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## Near-infrared spectroscopy for the determination of testosterone in thin-film composites

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### Abstract

More rapid, reproducible, and cost-effective methods to control product quality in the pharmaceutical industry continue to be a major emphasis, particularly with the FDA through its recent process analytical technologies (PAT) initiative. Many different methods have been used to determine the stability and content uniformity of a drug in various dosage forms; however, most of these methods include the destruction of the sample. Therefore, the development of nondestructive methods that allow the analysis of each individual dosage form has become the basis of much research. A new assay for the nondestructive determination of testosterone content in mucoadhesive bi-layer thin-film composites (TFCs) using near-infrared spectroscopy (NIR) was developed. Five sets of the circular films ( $n = 5$ ) with theoretical testosterone content of 0, 1, 2, 3, and 4 mg per 3/8th in. diameter disks were scanned in the near-infrared region of 1100–2500 nm to determine testosterone content. The NIR results were directly compared with those obtained using a previously developed ultraviolet assay for testosterone at 240 nm. Principal component regression (PCR) was performed to calibrate the NIR assay. This correlation produced  $r^2 = 0.99$  with a standard error of estimate (SEE) = 0.18 mg, and a standard error of performance (SEP) = 0.18 on cross validation with an equal number of samples ( $F$  test passed at  $P = 0.05$ ). Though the UV assay showed a slightly better  $r^2$  value, the NIR assay was much quicker, easier, and nondestructive. Therefore, the NIR assay may have significant potential for use in the quality control of pharmaceutical films containing drugs.

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**Keywords:** Principal component regression; Buccal; Mucoadhesive; Analytical method; Nondestructive

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## 1. Introduction

The pharmaceutical industry has been rapidly expanding into new and different types of delivery vehicles in the past decade. Many of these new delivery methods are presenting unique problems concerning the quality control of these products. Two of the most important parameters in the quality control of dosage forms are drug stability and content uniformity. The most common methods for determining stability and content uniformity involve the first step of dissolving the sample in a suitable medium. A multitude of analytical techniques can then be used to determine the drug concentration and/or stability. Some commonly used techniques are HPLC, gas chromatography (GC), capillary electrophoresis (CE), and ultraviolet (UV) analysis. All of these analytical methods require destruction of the sample in combination with a series of physical and/or chemical manipulations. For example, sample preparations can be very difficult and tedious due to multiple dilutions, filtrations and extractions. Moreover, depending on the analytical method employed, such techniques often require lengthy analysis times for each sample.

The development of more rapid, reproducible, cost-effective, and perhaps non-destructive methods to quality control products in the pharmaceutical industry continues to be a major emphasis. A potential method that may meet this criteria is near infrared spectroscopy (NIR) [1–5]. NIR is performed over a range of wavelengths that is capable of both quantitative and qualitative analysis. One important feature of NIR is that it can be performed on the raw product without destroying the sample. Secondly, the sample time can be reduced to only a few minutes per sample. Many companies that make solid dosage forms are turning to NIR analysis for quality control of their products. For example, utilizing NIR, a manufacturer of solid tablets may randomly sample from a population of tablets during a production run and quickly determine, in real time, whether the batch falls within acceptable limits for drug content and/or content uniformity. In fact, the Food and Drug Administration has strongly encouraged the use of NIR as on-line,

in-line, or at-line measurement tool for unit operations and/or as an alternative test [6].

NIR spectrometry and nonparametric multivariate analysis are a strong combination in solid dosage-form analysis, as demonstrated by analysis of intact tablets [7], detection of tampering in gelatin capsules [8], and detection of contamination in drug capsules [9]. NIR spectrometry and multivariate analysis has been further employed to discriminate between different tablet formulations inside blister packages [10]. NIR spectrometry and multivariate analysis have even been used to determine the moisture and salicylic acid content of degraded aspirin tablets [11].

Raman spectrometry with a NIR light source has also been used on drug formulations in gel capsules and on gel capsules inside blister packs [12]. Analysis of the Raman spectra collected from bucindolol capsules in the interior of the blister packs with multivariate calibration yielded a standard error of performance (SEP) of only 3.36% of the range of active ingredient. As is frequently the case in NIR reflectance spectrometry, the largest source of prediction error was sample inhomogeneity.

NIR cameras are being increasingly employed in hyperspectral imaging experiments. Imaging spectrometers based on framing array cameras have rapid scanning ability and high sensitivity. NIR imaging has been used in human stroke patients to discover atherosclerotic plaque by identifying the location of oxidized lipoprotein spectral signatures [13]. The InSb camera employed in these studies had a custom cold (77 K) bandpass filter for NIR use and could be fitted with a warm (298 K) tunable interference filter system or a warm filter disk. Probability density contours drawn in multi-dimensional standard deviations (S.D.s) were put to use to form pictures that exposed the locations of atherosclerotic plaque inside blood vessels. NIR multispectral imaging has been used to monitor solid-phase peptide synthesis [14]. An acousto-optic tunable filter and a NIR indium gallium arsenide (InGaAs) focal plane array camera maintained all the advantages of a traditional NIR spectrometer in noninvasive observation of reactions and identification of the products during the solid-phase peptide synthesis. The NIR hyperspec-

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128 tral imaging system added an important character-  
129 istic to the monitoring that traditional NIR  
130 spectrometers could not offer the ability to mea-  
131 sure spectra at different sites within a sample. In  
132 the peptide synthesis study, spectra recorded by  
133  $16 \times 16$  pixels were pooled to calculate an average  
134 spectrum for each sample. However, a qualitative  
135 spectrum could be gathered from a single pixel.

136 The kinetics of curing of an epoxy resin by  
137 amine was also studied using a NIR hyperspectral  
138 imaging spectrometer [15]. The kinetics of curing  
139 was calculated from data collected by a single pixel  
140 in the camera. The reaction rates inside the sample  
141 were not uniform. Because of this kinetic inhomog-  
142 eneity, differences in the degree of cure at  
143 different positions within the sample were as high  
144 as 37% when data from just a single pixel were  
145 employed for calculation. The inhomogeneity was  
146 not observed if the average of a large number of  
147 pixels were used. In a similar manner, ethylene/  
148 vinyl acetate copolymers were shown to display a  
149 high degree of chemical inhomogeneity [16].

150 Our laboratories have studied the potential of  
151 NIR to detect and quantify drug content and  
152 content uniformity of drugs in novel mucoadhesive  
153 bi-layer thin-film composites (TFCs) [17–19].  
154 These bioerodable TFCs ( $\sim 100$ – $200 \mu\text{m}$  in total  
155 thickness) are comprised of two layers, a drug  
156 containing mucoadhesive polymer layer, and an  
157 impermeable layer consisting of a pharmaceutical  
158 wax. The TFCs strongly adhere to a wet mucosal  
159 surface (i.e. buccal tissue) for up to 4 h and allow  
160 for uni-directional drug delivery through the tissue  
161 into the systemic circulation, or local delivery of  
162 mucosal vaccines. Recently, these TFCs have been  
163 used to deliver testosterone, salmon calcitonin,  
164 and (genetic) vaccines by the buccal routes in  
165 rabbits [17–19]. The current method to quantify  
166 testosterone content in the TFCs is destructive and  
167 time consuming involving the dissolution of the  
168 films containing testosterone in ethanol overnight  
169 followed by subsequent dilution with additional  
170 ethanol, filtration, and assay by UV.

171 The overall goal of this work was to develop a  
172 rapid, reproducible, and cost-effective quality  
173 control method for testosterone in the TFCs by  
174 employing NIR analysis.

## 2. Experimental 175

### 2.1. Materials 176

Polycarbophil (Noveon<sup>®</sup> AA-1, USP) was a 177  
generous gift from BF Goodrich (Cleveland, 178  
OH). Polymethacrylic acid-co-methyl methacry- 179  
late (Eudragit S-100) was obtained from Röhm 180  
America Inc. (Piscataway, NJ). Testosterone (4- 181  
androsten-17 $\beta$ -ol-3-one) was purchased from Al- 182  
drich Chemicals (Milwaukee, WI). DENTSPLY<sup>®</sup> 183  
Utility Wax was obtained from DENTSPLY 184  
International (York, PA). Ethanol (95%) USP, 185  
was purchased from Spectrum Laboratory Pro- 186  
ducts (Gardena, CA). 187

### 2.2. Preparation of thin-film composites [17–19] 188

The mucoadhesive TFCs were first produced as 189  
a semi-viscous gel and poured into molds and 190  
dried. Briefly, 95% ethanol USP (90.0 g), was 191  
added to a 300 ml stainless steel beaker. A 192  
Caframo Mixer (Model BDC 1850; Warton, 193  
Ontario) equipped with a 3 cm diameter dispersion 194  
blade was lowered into the solution and stirring 195  
was set at 250 rpm. Eudragit S-100 (1.333 g) was 196  
added to the solution over a period of 5 min and 197  
the solution turned an opaque bluish color. The 198  
solution was allowed to stir until the solution 199  
became clear. Next, Noveon<sup>®</sup> AA1 (4.0 g) was 200  
slowly added in small portions over 30 min. The 201  
gel was then stirred at 1000 rpm for 6 h and then 202  
q.s. to 100 g with 95% ethanol. The finished 203  
placebo gel was considered to be a stock placebo 204  
gel ( $1.333 \times$ ). 205

Gels containing testosterone were then prepared 206  
by aliquoting five separate stock placebo gels (15 g 207  
each) into separate 40 ml wide mouth I-Chem glass 208  
jars. The following accurately weighed testostero- 209  
ne powder was then added to each respective 210  
stock placebo gels stirring at 600 rpm; 0.0, 0.1803, 211  
0.3606, 0.5409 and 0.7212 g. The gels containing 212  
testosterone were mixed until all of the testostero- 213  
ne was dissolved in the gel at which point the gels 214  
were made to 20 g with 95% ethanol. The gels 215  
containing testosterone were cast into films by first 216  
fixing a plastic circular hollow ring (diameter = 6.2 217  
cm; total area =  $30.175 \text{ cm}^2$ ) onto a 4 in.  $\times$  4 in. 218

219 Mylar film. A volume of 7 ml of each gel was  
 220 dispensed from a 10 ml glass serological pipette  
 221 into the middle of the circular ring. The gels were  
 222 then dried overnight in an oven at a temperature  
 223 of 55 °C. Once dry, the plastic ring was removed  
 224 and the side of the film containing the muco-  
 225 adhesive film was rapidly dipped into melted  
 226 Dentsply wax and removed. The TFCs were  
 227 allowed to cool and then detached from the Mylar  
 228 film. A 3/8 in. Arch Punch (C.S. Osborne;  
 229 Harrison, NJ) was used to cut out circular disks  
 230 containing a theoretical testosterone dose of 0, 1,  
 231 2, 3, or 4 mg. The thickness and weight of placebo  
 232 TFCs and TFCs containing testosterone (n = 10)  
 233 were determined. A Marathon Electronic Digital  
 234 Micrometer Model 030025 EMD (0–25 mm,  
 235 resolution of 0.001 mm) was used to determine  
 236 the thickness of films.

### 237 2.3. Near infrared analysis of films

238 A Technicon InfraAlyzer 500 (Tarrytown, NY)  
 239 was used to obtain NIR spectra of all film samples.  
 240 Each concentration of testosterone stated above  
 241 was formed into 3/8 in. disks (n = 5) and assayed  
 242 by NIR separately. The scan was performed in the  
 243 range of 1100–2500 nm. The samples were  
 244 scanned using an aluminum sample focusing-cup  
 245 with a 135° liquid insert [20]. The entire sample  
 246 area was illuminated. A glass cover slip was placed  
 247 over the liquid insert and secured in place by black  
 248 electrical tape. Each disk was placed on top of the  
 249 glass cover slip and placed inside the spectro-  
 250 photometer. Each sample required a minute for  
 251 the sample placement and approximately 2 min for  
 252 the scan. One complete scan was obtained in 2 min  
 253 with 170-ms total signal integration at each  
 254 wavelength. Multiplicative scatter correction was  
 255 applied to the collected spectra of the films to  
 256 reduce the effect of any variations in film thickness  
 257 on the results. After each disk was scanned, it was  
 258 placed in a clean 7-ml scintillation vial and clearly  
 259 labeled.

### 260 2.4. Ultraviolet (UV) assay of the TFCs

261 A Beckman Instruments Model DU-7500i Spec-  
 262 trophotometer (Fullerton, CA) was used for UV

analysis of samples. After the NIR spectrum was 263  
 obtained for each disk, 1 ml of 95% ethanol was 264  
 added to each capped vial. The vials were then 265  
 vigorously shaken over night to ensure complete 266  
 dissolution of the films in the ethanol. The 267  
 solutions were diluted to a theoretical concentra- 268  
 tion of 20 mg/ml with ethanol and filtered using a 269  
 0.22 mm PTFE syringe filter to remove polymers 270  
 and wax. The UV absorbance was determined at 271  
 240 nm using the diode-array spectrophotometer. 272  
 The absorbance values were compared with a 273  
 standard curve of testosterone in ethanol ranging 274  
 from 1.5625 to 25 mg/ml. The results of the UV 275  
 assay were used to calculate the concentration of 276  
 testosterone in the film according to the dilution 277  
 factor. 278

### 279 2.5. Data correlation and analysis

In NIR spectrometry, the absorbance at any 280  
 single wavelength contains contributions from 281  
 many different sources (different analytes, physical 282  
 configurations of the sample, etc.). For this reason 283  
 any single wavelength in the spectrum may not 284  
 present a reliable linear correlation between the 285  
 absorbance and testosterone content. Therefore, 286  
 the statistical method of principal component 287  
 regression (PCR) was performed in order to 288  
 extract the testosterone concentration data from 289  
 the spectra. 290

The actual sample testosterone concentrations 291  
 according to the UV assay were paired with their 292  
 corresponding NIR spectra. PCR was employed to 293  
 analyze the spectra of intact films [11]. PCR uses 294  
 transformation of the spectra to principal axes to 295  
 convert the spectral absorbance values into princi- 296  
 pal component “scores” (coordinates in the new 297  
 PC coordinate system). Multiple linear regression 298  
 of the PC scores and testosterone concentrations is 299  
 used to create a calibration function for determin- 300  
 ing testosterone concentrations from spectra of 301  
 new unknown films. Principal-axis transformation 302  
 (PAT) of the spectra in **T** begins with standardiz- 303  
 ing the spectral data by subtracting the mean 304  
 absorbance of each column  $\mu(t_j)$  from the absor- 305  
 bances in each column  $t_{ij}$ , and dividing the 306  
 difference by the corresponding standard deviation 307  
 $\sigma_{S.D.}(t_j)$ : 308

$$309 \quad \mathbf{Z}_{ij} = [t_{ij} - \mu(t_j)] / \sigma_{S.D.}(t_j) \quad (1)$$

310 The normalized spectral matrix  $\mathbf{Z}$  is then trans-  
 311 posed and retained until the transformation matrix  
 312 is formed. Normalization gives information at  
 313 each wavelength equal weight in the post-trans-  
 314 formation spectral hyperspace. The transforma-  
 315 tion matrix  $\mathbf{L}^{-1}$  is formed from the eigenvalues  $\lambda$   
 and eigenvalues  $\mathbf{X}_\lambda$  of a correlation matrix  $\mathbf{R}$ :

$$316 \quad r_{jk} = \sum_{i=1}^n [t_{ij} - \mu(t_j)] \\ \times [t_{ik} - \mu(t_k)] / (n-1) \sigma_{S.D.}(t_j) \sigma_{S.D.}(t_k) \quad (2)$$

317 where  $n$  is the number of sample spectra and  $\mathbf{R}$  is  
 318 defined from  $k = 1$  to the number of columns in  $\mathbf{T}$ .  
 319 The square roots of the eigenvalues  $\lambda$  of  $\mathbf{R}$  are used  
 320 to diagonalize a square matrix. The matrix pro-  
 321 duct of the square root of these eigenvalues and  $\mathbf{X}_\lambda$   
 322 gives  $\mathbf{L}$ , which turns into the transformation  
 323 matrix upon inversion. The transformation matrix  
 324 effectively serves as a map joining the primary  
 325 spectral hyperspace to the new hyperspace, which  
 326 is ordinarily of smaller dimension. New spectral  
 327 coordinates, supplied in principal-axis space for  
 the sample spectra in  $\mathbf{T}$ , are given by:

$$328 \quad \mathbf{T}_p = \mathbf{L}^{-1} \mathbf{Z} \quad (3)$$

329 The new spectra are employed to good effect in  
 330 both qualitative and quantitative analysis of a  
 331 sample through application of least-squares re-  
 332 gression and discriminant analysis techniques. The  
 333 PAT process eliminates the collinearity problem in  
 334 the NIR spectra of samples, and reduces (often to  
 335 less than one-half dozen) the effective number of  
 336 wavelengths (dimensions in hyperspace) that need  
 337 to be taken into account in qualitative and  
 qualitative analysis of samples.

### 338 3. Results and discussion

339 Testosterone is most commonly used to treat  
 340 male hypogonadism, which is characterized by  
 341 delayed puberty, aplastic anemia, or protein wast-  
 342 ing diseases as well as diminished libido, depressed  
 343 mood, low energy, and depleted muscle mass [21–  
 344 23]. There are currently several marketed products

345 of testosterone indicated for androgen replacement  
 346 therapy including intramuscular oil-based depot  
 347 injections, transdermal systems, oral tablets, and  
 348 sublingual tablets [24–30]. The oral bioavailability  
 349 of testosterone has been reported to be from 1 to  
 350 6% due to extensive first pass metabolism and low  
 351 aqueous solubility [24,29]. The solubility of testos-  
 352 terone in water at 37 °C is only 46.3 µg/ml [24].  
 353 There are currently three marketed transdermal  
 354 products for delivering from 2.5 to 6 mg testoster-  
 355 one. However, these patches are as large as 44 cm<sup>2</sup>  
 356 and have been reported to cause skin irritation in  
 357 as high as 60% of patients due to the inclusion of  
 358 penetration enhancers [30–32]. Due to these limit-  
 359 ing factors, buccal delivery of testosterone may be  
 360 a plausible approach.

361 In previous studies in rabbits using TFCs  
 362 containing testosterone, Jay et al. reported that  
 363 the relative bioavailability for rabbits treated with  
 364 the testosterone (4 mg) TFCs was 50.2 ± 3.2% with  
 365 a CV of 6.4% [17]. It was concluded that these bi-  
 366 layer mucoadhesive TFCs disks could deliver  
 367 physiologically relevant amounts of insoluble  
 368 drugs such as testosterone across the buccal  
 369 mucosa.

370 In these current studies, the results from the  
 371 mass and thickness measurements showed that the  
 372 placebo TFCs had an average weight of 9.96 ± 1  
 373 mg and a thickness of 109 ± 6 µm. TFCs contain-  
 374 ing 4 mg testosterone had an average weight of  
 375 14 ± 2.6 mg and a thickness of 186 ± 34 µm. These  
 376 results confirmed that the manufacturing process  
 377 for the TFCs produced films having suitable  
 378 weight and thickness uniformity for these studies.

379 The UV assay was developed by first scanning a  
 380 solution of 12.5 mg of testosterone per ml of  
 381 ethanol over a range of 190–300 nm. The resulting  
 382 spectrum is shown in Fig. 1. The peak absorbance  
 383 was found at 240 nm and this value was used in the  
 384 formation of a standard curve. The standards were  
 385 in the range of 1.563–25 mg/ml resulting in  $r^2 =$   
 386 0.999 by least squares regression as shown in Fig.  
 387 2. In order for the amount of testosterone on the  
 388 disks to be quantified, they were dissolved in  
 389 ethanol and diluted to a theoretical concentration  
 390 of 20 mg/ml in ethanol. The absorbance of these  
 391 samples were determined at 240 nm and compared  
 392 with the standard curve. The actual quantity of

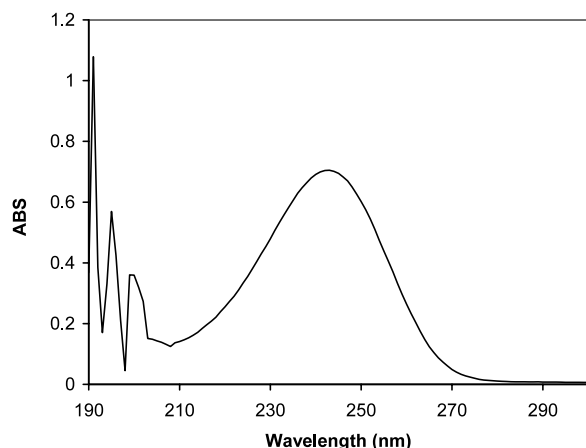


Fig. 1. UV spectrum of extracted testosterone from 3/8 in. TFCs.

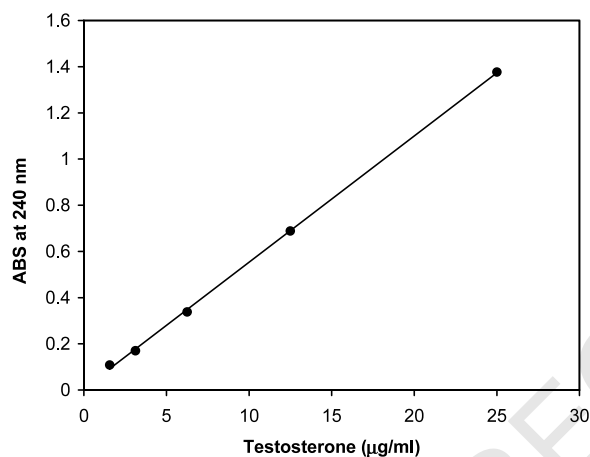


Fig. 2. UV standard curve of testosterone (1.5625–25 mg/ml) in ethanol.

3/8 in. disk. Further, the corresponding label strengths for testosterone in the TFCs were in the usually accepted limits of 90–110% except for the TFCs containing 3 mg testosterone. For reasons not known, TFCs containing 3 mg testosterone had average label strengths of 3.7 mg, which could not be explained by improper testosterone concentrations in the initial gels used to manufacture the TFCs. In general, these present results for testosterone content uniformity in TFCs containing testosterone agreed well with previous results [17].

The NIR of the disks containing testosterone were analyzed by first subtracting the spectrum of a background scan from each measurement. The resulting corrected NIR spectra are shown in Fig. 3. The univariate correlations by wavelength to testosterone concentration ranged from  $-0.96$  to  $+0.98$ . It is well known that calibrations created using a single wavelength are often not useful in mixtures of many constituents, however, and for that reason NIR spectrometry employs multivariate calibration techniques. PCR was performed on the NIR spectra and testosterone concentrations as determined by the UV assay. Four PCs were used in the calibration. The resulting concentrations of testosterone in each disk that were determined by the PCR were correlated with the concentrations found in the UV assay. The correlation ( $r^2 = 0.99$ ) is shown in Fig. 4. Cross validation was performed by applying the calibration developed on the training samples to the same number of additional samples that were not used to develop the calibration. The validation samples also covered the concentration range from 0 to 4 mg. The standard error of estimate (SEE) = 0.18 mg on the calibration samples, and the SEP = 0.18 mg on the validation samples, verifying cross validation by the  $F$  test at  $P = 0.05$ . The R.S.D. = 5% and the detection limit (three times the S.D. of the blank) = 0.50 mg. The R.S.D. of the NIR assay was comparable to that obtained by through UV spectrophotometry. Since any errors in the UV assay will be reflected in the NIR calibration, the equivalent R.S.D. are not surprising. The NIR assay is capable of determining the active content within the accepted limits of 90–110%.

testosterone in each disk was determined according to the standard curve and the dilution factor. The resulting testosterone weights for each of the corresponding TFCs are listed in Table 1. The results for the UV analysis for testosterone content uniformity demonstrate that the UV assay was suitable for meeting content uniformity requirements, with R.S.D. values between 3 and 9% for TFCs containing 1, 2, or 3 mg of testosterone per

Table 1

Content uniformity of testosterone in 3/8 in. TFCs disks as determined by the UV analysis method

Theoretical weight (mg)	0	1	2	3	4
Sample 1	0.0143	1.0092	2.4144	3.4158	3.7642
Sample 2	0.0228	1.0714	1.9484	3.4922	3.5163
Sample 3	0.0181	1.0463	1.9823	4.2133	3.7302
Sample 4	0.0219	1.0501	2.1848	3.6121	3.4775
Sample 5	0.0190	0.9942	2.3428	3.8369	4.7130
Mean $\pm$ S.D.	0.0192 $\pm$ 0.003	1.0343 $\pm$ 0.032	2.1746 $\pm$ 0.209	3.7141 $\pm$ 0.321	3.8402 $\pm$ 0.504
% R.S.D.	17.45	3.06	9.59	8.65	13.12

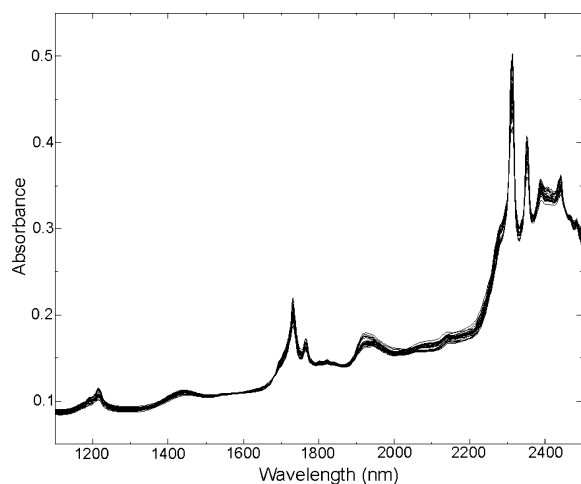


Fig. 3. Scatter-corrected NIR spectra of 3/8 in. TFCs containing testosterone. NIR was completed using a Technicon InfraAlyzer 500 (Tarrytown, NY). Scans were performed in the range of 1100–2500 nm.

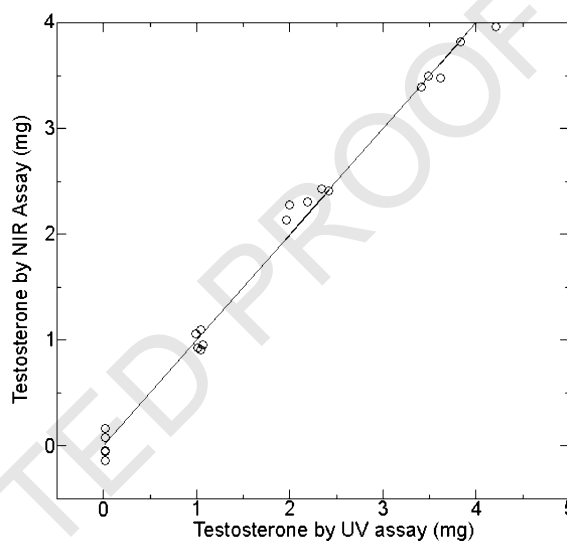


Fig. 4. Comparison of UV analytical method to the NIR analytical method in determining the content uniformity of testosterone in 3/8 in. TFCs (0–4 mg testosterone label strength). Validation samples are shown superimposed on the calibration line.

450 In conclusion, although the UV assay showed a  
 451 slightly better  $r^2$  value, the NIR assay was much  
 452 quicker, easier, and nondestructive. The difference  
 453 between  $r^2 = 0.99$  for the NIR assay and  $r^2 = 0.999$   
 454 for the UV assay corresponds to a change in SEE  
 455 of approximately 0.18–0.17 mg, a trivial difference  
 456 over the 0–4 mg calibration range. Patches could  
 457 be doubled up to increase path length, and such a  
 458 technique might enable a useful calibration func-  
 459 tion to be created in a lower concentration range  
 460 (e.g. 0–1 mg testosterone). The NIR assay may  
 461 have significant potential for use in the quality

control of pharmaceutical films containing testos- 462  
 terone. 463

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## References

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- [1] J.D. Kirsch, J.K. Drennen, Nondestructive tablet hardness testing by near-infrared spectroscopy: a new and robust spectral best-fit algorithm, *J. Pharm. Biomed. Anal.* 19 (1999) 351–362.
- [2] J.D. Wargo, J.K. Drennen, Near-infrared spectroscopic characterization of pharmaceutical powder blends, *J. Pharm. Biomed. Anal.* 14 (1996) 1415–1423.
- [3] J.D. Kirsch, J.K. Drennen, Determination of film-coated tablet parameters by near-infrared spectroscopy, *J. Pharm. Biomed. Anal.* 13 (1995) 1273–1281.
- [4] G. Buckton, E. Yonemochi, W.L. Yoon, A.C. Moffat, Water sorption and near IR spectroscopy to study the differences between microcrystalline cellulose and silicified microcrystalline cellulose before and after wet granulation, *Int. J. Pharm.* 181 (1999) 41–47.
- [5] L. Allen, Quantitative determination of carisoprodol, phenacetin, and caffeine in tablets by near-IR spectrometry and their identification by TLC, *J. Pharm. Sci.* 63 (1974) 912–916.
- [6] A.S. Hussain, The process analytical technology (PAT) initiative: progress report and next steps, Process Analytical Technologies Subcommittee of the Advisory Committee for Pharmaceutical Science, Food and Drug Administration, June 12, 2002, Briefing <http://www.fda.gov/ohrms/dockets/ac/02/briefing/3869b1.htm>.
- [7] R.A. Lodder, G.M. Hieftje, Analysis of intact tablets by near-infrared reflectance spectrometry, *Appl. Spectrosc.* 42 (1988) 556–558.
- [8] R.A. Lodder, M. Selby, G.M. Hieftje, Detection of capsule tampering by near-infrared reflectance analysis, *Anal. Chem.* 59 (1987) 1921–1930.
- [9] R.A. Lodder, G.M. Hieftje, Detection of subpopulations in near-infrared reflectance analysis, *Appl. Spectrosc.* 42 (1988) 1500–1512.
- [10] P.K. Aldridge, R.F. Mushinsky, M.M. Andino, C.L. Evans, Identification of tablet formulations inside blister packages by near infrared spectroscopy, *Appl. Spectrosc.* 48 (1994) 1272–1276.
- [11] J.K. Drennen, R.A. Lodder, Nondestructive near-infrared analysis of intact tablets for determination of degradation product, *J. Pharm. Sci.* 79 (1990) 622–627.
- [12] T.M. Niemczyk, M.M. Delgado-Lopez, F.S. Allen, Quantitative determination of bucindolol concentration in intact gel capsules using Raman spectroscopy, *Anal. Chem.* 70 (1998) 2762–2765.
- [13] R.J. Dempsey, D.G. Davis, R.G. Buice, Jr, R.A. Lodder, Biological and medical applications of near-infrared spectrometry, *Appl. Spectrosc.* 50 (1996) 18A–34A.
- [14] M. Fischer, C.D. Tran, Investigation of solid-phase peptide synthesis by the near-infrared multispectral imaging technique: a detection method for combinatorial chemistry, *Anal. Chem.* 71 (1999) 2255–2261.
- [15] M. Fischer, C.D. Tran, Evidence for kinetic inhomogeneity in the curing of epoxy using the near-infrared multispectral imaging technique, *Anal. Chem.* 71 (1999) 953–959.
- [16] C.D. Tran, Y. Cui, S. Smirnov, Simultaneous multispectral imaging in the visible and near-infrared region: applications in document authentication and determination of chemical inhomogeneity of copolymers, *Anal. Chem.* 70 (1998) 4701–4708.
- [17] S. Jay, W. Fountain, Z. Cui, R.J. Mumper, Transmucosal delivery of testosterone in rabbits using novel bi-layer mucoadhesive wax-film composite disks, *J. Pharm. Sci.* 91 (2002) 2016–2025.
- [18] Z. Cui, R.J. Mumper, Buccal transmucosal delivery of calcitonin in rabbits using thin-film composites, *Pharm. Res.* 19 (2002) 1901–1906.
- [19] Z. Cui, R.J. Mumper, Bi-layer films for mucosal (genetic) immunization via the buccal route in rabbits, *Pharm. Res.* 19 (2002) 947–953.
- [20] R.A. Lodder, G.M. Hieftje, A disposable liquid microcell for near-infrared reflectance analysis, *Appl. Spectrosc.* 42 (1988) 518–519.
- [21] A. Gambineri, R. Pasquali, Testosterone therapy in men: clinical and pharmacological perspectives, *J. Endocrinol. Invest.* 23 (2000) 196–214.
- [22] J.G. Rabkin, G.J. Wagner, R. Rabkin, A double-blind, placebo-controlled trial of testosterone therapy for HIV-positive men with hypogonadal symptoms, *Arch. Gen. Psychiatry* 57 (2000) 141–147.
- [23] S. Parker, M. Armitage, Experience with transdermal testosterone replacement therapy for hypogonadal men, *Clin. Endocrinol.* 50 (1999) 57–62.
- [24] J. Voorspoels, J.P. Remon, W. Eechaute, W. De Sy, Buccal absorption of testosterone and its esters using a bioadhesive tablet in dogs, *Pharm. Res.* 13 (1996) 1228–1232.
- [25] A.S. Dobs, D.R. Hoover, M.C. Chen, R. Allen, Pharmacokinetic characteristics, efficacy, and safety of buccal testosterone in hypogonadal males: a pilot study, *J. Clin. Endocrinol. Metab.* 83 (1998) 33–39.
- [26] S.J. Winters, Current status of testosterone replacement therapy in men, *Arch. Fam. Med.* 8 (1999) 257–263.
- [27] C.C. Slater, I. Souter, C. Zhang, C. Guan, F.Z. Stanczyk, D.R. Mishell, Pharmacokinetics of testosterone after percutaneous gel or buccal administration, *Fertil. Steril.* 76 (2001) 32–37.
- [28] S. Kim, W. Snipes, G.D. Hodgen, F. Anderson, Pharmacokinetics of a single dose of buccal testosterone, *Contraception* 52 (1995) 313–316.
- [29] C.B. Cutter, Compounded percutaneous testosterone gel: use and effects in hypogonadal men, *J. Am. Board Fam. Pract.* 14 (2001) 22–32.
- [30] W.P. Jordan, Allergy and topical irritation associated with transdermal testosterone administration: a comparison of scrotal and nonscrotal transdermal systems, *Am. J. Contact Dermatitis* 8 (1997) 103–113.
- [31] W.P. Jordan, L.E. Atkinson, Comparison of the skin irritation potential of two testosterone transdermal systems: an investigational system and a marketed product, *Clin. Ther.* 20 (1998) 80–87.
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- 582 [32] A.S. Dobs, A.W. Meikle, S. Arver, S.W. Sanders, K.E. system in comparison with bi-weekly injections of testos- 585  
583 Caramelli, N.A. Mazer, Pharmacokinetics, efficacy, and terone enanthate for the treatment of hypogonadal men, J. 586  
584 safety of a permeation-enhanced testosterone transdermal Clin. Endocrinol. Metab. 84 (1999) 3469–3478. 587  
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