Detection of Sulfur-Containing Metabolites of Asparagus in Urine by SBSE-GCxGC-TOFMS

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1. Introduction

Consumption of asparagus has been known to cause a distinct odor in the urine of humans. This odor can be present in urine in as little as 10 to 15 minutes after consumption. The compound that is unique to asparagus and is responsible for the odor is asparagusic acid. Subjects who consumed asparagusic acid, without consuming asparagus, produced the characteristic odor associated with asparagus consumption. The asparagusic acid is metabolized to S-methyl thioesters and ultimately to methanethiol, dimethyl sulfide, dimethyl disulfide, dimethyl sulfone, and dimethyl sulfoxide. Traditional extraction techniques have utilized heated solvent extraction over periods as long as 48 hours for sample prep. In this study, the goal was to eliminate the use of potentially hazardous solvents and reduce overall extraction time through the use of GERSTEL's Twister Stir Bar Sorption Extraction (SBSE).

2. Instruments and Methods

In this study, measurements were made with a LECO Pegasus® 4D GCxGC-TOFMS system. This system consists of an Agilent 6890 gas chromatograph equipped with a LECO dual-jet thermal modulator between the primary and secondary columns and a LECO Pegasus IV Time-of-Flight Mass Spectrometer (TOFMS) as a detector. For this study, the primary analytical column was a GERSTEL-MACH LTM 10.0 m x 0.18 mm ID x 0.20 μm df Rtx-5. The secondary column was a 1.00 m x 0.10 mm ID x 0.10 μm df DB-17ms and was housed in the GC oven. The modulator temperature offset for this study was +30°C. Helium was used as the carrier gas at a constant flow of 1.5 mL/minute. The transfer line to the TOFMS consisted of the last 20 cm of the analytical column and was kept at 280°C. For the one-dimensional study, the modulator was turned off and the GC oven was maintained isothermally at 280°C.

Urine samples of approximately 250 mL were collected prior to consumption of asparagus and approximately 90 to 120 minutes after consumption of asparagus. The samples were immediately placed in 250 mL amber glass bottles, each containing a 10 mm x 0.5 mm GERSTEL PDMS stir bar and extracted on a stir plate at 800 rpm. Upon completion of the extraction, the stir bar was removed, rinsed with DI water, and placed in a GERSTEL Twister Desorption Unit (TDU) tube for analysis.

The tube containing the stir bar was loaded into the TDU and the GERSTEL Cooled Inlet System (CIS 4) was cooled. The TDU was then heated, and the analytes were desorbed from the stir bar. The analytes were then trapped in the cooled CIS 4. The CIS 4 was then heated to desorb the analytes onto the GC column.

The TDU was operated in splitless mode. Its initial temperature was 30°C and was held at this temperature for an equilibration time of 120 seconds. It was then heated to 280°C at a rate of 700°C/minute and held at this temperature for 120 seconds. The CIS 4 was cooled to a temperature of -120°C. It was heated to 280°C at a rate of 12°C/second and held for 120 seconds.

3. Results

In this study, the urine samples were not derivatized. Analytes of interest in the post-asparagus samples are sulfur-containing compounds not present in the pre-asparagus urine samples. In Figure 1, the 1D traces of pre-asparagus and post-asparagus samples are overlaid to highlight their differences.

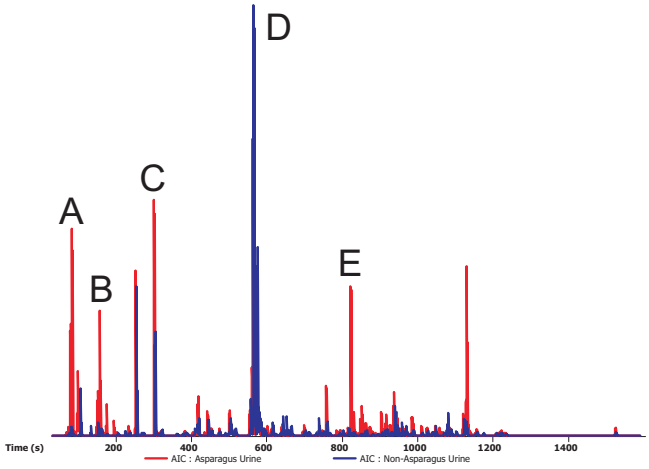

Figure 1: Overlay of pre- (blue) and post-asparagus (red) traces in 1D.

Some of the most prominent peaks, visible in red, include A) acetone, B) acetic anhydride, C) 4-heptanone, D) 5-methyl-2-(1-methylethyl)-cyclohexanone and E) 1-(1,5-dimethyl-4-hexenyl)-4-methyl benzene. Trace peaks not present in the pre-asparagus sample, but present in the post-asparagus sample include S-methyl propene thioate and 1,4-bis(methylthio)-butane. In the GCxGC-TOFMS analysis of pre-asparagus and post-asparagus samples, contour plots are shown in Figure 2 (back page).

The target compounds in this analysis are sulfur-containing metabolites of asparagusic acid. S-methyl thioesters are listed as being one of the primary metabolites. S-methyl 2-propenethioate had retention times of 205 seconds in the first dimension and 1.7 seconds in the second dimension. Its peak true and library spectra are shown in Figure 3 (back page).

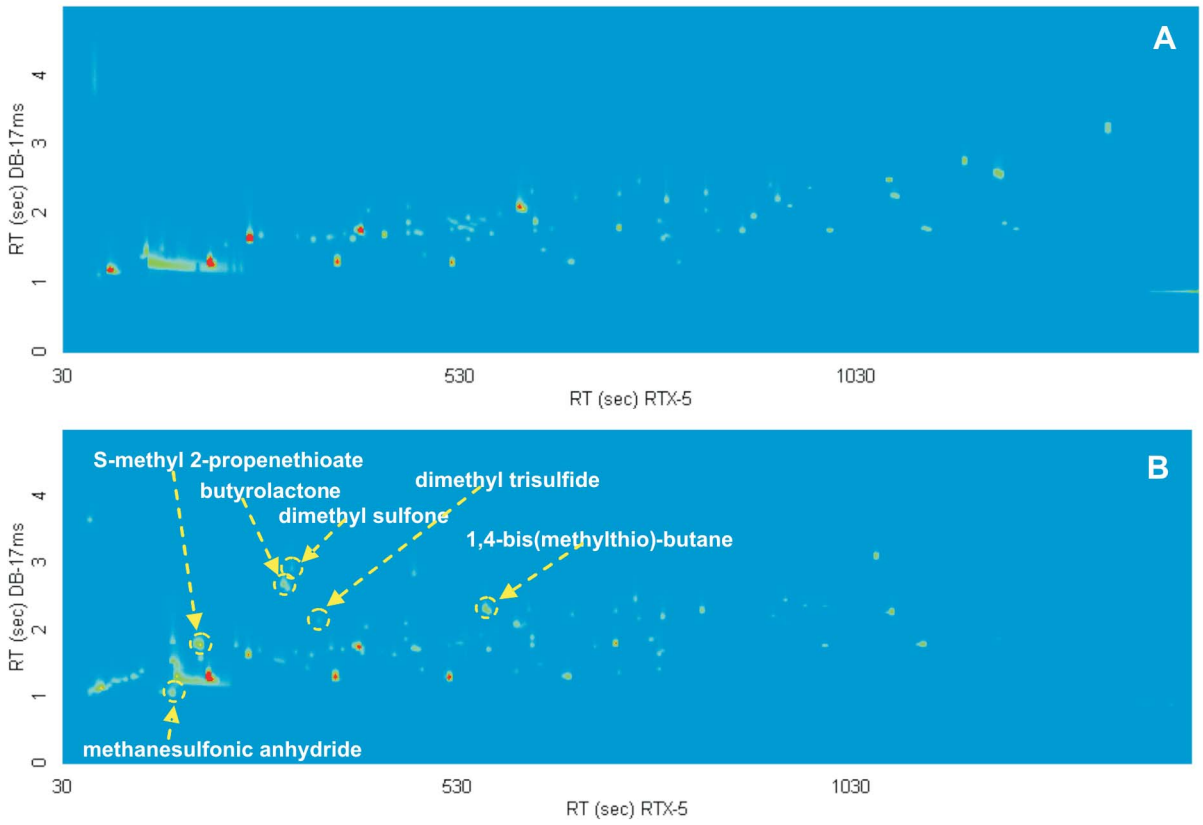

Figure 2: Contour plots of pre-asparagus urine (A) and post-asparagus urine (B). Relevant compounds not present in the pre-asparagus sample are highlighted in the post-asparagus sample.

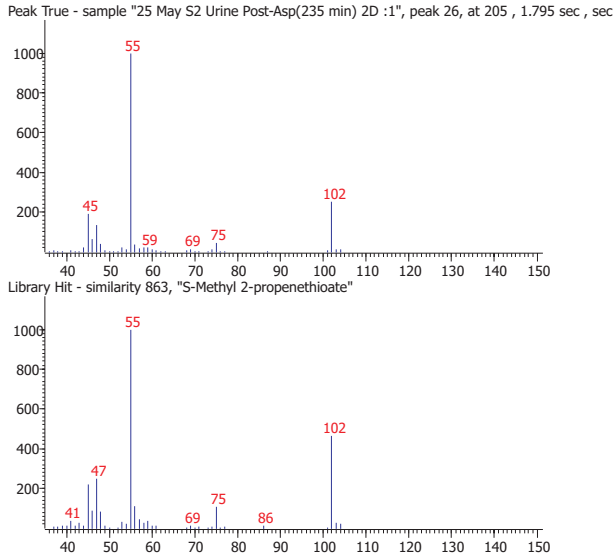

Figure 3: Peak True and Library Spectra for S-methyl 2-propenethioate.

4. Conclusions

This study has shown an improvement in not only overall analysis time, but in decreased complexity of sample preparation, as well as elimination of extraction solvents. The automated nature of the GERSTEL MPS 2–TDU–CIS4 system decreases the amount of labor necessary for each analysis by allowing for automation of the process. The use of GCxGC-TOFMS also allows for better resolution of analyte peaks and decreased coelutions. Any remaining coelutions can be resolved through True Signal Deconvolution, made possible by the high-speed, full-range spectra available from the LECO Pegasus TOFMS.

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