High resolution mass spectrometry for non-targeted environmental exposomics

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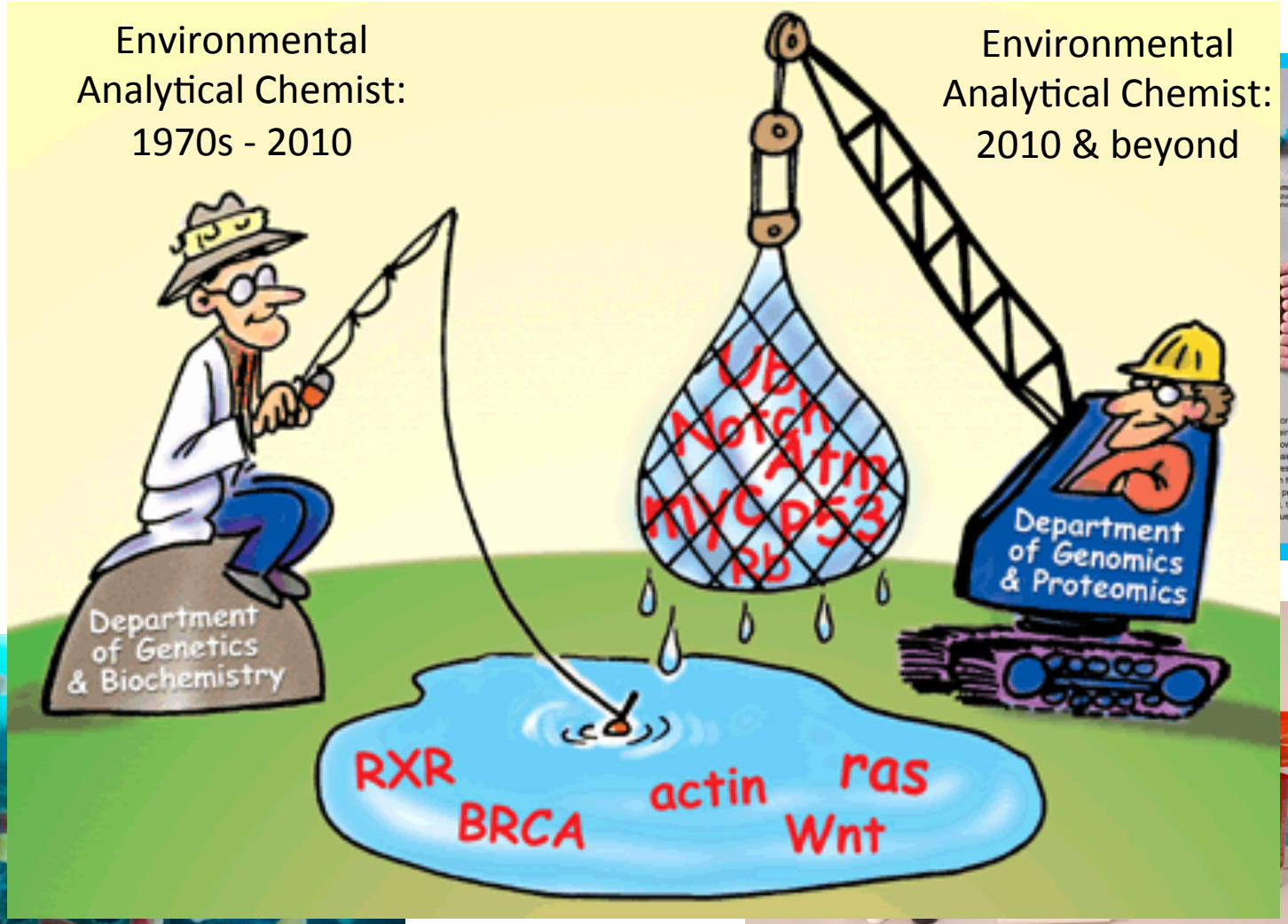
Duke UNIVERSITY

NICHOLAS SCHOOL OF THE ENVIRONMENT
DUKE UNIVERSITY
forging a sustainable future

What are the next emerging contaminants and how can we find them in the environment?

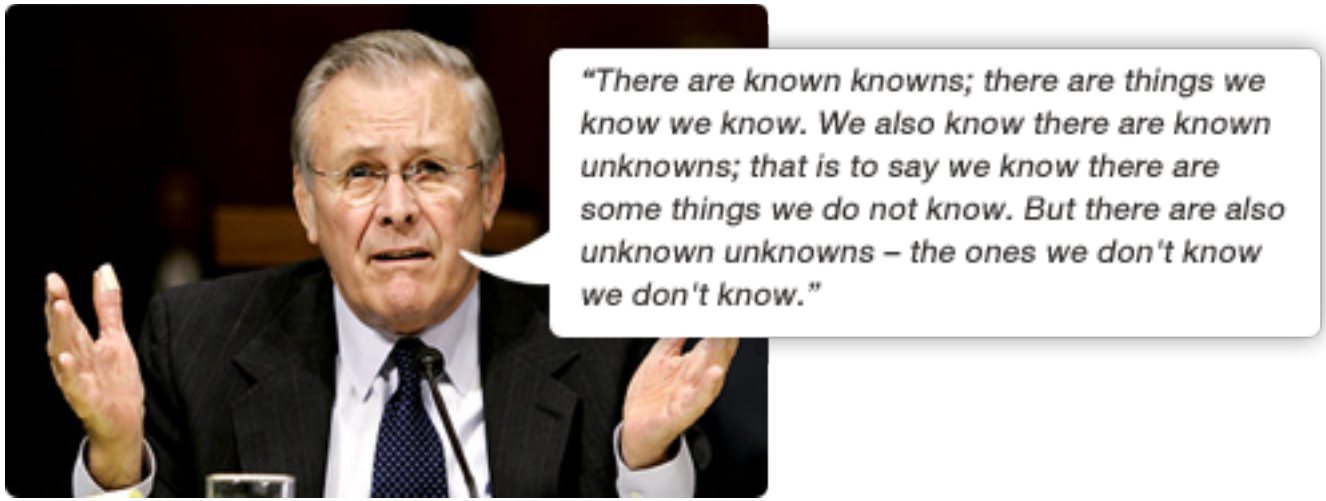


Science 16 February 2001: vol. 291 no. 5507 1221-1224

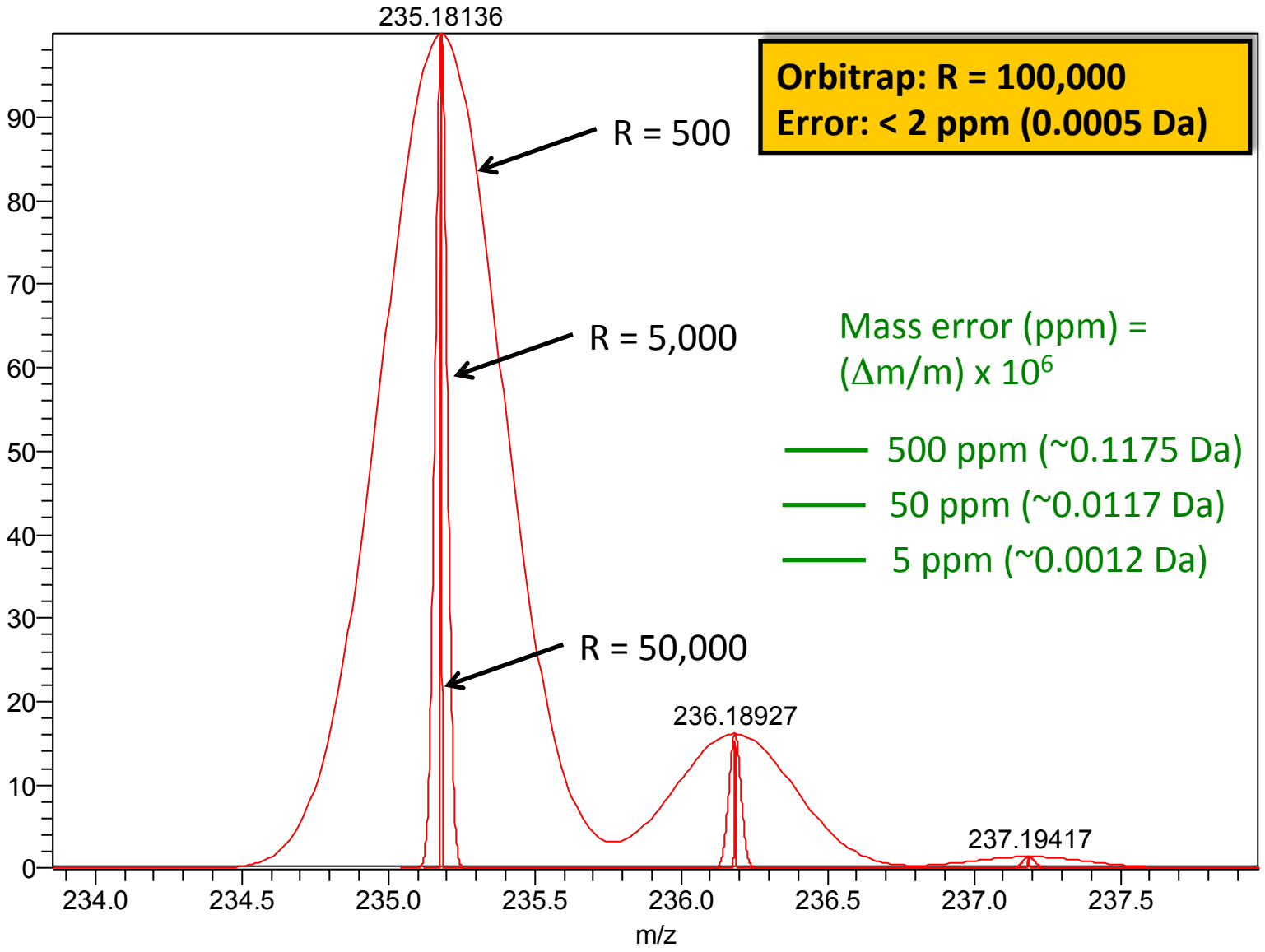
LC-HRMS: An emerging technique for environmental exposomics

LC-MS strategies for characterization of organic contaminants

Screening technique:	*Targeted*	*Suspect*	*Non-target*
Question:	*Are compounds x, y, & z present in this sample?*	*Which compounds of a defined list are present in this sample?*	*Which compounds are present in this sample?*
Compound Types:	*Known-knowns*	*Known-unknowns*	*Known-unknowns & unknown-unknowns*



Why do we use HRMS for non-targeted analysis of pollutants?



Semivolatile organic contaminants in the indoor environment: a challenging "exposome"

- Research on SVOCs has focused on occurrence and effects in the ambient environment – there have been few comprehensive studies on human exposure indoors
- SVOCs escape from household products over time and may accumulate in the indoor environment
- They are applied to consumer products to enhance performance or durability – such as:

Phthalates in personal care products

Flame retardants in furniture and electronics

Bisphenol A in waterbottles

Surfactants in cleaning agents

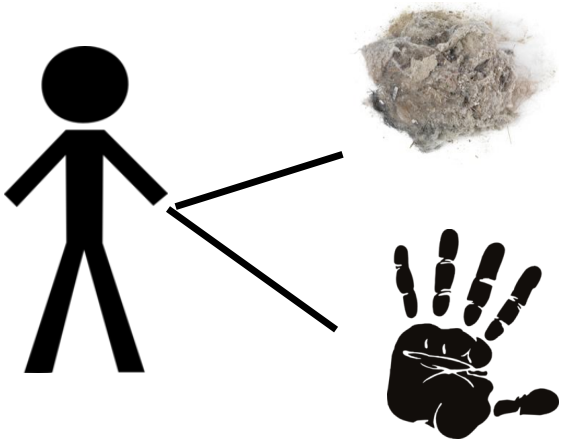
Antioxidants in food packaging

Why study SVOC's indoors?

87% of our time is spent indoors

Exposure through: inhalation, ingestion, dermal absorption,

- Some SVOC's are potential endocrine disrupters
  - Bisphenol A is a xenoestrogen
  - Flame retardants have been shown to act on the thyroid hormone receptor

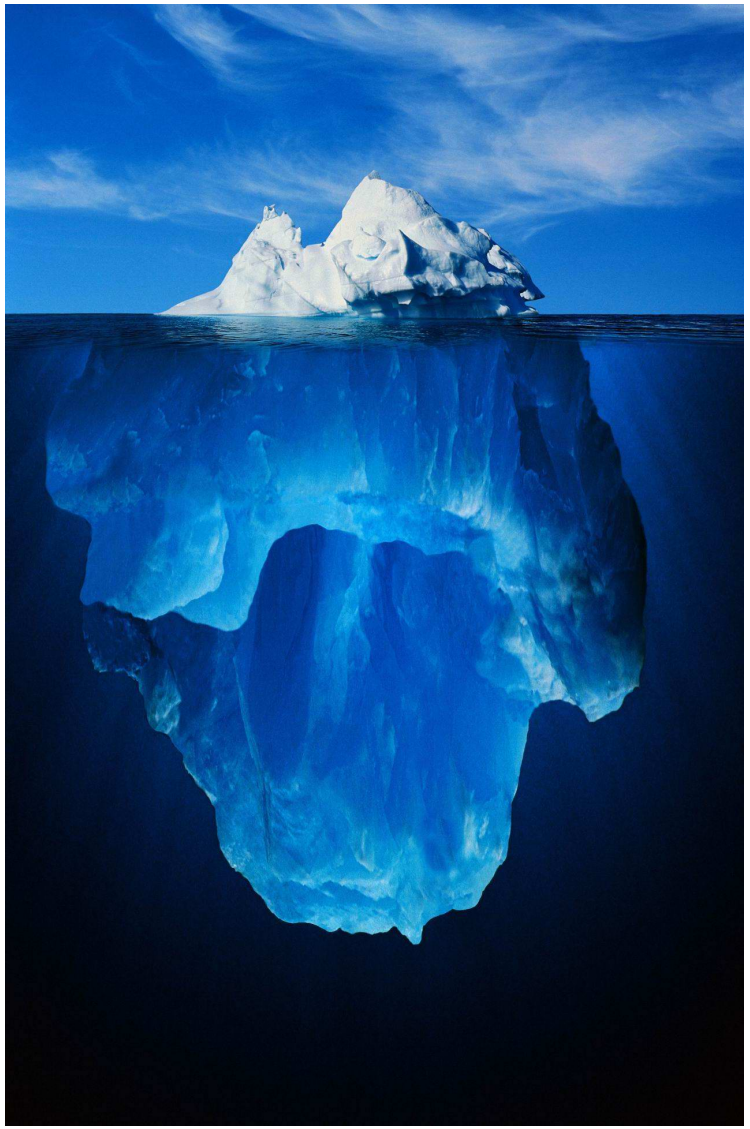


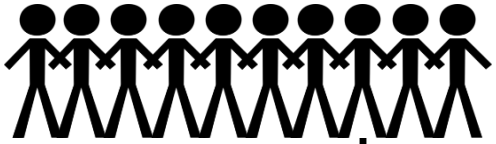
Objective:

Assess human exposure to SVOCs from the indoor environment through non-targeted analysis of paired house dust and hand wipes samples.

Analytical strategy for dust and handwipe samples

- Most indoor exposure analysis has applied gas chromatography mass spectrometry (focus on nonpolar organic contaminants)
- Liquid chromatography coupled with high resolution mass spectrometry can be used to characterize (semi)polar organic contaminants within indoor environments.
- Non-targeted data analytics allows *de novo* identificatio, prioritized by compounds with highest exposure potential.
- This approach complements more targeted, quantitative analysis of SVOCs by LC-MS/MS or GC-MS approaches.



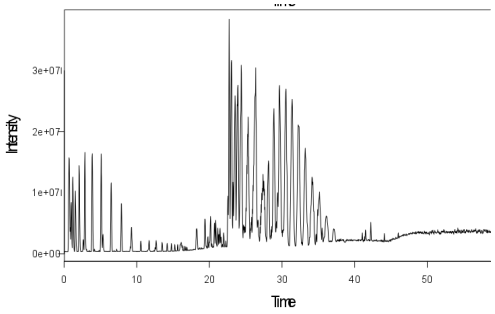


10 x dust and handwipes
+ dust blanks and wipe blanks

Sample preparation
Extraction by sonication in Hexane/Dichloromethane 1:1;
Solvent exchange to 10 % Acetonitrile in H_2O by speedvac, sonication and centrifugation.

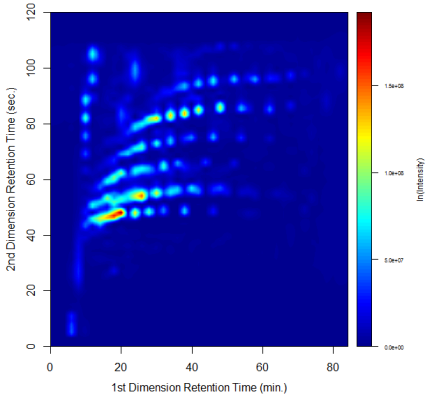
Liquid Chromatography
Reversed phase separation C18,
From 10 % Acetonitrile to 99% in 60 min

Orbitrap Velos
ESI(+) and ESI (-)
Resolution: 60’000 @ m/z 400
Top 4 data dependent MSMS
CID with 35 normalized energy

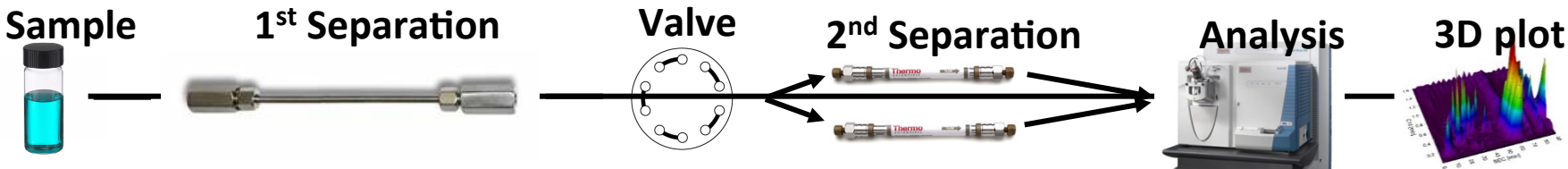


Comprehensive 2D Liquid Chromatography
Size exclusion X reversed phase separation
90 min run divided into 2 min segments

Orbitrap Velos
ESI(+)
Resolution: 60’000 @ m/z 400



Comprehensive 2D UHPLC (LC x LC)



- Slow separation
- isocratic

- Short, max 2 min
- Fast gradient
- 2 short columns
- used alternating

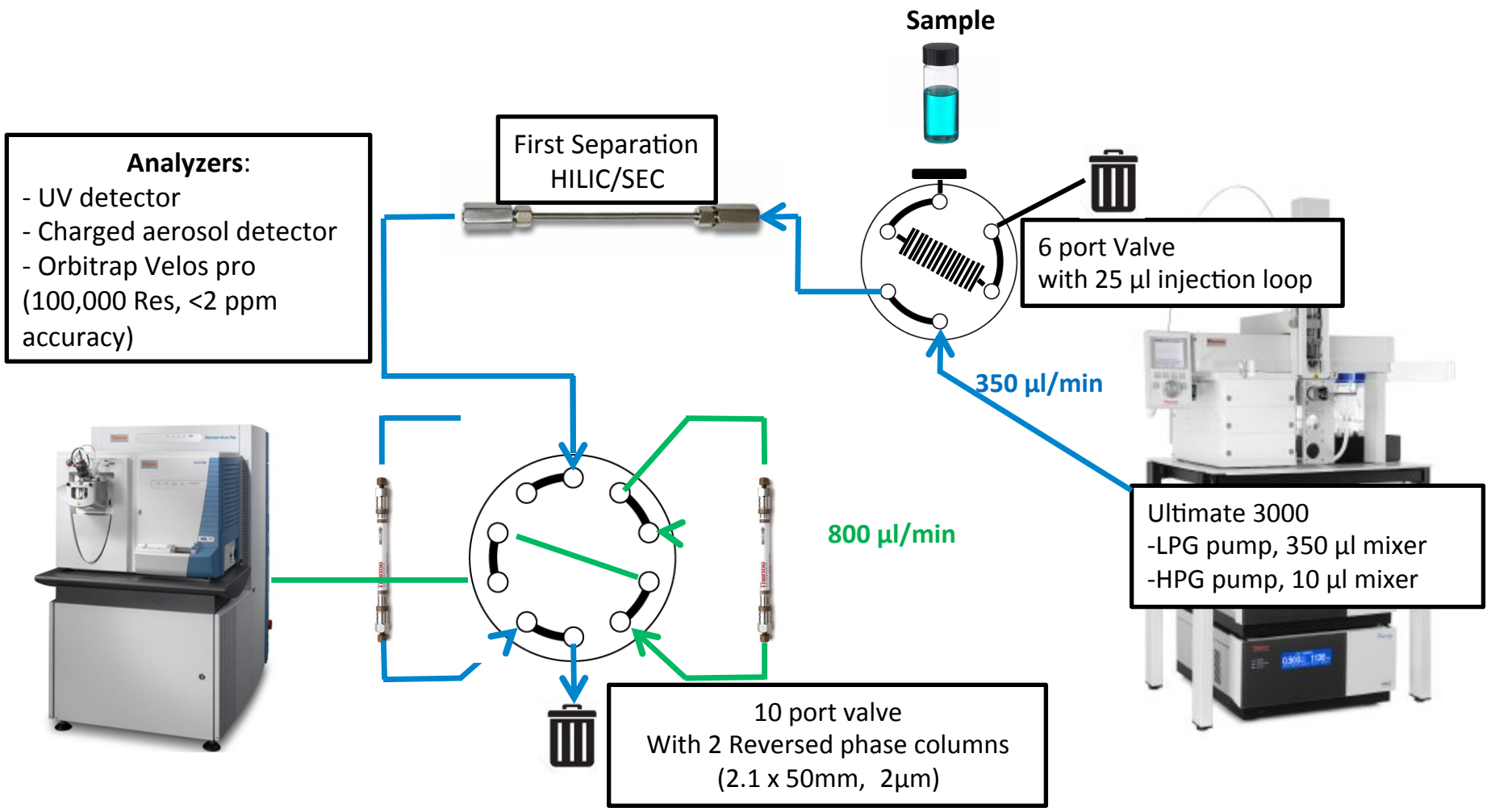
For effective separation:

-Separation mechanisms must be **orthogonal**.

-Example: Size and Hydrophobicity or Hydrophilic interaction and Hydrophobicity.

-While eluting from the first column – requires strong retention on the second column

2D UHPLC-HRMS configuration



Data processing starts with Thermo Compound Discoverer 2.0 for peak consolidation/filtering

Compound Discoverer 2.0

Peak detection consolidation

33,963 (+)
8,355 (-)

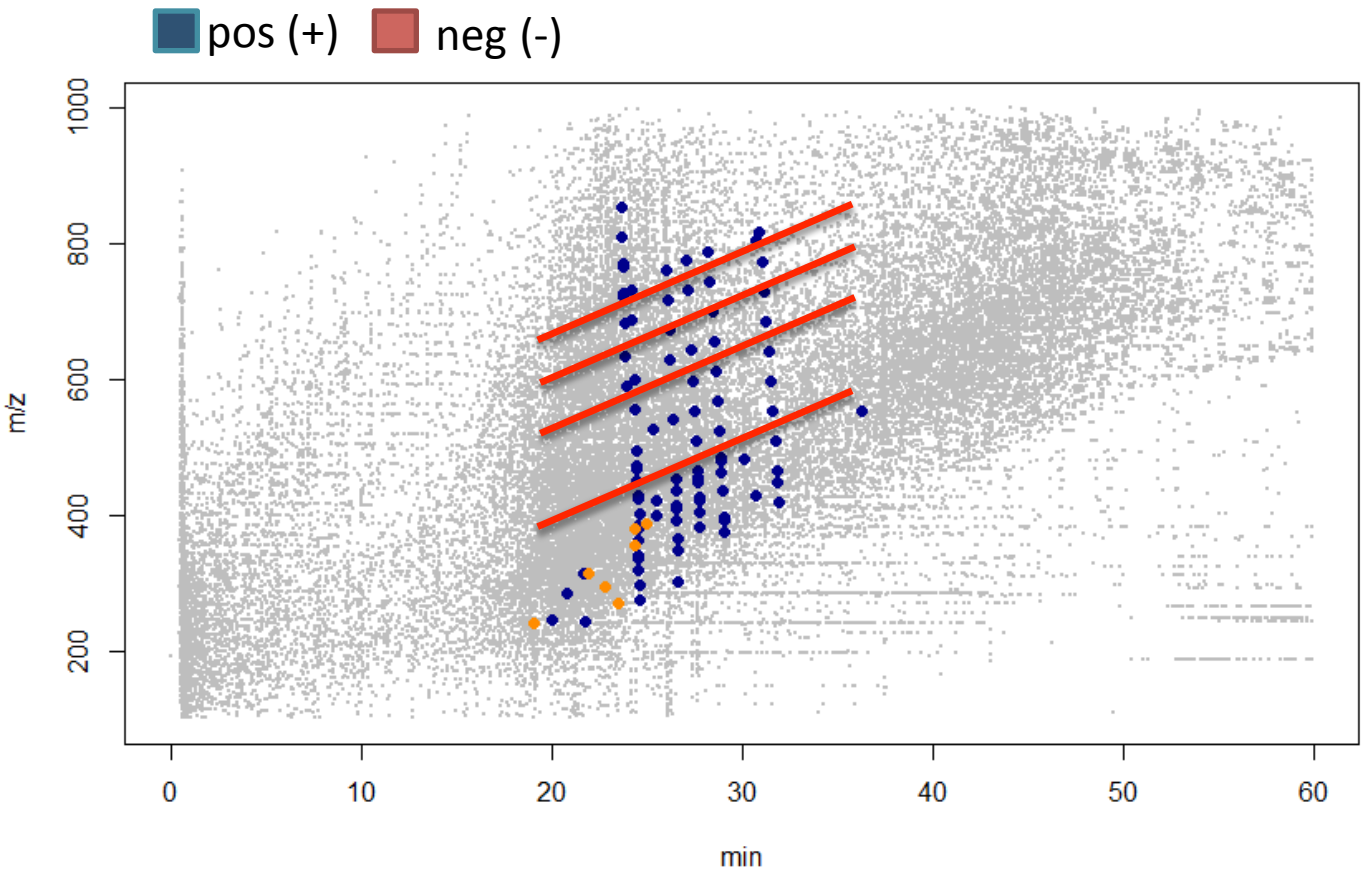
- blank area >5000

33,467 (+)
8,259 (-)

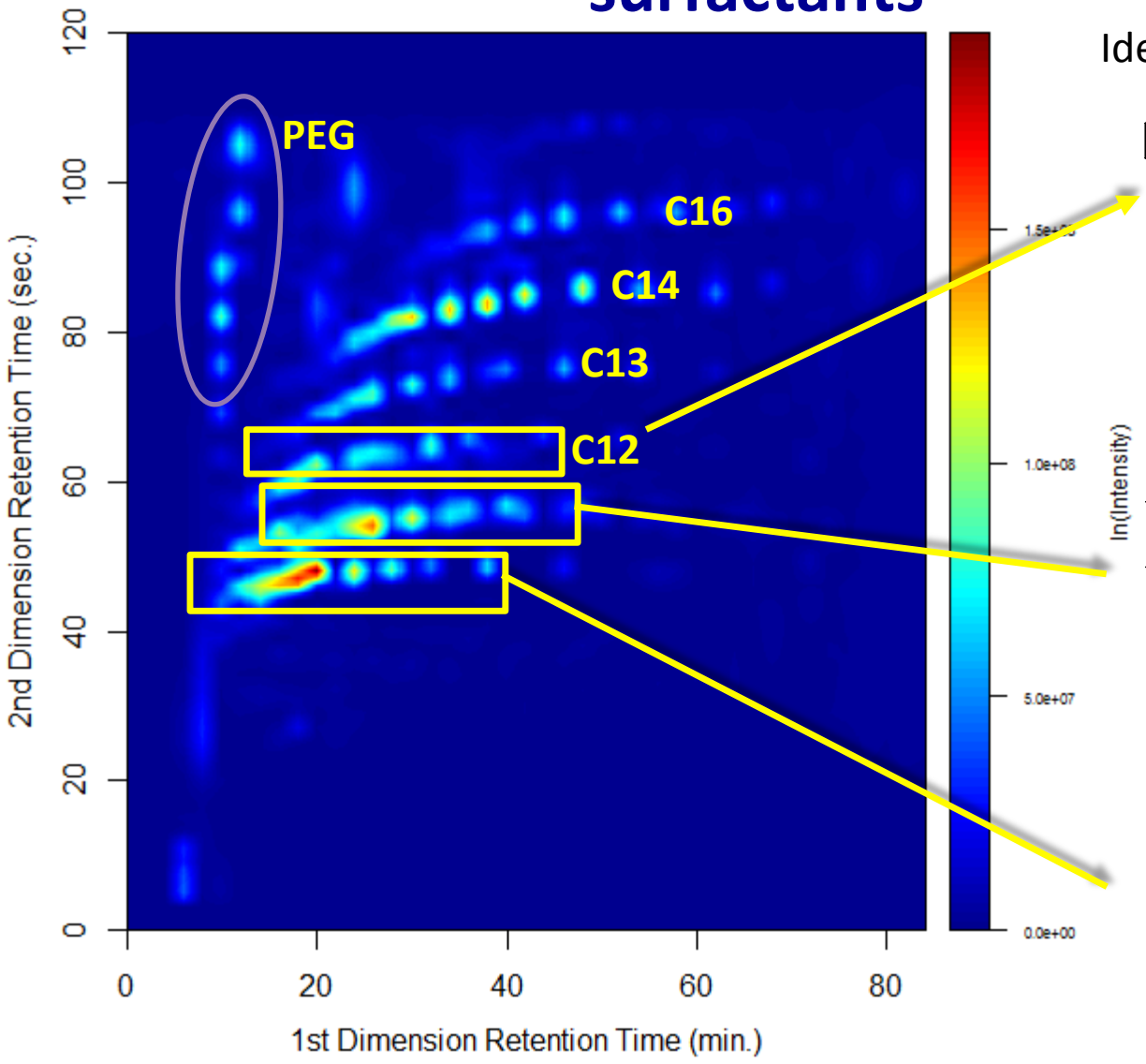
In all dust and all wipes

90 (+)
6 (-)

83% non ionic surfactants



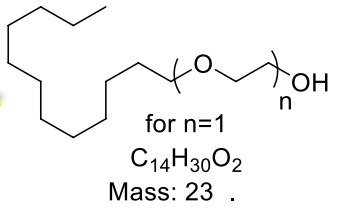
Comprehensive LC x LC-HRMS of dust reveals ethoxylated surfactants



Hydrophobicity – Alkyl length

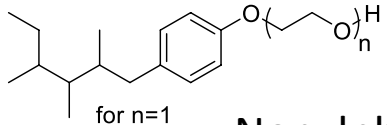
Size – Ethoxylate length

Identified with Standards:



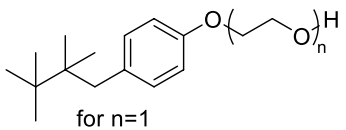
for n=1
$C_{14}H_{30}O_2$
Mass: 23 .

C12 alcohol ethoxylate (AE C12)



for n=1
$C_{17}H_{28}O_2$
Mass: 264.21

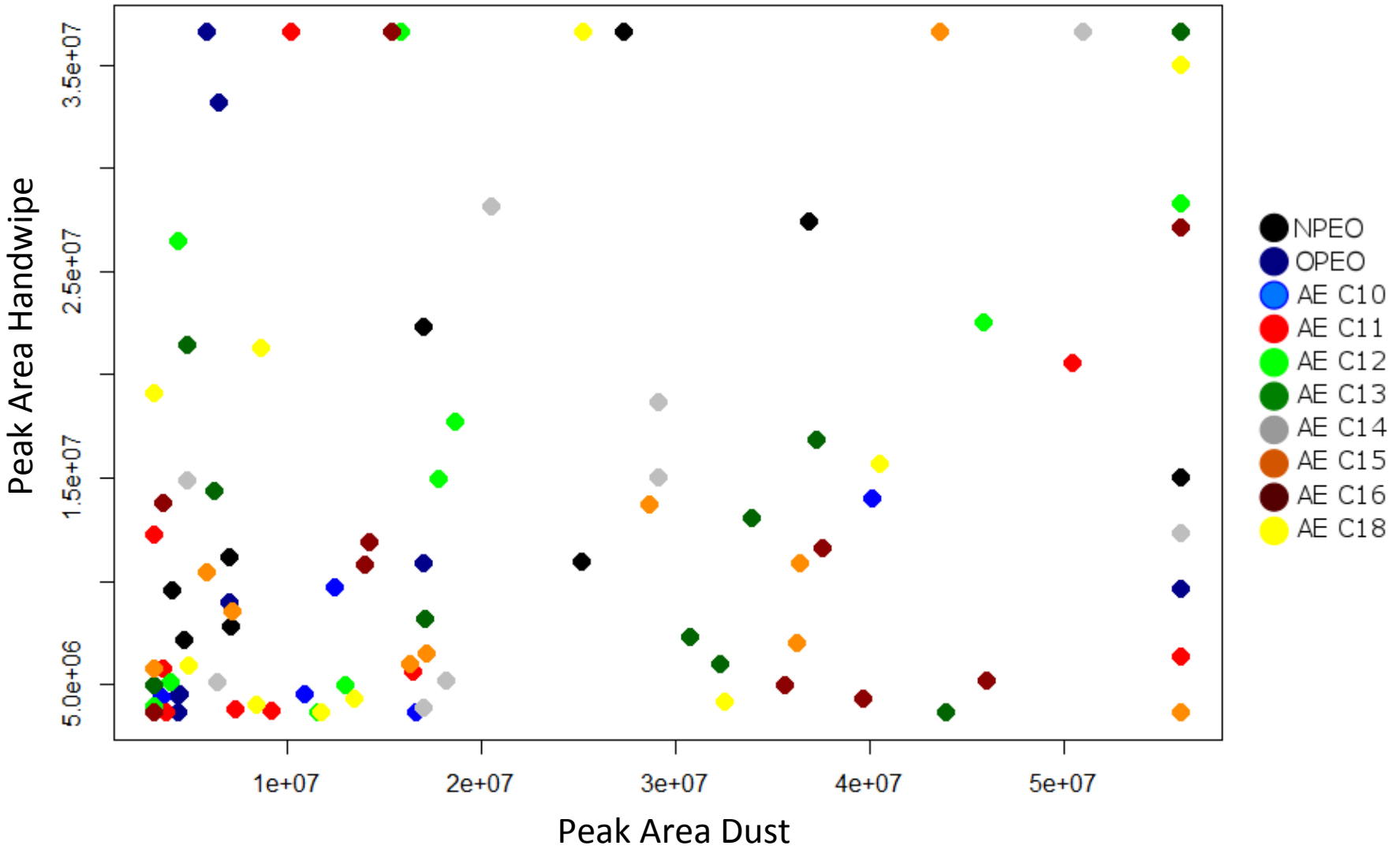
Nonylphenol ethoxylate (NPEO)



for n=1
$C_{16}H_{26}O_2$
Mass: 25 .

Octylphenol ethoxylate (OPEO)

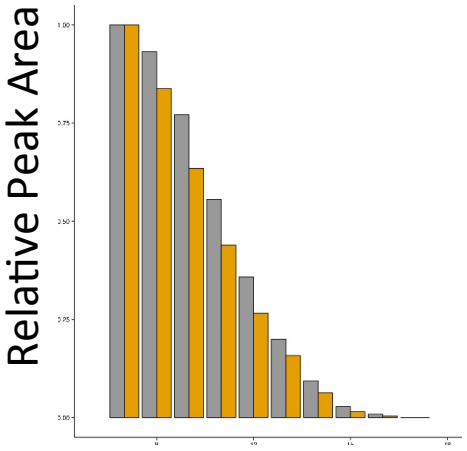
There was no correlation between ethoxylated surfactant peak areas in paired dust/handwipe samples (decoupled sources?)



Nonionic surfactant ethoxymer distributions in paired dust/handwipe samples

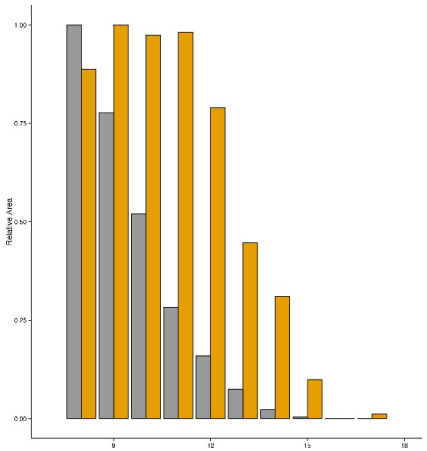
Example: NPEO

Person 2



Ethoxylate number
5-17

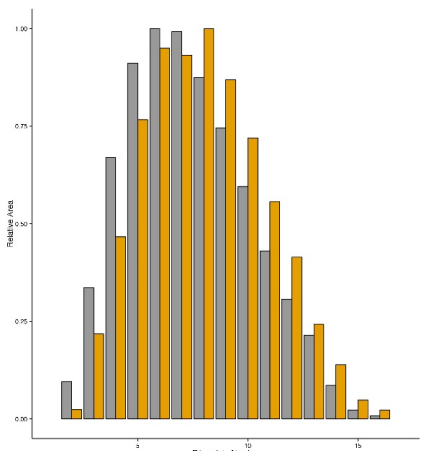
Person 7



Ethoxylate number
5 - 17

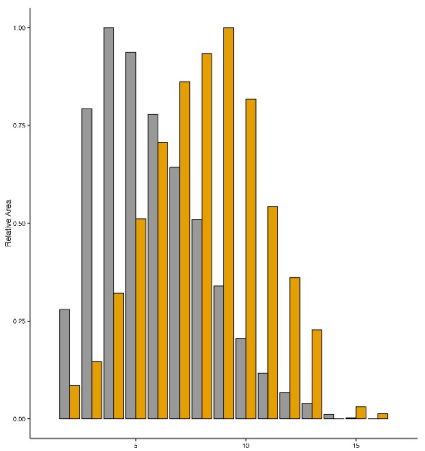
Example: Alcohol Ethoxylate C14

Person 4



Ethoxylate number
2 - 16

Person 6



Ethoxylate number
2 - 16

Ethoxymer distribution varied from surfactant to surfactant and person to person – this suggests different sources of ethoxylated surfactants in some cases.

Subraction of surfactant features prioritizes monomeric compounds for identification

Compound Discoverer 2.0

peak detection
consolidation

33,963 (+)
8,355 (-)

-blank
area >5000

33,467 (+)
8,259 (-)

In at least
1 dust/hand wipe
pair

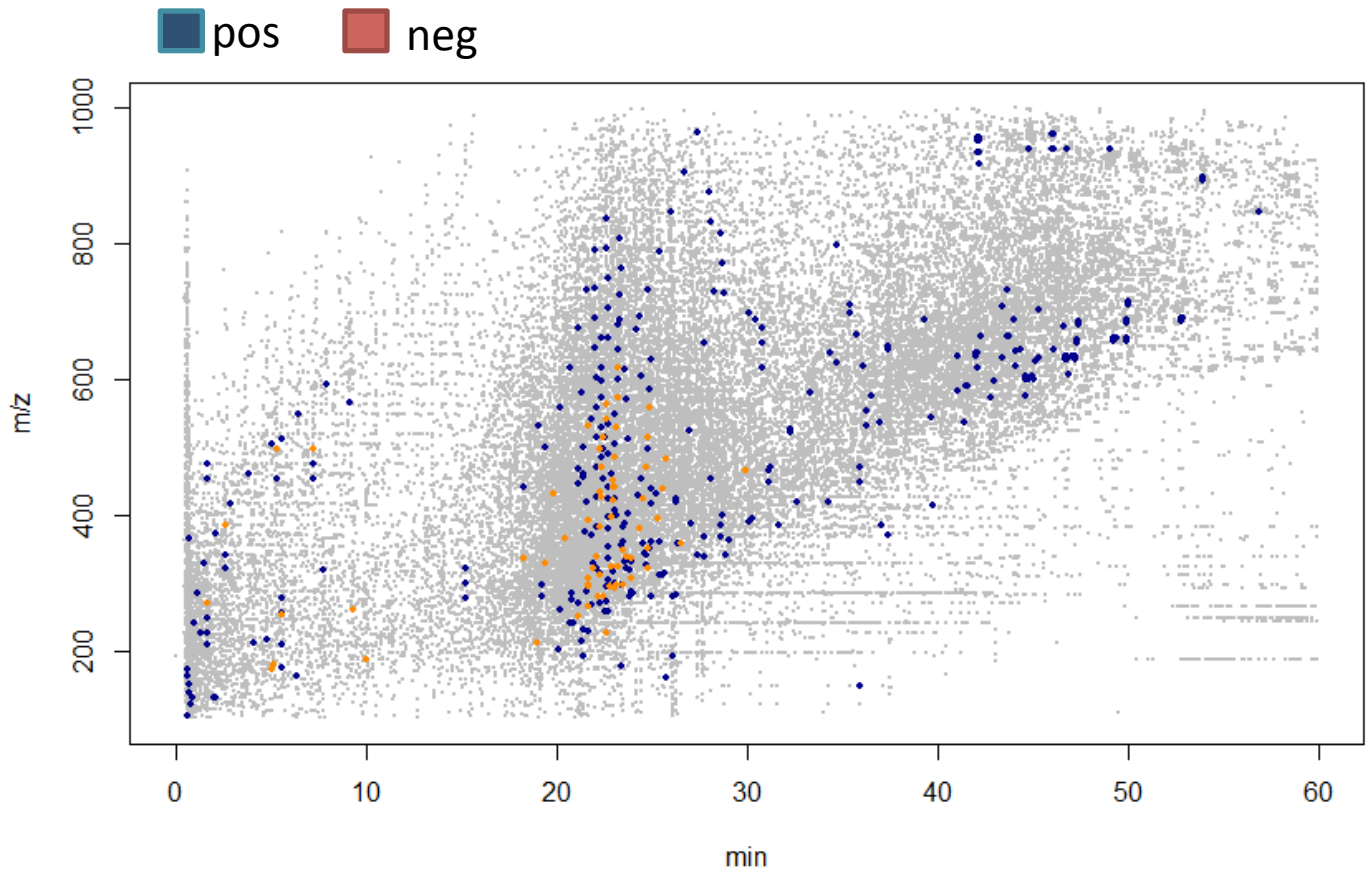
3,976 (+)
834 (-)

Max area > 55,000

501 (+)
67 (-)

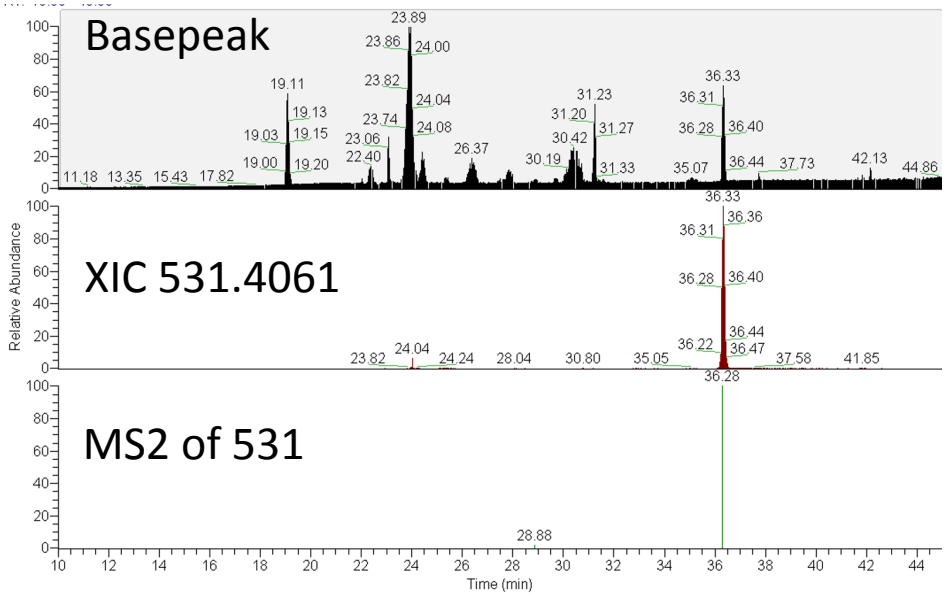
Subtract
surfactants

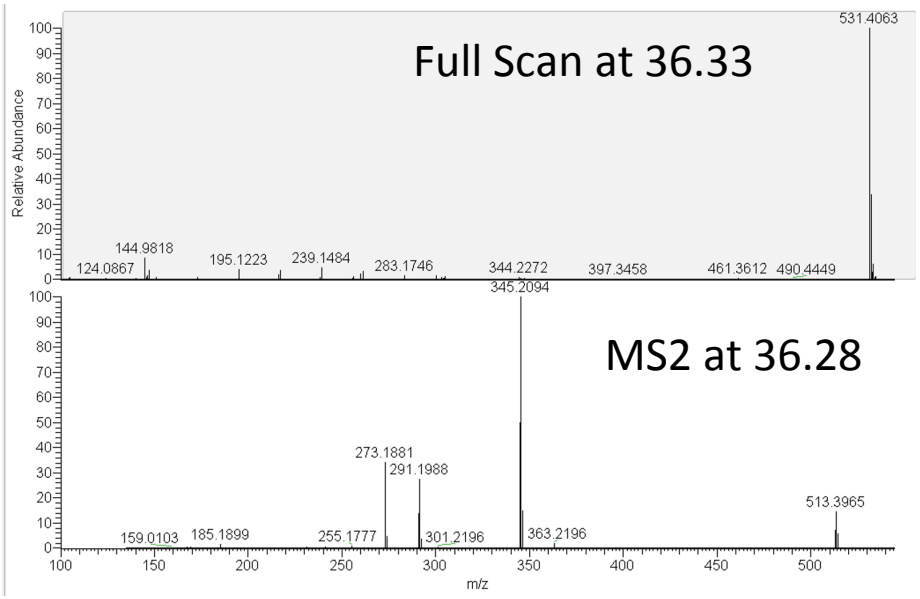
316 (+)
67 (-)



Workflow strategies for identifying compounds in dust/ handwipes from LC-HRMS data

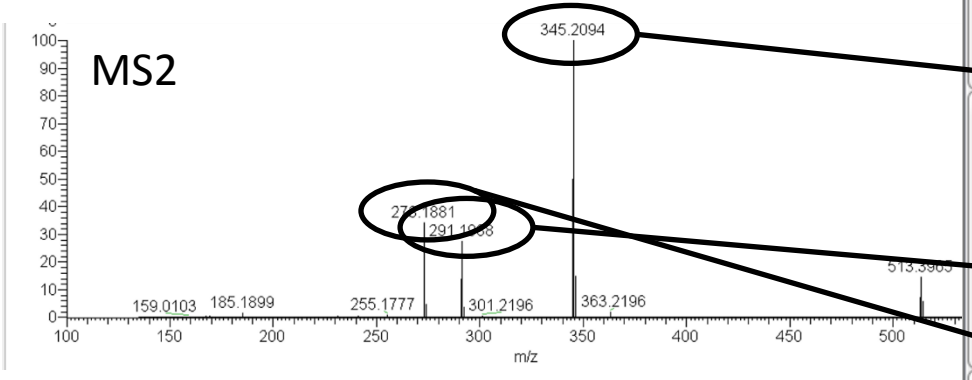
Exact Mass → **Identified!** **??**

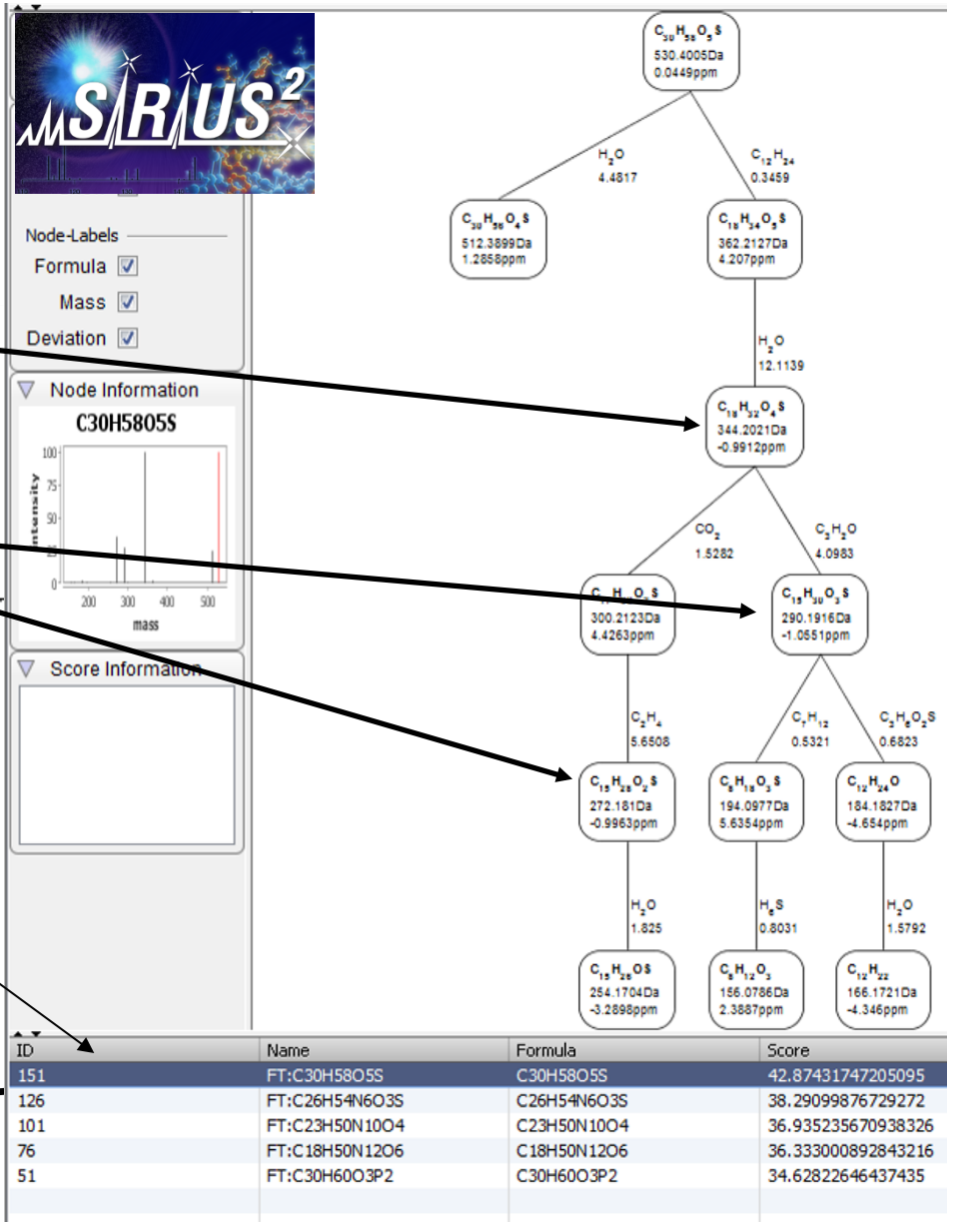




Molecular formula generation: Vital first step toward structural ID

SIRIUS (http://bio.informatik.uni-jena.de/software/sirius/)
Calculates molecular formula assuming that all fragments must be a subset of the parent formula



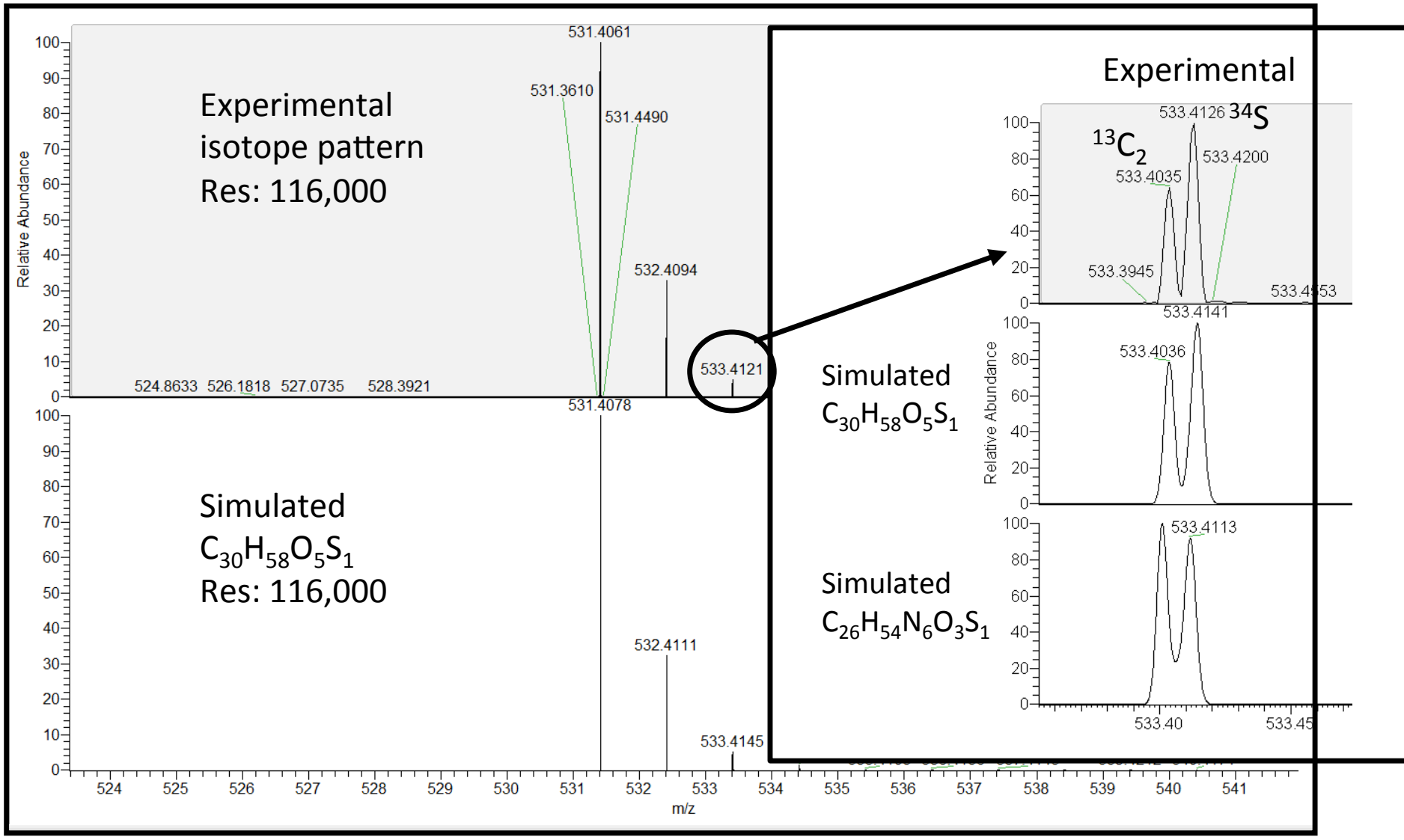


With 5 ppm mass range: **16 possibilities**
With fragment trees: limited to **5**

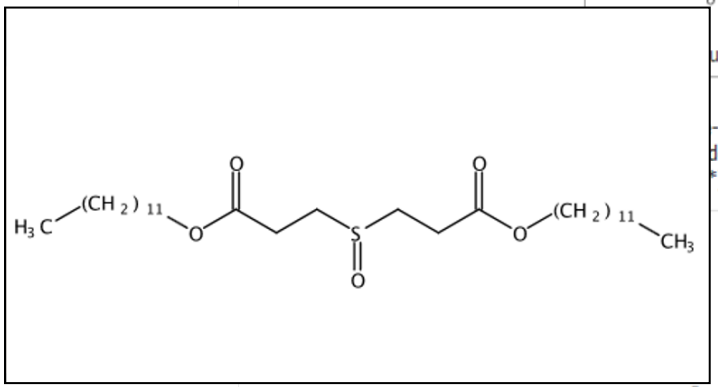
ID	Name	Formula	Score
151	FT:C30H58O5S	C30H58O5S	42.87431747205095
126	FT:C26H54N6O3S	C26H54N6O3S	38.29099876729272
101	FT:C23H50N10O4	C23H50N10O4	36.935235670938326
76	FT:C18H50N12O6	C18H50N12O6	36.333000892843216
51	FT:C30H60O3P2	C30H60O3P2	34.62822646437435

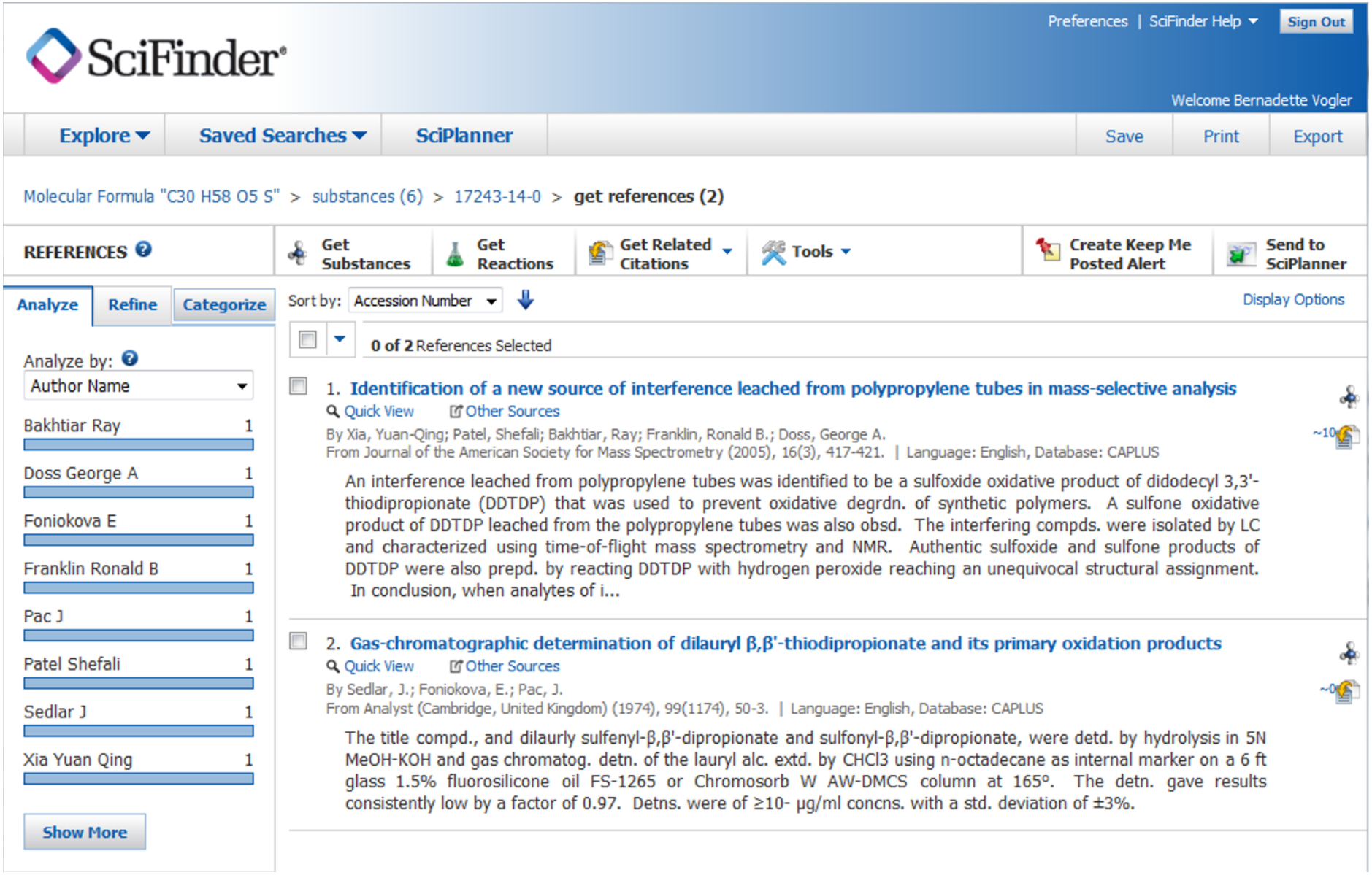
Highest scoring molecular formula for m/z 531.4061: $C_{30}H_{58}O_5S_1$

Ultra-high resolution allows molecular formula validation by isotope fine structure inspection



Molecular Formula $C_{30}H_{58}O_5S$ SciFinder database search

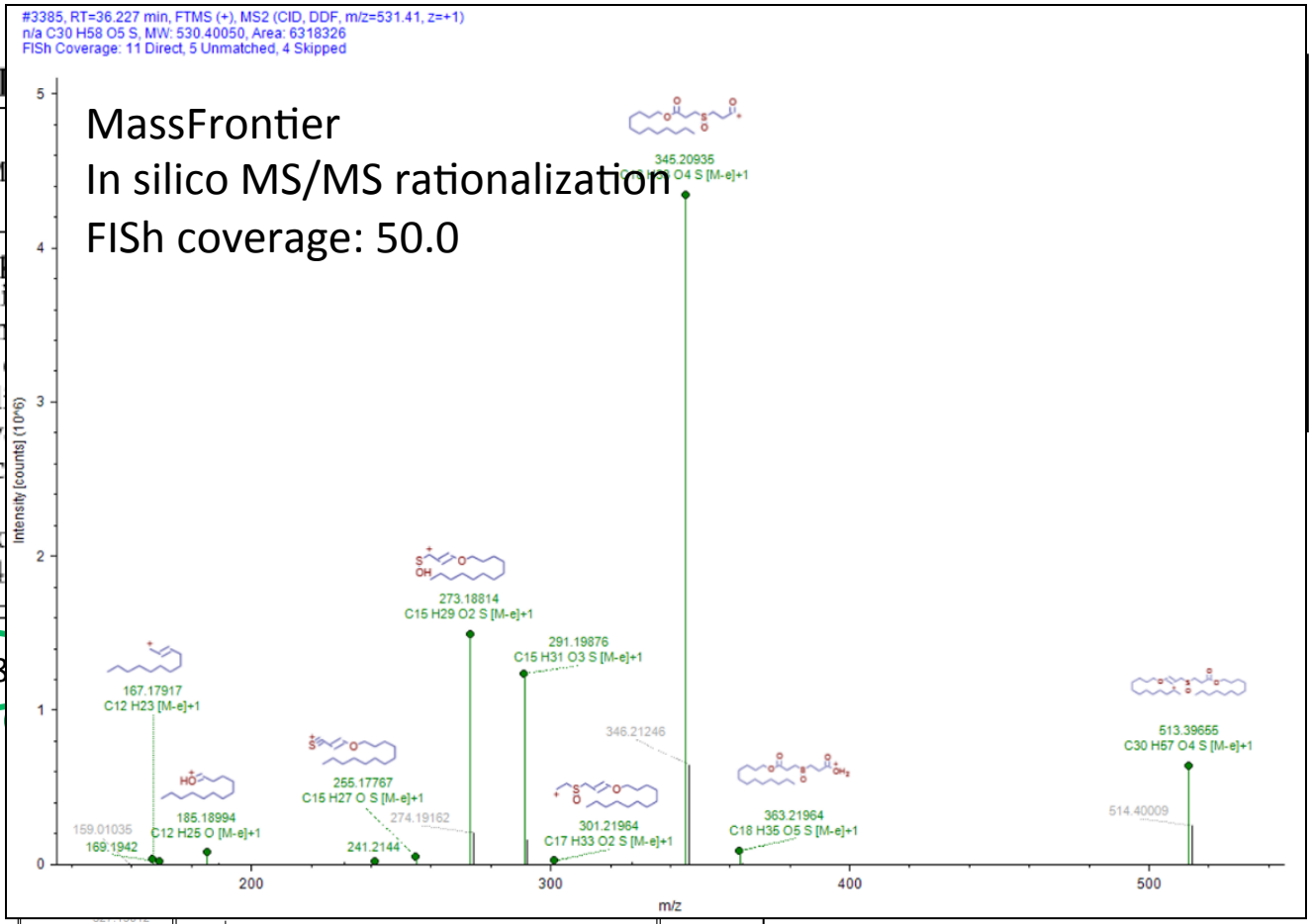



SciFinder
Preferences | SciFinder Help
Sign Out
Welcome Bernadette Vogler
Explore
Saved Searches
SciPlanner
Save
Print
Export
Molecular Formula "C30 H58 O5 S" > substances (6) > 17243-14-0 > get references (2)
REFERENCES
Get Substances
Get Reactions
Get Related Citations
Tools
Create Keep Me Posted Alert
Send to SciPlanner
Analyze
Refine
Categorize
Analyze by:
Author Name
Bakhtiar Ray 1
Doss George A 1
Foniokova E 1
Franklin Ronald B 1
Pac J 1
Patel Shefali 1
Sedlar J 1
Xia Yuan Qing 1
Show More
Sort by: Accession Number
Display Options
0 of 2 References Selected
1. Identification of a new source of interference leached from polypropylene tubes in mass-selective analysis
Quick View
Other Sources
By Xia, Yuan-Qing; Patel, Shefali; Bakhtiar, Ray; Franklin, Ronald B.; Doss, George A.
From Journal of the American Society for Mass Spectrometry (2005), 16(3), 417-421. | Language: English, Database: CAPLUS
An interference leached from polypropylene tubes was identified to be a sulfoxide oxidative product of didodecyl 3,3'-thiodipropionate (DDTDP) that was used to prevent oxidative degrdn. of synthetic polymers. A sulfone oxidative product of DDTDP leached from the polypropylene tubes was also obsd. The interfering compds. were isolated by LC and characterized using time-of-flight mass spectrometry and NMR. Authentic sulfoxide and sulfone products of DDTDP were also prepd. by reacting DDTDP with hydrogen peroxide reaching an unequivocal structural assignment. In conclusion, when analytes of i...
2. Gas-chromatographic determination of dilauryl β,β'-thiodipropionate and its primary oxidation products
Quick View
Other Sources
By Sedlar, J.; Foniokova, E.; Pac, J.
From Analyst (Cambridge, United Kingdom) (1974), 99(1174), 50-3. | Language: English, Database: CAPLUS
The title compd., and dilaurly sulfenyl-β,β'-dipropionate and sulfonyl-β,β'-dipropionate, were detd. by hydrolysis in 5N MeOH-KOH and gas chromatog. detn. of the lauryl alc. extd. by CHCl3 using n-octadecane as internal marker on a 6 ft glass 1.5% fluorosilicone oil FS-1265 or Chromosorb W AW-DMCS column at 165°. The detn. gave results consistently low by a factor of 0.97. Detns. were of ≥10- μg/ml concns. with a std. deviation of ±3%.

Identification of a New Source of Interference Leached from Polypropylene Tubes in Mass-Selective Analysis

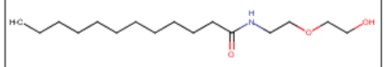
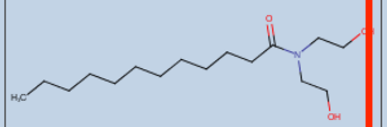
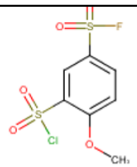
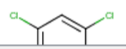
Yuan-Qing Xia, Shefali
and George A. Doss
Department of Drug Metabolism, M

An interference leached from
product of didodecyl 3,3′-thi
degradation of synthetic polyn
polypropylene tubes was also
characterized using time-of-fl
sulfone products of DDTDP w
reaching an unequivocal struc
solubilized in predominantly
possibility of contamination fr
Mass Spectrom 2005, 16, 417-4



Identifying features from an in-house curated suspect database (31,985 entries)

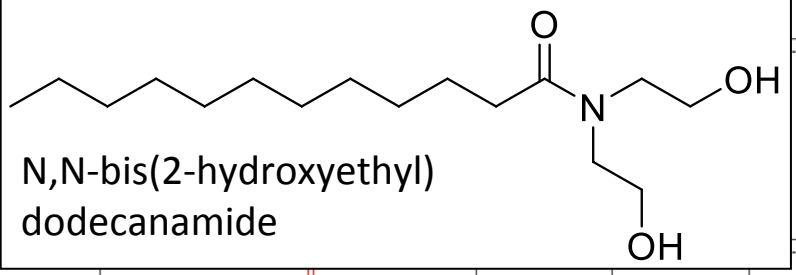
Grid view for DukeMasterDB

Duke University Unique Record #	Structure	Formula	Exact Mass (1)	CAS #	TSCA #	Pesticide Product Code #	Traditional Name	CAS Index Name	LogP	2012 National Production Volume	EFS
DU15264		C16H33NO3	287.24603	20138-28-7	TSCA21318	PC79067	N-[2-(2-hydroxyethoxy) ethyl]dodecanamide	Dodecanamide, N-[2-(2-hydroxyethoxy)ethyl]-	3.16		
DU3741		C16H33NO3	287.24603	120-40-1	TSCA2977	PC79018	N,N-bis(2-hydroxyethyl) dodecanamide	Dodecanamide, N,N-bis(2-hydroxyethyl)-		1,000,000 - 10,000,000	
DU177			287.86008	58-89-9	TSCA182	PC9001	lindane	Cyclohexane, 1,2,3,4,5,6-hexachloro-, (1.alpha.,2.alpha.,3.beta.,4.alpha.,5.alpha.,6.beta.)-	4.35		
DU18								cid, 2,2-dimethyl ester	1.22		
DU27			287.93292				methoxybenzenesulfonyl fluoride	yl fluoride, 3-(chlorosulfonyl)-4-methoxy-	1.42		
DU10120		C12H7Cl3O2		3380-34-5	TSCA11317	PC54901	triclosan	Phenol, 5-chloro-2-(2,4-dichlorophenoxy)-			EFS9

sterDB: 31,985 out of 31,985 rows.

2012 National Production Volume

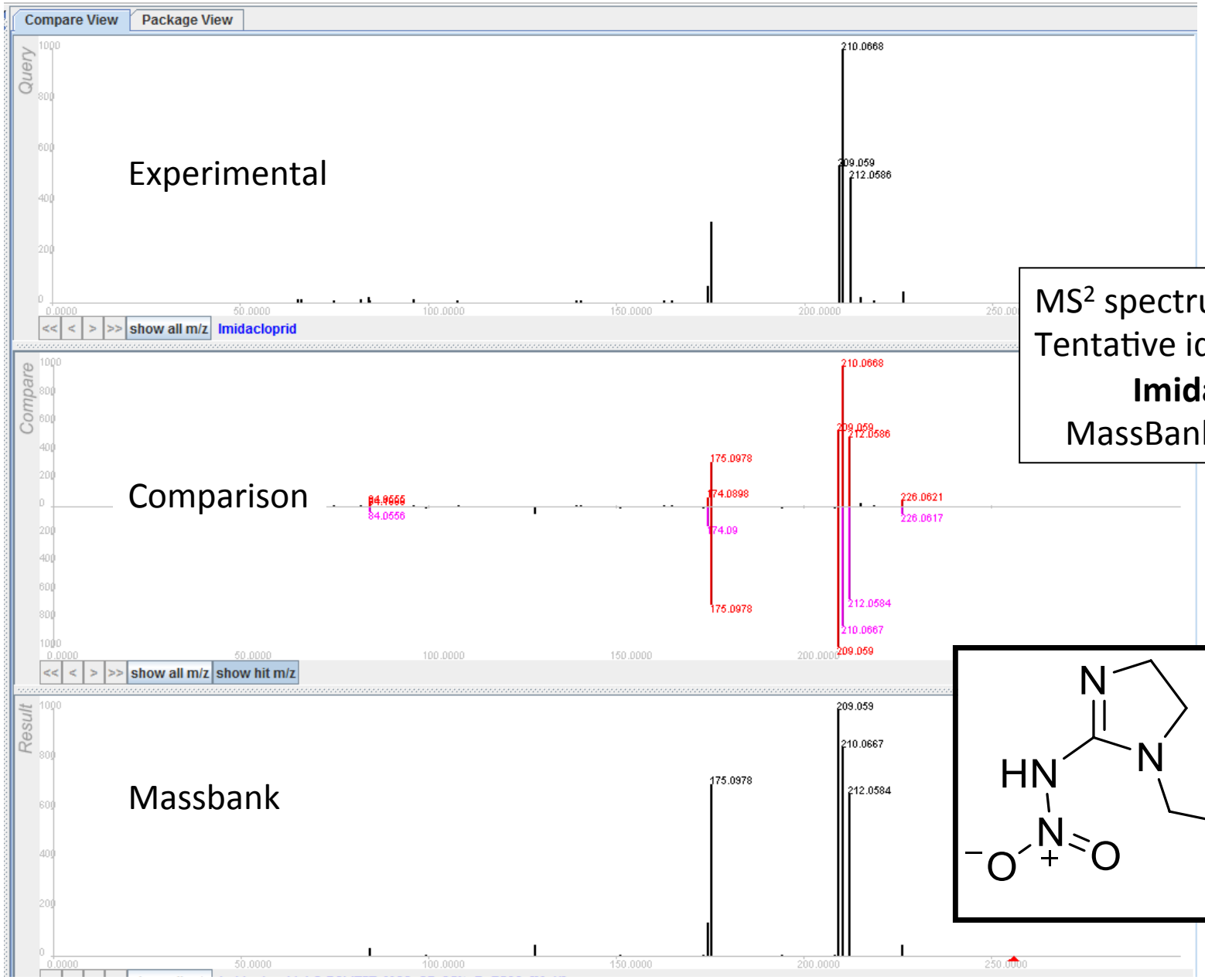
Search by formula



N,N-bis(2-hydroxyethyl) dodecanamide

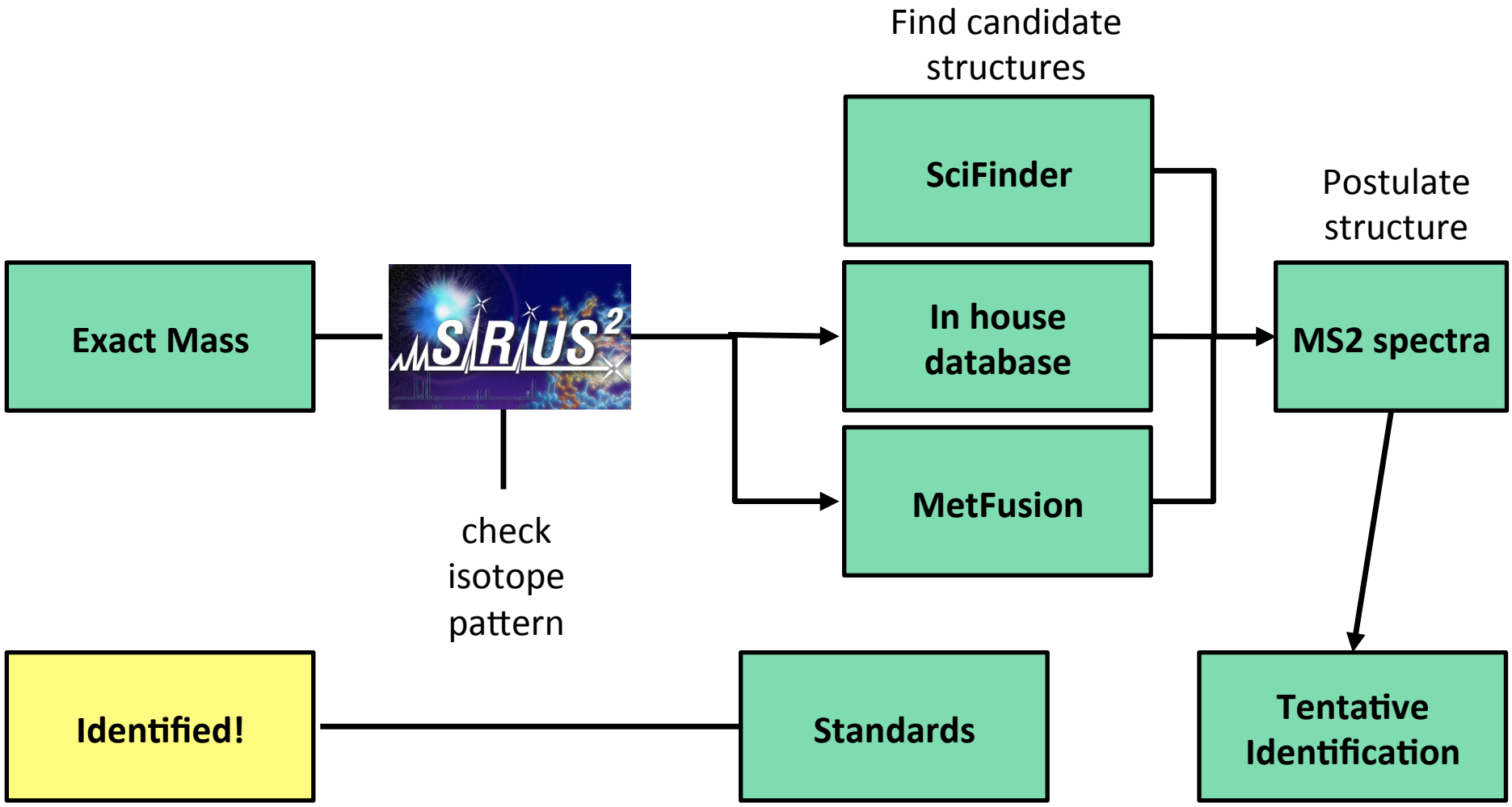
Tentative identification supported by in silico MS^2 prediction using Mass Frontier (FISh Score: 80)

MetFusion for compound ID from HRMS^2 data



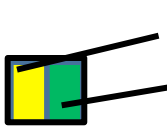
MS^2 spectrum is a match
Tentative identification:
Imidacloprid
MassBank Score: 0.96

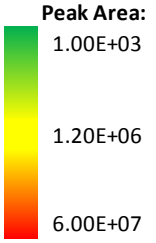
Generalized workflow strategies for identifying SVOC contaminants in paired dust/handwipes by LC-HRMS



Compounds identified in dust/ handwipes

34 compounds
- 10 identified with Standard
- 24 tentatively identified



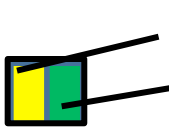


	Name	# of hits W	# of hits D	Paired samples 1	2	3	4	5	6	7	8	9	10	STD?
Organophosphates	di-tertbutyl triphenyl phosphate	4	5											
	tri-(2-butoxyethyl)-phosphate (TBOEP)	10	10											x
	tris (4-butyl-phenyl) phosphate (TBPP)	7	5											x
	tris (2-chloro-ethyl) phosphate (TCEP)	5	10											x
	tris (1-chloro-isopropyl) phosphate (TCPP)	10	10											x
	tricresyl phosphate	6	7											
	triphenyl phosphate (TPP)	10	10											x
	V6	2	4											x
Surfactants	dodecyl sulfate	2	5											
	tridecyl sulfate	2	6											
	tetradecyl sulfate	2	5											
	pentadecyl sulfate	3	6											
	hexadecyl sulfate	6	10											
	dodecylethanolamine	9	8											
	N-lauroyl sarcosine	6	6											
	perfluorooctanesulfonic acid (PFOS)	0	2											

Surfactants used in shampoo, cosmetics

Compounds identified in dust/ handwipes

34 compounds
- 10 identified with Standard
- 24 tentatively identified



Peak Area:
1.00E+03
1.20E+06
6.00E+07

		W	D	1	2	3	4	5	6	7	8	9	10	STD?
Polymer additives	acetyl butyl citrate	10	10											
	benzyl butyl phthalate	9	10											x
	caprolactam cyclic dimer	8	9											
	caprolactam	5	10											
	caprolactam	4	10											
	caprolactam cyclic pentamer	1	10											
	caprolactam cyclic hexamer	0	6											
	dilauryl sulfinyl-ß,ß'-dipropionate	10	9											
	N,N-bis(2-hydr y hy)	10	10											
	oleamide	8	6											
Pesticides	fipronil	6	9											x
	fipronil Sulfone	4	8											x
	imazalil	1	4											
	imidacloprid	4	9											x
	ketoconazol	1	1											
Others	1,3-dilinolein													
	alpha-tocopheryl nicotinate	2	5											
	piperine	10	10											

Byproduct of polymerization used for food packaging

Leaching from plastics

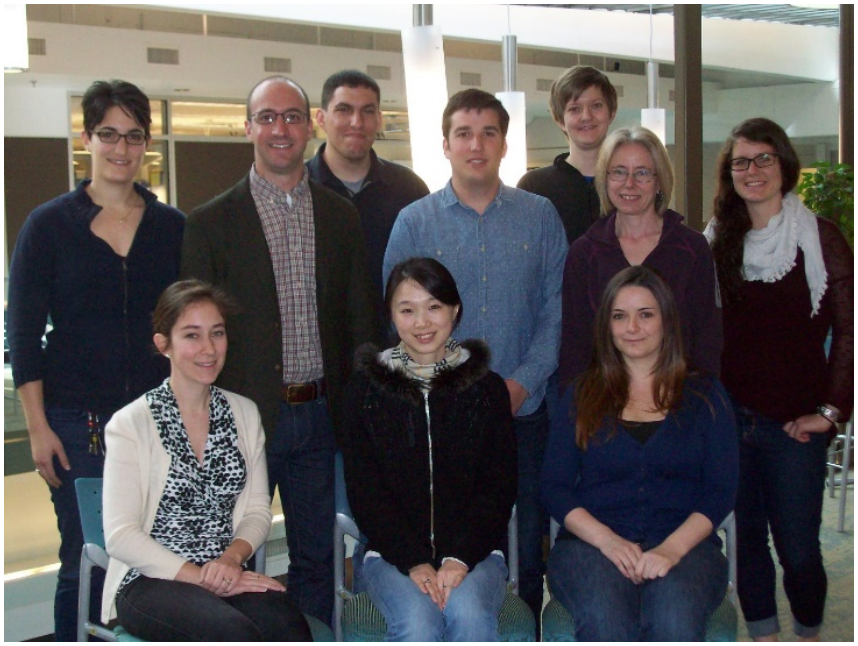
Oxidation product of cooking oil

Comes from black pepper

Conclusions: Exploring the indoor environment exposome using non-targeted analysis strategies

- (2D)LC-HRAM mass spectrometry is a powerful tool for analysis of SVOC compounds in dust and hand wipe samples.
- Non-targeted workflows allow a more holistic view of contaminant exposure in indoor environments relative to targeted analysis.
- 213 tentative and confirmed identifications were made from 567 filtered components in dust/wipes (37.5% of filtered features).
- The most dominant compounds in dust and handwipes were non ionic surfactants such as nonylphenol ethoxylates or alcohol ethoxylates.

Acknowledgement



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