



Phentolamine Adulteration in Dietary Supplements

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Introduction

Phentolamine is an alpha-adrenergic blocker used as an antihypertensive agent among other things. It had been investigated for the treatment of erectile dysfunction (ED) in the past. At that time, oral use was ruled out due to the inability to reliably control blood pressure. In the United States, this compound is available by prescription only. It is illegal to use this compound in dietary supplements and its presence in a supplement makes that product misbranded and adulterated.

Flora Research Laboratories (FRL) utilizes the Varian 500-MS ion trap mass spectrometer in TurboDDS™ mode to test male enhancement supplements for adulteration with clandestine PDE-5 inhibitors (ED drugs like Viagra®, Cialis® and Levitra®). TurboDDS is utilized because there are numerous novel analogues detected on an ongoing basis (homosildenafil, thiosildenafil, hydroxyhomosildenafil, amino-tadalafil, piperadino vardenafil, etc.). Using TurboDDS mode, FRL detected the first prodrug analogue of a PDE-5 inhibitor as well as several other novel analogues in this class. The Varian 500-MS is operated in tandem with a Varian 335 PDA (photo diode array) detector controlled with Galaxie™ chromatography software. Numerous analogues give characteristic ultra-violet (UV) spectra that can be traced back to the parent drug. The combination of PDA and TurboDDS data allows for faster compound detection and identification.

This note describes a situation in which phentolamine was detected during a routine screening for PDE-5 inhibitors in a dietary supplement. It clearly demonstrates the urgent need for a broad spectrum analysis, in which unknowns can be detected along with target compounds, to protect consumers from illegally adulterated and potentially harmful dietary supplements.

Instrumentation

- Varian 500-MS Ion Trap Mass Spectrometer with TurboDDS
- Varian 212-LC Binary Solvent Delivery Module (2)
- Varian 430 AutoSampler
- Varian 335 Photo Diode Array (PDA)

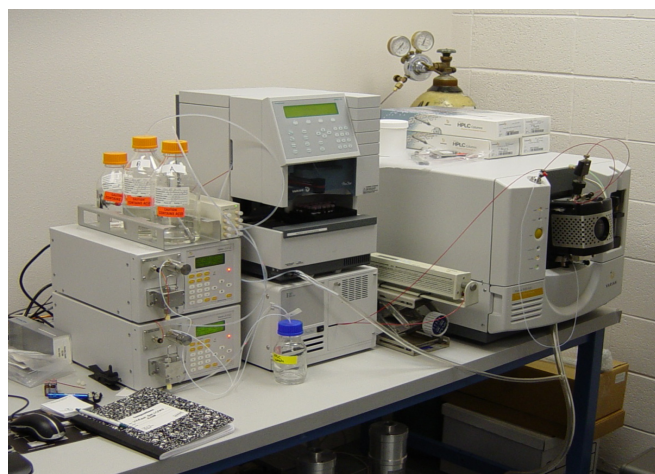


Figure 1. Varian 500-MS analytical system used at Flora Research Laboratories.

Results and Discussion

A great benefit of the TurboDDS mode is the ability to detect unknown compounds during routine screening. While analyzing a dietary supplement during a routine PDE-5 inhibitor screen, FRL detected a suspect compound giving an [M+H]⁺ ion at m/z 282 (Figure 2). The MS² product spectrum gave a dominant base peak at m/z 212 (Figure 3). In addition, the PDA spectra obtained from the Varian 335 PDA detector and Galaxie software showed an absorbance maxima at 278 nm. This compound did not match up to any of the declared ingredients, which included numerous botanicals.

The NIST 2008 library is the first to contain MS/MS spectra, which is a useful tool that can provide more information about the identity of the sample. A NIST MS/MS library search of the ESI(+) mode spectra was conducted. Phentolamine was in the library and the library spectra gave the characteristic m/z 212 product ion in MS/MS. Figure 4 shows the match obtained against the library. Further literature searches of this compound revealed that the UV spectra matched the published data.

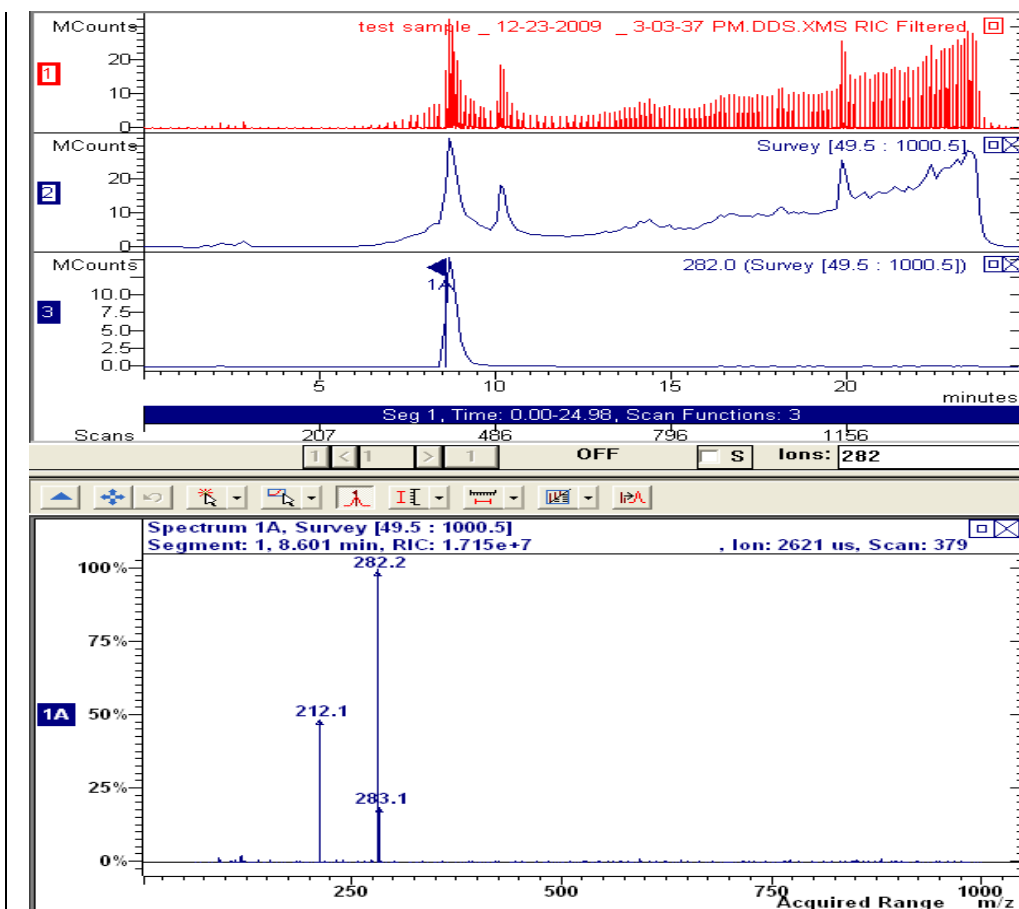
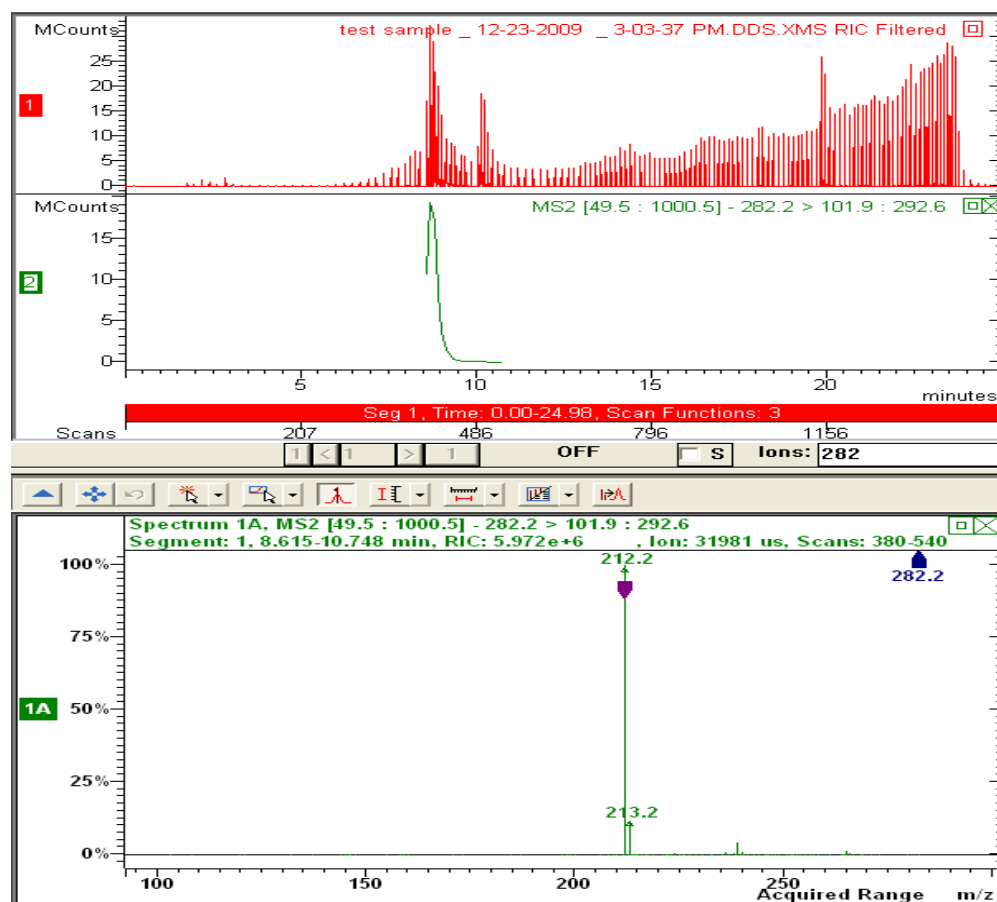


Figure 2. Dietary supplement sample analysis using TurboDDS™ data dependant scanning. The top trace shows the TurboDDS scan (red), with full scan survey reconstructed ion chromatograms in blue. A large M+H ion at m/z 282 was detected in the full scan survey scan, with the spectrum listed on the bottom.

Figure 3. The MS^2 spectrum for the peak at m/z 282 detected in the survey scan. A dominant peak at m/z 212.2 is observed.



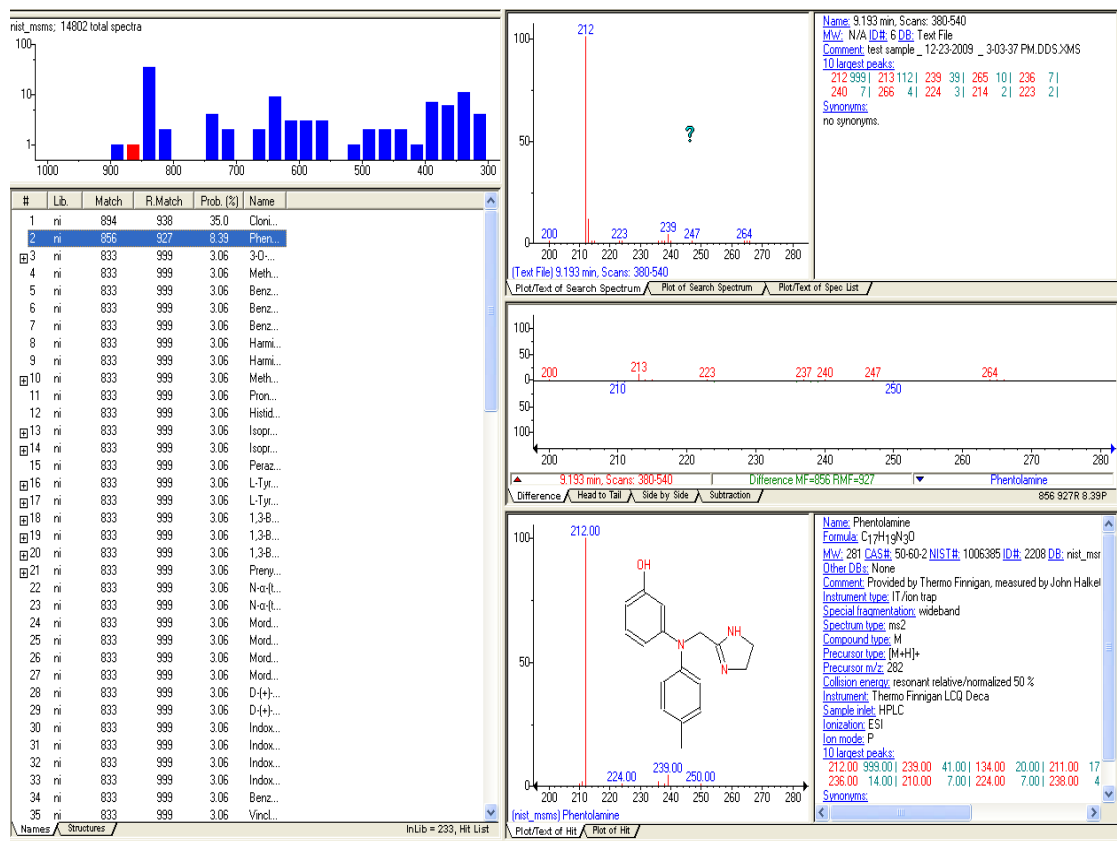
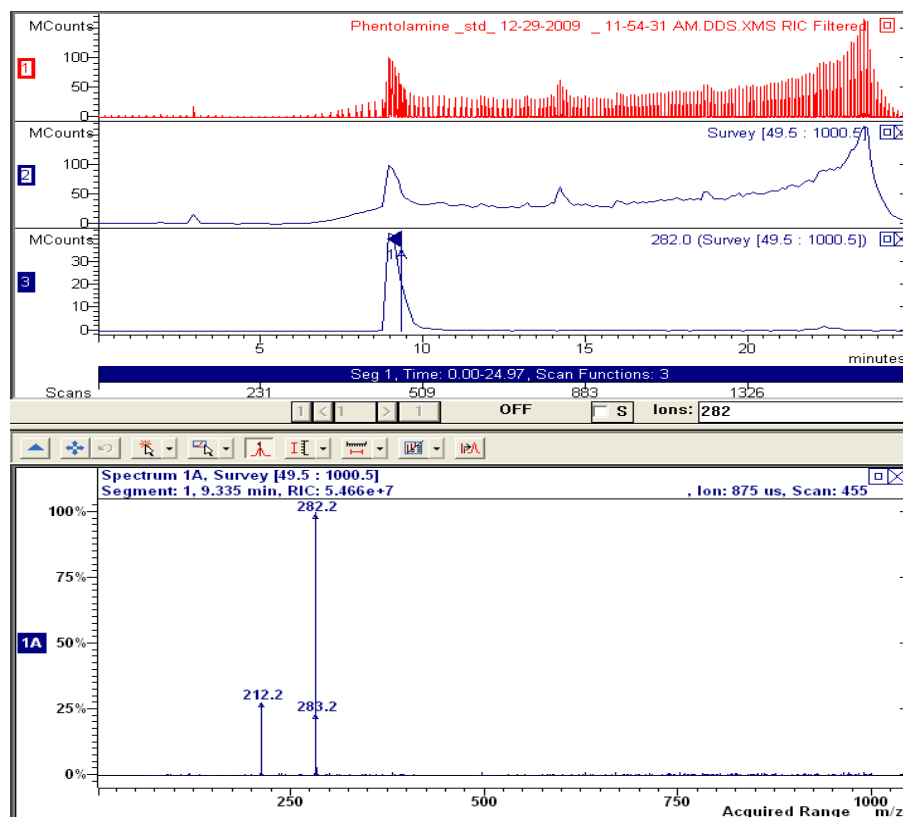


Figure 4. Phentolamine library match against the NIST MS/MS library. Good fit and reverse fit library match values obtained.

At this point, FRL was fairly certain that the compound was phentolamine. In order to further confirm the findings (even though we had PDA, MS, and NIST MS/MS match) a standard was obtained from Sigma-Aldrich Chemical Company. The retention time, PDA, MS and MS/MS spectra all matched the standard (Figure 5). The compound identity was confirmed as phentolamine. During this round of testing, three other samples were found to be adulterated with this compound.

Figure 5. Phentolamine standard from Sigma-Aldrich analyzed on the 500-MS with TurboDDSD. The standard showed identical retention time, MS/MS spectrum and PDA absorbance maxima to that of the test sample.



To the best of FRL's knowledge, this is the first time that phentolamine was identified as an adulterant in male enhancement products. In addition, this and the detection of testosterone derivatives suggests that screening is necessary not only for PDE-5 inhibitors but for steroids and alpha-adrenergic blockers as well.

Conclusion

Dietary supplement adulteration is becoming a more serious problem every day. These statements clearly demonstrate the importance of increased surveillance using the power of mass spectrometry: "If we did not have the power of TurboDDS, this compound could have easily eluded detection," said James Neal-Kababick, Director of Flora Research Laboratories and the pioneer of the emerging field of phytoforensic science. "How do you test for a compound you don't know exists? In our field, the evolution of Data Dependent Scanning (DDS) combined with the incredible robustness of the Varian 500-MS has allowed our laboratory to detect numerous pharmaceuticals and analogues [in dietary supplements], which eluded detection in other laboratories on prior occasions," said Neal-Kababick.

The detection of phentolamine is yet another incident in a long series demonstrating the importance of TurboDDS and ion trap LC/MS in natural products research and phytoforensic science. "Problems that would traditionally take us days or weeks to solve can now be solved in hours and that can mean the difference between life and death for consumers at risk," said Neal-Kababick.

Currently, Flora Research Laboratories is investigating the integration of analytical NMR and high resolution mass spectroscopy into the analytical testing laboratory environment as the natural companions to benchtop mass spectroscopy. "Each instrument provides a piece in a complex puzzle," said Neal-Kababick.

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These data represent typical results.

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