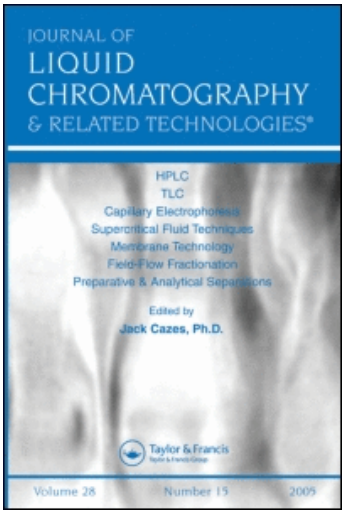

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SIMULTANEOUS DENSITOMETRIC DETERMINATION OF BETAMETHASONE VALERATE AND MICONAZOLE NITRATE IN CREAM, AND ITS VALIDATION

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ABSTRACT

A simple and rapid densitometric method has been developed for simultaneous determination of betamethasone valerate and miconazole nitrate in cream preparations. After extraction of the analyte with 96 % ethanol, the extracts were spotted on pre-coated silica gel plates, which were eluted two times with a mixture of chloroform-acetone-glacial acetic acid, 34+4+3 (v/v/v). Quantitative evaluation was performed by measuring the absorbance reflectance of the analyte spots at λ = 233 nm. The densitometric method is selective, precise, and accurate, and can be used for routine analysis of the cream preparations in pharmaceutical industry quality control laboratories.

INTRODUCTION

Many pharmaceutical preparations containing betamethasone valerate as the sole active ingredient, or in combination with other drugs, are marketed in Indonesia for topical application as cream or ointments.[1] Benoson-M® is a cream preparation recently produced in Indonesia by Bernofarm Pharmaceutical Company, Surabaya. This cream contains betamethasone valerate (1.2 mg g^{-1}) and miconazole nitrate (20 mg g^{-1}) as the active ingredients. The Indonesian Pharmacopoeia IV,[2] Indian Pharmacopoeia 1996,[3] USP 23 – NF 18,[4] USP 23 – NF 18, 3rd Supplement,[5] BP 1993,[6] and BP 1993 addendum 1995[7] contain no simultaneous assay for that combination. An on-line post-column photo-chemical derivatization in liquid chromatographic-diode-array detection analysis of betamethasone valerate or miconazole nitrate in combination with other drugs has been reported.[8] Densitometric determination of betamethasone valerate or miconazole nitrate with other drugs has been reported,[9,10] but no methods are available for the two drugs in combination.

The aim of this work was to develop a simple densitometric method for routine analysis of the combination of betamethasone valerate and miconazole nitrate in cream pharmaceutical preparations.

EXPERIMENTAL

Materials and Reagents

Betamethasone valerate (The Upjohn Company, Michigan, USA; Batch 13 AAP; Assay 100.20 %), miconazole nitrate (Baxanes Investment Est., Zurich, Switzerland; Batch 93001; Assay 99.45 %) were pharmaceutical grade. Both substances were used as received for preparing laboratory-made cream and standard solutions. Their UV, IR spectra and melting points were identical to those of the authentic standards (Sigma, St.Louis, Mo. USA).

Acetone, chloroform, glacial acetic acid (J. T. Baker) were analytical grade reagents; 96 % ethanol was extra pure quality (E. Merck). The solvents and reagents were used without further purification. Excipients for laboratory-made creams (isopropyl myristate, cetyl alcohol, emulgin B_1®, glycerin, nipagin, nipasol, citric acid, lavender oil, and distilled water) were pharmaceutical grade.

Laboratory-made creams were prepared containing five different concentrations of betamethasone valerate (0.96, 1.08, 1,20, 1.32, and 1.44 mg g^{-1}) and miconazole nitrate (16, 18, 20, 22 and 24 mg g^{-1}).

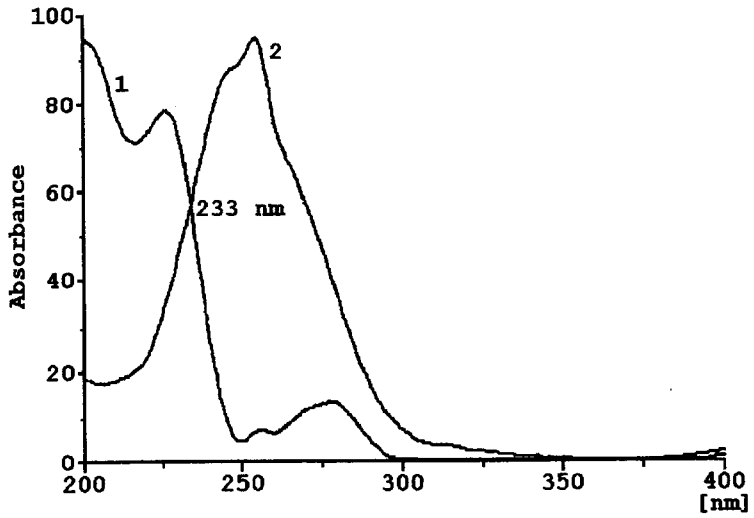

Figure 1. *In situ* absorbance reflectance spectra of miconazole nitrate (1) and betamethasone valerate (2) spots from λ = 200 to 400 nm, with their intersection point (at 233 nm). Stationary phase: precoated plate silica gel 60 F_{254} (E. Merck); mobile phase: chloroform–acetone -glacial acetic acid, 34+4+3 (v/v/v), two times elution.

Stock standard solutions were prepared daily by dissolving accurately weighed betamethasone valerate (24.0 mg) and miconazole nitrate (400.0 mg) in 96 % ethanol (50.0 mL). Various standard solutions were prepared from the stock solution by dilution with 96 % ethanol. For linearity studies, solutions were prepared containing 80, 96, 120, 144, 180, 192, 213, and 240 μg mL^{-1} betamethasone valerate and 1333, 1600, 2000, 2400, 3000, 3200, 3555, and 4000 μg mL^{-1} miconazole nitrate. 4 μL of these solutions was spotted on the TLC plate.

Sample Extraction

1 g of cream (accurately weighed) was transferred into a 10.0 mL volumetric flask and about 8 mL of 96 % ethanol was added, ultrasonicated for 15 minutes and diluted up to volume with 96 % ethanol. When the sample was not completely soluble, the mixture was centrifuged and filtered through Whatman type 40, and diluted to 10.0 mL with 96 % ethanol. Four μL of this solution was spotted on the TLC plates.

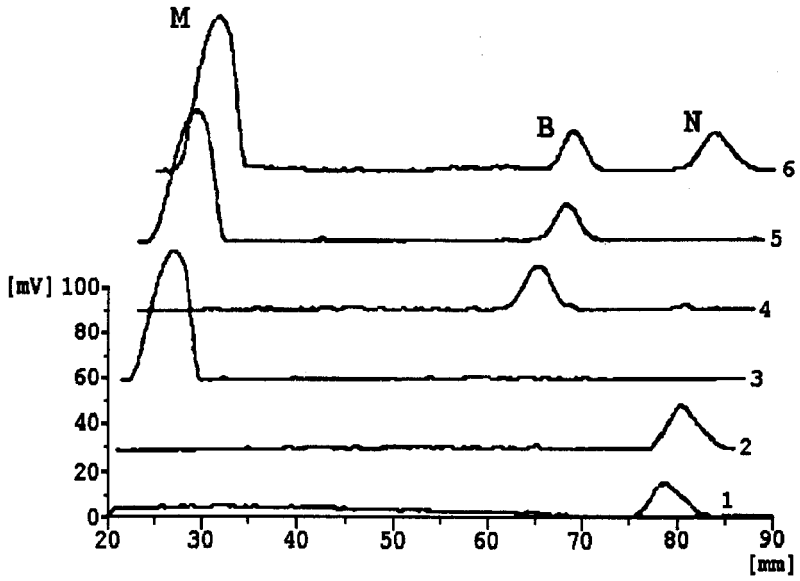

Figure 2. Densitograms (λ = 233 nm) obtained from: (1) extract from excipients; (2) solution of nipagin and nipasol; (3)standard solutions of miconazole nitrate, (4) betamethasone valerate and (5) its mixture; (6) extract of laboratory-made cream. Peak identities: (M) miconazole nitrate; (B) betamethasone valerate; (N) nipagin and nipasol.

Chromatography

Chromatography was performed on pre-coated silica gel 60 F_{254} plates (E. Merck. # 1.05554); a Nanomat III (Camag, Muttenz, Switzerland) was used for sample application. The mobile phase used in this experiment, chloroform – acetone - glacial acetic acid, 34 + 4 + 3 (v/v/v), was a modification of that used in previous work.[11] Ascending development was performed in a Camag twin-through chamber (20 x 10 cm); the mobile phase migration distance in all experiments was 8.0 cm. In this system, elution was performed two times (development time *ca.* 2 x 15 min at 25±1^{o} C).

Densitometric scanning was performed with a Camag TLC-Scanner II. The purity and identity of the analyte spots were determined by scanning the absorbance - reflectance mode from 200 to 400 nm. Quantitative evaluation was performed by measuring the absorbance reflectance of the analyte spots at 233 nm (See Figure 1). The densitometric scanning parameters 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, E = end spectrum) and quantification of the analyte spots were 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) and accuracy by the method of Funk et al.[12] The selectivity of the method was proved by identification and purity checks of the analyte spots. A five-point accuracy study (80 - 120 % of the expected value) was performed for the laboratory-made creams. The precision was evaluated by analyzing six different extract aliquots from laboratory-made creams containing 1.20 mg g^{-1} betamethasone valerate and 20 mg g^{-1} miconazole nitrate according to a modified method of Renger et al.[13]

RESULTS AND DISCUSSION

After the TLC-plate was eluted two times, the densitogram (Figure 2) showed the spots of miconazole nitrate (R_f 0.26), betamethasone valerate (R_f 0.67) well separated from the spots of nipagin and nipasol (R_f 0.81). These preservatives could not be separated in this system, so it appeared as one peak. In this work, the plate must be eluted two times due to the very low migration distance (R_f = 0.06) of miconazole nitrate by the first elution. For quantitative determination by TLC, the R_f- values of the analyte spots should be in the range of 0.3 to 0.8.[14] This TLC-system demonstrated that all the analyte spots of the cream extracts, furnished *in situ* UV spectra identical with those of standards (r>0.9999). Purity check of the analyte spots using CATS software also showed that all the analyte spots of the cream extracts were pure. The values of $r_{S,M}$ and $r_{M,E}$ were >0.9999, demonstrating that the proposed TLC method is highly selective.

The basic calibration plot of peak area against amount of analyte was constructed within the ranges 66 - 200 % of the expected values in cream preparations. Under this condition, linearity of betamethasone valerate was achieved from 320 to 960 ng $spot^{-1}$. The relative process standard deviation value V_{XO}[12] of betamethasone valerate was 0.92 % (n = 8; r = 0.9997). For miconazole nitrate, linearity was achieved from 5.3 to 16.0 μg $spot^{-1}$ (V_{XO} = 1.69 %; n = 8; r = 0.9991). ANOVA regression-test for testing linearity of the two regression lines showed very significant calculated F-values (10901.1 for betamethasone valerate; 3211.7 for miconazole nitrate; critical value $F_{1,6}$ = 13.74, α = 0.01).

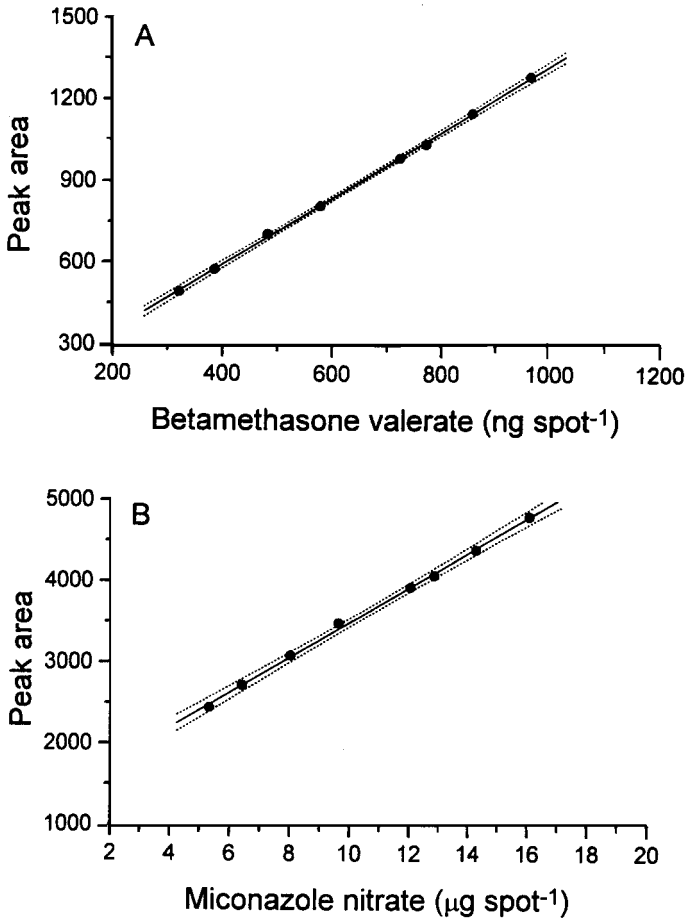

Figure 3. Basic calibration curves and their confident bands (α = 0.01) for (A) betamethasone valerate (regression equation Y = 104.95 + 1.23X) and (B) miconazole nitrate (regression equation Y = 1340.97 + 216.19 X).

The basic calibration curves are presented in Figure 3. The plots of the residuals against the quantities of the analyte confirmed the linearity of the basic calibration graphs (Figure 4). The residuals were distributed at random around the regression line, neither trend nor uni-directional tendency was found.

Those two basic calibration curves showed variance homogeneity over the whole range. The calculated parameter PW^{12} was 0.81 (for betamethasone valerate) and 3.35 (for miconazole nitrate). All the PW-values were less from

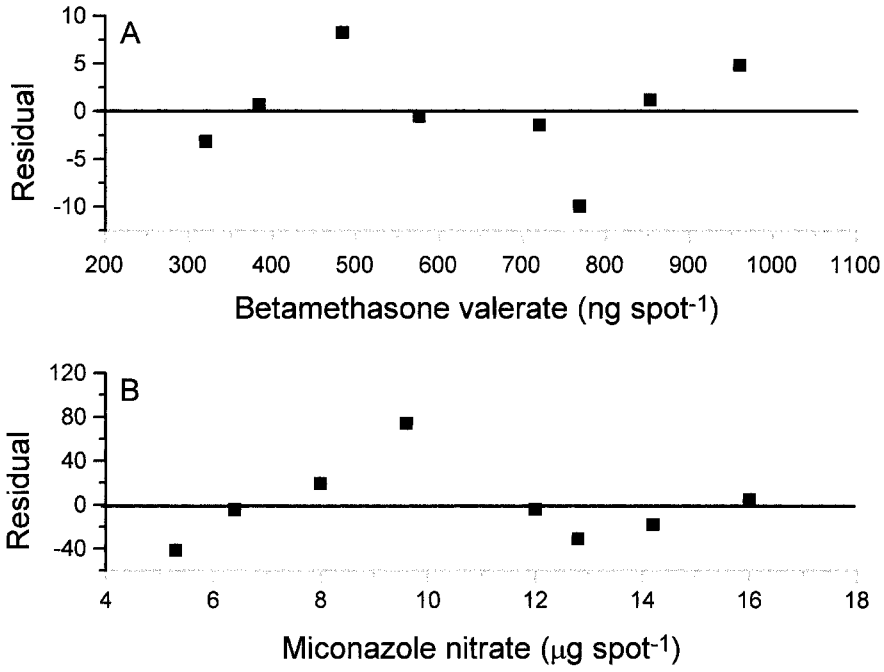

Figure 4. Residual plot (peak area) in the linear working range of (A) betamethasone valerate and (B) miconazole nitrate.

the F_{table}-value (5.35; for f_1=9, f_2=9; α=0.01). DL was determined by making a linear regression of relatively low concentration of betamethasone valerate (76 to 384 ng spot^{-1}; n=9; V_{XO} = 4.8 %; r = 0.997) and miconazole nitrate (1.3 to 6.4 μg spot^{-1}; n=9; V_{XO} = 3.9 %; r = 0.998).[12] By this method, the DL values were 50.9 ng spot^{-1} (betamethasone valerate) and 0.68 μg spot^{-1} (miconazole nitrate). According to Carr and Wahlich,[15] the value of quantitation limit (QL) could be estimated as 3 times the DL-value.

Table 1 demonstrated the high accuracy as revealed by the percentage of mean recovery data (100.1 and 100.5 %). To prove whether systemic errors occurred, linear regression of recovery curves of X_f (concentration of the analyte measured by the propose method) against X_c (nominal concentration of the analyte) were constructed.[12]

The confidence range data (α=0.05) of the intercept {VB(a_f)} and slope {VB(b_f)} from the recovery curves did not reveal the occurrence of constant- and proportional-systematic errors.

Table 1

Results from Determination of Accuracy on Laboratory-Made Cream

No.	Betamethasone Valerate (ng spot^{-1})		Miconazole Nitrate (µg spot^{-1})	
	X_c*	X_f*	X_c*	X_f*
1	384.0	373.9	6.40	6.35
2	384.0	386.9	6.40	6.42
3	432.0	435.4	7.20	7.25
4	480.0	479.7	8.00	8.13
5	480.0	479.1	8.00	8.19
6	528.0	520.9	8.80	8.67
7	528.0	526.0	8.80	8.91
8	576.0	590.0	9.60	9.74
9	576.0	583.8	9.60	9.50
Mean recovery ± SD (%)	100.08 ± 1.48		100.49 ± 1.36	
Recovery curve	X_f = -23.0 +1.05 X_C		X_f = 0.02 + 1.00 X_C	
$VB(a_f)^+$	-23.04 ±37.70		0.02 ± 0.67	
$VB(b_f)^+$	1.05 ±0.08		1.00 ± 0.08	

* X_C = nominal concentration of the analyte in the spotted solution; X_f = measured concentration of the analyte in the spotted solution. + For • = 0.05.

Table 2

Results from Evaluation of Precision

Measurement*	RSD-Value (n=6)	
	Betamethasone valerate**	Miconazole nitrate**
1	0.69 %	1.05 %
2	0.57 %	0.89 %
3	1.32 %	1.67 %

* Each measurement was performed by a different analyst on different days and on different plates. ** Evaluated on one plate by one analyst (repeatability).

All the RSD of the repeatability and intermediate precession evaluations have values less than 2 % (see Table 2). The three measurements were performed within one laboratory by different analysts on different plates and days. These results demonstrated that the accuracy and precision of the proposed method were satisfactory. Therefore the proposed method is suitable for the routine analysis of cream and products of similar composition in pharmaceutical industry quality control laboratories.

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REFERENCES

1. *IIMS, MIMS Indonesia, Vol. 25, No. 3.* Singapore: MIMS Asia (1996), pp. 349-351, 354, 355.

2. *Farmakope Indonesia Edisi IV*. Jakarta, Indonesia: Departemen Kesehatan Republik (1995), pp. 142-144, 564-566.

3. *Indian Pharmacopoeia 1996.* New Delhi: The Controller of Publication (1996), pp. 103-104, 492-494.

4. *The United States Pharmacopoeia 23 – National Formulary 18*. Rockville, *Maryland*: United States Pharmacopoeial Convention (1994), pp. 195-196, 1026-1028.

5. *The United States Pharmacopoeia 23 – National Formulary 18, 3rd Supplement.* Rockville, Maryland: United States Pharmacopoeial Convention (1995), pp. 2933-2935.

6. *British Pharmacopoeia 1993, Volume II.* London: HMSO (1993), pp. 795-798, 10144.

7. *British Pharmacopoeia 1993, Addendum 1995.* London: HMSO (1995), pp. 1632-1635.

8. A. M. Di Pietra, V. Andrisano, R. Gotti, V. Cavrini. *J. Pharm. Biomed. Anal*., **14**, 1191-1199 (1996).

9. *Camag Bibliography Service, Cumulative CD version 1.00.* Muttenz: Camag (1997).

10. G. Indrayanto, I. Wahyuningsih, R. K. Salim. *J. Planar Chromatogr*., **10**, 204-207 (1997).

11. G. Indrayanto, L. Aditama, W. Tanudjaja, S. Widjaja. *J. Planar Chromatogr*., (In press).

12. W. Funk, V. Damman, G. Donnervert. *Qualitätssicherung in der Analytischen Chemie*, Weinheim, New York, Basel, Cambridge: VCH (1992), pp. 12-36, 161-180.

13. B. Renger. H. Jehle, M. Fischer, W. Funk. *J. Planar Chromatogr*., **8**, 269-278 (1995).

14. H. P. Frey, K. Zieloff. *Qualitative und Quantitative Duennschicht-Chromatographie (Planar Chromatography)*, Weinheim, New York, Basel, Cambridge: VCH (1993), pp. 324-325.

15. G. P. Carr, J. C. Wahlich. *J. Pharm. Biomed. Anal*., **8**, 613-618 (1990).

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