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アプリケーションノート

Direct Analysis in Real time (DART) Mass Spectrometry of Adulterants in Herbal Slimming Products using Xevo TQ MS and Data Directed Analysis

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Abstract

Xevo TQ MS with a DART source enables the direct analysis of solids or liquids for simultaneous intact molecular ion and fragmentation information with rapid sample analysis.

Benefits

Use of Xevo TQ MS with a DART source enables the direct analysis of solids or liquids for simultaneous intact molecular ion and fragmentation information with rapid sample analysis.

Introduction

The use of herbal medicines (HM) or herbal dietary supplements (HDS) has become very popular worldwide. In the 2009, the total consumer sales of dietary supplements in the US expanded by 6.2% to $25.2 billion.[1] HM exist to treat a wide variety of ailments. Marketed as natural alternatives to pharmaceutical-based medications, they are available for purchase in many pharmacies, health food shops, and over the Internet.

There have been reports that these supposed natural alternatives have been illicitly adulterated with pharmaceutical products or their structurally modified analogues.[2-12] Given that a herbal medicine could contain undeclared synthetic drugs and can be obtained without prescription freely over the Internet, there is the potential for a threat to consumer health.

Herbal slimming products are heavily promoted online, with emails frequently sent directly to the consumer quoting prices and showcasing new products.

Sibutramine (brand names Meridia and Reductil) is used in the treatment of obesity. It is a serotonin and norepinephrine (NE) reuptake inhibitor (SNRI). Clinical trial data consistently demonstrate that when sibutramine is used in conjunction with a low-calorie diet it produces an initial and sustained weight loss effect[13] on patients that have been unable to lose weight using diet and exercise alone. It is available by prescription from a licensed physician and is a USDA schedule IV substance.

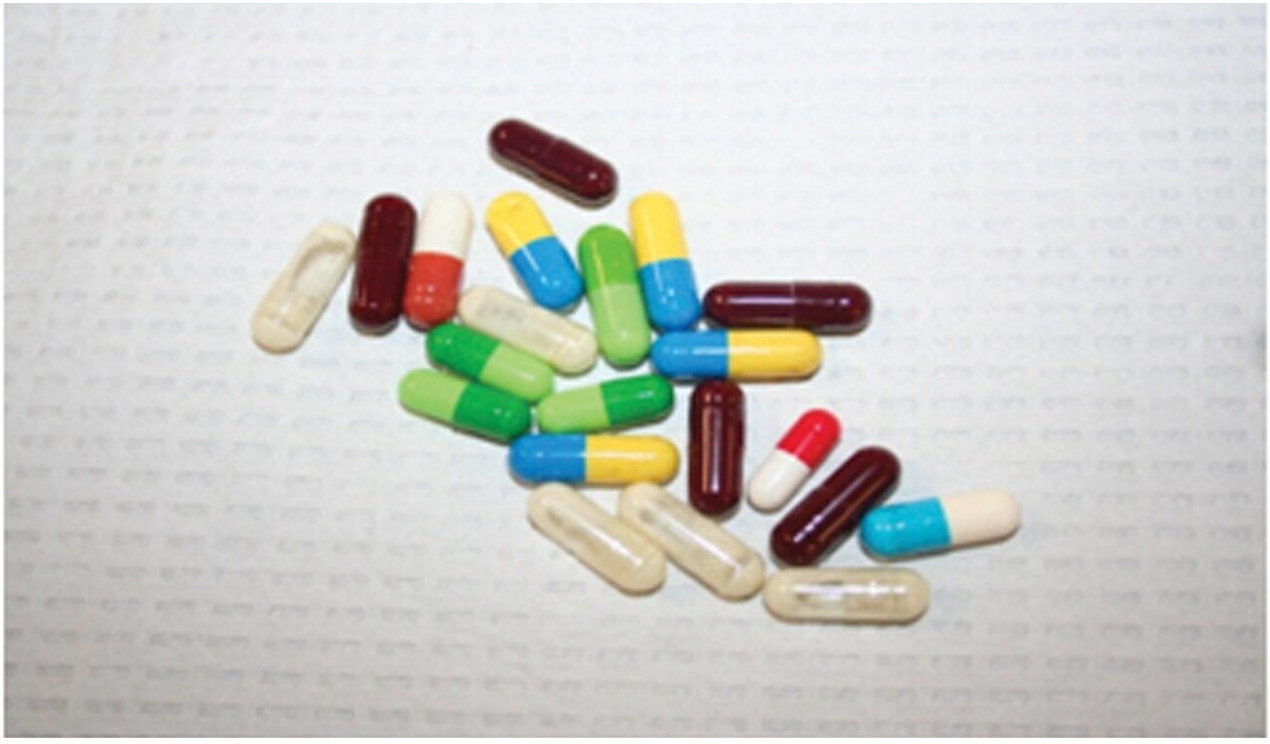


Figure 1. An increasing number of herbal medicines are available from a variety of sources.

Recent reports suggesting an increased risk of serious cardiovascular events (such as heart attack or stroke) in patients with known cardiovascular disease taking sibutramine[14] have prompted the European Medicines Agency (EMA) to recommend that the use of sibutramine be suspended.[15]

Several troubling studies showing the adulteration of herbal slimming products with sibutramine is a common occurrence.[16-20]

In this application note, we report on the adulteration of herbal slimming products purchased over the Internet, from store websites and auction sites. Samples were found to contain undeclared pharmaceutical substances with the main component being sibutramine. Figure 1 shows some of the products tested.

Experimental

Sample procurement

Herbal slimming products were purchased from on-line auction sites and Internet shops. Sample packaging and enclosed information declared the presence of many natural ingredients including: sweet potato fiber, gotu kola, marumi kumquat, guttigerae plant, as well as others. Neither the patient information nor packaging declared the presence of sibutramine or any other synthetic substances.

GC conditions

MS system:	Waters Xevo TQ Mass Spectrometer
Ionization mode:	DART SVP Standardized Voltage and Pressure Technology
Polarity:	Positive ion mode
Grid voltage:	350 V
Heater temperature:	400 °C
Helium gas:	2 L/min
Acquisition range:	100 to 900 Da
Scan speed:	10,000 amu/sec

Results and Discussion

Sample analysis using DART and the Xevo TQ MS

The samples were analysed directly using a DART source (IonSense, Saugus, MA, U.S.) designed to fit the Waters Xevo MS family of instruments.

DART[21] (Figure 2) is related to atmospheric pressure chemical ionization (APCI) employing an electrical discharge to create a plasma. Reactive ionizing species are produced from metastable reactive ions such as He, N_2, reacting with ambient water, oxygen, or other atmospheric components to produce hydronium ions. Protons are then transferred to the analyte molecules.

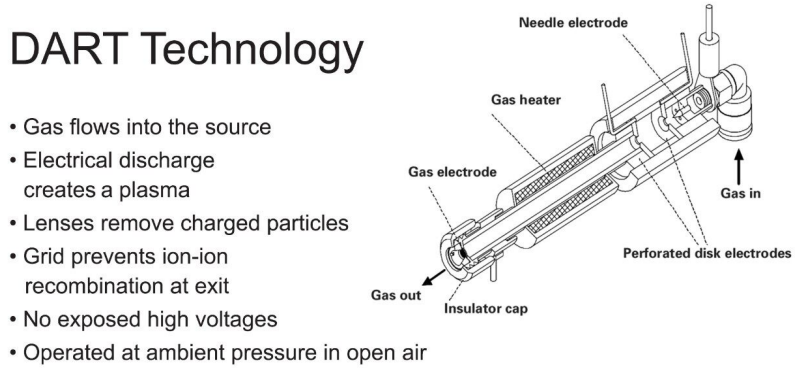
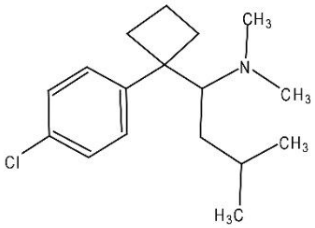
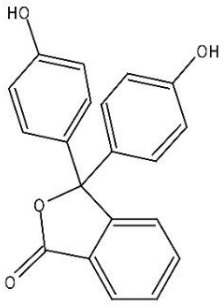


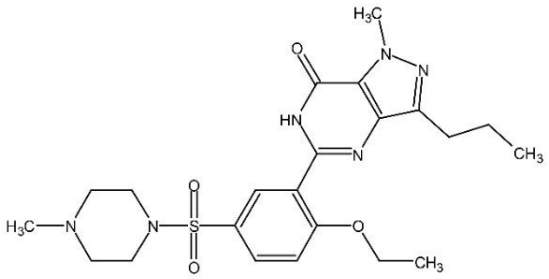
Figure 2. Summary of DART ionization process.



Sibutramine, $C_{17}H_{26}ClN$
$[M+H]^+$ = m/z 280



Phenolphthalein $C_{20}H_{14}O_4$
$[M+H]^+$ =m/z 319



Sildenafil, $C_{22}H_{30}N_6O_4S$
$[M+H]^+$ =m/z 475

Figure 3. Structures of sibutramine, sildenafil, and phenolphthalein.

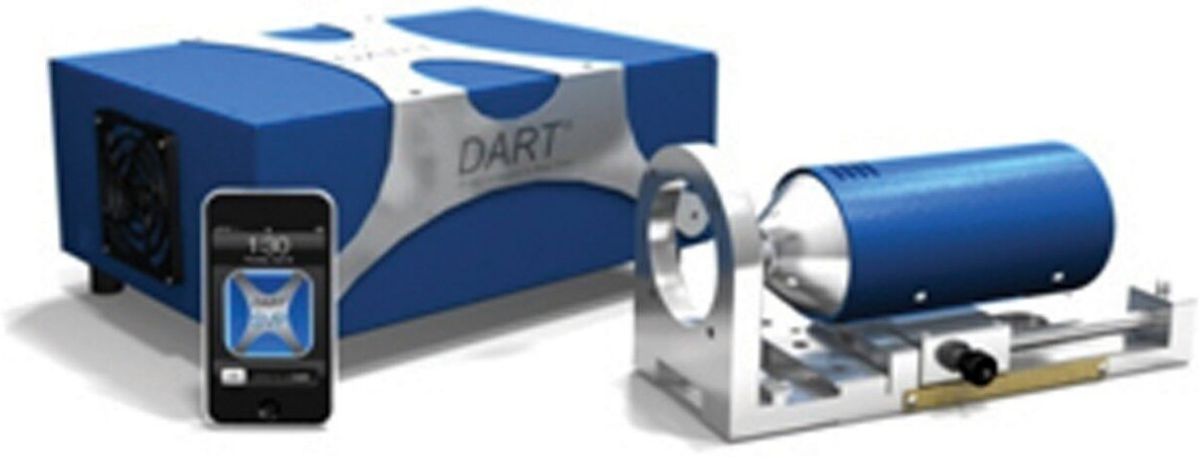


Figure 4. DART SVP and iPod controller.

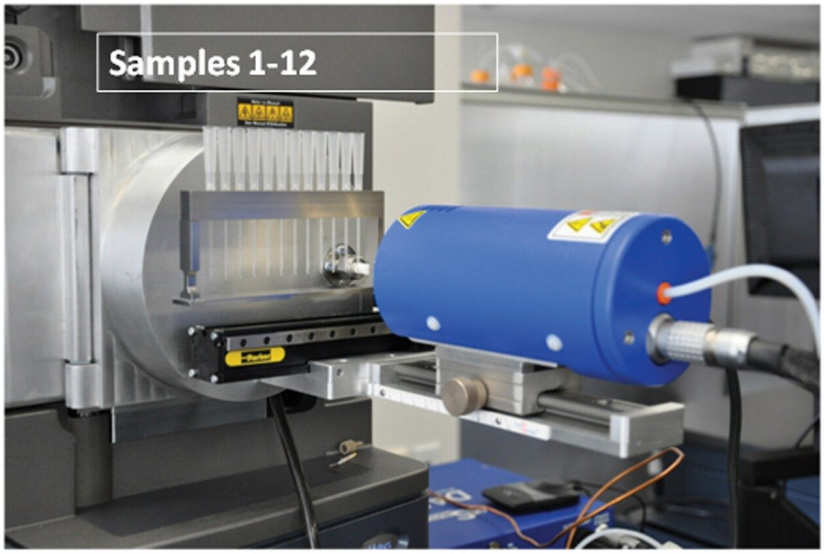


Figure 5. Dart SVP source for Xevo instruments shown with the 12 DIP-it sampler.

The herbal supplement samples were predominantly capsules containing fine powder. Each powdered sample was applied to separate glass melting point capillaries specially made to fit into the 12 DIP-it sample holder. The capillaries were then placed in the sampler and the ipod controller was programmed to move the sampler at a speed of 0.5 mm/s. This allowed greater throughput for analysis of multiple samples.

Data were acquired in survey mode. Survey scan capability on Xevo TQ MS intelligently switches from MS to

MS/MS data in one run, thus improving productivity. MS scanning experiments can be used to trigger MS/MS experiments in real time. A more targeted screen can also be performed using parent ion or neutral loss spectral acquisition, to screen for compounds that have common structural features.

From the analysis, it was possible to show that nine HDS samples (4-12) purchased from Internet suppliers were adulterated with sibutramine. Some contained other adulterants.

Data from a survey scan analysis experiment is shown in Figure 6. Each peak (1-12) represents a different standard/sample that has transversed the desorption ionization region of the DART gun. The data is acquired in a single file.

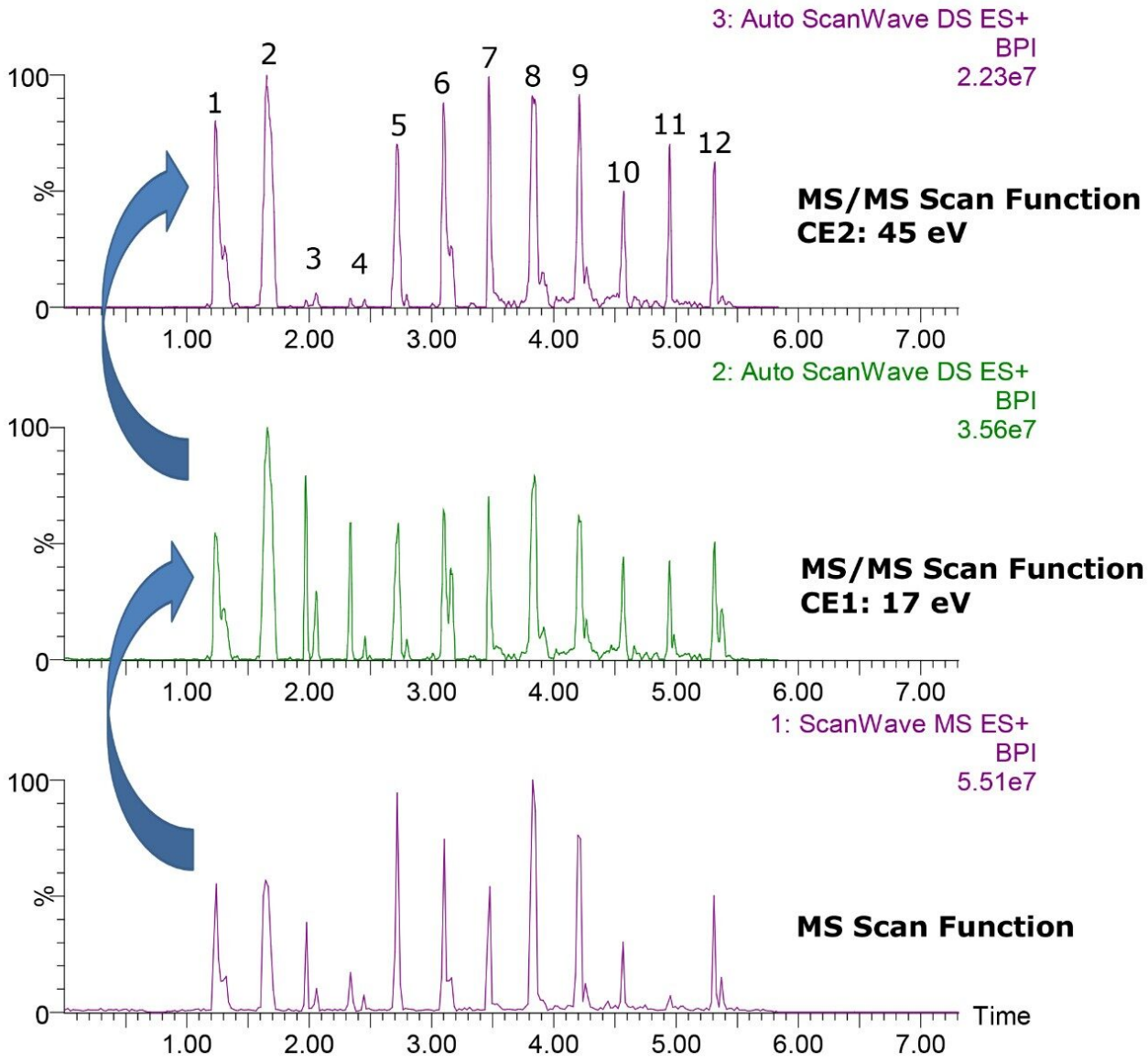


Figure 6. Data from a survey scan analysis experiment.

The data from the analysis of a solid drug substance for sibutramine, phenolphthalein, and sildenafil is shown in Figure 7, with $[M+H]^+$ ions of *m/z* 280, *m/z* 319, and *m/z* 475 respectively. Characteristic fragmentation patterns for each compound are also shown. The survey experiment was set up to collect two MS/MS functions, one at 17 eV and the other at 45 eV, which gives more opportunity to obtain useful fragmentation information for a wide range of compound types.

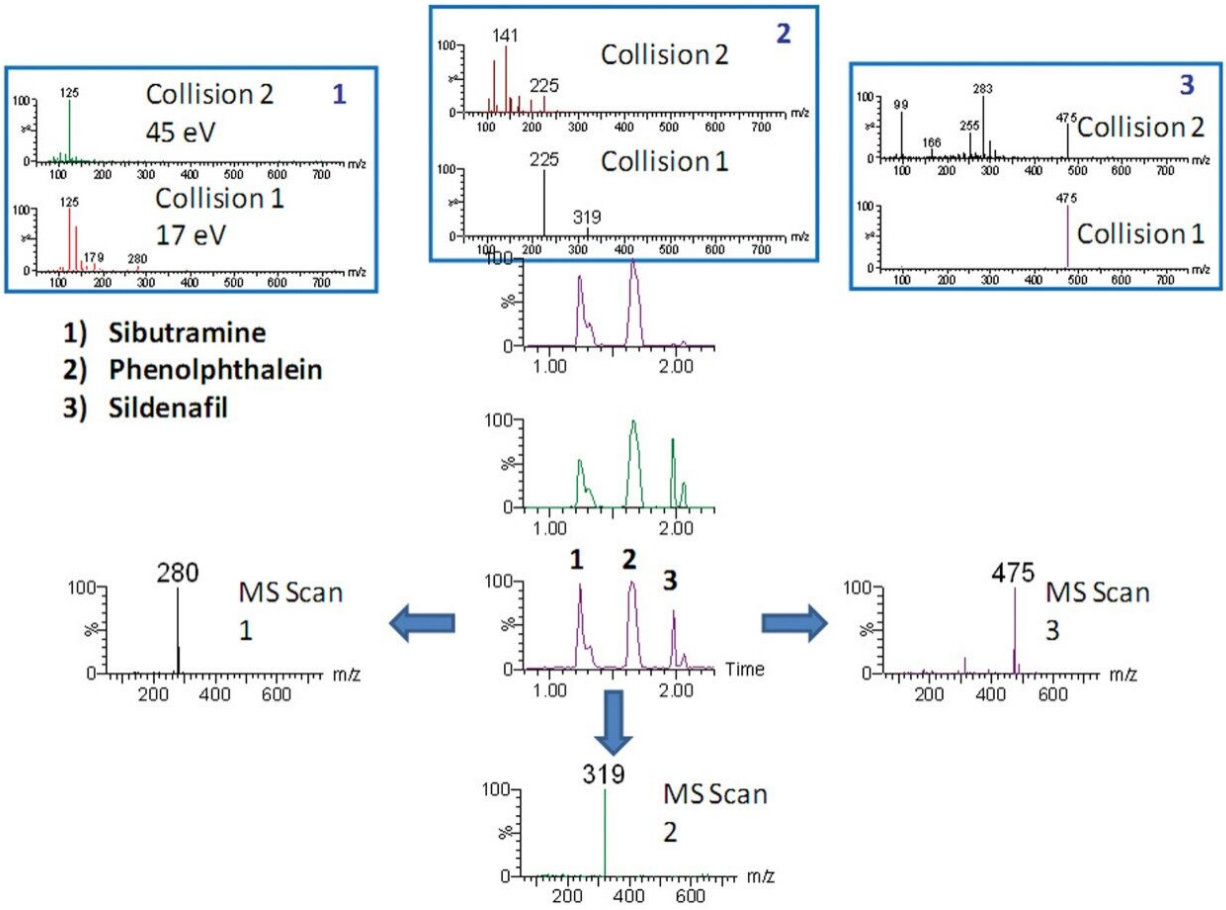


Figure 7. MS scan and dual collision energy data from a single DART-data directed MS/MS experiment for solid sibutramine, phenolphthalein and sildenafil drug substance.

Sample 6: Adulteration with sibutramine and phenolphthalein

Sample 6 was ordered from a popular online auction site. The patient information stated, "This product is made from purely natural herbal ingredients without any toxic or side effects." Two major components were found in sample 6 (Figure 8). Partial separation between the components is clearly visible during the ionization process. The *m/z* of the first component is *m/z* 280 and the fragmentation pattern gives a characteristic charged fragment of *m/z* 125 and *m/z* 139, typical of sibutramine.

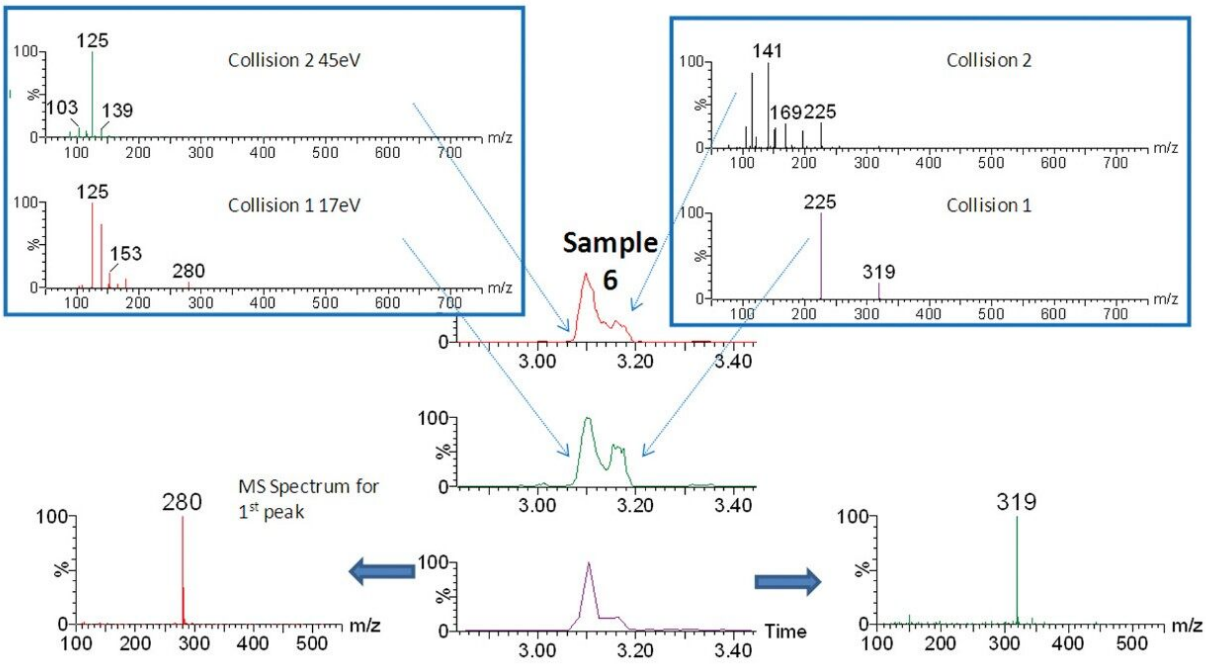


Figure 8. Full scan MS and dual collision energy data from a single DART-data directed MS experiment for Sample 6, showing partial separation of two components identified to be sibutramine and phenolphthalein.

This increases the confidence that this component is sibutramine. The second major component gave rise to *m/z* 319 in the MS function. The MS/MS fragmentation shows the major fragment in the low collision energy experiment to be *m/z* 225. There is excellent agreement between the fragmentation patterns from both the low and high collision energy spectra for the standard phenolphthalein and this component in the sample.

The use of dietary phenolphthalein as a laxative has been discontinued due to its links with multiple carcinogenic effects.[22,23]

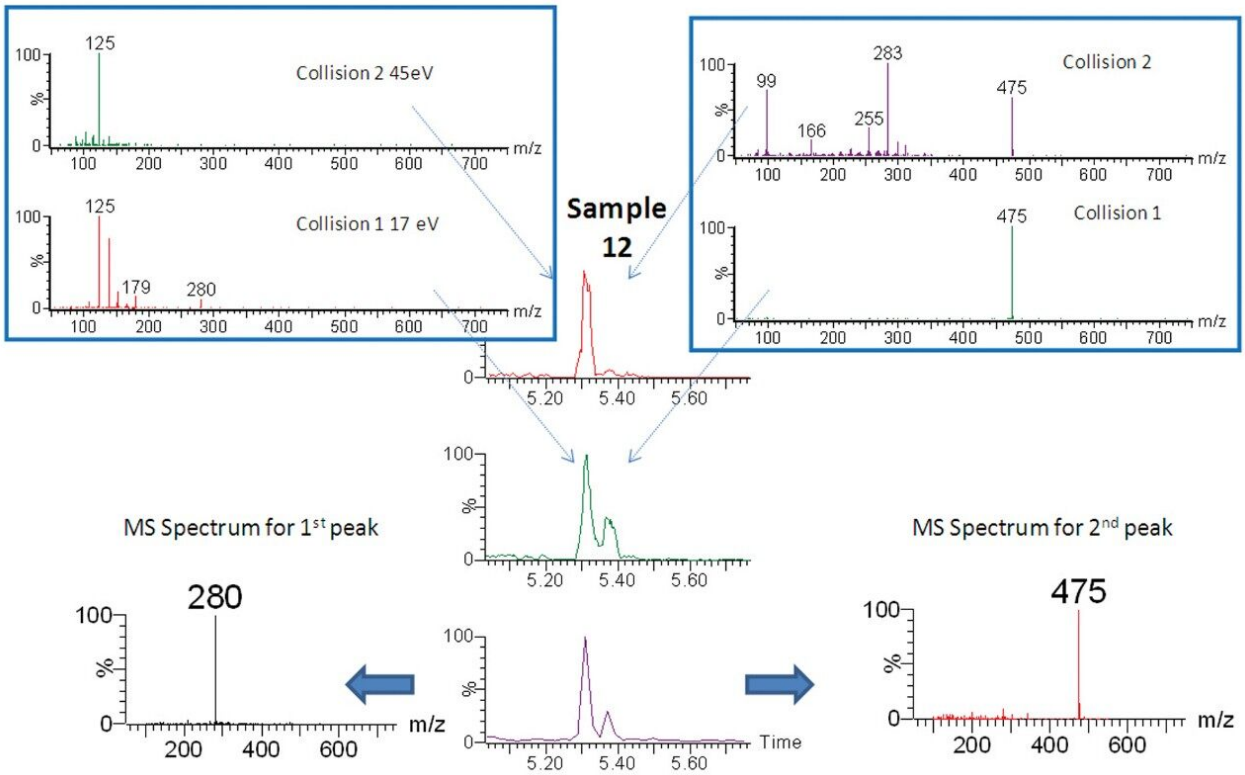


Figure 9. Full scan MS and dual collision energy data from a DART-data directed MS experiment of Sample 12. Showing partial separation of two components identified to be sibutramine and sildenafil.

Sample 12 was found to contain sibutramine and sildenafil (*m/z* 475), one of the three approved phosphodiesterase type 5 inhibitors used in the treatment of erectile dysfunction. Partial separation between the two components was also apparent, speculatively due to the slight volatility differences. The fragmentation pattern was consistent with that obtained for the standard drug substance of sildenafil (Figure 7, spectrum 3).

It is not clear why sildenafil might be used to adulterate a slimming product. It is possible that the slimming pills and herbal erectile dysfunction products are manufactured at the same site. Their occurrence together may be a consequence of poor cleaning validation techniques during the manufacturing process.

The techniques described here have been used to successfully identify likely adulterants in herbal slimming products. It is possible to generate and save representative spectra from the standard drug substance of known adulterants and other compounds for the purpose of comparing them with spectra generated from samples. The spectra can be archived in a library that can be searched, making this a rapid screening method that can be used without sample preparation.

Conclusion

- The testing of unlicensed herbal medicines and herbal dietary supplements are vital functions due to the possibility of illegal adulteration and/or contamination and the potential that exists for adverse health effects to unsuspecting consumers.
- The DART source module easily attaches and detaches to the MS instrument in seconds. This further increases the extensive source flexibility of the Xevo family of instruments.
- The direct analysis of solids or liquids using DART on a Xevo TQ provides simultaneous intact molecular ion and fragmentation information while allowing samples to be analyzed very rapidly and without the need for complex sample preparation or chromatography.

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