reduction in cortisol secretion, variable decrease in aldosterone secretion and a secondary increase in adreno-cortico-trophic-hormone (ACTH)-stimulated androgen production. The principal cause of adrenal destruction is autoimmune adrenalitis [1]. In the acquired immunodeficiency syndrome, the adrenal gland may be also destroyed by a variety of opportunistic infections [2,3]. However, congenital adrenal hyperplasia is the most common primary adrenal dysfunction in childhood [4]. In all cases, treatment includes hormonal replacement therapy with glucocorticoids as hydrocortisone. Patients should also receive life-long fludrocortisone replacement, in a single daily dose of 50–200 μg, as a substitution for aldosterone [4,5]. Fludrocortisone acetate is commercially available in compressed tablets only. A liquid dosage form would be highly desirable for patients with swallowing problems. In addition, an oral liquid form allows the dose to be easily adjusted. For very young children, usually tablets are crushed into powder to be mixed with food vehicles. This practice can not assure a good dissolution and hence therapeutic activity of fludrocortisone acetate. An oral solution would be more acceptable for pediatric patients. Because of the lack of published data on the stability of fludrocortisone acetate in oral liquid dosage form, we designed a study to determine the stability of the drug in solution prepared from commercially available tablets and powder at three controlled temperatures.

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2. Materials and methods

2.1. Materials

Fludrocortisone acetate powder and norethindrone (internal standard) were of analytical grade (Sigma, St Quentin, France, lot 55H11161 and lot 26H0455, respectively). Fludrocortisone acetate 0.05-mg tablets were manufactured by PCH (Paris, France, lot 89022). They were consisted of calcium hydrogenophosphate, magnesium stearate and corn starch. Sterile water for irrigation was obtained from Fresenius (Sèvres, France, lot 0146/36). Ethanol 90% v/v was obtained from Rhône-Poulenc-Rorer (Melun, France, lot A2943790/2). Acetonitrile and tetrahydrofuran were HPLC grade (Chromanorm®, Prolabo, Paris, France).

2.2. Formulations of solutions

The solution of fludrocortisone acetate 40 μg/ml was prepared by crushing 100 tablets of 0.05-mg fludrocortisone acetate using a glass mortar and pistil. Due to the incomplete recovery of fludrocortisone acetate from tablets, extra tablets were used to obtain a concentration of 40 μg/ml instead of the expected concentration of 50 μg/ml. Twenty ml of ethanol 90% v/v were added and the mixture was triturated to make a paste. After 30 min, 80 ml of sterile water were added and mixed for 20 min. The milky suspension was filtered through a 7-μm filter paper (Dumas®, Prolabo) to have a clear solution which was transferred in nine amber glass prescription bottles. The recovery of fludrocortisone acetate obtained from compressed tablets was also studied from five solutions prepared independently. Another solution of fludrocortisone acetate 40 μg/ml was prepared with fludrocortisone acetate powder. A sufficient quantity was weighed and mixed in a volumetric flask first with ethanol 90% and then with water in the same proportion. The solution was also transferred in nine amber glass prescription bottles. Both solutions obtained from tablets and powder were formulated with a final ethanol concentration of 17% v/v.

2.3. Storage of solutions

Three bottles from each formulation were stored in a dark place at the following temperatures: 4 ± 2, 23 ± 3 and 40 ± 3°C for the forced degradation.

2.4. Sampling

From each bottles a 50 μl samples were taken and diluted in 10 ml of mobile phase to be assayed in duplicate by high-performance liquid chromatography, immediately after preparation, 1, 7, 14, 30, 50 and 60 days. The physical appearance of the samples was assessed by visual observation against a black using a white backgrounds under normal light. The odor of each bottle was compared to a vehicle solution (ethanol 17%) stored at the same selected conditions. The apparent pH of each sample was measured by digital pH meter (Model 93313 pH meter Bioblock scientific, Ilkirch, France) at the beginning and at the end of the study.

2.5. HPLC analysis

Fludrocortisone acetate was quantified by the adapted Schrive et al. HPLC method [6]. The method required a liquid chromatograph with the ultraviolet light detector (LC spectrophotometer, Waters, Millford, MA, USA) set at a wavelength of 238 nm, an injector set (Rheodyne valve Model 7125, Touzart and Matignon, Vitry sur Seine, France) to deliver 100 μl, a recording integrator (Data Module 745, Waters) and a C18 reverse phase column (Novapack® C18 column, 4-μm, 15 cm, Waters) running at ambient temperature. The mobile phase pumped at a rate of 0.8 ml/min consisted of water, acetonitrile and tetrahydrofuran (60:33:7 v/v/v). The mobile phase was filtered and degassed with ultrasonic bath. The retention times for fludrocortisone and norethindrone were 5.1 and 6.5 min, respectively (Fig. 1).

A 1 mg/ml fludrocortisone acetate reference standard and a 1 mg/ml norethindrone stock solutions were prepared in ethanol 90% v/v and stored at +4°C. Calibration curves were performed with standard solutions diluted in mobile phase to yield concentrations of fludrocortisone acetate to 300, 250, 100, 50 and 25 ng/ml with 200 ng/ml of internal standard. The standard curve was constructed by plotting the peak-height ratio of fludrocortisone acetate to norethindrone against the fludrocortisone acetate concentration and was used for calculating the drug concentrations of the samples. In order to establish the stability-indicating nature of the assay, fludrocortisone acetate solutions obtained from powder and tablets were stored at +40°C until the chromatographic peak was not detected. We showed that a degradation product was eluted at 3.1 min without interfering with fludrocortisone and internal standard peaks (Fig. 1). The same degradation product peak was found in the stability studies for each formulation whatever the temperature conditions. With this HPLC method the quantification of fludrocortisone acetate was not influenced by degradation product.

2.6. Data analysis

For each formulation, mean concentrations for each time were determined and converted to percentages of initial concentration as the Anaizi et al. method [7]. The data were analyzed by linear regression analysis. The time t_{90} which represents a 90% of the initial concentration for the degradation of fludrocortisone acetate solutions was calculated from the linear regression analysis with 95%

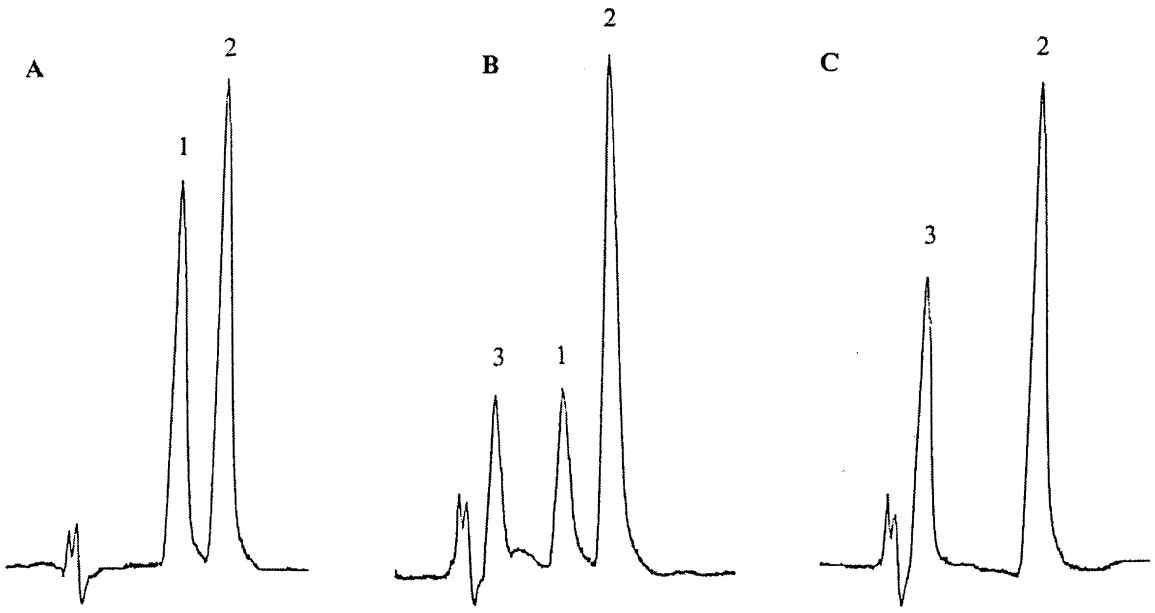

Fig. 1. Chromatograms of fludrocortisone acetate solutions, not degraded (A); partially degraded (B); and totally degraded (C). Peak 1: fludrocortisone acetate 200 ng/ml (retention time = 5.1 min), peak 2: internal standard (retention time = 6.5 min), peak 3: product of degradation (retention time = 3.1 min).

confidence limits (CL). The concentration for lower CL corresponded to that achieved on the expire date.

3. Results

The straight line of linear regression of fludrocortisone acetate HPLC assay was $y = 0.0098x + 0.00363$ (± 0.00015). The limits of detection and quantification of fludrocortisone acetate were 18 and 25 ng/ml, respectively (signal/noise = 3:1). The coefficient of correlation was of 0.998 ± 0.001. Intra-day and inter-day variations were 1.9% ($n = 10$) and 2.5% ($n = 5$), respectively. The recovery of fludrocortisone acetate from compressed tablets was of 78 ± 3% ($n = 5$) which explained the final concentration of 40 μg/ml in the solutions. The apparent initial pHs were significantly different between solutions prepared from tablets and those prepared from the powder, 7.7 and 6.9, respectively. No difference was found between initial and final pH values at the end of the study. Likewise, visual and organoleptics observations did not reveal any changes during the study period. Percentages of initial concentration of fludrocortisone acetate are represented in Table 1. Figs. 2 and 3 represented the fludrocortisone acetate degradation

Table 1
Stability of fludrocortisone acetate 40 μg/ml solutions from tablets and pure powder at +4, +23 and +40°C

Day	% Initial concentration remaining[a]					
	+4°C		+23°C		+40°C	
	Tablets	Powder	Tablets	Powder	Tablets	Powder
0	100.0 ± 1.6[b]	100.0 ± 1.8[c]	100.0 ± 1.7[d]	100.0 ± 1.7[e]	100.0 ± 1.6[f]	100.0 ± 1.7[g]
1	98.6 ± 1.9	100.0 ± 1.8	93.1 ± 1.9	98.7 ± 1.8	68.1 ± 1.4	78.3 ± 1.8
7	95.9 ± 1.7	100.0 ± 1.5	76.4 ± 1.8	91.7 ± 1.3	10.5 ± 1.7	32.0 ± 1.7
14	91.7 ± 1.8	98.8 ± 1.5	62.5 ± 1.4	85.9 ± 1.9	<LOD[h]	<LOD[h]
30	87.5 ± 1.4	97.4 ± 1.3	44.4 ± 1.9	78.2 ± 1.3		
50	80.6 ± 1.4	97.4 ± 1.3	27.8 ± 1.6	69.2 ± 1.3		
60	75.0 ± 1.4	97.4 ± 1.7	18.1 ± 1.2	60.3 ± 1.6		

[a] Reported as mean ± SD of duplicate determinations for three samples.
[b] The actual mean ± SD initial concentration was 38.1 ± 0.6 μg/ml, and the initial apparent pH was 7.7.
[c] The actual mean ± SD initial concentration was 40.6 ± 0.7 μg/ml, and the initial apparent pH was 6.9.
[d] The actual mean ± SD initial concentration was 38.2 ± 0.6 μg/ml, and the initial apparent pH was 7.7.
[e] The actual mean ± SD initial concentration was 40.5 ± 0.7 μg/ml, and the initial apparent pH was 6.9.
[f] The actual mean ± SD initial concentration was 38.1 ± 0.6 μg/ml, and the initial apparent pH was 7.7.
[g] The actual mean ± SD initial concentration was 40.6 ± 0.7 μg/ml, and the initial apparent pH was 6.9.
[h] Limit of detection.

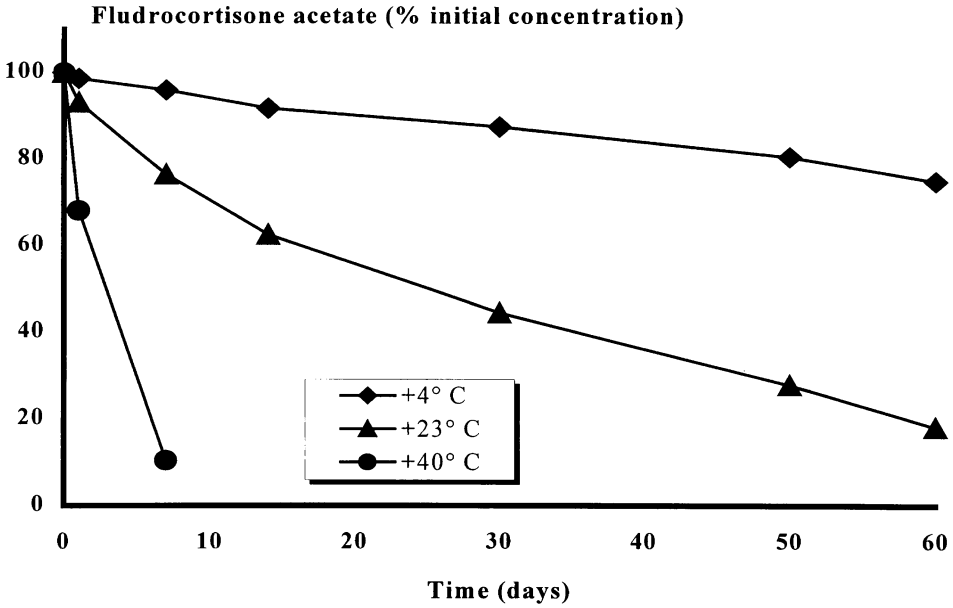

Fig. 2. Stability of fludrocortisone acetate solution prepared from tablets at +4, +23 and +40°C. Standard deviations were lower than represented symbols.

curves at the three temperatures chosen. Kinetic analysis showed that the order of fludrocortisone acetate degradation fit a first order kinetic model ($C = C_0 \exp(-kt)$) with C_0 values at 38.2, 39.3, 39.4 μg/ml; and k values at 0.0118, 0.0042, 0.0017 for tablets and powder solutions stored at 23°C and tablets solution at 4°C, respectively. As shown for tablets fludrocortisone acetate solutions, a 20°C temperature increase of the reaction led to a ~3-fold higher degradation rate. The t_{90} values with 95% CL were 2 days (1–3) at +23°C and 22 days (19–25) at +4°C for the solution prepared from tablets. The t_{90} values for the solution prepared from the powder were 11 days (9–13) at +23°C and at least 60 days at +4°C. A product of degradation was observed in 3.1 min with the forced degradation at +40°C which did not interfere with the other peaks. For every solution and whatever are the stocking conditions, the same degradation product peak was detected during the study which did not correspond to the hydrocortisone peak.

4. Discussion

The poor recovery of fludrocortisone acetate was related to its very low water solubility. Like numerous steroids,

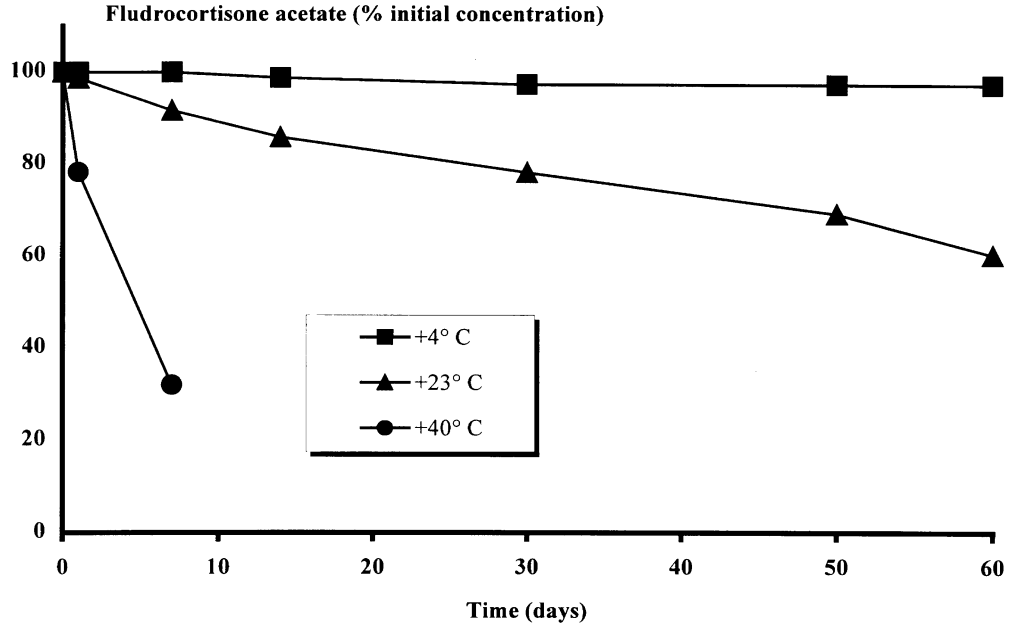

Fig. 3. Stability of fludrocortisone acetate solution prepared from powder at +4, +23 and +40°C. Standard deviations were lower than represented symbols.

fludrocortisone acetate was known to be practically insoluble in water (0.04 mg/ml) and soluble in ethanol (20 mg/ml) [8,9]. Thus, addition of ethanol increase the solubility of fludrocortisone acetate from tablets. Moreover, it was known that ethanol plays a preservative role at a concentration above 15% and whatever pH range [10] avoiding addition of preservative parabens. No other additives like propylene glycol were used to increase fludrocortisone acetate recovery from tablets that appeared useless for the liquid form prepared from pure powder. However, ethanol is well known for its potential adverse effects. Guidelines for its inclusion in liquid pharmaceutical have been laid down by American Academy of Pediatrics [11]. A single dose of drug that gives a blood ethanol level not exceeding 25 mg/100 ml can be considered acceptable. The blood ethanol concentration achieved with a single dose of this preparation was estimated. Infants with weight less than 3 kg can reached the limit of blood ethanol level after maximal fludrocortisone acetate dose of 200 μg. The chronic administration of the solution should be discussed notably for the very young children. Solutions prepared from tablets are less stable than those prepared from powder. Excipients and pH of the solution prepared from tablets could explain this difference in stability. It was shown for other steroids that the rate of instability was four to five times lower at pH 6.9–7.9 than that observed at pH 9.1 [12]. Taking into consideration that only 0.8-pH difference was observed between solutions, the real impact of this factor on the chemical stability could not be established.

5. Conclusion

Fludrocortisone acetate 40 μg/ml oral solutions prepared from 0.05-mg tablets was stable 19 days when stored at +4°C whereas those prepared from the pure powder was stable at least 60 days at +4°C. Variation in fludrocortisone acetate recovery from tablets and lower stability of this formulation suggest that oral solution made with pure powder are to be preferred. This oral solution appear to be an alternative to the administration of tablets for the pediatric patients or those unable to swallow dry forms.

References

[1] W. Oelkers, Adrenal insufficiency, N. Engl. J. Med. 335 (1996) 1206–1212.
[2] P.-U. Freda, S.-L. Wardlaw, K. Bradney, R.-S. Goland, Primary adrenal insufficiency in patients with the acquired immunodeficiency syndrome: has adjournment (transfer) of five accommodated, J. Clin. Endocrinol. Metab. 79 (1994) 1540–1545.
[3] O.-F. Sellmeyer, C. Grunfeld, Endocrine and metabolic disturbances in human immunodeficiency virus infection and the acquired immune deficiency syndrome, Endocr. Rev. 17 (1996) 518–532.
[4] I.-A. Hughes, A. Wilton, C. Lole, O.-P. Gray, Continuing need for mineralocorticoid therapy in salt-losing congenital adrenal hyperplasia, Arch. Dis. Child. 54 (1979) 350–355.
[5] Y.-J. Lim, J.-A. Batch, G.-L. Warne, Adrenal 21-hydroxylase deficiency in childhood: 25 years' experience, J. Paediatr. Child. Health 31 (1995) 222–227.
[6] I. Schrive, M. Dusseau, M. Freiermuth, J.-C. Plasse, Simple method for quantification of fludrocortisone and hydrocortisone in pharmaceutical forms, J. Pharm. Clin. 13 (1994) 45–48.
[7] H. Anaizi, C. Swenson, Instability of aqueous captopril solutions, Am. J. Hosp. Pharm. 50 (1993) 486–488.
[8] Fludrocortisone acetate, in: J.E.F. Reynolds (Ed.), Martindale: The Extra Pharmacopoeia, The Pharmaceutical Press, London, 1993.
[9] Fludrocortisone acetate, in: P.G. Stecher (Ed.), The Merck Index, Merck and Co. Inc, New Jersey, 1989.
[10] H.-C. Ansel, L.-V. Allen, N.-G. Popovich, Dosage form design, Pharmaceutical Dosage Forms and Drug Delivery Systems, Lippincott Williams and Wilkins, Philadelphia, 1999.
[11] American Academy of Pediatrics, Ethanol in liquid preparations intended for children, Pediatrics 73 (1984) 405–407.
[12] C. Monder, Stability of corticosteroids in aqueous solutions, Endocrinology 82 (1968) 318–326.