MULTIPLEXED LC-MRM-MS ANALYSIS OF CARDIOVASCULAR DISEASE BIOMARKERS USING NOVEL INTEGRATED MICROFLUIDICS

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of deaths worldwide, despite mortality rates have declined in many high-income countries. Cardiovascular deaths and disease have increased at a fast rate in low- and middle-income countries. The background of CVD, notably atherosclerosis, begins in early life, making primary prevention efforts necessary from childhood. The verification and translation of putative plasma markers to robust clinical diagnostic assays are challenged by innovation obstacles that have to be addressed. Targeted LC-MS based assays afford protein quantification with the reproducibility and throughput required to improve biomarker acceptance. MRM, using tandem quadrupole mass spectrometry, is one of the enabling technologies. Miniaturized LC systems offer improved mass-sensitivity but lack the required throughput, robustness and reproducibility. Here, the application of a novel microfluidics device and a tandem quadrupole MS/MS system for the quantification of CVD marker peptides and proteins is presented and considered for speed, sensitivity and selectivity.

METHODS

Materials

MS Qual/Quant QC Mix and human plasma were obtained from Sigma-Aldrich (St. Louis, MO, USA). MassPREP E. Coli digest standard was from Waters Corporation (Milford, MA, USA). Stable Isotopically Labeled (SIL) CVD peptides were from Pepscan (Lelystad, The Netherlands).

RESULTS

Sensitivity

The MS Qual/Quant mixture was spiked into E. Coli background to represent a high throughput validation study such that loads for a 1 µl injection ranged from 32 amol to 40 fmol peptide standards. This covers 3.2 amol for NLSVEDAA[R] — MRM chromatogram for an Xevo TQ-S based assay shown in Figure 2 — to 40 fmol for GGFPSDSY[R] and VLDALQAIR, spanning four orders of dynamic range.

LC conditions

- LC system
  - nanoACQUITY
- Sample loop
  - 5 µl
- Separation device
  - iKey Peptide BEH C18, 130Å, 1.7 µm, 150 µm X 100 mm
- Column temperature
  - ambient
- Flow rate
  - 1.2 µl/min
- Mobile phase A
  - 98.9/1/0.1% v/v water/acetonitrile/formic acid
- Mobile phase B
  - 98.9/1/0.1% v/v acetonitrile/water/formic acid in water
- Injection volume
  - 1 µl
- Gradient
  - 3 to 40% B in 45 min

MS conditions

- Mass spectrometer
  - Xevo TQ-S with IonKey — shown in Figure 1
- Acquisition mode
  - MRM — 13 CVD and 14 MS Qual/Quant QC Mix peptides were monitored; scheduled retention time windows were 2 min wide
- Resolution
  - 0.7 Da
- Ionization mode
  - ESI positive
- Capillary voltage
  - 3.6 kV
- Source temperature
  - 100 ºC

Informatics

MassLynx raw data were loaded and analyzed using TargetLynx and/or Skyline (University of Washington, Seattle, WA).

CONCLUSION

- Good linearity was observed over four orders of dynamic range for the analysis of MS Qual/Quant QC Mix and CVD SIL peptides combined with the integrated microfluidics interface
- Quantitative measurements for light to heavy SIL peptides showed excellent accuracy and precision in close agreement with expected values for the MRM analysis of MS Qual/Quant QC Mix with a limit of detection estimated to be smaller than 3.2 amol on column
- Candidate biomarker CVD SIL equivalent peptides were measured over at least three orders of dynamic range with LODs ranging from sub 20 to 100 amol in trypsin digested human plasma

References