# Analysis of Synthetic Phosphodiesterase Type-5 Inhibitors in Counterfeit Products by Data Directed LC/MS/MS, Accurate Mass MS/MS and LC-UV.

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#### INTRODUCTION

Pharmaceutical counterfeiting is a global problem which is more prevalent in developing countries but the impact is being increasingly felt in developed countries<sup>1-2</sup>. Counterfeit pharmaceuticals may contain the wrong active (pharmaceutical) ingredient (API) or contain the correct ingredient but the level of the API may be misrepresented, either too high or too low than what is indicated on the label. Counterfeit drugs may also be mislabelled with respect to the source and are packaged to appear to originate from a reputable vendor but in reality have come from a different source<sup>3</sup>. They may contain high levels of impurities and contaminants. There have been many incidents of counterfeiting resulting in death<sup>3,4</sup>. Even the US drug supply among the most secure in the world has not been immune<sup>5</sup>. These cases highlight a very serious public health concern.

The Center for Medicine in the Public Interest, predicts that counterfeit medicine sales will reach approx. €5.5 billion globally by 2010<sup>6</sup>

The market success of the three approved synthetic Phospodiesterase Type-5 (PDE-5) inhibitors for the treatment of erectile dysfunction (ED) has led to an explosion in the number of detected cases of counterfeiting of sildenafil citrate, vardenafil hydrochloride and tadalafil. Shown in figure 1 is a picture of some of the imitation brand and generic products analysed. The legitimate samples were compared with those samples obtained, without prescription from online and overseas pharmacies.

Various analytical techniques are used including sub 2µm particle LC and a tandem quadrupole MS capable of acquiring simultaneous MS and MS/MS data<sup>7</sup>. High resolution MS experiments were carried out on a quadrupole Time of Flight mass spectrometer. Photo diode array data was collected simultaneously to provide additional information.



Figure 1. Shown is a picture of some of the imitation brand and generic products.

#### EXPERIMENTAL LC conditions Solvent Delivery:

Sample Delivery: Column:

Column Temp: Sample Temp: Injection Volume: Flow Rate: Mobile Phase A: Mobile Phase B: Gradient:

**PDA** conditions Range: 210-400nm MS Conditions MS System:

**Ionisation Mode** Capillary Voltage (ESI):

Cone Voltage: Collision Energy (CE):

Desolvation Temp: Desolvation Gas: Source Temp: Acquisition Range: QTOF acquisition time: Quadrupole Scan Speed: Lock mass reference (ESI):

#### **Sample Procurement**

Imitation "brand" and "generic" samples of the drugs (there are currently no approved generic equivalents for the three approved synthetic PDE-5 inhibitors) were obtained, without prescription from internet pharmacies. The samples arrived with customs declarations that did not reflect the true contents of the package (Figure 2). Some samples were labeled as natural foods, candy or gifts. A few of the samples were packaged using boxes and logos from the originator. Patient information leaflets were often absent or when present had many spelling and other typographical mistakes in them.



Figure 2. Shown are pictures of products obtained with accompanying customs declarations.

#### **METHODS**

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ters <sup>®</sup> ACQUITY UPLC <sup>®</sup> Binary Solvent nager ters ACQUITY Sample Manager QUITY UPLC BEH C <sub>18</sub> 2.1 x 100 mm, ' μm ° C C IO μL	
<ul> <li>μL/min.</li> <li>mM Ammonium Acetate in Water</li> <li>50 Methanol/Acetonitrile</li> <li>min, 20-52% B, hold at 52% B until</li> <li>min, 10.5-12.5 min 85% B, hold at</li> <li>% B until 15 minutes return to</li> <li>ial conditions.</li> </ul>	! ! ! ! ! ! !
Waters <sup>®</sup> XEVO <sup>™</sup> Quadrupole Time of-flight MS or XEVO <sup>™</sup> TQ Triple Quadrupole MS ESI Positive 3.0 kV	
40 V 25-55eV	∎ ;
100-450°C 800 L/Hr 120 °C 100—700 amu or 50-1000 amu 0.05 sec 5000 amu/sec	(

5000 amu/sec Leucine Enkephalin

### **RESULTS AND DISCUSSION**

#### **Discovery of Mixed Counterfeit Tablets using a** XEVO<sup>™</sup> TQ MS with Survey Scanning

A sample obtained from an internet pharmacy was sold as a 20 mg Vardenafil tablet. The sample was analysed using a Waters Xevo<sup>™</sup> TQ MS in survey scan mode. In survey mode a full spectrum LC-MS run is collected, with a LC-MS/MS experiment triggered if the signal in the LC-MS survey meets preset criteria. The data showed that the sample did not contain vardenafil (m/z 489, Rt=8.70 min) it predominantly contained sildenafil (m/z 475, Rt = 8.30 min) and also contained tadalafil (m/z 390, Rt= 5.92 min). The expected diagnostic fragments were apparent in the sildenafil spectrum, m/z 311, m/z 283 and m/z 99. In addition to finding known compounds, it can be seen from the MS/MS fragmentation patterns in Figure 3, that there are other potentially related compounds in the sample. Many of them above 0.1% of the API peak (measured at 230nm in the UV).



Figure 3. Shows Survey ScanWave<sup>7</sup> MS (lower trace) switching to ScanWave DS. Product Ion spectra for peaks labeled 1-6 are also shown.

#### Impurity Profiles found in Imitation Sildenafil Citrate Tablet Samples

Sildenafil citrate tablet samples from five different internet or over seas pharmacies were analysed (n=6) by high resolution MS using a Waters XEVO<sup>™</sup> QToF with simultaneous UV. The impurity profiles were compared to those of sildenafil from the original manufacturer. They were found to contain significantly higher numbers of impurities, the levels of which were frequently above the reporting threshold of 0.05% (of the API peak, measured in the UV) Figure 4.



Authentic brand sildenafil citrate, vardenafil hydrochloride and tadalafil were obtained from reputable pharmaceutical wholesalers. The extracted tablet samples were analysed by UPLC and high resolution MS on a Waters XEVO QToF MS in MS<sup>E</sup> mode. An MS<sup>L</sup> experiment acquires a low and a high collision energy MS function allowing the collection of MS and all product ion fragmentation data simultaneously. This in combination with TOF MS can rapidly identify unknown compounds using exact mass measurement and elemental composition determination with isotope ratio comparison (iFit). Results are shown in (Figure







Figure 4. UV Chromatograms at 230nm shown for blank sample, authentic brand sildenafil and imitation brand sildenafil from an internet pharmacy.

Pharmacies

The impurity data was processed using MarkerLynx XS. Tablet samples purchased from one internet pharmacy were MarkerLynx XS integrates and aligns chemical and biological MS made to resemble the authentic sildenafil citrate, vardenafil data points and converts them into Exact Mass Retention Time hydrochloride and tadalafil tablets from the original (EMRT) pairs. Those EMRT pairs can then be used for multivariate manufacturers. Though the appearance of the tablets conformed statistical analysis to visualize and interpret complex MS data to the appearance of the genuine medicine, when the results sets<sup>8</sup> (Figure 7). Significant differences between those six were compared with the data from the authentic samples (Figure groups are clearly illustrated in the plot. 5) it showed that the vardenafil and tadalafil pills contained the wrong API. It could be seen that while the sildenafil citrate tablet Scores Num vs. Comp[1] vs. Comp[2]. colored by Group Imitation did contain the correct API (m/z 475.2128), the vardenafil (m/z Sildenafil tablets 489.2284) and tadalafil (m/z 390.1454) samples did not (Figure This case alone highlights the level of risk a consumer is 6). taking when purchasing drugs from random uncertified internet pharmacies







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## **Risk Associated With Using Uncertified Internet**

Figure 6.  $MS^{E}$  chromatograms for high collision energy MS function and associated spectra for imitation brand tadalafil, vardenafil and sildenafil, Photographs of the products are also shown

ceutical Counterfeiting. Analyst, 2005, 130, 271- 179 www.fda.gov/oc/initiatives/counterfeit/ga.html

www.gphf.org/web/en/minilab/ hintergrund\_arzneimittelfaelschungen.htm#Beispiele /35.pixelcms.com/ams/ assets/312296678531/455\_EAASM\_counterfeiting%

- t\_020608.pdf ring Qualitative Confirmation using Xevo TQ MS with Survey Scanning. Waters
- application Note 720002838en
- 8. L. Eriksson, Book "Multi- and Megavariate Data Analysis: Basic Principles and Applications", 2nd edition, 2008

#### MarkerLynx<sup>™</sup> XS Data Interpretation



Figure 7. OPLS-DA model 3-D score plot showing group difference between the authentic sildenafil (blue Group) and imitation sildenafil tablets (red, pink, turquoise, purple and green Groups)

#### CONCLUSION

Counterfeit pharmaceutical drugs were easily differentiated from the original drug manufacturer by utilizing a variety of Waters solutions.

#### ACQUITY UPLC provided:

high resolution and sensitivity to resolve and detect differences between imitation and the originator drug product impurity profiles.

#### Xevo TQ provided:

- the ability to do data directed analysis allowing simultaneous MS and MS/MS data acquisition in one injection.
- reduced number of experiments compared to other triple quadrupole instruments.

#### Xevo Q-ToF provided:

- accurate mass for confident compound identification and verification
- MS<sup>E</sup> for fragment analysis essential for structural confirmation of analytes.

#### Markerlynx XS provided:

Intelligent multivariate statistical tools aiding visualization of complex data.

Uncertified internet pharmacies are potentially unsafe

feit Medicines, An update on Estimates. World Health Organisation: Geneva

www.who.int/medicines/services/counterfeit/impact/

NewEstimatesCounterfeit.pdf