Comparison of 4 Different Multiclass, Multiresidue Analysis Methods for Veterinary Drugs in Fish and Other Food Matrices

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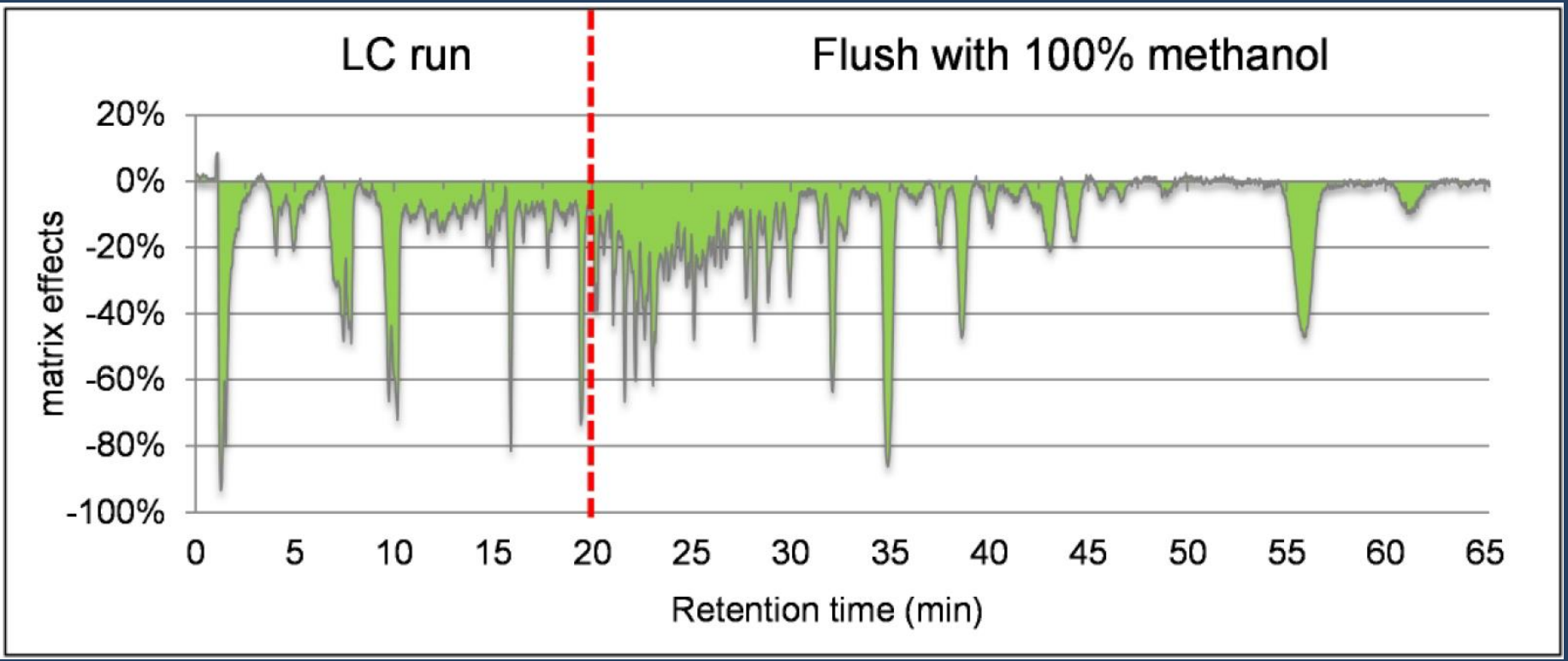
Conclusions

- The ARS Method **(USDA-FSIS CLG.MRM3)** is MUCH FASTER, EASIER, CHEAPER, and yields BETTER OVERALL RESULTS for the targeted analytes and levels in tilapia and other animal tissues than the other methods involving more complicated extraction and cleanup steps.
- Stay tuned for publications of completed validation studies of this and the QuEChERS Mega-Method.
- Try it yourself. **Analytical methods are not toothbrushes!**

Lessons Learned for Next Time

- Avoid Mistakes! **Take more time to spend less time!**
- Make the study design **less complicated!**
- Extract aminoglycosides with others in a **QuPPE-type method**
- Use triplicate **10X ng/g** stds for setting **reference ion ratios**
- Use a wider range of **calibration stds (0.1X to 10X)**
- Include **S/N > 3** for ions as another condition for **identification**
- Set default **peak integration baseline at 25%** rather than 50%
- Use the **QuEChERS Mega-Method** to include GC-analytes
- **Share the samples** for others to evaluate their own method, and ask them to conduct the QuEChERS Mega-Method, too.
- Employ **LC Column-Switching and Back-Flushing**

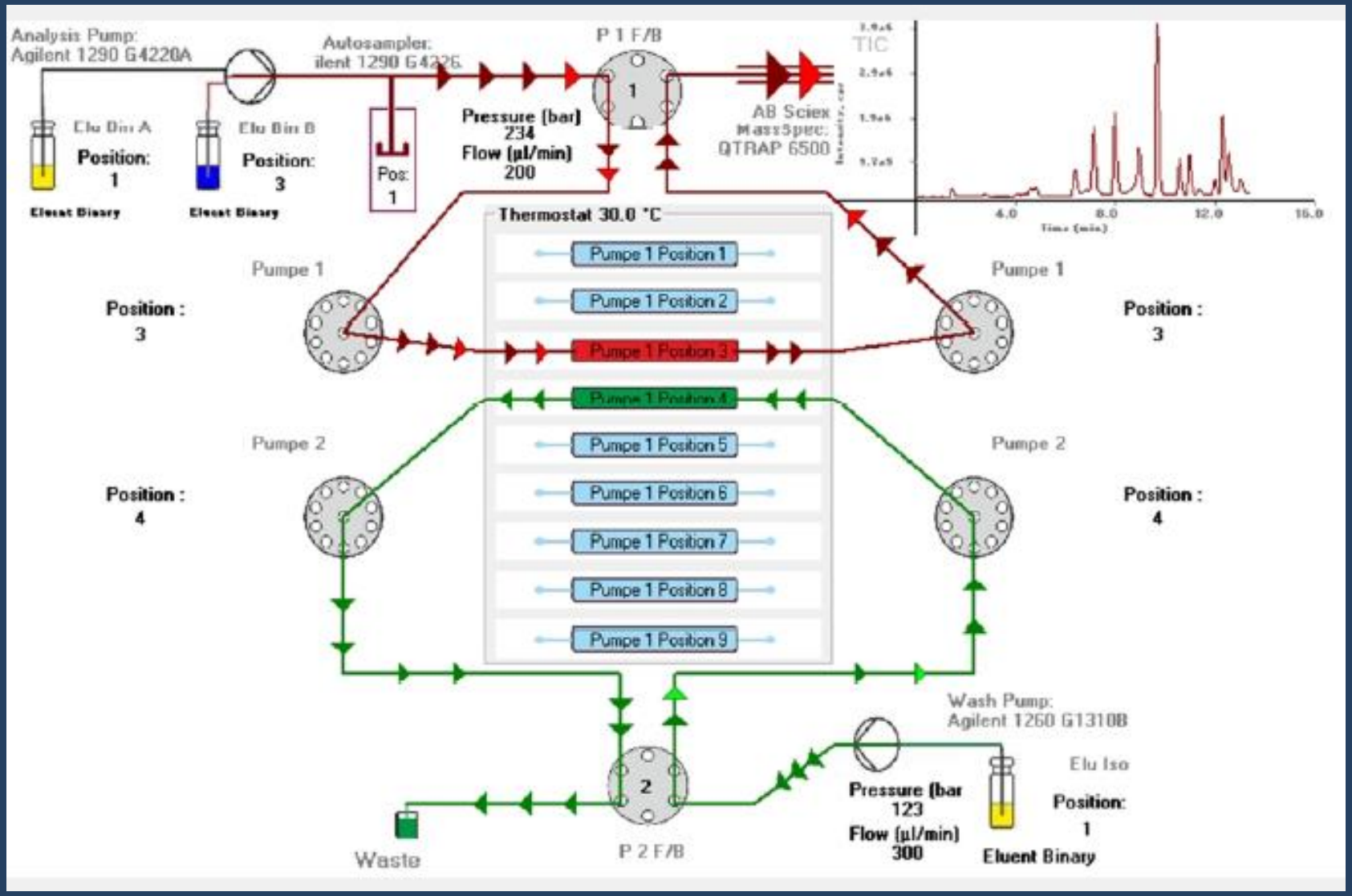
Ghost Peaks and Matrix Effects in LC-MS/MS



Analysis of arugula extract in 20 min by LC-MS/MS, but more than an hour is needed for matrix components to elute using 100% MeOH. Those components cause ghost peaks and induce matrix effects in subsequent injections.

From: Roussev et al., Sciex Application Note 230415-01 (2015)

Column Switching and Back-Flushing



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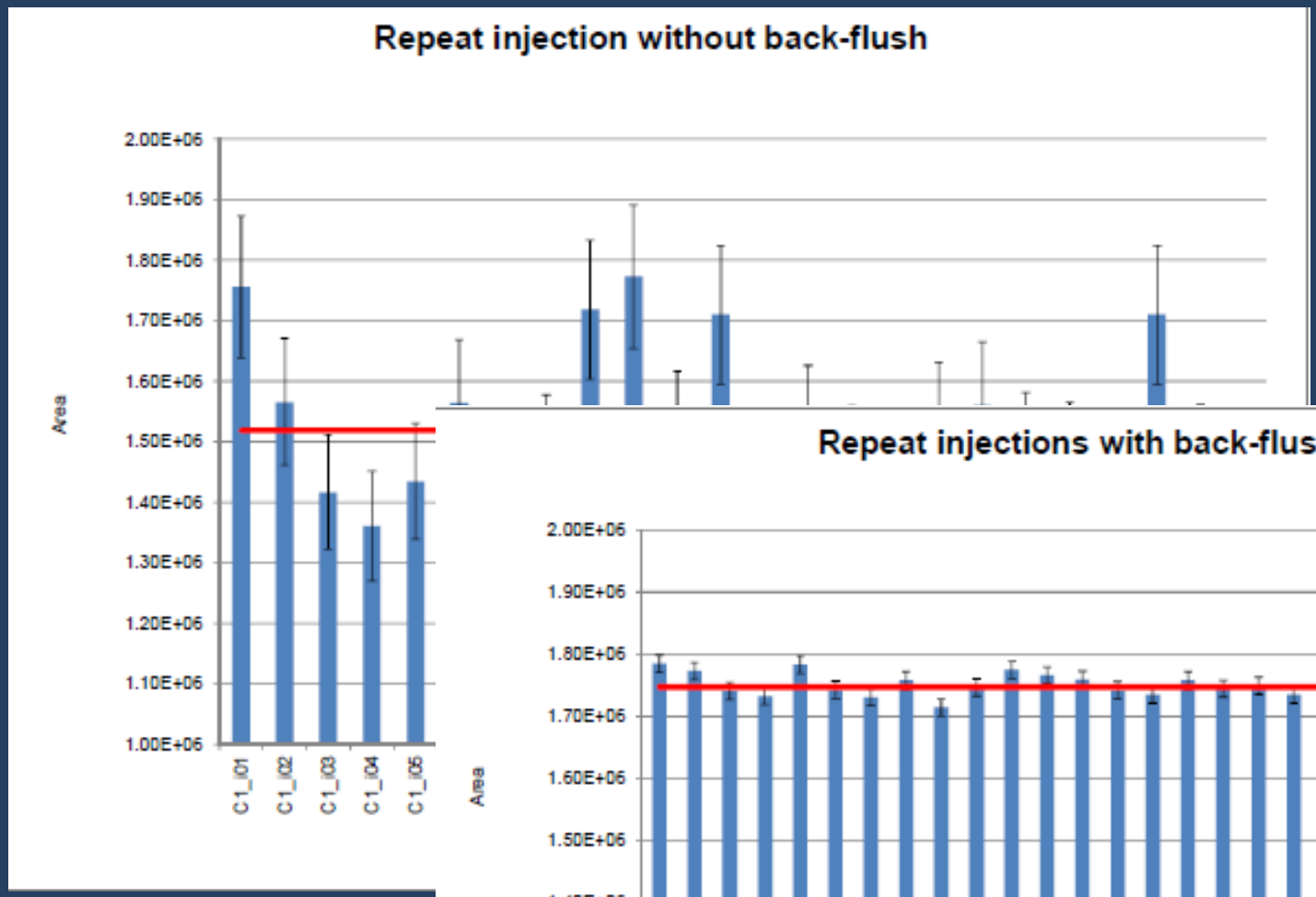
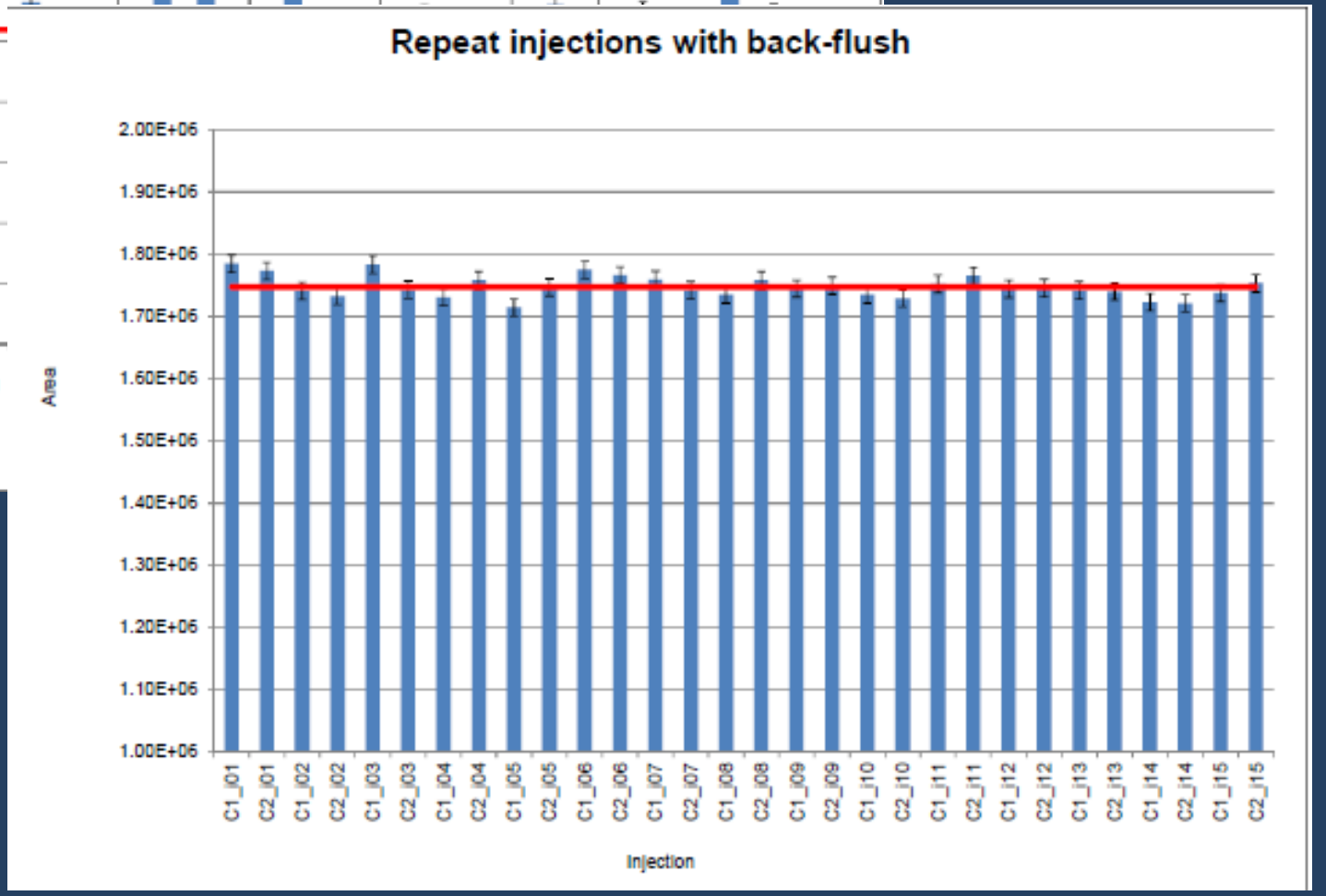




From: Roussev et al., Sciex Application Note 230415-01 (2015)

“What World Do You Live In?”

1) I live in the future of what should be, which could be now, and technology is no longer the limitation, if it ever was.

2) I’ve lived in that future my whole career, and I’ve seen how experts, dogma, and intuition are to be questioned.

3) I live in a world in which hundreds of diverse contaminants can be accurately and robustly quantidentified in 100 food samples per day without review (QC checks only).

4) Method validation can be done through blind batches of proficiency test samples using a template, and labs can evaluate more than one method easily within days.

TYM TAM = test your method, test another method

High Throughput QuEChERS Mega-Method

1) Sample Processing (using Liquid N_2?)

2) 2 g test portion + 10 mL 4/1 (v/v) MeCN/water

– Shake 10 min then centrifuge 3 min

3A) Transfer 200 µL extract to 2 mL tube (evaporate MeCN)

– Add 750 µL water and conduct ultracentrifugation for 5 min

– Transfer 500 µL to AS vial and inject 10 µL in UHPLC-MS/MS

3B) decant extract into 15 mL tube with 2 g 4/1 (w/w) $MgSO_4$/NaCl

– Shake 5 min then centrifuge 3 min

– Transfer 1 mL to AS vial, pass 300 µL through µ-SPE (ITSP)

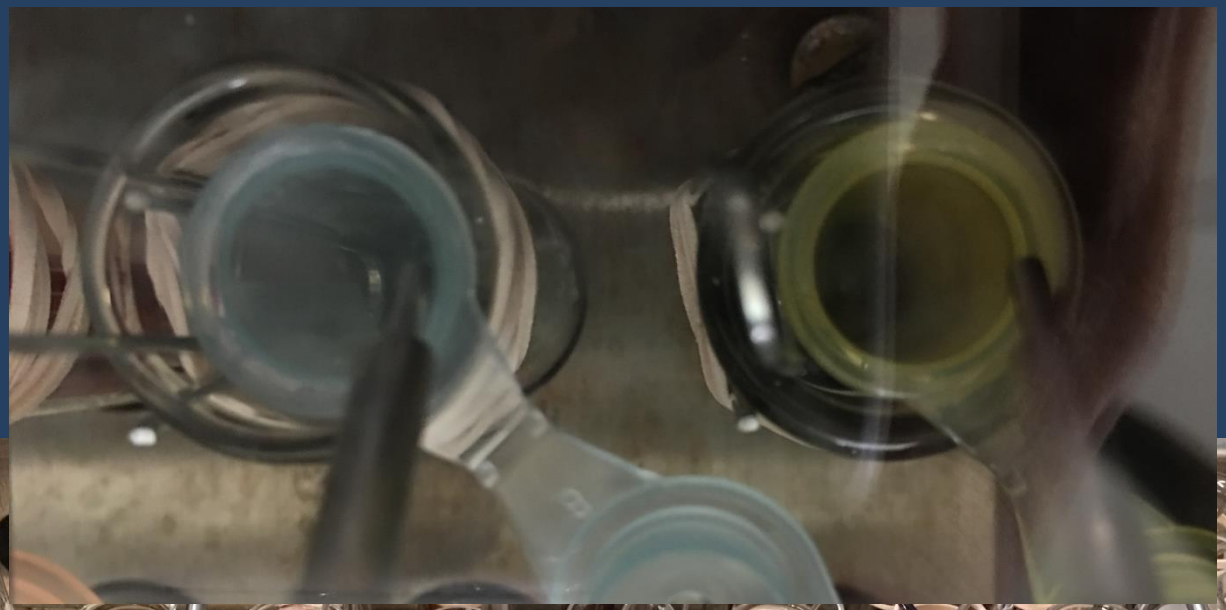
– 1.5 µL injected in low-pressure (LP)GC-MS/MS

4AB) Automatic summation peak integration and identifications

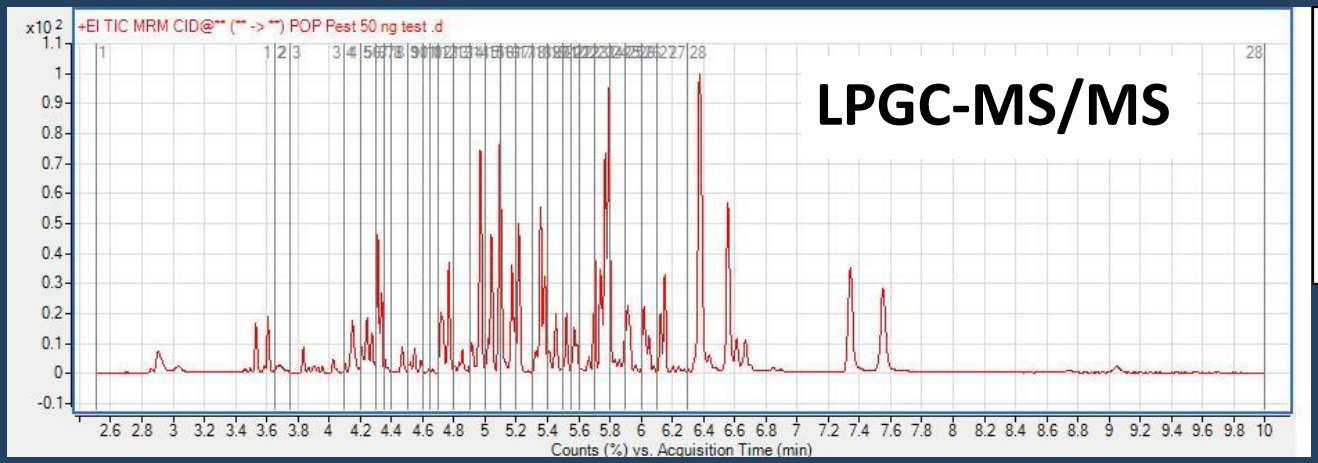
Comminution Using Liquid Nitrogen

See: Roussev et al., *J. Agric. Food Chem.* **67**, 9203-9209 (2019)

Rapid Evaporation of Small Volumes

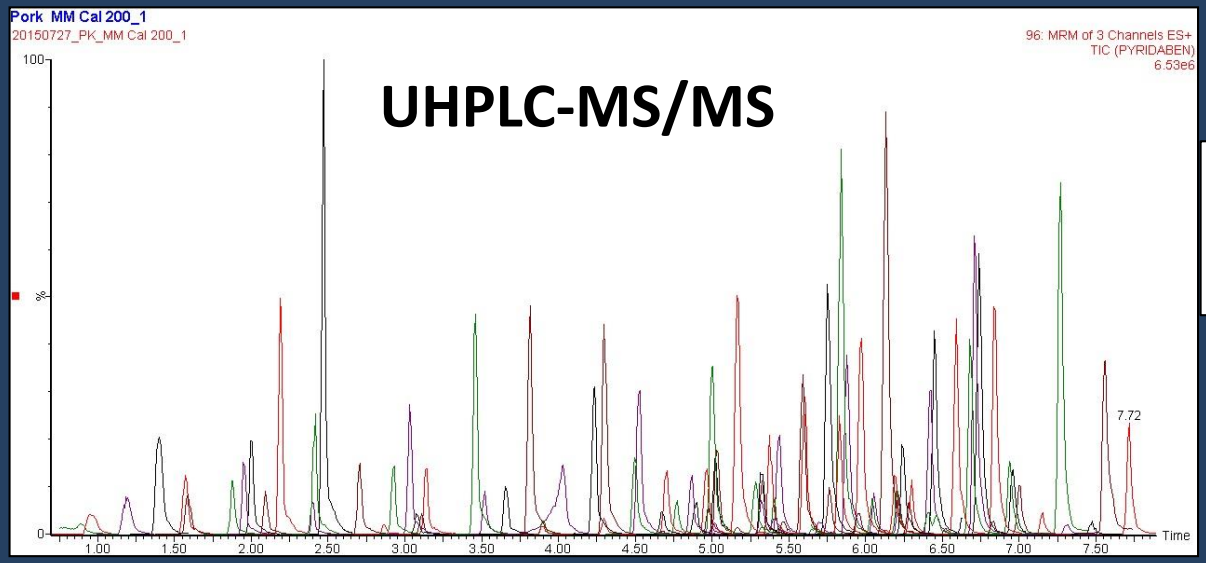


>260 Analytes in Parallel by 10 min Analyses



152 pesticides + 65 environmental & other contaminants

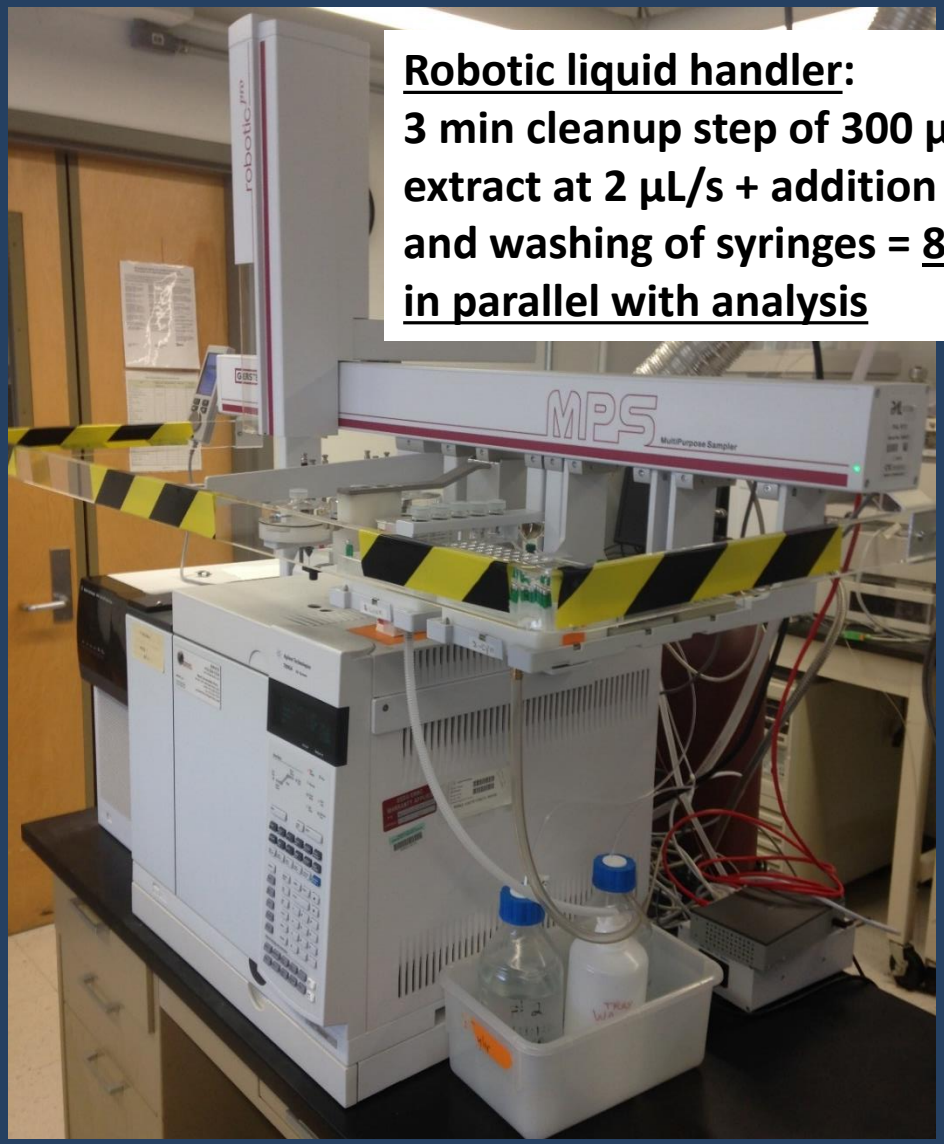
53 overlapping pesticides



101 pesticides + internal/QC standards

See: Sapozhnikova, *J. Chromatogr. A* **1572** (2018) 203-211

Automated ITSP Cleanup and LPGC-MS/MS



Robotic liquid handler:
3 min cleanup step of 300 µL extract at 2 µL/s + addition of APs and washing of syringes = 8 min in parallel with analysis

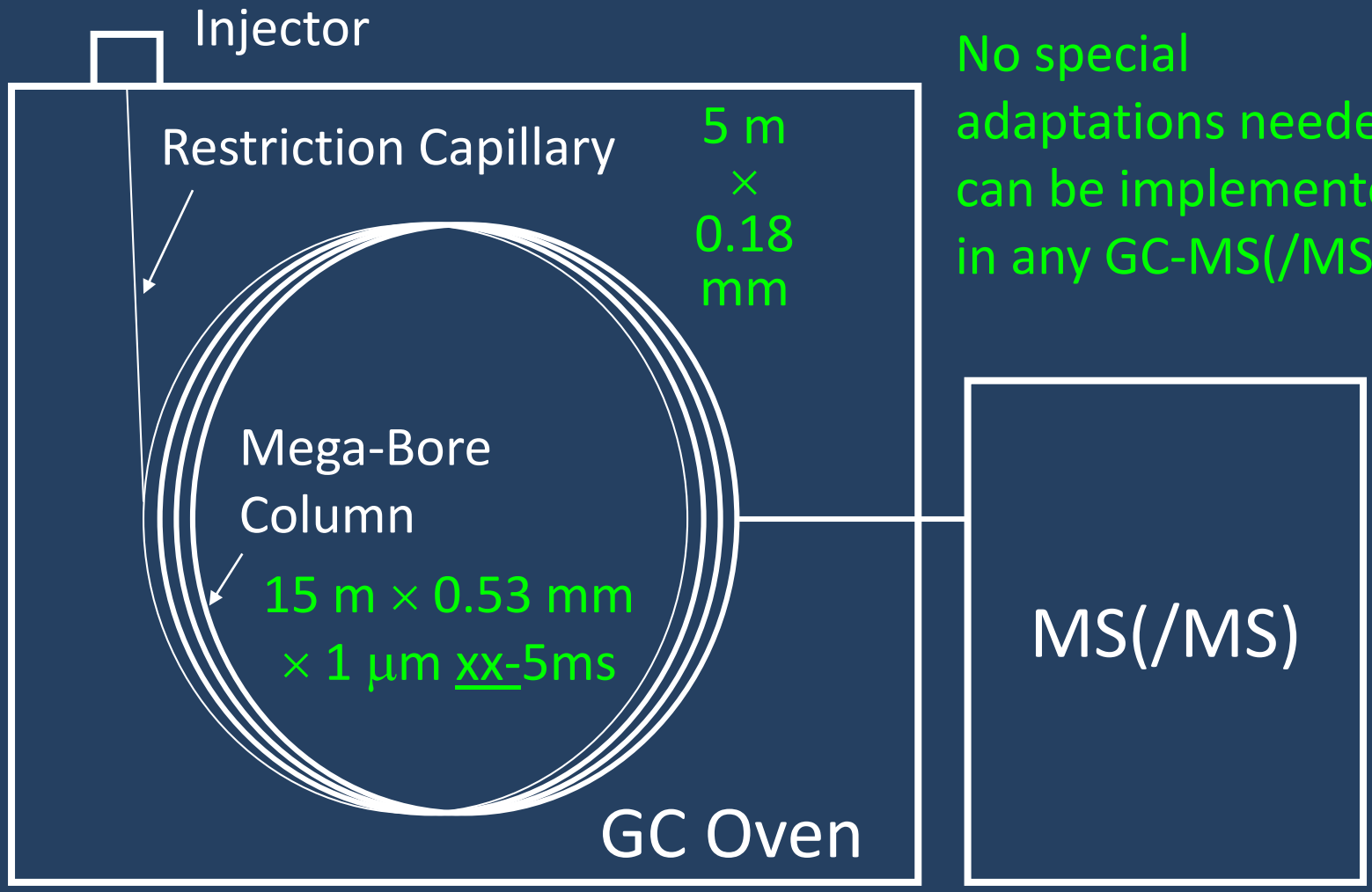
20 mg $MgSO_4$ + 12 mg PSA + 12 mg C18 + 1 mg CarbonX = 45 mg sorbent mixture

Mini-cartridges (used) showing removal of chlorophyll and other matrix components

Final extract volumes = 278 ± 5 µL (n = 255) after 25 µL each of APs and (MeCN or Std)

See: Lehotay *et al.*, *Chromatographia*, **79** (2016) 1113-1130

Fast Low-Pressure (LP)GC-MS/MS

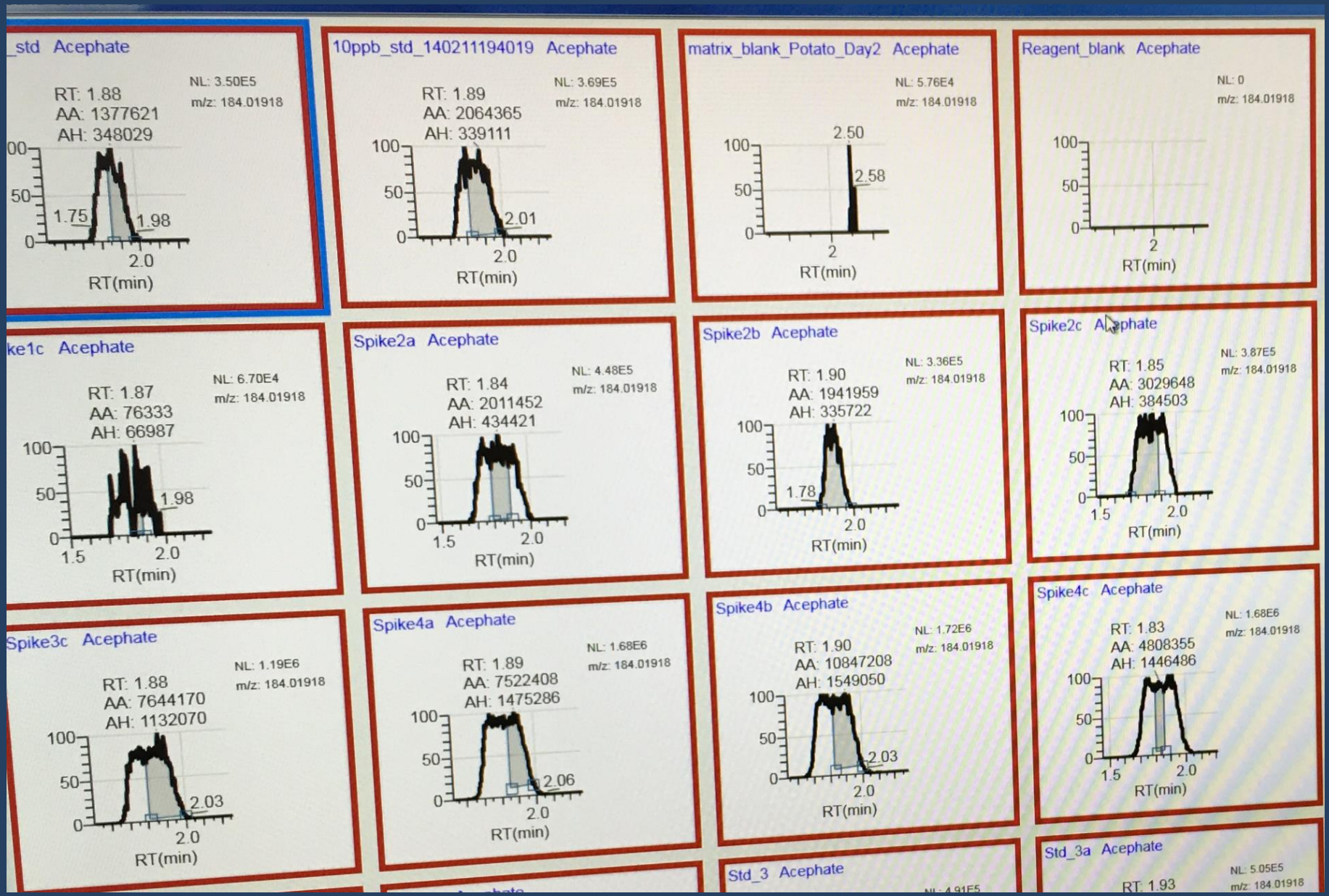


No special adaptations needed; can be implemented in any GC-MS(/MS).

Review of dozens of publications using LPGC-MS(/MS):

Sapozhnikova and Lehotay, *Anal. Chim. Acta* **899**, (2015) 13-22

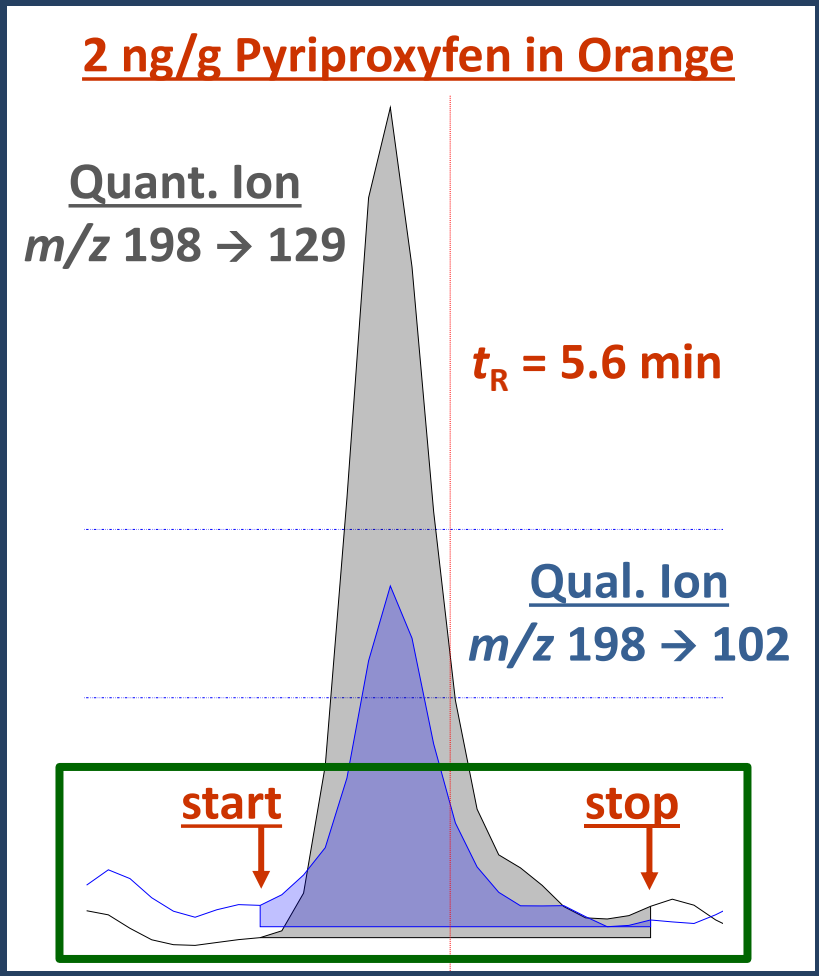
Poor integration undoes excellent detection



Summation Integration in Chromatography

SIMPLIFY, don't COMPLIFY!

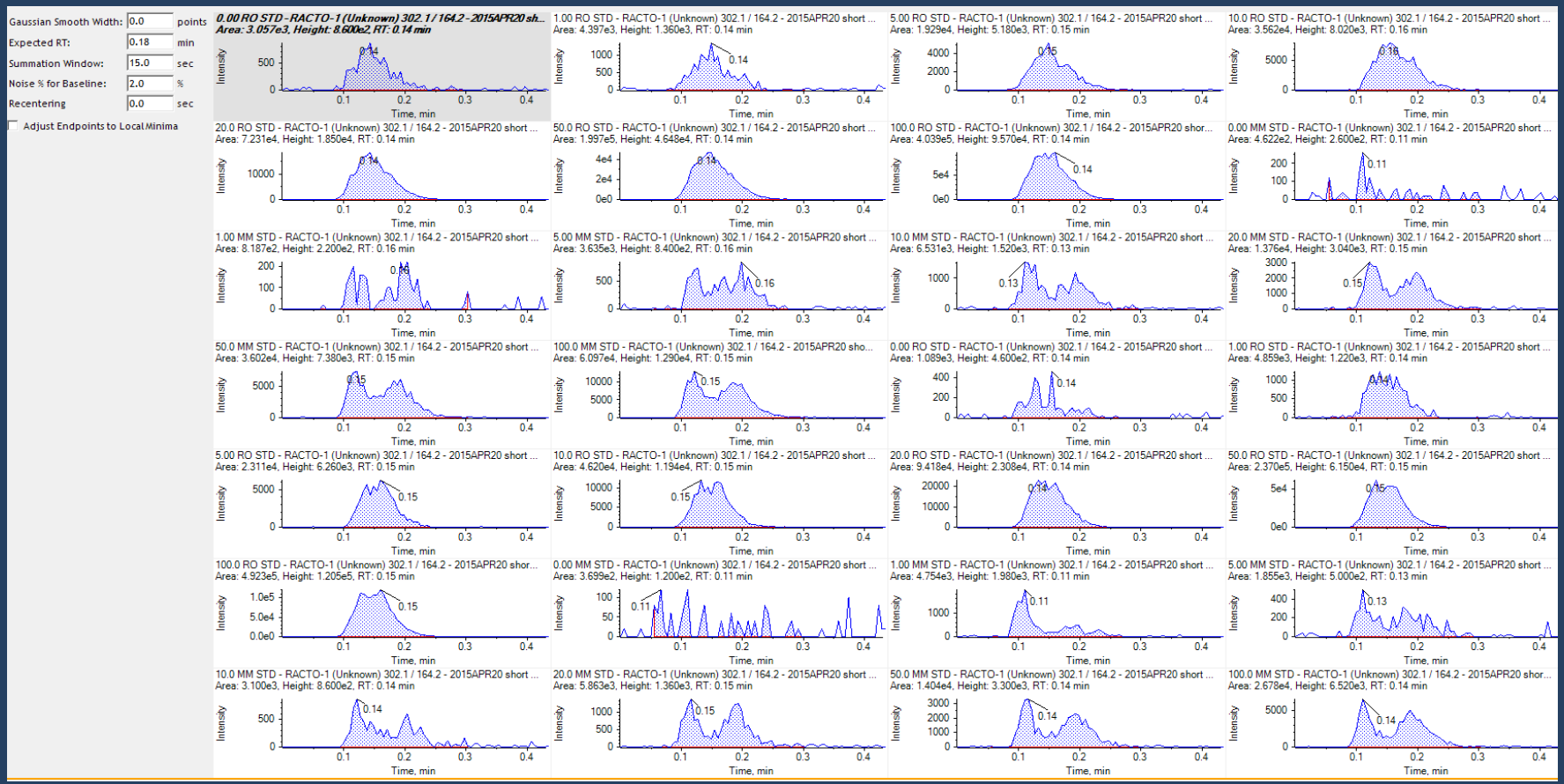
- Draw a straight line at the baseline just before the start of the expected peak to just after its expected end → **EASY PEASY!**
- **See:** Lehotay, *LCGC North America* **35** (2017) 391-402.
- **Advanced ≠ Better**
- **Function ≠ Beauty**
- **Time = Money**



Quant. and Qual. Ions Co-Elute with the Same t_R!

Summation Integration Function

- ≈1 min to integrate a batch of >60 samples of ≈660 MRMs per sample **WITHOUT REVIEW!**



Overall LPGC-MS/MS Results

Out of 195 analytes and 73 injections in 6 matrices = **14,235 decisions**

	"Advanced"	Summation
False Pos.	0.19%	**0.11%**
False Neg.	11.2%	**9.5%**
True	91.6%	**92.9%**

192 times net overall times that summation did not yield a false result *vs.* "advanced" = **1.3% improvement**

FDA/USDA Rules in Automatic Post-Run Ident.

(*e.g.* in Excel or Instrument Software)

But: <u>Any Set of Identification Criteria can be Applied</u>

1) Ret. time (t_R) for each ion (Quant. and Qual.) must be ≤|0.1| min from the contemporaneous t_R(ref.), which is the **avg t_R** from high conc. calibration stds in solvent in the same sequence.

2) Ion Ratio (IR) = (signal ion 2)/(signal ion 1), 3/1, 3/2, *etc.* (in %); IR(ref.) = **avg IR** of contemporaneous high conc. calibration stds in solvent **[note: IR(ref.) ≤ 110%]**

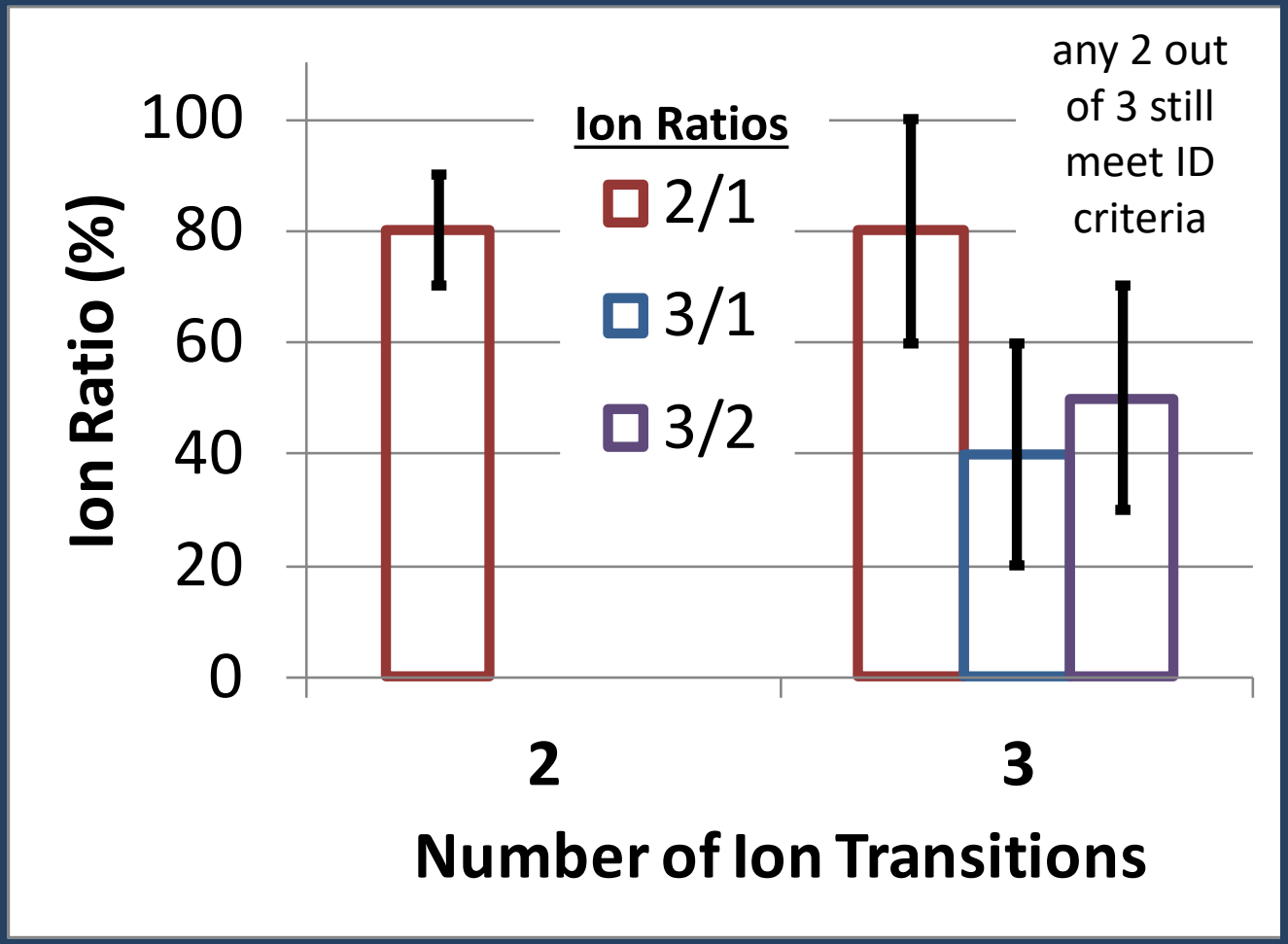
Ident. requires |±10%| for ≥1 IR or |±20%| for ≥2 IRs *vs.* IR(ref.)

3) Conc. must be > **reporting level** (*e.g.* LOQ, LOI, or MRL)

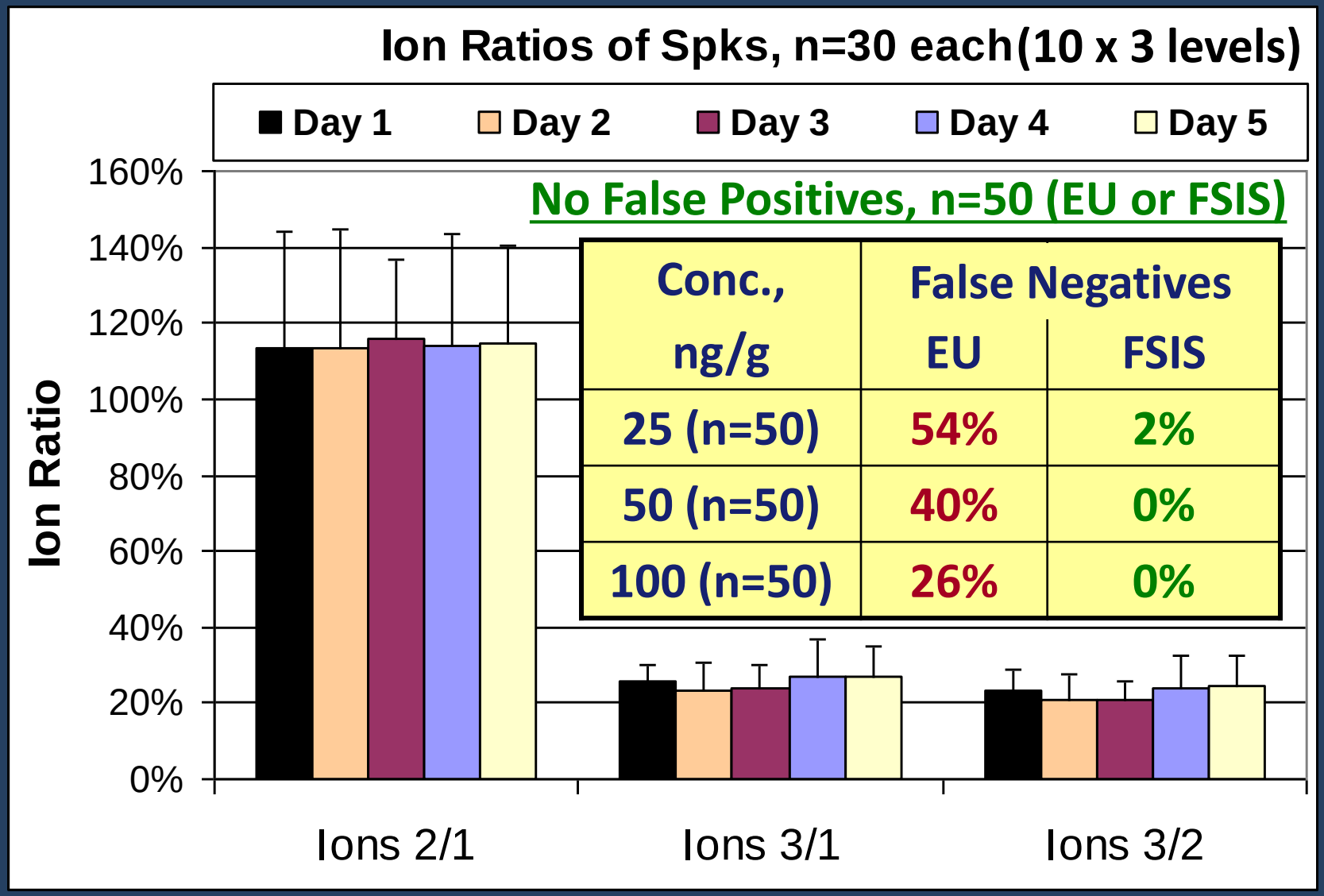
(S/N > 3 for ions used in ident, and no positives in reagent blanks)

Example MS/MS (or SIM) Ion Ratio Ranges

(collect 3 ion transitions per analyte!)



Ion Ratios for Ciprofloxacin in Kidney



QuEChERSER Results for 284 Analytes

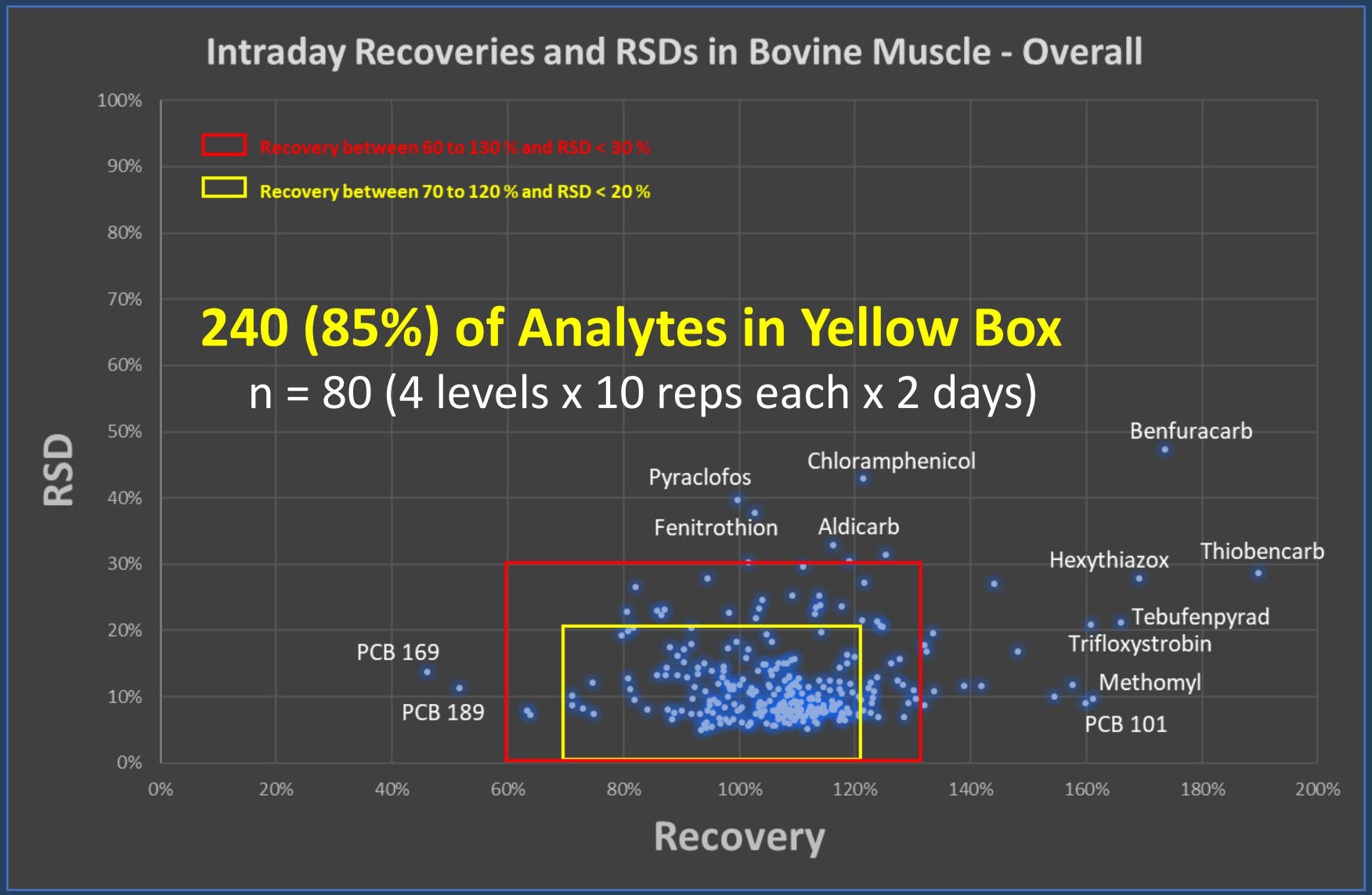
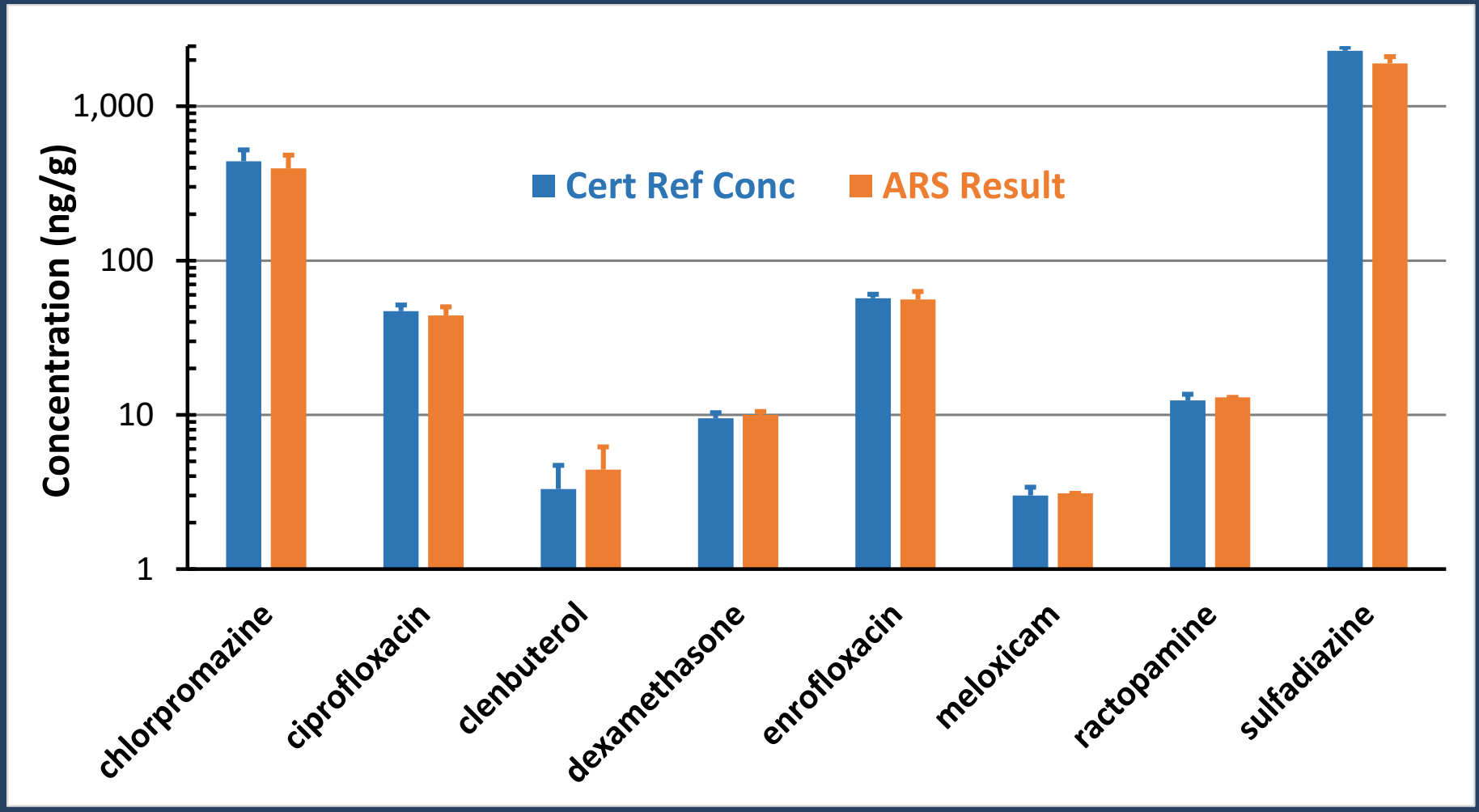


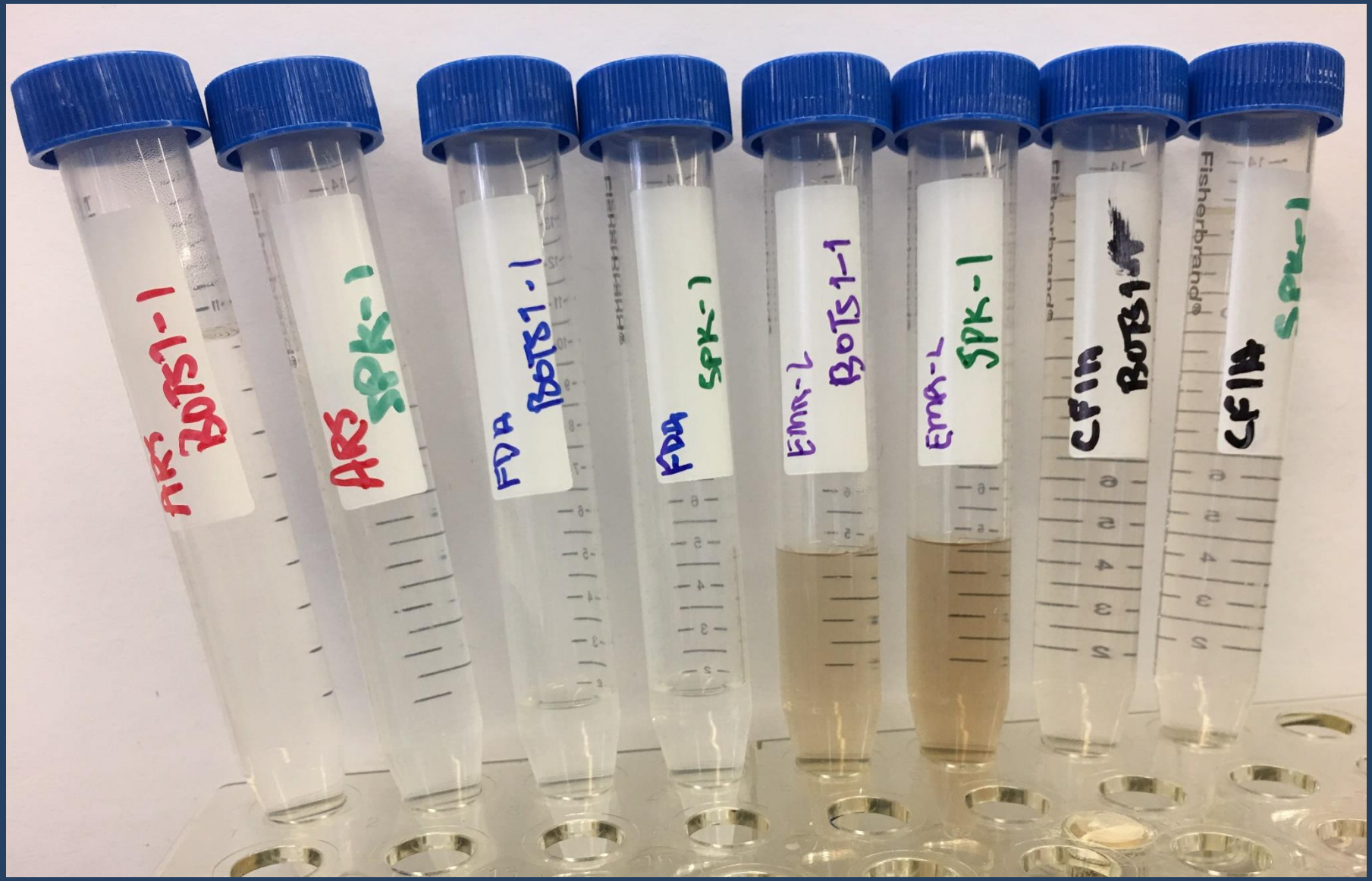
Figure by Sergio Monteiro

Comparison of Results with National Research Council Canada Certified Reference Material BOTS-1

veterinary drug residues in bovine muscle (freeze-dried)



Comparison of 4 Methods for Vet. Drugs



The 4 Methods in the Literature

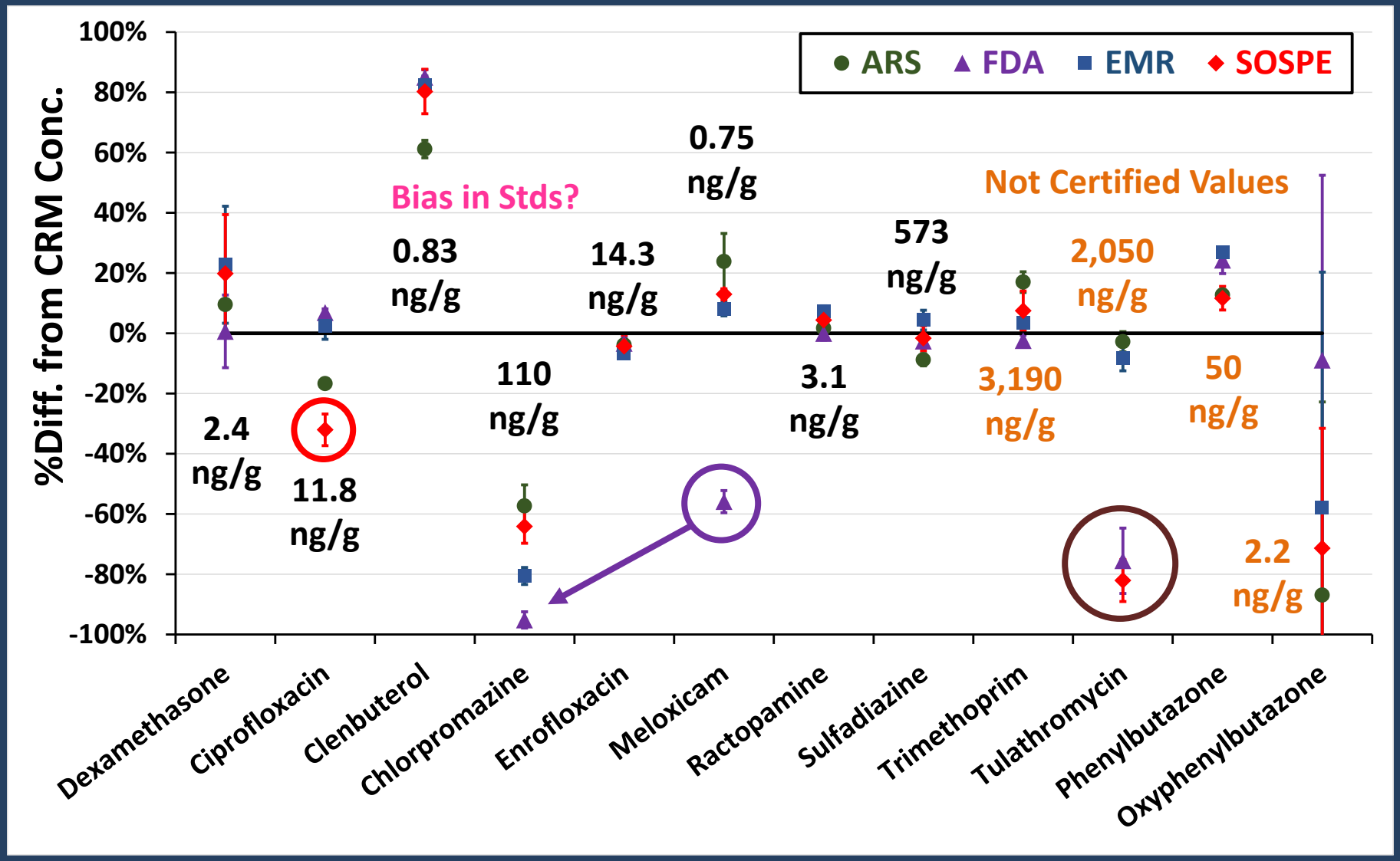
ARS Method: Lehotay & Lightfield "Simultaneous analysis of aminoglycosides with many other classes of drug residues in bovine tissues by [UHPLC-MS/MS] using an ion-pairing reagent added to final extracts" *Anal. Bioanal. Chem.* **410** (2018) 1095-1109

FDA Method: Turnipseed et al. "Extended [LC-HRMS] screening method for veterinary drug, pesticide and human pharmaceutical residues in aquaculture fish" *Food Addit. Contam. A* **(in press)** (or *J. Agric. Food Chem.* 2017)

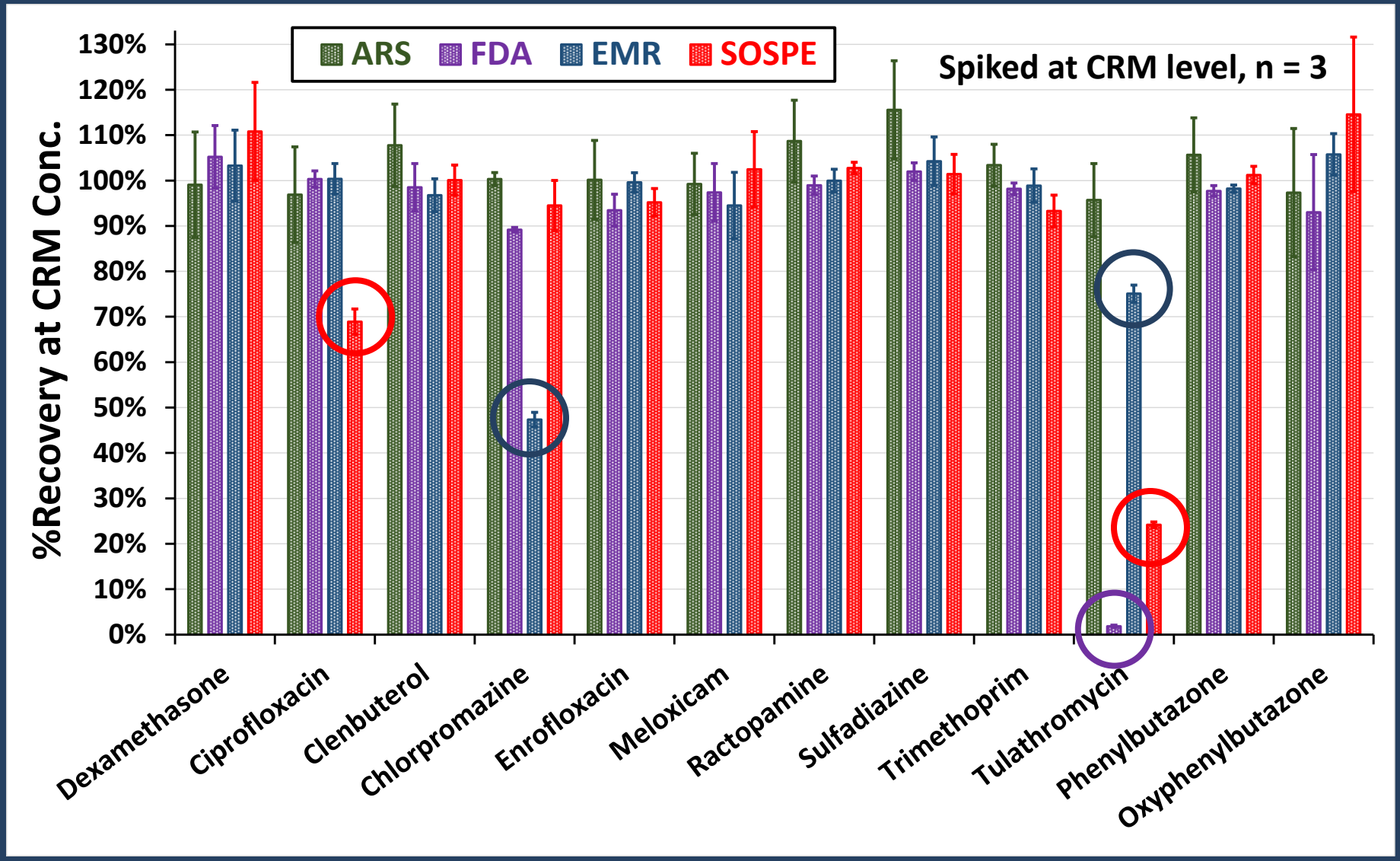
EMR Method: Zhao et al. "Multi-class multi-residue analysis of veterinary drugs in meat using enhanced matrix removal lipid cleanup and [LC-MS/MS]" *J. Chromatogr. A* **1549** (2018) 14-24

SOSPE Method: Wang et al. "Development and validation of a multiclass method for analysis of veterinary drug residues in milk using [UHPLC-ESI-Q/Orbitrap MS]" *J. Agric. Food Chem.* **63** (2015) 9175-9187

4 Methods for Vet. Drugs (in BOTS-1)



Recoveries of Spiked Samples (in Beef)



AOAC Int. SPSFAM VDR Working Group + FDA + China List of 79+ Analytes in Fish

Anthelmintic (8): (Aba)(Dora)(Ema)(Iver)mectin, Albendazole + Sulfones/Sulfoxide

Aminoglycoside (4+): Gentamicins, (Neo)(Paromo)(Spectino)mycin

Quinolone (8+): (Cipro)(Dano)(Di)(Enro)(Sara)floxacin, Flumaquine, (Nalidixic)(Oxolinic) Acid

β-Lactam (6+): (Amoxi)(Ampi)(Cloxa)(Dicloxa)(Oxa)(Peni)cillin

Macrolide (7): (Erythro)(Kitasa)(Linco)(Neo+Spira)mycin, (Tilmico)(Tylo)sin

Tetracycline (4): (Chlor)(Doxy)(Oxy)(Tetra)cycline

Sulfonamide (18): Sulfa(chloro)(ethoxy)(methoxy)pyridazine, Sulfanitran, Sulfanilamide, Sulfa(methi)(thia)(methoxa)zole, Sulfa(clo)(dia)(mera)(metha)zine, Sulfa(dimeth)(dox)(monometh)oxine, Sulfapyridine, Sulfaquinoxaline, Sulfisoxazole

Trimethane Dye (5): Brilliant Green; Malachite Green + Leuco, Crystal Violet + Leuco

Other (19+): Azamethiphos, Carprofen, p-Toluenesulfonamide, (Chlor)(Thi)amphenicol, Florfenicol + Amine, Colistin, Dapsone, (Dimetr)(Metron)idazole, Diminazine, (Hexaflum)(Lufen)uron, (Ormeto)(Trimetho)prim, Trichlorfon, Tricaine Methanesulfonate, Zeranol

Design of the Study

Divided the 79+ analytes into 3 groups and devised a semi-random spiking scheme for each group in 102 tilapia samples, which were the same for each of the 4 methods (408 samples total)

30 Blanks, 30 each of ½X and 1X spikes, and 12 ¼X spikes = 102

X = MRL or Target Levels in Fish from AOAC SMPR 2018.010 et al.

0.1X Level used as Identification Concentration Threshold

34 samples were analyzed each day by each method in batches

Protocols approved by Sherri Turnipseed, Jian Wang, Michael Young, and John Lee et al. Jian asked me to do experiments of his method for aminoglycosides. I offered to send them the samples for their analysis, but no takers.

Sherri sent me 7 incurred fish samples for blind analyses

Comparison of 4 Methods for Sherri's Fish

concentrations in ng/g in same analytical sequence

Drug	#	ARS	FDA	EMR	SOSPE	Avg
Sulfadiazine	O-7	300	315	488	447	388
	O-8	359	488	659	538	511
	O-9	1,009	1,149	1,395	1,281	1,208
	O-10	440	516	631	577	541
Ciprofloxacin	C-5	48	48	60	**19**	44
	C-6	66	70	107	29	68
Enrofloxacin	C-5	1,329	1,223	1,663	887	1,276
	C-6	1,360	1,165	1,873	769	1,292
Crystal Violet was also detected, but it was in blanks, too						

Not enough sample for method replicates -
Cannot eliminate biases unrelated to the methods

Experimental for the Study

Purchased 34 fresh/frozen, farm-raised/wild tilapia samples (> 1 kg) in Oct. 2018 originating from China (19), Cambodia, Malaysia, Taiwan, Vietnam, Colombia, Peru, Ecuador, and Honduras

Within 1-2 days, cut fish into chunks, weighed 500 g into Blixer, added 400 ng/g sulfabromomethazine, and chopped w/ dry ice

The same day, weighed twelve 2 g test portions of each fish into 50 mL centrifuge tubes (3 replicates each fish for 4 methods)

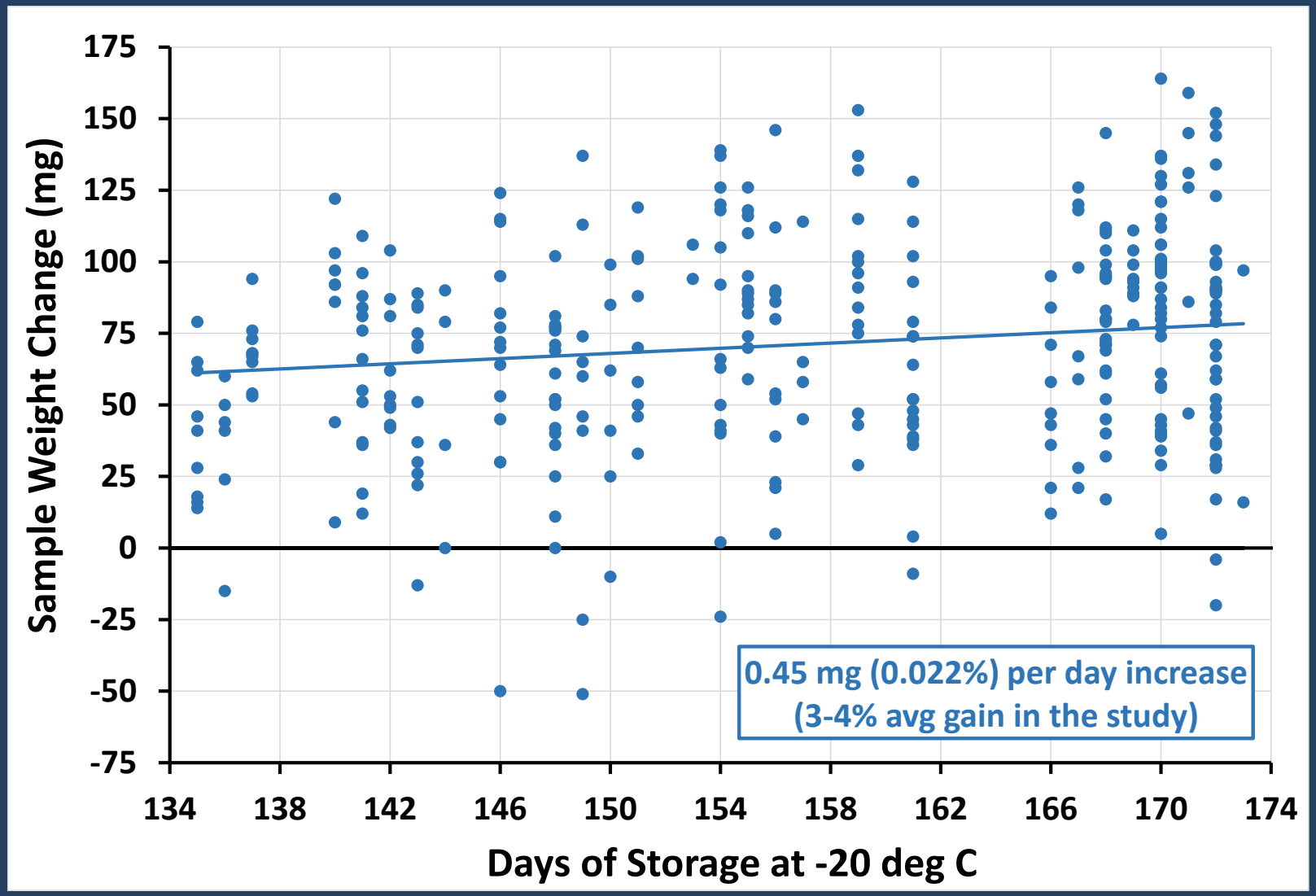
Stored tubes at -20˚C in freezer for 6-8 weeks, then on Dec. 13, thawed, uncapped, spiked, capped, vortexed, and stored in freezer

FEDERAL GOVERNMENT SHUTDOWN DEC. 22, 2018 – JAN. 25, 2019

Analyzed samples Mar. 12 – Apr. 20, 2019 (34 x 3 days per method) **but only conducted one day of SOSPE due to low throughput**

Samples Gained a Bit of Weight in the Freezer

No change in weight for matrix blanks stored < 14 days



Extraction Steps

ARS Method: 2 g sample + 10 mL 4/1 (v/v) MeCN/water
Shake for 5 min, Centrifuge for 3 min

FDA Method: 2 g sample + 8 mL MeCN w/ 2% HOAc +
0.2% *p*-toluenesulfonic acid monohydrate;
Shake for 30 min, Centrifuge for 3 min at 4˚C

EMR Method: 2 g sample + 2 mL 0.1 M EDTA Sol'n; Shake 2 min;
Centrifuge 3 min at 4˚C; Decant extract; To pellet, add 8 mL
cold MeCN w/ 2% HO_2CH + 2% DMSO; Shake 5 min,
Centrifuge for 3 min at 4˚C; Decant into initial extract

SOSPE Method: 2 g sample + 5 mL Sol'n w/ 0.86% oxalic acid +
0.74% EDTA + NH_4OAc to yield pH = 3 + 10 mL MeCN;
Shake 1 min; Centrifuge 3 min; Decant into 1 g $(NH_4)_2SO_4$;
Shake 2 min; Centrifuge 3 min; Transfer extract to a tube

Aminoglycoside Extraction Steps (or not)

(Official FDA/USDA Method)

To centrifugal pellets in each method,
add 10 mL of 10 mM NH_4OAc + 0.4 mM EDTA + 2% $HO_2C_2Cl_3$ + 0.5% NaCl; Shake 10 min; Centrifuge for 3 min;

Transfer 5 mL to a tube; Add 90-120 µL 15% NaOH Sol'n, then adjust with 0.2 M HCl/NaOH to pH 7.5±0.1 w/ pH meter

Add 0.25 g cation exchange sorbent to the tubes; Shake 1 min; Centrifuge for 3 min; Aspirate supernatant to waste

Add 1 mL 10% HO_2CH to tubes; Shake 1 min; Centrifuge for 3 min

Transfer 71 µL (71 mg equiv. sample) to combined final extracts

Cleanup and Final Steps

ARS Method: Transfer 407 µL + 71 µL AMGs extracts (71 mg each) + 146 µL 274 mM 1-heptanesulfonate + water = 0.8 mL; centrifuge at 12,000 rcf at 4˚C for 5 min; transfer 500 µL to AS vial for UHPLC-MS/MS injection of 4 µL (0.35 mg equiv)

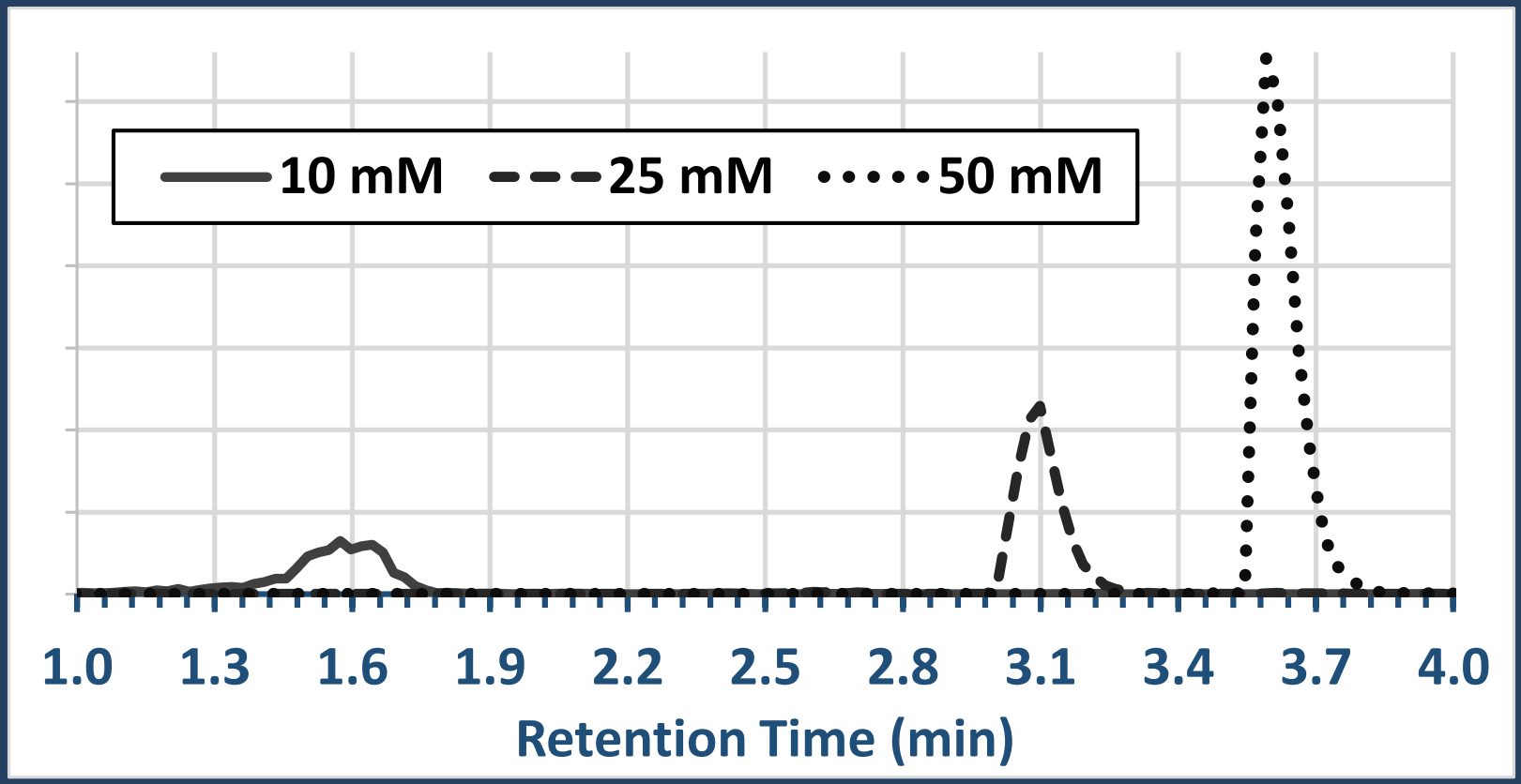
FDA Method: Transfer 3 mL extracts to 200 mg Oasis-Prime HLB cartridges; Elute via gravity, then apply pressure to elute all; Transfer 338 µL (71 mg) to vials and final steps as above

EMR Method: Transfer 5 mL extracts to 600 mg Captiva EMR-L cartridges; Elute via gravity; Add 1.25 mL 4/1 MeCN/water; Apply vacuum; Transfer 510 µL (71 mg) to vials, *etc.*

SOSPE Method: Pretreat 225 mg Oasis HLB cartridges; Add water layer extract to waste + 2 mL extraction solvent to waste; then collect 15 mL extracts at 1 mL/min + 4 mL MeOH; Transfer 533 µL (71 mg) to vials, *etc.*

Sodium 1-Heptanesulfonate in Final Extract

UHPLC of apramycin and amount of IP agent



Parameters Tracked and Evaluated

- Reagent-Only (RO) & Matrix-Matched (MM) stds @ 0, ¼, ½, 1, 2X
- High ROs averaged to set 3 ref. Ion Ratios & t_R (and SDs) for Ident.
- QC Comminution Spike and 5 Internal Stds, also evaluated
- MMs used for calculation of analyte concentrations, and setting of 0.1X cut-off reporting level for identifications
- Matrix Effects are %Diff in slopes of MM vs. RO calibrations
- S/N and peak widths (S/N used to **estimate** LOQ)
- Spiked samples at 1X in triplicate (Recovery and RSD)
- Recoveries and RSDs of 72 samples at ¼X, ½X and 1X
- Rates of False Positives and Negatives at 0 and each level
- Results for all 3 ions per analyte per Int. Std. were processed

Comparison of Results for Lincomycin

Group A, 1X = 100 g/g

Method	t_R (min)	Cal. R^2	Matrix Effect	Fresh 1X Spk %Recov. (%RSD)	Stored Samples %Recov. (%RSD)	False Pos.	False Neg.
ARS	3.63	0.998	13%	100 (4)	102 (16)	0%	0%
FDA	3.66	0.99	18%	88 (3)	86 (15)	0%	0%
EMR	3.72	0.99	12%	78 (6)	77 (17)	0%	0%
SOSPE	3.78	0.995	0%	83 (5)	12 (19)	0%	52%

EXAMPLE OF HOW SINGLE LAB FRESHLY-SPIKED MATRIX VALIDATION CAN BE DECEIVING

(and how methods designed for one matrix may not transfer into another)

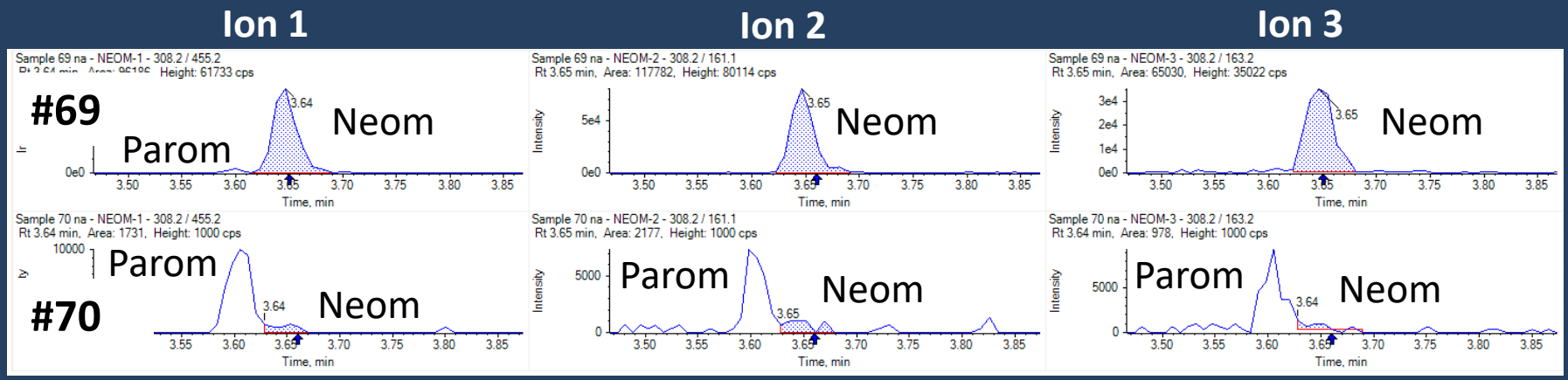
Analytes with Issues in the Study

- Chloramphenicol, 1X = 0.6 ng/g was too low for the analysis
- Crystal Violet (1X = 2 ng/g), found in Reagent Blanks
- Oxytetracycline (and Chlortetracycline?) incurred in fish?
- Doxycyline confused with Tetracycline, Dicloxacillin Δt_R non-ARS
- Azamethiphos, 1X = 20 ng/g – False Negatives > 70%
- Colistin A and B (sum), 1X = 150 ng/g – False Negatives > 70%
- *p*-Toluenesulfonamide (Chloramine-T), 1X = 900 ng/g
  gave only 2 ion transitions with high ion ratio variability
- Trichlorfon, 1X = 10 ng/g – degrades to dichlorvos
- Paromomycin and Neomycin conversions
  See: Lehotay et al., *J. Chromatogr. A* **1313** (2013) 103-112

Comparison of Results for Aminoglycosides

% False Positive / % False Negative

Analyte	0.1X (ng/g)	ARS	FDA	EMR	SOSPE (n=34)	w/o AMG Steps ARS (n=21)	w/o AMG Steps FDA (n=21)
Gentamicin (C_2+C_{2a})	5	16/17	3/11	0/44	8/90	0/91	0/91
Neomycin	50	60/3	80/64	72/79	33/76	0/84	0/100
Paromomycin	50	16/0	38/0	42/0	8/30	0/91	0/100
Spectinomycin	30	0/7	0/17	0/31	0/52	0/27	0/45
“ ”-Hydrate	“ ”	13/28	10/52	13/42	31/71	0/63	0/27



Overall Results (False Positives)

No. of Analytes out of 70-71 included in assessment

Analyte (0.1X Level, ng/g)	ARS (n = 31)	FDA (n = 30)	EMR (n = 29)	SOSPE (n = 9)
False Positives ≤ 10%	**68**	60	65	61
Thiamphenicol (**5**)	**20%**	3%	7%	**11%**
Chlortetracycline (**20**)	**20%**	**27%**	**41%**	**44%**
Oxytetracycline (**20**)	10%	**20%**	**66%**	**33%**
Gentamicin (**5**)	**16%**	3%	**0%**	8%
Amoxacillin (**5**)	**0%**	**24%**	**23%**	**15%**
Crystal Violet Leuco (**0.2**)	3%	**27%**	10%	**0%**
Malachite Green Leuco (**0.2**)	**0%**	**13%**	**14%**	**0%**
Abamectin (**10**)	**0%**	**20%**	**0%**	**0%**
Cloxacillin (**30**)	**0%**	**14%**	**0%**	**15%**
Ciprofloxacin (**10**)	**0%**	**17%**	**0%**	**0%**
Zeranol (**1**)	10%	**23%**	**14%**	**0%**

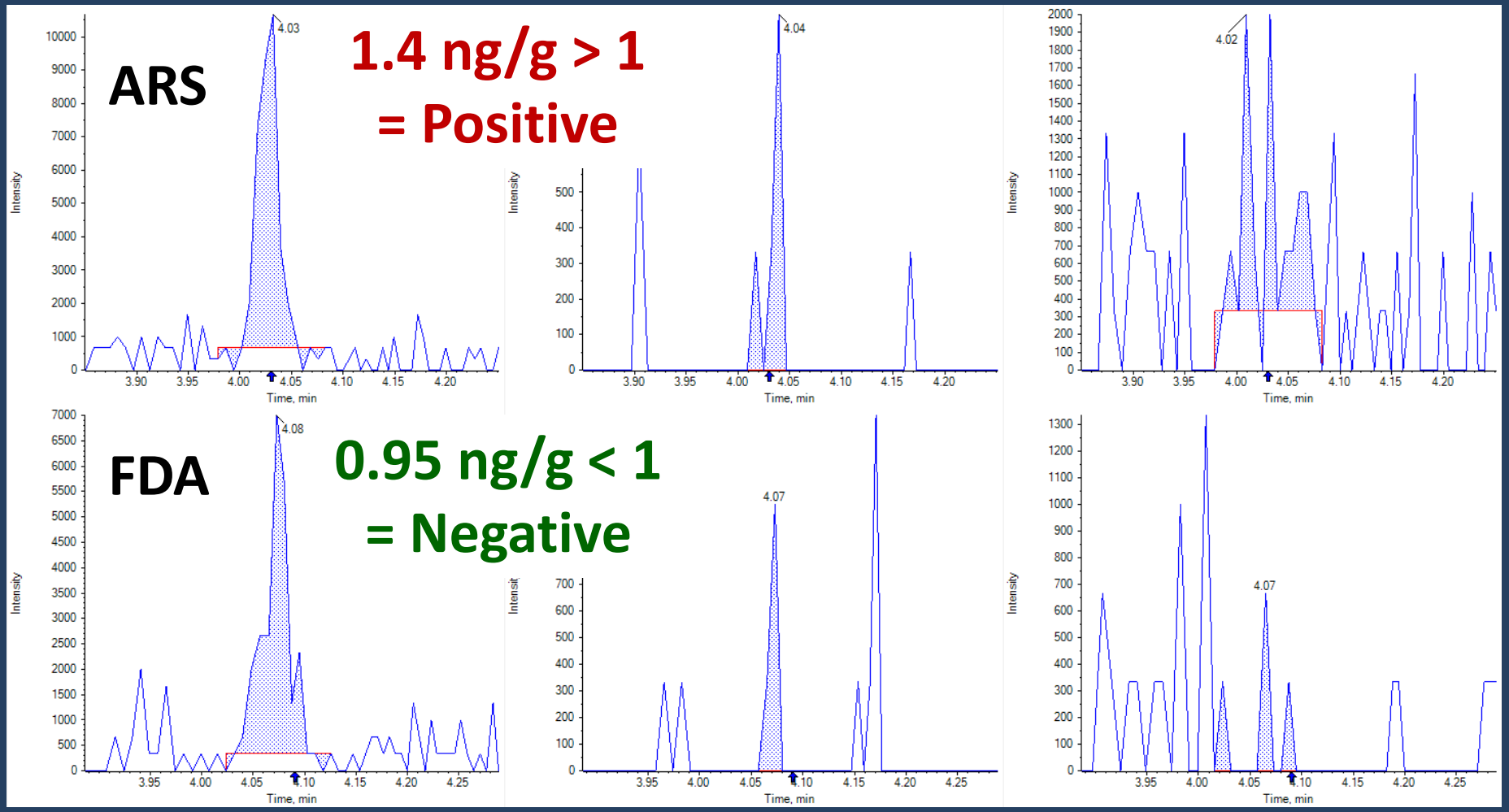
Overall Results (False Negatives at ½X)

No. of Analytes out of 70-71 included in assessment

Analyte (0.1X Level, ng/g)	ARS (n = 30)	FDA (n = 30)	EMR (n = 30)	SOSPE (n = 8)
False Negatives ≤ 10%	**56**	53	**56**	51
Teflubenzuron (**30**)	**50%**	**23%**	**27%**	**0%**
Tricaine methanesulfonate (**1**)	**43%**	**43%**	**50%**	**100%**
Thiamphenicol (**5**)	**27%**	**27%**	**23%**	**11%**
Sulfanilamide (**10**)	**30%**	**23%**	**0%**	**11%**
Brilliant Green(**0.2**)	7%	**0%**	**100%**	**0%**
Malachite Green (**0.2**)	**100%**	**0%**	**33%**	**0%**
Malachite Green Leuco (**0.2**)	**40%**	**13%**	10%	**0%**
Crystal Violet Leuco (**0.2**)	**37%**	**33%**	10%	**0%**
Dapsone (**0.5**)	7%	**23%**	3%	**0%**
Dimetridazole (**1**)	**0%**	3%	**0%**	**33%**
Diminazine (**50**)	**63%**	7%	**0%**	**100%**

Example of Sarafloxacin

0.1X = 1 ng/g – Tilapia #72

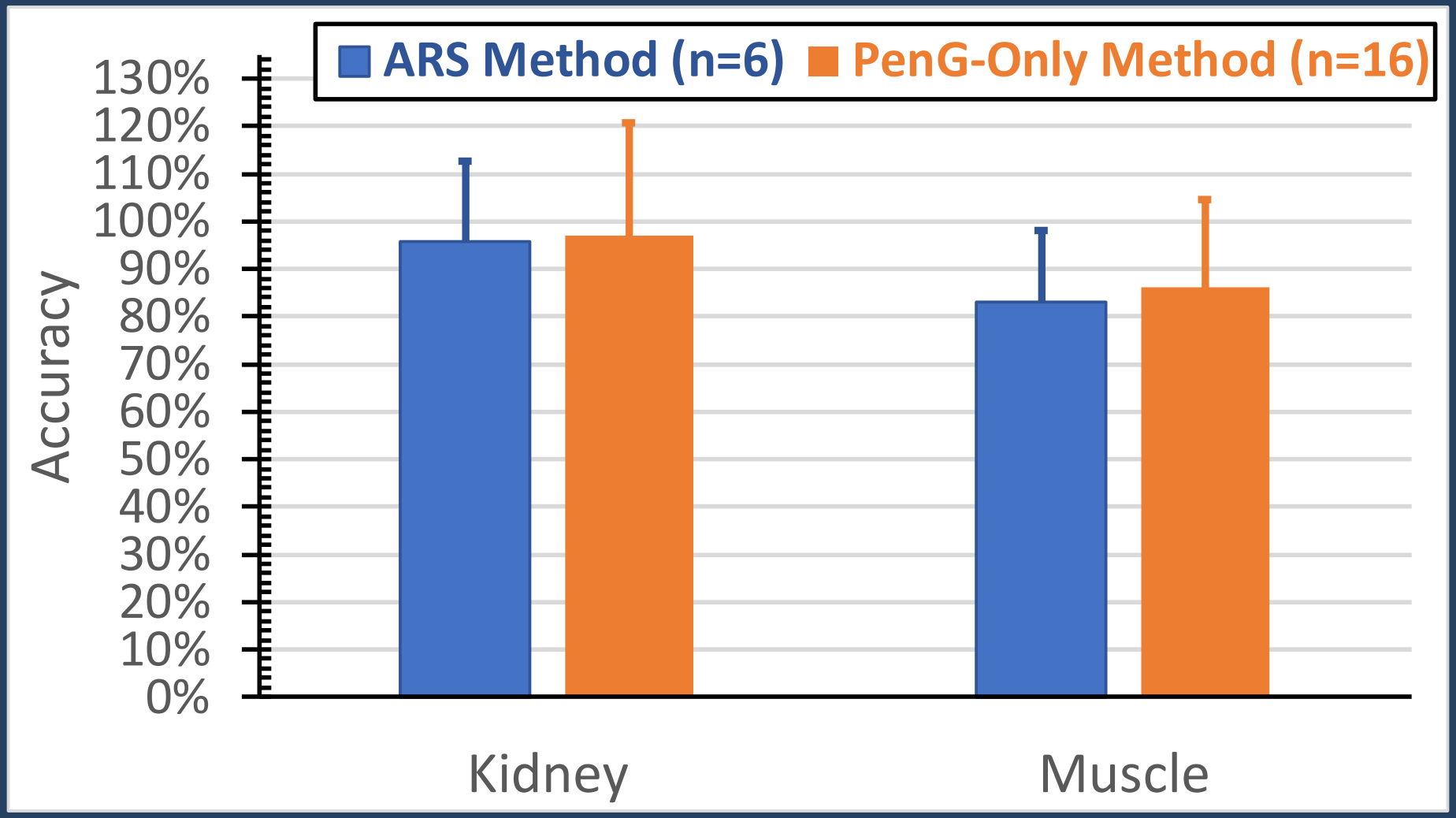


Comparison of Results for β-Lactams

% False Positive / % False Negative

Analyte	0.1X (ng/g)	ARS	FDA	EMR	SOSPE (n=34)	w/o AMG Steps: ARS (n=21)	w/o AMG Steps: FDA (n=21)
Amoxicillin	5	0/79	24/61	23/56	15/95	0/45	0/91
Ampicillin	5	7/14	10/60	3/66	11/80	0/38	0/69
Cloxacillin	30	0/0	14/0	3/1	15/0	0/0	20/0
Dicloxacillin	30	0/0	oops?	oops?	oops?	0/0	oops?
Oxacillin	30	0/0	7/0	3/6	8/14	0/0	0/0
Penicillin G	5	13/42	3/82	10/41	11/4	0/15	0/23
“ ” metabolite	“”	0/6	0/14	0/4	0/32	0/30	0/100

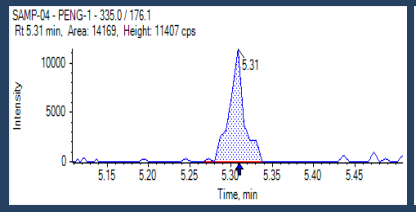
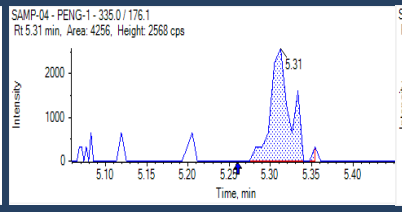
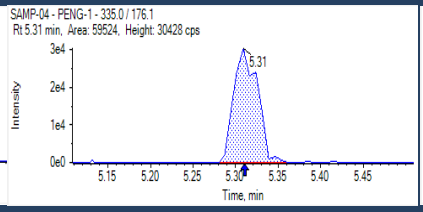
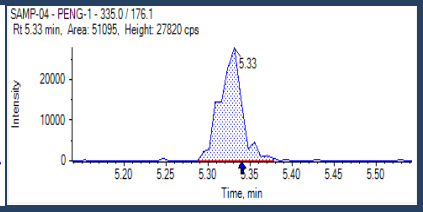
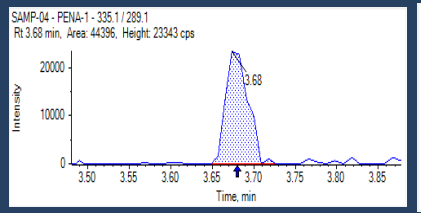
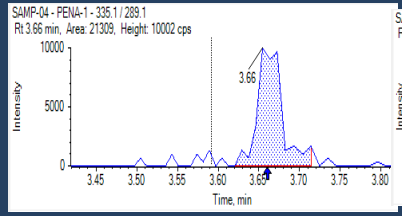
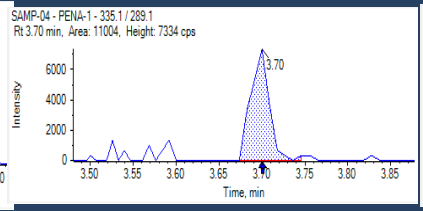
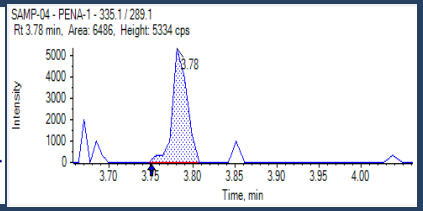
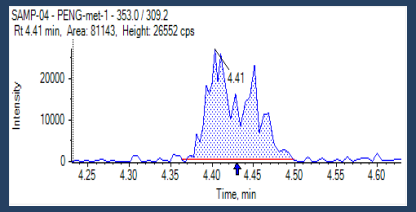
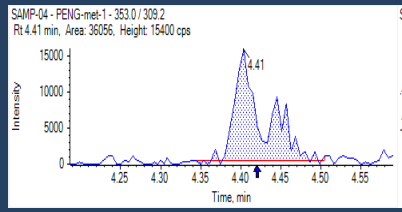
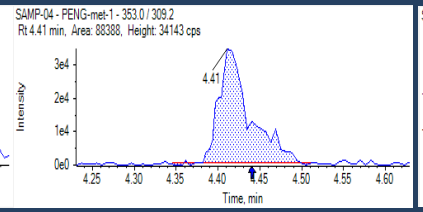
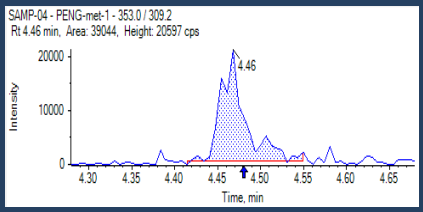
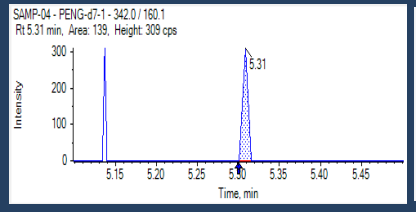
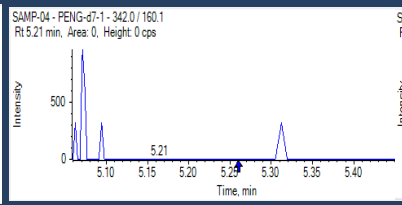
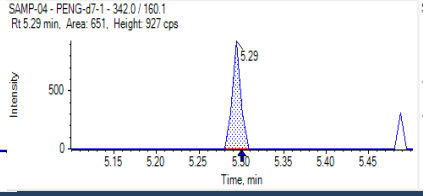
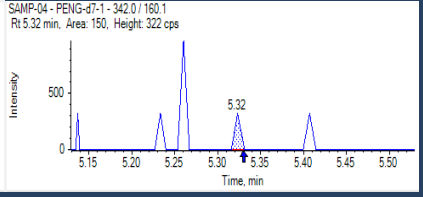
Analysis of Penicillin G in Prof. Test Samples



Freeze-dried PT samples analyzed among 4 labs monthly with blind conc. 20-200 ng/g (so far)

Analysis of Penicillin G and Metabolites

Sample #4 (50 ng/g Pen G added)

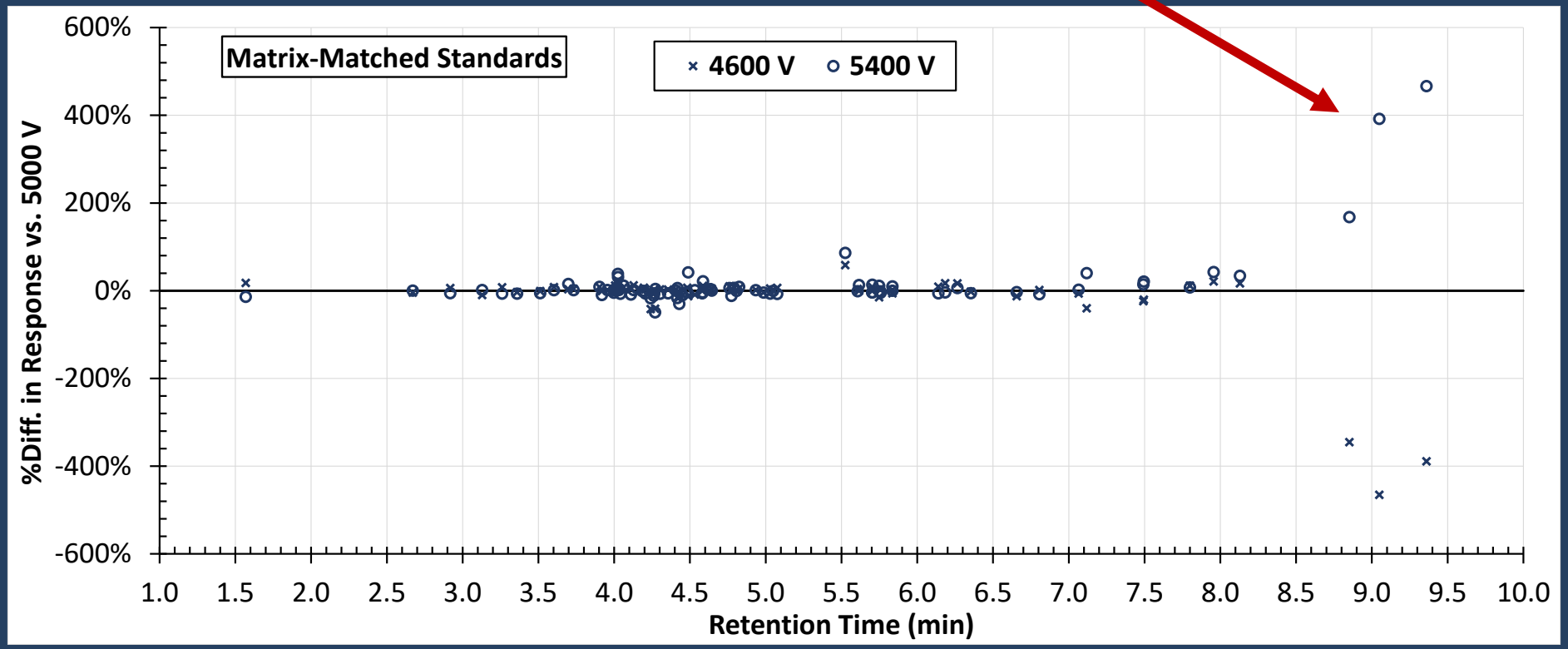
	ARS	FDA	EMR	SOSPE
Pen G				
Penillic Acid				
Penilloic Acid				
Pen G-d7				

Comparison of Results for Anthelmintics

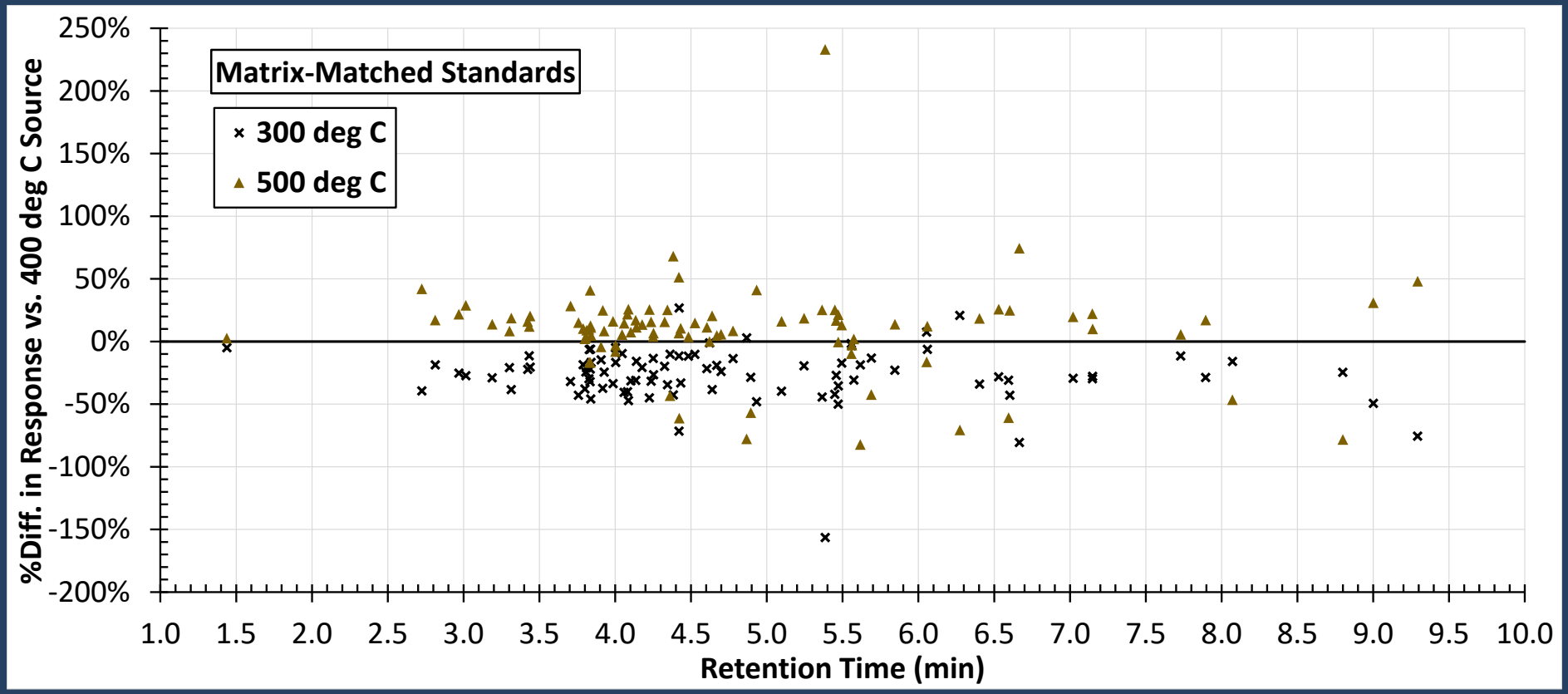
% False Positive / % False Negative

Analyte	0.1X (ng/g)	ARS	FDA	EMR	SOSPE (n=34)	w/o AMG Steps: ARS (n=21)	w/o AMG Steps: FDA (n=21)
Abamectin	10	0/0	20/0	0/0	0/0	0/0	0/8
Doramectin	10	0/1	10/1	0/0	0/0	0/0	0/8
Emamectin	10	0/0	3/0	0/0	0/0	0/0	0/0
Ivermectin	10	0/21	0/0	0/15	0/0	0/6	0/6
Albendazole	10	0/0	0/0	0/0	0/0	0/0	0/0
“ ” Sulfone	10	0/0	0/0	0/0	0/0	0/0	0/0
“ ” Sulfoxide	10	0/0	0/0	0/0	0/0	0/0	0/0
“ ” 2-Aminosulfone	10	0/0	0/0	0/0	0/29	0/0	0/0

Ion Spray Voltage Can Matter A LOT (for Mectins)



Source Temperature Matters Somewhat



Results for Triarylmethane Dyes

% False Positive / % False Negative

Analyte	0.1X (ng/g)	ARS	FDA	EMR	SOSPE (n=34)	w/o AMG Steps: ARS (n=21)	w/o AMG Steps: FDA (n=21)
Brilliant Green	0.2	3/13	10/0	0/94	0/0	0/9	0/0
Crystal Violet - Leuco	0.2	3/32	27/26	10/16	0/8	0/29	0/24
Malachite Green	0.2	0/28	0/0	3/27	0/29	0/73	0/18
“” - Leuco	0.2	0/28	13/11	14/12	0/0	0/8	0/8

Crystal Violet was found in Reagent Blanks, and some False Positives are probably actual positive in the samples

Results for Tetracyclines

% False Positive / % False Negative

Analyte	0.1X (ng/g)	ARS	FDA	EMR	SOSPE (n=34)	w/o AMG Steps: ARS (n=21)	w/o AMG Steps: FDA (n=21)
Chlortetracycline	20	20/0	27/3	41/0	44/0	0/0	25/0
Doxycycline	1	oops?	oops?	oops?	oops?	oops?	oops?
Oxytetracycline	20	10/0	20/0	66/0	33/0	0/0	0/0
Tetracycline	20	0/0	0/7	0/0	0/0	0/0	0/0

Some samples may contain incurred drugs, which affects calibration, too

Some small differences for the following among the 4 methods, but all mostly meet 90% true qualitative result criteria

Quinolone (8+): (Cipro)(Dano)(Di)(Enro)(Sara)floxacin, Flumaquine, (Nalidixic)(Oxolinic) Acid

Macrolide (7): (Erythro)(Kitasa)(Linco)(Neo+Spira)mycin, (Tilmico)(Tylo)sin

Sulfonamide: Sulfa(chloro)(ethoxy)(methoxy)pyridazine, Sulfanitran, Sulfa(methi)(thia)(methoxa)zole, Sulfa(clo)(dia)(mera)(metha)zine, Sulfa(dimeth)(dox)(monometh)oxine, Sulfapyridine, Sulfaquinoxaline, Sulfisoxazole

Other: Carprofen, Thiamphenicol, Florfenicol + Amine, Dapsone, (Dimetr)(Metron)idazole, Diminazine, (Hexaflum)(Lufen)uron, (Ormeto)(Trimetho)prim