

Extracting metabolite ions of peptide drugs from an *in vivo* matrix background

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Drug Safety Sciences

Analytical Sciences, Drug Metabolism & Pharmacokinetics



Outline

- Introduction
- Filtering peptide ions from an *in vivo* matrix:
 - selective ion extraction based on charge state
 - background subtraction using ion mobility
- Software tools – unbiased approaches:
 - small molecule software tools
 - large molecule software tools
- Conclusions

Introduction: Why study peptide metabolism?

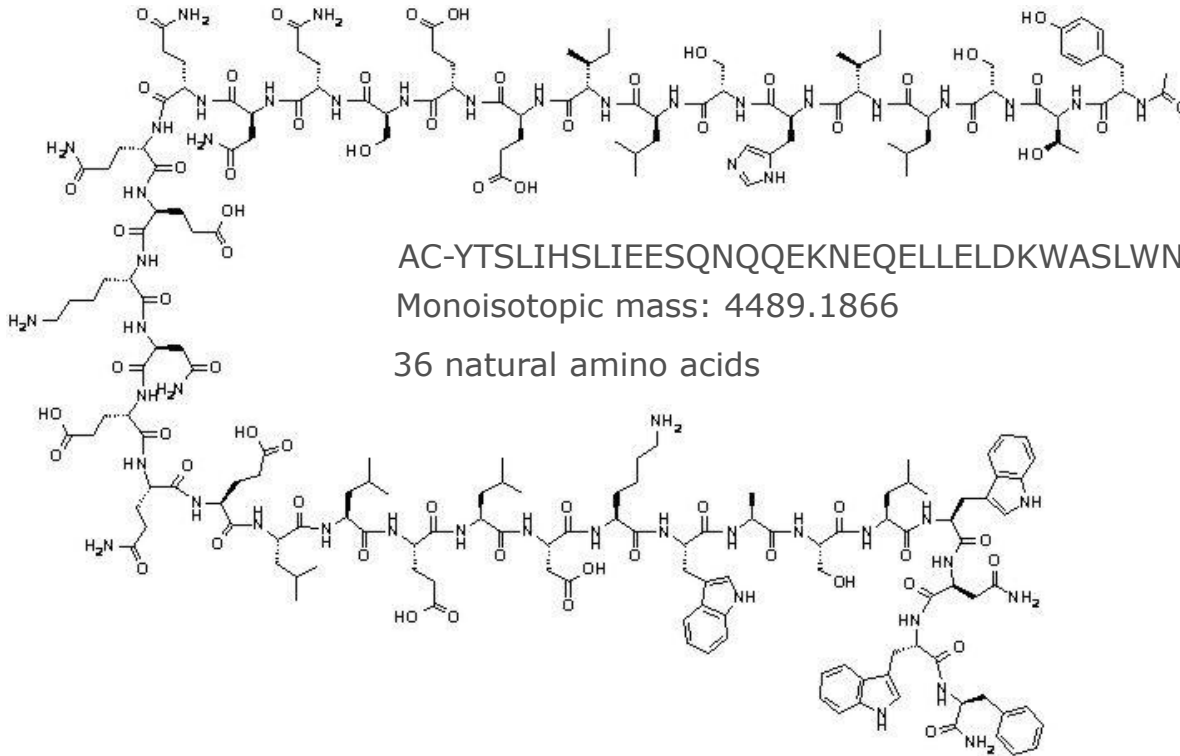
- Development of more stable peptide drugs
- In support of quantitative assays (LBA and LC/MS/MS)
- Safety evaluation?
 - peptides consisting of natural amino acids considered safe
 - level of modification allowed before additional safety evaluation is recommended?
 - increased regulatory attention

└───> peptides treated as small molecules



while different toolbox required (non-CYP mediated metabolism, AA recycled, etc.)

Model peptide: enfuvirtide



- IV dosed to rat at 2 mg/kg – vehicle dosed rat (controls)
- Plasma samples 8 min, 30 min, 120 min after dosing

Method

Sample preparation:

= sample prep used for parent drug quantification

Chromatography:

UHPLC: Waters Acquity UPLC or Shimadzu Nexera

Acquity BEH C18 2.1 x 150 mm (1.7 μm ; 120 \AA)

flow rate: 0.2 ml/min

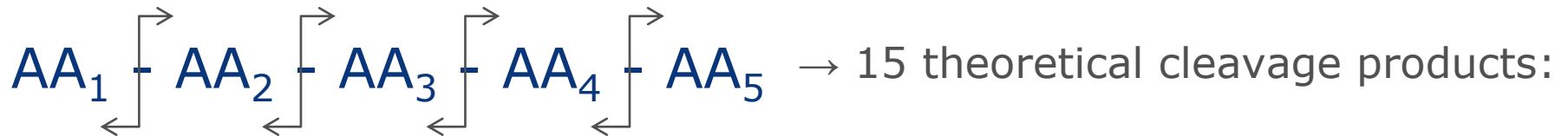
linear 45 min gradient with HCOOH (ESI+) or NH₄OH (ESI-)

Mass spectrometry:

Waters Qtof Synapt G2 (+ ion mobility)

AB Sciex TripleTof 5600

XIC's of theoretical cleavages?



$AA_1 - AA_2 - AA_3 - AA_4 - AA_5$	AA_2	AA_3	AA_4
$AA_1 - AA_2 - AA_3 - AA_4$	$AA_2 - AA_3$	$AA_3 - AA_4$	AA_5
$AA_1 - AA_2 - AA_3$	$AA_2 - AA_3 - AA_4$	$AA_3 - AA_4 - AA_5$	$AA_4 - AA_5$
$AA_1 - AA_2$	$AA_2 - AA_3 - AA_4 - AA_5$		
AA_1			

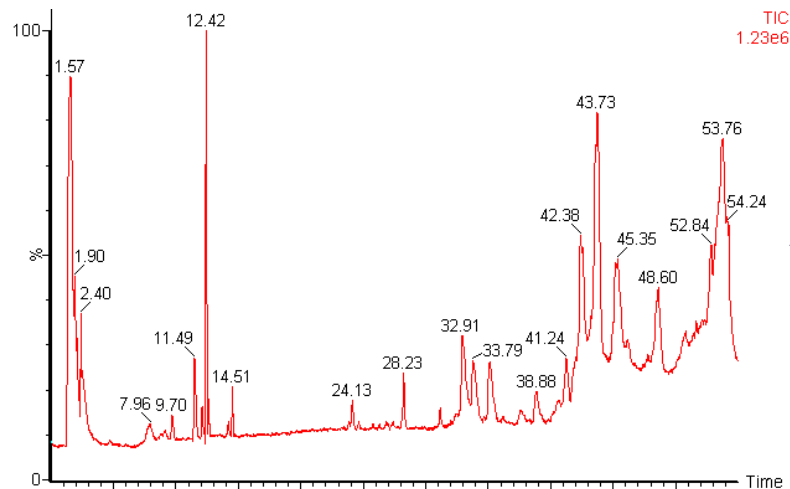
packages available to generate possible cleavage products in silico

enfuvirtide: 36 natural AA \rightarrow 666 theoretical cleavage products

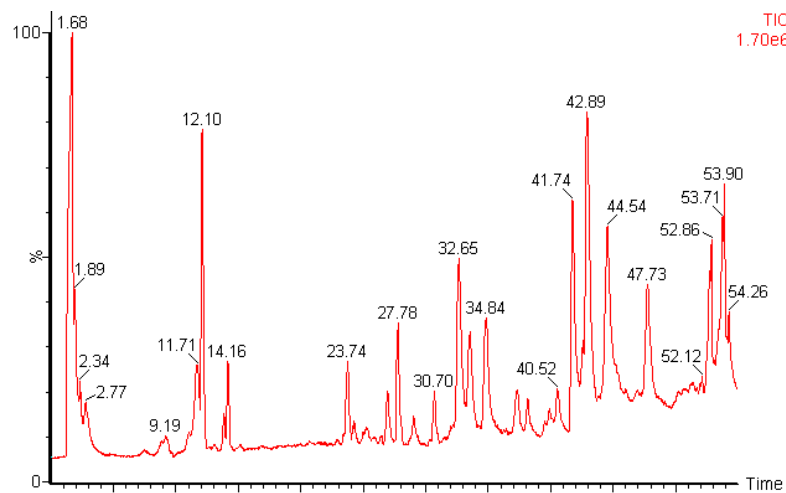
- time consuming, false positives
- theory down approach \rightarrow unexpected metabolites will be missed

non-biased approach: control – analyte comparison

120 min plasma samples



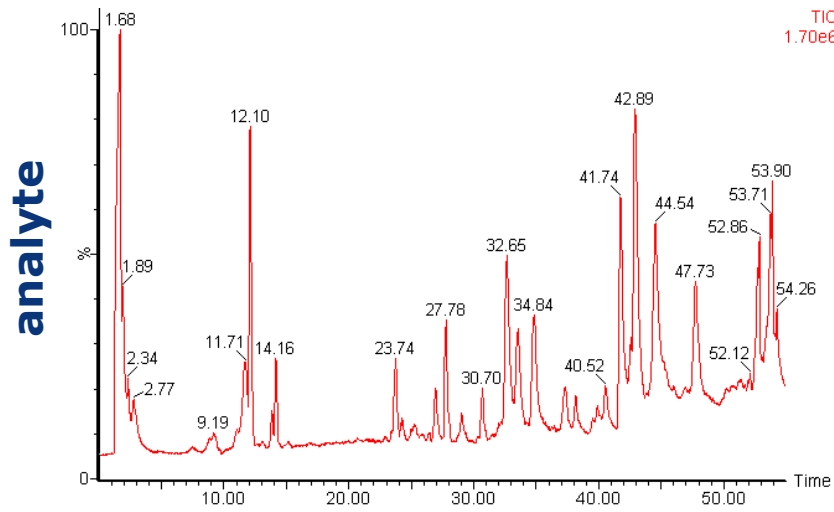
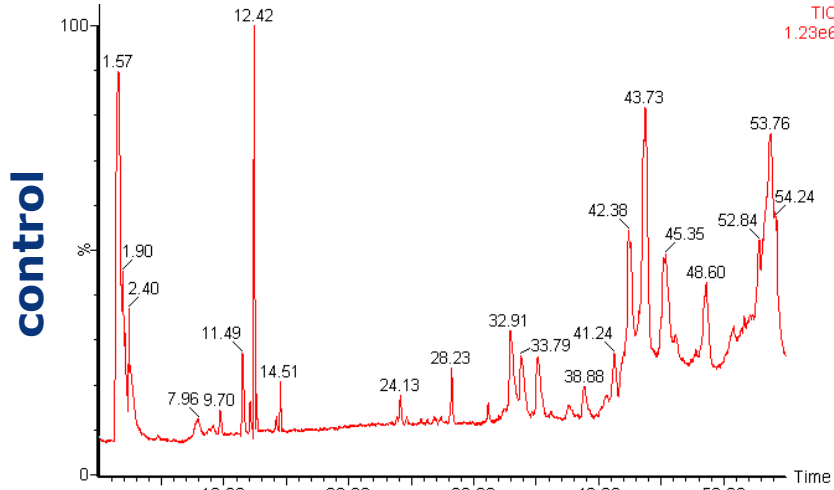
Control
vehicle dosed rat



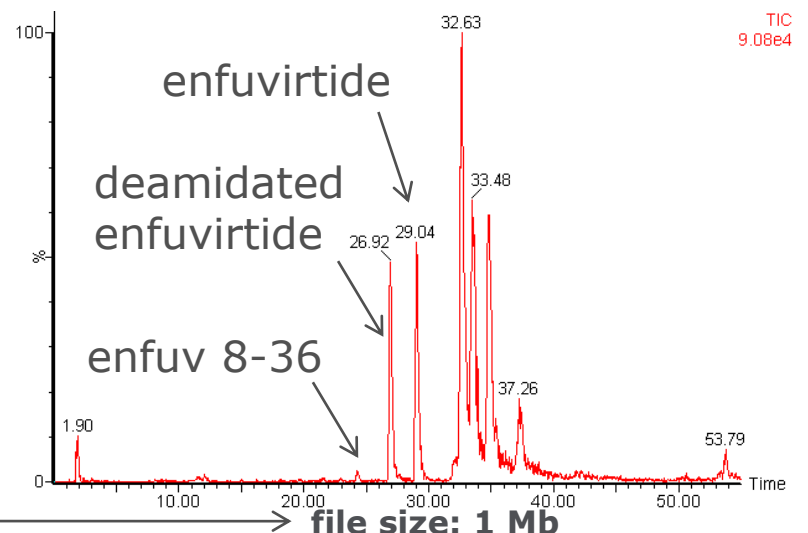
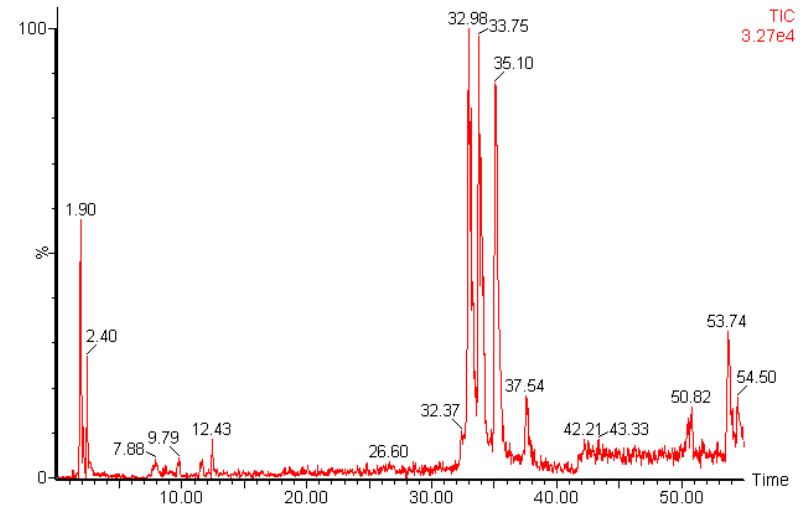
Analyte
peptide dosed rat

ion extraction based on charge state

original chromatograms



5- charge state filtered chromatogram



file size: 150 Mb

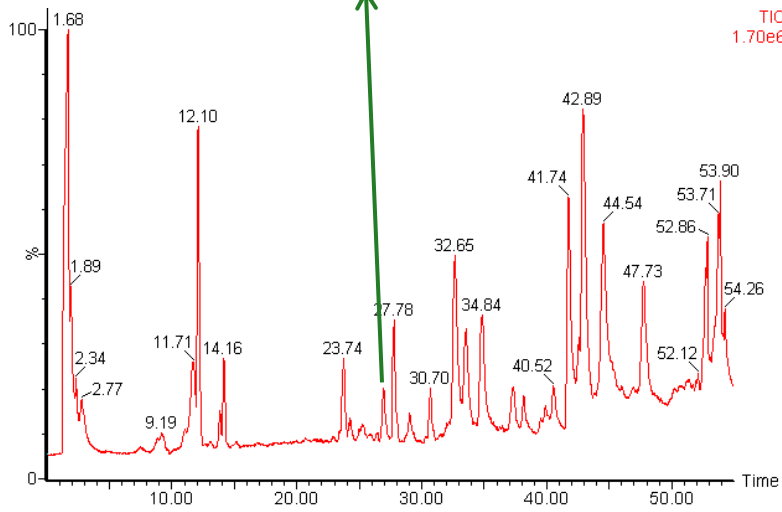
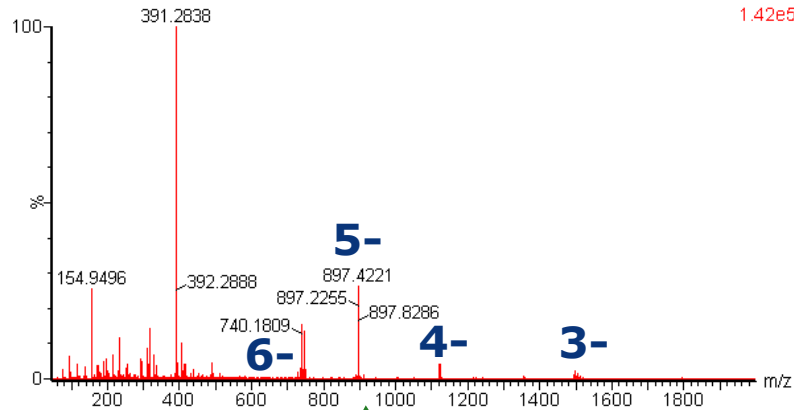
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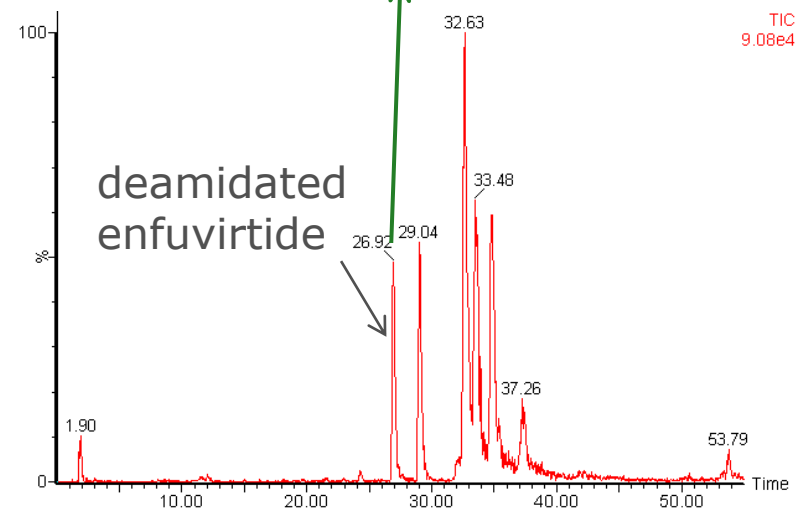
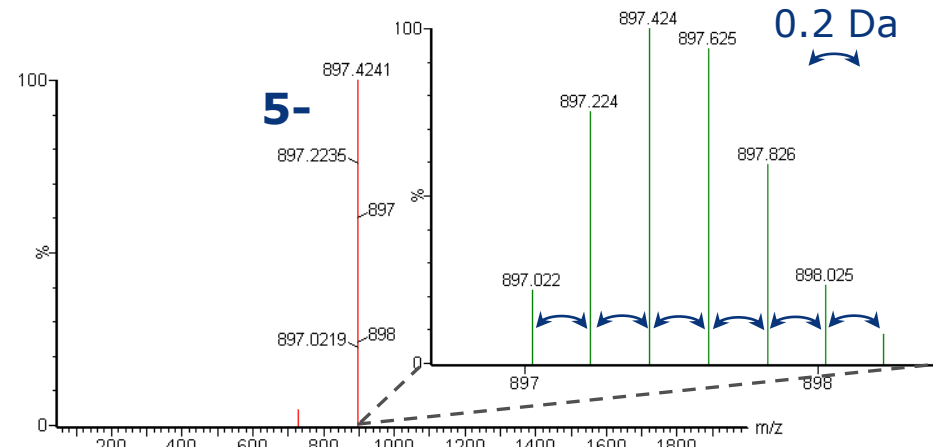
PHARMACEUTICAL COMPANIES
OF *Johnson & Johnson*

ion extraction based on charge state

original chromatograms

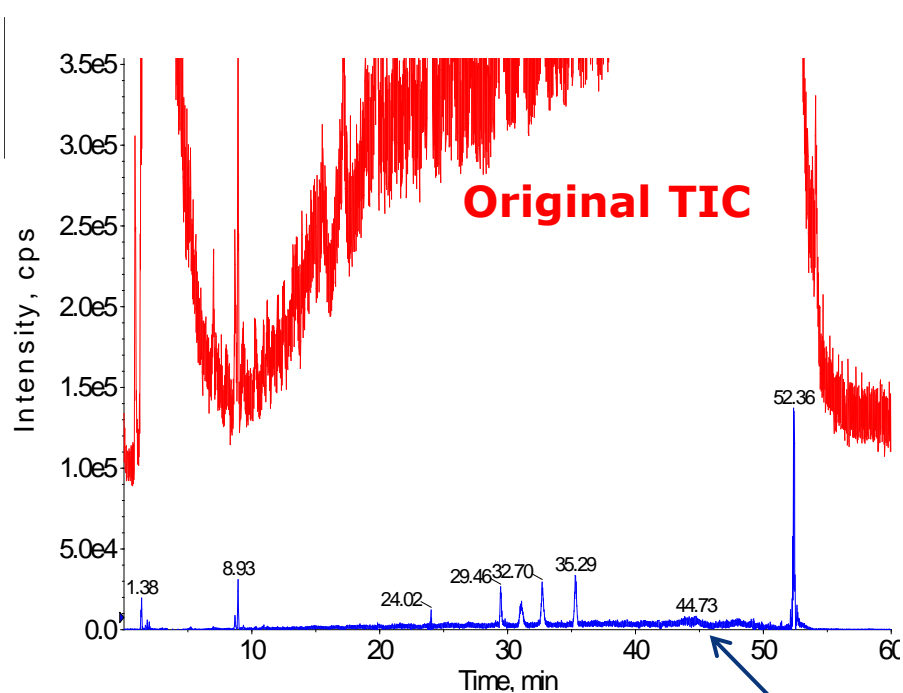


5- charge state filtered chromatogram

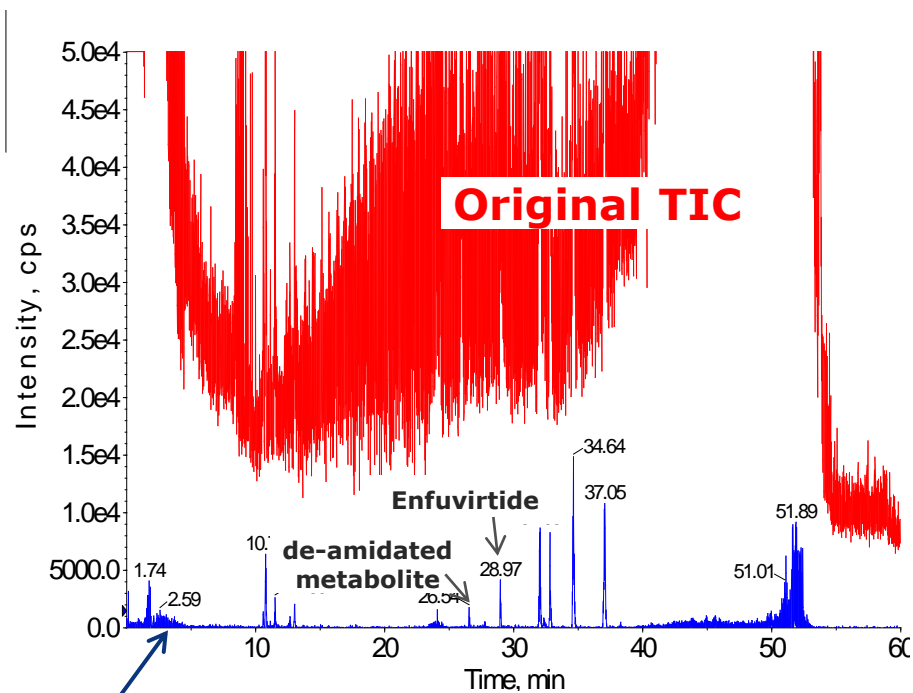


ion extraction based on charge state

control



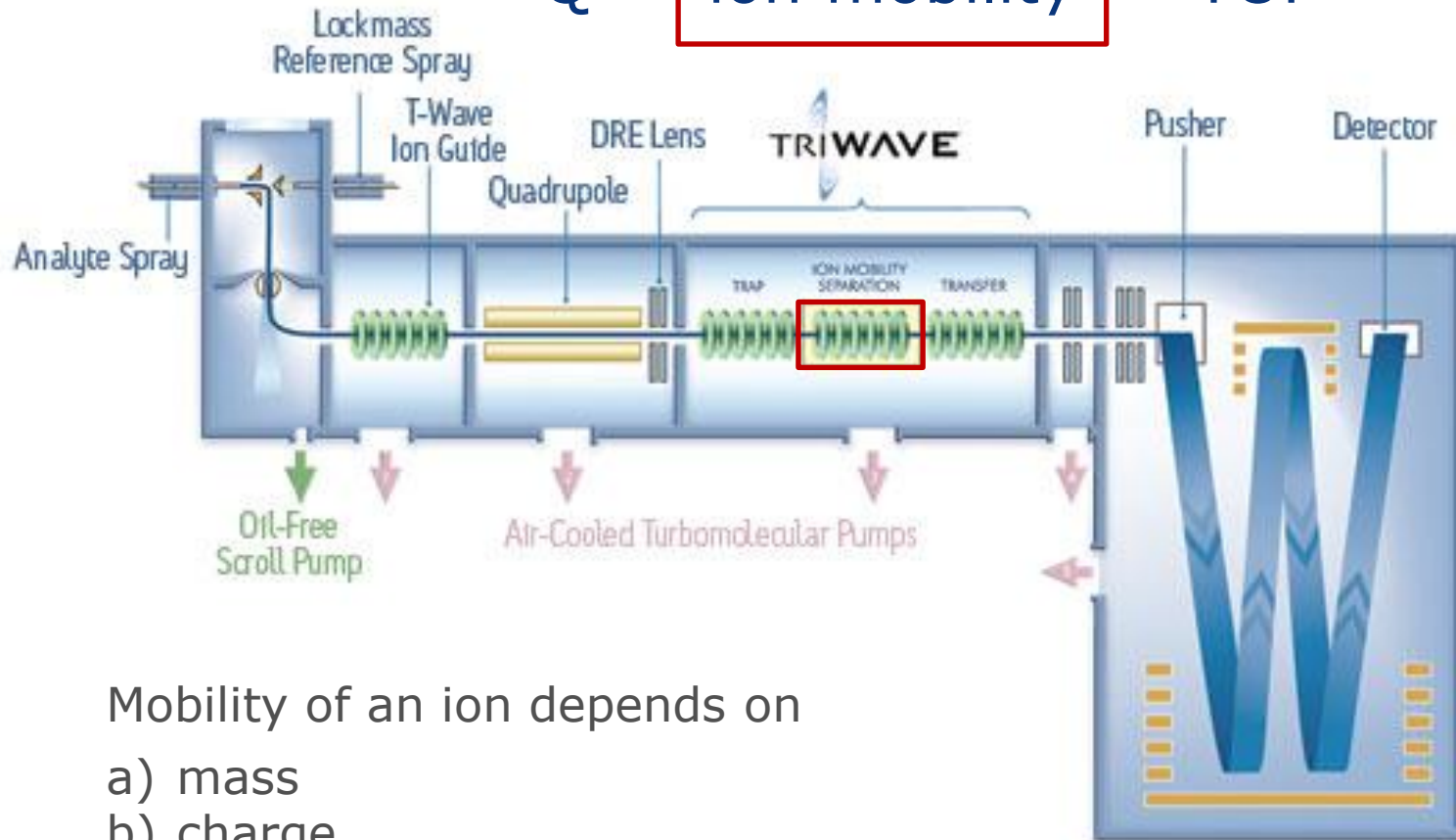
analyte



**Combined 2-, 3-, 4-, 5- and 6- charge states chromatogram
by data filtration on 0.2 + 0.33 + 0.5 mass difference**

ion extraction based on ion mobility

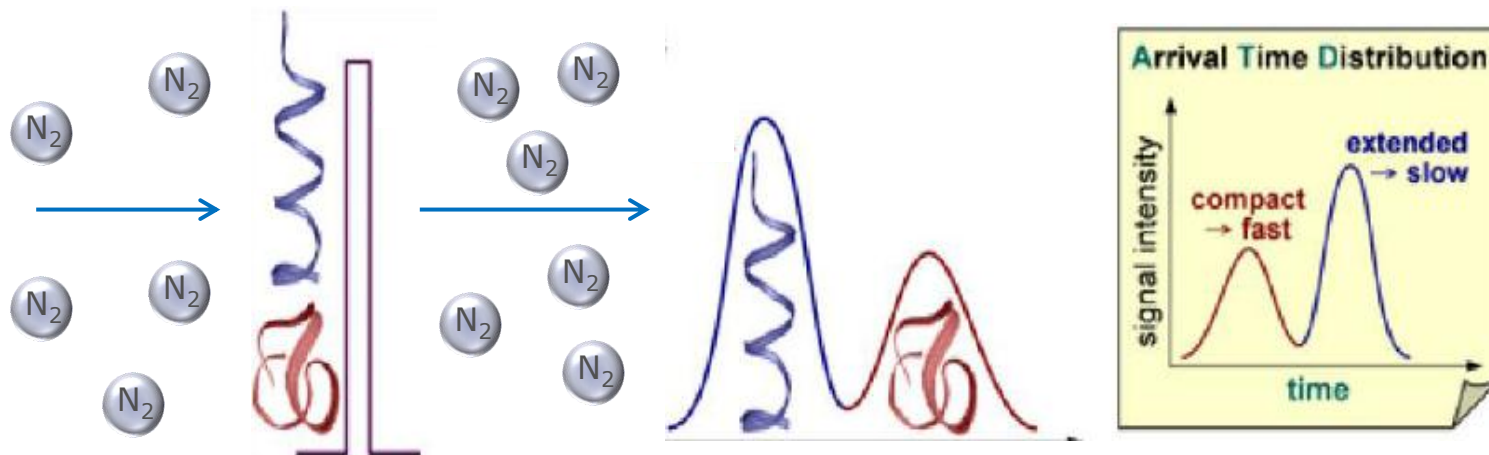
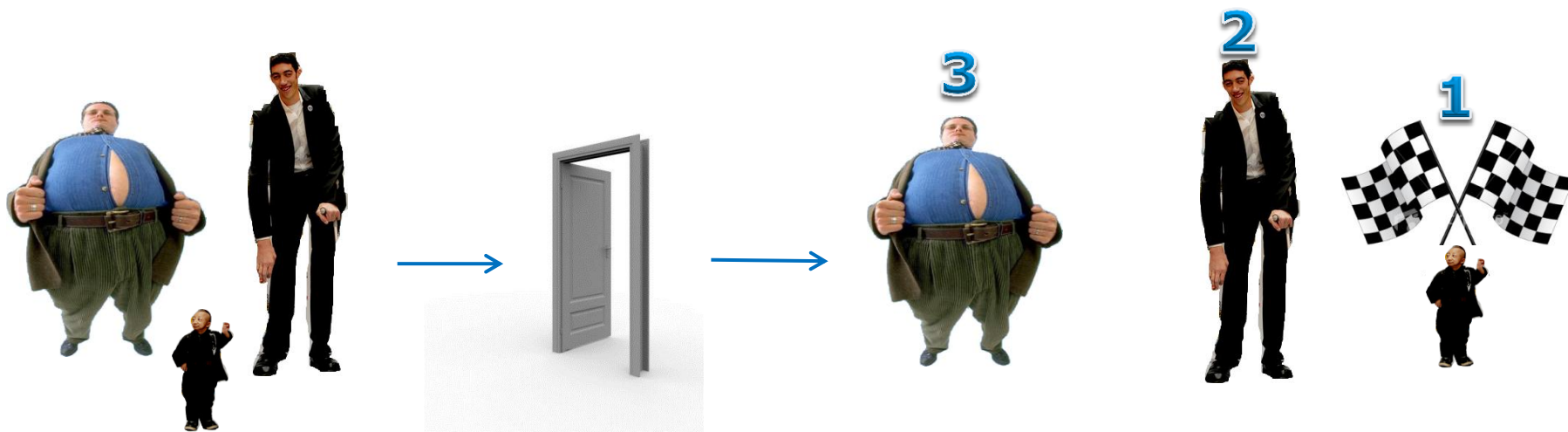
Q - ion mobility - TOF



Mobility of an ion depends on

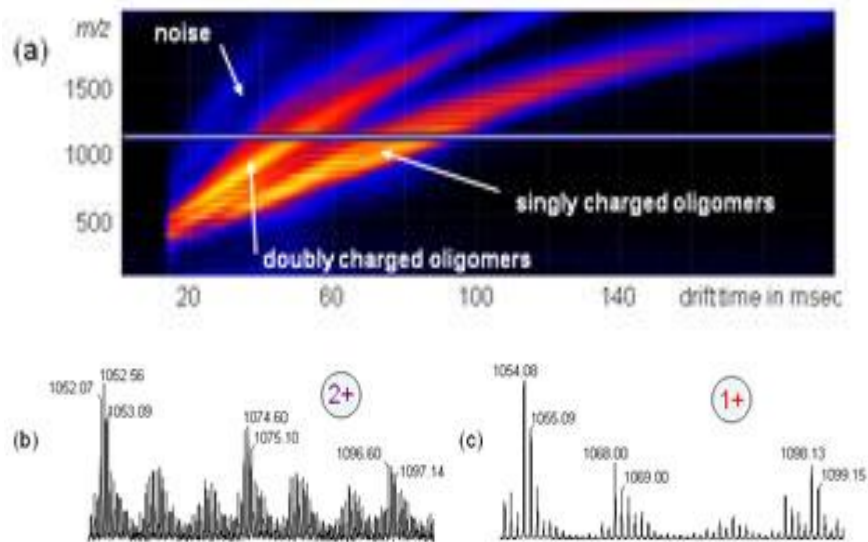
- a) mass
- b) charge
- c) cross-sectional area (shape)

ion extraction based on ion mobility



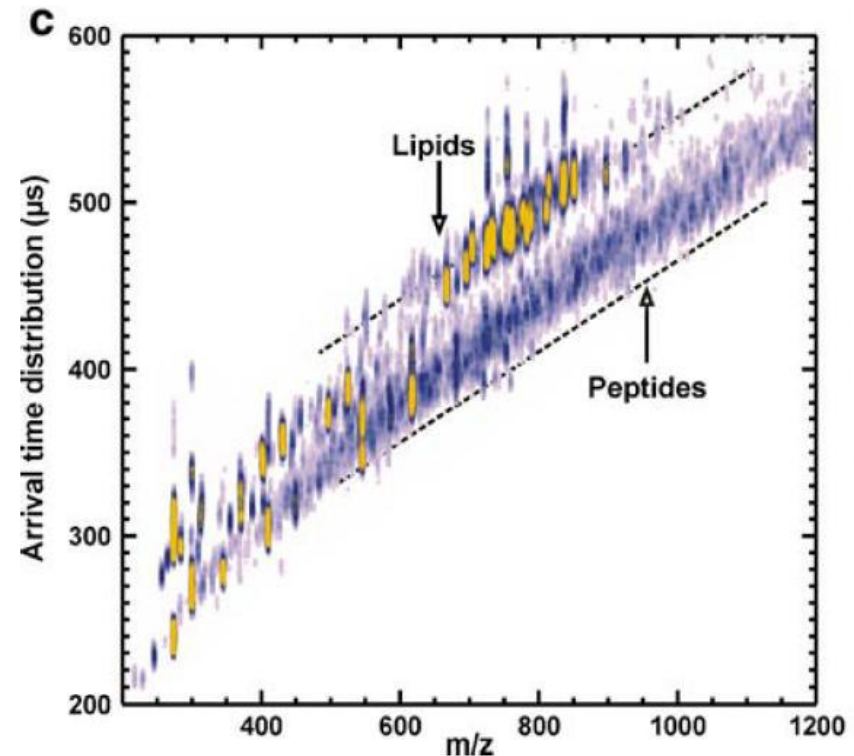
*The Bowers Group Website [Ion Mobility Theory]:
http://bowers.chem.ucsb.edu/theory_analysis/ion-mobility/index.shtml*

ion extraction based on ion mobility



ESI-IMS-MS of a pegylated sorbitan trioleate

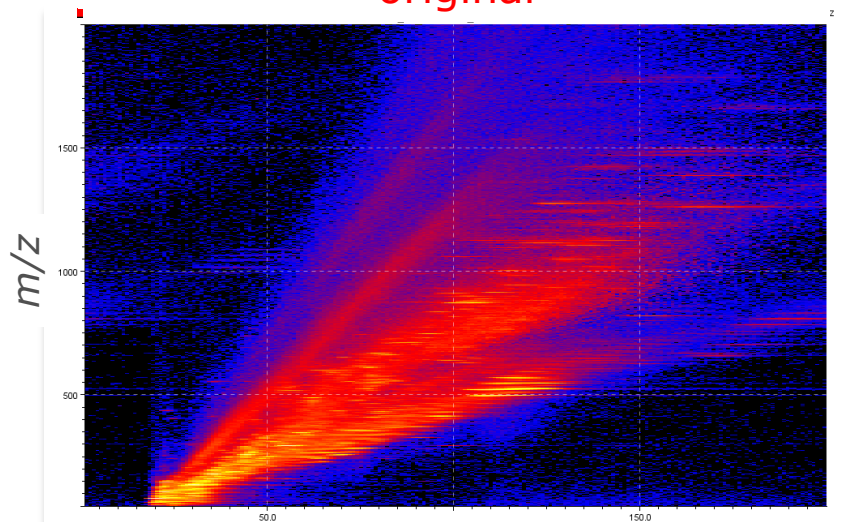
www.uakron.edu



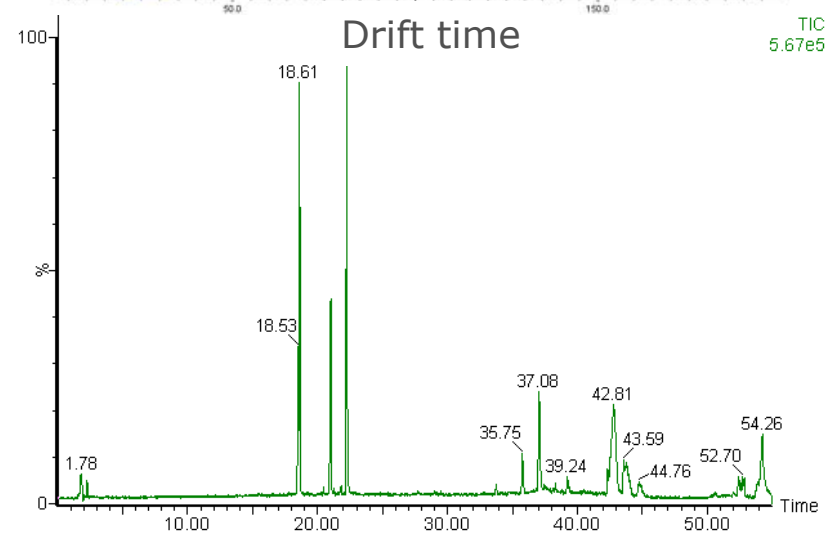
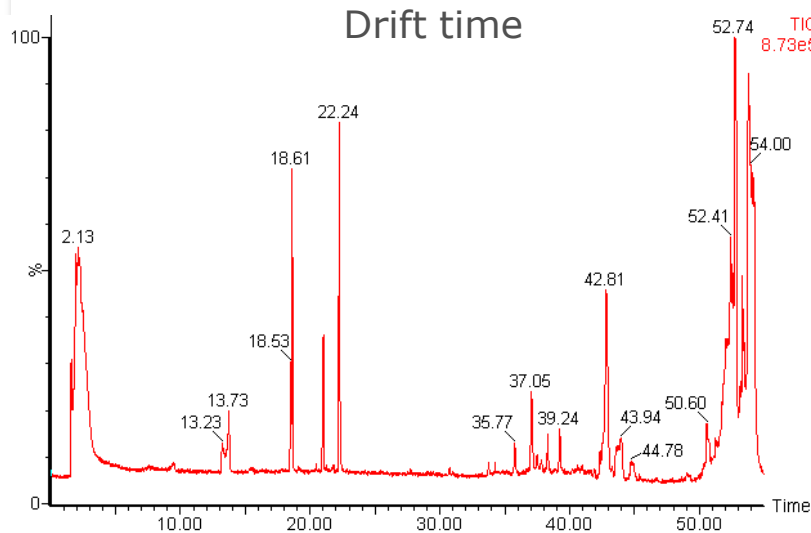
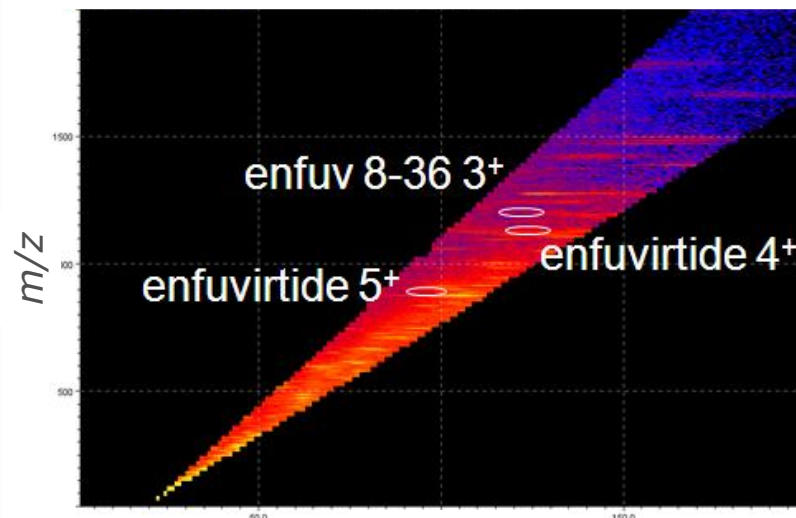
John A. McLean et al., J. Mass Spectrom. 2007; 42: 1099.

ion extraction based on ion mobility

original

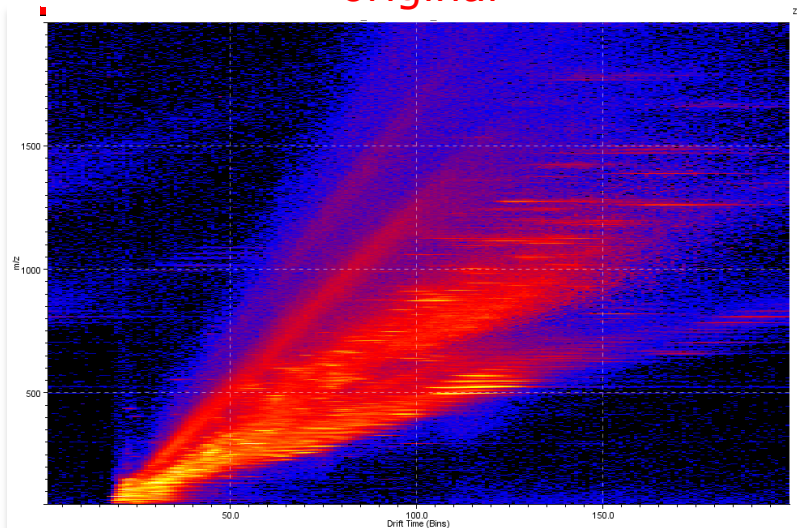


Ion mobility filtered

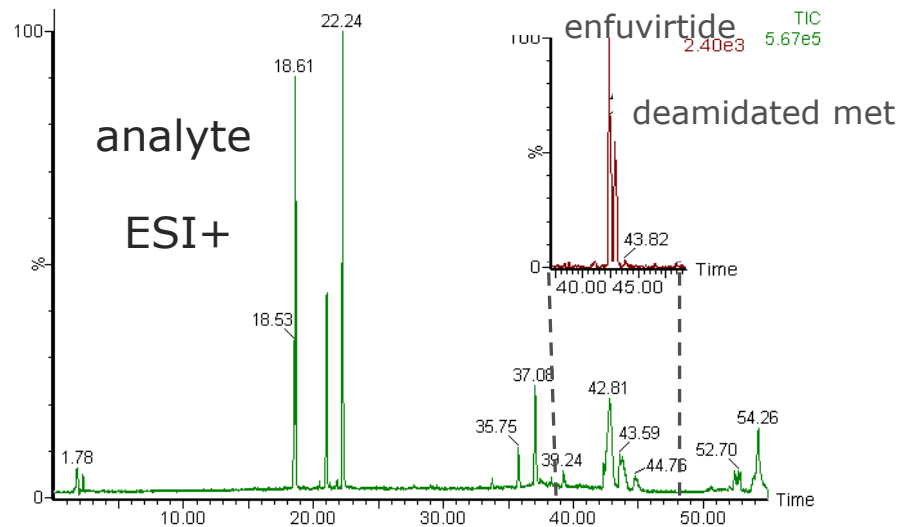
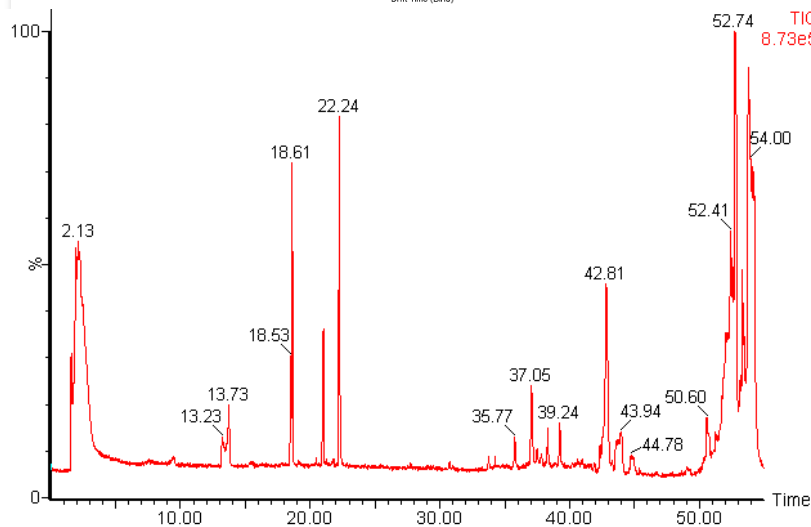
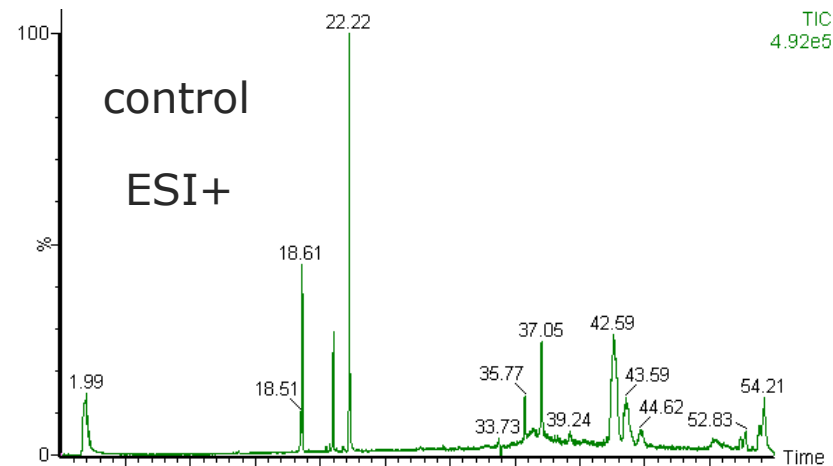


ion extraction based on ion mobility

original



Ion mobility filtered

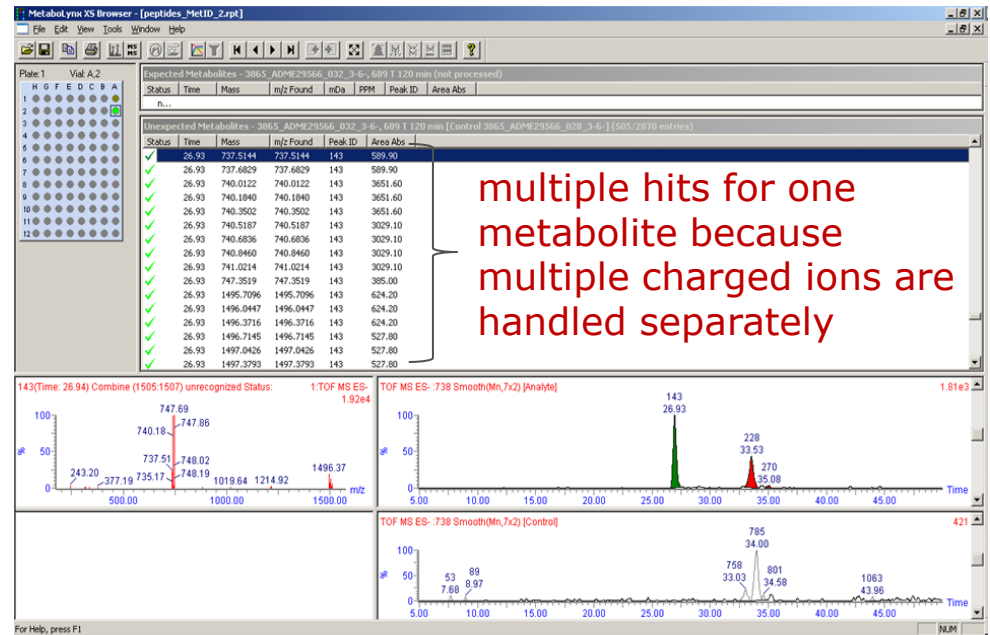


Small molecule software tools

- + Based on comparison control (vehicle dosed) and analyte sample
→ unbiased approach: unexpected metabolites detected
- + Use of charge state stripped files reduces processing time and allows the use of lower thresholds

e.g., Metabolynx

- can't handle multiple charged data
- no peptide identification



Large molecule software tools: biopharmalynx

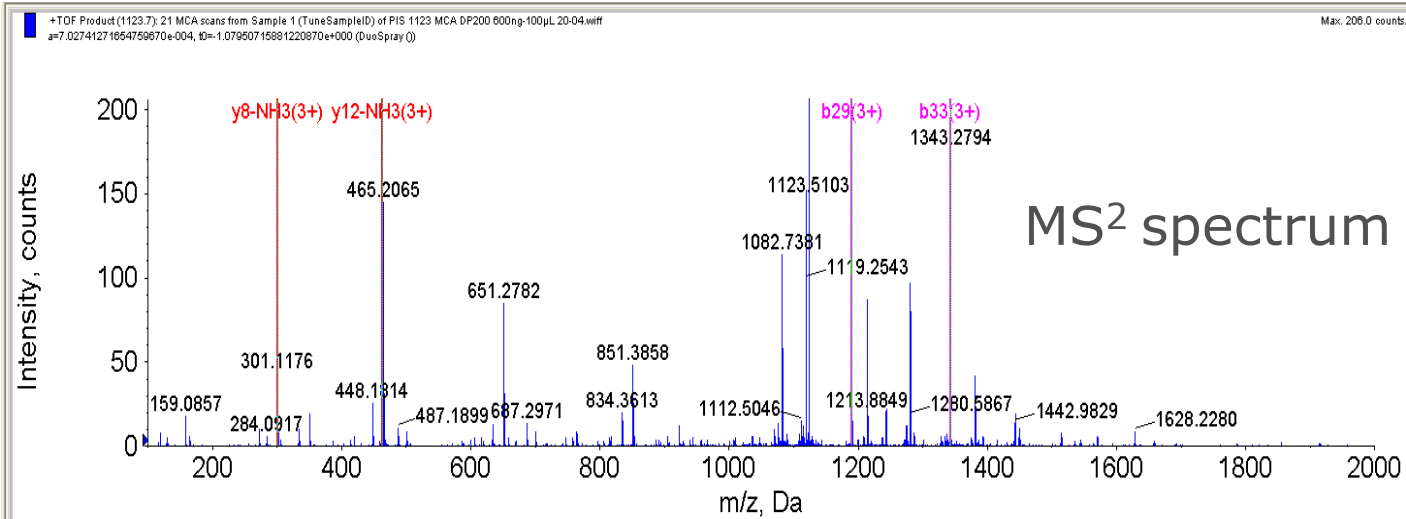
- + Based on comparison control (vehicle dosed) and analyte sample → unbiased approach: unexpected metabolites detected
- + Use of ion mobility filtered files reduces processing time and allows the use of lower thresholds
- + Identification of peptide cleavage products (increased confidence with MS^E and IMS)

- positive ion mode only
- many false positives

The screenshot displays the Biopharmalynx software interface, showing a table of peptide identifications. The table has the following columns: Protein, Peptide, Fragments, Start, End, Analyte m/z, Modifiers, Analyte Mass (Da), Calculated Pept..., Analyte RT..., and Analyte Intensi... The table lists 33 rows of data, with the 4th row highlighted in blue. The 4th row is: 4 enfuvt... YTSLSHSLIEESQSQEQNEQELLELDKQWASLWVWF 1-F1-36* 0 35 1123.5522 Acetyl N-TERM(1),Am... 3445.702 3445.722 33.2 2000.0. The interface also includes a 'Display Options' panel on the left and a 'Color Key' section below it.

Protein	Peptide	Fragmen...	Start	End	Analyte m/z	Modifiers	Analyte Mass (Da)	Calculated Pept...	Analyte RT...	*1 Analyte Intensi...
1 enfuvt...	YTSLSHSLIEESQSQEQNEQELLELDKQWASLWVWF	1-F1-36*	1	36	1123.3058	Acetyl N-TERM(1),Am...	4489.1914	4489.1865	42.4	39677.0
2 enfuvt...	YTSLSHSLIEESQSQEQNEQELLELDKQWASLWVWF	1-F1-36*	1	36	1123.5513	Acetyl N-TERM(1)	4490.1733	4490.1704	42.9	22060.0
3 enfuvt...	YTSLSHSLIEESQSQEQNEQELLELDKQWASLWVWF	1-F1-36*	1	36	1123.5521	Acetyl N-TERM(1)	4490.1768	4490.1704	42.5	20733.0
4 enfuvt...	YTSLSHSLIEESQSQEQNEQELLELDKQWASLWVWF	1-F1-36*	0	35	1123.5522	Acetyl N-TERM(1),Am...	3445.702	3445.722	33.2	2000.0
5 enfuvt...	SLIEESQSQEQNEQELLELDKQWASLWVWF	1-F7-34	7	34	1134.5582		3400.5509	3400.6423	43.5	1062.0
6 enfuvt...	SLIEESQSQEQNEQELLELDKQWASLWVWF	1-F8-28	8	28	1279.1324		2556.2490	2556.2554	42.6	1023.0
7 enfuvt...	SLHSLIEESQSQEQNEQELLELDKQWASLWVWF	1-F3-15	3	15	749.3824		1496.7490	1496.7471	18.6	967.0
8 enfuvt...	LDKQWASLWVWF	1-F26-34	26	34	566.7946		1131.5732	1131.5713	17.8	838.0
9 enfuvt...	SLHSLIEESQSQEQNEQELLELDKQWASLWVWF	1-F3-17	3	17	877.9353		1753.8547	1753.8483	36.5	828.0
10 enfuvt...	ELDKQWASLWVWF	1-F25-31	25	31	948.4197		847.4118	847.4076	20.5	794.0
11 enfuvt...	YTSLSHSLIEESQSQEQNEQELLELDKQWASLWVWF	1-F1-36*	1	36	890.4397	Amidation C-TERM(1)	4447.1507	4447.1763	43.1	733.0
12 enfuvt...	ESQSQEQNEQELLELDKQWASLWVWF	1-F11-28	11	28	1101.5306		2201.0454	2201.0447	44.8	707.0
13 enfuvt...	SLHSLIEESQSQEQNEQELLELDKQWASLWVWF	1-F6-15	6	15	592.7834		1183.5509	1183.5469	37.0	665.0
14 enfuvt...	SLHSLIEESQSQEQNEQELLELDKQWASLWVWF	1-F5-21	5	21	1027.4954		2052.9749	2052.9712	37.3	654.0
15 enfuvt...	SLHSLIEESQSQEQNEQELLELDKQWASLWVWF	1-F4-24	4	24	841.4265		2521.2556	2521.2659	22.5	651.0
16 enfuvt...	YTSLSHSLIEESQSQEQNEQELLELDKQWASLWVWF	1-F1-15	1	15	881.4329		1760.8500	1760.8500	18.4	639.0
17 enfuvt...	SLHSLIEESQSQEQNEQELLELDKQWASLWVWF	1-F4-23	4	23	1205.0966		2408.1814	2408.1819	43.6	633.0
18 enfuvt...	SLHSLIEESQSQEQNEQELLELDKQWASLWVWF	1-F14-26	14	26	807.9031		1613.7903	1613.7897	26.5	631.0
19 enfuvt...	SLHSLIEESQSQEQNEQELLELDKQWASLWVWF	1-F4-33	4	33	609.3105		3649.8154	3649.8264	25.1	606.0
20 enfuvt...	SLHSLIEESQSQEQNEQELLELDKQWASLWVWF	1-F12-27	12	27	648.9738		1943.8976	1943.9072	24.1	604.0
21 enfuvt...	ELDKQWASLWVWF	1-F25-36	25	36	797.8861		1593.7563	1593.7616	25.1	584.0
22 enfuvt...	SLHSLIEESQSQEQNEQELLELDKQWASLWVWF	1-F3-20	3	20	709.3486		2125.0220	2125.0288	44.9	580.0
23 enfuvt...	SLHSLIEESQSQEQNEQELLELDKQWASLWVWF	1-F3-14	3	14	685.3552		1368.6945	1368.6885	37.2	569.0
24 enfuvt...	KWASLWVWF	1-F28-33	28	33	790.4247		789.4168	789.4174	29.6	565.0
25 enfuvt...	YTSLSHSLIEESQSQEQNEQELLELDKQWASLWVWF	1-F2-21	2	21	785.7162		2354.1350	2354.1350	22.3	548.0
26 enfuvt...	QEQNEQELLELDKQWASLWVWF	1-F15-27	15	27	808.3911		1614.7662	1614.7737	29.0	520.0
27 enfuvt...	QEQNEQELLELDKQWASLWVWF	1-F14-31	14	31	551.2745		2201.0664	2201.0601	35.4	519.0
28 enfuvt...	QEQNEQELLELDKQWASLWVWF	1-F13-23	13	23	694.3252		1386.6345	1386.6376	26.6	506.0
29 enfuvt...	SLHSLIEESQSQEQNEQELLELDKQWASLWVWF	1-F3-29	3	29	1094.2114		3279.5104	3279.6260	39.1	502.0
30 enfuvt...	QEQNEQELLELDKQWASLWVWF	1-F19-36*	19	36	580.7936	Amidation C-TERM(1)	2319.1426	2319.1323	28.7	496.0
31 enfuvt...	YTSLSHSLIEESQSQEQNEQELLELDKQWASLWVWF	1-F1-36*	1	36	899.0441	Acetyl N-TERM(1)	4490.1807	4490.1704	40.3	479.0
32 enfuvt...	YTSLSHSLIEESQSQEQNEQELLELDKQWASLWVWF	1-F2-35	2	35	690.6810		4138.0381	4138.0283	42.9	478.0
33 enfuvt...	ELDKQWASLWVWF	1-F17-29...	17	29	552.6131		1654.8154	1654.8202	20.5	477.0

Structure identification



Controls: Reset to Default... Set as Default Sequence Tag

Sequences and Tags: +TOF Product (1123.7): 21 MCA scans from Sample 1 (TuneSampleID) of PIS 1123 MCA DP200 600ng-100µL 20-04.wiff

Fragments [Precursor MW = 4490.0102, Charge = 4] 30 of 249 matched - 12%

X Ace YTSLSLHSEESQNDQEKNEDELLELDKWASLWNW(-41.0265) Ami 188.9185 Ion charge: 3

	Residue	Mass	Immonium	a	a-NH3	b	b-NH3	y	y-NH3
14	N, Asn	114.0429	87.0553	543.9418	538.2663	553.2734	547.5979	920.4523	914.7768
15	Q, Gln	128.0586	101.0709	586.6280	580.9525	595.9596	590.2841	882.4380	876.7625
16	Q, Gln	128.0586	101.0709	629.3142	623.6386	638.6458	632.9703	839.7518	834.0763
17	E, Glu	129.0426	102.0550	672.3284	666.6528	681.6600	675.9845	797.0656	791.3901
18	K, Lys	128.0950	101.1073	715.0267	709.3512	724.3583	718.6828	754.0514	748.3759
19	N, Asn	114.0429	87.0553	753.0410	747.3655	762.3726	756.6971	711.3531	705.6776
20	E, Glu	129.0426	102.0550	796.0552	790.3797	805.3868	799.7113	673.3388	667.6633
21	Q, Gln	128.0586	101.0709	838.7414	833.0659	848.0730	842.3975	630.3246	624.6491
22	E, Glu	129.0426	102.0550	881.7556	876.0801	891.0872	885.4117	587.6384	581.9629
23	L, Leu	113.0841	86.0964	919.4503	913.7748	928.7819	923.1064	544.6242	538.9487
24	L, Leu	113.0841	86.0964	957.1450	951.4694	966.4766	960.8011	506.9295	501.2540
25	E, Glu	129.0426	102.0550	1000.1592	994.4836	1009.4908	1003.8153	469.2348	463.5593
26	L, Leu	113.0841	86.0964	1037.8538	1032.1783	1047.1855	1041.5100	426.2206	420.5451
27	D, Asp	115.0269	88.0393	1076.1962	1070.5206	1085.5278	1079.8523	388.5259	382.8504
28	K, Lys	128.0950	101.1073	1118.8945	1113.2190	1128.2261	1122.5506	350.1836	344.5081
29	W, Trp	186.0793	159.0917	1180.9209	1175.2454	1190.2526	1184.5770	307.4853	301.8098
30	A, Ala	71.0371	44.0495	1204.6000	1198.9244	1213.9316	1208.2561	245.4589	239.7833
31	S, Ser	87.0320	60.0444	1233.6106	1227.9351	1242.9423	1237.2667	221.7798	216.1043

Bioanalyst

Summary

- Described techniques can be applied for peptides with **MW 1000 – 10000**
- Vehicle dosed animal samples to be used as **control samples** are crucial for success
- Both **isotope filtering** and **ion mobility** can be applied to significantly reduce endogenous background ions
- A **combination** of the capabilities of existing **small molecule and large molecule software tools** would make an ideal software package

Acknowledgements

Janssen R&D, Belgium

Analytical Sciences DMPK

Lieve Dillen

Willy Cools

Marc Bockx

Liesbeth Vereyken

ADME-TOX

Russell Mortishire-Smith

Bart Stoops

Ron Gilissen

Nigel Austin

Koen Wuyts

Regulated bioanalysis

Ronald de Vries

Waters

Alex Muck, Jan Claereboudt

AB Sciex

Hesham Ghobarah