COGENT
Diamond Hydride™

Harmful Substances in Dietary Supplements DMAA

Extended Application Note

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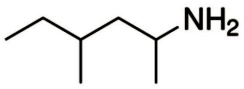


DMMA

Method 1:

Time (min.)	%B
0	20
6	90
8	90
10	20

Method 2:

Time (min.)	%B
0	95
1	95
3	70
4	70
6	30
8	30
10	95

Method 3:

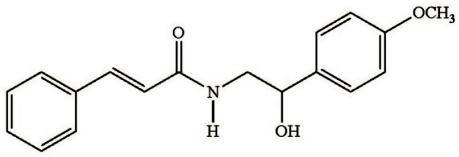
Time (min.)	%B
0	90
7	30
8	30
9	90

Introduction

A dietary supplement is a substance to be consumed for the purpose of providing additional nutrients to an otherwise deficient diet. In the United States, the Food and Drug Administration (FDA) regulates dietary supplements as they do food. Unlike a drug, the FDA does not need to verify a supplement's efficacy and safety claims prior to sale to the general public. Only after the FDA has demonstrated a supplement is unsafe can action be taken to halt production, distribution, and sale.

In recent years, there have been notable cases of an ingredient present in a dietary supplement that has caused serious health issues or even death. 1,3-Dimethylamylamine (DMAA) is one example. Purported to have energy-boosting effects, DMAA has been added to certain workout supplements and energy drinks. However, its effects on the constriction of blood vessels are believed to contribute to cardiovascular issues. With susceptible individuals under strenuous conditions, this may cause significant adverse health problems. Indeed, at least five deaths have been linked to DMAA use in dietary supplement formulations.

Another example is aegeline, found in aegle marmelos trees and used in traditional medicines. It has been implicated in cases of liver damage although it is claimed to produce weight-loss effects. It has been pulled from distribution and later recalled from shelves by the manufacturer after the FDA intended to impose a recall itself. Due to the potentially dangerous effects of these substances to the consumer, reliable analytical methods are needed for their analysis in dietary supplement formulations.



Aegeline

Experimental

Materials

Protein sport powder mixes were obtained from an anonymous source. Deionized water (DI H_2O) was prepared on a Milli-Q™ purification system from Millipore (Bedford, MA, USA). Acetonitrile (HPLC grade) was obtained from GFS Chemicals, Inc. (Powell, OH, USA).

Instrumentation

An Agilent (Little Falls, DE, USA) 1200SL Series LC system, including degasser, binary pump, temperature-controlled autosampler, and temperature-controlled column compartment was used. The mass spectrometer system was an Agilent (Santa Clara, CA, USA) Model 6210 MSD TOF with a dual sprayer electrospray source (ESI). The flow rate was 0.4 mL/min. Solvent A was DI H_2O + 0.1% formic acid and solvent B was acetonitrile + 0.1% formic acid. The columns used were as follows:

Method 1: Cogent Bidentate C18 2.o™, 2.2µm, 120Å, 2.1 x 50 mm

Methods 2 & 3: Cogent Diamond Hydride™, 4µm, 100Å, 2.1 x 150 mm

Sample Preparation

For the Methods 1 & 2 samples, two scoops (2.9456g) protein powder mix were dissolved in 50.00 mL DI water in a beaker. A portion was filtered (0.45µm, nylon) and 400µL filtrate was mixed with 400µL acetonitrile. The solution was centrifuged and the supernatant was collected and used for LC-MS injections.

For the Method 3 sample, 5.50 g supplement powder was added to a 100 mL volumetric flask and dissolved in a DI water diluent by sonication. This solution was filtered through a 0.45µm nylon syringe filter (MicroSolv Tech Corp) and diluted 1:200 with 50% DI water /50% ACN/ 0.1% formic acid (v/v) for LCMS injections.

Results and Discussion

Method 1: The aegeline peak is well-retained and symmetrical in the extracted ion chromatogram (EIC). The Cogent Bidentate C18 2.0™ column is suitable for analysis of this potentially dangerous compound in sports powder mix samples. Peak 1 in the EIC in **Figure 1** corresponds to aegeline. Use of MS allowed for the isolation of clean EIC for aegeline which was free of interferences. The small particle size of the stationary phase led to a high-efficiency peak

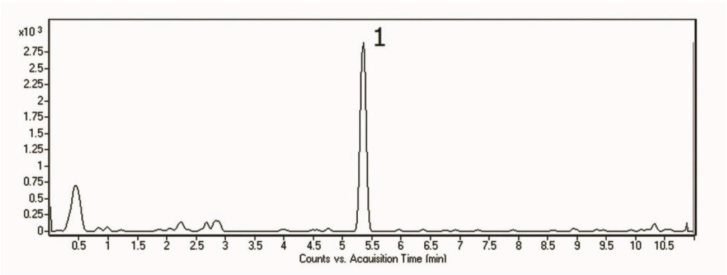


Figure 1

Method 2: In this protein powder mix, DMAA was not detected, but two compounds were found that were not listed among the label's ingredients. These were caffeine and creatine. This can be important for individuals who wish to avoid consuming these substances but are unaware of their presence in this powder mix. Although rare, overconsumption of caffeine can even be fatal for certain susceptible individuals. At lower doses, caffeine can still produce adverse effects such as restlessness, anxiety, and insomnia. Hence, the consumer should know what they are getting in a dietary supplement, and ingredients like these should be specified in the labeling so that informed decisions can be made by the consumer.

The other compounds observed in the EICs were identified as various vitamins. These included vitamin C, biotin, vitamin B6, and thiamine.

As for the chromatography, the Diamond Hydride™ column was an ideal choice for analysis of these polar compounds. All the compounds are retained past the solvent front and selectivity is good (**Figure 2**).

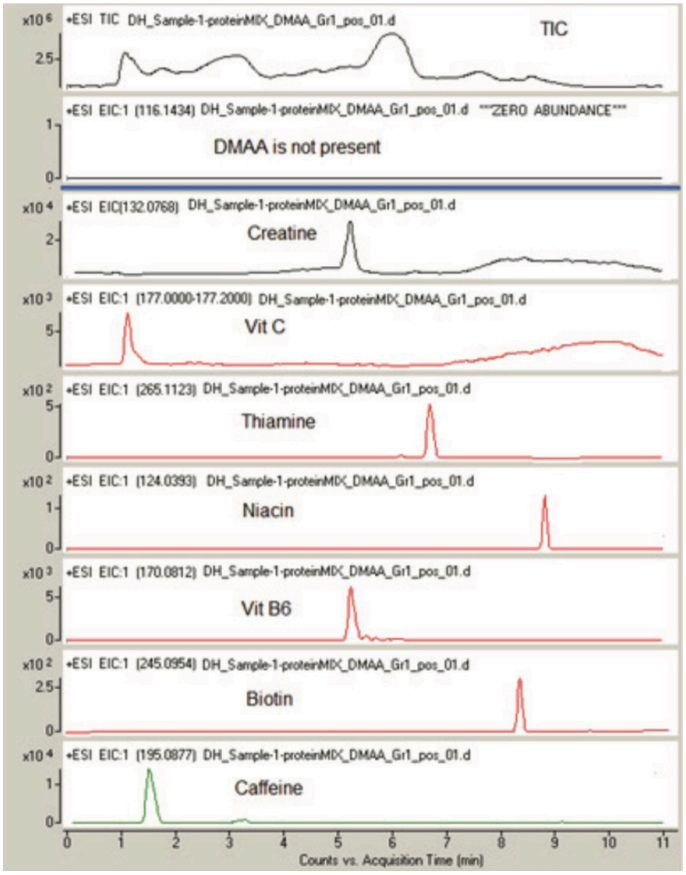


Figure 2

Indeed, when the analysis was tried in reversed phase, retention was low and the compounds were not well- separated chromatographically (data not shown).

Method 3: In this workout supplement mix, DMAA was observed in the EIC (peak 2). It was well-retained and the peak shape was symmetrical. Four other components of the protein mix were also detected. These were caffeine, beta- alanine, creatine, and L-arginine (**Figure 3**). In addition to MS- specificity, good chromatographic selectivity was obtained using the Diamond Hydride™ column.

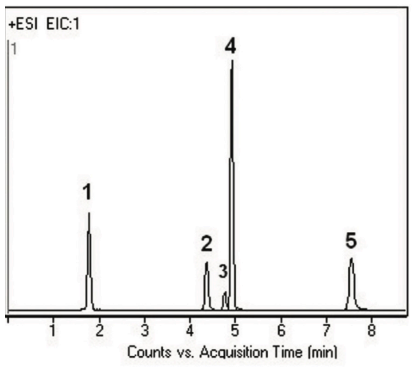


Figure 3

1. Caffeine
2. DMAA
3 β-alanine
4. Creatine
5. L-arginine

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Conclusion

Various sport protein supplement mixes were analyzed to assess their content, which may be used for safety investigations. In some cases, compounds were found that were not listed on the product label. In others, the potentially harmful compounds DMAA and aegeline were detected.

The Cogent Diamond Hydride™ column was a good choice for the polar analytes while the Cogent Bidentate C18 2.o™ produced a high-efficiency, well-retained peak for aegeline in reversed phase. Use of these columns can be applicable to important analyses pertaining to the tighter regulation of dietary supplements.

For more information visit www.mtc-usa.com

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