APPLICATION NOTE

FT-NIR Spectroscopy

Authors:

Kieran Evans

Kathryn Lawson-Wood

PerkinElmer, Inc.
Seer Green, UK

Dr. Sulaf Assi

Department of Archaeology, Anthropology and Forensic Science, Bournemouth University
Poole, UK

Identification of Counterfeit Phosphodiesterase Inhibitors using NIR Spectroscopy and Chemometrics

Introduction

The counterfeiting of pharmaceuticals for economic gain is a growing problem in a modern society within which medicines play a major role. There are four main types of pharmaceutical counterfeits:

- Incorrect or no Active Pharmaceutical Ingredient (API)
- Incorrect concentration of the API
- Illegitimate excipients
- Falsified expiry dates

The exact number of counterfeit medicines produced/consumed each year is difficult to determine due to the usually illegitimate methods by which they are distributed. However, the scale of the problem can be represented by the fact that 20 million pills, bottles, and sachets of counterfeit medicines were seized in a five-month operation conducted by the International Criminal Police Organization in China and South-East Asia in 2009.[1]

The medicines characterized in this study are selective Phosphodiesterase-5 (PDE_5) inhibitors. This class of medicines is used primarily for the treatment of Erectile Dysfunction with various other uses such as the treatment of Benign Prostatic Hyperplasia (BPH) and Pulmonary Arterial Hypertension.[2]

PerkinElmer®
For the Better

More specifically, counterfeiting of the most widely used of these three drugs (sildenafil) is a large scale problem, with nearly 80% of online sources that are claiming to sell Viagra® actually selling counterfeits. In the aforementioned study by Pfizer, materials found in the counterfeits included amphetamines, metronidazole, and illegitimate binding ingredients.[3]

The API and excipients found in the legitimate forms of the medicines are shown in Table 1.[4]

Table 1. Medicine constituents.

Medicine Brand Name	Active Ingredient	Tablet Core Excipients	Tablet Coating Excipients
Viagra®	Sildenafil citrate	• Microcrystalline cellulose • Calcium hydrogen phosphate • Croscarmellose sodium • Magnesium stearate	• Hypromellose • Titanium dioxide (E171) • Lactose monohydrate • Triacetin • Indigo carmine aluminum lake (E132)
Levitra™	Vardenafil hydrochloride	• Crospovidone • Magnesium stearate • Microcrystalline cellulose • Colloidal anhydrous silica	• Macrogel 400 • Hypromellose • Titanium dioxide (E171) • Ferric oxide yellow (E172) • Ferric oxide red (E172)
Cialis™	Tadalafil	• Lactose monohydrate • Microcrystalline cellulose • Sodium laurilsulfate • Magnesium stearate	• Lactose monohydrate • Hypromellose • Triacetin • Titanium dioxide (E131) • Iron oxide yellow (E171) • Iron oxide red (E171)

This application note demonstrates the use of chemometric models using PerkinElmer's Spectrum Two N™ FT-NIR spectrometer with AssureID™ and Spectrum 10™ software for determining the authenticity of sildenafil, tadalafil, and vardenafil tablets.

Experimental

NIR Spectra of authentic and counterfeit sildenafil, tadalafil, and vardenafil were measured using the PerkinElmer Spectrum Two N FT-NIR spectrometer with the Near-Infrared Reflectance Module (NIRM). Experimental parameters are shown in Table 2. The only sample preparation required in this analysis was the crushing of tablets into a fine powder.

Table 2. Experimental parameters for sample measurements.

Parameter	Value
Spectral Resolution	8 cm^{-1}
Number of Scans	32
Scan Range	10,000-4,000 cm^{-1}

Chemometric models of the three drugs, sildenafil (Viagra®), vardenafil (Levitra™), and tadalafil (Cialis™) were produced using a SIMCA algorithm in PerkinElmer AssureID software with first derivative pre-processing to eliminate effects from baseline shift. These models were used in Spectrum 10 software to identify possible counterfeits of these drugs.

Table 3. Number of authentic and counterfeit samples scanned for sildenafil, vardenafil, and tadalafil.

Material	No. Authentic Spectra	No. Counterfeit Spectra
Sildenafil (Viagra®)	103	120
Vardenafil (Levitra™)	20	42
Tadalafil (Cialis™)	30	45

The number of authentic and counterfeit spectra measured for each material is shown in Table 3.

The SIMCA models were validated in Spectrum 10 software using the counterfeit spectra and Verify command.

Results and Discussion

Sildenafil (Viagra®)

A SIMCA model of 93 authentic sildenafil samples is shown in Figure 1.

To validate the SIMCA model, 120 counterfeit sildenafil and 10 authentic samples were used. The Verify command in Spectrum 10 (for SIMCA methods developed in AssureID) was used to determine validation sample authenticity. Verify was successful in identifying 100% of samples correctly. As shown in Figure 2, a sharp peak at 7187 cm^{-1} is present in most of the counterfeit spectra which is not present in the mean fingerprint for the authentic sildenafil. This peak is likely due to the presence of talc being used as an excipient in the counterfeit materials.

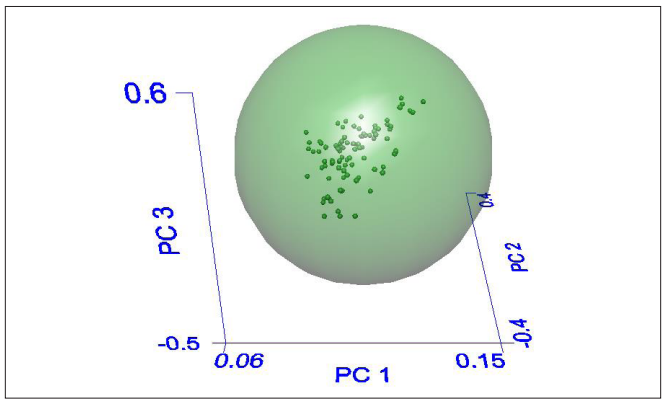

Figure 1. SIMCA model of authentic sildenafil and sildenafil citrate.

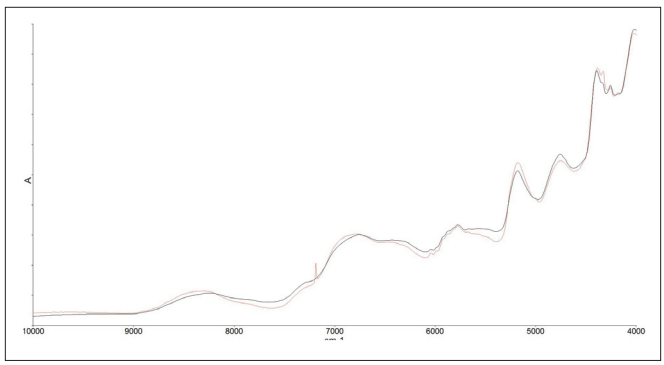

Figure 2. A typical spectrum of a counterfeit sildenafil sample (black) compared to the mean fingerprint of authentic sildenafil (red).

Vardenafil (Levitra™)

15 authentic vardenafil NIR spectra were used to produce a SIMCA model (Figure 3). Due to a small sample set, the number of principal components selected by AssureID software was two.

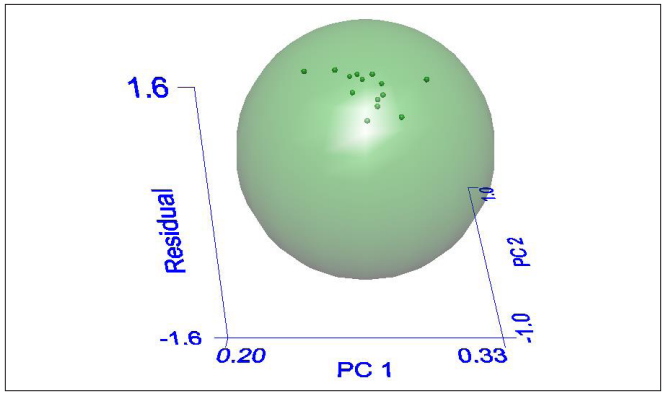

Figure 3. SIMCA model of authentic vardenafil.

The validation set included 42 counterfeit and five authentic vardenafil spectra. Unlike Sildenafil, the counterfeit vardenafil spectra did not contain any obviously different spectral features. However, this did not prevent Verify correctly identifying 100% of the validation samples. The lack of obvious spectral differences in this region may have been due to the counterfeits having a different concentration of API or altered expiry date.

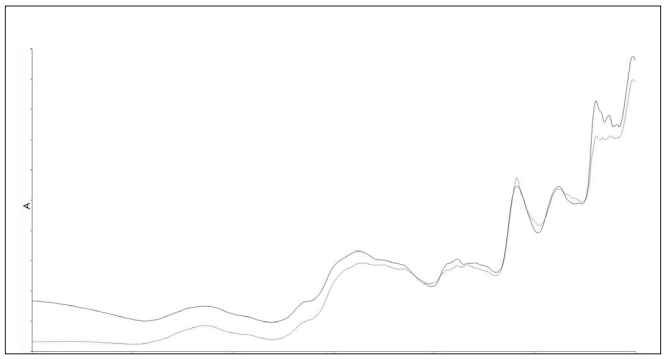

Figure 4. A typical example of a counterfeit vardenafil spectrum (green) compared to the mean fingerprint of authentic vardenafil (black).

Tadalafil (Cialis™)

30 authentic NIR Spectra of tadalafil were used to produce a SIMCA model (Figure 5). Again, due to a small sample set, the number of principal components selected by AssureID software was two.

The validation set contained 45 counterfeit and five authentic spectra of tadalafil. The Verify command was again successful in identifying 100% of the validation spectra correctly. As observed with counterfeit sildenafil samples, tadalafil counterfeits all contained talc, and thus a sharp peak at 7187 cm^{-1} was present in their spectra.

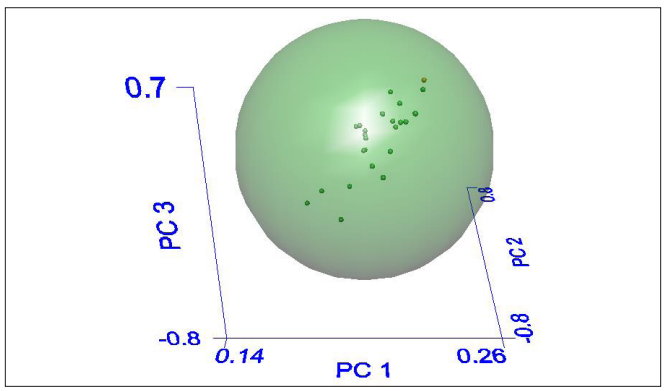

Figure 5. SIMCA model of authentic tadalafil.

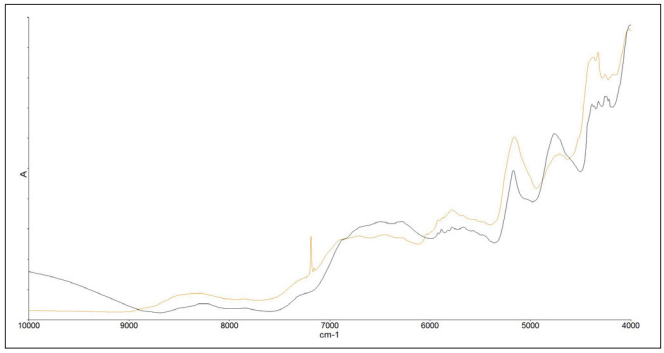

Figure 6. A typical example of a counterfeit tadalafil spectrum (orange) compared to the mean fingerprint of authentic tadalafil (black).

Model Distances

A more quantitative depiction of the validity of the models can be shown by the distance ratio of the validation samples. This value is the ratio of the total distance from the model against the critical distance, or threshold value, with a sample only being classified within the model if the ratio is less than 1.0000. Table 4 shows the mean model distances for the validation samples of each drug.

Table 4. Average distance ratios for the medicines of interest.

Medicine Brand Name	% Authentic Validation Samples Identified Correctly	% Counterfeit Validation Samples Identified Correctly	Average Authentic Distance Ratio (Range)	Average Counterfeit Distance Ratio (Range)
Sildenafil	100	100	0.8328 (0.75–0.92)	11.2338 (2.63–26.11)
Vardenafil	100	100	0.5374 (0.50–0.57)	3.6330 (3.19–4.70)
Tadalafil	100	100	0.6862 (0.61–0.84)	11.5445 (8.89–18.95)

The Verify command can be incorporated into Spectrum Touch™ methods, allowing a workflow approach and simple routine operation. To provide a more user-friendly interface, Spectrum Touch software enables users to access methods from a menu and run them with detailed step-by-step instructions. This eliminates the need for costly and time-consuming training. An example of the output created from a Spectrum Touch workflow for the analysis of a sample using the sildenafil model is shown in Figure 7. The results page shows an easy-to-read pass/fail result, with further details such as model distance. In this example, a counterfeit sample of sildenafil is correctly identified with the interface clearly displaying various detailed results from the Verify algorithm. Alternatively, the 'Scanalyze' function in Spectrum 10 allows a sample to be scanned and identified using the Verify algorithm in one click.

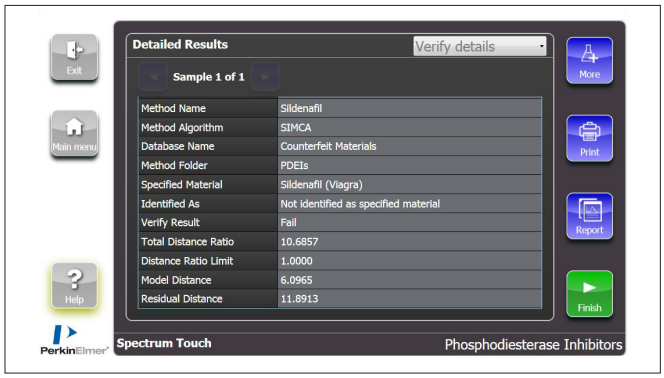

Figure 7. Example Verify Result from a Spectrum Touch method.

Conclusion

The PerkinElmer Spectrum Two N™ FT-NIR Spectrometer allows for quick analysis of pharmaceutical materials with minimal sample preparation. PerkinElmer AssureID and Spectrum 10 software with the Verify command allow for simple yet accurate characterization of counterfeit pharmaceutical products. Additionally, for increased simplicity and a workflow-based approach, Verify can be incorporated into Spectrum Touch methods, allowing quick and simple screening of counterfeit medicines.

References

1. http://www.who.int/bulletin/volumes/88/4/10-020410/en/ (Accessed 10/06/17).
2. Huang SA, Lie JD. Phosphodiesterase-5 (PDE5) Inhibitors in the Management of Erectile Dysfunction. Pharmacy and Therapeutics. 2013; 38(7):407-419.
3. https://www.viagra.com/getting/avoid-counterfeits (Accessed 10/09/17).
4. The electronics Medicine Compendium (eMC), https://www.medicines.org.uk/emc/, (Accessed 10/20/17).

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

013874A_01 PKI