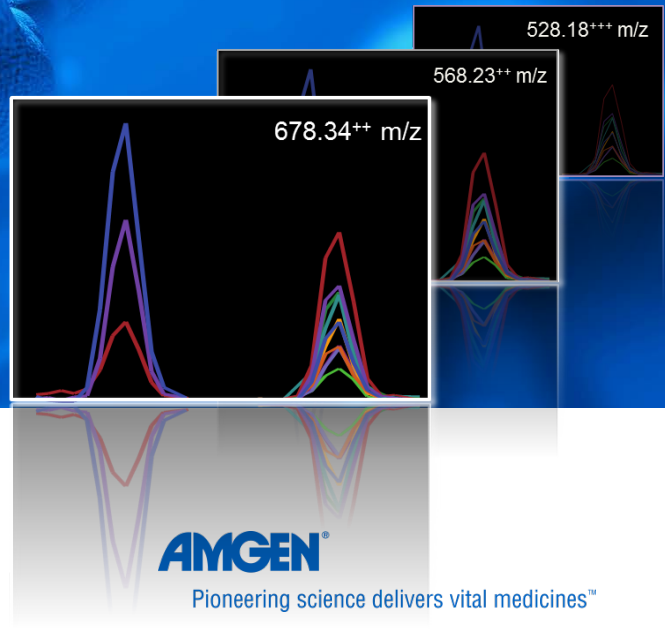
Rapid Identification of Contaminants and Interferences using Skyline

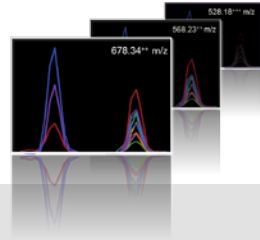


MATT RARDIN

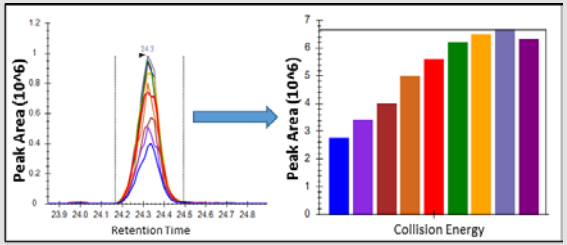
ASMS SKYLINE USER MEETING, INDIANAPOLIS, IN
JUNE 4TH, 2017

AMGEN®
Pioneering science delivers vital medicines™

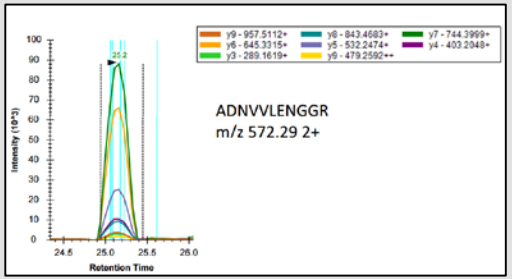
SKYLINE APPLICATIONS AT AMGEN



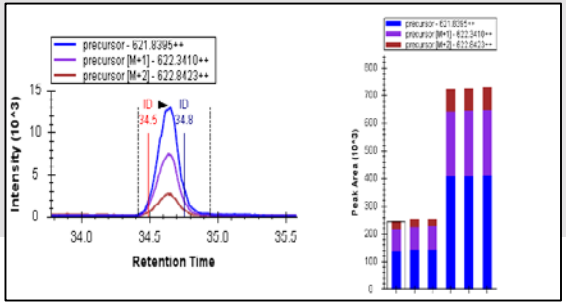
SRM



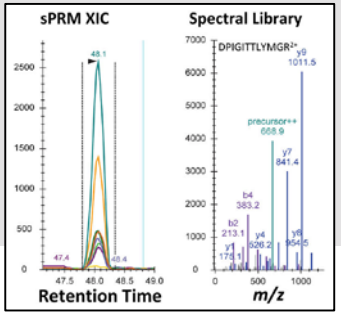
DIA / SWATH



MS1 Filtering



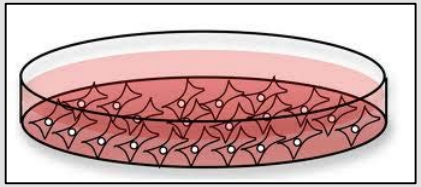
PRM / MRM-HR



AMGEN

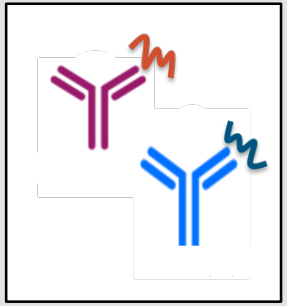
WORKFLOW DEVELOPMENT

Tissue culture cells



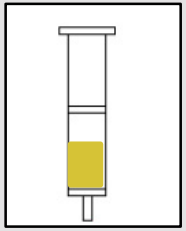
Lysis & Digestion

Affinity Enrichment



Acid elution

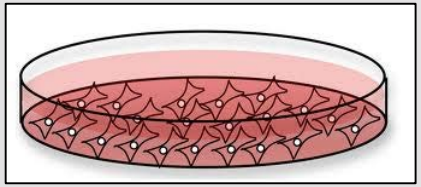
SPE



Orbitrap Fusion LC-MS/MS

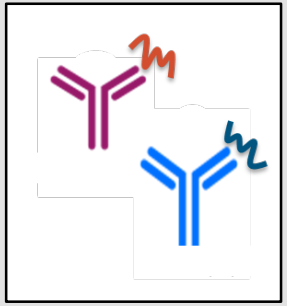
WORKFLOW DEVELOPMENT

Tissue culture cells



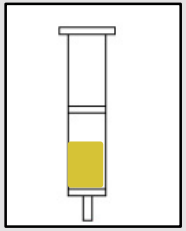
Lysis & Digestion

Affinity Enrichment

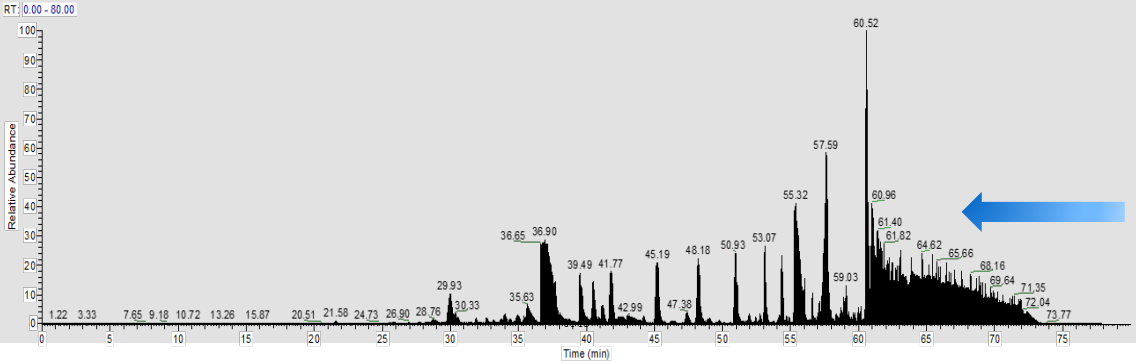


Acid elution

SPE

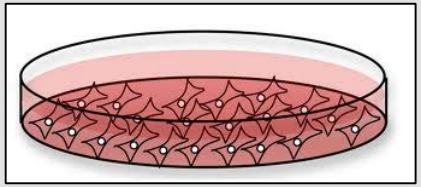


Orbitrap Fusion LC-MS/MS



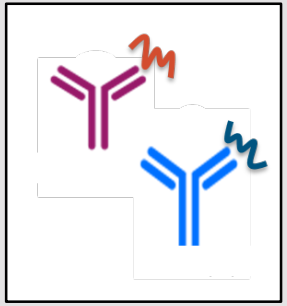
WORKFLOW DEVELOPMENT

Tissue culture cells



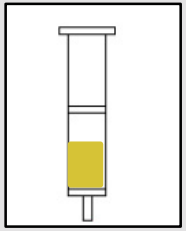
Lysis & Digestion

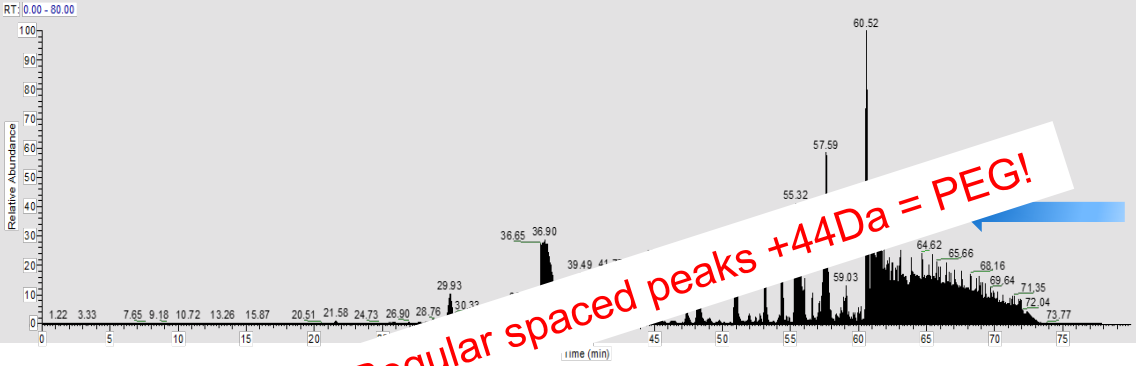
Affinity Enrichment



Acid elution

SPE



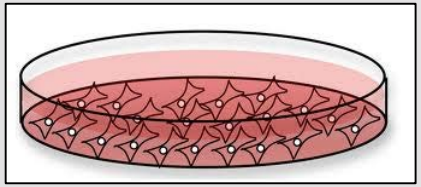


Regular spaced peaks +44Da = PEG!

Orbitrap Fusion LC-MS/MS

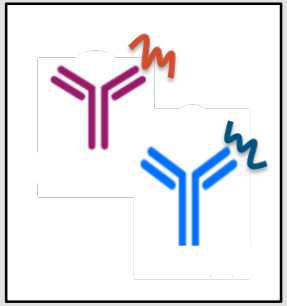
TRACKING DOWN THE SOURCE

Tissue culture cells



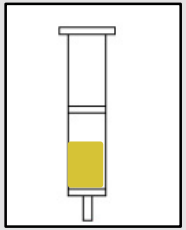
Lysis & Digestion

Affinity Enrichment



Acid elution

SPE

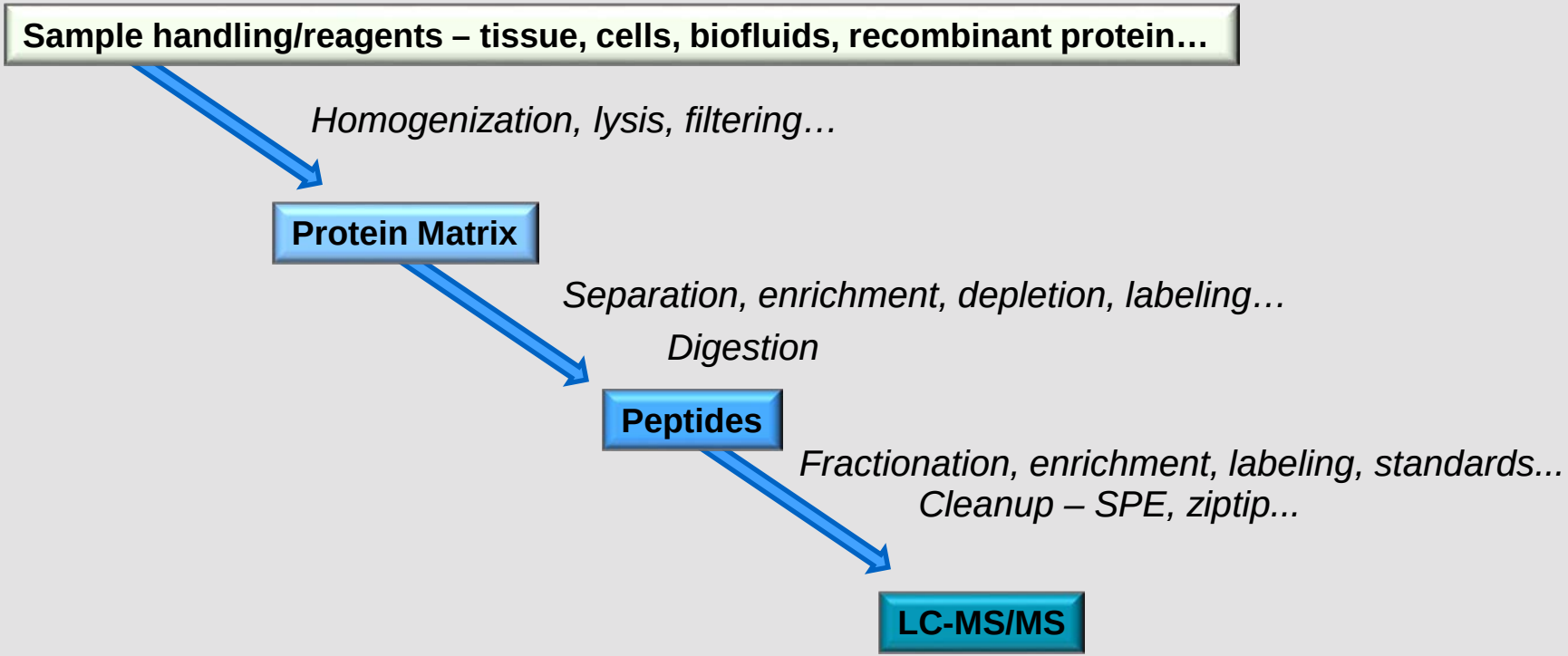


Orbitrap Fusion LC-MS/MS

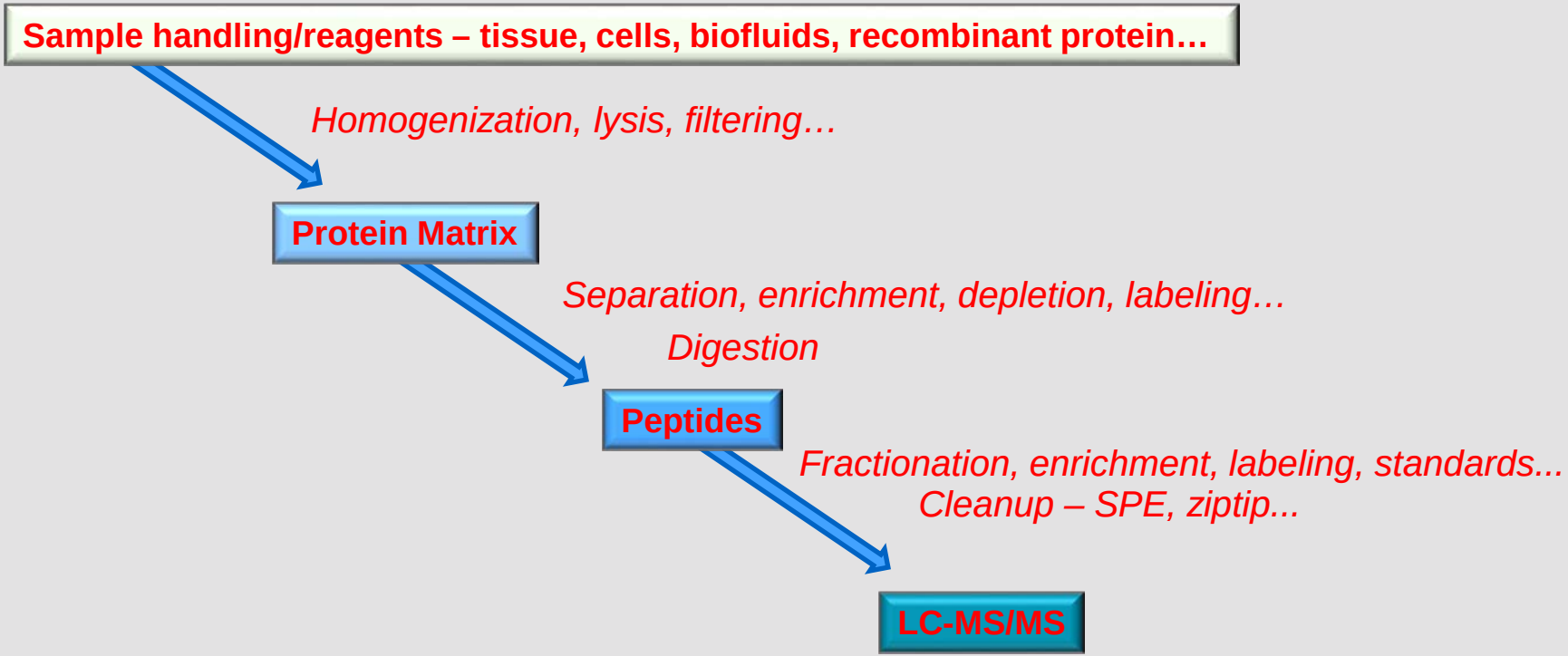
Media?
Buffers?
Plastics?
Antibodies?
Reagents?
Contaminated solvents?

AMGEN

PROTEOMIC WORKFLOWS

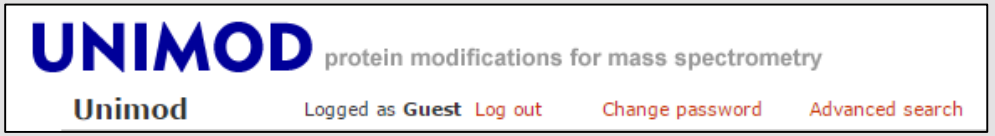


PROTEOMIC WORKFLOWS *ARE* A SOURCE OF CONTAMINATION



AMGEN

RESOURCES FOR CONTAMINANTS AND INTERFERENCES



AMGEN

DEVELOPING A SKYLINE LIBRARY OF CONTAMINATES AND INTERFERENCES USING MS1 AND SMALL MOLECULE FEATURES

GCB Duke Center for Genomic and Computational Biology

Skyline for small molecules: a flexible tool for cross-platform LC-MS/MS method creation and data analysis for metabolomics.

Genome Sciences UNIVERSITY OF WASHINGTON

J. Will Thompson[1], Brian Pratt[2], Max Horowitz-Gelb[2], Laura G. Dubois[1], Lisa St.John-Williams[1], Giuseppe Astarita[3], M. Arthur Moseley[1], Michael MacCoss[2], and Brendan MacLean[2]

[1]Duke Proteomics and Metabolomics Core, Center for Genomic and Computational Biology, School of Medicine, Duke University, Durham, NC; [2]Department of Genome Sciences, School of Medicine, University of Washington, Seattle, WA; [3]Waters Corporation, Milford, MA

Recent Advances in Skyline: Small Molecule Targets and Ion Mobility Filtering

Brian Pratt[1], Max Horowitz-Gelb[1], J. Will Thompson[2], Erin Baker[3], J. Will Thompson[4], Michael J. MacCoss[1], Brendan MacLean[1]

[1]University of Washington, Seattle WA, [2]Duke University School of Medicine, Durham NC, [3]Pacific Northwest National Laboratory, Richland WA

Monoisotopic ion mass (singly charged)	Ion type	Formula for M or subunit or sequence	Compound ID or species	Possible origin and other comments	ESI	MALDI	References
33.03349	$[M+H]^+$	CH_4O	Methanol	Acetonitrile, solvent	X		A
42.03383	$[M+H]^+$	CH_3CN	ACN	Acetonitrile, solvent	X		A
59.06037	$[M+NH_4]^+$	CH_3CN	ACN	Acetonitrile, solvent	X		A, F
63.04406	$[A_1B+H]^+$	$[C_2H_4O]_nH_2O$	PEG	Polyethylene glycol, ubiquitous polyether	X		D, K
64.01577	$[M+Na]^+$	CH_3CN	ACN	Acetonitrile, solvent	X		A, F
65.05971	$[M_2+H]^+$	CH_3OH	Methanol	Methanol, solvent	X		A
74.06004	$[M+H]^+$	C_3H_7NO	Dimethyl formamide	solvent	X		A, C, F
74.06004	$[A_1B_1+H]^+$	$(CH_3CN)_n(CH_3OH)_m$	Acetonitrile/Methanol	ESI solvents	X		K
77.05971	$[A_1B+H]^+$	$[C_3H_6O]_nH_2O$	PPG	Polypropylene glycol, ubiquitous polyether	X		D, K
79.02121	$[M+H]^+$	C_2H_6OS	DMSO	Dimethylsulfoxide, solvent	X		A
83.06037	$[M_2+H]^+$	CH_3CN	Acetonitrile	ESI solvents	X		A, C
85.02600	$[A_1B+Na]^+$	$[C_2H_4O]_nH_2O$	PEG	Polyethylene glycol, ubiquitous polyether	X		D, K
85.05887	$[M+H]^+$	C_2D_6OS	d6-DMSO	d_6-Dimethylsulfoxide, solvent	X		A
88.03931	$[A_1B_1+H]^+$	$(CH_3CN)_n(HCOOH)_m$	Acetonitrile/Formic Acid	ESI solvents	X		A, C

Expanding Skyline's Capabilities to Small Molecule Data Analysis

Laura G. Dubois

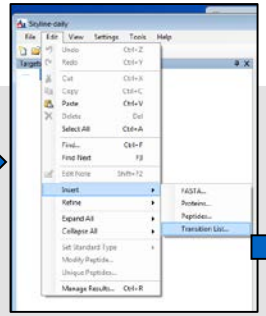
Duke Proteomics and Metabolomics Core Facility

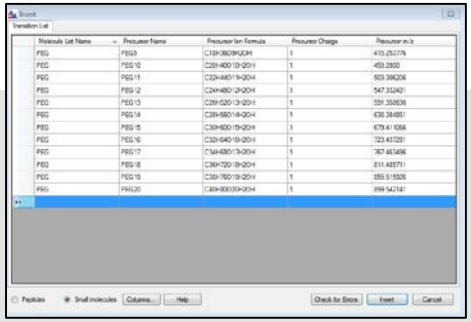
AMGEN

CREATING A MOLECULAR CONTAMINATION LIBRARY

Molecule List Name	Precursor Name	Precursor Formula	Precursor Charge	Precursor m/z
PEG	PEG9	C18H36O9H2OH	1	415.2538
PEG	PEG10	C20H40O10H2OH	1	459.2800
PEG	PEG11	C22H44O11H2OH	1	503.3062
PEG	PEG12	C24H48O12H2OH	1	547.3324
PEG	PEG13	C26H52O13H2OH	1	591.3586
PEG	PEG14	C28H56O14H2OH	1	635.3849
PEG	PEG15	C30H60O15H2OH	1	679.4111
PEG	PEG16	C32H64O16H2OH	1	723.4373
PEG	PEG17	C34H68O17H2OH	1	767.4635
PEG	PEG18	C36H72O18H2OH	1	811.4897
PEG	PEG19	C38H76O19H2OH	1	855.5159
PEG	PEG20	C40H80O20H2OH	1	899.5421

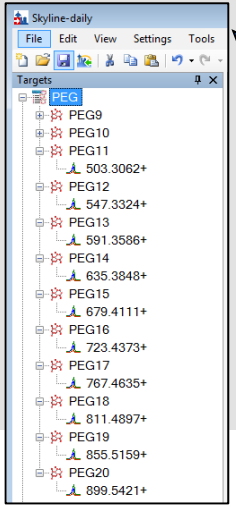
Transition List



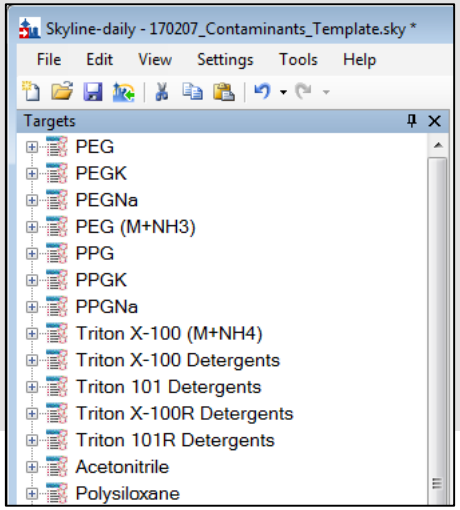


Import List into Skyline

Populate your molecular tree



Add additional groups to expand your library as needed

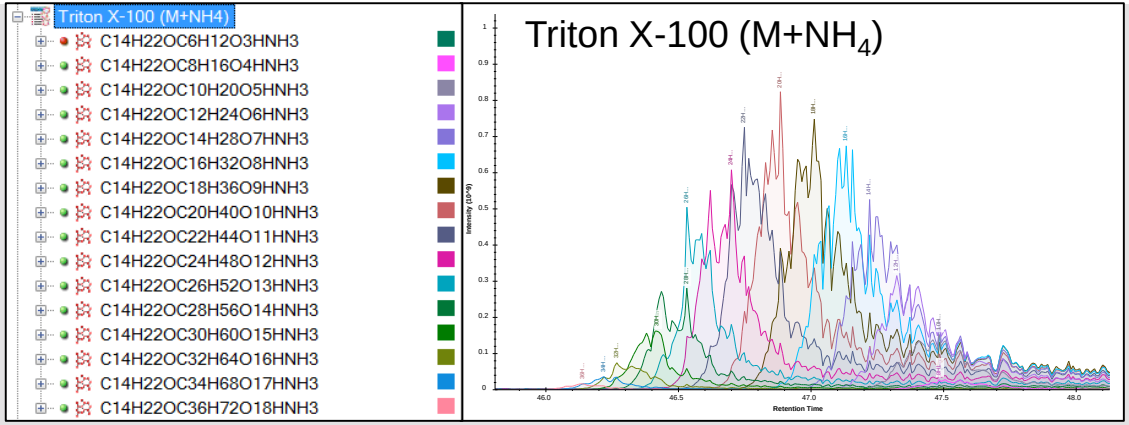


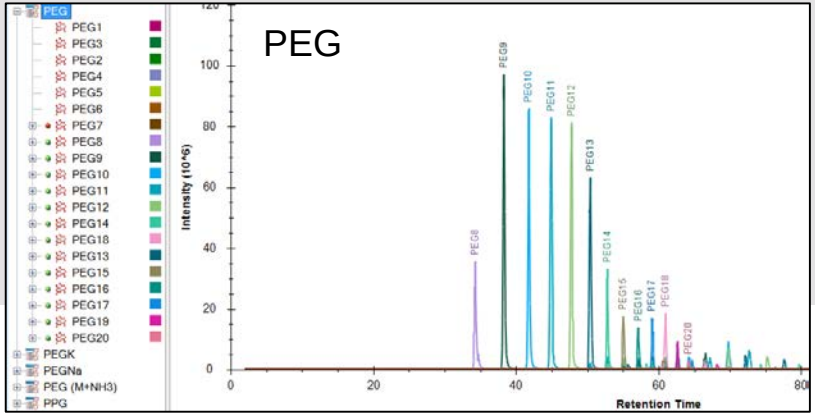
Contamination Library

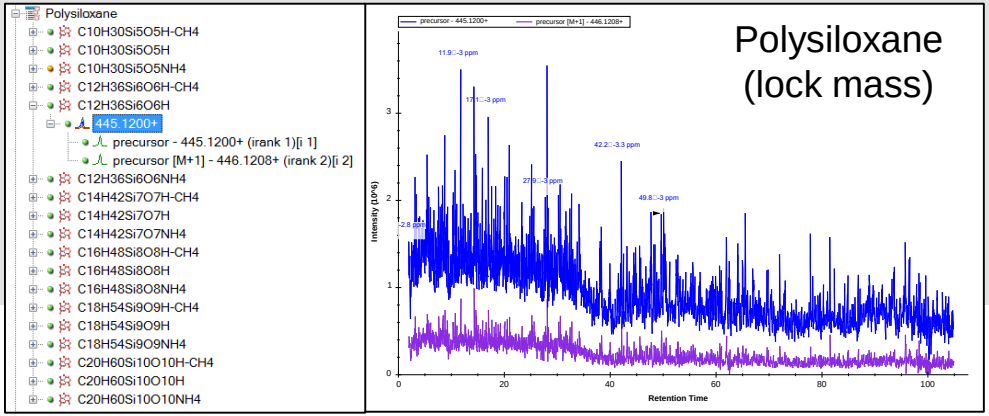
- 64 molecular groups
- 641 molecules

MONITORING COMMON CONTAMINANTS USING MS1 FILTERING

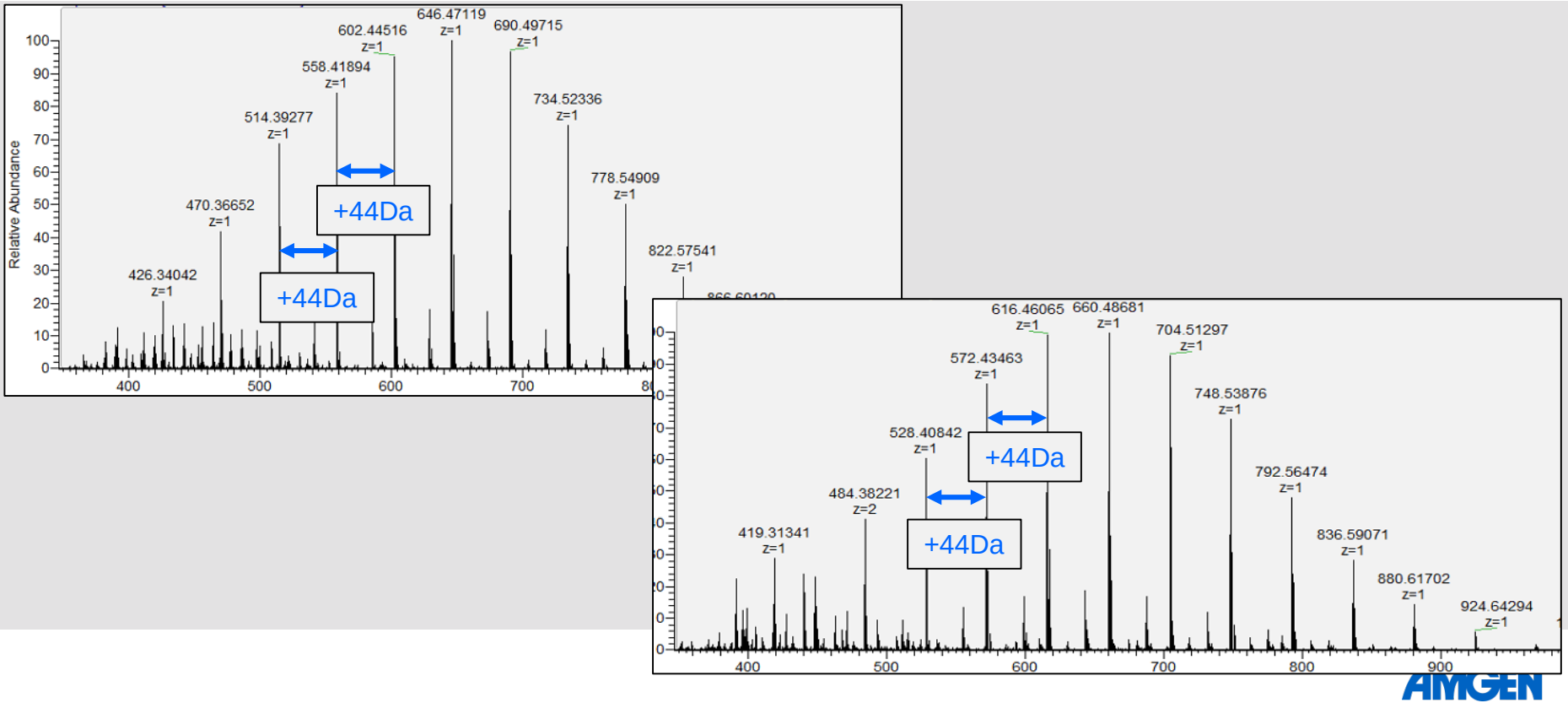
Contamination never looked so GOOD…



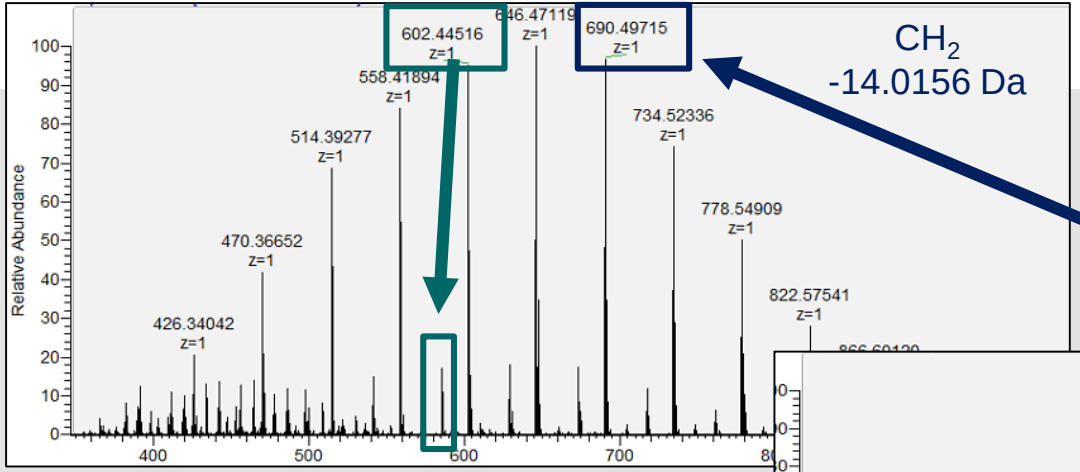




IDENTIFYING AN UNKNOWN SPECIES OF (PEG)?

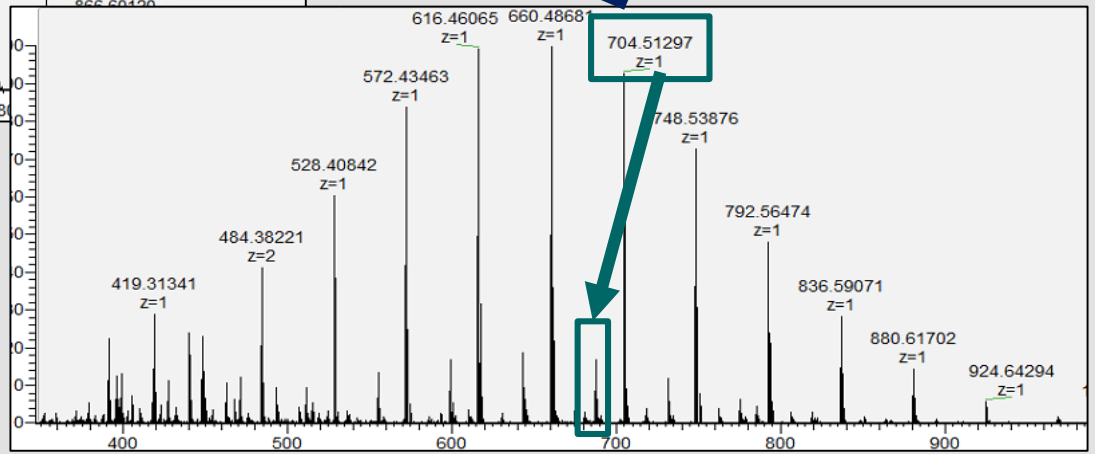


IDENTIFYING AN UNKNOWN SPECIES OF (PEG)?



CH_2
-14.0156 Da

NH_3
-17.0265 Da



NH_3
-17.0265 Da

AMGEN

IDENTIFYING UNKNOWN SPECIES USING mMASS

http://www.mmass.org/

Rapid Communications in Mass Spectrometry

Explore this journal >

Letter to the Editor

mMass **data miner: an open source alternative for mass spectrometric data analysis**

Martin Strohalm ✉, Martin Hassman, Bedřich Košata, Milan Kodíček

First published: 22 February 2008 Full publication history

DOI: 10.1002/rcm.3444 View/save citation

Cited by (CrossRef): 104 articles Check for updates | Citation tools

Mass to formula tool

mMass

File View Processing Sequence Tools Libraries Links Window Help

Mass To Formula

Mo. mass: 220.227653 Charge: 1 NH4+ Tolerance: 5 Da ppm Generate

Minimal formula: Maximal formula:

Composition rules: H/C NOPS/C NOPS RDBE Integer RDBE Check isotopic pattern

neutral formula	mass	m/z	error	H/C	rdbe	pattern
C12H26O2	202.1933	220.2271	2.5	2.2	0.0	n/a

Show Isotopic Pattern
Search in PubChem
Search in ChemSpider
Search in METLIN
Search in HMDB
Search in Lipid MAPS
Copy Formula
Copy List

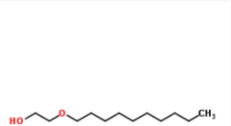
ChemSpider

Search and share chemistry

Simple Structure Advanced History

Found 386 results

Search term: **C12H26O2** (Found by molecular formula)



2-(Decyloxy)ethanol

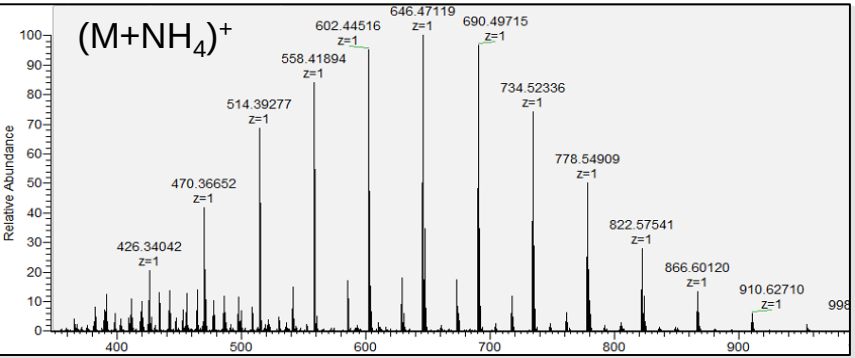
Molecular Formula $C_{12}H_{26}O_2$

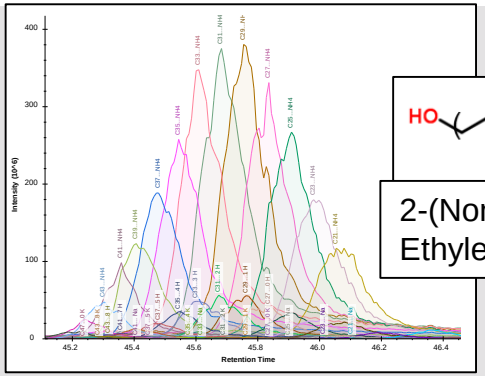
Average mass 202.334 Da

Monoisotopic mass 202.193283 Da

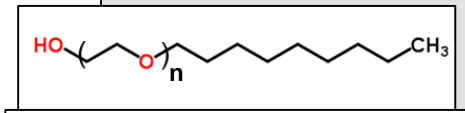
ChemSpider ID 56542

VISUALIZING NEW CONTAMINANTS IN SKYLINE



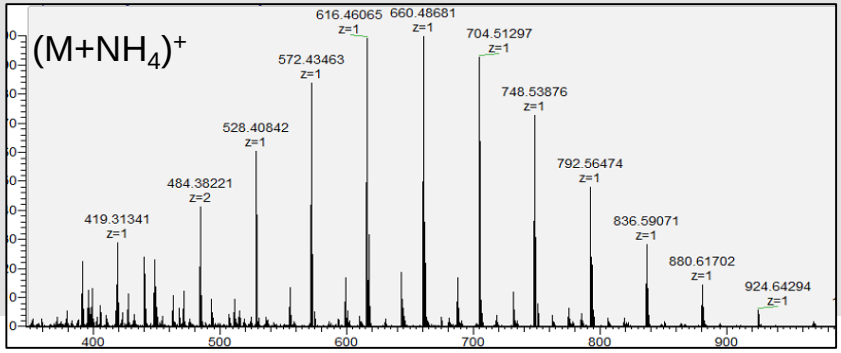


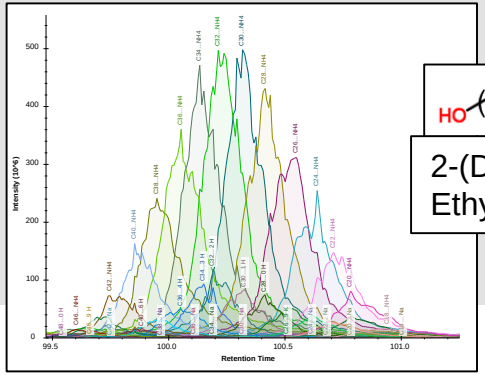
$C_9H_{20}O(C_2H_4O)_n$



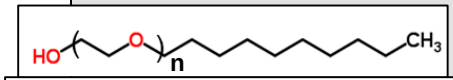
2-(Nonyloxy)ethanol
Ethylene glycol mono-n-nonyl ether

CH_2
+14.0156 Da





$C_{10}H_{22}O(C_2H_4O)_n$



2-(Decyloxy)ethanol
Ethylene glycol mono-n-decyl ether

AMGEN

SO WHAT WAS THE SOURCE OF CONTAMINATION?

Antibody columns were stored wrapped in parafilm…

SO WHAT WAS THE SOURCE OF CONTAMINATION?

Antibody columns were stored wrapped in parafilm…

Home › Proteomics Blog › This is why you do not let artificially smelly people stand next to the Q-Exactive

This is why you do not let artificially smelly people stand next to the Q-Exactive

Posted on October 7, 2014 by Brett Phinney — 7 Comments ↓

The Q-exactives seem to be much more sensitive to environmental contaminants than our pervious instruments (LTQ's and LTQ-FT's). Here is an example of two BSA QC's run back to back .The only difference is that for one of them a HPLC service engineer with a lot of cologne was standing next to the Q-exactive. Notice the 371 background ion goes from 10^5 to 10^8 and totally swamps out the base peak ion chromatograms!

ANOTHER SOURCE OF CONTAMINATION – MS STANDARDS?

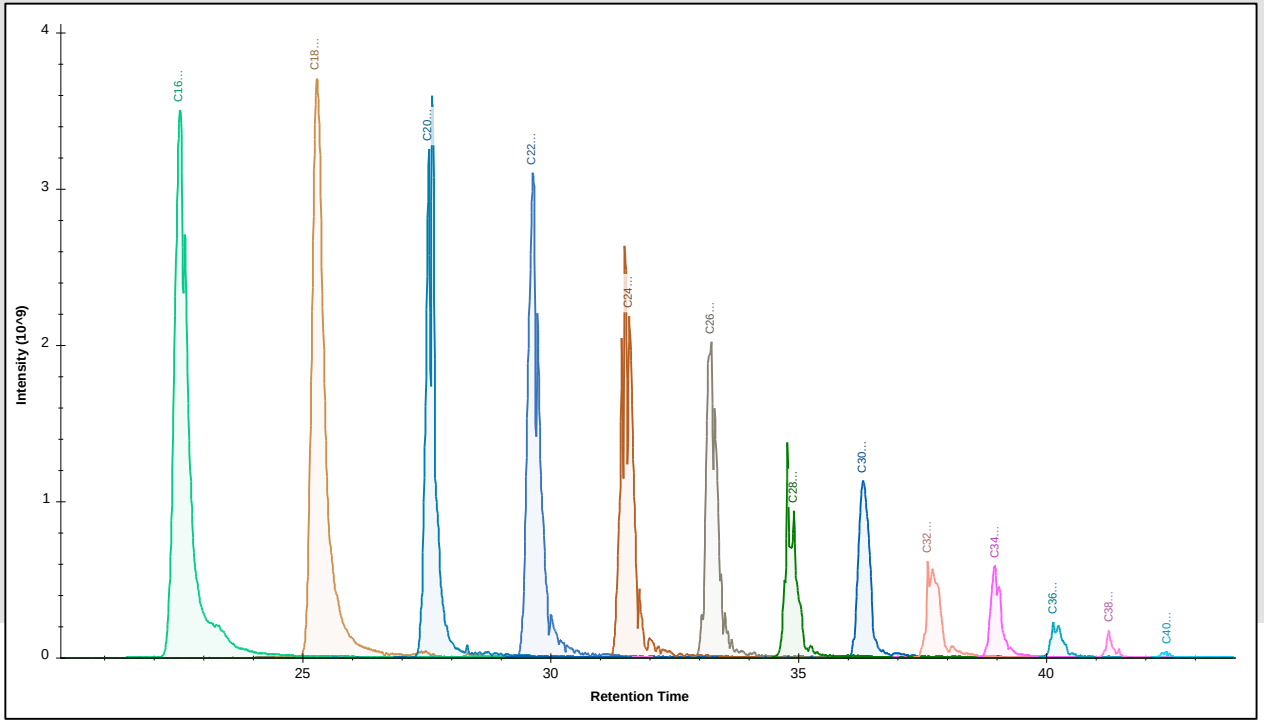
Many vendors now sell various mass spec standards… peptides, peptide mixture, proteins, antibodies, heavy labeled peptides and proteins

- **We purchases 10ug of a heavy labeled (Lys+8, Arg+10) protein purified from HELA cells (~$1500)**

ANOTHER SOURCE OF CONTAMINATION – MS STANDARDS?

Many vendors now sell various mass spec standards… peptides, peptide mixture, proteins, antibodies, heavy labeled peptides and proteins

- **We purchases 10ug of a heavy labeled (Lys+8, Arg+10) protein purified from HELA cells (~$1500)**
- **Peptide mapping of the protein standard revealed wide spread contamination with PEG**
- **Vendor was using 1% NP40 in their lysis and did not effectively remove associated PEG contamination nor did they even realize the problem…**



CONCLUSIONS

- **Using Skyline's small molecule tools we developed a library of common contaminants and interferences that can easily be monitored using MS1 Filtering**
- **Currently have a list of 64 molecular groups and over 641 molecules in our library**
- **Allows one to rapidly assess contamination of samples**
- **Provided an approach for identifying unknown molecular species**

AMGEN

ACKNOWLEDGING THE CONTAMINATION CREW

- No one volunteered to be on this list…