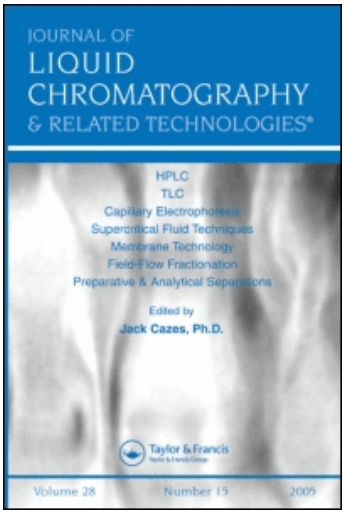
This article was downloaded by:
On: *23 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

TLC Densitometric Determination of Mometasone Furoate in Topical Preparations: Validation

Lestyo Wulandari[a]; Tan Kiauw Sia[a]; Gunawan Indrayanto[b]
[a] R & D Laboratory, Bernofarm Pharmaceutical Company, Surabaya, Indonesia [b] Department of Natural Product Chemistry, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia

Online publication date: 30 January 2003

To cite this Article Wulandari, Lestyo , Sia, Tan Kiauw and Indrayanto, Gunawan(2003) 'TLC Densitometric Determination of Mometasone Furoate in Topical Preparations: Validation', Journal of Liquid Chromatography & Related Technologies, 26: 1, 109 — 117
To link to this Article: DOI: 10.1081/JLC-120017156
URL: http://dx.doi.org/10.1081/JLC-120017156

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016
©2002 Marcel Dekker, Inc. All rights reserved. This material may not be used or reproduced in any form without the express written permission of Marcel Dekker, Inc.

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 26, No. 1, pp. 109–117, 2003

TLC Densitometric Determination of Mometasone Furoate in Topical Preparations: Validation

Lestyo Wulandari,[1] Tan Kiauw Sia,[1] and Gunawan Indrayanto[2,*]

[1]R & D Laboratory, Bernofarm Pharmaceutical Company, Surabaya, Indonesia
[2]Department of Natural Product Chemistry, Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia

Downloaded At: 20:27 23 January 2011

ABSTRACT

A simple and rapid densitometric method has been developed for determination of mometasone furoate in the topical pharmaceutical preparations. After extraction of the analyte with 96% ethanol, the extracts were spotted on pre-coated silica gel plates, which were then developed with a mixture of dichloromethane-diethyl ether (3 : 1). Quantitative evaluation was performed by measuring the absorbance reflectance of the analyte spots at 260 nm. The densitometric method is selective, precise, and accurate and can be used for routine analysis of the topical preparations in pharmaceutical industry quality control laboratories.

*Correspondence: Gunawan Indrayanto, Department of Natural Product Chemistry, Faculty of Pharmacy, Airlangga University, Jl. Dharmawangsa dalam, Surabaya 60286, Indonesia; E-mail: indrayanto@hotmail.com.

DOI: 10.1081/JLC-120017156 1082-6076 (Print); 1520-572X (Online)
Copyright © 2003 by Marcel Dekker, Inc. www.dekker.com

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016
©2002 Marcel Dekker, Inc. All rights reserved. This material may not be used or reproduced in any form without the express written permission of Marcel Dekker, Inc.

Key Words: Mometasone furoate; TLC; Topical preparations; Validation.

INTRODUCTION

Some topical pharmaceutical preparations containing mometasone furoate, 9α, 21-dichloro-11β, 17α-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione 17-(2-furoate) as the sole active ingredient, are marketed now in Indonesia.[1] Mometasone furoate is a high potent chlorinated glucocorticoid with a favorable ratio between local and systemic side effects.[2] The USP 25-NF 20,[3] describes an HPLC method for assaying mometasone furoate. A spectrophotometric method was described in the BP 2000[4] for the determination of mometasone furoate. Teng et al.[2] reported the determination of mometasone furoate and its degradation products in biological fluids using HPLC. Wang et al.[5] published a competitive enzyme immunoassay for the direct determination of mometasone furoate in human plasma. The determination of clotrimazole and mometasone furoate in pharmaceutical preparation, using HPLC overloaded dual wavelength detection, was also reported.[6] USP 25-NF 20[3] and BP 2000[4] described TLC methods for purity determination and qualitative identification of mometasone furoate.

The aim of this work was to develop a simple and rapid TLC densitometric method for routine analysis of mometasone furoate in the topical pharmaceutical preparations.

Downloaded At: 20:27 23 January 2011

EXPERIMENTAL

Materials and Reagents

Mometasone furoate (Sterling S.N.I.F.F., Corciano Ferugia, Italia; Batch MTC15-023/01; Assay 100.4%; Conforms to USP 24) was pharmaceutical grade. The substance was used as received for preparing laboratory-made pharmaceutical preparations and standard solutions.

Dichloromethane, (E. Merck, Germany), 96% ethanol, diethyl ether (JT Baker, USA), were analytical grade reagents. The solvents were used without further purification. Excipients for laboratory-made cream preparations (isopropyl myristate, cetyl alcohol, emulgin $B_1^{®}$, propylene glycol, nipagin, nipasol, and distilled water) were pharmaceutical grade.

Laboratory-made cream preparations were prepared containing five different concentrations of mometasone furoate (0.8, 0.9, 1.0, 1.1, and 1.2 mg g^{-1}); these were used for accuracy determination.

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016
©2002 Marcel Dekker, Inc. All rights reserved. This material may not be used or reproduced in any form without the express written permission of Marcel Dekker, Inc.

Commercial mometasone furoate preparations containing $1\,mg\,g^{-1}$ were purchased in December 2001 from a local pharmacy in Surabaya (cream-1, cream-2, ointment-1, oinment-2, and lotion). All the commercial pharmaceutical preparations were produced in Indonesia.

Stock standard solutions were prepared daily by dissolving accurately weighed mometasone furoate (25.0 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 200, 188, 150, 125, 100, 75, 63, 50, and $42\,\mu g\,mL^{-1}$, and $2\,\mu L$ of these solutions was spotted on the TLC plate.

Sample Extraction

One thousand milligram of the topical preparations (accurately weighed) was transferred into a 10.0 mL volumetric flask and about 8 mL of 96% ethanol was added, ultrasonicated for 15 min, mixed with a vortex-mixer for 5 min, 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. Two μL of this solution was spotted on the TLC plates.

Chromatography

Chromatography was performed on pre-coated silica gel 60 F_{254} TLC aluminium-backed sheets (E. Merck. # 1.05554); a Nanomat III equipped with a dispenser magazine containing $2\,\mu L$ glass capillaries (Camag, Muttenz, Switzerland), was used for sample application. The mobile phase used was a mixture of dichloromethane-diethyl ether (3:1), this composition was modified from that described in BP2000.[4] Ascending development was performed in a Camag twin-through chamber (for 20×10 cm plates); the mobile phase migration distance in all experiments was 8.0 cm (development time *ca*. 15 min. at $25 \pm 2^{\circ}C$; R_f of mometasone furoate was *ca*. 0.38).

Densitometric scanning was performed with a Camag TLC-Scanner II. The purity and identity of the analyte spots were determined by scanning, in the absorbance-reflectance mode from 200 to 400 nm. Quantitative evaluation was performed by measuring the absorbance reflectance of the analyte spots at $\lambda_{maximum}$ 260 nm (See Fig. 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), sdv (relative standard deviation), and

Downloaded At: 20:27 23 January 2011

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016
©2002 Marcel Dekker, Inc. All rights reserved. This material may not be used or reproduced in any form without the express written permission of Marcel Dekker, Inc.

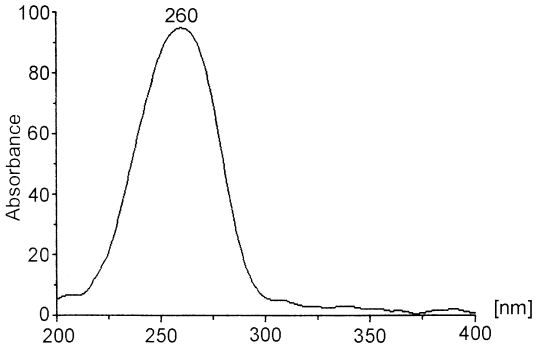


Figure 1. In situ absorbance reflectance spectrum of mometasone furoate from 200 to 400 nm, with its λ maximum at 260 nm. Stationary phase: pre-coated TLC plate silica gel 60 F_{254} (E. Merck); mobile phase: a mixture of dichloromethane–diethyl ether (3 : 1).

Downloaded At: 20:27 23 January 2011

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), accuracy, and range by the method of Funk et al.[7] and Hahn-Dienstrop.[8] The selectivity of the method was proven by identification and purity checks of the analyte spots. A five-point accuracy study (80–120% of the expected value) was performed on the laboratory-made cream preparations. For commercial preparations, accuracy studies were performed using single-point standard addition methods (30% of label claim). The precision was evaluated by analyzing six different extract aliquots from laboratory-made creams containing mometasone furoate (0.8, 1.0, and 1.2 mg g^{-1}), according to a modified method of Renger et al.[9]

RESULTS AND DISCUSSION

After the TLC plate was developed, the densitograms (Fig. 2) showed that the spot of mometasone furoate (R_f 0.38) could be well separated with the spot of the preservatives nipagin and nipasol (R_f 0.59). In this system, spots of nipagin and nipasol could not be separated. An unknown spot (R_f 0.83) was

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016
©2002 Marcel Dekker, Inc. All rights reserved. This material may not be used or reproduced in any form without the express written permission of Marcel Dekker, Inc.

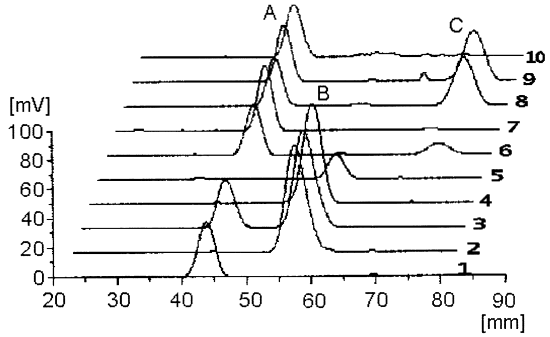


Figure 2. Densitograms ($\lambda = 260$ nm) obtained from: (1) solution of standard mometasone furoate, (2) extract from excipients of laboratory-made cream, (3) extract of laboratory-made cream, (4) solution of nipagin, (5) solution of nipasol, (6) extract of commercial cointment-1, (7) extract of commercial lotion, (8) extract of commercial cream-1, (9) extract of commercial ointment-2, and (10) extract of commercial cream-2. Peak identities: (A) mometasone furoate, (B) nipagin and nipasol, (C) unknown.

Downloaded At: 20:27 23 January 2011

detected in extracts of commercial ointment-1, ointment-2, and cream-1. This TLC system demonstrated that all the analyte spots of the laboratory-made cream and commercial extracts yielded in situ UV absorption spectra that are identical to the standard ($r > 0.9999$). Purity checks using CATS software also showed that all of the analyte spots of the laboratory-made cream and commercial extracts were pure. The values of $r_{S,M}$ and $r_{M,E}$ were >0.9999, demonstrating that the proposed TLC method is selective.

The basic calibration plot of peak area against amount of analyte was constructed within the ranges 40–200% of the expected values in the pharmaceutical preparations. Under this condition, linearity of mometasone furoate was achieved from 85 to 400 ng spot^{-1} with line equation $Y = 265.16 + 4.36$ X. The relative process standard deviation (V_{XO}) and X_P values[7] of mometasone furoate were 3.5% and 37 g spot^{-1}, respectively ($n = 9$; sdv $= 3.2$; $r = 0.9981$). ANOVA regression-test for testing linearity of the regression line showed significant calculated F-value (1854.98 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 PW[7] was 0.61. The PW-value was less from the F_{table}-value (5.35; for $f_1 = 9, f_2 = 9$; $\alpha = 0.01$).

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016
©2002 Marcel Dekker, Inc. All rights reserved. This material may not be used or reproduced in any form without the express written permission of Marcel Dekker, Inc.

Downloaded At: 20:27 23 January 2011

Table 1. Results from determination of the accuracy of analysis of the laboratory-made (LM) and commercial preparations.

Sample	Amount found[a] (mean ± SD)[e]	Amount added[a]	%Recovery (mean ± SD)	Recovery curve[b]	$V_{B(af)}$[c]	$V_{B(bf)}$[c]
LM-cream	–	–	100.4 ± 0.8[d]	$X_f = -2.52 + 1.01\ X_c$	-2.52 ± 17.38	1.01 ± 0.09
Cream-1	100.5 ± 1.3	30	100.1 ± 1.5[e]	–	–	–
Cream-2	99.5 ± 1.8	30	100.3 ± 0.4[e]	–	–	–
Ointment-1	100.6 ± 1.1	30	100.2 ± 0.9[e]	–	–	–
Ointment-2	99.6 ± 0.7	30	100.7 ± 1.5[e]	–	–	–
Lotion	100.5 ± 1.1	30	100.3 ± 0.7[e]	–	–	–

[a]% of label claim.
[b]X_f and X_c are, respectively, the measured and nominal amount of the analyte spotted (ng spot^{-1}).
[c]For $p = 0.05$.
[d]$n = 5$.
[e]$n = 4$.

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016
©2002 Marcel Dekker, Inc. All rights reserved. This material may not be used or reproduced in any form without the express written permission of Marcel Dekker, Inc.

DL was determined by making a linear regression of relatively low concentrations[7] of mometasone furoate (10 to 125 ng spot^{-1}; $n = 6$; $V_{XO} = 2.0\%$; sdv = 2.5; $r = 0.9997$; line equation $Y = 1598 + 14.58$ X). ANOVA regression-test showed significant F-value (6679.6 for $p < 0.0001$). By this method, the calculated X_p[7] value was 6.75 ng spot^{-1}, in this case DL = X_p.[7] According to Carr and Wahlich,[10] the value of quantitation limit (QL) could be estimated as 3 times the DL-value (20 ng spot^{-1}).

Table 1 demonstrates the high accuracy as revealed by the percentage of mean recovery data (100.1–100.7%). To prove whether systemic errors did not occur, linear regression of recovery curve of X_f (concentration of the analyte measured by the proposed method) against X_c (nominal concentration of the analyte) of the laboratory-made cream was constructed.[7] The confidence range data ($p = 0.05$) 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.

All the RSD values of the repeatability and intermediate precision evaluations were less than 2% (see Table 2). These values were also less than the required values that were described by Ermer[11] (1.44%; specification range of 95–105%; basic lower limit 97.5%; $n = 6$). The three measurements were performed within one laboratory by different analysts on the different plates and days. These results demonstrated that the accuracy and precision of the proposed method were satisfactory in the range[8] of 80 to 120% of the expected value of the topical preparation.

Downloaded At: 20:27 23 January 2011

Table 2. Results from evaluation of precision of laboratory-made cream.

	RSD-value ($n = 6$)[b]		
Measurement[a]	Laboratory-made cream A[c]	Laboratory-made cream B[d]	Laboratory-made cream C[e]
1	0.72	1.13	0.75
2	1.04	1.18	1.0
3	1.2	0.77	0.77

[a]Each measurement was performed by a different analyst on different days and on different plates.
[b]Evaluated on one plate by one analyst (repeatability).
[c]Content of mometasone furoate was 0.8 mg g^{-1}.
[d]1.0 mg g^{-1}.
[e]1.2 mg g^{-1}.

©2002 Marcel Dekker, Inc. All rights reserved. This material may not be used or reproduced in any form without the express written permission of Marcel Dekker, Inc.

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 TLC method is relatively cheaper, simpler, and faster, compared to the HPLC methods.[2,,3]

ACKNOWLEDGMENTS

The authors are very grateful to Achmad Toto Poernomo (Faculty of Pharmacy, Airlangga University), Aloysius Indrayanto (ITS, Surabaya), and Koencoro Foe (UWM, Surabaya) for their nice technical assistance and for providing reference 2.

REFERENCES

1. ISO (Informasi Spesialite Obat) Indonesia, Volume XXXV, Ikatan Sarjana Farmasi Indonesia, Jakarta, Indonesia, 2001, 16 pp., 370–371.
2. Teng, X.W.; Foe, K.; Brown, K.F.; Cutler, D.J.; Davies, N.M. High-performance liquid chromatographic analysis of mometasone furoate and its degradation products: application to in vitro degradation studies. J. Pharm. Biomed. Anal. **2001**, *26*, 313–319.
3. *The United States Pharmacopoeia 25—National Formulary 20*, Asian Ed.; United States Pharmacopoeial Convention: Rockville, MD, 2001; 1166–1168.
4. *British Pharmacopoeia 2000*; The Stationary Office: London, 2000; Vol 1, 1065–1066.
5. Wang, C.J.; Tian, Z.; Brynes, K.; Lin, C.C. A competitive enzyme immunoassay for the direct determination of mometasone furoate (SCH 32088) in human plasma. J. Pharm. Biomed. Anal. **1992**, *10*, 473–479.
6. Zhu, J.; Coscolluella, C. Chromatographic assay of pharmaceutical compounds under column overloading. J. Chromatogr. B: Biomed. Sci. and Applic. **2000**, *741*, 55–65.
7. Funk, W.; Damman, V.; Donnervert, G. *Qualitätssicherung in der Analytischen Chemie*; VCH: Weinheim, New York, Basel, Cambridge, 1992; 12–36, 161–180.
8. Hahn-Dienstrop, E. *Applied Thin Layer Chromatography*; Wiley-VCH Verlag GmbH: Weinheim, 2000; 198–205.
9. Renger, B.; Jehle, H.; Fischer, M.; Funk, W. Validation of analytical procedures in pharmaceutical analytical chemistry: HPTLC assay of theophylline in an effervecent tablets. J. Planar Chromatogr. **1995**, *8*, 269–278.

Downloaded At: 20:27 23 January 2011

©2002 Marcel Dekker, Inc. All rights reserved. This material may not be used or reproduced in any form without the express written permission of Marcel Dekker, Inc.

10. Carr, G.P.; Wahlich, J.C. A practical approach to method validation in pharmaceutical analysis. J. Pharm. Biomed. Anal. **1990**, *8*, 613–618.
11. Ermer, J. Validation in pharmaceutical analysis. Part 1: An integrated approach. J. Pharm. Biomed. Anal. **2001**, *24*, 755–767.

Received April 26, 2002
Accepted August 9, 2002
Manuscript 5855

Downloaded At: 20:27 23 January 2011