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Screening of PDE-5 Inhibitors in Illicit Dietary Supplements using a DART® Source Equipped Waters ACQUITY® QDa® Mass Detector

Introduction

Phosphodiesterase type 5 (PDE-5) inhibitors, notably Sildenafil, Tadalafil and Vardenafil, are predominately used to treat erectile dysfunction (ED). Their widespread use and popularity has led to an increase in the prevalence of illicit sexual performance enhancement products, most notably in the form of adulterated herbal supplements. Supplements are widely perceived as being "safe and natural", as well as more affordable than prescription drugs, and thus have become a target for criminals looking to profit from their sale.

Due to the serious side effects and health risks of ingesting PDE-inhibitor adulterated supplements, especially when co-administered with other drugs, the Association of Official Analytical Chemists (AOAC) issued a call, effective on October 16, 2014, to develop analytical methods for identification, determination and screening of PDE-5 inhibitors in both dietary ingredients and supplements. Their method performance requirements include a target minimum concentration of 100 ppm and a high concentration of 1000 ppm.

DART is a technique that is capable of rapidly screening herbal dietary supplements for PDE-5 inhibitors by permitting direct sample interrogation and analysis in seconds per sample. The analysis was performed using a DART source interfaced to a Waters ACQUITY QDa detector as a means of providing a cost-effective instrument with the capability of in-source fragmentation for increased specificity.

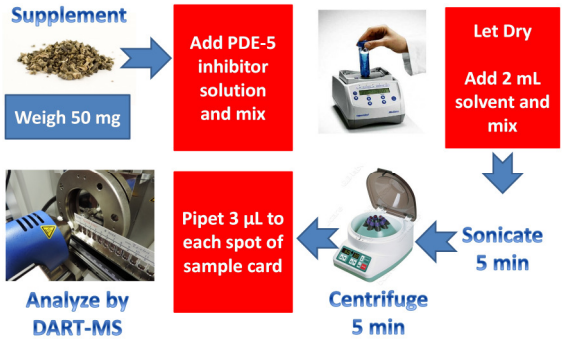

Figure 1: Spiked herbal dietary supplement extract preparation workflow.

Experiment

Simulated adulterated dietary supplements were prepared using the workflow shown in **Figure 1**. Sildenafil (Viagra), Tadalafil (Cialis) and Vardenafil (Levitra) were spiked into 50 mg of four different dietary supplements at concentrations of 100 ppm and 1000 ppm: Black Cohosh, St. John's Wort, G.N. Cinnamon and V.S. Pomegranate. A mixture of all three PDE-5 inhibitors in each of the four dietary supplements at both 100 ppm and 1000 ppm was also prepared.

The PDE-5 inhibitors were extracted from the supplements using 2 mL of 9:1 Acetonitrile:Water. The extract was sonicated and centrifuged for 5 minutes each. The supernatant was collected and analyzed directly by DART-QDa using a 12-spot wire mesh card (QuickStrip™) with 5 µL deposition volumes. The samples were introduced into the DART ionization region using a motorized rail set at 0.5 mm/s. Each sample was analyzed using a DART gas temperature of 350°C and cone voltages of +15, 30, 50, and 70 V for the QDa detector.

Results

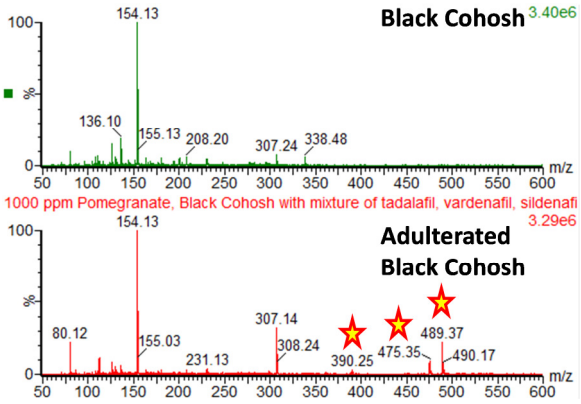

Figure 2: +15 V spectrum of black cohosh supplement (top) adulterated with sildenafil, tadalafil and vardenafil (bottom).

Each PDE-5 inhibitor was detected in the acetonitrile:water extracts of all four supplements at 1000 ppm when each supplement was spiked with one PDE-5 inhibitor (**Table 1**). Sildenafil and vardenafil were both detected in each supplement at 100 ppm concentration whereas tadalfil was only detected in the V.S. Pomegranate supplement at 100 ppm.

Table 1: Detected PDE-5 inhibitors in dietary supplements.

PDE-5	Conc.	Black Cohosh	St. John's Wort	G.N. Cinnamon	V.S. Pomegranate
Sildenafil (MW: 474.6)	100 ppm	Detected	Detected	Detected	Detected
Sildenafil	1000 ppm	Detected	Detected	Detected	Detected
Tadalafil (MW: 389.4)	100 ppm	N.D.	N.D.	N.D.	Detected
Tadalafil	1000 ppm	Detected	Detected	Detected	Detected
Vardenafil (MW: 488.6)	100 ppm	Detected	Detected	Detected	Detected
Vardenafil	1000 ppm	Detected	Detected	Detected	Detected

The results for the supplements that were spiked with mixtures of all three PDE-5 inhibitors were the same with the exception of tadalafil, which was not detected in St. John's Wort at both 1000 ppm and100 ppm. This suggests that there is interference in the detection of PDE-5 inhibitors when multiple PDE-5 inhibitors are present in a sample at high concentrations.

The detection of each PDE-5 inhibitor was confirmed using in-source CID. Characteristic fragment ions for each PDE-5 inhibitor were detected when the voltage applied to the sample cone was increased. Individual characteristic fragment ions for each PDE-5 inhibitor were also detected when all three inhibitors were spiked in one supplement (**Figure 3)**.

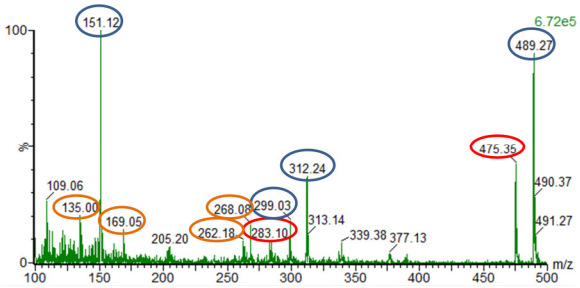

Figure 3: +70 V spectrum of sildenafil (red), tadalafil (orange) and vardenafil (blue) and their fragment ions in V.S. Pomegranate supplement.

Conclusion

The presented method demonstrated the rapid detection of Sildenafil, Tadalafil and Vardenafil PDE-5 inhibitors from dietary supplements. Fragmentation by in-source CID provided confirmation for the presence of PDE-5. This method met the performance criteria defined by AOAC and can be applied to the analysis of other PDE-5 inhibitors. The analysis of each extract was achieved in less than a minute, providing a rapid screening technique that can be utilized for dietary supplement authenticity or food safety applications.

Revision History			
REV	DCR #	Description of Change	Effective Date
1	361	Initial Release	09/15/2016