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# Development and evaluation of Nano-ESI coupled to a triple quadrupole mass spectrometer for quantitative proteomics research

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# Development and evaluation of Nano-ESI coupled to a triple quadrupole mass spectrometer for quantitative proteomics research

## 1. Introduction

Recently, the promotion of proteomic analysis has resulted in the identification of many proteins. Analyzing the proteins function, such as its various signaling pathways, is a clue to understanding disease. Historically, quadrupole time-of-flight mass spectrometry has been preferred over more quantitative, but less sensitive, triple quadrupole based mass spectrometric methodologies. Our aim is to develop a triple quadrupole based system to quantitatively analyze peptide fragments through a shotgun proteomics approach. We developed both a conventional and nano-flow quantitative system for proteomic analyses utilizing liquid chromatography (LC) coupled to a triple quadrupole mass spectrometer (triple Q MS). We also demonstrate the sensitivity enhancement of the triple Q MS as a function of the MS front-end LC at analytical and nano-flow rates (Fig. 1).

Fig. 1 Nano-flow LC and LCMS-8040 triple quadrupole mass spectrometer

## 2. Methods and Materials

### 2-1. Methods

Samples were measured by ESI-MS coupled to either an UHPLC system or a nano-flow HPLC system.

**Analysis by UHPLC coupled to triple Q MS (conventional LC-MS)**

**UHPLC conditions (Nexera system)**

| | |
|---|---|
| Column | : Shim-pack XR-ODS III 2 mm I.D. × 50 mm L., 1.6 µm |
| Mobile phase A | : 0.5% acetic acid in aq. |
| B | : acetic acid / Water / Acetonitrile (0.5/19.5/80) |
| Flow rate | : 0.2 mL/min |
| Time program | : B conc.5%(0 min) - 60%(10 min) - 100%(10.01-20 min) - 5%(20.01-30 min)(Fig. 2) |
| Injection vol. | : 1 µL |
| Column temperature | : 40°C |

**MS conditions (LCMS-8040)**

| | |
|---|---|
| Ionization | : ESI |
| Events | : Positive MRM mode |
| Applied Voltage | : 4.5 kV |
| Desorption Liquid temp. | : 300°C |
| Heat Block temp | : 500°C |
| Nebulizer Gas flow rate | : 3 L/min |
| Drying Gas flow rate | : 10 L/min |

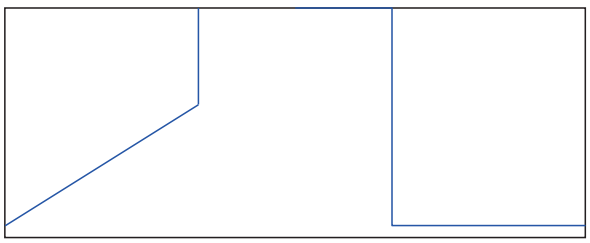

Fig. 2 The time program of conc.B gradient curve of the UHPLC system

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**Analysis by nano-flow HPLC coupled to triple Q MS (nano-flow LC-MS)**

**nanoLC conditions (Prominence system)**

| | |
|---|---|
| Column | : column with nano spray tip 100 µm I.D. × 150 mm L., 3 µm (Reprosil$^{R}$ particles) |
| Mobile phase A | : 0.5% acetic acid in aq. |
| B | : acetic acid / Water / Acetonitrile (0.5/19.5/80) |
| Flow rate | : 500 nL/min |
| Time program | : B conc.5%(0 min) - 60%(30 min) - 100%(31-40 min) - 5%(45-65 min) (Fig. 3) |
| Injection vol. | : 1 µL |
| Column temperature | : Room Temp. |

**MS conditions (LCMS-8040)**

| | |
|---|---|
| Ionization | : nano-ESI (sprayed by nano spray tip) |
| Events | : Positive MRM mode |
| Applied Voltage | : 2.5 kV |
| Desorption Liquid temp. | : 250°C |
| Heat Block temp. | : 200°C |
| Nebulizer Gas flow | : None |
| Drying Gas flow | : None |

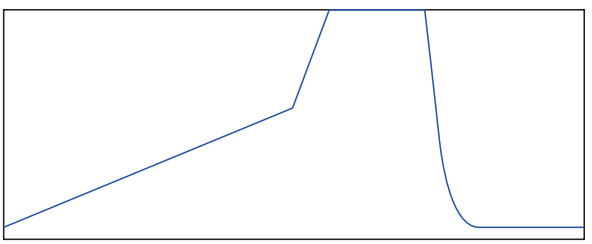

Fig. 3 The time program of conc.B gradient curve of the nanoLC system

The nano-flow HPLC system consists of binary pumps and autosampler. The flow channel is illustrated below (Fig. 4).

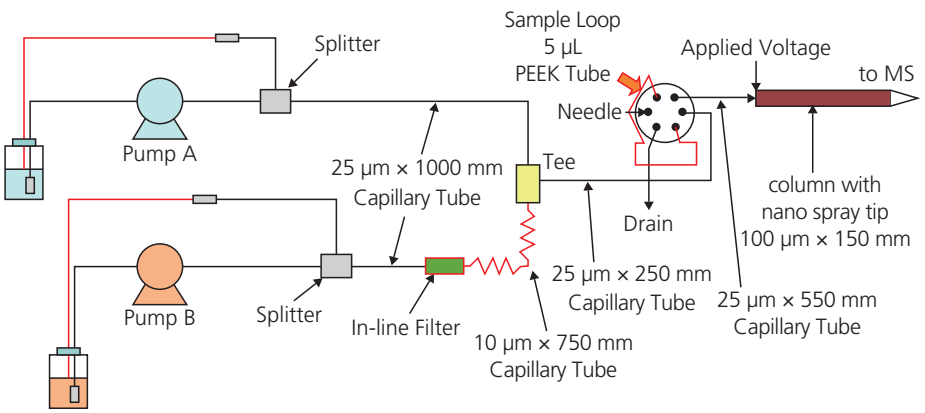


Fig. 4 The flow channel configuration of the nano-flow HPLC system

## 2-2. Materials

Digested BSA (Bovine Serum Albumin) was analyzed to optimize the conditions of the LC and the triple Q MS parameters.

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**Digestion of BSA**

1 mg/mL of BSA in 50 mM Ammonium Bicarbonate (ABC) and 8 M Urea solution
↓ Add 10 mM of dithiothreitol, 37°C 30 min
↓ Add 50 mM of 2-iodoacetamide, 37°C 30 min
↓ Add 10 µL of LysC (1 µg/µL), 37°C 4h
↓ Diluted by 3 mL of 50 mM ABC
↓ Add 20 µg of Trypsin, 37°C overnight
Quenched by 10% trifluoroacetic acid (TFA) in aq.

**Desalination of peptides**

Add 1 mL of Buffer B to Stage Tip
↓ Centrifuge 500 g 3 min
Add 1 mL of Buffer A to Stage Tip
↓ Centrifuge 500 g 3 min
Add 1 mL of BSA Digestion to Stage Tip
↓ centrifuge 500 g 4 min
Add 1 mL of Buffer A to Stage Tip
↓ centrifuge 500 g 4 min
Add 250 µL of Buffer B to Stage Tip
↓ Centrifuge 300 g 2 min
Preserve elution buffer at -20°C

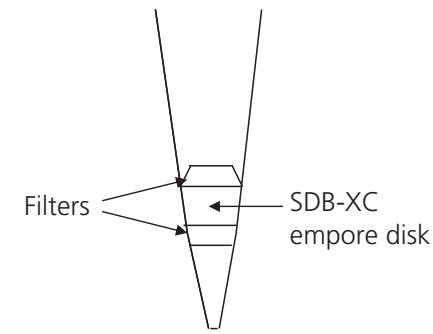


Fig. 5 Structure of Stage Tip.
Buffer A: TFA / $H_2O$ / ACN (0.1/4.9/95),
Buffer B: TFA / $H_2O$ / ACN (0.1/19.9/80)

# 3. Results

Most peptides in digested BSA were detected as protonated divalent or trivalent molecules($[M+2H]^{2+}$ or $[M+3H]^{3+}$) in ESI positive ion mode. The precursor ions were monitored by Q1 SIM measurement. Mass spectrometric parameters for MRM analysis, such as product ion and collision energy, were optimized by an automatic MS optimization procedure. The chromatographic and automatic MS optimization produced 107 MRM transitions for 11 peptides for both the nano-flow LC-MS method and the conventional LC-MS methods. Quantitative analyses were performed for peptides containing at least 7 amino acids (Table 1).

Calibration curves were generated from each MRM transition with linearity improving when analyzed by nano-flow LC-MS as opposed to conventional LC-MS. Concentration ranges of the conventional LC-MS method and the nano-flow LC-MS method were 1-50 pmol and 10-500 fmol. Additionally, the LOD and LOQ of each transition analyzed by the nano-flow LC-MS method is several hundred fold lower than the conventional LC-MS method (Table 1, Fig. 6). In summary, we improved the sensitivity of a triple Q MS by reducing the LC flow rate to nL/min. A few femtomoles of injected peptides could be analyzed by nano-flow LC-MS.

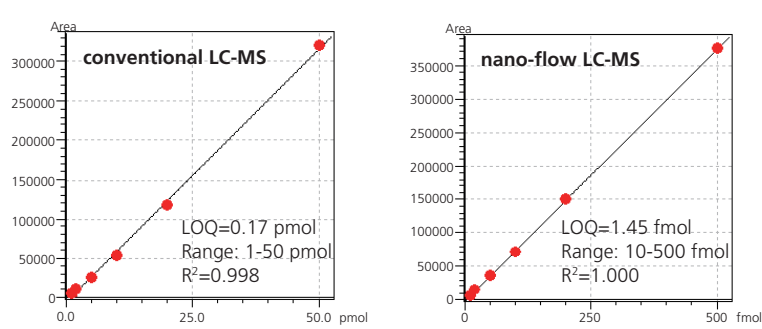


Fig. 6 Representative calibration curve [DDSPDLPK>b2(443.70>230.90)]

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## 4. Conclusions

- Nano-flow LC enhanced the sensitivity of a triple Q MS, as a function of the MS front-end LC, as opposed to conventional LC while improving the linearity of calibration curves.
- We developed and evaluated a novel quantitative system for proteomics with the results suggesting that this system could be applicable as a tool for shotgun proteomics.

Table 1 MRM transitions of BSA Digests and the LOD, LOQ, and relation coefficient ($R^2$) of each transition for both the nano-flow LC-MS method and the conventional LC-MS method.

| | | conventional LC-MS | | | nano-flow LC-MS | | |
|---|---|---|---|---|---|---|---|
| MRMtransition | *m/z* | LOD(pmol) | LOQ(pmol) | coefficient($R^2$) | LOD(fmol) | LOQ(fmol) | coefficient($R^2$) |
| ECCDKPLLEK>y2++-H2O | 646.30>129.30 | 0.41 | 1.24 | 0.996 | 0.58 | 1.74 | 1.000 |
| ECCDKPLLEK>y1 | 646.30>147.00 | 0.36 | 1.08 | 0.999 | 1.67 | 5.02 | 1.000 |
| ECCDKPLLEK>y10++-H2O | 646.30>637.35 | 0.08 | 0.24 | 0.995 | 0.27 | 0.80 | 0.999 |
| ECCDKPLLEK>y3+++ | 646.30>130.20 | 0.29 | 0.86 | 0.996 | 3.92 | 11.75 | 0.999 |
| ECCDKPLLEK>b4+++-H2O | 646.30>183.30 | 0.30 | 0.91 | 0.997 | 2.25 | 6.75 | 0.999 |
| ECCDKPLLEK>y2-H2O | 646.30>258.10 | 0.19 | 0.56 | 0.997 | 1.94 | 5.83 | 0.995 |
| CCTESLVNR>b4+++-H2O | 569.75>178.00 | 0.03 | 0.10 | 0.998 | 0.41 | 1.24 | 1.000 |
| CCTESLVNR>y9++-H2O | 569.75>560.95 | 0.03 | 0.09 | 0.998 | 1.20 | 3.61 | 0.999 |
| CCTESLVNR>y7 | 569.75>818.45 | 0.02 | 0.06 | 0.997 | 0.32 | 0.97 | 0.999 |
| CCTESLVNR>y1 | 569.75>175.20 | 0.03 | 0.09 | 0.996 | 1.91 | 5.72 | 0.998 |
| CCTESLVNR>b2 | 569.75>320.85 | 0.06 | 0.17 | 0.994 | 0.37 | 1.12 | 1.000 |
| CCTESLVNR>y2 | 569.75>289.05 | 0.09 | 0.28 | 0.999 | 1.58 | 4.73 | 1.000 |
| CCTESLVNR>y5 | 569.75>588.15 | 0.06 | 0.17 | 0.996 | 0.29 | 0.88 | 0.998 |
| CCTESLVNR>y6 | 569.75>717.15 | 0.05 | 0.15 | 0.996 | 0.58 | 1.74 | 0.998 |
| CCTESLVNR>y3 | 569.75>388.35 | 0.09 | 0.26 | 0.995 | 1.81 | 5.42 | 0.998 |
| CCTESLVNR>b5+++ | 569.75>213.10 | 0.11 | 0.32 | 0.992 | 1.01 | 3.02 | 0.998 |
| DDSPDLPK>b2 | 443.70>230.90 | 0.06 | 0.17 | 0.998 | 0.48 | 1.45 | 1.000 |
| DDSPDLPK>y2 | 443.70>244.20 | 0.06 | 0.17 | 0.998 | 0.29 | 0.88 | 1.000 |
| DDSPDLPK>y6 | 443.70>656.35 | 0.05 | 0.16 | 0.998 | 0.07 | 0.20 | 1.000 |
| DDSPDLPK>b2-H2O | 443.70>212.70 | 0.15 | 0.45 | 0.995 | 0.79 | 2.37 | 0.999 |
| DDSPDLPK>y5 | 443.70>569.20 | 0.11 | 0.34 | 0.999 | 0.27 | 0.82 | 1.000 |
| DDSPDLPK>y1 | 443.70>147.25 | 0.13 | 0.40 | 0.999 | 1.65 | 4.95 | 1.000 |
| DDSPDLPK>y6++-H2O | 443.70>319.90 | 0.08 | 0.24 | 0.996 | 3.12 | 9.37 | 0.998 |
| DDSPDLPK>y5++ | 443.70>285.30 | 0.21 | 0.64 | 0.996 | 1.14 | 3.42 | 1.000 |
| DDSPDLPK>b3-H2O | 443.70>300.20 | 0.12 | 0.36 | 0.993 | 2.22 | 6.66 | 0.999 |
| DDSPDLPK>y6+++-H2O | 443.70>213.40 | 0.51 | 1.54 | 0.999 | 1.88 | 5.64 | 0.997 |
| DDSPDLPK>b4+++ | 443.70>139.10 | 0.30 | 0.89 | 0.998 | 3.33 | 9.99 | 1.000 |
| DLGEEHFK>y6 | 487.75>746.20 | 0.05 | 0.14 | 0.989 | 0.36 | 1.07 | 1.000 |
| DLGEEHFK>b2 | 487.75>229.05 | 0.05 | 0.15 | 0.988 | 0.27 | 0.82 | 0.999 |
| DLGEEHFK>b3+++ | 487.75>96.45 | 0.21 | 0.62 | 0.981 | 1.26 | 3.79 | 0.994 |
| DLGEEHFK>y2+++ | 487.75>98.45 | 0.86 | 2.57 | 0.965 | 2.46 | 7.37 | 0.997 |
| DLGEEHFK>y6+++ | 487.75>249.20 | 0.08 | 0.24 | 0.992 | 0.37 | 1.11 | 0.995 |
| DLGEEHFK>y6++ | 487.75>373.45 | 0.11 | 0.32 | 0.983 | 0.38 | 1.15 | 0.999 |
| GACLLPK>y2 | 379.70>244.10 | 0.05 | 0.14 | 0.991 | 0.40 | 1.19 | 1.000 |
| GACLLPK>b2 | 379.70>129.20 | 0.03 | 0.08 | 0.990 | 0.35 | 1.04 | 1.000 |
| GACLLPK>y5++ | 379.70>315.70 | 0.04 | 0.12 | 0.990 | 0.37 | 1.11 | 1.000 |
| GACLLPK>y5 | 379.70>630.35 | 0.04 | 0.13 | 0.990 | 0.38 | 1.13 | 1.000 |
| GACLLPK>y1 | 379.70>147.20 | 0.04 | 0.12 | 0.992 | 0.45 | 1.34 | 1.000 |
| GACLLPK>b2 | 379.70>289.10 | 0.04 | 0.12 | 0.990 | 0.39 | 1.16 | 1.000 |
| GACLLPK>y3 | 379.70>357.15 | 0.05 | 0.14 | 0.991 | 0.32 | 0.95 | 1.000 |
| GACLLPK>y4 | 379.70>470.40 | 0.05 | 0.14 | 0.993 | 0.39 | 1.16 | 1.000 |
| GACLLPK>y6++ | 379.70>351.15 | 0.04 | 0.12 | 0.991 | 0.31 | 0.94 | 0.999 |
| GACLLPK>b4 | 379.70>402.25 | 0.03 | 0.08 | 0.994 | 0.57 | 1.71 | 0.999 |
| GACLLPK>b4++ | 379.70>201.25 | 0.03 | 0.10 | 0.992 | 0.68 | 2.03 | 0.999 |
| LVTDLTK>y5 | 395.25>577.35 | 0.03 | 0.09 | 0.991 | 0.37 | 1.10 | 1.000 |
| LVTDLTK>b2 | 395.25>212.90 | 0.04 | 0.11 | 0.991 | 0.38 | 1.14 | 1.000 |
| LVTDLTK>y7++-H2O | 395.25>386.20 | 0.02 | 0.05 | 0.989 | 0.50 | 1.51 | 1.000 |
| LVTDLTK>y2 | 395.25>248.05 | 0.05 | 0.14 | 0.989 | 0.40 | 1.20 | 0.999 |
| LVTDLTK>y1 | 395.25>147.30 | 0.04 | 0.11 | 0.991 | 0.64 | 1.93 | 0.999 |
| LVTDLTK>y6 | 395.25>676.35 | 0.03 | 0.08 | 0.991 | 0.17 | 0.52 | 0.999 |
| LVTDLTK>y3 | 395.25>361.10 | 0.05 | 0.16 | 0.991 | 0.20 | 0.59 | 0.999 |
| LVTDLTK>y2-H2O | 395.25>230.10 | 0.25 | 0.75 | 0.990 | 0.35 | 1.04 | 0.998 |
| AEFVEVTK>b2 | 461.75>201.15 | 0.04 | 0.11 | 0.991 | 0.27 | 0.81 | 1.000 |
| AEFVEVTK>y6 | 461.75>722.40 | 0.03 | 0.10 | 0.992 | 0.41 | 1.24 | 1.000 |
| AEFVEVTK>y2 | 461.75>248.30 | 0.03 | 0.10 | 0.993 | 0.34 | 1.01 | 0.999 |
| AEFVEVTK>y5 | 461.75>575.30 | 0.05 | 0.16 | 0.994 | 0.29 | 0.88 | 0.999 |
| AEFVEVTK>y6++ | 461.75>361.60 | 0.06 | 0.17 | 0.991 | 0.25 | 0.74 | 0.999 |
| AEFVEVTK>y4 | 461.75>476.30 | 0.03 | 0.08 | 0.991 | 0.36 | 1.07 | 0.999 |
| AEFVEVTK>y3 | 461.75>346.95 | 0.04 | 0.11 | 0.989 | 0.26 | 0.77 | 0.998 |
| AEFVEVTK>y2-H2O | 461.75>230.40 | 0.04 | 0.11 | 0.993 | 0.23 | 0.70 | 0.998 |
| AEFVEVTK>b2-H2O | 461.75>183.00 | 0.04 | 0.12 | 0.996 | 0.22 | 0.65 | 0.997 |
| AEFVEVTK>y3+++-H2O | 461.75>110.05 | 0.08 | 0.25 | 0.988 | 0.38 | 1.14 | 0.997 |
| EACFAVEGPK>y1 | 554.25>147.25 | 0.05 | 0.16 | 0.996 | 1.06 | 3.19 | 0.996 |
| EACFAVEGPK>y2 | 554.25>244.25 | 0.04 | 0.13 | 0.994 | 0.39 | 1.18 | 1.000 |
| EACFAVEGPK>b3-H2O | 554.25>342.95 | 0.04 | 0.12 | 0.991 | 0.36 | 1.09 | 1.000 |
| EACFAVEGPK>y6 | 554.25>600.25 | 0.03 | 0.08 | 0.991 | 0.41 | 1.22 | 1.000 |
| EACFAVEGPK>y3 | 554.25>301.00 | 0.04 | 0.12 | 0.993 | 0.50 | 1.49 | 0.999 |
| EACFAVEGPK>y7 | 554.25>747.20 | 0.02 | 0.07 | 0.992 | 0.67 | 2.02 | 0.999 |
| EACFAVEGPK>b2-H2O | 554.25>182.95 | 0.05 | 0.16 | 0.992 | 0.49 | 1.47 | 0.999 |
| EACFAVEGPK>y5 | 554.25>529.35 | 0.02 | 0.05 | 0.990 | 0.30 | 0.90 | 1.000 |
| EACFAVEGPK>y4 | 554.25>430.05 | 0.03 | 0.10 | 0.989 | 0.35 | 1.06 | 0.999 |
| EACFAVEGPK>y8++ | 554.25>454.25 | 0.04 | 0.12 | 0.991 | 0.39 | 1.17 | 1.000 |
| EACFAVEGPK>y9++ | 554.25>490.05 | 0.02 | 0.06 | 0.988 | 0.34 | 1.02 | 0.999 |

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