Comparison of LC-MS Pesticide Screening Results Using Different Reversed-Phase Superficially Porous Columns

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Pittcon

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Outline

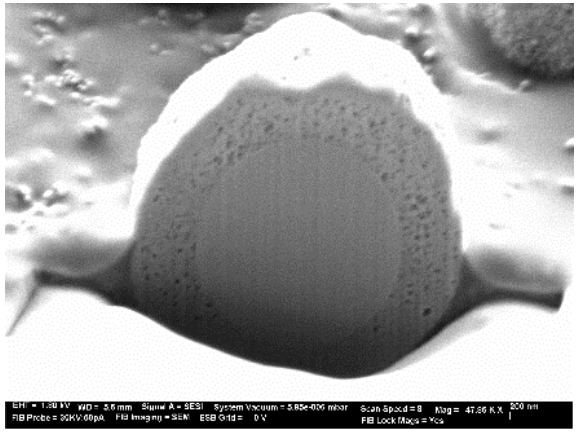
- Pesticides
- Superficially Porous Particles (SPPs)
- HALO® Column Chemistries used in this Study
- Screening Results
- Summary

Pesticides – Facts and Figures

- Substance or mixture of substances intended for:
  - Preventing, destroying, repelling or mitigating any pest
  - Use as a plant regulator, defoliant, or desiccant
  - Use as a nitrogen stabilizer
- > 20 different types of pesticides
  - Most well-known types are herbicides, insecticides, fungicides, and rodenticides
- Why is it important to monitor pesticides?
  - Crop safety
  - Drinking water and wastewater safety
  - Runoff

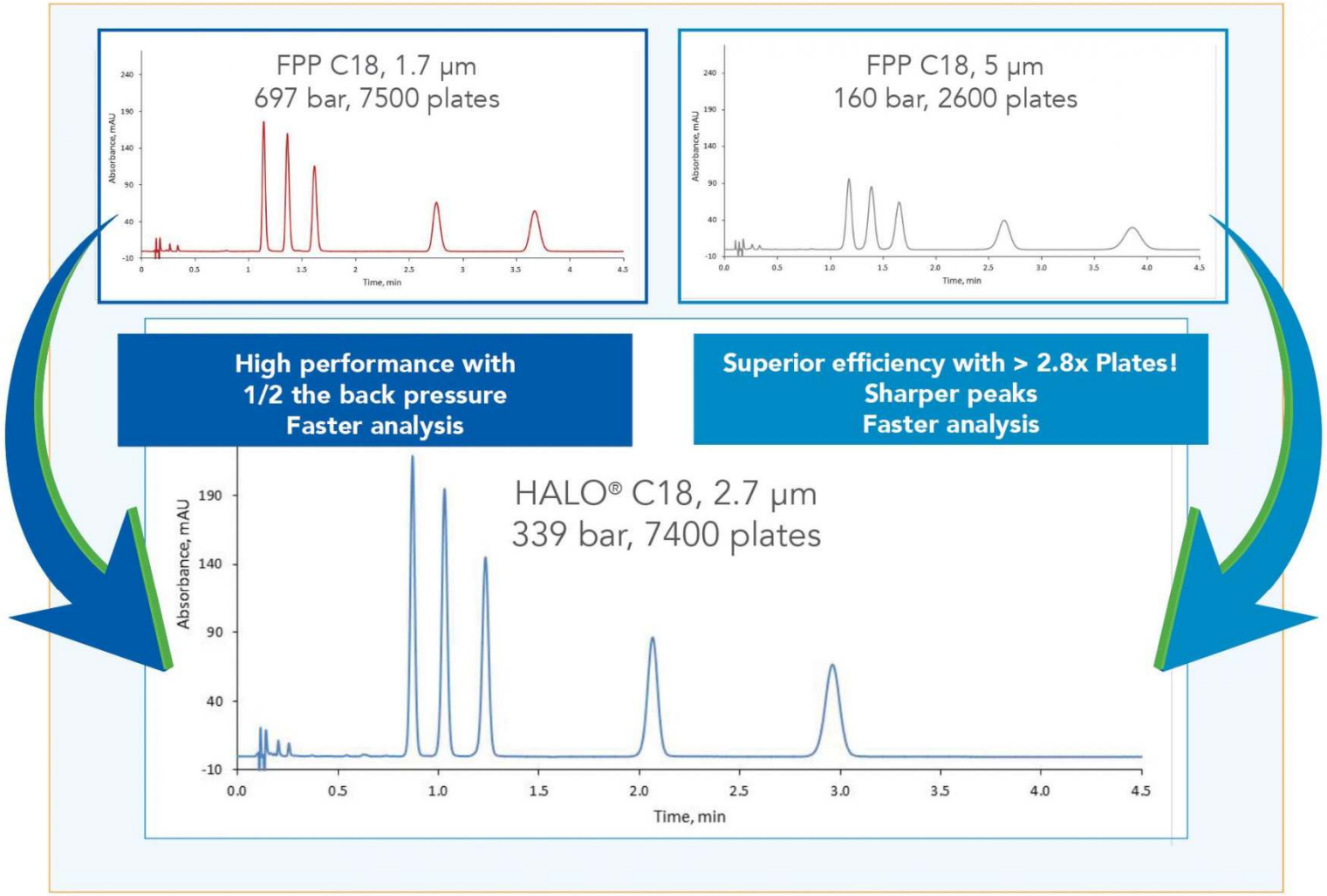
Superficially Porous Particles

HALO 90 Å, 2.7 µm



HALO Advantages

- Fast separations
- Lower back pressure compared to sub-2-µm particle columns
- Increased efficiency/resolution



Hydrophobic Subtraction Model Characterization

F_s	Name	H	S*	A	B	C (pH 2.8)	C (pH 7.0)	EB retention factor	USP type	Phase type
0	Halo C18	1.1	0.04	0	-0.05	0.05	0.04	6.1	L1	C18
10.04	Halo C8	0.91	0.02	-0.13	0	-0.01	0.18	4.3	L7	C8
12.07	Halo AQ-C18	1	-0.036	0.099	-0.048	0.156	0.864	6.7	L1	C18
15.56	Halo LPH-C18	0.99	-0.036	0.33	0.0055	0.106	1.02	6.2	L1	C18
17.35	Halo Phenyl-Hexyl	0.78	-0.09	-0.23	0	0.1	0.45	3.5	L11	Phenyl
17.43	Halo C30	0.938	-0.046	-0.14	0.023	0.17	0.35	4.5	L62	C30
22.78	Halo ES-CN	0.566	-0.11	-0.344	0.021	0.126	1.15	1.88	L10	CN
26.76	Halo Biphenyl	0.708	-0.183	-0.279	0.028	0.047	0.99	3.1	L11	phenyl
52.83	Halo RP-Amide	0.85	0.08	-0.38	0.19	-0.41	0.31	4.6	L60	EP
94.45	HALO PFP	0.702	-0.117	-0.073	-0.062	1.17	0.972	2.3	L43	F

https://www.hplccolumns.org/database/compare.php

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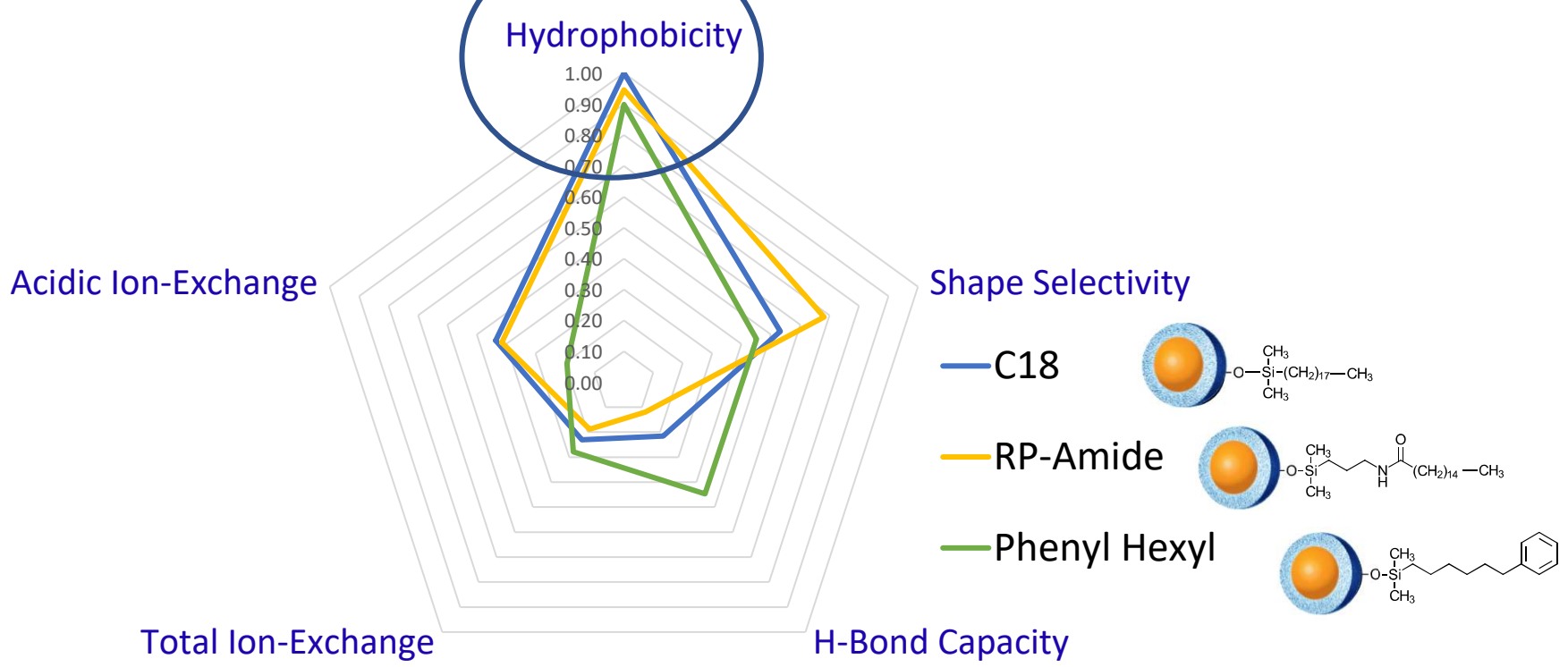
$$F_s = \sqrt{\left(w_H(H_1 - H_2)\right)^2 + \left(w_S(S^*_1 - S^*_2)\right)^2 + \left(w_A(A_1 - A_2)\right)^2 + \left(w_B(B_1 - B_2)\right)^2 + \left(w_{C_{2.8}}(C_{2.8_1} - C_{2.8_2})\right)^2}$$

If Fs is < 12, then similar selectivity
If Fs is > 12, then different selectivity

Euerby-Modified Tanaka Characterization

Ranking	Hydrophobicity	Shape Selectivity	Hydrogen Bond Capacity	Acidic Ion-Exchange	Total Ion-Exchange
1	C18	RP-Amide	Phenyl-Hexyl	C18	Phenyl-Hexyl
2	RP-Amide	C18	C18	RP-Amide	C18
3	Phenyl-Hexyl	Phenyl-Hexyl	RP-Amide	Phenyl-Hexyl	RP-Amide



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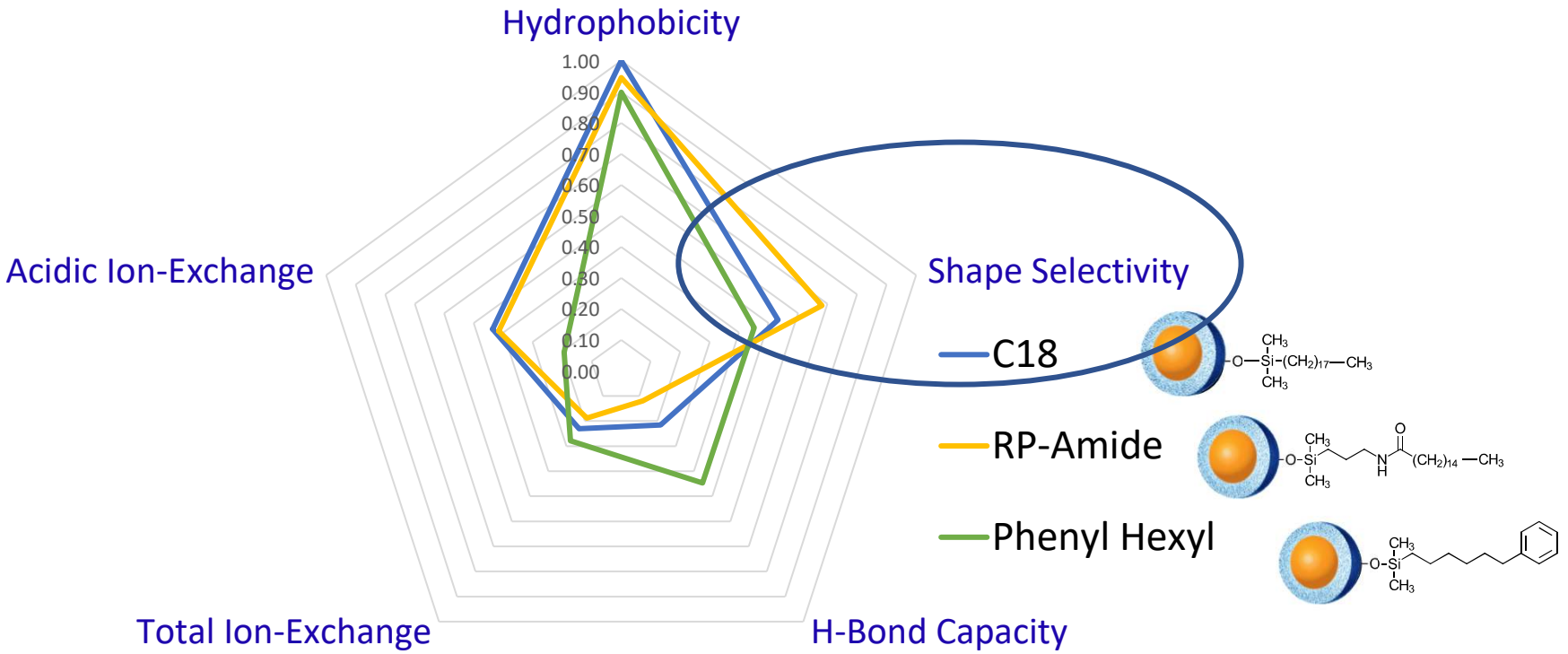
3	Phenyl-Hexyl	Phenyl-Hexyl	RP-Amide	Phenyl-Hexyl	RP-Amide



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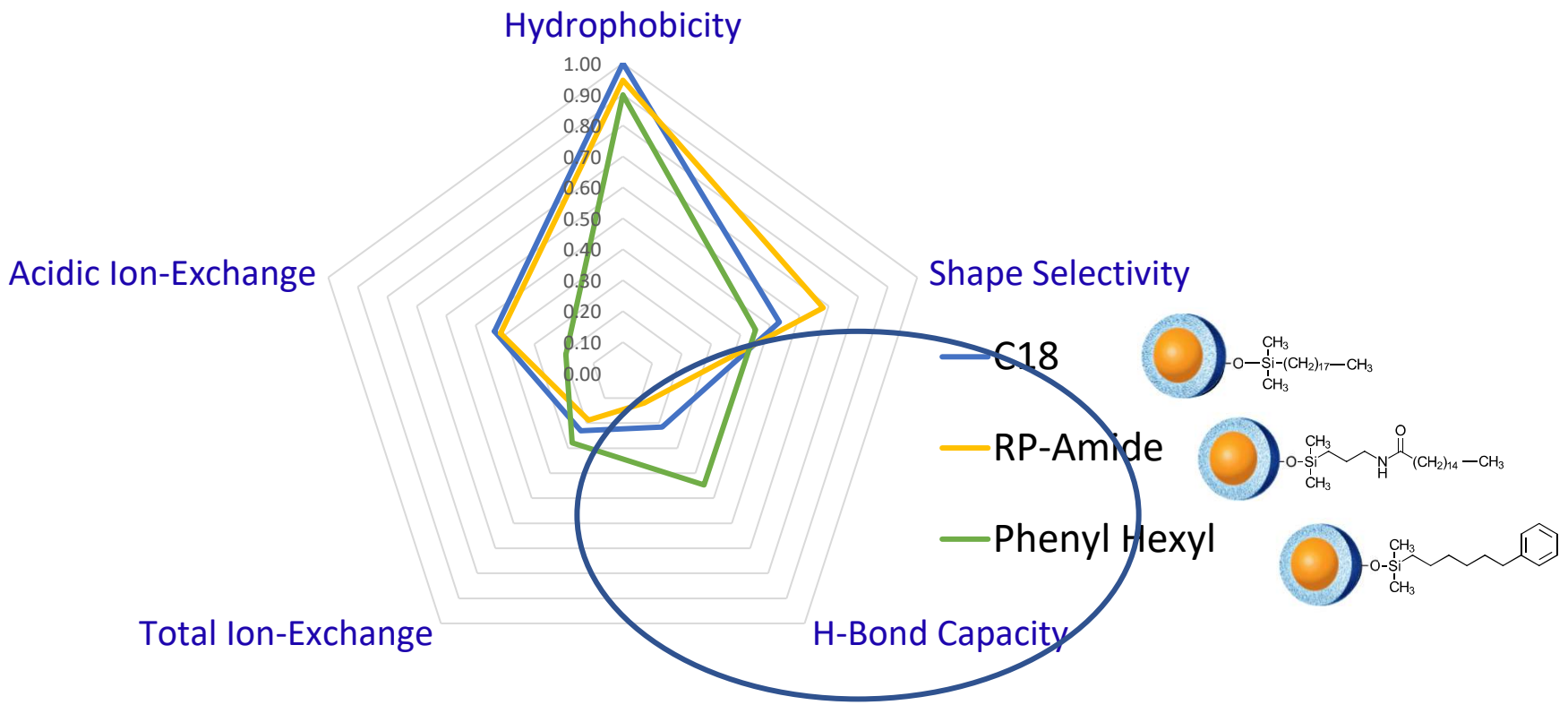
3	Phenyl-Hexyl	Phenyl-Hexyl	RP-Amide	Phenyl-Hexyl	RP-Amide



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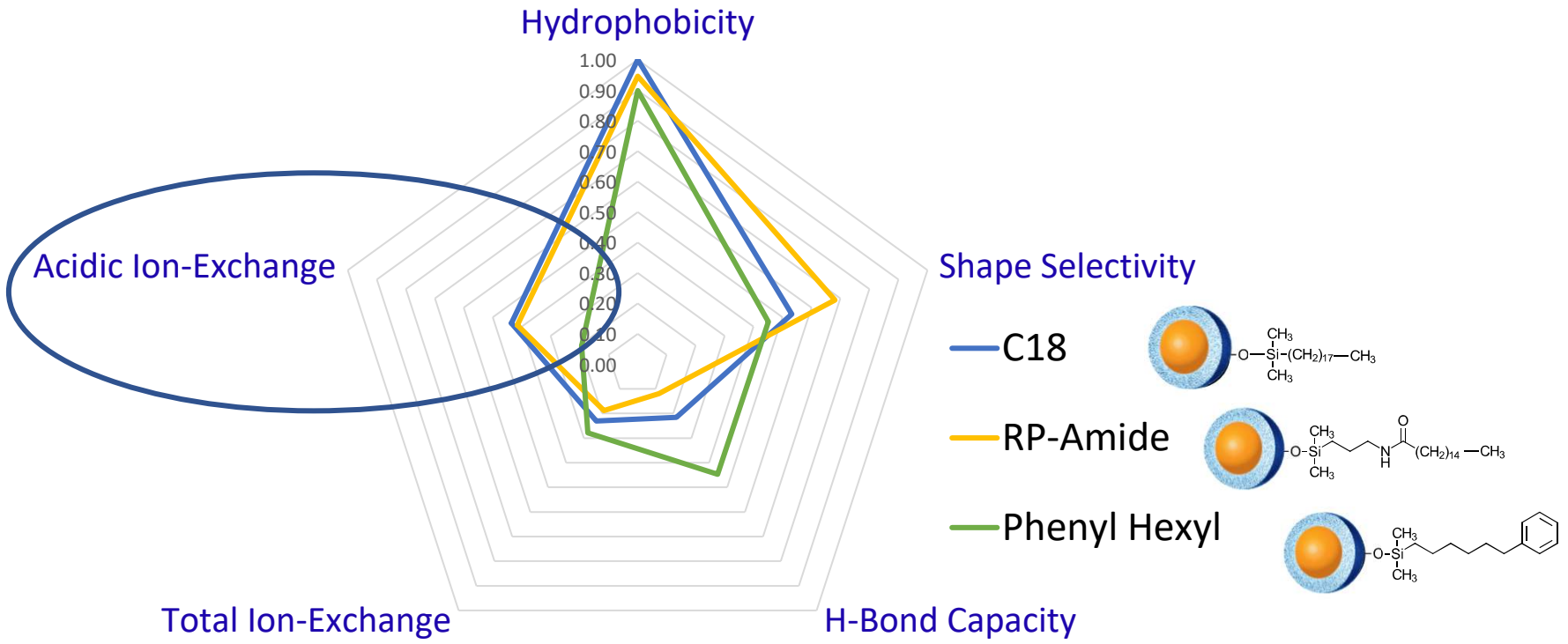
3	Phenyl-Hexyl	Phenyl-Hexyl	RP-Amide	Phenyl-Hexyl	RP-Amide



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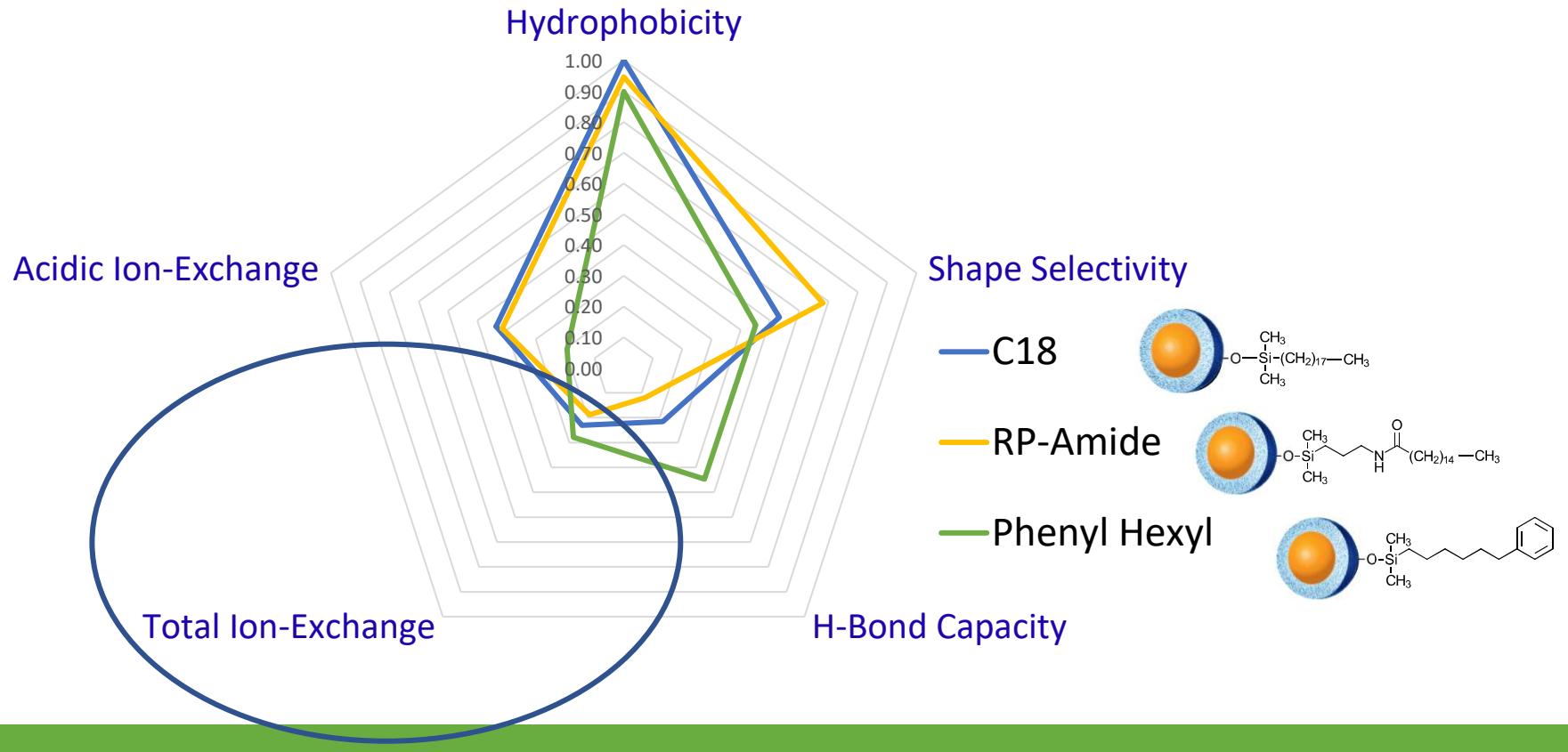
2	RP-Amide	C18	C18	RP-Amide	C18
3	Phenyl-Hexyl	Phenyl-Hexyl	RP-Amide	Phenyl-Hexyl	RP-Amide



Screening Method Conditions

- Columns:
  - HALO 90 Å C18, 2.7 µm, 2.1 x 100 mm
  - HALO 90 Å RP-Amide, 2.7 µm, 2.1 x 100 mm
  - HALO 90 Å Phenyl-Hexyl, 2.7 µm, 2.1 x 100 mm
- Mobile Phase:
  - A: 5 mM Ammonium Formate, 0.1% Formic Acid in water
  - B: 5mM Ammonium Formate, 0.1% Formic Acid in methanol
- Flow Rate: 0.4 mL/min
- Gradient: 2 min hold at 5% B; 5-95% B in 15 min
- Temperature: 35 ^{o}C
- Starting Back Pressure: 495 bar
- Injection: 2 µL of 1 µg/mL solution of each analyte
- Sample: LC PestiMix 5 from LGC Standards
- Sample Solvent: 80/20 Mobile Phase A/ACN
- Instrument: Shimadzu Nexera and ThermoFisher Q Exactive HF

MS Conditions

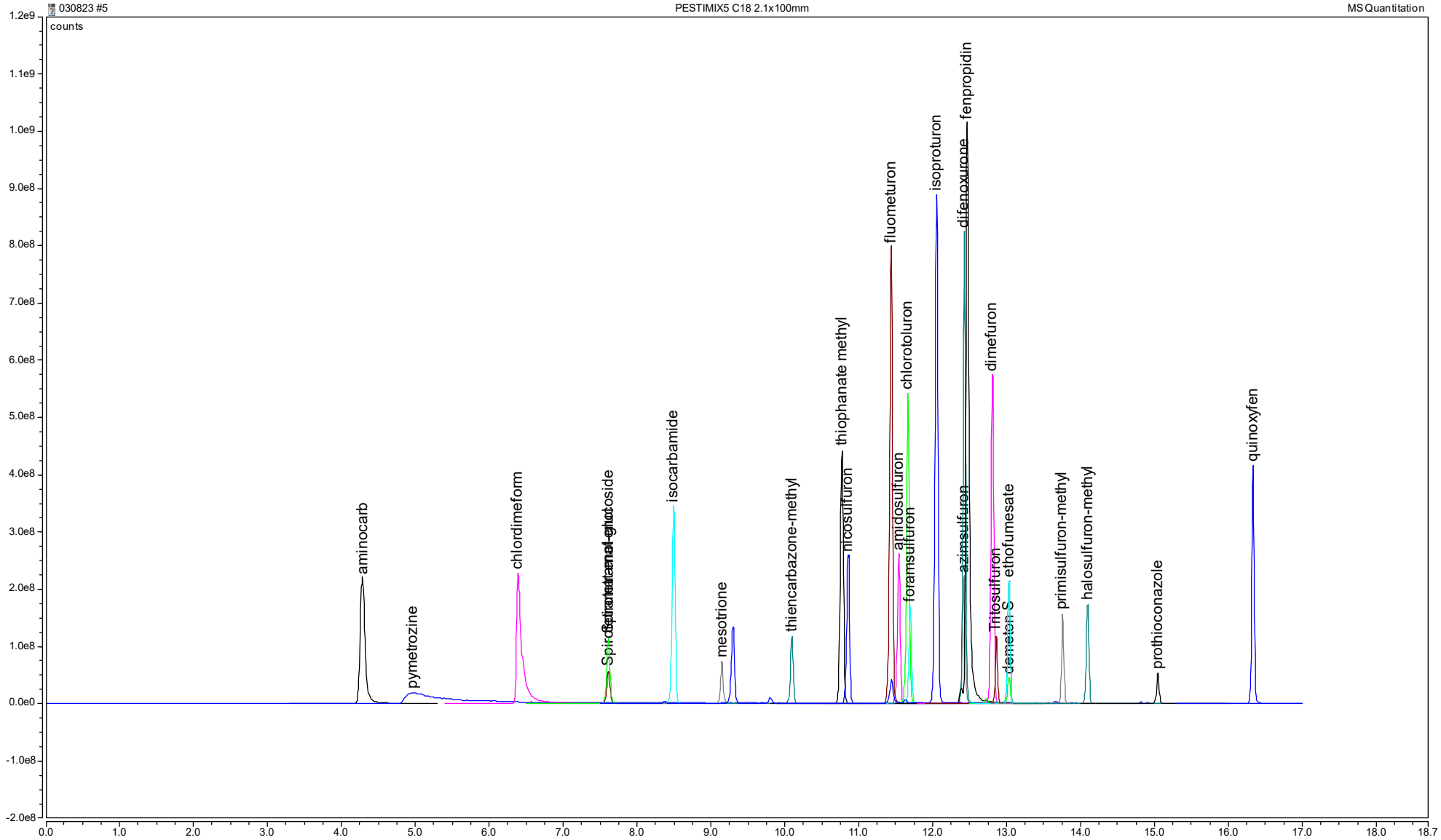
- Spray Voltage (kV): 3.5
- Capillary temperature: 350 °C
- Sheath gas: 40
- Aux gas: 20
- RF lens: 40

Pesticide Properties

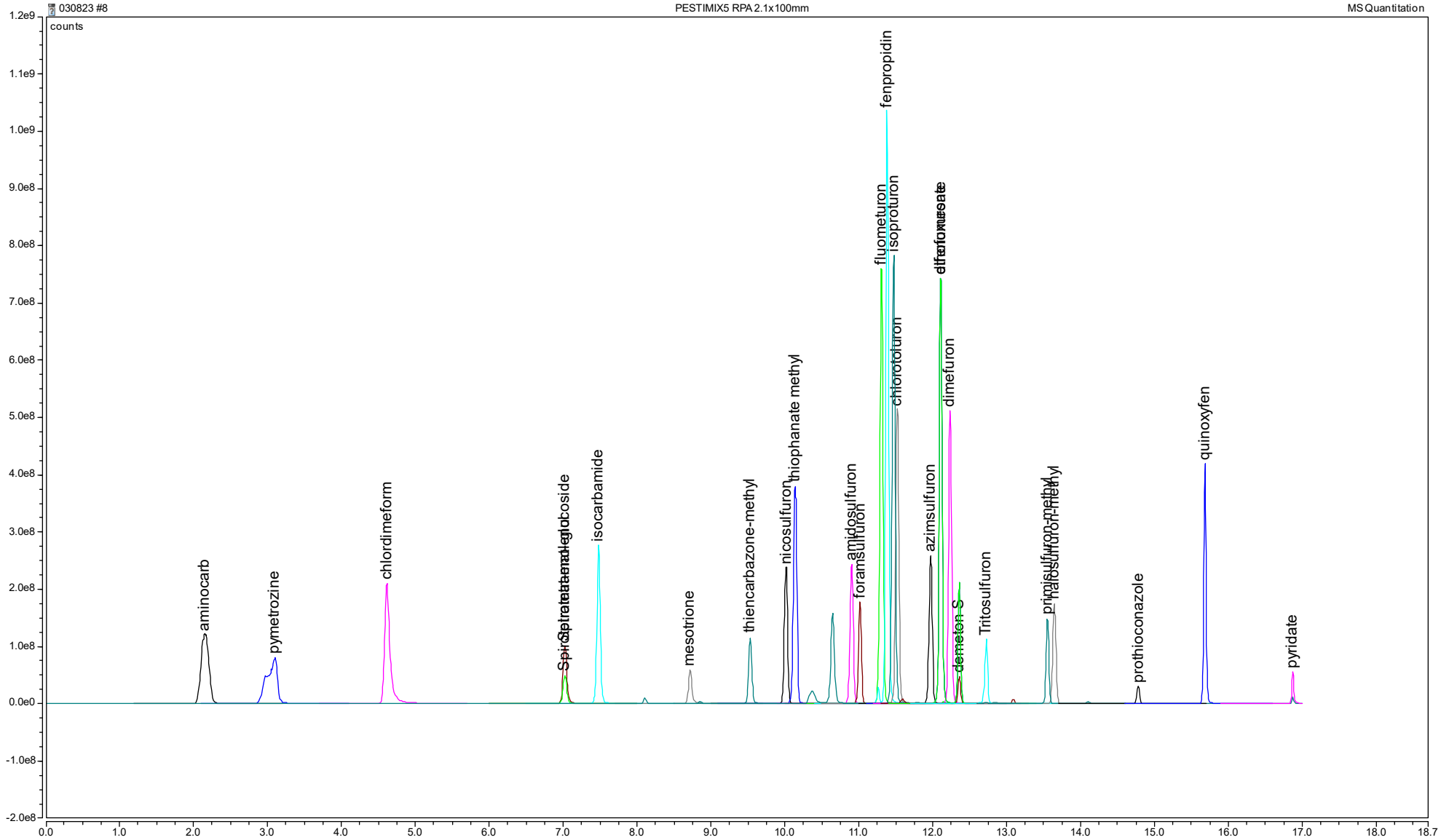
Name	Pesticide Class	log P	Formula	Monoisotopic Mass	Functional Groups
Isocarbamide	herbicide	0.8	C8H15N3O2	185.11642673	imidazolidinone, carboxamide
Chlordimeform	insecticide	2.9	C10H13ClN2	196.07672610	carboxamidine
Isoproturon	herbicide	2.9	C12H18N2O	206.14191320	phenylurea
Aminocarb	insecticide	1.9	C11H16N2O2	208.12117776	carbamate ester
Chlorotoluron	herbicide	2.4	C10H13ClN2O	212.07164070	phenylurea
Pymetrozine	insecticide	0	C10H11N5O	217.09635999	1,2,4-triazine and a member of pyridines
Fluometuron	herbicide	2.4	C10H11F3N2O	232.08234746	phenylurea
Demeton-s	insecticide	2.3	C8H19O3PS2	258.05132381	organothiophosphorus
Fenpropidin	fungicide	5.5	C19H31N	273.24564999	piperidine
Ethofumesate	herbicide	2.7	C13H18O5S	**286.08749484**	benzofuran methanesulfonate
Difenoxurone	herbicide	2.4	C16H18N2O3	**286.13174244**	diphenylurea
Quinoxyfen	fungicide	5.1	C15H8Cl2FNO	306.99669740	quinoline
Dimefuron	herbicide	2.6	C15H19ClN4O3	338.11456820	phenylurea
Mesotrione	herbicide	0.7	C14H13NO7S	339.04127293	sulfone, a C-nitro compound, an aromatic ketone and a beta-triketone
Thiophanate-methyl	fungicide	2.6	C12H14N4O4S2	342.04564729	thiourea
Prothioconazole	fungicide	2.7	C14H15Cl2N3OS	343.03128870	monochlorobenzene, triazole, tertiary alcohol, cyclopropane, thiocarbonyl
Amidosulfuron	herbicide	-0.2	C9H15N5O7S2	369.04129018	aromatic ether sulfonylurea, pyrimidine
Thiencarbazone-methyl	herbicide	1.2	C12H14N4O7S2	390.03039115	sulfonylurea ,thiophene, methyl ester, triazoles, ether
Nicosulfuron	herbicide	0.6	C15H18N6O6S	410.10085349	sulfonylurea
Azimsulfuron	herbicide	0.3	C13H16N10O5S	424.10258482	sulfonylurea
Halosulfuron-methyl	herbicide	1.3	C13H15ClN6O7S	434.04114570	pyrazole
Tritosulfuron	herbicide	3.1	C13H9F6N5O4S	445.02794393	benzenesulfonamide, sulfonylurea, triazine, trifluoromethyl
Foramsulfuron	herbicide	0.5	C17H20N6O7S	452.11141817	sulfonylurea, benzamide
Spirotetramat-enol-glucoside	insecticide metabolite	0.5	C24H33NO8	463.22061701	benzene, azaspiro compound, carbonate ester, gamma-lactam, pyrroles
Primisulfuron-methyl	herbicide	3.6	C15H12F4N4O7S	468.03628256	sulfonylurea, difluoromethyl, pyrimidine, benzylester

Data obtained from https://pubchem.ncbi.nlm.nih.gov/

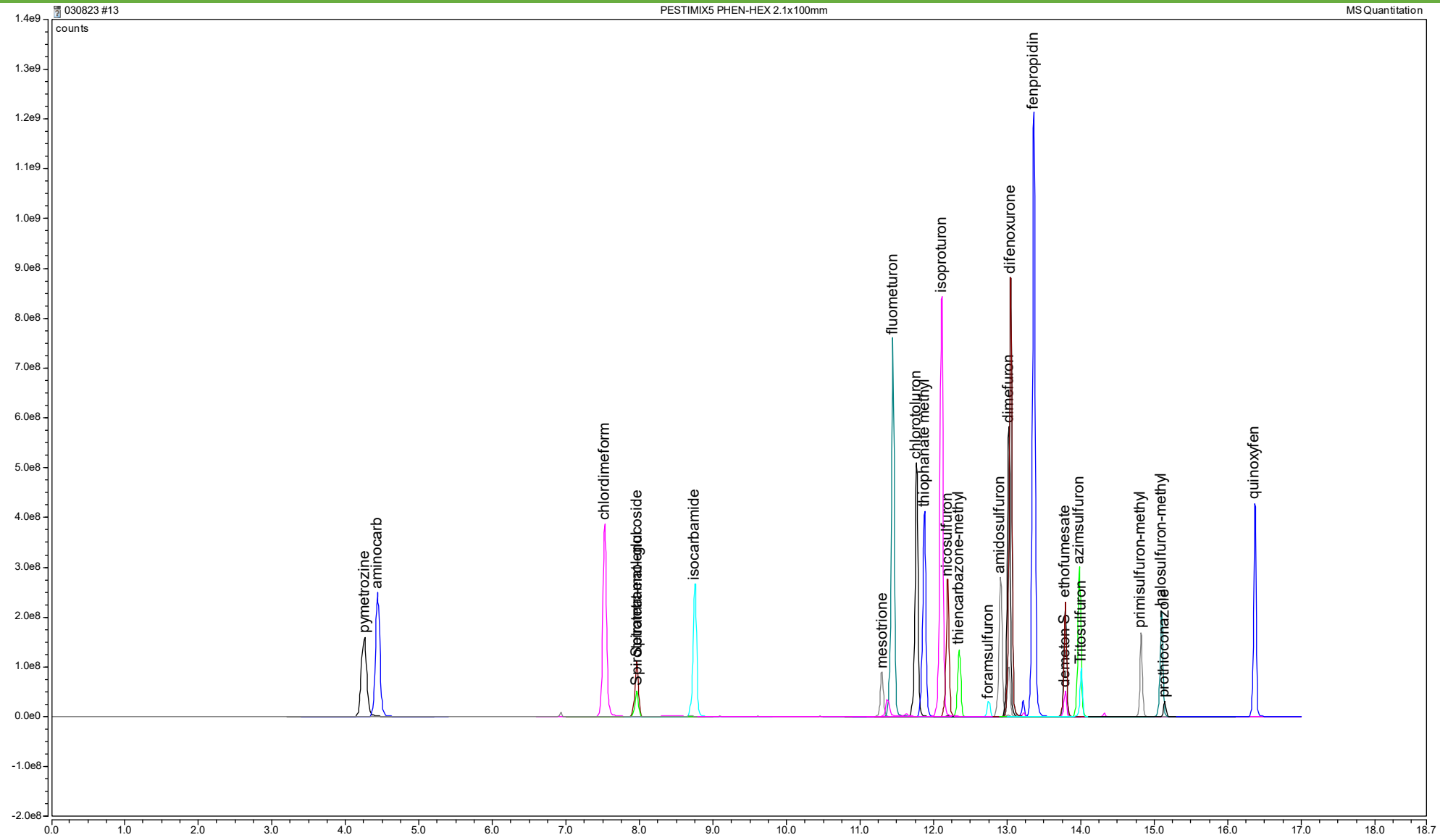
HALO® C18 Results



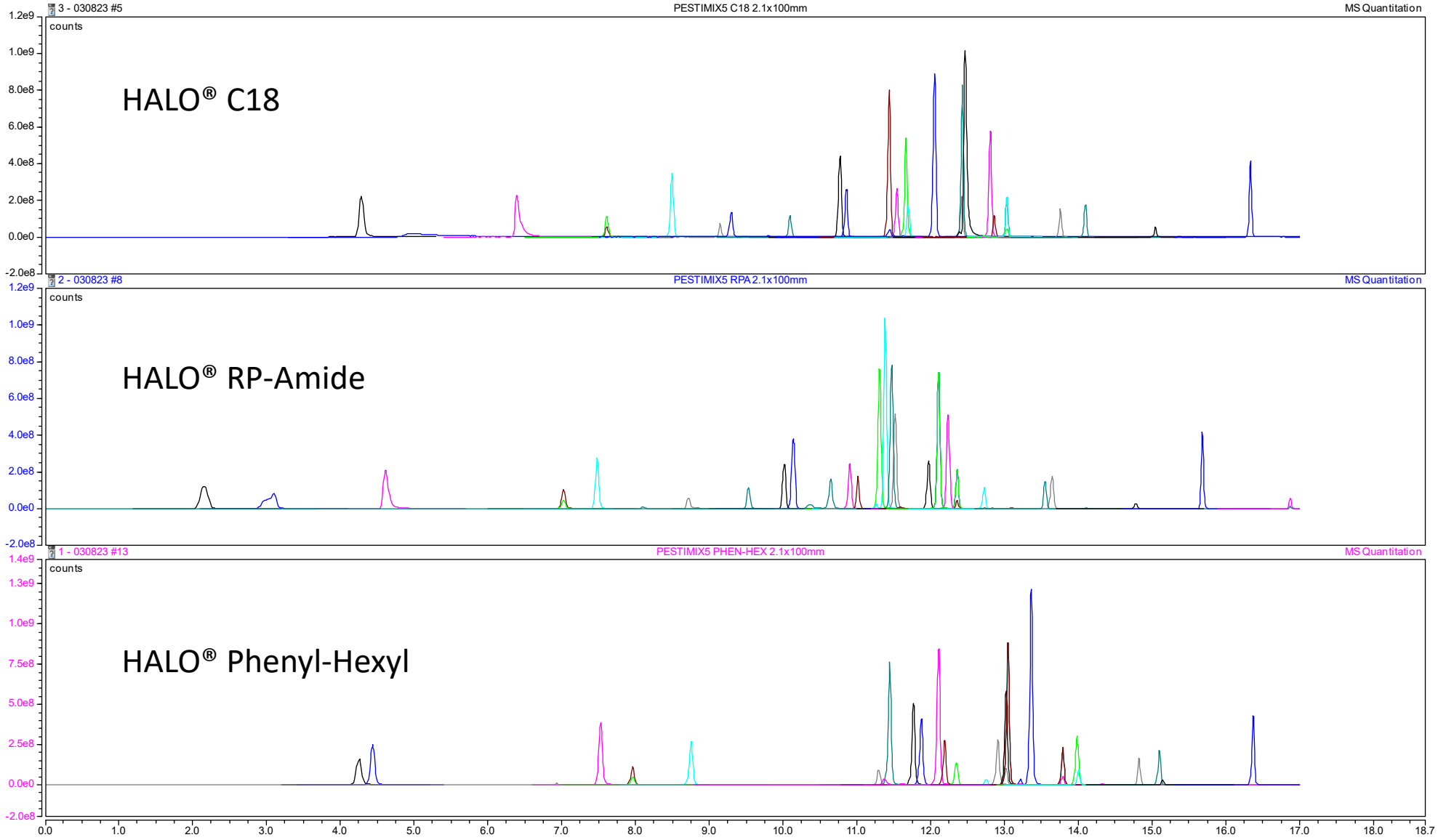
HALO® RP-Amide Results



HALO® Phenyl-Hexyl Results



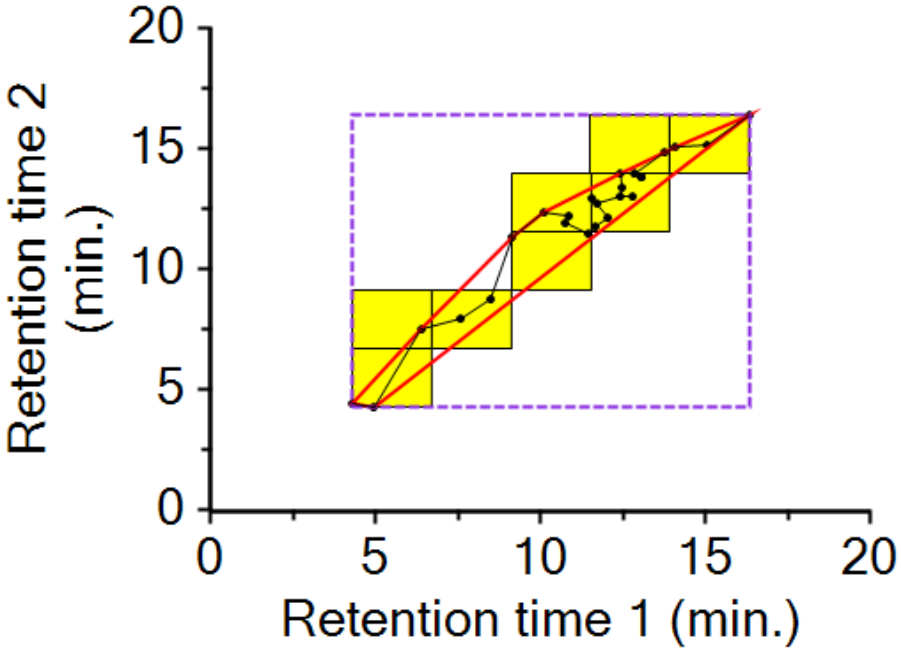
Comparison across 3 Phases



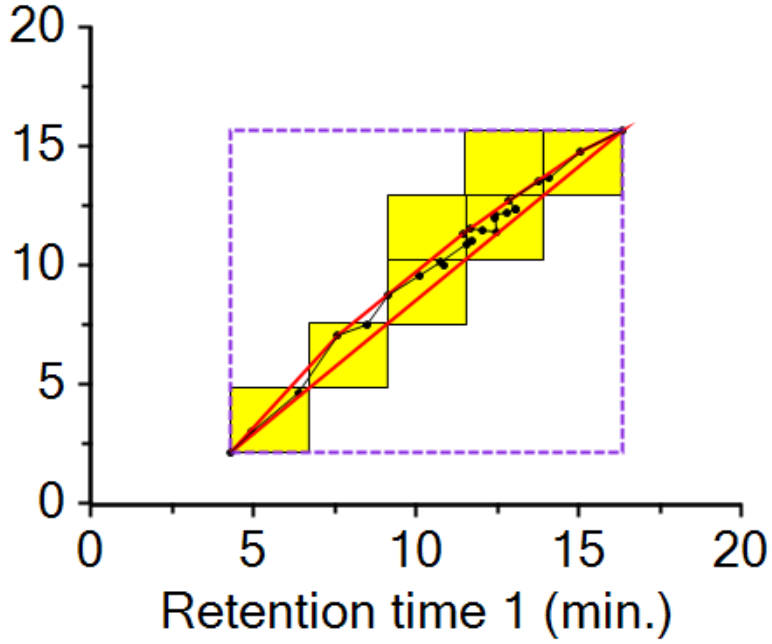
- Asymmetry: C18 > RP-Amide > Phenyl-Hexyl
- Peak Width: similar on all 3 phases

Pair-wise analysis of retention times used to probe orthogonality

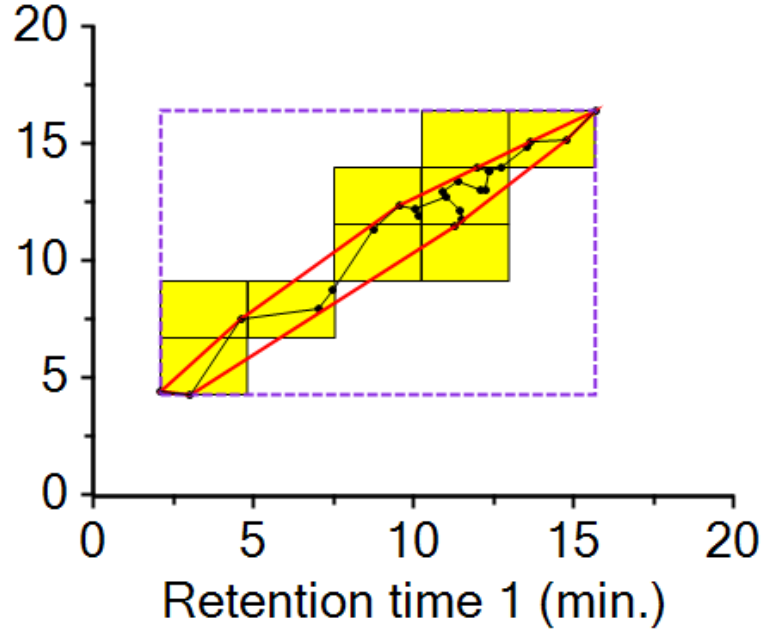
C18 vs Phenyl-Hexyl



C18 vs RP-Amide

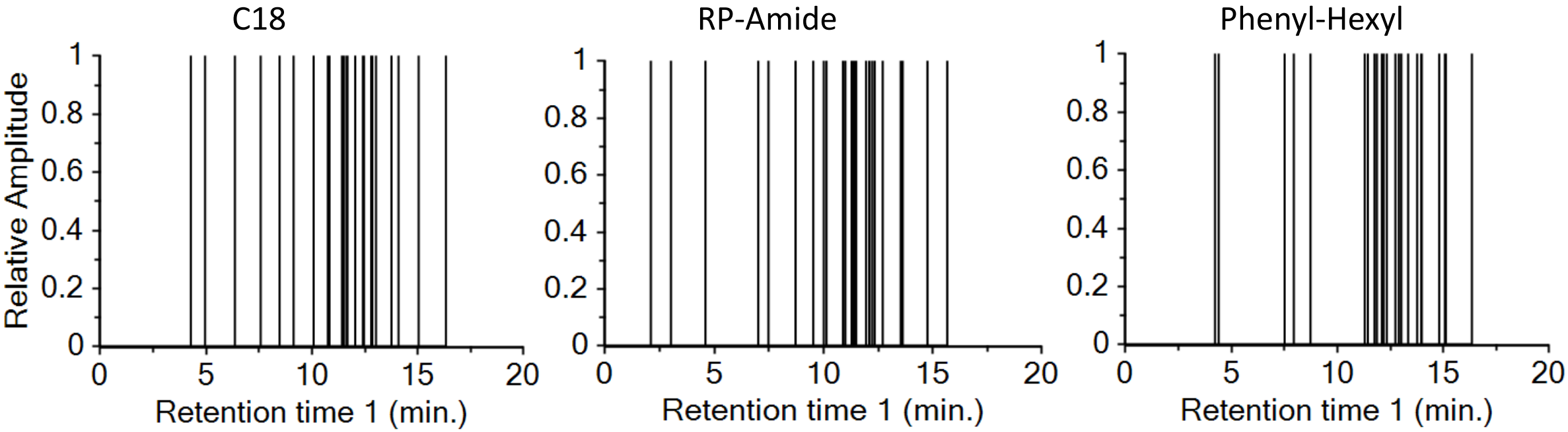


RP-Amide vs Phenyl-Hexyl



The retention times are analyzed with Orca®, an orthogonality calculator written by Mark Schure at Kroungold Analytical, Inc. which gives fractal dimension (D), correlation coefficients and other metrics for 1D and 2D chromatography.

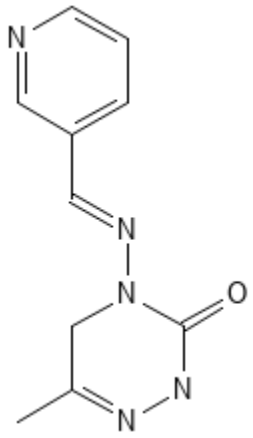
1D Fractal Dimension Analysis



D≈1 D≈1 D≈0.9

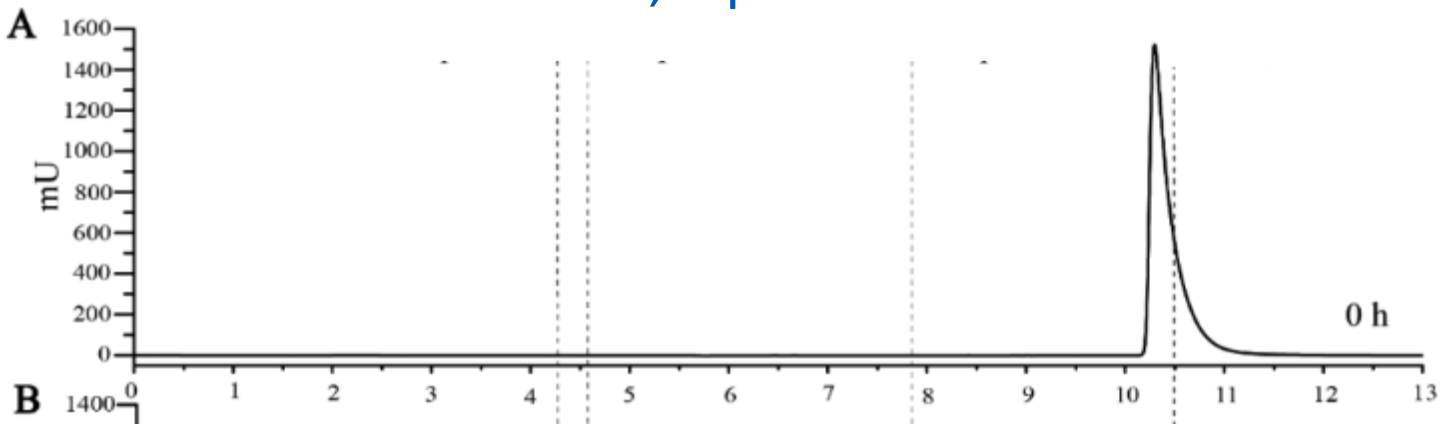
- Ordering refers to the distance between peaks being similar
- C18 has higher ordering, RP-Amide is roughly as ordered and Phenyl-Hexyl is less ordered

Impact of Phase on Pymetrozine Peak Shape

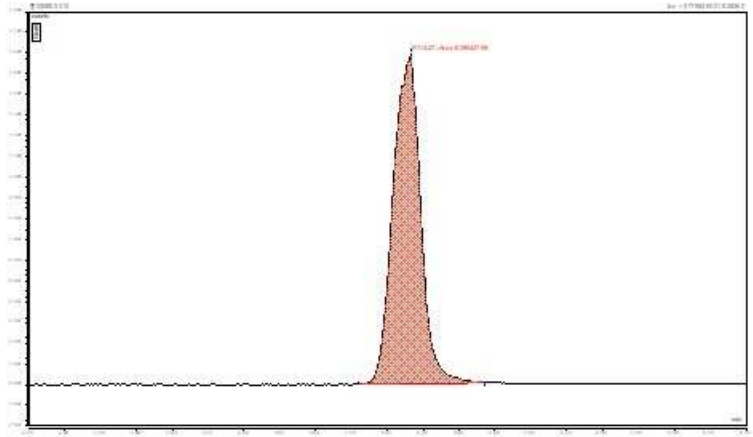


- Insecticide used to prevent aphids and whiteflies
- 1,2,4-triazines and a member of pyridines

C18, 5 µm



Asymmetry = 0.93 on HALO® Phenyl-Hexyl



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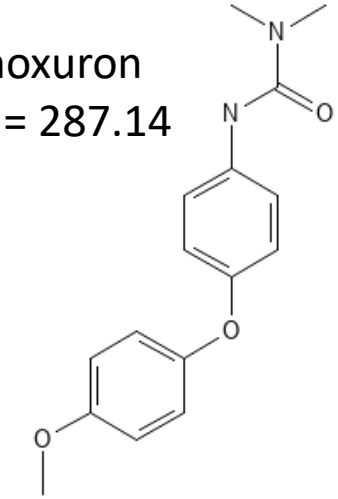
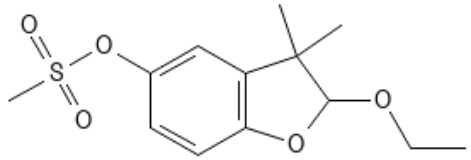
Resolution of Isobaric Compounds

Difenoxuron
M+1 = 287.14

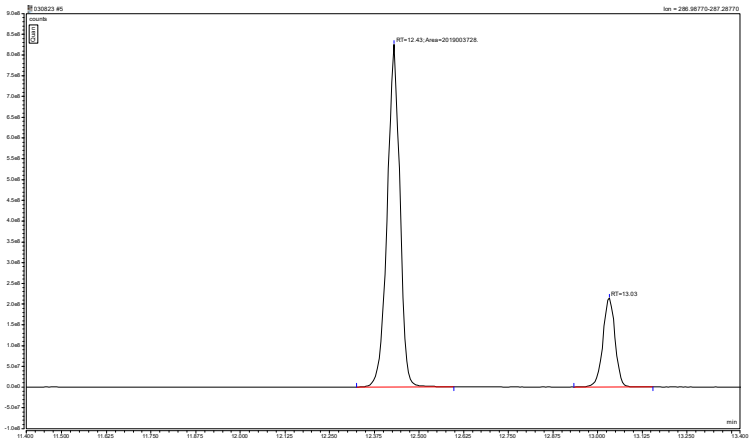




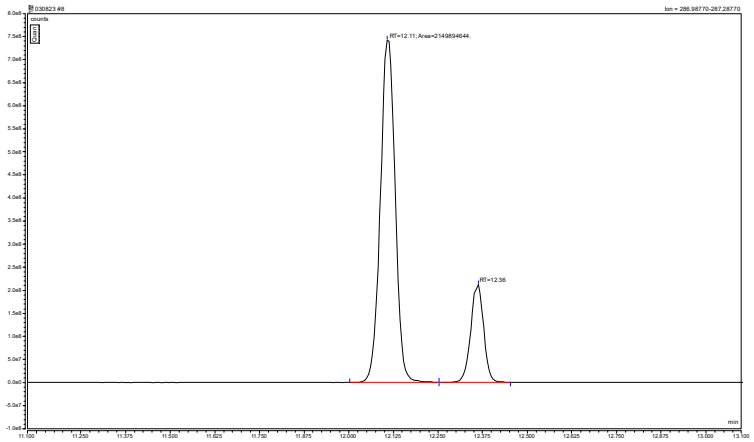
Ethofumesate
M+1 = 287.09

- Important to chromatographically resolve isobaric compounds to minimize ion suppression
- All 3 HALO® phases used in this study baseline resolved the two compounds with the same mass
- Difenoxuron elutes first followed by ethofumesate

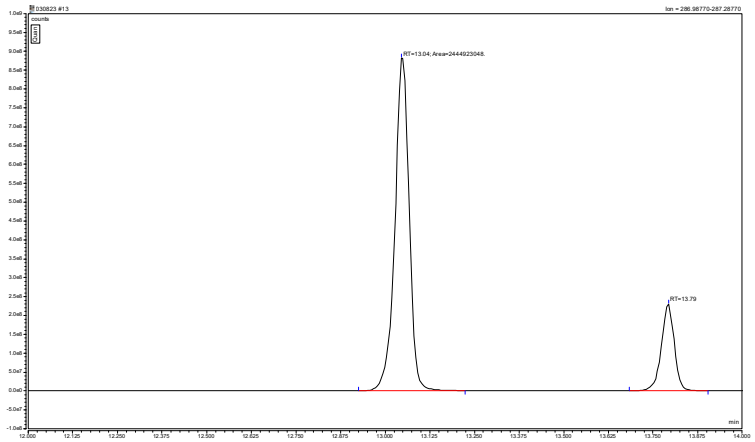
HALO® C18



HALO® RP-Amide



HALO® Phenyl-Hexyl



Future Work

- Screen additional HALO® stationary phases using short columns and/or 1.5 mm ID
- Expand the panel of pesticides investigated
- Decrease the run time of the analysis

Summary

- No phase identified to be best for all pesticides
  - Phenyl-Hexyl provided best peak shapes
  - C18 provided most ordered separation
- Recommended strategy to screen multiple phases to find which is best for the pesticide set that is being targeted
- HALO phases offer robust separations for a variety of pesticides
- Screen with short columns and change to longer columns and/or smaller particles if needed with best selectivity/symmetry
- Pressure drop advantage for HALO® (vs FPP) always allows faster runs with short columns and improved resolution with longer columns as needed

Acknowledgements

- AMT
  - Conner McHale
  - Stephanie Rosenberg
- Dr. Thomas Waeghe
- Dr. Richard Henry
- Dr. Mark Schure

Questions

support@advanced-materials-tech.com

Photo by Alexander Grey on Unsplash