Detection of Adulterants in "All Natural" Weight Loss Supplements Using the Agilent 500 Ion Trap LC/MS with TurboDDS

Application Note

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Abstract

This application note presents a method for the analysis of adulterants in weight loss supplements marketed as "all natural." The analysis is performed using the Agilent 500 Ion Trap LC/MS with Turbo Data Dependent Scanning (TurboDDS), which performs full scan and MS/MS analysis in a single injection. Target adulterants were observed and yielded spectra with excellent library matches to the NIST MS Library when spiked into a dietary supplement matrix at 10 µg/mL and then diluted 1:10 in water. A real incurred weight loss product was also analyzed and found to be adulterated with Bumetanide. Unlike other techniques that use targeted analysis to detect known adulterants in a dietary supplement of interest, TurboDDS screens and identifies both known and unanticipated adulterants in a single experiment.

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Introduction

In June 2007, the U.S. Federal Drug Administration issued the *Dietary Supplement Current Good Manufacturing Practice (cGMP) Final Rule (21 CFR Part 111)*. In essence, the final rule requires that the proper controls be in place for dietary supplements during manufacturing, packaging, labeling, and holding operations. As of June 2010, all companies that manufacture non-prescription dietary supplements for sale in the United States must comply with these regulations.[1,2]

In this application note, the Agilent 500 Ion Trap LC/MS is paired with the Agilent 1260 Infinity LC for the detection of eight common adulterants in weight loss supplements that have been marketed as "all natural." These adulterants include loop diuretics (Bumetanide and Furosemide), appetite suppressants (Sibutramine, Rimonabant, and NIDA), a respiratory drug (Theophylline), a laxative (Phenolphthalein), and an antiepileptic drug (Phenytoin).

In targeted LC/MS analysis, an MS/MS instrument is programmed with MRM transitions that correspond to certain known target compounds. Targeted analysis is sensitive and specific, but if unknown or novel adulterants have been added to a sample of interest, those adulterants will go undetected. The use of TurboDDS in this analysis allows for simultaneous full scan surveying and MS/MS data collection, which is then used for NIST library confirmation. For analysis of weight loss supplements, TurboDDS analysis is ideal because known common adulterant compounds can be given priority over matrix ions with the use of an "include list," but the survey scans will still capture and analyze any other prevalent components present in the sample.

Figure 1. Agilent 1260 Infinity LC and Agilent 500 Ion Trap LC/MS.

Experimental

Sample preparation

Clean matrix samples, neat standards, and incurred samples were supplied by Flora Research Laboratories. Matrices and incurred samples were prepared for injection by adding 500 mg (or the contents of one capsule) to 30 mL of 50:50 acetronitrile:water in a 50 mL volumetric flask. The flask was vortexed, sonicated for 10 minutes, and then brought to volume with 50:50 acetonitrile:water. The contents of the flask were then transferred to a centrifuge tube and centrifuged for 30 minutes at 10,000 rpm. Finally, the supernatant was filtered through a 0.2 µ syringe filter and diluted 1:10 in water for injection. The standard samples were made at 10 µg/mL and then diluted 1:10 in water for a final concentration of 1 µg/mL. The concentration chosen for preparation was 10 µg/mL because it is the lowest point for quantitation of Bumetanide required by the FDA (the compound which has the smallest effective dose of all of the adulterants analyzed).

LC/MS analysis

Table 1 shows the LC parameters used for analysis of adulterants in weight loss dietary supplements. MS analysis was performed on a 500 Ion Trap LC/MS in TurboDDS mode. **Table 2** shows the MS parameters used for the analysis of adulterants in weight loss dietary supplements.

Table 1. LC Conditions

LC column	2.0 mm x 150 mm Pursuit XRs 3 µ C18 (A6001250X020)
Mobile phase	A = 0.1% formic acid in water B = 0.1% formic acid in acetonitrile
Gradient program	5% B for 3 min; ramp up to 95% B over 15 min; hold for 7 min; bring to 5% B; hold for 8 min
Flow rate	0.200 mL/min
Injection volume	10 µL

Table 2. MS Conditions

Ionization mode	ESI positive and negative
API drying gas	30 psi at 350 °C
API nebulizing gas	50 psi
Needle voltage	± 5000 V
Shield voltage	± 600 V
Capillary voltage	± 80 V
RF loading	95%
Scan range	50-500 *m/z*
MS^n depth	n = 2
TurboDDS breadth	N = 6
Trigger threshold	+ mode: 10,000; - mode: 1,000
Include list	Yes
Positive mode include list	180.5-181.5 252.5-253.5 279.5-280.5 318.5-319.5 330.0-332.0 364.8-365.6 457.8-459.5 464.0-466.0
Negative mode include list	178.0-180.0 250.0-252.0 316.0-318.0 328.0-330.0 362.0-364.0

Results and Discussion

This LC/MS method separates and detects adulterants in very complex matrices and provides the assurance of full scan analysis with the confirmation of MS/MS identification with NIST library matching.

Matrix injections

Standards were spiked into complex dietary supplement matrices at 10 µg/mL and diluted 1:10 in water for injection. Diluting the matrix by a factor of 10 helps reduce matrix effects and increases the uptime of the instrument between cleanings. **Figure 2** and **3** show the extracted ion chromatograms in positive and negative mode for the compounds of interest after they were spiked into a clean matrix blank. The matrix was also run before spiking to confirm that none of the adulterants listed here were present.

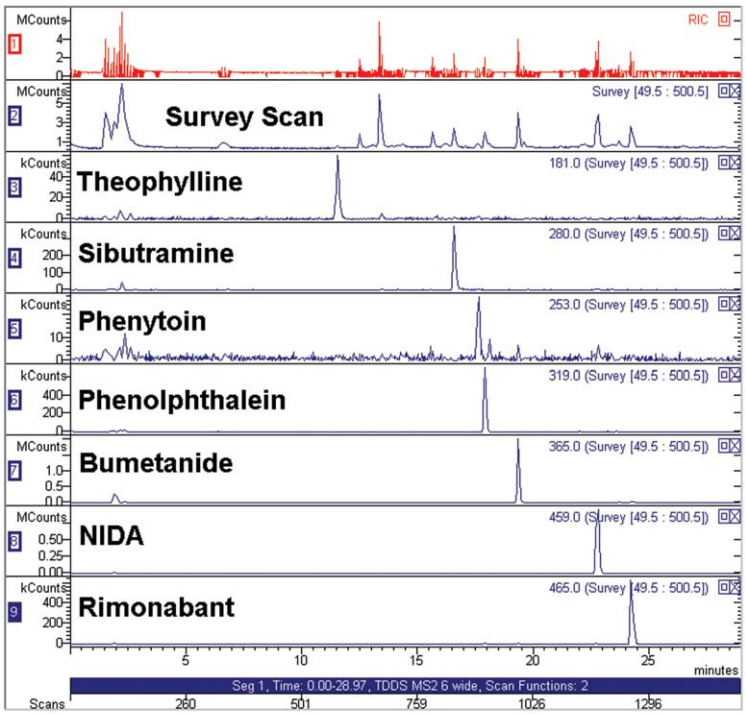

Figure 2. Extracted ion chromatogram in positive mode for a spiked blank matrix injection at 1 ppm.

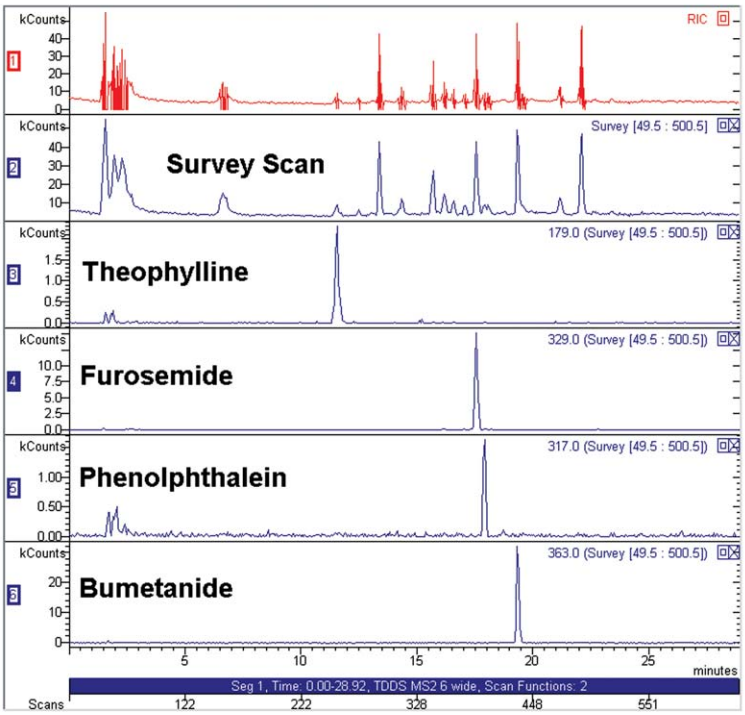

Figure 3. Extracted ion chromatogram for a spiked dietary supplement matrix analyzed using negative mode electrospray and TurboDDS.

Real sample injection

The analytical method was also used to analyze real world samples that had been adulterated with Bumetanide. **Figure 4** shows the chromatogram for Bumetanide analyzed in negative mode. The chromatogram on the bottom represents the triggered MS/MS data points.

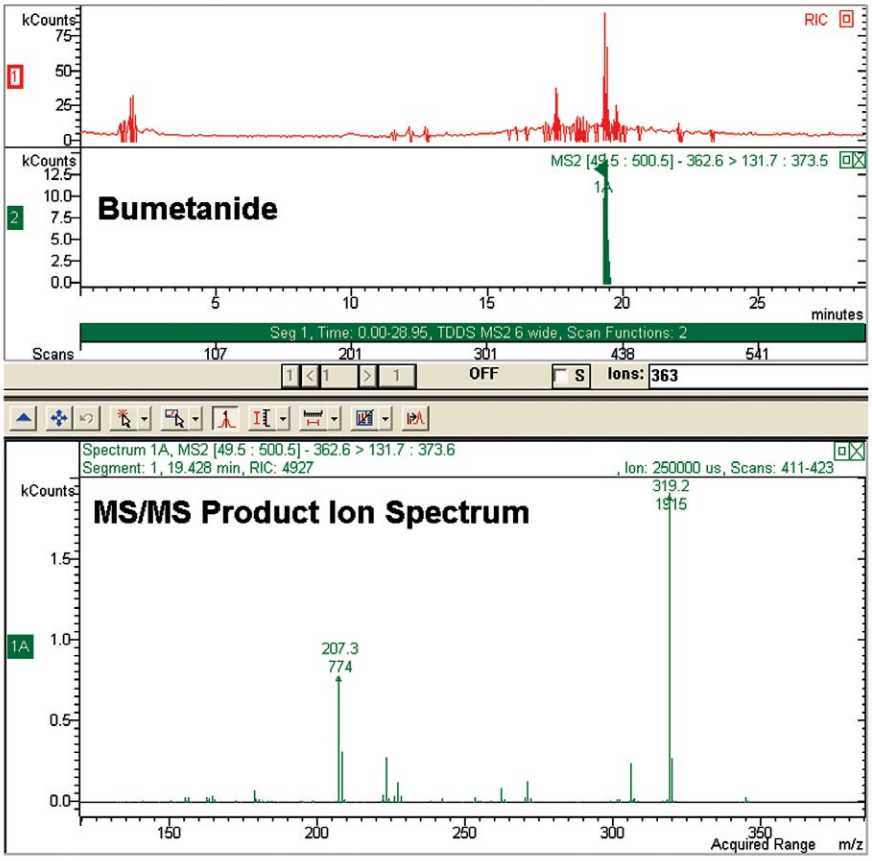

Figure 4. MS/MS data for a real dietary supplement adulterated with Bumetanide.

The MS/MS product ion spectrum of the sample adulterated with Bumetanide was exported to the NIST library and searched against a user library created from weight loss supplement adulterant standards, as well as the NIST MS Library. The results are shown in **Figure 5**.

In the MS/MS library search, the Bumetanide spectrum from the NIST MS Library was the top match, and other Bumetanide entries made up the top six matches. The direct match score for the top match was 925 out of 1000, and the reverse match (Dot Product) score was 967 out of 1000, a very strong spectral match against the library, and hence strong specificity for identifying Bumetanide.

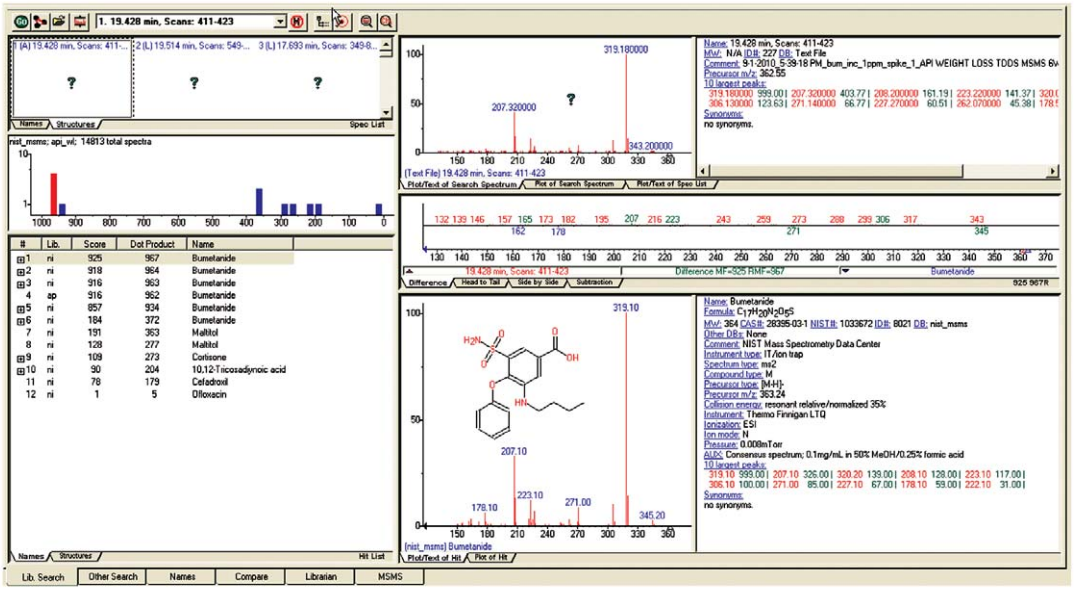

Figure 5. NIST MS Library match for Bumetanide in an incurred sample.

Conclusions

In this analysis, TurboDDS software provided screening, identification, and confirmation for common weight loss supplement adulterants in a single injection. In order to do this, the compounds of interest were analyzed in full scan, and subsequently fragmented to give MS/MS data used for NIST library confirmation searching. The advantage of this type of screening is that the known targets are identified, while any unanticipated contaminants are also found as part of the survey scan. TurboDDS analysis provides confidence in the entire contents of a sample, not just the compounds that would be part of a target screen. The MS/MS data generated from injections in complex matrices were well matched by a NIST user library, as well as the NIST MS Library, providing an additional level of identity confirmation. This method has high specificity for target analytes without compromising the value of full scan screening.

References

1. Dietary supplement regulation facts. Downloaded September 8, 2010, from the following URL: http://www.fda.gov/Food/DietarySupplements/default.htm

2. "White Paper: Final FDA Rule on Dietary Supplements (21 CFR Part 111)" Generated by MasterControl. Downloaded September 8, 2010, from the following URL: http://www.mastercontrol.com/resource/

www.agilent.com/chem/

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