

## Overview

To compare the sensitivity of traditional HPLC/MS/MS instrumentation to micro flow LC/MS/MS (MFLC/MS/MS) for the detection of large biological molecules.

## Introduction

Trypsin digestions of three proteins, myoglobin from horse skeletal muscle, human somatotropin, and human ceruloplasmin, in buffer solution (100 µg/mL before digestion) were analyzed by both HPLC/MS/MS and MFLC/MS/MS to compare sensitivity of the two techniques. A Shimadzu system equipped with LC-20AD pumps was used for traditional HPLC/MS/MS analysis, and an Eksigent Express HT-Ultra LC<sup>®</sup> system was used for MFLC/MS/MS analysis. Both systems were coupled to the same AB SCIEX QTRAP<sup>®</sup> 5500 mass spectrometer and utilized to analyze the same tryptic digestion samples to provide a direct sensitivity comparison between the two systems. The same mobile and stationary phases and an injection volume of 1 µL were used for all analyses. Column inside diameters of 2 mm and 0.5 mm and flow rates of 700 and 45 µL/min, respectively, were used. In order to show the relationship between flow rate and instrument signal, analysis of one tryptic digestion was also performed using three column diameters with flow rates ranging from 10 - 400 µL/min, according to column diameter.

**Table 1.** MFLC/MS/MS (45 µL/min) vs. HPLC/MS/MS (700 µL/min) instrument signal for signature tryptic peptides of large biological molecules.

Protein	Average Peak Area (HPLC)	Average Peak Height (HPLC)	Average Peak Area (MFLC)	Average Peak Height (MFLC)	MFLC Signal Gain (peak area)	MFLC Signal Gain (peak height)
<b>Ceruloplasmin</b>	4.01E+04	2.87E+04	1.96E+05	1.64E+05	4.9X	5.7X
<b>Somatotropin</b>	3.69E+03	2.43E+03	2.21E+04	1.58E+04	6.0X	6.5X
<b>Myoglobin</b>	4.14E+04	3.39E+04	1.89E+05	1.61E+05	4.6X	4.7X

**Table 2.** Instrument signal for myoglobin signature tryptic peptide using scaled flow rates.

Flow Rate (µL/min)	Column ID (mm)	Average Analyte Peak Area (cps)	Average Analyte Peak Height (cps)	Average Peak Width (min)	MFLC Signal Gain (peak area)	MFLC Signal Gain (peak height)
400	2.00	3.19E+04	1.61E+04	0.093	—	—
30.0	0.500	1.70E+05	1.48E+05	0.093	5.3X	9.2X
10.0	0.300	4.32E+05	2.08E+05	0.15	13.5X	12.9X

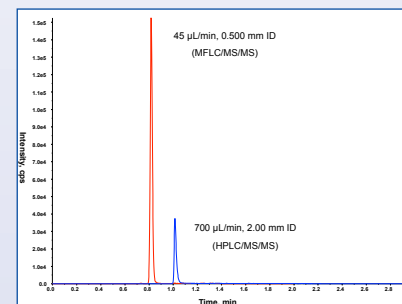
## Results

Tryptic digestions of the three proteins provided signature peptides for HPLC/MS/MS and MFLC/MS/MS analysis. Under the same instrumental conditions, MFLC/MS/MS showed an increase in sensitivity between 300 and 500 percent over HPLC/MS/MS for the three protein analytes.

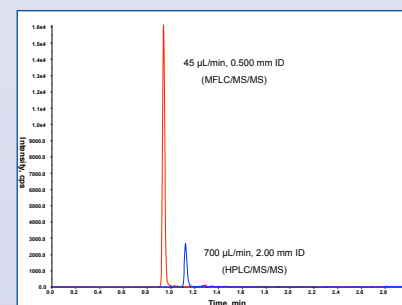
Using three different column inside diameters and flow rates ranging from 10 - 400 µL/min, and an overall sensitivity gain of over 12 fold was achieved using the lowest flow rate as compared to the highest flow rate.

## Conclusions

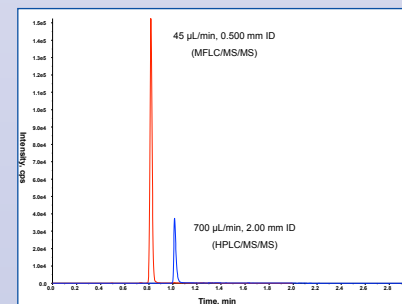
Here we have demonstrated the sensitivity advantage of MFLC/MS/MS over HPLC/MS/MS for large biological molecule analysis using the same injection volume and instrument conditions. While MFLC/MS/MS may not be superior to HPLC/MS/MS for every application, its increased sensitivity gives it value as a large molecule analytical technique, especially when limited solvent consumption and sample volume are a factor.



**Figure 1.** Instrument signal comparison of trypsin-digested horse skeletal myoglobin.



**Figure 2.** Instrument signal comparison of trypsin-digested human somatotropin.



**Figure 3.** Instrument signal comparison of trypsin-digested human ceruloplasmin.



**Figure 4.** AB SCIEX QTRAP<sup>®</sup> 5500 mass spectrometer with Eksigent Express HT-Ultra LC<sup>®</sup> MFLC system.