DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY®
Vol. 30, No. 5, pp. 505–511, 2004

RESEARCH PAPER

The Use of Near-Infrared Spectroscopy for the Quantitation of a Drug in Hot-Melt Extruded Films

Sri Venkata S. Tumuluri,[1] Suneela Prodduturi,[1] Michael M. Crowley,[3] Steven P. Stodghill,[1,2] James W. McGinity,[3] Michael A. Repka,[1,2,*] and Bonnie A. Avery[1,2]

[1]Department of Pharmaceutics, School of Pharmacy and [2]Research Institute of Pharmaceutical Sciences, The University of Mississippi, University, Mississippi, USA
[3]Division of Pharmaceutics, College of Pharmacy, The University of Texas at Austin, Austin, Texas, USA

ABSTRACT

The objective of the study was to demonstrate the utility of near-infrared spectroscopy (NIRS) for quantitative analysis of a model drug in hot-melt extruded film formulations. Polyethylene oxide (PEO) films with clotrimazole (CT) as a model drug were prepared by hot-melt extrusion (HME) incorporating drug concentrations ranging from 0–20% and analyzed using a Fourier transform near-infrared (FT-NIR) spectrophotometer in the reflectance mode. High performance liquid chromatography (HPLC) was the reference method used for this study. The NIR calibration model derived for CT was composed of 21 frequency ranges that were correlated to the values quantified using the HPLC reference method. The NIR method developed resulted in an assayed CT amount in the film matrix to be within 3.5% of the quantity determined by the reference method. These studies clearly demonstrate that NIRS is a powerful method for the quantitation of active drug substances contained in films produced by HME and warrants further investigation.

Key Words: Clotrimazole; Extrusion; Near-infrared spectroscopy; Reflectance spectroscopy.

*Correspondence: Michael A. Repka, B.S.Pharm., D.D.S., Ph.D., Department of Pharmaceutics, School of Pharmacy, The University of Mississippi, P.O. Box 1848, University, MS 38677-1848, USA; Fax: (662) 915-1177; E-mail: marepka@olemiss.edu.

DOI: 10.1081/DDC-120037481
0363-9045 (Print); 1520-5762 (Online)

INTRODUCTION

The application of near-infrared (NIR) reflectance spectroscopy to pharmaceutical samples is proving to be a valuable means of providing fast and reliable drug information. Researchers have applied NIR reflectance spectroscopy to quantify the moisture content in various formulations, to distinguish placebo tablets from those containing active drug, and to classify granulations according to physical characteristics.[1,2] Buchanan et al. successfully used NIRS to evaluate the active drug component in film-coated tablets.[3] Various parameters of different polyethylenes such as density,[4] molecular weight, and crystallinity[5] have also been determined using NIRS. Workman used NIRS for the quantification of low density polyethylene, linear low density polyethylene, and high density polyethylene in polymer film mixtures.[6] The use of Fourier transform NIR (FT-NIR) spectroscopy for the quantitative analysis of an active ingredient in complex matrices such as translucent gels has also been demonstrated.[7] Another study has used NIRS for the identification of PVC-based films.[8] In addition, NIR spectroscopy has been employed to determine the matrix composition and to monitor hydration, degradation, and drug release kinetics.[9,10]

For pharmaceutical systems, several research groups have recently demonstrated that hot-melt extrusion (HME) techniques are valuable methods to prepare granules, pellets, sustained release tablets, and transdermal and transmucosal drug delivery systems.[11–18] More recently, researchers have proposed HME as a promising technique for various novel drug delivery systems, including films.[15,19–21] For pharmaceutical applications, hot-melt extrusion offers many advantages over traditional processing techniques.[14] These advantages include: 1) no organic solvents used, 2) fewer processing steps and equipment demands, 3) potential for a continuous process, and 4) lower processing costs. To produce pharmaceutical dosage forms via hot-melt extrusion, a pharmaceutical grade polymer must be selected that can be processed at a relatively low temperature due to the thermal sensitivity of many drugs.[14] All components must be thermally stable at the processing temperature during the short duration (approximately 30–120 seconds) of the heating process.

Polyethylene oxide (PEO) was chosen as the film former due to its low melting point, stability, and thermoplasticity. Clotrimazole (CT) was used the model drug due to its high thermal stability and its clinical applicability in the treatment of oral candidiasis.[22] Clotrimazole is an ideal antifungal agent for incorporation into film dosage forms for potential intraoral drug delivery. Its structure is illustrated in Fig. 1.

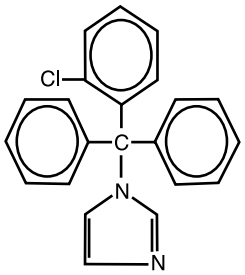

Figure 1. Chemical structure of clotrimazole.

Traditionally, the analytical method of choice for drug content determination has been high-performance liquid chromatography (HPLC). Although this method is widely used in the pharmaceutical industry, it has several inherent drawbacks. An invasive and destructive technique, HPLC is also expensive (solvents and disposal) and often requires extensive sample preparation and long run times. Alternatively, the proposed NIR method of analysis is nondestructive, allowing a film sample to be analyzed by both NIR and HPLC techniques.

NIR spectroscopy offers many advantages for the acquisition of specific spectral information. These advantages include, in part, the elimination of special optical components, the convenience of atmospheric transparency, and the extension of the linear dynamic range to fairly large concentrations of analyte.[23,24] Also, NIR spectroscopy offers the advantage of minimizing or eliminating sample preparation.[23,24] In the pharmaceutical industry, material identification and quantification is an important analytical procedure that often requires the use of time-consuming HPLC and other spectroscopic analytical procedures. The advantages of using FT-NIR spectroscopy for quantification include: 1) samples can be analyzed directly through glass and other packaging materials, thus reducing sample preparation requirements; 2) FT-NIR can provide data analysis and interpretation in a matter of seconds; and 3) samples can be retained in their original packages for future measurements, which is a major advantage of this nondestructive analysis.

Process analytical technology (PAT) is the use of on-line sensors to effect the timely measurement and control of an industrial process.[25] Applications of near-infrared spectroscopy and its feasibility as a process analytical technique have been reported, but have not been exploited to its fullest extent. The use of PAT to improve the control and understanding of some

important steps in the production of pharmaceutical products has gained tremendous importance. However, limited research has been reported to date on the quantification of actives in film matrices (either on-line or off-line) by NIRS. In addition, since HME technology is a continuous process, NIRS quantification for films produced via this technique may also have applicability to on-line quantification.

The objective of the present study is to demonstrate the use of NIRS for the off-line quantitative analysis of clotrimazole in HME film matrices. Hence, this study is aimed at developing a method to quantify a model drug incorporated into hot-melt extruded polymeric matrices.

EXPERIMENTAL

Materials

Polyethylene oxide (M.W. 200,000) was purchased from Dow Chemical Company, Danbury, CT. Clotrimazole was obtained from Spectrum Chemical, Inc., Gardena, CA. Other reagents (HPLC grade) were purchased from Fisher Chemicals, Fair Lawn, NJ.

Methods

Formulation and Hot-Melt Extrusion

Based on previous studies,[26–28] polyethylene oxide (PEO) was chosen as the polymeric carrier for the matrix film formulations. Prior to the extrusion process, 200-g batches containing the model drug and the polymer in the formulations were subjected to particle size reduction in a mortar and pestle for 15 minutes. Final blending was achieved using a V-blender for 20 min (100 rpm).[15] The blended powders were then dried in an oven at 50°C for 24 hr to minimize moisture content.[14]

The powder blend was fed into a single-screw extruder (RCP-750, Randcastle Corp., Cedar Grove, NJ) equipped with a Nitralloy 135M screw (3:1 compression ratio with flight configuration containing feed, compression, and mixing sections) and a 6-inch flex-lip die. The extrusion temperatures ranged from 80–90°C with a screw speed of 70 rpm. The three heating zones and die temperature zone were set and allowed to equilibrate. After equilibration, the samples were processed at temperature set points of 80°, 85°, 85°, and 90°C. The residence time of the materials in the extruder was approximately 1–2 minutes. In the present study, the extruded films had a thickness range of 0.34–0.36 mm. The films were collected in a roll, labeled, and sealed in foil-lined 5-mil polyethylene bags (1 mil=25.4 μm).

Determination of Clotrimazole Content by HPLC

The chromatographic system consisted of a Waters 600 pump and a dual wavelength Waters 2487 UV detector (Waters Corporation). The column used was a Novapak® C18 (Waters Corporation) (3.9×150 mm ID and 4 μ particle size). The HPLC method developed by Hoogerheide et al.[29] was used for the quantitation of CT as published, with the exception that the wavelength utilized for detection was 225 nm. The relative standard deviation for this method was reported to range from 0.3% to 1.8%. The mobile phase consisted of methanol and 0.025 M potassium dihydrogen phosphate in the ratio of 3:1 and the flow rate was 1 mL/min. The injection volume for the standard and the sample preparations was 20 μL. Stock solutions of CT were prepared using methanol. Five calibration standards were prepared for CT by diluting the stock solutions with the solvent in appropriate quantities, which were then injected into the HPLC system. Regression analysis was performed on the data points generating the calibration curve, which was determined to be linear in the concentration range of 1 to 100 μg/mL. All analyzed samples in this study fell within this range.

Random samples (n=6) were taken from the extruded film using a die punch. The samples were weighed and placed in a 25 ml volumetric flask. Fifteen mL of methanol was added to the flask and sonicated for 10 min or until the entire film was dissolved. The samples were then diluted up to 25 mL using methanol and centrifuged for 10 min. The supernatant

Table 1. The theoretical concentrations of clotrimazole (%) incorporated into the HME films.

HME film	Theoretical concentration of clotrimazole (%)
A(PEO only)	0
B	2
C	4
D	5
E	7.5
F	10
G	12.5
H	15
I	20

Table 2. Validation samples as predicted by the model.

Film	Actual value of clotrimazole in films (%)[a]	Predicted value of clotrimazole in films (%)	% Difference between actual and predicted value of clotrimazole
V-1	2.04 (0.17)	1.97	−3.43
V-2	4.96 (0.21)	5.06	2.01
V-3	12.88 (0.32)	12.76	−0.93
V-4	15.62 (0.39)	15.59	−0.19

Note: Actual value determined by HPLC and predicted value determined by NIR.
[a]The values represent the mean of n=6 (HPLC) samples. Standard deviations enclosed in parentheses.

was removed and filtered through a 0.45 μ nylon filter prior to injection into the chromatographic system. The content of CT was calculated from the equation obtained from the regression analysis.

NIRS Instrumentation

The samples were scanned using a Bruker EQUINOX© 55/S (Model # 502, Bruker Optics Inc., Billerica, MA) FT-IR Spectrophotometer equipped with OPUS© 2.2 software. The spectra were collected in the NIR mode using a tungsten lamp source and an air-cooled GE-Diode detector with a quartz beam splitter.

NIR Data Collection Process

Nine different PEO films containing concentrations of clotrimazole in the range of 0–20% were utilized for the calibration model (Table 1). Near infrared scans of the films were obtained prior to analysis by HPLC. Six random disc-shaped samples were taken from each of the nine films using a standardized die punch. Four additional samples were arbitrarily chosen and used as test samples for the external validation. These test samples were taken such that two samples (B and D) represented the lower CT concentration range and two (G and H) the higher drug concentration range. The fiber optic probe was arranged in a fixed position using a standard king stand with a clamp. The aperture of the probe (2.4 mm) was approximately of the same size as that of the punched film. Each sample was scanned 25 times and averaged in the wavelength region from 10000–5000 cm^{-1} using a resolution of 2 cm^{-1}. Each of the disc shaped films (total=58), after being scanned, were analyzed using the HPLC method previously described. For model derivation, the spectra from each sample were assigned the concentration values obtained by the HPLC analysis. Fifty-four samples were used for developing the calibration model and four for the validation set. Calibration equations were obtained using Partial Least Squares (PLS) regression and spectral data from 10000–5000 cm^{-1}.

Validation

For any type of modeling the issue of validation is of utmost importance. In calibrating the model utilized in this study, the robustness was assessed in two ways:

- Cross validation—internal validation.
- External validation—utilization of test samples.

In the first assessment, cross validation was performed by omitting one concentration range from the sample sets and the root mean squared error of cross validation was calculated. The samples that were omitted served as validation samples for the calibration model. In the second assessment, the constructed

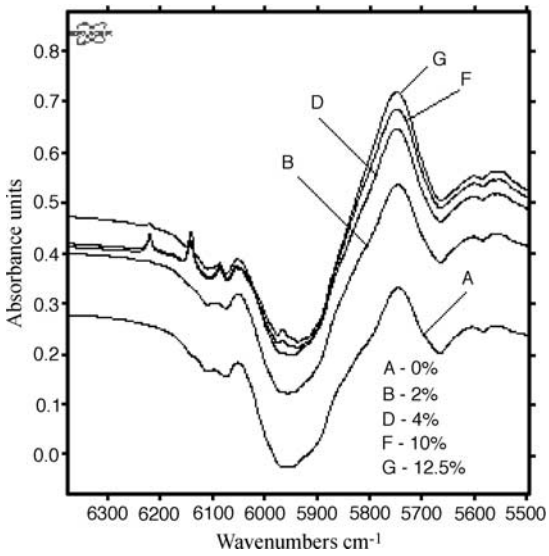

Figure 2. Raw spectra of the PEO-only and clotrimazole incorporated HME films (unprocessed). (*View this art in color at www.dekker.com.*)

model was validated by checking the predicted values of test samples from the films B, D, G, and H. The values are provided in Table 2.

RESULTS AND DISCUSSION

Chemometric modeling was performed using OPUS/QUANT 2 version 2.2 software, and PLS algorithm was used for the calibration models. The complexity of the raw spectral data, which necessitated preprocessing, is illustrated in Fig. 2. There are a number of preprocessing tools such as normalization, weighting, smoothing, and baseline corrections.[30] Normalization places all of the samples on the same scale by dividing by a constant. This process is performed to remove systematic variation, usually associated with sample quantity. Due to variations in the path length, the spectra vary in intensity. Normalization to unit area reduces this path length variation[30] and thus, the remaining spectral differences are attributed to chemical variations in the samples. In the present study, since the HME films may vary in thickness, the path length might also vary. Therefore, in order to minimize the path length variable, vector normalization was chosen as the preprocessing method and the spectra were mean-centered.

Twenty-one frequency ranges between 6234 and 5483 cm were utilized to develop the calibration model. These frequency ranges were selected to account for the deviations in the drug concentrations.

The regression vector was highly influenced by the responses from CT and the film matrix. The number of factors/ranks used in the model was 9. The factor number used in the calibration model was determined using PRESS analysis. The calibration curve (Fig. 3) yielded a correlation coefficient (R^2) of 0.999 with a root mean square error of estimation (RMSEE) of 0.195 and a root mean square error of cross validation (RMSECV) of 0.298. The slope of 1.0012 indicated that the absolute value of the predicted percentage and the actual percentage of clotrimazole were well correlated. As discussed previously, the constructed calibration model was validated using internal validation and external validation. These data indicate not only the accuracy of the calibration model, but also the robustness of the model to predict an unknown within a margin of error of less than 3.5%. As shown in Table 2, the model is more accurate at higher concentrations when compared to the predicted error in lower concentrations. This may be explained by the following discussion.

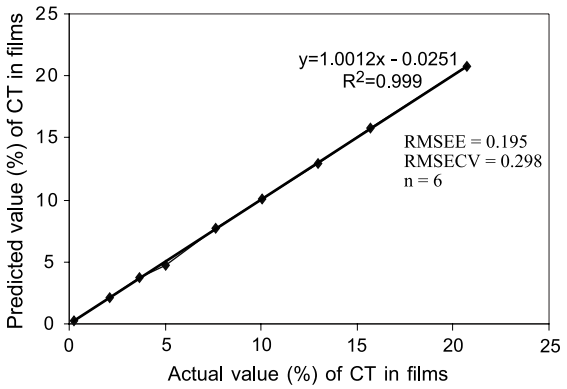

Figure 3. Calibration curve of clotrimazole in the HME films. (*View this art in color at www.dekker.com.*)

During HME, polymeric materials soften and become flexible due to the shearing effect of the rotating screw and from heat via the thermal devices attached to the barrel.[14] At lower concentrations, uniform dispersion of the drug in the polymer may be problematic, especially in single-screw type hot-melt extruders.[21] Indeed, it has been reported that content uniformity in HME films containing higher concentrations of CT (10%) was better than those CT-containing films at the 2% level.[31] Therefore, blend uniformity may have contributed to the lower correlation in the lower concentration ranges. Hence, this may be a reason for the greater deviation from the actual value at these lower concentrations. By the utilization of more training sets, the accuracy level may be improved. In addition, partial least squares (PLS), which is a quantitative spectral decomposition technique, may cause spectra containing higher constituent concentrations to be weighted more than those with lower concentrations. Regardless, the current level of accuracy of less than 3.5% demonstrates that NIRS can be used as an effective tool to quantify a model drug in PEO films produced by hot-melt extrusion.

CONCLUSIONS

It can be concluded that the described NIRS technique is a means to provide an accurate and nondestructive assessment of the amount of drug present in HME film matrices. The predicted value obtained by the calibration model in FT-NIR spectroscopy exhibited good correlation with the actual value obtained by the HPLC analysis. Thus, NIRS was demonstrated to be a versatile technique with minimal sample preparation, and decreased cost and analysis time for the quantitation of a model drug contained in

films produced by hot-melt extrusion techniques. The successful use of NIRS in this off-line study is encouraging for future studies utilizing this technique for the on-line quantification of drugs incorporated into HME dosage forms.

ACKNOWLEDGMENTS

The authors wish to thank Burroughs Welcome Fund and The American Foundation for Pharmaceutical Education for its support for this project via the American Association of Colleges of Pharmacy (AACP). The authors also wish to thank Ms. Hui Li, Bruker Optics, for her technical support for the OPUS software.

REFERENCES

1. Dreassi, E.; Ceramelli, G.; Savini, L.; Corti, P.; Perruccio, P.L.; Lonardi, S. Application of near-infrared reflectance analysis to the integrated control of antibiotic tablet production. Analyst **1995**, *120*, 319–323.
2. Dempster, M.A.; Jones, J.A.; Last, I.R.; McDonald, B.F.; Prebble, A. Near-infrared methods for the identification of tablets in clinical trial supplies. J. Pharm. Biomed. Anal. **1993**, *11*, 1087–1092.
3. Buchanan, B.R.; Baxter, M.A.; Chen, T.S.; Qin, X.; Robinson, P.A. Use of near-infrared spectroscopy to evaluate an active in a film coated tablet. Pharm. Res. **1996**, *13* (4), 616–621.
4. Nagata, T.; Ohshima, M.; Tanigaki, M. In-line monitoring of polyethylene density using near infrared spectroscopy. Polym. Eng. Sci. **2000**, *40* (5), 1107–1113.
5. Camacho, W.; Karlsson, S. Simultaneous determination of molecular weight and crystallinity of recycled HDPE by infrared spectroscopy and multivariate calibration. J. Appl. Polym. Sci. **2002**, *85*, 321–327.
6. Workman, J.J. Quantification of LDPE [low density poly(ethylene)], LLDPE [linear low density poly(ethylene)], and HDPE [high density poly(ethylene)] in polymer film mixtures ''as received'' using multivariate modeling with data augmentation (data fusion) and infrared, raman and near-infrared spectroscopy. Spectrosc. Lett. **1999**, *32* (6), 1057–1071.
7. Kemper, M.S.; Magnuson, E.J.; Lowry, S.R.; McCarthy, W.J.; Aksornkoae, N.; Watts, D.C.; Johnson, J.R.; Shukla, A.J. Use of FT-NIR transmission spectroscopy for the quantitative analysis of an active ingredient in a translucent pharmaceutical topical gel formulation. AAPS PharmSci **2001**, *3* (3), 1–5, article 23.
8. Laasonen, M.; Rantanen, J.; Harmia-Pulkkinen, T.; Michiels, E.; Hiltunen, R.; Räsänen, M.; Vuorela, H. Near infrared reflectance spectroscopy for the fast identification of PVC-based films. Analyst **2001**, *126*, 1122–1128.
9. Reich, G. In *Use of DSC and NIR Spectroscopy to Study the Hydration, Degradation and Drug Release Behavior of PLA/PLGA Microparticles and Films with Free and Blocked Carboxylic End Groups*, Proceedings of the 27th International Symposium on Controlled Release of Bioactive Materials, Controlled Release Society: Minneapolis, 2000; 642–643.
10. Reich, G.; Schönbrodt, T. In *Fast and Noninvasive Near Infrared Analysis of PLA and PLGA Microparticles, Films and Tablets*, Proceedings of the 28th International Symposium on Controlled Release of Bioactive Materials, Controlled Release Society: Minneapolis, 2001; 387–388.
11. Aitken-Nichol, C.; Zhang, F.; McGinity, J.W. Hot melt extrusion of acrylic films. Pharm. Res. **1996**, *13*, 804–808.
12. Follonier, N.; Doelker, E.; Cole, E.T. Evaluation of hot-melt extrusion as a new technique for the production of polymer-based pellets for sustained release capsules containing high loadings of freely soluble drugs. Drug Dev. Ind. Pharm. **1994**, *20*, 1323–1339.
13. Repka, M.A.; McGinity, J.W. Bioadhesive properties of hydroxypropylcellulose topical films produced by hot-melt extrusion. J. Control. Release **2001**, *70*, 341–351.
14. Repka, M.A.; McGinity, J.W.; Zhang, F.; Koleng, J.J. *Encyclopedia of Pharmaceutical Technology*; Swarbrick, J., Boylan, J., Eds.; Marcel Dekker, Inc.: New York, 2002.
15. Repka, M.A.; McGinity, J.W. Influence of chlorpheniramine maleate on topical films produced by hot-melt extrusion. Pharm. Dev. Technol. **2001**, *6* (3), 295–302.
16. Repka, M.A.; McGinity, J.W. Physical-mechanical, moisture absorption and bioadhesive properties of hydroxypropylcellulose hot-melt extruded films. Biomaterials **2000**, *21* (14), 1509–1517.
17. Zhang, F. Dissertation; The University of Texas at Austin, 1999.
18. Zhang, F.; McGinity, J.W. Properties of sustained-release tablets prepared by hot-melt extrusion. Pharm. Dev. Technol. **1999**, *4*, 241–250.

19. Repka, M.A.; Gerding, T.G.; Repka, S.L.; McGinity, J.W. Influence of plasticizers and drugs on the physical-mechanical properties of hydroxypropylcellulose films prepared by hot-melt extrusion. Drug Dev. Ind. Pharm. **1999**, *25*, 625–633.
20. McGinity, J.W.; Zhang, F.; Repka, M.A.; Koleng, J.J. Thermal processing of pharmaceutical powders. Pharm. Technol. Jpn. **2000**, *16* (6), 897–913.
21. McGinity, J.W.; Zhang, F.; Repka, M.A.; Koleng, J.J. Hot-melt extrusion as a pharmaceutical process. Am. Pharm. Rev. **2001**, *4* (2), 25–36.
22. Powderly, W.G.; Mayer, K.H.; Perfect, J.R. Diagnosis and treatment of oropharyngeal candidiasis in patients infected with HIV: a critical reassessment. AIDS Res. Hum. Retrovir. **1999**, *15* (16), 1405–1412.
23. Ciurczak, E.W.; Drennen, J.K., III. *Pharmaceutical and Medical Applications of Near-Infrared Spectroscopy*; Marcel Dekker, Inc.: New York, 2002.
24. Siesler, H.W.; Ozaki, Y.; Kawata, S.; Heise, H.M. *Near-Infrared Spectroscopy: Principles, Instruments, Applications*; Wiley-VCH, 2002.
25. Hammond, S.; Warman, M.The use of process analytical technology in Pfizer manufacturing plants. 225th ACS National Meeting.
26. Crowley, M.M.; Fredersdorf, A.; Schroeder, B.; Prodduturi, S.; Repka, M.A.; McGinity, J.W. The influence of guaifenesin and ketoprofen on the properties of hot-melt extruded polyethylene oxide films. Eur. J. Pharm. Sci., *in press*.
27. Crowley, M.M.; Zhang, F.; Koleng, J.J.; McGinity, J.W. Stability of polyethylene oxide in matrix tablets prepared by hot-melt extrusion. Biomaterials **2002**, *23* (21), 4241–4248.
28. Repka, M.A.; McGinity, J.W. Influence of vitamin E TPGS on the properties of hydrophilic films produced by hot-melt extrusion. Int. J. Pharm. **2000**, *202* (1–2), 63–70.
29. Hoogerheide, J.G.; Strusiak, S.H.; Taddei, C.R.; Townley, E.R.; Wyka, B. High performance liquid chromatographic determination of clotrimazole in pharmaceutical formulations. J. Assoc. Off. Anal. Chem. **1981**, *64* (4), 864–869.
30. Beebe, K.R.; Pell, R.J.; Seasholtz, M.B. *Chemometrics: A Practical Guide*; John Wiley & Sons, Inc., 1998.
31. Prodduturi, S.; Repka, M.A. *Drug Content and Adhesive Uniformity of Hot-Melt Extruded Films Containing Clotrimazole*; American Association of Pharmaceutical Scientists: Toronto, Canada, 2002.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016