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應用手冊

Metabonomics Analysis of Zucker Rat Urine Using UPLC-MS (Tof): A Time-Course Study

Ian D. Wilson, Rebecca Williams, Jennifer H. Granger, Robert S. Plumb

AstraZeneca, Waters Corporation

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Abstract

In this application note, we take advantage of the merits of UPLC-MS (Tof) to study rat urine samples in a disease state model for obesity.

Benefits

Enhanced sensitivity and resolution

Introduction

UPLC for Metabonomics

UltraPerformance LC (UPLC) coupled with time-of-flight (Tof) mass spectrometry has recently been applied to several areas of research where improved chromatographic resolution and mass spectral sensitivity are

advantageous. These features are particularly relevant to the field of metabonomics as the analytesof interests are found in complex biological matrices such as urine, plasma, and tissue extracts. In metabonomics applications, an increase in resolution and sensitivity not only facilitates the discovery ofmore biomarkers, but also the detection of low-level metabolites not previously observed with conventional LC-MS strategies. In this application, we take advantage of the merits of UPLC-MS (Tof) to study rat urine samples in a disease state model for obesity.

Experimental

LC Conditions

UPLC system:	Waters ACQUITY UPLC System
Column:	2.1 x 100 mm, 1.7 µm ACQUITY UPLC BEH C_{18} Column
Flow rate:	0.600 mL/min. (~0.400 mL/min. to MS)
Gradient:	0–20% B, 20–95% B in 10 min.
Mobile phase:	A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile
Injection volume:	5 µL
Sample temp.:	10 °C
Column temp.:	40 °C

MS Conditions

MS system:	Waters Micromass LCT Premier Mass Spectrometer
Mode:	ESI+
Acquisition range:	50–850 *m/z* with Woptics resolution
Cone voltage:	80 V
Capillary voltage:	2800 V
Scan time:	0.4 s
Interscan delay:	0.1 s
LockSpray:	25 fmol/µL Leucine enkephalin at 30 µL/min.

Sample Set

Urine samples were collected at two time periods (am and pm) from male and female (fa/fa) obese Zucker rats (+/+, +/-, and -/-) and control AlderleyPark (AP) rats (Wistar-derived) at ages 6, 8, 10, 12, 14, 16, 18, and 20 weeks. Prior to analysis by UPLC-MS (Tof), all samples were centrifuged at 13,000 rpm and an aliquot of the supernatant was diluted 1:4 with deionized water.

Data Processing

All UPLC-MS (Tof) data was subjected to chemometric analysis after data reduction using the Waters MarkerLynx Application Manager for MassLynx Software. Further statistical analysis was conducted using SIMCA–P+ Software (Umetrics) and an exported multivariate data array from MarkerLynx.

Results and Discussion

The Zucker rat presents an excellent disease state model for obesity and diabetes, both growing concerns in Western societies. In this study, urine samples from three Zucker phenotypes, (+/+), (+/-) and (-/-), collected over the course of 20 weeks were analyzed using UPLC-MS (Tof). The analogous data was also acquired for control rat urine, also collected as the animals aged, for comparative purposes. A representative total ion current chromatogram for a (+/+) Zucker rat and a control rat urine sample collected at 6 weeks is shown in Figure 1.

Although some differences can be visually noted between the two pieces of data shown in Figure 1, more subtle changes can be facilitated using a pattern recognition approach, such as principal components analysis (PCA).

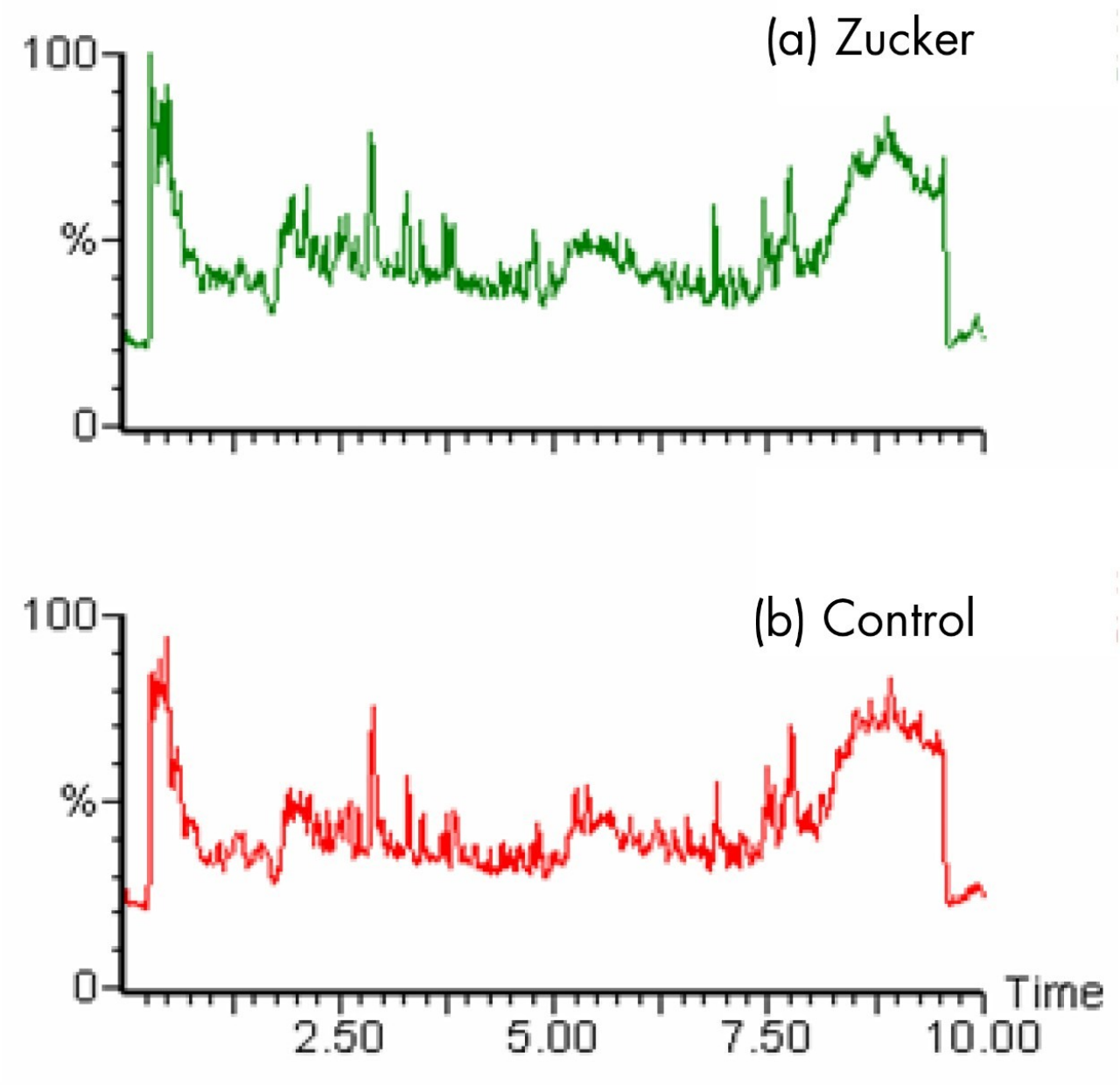

Figure 1. Total ion current chromatograms for UPLC-MS (Tof) analysis of (a) (+/+) Zucker rat urine and (b) control AlderleyPark (AP) rat urine collected from animals at 6 weeks.

To this end, Figure 2 presents the results from PCA of all week six samples. From the scores plot in Figure 2a, one notes a distinct clustering of all three Zucker phenotypes and the control AlderleyPark (AP) urine samples. Furthermore, clustering due to gender and diurnal variation is also observed. The corresponding loadings plot,

Figure 2b, indicates which ions and their chromatographic retention time are attributable to the clustering observed in the scores plot. In this case, the loadings plot contains 25,794 retention time and *m/z* pairs, or markers. This number is twelve times the amount observed from the equivalent analysis using conventional LC-MS (Tof) data for the same sample set.

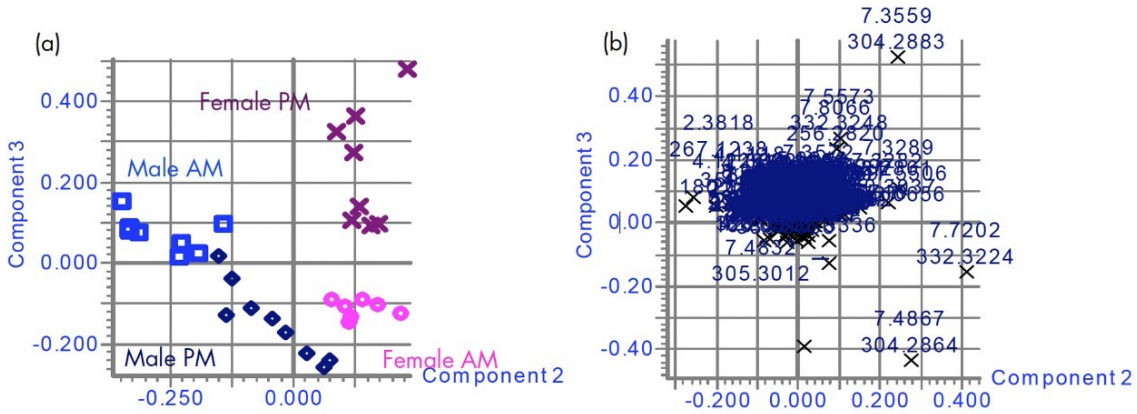

Figure 2. Chemometric analysis of the UPLC-MS (Tof) data for Zucker and AP rat urine using PCA results in the (a) scores and (b) loadings plots shown above.

Aging of Animals Under Study

Since the sample set was collected over a 20-week period, one can investigate metabolic effects as the animals age. In this example, two clusters are readily identified from the scores plot (Figure 3a); one for samples from weeks 6–12, and one for samples from weeks 14–20. One can therefore conclude that after week 12, both the Zucker rat and AP rat undergo a distinctive age-related metabolic change. The rationale for this change, perhaps, lies in the identity of the significant ions from the loadings plot (Figure 3b). One dominant ion (circled in red in the loadings plot) is *m/z* = 332 at a retention time of 7.17 minutes. The trend line for the *m/z* = 332 ion is shown in Figure 4.

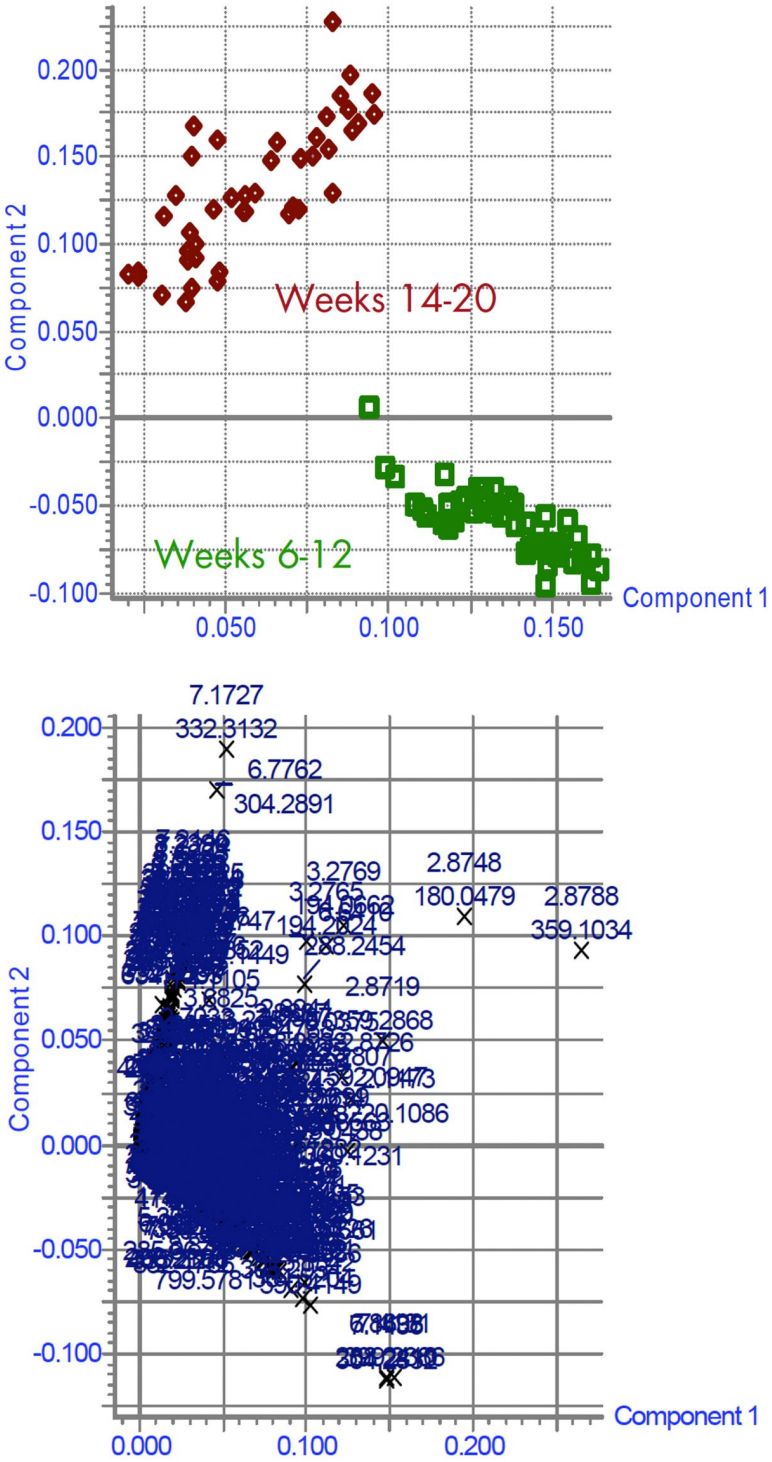

Figure 3. Resulting (a) scores and (b) loadings plots from PCA of UPLC-MS (Tof) data from (+/+) Zucker and

AP rat urine collected from weeks 6–20.

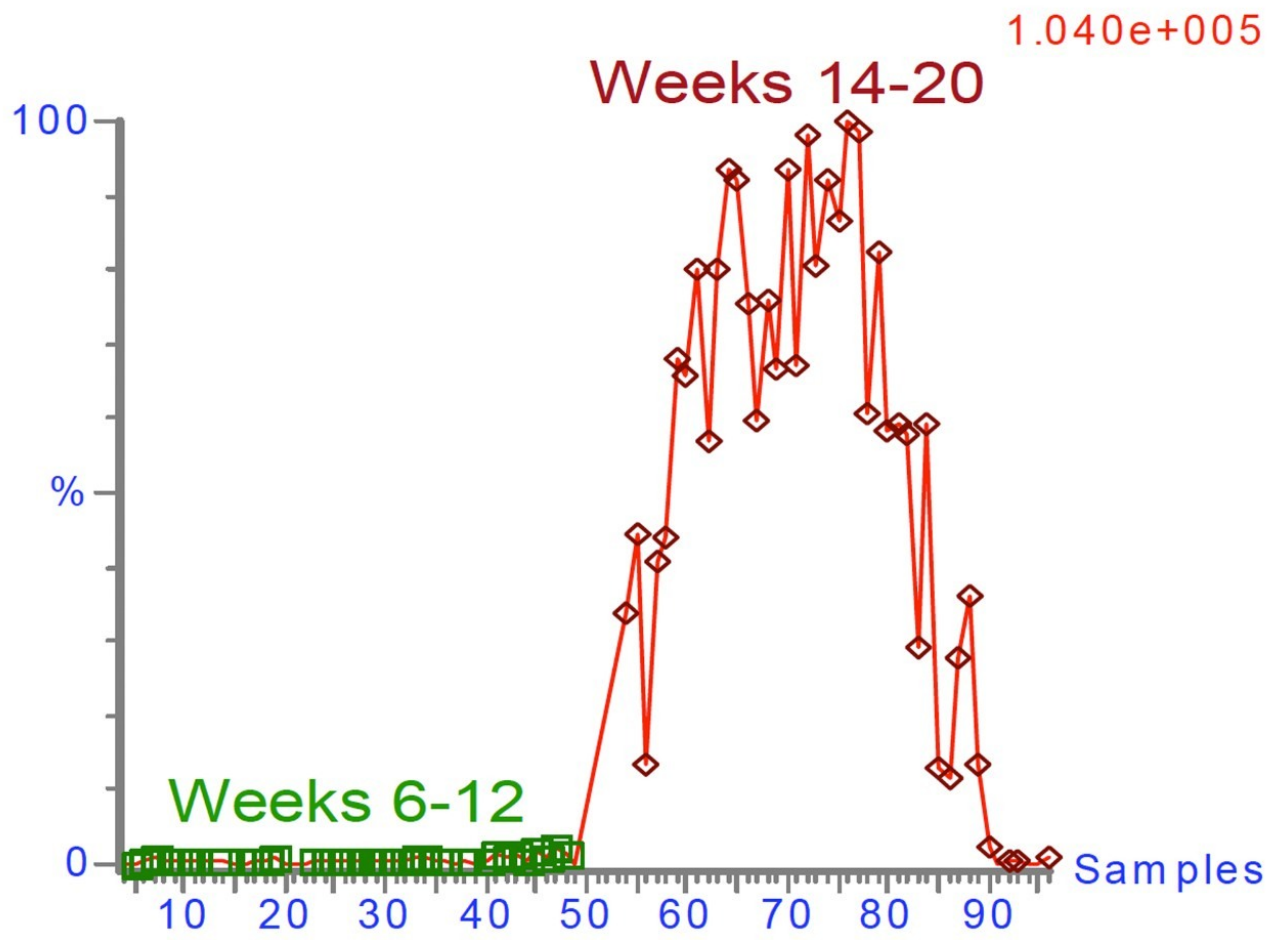

Figure 4. Trend line for the m/z= 332 ion for (+/+) Zucker and AP rat urine samples over 20 weeks.

Comparing HPLC and UPLC Data

A direct comparison was made between data acquired using HPLC-MS (Tof) and UPLC-MS (Tof) data for the week 6 sample set. The 3-D scores plots from partial least squares-discriminant analysis (PLS-DA) are presented in Figure 5. A closer clustering is observed for the UPLC data (Figure 5b), which may be attributed to the increased amount of markers detected. Additionally, clustering due to strain differences is evident.

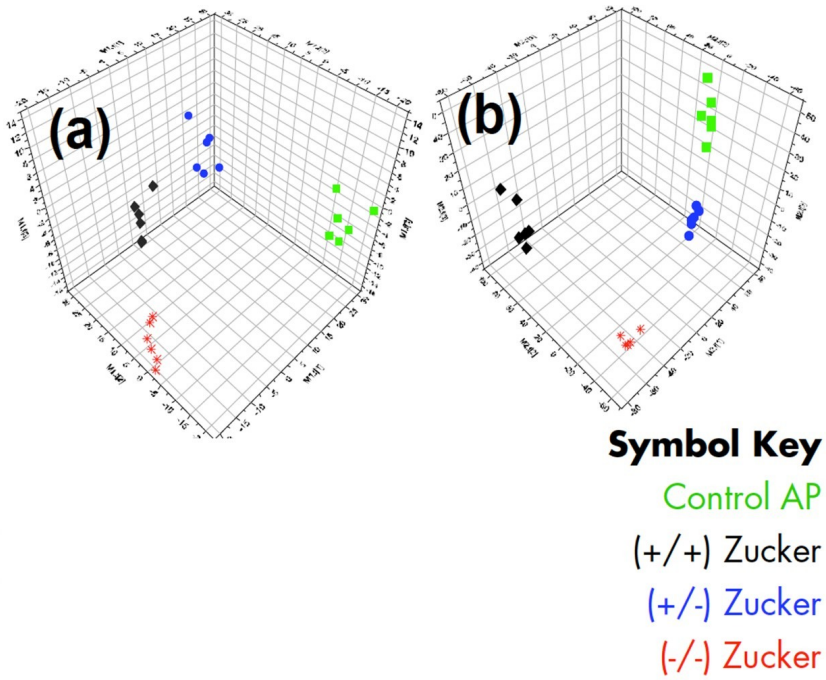

Figure 5. Scores plots from PLS-DA of (a) HPLC-MS (Tof) and (b) UPLC-MS (Tof) data from the analysis of week 6 Zucker and AP rat urine.

Conclusion

UltraPerformance Liquid Chromatography (UPLC) offers several advantages for the metabonomics analysis of biological samples, amongst which are enhanced sensitivity and resolution. As has been illustrated by this temporal exploration of Zucker rat urine, improved chromatographic resolution leads to the determination of more biomarkers. This is demonstrated by the comparison of UPLC-MS (Tof) to the HPLC-MS (Tof) analysis of the same sample set. In this instance, UPLC generates twelve times the number of detected markers found with HPLC. Furthermore, not only are the same markers contained within the HPLC-MS dataset also found by using UPLC, but new, previously unresolved components can also be detected.

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