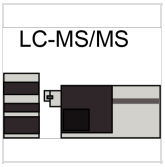

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Targeted proteomic LC-MS/MS analysis

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1 Works for me dx.doi.org/10.17504/protocols.io.bf9xjr7n

LBNL-omics

Yan Chen

ABSTRACT

This protocol details steps in targeted proteomic data acquisition with a standard-flow UHPLC-QQQ system. It was adapted from Chen, Y. et al. “A rapid methods development workflow for high-throughput quantitative proteomic applications.” PloS ONE 14,2 e0211582. 14 Feb. 2019, doi:10.1371/journal.pone.0211582.

EXTERNAL LINK

https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0211582

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KEYWORDS

LC-MS, Targeted proteomics, MRM

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MATERIALS

NAME	CATALOG #	VENDOR
Acetonitrile LCMS quality	9829-02	JT Baker
Pierce™ Formic Acid	TS-28905	Thermo Fisher
LCMS grade water	BJLC365-2.5	VWR International
Isopropanol	BJ650447-4L	VWR International

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Agilent Tune Mix: G1969-85000	G1969-85000	

MATERIALS TEXT

Analytical column: Ascentis Express Peptide C18 column (**2.7 µm** particle size, 160-Å pore size, 5-cm length × **2.1 mm** internal diameter).

Guard column: Ascentis guard column (5-mm × **2.1 mm** ID with **2.7 µm** particle size, 160-Å pore size)

LC-MS system: Agilent 6460 QQQ mass spectrometer system coupled with an Agilent 1290 Infinity II UHPLC system (Agilent Technologies, Santa Clara, CA)

EQUIPMENT

NAME	CATALOG #	VENDOR
6460QQQ	6460QQQ	Agilent Technologies
1290 UHPLC	1290UHPLC	Agilent Technologies

SAFETY WARNINGS

Wear proper PPE (gloves, safety goggle, and lab coat), and prepare solvents in a chemical hood.
Store organic solvents in a flammable storage cabinet.

BEFORE STARTING

Prepare the following solvents:

Needle wash solvents: Add **100 mL** isopropanol into **900 mL** water.

Solvent A: Add **0.1 % volume** formic acid into LC-MS grade water.

Solvent B: Add **0.1 % volume** formic acid into LC-MS grade acetonitrile

Proteomics: HPLC and Mass Spectromtery

1 Thaw peptide samples **On ice** , and transfer **30 µl** of each sample to LC autosampler vials (Agilent, Cat.# 5182-0567, 5182-0564) or 96-well plate (Bio-Rad, Cat.# HSP9655).

2 Liquid chromatography tandem-mass spectrometry (LC-MS/MS) analysis is performed with an Agilent 6460 QQQ mass spectrometer system coupled to an Agilent 1290 Infinity II UHPLC system (Agilent Technologies, Santa Clara, CA).

6460QQQ
Triple quadrupole mass spectrometer
Agilent Technologies 6460QQQ

1290 UHPLC
Ultra-high performance liquid chromatography system
Agilent Technologies 1290UHPLC

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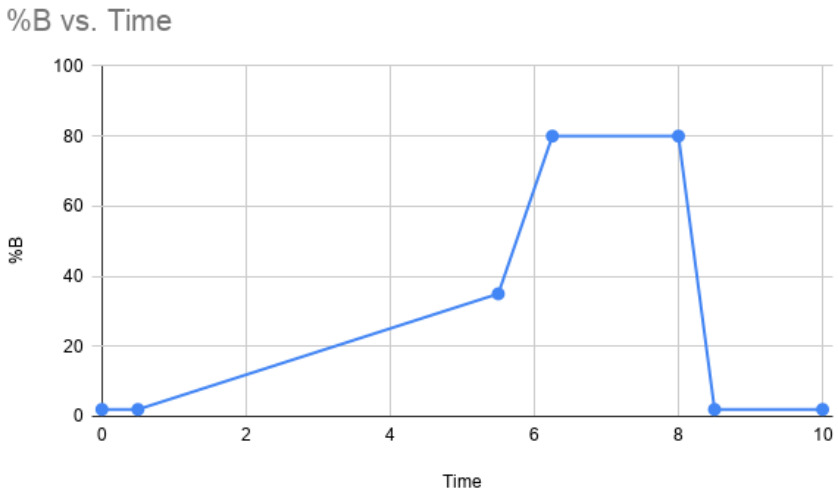
3 Samples were loaded on the temperature controlled autosampler at **4 °C** . The Agilent 1290 Infinity II UHPLC is equipped with a Sigma–Aldrich Ascentis Peptides ES-C18 analytical column (**2.1 mm ID** , **50 mm length** , **2.7 µm particle size** , and 160-Å pore size (Sigma-Aldrich, Cat.# 53301-U)) coupled to a **2.1 mm ID** , **5 mm length** guard column (Sigma-Aldrich, Cat.# 53536-U) with the same particle and pore size. The column is operated at **60 °C** .

4 Twenty micrograms **20 µg** of peptides are loaded onto the column from each sample and separated using a linear mobile-phase gradient consisting of 0.1% formic acid in water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B) operating at a flow rate of 0.4 ml/min. A 10 minutes gradient of chromatographic separation is as follows:

Step	%A	%B	Time (min)
1	98	2	0.00
2	98	2	0.50
3	65	35	5.50
4	20	80	6.25
5	20	80	8.00
6	98	2	8.50
7	98	2	10.00

Chromatographic gradient table



Chromatographic gradient

The gradient length depends on the application of interest and the number of target peptides for detection.

5 The eluted peptides were ionized via an Agilent Jet Stream ESI source operating in positive ion mode with the following source parameters:

Gas temperature	250 °C

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Gas flow	13 liters/min
Nebulizer pressure	35 psi
Sheath gas temperature	250 °C
Sheath gas flow	11 liters/min
Capillary voltage	3500 V
Nozzle voltage	0 V

6 SRM transitions were monitored through either scanning or scheduled MRM mode with the following criteria:

- Minimum dwell time of a transition is 2 ms
- Maximum number of concurrent transitions are 200
- Total cycle time less than 1.5 sec.

7 The MS raw data were acquired using Agilent MassHunter version B.08.02

MassHunter B.08.02
by Agilent Technologies, Inc

8 Acquired SRM data were imported and analyzed by Skyline software version 20.1 (MacCoss Lab Software).

Skyline 20.1
by University of Washington

9 For a small sample set, peak integration is visually checked and validated by browsing replicate peak area and retention time tabs. For a large set of more than 100 samples, peak integration is completed by using skyline advanced peak picking models (e.g., mProphet).

Reiter L, Rinner O, Picotti P, Hüttenhain R, Beck M, Brusniak MY, Hengartner MO, Aebersold R (2011). mProphet: automated data processing and statistical validation for large-scale SRM experiments.. Nature methods.
https://doi.org/10.1038/nmeth.1584

10 A peptide quantitative report was exported to a .csv file for further analysis and sharing. The skyline file with the data and SRM methods information was uploaded to a project folder on the PanoramaWeb server. The project information (data and SRM methods) is made public when a manuscript is accepted for journal publication.

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Sharma V, Eckels J, Taylor GK, Shulman NJ, Stergachis AB, Joyner SA, Yan P, Whiteaker JR, Halusa GN, Schilling B, Gibson BW, Colangelo CM, Paulovich AG, Carr SA, Jaffe JD, MacCoss MJ, MacLean B (2014). Panorama: a targeted proteomics knowledge base.. Journal of proteome research. https://doi.org/10.1021/pr5006636

Sharma V, Eckels J, Schilling B, Ludwig C, Jaffe JD, MacCoss MJ, MacLean B (2018). Panorama Public: A Public Repository for Quantitative Data Sets Processed in Skyline.. Molecular & cellular proteomics : MCP. https://doi.org/10.1074/mcp.RA117.000543

UHPLC-QQQ performance monitoring and QC

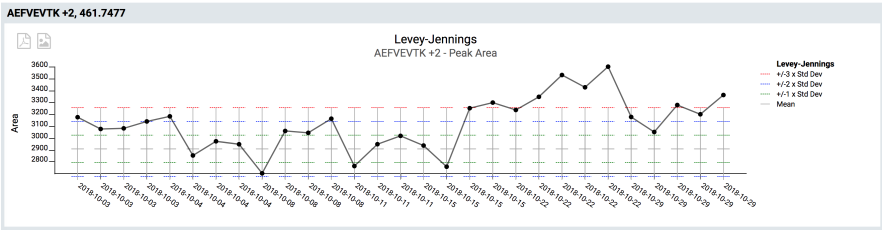
11 The mass spectrometer is subjected to a full autotune at least quarterly (and more frequently, if necessary) to optimize ion transmission and update EM voltage.

Agilent Tune Mix: G1969-85000
Catalog #: G1969-85000

12 Agilent 6460 QQQ mass spectrometer is subjected to a check tune prior to analyzing a batch of samples for resolution and mass accuracy verification.

13 The UHPLC-QQQ performance is tracked **daily** by injecting 50 fmol BSA tryptic digest standard and monitoring eight BSA peptides using a scheduled MRM assay. Peptide retention time, peak shape, and peak intensity of these peptides are monitored to determine if maintenance is required.

14 The performance tracking is automated and monitored via PanoramaWeb server through an established AutoQC pipeline.



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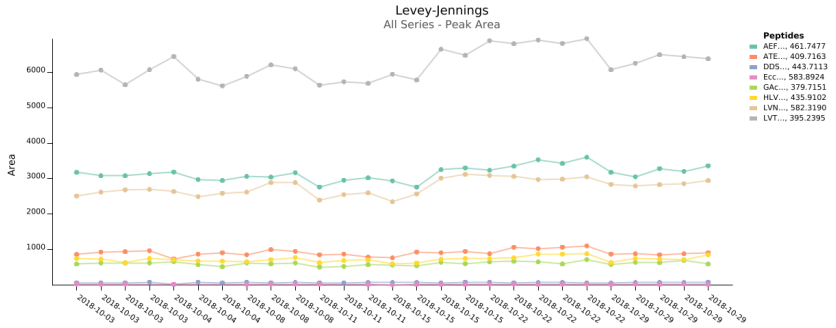

An example of autoQC instrument performance track on the peak area of eight BSA tryptic peptide measurements

AutoQC loader
by University of Washington

Bereman MS, Beri J, Sharma V, Nathe C, Eckels J, MacLean B, MacCoss MJ (2016). An Automated Pipeline to Monitor System Performance in Liquid Chromatography-Tandem Mass Spectrometry Proteomic Experiments. Journal of proteome research.
https://doi.org/10.1021/acs.jproteome.6b00744

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