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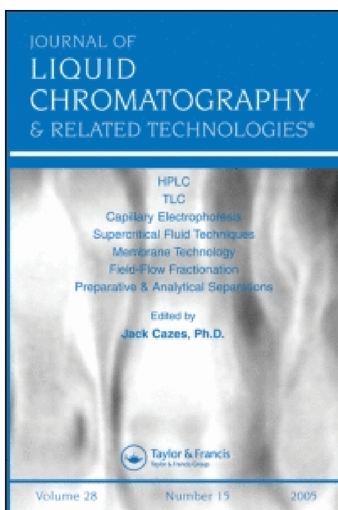

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THIN-LAYER CHROMATOGRAPHIC METHOD FOR THE QUALITY CONTROL OF DEXAMETHASONE AND BETAMETHASONE TABLETS

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SUMMARY

A rapid, sensitive and specific method for the assay and identification of corticosteroids (dexamethasone and betamethasone) in tablet forms is described. The method involves a rapid single step extraction of the steroids with extremely low volume of solvents (0.1 ml water + 0.4 ml ethanol) followed by identification and estimation by thin layer chromatography using the solvent system chloroform : methanol : water :: 180 : 15 : 1, v/v). The steroids, extracted from the chromatogram by ethanol , were assayed directly by measuring the extinction of the extract at the respective absorption maxima (around 240 nm). The results agree with those obtained by the BP, 1980 and IP, 1966 methods.

INTRODUCTION

Determination of different corticosteroids in pharmacopoeial preparations (tablets) has been described by the United States Pharmacopoeia (1,2), the

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British Pharmacopoeia (3,4) and the Indian Pharmacopoeia (5,6). The assay procedure described by United States Pharmacopoeia (U.S.P) involves the separation of corticosteroids by high performance liquid chromatography followed by ultraviolet absorption or by color reaction. The method described by British Pharmacopoeia (B.P) and Indian Pharmacopoeia (I.P) involves extraction of the corticosteroid from the pharmaceutical preparation with chloroform followed by colorimetric estimation of the extracted material. The present investigation describes a simple and rapid screening method for corticosteroids using thin layer chromatography (TLC), a technique devoid of costly instrumental or time consuming procedural variables described by different pharmacopoeias. The method permits the identification and determination of a large number of sample in a very short period.

METHOD

Apparatus

(a) TLC plates - 20x20 cm, coated with 0.40 mm thick layer of silica gel (silica gel 60 G : silica gel HF 254 :: 10 : 3, w/w) activated at 110 C for 1h.

(b) Parkin Elmer spectrophotometer , Model - Hitachi 200.

(c) Ultraviolet viewer - Desaga UVIS system.

Reagents:

(a) Ethanol - Dehydrated, distilled and absorption free (at around 240 nm).

(b) Developing solvents - Freshly distilled certified grade solvents were used. The solvent compositions (v/v) used were :

A. Chloroform : Ethylacetate : water :: 10 : 90 : 1,

B. Chloroform : Ethylacetate :: 1 : 1,

C. Dichloromethane : Diethylether : methanol : water :: 77 : 15 : 8 : 1,

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D. Chloroform : Ethylacetate : Methanol : Water :: 40 : 30 : 4 : 1,

E. Chloroform : Methanol : Water :: 100 : 15 : 1 ,

and F. Ethylacetate : Ethanol : Water :: 94 :4 :2.

For the assay of steroids, the solvent system 'E' was used for TLC.

(c) Corticosteroid standard solution - 1.25 mg/ml; 125 mg of International Reference standard of dexamethasone or betamethasone was dissolved in 100 ml of ethanol . The working solution was made by mixing 0.4 ml of the standard solution with 0.1 ml of distilled water.

Procedure

The procedure described here is meant for dexamethasone or betamethasone tablets of usual strength i.e., 0.5 mg of the steroid per tablet. For tablets of higher strength, volume of alcohol and water should be adjusted to produce an extractive containing about 1 mg of steroid per ml (water : ethanol ratio should be 1 : 4, v/v).

Twenty tablets were weighed and ground to a fine powder. Powder equivalent to one tablet (average weight) was weighed accurately and transferred into a stoppered centrifuge tube (for uniformity of content determination , an intact tablet was taken in the centrifuge tube) and 0.1 ml of water was added followed by shaking on a vortex mixer for one minute (or till the tablet was disintegrated). Ethanol (0.4 ml) was then added to each tube followed by intermittent shaking for 10 minutes. The clear supernatant was ready for TLC.

On an imaginary line, 2 cm from the bottom of the previously activated (110 °C, 1h) TLC plates, 30 µl of the test solution or standard solution was applied as a 1 cm band. The bands were dried with a hair drier and the plate was placed in the developing tank previously saturated with the developing solvent 'E'. The solvent front was allowed to ascend upto 12 cm from the starting point.

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After development the plate was examined under short wave (254 nm) ultraviolet light. The steroids appearing as dark blue bands were marked and, in a draught free place , gel bands containing the steroids were scrapped from the plate on a glazed paper with the help of a sharp razor blade and were transferred to separate 10ml stoppered centrifuge tubes. The steroids were then extracted from silica gel by shaking with 3 ml of alcohol on a vortex mixer followed by centrifugation at about 800 x g for 10 minutes. Optical density (O.D) of the clear supernatant were measured at 240 nm against an appropriate silica gel blank. The OD of the silica gel blank is generally negligible. The amount of the steroid was calculated from the extinction value obtained for standard. For comparison, the corticosteroids were also assayed by the triphenyltetrazolium reduction method described in B.P. (3,4) and I.P. (5,6).

RESULT AND DISCUSSION

Identification and seperation of corticosteroids from related foreign steroids may be achieved by using either adsorption TLC or partition TLC. However , for quantitative analysis , partition TLC is generally not suitable as nondestructive visualisation of the steroids on (impregnated) partition TLC plates is difficult. Also , the impregnating liquids sometimes interfere with the subsiquent assay of the steroids eluted from such plates. Thus adsorption chromatography was our method of choice for the quantitative TLC of the corticosteroids. Since the corticosteroids tested were moderately polar and were neutral to acids or bases , we used neutral but moderately nonpolar developing solvent systems against activated silica gel plates for the chromatography . For the purpose of selection of a suitable solvent system, 5 ul of 0.1% ethanolic solutions of the authentic steroids (Central Drugs Laboratory reference standard) were applied on the TLC plates and developed with the desired solvent system once or , after briefly drying in a current of air for 10 minutes, twice in the same direction and up to the same hight. Table 1 shows the mobility of

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some of the pharmaceutically important corticosteroids and their esters on such systems. It is apparent from Table 1 that the solvent system 'E' separates dexamethasone and betamethasone from each other and from the related steroids effectively. The separation is excellent when the development was carried out twice, though the recovery of the steroids after the second development was slightly less (about 96%, not shown in this paper). Thus the solvent system 'E' (chloroform : methanol : water :: 180 : 15 : 1 ; v/v , single development) was used for the identification and assay of the steroids. This system does not separate dexamethasone from hydrocortisone which may, however, be achieved by using the system A or B or D if required.

Table 2 shows the result of analysis of four dexamethasone tablets and one betamethasone tablet sample by the TLC method and IP/BP method. There is a good agreement in the assay values for all the samples obtained by these two methods (evident by the extremely low 't' value). We have assayed hundreds of commercially available dexamethasone tablets and betamethasone tablets containing 0.5mg of the steroid by the method described without any difficulty. Results obtained for dexamethasone tablets and betamethasone tablets of known steroid content showed a percent recovery of 100.18 + 1.13 (n=30) and 99.99 + 1.79 (n=20) respectively which is excellent considering the ease of the operation.

The method described here has several advantages over the pharmacopoeial methods concerned. The HPLC arrangement as suggested by USP is not easily available in all laboratories and also it is handelled only by experts. Moreover, the sample needs time consuming treatment before it could be subjected to HPLC (however , sample prepared by our method may be used for HPLC without any additional work). The present method is very simple and rapid and uses a minimal volume of solvents when compared with the IP, BP or USP method. The efficiency of the extraction step is excellent . The solvent (0.1 ml of water and 0.4 ml of alcohol) required for extraction is very small and the extraction is complete within 5 minutes. By this method about 50 samples can be analysed within 7 hours

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Table 1. Mobility of corticosteroids and their esters on silica gel thin layer chromatogram using different solvent systems.

Steroids	hRf *					R Dexa **					
	A	B	C	D	E	A	B	C	D	E	Fa
Betamethasone	39	13	29	45	16	101	102	98	106	87	100
Dexamethasone	38	12	29	45	19	100	100	100	100	100	100
Fluocortolone	–	–	–	49	30	–	–	–	119	160	95
Hydrocortisone	32	10	30	39	17	89	82	100	94	95	92
Prednisolone	30	9	25	37	14	65	67	85	81	75	66
Prednisone	31	12	39	47	30	87	102	118	119	132	88
Triamcinolone	–	–	–	16	6	–	–	–	31	32	50
Beclomethasone dipropionate	–	–	–	–	56	–	–	–	–	215	118
Desoxycortisone phenylpropionate	–	–	–	–	58	–	–	–	–	220	121
Desoxycortisone acetate	–	–	–	–	57	–	–	–	–	217	112
Hydrocortisone acetate	47	24	56	–	50	120	184	148	–	19	107

* h Rf = Rf x 100 ; Solvents : see text

$$\text{** R Dexa is } \frac{\text{Rf steroid}}{\text{Rf Dexamethasone}} \times 100$$

Developed twice in the same direction

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Table 2. Comparison of corticosteroid values obtained by TLC - UV absorption procedures with the pharmacopoeial methods in commercial corticosteroid tablets.

Sample No.	Steroid contained	Steroid content found per tablet in µg		"t" b
		TLC - UV	IP / BP	
1.	Dexamethasone	423.3 + 3.6	415.3 + 8.05	2.03 c
2.	Do	493.5 + 19.8	485.2 + 5.05	0.91 d
3.	Do	482.0 + 11.5	490.4 + 13.60	1.05 d
4.	Do	245.6 + 4.4	248.1 + 8.05	0.61 d
5.	Betamethasone	479.8 + 14.1	475.0 + 17.70	0.47 d

a Avarage of 5 independent determinations + S.D

b "t" = Students t

c $p << 0.05$

d $p < 0.1$

by a single person which is practically impossible by the IP, BP, or USP method. "Uniformity of contents" test of dexamethasone or betamethasone tablets by BP or USP method is laborious and time consuming. The test can be easily done by the present method . This method is also highly suitable for the monitoring and quality control of the drug produced by the different manufacturers.

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REFERENCES

1. United States Pharmacopoeia (1980) XX Rev., U.S. Pharmacopoeial Convention Inc. Rockville, MD, pp. 209-210.

2. United States Pharmacopoeia (1980) XX Rev., U.S.Pharmacopoeial Convention Inc. Rockville, MD, p. 61.

3. British Pharmacopoeia (1980). The Pharmaceutical Press, London,UK., pp. 739-740.

4. British Pharmacopoeia (1980). The Pharmaceutical Press, London, UK., p. 757.

5. The Pharmacopoeia of India (1966) Supplement (1975), Government of India Press, Nasik, India., pp. 58-59.

6. The Pharmacopoeia of India (1966) Supplement (1975), Government of India Press, Nasik, India. p. 95.

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