

## Mass Spectrometry

Author:

MS Applications Team

PerkinElmer, Inc.

Waltham, MA

# Analysis of Phosphodiesterase Type 5 Inhibitors Extracted from Herbal Preparations by DSA/TOF MS

## Introduction

Dietary supplement use is becoming more prevalent among adults<sup>1</sup>. With this increased use there has been a concomitant rise in dietary supplement adulteration

by fraudulent manufacturers<sup>2</sup>. One example of this is the adulteration of supplements labeled as "herbal" or "botanical" mixtures with synthetic prescription drugs, their chemical analogs, drugs removed from the market due to safety concerns, or combinations of these classes<sup>2,3,4</sup>. These compounds are typically added to supplements to produce a biological effect or enhance the action of the natural products. A case in point of this is the addition of prescription phosphodiesterase type 5 (PDE-5) inhibitor drugs and their structural analogs to herbal supplements marketed as treatments for erectile dysfunction<sup>5</sup>. The presence of undeclared prescription drugs and untested or banned compounds can cause detrimental health effects either directly or by interactions with other drugs being taken by the user. Methods to detect these compounds are important to ensure the safety of those consuming dietary supplements. To this end, the U.S. Pharmacopeial Convention is developing a proposed general chapter, 2251, which outlines analytical methods for detection of dietary supplement adulteration<sup>5</sup>.

Ambient ionization methods such as direct sample analysis are gaining in popularity. These methods offer the possibility of drastically reducing sample analysis times from minutes to seconds. However, the success of these approaches is dependent upon many factors; in particular, the analyte matrix and the intensity of confounding background signals are crucial in determining viability. The work presented here was undertaken to determine the feasibility of detecting three PDE-5 inhibitors in an herb matrix using direct sample analysis/time-of-flight (TOF) mass spectrometry (MS), with the goal of determining if this approach can be used as a screening method for their qualitative identification. Analysis of the same analytes using conventional LC/TOF MS methodology is the subject of a separate note.

## Experimental

Drug standards for tadalafil, sildenafil, and vardenafil (Figure 1) were obtained from Cerilliant®. Herbal matrix (dried organically grown mint leaves) was obtained locally and finely crushed. Spiked herbal mixtures were prepared and drugs were extracted as outlined in Table 1. The AxION® Direct Sample Analysis™ (DSA™) TOF MS system was used to perform the analysis, as outlined in Table 2. The AxION DSA ion source was operated without the capillary extension. The instrument acquisition sequences were run using AxION® DSA Controller software. A calibrant solution (APCI tuning mix diluted 1:10 with 50% acetonitrile in water) was infused to the DSA source at 15 µL/min. during sample acquisition. Samples were acquired immediately after their application to the DSA sample mesh. Data files were recalibrated post-acquisition using calibrant ions infused during data acquisition. Post-acquisition data processing was performed using AxION Solo™ software (Table 3).

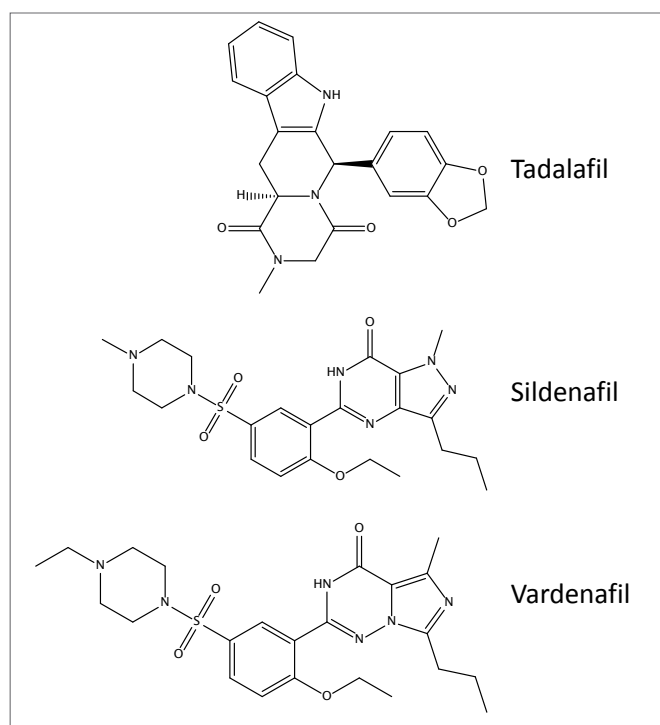


Figure 1. PDE-5 Inhibitors examined in this study.

Table 1. Sample Preparation.

Procedure
10 mg of finely crushed dried mint leaves per sample
1 mg/mL stock solutions of tadalafil, sildenafil and vardenafil
Mint spiked with 50 (L1), 25 (L2), 12.5 (L3) µg each drug or untreated
Spiked samples were dried overnight at RT
Samples were extracted 15 min. at RT with 1 mL methanol
Extracts were centrifuged 5 min. at 1,500 x g
1 µL of extract was applied to the mesh for DSA analysis

Table 2. Mass Spectrometry.

DSA-TOF Parameters
AxION 2 TOF mass spectrometer
AxION Direct Sample Analysis (DSA) source
AxION DSA Controller and AxION Solo software
Positive pulse mode
5 spectra per second acquisition rate
Low $m/z$ 50, high $m/z$ 2000
Drying gas 4 L/min. at 300 °C
Auxillary gas 4 L/min. at 300 °C
Nebulizer gas pressure 80 psi
Endplate heater: medium
Capillary exit 90V, Skimmer 25 V
Endplate -2000 V, Capillary entrance -3000 V, Corona 4
Acquisition time 6 seconds per sample

Table 3. AxION Solo Settings.

Method Parameters
Isotope search window $\pm$ 0.005 u
Monoisotopic weight 7
Isotope ratio tolerance 300%
Bin window $\pm$ 0.025 u
MS strong signal 3000 counts
F-M-3 1% strong signal

## Results

Figure 2 shows an averaged DSA/TOF mass spectrum of the L1 spiked sample first replicate. This spectrum shows that all three PDE-5 inhibitor drugs were detectable at a loading of 50 ng, assuming a 100% sample recovery in the preparation. The assigned masses for each signal were within  $\pm$  2 parts-per-million of the calculated masses for each analyte ion peak.

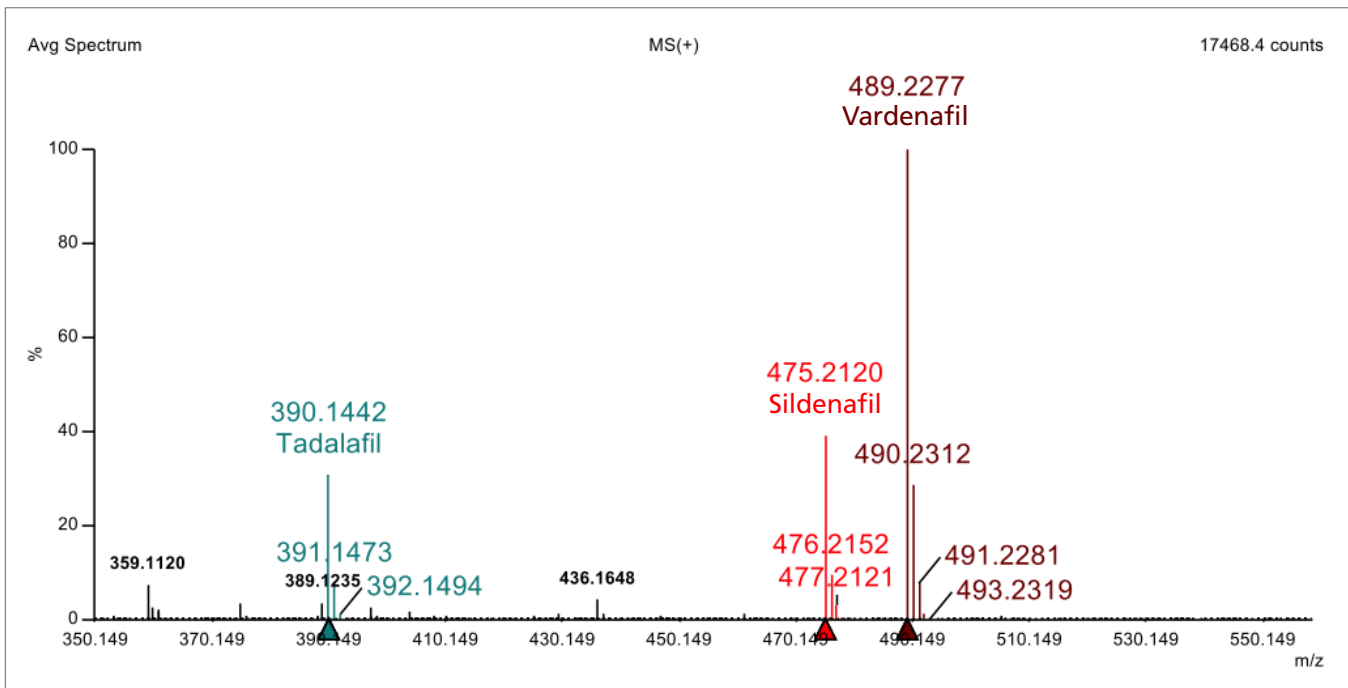


Figure 2. Averaged mass spectrum of L1 extract replicate 1. The effective loading onto the DSA support was 50 ng per analyte, assuming 100% recovery from the sample.

Figure 3 shows a summary of AxION Solo analysis for the first replicate at each spiked level as well as untreated control matrix. The coloration of each spot indicates that the analyte was detected in that sample. These results demonstrated good specificity in that all three drugs were positively detected at all three spiked levels and were undetectable in matrix blanks.

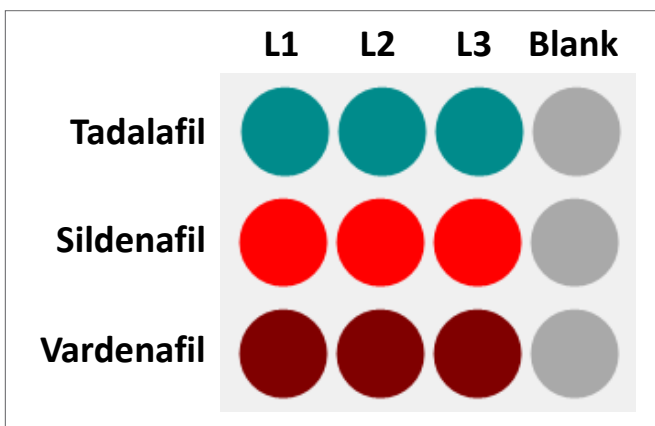


Figure 3. Example of screening results for the first replicate of each level.

Table 4 shows a summary of replicate analysis in which all three drugs were detectable at all spiked levels and undetectable in blank matrix samples. The ppm mass deviations from calculated masses for detected ions indicated in Table 4 show good mass accuracy for all analytes at all levels.

Table 4. Summary of results for all replicates at all levels, ppm mass deviations from calculated masses in green, nd = not detected.

Analyte	Level	Rep 1	Rep 2	Rep 3
Tadalafil	1	-1.6	-0.3	-2.7
	2	-1	1.2	-1.7
	3	-1.1	1.4	-2.1
	Blank	nd	nd	nd
Sildenafil	1	-0.4	0.7	-0.7
	2	0.5	1.8	0.3
	3	0.4	2.3	0.7
	Blank	nd	nd	nd
Vardenafil	1	-0.4	0.4	1.4
	2	0.9	1.6	2.6
	3	0.1	2.7	4
	Blank	nd	nd	nd

## Conclusion

The data presented here demonstrates the feasibility of detecting PDE-5 inhibitors from an herbal matrix by DSA/TOF MS analysis. With the simple preparation procedure, rapid sample acquisition and good specificity, the DSA/TOF MS method has the potential to screen for PDE-5 inhibitor adulteration of dietary supplements. Time to analysis with DSA is significantly faster than the comparable LC/TOF MS analysis, however, other sample matrices can potentially play a major impact on the effectiveness of the technique.

## References

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