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Rapid Detection of Undeclared Active Ingredients in Online Health Supplements Using DART (Direct Analysis in Real Time) Open Ambient Ionization Source Coupled to ACQUITY QDa Mass Detector

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APPLICATION BENEFITS

- Rapid detection of undeclared pharmaceuticals in herbal supplements with little or no sample preparation
- In-source fragmentation to provide additional confidence in results
- Relatively simple operation for non-expert mass spectrometry users
- Potential for routine screening using pre-built libraries and/or profiling software

WATERS SOLUTIONS

DART™ QDa™ System

MassLynx™ v4.2 Software

KEYWORDS

DART QDa System, undeclared pharmaceuticals, online supplements, routine screening

INTRODUCTION

Dietary supplements are used by millions of consumers to improve health, maintain wellness, or support a more challenging lifestyle. Some of these supplements address conditions that many regard as shameful, awkward, or otherwise difficult to discuss with a physician. These conditions can include sexual dysfunction or excessive weight gain.[1]

Often, consumers choose supplements because they want a safe and natural alternative to drugs that are contraindicated for health reasons such as a heart condition. An example that illustrates this situation is that, almost exclusively, erectile dysfunction medication approved by the FDA are phosphodiesterase type 5 (PDE5) enzyme inhibitors,[2] such as Sildenafil (Viagra,™ Pfizer), which can be fatal when taken with nitrate vasodilators (e.g., nitroglycerin).

DART (Direct Analysis in Real Time) by IonSense is an ambient ionization technique that allows for quick sampling of compounds without sample surface contact. This ionization technique, combined with the Waters™ ACQUITY™ QDa Mass Detector, enables a rapid, sample preparation-free technique for sample screening. The system further generates easy-to-interpret mass spectral information in seconds.

This application note shows the utilization of the DART QDa System for the direct analysis of a variety of online dietary supplements that are claimed to be 100% herbal treatments for a variety of conditions including impotence, obesity, and rheumatism. All but one of the examples were cited as containing undeclared pharmaceutical ingredients on the FDA website. Here we were able to show the detection of undeclared compounds in all cases. Further, we show that by inducing in-source fragmentation, we were able to produce additional specificity to aid in compound identification.

EXPERIMENTAL

Sample description

The following samples were analyzed:

Table 1. Analyzed herbal supplements and the suspected undeclared pharmaceutically active compounds with potential contraindications.

Type	"Brand"	Undeclared compound	Potential impact
Male potency supplements	Royal Honey*	Tadalafil (Cialis™)	Can interact with medications containing nitrates which can dangerously lower blood pressure
Slimming aids*	NuVitra King dietary supplement	Sibutramine Fluoxetine (Prosac™)	Sibutramine: widely banned appetite suppressant – linked with strokes and adverse cardiovascular events. Fluoxitine: Diabetes, decreased Na, K, and Mg in blood. Increased risk of bleeding
Rheumatism/arthritis/ general wellbeing	Ortiga joint remedy	Diclofenac	NSAID: Duodenal ulceration, can produce allergic reaction following the use of asperin

**Detailed on FDA website.*

Table 2. Analytical standards.

Standard	Reference
Tadalafil	European Pharmacopeia EP00TLG5 Batch 2.0
Sibutramine	Sigma, Lot: 109K4605V
Acetaminophen	Sigma, Lot: SLBM5923V
Diclofenac	Sigma, Lot: BCBW5662
Fluoxetine	Sigma, Lot: LRAA9180

Method conditions

Sample introduction techniques employed:

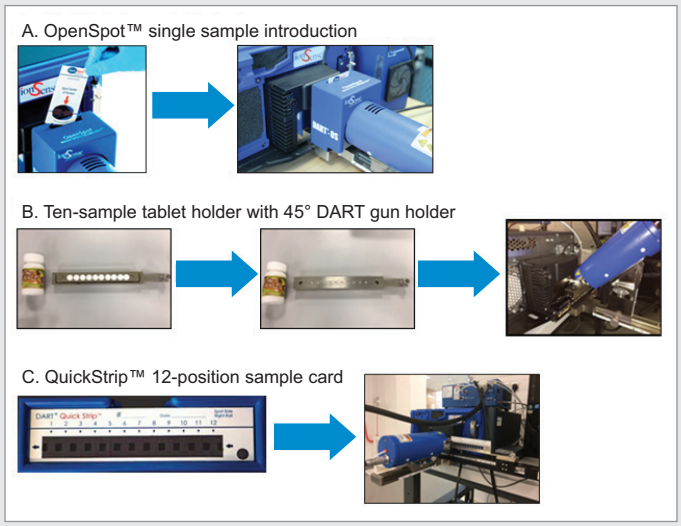

Figure 1. Sample introduction techniques utilized by the DART QDa System.
(A) OpenSpot sample introduction employed for the analysis of Nuvitra.
(B) Ten-tablet sample holder employed for the analysis of Ortiga joint remedy.
(C) Twelve-position QuickStrip card with linear rail for the analysis of Royal Honey.

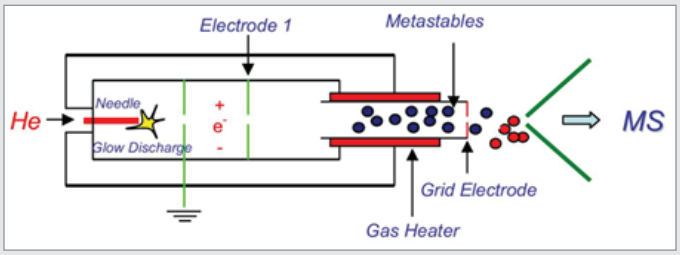

Figure 2. Schematic of DART mechanism in action.

Instrument parameter		Sample tested	
		Royal Honey	Ortiga joint remedy Nuvitra
DART temp. (°C)	450	200/250	250
QDa polarity	POS	POS	POS
QDa mass range (Da)	50–600	100–600	100–600
QDa cone voltage (V)	5/50	5	5
Sampling technique	Solvent swap using ether followed by spotting on QuickStrip card	Apply small amount of powder on OpenSpot card	Analyzed directly using IonSense tablet holder
Sample introduction	Quickstrip	OpenSpot	Tablet holder and 45° DART Holder

Data management

Informatics software: MassLynx v4.2

RESULTS AND DISCUSSION

MALE POTENCY

Royal Honey

Initially, direct analysis was attempted for the honey; however, the matrix complexity resulted in spectra dominated by compounds native to the honey.

A simple "solvent swap" sample preparation was devised by measuring approximately 2 mL of the honey and adding 2 mL of tertiary butyl ether. The container was shaken vigorously for a few seconds, and the supernatant was spotted onto the QuickStrip card for analysis (Figure 3).

The polar compounds, such as saccharides contained in honey, are immiscible with the highly hydrophobic ether removing the vast majority of matrix interference from the honey. Analysis of the supernatant at 450 °C using a 5 V cone voltage shows spectra consistent with the erectile dysfunction drug Tadalafil (*m/z* 390) when compared with Tadalafil standard (Figure 4). Increasing the cone voltage to 50 V induces similar fragmentation patterns for both the Tadalafil standard and the honey supernatant (*m/z* 135, 268).

Nuvitra

A Nuvitra capsule was split and a small amount of the contents were applied to an OpenSpot card which was directly introduced to DART (Figure 5). The method was initially run using a DART temperature of 200 °C with a QDa cone voltage of 5 V. Increasing the temperature to 250 °C promoted the ionization of fluoxetine while inducing fragmentation of both fluoxitine and sibutramine (fragments at *m/z* 125 and 195, respectively [Figure 6]).

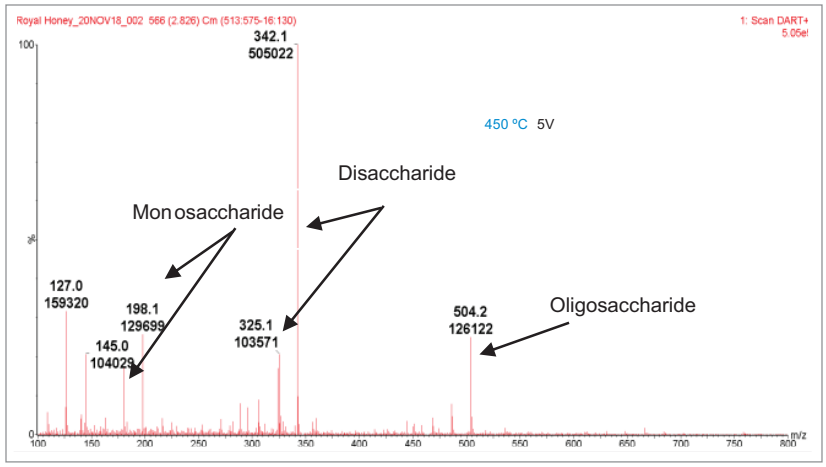

Figure 3. Spectra of the direct analysis of Royal Honey using the DART QDa System showing possible compounds commonly found in honey.

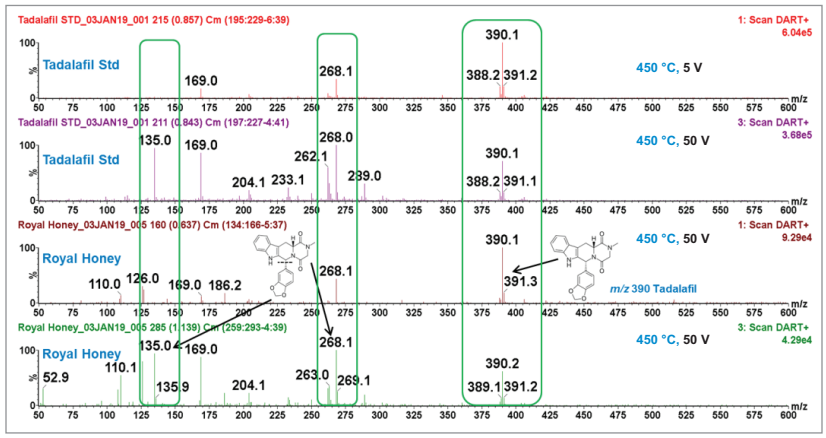

Figure 4. Analysis of "Royal Honey" supernatant showing spectra consistent with Tadalafil (Cialis,™ Eli Lilly).

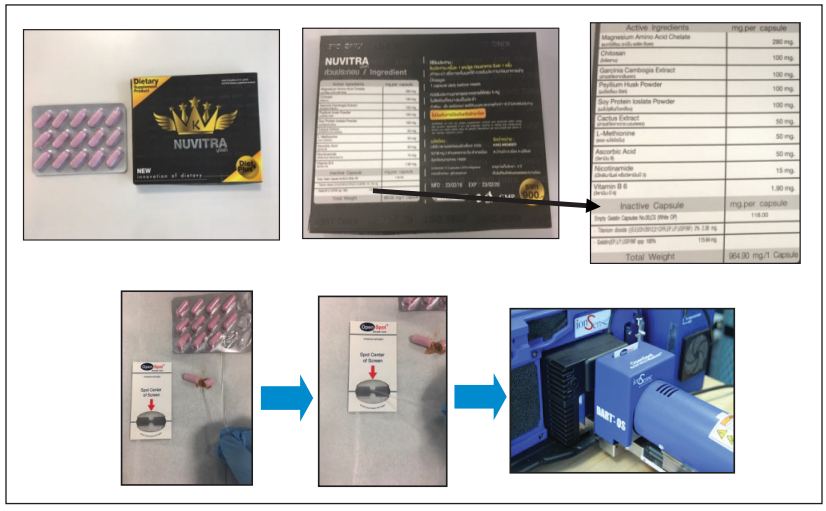

Figure 5. Details for Nuvitra-declared ingredients and the sample introduction method.

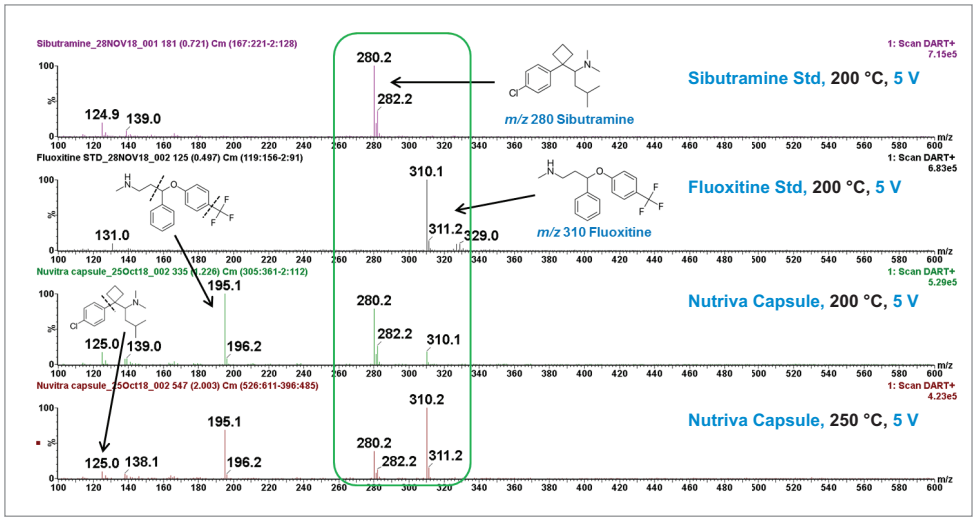

Figure 6. Analysis of Nuvitra capsule contents producing spectra consistent with sibutramine (a widely banned appetite suppressant) and fluoxetine (an antidepressant: Prozac,™ Eli Lilly). Increasing the DART temperature promotes the ionization of fluoxetine.

Ortiga joint remedy

Ortiga joint remedy tablets are uncoated and therefore can be introduced directly to the DART source using the DART tablet holder and linear rail. Ten separate tablets were analyzed in Figure 7.

The FDA website states that Ortiga tablets are suspected to contain the NSAID (non-steroidal anti-inflammatory drug) dicolfenac (*m/z* 296). Tablet analysis indicated that diclofenac was not present (Figure 8). The tablet analysis gave a base peak signal at *m/z* 152 which does correspond to the molecular ion of acetaminophen. When compared to the acetaminophen standard, both standards produced fragments at *m/z* 110 indicating the loss of the acetyl group of acetaminophens.

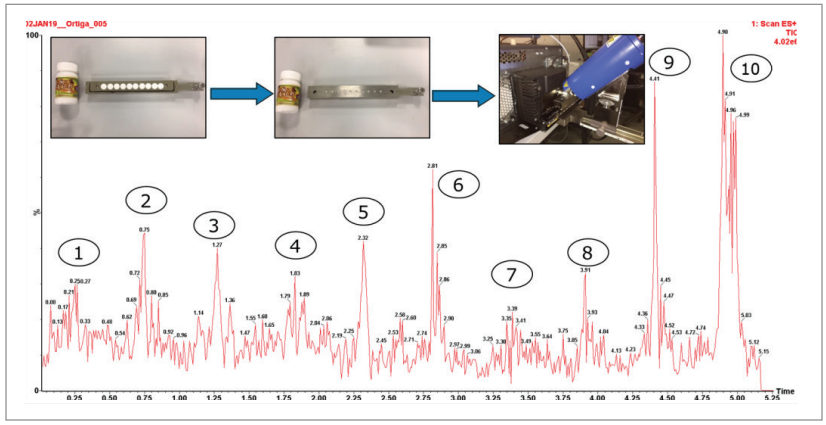

Figure 7. Total ion count (TIC) of 10 Ortiga tablets and the loading of tablets onto the DART tablet holder (inset).

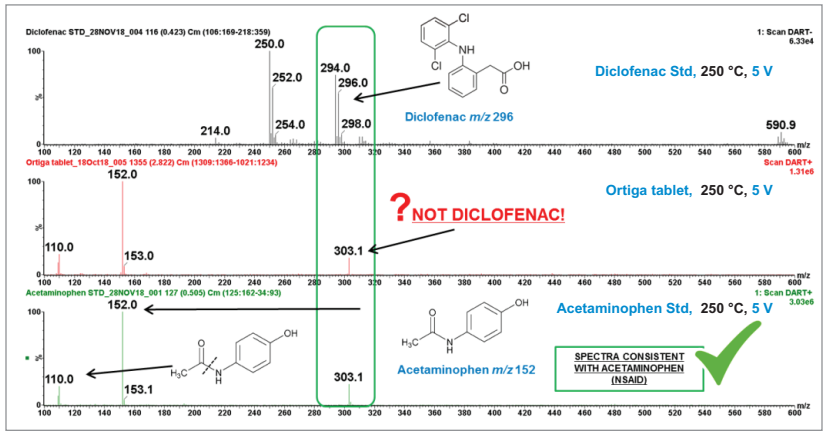

Figure 8. Comparison of Ortiga tablet with diclofenac and acetaminophen standard showing spectra consistent with the latter.

CONCLUSIONS

The DART QDa System can provide a rapid screening technique for suspect supplements or over the counter medicines (OTCs). This may be routinely performed using library matching or profile matching software.

The DART QDa System was able to detect multiple, undeclared, active, pharmaceutical ingredients within supplements purchased online, in multiple dosage forms (i.e., tablets, powders, and honey).

By utilizing in-source fragmentation with the QDa, and temperature manipulation of the DART helium gas stream, more information was derived from the sample in terms of specificity.

The discrepancy between the suspected, undeclared compound and the compounds detected in Ortiga joint remedy serves to highlight the lack of consistency and responsibility of the producers of these products, and the need to rapidly screen these products for consumer protection.

High resolution mass spectrometry (HRMS) would still be required for full spectral confirmation; however, the specificity provided by the single quadrupole detector will decrease the likelihood of false positives.

References

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3. Cody, R. B.; Laramee, J. A.; Nilles, J. M.; Durst, H. D. Direct Analysis in Real Time (DART) Mass Spectrometry. *JEOL News*. **2005**, *40* (1), 11.

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