Quality Assurance Issues for DON Testing

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USWBSI Diagnostic Laboratories

- The USWBSI funds 4 diagnostic labs
  - University of Minnesota: Dr. Yanhong Dong
  - North Dakota State University (wheat): Dr. Michelle Mostrum
  - North Dakota State University (barley): Dr. Paul Schwarz
  - Virginia Tech.: Dr David Schmale.
- > $500,0000 in annual funding
- >50,000 samples tested/year
- These labs are an integral part of the USWBSI Goal: *to develop as quickly as possible effective control measures that minimize the threat of FHB), including the reduction of mycotoxins, to the producers, processors, and consumers of wheat and barley.*

So Why be Concerned About Quality Assurance and Control?

- Laboratory managers: assure that proper procedures are being followed, methods are adequate, and instrumentation is performing properly. Researchers: Important in the design of experiments, interpretation of data, and understanding of limitations.
- Administration: Need a mechanism for oversight in this area of considerable investment.

Sources of Error in DON Testing

- Nature and Sampling
  - Larger source of error!

- Analytical
  - Smaller source of error!

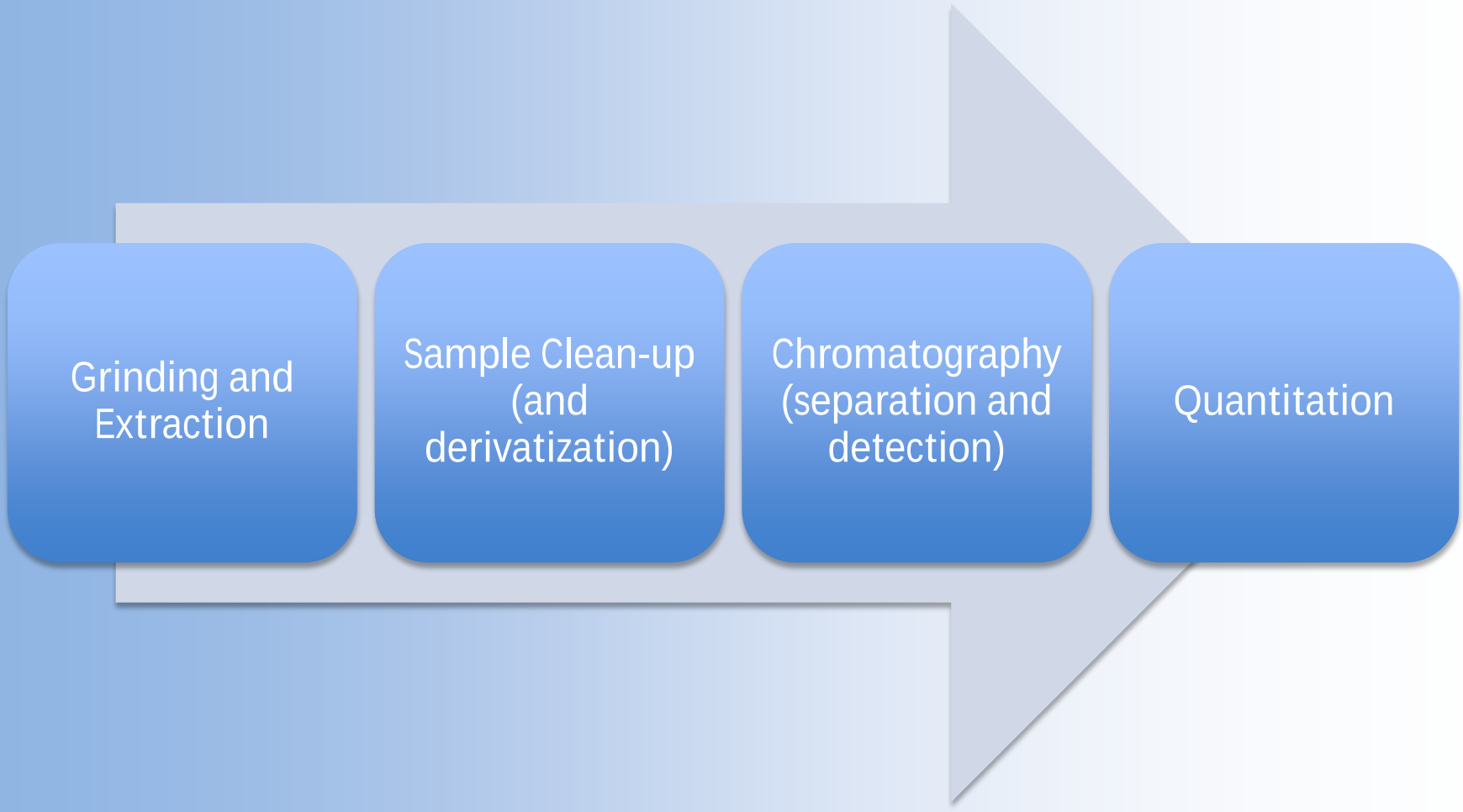
Sampling

- Sampling was covered at 2007 Forum.
  - Information at: http://www.scabusa.org/
- We know that DON can vary significantly from
  - spikelet to spikelet
  - spike to spike
  - Location in plot/field
- Solution is to obtain a large and more representative sample, and then reduce for analysis

Practical Limitations on Sample Size and Testing

- **10 g samples**
  - 2 min for grinding and cleaning (mill).
  - 2 min x 10,000 samples = 2 months technician labor.
    - $10,000
- **200 g samples**
  - 15 min for grinding, “splitting”, and cleaning (mill and divider).
  - 15 min x 10,000 samples = 16 months technician labor
    - $80,000
    - Additional $7.0/sample cost
    - Slows return of data
- **The burden for proper sampling falls to the researcher!**

Analysis of DON

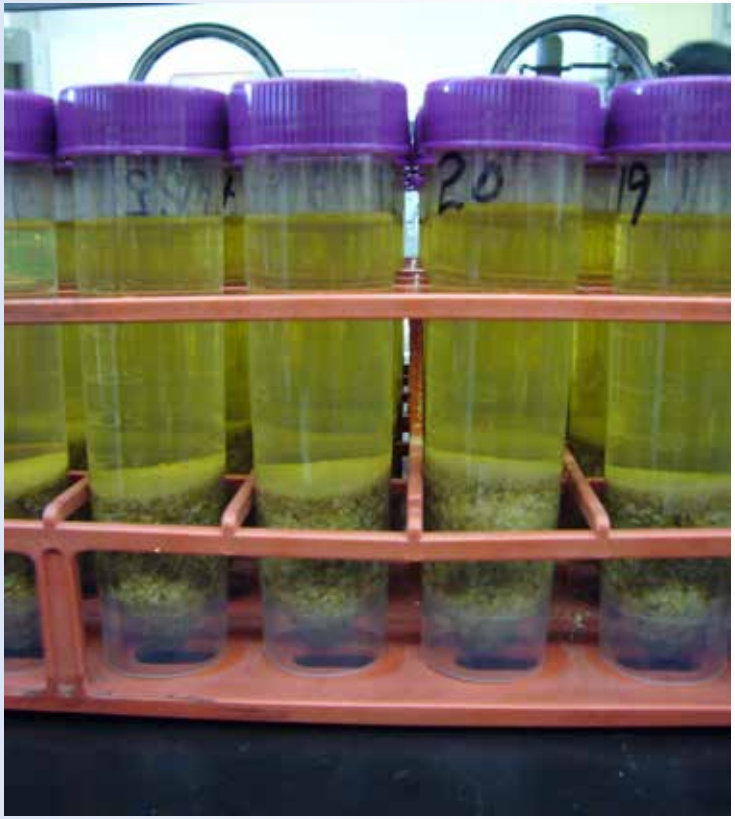


Grinding

- Grinding is needed to improve extraction efficiency
  - Not a large source of error.
  - Error can be largely eliminated with:
    - Consistent and uniform particle size
    - Cleaning between samples to avoid carry-over
    - Important for researchers grinding their own samples!

Extraction

- DON is typically extracted with acetonitrile:water.
  - Can be a source of analytical error.
  - Minimize error with:
    - Accurate weighing and pipetting . Moisture correction?
    - Larger vs. smaller weights and volumes
      - 2g -5 g/ ml are common.
      - Balance against chemical cost

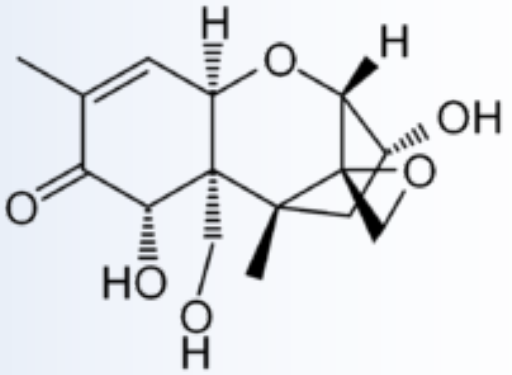


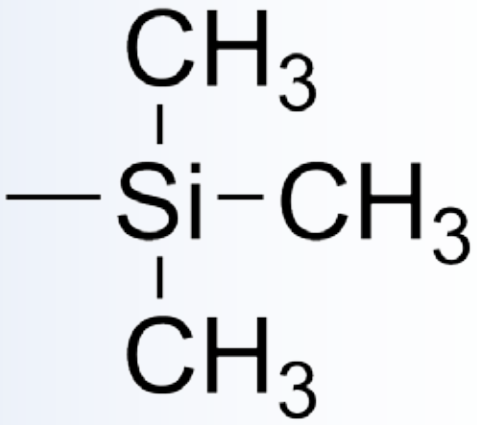
Sample Clean-Up

- Several 100 compounds may be extracted
- Removal of compounds that are not of interest can improve chromatographic separation and quantitation of DON
  - Not always needed, but is especially important if “low” limits of detection are needed.

Derivatization

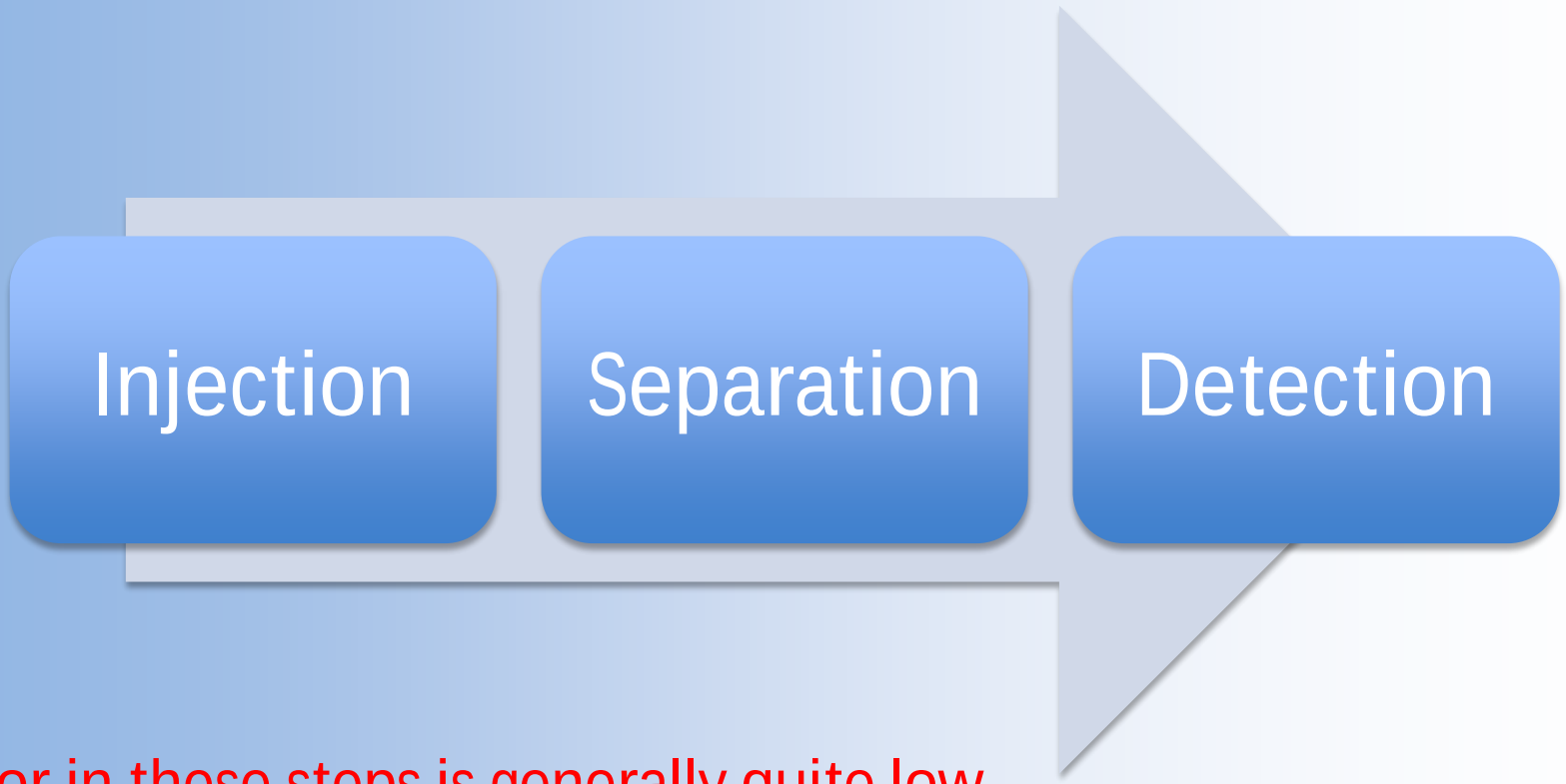
- Required for analysis by GC
  - "Gas" chromatography requires a "volatile" sample
    - Sample is in gas phase
    - DON is not volatile
  - Sample is treated with a "Silylating Agent" (TMS, TMCS)
  - Generally not a source of error
    - Pipetting and quality of chemicals
- Not needed for HPLC
    - Sample is in liquid phase





Trimethylsilyl (TMS) group

Analysis



- Error in these steps is generally quite low
  - Columns are monitored for loss in separation efficiency
  - Detector response may decrease with time.
  - Detector specific calibrations

Sample Injection

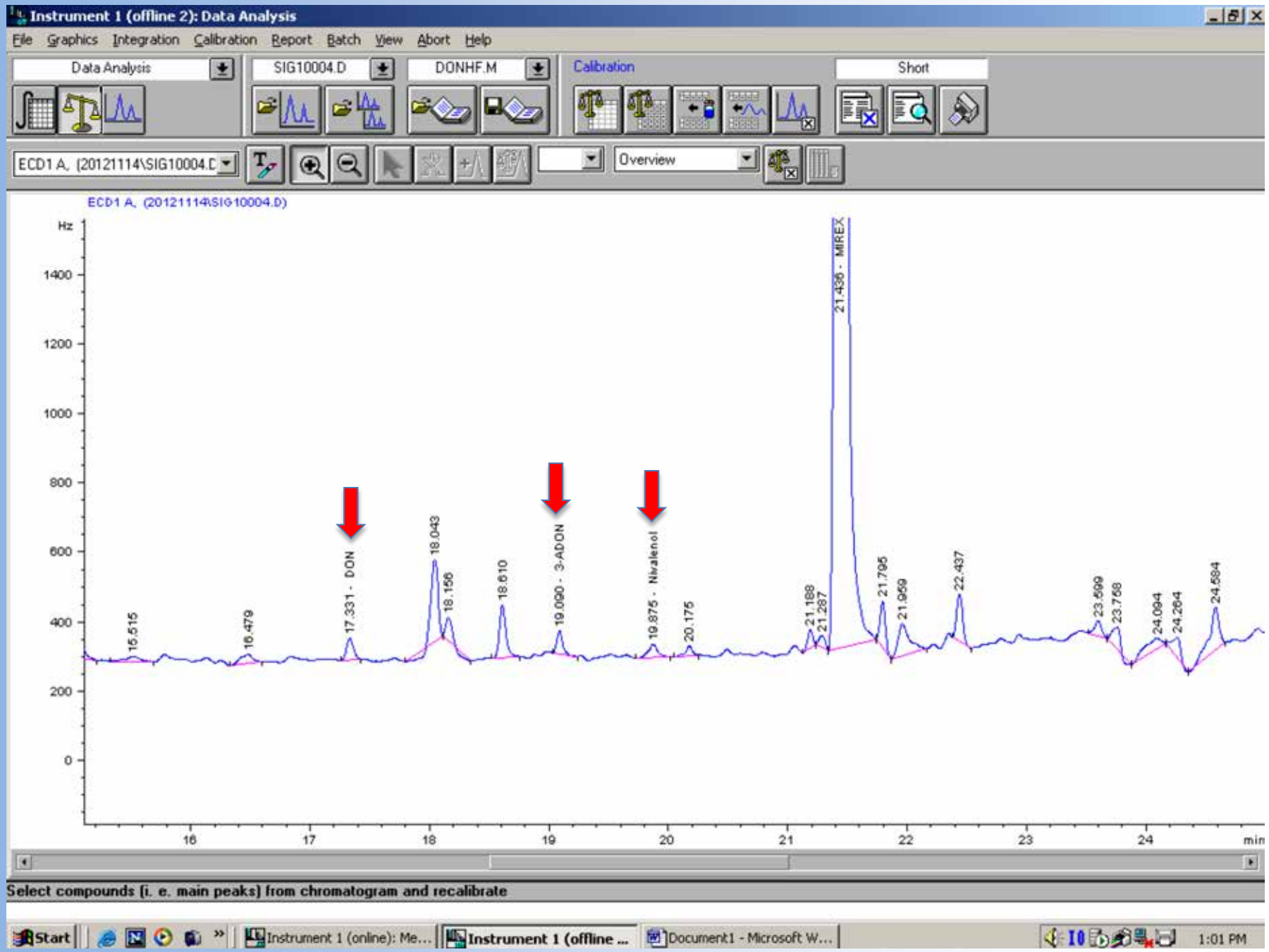
- Auto-sampler: Automated sample introduction reduces errors
- Use of external standard (Mirex) to monitor injection volume

Chromatographic Separation

- The sample extract contains a mixture of compounds.
- The extract is dissolved into a liquid (mobile phase), or volatilized into the gas phase, which carries it through a column containing the stationary phase.
- The compounds in the extract travel through the column at different speeds, causing them to separate.
  - Based upon on differential partitioning between the mobile and stationary phases.

GC Column

HPLC Column


Instrument 1 (offline 2): Data Analysis
File Graphics Integration Calibration Report Batch View Abort Help
Data Analysis
SIG10004.D
DONHF.M
Calibration
Short
ECD1 A, (20121114\SIG10004.D
Overview
ECD1 A, (20121114\SIG10004.D)
Hz
1400
1200
1000
800
600
400
200
0
15.515
16.479
17.331 - DON
18.043
18.156
18.610
19.090 - 3-ADON
19.875 - Nivalenol
20.175
21.188
21.287
21.436 - MIREX
21.795
21.959
22.437
23.599
23.758
24.094
24.264
24.584
16
17
18
19
20
21
22
23
24
min
Select compounds (i. e. main peaks) from chromatogram and recalibrate
Start
Instrument 1 (online): Me...
Instrument 1 (offline ...
Document1 - Microsoft W...
1:01 PM

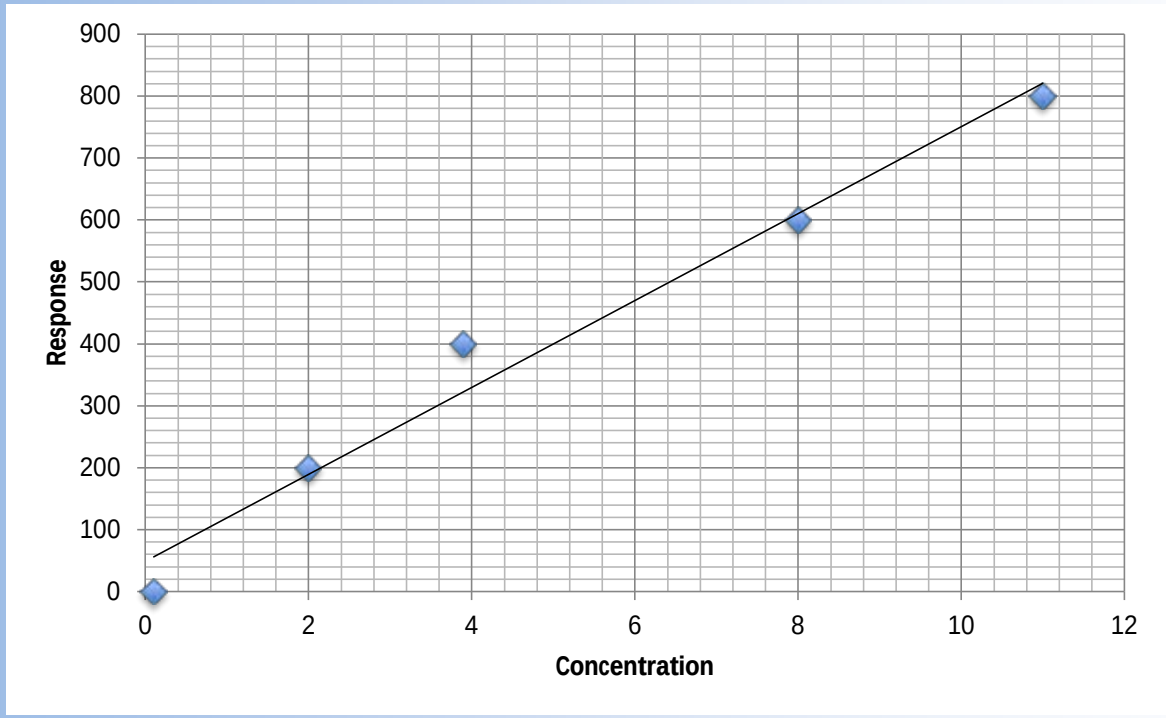
Chromatographic Separation and Detection

- USWBSI labs use
  - Gas Chromatography
    - GC-MS (Mass Selective Detection)
    - GC-ECD (Electron Capture Detection)
- Commercial labs also use
  - ELISA (test kits)
  - HPLC with UV detection

Detection of DON

- GC-ECD (electron capture detector)
  - Very sensitive to halogen ions (TMCS derivatives)
- GC-MS (mass selective detector)
  - Compounds are fragmented and ionized
  - Monitor for “select” ions that are diagnostic of DON or other specific compounds of interest
  - Can be used for positive identification of compounds

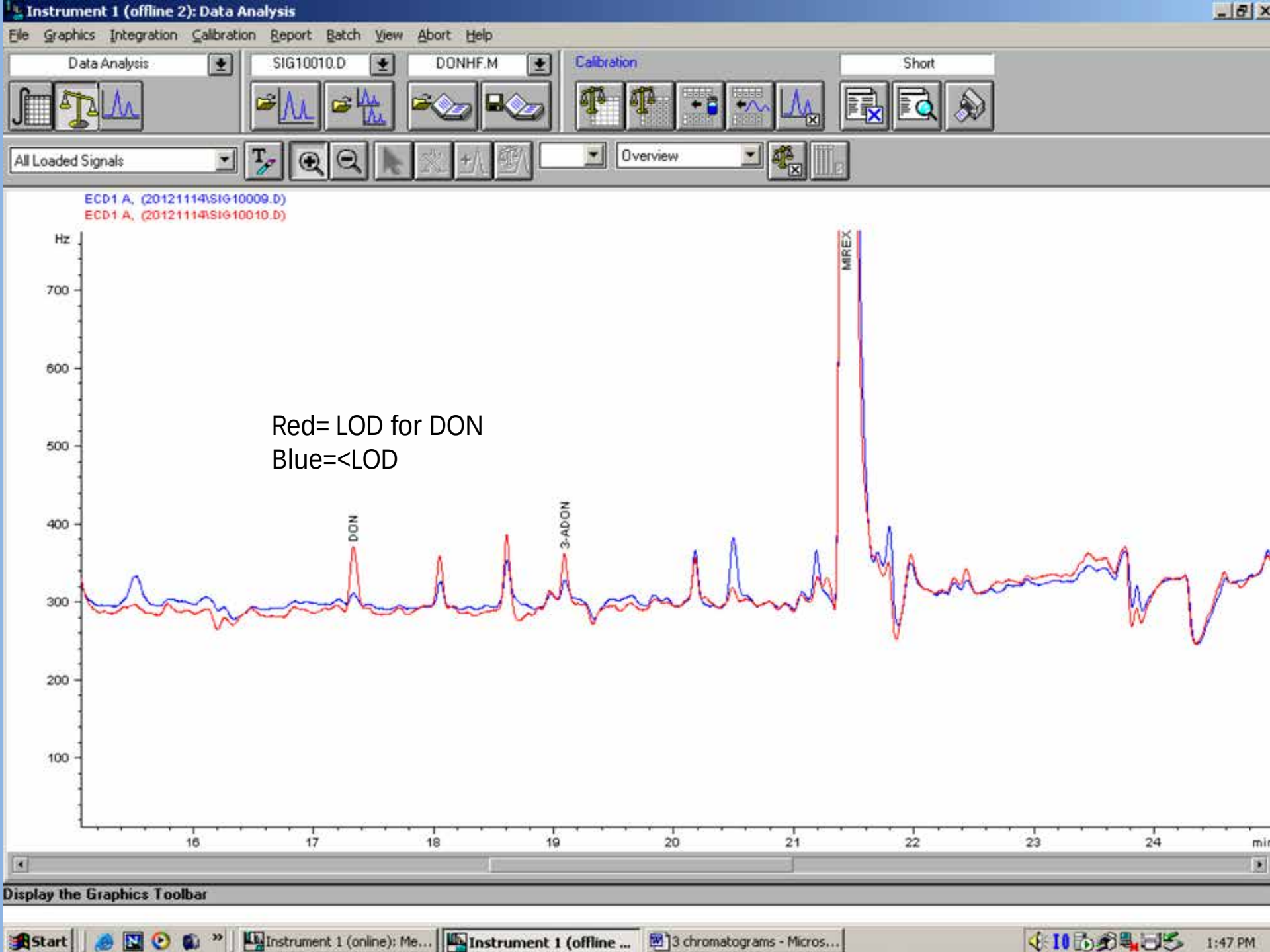
Quantitation

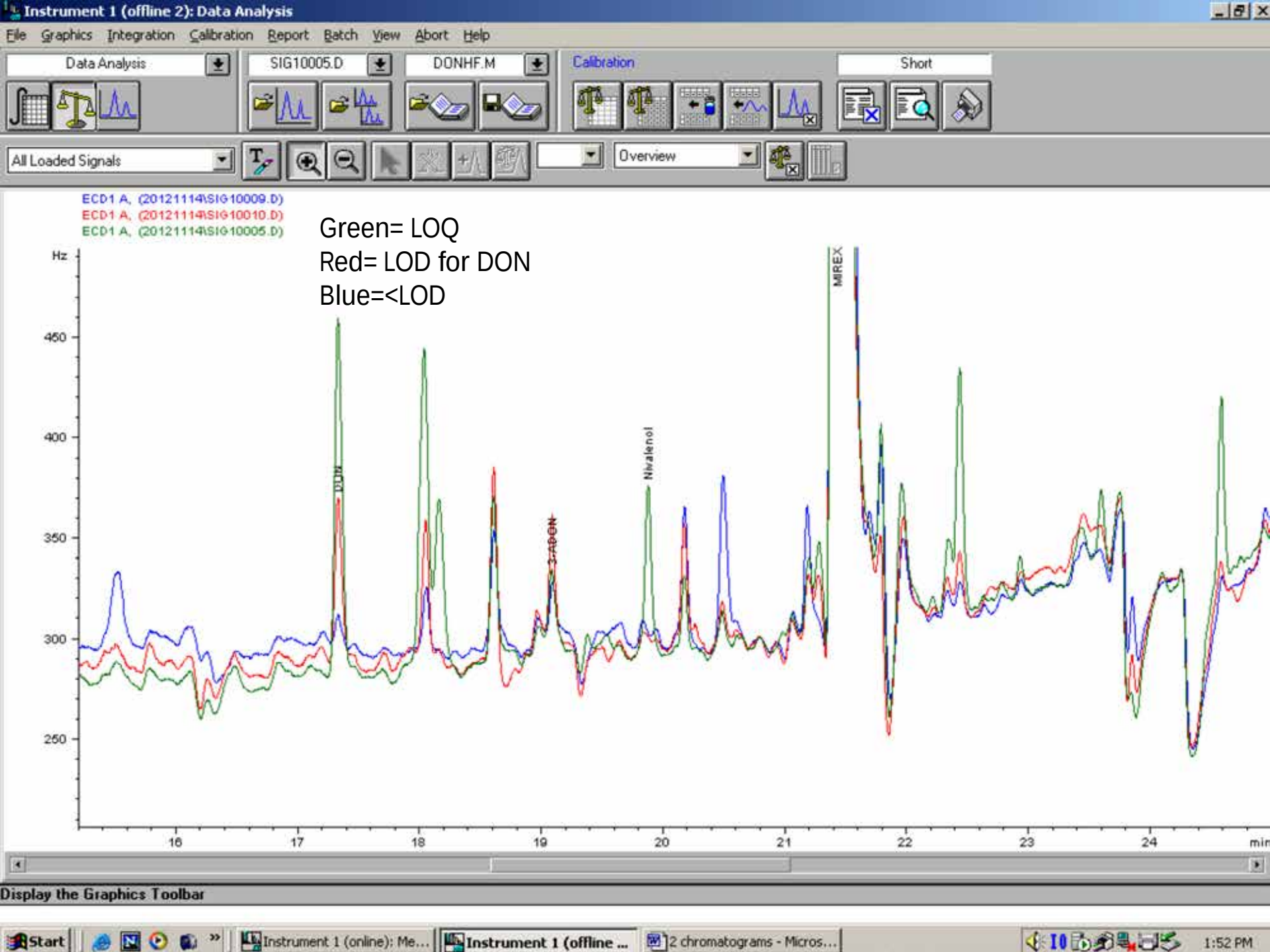


- Standard curve using “known” concentrations of DON
- Curve has specific range
- Curve for each instrument/detector
- Likely a source of “inter-lab” differences

LOD and LOQ

- Limit of Detection: is the lowest concentration an analyte can be detected (but not quantitated).
- Limit of Quantitation: is the lowest concentration of an analyte that can be determined with accuracy and precession
- Both are complicated concepts
- LOD: the signal of the analyte should be at least 3 x greater than the background noise
- LOQ: the signal of the analyte should be at least 5-6 x greater than the background noise

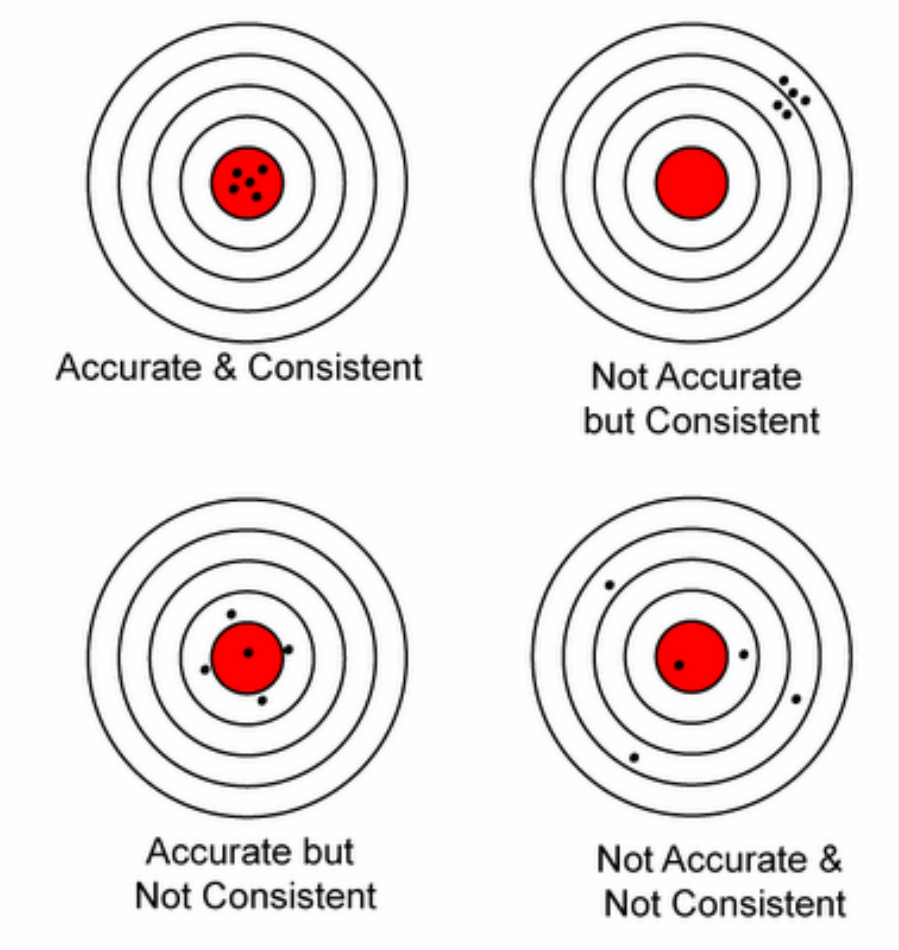

Instrument 1 (offline 2): Data Analysis
File Graphics Integration Calibration Report Batch View Abort Help
Data Analysis
SIG10010.D
DONHF.M
Calibration
Short
All Loaded Signals
Overview
ECD1 A, (20121114\SIG10009.D)
ECD1 A, (20121114\SIG10010.D)
Hz
700
600
500
400
300
200
100
DON
3-ADON
MIREX
Red= LOD for DON
Blue=<LOD
16
17
18
19
20
21
22
23
24
min
Display the Graphics Toolbar
Start
Instrument 1 (online): Me...
Instrument 1 (offline ...
3 chromatograms - Micros...
1:47 PM


Instrument 1 (offline 2): Data Analysis
File Graphics Integration Calibration Report Batch View Abort Help
Data Analysis
SIG10005.D
DONHF.M
Calibration
Short
All Loaded Signals
Overview
ECD1 A, (20121114\SIG10009.D)
ECD1 A, (20121114\SIG10010.D)
ECD1 A, (20121114\SIG10005.D)
Green= LOQ
Red= LOD for DON
Blue=<LOD
Hz
460
400
350
300
250
DON
3-ADON
Nivalenol
MREX
16
17
18
19
20
21
22
23
24
min
Display the Graphics Toolbar
Start
Instrument 1 (online): Me...
Instrument 1 (offline ...
2 chromatograms - Micros...
1:52 PM

LOQ

- USWBSI Labs
  - LOQ: 0.05 to 0.1 ppm
    - Reported as Non-detectable or <0.1 ppm
  - Some cooperators request that numbers below LOQ be reported (statistical analysis)

Accuracy and Consistency



http://spcpchemroom9.blogspot.com

Intra-Lab Checks (consistency)

Are results consistent over time?

- Labs run multiple checks with each set of analysis
- These provide SD or CV
- Too large a deviation from mean suggests that analyses be repeated and source(s) of error be identified

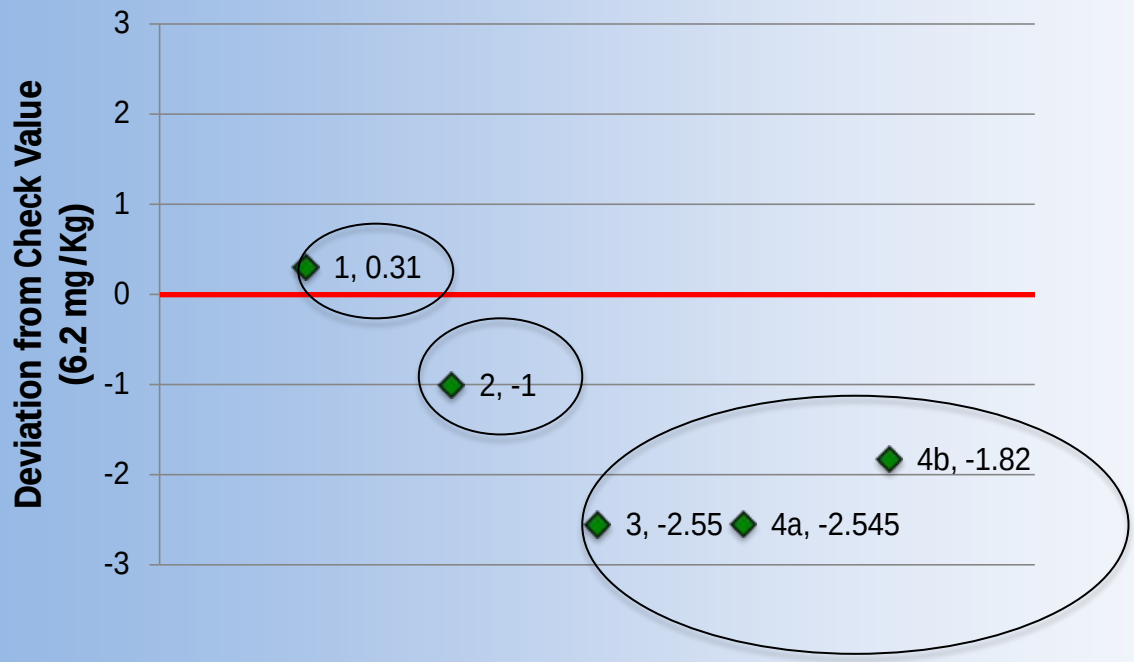
USWBSI labs

- Each lab runs 500-1000 check/year
- 3-8 samples (low to high DON, wheat, barley and maize)
- CVs vary with lab, but average 10-16%
  - Highest CVs on on samples close to LOQ

Inter-Lab Checks

Provided by Trilogy Labs

- 3 samples/month:
  - Barley and wheat
  - Low, medium and high
- Samples were sent to the four labs in March, April , September and October.
- Provides a comparison between labs
  - However this is not a measure of accuracy


Deviation from Check Value
(6.2 mg/Kg)
3
2
1
0
-1
-2
-3
1, 0.31
2, -1
4b, -1.82
3, -2.55
4a, -2.545

Low, Medium and High DON check samples (Trilogy). March 2012

Inter-Lab Checks

- There are differences between labs. However:
  - Sample rank (high, medium, low) does not change across labs.
  - Differences (%) between high and low-medium samples are consistent across labs
  - Complete experiments should be sent to the same lab

		Trilogy		Lab 1		Lab 2		Lab 3		Lab 4	
March		Value	Diff %	Value	Diff %	Value	Diff %	Value	Diff %	Value	Diff %
	Low	0.70	-89	0.71	-89	0.60	-88	0.45	-88	0.42	-89
	Med	3.50	-44	3.44	-47	2.70	-48	1.97	-68	2.32	-63
	High	6.20		6.50		5.20		3.65		3.66	
April											
	Low	0.50	-92	0.50	-92	0.50	-91	0.53	-91	0.49	-91
	Med	3.90	-39	3.90	-39	3.00	-53	3.14	-51	2.84	-56
	High	6.40		6.40		5.60		6.02		5.20	
September											
	Low	0.50	-92	0.46	-92	0.50	-91	0.46	-93	0.44	-90
	Med	3.50	-45	2.49	-55	2.90	-55	3.29	-47	2.59	-60
	High	6.40		5.51		5.70		6.16		4.59	
October											
	Low	1.40	-77	0.86	-79	1.10	-78	1.17	-80	1.07	-77
	Med	4.90	-21	2.90	-30	4.40	-14	4.86	-21	3.81	-17
	High	6.20		4.13		5.10		5.89		4.61	