

Target Analyses in Parallel Reaction Monitoring Mode (PRM)

Skyline Webinar

January 13, 2015

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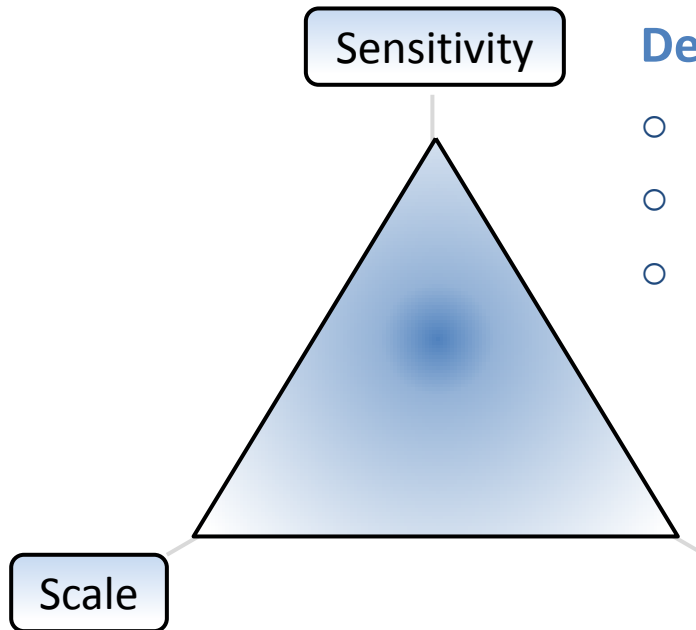
*Head Luxembourg Clinical Proteomics Center
Invited Professor University of Luxembourg*



INTRODUCTION

TARGET QUANTITATIVE ASSAYS

Characteristics of Quantitative Assays



Detection of abundance components

- Wide range of protein concentrations
- Need for low LoD / LoQ
- Wide dynamic range

Biological variability

- Need to perform large studies
- Throughput, *i.e.* robust platform
- Multiplexing capability

Selectivity

Complexity of proteomic samples

- Reduce sample complexity
(*interferences in measurements*)
- High resolution instruments: LC + MS

Types of Targeted Experiments

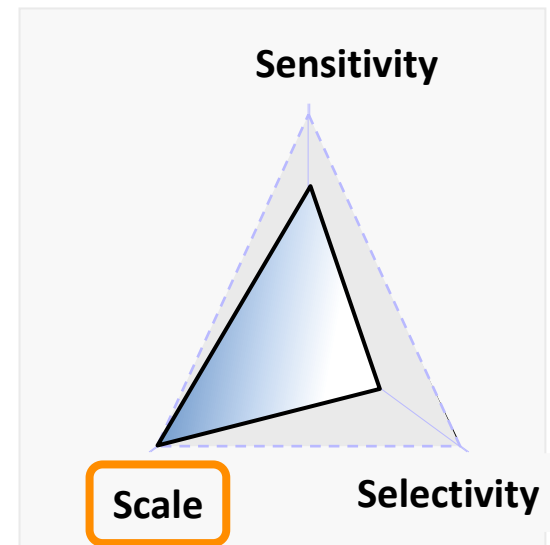
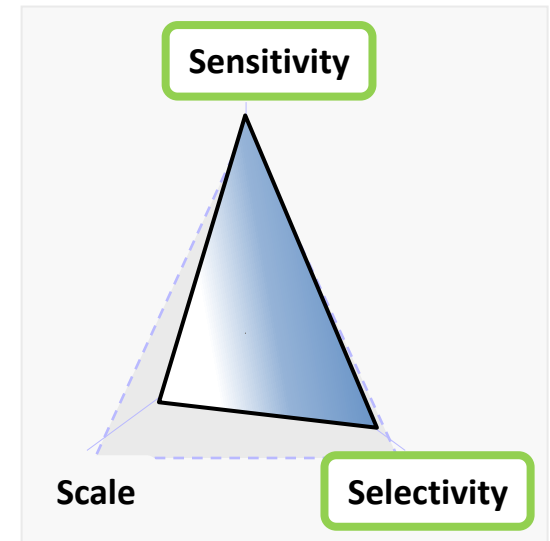
- **Classical Quantitative Experiment**

- **Precise quantification** (*biomarkers*)
- **Internal standards** (calibrated amount)
- Limited number of analytes

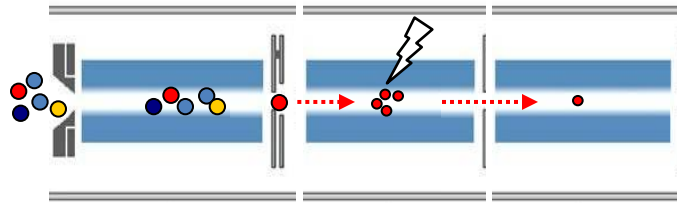
- **Screening Experiment**

- **Detection** of peptides in complex matrix (*e.g. blood or urine samples*)
- **Large scale** (*hundred of candidates*)
- **Multiplexing** capability

Gallien et al., J. Mass Spectrom. 2011

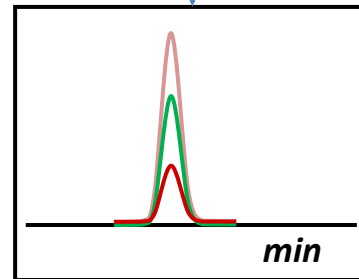


Selected Reaction Monitoring (SRM)

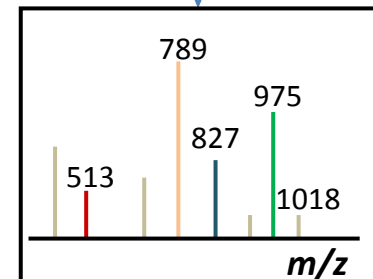


Triple quadrupole instrument

Quantification
(Traces; AUC)



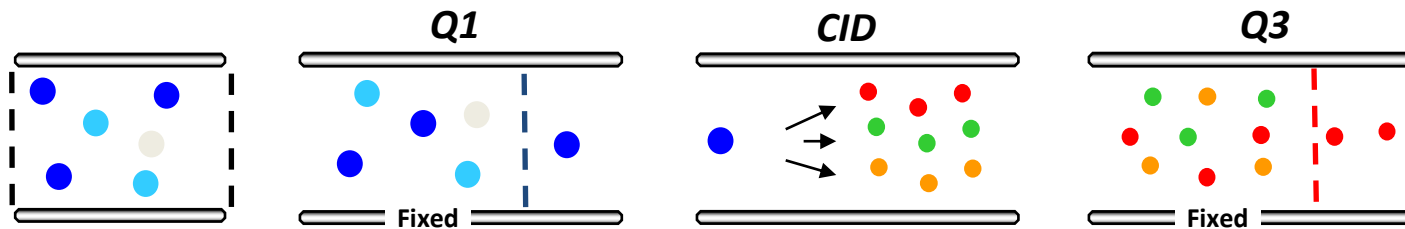
Identity confirmation



Kim et al., Proteomics Clin. Appl. 2013

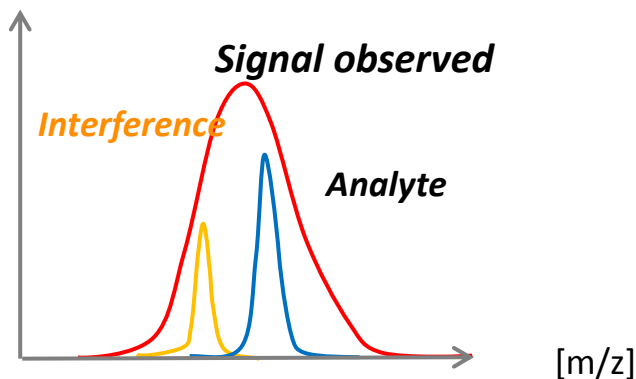
Targeted Proteomics

- **SRM experiments:** triple quadrupole instrument - reference method



- **Limitations:**

- Actual number of transitions to be monitored
- **Low resolution** mass analyzers (both Q1 and Q3)
 - > co-isolation of **interferences** along with the precursor ion

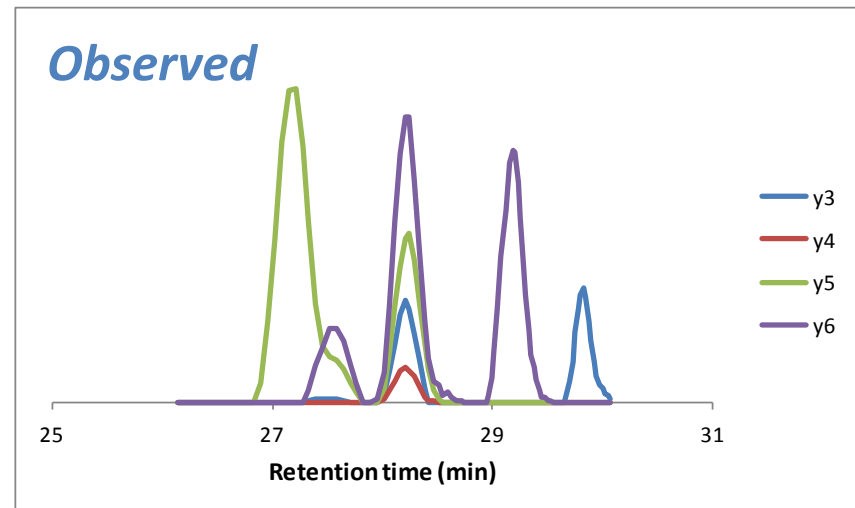
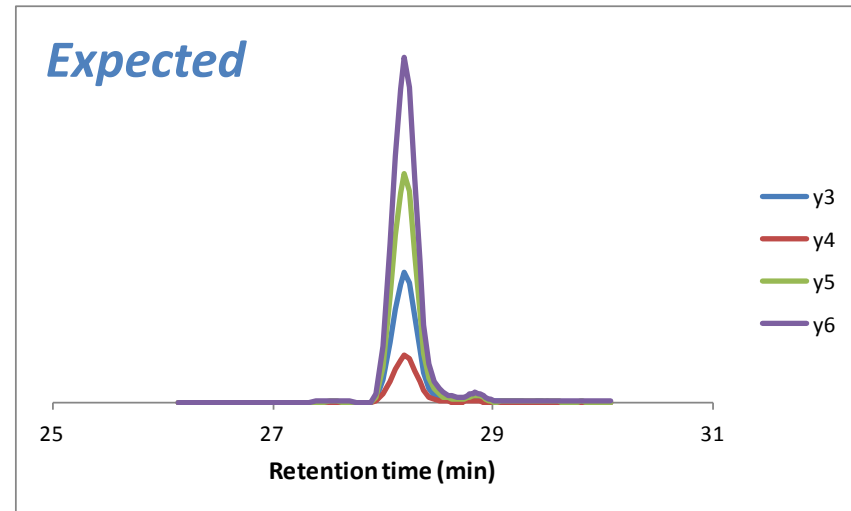


Gallien et al., J. Mass Spectrom. 2011

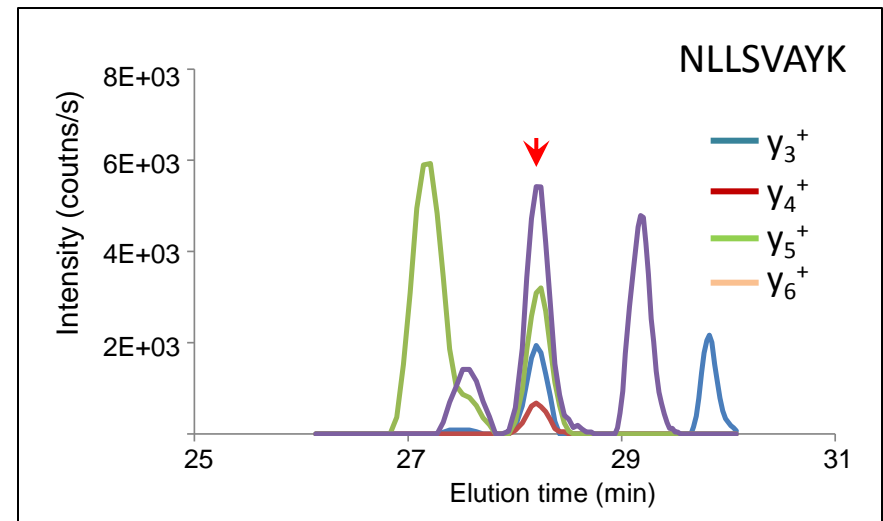
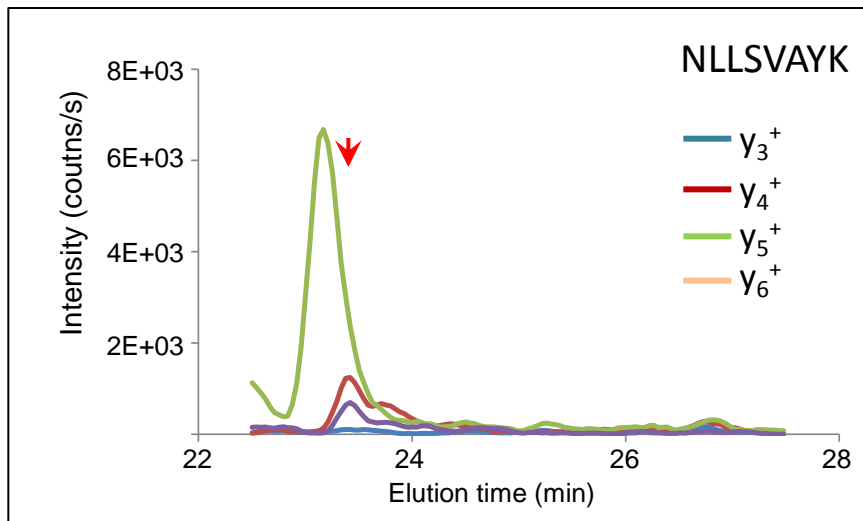
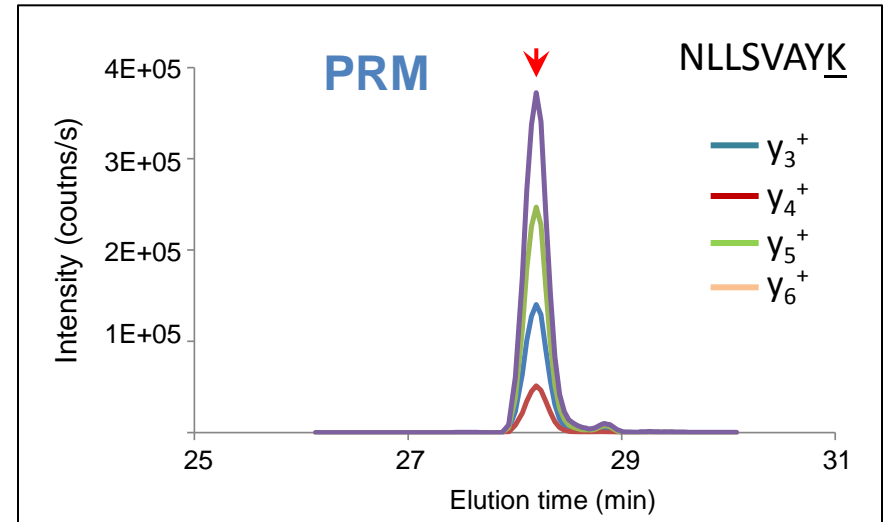
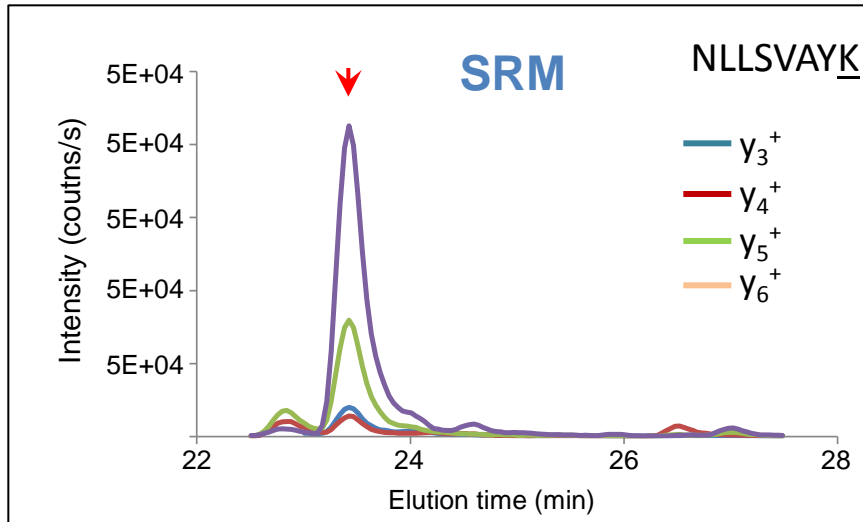
Selectivity is an Issue



*"One train can hide another one !"
Check twice !*



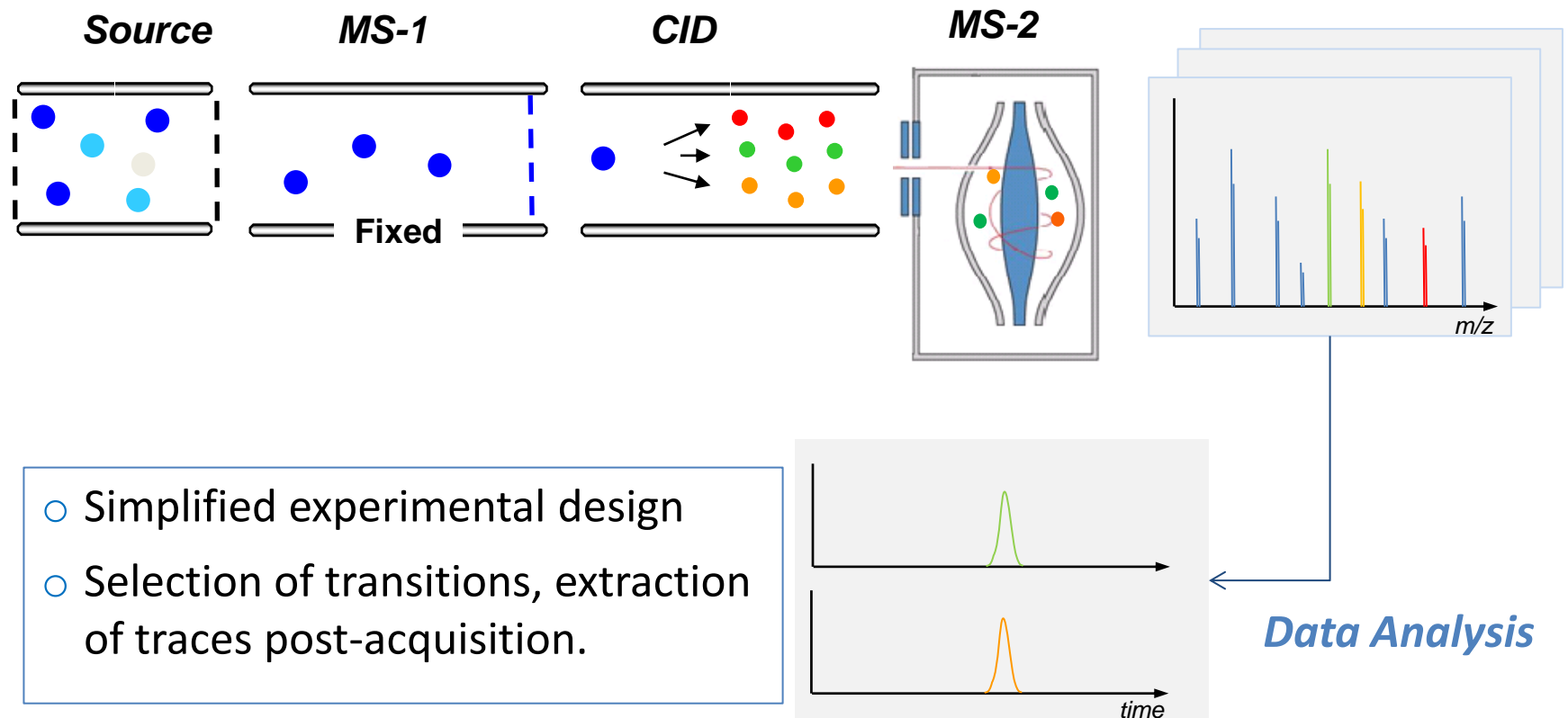
Selectivity of Measurements



PARALLEL REACTION MONITORING (PRM)

Parallel Reaction Monitoring (PRM)

- Performed on a quadrupole / orbitrap instrument (high-resolution)

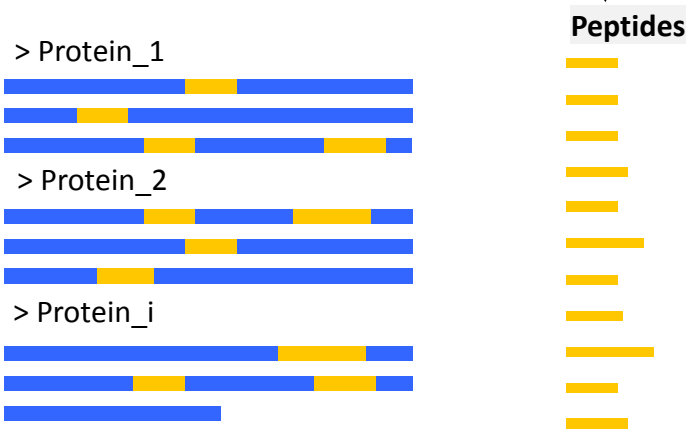
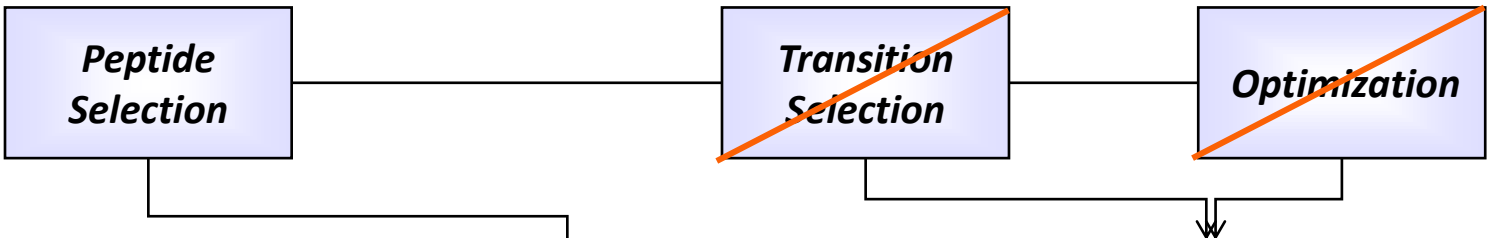
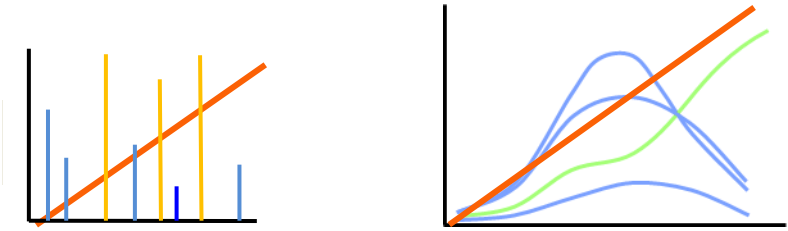


Gallien et al., J. Proteomics 2014

Design of a Targeted Experiment: PRM Mode

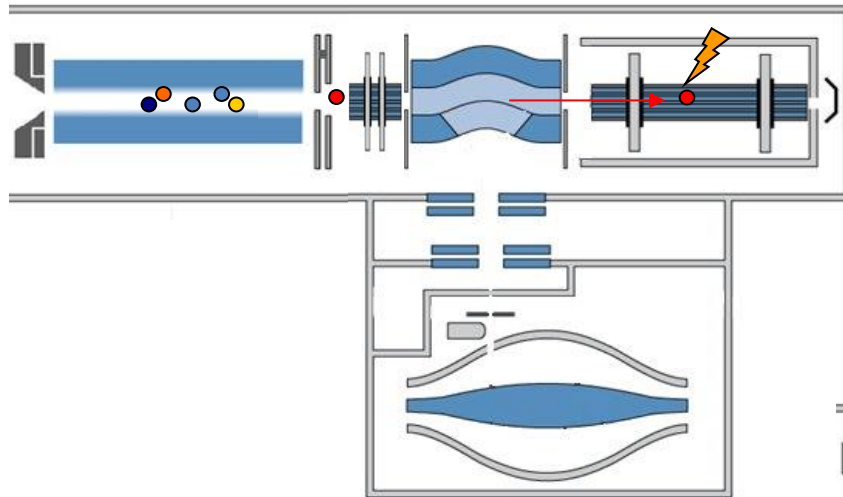
Quadrupole – Orbitrap / PRM mode

- *Simplified Method Design*
- *Flexible Data Analysis*

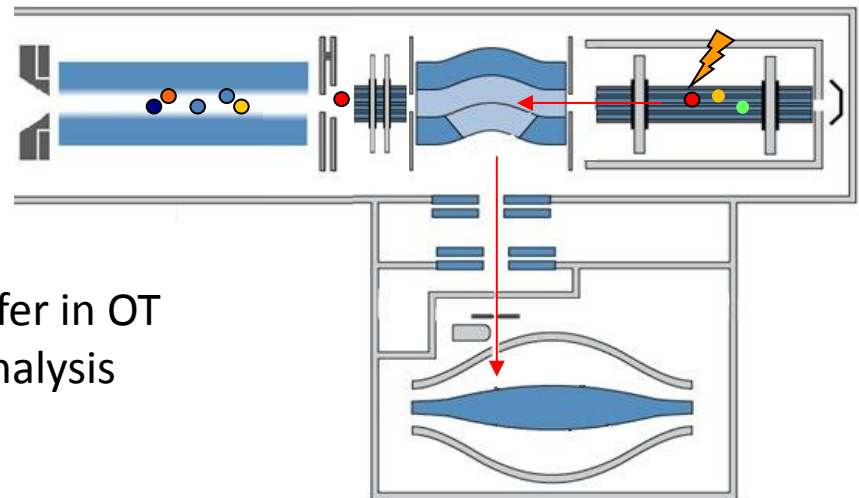


Pep	Q1	Frag	CE	Time	Win	Fill time	Area
Pep_A	724	786	30	25.3	0.7	50	
Pep_A		887	30				
Pep_A		966	30				
Pep_A		108	30				
Pep_B	863	757	35	17.5	0.7	50	
Pep_B		97	35				
Pep_B		108	35				
Pep_B		112	35				
.....							
Pep_N	563	797	25	24.3	0.7	50	
Pep_N		899	25				
Pep_N		999	25				
Pep_N		1101	25				

Parallel Reaction Monitoring Mode (PRM)

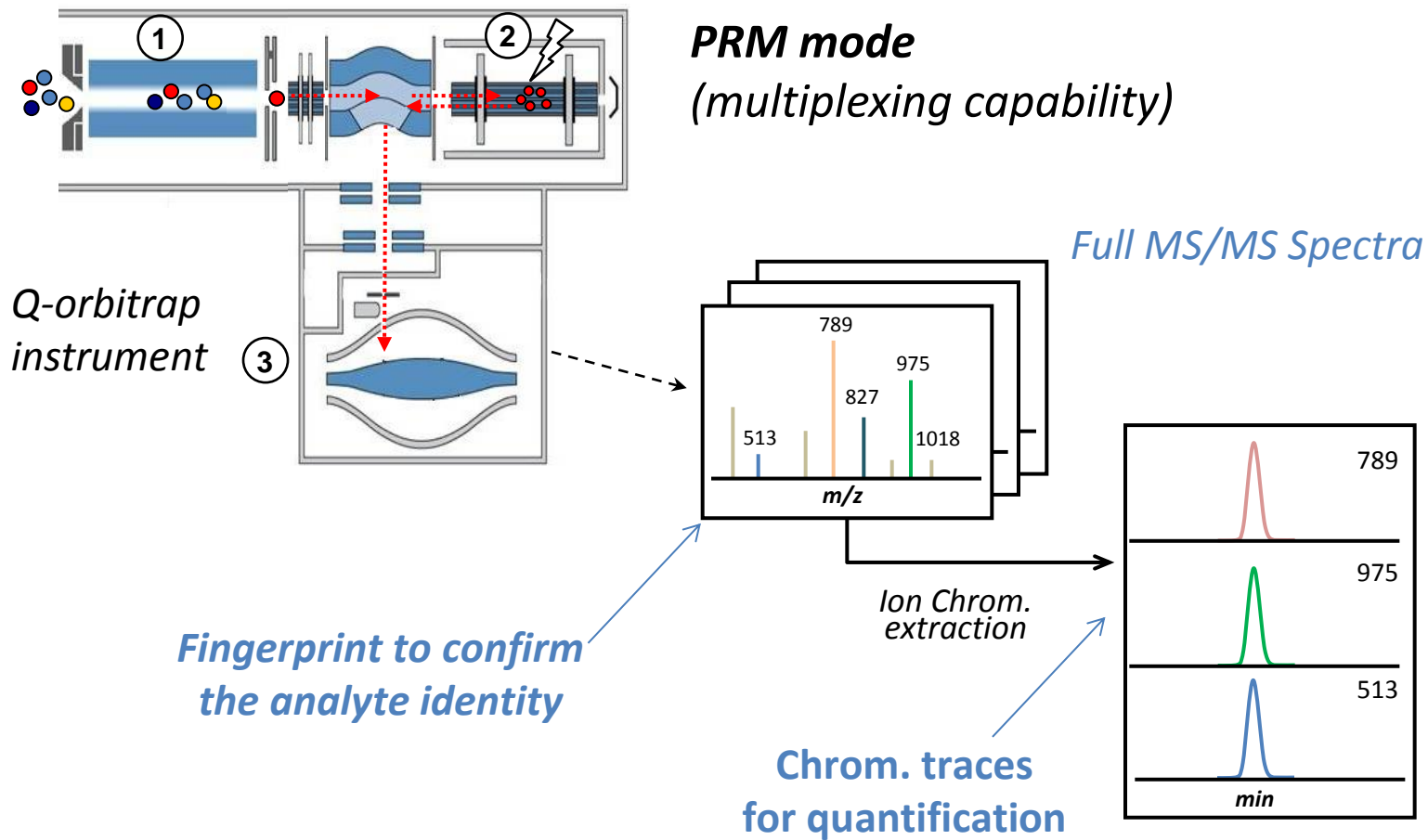


1. Isolation
2. Fragmentation



3. Transfer in OT
4. HR Analysis

Parallel Reaction Monitoring Experiment

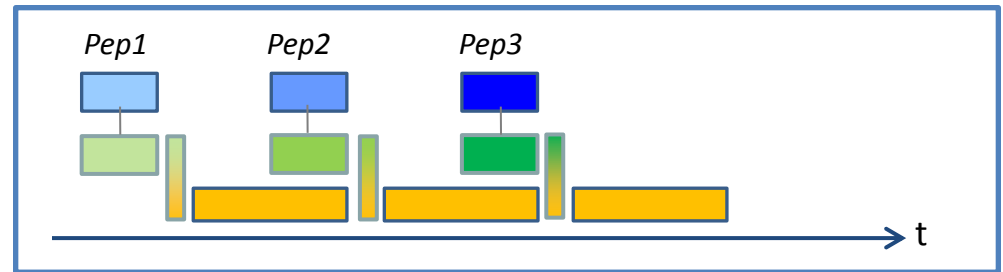


Kim et al., Proteomics Clin. Appl. 2013

Quantification Methods in PRM

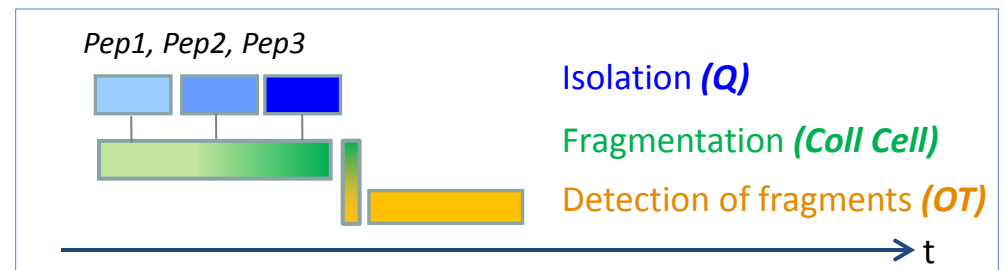
Sequential: Iterative analyses

- Sequential isolation / fragmentation events
- Multiple detection scans



Multiplexed: Parallel analysis

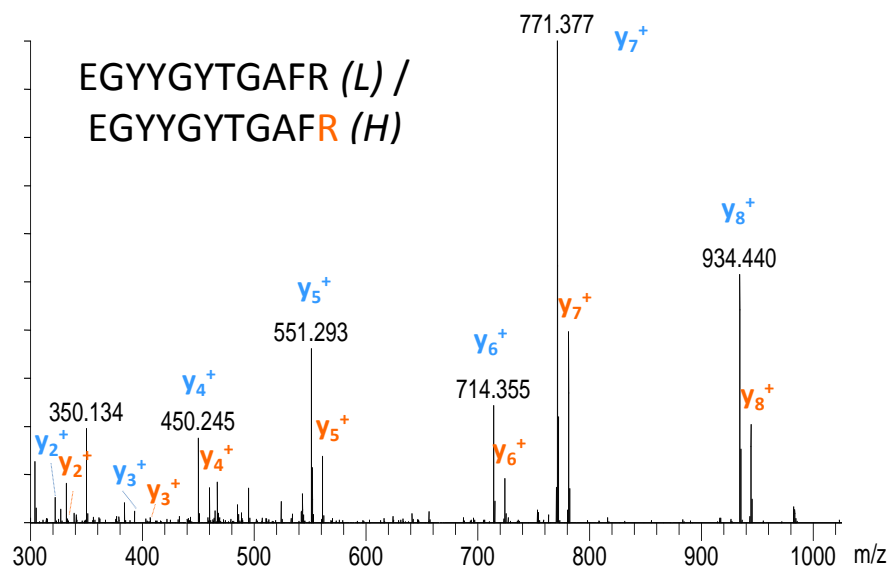
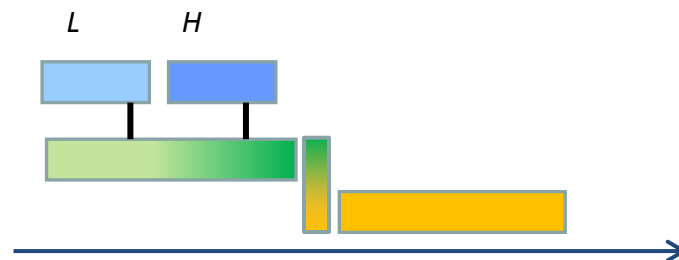
- Sequential isolation and fragmentation
- Intermediate storage
- **Single detection scan**



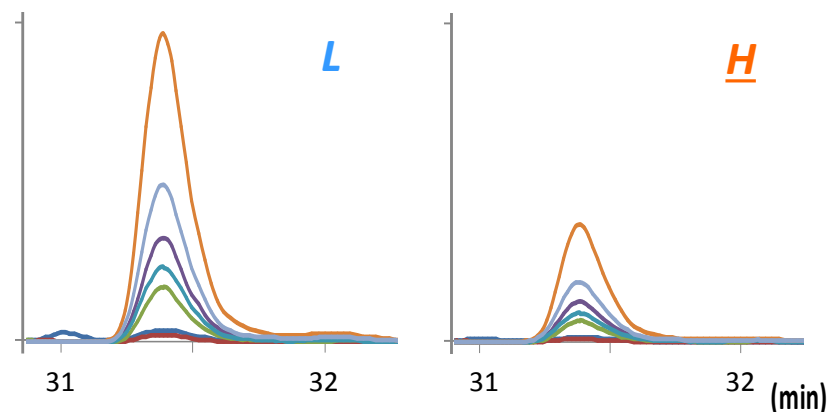
Gallien et al., Mol. Cell. Proteomics 2012

PRM Mode: Multiplexed Analysis

- Sequential isolation of L/H precursors
- Fragmentation and storage in HCD cell
- One orbitrap detection scan



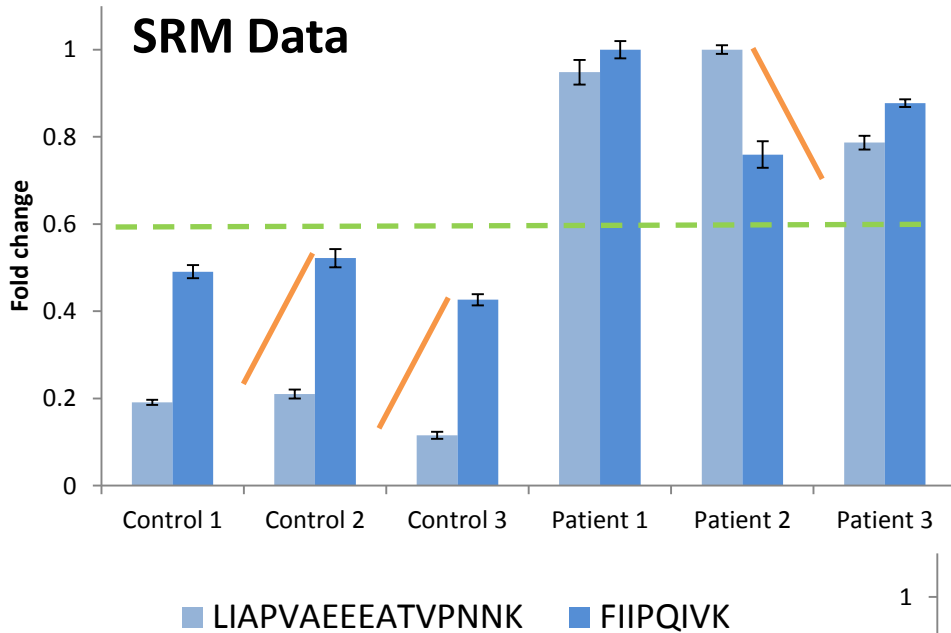
- Quantification based fragment ions
- Selection of ions post-acquisition



Quantification similar to SRM, but using high resolution fragment ions

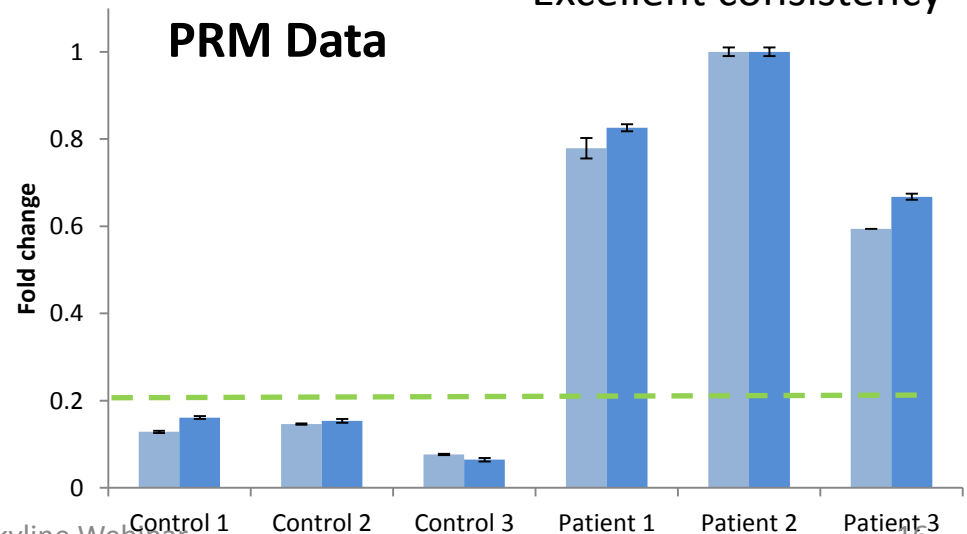
PRM Analyses of Plasma Samples

✘ Poor correlation



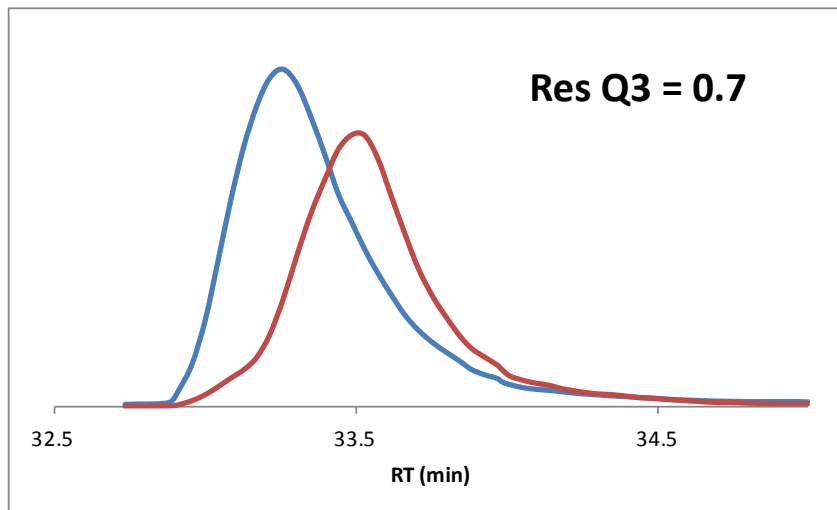
- Peptides **FIIPQIVK** and **LIAPVAEEEEATVPNNK**
- Surrogates: *L-lactate dehydrogenase*
- Plasma samples (*Alb+IgG depleted*)
- **Pilot study:** 3 controls and 3 disease

✔ Excellent consistency

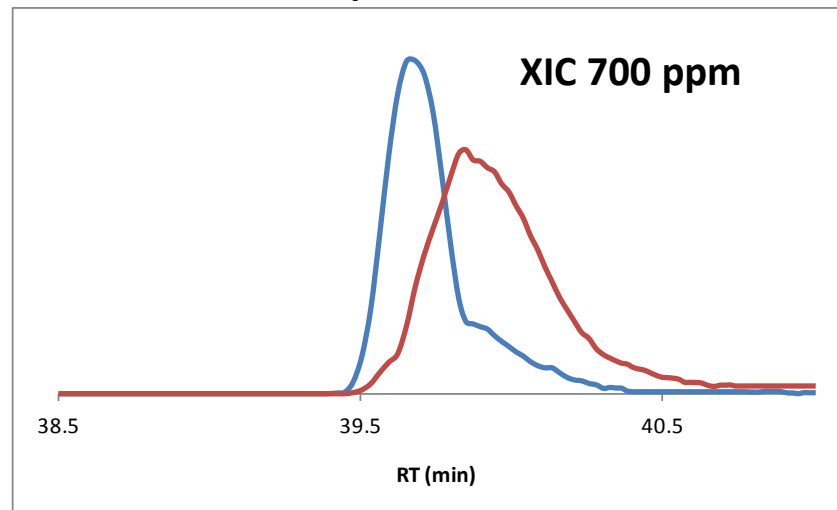


Selectivity in HR/AM Mode /1

SRM on Triple quadrupole



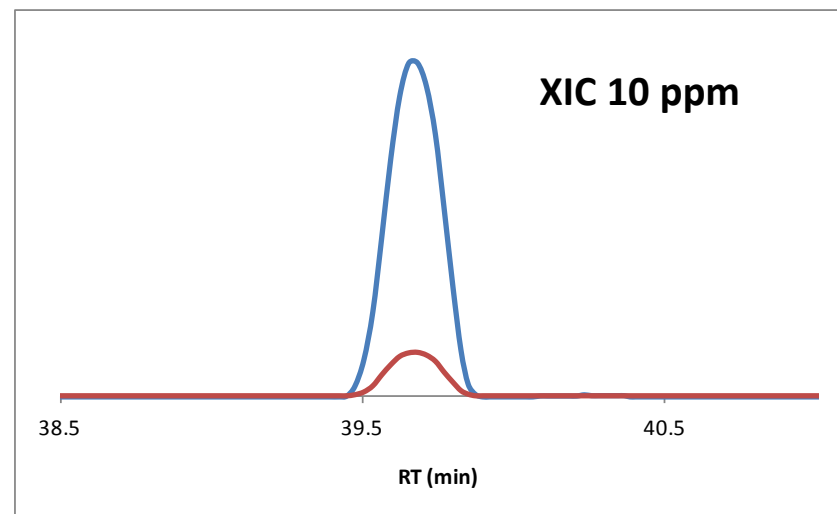
PRM on Q-Orbitrap



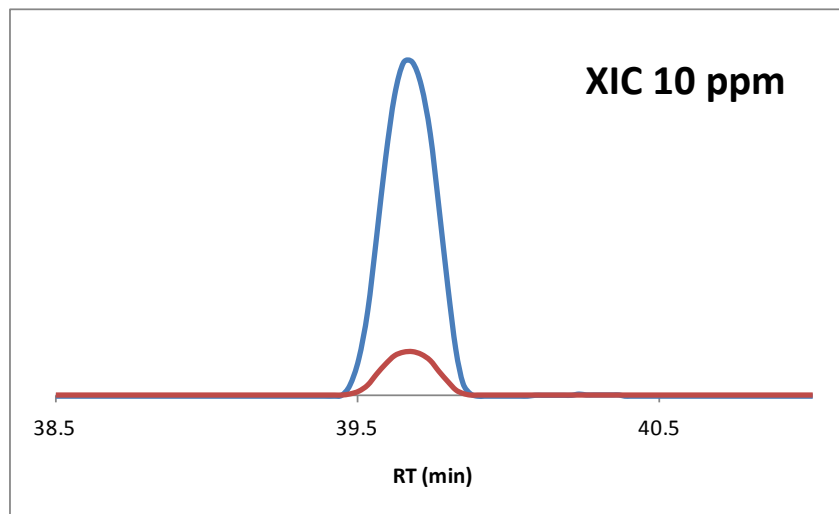
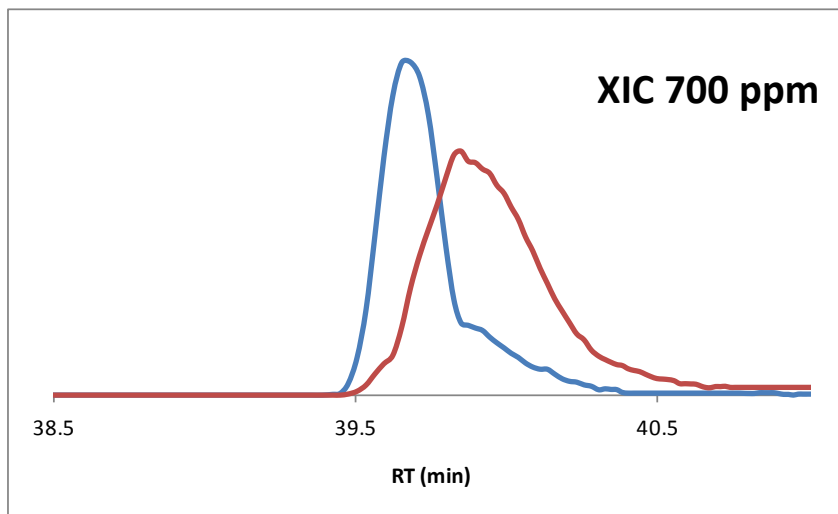
SDLAVPSELALLKYK
spiked in urine samples

Transitions : — 682.40->878.54
— 682.40->977.61

Gallien et al., J. Proteomics 2013



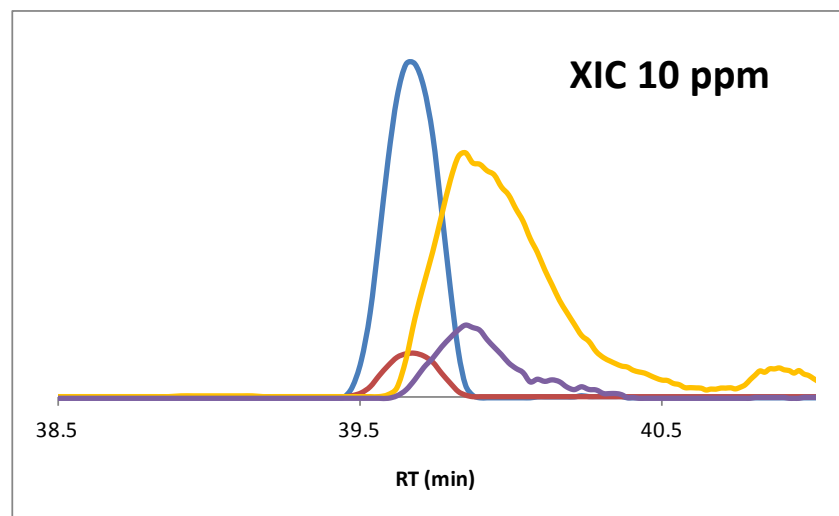
Selectivity in HR/AM Mode /2



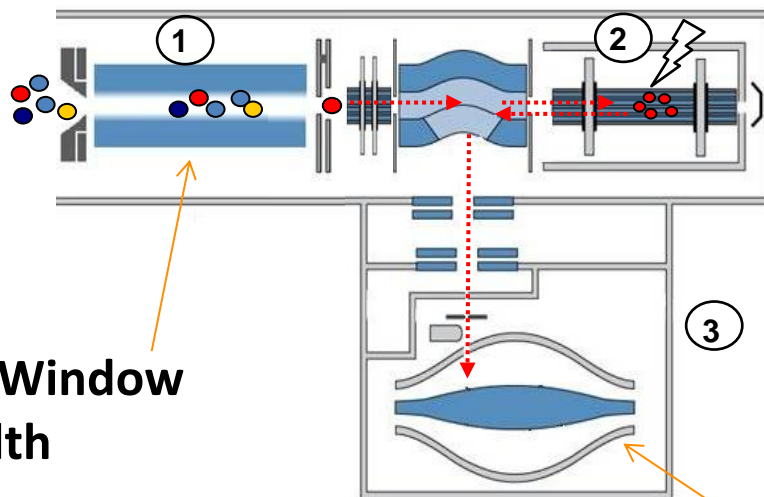
SDLAVPSELALLKYK
spiked in urine samples

Transitions

- 682.40 -> 878.54
- xxx -> 878.45 , **Interference**
- 682.40 -> 977.61
- yyy -> 977.52, **Interference**



Selectivity of PRM Measurements



**Selection Window
Width**

[Typically: 1.0 (2.0) m/z

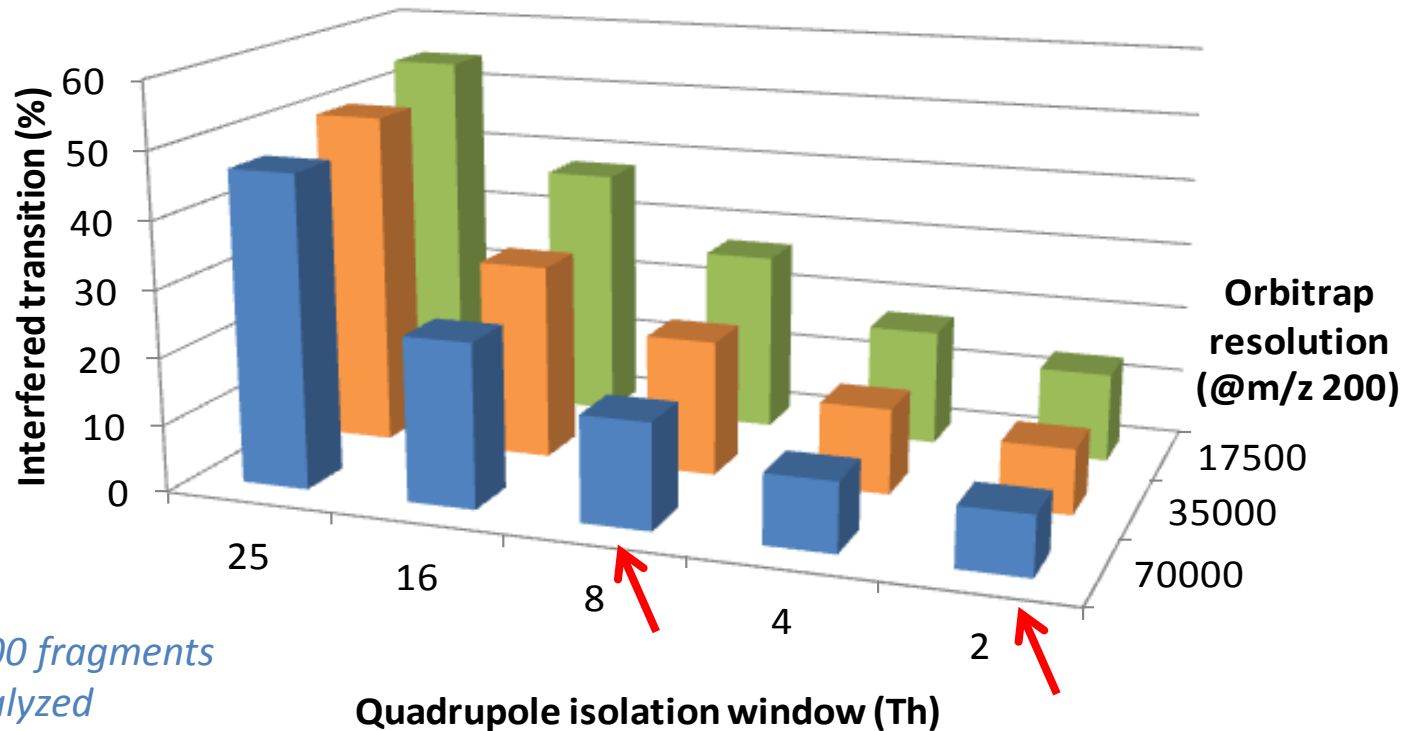
High selectivity: 0.7 (0.4) m/z]

(High performance Quad)

High Resolution (OT)

[35,000 – 140,000]

Selectivity of MS/MS Analyses



2200 fragments
analyzed

- > Selectivity of measurements is affected by the precursor isolation window
- > Increased (nominal) orbitrap resolution (17 k to 70k) partially compensate

Conclusion

- **Parallel Reaction Monitoring**
 - High-resolution accurate mass quantification is an alternative to conventional SRM
- **Simple experimental design**
 - Acquisition and data analysis are decoupled
 - Only precursor m/z and elution times are required *a priori*
- **Iterative data processing: selection of fragment ions post-acquisition**
 - Data analysis is performed using conventional tools: *Skyline*
- **Improved data quality**
 - High confidence assignments: *accurate mass; reference MS/MS spectra*
 - Increased analytical precision: *high selective measurements.*