

Statistical Process Control for Assessing LC MS/MS Performance

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June 15, 2014

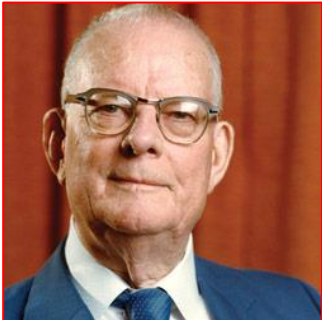
Skyline Users Group Meeting
2014 American Society for Mass Spectrometry Conference

Baltimore, Maryland

What is Statistical Process Control (SPC)?

A powerful collection of tools used in achieving process stability and improving capability through the identification and reduction of assignable causes of variation

Walter Shewhart



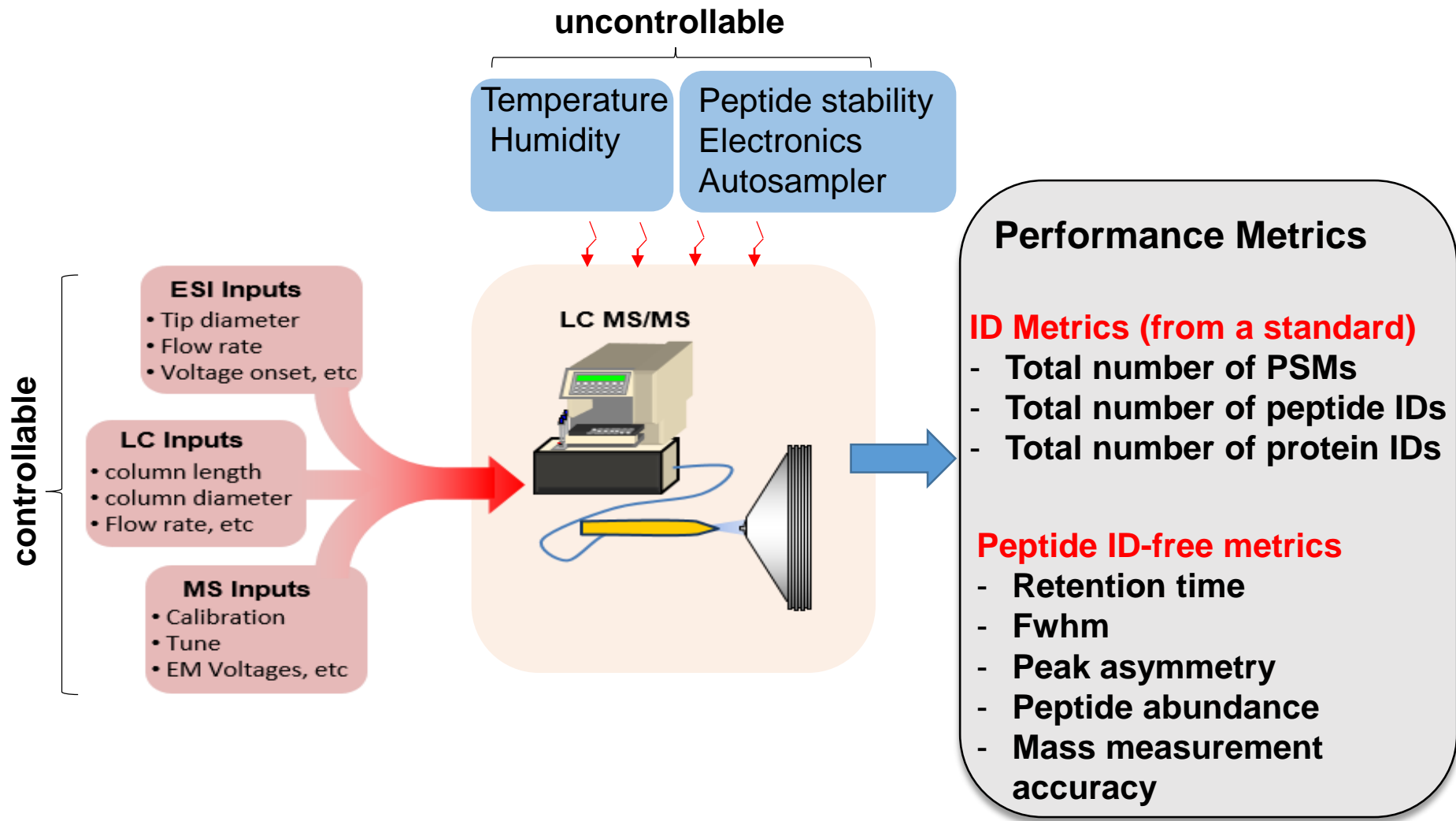
(1891-1967)

- **Special cause vs. chance cause variation**
- **1st implemented in Japan post WWII**
- **Revived in USA by the automotive industry in the 1980's**
- **Sales, marketing, finance, clinical diagnostics**

No definition of quality - SPC describes “quality” as inversely proportional to variance

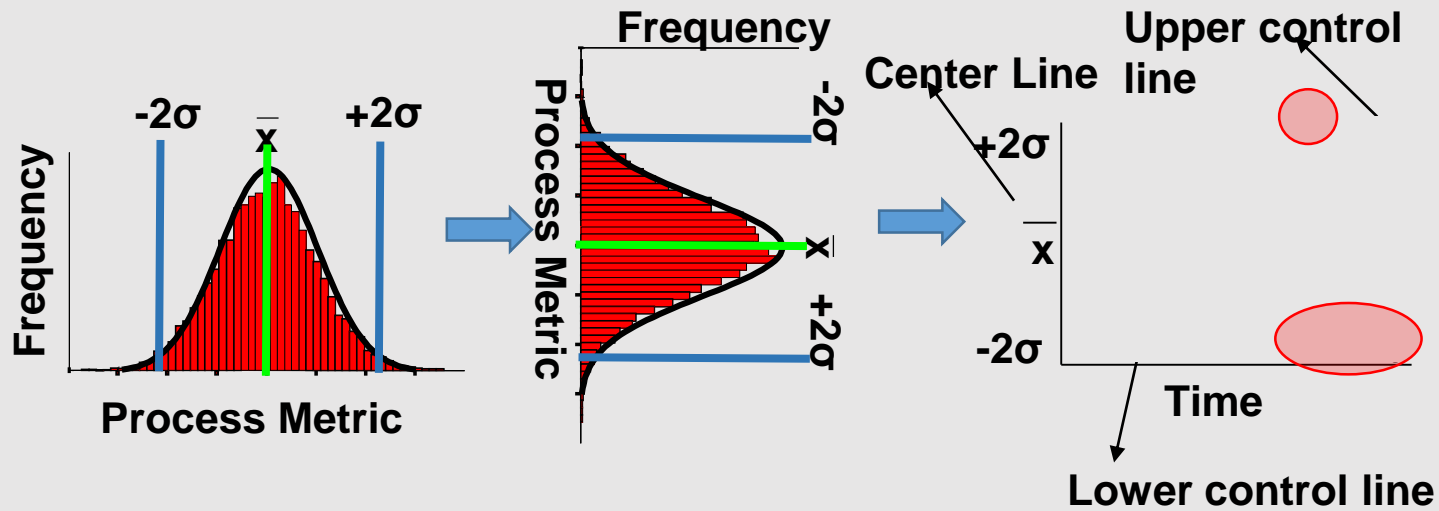
LC MS/MS is a Process

A process is everything required to turn an input(s) into the desired output



Shewhart Control Charts

Shewhart, W.A. J. Frankl. Inst. 226, 163, 1938

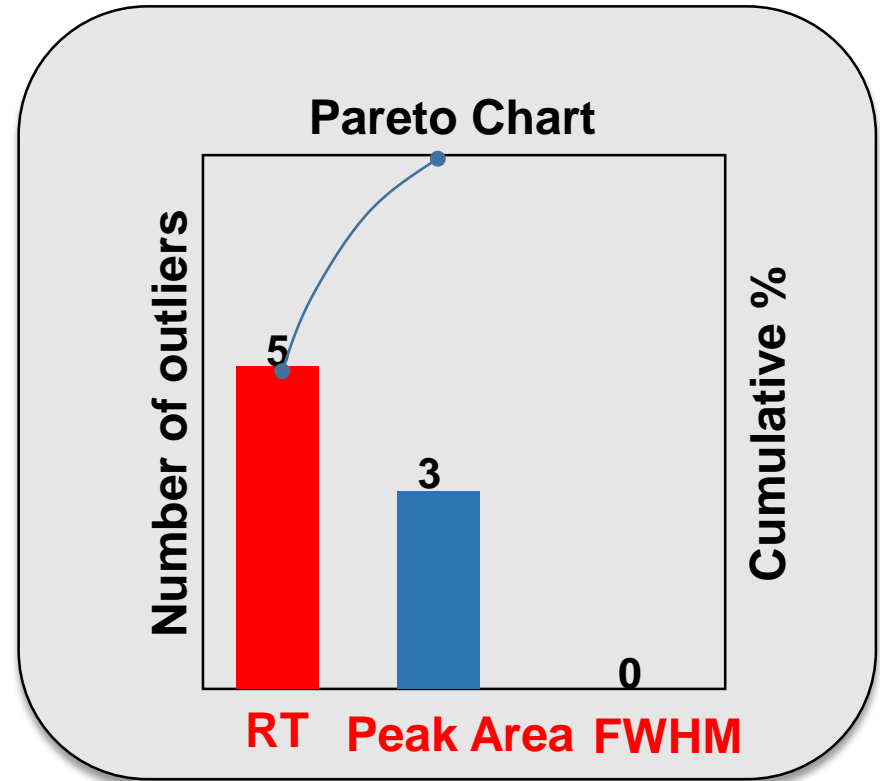
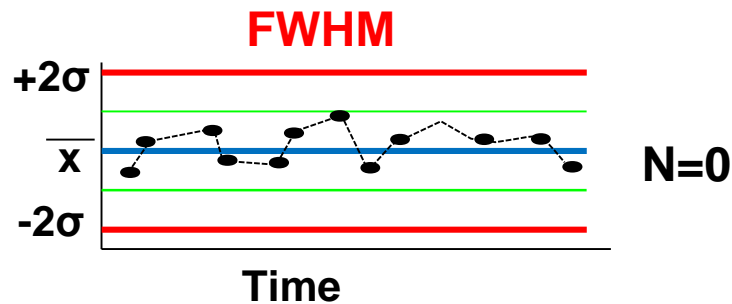
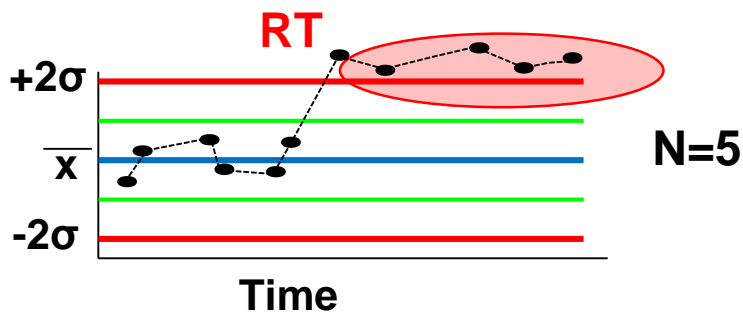
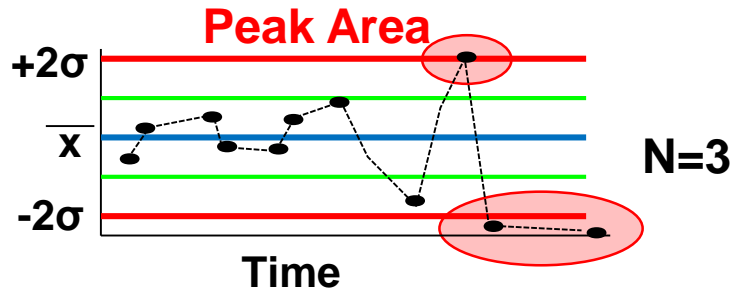


Primary tool in SPC – used for monitoring process output

Pareto Analysis

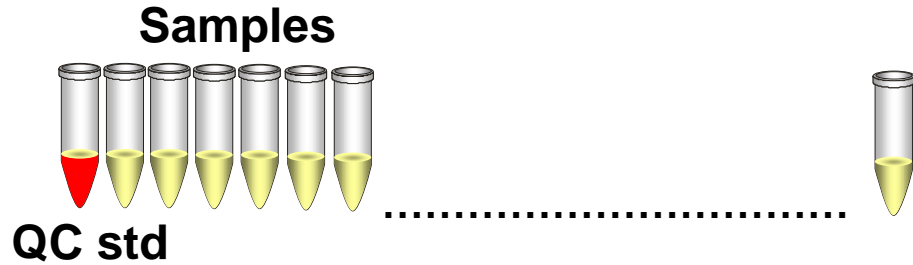
80/20 Principle - Identifies the most significant problems – vital few

Juran, J.M., Quality Control Handbook 1974

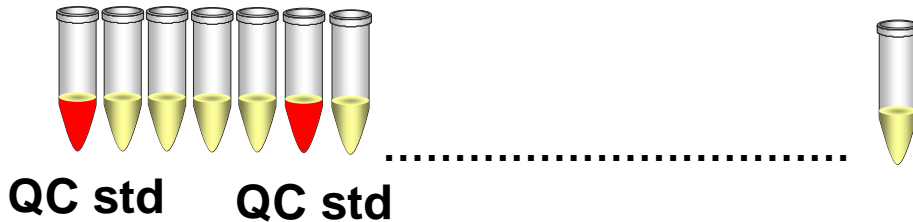


How Does One Monitor Performance in LC MS/MS

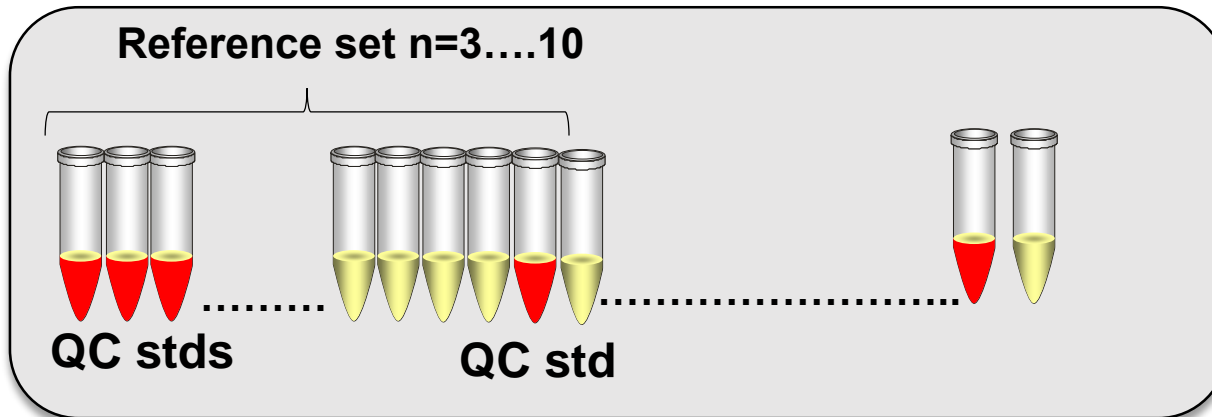
1. Run a standard before each analysis or at the beginning of each day



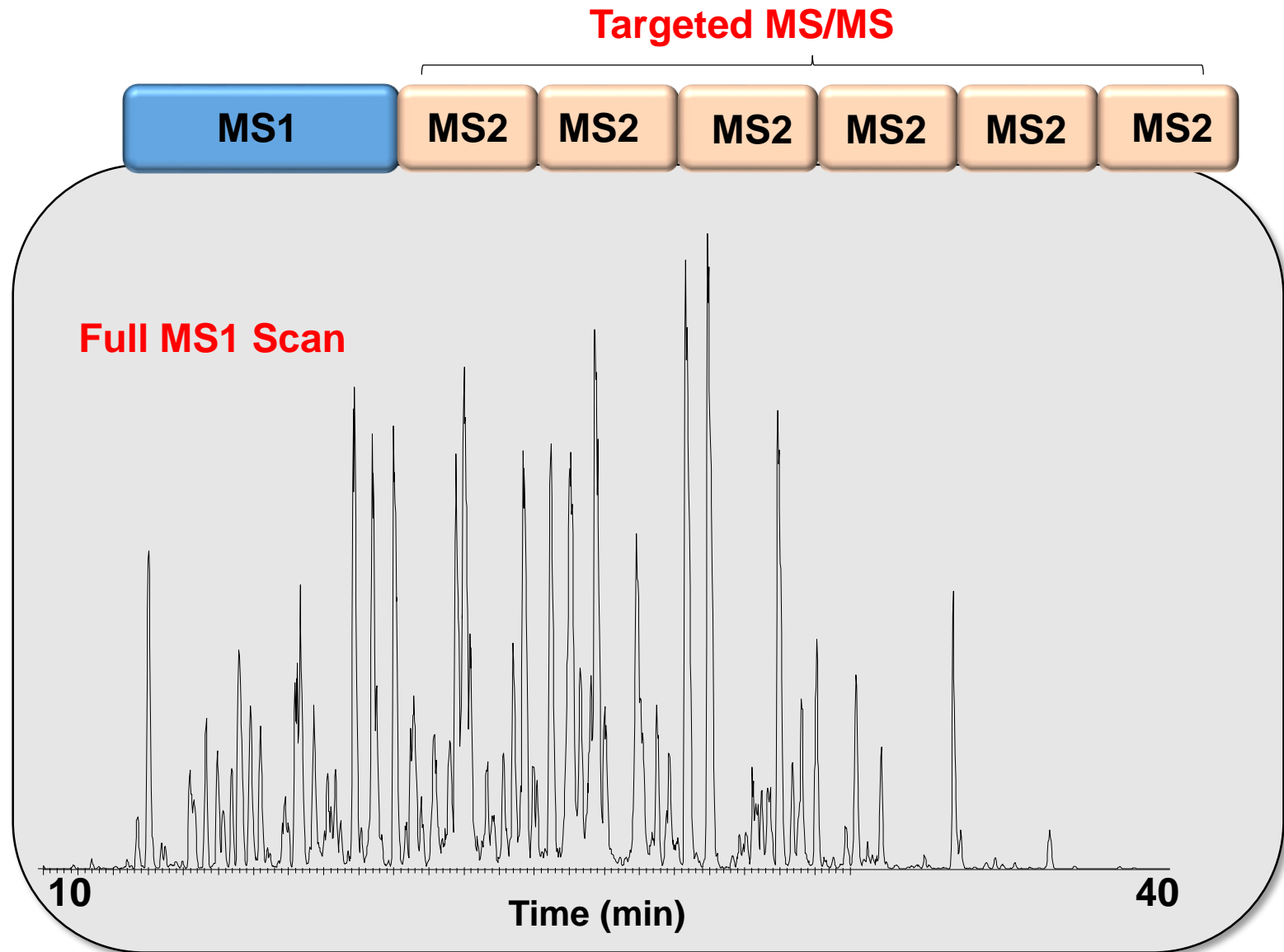
2. Run a standard systematically throughout a study



3. Run a reference set

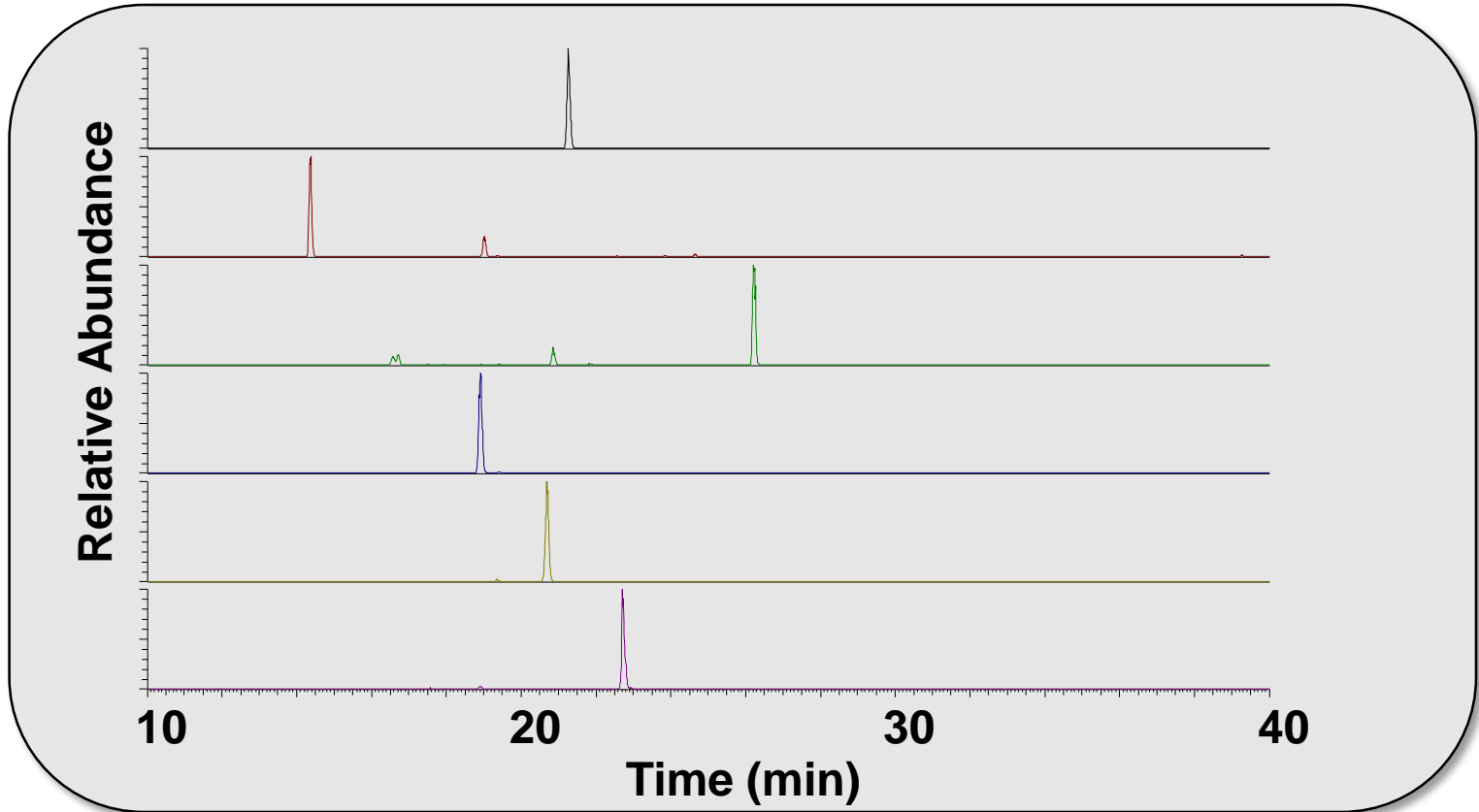


Scan Cycle for Quality Control Standards



Targeted MS/MS

Monitor several peptides across the gradient



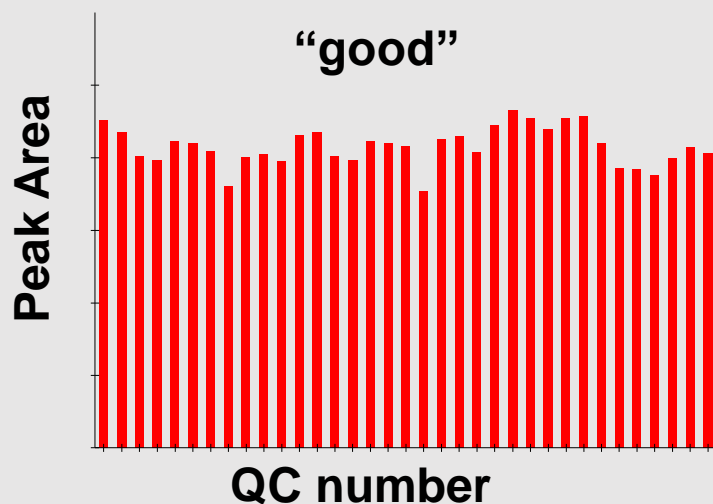
LC related {
1. RT reproducibility
2. Peak asymmetry
3. FWHM

MS related {
4. Peptide abundance
5. Mass measurement accuracy

Motivation for Tool Development

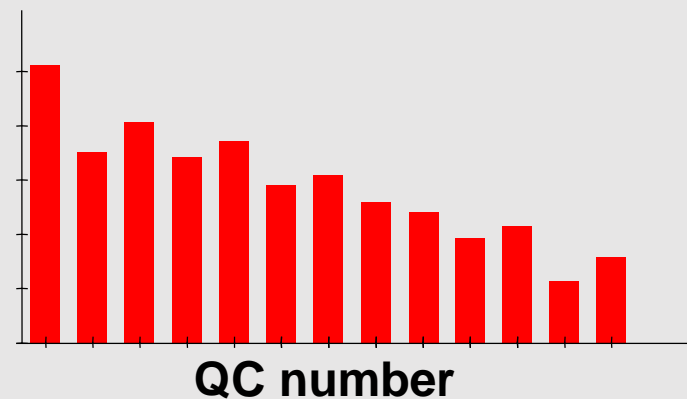
K.AEFVEVTK.L

“good”



K.AEFVEVTK.L

“bad”



1. Quantitative method to assess instrument performance
2. Visual tools
3. Methods for process monitoring
4. Versatile (SRM, MS1, Targeted MS/MS, high RP, low RP, and vendor neutral)
5. Easy and fast!

Statistical Process Control in Proteomics (SProCoP)

Bereman, MS *et. al.*, JASMS 25(4) 581. 2014

Settings Tools Help

- SRM Collider
- SProCoP
- Tool Store...**
- External Tools...
- Immediate Window
- Options...

MS1Probe
MSstats
Population Variation
Protter
QuaSAR
SProCoP

Organization:
Bereman Lab, NCSU

Authors:
Michael S. Bereman, Ph.D., Nick Shulman, Yuval Boss

Languages:
R(3.0.2), C#

More Information:
<http://www4.ncsu.edu/~msberema/>

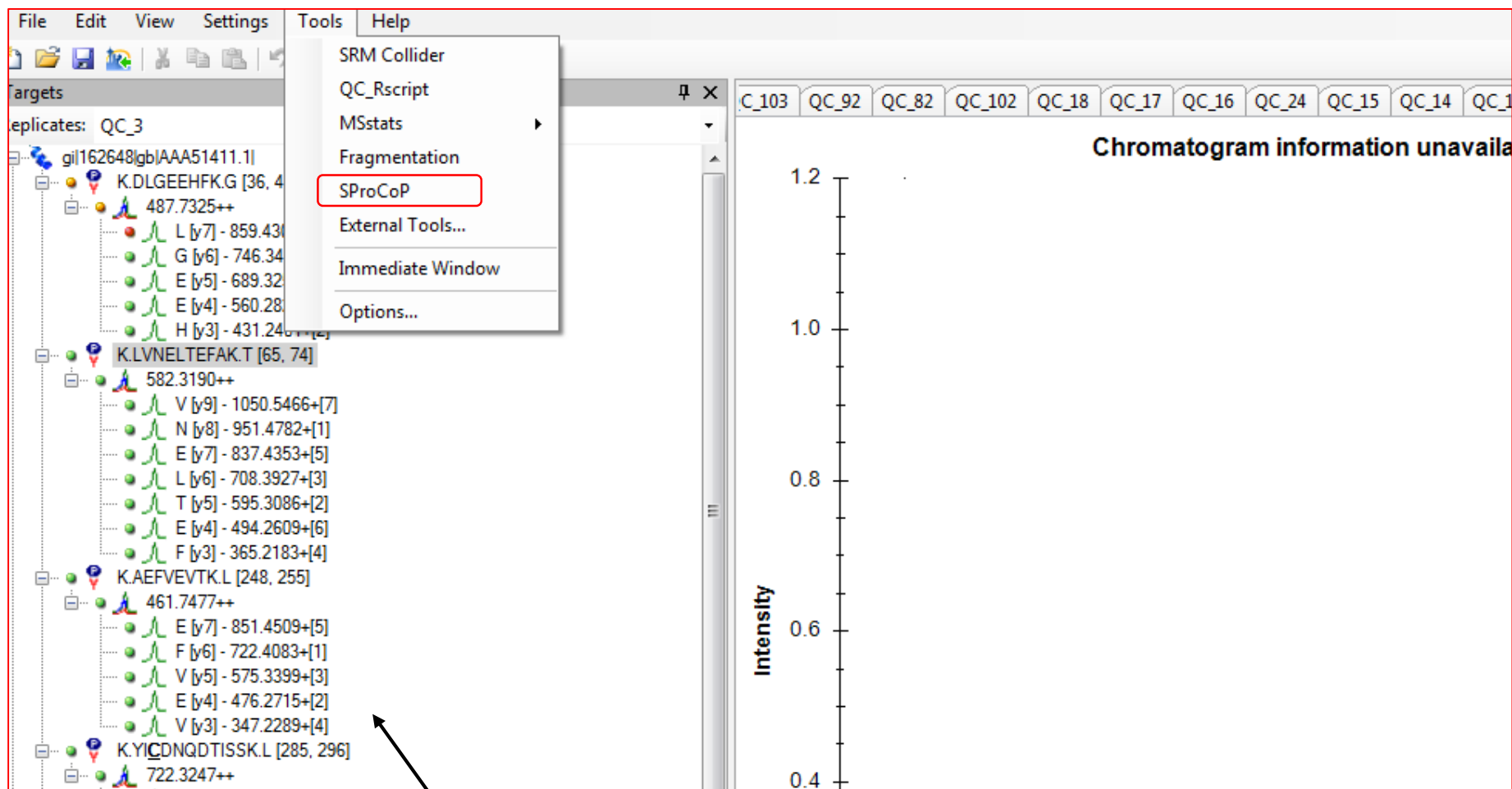
Status:
Not currently installed. Version: 1.0 is available

Description:
Statistical process control (SPC) is a robust set of tools that aids in the visualization, detection, and identification of assignable causes of variation in any process that creates products, services, or information. A tool has been developed termed Statistical Process Control in Proteomics (SProCoP) which implements aspects of SPC (e.g., control charts and Pareto analysis) into the Skyline software. It provides real time evaluation of the chromatographic performance (e.g., retention time reproducibility, peak asymmetry, and resolution); and mass spectrometric performance (targeted peptide ion intensity and mass measurement accuracy for high resolving power instruments) via control charts and

Tool Store... Install Close

Using SProCoP

Step 1 - Import QC standards



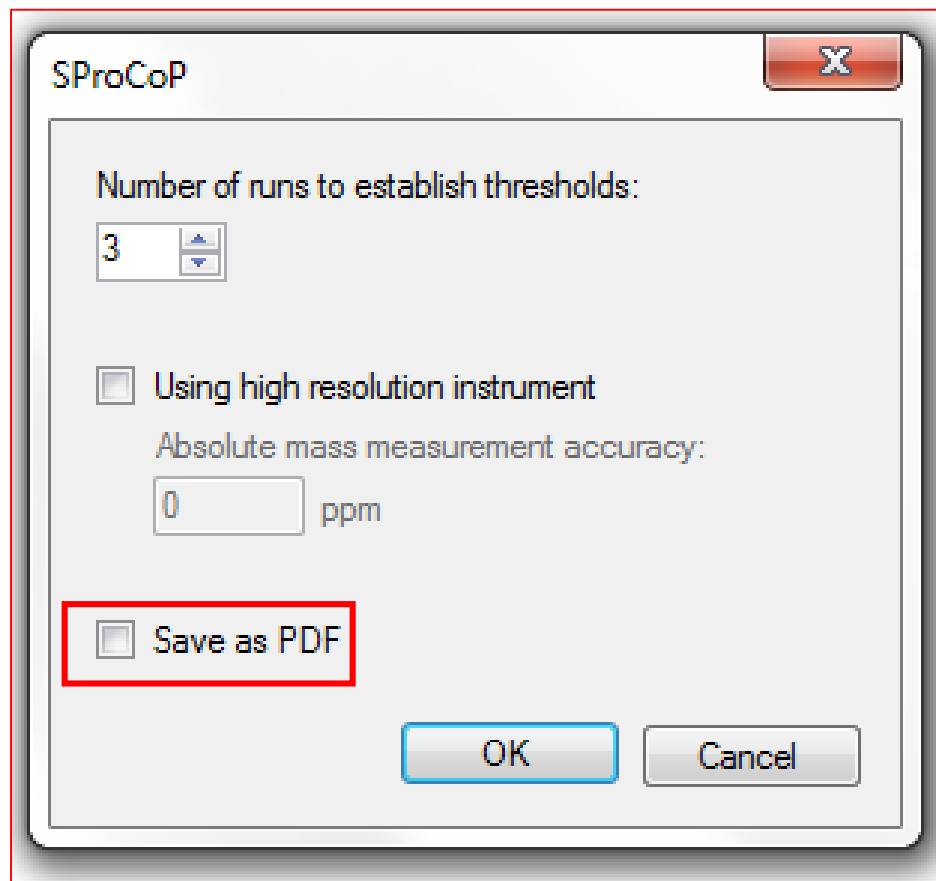
Targeted peptides

Step 2 – Defining Output

User can specify number of runs (i.e., Reference Set)
Default = 3

MMA in ppm
(only for high RP instruments)

Saves individual charts

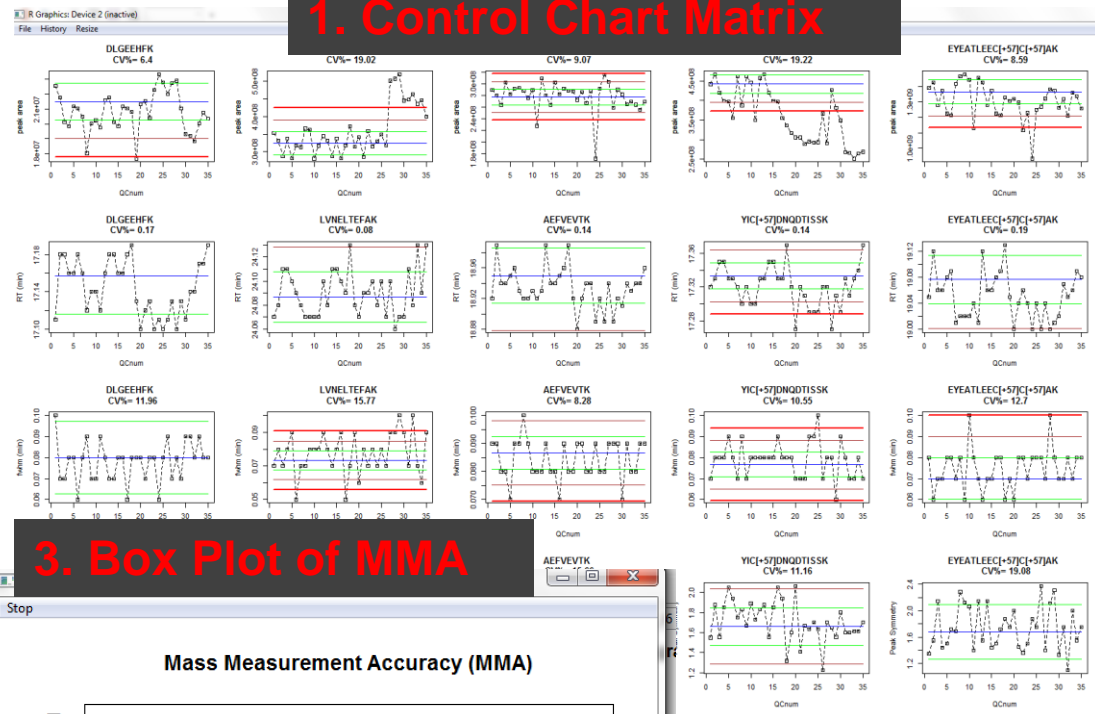


The image shows a software dialog box titled "SProCoP" with a close button (X) in the top right corner. The dialog contains the following elements:

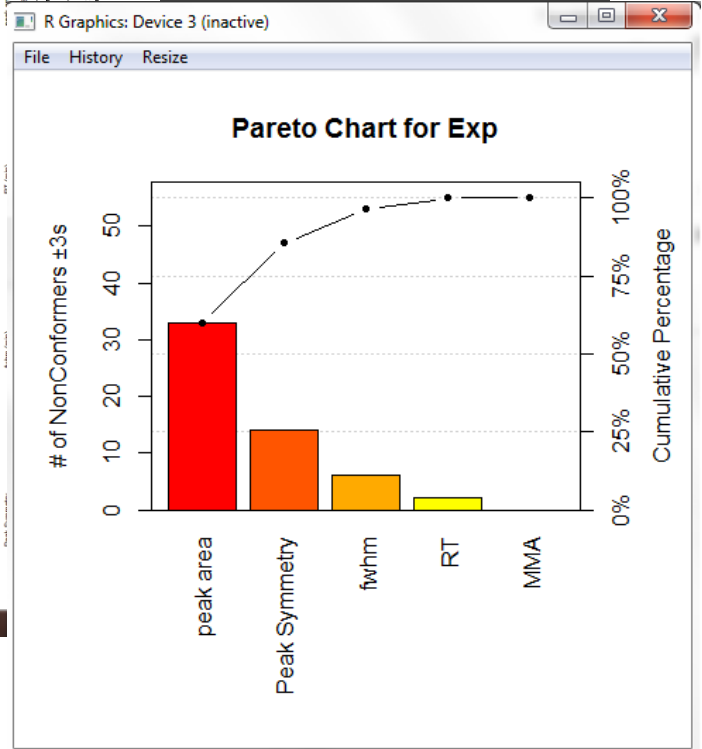
- A label "Number of runs to establish thresholds:" followed by a spin box containing the value "3". A red arrow points from the text "User can specify number of runs (i.e., Reference Set) Default = 3" to this spin box.
- A checkbox labeled "Using high resolution instrument".
- A label "Absolute mass measurement accuracy:" followed by a text input field containing "0" and the unit "ppm". A red arrow points from the text "MMA in ppm (only for high RP instruments)" to this input field.
- A checkbox labeled "Save as PDF" which is highlighted with a red rectangular box. A red arrow points from the text "Saves individual charts" to this checkbox.
- At the bottom right, there are two buttons: "OK" and "Cancel".

Simple – Yet Powerful Charts to Assess Performance

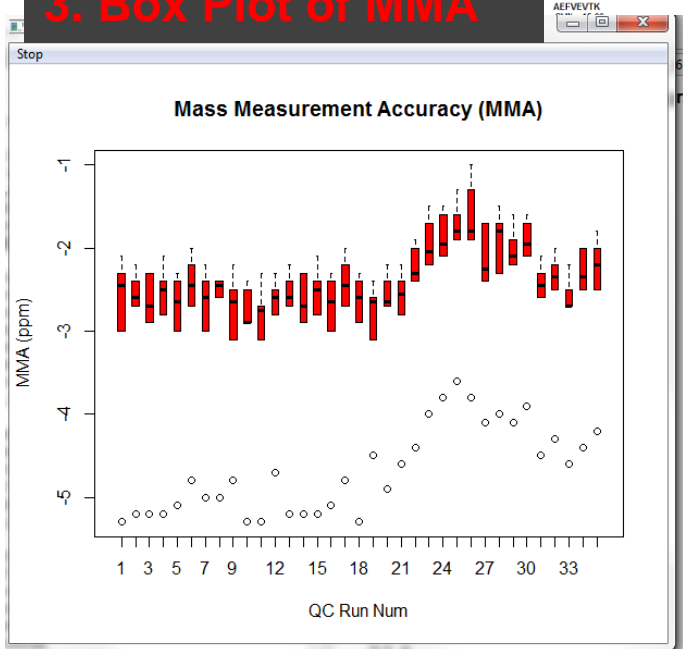
1. Control Chart Matrix



2. Pareto Chart



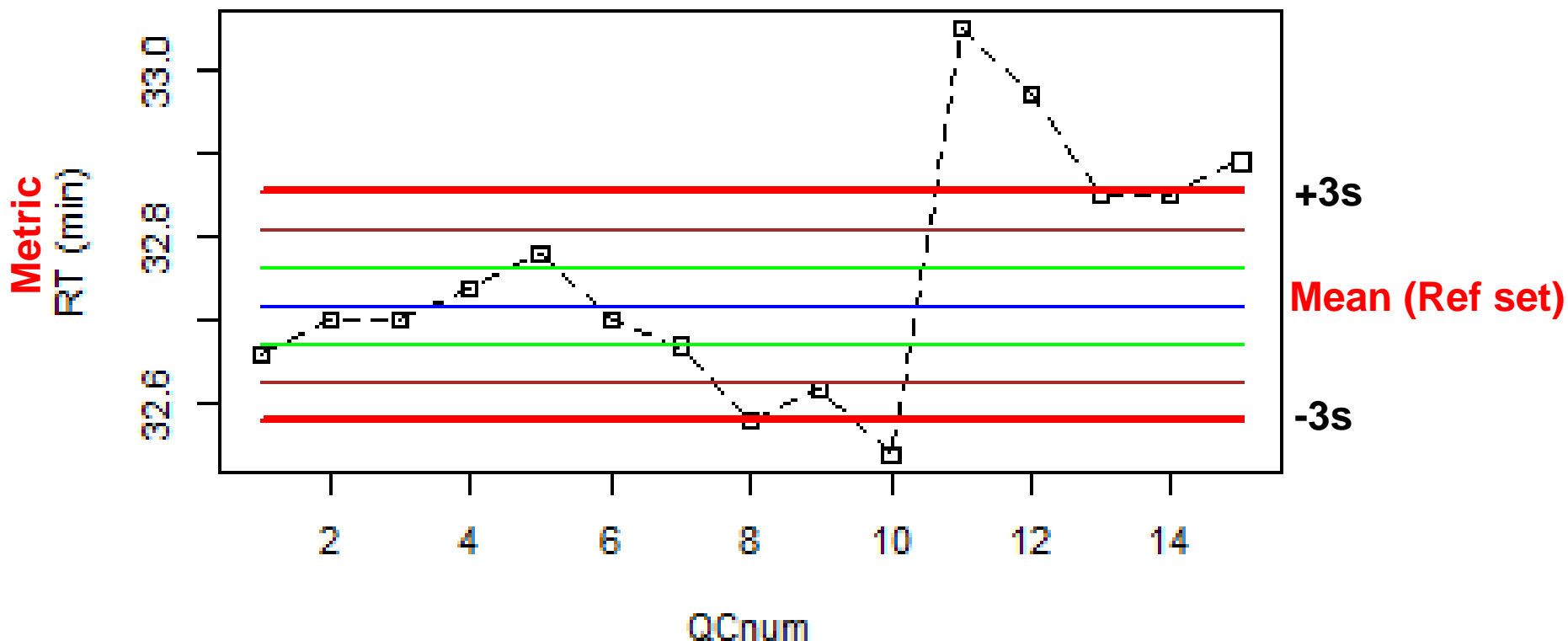
3. Box Plot of MMA



Shewhart Control Charts in SProCoP

3 - Sigma Quality Performance (~1 out of 370 to be a false positive)

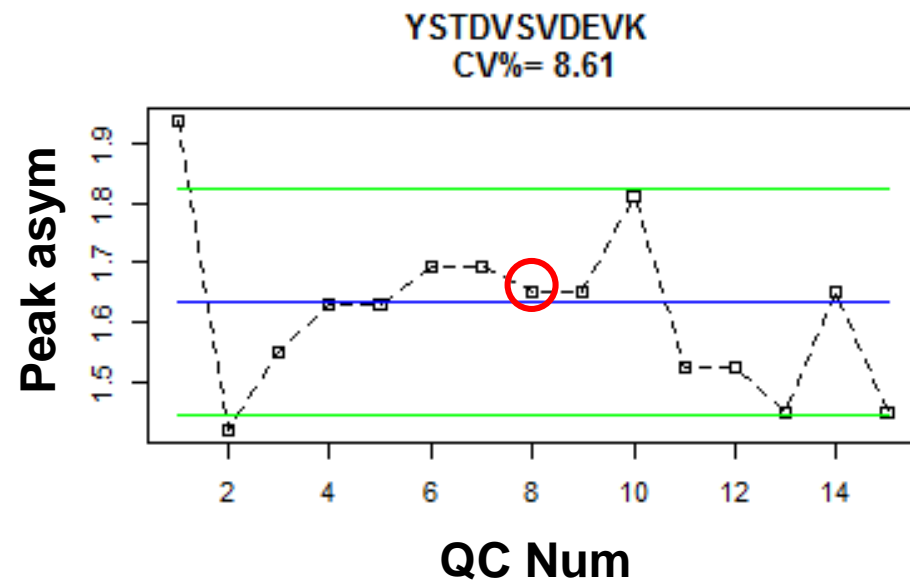
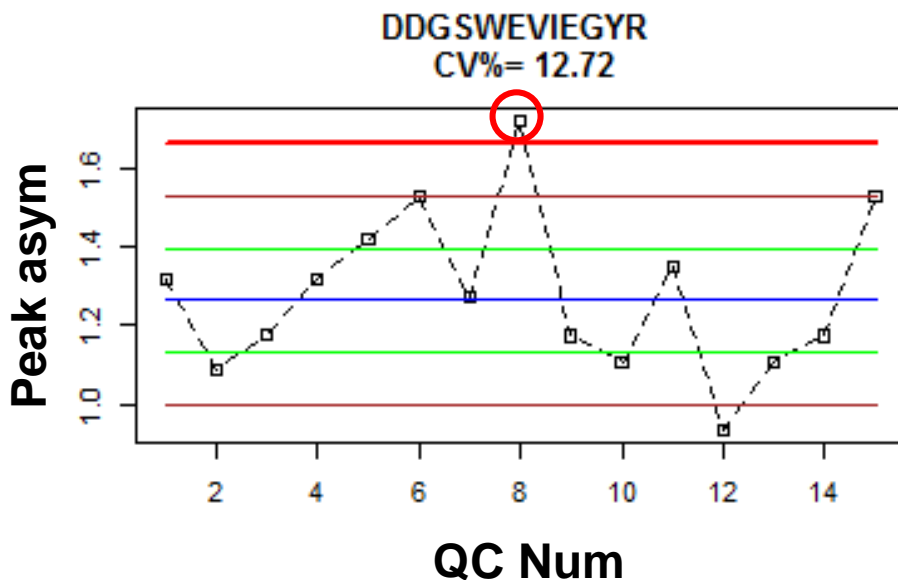
Peptide sequence → VLVLDTDYK
CV%= 0.44 ← CV across all runs



Interpretation of Control Charts

Detect systematic trends – instead of single semi random occurrences

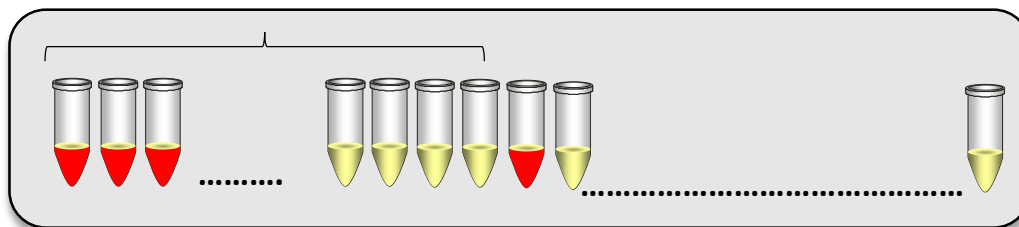
1. Do not rely on single peptide/metric measurements
2. How do the other peptides for that particular metric perform?
3. Is there an obvious systematic trend?
4. How does the next QC run perform?



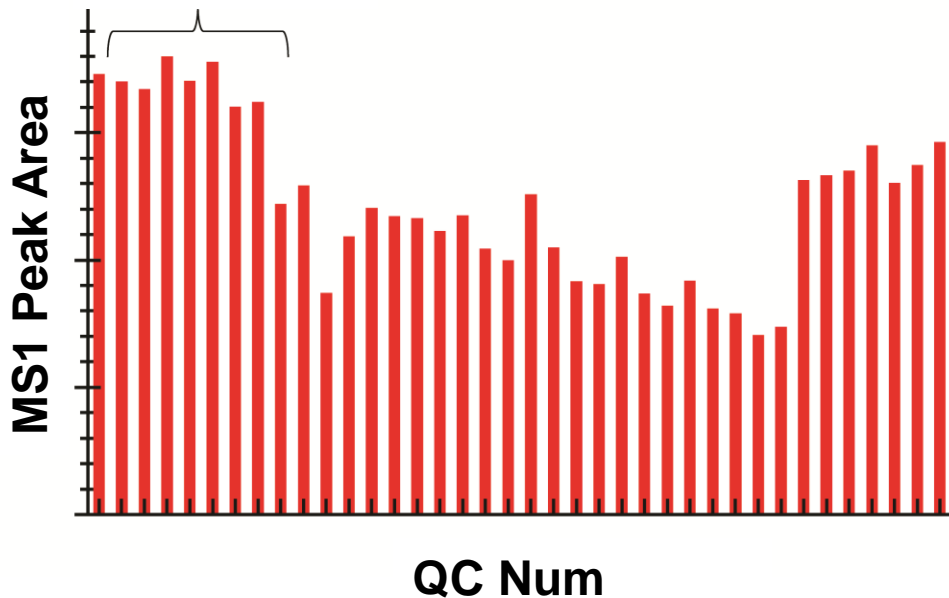
1. Utilizing SProCoP – Monitoring LC MS/MS – System Suitability

Bereman, MS *et. al.*, JASMS 25(4) 581 2014

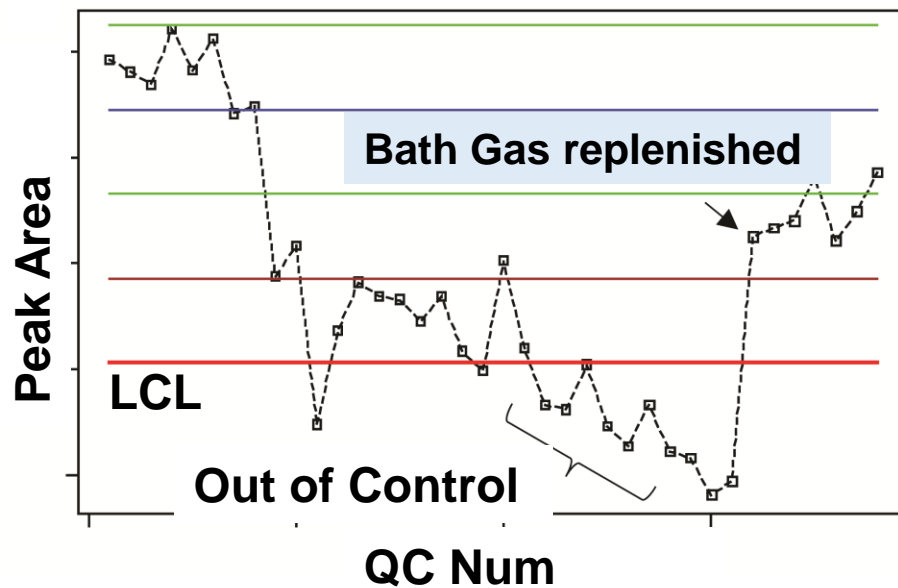
Reference set n=10



In Control

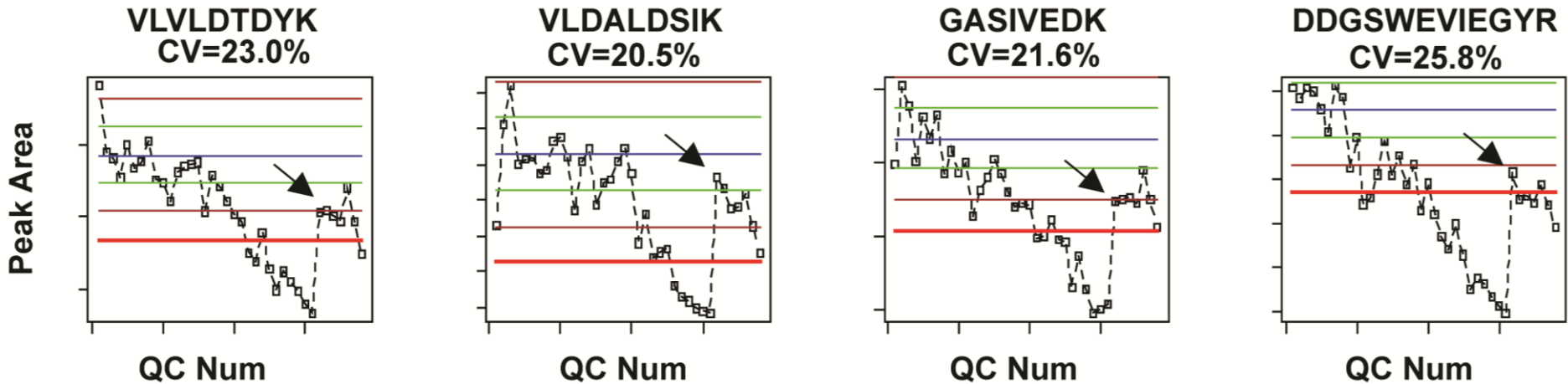


FFVAPFPEVFGK
CV=26.3%



1. Utilizing SProCoP – Monitoring LC MS/MS – System Suitability

Bereman, MS *et. al.*, JASMS 25(4) 581 2014



Importance of Systematic Evaluation – Problem may have not been diagnosed as quickly

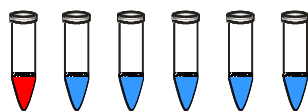
2. Utilizing SProCoP – Identification of Sources of Variation

Do self packed traps affect the LC MS/MS process?

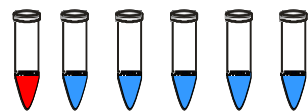
Bereman *et al.*, MCP 2013



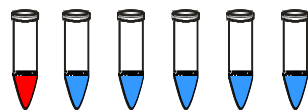
1 lysate + 5 QC stds



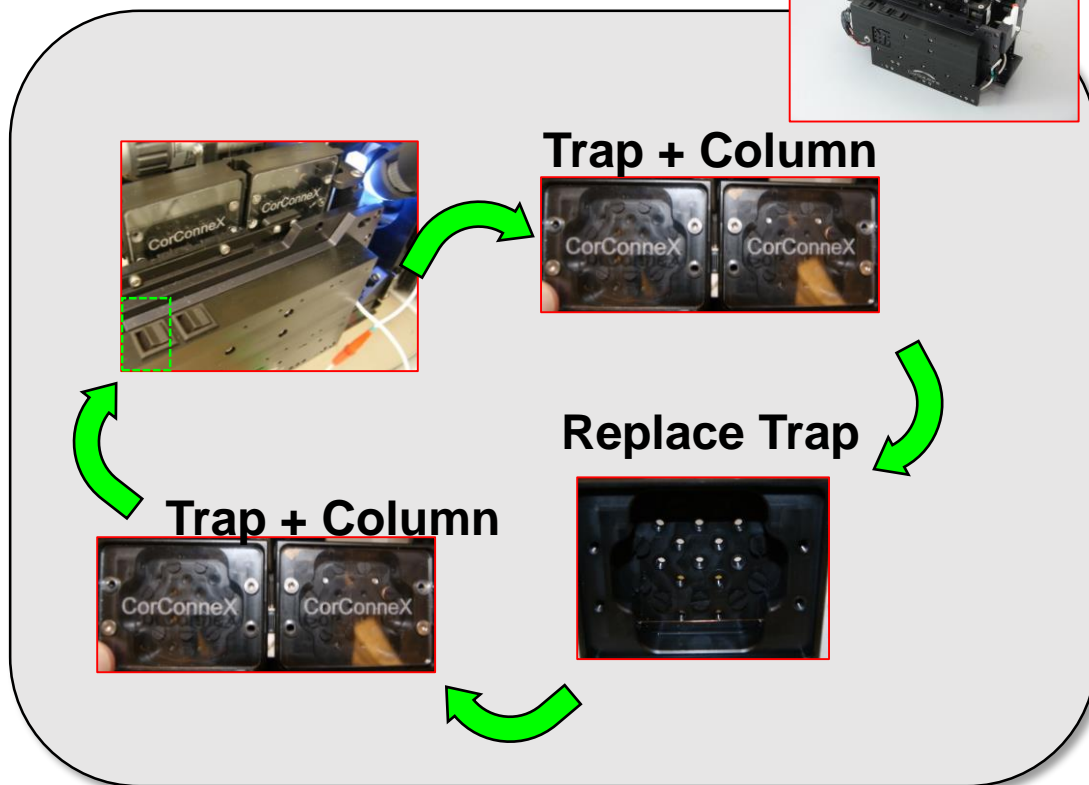
Trap 1 (4.0 cm)



Trap 2 (3.9 cm)



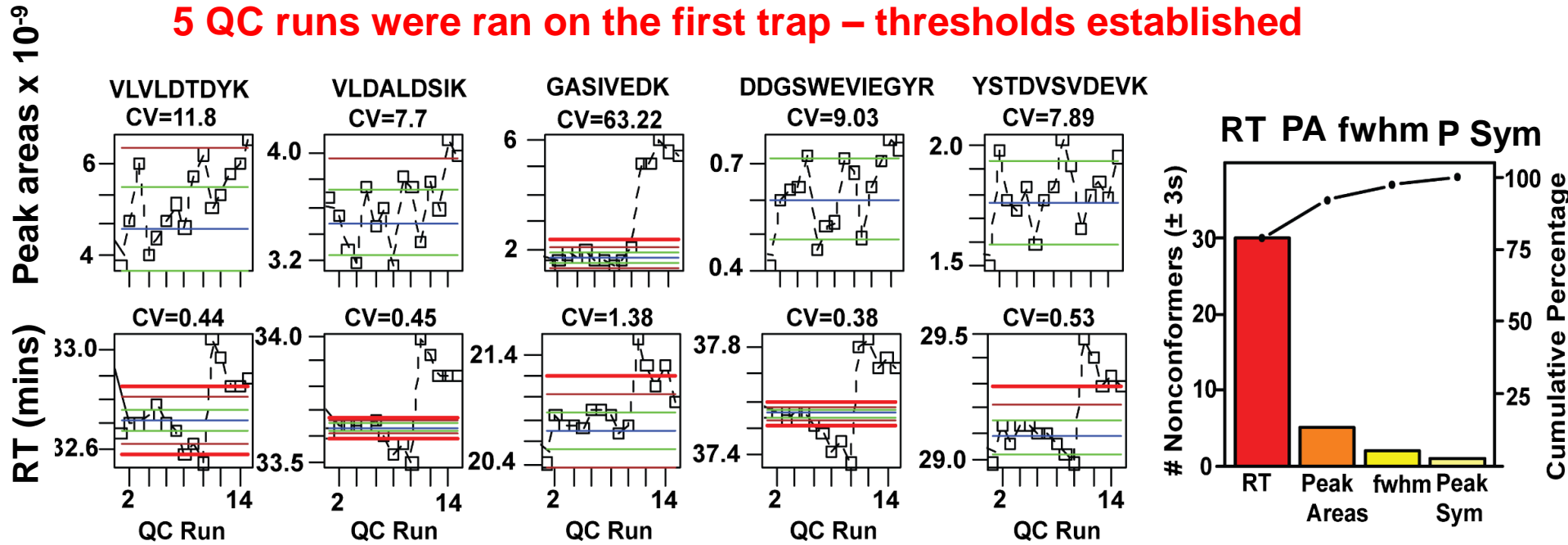
Trap 3 (4.1 cm)



2. Utilizing SProCoP – Identification of Sources of Variation

Bereman, MS *et. al.*, JASMS 25(4) 581 2014

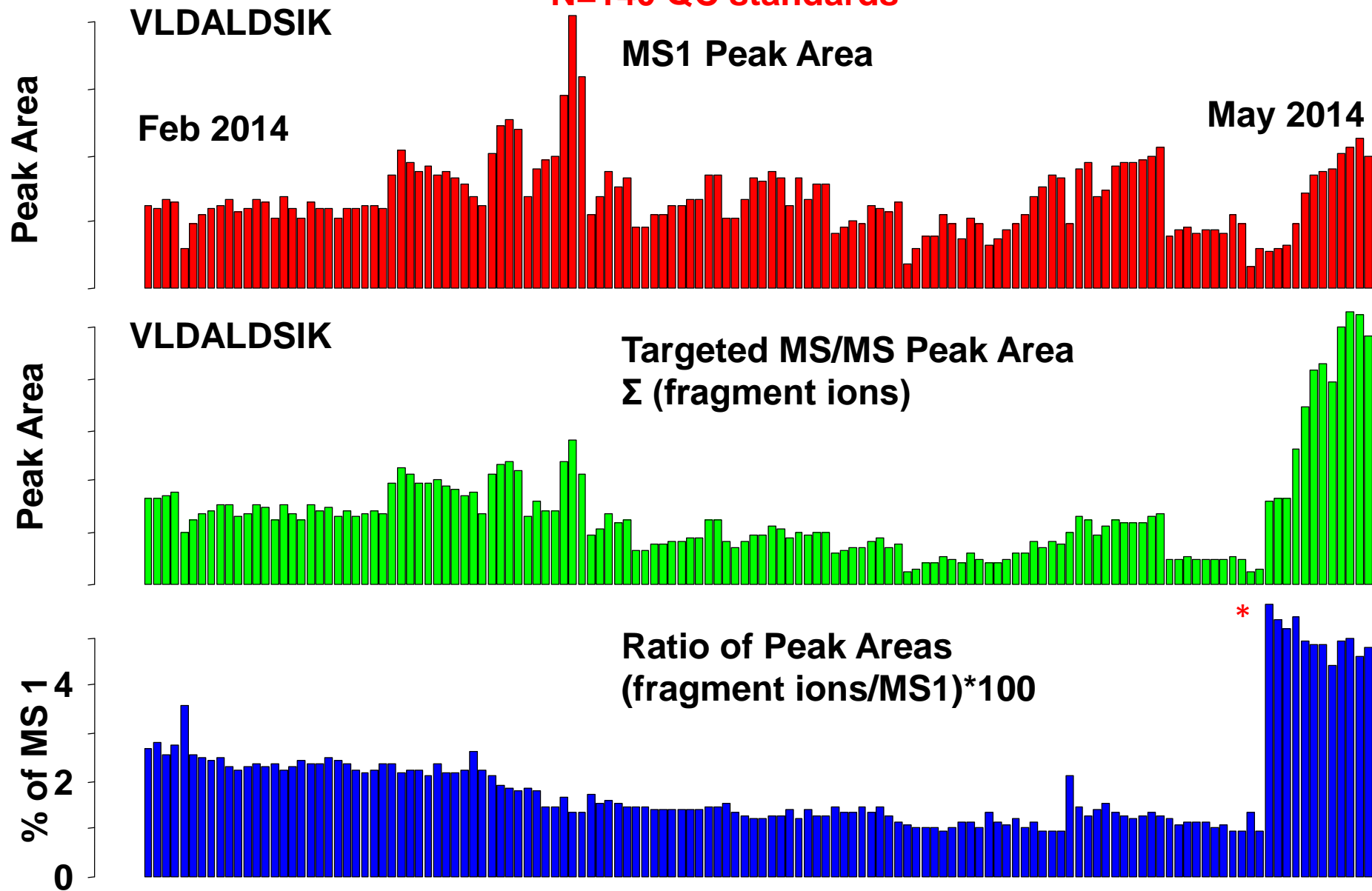
5 QC runs were ran on the first trap – thresholds established



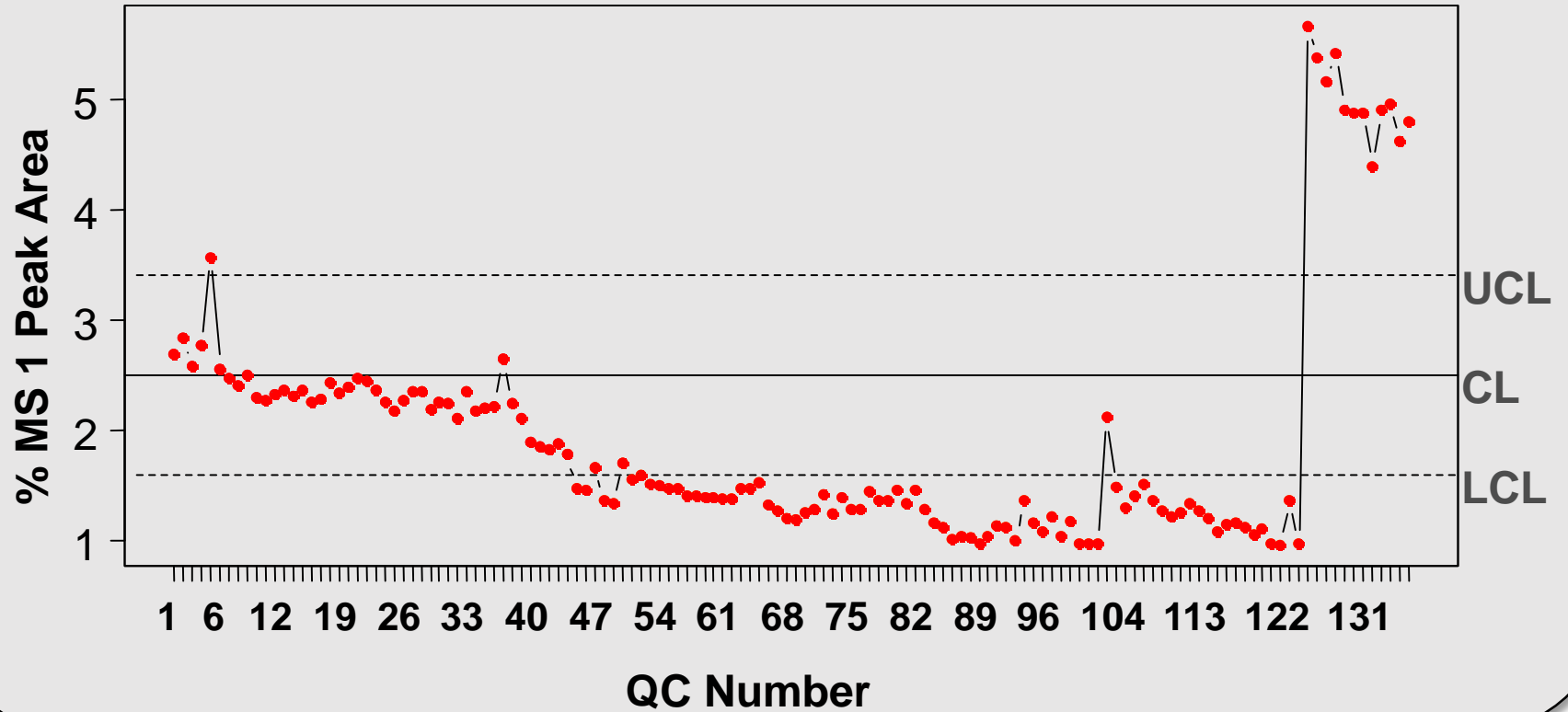
Pareto chart provides nice summary of data – and points to which metric is most variable

3. Longitudinal Instrument Tracking - SProCoP

N=140 QC standards

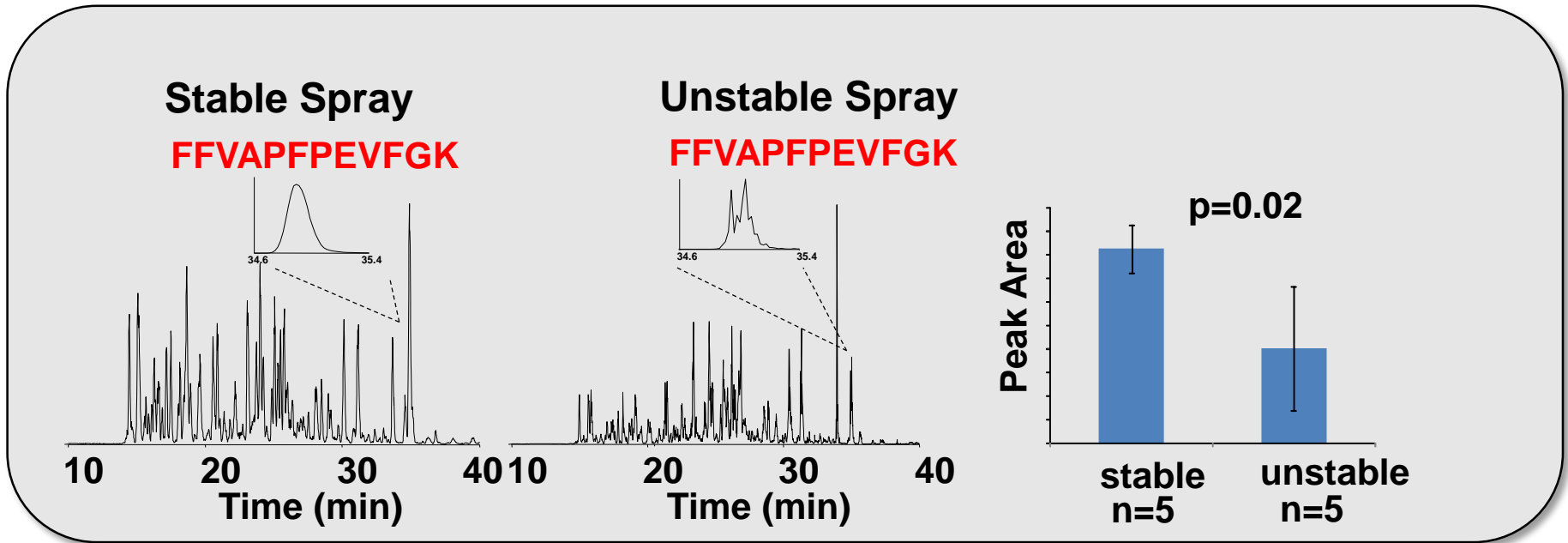


Longitudinal Instrument Tracking - SproCoP



Future Metric – Spray Stability

Difficult to quantify

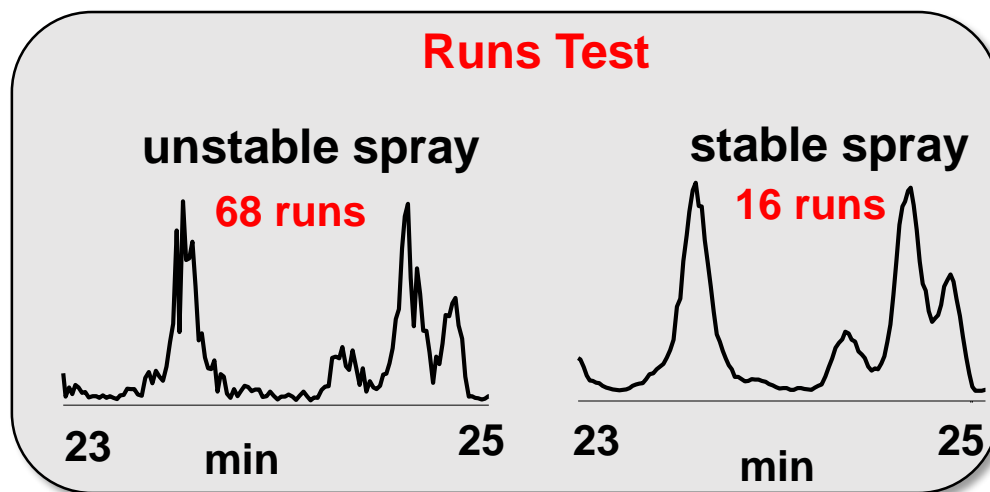
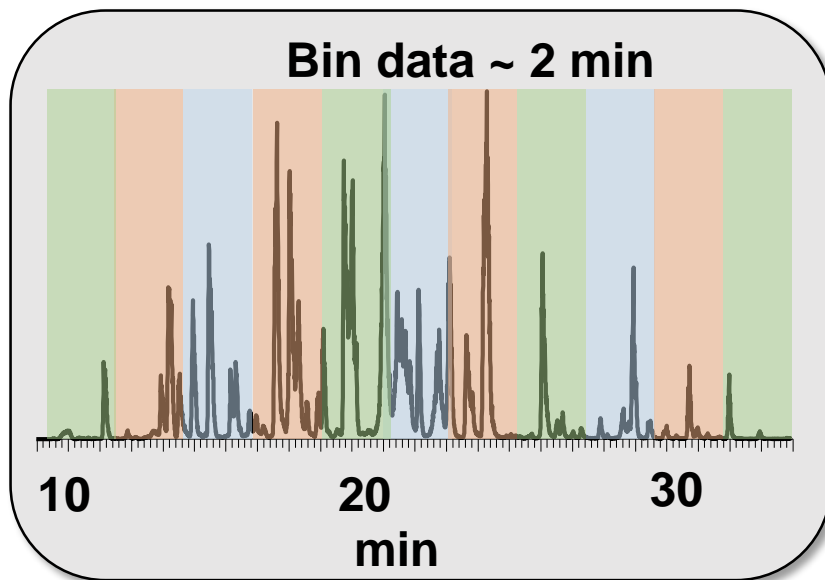
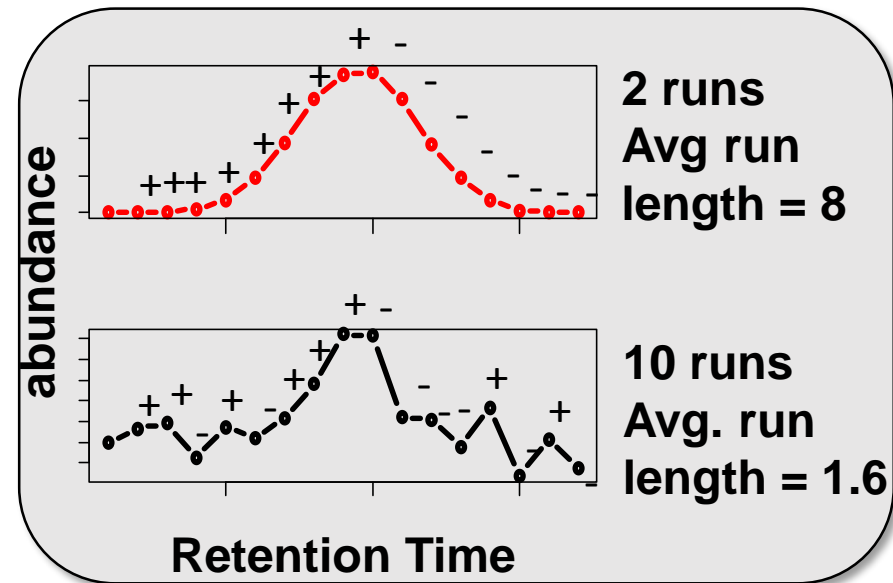


Increased variance in abundances – decreased power to identify differences and can lead to false positives

Qualitative detection is based on duty cycle

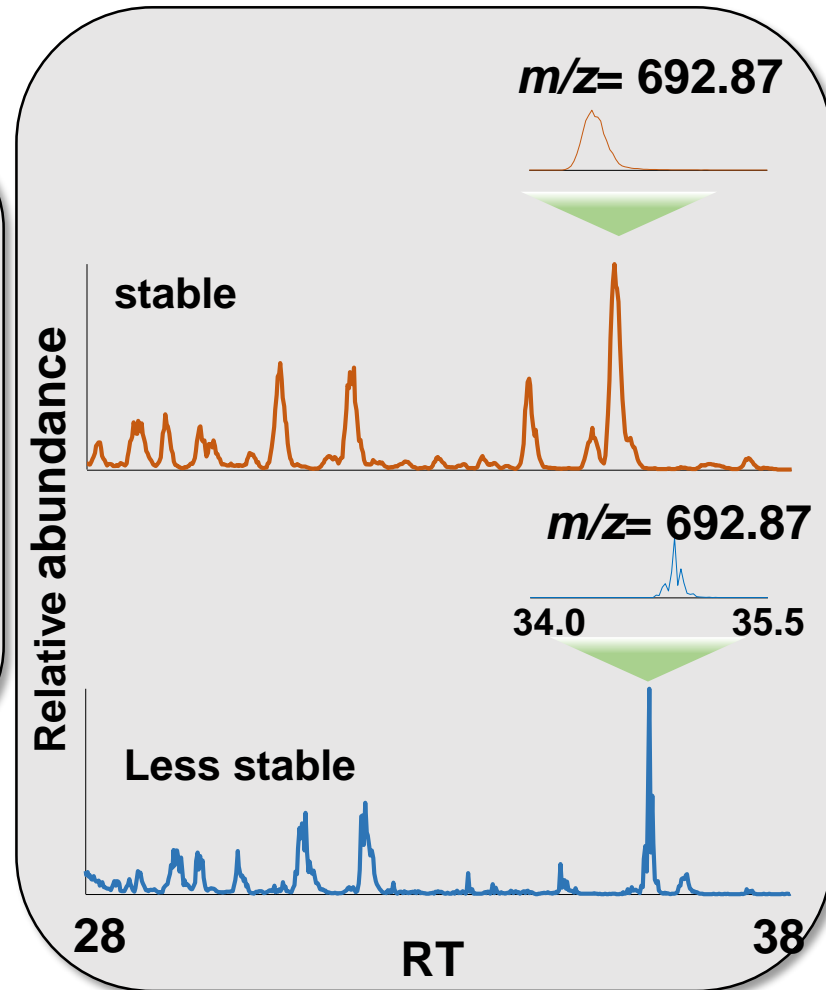
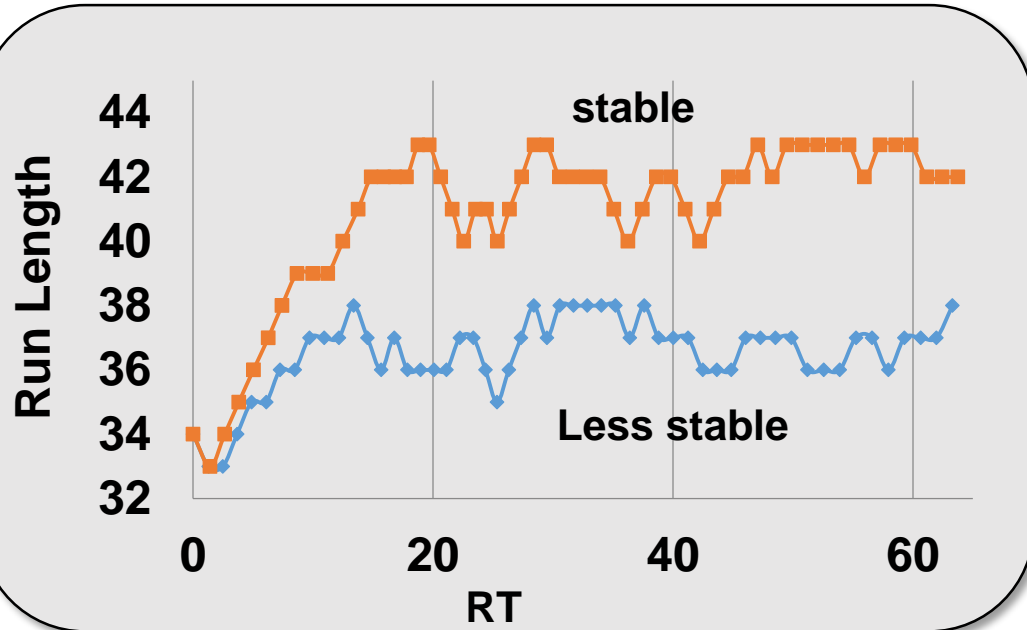
Runs Test to Determine When ESI Stability Changes

$$\text{Sign} = I_{N+1} - I_N$$



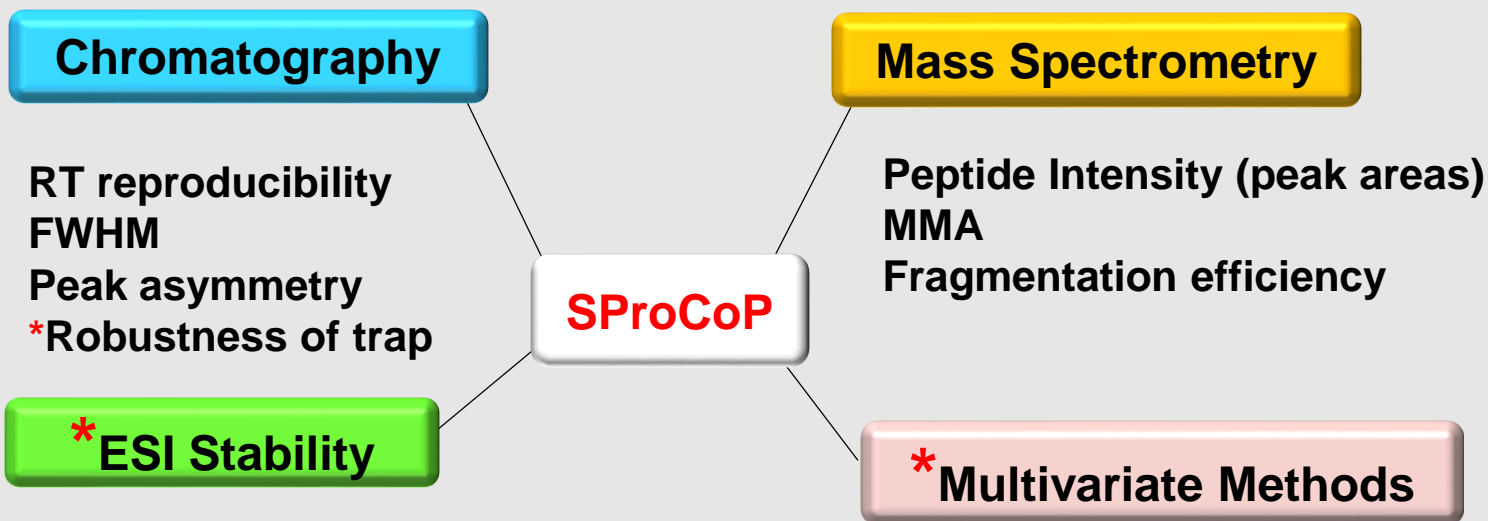
Unstable spray was smoothed (Boxcar n=3) to mimic stability

Average Run Length Differentiates ESI Stability



- ❑ Define spray stability based on reference set – track metric in control chart

Summary – What Can SProCoP do for YOU – ?



***Features will be available in next version**

***Thursday Poster #288**

All from a couple of mouse clicks!

Acknowledgements

N.C. State University

**Center for Human Health and the
Environment
Dr. Emily Griffith - Department of
Statistics**

Skyline Team

**Michael MacCoss
Brendan MacLean
Nick Shulman
Yuval Boss**