Utilizing MFLC-MS/MS for Large Molecule Quantitative Bioanalysis

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OVERVIEW

Purpose

Quantitative bioanalysis of a large molecule utilizing microflow liquid chromatography coupled with tandem mass spectrometry (MFLC-MS/MS)

Methods

Human MAOB extracted from dog plasma by organic precipitation and trypsin pellet digestion, followed by analysis by MFLC-MS/MS (Eksigent ekspert™ microLC 200 system coupled with an ABSciex 5500® QTRAP) and HPLC-MS/MS (Shimadzu LC20AD pumps coupled with an ABSciex 5500® QTRAP). Samples were also analyzed using a novel in-source column (New Objective PicoFuze™).

Results

MFLC-MS/MS analysis resulted in acceptable accuracy and precision for use as a routine quantitative bioanalytical technique. The PicoFuze™ column also demonstrated comparable separation and signal to MFLC analysis.

INTRODUCTION

With the growing development of large biological molecules as therapeutics, as well as the identification of large molecule biomarkers, there is an increasing need for sensitive and selective techniques for quantitative bioanalysis of these molecules. Here we have demonstrated a high throughput method for quantitative bioanalysis of human monoamine oxidase B (MAOB), a protein that is of interest as a biomarker for Parkinson's disease, utilizing MFLC-MS/MS. This method demonstrated comparable performance to traditional HPLC-MS/MS analysis. We also have shown here the use of a novel in-source HPLC column called PicoFuze™ (New Objective, Inc.) that utilizes micro flow rates. The data suggests that the increased sensitivity of MFLC-MS/MS, along with its accuracy, precision, and robustness, gives it value as a large molecule bioanalytical technique with application to the analysis of large molecule therapeutics and biomarkers.

METHODS

Extraction

- MAOB spiked into dog plasma from 1-100 µg/mL and extracted (n=6 at each level)
- Sample volume: 25 µL
- Organic precipitation: 75 µL methanol
- Supernatant discarded after centrifugation
- Trypsin digestion of plasma pellet
- LC/MS analysis of a surrogate peptide and confirmatory peptide

MFLC

- Eksigent ekspert™ microLC 200 System
- Gradient using acetonitrile and water with 1% formic acid
- Flow rate: 44 µL/minute
- Stationary Phase: ProntoSIL 120-3-C18 (MAC-MOD)
- Column temperature: 50°C

HPLC

- Shimadzu LC20AD HPLC pumps
- Gradient using acetonitrile and water with 1% formic acid
- Flow rate: 700 µL/minute
- Stationary Phase: ProntoSIL 120-3-C18 (MAC-MOD)
- Column temperature: 50°C

PicoFuze™

- Standard ABSciex ESI probe enabled with a PicoFrit column
- Eksigent ekspert™ microLC 200 System
- Gradient using acetonitrile and water with 1% formic acid
- Flow rate: 7 µL/minute
- Stationary Phase: ProntoSIL 120-3-C18 (MAC-MOD)
- Column temperature: 50°C

Mass Spectrometry

- ABSciex 5500® QTRAP operating in MRM mode
- ESI
- Positive ion mode
- MRM transitions:

  Surrogate Peptide IMDLLGDR:
  m/z 466.7 → 688.3 (MH+2)

  Confirmatory Peptide LLHDSGLNVVVLEAR:
  m/z 545.6 → 587.1 (MH+3)

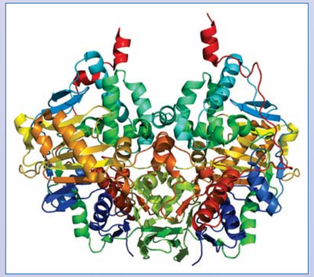

Figure 1. 3D Structure of Human MAOB

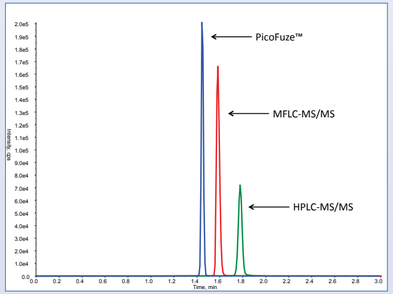

Figure 2. PicoFuze™, MFLC-MS/MS, and HPLC-MS/MS Analysis of MAOB Digest

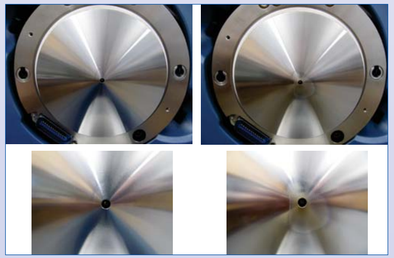

Figure 3. 5500 MS interface plate after approximately 400 injections from the MFLC system (left) and after approximately 150 injections from the HPLC system (right).

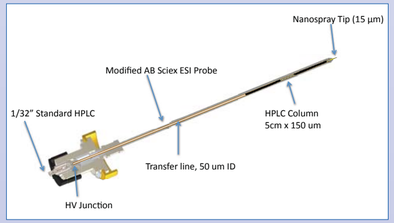

Figure 4. PicoFuze™ Column

Table 1. Chromatographic Conditions for HPLC, MFLC, and PicoFuze™

	HPLC	MFLC	PicoFuze™
Flow Rate (µL/min)	700	44.0	7.00
Column ID (mm)	2.00	0.500	0.200
Stationary Phase	C-18	C-18	C-18
Column Temperature (°C)	50	50	50
Injection Volume (µL)	1.00	1.00	1.00

Table 2. Accuracy and Precision for HPLC-MS/MS and MFLC-MS/MS Analysis of MAOB (Surrogate Peptide IMDLLGDR)

Sample Name	Concentration (µg/mL)	HPLC		MFLC	
		Average Accuracy (%)	%CV	Average Accuracy (%)	%CV
LLOQ	1.00	101	9.6	92.4	8.2
LQC	3.00	98.1	6.2	95.8	7.5
MQC	50.0	90.8	5.0	104	13.7
HQC	80.0	105	5.7	94.7	7.9
ULOQ	100	111	11.7	92.6	14.6

Table 3. Instrument Signal for HPLC, MFLC, and PicoFuze™ Analysis of MAOB (Surrogate Peptide IMDLLGDR)

	Average Analyte Peak Area	Average Analyte Peak Height	Average Analyte Signal/Noise	Average Analyte Peak Width (min)
HPLC-MS/MS	1.34E+05	7.81E+04	5.97E+03	0.371
MFLC-MS/MS	3.08E+05	1.61E+05	1.64E+04	0.397
PicoFuze™	2.71E+05	1.69E+05	2.25E+04	0.321

CONCLUSIONS

- MFLC-MS/MS analysis demonstrated accuracy and precision for the protein analyte within ± 20% at the LLOQ and within ±15% at all other levels.
- MFLC-MS/MS instrument signal was >2X higher than HPLC-MS/MS.
- Carryover evaluated on the MFLC-MS/MS system for the surrogate peptide was <20% of the LLOQ.
- Ruggedness of the MFLC-MS/MS system was shown over hundreds of sample injections as compared to HPLC-MS/MS, which required cleaning of MS source more frequently.
- PicoFuze™ column gave adequate separation and signal for both surrogate peptide and confirmatory peptide.