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THIN-LAYER CHROMATOGRAPHIC DETERMINATION OF BETAMETHASONE DIPROPIONATE IN SEMI-SOLID PHARMACEUTICAL PREPARATIONS

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SUMMARY

A novel and specific thin-layer chromatographic method for determination of betamethasone dipropionate in semi-solid pharmaceutical preparations is described. Thin-layer chromatography was carried out on silica gel K6F-plates using cream or ointment extracts obtained with chloroform or ethanol respectively. A direct determination was performed by a chromatogram-scanner in the reflectance detection mode with zigzag scanning. Betamethasone dipropionate was determined at λ_{max} 240 nm. The method described is rapid, enabling a good reproducibility; it is suitable for quality control and stability investigations. The relative standard deviation is 1.3%.

INTRODUCTION

Interest in developing versatile techniques for corticosteroid determination in semi-solid preparations has grown continually over the last 30 years. The production of new pharmaceutical formulations containing new corticosteroids provides a challenge to the development of better analytical techniques than those available. Among many methods devised for corticosteroid determination[1,2] three are included in Pharmacopoeias and followed by manufacturers. These methods are based on the reactions between corticosteroids and (a) tetrazole salts[3], (b) phenylhydrazone[4] and (c) isoniazide[5].

Some problems appear when applying these methods owing to certain ingredients and decomposition products. Therefore, Graham *et al.*[6] recommended a column procedure to avoid or at least decrease the interferences present. Graham *et al.*[7] employed a similar procedure for analysis of betamethasone and its monoesters in pharmaceutical preparations. According to ref. 8, high-performance liquid chromatography should be used for betamethasone dipropionate analysis.

This paper reports a thin-layer chromatographic (TLC) method for determination of betamethasone dipropionate in semi-solid preparations. Betamethasone dipropionate can be determined directly on the TLC plate by a chromatogram-scanner after separation of the compound from interfering components.

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EXPERIMENTAL

Materials

All solvents, tetrazolium blue, triphenyltetrazolium chloride and isoniazide were of analytical reagent grade (E. Merck, Darmstadt, G.F.R.). Commerical silica gel K6F TLC plates, 20 × 20 cm (Whatman, Clifton, NJ, U.S.A.), layer thickness 0.25 mm, were used. Solutions were spotted on to the TLC plates using micropipettes.

Drug and formulations

Betamethasone dipropionate was a gift from Schering Corporation (U.S.A.). Betamethasone dipropionate ointment (Diprogent Ointment; Belupo Pharmaceutical Works, Yugoslavia; licenced by Schering Corporation) and betamethasone dipropionate cream (Beloderm Cream and Diprogent Cream, Belupo, licensed by Schering) were purchased. Ointment and cream contained 0.05% betamethasone in the form of dipropionate. Diprogent Ointment and Diprogent Cream contained 1000 international units per g of gentamicin in the sulphate form.

Apparatus

The following were used: a Shimadzu dual-wavelength TLC scanner, Model CS-910, with a dual-pen recorder (Philips, Model PM 8222); Varian Techtron spectrophotometer, Model 635; M4020 shaker from Köttermann (Hanigsen, G.F.R.); "Superspeed" centrifuge from Sorvall (Newtown, CN, U.S.A.) and a Grant BT3 block thermostat (Cambridge, Great Britain).

Sample preparation

Ointments and creams —Method A. The procedure of Graham *et al.*[6] was followed except that an amount of ointment (or cream) containing the equivalent of 1 mg betamethasone was used instead of 5 mg betamethasone.

Ointments —Method B. The procedure given in ref. 9 for assay preparation was followed, except that: (a) 30 ml of ethanol were used instead 10.0 ml of ethanol and 5.0 ml of internal standard solution, (b) the extraction was repeated once with 15 ml of ethanol and (c) the sample extracts were combined into a 50-ml volumetric flask and diluted to volume with ethanol.

Creams —Method B. An accurately weighed amount of cream, equivalent to about 1 mg betamethasone, was transferred to a separator, 150 ml of 10% hydrochloric acid were added and mixed. The mixture was extracted with four 20-ml portions of chloroform, shaking each portion for about 2 min. The chloroform phase was filtered through about 3 g anhydrous sodium sulphate into a 100-ml volumetric flask and made up to volume with chloroform. A 10.0-ml volume of the solution was transferred to a 20-ml conical test-tube and evaporated to dryness at 50°C in a block thermostat under a nitrogen stream, whereupon 5.0 ml of methanol were added to the residue. The tube was heated for 5 min in a 60°C water-bath, then agitated vigourously for 3 min, adjusted to room temperature and centrifuged for 5 min at 2611 × *g*.

Determinations

Proposed method. Sample solution and standard solution (50 μl or 30 μl of ointment sample obtained by *Method B* respectively) were spotted successively on the

TLC plate. The TLC tank was lined with filter-paper saturated with the solvent mixture chloroform–acetone (70:10) and the system was allowed to equilibrate for 60 min. After placing the TLC plate into the tank the solvent front was allowed to migrate 15 cm from the origin (*ca.* 45 min). The developed plate was dried in a stream of air for 30 min. The spots were located under short-wavelength UV-light.

The TLC plate was scanned at 20 mm/min in a direction perpendicular to the direction of development, using the following operating conditions: photometric mode, dual-wavelength, λ_s = 240 nm, λ_r = 400 nm; detection mode, reflection; measuring mode, absorbance; stage scanning mode, zigzag; working curve linearizer, channel 1. The speed of the recorder was 20 mm/min. The profile and integration curves were recorded for each spot on the TLC plate. The peak heights were measured for the integrated values of spots of sample solution and standard solution. The betamethasone content in the preparation was calculated from[10]

$$S\,(\%) = \frac{Y_p X_s \cdot 100}{Y_s X_p}$$

where S = percentage of betamethasone in preparation, X_s = mass of standard applied (μg), X_p = mass of sample applied (μg) (calculated from the declared quantity in the preparation), Y_p = mean of heights (cm) of the integrated values of the sample spots and Y_s = mean of heights (cm) of the integrated values of the standard spots.

Tetrazole method. The procedure as described previously[11] was followed, except that triphenyltetrazolium chloride was used instead of tetrazolium blue. The same procedure was used with 20.0 ml of standard solution (concentration 10 μg/ml) and 20 ml of ethanol as blank.

This procedure was used for determination of betamethasone dipropionate in ointment and cream after extraction by *Method A*.

Isoniazide method. Five millilitres of a chloroform extract of cream, obtained by *Method B*, was used. The procedure of Umberger[5] was used, and repeated with 5.0 ml of standard solution (concentration 10 μg/ml).

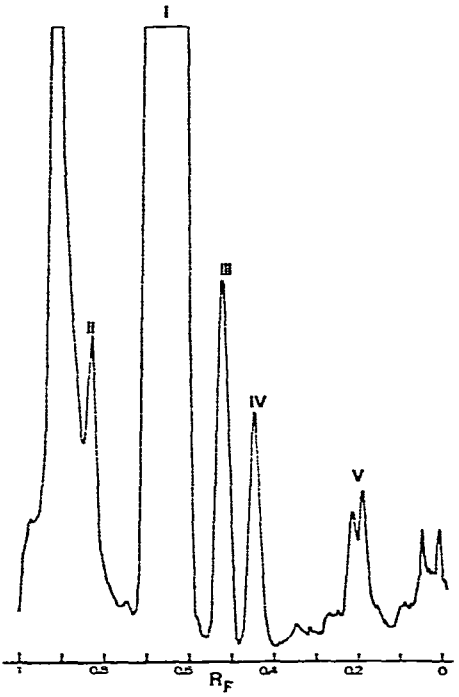
RESULTS AND DISCUSSION

Specificity of the determination

Two solvent systems from other studies[11,12] were tested: (a) chloroform–acetone (70:10) and (b) chloroform–ethyl acetate (50:50); the former was more satisfactory, giving complete resolution of betamethasone dipropionate and accompanying substances (Fig. 1).

Ultraviolet absorption spectra of betamethasone dipropionate and accompanying substances on the TLC plate were constructed by plotting absorbance at different wavelengths (Fig. 2). The maximum absorbance of betamethasone dipropionate was at 240 nm. In selecting the dual-wavelength settings this wavelength was used for the sample side, while 400 nm —a wavelength at which no absorption occurs— was used for the reference side.

When the developed chromatogram was sprayed with tetrazolium blue in alkaline methanol[13] and triphenyltetrazolium chloride in alkaline methanol (prepared as tetrazolium blue in alkaline methanol) positive reactions were obtained with ac-



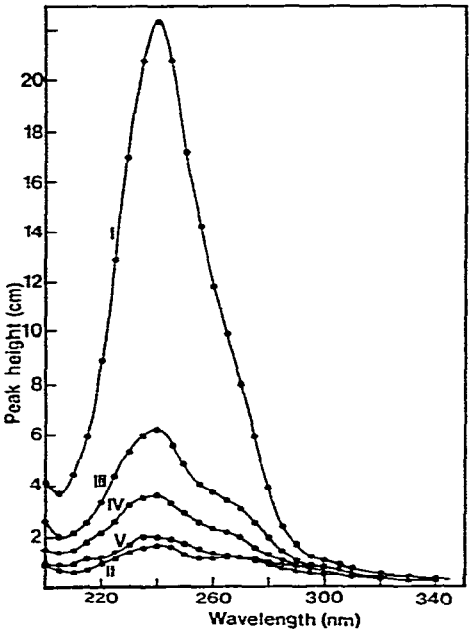


Fig. 1. Chromatogram of betamethasone dipropionate and accompanying substances obtained by linear scanning of the TLC plate. Peaks: I = betamethasone dipropionate; II–V = accompanying substances. 400 μg of betamethasone dipropionate were spotted.

Fig. 2. Ultraviolet absorption spectra of betamethasone dipropionate and accompanying substances obtained by scanning the TLC plate after separation in the solvent mixture chloroform–acetone (70:10). Spectra: I = betamethasone dipropionate, hR_F = 67; II = accompanying substance, hR_F = 85; III = accompanying substance, hR_F = 52; IV = accompanying substance, hR_F = 45; V = accompanying substance, hR_F = 20.

companying substances. Therefore, these substances can interfere in the colorimetric determination of betamethasone dipropionate with tetrazolium blue or triphenyltetrazolium chloride reagents.

The interference of ingredients in cream was investigated. Extracts of cream were spotted on the TLC plates and developed with the solvent mixture chloroform–acetone (70:10). Inspection of the developed chromatograms under short-wavelength UV-light revealed only spots of betamethasone dipropionate. Then the developed chromatograms were sprayed with the previously mentioned reagents or with isoniazide in acidic methanol[5]. Two spots from ingredients (Fig. 3) were found to interfere in colorimetric determinations of betamethasone dipropionate.

Separation of betamethasone dipropionate from accompanying substances and excipient interferences by TLC in the mobile system chloroform–acetone (70:10) offers a specific assay of betamethasone dipropionate on the TLC plate by use of a chromatogram scanner.

Linearity of response

Using the curve linearizer, a linear relation between integrated values and the

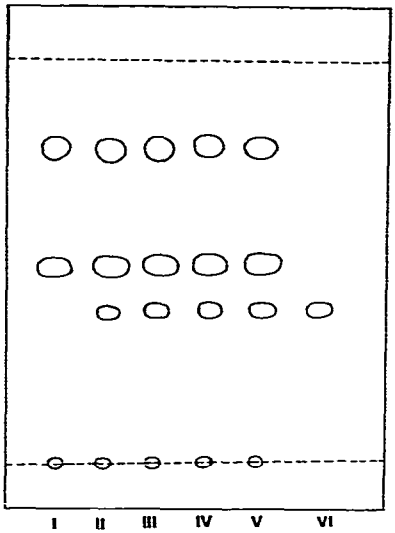


Fig. 3. Separation of betamethasone dipropionate and excipient interferences from cream. Bands: I = extract of cream without betamethasone dipropionate; II and V = extracts of cream obtained by *Method B*; III and IV = extracts of cream obtained by *Method A*; VI = betamethasone dipropionate.

amounts of betamethasone dipropionate spotted was obtained. Since not more than 1.5 μg per spot were placed on the TLC plates, the procedure was found to be satisfactory.

Accuracy and reproducibility of determination

It is known that reproducibility depends on local variations on a individual TLC plate as well as variations between plates. In order to determine which factors are dominant the following tests were carried out. Four TLC plates were spotted with the cream extracts obtained according to *Method B*. Onto each TLC plate three spots of sample solution and three spots of standard solution (concentration 1 μg per spot) were spotted alternatively. The found concentration (Table I) for each spot of sample was calculated by the mean of the integrated values of three spots of standard solution. The mean betamethasone contents, S.D. and R.S.D. were calculated for each plate and these results were used for calculation of the corresponding mean values for four TLC plates. Variations within a plate are found to be 1.03–4.38%, whereas variations between plates were 1.06%. The R.S.D. data clearly show the existence of local variations within a plate and in spotting. In order to avoid these variations three spots of sample solution and three spots of standard solution should be spotted on the TLC plate one after another. The content of betamethasone should be calculated from mean integrated values of standard and sample.

The method proposed was compared with the tetrazole and isoniazide methods. Samples of betamethasone dipropionate cream, prepared in the laboratory, were assayed in quintuplicate (Table II). TLC determinations were carried out with extracts obtained by both *Methods A* and *B*. In addition, the same extracts were analysed by the tetrazole or isoniazide method. The highest recovery was obtained by TLC determination with extracts from *Method B* (99.2%) and R.S.D. was 1.3%.

TABLE I

REPRODUCIBILITY OF TLC DETERMINATIONS ON SAME AND DIFFERENT TLC PLATES

Sample solution and standard solution (concentration 1 μg per spot) were spotted on four TLC plates, one after another.

Plate No.	*Spot No.*	*Found concentration (% of added)*	*Mean*	*S.D.*	*R.S.D. (%)*
1	1	97.9			
	2	99.8	98.3	1.27	1.29
	3	97.4			
2	1	100.4			
	2	96.5	99.8	3.04	3.05
	3	102.5			
3	1	97.0			
	2	95.0	98.5	4.31	4.38
	3	103.4			
4	1	99.3			
	2	101.1	100.5	1.04	1.03
	3	101.1			
		Mean	99.3		
		S.D.	1.05		
		R.S.D. (%)	1.06		

TABLE II

ACCURACY OF DETERMINATIONS BY THE PROPOSED METHOD AND COLORIMETRIC METHODS

0.5 mg of betamethasone in the form of dipropionate were added per 1 g of cream base.

Sample No.	*Found concentration (% of added)*			
	By proposed method		*By tetrazole method*	*By isoniazide method*
	Extract by Method A	*Extract by Method B*		
1	83.9	98.4	82.4	90.1
2	103.1	101.1	91.1	100.5
3	95.6	99.9	90.8	91.5
4	109.4	98.7	104.1	96.9
5	103.1	97.8	107.8	96.5
Mean	99.02	99.18	95.24	95.10
S.D.	9.76	1.32	10.46	4.25
R.S.D. (%)	9.80	1.33	10.98	4.47

TLC determination of betamethasone dipropionate extracted by *Method A* shows high R.S.D. (9.8%); even worse results were obtained employing the tetrazole method (R.S.D. 11.0%). These could be ascribed to variations in the extraction procedure of *Method A*. Additional interferences in tetrazole method arise from excipient interferences and accompanying substances. In the TLC determinations

these interferences have been avoided. The recovery of the isoniazide method is as high as in the tetrazole method, while reproducibility is markedly better (R.S.D. 4.5%).

Results of the analysis of commercial preparations of ointments and creams, containing only betamethasone dipropionate as active component, or in combination with the antibiotic gentamicin sulphate, are given in Table III. The determinations were carried out with several cream and ointment batches as well as with several samples of the same batch. Results for extracts obtained by *Method B* and applying the proposed method were compared with results for the tetrazole and isoniazide methods. The best reproducibility in respect of the declared amounts was achieved by the proposed method, whereas the worst results were obtained by the tetrazole method. Hence, the latter is not reliable for determination of betamethasone dipropionate in creams and ointments if not preceded by sample clean-up.

TABLE III

ANALYSIS OF COMMERCIAL OINTMENTS AND CREAMS CONTAINING BETAMETHASONE DIPROPIONATE

Type of sample	*Batch*	*Sample from tube No.*	*Found concentration (% of declared)*		
			By proposed method	*By tetrazole method*	*By isoniazide method*
Beloderm	A	1	104.6	107.0	110.6
Cream	A	1	101.9	104.3	103.5
	A	1	104.9	84.4	100.0
	B	1	100.8	106.1	100.5
Diprogent	C	1	96.9	96.8	98.1
Cream	C	2	98.4	78.0	98.0
	D	1	101.2	–	--
Diprogent	E	1	99.4	108.0	
Ointment	E	2	100.8	110.0	
	F	1	99.3	100.1	
	F	2	101.5	92.7	
	G	1	99.0	106.1	
	G	1	100.9	110.4	
	G	2	102.3	116.0	
	H	1	102.1	97.8	
	J	1	99.6	73.2	
	J	1	101.4	93.5	

In conclusion, the proposed TLC determination is suitable for the quality control of betamethasone dipropionate ointments and creams. It is rapid and specific, and the precision and accuracy of assay are better than with existing colorimetric methods.

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