Therapeutic peptides are highly potent, selective, relatively safe and well tolerated by humans which differentiate them from typical small molecule drugs. Based on recent data, more than 60 peptide drugs have reached the market for treatment of a wide range of diseases including bone and prostate cancer, type 2 diabetes, osteoporosis, heart failure and several others with increasing market potential evidenced by ~140 peptide development. The purpose of this study was to develop an integrated microflow LC-MS/MS solution coupled to the SCIEX Advanced Biotransform Solution to take advantage of the gains in sensitivity from microflow LC and to identify peptide catabolites at low levels. The increased sensitivity is critical when the sample volume is limited as in small animal studies.

Microflow Liquid Chromatography: A SCIEX MS MonoLC system, with an integrated autosampler, was used in direct injection mode in combination with a source mounted column (SCIEX) A 50 x 0.3 mm HILIC Peptide SCX C18 3.7 µm 100 A column was used (SCIEX). Mobile Phase A, water with 0.1% formic acid, and mobile phase B, acetonitrile with 0.1% formic acid was used at a flow rate of 10 µl/min. The column temperature was set to 35°C. Injection volume was 5 µl, and the autosampler needle and valve connected 1 cycle using mobile phase B, followed by two cycles using mobile phase A.

MS/MS Conditions: For the microflow LC experiments, the standard electrode (100 µl) was placed in a 25 µl iK electronics Detector with a fixed window of 50 Da. SWATH® and SWATH® acquisition (m/z 300-1600, SWATH with fixed window of 50 Da, optimized source parameter were used for each flow LC column).

Data Analysis (MetabolitePilot®): 2.0 software was used to compare the sensitivity of the microflow and analytical LC-MS/MS data for the peptides top three catabolites.

Results
Most LC-MS/MS methods for bioanalytical studies use analytical flow chromatography of 350-500 µl min to profile peptide catabolites and often lack sensitivity to identify low level catabolites. The microflow LC method at 10 µl/min showed on average 15X improvement in signal-to-noise (S/N) ratio when compared to analytical flow LC for the top three major peptide catabolites detected after 60 min of incubation in rat plasma. This improvement was required to monitor peptide catabolism in small animal studies, as shown in Figure 4-6.

Improved Signal to Noise Ratio (S/N)

Table 2. The therapeutic peptide and their top three catabolites products identified. The XIC of these catabolite were monitored to check sensitivity gain by microflow LC-MS/MS.

Microflow LC Improved Sensitivity. The Peakiew® extracted ion chromatogram (XIC) shows improved sensitivity of microflow LC-MS/MS for the top three major peptide catabolites. Table 2 shows the S/N ratio for each peptide catabolite detected in the analytical flow LC-MS/MS data.

Conclusions
- A fast, robust, and reliable method, for the monitoring peptide catabolism in plasma.
- Signal-to-noise ratio improvement of up to 46X enables detection of catabolites at low abundance levels.
- Microflow LC method coupled to the high resolution TripleTOF® 6600 system provides optimal sensitivity and confidence to identify the low-level metabolites in complex samples when sample volume is limiting factor.

References
2. Ian Moore and Jinal Patel. Rapid Peptide Catabolite ID using the SCIEX Routine Bioanalytical Solution. SCIEX Technical Note, Document number: RUO-MKT-02-6000

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