Metabolomics in diabetic mouse model using highly sensitive ZenoTOF 7600 system

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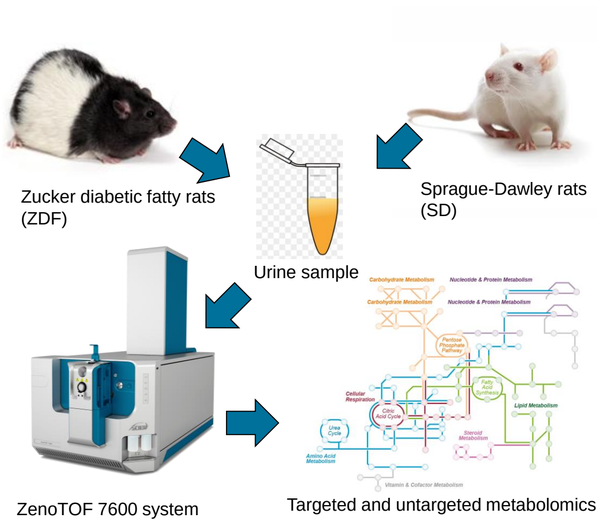
INTRODUCTION



MATERIALS AND METHODS

Sample preparation: Urine samples were collected from four distinct rat groups: Zucker diabetic fatty (ZDF) rats, male and female; Sprague Dawley (SD) rats, male and female. Urine was collected from N=5 rats per group. 20µL of urine was aliquoted and diluted 10-fold with mobile phase A prior to LC-MS/MS analysis.

Chromatography: An ExionLC AD HPLC system (SCIEX) with a Phenomenex Luna Omega Polar C18, 3 µm 150 x 2.1 mm (00F-4760-AN) was used for sample separation. A simple linear gradient from 0 to 95% B was used with standard reverse phase mobile phases (A = 0.1% formic acid in water and B = 0.1% formic acid in acetonitrile) with a flow rate of 300 µL/min. Either a 0.2 or 2 µL injection volume was used and the column temperature was maintained at 40 ˚ C throughout the analysis. The total run time was 13.1 min including 2 min of equilibration.

Mass spectrometry: MRM[HR] data was acquired on the SCIEX ZenoTOF 7600 system in positive ESI mode using SCIEX OS software. The ion source conditions were as follows: CUR 35, GS1 55, GS2 55, ISVF 5500, TEM 600 ºC. High resolution MS/MS was collected for each metabolite using an accumulation time of 10 msec. A collision energy (CE) of 30 was used for each MS/MS. Methods were built with the Zeno trap both activated and deactivated to enable the sensitivity comparisons. Three replicates were collected on each sample with each method.

Data processing: MS/MS interpretation, peak integration, and quantitative analysis were conducted in SCIEX OS software, then results were imported into MarkerView software for multivariate statistical analysis (Figure 1). To build a processing method for MRM[HR] data, the MS/MS spectrum was first examined in the Explorer module to select the best fragment ion. This was also compared to the library spectrum from LibraryView software using the SCIEX Accurate Mass Metabolite Library (AMMSL 2.0). Structural information from ChemSpider was also used to confirm the identity of the fragment and obtain the theoretical m/z of fragment of interest to be used. This fragment accurate mass information obtained in Explorer mode was then used to build a final processing method in the Analytics module of SCIEX OS software. Peak areas of the fragment ions were then imported into MarkerView software for statistical analysis.

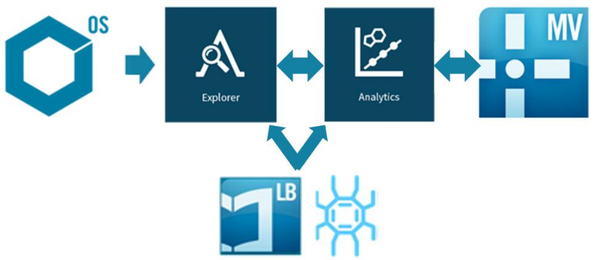


Figure 1. Workflow diagram. Data was both acquired and processed using SCIEX OS software. MS/MS was interpreted using both Explorer and Analytics, library searching was performed using the Library View and ChemSpider.

RESULTS

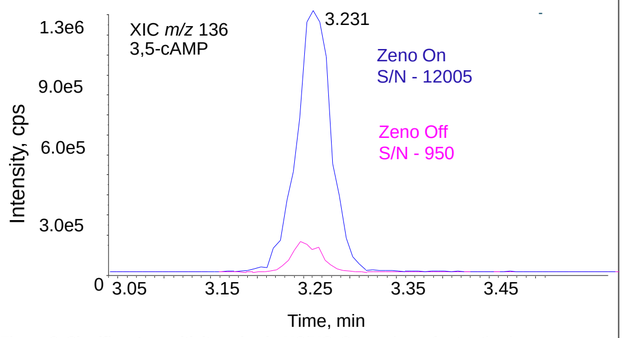


Figure 2. Significant sensitivity gains in MS/MS. Comparison of extraction ion chromatograms (XICs) for cAMP fragments obtained from MS/MS collect with Zeno trap on (blue) vs. Zeno trap off (pink). Signal/noise ratio improved ~12.5 fold when using the Zeno trap. This XIC is from a 2 µL injection of diluted urine.

Table 1. Increased quality of MS/MS spectra due to Zeno trap. Activation of the Zeno trap provided significant MS/MS signal increase and therefore large increases in fragment ion XICs (average of 13.6-fold). High mass accuracy and very good library hits were observed for the resulting Zeno MS/MS spectra.

Metabolite	Fragment ion (m/z)	Library match	MS/MS fragment mass error (ppm)	Area gain with Zeno trap on (on/off)
Acetylglutamate	*84.0444*	*√*	*-0.14*	*12.51*
Arginine	*70.0651*	*√*	*3.73*	*13.18*
Carnitine	*103.0401*	*√*	*-4.52*	*11.12*
Creatine	*43.0291*	*√*	*3.87*	*18.11*
Cyclic AMP	*136.0618*	*√*	*2.56*	*10.00*
Glutamine	*84.0444*	*√*	*1.72*	*18.08*
Histidine	*110.0713*	*√*	*0.24*	*26.72**
Leucine	*69.0699*	*√*	*0.56*	*15.83*
Methyladenosine	*150.0778*	*√*	*1.28*	*10.28*
Phenylalanine	*120.0808*	*√*	*2.37*	*10.38*
Tryptophan	*118.0651*	*√*	*0.26*	*11.93*
Tyrosine	*119.0495*	*√*	*-3.81*	*8.89*
Uric acid	*141.0407*	*√*	*4.24*	*10.29*
			Average area gain:	***13.64***

**Zeno trap off peak area very low, hard to measure*

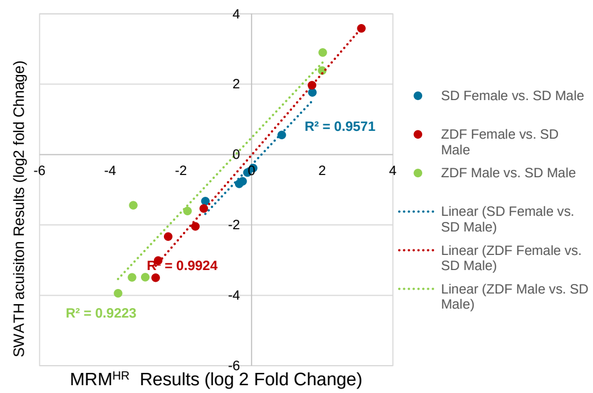
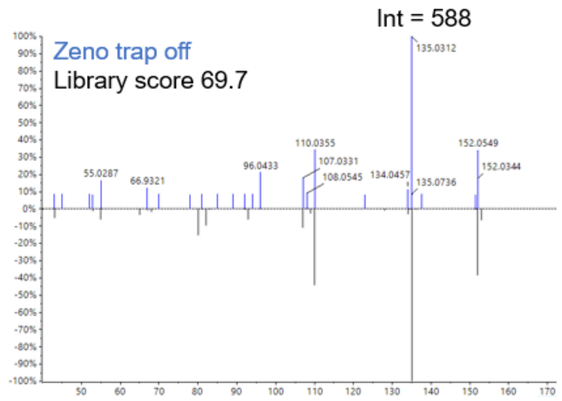


Figure 3. Correlation of quantitative results from SWATH acquisition and MRM[HR] workflow. The log2 fold change results were computed for eight compounds across the three groups of comparisons: SD female/SD male, ZDF female/SD male and ZDF male/SD male.



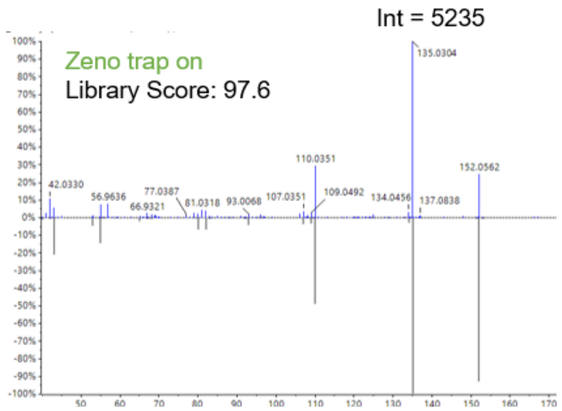


Figure 4. Improved spectral quality yields higher library scores. Improved spectral quality in turn provides improved library matching, as highlighted here for guanine. (Top) library score 69.7 for guanine for MS/MS collected with Zeno trap off (Bottom) library score of 97.6 for to Zeno trap on. Peak intensity of the m/z = 135.03 ion is shown on the top of each spectrum highlighting the much higher sensitivity with Zeno trap on.

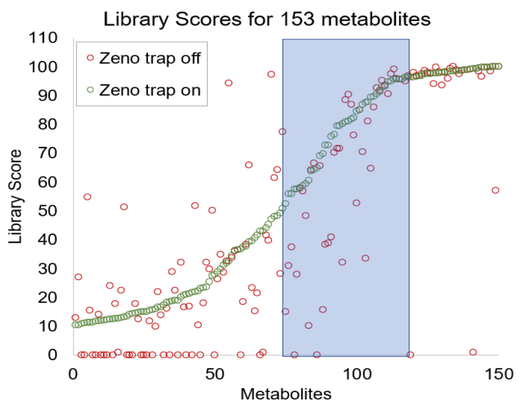


Figure 5. A total of 153 metabolites were identified with Zeno trap turned on and off. These metabolites were separated into three groups. Group one had poor library scores, <50 in Zeno trap on data set. Group two has library scores between 50 and 90 with Zeno trap on compared to Zeno trap off, and with greater confidence in library match. Group three had the highest library scores, >90.

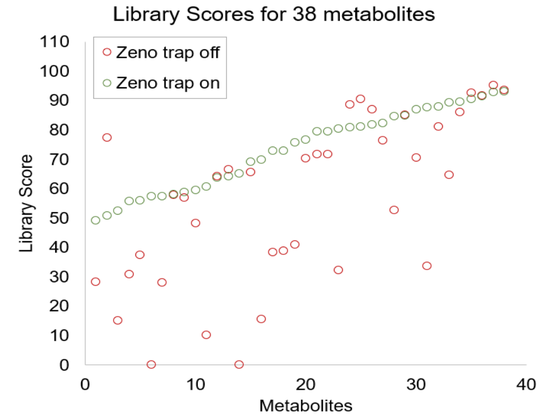


Figure 6. Large sensitivity gains observed in Zeno MS/MS resulted in higher library scores. Urine metabolites with library scores between 50 and 90 in the Zeno trap on data (green) were aligned with the Zeno trap off data (red).

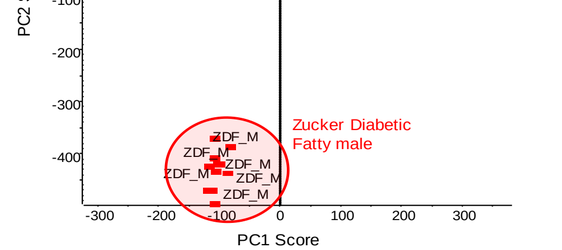


Figure 7. Principal component analysis clearly separates groups. The interest list of 4222 significant features (p-value ≤ 0.01, ± Log fold change ≥ 0.5) was used for principal component analysis.

CONCLUSIONS

- Zeno MS/MS provided a 13-fold average increase in MS/MS sensitivity, thus providing both high-quality, full-scan MS/MS data for each metabolite for confident compound identification.
- Non-targeted SWATH acquisition studies to targeted MRM[HR] workflow on a single MS instrument was demonstrated.
- Zeno MS/MS has a larger impact on library scoring when the analyte abundance is lower and yields weaker MS/MS spectra

REFERENCES

1. Rapid analysis and interpretation of metabolomics SWATH acquisition data using a cloud-based processing pipeline. SCIEX technical note RUO-MKT-02-13056-A.

TRADEMARKS/LICENSING