Targeted Analyte Identification in Chromatography – Triple Quadrupole MS/MS

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Parameters Assessed in Method Validation

Random Error: Deviation of a measured value due to normally distributed variation around the mean.

Systematic Error: Deviation above or below the actual value due to bias in the tools or materials used to make the measurement.

Spurious or Gross Error: Misteaks!
(can be reduced with care and precautions, but never eliminated)

Some Background Publications

- Bethem *et al.* (2003) "Establishing the fitness for purpose of mass spectrometric methods" *J. Am. Soc. Mass Spectrom.*, **14**, 528-41.
- Lehotay *et al.* (2008) "Identification and confirmation of chemical residues by chromatography-mass spectrometry and other techniques" *Trends Anal. Chem.* **27**, 1070-90.
- Heller *et al.* (2010) "Issues in mass spectrometry between bench chemists and regulatory laboratory managers" *J. AOAC Int.* **93**, 1625-32.
- Lehotay *et al.* (2015) "Current issues involving screening and identification of chemical contaminants in foods by mass spectrometry" *Trends Anal. Chem.* **69**, 62-75
- Mol *et al.* (2015) "Identification in residue analysis based on liquid chromatography with tandem mass spectrometry: Experimental evidence to update performance criteria" *Anal. Chim Acta* **873**, 1-13

Definitions

- ***Indication*** = result of a screening method (*i.e.* "presumed" positive or negative)

- ***Determination*** = result from an analytical quantitative method (*e.g.* GC/PFPD, LC/UV)

- ***Identification*** = qualitative result from a highly selective method (*e.g.* GC-MS, LC-MS^n) that meets given criteria

- ***Confirmation*** = result from 2 or more independent analyses in agreement (ideally, one of which uses a different chemical mechanism or approach and meets identification criteria)

Possible New Terms

- ***Quantidentification*** = analyte quantitative result when MS identification criteria have been met

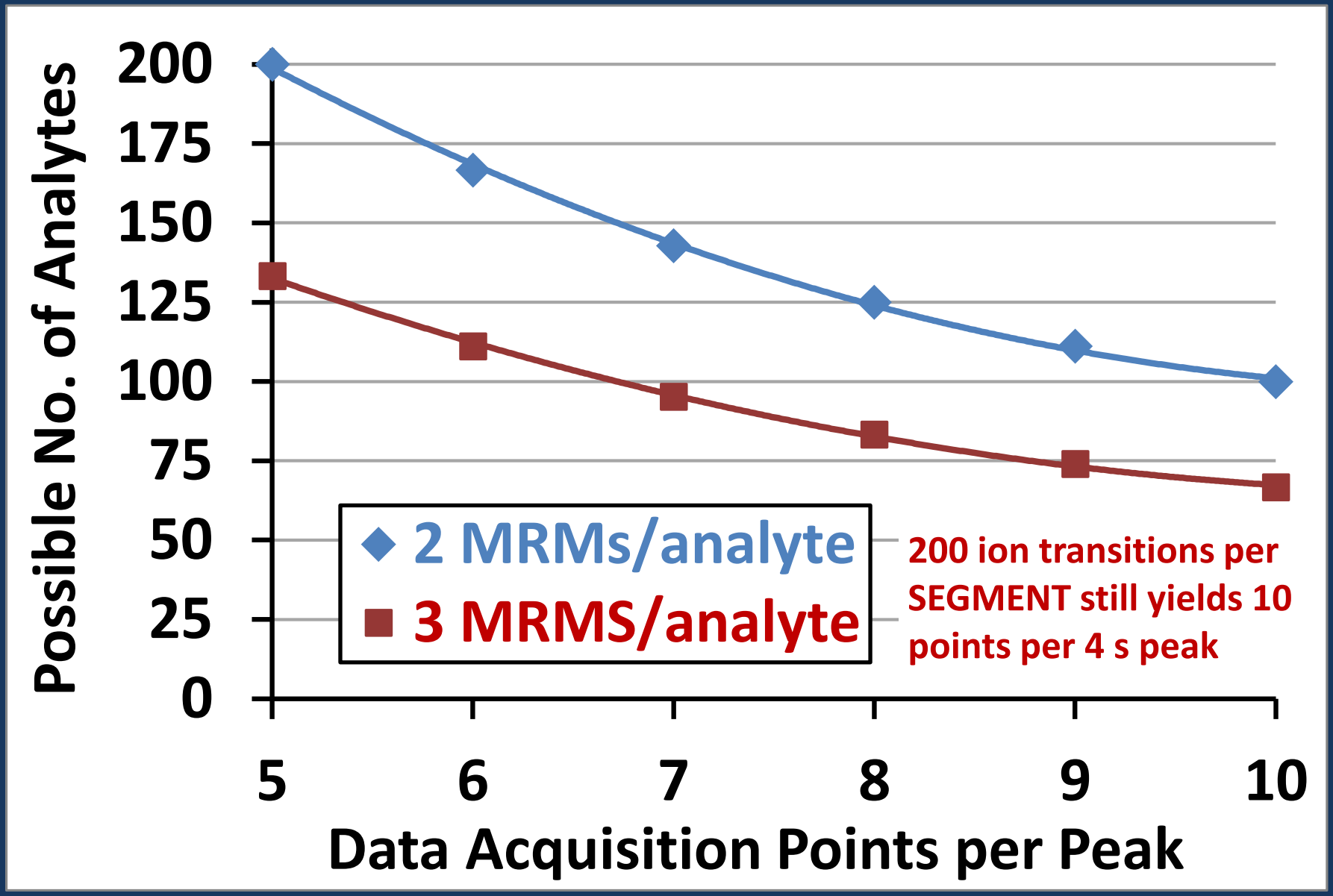
- ***Quanticonfirmation*** = quantitative result when analyte confirmation has been achieved

By the way,

- **Identify** → ***Identification***
- *ergo*, **Quantify** → ***Quantification***
- Neither ***qualitate*** **nor** ***quantitate*** are verbs, thus ***quantitation*** **is not a noun!**

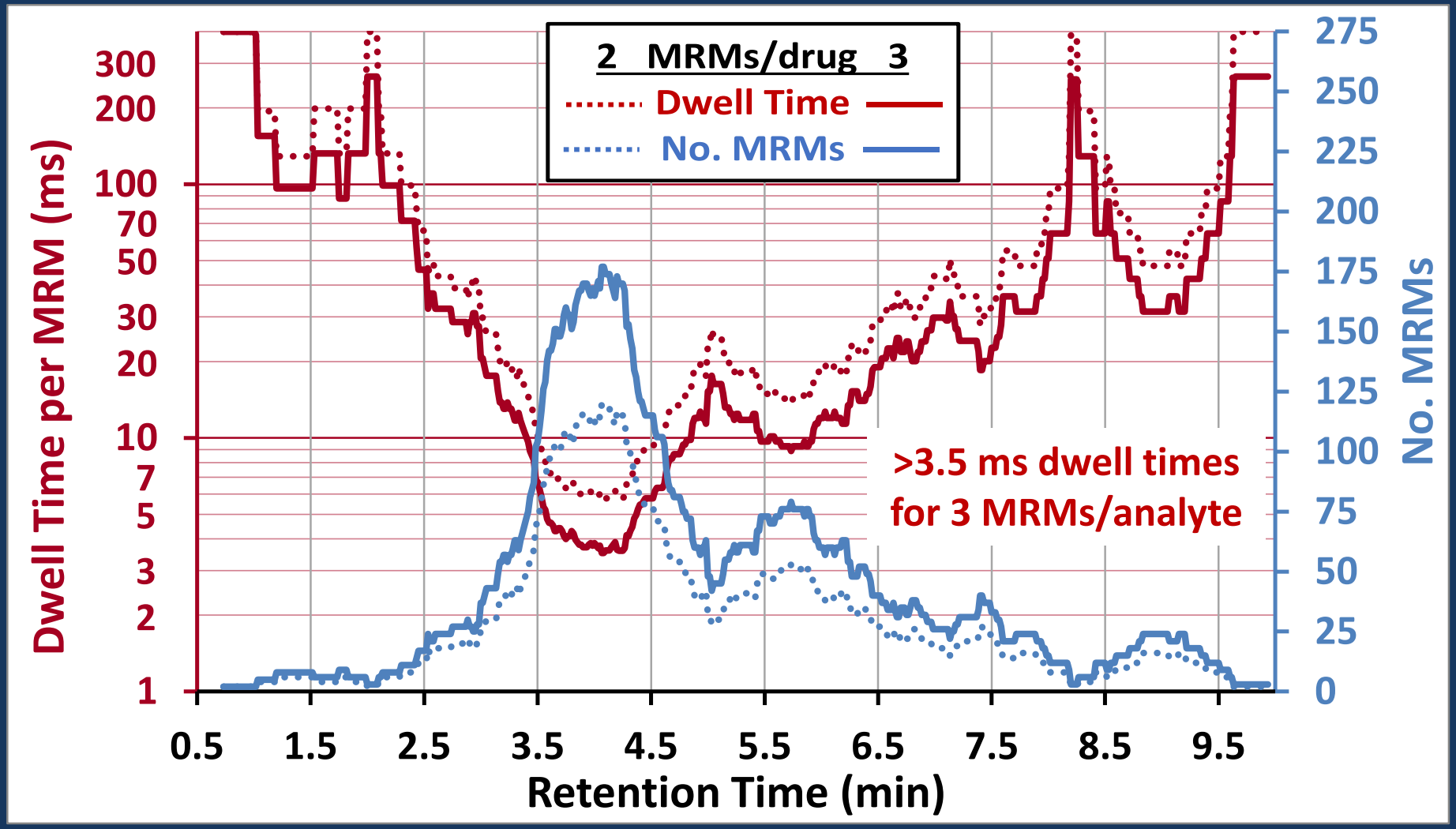
(whereas ***quantitative*** and ***qualitative*** are fine since they derive from ***quantity*** and ***quality***)

How many MS/MS ion transitions to acquire?



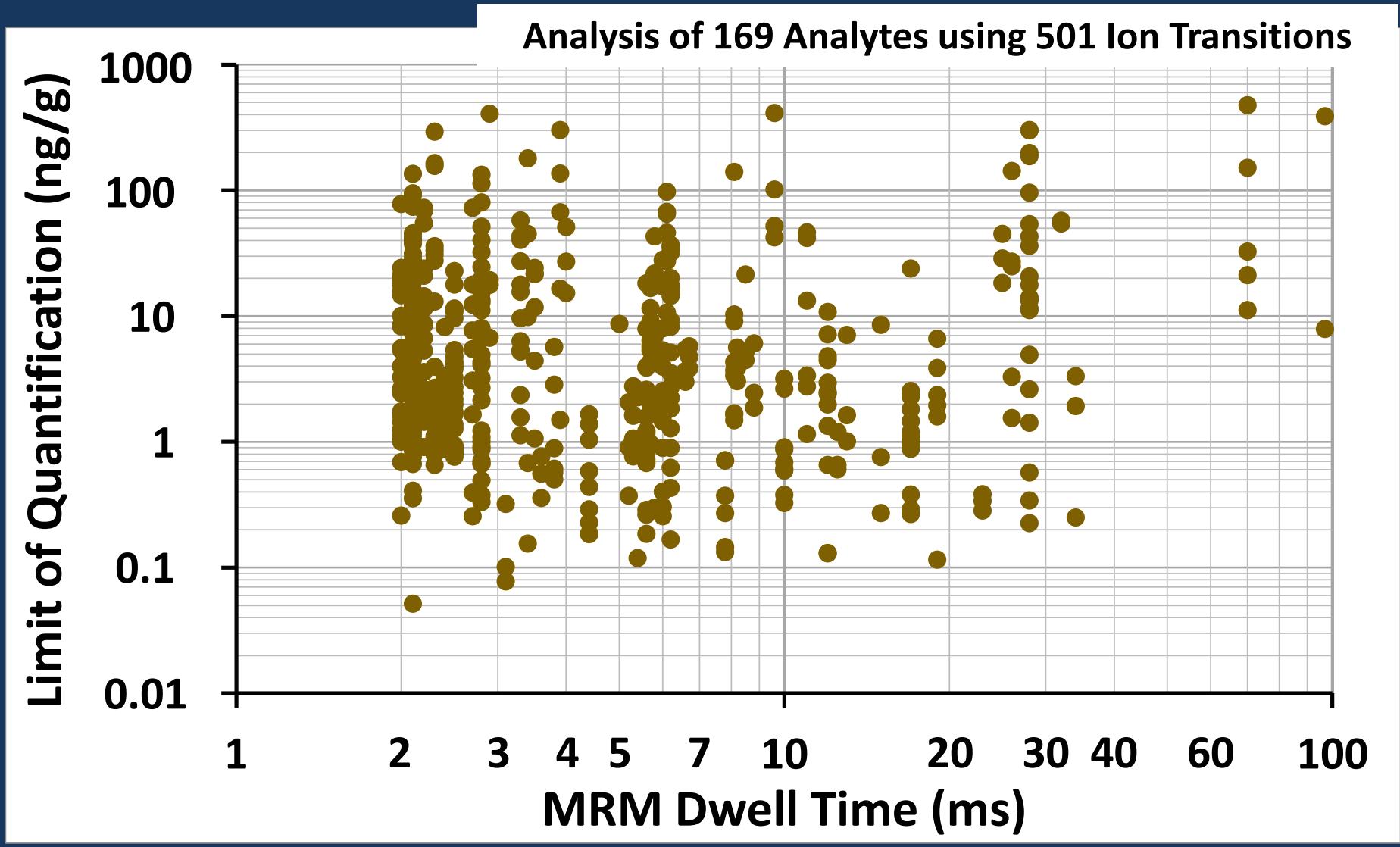
Example of sMRM for 169 Analytes in 9 min

5 points per 4 s peak, 24 s t_R windows including 20 ms ESI(+/-) switching!



Dwell time is not the main factor in LOQs

Analyte Properties are MUCH More Prominent



Rules for Identification in 2002/657/EC (EU)

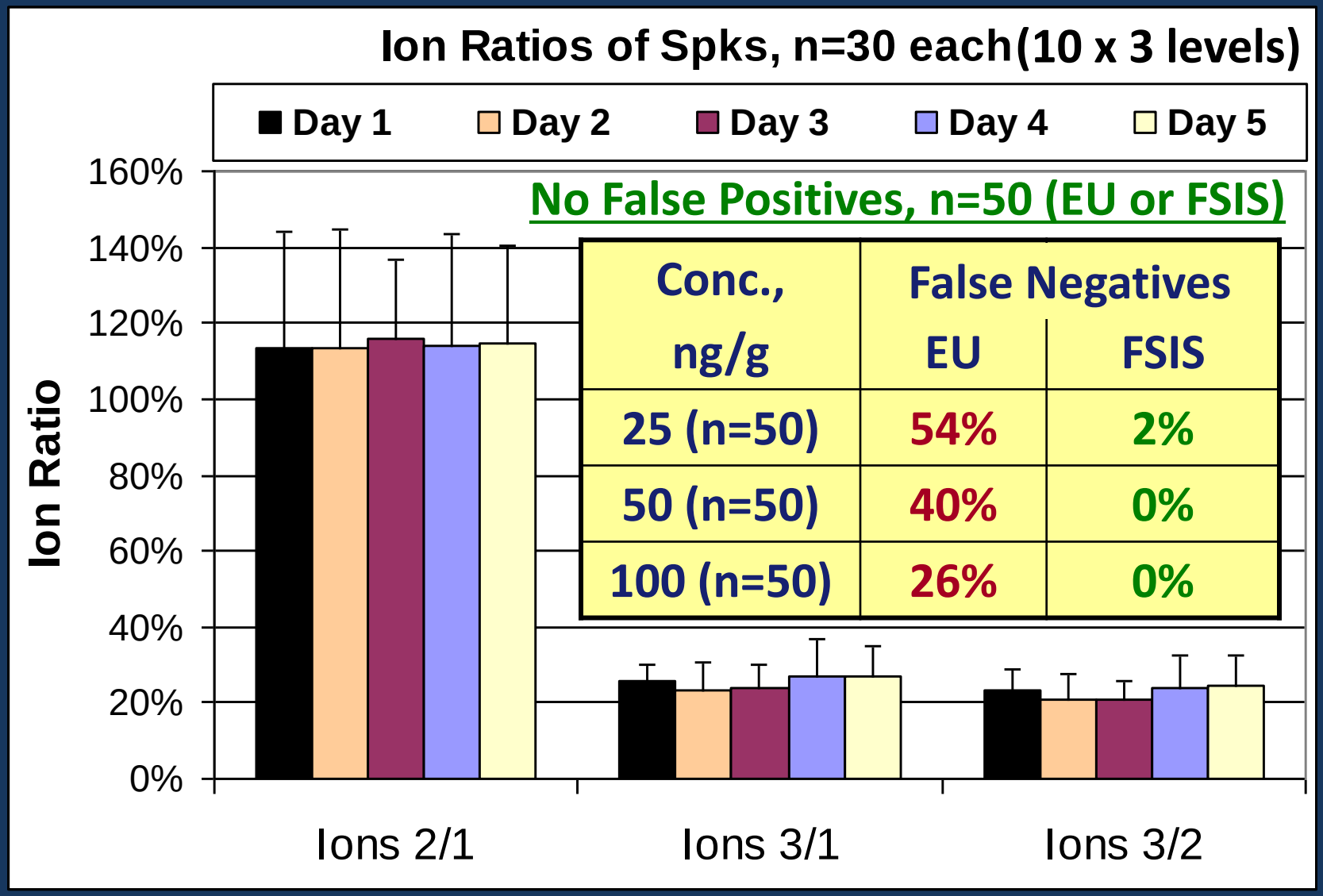
Low Resolution MS	Ident. Points
Ion or MS^n precursor	1.0
MS^n product ion	1.5
High Resolution MS	
Ion or MS^n precursor	2.0
MS^n Product Ion	2.5

Rel. Abundance *vs.* Base Peak	Acceptable Deviation GC/EI-MS	Other
>50%	± 10%	± 20%
>20-50%	± 15%	± 25%
>10-20%	± 20%	± 30%
≤10%	± 50%	± 50%

FDA/USDA (Doc. #118) MS/MS Ident. Criteria

- ✓ Retention time (t_R) is within ± 0.1 min of average t_R and peak shape matches that of reference std
- ✓ t_R and peak shape of qualifier ion(s) matches those of the quantification ion
- ✓ 2 qualifier ions ≤|20%| or 1 qualifier ion ≤|10%| of avg. ion ratio from contemporaneous reference stds
- ✓ Absence of positive findings in known blanks
- ✓ Signal > "reporting level" calibration stds in matrix, which could be LOQ, LOI, S/N, MRL, or other threshold
- ✓ The ion transitions used make structural sense

Ion Ratios for Ciprofloxacin in Kidney



No False Positives, n=50 (EU or FSIS)

Conc., ng/g	False Negatives	
	EU	FSIS
25 (n=50)	54%	2%
50 (n=50)	40%	0%
100 (n=50)	26%	0%

Guidelines in SANTE/12682/2019

Acceptable Diff. vs. Ref.

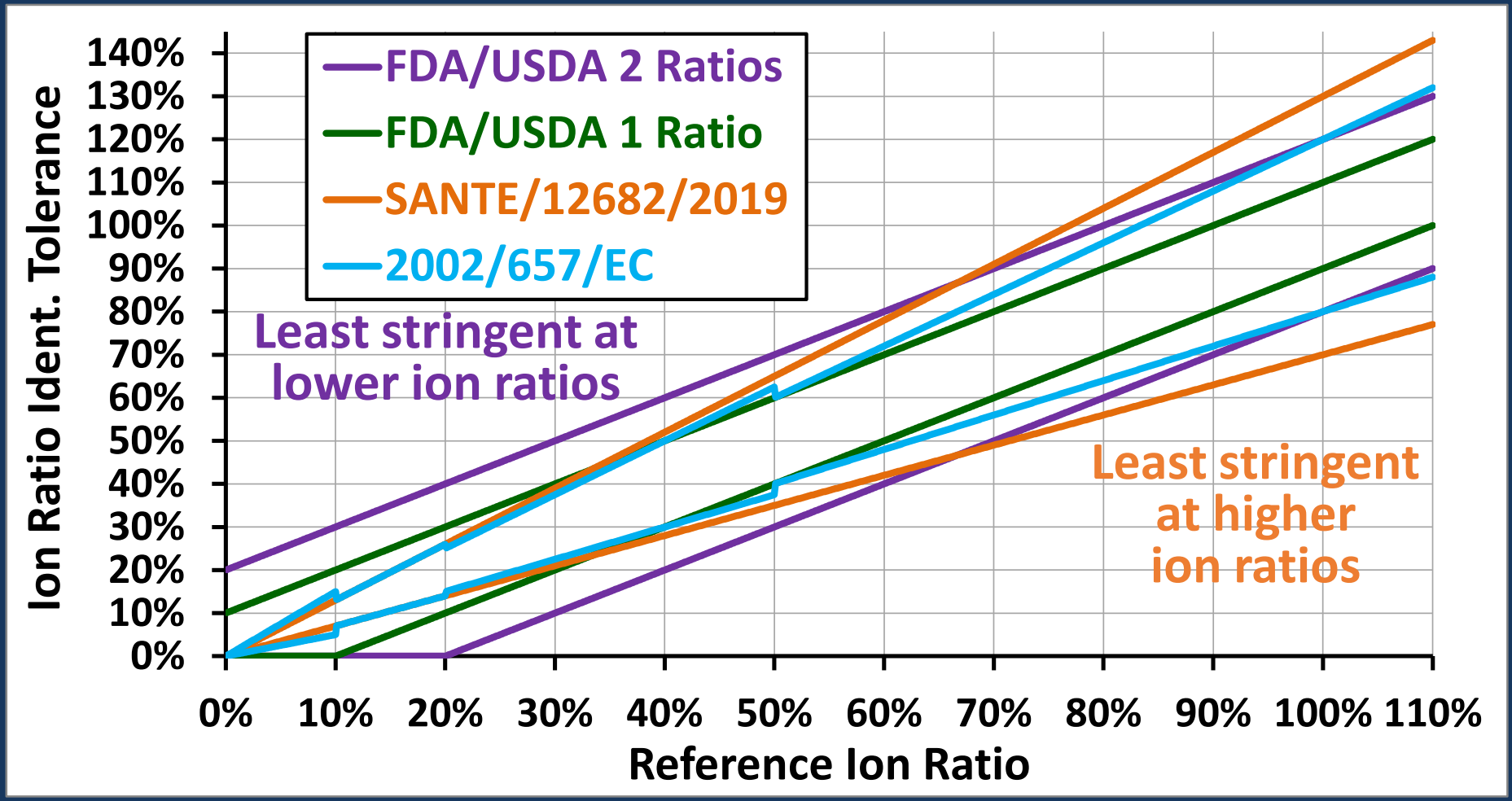
EI-MS (≥3 ions) MS/MS (≥2 ions)

±30% Rel

Ref. Ion Ratio	Sample Ion Ratio
70%	49 – 91%
24%	16.8 – 31.2%
12%	8.4 – 15.6%
4%	2.8 – 6.2%

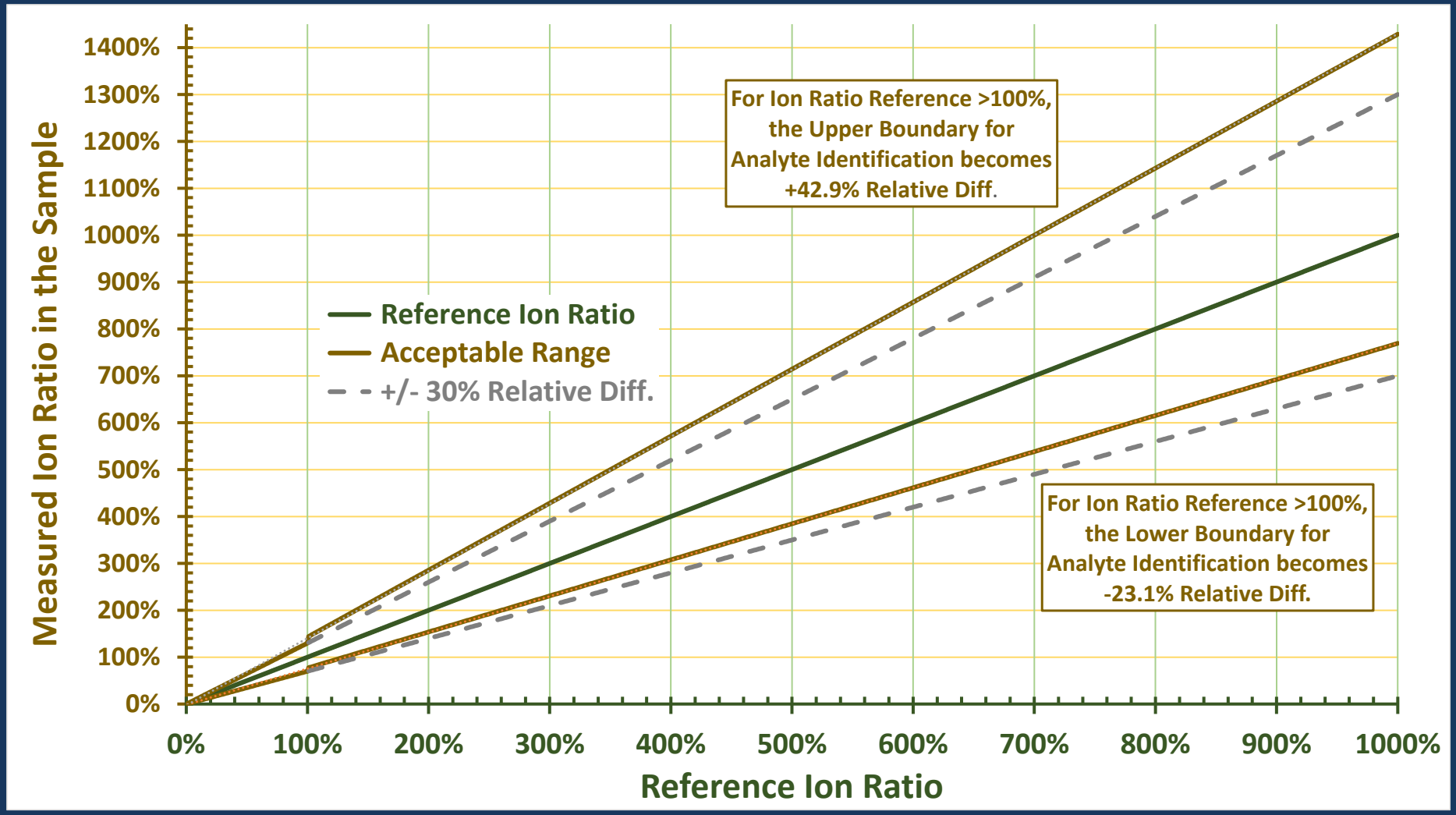
≥2 ions in high resolution MS with mass accuracy ≤5 ppm

Which MS/MS Identification Criteria to Use?



Note: 2002/657/EC ion ratio tolerances plotted for LC-MS (its GC-MS tolerances are twice as narrow)

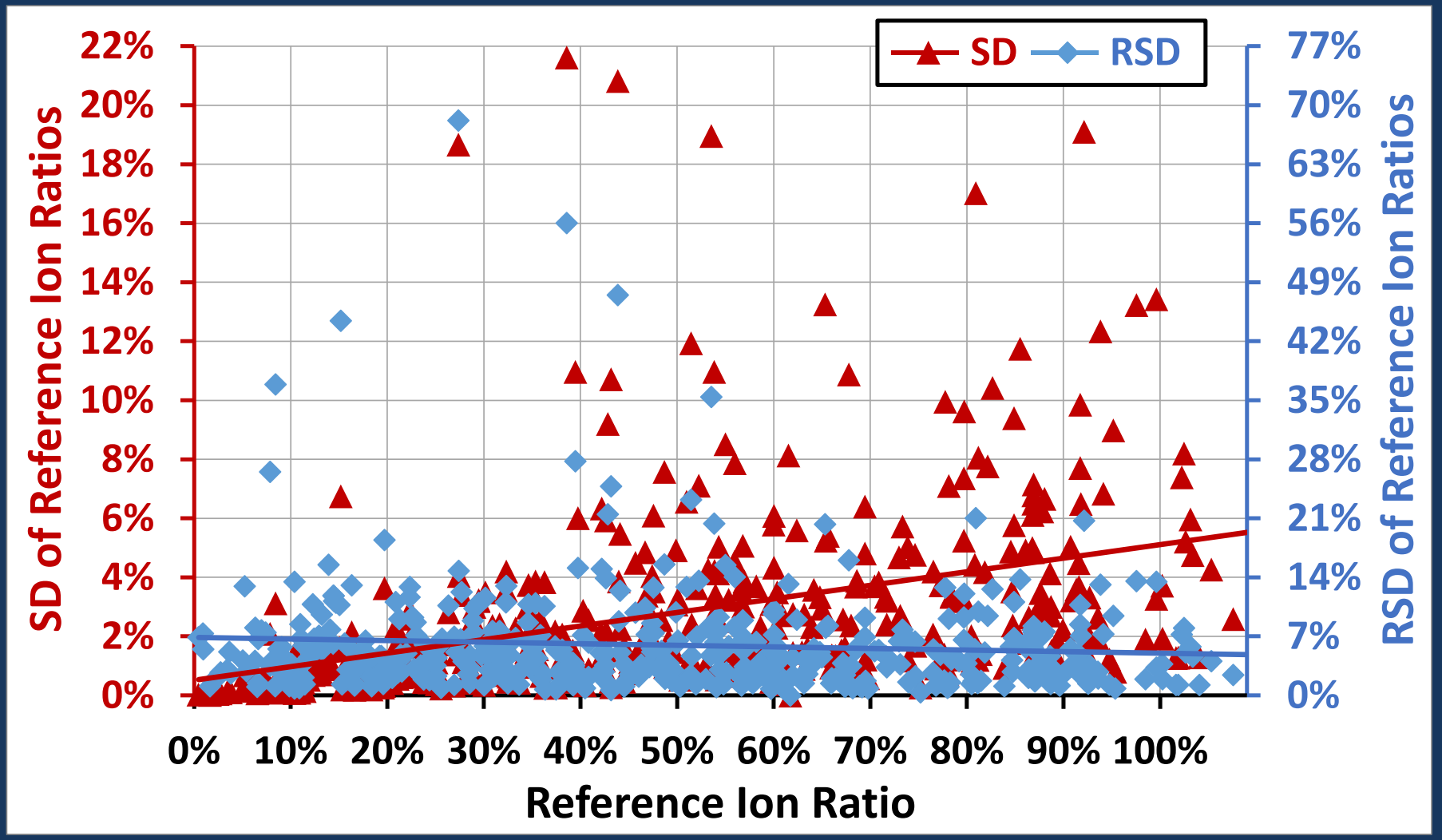
Note: if the Reference Ion Ratio is > 100%:



e.g.: Ion 1/Ion 2 = 60% (±30% = 42-78% identification window)

Ion 2/Ion 1 = 167% (with 128-238% for the same window)

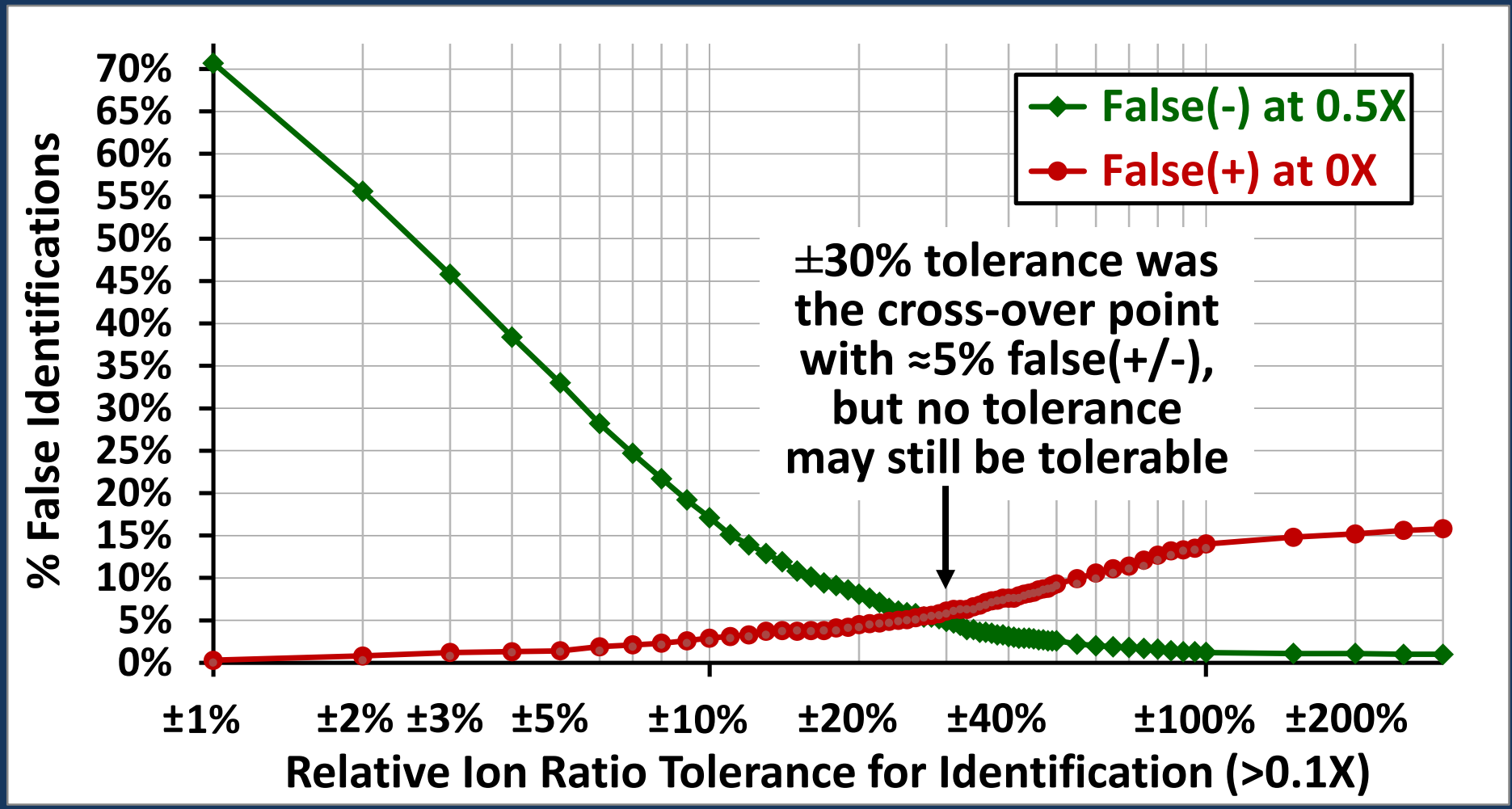
Which MS/MS Identification Criteria to Use?



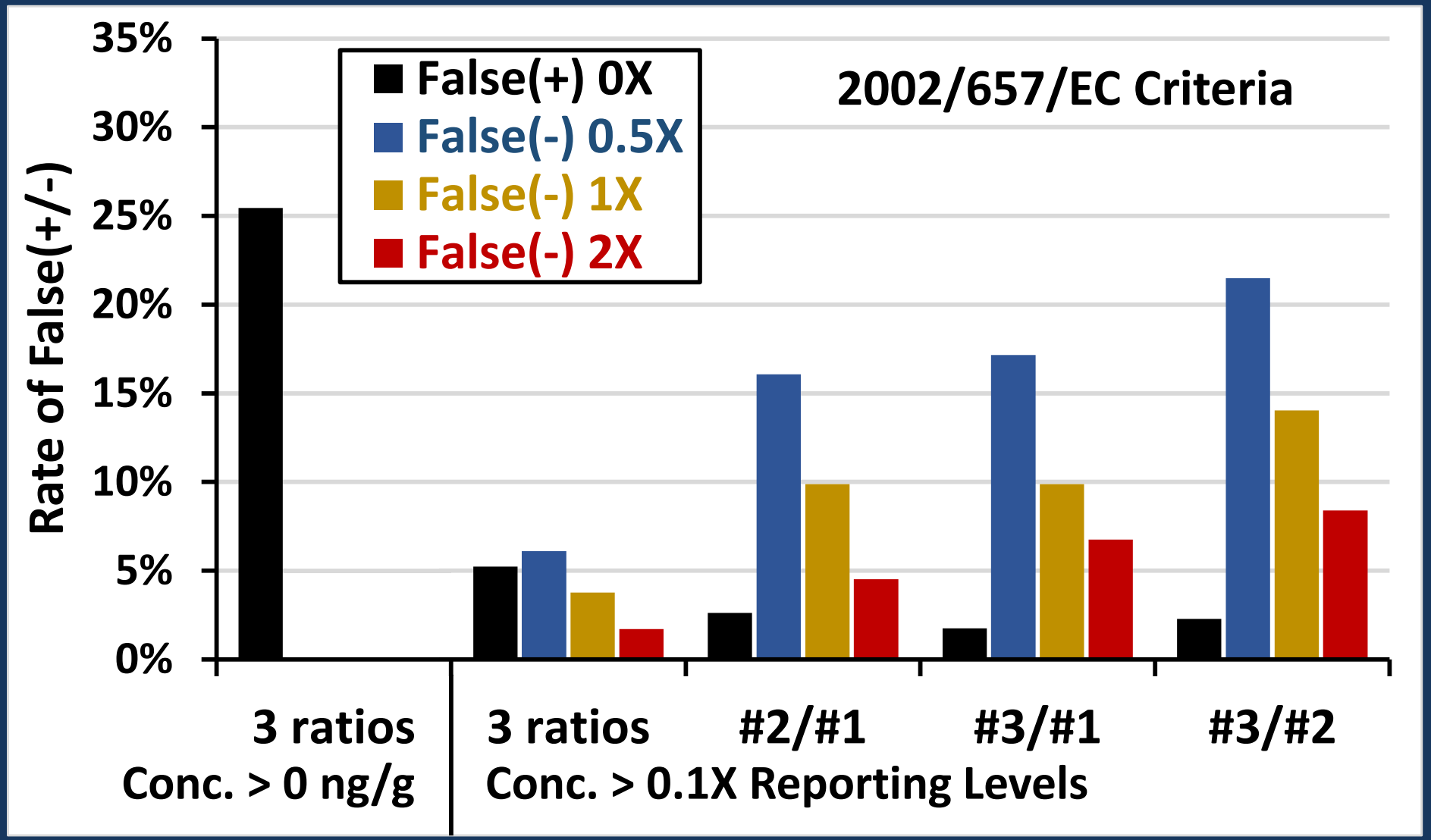
Relative ion ratio tolerances from the reference ion ratio look to be more appropriate than absolute ion ratio windows

SANTE/12682/2019 criteria work well

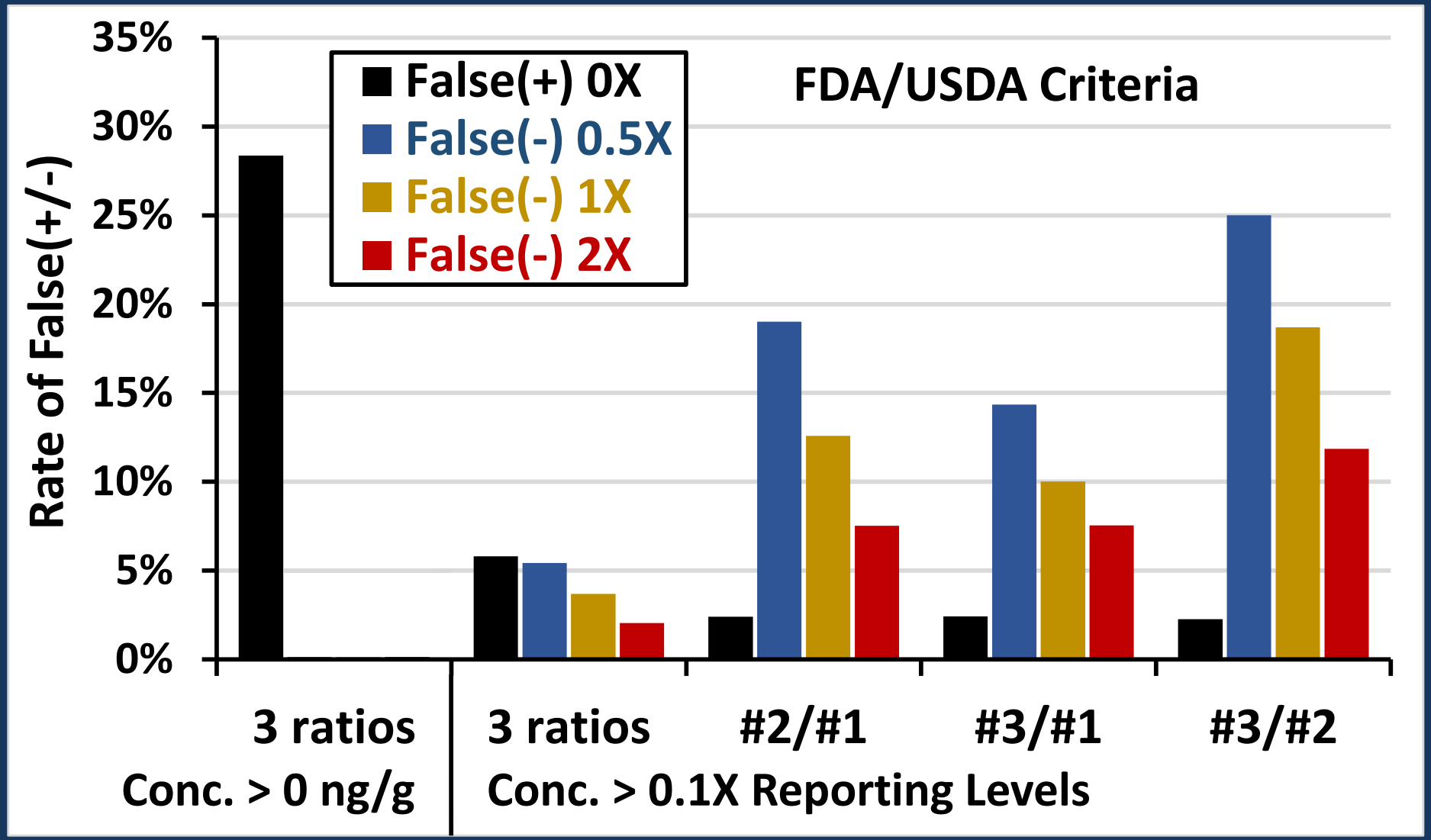
and are simplest in concept and practice



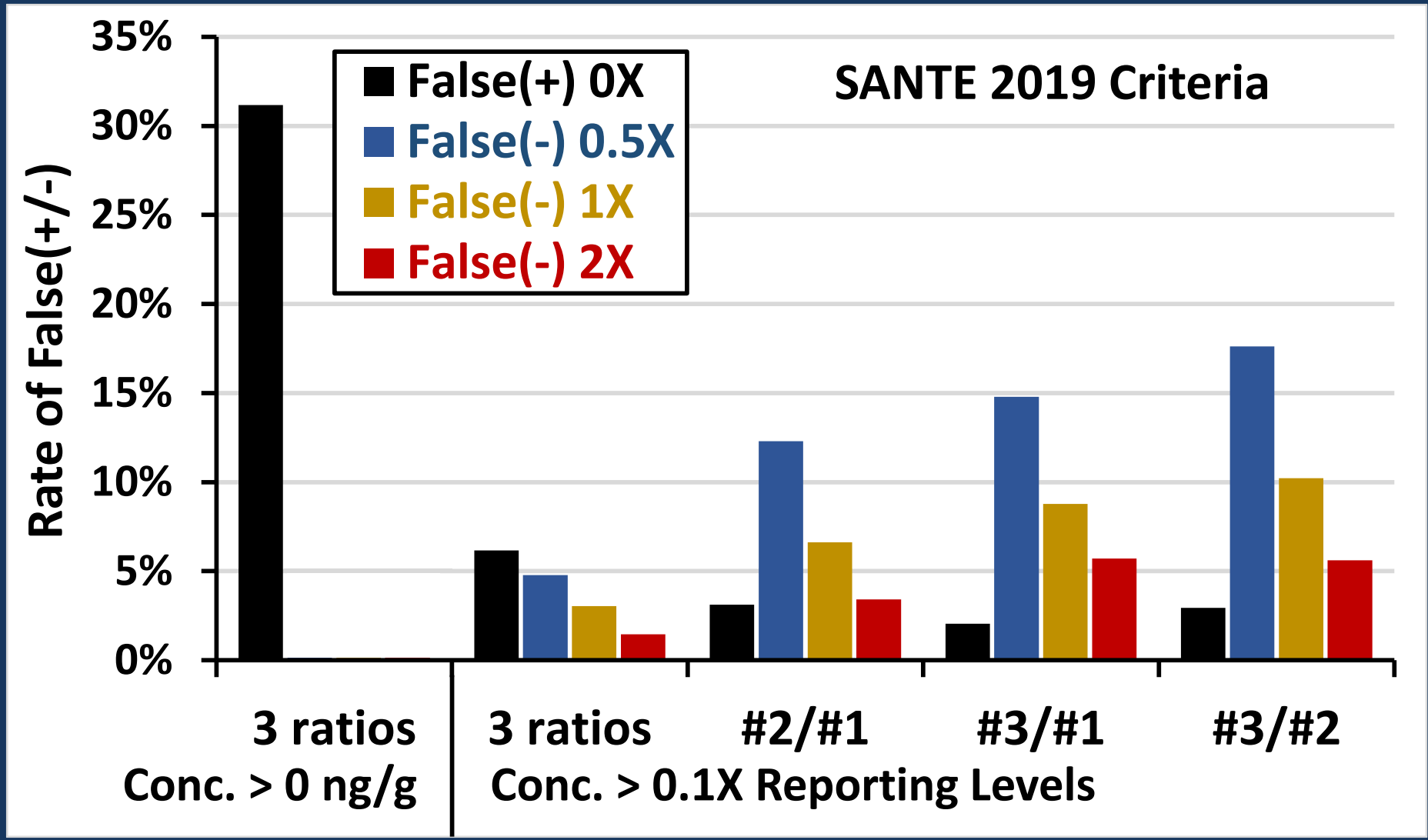
SANTE/2019, FDA/USDA, and 2002/657/EC Criteria Yielded Similar Rates of False(+) and False(-) Results



False(+/-) Results are reduced by using 3 MRMs/analyte in MS/MS



Occam's Razor in Analytical Chemistry: If needs are met by multiple methods, use the simplest!



Conclusions

1) The SANTE/12682/2019 identification criteria are simpler to implement and work just as well as the other criteria evaluated.

2) Acquisition of 3 ion transitions in MS/MS reduces rates of false negatives than when acquiring just 2 transitions, without significant increase in the rates of false positives.

3) To further reduce rates of false positives, set a concentration or S/N threshold for identification based on the **need** for the analysis.

See: Lehotay, "Comparison of analyte identification criteria and other aspects in triple quadrupole tandem mass spectrometry: Case study using UHPLC-MS/MS for regulatory analysis of veterinary drug residues in liquid and powdered eggs" *Anal. Bioanal. Chem.* (in press).