EVALUATION OF AN OPEN AMBIENT IONISATION SOURCE COUPLED TO A PORTABLE MASS DETECTOR AS A TOOL FOR THE RAPID DETECTION OF UNDECLARED ACTIVE INGREDIENT(S) IN ONLINE HEALTH SUPPLEMENTS

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INTRODUCTION

Dietary supplements are used by millions of consumers to improve health, maintain wellness, or to 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, like sexual dysfunction or excessive weight gain. [1]

Consumers can sometimes choose supplements because they want a 'safe' and 'natural' alternative to drugs that are contraindicated for health reasons - such as a heart condition. Almost exclusively, FDA approved erectile dysfunction medication are phosphodiesterase type 5 (PDE5) enzyme inhibitors[2], e.g. Sildenafil (Viagra™, Pfizer). This class of drug can be fatal when taken with nitrate vasodilators like nitroglycerin.

The DART® QDa™ System integrates the Waters™ ACQUITY™ QDa Mass Detector with the DART Ion Source from IonSense® to enable a rapid, sample preparation-free solution screening of uncontrolled dietary supplements. The DART QDa System generates mass spectral information in seconds.

Within this body of work we tested a variety of dietary supplements obtained online (Refer Table1.), claiming 100% herbal treatments for a variety of conditions including impotence, obesity and rheumatism. All but one of the examples were cited on the FDA website as containing undeclared pharmaceutical ingredients. We were able to detect undeclared compounds in all cases using in-source fragmentation for additional specificity.

Type	'Brand'	Undeclared Compound	Potential Impact
Male potency supplements	Green Devil	Sildenafil (Viagra™)	Can Interact with medications containing nitrates which can dangerously lower blood pressure
	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™)	As detailed above (Sibutramine), Fluoxitine: Diabetes, decreased Na, K and Mg in blood. Increased risk of bleeding
	Asia Slim Brand	Bisacodyl	Laxative: Atonic colon (prolonged use), abdominal cramps, inflamed colon
Rheumatism/* Arthritis/General Wellbeing	Linsen Double Caulis Plus	Dexamethasone	Steroid: Osteoporosis, infectious diseases e.g. herpes simplex, diabetes, hypertension
	Ortiga Joint Remedy	Diclofenac	NSAID: Duodenal ulceration, can produce allergic reaction following use of aspirin

Table1. Online supplements analysed detailing the suspected

** Detailed on FDA website*

METHODS

The DART source utilises heated gas (for this work, helium) exposed to an electrode discharge to create metastable species. In positive mode using helium, ionisation of the sample is enabled by the ionisation of water clusters followed by proton transfer to the sample. In negative mode, ionisation is possible through surface Penning ionisation electron capture by atmospheric oxygen which reacts with the sample to produce anions[4].

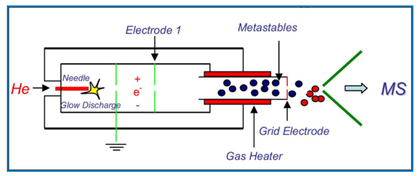


Figure 1. Schematic of DART mechanism of action

The DART source was operated with a fixed helium gas flow of 1.5mL/min. All other analysis parameters are detailed in *Table 2.*

Where possible, the supplement packaging claiming no drugs present have been presented.

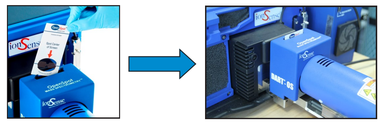
Instrument parameter	Sample Tested					
	Green Devils	Royal Honey	Nuvitra	Asia Slim Brand	Linsen Double Caulis	Ortiga Joint Remedy
DART Temperature °C	400	450	200/250	150	250	250
QDa Polarity	POS	POS	POS	POS	NEG	POS
QDa mass Range (Da)	100-600	50-600	100-600	100-600	100-600	100-600
QDa Cone Voltage	15/100V	5/50V	5V	8/60V	5/50V	5V
Sample Introduction	OpenSpot	Quickstrip	OpenSpot	OpenSpot	OpenSpot	Tablet holder and 45° DART Holder

Table 2. Analysis parameter for the samples tested

METHODS (CONTINUED)

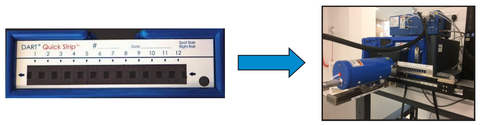
Sample Introduction Techniques Employed

OpenSpot™ single sample introduction



10 sample tablet holder with 45º DART gun holder

QuickStrip™ 12 position sample card



Standards Used

Standard	Reference
Sildenafil	European Pharmacopeia EP00HHW2 Batch 1.1
Tadalafil	European Pharmacopeia EP00TLG5 Batch 2.0
Sibutramine	Sigma, Lot :109K4605V
Bisacodyl	Sigma, Lot :104K1274V
Acetaminophen	Sigma, Lot SLBM5923V
Dexamethasone	Sigma, Lot :BCBW8446
Diclofenac	Sigma, Lot :BCBW5662
Fluoxetine	Sigma, Lot :LRAA9180

Table 3. List of standards used for comparison with online samples

RESULTS

MALE POTENCY

"Green Devils"

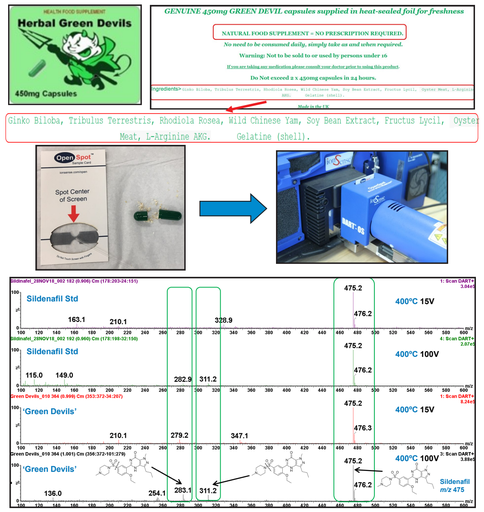


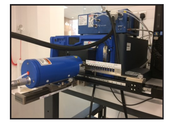
Figure 2. Analysis of 'Green Devil' capsule contents showing spectra consistent with Sildenafil (Viagra™, Pfizer). Cone voltage ramping employed producing fragments for additional confidence

RESULTS

"Royal Honey"

Rapid analysis of complex mixtures

2mL Sample + 2mL Tert Butyl Ether → Manually Shake <5mins → Analyse supernatant by DART QDa System (QuickStrip)



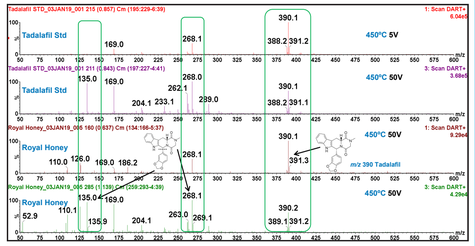
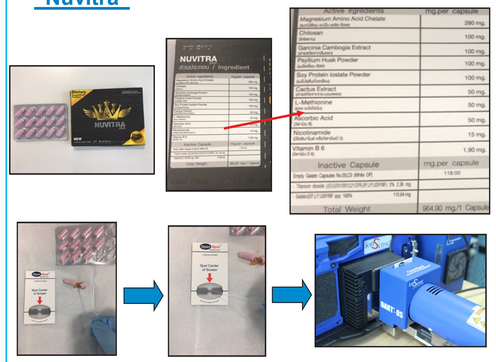


Figure 3. Comparison of Royal Honey supernatant with Tadalafil standard

SLIMMING AIDS

"Nuvitra"



Effect of temperature

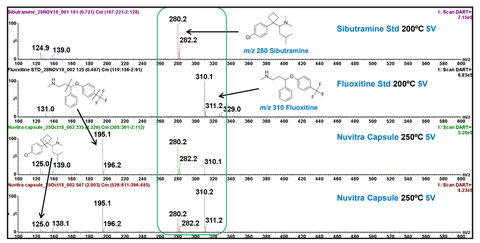


Figure 4. Analysis of Nuvitra capsule contents producing spectra consistent with Sibutramine (widely banned appetite suppressant), and fluoxetine (antidepressant : Prozac™, Eli Lilly). Increasing the DART temperature promotes the ionisation of fluoxitine

RESULTS

SLIMMING AIDS (continued)

"Asia Slim Brand"

Tablet coating manually removed before introduction to DART QDa source

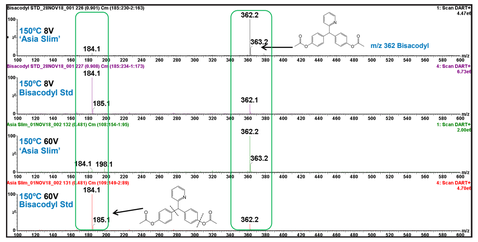
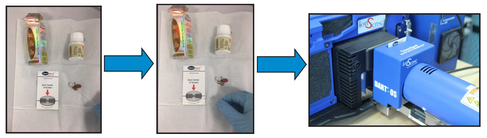


Figure 5. Analysis of "Asia Slim" tablet producing spectra consistent with bisacodyl (laxative)

JOINT / RHEUMATIC AIDS

"Linsen Double Caulis"



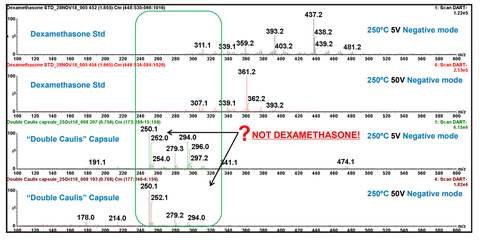


Figure 6. Analysis of "Linsen Double Caulis" capsule contents producing spectra NOT consistent with dexamethasone (as declared in FDA website)

Compared with Diclofenac Standard

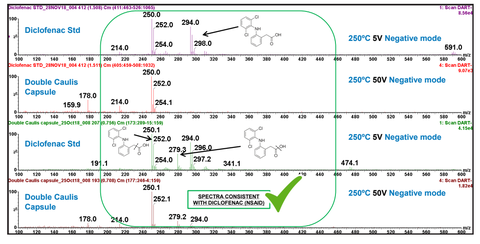


Figure 7. Comparison of "Linsen Double Caulis" spectra with Diclofenac (NSAID) standard shows common fragmentation patterns.

RESULTS

JOINT / RHEUMATIC AIDS (continued)

"Ortiga Joint Remedy"

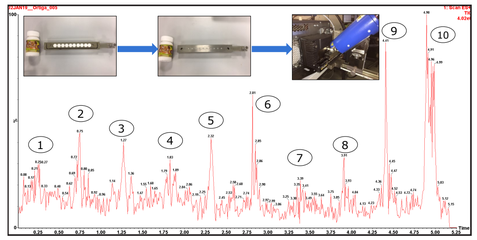


Figure 8. TIC generated by analysis of the "Ortiga Joint Remedy" using the IonSense tablet holder, enabling multiple sample analysis (10 tablets).

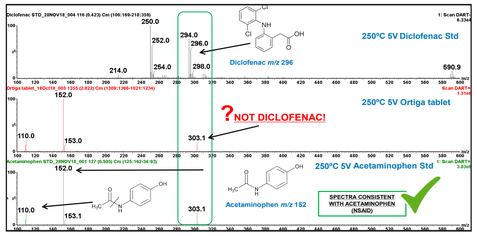


Figure 9. Comparison of Ortiga samples with Diclofenac std and acetaminophen standard shows spectra consisten with the latter.

DISCUSSION

The DART QDa System was able to detect multiple undeclared active pharmaceutical ingredients within the 'supplements' purchased online.

Comparison consistent with the spectra produced by analysing analytical standards under identical conditions

By utilising in-source fragmentation and temperature manipulation further interrogation of the spectra was possible providing increased confidence for compound identification.

The relatively simple operation of the DART QDa System could provide a rapid screening technique for suspect supplements/over the counter medicines (OTC's). This could be carried out routinely using library matching or profile matching software

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' compared to for example, UV or ion mobility based detectors for routine screening.

CONCLUSION

- **The DART QDa System successfully detected undeclared pharmaceutically active compounds within 'herbal supplements' purchased online.**
- **This was achieved using minimal/no sample preparation involving several sample forms i.e. tablet, powder and natural product (i.e honey).**
- **In source fragmentation provided more in-depth spectral data for increased confidence in results.**
- **The DART QDa System could potentially provide rapid 'first line of defence' for the identification of potentially harmful pharmaceutically active compounds with little or no sample preparation.**
- **Screening of products could be carried out utilising pre-built library matching or profiling software for rapid data**

References

1, 2 www.USP.org

3 https://www.fda.gov/Drugs/ResourcesForYou/Consumers/BuyingUsingMedicineSafely/MedicationHealthFrauddefault.htm

4 Robert B. Cody, James A. Laramee, J. Michael Nilles, and H. Dupont Durst : Direct Analysis in Real Time (DART™), Mass Spectrometry

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