# Pesticide Analysis FAQs

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# goal is to provide practice information


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# focus on

## GC and LC tandem mass spectrometry

- QQQ
- MRM or transitions

## Matrix-matched calibration

# important references

## Document Nº SANTE/12682/2019

ANALYTICAL QUALITY CONTROL AND METHOD VALIDATION PROCEDURES
FOR PESTICIDE RESIDUES ANALYSIS IN FOOD AND FEED

https://www.eurl-pesticides.eu/userfiles/file/EurlALL/AqcGuidance_SANTE_2019_12682.pdf

**Guidelines for the Validation of Chemical Methods for the FDA FVM Program, 3rd Ed.**

**US Food & Drug Administration**
**Office of Foods and Veterinary Medicine**

**Guidelines for the Validation of Chemical Methods for the FDA FVM Program, 3rd *Edition***

https://www.fda.gov/media/81810/download

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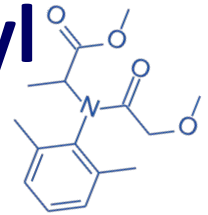
# product ion peaks

**Q1 ion** → **Q3 ion**

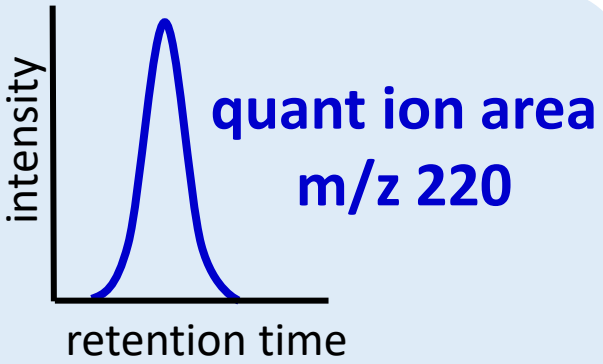
precursor ion — product ion

**Metalaxyl**



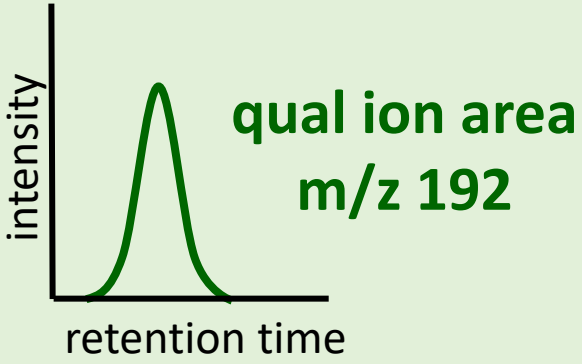
**280** —MRM 1→ **220**

precursor ion — product ion

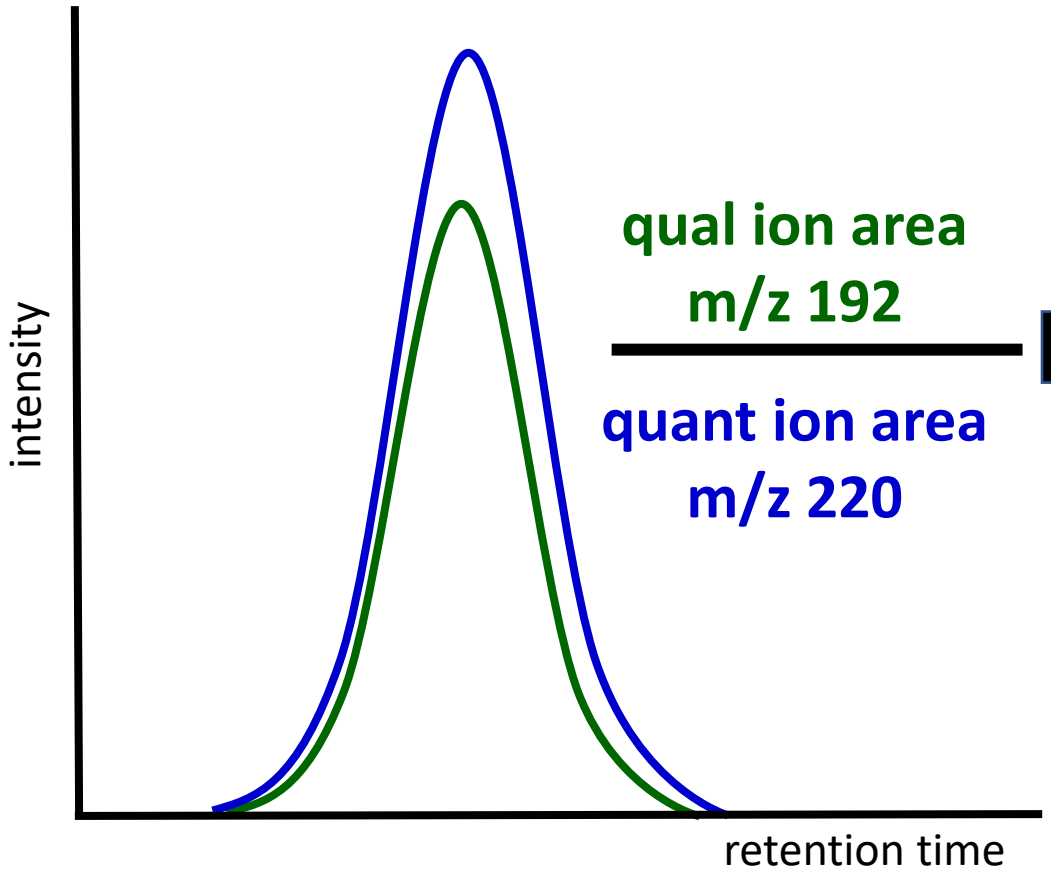



**280** —MRM 2→ **192**

precursor ion — product ion



# identification data




qual ion area
m/z 192

quant ion area
m/z 220

ion ratio

**82%**

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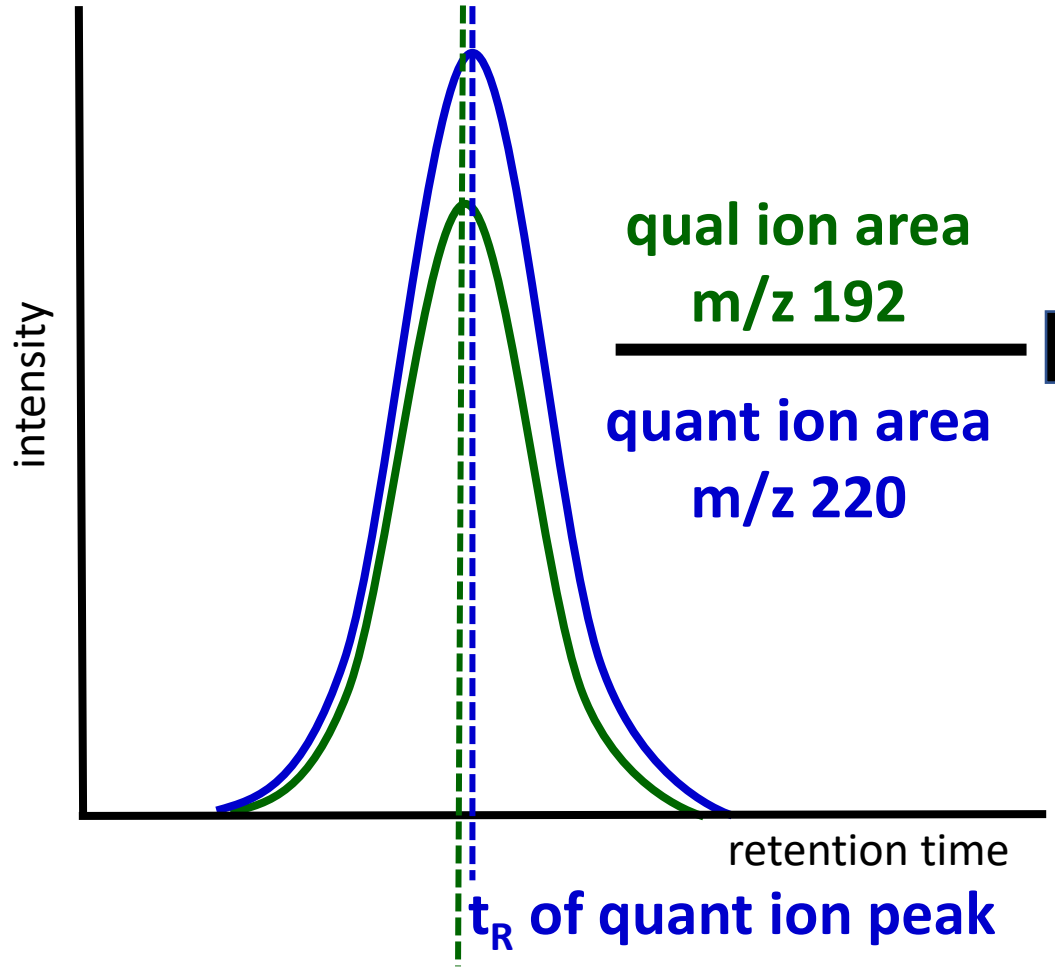
# identification data

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m/z 220

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**82%**

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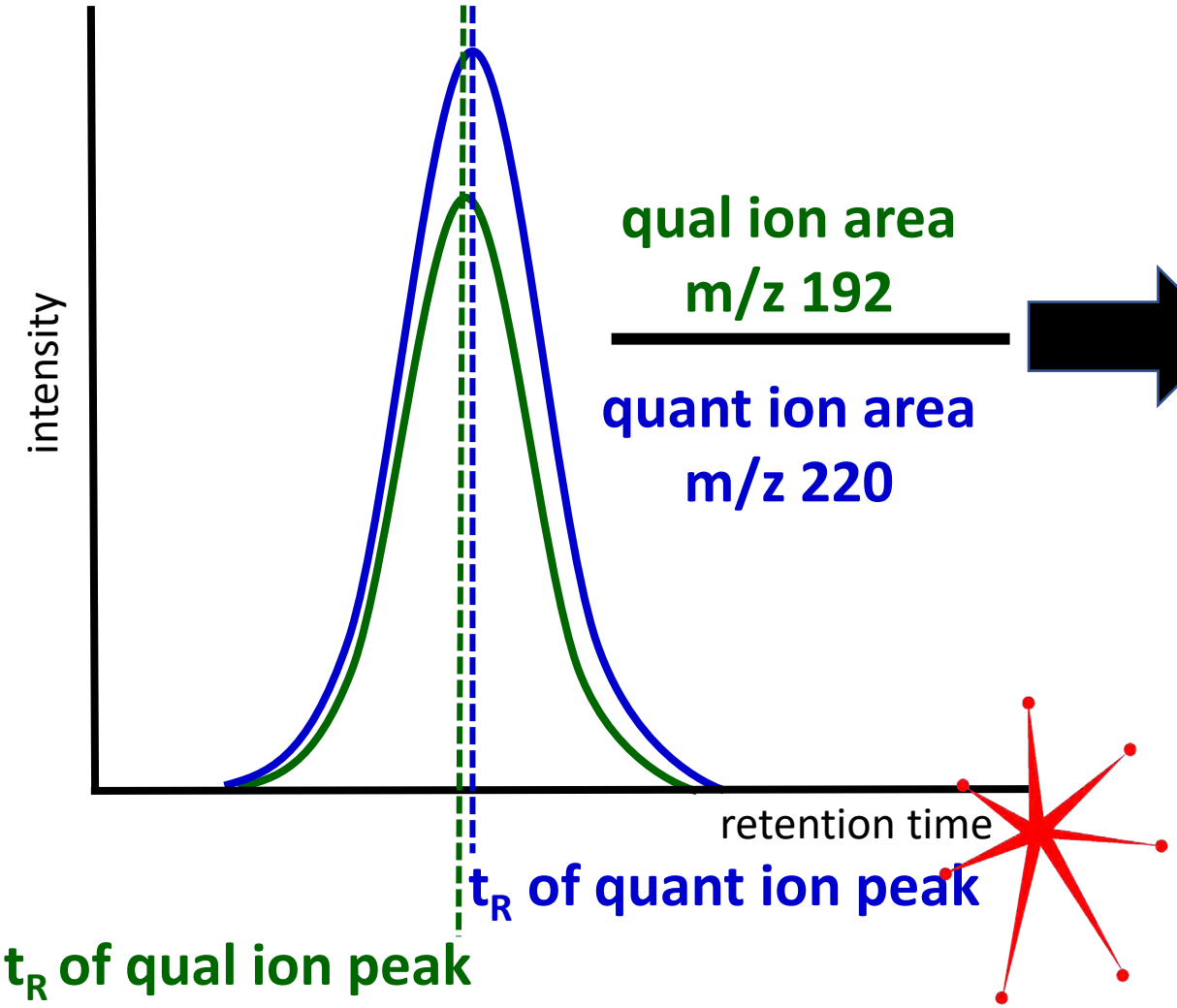


qual ion area
m/z 192

quant ion area
m/z 220

ion ratio

**82%**

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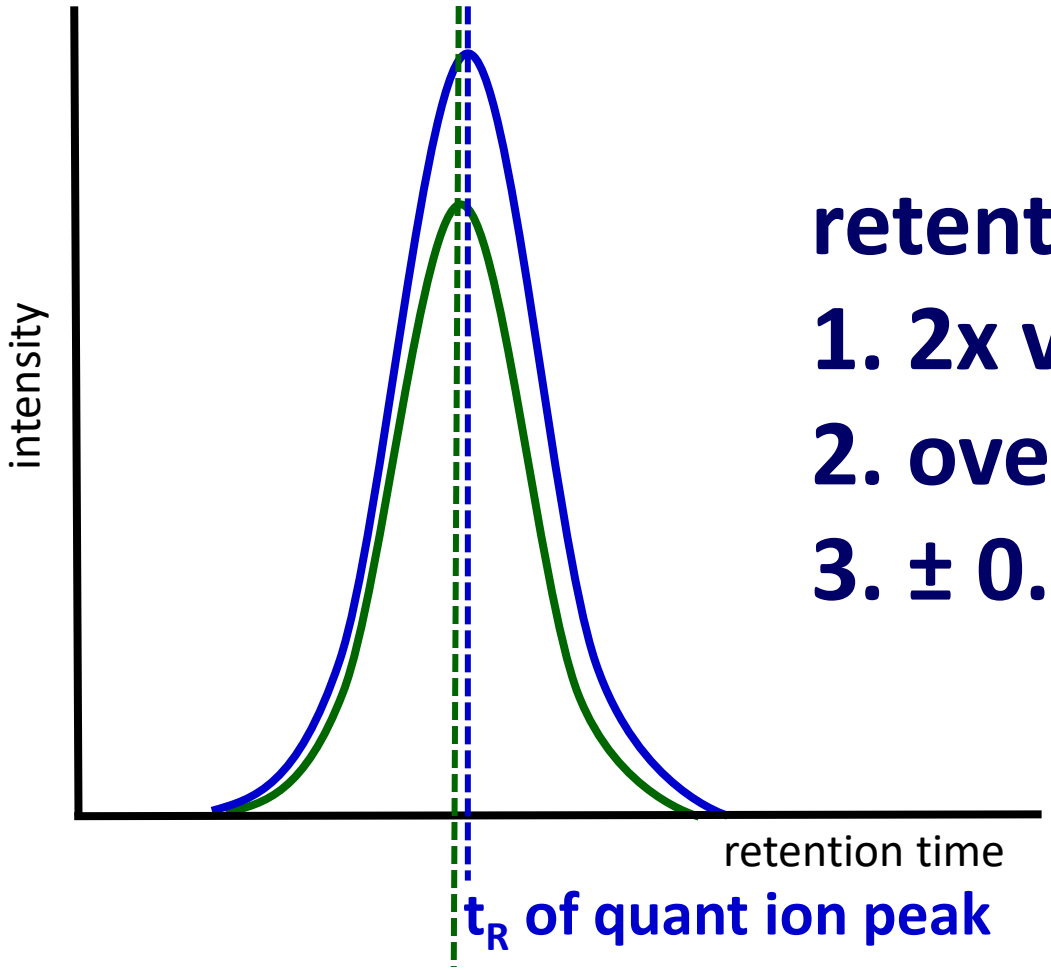
# identification data –retention time

quant ion

qual ion




**retention time**

**1. 2x void**

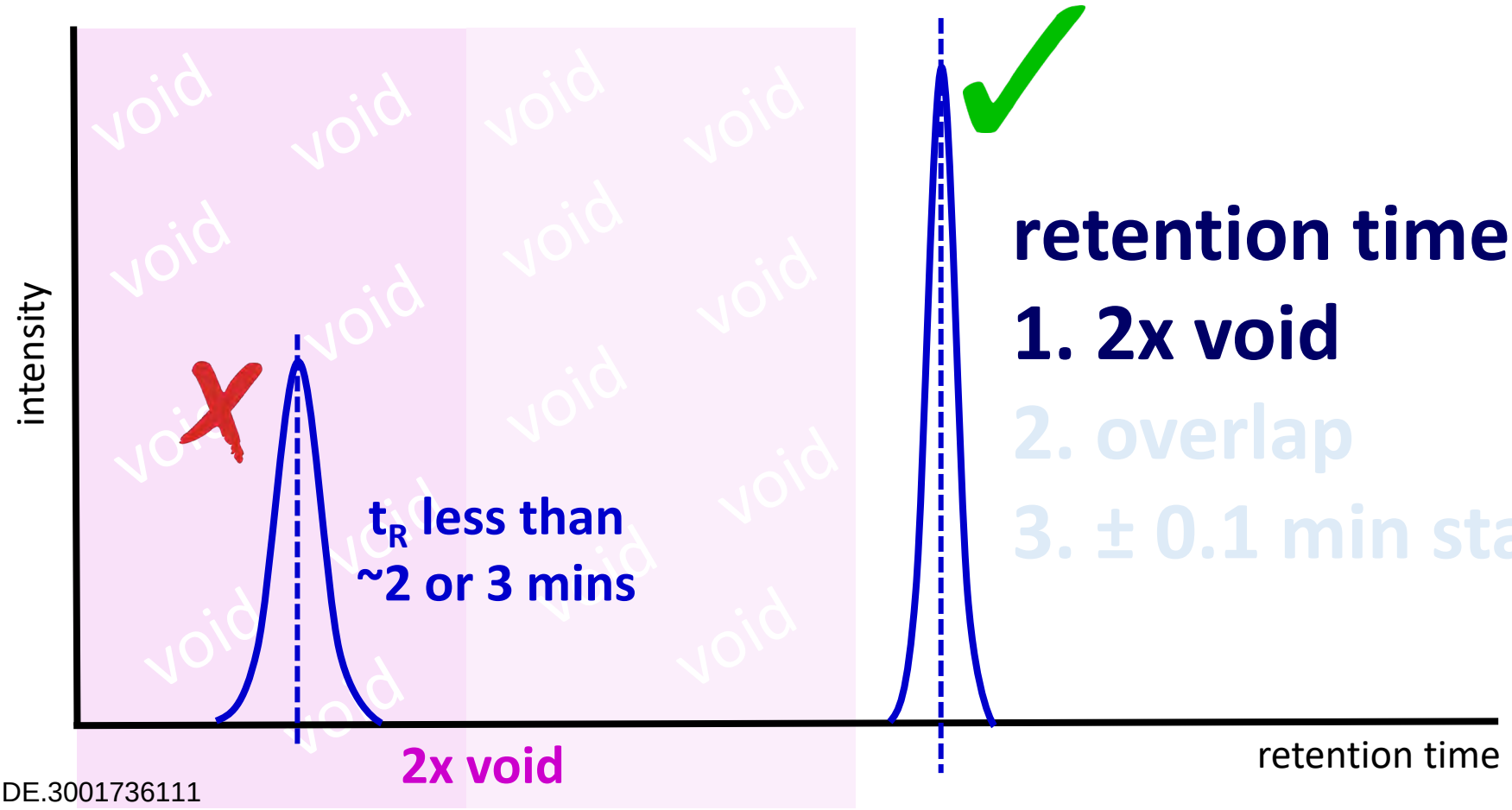
**2. overlap**

**3. ± 0.1 min standard**

# identification data –retention time

**“real” retention for polar pesticides**

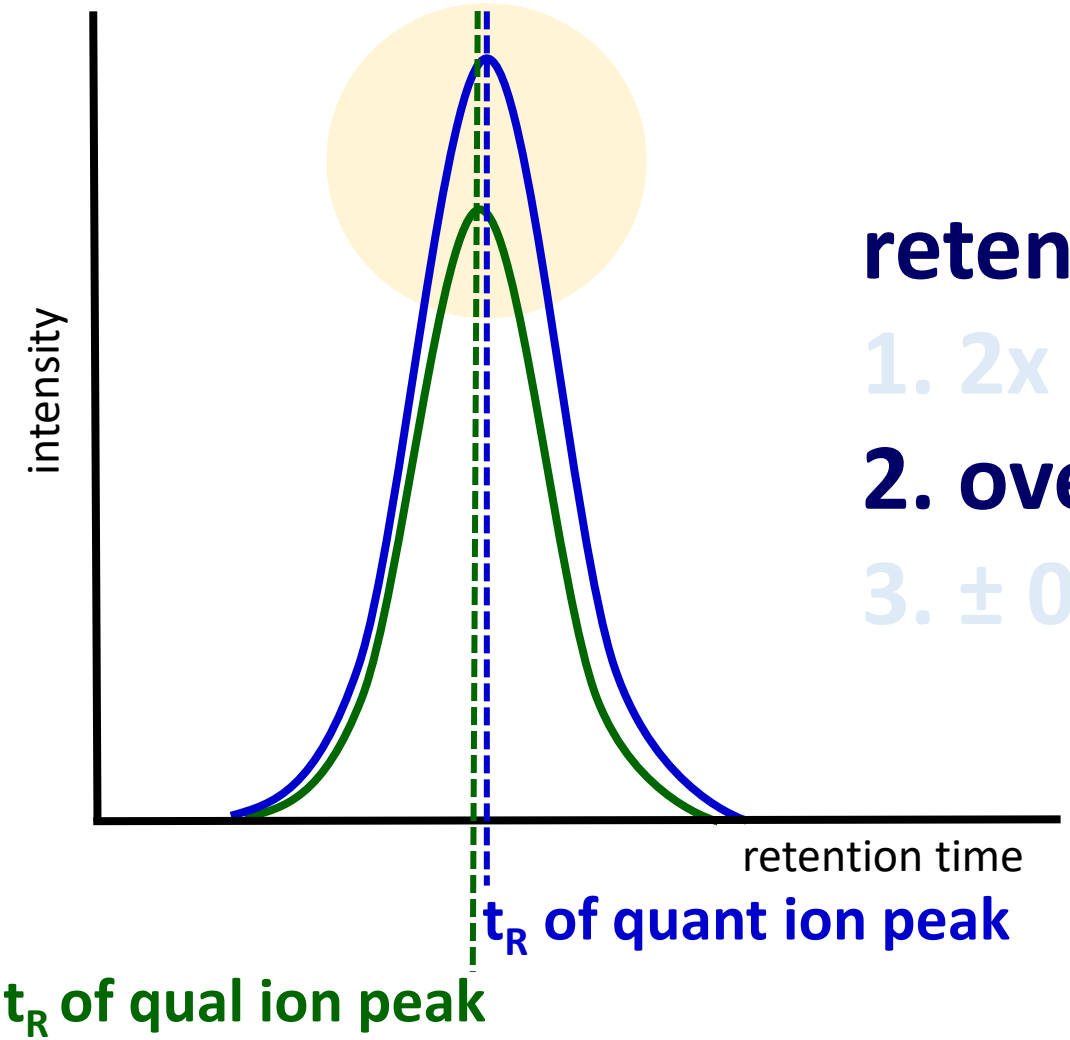
**daminozide, chlormequat chloride, methamidophos, acephate**



# identification data –retention time

quant ion

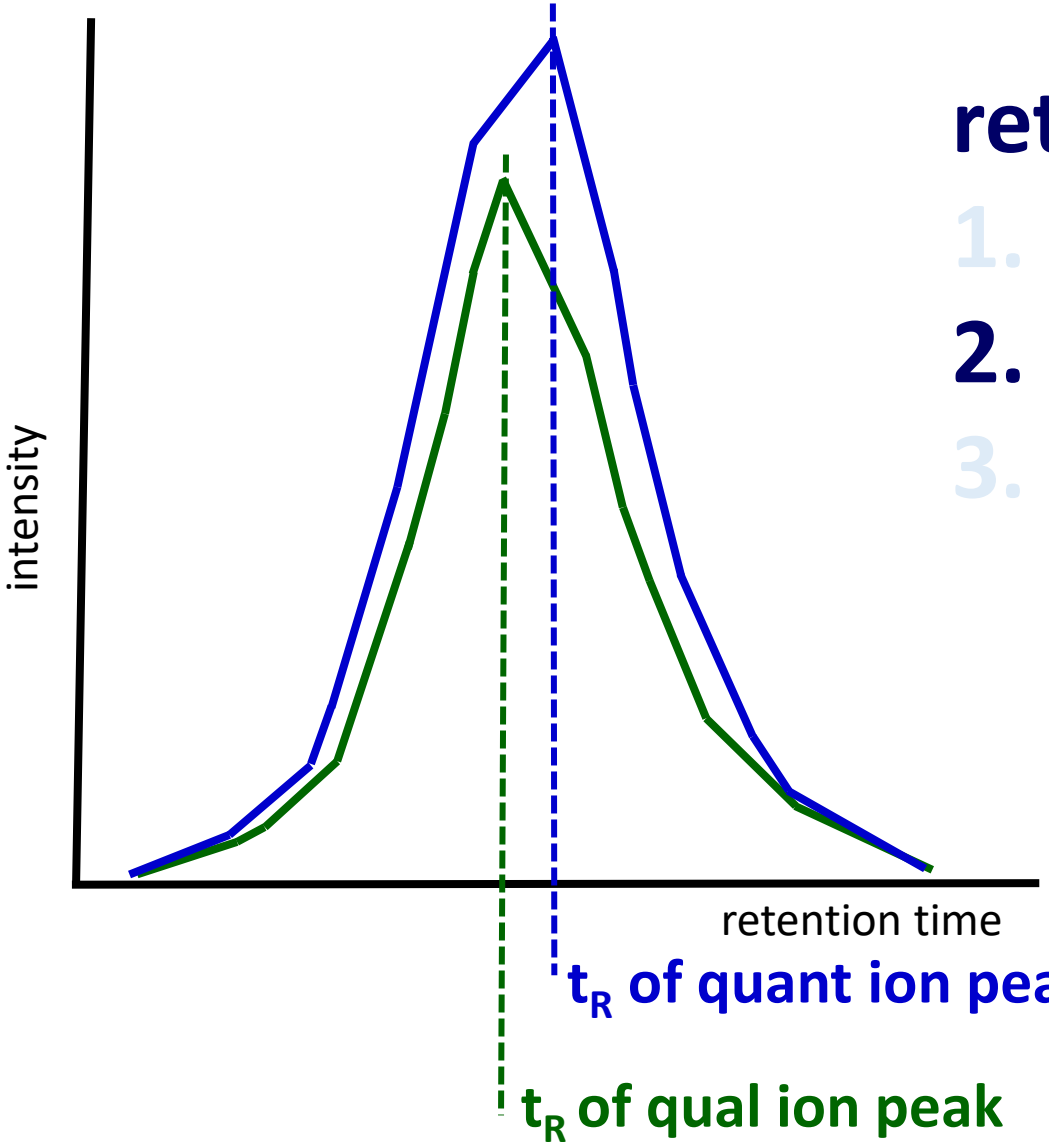
qual ion




## retention time

1. 2x void
2. overlap
3. ± 0.1 min standard

# identification data –retention time




**retention time**

1. 2x void
2. overlap
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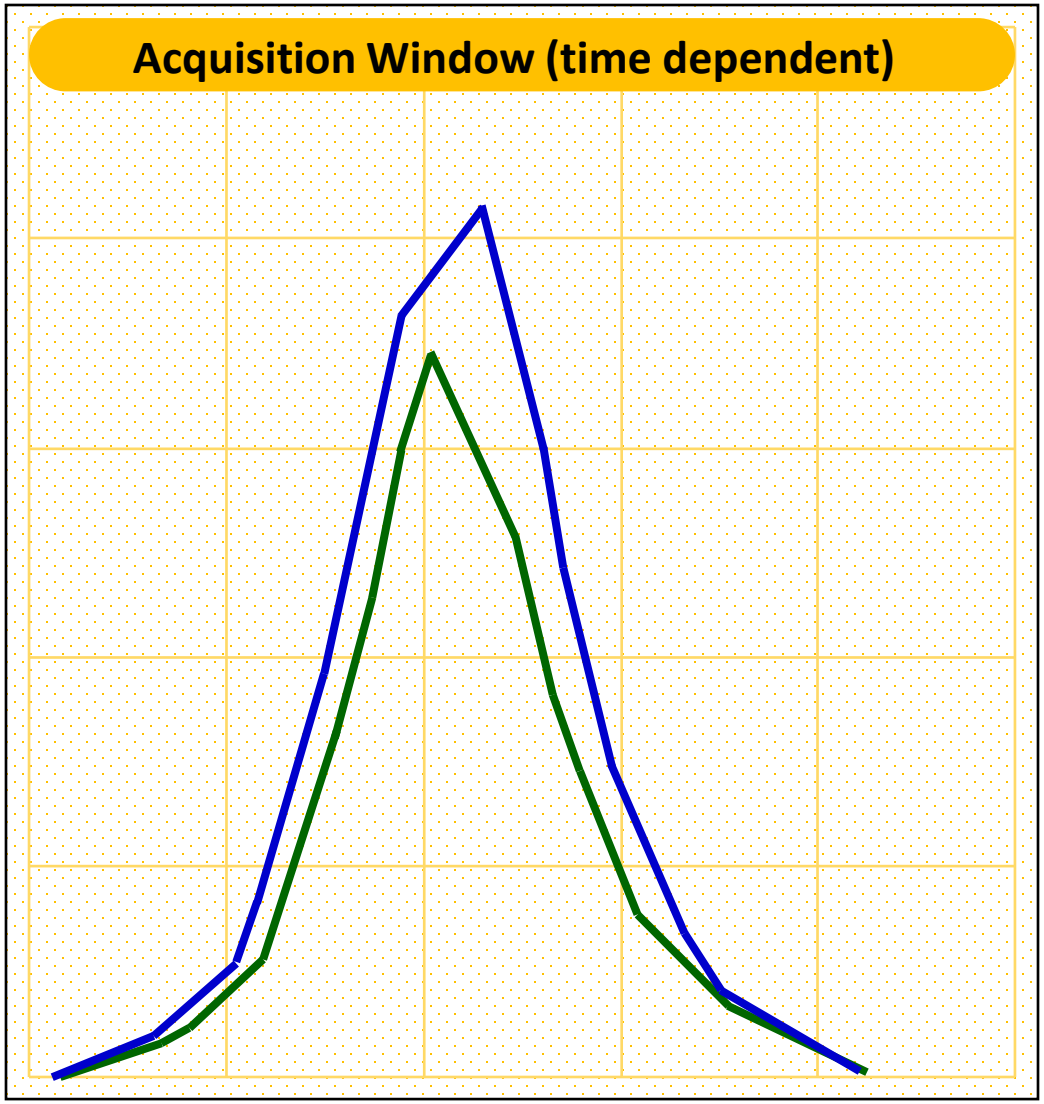
# identification data –retention time




**sensitivity**
**&**
**robustness**

**recommend a conservative approach**

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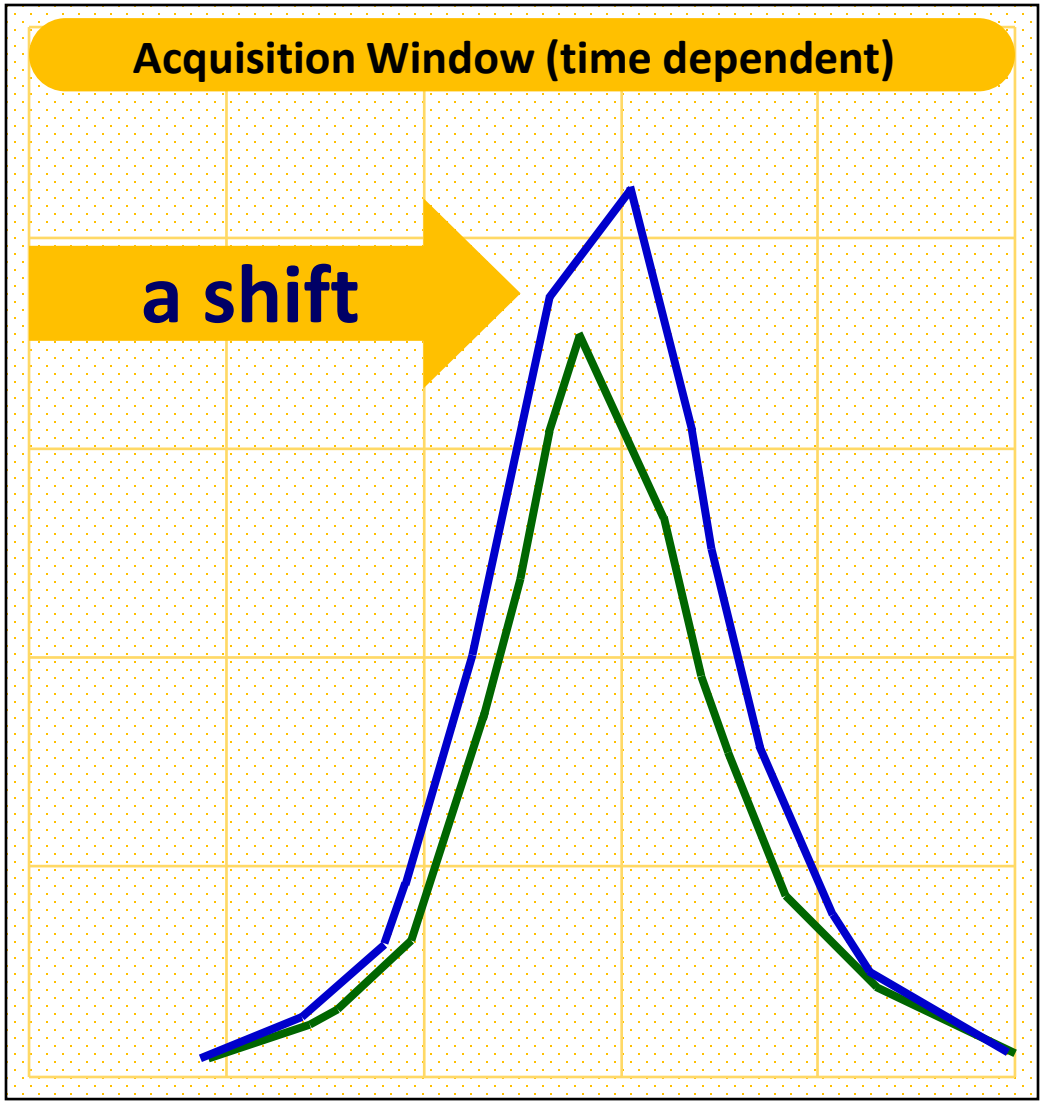
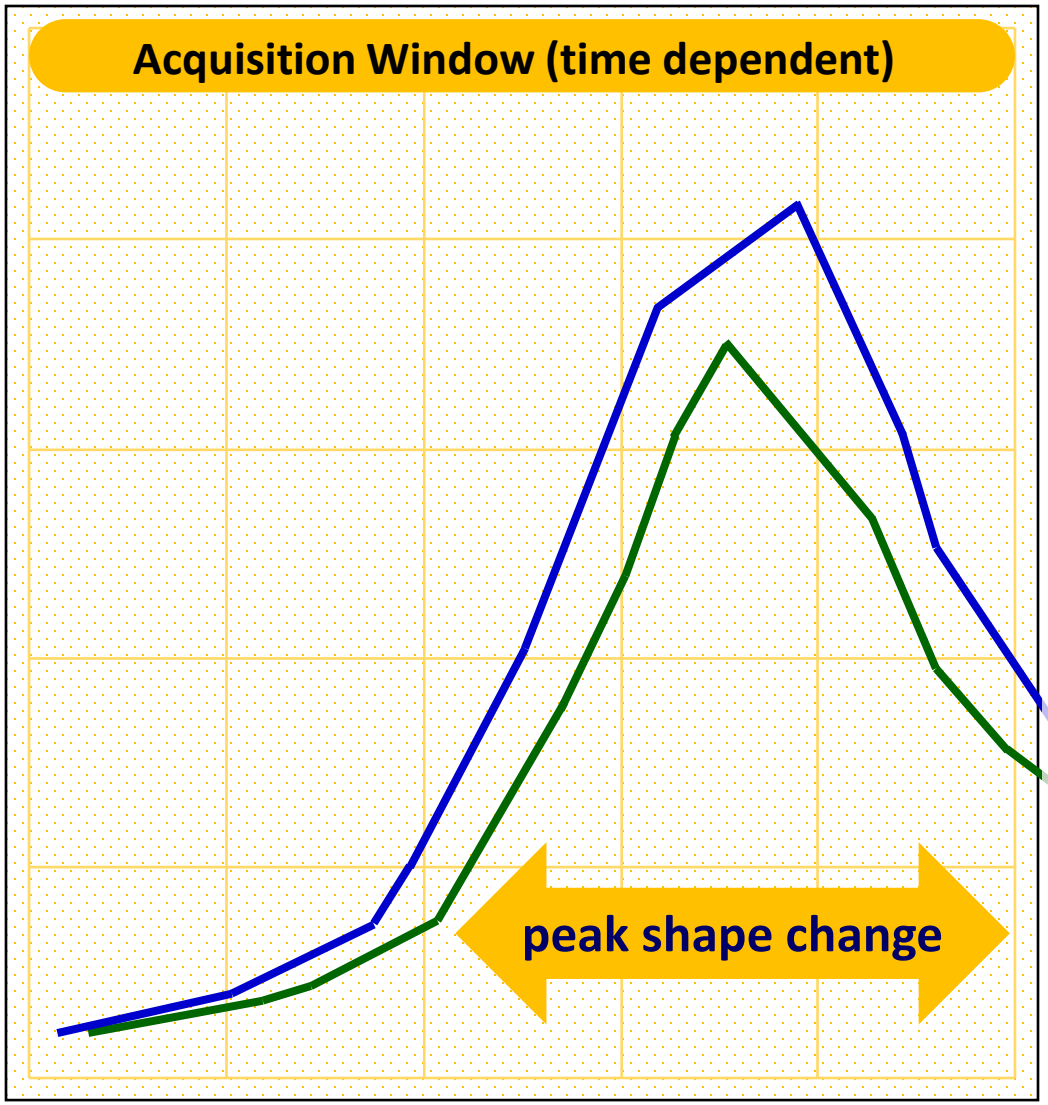
# identification data –retention time




**after a few batches…**

# identification data –retention time




**after a**
**few more batches…**

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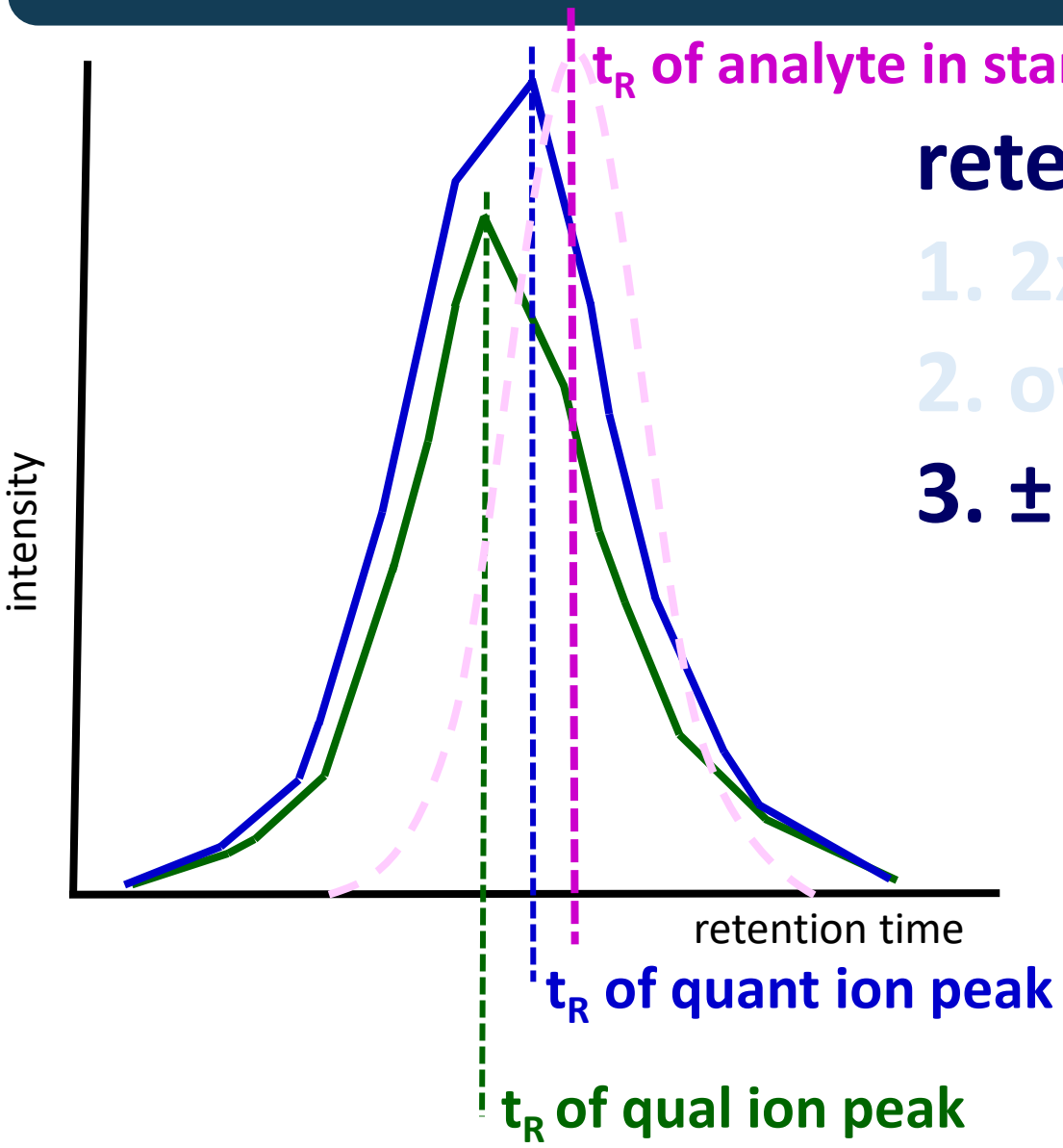
# identification data –retention time




**retention time**

1. 2x void
2. overlap
3. ± 0.1 min standard

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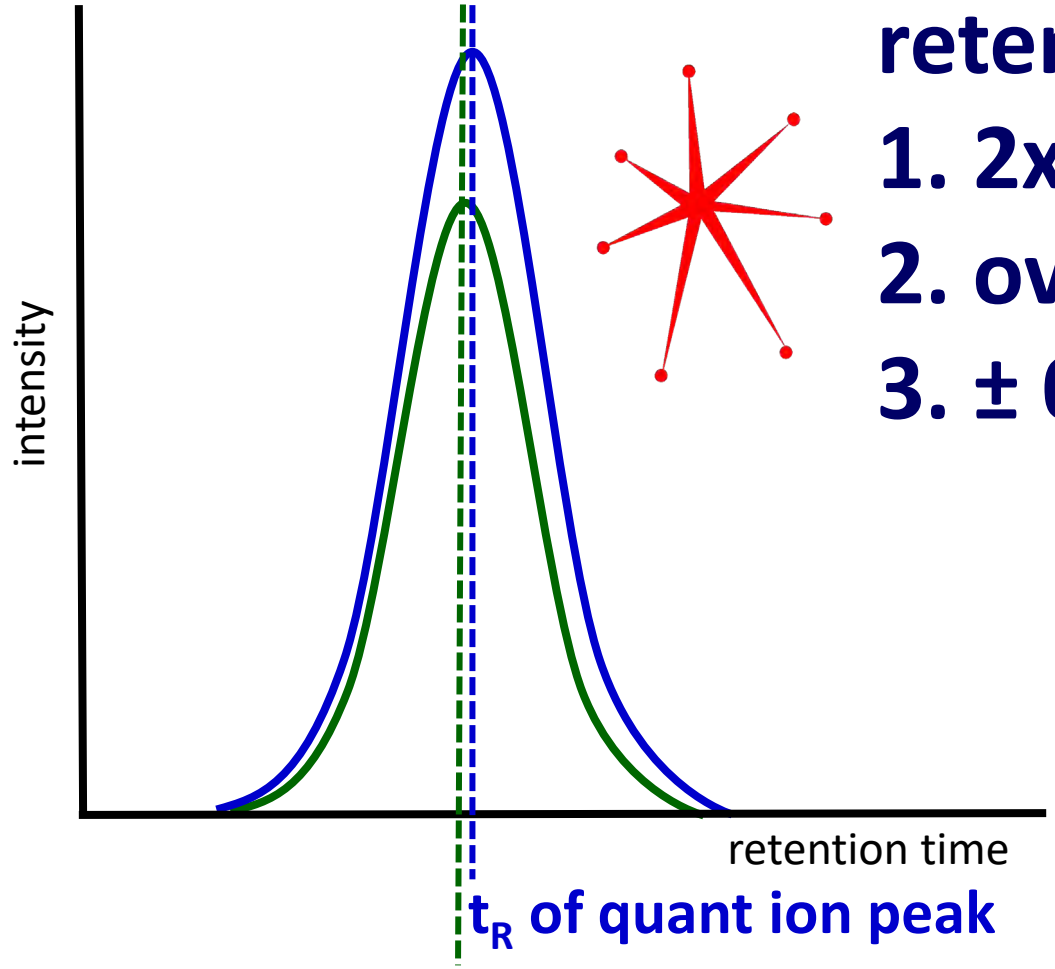
# identification data- retention time



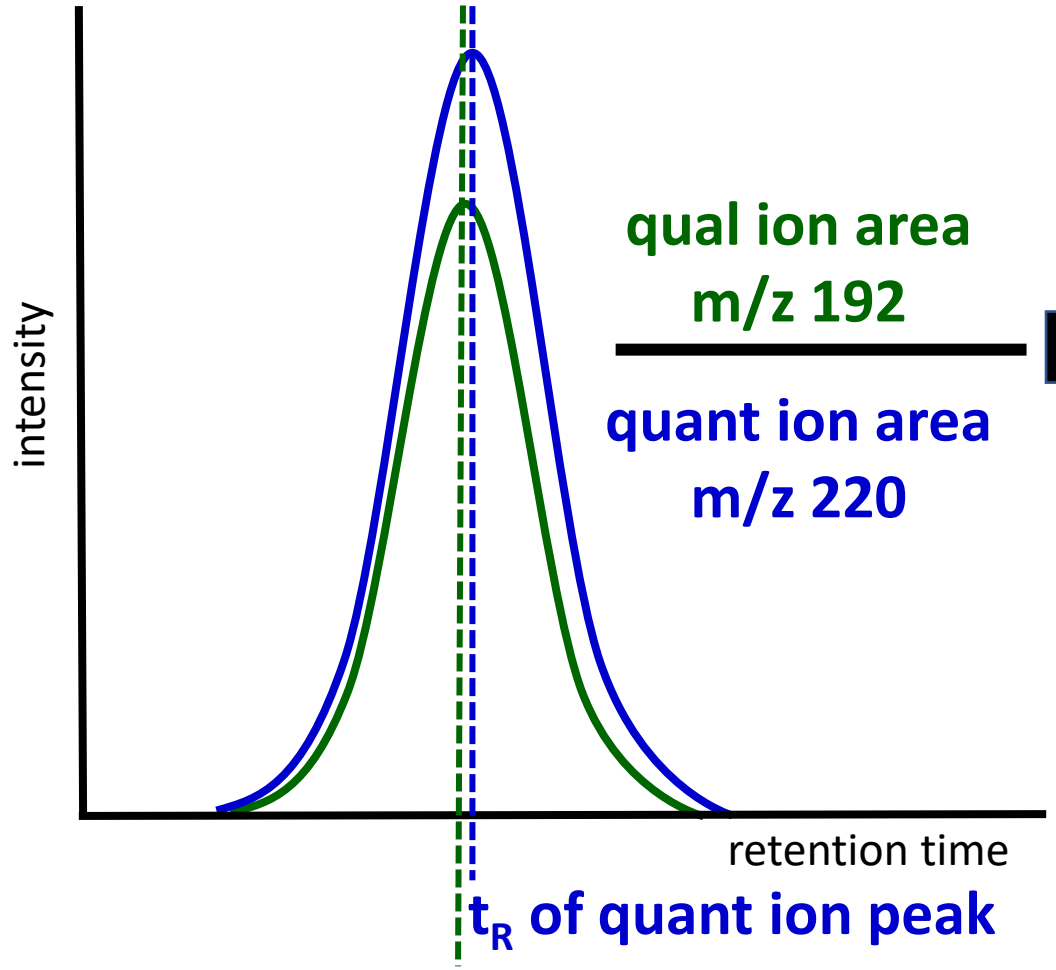

**retention time**

**1. 2x void**

**2. overlap**

**3. ± 0.1 min standard**

# identification data- ion ratio




qual ion area
m/z 192

quant ion area
m/z 220

ion ratio

**82%**

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# identification data- ion ratio

**Table 3.** Identification requirements for different MS techniques.[1]

| MS detector/Characteristics | | Acquisition | Requirements for identification | |
|---|---|---|---|---|
| Resolution | Typical systems (examples) | | minimum number of ions | other |
| Unit mass resolution | Single MS quadrupole, ion trap, TOF | full scan, limited m/z range, SIM | 3 ions | S/N ≥ 3[d]<br><br>Analyte peaks from both product ions in the extracted ion chromatograms must fully overlap.<br><br>Ion ratio from sample extracts should be within **±30 % (relative)** of average of calibration standards from same sequence |
| | MS/MS triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap | selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution | 2 produc ions | |
| Accurate mass measurement | High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS | full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof | 2 ions with mass accuracy ≤ 5 ppm[a, b, c] | S/N ≥ 3[d]<br><br>Analyte peaks from precursor and/or product ion(s) in the extracted ion chromatograms must fully overlap.<br><br>Ion ratio: see D12 |

[a] preferably including the molecular ion, (de)protonated molecule or adduct ion
[b] including at least one fragment ion
[c] < 1 mDa for m/z < 200
[d] in case noise is absent, a signal should be present in at least 5 subsequent scans

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# identification data- ion ratio

## target ion ratio

**(average from calibration for batch)**

# 80% ± 30%

# identification data- ion ratio

Let’s put the breaks on

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# identification data- ion ratio

## Setting up method or adding compounds

- Ion ratios determined for individual mixes

  (without coeluting compounds)

- Check ion ratio for combined mix

# identification data- ion ratio

**Metalaxyl**

**Q1/Q3**

**quant: 234/146**

**qual: 132/117**

**ion ratio: 70%**

# identification data- ion ratio

**Metalaxyl**

**Q1/Q3**

**quant: 234/146**

**qual: 132/117**

**ion ratio: 70%**

# identification data- ion ratio

**Metalaxyl**

**Q1/Q3**

**quant: 234/146**

**qual: 132/117**

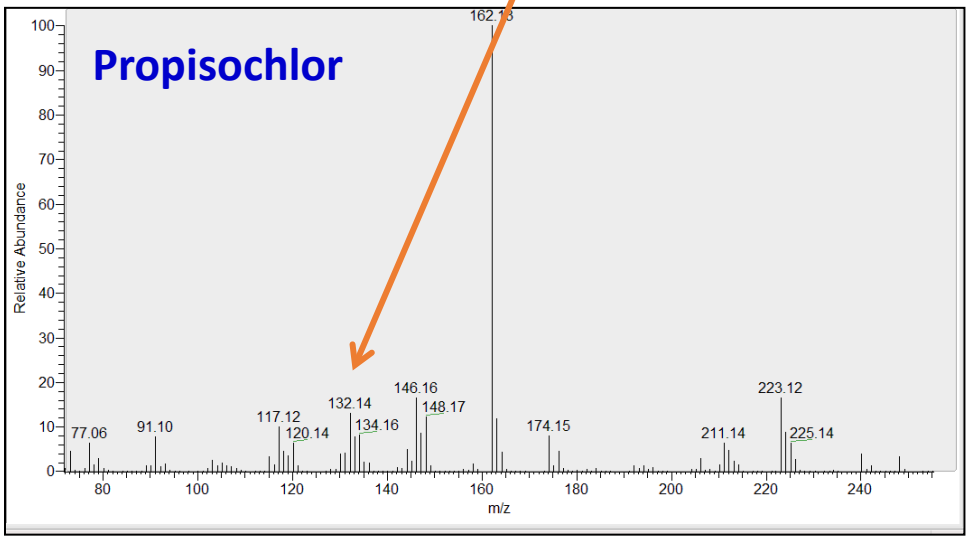
**ion ratio: 70%**


**Propisochlor**

**0.02 min different $t_R$**

**Corruption**

**of 132/117 MRM**

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# identification data- ion ratio

## target ion ratio

(average from calibration for batch)

# 80% ± 30%

## absolute

**Simply add/subtract 30**

**80% - 30% = 50%**

**80% + 30% = 110%**

**50 to 110% = “pass”**

# identification data- ion ratio

## target ion ratio

(average from calibration for batch)

# 80% ± 30%

## absolute

**Simply add/subtract 30**

**80% - 30% = 50%**

**80% + 30% = 110%**

**50 to 110% = “pass”**

## relative

**80% (0.3) = 24%**

**80% - 24% = 56%**

**80% + 24% = 104%**

**56 to 104% = “pass”**

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# identification data- ion ratio

## target ion ratio

(average from calibration for batch)

80% + 30%

abs

Simply a %

80% - 6%

80% + 4%

50 to 110% = “pass”  56 to 104% = “pass”

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# identification data- ion ratio

## target ion ratio

(average from calibration for batch)

# 20% ± 30%

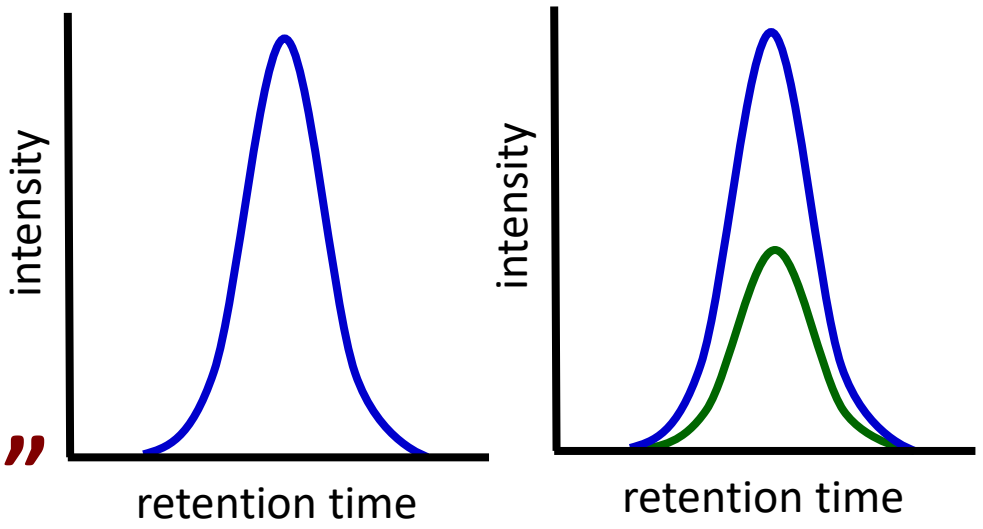
## absolute

**Simply add/subtract 30**

**20% - 30% = -10%**

**20% + 30% = 50%**

**“0” to 50% = “pass”**

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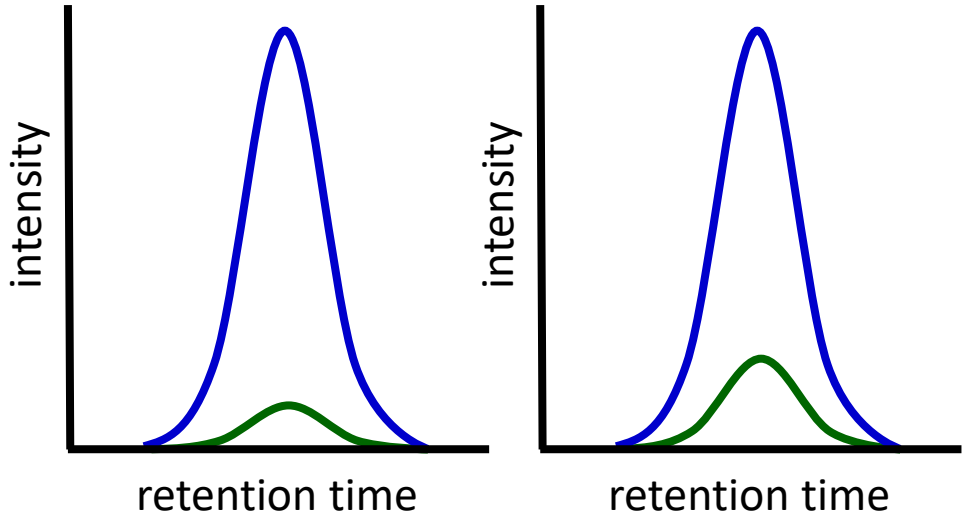
# identification data- ion ratio

## target ion ratio

**(average from calibration for batch)**

# 20% ± 30%




## relative

**20% (0.3) = 6%**

**20% - 6% = 14%**

**20% + 6% = 26%**

**14 to 26% = “pass”**

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# identification data- ion ratio

ion ratio range = false – and false + control

too wide (absolute) = false positives

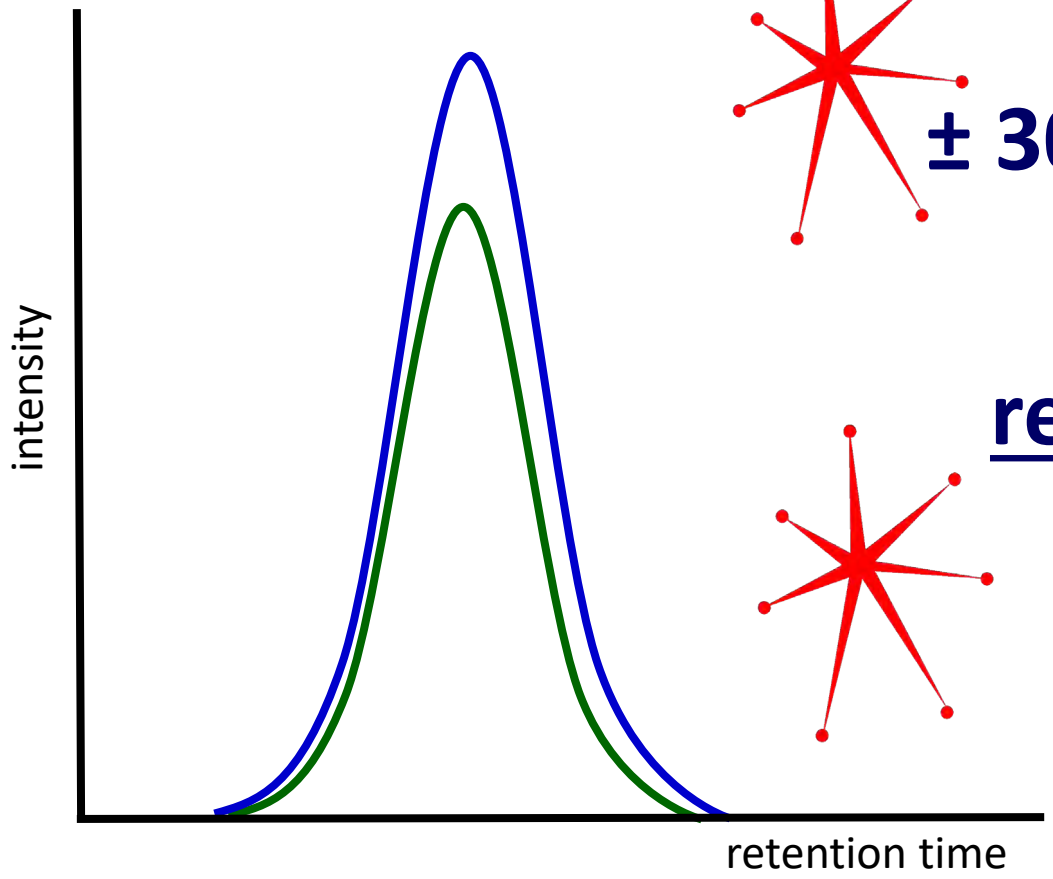
too narrow = false negative

**Why?**

appear more sensitivity (lower LODs)

easier to say meeting regulatory limits

# identification criteria




**ion ratio**

**± 30% (relative)**

**retention time**

**not in void**

**overlap**

**± 0.1 min**

# limit of detection

different ways
disagreement
fit-for-purpose

Several:
Instrument –solvent
Matrix – matrix without prep
Method – all method steps

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# limit of detection

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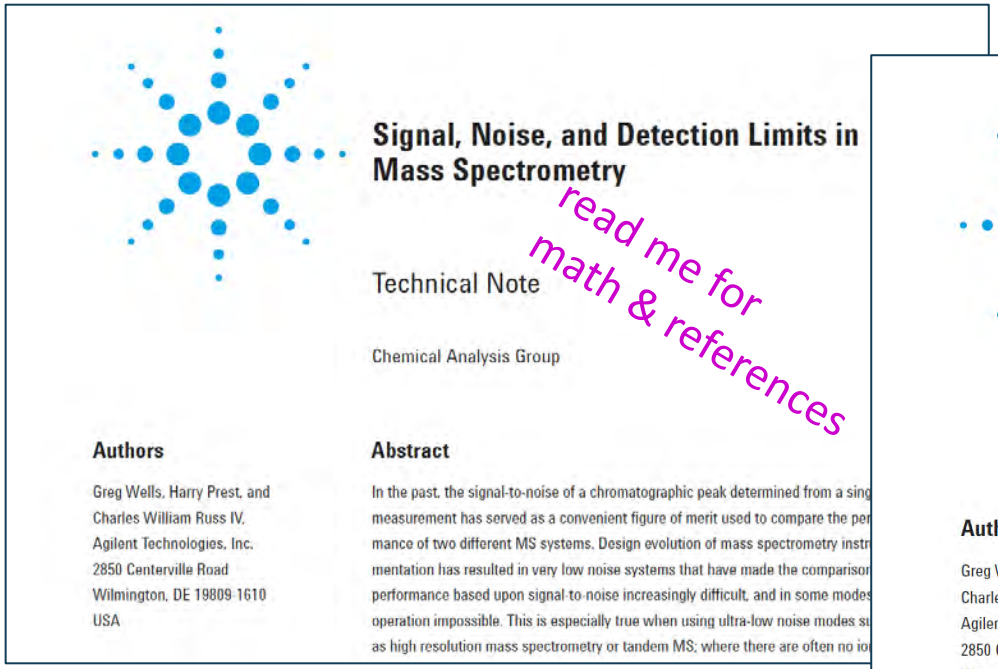
## S/N popular but…

**S/N is not really appropriate for estimating detection limits**

**Modern MS/MS systems have very low noise…**

**A statistical approach will yield more robust calculations of detection limits**



**Signal, Noise, and Detection Limits in Mass Spectrometry**

read me for math & references

Technical Note

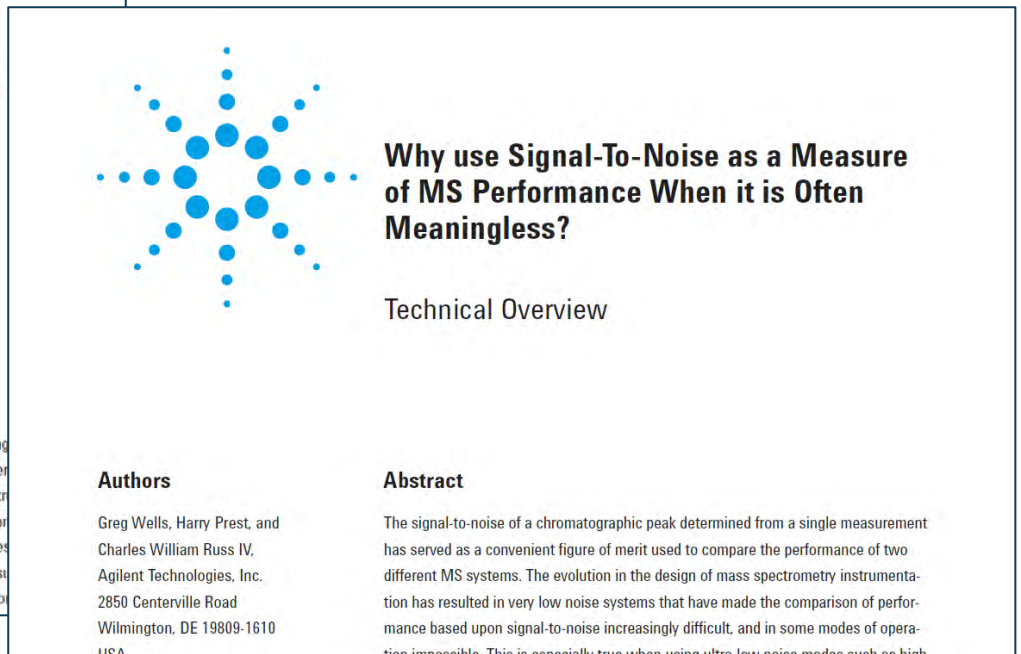
Chemical Analysis Group

**Authors**

Greg Wells, Harry Prest, and Charles William Russ IV,
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19809-1610
USA

**Abstract**

In the past, the signal-to-noise of a chromatographic peak determined from a sing
measurement has served as a convenient figure of merit used to compare the per
mance of two different MS systems. Design evolution of mass spectrometry instr
mentation has resulted in very low noise systems that have made the comparison
performance based upon signal-to-noise increasingly difficult, and in some modes
operation impossible. This is especially true when using ultra-low noise modes su
as high resolution mass spectrometry or tandem MS; where there are often no io



**Why use Signal-To-Noise as a Measure of MS Performance When it is Often Meaningless?**

Technical Overview

**Authors**

Greg Wells, Harry Prest, and Charles William Russ IV,
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19809-1610

**Abstract**

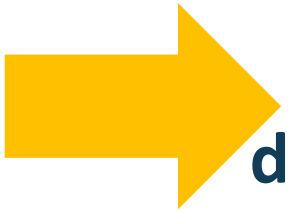
The signal-to-noise of a chromatographic peak determined from a single measurement has served as a convenient figure of merit used to compare the performance of two different MS systems. The evolution in the design of mass spectrometry instrumentation has resulted in very low noise systems that have made the comparison of performance based upon signal-to-noise increasingly difficult, and in some modes of opera-

# limit of detection

$$DL = t_{(n-1,\ 1-\alpha=0.99)}\ (S)$$

$t_{(n-1,\ 1-\alpha=0.99)}$: students' t value

$(S)$: standard deviation of area counts



**calculated detection limit**

1. replicate determinations (n=8)
2. calculate standard deviation of area counts (S)
3. determine t value from table (for n-1=7, t=2.998)
4. convert DL area counts to DL pg

   DL as pg = (DL)*(pg on column/average area counts)

# limit of detection

$$DL = t_{(n-1,\ 1-\alpha=0.99)}\ (S)$$

$t_{(n-1,\ 1-\alpha=0.99)}$: students' t value

$(S)$: standard deviation of area counts

**calculated detection limit**

Verify

1. replicate determinations (n=8)
2. calculate standard deviation of area counts (S)
3. determine t value from table (for n-1=7, t=2.998)
4. convert DL area counts to DL pg

   DL as pg = (DL)*(pg on column/average area counts)

# limit of detection

$DL = t_{(n-1,\ 1-\alpha=0.99)} (S)$

$t_{(n-1,\ 1-\alpha=0.99)}$: students' t value

$(S)$: standard deviation of area counts

**calculated detection limit**

1. replicate determinations (n=8)

**Must be near DL concentration**

**Different concentrations for different analytes?**

**Identification criteria must be met**

**Quant ion or qual ion?**

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# What do I need to know?

# limit of detection

Pesticides: different signal → different levels needed

## Lowest level meeting ion ratio criteria

### Metalaxyl

| | 0.1 ppb | 1 ppb | 10 ppb | 100 ppb | 1000 ppb |
|---|---|---|---|---|---|
| Injection 1 | ✗ | ✓ | ✓ | ✓ | ✓ |
| Injection 2 | ✓ | ✗ | ✓ | ✓ | ✓ |
| Injection 3 | ✗ | ✗ | ✓ | ✓ | ✓ |
| Injection 4 | ✗ | ✓ | ✓ | ✓ | ✓ |
| Injection 5 | ✓ | ✓ | ✓ | ✓ | ✓ |
| Injection 6 | ✗ | ✓ | ✓ | ✓ | ✓ |
| Injection 7 | ✓ | ✓ | ✓ | ✓ | ✓ |
| Injection 8 | ✗ | ✓ | ✓ | ✓ | ✓ |

# limit of detection

Pesticides: different signal → different levels needed

## Lowest level meeting ion ratio criteria

### Metalaxyl

| | 0.1 ppb | 1 ppb | 10 ppb | 100 ppb | 1000 ppb |
|---|---|---|---|---|---|
| Injection 1 | ✗ | ✓ | ✓ | ✓ | ✓ |
| Injection 2 | ✓ | ✗ | ✓ | ✓ | ✓ |
| Injection 3 | ✗ | ✗ | ✓ | ✓ | ✓ |
| Injection 4 | ✗ | ✓ | ✓ | ✓ | ✓ |
| Injection 5 | ✓ | ✓ | ✓ | ✓ | ✓ |
| Injection 6 | ✗ | ✓ | ✓ | ✓ | ✓ |
| Injection 7 | ✓ | ✓ | ✓ | ✓ | ✓ |
| Injection 8 | ✗ | ✓ | ✓ | ✓ | ✓ |

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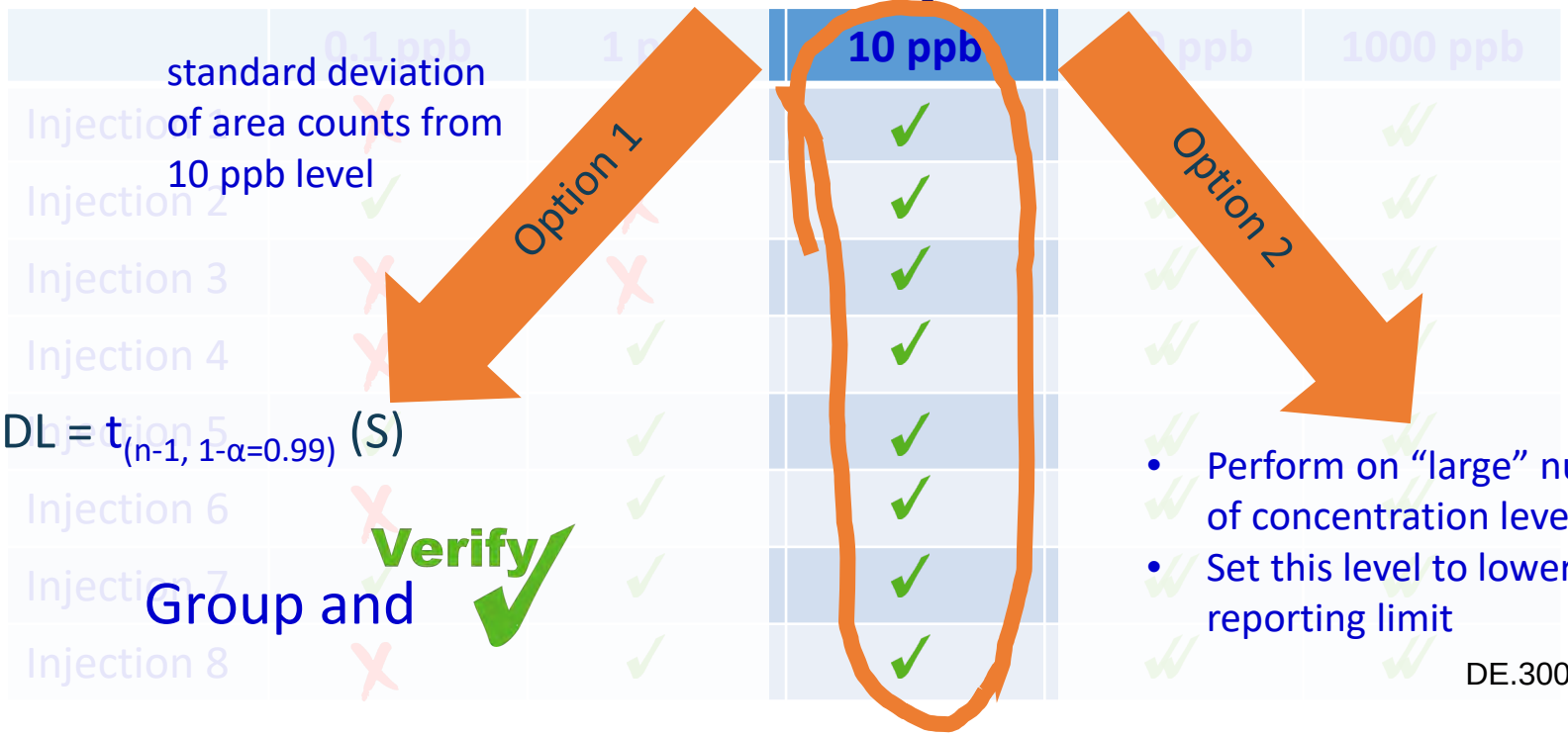
# limit of detection

Pesticides: different signal → different levels needed

## Lowest level meeting ion ratio criteria

### Metalaxyl

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# recovery

Known **200 ppb**

**loss of analyte during all steps of the method procedure**

$$\text{Recovery} = \frac{\text{(calculated) } 146}{\text{(known) } 200} \times 100 = 73\%$$

Calculated **146 ppb**

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# recovery

80 – 120%

low levels

difficult pesticides

difficult matrices

See SANTE for recommendations on recovery correction
See FDA for concentration dependent ranges

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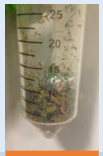
# recovery- matrix matching

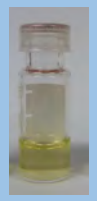
add pesticides



spike test portion with pesticides

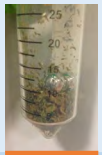
sample preparation

final extract



analyte loss due to method processing & remaining concentration determined by calibration curve

recovery standard



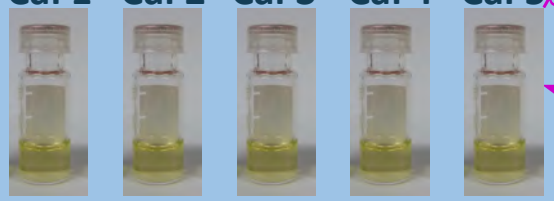
test portion
NO pesticides added

sample preparation

bulk extract

cal. standards

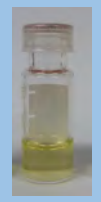
Cal 1 Cal 2 Cal 3 Cal 4 Cal 5



add pesticides

calibration curve
(pesticides did not go through prep steps so no loss)

matrix blank



incurred pesticides

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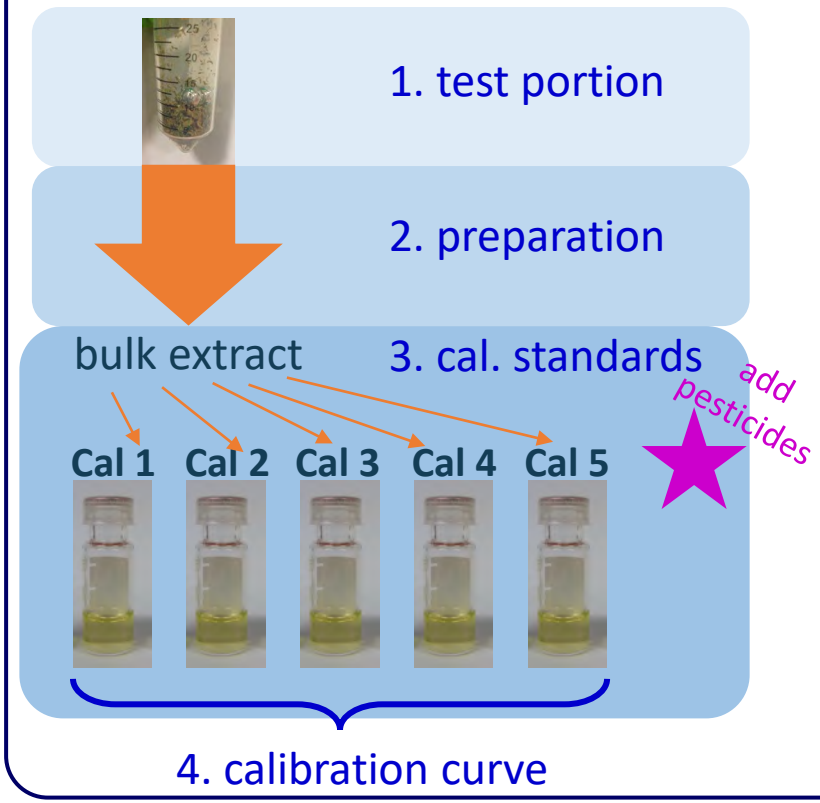
# recovery ≠ procedural calibration

## Typical Matrix Matched Calibration

pesticides added to aliquoted final extract

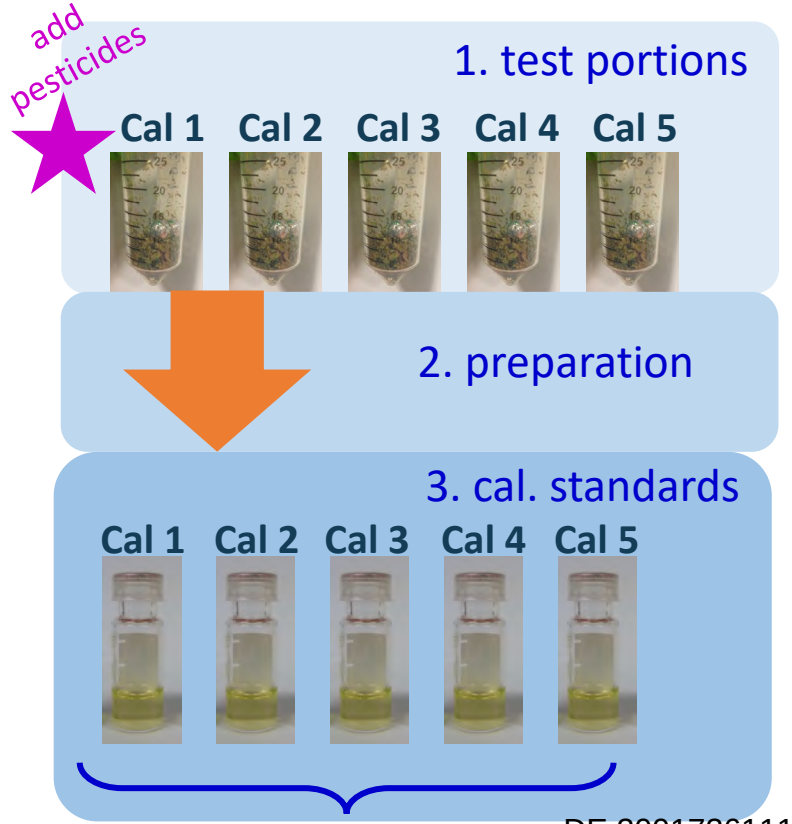
- recovery not compensated
- matrix effects are compensated
- can use for recovery calculation




## Procedural Calibration

pesticides added to individual test portions

- recovery compensated
- matrix effects are compensated

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# conclusions

## Identification

Retention time
- 2x void
- overlap
- ± 0.1 min of standard

Ion Ratio
- ± 30% Relative
- no coelutions (setup)

## Detection Limits

~~S/N~~

Lowest Level ID confirmed
- repeat determinations
- statistical determination
- combine for several DL
- verify

## Recovery

Recovery definition
- loss due to prep

Calibration
- matrix-matched
- procedural

FDA and
EU SANTE references

DE.3001736111

# Thank you!


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