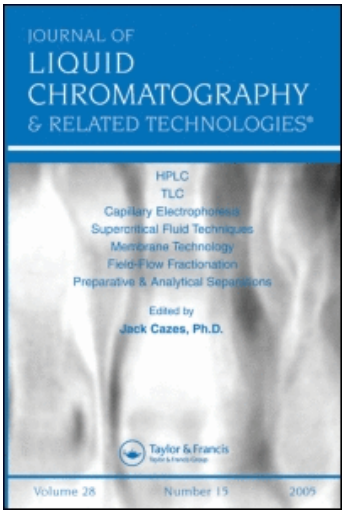

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THIN LAYER CHROMATOGRAPHIC METHOD FOR RAPID IDENTIFICATION AND QUANTIFICATION OF CORTICOSTEROID SODIUM PHOSPHATES IN PHARMACEUTICAL PREPARATIONS

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ABSTRACT

A rapid and inexpensive thin layer chromatography (TLC) procedure for the assay of dexamethasone sodium phosphate (DSP) and betamethasone sodium phosphate (BSP) persent in pharmaceutical preparations is described. Free steroids liberated after alkaline phosphatase reaction is isolated by TLC on silica gel layer and estimated after elution with ethanol. Assay of unesterified steroids and identification of the preservatives like methyl or propyl parabenzoates, phenol, benzyl alcohol etc. may also be carried out by this method without any additional cost. Possibility of using this method for other corticosteroid sodium phosphates (CSP) are discussed.

INTRODUCTION

The various pharmacopoeial methods for the identification and quantitation of different corticosteroid sodium phosphates (CSP) such as dexamethasone

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sodium phosphate (DSP) and betamethasone sodium phosphate (BSP) when present in injections, eye drops, lotions etc. are tedious and time consuming. They generally involve repeated solvent extraction of the sample followed by color reaction of either the intact steroid phosphates (1,2,3) or of the free corticosteroid released after reaction with alkaline phosphatase (4). HPLC methods for the assay of DSP injections (5,6) , ointments and eye drops (5) and BSP (bulk drug) have also been reported. However these methods lack in specificity as they can not defferentiate between BSP and DSP. Thus we have saught an inexpensive, simple and accurate method suitable for the rapid identification and quantitation of the CSPs.

MATERIALS

Apparatus

(a) TLC plates - 20 x 20 cm, coated with 0.4mm thick layer of silica gel (Silica gel 60 G : Silica gel GF 254 :: 10:3, w/w) activated at 110 C for 1h.

(b) Parkin Elmer recording spectrophotometer model Hitachi 200.

(c) Ultraviolet viewer,- Desaga UVIS system.

(d) Table centrifuge (swing head, 5000 rpm) - Model Remi R8C .

Reagents

(a) Standerd solutions -

  (i) Stock : Dexamethasone / Betamethasone, 2mg/ml in ethanol.

  (ii) Working standerd : 0.1 ml of the stock is diluted with 0.1 ml of borate buffer.

  (iii) BSP/DSP, 4.4 mg of the steroid / ml borate buffer.

(b) Borate buffer - 0.1 M borate buffer pH 9.0 containing 100mg of $MgCl_2 2H_2O$.

(c) Alkaline phosphatase (Sigma Chemicals, 1.1 unit/mg) - 2mg/ml in borate buffer.

(d) Developing solvent -

  (i) Chloroform : methanol : water :: 180 : 15 : 1 (v/v)

  (ii) Dichloromethane : diethyl ether : methanol : water :: 77 : 15 : 8 : 1 (v/v)

METHODS

Concentration of CSP in the formulation is adjusted to a nominal 4.4 mg/ml if necessary by the addition of borate buffer. To the standerd solution

or sample (0.05ml) in a 5 ml stoppered centrifuge tube , phosphatase enzyme (0.05ml) is added and incubated (1h, 37 C). A control contained of sample (0.05 ml) with borate buffer only. After incubation, ethanol (0.1 ml) is then added to all the tubes and mixed to dissolve any precipitated steroid. Aliquots (0.03 ml) from each tube are then applied as 1 cm bands to the TLC plates which are then developed (12 cm) using the developing solvent (1). After location under short wave ultraviolet lamp, the bands corresponding to the free steroid are scrapped off, extracted with ethanol (3 ml) by centrifugation at 1000 x g for 10 min and the absorbance of the clear supernate is measured at 240 nm using 1 cm cell path. A silica gel band of equivalent area and containing no sample was extracted in an identical manner and used in the reference beam. When the control containing no enzyme produces a spot corresponding to the free steroid, the difference in absorbance between the enzyme control and the assay sample determines the content of steroid in the original formulation . The absorbance of the enzyme control determines the content of free steroid in the sample.

RESULTS AND DISCUSSION

To ascertain the enzyme requirement for the hydrolysis of corticosteroid sodium phosphate, the substrate (DSP) was incubated with the varying amounts of enzyme. As complete hydrolysis of the CSP was effected at an enzyme concentration 1 mg ml, a concentration of 2 mg ml was considered adequate (Table 1).

The average recoveries of DSP and BSP using our present method are shown in Table 2. The average percentage of recovery was 100.56 for DSP and 100.46 for BSP. Some commercially available DSP and BSP injections were also analysed (Table 3) and good agreement was noted between the results obtained by the present method and by some official methods (British Pharmacopoeia, 1980, Indian Pharmacopoeia Suppl. 1975).

The TLC system used were described earlier (7) which allows clearcut separation of dexamethasone from betamethasone , prednisolone and hydrocortisone, the hRf values (Rf x 100) being 19, 16, 14 and 17 respectively. If required, an improved separation may be obtained by developing the plate twice

TABLE 1.

Enzyme Requirement for the Complete Hydrolysis of Dexamethasone Sodium Phosphate

Concentration of enzyme solution used (mg/ml)	Amount of enzyme in reaction mixture (mcg)	Weight of sample* determined** in reaction mixture (mcg)		Percent recovery	
		Mean	SD	Mean	SD
0.25	12.5	189.20	9.67	86.00	4.40
0.50	25.0	205.80	8.87	93.55	4.03
1.00	50.0	219.40	8.87	99.73	4.03
2.00	100.0	220.93	6.93	100.42	3.15
4.00	200.0	218.13	4.87	99.15	2.21

* The nominal weight of steroid sodium phosphate for each assay is 220 mcg (= 0.05 ml standard solution).

** Average of 4 independent determinations.

TABLE 2.

Recovery of Dexamethasone Sodium Phosphate (DSP) and Betamethasone Sodium Phosphate (BSP) by the Thin Layer Chromatographic Procedure

Steroids	Free steroid recovered (mcg)		Calculated amount of CSP (mcg)		Percent recovery*	
	Mean	SD**	Mean	SD	Mean	SD
DSP	168.16	3.14	221.24	4.13	100.56	1.88
BSP	167.98	3.44	221.00	4.52	100.46	2.05

* Standard solution (0.05ml) = 220 mcg; ** Average of 8 determinations.

TABLE 3.

Assay of some Commercially Available BSP and DSP Injections

Sample*	Percent recovery by official method**		Percent recovery by TLC method	
	Mean	SD	Mean	SD
1	100.64	0.65	101.94	3.59
2	95.61	2.79	93.50	1.46
3	96.69	0.74	96.50	2.39
4	101.08	1.92	101.68	3.03
5	95.55	0.99	95.84	3.03
6	96.37	0.66	98.20	3.30
7	100.40	2.11	99.61	1.50
8	103.88	1.06	103.84	2.65

* Sample 1-3 were BSP, sample 4-8 were DSP

** Sample 1 was assayed by BP 1980 method, others were assayed by the IP 1966, suppliment 1975 method.

in the same solvent (Table 4) . Betamethasone and prednisolone move together in this system but may be separated using the solvent system (ii) under identical conditions - the hRf values being 41.9 and 33.3 for betamethasone and prednisolone respectively (Table 4).

The present method is simple and inexpensive with solvent and enzyme requirements at a minimum. It is also highly specific as hydrolysis of the test compound by an alkaline phosphatase ensures that the compound is a phosphate ester and TLC identification of a corticosteroid in the hydrolysate indicates that the compound is a CSP. Also this method can differentiate between DSP and

TABLE 4.

The hRf Values of the Free Corticosteroids and the Preservatives After TLC on Silicagel Layers.

Name of compound	hRf* values	
	Solvent A	Solvent B
Dexamethasone	19 (100)**	29
Betamethasone	16 (87)	29
Prednisolone	14 (75)	25
Hydrocortisone	17 (95)	30
Phenol	60 -	85
Methyl parabenzoate	54 -	81
Propyl parabenzoate	61 -	86

* Avarage of 4 determinations

** hRf after 2nd development are in parentheses. Solvent A - Chloroform : methanol : water :: 180 : 15: 1, v/v; solvent B - Dichloromethane : diethyl ether : methanol : water :: 77 : 15 : 8 : 1, v/v .

BSP as it involves TLC of the free steroid instead of the intact CSP. A large number of samples can be run simultaneously . The free cortical steroids in the sample can be detected and determined from the control experiments thus functioning as a limit test. The perservatives usually added in the samples like methyl and propyl parabenzoate or phenol are also separated (Table 4) and identified in the chromatogram and easily be estimated if required, after elution. This method may also be used for the estimation of steroid esters like prednisolone sodium phosphate, hydrocortisone sodium phosphate etc. possibly without any modification because of their similar behaviour under the experimenttal conditions described.

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