APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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LC/TOF MS Analysis of Phosphodiesterase Type 5 Inhibitors Extracted from Herbal Preparations

Introduction

Dietary supplement use is becoming more prevalent among adults[1]. With this increased use there has been a concomitant rise in dietary supplement adulteration by fraudulent manufacturers[2]. 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[2,3,4]. 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[5]. 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[5]. The work presented here was undertaken to determine the feasibility of detecting three PDE-5 inhibitors in an herb matrix using liquid chromatography (LC) 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.

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Experimental

Drug standards for tadalafil, sildenafil, and vardenafil (Figure 1) were obtained from Cerilliant®. Herbal matrix (organically grown dried mint leaves) was obtained locally and finely crushed. The spiked herbal mixtures were prepared and drugs were extracted as outlined in Table 1. LC/TOF MS was performed as outlined in Tables 2 through 4.

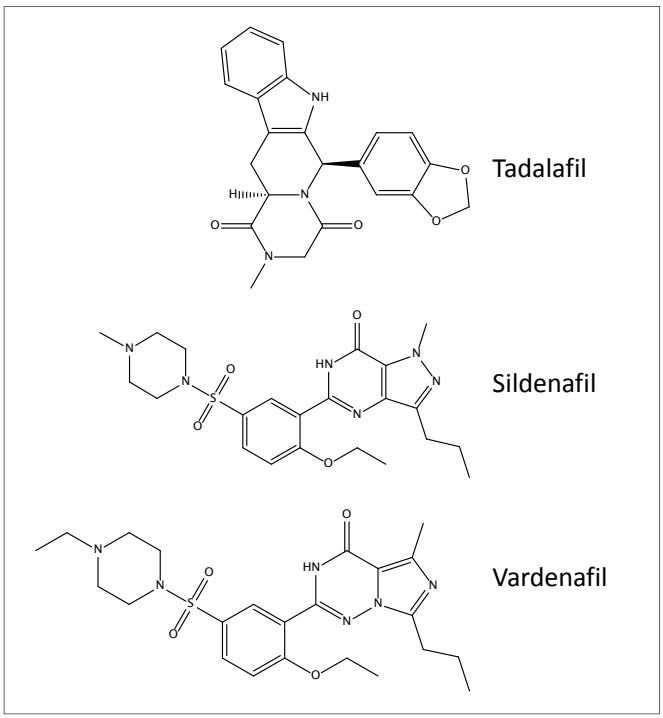

Figure 1. PDE-5 Inhibitors examined in this study.

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
Extracts were diluted 1:10 with methanol
2 µL of extract was injected for LC/TOF MS analysis

Table 2. Liquid Chromatography.

FX-15 UHPLC Pump, Autosampler, and Column Oven	
Mobile phase A	Water containing 0.1% formic acid
Mobile phase B	Acetonitrile containing 0.1% formic acid
Autosampler needle wash	80:20 acetonitrile:water
Sample injection	2 µL fixed loop, 3 injections
Flow rate	0.4 mL/min
Column temperature	45 °C
Brownlee™ SPP RP-Amide column	2.1 x 100 mm, 2.7 µm, p/n N9308501
Pre-column filter	0.5 µm porosity stainless steel

Table 3. Liquid Chromatography Gradient.

Time (min.)	% B
0	5
10	95
11	95
11.1	5
3 min. equilibration at 5% B	

Table 4. Mass Spectrometry.

AxION® 2 TOF MS Parameters
PerkinElmer AxION 2 TOF mass spectrometer
Chromera® and TOF MS Driver software
Ultraspray™ 2 dual probe electrospray source
Positive pulse mode
4 spectra per second acquisition rate
Low *m/z* 70, high *m/z* 2000
Drying gas 12 L/min. at 350 °C
Endplate heater, medium
Lockmass calibrants melamine and reserpine: 6 µg/mL each
Lockmass solution: methanol containing 0.1% formic acid
Lockmass mode: 127.0727, 609.2807, Search span 50 mmu
Left ESI probe (lockmass): 20 psi, right probe (column): 80 psi
Capillary exit 80V, Skimmer 25 V
Extracted ion chromatogram (EIC) tolerance: ± 0.03 u

Results

Figure 2 shows an example extracted ion chromatogram overlaid with the base-peak ion chromatogram (BIC, *m/z* 130 – 600) for the first replicate of the L1 and blank sample extracts. These results demonstrated good chromatographic resolution and the absence of any detectable analyte signal in blank matrix.

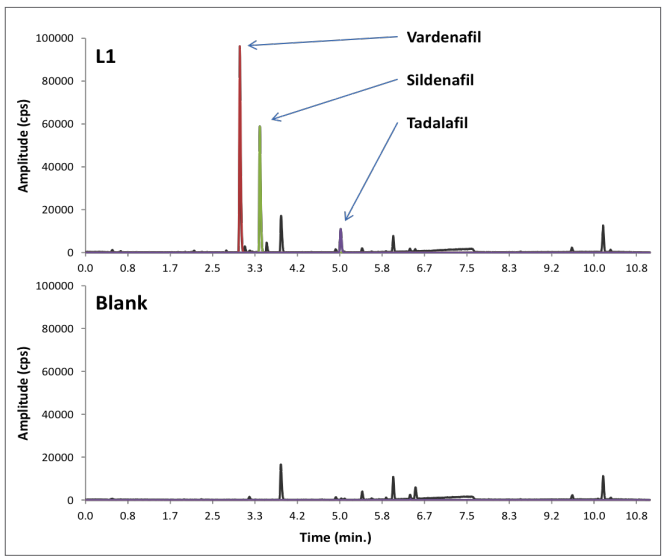

Figure 2. Example EICs and BIC for Level 1 and Blank first replicates. Level 1 preparation contains 50 µg of each analyte.

The results for all sample replicates at all levels are summarized in Table 5. The average signal-to-noise ratio for analyte peaks was 6537 (range 1144 to 19215). Average imprecision was 2.1 %CV (range 0.8 to 4.2). No analytes were detected in untreated blank matrix samples.

Table 5. Summary of all sample replicates (n=3), nd = not detected.

Analyte	Sample	Ave. Area	Ave. S/N	Area %CV
Tadalafil	L1	3.70E+07	4118	3.7
	L2	1.80E+07	2439	4.2
	L3	1.20E+07	1144	2.0
	Blank	nd	nd	nd
Sildenafil	L1	2.00E+08	19215	1.3
	L2	9.60E+07	9863	1.1
	L3	5.70E+07	6253	3.6
	Blank	nd	nd	nd
Vardenafil	L1	1.90E+08	7679	0.8
	L2	1.10E+08	4551	1.4
	L3	7.20E+07	3573	1.0
	Blank	nd	nd	nd

Conclusion

The data presented here demonstrate the feasibility of detecting PDE-5 inhibitors from an herbal matrix by LC/TOF MS analysis. The sample preparation procedure is simple and liquid chromatography separation allows for resolution of analytes from herbal matrix components. This LC/TOF MS method has the potential for screening of PDE-5 inhibitor adulteration in dietary supplements.

References

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