Rapid Detection and Identification of Synthetic Phosphodiesterase Type-5 Inhibitors in Counterfeit and Adulterated products using the Atmospheric Solids Analysis Probe

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INTRODUCTION

When hyphenated methods such as LC/MS and GC/MS are used in situations that require high throughput sample screening, sample extraction and chromatographic separation are frequently required and this can create a sample bottleneck. In recent years novel ambient desorption ionisation techniques for surface analysis of solid and liquid samples with subsequent MS detection have been reported. The techniques include desorption electrospray ionisation[1] (DESI), Direct analysis in real time[2] (DART) and atmospheric solids analysis probe[3] (ASAP).

The advantage of using these direct ionisation methods is that sample preparation is often minimal or absent altogether. The total analysis time can be decreased significantly due to the elimination of the chromatographic separation facilitating fast analysis times.

The ASAP, was invented by McEwen et al[3]., and can be used to analyse volatile or semi-volatile solid or liquid samples using atmospheric pressure ionisation (API) (Figure 1A). We investigated the Waters® Atmospheric Pressure Solids Analysis Probe (Figure 1B) for the rapid determination of synthetic phosphodiesterase type-5 inhibitors in counterfeit tablet samples (Figure 2A) and adulterated herbal supplements (Figure 2B).

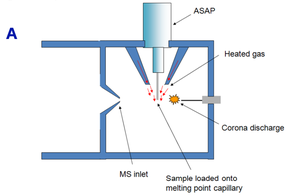

Figure 1. Illustration of ionisation using ASAP (top) and the ASAP probe (beneath).

METHODS

EXPERIMENTAL

MS Conditions

MS System:	Waters® LCT Premier™ XE Time-of-flight MS or Xevo™ QTof ™ MS
Mode:	ESCI Positive
Capillary Voltage (ESI):	3.0 kV
Corona current (APCI):	5µA
Cone Voltage:	40 V
Collision Energy Ramp:	35-55eV
Desolvation Temp:	100-450˚C
Desolvation Gas:	600 L/Hr
Source Temp:	120 ˚C
Acquisition Range:	50—1000 amu
Scan time:	0.5 sec
Lock mass reference (ESI):	Leucine Enkephalin

The instrument was operated in combined ESI/APCI mode (ESCI). This enabled the acquisition of analyte data in APCI mode and reference data in ESI mode.

Phosphodiesterase Type-5 Counterfeiting and Adulteration

Pharmaceutical counterfeiting is a global phenomenon and the number of detected cases continues to grow[4-7]. The Center for Medicine in the Public Interest, predicts that counterfeit medicine sales will reach approx. €55.5 billion globally by 2010[7]. The pervasive 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 2A is a picture of some of the imitation brand and generic products analysed. Recently a number of alternative herbal dietary supplements (HDS) used to treat ED have been reported to be adulterated with the pharmaceutical products or their structurally modified analogues[8-17] (Figure 2B).

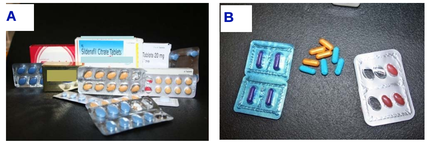

Figure 2A. Shows a picture of some of the imitation brand and generic products. 2B shows some of the adulterated herbal products obtained over the internet.

RESULTS AND DISCUSSION

Sildenafil Citrate, Vardenafil Hydrochloride and Tadalafil tablet Samples

Authentic brand sildenafil citrate, vardenafil hydrochloride and tadalafil were obtained from reputable pharmaceutical wholesalers. Sample vaporization profiles from 13 legitimate products and the internet pharmacy samples are shown in Figure 3A. Each peak represents a different sample and the data is acquired in a single file. Tablet samples purchased from one internet pharmacy were made to look like the authentic sildenafil citrate, vardenafil hydrochloride and tadalafil tablets from the original manufacturers. Though the appearance of the tablets conformed to the appearance of the genuine medicine, results from ASAP with Time-of-Flight (TOF) MS detection showed that the vardenafil and tadalafil pills contained the wrong active pharmaceutical ingredient (API). It could be seen that while the sildenafil citrate tablet did contain the correct API (m/z 475), the vardenafil (m/z 489) and tadalafil (m/z 390) samples did not (Figures 3B, 3C). This case alone highlights the level of risk a consumer is taking when purchasing drugs from random uncertified internet pharmacies.

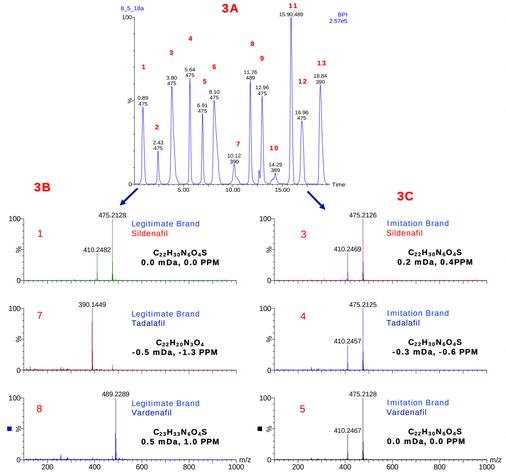

Figure 3. Spectra and Elemental composition results for legitimate, sildenafil, tadalafil and vardenafil and imitation brand sildenafil, tadalafil and vardenafil .

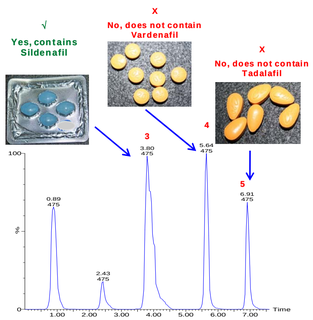

Figure 4. Sample vaporization profiles for five counterfeit tablet samples. ASAP with TOF MS detection identified the vardenafil and tadalafil to be fraudulent counterfeit tablets.

Adulteration of HDS samples with Synthetic PDE-5 Inhibitors

Five herbal products purchased on the internet were analysed using ASAP with TOF MS detection.

From the analysis it was possible to show that all five were adulterated with tadalafil and/or sildenafil or suspected analogues.

- **Sample 1** was found to be adulterated with tadalafil (m/z 390) as can be seen from spectrum 1 in figure 5.
  This sample declared the presence of many natural ingredients including; Dioscorea spinosina (wolfberry fruit, Glycyrrhiza glabra (liquorice root) as well as others. Neither the patient information nor the packaging declared the presence of tadalafil.
- **Sample 2** showed that it was adulterated with sildenafil, (Figure 5, No 2) interestingly this sample shared the same product name as sample 1 but shipped from a different geographical location (Europe versus Asia)
- **Samples 3 and 4** (Figure 5, No 3 and No 4) mixed adulteration was identified using ASAP. Sample 3 was adulterated with tadalafil and a higher level of sildenafil. Sample 4 had tadalafil as the major adulterant with less sildenafil.
- Quantitation of the samples using LC/MRM (data not shown) revealed that the doses of the sildenafil and tadalafil are sufficiently high to be therapeutic.

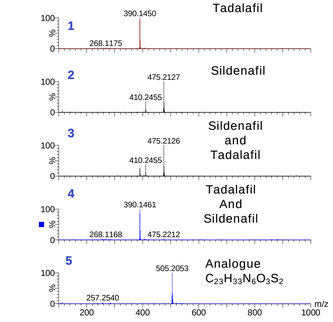

Figure 5. Spectra from the direct analysis of five HDS samples.

- **Sample 5:** The ASAP in combination with accurate mass MS/MS detection identified the presence of a suspected analogue, with suggested elemental composition of $C_{23}H_{33}N_6O_3S_2$ (-0.3 mDa, -0.6 PPM).

It is suspected that this analogue is thiohomosildenafil (m/z 505) where one oxygen is substituted with a sulphur atom and an ethyl group replaces the methyl group attached to the piperazinyl nitrogen[16]. When MS/MS fragmentation of this sample was carried out the major fragments were m/z 113, 299, 327 and 393 (Figure 6). These fragments have been reported in certain analogues including thiohomosildenafil in the literature [15,16,17,18]

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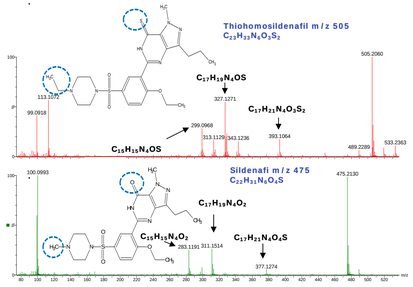

Figure 6. Spectra from the direct analysis of Sample 5 (top) using a high collision energy experiment on the Waters Xevo QTOF mass spectrometer. Spectrum for sildenafil standard is also shown (beneath)

CONCLUSION

- **ASAP does not require sample extraction or chromatographic separation therefore it provides a very useful tool in the rapid screening of large amounts of solid or liquid samples, providing that they are sufficiently volatile.**
- **The probe can be fitted very quickly to an API source by simply replacing the electrospray (ESI) or atmospheric pressure chemical ionisation (APCI) probes and ensuring a corona pin is installed.**
- **The probe inserts into the source through a lock. When removed the source is closed to atmosphere. Once the probe is installed the sample is completely enclosed by the source. With other direct ionisation methods the sample is exposed to the environment. This leads to safety concerns about the sample vapors reaching the surrounding air and the impact the ambient conditions may have on the sample itself.**
- **Using ASAP the turn around time from sample receipt to structural identification and unknown compound determination is accelerated.**
- **ASAP when used with TOF MS or MS/MS can rapidly identify unknown compounds using exact mass measurement and elemental composition determination with isotope ratio comparison (iFit).**

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