SRM vs HR-MS quantitative proteomics: matrix effect and LOQ in serum

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Overview

2-D clean-up kit

Trypsin digestion

→ Spike of 4 proteins digest

→ Triple quadrupole (SRM) / HR-MS t-MS² / t-SIM

→ HR-MS t-MS²: Obvious choice of the right peak

Introduction

Serum is one of the gold standards matrix for diagnosis or prognosis purposes

→ Most of proteins that are indicators of disease state or progression lies in the low serum concentration range

→ Serum is very complex due to :
- Large protein concentration dynamic range
- High number of different proteins present depending on the physiological state

→ Matrix effects ? LOD / LOQ ?

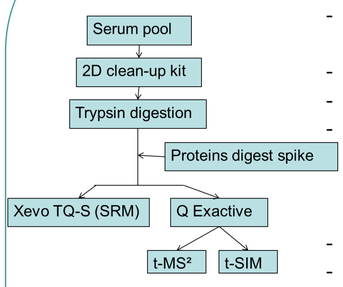
→ **GOAL OF THIS STUDY**: compare triple quadripole versus HR-MS technologies
- matrix effects
- LOQ

→ Triple quadrupole: Xevo TQ-S (Waters) / HR-MS (Orbitrap): Q Exactive (Thermo)

→ Comparison of results obtained with the 2 insruments

Methods

Experimental scheme



- 2D clean-up kit (GE) was applied according to the manufacturer recommendations for protein precipitation
- Protein digestion was performed using trypsin, after reduction and alkylation
- Samples were dried under vacuum
- Samples were reconstituted in water 0.1% formic acid (v/v) and spiked with the 4 exogenous proteins digest mix (MassPrep Digestion Standard Mix2, Waters): Bovine Serum Albumine (BSA, P02769), Glycogen Phosphorylase B from rabbit (GPB, P00489), Enolase 1 (ENO, P00924) and Alcohol Deshydrogenase from *Saccharomyces cerevisiae* (ADH, P00330)
- Samples were splitted in 2 and stored at -20°C until injection
- Each samples was injected on triple quadrupole (TQ) and High Resolution – Mass Spectrometry (HR-MS) instruments

Selected peptides

Peptides were selected according the following criteria:
- Already observed in discovery method using MPDS Mix
- Absence of the peptide sequence in human proteome (BLAST)
  - Not possible for GPB peptides
- Length between 4 and 40 amino acids
- No cysteine, no methionine in peptide sequence
- No potential glycosylation site (NXT, NXS)
- No miss cleavage (no RP/KP for cleavage site to avoid partial digestion)

Protein	Peptide	Parent (*m/z*)
BSA	AEFVEVTK	461.7
	HLVDEPQNLIK	653.4
	LVNELTEFAK	582.3
	QTALVELLK	507.8
	LGEYGFQNALIVR	740.4

Protein	Peptide	Parent (*m/z*)
GPB	VAAAFPGDVDR	559.3
	TNFDAFPDK	527.7
	VLVDLER	422.3
	VIFLENYR	527.3
	VLYPNDNFFEGK	721.9

Protein	Peptide	Parent (*m/z*)
ENO	TFAEALR	404.2
	YDLDFK	400.7
	VNQIGTLSESIK	644.9
	AADALLLK	407.8
	NVNDVIAPAFVK	643.9

Protein	Peptide	Parent (*m/z*)
ADH	IGDYAGIK	418.7
	VLGIDGGEGK	472.8
	ANELLINVK	507.3
	DIVGAVLK	407.7
	EALDFFAR	484.7
	VVGLSTLPEIYEK	724.4

UPLC:

Acquity I-Class (Waters): HSS T3 2.1*150 mm, 1.7 µm
- Column temperature: 40°C, Flow rate: 0.250 mL/min
- 43 min linear gradient from 0% to 35% ACN 0.1% formic acid (v/v) (total run time: 60min)
- Volume of injection: 9 µL

Mass spectrometry:

Xevo TQ-S (Waters): Selected Reation Monitoring (SRM)
- Source parameters: capillary 2.0 kV, cone 35 V, source offset: 50 V, Desolvatation gas flow: 1000 L/h
- LM 1 / 2 Resolution: 2.8 / 2.8, HM 1 / 2 Resolution: 14.9 / 14.8
- 3 transitions (y ions) per peptide
- 3 time windows with 24, 15 and 24 transitions : auto-dwell time (12 points per peak , FWHH of 8 s)

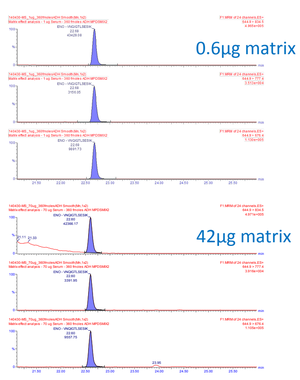
Q Exactive (Thermo):
- Targeted MS²: resolution: 35000, AGC target: 5 10^5, max accu time : 125 ms, isolation window: 3 *m/z*, isolation offset: 0.5 *m/z*.
- Targeted SIM: resolution: 140000, AGC target: 2 10^5, max accu time: 125 ms, isolation window: 3 *m/z*, isolation offset: 0.5 *m/z*, MSX count (multiplex): 4.

Results

Comparison of SRM (Xevo TQ-S), t-MS² and t-SIM (Q Exactive)

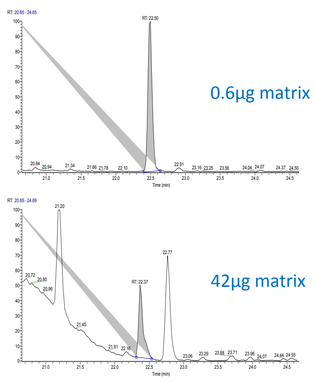
Peptide VNQIGTLSESIK of Enolase (465 fmoles – 22 ng)

SRM

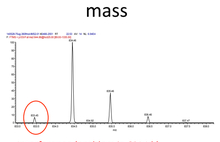


- Interference on transition 644.9>834.5: $(M+2H)^{2+} > y8^{+}$
  - Chromatogram 42µg of matrix
  - Area increases when amount of matrix increases
  - Not suitable for quantification
- 2 other transitions are rather constant when matrix amount is increased
  - Suitable for quantification

t-MS²

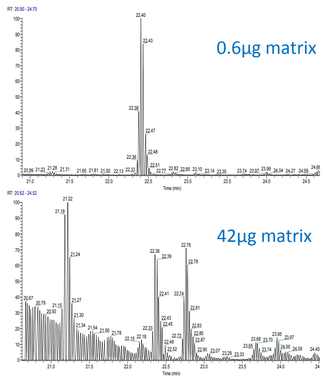


- Interference on 834.4562 ($y8^{+}$)
  - Can be avoided with the fragment exact mass



- Reliable quantification of the peptide when the matrix amount increases
- No matrix effect observed!

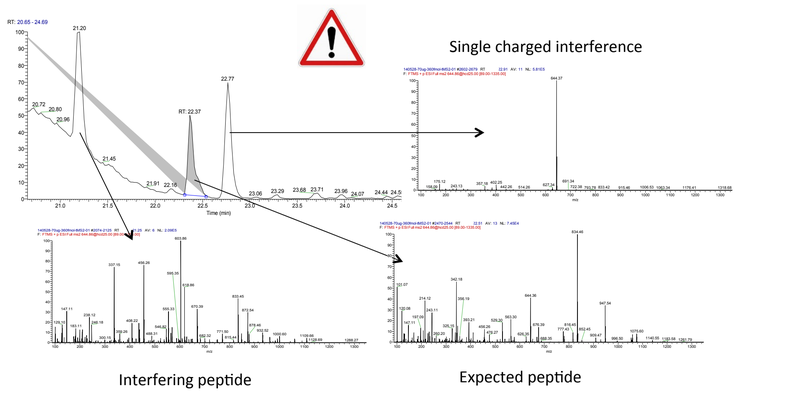
t-SIM



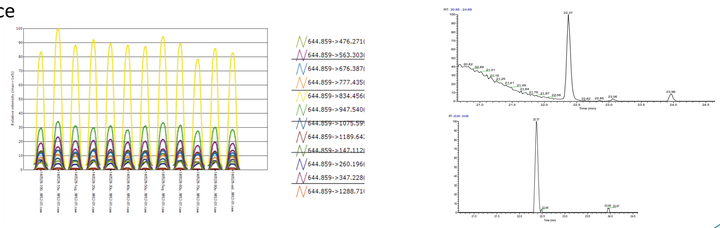
- Suppression effect observed when matrix amount increases !
- Not reliable quantification

All the selected peptides give the same conclusion (except for SIM, for which results are very variable): matrix effect is very dependent of the selected transition and does not seem to be predictable (not related to peptide length, hydrophobicity,…)

HR-MS spectra (t-MS²) for VNQIGTLSESIK with 42 µg matrix



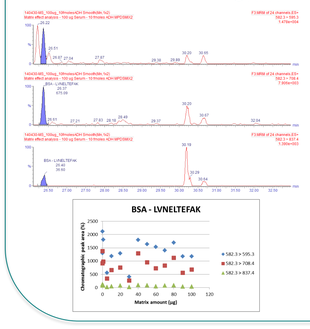
- Targeted MS² allows to easily identify the chromatographic peak corresponding to the target peptide
- Provides also information on interfering compounds
- It allows to easily select the most intense fragments and to check for matrix interference



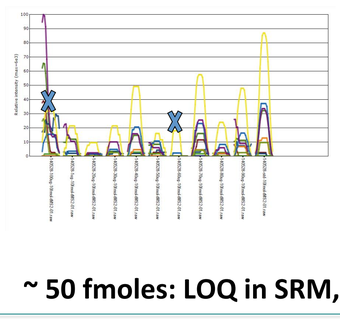
LOD of SRM (Xevo TQ-S) and t-MS² (Q Exactive)

Peptide of BSA (58 fmoles – 4 ng)

SRM



t-MS²



- In SRM
  - the 3rd transition (y7) is not detected
  - Ion ratio (y5/y6) is OK in all samples except 2 (60 µg and 90 µg matrix)
- In targeted MS²
  - Detection in all samples except 2(X) (60 µg and 100 µg matrix)
  - Ion ratio not constant through samples series

~ 50 fmoles: LOQ in SRM, LOD in t-MS²

Conclusions

- **In triple quadrupole, matrix interferences are observed on several transitions (predicted as the most intense)**
  - **Transitions dependent, not predictable**
- **t-MS² with HR-MS is the less senstitive to the matrix effect of serum**
  - **All peptides are detected with equivalent area whatever the matrix amount**
- **Use of HR-MS in t-MS² mode for transitions selection for triple quadrupole**
  - **Most intense fragments are for the majority (~66%) the highest ones obtained using Xevo TQ-S**

Acknowledgements

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