# DEVELOPMENT OF A ROBUST, ACCURATE AND REPRODUCIBLE PROCEDURE FOR QUANTITATIVE ANALYSIS OF CARDIAC TROPONIN T USING A CHIP-BASED NANOSPRAY SOURCE


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## INTRODUCTION

Liquid chromatography/tandem mass spectrometry offers great potential for quantitative proteomics, but optimal workflows and system configurations still needs to be established. In this study we developed, validated and compared three procedures for absolute quantitation of cardiac troponin T (cTnT) using microflow LC/MS or chip-based nanospray source with direct injection or preconcentration nanoflow LC/MS. Troponin T content was evaluated in hearts of C57BL/6 and ApoE/LDLR double knock-out mice.

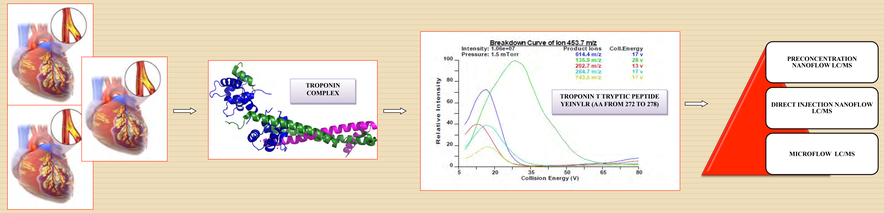


Fig. 1. *Our workflow for quantification of cardiac troponin T.*

## METHODS

A triple quadrupole (TSQ Vantage, Thermo) mass detector equipped with HESI II electrospray source or chip-based nanospray source (PicoChip, New Objective) connected to standard flow HPLC (Surveyor, Thermo) or nanoflow HPLC (Dionex RSLCnano, Thermo) were used. Specific parameters of the LC/MS analysis are shown in Table 1.

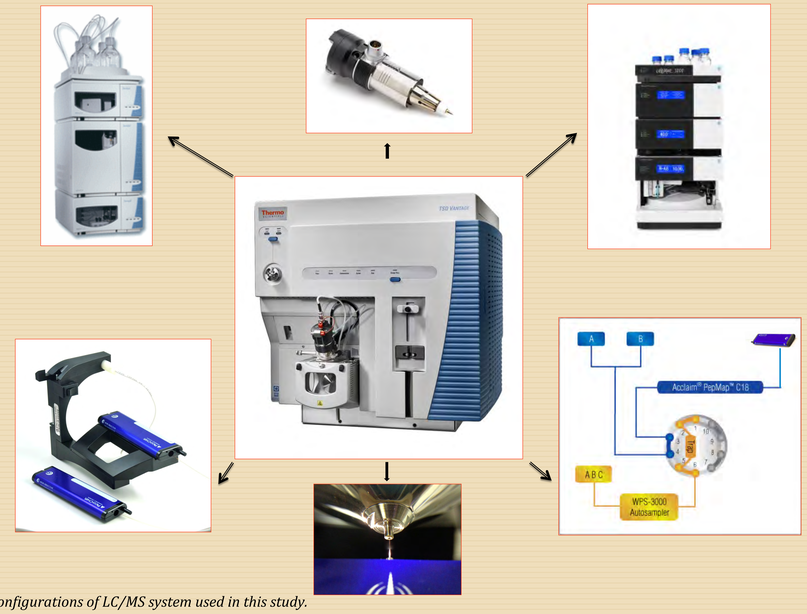


*Fig. 2. Configurations of LC/MS system used in this study.*

Troponin T tryptic digestion product (YEINVLR) and a synthetic structural analogue (internal standard; YEIQVLR) were monitored by MS detector in a multiple reaction monitoring mode. Three transitions from doubly charged ion of YEINVLR peptide were recorded: 453.75→136.1, 453.75→293.0 and 453.75→614.7, whereas the structural analogue produced three following transitions: 460.75→136.1, 460.75→292.9 and 460.75→628.7. A standard addition calibration curve was used for quantitation. The amount of peptide in biological sample was used as a representation of the amount of parent protein. Microbore, direct injection nanoflow and preconcentration nanoflow LC/MS methods were compared in terms of feasibility, linearity, sensitivity, accuracy and precision.

## METHODS

*Table 1. LC/MS parameters of the tested methods.*

| | I SETUP (microflow LC/MS) | II SETUP (direct injection nanoflow LC/MS) | III SETUP (preconcentration nanoflow LC/MS) |
|---|---|---|---|
| System configuration | HPLC system; HESI II interface; TSQ Vantage | nanoRSLC system; PicoChip system (used for separation and as ion source); TSQ Vantage | nanoRSLC system; PicoChip system (used as ion source); TSQ Vantage |
| Analytical column | Hypersil BDS C18 (100 x 1 mm I.D., 3 µm, 130 Å, Thermo Scientific) | ProteoPep™ II, C18 (100 mm x 75 µm I.D., 5µm, 300Å, New Objective) | Acclaim PepMap100 RSLC C18 (15 cm x 75 µm I.D., 2 µm, 100 Å, Thermo Scientific) |
| Mobile phase | A – FA (0.1%, v/v) in $H_2O$; B – FA 0.1%, v/v) in AcN | A – FA (0.1%, v/v) in $H_2O$; B – FA (0.1%, v/v) in AcN/ H2O (80:20, v/v) | A – FA (0.1%, v/v) in$H_2O$; B – FA (0.1%, v/v) in AcN/H2O (80:20, v/v) |
| Gradient elution profile/ program | 25% B, 0–1 min; 25%-95% B, 1-6 min | 15% - 80% B in 8 min | 4%-25% B in 10 min; 25%-95% B in 22.5 min |
| Flow rate | 80 µL $min^{-1}$ | 600 nL $min^{-1}$ | 300 nL $min^{-1}$ |
| Injection volume | 2.5 µL | 0.5 µL | 2.5 µL |
| Ion monitoring mode | MRM | MRM | MRM |
| Capillary voltage (kV) | 3.0 | 1.8 | 1.8 |
| ESI capillary I.D./tip (µm) | 100/100 | 75/15 | 75/15 |
| Nebulising gas flow (au.) | 15 | - | - |
| Ion tube temperature (°C) | 320 | 220 | 220 |
| Vaporizer temp. (°C) | 100 | - | - |
| Desolvation gas pres. (psi) | 5 | - | - |

## RESULTS

This study developed and compared three analytical procedures for the absolute quantification of troponin T in mouse hearts. All three procedures: microbore, direct injection nano- and preconcentration nanoLC-MS/MS provided excellent linearity, precision and accuracy.

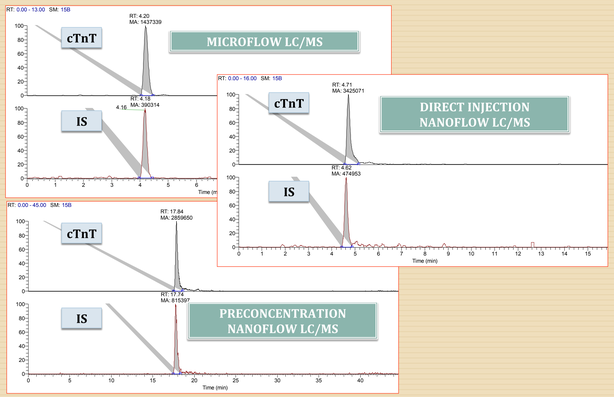


*Fig. 3. Representative LC-MRM-MS chromatograms of cTnT-specific peptide (m/z = 453.75) and internal standard (m/z = 460.75) obtained by analysis of a selected unspiked sample using microflow, direct injection nanoflow, and preconcentration nanoflow LC-MS/MS.*

## RESULTS

### LINEARITY AND SENSITIVITY OF THE METHODS

The chip-based preconcentration nanoflow LC/MS method offered the highest sensitivity (LLOQ = 0.25 fg $\mu L^{-1}$) and a minimal matrix effect generating the most reliable quantitative results (Table 2). This LLOQ value was 8 times better than in direct injection nanoflow LC/MS and 200 better than in microflow LC/MS.

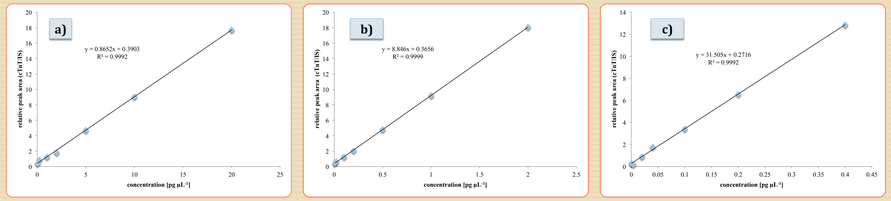


*Fig. 4. Standard addition calibration curves for (a) microflow, (b) direct injection nanoflow and (c) preconcentration nanoflow LC/MS.*

*Table 2. Comparison between the microflow, direct injection nano-, preconcentration nanoflow LC/MS method in terms of linear range, slopes and Y-intercepts of the weighted calibration curves. Values are mean ± SD, n=5.*

| | Microflow | Direct injection nanoflow | Preconcentration nanoflow |
|---|---|---|---|
| Linear range [pg $\mu L^{-1}$] | 0.05-20 | 0.01-2 | 0.00025-0.4 |
| Slope | 0.8652 ± 0.0523 | 8.846 ± 0.4743 | 31.505 ± 1.5661 |
| Y-intercept | 0.3903 ± 0.0191 | 0.3656 ± 0.0185 | 0.2716 ± 0.0182 |
| LOD [pg $\mu L^{-1}$] | 0.02 | 0.003 | 0.0001 |
| LOQ [pg $\mu L^{-1}$] | 0.05 | 0.01 | 0.00025 |

### ACCURACY AND PRECISION OF THE METHODS

The differences in method accuracy or precision for microflow, direct injection and preconcentration nanoflow methods, respectively, were not markedly different (Table 3).

*Table 3. Intra-batch and inter-batch accuracy and precision determined for chip-based preconcentration nanoflow LC/MS method.*

| QC ID | Nominal conc. [pg $\mu L^{-1}$] | Intra-batch n | Mean conc. found [pg $\mu L^{-1}$] | Accuracy (%) | CV (%) | Inter-batch n | Mean conc. found [pg $\mu L^{-1}$] | Accuracy (%) | CV (%) |
|---|---|---|---|---|---|---|---|---|---|
| Preconcentration nanoflow | | | | | | | | | |
| LLOQ QC | 0.00025 | 5 | 0.00026 | 104 | 6 | 15 | 0.00024 | 96 | 7 |
| LQC | 0.004 | 5 | 0.0041 | 102.5 | 5 | 15 | 0.00395 | 98.7 | 5 |
| MQC | 0.1 | 5 | 0.095 | 95 | 4 | 15 | 0.096 | 96 | 3 |
| ULOQ QC | 0.4 | 5 | 0.39 | 97.5 | 4 | 15 | 0.39 | 97.5 | 4 |

Troponin T content in hearts of C57BL/6 and ApoE/LDLR double knock-outs established with preconcentration nanoflow method was: 0.28 ±0.02 and 0.30 ± 0.03 µg $mg^{-1}$ tissue, respectively.

## CONCLUSIONS

- **Chip-based nanospray LC/MS offers massive sensitivity gain with accuracy, reproducibility and separation time similar to microflow methods.**
- **The proposed setup for absolute quantification of cTnT could be a useful template for other targets in quantitative proteomics.**


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