

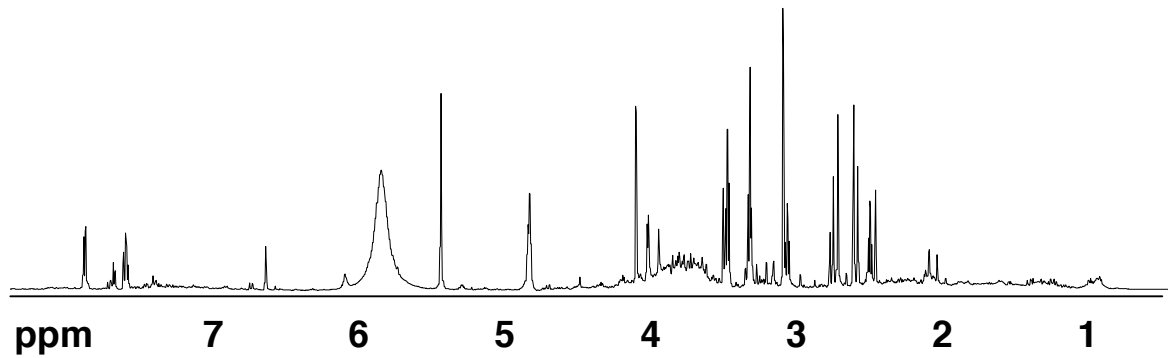
Trends in Quantitative Metabolomics

David Wishart

University of Alberta, Edmonton, Canada

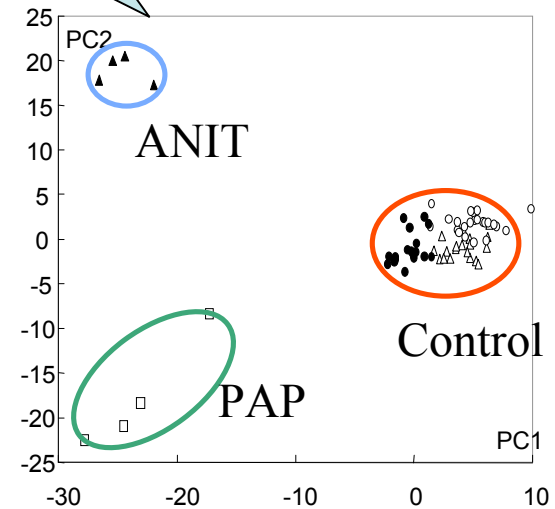
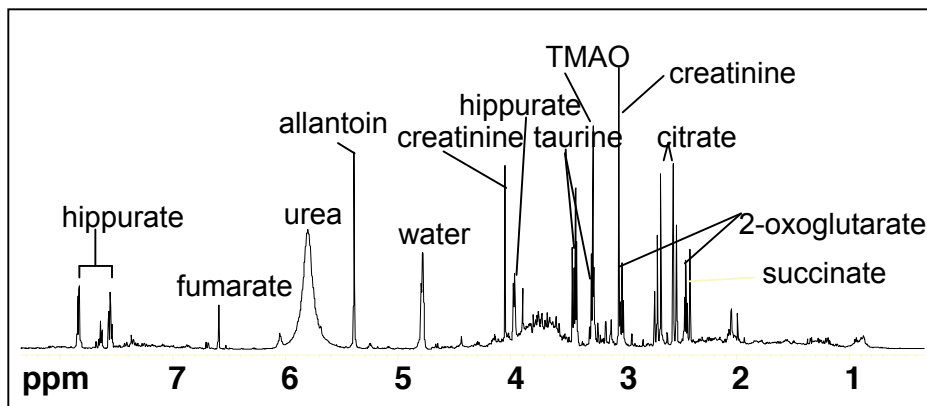
ABRF 2010, Sacramento CA, March 23, 2010

2 Routes to Metabolomics



**Quantitative
Or Targeted
Methods**

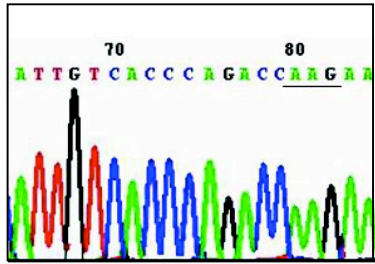
**Chemometric or
Non-targeted
Methods**



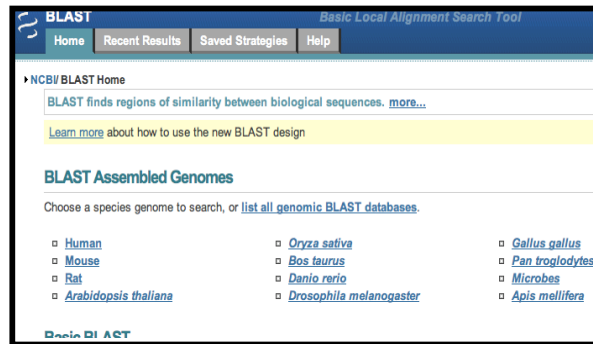
Quantitative Metabolomics

- *Compounds are identified and quantified absolutely*
- Data is independent of platform or technology (NMR, MS, HPLC)
- Data is uniformly formatted, easily shared & easily understood
- Biomarkers can be approved/translated to practice more easily
- Biomarkers can be interpreted or understood more easily
- No need for spectral alignment or binning
- No need to worry about the effects of artifactual peaks (adducts, contaminants, decomposition products)
- Easily adapted to robust statistical methods developed for transcriptomics and proteomics
- Takes time to ID and quantify compounds
- Not all compounds can be quantified or ID'd (i.e. missing data)

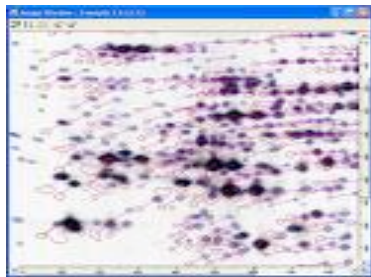
The Problem With Metabolomics



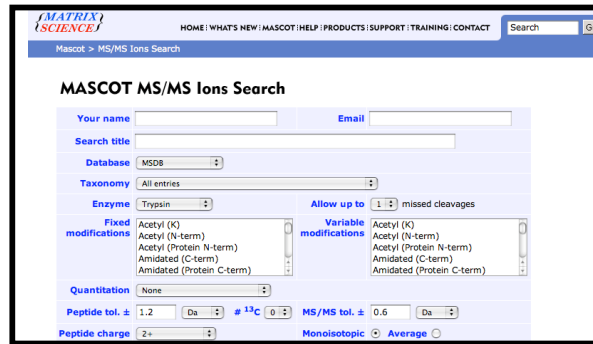
Genomics



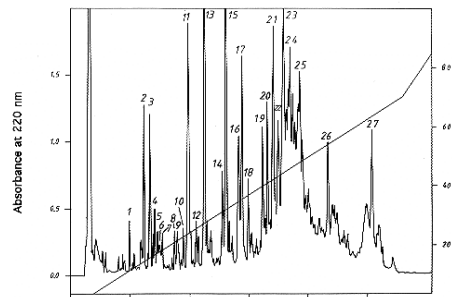
Gene IDs +
Transcript
Abundance



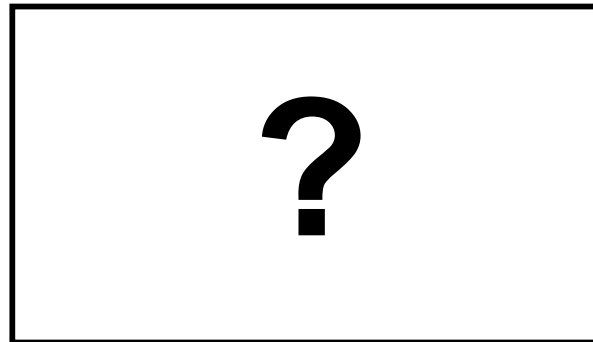
Proteomics



Protein IDs +
Concentrations



Metabolomics



Metabolite IDs +
Concentrations

The Human Metabolome Project



- **\$7.5 million Genome Canada Project launched in Jan. 2005 - still ongoing**
- **Mandate to quantify and identify all metabolites in biofluids such as urine, CSF and blood as well as tissues using HT experiments and text analysis (~8000 cmpds to date)**
- **Collect reference compound spectra (NMR, GC-MS and MS/MS) for as many metabolites as possible**
- **Make all data freely and electronically accessible (HMDB, DrugBank, FooDB, T3DB)**
- **Develop novel technologies and software to improve metabolome coverage and metabolomic throughput**

Human Metabolomes

2900 (T3DB)

Toxins/Env. Chemicals

1500 (DrugMet)

Drug metabolites

30000 (FooDB)

Food additives/Phytochemicals

1450 (DrugBank)

Drugs

8000 (HMDB)

Endogenous metabolites



Meet the Metabolomes...

The screenshot shows the HMDB homepage with a navigation menu (Home, Browse, Search, About, Downloads, Contact Us) and a search bar. The main content area features the HMDB logo and a detailed description of the database, including its scope and supported data types. A 'What's New?' section at the bottom highlights the release of version 2.5 on November 5, 2009.

<http://www.hmdb.ca>

The screenshot displays the T3DB homepage, featuring a navigation menu and a search bar. The main content area includes the T3DB logo and a comprehensive description of the database, detailing its focus on toxin-target interactions and the types of data it provides. A 'What's New?' section notes the release of T3DB Version 1.0.

<http://www.T3DB.org>

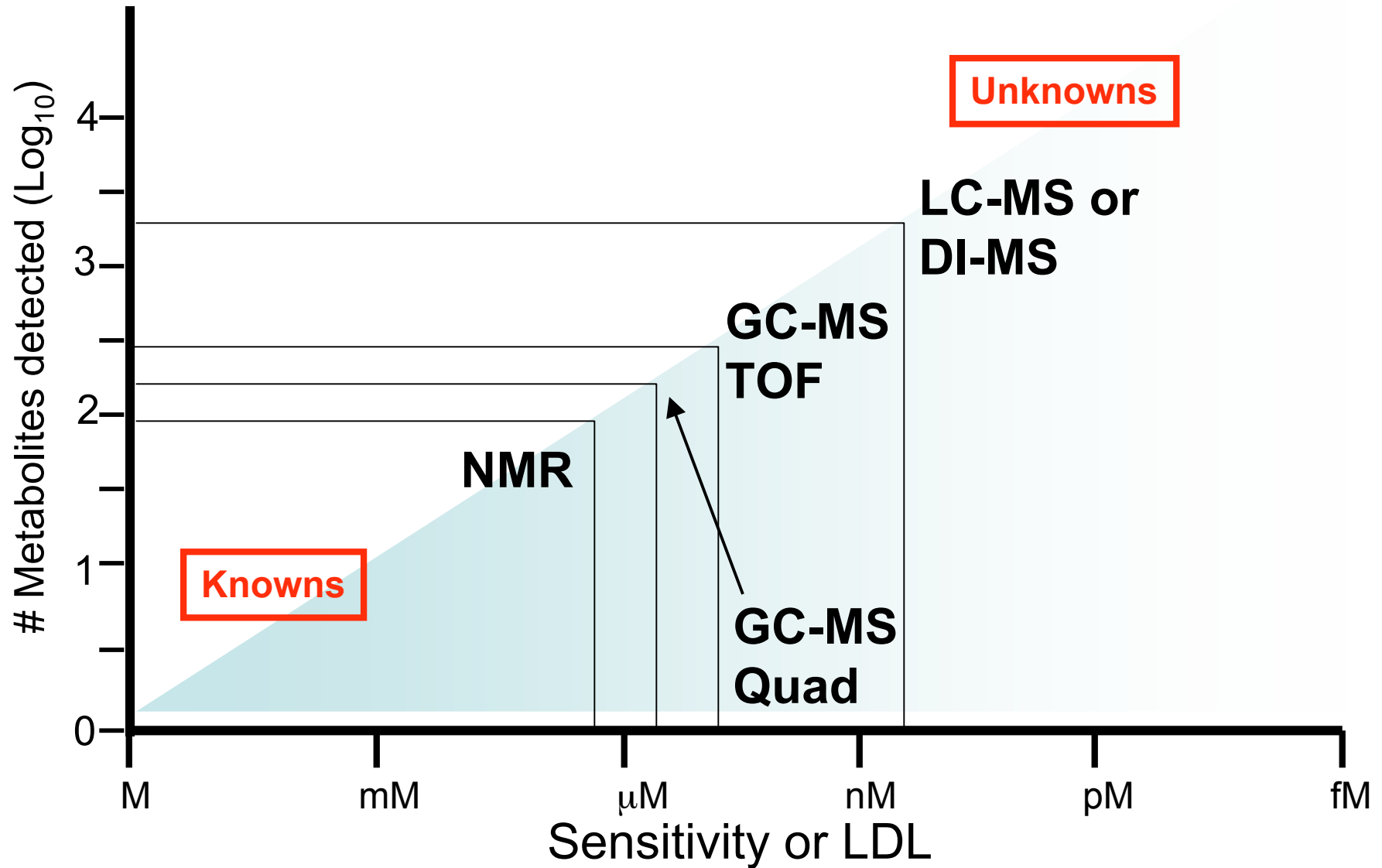
The screenshot shows the FoodB homepage with a navigation menu and a search bar. The main content area features the FoodB logo and a welcome message, followed by a detailed description of the database's purpose and the data it contains. A 'What's New?' section at the bottom lists recent updates and testing information.

<http://www.foodbs.org/foodb>

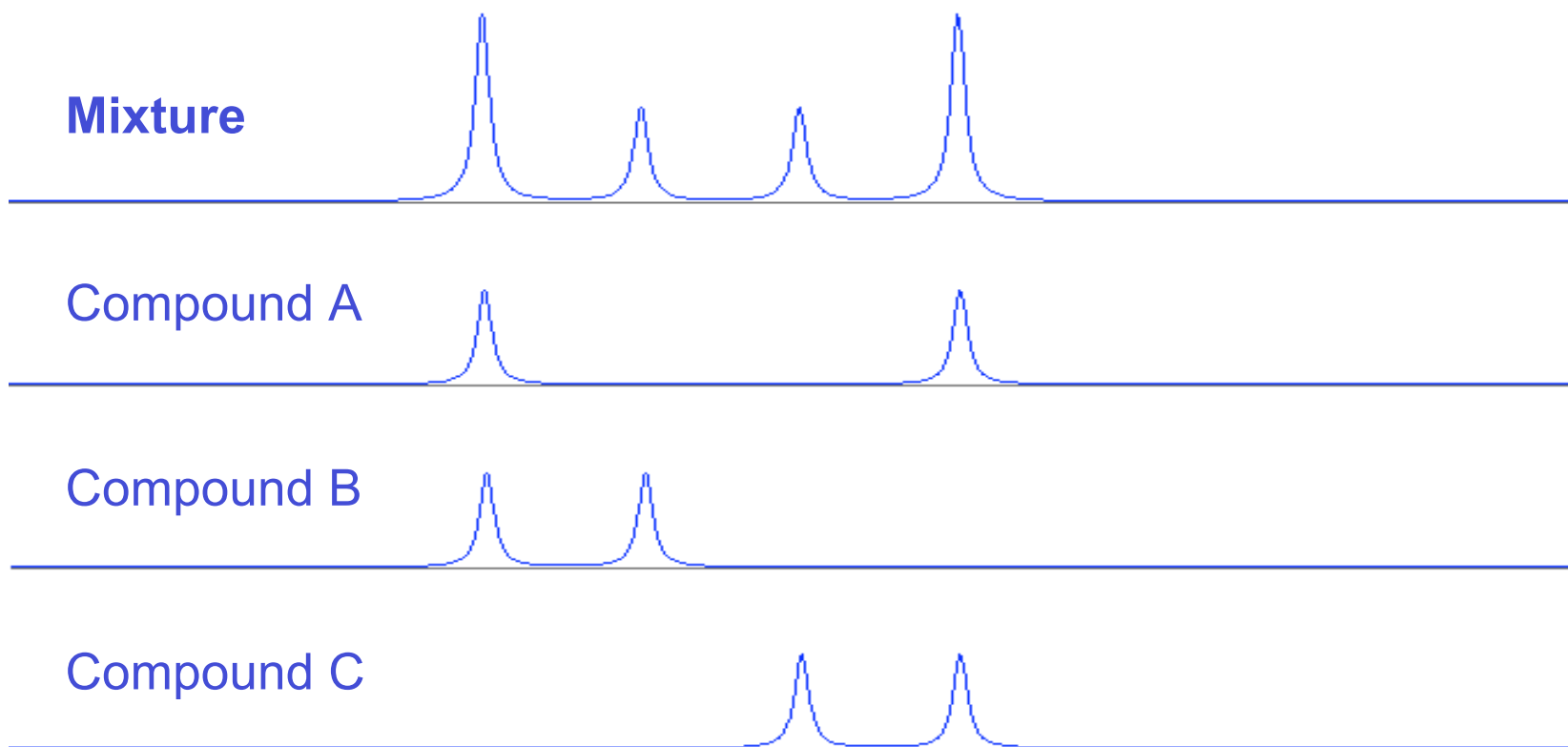
The screenshot displays the DrugBank homepage, featuring a navigation menu and a search bar. The main content area includes the DrugBank logo and a detailed description of the database, highlighting its focus on drug-target interactions and the types of data it provides. A 'What's New?' section at the bottom lists recent updates and testing information.

<http://www.drugbank.ca>

Technology & Sensitivity



Metabolite ID by Spectral Deconvolution (NMR)



NMR Spectral DBs

SBDS (<http://riodb01.ibase.aist.go.jp>)

The screenshot shows the SBDS website interface. At the top, there is a navigation bar with links for 'Home', 'Introduction', 'Disclaimer', 'HELP', 'Contact', 'What's New', 'SDBS', and 'LINKS'. Below this, a green banner reads 'Spectral Database for Organic Compounds SBDS'. The main content area includes a welcome message, a list of developers (NMR: T. Saito, K. Hayamizu, M. Yanagisawa and O. Yamamoto; MS: N. Wasada; ESR: K. Somino; IR: S. Kitagawa, K. Tanabe and T. Tamura; Raman: K. Tanabe and J. Hiraiishi), and a 'What's New' section with dates and updates. A disclaimer is also present, along with a red box containing the text: 'Please do not access or download data by using automated processes. Please check Javascript and Cookie settings if you can not proceed to search page. The settings have to be active in order to use SBDS. We had a numerous access from one particular site.'

NMRShiftDB (www.ebi.ac.uk/nmrshiftdb/)

The screenshot shows the NMRShiftDB website. It features a search bar at the top with the text 'NMRShiftDB - open nmr database on the web'. Below the search bar, there is a login section with fields for 'Username:' and 'Password:', and a 'Login' button. A 'Create New Account' link is also visible. The main content area includes a 'Home' button, a 'Search' button, and a 'Results' section. A table titled 'Current usage of NMRShiftDB is:' shows 'Registered Users: 1815', 'Structures which can be searched: 21540', and 'Spectra: Measured 25137, calculated 550'. There is also a 'Hall of Fame' section with a table listing names and contributions.

Name	Contributions
1. E. Wilmshagen	921
2. S. Dathie	505
3. P. Braetigam	439
4. S. Kuhn	390
5. N. Prakash	350
6. B. Pügel	305
7. M. Genske	181
8. N. Kuznik	120
9. K. Bohn	111
10. R. Ellinger	76
11. A. Dransfeld	56
12. K. Barussek	26
13. M. Mitchell	20
14. J. Btzer	19

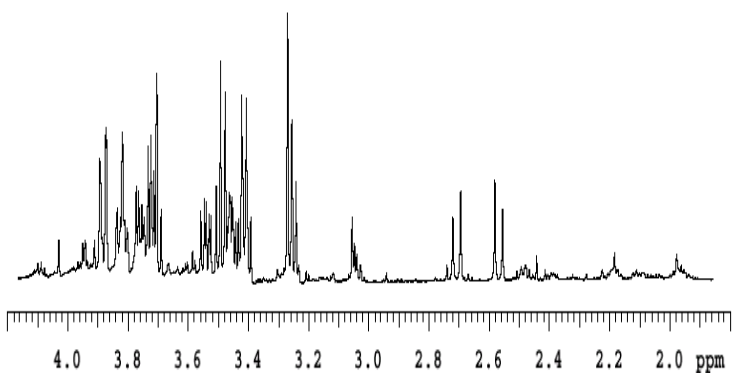
HMDB (www.hmdb.ca)

The screenshot shows the HMDB website. At the top, there is a navigation bar with links for 'Home', 'Search', 'About', 'Downloads', and 'Contact Us'. Below this, a green banner reads 'Human Metabolome Database'. The main content area includes a search bar with the text 'Search HMDB' and a 'Search' button. A description of the database is provided, along with a 'What's New' section.

BMRB (www.bmrw.wisc.edu)

The screenshot shows the BMRB website. It features a search bar at the top with the text 'Biological Magnetic Resonance Data Bank'. Below the search bar, there is a navigation bar with links for 'Search Archive', 'Deposit Data', 'NMR Statistics', 'Spectroscopists' Corner', and 'Programmer's Corner'. The main content area includes a 'Metabolomics/Metabonomics' section with a description of the field and a 'Metabolomics/Metabonomics' section with a description of the database.

NMR Compound ID - HMDB



NMR spectrum of mixture

The screenshot shows the HMDB Spectra Search interface. The search criteria are as follows:

- Search By: NMR Peaklist Data
- Spectral Database: All
- NMR Experiment Type: 1D_1H
- Biofluid: No Restriction
- Top Matches Returned: 10
- 1H Shift Tolerance (+/-): 0.01
- 13C Shift Tolerance (+/-): 0.03
- Chemical Shift Type: 1H
- Input Peak List: 2.31, 2.85, 2.86, 2.87, 2.88, 3.09, 3.08, 4.25, 4.26

Peak list to HMDB

Phenyllactate
Phenylpyruvate
Phenylacetic acid
Tropic acid
Benzyl alcohol

...

The screenshot shows the search results for the peak list. The results are as follows:

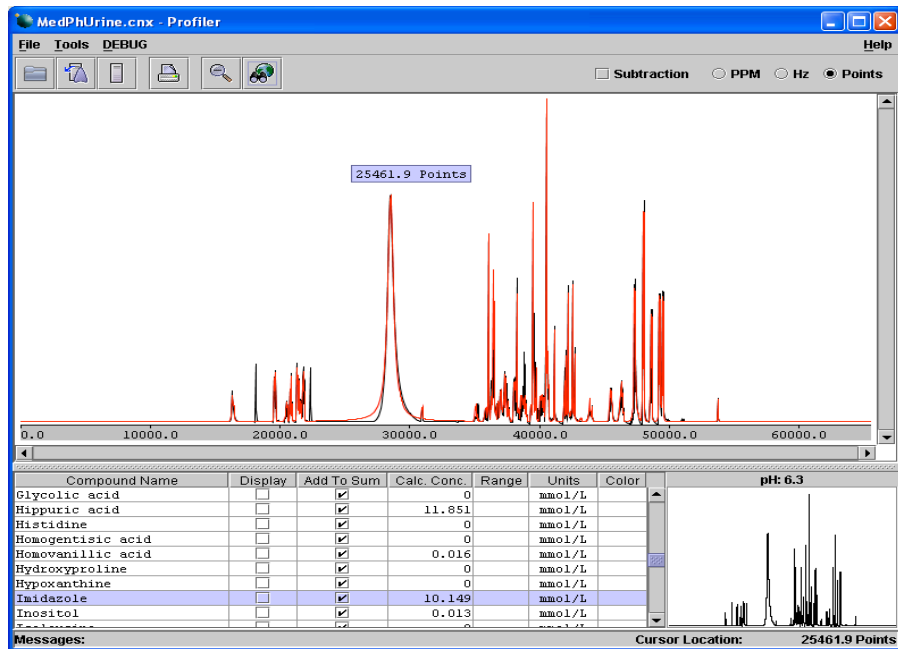
HMDB ID	Name	Peaklist	Spectral Image	Spectral DB	Score
HMDB00045	Phenylpyruvic acid	View	View	experimental	80.23
HMDB01326	Phenyl acetate	View	View	experimental	81.10
HMDB01318	Benzyl alcohol	View	View	experimental	77.10
HMDB00272	D-Phenylacetic acid	View	View	experimental	74.21
HMDB00563	D-Phenylacetic acid	View	View	experimental	73.20
HMDB01337	D-Hydroxyphenylamine	View	View	experimental	71.13
HMDB00038	Galactaric acid	View	View	experimental	71.2
HMDB00489	Tropic acid	View	View	experimental	65.20
HMDB00031	Malic acid	View	View	experimental	61.2
HMDB02222	3-Methylphenylacetic acid	View	View	experimental	51.2

High scoring matches

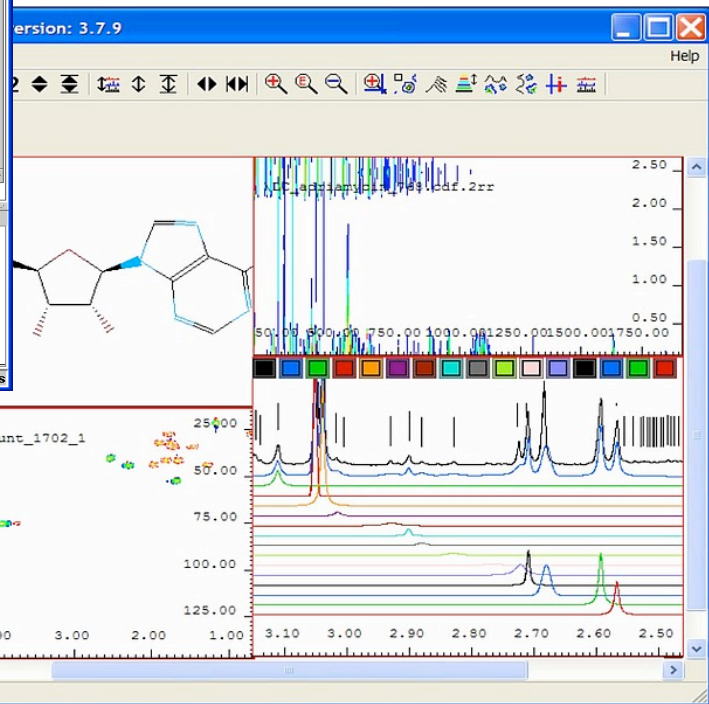
NMR Compound ID - HMDB

- Database of 1000 experimental ^1H NMR and 1000 ^{13}C HSQC NMR reference spectra (in H_2O) + ~500 TOCSY spectra
- Database of 3000 predicted ^1H NMR and ^{13}C NMR spectra
- Accepts 1D ^1H NMR ^{13}C NMR, TOCSY and HSQC queries
- Supports different chemical shift tolerances for ^1H and ^{13}C , biofluid selection, database selection and top match filtering

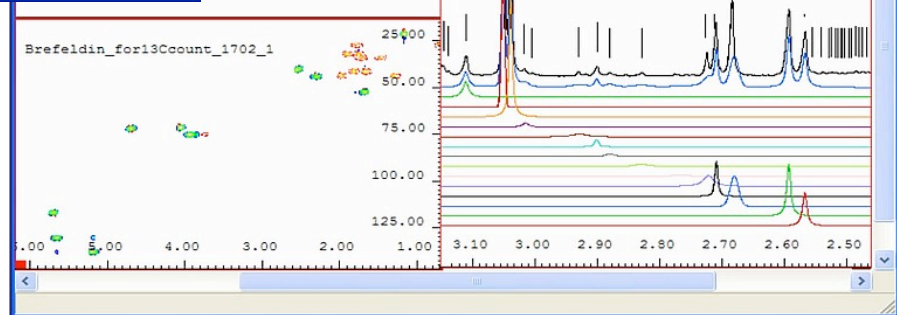
Commercial Tools For NMR-based Quant. Metabolomics



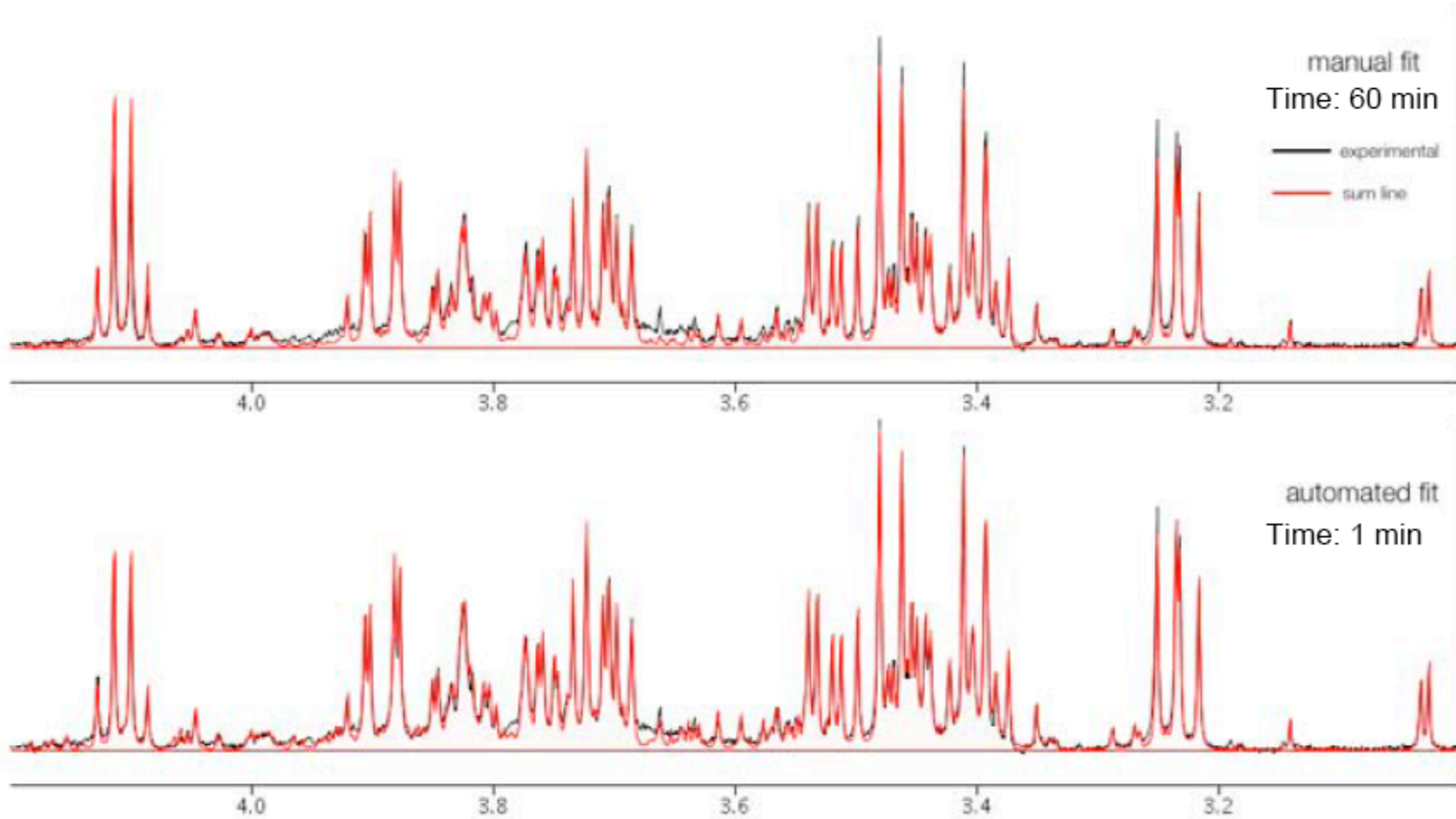
www.bruker-biospin.com



www.chenomx.com

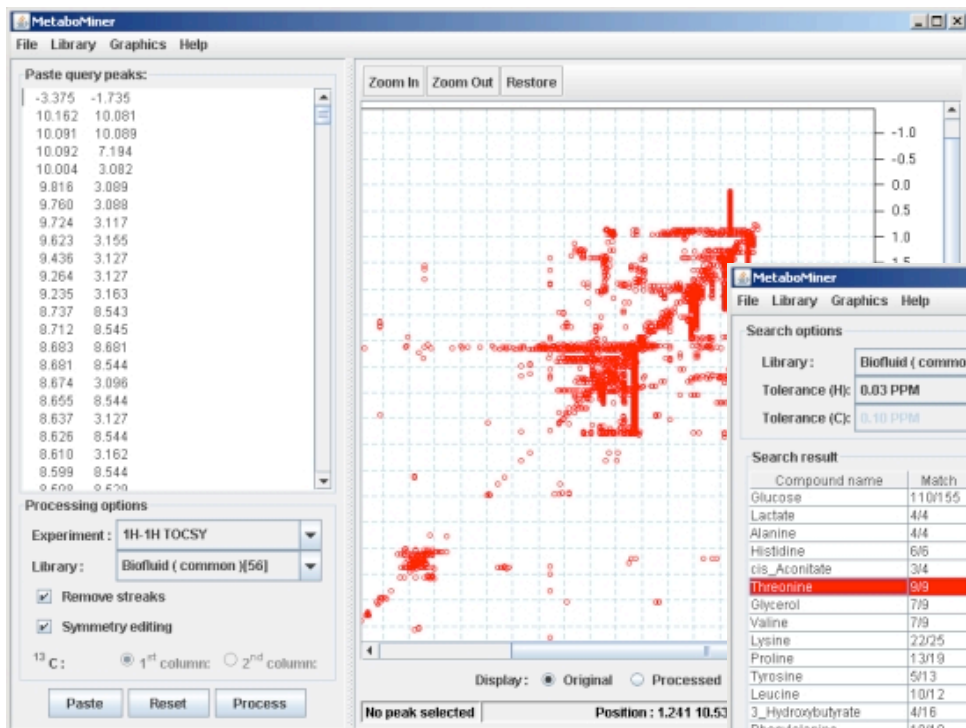


Automated vs Manual Fitting



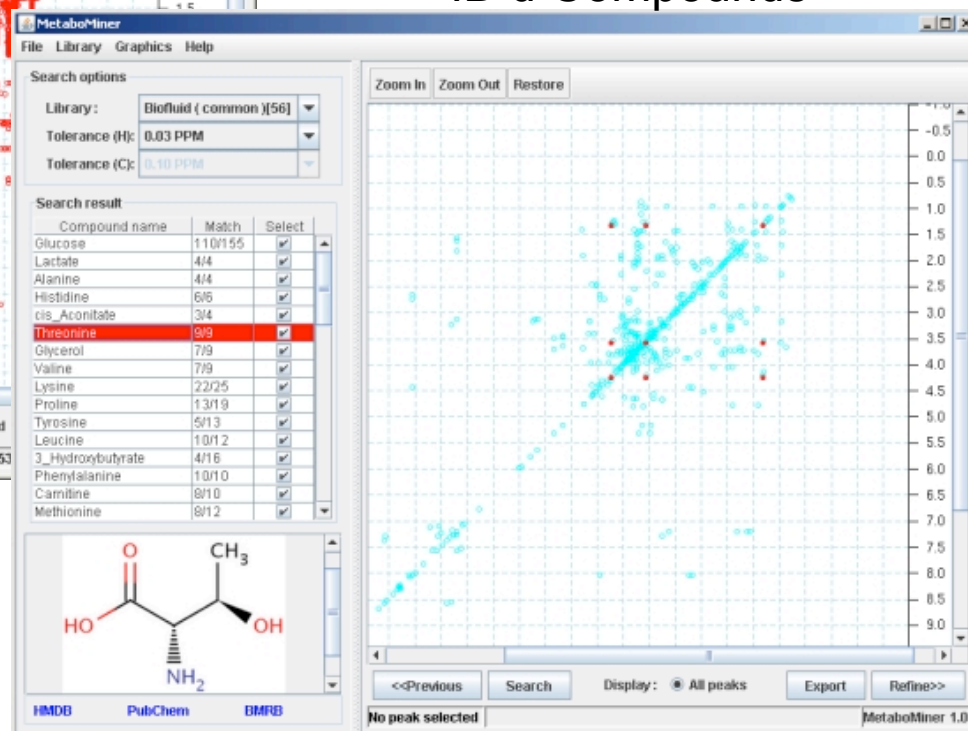
Cerebrospinal Fluid (cmpd ID correlation ~95%)

NMR Compound ID from Mixtures - MetaboMiner



Raw TOCSY Spectrum

ID'd Compounds



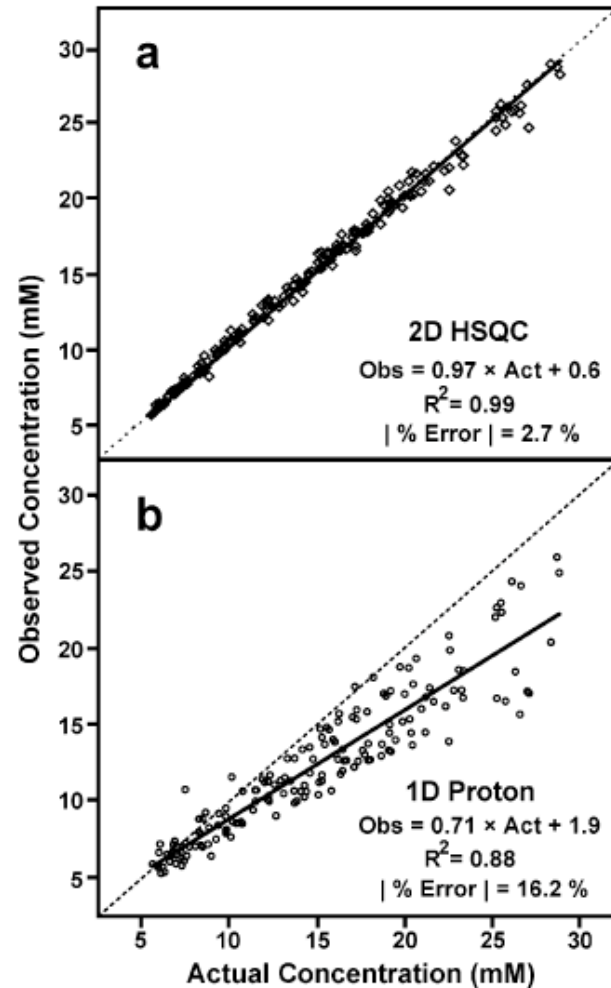
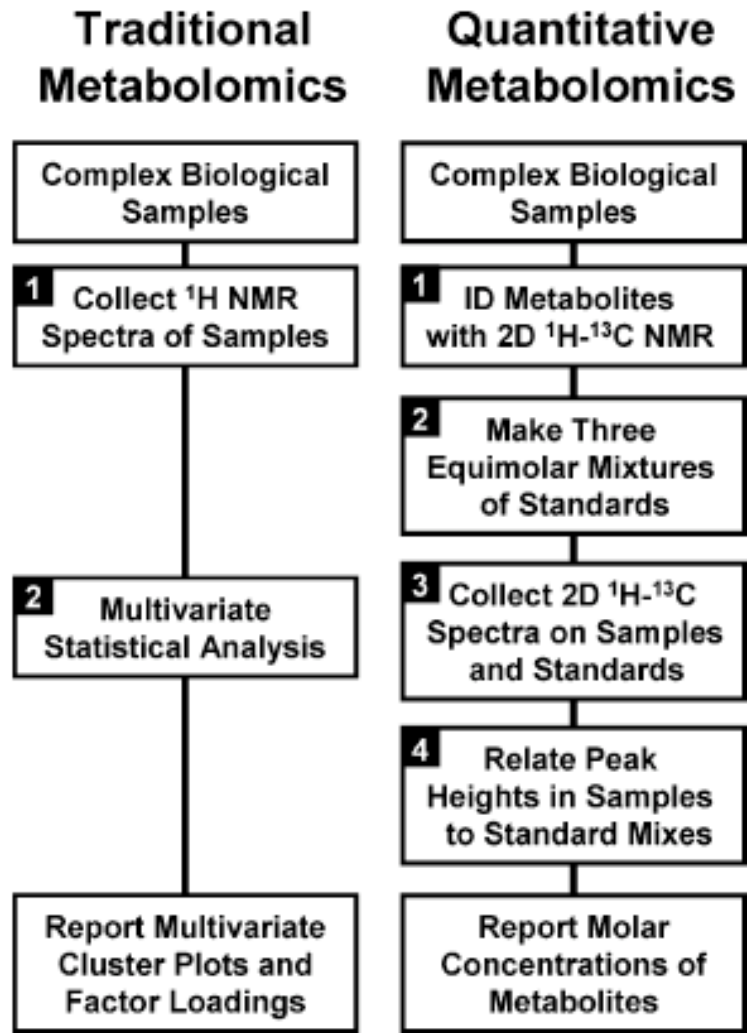
<http://wishart.biology.ualberta.ca/metabominer/>

MetaboMiner Performance

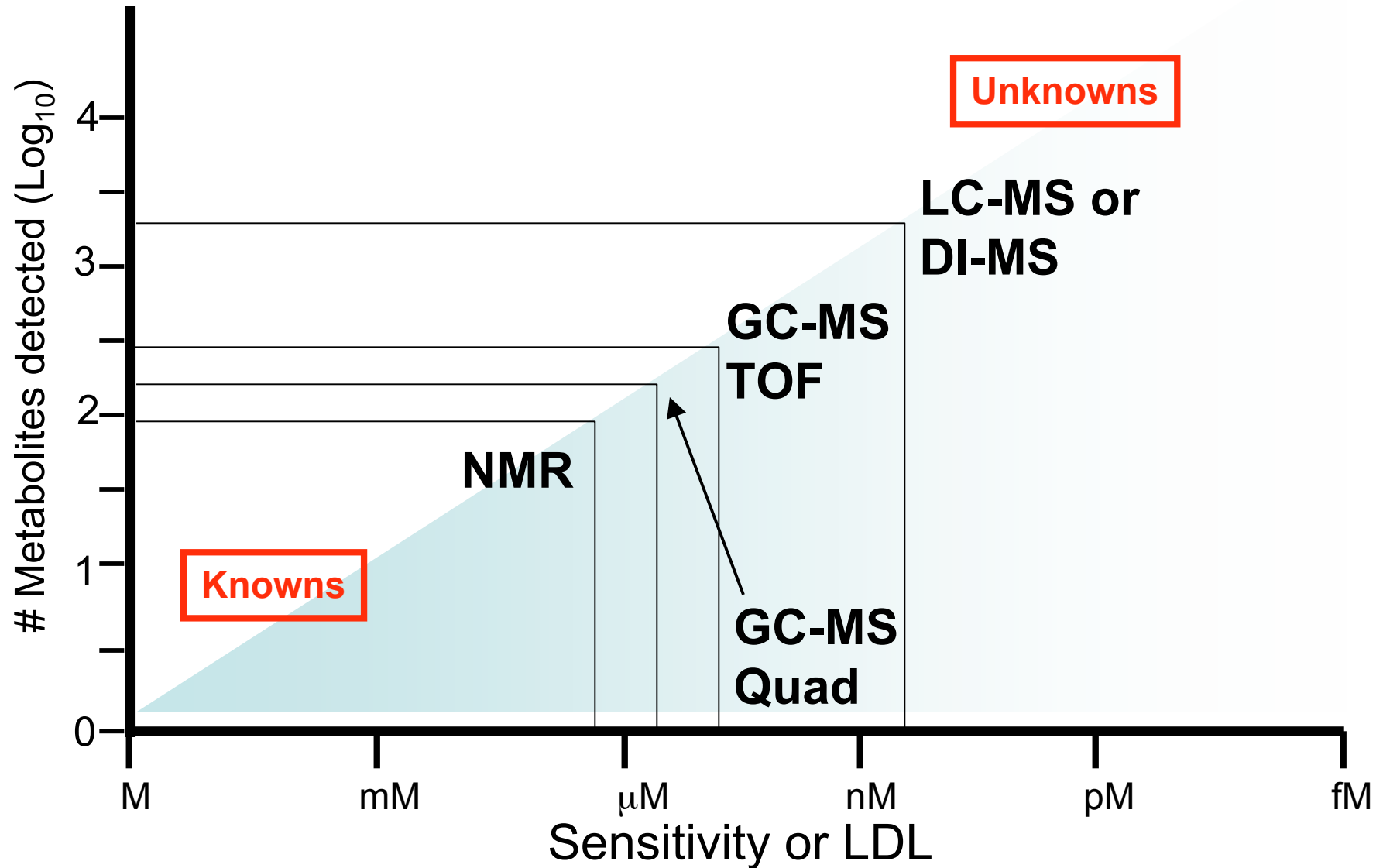
Sample	pH	TOCSY			HSQC		
		Identifiable*	Identified	Correct	Identifiable*	Identified	Correct
Cocktail	7.2	28	31	27	28	31	26
	4.2		28	24		30	22
Plasma	7.3	28	32	27	31	32	27
	8.8		25	22		29	24

Sample	pH	TOCSY			HSQC		
		Recall (%)	Precision (%)	F-measure (%)	Recall (%)	Precision (%)	F-measure (%)
Cocktail	7.2	96	87	92	93	84	88
	4.2	86	86	86	79	73	76
Plasma	7.3	96	84	90	87	84	86
	8.8	79	88	83	77	83	80

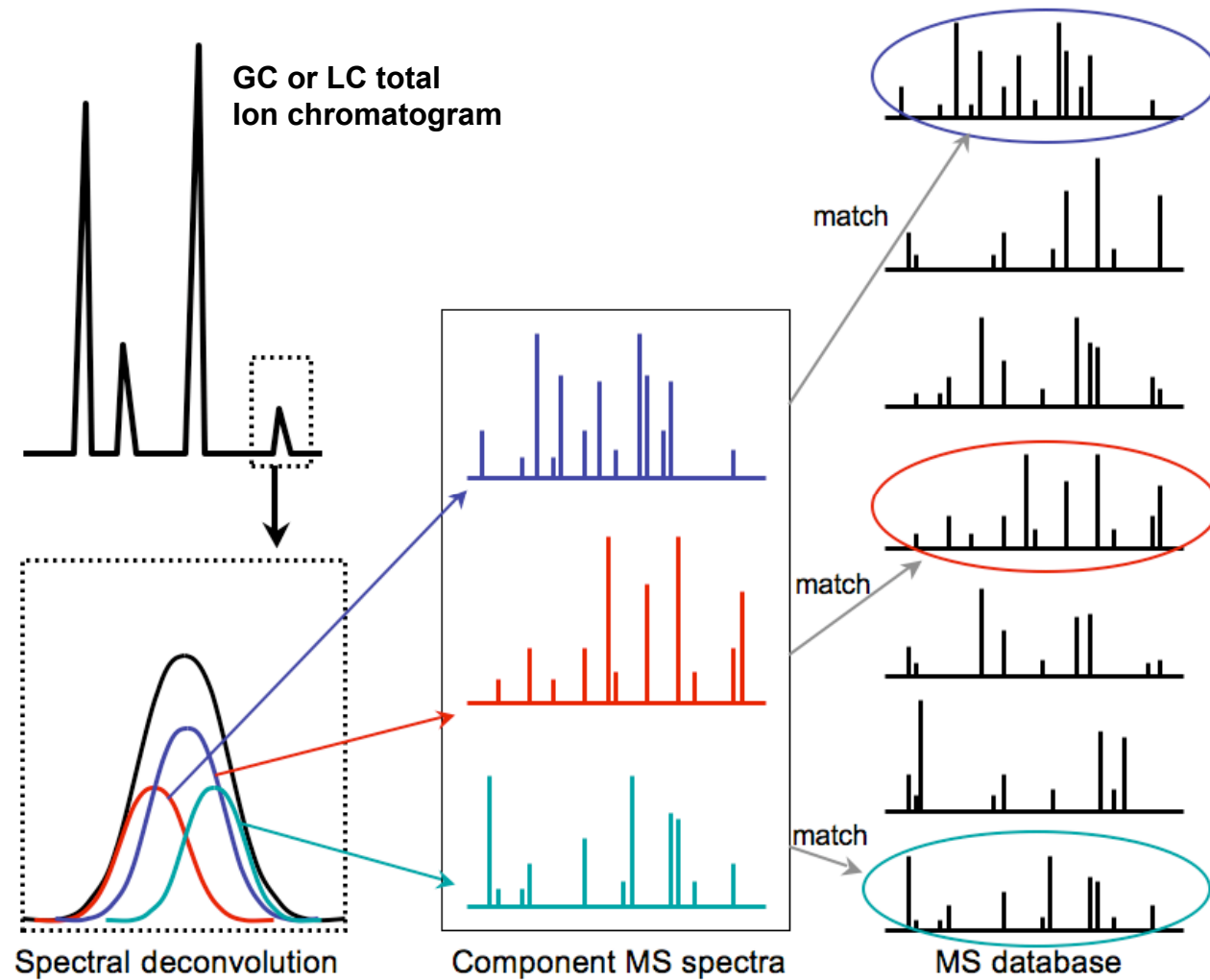
Quantitative Metabolomics (2D NMR)



Technology & Sensitivity



Metabolite ID by GC/LC-MS



MS Spectral DBs

NIST/AMDIS (http://chemdata.nist.gov)

Scientific Instrument Services, Inc.
Apparatus and Services for Mass Spectrometry, Gas Chromatography and Liquid Chromatography

The NIST 08 Mass Spectral Library (NIST/EPA/NIH)--New 2008 Version

--The fully-evaluated, most widely used mass spectral reference library. Compiled by the National Institute of Standards and Technology (NIST).

Summary: The NIST 08 mass spectral database, the successor to the NIST 05, is a fully evaluated collection of electron impact (EI) mass spectra. It also contains MS/MS spectra and GC data. It is the product of a two decade, comprehensive evaluation and expansion of the world's most widely used mass spectral reference library by a team of experienced mass spectrometrists in which each spectrum was examined for correctness.

NIST Components

NIST is not just a mass spectral library. It contains these components:

- UPDATED) Electron Impact (EI) mass spectral library** - 220,460 spectra of 192,108 unique compounds, with identifications and usually chemical structures. You may search names of compounds online.
- UPDATED) MS/MS library** - 14,802 spectra of 5,308 precursor ions (3,898 cations and 1,410 anions).
- UPDATED) Gas Chromatography (GC) data library** - 224,038 Kovats retention index values for 21,847 compounds in the EI library, now on both polar and non-polar columns. Includes retention indices with GC column conditions and literature citations.
- UPDATED) NIST MS Search software** - software for searching (identifying) compounds from their mass spectra and for browsing mass spectral libraries. Also includes MS interpretation programs for analyzing mass spectra on the basis of chemical structure, molecular formula, isotopic patterns, and more.
- UPDATED) AMDIS software** - software for deconvoluting gas/liquid chromatograms
- UPDATED) Documentation** - Approximately 50 page printed and electronic manual on

Figure: EI spectra, structure, and corresponding data for a sample compound in the NIST database.

Metlin (http://metlin.scripps.edu/)

Scripps Center for Mass Spectrometry

METLIN Overview

The METLIN Metabolite Database is a repository for mass spectral metabolite data. All metabolites are neutral or free acids. It is a collaborative effort between the Scripps and Oregon groups and centers for Mass Spectrometry at The Scripps Research Institute.

- Overview
- About
- Metabolites
- Health Resources
- MSMS
- LCMS
- FTMS
- Contact
- Download
- Admin

HMDB (www.hmdb.ca)

Human Metabolome Database

Version 2.0 | Version 1.0

The Human Metabolome Database (HMDB) is a freely available electronic database containing detailed information about small molecule metabolites found in the human body. It is intended to be used for applications in metabolomics, clinical chemistry, biomarker discovery and general education. The database is designed to contain or link three kinds of data: 1) chemical data, 2) clinical data, and 3) molecular biology/biochemistry data. The database (version 2.0) contains over 6500 metabolite entries including both water-soluble and lipid soluble metabolites as well as metabolites that would be regarded as either abundant (> 1 uM) or relatively rare (< 1 nM). Additionally, approximately 1500 protein (and DNA) sequences are linked to these metabolite entries. Each Metabocard entry contains more than 100 data fields with 2/3 of the information being devoted to chemical/clinical data and the other 1/3 devoted to enzymatic or biochemical data. Many data fields are hyperlinked to other databases (KEGG, PubChem, MetaCyc, CHEBI, PDB, Swiss-Prot, and GenBank) and a variety of structure and pathway viewing applets. The HMDB database supports extensive text, sequence, chemical structure and relational query searches. Two additional databases, DrugBank and FoodB are also part of the HMDB suite of databases. DrugBank contains equivalent information on ~1500 drugs while FoodB contains equivalent information on ~2000 food components and food additives.

HMDB is supported by David Wishart, Departments of Computing Science & Biological Sciences, University of Alberta.

More about the HMDB

What's New?

- PathBrowse and the associated HMDB pathways have been updated to automatically highlight the given metabolite in a pathway. For example, see the [Alanine and aspartate metabolism pathway with Liponamide highlighted](#). Pathways have also been unified so that the pathways from the HMDB, KEGG, and SimCell all match for each metabolite.

MassBank (www.massbank.jp)

MassBank.jp

High Resolution Mass Spectral Database

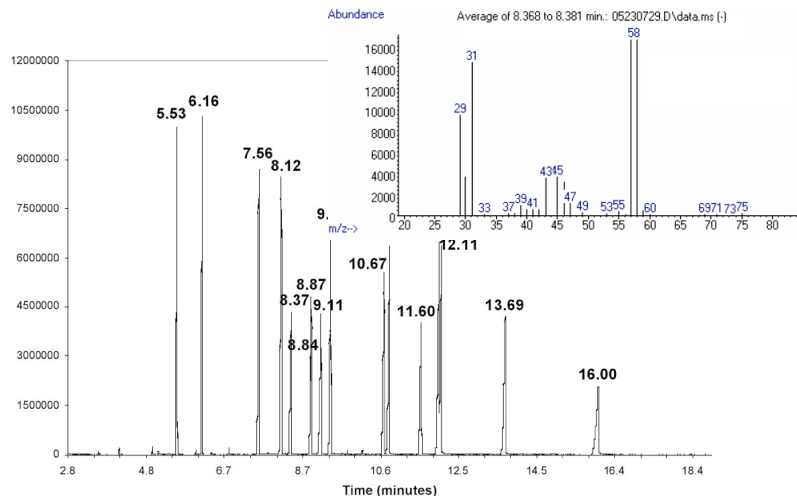
This site presents the database of comprehensive, high-resolution mass spectra of metabolites. Supported by the JST BRD project, it offers various query methods for standard spectra from Keio Univ., RIKEN PSG, and others.

The Mass Spectrometry Society of Japan officially supports MassBank.

Database

- Search Page
- Quick Search Page
- Substructure Search
- Browse Page
- Peak Search Page
- Spectral Browser
- Batch Search Service

MS Compound ID - HMDB



LC-MS Spectrum

Phenyllactate
 Phenylpyruvate
 Atrolactic acid
 Homovanillin
 Coumaric acid

...

Human Metabolome Database
 Version 2.0 | Version 1.0

Spectra Search

MS Search

Database: HMDB FoodDB DrugBank

Molecular Species: Positive Mode Negative Mode Neutral Molecule

MW (Da): 166.17394, 212.16982, 132.28712 (Da). Enter a list of MW (ie. 853.3309)

MW Tolerance (±): 0.001 (Da)

Peak list to HMDB

MS Search Result

14 results found, displaying 1 to 14

HMDB ID	Common Name	Chemical Formula	Adduct MW (Da)	[Matching HMDB MW]	MW Difference (Da)	Adduct
HMDB000225	3-(3-Hydroxyphenyl)propionic acid	C ₉ H ₁₀ O ₃	166.083	[166.062988]	1.9E-5	N/A [0+]
HMDB000522	Atrolactic acid	C ₉ H ₁₀ O ₃	166.083	[166.062988]	1.9E-5	N/A [0+]
HMDB000563	D-Phenylactic acid	C ₉ H ₁₀ O ₃	166.083	[166.062988]	1.9E-5	N/A [0+]
HMDB000258	L-3-Phenylactic acid	C ₉ H ₁₀ O ₃	166.083	[166.062988]	1.9E-5	N/A [0+]
HMDB000229	Phenylacetic acid	C ₉ H ₁₀ O ₂	166.083	[166.062988]	1.9E-5	N/A [0+]
HMDB000272	4-Methoxyphenylacetic acid	C ₉ H ₁₀ O ₃	166.083	[166.062988]	1.9E-5	N/A [0+]
HMDB000229	Disaminotyrosine	C ₉ H ₁₀ O ₃	166.083	[166.062988]	1.9E-5	N/A [0+]
HMDB000229	3-Phenylpropionic acid	C ₉ H ₁₀ O ₂	166.083	[166.062988]	1.9E-5	N/A [0+]
HMDB051273	Homovanillin	C ₉ H ₁₀ O ₃	166.083	[166.062988]	1.9E-5	N/A [0+]
HMDB000205	Phenylpyruvic acid	C ₉ H ₈ O ₃	164.047	[164.047348]	3.51E-4	N/A [0+]
HMDB001113	m-Coumaric acid	C ₉ H ₈ O ₃	164.047	[164.047348]	3.51E-4	N/A [0+]
HMDB000203	4-Hydroxycinnamic acid	C ₉ H ₈ O ₃	164.047	[164.047348]	3.51E-4	N/A [0+]
HMDB000201	2-Hydroxycinnamic acid	C ₉ H ₈ O ₃	164.047	[164.047348]	3.51E-4	N/A [0+]
HMDB122823	Enol-phenylpyruvate	C ₉ H ₈ O ₃	164.047	[164.047348]	3.51E-4	N/A [0+]

High scoring matches

MS/MS Compound ID - HMDB

- Database of 1000 experimental MS/MS spectra (low, medium and high collision energies) collected on QqQ - but largely valid for ion trap instruments as well
- Allows selection of different instruments (QqQ, ion trap, FT-MS qTOF), collision energies, ionization modes, parent ion mass tolerance and fragment ion mass tolerance
- Designed for identification of a single compound at a time

BioCrates IDQ Kit

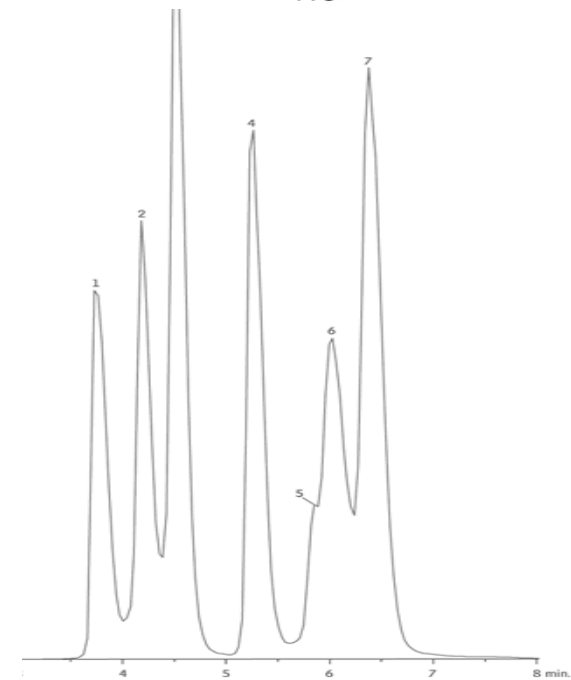
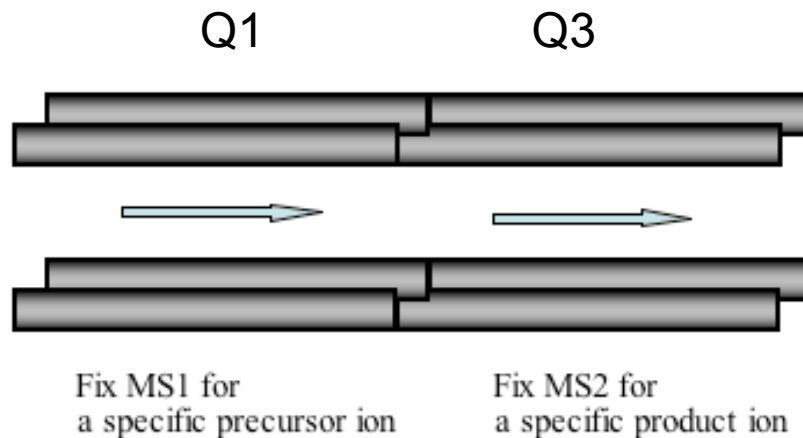
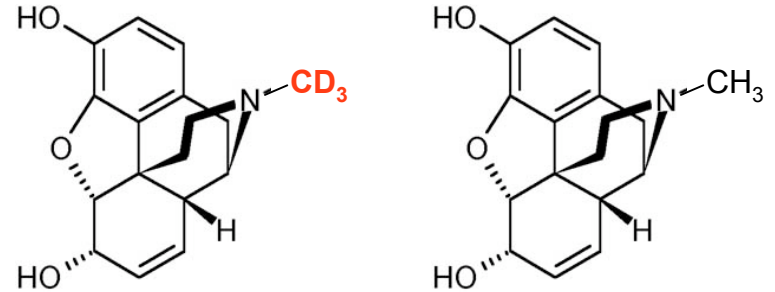


40 acylcarnitines, 13 amino acids, 15 LysoPCs, 77 PCs, 15 SMs = 160

Multiple Reaction Monitoring

Table I +MRM Transitions for Opiates.

Mass Spectrometer Experiments:				
Compound	Q1	Q3	Decustering Potential (V)	Collision Energy (V)
Morphine	286	152	46	79
Morphine	286	165	46	51
Hydromorphone	286	185	46	41
Hydromorphone	286	157	46	55
Oxymorphone	302	227	36	37
Oxymorphone	302	198	36	55
Codeine	300	152	46	85
Codeine	300	115	46	89
Hydrocodone	300	199	46	39
Hydrocodone	300	128	46	39
Oxycodone	316	240	31	39
Oxycodone	316	256	31	33
6-Monoacetylmorphine	328	211	51	55
6-Monoacetylmorphine	328	193	51	35



Sample Urine Metabolite List

Concentration range from 10 nM to 7.2 mM (1,000,000 X concentration)

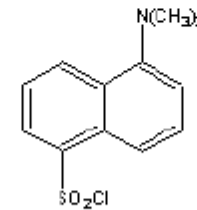
Arginine 38.7 uM	Tyrosine 204.0 uM	C14:2 Carn 0.03 uM	C4:1 Carn 0.235 uM	C8 Carnitine 1.05 uM	PC(36:5) aa 0.011 uM	LysoPC-20:4 0.039 uM	SM(22:3) 0.016 uM
Glutamine 531.0 uM	Valiine 37.0 uM	C14:2-OH 0.02 uM	C5 Carnit 4.39 uM	C9 Carnitine 1.37 uM	PC(38:5) aa 0.016 uM	LysoPC-6:0 0.073 uM	SM(24:0) 0.342 uM
Glycine 922.0 uM	Leu/Ile 128.0 uM	C16 Carn 0.021 uM	C6-OH Carn 0.703 uM	PC(28:1) aa 0.059 uM	PC(42:4) aa 0.010 uM	SM(OH)16:1 0.020 uM	SM(24:1) 0.206 uM
Histidine 1146.0 uM	Carnitiine 73.2 uM	C16-OH Cr 0.035 uM	C5-M-DC 0.531 uM	PC(30:2) aa 0.009 uM	PC(38:3) ae 0.021 uM	SM(OH)22:1 0.065 uM	SM(26:0) 0.020 uM
Methionine 15.6 uM	C10 Carn 0.324 uM	C16:1-OH 0.035 uM	C5-OH Carn 1.46 uM	PC(34:1) aa 0.094 uM	PC(38:4) ae 0.025 uM	SM(OH)22:2 0.060 uM	SM(26:1) 0.014 uM
Phenylalanin 52.7 uM	C10:1 Carn 1.83 uM	C2 Carnitine 45.2 uM	C5:1 Carn 1.84 uM	PC(34:2) aa 0.087 uM	PC(38:5) ae 0.092 uM	SM(OH)24:2 0.015 uM	Glucose 2264 uM
Proline 42.9 uM	C10:2 Carn 0.796 uM	C3 Carnitine 2.12 uM	C5:1-OH 0.367 uM	PC(34:4) aa 0.009 uM	PC(38:6) ae 0.068 uM	SM(16:0) 0.352 uM	Creatinine 7222 uM
Serine 408.0 uM	C12 Carn 0.203 uM	C3-OH Carn 0.163 uM	C6 Carnitine 0.814 uM	PC(36:1) aa 0.053 uM	PC(40:5) ae 0.014 uM	SM(16:1) 0.001 uM	
Threonine 220.0 uM	C14 Carn 0.063 uM	C4 Carnint 11.0 uM	C6:1 Carnt 0.294 uM	PC(36:3) aa 0.054 uM	PC(42:3) ae 0.012 uM	SM(18:1) 0.023 uM	
Tryptophan 15.0 uM	C14:1-OH 0.016 uM	C4-OH Carn 0.405 uM	C8-OH Carn 0.509 uM	PC(36:4) aa 0.051 uM	PC(44:3) ae 0.014 uM	SM(20:2) 0.020 uM	

Quantitative MS Metabolomics With Chemoselective Labeling

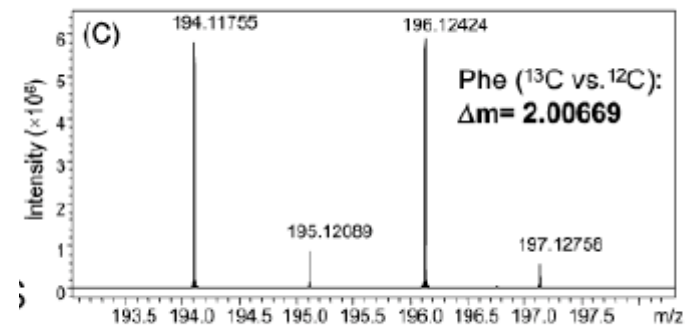
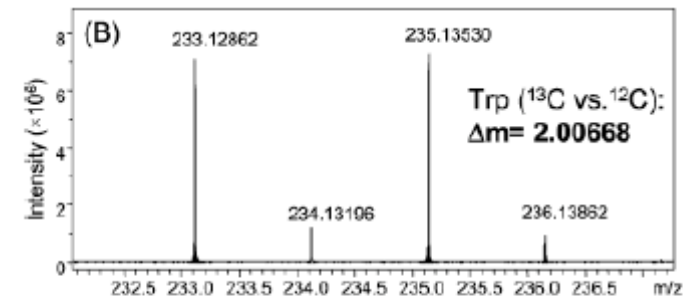
Peak Pair Table_C13/C12-Dansylation-Labeled Urine_#9-Sep0707-FTMS
File:RPLC-FTMS: #9-Sept0707-Run-6

Mass Diff. Error is ppm between theoretical mass difference and determined mass difference.
Listed pairs are the partial pairs that S/N > 20 counted manually. > 5.0E5 (Background ~2-2.5E4)
Signal that > 1.0E6 are the S/N > 50.

	RT (min.)	¹² C-Dns-labeled (m/z)	¹³ C-Dns-labeled (m/z)	Mass Differences	Mass Diff. Error (ppm)	Ion Int.	Comment
1	1.02	387.068162	389.074675	2.00651	0.51	1.2E+06	Good Pair
2	1.02	521.170220	523.177238	2.00702	-0.59	1.2E+06	Good Pair
3	1.06	389.128016	391.134692	2.00668	0.09	8.0E+06	Good Pair
4	1.06	424.117491	426.124175	2.00668	0.06	1.7E+06	Good Pair
5	1.06	375.077670	377.084285	2.00662	0.25	1.0E+06	Good Pair
6	1.09	389.127995	391.134601	2.00661	0.27	4.0E+06	Very Good Pair
7	1.16	388.107705	390.114456	2.00675	-0.11	1.8E+06	Good Pair
8	1.16	495.089348	497.096414	2.00707	-0.72	2.0E+06	Good Pair
9	1.16	517.071236	519.078356	2.00712	-0.79	2.0E+06	Good Pair
10	1.16	526.131270	528.138634	2.00736	-1.24	2.5E+06	Good Pair
11	1.16	533.044803	535.051978	2.00737	-1.24	5.0E+05	
12	1.16	555.121314	557.128303	2.00699	-0.50	3.5E+06	Good Pair
13	1.16	569.191220	571.197888	2.00667	0.07	3.5E+06	Good Pair
14	1.20	359.072965	361.079792	2.00683	-0.32	1.5E+07	Super Good Pair
15	1.20	381.055037	383.061828	2.00679	-0.21	1.4E+06	Good Pair
16	1.20	390.115176	392.122062	2.00689	-0.45	4.0E+06	Very Good Pair
17	1.20	560.113434	562.120222	2.00679	-0.14	1.1E+06	Good Pair
18	1.26	403.141909	405.148411	2.00650	0.51	2.2E+07	Super Good Pair
19	1.26	421.156070	423.162728	2.00666	0.12	5.5E+05	
20	1.26	501.154144	503.160581	2.00644	0.54	1.0E+06	Good Pair
21	1.26	512.207250	514.213198	2.00595	1.48	5.0E+05	
22	1.26	719.145138	721.150728	2.00559	1.55	1.0E+06	Good Pair
23	1.26	763.215346	765.221011	2.00567	1.37	1.5E+06	Good Pair
24	1.26	779.177121	781.183454	2.00633	0.48	5.0E+05	
25	1.33	410.090287	412.097029	2.00674	-0.08	1.3E+06	Good Pair
26	1.33	425.126006	427.132314	2.00631	0.94	6.0E+05	
27	1.33	452.185226	454.191871	2.00664	0.14	5.0E+05	



¹²C and ¹³C Dansyl labeling



Quantitative MS Metabolomics in Human Urine

Compound	Retention Time (min)	Conc. in Urine (µM)	Compound	Retention Time (min)	Conc. in Urine (µM)
Dns-o-phospho -L-serine	0.92	<D.L. *	Dns-Ile	6.35	25
Dns-o-phospho -L-tyrosine	0.95	<D.L.	Dns-3-aminosalicylic acid	6.44	0.5
Dns-adenosine monophosphate	0.99	<D.L.	Dns-pipecolic acid	6.50	0.5
Dns-o-phosphoethanolamine	1.06	16	Dns-Leu	6.54	54
Dns-glucosamine	1.06	22	Dns-cystathionine	6.54	0.3
Dns-o-phospho -L-threonine	1.09	<D.L.	Dns-Leu-Pro	6.60	0.4
Dns-6-dimet hylamine purine	1.20	<D.L.	Dns-5-hydroxylysine	6.65	1.6
Dns-3-methyl -histidine	1.22	80	Dns-Cystine	6.73	160
Dns-taurine	1.25	834	Dns-N-norleucine	6.81	0.1
Dns-carnosine	1.34	28	Dns-5-hydroxydopamine	7.17	<D.L.
Dns-Arg	1.53	36	Dns-dimethylamine	7.33	293
Dns-Asn	1.55	133	Dns-5-HIAA	7.46	18
Dns-hypotaurine	1.58	10	Dns-umbelliferone	7.47	1.9
Dns-homocarnosine	1.61	3.9	Dns-2,3-diaminopropionic acid	7.63	<D.L.
Dns-guanidine	1.62	<D.L.	Dns-L-ornithine	7.70	15
Dns-Gln	1.72	633	Dns-4-acetyamidophenol	7.73	51
Dns-allantoin	1.83	3.8	Dns-procaine	7.73	8.9
Dns-L-citrulline	1.87	2.9	Dns-homocystine	7.76	3.3
Dns-1 (or 3 -)-methylhistamine	1.94	1.9	Dns-acetaminophen	7.97	82
Dns-adenosine	2.06	2.6	Dns-Phe-Phe	8.03	0.4
Dns-methylguanidine	2.20	<D.L.	Dns-5-methyo xysalicylic acid	8.04	2.1
Dns-Ser	2.24	511	Dns-Lys	8.16	184
Dns-aspartic acid amide	2.44	26	Dns-aniline	8.17	<D.L.
Dns-4-hydroxy -proline	2.56	2.3	Dns-leu -Phe	8.22	0.3
Dns-Glu	2.57	21	Dns-His	8.35	1550
Dns-Asp	2.60	90	Dns-4-thialysine	8.37	<D.L.
Dns-Thr	3.03	157	Dns-benzylamine	8.38	<D.L.
Dns-epinephrine	3.05	<D.L.	Dns-1-ephedrine	8.50	0.6
Dns-ethanolamine	3.11	471	Dns-tryptamine	8.63	0.4
Dns-aminoadipic acid	3.17	70	Dns-pyridoxamine	8.94	<D.L.
Dns-Gly	3.43	2510	Dns-2-methyl -benzylamine	9.24	<D.L.
Dns-Ala	3.88	593	Dns-5-hydroxytryptophan	9.25	0.12
Dns-aminolevulinic acid	3.97	30	Dns-1,3-diaminopropane	9.44	0.23
Dns-r-amino -butyric acid	3.98	4.6	Dns-putrescine	9.60	0.5
Dns-p-amino -hippuric acid	3.98	2.9	Dns-1,2-diaminopropane	9.66	0.1
Dns-5-hydro xymethyluricil	4.58	1.9	Dns-tyrosinamide	9.79	29
Dns-tryptophanamide	4.70	5.5	Dns-dopamine	10.08	140
Dns-isoguarine	4.75	<D.L.	Dns-cadaverine	10.08	0.08
Dns-5-aminopentanoic acid	4.79	1.6	Dns-histamine	10.19	0.4
Dns-sarcosine	4.81	7.2	Dns-3-methoxy -tyramine	10.19	9.2
Dns-3-amino -isobutyrate	4.81	85	Dns-Tyr	10.28	321
Dns-2-aminobutyric acid	4.91	17	Dns-cvsteamine	10.44	<D.L.

Compound	Retention Time (min)	Conc. in Urine (µM)	Compound	Retention Time (min)	Conc. in Urine (µM)
Dns-Ser-Leu	5.06	<D.L.	Dns-phenol	10.52	1.0
Dns-Pro	5.07	13	Dns-desipramine	10.57	<D.L.
Dns-pyridoxine	5.27	<D.L.	Dns-3-chloro tyrosine	10.58	<D.L.
Dns-Val	5.35	75	Dns-2,3-diaminosalicylic acid	10.60	0.6
Dns-Met	5.40	16	Dns-octopamine	10.75	<D.L.
Dns-Thr-Leu	5.40	0.6	Dns-serotonin	10.85	1.0
Dns-3-hydroxypicolinic acid	5.47	44	Dns-o-(p or m)-cresol	10.93	2.1
Dns-salicylic acid	5.51	7.6	Dns-metanephrine	10.97	0.04
Dns-Trp	5.59	120	Dns-propranolol	11.00	0.04
Dns-kynurenine	5.66	6.3	Dns-4-aminophenol	11.04	<D.L.
Dns-Gly-Leu	5.79	1.1	Dns-synephrine	11.06	0.27
Dns-Gly-Trp	5.85	<D.L.	Dns-phenylephrine	11.17	0.03
Dns-norvaline	5.89	0.3	Dns-tyramine	11.22	5.1
Dns-Ala-leu	5.89	<D.L.	Dns-hydroquinone	11.28	<D.L.
Dns-ethylamine	5.90	25	Dns-spermidine	11.37	0.4
Dns-4-aminobenzoic acid	5.99	1.6	Dns-diidodthyronine	11.59	<D.L.
Dns-Ala-Trp	6.00	0.5	Dns-4-isopropylphenol	11.81	<D.L.
Dns-3-aminobenzoic acid	6.08	1.2	Dns-spermine	12.05	0.3
Dns-Phe	6.20	90			

672 peaks by amino labeling
 120 standards spiked
 92 peaks identified/quantified
 30 nM - 2.51 mM

820 peaks by carboxy labeling
 Still assessing

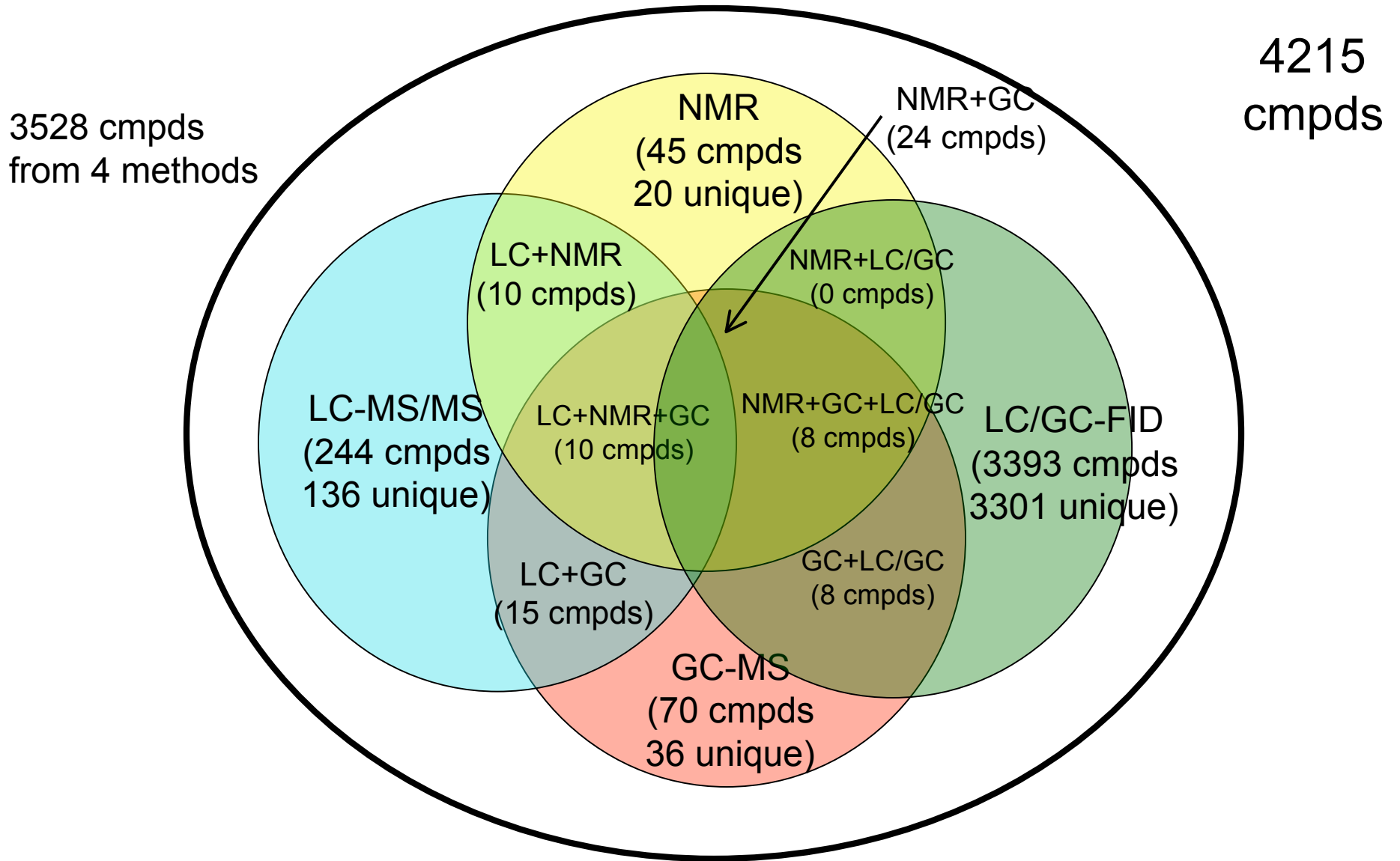
Advantages to Derivatization

- Tags can convert non-UV active compounds into UV or fluorescently detectable cmpds
- Tags improve ionization efficiency and lower limit of detection
- Tags permit affinity purification and concentration
- Tags make polar molecules hydrophobic, leading to better LC separations
- Tags permit isotope based quantification
- Tags greatly increase # compounds detected
- Tags allow independent confirmation of “real” peaks
- Best route to automated ID & quantification by LC-MS

What's Possible

- NMR-based metabolomics (~50 metabolites identified/quantified, μM limit, 500 μL)
- GC-MS based metabolomics (~90 metabolites identified/quantified, $<\mu\text{M}$ limit, 50 μL)
- DI-MS based metabolomics (160 metabolites identified/quantified, nM limit, 20 μL)
- LC-MS based metabolomics (300 metabolites identified/quantified, nM limit, 50 μL)
- Lipidomics (3000 lipids identified and semi-quantified, nM sensitivity, 1 mL)

The Serum Metabolome



Conclusions & Trends

- **Quantitative metabolomics is becoming more the “norm”**
- **Possible to ID and quantify >3000 compounds down to pM sensitivity**
- **NMR-based quantitative metabolomics**
 - Depends critically on existence of searchable spectral DBs
 - Moving from 1D NMR to 2D NMR
 - Becoming increasingly automated or semi-automated
 - Moving from purely commercial realm to open source/access
- **MS-based quantitative metabolomics**
 - Slower in uptake than NMR-based methods
 - Depends critically on existence of searchable spectral DBs
 - Depends on having labeled standards for quantification
 - More easily done with lipids or lipid classes

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