Application Note 02101

Analysis of Bumetanide in a Dietary Supplement Using the Varian 500-MS Ion Trap Mass Spectrometer

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Introduction

Bumetanide is a loop diuretic used to treat heart failure. The World Anti-Doping Agency (WADA) and National Football League (NFL) consider the supplement a banned substance for athletes. Its alleged use is to mask steroids by increasing urine output. Recently, some NFL football players tested positive for bumetanide (Figure 1).

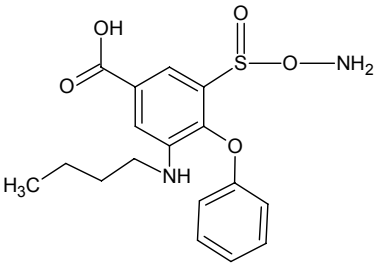

Figure 1. Molecular structure of bumetanide, MW 364.4 g/mol.

The US Food and Drug Administration (FDA) is examining dietary supplements for bumetanide adulteration. Loop diuretics can be illegally added to dietary supplements used for weight loss to enhance their "apparent" effectiveness in reducing body weight.

In this application, the Varian 500-MS LC/MS Ion Trap is used in positive and negative ionization mode to analyze bumetanide in an adulterated dietary supplement.

Figure 2. Varian 500-MS LC/MS/MS Ion Trap.

Instrumentation

- Varian 500-MS LC/MS Ion Trap with ESI source
- Varian 212-LC Binary Solvent Delivery Modules
- Varian ProStar™ 430 Autosampler
- Varian MetaTherm™ Column Heater

Sample Preparation

The dietary supplement sample was prepared in 50% acetonitrile. Approximately 100 mg was taken from a composited sample and weighed into a 100-mL volumetric flask. 80 mL of extraction solvent was added, the sample was shaken for 5 minutes, and then sonicated for 5 minutes. After cooling and diluting to volume, a 10-mL aliquot was decanted into a 15-mL polypropylene centrifuge tube and centrifuged at 3500 RPM for 15 minutes. The sample was then filtered with a 0.45-µm PTFE syringe filter into an amber autosampler vial for analysis on the Varian 500-MS using ESI-LC/MS/MS.

HPLC Conditions

Column: Pursuit™ XRs C18, 3 µm, 150 x 2.0 mm (Varian Part Number A6001150X020)
Solvent A: 0.1% formic acid in water
Solvent B: 0.1% formic acid in acetonitrile
Injection Volume: 5 µL
Column Temp: 30 °C

LC Program:

Time (min:sec)	%A	%B	Flow (µL/min)
00:00	90	10	200
20:00	10	90	200
20:06	90	10	200
25:00	90	10	200

MS Parameters

Ionization Mode: ESI (positive and negative)
Needle: ±5000 V
Shield: ±600 V
Nebulizing Gas: 50 psi
Drying Gas: 30 psi at 350 °C

Table 1. MS segment parameters.

Analyte	Polarity	Transition	Retention Time (min)	Capillary Voltage (V)	Excitation Amplitude (V)	RF Load (%)
Bumetanide	Positive	365 → 114-375	15.89	80	1.49	100
	Negative	363 → 113-373			1.48	

Results and Discussion

In this example, a bumetanide standard at 540 µg/mL and an herbal dietary supplement adulterated with bumetanide are analyzed. In both cases bumetanide was identified with good peak shape and signal-to-noise (S/N). The analysis was performed in positive and negative mode simultaneously. The protonated and deprotonated molecules were isolated, fragmented in the ion trap and then a range of product ions were detected.

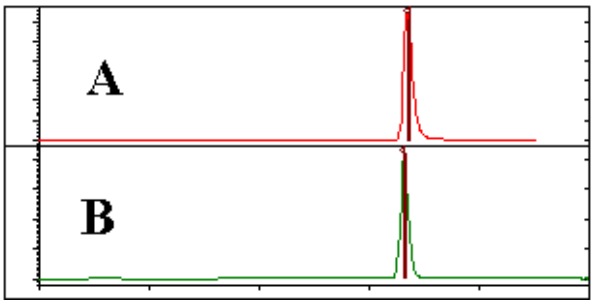

Figure 3. Bumetanide standard (A) and bumetanide in a dietary supplement extract (B) in positive mode ESI.

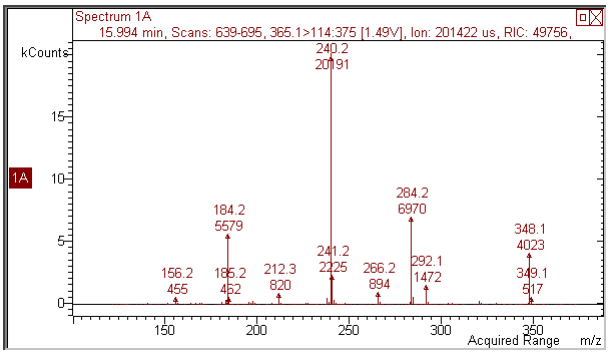

Figure 4. Product ion spectrum for bumetanide in positive mode ESI precursor *m/z* 365.

The ability to use *tandem ESI MS/MS* allows for a rapid analysis with excellent specificity for complete confidence in results. Since both ESI positive and ESI negative can be acquired simultaneously (see Figures 4 and 5), additional strong evidence is obtained to help confirm the identity.

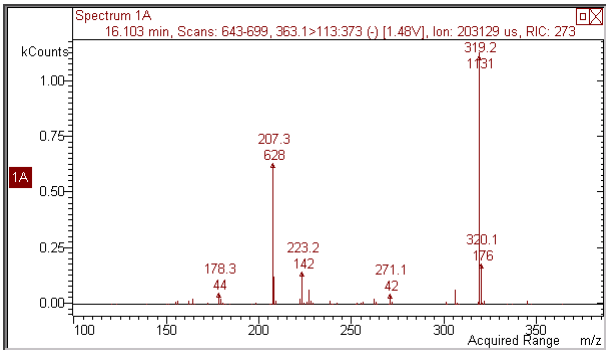

Figure 5. Product ion spectrum for bumetanide in negative mode ESI, precursor *m/z* 363.

The 500-MS also provides quantitative data for reporting the concentration of bumetanide. Figure 6 displays a calibration curve from 1 to 20 µg/mL.

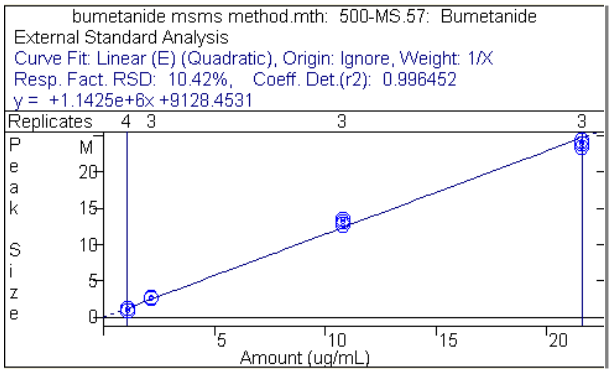

Figure 6. Calibration curve for bumetanide from 1 to 20 µg/mL.

Using the Varian 500-MS LC/MS/MS Ion Trap provides confirmation of compound identity in a single run. There is no need to set up a second experiment for confirmation. The secondary confirmation data is gathered simultaneously without loss of data quality or use of additional run time.

Many pharmaceutical adulterants are amenable to both positive and negative ionization by electrospray ionization mass spectroscopy. Furthermore, due to the significant advances in source design, many more compounds will ionize by ESI than are declared in the literature.

Conclusion

The Varian 500-MS LC Ion Trap Mass Spectrometer provides sensitive and accurate analysis of prohibited drugs and adulterants, such as bumetanide, in dietary supplements. This sample was analyzed in positive and negative mode simultaneously, providing detection and confirmation of bumetanide in a single run. The 500-MS is easy to use and provides quantitative results with minimal sample preparation. The MS/MS method has far more specificity than traditional HPLC methods using conventional detectors.

These data represent typical results.
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