

## Metabolomics Differentiates Classified Cancer Samples

*Microflow Chromatography coupled with Targeted Metabolomics on QTRAP® 6500+ LC-MS/MS System*

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Identification of metabolites from urine and plasma is necessary for validating potential disease biomarkers for research and interrogating the affected metabolic pathways to further understand their biological implications. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis is an essential tool for identification and quantitation of metabolites in complex sample matrices due to its inherent sensitivity gains. Using the previously described single injection microflow hydrophilic interaction liquid chromatography (HILIC) and multiple reaction monitoring (MRM) workflow, 263 unique metabolites out of 312 targets (296 out of 363 Q1/Q3 transitions monitored) were identified from plasma/urine and MDCK (Madin-Darby Canine Kidney) cell line extracts, covering all major metabolic pathways. This microflow HILIC-MRM method on average provided 10 times more sensitivity compared to similar analytical flow methods<sup>1</sup>.

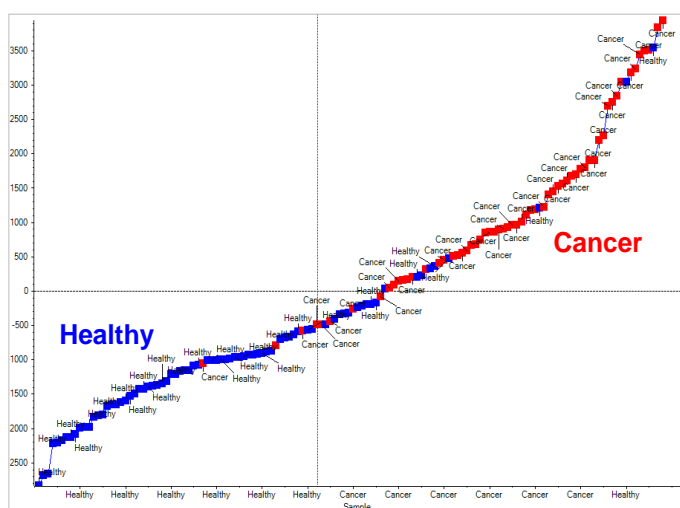
As a case study, plasma extracted metabolites from a group of pre-classified colon cancer patients and healthy individuals were analyzed using this method and 312 polar metabolites covering



all major metabolic pathways were monitored. Using this robust targeted metabolomics method and improved sensitivity provided by microflow LC, pre-classified colon cancer patients from healthy individuals were successfully differentiated indicating the power of the method.

### Key Benefits of Targeted HILIC Metabolomics Assay

- A single microflow LC-MS/MS targeted method allowing detection of 312 polar metabolites across multiple biochemical pathways
- Microflow Luna-NH2 HILIC chromatography provides excellent chromatographic separation of polar, hydrophilic metabolites.
- Improved sensitivity with S/N improvement of up to 60X with microflow LC
- Microflow LC reduces solvent consumption and costs
- The sensitivity and speed of the QTRAP® 6500+ with IonDrive™ Technology allows an efficient high throughput assay by using +/- polarity switching (5 msec) in a single sample injection
- Method enabled the identification of 14 differentiating metabolites between cancer and healthy cases



**Figure 1: Multivariate Analysis of a Pre-Classified Colon Cancer Sample Set.** Principal Component Analysis (PCA) of two groups of pre-classified samples, namely healthy and diseased (Cancer). PCA scores plot shows the differentiation between the two groups across PC1 which highlights the largest variation of the dataset. In this case, this happens to be the biological effect we were expecting.

## Material and Methods

**Sample Preparation:** 100  $\mu$ L of a plasma metabolite extract from 40 subjects (20 healthy and 20 pre-classified with colon cancer) was transferred to a 1.5 mL microtube. The sample was dried using a TurboVap evaporator to a pellet using no heat. The pellet was dissolved in 100  $\mu$ L of HILIC sample resuspension buffer, mixed well by vortexing and centrifuged at 14,000 RPM for 10 min. Ninety microliters of supernatant was transferred to deactivated QsertVials (Waters) for LC-MS/MS analysis. Injection volume was 2  $\mu$ L with 5 replicate injections.

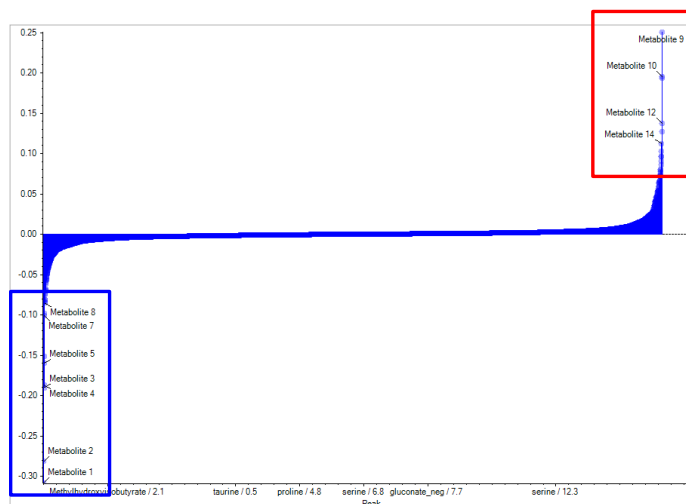
**Microflow Liquid Chromatography:** A SCIEX M3 MicroLC system, with an integrated autosampler, was used in direct injection mode, in combination with a source mounted column oven (SCIEX). A Luna 3  $\mu$ m NH<sub>2</sub> 100 Å, 150 x 0.3 mm analytical column (Phenomenex) was used with a micro filter 1  $\mu$ m SS (Upchurch Scientific) before the column to prolong column life time using the method previously described<sup>1</sup>.

**Mass Spectrometry:** A SCIEX QTRAP® 6500+ with IonDrive™ Turbo V source plumbed with the 25  $\mu$ m ID hybrid electrode was used. A total of 187 positive ion mode MRM's and 176 negative ion mode MRM's for a total 312 unique polar metabolites were combined into a single +/- switching experiment (363 total MRM transitions) with 3 msec dwell time and 50 ms settling time for polarity switching as previously described<sup>1</sup>, to monitor these endogenous metabolites across different metabolic pathways.

**Data Processing:** MultiQuant™ 3.0.2 Software (SCIEX) was used for MRM data analysis with MQ4 peak integration algorithm. Results were then processed using MarkerView™ Software for Principal Component Analysis (PCA).

## Results

As a case study, this microflow LC-MRM method was used to analyze the extracted metabolites from plasma from samples that have been pre-classified as either colon cancer and healthy. 312 polar metabolites across multiple biochemical pathways were profiled and the added sensitivity provided by the method enabled the clear and expected differentiation of the samples. Using PCA, the samples were clearly separated between healthy and diseased (cancer) across PC1 (Figure 1 Scores plot) .



**Figure 2: Metabolites Responsible for the Differentiation of the Pre-Classified Colon Cancer Sample Set.** The Loadings plot highlights the metabolites responsible for the differentiation of the sample grouping on the left. It can clearly be seen that separation is due to a handful (14) metabolites. Note: Metabolites have been blinded from this study to protect any IP for UW and as requested by the Principal Scientist (D. Rafferty).

The Loadings plot (Figure 2) highlights the metabolites responsible for the differentiation of the samples. It can clearly be seen that separation is due to a handful (14) metabolites.

Using this robust targeted metabolomics method and improved sensitivity provided by microflow LC on QTRAP 6500+ system, colon cancer samples could be clearly differentiated from healthy samples, highlighting the power of this complete solution.

## References

1. Microflow Chromatography Provides Improved Sensitivity and Coverage of Polar Metabolites for Targeted Metabolomics (2017). Khatereh Motamedchaboki and Baljit Ubhi. <https://sciex.com/x100586>.

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