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Adventures in Metabolite Profiling with an Accurate Mass QTof

David Johnson, Ph.D.
Director, DMPK | MicroConstants

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Background on Drug Metabolism

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What is Metabolite Profiling?

- Determining the metabolites of a drug candidate when it's dosed to a subject or *in vitro* system
- Usually involves a species comparison

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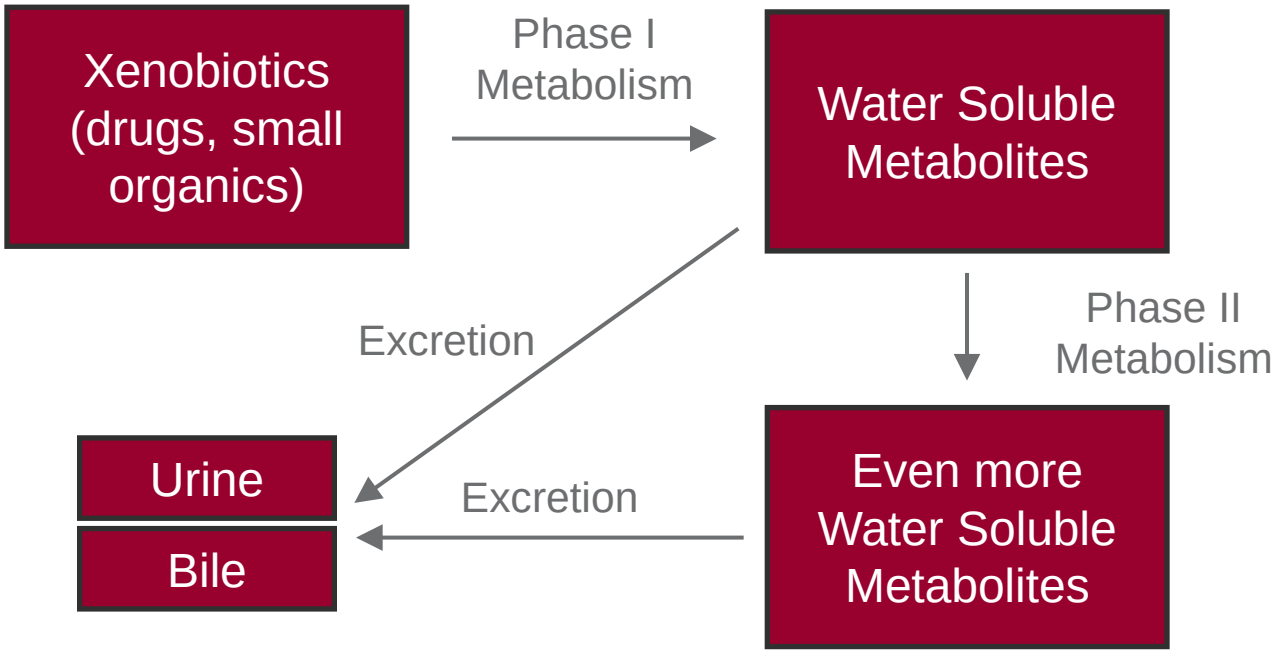
Overview of Drug Metabolism

- The major effect of drug metabolism by the body is to convert lipophilic compounds that favor absorption into hydrophilic compounds that favor excretion in the urine or bile.



Common Phase I Reactions

- Oxidation
  - Most common metabolic reaction
  - Most important Phase I reaction
  - Responsible for a broad array of transformations
  - Hydroxylation (M+16), dealkylation, epoxidation, etc.
- Hydrolysis
  - Ester and amide bonds, epoxiedes, sulfates, glucuronides
- Reduction
  - Carbonyls, nitro groups, N-oxide/sulfoxide

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Phase II Reactions

- Can happen to parent compound or Phase I metabolite
  - Cofactors are always involved
  - Can usually tell which atoms can be conjugated
- Examples:
  - Glucuronidation (M+176)
  - Sulfation (M+80)
  - Glutathione conjugation (M+305, 307)
  - Methylation (M+14)
  - Amino acid conjugation (M+57; +107)

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Relevance of Metabolite Profiling

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Why Do Metabolite Profiling?

- Identify the candidate's metabolic pathway to understand the fate of the drug and metabolites
  - Toxic metabolites
- Seek out pharmacologically active metabolites for patent protection
- Help select species for toxicological studies
  - Avoid unique human metabolites
- To save time and money!

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Methods for Metabolite Profiling

- Definitive radiolabel ADME study
  - Expensive
  - Usually performed just before NDA
- Cold compound profiling
  - Inexpensive
  - Performed early in discovery setting

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Metabolite Profiling Workflow

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Overview of Profiling Work

- Generate samples
  - Hepatocyte incubation
  - Plasma from dosed subjects
- Develop analytical method
- Analyze samples with QTof
  - Collect product ion spectra
- Search all species for metabolites using MetaboLynx
- Program QuanLynx with all metabolites
- Report peak heights for all species
- Draw hypothetical structures based on accurate mass

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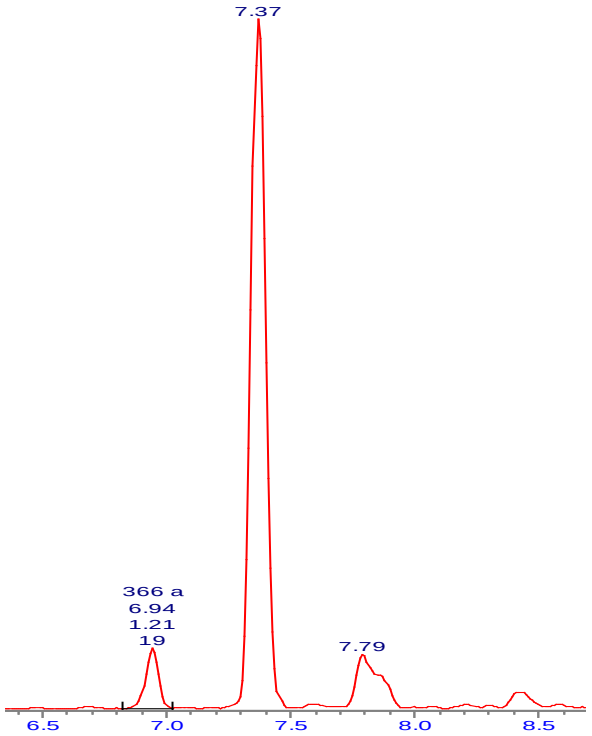
Analytical Method Highlights

- Uses Waters Acquity UPLC system
  - Excellent gradient control and solvent mixing
  - Minimal dead volume
  - Reproducible retention times
- Small particle size columns
  - High theoretical plates
  - Better separation of regioisomers
- 15 minute gradient
- Includes in-line photodiode array



Hydroxylated Metabolites of Client Compound

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Analytical Method Highlights (cont.)

Data table emphasizing the importance of using high resolution chromatography with very reproducible retention times

Name	Found *m/z*	TR, min	Assigned Identity	Molecular Formula	Theoretical *m/z*	Mass Difference		
						mDa	PPM	From Parent
Parent	350.1416	7.99	Client compound	C19H16FN5O	350.1417	-0.1	-0.3	-0.0001
M4	366.1364	6.93	Hydroxylation	C19H16FN5O2	366.1366	-0.2	-0.6	15.9948
M5	366.1367	7.37	Hydroxylation	C19H16FN5O2	366.1366	0.1	0.3	15.9950
M6	366.1377	7.79	Hydroxylation	C19H16FN5O2	366.1366	1.1	3.0	15.9960
M7	366.1368	7.88	Hydroxylation	C19H16FN5O2	366.1366	0.2	0.5	15.9951
M8	366.1375	8.18	Hydroxylation	C19H16FN5O2	366.1366	0.9	2.5	15.9958
M9	366.1333	8.42	Hydroxylation	C19H16FN5O2	366.1366	-3.3	-9.0	15.9916

Notice the baseline resolution of isobaric regioisomers!

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Importance of Accurate Mass

Data table emphasizing the need for accurate mass to assign identities to metabolites

Name	Found *m/z*	T_R, min	Assigned Identity	Molecular Formula	Theoretical *m/z*	Mass Difference		
						mDa	PPM	From Parent
Parent	350.142	7.99	Client compound	C19H16FN5O	350.1417	-0.1	-0.3	-0.0001
M3	364.126	8.82	**Hydroxylation + Desaturation?**	**C19H14FN5O2**	**364.1210**	**4.5**	**12.5**	**13.9838**
			Methylation - definitely not	C20H18FN5O	364.1574	-31.9	87.6	14.0157
M15	526.173	6.95	Glucuronide conjugation	C25H24FN5O7	526.1738	-0.6	-1.1	176.0315
M16	526.165	7.16	**Epoxidation + Glutathione - Glutamic acid + Dehydration**	**C24H24FN7O4S**	**526.1673**	**-2.0**	**-3.8**	**176.0237**
			Glucuronide conjugation - not likely	C25H24FN5O7	526.1738	-8.5	-16.2	
M17	526.173	7.59	Glucuronide conjugation	C25H24FN5O7	526.1738	-0.6	-1.1	176.0315
M18	526.172	7.78	Glucuronide conjugation	C25H24FN5O7	526.1738	-2.2	-4.1	176.0299

Accurate mass was needed to tell the difference between different possible metabolites with the same nominal mass shift

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MetaboLynx XS Features

- Powerful software for visualizing full scan data sets and identifying metabolites
  - Structure-based metabolite search
  - Lists both expected and unexpected metabolites
  - Includes dealkylation tool for generating multiple base structures for the search
  - Visualize chromatograms of both analyte and control
  - Generates fragment ion trees using MS^E data
  - Permits easy viewing of product ion spectra

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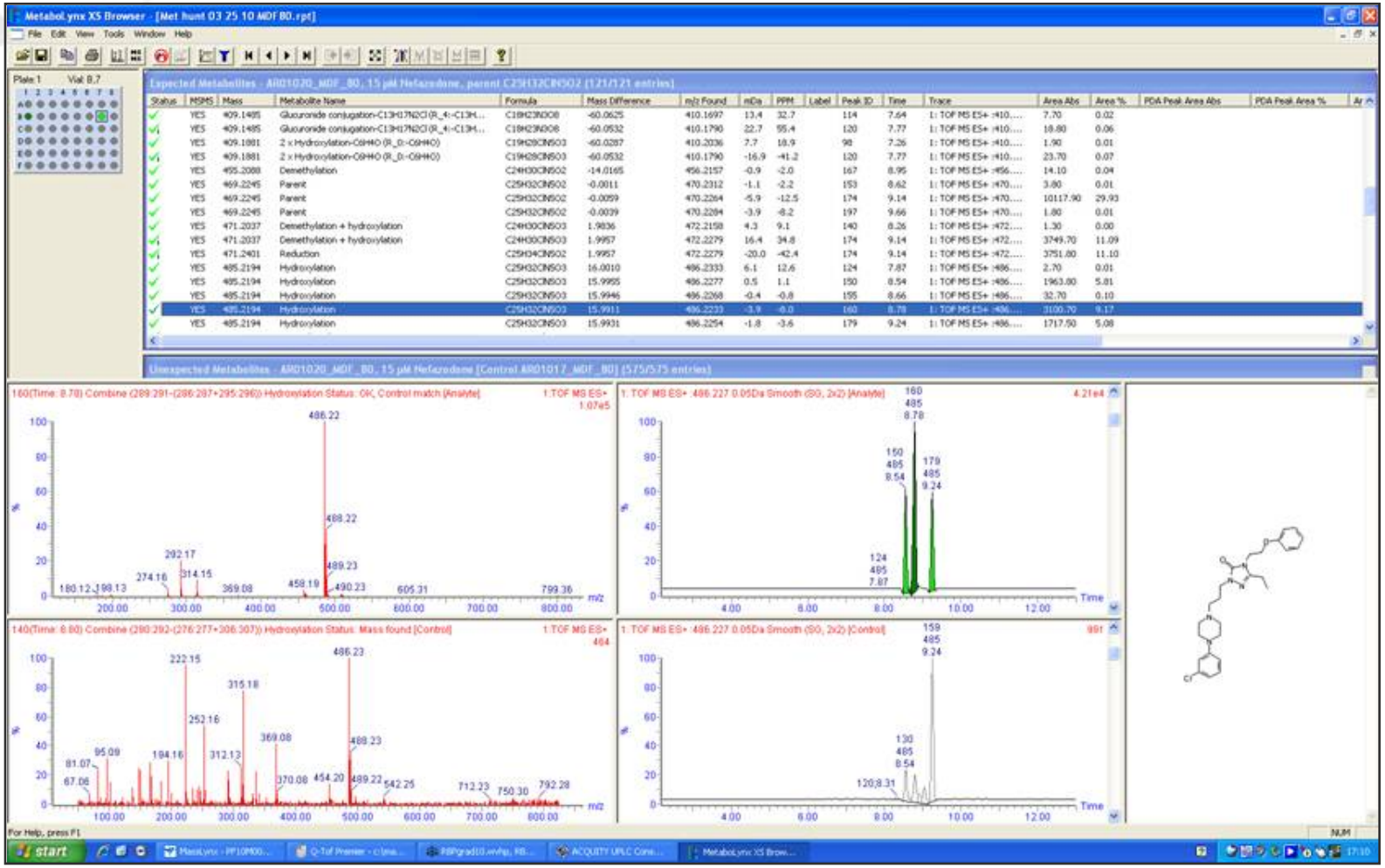
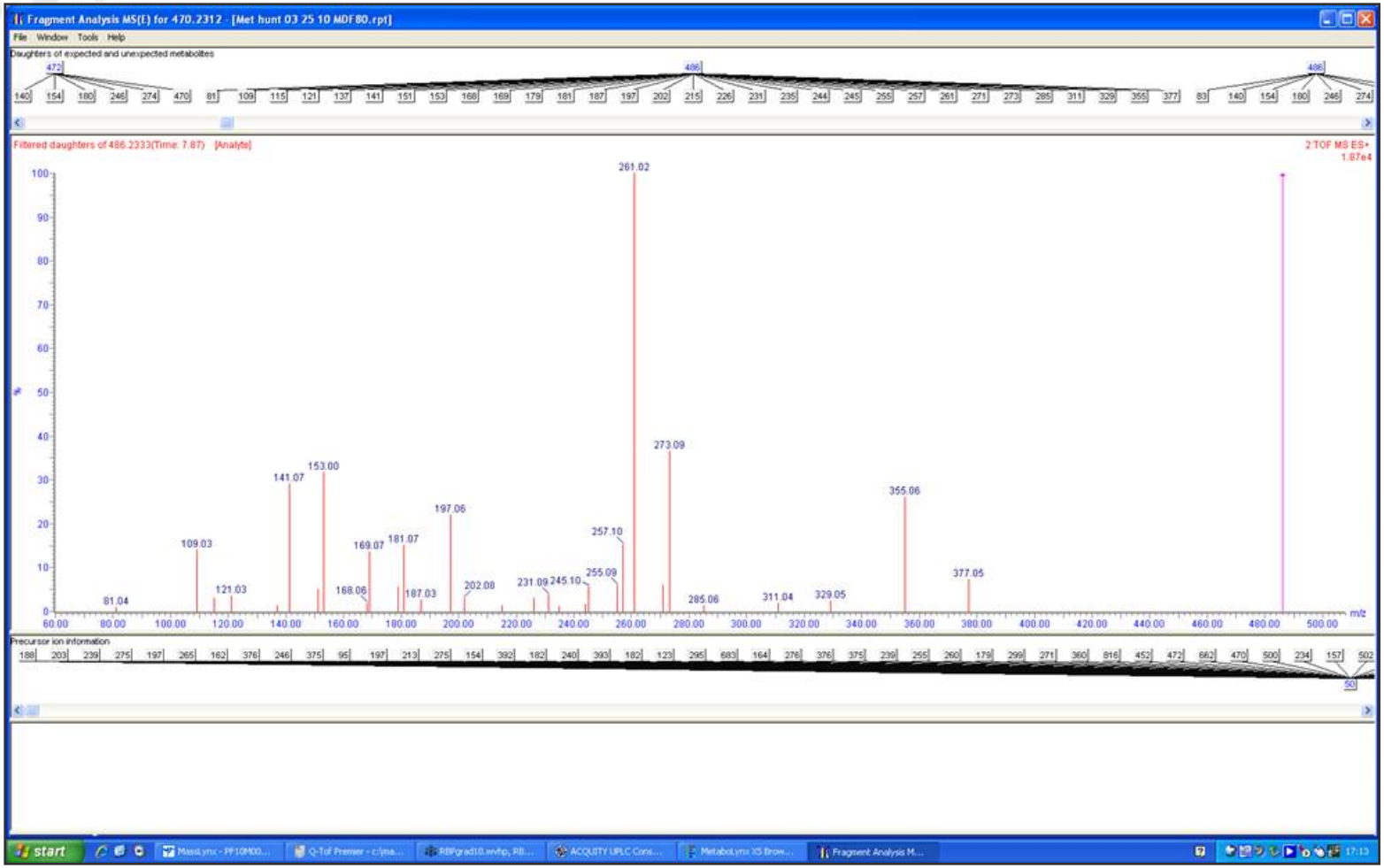
MetaboLynx XS Screen Shot



MetaboLynx XS Screen Shot



Example of Metabolite Profiling Data

Species	Time, h	Peak Height								
		M1	M2	Parent	M3	M5	M6	M7	M14	M17
Rat	0	0	10	15,600	**0**	10	0	0	0	2
	0.5	0	21	13,100	**6**	436	196	0	0	242
	1	0	38	12,000	**10**	655	368	0	3	469
	2	0	76	9,880	**13**	921	717	0	2	850
	4	0	138	5,190	**16**	1,100	1,160	0	5	1,380
Dog	0	**0**	0	12,100	0	2	**0**	**0**	0	0
	0.5	**5**	0	12,200	0	91	**0**	**22**	5	7
	1	**12**	3	9,440	0	161	**0**	**26**	29	50
	2	**22**	7	6,580	0	229	**0**	**17**	53	213
	4	**40**	7	4,140	0	314	**0**	**8**	91	674
Monkey	0	0	7	14,800	0	1	0	0	0	0
	0.5	0	11	13,600	0	218	196	2	5	13
	1	0	12	11,400	1	305	476	3	8	24
	2	0	14	9,520	2	419	1,010	3	6	74
	4	0	20	6,710	0	460	1,830	0	12	187
Human	0	0	6	14,600	0	3	0	0	**5**	0
	0.5	0	25	11,900	0	224	464	0	**178**	31
	1	0	31	8,980	0	322	952	0	**689**	76
	2	0	30	5,660	0	377	1,790	0	**1,020**	161
	4	0	48	3,200	0	297	2,390	0	**2,780**	289
m/z		337.11	338.14	350.14	364.13	366.14	366.14	366.14	446.10	526.17
T_R (min)		6.83	9.47	7.99	8.82	7.37	7.79	7.88	8.22	7.59

Summary

- Metabolite profiling is a vital part of drug development
- Accurate mass spectrometry is a powerful tool
  - Metabolite identification
  - Proposing structures
- Empowers metabolism experts to be better experts!

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