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Quality assessment of internet pharmaceutical products using traditional and non-traditional analytical techniques

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Abstract

This work investigated the use of non-traditional analytical methods to evaluate the quality of a variety of pharmaceutical products purchased via internet sites from foreign sources and compared the results with those obtained from conventional quality assurance methods. Traditional analytical techniques employing HPLC for potency, content uniformity, chromatographic purity and drug release profiles were used to evaluate the quality of five selected drug products (fluoxetine hydrochloride, levothyroxine sodium, metformin hydrochloride, phenytoin sodium, and warfarin sodium). Non-traditional techniques, such as near infrared spectroscopy (NIR), NIR imaging and thermogravimetric analysis (TGA), were employed to verify the results and investigate their potential as alternative testing methods. Two of 20 samples failed USP monographs for quality attributes. The additional analytical methods found 11 of 20 samples had different formulations when compared to the U.S. product. Seven of the 20 samples arrived in questionable containers, and 19 of 20 had incomplete labeling. Only 1 of the 20 samples had final packaging similar to the U.S. products. The non-traditional techniques complemented the traditional techniques used and highlighted additional quality issues for the products tested. For example, these methods detected suspect manufacturing issues (such as blending), which were not evident from traditional testing alone.
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Keywords: Analytical chemistry; Chromatography; Chemometrics; Dissolution; Image analysis; Near infrared spectroscopy; Thermal analysis

Abbreviations: CV, coefficient of variance; FDA, Food and Drug Administration; NIR, near infrared spectroscopy; PLS, partial least squares; RPM, revolutions per minute; R.S.D., relative standard deviation

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1. Introduction

With the continually increasing number of ways to purchase pharmaceutical products, ensuring the quality of those products becomes ever more important and challenging. The internet in particular has revolutionized the way in which consumers purchase goods. Consumers are turning to online pharmacies with increasing frequency as a source for their medications. Many consumers believe that online pharmacies are more convenient than traditional pharmacies and offer cost savings. While the United States Food and Drug Administration (FDA) recognizes the benefits that legitimate online pharmacies offer to consumers, the agency is concerned with the potential for patient harm that exists from purchasing medications on the internet.

The internet provides ample opportunity for circumventing established FDA safeguards. FDA cannot provide adequate assurance that a product imported through the internet is manufactured in accordance with current good manufacturing practices (cGMP), contains the appropriate active ingredient, is free of potentially harmful contaminants, or was properly stored and shipped. Generally, products imported through the internet are unapproved by the FDA, and there are concerns about their safety and effectiveness.

The Office of Compliance in FDA's Center for Drug Evaluation and Research initiated this study to determine the quality of a select group of pharmaceutical products purchased via the internet from foreign sources. Traditional testing methods, such as potency, content uniformity, chromatographic purity and drug release rate, were used to determine compliance, and non-traditional techniques, such as NIR, NIR imaging and TGA, were investigated for their ability to differentiate products and provide additional quality information.

2. Materials and methods

2.1. Materials

Five drug products – fluoxetine hydrochloride capsules (Prozac), levothyroxine sodium tablets (Synthroid), metformin hydrochloride tablets (Glucophage), phenytoin sodium capsules and tablets (Dilantin), and warfarin sodium tablets (Coumadin) – were selected by the FDA Office of Compliance for inclusion in this study. One sample of each drug product manufactured in the United States was purchased from a local supplier, to be used as a reference. A total of twenty samples were purchased. The internet samples were purchased from eight different internet pharmacy sites—five samples of fluoxetine hydrochloride capsules (20 mg), two samples of levothyroxine sodium tablets (100 μg), four samples of metformin hydrochloride tablets (500 mg), three samples of phenytoin sodium capsules (100 mg), two samples of phenytoin sodium tablets (100 mg), and four samples of warfarin sodium tablets (5 mg).

2.2. Methods

2.2.1. Physical characterization

The physical dimensions of each sample (length, width, geometric shape), color (both internal and external) and any external markings were recorded. Each sample, along with its original packaging, was photographed using a Nikon CoolPix 990 Digital Camera.

To measure weight variation, 10 capsules or tables were randomly selected from each lot and individually weighed. The average weight and relative standard deviation (R.S.D.) were determined. If the R.S.D. was greater than 6.0%, an additional 20 units were randomly selected, weighed and the average weight of the 30 units was reported.

2.2.2. Assay and chromatographic purity

Ten tablets or capsules were randomly selected from each sample, and each unit was analyzed by the compendia or approved manufacturer's method. The average of all 10 units was reported. If the USP monograph did not have a chromatographic purity method specified, one high concentrated (10× assay) sample solution was analyzed to assess impurities (Inman and Tenbarge, 1988) (Table 1).

2.2.3. Dissolution

Four drugs, fluoxetine hydrochloride, levothyroxine sodium, phenytoin sodium and warfarin sodium, were analyzed according to the appropriate USP method

Table 1
Assay method

	Method of analysis	Equipment	Column
Fluoxetine capsules	US Pharmacopeia 26 (2003, p. 816)	Shimadzu LC-10ADvp, SIL-10ADvp and SPD-10AVvp	Zorbax RX-C8 (4.6 mm × 250 mm, 5 μm) and Zorbax SB-CN (4.6 mm × 250 mm, 5 μm)
Levothyroxine sodium tablets	US Pharmacopeia 26 (2003, p. 1076)	HP 1050 HPLC system	Zorbax CN (4.6 mm × 250 mm, 5 μm)
Metformin hydrochloride tablets	NDA[a]	Agilent 8453 spectrophotometer with 1 cm and 0.5 mm flow cell Waters 2690 separation module and 2487 detector	Waters μ-Bondapak C18 (3.9 mm × 150 mm, 10 μm)
Phenytoin sodium tablets	US Pharmacopeia 26 (2003, p. 1467) (phenytoin tablet monograph)	Shimadzu LC-10ADvp, SIL-10ADvp and SPD-10Avvp Agilent 1100 HPLC system (HP G1313A, G1314A and G1311A)	Ultrasphere ODS (4.6 mm × 250 mm, 5 μm)
Phenytoin sodium capsules	US Pharmacopeia 26 (2003, p. 1469) (extended phenytoin sodium capsule monograph)	Agilent 1100 HPLC system (HP G1313A, G1314A and G1311A)	Ultrasphere ODS (4.6 mm × 250 mm, 5 μm) and Symmetry C18 (4.6 mm × 250 mm, 5 μm)
		Waters 2690 separation module and 2487 detector Shimadzu LC-10ADvp, SIL-10ADvp and SPD-10AVvp	
Warfarin sodium tablets	US Pharmacopeia 26 (2003, p. 1938)	Agilent 1100 HPLC system (HP G1313A, G1314A and G1311A)	Zorbax CN (4.6 mm × 250 mm, 5 μm)

[a] Private communication. New Drug Application Submission to the U.S. FDA. 20-357; 1995.

(US Pharmacopeia 26, 2003). Metformin hydrochloride was analyzed according to the approved manufacturer's method (Private Communication, New Drug Application Submission to the U.S. FDA. 20-357; 1995). A Distek Dissolution System 2100A with either a basket or paddle was used for all dissolution analyses. A volume of 500 or 900 mL and a speed of 50 or 100 rpm were used as specified in each method.

The dissolution medium was degassed by pulling a vacuum at 140–150 mm of mercury on a carboy filled with 18 l of medium for 15 min, while the medium is agitated by allowing a stream of air to bubble off the bottom of the carboy.

Original analysis samples were collected using Distek DS 4300 Autosampler with Distek filters. The check analysis sample aliquots were collected manually using 5 mL syringes with 0.45 μm nylon membrane filters. In addition to the sampling times specified in each method, aliquots were taken at several other sampling times in order to obtain dissolution profiles.

2.2.4. Thermogravimetric analysis (TGA)

TGA samples were prepared according to their finished dosage forms. For capsules with powdered contents, the contents were passed through a 60-mesh sieve. For capsules with beaded contents or uncoated tablets, the contents were loaded into a WIG-L-BUG (Crescent Dental Mfg. Co.) vial and processed for 5 s, and then the powder was passed through a 60-mesh sieve. For coated tablets, the coating was removed using a razor blade, then the uncoated tablet was placed into a WIG-L-BUG vial and processed for 5 s and the powder was passed through a 60-mesh sieve.

After preparation, each sample was analyzed using a Shimadzu TGA-50H equipped with platinum pan and TA60 software version 1.40. The sample was allowed to stabilize for 2 min in an atmosphere of 50 mL/min nitrogen and then was heated at a rate of 10 °C/min to at least 600 °C.

2.2.5. Near infrared spectroscopy (NIR)

For samples in tablet form, 20 tablets were removed from the packaging with forceps and positioned on the

spectrometer sample stage. Each individual tablet was oriented in the same position according to distinguishing marks (when applicable). All samples were scanned intact.

For samples in the form of tablets with thick coatings, five tablets were removed from the packaging. If they were round and exhibited a smooth convex surface with no distinguishing marks, the coating was removed from one side of the tablet using a razor blade to produce a smooth flat surface. Each individual tablet was positioned by allowing the portion of the inner core to be exposed to reflectance for data collection.

For samples in capsule form, 20 capsules were removed from the packaging with forceps and were positioned on the spectrometer sample stage. Each individual capsule was oriented for data collection in the same position according to distinguishing marks (when applicable).

All samples were scanned using a Thermo-Nicolet Antaris – Method Development System – Fourier Transform Near Infrared Spectrophotometer using Nicolet 'RESULT Integration' software version 1.2 (Build 239). Scans were made in the Integrating Sphere Reflectance mode at a resolution of 8.0 cm^{-1} through the range 4000–10,000 cm^{-1} (2500–1000 nm). Thirty-two scans were performed on each sample, with a gain optimized to 2× or 4× depending on the sample. No beam attenuation was used.

2.2.6. NIR imaging

The two high dosage products, metformin hydrochloride (500 mg) and phenytoin sodium (100 mg) were also studied by NIR imaging. Five specimens (tablets or capsules) from each lot were evaluated by NIR imaging. Four different lots of metformin product (all tablets) were compared to one lot of innovator product (tablets), and five different lots of phenytoin product (three lots of capsules, two lots of tablets) were compared to one lot of innovator product (capsules). For capsules, the powder was removed from the capsule to avoid interference from the shell.

The specimens were scanned on a MatrixNIR chemical imaging system (Spectral Dimensions, Inc., Olney, MD), using Matrix Acquire data acquisition software (version 1.0, Spectral Dimensions). One scan of eight coadds from 1050 to 1650 nm (5 nm steps) was performed on each tablet (i.e., each was scanned eight times, and the scans were averaged). This represents nearly the full effective range of the instrument employed.

Pure reference compacts (320 mg) were prepared (2000 lb for 1 min) using a Carver press (Carver, Inc., Wabash, IN) for each drug substances and for each major excipient. These compacts were scanned in the same manner as the samples to facilitate the creation of a component spectral library. For metformin product comparison, metformin and povidone compacts were created. For phenytoin product comparison, phenytoin, confectioners' sugar, and lactose monohydrate compacts were created.

Analysis of the data was conducted using ISys data analysis software (version 3.0, Spectral Dimensions). The reflectance spectra were modified in the following manner. First, any background signal due to ambient light or other sources was removed by subtracting a "dark cube" (i.e., a scan with no sample present). Next, each pixel's spectrum was divided by a maximum performance spectrum for that pixel (created by scanning a high-reflectivity standard, Spectralon-99) to normalize the data. This minimizes any artificial interpixel differences created by inherent electronics of the detector array. Finally, reflectance values (R) were converted to absorbance (A) by the equation:

$$A = \log\left(\frac{1}{R}\right)$$

As is typical when applying chemometrics to NIR spectral data of pharmaceuticals to obtain chemical information (e.g., chemical identity), a second derivative algorithm (Savitzky–Golay algorithm, using third-order polynomials across 15 data points) (Savitzky and Golay, 1964) was applied to each spectrum to minimize physical product features (e.g., hardness and particle size). For each product, a partial least squares (PLS) model was created based on the expected major component of that product and the model applied to each sample. Final images of each sample depict the concentration of drug substance or excipient at each pixel. Images from each sample were visually compared to images of the control (innovator) product and rated for similar degree of blending.

Table 2
Weight variation (average weight and R.S.D.)

Fluoxetine	F1: 0.30210 g, 2.2%; F2: 0.32547 g, 4.0%; F3: 0.28334 g, 1.0%; F4: 0.17844 g, 3.8%; F5: 0.35886 g, 3.0%; U.S. product: 0.27743 g, 1.6%
Levothyroxine	L1: 0.10223 g, 1.1%; L2: 0.10238 g, 1.4%; U.S. product: 0.13238 g, 1.0%
Metformin	M1: 0.55928 g, 0.6%; M2: 0.52986 g, 1.6%; M3: 0.53377 g, 1.3%; M4: 0.54590 g, 0.6%; U.S. product: 0.53298 g, 1.0%
Phenytoin	
Capsules	PC1: 0.30746 g, 0.9%; PC2: 0.28458 g, 0.7%; PC3: 0.29278 g, 2.8%; U.S. product: 0.27925 g, 0.8%
Tablets	PT1: 0.25654 g, 8.3%; PT2: 0.20312 g, 1.3%
Warfarin	W1: 0.20245 g, 1.3%; W2: 0.13930 g, 0.7%: W3: 0.22175 g, 0.7%; W4: 0.20046 g, 1.6%; U.S. product: 0.22208 g, 0.9%

One sample of phenytoin sodium was sugar-coated tablets and the other was film-coated tablets. Although there is no compendia weight variation requirement for sugar-coated tablets, the test was conducted for comparison purposes.

3. Results

3.1. Physical characterization

All tablets and capsules passed USP criteria for weight variation. Weight variation results indicated all products within a given drug category were uniform in weight (Table 2). The percentage R.S.D.s obtained from weight variation tests were comparable to those obtained from dissolution and content uniformity studies.

One sample of phenytoin sodium was sugar-coated tablets and the other was film-coated tablets. Although there is no compendial weight variation requirement for sugar-coated tablets, the test was conducted for comparison purposes.

The final packaging of these products was of significant importance. Products purchased from U.S. sources, in compliance with FDA labeling requirements, were received in plastic opaque bottles with labels containing the trade name and the chemical name of the drug product, the amount of active ingredient per dosage unit, the manufacturer's name, expiration date, and lot number. Package inserts were also included.

However, for products purchased from internet sites, only one of the 20 had final packaging, including the package insert, similar to that of the U.S. products. The final packaging of the 19 remaining internet samples was bubble wrap inside a paper envelope (two samples), a Styrofoam sheet inside a paper envelope (one sample), loose blister packs (six samples), capsules or tablets in clear plastic bags without labels (three samples), and tablets or capsules in opaque plastic containers or boxes with labels (seven samples) (examples in Figs. 1–5).

3.2. Dissolution

Dissolution studies were conducted according to USP or approved manufacturer's protocols (Table 3).

3.3. Assay and chromatographic purity

HPLC analysis results of all samples were conducted according to USP or approved manufacturer's protocols. Results are detailed in Tables 4 and 5.

3.4. TGA

TGA was conducted on all 20 samples. Eleven of the 20 internet samples were found to have different formulations when compared to the U.S. product.

All four samples of metformin HCl (M1, M2, M3, and M4) yielded similar TGA curves to that of Bristol–Myers Squibb's Glucophage (Fig. 6).

The TGA curves of the three samples of levothyroxine sodium were similar to each other in solvent losses; however, the two internet products (L1 and L2) were different from the U.S. approved levothyroxine sodium product, Synthroid, in decomposition.

One sample of warfarin sodium, W3, had a derivative thermogravimetric (DTG) curve similar to that of Coumadin (U.S. product). The DTG curves of the other three samples (W1, W2, and W4) were different from Coumadin. The DTG curves of two of these

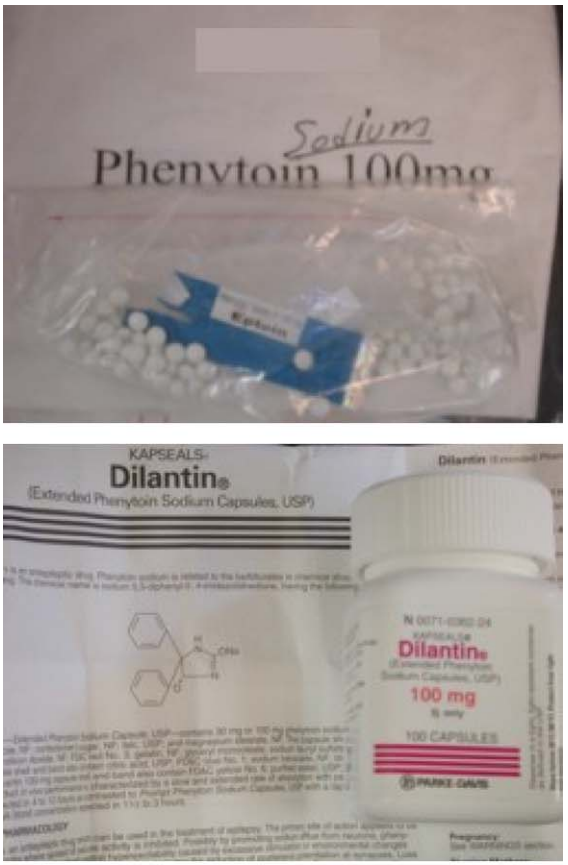

Fig. 1. Phenytoin sodium tablets and capsules. (a) Internet product phenytoin sodium tablets. (b) U.S. product phenytoin sodium capsules (Dilantin). (c) Internet product phenytoin sodium capsules.

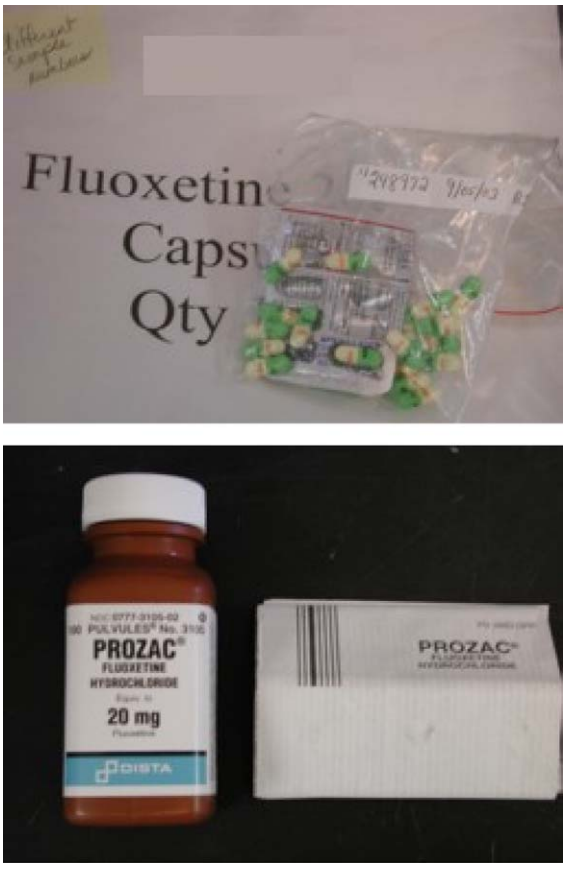

Fig. 2. Fluoxetine hydrochloride capsules. (a) Internet product fluoxetine capsules. (b) U.S. product fluoxetine tablets (Prozac).

three samples (W1 and W4) were similar to each other.

Two of the five samples of fluoxetine HCl (F2 and F3) yielded similar TGA curves to that of Prozac. The TGA curves of the remaining three samples (F1, F4, and F5) were different from Prozac (Fig. 7).

As expected since these were two different dosage forms, the TGA curves of the two phenytoin sodium tablet samples (PT1 and PT2) were different to that of Parke-Davis' Dilantin, a capsule. Two of the three phenytoin sodium capsule samples (PC1 and PC2) yielded similar TGA curves to that of Dilantin. The TGA curve of the other capsule sample (PC3) is different from Dilantin.

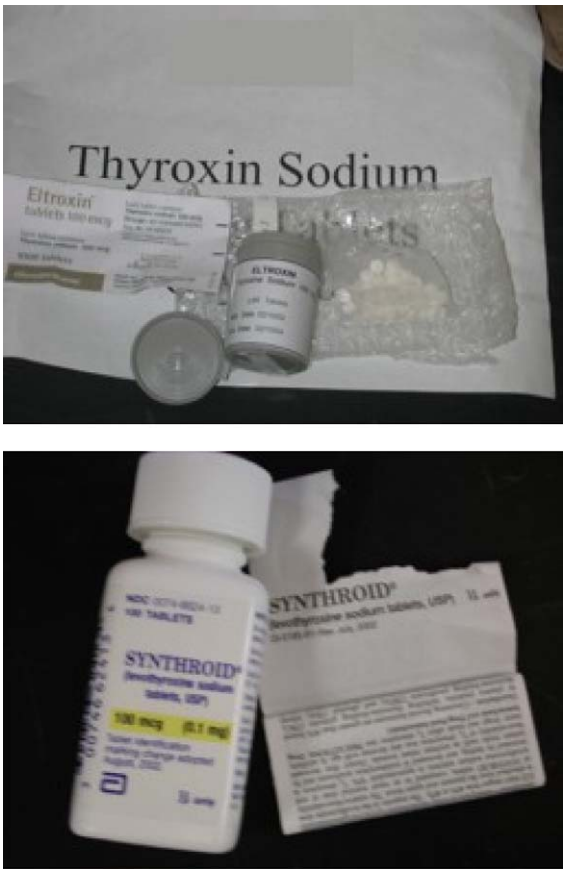

Fig. 3. Levothyroxine sodium tablets. (a) Internet product thyroxin sodium tablets. (b) U.S. product levothyroxine sodium tablets (Synthroid).

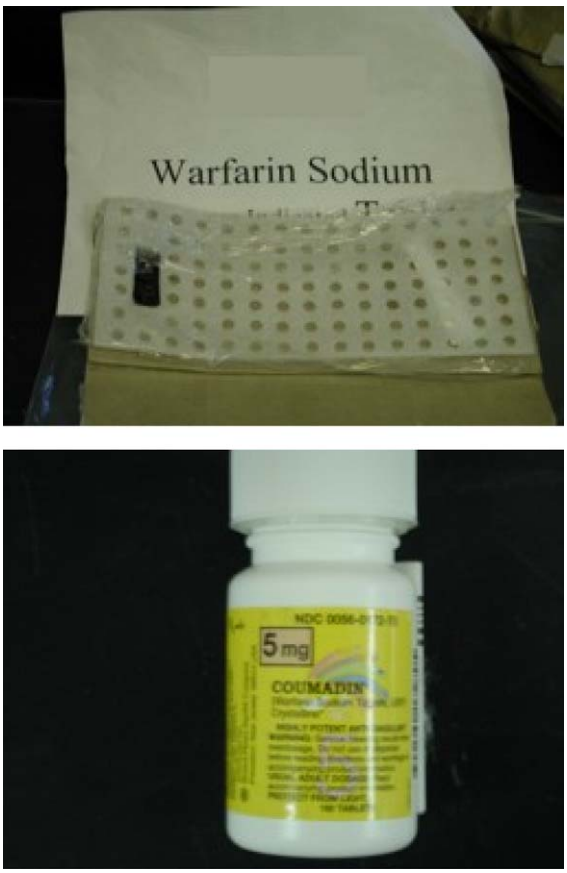

Fig. 4. Warfarin sodium tablets. (a) Internet product warfarin sodium tablets. (b) U.S. product warfarin sodium tablets (Coumadin).

3.5. NIR

NIR spectra allow a qualitative assessment of the uniformity of these pharmaceutical products. An example is warfarin sodium in which the variability of the internet sample can be seen to be greater than the U.S. sample (Fig. 8). Chemometric analyses of the second derivatives yield principle component plots showing distinct groupings (Fig. 9), indicating all products are distinct in either formulation and/or processing method/location. Furthermore, these plots give some sense of the variance within each grouping. The two phenytoin tablet samples were compared to each other since the U.S. product was a capsule. Chemometric analysis of the second derivative of the two samples showed the two products to have different variances which was corroborated by the weight variation test.

3.6. NIR imaging

Post-normalization images display absorbance values at a chosen wavelength. Control and sample specimens were judged based on visual comparison of processed PLS images. The reason for using subjective judgment is discussed below. Processed PLS images of a representative metformin control specimen are shown in Fig. 10. Note that tablet edges and imprinted designs can affect the image and should be ignored. Fig. 11 shows metformin and povidone intensities for two samples, one well blended and one poorly blended. Table 6 provides the overall results for metformin products.

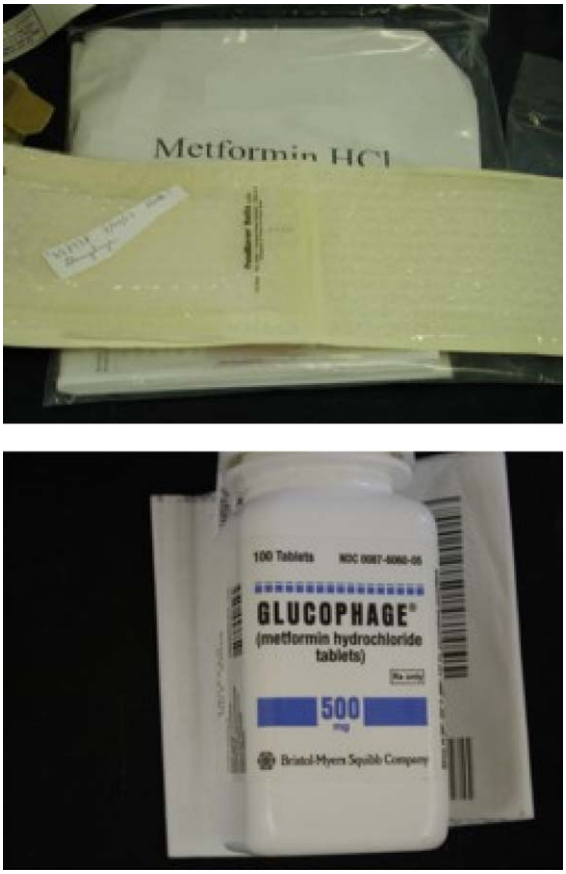

Fig. 5. Metformin hydrochloride tablets. (a) Internet product metformin hydrochloride tablets. (b) U.S. product metformin hydrochloride tablets (Glucophage).

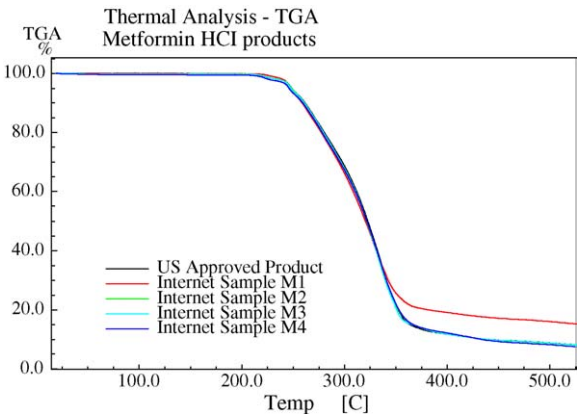

Fig. 6. TGA curves for metformin HCl products.

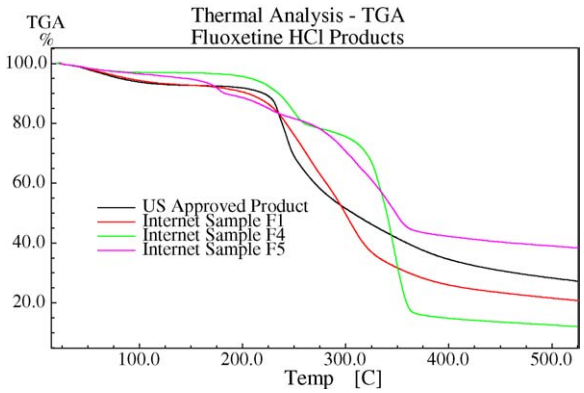

Fig. 7. TGA curves for fluoxetine HCl products.

Generally, all studied samples from a given lot were similar.

Processed PLS images of a representative phenytoin control sample are shown in Fig. 12. The left image displays phenytoin density, the middle image displays density of the excipient confectioners' sugar, and the right image displays density of the excipient, lactose monohydrate (again, red indicates high density, blue indicates low density). Fig. 13 shows phenytoin, confectioners' sugar, and lactose monohydrate intensities for three samples, and one in which

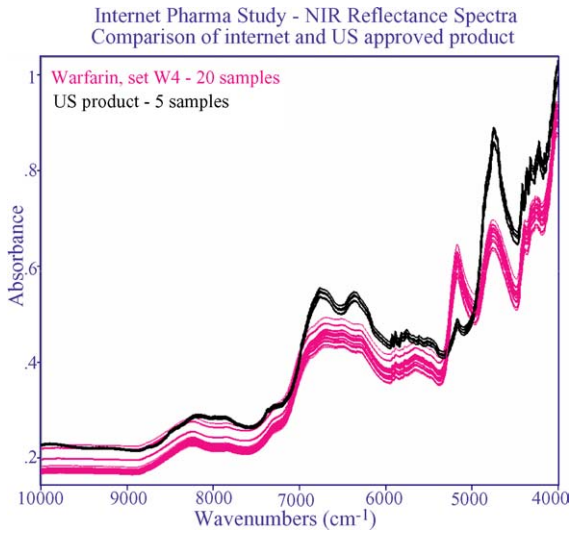

Fig. 8. NIR spectra of warfarin sodium products. NIR of warfarin sodium: more variability is seen with the internet product (pink) than the U.S. product (black). The difference in peak intensities suggests the same formulation but in different proportions.

Table 3
Dissolution results (% dissolved); range of six tablets or capsules unless indicated as stage 2

Fluoxetine	
Q = 75 at 30 min	F1: 89–99; F2: 101–104; F3: 95–98; F4: 84–99; F5: 82–92; U.S. product: 93–97
Levothyroxine	
Test 1, Q = 70 at 45 min	L1: 84–92; L2: 88–94; U.S. product: 88–94
Test 2, Q = 80 at 15 min	L1: 51–83; L2: 44–68; U.S. product: 81–86
Metformin	
	M1: 97–99; M2: 96–103; M3: 96–99; M4: 94–99; U.S. product: 96–99
Phenytoin	
Capsules	
20–40 at 30 min	PC1: 36–40; PC2; 33–36; PC3: 33–57; U.S. product: 34–36
40–80 at 60 min	PC1: 64–70; PC2: 57–61; PC3: 76–98; U.S. product: 56–61
NLT 75 at 120 min	PC1: 83–87; PC2: 81–86; PC3: 78–101; U.S. product: 78–84
Stage 2	
Average (12) 25–45	PC3: 48
Average (12) 45–75	PC3: 94
Average (12) NLT 70	PC3: 96
Tablets	
Q = 70 at 120 min	PT1: 91–98; PT2: 97–102
Warfarin	
Q = 80 at 30 min	W1: 89–98; W2: 96–100; W3: 99–101; W4: 88–100; U.S. product: 101–106

a component, lactose monohydrate, is absent. This particular sample failed the dissolution requirement (see Table 3) and the lack of lactose monohydrate may have been a contributing factor. Table 6 provides the overall results for phenytoin products. Generally, all studied samples from a given lot were similar.

4. Discussion

Sample packaging was a significant problem with virtually all the internet purchased samples. Many had either no or minimal labeling information for proper usage or testing of these drugs. Some samples had packaging with labeling in foreign languages. Some samples were shipped loosely in an unlabeled plastic bag.

Most samples passed the dissolution test, with two exceptions. The levothyroxine sodium tablets monograph lists two tests, with different sampling times and compliance tolerances. If the labeling states that it meets USP Dissolution Test 2, then the product must comply with Test 2; otherwise the product must comply with Test 1. Since both samples came in plastic bags with no description of the labeling, the Test 1 criteria were applied and both samples met the dissolution requirement. However, this is an example of the lack of labeling and the resulting problems that are encountered by the regulatory chemist. If these products were manufactured in a manner which was consistent with the Test 2 dissolution criteria, then both samples would have failed the dissolution requirement (see Table 3). Without proper labeling, the correct tests and acceptance criteria cannot be applied to a sample and therefore the quality of the product cannot be assured.

Additionally, the extended phenytoin sodium capsules monograph lists three tests. The bottle labeling must indicate if the product should comply with Test 1, 2, or 3. If there is no indication, it implies that Test 1 criteria must be met. One phenytoin sodium capsule sample failed to meet the USP dissolution tolerance for both stage 1 and stage 2 tests (Table 3). Stage 3 testing was not conducted since the results obtained after stage 2 would have caused the sample to be a failure at stage 3 also. If this sample had been treated as prompt phenytoin sodium capsules,

Table 4
Assay (average, R.S.D. and range of 10 units)

Fluoxetine	
Average	F1: 99.0%; F2: 102.0%; F3: 98.1%; F4: 100.8%; F5: 95.0%; U.S. product: 95.6%
R.S.D.	F1: 2.9%; F2: 2.2%; F3: 2.0%; F4: 5.7%; F5: 2.1%; U.S. product: 1.8%
Range	F1: 94.6–104.0; F2: 96.7–104.5; F3: 94.9–101.1; F4: 85.9–106.8; F5: 92.2–98.3; U.S. product: 93.1–97.6
Levothyroxine	
Average	L1: 93.0%; L2: 91.9%; U.S. product: 96.1%
R.S.D.	L1: 1.4%; L2: 1.4%; U.S. product: 1.8%
Range	L1: 90.7–95.2; L2: 90.3–94.7; U.S. product: 93.7–97.9
Metformin	
Average	M1: 495.0 mg[a] ; M2: 101.3%: M3: 99.6%; M4: 98.8%; U.S. product: 100.1%
R.S.D.	M1: 0.8%; M2: 1.5%; M3: 1.5%; M4: 1.6%; U.S. product: 1.2%
Range	M1: 490.9–500.3; M2: 98.5–103.5; M3: 98.7–102.5; M4: 95.0–100.6; U.S. product: 98.5–101.7
Phenytoin	
Capsules	
Average	PC1: 101.9%; PC2: 100.7%; PC3: 103.6%; U.S. product: 97.0%
R.S.D.	PC1: 4.1%; PC2: 3.7%; PC3: 4.6%; U.S. product: 3.9%
Range	PC1: 91.0–106.1; PC2: 95.8–106.6; PC3: 93.6–109.3; U.S. product: 90.8–102.3
Tablets	
Average	PT1: 95.4%; PT2: 95.5%
R.S.D.	PT1: 3.5%; PT2: 1.7%
Range	PT1: 90.7–101.4; PT2: 91.6–97.3
Warfarin	
Average	W1: 98.3%; W2: 96.4%; W3: 99.6%; W4: 96.8%; U.S. product: 99.9%
R.S.D.	W1: 3.1%; W2: 1.9%; W3: 1.7%; W4: 2.2%; U.S. product: 0.9%
Range	W1: 93.6–103.8; W2: 94.2–99.2; W3: 97.4–102.8; W4: 91.8–99.2; U.S. product: 98.0–101.0

[a] Sample received with no declared strength, therefore result reported as mg found/unit.

it still would have failed the USP dissolution tolerance.

The traditional test for purity is HPLC analysis. In this study, assay results are reported as % of label amount. One sample of metformin HCl tablets had no label declaration, so results were reported as mg found in the tablet. All 20 samples met the compendia assay limits (Table 4). There were some issues, however. One fluoxetine HCl capsule sample failed to meet the USP chromatographic purity requirement for individual impurity and total impurities (Table 5). Additionally, there is no USP monograph for phenytoin sodium tablets; as a result, the two tablet samples were evaluated using the phenytoin tablets monograph.

Thermogravimetric analysis is a viable application for fingerprinting pharmaceutical formulations and can be used to determine the "sameness" or "differences" between samples (Wendlandt and Collins, 1974; Wendlandt, 1974, 1986; Layloff, 1991). The variety of manufacturing procedures and the wide selection of excipients in the manufacture of various products, even with the same active pharmaceutical ingredient (API) means that TGA curves can differ for similar tablets (or capsules) manufactured by different producers. Different excipients and formulations can affect product stability and performance.

Several properties may serve as a fingerprint for a given manufacturer's product, making it difficult to make comparisons of each property separately. The difficulty with most approaches is the resource intensive nature of the analysis. Near infrared spectroscopy is a sophisticated and selective technique that offers an excellent opportunity for use as a screening tool to detect counterfeit samples (Olsen, 2002). NIR is sensitive to both chemical and physical nature of sample constituents and can be performed rapidly with minimal sample preparation. For our purposes NIR is

Table 5
Related substances or impurity profile

Fluoxetine	
Total number of peaks	F1: 9; F2: 6; F3: 4; F4: 5; F5: 6; U.S. product: 4
Total impurities	F1: 5.31%; F2: 0.25%; F3: 0.31%; F4: 0.11%; F5: 0.39%; U.S. product: 0.23%
Levothyroxine	
% Liothyronine	L1: 0.25; L2: 0.20; U.S. product: 0.35
Within limit	L1: yes; L2: yes; U.S. product: yes
Metformin	
Total number of peaks	M1: 2; M2: 2; M3: 2; M4: 2; U.S. product: 2
Total impurities	M1: LT 0.01%; M2: LT 0.01%; M3: LT 0.01%; M4: LT 0.01%; U.S. product: LT 0.01%
Phenytoin	
Capsules	
Related cmp. A	PC1: 0.1%; PC2: trace; PC3: trace; U.S. product: trace
Related cmp. B	PC1: 0.1%; PC2: 0.1%; PC3: 0.1%; U.S. product: 0.1%
Tablets	
Related cmp. A	PT1: 0.1%; PT2: trace
Related cmp. B	PT1: 0.4%; PT2: 0.2%
Warfarin	
Total number of peaks	W1: 6; W2: 8; W3: 3; W4: 5; U.S. product: 2
Total area (%)	W1: 0.2; W2: 0.3; W3: 0.1; W4: 0.2; U.S. product: 0.1

demonstrated as a helpful technique to distinguish one product from others of the same type. Because nearly all compounds important for formulation exhibit some NIR spectrum, it is possible to determine the similarities of the components in a sample instead of assuming total uniformity based on the active ingredient alone (Filmore, 2003).

In this study, NIR confirmed the findings of the content uniformity testing—some internet products had a larger tablet-to-tablet variability than their U.S. counterparts. In addition, the TGA finding that some products had different compositions (excipients) was confirmed. This technique very rapid—all 20 samples were scanned over 2 days.

The NIR imaging results demonstrate the ability of the imaging system to detect the drug substance and display its density pattern within the drug product. For the innovator products of metformin and phenytoin (see Figs. 10 and 12), the drug substances seem well distributed throughout the product. The excipient povidone in the metformin product also seems well distributed. The sugars in the phenytoin product exhibit some regions of high intensity, possibly due to the presence of large particles or slight clumping of the excipients.

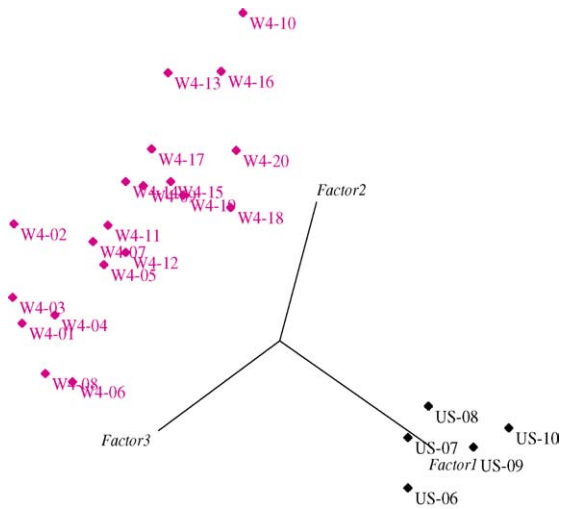

Fig. 9. Chemometric analysis of warfarin sodium. Chemometrics (PCA, mean centered, second derivative): mathematical treatment of the NIR data gives a graphical representation of the spectra. The increased variability of the internet product (pink) is very visible and the two products are seen to be dissimilar (occupying different areas in this 3D space).

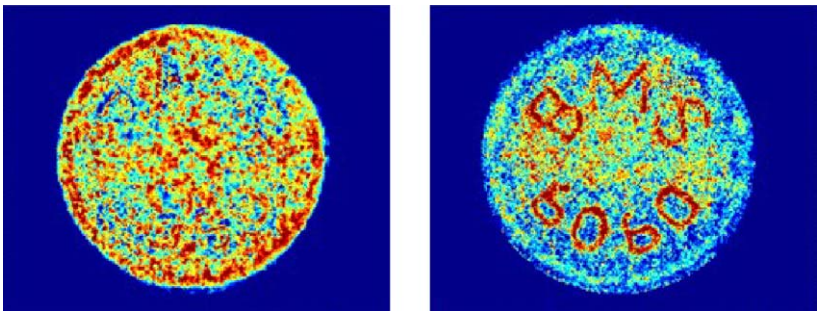

Fig. 10. PLS images of a metformin control sample. The image on the left displays metformin density—red indicates high density and blue indicates low density. The image on the right shows povidone density, again with red indicating high density.

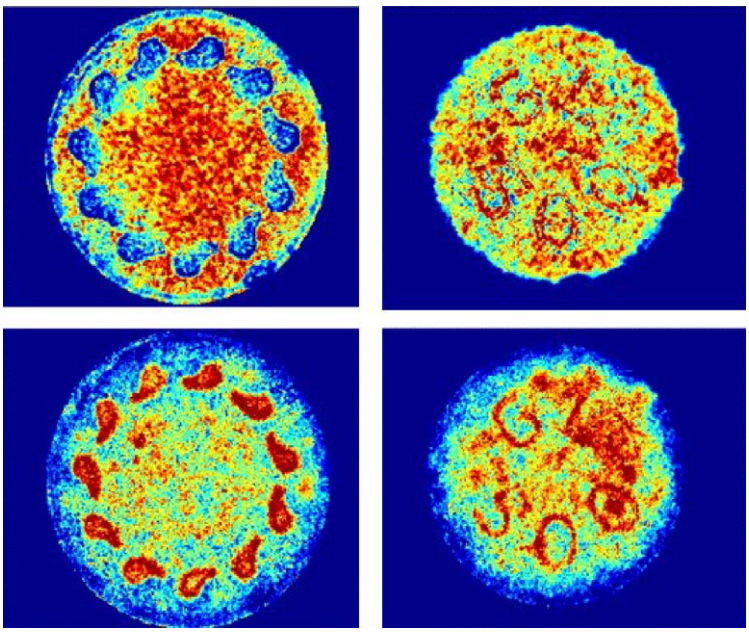

Fig. 11. PLS images of two metformin samples. The left images show metformin (upper) and povidone (lower) densities where red indicates high density. The right images show metformin and povidone densities for a sample with poorer blending.

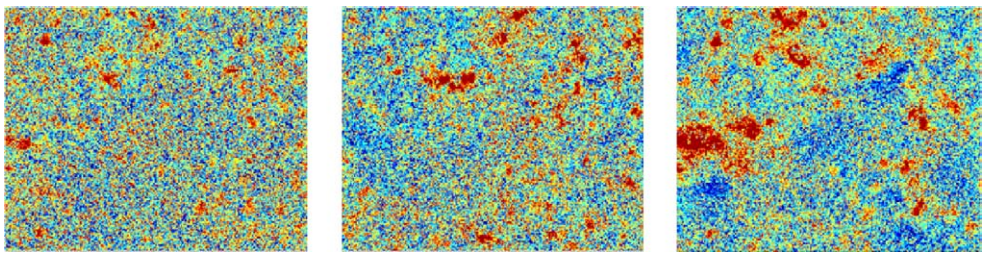

Fig. 12. PLS images of a phenytoin control specimen sample. These are magnified (10×) images of powder removed from the capsule shells. The image on the left displays phenytoin density. As in earlier figures, red indicates high density and blue indicates low density. The middle image shows confectioners' sugar density, and the image on the right shows lactose monohydrate density.

Table 6
NIR imaging on intra-dose homogeneity

Metformin	
Presence of API	M1: yes; M2: yes; M3: yes; M4: yes; U.S. product: yes
Intra-dose homogeneity	M1: no; M2: no; M3: no; M4: yes; U.S. product: yes
Phenytoin	
Capsules	
Presence of API	PC1: yes; PC2: yes; PC3: yes; U.S. product: yes
Intra-dose homogeneity	PC1: somewhat; PC2: yes; PC3; somewhat; U.S. product: yes
Tablets	
Presence of API	PT1: yes; PT2: yes
Intra-dose homogeneity	PT1: no; PT2: no

Regions of high concentration, indicating a lack of mixing, were more prevalent in the internet products than in the U.S. products. Poor mixing and different (absent) excipients could explain the dissolution failure.

Additionally, these types of images permit the direct qualitative comparison of sample drug products to the innovator (control) product. The left images in Fig. 11 show uniformity of density for both metformin and povidone, similar to the control (ignoring edges and imprinted designs). The right images of Fig. 11, however, show large distinct regions of high density of metformin and povidone, suggesting poor blending. For the phenytoin products (Fig. 13), the top product is well blended (there are some regions of high density for the sugars, but this is also observed in the control). The middle product is slightly less well blended, but not severely so. Note that for the middle product densities of phenytoin and lactose monohydrate coincide somewhat, perhaps suggesting that the two are blended prior

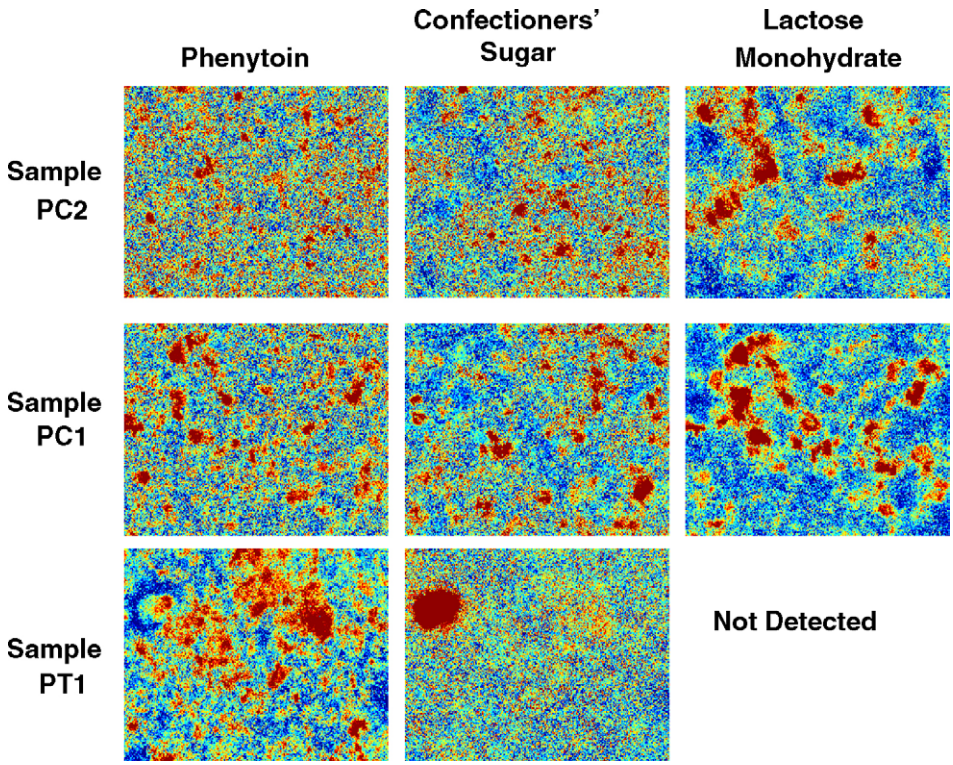

Fig. 13. PLS images of three phenytoin sample specimens. Each row is a separate specimen. The left images show phenytoin in red, the middle images show confectioners' sugar in red, and the right images show lactose monohydrate in red.

to mixing with confectioners' sugar. The lower product (a tablet), shows large distinct regions of density for both drug and confectioners' sugar, showing that the product is not as homogeneous as the control.

Finally, it should be noted that the imaging results here are presented in a subjective manner. An effective objective measurement of intra-dose homogeneity could be carried out as in Lyon et al. (2002) by recording the average and standard deviation of density for a given component within a specimen. Homogeneous tablets will have small standard deviations (or at least, small CV), while heterogeneous tablets will have larger standard deviations (or CV). However, to identify an entire sample lot as less homogeneous than the innovator, it would be necessary to examine multiple lots of innovator product to determine a range of acceptable variability. Such a large-scale study is beyond the scope of the current work but could be the basis for future study.

5. Conclusions

Most internet samples collected during this study did not physically resemble their approved U.S. counterparts, did not have package inserts, and contained minimal labeling information. This makes proper identification and usage difficult for U.S. consumers. This lack of information also made it difficult for the FDA laboratory to determine which monographs to apply in the regulatory testing of these products. Seven of the samples were in questionable containers such as zip-lock bags and folders. These types of containers would lead one to believe that the internet supplier is repackaging from a bulk container. If this assumption is correct, then the bulk container could contain product from various manufacturers; thereby, compounding the issue of quality since one manufacturer's product may pass quality attribute tests while a second manufacturer's product could fail the very same tests.

Of the 20 samples received and tested, 2 failed USP monographs for quality attributes. One failure (phenytoin) was for dissolution, which raises the question of bioavailability of this product. The second failure (fluoxetine) was for chromatographic purity, a potential safety issue. Although this was a limited study, it is indicative of a higher failure rate than what is observed in products manufactured in the United States (Yorke, 1995). Both of these quality failures could be due to formulation problems, manufacturing processes, improper storage or shipping conditions. TGA, NIR and NIR imaging suggest that formulation differences may be at least one cause for the phenytoin and fluoxetine failures.

Non-traditional analytical techniques complemented the traditional techniques and highlighted additional quality issues for these products. TGA and NIR identified 11 of the 20 products as having different formulations from the approved U.S. counterpart. NIR highlighted three products as being more variable than the U.S. product. TGA and NIR indicated that many of the samples had excipients different from the U.S. approved product, which can affect the shelf life of the product. NIR imaging also highlighted formulation differences and was able to detect manufacturing issues (such as non-uniform blending), which could lead to quality issues for future lots. Most of these formulation and manufacturing issues would not have been evident with traditional testing alone. Both NIR and NIR imaging can be nondestructive so tablets can be scanned and then used for further testing.

The failures and concerns identified in this study could be further explored in a larger study. If the problems encountered with 20 samples continue to be prevalent in a larger study, this would demonstrate that internet sources of foreign prescription pharmaceuticals could pose significant safety and/or efficacy risks to U.S. consumers.

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