TNO triskelion bv

*i*HUMITE®

cost efficient, systematic and accurate human drug metabolite profiling

Drug metabolism =

biochemical modification of pharmaceutical substances by living organisms, usually through specialized enzymatic systems

- converts drugs into more readily excreted polar products
- can result in **toxication** or detoxication
- therefore all circulating “>10%” metabolites must be characterized and considered for safety assessment

Issues in metabolite ID:

- **Must:** FDA/ICH MIST require all “>10%” human plasma metabolites to be profiled
  - as early as possible
  - with coverage checked in tox species
- **Inefficient**: Low predictive value of early human metabolite ID studies
  - In vitro (incomplete biotransformation)
  - cold compound in First-in-Man (operator dependent profiling)
- **Time and $ loss:** Serious risk of exceeding timelines due to new metabolite findings in mass balance study
  - Additonal metabolite id work
  - Additional tox studies

iHumite® workflow

Deliverables:

- accurate profiling of major plasma metabolites already in First-in-Man study
- according to FDA/ICH MIST guidelines
- reduced risk of new findings and delay in clinical phase 3

Outline *i*Humite®

- FDA and ICH Guidelines
- *i*Humite® Workflow
  - Phase 1: Drug Metabolite Prediction
  - Phase 2: Accurate MS measurements
  - Phase 3: MS Data Processing
- *i*Humite® in the Drug Development Process
- Conclusions

FDA and ICH Guidelines

FDA 2008: focus on major human plasma metabolites (>10% of parent)

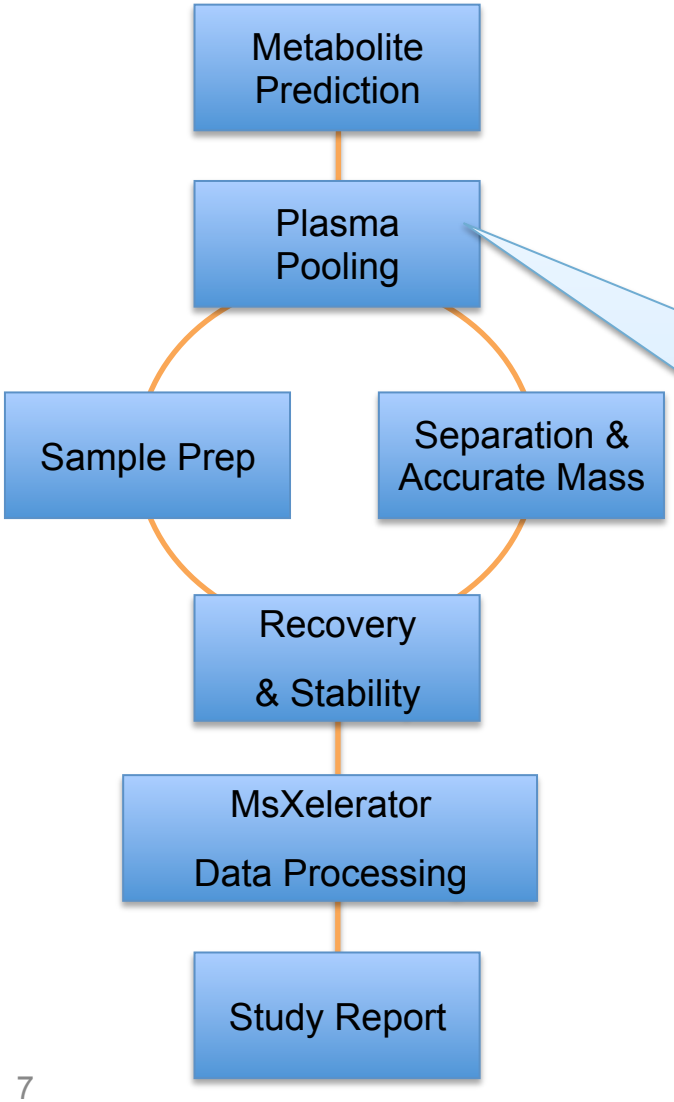
- *as early as possible in drug development*
- *when present coverage in tox species to be checked*
- *when disproportionate (rel. and abs. amount) potential tox issue*

ICH 2010: major defined as >10% of the sum of parent and metabolites

- *consider unique human metabolite case-by-case*
- *most glutathione conjugates are not of tox concern*
- *when needed: follow-up safety studies before clinical phase 3*

FDA Feb 2008: http://www.fda.gov/cder/guidance/index.htm
ICH M3(R2) Jan 2010: http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm

*i*Humite® Workflow



Optimized for first-in-man studies (no radiolabel):

- single dose, rising multiple dose

  major metabolites in plasma?
  any metabolite accumulation?

At therapeutic dose:

- *steady state plasma $AUC_{0\text{-}24}$*
- *coverage in tox species*

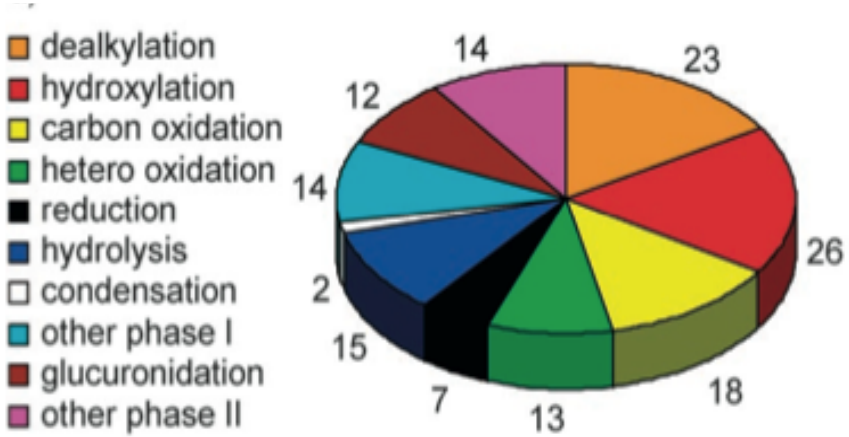
  (banked plasma)

*i*Humite® phase 1: Drug Metabolite Prediction

Prediction algorithm developed at Organon/Schering-Plough/MSD based on:

Metabolite Literature Database

(MDL Elsevier now Accelrys)



144 rules (2008)

Rules

① derived from published data (rat, human; various matrices)

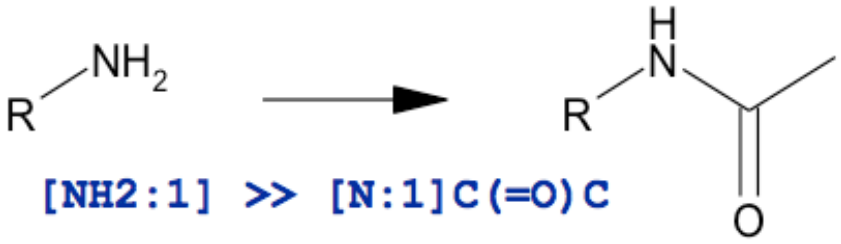
② extended at Netherlands eScience Center (Lars Ridder)

③ probability scores are calculated to rank metabolites and put main metabolites at top

④ allow a systematic approach

Rule Based Metabolite Prediction

- Rule example: N-acetylation



- Probabilities based on experimental data in literature database

human phase 1, phase 2:

$$probability = \frac{correct\ predictions}{metabolites\ generated}$$

- Rules cover 70 % of experimental reactions in the training set*

➔ targeted search: saves time and improves detection limit

**Combining Expert Knowledge and Empirical Scoring in the Prediction of Metabolites, Ridder L et al, ChemMedChem 2008, 3, 821 – 832*

Success rate iHumite® Metabolite Prediction

Comparison for human plasma

14 compounds/different therapeutic targets/7 pharma companies:

- ^{14}C ADME phase 3 studies*: 20 major plasma metabolites
- Human metabolites have been predicted

*Drug Metabolism and Disposition 2008-2012

Selected ^{14}C labeled compounds with human plasma data published

(^{14}C in title of publication in Drug Metabolism and Disposition, 2008-2012)

#	Compound	Company	DMD	First author	Mode of Action	Main plasma metabolite reported
1	Mirabegron	Astellas Pharma	2012	Takusagawa	β3-Adrenoceptor agonist	O-glucuronide
2	Brivanib Alaninate	BMS	2011	Gong	Dual inhibitor of vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF)	O-dealkylation, sulfation, carboxylation
3	Peliglitazar	BMS	2011	Wang	Dual α/γ peroxisome proliferator-activated receptor activator	1-O-β-acyl-glucuronide
4	SB-649868	GSK	2011	Renzulli	Orexin 1 and 2 receptor antagonist	hemiaminal
5	Sunitinib	Pfizer	2011	Speed	Oral multi-targeted tyrosine kinase inhibitor	des-ethyl
6	BMS-690514	BMS	2010	Christopher	ErbB/vascular endothelial growth factor receptor inhibitor	O-glucuronide
7	INCB018424	Incyte	2010	Shilling	Selective janus tyrosine kinase 1/2 Inhibitor	2-hydroxy-cyclopentyl
8	Lersivirine	Pfizer	2010	Vourvahis	Next-generation non-nucleoside reverse transcriptase inhibitor	glucuronide
9	Stavudine	BMS	2010	Zhou	Orally active nucleoside reverse transcriptase inhibitor	+O +Glucuronide
10	Apixaban	BMS	2009	Zhang	Reversible and direct inhibitor of coagulation factor Xa	O-demethyl sulfate
11	Bazedoxifene	Wyeth	2009	Chandrasekaran	SERM	Indole glucuronide
12	Vildagliptin	Novartis	2009	He	Dipeptidyl peptidase 4 inhibitor	carboxylic acid
13	Brasofensine	BMS	2008	Zhu	Inhibitor of the synaptic dopamine transporter	O-demethylation
14	Brivaracetam	UCB Pharma	2008	Sargentini-Maier	SV2A ligand	n-propyl side chain hydroxylation

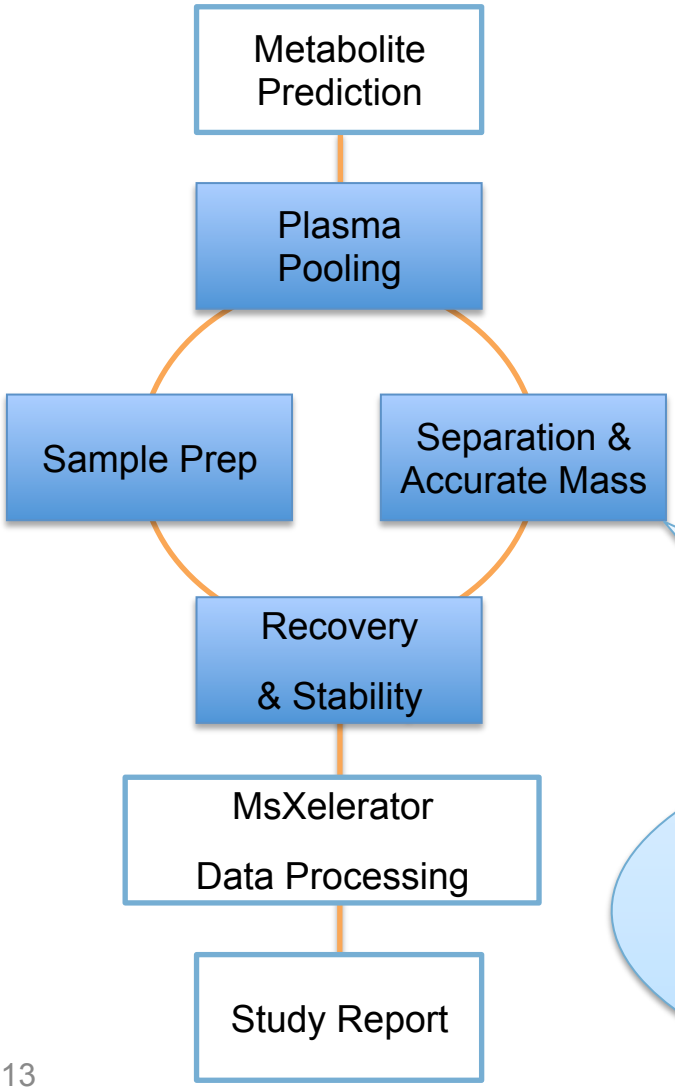
Success rate *i*Humite® Metabolite Prediction

Result

- 75% of these metabolite structures (15/20) predicted including all of the 1-step metabolites**
- 85% of metabolites (17/20) in a targeted search using elemental composition formula from the predicted set of metabolites

**ASMS 2012, WP475

*i*Humite® phase 2: Accurate Mass LC-MS/MS



LTQ-Orbitrap:

- *elemental formula of metabolites*
  - *ppm mass accuracy*
  - *ultrahigh mass resolution*
- *fragmentation, structure assignment*

*i*Humite® phase 3: MS Data Processing

MsXelerator software*:

Reads

- the predicted metabolite set
- All MS vendor data files
  - incl. Thermo Xcalibur (LTQ Orbitrap)

Is used

- for the targeted (predicted) metabolite search
- to compare peaks detected in drug dosed and placebo dosed plasma pools in the non-targeted approach

Combines

- results of both targeted and non-targeted searches to facilitate reporting

* msmetrix.com

*i*Humite® phase 3: MS Data Processing

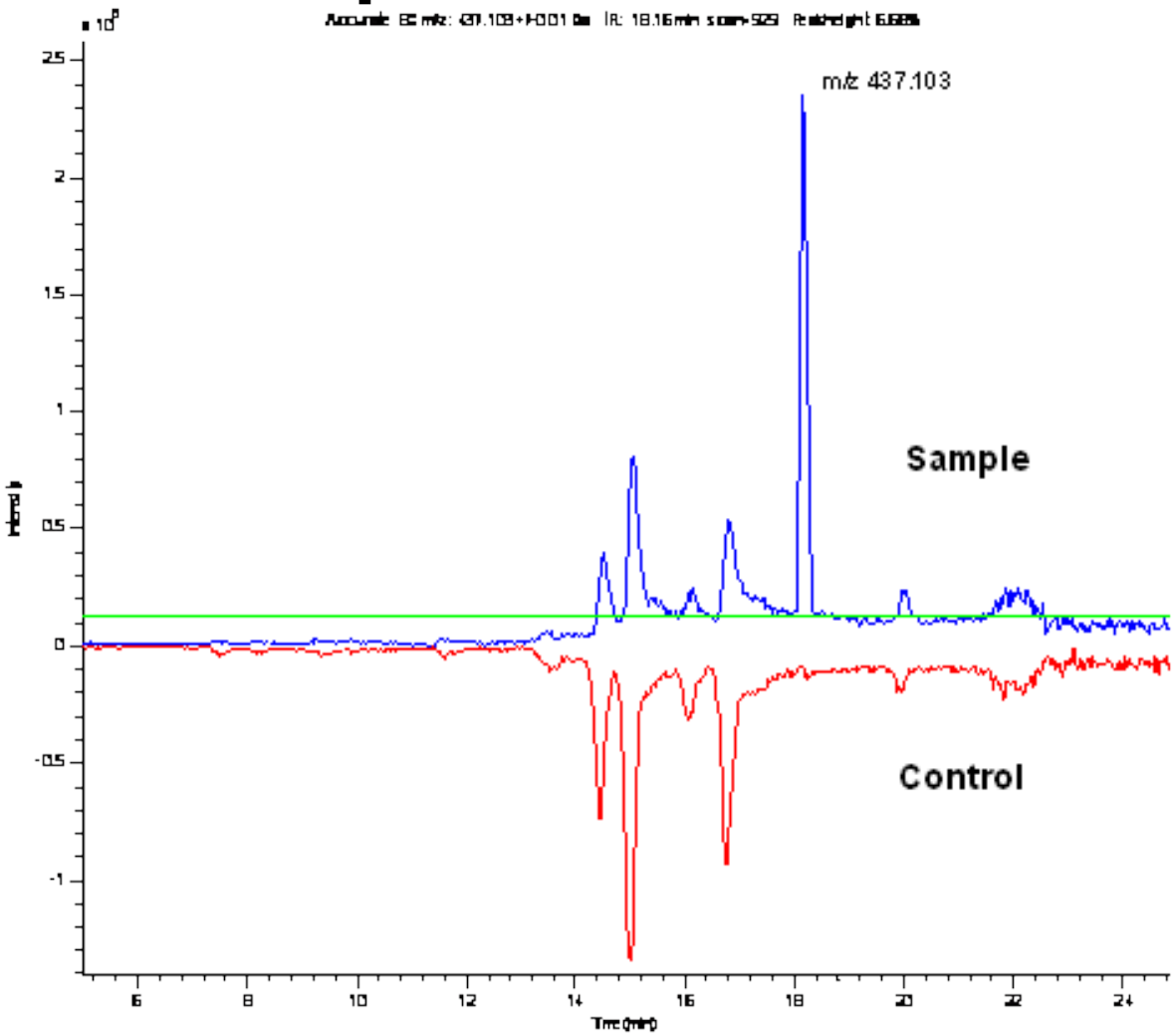
MsXelerator software:

Optional

- Mass defect filter
- Isotope pattern filter
- Neutral loss filtering (biotransformation in fragment)
- Product ion filter (biotransformation in neutral)

* msmetrix.com

*i*Humite® phase 3: MS Data Processing



* msmetrix.com

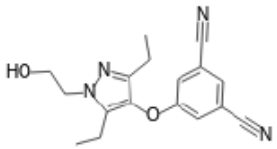
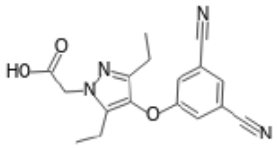
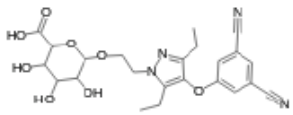
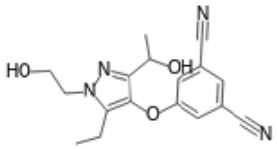
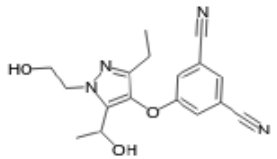
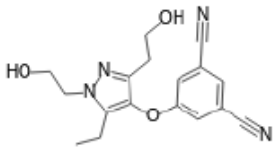
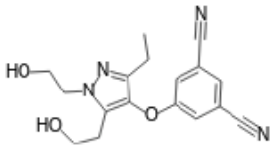
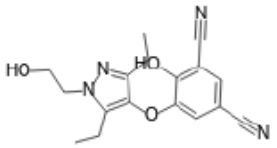
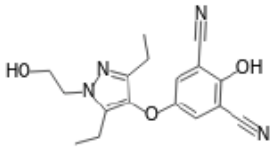
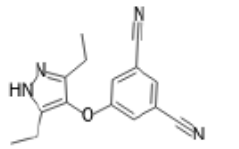
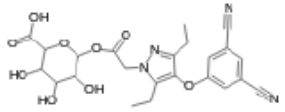
Why *i*Humite®?

Drug development before iHumite®:

- **Bad** case: prediction require more MS data processing
  - More time/budget required to identify all “>10%” metabolites
  - Budget 5.000-20.000 $
- **Worse** case: ^{14}C phase 3 study results in new human metabolites already observed in tox animals
  - Additional LC-MS ID study
  - Budget 10.000-30.000 $
- **Worst** case: ^{14}C phase 3 study reveals new human metabolites not observed in tox animals
  - Additional tox study
  - Additional investment of 50.000-200.000 $
  - Delay of drug registration ($$$)

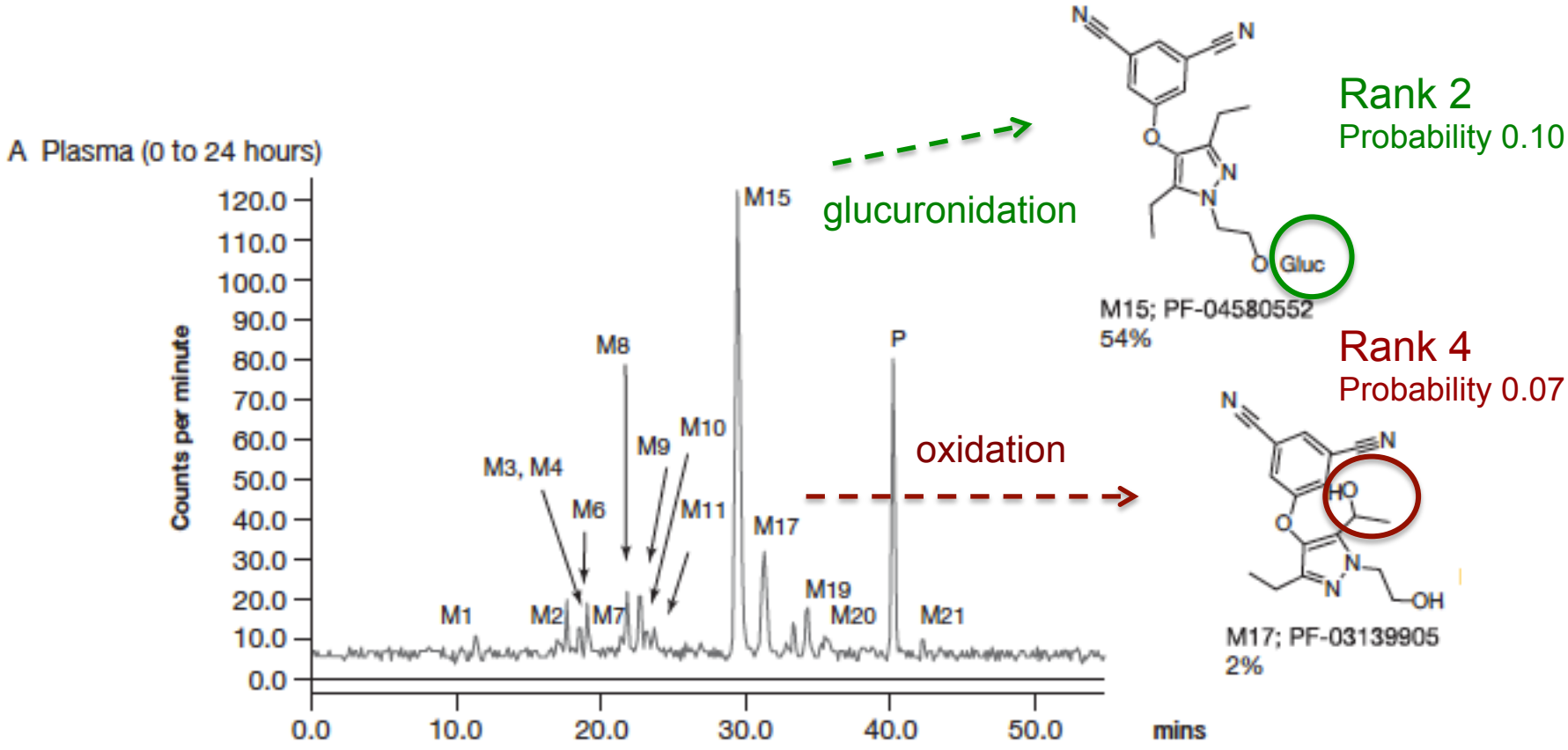
Example: Predicted metabolites of Lersivirine (rank 1-10)

TNO triskelion bv

Structure	Priority score	N steps	Reaction sequence
	1.0	0	PARENT
	0.199536	1	primary alcohol oxidation
	0.102502	1	O-glucuronidation
	0.073395	1	benzylic hydroxylation
	0.073395	1	benzylic hydroxylation
	0.063228	1	aliphatic hydroxylation
	0.063228	1	aliphatic hydroxylation
	0.059605	1	aromatic hydroxylation
	0.058762	1	aromatic hydroxylation
	0.048508	1	N-dealkylation
	0.031946	2	primary alcohol oxidation O-glucuronidation

Example: ^{14}C-Lersivirine in Human Plasma

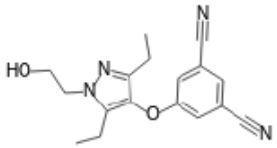
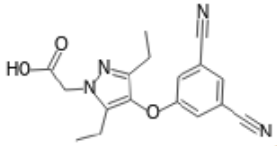
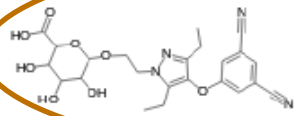
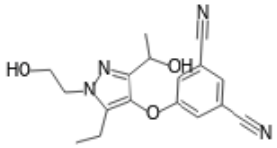
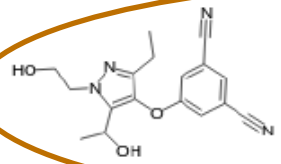
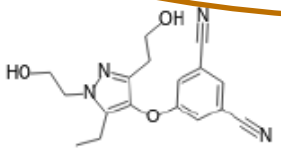
(Prediction on Pfizer published data)

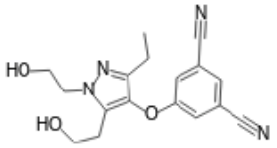
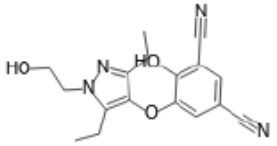
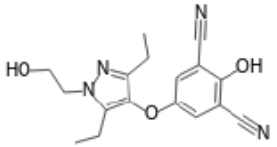
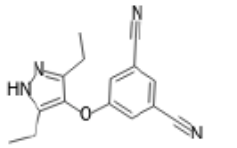
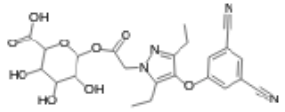


Vourvahis et al, DMD, 2010, 38, 5, 789–800

Example: Predicted metabolites of Lersivirine (rank 1-10)

TNO triskelion bv

Structure	Priority score	N steps	Reaction sequence
	1.0	0	PARENT
	0.199536	1	primary alcohol oxidation
	0.102502	1	O-glucuronidation
	0.073395	1	benzylic hydroxylation
	0.073395	1	benzylic hydroxylation
	0.063228	1	aliphatic hydroxylation

Structure	Priority score	N steps	Reaction sequence
	0.063228	1	aliphatic hydroxylation
	0.059605	1	aromatic hydroxylation
	0.058762	1	aromatic hydroxylation
	0.048508	1	N-dealkylation
	0.031946	2	primary alcohol oxidation O-glucuronidation

Conclusions

*i*Humite® Workflow for Drug Metabolite Profiling:

- systematic
- cost efficient
- complete
- effective
- **applications**

- *In vitro* species comparison
- First-in-man plasma profiling
- Coverage in preclinical studies
- ^{14}C human ADME

*i*Humite® Workflow

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