Racing to Zero and Infinity: Analytical Advances and MRLs

Wiley Hall 4th,Ph.D.

USDA-ARS-SJVASC

Racing to Zero and Infinity: Analytical Advances and MRLs

Cost Increase

Trade Barrier (?)

Cost Reduction

~~Trade Barrier (?)~~

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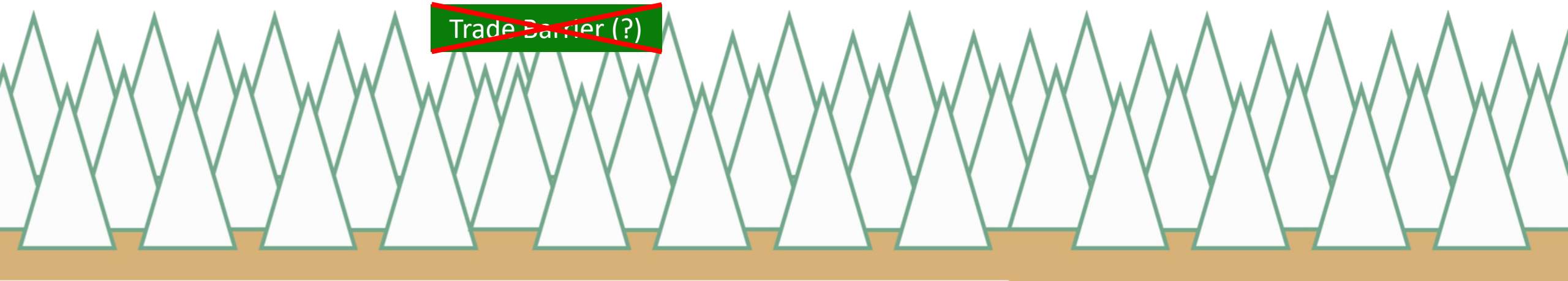


Analytical Advances and MRLs

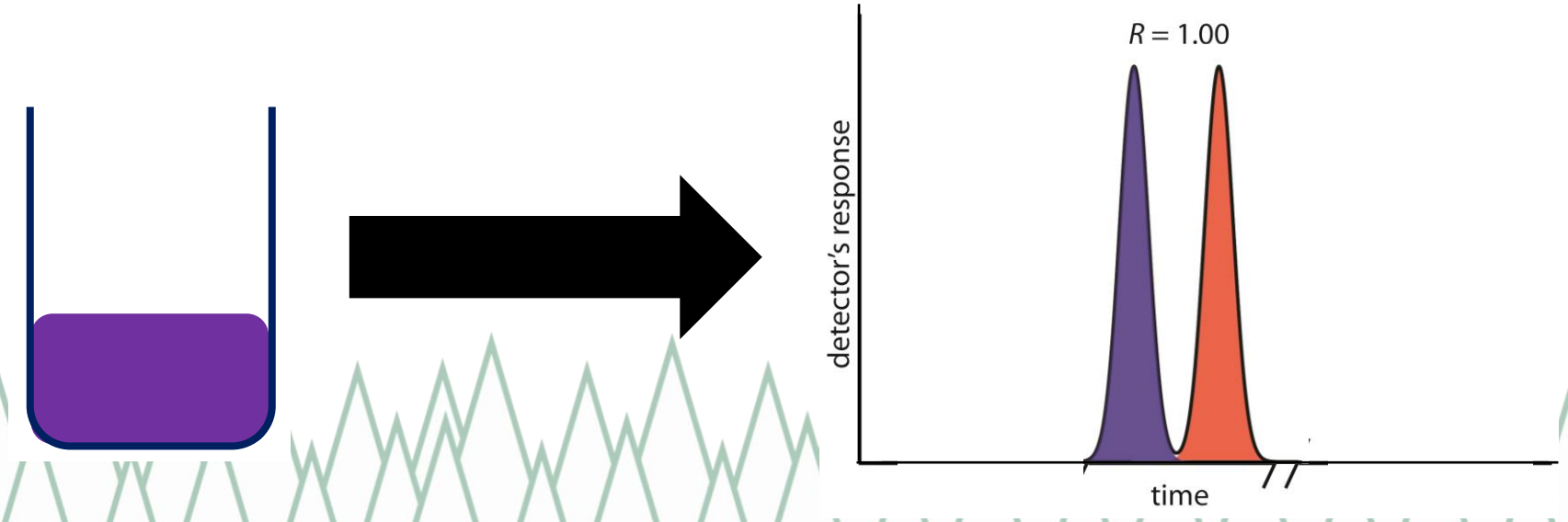
I. A Quick Intro to Analytical Chemistry

II. Advance #1: nDATA Multi-Residue Screens

III. Advance #2: Alternative Separation Methods

How Pesticide Analyses are Performed

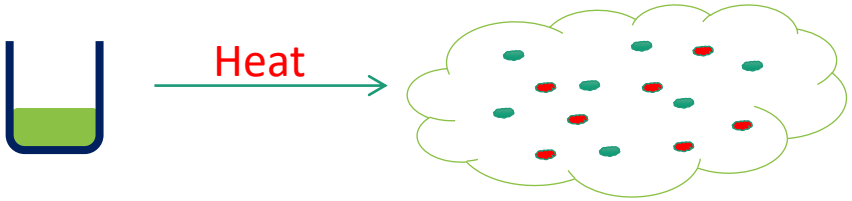
- Most pesticide residue analysis is performed with chromatography (separates components of a mixture) and mass spectrometry (IDs and measures amt)



Pesticide Analyses: Chromatography

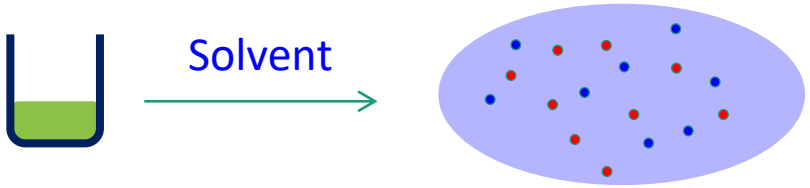
Chromatography starts with dissolving your sample into mobile phase:

Gas Chromatography: the mobile phase is a gas



To analyze a chemical with GC, you must be able to vaporize it without it falling apart

Liquid Chromatography: the mobile phase is a liquid



To analyze a chemical with LC, it must be able to dissolve in the mobile phase (usually water soluble)

The mobile phase is then used to move the sample through a column: a tube filled with a material called the stationary phase…

Pesticide Analyses: Chromatography

Different chemicals will move through a chromatography column at different speeds

How quickly a compound moves through a column depends on the strength of the chemical interaction between that compound and the stationary phase (relative to the mobile phase)

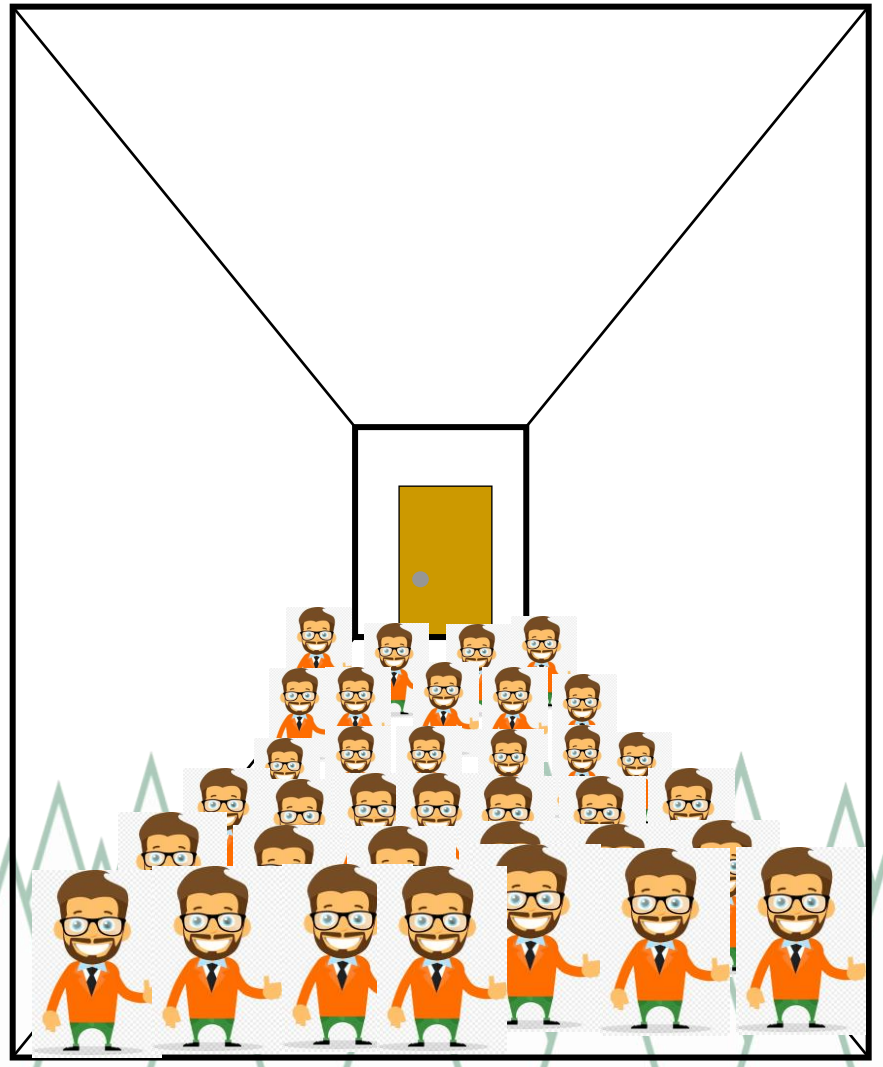
Pesticide Analyses: Chromatography

Different chemicals will move through a chromatography column at different speeds

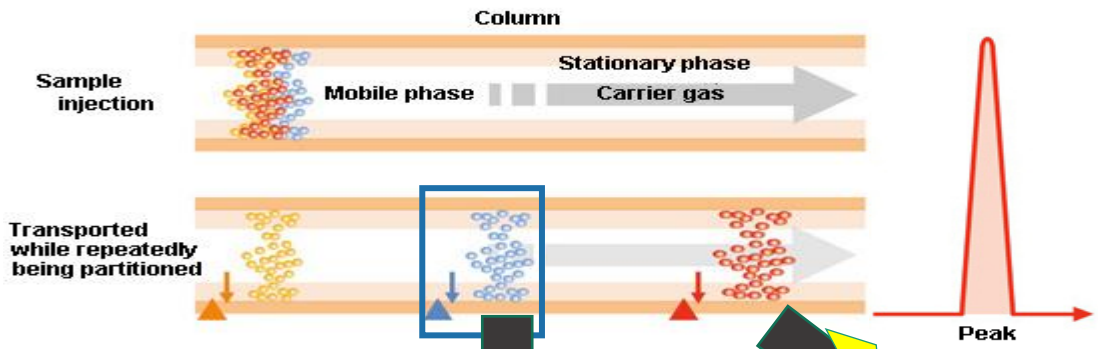
Think of the column as a hallway filled with people (stationary phase)

If you like the people, you'll spend some time talking to them as you walk through the hallway

If you don't like the people in the hallway, you'll move through it as quickly as you can

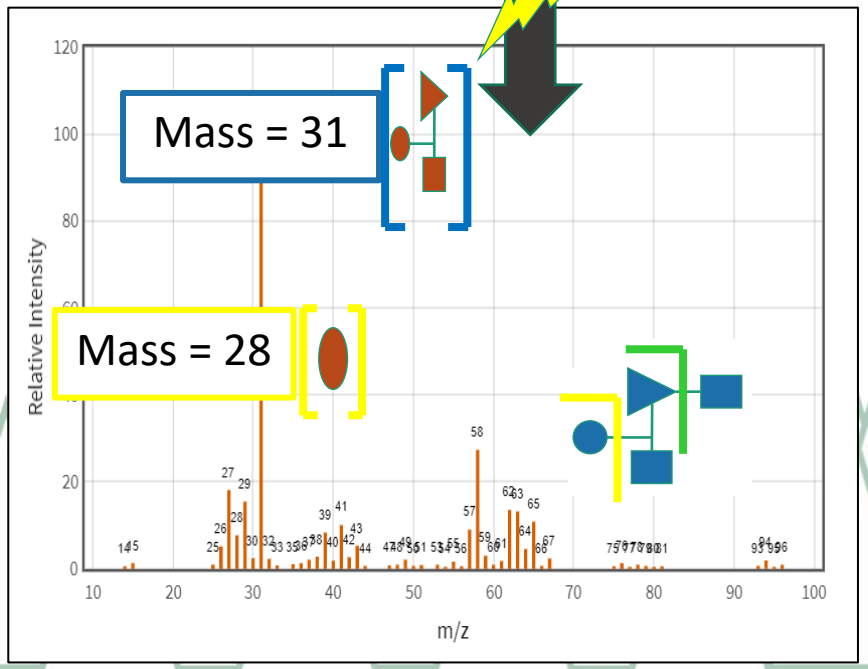


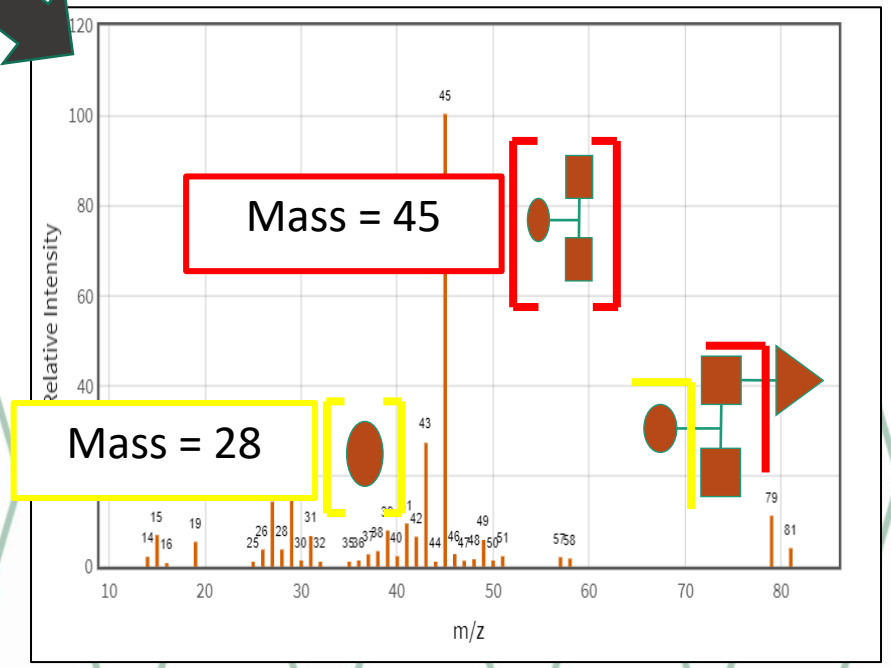
Pesticide Analyses: Mass Spectrometry



As compounds exit the column, they enter the mass spectrometer…

…which gives a signal based on how much of the compound is present.





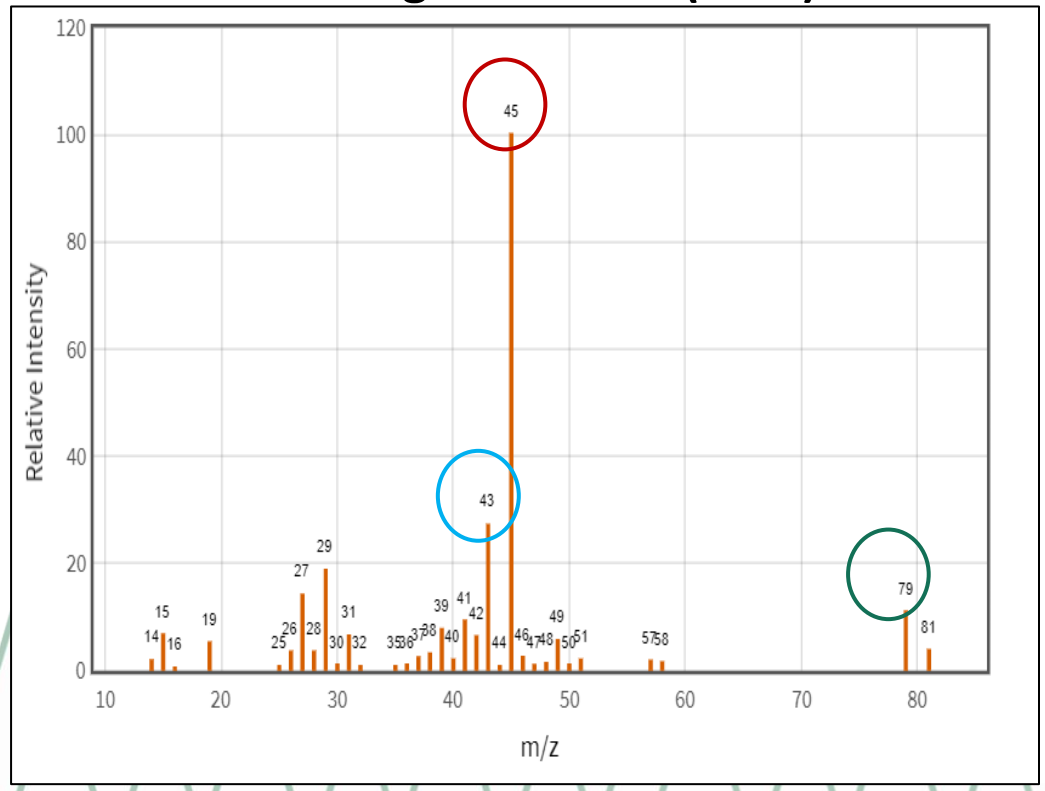
The mass spectrometer will also break the compound into fragments, giving a unique(ish) pattern for each analyte (mass spectrum)

A compound is ID based on its retention time and mass spectrum

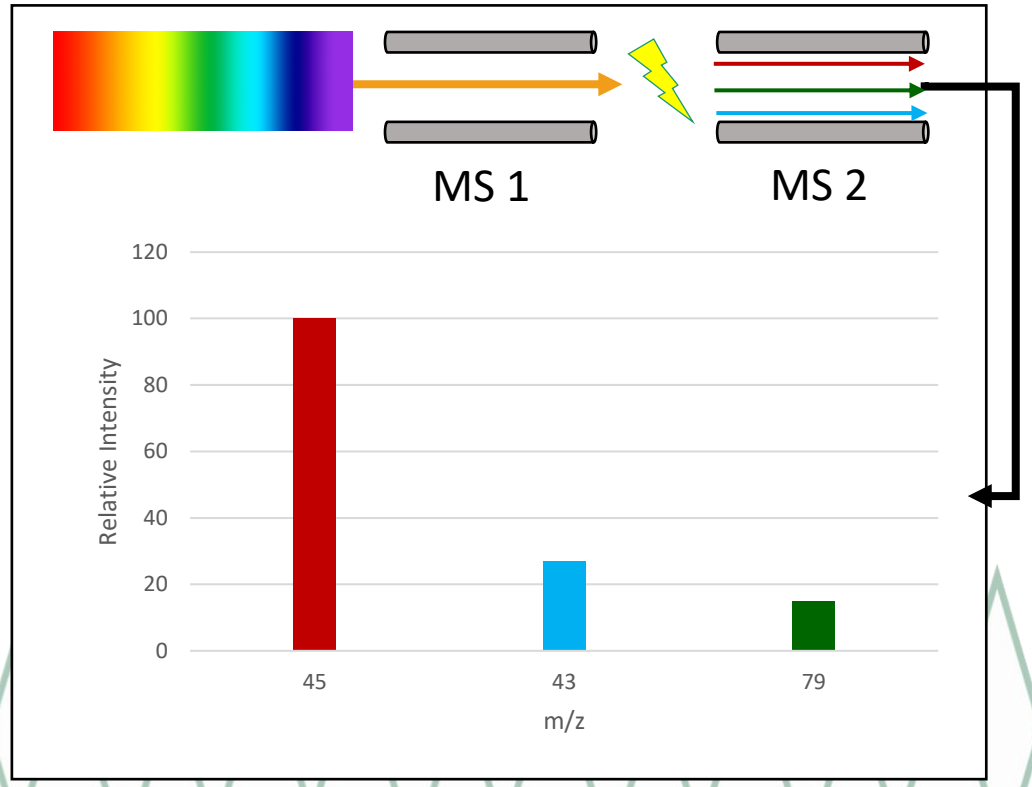
Pesticide Analyses: Mass Spectrometry

Mass Spectrometers can Operate in Targeted Mode for Increased Sensitivity

Non-Targeted Mode (Scan)



Targeted Mode (MRM)



Pesticide Analyses: Mass Spectrometry

Mass Spectrometers can Operate in Targeted or Non-Targeted Mode

Non-Targeted Mode (Scan)

- All the signal, all the time (full spectrum)
- Don't need to pre-knowledge of what to target
- Less time per fragment = lower signal
- Low* specificity = higher noise
- Most instruments that work in Scan mode can also measure mass with high accuracy (HRAM*)

Targeted Mode (MRM)

- A specific signal at a specific time
- The MS acts as a "mass filter"
- Only looks for 2 or 3 masses (fragments) at a time
- More time per fragment = higher signal
- Higher specificity = reduced noise

Analytical Advances and MRLs

I. A[illegible]tical C[illegible]

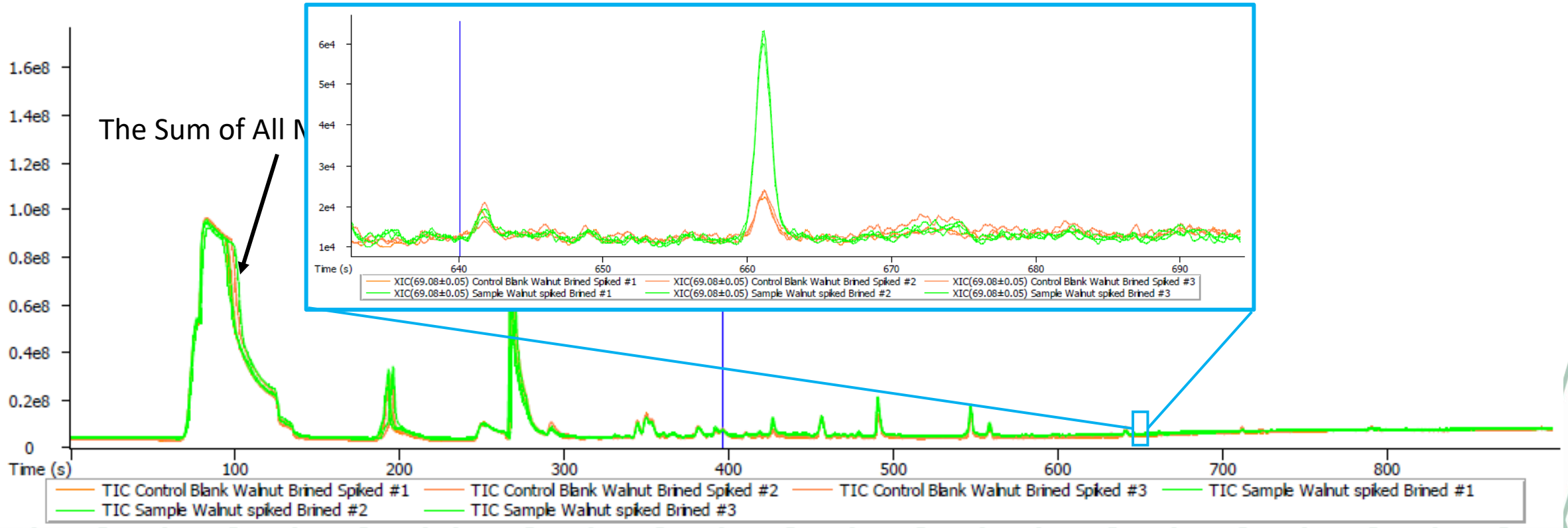
See me later to collect your diplomas!!

II. Advance #1: nDATA Multi-Residue Screens

III. Advance #2: Alternative Separation Methods

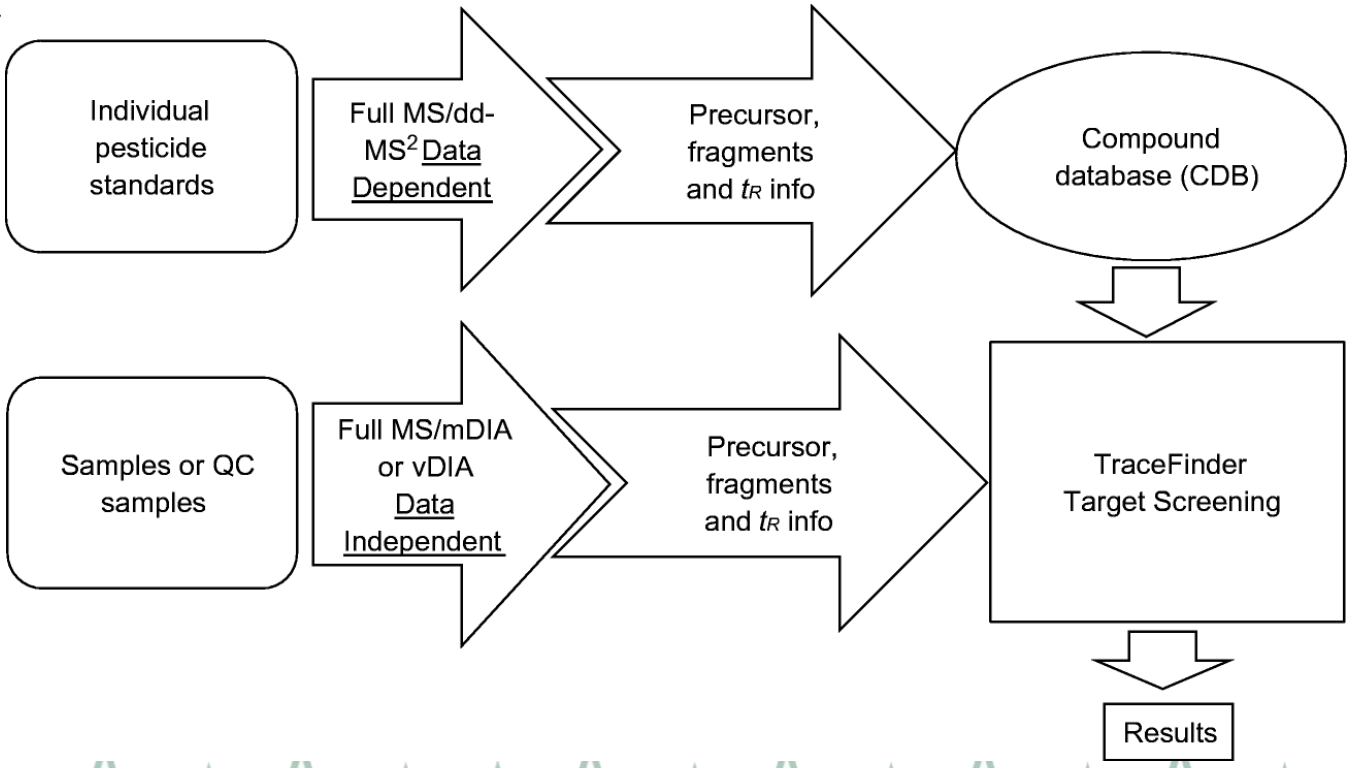
nDATA: non-target Data Acquisition for Target Analysis

- Non-Targeted analyses provide a lot more data, but the analysis can be more difficult…



nDATA: non-target Data Acquisition for Target Analysis

- Hybrid Non-Targeted (acquisition) / Targeted (Data Searching)
- Mass Spec. operates in scan mode, auto-selecting masses to fragment (for ID confirmation)
- After data is collected, a targeted search is made based on a database of known pesticides – now up to 1200 compounds
- Possible due to advances in detector sensitivity and speed and the use of a standardized method



Wang, J., Chow, W., Wong, J.W. *et al.* Non-target data acquisition for target analysis (nDATA) of 845 pesticide residues in fruits and vegetables using UHPLC/ESI Q-Orbitrap. *Anal Bioanal Chem* **411**, 1421–1431 (2019). https://doi.org/10.1007/s00216-019-01581-z

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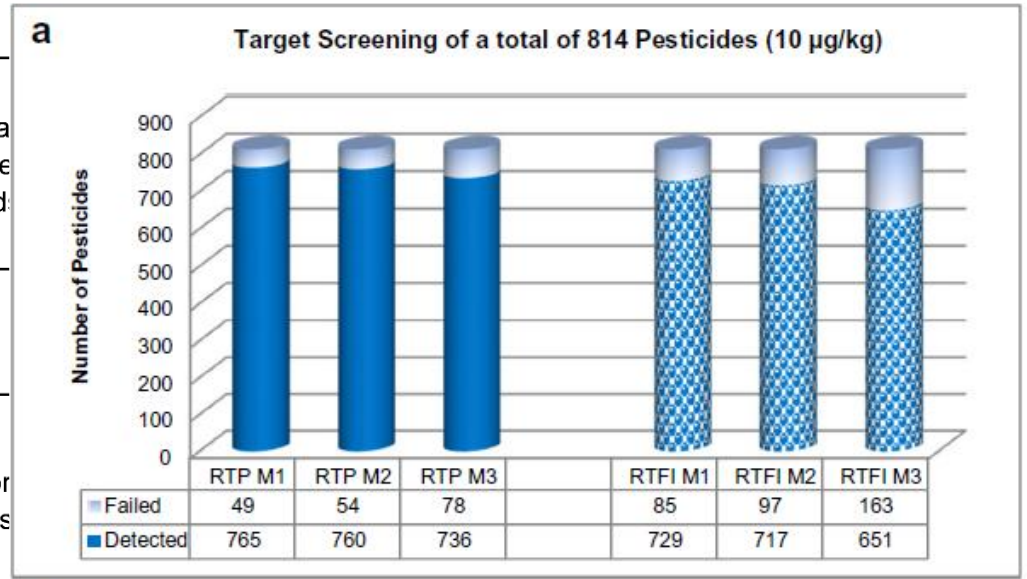

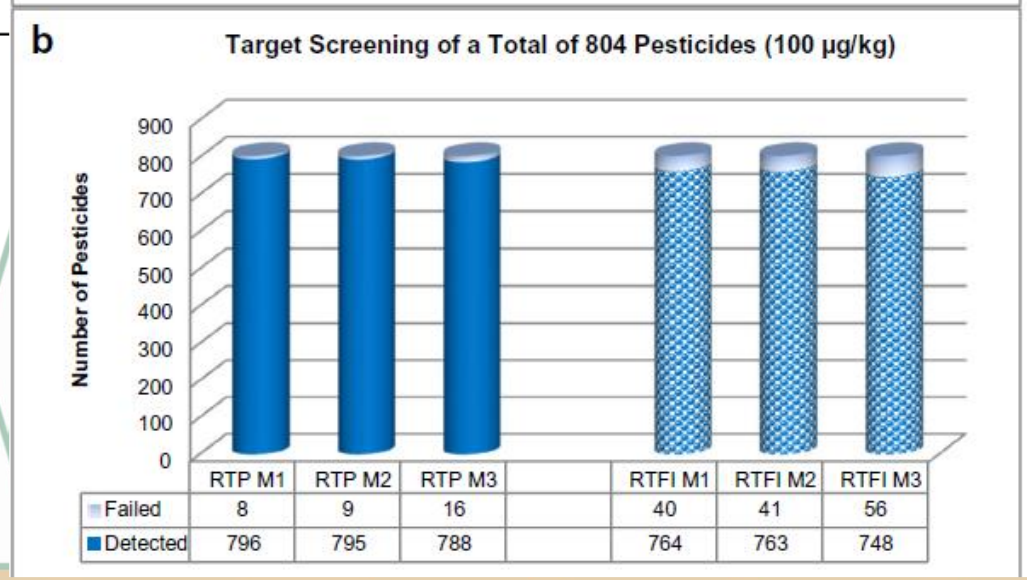

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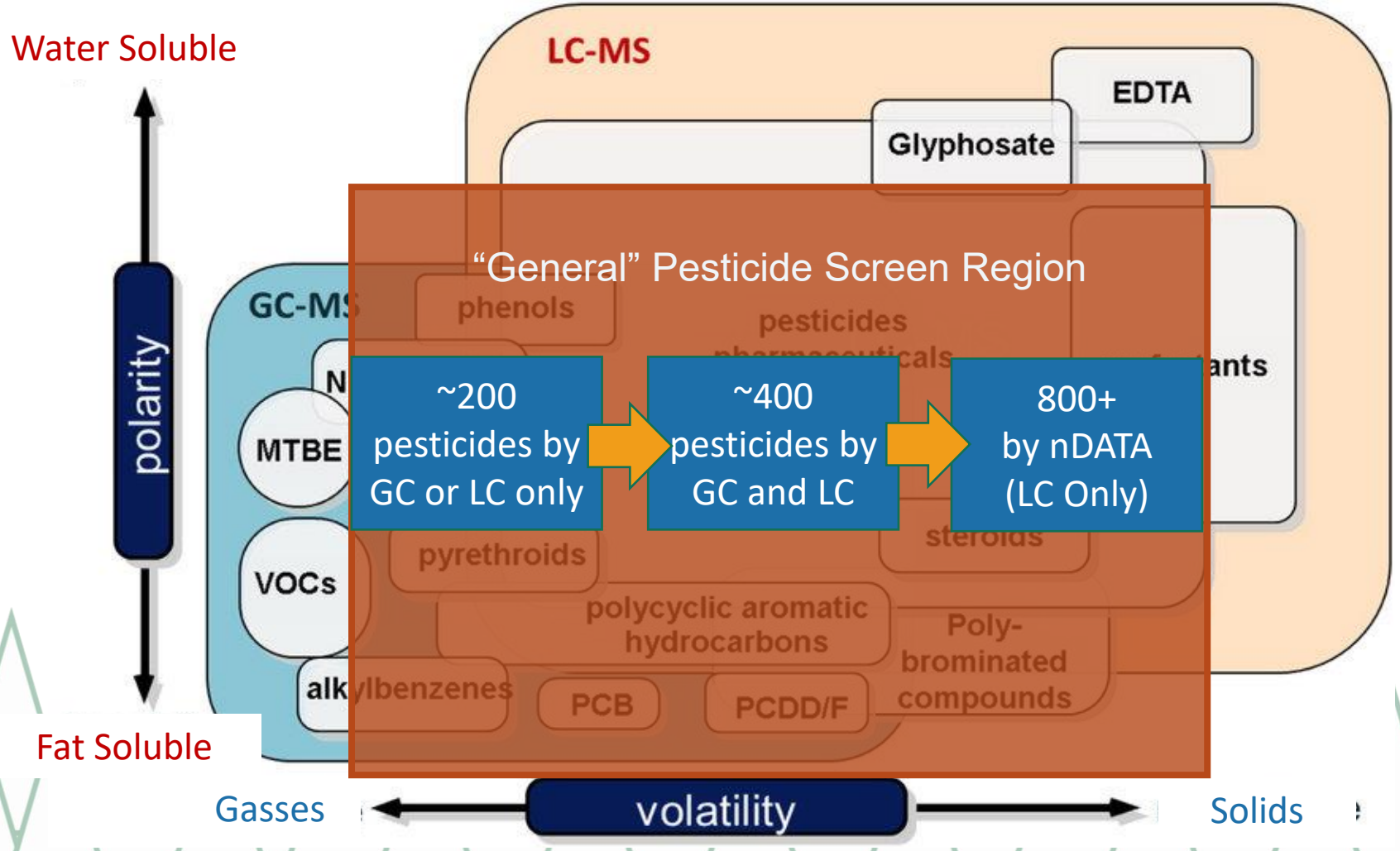
nDATA: non-target Data Acquisition for Target Analysis

- Easy and relatively quick and inexpensive to increase the number of compounds in a screen **Cost Reduction**
- Flexible – part or all of the CDB can be used
- Standardizes testing methodology **Cost Reduction**
- Data can be reviewed later for new compounds of interest (*not quantitatively*) ~~Trade Barrier (?)~~

- (relatively) High barrier to entry **Cost Increase**
  - will require new instrumentation for most labs (q-TOF or q-Orbitrap)
  - Chemical standards required for validations
- Could lead to unreasonable client expectations? **Trade Barrier (?)**
- Still leaves out some high use compounds
- A "deep" method, but not very "wide"

RESIDUE ANALYSIS: THE (2-D) UNIVERSE OF CHEMICALS



https://www.researchgate.net/figure/Domains-of-GC-MS-and-LC-MS-techniques-in-the-two-dimensions-of-hydrophobicity-and_fig10_290221006

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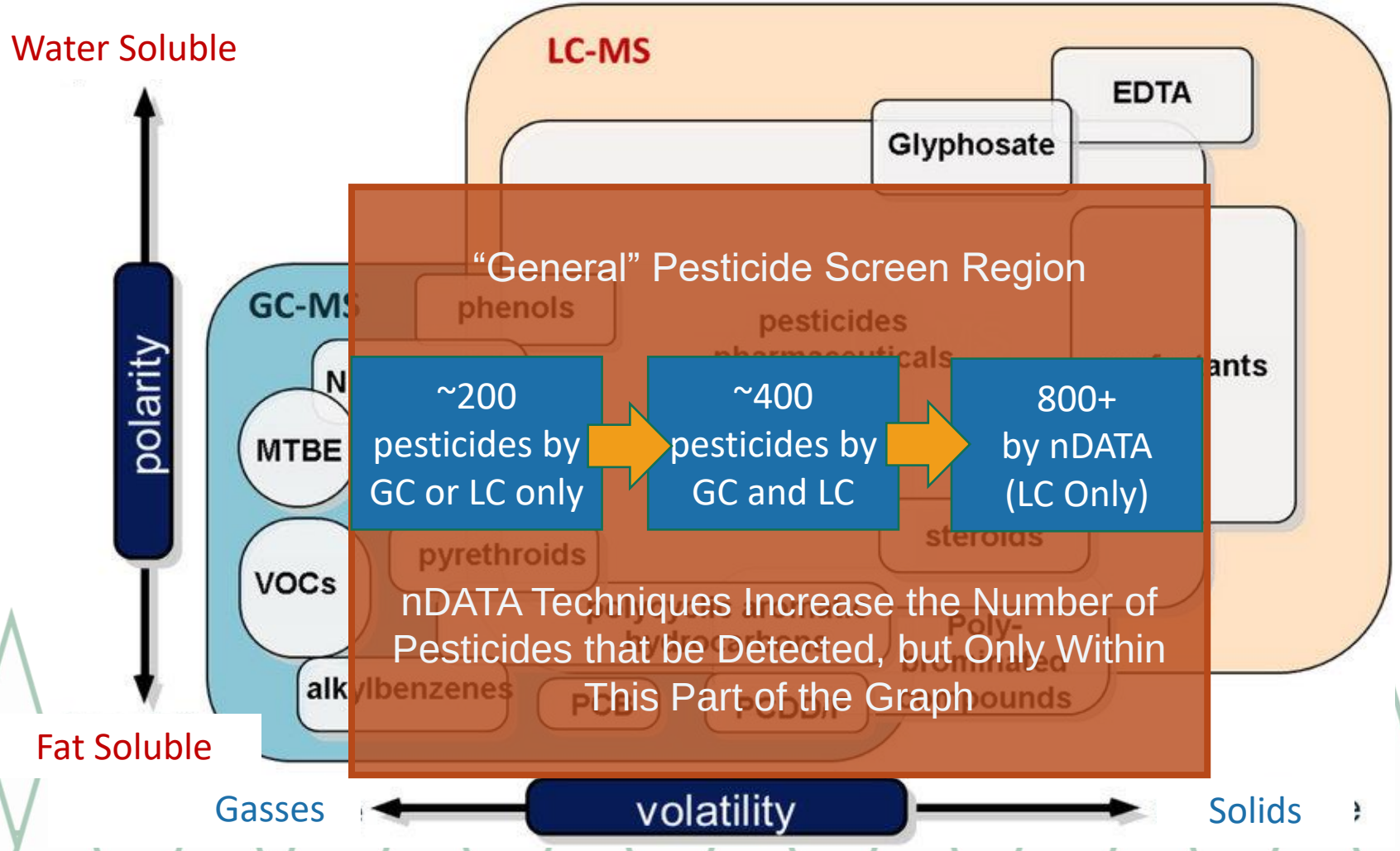

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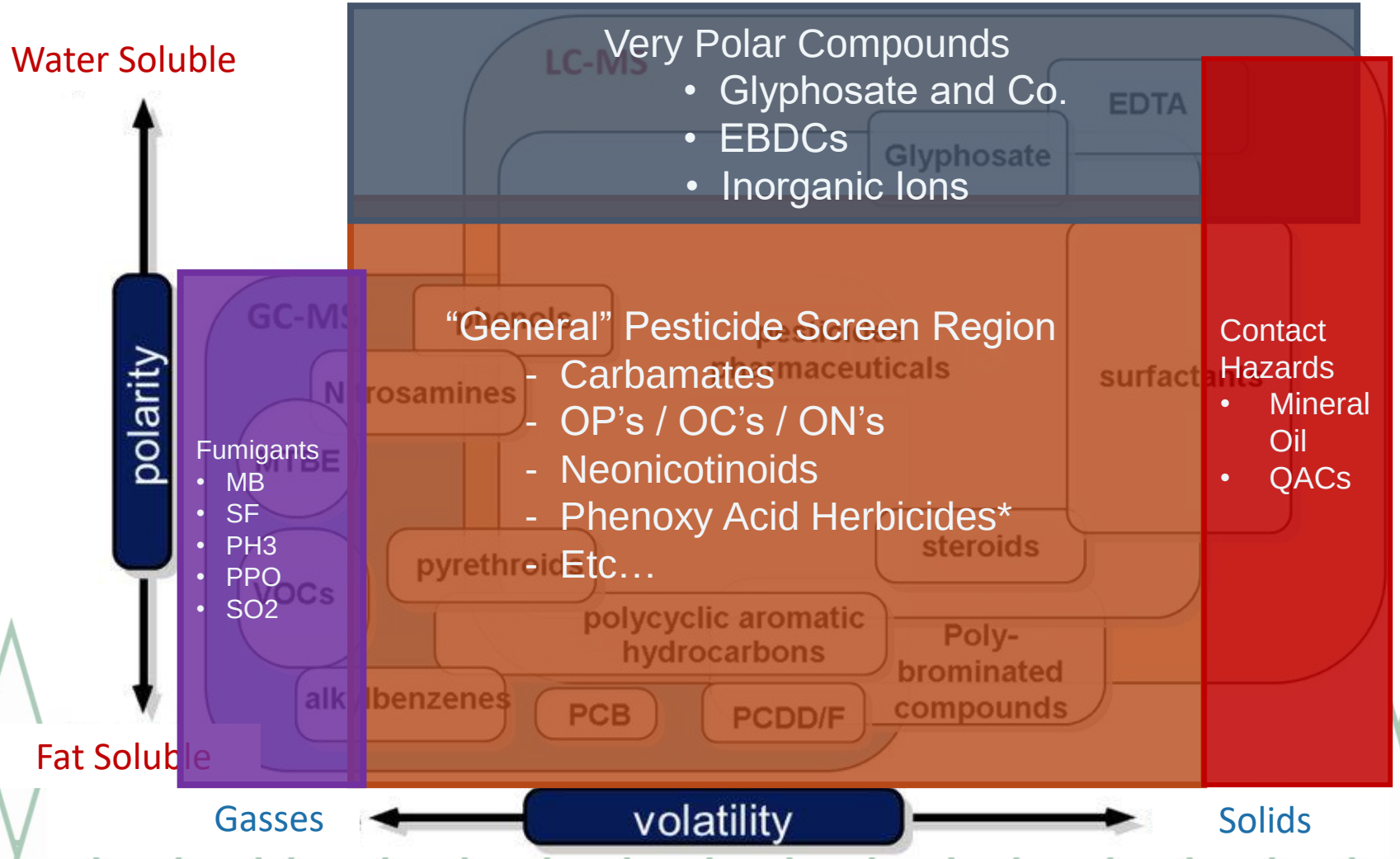

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The "Top" CA Pesticides: "General" screens

Rank	Compound	Rank	Compound	Rank	Compound	Rank	Compound
1	GLYPHOSATE	22	ABAMECTIN	42	2,4-D, DIMETHYLAMINE SALT	59	RIMSULFURON
2	OXYFLUORFEN	22	CYPRODINIL	43	MALATHION	60	QUINOXYFEN
3	PENDIMETHALIN	23	METAM-SODIUM	43	SAFLUFENACIL	61	DIFENOCONAZOLE
4	PARAQUAT DICHLORIDE	24	SULFURYL FLUORIDE	44	TRIFLOXYSTROBIN	61	EPTC
5	IMIDACLOPRID	25	METHYL BROMIDE	45	S-METOLACHLOR	62	THIAMETHOXAM
6	GLUFOSINATE-AMMONIUM	26	METHOMYL	46	NALED	63	DIURON
7	MANCOZEB	27	LAMBDA-CYHALOTHRIN	47	ETOXAZOLE	64	METCONAZOLE
8	METHOXYFENOZIDE	28	CHLORANTRANILIPROLE	47	ORYZALIN	65	CARBARYL
9	BIFENTHRIN	29	POTASSIUM PHOSPHITE	48	FLUOPYRAM	65	MYCLOBUTANIL
10	CHLORPYRIFOS	30	PYRACLOSTROBIN	49	COPPER ETHANOLAMINE COMPLEXES	66	FLONICAMID
11	CHLOROTHALONIL	31	ZIRAM	49	PROPICONAZOLE	67	INDAZIFLAM
12	PROPANIL	32	SPINETORAM	50	(S)-CYPERMETHRIN	68	PYRAFLUFEN-ETHYL
13	AZOXYSTROBIN	32	THIOBENCARB	50	BENSULIDE	69	ACETAMIPRID
14	BOSCALID	33	CALCIUM HYPOCHLORITE	51	SODIUM BROMIDE	69	PROPARGITE
15	ETHEPHON	34	CAPTAN	52	SULFUR DIOXIDE	70	CHLORTHAL-DIMETHYL
16	IPRODIONE	35	CHLORINE	53	TEBUCONAZOLE	71	FLUXAPYROXAD
17	1,3-DICHLOROPROPENE	36	SODIUM HYPOCHLORITE	54	FIPRONIL	71	SODIUM CHLORATE
18	DIMETHOATE	37	HYDROGEN CYANAMIDE	55	ESFENVALERATE	72	BETA-CYFLUTHRIN
19	POTASSIUM N-METHYLDITHIOCARBAMATE	38	SPIROTETRAMAT	56	FLUBENDIAMIDE		
20	CHLOROPICRIN	39	TRIFLURALIN	57	FOSETYL-AL		
20	PHOSPHORIC ACID	40	CARFENTRAZONE-ETHYL	57	RIMSULFURON		
21	PERMETHRIN	41	PROPYLENE OXIDE	58	QUINOXYFEN		

The "Top" CA Pesticides: "General" screens

12,412,791 lbs →

Rank	Compound	Rank	Compound	Rank	Compound	Rank	Compound	Pounds (2020)
1	GLYPHOSATE	22	ABAMECTIN	42	2,4-D, DIMETHYLAMINE SALT	59	RIMSULFURON	38905
2	OXYFLUORFEN	22	CYPRODINIL	43	MALATHION	60	QUINOXYFEN	40195
3	PENDIMETHALIN	23	METAM-SODIUM	43	SAFLUFENACIL	61	DIFENOCONAZOLE	63753
4	PARAQUAT DICHLORIDE	24	SULFURYL FLUORIDE	44	TRIFLOXYSTROBIN	61	EPTC	173257
5	IMIDACLOPRID	25	METHYL BROMIDE	45	S-METOLACHLOR	62	THIAMETHOXAM	50627
6	GLUFOSINATE-AMMONIUM	26	METHOMYL	46	NALED	63	DIURON	16033
7	MANCOZEB	27	LAMBDA-CYHALOTHRIN	47	ETOXAZOLE	64	METCONAZOLE	59226
8	METHOXYFENOZIDE	28	CHLORANTRANILIPROLE	47	ORYZALIN	65	CARBARYL	110231
9	BIFENTHRIN	29	POTASSIUM PHOSPHITE	48	FLUOPYRAM	65	MYCLOBUTANIL	42386
10	CHLORPYRIFOS	30	PYRACLOSTROBIN	49	COPPER ETHANOLAMINE COMPLEXES	66	FLONICAMID	39092
11	CHLOROTHALONIL	31	ZIRAM	49	PROPICONAZOLE	67	INDAZIFLAM	41662
12	PROPANIL	32	SPINETORAM	50	(S)-CYPERMETHRIN	68	PYRAFLUFEN-ETHYL	2845
13	AZOXYSTROBIN	32	THIOBENCARB	50	BENSULIDE	69	ACETAMIPRID	68377
14	BOSCALID	33	CALCIUM HYPOCHLORITE	51	SODIUM BROMIDE	69	PROPARGITE	225197
15	ETHEPHON	34	CAPTAN	52	SULFUR DIOXIDE	70	CHLORTHAL-DIMETHYL	210653
16	IPRODIONE	35	CHLORINE	53	TEBUCONAZOLE	71	FLUXAPYROXAD	54733
17	1,3-DICHLOROPROPENE	36	SODIUM HYPOCHLORITE	54	FIPRONIL	71	SODIUM CHLORATE	434516
18	DIMETHOATE	37	HYDROGEN CYANAMIDE	55	ESFENVALERATE	72	BETA-CYFLUTHRIN	34253
19	POTASSIUM N-METHYLDITHIOCARBAMATE	38	SPIROTETRAMAT	56	FLUBENDIAMIDE		Total Pounds →	1,852,941
20	CHLOROPICRIN	39	TRIFLURALIN	57	FOSETYL-AL			
20	PHOSPHORIC ACID	40	CARFENTRAZONE-ETHYL	57	RIMSULFURON			
21	PERMETHRIN	41	PROPYLENE OXIDE	58	QUINOXYFEN			

Where is Our Effort Best Focused?

Take Grapes as an Example:

- Grapes / Wine / Raisins have MRLs for ~175 A.I.'s Worldwide
- In 2018, 175 different pesticides were applied to grapes (CA PUR) in California (only 103 with more than 1000 lbs reported statewide)
- Across the three most recent years of FDA testing (PDP – 2016, 2015 and 2010) only 78 different compounds were found.
- Of course, that's based on current testing schemes
- It may be that the presence of "unexpected" pesticides residues indicates a larger problem

Analytical Advances and MRLs

I. A Quick Intro to Analytical Chemistry

II. Advance #1: nDATA Multi-Residue Screens

III. Advance #2: Alternative Separation Methods

The “Top” CA Pesticides: Polar compounds

Quick Method for the Analysis of numerous Highly Polar Pesticides in Foods of Plant Origin via LC-MS/MS involving Simultaneous Extraction with Methanol (QuPPe-Method)

- **Version 9.2** (October 2016, Document History, see page **65**)

Authors: M. Anastassiades; D. I. Kolberg; A. Benkenstein; E. Eichhorn; S. Zechmann; D. Mack; C. Wildgrube; I. Sigalov; D. Dörk; A. Barth

Method 1.1 “Glyphosate & Co. AS 11”
Method 1.2 “Glyphosate & Co. AS 11-HC”
Method 1.3 “Glyphosate & Co. Hypercarb”
Method 1.4 “PerChloPhos”
Method 2 “Fosetyl and Maleic Hydrazide”
Method 3 “Amitrole & Co”
Method 4.1 “Quats & Co Obelisc R”
Method 4.2 “Quats & Co BEH Amide”
Method 5 “Quats & Co. MonoChrom MS”
Method 6 “Streptomycin and Kasugamycin”
Method 7 “Morpholine, Diethanolamine and Triethanolamine”
Method 8 “Triazole derivative metabolites (TDMs)”

- Simple, unified method for the extraction
- Each Sub-Method Requires a Different instrument Setup

Polars analysis: The "QuPPe"

Using Liquid Chromatography Multiple Methods / Columns are Required to Analyze These Heavily Used Polar (Charged) Pesticides

From the QuPPe v11: | From my own work:

	Hypercarb	AS 11-HC	Obelisc-R	Diol-Type C
Phosphonate	Y	Y*	?	Y
Fosetyl	Y	Y	?	Y
AMPA	Y	Y	?	Y
Glyphosate	Y	Y	?	Y
Glufosinate	Y	Y	?	Y
Ethephon	Y	Y	?	Y
Diquat	N	N	Y	Y**
Paraquat	N	N	Y	Y**
Mepiquat	Y	N	Y	Y**
Chlormequat	Y	N	Y	Y**
Chlorate	Y	?	?	Y
Perchlorate	Y	?	Y	Y
Bromate	Y*	?	?	?

* Poor performance

** Same column, different method

What if we *didn't use LC-MSMS?*

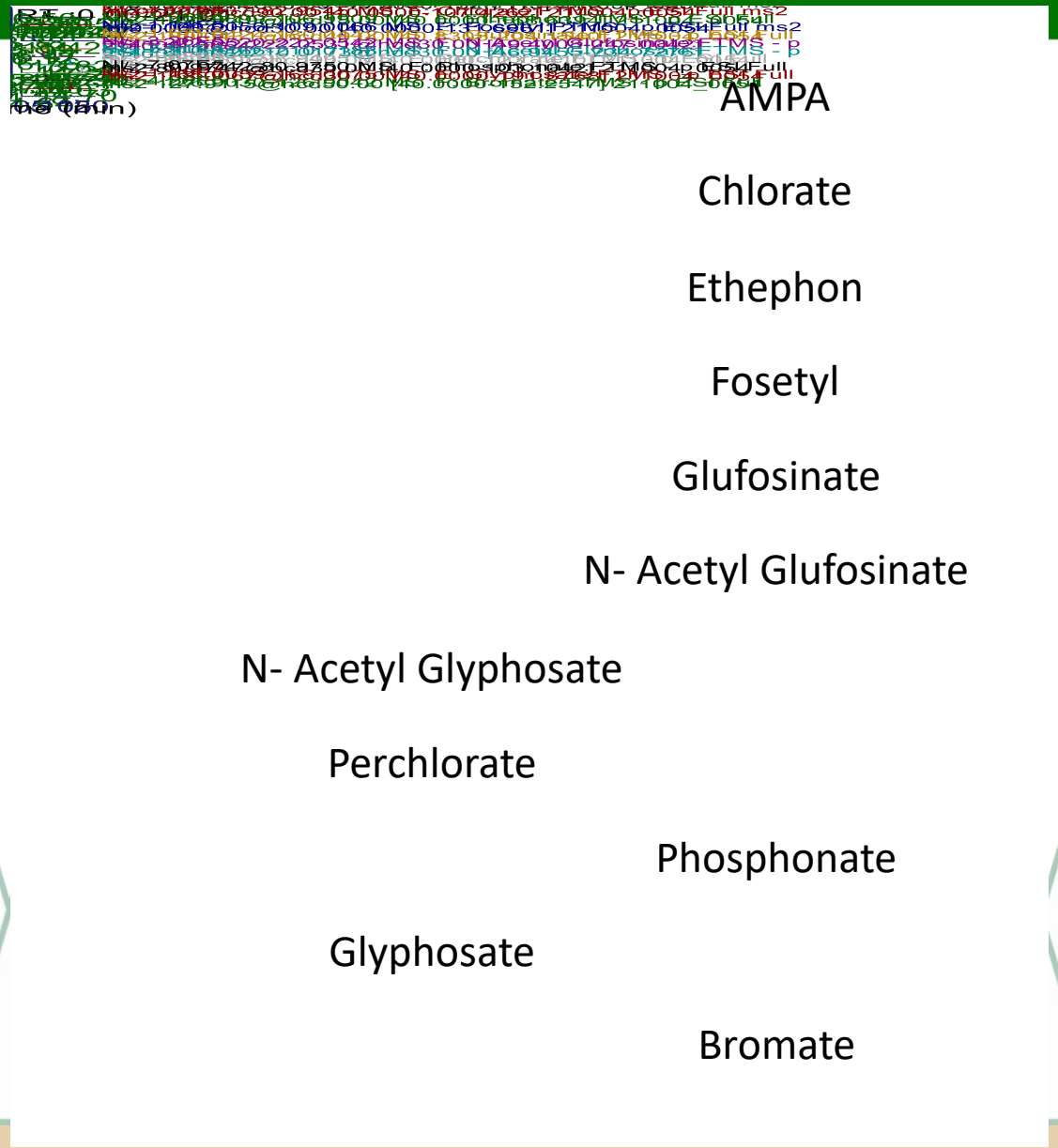
Ion Chromatography-MSMS for the Analysis of Polar Pesticides

- Electrolytic ion suppression allows the use of KOH as an eluent
- Eluent generator saves space and allows for the use of a single pump
- No carry over
- Excellent resolution and sensitivity

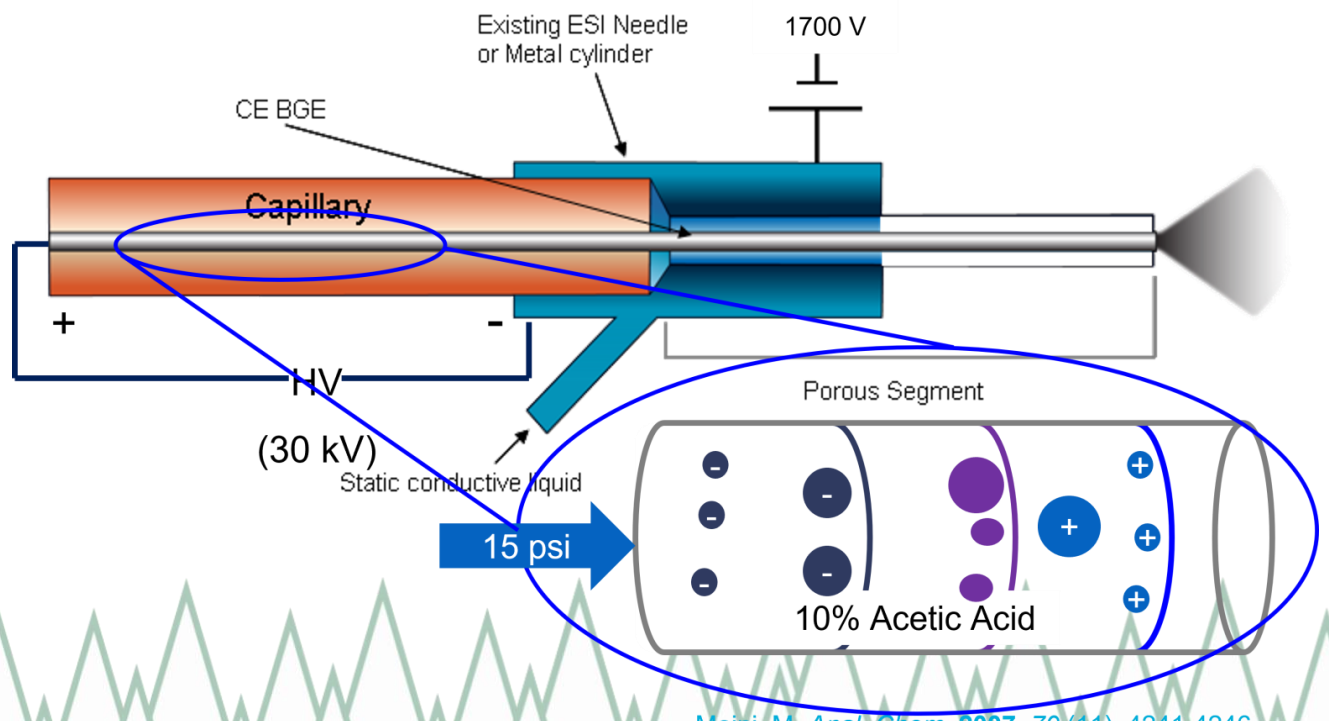
Li, Y. et al. (2019). Application Report No. 73204. Thermo Fisher Scientific.

Ion Chromatography-MSMS for the Analysis of Polar Pesticides

- No carry over
- Excellent resolution and sensitivity
- Large matrix effect
- Only anions or cations w/in a single method
- Potential MS damage with suppressor failure



CESI-MSMS Analysis of Polar Pesticides



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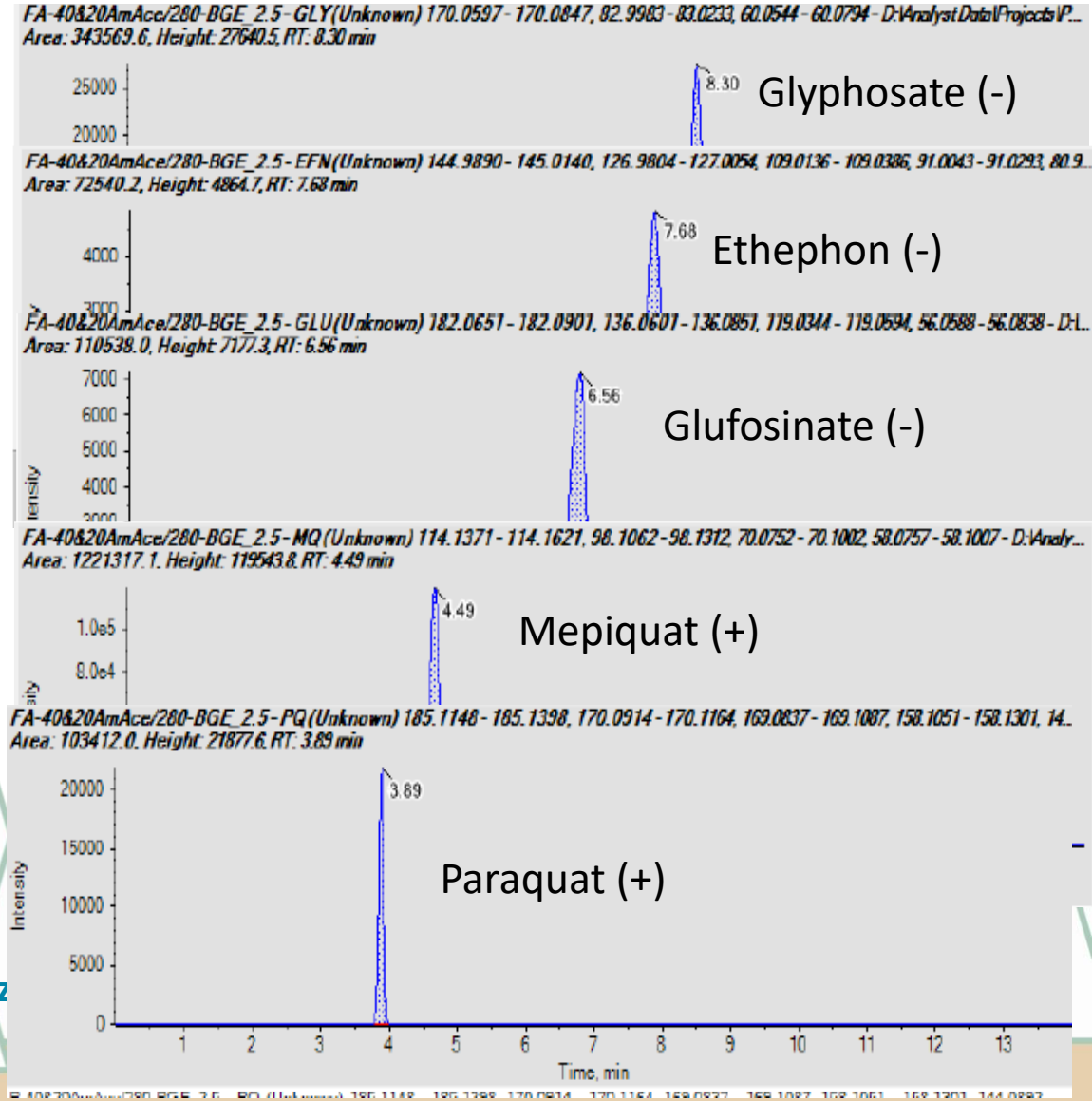
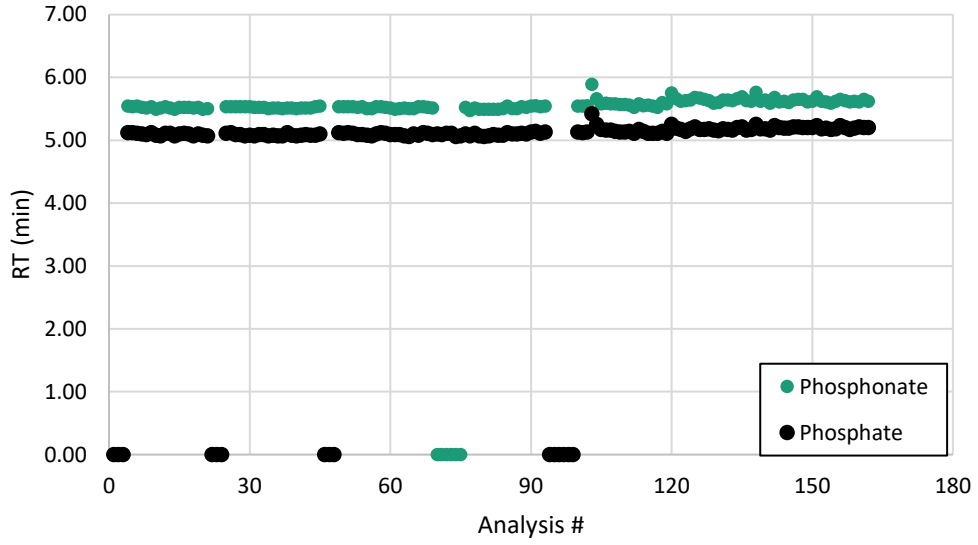
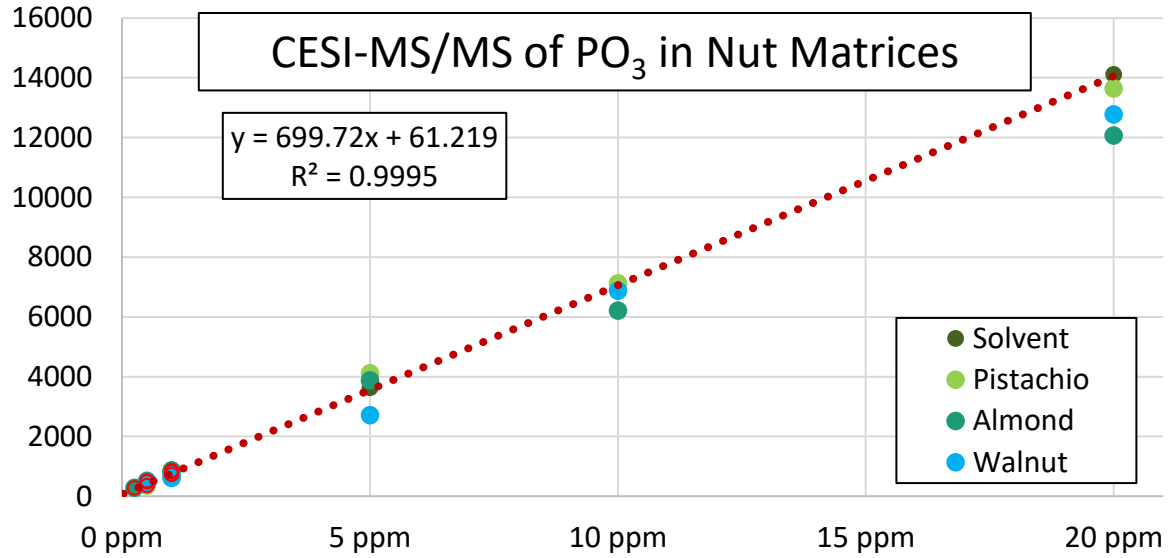


CESI - "The Integration of Capillary Electrophoresis (CE) with Electrospray Ioniz
Single Dynamic Process Within the Same Device"

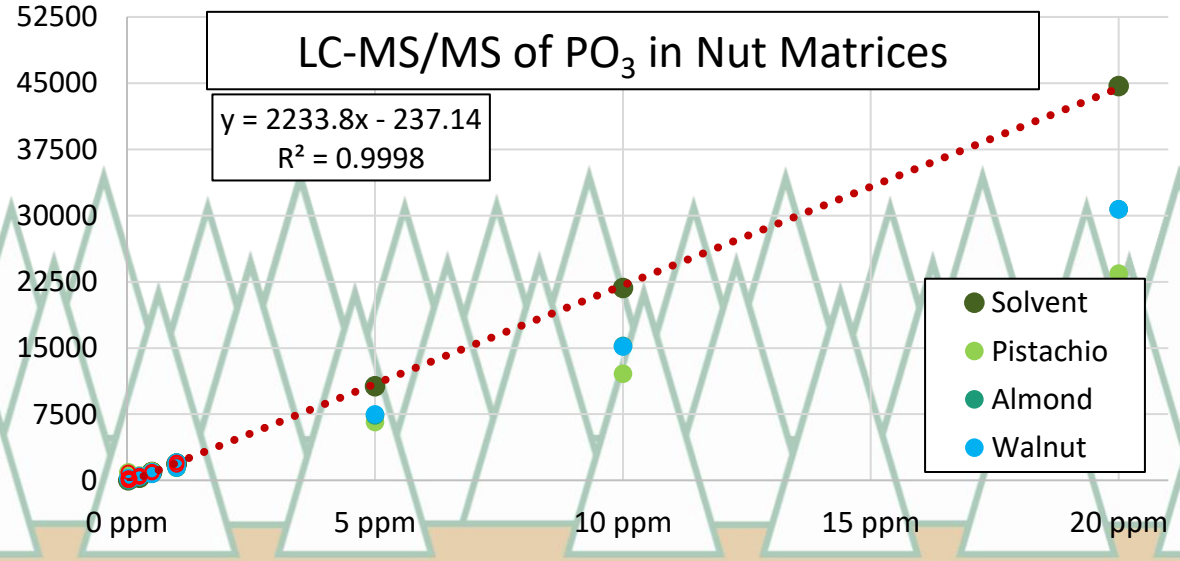
CESI-MSMS Analysis of Polar Pesticides





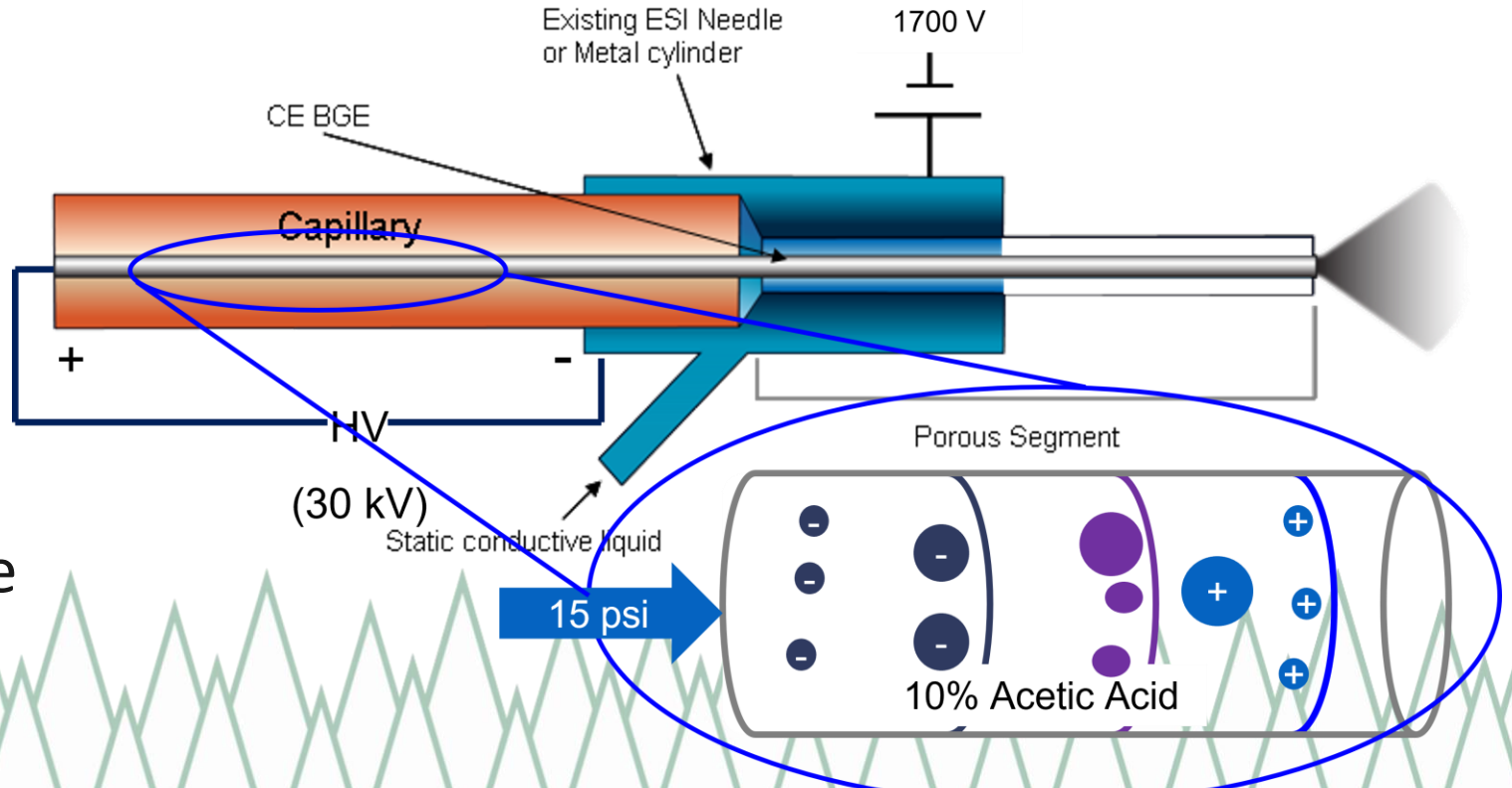
- Consistent Retention (Migration) Times and Resolution

- Little to No Ion Suppression



CESI-MSMS Analysis of Polar Pesticides

- Consistent Retention (Migration) Times and Resolution
- Little to No Ion Suppression
- New Information
- Anions and Cations in a Single Run
- Expensive and Fragile



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Alternate Separation Methods

- Expands the "breadth" of pesticides being tested
  - High Efficiency (lbs usage / pesticide)

Cost Reduction

- Turns single residue meth multi residue methods

Cost Reduction

- Requires additional instrumentation

Cost Increase

- Relatively few compounds per injection
- Still not as comprehensive as other multi-residue methods

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8	METHOXYFENOZIDE	28	CHLORANTRANILIPROLE	47	ORYZALIN	65	CARBARYL
9	BIFENTHRIN	29	POTASSIUM PHOSPHITE	48	FLUOPYRAM	65	MYCLOBUTANIL
10	CHLORPYRIFOS	30	PYRACLOSTROBIN	49	COPPER ETHANOLAMINE COMPLEXES	66	FLONICAMID
11	CHLOROTHALONIL	31	ZIRAM	49	PROPICONAZOLE	67	INDAZIFLAM
12	PROPANIL	32	SPINETORAM	50	(S)-CYPERMETHRIN	68	PYRAFLUFEN-ETHYL
13	AZOXYSTROBIN	32	THIOBENCARB	50	BENSULIDE	69	ACETAMIPRID
14	BOSCALID	33	CALCIUM HYPOCHLORITE	51	SODIUM BROMIDE	69	PROPARGITE
15	ETHEPHON	34	CAPTAN	52	SULFUR DIOXIDE	70	CHLORTHAL-DIMETHYL
16	IPRODIONE	35	CHLORINE	53	TEBUCONAZOLE	71	FLUXAPYROXAD
17	1,3-DICHLOROPROPENE	36	SODIUM HYPOCHLORITE	54	FIPRONIL	71	SODIUM CHLORATE
18	DIMETHOATE	37	HYDROGEN CYANAMIDE	55	ESFENVALERATE	72	BETA-CYFLUTHRIN
19	POTASSIUM N-METHYLDITHIOCARBAMATE	38	SPIROTETRAMAT	56	FLUBENDIAMIDE		
20	CHLOROPICRIN	39	TRIFLURALIN	57	FOSETYL-AL		
20	PHOSPHORIC ACID	40	CARFENTRAZONE-ETHYL	57	RIMSULFURON		
21	PERMETHRIN	41	PROPYLENE OXIDE	58	QUINOXYFEN		

Heavy Metal Screen

- General Screen
- Polar Suite (Anions)
- EBDC Screen
- Volatiles
- Cationic Pesticides

Final Thoughts

The Race to Zero

- Less focus on decreasing reporting limits
- Instead, higher sensitivity is being used:
  - Faster Scanning (more pesticides)
  - Less Sample per Analysis (reduced downtime)

Cost Reduction

The Race to Infinity

- Fewer technological restraints on the number of pesticides per analysis

Cost Increase

- Which pesticides do we want to focus on?

Trade Barrier (?)

~~Trade Barrier (?)~~

- Analytical arms race continues…even worsens?

Cost Increase

THANK YOU!

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1. Fast Response to Industry / Scientific Residue Issues
2. Residue Analysis Regulatory Purposes
3. Agrochemical Residue Method Simplification and Publication
4. Harm Reduction for Agrochemical Use

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