Rapid Screening and Confirmation of PDE-5 Inhibitors in Dietary Ingredients by DART-MS Ambient Ionization

Robert Goguen[1], Joseph LaPointe[1], Julie Carbonello[1], Elizabeth Crawford[1], and Brian Musselman[1]
[1] IonSense, Inc., Saugus, MA, USA

Introduction

Phosphodiesterase type 5 inhibitors (PDE-5 Inhibitors) are used to treat erectile dysfunction (ED). Numerous different PDE-5s have been detected in herbal supplements, vitamin tablets and adulterated pharmaceuticals. In response to this adulteration problem, the Association of Official Agricultural Chemists (AOAC) has issued a call for methods focused on developing technologies for rapid determination of the presence of various PDE-5s in dietary ingredients. This work describes the method we have developed to address this adulteration problem.

Methods

PDE-5 Inhibitors: Three different PDE-5 inhibitors were used for the development of this method. They were Sildenafil, Tadalafil, and Vardenafil. Sildenafil and Tadalafil samples were prepared using United States Pharmacopeia (USP) Reference Standards. In the case of Tadalafil, the non-standard sample was dissolved in acetonitrile / water with 1% formic acid while Vardenafil and Sildenafil were dissolved in acetonitrile. A mixture of Sildenafil and Tadalafil was also made. Molecular weights for the protonated molecules are shown in Figure 1.

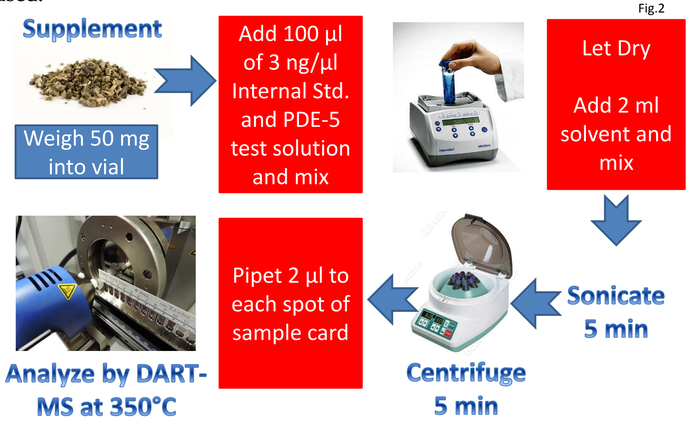
Fig.1

Compound	$[M+H]^+$	Exact Mass
Tadalafil	$C_{22}H_{19}N_3O_4$	390.1453
Sildenafil	$C_{22}H_{30}N_6O_4S$	475.2127
Vardenafil	$C_{23}H_{32}N_6O_4S$	489.2284

Dietary Supplements: Thousands of dietary supplements exist throughout the world. Black Cohosh and St. Johns Wort were chosen for their widespread use here in the US. Both supplements were purchased from local stores formulated in capsule form. The supplement materials, present as a powder, was removed from the capsule and processed for analysis without further manipulation.

Internal Standard: The internal standard used was a PDE-5 inhibitor that was different from the targeted PDE-5. For the Sildenafil, Tadalafil, and the mix of the two, Vardenafil was used as the internal standard. For the Vardenafil the Sildenafil was used.

Fig.2



Spiked Sample Preparation: Spiked samples were prepared by depositing an aliquot of the PDE-5 inhibitor solution onto a measured 50 mg quantity of supplement powder. The internal standard was also spiked onto the powder. The wet powder was vortexed for 10 seconds and then allowed to dry. Samples were prepared at concentrations called for in the AOAC method solicitation, specifically 0, 50, 100, and 1000 ppm for each PDE-5 inhibitor.

Sample Preparation for Analysis: Sample preparation steps are outlined in Figure 2. Preparation is limited to extraction of the spiked supplement with an organic solvent, acetonitrile, in which the PDE-5 inhibitors are known to be soluble, followed by rapid mixing with a vortex, followed by centrifuge to provide a clear liquid for pipetting onto the QuickStrip.

AOAC Method Requirements:

AOAC SMPR 2014_012			
Parameter	**Parameter Requirements**	**Target Test Concentration**	**Minimum Acceptable Results**
Probability of Detection (POD) at Low Concentration	Minimum of 33 replicates per matrix type, spiked at or below the designated low level target test concentration	100 ppm	90% POD
POD at High Concentration	Minimum of 33 replicates per matrix type, spiked at 10X the designated low level target test concentration	10X Low Concentration	100%
POD at 0 Concentration	Minimum of five replicates per matrix type	0 ppm	

Experiment

The AOAC solicitations is for three methods; one each for determination **SPMR 2014_010**, identification **SPMR 2014_11** and screening **SPMR 2014_12** of PDE-5 Inhibitors 5 different Dietary Supplements. Two screening methods were developed as well as a combined method for use in determination.

High Resolution Screening Method: The HR screening method was developed using a high resolution mass spectrometer coupled to a DART source configured to run QuickStrips. 2 µl aliquots of an extract spiked with and inhibitor and standard were spotted onto a QuickStrip and analyzed by DART. When a peak indicating an inhibitor was detected, it would be integrated. Peak area of each inhibitor would be used to generate a ratio based on the area of the internal standard. An inhibitor was considered detected if a ratio greater than 0 was reported. Individual scan filters were created in the processing software so that the inhibitor that triggered the ratio could be identified.

MS/MS Screening Method: The MS/MS screening method was developed using a QQQ LC/MS system coupled to a DART source. 2 µl aliquots of an extracted spiked sample were spotted onto a QuickStrip and analyzed with a MS/MS method. Figure 3 shows the fragments of the parent ions and the optimized MS/MS parameters. The ratios of the product ions were used to confirm the detection of a PDE-5 Inhibitor.

Fig.3

PDE-5 Inhibitor	Parent Mass (m/z)	Product Mass (m/z)	Collision Energy (V)	Tube Lens (V)
Tadalafil	390	168.73	38	70
	390	268.021	12	70
Sildenafil	475	282.998	31	111
	475	311.119	29	111
Vardenafil	489	151.048	41	142
	489	169.043	34	111

Results

All three PDE-5s were detected when extracted at concentrations ranging from 50ppm to 1000ppm in dietary supplements. Figure 4 shows the partial spectrum for the PDE-5s and Figure 5 contains the results from the black cohosh analysis. The screening method detected ions at the expected mass of each PDE-5 inhibitor. Visual inspection of the M+2 and M+3 isotope ratios confirmed the expected ratios were present.

Fig.4

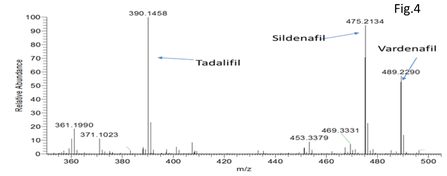


Fig.5

PDE-5 inhibitor	Concentration	# of Samples	# Detected	%RSD
Sildenafil	50 ppm	10	7	18
Tadalafil	50 ppm	10	10	33
Vardenafil	50 ppm	10	10	6
Mixture of 2 (S+T)	50 ppm	10	10	16
Blank		20	none	NA
Sildenafil	100 ppm	10	10	25
Tadalafil	100 ppm	10	10	14
Vardenafil	100 ppm	10	10	14
Mixture of 2 (S+T)	100 ppm	10	10	10
Sildenafil	1000 ppm	10	10	8
Tadalafil	1000 ppm	10	10	17
Vardenafil	1000 ppm	10	10	7
Mixture of 2 (S+T)	1000 ppm	10	10	9

Blind Study: A blind study was conducted to test the validity of the method. Samples were prepared and analyzed by two different chemists. Figure 6 shows the results.

One Sildenafil sample in the blind study was misidentified as Vardenafil. This is most likely due to Vardenafil being used as the internal standard. The use of an isotopically labeled standard would prevent this from happening.

Fig.6

PDE-5 inhibitor	# of Samples	# Detected
Sildenafil	11	10
Tadalafil	3	3
Vardenafil	4	5
Sildenafil & Tadalafil	7	7
Blank	17	none

Conclusions

- The method developed here meets and exceeds the requirements set forth by AOAC SPMR 2014_10, and 11.
- The method can quickly identify the presence of a PDE-5 and confirm it with exact mass and MS/MS with minimal sample prep allowing us to achieve the required time limit for the method.

Future Research

The next step in this method development is to expand the method to include other inhibitors and types of supplements. This includes pressed tablets, softgels, gelcaps, liquid drinks, and extracts. An isotopically labelled PDE-5 inhibitor is to be used as the internal standard.