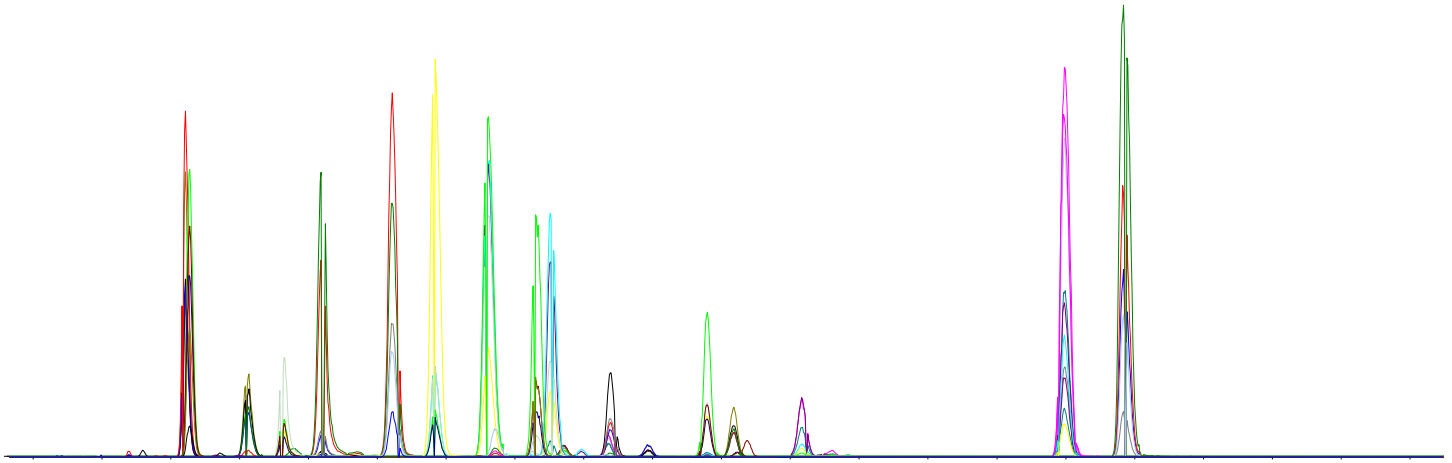
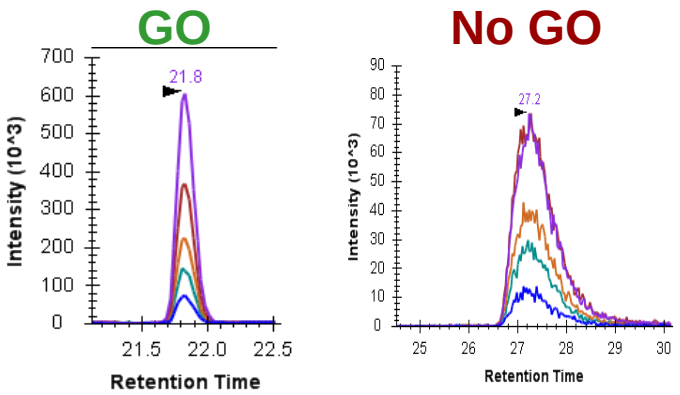
# System Suitability for LC-MS



# What is System Suitability?

- "System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated". (FDA)
- “The checking of a system, before or during analysis of unknowns, to ensure system performance” (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceutical for Human Use [ICH])
- **Simply put: Analysis of a known sample to assess system performance**
  - Helps to identify when the system is not working




**Define Pass/Fail Criteria**

# Is System Suitability the same as “QC”

- No!
- “QC” = Quality Control of your method
  - Takes into account sample prep
  - L, M, H QC standards used to show the entire workflow gives “expected” responses
- QC is method specific!!
- System suitability is designed for long-term system evaluation

# When should you run a system suitability evaluation?

- All the time!
  - Before unknown samples
  - Periodically (daily to weekly)
  - Before and after instrument repair
  - Before and after changes to hardware/plumbing
  - Before and after changes to software
  - Anytime you think your system is not performing optimally
  - When you think your system IS performing optimally, for longitudinal comparison

# What are metrics of a system suitability protocol?

**MS**

- MS response, sensitivity
- MS background, noise
- SRM Transition Ratio
- Precision

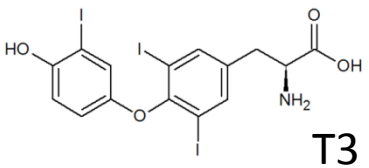
**LC**

- Retention time
- Peak shape, FWHM
- Chromatographic resolution
- Carryover

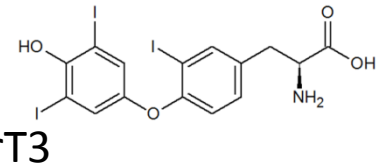
Goal: to confirm adequate signal/noise and reproducibility prior to analysis of real samples

# Resolution of Critical Pairs

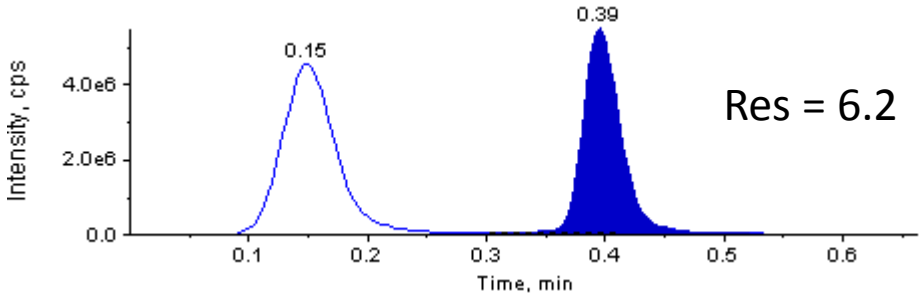
"critical pair"



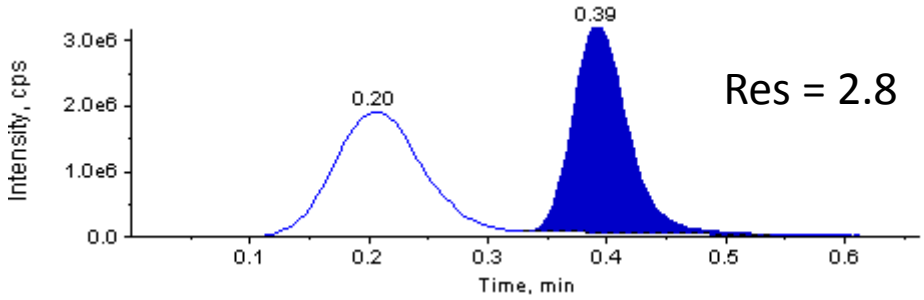

T3




rT3




Res = 6.2

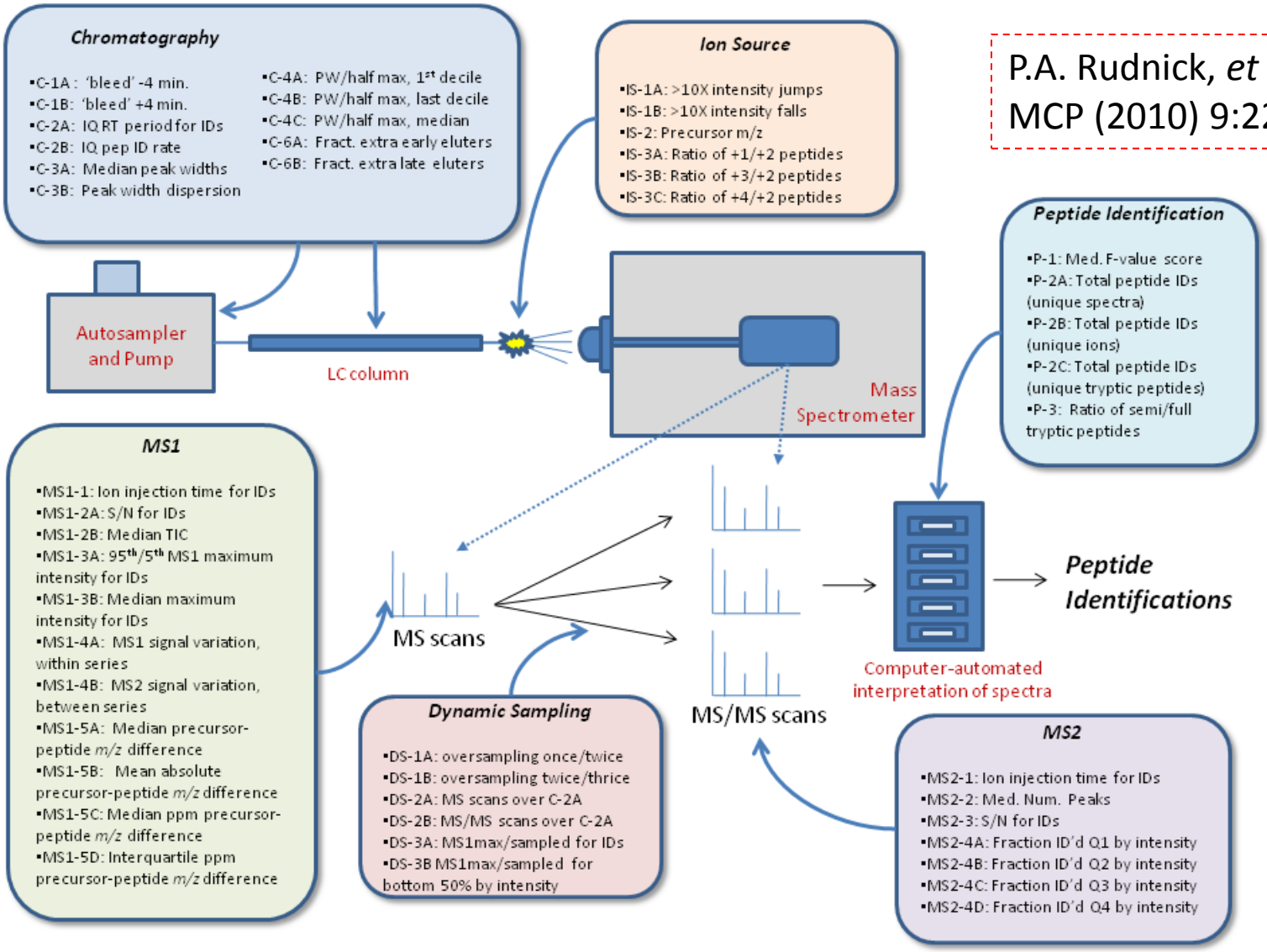



Res = 2.8

$$\text{Resolution} = \frac{t_{R1} - t_{R2}}{\frac{1}{2} \times (t_{W1} - t_{W2})}$$

| **Empirical Acceptance Limit** |
| --- |
| Resolution > 1.705 |

# Examples of Performance Metrics for "Discovery" LC-MS/MS performance




P.A. Rudnick, *et al*, MCP (2010) 9:225-241

http://peptide.nist.gov/metrics/

# What can you use as a system suitability sample?

- Stable sample that doesn't change with time
- Similar to your actual samples
  - Small molecule
  - Peptides
  - Proteins
- Minimal carryover
- Inexpensive
- Easy to prepare
- Mixture of several analytes
- Commercially available and QC'd

**Examples:**

- Cell lysate digested in bulk and aliquoted
  - Yeast, HeLa, etc
- Synthetic peptide standards
- Simple protein digest
- NIST standards
- Isotopically labeled compounds
- Peptides spiked into a complex background

# Fit-for-Purpose or Universal SST

Fit-for-purpose:

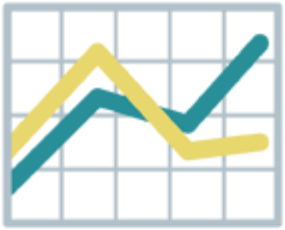
- Identical to target analyte
- Reflects assay performance
  - Noise
  - Carryover
  - Retention Time
  - LC Resolution

Universal:

- Common Setup
  - Easy, Robust Method
  - Common Ionization Mode (ESI/APCI)
- Reflects LCMS performance
  - Isolates if issues are related to the assay performance or LCMS System performance

# How can you assess “normal” system performance?


- Tune/calibrate LC and MS components
- Monitor variability of metrics over several injections
- Always use the same method for LC and MS, even if it is different than your sample method
- Ask the vendor for performance specifications for both LC and MS parameters
- Note when system performance deviates
- Compare to other systems running the same system suitability protocol

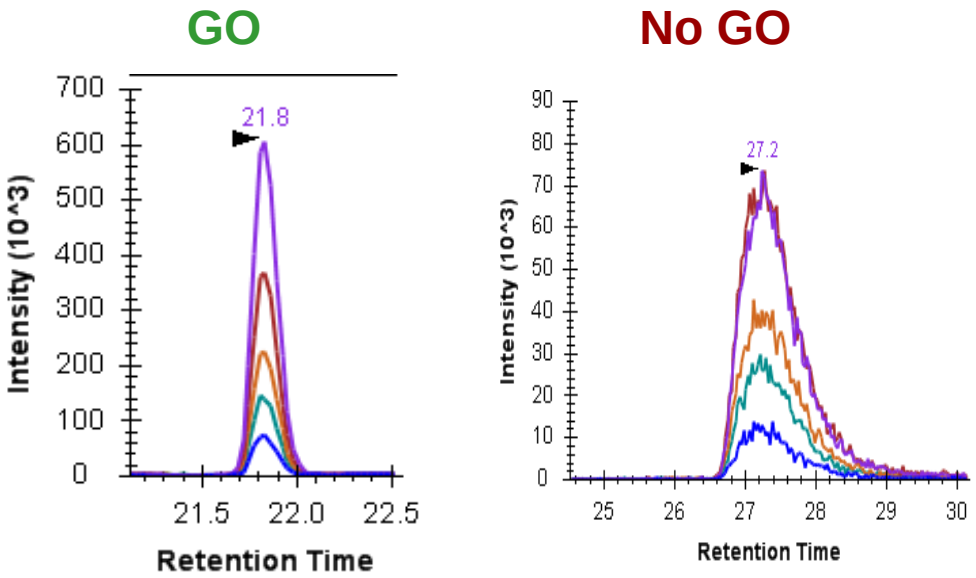


# How can you evaluate system suitability data (for free)?

- RawMeat.exe*: http://proteomicsresource.washington.edu/protocols06/
- NIST metrics: peptide.nist.gov/metrics/
- Skyline: proteome.gs.washington.edu/software/skyline
  - SProCoP (Bereman et al, JASMS 2014)
- Panorama: www.panoramaweb.org
- AutoQC: http://skyline.gs.washington.edu/software/AutoQC/daily/index.html
- Retention Time Viewer: gibsonproteomics.org/resources/rt-viewer
- Database searches for peptide ID
- Excel
- Any longitudinal data tracking system
- Check with your instrument vendor

* Thermo instruments only; not currently supported

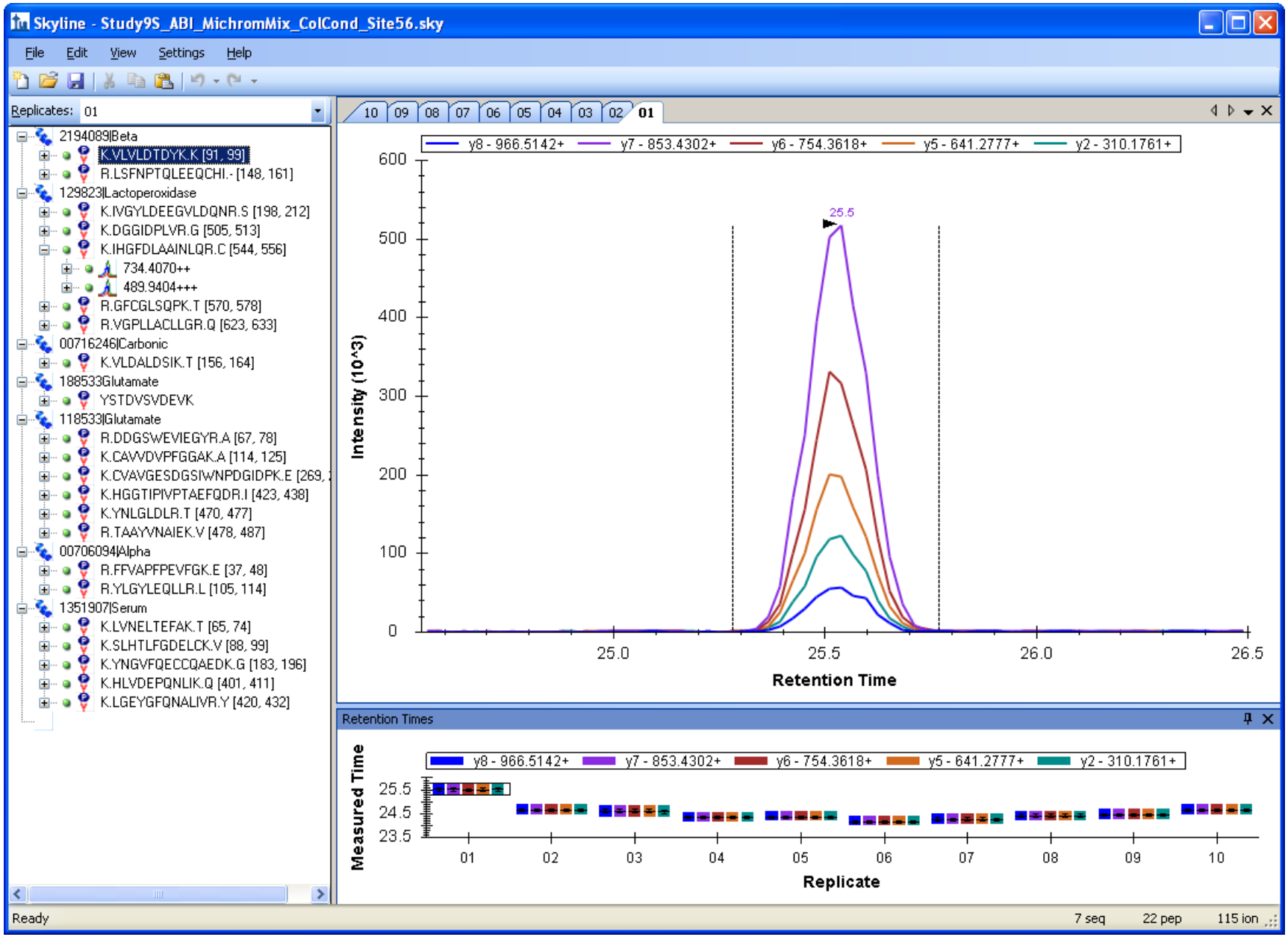
# QUALitiative SST Review



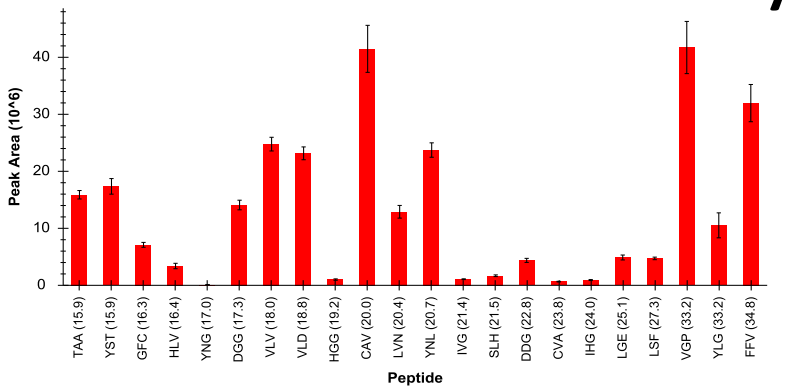

**It's not always this obvious!!**

**Use pre-define QUANtititative acceptance limits**

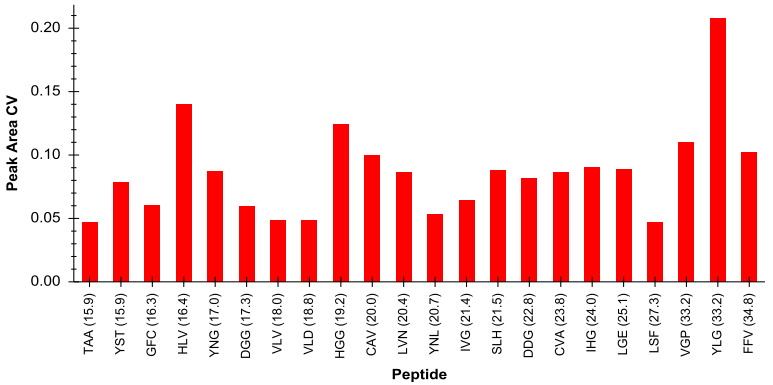
# Data Processing in Skyline



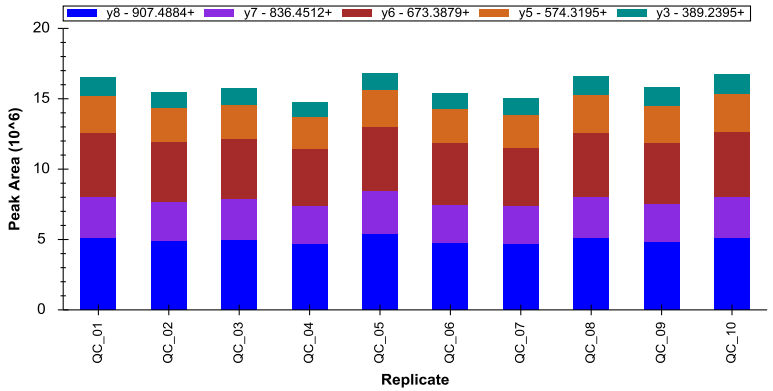
# Data Analysis in Skyline




- **Peptide Area View**
  - Mean peak area over 10 replicate injections
  - Error bars are 1 standard deviation
  - Peptides sorted by retention time



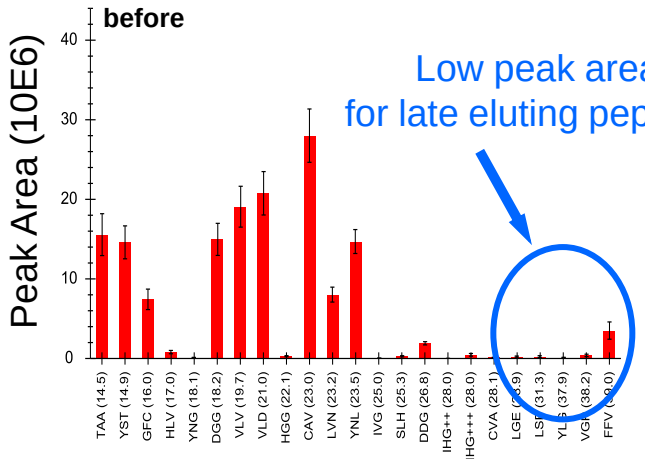

- **Peptide Area CV Over 10 Replicates**
  - Peak area variability should be low (<0.2)
  - Gives overview of poorly performing peptides
  - Peptides with low peak areas often have higher CVs

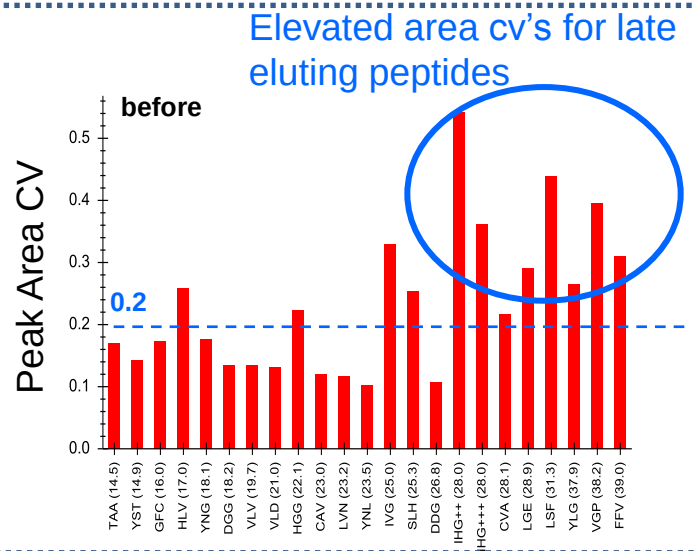



- **Peptide Replicate View for Peptide TAA**
  - Peak area variability should be low (<0.2)
  - Gives overview of poorly performing peptides
  - Peptides with low peak areas often have higher CVs

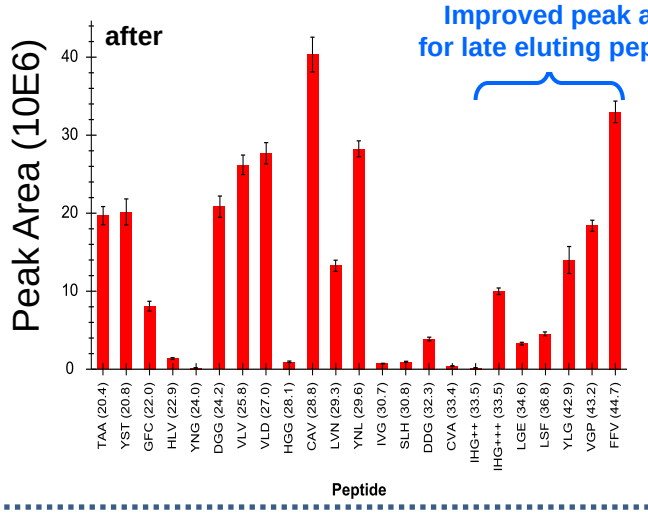
# Use of System Suitability to Recognize Problems

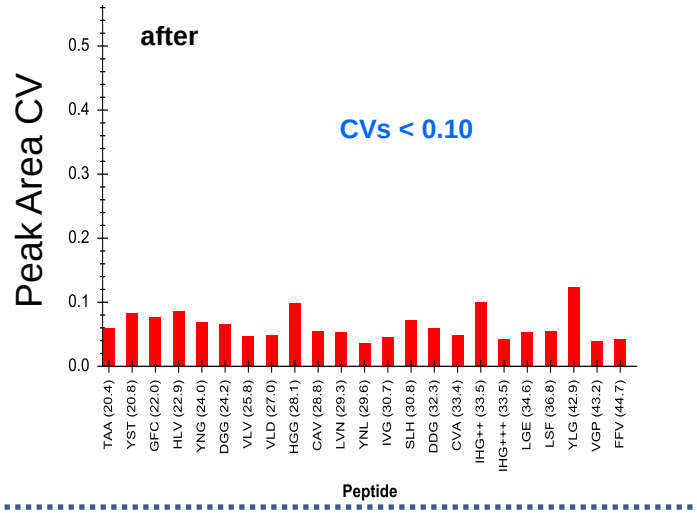
BEFORE



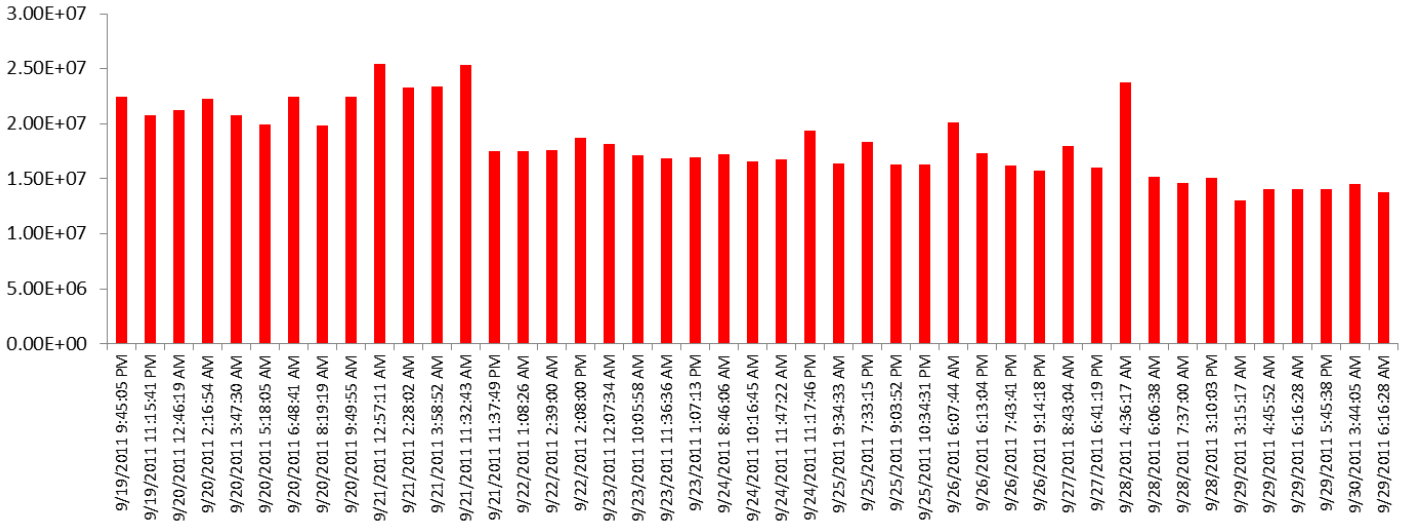




AFTER



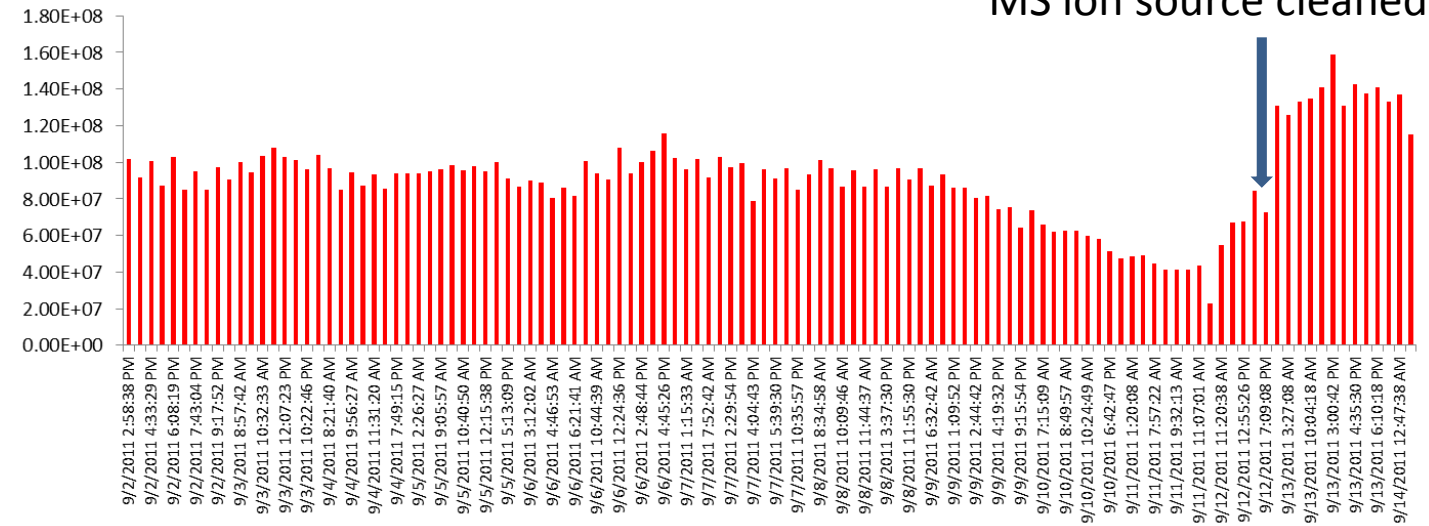
# Longitudinal System Performance




Study 1:

- 10 days to complete
- SSS run every 8-12 hours
- Stable performance with slight decrease in peak area

MS ion source cleaned

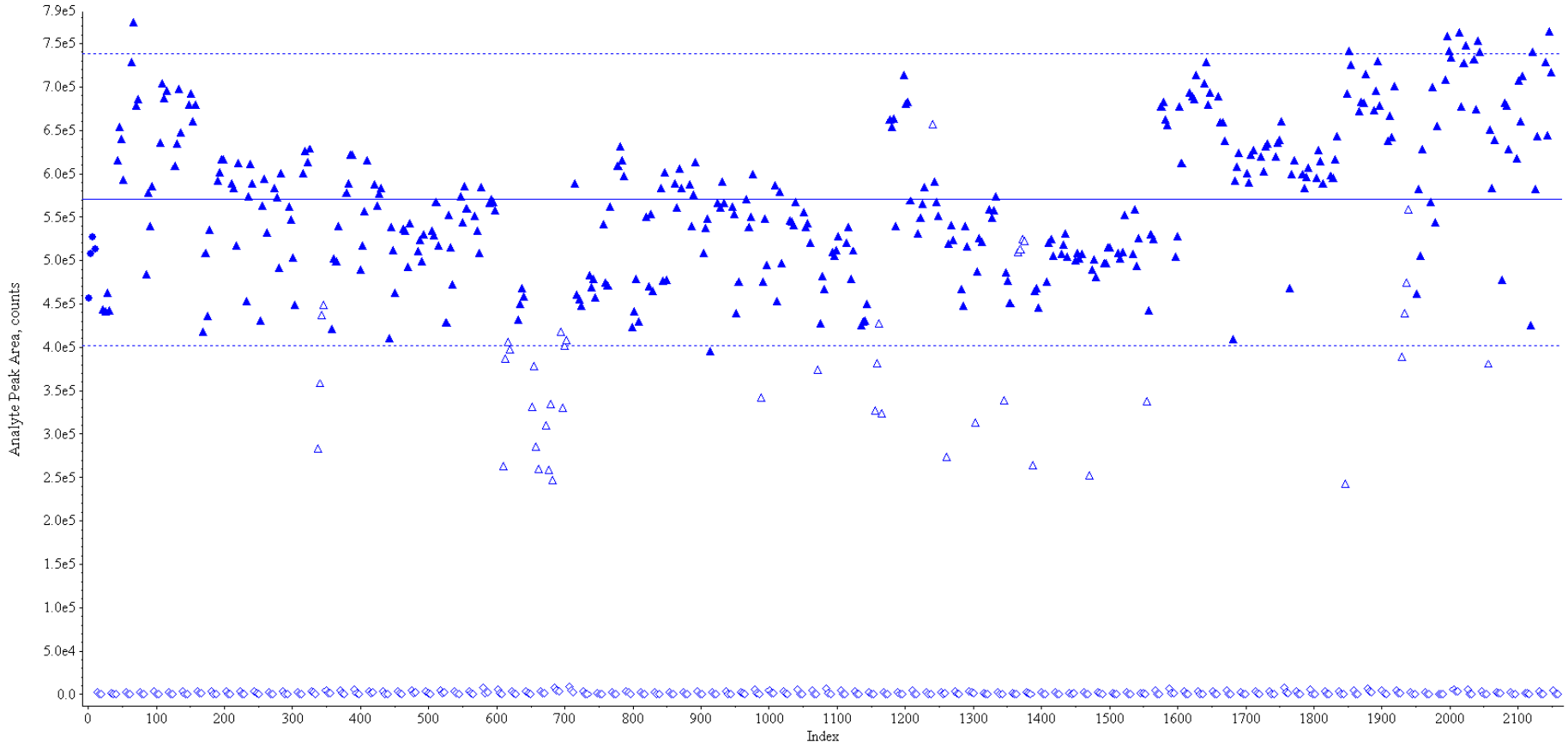



Study 2:

- 12 days to complete
- SSS run every 8-12 hours
- Stable performance with steady decrease in peak area
- Source cleaning fixed problem

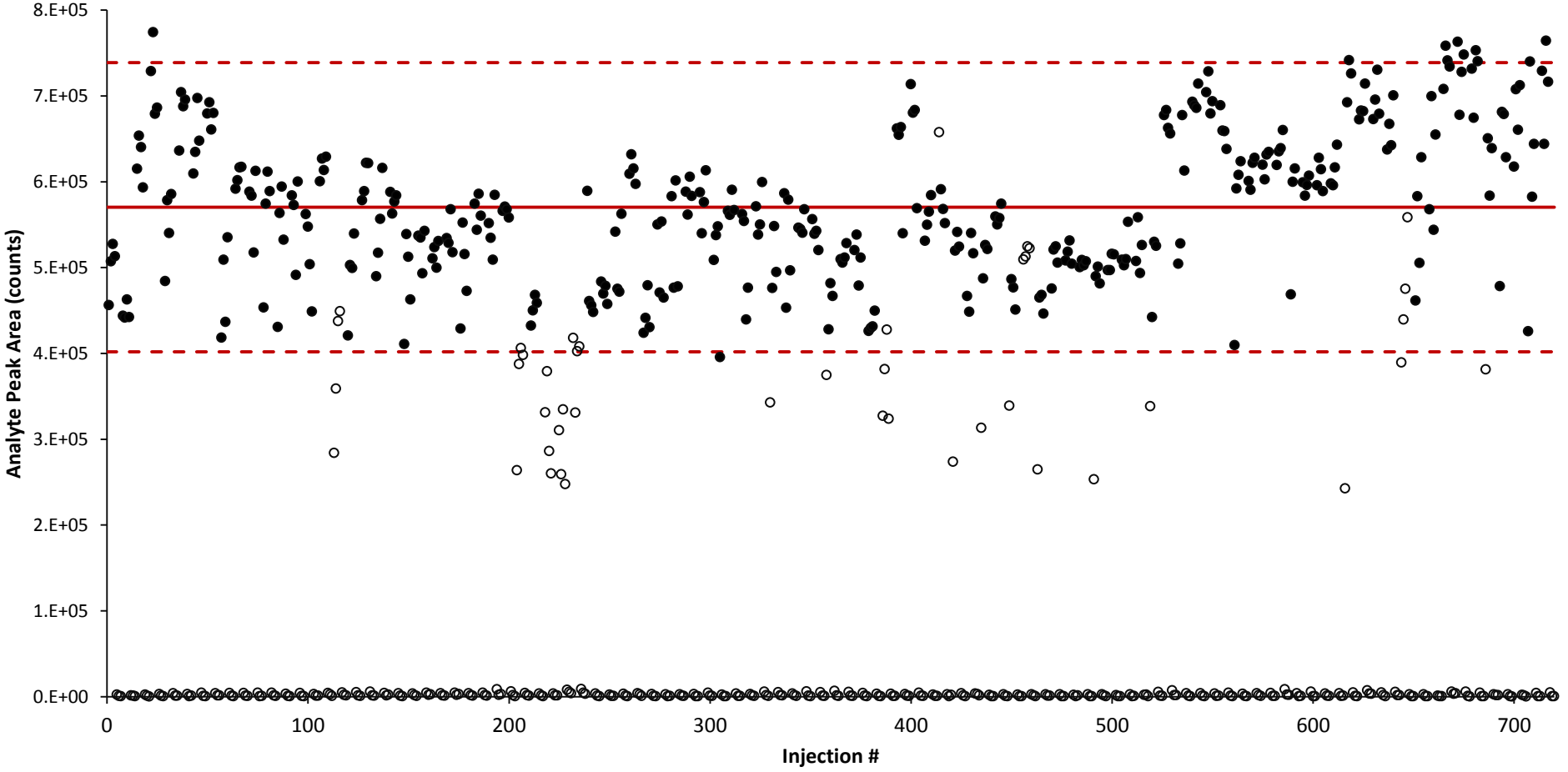
# Longitudinal Monitoring

## Analyst® (“Metric Plots”)

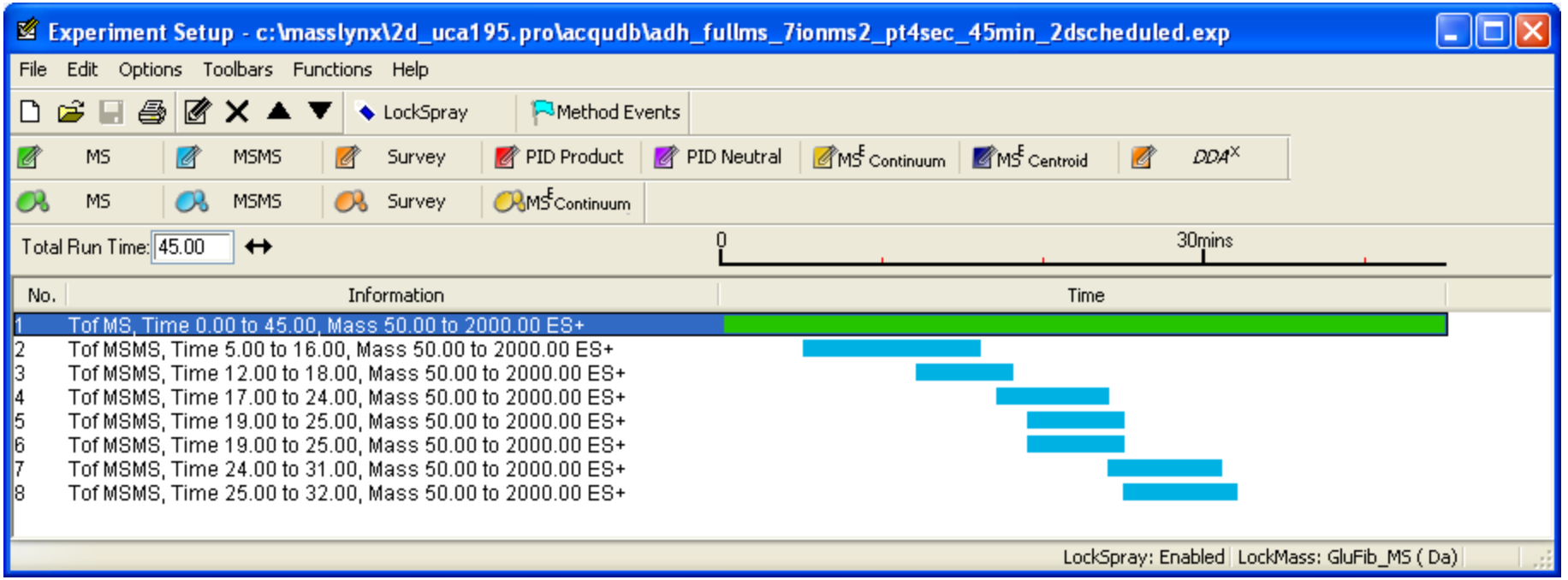


# Longitudinal Monitoring

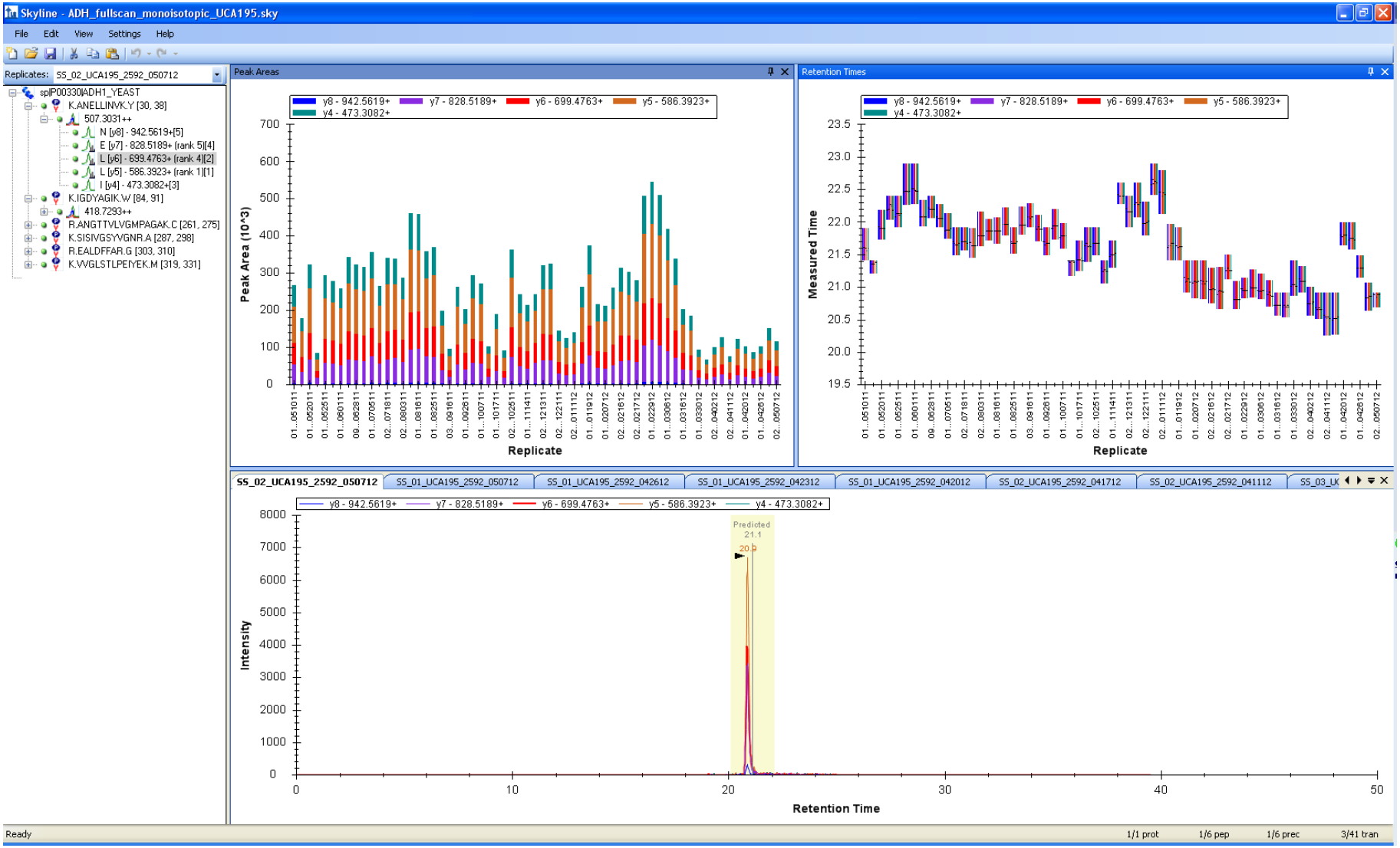
## Excel



# Targeted MS/MS (Pseudo-MRM) Method on a QToF



# Longitudinal Measurements over 1 Year



# Peak Intensity versus Sequence Coverage

Nominal mass ($M_r$): **37282**; Calculated pI value: **6.21**
NCBI BLAST search of ADH1_YEAST against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Saccharomyces cerevisiae

Fixed modifications: Carbamidomethyl (C)
Variable modifications: Deamidated (NQ),Oxidation (M)
Semi-specific cleavage, (peptide can be non-specific at one ter
Cleavage by semiTrypsin: cuts C-term side of KR unless next res
Sequence Coverage: **48%**

Matched peptides shown in **Bold Red**

```
    1 MSIPETQKGV IFYESHGKLE YKDIPVPKPK ANELLINVKY SGVCHTDLHA
   51 WHGDWPLPVK LPLVGGHEGA GVVVGMGENV KGWKIGDYAG IKWLNGSCMA
  101 CEYCELGNES NCPHADLSGY THDGSFQQYA TADAVQAAHI PQGTDLAQVA
  151 PILCAGITVY KALKSANLMA GHWVAISGAA GGLGSLAVQY AKAMGYRVLG
  201 IDGGEGKEEL FRSIGGEVFI DFTKEKDIVG AVLKATDGGA HGVINVSVSE
  251 AAIEASTRYV RANGTTVLVG MPAGAKCCSD VFNQVVKSIS IVGSYVGNRA
  301 DTREALDFFA RGLVKSPIKV VGLSTLPEIY EKMEKGQIVG RYVVDTSK
```

Nominal mass ($M_r$): **37282**; Calculated pI value: **6.21**
NCBI BLAST search of ADH1_YEAST against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Saccharomyces cerevisiae

Fixed modifications: Carbamidomethyl (C)
Variable modifications: Deamidated (NQ),Oxidation (M)
Semi-specific cleavage, (peptide can be non-specific at one termi
Cleavage by semiTrypsin: cuts C-term side of KR unless next resid
Sequence Coverage: **46%**

Matched peptides shown in **Bold Red**

```
    1 MSIPETQKGV IFYESHGKLE YKDIPVPKPK ANELLINVKY SGVCHTDLHA
   51 WHGDWPLPVK LPLVGGHEGA GVVVGMGENV KGWKIGDYAG IKWLNGSCMA
  101 CEYCELGNES NCPHADLSGY THDGSFQQYA TADAVQAAHI PQGTDLAQVA
  151 PILCAGITVY KALKSANLMA GHWVAISGAA GGLGSLAVQY AKAMGYRVLG
  201 IDGGEGKEEL FRSIGGEVFI DFTKEKDIVG AVLKATDGGA HGVINVSVSE
  251 AAIEASTRYV RANGTTVLVG MPAGAKCCSD VFNQVVKSIS IVGSYVGNRA
  301 DTREALDFFA RGLVKSPIKV VGLSTLPEIY EKMEKGQIVG RYVVDTSK
```

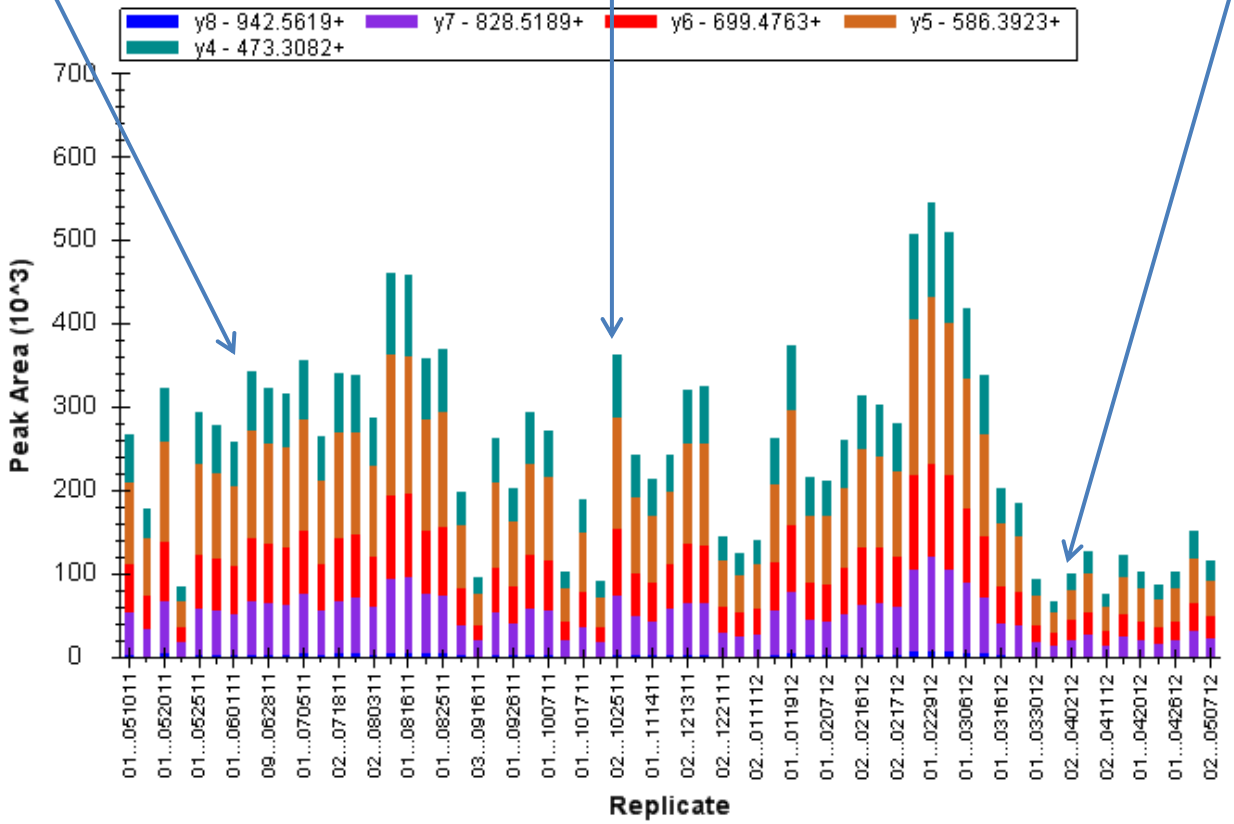
Nominal mass ($M_r$): **37282**; Calculated pI value: **6.21**
NCBI BLAST search of ADH1_YEAST against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Saccharomyces cerevisiae

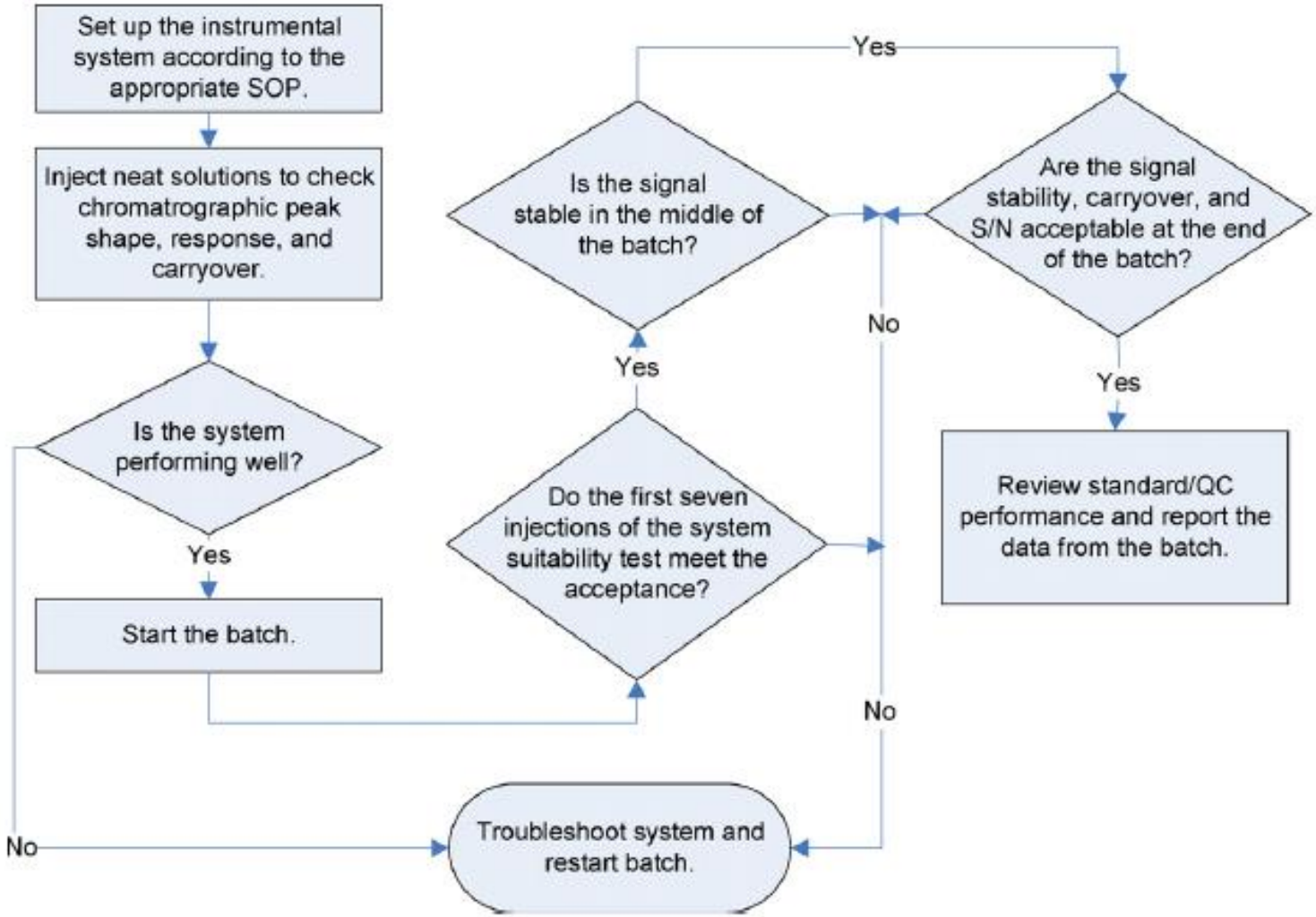
Fixed modifications: Carbamidomethyl (C)
Variable modifications: Deamidated (NQ),Oxidation (M)
Semi-specific cleavage, (peptide can be non-specific at one term
Cleavage by semiTrypsin: cuts C-term side of KR unless next resi
Sequence Coverage: **49%**

Matched peptides shown in **Bold Red**

```
    1 MSIPETQKGV IFYESHGKLE YKDIPVPKPK ANELLINVKY SGVCHTDLHA
   51 WHGDWPLPVK LPLVGGHEGA GVVVGMGENV KGWKIGDYAG IKWLNGSCMA
  101 CEYCELGNES NCPHADLSGY THDGSFQQYA TADAVQAAHI PQGTDLAQVA
  151 PILCAGITVY KALKSANLMA GHWVAISGAA GGLGSLAVQY AKAMGYRVLG
  201 IDGGEGKEEL FRSIGGEVFI DFTKEKDIVG AVLKATDGGA HGVINVSVSE
  251 AAIEASTRYV RANGTTVLVG MPAGAKCCSD VFNQVVKSIS IVGSYVGNRA
  301 DTREALDFFA RGLVKSPIKV VGLSTLPEIY EKMEKGQIVG RYVVDTSK
```

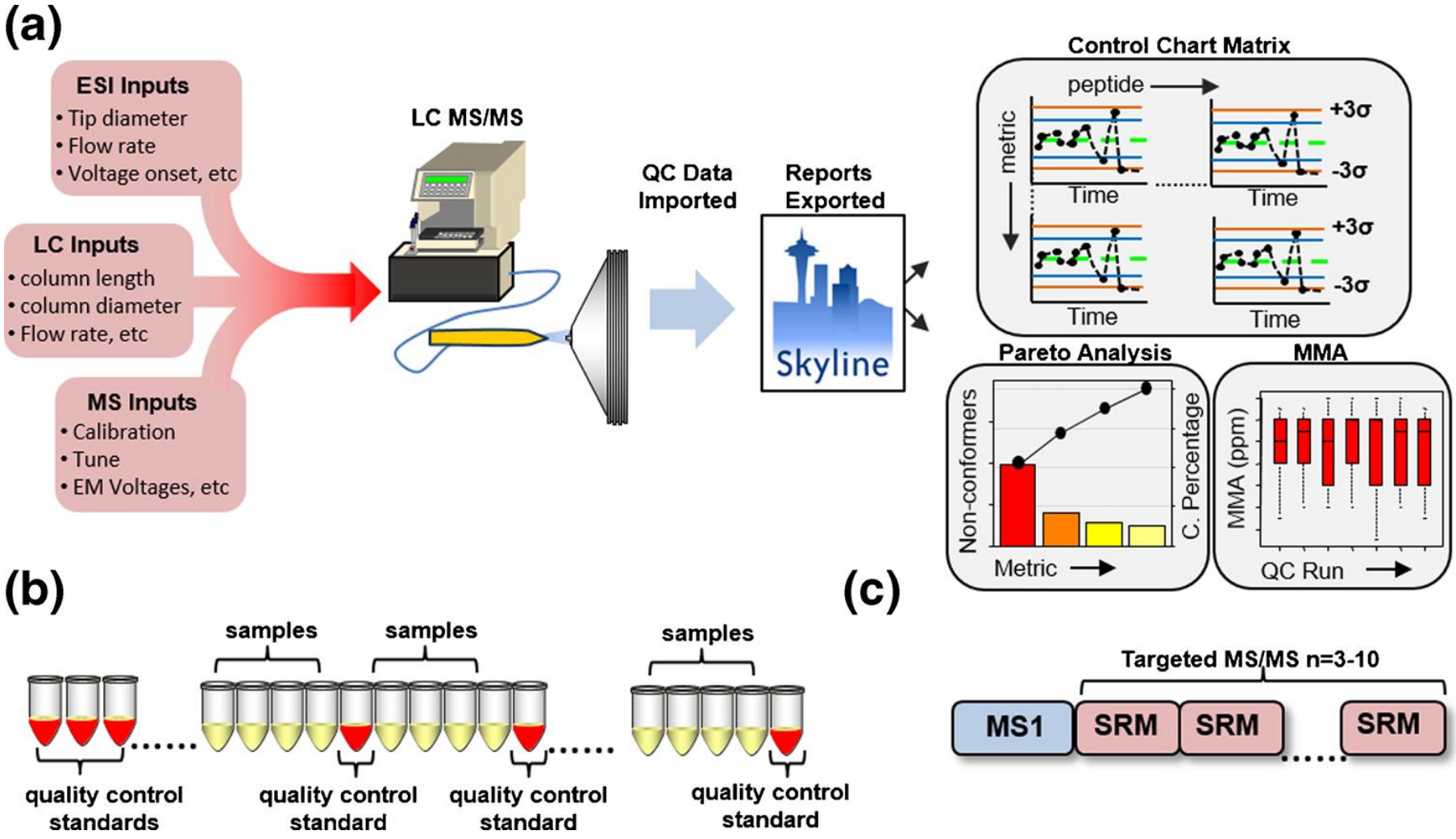


# How do you apply system suitability in everyday use?

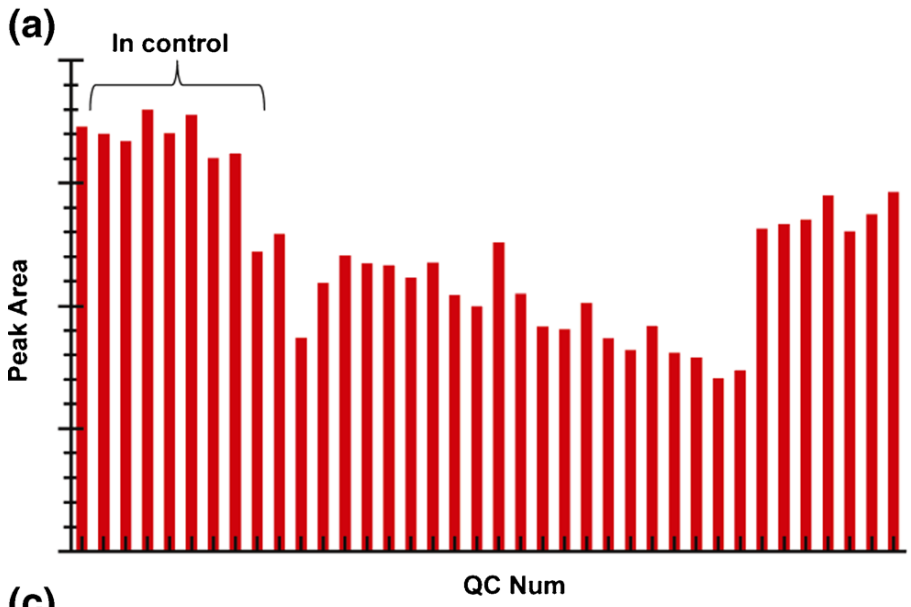


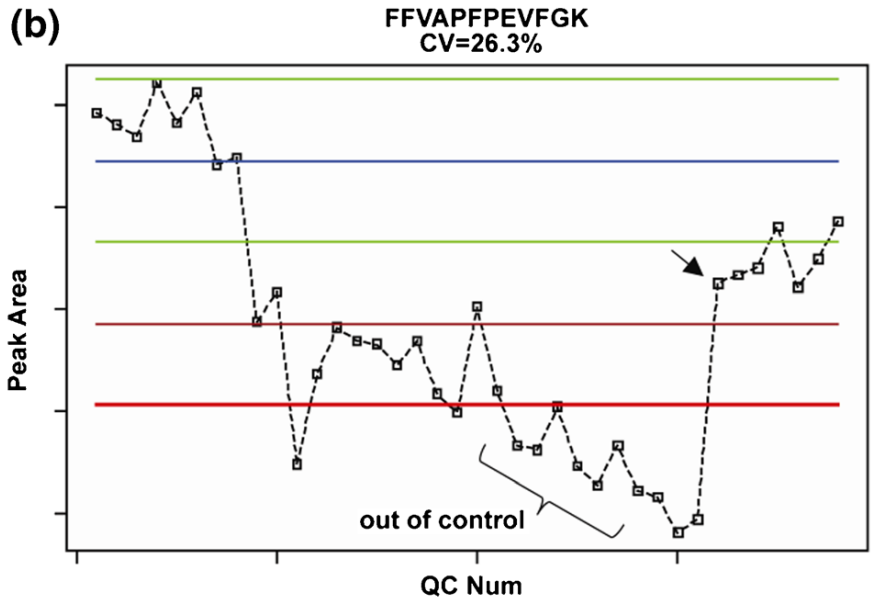

*C.J. Briscoe et al. / Journal of Pharmaceutical and Biomedical Analysis 44 (2007) 484–491*

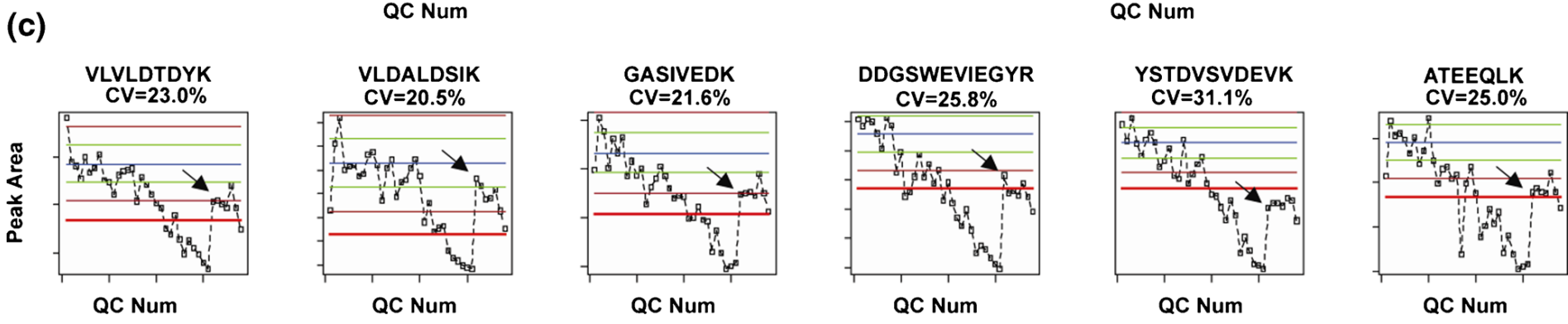
# Statistical Process Control for Proteomic Experiments
# Bereman *et al*, JASMS 2014



# Longitudinal Performance Tracking



# Summary

- Set up a system suitability protocol that works for you and use it
- Don’t waste precious sample and time without making sure your LC-MS system is working
- Use system suitability to monitor changes in hardware, software, any changes at all
- Keep examples of poor system suitability data to help trouble shoot future issues

# Resources

- **Using Skyline to Monitor Long-Term Performance Metrics of High-Resolution Mass Spectrometers** *(presentation at Skyline User's meeting, ASMS 2012)*
  - https://skyline.gs.washington.edu/labkey/wiki/home/software/Skyline/events/2012%20User%20Group%20Meeting%20at%20ASMS/page.view?name=thompson
- **Effectively Dealing with Transition Selection and Data Analysis for Multiplexed Quantitative SRM-MS Assays across Multiple Vendor Instruments** *(presentation at Skyline User's meeting, ASMS 2012)*
  - https://skyline.gs.washington.edu/labkey/wiki/home/software/Skyline/events/2012%20User%20Group%20Meeting%20at%20ASMS/page.view?name=abbatiello
- Burkhart, J.M., Premsler, T., and Sickmann, A. (2011). Quality control of nano-LC-MS systems using stable isotope-coded peptides. *Proteomics* 11, 1049-1057.
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- Rudnick, P.A., et al, (2010). Performance Metrics for Liquid Chromatography-Tandem Mass Spectrometry Systems in Proteomics Analyses. *Molecular & cellular proteomics : MCP* 9, 225-241.
- Careri, M., Mangia, A. (2006). Validation and qualification: the fitness for purpose of mass spectrometry-based analytical methods and analytical systems. Anal. Bioanal. Chem, 386:38-25.
- Mutton, I., Boughtflower, B., Taylor, N., Brooke, D. (2011) The design and use of a simple Sytem Suitability Test Mix for generic reverse phase high performance liquid chromatography-mass spectrometry systems and the implications for automated system monitoring using global software tracking. Journal of Chrom A, 1218:3711-3717.
- Bereman, M.S., Johnson, R., Bollinger, J., Boss, Y.; Schulman, N.; MacLean, B.;Hoofnagle, A.N.; MacCoss, M.J. (2014) Implementation of Statistical Process Control for Proteomic Experiments Via LC MS/MS. JASMS 25:581-587.