Technologies for detection of chemical and biological contaminants in foods

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Outline

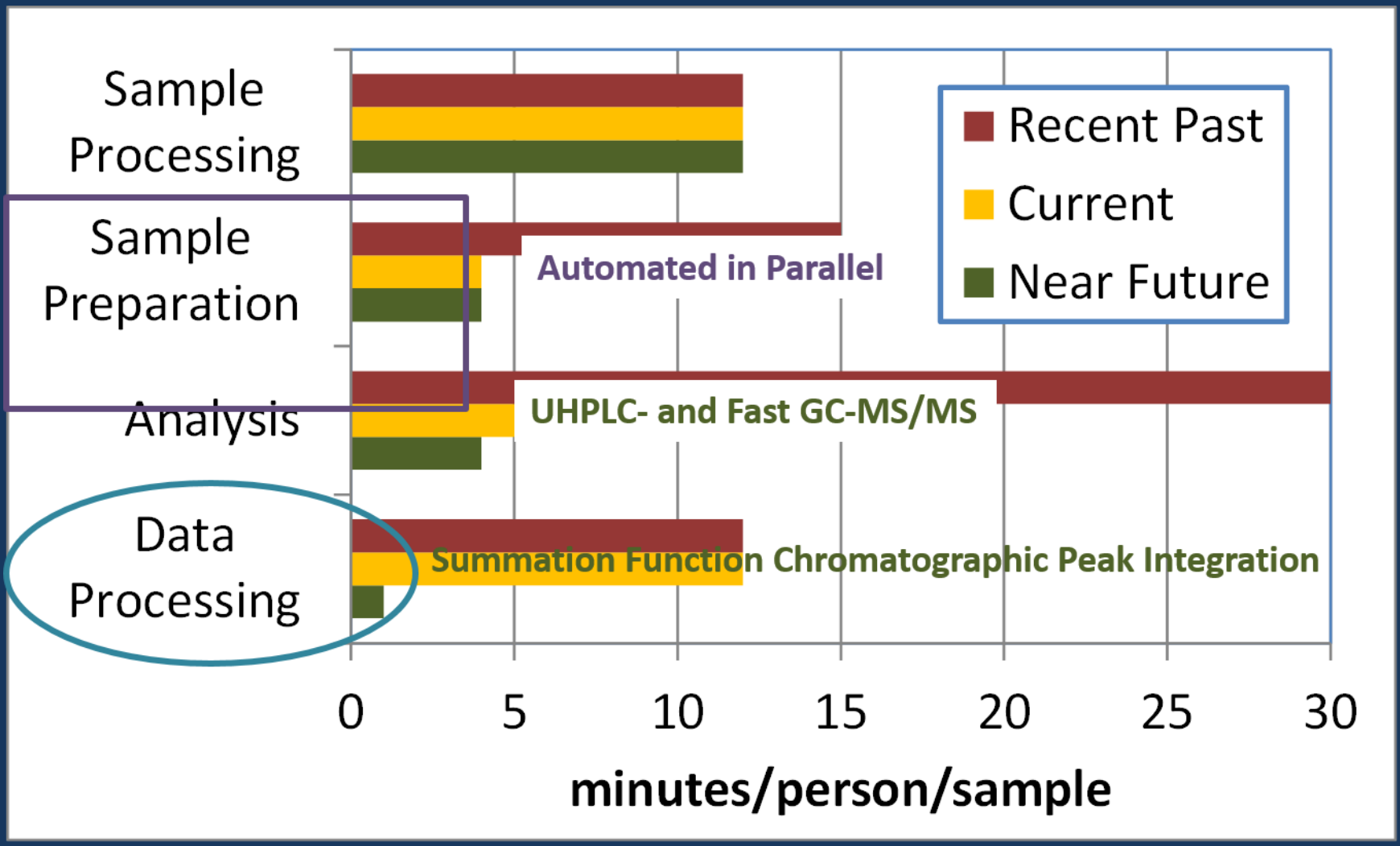
1) **Project Overview**
2) **Steven Lehotay and Yelena Sapozhnikova**
    a) veterinary drug residue analysis in meats and eggs
    c) pesticides and environmental contaminants analysis
    d) sample processing and test portions study
    b) automated sample prep and analysis,
        including data handling and identification
3) **Guoying Chen**
    mercury analysis
4) **Johnny Perez**
    mass spectrometry in antimicrobial resistance research

Mission Statement and Goal

Develop and transfer to stakeholders effective, efficient, and useful analytical approaches for the screening, quantification, and/or identification of chemicals of concern in food and food-related matrices, including aspects related to antimicrobial resistance.

The goal of our work is to better protect the food supply for the benefit of human health, the environment, and agriculture, and conduct outstanding research, disseminate the findings, transfer the technologies, and have rewarding interactions in the process.

Sample Throughput to analyze Chemical Residues in foods → QuEChERS + LC-& GC-MS (/MS)

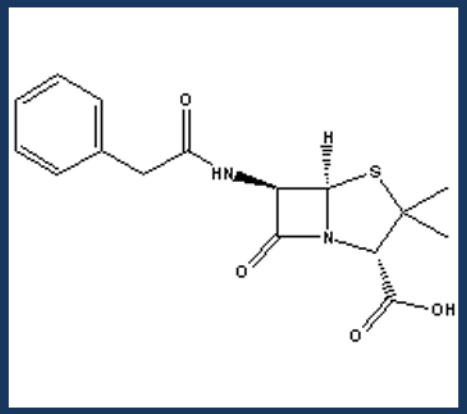


Some Recent Publications of Note

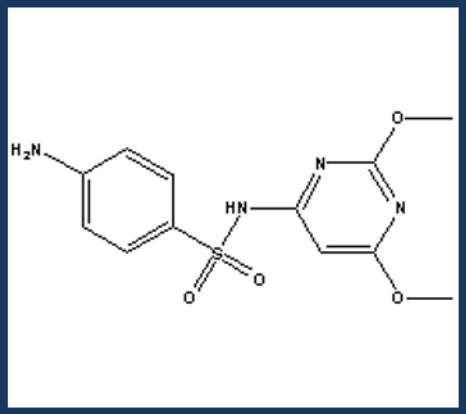
- Lehotay *et al.* (2016) "Automated mini-column solid-phase extraction cleanup for high-throughput analysis of chemical contaminants in foods by low-pressure gas chromatography – tandem mass spectrometry" *Chromatographia*, **79**, 1113-1130
- Han et al. (2016) “Method validation for 243 pesticides and environmental contaminants in meats and poultry by tandem mass spectrometry coupled to low-pressure gas chromatography and ultrahigh performance liquid chromatography” *Food Control* **66**, 270-282
- Sapozhnikova and Lehotay (2015) “Review of recent developments and applications in low-pressure (vacuum outlet) gas chromatography” *Anal. Chim. Acta* **899**, 13-22
- Lehotay *et al.* (2015) “Current issues involving screening and identification of chemical contaminants in foods by mass spectrometry” *Trends Anal. Chem.* **69**, 62-75
- Lehotay and Cook (2015) “Sampling and sample processing in pesticide residue analysis” *J. Agric. Food Chem.* **63**, 4395-4404
- Sapozhnikova and Lehotay (2015) “Evaluation of different parameters in the extraction of incurred pesticides and environmental contaminants in fish” *J. Agric. Food Chem.* **63**, 5163-5168

Major Classes of Antibiotics

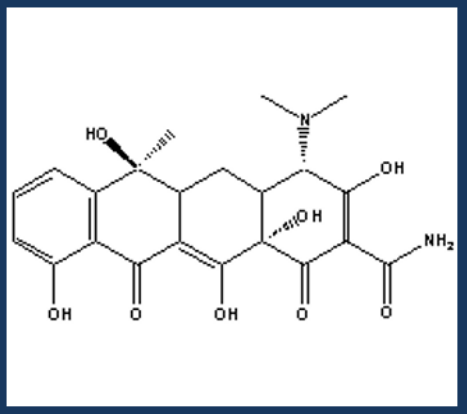
β-Lactams



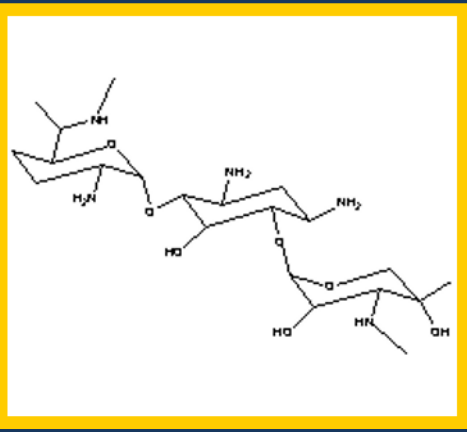
Sulfonamides



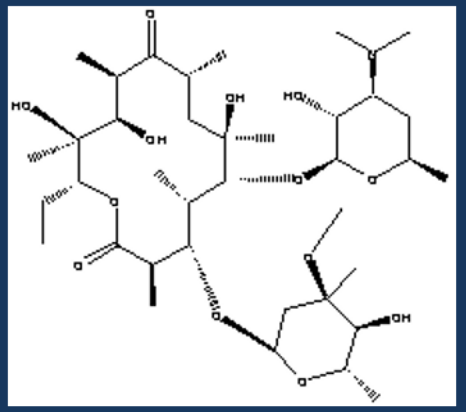
Tetracyclines



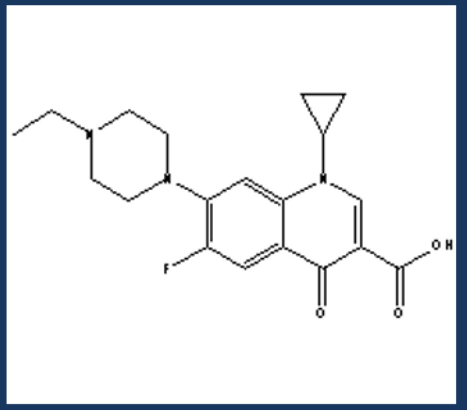
Aminoglycosides



Macrolides

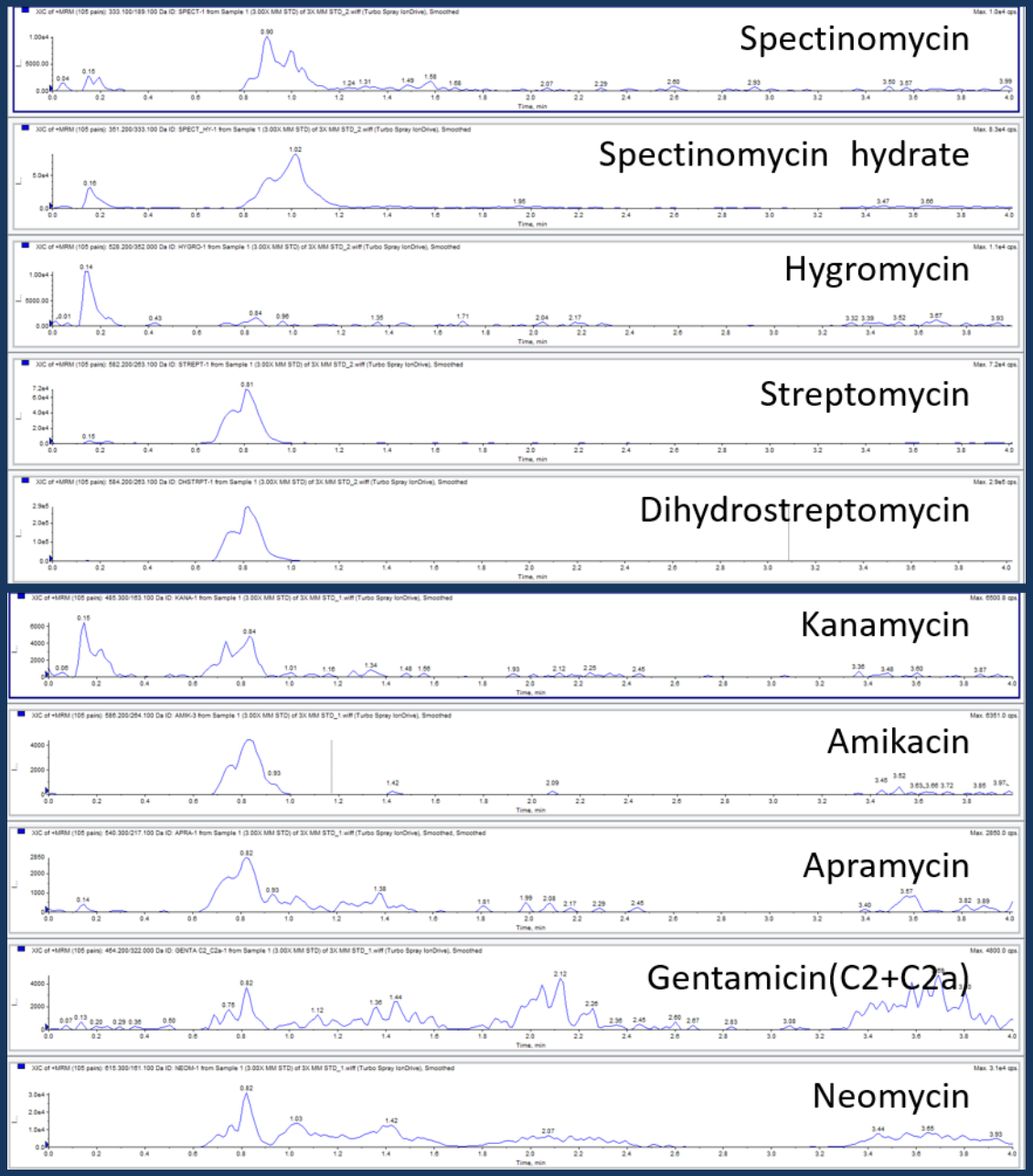


Quinolones

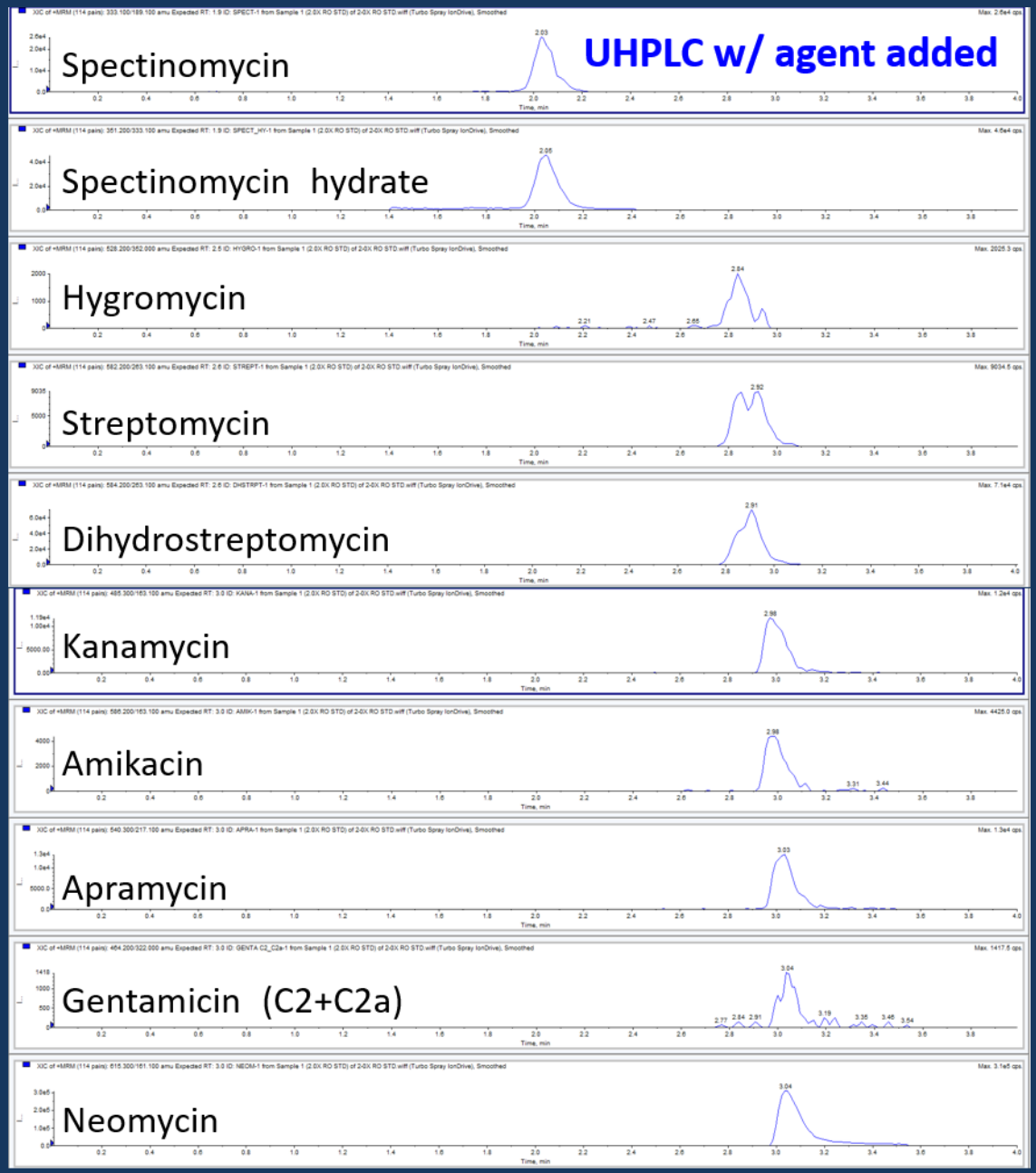


- Currently, 219 vet. drugs (including >100 antibiotics) are on our list, but have targeted and evaluated ≈180 so far in (UHP)LC-MS/MS.

UHPLC-MS/MS of AMGs w/o Ion-Pairing Agent

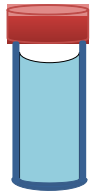


50 mM sodium 1-heptanesulfate in final extract



Updated Vet. Drug Residue Method for FSIS

Aminoglycosides	Multiclass, Multiresidues
2 g tissue + 20 mL of 10 mM NH_4OAc, 0.4 mM EDTA, 2% trichloroacetic acid, and 0.5% NaCl in water + IS	2 g tissue + 10 mL 4/1 (v/v) acetonitrile/water + IS
Shake 5 min on pulsed vortex platform shaker (80% setting, max pulsation)	
Centrifuge 3 min at 3700 rcf	
Transfer 10.75 mL (1 g equiv. sample) to 15 mL tube	Centrifuge 3 min at 3700 rcf
Adjust pH to 6.5 ± 0.1 using a pH meter	Tissue equivalence 0.174 g/mL
Condition 50 mg WCX* DPX† tips with 3 mL each of methanol and water	**(no cleanup)**
Load extract in 3 portions onto 50 mg WCX DPX tips	407 µL extract (71 mg sample equiv.)
Wash DPX tips with 5 mL water	
Elute DPX tips with 1 mL 10% formic acid in water	71 µL extract (71 mg sample equiv.)



+ 272 µL 138 mM sodium 1-heptanesulfate ion-pairing (IP) reagent in water/acetonitrile

*__WCX = weak cation exchange sorbent__

†__DPX = dispersive pipette extraction__

Yields 95 mg/mL final extract for each method in 34/66 (v/v) acetonitrile/water containing 50 mM IP reagent and 0.85% HO_2CH → 4 µL injection = 0.38 mg equiv. sample on column

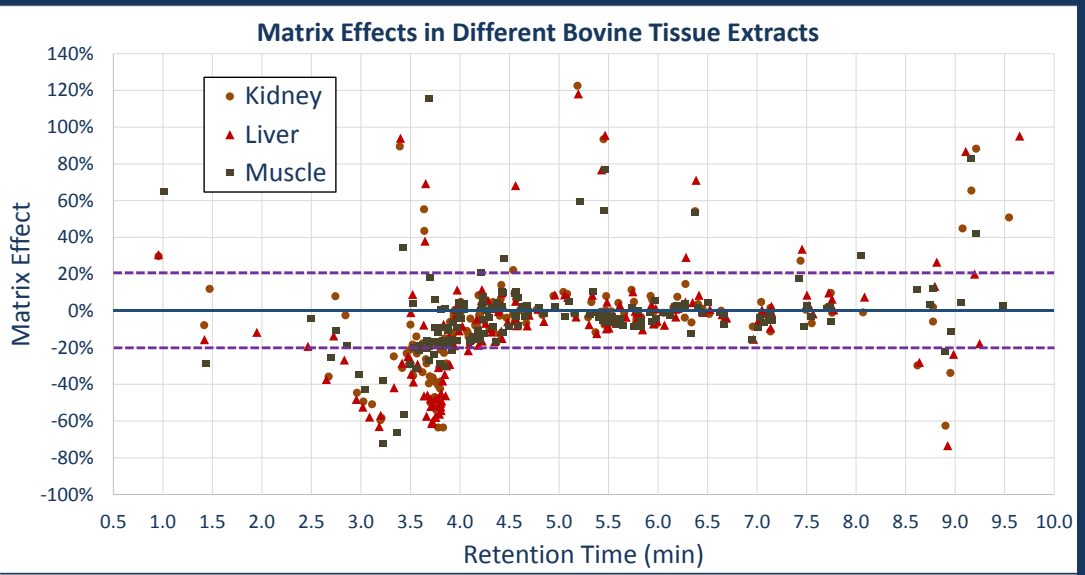
Validation Results

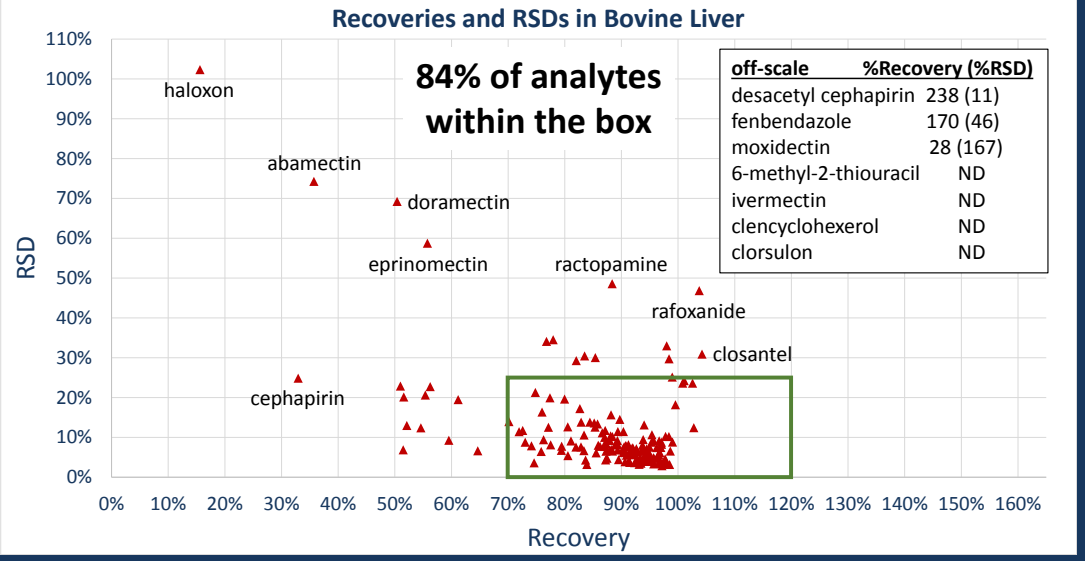
Table 1: Results for the veterinary drugs spiked at 0.5X, 1X, and 2X levels, n=10 each, in the bovine tissues; (t_R = retention time); aminoglycosides in blue text. Red = <50 or >150% Recovery or >40% RSD

Gold = 80-110% Recovery, ≤15% RSD | Silver = 70-120% Recovery, ≤25% RSD | Bronze = 50-150% Recovery, ≤40% RSD

Drug Analyte	t_R (min)	1X Level (ng/g)	Kidney	Liver	Muscle
13C6-Sulfamethazine	3.75	200			
2-Mercaptobenzimidazole	3.66	25			
2-Mercapto-1-methylimidazole	1.95	200			
Quinoxyaline-2-caboxylic acid	3.82	100			
2-Thiouracil	0.96	400			
Abamectin (Avermectin B1a)	8.80	50			
Albendazole-2-amino sulfone	3.81	50			
Albendazole sulfoxide	4.13	50			
Albendazole	5.45	50			
Albendazole sulfone	4.57	50			
Amikacin	3.71	100			
Amoxacillin	3.50	50			
Ampicillin	3.89	20			
Apramycin	3.78	100			
Acetopromazine	5.09	10			
Azaperone	4.21	10			
Bacitracin	4.68	1000			
Beclomethasone	6.07	100			
Betamethasone	5.96	100			
Bithionol	8.09	10			
Bromchlorobuterol	4.29	10			
Brombuterol	4.35	10			
Cambendazole	4.55	10			
Chloramphenicol	4.72	50			
Carazolol	4.43	10			
Carbadox	3.74	30			
Carprofen	6.97	50			
Cefazolin	3.81	100			
Cephapirin	3.48	100			
Cimaterol	3.57	10			
Ciprofloxacin	3.96	50			
Clencyclohexerol	3.88	10			
Clenbuterol	4.22	10			
Clenbuterol-d9	4.20	200			
Clenpenterol	4.43	10			
Clindamycin	4.58	100			
Clorsulon	4.54	100			
Closantel	8.82	50			
Cloxacillin	6.20	10			
Chlorpromazine	5.58	10			
Cortisone	5.48	100			
Chlortetracycline	4.39	1000			
Danofloxacin	3.99	200			
Dapson	3.86	100			
DCCD	3.40	400			
Desacetyl-cephapirin	2.65	100			
Desethylene ciprofloxacin	3.86	100			
Diclofenac	7.10	200			
Dicloxacillin	6.53	100			
Difloxacin	4.17	50			
Dipyrone (metabolite)	3.64	200			
Dimetridazole	3.19	50			
Dimetridazole-hydroxy	2.73	50			
Doramectin	8.99	100			
Doxycycline	4.56	100			
Dihydrostreptomycin	3.66	500			
Emamectin B1a	7.14	50			
Enrofloxacin	4.03	100			
Eprinomectin	8.64	100			
Erythromycin A	5.20	100			
Fenbufen	6.46	50			
Fenbendazole	6.18	400			
Fenbendazole sulfone	5.17	400			
Fenoterol	3.67	50			
Florfenicol	4.31	300			
Florfenicol Amine	3.09	300			
Flubendazole	5.68	10			
Flubendazole-2-amino	4.43	10			
Flumethasone	5.85	100			
Flumequine	5.62	300			
Flunixin	6.69	25			
Flunixin-d3	6.69	200			
Gamithromycin	4.56	100			
Gentamicin C1	3.80	300			
Gentamicin C1a	3.81	300			
Gentamicin C2+C2a	3.81	300			
Haloperidol	4.96	10			
Haloxon	6.65	100			
Hygromycin	3.64	100			
Indoprofen	5.94	50			
Ipronidazole	4.58	10			
Ipronidazole-hydroxy	3.95	10			
Ivermectin	9.25	50			
Josamycin	5.82	100			
Kanamycin	3.72	100			
Ketoprofen	6.28	50			
Lasalosid A	9.65	100			
Levamisole	3.83	100			

Drug Analyte	t_R (min)	1X Level (ng/g)	Kidney	Liver	Muscle
Lincomycin	3.78	100			
Mabuterol	4.42	10			
Marbofloxacin	3.85	100			
Mebendazole	5.47	10			
Mebendazole-2-amino	4.32	10			
Meclofenamic acid	7.53	200			
Meloxicam	6.42	100			
6-Methyl-2-thiouracil	1.36	400			
Melengesterol acetate	7.57	25			
Morantel	4.22	100			
Moxidectin	8.93	100			
Metronidazole	2.83	10			
Metronidazole-hydroxy	2.47	10			
Nafcillin	6.39	100			
Nalidixic acid	5.48	200			
Naproxen	6.35	100			
Neomycin	3.84	1000			
Niclosamide	7.76	10			
Niflumic acid	7.15	200			
Nitroxynil	5.75	50			
Norfloxacin	3.91	50			
Novobiocin	7.78	1000			
Oxyphenylbutazone	6.18	100			
Orbifloxacin	4.10	50			
Oxytetracycline	3.96	1000			
Oxacillin	5.98	100			
Oxbendazole	4.63	10			
Oxyclozanide	7.46	10			
Oxfendazole	4.70	800			
Phenylbutazone	7.05	100			
Phenylbutazone-d10	7.02	200			
Penicillin G	5.47	50			
Penicillin G d7	5.43	200			
6-Phenyl-2-thiouracil	4.23	400			
Pirlimycin	4.48	300			
Piroxicam	5.77	100			
Propionylpromazine	5.48	10			
Prednisone	5.38	100			
Prednisolone	5.51	100			
Promazine	5.06	10			
Procaterol	3.58	100			
Propyphenazone	5.80	100			
6-Propyl-2-thiouracil	3.53	50			
Pyrantel	3.97	100			
Ractopamine	3.98	30			
Ractopamine-d3	3.96	200			
Rafoxanide	9.11	10			
Ritodrine	3.76	10			
Ronidazole	2.96	10			
Salbutamol	3.51	10			
Sarafloxacin	4.18	50			
Sulfabromomethazine	5.54	100			
Sulfachloropyridazine	4.09	100			
Sulfadiazine	3.02	100			
Sulfadimethoxine	4.79	100			
Sulfadoxine	4.26	100			
Selamectin	9.20	200			
Sulfaethoxypyridazine	4.42	100			
Sulfisoxazole	4.35	100			
Sulfamethizole	3.72	100			
Sulfamethoxypyridazine	3.79	100			
Sulfamerazine	3.42	100			
Sulfamethoxazole	4.19	100			
Sulfamethazine	3.76	100			
Sulfanilamide	1.42	100			
Sulfanitran	5.49	100			
Spectinomycin	3.52	100			
Sulfapyridine	3.34	100			
Sulfaquinoxaline	4.85	100			
Streptomycin	3.65	500			
Sulfathiazole	3.20	100			
Thiabendazole	3.87	100			
5-Hydroxythiabendazole	3.71	100			
Tetracycline	4.03	1000			
Triclabendazole	7.51	50			
Triclabendazole sulfoxide	7.15	50			
Triflupromazine	5.79	10			
Tildipirosin	3.90	500			
Tilmicosin	4.64	100			
Tiamulin	5.31	600			
Tobramycin	3.78	500			
Tolfenamic acid	7.73	200			
Tulathromycin	4.11	1000			
Tylosin	5.34	200			
Virginiamycin M1	6.28	100			
Xylazine	4.22	10			
Zeranol	5.99	100			
Zilpaterol	3.51	12			





Multi-Application, Multiresidue Analysis

Goal: Develop a multi-class, multi-residue method for analysis of pesticides as well as legacy and emerging environmental contaminants in food:

- Pesticides
- Polychlorinated biphenyls (PCBs), including dioxin-like PCB congeners
- Polycyclic aromatic hydrocarbons (PAHs)
- Polybrominated diphenyl ethers (PBDEs)
- Novel alternate flame retardants (FRs)

High Throughput Efficiency Start-to-Finish

1) Sample processing (Blixer → 2-5 g test portions)

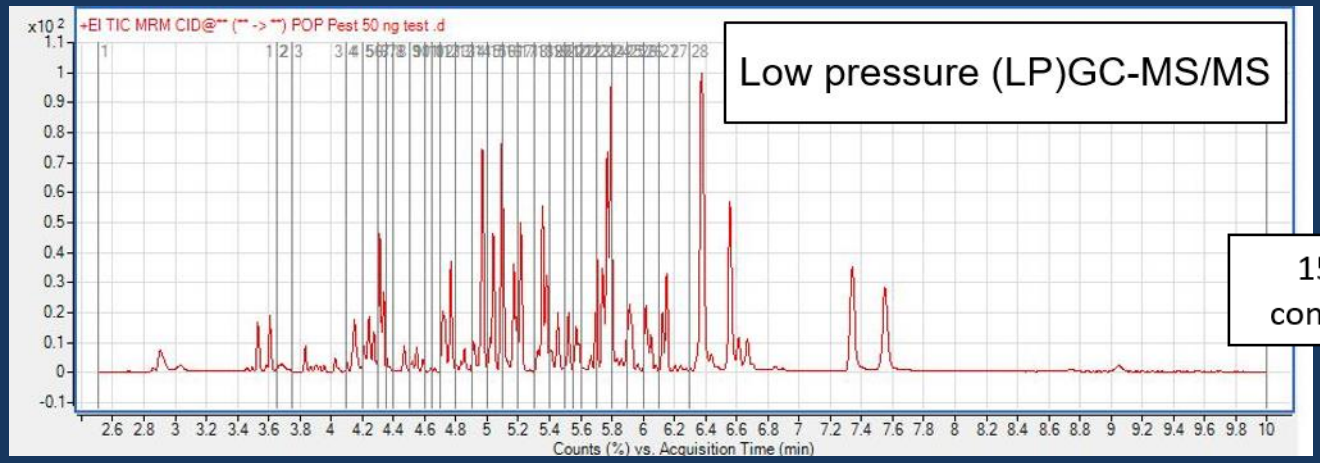
2) QuEChERS batch extraction by platform pulsed vortexing followed by centrifugation

3a) UHPLC-MS/MS analysis of LC-amenable analytes

3b) Automated cleanup + fast, low-pressure (LP) GC-MS/MS

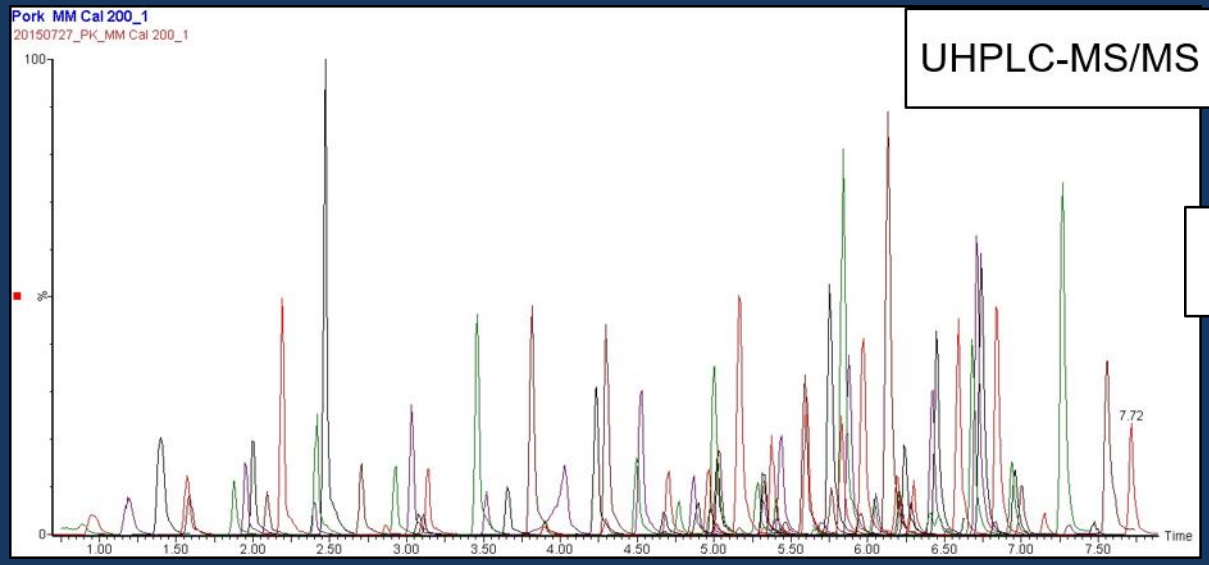
4a+b) Trustworthy automatic peak integrations and analyte identifications without human review

>240 Analytes in Parallel 10 min Analyses



Low pressure (LP)GC-MS/MS

150 pesticides, > 50 environmental contaminants & internal/QC standards



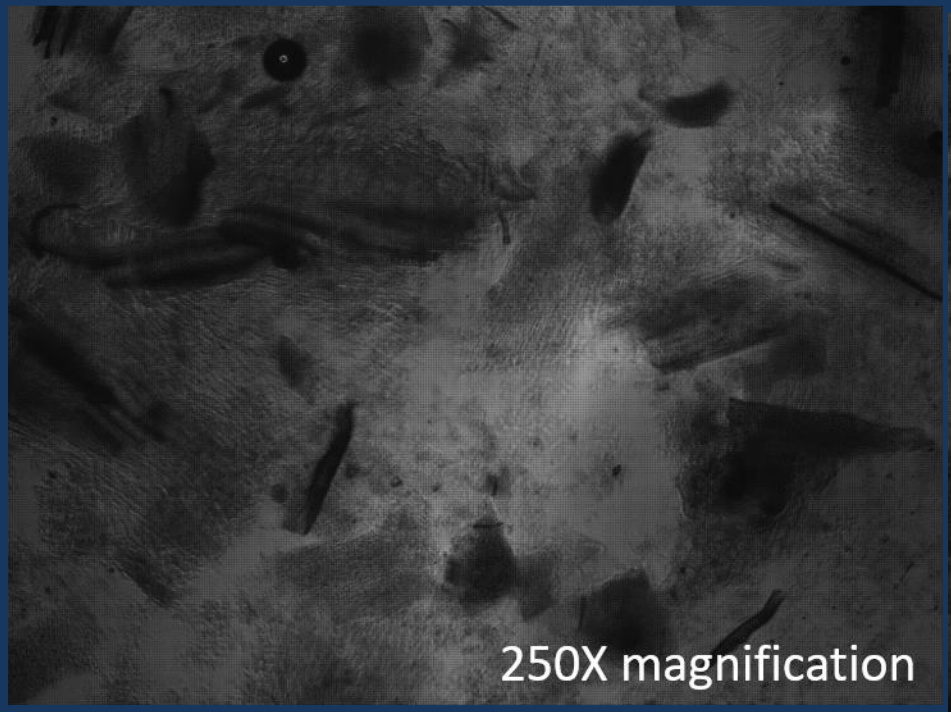
UHPLC-MS/MS

55 overlapping pesticides

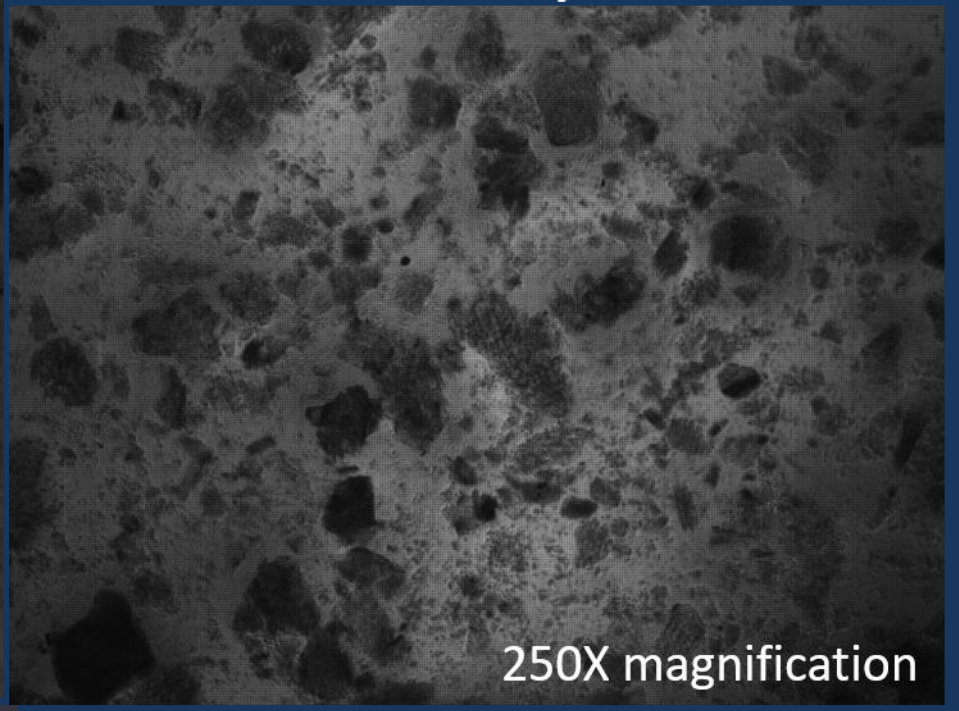
99 pesticides & internal/ QC standards

Comminuted Broccoli

Blixer



Blixer + Cryomill

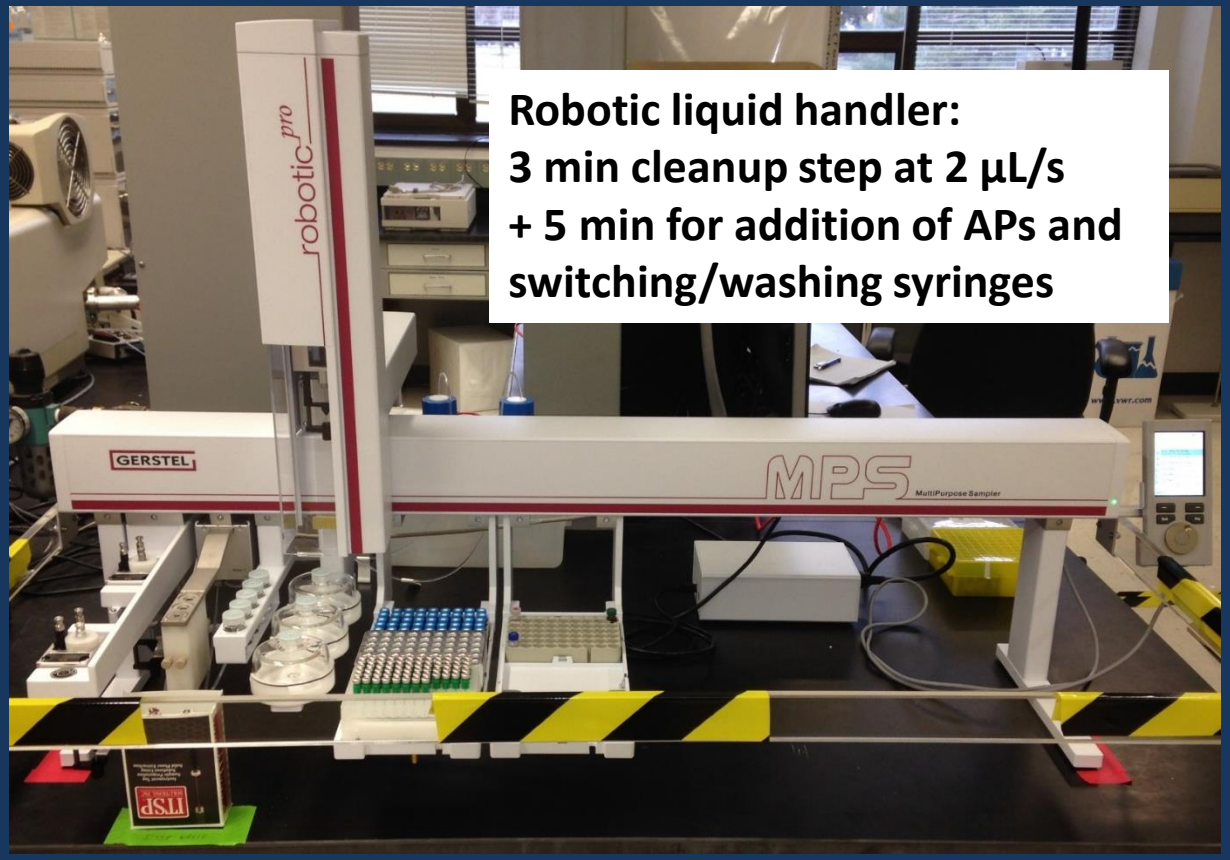


Robot Coupe Blixer has a spatula in the lid to ease and improve comminution

Conclusion: Cryomill = Overkill

Instrument Top Sample Preparation (ITSP)

Determined performance results in the use of automated mini-SPE cleanup in the LPGC-MS/MS analysis of pesticides and other contaminants in QuEChERS extracts of 10 different matrices.



Robotic liquid handler:
3 min cleanup step at 2 µL/s
+ 5 min for addition of APs and switching/washing syringes

Used mini-cartridges showing removal of chlorophyll and other matrix components

Final extract volumes = 278 ± 5 µL (n = 255) after 50 µL addition of APs (and/MeCN) solution

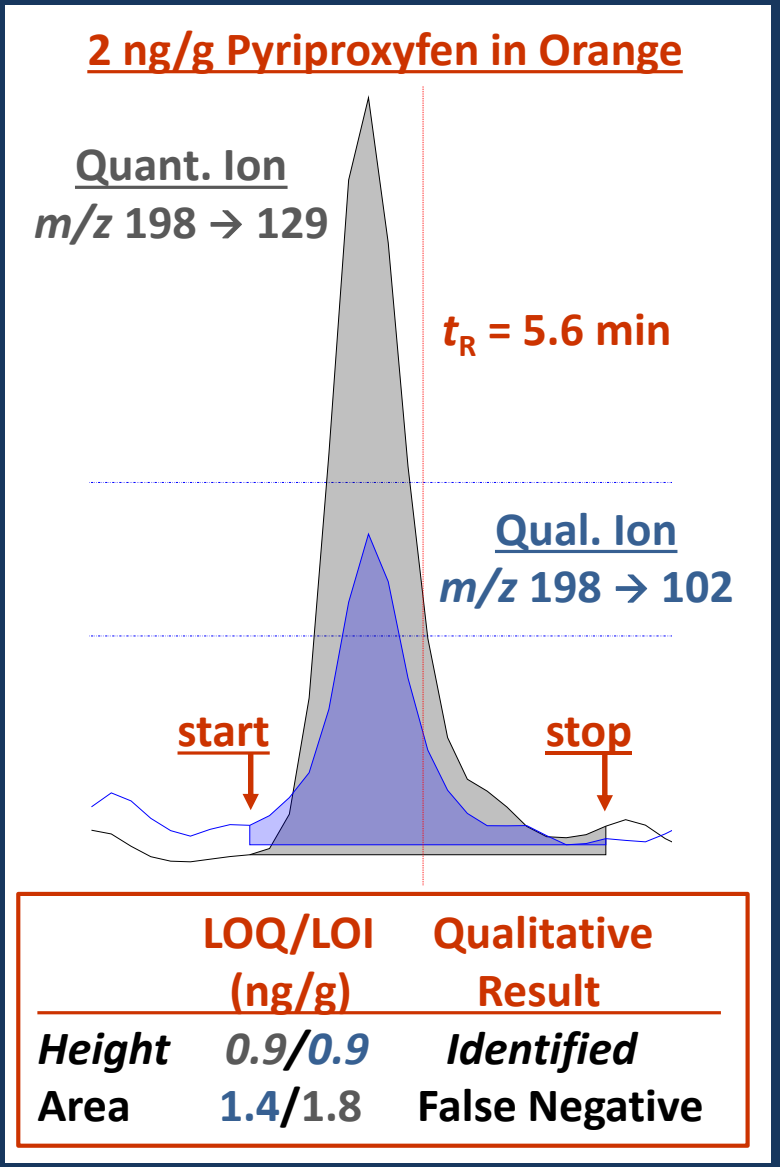
ITSP+LPGC-MS/MS takes 13 min per injection cycle

Agilent 7010 enabled 1:9 split injection (0.1 mg sample equivalent) rather than 10-fold higher amount to still achieve <10 ng/g LOQs and LOIs (quantification and identification) for nearly all analytes in LPGC-MS/MS, entailing hundreds of injections over many days before maintenance is needed.

Summation Integration in Chromatography

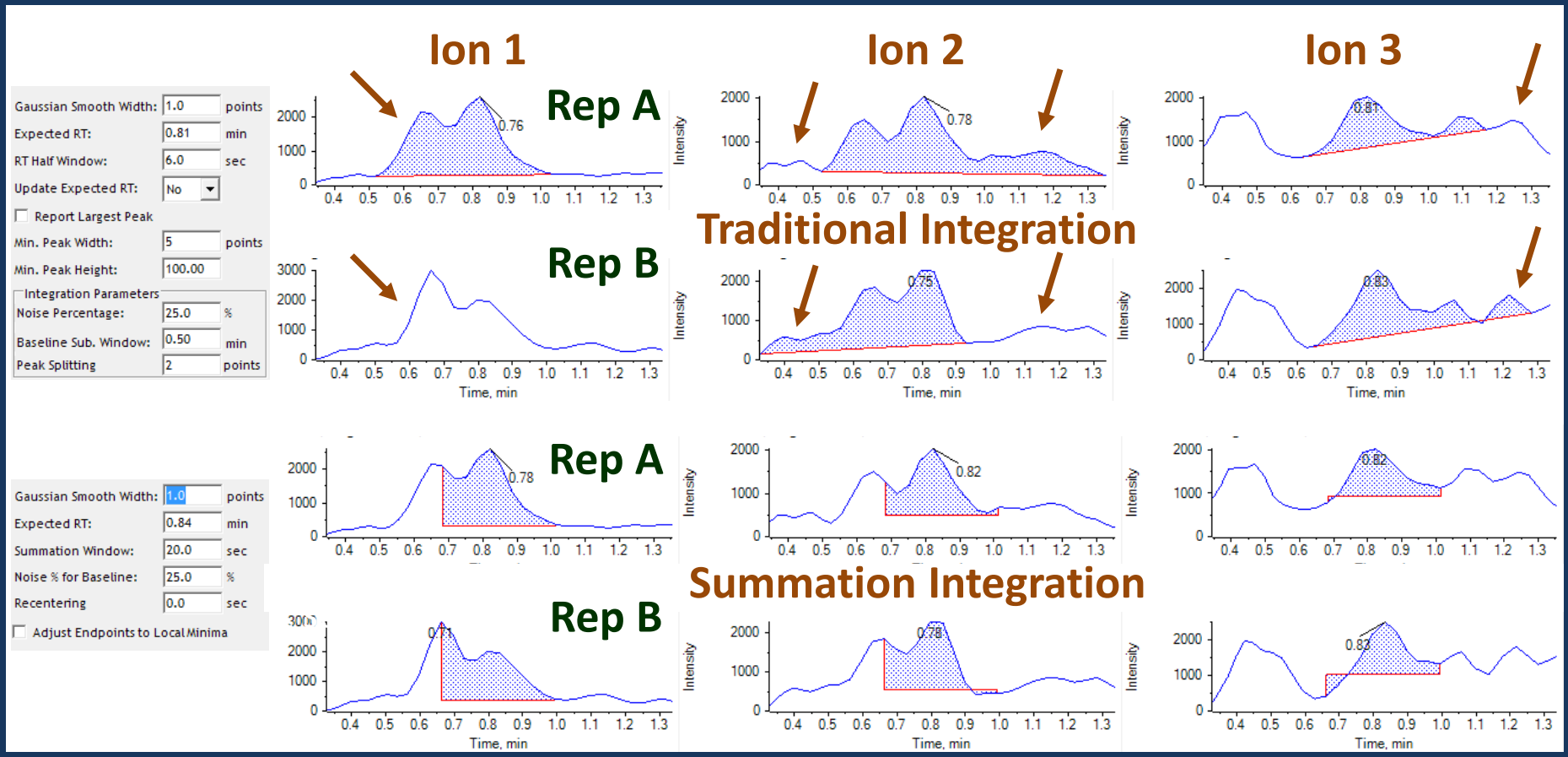
SIMPLIFY, don't COMPLIFY!

- Draw a straight line at the baseline just before the start of the expected peak to just after its expected end → **EASY PEASY!**
- *e.g.* Elkin *et al.* "Computer-controlled mass fragmentography with digital signal processing" *J. Chromatogr.* **81** (1973) 47-55
- **Advanced ≠ Better**
- **Function ≠ Beauty**

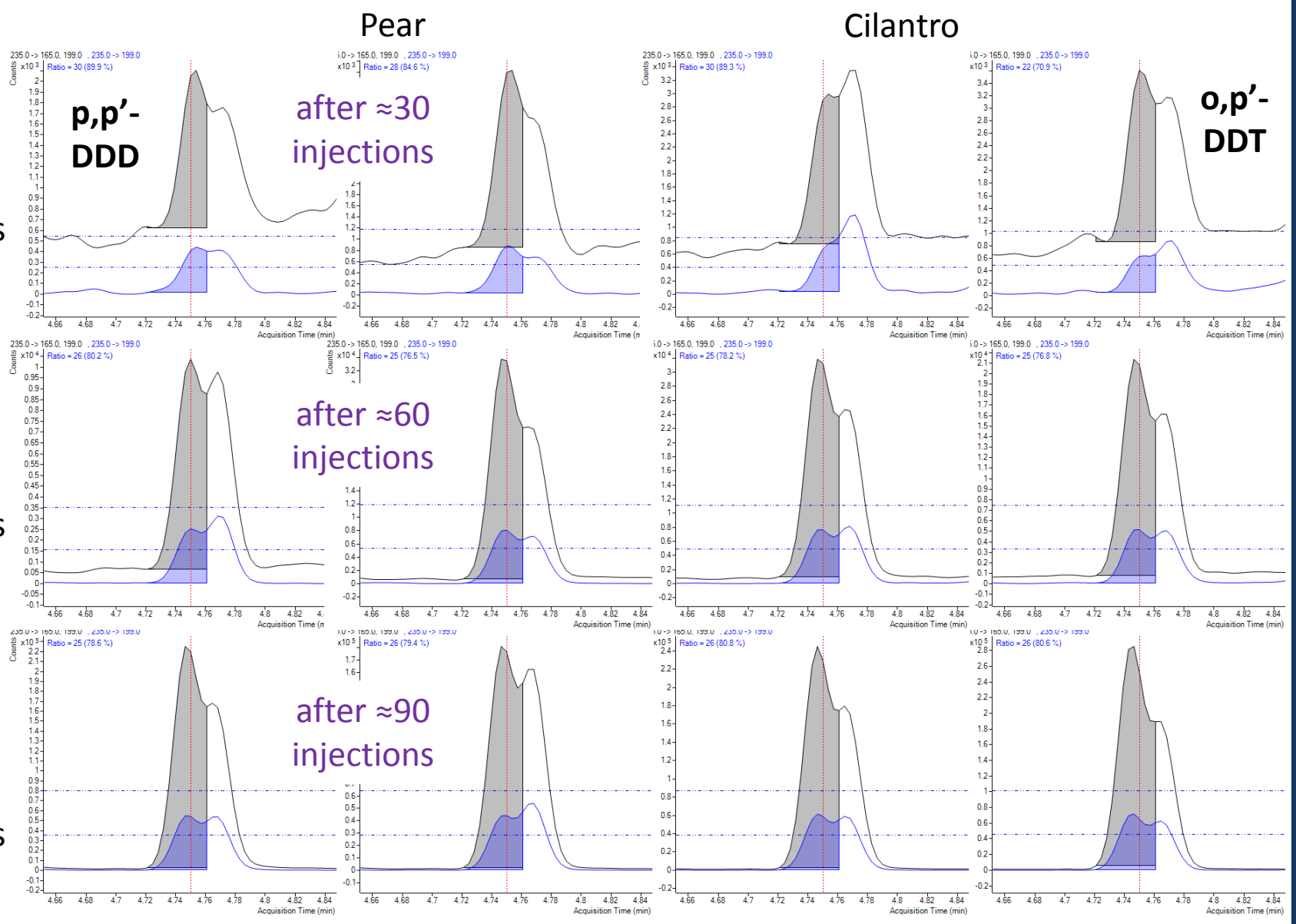


	LOQ/LOI (ng/g)	Qualitative Result
Height	*0.9/0.9*	*Identified*
Area	1.4/1.8	False Negative

Summation integration is consistent and reliable



p,p’-DDD and o,p’-DDT partially co-elute but can be consistently integrated individually



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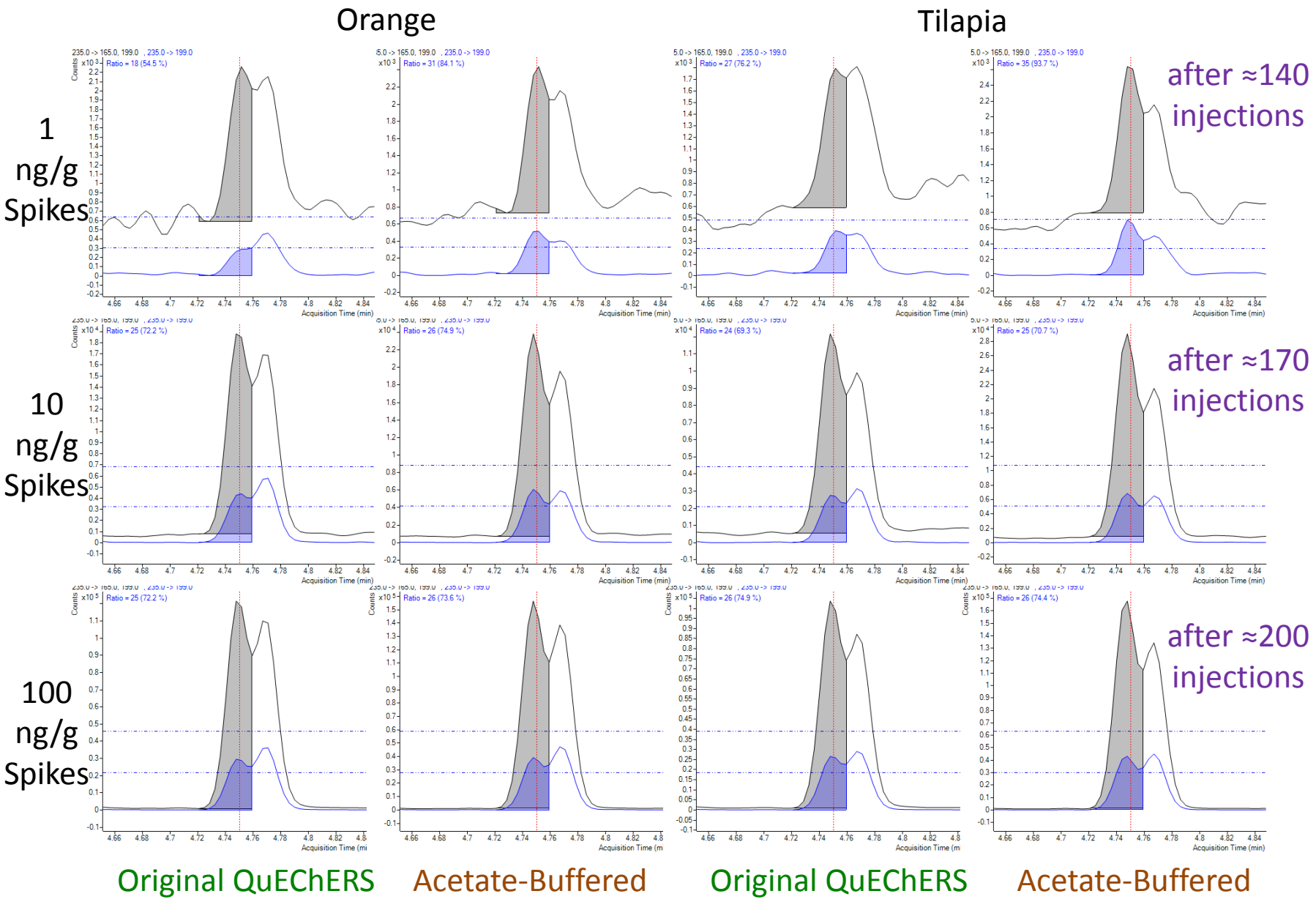

Orange
Tilapia
1 ng/g Spikes
10 ng/g Spikes
100 ng/g Spikes
after ≈140 injections
after ≈170 injections
after ≈200 injections
Original QuEChERS
Acetate-Buffered
Original QuEChERS
Acetate-Buffered

Rules in Automatic Post-Run Identification (*e.g.* in Excel or Instrument Software)

1) Ret. time (t_R) for each ion (Quant. and Qual.) must be ≤|0.1| min from the contemporaneous t_R(ref.)

2) Ion Ratio (IR) = (peak area ion 2)/(peak area ion 1), 3/1, 4/1, *etc.* (in %); IR(ref.) and t_R(ref.) = avg. of contemporaneous high conc. calibration stds in solvent **[note: IR(ref.) ≤ 110%]**

IR must be |±10| for ≥1 ion or |±20| for ≥2 ions vs. IR(ref.)

3) Conc. must be > reporting level

Conclusions

- Smaller test portions are possible using the Blixer for many food samples.

- High quality, rugged results can be achieved for hundreds of ultratrace analytes in diverse foods using automated high-throughput analysis by QuEChERS + ITSP + LPGC-MS/MS and UHPLC-MS/MS without matrix-matched calibration followed by summation function chromatographic peak integrations + defined post-run processing to yield accurate determinations and identifications with minimal need for human review.

Other Current Work

- Identification and monitoring of food packaging components in processed foods
- Evaluation of EMR-Lipid, Chlorofiltr, and other sorbents for cleanup
- Analysis of seafoods for veterinary drugs and other chemical contaminants
- Analyses to establish Certified Reference Material for veterinary drugs in bovine muscle
- Interlaboratory study report on rapid method to monitor inorganic arsenic in rice
- Flow-injection analysis for mass spectrometric detection

Speciation of trace mercury (Hg) impurities in fish oil by differential photochemical vapor generation-atomic fluorescence spectrometry (PVG-AFS)

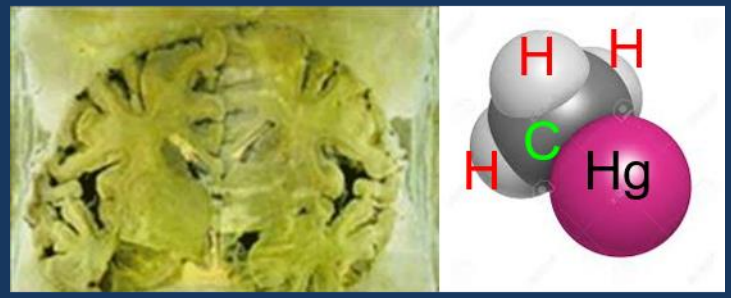
Guoying Chen and Bunhong Lai

Summary

1. Differential photochemical vapor generation using UV-B and UV-C
2. Math-based approach avoided chemical or chromatographic separation
3. 0.4% anthranilic acid in 20% formic acid was an effective PVG solution
4. Cost-effective instrumentation, operation, and chemical reagents
5. Issue: cleanup of cysteine in fish muscle that interfered in the analysis

Methylmercury ($MeHg^+$) toxicity: Minamata disease

- Chisso Factory discharged 70-150 ton $MeHg^+$ to Minamata Bay, Japan (1932-68)
- $MeHg^+$, lipophilic and hydrophilic, easily passes blood-brain and placental barriers
- $MeHg^+$ is especially damaging to brain development for fetus and children
- 11,540 fell victim by consuming local fish/shellfish, total damage: 12.6B Yen
- Symptoms:
  - uncontrollable tremors
  - loss of motor control
  - loss of auditory and visual senses
  - ataxia: loss of muscle control during voluntary movements
  - numbness in the extremities like hands and feet
  - speech impairment
  - children with congenital disease
  - paralysis, coma, even death



Damaged brain $MeHg^+$

Mother bathing a 16-year-old daughter

Minamata, Japan

Human exposure to Hg

Background

- Ubiquitous presence
- Highly toxic Class 1 metals
- Human Hg exposure: seafood consumption
- 70-95% of Hg in fish is $MeHg^{+}$
- Species-dependent toxicity: $MeHg^{+} > Hg^{++}$

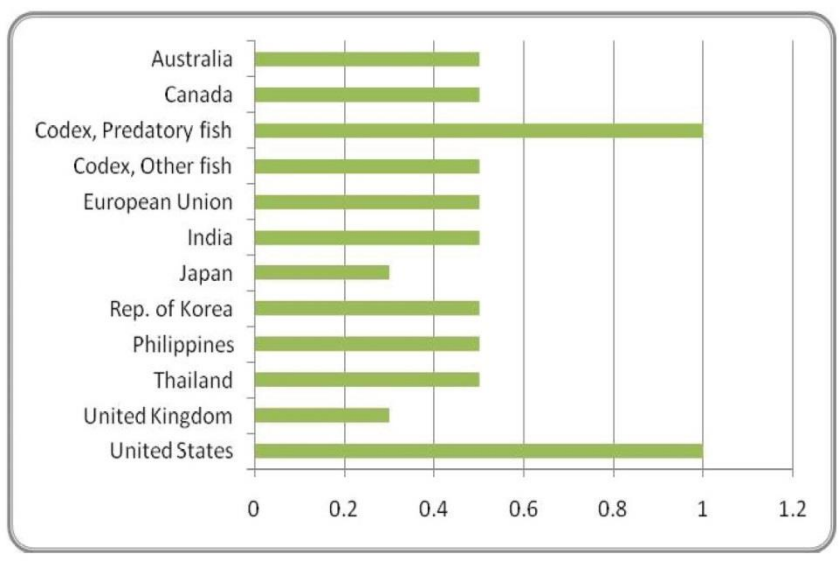
Table 4.1: Elemental Impurity Classification

	Included Elemental Impurities	Include in Risk Assessment?
Class 1	As, Pb, Cd, Hg	Yes
Class 2A	V, Mo, Se, and Co	Yes
Class 2B	Ag, Au, Tl, Pd, Pt, Ir, Os, Rh, and Ru	Yes only if intentionally added
Class 3	Sb, Ba, Li, Cr, Cu, Sn, Ni	Dependent upon route of administration – see Class 3 description
Class 4	B, Fe, Zn, K, Ca, Na, Mn, Mg, W, Al	No

Regulations

$MeHg^{+}$:

- FAO/WHO Joint Expert Committee on Food Additives (JECFA) provisional tolerable weekly intakes (PTWI): **1.6 µg/kgbw**
- JECFA warned a greater risk for pregnant/breas feeding women.
- FAO/WHO Codex Alimentarius guideline: **1 mg/kg in predatory fish; 0.5 mg/kg in other fish**
- Most countries: **0.5 mg/kg**



Hg^{++}: PTWI: **4 µg iHg/kgbw**

HPLC–ICPMS *vs.* non-HPLC-MS methods

1. HPLC-MS methods: expensive instrumentation, operation, and personnel

- Separation by HPLC: slow and expensive instrumentation
- Quantification by ICPMS: $200/sample

  Regulations only target iAs or $MeHg^{+}$; complete speciation is not needed

2. Non-HPLC-MS methods: low-cost, sensitive, rapid, green chemistry

- Separation: 1. stepwise chemical reduction
  2. mathematical approach by UV vapor generation (UVG) (green chemistry)
  3. cryogenic trapping (CT) using sorbent (green chemistry)
- Sample Introduction: cold vapor (CVG) or hydride generation (HG)
- Quantification: atomic absorption or fluorescence spectrometry (AAS or AFS)

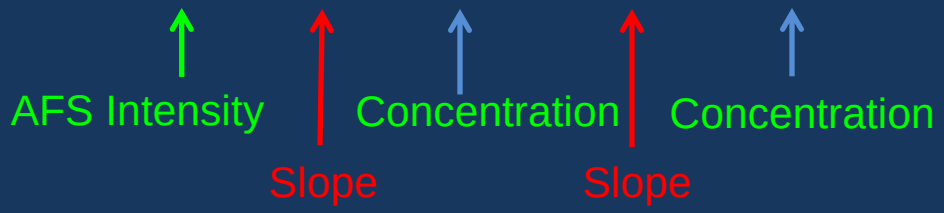
Hg^{++}/$MeHg^{+}$ speciation by PVG under UV-B and UV-C

- **Prerequisites:**
  1. Hg^{++} and $MeHg^{+}$ are the only detectable species in fish (observed globally)
  2. AFS responses are linear (under adequate conditions)
  3. AFS responses are additive (theoretically valid)
  4. Prior elimination of interfering cysteine

- **Linear equations:**

@UV-B: $$I_B = m_B[Hg^{++}] + n_B[MeHg^{+}] \quad (1)$$

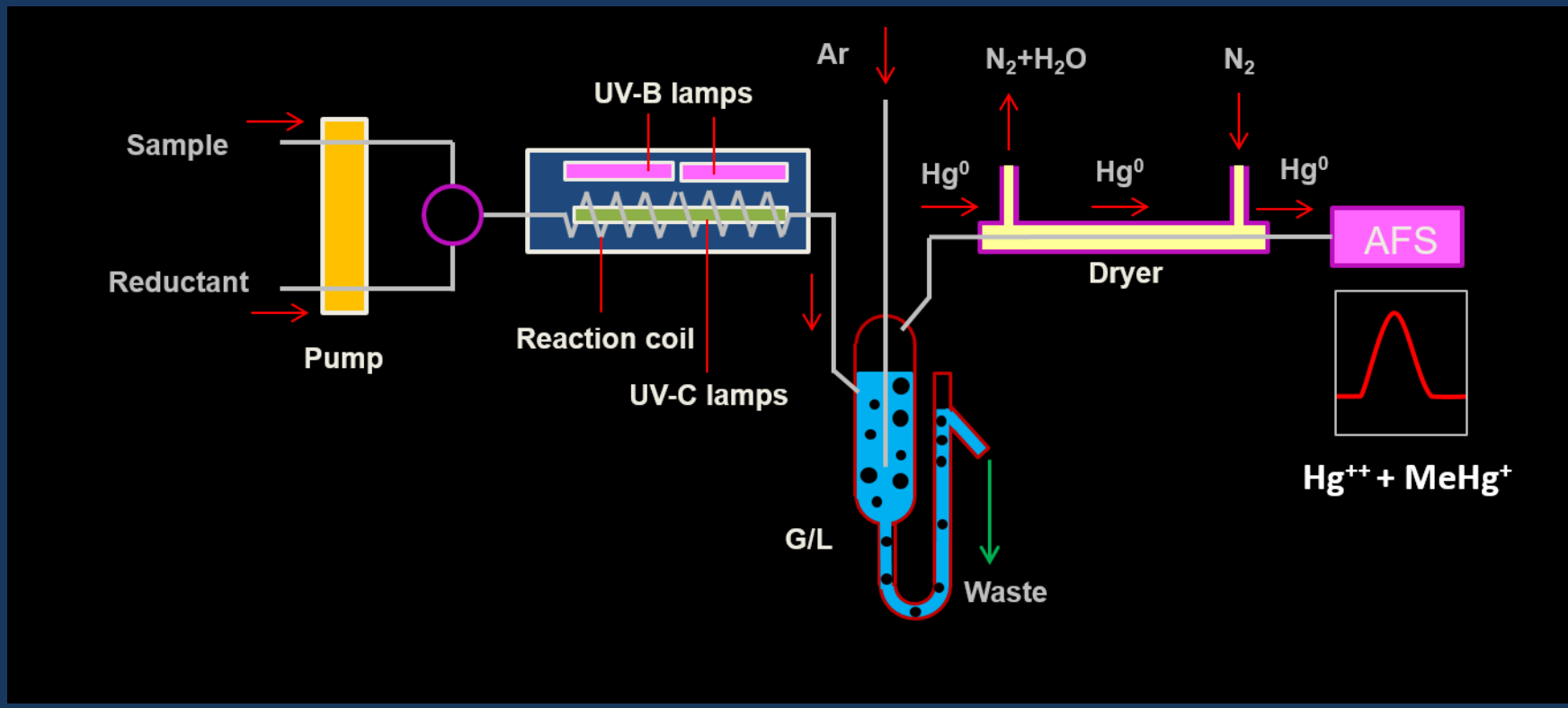
@UV-C: $$I_C = m_C[Hg^{++}] + n_C[MeHg^{+}] \quad (2)$$



Solved using junior high algebra

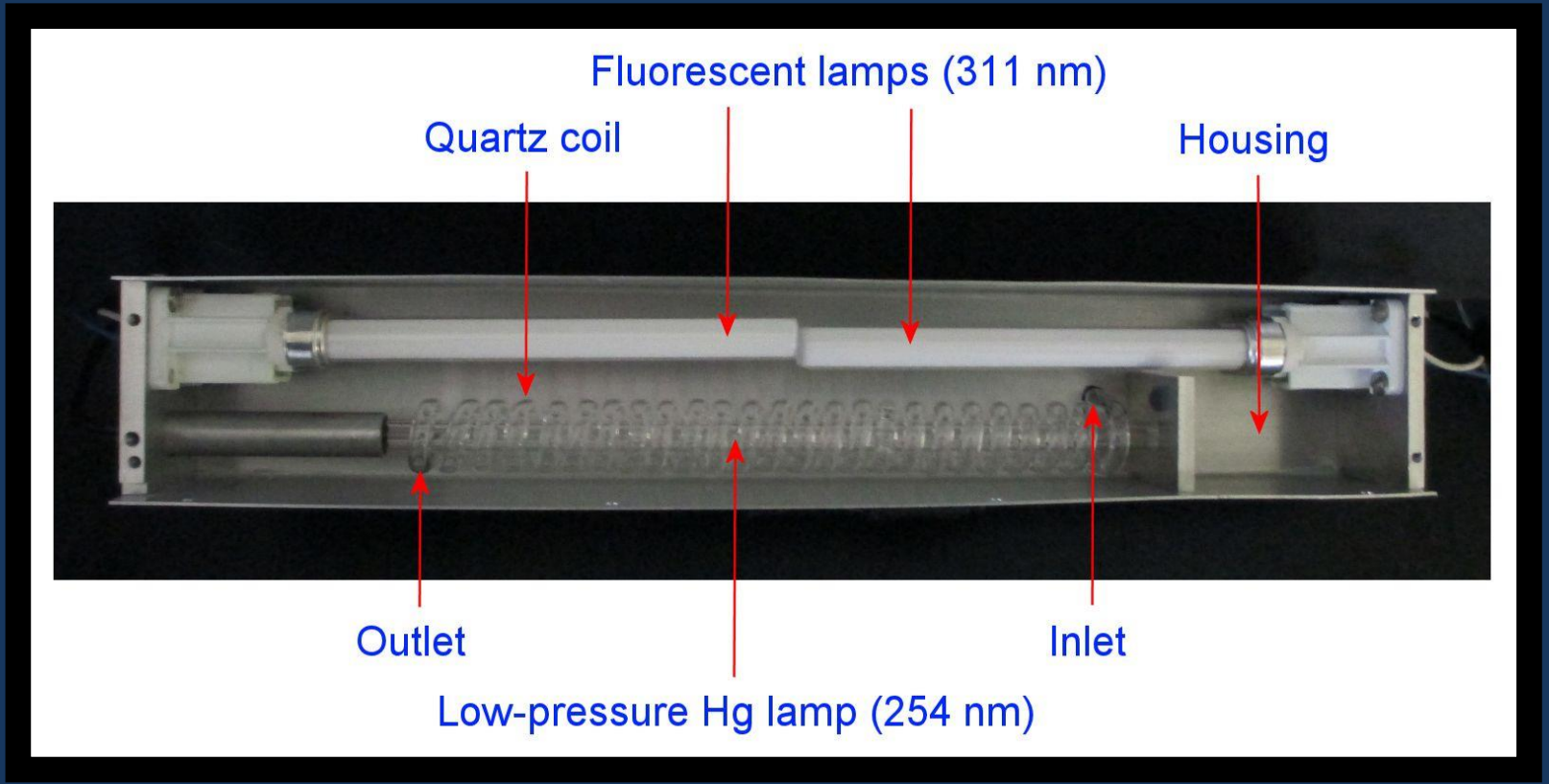
PVG–AFS instrumentation setup

- **Photoreactor coil** is illuminated by UV-C or UV-B lamps
- **Gas-liquid separator** separates Hg vapor from matrix components
- **Dryer** eliminates moisture from Hg vapor
- **AFS** registers atomic fluorescence signal



Design of a dual-source photochemical reactor

Light source	UV-B	Philips fluorescent lamp PL-S 9W/01 (311 nm)
	UV-C	UVP low-pressure mercury lamp 3SC-9 (254 nm)
Reductant	20% formic acid – 0.4% anthranilic acid	
Photoreactor	Quartz coil of 16.2 mL, 110 s exposure time	



Significance of Hg speciation in fish oil supplement

- **Health benefits of fish oil supplement**
  - Rich omega-3 fatty acids especially EPA and DHA
  - FDA approval to lower triglycerides levels
  - Benefits for >60 conditions especially cardiovascular system
  - Global fish oil production: 1-1.25 million tons (2010)
- **Raw material of fish oil**
  - Mackerel, herring, tuna, anchovy, salmon, sardine, cod liver, krill, etc.
- **Widespread concerns on impurities**
  - $MeHg^{+}$ and Hg^{++} or other environmental contaminants
- **Challenges in Hg speciation in fish oil**
  - Low-level presence at ppb to sub-ppb level
  - So far only total Hg is measured in fish oil; speciation is not carried out.
  - Speciation data will shed light on purification practice

Procedure

1. **Liquid-liquid extraction (LLE)**
   - Mix 2 mL of fish oil with 40 mL of water in a 50 mL flask
   - Shake for 10 min on a platform vortexer
   - Centrifuge at 4000 rpm for 10 min
   - Separate aqueous extract

2. **Photochemical vapor generation (PVG)**
   - Mix aqueous extract with 20% FA–0.4% AA in a quartz coil
   - Expose to 254 nm (UV-C) or 311 nm (UV-B)
   - Sweep the resulting Hg^0 vapor with high-purity Ar to gas/liquid separator (G/L)
   - Remove moisture from Hg^0 using a Nafion membrane dryer

3. **Atomic fluorescence spectrometry (AFS)**
   - Illuminate Hg^0 with a Hg hollow cathode lamp at 254 nm
   - Detect resulting resonance fluorescence with a photomultiplier tube

4. **Calculation**
   - Obtain 4 slopes from 4 calibration curves: m_B, n_B, m_C, and n_C
   - Solve the following equation set:

$$I_B = m_B[Hg^{++}] + n_B[MeHg^{+}] \quad (1)$$

$$I_C = m_C[Hg^{++}] + n_C[MeHg^{+}] \quad (2)$$

Performance and results

1. **Ultra-High sensitivity**
   - LOD: Hg^{++}: 0.3 ng/mL; $MeHg^{+}$: 1.0 ng/mL
   - LOQ: Hg^{++}: 1.7 ng/mL; $MeHg^{+}$: 5.6 ng/mL
2. **Rapid LLE with reasonable recoveries**
   - $MeHg^{+}$: ≈73%
   - Hg^{++}: should be higher because K_{ow} <1
3. **Green chemistry**
   - No strong or unstable reductant
   - No strong acid or base for digestion
4. **Ultralow Hg impurities**
   - Average total Hg = 2.54 ng/mL
   - $MeHg^{+}/Hg^{++}$ ratio is 3.5
5. **Conclusion:**
   - Hg binds to fish meal rather than fish oil
   - Effective purification by: (a) water washing, (b) bleaching, and (c) molecular distillation.

Hg impurities in fish oil

#	iHg	MeHg	#	iHg	MeHg
1	0.70	<LOD	21	0.30	<LOD
2	0.33	<LOD	22	0.45	<LOD
3	3.18	<LOD	23	<LOD	<LOD
4	<LOD	<LOD	24	0.99	5.07
5	0.78	<LOD	25	0.84	2.13
6	<LOD	<LOD	26	0.44	<LOD
7	<LOD	<LOD	27	0.98	6.51
8	<LOD	2.75	28	0.38	<LOD
9	0.40	<LOD	29	0.38	<LOD
10	<LOD	<LOD	30	0.57	<LOD
11	0.63	<LOD	31	<LOD	<LOD
12	0.76	<LOD	32	<LOD	<LOD
13	0.87	<LOD	33	0.58	<LOD
14	0.63	<LOD	34	0.34	<LOD
15	0.75	<LOD	35	<LOD	<LOD
16	0.94	<LOD	36	0.70	3.03
17	0.31	<LOD	37	0.36	<LOD
18	0.38	<LOD	38	<LOD	<LOD
19	0.40	<LOD	Average	0.57	1.97
20	0.38	<LOD	LOD	0.30	1.68

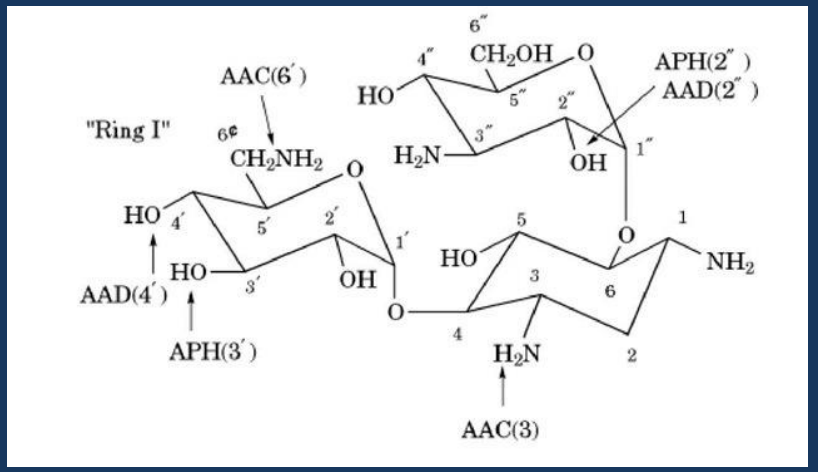
Here’s Johnny……

Rapid Detection for Aminoglycoside Resistance using UHPLC-MS/MS

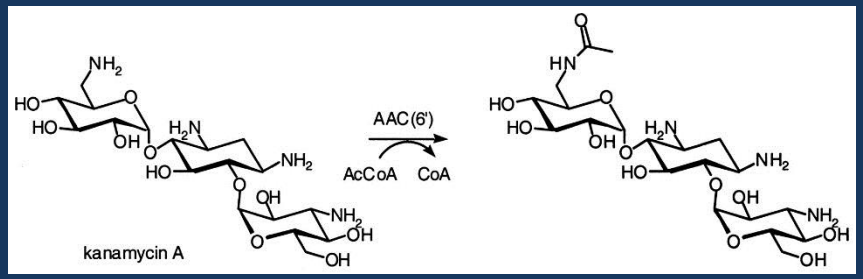
- Last couple years, HPLC-MS was employed for rapid detection of β-lactam resistance by the appearance of the modified antibiotic
- Due to the surge in resistance to aminoglycosides, another highly essential antibacterial treatment agent, we wanted to develop a rapid detection UHPLC method for modified aminoglycosides
- Currently, no reporting of LC-MS methods for modified aminoglycosides

Aminoglycoside Modification

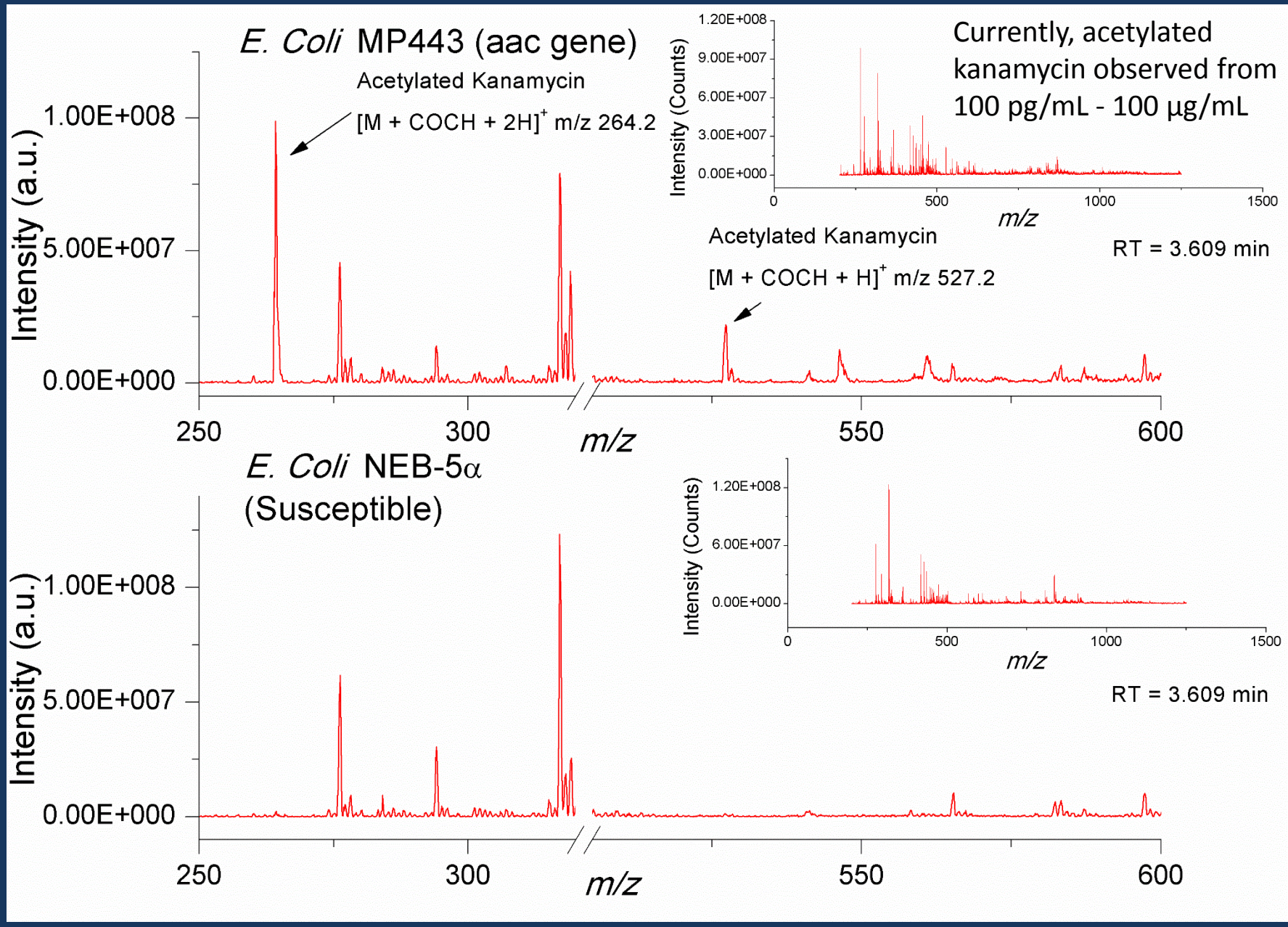
- **Acetyltransferases (AAC)**
- Phosphotransferases (APH)
- Nucleotidyltranferases (AAD)



Kanamycin + Acetyl-CoA $\xrightarrow{Acetyltransferase}$ Acetyl-Kanamycin + CoA



1. Acquired the MP443 (aac) plasmid from Dr. Martin Pavelka (University of Rochester Medical School)
2. Transformation to a competent E-Coli strain
3. Grew bacteria overnight in LB broth and diluted to an OD = 1
4. Pelleted the bacteria and inoculated 10 ug/mL Kanamycin (aq)
5. Incubated for 5 hours prior to injection of the supernatant

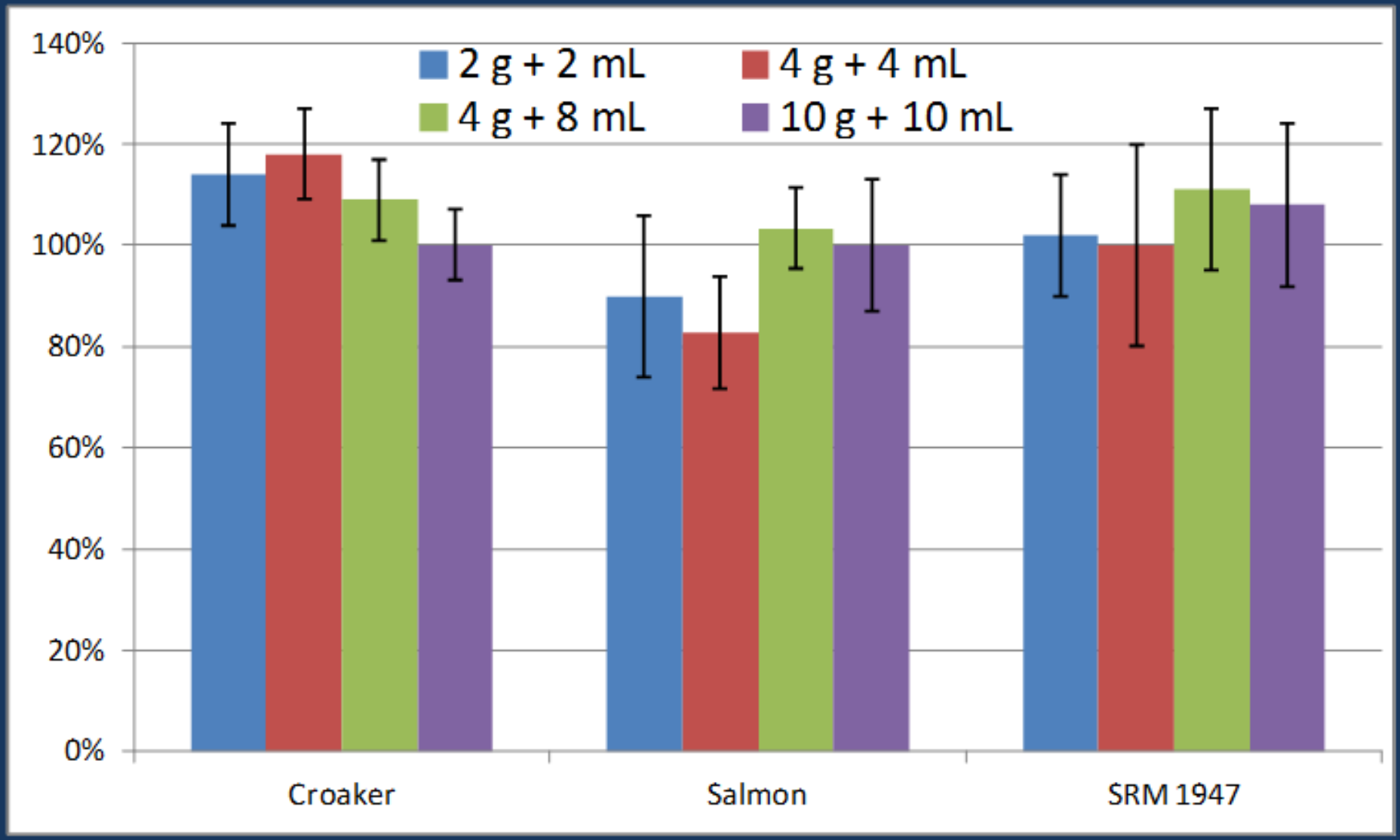

E. Coli MP443 (aac gene)
Acetylated Kanamycin
[M + COCH + 2H]+ m/z 264.2
Acetylated Kanamycin
[M + COCH + H]+ m/z 527.2
RT = 3.609 min
Intensity (a.u.)
1.00E+008
5.00E+007
0.00E+000
250
300
550
600
m/z
Intensity (Counts)
1.20E+008
9.00E+007
6.00E+007
3.00E+007
0.00E+000
0
500
1000
1500
m/z
Currently, acetylated kanamycin observed from 100 pg/mL - 100 µg/mL
E. Coli NEB-5α
(Susceptible)
RT = 3.609 min

Conclusions (2)

- Further streamlined sample preparation to “dilute and shoot”
- Feasibility of FI-MS/MS demonstrated for veterinary drug residue monitoring at tolerance levels in bovine muscle and kidney
- Refinements needed to improve results for some drugs at 10 ng/g (can we inject more equivalent sample?)
- Simple screening/identification approach devised needs further evaluation to assess rates of false positives/negatives

…. to be continued…

Sample Size and Extract Volume



Additional Future Work (2015)

Evaluate flow-injection tandem mass spectrometry (FI-MS/MS) to provide 3 min screening analysis of 130+ drugs (and other electrospray-amenable contaminants, such as many pesticides) with automatic software identifications of positives.

Investigate new automated sample cleanup tool (ITSP) for use in FI-MS/MS and QuEChERS applications.

Assess new chromatographic column stationary phases to include aminoglycosides in the same analysis as other veterinary drugs.

Possible Future Plans (2015-2020)

- Develop better methods for speciation analysis of arsenic, and mercury
- Develop rapid analytical methods for emerging contaminants of concern
- Validated criteria for identification purposes in FI-MS/MS and other types of analyses
- Collaborate in studies involving antibiotic resistance
- Investigate chemical/MS-based methods for the monitoring of biological analytes and processes (*e.g.* metabolomics, exposomics)

Updated LC-MS/MS Method Logistics

10 min sample prep for a few samples, or 1 chemist was able to process 60 pre-homogenized samples in 3 hours (for overnight LC-MS/MS run)

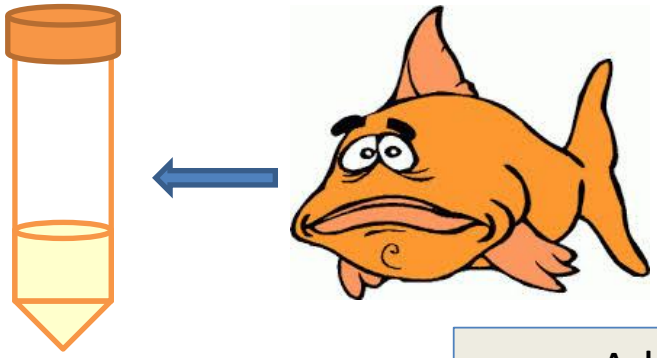
(longest steps involved labeling tubes/vials, weighing, and preparing calibration standards)

No glassware to be cleaned afterwards

Waste = 10 mL MeCN, pipet tips, and a 50 mL tube

Review of results for 135 drugs x 3 transitions x 67 injections (>27,000 data points) took 8 hours

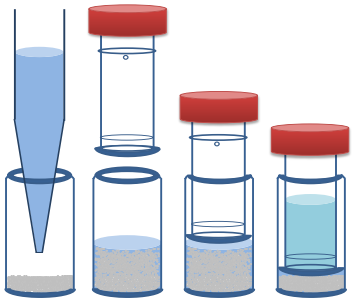
Sample Preparation



10 g homogenized fish + internal standards

Add 10 mL MeCN and shake 10 min on vortex shaker at 80% setting with max. pulsing

Add 5 g HCO_2NH_4, shake 1 min, centrifuge 2 min at 3700 rcf



Filter-vial dispersive-SPE:

- Add 0.5 mL extract to the PVDF (0.2 µm) filter-vial shell containing 75 mg each anh. $MgSO_4$ + 1/1/1 PSA/C18/Z-Sep
- Partially depress the filter-vial plunger and shake for 30 s in an autosampler vial tray
- Fully depress the plunger into the filter-vial shell

We are currently evaluating the approach for analysis of beef, pork, and chicken muscle for possible implementation by FSIS

Conclusions of Extraction Study

- 1 min extraction with the pulsed vortex shaker is sufficient for extraction of many incurred contaminants in the homogenized fish tissues, but 10 min extraction time is better as a precaution – **batch analysis of 50 samples at a time**
- Extraction with a probe blender was rapid and complete, but it limited sample throughput and was inconvenient
- 1:1 sample:MeCN (g:mL) ratio was sufficient to achieve full extraction, and 2 g homogenized sample gave equivalent results as 4 and 10 g samples
- Spiking with an int. std. does not compensate for lower extraction efficiency in incurred samples vs. spikes

Updated LC-MS/MS Method for Veterinary Drugs

	2 g tissue in a 50 mL tube add IS mix (SMZ-IS; flunixin-d3)
extraction	add 10 mL of 4/1 (*v/v*) MeCN/water vortex briefly, shake for 5 min centrifuge for 5 min >3500 rcf
clean-up	0.4 mL supernatant + 25 mg C18 in filter-vial d-SPE; vibrate AS tray for 30 s and filter through 0.2 µm PVDF by pressing plungers to seal the vials
	Inject 1 µL in LC-MS/MS

The sensitive MS/MS instrument allows >100-fold less injected sample equivalent (0.17 mg)!

3-Day Validation Experiment

Day 1:

- **Analyst 1, Reagents Lot A, 10 matrix blanks from different sources, 6 spikes at 3 levels each in 6 matrices + 4 spikes each at same levels in mixed matrices (using filter-vial d-SPE); 6-point calibration in mixed-matrix and reagent-only stds**

Days 2 & 3:

- **Analysts 2 & 3 repeat using Reagents Lot B with different sources of matrices**

Status of LC-MS/MS Study

- Validated the method using core-shell Kinetex column for 134 vet. drugs in bovine muscle (submitted paper).
- Validated method using unique “Select DA” column for 134 vet. drugs in bovine kidney, liver, and real samples (experiments completed, data review pending).
- UHPLC-MS/MS instrument was purchased, and method will be optimized for implementation by FSIS.

Chemical Residues Research Group

Guoying Chen

Steven J. Lehotay

Johnny J. Perez

Yelena Sapozhnikova

Bunhong Lai

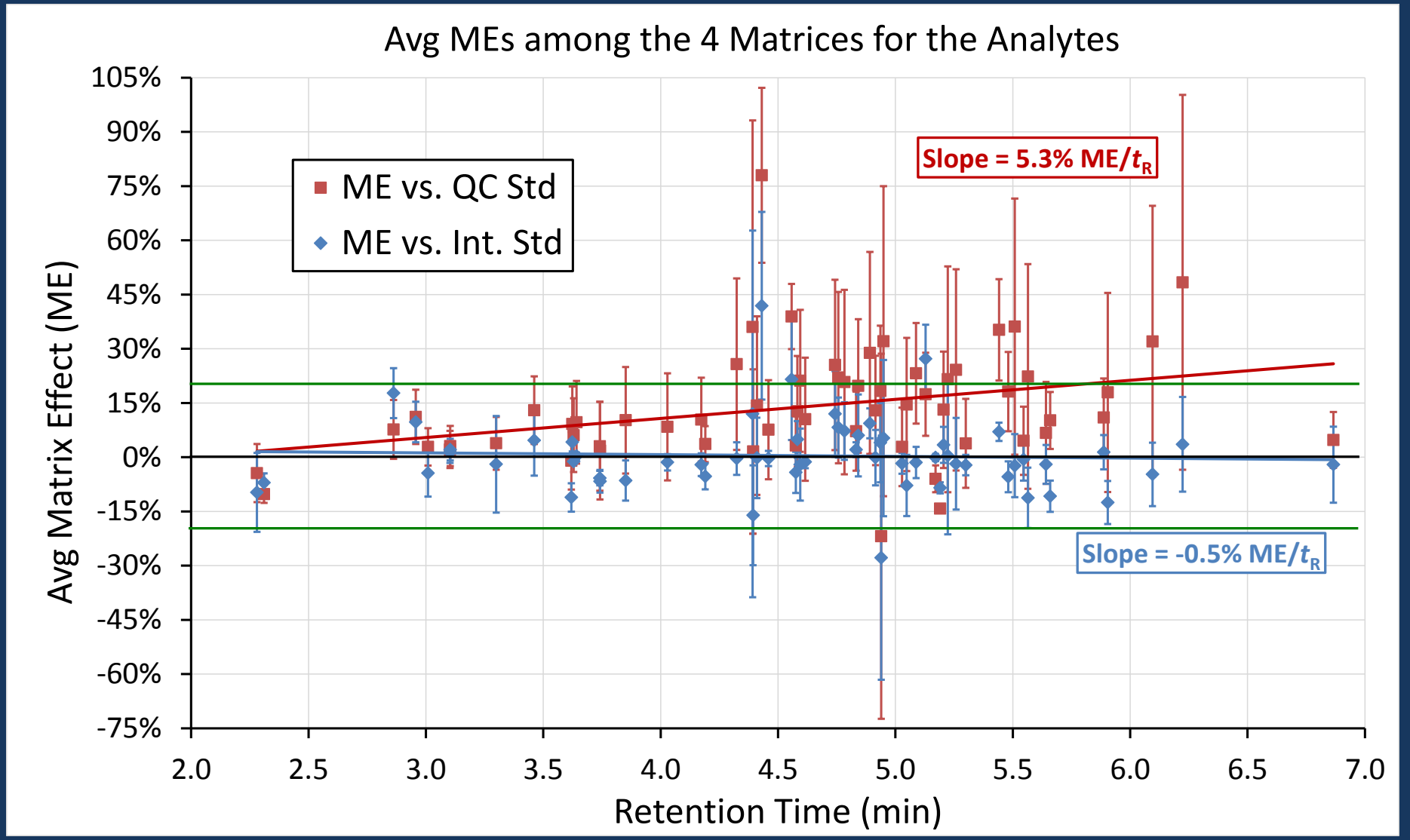
Alan R. Lightfield

Robyn Moten

Tawana Simons

Limei Yun

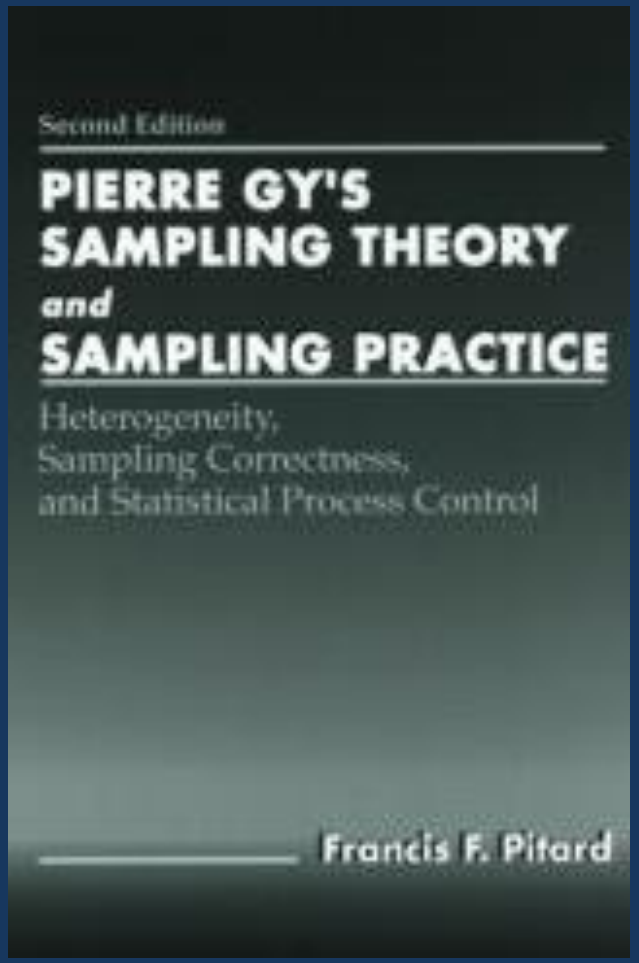
Oh, and we may have eliminated matrix effects in GC-MS …



… via use of appropriate int. stds + analyte protectants in split inj'n

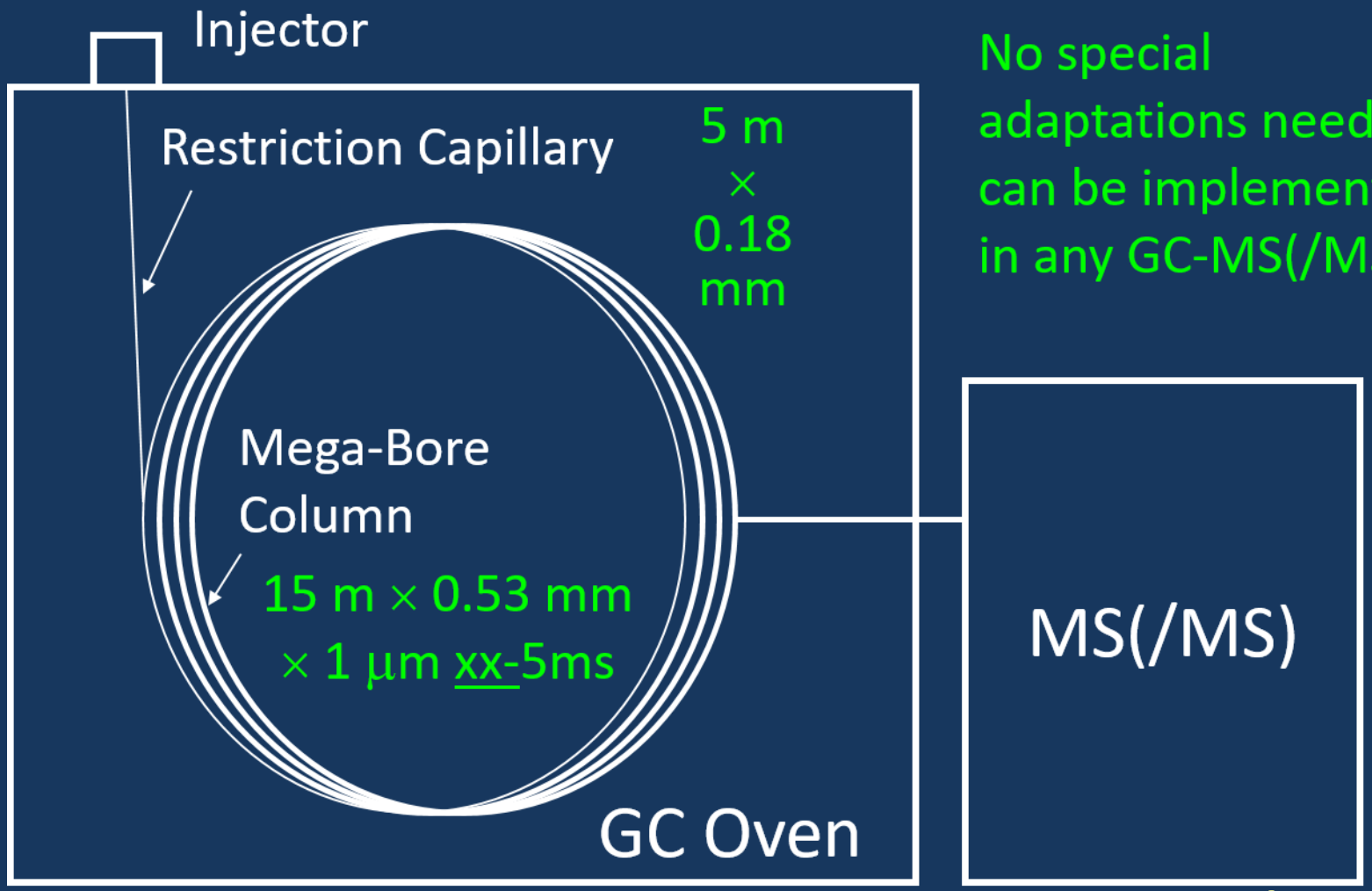
Sampling and Sample Processing

- For particulate materials
- Finite Elements
- Infinite Elements & Increments
- Compositional Heterogeneity and Fundamental Error
- Distributional Heterogeneity
- Sample Correctness and Tools



Slide adapted from Jo Marie Cook

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

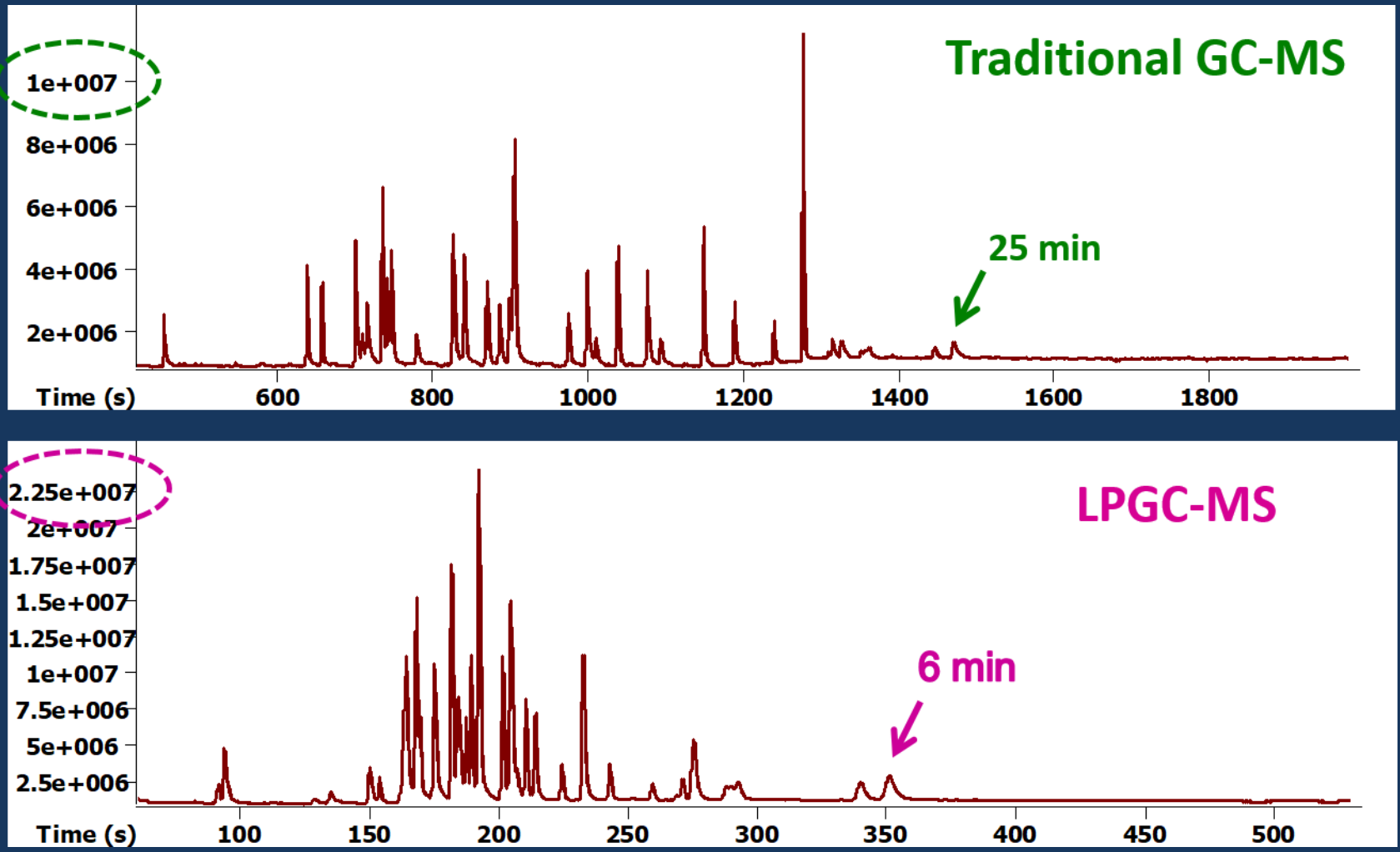


No special adaptations needed; can be implemented in any GC-MS(/MS).

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

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

LPGC-MS is Much Faster

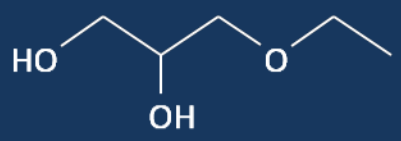


and more sensitive

Analyte Protectants

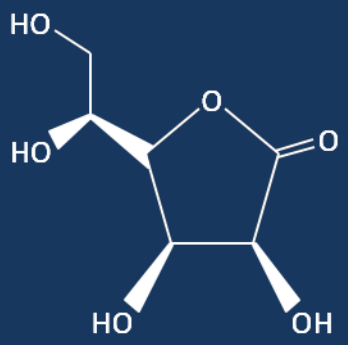
Strongly interact with active sites in GC system (inlet, column and ion source) to decrease degradation and adsorption of co-injected analytes.

Sharper peaks, less tailing, more ruggedness, lower LOD



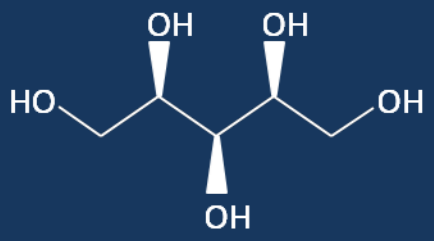
ethylglycerol

1 mg/ mL



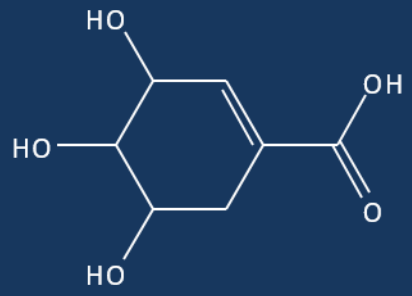
gulonolactone

0.1 mg/ mL



sorbitol

0.1 mg/ mL

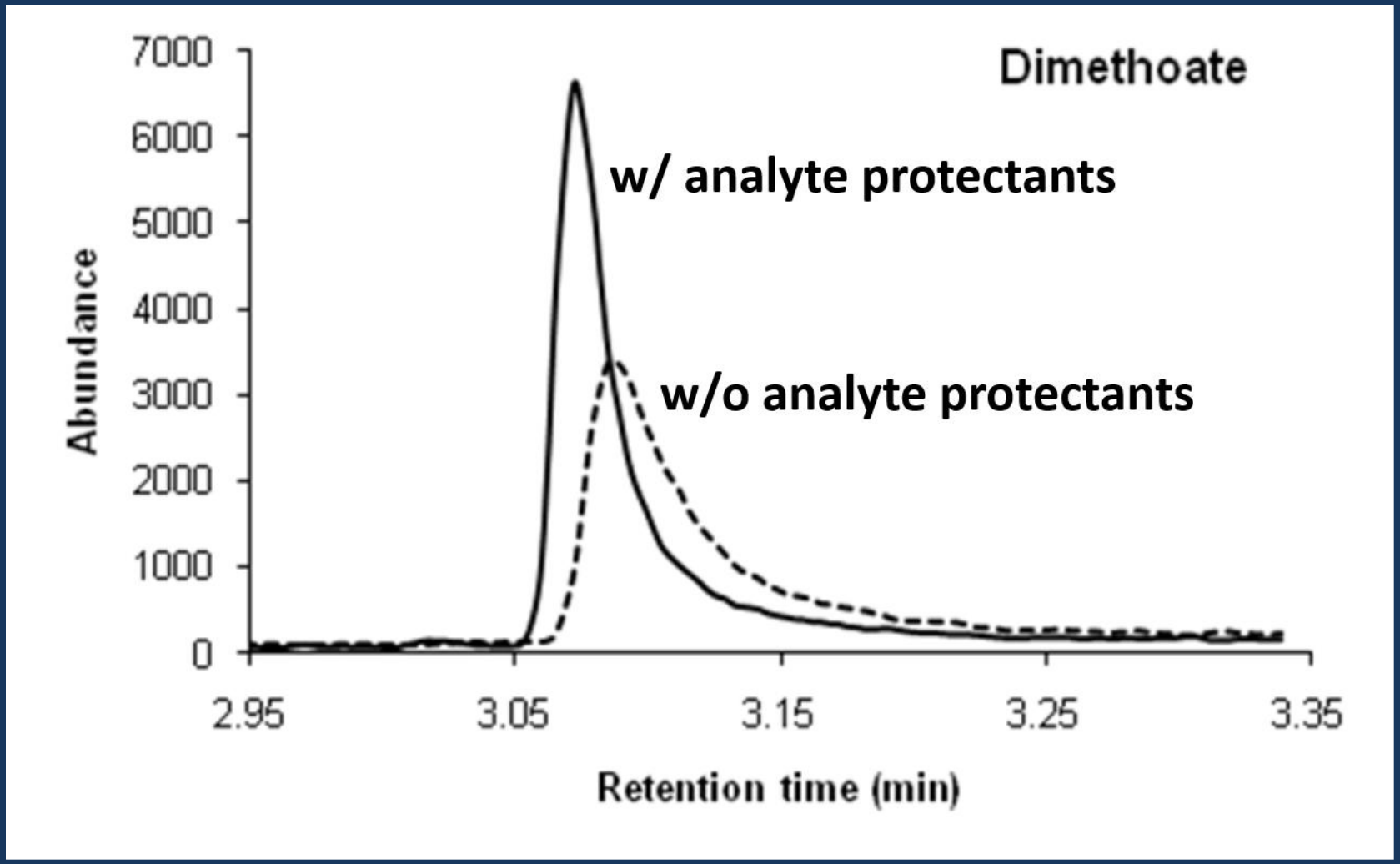


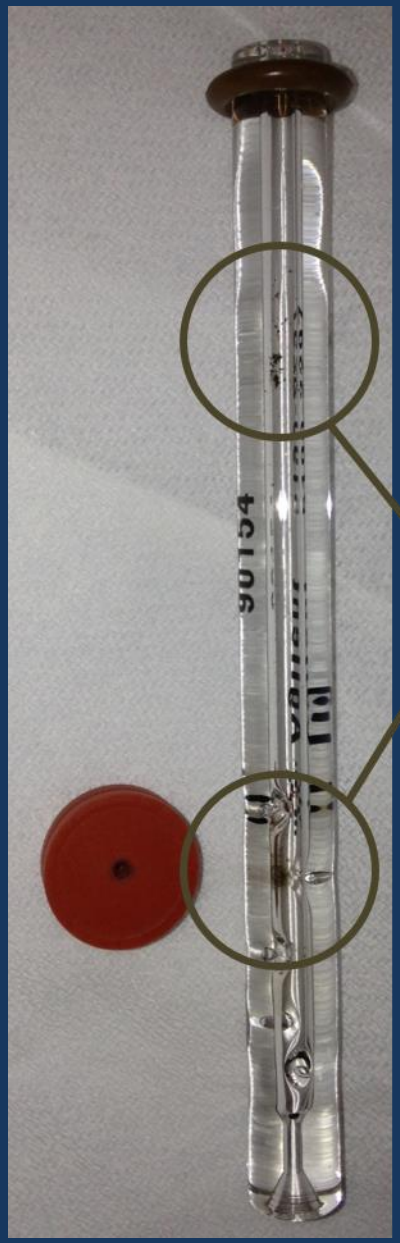
shikimic acid

0.05 mg/ mL

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

Effect of Analyte Protectants





Injection liner and septum after 325 injections in 5 days including 230 matrix extracts (1 mg equiv.) of 10 diverse food commodities

A little “dirt” here and there, but the analyte protectants did their job and results still looked great from start to finish.

Acknowledgments

CTC Analytics
ITSP Solutions
Gerstel
Restek

Jessie Matarrita
Robyn Moten
Tawana Simons
Yelena Sapozhnikova

Alan Lightfield
Limei Yun
Lijun Han

Disclaimer

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

Thank You!

Contact: Steven. Lehotay@ars.usda.gov

FDA Sampling for Pesticides

• < 25 g units (berries)	1 kg (2.2 lbs)
• 25 – 250 g (apples)	1 kg (≥ 10 units)
• > 250 g (cabbage)	2 kg (≥ 5 units)
• Grains, Tree Nuts	1 kg
• Herbs	0.5 kg
• Spices	0.1 kg

CODEX: 1 kg (2.2 lbs)

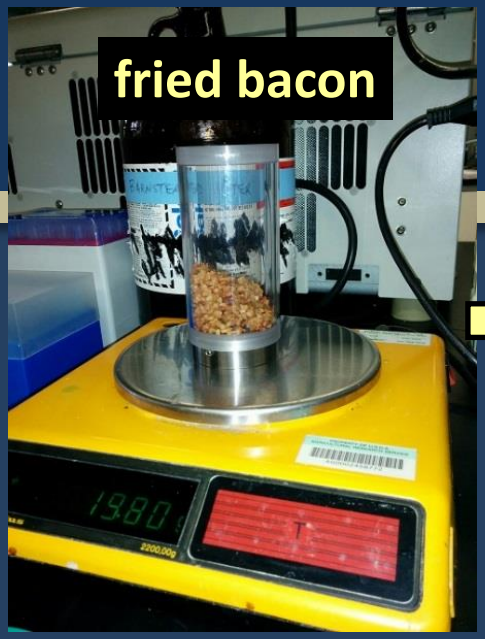
Pesticide Data Program: 3–5 lbs fresh, 2 lbs processed

USDA-FSIS: 1 lb meat, poultry, fish

Slide adapted from Jo Marie Cook

Cryogenic Sample Processing

Spex FreezerMill
(Cryomill)



Instrument Top Sample Preparation (ITSP) (2)

Morris and Schriner (2015) "Development of an automated column solid-phase extraction cleanup of QuEChERS extracts, using a zirconia-based sorbent, for pesticide residue analyses by LC-MS/MS" *J. Agric. Food Chem.* **63**, 5107-5119

Hill Laboratories
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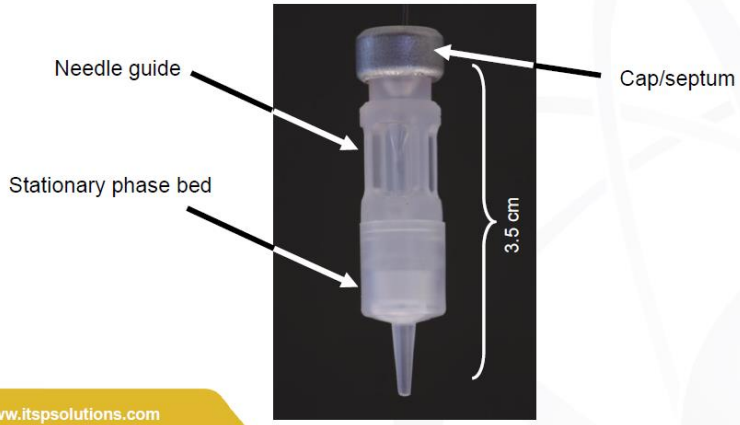
www.hill-laboratories.com

Robotic SPE clean-up Cartridges.

- A miniaturised SPE cartridge invented by ITSP Solutions.
- Stationary phase mixtures co-developed and trialled for QuEChERS at Hill Labs.
  - **30 – 45mg of stationary phase, depending on mixture.**



www.itspsolutions.com

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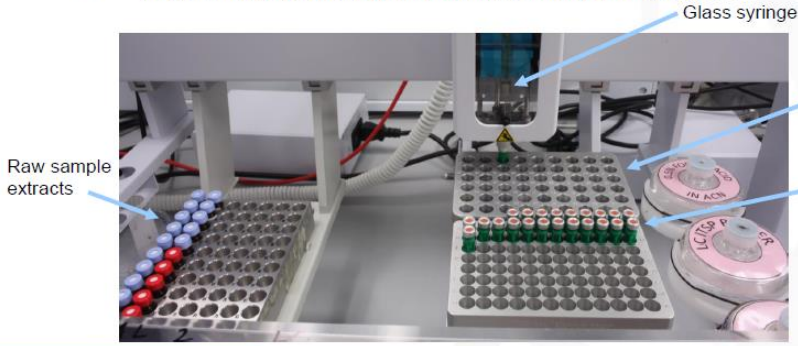
BETTER TESTING BETTER RESULTS
www.hill-laboratories.com

Robotic SPE clean-up - CTC autosampler

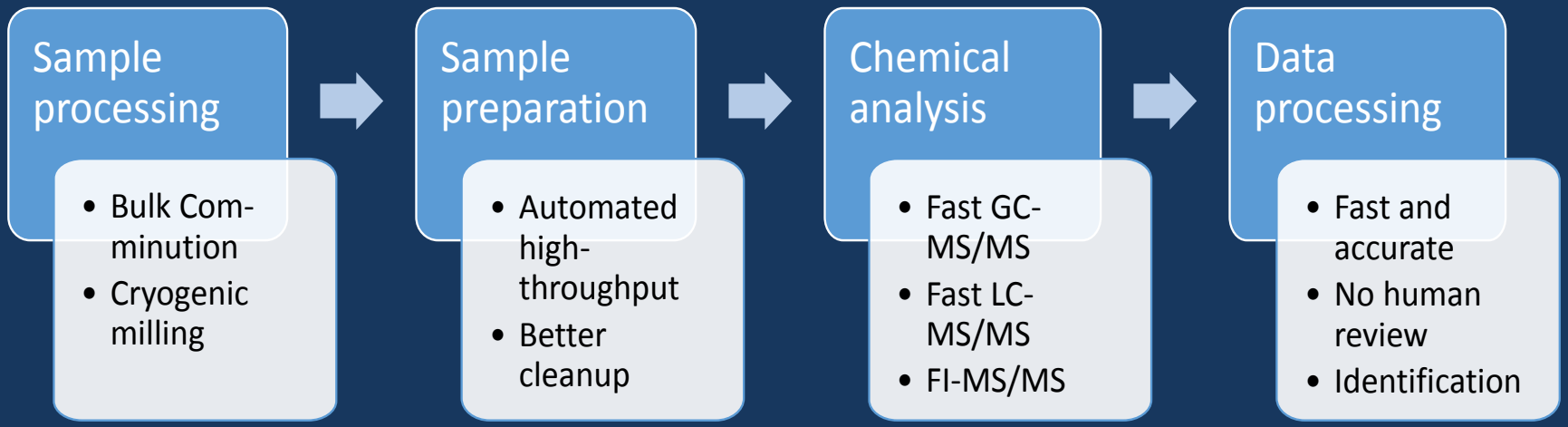
- Robotized on a CTC autosampler
  - **Hence ITSP "Instrument Top Sample Prep", although it can also be done stand-alone on a bench top.**
  - **Well-plate dimensions, although 2-mL vials used rather than wells.**



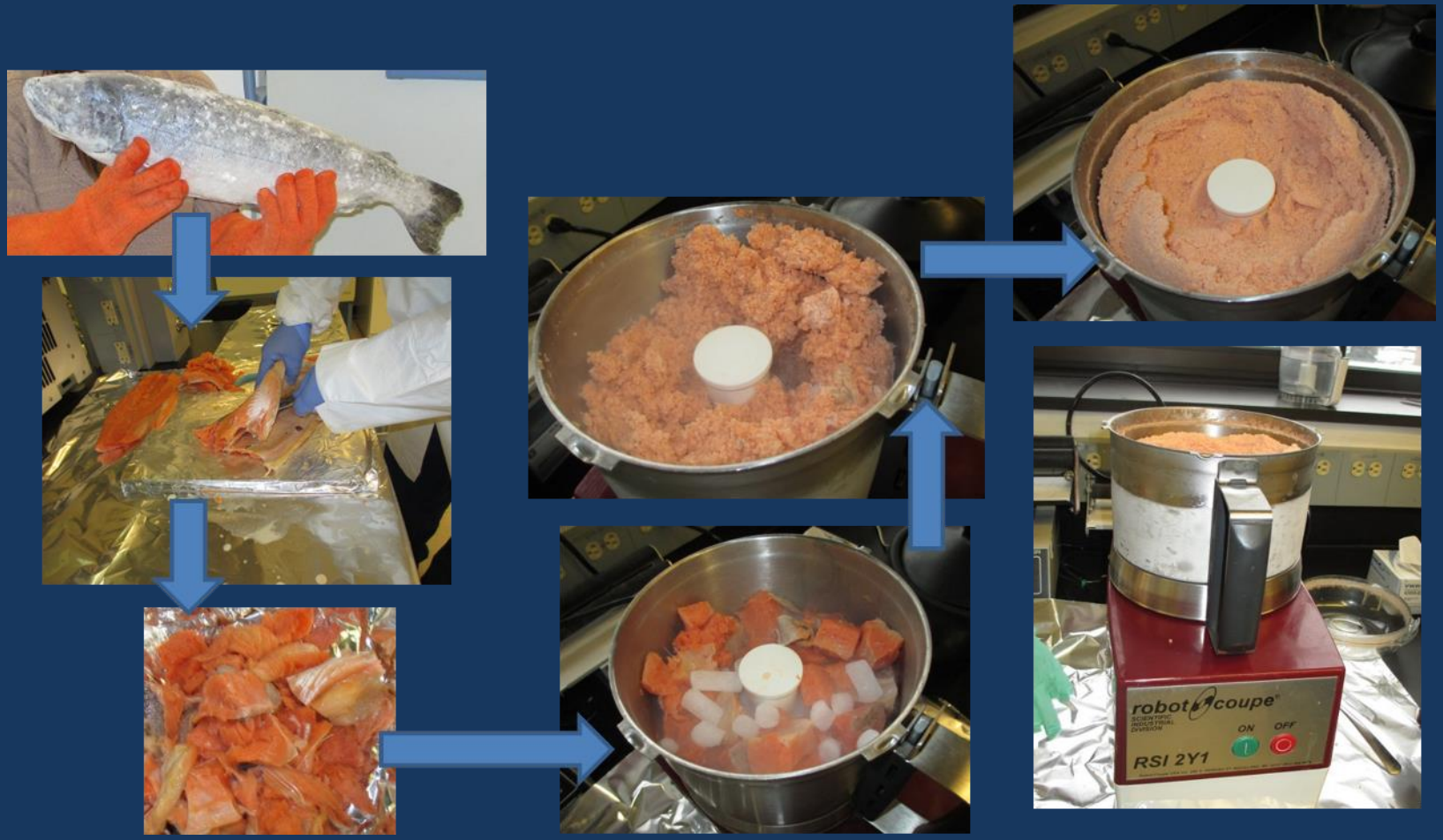
www.itspsolutions.com

www.nacrw.org/2014/presentations/O21-Morris.pdf

5-year project plan



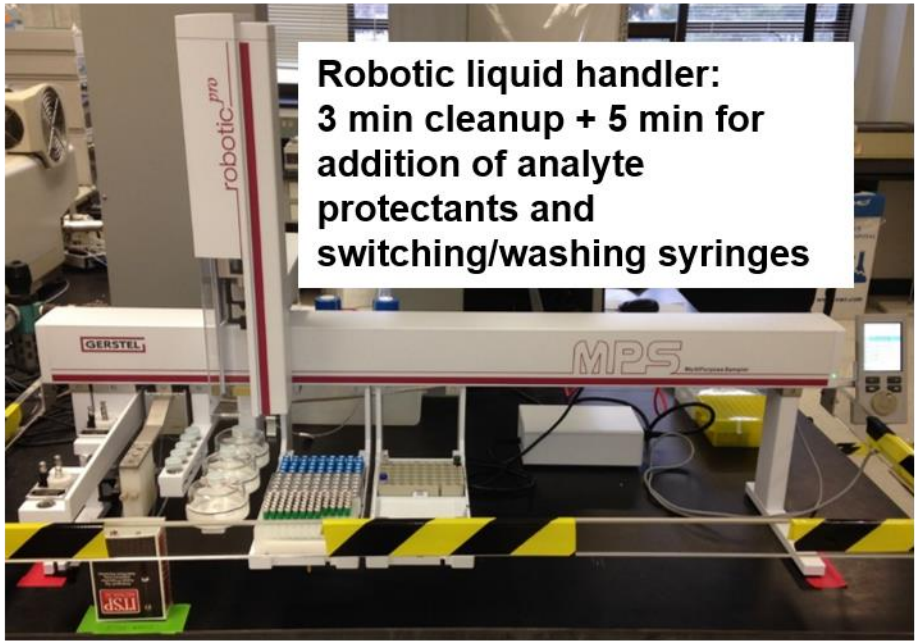
Sample processing



Cryogenic milling

Automated high-throughput sample preparation and data processing to monitor veterinary drugs, pesticides, and environmental contaminants

Automated Sample Cleanup – Instrument Top Sample Preparation (ITSP)



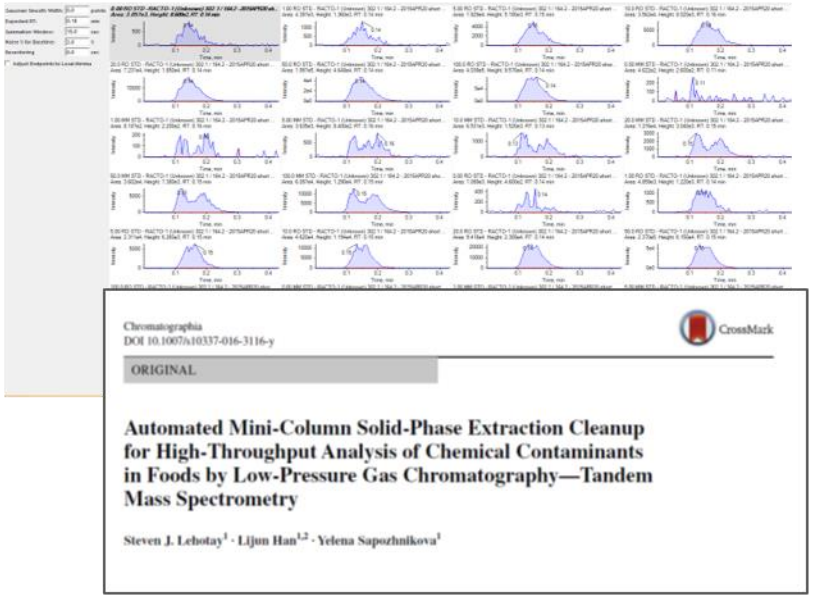
Robotic liquid handler: 3 min cleanup + 5 min for addition of analyte protectants and switching/washing syringes

Used mini-cartridges showing removal of chlorophyll and other matrix components

Automated Data Processing

Summation Integration Function

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



Chromatographia
DOI 10.1007/s10337-016-3116-y

ORIGINAL

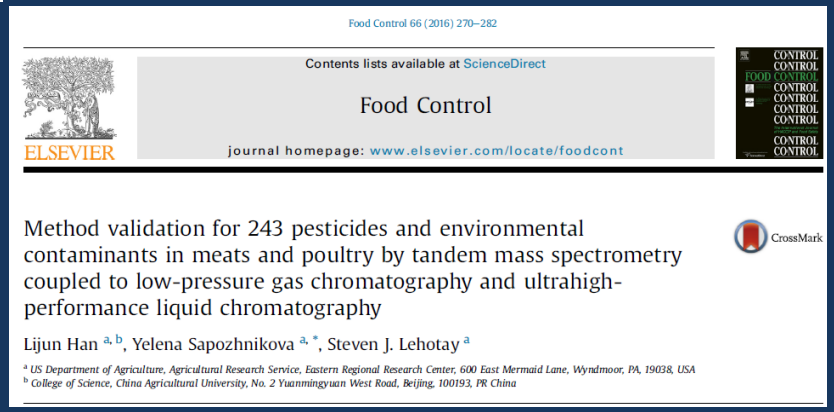
Automated Mini-Column Solid-Phase Extraction Cleanup for High-Throughput Analysis of Chemical Contaminants in Foods by Low-Pressure Gas Chromatography—Tandem Mass Spectrometry

Steven J. Lehotay[1] · Lijun Han[1,2] · Yelena Sapozhnikova[1]

Multiclass, multiresidue analysis of pesticides, and environmental and emerging contaminants in foods

Simultaneous analysis method for diverse pesticides, legacy and emerging environmental contaminants in meats:

- Pesticides & environmental contaminants: PAHs, PCBs, PBDEs, flame retardants (≈300 total)
- High throughput, fast, simple sample preparation based on QuEChERS extraction and streamlined clean-up
- Cost of materials ≈$3/per sample
- Fast Gas & Liquid chromatography tandem mass spectrometry analysis, 10 min each in parallel



Food Control 66 (2016) 270–282

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

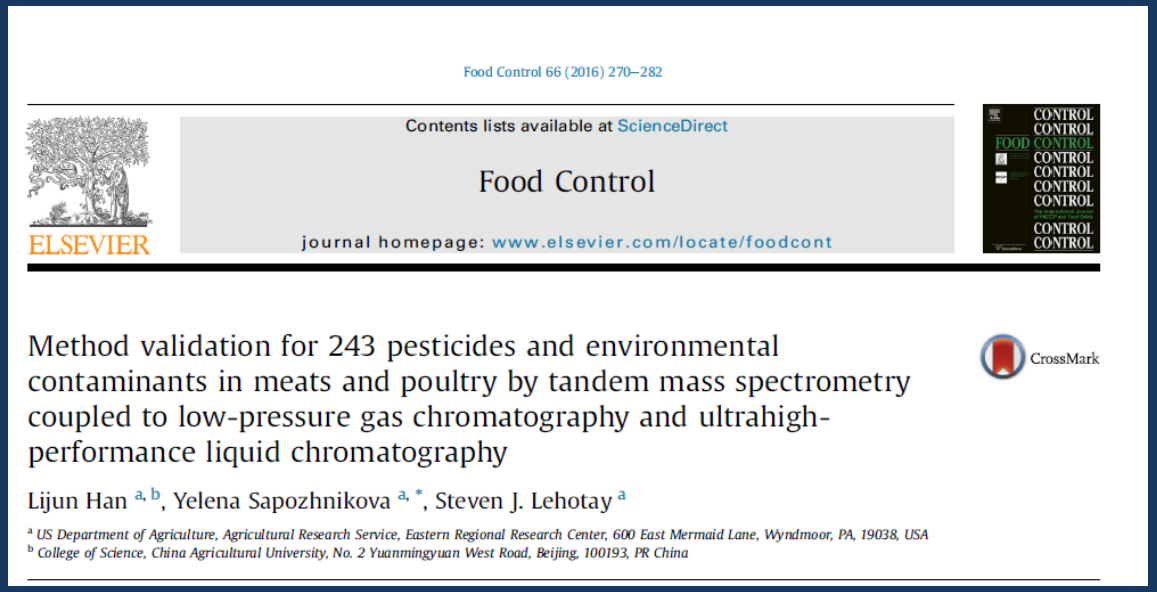
Method validation for 243 pesticides and environmental contaminants in meats and poultry by tandem mass spectrometry coupled to low-pressure gas chromatography and ultrahigh-performance liquid chromatography

Lijun Han [a, b], Yelena Sapozhnikova [a, *], Steven J. Lehotay [a]

[a] US Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 East Mermaid Lane, Wyndmoor, PA, 19038, USA
[b] College of Science, China Agricultural University, No. 2 Yuanmingyuan West Road, Beijing, 100193, PR China

Simultaneous analysis method for diverse pesticides, legacy and emerging environmental contaminants in meats

- Pesticides (EPA list) & environmental contaminants (≈300 total)
- QuEChERS extraction & d-SPE clean-up
- Fast GC & LC-MS/MS analysis, 10 min each



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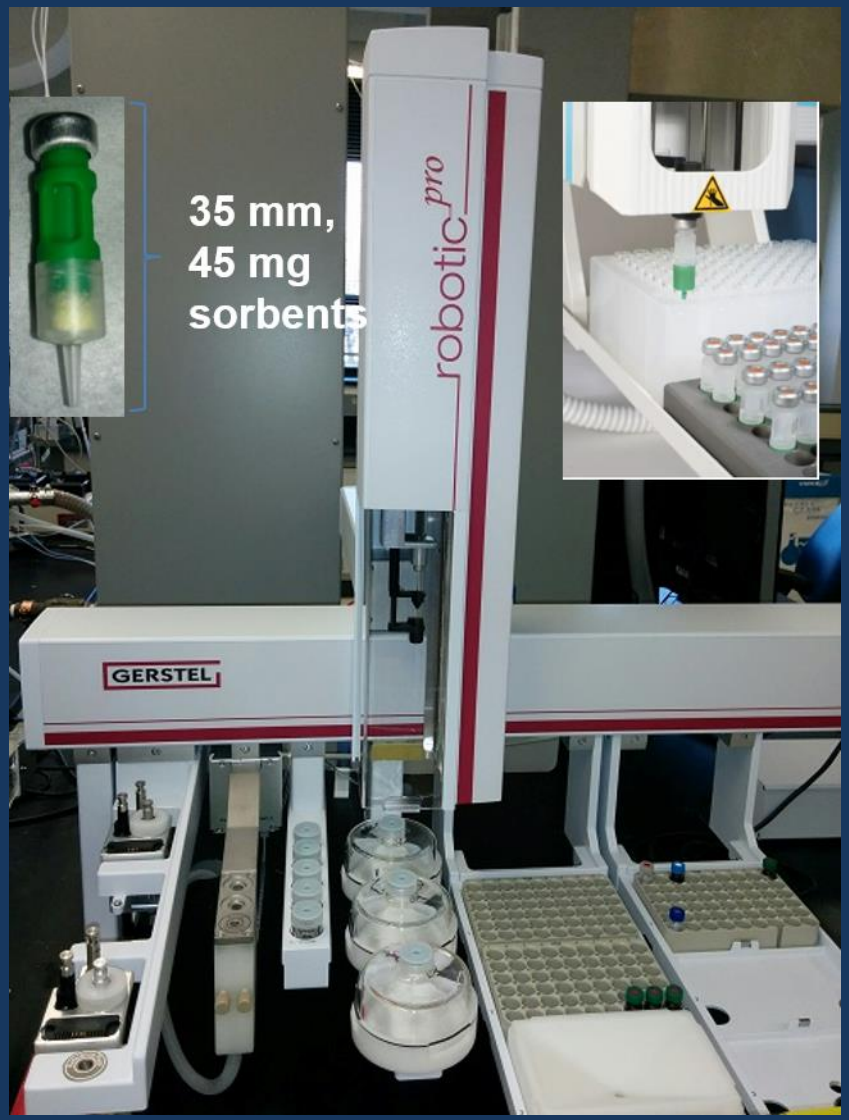
[b] College of Science, China Agricultural University, No. 2 Yuanmingyuan West Road, Beijing, 100193, PR China

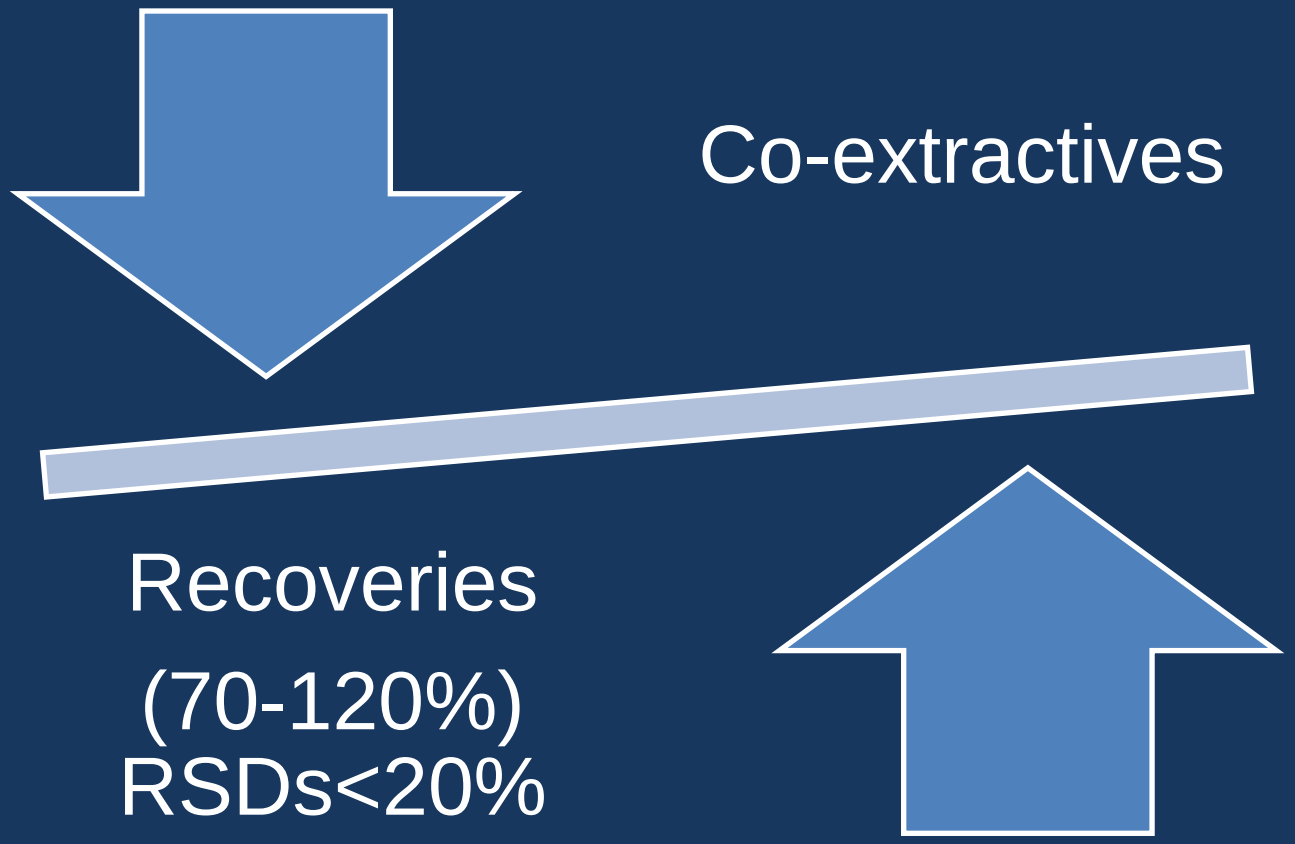
Automated SPE cleanup



- ITSP = Instrument Top Sample Preparation
- Mini-SPE cleanup
- 45 mg anh. $MgSO_4$/ PSA/C_{18}/Z-Sep/CarbonX

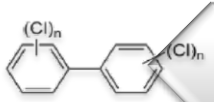
S.J. Lehotay, L. Han, Y. Sapozhnikova, Chromatographia, (2016) 1-18.

Efficient cleanup



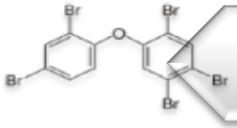
Multi-class, multiresidue method

Pesticides



Polychlorinated biphenyls (PCBs)

Polycyclic aromatic hydrocarbons (PAHs)

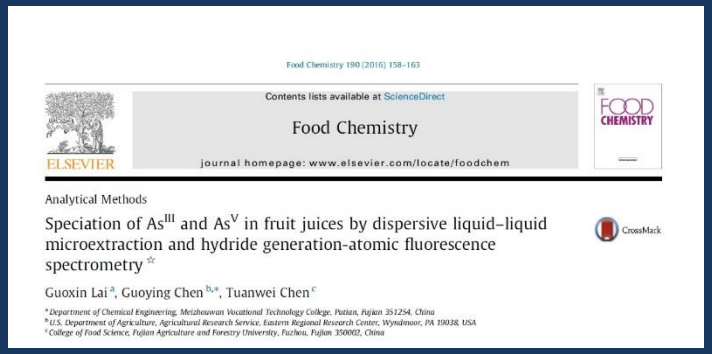


Polybrominated diphenyl ethers (PBDEs)

Novel alternate flame retardants (FRs)

Novel analytical methods for inorganic and organometallic toxic metals: mercury (Hg) and arsenic (As)

- Speciation of As and Hg
- Solid phase extraction (SPE) for cleanup and enrichment of inorganic As
- Hg^{++} and $MeHg^{+}$ speciation in fish oil supplement by photochemical vapor generation (PVG)
- Atomic fluorescence spectrometric quantification – sensitive, rapid, low cost
- Patent filing on a cryogenic trap system for As speciation


Food Chemistry 190 (2016) 158–163

Contents lists available at ScienceDirect

Food Chemistry

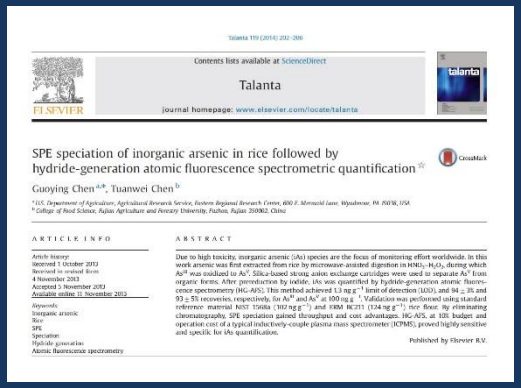
journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Speciation of As^{III} and As^{V} in fruit juices by dispersive liquid–liquid microextraction and hydride generation-atomic fluorescence spectrometry ☆

Guoxin Lai [a], Guoying Chen [b,*], Tuanwei Chen [c]

[a] Department of Chemical Engineering, Meizhouwan Vocational Technology College, Putian, Fujian 351254, China
[b] U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA 19038, USA
[c] College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China


Talanta 119 (2014) 202–206

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

SPE speciation of inorganic arsenic in rice followed by hydride-generation atomic fluorescence spectrometric quantification ☆

Guoying Chen [a,*], Tuanwei Chen [b]

[a] U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA
[b] College of Food Science, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China

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Atomic fluorescence spectrometry

ABSTRACT

Due to high toxicity, inorganic arsenic (iAs) species are the focus of monitoring effort worldwide. In this work arsenic was first extracted from rice by microwave-assisted digestion in HNO_3–H_2O_2, during which As^{III} was oxidized to As^{V}. Silica-based strong anion exchange cartridges were used to separate As^{V} from organic forms. After prereduction by iodide, iAs was quantified by hydride-generation atomic fluorescence spectrometry (HG-AFS). This method achieved 1.3 ng g^{-1} limit of detection (LOD), and 94 ± 3% and 93 ± 5% recoveries, respectively, for As^{III} and As^{V} at 100 ng g^{-1}. Validation was performed using standard reference material NIST 1568a (102 ng g^{-1}) and IRMM BCR#211 (124 ng g^{-1}) rice flour. By eliminating chromatography, SPE speciation gained throughput and cost advantages. HG-AFS, at 10% budget and operation cost of a typical inductively-couple plasma mass spectrometer (ICPMS), proved highly sensitive and specific for iAs quantification.

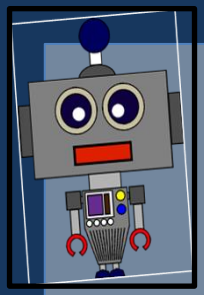
Published by Elsevier B.V.

Bioanalytical methods to monitor for antibiotic resistant organisms and/or their biomarkers

- Developing bioanalytical methods (including mass spectrometry) to monitor for **antibiotic resistant** organisms and/or their biomarkers in conjunction with antibiotic residues in seafood and meats.
- Developing rapid **antimicrobial resistance** (AMR) assays based on high resolution mass spectrometry (HRMS)

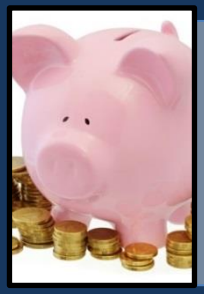
QuEChERS extraction with acetonitrile

Batch of 12 pre-homogenized samples can be prepared in 1 hr

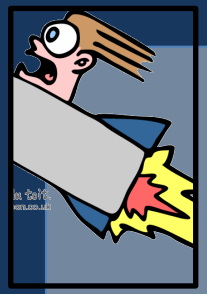


Automated SPE cleanup

Waste = 1-2 mL acetonitrile & disposable pp tube



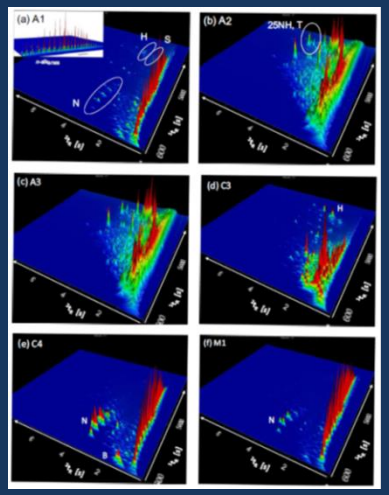
Cost of materials ≈ $3-4/sample



LPGC & UHPLC-MS/MS:

10 min run in parallel

Multiresidue method for food packaging contaminants in packaged foods



Phase 1. Identification of food packaging contaminants leaching from stretch plastic films used as food packaging:

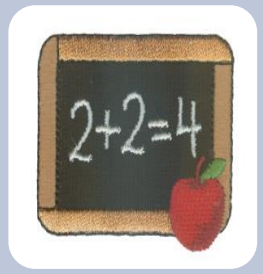
- In food simulants
- In packaged food (e.g. ground beef, pork, chicken)

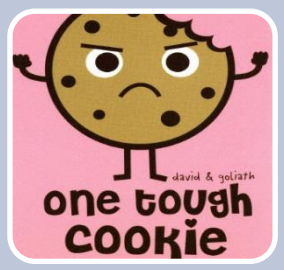
Non-targeted analysis by GCxCG-TOF-MS

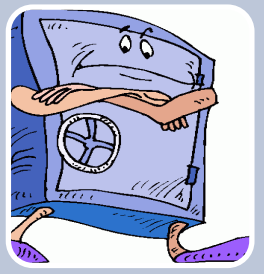
Phase 2. Method development

Phase 3. Market survey & data for risk assessment

Sample preparation - QuEChERS



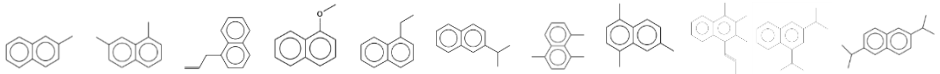
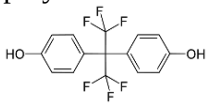
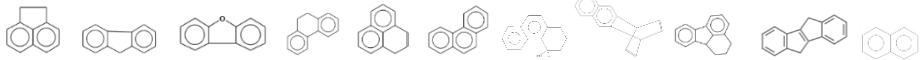
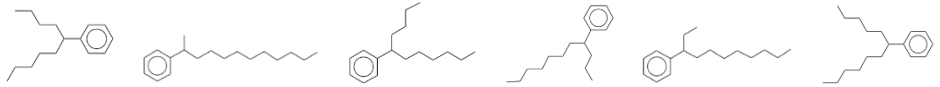
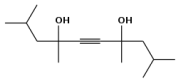
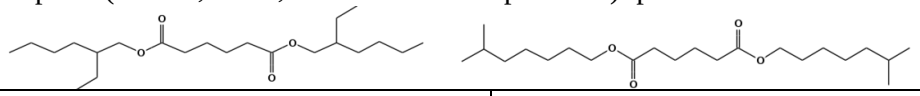
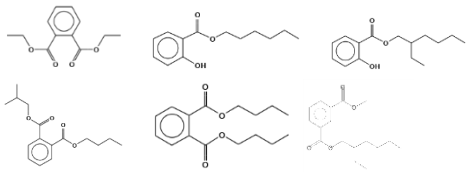
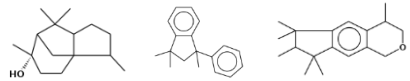
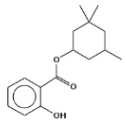
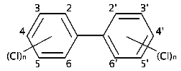
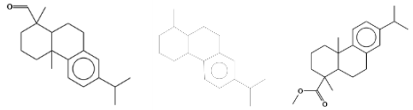
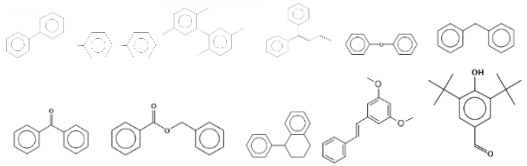




Quick	Easy	Cheap	Effective	Rugged	Safe

93 organic chemicals identified in food simulants

(with >80% match similarity to the standard NIST mass spectral library)

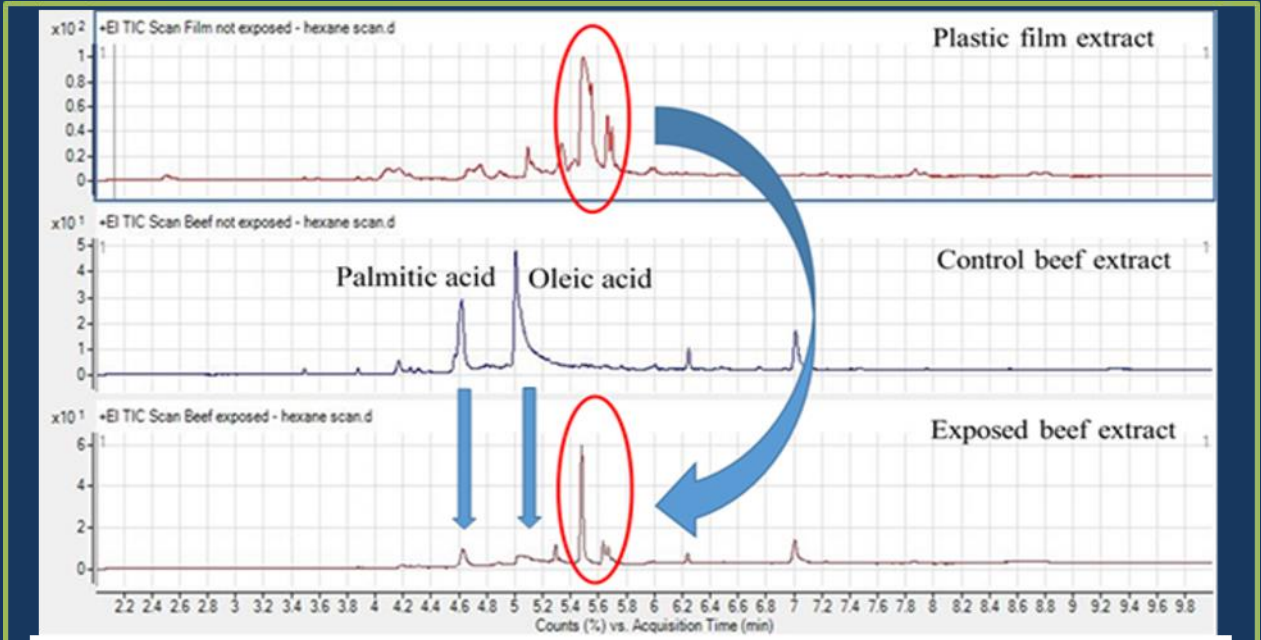
Chemical Class: Uses/Sources		
Alkylated naphthalene: lubricant additive 		Hexafluorobisphenol A: polymer additive 
Polycyclic aromatic hydrocarbon (PAH): combustion, biogenic, petroleum 		
Linear alkylbenzene (LAB): precursor of biodegradable detergents 		2,4,7,9-Tetramethyl-5-decyn-4,7-diol: adhesive, surfactant, plastic additive 
Adipates (DEHA, DOA, and five other adipic acids): plasticizer 		
Phthalates and salicylates: plasticizer 	Cedrol, 1H-Indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-, and Galaxolide (musk): fragrance 	Homosalate: UV filter 
PCBs (two isomers of di-chloro and three isomers of tri-chloro) 	13-Isopropylpodocarpa-8,11,13-trien-19-al, 10,18-Bisnorabieta-8,11,13-triene, and Methyl dehydroabietate: thermal degradation 	Phenyl/Biphennyl/diphenyl compounds (miscellaneous) 

Some examples of identified chemicals

Identified compound	Use	Concern
Benzyl chloride	Manufacturing of plasticizers	Probable human carcinogen
Benzyl benzoate	Flavor and fragrance agent	Endocrine disruptor
Furan, 2-pentyl	Flavoring agent	Suspected genotoxicity
Benzophenone	UV blocker	Endocrine disruptor
2,4-trimethyl-1,3-pentanediol diisobutyrate (TXIB)	Low-viscosity plasticizer	Reproductive/ developmental toxicity
2-ethylhexyl methyl isophthalate	Commonly used plasticizer	Genetic mutation, reproduction toxicity

Current work

- Currently identifying chemicals leaching into meats



Scan chromatograms of plastic film extract, control beef extract and beef exposed to plastic film packaging for 24 hours

Retention Times and Peak Widths are Rock Solid in UHPLC-MS/MS

3-Day Validation Experiment of 101 Pesticides analyzed by UHPLC-MS/MS

40 matrix (muscle) spks and blks + QC = **65 injections per day**

Avg t_R (min) of reagent stds and matrices throughout the run (SD <0.020)

#	Analyte	Day 1 = 7/17/15		Day 2 = 7/22/15		Day 3 = 7/28/15	
		Rgt	Cattle	Rgt	Chicken	Rgt	Pork
1	Methamidophos	0.965	0.963	0.950	0.955	0.962	0.963
8	Oxamyl	1.977	1.970	1.950	1.950	1.962	1.963
18	Dimethoate	3.055	3.055	3.030	3.030	3.045	3.045
28	Oxadixyl	4.030	4.030	4.012	4.010	4.020	4.022
42	Metalaxyl	5.023	5.017	5.000	5.000	5.008	5.010
67	Azinphos	6.067	6.065	6.048	6.045	6.052	6.057
90	Profenophos	7.023	7.018	7.003	7.007	7.017	7.018
100	Methoprene	8.013	8.013	8.000	8.005	8.010	8.010

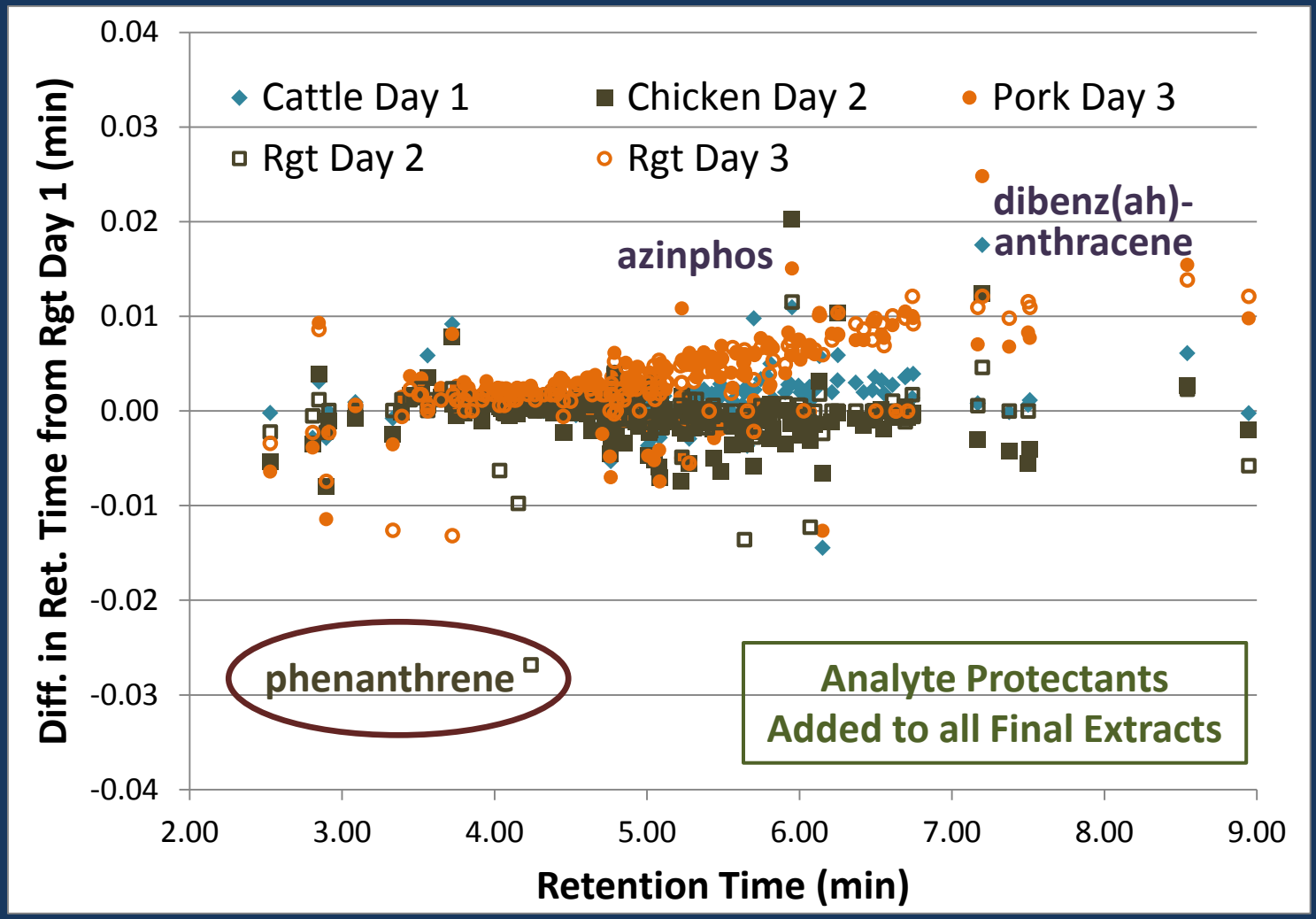
New mobile phase added for each sequence

And Fast, Low-Pressure GC-MS/MS, Too

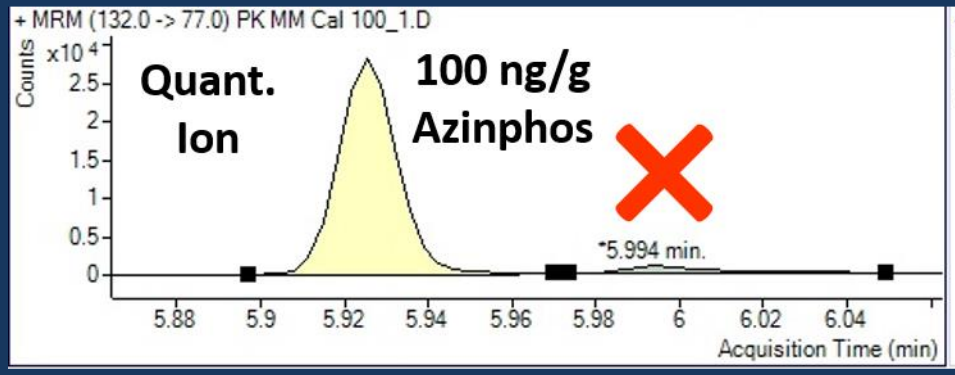
3-Day Validation Experiment of 202 Pesticides analyzed by LPGC-MS/MS

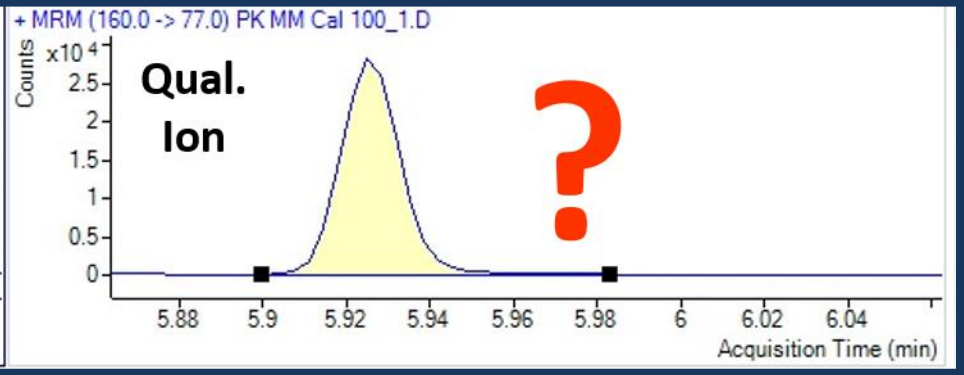
40 matrix (muscle) spks and blks + QC = **70 injections per day**

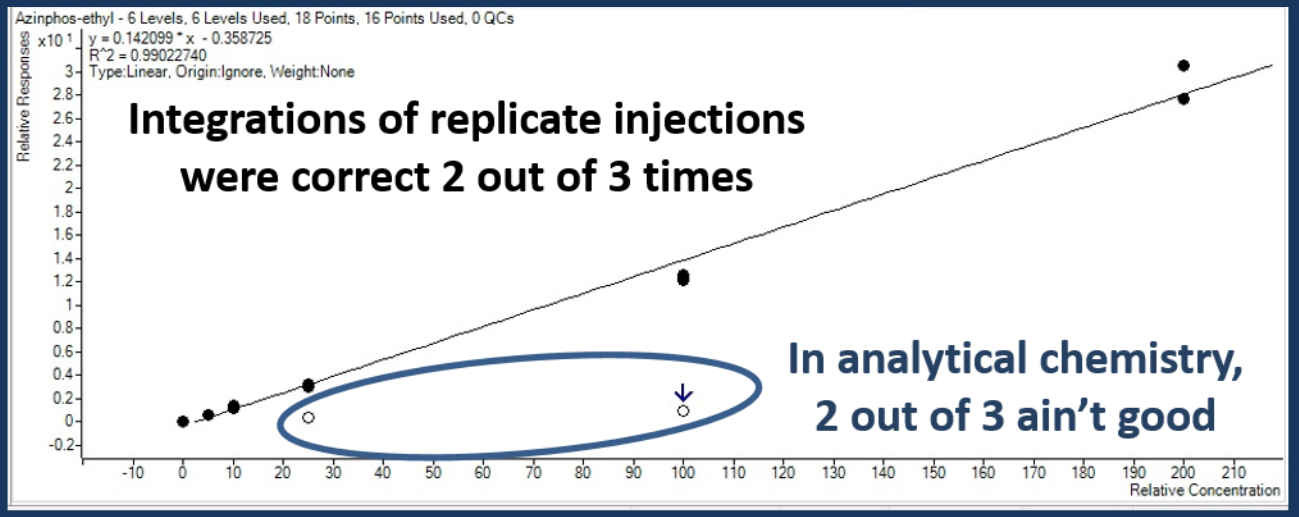
Avg t_R (min) of reagent stds and matrices throughout the run (SD <0.040)



What the Heck?





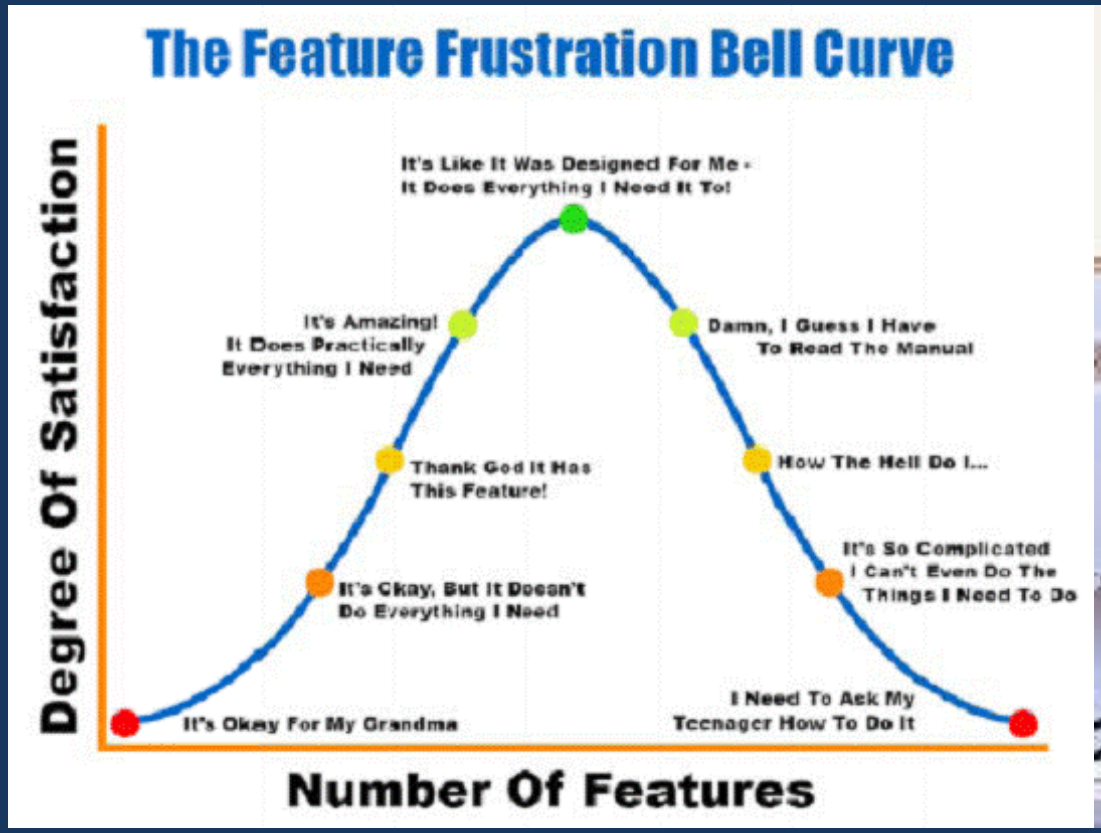


Analytical chemists are hard-working lazy people who look for short-cuts rather than make manual re-integrations.

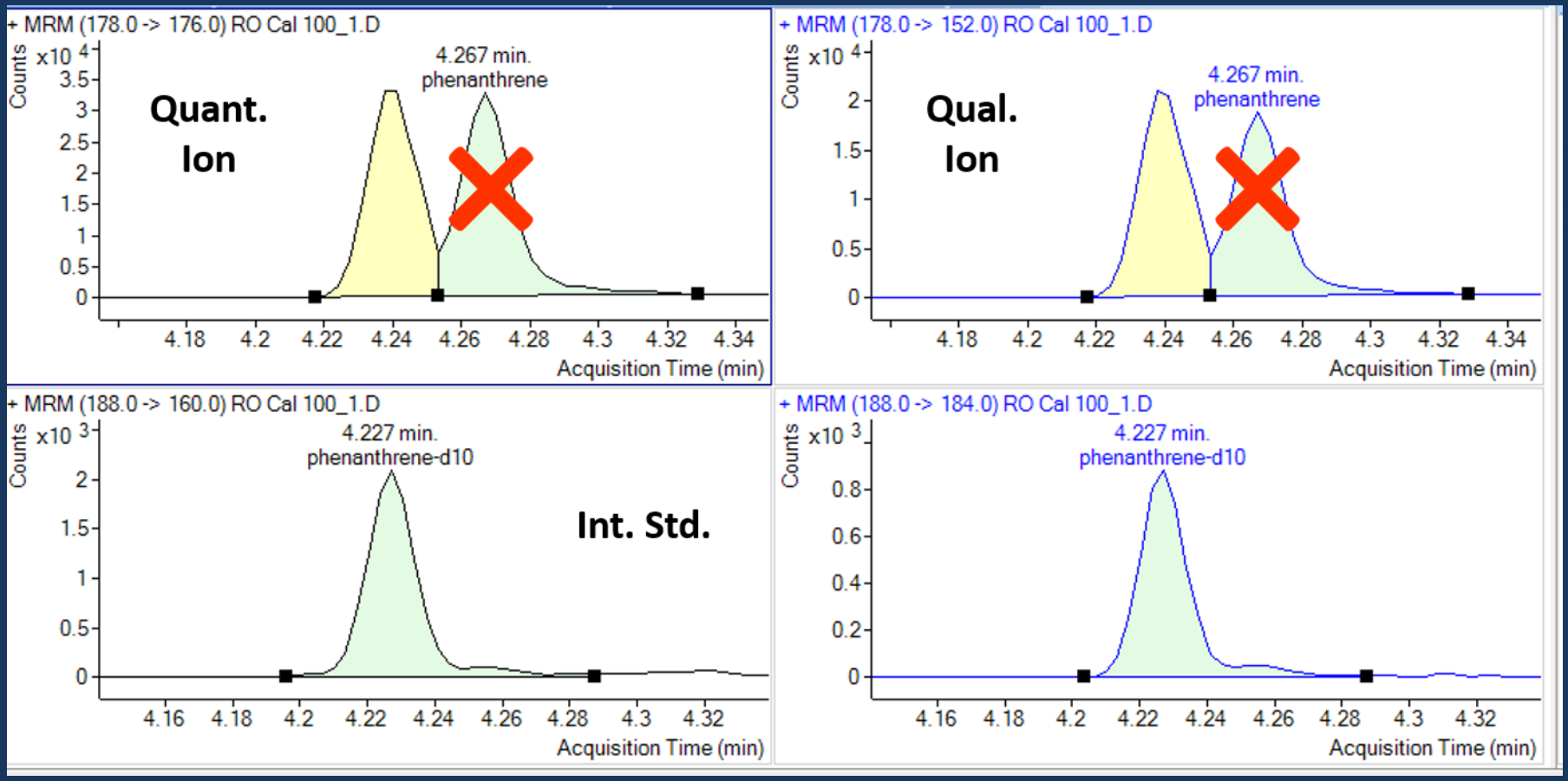
Real samples can't be dropped as outliers.

In chromatography, the primary parameters are ret. time (t_R) and peak shape (width, height/area)

If t_R and peak widths are so important and consistent in good methods, why do most (all?) sophisticated (and expensive) chromatographic peak integration software programs so often choose peaks at the wrong t_R with quite variable peak shapes?



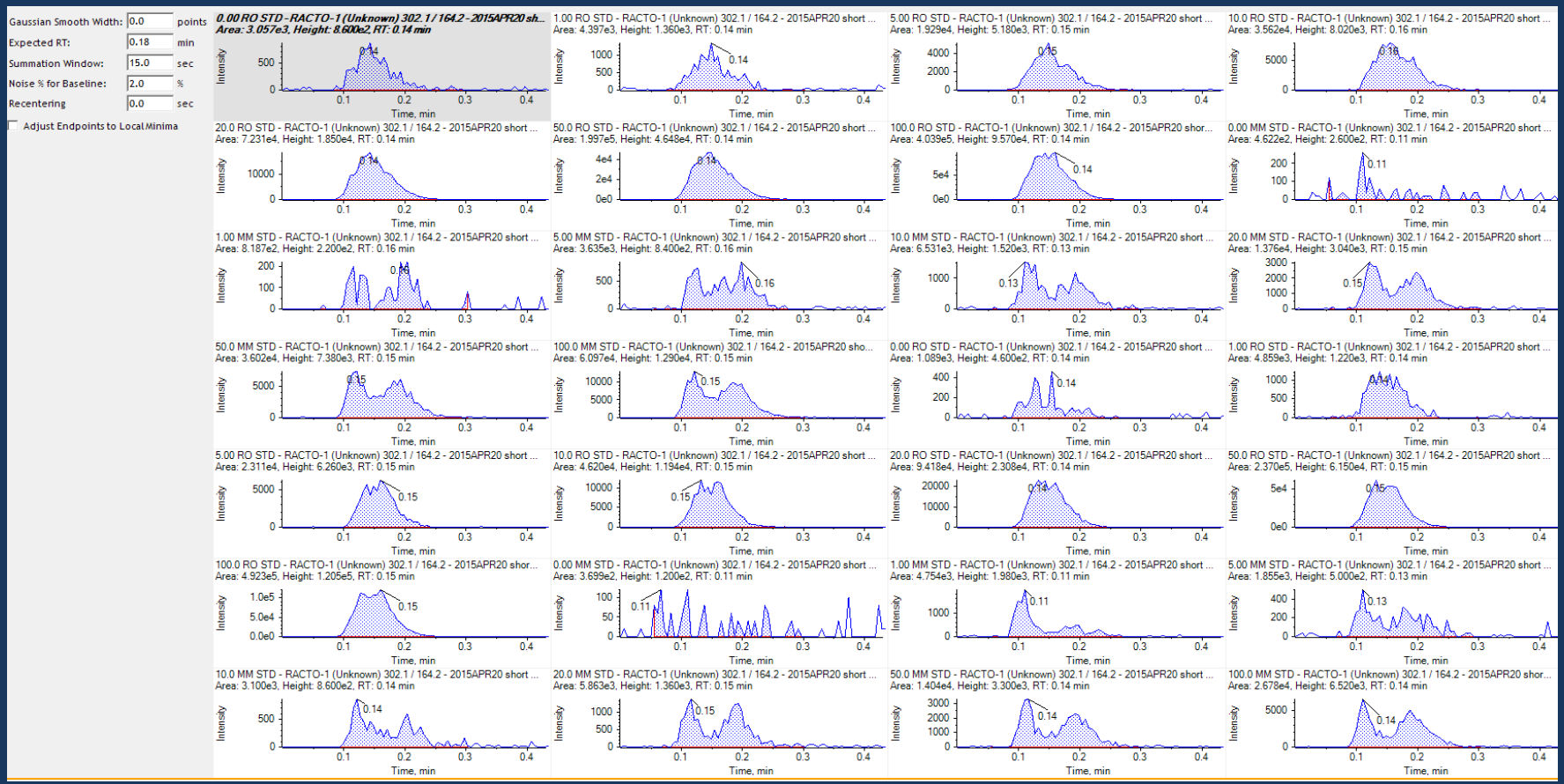
Don’t Trust the “Advanced” Software



And don’t trust the analyst, either. This mistake was caught after preparing the previous slide for this presentation.

Summation Integration Function

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

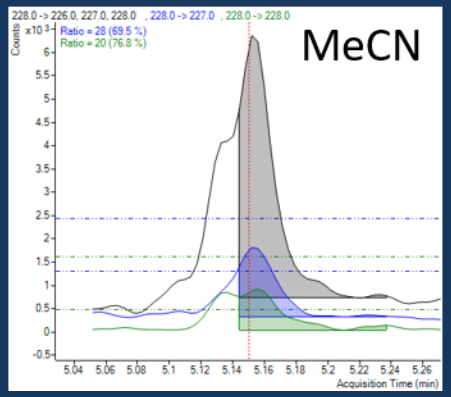


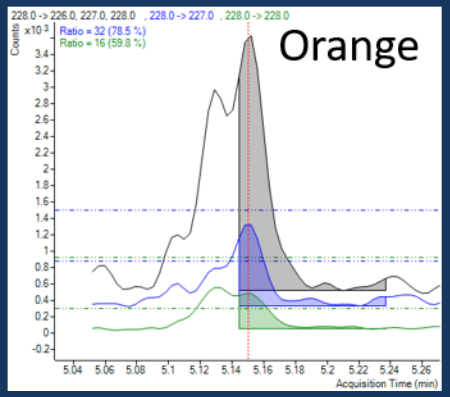
- This is a >40 year-old integration function, but **LACKING IN SOME DATA PROCESSING SYSTEMS!**

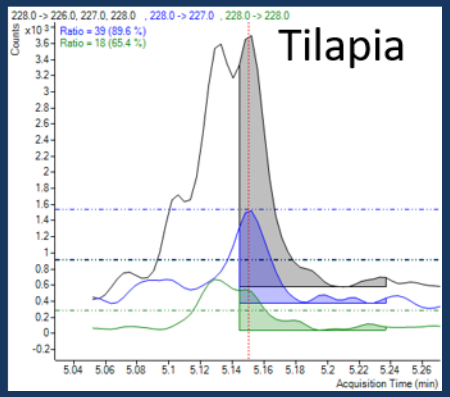
chrysene 1 µL 9:1 split injection after ITSP

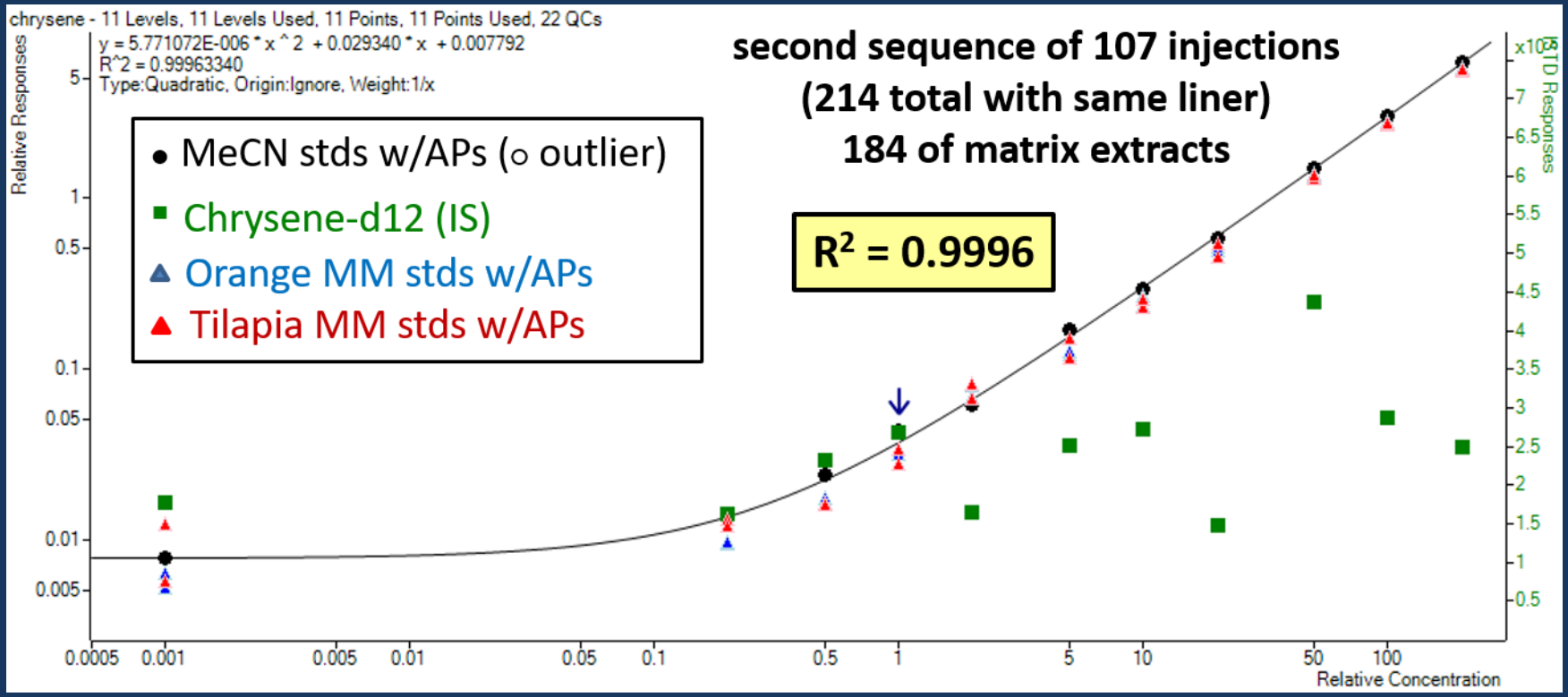
partial co-elution with benz(a)anthracene – summation integration at mid-point

1 ng/g Stds









RESOLVED: Garbage In = Garbage Out

Correct and consistent chromatographic peak integration is essential to achieving high quality final results.

RESOLVED: Despite technology and software advancements, no set of peak integration parameters works consistently for all analytes, concentrations, and matrices in the real-world (at least not yet in my experience).

RESOLVED: Good analysts are able to conduct peak integrations better than current advanced software tools (but good analysts are hard to find, earn wages, get bored reviewing data, and still make misteaks).

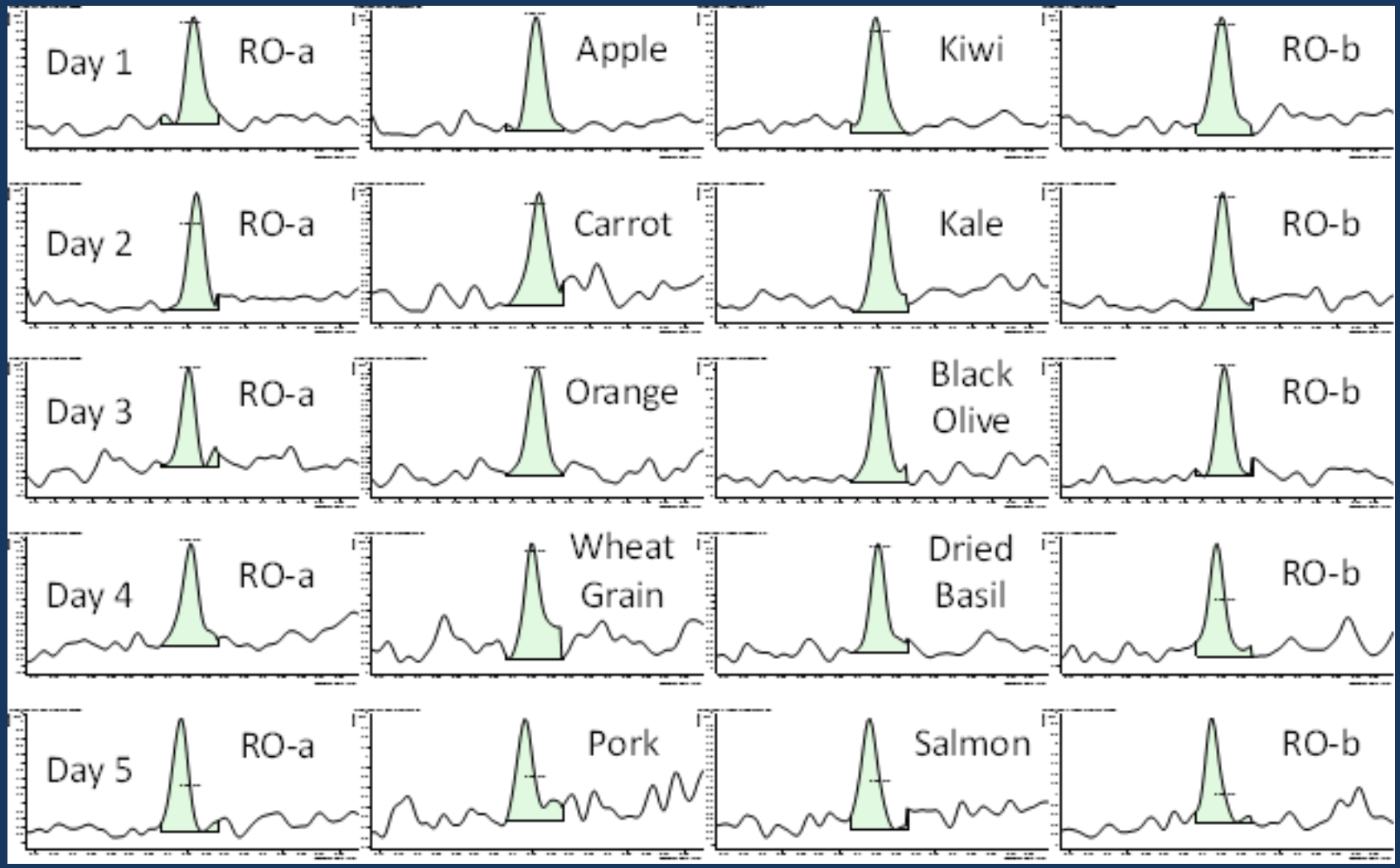
RESOLVED: Human review takes too long!

High-throughput (or even low-throughput) multi-analyte monitoring applications:

G.F. Pang *et al.* (Beijing, China) include 1,138 pesticides in their GC- and LC- MS/MS monitoring approach. Large team of chemists conduct analyses and review results.

USDA: 240 analytes × 2-4 ion transitions × 50 samples/batch = 36,000 peaks!
Analyst review and re-integration at 1 s per peak = 10 hours w/o breaks on each instrument!

5 ng/mL endosulfan sulfate in reagent-only and matrix-matched calibration standards



LOQ ≈2 ng/mL in all matrices; even after 325 injections, including 230 food extracts

Mathematical approaches to speciate As or Hg

Comparison

<table>
<tr><td rowspan="2">As hydrides</td><td colspan="5">$I(A) = m_a[As(III)] + n_a[As(V)] + p_a[MMA] + q_a[DMA]$,
$I(B) = m_b[As(III)] + n_b[As(V)] + p_b[MMA] + q_b[DMA]$,
$I(C) = m_c[As(III)] + n_c[As(V)] + p_c[MMA] + q_c[DMA]$,
$I(D) = m_d[As(III)] + n_d[As(V)] + p_d[MMA] + q_d[DMA]$,
As speciation by HG-AFS under 4 sets of HG conditions (Cava-Montesinos, et al., Talanta, 2005, 66, 895-901)</td></tr>
<tr><td>4</td><td>4</td><td>4</td><td>n/a</td><td>16</td></tr>
<tr><td>Volatile species</td><td>Analytes</td><td>Equations</td><td>Reductants</td><td>Wavelengths</td><td>Calibration curves</td></tr>
<tr><td rowspan="2">Hg^0 vapor</td><td>2</td><td>2</td><td>1</td><td>2</td><td>4</td></tr>
<tr><td colspan="5">$I_B = m_B[Hg^{++}] + n_B[MeHg^+]$
$I_C = m_C[Hg^{++}] + n_C[MeHg^+]$
(Hg speciation by PVG-AFS at 2 UV wavelengths, this work)</td></tr>
</table>