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

William Fountain [a], Karen Dumstorf [b], Amanda E. Lowell [a,b], Robert A. Lodder [a,b], Russell J. Mumper [a,*]

[a] *Division of Pharmaceutical Sciences, Center for Pharmaceutical Science and Technology, College of Pharmacy, University of Kentucky, Lexington, KY 40536-0082, USA*
[b] *Department of Chemistry, University of Kentucky, Lexington, KY 40536-0082, USA*

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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.

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

* Corresponding author. Tel.: +1-859-257-2300x258; fax: +1-859-323-5985.
E-mail address: rjmump2@uky.edu (R.J. Mumper).

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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 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 multidimensional 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-

tral imaging system added an important characteristic to the monitoring that traditional NIR spectrometers could not offer the ability to measure spectra at different sites within a sample. In the peptide synthesis study, spectra recorded by 16×16 pixels were pooled to calculate an average spectrum for each sample. However, a qualitative spectrum could be gathered from a single pixel.

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

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

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

2. Experimental

2.1. Materials

Polycarbophil (Noveon® AA-1, USP) was a generous gift from BF Goodrich (Cleveland, OH). Polymethacrylic acid-co-methyl methacrylate (Eudragit S-100) was obtained from Röhm America Inc. (Piscataway, NJ). Testosterone (4-androsten-17ß-ol-3-one) was purchased from Aldrich Chemicals (Milwaukee, WI). DENTSPLY® Utility Wax was obtained from DENTSPLY International (York, PA). Ethanol (95%) USP, was purchased from Spectrum Laboratory Products (Gardena, CA).

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

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

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

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

2.3. Near infrared analysis of films

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

2.4. Ultraviolet (UV) assay of the TFCs

A Beckman Instruments Model DU-7500i Spectrophotometer (Fullerton, CA) was used for UV analysis of samples. After the NIR spectrum was obtained for each disk, 1 ml of 95% ethanol was added to a vial containing a single disk previously scanned by NIR. The vials were then vigorously shaken over night to ensure complete dissolution of the films in the ethanol. The solutions were diluted to a theoretical concentration of 20 μg/ml with ethanol and filtered using a 0.22 μm PTFE syringe filter to remove polymers and wax. The UV absorbance was determined at 240 nm using the diode-array spectrophotometer. The absorbance values were compared with a standard curve of testosterone in ethanol ranging from 1.5625 to 25 μg/ml. The results of the UV assay were used to calculate the concentration of testosterone in the film according to the dilution factor.

2.5. Data correlation and analysis

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

The actual sample testosterone concentrations according to the UV assay were paired with their corresponding NIR spectra. PCR was employed to analyze the spectra of intact films [11]. PCR uses transformation of the spectra to principal axes to convert the spectral absorbance values into principal component "scores" (coordinates in the new PC coordinate system). Multiple linear regression of the PC scores and testosterone concentrations is used to create a calibration function for determining testosterone concentrations from spectra of new unknown films. Principal-axis transformation (PAT) of the spectra in **T** begins with standardizing the spectral data by subtracting the mean absorbance of each column $\mu(t_j)$ from the absorbances in each column t_{ij}, and dividing the difference by the corresponding standard deviation $\sigma_{S.D.}(t_j)$:

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

The normalized spectral matrix **Z** is then transposed and retained until the transformation matrix is formed. Normalization gives information at each wavelength equal weight in the post-transformation spectral hyperspace. The transformation matrix $\mathbf{L}^{-1}$ is formed from the eigenvalues λ and eigenvalues $\mathbf{X}_\lambda$ of a correlation matrix **R**:

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

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

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

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

3. Results and discussion

Testosterone is most commonly used to treat male hypogonadism, which is characterized by delayed puberty, aplastic anemia, or protein wasting diseases as well as diminished libido, depressed mood, low energy, and depleted muscle mass [21–23]. There are currently several marketed products of testosterone indicated for androgen replacement therapy including intramuscular oil-based depot injections, transdermal systems, oral tablets, and sublingual tablets [24–30]. The oral bioavailability of testosterone has been reported to be from 1 to 6% due to extensive first pass metabolism and low aqueous solubility [24,29]. The solubility of testosterone in water at 37 °C is only 46.3 μg/ml [24]. There are currently three marketed transdermal products for delivering from 2.5 to 6 mg testosterone. However, these patches are as large as 44 cm^2 and have been reported to cause skin irritation in as high as 60% of patients due to the inclusion of penetration enhancers [30–32]. Due to these limiting factors, buccal delivery of testosterone may be a plausible approach.

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

In these current studies, the results from the mass and thickness measurements showed that the placebo TFCs had an average weight of 9.96 ± 1 mg and a thickness of 109 ± 6 μm. TFCs containing 4 mg testosterone had an average weight of 14 ± 2.6 mg and a thickness of 186 ± 34 μm. These results confirmed that the manufacturing process for the TFCs produced films having suitable weight and thickness uniformity for these studies.

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

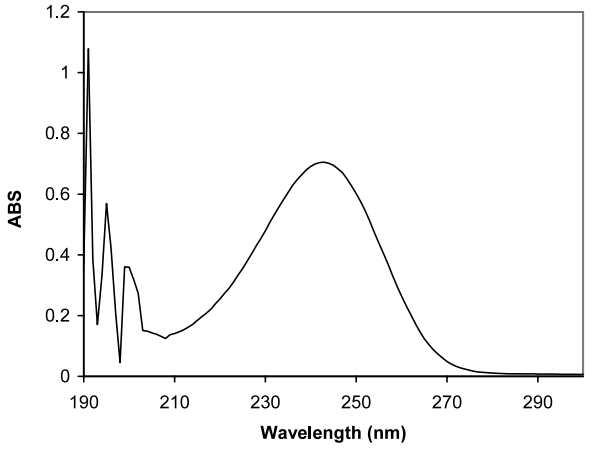

Fig. 1. UV spectrum of extracted testosterone from 3/8 in. TFCs.

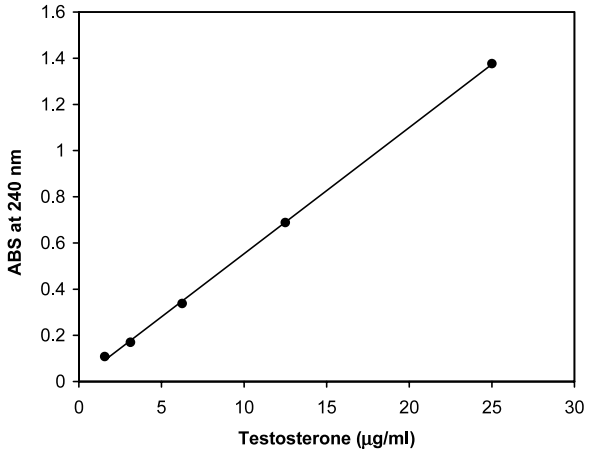

Fig. 2. UV standard curve of testosterone (1.5625–25 μg/ml) in ethanol.

quantity of 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 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

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 ± S.D.	0.0192 ± 0.003	1.0343 ± 0.032	2.1746 ± 0.209	3.7141 ± 0.321	3.8402 ± 0.504
% R.S.D.	17.45	3.06	9.59	8.65	13.12

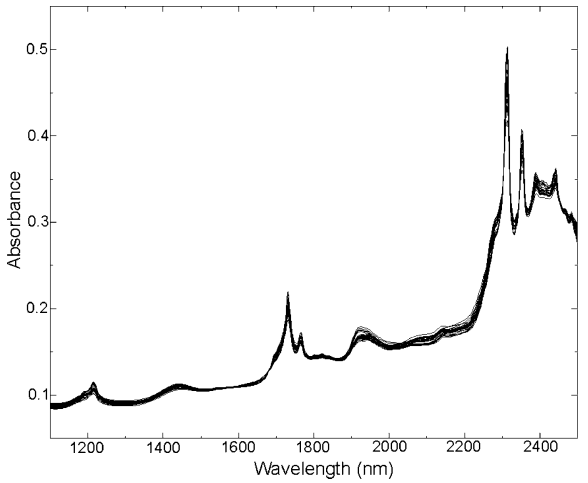

Fig. 3. Scatter-corrected NIR spectra of 3/8 in. TFCs containing testosterone. NIR was completed using a Technicon InfraAlyzer 500 (Tarrytown, NY). Scans were performed in the range of 1100–2500 nm.

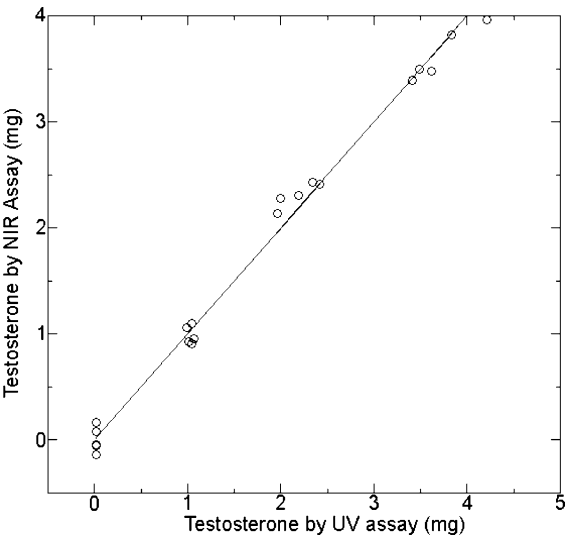

Fig. 4. Comparison of UV analytical method to the NIR analytical method in determining the content uniformity of testosterone in 3/8 in. TFCs (0–4 mg testosterone label strength). Validation samples are shown superimposed on the calibration line.

active content within the accepted limits of 90–110%.

In conclusion, although the UV assay showed a slightly better r^2 value, the NIR assay was much quicker, easier, and nondestructive. The difference between $r^2 = 0.99$ for the NIR assay and $r^2 = 0.999$ for the UV assay corresponds to a change in SEE of approximately 0.18–0.17 mg, a trivial difference over the 0–4 mg calibration range. Patches could be doubled up to increase path length, and such a technique might enable a useful calibration function to be created in a lower concentration range (e.g. 0–1 mg testosterone). The NIR assay may have significant potential for use in the quality control of pharmaceutical films containing testosterone.

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