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Assay of Some Adreno-corticosteroid Drugs with Pyridinium Fluorochromate Reagent

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ABSTRACT

In the present paper, we have reported a simple and convenient titrimetric method for determination of adreno-corticosteroid drugs e.g. betamethasone sodium phosphate, dexamethasone sodium phosphate, hydrocortisone sodium succinate, prednisolone sodium succinate and triamcinolone in pure form and in their pharmaceutical preparations such as betnesol (Inj), cortil (Tab), decdon (Inj), dexasone (Tab), Primacprt (Inj), wycort (Inj), wysolone (Tab), predone forte (Syrup), tricort (Inj) and ledercort (Tab) with pyridinium fluorochromate (PFC) reagent. It is a versatile oxidizing agent of chromium (VI) and it is being increasingly used as an oxidant for several classes of organic compounds. The value of percentage error, coefficient of variation (CV) and standard deviation (SD) prove the method to be precise and reproducible. To establish authenticity of the method, recovery experiments were also carried out by standard drug addition method. It was noted that the excipients present in pharmaceutical preparations do not interfere in the estimation.

Key words: Adreno-corticosteroid drugs, pharmaceutical preparations, pyridinium fluorochromate, oxidizing agent, titration.

INTRODUCTION

A variety of compounds containing chromium (VI) have proved to be versatile oxidizing reagents[1]. Extensive work has led to the development of a good number of these oxidants such as the Collins reagent[2], chromium trioxide-3,5-dimethylpyrazole complex[3], pyridinium chlorochromate[4], pyridinium dichromate[5], 2,2'-bipyridinium chlorochromate[6], pyridinium fluorochromate[7,8], quinolinium fluorochromate[9],quinolinium chlorochromate[10], 3,5-dimethyl pyrazolium fluorochromate[11], 2,6-dicarboxypyridinium chlorochromate[12,13], N-methylpiperidinium chlorochromate[14], tetramethylammonium fluorochromate[15] and benzyltrimethylammonium fluorochromate[16].

Industrial demand has lead many workers to search for more ideal oxidants with a number of specifications including: lower cost, higher yields, better selectivity, easier preparations, high solubility, less toxicity and short reaction times. Among the above mentioned reagents[2-16], PFC has an edge over others for rendering higher yields. In the present manuscript we have used pyridinium fluorochromate (PFC) as an oxidizing reagent, it has been synthesized in our laboratory by reported method[17].

Adrenocorticoids are synthesized in the cortex (shell) of adrenal glands. They are named as glucocorticoids (hydrocortisol, cortisone) and mineralocorticoids (aldosterone) on the basis of their activity. Mineralocorticoids control salt and water balance in the renal tubes whereas glucocorticoids regulate biosynthesis and metabolism of carbohydrates, proteins and lipids. Addison's disease, Cushing's disease and Conn's syndrome are pathologic conditions related to adrenal cortex and the hormones produced in them. Importance of adrenocorticoids is most dramatically observed in adrenalectomized animals. There is an increase of urea in blood, muscle weakness, decreased liver glycogen and decreased resistance to insulin, lowered resistance to trauma (cold, chemical shock, glucocorticoid activity) and electrolyte disturbances (mineralocorticoid activity). Betamethasone and Dexamethasone are usually used as an anti-inflammatory and immunosuppressants. Hydrocortisone is mostly taken in the sensitive case of inflammation, allergy, collagen disease, asthma, adrenocortical deficiency, shock and some neoplastic conditions. Predinosolone is used for the treatment of a wide range of inflammatory and auto-immune conditions such as asthma, uveitis, rheumatoid arthritis, ulcerative colitis and Crohn's disease whereas Triamcinolone is mostly used for the treatment of hayfever.

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MATERIALS AND METHODS

To prepare the solution of pyridinium fluorochromate (0.03N), 0.495 g of pyridinium fluorochromate was dissolved in 150 mL of glacial acetic acid (Merck) and made up to the volume with distilled water in 250 mL volumetric flask. The solution was standardized iodometrically.

A Stock solution of sodium thiosulphate (0.01N) was prepared by dissolving 2.481 g of sodium thiosulphate in distilled water in 1000 mL volumetric flask and made up to the mark with distilled water. The

solution was standardized by 0.01N potassium dichromate solution iodometrically.

For the preparation of Potassium dichromate solution (0.01N), 0.245 g of $K_2Cr_2O_7$ was dissolved in distilled water in 500 mL volumetric flask. 10% w/v aqueous solution Potassium iodide (Baker analysed reagent) was prepared in distilled water.

1% w/v aqueous solution of starch was prepared in distilled water. For preparation of sample solution, accurately weighed 100 mg pure samples as well as pharmaceutical samples of betamethasone sodium phosphate, dexamethasone sodium phosphate, hydrocortisone sodium succinate, prednisolone and sodium succinate were dissolved in minimum amount of distilled water and triamcinolone in glacial acetic acid. After getting a clear solution the solution was made up to the mark with distilled water. While diluting with distilled water every care was taken to keep the solution homogeneous.

To prepare tablet solution, twenty tablets of pharmaceutical products were crushed to a fine powder. The powder equivalent to 100 mg of sample was taken in 100 mL calibrated volumetric flask and dissolved in the same way as described for the pure sample.

Injections equivalent to 100 mg of the pure sample were taken and dissolved in distilled water in 100 mL volumetric flask to get a concentration of 1 mg/mL.

Aliquots containing 1-5 mg of the samples were taken in 100 mL stoppered conical flask and 5 mL of 0.03N pyridinium fluorochromate reagent and 10 mL of 5N H_2SO_4 was added to it. The reaction mixture was shaken thoroughly and allowed to react for required reaction time e.g. for betamethasone sodium phosphate, dexamethasone sodium phosphate, hydrocortisone sodium succinate, prednisolone sodium succinate, 10 minutes whereas triamcinolone for 15 minutes at room temperature (25-30°C). After the reaction was over 5 mL of 10% KI solution was added to it and contents shaken thoroughly and allowed to stand for one minute. The liberated iodine was titrated with 0.01N sodium thiosulphate using starch as indicator. A blank experiment was also run under identical conditions using all the reagents except the sample. The amount of the sample recovered was calculated by the difference in the titre values of sodium thiosulphate solution for blank and actual experiment.

For each experiment, the amount of the compound was calculated by following expression

$$\text{Weight (mg) of sample} = \frac{M \times N(B-S)}{n}$$

Where, M = Molecular weight of the sample, N = Normality of sodium thiosulphate solution, B = Volume of sodium thiosulphate solution for blank, S = Volume of sodium thiosulphate solution for sample, n = Stoichiometry of the reaction.

For testing quantitative validity of the recommended method, standard deviation (SD) and coefficient of variation (CV) were also calculated for each sample size. At least nine determinations were carried out and the results were noted. The determinations were done with varying sample size (i.e. 1-10 mg) but for convenience, results have been shown only with 1, 3 and 5 mg sample size (Table-1). For every sample of adrenocorticosteroids e.g. betamethasone sodium phosphate, dexamethasone sodium phosphate, hydrocortisone sodium succinate, prednisolone sodium succinate and triamcinolone, recovery experiments were done by the Standard drug-addition method (Table-2-6).

A known amount of the pure compound was taken and to this, varying amounts of the pharmaceutical preparations of the same compound were added. The total amount of the sample was found by following expression:

$$\% \text{ Recovery} = \frac{N(\sum XY) - (\sum X)(\sum Y)}{N(\sum X^2) - (\sum X)^2} \times 100$$

Where, N = Σ N = Total number of observations, X = Amount of drug added, Y = Amount of drug obtained by calculation, ΣX = ΣNX, ΣY = ΣNY, ΣXY = Σ (NX) (Y), ΣX^2 = Σ (NX) (X).

RESULTS AND DISCUSSION

It was found that the stoichiometric ratio between PFC reagent and adreno-corticosteroids varies. Betamethasone sodium phosphate (1:4), dexamethasone sodium phosphate (1:2), hydrocortisone sodium succinate (1:4), prednisolone sodium succinate (1:4) and triamcinolone (1:2) in pure form and in their pharmaceutical preparations. The ratio remains constant even under varying reaction conditions i.e. change in reaction time, concentration of the reagent and temperature etc. It was observed that all the adreno-coticosteroid drugs studied need 10 minutes to complete the reaction except triamcinolone which needs 15 minutes. All the compounds at more than the described reaction time (10-15 min.) do not give any improvement in the results. A lesser reaction time (below 10 min) gives higher percentage of error because of incomplete reaction. A variation of concentration of pyridinium fluorochromate reagent was also studied and it was found that the recommended concentration (0.03N) was suitable for accurate and concordant results.

The effect of sulphuric acid reaction was also studied and it was found that in the absence of acid the reaction was very slow. At very low concentration of sulphuric acid (1-4 N), it gives very slow reaction whereas higher concentrated acid (5-10 N) has no improvement over the results. The next variation to be studied was reaction temperature. The reactivity of the sample was very slow at ice cold temperature but increases with the rise in temperature up to room temperature (25-30^{0}C). Beyond this temperature (35-95^{0}C) there was no improvement over the results. There is no equivalent method reported in Indian phamacopoeia[18]. Here we describe determination of adrenocarticosteroid drugs.

Table-1: Milligram determination of some adrenocorticosteroids (pure sample) and its pharmaceutical preparations with pfc reagent in acidic medium

S. No.	Sample	Aliquots Taken (mL)	Amount present* (mg)	Reaction time (min.)	Molecularity	Amount obtained by calculation** (mg)	Error (%)	SD	CV
1	Betamethasone sodium	1.00	0.972	10	4	0.962	-1.03	0.0026	0.2703
	phosphate (Pure)	3.00	2.916	10	4	2.893	-0.79	0.0035	0.1210
		5.00	4.860	10	4	4.838	-0.45	0.0022	0.0455
A	Betnesol (Inj)GSK	1.00	0.943	10	4	0.933	-1.06	0.0041	0.4394
		3.00	2.829	10	4	2.806	-0.81	0.0025	0.0891
		5.00	4.715	10	4	4.695	-0.42	0.0025	0.0532
B	Cortil (Tab)Micro Labs	1.00	0.953	10	4	0.943	-1.05	0.0038	0.4030
		3.00	2.859	10	4	2.839	-0.71	0.0021	0.0740
		5.00	4.765	10	4	4.747	-0.38	0.0022	0.0463
2	Dexamethasone sodium	1.00	0.996	10	2	0.986	-1.00	0.0015	0.1521
	phosphate (Pure)	3.00	2.988	10	2	2.968	-0.67	0.0017	0.0573
		5.00	4.950	10	2	4.960	-0.40	0.0022	0.0444
A	Decdon (Inj) Wookhardt	1.00	0.937	10	2	0.927	-1.08	0.0025	0.2697
		3.00	2.811	10	2	2.790	-0.75	0.0030	0.1075
		5.00	4.685	10	2	4.670	-0.32	0.0019	0.0407
B	Dexasone (Tab) Cadila	1.00	0.948	10	2	0.938	-1.05	0.0019	0.2026
		3.00	2.844	10	2	2.826	-0.63	0.0030	0.1063
		5.00	4.740	10	2	4.722	-0.38	0.0025	0.0529
3	Hydrocortisone sodium	1.00	0.934	10	4	0.924	-1.07	0.0023	0.2489
	succinate (pure)	3.00	2.802	10	4	2.785	-0.61	0.0028	0.1005
		5.00	4.670	10	4	4.656	-0.30	0.0025	0.0537
A	Primacort (Inj) Macleods	1.00	0.893	10	4	0.884	-1.01	0.0025	0.2828
		3.00	2.679	10	4	2.660	-0.71	0.0028	0.1053
		5.00	4.465	10	4	4.446	-0.43	0.0030	0.0675
B	Wycort (Inj) Wyeth	1.00	0.889	10	4	0.880	-1.01	0.0022	0.2500
		3.00	2.667	10	4	2.650	-0.64	0.0030	0.1132
		1.00	0.893	10	4	0.884	-1.01	0.0025	0.2828
4	Prednisolone sodium	1.00	0.958	10	4	0.948	-1.04	0.0031	0.3270
	succinate (pure)	3.00	2.874	10	4	2.851	-0.80	0.0026	0.0912
		5.00	4.790	10	4	4.763	-0.56	0.0027	0.0567
A	Wysolone (Tab) Wyeth	1.00	0.919	10	4	0.909	-1.09	0.0014	0.1540
		3.00	2.757	10	4	2.734	-0.83	0.0011	0.0402
		5.00	4.595	10	4	4.574	-0.46	0.0013	0.0284
B	Predone Forte (Syrup) Cipla	1.00	0.903	10	4	0.894	-1.00	0.0032	0.3579
		3.00	2.709	10	4	2.689	-0.74	0.0026	0.0967
		5.00	4.515	10	4	4.492	-0.51	0.0020	0.0445
5	Triamcinolone(pure)	1.00	0.937	15	2	0.930	-0.96	0.0024	0.2581
		3.00	2.817	15	2	2.798	-0.67	0.0021	0.0751
		5.00	4.695	15	2	4.675	-0.43	0.0023	0.0492
A	Tricort (Inj)Cadila	1.00	0.907	15	2	0.898	-0.99	0.0024	0.2673
		3.00	2.721	15	2	2.701	-0.74	0.0025	0.0926
		5.00	4.535	15	2	4.511	-0.53	0.0030	0.0665
B	Ledercort (Tab) Wyeth	1.00	0.896	15	2	0.887	-1.00	0.0029	0.3269
		3.00	2.688	15	2	2.669	-0.71	0.0019	0.0712
		5.00	4.480	15	2	4.457	-0.51	0.0026	0.0583

*Tab = Tablet, Inj = Injection, * In each sample nine estimations were done, ** Average of nine determinations*

Table-2: Recovery studies of betamethasone sodium phosphate by standard drug addition method

S.No.	No. of observations (N)	Amount present (Pure) (mg)	Amount of drug added (mg) X	Total amount of drug obtained by calculation (mg)	Amount of drug obtained by calculation (mg)Y	XY	X^2	Recovery (%)
1	3	0.972	0.986	1.978	0.970	0.956	0.972	99.45
2	3	0.972	1.978	2.984	1.984	3.924	3.912	
3	3	0.972	2.984	3.938	2.951	8.806	8.904	
4	3	0.972	3.978	4.964	3.958	15.745	15.824	
	ΣN=12		ΣX=9.935		ΣY=9.863	ΣXY=29.435	ΣX^2=29.612	

Table-3: Recovery studies of dexamethasone sodium phosphate by standard drug addition method

S.No.	No. of observations (N)	Amount present (Pure) (mg)	Amount of drug added (mg) X	Total amount of drug obtained by calculation (mg)	Amount of drug obtained by calculation (mg)Y	XY	X^2	Recovery (%)
1	3	0.996	0.977	1.970	0.975	0.953	0.955	99.14
2	3	0.996	1.980	2.966	1.982	3.924	3.920	
3	3	0.996	2.981	3.982	2.961	8.827	8.886	
4	3	0.996	3.979	4.985	3.962	15.765	15.832	
	ΣN=12		ΣX=9.917		ΣY=9.880	ΣXY=29.469	ΣX^2=29.593	

Table-4: Recovery studies of hydrocortisone sodium succinate by standard drug addition method

S.No.	No. of observations (N)	Amount present (Pure) (mg)	Amount of drug added (mg) X	Total amount of drug obtained by calculation (mg)	Amount of drug obtained by calculation (mg)Y	XY	X^2	Recovery (%)
1	3	0.958	0.977	1.983	0.993	0.970	0.955	99.72
2	3	0.958	1.983	2.976	1.939	3.845	3.932	
3	3	0.958	2.970	3.980	2.962	8.797	8.821	
4	3	0.958	3.987	4.991	3.989	15.904	15.896	
	?N=12		?X=9.917		?Y=9.883	?XY=29.516	$?X^2$=29.604	

Table-5: Recovery studies of prednisolone sodium succinate by standard drug addition method

S.No.	No. of observations (N)	Amount present (Pure) (mg)	Amount of drug added (mg) X	Total amount of drug obtained by calculation (mg)	Amount of drug obtained by calculation (mg)Y	XY	X^2	Recovery (%)
1	3	0.958	0.977	1.983	0.993	0.970	0.955	99.72
2	3	0.958	1.983	2.976	1.939	3.845	3.932	
3	3	0.958	2.970	3.980	2.962	8.797	8.821	
4	3	0.958	3.987	4.991	3.989	15.904	15.896	
	ΣN=12		ΣX=9.917		ΣY=9.883	ΣXY=29.516	ΣX^2=29.604	

Table-6: Recovery studies of triamcinolone by standard drug addition method

S.No.	No. of observations (N)	Amount present (Pure) (mg)	Amount of drug added (mg) X	Total amount of drug obtained by calculation (mg)	Amount of drug obtained by calculation (mg)Y	XY	X^2	Recovery (%)
1	3	0.939	0.984	1.969	0.928	0.913	0.968	99.48
2	3	0.939	1.977	2.971	1.961	3.877	3.909	
3	3	0.939	2.960	3.978	2.941	8.705	8.762	
4	3	0.939	3.962	4.980	3.949	15.646	15.697	
	ΣN=12		ΣX=9.883		ΣY=9.779	ΣXY=29.141	ΣX^2=29.336	

Excipients like starch, calcium carbonate, sodium carbonate, cellulose, magnesium trisilicate, tricalcium phosphate and gum acacia if present in the pharmaceutical preparations do not interfere in the estimation.

On the basis of available literature and stoichiometry established between Adrenocorticosteroid samples drug and PFC reagent i.e., Betamethasone sodium phosphate (1:4), Dexamethasone sodium phosphate (1:2), Hydrocortisone sodium succinate (1:4), Prednisolone sodium succinate (1:4) and Triamcinolone (1:2), a possible course of reaction may also be suggested. It has been reported that Adrenocorticosteroids are oxidized to corresponding products. The most oxidizable positions are C-17 and C-11. Where the loss of side chain gives rise to ketonic products[19]. It is possible that the present reagent also behaves similarly. We have not studied the mechanism in the detail.

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