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アプリケーションノート

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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Abstract

In this application note we examine the utility of the Atmospheric Pressure Solids Analysis Probe (ASAP) for the determination of synthetic PDE-5 inhibitors in counterfeit tablet samples and adulterated herbal supplements.

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 can create a processing bottleneck. In recent years, novel ambient desorption ionization techniques for surface analysis of solid and liquid samples with subsequent MS detection have been reported. Techniques include desorption electrospray ionization1 (DESI), direct analysis in real time[2] (DART), and use of an atmospheric solids analysis probe[3] (ASAP).

The advantage of using these direct ionization methods is that sample preparation is often minimal or absent altogether. Total analysis time can be decreased significantly due to the elimination of the chromatographic separation. Additionally, direct ionization techniques are a good first step to determine the necessity of engaging in chromatographic separation.

ASAP was invented by McEwen *et al*[3] and can be used to analyze volatile or semi-volatile solid or liquid samples using atmospheric pressure ionization (API). The sample is applied to a glass melting point capillary and vaporizes when inserted into a heated stream of gas (100 to 500 °C). A corona discharge is used for ionization (Figure 1). The probe is readily fitted to an API source by replacing the electrospray (ESI) or atmospheric pressure chemical ionization (APCI) probe and ensuring that a corona pin is installed. The probe consists of two parts: an outer assembly and an inner probe (Figure 2) that holds the melting point capillary securely in place.

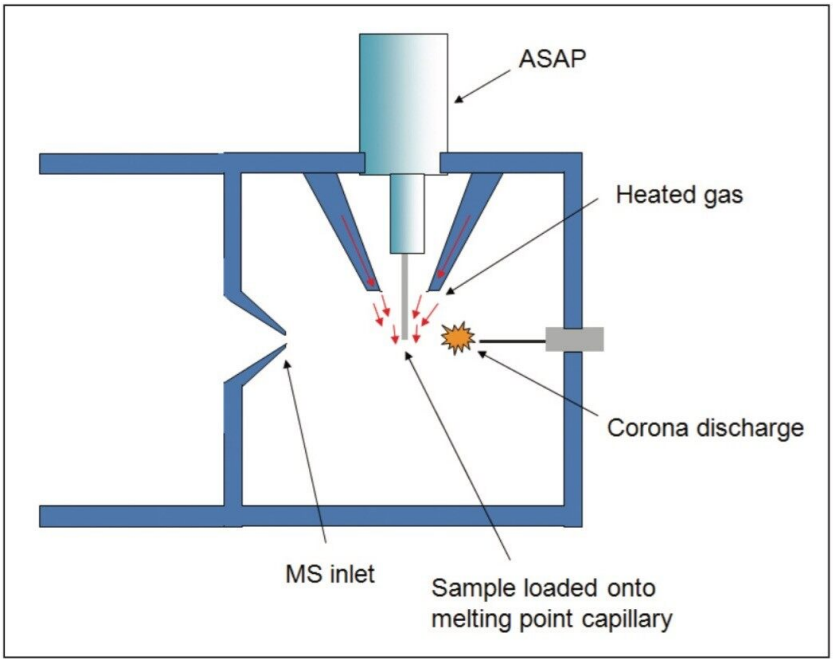

Figure 1. Illustration of ionization using ASAP. Used with permission.[19]

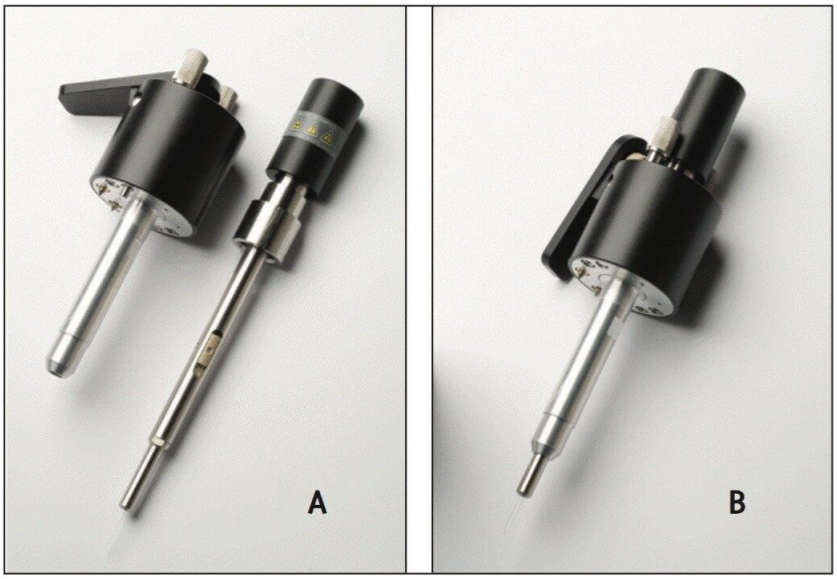

Figure 2. The ASAP probe. A shows outer assembly and inner probe. B shows the assembled device.

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 ionization methods, the sample is exposed to the environment. This leads to safety concerns about sample vapors

reaching the surrounding air, and analytical concerns about the impact that ambient conditions may have on the sample itself. In this application note we examine the utility of the Waters Atmospheric Pressure Solids Analysis Probe for the rapid determination of synthetic phosphodiesterase type-5 inhibitors in counterfeit tablet samples (Figure 3) and adulterated herbal supplements (Figure 4).

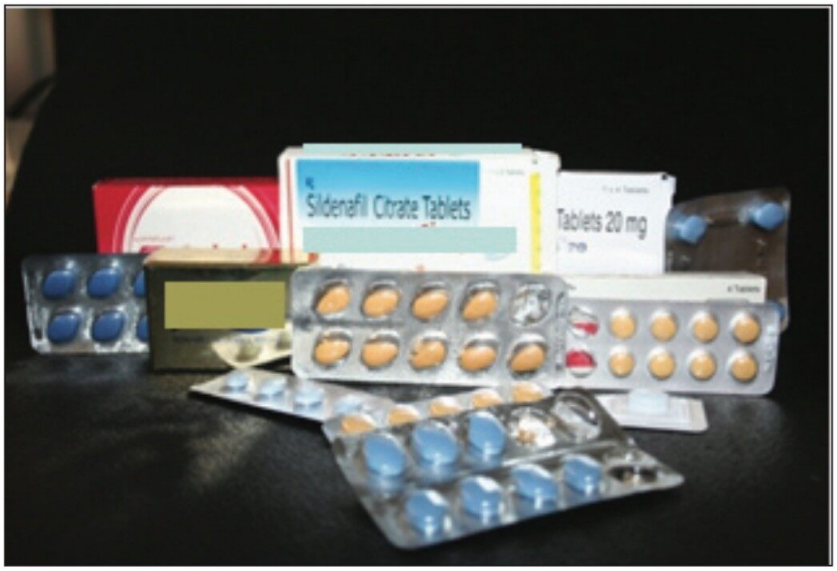

Figure 3. Shown is a picture of some of the imitation brand and generic products for treatment of ED.

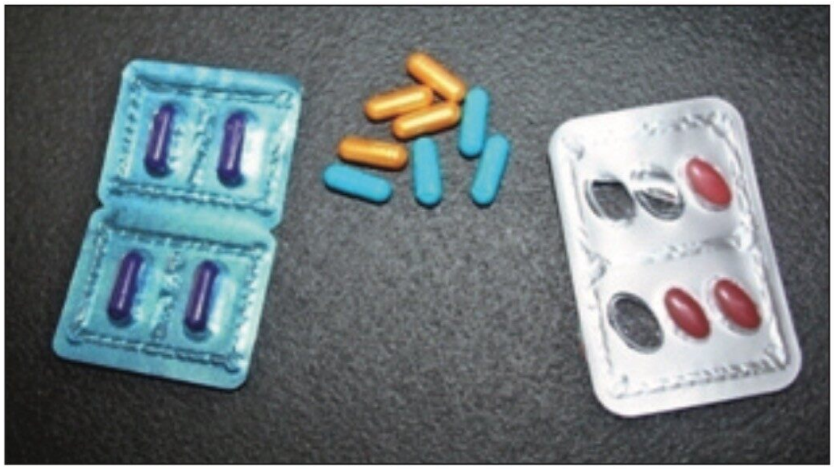

Figure 4. Shown is a picture of some of the adulterated products obtained over the Internet.

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.[8]

The pervasive success of the three approved synthetic phosphodiesterase type-5 (PDE-5) inhibitors for the treatment of erectile dysfunction (ED) has led to an explosion in the number of detected cases of counterfeit sildenafil citrate (brand name Viagra), vardenafil hydrochloride (brand name Levitra), and tadalafil (brand name Cialis) (Figure 5). In addition to the reported detection of counterfeit tablet forms of these products, herbal dietary supplements (HDS) that claim to be all-natural alternatives to the PDE-5 inhibitors are being heavily advertised on the Internet.

Recently, there have been reports that these supposed natural alternatives to the drugs used to treat ED have been illicitly adulterated with the pharmaceutical products or their structurally-modified analogues.[9-17] When a HDS product is labelled as natural, there is a also an implicit assumption that it is safe. Given that the HDS could contain undeclared synthetic drugs and can be obtained without prescription easily over the Internet, there is the potential for a threat to public health.

Experimental

Sample Procurement

Counterfeit tablet samples: Imitation "brand" and "generic" samples of these drugs (there are currently no approved generic equivalents for the three approved synthetic PDE-5 inhibitors) were obtained from Internet pharmacies.

Herbal dietary supplements (HDS): Five products were obtained over the Internet and analyzed by ASAP with high-resolution mass spectrometry. Four capsules and one tablet were purchased. All five were found to be adulterated, containing sildenafil, and/or tadalafil, or analogues of these drugs – none of which were declared on the box or enclosed information.

MS Conditions

MS system:	Waters LCT Premier XE Mass Spectrometer
Ionization mode:	ESCi positive
Capillary voltage:	3.0 kV (ESI)

Corona current:	5 µA (APcI)
Cone voltage:	40 V
Aperture 1:	15 V
Desolvation temp.:	100 to 450 °C
Desolvation gas:	500 L/Hr
Source temp.:	120 °C
Acquisition range:	100 to 1000 amu
Scan time:	0.5 sec
Lock Mass reference:	Leucine Enkephalin (ESI)

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.

Sample Loading

Tablet samples were loaded onto the glass capillary by first exposing the inside of the tablet and making contact between the glass capillary and the inner pill.

The herbal supplement samples were predominantly capsules containing fine powder. Once the sample was applied to the glass capillary, excess was then removed using a stream of nitrogen.

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.

Thirteen sample profiles from legitimate products and Internet pharmacy samples are shown in Figure 6.

Tablet samples purchased from one Internet pharmacy were manufactured to look like authentic sildenafil citrate, vardenafil hydrochloride, and tadalafil tablets. While the appearance of these tablets conformed to the appearance of the genuine medicines, results from using ASAP with time-offlight (TOF) MS detection showed that the vardenafil and tadalafil pills contained the wrong active pharmaceutical ingredient (API). Data showed that the sildenafil citrate tablet did contain the correct API (*m/z* 475); however the vardenafil (*m/z* 489) and tadalafil (*m/z* 390) samples did not (Figures 7 and 8).

This case alone highlights the level of risk a consumer is taking when purchasing drugs from random and uncertified Internet pharmacies.

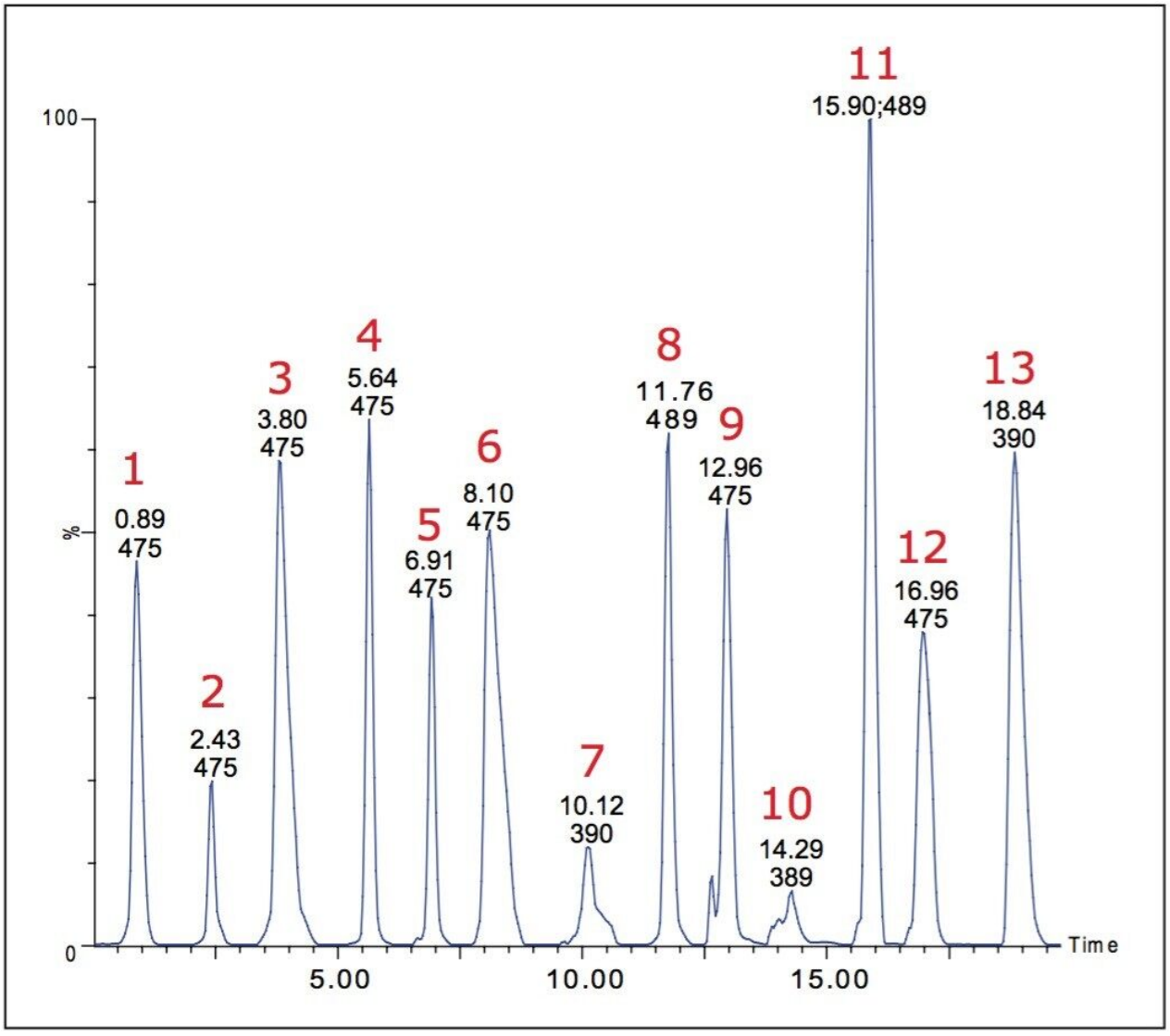

Figure 6. ASAP sample vaporization profiles (desolvation temperature is ramped from 100 to 450 °C) for 13 tablet samples obtained over the Internet and authentic products obtained from reputable sources.

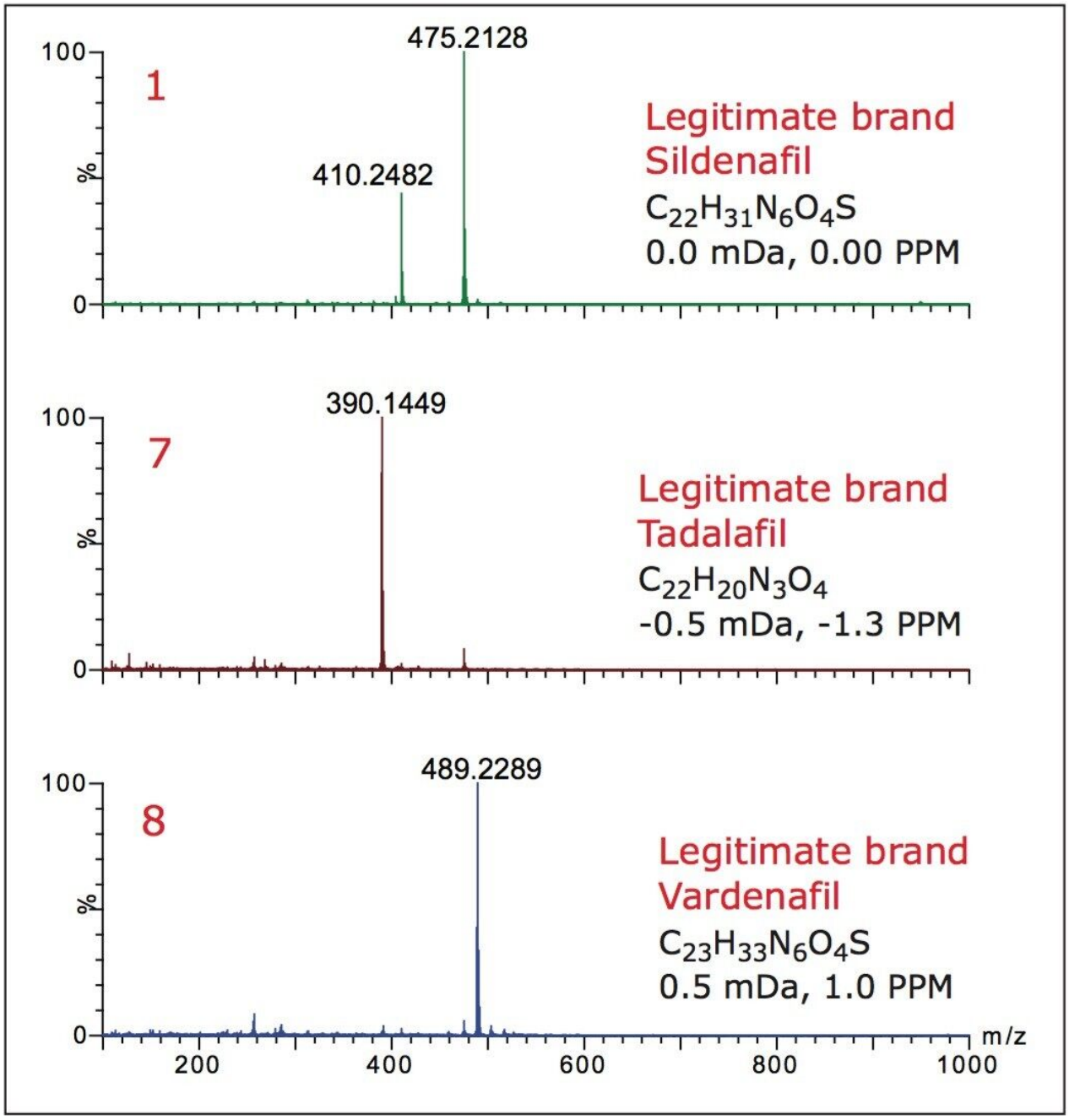

Figure 7. Elemental composition results for legitimate sildenafil, tadalafil, and vardenafil.

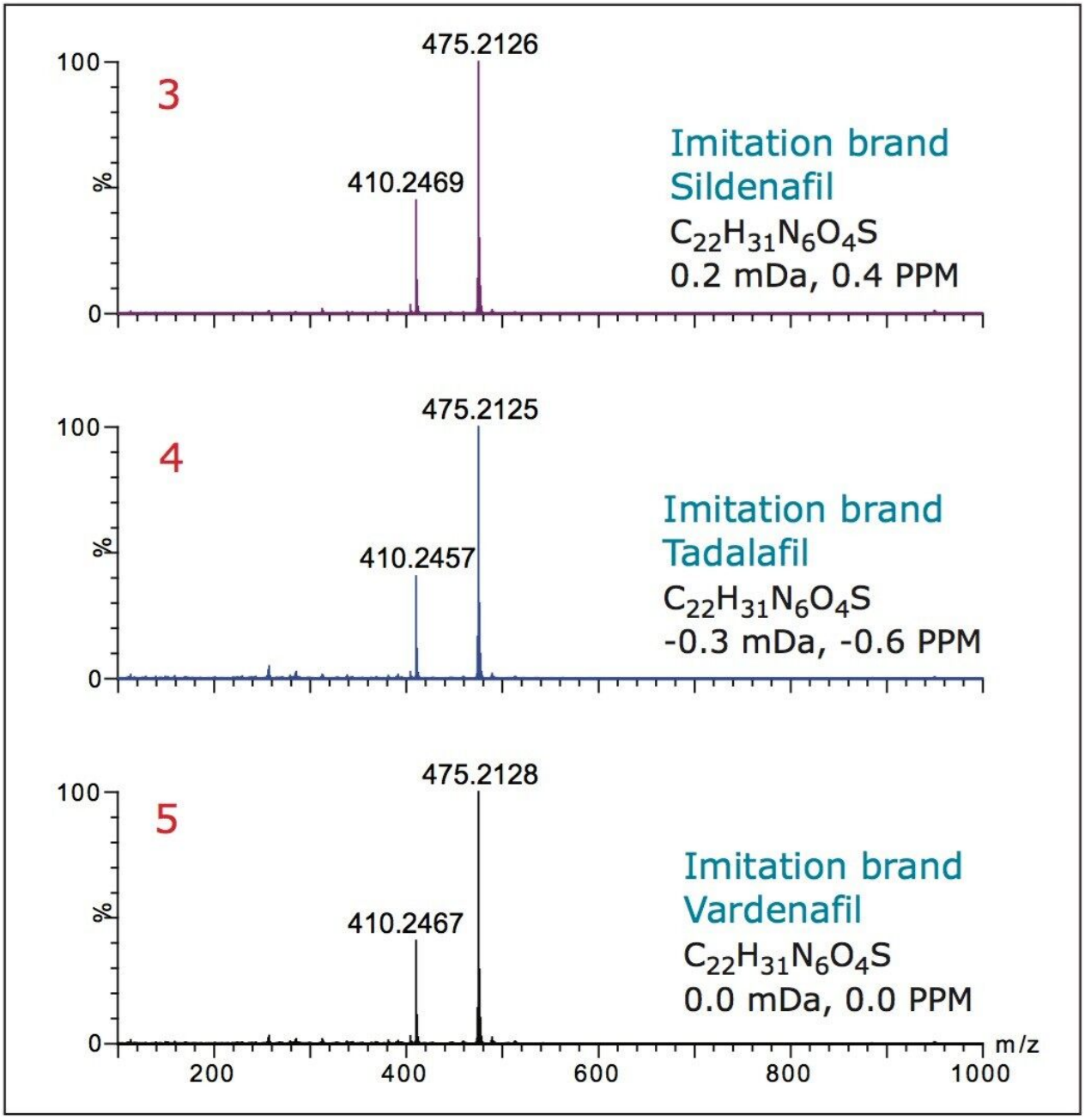

Figure 8. Elemental composition results for imitation-brand sildenafil, tadalafil, and vardenafil. See also Figure 9.

The principal active component in 13 tablet samples was identified by ASAP in less than 20 minutes. Sample extraction was not necessary and chromatographic separation was not required to determine if the sample contained what it claimed.

In certain analyses, it is sometimes sufficient to get a yes/no answer before the sample is passed for further characterization (Figure 9). ASAP used in conjunction with TOF-MS detection provides a simple way to obtain this answer rapidly.

Detailed results obtained from subsequent analysis by UPLC coupled to TOF-MS or MS/MS data and PDA spectral comparisons agree with the results obtained by ASAP for all of the samples analyzed.

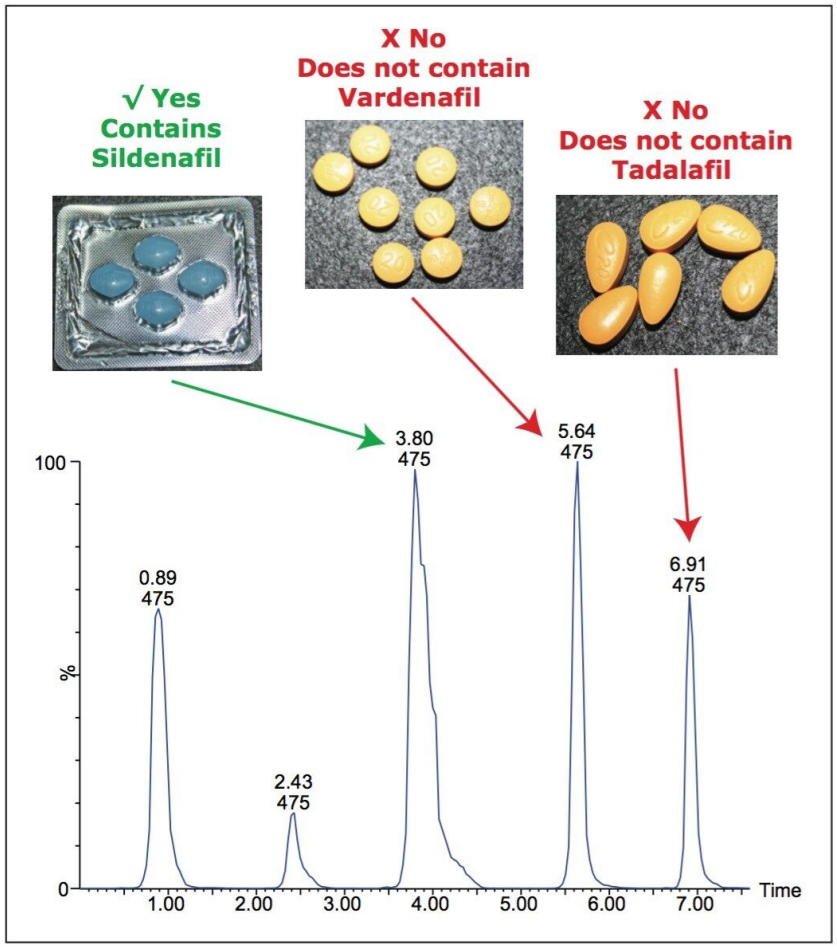

Figure 9. Profiles for the counterfeit tablet samples. ASAP with TOF-MS detection identified the vardenafil and tadalafil to be fraudulent, counterfeit tablets.

Adulteration of HDS Samples with Synthetic PDE-5 Inhibitors

Five herbal products purchased on the Internet were analyzed using ASAP with TOF-MS detection.

From the analyses, it was possible to show that all five were adulteratedwith 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 11. Package information for this sample declared the presence of many natural ingredients, including dioscorea spinosina (wolfberry fruit) and glycyrrhiza glabra (liquorice root). Neither the patient information nor its packaging declared the presence of tadalafil.

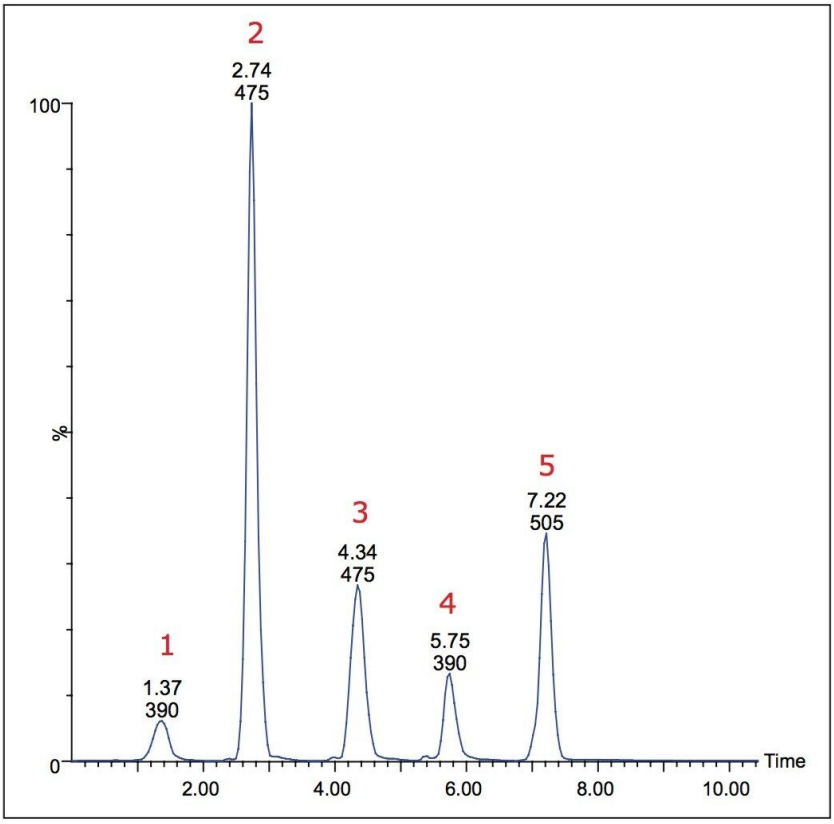

Figure 10. Vaporization profiles of the five HDS samples.

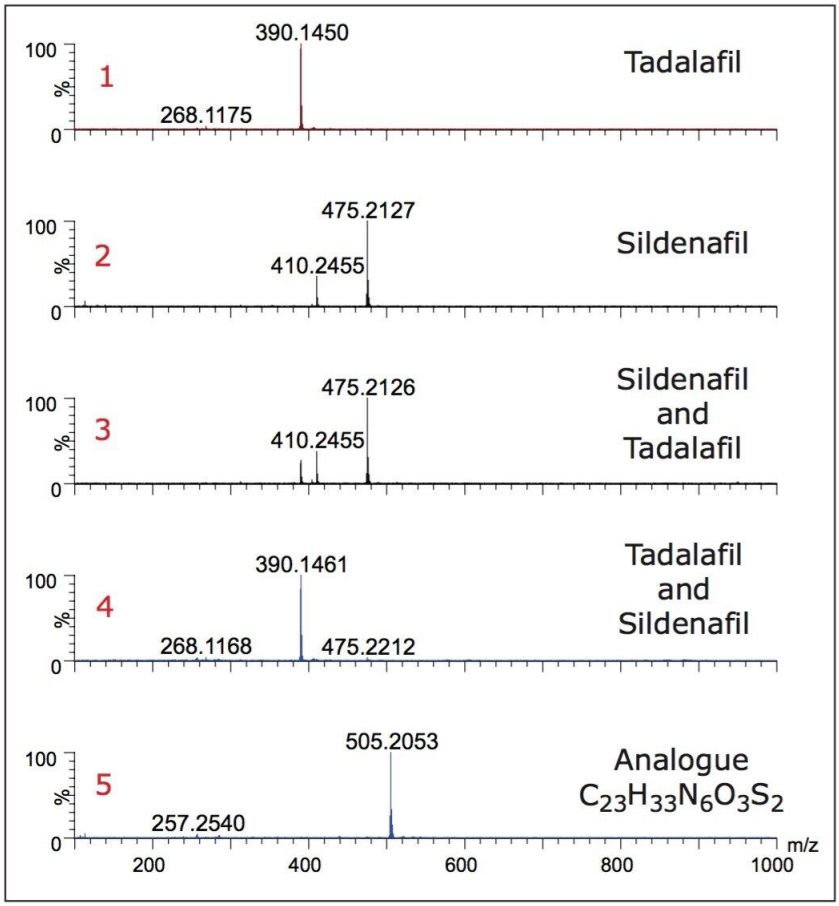

Figure 11. Spectra from the direct analysis of five HDS samples.

In each case, the principle component identified in the sample agreed with that determined when the same HDS sample was analyzed by UPLC-MS/MS utilizing a Waters Xevo TQ MS with simultaneous UV detection.[20] Sample 2 showed that it was adulterated with sildenafil; interestingly this sample shared the same product name as sample 1 but was shipped from a different geographical location (Europe versus China). In samples 3 and 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-MS revealed that the doses of the sildenafil and tadalafil are sufficiently high to be therapeutic.

The use of ASAP in combination with accurate mass 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[17].

When MS/MS fragmentation of this sample was carried out in previous work (data not shown), a characteristic fragment of sildenafil, *m/z* 99, and related compounds were observed. Other fragments

observed included *m/z* 113, 299, 327, and 393. These fragments have been reported in certain analogues including thiohomosildenafil in the literature.[13, 16, 17, 18]

Sample 5 was also declared to be all-natural. Packaging stated its ingredients had helped to “support male performance” for centuries. It was said to contain wild yam extract, Siberian Ginseng extract, jujube extract, and cayenne extract, as well as others.

Conclusion

The illegal counterfeiting of pharmaceuticals and adulteration of herbal dietary supplements with synthetic pharmaceuticals has been recognized as a growing global problem, with the number of detected cases rising every year. This has created a demand for high-throughput analytical techniques. When hyphenated methods such as LC-MS and GC-MS are used to analyse these samples, the need for chromatographic separation and sample extraction often creates a sample processing bottleneck.

The ASAP probe enables direct sample analysis by mass spectrometry, therefore providing a very useful tool for rapidly screening large amounts of solid or liquid samples, providing that they are sufficiently volatile.

The probe can be fitted very quickly to a mass spectrometer’s API source by simply replacing the electrospray (ESI) or atmospheric pressure chemical ionization (APCI) probes and ensuring that a corona pin is installed.

When paired with TOF-MS, the ASAP probe enables analysts to rapidly identify unknown compounds using exact mass measurement and elemental composition determination with isotope ratio comparison (iFit). Using ASAP, the turnaround time from sample receipt to structural identification and unknown compound determination is greatly accelerated.

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