A Comparison of UPLC™/MS(TOF) and ¹H-NMR Metabonomics Strategies For the Analysis of Urine From Zucker Rats

Introduction

Metabolomics is a rapidly expanding area of scientific research, which has the potential to improve our understanding of disease and to provide insights into the mechanisms of toxicity and efficacy. The approach relies on comparing the metabolomic profiles of treatment groups. Following exposure to an external stimuli, e.g. a candidate pharmaceutical or environmental stress using chemometric data analysis strategies. Such recent interest has been focused on LC/MS analysis methods as an alternative to ¹H-NMR. An additional incentive for LC/MS based metabolomics has been found in Ultra Performance Liquid Chromatography (UPLC) where small particle sizes (< 2μm) and improved chromatographic resolution, increased sensitivity and reduced analysis times. This has allowed the use of small particle sizes (< 2μm) and pumping-injection systems capable of operating under such excising pressure regimes (up to 15,000 psi). When coupled with time of flight mass spectrometry, UPLC presents a means to achieve high sample throughput with reduced LC/MS detection. Increased sensitivity and mass spectrometry detection capabilities. This approach of UPLC(TOF) is particularly attractive for metabolomics applications where the rapid and accurate detection of metabolites is required. Metabolomics analysis of urine from male Zucker rats using UPLC-MS(TOF) data generated from the collection of urine samples from prediabetic male and female Zucker rats. The data will be divided into control and gender differences amongst prediabetic Zucker rats. The same group clustering is observed for both the UPLC/MS and ¹H-NMR data illustrating the complementary nature of the two analytical approaches.

Methods

Urine samples were collected from male and female Zucker rats (n=10 each) at two time periods, morning and evening for a total of 40 samples per male and female rat. All samples were then centrifuged at 15,000 g for 5 minutes at 10°C. The supernatant was transferred to a 1.5ml Eppendorf vial and cooled to 4°C. All samples were collected from male and female Zucker rats. The separation shown in Figure 7a for PCA of the ¹H-NMR data is based on a higher level of diabetics, hypertensive and boric acid in the Zucker rat and control urine samples. The same group clustering is observed for both the UPLC/MS and ¹H-NMR data illustrating the complementary nature of the two analytical approaches.

Results & Discussion

The collected UPLC/MS data was analyzed using MarkerLynx™ Application Manager (Umetrics) from an exported MarkerLynx markers table. The processed data file was then analyzed by Principal Components Analysis (PCA), within the MarkerLynx program. An example of the MarkerLynx browser for male and female Zucker rat urine samples is presented in Figure 1.

When considering diurnal variation, PCA of the UPLC/MS data alone is not sufficient to view clustering in the scores plot as illustrated in Figure 3a. However, using a supervised method, such as PLS-DA, results in more discernible clustering (Figure 3c).

In addition to samples from Zucker rats, control urine samples were also analyzed using the same approach and can be incorporated into the chemometric analysis. Visual inspection of the UPLC/MS data shows observable differences between control and Zucker urine samples.

Figure 3. Scores plots from PLS-DA of ¹H-NMR data comparing male Zucker rats during the AM and PM regimes (a) and control rats during the AM and PM regimes (b) (Umetrics) from an exported MarkerLynx markers table. The processed data file was then analyzed by Principal Components Analysis (PCA), within the MarkerLynx program. An example of the MarkerLynx browser for male and female Zucker rat urine samples is presented in Figure 1.

Visual inspection of representative ¹H-NMR spectra of the same samples also results in observable differences between control and Zucker urine samples.

Figure 4. Representative total ion chromatograms from UPLC/MS analysis of urine from Zucker rats.

Figure 5. Comparison of UPLC™/MS(TOF) and ¹H-NMR Metabonomics Strategies for the Analysis of Urine From Zucker Rats.