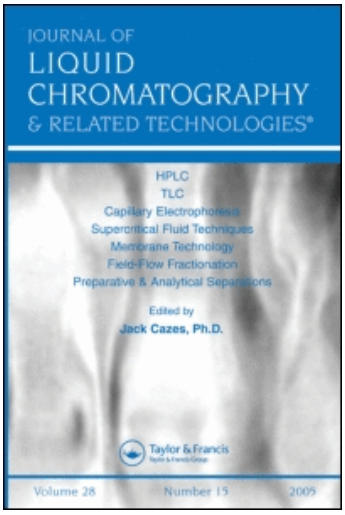

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HPTLC Determination of Betamethasone in Tablets and Its Validation

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ABSTRACT

A simple and rapid high performance thin layer chromatography (HPTLC) densitometric method has been developed for determination of betamethasone base in the tablets. After extraction of the analyte with 96% ethanol, the extracts were spotted on precoated HPTLC silica gel plates, which were then developed with a mixture of chloroform–methanol–water (18 : 5 : 0.5). Quantitative evaluation was performed by measuring the absorbance reflectance of the analyte spots at 245 nm. The densitometric method is selective, precise, and accurate and can be used

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for routine analysis of the tablet preparations in pharmaceutical industry quality control laboratories.

Key Words: Betamethasone; High performance thin layer chromatography; Tablet; Validation.

INTRODUCTION

Some tablet pharmaceutical preparations containing betamethasone base as the sole active ingredient are marketed now in Indonesia.[1] The USP 25-NF 20,[2] BP 2000,[3] and Indonesian Pharmacopoeia[4] described column liquid chromatography (LC) methods for the determination of betamethasone. Indrayanto et al.[5–7] reported some thin layer chromatography (TLC) methods for determination of betamethasone valerate and other drugs in the topical preparations. Das et al.[8] described the quantitative determination of betamethasone tablets by measuring the extinction at 240 nm after scraping zones from the TLC plate, while Unterhalt and Sanatgar[9] reported a qualitative TLC method for identification of betamethasone.

The aim of this work was to develop a simple, cheap, rapid, and validated high performance TLC (HPTLC) densitometric method for routine analysis of betamethasone in tablets.

EXPERIMENTAL

Materials and Reagents

Betamethasone base (Tianjin Tianyao Pharmaceutical Co. Ltd., China; Batch No. BE020701; Assay 99.52%; Conforms to USP 23/BP 93) was pharmaceutical grade. The substance was used as received for preparing Benoson® tablets. The substance fulfills the requirement of the Indonesian Pharmacopoeia[4] and had identical ultraviolet (UV), infrared (IR), and melting point properties compared to an authentic standard of betamethasone (Sigma, St. Louis, USA).

Ethanol (96%), chloroform, and methanol (Mallinckrodt Baker, Inc., Phillipsburg, NJ) were analytical grade reagents. The solvents were used without further purification.

Benoson® tablets containing 0.5 mg betamethasone tablet^{-1} (average weight of tablet was ca. 225 mg) were provided by the Department of Production, Bernofarm Pharmaceutical Company (Buduran, Sidoarjo, Surabaya, Indonesia) for accuracy determination. Calcium diphosphate,

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Avicel®, magnesium stearate, talcum, Primojel®, Tween 80, and glycine were pharmaceutical grade substances used as excipients for Benoson® tablets.

Two commercial tablets (T-1 and T-2) containing 0.5 mg betamethasone tablet^{-1} were purchased in August 2002 from a local Pharmacy in Surabaya. T-1 and T-2 were produced in Indonesia.

Stock standard solutions were prepared daily by dissolving accurately weighed betamethasone (20.0 mg) in 96% ethanol (100.0 mL). Various standard solutions were prepared from the stock solution by dilution with 96% ethanol. For linearity studies, solutions were prepared containing 10.0, 20.0, 33.0, 40.0, 50.0, 60.0, 66.0, 80.0, 100, and 120 μg mL^{-1}, and 2×2.0 μL of these solutions was spotted on the HPTLC plate.

Sample Extraction

Twenty tablets were weighed and their average weight was determined. The tablets were mixed and finely powdered, 270 mg (or equivalent to 0.6 mg of betamethasone; accurately weighed) of it was transferred into a 10.0 mL volumetric flask that contained ca. 8 mL of 96% ethanol, ultrasonicated for 15 min, and then diluted to 10.0 mL with 96% ethanol. The solution was filtered through Whatman type 40 paper before spotting (2×2.0 μL) on the HPTLC plate.

Chromatography

Chromatography was performed on precoated 20×10 cm HPTLC LiChrospher Si 60 F 254 aluminum-backed sheets (E. Merck, Darmstadt, Germany, #1.05586); a Nanomat III (Camag, Muttenz, Switzerland) equipped with a dispenser magazine containing 2.0 μL glass capillaries (Camag, Cat. 022.7772, CV $\leq$ 0.6%, $R \leq 0.25\%$) was used for sample application. The mobile phase used was a mixture of chloroform–methanol–water (18 : 5 : 0.5); this composition was modified from that described previously.[8] Ascending development was performed in a Camag HPTLC twin-through chamber; the mobile phase migration distance in all experiments was 8.0 cm (development time ca. 15 min at 25°C ± 2°C). Each HPTLC plate was developed with the mobile phase, then air dried for 60 min prior to the analysis.

Densitometric scanning was performed with a Camag TLC-Scanner II. The purity and identity of the analyte spots were determined by scanning a spectrum in the absorbance-reflectance mode from 200 to 400 nm. Quantitative evaluation was performed by measuring the absorbance reflectance of the analyte spots at 245 nm (see Fig. 1). The densitometric scanning parameters

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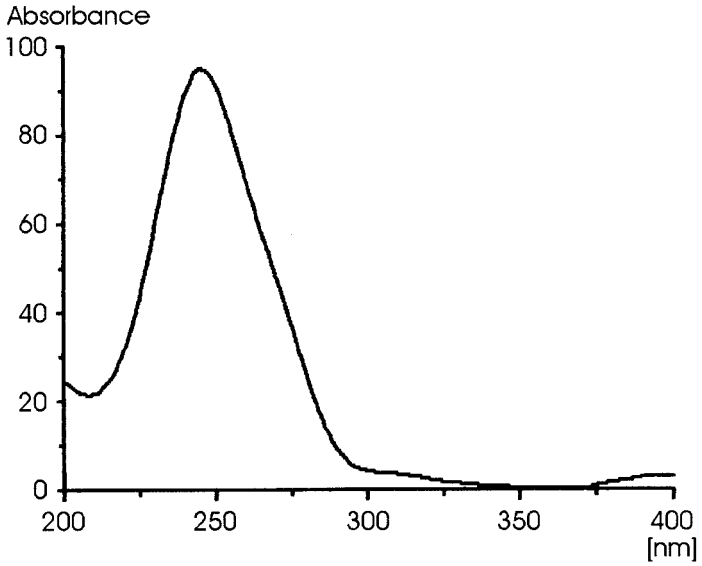

Figure 1. In situ absorbance reflectance spectrum of betamethasone from 200 to 400 nm, with its absorption maximum at 245 nm. Stationary phase: precoated HPTLC LiChrospher Si 60 F 254 aluminum-backed sheets; mobile phase: chloroform–methanol–water (18 : 5 : 0.5).

were: bandwidth 10 nm, slit width 4, slit length 6, and scanning speed 4 mm s^{-1}. Calculations for identity, purity checks ($r_{S,M}$ and $r_{M,E}$ where S = start; M = center; and E = end of spectrum), sdv (relative standard deviation of the linear curve), and quantification of the analyte spots was performed by CATS version 3.17 (1995) software (Camag). Routine quantitative evaluations were performed via peak areas with linear regression, using at least four-point calibration on each plate.

Validation

The method was validated for linearity, homogeneity, detection limit (DL), accuracy, and range by the methods of Funk et al.[10] and Hahn-Dienstrop.[11] The selectivity of the method was proven by identification and purity checks of the analyte spots. A five-point standard addition accuracy study (added with 30.0%, 40.0%, 50.0%, 60.0%, and 70.0% of the label claim) was performed on the Benoson® tablets. For commercial preparations, accuracy studies were performed using one single-point standard addition method (30.0% of label

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claim). The precision was evaluated by analyzing five different extract aliquots from Benoson® tablets according to a modified method of Renger et al.[12]

RESULTS AND DISCUSSION

After the HPTLC-plate was developed, the densitogram (Fig. 2) showed only the spots of betamethasone (R_f 0.52), while other components were not detected or developed in this proposed method. The HPTLC system demonstrated that all analyte spots of the extracts of the pharmaceutical preparation, furnished in situ, had UV spectra identical with those of standards ($r \geq 0.9999$). Purity check of the analyte spots using CATS software also showed that all analyte spots of the extracts were pure. The values of $r_{S,M}$ and $r_{M,E}$ were ≥ 0.9999, demonstrating that the proposed method is highly selective.

The basic calibration plot of peak area against amount of analyte was constructed within the ranges 16–200% of the expected values in the pharmaceutical preparations. Under this condition, linearity of betamethasone was achieved from 40.0 to 480 ng spot^{-1} with the line equation $Y = 57.79 + 3.56X$. The calculated relative process standard deviation (V_{XO}) and $X_p$values[10] of betamethasone were 2.9% and 29.3 ng spot^{-1}, respectively ($n = 10$; sdv = 2.5; $r = 0.99896$). ANOVA regression testing of the linearity of

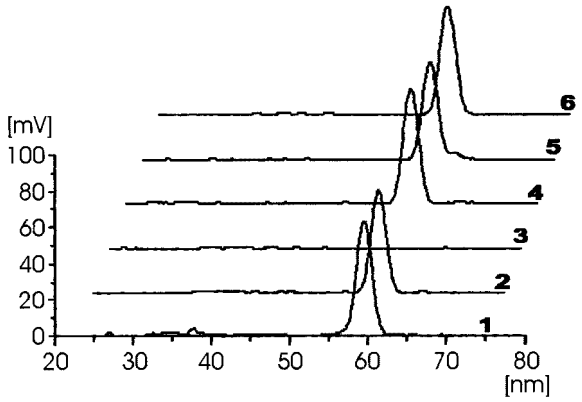

Figure 2. Densitograms (at 245 nm) obtained from: (1) solution of standard betamethasone (Sigma), (2) solution of betamethasone from Tianjin Tianyao Pharmaceutical Co. Ltd., (3) extract of excipient of Benoson® tablets, (4) extract of Benoson® tablets, (5) extract of commercial tablets T-1, and (6) extract of commercial tablets T-2.

the regression line showed a significant calculated F-value (3838.5 for $p < 0.0001$). The plots of the residuals against the quantities of the analyte confirmed the linearity of the basic calibration graphs (data not shown). The residuals were distributed at random around the regression line; neither trend nor uni-directional tendency was found.

The basic calibration curve showed variance homogeneity over the whole range. The calculated parameter *Prüftwert* (PW)[10] was 4.15. The PW-value was less from the F_{table}-value (5.35; for $f_1 = 9$, $f_2 = 9$; $\alpha = 0.01$).

Detection limit was determined by making a linear regression of relatively low concentrations[10] of betamethasone (6.00 to 80.0 ng spot^{-1}; $n = 8$; $V_{XO} = 3.0\%$; sdv = 3.2; $r = 0.9993$; line equation $Y = 74.2 + 17.0X$). The ANOVA regression-test showed a significant F-value (4499.4 for $p < 0.0001$). By this method, the calculated X_p[10] value was 4.80 ng spot^{-1}; in this case, DL $= X_p$.[10] According to Carr and Wahlich,[13] the value of quantitation limit (QL) could be estimated as three times the DL-value (14.4 ng spot^{-1}).

Table 1 demonstrates the high accuracy of the new method as revealed by the percentage of mean recovery data (99.2–100.1%). To prove whether systematic errors occurred, a linear regression of the recovery curve of X_f (percentage of label claim of the analyte found by the proposed method) against X_c (nominal percentage of label claim of the analyte after addition with the standard) of the Benoson® tablets was constructed.[10] In this case, the recovery curve equation was $X_f = 15.5 + 0.894X_c$. The confidence range data ($p = 0.05$) of the intercept VBa_f (15.5 ± 25.4) and slope VBb_f (0.894 ± 0.172) from the recovery curves did not reveal the occurrence of constant- or proportional-systematic errors.[10]

All of the values of the repeatability and intermediate precision evaluations were less than 2% (see Table 2). These values were also less than the required values as described by Ermer[14] (2.3%; specification range of 95–105%; basic lower limit 99.0%; $n = 6$). The three measurements were performed within one laboratory by different analysts on different plates and days.

Due to the higher price of HPTLC plates compared to conventional TLC plates (in Indonesia), the application of "re-used" HPTLC plates has been tested. After being used for analyzing samples, the plates were developed again with the mobile phase, then air dried for 60 min before using for the next measurements. Although statistical calculation using one-way ANOVA showed no significant difference ($p = 0.70$) between the percentage of the label claim found, the RSD of the precision-testing of the plates reused three times showed a value >2% (Table 3). This showed that the same HPTLC plates can be used three times in this system, so the operation cost for the measurement can be reduced.

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Table 1. Accuracy results of pharmaceutical preparations.

Pharmaceutical preparations	Ingredient	Label claim (%)				
		Original[a]	Added	Theory	Found	Recovery (%)
T-1	Betamethasone	100.6 ± 1.88	30.0[b]	130.6	129.6	99.2[c]
T-2	Betamethasone	79.3 ± 0.97	30.0[b]	109.2	109.0	99.8[c]
Benoson® tablets	Betamethasone	96.5 ± 0.78	30.0	126.5	126.9	100.3
			40.0	136.5	139.7	102.3
			50.0	146.5	147.1	100.4
			60.0	156.5	154.5	98.7
			70.0	166.5	164.2	98.6
Mean Recovery (mean ± SD)						100.1 ± 1.51

[a]Mean ± SD ($n = 5$).
[b]$n = 2$.
[c]Average of two measurements.

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Table 2. Results from evaluation of precision of Benoson® tablets.

Measurement[a]	RSD value (%, $n = 6$)[b]
1	1.12
2	1.39
3	1.60

[a]Each measurement was performed by a different analyst and on a different plate on different days.
[b]Evaluated by one analyst on one plate (repeatability).

Table 3. Results from evaluation of Benoson® tablets using new and "re-used" HPLTC plates.

HPLTC plate[a]	% of label claim (mean ± SD; $n = 5$)	RSD (%)
New-plate	96.1 ± 1.37	1.42
Re-used once	96.2 ± 1.85	1.92
Re-used twice	96.6 ± 1.36	1.41
Re-used three times	97.1 ± 2.85	2.94

RSD = relative status deviation.

Therefore, the proposed method is suitable for the routine analysis of products of similar composition in pharmaceutical industry quality control laboratories. Our experiences also showed that the HPTLC method is relatively cheaper, simpler, and faster compared to the LC methods.[4]

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