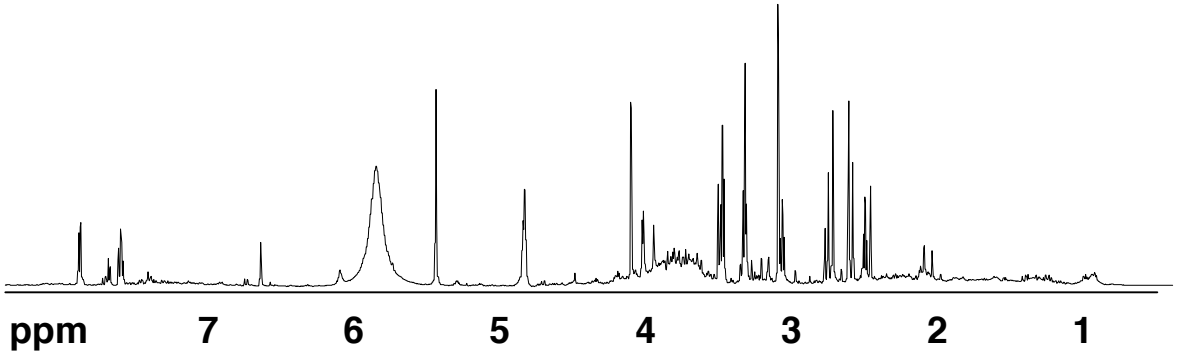
Trends in Quantitative Metabolomics

David Wishart

University of Alberta, Edmonton, Canada

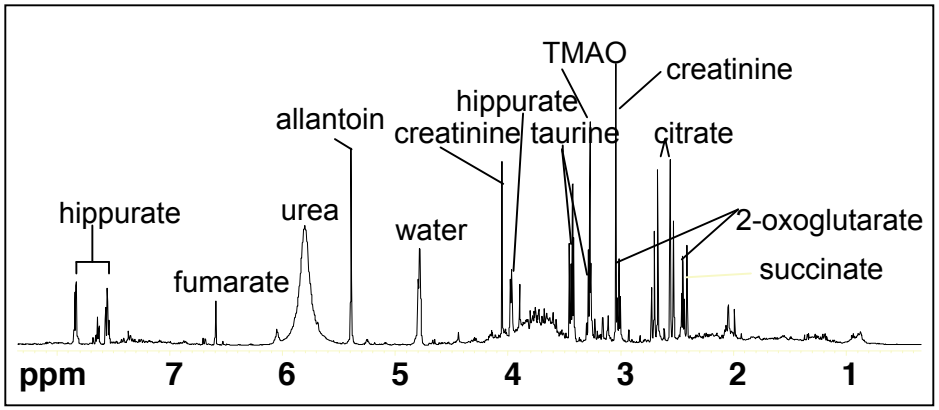
ABRF 2010, Sacramento CA, March 23, 2010

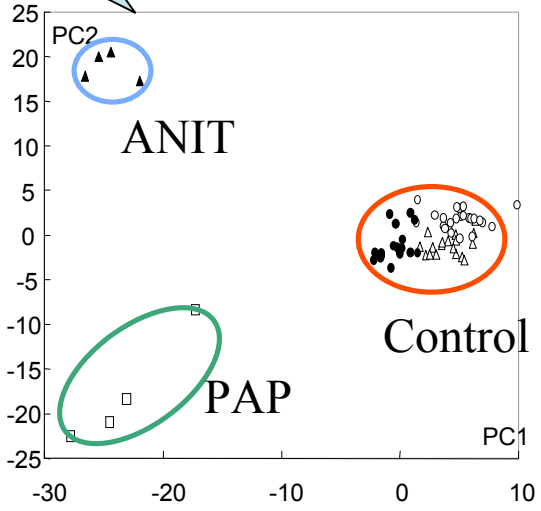
2 Routes to Metabolomics



Quantitative Or Targeted Methods

Chemometric or Non-targeted Methods

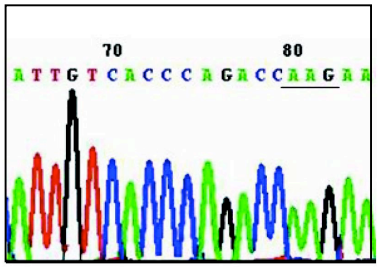




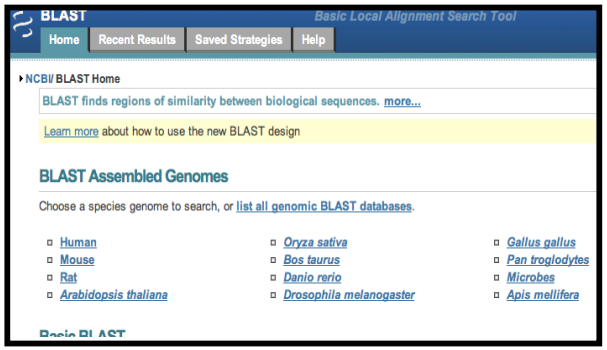
Quantitative Metabolomics

- ***Compounds are identified and quantified absolutely***
- **Data is independent of platform or technology (NMR, MS, HPLC)**
- **Data is uniformly formated, 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)**

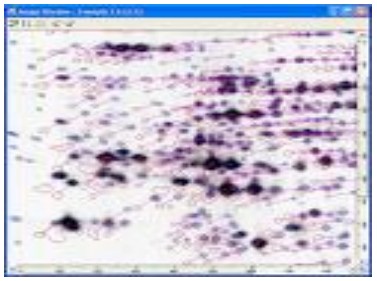
The Problem With Metabolomics



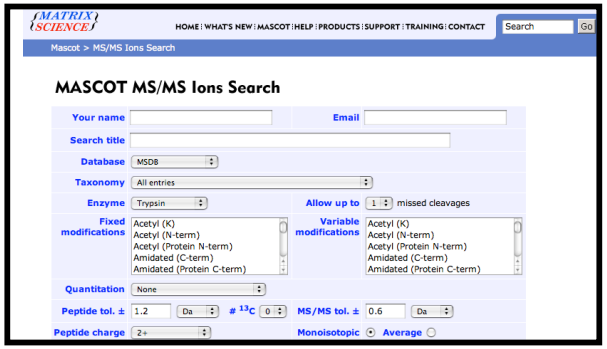
Genomics



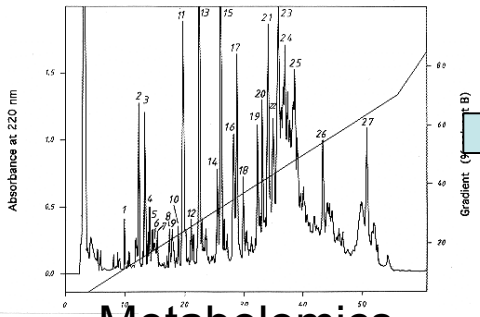
Gene IDs +
Transcript
Abundance



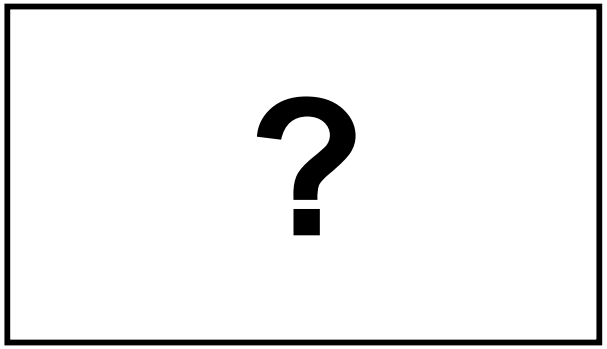
Proteomics



Protein IDs +
Concentrations



Metabolomics

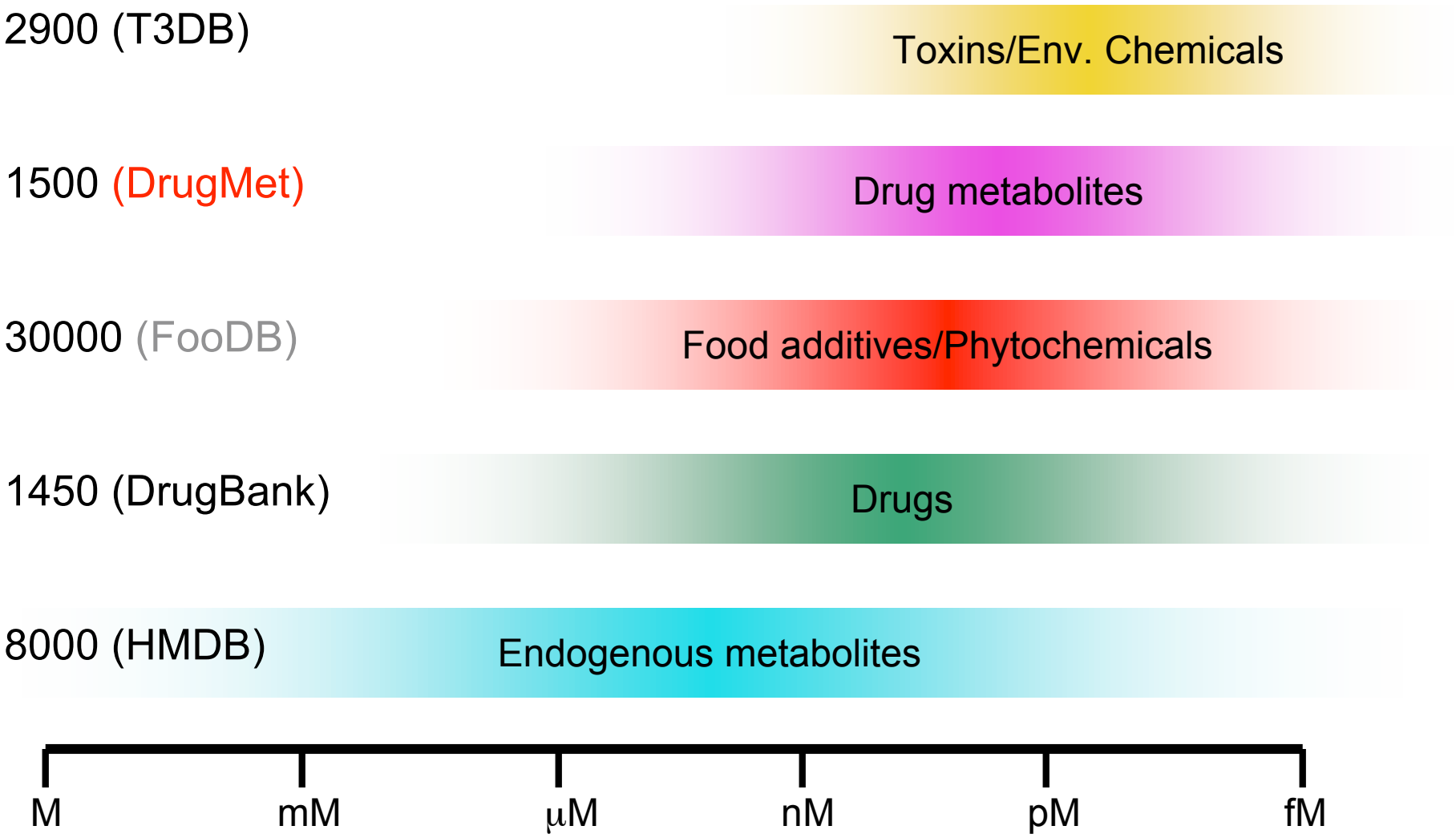


Metabolite IDs +
Concentrations

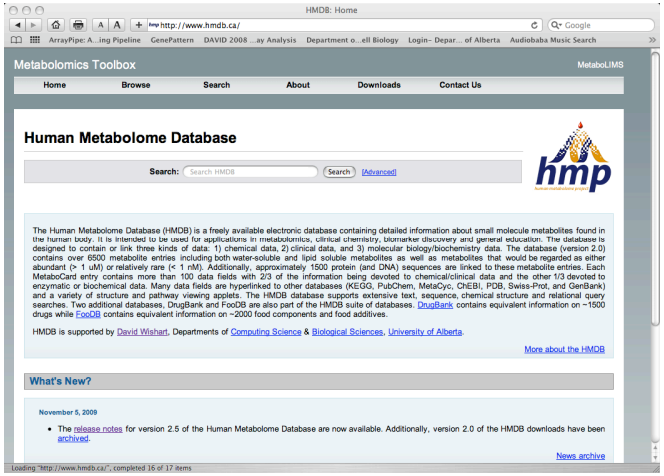
The Human Metabolome Project

- $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

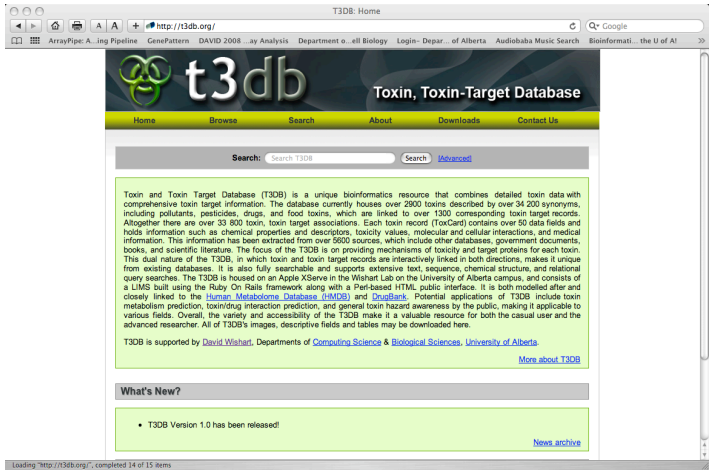
Human Metabolomes



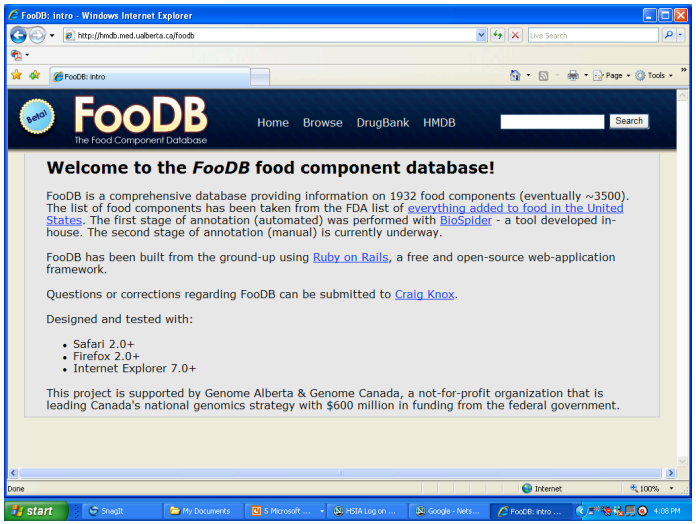
Meet the Metabolomes…



http://www.hmdb.ca



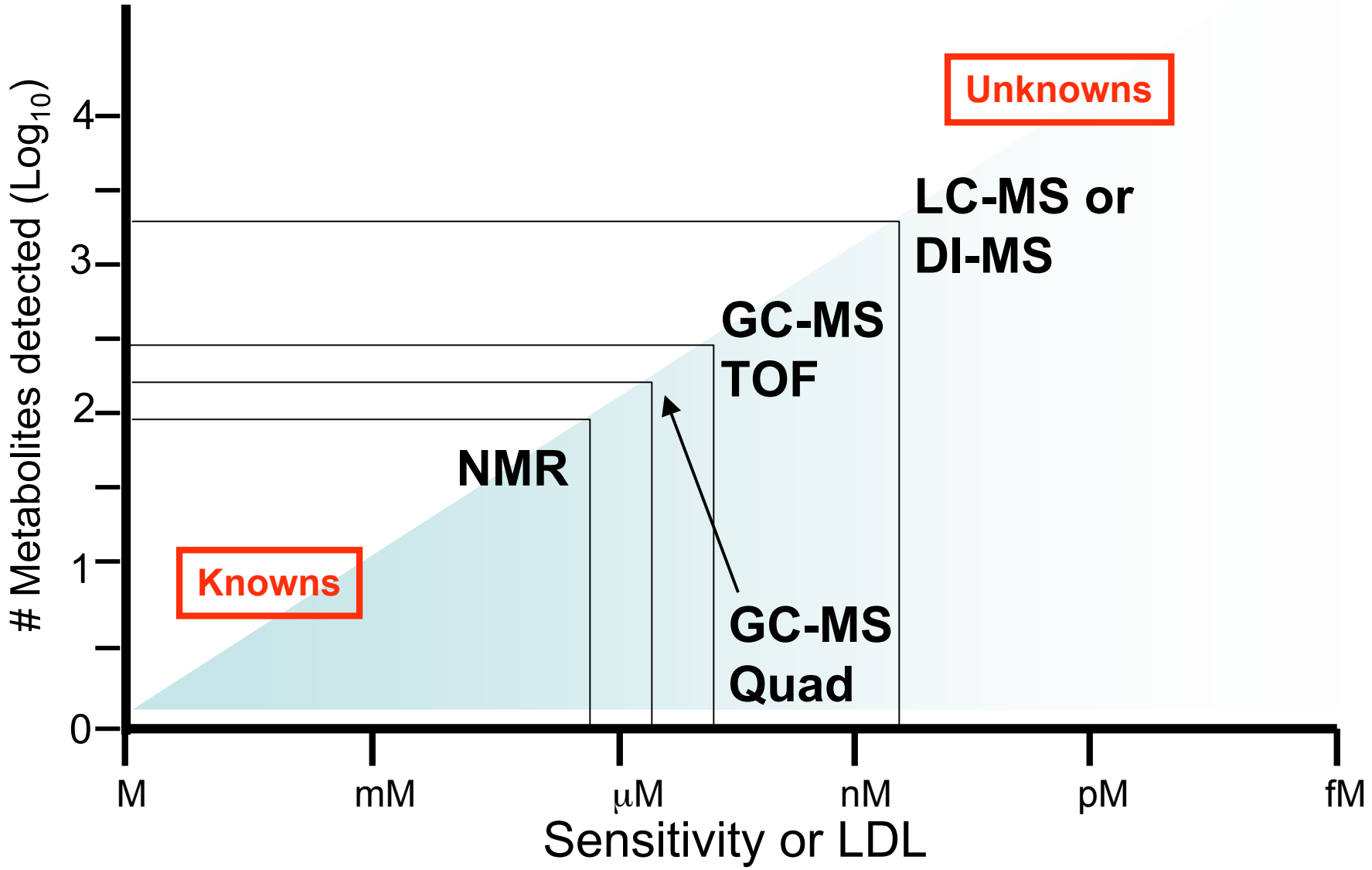
http://www.T3DB.org



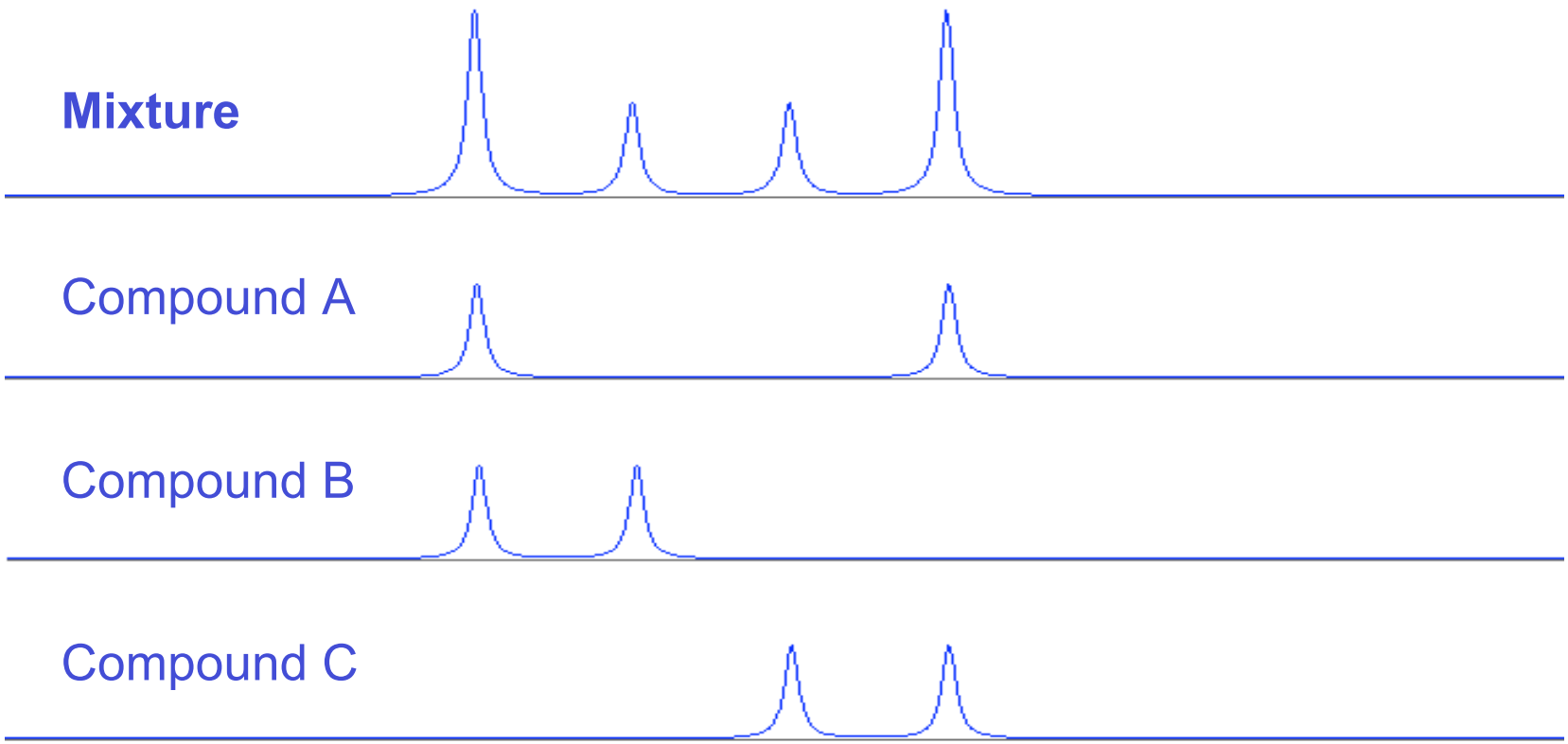
http://www.foodbs.org/foodb

http://www.drugbank.ca

Technology & Sensitivity

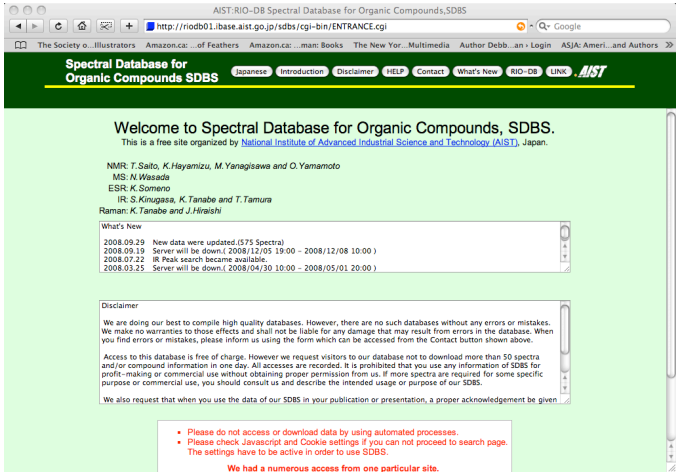


Metabolite ID by Spectral Deconvolution (NMR)

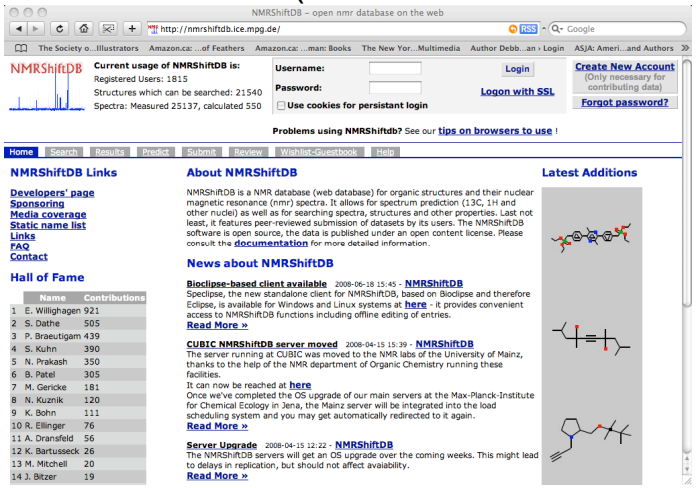


NMR Spectral DBs

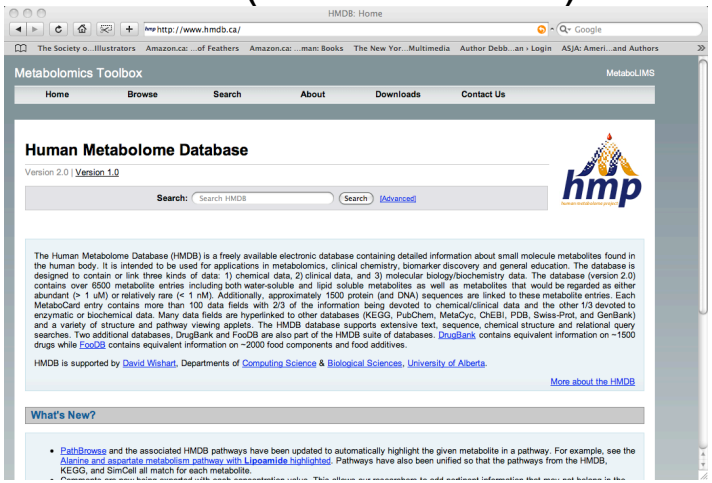
SBDS (http://riodb01.ibase.aist.go.jp)



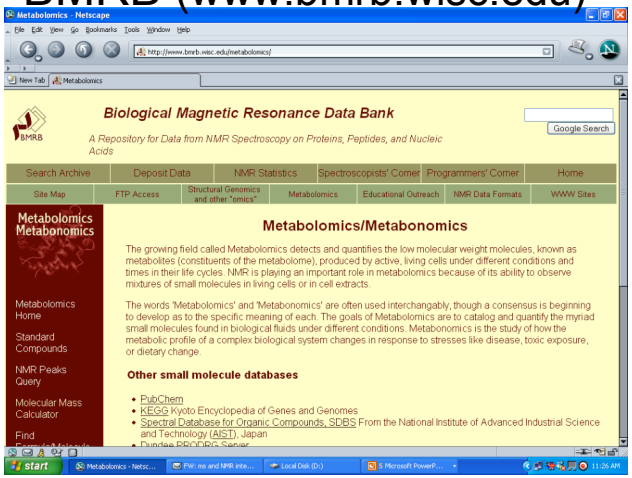
NMRShiftDB (www.ebi.ac.uk/nmrshiftdb/)



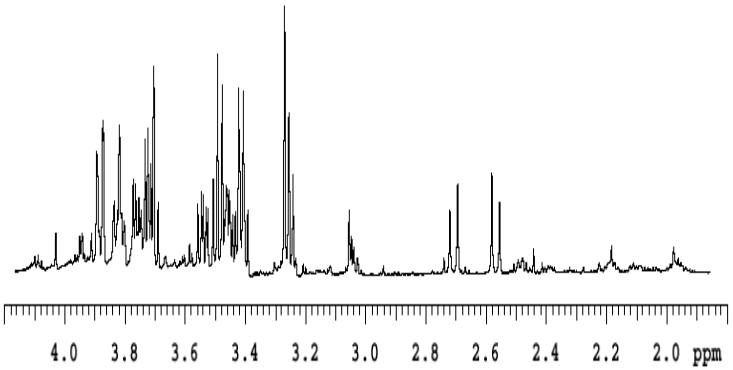
HMDB (www.hmdb.ca)



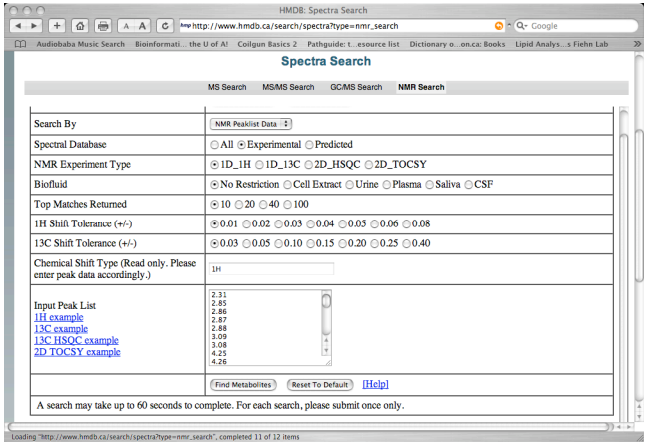
BMRB (www.bmrb.wisc.edu)



NMR Compound ID - HMDB

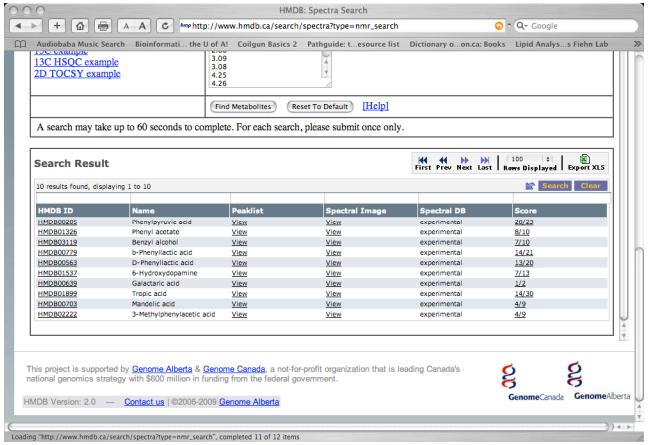


NMR spectrum of mixture



Peak list to HMDB

Phenyllactate
Phenylpyruvate
Phenylacetic acid
Tropic acid
Benzyl alcohol
…

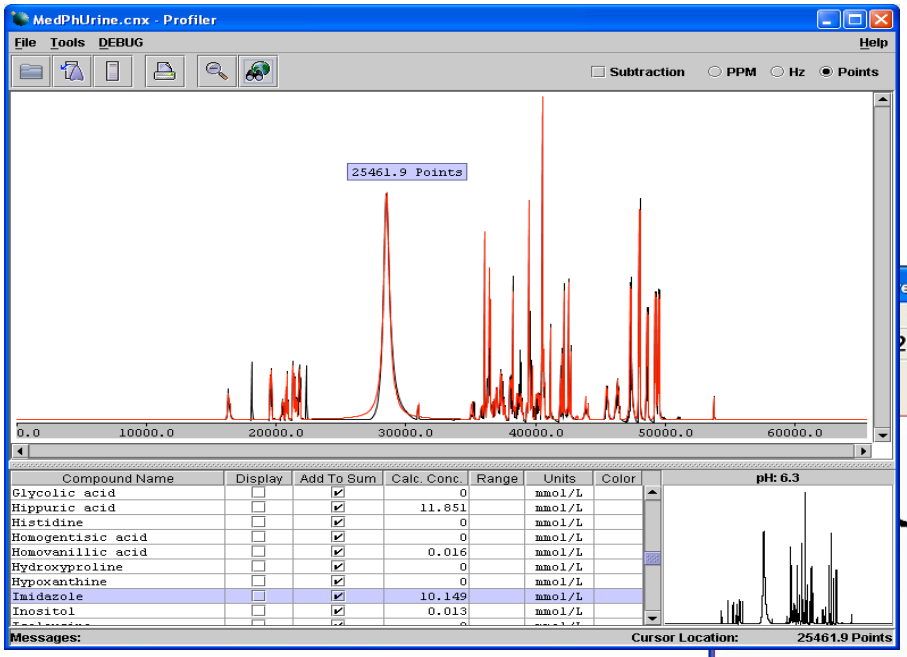


High scoring matches

NMR Compound ID - HMDB

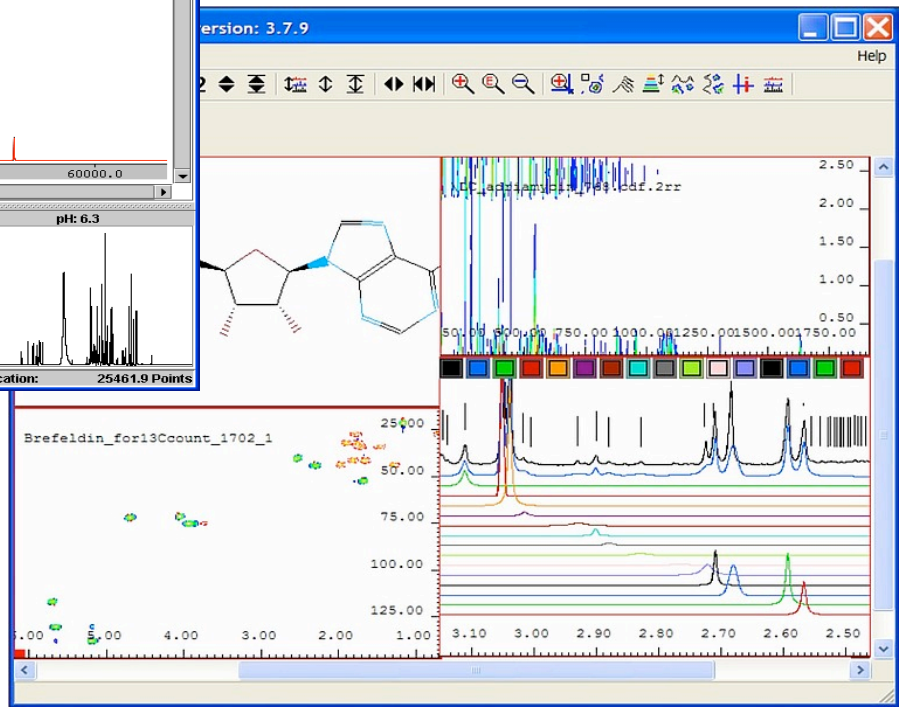
- Database of 1000 experimental ^{1}H NMR and 1000 ^{13}C HSQC NMR reference spectra (in H_2O) + ~500 TOCSY spectra
- Database of 3000 predicted ^{1}H NMR and ^{13}C NMR spectra
- Accepts 1D ^{1}H NMR ^{13}C NMR, TOCSY and HSQC queries
- Supports different chemical shift tolerances for ^{1}H and ^{13}C,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

Commercial Tools For NMR-based Quant. Metabolomics

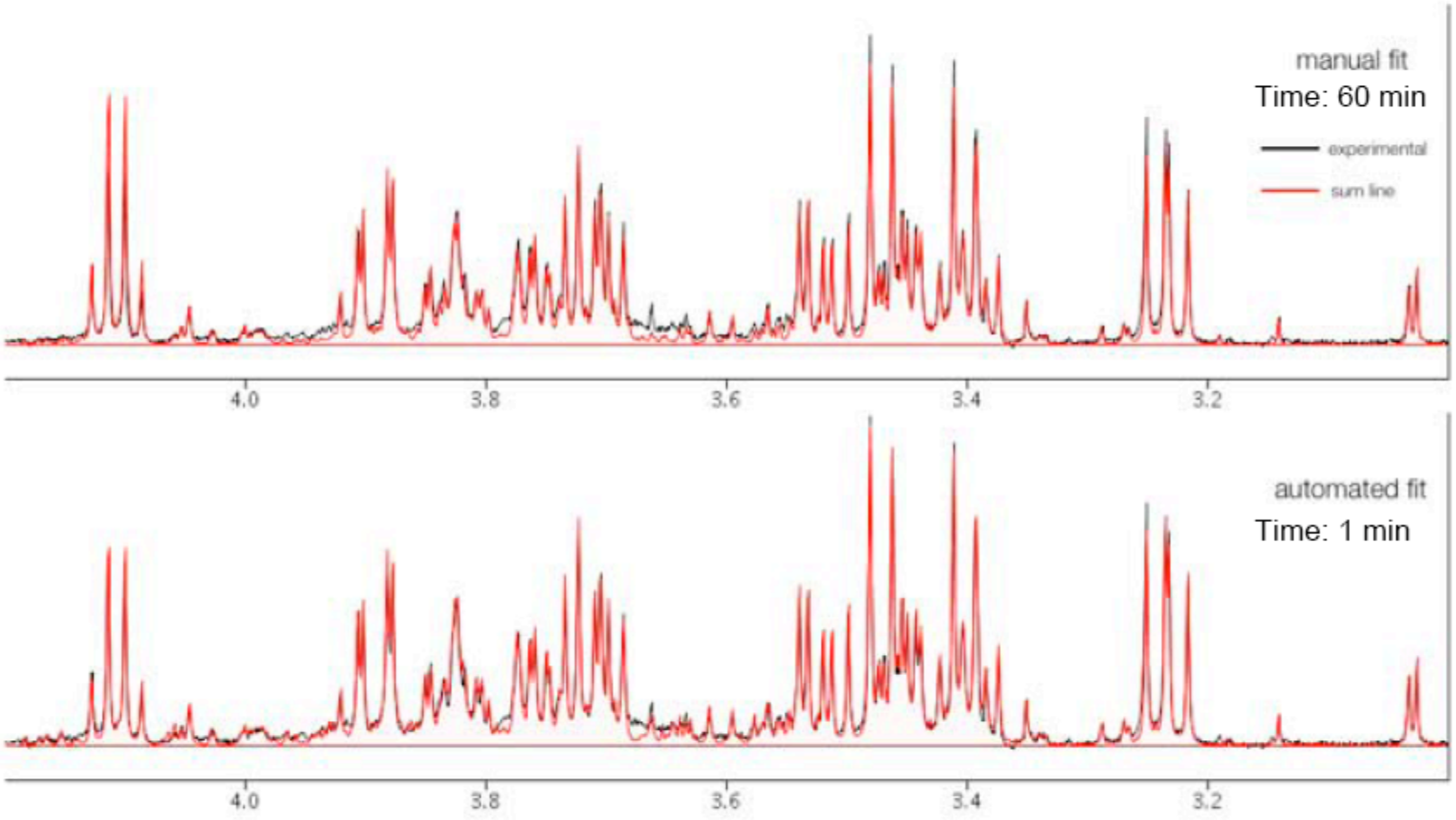


www.chenomx.com

www.bruker-biospin.com

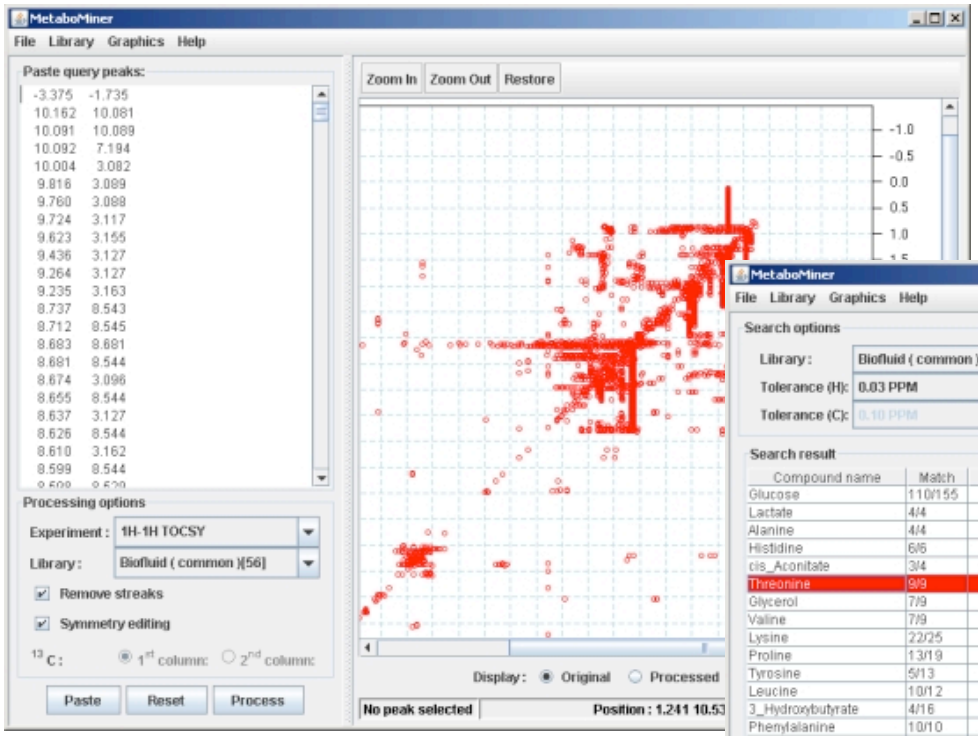


Automated vs Manual Fitting



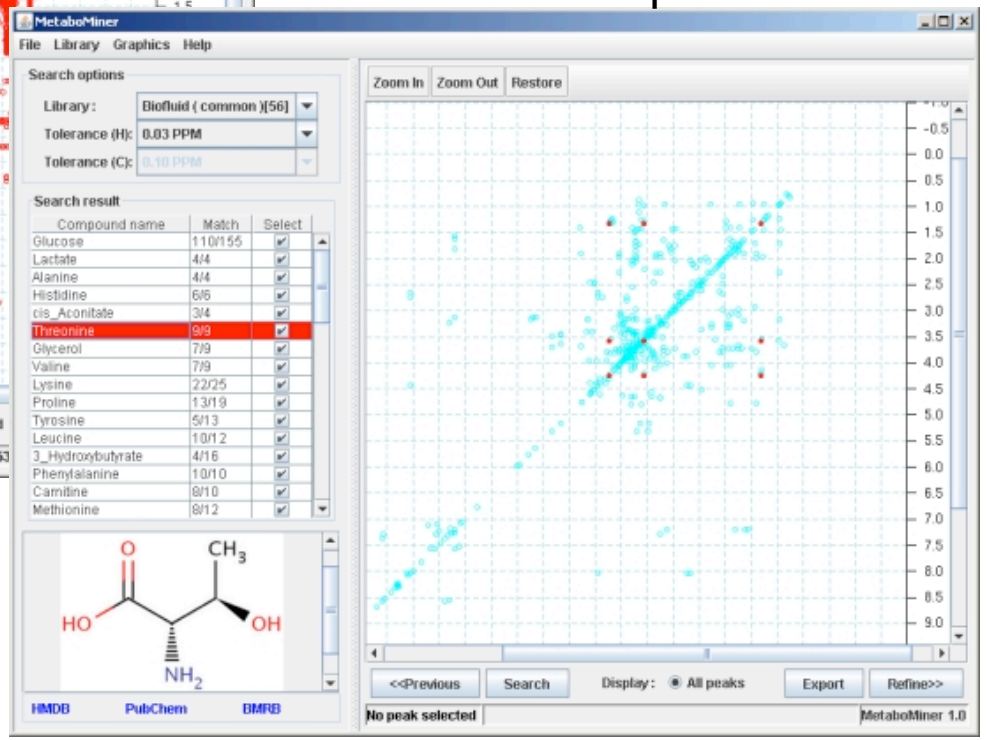
Cerebrospinal Fluid (cmpd ID correlation ~95%)

NMR Compound ID from Mixtures - MetaboMiner



Raw TOCSY Spectrum

ID'd Compounds



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

MetaboMiner Performance

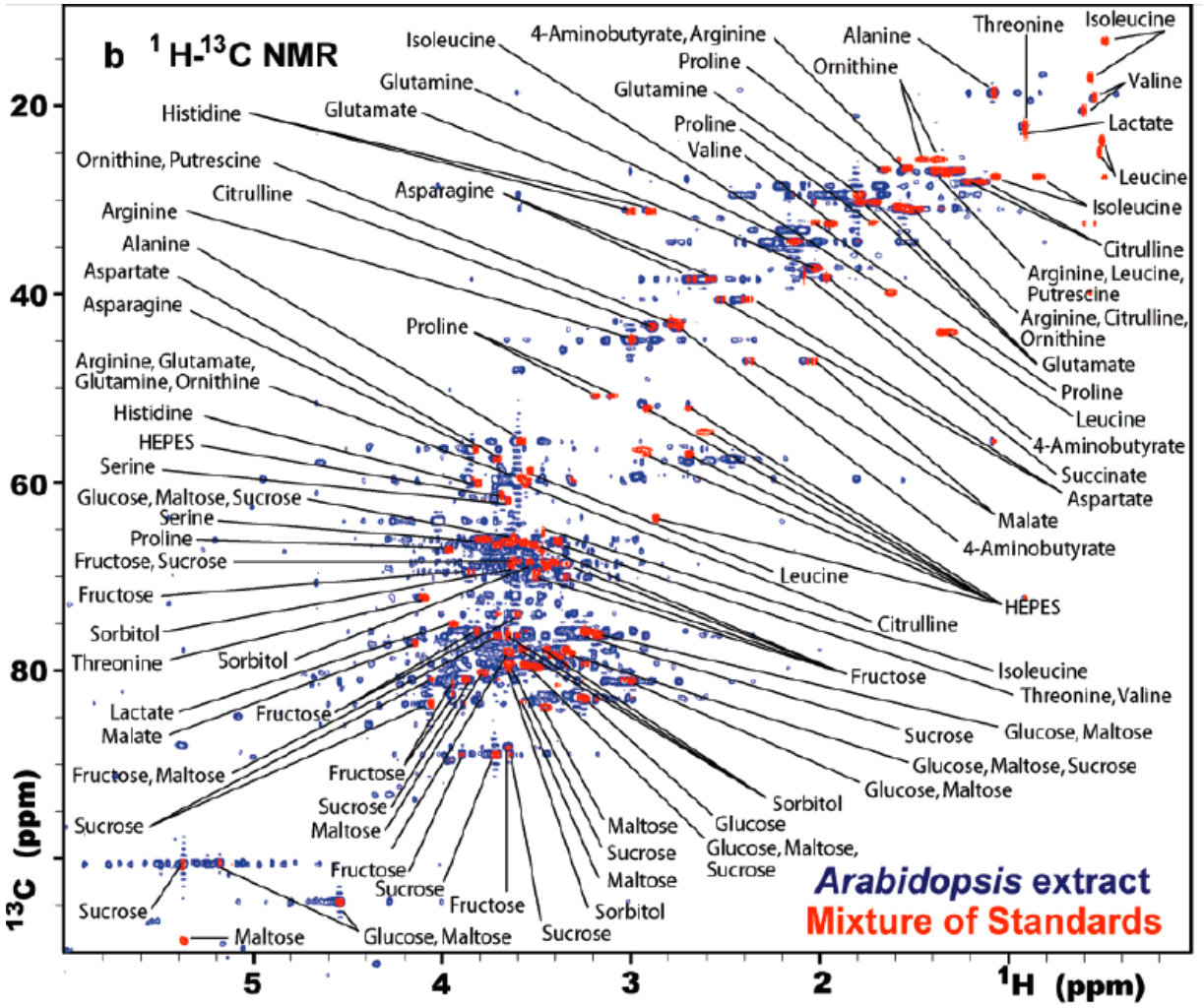
Sample	pH	TOCSY			HSQC		
		Identifiable*	Identified	Correct	Identifiable*	Identified	Correct
Cocktail	7.2	28	31	27	28	31	26
	4.2		28	24		30	22
Plasma	7.3	28	32	27	31	32	27
	8.8		25	22		29	24

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

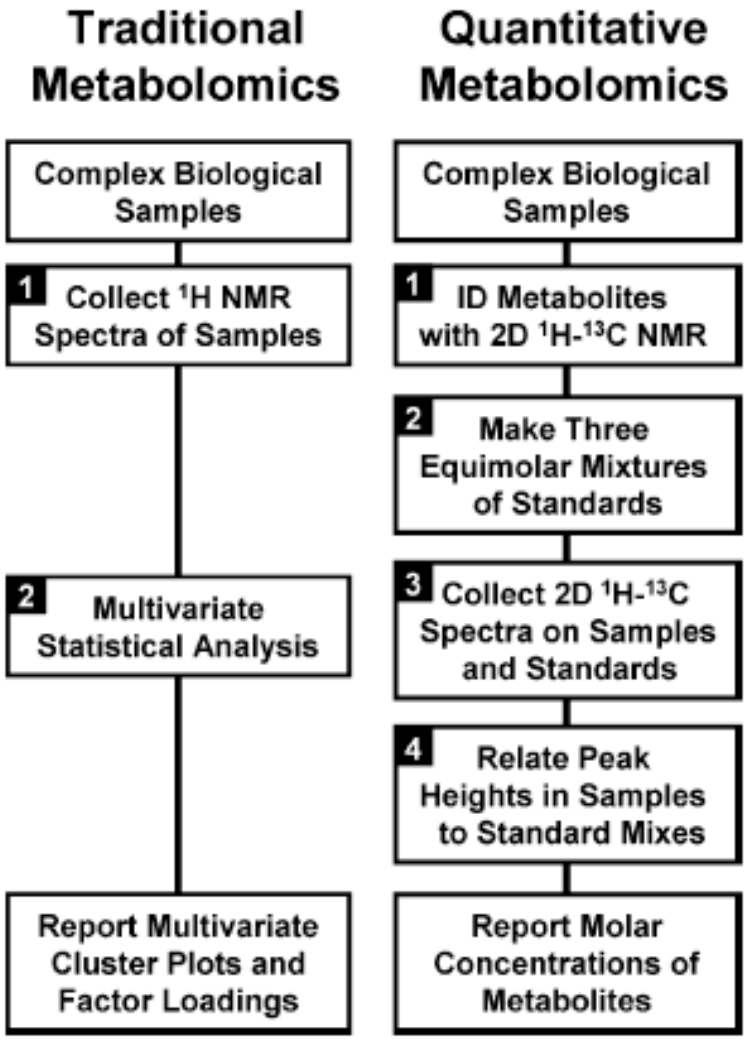
Anal. Chem. **2007,** *79,* 9385–9390

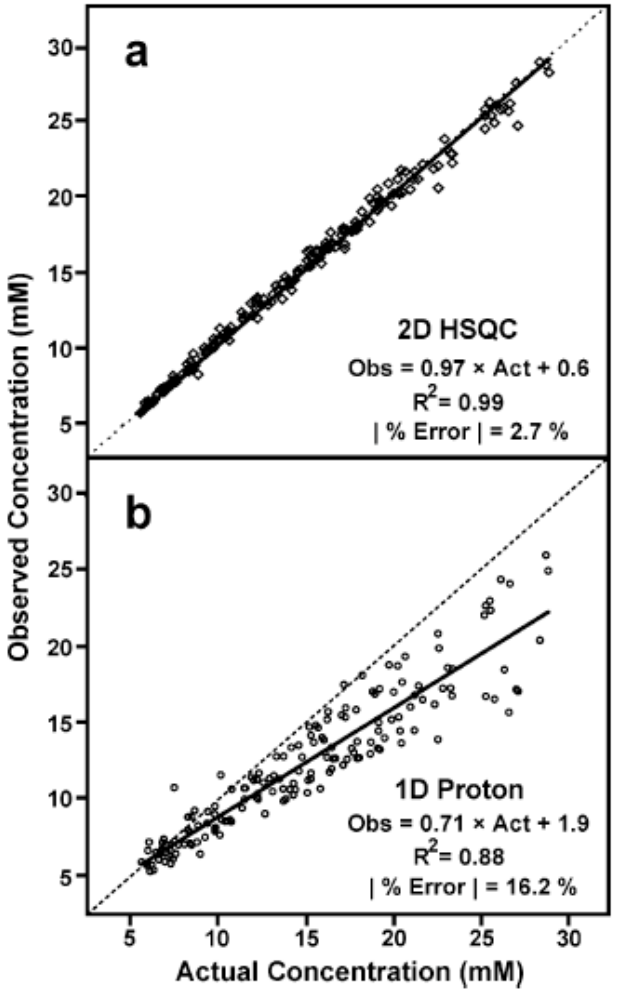
Method for Determining Molar Concentrations of Metabolites in Complex Solutions from Two-Dimensional $^{1}H-^{13}C$ NMR Spectra

Ian A. Lewis, Seth C. Schommer, Brendan Hodis, Kate A. Robb, Marco Tonelli, William M. Westler, Michael R. Sussman, and John L. Markley*



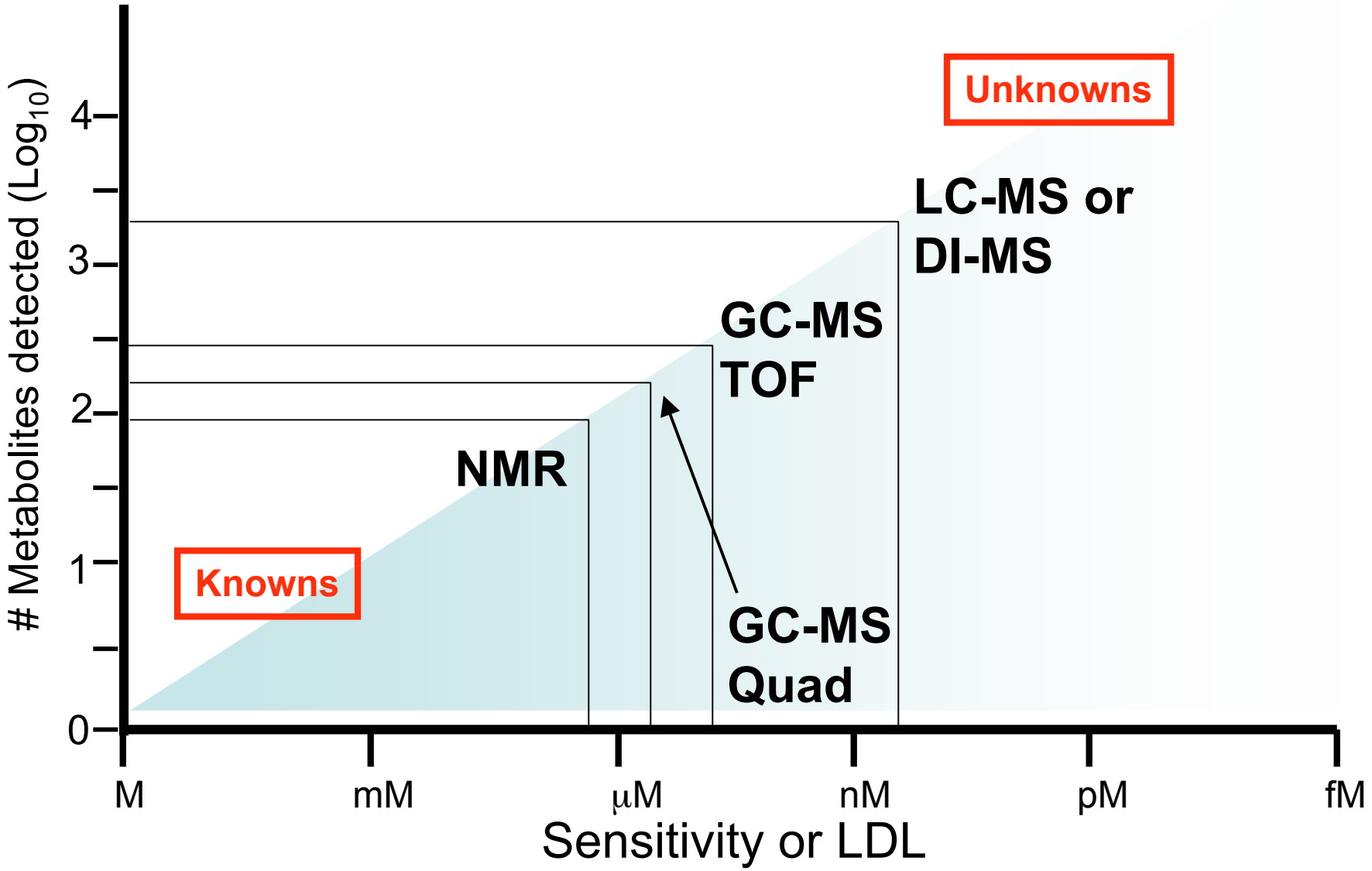
Quantitative Metabolomics (2D NMR)



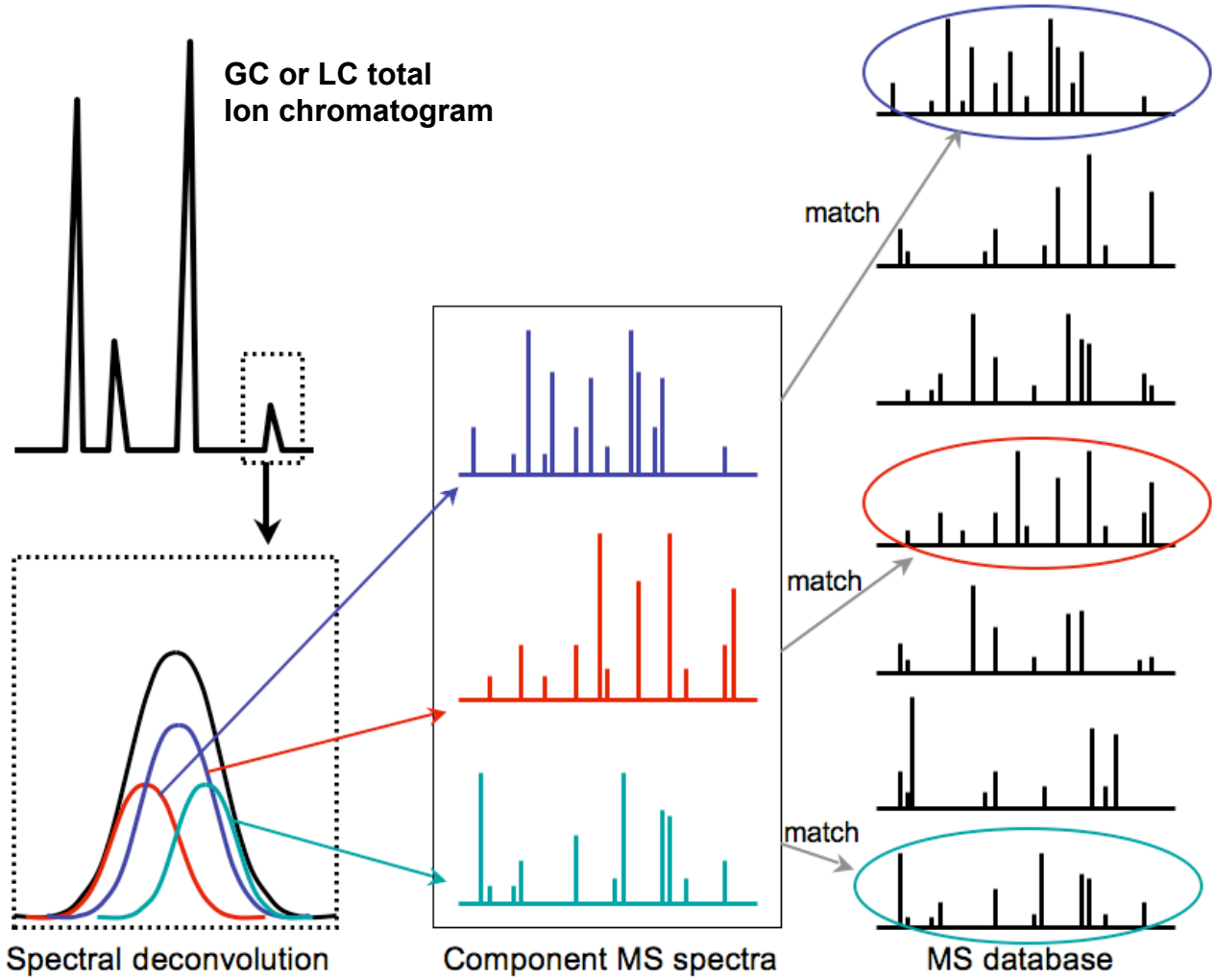


Lewis IA et al. Anal. Chem. 79:9385 (2007)

Technology & Sensitivity

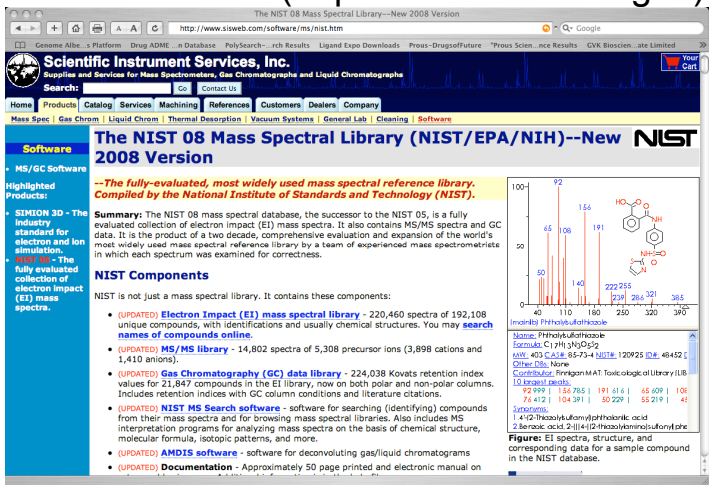


Metabolite ID by GC/LC-MS

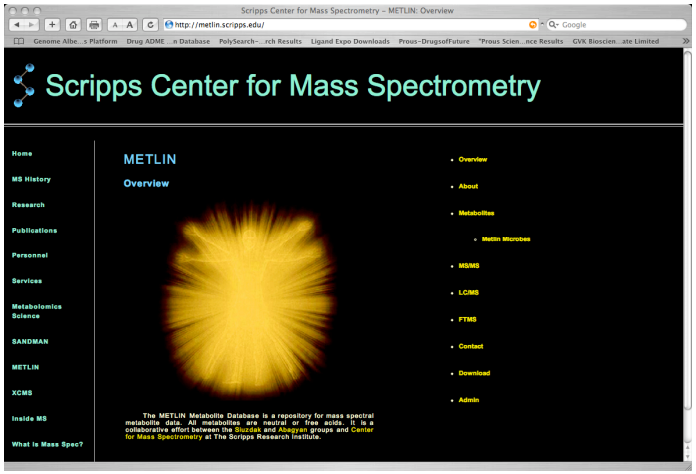


MS Spectral DBs

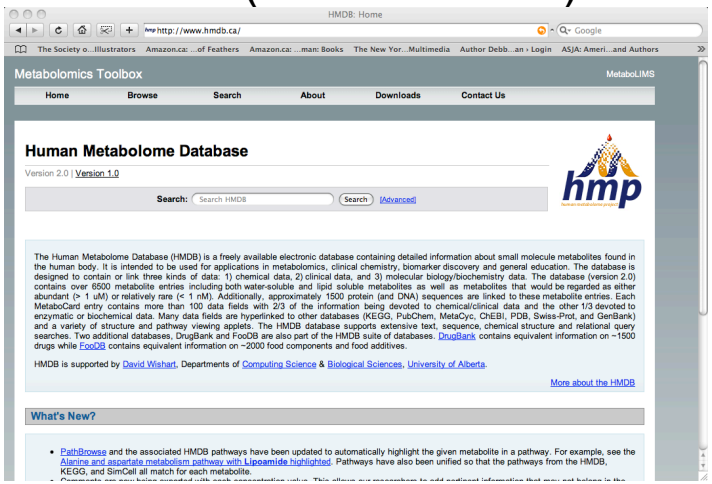
NIST/AMDIS (http://chemdata.nist.gov)



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

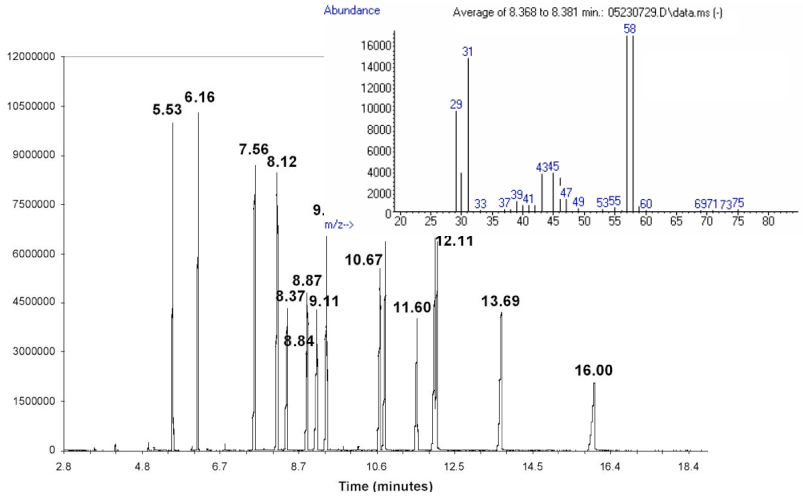


HMDB (www.hmdb.ca)

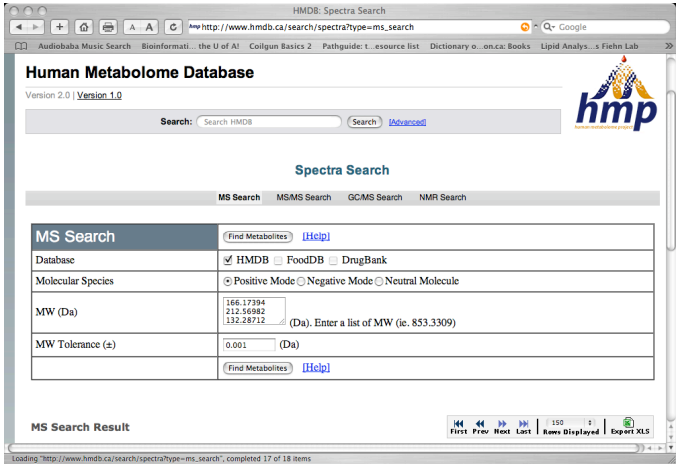


MassBank (www.massbank.jp)

MS Compound ID - HMDB

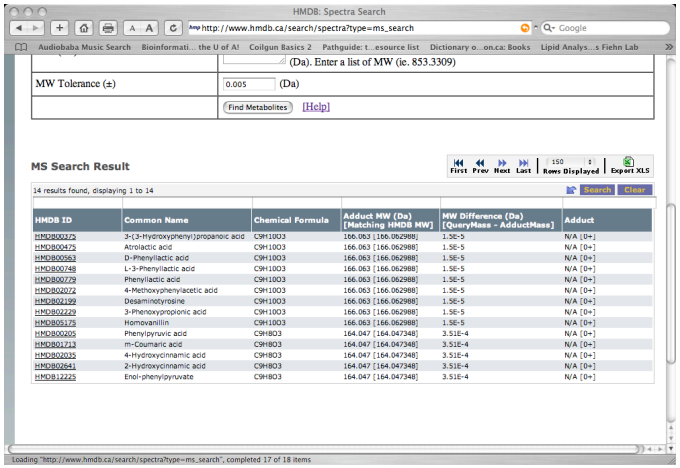


LC-MS Spectrum



Peak list to HMDB

Phenyllactate
Phenylpyruvate
Atrolactic acid
Homovanillin
Coumaric acd
…



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

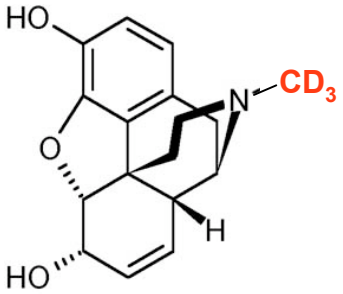
40 acylcarnitines, 13 amino acids, 15 LysoPCs, 77 PCs, 15 SMs = 160

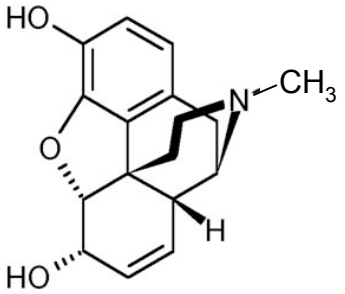
Multiple Reaction Monitoring

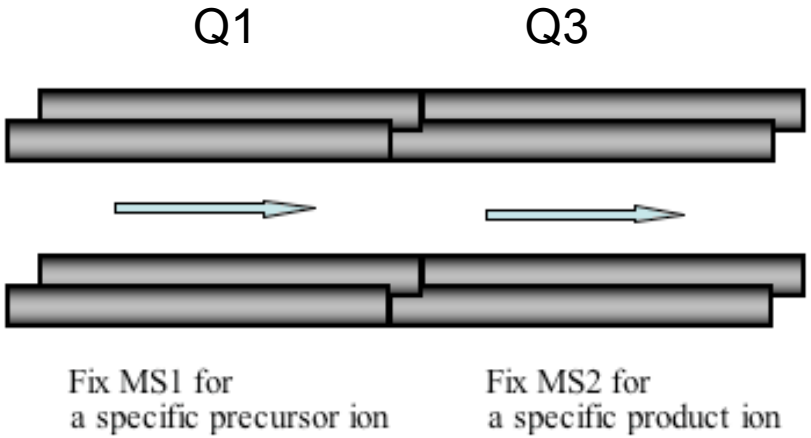
Table I +MRM Transitions for Opiates.

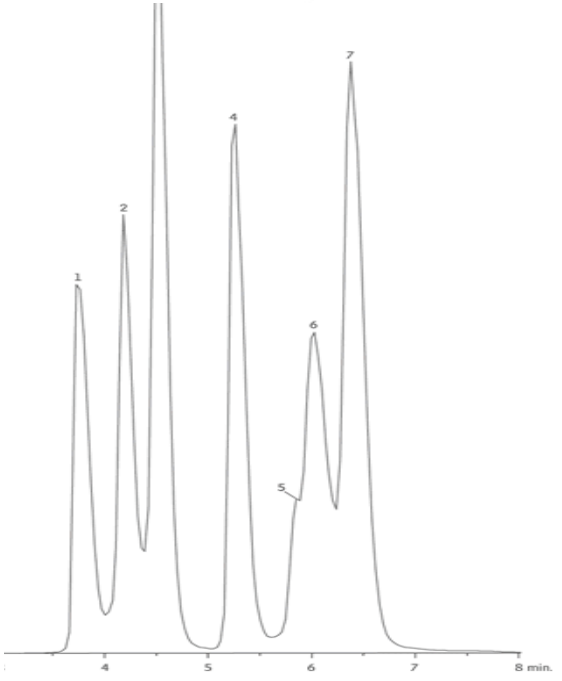
Mass Spectrometer Experiments:

Compound	Q1	Q3	Declustering 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	

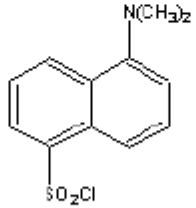
Quantitative MS Metabolomics With Chemoselective Labeling

Peak Pair Table__C13/C12-Dansylation-Labeled Urine_#9-Sep0707-FTMS

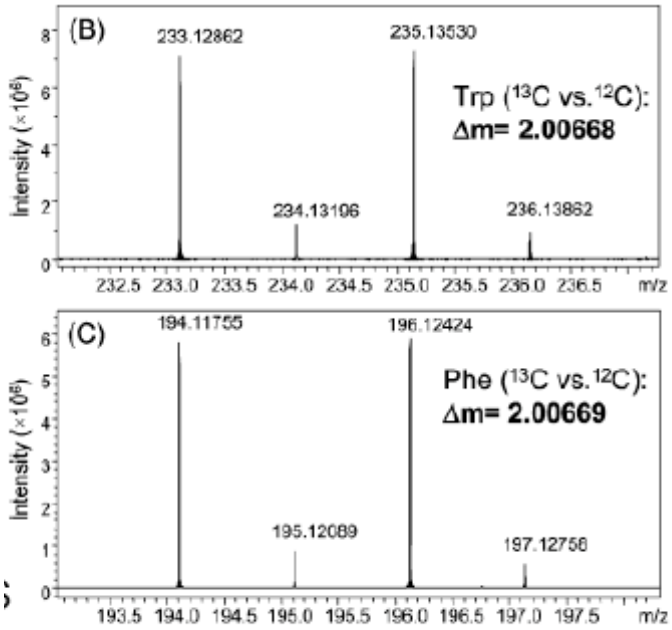
File:RPLC-FTMS: #9-Sept0707-Run-6

Mass Diff. Error is ppm between therotical mass difference and determined mass difference.
Listed pairs are the patial 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	533.044603	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	



^{12}C and ^{13}C Dansyl labeling



Quantitative MS Metabolomics in Human Urine

30 nM →

2.51mM →

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-adnosine 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-diaminoproprionic 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-pyrydoxamine	8.94	<D.L.
Dns-Gly	3.43	2510	Dns-2-methyl -benzylamine	9.24	<D.L.
Dns-Ala	3.88	593	**Dns-5-hydroxytrptophan**	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-hydroxymethyluricil	4.58	1.9	**Dns-tyrosinamide**	9.79	29
Dns-tryptophanamide	4.70	5.5	**Dns-dopamine**	10.08	140
Dns-isoguanine	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-cysteamine	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-chlorotyrosine	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-salicyluric ac id	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-diiodothyronine	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

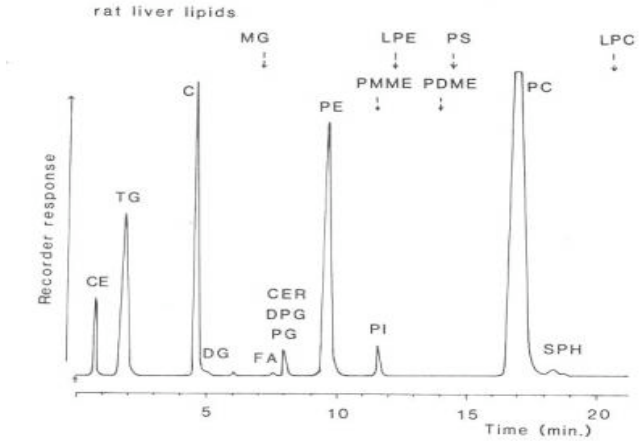
820 peaks by carboxy labeling
Still assessing

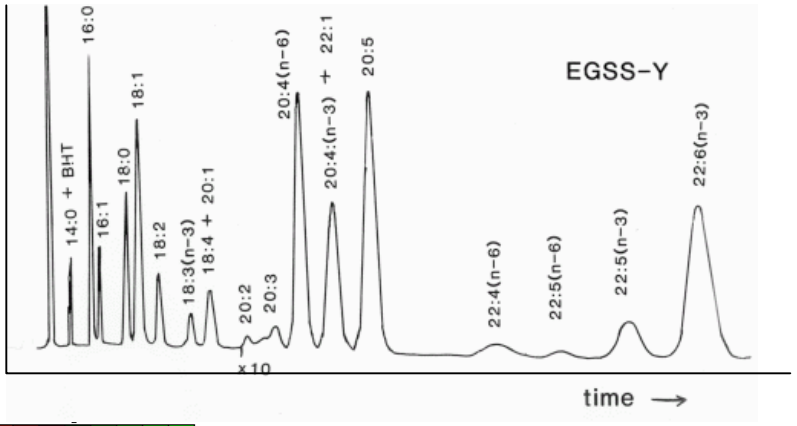
Guo K. & Li L. Anal Chem. 2009 May 15;81(10):3919-32.

Advantages to Derivitization

- 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

Lipomics/Tethys TrueMass Technologies





Lipomics Surveyor

Update >

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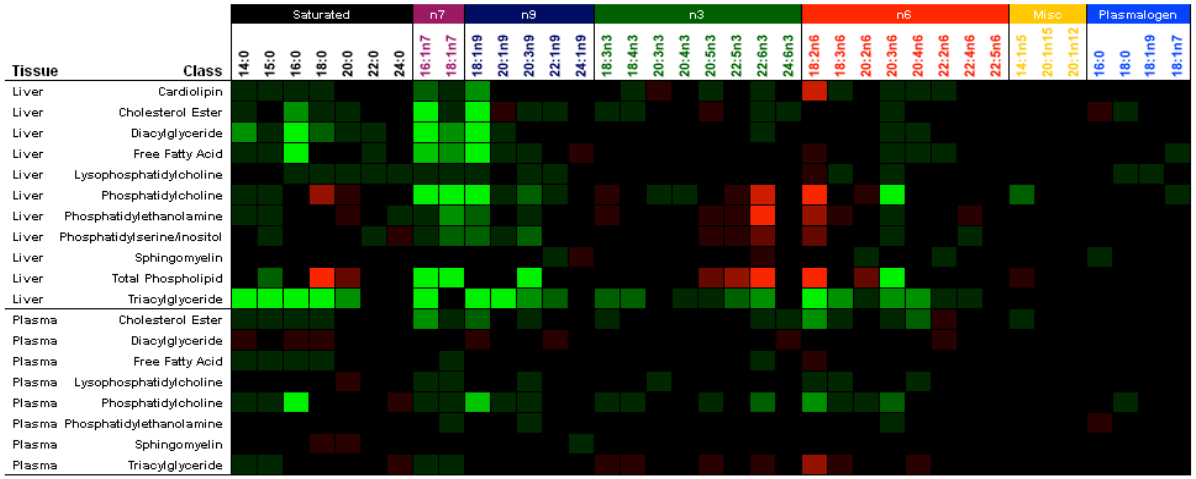

1) Lipid separation
2) Lipid quantification
3) FAMES
4) GC-MS of FAMES
5) FAMES quantification
6) Lipid comp. & quant.

What's Possible

- NMR-based metabolomics (~50 metabolites identified/quantified, uM limit, 500 uL)
- GC-MS based metabolomics (~90 metabolites identified/quantified, <uM limit, 50 uL)
- DI-MS based metabolomics (160 metabolites identified/quantified, nM limit, 20 uL)
- LC-MS based metabolomics (300 metabolites identified/quantified, nM limit, 50 uL)
- Lipidomics (3000 lipids identified and semi-quantified, nM sensitivity, 1 mL)

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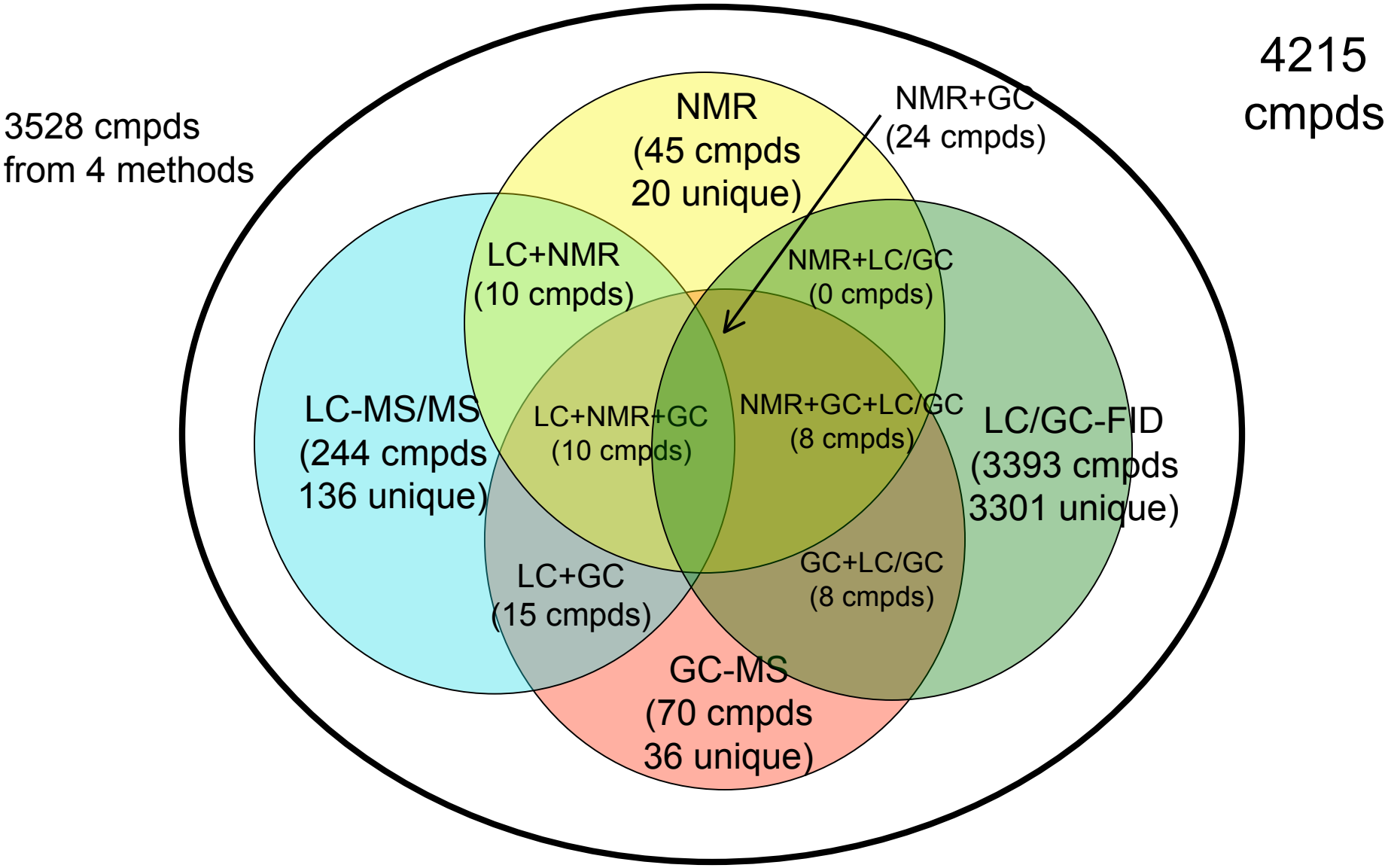
The Serum Metabolome



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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Alberta Cancer Foundation

GenomeCanada

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CHENOMX