Jun 08, 2020
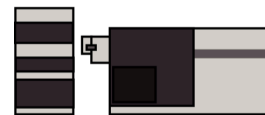
🌐 Targeted proteomic LC-MS/MS analysis

LC-MS/MS

DOI

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Protocol status: Working
We use this protocol and it's working

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Last Modified: June 08, 2020

Protocol Integer ID: 36887

Keywords: LC-MS, Targeted proteomics, MRM, targeted proteomic data acquisition, targeted proteomic lc, throughput quantitative proteomic application, quantitative proteomic application, proteomic data, ms analysis this protocol details step, ms analysis this protocol, ms analysis, m, flow uhplc

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.

Materials

MATERIALS

⊗ Acetonitrile LCMS quality **JT Baker Catalog** #9829-02

⊗ Pierce™ Formic Acid **Thermo Fisher Catalog** #TS-28905

⊗ LCMS grade water **VWR International (Avantor) Catalog** #BJLC365-2.5

⊗ Isopropanol **VWR International (Avantor) Catalog** #BJ650447-4L

STEP MATERIALS

⊣⊢ 2.7 μm

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)

Protocol materials

⊗ Acetonitrile LCMS quality **JT Baker Catalog #**9829-02

⊗ Pierce™ Formic Acid **Thermo Fisher Catalog #**TS-28905

⊗ LCMS grade water **VWR International (Avantor) Catalog #**BJLC365-2.5

⊗ Isopropanol **VWR International (Avantor) Catalog #**BJ650447-4L

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

Troubleshooting

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 start

Prepare the following solvents:

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

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

Solvent B: Add [M] 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).

Equipment	
6460QQQ	NAME
Triple quadrupole mass spectrometer	TYPE
Agilent Technologies	BRAND
6460QQQ	SKU
https://www.agilent.com/en/products/liquid-chromatography-mass-spectrometry-lc-ms/lc-ms-instruments/triple-quadrupole-lc-ms	LINK

| Equipment	
1290 UHPLC	NAME
Ultra-high performance liquid chromatography system	TYPE
Agilent Technologies	BRAND
1290UHPLC	SKU
www.agilent.com	LINK

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

%B vs. Time
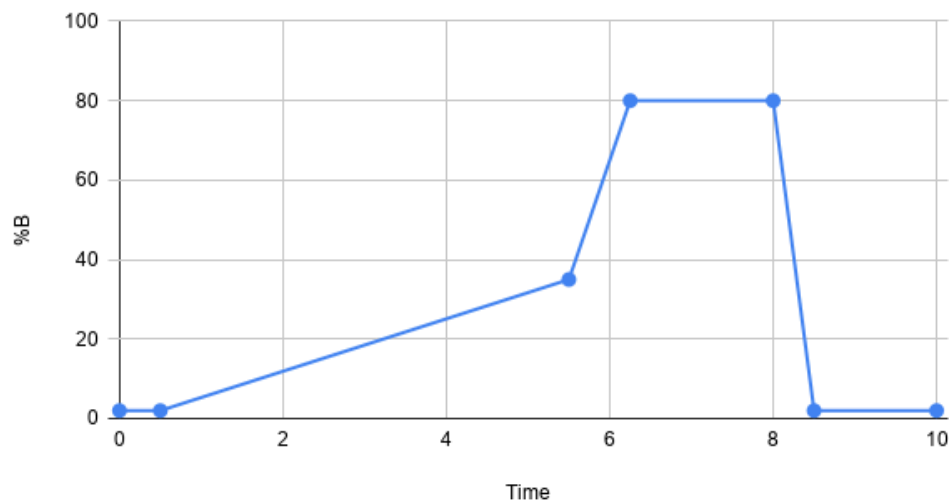
Chromatographic gradient

> **Note**
>
> 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
	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

Software	
MassHunter	NAME
Agilent Technologies, Inc	DEVELOPER

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

Software	
Skyline	NAME
University of Washington	DEVELOPER

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).

Citation

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   LINK

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.

Citation

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   LINK

Citation

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   LINK

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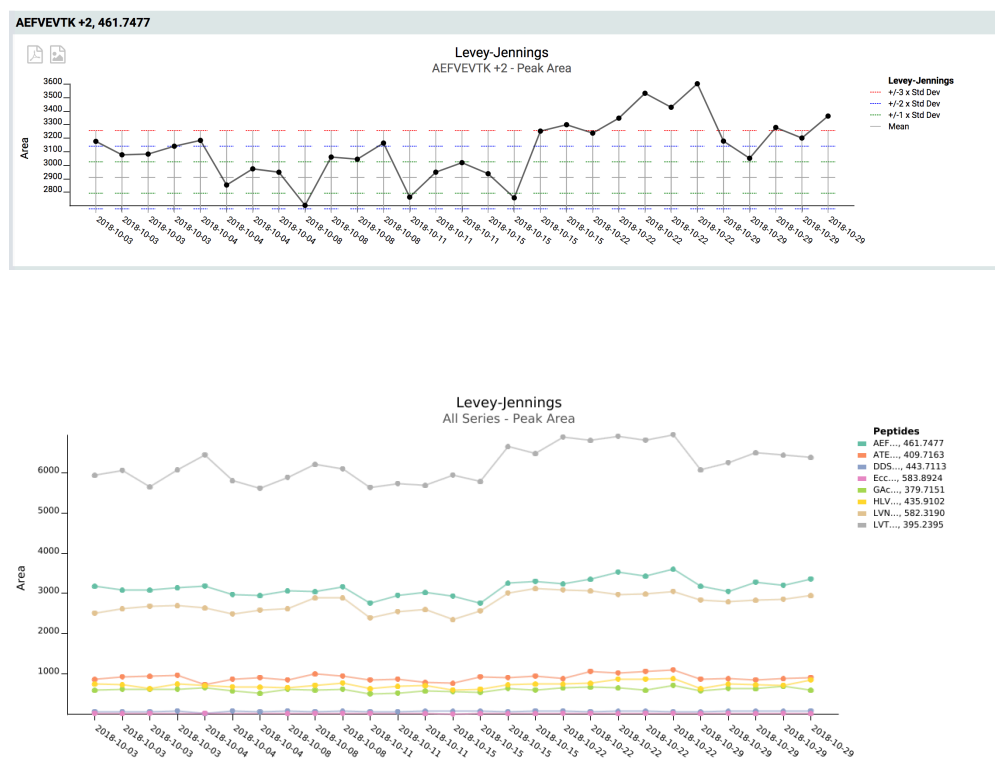
An example of autoQC instrument performance track on the peak area of eight BSA tryptic peptide measurements

Software

AutoQC loader	NAME
University of Washington	DEVELOPER

Citation

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   LINK

Citations

Step 10

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. Panorama: a targeted proteomics knowledge base.
https://doi.org/10.1021/pr5006636

Step 10

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

Step 14

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

Step 9

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