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應用手冊

The Application of GC-MS for the Metabonomic Analysis of Rat Urine

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Abstract

This application note demonstrates how GC-MS (ToF) using the Waters Micromass GCT Mass Detector has been employed with both EI and CI detection for the analysis of rat urine in a metabonomics study.

Introduction

Metabonomics is the study of changes in the endogenous metabolites in biological fluids such as urine and plasma as a result of a toxic insult, disease state, or genetic difference. In order to detect these changes, information-rich techniques such as mass spectrometry coupled with a liquid or gas chromatographic separation process are employed. The greater the sensitivity and resolving power of the system, the more analytes detected and hence a greater chance of finding a biomarker of disease or toxicity. Capillary GC coupled to time-of-flight mass spectrometry, while requiring sample pre-treatment via derivatization, is relatively simple to use and provides the highly sensitive analyses required for multivariate statistical processing, as with Principal Components Analysis (PCA). Here we show how GC-MS (ToF) using the Waters Micromass GCT Mass Detector has been employed with both EI and CI detection for the analysis of rat urine in a metabonomics study.

Experimental

GC Conditions

Column:	DB5MS 30 m x .25 mm ID x .25 µm film
Flow Rate:	1.5 mL/min.
Helium Injection Volume:	1 µL, split 10:1
Injector Temp:	275 °C
Temp Program:	50 °C for 4 min., ramp to 300 °C at 15 °C/min., hold for 5 min.
Solvent Delay:	6 min.

MS Conditions

Acquisition Range:	50 -700 Da EI, 100 -700 Da CI
Scan Time:	0.15 s
InterscanDelay:	0.05 s
Ionization:	EI^+, CI^+ using ammonia
Lock Mass:	tris-trifluoromethlytriazine(metri)
Source Temp:	180 °C EI, 175 °C CI
Transfer Line Temp:	250 °C

Animal Samples

Urine samples were collected from male and female obese (fa/fa) Zucker rats (BABU, Alderley Park; n = 10 of each sex; males age 13 weeks and females age 8 weeks) at two time periods, morning (AM) and evening (PM) to give a total of 40 samples. Samples were stored at -20 °C prior to analysis.

Sample Preparation

The urine samples were thawed and a 50 µL aliquot of each sample evaporated to dryness. A 250 µL aliquot of pyridine was added to each. This was immediately followed by the addition of 50 µL of BSTFA with 1% TCMS. Samples were then vortexed for 15 seconds and then heated to 80 °C for one hour. Each 300 µL derivatized sample aliquot was transferred to an auto sampler vial with a 400 µL low-volume insert. Each sample was analyzed in triplicate.

Results and Discussion

Its is projected that type II diabetes will be the biggest killer in the western world by the year 2020. Thus, the biomarker information that can be derived from the study of Zucker rats (a naturally diabetic genetic strain) may

hold important clues to controlling the disease.

Successful biomarker discovery is dependent upon high quality analytical data. The capillary gas chromatographic analysis of the rat urine samples produces a very information-rich complex EI chromatogram, generated by the GCT. The GCT is a time-of-flight (ToF) mass detector optimized for high resolution (7000 FWHM) and sensitivity for exact mass measurement. When operated in EI mode, the system is capable of generating library-searchable spectra with a high mass accuracy (<5 ppm error). In the event that an analyte is not contained in a library search, the elemental composition deduction capabilities of the GCT assist in the successful determination of unknowns.

A representative chromatogram from each of the Zucker and control animal groups is shown in Figure 1. While there are some subtle qualitative differences (e.g., the intensity of the peak at retention time 8.75 min.), the chromatograms are very similar. The CI GC-MS chromatogram from the same two samples is given in Figure 2. As we can see from the two data sets, the EI chromatogram is more information-rich than the CI analysis; however, the CI data provides molecular weight information which is not present in the EI data. The average peak width at the base for the EI GC-MS data was 0.05 min. with a peak capacity of approximately 400 for the twenty-minute separation.

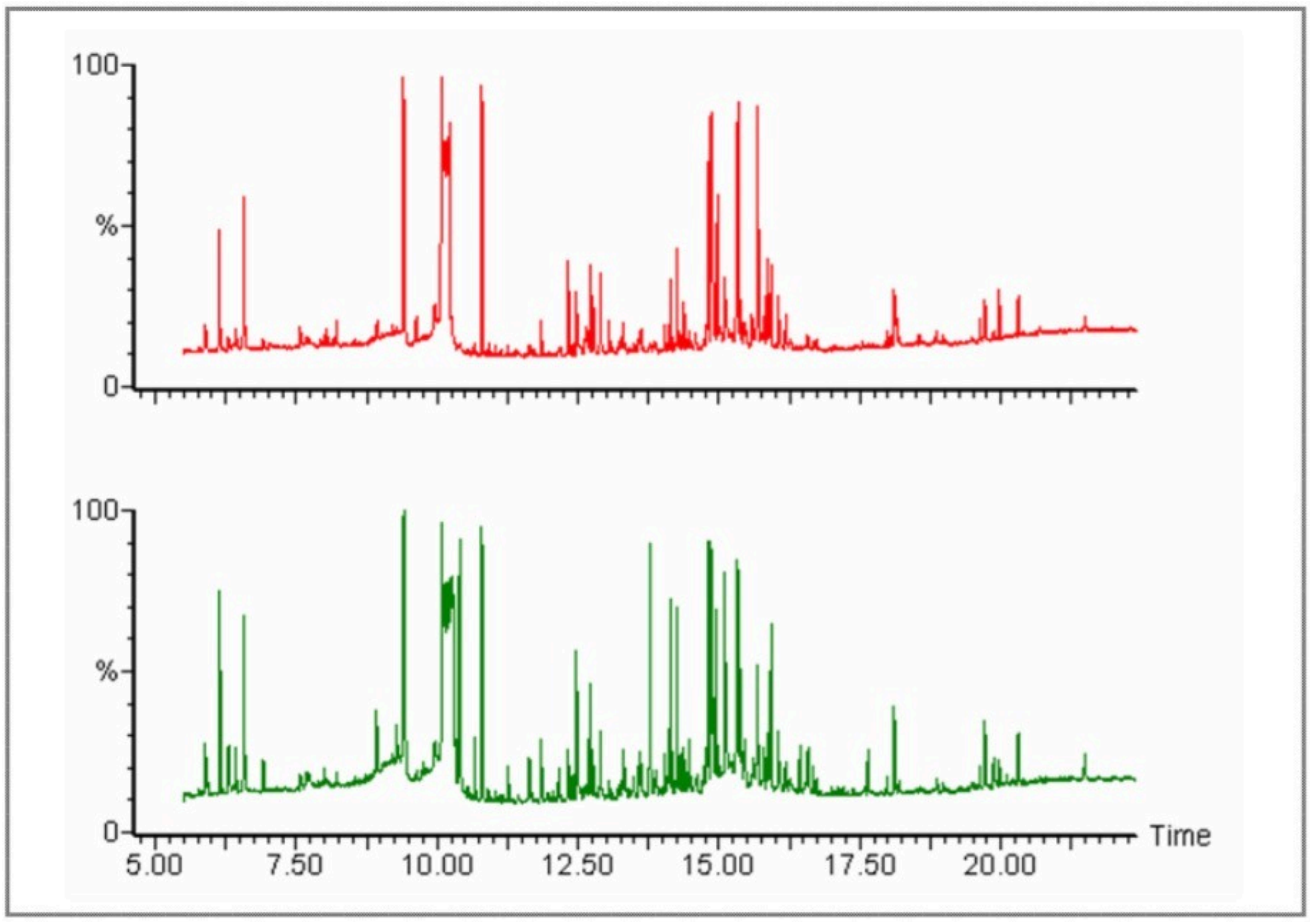

Figure 1. A representative chromatogram from each of the Zucker and control animal groups.

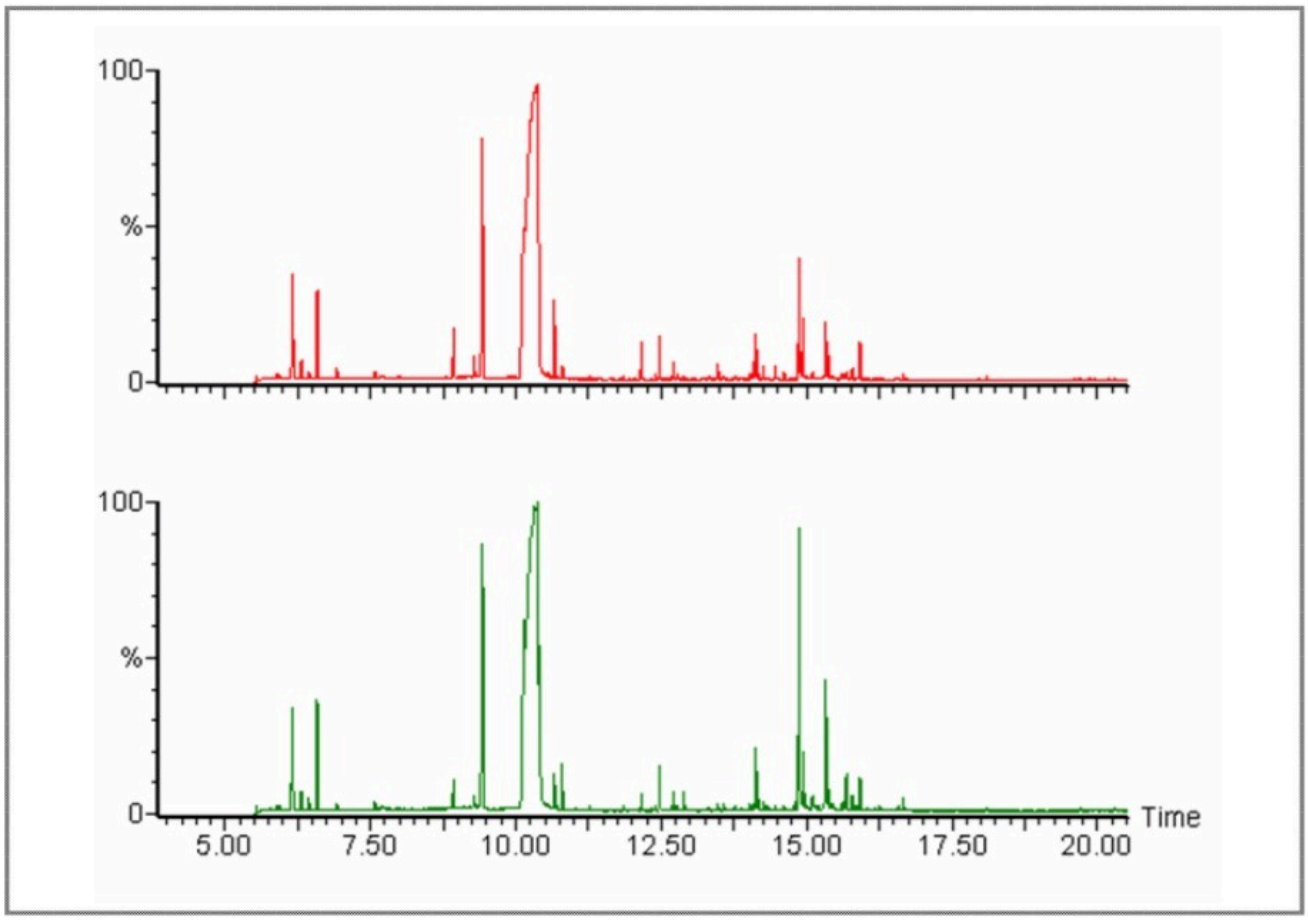

Figure 2. CI GC-MS chromatogram.

The EI GC-MS data was subjected to peak deconvolution and integration followed by PCA using the MarkerLynx Application Manager for MassLynx Software, Figure 3. A total of 1282 markers were detected in the EI GC-MS data set. The subsequent PCA scores plot of component 1 versus component 2 is displayed in Figure 4. We can see from this data that the four animal groups (control week 6, Zucker week 6, control week 20, and Zucker week 20) are easily separated into distinct regions. This result demonstrates that GC-MS can be used in metabonomics applications to determine the differences between biological and temporal groups. The resulting loadings plot from the multivariate analysis of the EI GC-MS data is given in Figure 5. In this example, each analyte is labeled with its retention time and *m/z* value. The analytes furthest away from the origin of the plot are those which contribute most strongly to the variance observed in the data set. One such ion is *m/z*= 457. This ion was subjected to elemental composition analysis using the exact mass value of 457.1943, giving a result of $C_{17}H_{37}N_4O_3Si_4$ with a mass error of 3.3 ppm. This data was then submitted to a NIST library search which resulted in a hit for TMS-derivatized uric acid (match score of 792, probability of 82%) shown in Figure 6. The agreement between the exact mass measurement and library search results is excellent and is supported by the CI data.

Figure 3. EI GC-MS data subjected to peak deconvolutionand integration followed by PCA using the MarkerLynx Application Manager for MassLynx Software.

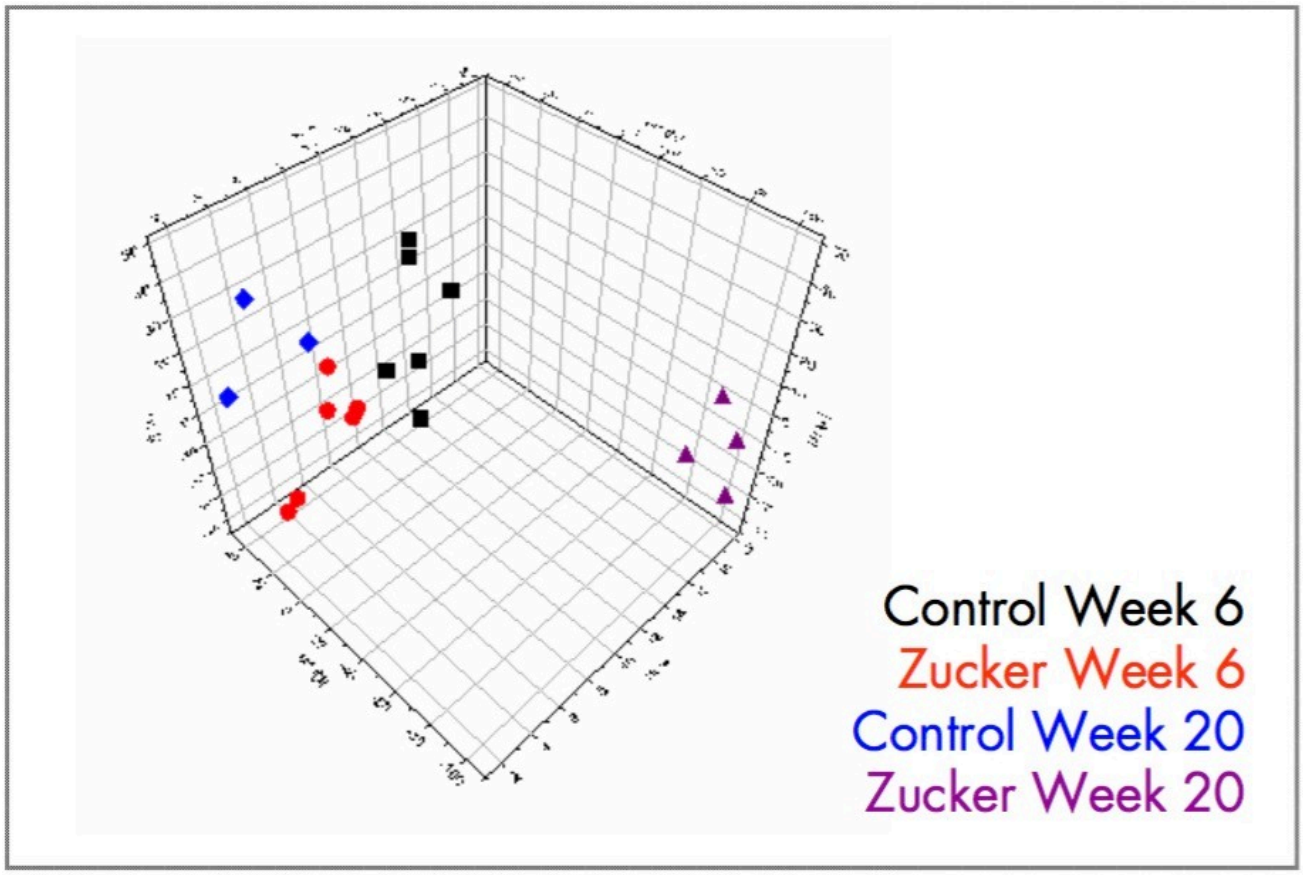

Figure 4. PCA scores plot of component 1 versus component 2

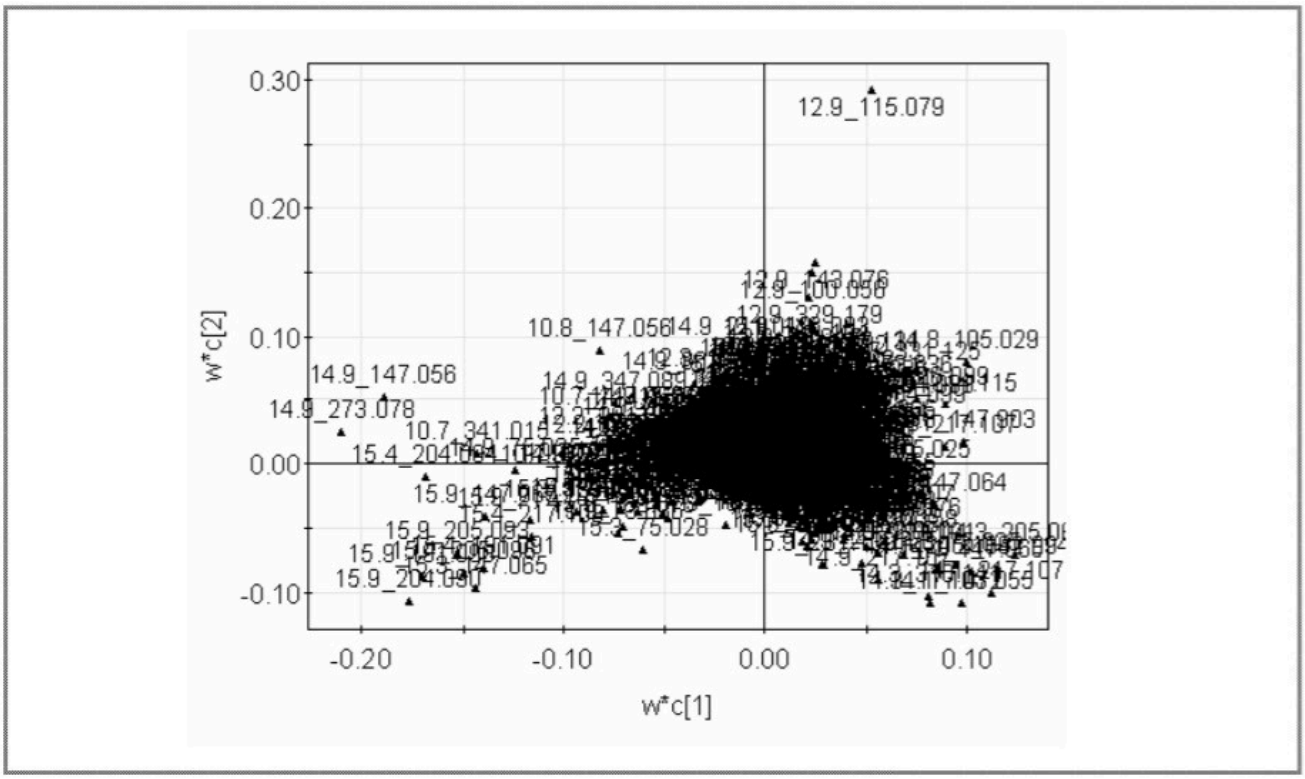

Figure 5. The resulting loadings plot from the multivariate analysis of the EI GC-MS data.

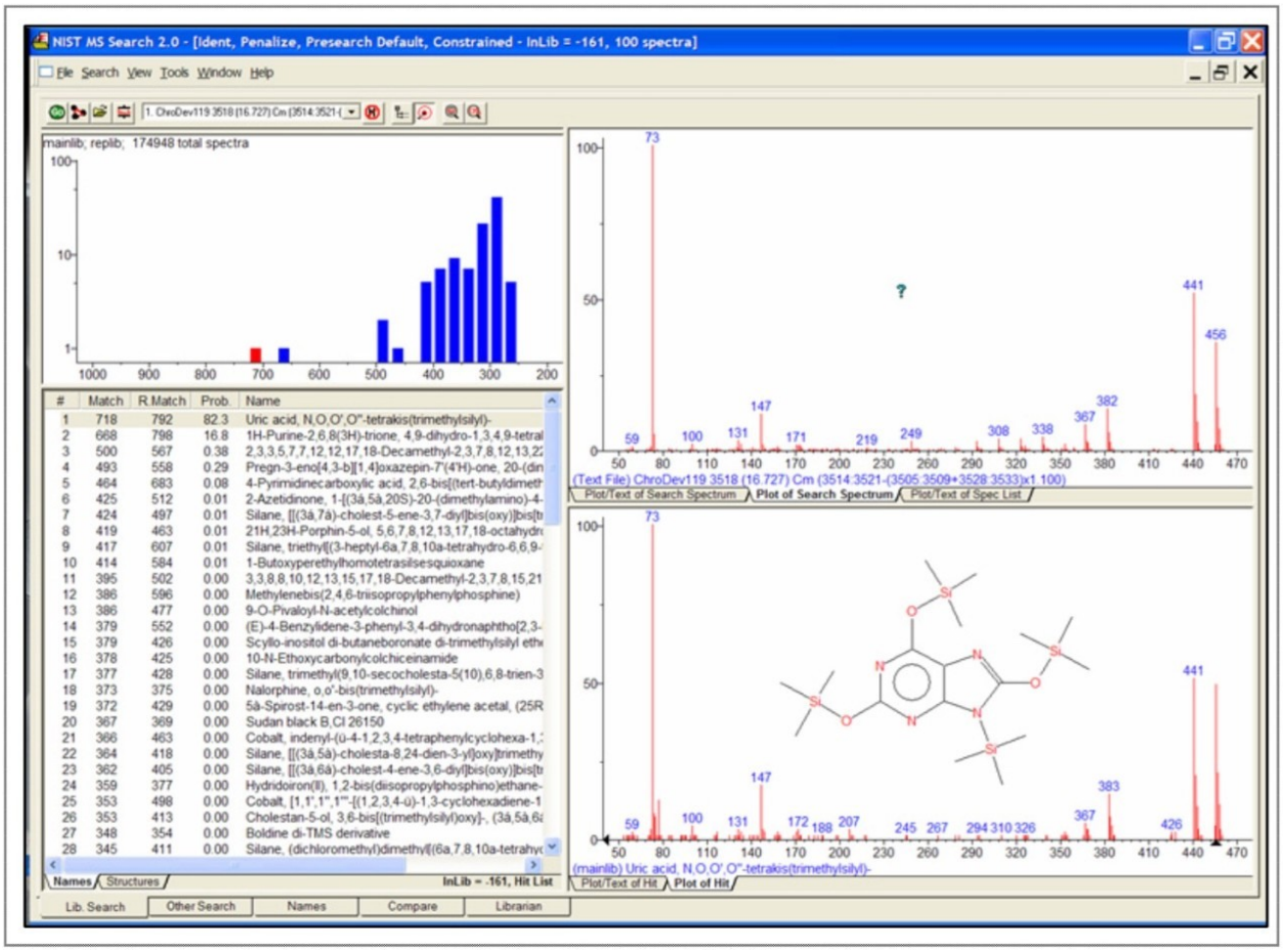

Figure 6. Data submitted to a NIST library search which resulted in a hit for TMS-derivatizeduric acid.

Conclusion

We have demonstrated the utility of GC-MS(ToF) for metabonomic studies of biological fluids following sample derivatization. After data deconvolution and integration, the resulting statistical data indicates differences as a result of disease or toxic insult. One of the significant advantages of GC-MS is that the ions of interest identified in the loadings plot can be subsequently searched using the NIST database for analyte identification.

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