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Technological Advancements in Screening Platforms for Metabolite Data Access and Evaluation

Russell Mortishire-Smith, PhD

Waters Corporation

Waters 60 DIAMOND ANNIVERSARY

Outline

- Introduction
- Metabolite Identification Challenges
- Benefits of Ion Mobility in Metabolite Identification
- Integration of Ion Mobility into DMPK using Software
- Future Opportunities

Acquiring Data is the First Step!

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The Discovery LC-MS Workflow for Metabolite Identification

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Design

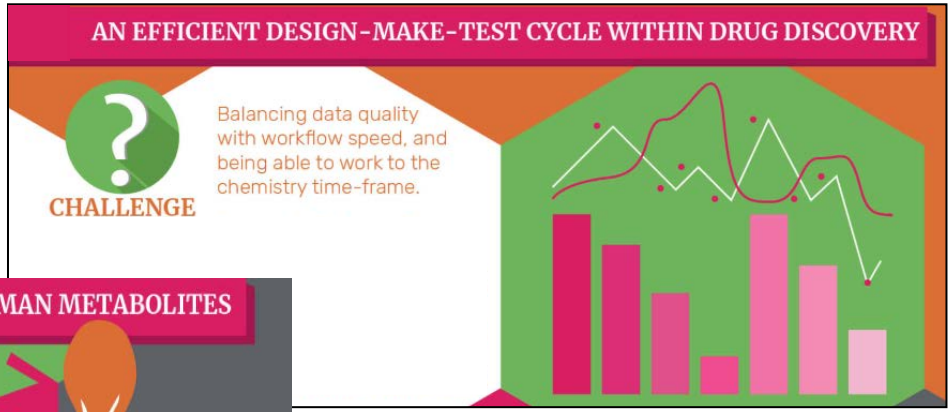
Make

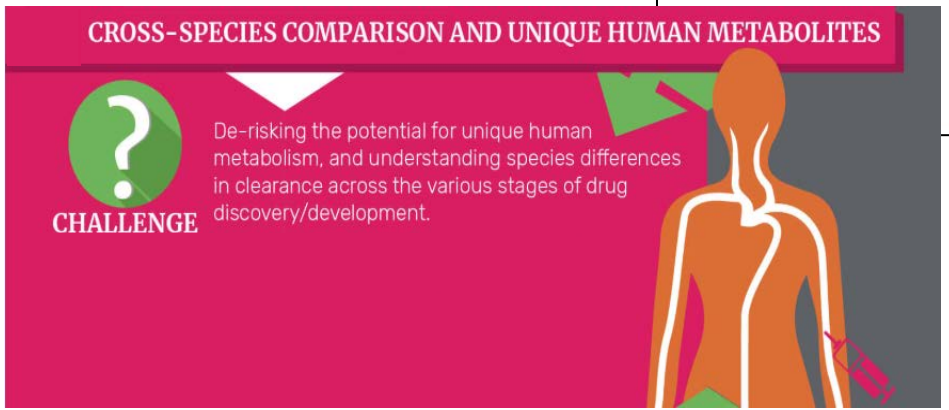
Test

Analyze

Compound	Clearance	Soft Spot?	Enzyme Interaction	Overall
A	Low	N	Low	Good candidate
B	Medium	Y	Low	Possible candidate
C	High	Y	High	Poor candidate

Metabolite Identification Challenges - Workflow





https://www.bioanalysis-zone.com/2018/07/30/in-the-zone-biotransformation_inz_biot_waters/

Metabolite Identification Challenges - Technical

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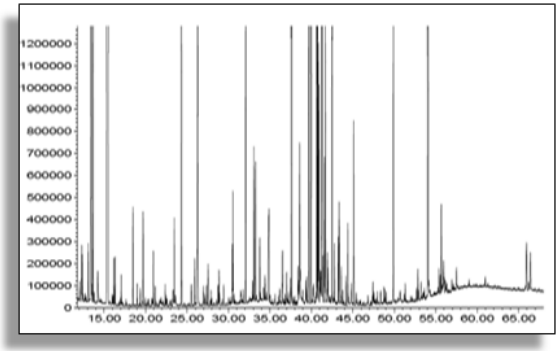
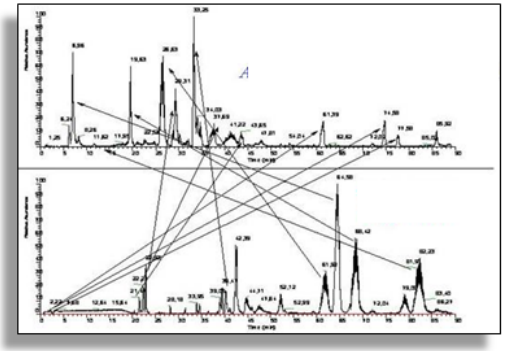
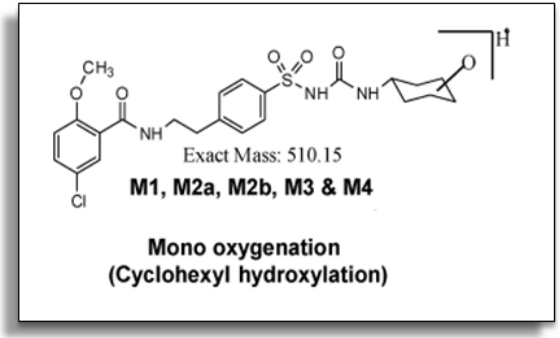


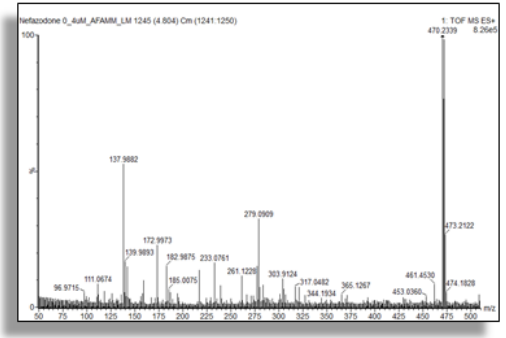
Complex biological backgrounds



Retention time shifting



Localization of sites of metabolism



Spectral Clarity

A Technological Advancement – IMS for Met ID

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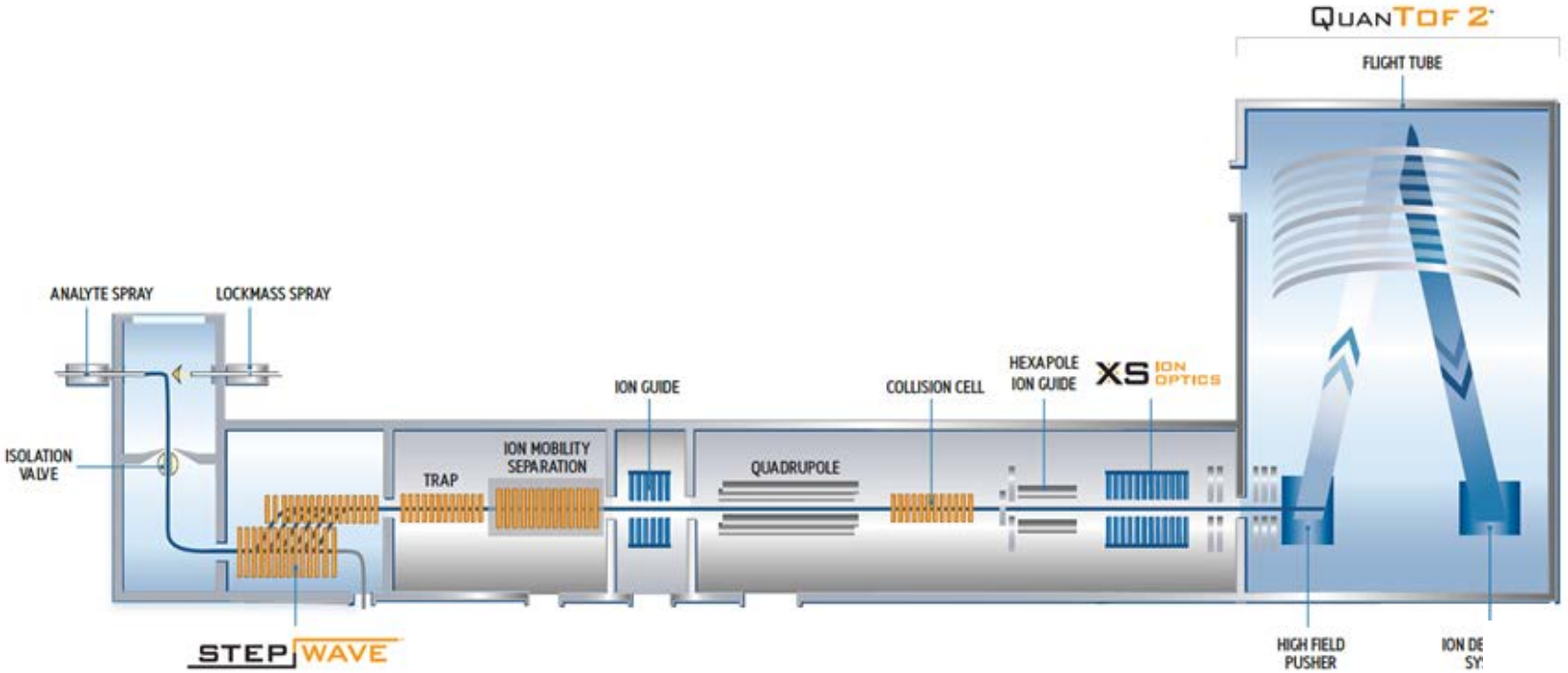
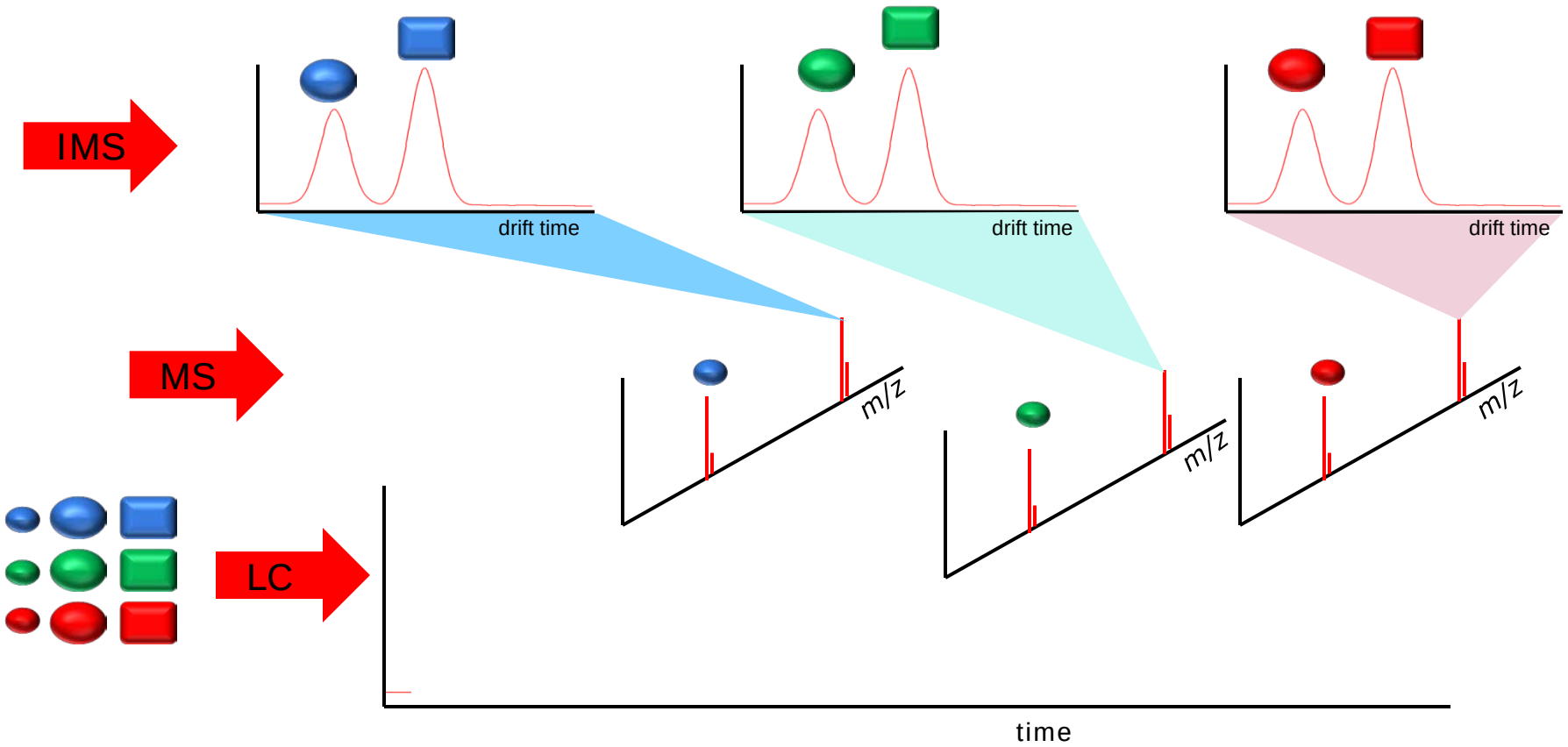
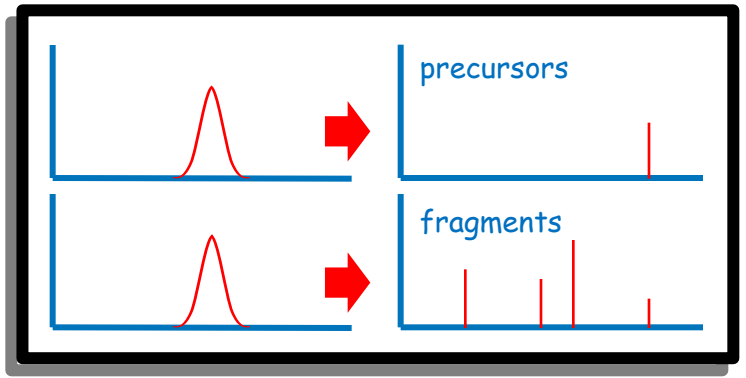


LC-IMS-MS

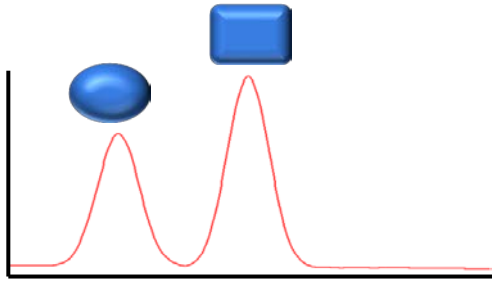


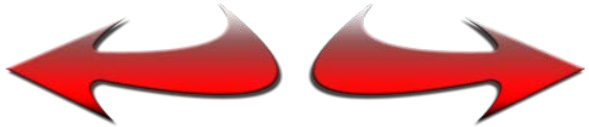
Data Independent Acquisitions

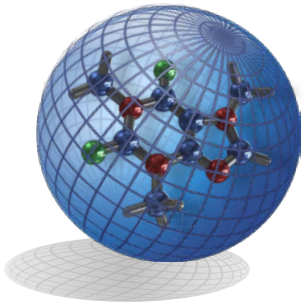


- Single sample injection
- Alternating low and high energy spectra
  - Precursor and fragment ions
  - MW information
  - Structural information
  - Confirmatory ions
- All of the data all of the time
  - No data dependent switching where information can be missed
- MS^E

IMS







Orthogonal Separation

- The ability to see more
- Resolution of isomers
- Cleaner spectra

Collision Cross Section

- IMS drift time ⇒ CCS
- Additional identification point
- Matrix independent
- System independent

Ion Mobility for Spectral Clarity in Metabolite Identification

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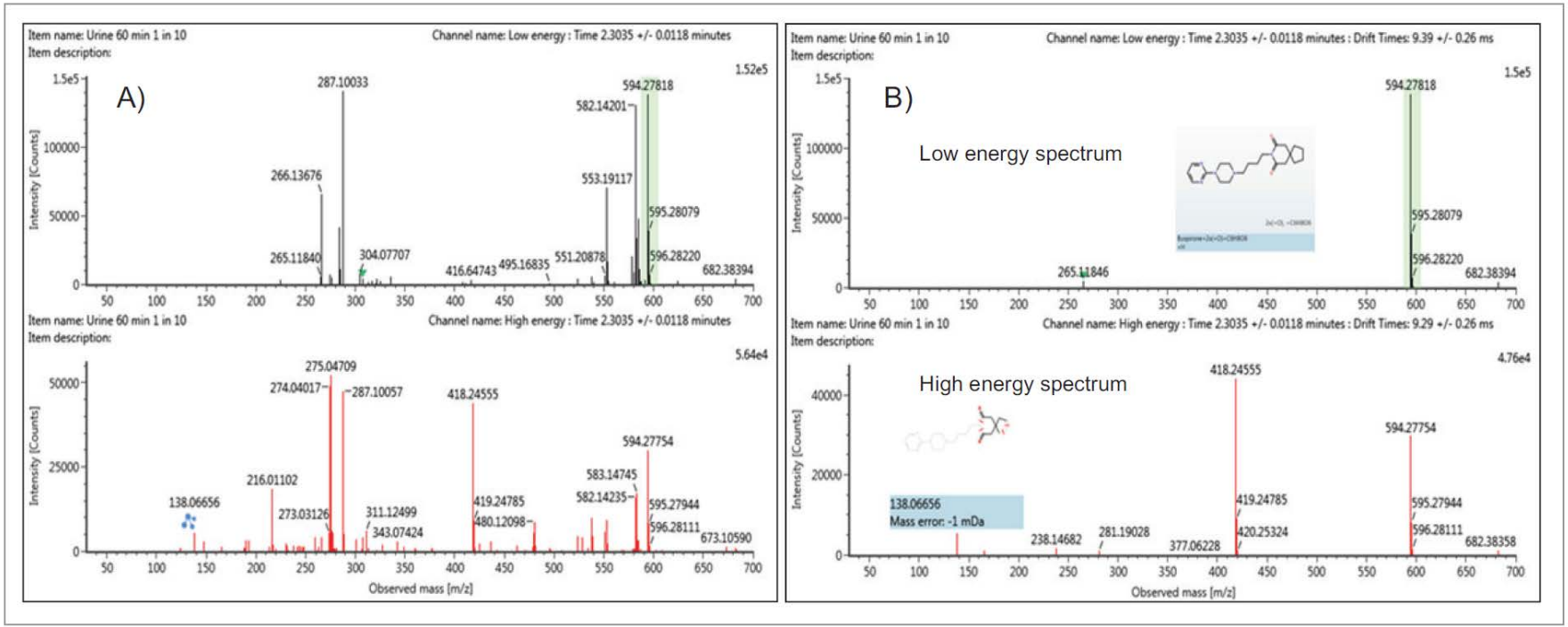

Low and high energy (HDMS^E) spectra for the dihydroxylated glucuronide metabolite of buspirone without ion mobility showing many precursors and fragment ions at the same t_R (A) and with ion mobility (B).

Kirk, Jayne, Russell Mortishire-Smith and Mark Wrona. Integrating Ion Mobility into Routine Metabolite Identification Studies using the Vion IMS QTof Mass Spectrometer. Waters Application Note 720006121EN. 2017.

CCS Values to Resolve Co-Eluting Metabolites

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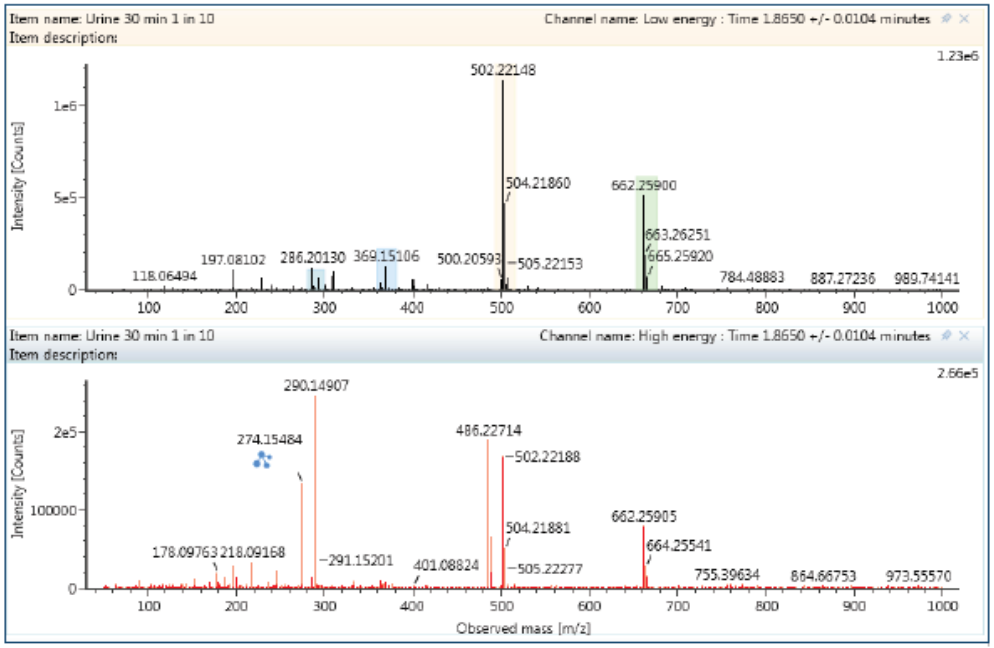
A)

Component name	Label	Formula	Observed m/z	Observed RT (min)	Observed CCS (Å²)
Nefazodone+O+C6H8O6		C31H40ClN5O9	662.2590	1.86	261.30
Nefazodone+2x(+O)		C25H32ClN5O4	502.2215	1.87	220.95

B)



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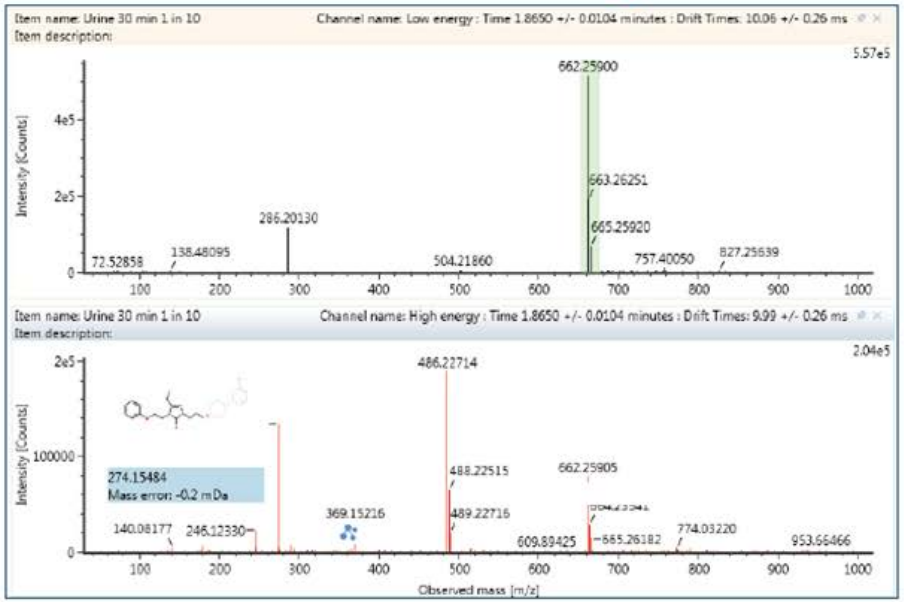
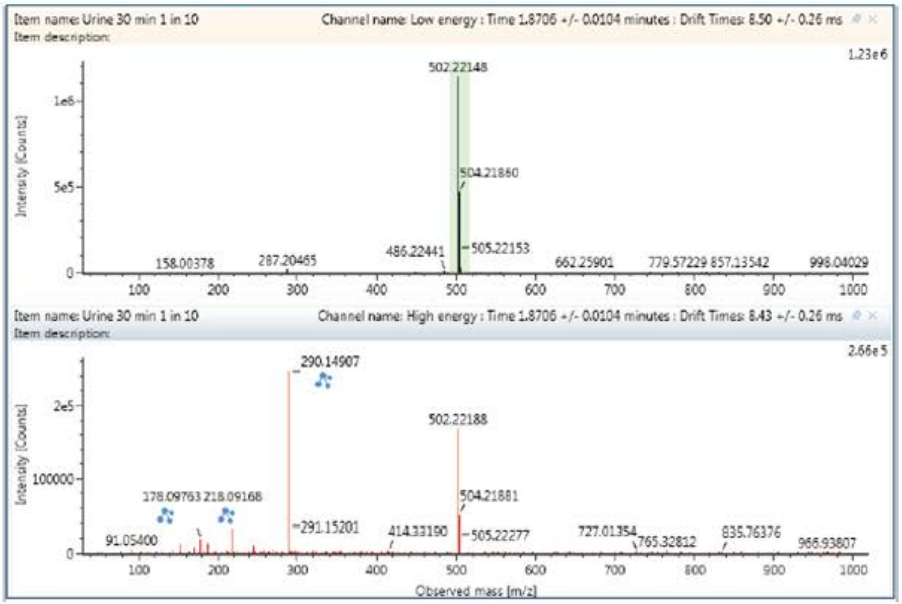
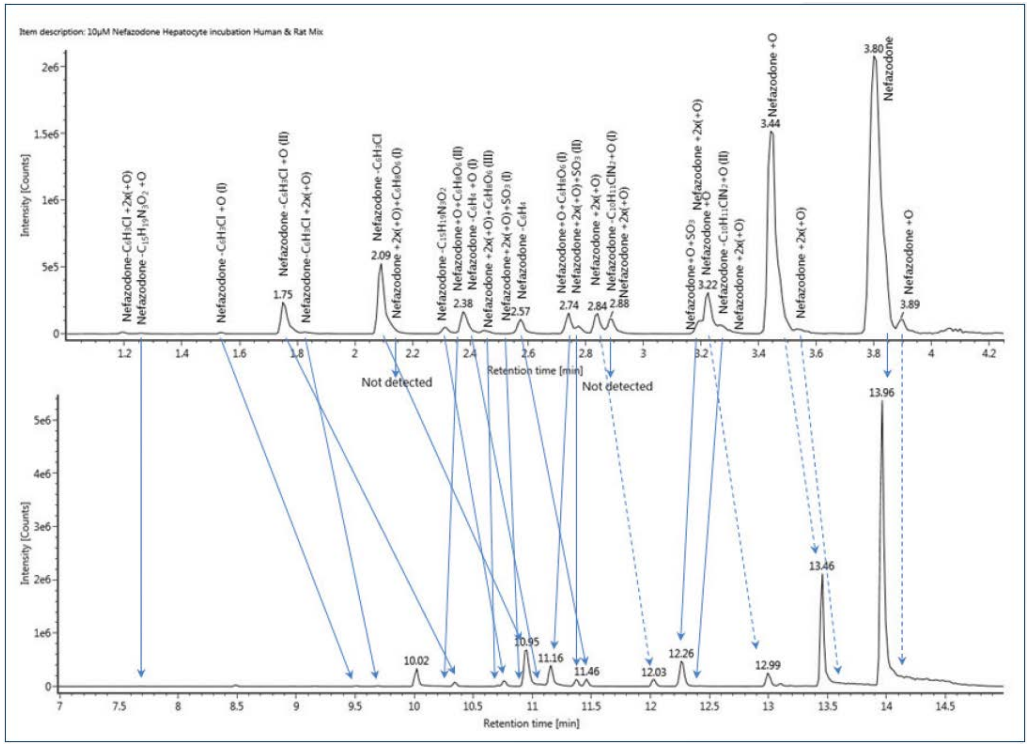


D)



Kirk, Jayne, Russell Mortishire-Smith and Mark Wrona. Integrating Ion Mobility into Routine Metabolite Identification Studies using the Vion IMS QTof Mass Spectrometer. Waters Application Note 720006121EN. 2017.

CCS Values for Condition Independent Metabolite Tracking



Corresponding Nefazodone Metabolites Matched across Method 1 and Method 2 Samples by Comparison of CCS Measurement

		Method 1 UPLC		Method 2 HPLC		
Metabolite	Theoretical $[M+H]^+$	Mean RT (min)	Mean CCS (Å²)	Mean RT (min)	Mean CCS (Å²)	Δ% CCS
Nefazodone	470.2317	3.81	210.28	13.97	210.65	0.18
Nefazodone +O+$C_6H_8O_6$ (I)	662.2587	2.75	255.40	11.17	254.88	-0.21
Nefazodone +O+$C_6H_8O_6$ (II)	662.2587	nd	nd	11.39	239.72	-
Nefazodone +2x(+O)+$C_6H_8O_6$ (I)	678.2536	2.19	252.41	nd	nd	-
Nefazodone +2x(+O)+$C_6H_8O_6$ (II)	678.2536	2.38	258.59	10.35	257.38	-0.47
Nefazodone +2x(+O)+$C_6H_8O_6$ (III)	678.2536	2.46	242.99	10.76	242.72	-0.11
Nefazodone +O+SO_3	566.1834	3.19	228.23	12.27	229.04	0.35
Nefazodone +2x(+O)+SO_3 (I)	582.1783	2.55	224.18	10.94	224.46	0.12
Nefazodone +2x(+O)+SO_3 (II)	582.1783	2.78	232.68	11.38	233.44	0.33
Nefazodone -C_6H_4	394.2004	2.58	196.10	11.47	196.13	0.02
Nefazodone -C_6H_4 +O (I)	410.1953	2.38	198.24	10.98	198.49	0.13
Nefazodone -C_6H_4 +O (II)	410.1953	nd	nd	11.21	198.77	-
Nefazodone -C_6H_3Cl	360.2394	2.10	179.46	10.95	179.82	0.20
Nefazodone -C_6H_3Cl +O (I)	376.2343	1.54	183.86	9.52	183.19	-0.36
Nefazodone -C_6H_3Cl +O (II)	376.2343	1.76	182.49	10.42	182.53	0.02
Nefazodone -C_6H_3Cl +2x(+O)	392.2292	1.83	185.12	9.70	185.00	-0.07
Nefazodone -$C_{10}H_{11}ClN_2$+O (I)	292.1655	2.89	199.40	nd	nd	-
Nefazodone -$C_{10}H_{11}ClN_2$+O (II)	292.1655	3.28	164.07	12.28	164.09	0.01
Nefazodone -$C_{15}H_{19}N_3O_2$	197.0840	2.32	143.13	10.78	142.62	-0.36
Nefazodone -$C_{15}H_{19}N_3O_2$ +O	213.0789	1.27	145.05	7.69	144.58	-0.33

Holdsworth, Catherine, Richard Clayton, Helen Robinson, Callum Lord-Mears and John Kendrick. Utilisation of Ion Mobility Enabled Collisional Cross Section Measurements for the Comparison of Metabolites across Differing Chromatographic Methods. Poster presented at the Joint DMDG/GMP Open Meeting 2016.

Using Software to Take Advantage of IMS-LC-MS in DMPK

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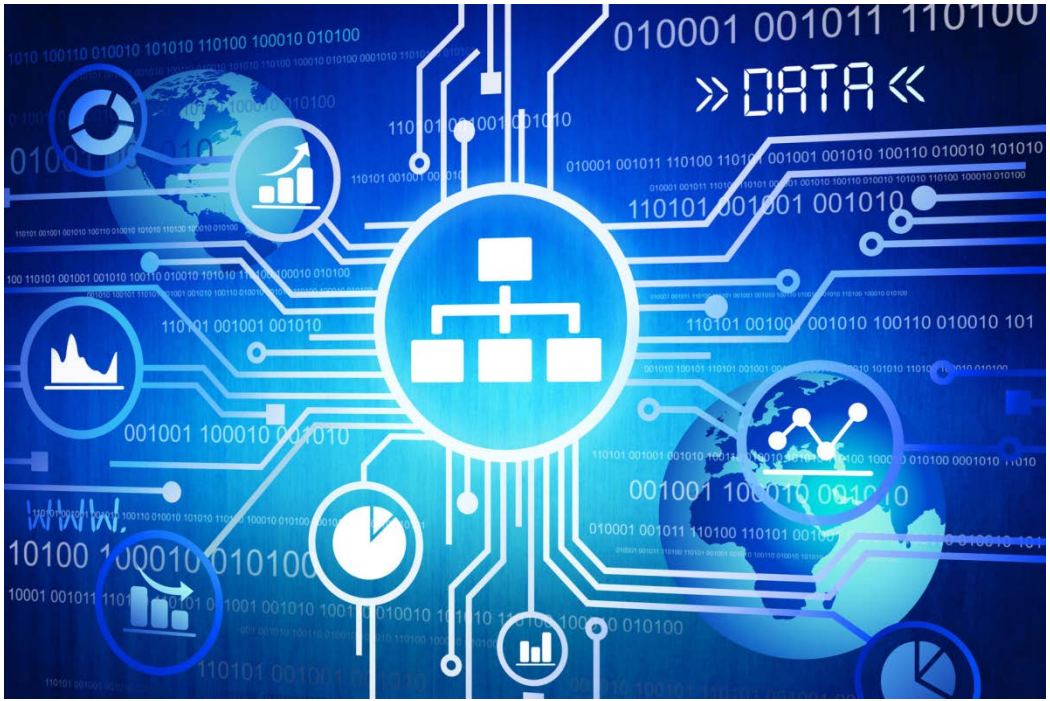

UNIFI Met ID Application for Small Molecules

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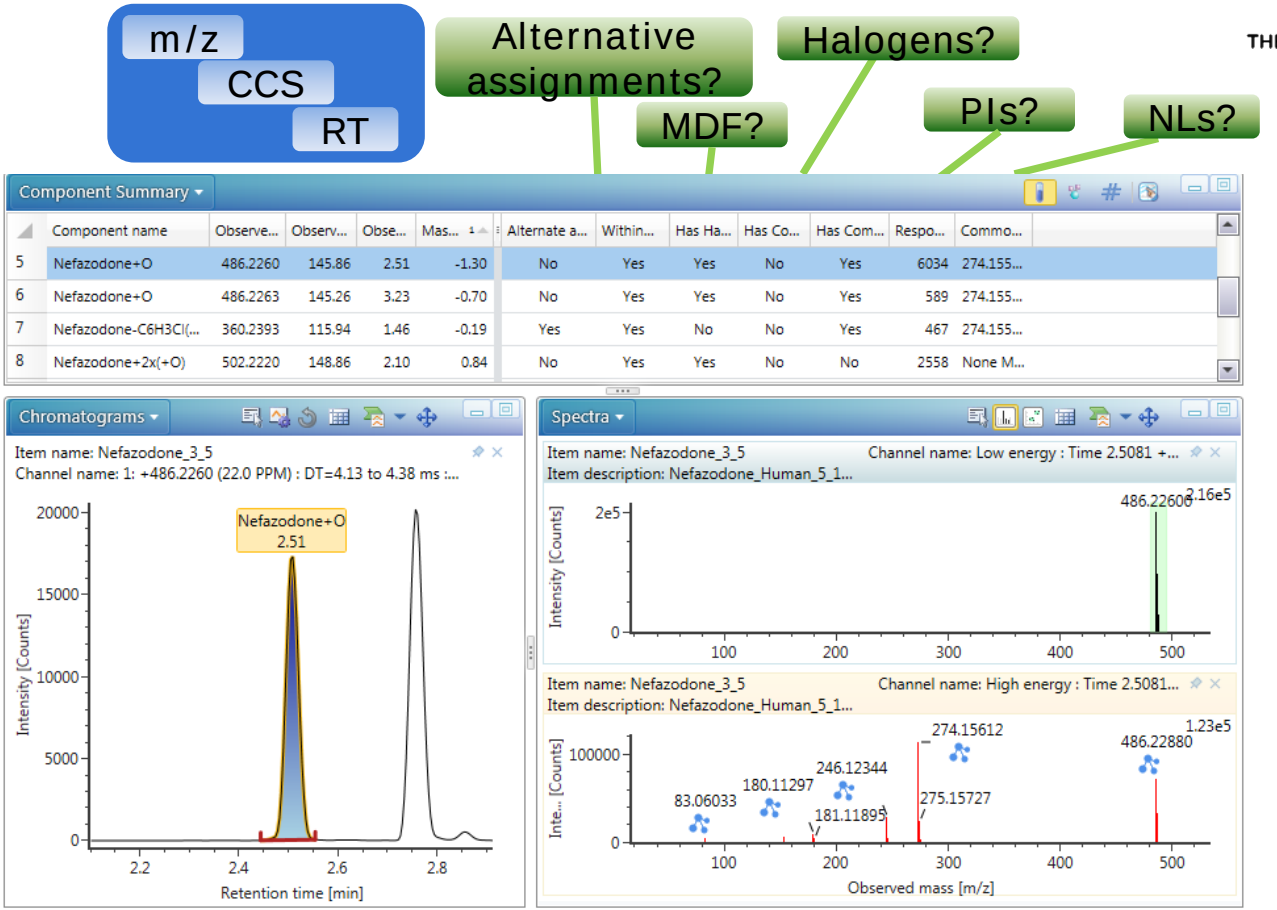

Intelligent Software Tools for Peptide Metabolite Profiling

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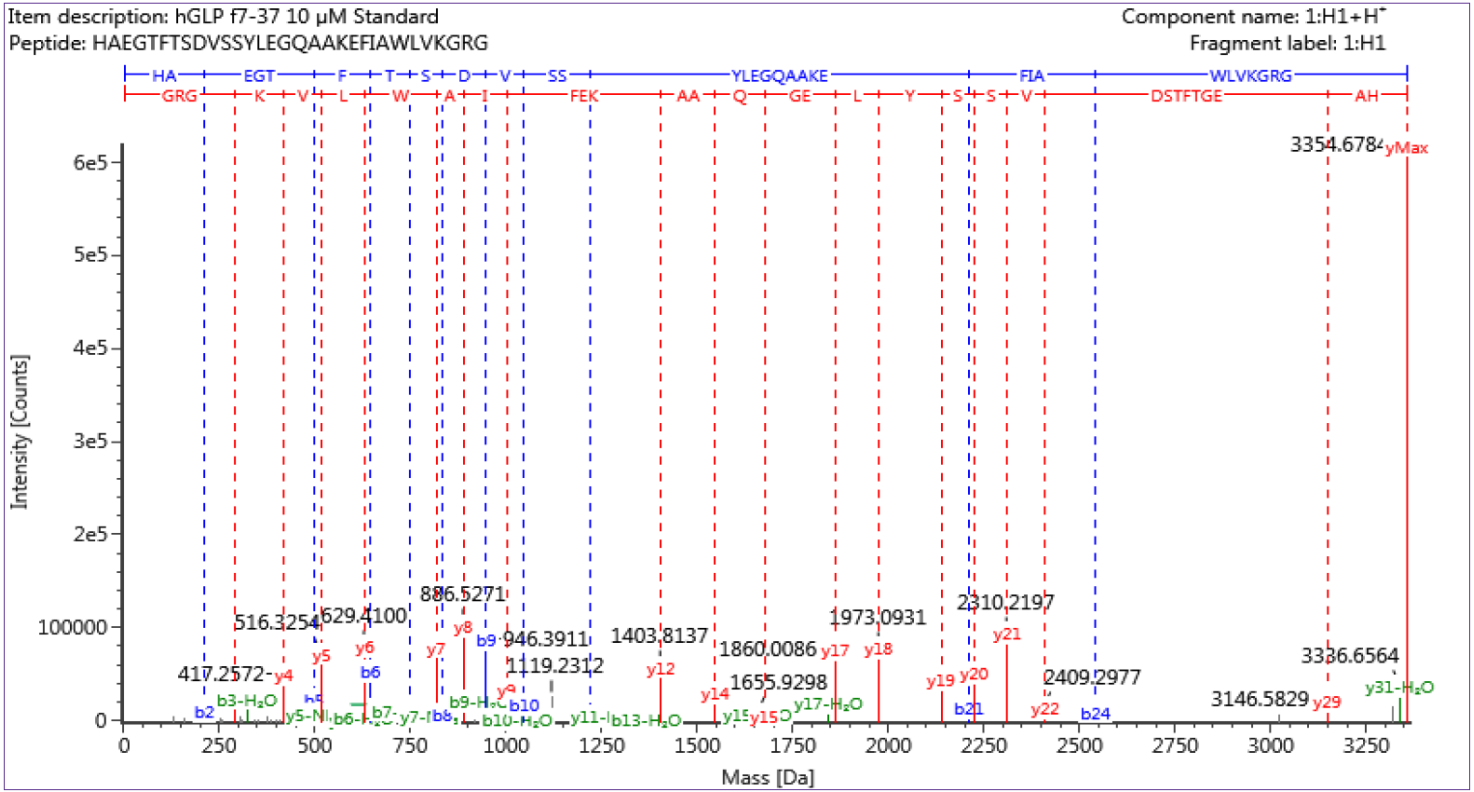
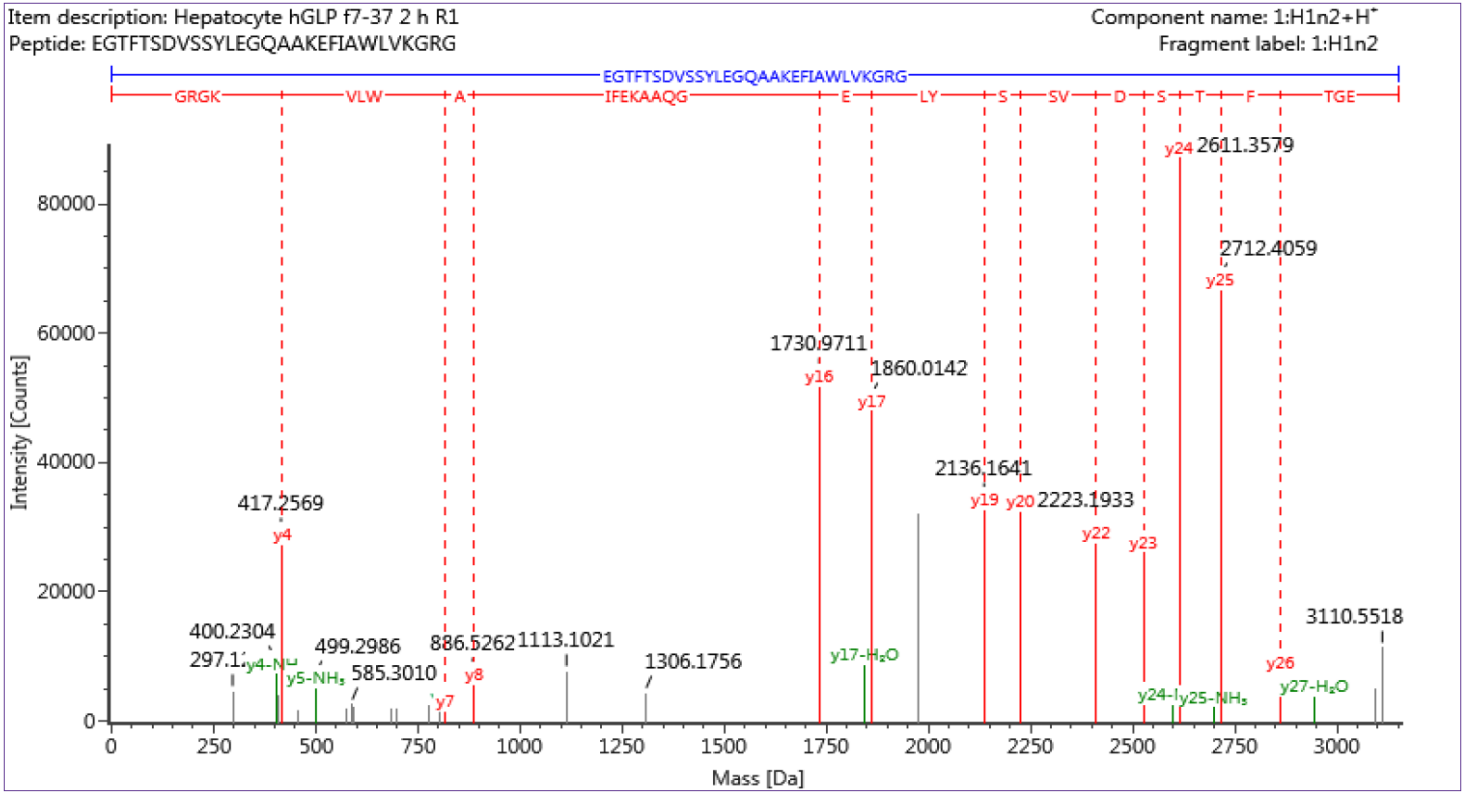


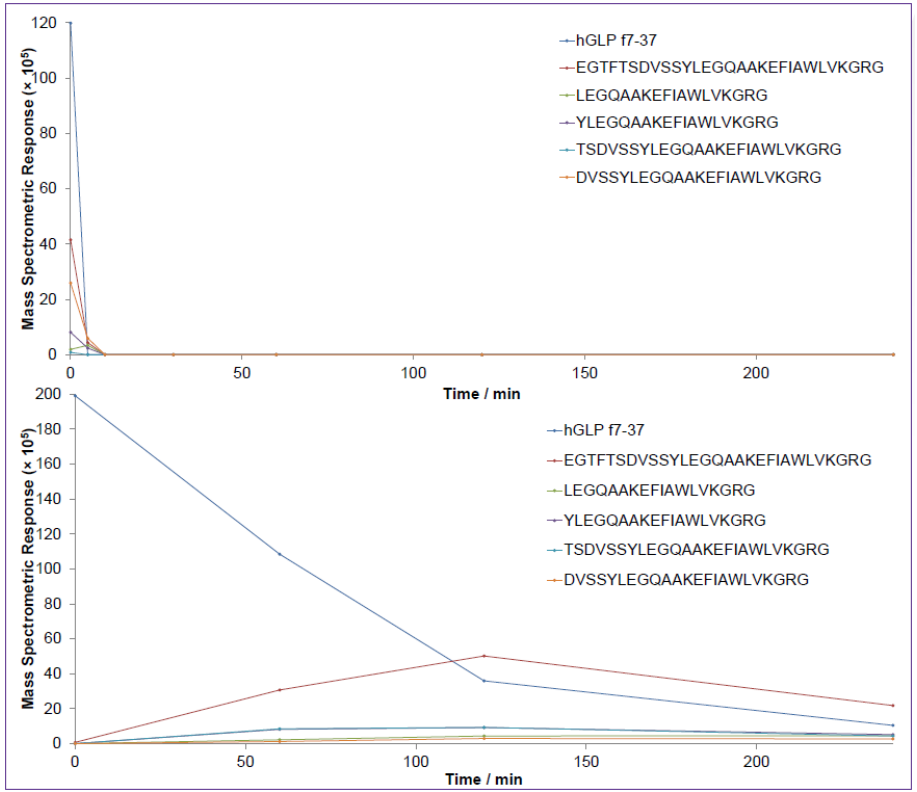
H Robinson, R Clayton, S Johnson, J Kirk, and M Wrona. Evaluation of Intelligent Software Tools for the Metabolite Profiling and Identification of Peptide-Based Large Molecules. Poster presented at ASMS, 2018.

Assessing the Feasibility of Each Potential Metabolite



H Robinson, R Clayton, S Johnson, J Kirk, and M Wrona. Evaluation of Intelligent Software Tools for the Metabolite Profiling and Identification of Peptide-Based Large Molecules. Poster presented at ASMS, 2018.

Summary Plot Comparisons



H Robinson, R Clayton, S Johnson, J Kirk, and M Wrona. Evaluation of Intelligent Software Tools for the Metabolite Profiling and Identification of Peptide-Based Large Molecules. Poster presented at ASMS, 2018.

What Value Can the Right Evaluation Software Add?

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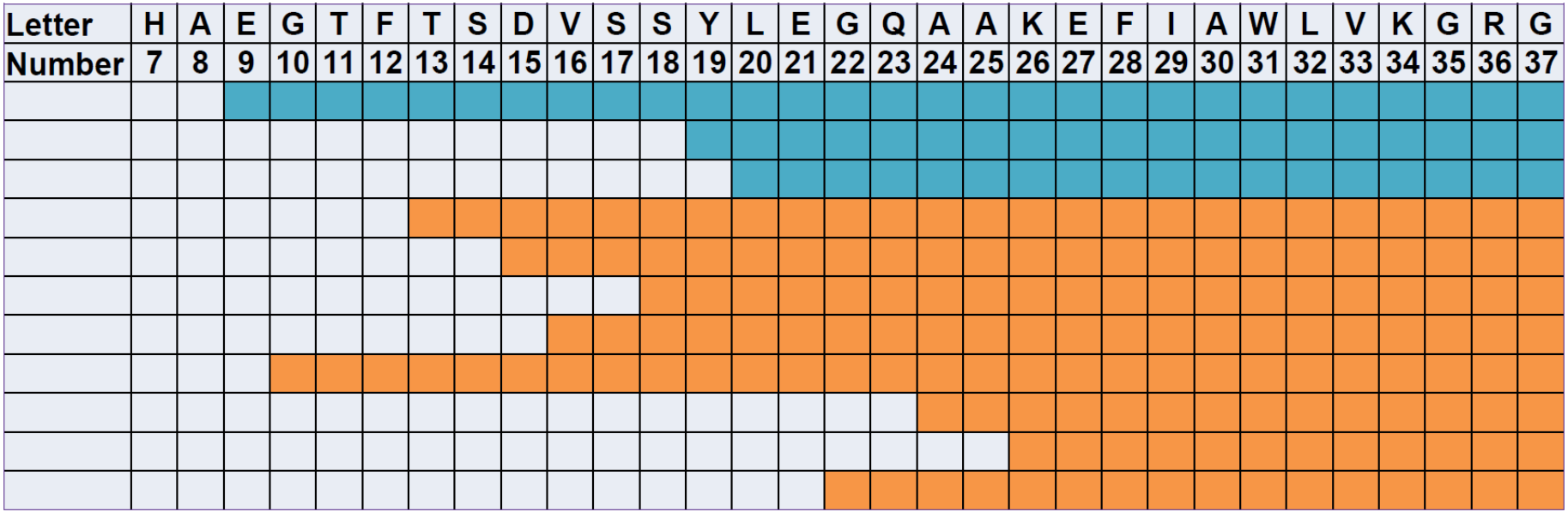

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UNIFI API for Third Party Partnerships

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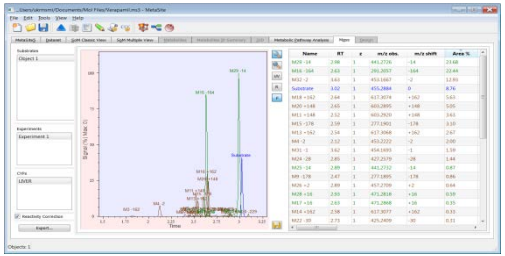
Vion™ IMS QTof

UNIFI®
SCIENTIFIC INFORMATION SYSTEM



MASSMETASITE
WebMetabase

What are Mass-MetaSite and WebMetabase?

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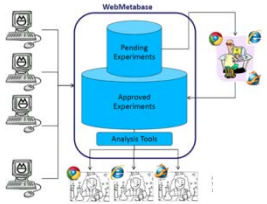
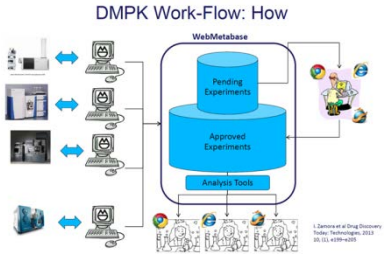
- Mass-MetaSite
  - engine that processes LC-MS data to identify drug metabolites in biological samples

- WebMetabase
  - processes MMS data from multiple compounds and conditions for databasing and visualization



- Together, MMS and WMB capture all in vitro and in vivo biotransformations into a database, enabling data sharing, mining and new chemistry design.



The Vion/UNIFI/Mass-MetaSite and WebMetabase Workflow

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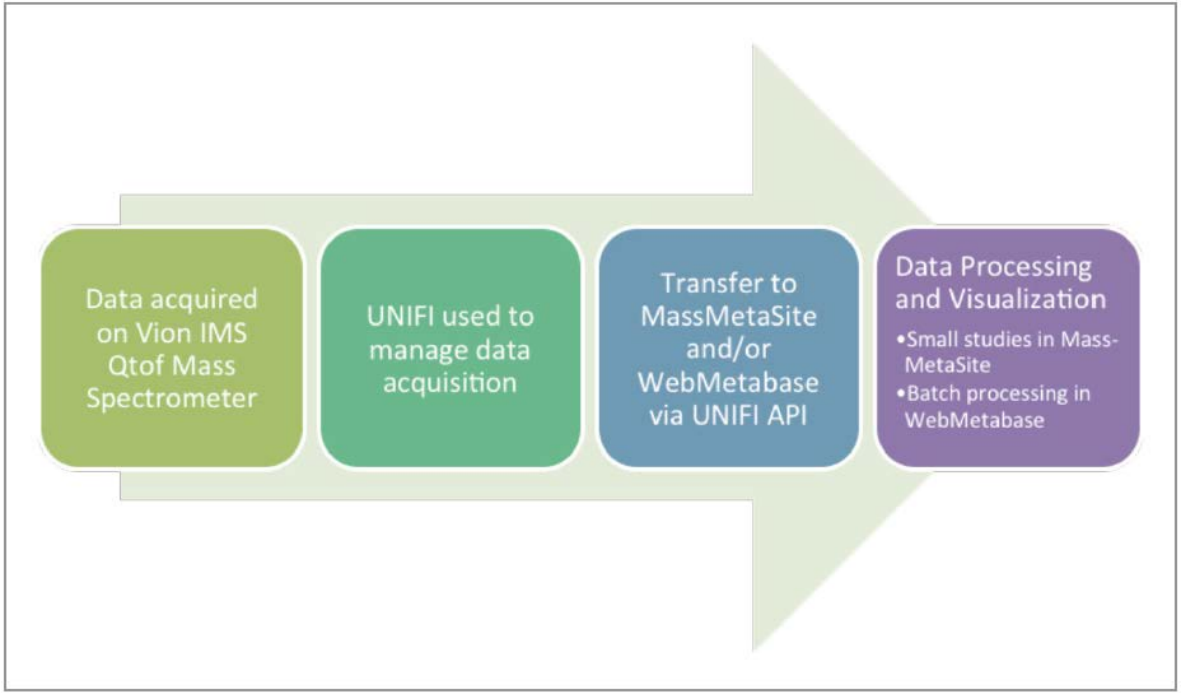

What Does This All Mean for the DMPK Lab?

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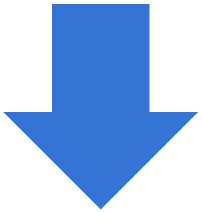
Capacity

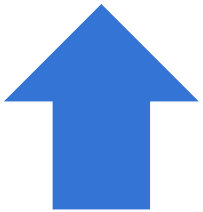
- **Better spectral quality by IMS** means increased **confidence** in identification, structural elucidation, distinguishing co-eluting metabolites

Time



- **CCS values** means another separation dimension acquired at the same time that is **matrix, ion concentration** and **chromataographic condition independent**

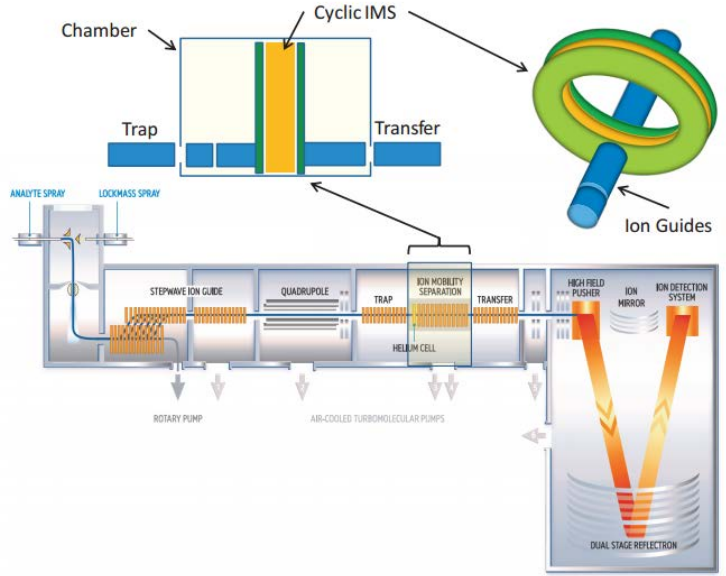
Automation

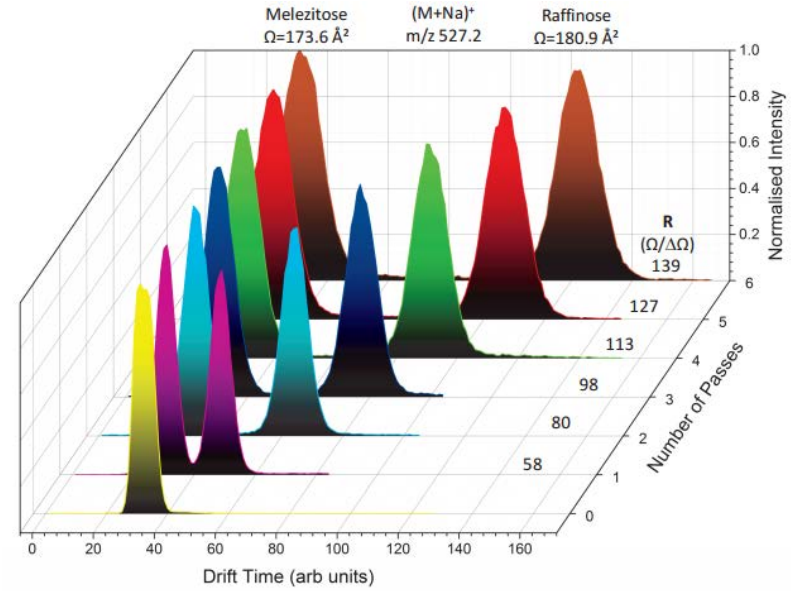


- Integration of ion mobility data with software means a **less manual workflow**, allowing scientists to work on value added projects

What are Future Possibilities for IMS and CCS?

Where Do We Go From Here – Cyclic IMS?





- Improvements in IMS resolution will come with time
- Significant opportunity if we can make CCS modelling routine
- CCS libraries will provide fuel for modelling

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ISiCLE: A molecular collision cross section calculation pipeline for establishing large in silico reference libraries for compound identification

Sean M. Colby[1], Dennis G. Thomas[1], Jamie R. Nunez[1], Douglas J. Baxter[1], Kurt R. Glaesemann[2], Joseph M. Brown[1], Meg A Pirrung[3], Niranjan Govind[1], Justin G. Teeguarden[1,4], Thomas O. Metz[1,*], Ryan S. Renslow[1,*]

[1] Earth and Biological Sciences Directorate, Pacific Northwest National Laboratory, Richland, WA, USA.

[2] Communications and Information Technology Directorate, Pacific Northwest National Laboratory, Richland, WA, USA.

[3] National Security Directorate, Pacific Northwest National Laboratory, Richland, WA, USA.

[4] Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR, USA

KEYWORDS: metabolomics, standards-free, collision cross section, high-performance computing, computational chemistry, density functional theory, molecular dynamics, ion mobility

Thank You!

- Covance
  - Richard Clayton
  - Catherine Holdsworth
  - Sarah Johnson
  - John Kendrick
  - Callum Lord-Mears
  - Helen Robinson

- Waters
  - Nathan Anderson
  - Yun Alelyunas
  - Jayne Kirk
  - Mark Wrona

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