What is more than QuEChERS? The QuEChERSER mega-method for the analysis of pesticides, veterinary drugs, and environmental contaminants

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Disclaimer

Mention of brand or firm name does not constitute an endorsement by the USDA above others of a similar nature not mentioned.

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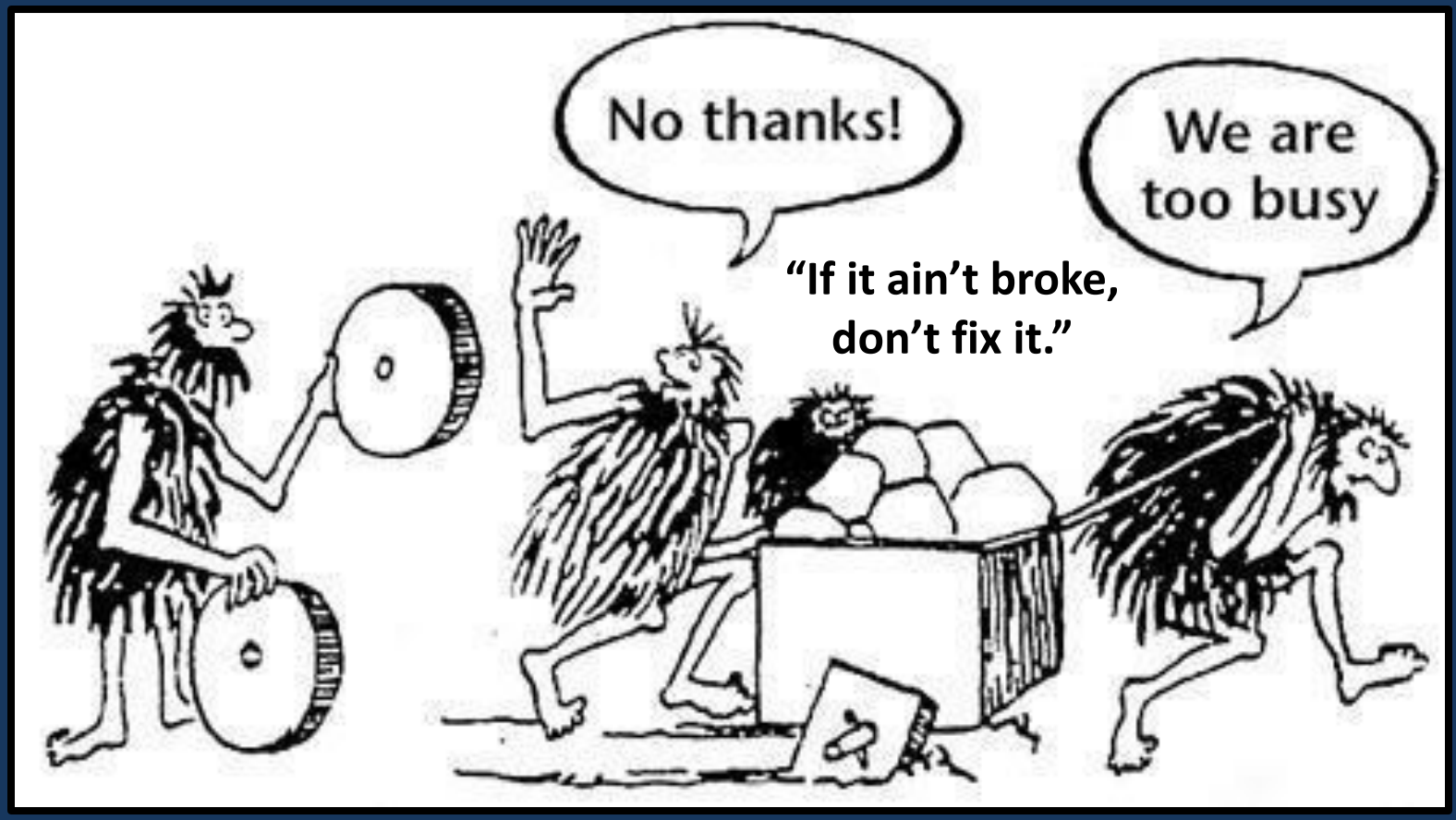
Conclusions

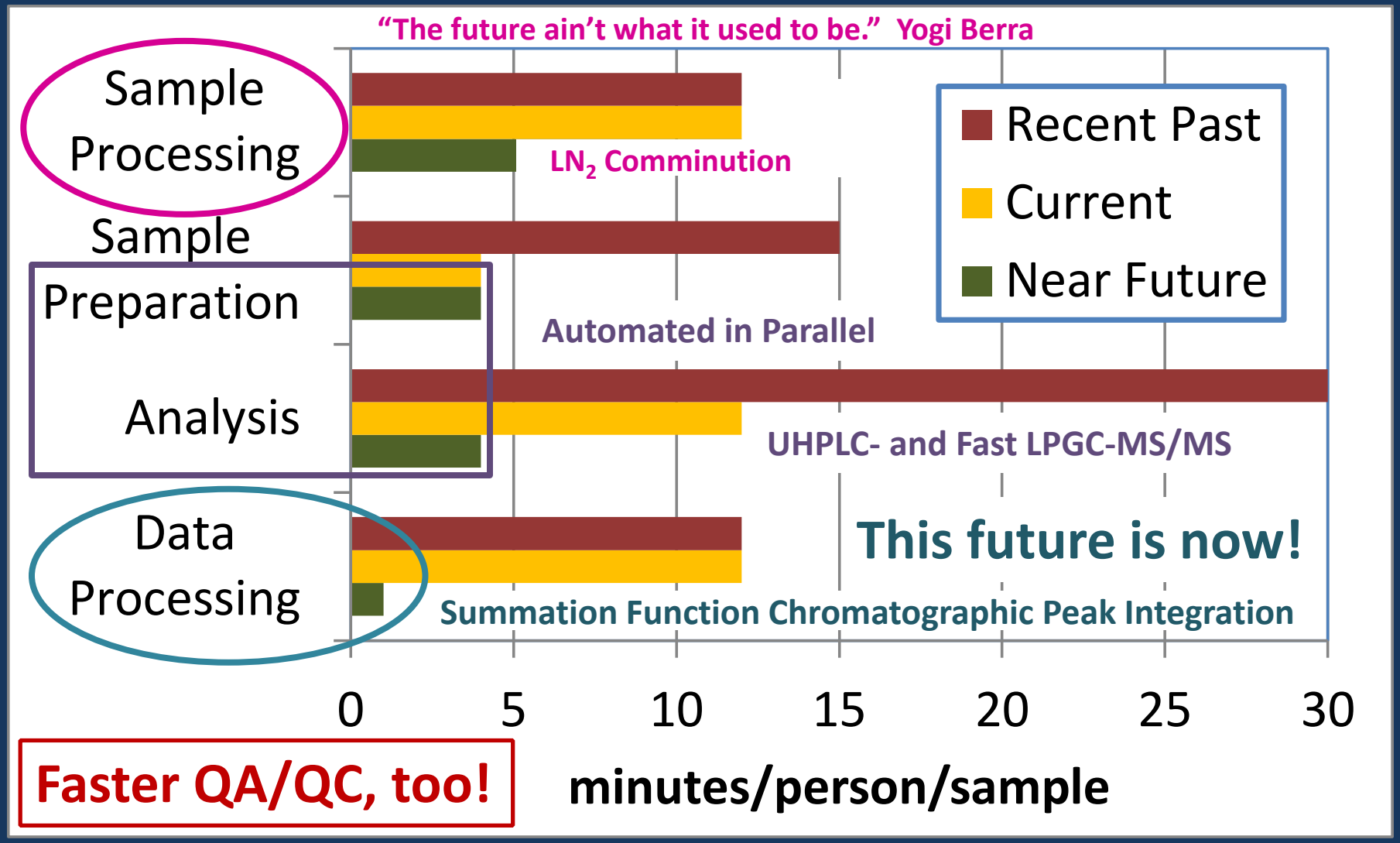
1) Advantages abound in the QuEChERSER mega-method.
2) Reliable high-quality results can be achieved from **start-to-finish** for **hundreds** of targeted ultratrace multi-application contaminants in diverse foods using **semi-automated high-throughput** analysis by the **QuEChERSER mega-method** with back-flushing UHPLC-MS/MS + ITSP+LPGC-MS/MS in parallel followed by summation function peak integration and post-run processing to yield accurate and trustworthy **quantidentifications** with little need for human review.
3) Measurement uncertainty for each step and overall can be easily determined in every batch, too.

The Status Quo is to be Questioned

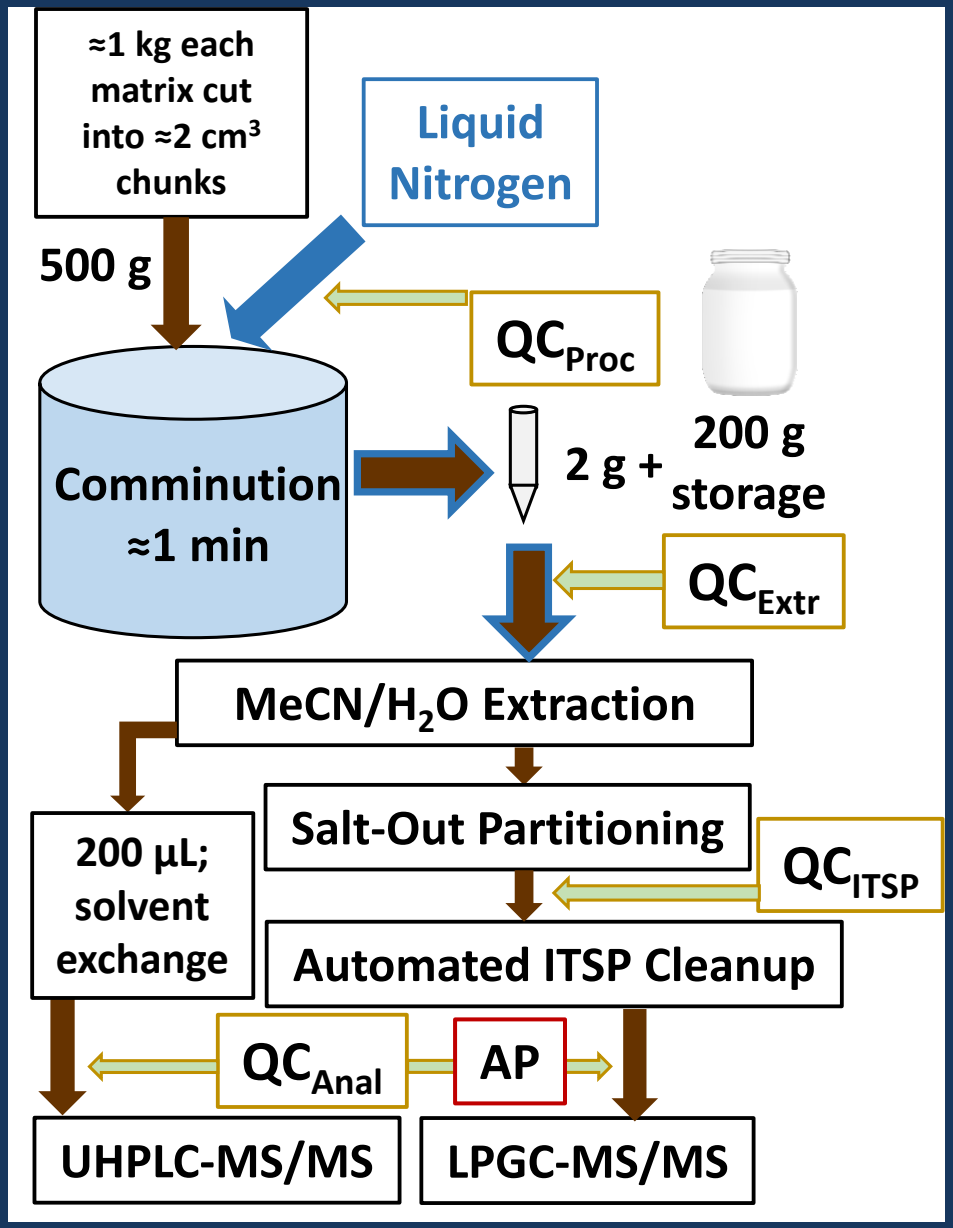
"The problem in this business isn't to keep people from stealing your ideas; it's making them steal your ideas!"
Howard Aiken



Sample Throughput to Analyze Chemical Residues → QuEChERSER + UHPLC- & ITSP+LPGC- MS(/MS)



High-Throughput QuEChERSER Approach



Addition of Quality Control Spikes

1) QC analyte(s) spiked during sample comminution –

   e.g., chlorpyrifos-methyl?

2) Int. stds added prior to extraction –

   e.g., atrazine-d_5, malathion-d_{10}, pyridaben-d_{13},

   $^{13}C_{12}$-p,p'-DDE, $^{13}C_{12}$-PCB 153?, PBDEs?, PAHs?

   $^{13}C_6$-sulfamethazine, flunixin-d_3, ractopamine-d_3,

   clenbuterol-d_9, phenylbutazone-d_{10}, pen G-d_7

3) QC_{ITSP} added before ITSP – *e.g.,* fenthion-d_6

4) *p*-terphenyl-d_{14} for GC and $^{13}C_1$-phenacetin for LC added to final extracts before analysis

Assessment of Quality Control Spikes

For this purpose,

CV = calculated; RSD = measured

For LC: $CV^2_{Overall} = CV^2_{Anal} + CV^2_{Extr} + CV^2_{Proc}$

$CV_{Overall} = RSD_{Overall} = RSD_{Proc}$; $RSD_{Anal} = CV_{Anal}$

$CV^2_{Extr} = RSD^2_{Extr} - RSD^2_{Anal}$

$CV_{Proc} = \sqrt{(RSD^2_{Proc} - RSD^2_{Extr})}$

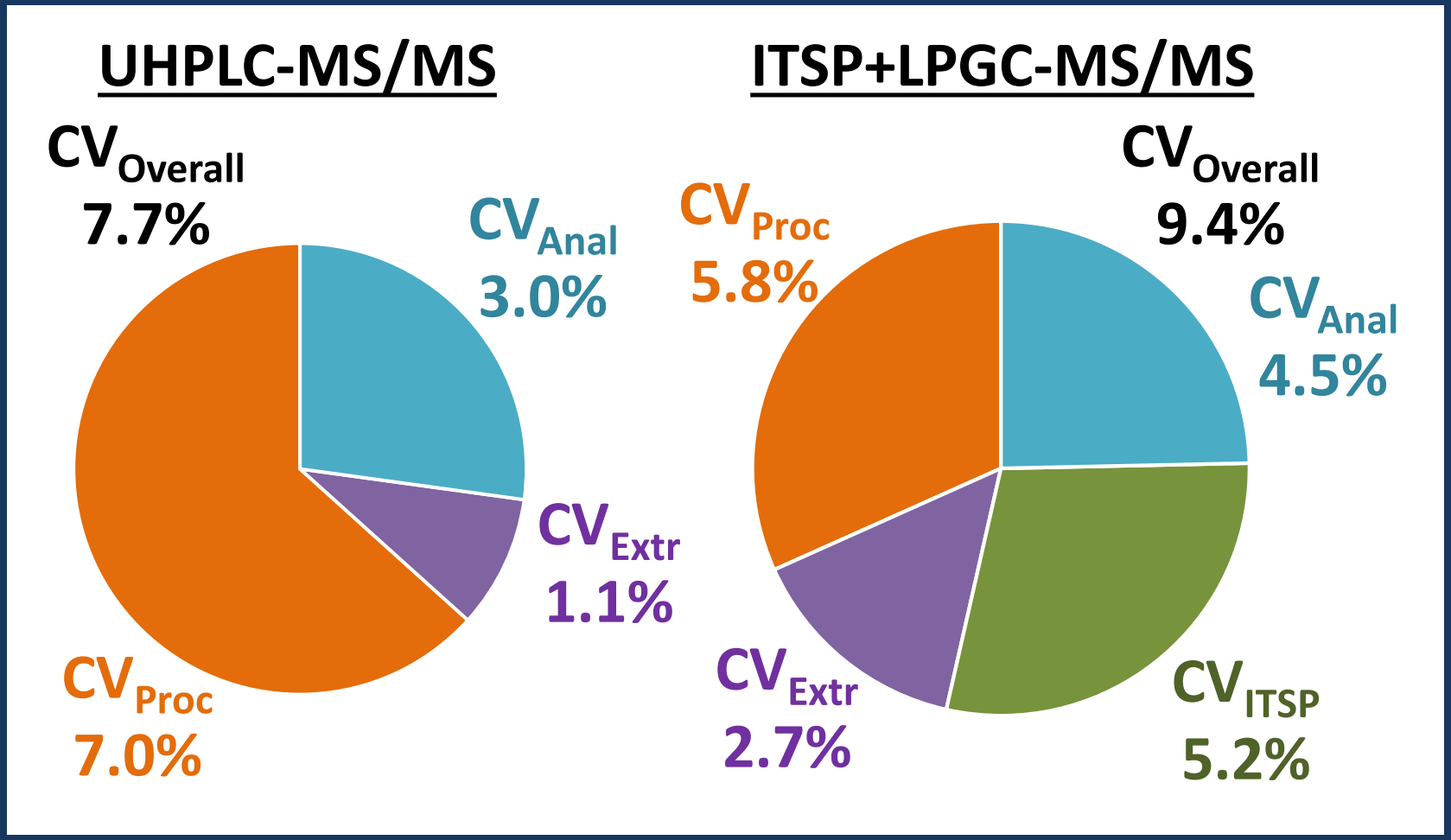
Pinpointing a Method's Weak Link

Calculated CV of each step from labs in a study, n ≈ 21

Lab	Anal	Extr	Proc	Overall
Avg	7%	8%	10%	15%
Best	3%	6%	6%	9%
A	**19%**	*i*	**21%**	28%
B	11%	**38%**	**41%**	**57%**
C	**20%**	**47%**	15%	**51%**
D	7%	**16%**	10%	20%
E	9%	14%	**20%**	26%

QuEChERSER with LN_2 Comminution

0.25 – 15 g Test Portions!



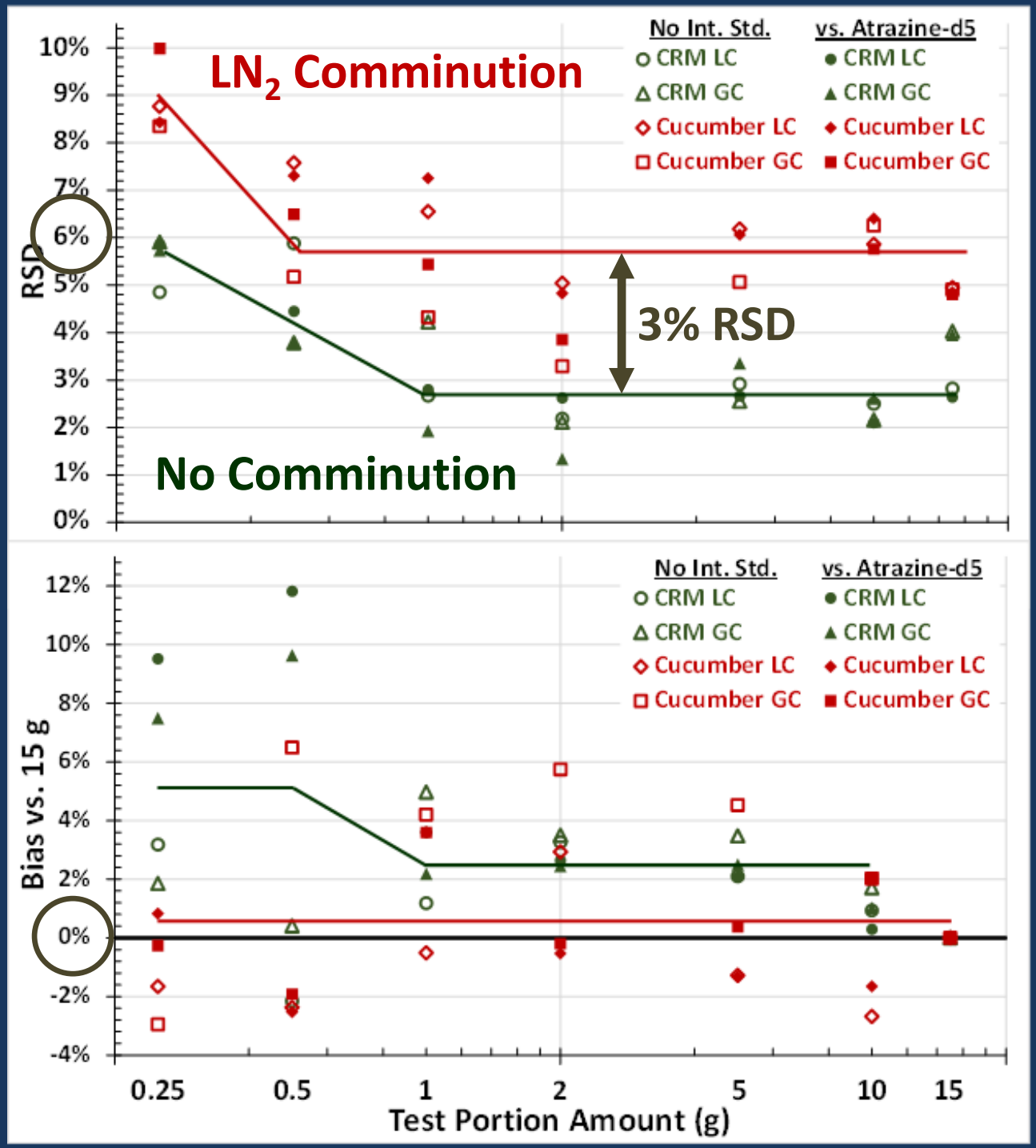
See: Lehotay et al., *J. Agric. Food Chem.* **68** (2020) 1468-1479

Comminution using Liquid Nitrogen (LN_2)

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

Comminution using LN_2

Picture from Manol Roussev



LN_2 Comminution

Accuracy vs. Test Portion Size (g)

No Effect on Precision down to 0.5-1 g

No effect on Bias down to 0.25-0.5 g

See: Lehotay et al., *J. Agric. Food Chem.* **68** (2020) 1468-1479

Benefits and Implications of LN_2 Comminution

REDUCING THE SAMPLE TEST PORTION SIZE *VIA* SAFE, CONVENIENT, INEXPENSIVE, AND HIGH-THROUGHPUT USE OF STANDARD CHOPPERS WITH LN_2 LEADS TO KEY BENEFITS:

- ✓ **2 g test portions are consistently representative of the original collected bulk sample cut into ≈4 cm^3 chunks WITHOUT PRE-FREEZING for comminution in a SINGLE STEP < 5 MINUTES!**
- ✓ **Volatile pesticides are not vaporized, and degradation is halted.**
- ✓ **Weighing of frozen powder is easy, and no wait is needed for dry ice to sublime (LN_2 is colder, cheaper, and cleaner than dry ice, and water freezes and sinks, not condenses onto pieces of it to cause weight bias).**
- ✓ **The tinier extracted sample particles and larger volume of extraction solvent per sample leads to better extraction efficiencies and more volume for pipetting in subsequent steps.**
- ✓ **Use of 15 mL tubes vs. 50 mL lowers cost, doubles (at least) batch sizes on shakers and centrifuges, and generates less plastic waste.**

High-Throughput QuEChERSER Mega-Method

1) Cryogenic Sample Comminution (add QC?)

2) 2 g test portions in 15 mL cent. tubes in tray(s) (add IS mix)

make 3? QC spikes at 1X in each batch (add IS mix)

include matrix and reagent blanks for cal stds (no IS)

3) Add 10 mL 4/1 (v/v) MeCN/water

(use dispenser; prepare fresh extraction solvent weekly?)

4) Shake in batch (of up to 100) 10 min, then centrifuge >3700 rcf for 3 min (up to 48 at a time)

QuEChERSER Steps for UHPLC Analysis

5A) Transfer 200 µL extract to 2 mL tube
equivalent to ≈35 mg sample, depending on water content

6A) Evaporate to just dryness at 40°C under N_2 flow
≈5 min needed (remove dry tubes right away)

7A) Add 750 µL initial mobile phase and 50 µL QC/cal stds

8A) Ultracentrifuge for 5 min at ≈13,000 rcf (at 4°C)

9A) Transfer ≈500 µL to polypropylene autosampler vials
and inject 10 µL in UHPLC-MS/MS
(≈0.44 mg sample equivalent injected; increase inj. vol. if needed)

QuEChERSER for ITSP+LPGC-MS/MS Analysis

5B) Decant remaining initial extract into 15 mL cent. tubes containing 2 g 4/1 (w/w) anh. $MgSO_4$/NaCl
(pre-weighed salts available from at least 2 vendors)
- shake briefly by hand to break up chunks of salt

6B) Shake 1 min in tray(s) then centrifuge 3 min as in Step 4

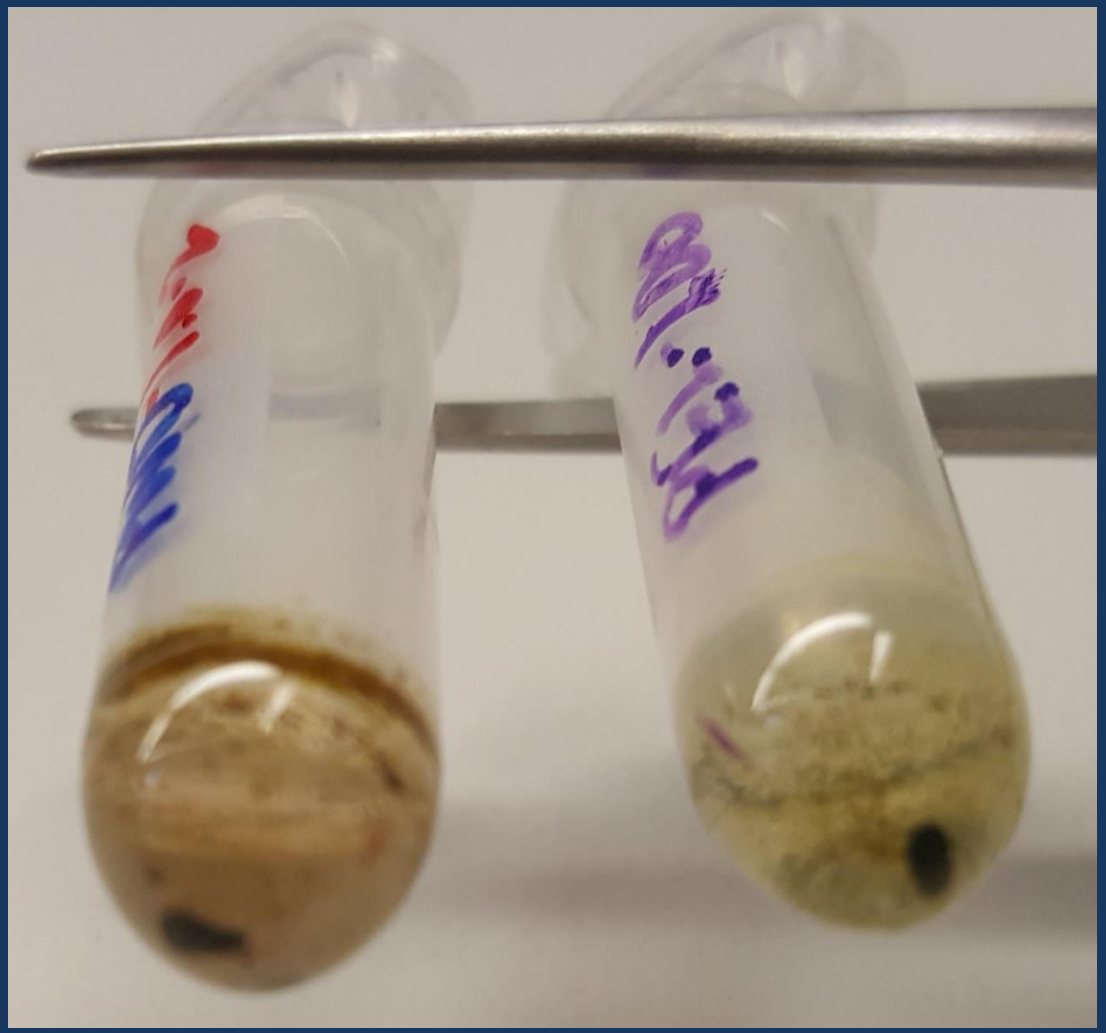
7B) Transfer 1 mL to dark glass autosampler vials (add QC)
- add 50 µL QC/cal in MeCN w/ formic acid to receiving vials

8B) Conduct ITSP+LPGC-MS/MS: inject 3 µL final extract
+ 1 µL in syringe of 1 µg/µL shikimic acid in 9/1 MeCN/water

3A) Evaporation of Small Volumes (≈5 MIN!)

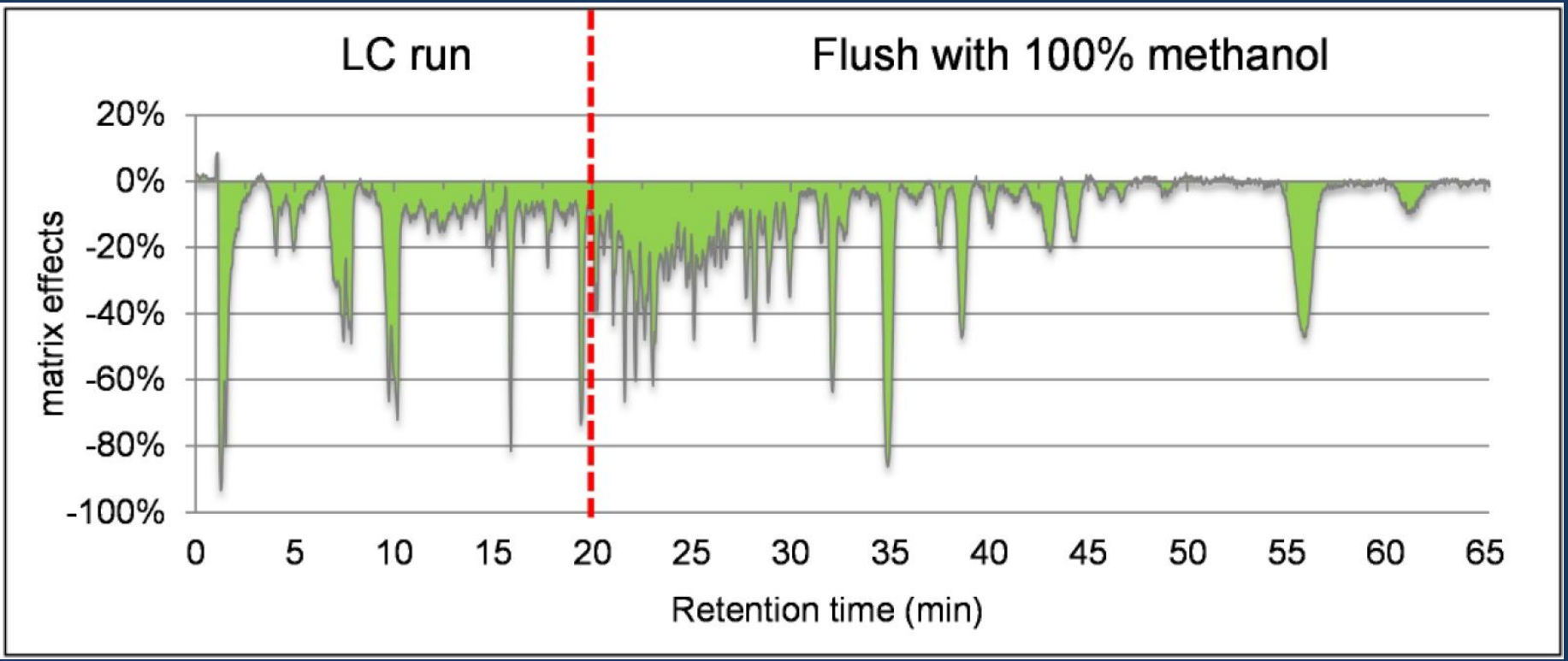
Solvent Exchange and Ultracentrifugation

1) Evaporation of MeCN precipitates nonpolar matrix components.
2) Ultracentrifugation is better than filtration that clogs membranes, adds components, and removes analytes.
3) Final extracts match initial mobile phase to give good peaks for early eluters.



Cleanup of hemp powder and plant extracts prior to UHPLC

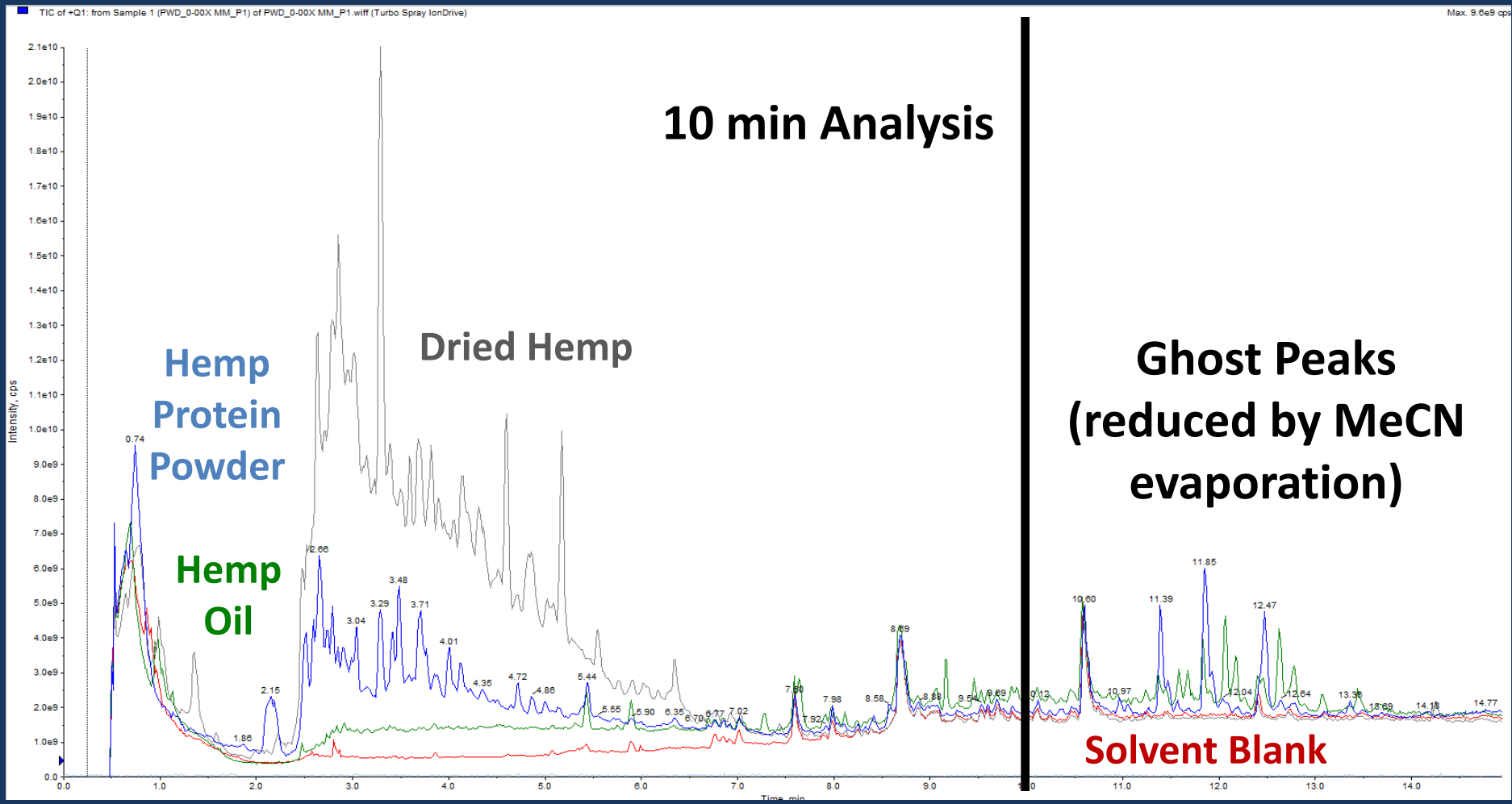
Ghost Peaks and Matrix Effects in LC-MS/MS



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)

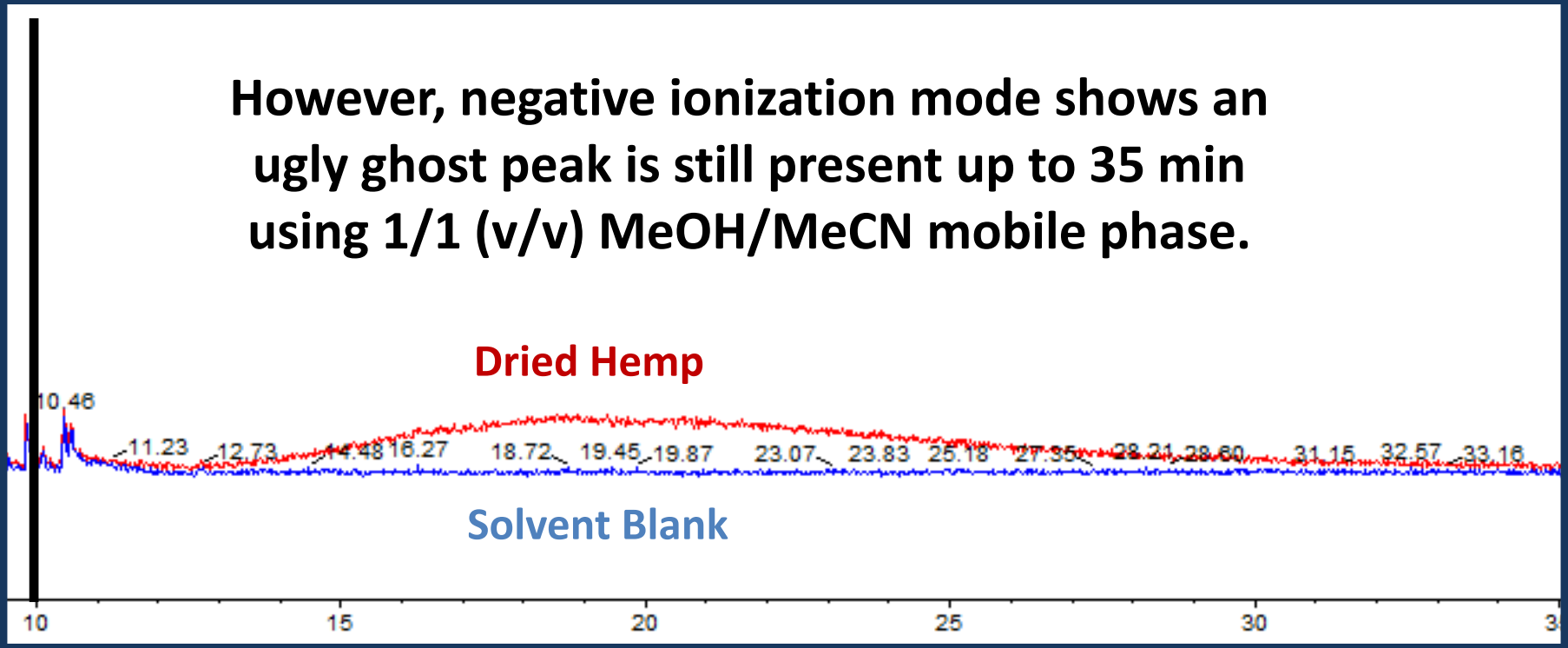
UHPLC-MS ESI^{+} without Backflush of Hemp



2 4 6 8 10 12 14

Retention Time (min)

UHPLC-MS ESI$^-$ without Backflush of Hemp



Backflushing of the analytical column sweeps all matrix components to waste after every injection, leaving a clean column free of ghost peaks in every analysis.

Dual Column Back-Flushing UHPLC

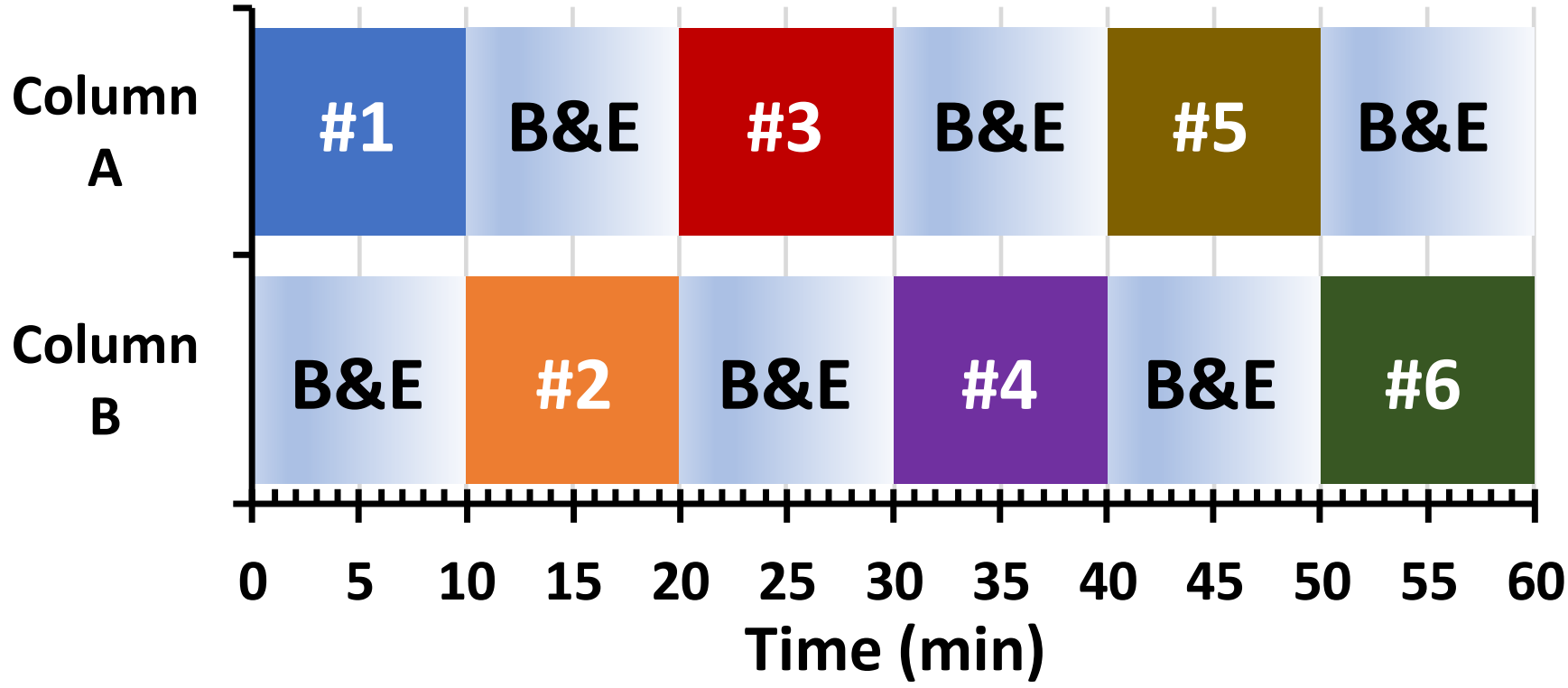
Column B is back-flushed to waste with 1.3 mL total organic mobile phase component as Column A undergoes 10 min gradient for analysis, then *vice versa* in alternate injections.

Retention times and analyte signals were indistinguishable between columns when re-equilibrated 3 min.

Only inject samples or blanks that match the mobile phase buffer/acid or else signals for acid/base analytes will be affected in the next injection.

UHPLC-MS/MS Alternating Column Backflush

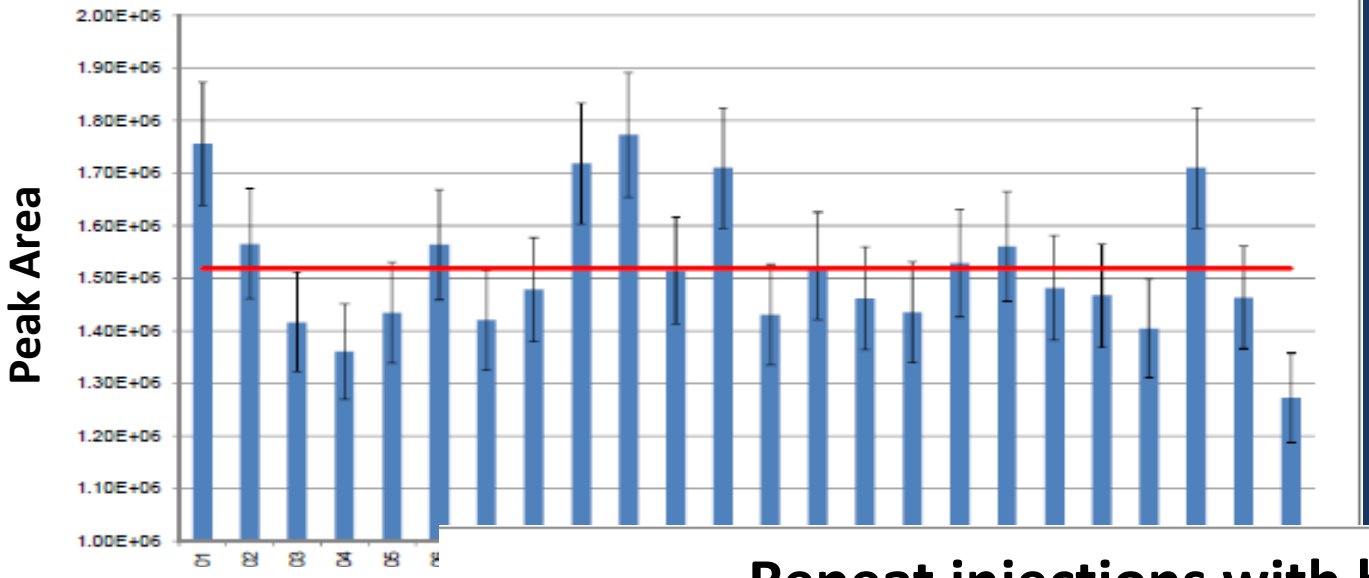
6 Samples Analyzed per hour



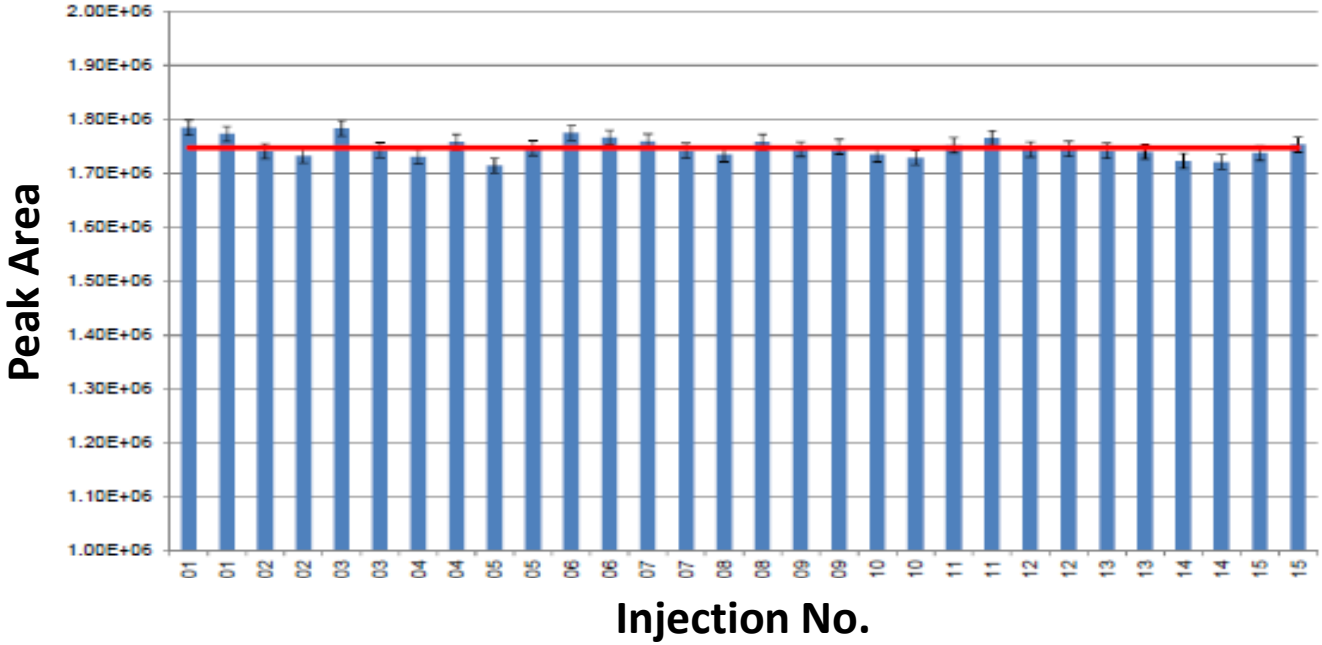
B&E

5 min backflush with organic solvent,
then 5 min equilibration to initial conditions

Repeat injections without back-flush



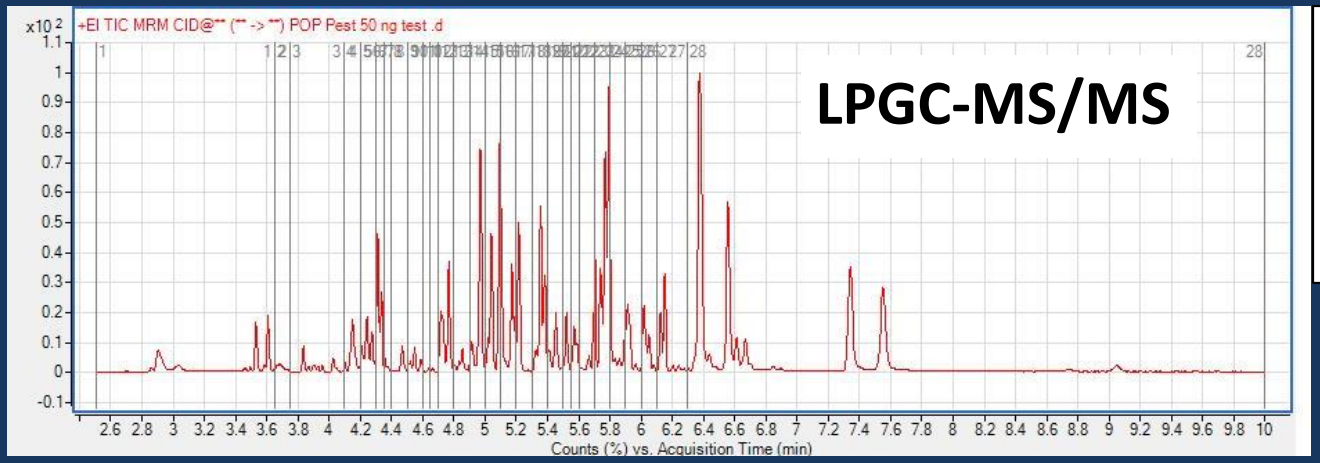
Repeat injections with back-flush



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

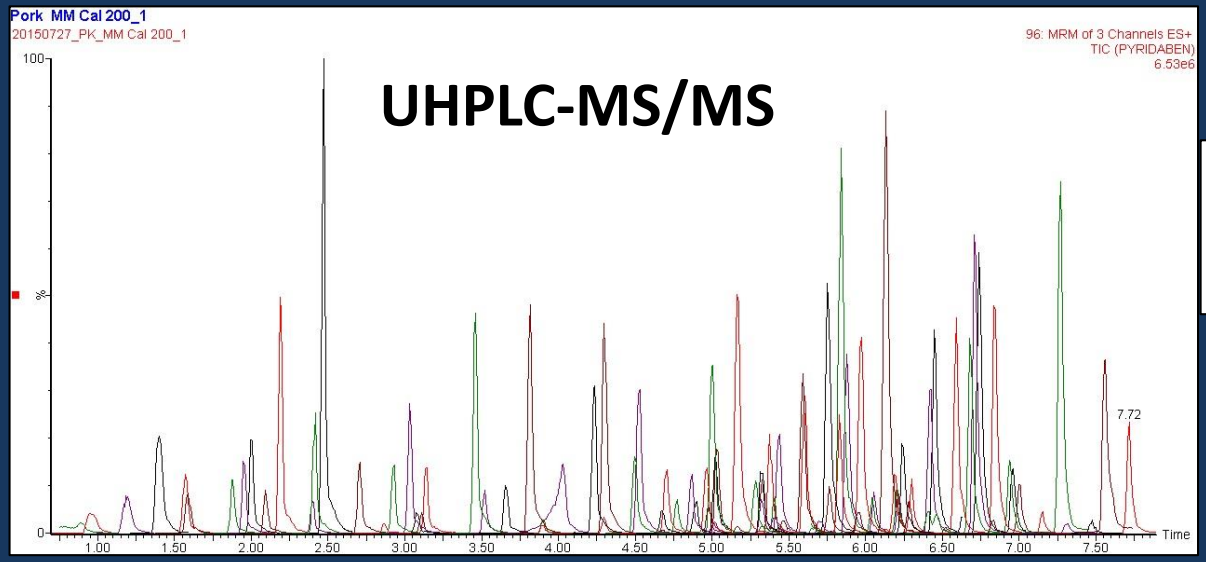
>260 Analytes in Parallel by 10 min Analyses

(>325 including veterinary drugs now in QuEChERSER)



152 pesticides + 65 environmental & other contaminants

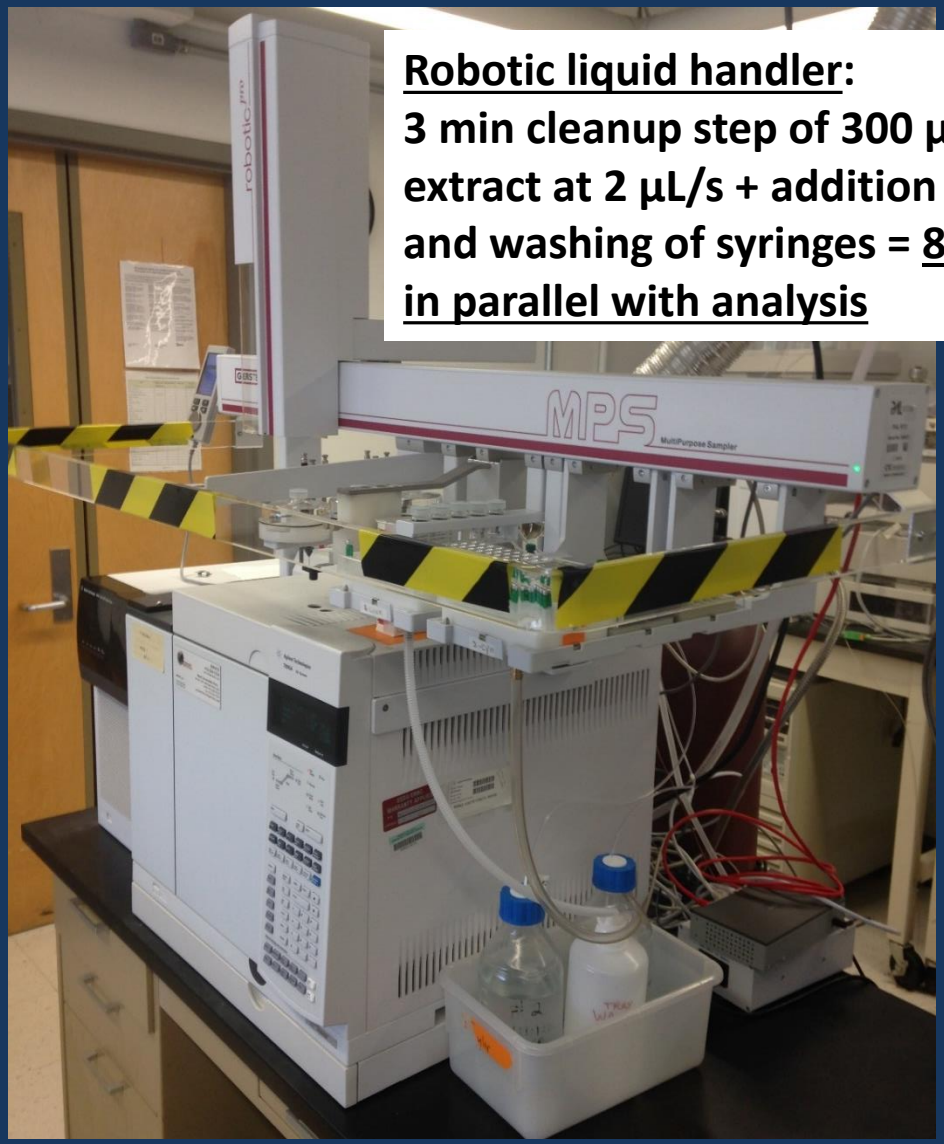
53 overlapping pesticides



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

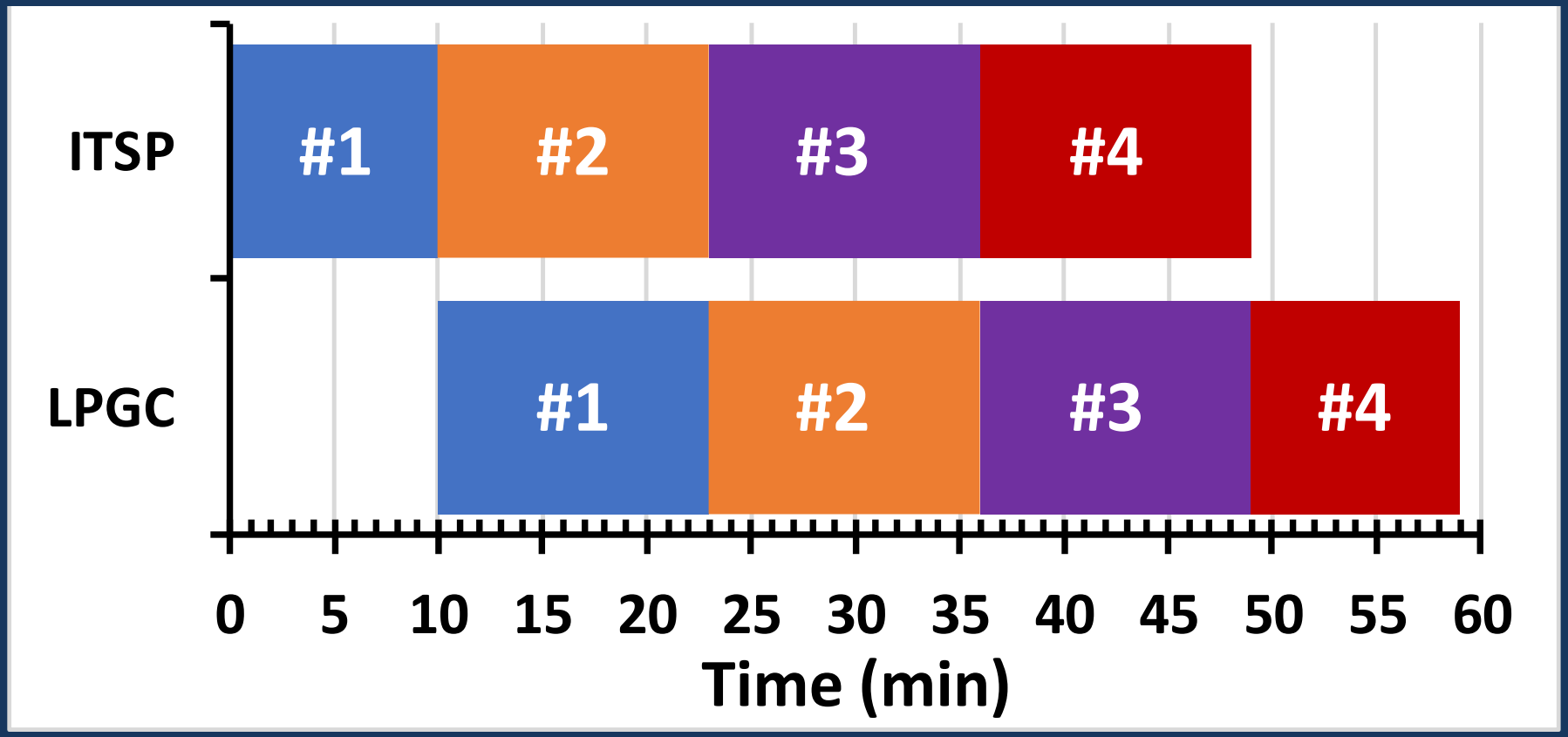
Final extract volumes = 278 ± 5 µL (n = 255) after 25 µL each of APs and (MeCN or Std)

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

Automated µSPE+LPGC-HRMS (Orbitrap)

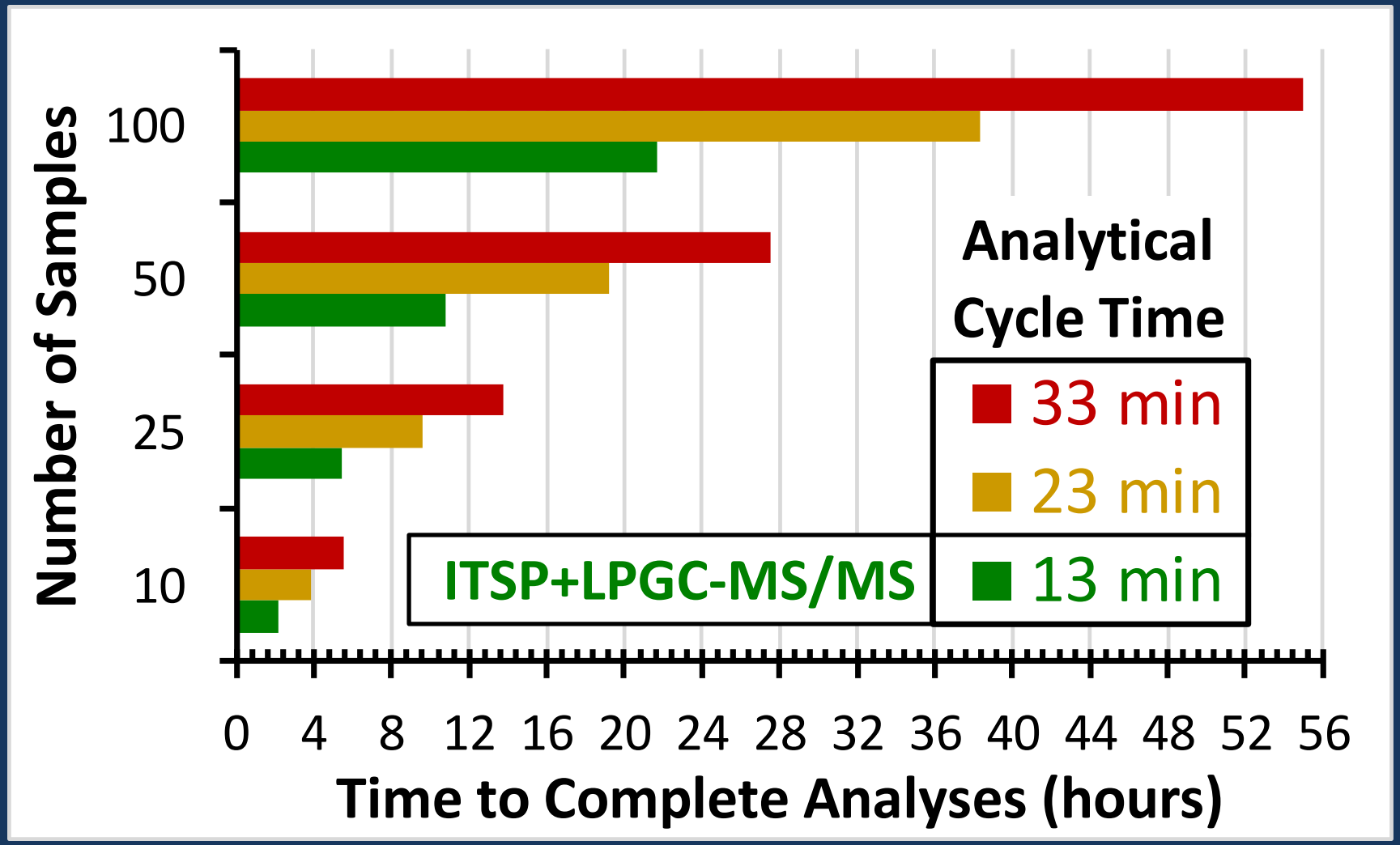
ITSP+LPGC-(HR)MS(/MS) Operates in Parallel and in Parallel with UHPLC-MS/(HR)MS

4 Samples Analyzed in 59 min

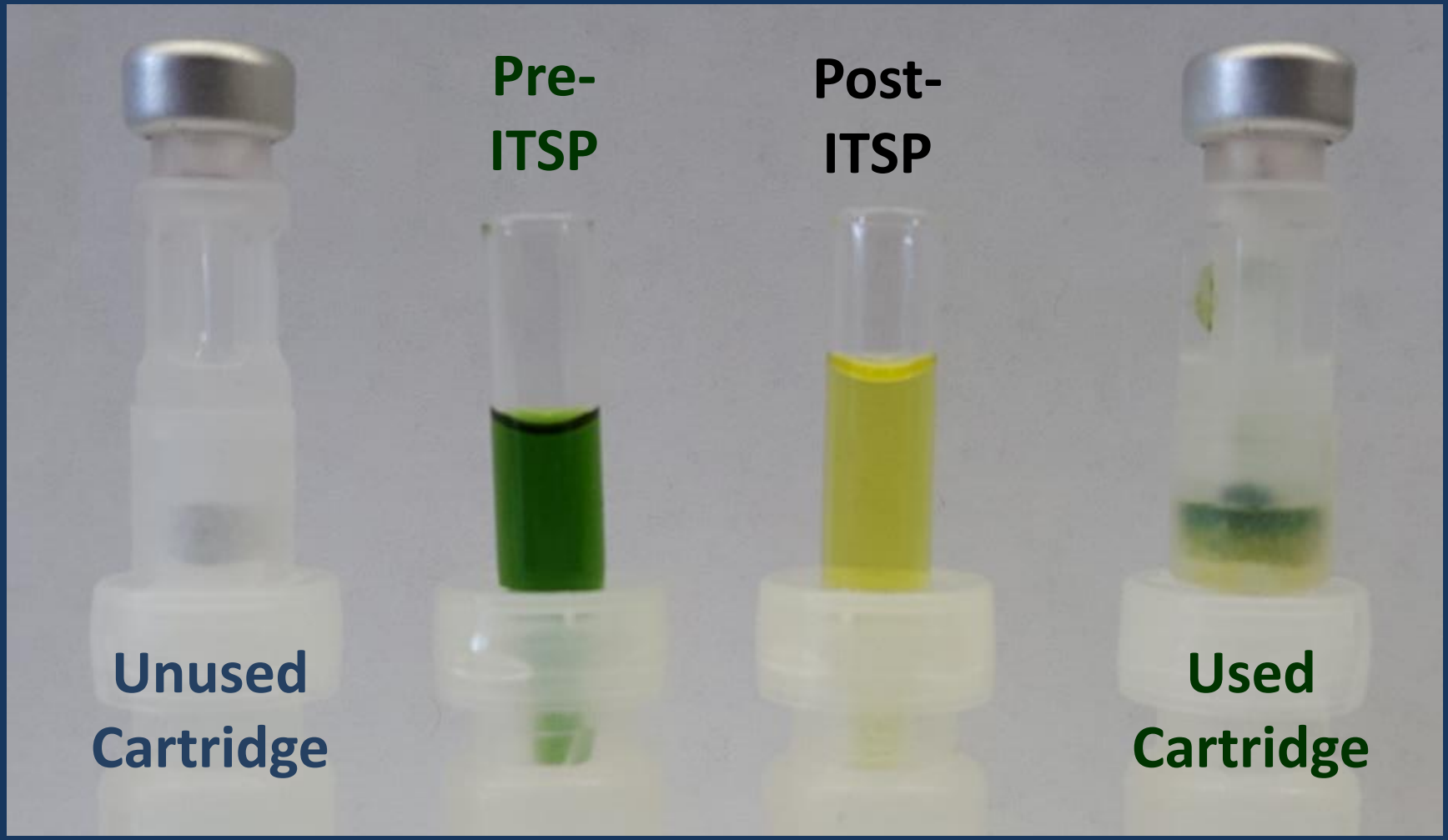


ITSP+LPGC-MS/MS Increases Batch Sizes

100 Samples Analyzed in 21.8 hours



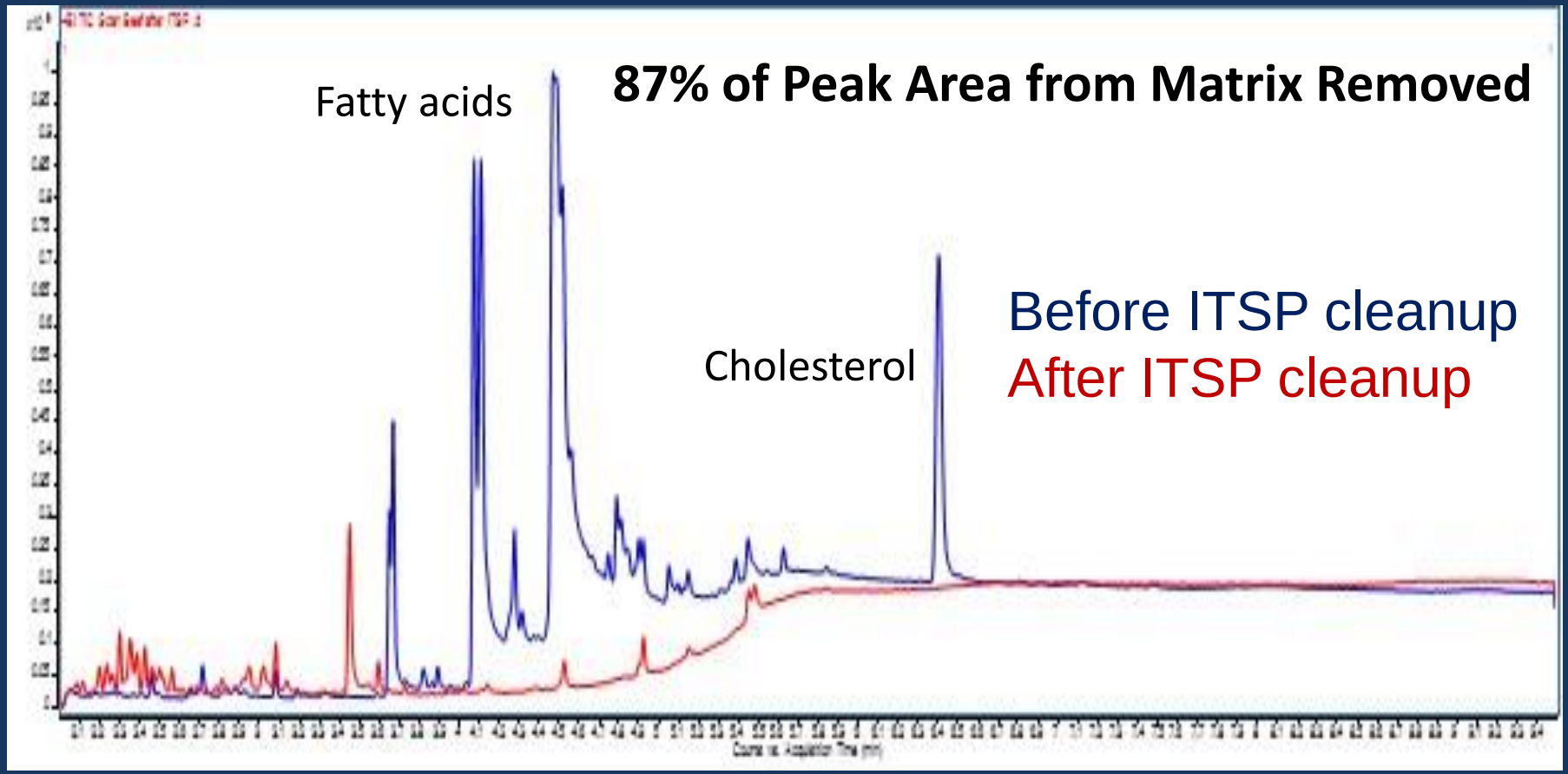
ITSP Cleanup of Hemp Plant Extract for GC



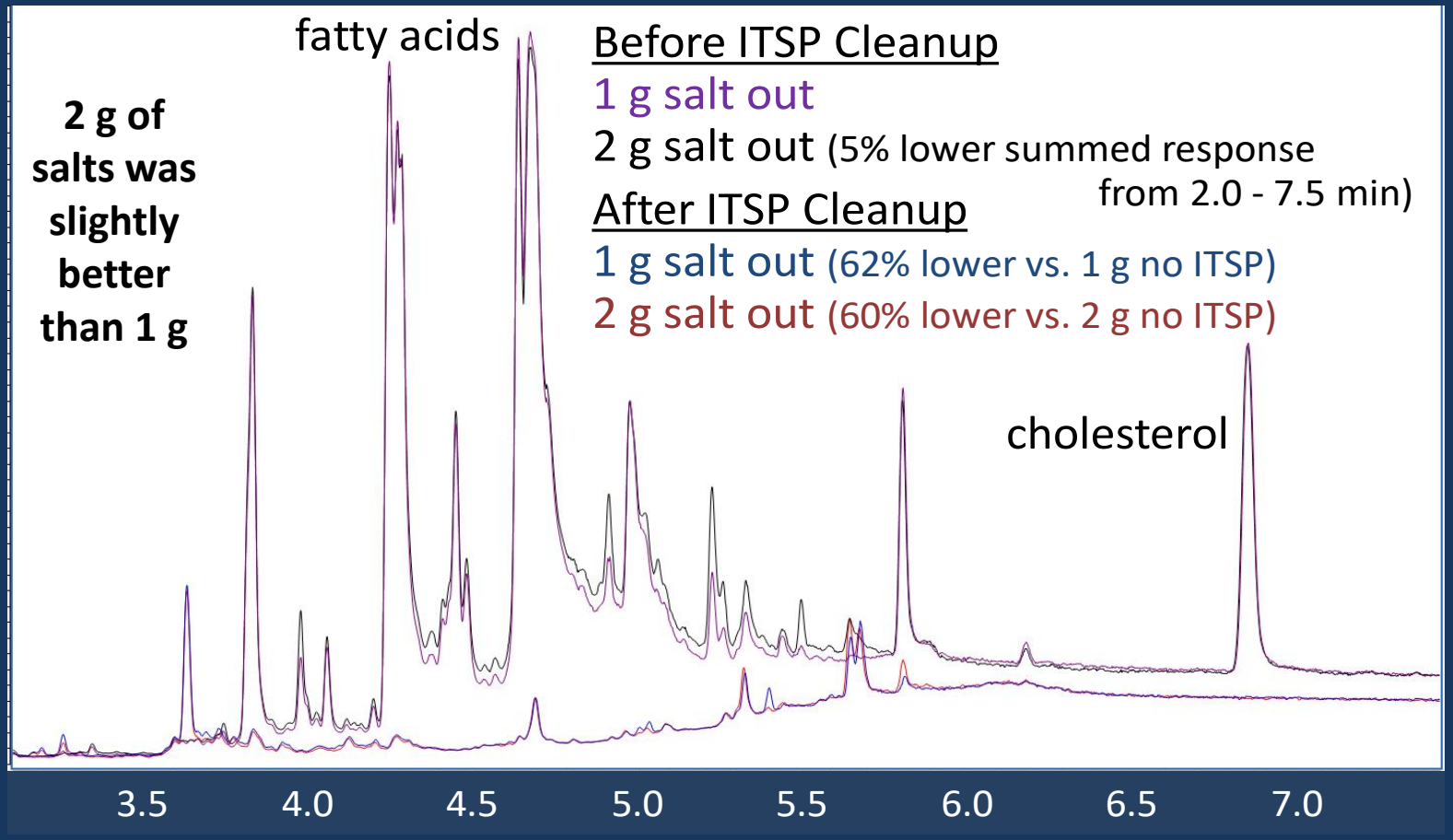
In QuEChERSER, the extracts after the salt-out step are 4-fold more dilute than in QuEChERS, which provides better cleanup by not exceeding capacity of the sorbents.

ITSP Cleanup is Very Effective (including fatty samples!)

Full Scan LPGC-MS of Beef Extracts

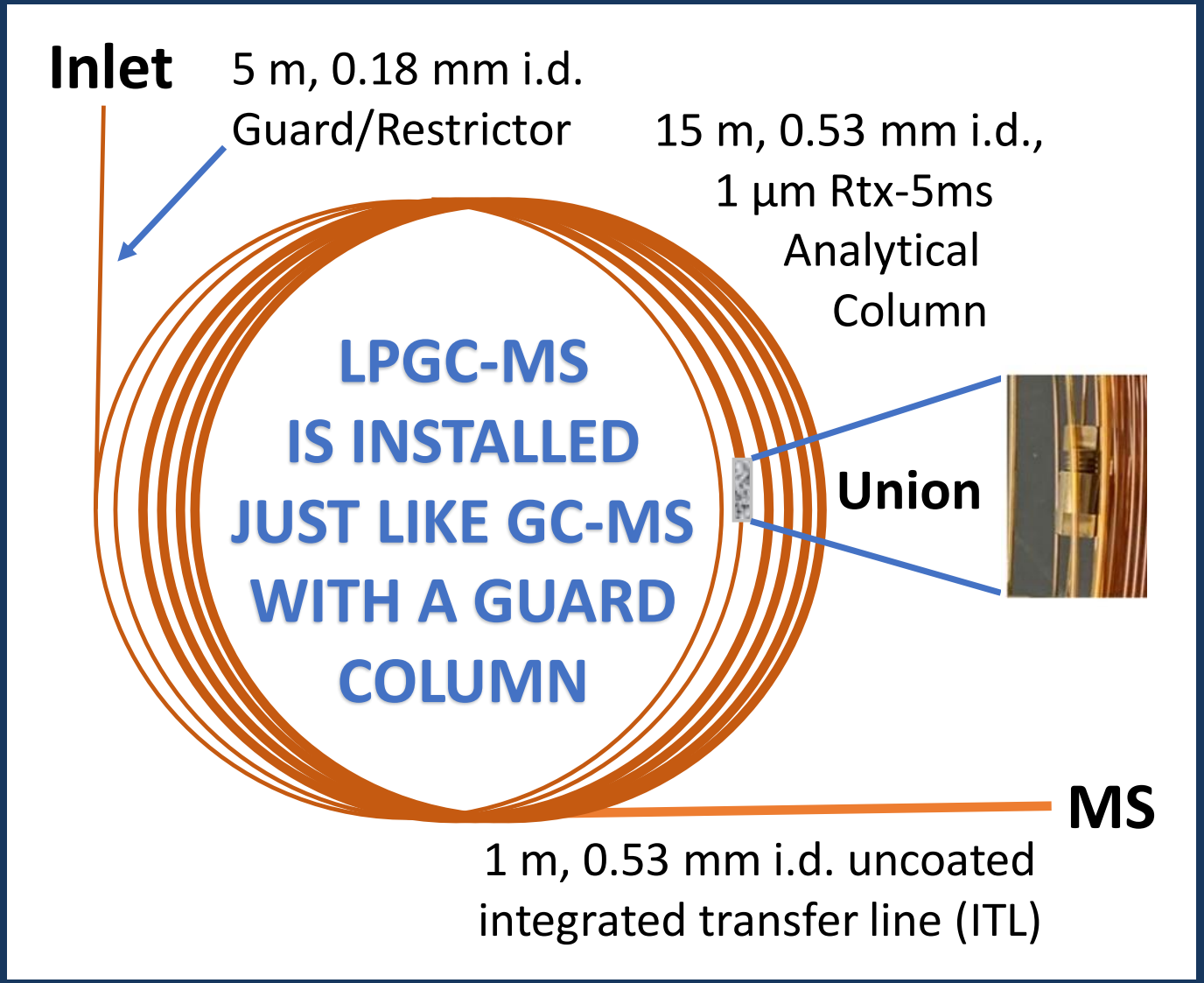


Full Scan LPGC-MS of Lamb Extracts



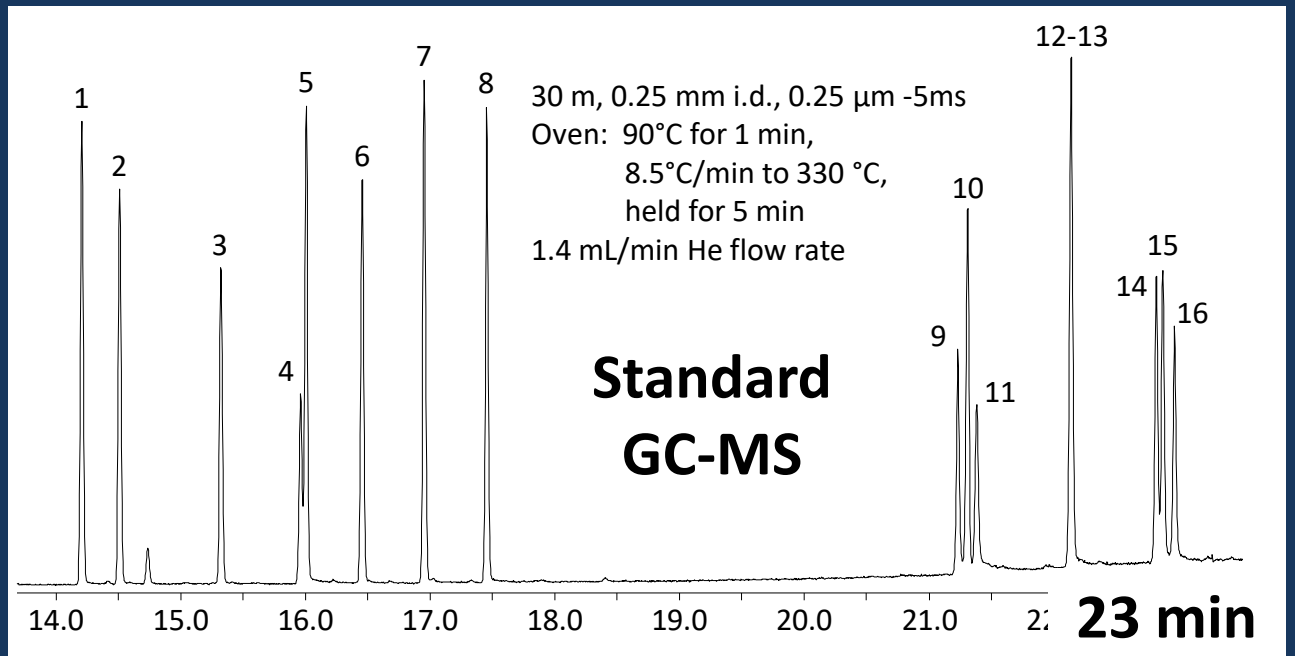
In QuEChERSER, the initial liquid extract is decanted into the pre-weighed salts in a 15 mL tube, which is more consistent and easier than adding the salts to the sample+extract in QuEChERS.

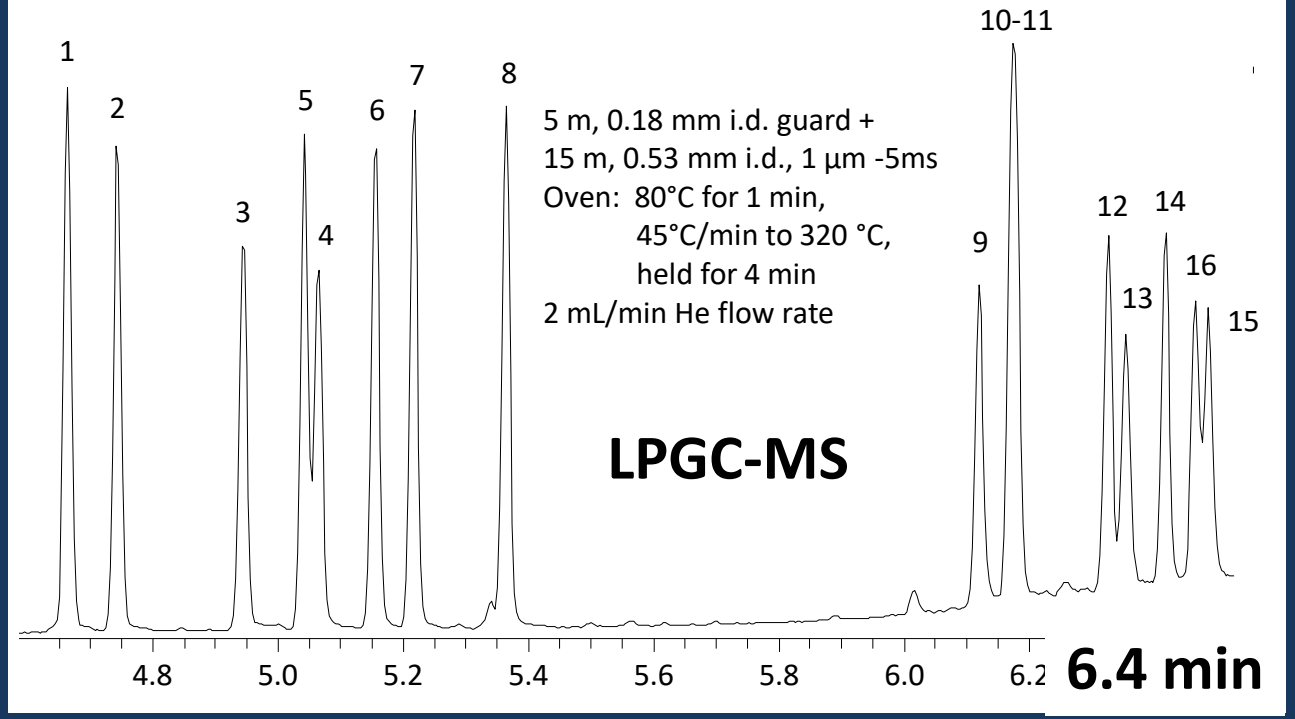
Fast Low-Pressure (LP)GC-MS/MS



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

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



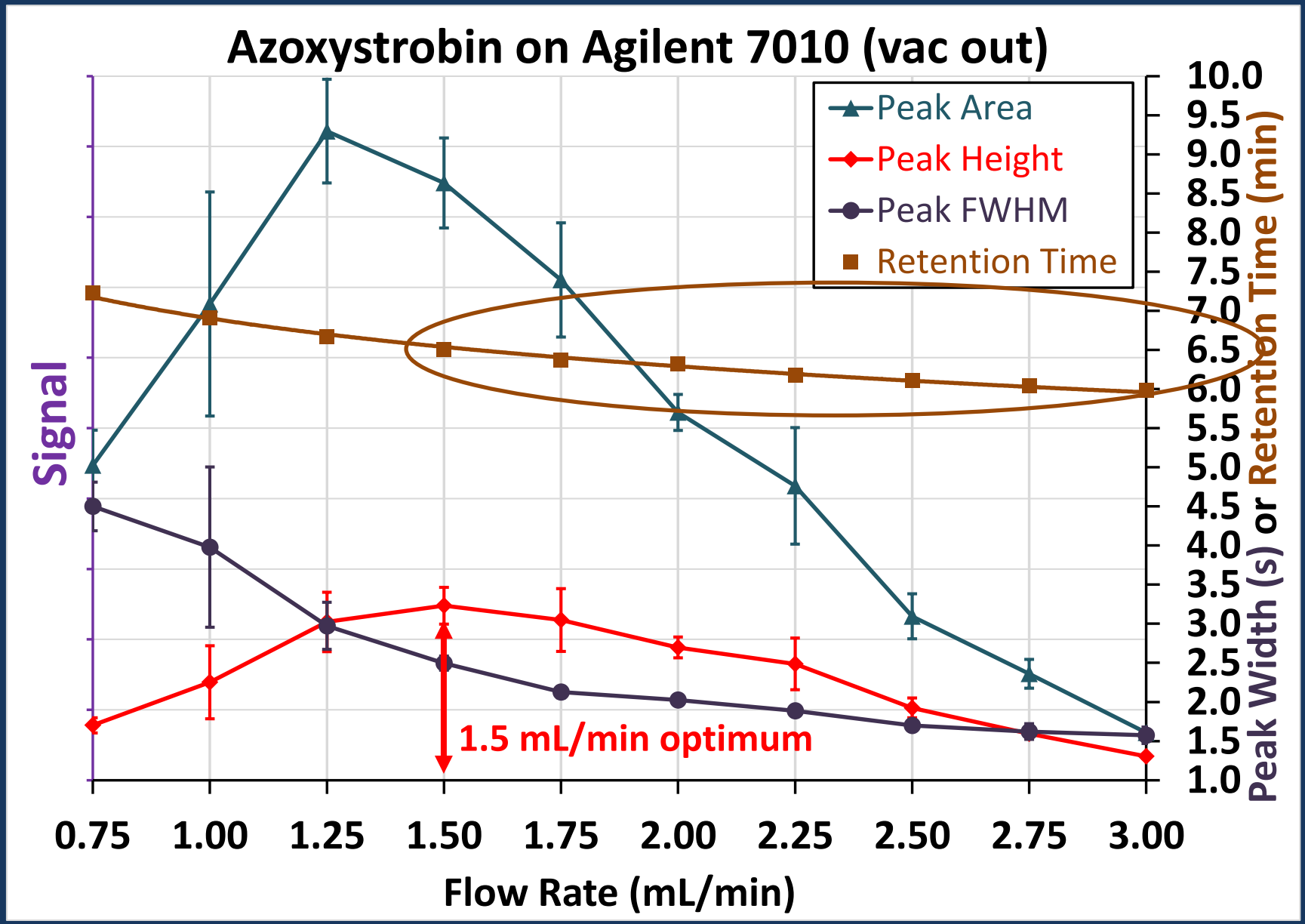


Peak widths in LPGC are ≈2.5 s vs. ≈ 5 s in std GC, which is why separations are similar but 3-4 times faster!

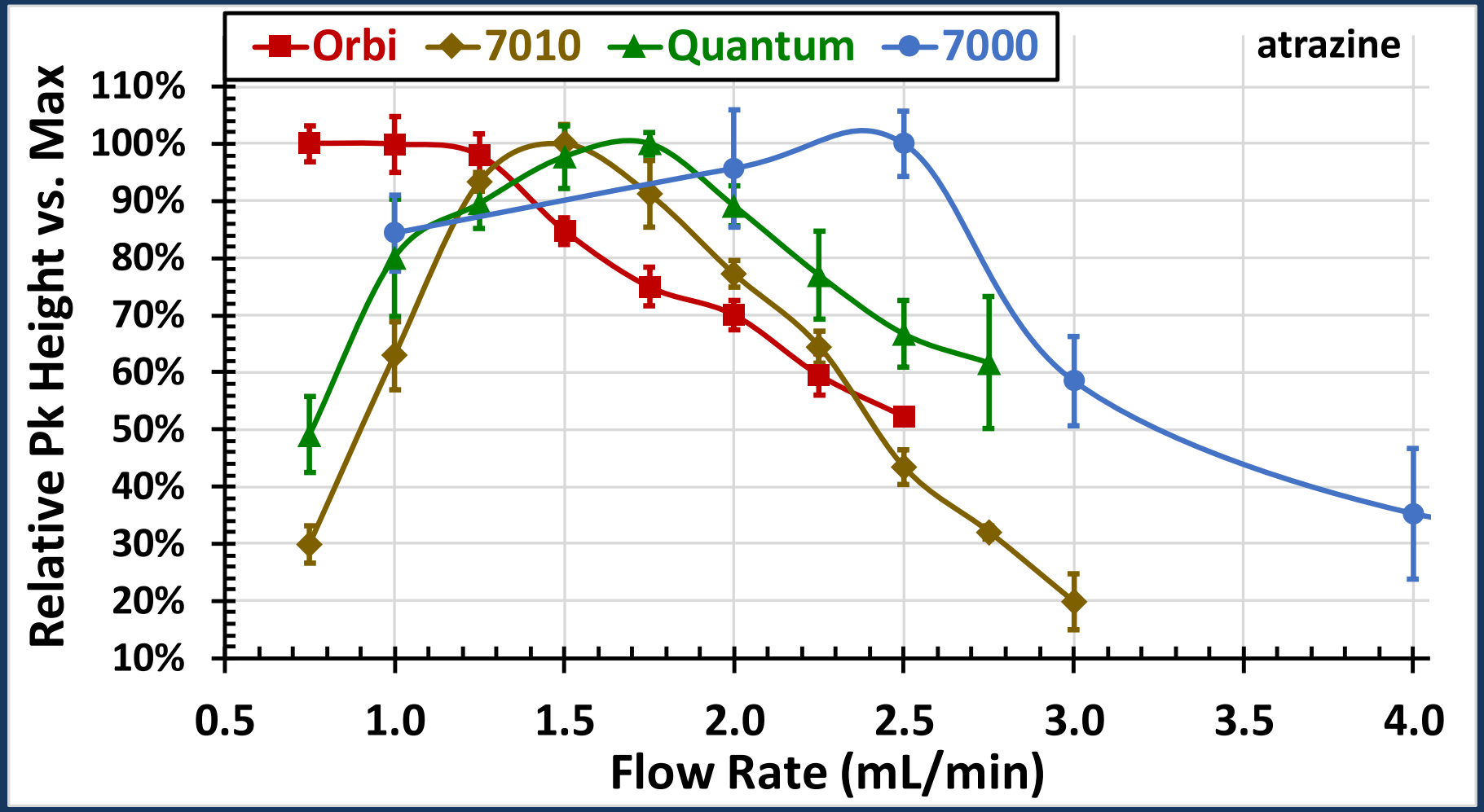
Co-elutions of isobaric analytes occur in std GC, too, and it's no big deal with many practical solutions.

Analyses by Jana Rousova Hepner

Optimization of LPGC-MS Flow Rate

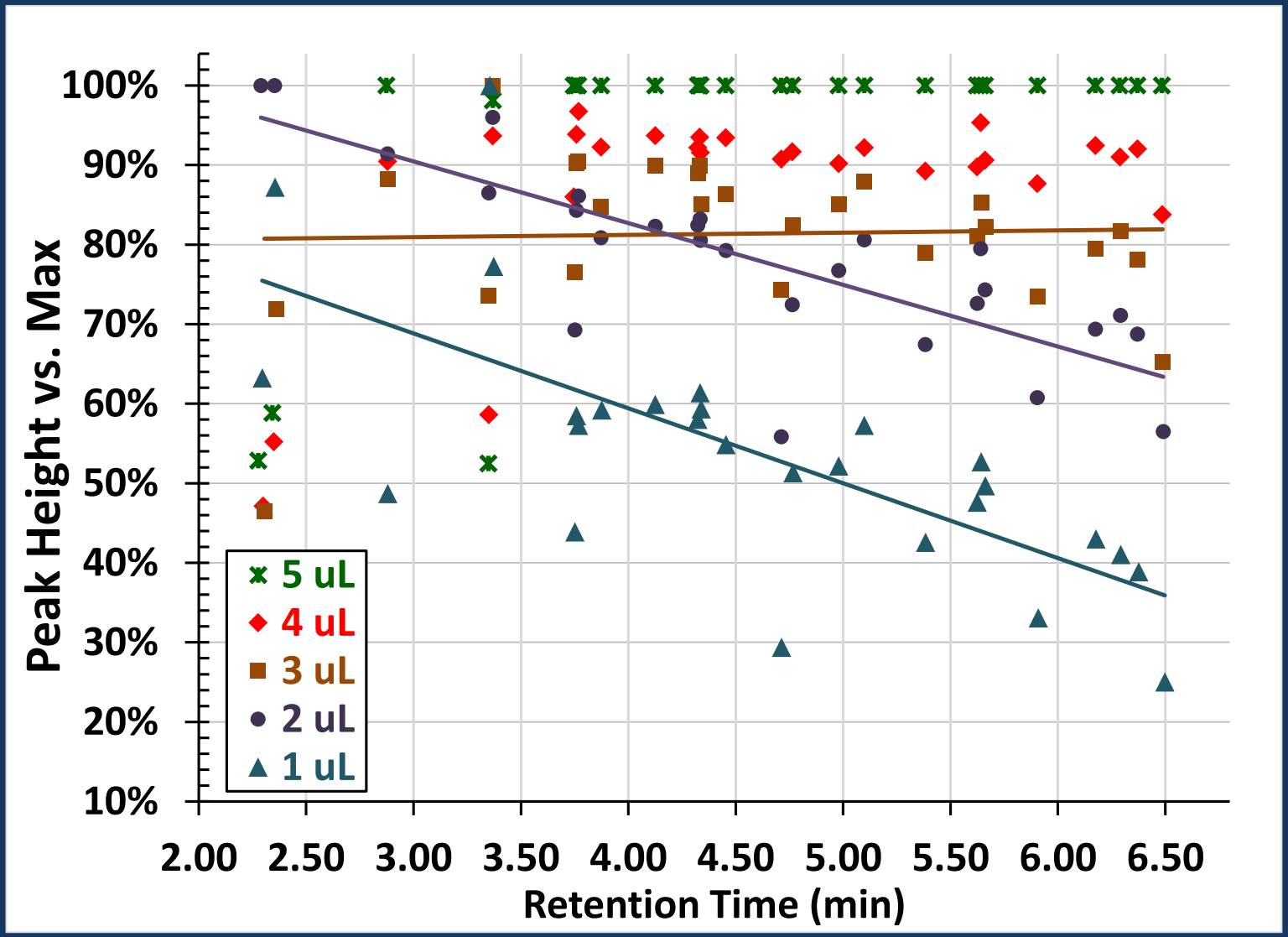


Flow rate with max sensitivity in LPGC-MS (megabore) depends on the instrument



Large-Volume Injection in LPGC with Standard Injector!

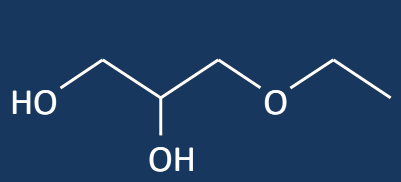
40 psi pressure pulse for MeCN extracts, 280°C inlet



Analyte Protectants (APs)

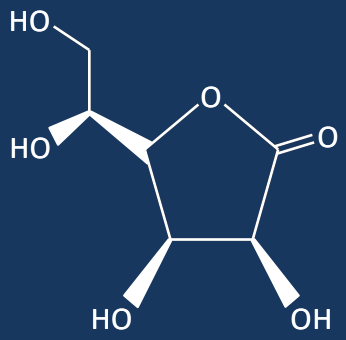
Strongly interact with active sites in GC inlet, column, and MS ion source to reduce adsorption of analytes.

Sharper peaks, less tailing, more ruggedness, lower LOD



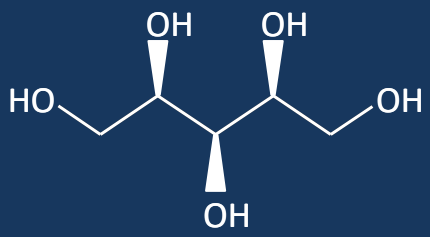
ethylglycerol

1 mg/ mL



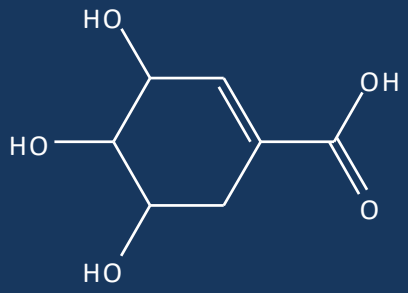
gulonolactone

0.1 mg/ mL



sorbitol

0.1 mg/ mL

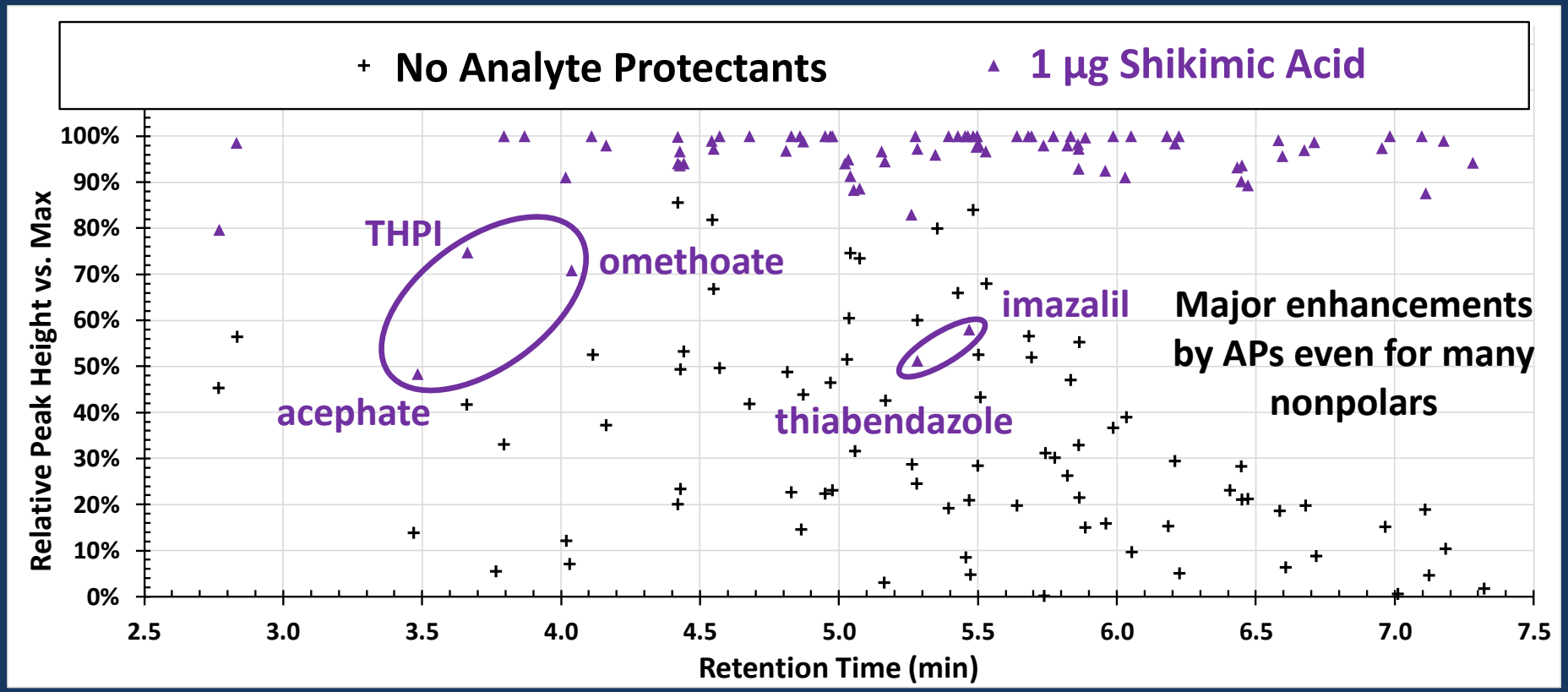


shikimic acid

0.05 mg/ mL

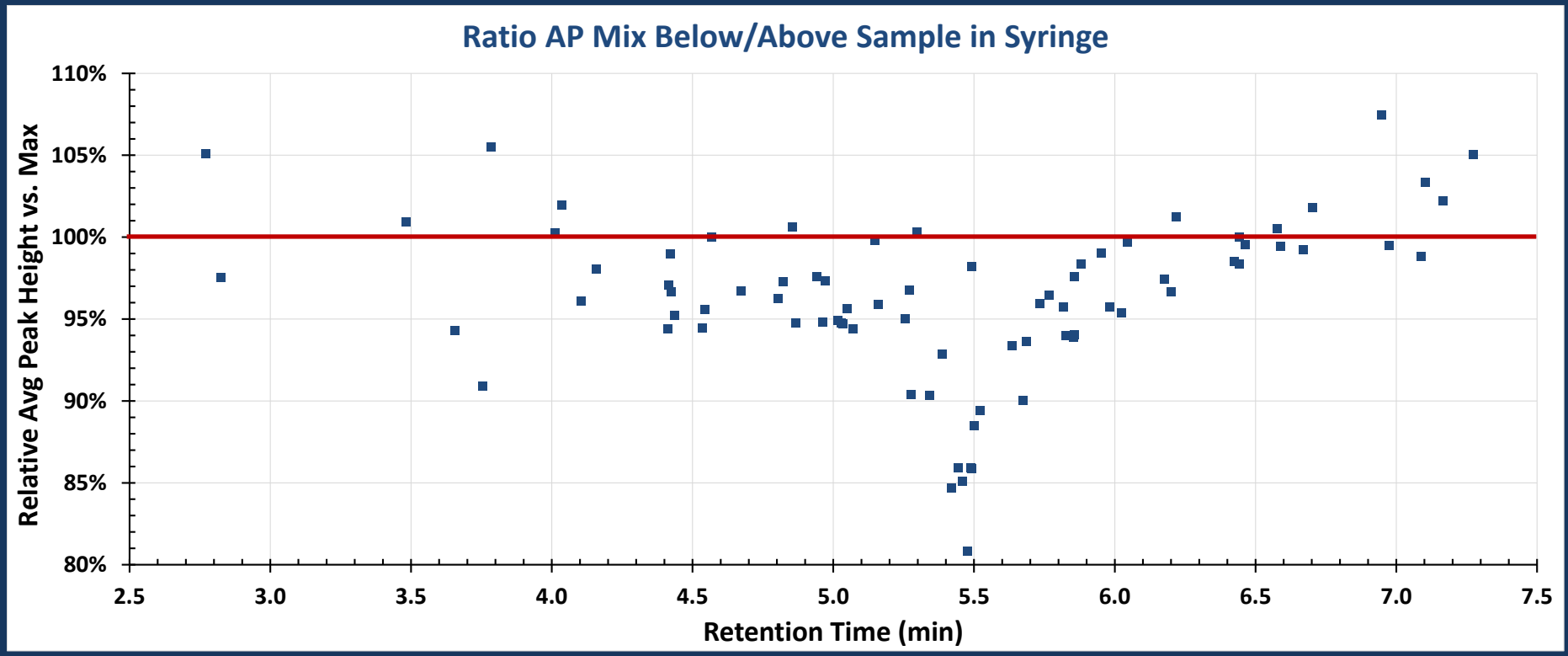
Mastovska *et al.*, *Anal. Chem.* **77** (2005) 8129-8137

Re-Assessment of APs with the Agilent 7010



Only shikimic acid is needed on the 7010, with 1 µg co-injected on column

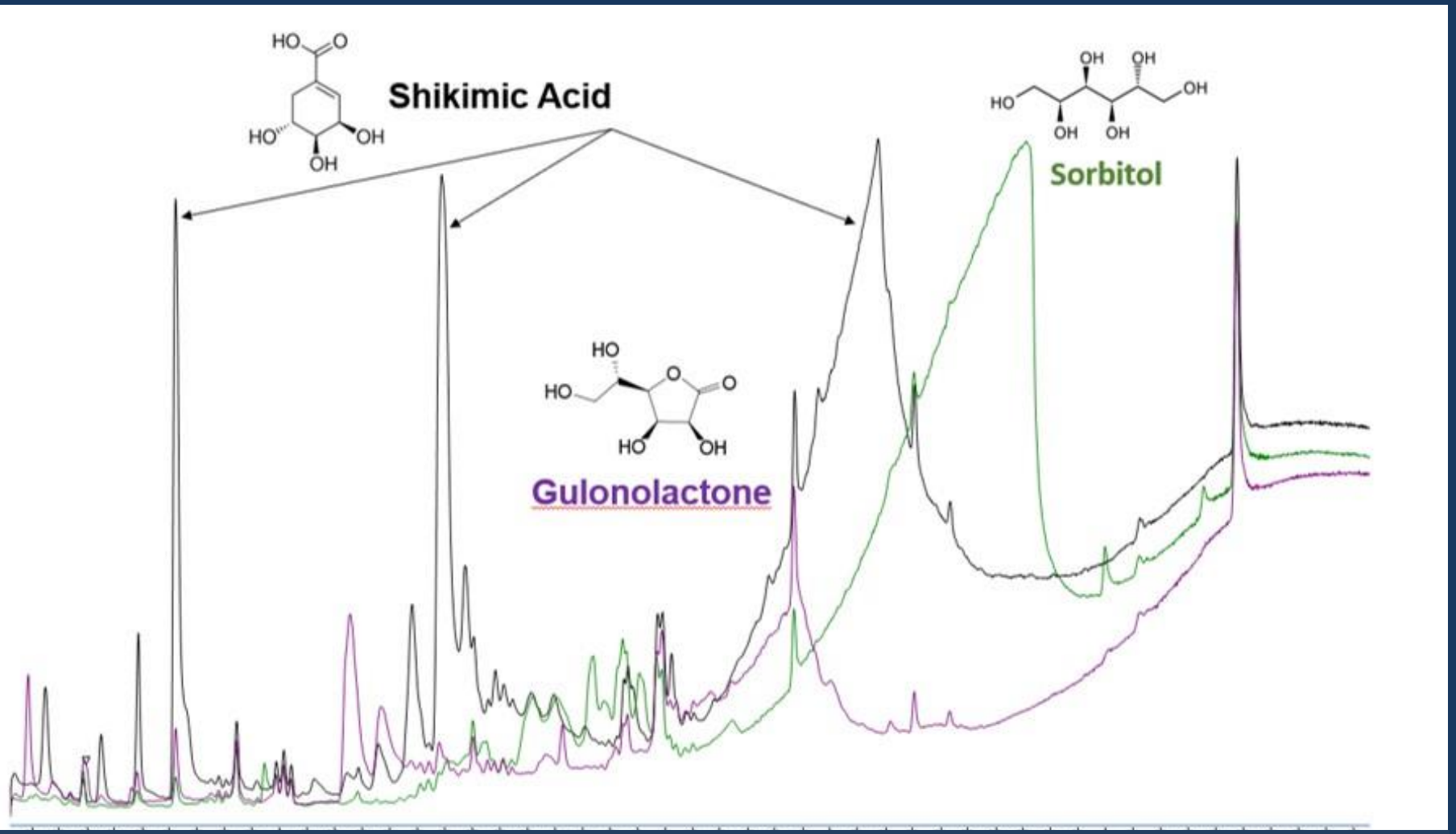
Addition of APs within the Injection Syringe



It is equal or better to add the APs Above the Sample rather than Below it within the Syringe – Due to Carry-Over Concerns, too

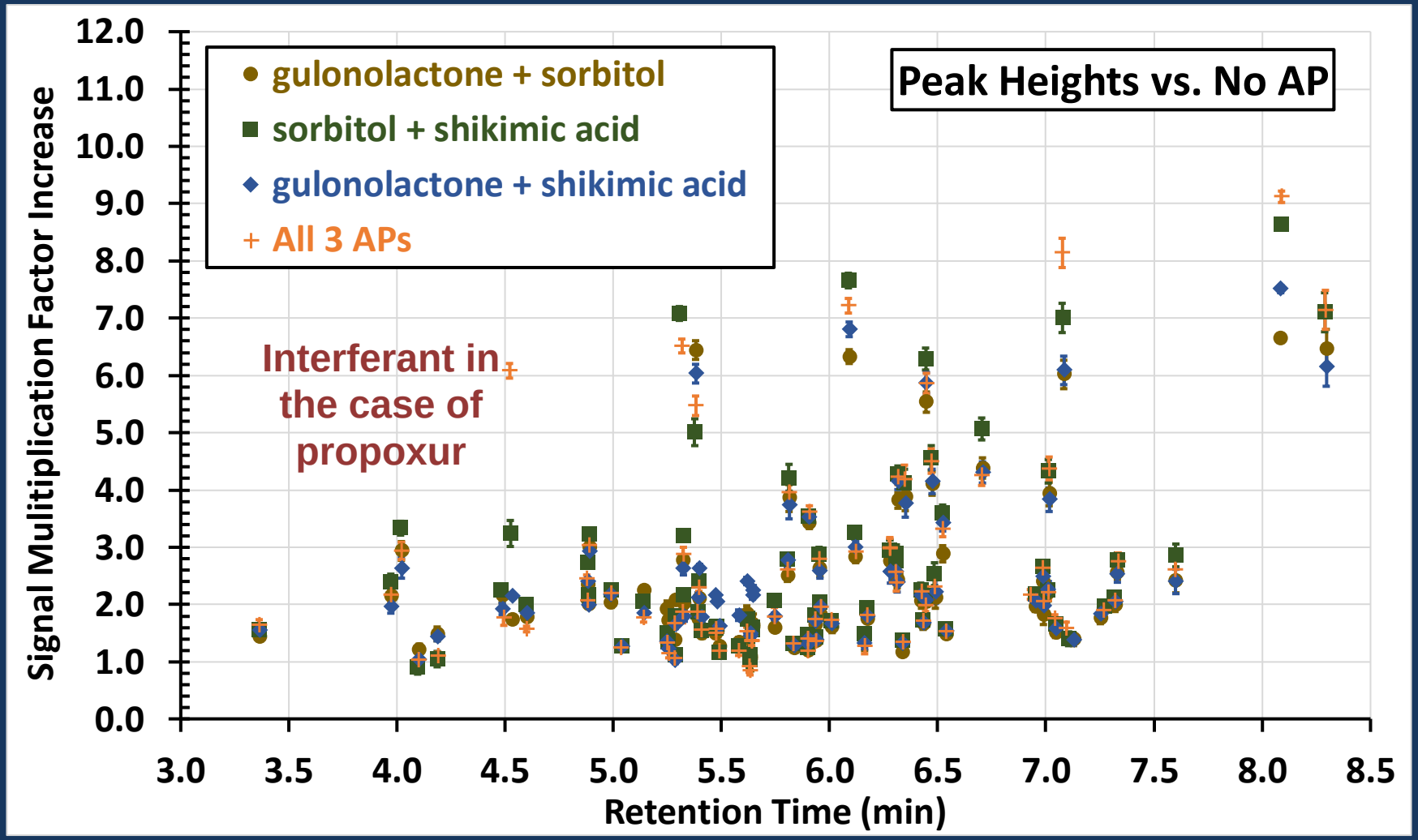
Unlike other models, “suppression” occurs when co-elutions are excessive on the 7010

Elution of APs in LPGC-MS



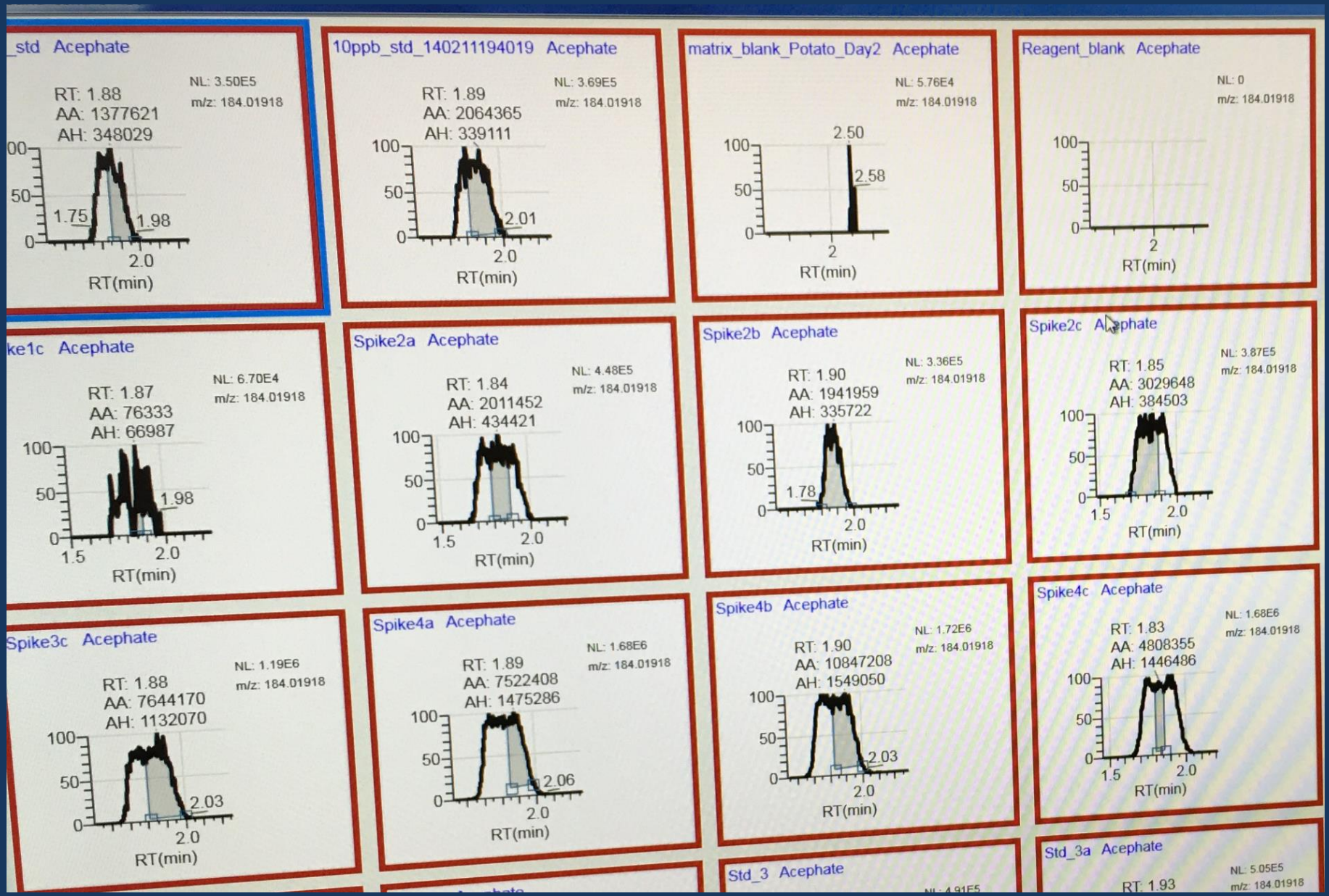
Jaap de Zeeuw added structures and colors

Mixture of APs in LPGC-Orbitrap is Fine



1 µg each of sorbitol and shikimic acid seem best

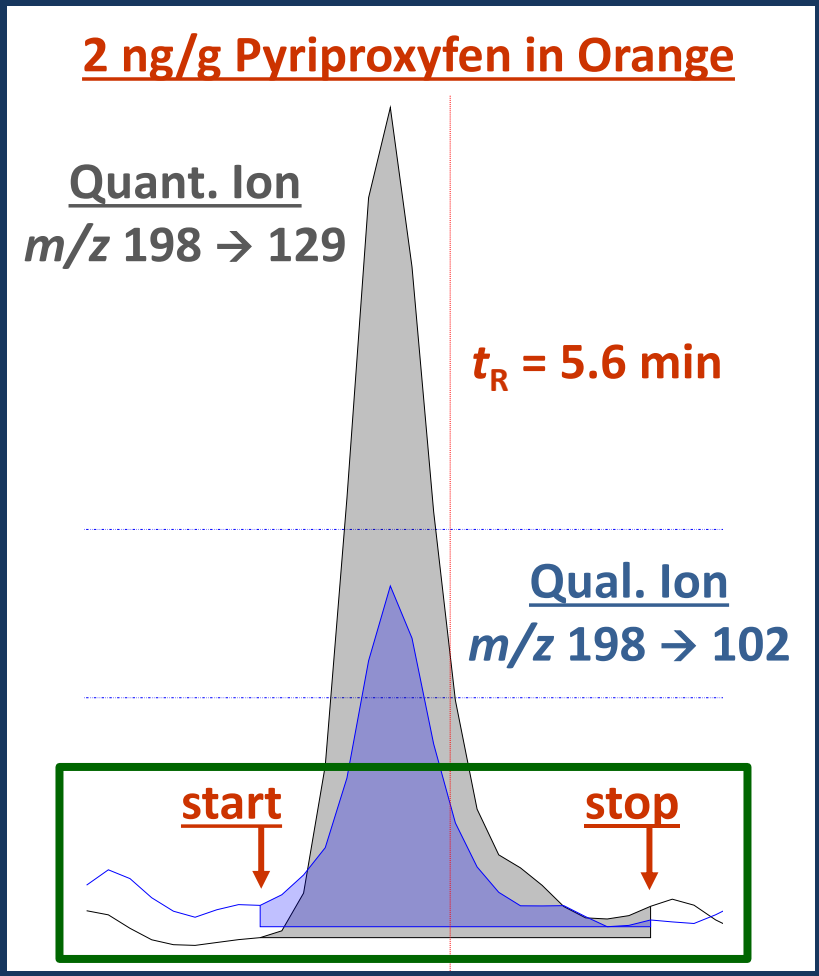
Poor integration undoes excellent detection



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!

ITSP+LPGC-MS/MS Robustness

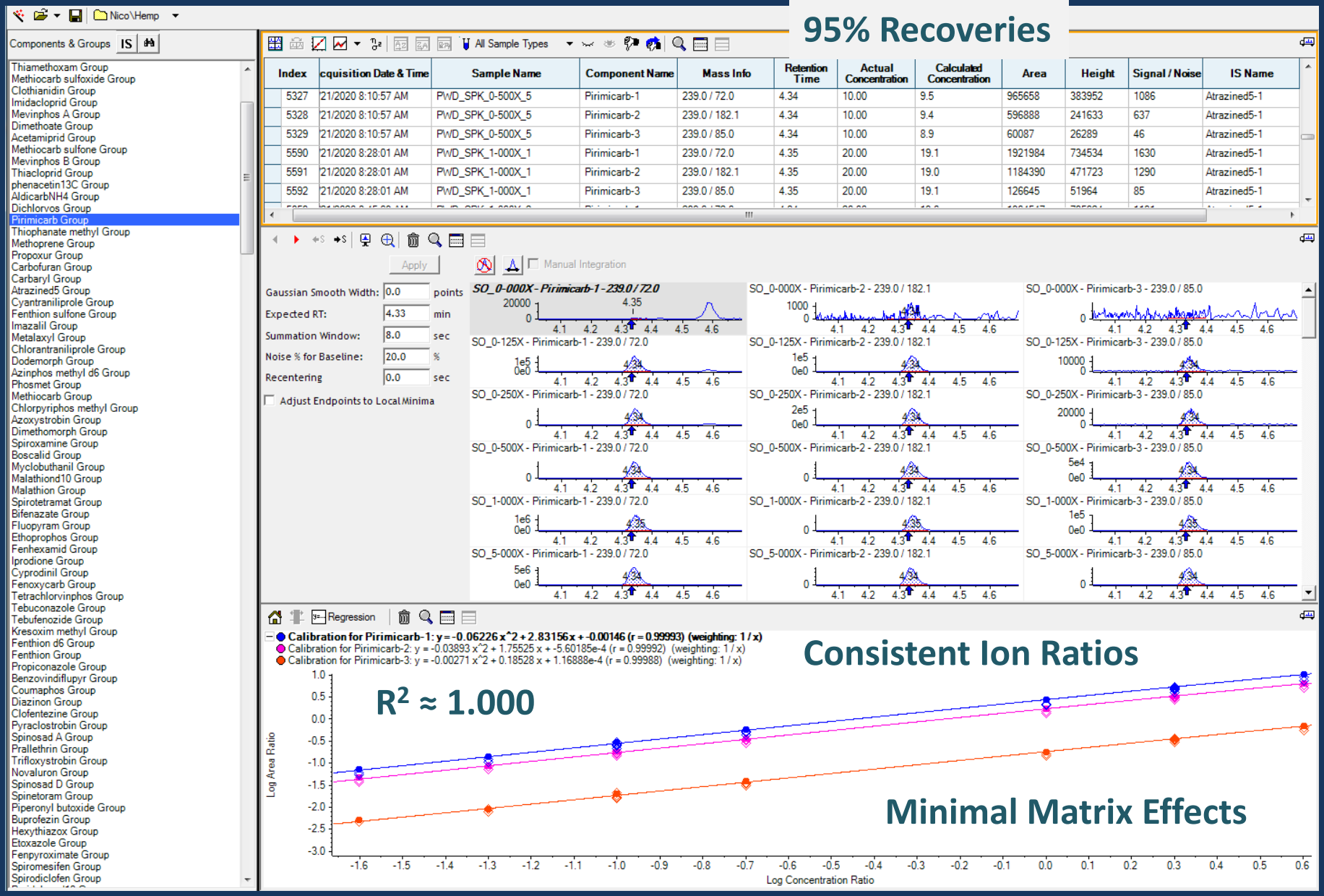
Analyte	Initial <50 injections		After >250 injections	
	Avg ± SD t_R (min)	Analytical RSD	Avg ± SD t_R (min)	Analytical RSD
Dichlorvos	2.818 ± 0.004	6.6%	2.824 ± 0.002	5.2%
Ethoprophos	4.110 ± 0.002	6.0%	4.106 ± 0.002	3.4%
Endosulfan I	5.405 ± 0.002	9.3%	5.398 ± 0.003	9.2%
Azoxystrobin	7.249 ± 0.004	9.4%	7.255 ± 0.010	7.5%

Results for pesticides spiked into hemp powder and oil (Day 1 sequence of 62 injections) and in hemp pellets (Day 5 sequence totalling 298 injections) using QuEChERSER sample preparation. The inlet liner was changed after 140 injections.

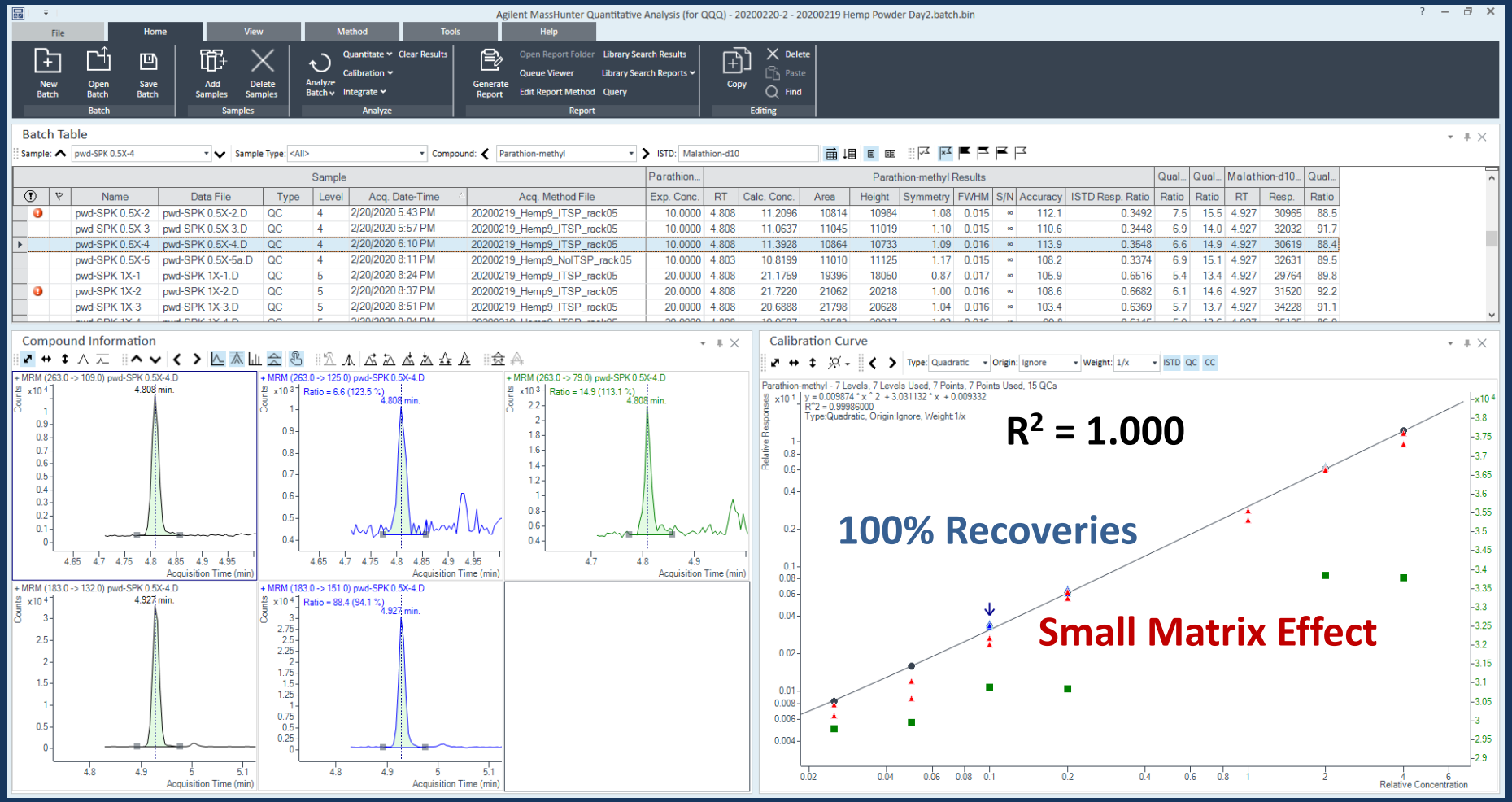
Don't trim the columns, just change the inlet liner and septum.

Keep oven 150-250°C between sample sequences, 1.5 mL/min.

Quick Data Review in UHPLC-MS/MS



Quick Data Review in ITSP+LPGC-MS/MS



Good Integrations, No Interferences, and Consistent Ion Ratios

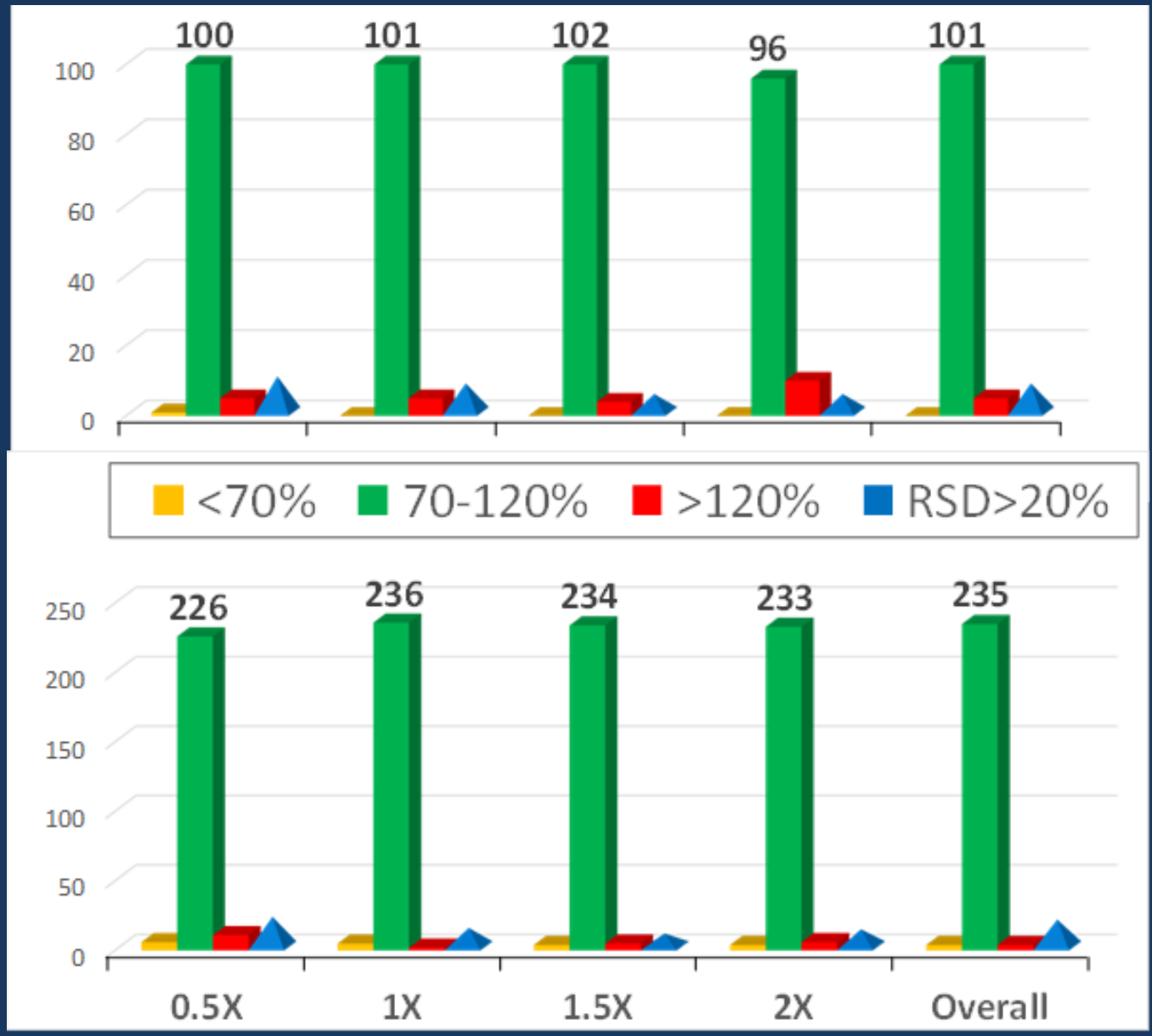
QuEChERSER Results for Analytes in Catfish

Validation out of 106 Vet. Drugs

and

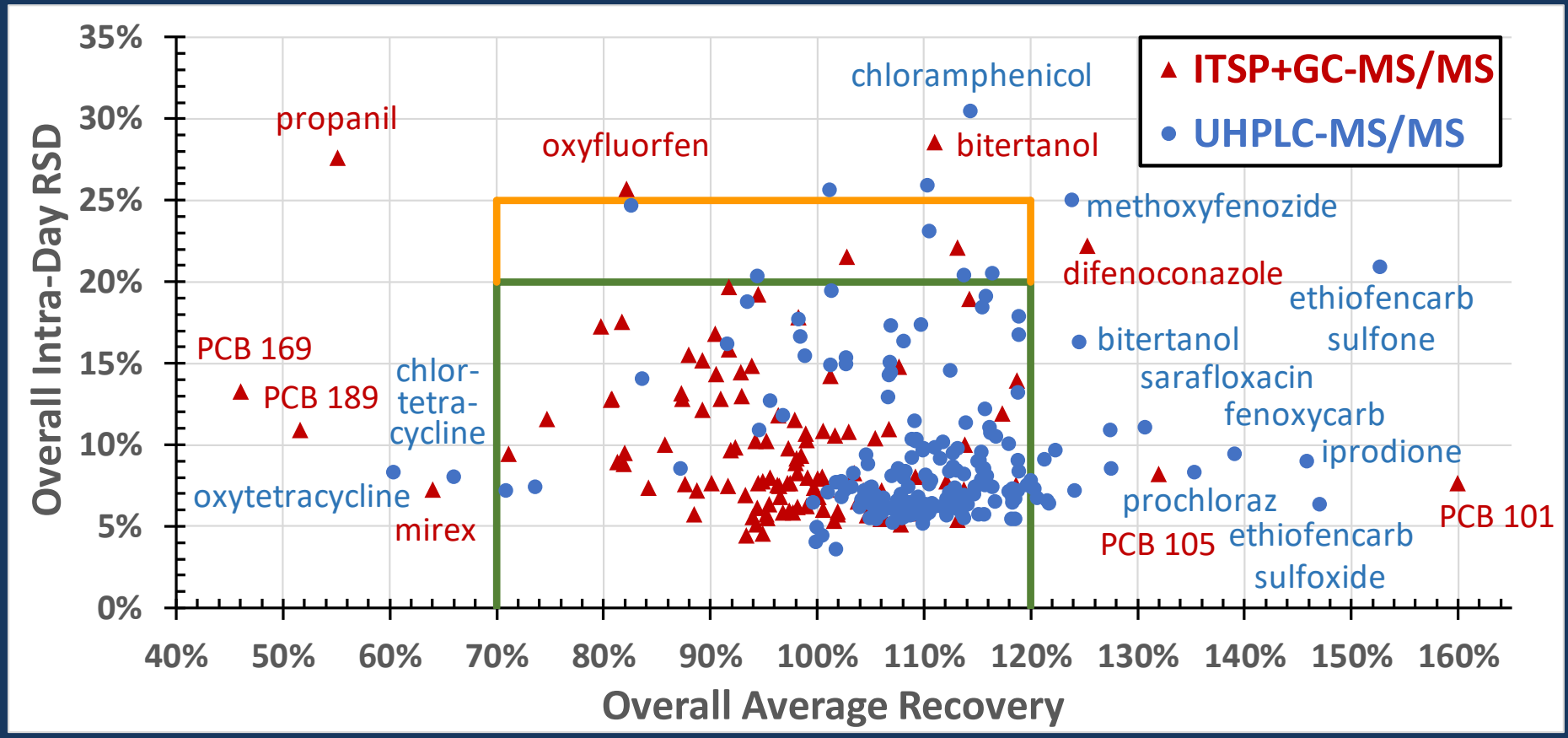
243 Pesticides and (16) PCBs

n = 40
(4 levels x 10 reps each)



Validation of QuEChERSER in Beef

221 out of 259 (85%) of Analytes in the Green Box

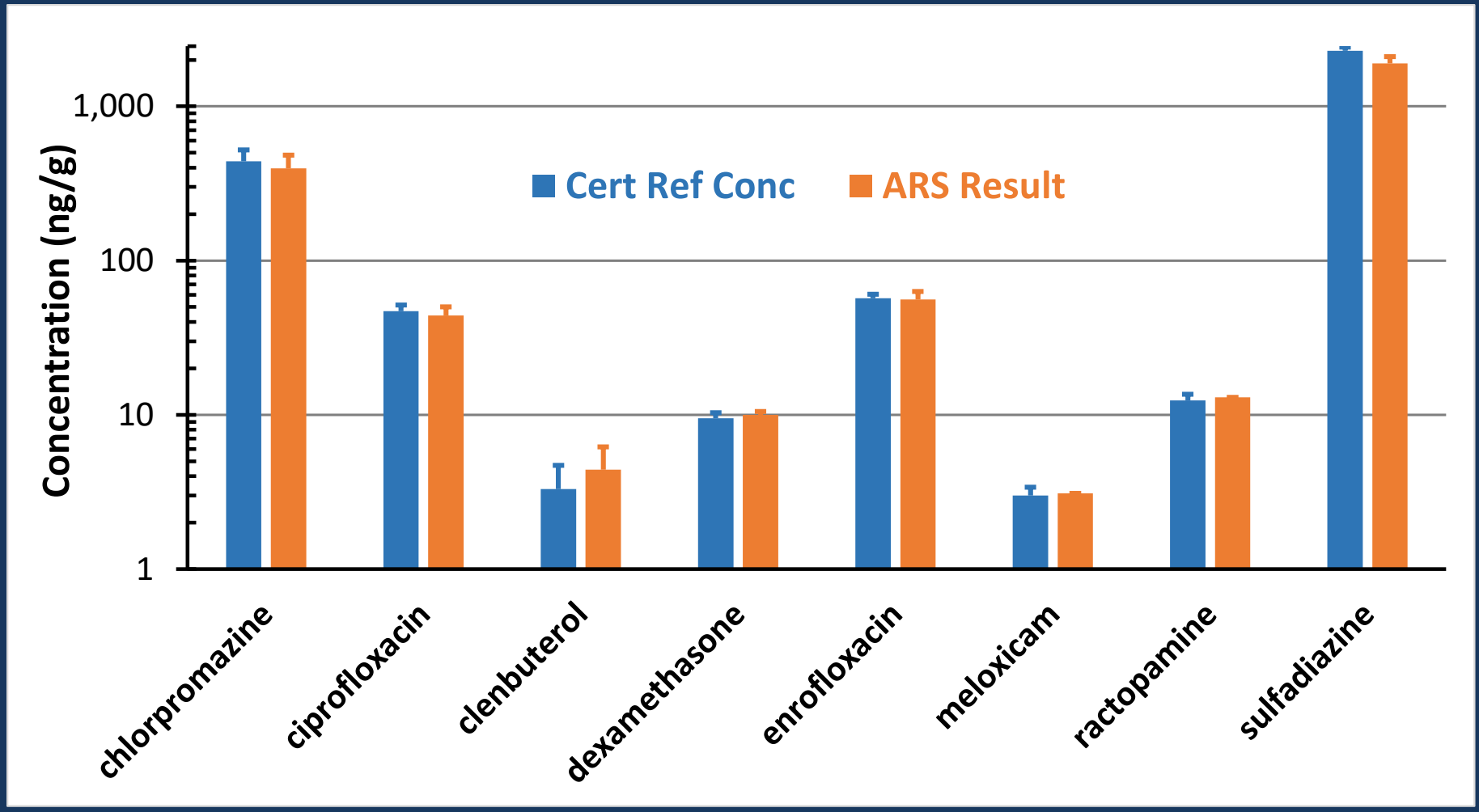


0.5X, 1X, 1.5X, and 2X spiking levels (X typically 20 ng/g)

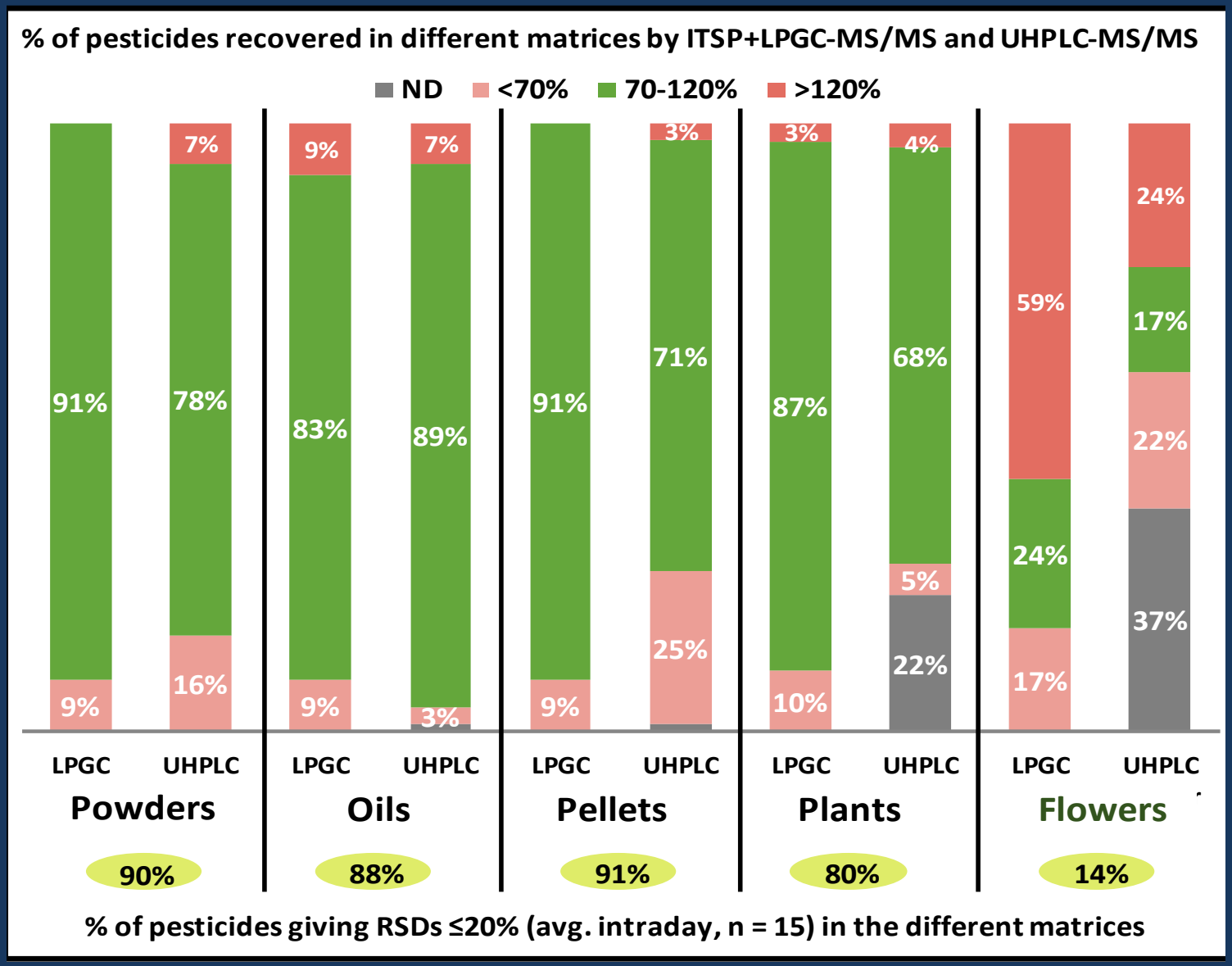
10 replicates each times 2 Days (n = 80)

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

veterinary drug residues in bovine muscle (freeze-dried)



QuEChERSER of Pesticides in Hemp Matrices



The mini/mega-method worked well except for dried hemp flowers.

Conclusions

1) Advantages abound in the QuEChERSER mega-method.
2) Reliable high-quality results can be achieved from **start-to-finish** for **hundreds** of targeted ultratrace multi-application contaminants in diverse foods using **semi-automated high-throughput** analysis by the **QuEChERSER mega-method** with back-flushing UHPLC-MS/MS + ITSP+LPGC-MS/MS in parallel followed by summation function peak integration and post-run processing to yield accurate and trustworthy **quantidentifications** with little need for human review.
3) Measurement uncertainty for each step and overall can be easily determined in every batch, too.

QuEStIONS ER?

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