Identification of xenobiotic metabolites using *in silico* tools and non-targeted analysis

Matthew W. Boyce[a,b], Kristin A. Favela[c], Alex Chao[b], Grace Patlewicz[b], Jon R. Sobus[b], Antony Williams[b], John F. Wambaugh[b]

[a]Oak Ridge Associated University, Oak Ridge, TN 37830, USA
[b]Center for Computational Toxicology and Exposure, US EPA, RTP, NC 27711, USA
[c]Forensics and Specialty Analysis, Southwest Research Institute, San Antonio, TX 78228, USA

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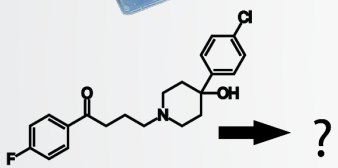
Guiding NTA with *in silico* predictions

Sample Preparation | *In Silico* Data Generation | Data Acquisition | Data Processing | Data Analysis

In Vitro Assay



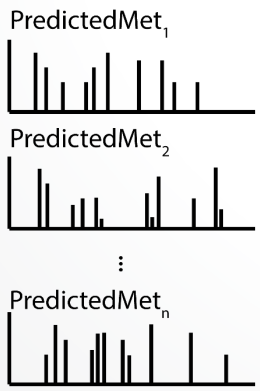
In Silico data

Aggregate Metabolite Predictions

Software: TIMES, Meteor, BioTransformer, QSAR Toolbox

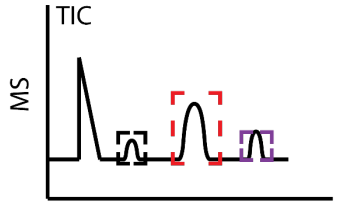
Suspect List: $PredictedMet_1$, $PredictedMet_2$, ⋮, $PredictedMet_n$

CFM-ID Predictions

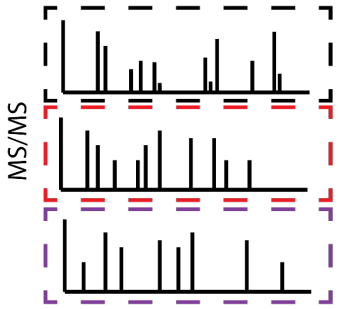


Non-Targeted Analysis

Parent ion selection



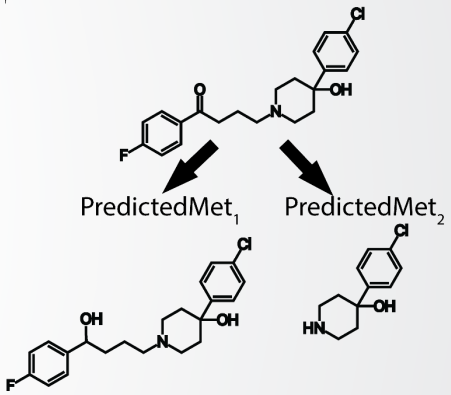
Parent ion fragmentation



Feature Selection & Data Cleaning

1) Peak Selection
2) Feature Identification
3) Data Cleaning

Metabolite Assignment



In vitro assay

Sample Preparation | *In Silico* Data Generation | Data Acquisition | Data Processing | Data Analysis

Metabolite Generation

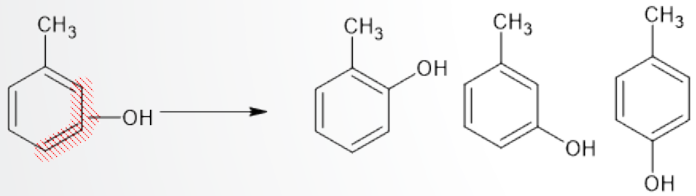
- Starting compounds metabolized via pooled primary human hepatocytes (10 donors)
  - Three time points: 0, 1, 4h
  - Three sample treatments: Supernatant (post lysis), B-glucuronidase treated, cell pellet
- Standards/Controls
  - Vehicle blank – DMSO
    - Used as blank for MS analysis
  - Standard control – Cell free solution with compound
    - Used to identify retention time window and mass error

Compiling a suspect screening list

Sample Preparation | *In Silico* Data Generation | Data Acquisition | Data Processing | Data Analysis

Known Metabolites

- Pulled 438 metabolites from 49 papers
- Markush structures were enumerated



Predicted Metabolites

- Compiled predicted structures from:
  - TIMES
  - BioTransformer
  - QSAR Toolbox
  - Meteor Nexus
- 1,666 predictions in total

Suspect Screening List

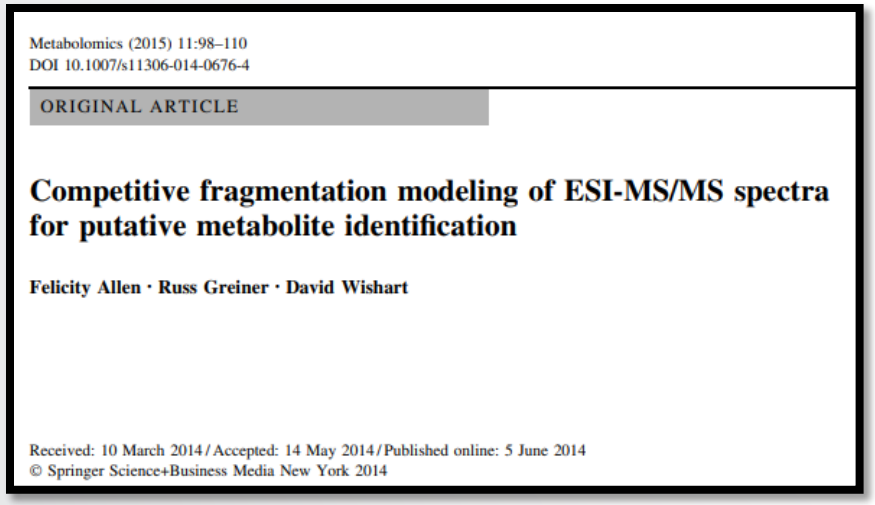
- 1,808 unique structures used to generate *in silico* MS^2 spectra
- 490 unique molecular formulae for MS^1 formula assignment

Generating database of *in silico* MS^2 spectra

Sample Preparation | *In Silico* Data Generation | Data Acquisition | Data Processing | Data Analysis

Fragmentation spectra were generated for each predicted metabolite

Competitive Fragmentation Modeling-ID (CFM-ID)



Metabolomics (2015) 11:98–110
DOI 10.1007/s11306-014-0676-4

ORIGINAL ARTICLE

Competitive fragmentation modeling of ESI-MS/MS spectra for putative metabolite identification

Felicity Allen · Russ Greiner · David Wishart

Received: 10 March 2014 / Accepted: 14 May 2014 / Published online: 5 June 2014

Spectra were generated using CFM-ID

- Reference spectra were generated at three collision energies (CE)
- Data were stored in database to query against for comparisons
- Validated against CASMI datasets for HRMS identification
  DOI: 10.3390/metabo10060260
- Applied to ~700,000 chemicals in EPA's CompTox Dashboard
  DOI: 10.1038/s41597-019-0145-z

MS^1 and MS^2 data collection

Sample Preparation | *In Silico* Data Generation | **Data Acquisition** | Data Processing | Data Analysis

LC-qTOF was used to collect high resolution MS^1 and MS^2 data

MS^1
- ESI+ and ESI-
- Range 100 – 1700 m/z
- Used to collect features for identification

MS^2
- Data-dependent acquisition (using suspect screening list)
- 1 replicate per treatment per time point
- Used to identify a feature's probable structure

Data processing steps

Sample Preparation → *In Silico* Data Generation → Data Acquisition → **Data Processing** → Data Analysis

MS^1 : Formula-level identification

MS^1 Data → **Feature Extraction & Alignment** (Agilent Profinder) → **Molecular Formula Identification** (Agilent Mass Profiler Professional) → **Data Cleaning (EPA NTA WebApp)** (Reproducibility Filtering, Feature Deduplication, Background Subtraction) → ***Output for data analysis***

Data processing steps

Sample Preparation → *In Silico* Data Generation → Data Acquisition → **Data Processing** → Data Analysis

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***Output of MS^1 processing*: Annotated features**

Suspect-Screening matches

- Identified using suspect list
- Molecular formula with suspected structural assignments

Features without suspect matches

- Formula proposed using Agilent's Molecular-Formula generator
- Formulae with no known structural assignments

Data processing steps

Sample Preparation → *In Silico* Data Generation → Data Acquisition → **Data Processing** → Data Analysis

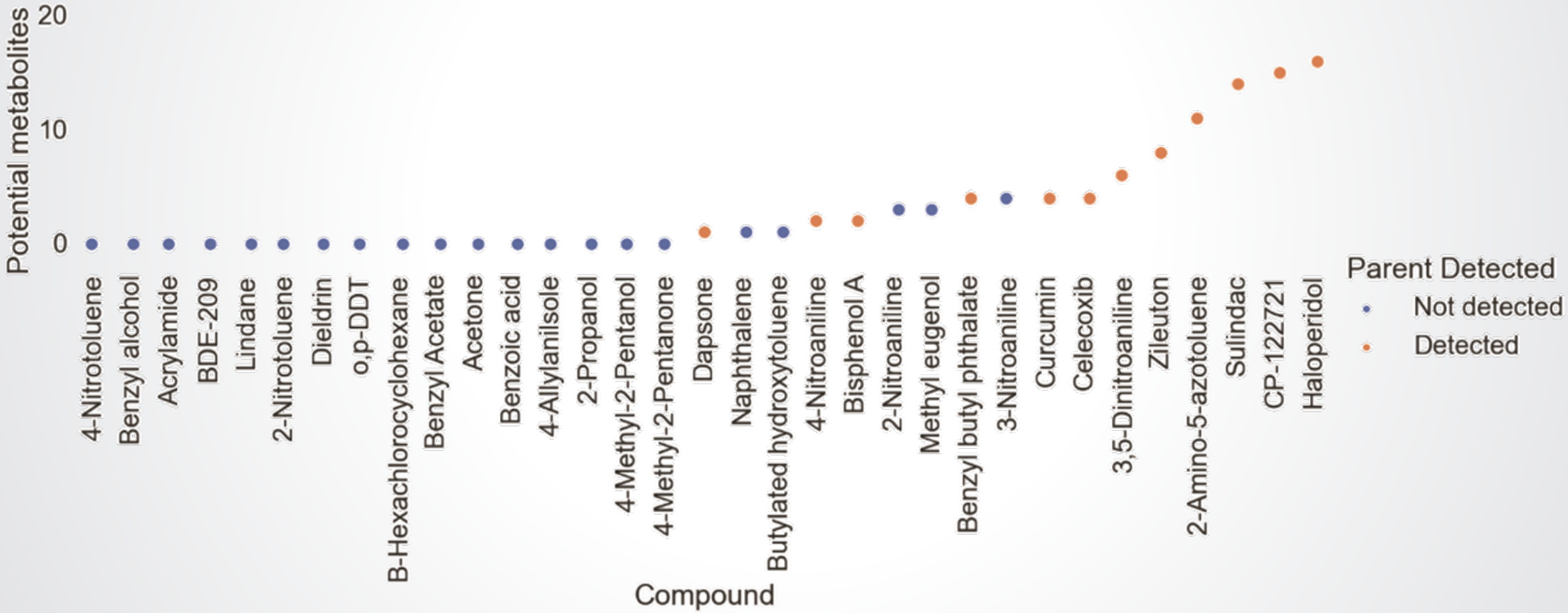
MS^1 : Formula-level identification

MS^1 Data → **Feature Extraction & Alignment** (Agilent Profinder) → **Molecular Formula Identification** (Agilent MPP) → **Data Cleaning (EPA NTA WebApp)** (Reproducibility Filtering, Feature Deduplication, Background Subtraction) → **Data Analysis** → **Feature List** (Formula)

MS^2 : Structure-level identification

MS^2 Data (Data-Dependent Acquisition) → ***In silico* MS^2 Spectra Library Matching** ($CFMID_{Metabolite\text{-}Library}$) → **Matched Spectra Ranking** (Calculate quotient score and filter scores that fail to meet a threshold) → **Feature List** (Structure)

Which parents have tentative metabolites?



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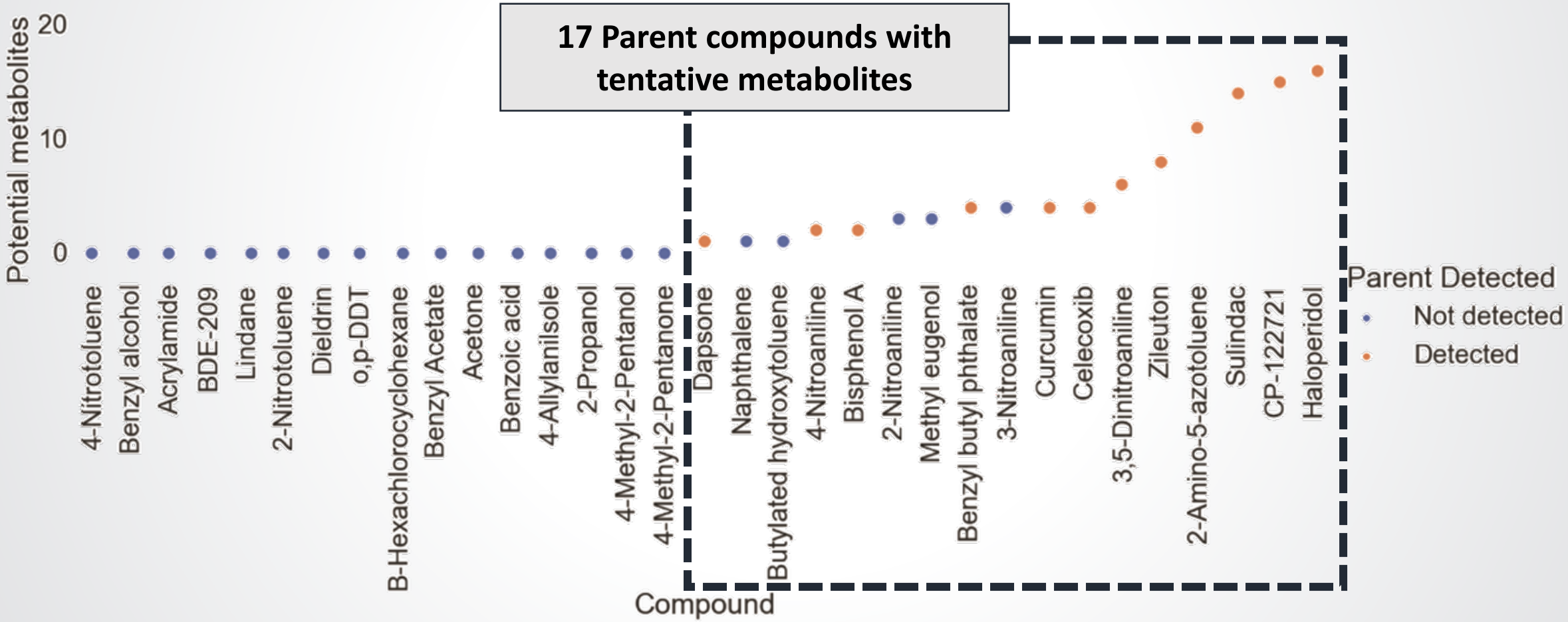
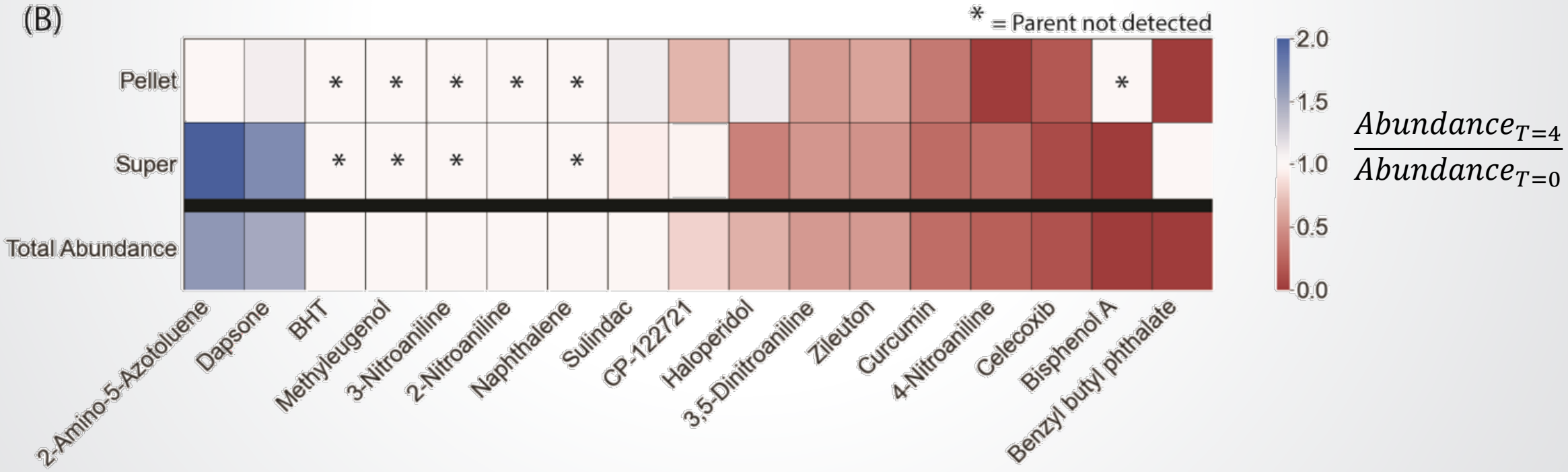


Which parents are being metabolized?

Sample Preparation | *In Silico* Data Generation | Data Acquisition | Data Processing | Data Analysis

Relative change in parent signal over 4h

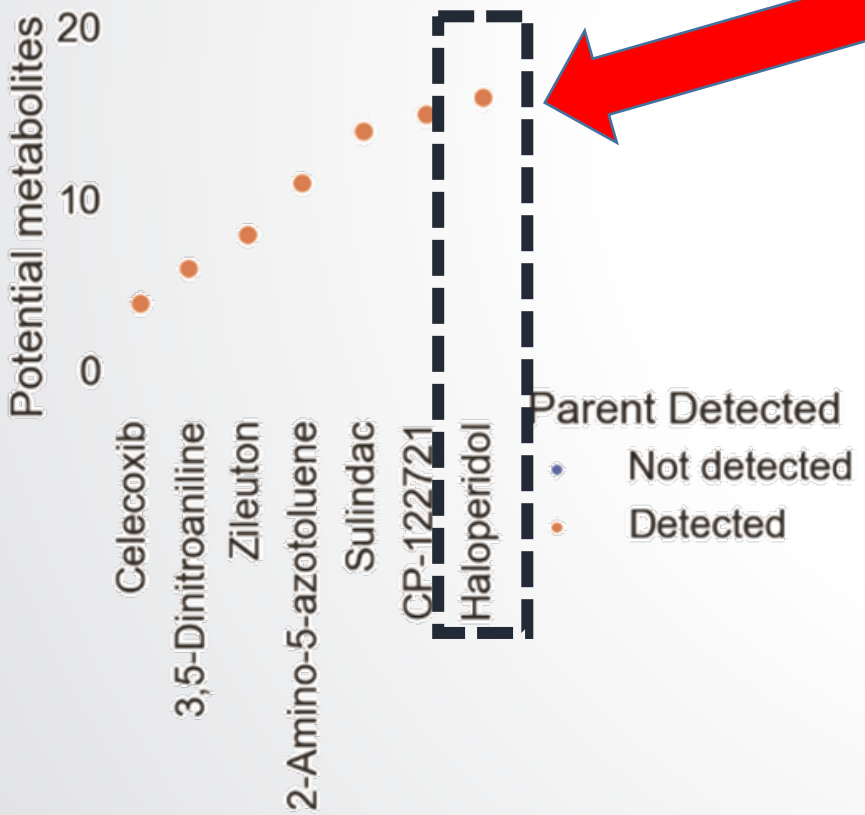
No Change ⟵ ⟶ Greatest Decrease

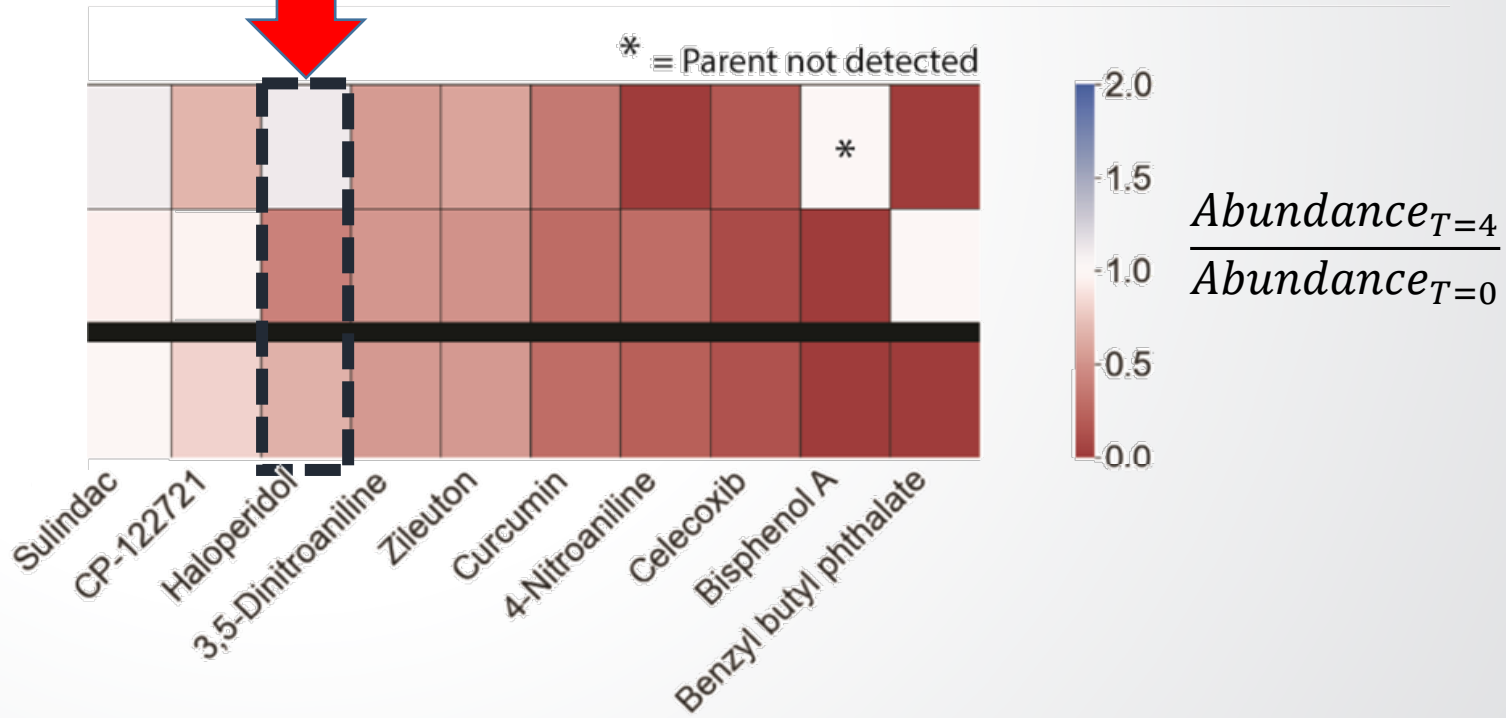


Which parents are being metabolized?

Sample Preparation | *In Silico* Data Generation | Data Acquisition | Data Processing | Data Analysis

Used to develop data analysis steps





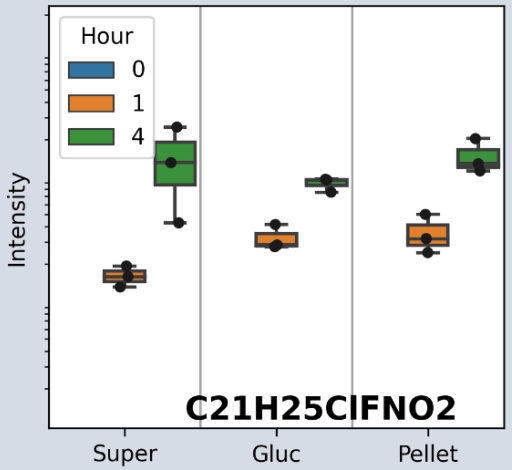
$$\frac{Abundance_{T=4}}{Abundance_{T=0}}$$

Identifying relevant features

Sample Preparation | *In Silico* Data Generation | Data Acquisition | Data Processing | Data Analysis

MS^1 Analysis Workflow

1) Broad feature filtering



Criteria for selecting features:

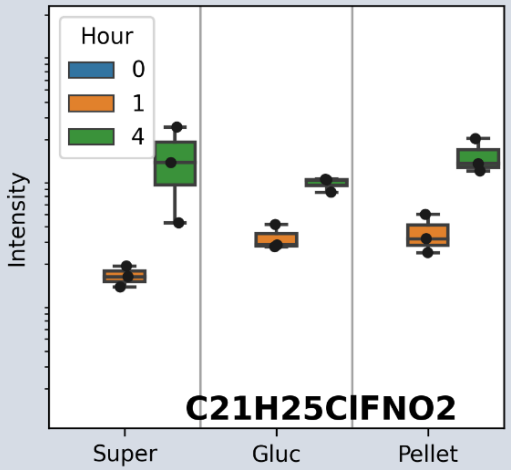
1. Fold-change increase ≥ 1.5
2. Appears in a minimum of two time points

Identifying relevant features

Sample Preparation → *In Silico* Data Generation → Data Acquisition → Data Processing → Data Analysis

MS^1 Analysis Workflow

1) Broad feature filtering



Criteria for selecting features:

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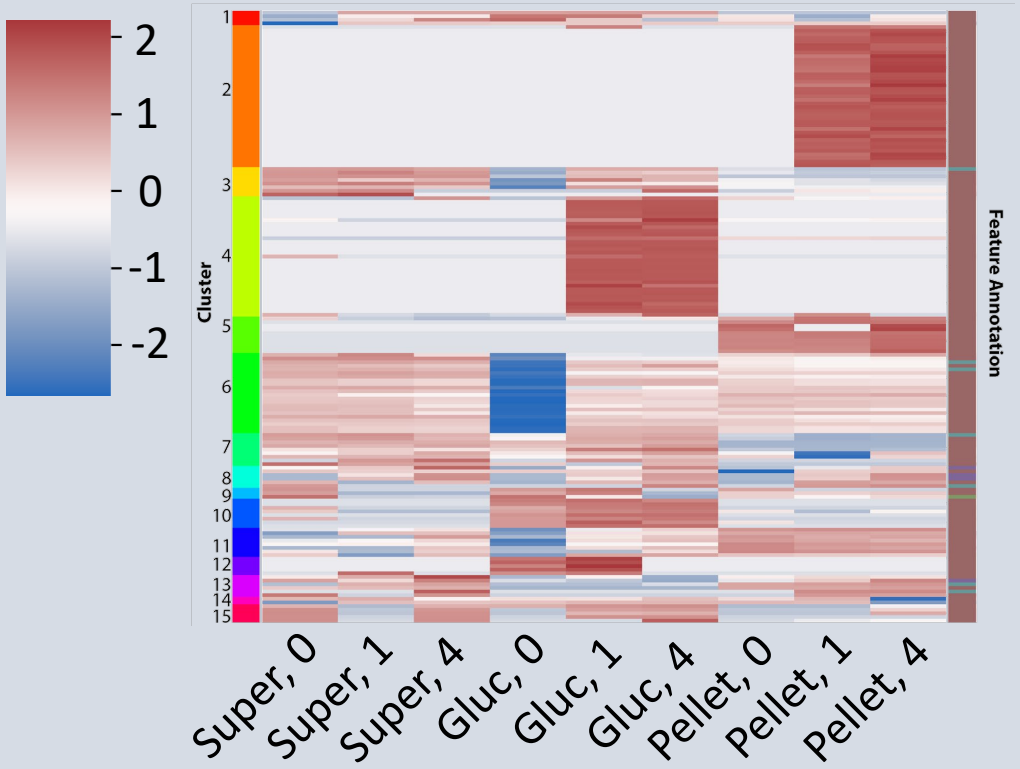
2) Cluster similar features



Feature Annotation Source

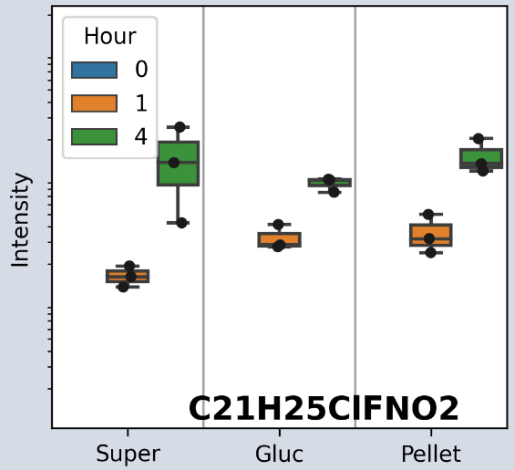
- Molecular Formula Generator
- Haloperidol (parent)
- Haloperidol metabolite
- Endogenous metabolites (human blood plasma)

Identifying relevant features

Sample Preparation → *In Silico* Data Generation → Data Acquisition → Data Processing → **Data Analysis**

MS^1 Analysis Workflow

1) Broad feature filtering



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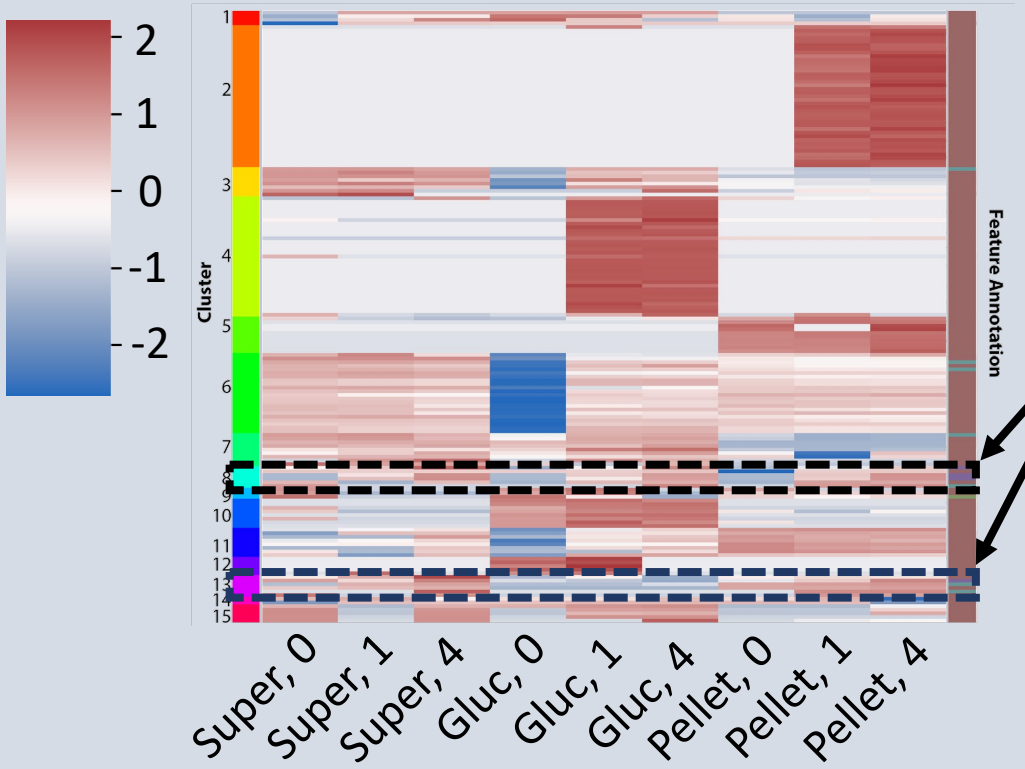

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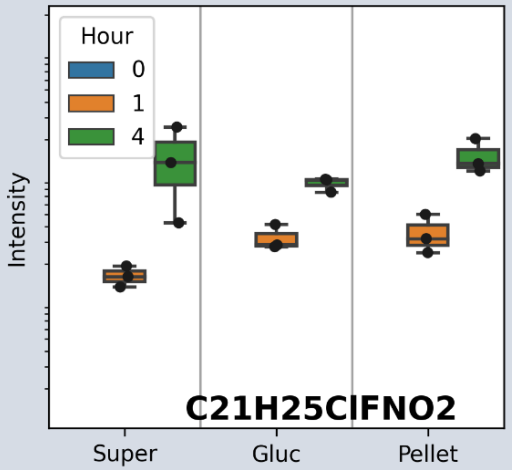
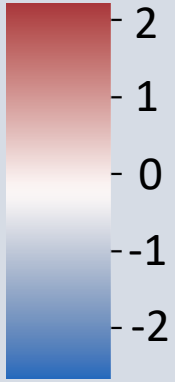
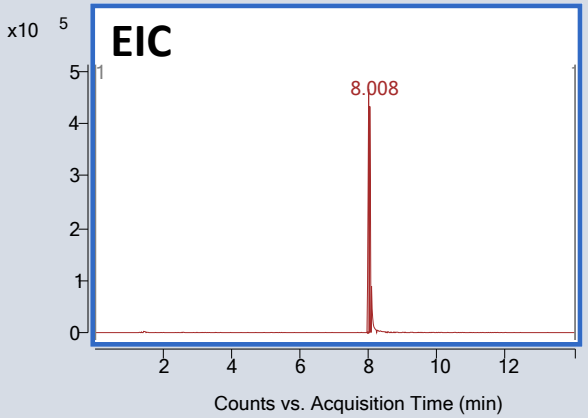
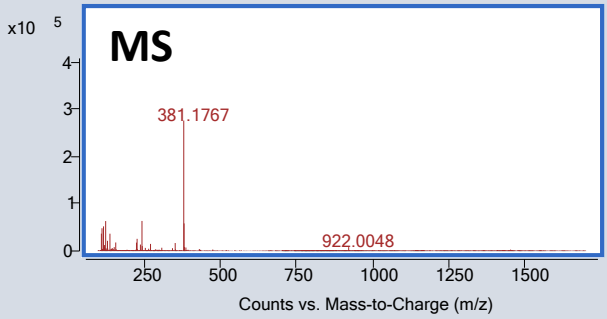


2) Cluster similar features



3) Manual Review





Criteria for selecting features:

1. Fold-change increase ≥ 1.5
2. Appears in a minimum of two time points

Super, 0 | Super, 1 | Super, 4 | Gluc, 0 | Gluc, 1 | Gluc, 4 | Pellet, 0 | Pellet, 1 | Pellet, 4

Assigning structure to features

Sample Preparation | *In Silico* Data Generation | Data Acquisition | Data Processing | Data Analysis

MS^2 Analysis Workflow

CFM-ID Comparisons

1. Precursor ions filtered using features from MS analysis
2. MS^2 data matched against CFM-ID database
3. CFM-ID matches ranked based on similarity values and normalized as a 'Q-Score' (ranging from 0 – 1)

Assigning structure to features

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MS^2 Analysis Workflow

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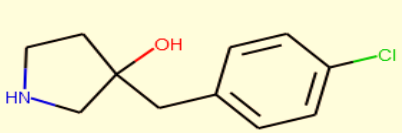
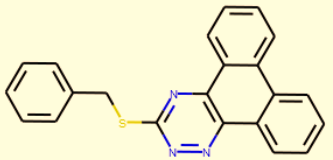
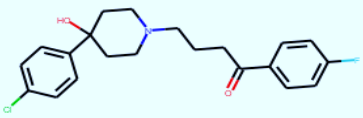
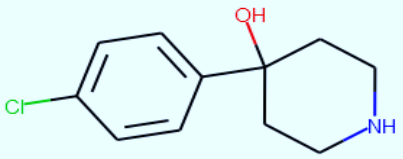
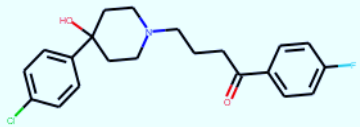
Q-Score ≥ 0.75

Structure Assignment

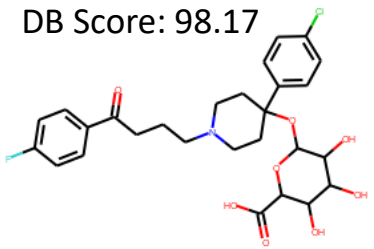
	$C_{11}H_{14}ClNO$ 8 matches	$C_{22}H_{15}N_3S$ 1 match	$C_{21}H_{23}ClFNO_2$ 2 matches
Top Match	Q-Score: 1.0 	Q-Score: 1.0 	Q-Score: 1.0 
Suspect List Match	Q-Score = 0.90 	None	Q-Score: 1.0 

Metabolite identifications

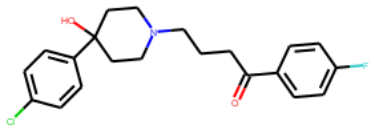
Sample Preparation | *In Silico* Data Generation | Data Acquisition | Data Processing | Data Analysis

CFM-ID Match (level 2b):

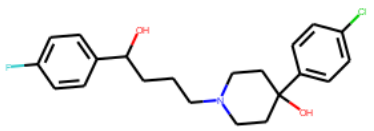
Q-Score: 1.0
DB Score: 98.17



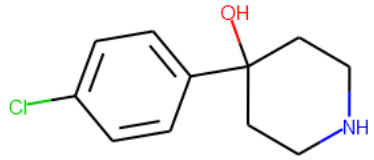
Q-Score: 1.0
DB Score: 85.04



Q-Score: 1.0
DB Score: 98.43

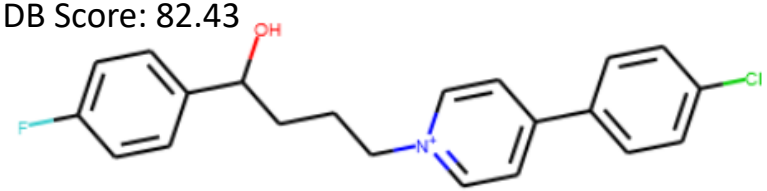


Q-Score: 0.9
DB Score: 95.83

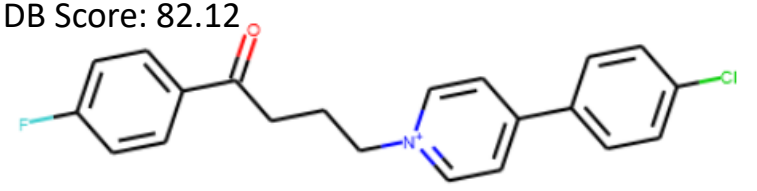


Suspect Match (level 3):

DB Score: 82.43



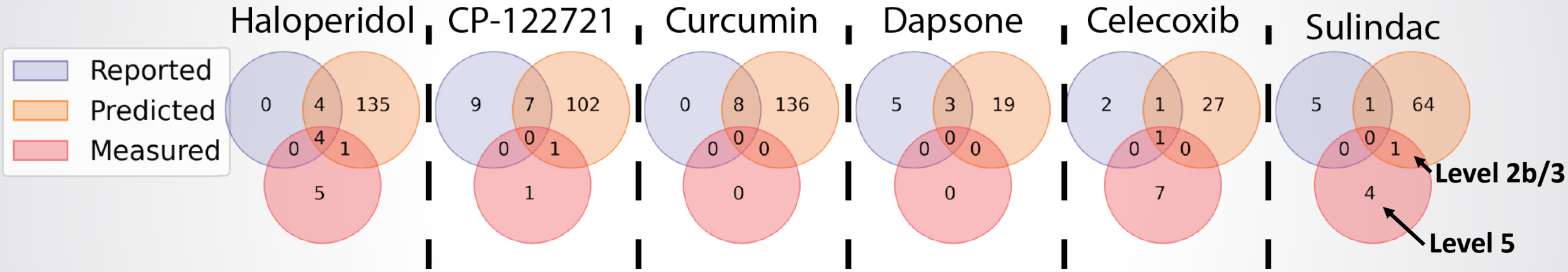
DB Score: 82.12



Predicted Formula (level 5):

- $C_9H_{13}ClN_2O_6$ (278.0816)
- $C_9H_{11}NO_3$ (181.0738)
- $C_6H_{10}O_3$ (130.0632)
- $C_{24}H_30N_2O_{11}$ (522.1851)

Distribution of tentative identifications



Summary:

- 3 metabolites not reported in literature, but identified via *in silico* tools
- 5 metabolites identified through all sources
- 17 features without known structures, but related to metabolites

Acknowledgements

Environmental Protection Agency

- Grace Patlewicz
- John Wambaugh
- Lucina Lizarraga
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- Brian Meyer
- Vicente Samano
- Nancy Baker
- Daniel Chang
- Elin Ulrich

Southwest Research Institute

- Kristin Favela

Thermo Scientific

- Jessica A. Bonzo

Where to reach me

Boyce.matthew@epa.gov

linkedin.com/in/mwboyce16/

orcid.org/0000-0002-3794-1678